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Robbins and Cotran PATHOLOGIC BASIS OF DISEASE

Seventh Edition

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IN MEMORY OF
Dr. Stanley L. Robbins (1915–2003)
and
Dr. Ramzi S. Cotran (1932–2000)
Dear friends, respected colleagues, and dedicated teachers
They leave a legacy of excellence
that will enrich the lives of generations
of future physicians.

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Preface

We launch the seventh edition of *Pathologic Basis of Disease* with mixed emotions, excitement and enthusiasm, as we enter the new millennium, tempered by sadness over the loss of our dear colleagues Drs. Stanley Robbins and Ramzi Cotran. To acknowledge their immeasurable and everlasting contribution to this text, the book is now renamed *Robbins and Cotran Pathologic Basis of Disease*.

This edition, like all previous ones, has been extensively revised, and some areas completely rewritten. Some of the more significant changes are as follows:

- Chapter 1 has been completely reorganized to include the entire spectrum of cellular responses to injury, from adaptations and sublethal injury to cell death. This was accomplished by combining the first two chapters of the sixth edition. We believe that this integrated and extensively revised chapter will allow a better understanding of cell injury, the most fundamental process in disease causation.
- Chapter 3 , covering tissue repair and wound healing, has been extensively revised to include new and exciting information in stem cell biology and the emerging field of regenerative medicine.
- Chapter 8 , dealing with infectious diseases, has been organized taxonomically with emphasis on mechanisms of tissue injury by different categories of infectious agents. While examples of infections by prototypic microorganisms have been retained, most of the organ-specific infectious diseases have been moved to later chapters where other diseases of the organ are described.
- Discussion of diabetes mellitus has been moved from the chapter on pancreatic diseases to the chapter on endocrine disorders, where it blends more logically with other hormonal diseases.
- Discussions of the lower urinary tract and the male genital system have been combined and grouped into a single chapter in recognition of the fact that there is overlap in diseases and diagnostic considerations.
- A new feature best described as "boxes" has been introduced in selected chapters. For boxes, we have selected topics at the cutting edge of science that are worthy of a more detailed presentation than is essential for a student textbook. In doing so, we hope that we have presented the excitement of the topic without encumbering the body of the text with details that may appear overwhelming to the beginning reader.
- The chapter on ocular diseases has been rewritten and reorganized to facilitate an understanding of ophthalmic pathology by the non-specialist.
- In addition to the revision and reorganization of the text, there have been significant changes in illustrations. Many new photographs and schematics have been added and a large number of the older "gems" have been enhanced by digital technology. Thus we hope that even the veterans of the Robbins Pathology titles who have seen many previous editions of the book will find the color illustrations more sparkling and fresh. Approximately 50 new pages of illustrations have been added.

In the 5 years since the previous edition, spectacular advances, including the completion of the human genome project, have occurred. Whenever appropriate we have blended the new discoveries into the discussion of pathogenesis and pathophysiology, yet never losing sight

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that the "state of the art" has little value if it does not enhance the understanding of disease mechanisms. As in the past, we have not avoided discussions of "unsolved" problems because of our belief that many who read the text might be encouraged to embark on a path of discovery.

Despite the changes outlined above and extensive revisions, our goals remain essentially the same.

- To integrate into the discussion of pathologic processes and disorders the newest established information available—morphologic and molecular.
- To organize the presentations into logical and uniform approaches, thereby facilitating readability, comprehension, and learning.
- To maintain a reasonable size of the book, and yet to provide adequate discussion of the significant lesions, processes, and disorders, allotting space in proportion to their clinical and biologic importance.
- To place great emphasis on clarity of writing and good usage of language in the recognition that struggling to comprehend is time-consuming and wearisome and gets in the way of the learning process.
- To make this first and foremost a student text—used by students throughout their 4 years of medical school and into their residencies—but, at the same time, to provide sufficient detail and depth to meet the needs of more advanced readers.

We have been repeatedly told by the readers that one of the features they value most in this book is its up-to-dateness. We have strived to maintain such timeliness by providing references from recent literature, many published in 2003 and some from the early part of 2004. However, older classics have also been retained to provide original source material for advanced readers.

With this edition, we also move into the digital age: the text will be available online to those who own the print version. This online access gives the reader the ability to search across the entire text, bookmark passages, add personal notes, use PubMed to view references, and many other exciting features, including timely updates. In addition, included in the text is a CD-ROM of case studies, previously available separately as the Interactive Case Study Companion developed by one of us (VK) in collaboration with Herb Hagler, PhD, and Nancy Schneider, MD, PhD, at the University of Texas, Southwestern Medical School in Dallas. This will enhance and reinforce learning by challenging students to apply their knowledge in solving clinical cases. A virtual microscope feature enables the viewing of selected images at various powers.

This edition is also marked by the addition of two new "seasoned" coauthors. All three of us have reviewed, critiqued, and edited each chapter to ensure uniformity of style and flow that have been the hallmarks of the text. Together, we hope that we have succeeded in bringing to the reader the excitement of the study of disease mechanisms and the desire to learn more than what can be offered in any textbook.

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Acknowledgments

The authors are grateful to a large number of individuals who have contributed in many ways toward the completion of this textbook.

First and foremost, all three of us offer our tributes and gratitude to two stalwarts of American pathology, Dr. Stanley Robbins and Dr. Ramzi Cotran. Their passion for excellence and uncompromising standards have made this book what it is. While neither of the two will see this edition in its completed form, their stamp on *Pathologic Basis of Disease* is indelible. Second, we thank our contributing authors for their commitment to this textbook. Many are veterans of previous editions; others are new to the seventh edition. All are acknowledged in the Table of Contents. Their names lend authority to this book, for which we are grateful.

Many colleagues have enhanced the text by reading various chapters and providing helpful critiques in their area of expertise. They include (at the University of Chicago): Drs. Todd Kroll,

Michelle LeBeau, Olaf Schneewind, Josephine Morello, Megan McNerney, Fred Wondisford, Aliya Husain, Jonathan Miller, Julian Solway, John Hart, Amy Noffsinger, Thomas Krausz, Raminder Kumar, Joanne Yocum, Christopher Weber, Elizabeth McNally, and Manny Utset; (at the University of California at San Francisco): Drs. Steve Gitelman, Jonathan Lin, David Wofsy, Patrick Treseler, Mark Anderson, and Aaron Tward; (at the University of Washington, Seattle): Drs. Zsolt Argenyi, Peter Beyers, Ann DeLancey, Charles Murry, Thomas Norwood, Brian Rubin, Paul Swanson, Melissa Upton, and Mathew Yeh. Dr. David Walker, at the University of Texas Medical Branch at Galveston provided a thorough critique of the chapter on infectious disease. Dr. Lora Hendrick Ellenson at Cornell University (Weill Medical College) provided a critique of the chapter on the female genital tract. Dr. Arlene Herzberg and Kelly McGuigan provided help with the chapter on skin diseases.

Special thanks are owed to Dr. Henry Sanchez at the University of California at San Francisco for his painstaking review and revision of the older color illustrations and for his magic touch in enhancing them digitally. Their freshness will be obvious to the readers. Many colleagues provided photographic gems from their collection. They are individually acknowledged in the text.

Our administrative staff needs to be acknowledged since they maintain order in the chaotic lives of the authors and have willingly chipped in when needed for multiple tasks relating to the text. At the University of Chicago, they include Ms. Vera Davis and Ms. Ruthie Cornelius; at The University of California at San Francisco, Ms. Ana Narvaez; and at the University of Washington, Seattle, Ms. Catherine Alexander, Steven Berard, Carlton Kim, Ms. Genevieve Thomas, and Ms. Vicki Tolbert. Ms. Beverly Shackelford at the University of Texas at Dallas, who has helped one of us (VK) for 21 years, deserves special mention since she coordinated the submission of all manuscripts, proofread many of them, and maintained liaison with the contributors and publisher. Without her dedication to this book and her meticulous attention to detail, our task would have been much more difficult. Most of the graphic art in this book was created by Mr. James Perkins, Assistant Professor of Medical Illustration at Rochester Institute of Technology. His ability to convert complex ideas into simple and aesthetically pleasing sketches has considerably enhanced this book.

Many individuals associated with our publisher, Elsevier (under the imprint of W.B. Saunders), need our special thanks. Outstanding among them is Ellen Sklar, Production Editor, supervising the production of this book. Her understanding of the needs of the authors and the complexity of publishing a textbook went a long way in making our lives less complicated.

Mr. William Schmitt, Publishing Director of Medical Textbooks, has always been our cheerleader and is now a dear friend. Our thanks also go to Managing Editor Rebecca Gruliow and Design Manager Ellen Zanolle at Elsevier. Undoubtedly there are many other "heroes" who may have been left out unwittingly—to them we say "thank you" and tender apologies for not acknowledging you individually.

Efforts of this magnitude take a heavy toll on the families of the authors. We thank our spouses Raminder Kumar, Ann Abbas, and Ann DeLancey for their patience, love, and support of this venture, and for their tolerance of our absences.

Finally, Vinay Kumar wishes to express his deep appreciation to Drs. Abul Abbas and Nelson Fausto for joining the team, and together we salute each other for shared vision and dedication to medical education. Despite differences in our vantage points, opinions, and individual styles, our common goal made this an exciting and rewarding partnership.

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Section I - General Pathology

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Chapter 1 - Cellular Adaptations, Cell Injury, and Cell Death

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Introduction to Pathology

Pathology is literally the study *(logos)* of suffering *(pathos)*. More specifically, it is abridging discipline involving both basic science and clinical practice and is devoted to the study of the structural and functional changes in cells, tissues, and organs that underlie disease. By the use of molecular, microbiologic, immunologic, and morphologic techniques, pathology attempts to explain the whys and wherefores of the signs and symptoms manifested by patients while providing a sound foundation for rational clinical care and therapy.

Traditionally, the study of pathology is divided into general pathology and special, or systemic, pathology. The former is concerned with the basic reactions of cells and tissues to abnormal stimuli that underlie all diseases. The latter examines the specific responses of specialized organs and tissues to more or less well-defined stimuli. In this book, we first cover the principles of general pathology and then proceed to specific disease processes as they affect particular organs or systems.

The four aspects of a disease process that form the core of pathology are its cause *(etiology)*, the mechanisms of its development *(pathogenesis)*, the structural alterations induced in the cells and organs of the body *(morphologic changes)*, and the functional consequences of the morphologic changes *(clinical significance)*.

Etiology or Cause.

The concept that certain abnormal symptoms or diseases are "caused" is as ancient as recorded history. For the Arcadians (2500 BC), if someone became ill, it was the patient's own fault (for having sinned) or the makings of outside agents, such as bad smells, cold, evil spirits, or gods.[1] In modern terms, there are two major classes of etiologic factors: intrinsic or genetic, and acquired (e.g., infectious, nutritional, chemical, physical). The concept, however, of one etiologic agent for one disease — developed from the study of infections or single-gene disorders — is no longer sufficient. Genetic factors are clearly involved in some of the common environmentally induced maladies, such as atherosclerosis and cancer, and the environment may also have profound influences on certain genetic diseases. Knowledge or discovery of the primary cause remains the backbone on which a diagnosis can be made, a disease understood, or a treatment developed.

Pathogenesis.

Pathogenesis refers to the sequence of events in the response of cells or tissues to the etiologic agent, from the initial stimulus to the ultimate expression of the disease. The study of pathogenesis remains one of the main domains of pathology. Even when the initial infectious or molecular cause is known, it is many steps removed from the expression of the disease. For example, to understand cystic fibrosis is to know not only the defective gene and gene product, but also the biochemical, immunologic, and morphologic events leading to the formation of cysts and fibrosis in the lung, pancreas, and other organs. Indeed, as we shall see throughout the book, the molecular revolution has already identified mutant genes underlying a great number of diseases, and the entire human genome has been mapped. Nevertheless, the functions of the encoded proteins and how mutations induce disease are often still obscure. Because of technologic advances, it is becoming increasingly feasible to link specific molecular abnormalities to disease manifestations and to use this knowledge to design new therapeutic approaches. For these reasons, the study of pathogenesis has never been more exciting scientifically or more relevant to medicine.

Morphologic Changes.

The morphologic changes refer to the structural alterations in cells or tissues that are either characteristic of the disease or diagnostic of the etiologic process. The practice of diagnostic pathology is devoted to identifying the nature and progression of disease by studying morphologic changes in tissues and chemical alterations in patients. More recently, the limitations of morphology for diagnosing diseases have become increasingly evident, and the field of diagnostic pathology has expanded to encompass molecular biologic and immunologic approaches for analyzing disease states. Nowhere is this more striking than in the study of tumors — breast cancers and tumors of lymphocytes that look morphologically identical may have widely different courses, therapeutic responses, and prognosis. Molecular analysis by techniques such as DNA microarrays has begun to reveal genetic differences that bear on the behavior of the tumors. Increasingly, such techniques are being used to extend and even supplant traditional morphologic methods.

Functional Derangements and Clinical Manifestations.

The nature of the morphologic changes and their distribution in different organs or tissues influence normal function and determine the clinical features (symptoms and signs), course, and prognosis of the disease.

Virtually all forms of organ injury start with molecular or structural alterations in cells, a concept first put forth in the nineteenth century by Rudolf Virchow, known as the father of modern pathology. We therefore begin our consideration of pathology with the study of the origins, molecular mechanisms, and structural changes of cell injury. Yet different cells in tissues constantly interact with each other, and an elaborate system of *extracellular matrix* is necessary for the integrity of organs. Cell-cell and cell-matrix interactions contribute significantly to the response to injury, leading collectively to *tissue and organ injury*, which are as important as cell injury in defining the morphologic and clinical patterns of disease.

Overview: Cellular Responses to Stress and Noxious Stimuli

The normal cell is confined to a fairly narrow range of function and structure by its genetic programs of metabolism, differentiation, and specialization; by constraints of neighboring cells; and by the availability of metabolic substrates. It is nevertheless able to handle normal physiologic demands, maintaining a steady state called *homeostasis*. More severe physiologic stresses and some pathologic stimuli may bring about a number of physiologic and morphologic *cellular adaptations*, during which new but altered steady states are achieved, preserving the viability of the cell and modulating its function as it responds to such stimuli (Fig. 1-1 and Table 1-1). The adaptive response may consist of an increase in the number of cells, called *hyperplasia*, or an increase in the sizes of individual cells, called *hypertrophy*. Conversely, *atrophy* is an adaptive response in which there is a decrease in the size and function of cells.

Figure 1-1 Stages in the cellular response to stress and injurious stimuli.

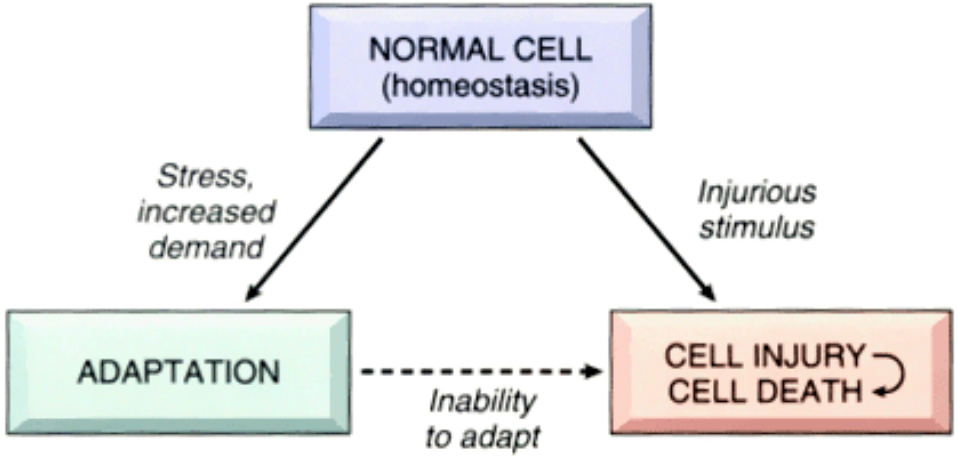


TABLE 1-1 -- Cellular Responses to Injury

Nature and Severity of Injurious Stimulus	**Cellular Response**
Altered physiologic stimuli:	**Cellular adaptations:**
• Increased demand, increased trophic stimulation (e.g. growth factors, hormones)	• Hyperplasia, hypertrophy
• Decreased nutrients, stimulation	• Atrophy
• Chronic irritation (chemical or physical)	• Metaplasia
Reduced oxygen supply; chemical injury; microbial infection	**Cell injury:**
• Acute and self-limited	• Acute reversible injury
• Progessive and severe (including DNA damage)	• Irreversible injury → cell death
	•••••Necrosis
	•••••Apoptosis
• Mild chronic injury	• Subcellular alterations in various organelles
Metabolic alterations, genetic or acquired	**Intracellular accumulations; calcifications**
Prolonged life span with cumulative sublethal injury	**Cellular aging**

hormone. It also occurs in certain pathologic conditions, when cells are damaged beyond repair, and especially if the damage affects the cell's nuclear DNA. We will return to a detailed discussion of these pathways of cell death later in the chapter.

Stresses of different types may induce changes in cells and tissues other than adaptations, cell injury, and death (see Table 1-1). Cells that are exposed to sublethal or chronic stimuli may not be damaged but may show a variety of *subcellular alterations*. Metabolic derangements in cells may be associated with *intracellular accumulations* of a number of substances, including proteins, lipids, and carbohydrates. Calcium is often deposited at sites of cell death, resulting in *pathologic calcification*. Finally, *cell aging* is also accompanied by characteristic morphologic and functional changes.

In this chapter, we discuss first how cells adapt to stresses, and then the causes, mechanisms, and consequences of the various forms of acute cell damage, including cell injury and cell death. We conclude with subcellular alterations induced by sublethal stimuli, intracellular accumulations, pathologic calcification, and cell aging.

Cellular Adaptations of Growth and Differentiation

Cells respond to increased demand and external stimulation by *hyperplasia* or *hypertrophy*, and they respond to reduced supply of nutrients and growth factors by *atrophy*. In some situations, cells change from one type to another, a process called *metaplasia*. There are numerous molecular mechanisms for cellular adaptations. Some adaptations are induced by direct stimulation of cells by factors produced by the responding cells themselves or by other cells in the environment. Others are due to activation of various cell surface receptors and downstream signaling pathways. Adaptations may be associated with the induction of new protein synthesis by the target cells, as in the response of muscle cells to increased physical demand, and the induction of cellular proliferation, as in responses of the endometrium to estrogens. Adaptations can also involve a switch by cells from producing one type of proteins to another or markedly overproducing one protein; such is the case in cells producing various types of collagens and extracellular matrix proteins in chronic inflammation and fibrosis (Chapter 2 and Chapter 3).

Figure 1-2 The relationships between normal, adapted, reversibly injured, and dead myocardial cells. The cellular adaptation depicted here is hypertrophy, and the type of cell death is ischemic necrosis. In reversibly injured myocardium, generally effects are only functional, without any readily apparent gross or even microscopic changes. In the example of myocardial hypertrophy, the left ventricular wall is more than 2 cm in thickness (normal is 1 to 1.5 cm). In the specimen showing necrosis, the transmural light area in the posterolateral left ventricle represents an acute myocardial infarction. All three transverse sections have been stained with triphenyltetrazolium chloride, an enzyme substrate that colors viable myocardium magenta. Failure to stain is due to enzyme leakage after cell death.

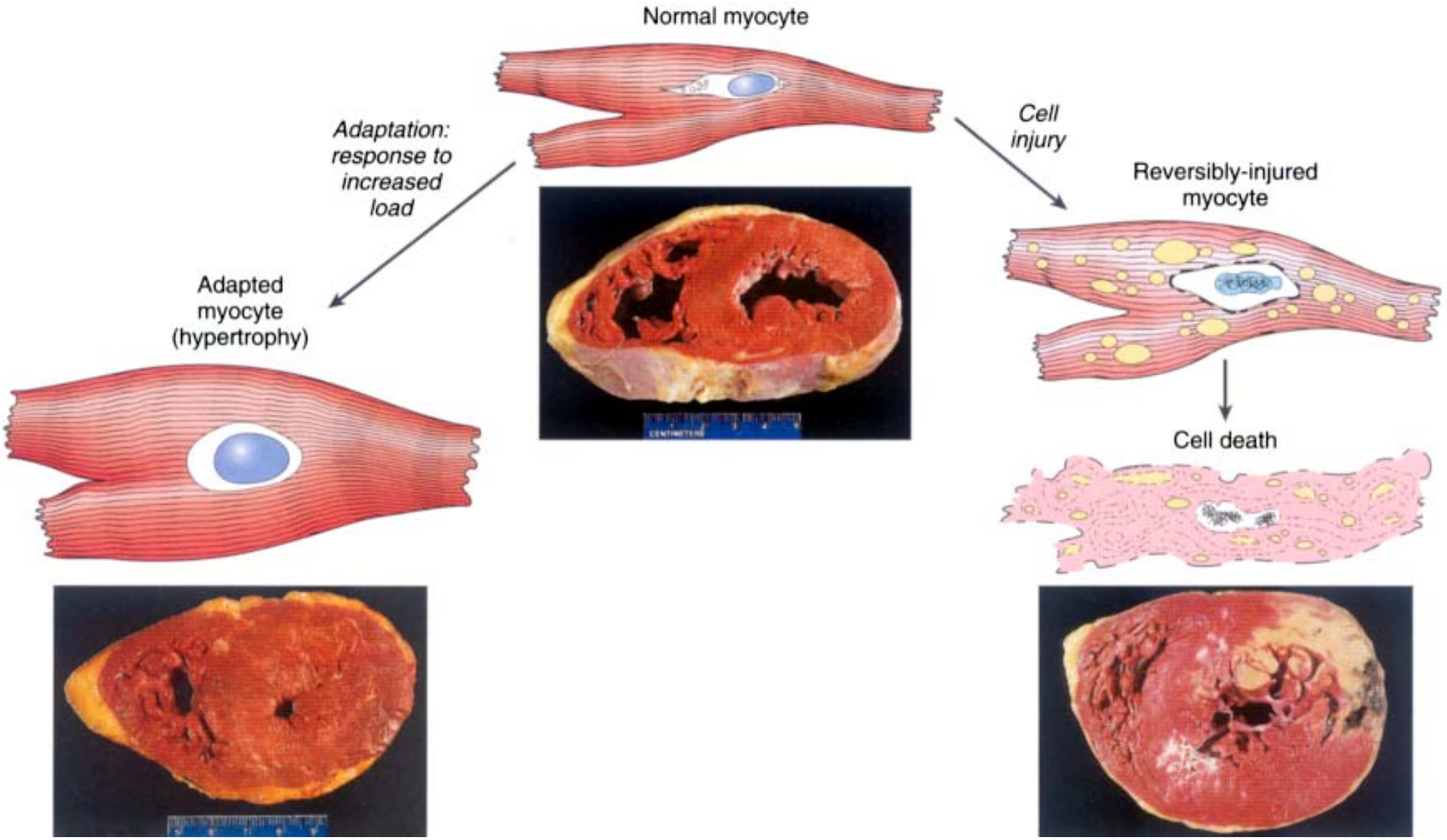


Figure 1-3 Physiologic hypertrophy of the uterus during pregnancy. *A,* Gross appearance of a normal uterus *(right)* and a gravid uterus (removed for postpartum bleeding) *(left). B,* Small spindle-shaped uterine smooth muscle cells from a normal uterus *(left)* compared with large plump cells in gravid uterus *(right)*.

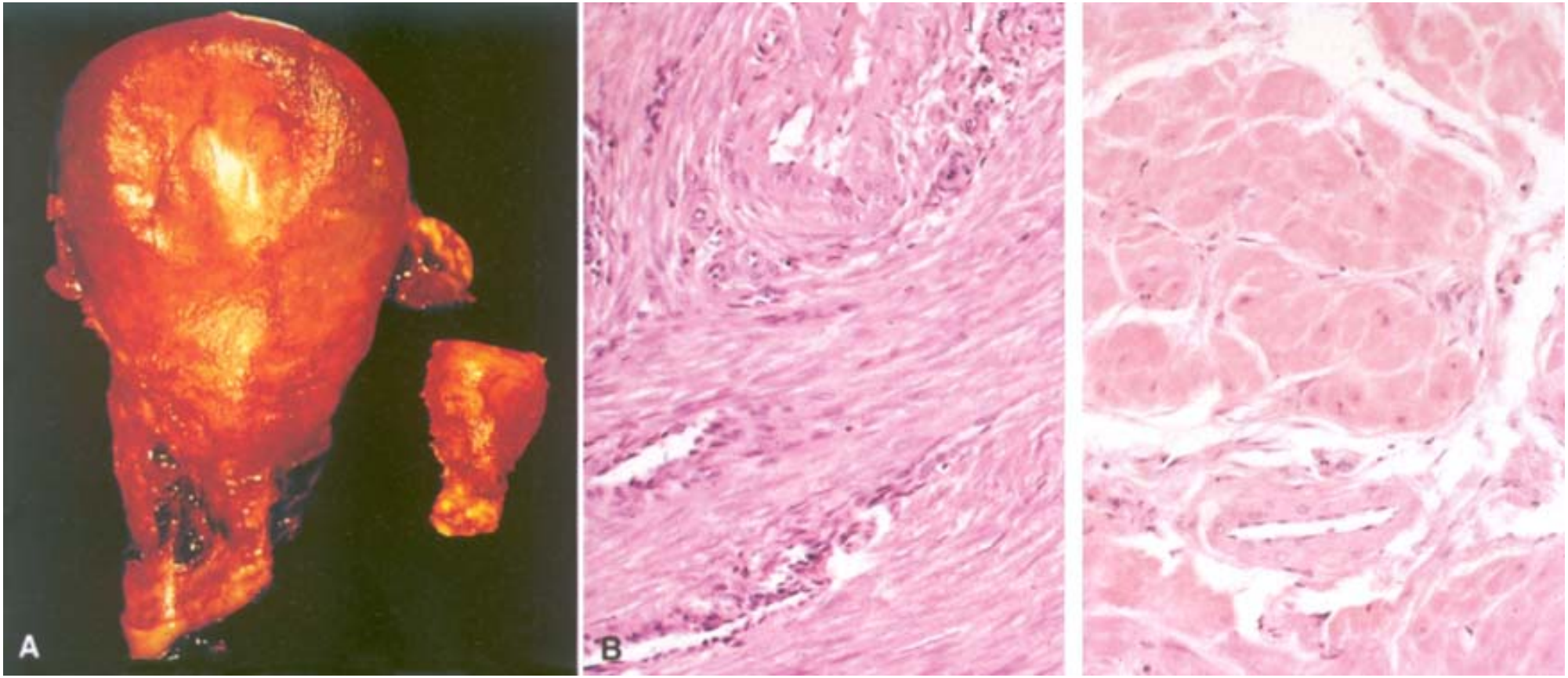


Figure 1-4 Changes in the expression of selected genes and proteins during myocardial hypertrophy.

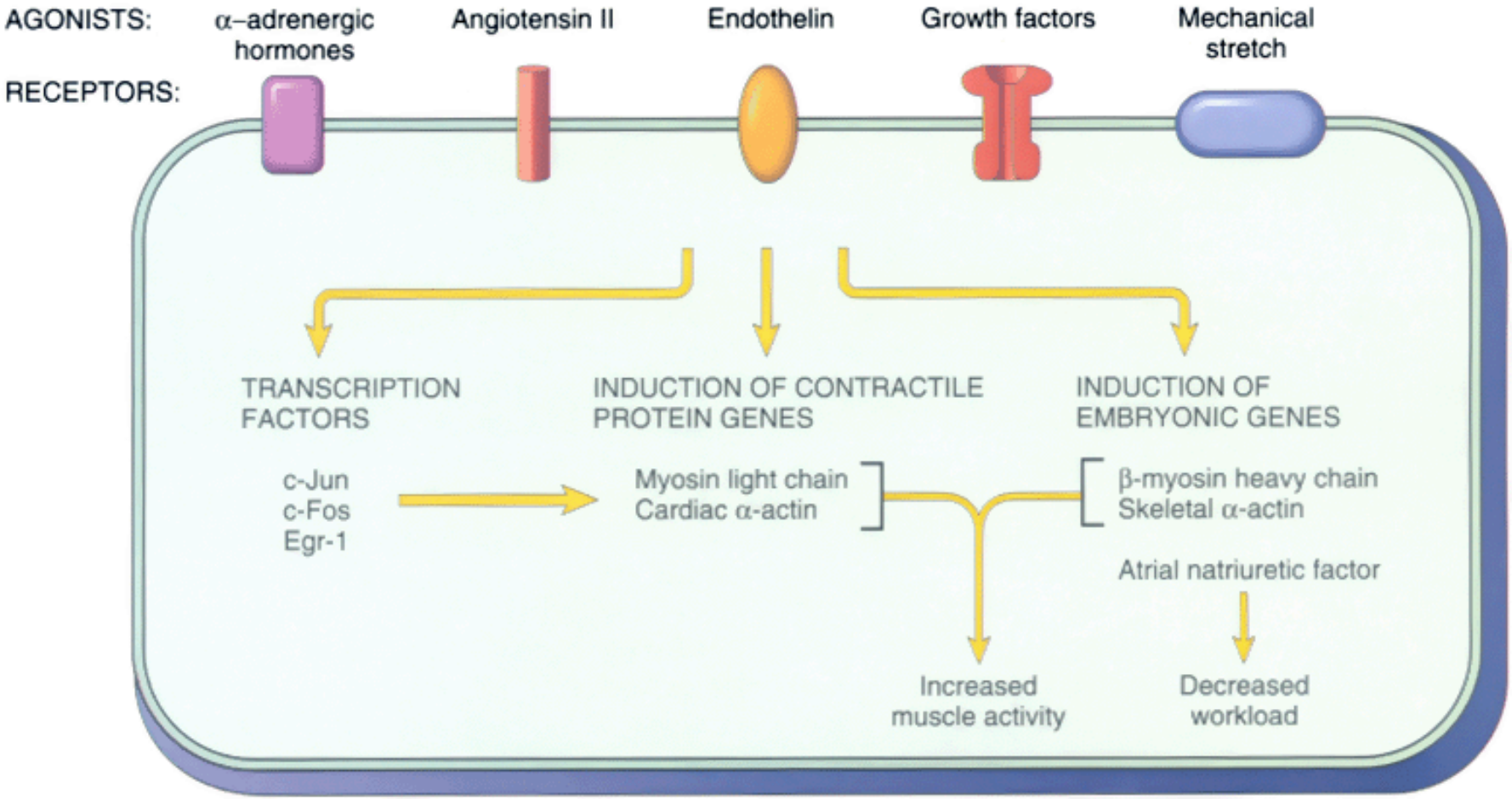


Figure 1-5 *A,* Atrophy of the brain in an 82-year-old male with atherosclerotic disease. Atrophy of the brain is due to aging and reduced blood supply. The meninges have been stripped. *B,* Normal brain of a 36-year-old male. Note that loss of brain substance narrows the gyri and widens the sulci.

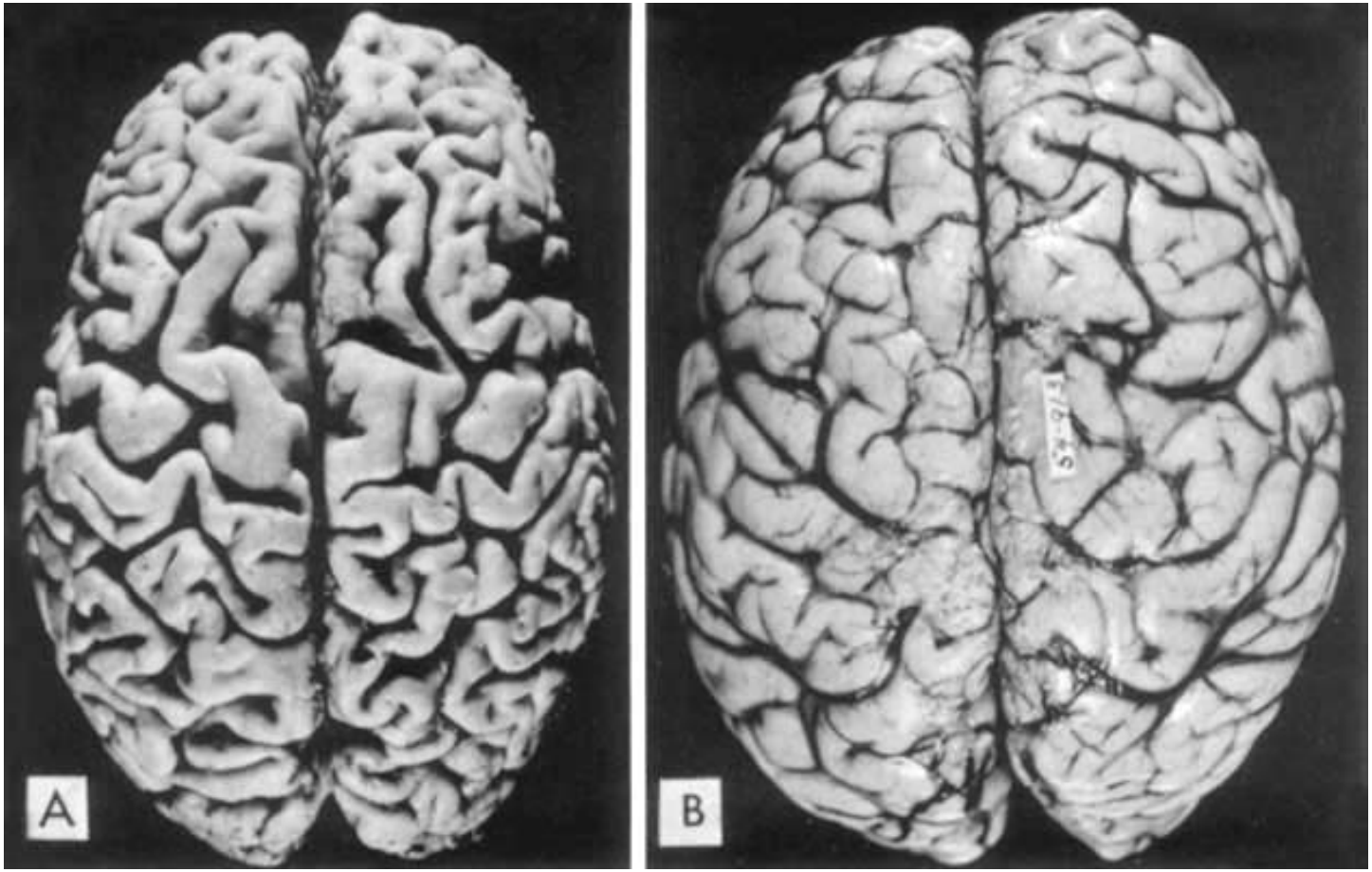
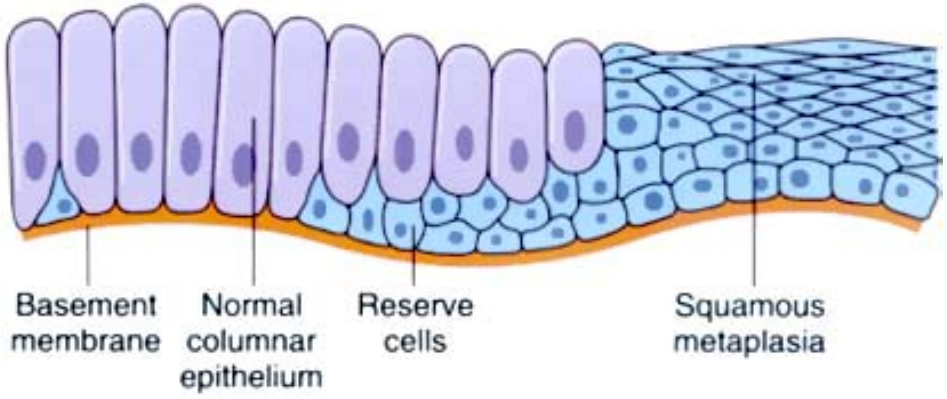


Figure 1-6 Metaplasia. *A,* Schematic diagram of columnar to squamous metaplasia. *B,* Metaplastic transformation of esophageal stratified squamous epithelium *(left)* to mature columnar epithelium (so-called Barrett metaplasia).



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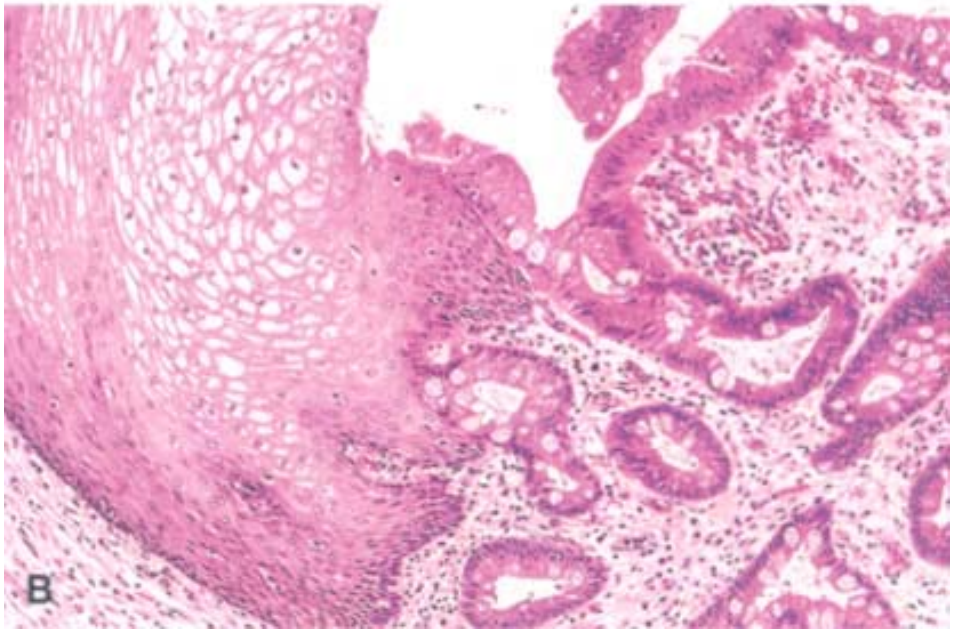


Irreversible injury and cell death. With continuing damage, the injury becomes irreversible, at which time the cell cannot recover. Is there a critical biochemical event (the "lethal hit") responsible for the point of no return? There are no clear answers to this question. However, as discussed later, in ischemic tissues such as the myocardium, certain structural changes (e.g., amorphous densities in mitochondria, indicative of severe mitochondrial damage) and functional changes (e.g., loss of membrane permeability) are indicative of cells that have suffered irreversible injury.

• *Irreversibly injured cells invariably undergo morphologic changes that are recognized as cell death.* There are two types of cell death, necrosis and apoptosis, which differ in their morphology, mechanisms, and roles in disease and physiology (Fig. 1-9 and Table 1-2). When damage to membranes is severe, lysosomal enzymes enter the cytoplasm and digest the cell, and cellular contents leak out, resulting in *necrosis*. Some noxious stimuli, especially those that damage DNA, induce another type of death, *apoptosis*, which is characterized by nuclear dissolution without complete loss of membrane integrity. *Whereas necrosis is always a pathologic process, apoptosis serves many normal functions and is not necessarily associated with cell injury.* Although we emphasize the distinctions between necrosis and apoptosis, there may be some overlaps and common mechanisms between these two pathways. In addition, at least some types of stimuli may induce either apoptosis or necrosis, depending on the intensity and duration of the stimulus, the rapidity of the death process, and the biochemical derangements induced in the injured cell. The mechanisms and significance of these two death pathways are discussed later in the chapter.

Figure 1-7 Stages in the evolution of cell injury and death.

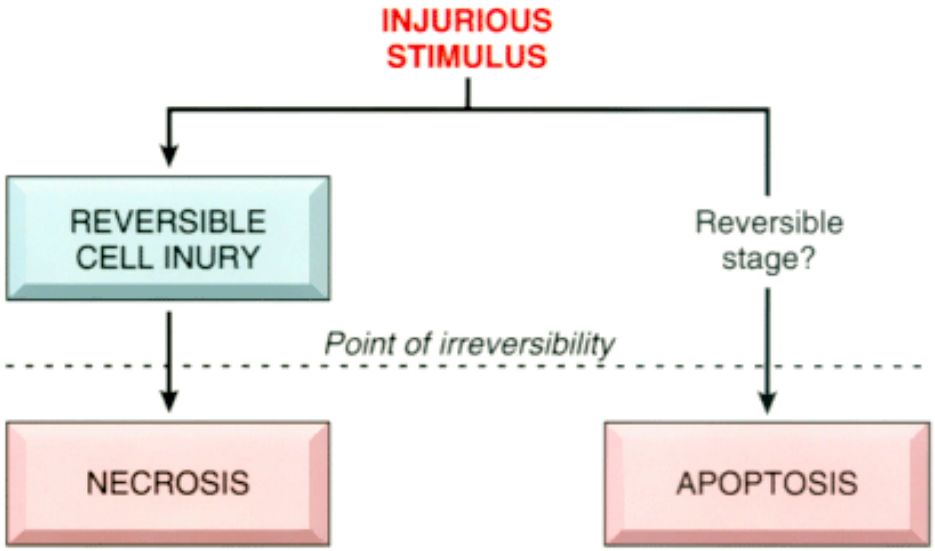
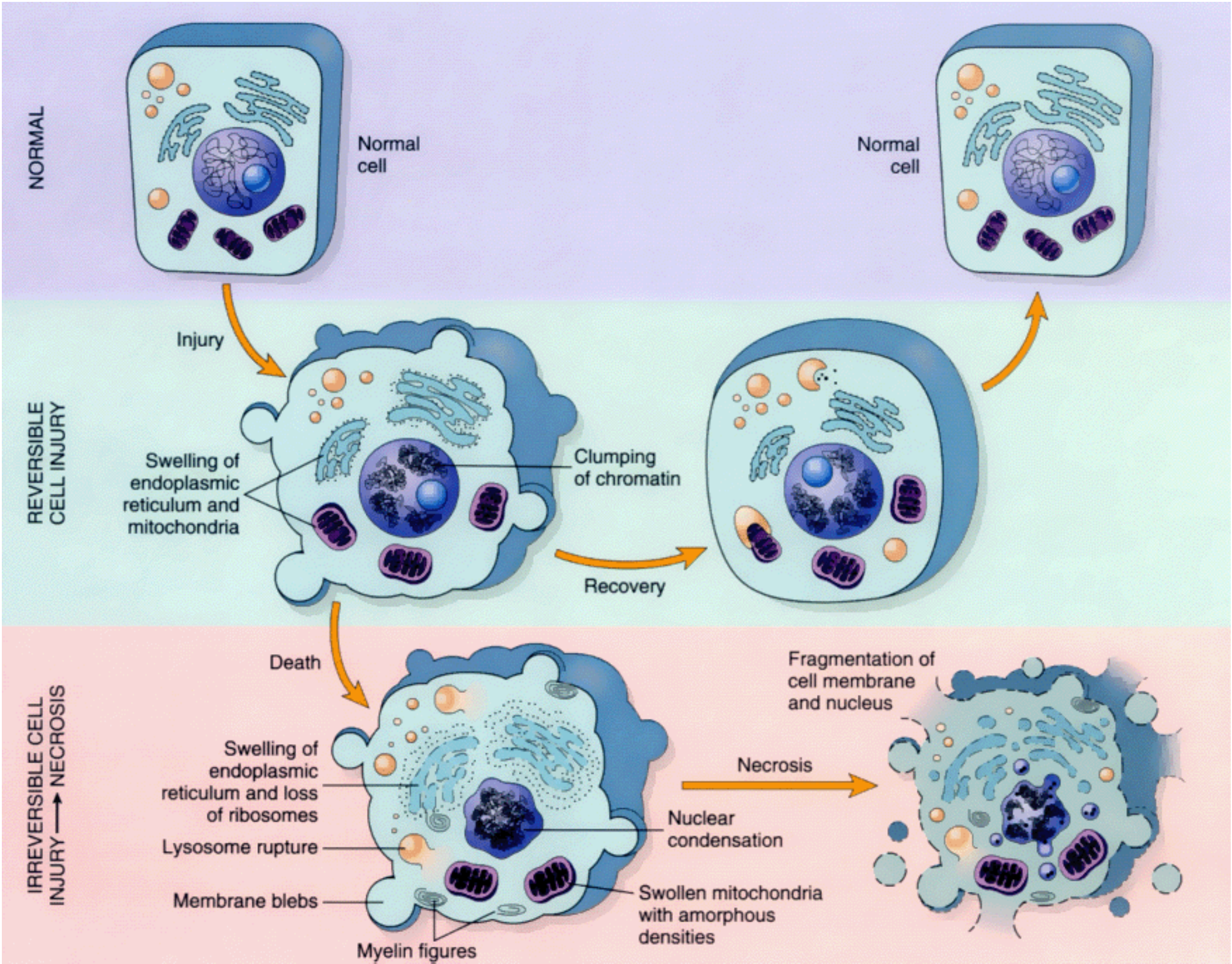


Figure 1-8 Schematic representation of a normal cell and the changes in reversible and irreversible cell injury. Depicted are morphologic changes, which are described in the following pages and shown in electron micrographs in Figure 1-17 . Reversible injury is characterized by generalized swelling of the cell and its organelles; blebbing of the plasma membrane; detachment of ribosomes from the endoplasmic reticulum; and clumping of nuclear chromatin. Transition to irreversible injury is characterized by increasing swelling of the cell; swelling and disruption of lysosomes; presence of large amorphous densities in swollen mitochondria; disruption of cellular membranes; and profound nuclear changes. The latter include nuclear codensation (pyknosis), followed by fragmentation (karyorrhexis) and dissolution of the nucleus (karyolysis). Laminated structures (myelin figures) derived from damaged membranes of organelles and the plasma membrane first appear during the reversible stage and become more pronounced in irreversibly damaged cells. The mechanisms underlying these changes are discussed in the text that follows.


NORMAL
Normal cell
Normal cell
REVERSIBLE CELL INJURY
Injury
Swelling of endoplasmic reticulum and mitochondria
Clumping of chromatin
Recovery
IRREVERSIBLE CELL INJURY → NECROSIS
Death
Swelling of endoplasmic reticulum and loss of ribosomes
Lysosome rupture
Membrane blebs
Myelin figures
Nuclear condensation
Swollen mitochondria with amorphous densities
Necrosis
Fragmentation of cell membrane and nucleus

Myelin figures densities

Figure 1-9 The sequential ultrastructural changes seen in necrosis *(left)* and apoptosis *(right)*. In apoptosis, the initial changes consist of nuclear chromatin condensation and fragmentation, followed by cytoplasmic budding and phagocytosis of the extruded apoptotic bodies. Signs of cytoplasmic blebs, and digestion and leakage of cellular components. *(Adapted from Walker NI, et al: Patterns of cell death. Methods Archiv Exp Pathol 13:18–32, 1988. Reproduced with permission of S. Karger AG, Basel.)*

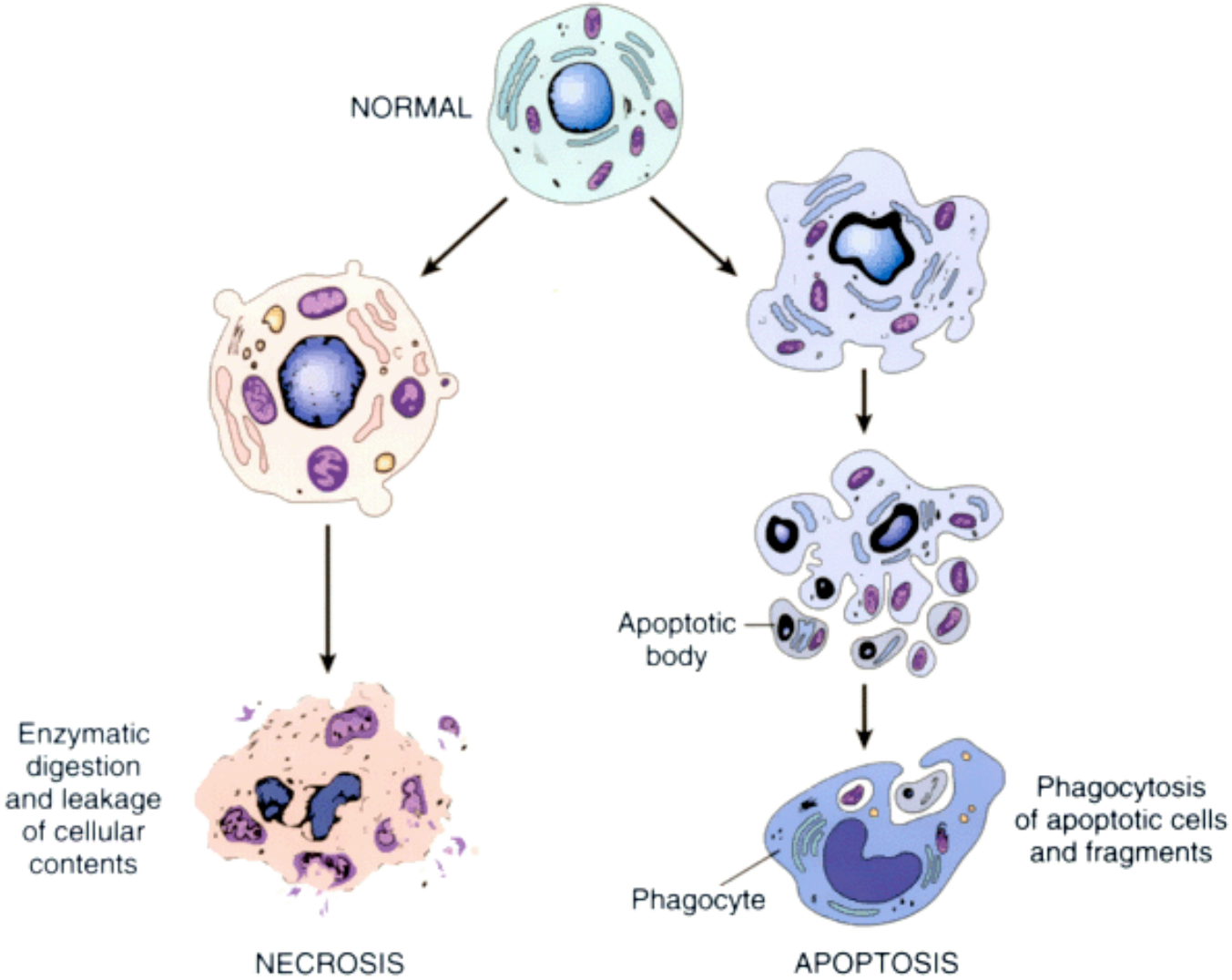


TABLE 1-2 -- Features of Necrosis and Apoptosis

Feature	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucelus	Pyknosis → karyorrhexis → karyolysis	Fragmentation into nucleosome size fragments

Plasma membrane	Disrupted	Intact; altered structure, especially orientation of lipids
Cellular contents	Enzymatic digestion; may leak out of cell	Intact; may be released in apoptotic bodies
Adjacent inflammation	Frequent	No
Physiologic or pathologic role	Invariably pathologic (culmination of irreversible cell injury)	Often physiologic, means of eliminating unwanted cells; may be pathologic after some forms of cell injury, especially DNA damage

however, are our daily companions: environmental and air pollutants, insecticides, and herbicides; industrial and occupational hazards, such as carbon monoxide and asbestos; social stimuli, such as alcohol and narcotic drugs; and the ever-increasing variety of therapeutic drugs.

Infectious Agents.

These agents range from the submicroscopic viruses to the large tapeworms. In between are the rickettsiae, bacteria, fungi, and higher forms of parasites. The ways by which this heterogeneous group of biologic agents cause injury are diverse and are discussed in Chapter 8 .

Immunologic Reactions.

Although the immune system serves an essential function in defense against infectious pathogens, immune reactions may, in fact, cause cell injury. The anaphylactic reaction to a foreign protein or a drug is a prime example, and reactions to endogenous self-antigens are responsible for a number of autoimmune diseases (Chapter 6).

Genetic Derangements.

Genetic defects as causes of cell injury are of major interest to scientists and physicians today (Chapter 5). The genetic injury may result in a defect as severe as the congenital malformations associated with Down syndrome, caused by a chromosomal abnormality, or as subtle as the decreased life of red blood cells caused by a single amino acid substitution in hemoglobin S in sickle cell anemia. The many inborn errors of metabolism arising from enzymatic abnormalities, usually an enzyme lack, are excellent examples of cell damage due to subtle alterations at the level of DNA. Variations in genetic makeup can also influence the susceptibility of cells to injury by chemicals and other environmental insults.

Nutritional Imbalances.

Nutritional imbalances continue to be major causes of cell injury. Protein-calorie deficiencies cause an appalling number of deaths, chiefly among underprivileged populations. Deficiencies of specific vitamins are found throughout the world (Chapter 9). Nutritional problems can be self-imposed, as in anorexia nervosa or self-induced starvation. Ironically, nutritional excesses have also become important causes of cell injury. Excesses of lipids predispose to atherosclerosis, and obesity is a manifestation of the overloading of some cells in the body with fats. Atherosclerosis is virtually endemic in the United States, and obesity is rampant. In addition to the problems of undernutrition and overnutrition, the composition of the diet makes a significant

Figure 1-10 Cellular and biochemical sites of damage in cell injury.

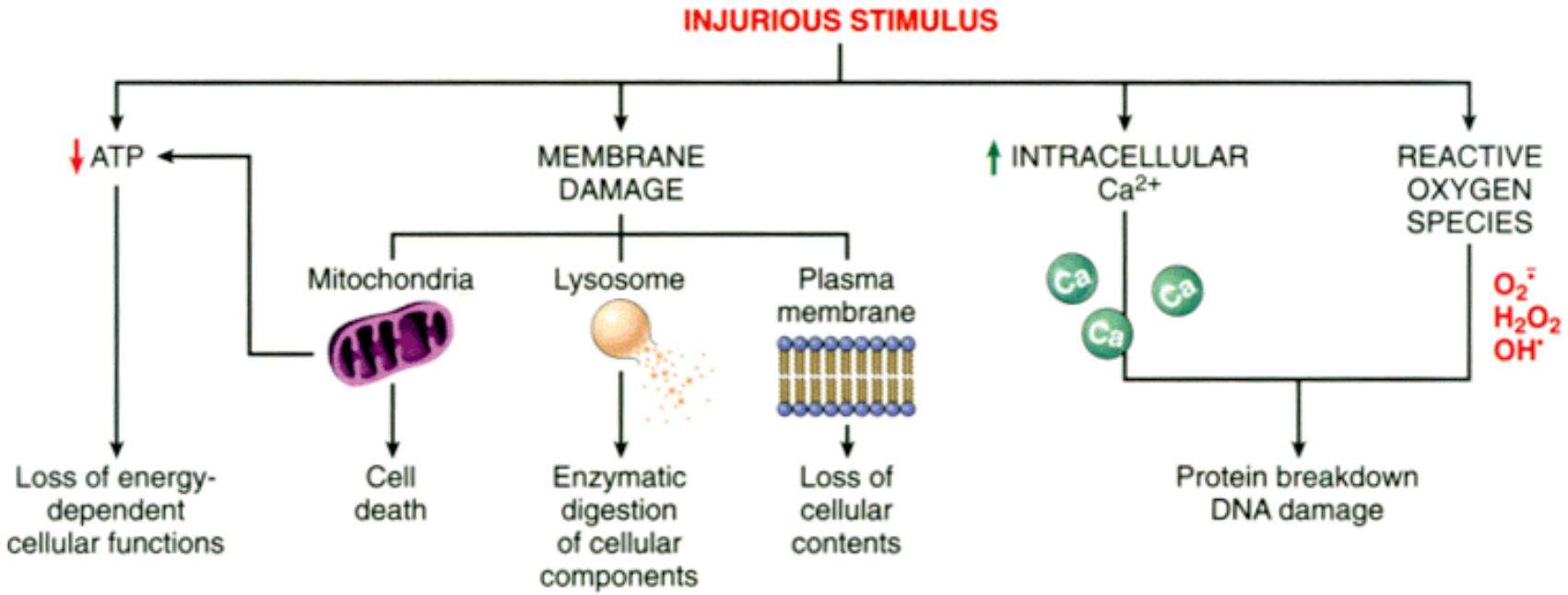


Figure 1-11 Functional and morphologic consequences of decreased intracellular ATP during cell injury.

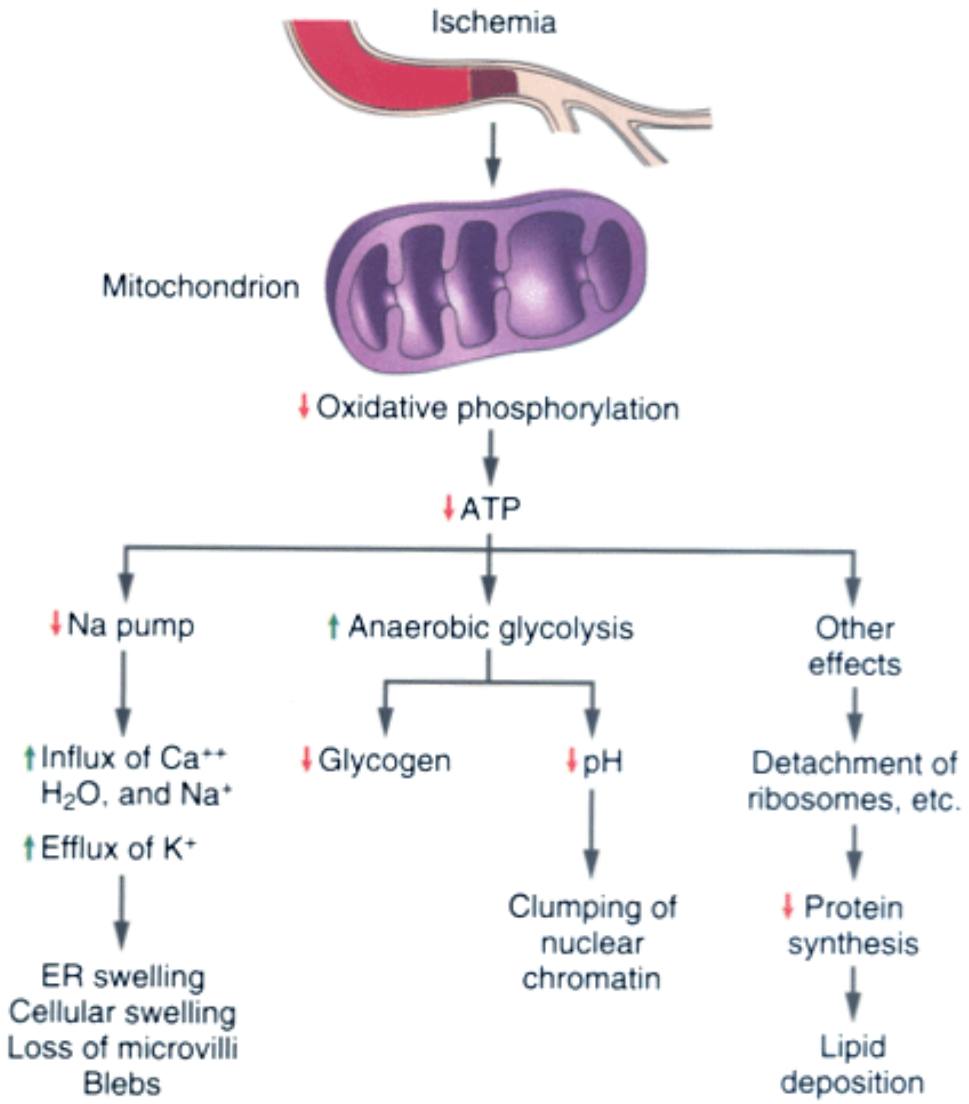


Figure 1-12 Mitochondrial dysfunction in cell injury.

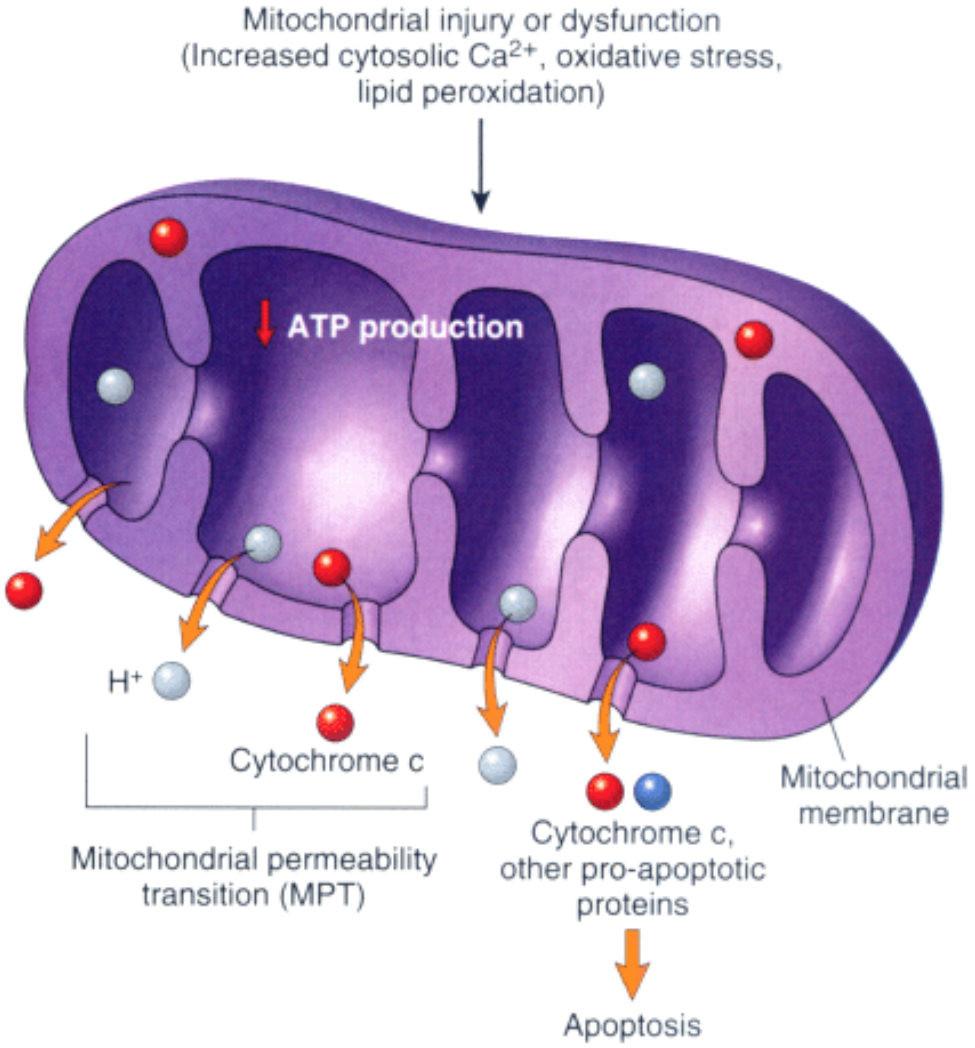


Figure 1-13 Sources and consequences of increased cytosolic calcium in cell injury. ATP, adenosine triphosphate.

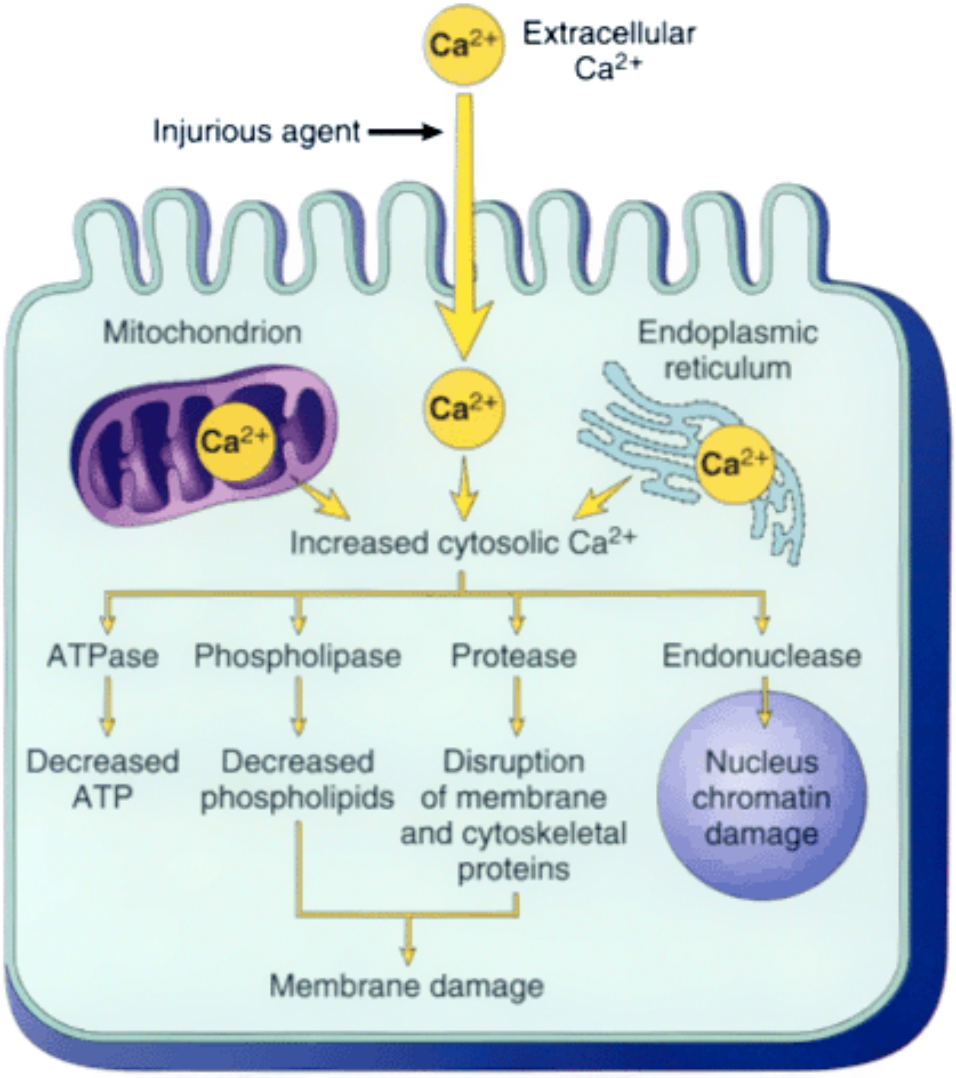


Figure 1-14 The role of reactive oxygen species in cell injury. O_2 is converted to superoxide (O_2^-) by oxidative enzymes in the endoplasmic reticulum (ER), mitochondria, plasma membrane, peroxisomes, and cytosol. O_2^- is converted to H_2O_2 by dismutation and thence to OH by the Cu^{2+} /Fe^{2+} -catalyzed Fenton reaction. H_2O_2 is also derived directly from oxidases in peroxisomes. Not shown is another potentially injurious radical, singlet oxygen. Resultant free radical damage to lipid (peroxidation), proteins, and DNA leads to various forms of cell injury. Note that superoxide catalyzes the reduction of Fe^{3+} to Fe^{2+} , thus enhancing OH generation by the Fenton reaction. The major antioxidant enzymes are superoxide dismutase (SOD), catalase, and glutathione peroxidase. GSH, reduced glutathione; GSSG, oxidized glutathione; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate.

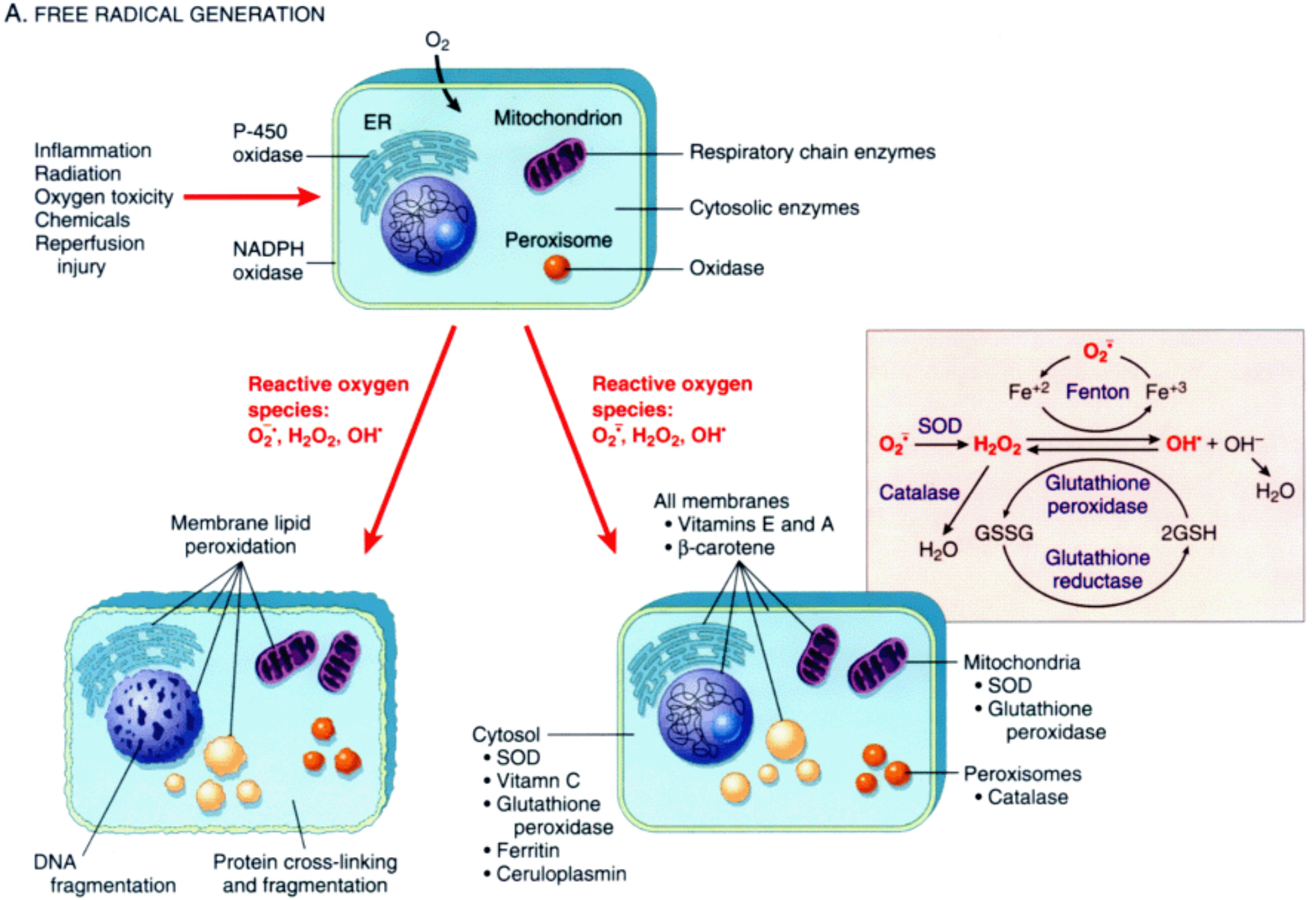

A. FREE RADICAL GENERATION
O_2
ER
Mitochondrion
P-450 oxidase
Inflammation
Radiation
Oxygen toxicity
Chemicals
Reperfusion injury
NADPH oxidase
Respiratory chain enzymes
Cytosolic enzymes
Peroxisome
Oxidase
Reactive oxygen species:
$O_2^{-\bullet}$, H_2O_2, $OH^\bullet$
Reactive oxygen species:
$O_2^{-\bullet}$, H_2O_2, $OH^\bullet$
$O_2^{-\bullet}$
Fe^{+2} Fenton Fe^{+3}
SOD
$O_2^{-\bullet}$ → H_2O_2 ⇌ $OH^\bullet$ + OH^-
Catalase
H_2O
Glutathione peroxidase
GSSG
2GSH
Glutathione reductase
H_2O
Membrane lipid peroxidation
DNA fragmentation
Protein cross-linking and fragmentation
All membranes
• Vitamins E and A
• β-carotene
Mitochondria
• SOD
• Glutathione peroxidase
Peroxisomes
• Catalase
Cytosol
• SOD
• Vitamn C
• Glutathione peroxidase
• Ferritin
• Ceruloplasmin
B. CELL INJURY BY FREE RADICALS
C. NEUTRALIZATION OF FREE RADICALS – NO CELL INJURY

Figure 1-15 Mechanisms of membrane damage in cell injury. Decreased O_2 and increased cytosolic Ca^{2+} are typically seen in ischemia but may accompany other forms of cell injury. Reactive oxygen species, which are often produced on reperfusion of ischemic tissues, also cause membrane damage (not shown).

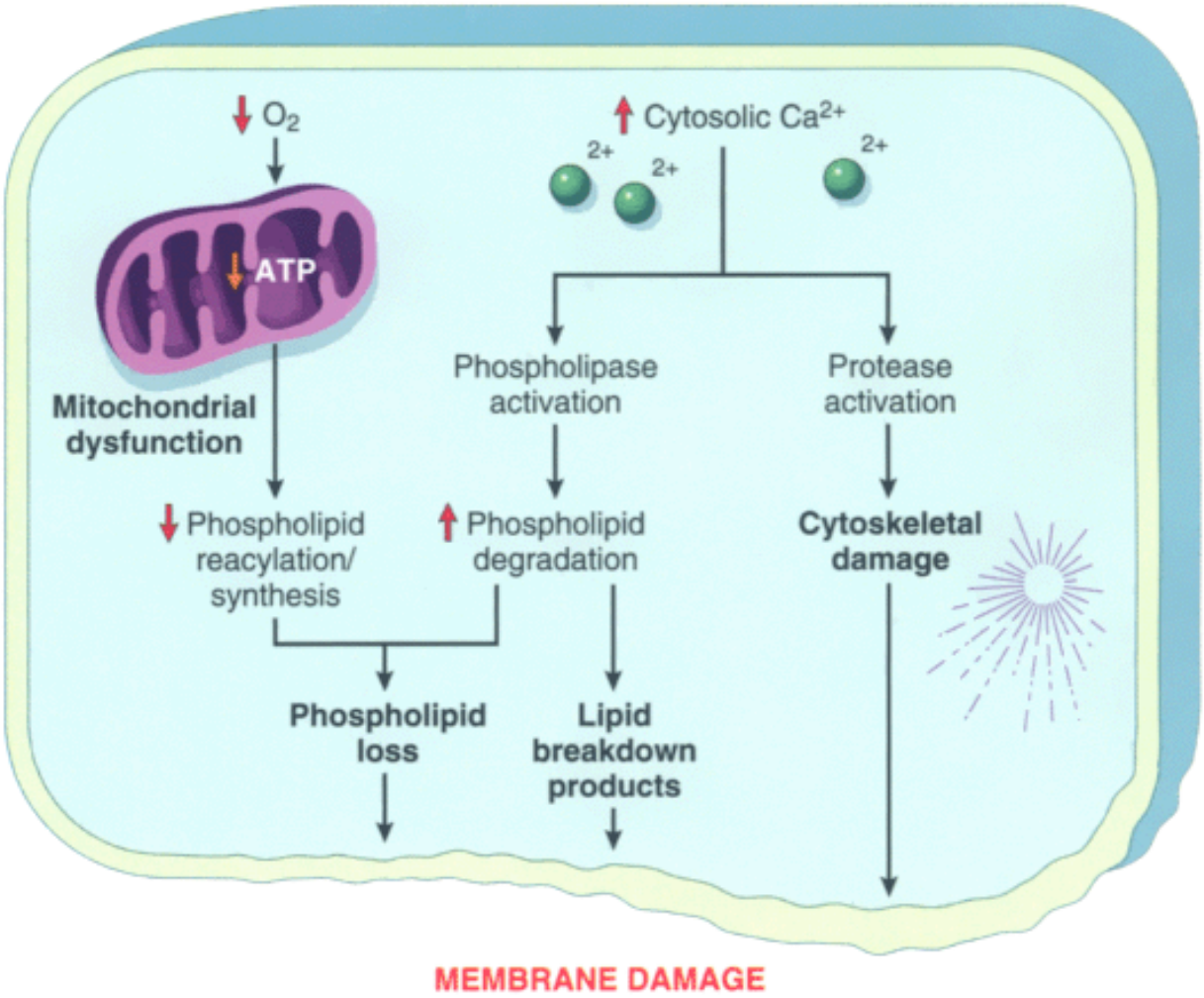


Figure 1-16 Timing of biochemical and morphologic changes in cell injury.

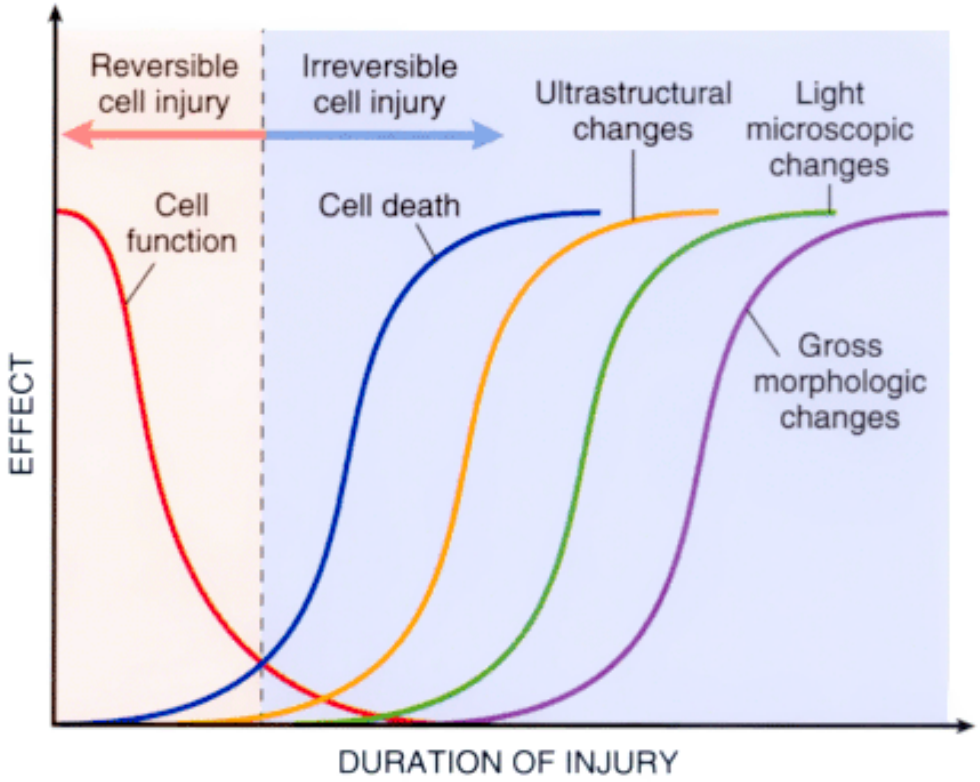
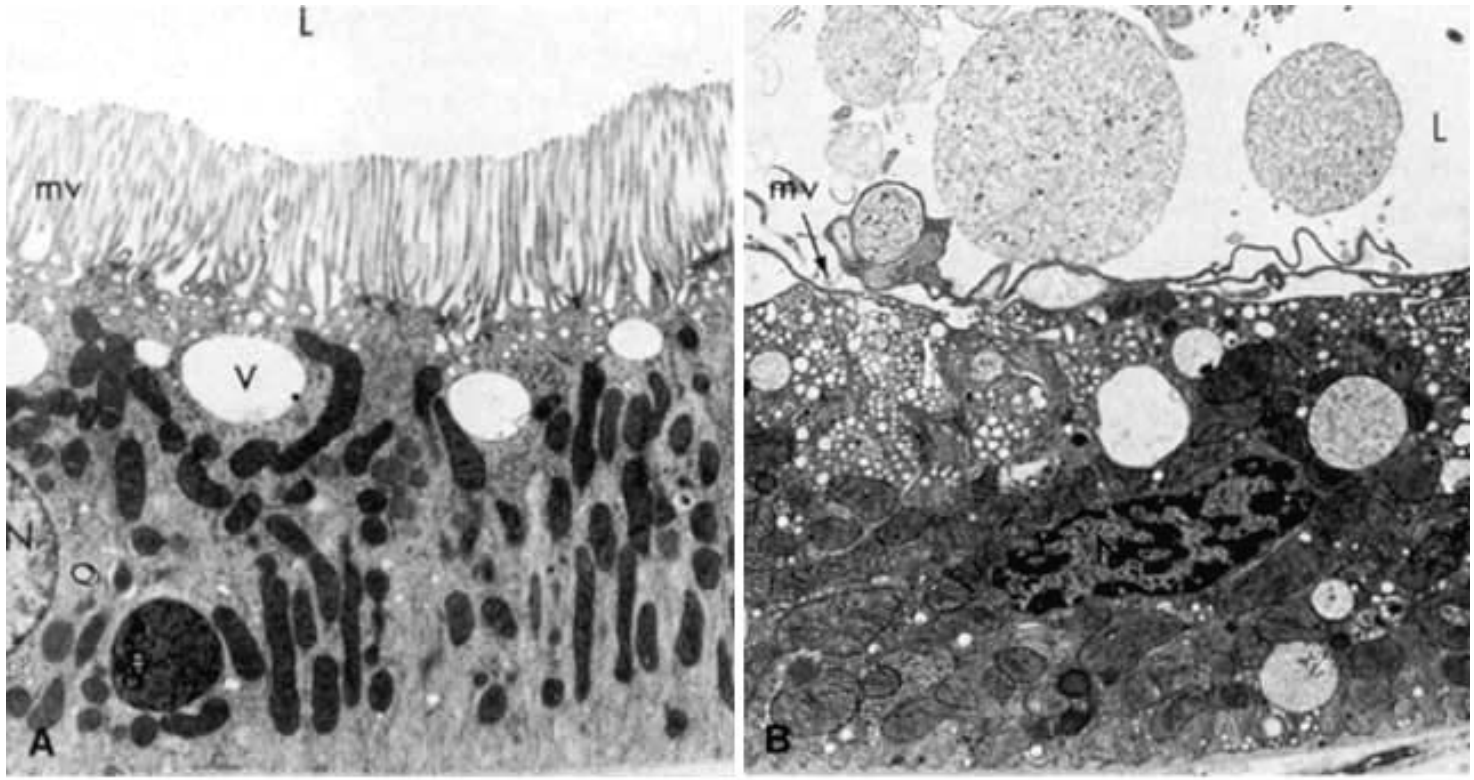


Figure 1-17 Morphologic changes in reversible and irreversible cell injury. *A*, Electron micrograph of a normal epithelial cell of the proximal kidney tubule. Note abundant microvilli (mv), lining the lumen (L). N, nucleus; V, apical vacuoles (which are normal structures in this cell type). *B*, Epithelial cell of the proximal tubule showing reversible ischemic changes. The microvilli (mv) are lost and have been incorporated in apical cytoplasm; blebs have formed and are extruded in the lumen (L). Mitochondria are slightly dilated. (Compare with *A*.) *C*, Proximal tubular cell showing irreversible ischemic injury. Note the markedly swollen mitochondria containing amorphous densities, disrupted cell membranes, and dense pyknotic nucleus. *(Courtesy of Dr. M. A. Venkatachalam, University of Texas, San Antonio, TX.)*


L
mv
V
N
A
mv
L
B

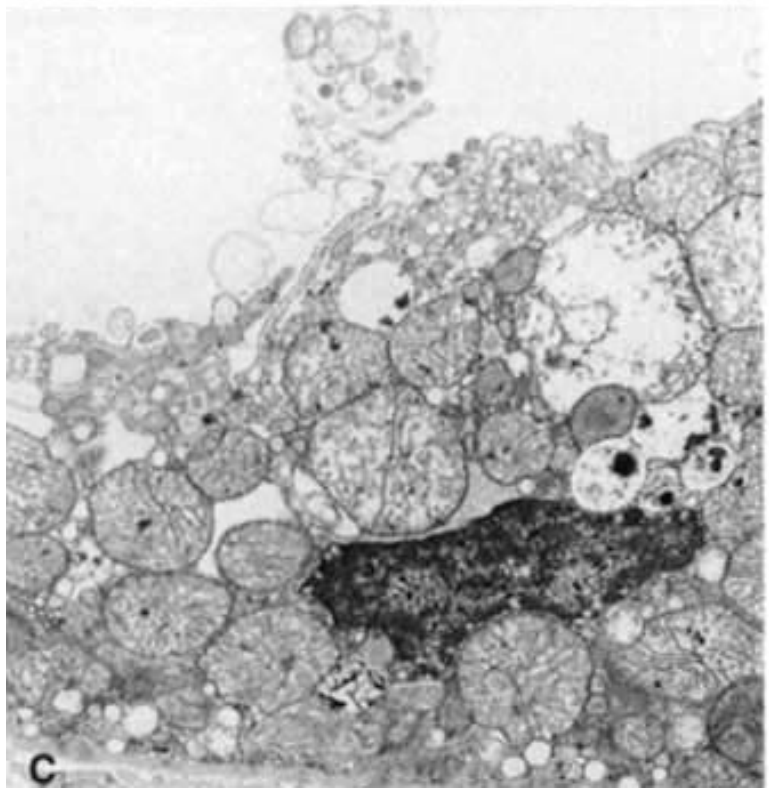

C

Figure 1-18 Ischemic necrosis of the myocardium. *A*, Normal myocardium. *B*, Myocardium with coagulation necrosis (upper two thirds of figure), showing strongly eosinophilic anucleate myocardial fibers. Leukocytes in the interstitium are an early reaction to necrotic muscle. Compare with *A* and with normal fibers in the lower part of the figure.

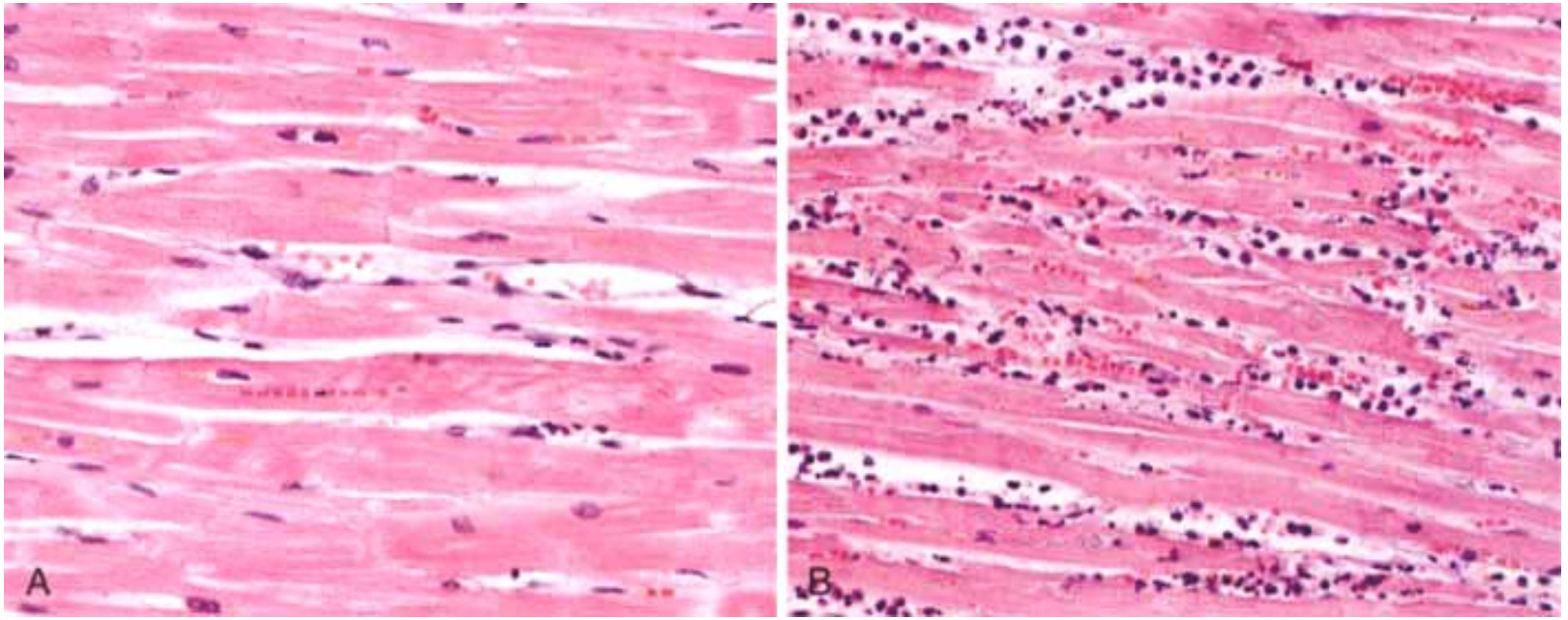


Figure 1-19 Coagulative and liquefactive necrosis. *A*, Kidney infarct exhibiting coagulative necrosis, with loss of nuclei and clumping of cytoplasm but with preservation of basic outlines of glomerular and tubular architecture. *B*, A focus of liquefactive necrosis in the kidney caused by fungal infection. The focus is filled with white cells and cellular debris, creating a renal abscess that obliterates the normal architecture.

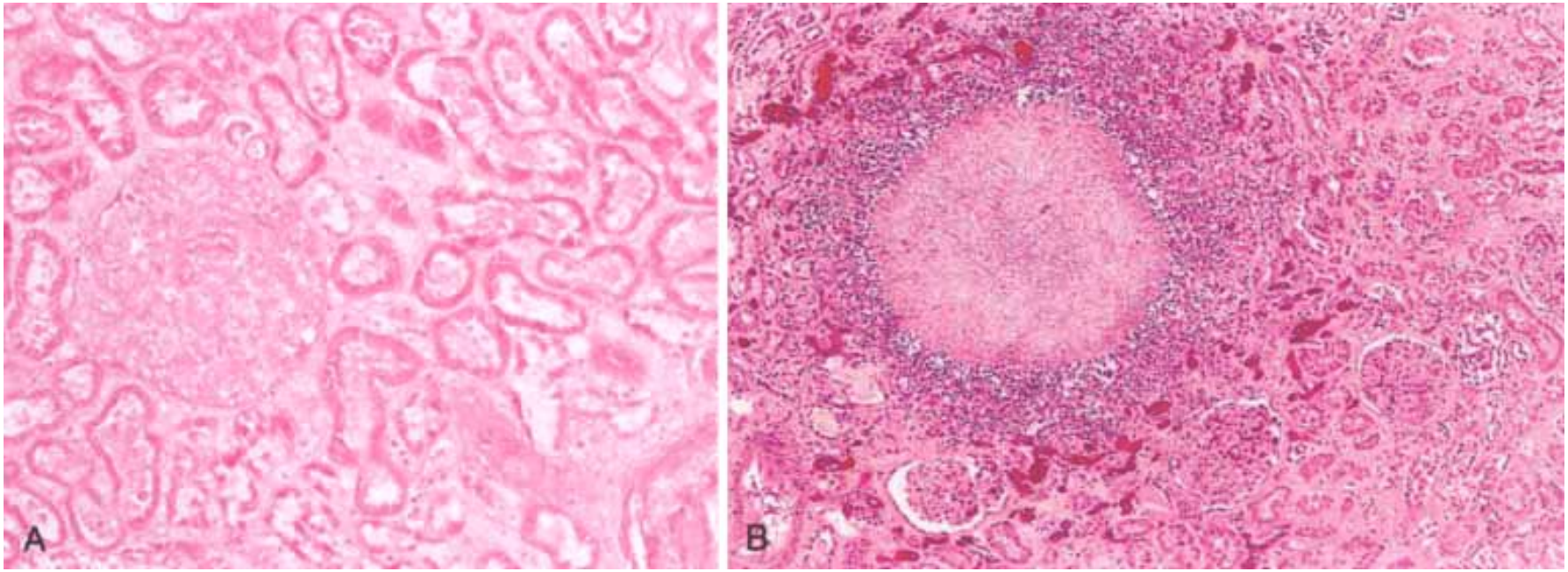


Figure 1-20 A tuberculous lung with a large area of caseous necrosis. The caseous debris is yellow-white and cheesy.

Figure 1-21 Foci of fat necrosis with saponification in the mesentery. The areas of white chalky deposits represent calcium soap formation at sites of lipid breakdown.

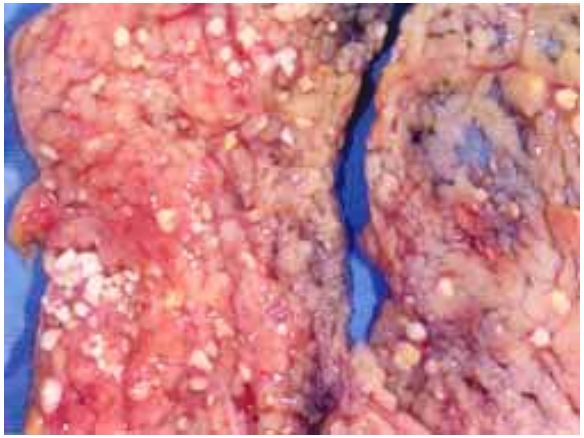


Figure 1-22 Postulated sequence of events in reversible and irreversible ischemic cell injury. Note that although reduced oxidative phosphorylation and ATP levels have a central role, ischemia can cause direct membrane damage. ER, endoplasmic reticulum; CK, creatine kinase; LDH, lactate dehydrogenase; RNP, ribonucleoprotein.

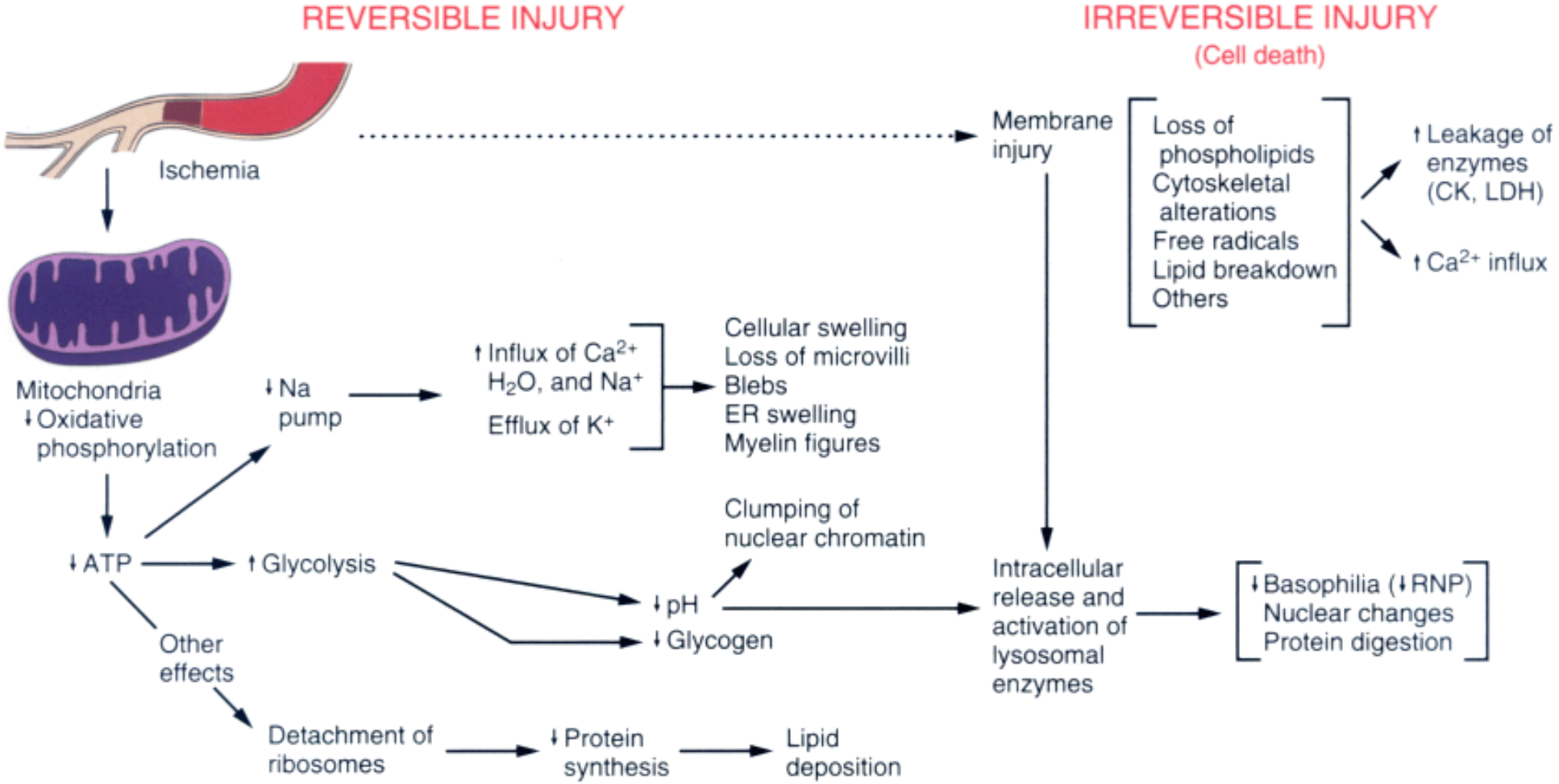


Figure 1-23 Sequence of events leading to fatty change and cell necrosis in carbon tetrachloride (CCl_4) toxicity. RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum.

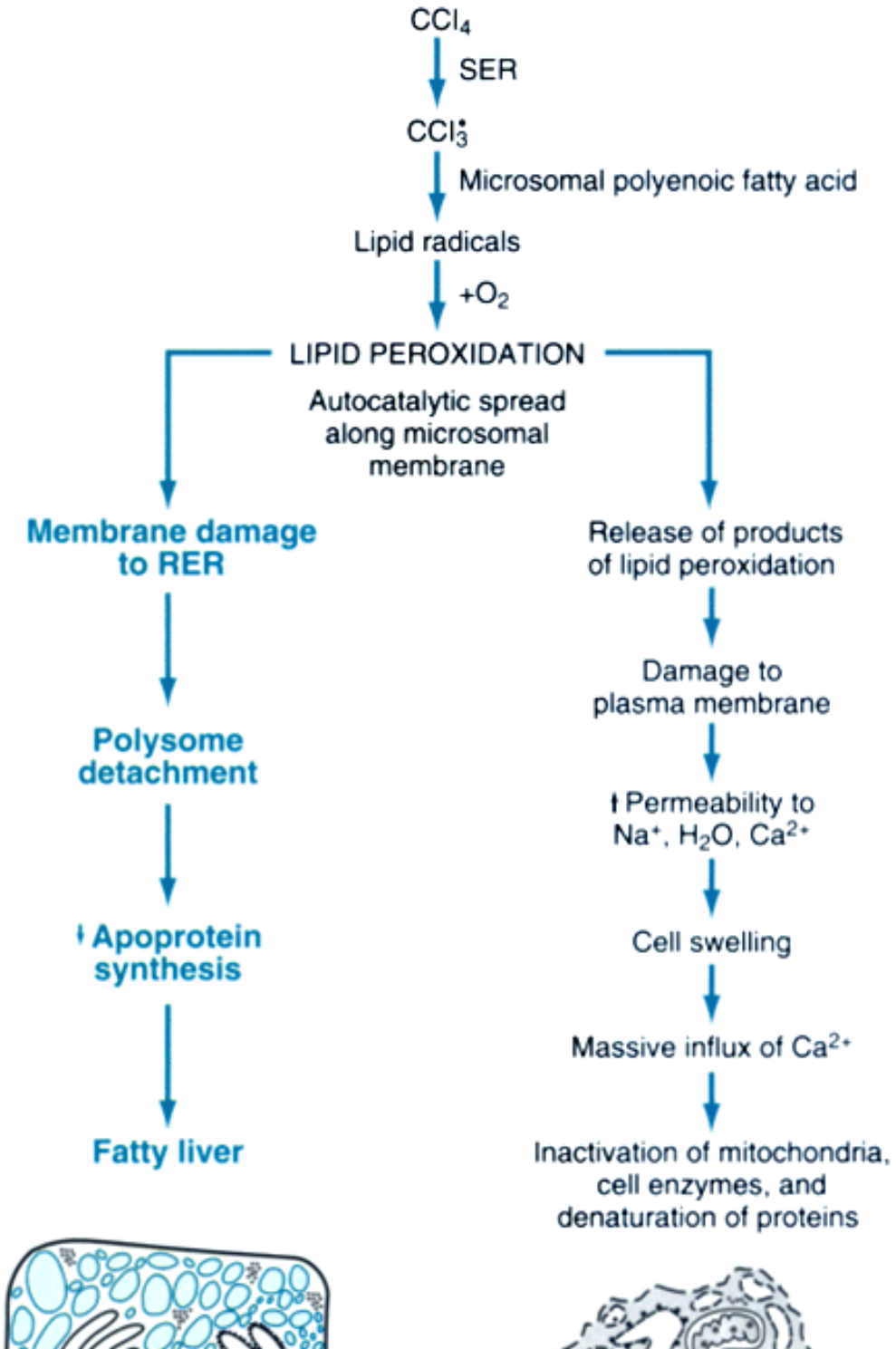


Figure 1-24 Rat liver cell 4 hours after carbon tetrachloride intoxication, with swelling of endoplasmic reticulum and shedding of ribosomes. At this stage, mitochondria are unaltered. *(Courtesy of Dr. O. Iseri, University of Maryland, Baltimore, MD.)*

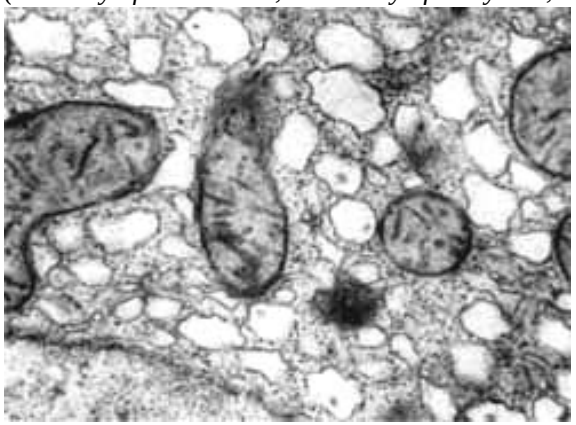


Figure 1-25 Ultrastructural features of apoptosis. Some nuclear fragments show peripheral crescents of compacted chromatin, whereas others are uniformly dense. *(From Kerr JFR, Harmon BV: Definition and incidence of apoptosis: a historical perspective. In Tomei LD, Cope FO (eds): Apoptosis: The Molecular Basis of Cell Death. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1991, pp 5–29.)*

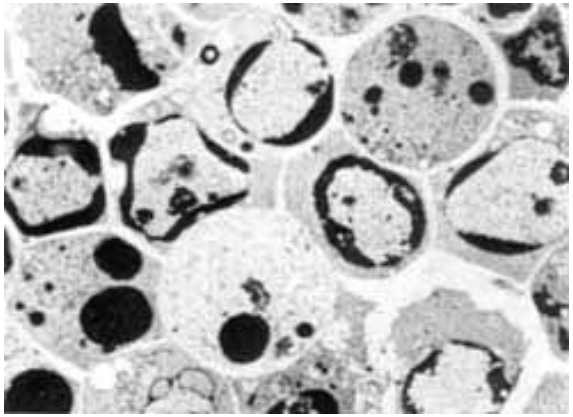


Figure 1-26 *A,* Apoptosis of epidermal cells in an immune-mediated reaction. The apoptotic cells are visible in the epidermis with intensely eosinophilic cytoplasm and small, dense nuclei. H&E stain. *(Courtesy of Dr. Scott Granter, Brigham and Women's Hospital, Boston, AM.) B,* High power of apoptotic cell in liver in immune-mediated hepatic cell injury. *(Courtesy of Dr. Dhanpat Jain, Yale University, New Haven, CT.)*

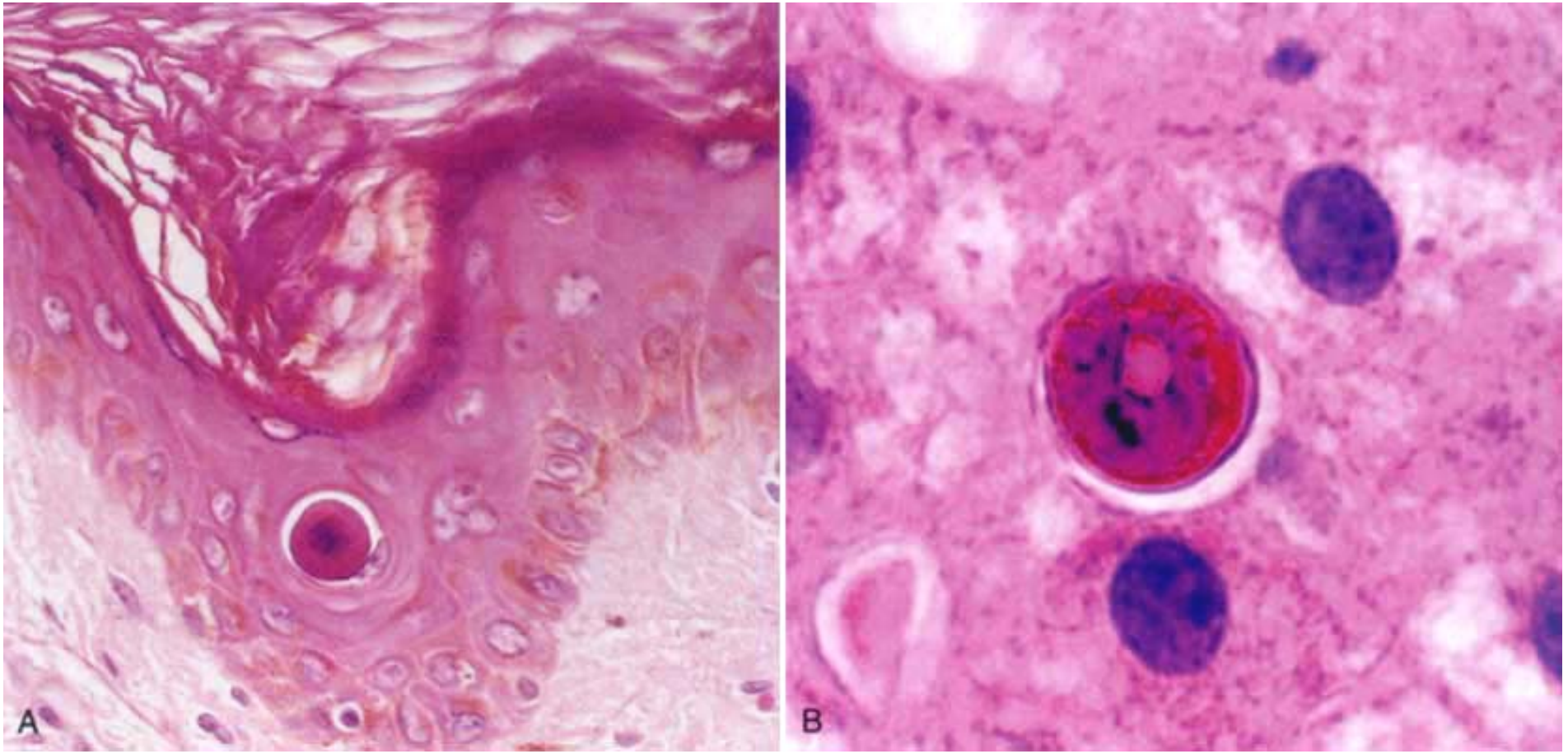


Figure 1-27 Agarose gel electrophoresis of DNA extracted from culture cells. Ethidium bromide stain; photographed under ultraviolet illumination. *Lane A*, Control culture. *Lane B*, Culture of cells exposed to heat showing extensive apoptosis; note ladder pattern of DNA fragments, which represent multiples of oligonucleosomes. *Lane C*, Culture showing massive necrosis; note diffuse smearing of DNA. The ladder pattern is produced by enzymatic cleavage of nuclear DNA into nucleosome-sized fragments, usually multiples of 180–200 base pairs. *These patterns are characteristic of but not specific for apoptosis and necrosis, respectively. (From Kerr JFR, Harmon BV: Definition and incidence of apoptosis: a historical perspective. In Tomei LD, Cope FO [eds]: Apoptosis: The Molecular Basis of Cell Death. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1991, p 13.)*

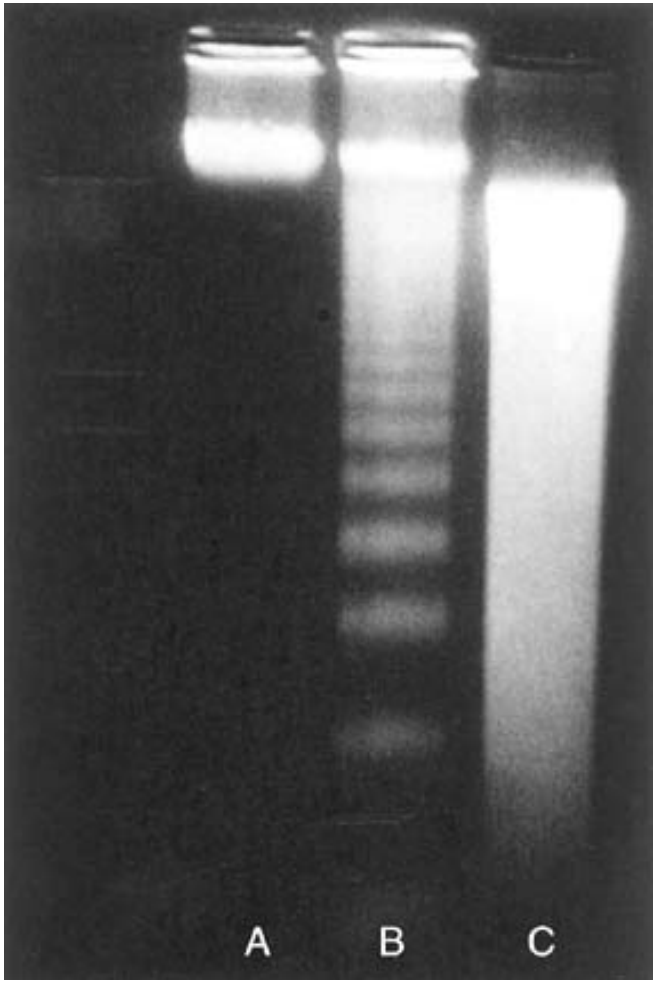


Figure 1-28 Mechanisms of apoptosis. Labeled (1) are some of the major inducers of apoptosis. These include specific death ligands (tumor necrosis factor [TNF] and Fas ligand), withdrawal of growth factors or hormones, and injurious agents (e.g., radiation). Some stimuli (such as cytotoxic cells) directly activate execution caspases *(right)*. Others act by way of adapter proteins and initiator caspases, or by mitochondrial events involving cytochrome *c*. (2) Control and regulation are influenced by members of the Bcl-2 family of proteins, which can either inhibit or promote the cell's death. (3) Executioner caspases activate latent cytoplasmic endonucleases and proteases that degrade nuclear and cytoskeletal proteins. This results in a cascade of intracellular degradation, including fragmentation of nuclear chromatin and breakdown of the cytoskeleton. (4) The end result is formation of apoptotic bodies containing intracellular organelles and other cytosolic components; these bodies also express new ligands for binding and uptake by phagocytic cells.

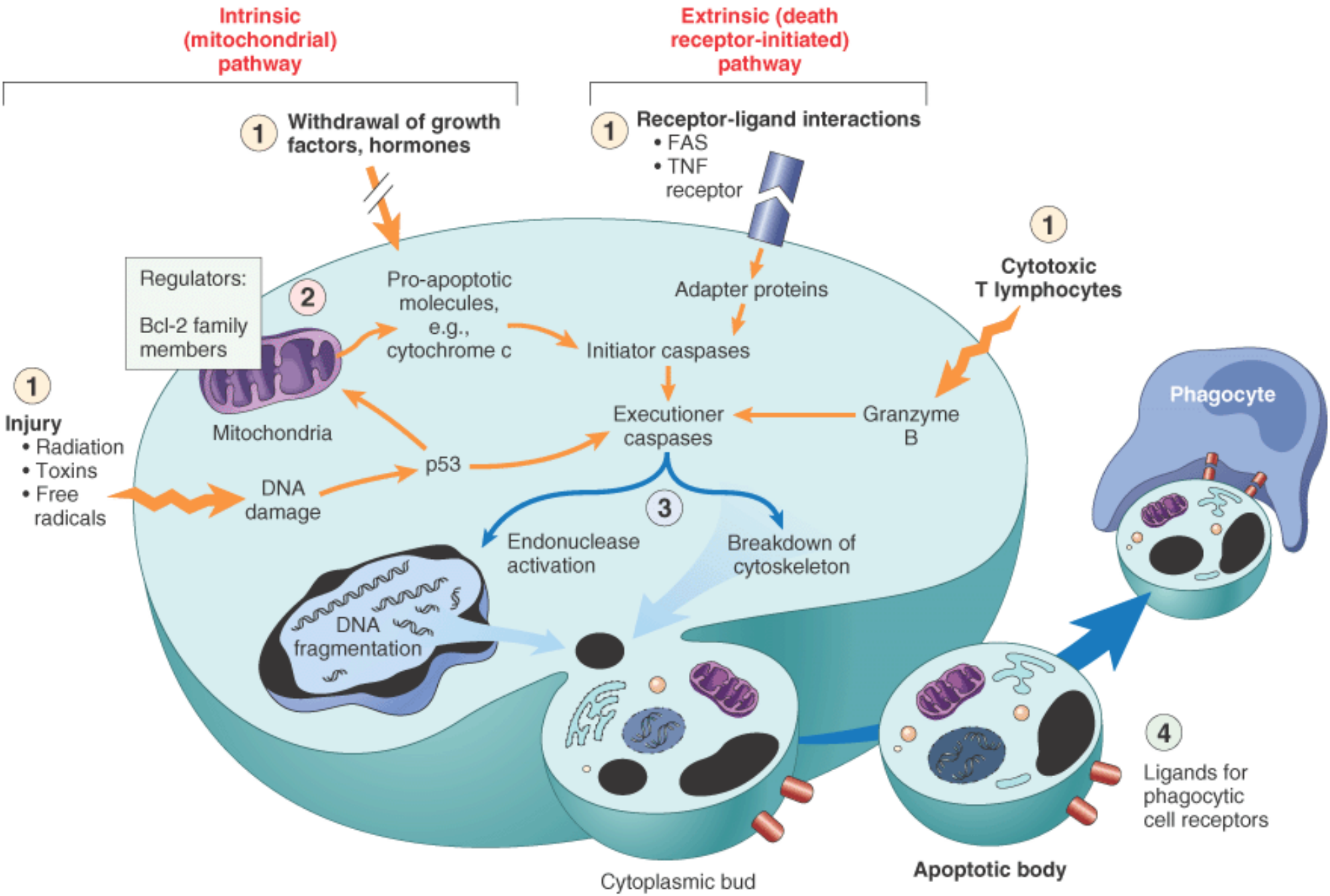


Figure 1-29 The extrinsic (death receptor-initiated) pathway of apoptosis, illustrated by the events following Fas engagement (see text).

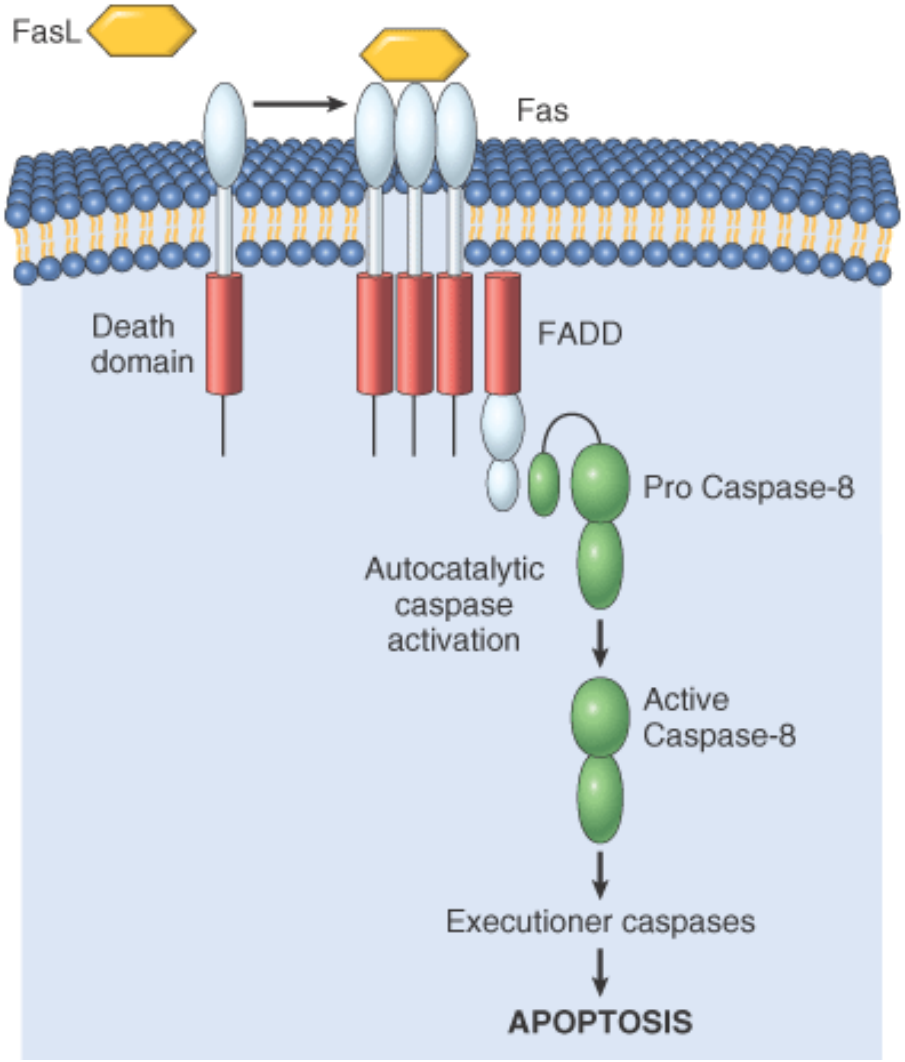


Figure 1-30 The intrinsic (mitochondrial) pathway of apoptosis. Death agonists cause changes in the inner mitochondrial membrane, resulting in the mitochondrial permeability transition (MPT) and release of cytochrome *c* and other pro-apoptotic proteins into the cytosol, which activate caspases (see text).

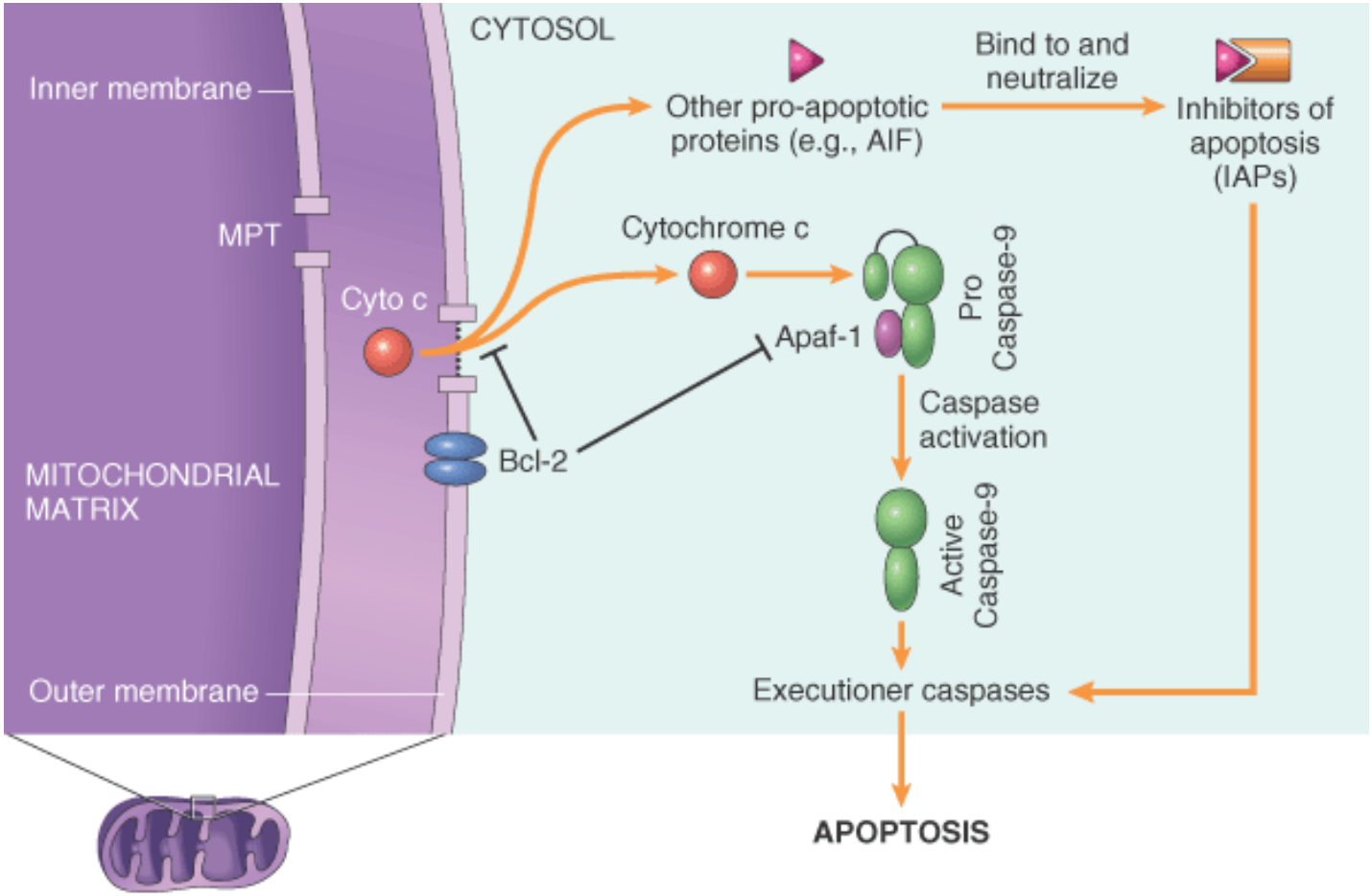
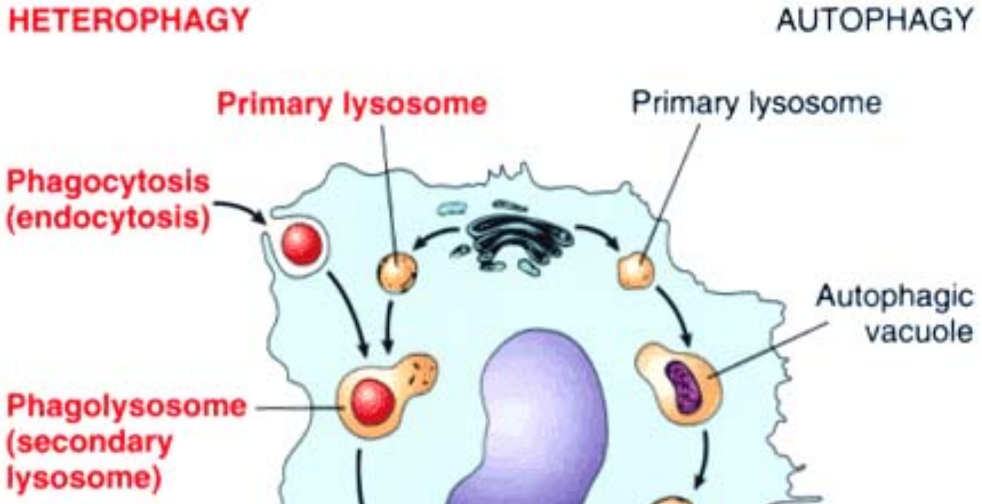


Figure 1-31 *A*, schematic representation of heterophagy *(left)* and autophagy *(right). (Redrawn from Fawcett DW: A Textbook of Histology, 11th ed. Philadelphia, WB Saunders, 1986, p 17.) B,* Electron micrograph of an autophagolysosome containing a degenerating mitochondrion and amorphous material.



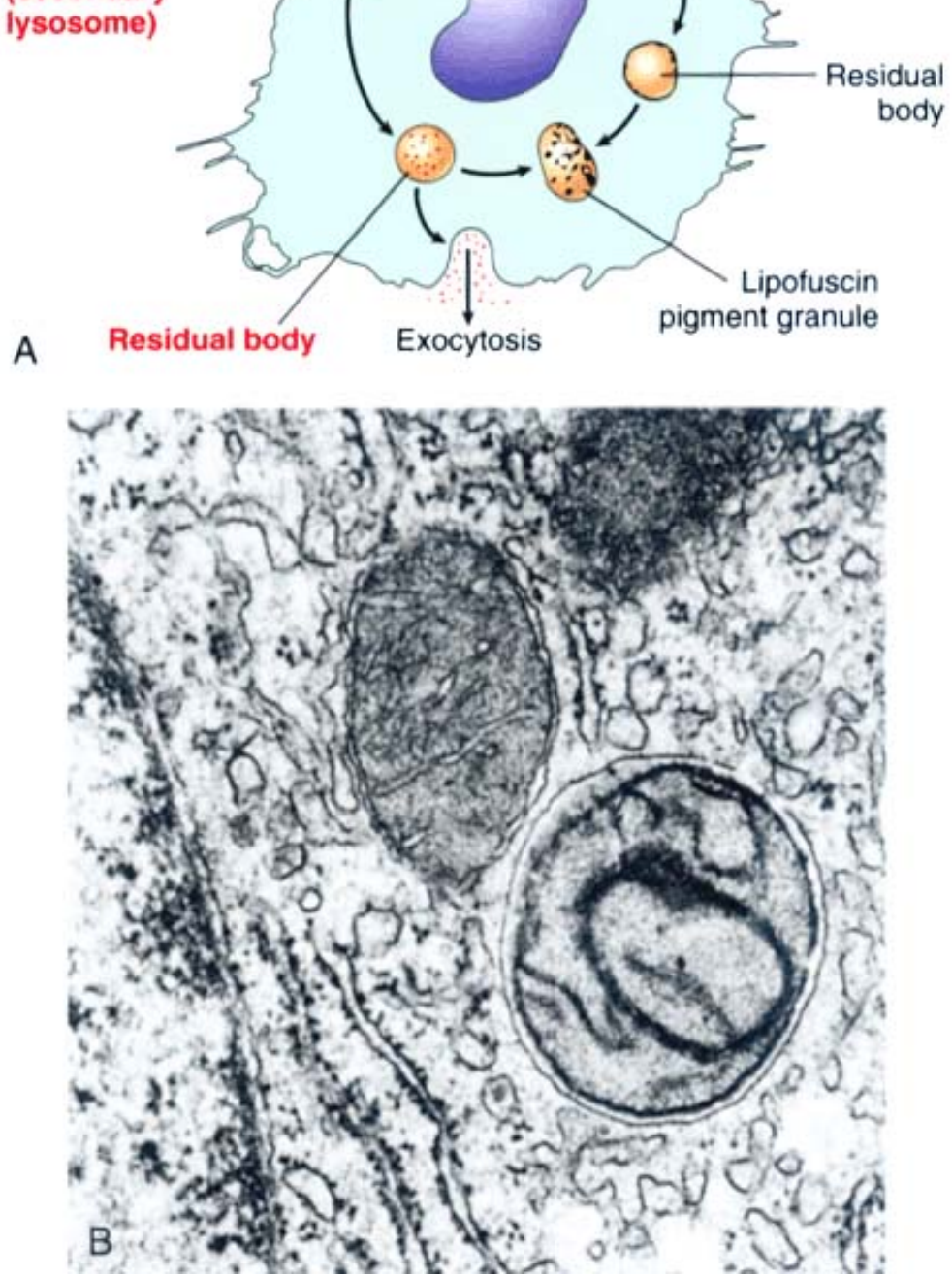


Figure 1-32 Electron micrograph of liver from phenobarbital-treated rat showing marked increase in smooth endoplasmic reticulum. *(From Jones AL, Fawcett DW: Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by Phenobarbital. J Histochem Cytochem 14:215, 1966. Courtesy of Dr. Fawcett.)*

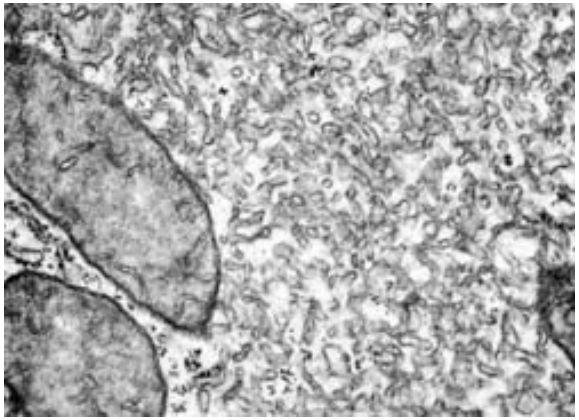


Figure 1-33 Enlarged, abnormally shaped mitochondria from the liver of a patient with alcoholic cirrhosis. Note also crystalline formations in the mitochondria.

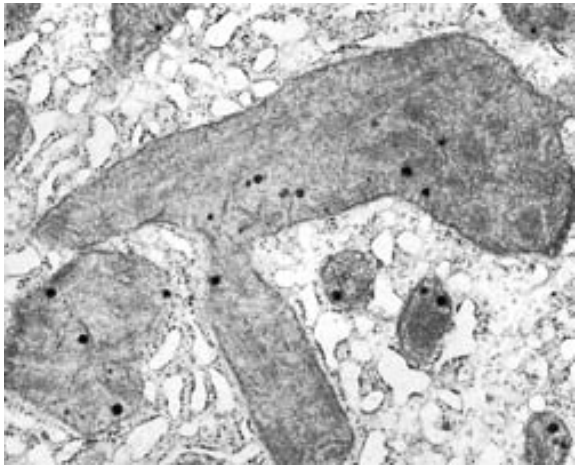


Figure 1-34 *A,* The liver of alcohol abuse (chronic alcoholism). Hyaline inclusions in the hepatic parenchymal cell in the center appear as eosinophilic networks disposed about the nuclei *(arrow)*. *B,* Electron micrograph of alcoholic hyalin. The material is composed of intermediate (prekeratin) filaments and an amorphous matrix.

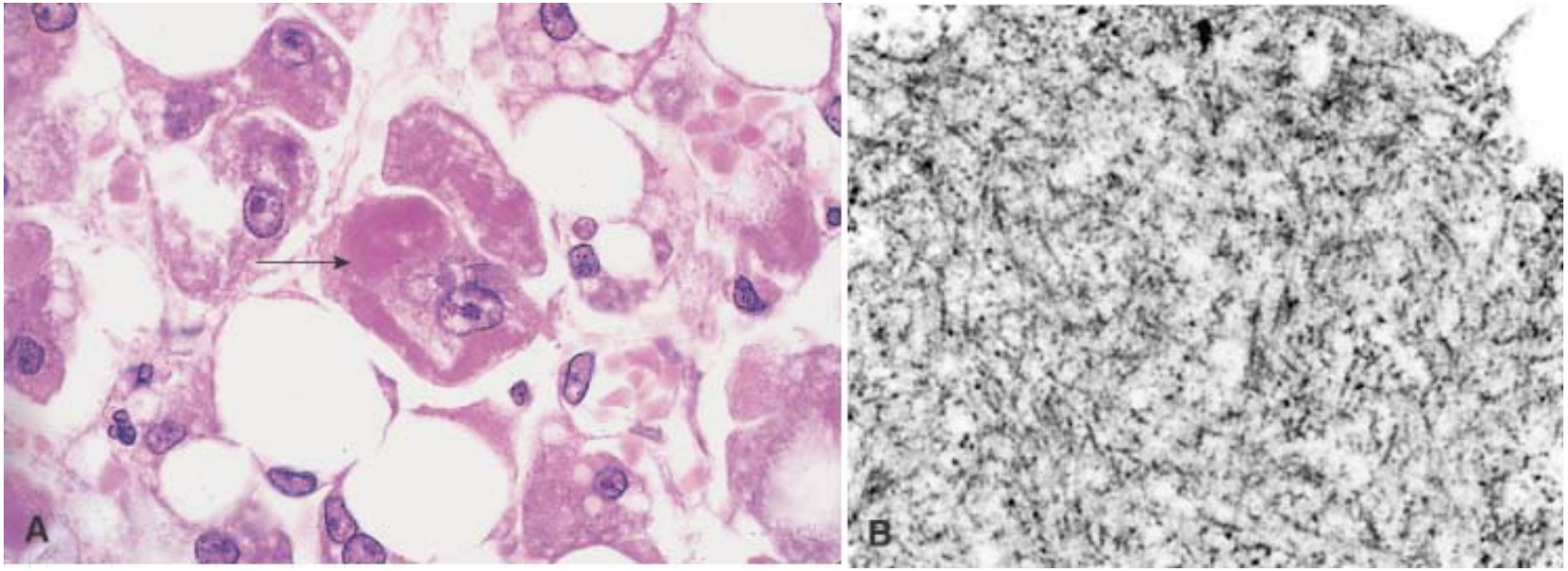
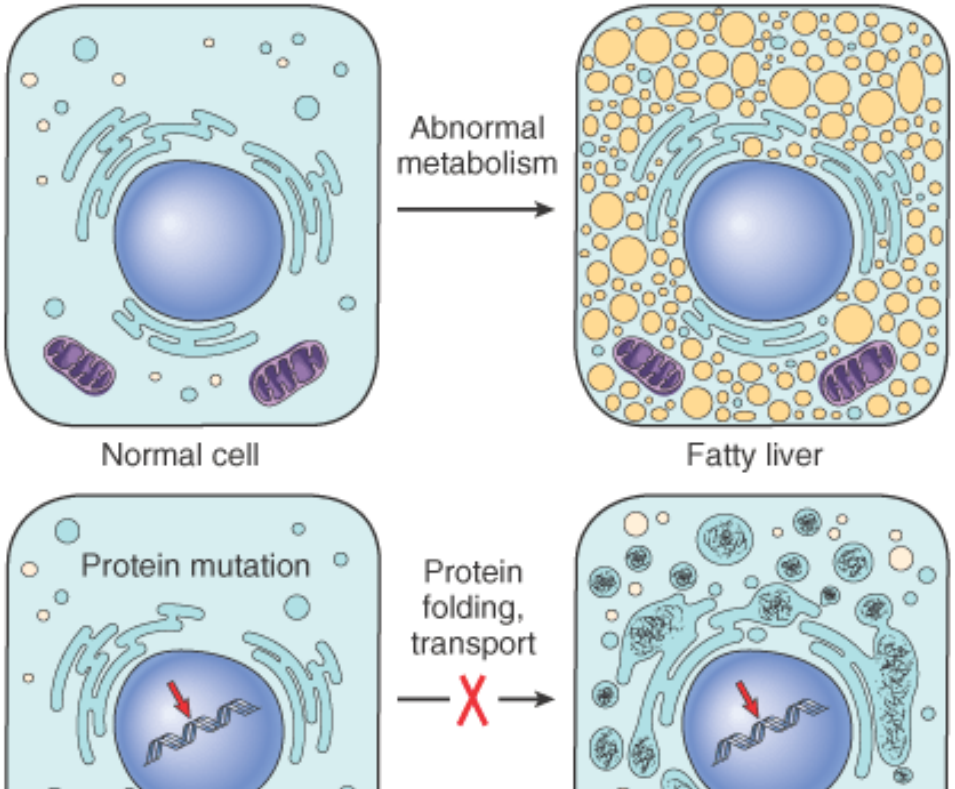


Figure 1-35 Mechanisms of intracellular accumulations: (1) abnormal metabolism, as in fatty change in the liver; (2) mutations causing alterations in protein folding and transport, as in alpha$_1$ -antitrypsin deficiency; (3) deficiency of critical enzymes that prevent breakdown of substrates that accumulate in lysosomes, as in lysosomal storage diseases; and (4) inability to degrade phagocytosed particles, as in hemosiderosis and carbon pigment accumulation.



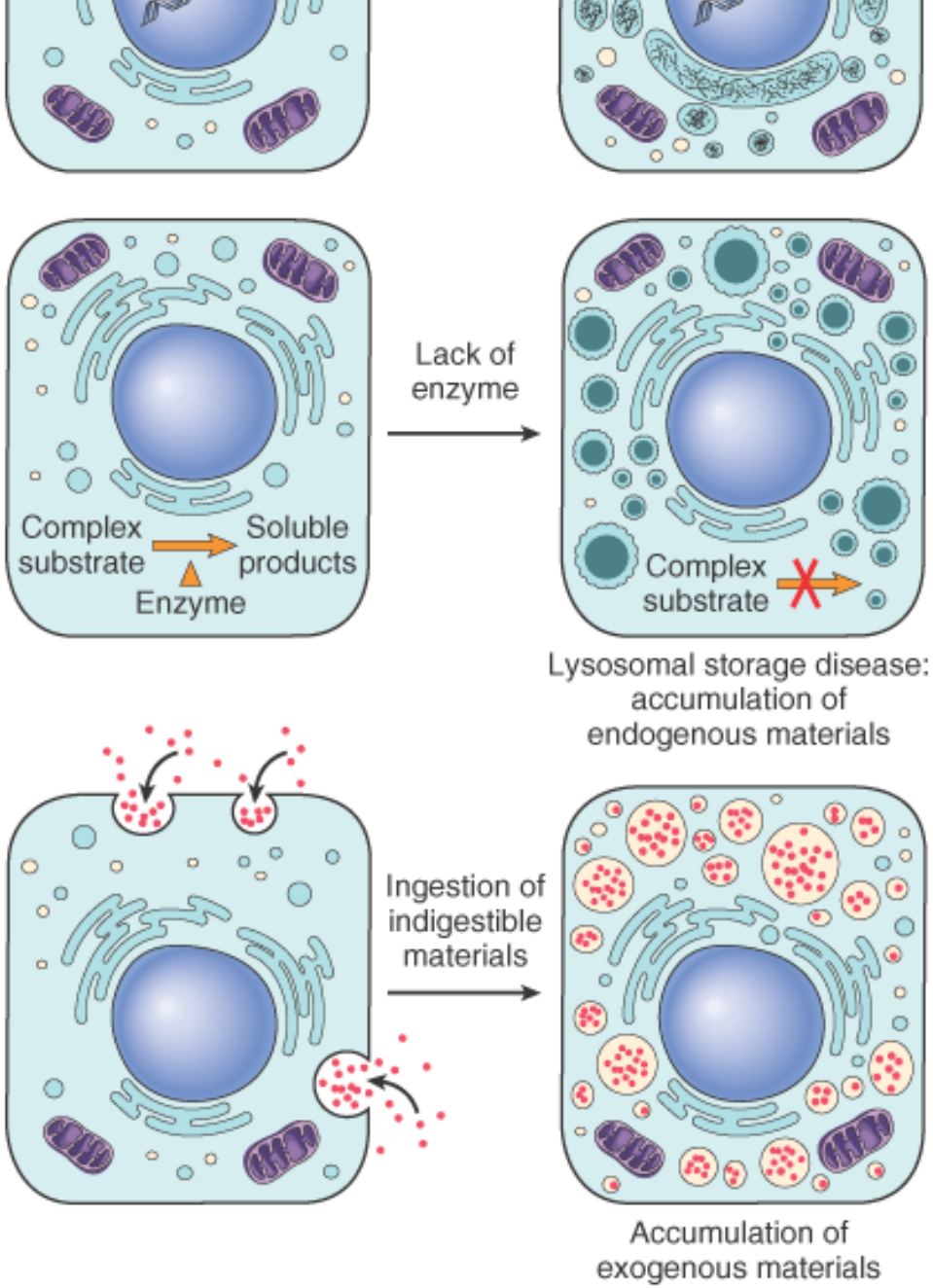
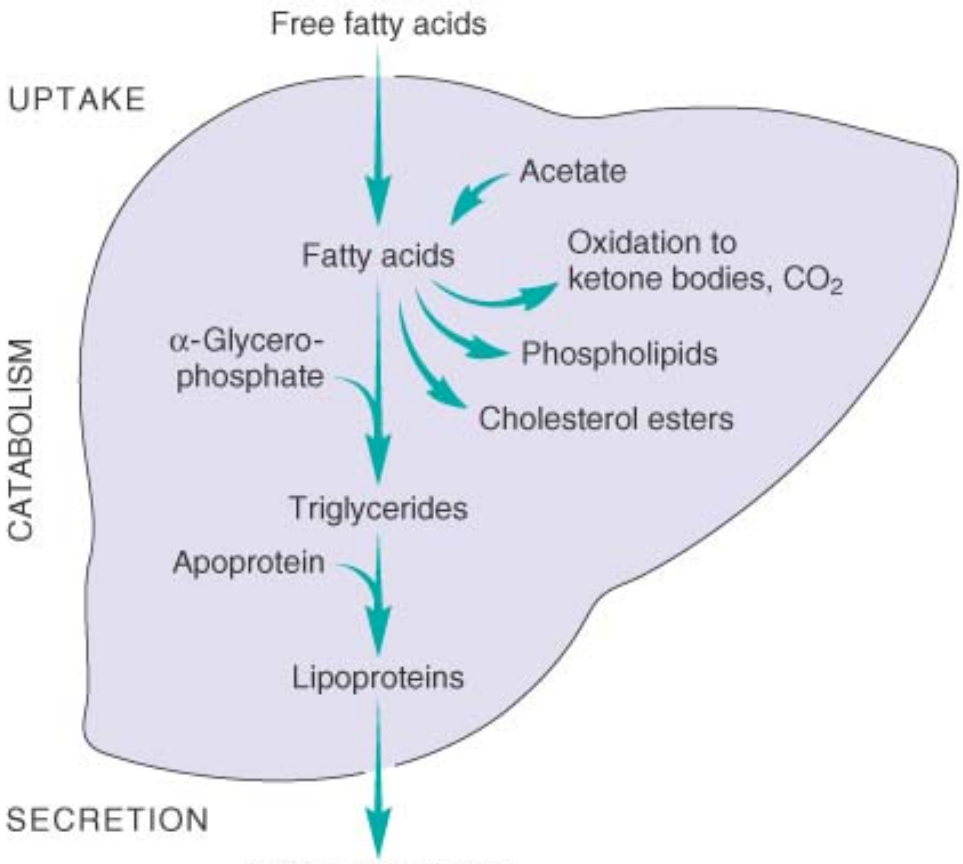


Figure 1-36 Fatty liver. *A,* Schematic diagram of the possible mechanisms leading to accumulation of triglycerides in fatty liver. Defects in any of the steps of uptake, catabolism, or secretion can result in lipid accumulation. *B,* High-power detail of fatty change of the liver. In most cells, the well-preserved nucleus is squeezed into the displaced rim of cytoplasm about the fat vacuole. *(B, Courtesy of Dr. James Crawford, Department of Pathology, Yale University School of Medicine, New Haven, CT.)*


Free fatty acids
UPTAKE
Acetate
Fatty acids
Oxidation to ketone bodies, CO_2
α-Glycero-phosphate
Phospholipids
Cholesterol esters
CATABOLISM
Triglycerides
Apoprotein
Lipoproteins
SECRETION
Lipid accumulation

A

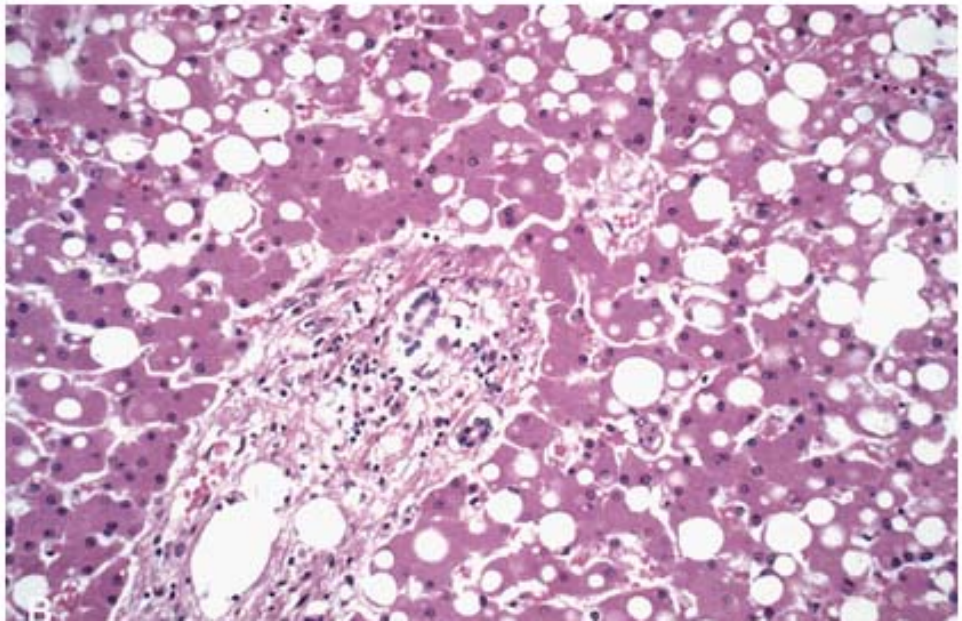


Figure 1-37 Cholesterolosis. Cholesterol-laden macrophages (foam cells) from a focus of gallbladder cholesterolosis (*arrow*). *(Courtesy of Dr. Matthew Yeh, University of Washington, Seattle, WA.)*

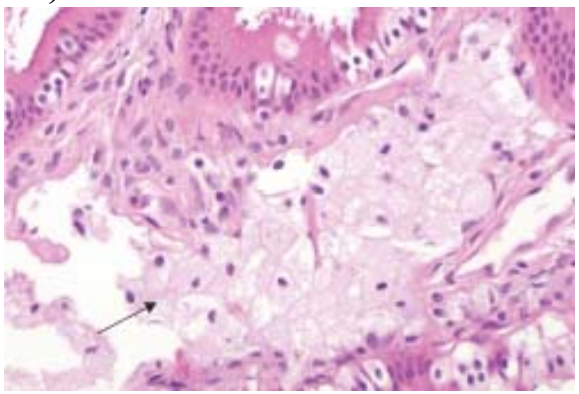


Figure 1-38 Protein reabsorption droplets in the renal tubular epithelium. *(Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

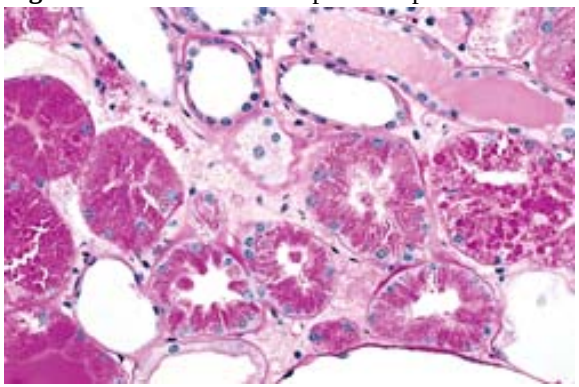


Figure 1-39 Mechanisms of protein folding and the role of chaperones. *A,* Chaperones, such as heat shock proteins (Hsp), protect unfolded or partially folded protein from degradation and guide proteins into organelles. *B,* Chaperones repair misfolded proteins; when this process is ineffective, proteins are targeted for degradation in the proteasome, and if misfolded proteins accumulate they trigger apoptosis.

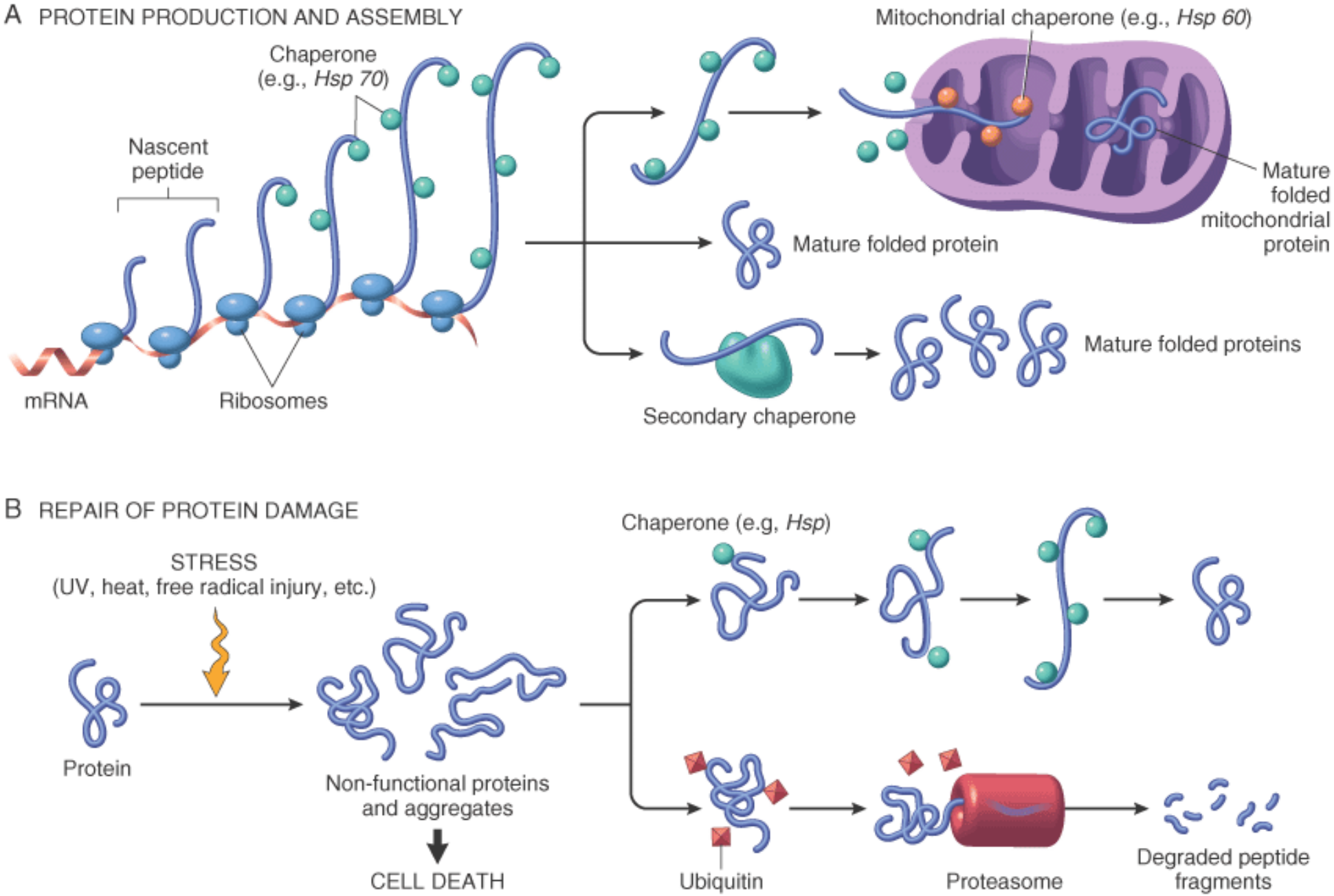


Figure 1-40 Lipofuscin granules in a cardiac myocyte as shown by *A*, light microscopy (deposits indicated by *arrows*), and *B*, electron microscopy (note the perinuclear, intralysosomal location).

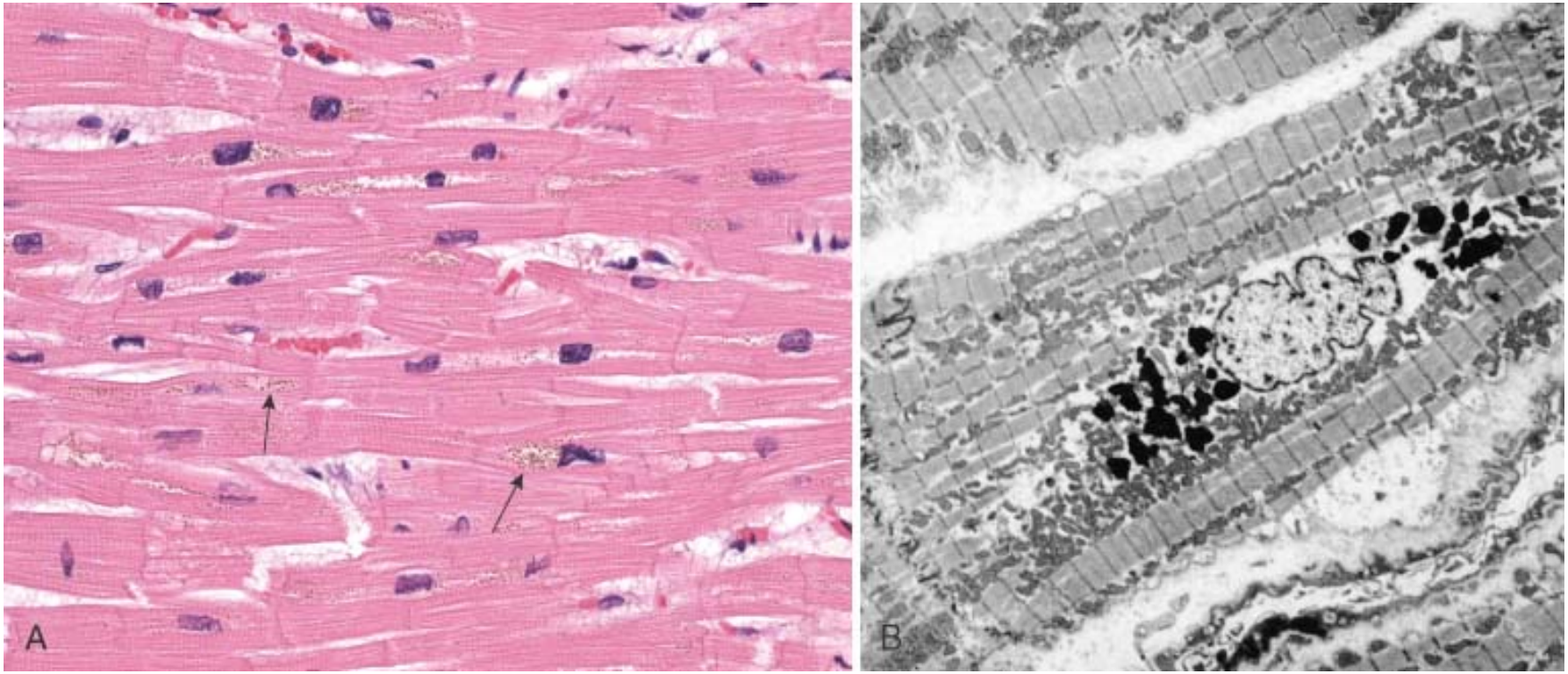


Figure 1-41 Hemosiderin granules in liver cells. *A*, H&E section showing golden-brown, finely granular pigment. *B*, Prussian blue reaction, specific for iron.

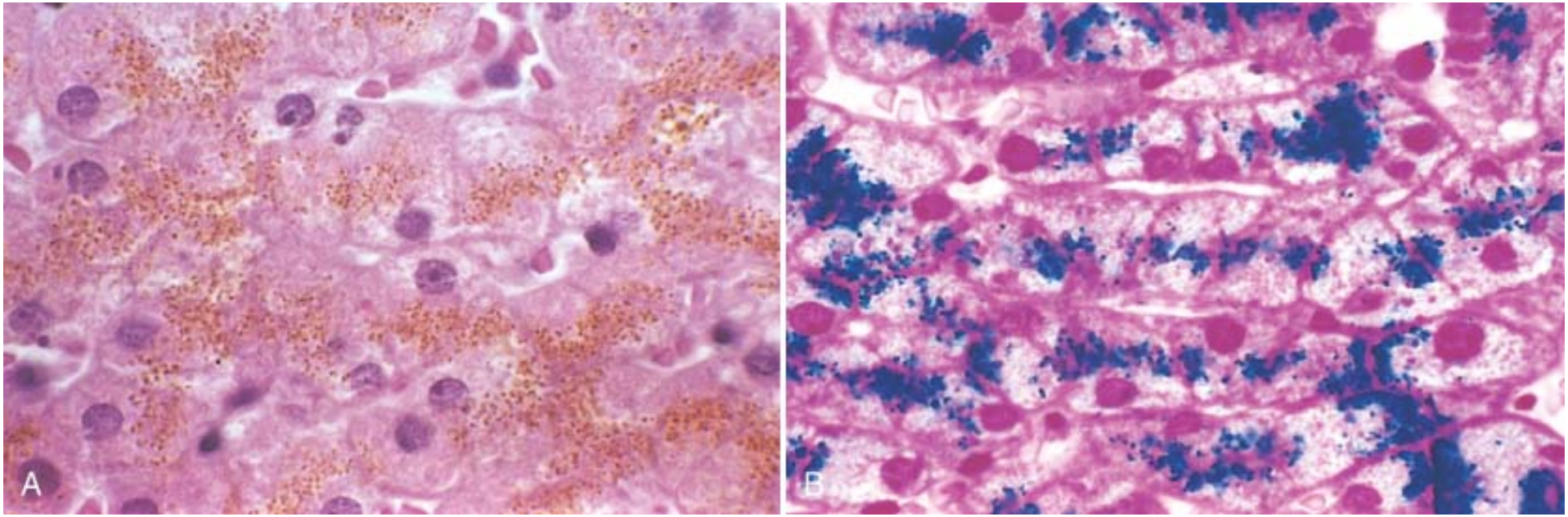


Figure 1-42 View looking down onto the unopened aortic valve in a heart with calcific aortic stenosis. The semilunar cusps are thickened and fibrotic. Behind each cusp are seen irregular

masses of piled-up dystrophic calcification.

Figure 1-43 Mechanisms of cellular aging. Genetic factors and environmental insults combine to produce the cellular abnormalities characteristic of aging.

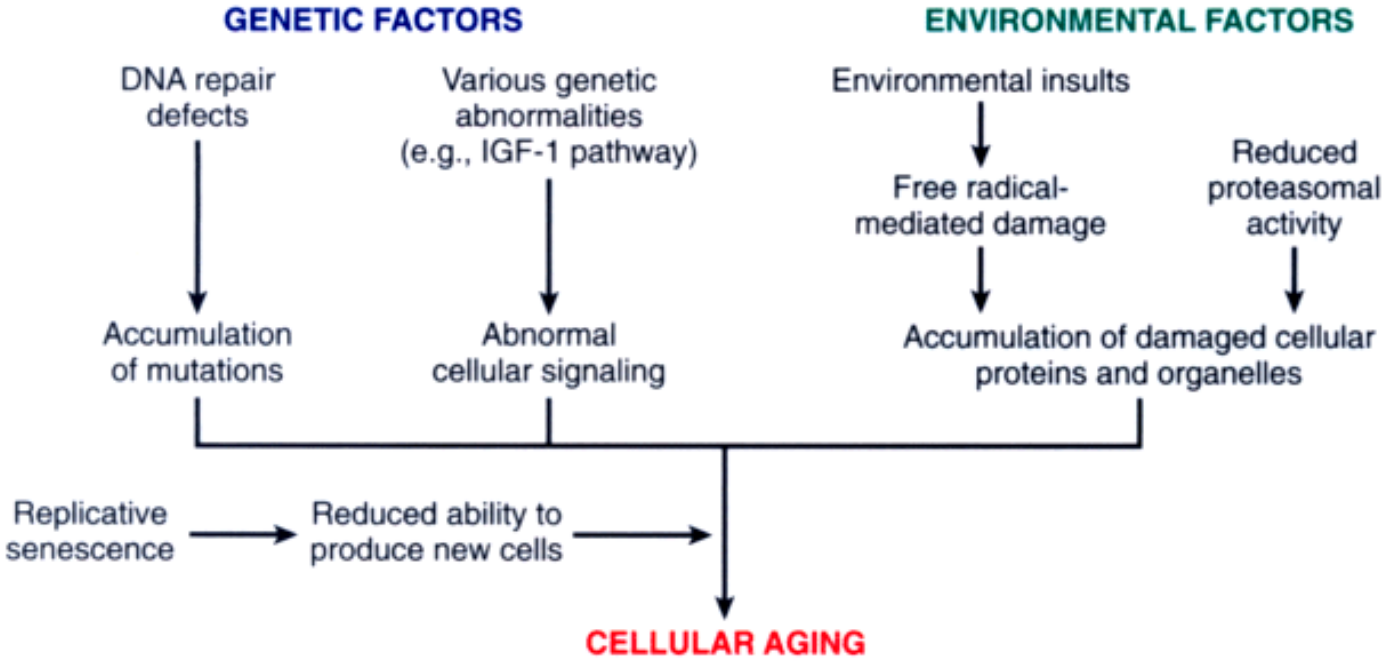


Figure 1-44 Finite population doublings of primary human fibroblasts derived from a newborn, a 100-year-old person, and a 20-year-old patient with Werner's syndrome. The ability of cells to grow to a confluent monolayer decreases with increasing population-doubling levels. *(From Dice JF: Cellular and molecular mechanisms of aging. Physiol Rev 73:150, 1993.)*

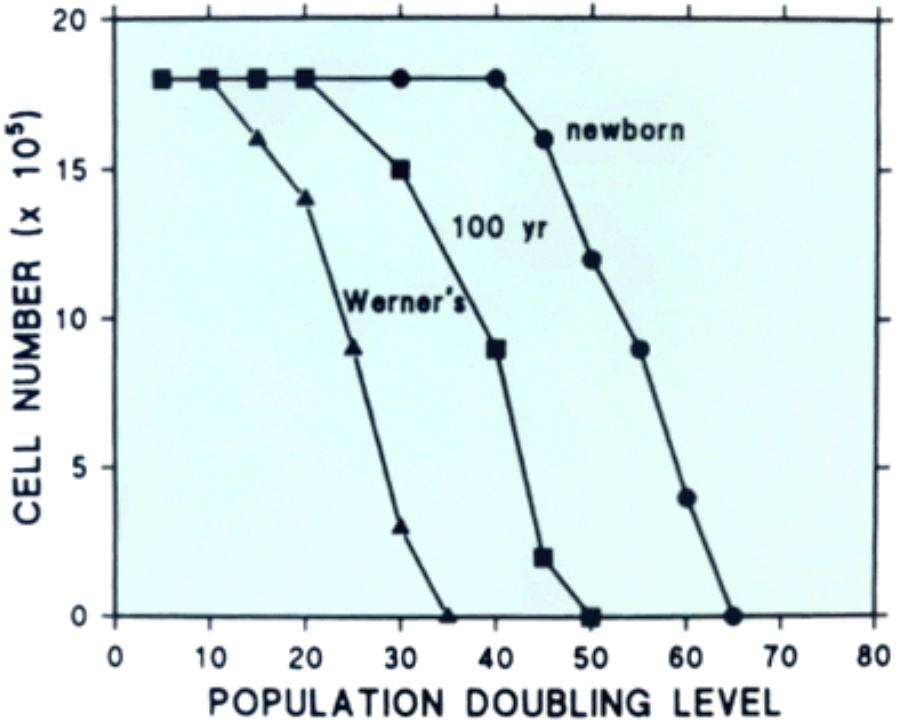
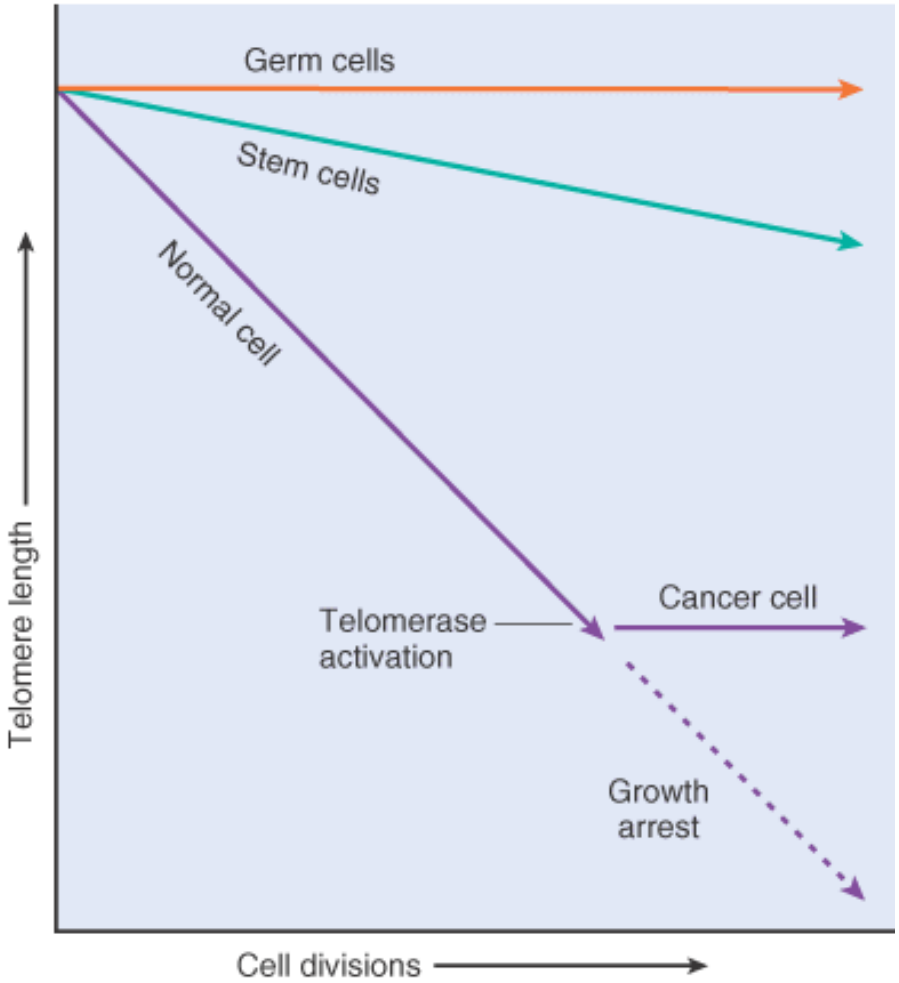


Figure 1-45 The role of telomeres and telomerase in replicative senescence of cells. *A*, Telomerase directs RNA template-dependent DNA synthesis, in which nucleotides are added to one strand at the end of a chromosome. The lagging strand is presumably filled in by DNA polymerase α. The RNA sequence in the telomerase is different in different species. *(Modified from Alberts BR, et al: Molecular Biology of the Cell, 2002, Garland Science, New York.) B*, Telomere-telomerase hypothesis and proliferative capacity. Telomere length is plotted against the number of cell divisions. In normal somatic cells, there is no telomerase activity, and telomeres progressively shorten with increasing cell divisions until growth arrest, or senescence, occurs. Germ cells and stem cells both contain active telomerase, but only the germ cells have sufficient levels of the enzyme to stabilize telomere length completely. Telomerase activation in cancer cells inactivates the teleomeric clock that limits the proliferative capacity of normal somatic cells. *(Modified and redrawn with permission from Holt SE, et al.: Refining the telomer-telomerase hypothesis of aging and cancer. Nature Biotech 14:836, 1996. Copyright 1996, Macmillan Magazines Limited.)*



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Chapter 2 - Acute and Chronic Inflammation

General Features of Inflammation

In Chapter 1 , we saw how various exogenous and endogenous stimuli can cause cell injury. In vascularized tissues, these same stimuli also provoke a host response called *inflammation. Inflammation agents such as microbes and damaged, usually necrotic, cells that consists of vascular responses, migration and activation of leukocytes, and systemic reactions.* Invertebrates with no vascular system, and even single-celled organisms, are able to get rid of injurious agents such as microbes by a variety of mechanisms. These mechanisms include entrapment and

phagocytosis of the offending agent, sometimes by specialized cells (hemocytes), and neutralization of noxious stimuli by hypertrophy of the host cell or one of its organelles. These cellular reactions have been retained through evolution, and the more potent defensive reaction of inflammation has been added in higher species. The unique feature of the inflammatory process is the *reaction of blood vessels, leading to the accumulation of fluid and leukocytes in extravascular tissues*.

The inflammatory response is closely intertwined with the process of repair. Inflammation serves to destroy, dilute, or wall off the injurious agent, and it sets into motion a series of events that try to heal and reconstitute the damaged tissue. Repair begins during the early phases of inflammation but reaches completion usually after the injurious influence has been neutralized. During repair, the injured tissue is replaced through *regeneration* of native parenchymal cells, by filling of the defect with fibrous tissue *(scarring)* or, most commonly, by a combination of these two processes.

Figure 2-1 The components of acute and chronic inflammatory responses: circulating cells and proteins, cells of blood vessels, and cells and proteins of the extracellular matrix.

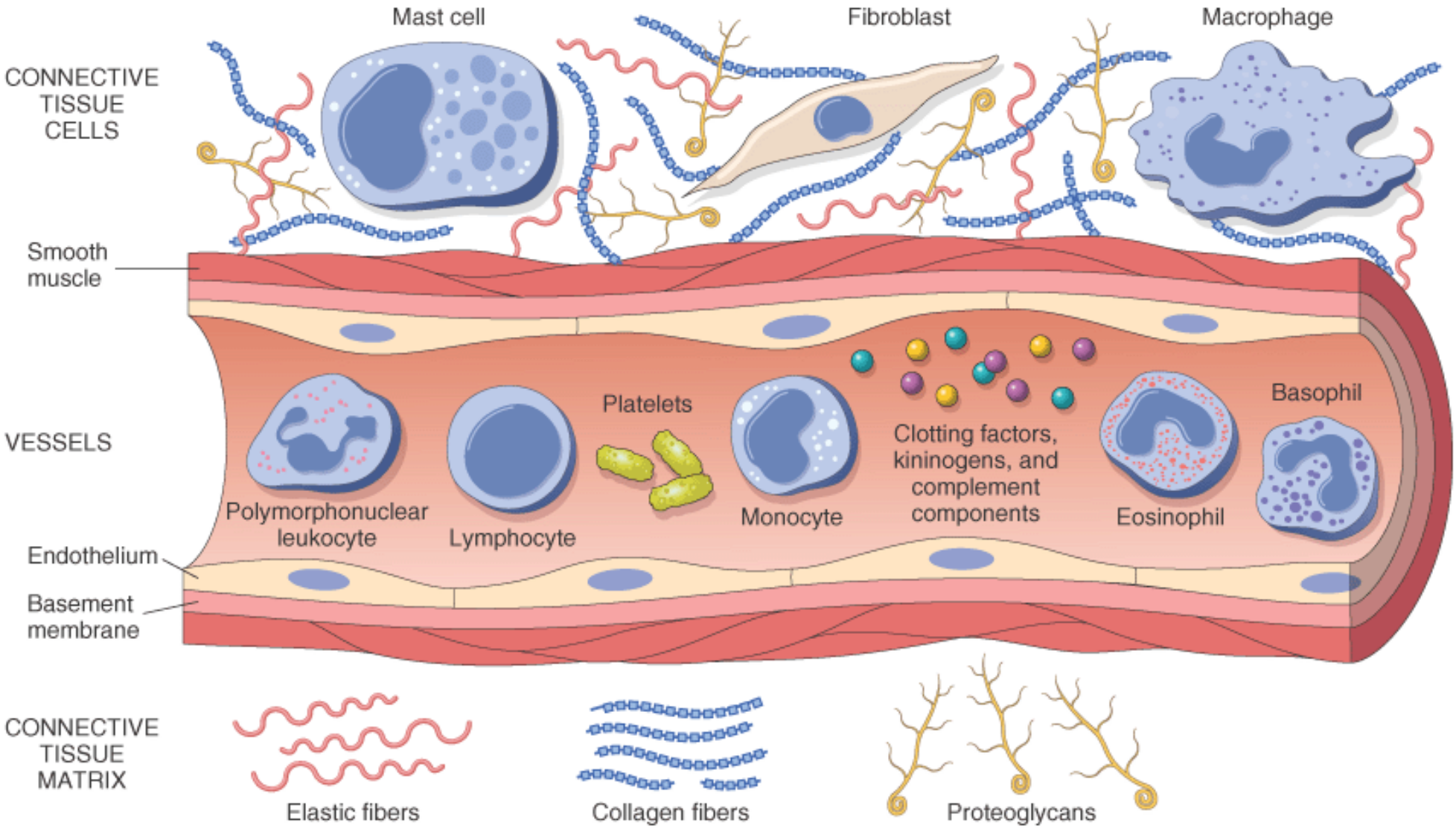
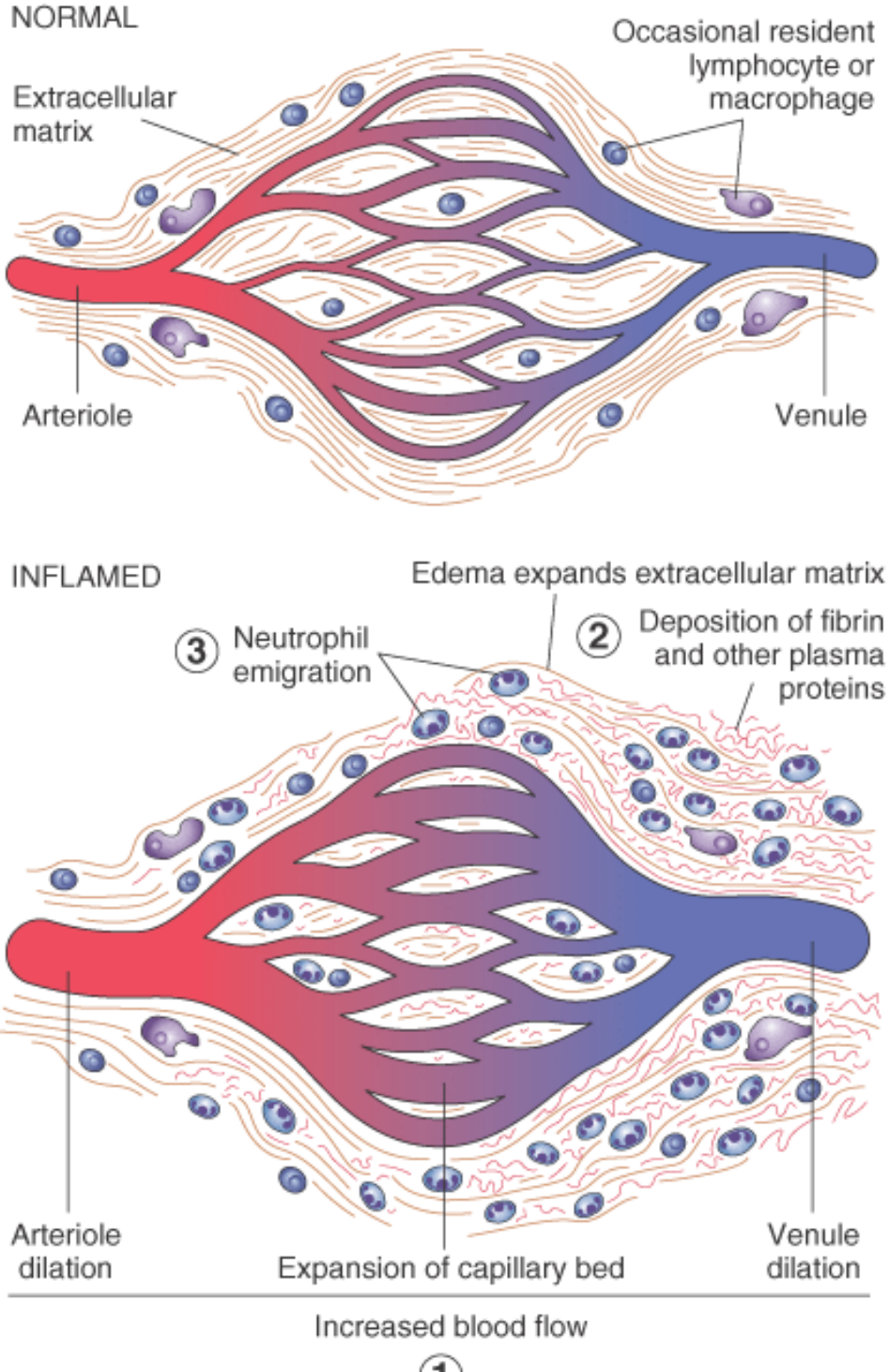


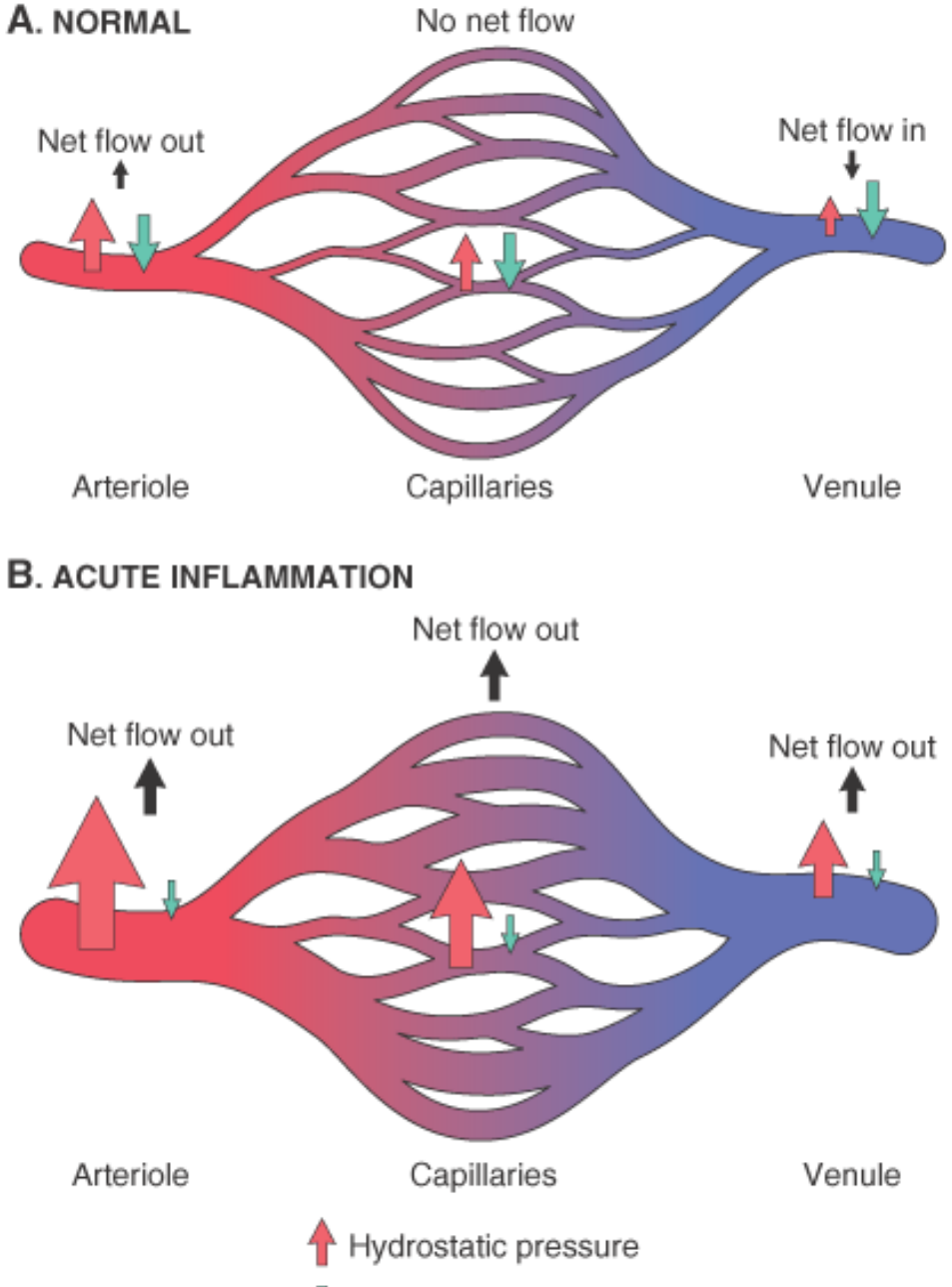
Figure 2-2 The major local manifestations of acute inflammation, compared to normal. (1) Vascular dilation and increased blood flow (causing erythema and warmth), (2) extravasation and deposition of plasma fluid and proteins (edema), and (3) leukocyte emigration and accumulation in the site of injury.



Increased blood flow

1

Figure 2-3 Blood pressure and plasma colloid osmotic forces in normal and inflamed microcirculation. *A*, Normal hydrostatic pressure (*red arrows*) is about 32 mm Hg at the arterial end of a capillary bed and 12 mm Hg at the venous end; the mean colloid osmotic pressure of tissues is approximately 25 mm Hg (*green arrows*), which is equal to the mean capillary pressure. Although fluid tends to leave the precapillary arteriole, it is returned in equal amounts via the postcapillary venule, so that the net flow (*black arrows*) in or out is zero. *B*, Acute inflammation. Arteriole pressure is increased to 50 mm Hg, the mean capillary pressure is increased because of arteriolar dilation, and the venous pressure increases to approximately 30 mm Hg. At the same time, osmotic pressure is reduced (averaging 20 mm Hg) because of protein leakage across the venule. The net result is an excess of extravasated fluid.



Hydrostatic pressure

Colloid osmotic pressure

Figure 2-4 Diagrammatic representation of five mechanisms of increased vascular permeability in inflammation (see text).

Gaps due to endothelial contraction

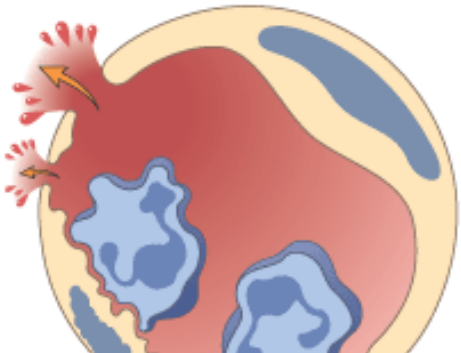
- Venules
- Vasoactive mediators (histamine, leukotrienes, etc.)
- Most common
- Fast and short-lived (minutes)

Direct injury

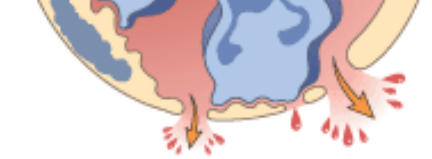
- Arterioles, capillaries, and venules
- Toxins, burns, chemicals
- Fast and may be long-lived (hours to days)

Leukocyte-dependent injury

- Mostly venules
- Pulmonary capillaries
- Late response
- Long-lived (hours)

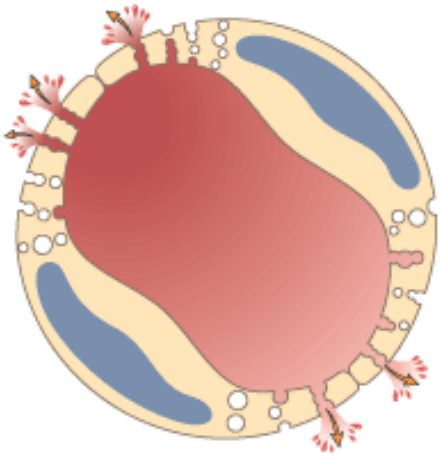


• Long-lived (hours)



Increased transcytosis

• Venules
• Vascular endothelium–derived growth factor



New blood vessel formation

• Sites of angiogenesis
• Persists until intercellular junctions form

Figure 2-5 Vascular leakage induced by chemical mediators. *A*, This is a fixed and cleared preparation of a rat cremaster muscle examined unstained by transillumination. One hour before sacrifice, bradykinin was injected over this muscle, and colloidal carbon was given intravenously. Plasma, loaded with carbon, escaped, but most of the carbon particles were retained by the basement membrane of the leaking vessels, with the result that these became "labeled" black. Note that not all the vessels leak—only the venules. In *B*, a higher power, the capillary network is faintly visible in the background. *(Courtesy of Dr. Guido Majno, University of Massachusetts Medical School, Worcester, MA.)*

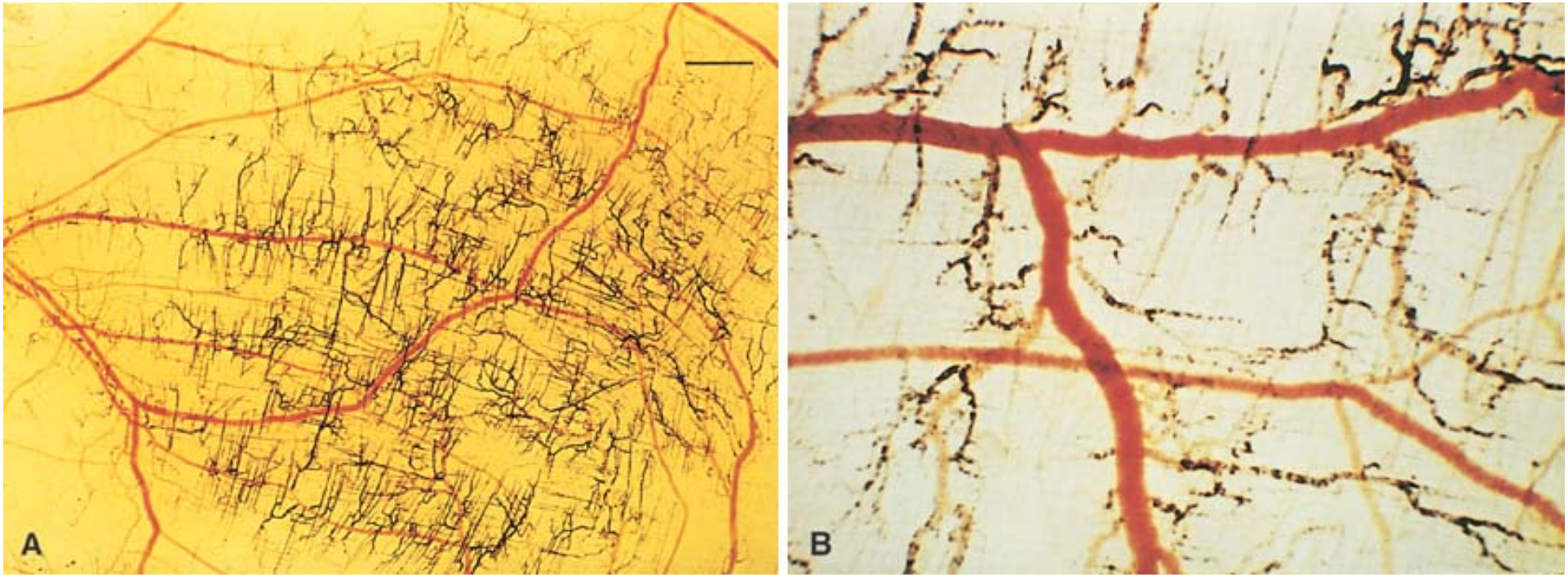


Figure 2-6 The multistep process of leukocyte migration through blood vessels, shown here for neutrophils. The leukocytes first roll, then become activated and adhere to endothelium, then transmigrate across the endothelium, pierce the basement membrane, and migrate toward chemoattractants emanating from the source of injury. Different molecules play predominant roles in different steps of this process—selectins in rolling; chemokines in activating the neutrophils to increase avidity of integrins (in green); integrins in firm adhesion; and CD31 (PECAM-1) in transmigration.

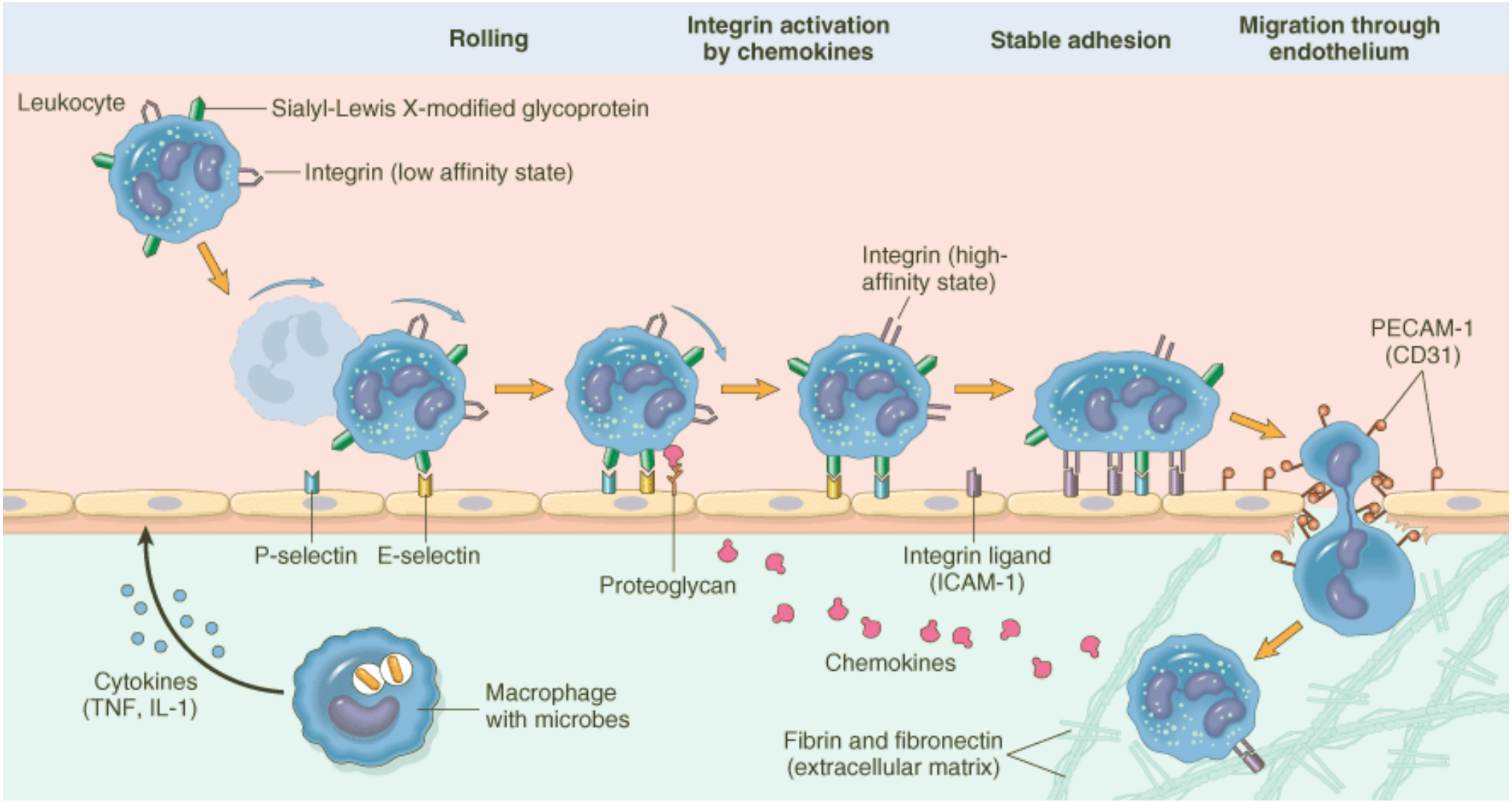


TABLE 2-1 -- Endothelial/Leukocyte Adhesion Molecules

Endothelial Molecule	Leukocyte Receptor	Major Role
P-selectin	Sialyl-Lewis X	Rolling (neutrophils, monocytes, lymphocytes)
	PSGL-1	
E-selectin	Sialyl-Lewis X	Rolling, adhesion to activated endothelium (neutrophils, monocytes, T cells)
ICAM-1	CD11/CD18 (integrins)	Adhesion, arrest, transmigration (all leukocytes)
	(LFA-1, Mac-1)	
VCAM-1	α4β1 (VLA4) (integrins)	Adhesion (eosinophils, monocytes, lymphocytes)

	α4β7 (LPAM-1)	
GlyCam-1	L-selection	Lymphocyte homing to high endothelial venules
CD31 (PECAM)	CD31	Leukocyte migration through endothelium
*ICAM-1, VCAM-1, and CD31 belong to the immunoglobulin family of proteins; PSGL-1, P-selectin glycoprotein ligand 1.		

Leukocyte Adhesion and Transmigration

Leukocyte adhesion and transmigration are regulated largely by the binding of complementary adhesion molecules on the leukocyte and endothelial surfaces, and chemical mediators—chemoattractants and certain cytokines—affect these processes by modulating the surface expression or avidity of such adhesion molecules.[16] [17] The adhesion receptors involved belong to four molecular families—the *selectins*, the *immunoglobulin superfamily*, the *integrins*, and *mucin-like glycoproteins*. The most important of these are listed in Table 2-1 .

- *Selectins*, so called because they are characterized by an extracellular N-terminal domain related to sugar-binding mammalian lectins, consist of E-selectin (CD62E, previously known as ELAM-1), which is confined to endothelium; P-selectin (CD62P, previously called GMP140 or PADGEM), which is present in endothelium and platelets; and L-selectin (CD62L, previously known by many names, including LAM-1), which is expressed on most leukocyte types (Box 2-1).[18] [19] Selectins bind, through their lectin domain, to sialylated forms of oligosaccharides (e.g., sialylated Lewis X), which themselves are covalently bound to various *mucin-like glycoproteins* (GlyCAM-1, PSGL-1, ESL-1, and CD34).
- The *immunoglobulin family* molecules include two endothelial adhesion molecules: ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1). Both these molecules serve as ligands for integrins found on leukocytes.
- *Integrins* are transmembrane heterodimeric glycoproteins, made up of α and β chains, that are expressed on many cell types and bind to ligands on endothelial cells, other leukocytes, and the extracellular matrix (Box 2-1).[20] The β_2 integrins LFA-1 and Mac-1 (CD11a/CD18 and CD11b/CD18) bind to ICAM-1, and the β_1 integrins (such as VLA-4) bind VCAM-1.
- *Mucin-like glycoproteins*, such as heparan sulfate, serve as ligands for the leukocyte adhesion molecule called CD44. These glycoproteins are found in the extracellular matrix and on cell surfaces.

The recruitment of leukocytes to sites of injury and infection is a multistep process involving attachment of circulating leukocytes to endothelial cells and their migration through the endothelium (see Fig. 2-6). The first events are the induction of adhesion molecules on endothelial cells, by a number of mechanisms (Fig. 2-7). Mediators such as histamine, thrombin, and platelet activating factor (PAF) stimulate the redistribution of P-selectin from its normal intracellular stores in granules (Weibel-Palade bodies) to the cell surface. Resident tissue macrophages, mast cells, and endothelial cells respond to injurious agents by secreting the cytokines TNF, IL-1, and chemokines (chemoattractant cytokines). (Cytokines are described in more detail below and in Chapter 6 .) TNF and IL-1 act on the endothelial cells of postcapillary venules adjacent to the infection and induce the expression of several adhesion molecules. Within 1 to 2 hours, the endothelial cells begin to express E-selectin. Leukocytes express at the tips of their microvilli carbohydrate ligands for the selectins, which bind to the endothelial selectins. These are low-affinity interactions with a fast off-rate, and they are easily disrupted by the flowing blood. As a result, the bound leukocytes detach and bind again, and thus begin to roll along the endothelial surface.

TNF and IL-1 also induce endothelial expression of ligands for integrins, mainly VCAM-1 (the ligand for the VLA-4 integrin) and ICAM-1 (the ligand for the LFA-1 and Mac-1 integrins). Leukocytes normally express these integrins in a low-affinity state. Meanwhile, chemokines that were produced at the site of injury enter the blood vessel, bind to endothelial cell heparan sulfate glycosaminoglycans (labeled "proteoglycan" in Figure 2-6), and are displayed at high concentrations on the endothelial surface. [21] These chemokines act on the rolling leukocytes and activate the leukocytes. One of the consequences of activation is the conversion of VLA-4 and LFA-1 integrins on the leukocytes to a high-affinity state. The combination of induced expression of integrin ligands on the endothelium and activation of integrins on the leukocytes results in firm integrin-mediated binding of the leukocytes to the endothelium at the site of infection. The leukocytes stop rolling, their cytoskeleton is reorganized, and they spread out on the endothelial surface.

The next step in the process is migration of the leukocytes through the endothelium, called transmigration or *diapedesis*. Chemokines act on the adherent leukocytes and stimulate the cells to migrate through interendothelial spaces toward the chemical concentration gradient, that is, toward the site of injury or infection. Certain homophilic adhesion molecules (i.e., adhesion

molecules that bind to each other) present in the intercellular junction of endothelium are involved in

Box 2-1. Selectins and Integrins: Adhesion Molecules Involved in the Inflammatory Response

The specific (nonrandom) adhesion of cells to other cells or to extracellular matrices is a basic component of cell migration and recognition and underlies many biologic processes, including embryogenesis, tissue repair, and immune and inflammatory responses. It is, therefore, not surprising that many different genes have evolved that encode proteins with specific adhesive functions. Two families of adhesive proteins that are especially important in inflammation are the *selectins* and the *integrins*.

Selectins

The selectins are a family of three closely related proteins that differ in their cellular distribution but all function in adhesion of leukocytes to endothelial cells. All selectins are single-chain transmembrane glycoproteins with an amino terminus that is related to carbohydrate-binding proteins known as C-type lectins. Like other C-type lectins, ligand binding by selectins is calcium-dependent (hence the name C-type). The binding of selectins to their ligands has a fast on rate but also has a fast off rate and is of low affinity; this property allows selectins to mediate initial attachment and subsequent rolling of leukocytes on endothelium in the face of flowing blood.

L-selectin, or CD62L, is expressed on lymphocytes and other leukocytes. It serves as a homing receptor for lymphocytes to enter lymph nodes by binding to high endothelial venules (HEVs). It also serves to bind neutrophils to cytokine-activated endothelial cells at sites of inflammation. L-selectin is located on the tips of microvillus projections of leukocytes, facilitating its interaction with ligands on endothelium. At least three endothelial cell ligands can bind L-selectin—glycan-bearing cell adhesion molecule-1 (GlyCAM-1), a secreted proteoglycan found on HEVs of lymph node; mucosal addressin cell adhesion molecule-1 (MadCAM-1), expressed on endothelial cells in gut-associated lymphoid tissues; and CD34, a proteoglycan on endothelial cells (and bone marrow cells). The protein backbones of all these ligands are modified by specific carbohydrates, which are the molecules actually recognized by the selectin.

E-selectin, or CD62E, previously known as endothelial leukocyte adhesion molecule-1 (ELAM-1), is expressed only on cytokine-activated endothelial cells, hence the designation E. E-selectin recognizes complex sialylated carbohydrate groups related to the Lewis X or Lewis A family found on various surface proteins of granulocytes, monocytes, and previously activated effector and memory T cells. E-selectin is important in the homing of effector and memory T cells to some peripheral sites of inflammation, particularly in the skin. Endothelial cell expression of E-selectin is a hallmark of acute cytokine-mediated inflammation, and antibodies to E-selectin can block leukocyte accumulation in vivo.

P-selectin (CD62P) was first identified in the secretory granules of platelets, hence the designation P. It has since been found in secretory granules of endothelial cells, called Weibel-Palade bodies. When endothelial cells or platelets are stimulated, P-selectin is translocated within minutes to the cell surface. On reaching the cell surface, P-selectin mediates binding of neutrophils, T lymphocytes, and monocytes. The complex carbohydrate ligands recognized by P-selectin appear similar to those recognized by E-selectin.

The essential physiologic roles of selectins have been reinforced by studies of gene knockout mice. L-selectin-deficient mice have small, poorly formed lymph nodes with few T cells. Mice lacking either E-selectin or P-selectin have only mild defects in leukocyte recruitment, suggesting that these two molecules are functionally redundant. Double knockout mice lacking both E-selectin and P-selectin have significantly impaired leukocyte recruitment and increased susceptibility to infections. Humans who lack one of the enzymes needed to express the carbohydrate ligands for E-selectin and P-selectin on neutrophils have similar problems, resulting in a syndrome called leukocyte adhesion deficiency-2 (LAD-2) (see text).

Integrins

The integrin superfamily consists of about 30 structurally homologous proteins that promote cell-cell or cell-matrix interactions. The name of this family of proteins derives from the hypothesis that they coordinate (i.e., "integrate") signals from extracellular ligands with cytoskeleton-dependent motility, shape change, and phagocytic responses.

All integrins are heterodimeric cell surface proteins composed of two noncovalently linked polypeptide chains, α and β. The extracellular domains of the two chains bind to various ligands, including extracellular matrix glycoproteins, activated complement components, and proteins on the surfaces of other cells. Several integrins bind to Arg-Gly-Asp (RGD) sequences in the fibronectin and vitronectin molecules. The cytoplasmic domains of the integrins interact with cytoskeletal components (including vinculin, talin, actin, α-actinin, and tropomyosin).

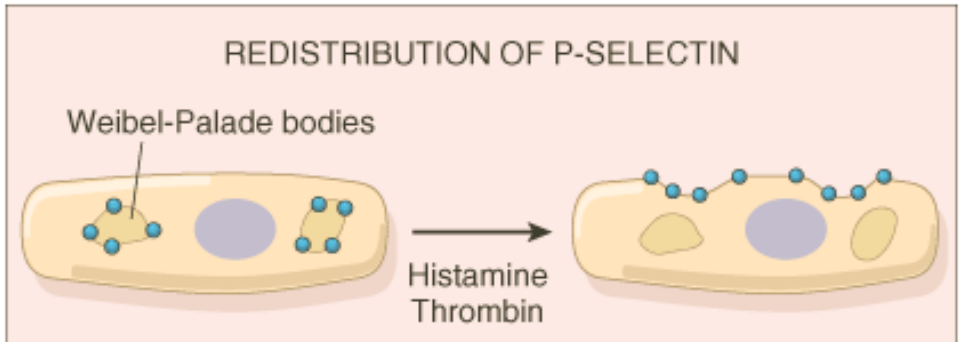
Three integrin subfamilies were originally defined on the basis of which of three β subunits were used to form the heterodimers. More recently, five additional β chains have been identified.

The β_1 -containing integrins are also called VLA molecules, referring to "very late activation" molecules, because α_1 β_1 and α_2 β_1 were first shown to be expressed on T cells 2 to 4 weeks after repetitive stimulation in vitro. In fact, other VLA integrins are constitutively expressed on some leukocytes and rapidly induced on others. The β_1 integrins are also called CD49a-hCD29, CD49a-h referring to different α chains (α_1 -α_8) and CD29 referring to the common β_1 subunit. Most of the β_1 integrins are widely expressed on leukocytes and other cells and mediate attachment of cells to extracellular matrices. VLA-4 (α_4 β_1) is expressed only on leukocytes and can mediate attachment of these cells to endothelium by interacting with vascular cell adhesion molecule-1 (VCAM-1). VLA-4 is one of the principal surface proteins that mediate homing of lymphocytes to endothelium at peripheral sites of inflammation.

The β_2 integrins are also called CD11a-cCD18, or the leukocyte function-associated antigen-1 (LFA-1) family, CD11a-c referring to different α chains and CD18 to the common β_2 subunit. LFA-1 (CD11aCD18) plays an important role in the adhesion of lymphocytes and other leukocytes with other cells, such as antigen-presenting cells and vascular endothelium. Other members of the family include CD11bCD18 (Mac-1 or CR3) and CD11cCD18 (p150,95 or CR4), which mediate leukocyte attachment to endothelial cells and subsequent extravasation. CD11bCD18 also functions as a fibrinogen receptor and as a complement receptor on phagocytic cells, binding particles opsonized with a by-product of complement activation called the inactivated C3b (iC3b) fragment.

The other integrins are expressed on platelets and other cell types, and bind to extracellular matrix proteins as well as proteins involved in coagulation.

Figure 2-7 Regulation of endothelial and leukocyte adhesion molecules. *A*, Redistribution of P-selectin. *B*, Cytokine activation of endothelium. *C*, Increased binding avidity of integrins (see text).



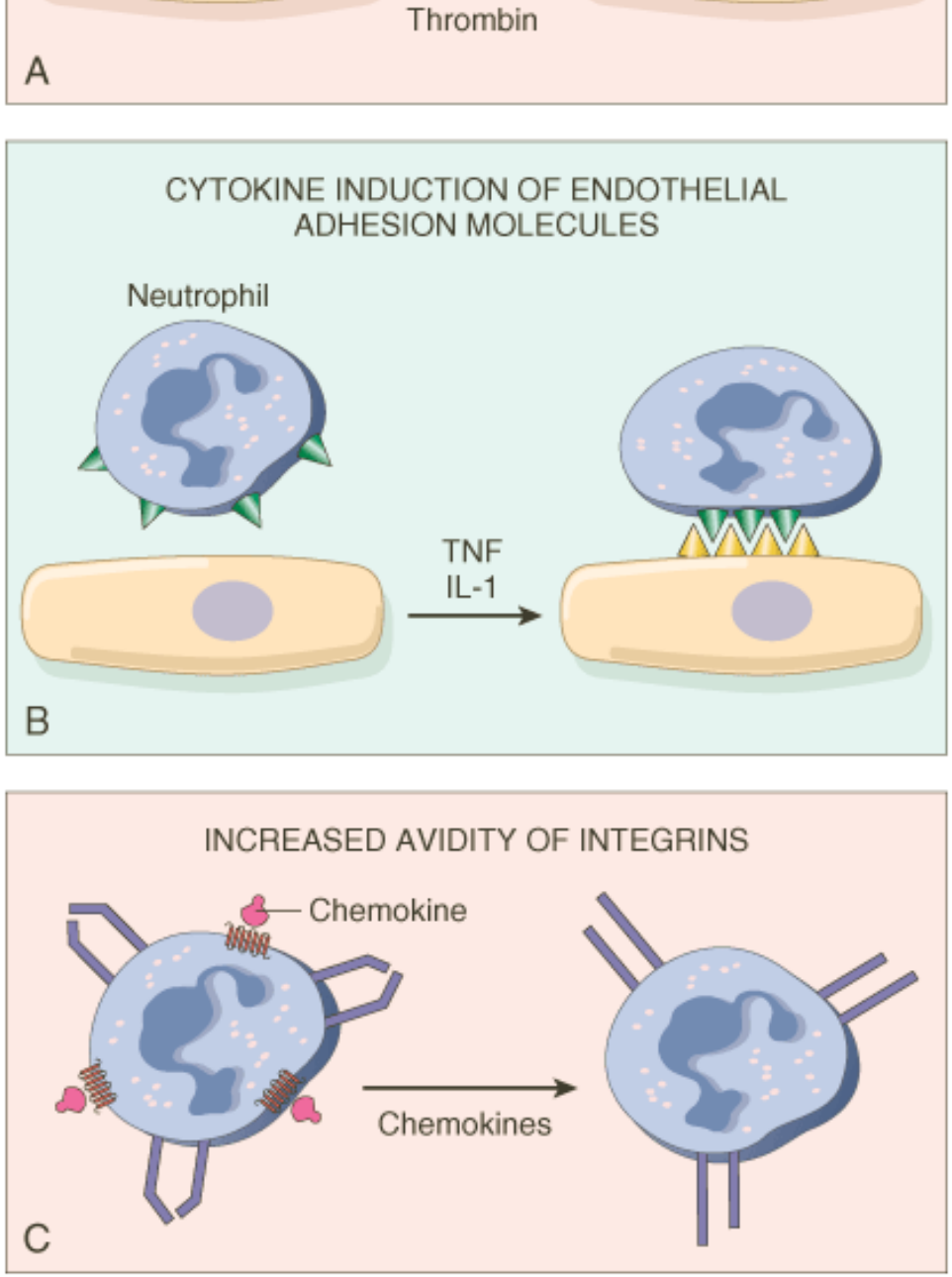


Figure 2-8 Schematic and histologic sequence of events following acute injury. The photomicrographs are representative of the early (neutrophilic) (*left*) and later (mononuclear) cellular infiltrates (*right*) of infarcted myocardium. The kinetics of edema and cellular infiltration are approximations. For sake of simplicity, edema is shown as an acute transient response, although secondary waves of delayed edema and neutrophil infiltration can also occur.

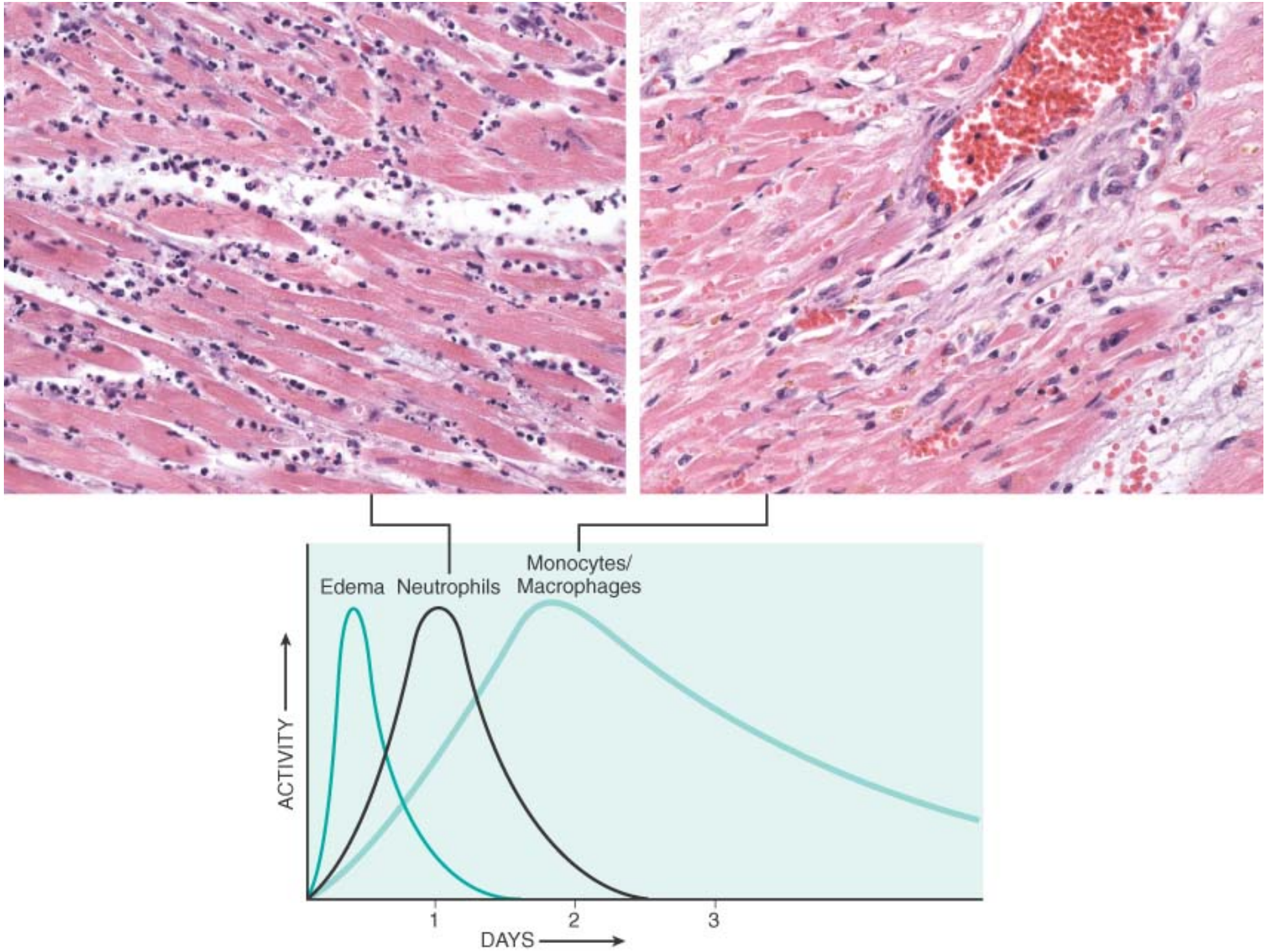

Edema
Neutrophils
Monocytes/
Macrophages
ACTIVITY
1
2
3
DAYS

Figure 2-9 Scanning electron micrograph of a moving leukocyte in culture showing a filopodium (*upper left*) and a trailing tail. *(Courtesy of Dr. Morris J. Karnovsky, Harvard Medical School, Boston, MA.)*

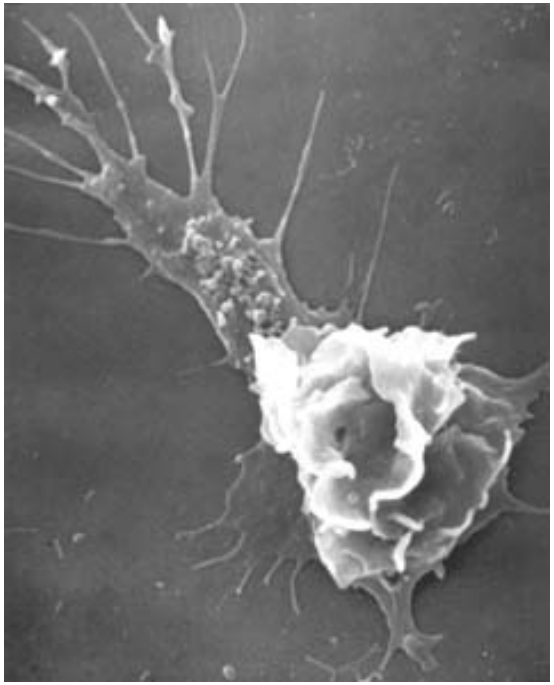


Figure 2-10 Leukocyte activation. Different classes of cell surface receptors of leukocytes recognize different stimuli. The receptors initiate responses that mediate the functions of the leukocytes. Only some receptors are depicted (see text for details).

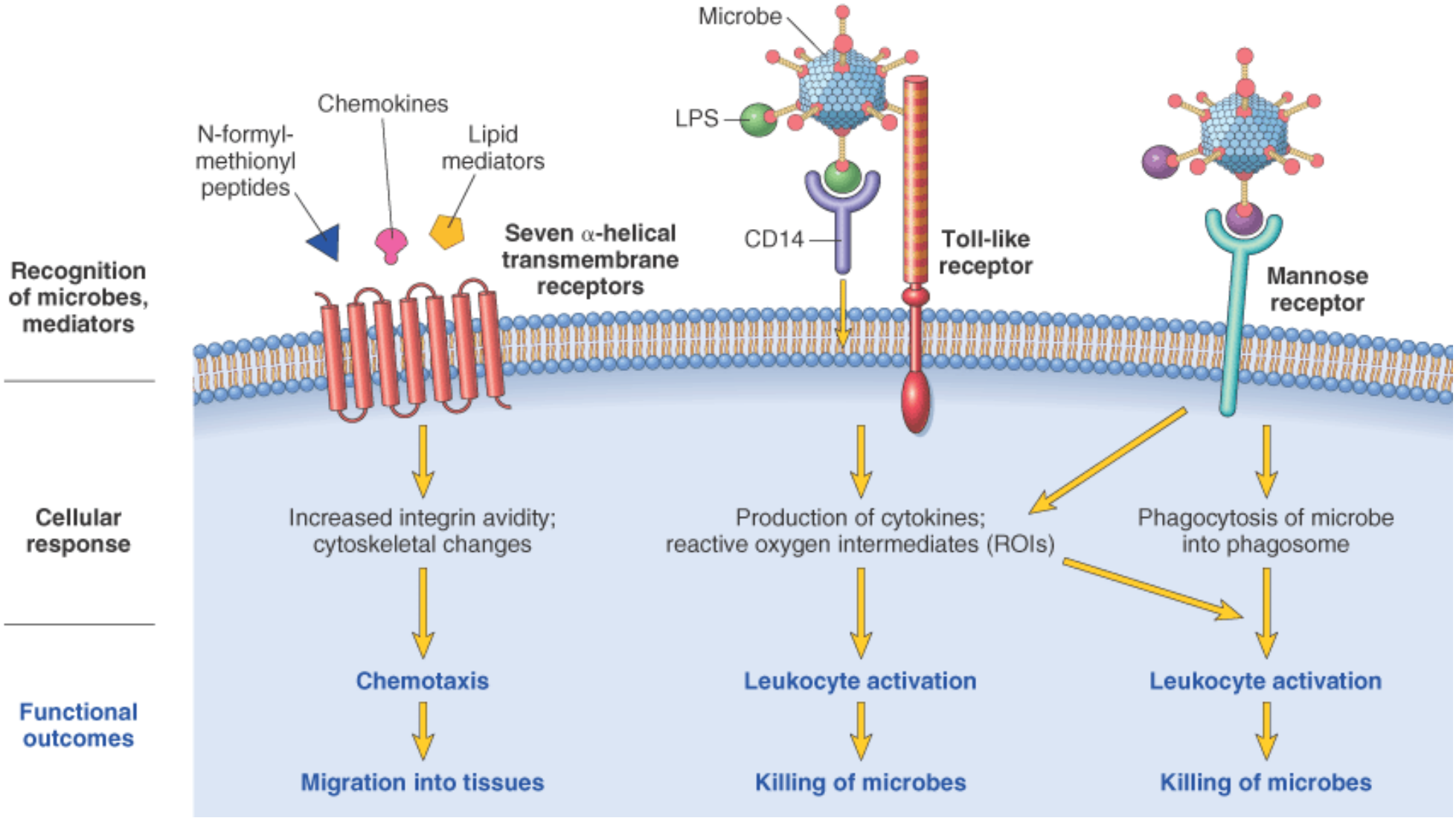
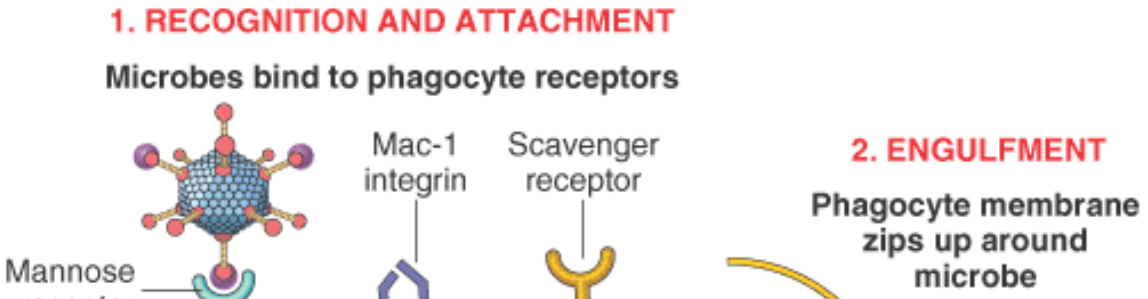
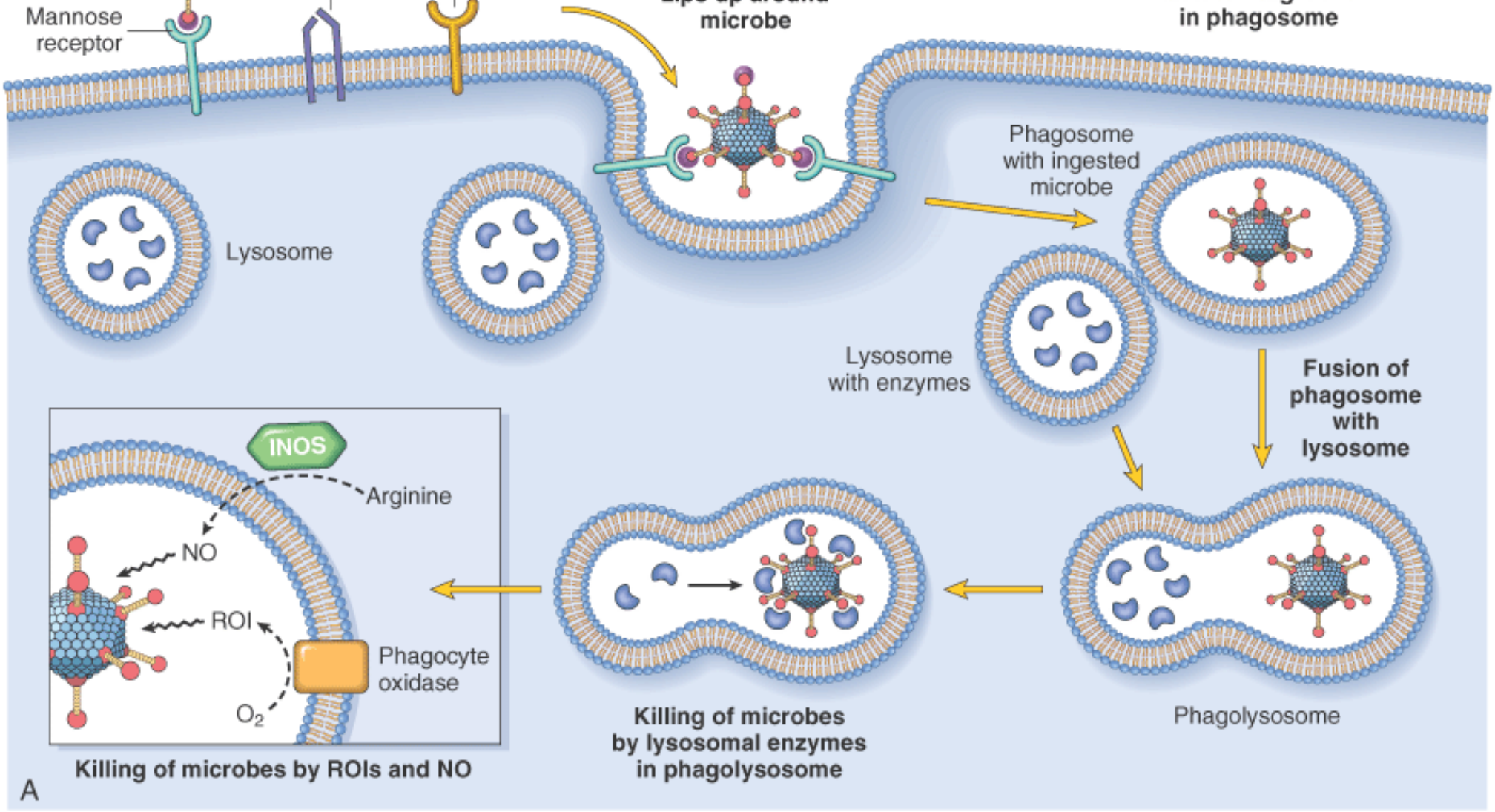
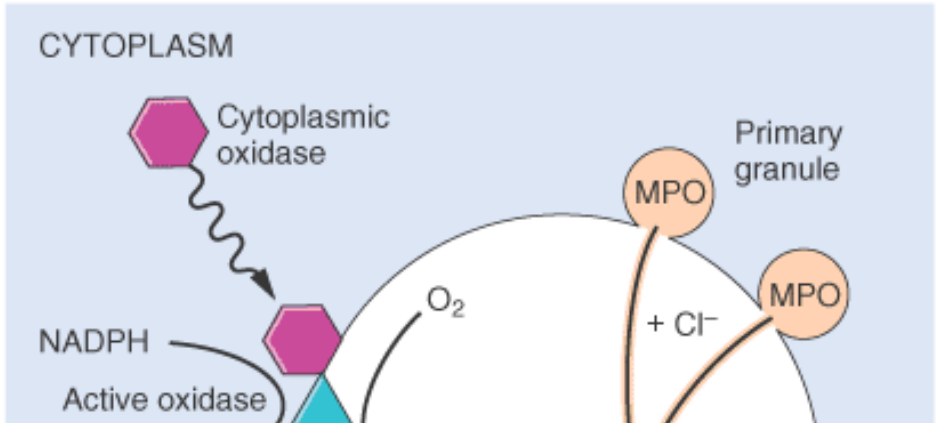


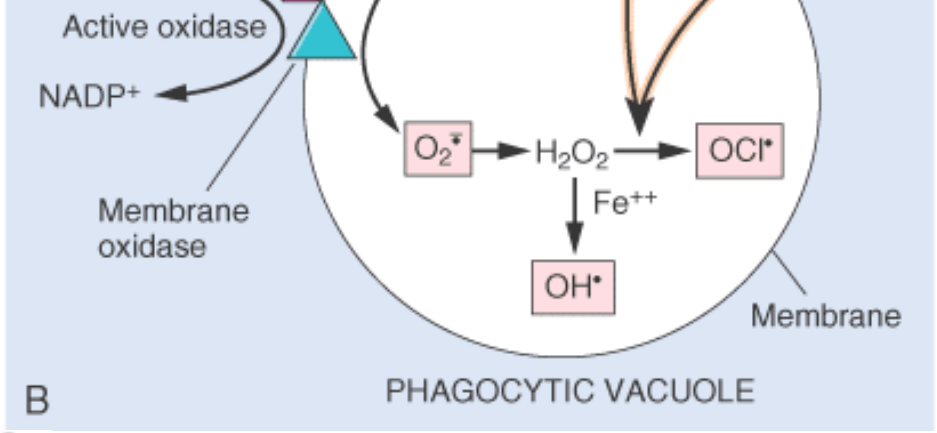
Figure 2-11 *A*, Phagocytosis of a particle (e.g., bacterium) involves attachment and binding of Fc and C3b to receptors on the leukocyte membrane, engulfment, and fusion of lysosomes with phagocytic vacuoles, followed by destruction of ingested particles within the phagolysosomes. Note that during phagocytosis, granule contents may be released into extracellular tissues. *B*, Production of microbicidal reactive oxygen intermediates within phagocytic vesicles.





3. KILLING AND DEGRADATION





$O_2^{\bullet}$

). Superoxide is then converted into hydrogen peroxide (H_2O_2), mostly by spontaneous dismutation. Hydrogen peroxide can also be further reduced to the highly reactive hydroxyl radical (OH). Most of the H_2O_2 is eventually broken down by catalase into H_2O and O_2, and some is destroyed by the action of glutathione oxidase. This process and its regulation were described in detail in Chapter 1 .

NADPH oxidase is an enzyme complex consisting of at least seven proteins.[32] In resting neutrophils, different NADPH

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oxidase protein components are located in the plasma membrane and the cytoplasm. In response to activating stimuli, the cytosolic protein components translocate to the plasma membrane or phagosomal membrane, where they assemble and form the functional enzyme complex (see Fig. 2-11*B*). Thus, the *reactive oxygen intermediates are produced within the lysosome* where the ingested substances are segregated, and the cell's own organelles are protected from the harmful effects of the ROIs. A similar enzyme system generates reactive nitrogen intermediates, notably nitric oxide, which also helps to kill microbes.

The H_2O_2 generated by the NADPH oxidase system is generally not able to efficiently kill microbes by itself. However, the azurophilic granules of neutrophils contain the enzyme *myeloperoxidase* (MPO), which, in the presence of a halide such as Cl^-, converts H_2O_2 to hypochlorite (HOCl). The latter is a potent antimicrobial agent that destroys microbes by *halogenation* (in which the halide is bound covalently to cellular constituents) or by oxidation of proteins and lipids (lipid peroxidation).[33] *The H_2O_2-MPO-halide system is the most efficient bactericidal system in neutrophils.* MPO-deficient leukocytes are capable of killing bacteria (albeit more slowly than normal cells), by virtue of the formation of superoxide, hydroxyl radicals, and singlet-oxygen.

Bacterial killing can also occur by *oxygen-independent mechanisms*, through the action of substances in leukocyte granules.[34] These include *bactericidal permeability increasing protein* (BPI), a highly cationic granule-associated protein that causes phospholipase activation, phospholipid degradation, and increased permeability in the outer membrane of the microorganisms; *lysozyme*, which hydrolyzes the muramic acid-*N*-acetyl-glucosamine bond, found in the glycopeptide coat of all bacteria; *lactoferrin*, an iron-binding protein present in specific granules; *major basic protein*, a cationic protein of eosinophils, which has limited bactericidal activity but is cytotoxic to many parasites; and *defensins*, cationic arginine-rich granule peptides that are cytotoxic to microbes (and certain mammalian cells).[35] In addition, neutrophil granules contain many *enzymes*, such as elastase, that also contribute to microbial killing (discussed later in the chapter).

After killing, acid hydrolases, which are normally stored in lysosomes, degrade the microbes within phagolysosomes. The pH of the phagolysosome drops to between 4 and 5 after phagocytosis, this being the optimal pH for the action of these enzymes.

Release of Leukocyte Products and Leukocyte-Induced Tissue Injury

During activation and phagocytosis, leukocytes release microbicidal and other products not only within the phagolysosome but also into the extracellular space. The most important of these substances in neutrophils and macrophages are *lysosomal enzymes*, present in the granules; *reactive oxygen intermediates*; and *products of arachidonic acid metabolism*, including prostaglandins and leukotrienes. These products are capable of causing endothelial injury and tissue damage and may thus amplify the effects of the initial injurious agent. Products of monocytes/macrophages and other leukocyte types have additional potentially harmful products, which are described in the discussion of chronic inflammation. Thus, if persistent and unchecked, the leukocyte infiltrate itself becomes the offender,[36] and leukocyte-dependent tissue injury underlies many acute and chronic human diseases (Table 2-2). This fact becomes evident in the discussion of specific disorders throughout this book.

Regulated secretion of lysosomal proteins is a peculiarity of leukocytes and other hematopoietic cells. (Recall that in most secretory cells, the proteins that are secreted are not stored within lysosomes.) The contents of lysosomal granules are secreted by leukocytes into the extracellular milieu by diverse mechanisms. Release may occur if the phagocytic vacuole remains transiently open to the outside before complete closure of the phagolysosome *(regurgitation during feeding)*. If cells are exposed to potentially ingestible materials, such as immune complexes deposited on immovable flat surfaces (e.g., glomerular basement membrane), attachment of leukocytes to the immune complexes triggers leukocyte activation, but the fixed immune complexes cannot be phagocytosed, and lysosomal enzymes are released into the medium *(frustrated phagocytosis)*. *Cytotoxic release* occurs after phagocytosis of potentially membranolytic substances, such as urate crystals, which damage the membrane of the phagolysosome. In addition, there is some evidence that proteins in certain granules, particularly the specific (secondary) granules of neutrophils, may be directly secreted by *exocytosis*.[37] [38]

After phagocytosis, neutrophils rapidly undergo *apoptotic cell death* and are ingested by macrophages.

Defects in Leukocyte Function

From the preceding discussion, it is obvious that leukocytes play a central role in host defense. Not surprisingly, therefore, defects in leukocyte function, both genetic and acquired, lead to increased vulnerability to infections (Table 2-3). Impairments of virtually every phase of leukocyte function—from adherence to vascular endothelium to microbicidal activity—have been identified, and the existence of clinical genetic deficiencies in each of the critical steps in the process has been described. These include the following:

- *Defects in leukocyte adhesion.* We previously mentioned the genetic deficiencies in leukocyte adhesion molecules (LAD types 1 and 2). LAD 1 is characterized by recurrent bacterial infections and impaired wound healing. LAD 2 is clinically milder than LAD 1 but is also characterized by recurrent bacterial infections.
- *Defects in phagolysosome function.* One such disorder is *Chédiak-Higashi syndrome*, an autosomal recessive condition

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characterized by neutropenia (decreased numbers of neutrophils), defective degranulation, and delayed microbial killing. The neutrophils (and other leukocytes) have *giant granules*, which can be readily seen in peripheral blood smears and which are thought to result from aberrant organelle fusion.[39]In this syndrome, there is reduced transfer of lysosomal enzymes to phagocytic vacuoles in phagocytes (causing susceptibility to infections) and abnormalities in melanocytes (leading to albinism), cells of the nervous system (associated with nerve defects), and platelets (generating bleeding disorders). The gene associated with this disorder encodes a large cytosolic protein that is apparently involved in vesicular traffic but whose precise function is not yet known. The secretion of granule proteins by cytotoxic T cells is also affected, accounting for part of the immunodeficiency seen in the disorder.

- *Defects in microbicidal activity.* The importance of oxygen-dependent bactericidal mechanisms is shown by the existence of a group of congenital disorders with defects in bacterial killing called *chronic granulomatous disease*, which render patients susceptible to recurrent bacterial infection. Chronic granulomatous disease results from *inherited defects in the genes encoding several components of NADPH oxidase*, which generates superoxide. The most common variants are an *X-linked defect* in one of the plasma membrane-bound

components (gp91phox) and *autosomal recessive* defects in the genes encoding two of the cytoplasmic components (p47phox and p67phox).[40] [41]
• Clinically, the most frequent cause of leukocyte defects is *bone marrow suppression*, leading to reduced production of leukocytes. This is seen following therapies for cancer (radiation and chemotherapy) and when the marrow space is compromised by tumor metastases to bone.

TABLE 2-2 -- Clinical Examples of Leukocyte-Induced Injury

Acute	**Chronic**
Acute respiratory distress syndrome	Arthritis
Acute transplant rejection	Asthma
Asthma	Atherosclerosis
Glomerulonephritis	Chronic lung disease
Reperfusion injury	Chronic rejection
Septic shock	
Vasculitis	

TABLE 2-3 -- Defects in Leukocyte Functions

Disease	**Defect**
Genetic	
Leukocyte adhesion deficiency 1	β chain of CD11/CD18 integrins
Leukocyte adhesion deficiency 2	Fucosyl transferase required for synthesis of sialylated oligosaccharide (receptor for selectin)
Chronic granulomatous disease	Decreased oxidative burst
••X-linked	••NADPH oxidase (membrane component)
••Autosomal recessive	••NADPH oxidase (cytoplasmic components)
Myeloperoxidase deficiency	Absent MPO-H_2O_2 system
Chédiak-Higashi syndrome	Protein involved in organelle membrane docking and fusion
Acquired	
Thermal injury, diabetes, malignancy, sepsis, immunodeficiencies	Chemotaxis
Hemodialysis, diabetes mellitus	Adhesion
Leukemia, anemia, sepsis, diabetes, neonates, malnutrition	Phagocytosis and microbicidal activity
Data from Gallin JI: Disorders of phagocytic cells. In Gallin JI, et al (eds): Inflammation: Basic Principles and Clinical Correlates, 2nd ed. New York, Raven Press, 1992, pp 860, 861.	

Although we have emphasized the role of leukocytes recruited from the circulation in the acute inflammatory response, *cells resident in tissues also serve important functions in initiating acute inflammation*. The two most important of these cell types are *mast cells* and tissue *macrophages*. Mast cells react to physical trauma, breakdown products of complement, microbial products, and neuropeptides. The cells release histamine, leukotrienes, enzymes, and many cytokines (including TNF, IL-1, and chemokines), all of which contribute to inflammation. The functions of mast cells are discussed in more detail in Chapter 6 . Macrophages recognize microbial products and secrete most of the cytokines important in acute inflammation. These cells are stationed in tissues to rapidly recognize potentially injurious stimuli and initiate the host defense reaction.

TERMINATION OF THE ACUTE INFLAMMATORY RESPONSE

It is predictable that such a powerful system of host defense, with its inherent capacity to cause tissue damage, needs tight controls to minimize the damage. In part, inflammation declines simply because the mediators of inflammation have short half-lives, are degraded after their release, and are produced in quick bursts, only as long as the stimulus persists. In addition as inflammation develops, the process also triggers a variety of stop signals that serve to actively terminate the reaction.[42] These active mechanisms include a switch in the production of pro-inflammatory leukotrienes to anti-inflammatory lipoxins from arachidonic acid (described below); the liberation of an anti-inflammatory cytokine, transforming growth factor-β (TGF-β), from macrophages and other cells; and neural impulses (cholinergic discharge) that inhibit the production of TNF in macrophages.[43] There are, in addition, many other controls whose existence is suspected from the phenotypes of mice in which genes encoding putative regulatory molecules have been knocked out—these mice develop uncontrolled inflammation, but precisely how the regulation works normally is not yet defined.[42] Not surprisingly, there is great interest in defining the molecular basis

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of the brakes on inflammation, since this knowledge could be used to design powerful anti-inflammatory drugs.

Chemical Mediators of Inflammation

Having described the events in acute inflammation, we can now turn to a discussion of the chemical mediators that are responsible for the events. Many mediators have been identified, and how they function in a coordinated manner is still not fully understood. Here we review general principles and highlight some of the major mediators (Fig. 2-12).

- *Mediators originate either from plasma or from cells.* Plasma-derived mediators (e.g., complement proteins, kinins) are present in plasma in *precursor forms that must be activated*, usually by a series of proteolytic cleavages, to acquire their biologic properties. Cell-derived mediators are normally *sequestered in intracellular granules* that need to be secreted (e.g., histamine in mast cell granules) or are *synthesized de novo* (e.g., prostaglandins, cytokines) in response to a stimulus. The major cellular sources are platelets, neutrophils, monocytes/macrophages, and mast cells, but mesenchymal cells (endothelium, smooth muscle, fibroblasts) and most epithelia can also be induced to elaborate some of the mediators.
- *The production of active mediators is triggered by microbial products or by host proteins, such as the proteins of the complement, kinin, and coagulation systems, that are themselves activated by microbes and damaged tissues.*
- Most mediators perform their biologic activity by initially *binding to specific receptors on target cells*. Some, however, have direct enzymatic activity (e.g., lysosomal proteases) or mediate oxidative damage (e.g., reactive oxygen and nitrogen intermediates).
- *One mediator can stimulate the release of other mediators by target cells themselves.* These secondary mediators may be identical or similar to the initial mediators but may also have opposing activities. They provide mechanisms for amplifying—or in certain instances counteracting—the initial mediator action.
- Mediators can act on one or few target cell types, have diverse targets, or may even have differing effects on different types of cells.
- *Once activated and released from the cell, most of these mediators are short-lived.* They quickly decay (e.g., arachidonic acid metabolites) or are inactivated by enzymes (e.g., kininase inactivates bradykinin), or they are otherwise scavenged (e.g., antioxidants scavenge toxic oxygen metabolites) or inhibited (e.g., complement regulatory proteins break up and degrade activated complement components). There is thus a system of checks and balances in the regulation of mediator actions.
- Most mediators have the potential to cause harmful effects.

Figure 2-12 Chemical mediators of inflammation. EC, endothelial cells.

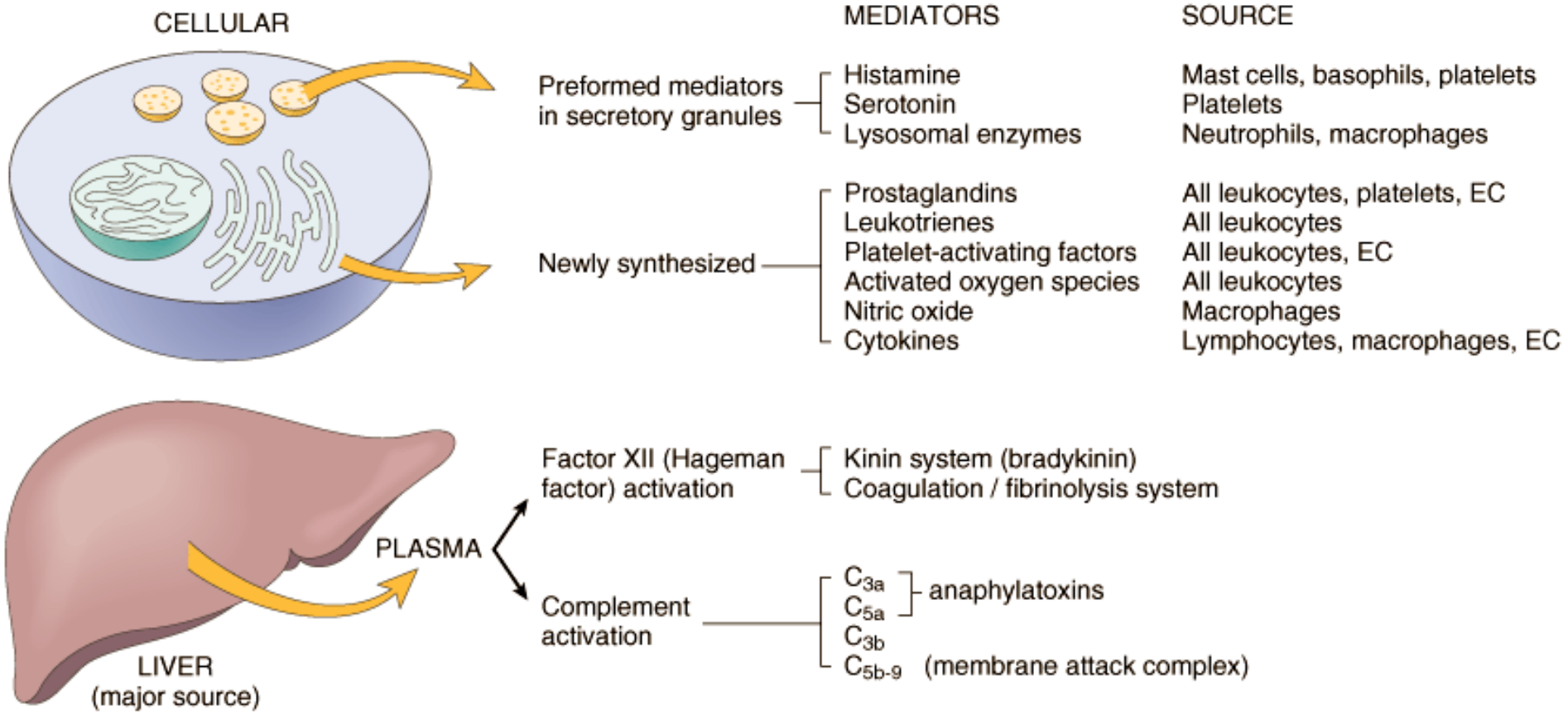


Figure 2-13 A flat spread of omentum showing mast cells around blood vessels and in the interstitial tissue. Stained with metachromatic stain to identify the mast cell granules (dark blue or purple). The red structures are fat globules stained with fat stain. *(Courtesy of Dr. G. Majno, University of Massachusetts Medical School, Worcester, MA.)*

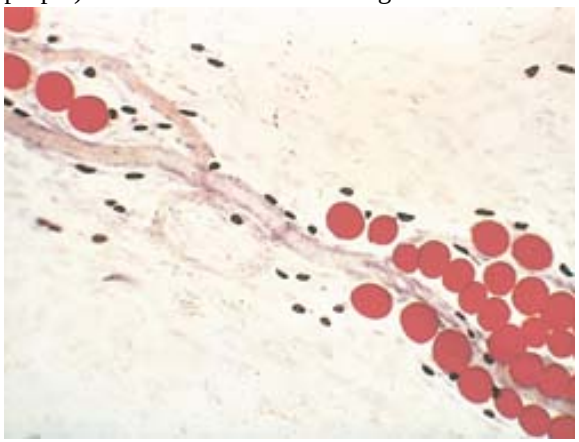
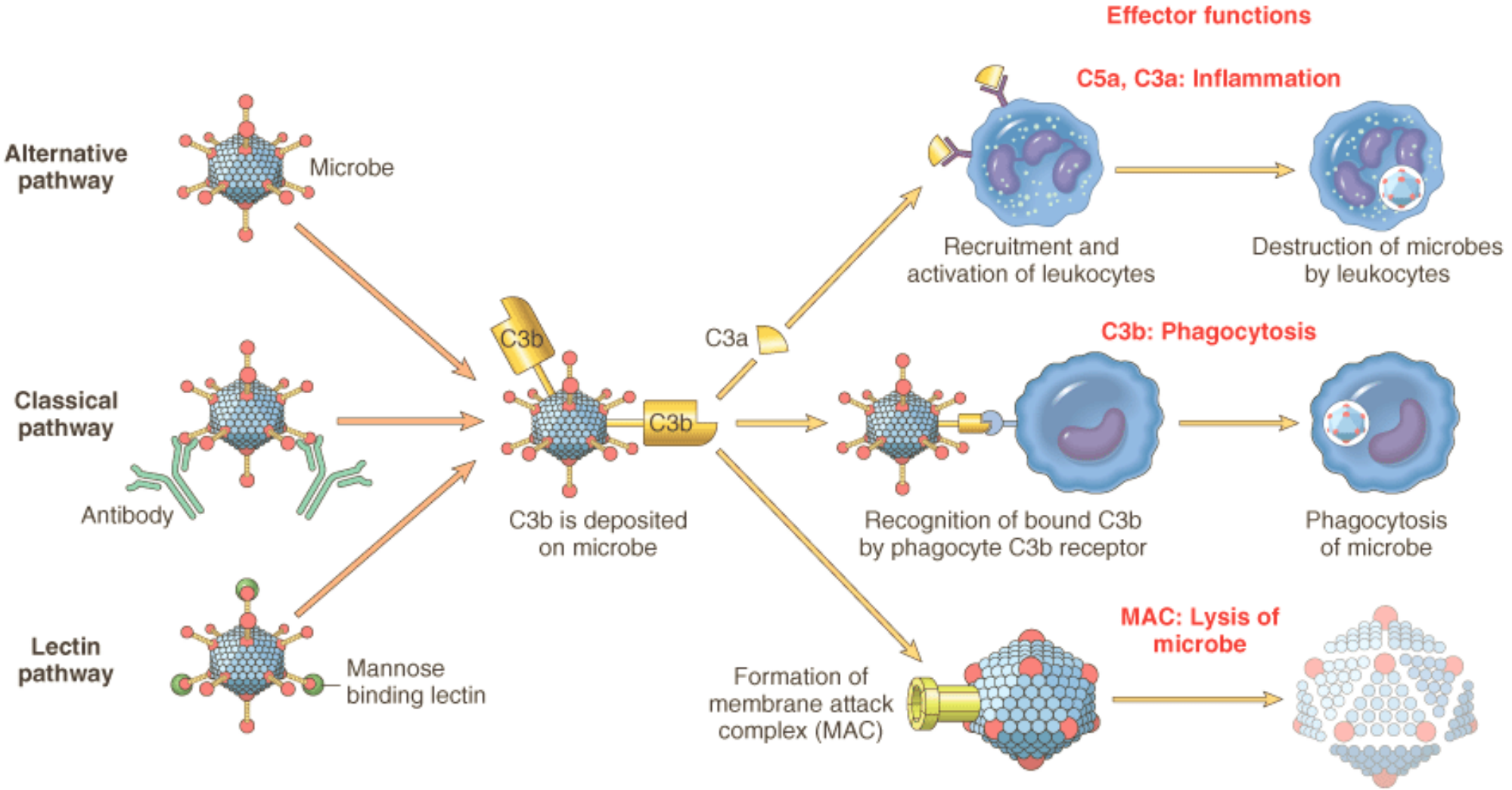


Figure 2-14 The activation and functions of the complement system. Activation of complement by different pathways leads to cleavage of C3. The functions of the complement system are mediated by breakdown products of C3 and other complement proteins, and by the membrane attack complex (MAC). The steps in the activation and regulation of complement are described in Box 2-2 .



Box 2-2. The Complement System in Health and Disease

The activation of the complement cascade may be divided into early and late steps. In the early steps, three different pathways lead to the proteolytic cleavage of C3. In the late steps, all three pathways converge, and the major breakdown product of C3, C3b, activates a series of other complement components.

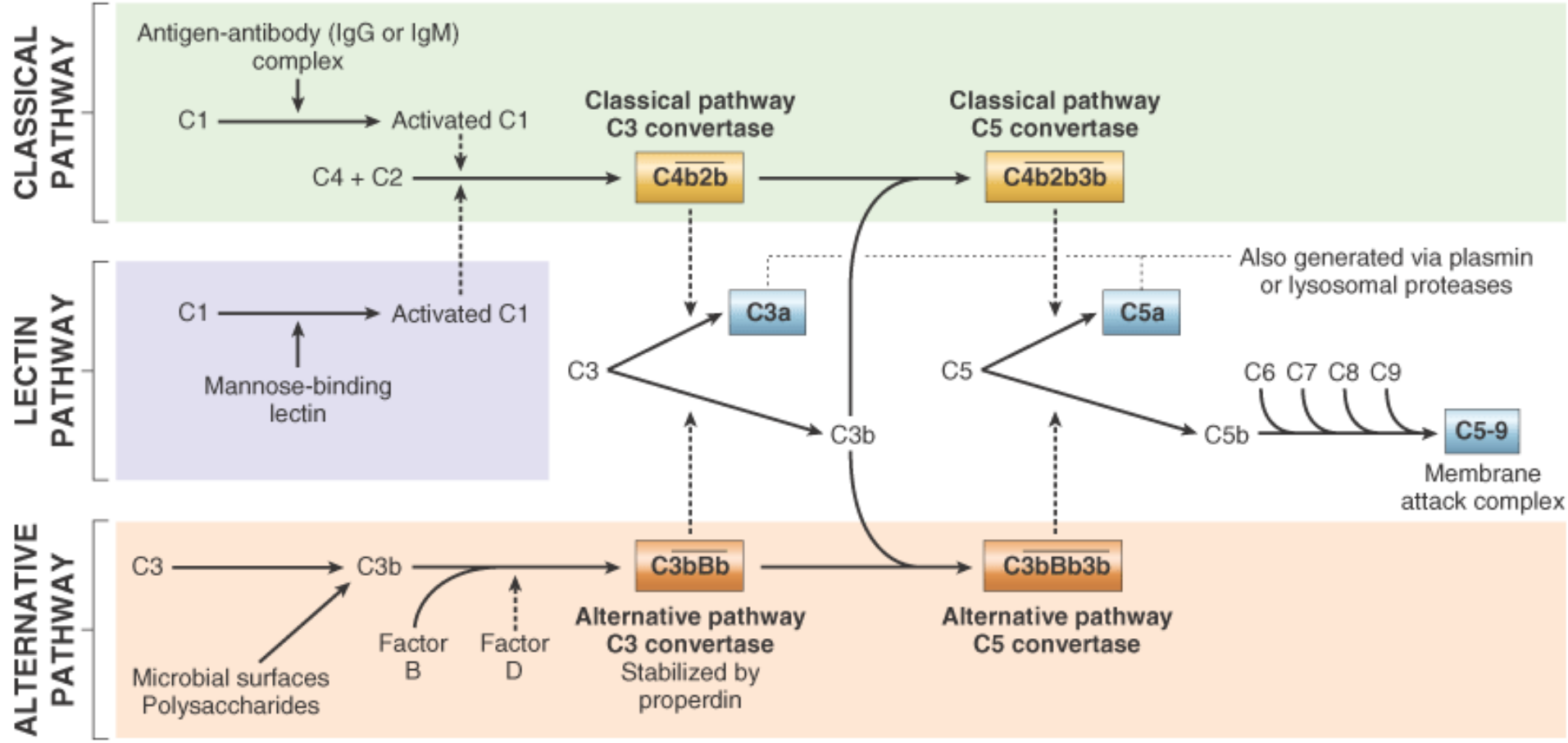
The Early Steps of Complement Activation

The pathways of early complement activation are the following (see Figure): The *classical pathway* is triggered by fixation of C1 to antibody (IgM or IgG) that has combined with antigen, and proteolysis of C2 and C4, and subsequent formation of a C4b2b complex that functions as a C3 convertase. The *alternative pathway* can be triggered by microbial surface molecules (e.g., endotoxin, or LPS), complex polysaccharides, and cobra venom. It involves a distinct set of plasma components (properdin, and factors B and D). In this pathway, the spontaneous cleavage of C3 that occurs normally is enhanced and stabilized by a complex of C3b and a breakdown product of Factor B called Bb; the C3bBb complex is a C3 convertase. In the *lectin pathway*, mannose-binding lectin, a plasma collectin, binds to carbohydrate-containing proteins on bacteria and viruses and directly activates C1; the remaining steps are as in the classical pathway. The C3 convertases break down C3 into C3b, which remains attached to the surface where complement is activated, and a smaller C3a fragment that diffuses away.

The Late Steps of Complement Activation

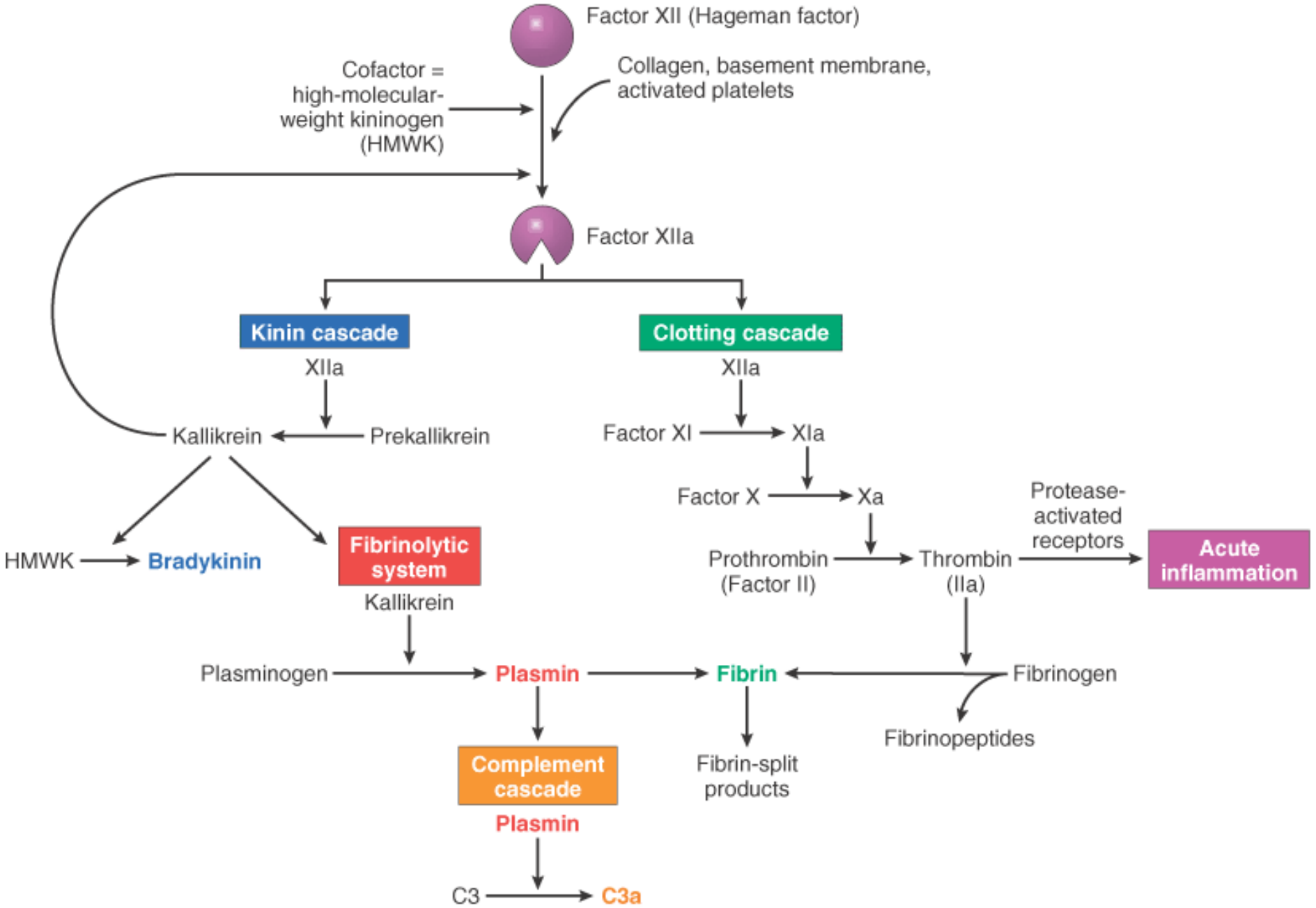
The C3b that is generated by any of the pathways binds to the C3 convertase and produces a C5 convertase, which cleaves C5. C5b remains attached to the complex and forms a substrate for the subsequent binding of the C6-C9 components. Polymerized C9 forms a channel in lipid membranes, called the

Figure 2-



receptors (PARs) because they bind multiple trypsin-like serine proteases in addition to thrombin.[49] These receptors are seven-transmembrane G protein-coupled receptors that are expressed on platelets, endothelial and smooth muscle cells, and many other cell types. Engagement of the so-called type

Figure 2-15 Interrelationships between the four plasma mediator systems triggered by activation of factor XII (Hageman factor). Note that thrombin induces inflammation by binding to protease-activated receptors (principally PAR-1) on platelets, endothelium, smooth muscle cells, and other cells.



The prostaglandins are also involved in the pathogenesis of *pain* and *fever* in inflammation. PGE_2 is hyperalgesic in that it makes the skin hypersensitive to painful stimuli. It causes a marked increase in pain produced by intradermal injection of suboptimal concentrations of histamine and bradykinin and is involved in cytokine-induced fever during infections (described later). PGD_2 is the major metabolite of the cyclooxygenase pathway in mast cells;

along with PGE_2 and $PGF_{2\alpha}$ (which are more widely distributed), it causes vasodilation and increases the permeability of postcapillary venules, thus potentiating edema formation.

There has been great interest in the COX-2 enzyme because it is induced by a variety of inflammatory stimuli and is absent in most tissues under normal "resting" conditions. COX-1, by contrast, is produced in response to inflammatory stimuli and is also constitutively expressed in most tissues. This difference has led to the notion that *COX-1 is responsible for the production of prostaglandins that are involved in inflammation but also serve a homeostatic function* (e.g., fluid and electrolyte balance in the kidneys, cytoprotection in the gastrointestinal tract). In contrast, *COX-2 stimulates the production of the prostaglandins that are involved in inflammatory reactions*.

• In the *lipoxygenase pathway*, the initial products are generated by three different lipoxygenases, which are present in only a few types of cells. 5-lipoxygenase (5-LO) is the predominant enzyme in neutrophils. The main product, 5-HETE, which is chemotactic for neutrophils, is converted into a family of compounds collectively called *leukotrienes*. LTB_4 is a potent chemotactic agent and activator of neutrophil functional responses, such as aggregation and adhesion of leukocytes to venular endothelium, generation of oxygen free radicals, and release of lysosomal enzymes. The cysteinyl-containing leukotrienes C_4 , D_4 , and E_4 (LTC_4 , LTD_4 , and LTE_4) cause intense vasoconstriction, bronchospasm, and increased vascular permeability. The vascular leakage, as with histamine, is restricted to venules. Leukotrienes are several orders of magnitude more potent than histamine in increasing vascular permeability and causing bronchospasm. Leukotrienes mediate their actions by binding to cysteiny leukotreine 1 (CysLT1) and CysLT2 receptors. They are important in the pathogenesis of bronchial asthma.

• *Lipoxins* are a recent addition to the family of bioactive products generated from AA, and transcellular biosynthetic mechanisms (involving two cell populations) are key to their production. Leukocytes, particularly neutrophils, produce intermediates in lipoxin synthesis, and these are converted to lipoxins by platelets interacting with the leukocytes. Lipoxins A_4 and B_4 (LXA_4 , LXB_4) are generated by the action of platelet 12-lipoxygenase on neutrophil-derived LTA_4 (Fig. 2-17). Cell-cell contact enhances transcellular metabolism, and blocking adhesion inhibits lipoxin

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production. *The principal actions of lipoxins are to inhibit leukocyte recruitment and the cellular components of inflammation.* They inhibit neutrophil chemotaxis and adhesion to endothelium.[55] There is an inverse relationship between the amount of lipoxin and leukotrienes formed, suggesting that the lipoxins may be endogenous negative regulators of leukotriene action and may thus play a role in the resolution of inflammation.

• A new class of arachidonic acid-derived mediators, called *resolvins*, have been identified in experimental animals treated with aspirin.[54] These mediators inhibit leukocyte recruitment and activation, in part by inhibiting the production of cytokines. Thus, the anti-inflammatory activity of aspirin is likely attributable to its ability to inhibit cyclooxygenases (see below) and, perhaps, to stimulate the production of resolvins.

Figure 2-16 Generation of arachidonic acid metabolites and their roles in inflammation. The molecular targets of action of some anti-inflammatory drugs are indicated by a red X. COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid.

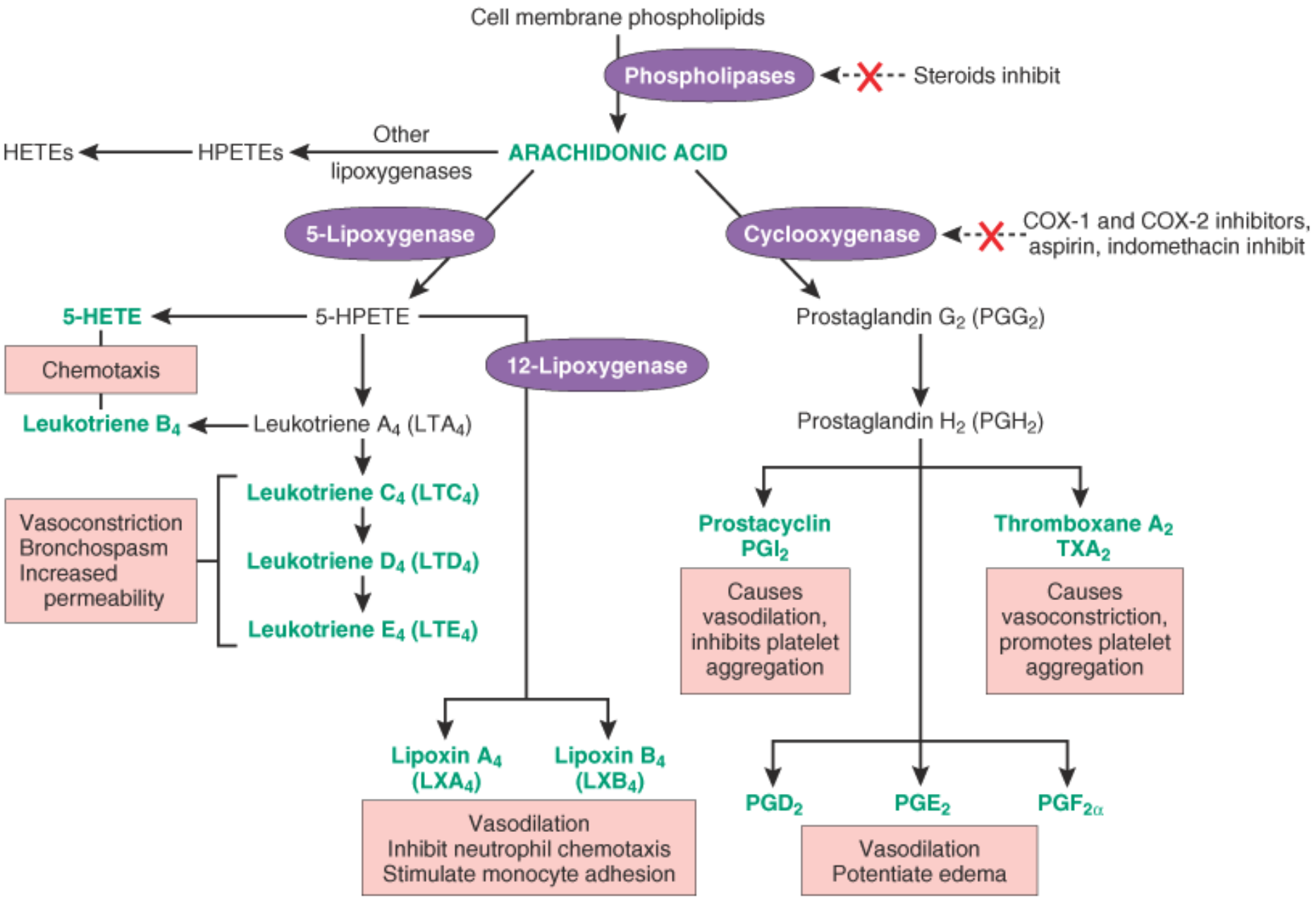


TABLE 2-4 -- Inflammatory Actions of Eicosanoids

Action	Metabolite
Vasoconstriction	Thromboxane A_2 , leukotrienes C_4 , D_4 , E_4

Vasodilation	PGI_2 , PGE_1 , PGE_2 , PGD_2
Increased vascular permeability	Leukotrienes C_4 , D_4 , E_4
Chemotaxis, leukocyte adhesion	Leukotriene B_4 , HETE, lipoxins

Figure 2-17 Biosynthesis of leukotrienes and lipoxins by cell-cell interaction. Activated neutrophils generate LTB_4 from arachidonic acid-derived LTA_4 by the action of 5-lipoxygenase, but they do not possess LTC_4 -synthase activity and consequently do not produce LTC_4 . In contrast, platelets cannot form LTC_4 from endogenous substrates, but they can generate LTC_4 and lipoxins from neutrophil-derived LTA_4 . *(Courtesy of Dr. C. Serhan, Brigham and Women's Hospital, Boston, MA.)*

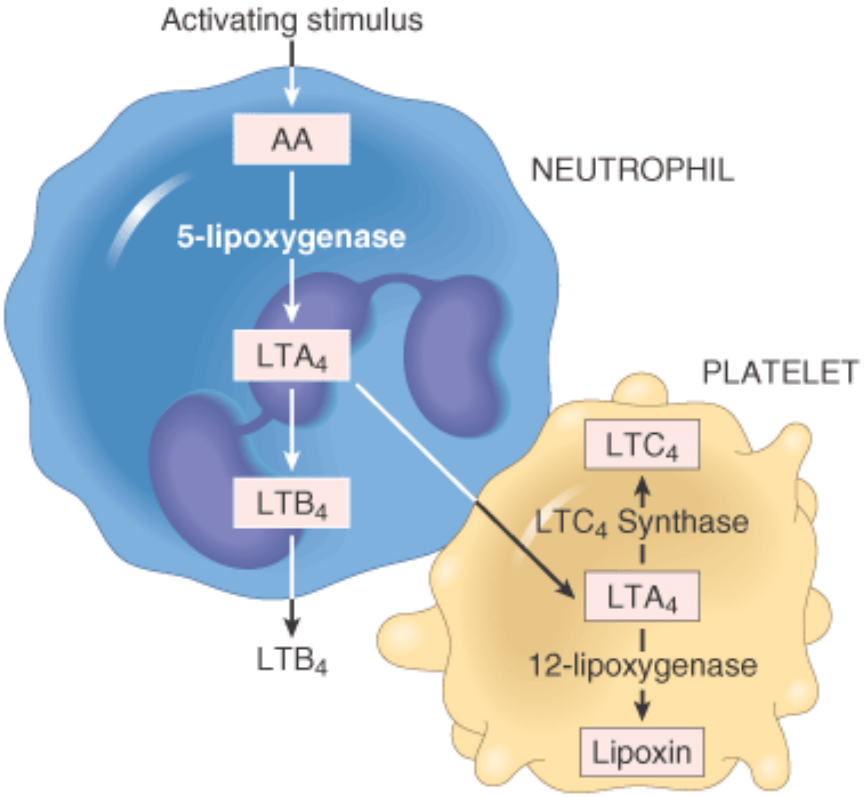


Figure 2-18 Major effects of interleukin-1 (IL-1) and tumor necrosis factor (TNF) in inflammation.

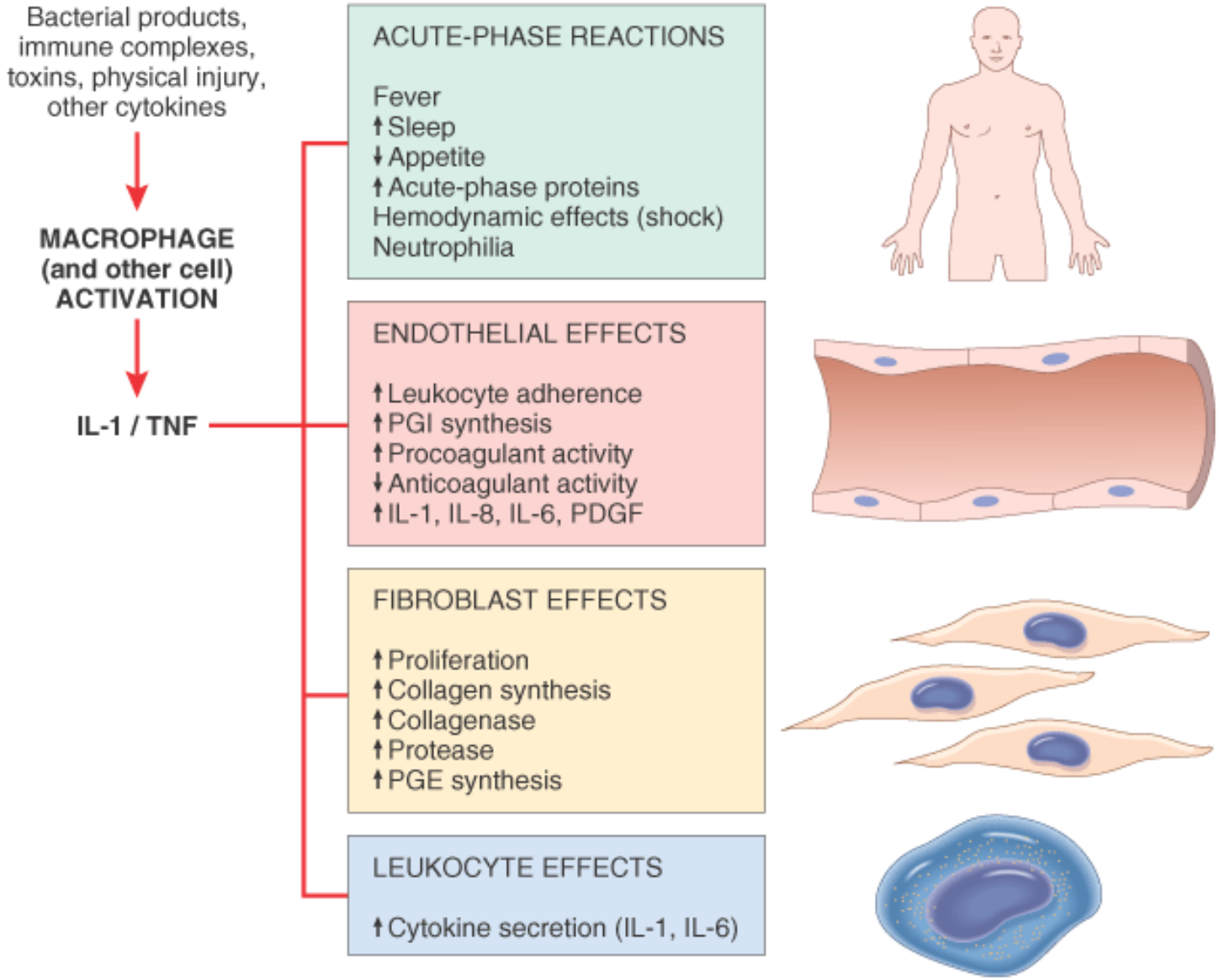


Figure 2-19 Functions of nitric oxide (NO) in blood vessels and macrophages, produced by two NO synthase enzymes. NO causes vasodilation, and NO free radicals are toxic to microbial and mammalian cells. NOS, nitric oxide synthase.

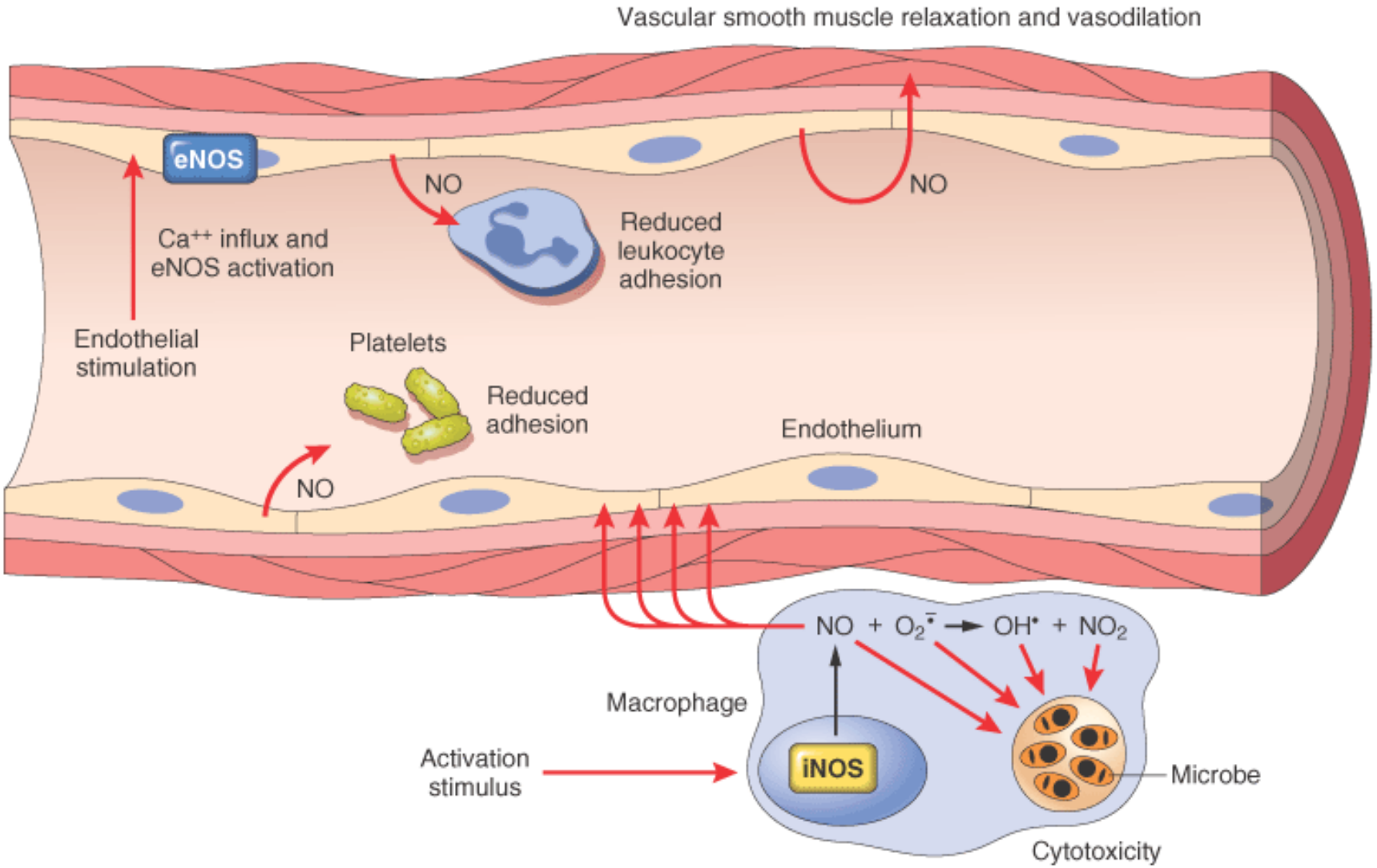
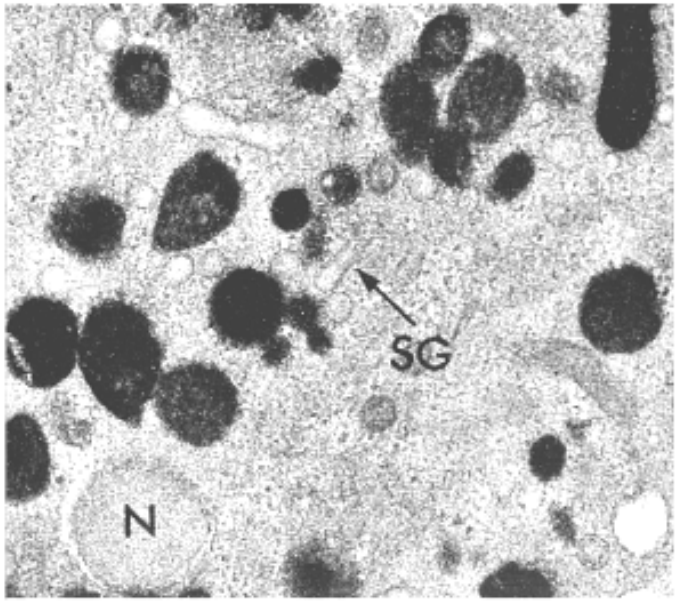


Figure 2-20 Ultrastructure and contents of neutrophil granules, stained for peroxidase activity. The large peroxidase-containing granules are the azurophil granules; the smaller peroxidase-negative ones are the specific granules (SG). N, portion of nucleus; BPI, bactericidal permeability increasing protein.



SPECIFIC GRANULES
- Lactoferrin
- Lysozyme
- Alkaline phosphatase
- Type IV collagenase
- Leukocyte adhesion molecules
- Plasminogen activation
- Phospholipase A_2

AZUROPHIL GRANULES
- Myeloperoxidase
- Lysozyme ← Bactericidal factors
- Cationic proteins
- Acid hydrolases
- Elastase
- Nonspecific collagenase
- BPI
- Defensins
- Cathepsin G
- Phospholipase A_2

O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) are the major species produced within the cell, and these metabolites can combine with NO to form other reactive nitrogen intermediates.[70] Extracellular release of low levels of these potent mediators can increase the expression of chemokines (e.g., IL-8), cytokines, and endothelial leukocyte adhesion molecules, amplifying the cascade that elicits the inflammatory response.[71] As mentioned earlier, the physiologic function of these reactive oxygen intermediates is to destroy phagocytosed microbes. At higher levels, release of

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these potent mediators can be *damaging* to the host. They are implicated in the following responses:

- *Endothelial cell damage, with resultant increased vascular permeability.* Adherent neutrophils, when activated, not only produce their own toxic species, but also stimulate xanthine oxidation in endothelial cells themselves, thus elaborating more superoxide.
- *Inactivation of antiprotease*, such as α_1 -antitrypsin. This leads to unopposed protease activity, with increased destruction of extracellular matrix.
- *Injury to other cell types* (parenchymal cells, red blood cells).

Serum, tissue fluids, and host cells possess *antioxidant mechanisms* that protect against these potentially harmful oxygen-derived radicals. These antioxidants were discussed in Chapter 1 ; they include: (1) the copper-containing serum protein *ceruloplasmin*; (2) the iron-free fraction of serum, *transferrin*; (3) the enzyme *superoxide dismutase*, which is found or can be activated in a variety of cell types; (4) the enzyme *catalase*, which detoxifies H_2O_2 ; and (5) *glutathione peroxidase*, another powerful H_2O_2 detoxifier.

Thus, the influence of oxygen-derived free radicals in any given inflammatory reaction depends on the *balance* between the production and the inactivation of these metabolites by cells and tissues.

NEUROPEPTIDES

Neuropeptides, similar to the vasoactive amines and the eicosanoids previously discussed, play a role in the initiation and propagation of an inflammatory response. The small peptides, such as *substance P* and neurokinin A, belong to a family of tachykinin neuropeptides produced in the central and peripheral nervous systems.[72] Nerve fibers containing substance P are prominent in the lung and gastrointestinal tract. Substance P has many biologic functions, including the transmission of pain signals, regulation of blood pressure,

TABLE 2-5 -- Summary of Mediators of Acute Inflammation

		Action		
Mediator	**Source**	***Vascular Leakage***	***Chemotaxis***	***Other***
Histamine and serotonin	Mast cells, platelets	+	-	
Bradykinin	Plasma substrate	+	-	Pain
C3a	Plasma protein via liver	+	-	Opsonic fragment (C3b)
C5a	Macrophages	+	+	Leukocyte adhesion, activation
Prostaglandins	Mast cells, from membrane phospholipids	Potentiate other mediators	-	Vasodilation, pain, fever
Leukotriene B_4	Leukocytes	-	+	Leukocyte adhesion, activation
Leukotriene C_4 , D_4 , E_4	Leukocytes, mast cells	+	-	Bronchoconstriction, vasoconstriction
Oxygen metabolites	Leukocytes	+	-	Endothelial damage, tissue damage
PAF	Leukocytes, mast cells	+	+	Bronchoconstriction, leukocyte priming
IL-1 and TNF	Macrophages, other	-	+	Acute-phase reactions, endothelial activation
Chemokines	Leukocytes, others	-	+	Leukocyte activation
Nitric oxide	Macrophages, endothelium	+	+	Vasodilation, cytotoxicity

stimulation of secretion by endocrine cells, and increasing vascular permeability. [73] Sensory neurons appear to produce other pro-inflammatory molecules, which are thought to link the sensing of dangerous stimuli to the development of protective host responses.[74]

OTHER MEDIATORS

The mediators described above account for inflammatory reactions to microbes, toxins, and many types of injury, but may not explain why inflammation develops in some specific situations. Recent studies are providing clues about the mechanisms of inflammation in two frequently encountered pathologic conditions.

• *Response to hypoxia.* In Chapter 1 we described the role of hypoxia in causing cell injury and necrosis. Hypoxia by itself is also an inducer of the inflammatory response. This response is mediated largely by a protein called hypoxia-induced factor 1α, which is produced by cells deprived of oxygen and activates many genes involved in inflammation, including VEGF, which increases vascular permeability.[75]

• *Response to necrotic cells.* Although it has been known for many years that necrotic cells elicit inflammatory reactions that serve to eliminate these cells, the molecular basis of this reaction has been largely unknown. One participant may be uric acid, which is a product of DNA breakdown, and crystallizes when present at sufficiently high concentrations in extracellular tissues. Uric acid crystals stimulate inflammation and subsequent immune response. [76] This proinflammatory action of uric acid is the basis of the disease gout, in which excessive amounts of uric acid are produced and crystals deposit in joints and other tissues.

SUMMARY OF CHEMICAL MEDIATORS OF ACUTE INFLAMMATION

Table 2-5 summarizes the major actions of the principal mediators. When Lewis discovered the role of histamine in

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inflammation, one mediator was thought to be enough. Now, we are wallowing in them! Yet, from this menu of substances we can emphasize a few mediators that may be particularly relevant in vivo (Table 2-6). Vasodilation, an early event in inflammation, is caused by histamine, prostaglandins, and nitric oxide. Increased vascular permeability is caused by histamine; the anaphylatoxins (C3a and C5a); the kinins; leukotrienes C, D, and E; PAF; and substance P. For chemotaxis, the most likely contributors are complement fragment C5a, lipoxygenase products (LTB_4), and chemokines. Prostaglandins play an important role in vasodilation, pain, and fever, and in potentiating edema. IL-1 and TNF are critical for endothelial-leukocyte interactions and subsequent leukocyte recruitment, and for the production of acute-phase reactants. Lysosomal products and oxygen-derived radicals are the most likely candidates responsible for the ensuing tissue destruction. NO is involved in vasodilation and also causes tissue damage.

Outcomes of Acute Inflammation

The discussion of mediators completes the description of the basic, relatively uniform pattern of the inflammatory reaction encountered in most injuries. Although hemodynamic, permeability, and leukocyte changes have been described sequentially and may be initiated in this order, all these phenomena may be concurrent in the fully evolved reaction to injury. As might be expected, many variables may modify this basic process, including the nature and intensity of the injury,

Figure 2-21 Outcomes of acute inflammation: resolution, healing by fibrosis, or chronic inflammation (see text).

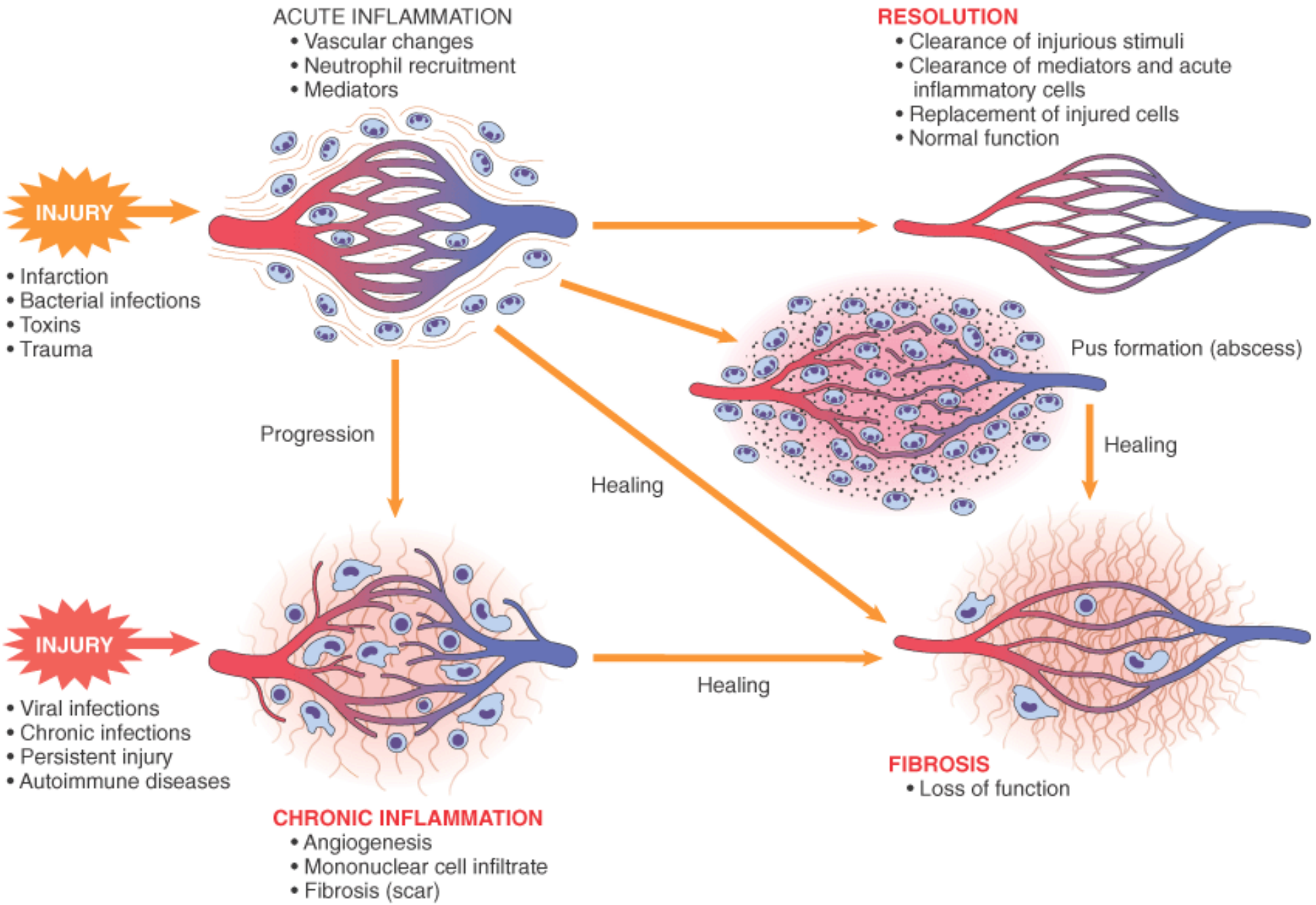


TABLE 2-6 -- Role of Mediators in Different Reactions of Inflammation

Vasodilation	Prostaglandins
	Nitric oxide

	Histamine
Increased vascular permeability	Vasoactive amines
	C3a and C5a (through liberating amines)
	Bradykinin
	Leukotrienes C_4 , D_4 , E_4
	PAF
	Substance P
Chemotaxis, leukocyte recruitment and activation	C5a
	Leukotriene B_4
	Chemokines
	IL-1, TNF
	Bacterial products
Fever	IL-1, TNF
	Prostaglandins
Pain	Prostaglandins
	Bradykinin
Tissue damage	Neutrophil and macrophage lysosomal enzymes
	Oxygen metabolites
	Nitric oxide

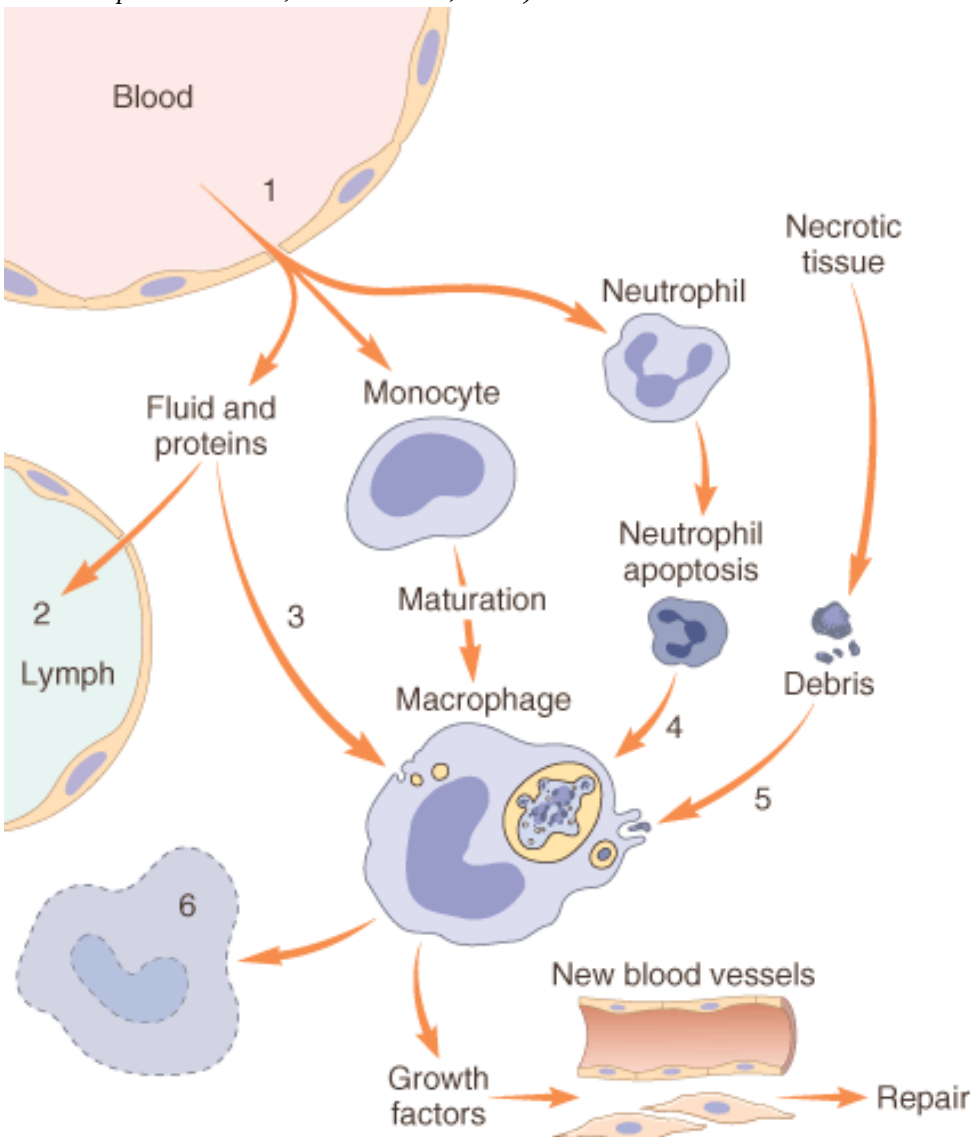
the site and tissue affected, and the responsiveness of the host. In general, however, *acute inflammation may have one of three outcomes* (Fig. 2-21):

1. *Complete resolution.* In a perfect world, all inflammatory reactions, once they have succeeded in neutralizing and eliminating the injurious stimulus, should end with restoration of the site of acute inflammation to normal. This is called *resolution* and is the usual outcome when the injury is limited or short-lived or when there has been little tissue destruction and the damaged parenchymal cells can regenerate. Resolution involves neutralization or spontaneous decay of the chemical mediators, with subsequent return of normal vascular permeability, cessation of leukocytic infiltration, death (largely by apoptosis) of neutrophils, and finally removal of edema fluid and protein, leukocytes, foreign agents, and necrotic debris from the site (Fig. 2-22). Lymphatics and phagocytes play a role in these events, as described later in this Chapter and in Chapter 3 .
2. *Healing by connective tissue replacement (fibrosis).* This occurs after substantial tissue destruction, when the inflammatory injury involves tissues that are incapable of regeneration, or when there is abundant fibrin exudation. When the fibrinous exudate in tissue or serous cavities (pleura, peritoneum) cannot be adequately cleared, connective tissue grows into the area of exudate, converting it into a mass of fibrous tissue—a process also called *organization*. In many pyogenic infections there may be intense neutrophil infiltration and

liquefaction of tissues, leading to pus formation. The destroyed tissue is resorbed and eventually replaced by fibrosis.

3. Progression of the tissue response to *chronic inflammation* (discussed below). This may follow acute inflammation, or the response may be chronic almost from the onset. Acute to chronic transition occurs when the acute inflammatory response cannot be resolved, owing either to the persistence of the injurious agent or to some interference with the normal process of healing. For example, bacterial infection of the lung may begin as a focus of acute inflammation (pneumonia), but its failure to resolve may lead to extensive tissue destruction and formation of a cavity in which the inflammation continues to smolder, leading eventually to a chronic lung abscess. Another example of chronic inflammation with a persisting stimulus is peptic ulcer of the duodenum or stomach. Peptic ulcers may persist for months or years and, as discussed below, are manifested by both acute and chronic inflammatory reactions.

Figure 2-22 Events in the resolution of inflammation: (1) return to normal vascular permeability; (2) drainage of edema fluid and proteins into lymphatics or (3) by pinocytosis into macrophages; (4) phagocytosis of apoptotic neutrophils and (5) phagocytosis of necrotic debris; and (6) disposal of macrophages. Macrophages also produce growth factors that initiate the subsequent process of repair. Note the central role of macrophages in resolution. *(Modified from Haslett C, Henson PM: In Clark R, Henson PM (eds): The Molecular and Cellular Biology of Wound Repair. New York, Plenum Press, 1996.)*



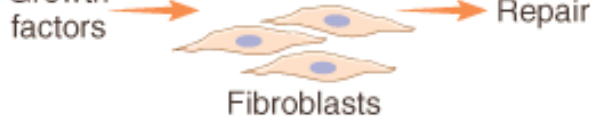


Figure 2-23 Serous inflammation. Low-power view of a cross-section of a skin blister showing the epidermis separated from the dermis by a focal collection of serous effusion.

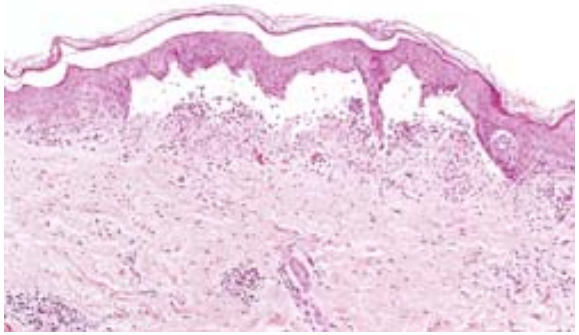


Figure 2-24 Fibrinous pericarditis. *A*, Deposits of fibrin on the pericardium. *B*, A pink meshwork of fibrin exudate (F) overlies the pericardial surface (P).

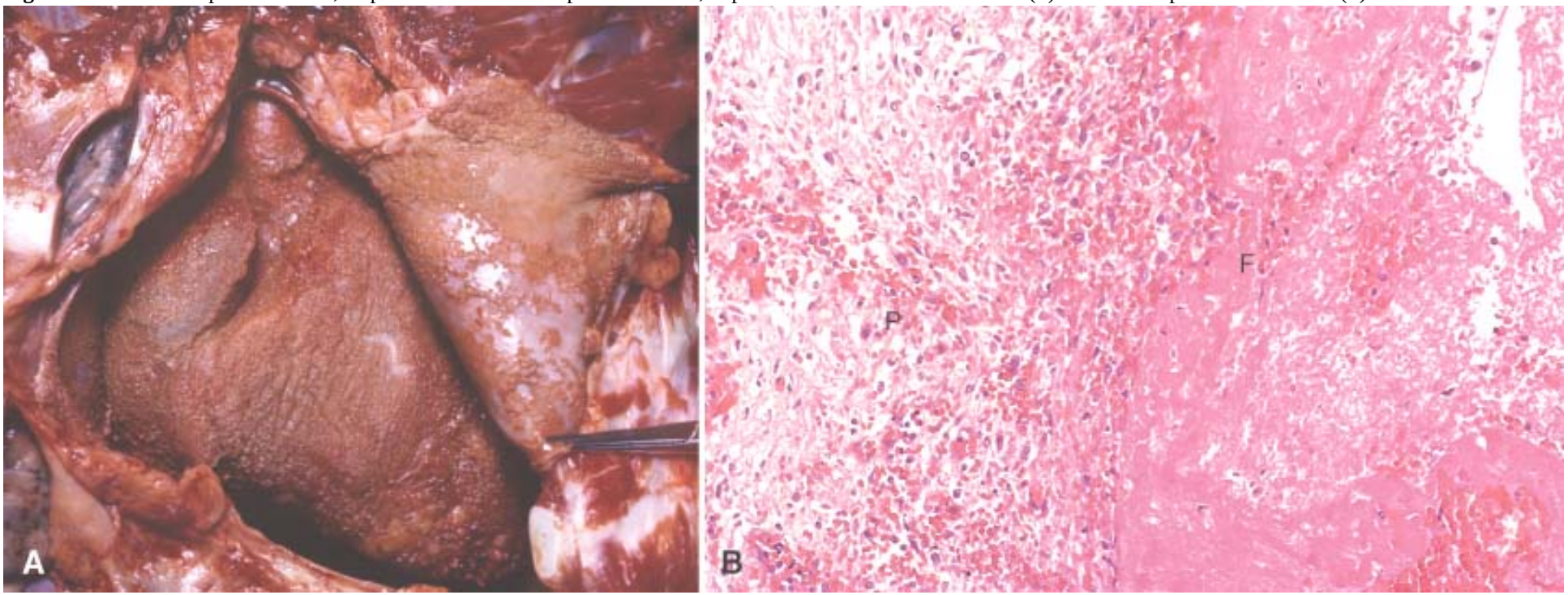


Figure 2-25 Suppurative inflammation. *A*, A subcutaneous bacterial abscess with collections of pus. *B*, The abscess contains neutrophils, edema fluid, and cellular debris.

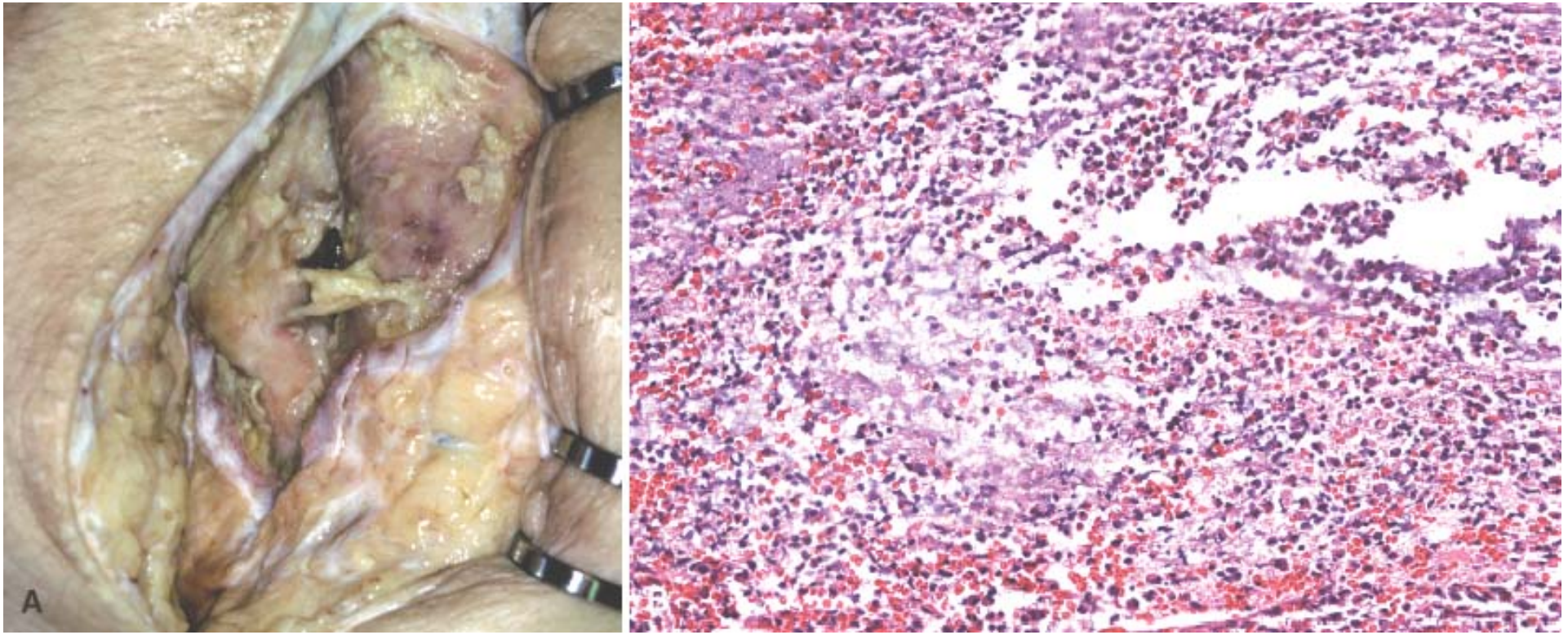


Figure 2-26 The morphology of an ulcer. *A,* A chronic duodenal ulcer. *B,* Low-power cross-section of a duodenal ulcer crater with an acute inflammatory exudate in the base.

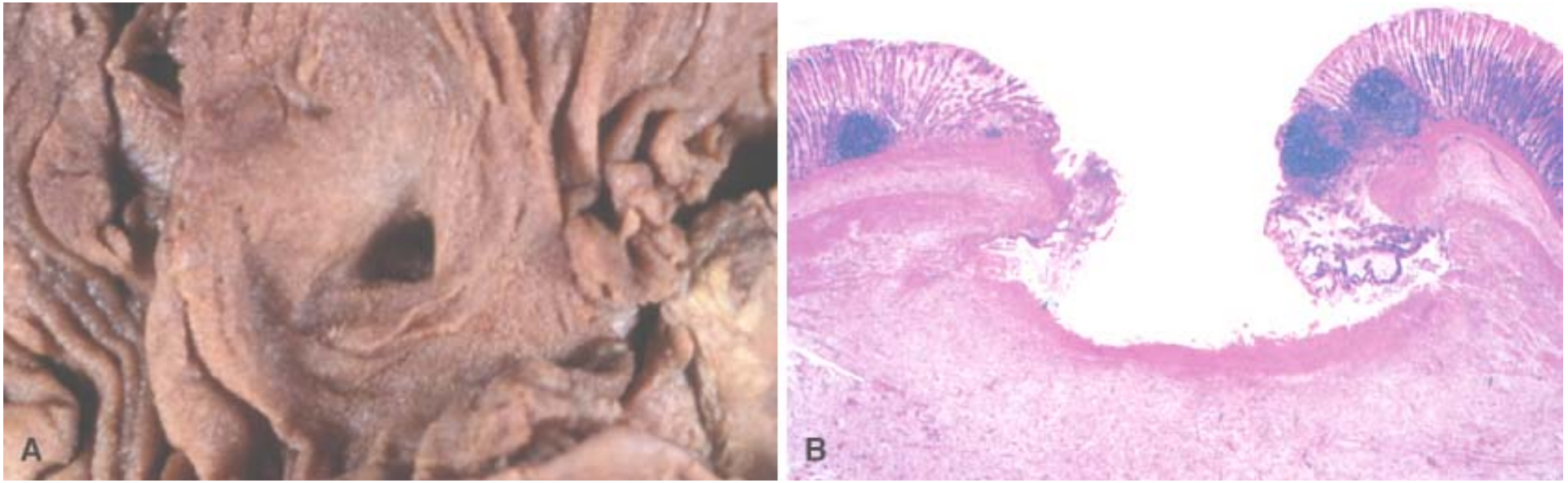


Figure 2-27 Maturation of mononuclear phagocytes. *(From Abbas AK, et al: Cellular and Molecular Immunology, 5th ed. Philadelphia, Saunders, 2003.)*

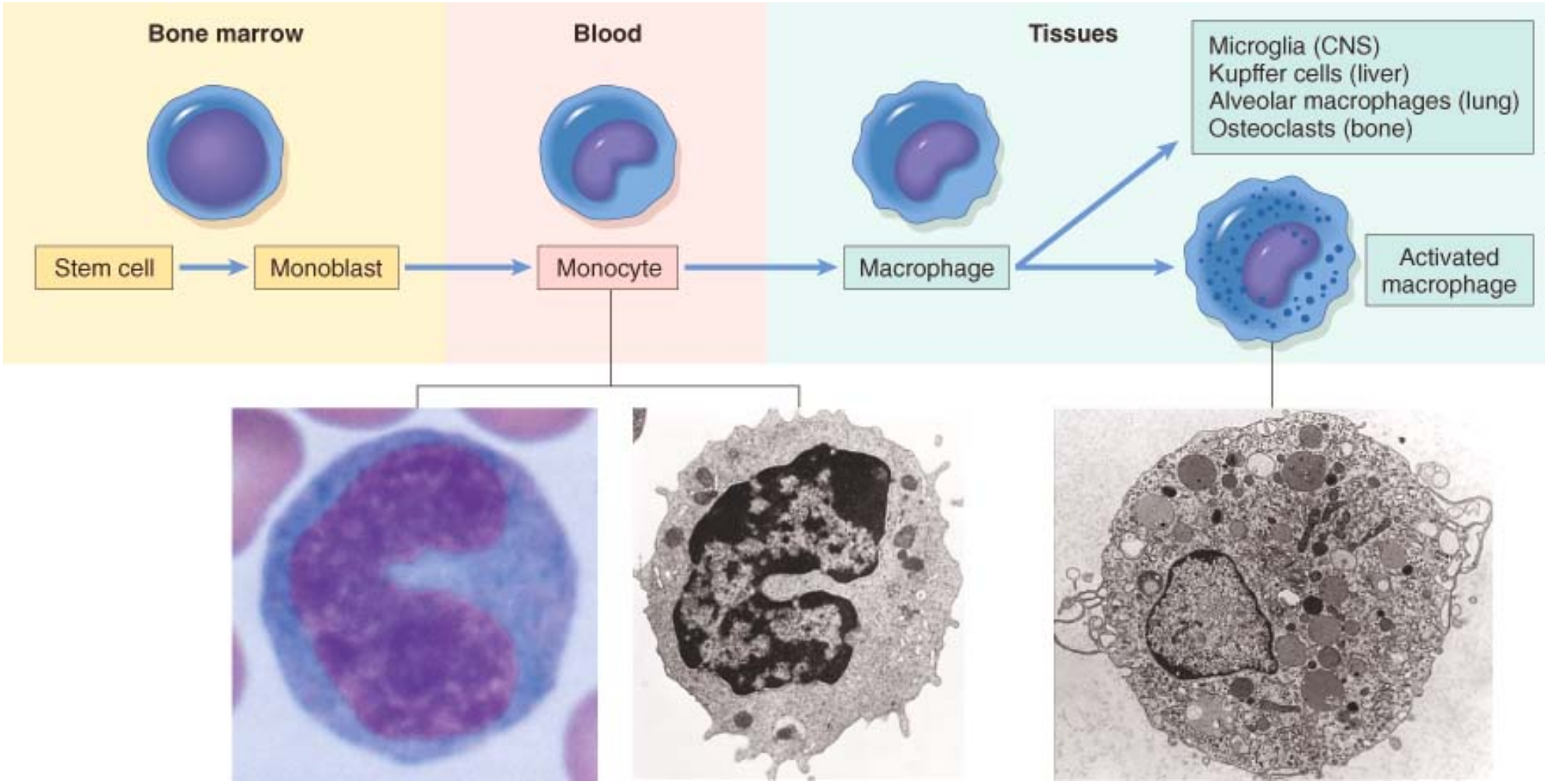


Figure 2-28 The roles of activated macrophages in chronic inflammation. Macrophages are activated by cytokines from immune-activated T cells (particularly IFN-γ) or by nonimmunologic stimuli such as endotoxin. The products made by activated macrophages that cause tissue injury and fibrosis are indicated. AA, arachidonic acid; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; TGFβ, transforming growth factor β.

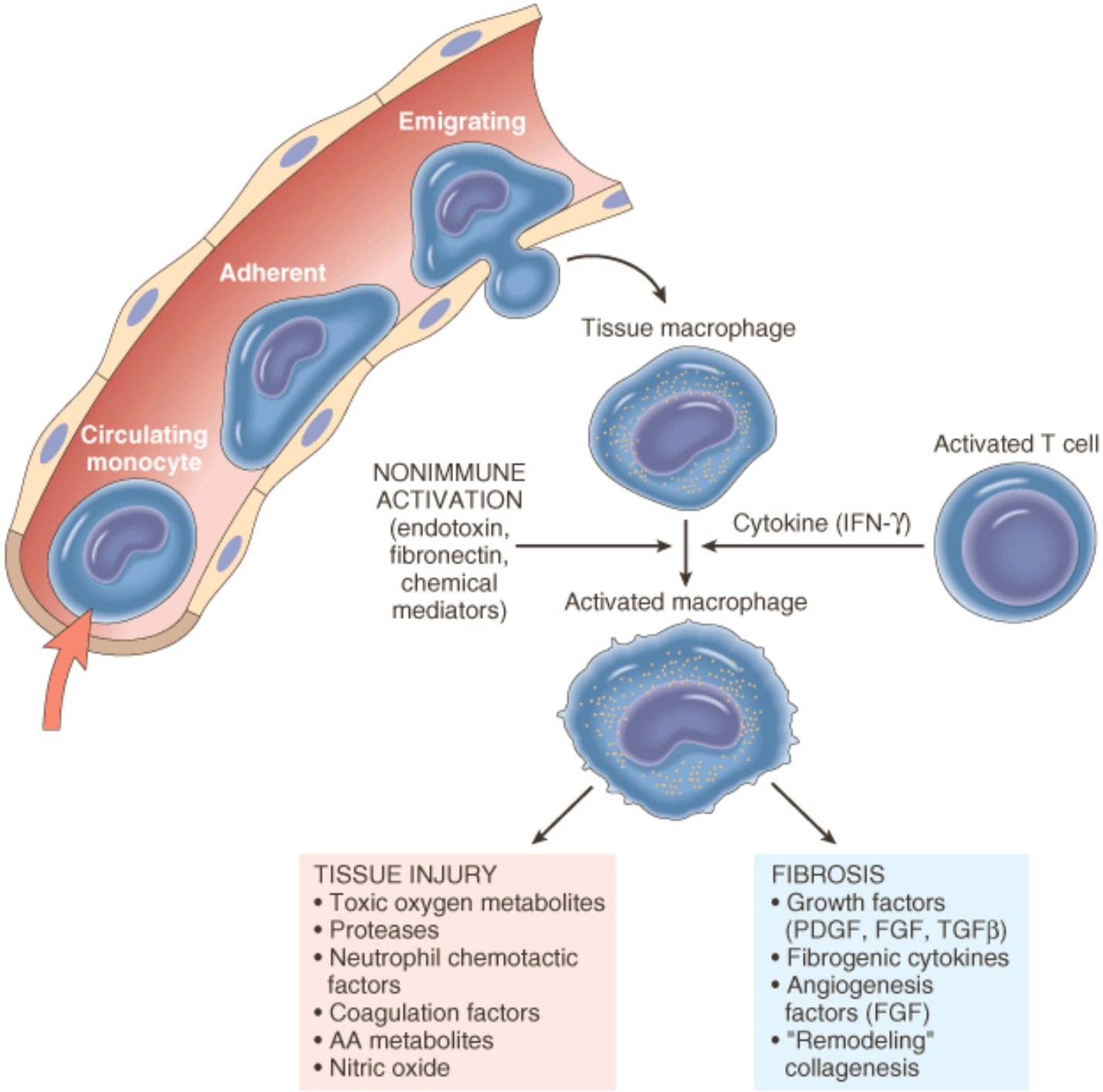


Figure 2-29 *A*, Chronic inflammation in the lung, showing all three characteristic histologic features: (1) collection of chronic inflammatory cells (*), (2) destruction of parenchyma (normal alveoli are replaced by spaces lined by cuboidal epithelium, *arrowheads*), and (3) replacement by connective tissue (fibrosis, *arrows*). *B*, By contrast, in acute inflammation of the lung (acute

bronchopneumonia), neutrophils fill the alveolar spaces and blood vessels are congested.

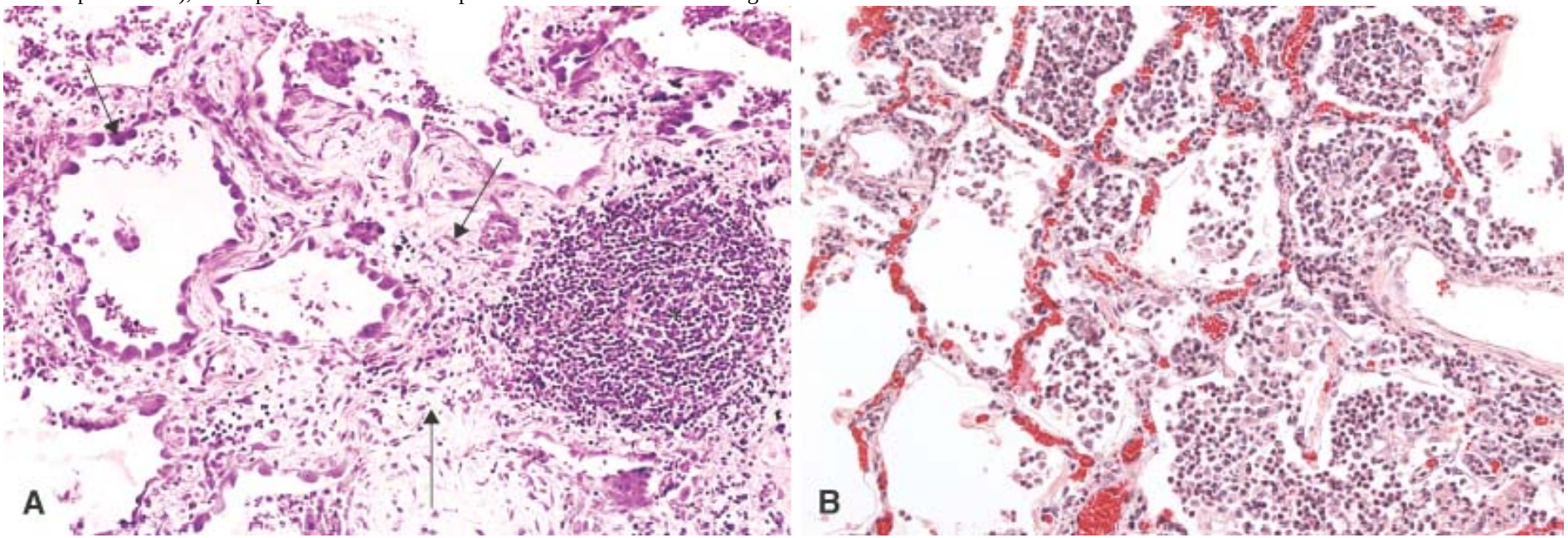
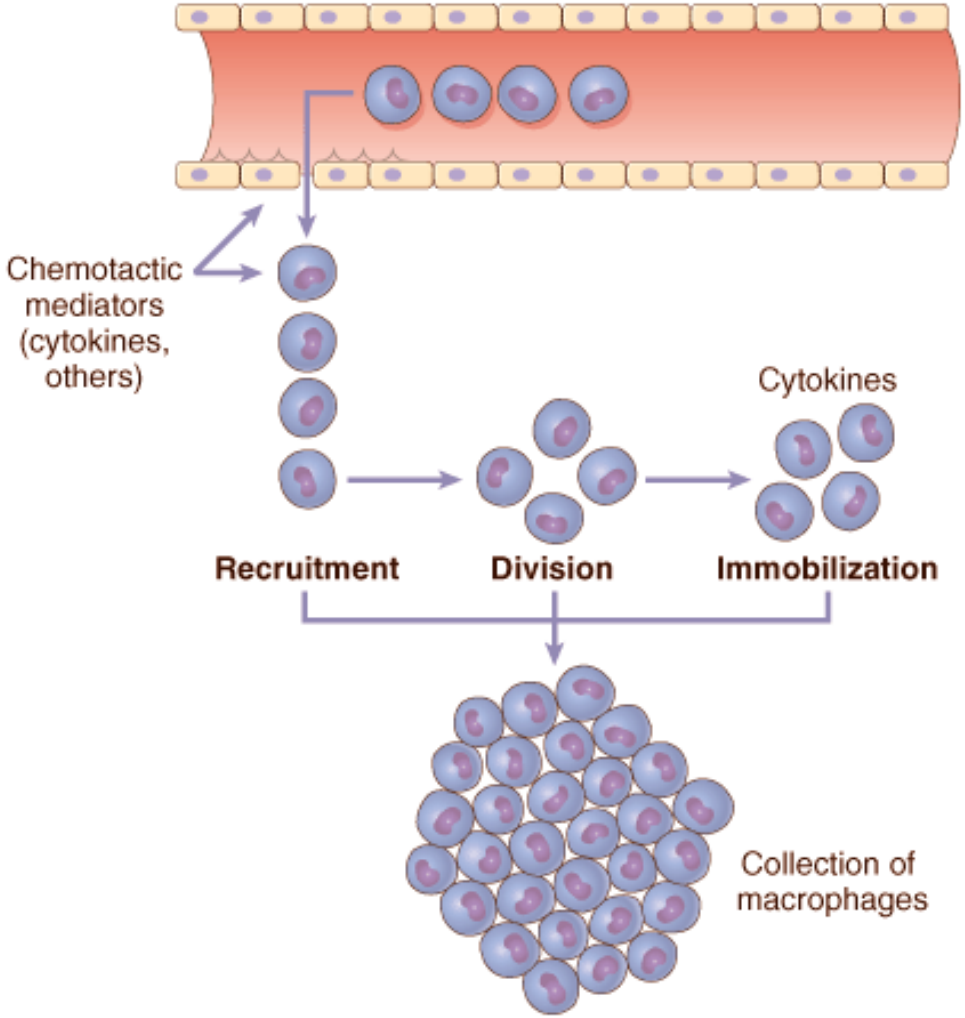


Figure 2-30 Mechanisms of macrophage accumulation in tissues. The most important is continued recruitment from the microcirculation. *(Adapted from Ryan G, Majno G: Inflammation. Kalamazoo, MI, Upjohn, 1977.)*



Lymphocytes and macrophages interact in a bidirectional way, and these reactions play an important role in chronic inflammation (Fig. 2-31). Macrophages display antigens to T cells, and produce membrane molecules (costimulators) and cytokines (notably IL-12) that stimulate T-cell responses (Chapter 6). Activated T lymphocytes produce cytokines, and one of these, IFN-γ, is a major activator of macrophages. *Plasma cells* develop from activated B lymphocytes and produce antibody directed either against persistent antigen in the inflammatory site or against altered tissue components. In some strong chronic inflammatory reactions, the accumulation of lymphocytes, antigen-presenting cells, and

plasma cells may assume the morphologic features of lymphoid organs, particularly lymph nodes, even containing well-formed germinal centers. This pattern of *lymphoid organogenesis* is often seen in the synovium of patients with long-standing rheumatoid arthritis.

• *Eosinophils* are abundant in immune reactions mediated by IgE and in parasitic infections (Fig. 2-32). The recruitment of eosinophils involves extravasation from the blood and their migration into tissue by processes similar to those for other leukocytes. One of the chemokines that is especially important for eosinophil recruitment is eotaxin. Eosinophils have granules that contain *major basic protein*, a highly cationic protein that is toxic to parasites but also causes lysis of mammalian epithelial cells. They may thus be of benefit in controlling

parasitic infections but they contribute to tissue damage in immune reactions (Chapter 6).[79]

• *Mast cells* are widely distributed in connective tissues and participate in both acute and persistent inflammatory reactions. Mast cells express on their surface the receptor that binds the Fc portion of IgE antibody (FcepsilonRI). In acute reactions, IgE antibodies bound to the cells' Fc receptors specifically recognize antigen, and the cells degranulate and release mediators, such as histamine and products of AA oxidation (Chapter 6). This type of response occurs during anaphylactic reactions to foods, insect venom, or drugs, frequently with catastrophic results. When properly regulated, this response can benefit the host. Mast cells are also present in chronic inflammatory reactions, and may produce cytokines that contribute to fibrosis.

Figure 2-31 Macrophage-lymphocyte interactions in chronic inflammation. Activated lymphocytes and macrophages influence each other and also release inflammatory mediators that affect other cells.

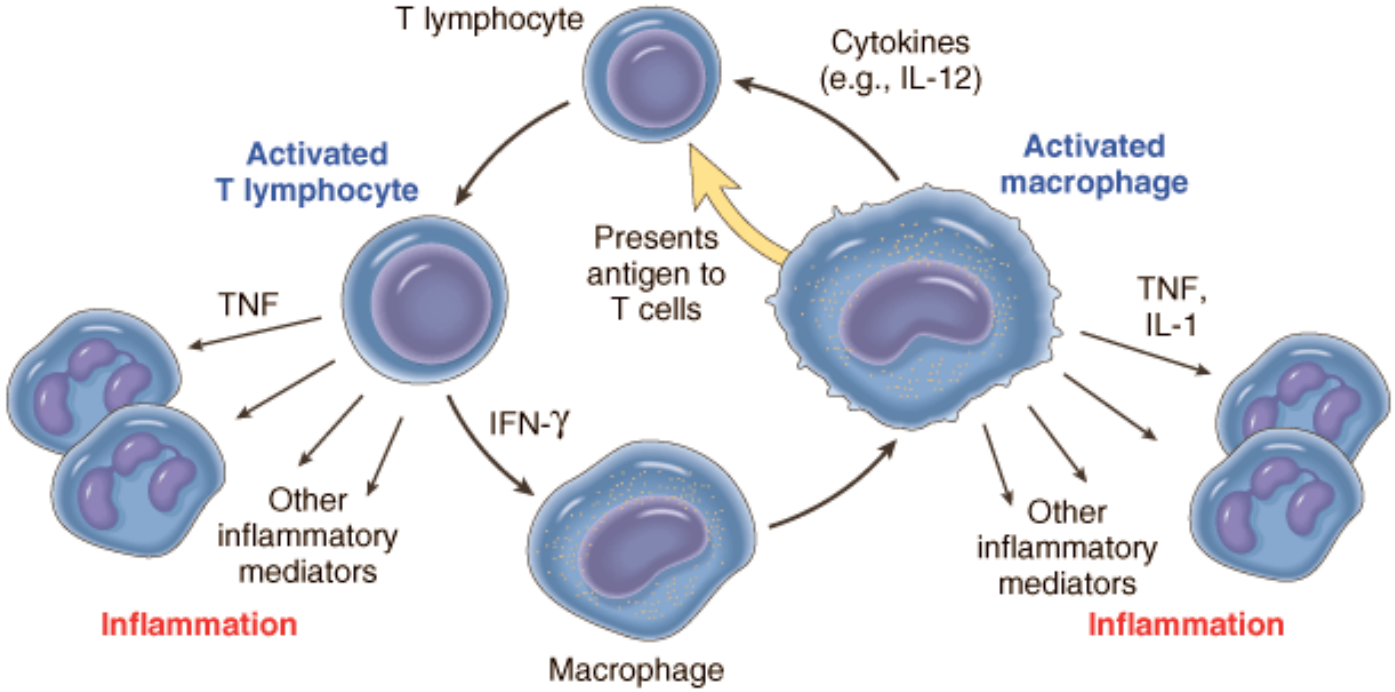


Figure 2-32 A focus of inflammation showing numerous eosinophils.

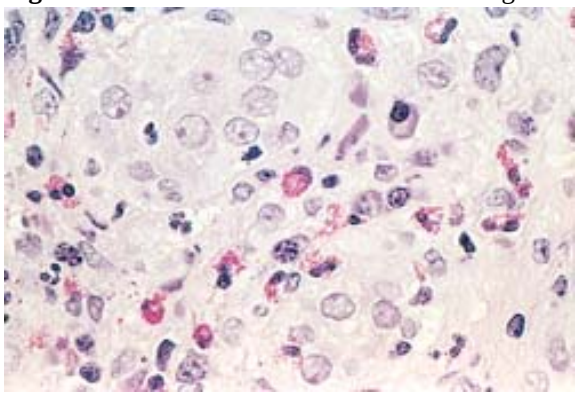


TABLE 2-7 -- Examples of Diseases with Granulomatous Inflammations

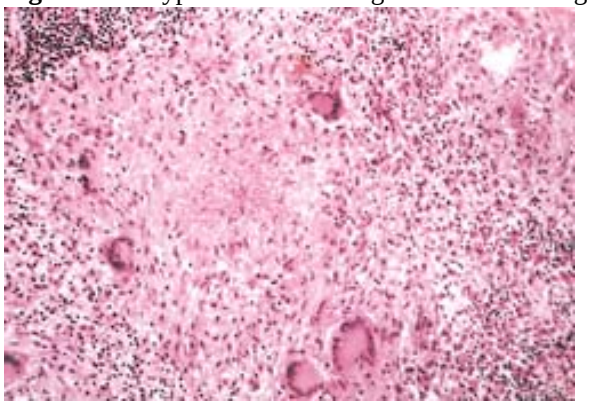
Disease	Cause	Tissue Reaction
Tuberculosis	*Mycobacterium tuberculosis*	Noncaseating tubercle (granuloma prototype): a focus of epithelioid cells, rimmed by fibroblasts, lymphocytes, histiocytes, occasional Langhans giant cell; caseating tubercle: central amorphous granular debris, loss of all cellular detail; acid-fast bacilli
Leprosy	*Mycobacterium leprae*	Acid-fast bacilli in macrophages; non-caseating granulomas
Syphilis	*Treponema pallidum*	Gumma: microscopic to grossly visible lesion, enclosing wall of histiocytes; plasma cell infiltrate; central cells are necrotic without loss of cellular outline
Cat-scratch disease	Gram-negative bacillus	Rounded or stellate granuloma containing central granular debris and recognizable neutrophils; giant cells uncommon

fibroblasts and connective tissue. Frequently, epithelioid cells fuse to form *giant cells* in the periphery or sometimes in the center of granulomas. These giant cells may attain diameters of 40 to 50 µm. They have a large mass of cytoplasm containing 20 or more small nuclei arranged either peripherally (Langhans-type giant cell) or haphazardly (foreign body-type giant cell) (Fig. 2-33). There is no known functional difference between these two types of giant cells, a fact that does not deter students from remembering the morphologic differences!

There are two types of granulomas, which differ in their pathogenesis. *Foreign body granulomas* are incited by relatively inert foreign bodies. Typically, foreign body granulomas form when material such as talc (associated with intravenous drug abuse) (Chapter 9), sutures, or other fibers are large enough to preclude phagocytosis by a single macrophage and do not incite any specific inflammatory or immune response. Epithelioid cells and giant cells form and are apposed to the surface and encompass the foreign body. The foreign material can usually be identified in the center of the granuloma, particularly if viewed with polarized light, in which it appears refractile.

Immune granulomas are caused by insoluble particles, typically microbes, that are capable of inducing a cell-mediated immune response (Chapter 6). This type of immune response does not necessarily produce granulomas but it does so when

Figure 2-33 Typical tuberculous granuloma showing an area of central necrosis, epithelioid cells, multiple Langhans-type giant cells, and lymphocytes.



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Chapter 3 - Tissue Renewal and Repair: Regeneration, Healing, and Fibrosis

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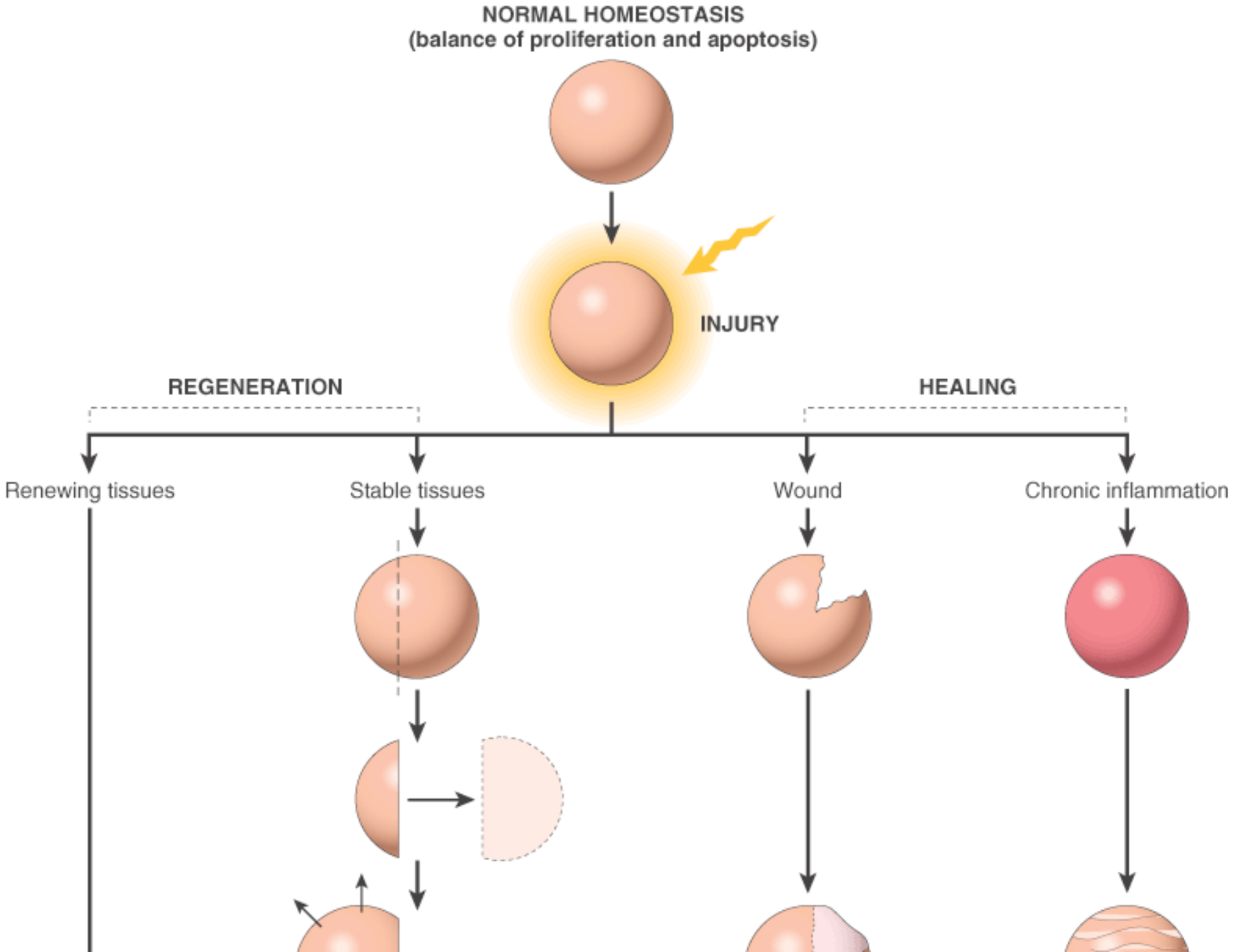
The body's ability to replace injured or dead cells and to repair tissues after inflammation is critical to survival. When injurious agents damage cells and tissues, the host responds by setting in motion a series of events that serve to eliminate these agents, contain the damage, and prepare the surviving cells for replication. The repair of tissue damage caused by surgical resection, wounds, and diverse types of chronic injury can be broadly separated into two processes, *regeneration* and *healing* (Fig. 3-1). Regeneration results in restitution of lost tissues; healing may restore original structures but involves collagen deposition and scar formation.

Definitions

The mechanisms of regeneration and healing will be discussed later in this chapter, but it is important from the outset to establish some important distinctions between these processes and to become familiar with terms used to describe them.

Figure 3-1 Tissue response to injury. Repair after injury can occur by regeneration, which restores normal tissue, or by healing, which leads to scar formation and fibrosis.



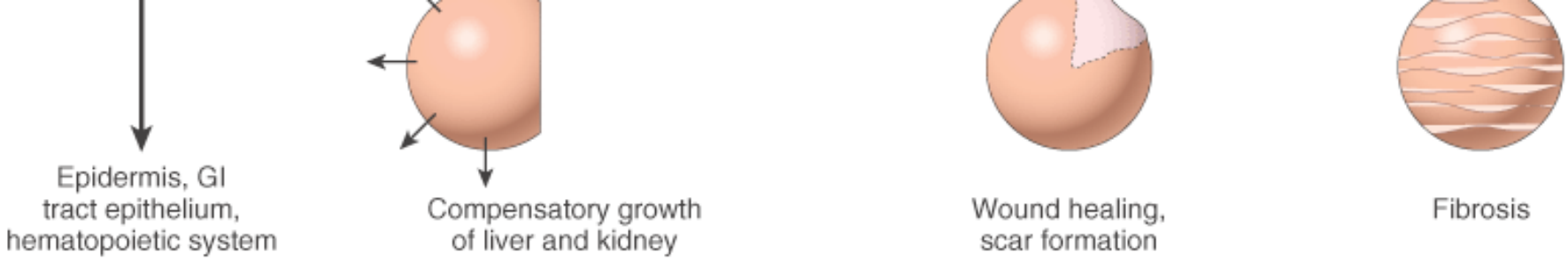


Figure 3-2 Mechanisms regulating cell populations. Cell numbers can be altered by increased or decreased rates of stem cell input, by cell death due to apoptosis, or by changes in the rates of proliferation or differentiation. *(Modified from McCarthy NJ et al: Apoptosis in the development of the immune system: growth factors, clonal selection and bcl-2. Cancer Metastasis Rev 11:157, 1992.)*

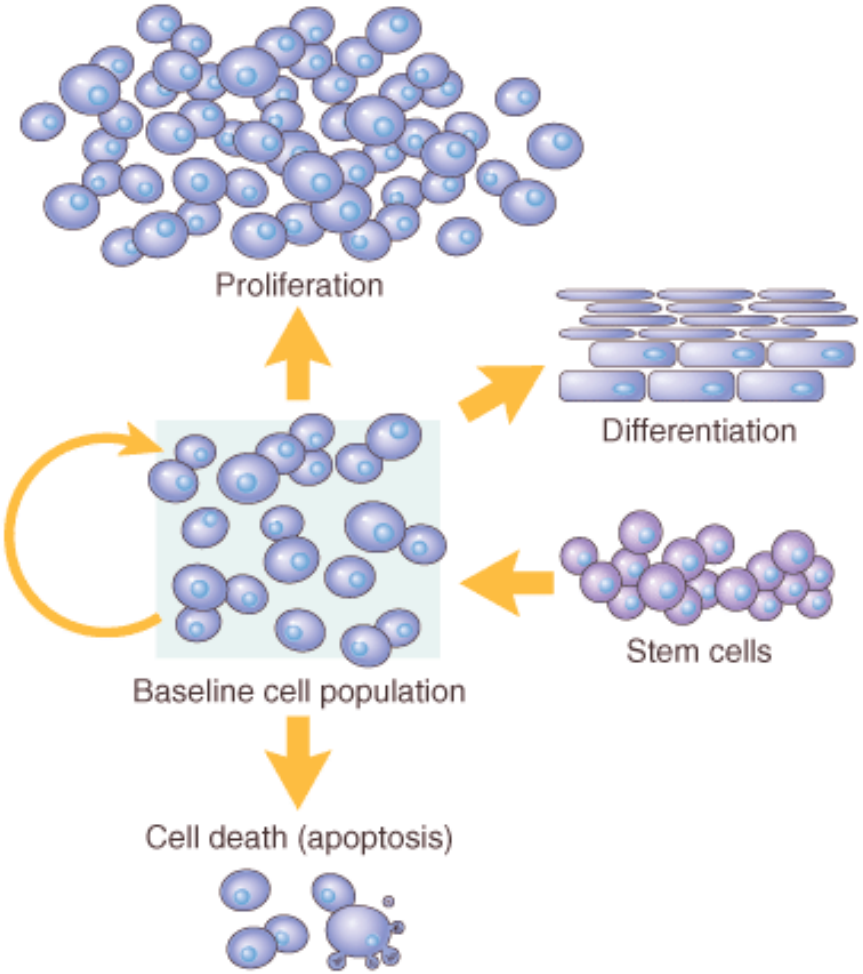


Figure 3-3 Cell-cycle landmarks. The figure shows the cell-cycle phases (G_0 , G_1 , G_2 , S, and M), the location of the G_1 restriction point, and the G_1 /S and G_2 /M cell-cycle checkpoints. Cells from labile tissues such as the epidermis and the gastrointestinal tract may cycle continuously; stable cells such as hepatocytes are quiescent but can enter the cell cycle; permanent cells

such as neurons and cardiac myocytes have lost the capacity to proliferate. *(Modified from Pollard TD and Earnshaw WC: Cell Biology. Philadelphia, Saunders, 2002.)*

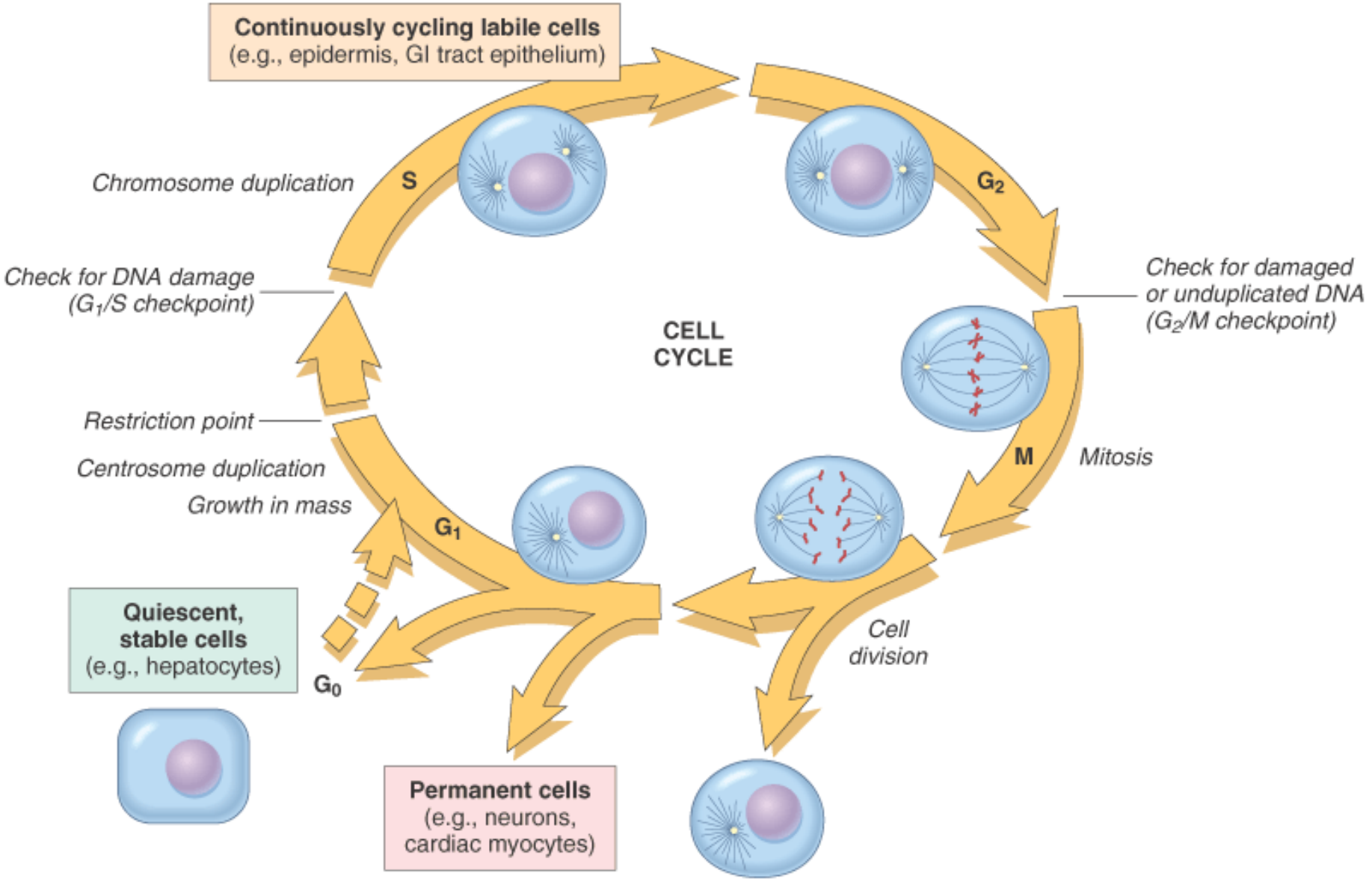
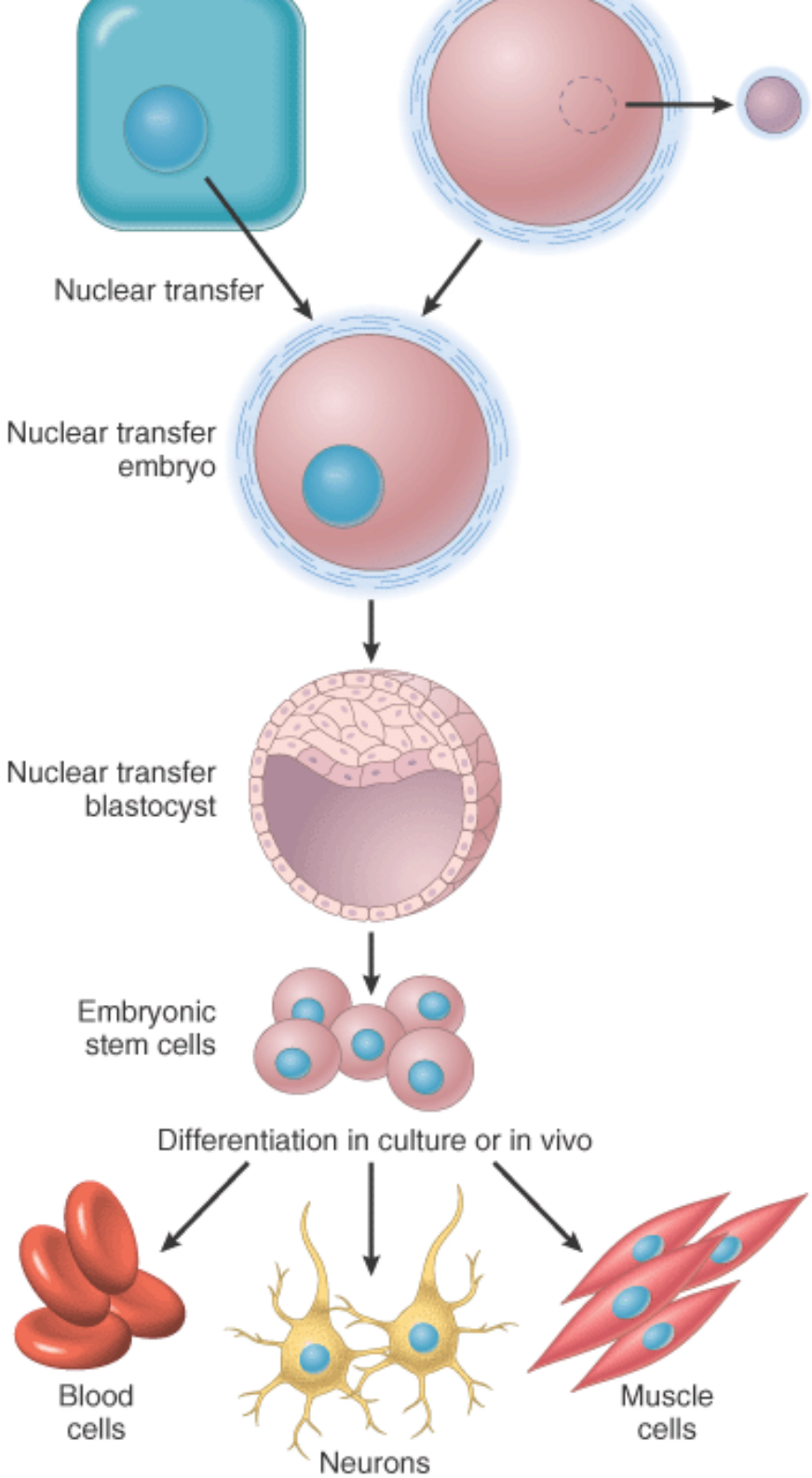


Figure 3-4 Steps involved in therapeutic cloning, using embryonic stem cells (ES cells) for cell therapy. The diploid nucleus of an adult cell from a patient is introduced into an enucleated oocyte. The oocyte is activated, and the zygote divides to become a blastocyst that contains the donor DNA. The blastocyst is dissociated to obtain ES. These cells are capable of differentiating into various tissues, either in culture or after transplantation into the donor. The goal of the procedure is to reconstitute or repopulate damaged organs of a patient, using the cells of the same patient to avoid immunologic rejection. *(Modified from Hochedlinger K, Jaenisch R: Nuclear transplantation, embryonic stem cells, and the potential for cell therapy. N Engl J Med 349:275–286, 2003.)*


Nuclear transfer
Nuclear transfer embryo
Nuclear transfer blastocyst
Embryonic stem cells
Differentiation in culture or in vivo
Blood cells
Neurons
Muscle cells

Neurons

Figure 3-5 Stem-cell niches in various tissues. *A,* Epidermal stem cells located in the bulge area of the hair follicle serve as a stem cells for the hair follicle and the epidermis. *B,* Intestinal stem cells are located at the base of a colon crypt, above Paneth cells. *C,* Liver stem cells (commonly known as oval cells) are located in the canals of Hering *(thick arrow),* structures that connect bile ductules *(thin arrow)* with parenchymal hepatocytes (bile duct and Hering canals are stained for cytokeratin 7; *courtesy of Tania Roskams, M.D., University of Leuven*). *D,* Corneal stem cells are located in the limbus region, between the conjunctiva and the cornea. *(Courtesy of T-T Sun, New York University, New York, NY.)*

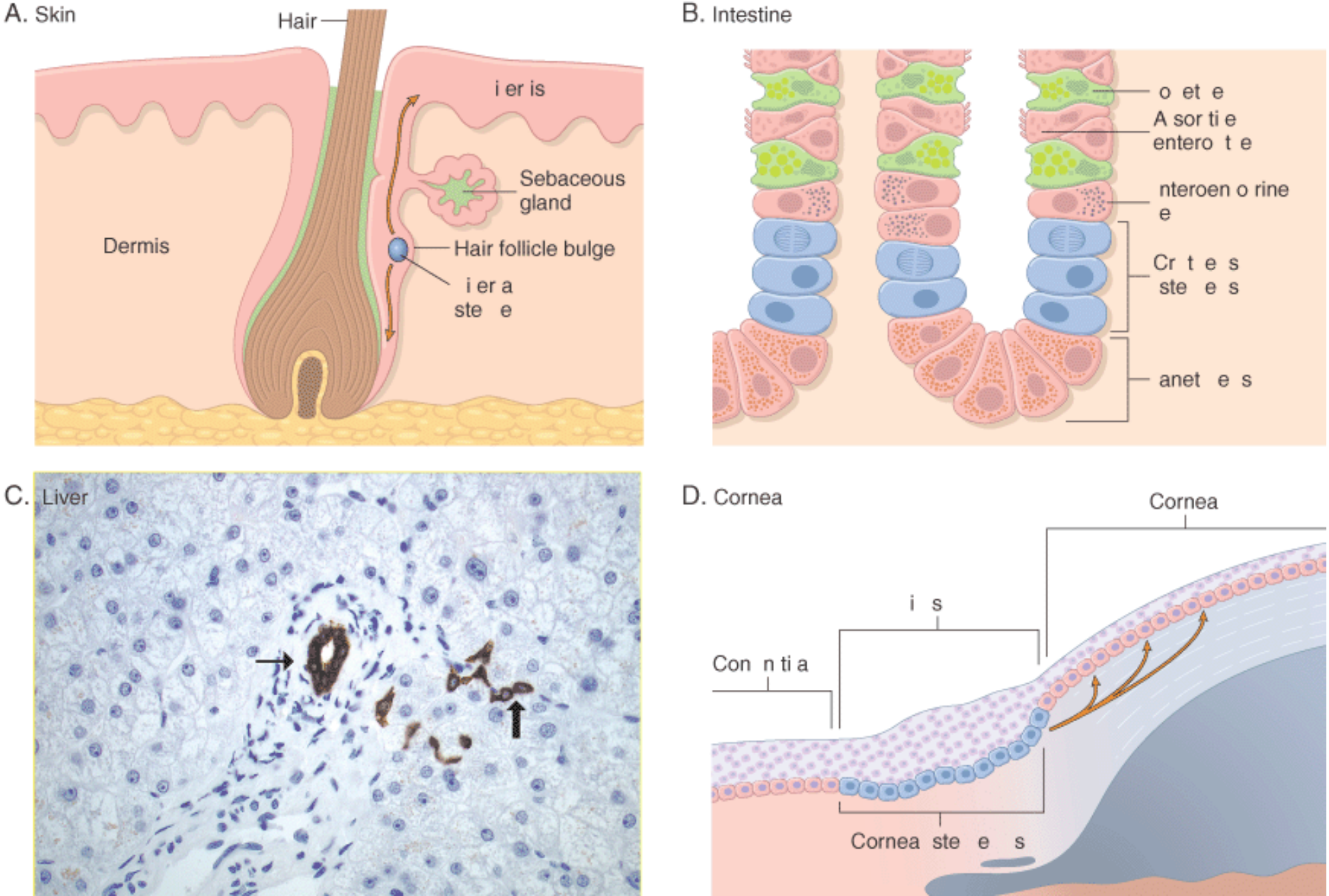


Figure 3-6 Differentiation pathways for pluripotent bone marrow stromal cells. Activation of key regulatory proteins by growth factors, cytokines, or matrix components leads to commitment of stem cells to differentiate into specific cellular lineages. Differentiation of myotubes requires the combined action of several factors (e.g., myoD, myogenin); fat cells require PPARγ, the osteogenic lineage requires CBFA1 (also known as RUNX2), cartilage formation requires Sox9, and endothelial cells require VEGF and FGF-2. *(Adapted and redrawn from Rodan GA, Harada S: The missing bone. Cell 89:677, 1997.)*

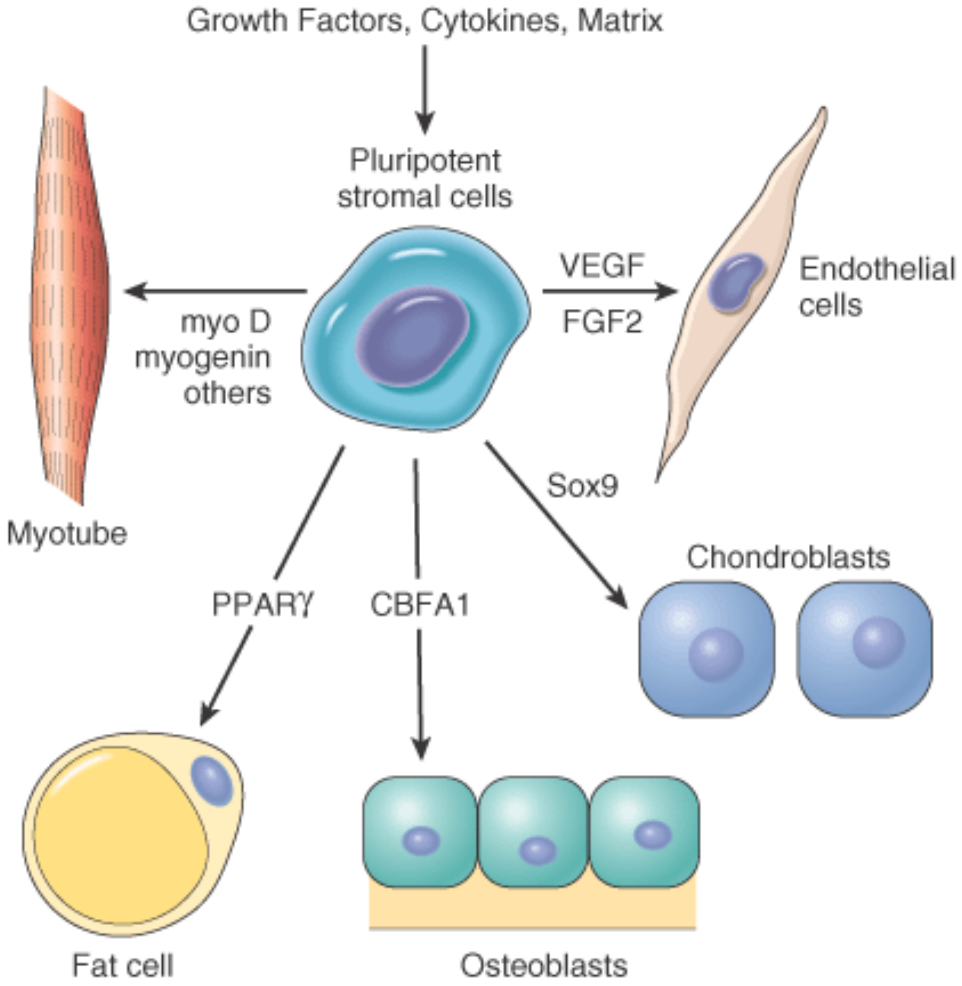


Figure 3-7 Differentiation of embryonic cells and generation of tissue cells by bone marrow precursors. During embryonic development the three germ layers—endoderm, mesoderm, and ectoderm—are formed, generating all tissues of the body. Adult stem cells localized in organs derived from these layers produce cells that are specific for the organs at which they reside. However, some adult bone marrow stem cells, in addition to producing the blood lineages (mesodermal derived), can also generate cells for tissues that originated from the endoderm and ectoderm (indicated by the *red lines*). *(Modified from Korbling M, Estrov Z: Adult stem cells for tissue repair—a new theropeutic concept? N Engl J Med 349:570–582, 2003.)*

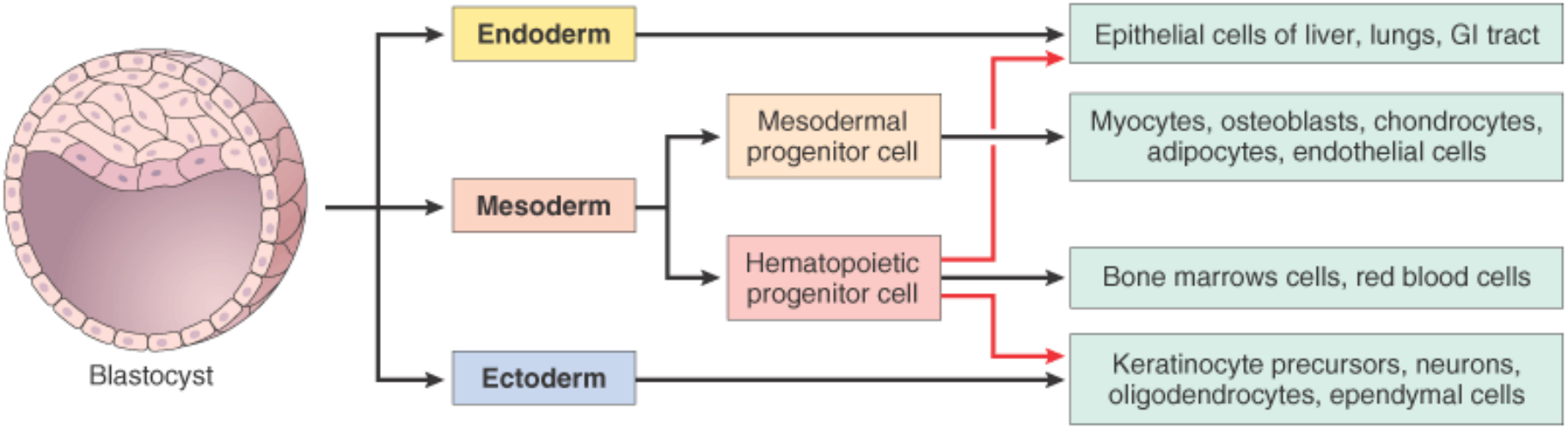


TABLE 3-1 -- Growth Factors and Cytokines Involved in Regeneration and Wound Healing

Cytokine	Symbol	Source	Functions
Epidermal growth factor	EGF	Platelets, macrophages, saliva, urine, milk, plasma	Mitogenic for keratinocytes and fibroblasts; stimulates keratinocyte migration and granulation tissue formation
Transforming growth factor alpha	TGF-α	Macrophages, T lymphocytes, keratinocytes, and many tissues	Similar to EGF; stimulates replication of hepatocytes and certain epithelial cells
Hepatocyte growth factor/scatter factor	HGF	Mesenchymal cells	Enhances proliferation of epithelial and endothelial cells, and of hepatocytes; increases cell motility
Vascular endothelial cell growth factor (isoforms A, B, C, D)	VEGF	Mesenchymal cells	Increases vascular permeability; mitogenic for endothelial cells (see Table 3-3)
Platelet-derived growth factor (isoforms A, B, C, D)	PDGF	Platelets, macrophages, endothelial cells, keratinocytes, smooth muscle cells	Chemotactic for PMNs, marcrophages, fibroblasts, and smooth muscle cells; activates PMNs, macrophages, and fibroblasts; mitogenic for fibroblasts, endothelial cells, and smooth muscle cells; stimulates production of MMPs, fibronectin, and HA; stimulates angiogenesis and wound contraction; remodeling; inhibits platelet aggregation; regulates integrin expression
Fibroblast growth factor-1 (acidic), -2 (basic) and family	FGF	Macrophages, mast cells, T lymphocytes, endothelial cells, fibroblasts, and many tissues	Chemotactic for fibroblasts; mitogenic for fibroblasts and keratinocytes; stimulates keratinocyte migration, angiogenesis, fam wound contraction and matrix deposition
Transforming growth factor beta (isoforms 1, 2, 3); other members of the family are BMP and activin	TGF-β	Platelets, T lymphocytes, macrophages, endothelial cells, keratinocytes, smooth muscle cells, fibroblasts	Chemotactic for PMNs, macrophages, lymphocytes, fibroblasts, and smooth muscle cells; stimulates TIMP synthesis, keratinocyte migration, angiogenesis, and fibroplasia; inhibits production of MMPs and keratinocyte proliferation; regulates integrin expression and other cytokines; induces TGF-β production
Keratinocyte growth factor (also called FGF-7)	KGF	Fibroblasts	Stimulates keratinocyte migration, proliferation, and differentiation

Insulin-like growth factor-1	IGF-1	Macrophages, fibroblasts and other cells	Stimulates synthesis of sulfated proteoglycans, collagen, keratinocyte migration, and fibroblast proliferation; endocrine effects similar to growth hormone
Tumor necrosis factor	TNF	Macrophages, mast cells, T lymphocytes	Activates macrophages; regulates other cytokines; multiple functions
Interleukins	IL-1, etc.	Macrophages, mast cells, keratinocytes, lymphocytes, and many tissues	Many functions. Some examples: chemotactic for PMNs (IL-1) and fibroblasts (IL-4), stimulation of MMP-1 synthesis (IL-1), angiogenesis (IL-8), TIMP synthesis (IL-6); regulation of other cytokines
Interferons	IFN-α, etc.	Lymphocytes and fibroblasts	Activates macrophages; inhibits fibroblast proliferation and synthesis of MMPs; regulates other cytokines
BMP, bone morphogenetic proteins; PMNs, polymorphonuclear leukocytes; MMPs, matrix metalloproteinases; HA, hyaluronic acid; TIMP, tissue inhibitor of matrix metalloproteinase.			
Modified from Schwartz SI: Principles of Surgery, McGraw Hill, New York, 1999.			

those that have major roles in these processes. Other growth factors are alluded to in various sections of the book.

Epidermal Growth Factor (EGF) and Transforming Growth Factor-α (TGF-α).

These two factors belong to the EGF family and share a common receptor. EGF was discovered by its ability to cause precocious tooth eruption and eyelid opening in newborn mice. EGF is mitogenic for a variety of epithelial cells, hepatocytes, and fibroblasts. It is widely distributed in tissue secretions and fluids, such as sweat, saliva, urine, and intestinal contents. In healing wounds of the skin, EGF is produced by keratinocytes, macrophages, and other inflammatory cells that migrate into the area. EGF binds to a receptor (EGFR) with intrinsic tyrosine kinase activity, triggering the signal transduction events described later. TGF-α was originally extracted from sarcoma

virus-transformed cells and is involved in epithelial cell proliferation in embryos and adults and malignant transformation of normal cells to cancer. TGF-α has homology with EGF, binds to EGFR, and produces most of the biologic activities of EGF. The "EGF receptor" is actually a family of membrane tyrosine kinase receptors that respond to EGF, TGF-α, and other ligands of the EGF family.[44] The main EGFR is referred to as EGFR1, or ERB B1. The ERB B2 receptor (also known as HER-2/Neu) has received great attention because it is overexpressed in breast cancers and is a therapeutic target.

Hepatocyte Growth Factor (HGF).

HGF was originally isolated from platelets and serum. Subsequent studies demonstrated that it is identical to a previously identified growth factor known as *scatter factor* (HGF is also referred to as HGF/scatter factor). It has mitogenic effects in most epithelial cells, including hepatocytes and cells of the biliary epithelium in the liver, and epithelial cells of the lungs, mammary gland, skin, and other tissues.[45] Besides its mitogenic effects, HGF acts as a morphogen in embryonic development and promotes cell scattering and migration. This factor is produced by fibroblasts, endothelial cells, and liver nonparenchymal cells. The receptor for HGF is the product of the proto-oncogene c-*MET*, which is frequently overexpressed in human tumors. HGF signaling is required for survival during embryonic development, as demonstrated by the lethality of knockout mice lacking c-*MET*.

Vascular Endothelial Growth Factor (VEGF).

VEGF is a family of peptides that includes VEGF-A (referred throughout as VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor. VEGF is a potent inducer of blood vessel

formation in early development *(vasculogenesis)* and has a central role in the growth of new blood vessels *(angiogenesis)* in adults (see Table 3-3). [46] It promotes angiogenesis in tumors, chronic inflammation, and healing of wounds. Mice that lack a single allele of the gene (heterozygous VEGF knockout mice) die during embryonic development with defective vasculogenesis and hematopoiesis. VEGF family members signal through three tyrosine kinase receptors: VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR-2 is located in endothelial cells and is the main receptor for the vasculogenic and angiogenic effects of VEGF. The role of VEGFR-1 is less well understood, but it may facilitate the mobilization of endothelial stem cells and has a role in inflammation. VEGF-C and VEGF-D bind to VEGFR-3 and act on lymphatic endothelial cells to induce the production of lymphatic vessels (lymphangiogenesis). VEGF-B binds exclusively to VEGFR-1. It is not required for vasculogenesis or angiogenesis, but may play a role in maintenance of myocardial function.

Platelet-Derived Growth Factor (PDGF).

PDGF is a family of several closely related proteins, each consisting of two chains designated *A* and *B*. All three isoforms of PDGF (AA, AB, and BB) are secreted and are biologically active. Recently, two new isoforms—PDGF-C and PDGF-D—have been identified. PDGF isoforms exert their effects by binding to two cell-surface receptors, designated PDGFR α and β, which have different ligand specificities.[47] PDGF is stored in platelet α granules and is released on platelet activation. It can also be produced by a variety of other cells, including activated macrophages, endothelial cells, smooth muscle cells, and many tumor cells. PDGF causes migration and proliferation of fibroblasts, smooth muscle cells, and monocytes, as demonstrated by defects in these functions in mice deficient in either the A or the B chain of PDGF. It also participates in the activation of hepatic stellate cells in the initial steps of liver fibrosis (Chapter 18).

Fibroblast Growth Factor (FGF).

This is a family of growth factors containing more than 10 members, of which acidic FGF (aFGF, or FGF-1) and basic FGF (bFGF, or FGF-2) are the best characterized. FGF-1 and FGF-2 are made by a variety of cells. Released FGFs associate with heparan sulfate in the ECM, which can serve as a reservoir for storing inactive factors. FGFs are recognized by a family of cell-surface receptors that have intrinsic tyrosine kinase activity. A large number of functions are attributed to FGFs, including the following:

- *New blood vessel formation (angiogenesis):* FGF-2, in particular, has the ability to induce the steps necessary for new blood vessel formation both in vivo and in vitro (see below).
- *Wound repair:* FGFs participate in macrophage, fibroblast, and endothelial cell migration in damaged tissues and migration of epithelium to form new epidermis.
- *Development:* FGFs play a role in skeletal muscle development and in lung maturation. For example, FGF-6 and its receptor induce myoblast proliferation and suppress myocyte differentiation, providing a supply of proliferating myocytes. FGF-2 is also thought to be involved in the generation of angioblasts during embryogenesis. FGF-1 and FGF-2 are involved in the specification of the liver from endodermal cells.[48]
- *Hematopoiesis:* FGFs have been implicated in the differentiation of specific lineages of blood cells and development of bone marrow stroma.

TGF-β and Related Growth Factors.

TGF-β belongs to a family of homologous polypeptides that includes three TGF-β isoforms (TGF-β1, TGF-β2, TGF-β3) and factors with wide-ranging functions, such as bone morphogenetic proteins (BMPs), activins, inhibins, and mullerian inhibiting substance.[49] TGF-β1 has the most widespread distribution in mammals and will be referred to as TGF-β. It is a homodimeric protein produced by a variety of different cell types, including platelets, endothelial cells, lymphocytes, and macrophages. Native TGF-βs are synthesized as precursor proteins, which are secreted and then proteolytically cleaved to yield the biologically active growth factor and a second *latent* component. Active TGF-β binds to two cell surface receptors (types I and II) with serine/threonine kinase activity and triggers the phosphorylation of cytoplasmic transcription factors called *Smads*. [50] TGF-β first binds to a type II receptor, which then forms a complex with a type I receptor, leading to the phosphorylation of Smad 2 and 3. Phosphorylated Smad2 and 3 form heterodimers with Smad4, which enter the nucleus and associate with other DNA-binding proteins to activate or inhibit gene transcription. TGF-β has multiple and often opposing effects depending on the tissue and the type of injury. Agents that have multiple effects are called pleiotropic; because of the large diversity of TGF-β effects, it has been said that TGF-β is pleiotropic with a vengeance.

- *TGF-β is a growth inhibitor for most epithelial cell types and for leukocytes.*[51] It blocks the cell cycle by increasing the expression of cell-cycle inhibitors of the Cip/Kip and INK4/ARF families (see Chapter 7). Loss of TGF-β receptors

frequently occurs in human tumors, providing a proliferative advantage to tumor cells.

- The effects of TGF-β on mesenchymal cells depend on concentration and culture conditions, it generally *stimulates the proliferation of fibroblasts and smooth muscle cells*.
- *TGF-β is a potent fibrogenic agent* that stimulates fibroblast chemotaxis, enhances the production of collagen, fibronectin, and proteoglycans. It inhibits collagen degradation by decreasing matrix proteases and increasing protease inhibitor activities. TGF-β is involved in the development of fibrosis in a variety of chronic inflammatory conditions particularly in the lungs, kidney, and liver.
- *TGF-β has a strong anti-inflammatory effect.* Knockout mice lacking the *TGF-β1* gene have widespread inflammation and abundant lymphocyte proliferation, presumably because of unregulated T-cell proliferation and macrophage activation.

Cytokines.

Cytokines have important functions as mediators of inflammation and immune responses (Chapter 6). Some of these proteins can be placed into the larger functional group of polypeptide growth factors because they have growth-promoting activities for a variety of cells. These are discussed in the appropriate chapters.

SIGNALING MECHANISMS IN CELL GROWTH

All growth factors function by binding to specific receptors, which deliver signals to the target cells. These signals have two general effects: (1) they stimulate the transcription of many genes that were silent in the resting cells, and (2) several of these genes regulate the entry of the cells into the cell cycle and their passage through the various stages of the cell cycle. In this section we review the process of receptor-initiated signal transduction as it applies to growth factors and signaling molecules in general, and their role in regulating the cell cycle.

Cell proliferation is a tightly regulated process that involves a large number of molecules and interrelated pathways. The first event that initiates cell proliferation is, usually, the binding of a signaling molecule, the *ligand*, to a specific cell *receptor*. As we shall see, typical ligands are growth factors and proteins of the ECM. We describe different classes of receptor molecules and the pathways by which receptor activation initiates a cascade of events leading to expression of specific genes. We end this section with brief comments about transcription factors.

Based on the source of the ligand and the location of its receptors—in the same, adjacent, or distant cells—three general modes of signaling, named *autocrine*, *paracrine*, and *endocrine*, can be distinguished (Fig. 3-8).

- *Autocrine signaling:* Cells respond to the signaling molecules that they themselves secrete, thus establishing an *autocrine loop*. Several polypeptide growth factors and cytokines act in this manner. Autocrine growth regulation plays a role in liver regeneration, proliferation of antigen-stimulated lymphocytes, and the growth of some tumors. Tumors frequently overproduce growth factors and their receptors, thus stimulating their own proliferation through an autocrine loop.
- *Paracrine signaling:* One cell type produces the ligand, which then acts on adjacent target cells that express the appropriate receptors. The responding cells are in close proximity to the ligand-producing cell and are generally of a different type. Paracrine stimulation is common in connective tissue repair of healing wounds, in which a factor produced by one cell type (e.g., a macrophage) has its growth effect on adjacent cells (e.g., a fibroblast). Paracrine signaling is also necessary for hepatocyte replication during liver regeneration (see below). A special type of paracrine signaling, called *juxtacrine*, occurs when the signaling molecule (e.g., tumor necrosis factor, TGF-α, and heparin-binding epidermal growth factor) is anchored in the cell membrane and binds a receptor in the plasma membrane of another cell. In this type of signaling, receptor-ligand

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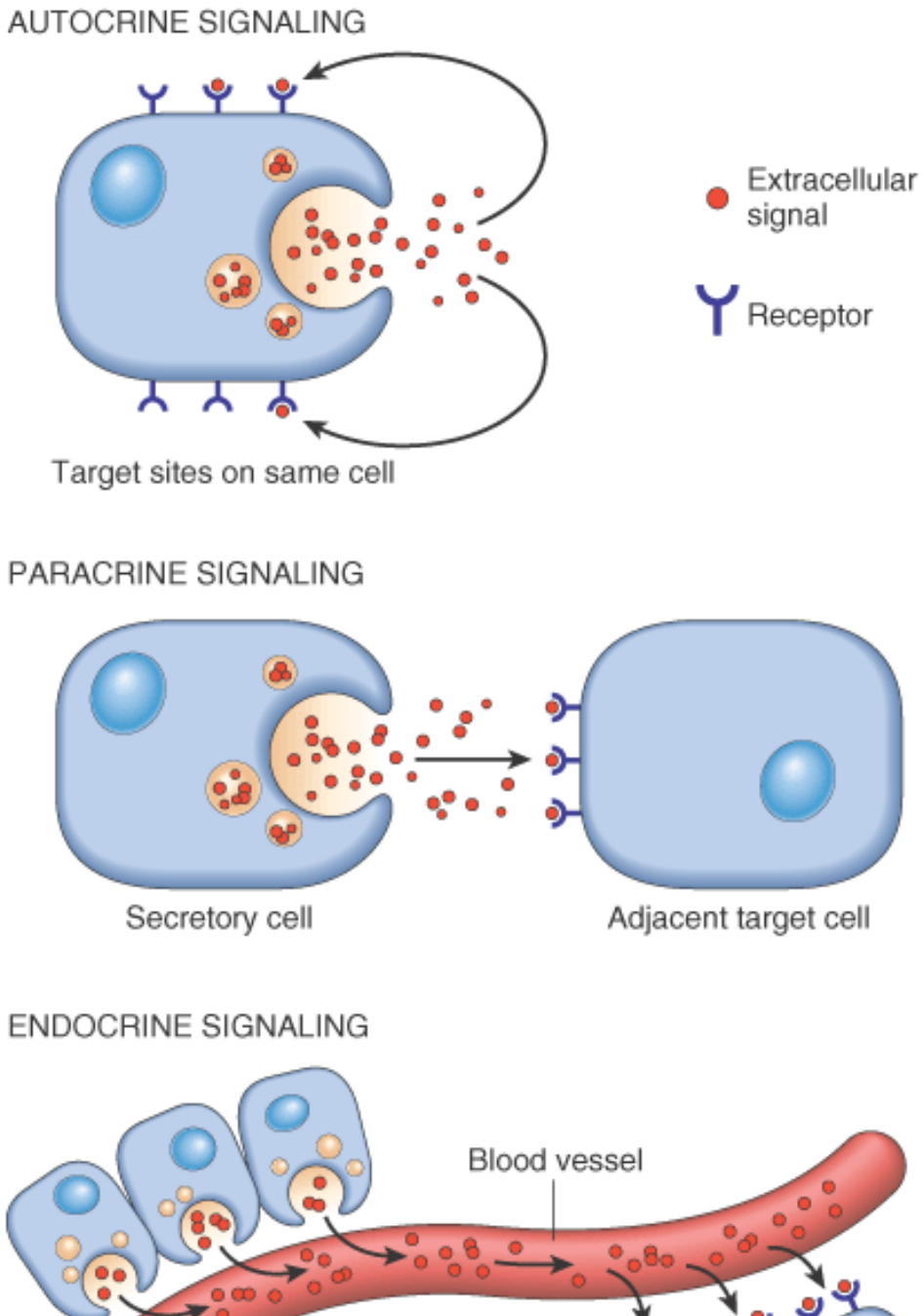
interaction is dependent on and promotes cell-cell adhesion.

- *Endocrine signaling:* Hormones are synthesized by cells of endocrine organs and act on target cells distant from their site of synthesis, being usually carried by the blood. Growth

factors may also circulate and act at distant sites, as is the case for HGF. Several cytokines, such as those associated with the systemic aspects of inflammation discussed in Chapter 2 , also act as endocrine agents.

Figure 3-8 General patterns of intercellular signaling demonstrating autocrine, paracrine, and endocrine signaling (see text). *(Modified from Lodish H, et al. [eds]: Molecular Cell Biology, 3rd ed. New York, WH Freeman, 1995, p. 855. © 1995 by Scientific American Books. Used with permission of WH Freeman and Company.)*



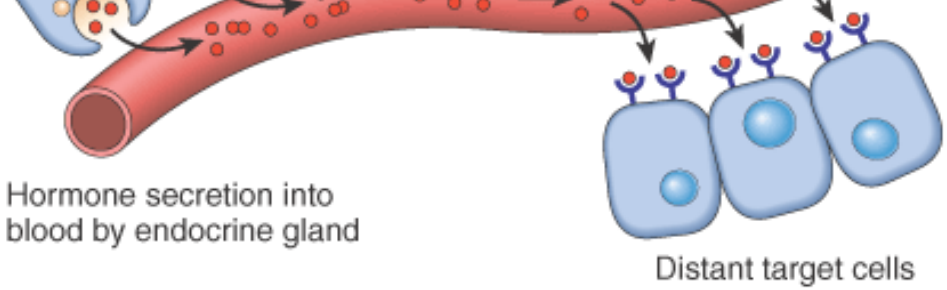
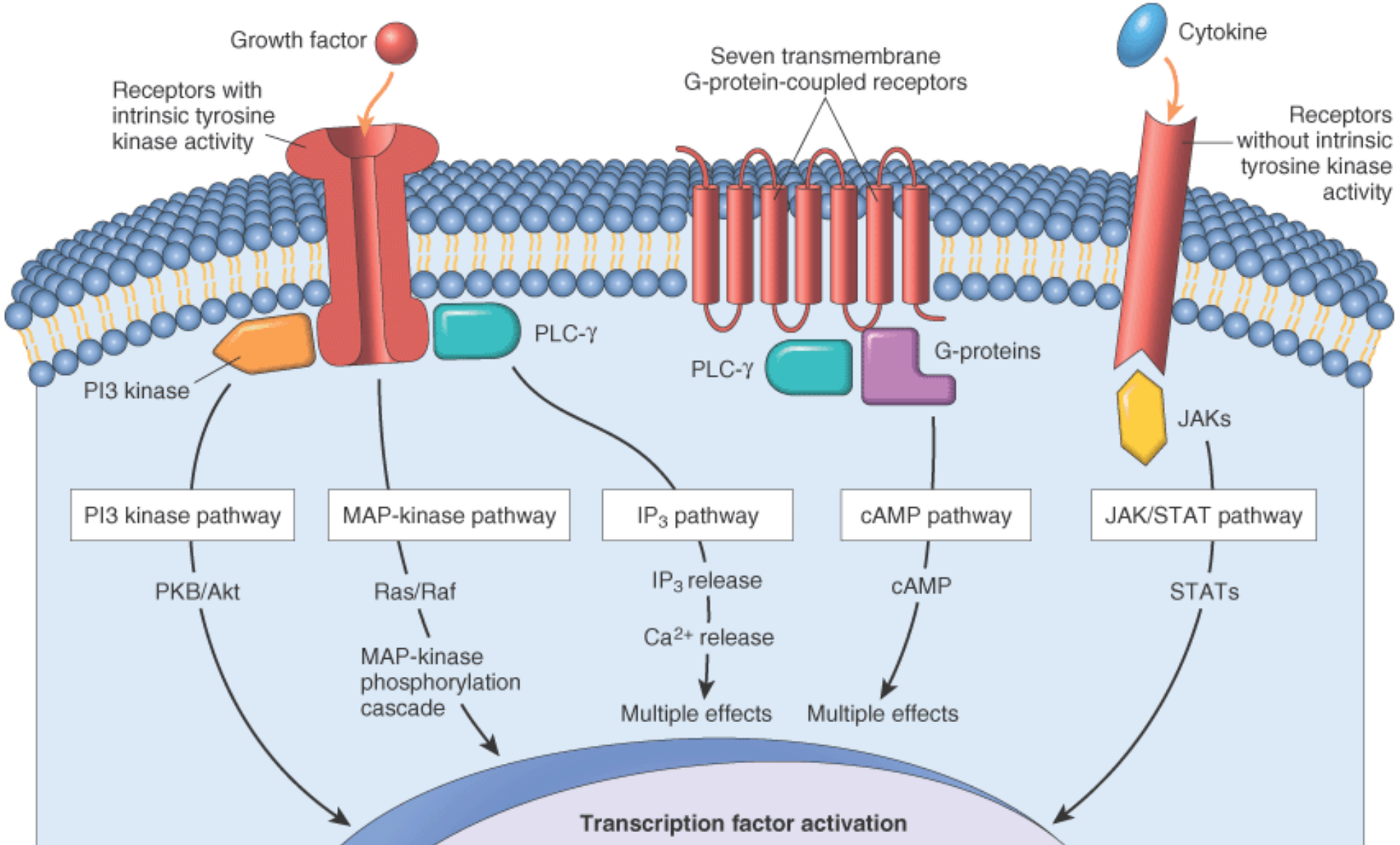


Figure 3-9 Examples of signal transduction systems that require cell-surface receptors. Shown are receptors with intrinsic tyrosine kinase activity, seven transmembrane G-protein-coupled receptors, and receptors without intrinsic tyrosine kinase activity. The figure also shows important signaling pathways transduced by the activation of these receptors through ligand binding.



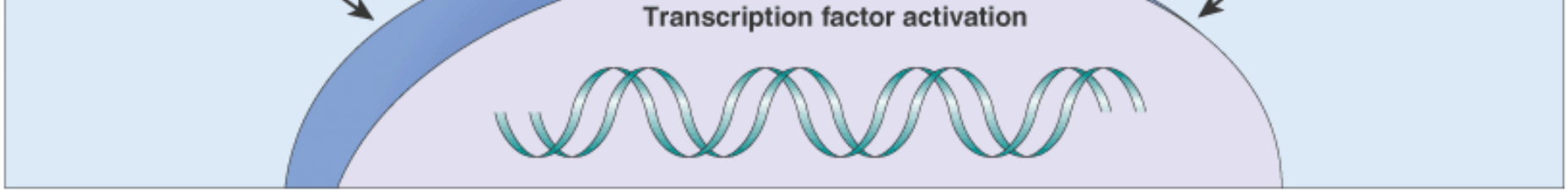


Figure 3-10 Signaling from tyrosine kinase receptors. Binding of the growth factor (ligand) causes receptor dimerization and autophosphorylation of tyrosine residues. Attachment of adapter (or bridging) proteins (e.g., GRB2 and SOS) couples the receptor to inactive RAS. Cycling of RAS between its inactive and active forms is regulated by GAP. Activated RAS interacts with and activates RAF (also known as MAP kinase kinase kinase). This kinase then phosphorylates a component of the MAP kinase signaling pathway, MEK (also known as MAP kinase kinase), which then phosphorylates ERK (MAP kinase). Activated MAP kinase phosphorylates other cytoplasmic proteins and nuclear transcription factors, generating cellular responses. The phosphorylated tyrosine kinase receptor can also bind other components, such as PI-3 kinase, which activates distinct signaling systems.

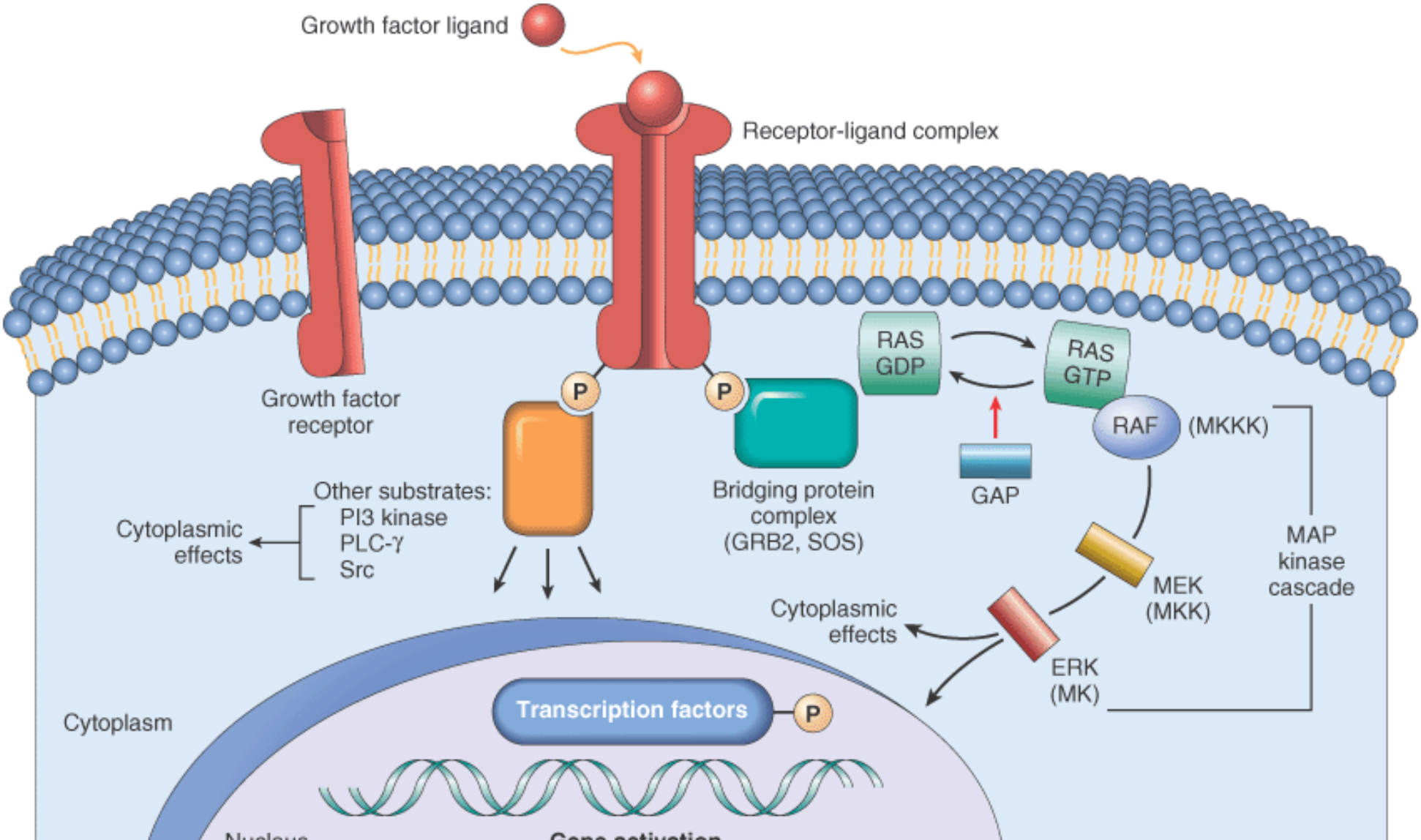
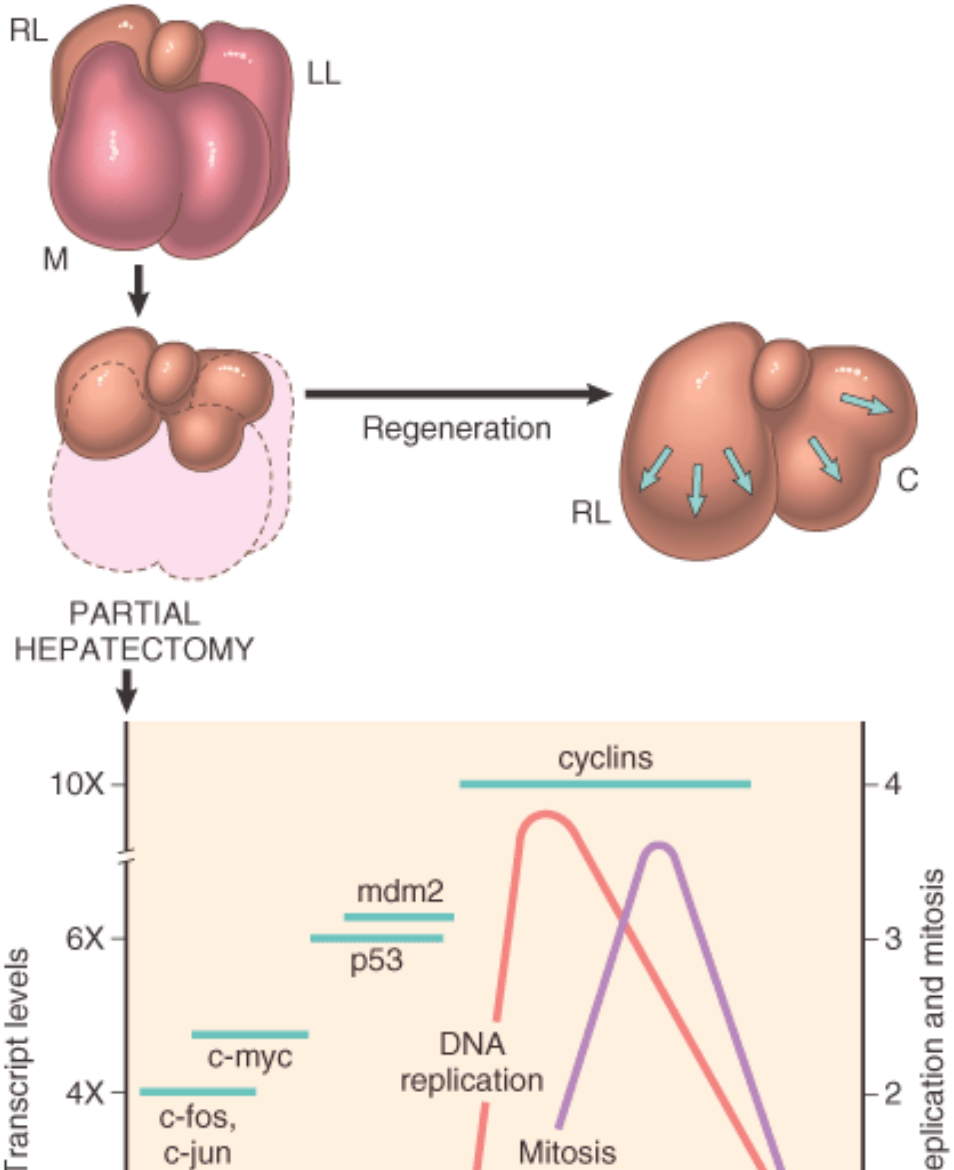


Figure 3-11 Liver regeneration after partial hepatectomy. *Upper panel,* The lobes of the liver of a rat are shown (M, median; RL and LL, right and left lateral lobes; C, caudate lobe). Partial hepatectomy removes two thirds of the liver (median and left lateral lobes), and only the right lateral and caudate lobes remain. After 3 weeks, the right lateral and caudate lobes enlarge to reach a mass equivalent to that of the original liver. Note that there is no regrowth of the median and left lateral lobes removed after partial hepatectomy. *(From Goss RJ: Regeneration versus repair. In Cohen IK, Diegelman RF, Lindblad WJ (eds): Wound Healing. Biochemical and Clinical Aspects. Philadelphia, W. B. Saunders Co., 1992, pp. 20–39.) Lower panel,* Timing of hepatocyte DNA replication, hepatocyte mitosis, and expression of messenger RNAs during liver regeneration. DNA replication is shown as the incorporation of tritiated thymidine × 10^{-4} *(right-side scale)*. Mitosis presented as the percentage of hepatocytes undergoing mitosis *(right-side scale)*. The expression of some of the many mRNAs in the regenerating rat liver is presented as fold elevation above normal *(left-side scale)*. Expression of the proto-oncogenes c-*fos*, c-*jun*, and c-*myc* corresponds to the immediate early gene phase of gene expression during liver regeneration.



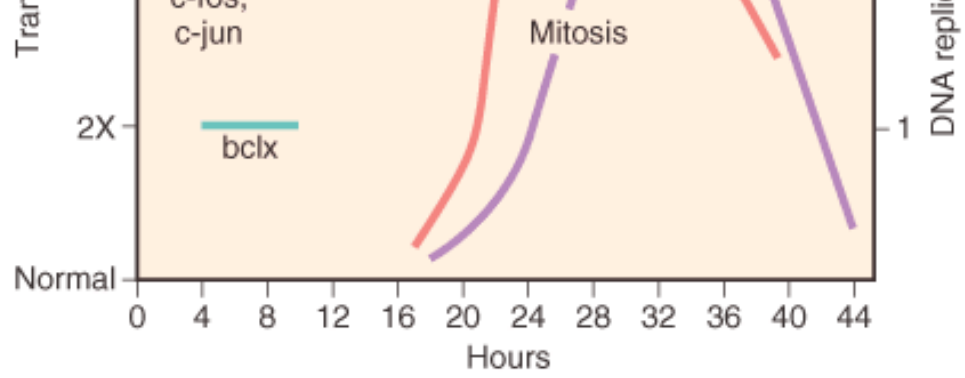


Figure 3-12 Regeneration of human liver. Computed tomography (CT) scans of the donor liver in living-donor hepatic transplantation. *Upper panel,* The liver of the donor before the operation. The right lobe, which will be used as a transplant, is outlined. *Lower panel,* A scan of the liver 1 week after performance of partial hepatectomy to remove the right lobe. Note the great enlargement of the left lobe (outlined in the panel) without regrowth of the right lobe *(Courtesy of R. Troisi, M.D. Ghent University city; reproduced in part from Fausto; Liver Regeneration. In Arias, et al: The Liver: Biology and Pathobiology, 4th ed. Philadelphia, Lippincott Williams & Wilkins, 2001.)*

Figure 3-13 Priming and cell-cycle progression in hepatocyte replication during liver regeneration. Quiescent hepatocytes become competent to enter the cell cycle through a priming phase mostly mediated by the cytokines TNF and IL-6 *(upper panel)*. Growth factors, mainly HGF and TGF-α, act on primed hepatocytes to make them progress through the cell cycle and undergo DNA replication *(lower panel)*. Norepinephrine, insulin, thyroid hormone, and growth hormone act as adjuvants for liver regeneration. The factors that determine the termination of cell replication are not known but are likely to involve cell cycle inhibitors, shut-off of growth factor production, and decreased metabolic demand on the liver.

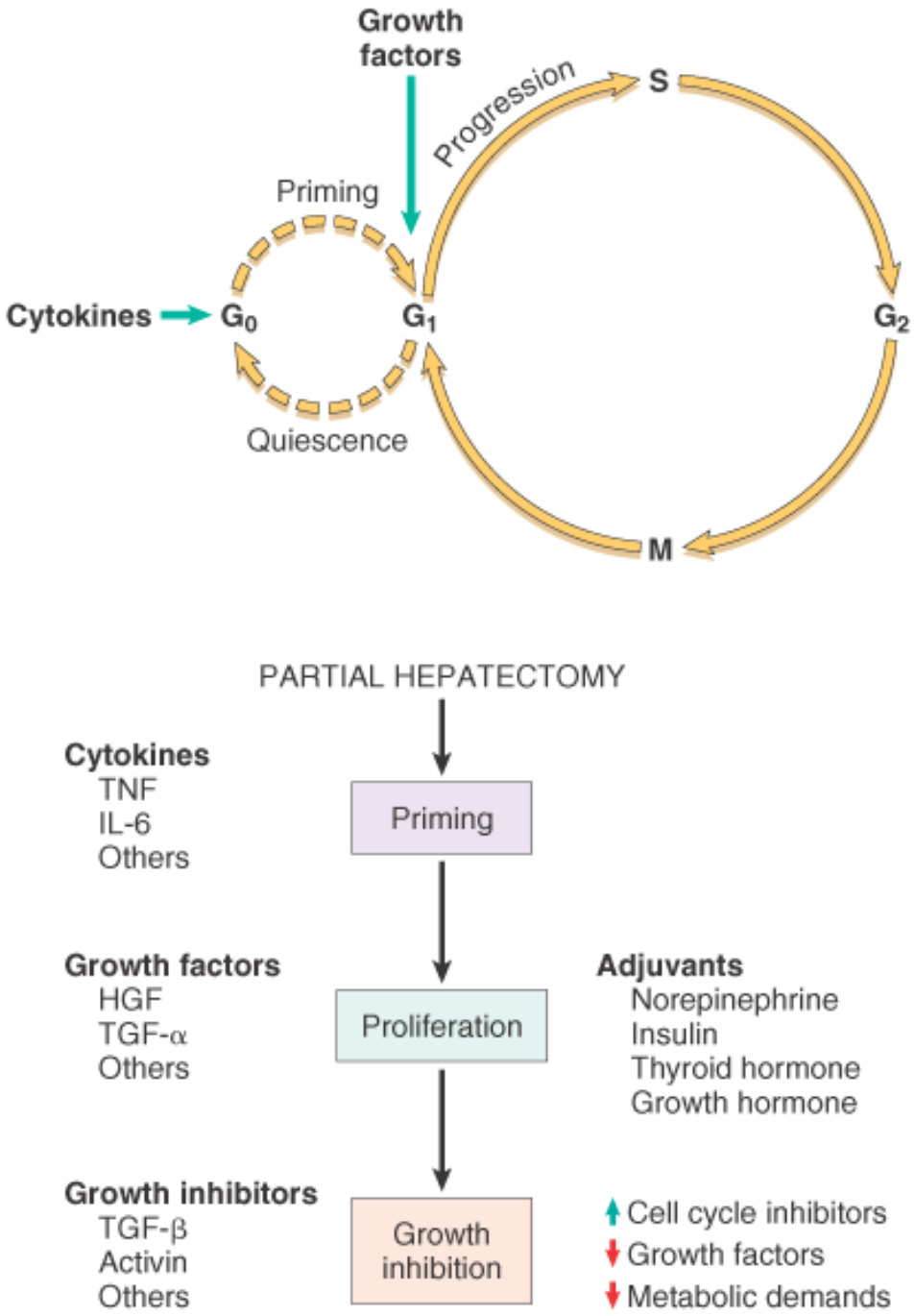


Figure 3-14 Major components of the extracellular matrix (ECM), including collagens, proteoglycans, and adhesive glycoproteins. Both epithelial and mesenchymal cells (e.g., fibroblasts) interact with ECM via integrins. To simplify the diagram, many ECM components (e.g., elastin, fibrillin, hyaluronan, syndecan) are not included.

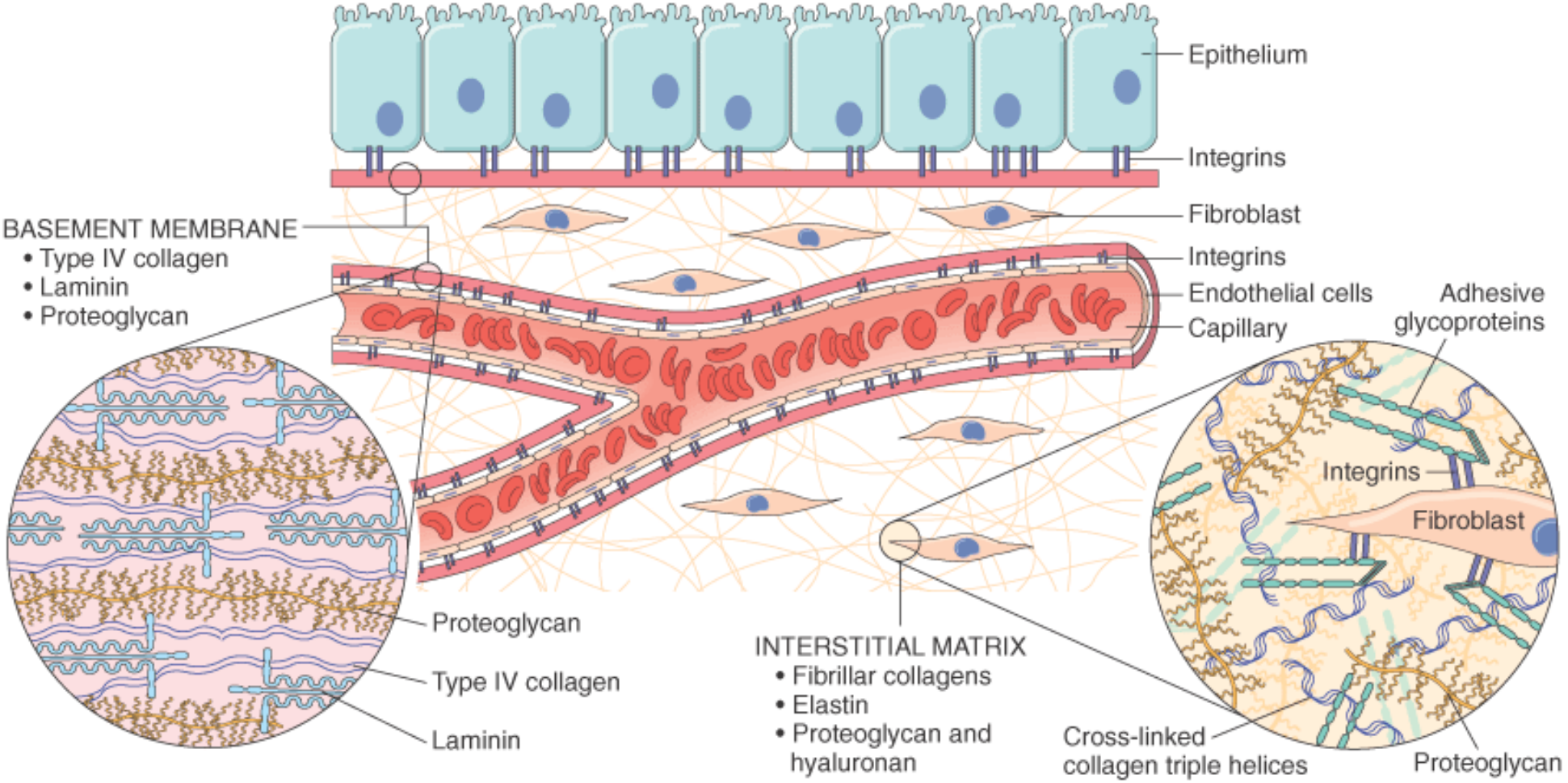


TABLE 3-2 -- Main Types of Collagens, Tissue Distribution, and Genetic Disorders

Collagen Type	Tissue Distribution	Genetic Disorders
Fibrillar Collagens		
I	Ubiquitous in hard and soft tissues	Osteogenesis Imperfecta
		Ehlers-Danlos syndrome—arthrochalasias type
II	Cartilage, intervertebral disk, vitreous	Achondrogenesis type II, spondyloepiphyseal dysplasia syndrome
III	Hollow organs, soft tissues	Vascular Ehlers-Danlos syndrome
V	Soft tissues, blood vessels	Classical Ehlers-Danlos syndrome
IX	Cartilage, vitreous	Stickler syndrome
Basement Membrane Collagens		

IV	Basement membranes	Alport syndrome
Other Collagens		
VI	Ubiquitous in microfibrils	Bethlem myopathy
VII	Anchoring fibrils at dermal-epidermal junctions	Dystrophic epidermolysis bullosa
IX	Cartilage, intervertebral disks	Multiple epiphyseal dysplasias
XVII	Transmembrane collagen in epidermal cells	Benign atrophic generalized epidermolysis bullosa
XV and XVIII	Endostatin-forming collagens, endothelial cells	Knobloch syndrome (type XVIII collagen)
Courtesy of Dr. Peter H. Byers, Department of Pathology, University of Washington, Seattle, WA		

original size after release of the tension. Morphologically, elastic fibers consist of a central core made of elastin, surrounded by a peripheral network of microfibrils. Substantial amounts of elastin are found in the walls of large blood vessels, such as the aorta, and in the uterus, skin, and ligaments. The peripheral microfibrillar network that surrounds the core consists largely of *fibrillin*, a 350-kD secreted glycoprotein, which associates either with itself or with other components of the ECM. The microfibrils serve as scaffolding for deposition of elastin and the assembly of elastic fibers. Inherited defects in fibrillin[86] result in formation of abnormal elastic fibers in a fairly common familial disorder, Marfan syndrome, manifested by changes in the cardiovascular system (aortic dissection) and the skeleton (Chapter 5).

CELL ADHESION PROTEINS

Most adhesion proteins, also called CAMs (cell adhesion molecules), can be classified into four main families: immunoglobulin family CAMs, cadherins, integrins, and selectins. These proteins are located in the cell membrane, where they function as receptors, or they are stored in the cytoplasm. As receptors, CAMs can bind to similar or different molecules in other cells, providing for interaction between the same cells (homotypic interaction) or different cell types (heterotypic interaction). Cadherins are generally involved in calcium-dependent homotypic interactions, while immunoglobulin family CAMs, because of the types of ligands they can bind, participate in both homotypic and heterotypic cell-to-cell interactions. The integrins have broader ligand specificity and are responsible for many events involving cell adhesion.[87] [88]

Integrins bind both to matrix proteins such as fibronectin and laminin, mediating adhesiveness between cells and ECM, as well as to adhesive proteins in other cells, establishing cell-to-cell contacts (see Box 2-1 , Chapter 2). *Fibronectin* is a larger protein that binds to many molecules, such as collagen, fibrin, proteoglycans, and cell-surface receptors. It consists of two glycoprotein chains, held together by disulfide bonds. Fibronectin mRNA has two splice forms, giving rise to tissue fibronectin and plasma fibronectin. The tissue fibronectin forms fibrillar aggregates at wound healing sites. The plasma form binds to fibrin, forming the provisional blood clot that

Figure 3-15 Steps in collagen synthesis (see text).

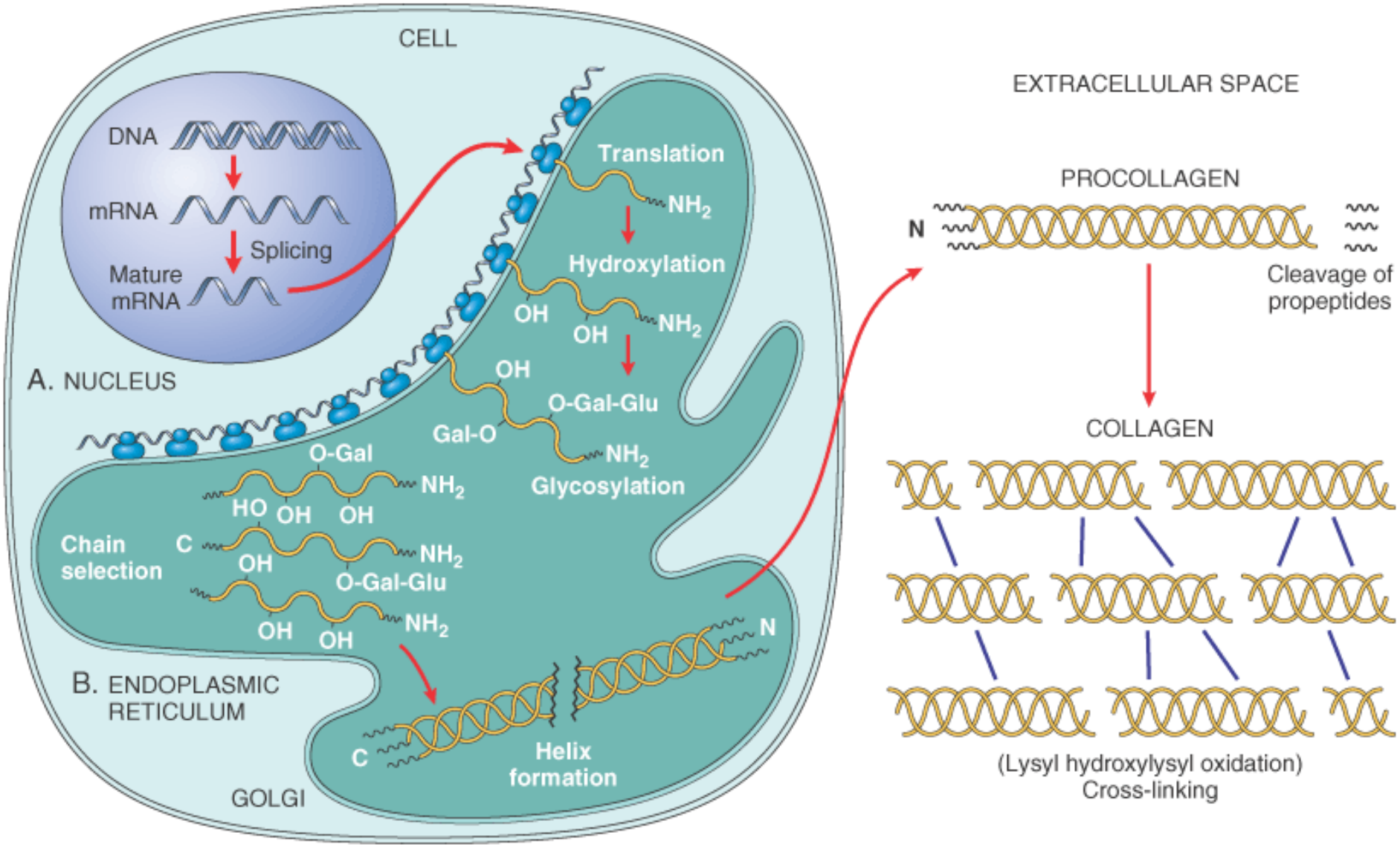
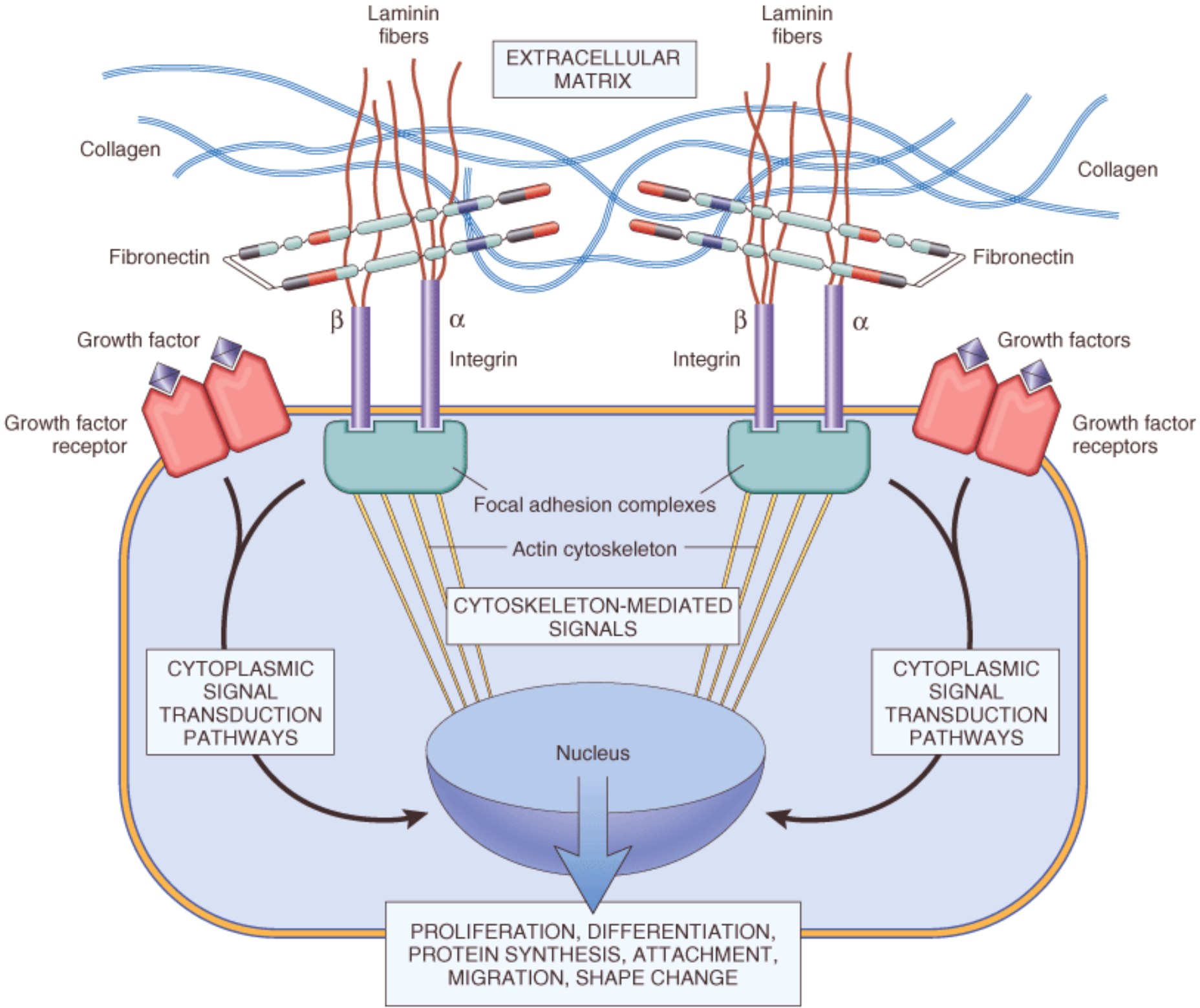


Figure 3-16 Mechanisms by which ECM (e.g., fibronectin and laminin) and growth factors can influence cell growth, motility, differentiation, and protein synthesis. Integrins bind ECM components and interact with the cytoskeleton at focal adhesion complexes (protein aggregates that include vinculin, α-actin, and talin). This can initiate the production of intracellular messengers or can directly mediate nuclear signals. Cell-surface receptors for growth factors may activate signal transduction pathways that overlap with those activated by integrins. Collectively, these are integrated by the cell to yield various responses, including changes in cell growth, locomotion, and differentiation.


Laminin fibers
EXTRACELLULAR MATRIX
Laminin fibers
Collagen
Collagen
Fibronectin
Fibronectin
β
α
Integrin
β
α
Integrin
Growth factor
Growth factors
Growth factor receptor
Growth factor receptors
Focal adhesion complexes
Actin cytoskeleton
CYTOSKELETON-MEDIATED SIGNALS
CYTOPLASMIC SIGNAL TRANSDUCTION PATHWAYS
CYTOPLASMIC SIGNAL TRANSDUCTION PATHWAYS
Nucleus
PROLIFERATION, DIFFERENTIATION, PROTEIN SYNTHESIS, ATTACHMENT, MIGRATION, SHAPE CHANGE

MIGRATION, SHAPE CHANGE

Figure 3-17 *A*, Granulation tissue showing numerous blood vessels, edema, and a loose ECM containing occasional inflammatory cells. This is a trichrome stain that stains collagen blue; minimal mature collagen can be seen at this point. *B*, Trichrome stain of mature scar, showing dense collagen, with only scattered vascular channels.

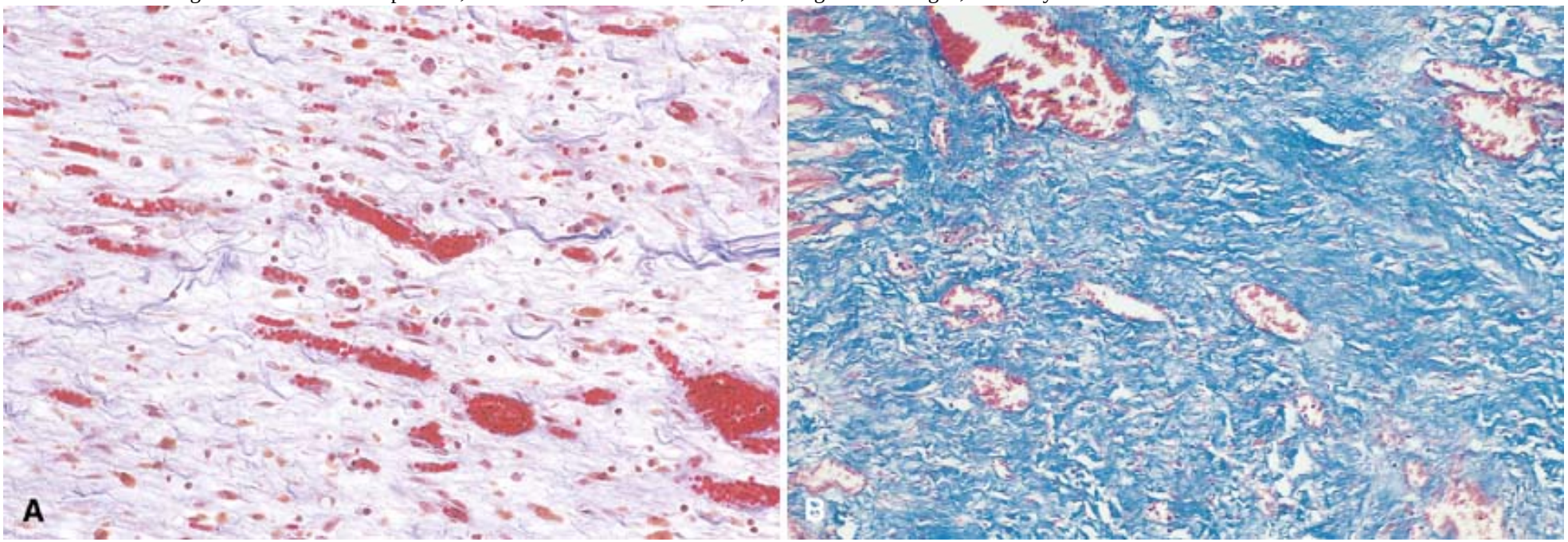


Figure 3-18 Angiogenesis by mobilization of endothelial precursor cells (EPCs) from the bone marrow and from pre-existing vessels (capillary growth). EPCs are mobilized from the bone marrow and may migrate to a site of injury or tumor growth *(upper panel)*. The homing mechanisms have not yet been defined. At these sites, EPCs differentiate and form a mature network by linking with existing vessels. In angiogenesis from pre-existing vessels, endothelial cells from these vessels become motile and proliferate to form capillary sprouts *(lower panel)*. Regardless of the initiating mechanism, vessel maturation (stabilization) involves the recruitment of pericytes and smooth muscle cells to form the periendothelial layer. *(Modified from Conway EM, Collen D, Carmeliet P: Molecular mechanisms of blood vessel growth. Cardiovasc Res 49:507, 2001.)*

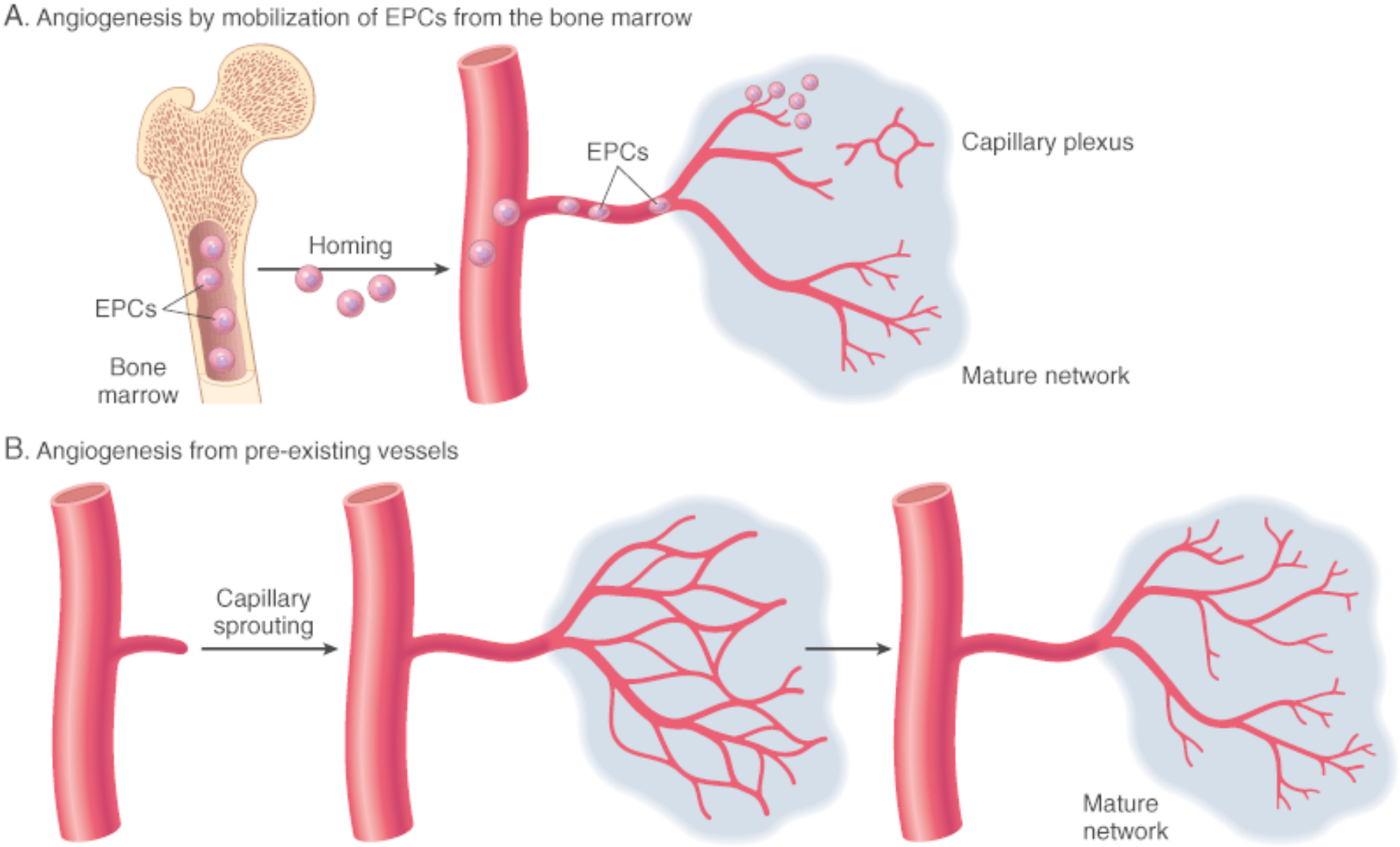


TABLE 3-3 -- Vascular Endothelial Growth Factor (VEGF)

Proteins	Family members: VEGF (VEGF-A), VEGF-B, VEGF-C, VEGF-D
	Dimeric glycoprotein with multiple isoforms
	Targeted mutations in VEGF result in defective vasculogenesis and angiogenesis
Production	Expressed at low levels in a variety of adult tissues and at higher levels in a few sites, such as podocytes in the glomerulus and cardiac myocytes
Inducing Agents	Hypoxia
	TGF-β

	PDGF
	TGF-α
Receptors	VEGFR-1
	VEGFR-2 (restricted to endothelial cells)
	VEGFR-3 (lymphatic endothelial cells)
	Targeted mutations in the receptors result in lack of vasculogenesis
Functions	Promotes angiogenesis
	Increases vascular permeability
	Stimulates endothelial cell migration
	Stimulates endothelial cell proliferation
	VEGF-C selectively induces hyperplasia of lymphatic vasculature
	Up-regulates endothelial expression of plasminogen activator, plasminogen activator inhibitor-1, tissue factor, and interstitial collagenase

in the absence of VEGF, more responsive to inhibitors of angiogenesis. A telling proof of the importance of these molecules is the existence of a genetic disorder caused by mutations in *Tie2* that is characterized by venous malformations.[103] Both physiologic and pathologic angiogenesis can be influenced by agents or conditions that stimulate VEGF expression, such as certain cytokines and growth factors (e.g., TGF-β, PDGF, TGF-α) and, notably, tissue hypoxia, which has long been associated with angiogenesis (see Table 3-3).

Despite the diversity of factors that may participate at various steps in angiogenesis, VEGF emerges as the most important growth factor in adult tissues undergoing physiologic angiogenesis (e.g., proliferating endometrium) as well as pathologic angiogenesis seen in chronic inflammation, wound healing, tumors, and diabetic retinopathy.

ECM Proteins as Regulators of Angiogenesis

A key component of angiogenesis is the motility and directed migration of endothelial cells, required for the formation of new blood vessels. These processes are controlled by several classes of proteins, including (1) *integrins*, especially $\alpha_v \beta_3$, which is critical for the formation and maintenance of newly formed blood vessels,[104] (2) *matricellular proteins*, including thrombospondin 1, SPARC, and tenascin C, which destabilize cell-matrix interactions and therefore promote angiogenesis,[105] and (3) *proteinases*, such as the plasminogen activators and *matrix metalloproteinases*, which are important in tissue remodeling during endothelial invasion. Additionally, these proteinases cleave extracellular proteins, releasing matrix-bound growth factors such as VEGF and FGF-2 that stimulate angiogenesis. Proteinases can also release inhibitors such as endostatin, a small fragment of collagen that inhibits endothelial proliferation and angiogenesis.[106] $\alpha_v \beta_3$ integrin expression in endothelial cells is stimulated by hypoxia. This integrin has multiple effects on angiogenesis: it directly interacts with a metalloproteinase (MMP-2, discussed below), it binds to and regulates the activity of VEGFR-2, and it mediates adhesion to ECM components such as fibronectin, thrombospondin, and osteopontin. [104]

SCAR FORMATION

Growth factors and cytokines released at the site of injury induce fibroblast proliferation and migration into the granulation tissue framework of new blood vessels and loose ECM that initially forms at the repair site. We discuss three processes that participate in the formation of a scar: (1) *emigration and proliferation of fibroblasts in the site of injury*, (2) *deposition of ECM*, and (3) *tissue remodeling*.

Fibroblast Migration and Proliferation

Granulation tissue contains numerous newly formed blood vessels. As discussed previously, VEGF promotes angiogenesis but is also responsible for a marked increase in vascular permeability (VEGF was first named vascular permeability factor).[107] The latter activity leads to exudation and deposition of plasma proteins, such as fibrinogen and plasma fibronectin, in the ECM and provides a provisional stroma for fibroblast and endothelial cell ingrowth. *Migration* of fibroblasts to the site of injury and their subsequent *proliferation* are triggered by multiple growth factors, including TGF-β, PDGF, EGF, FGF, and the cytokines IL-1 and TNF (see Table 3-5). The sources of these growth factors and cytokines include platelets, a variety of inflammatory cells (notably macrophages), and activated endothelium. Macrophages are important cellular constituents of granulation tissue, clearing extracellular debris, fibrin, and other foreign material at the site of repair. These cells also elaborate TGF-β, PDGF, and FGF and there-fore promote fibroblast migration and proliferation.[108] If the appropriate chemotactic stimuli are present, mast cells, eosinophils, and lymphocytes may also accumulate. Each of these cells can contribute directly or indirectly to fibroblast migration and proliferation. Of the growth factors involved in inflammatory fibrosis, TGF-β appears to be the most important because of the multitude of effects that favor fibrous tissue deposition. TGF-β is produced by most of the cells in granulation tissue and causes *fibroblast migration and proliferation, increased synthesis of collagen and fibronectin, and decreased degradation of ECM by metalloproteinases* (discussed later). TGF-β is also chemotactic for monocytes and causes angiogenesis in vivo, possibly by inducing macrophage influx. TGF-β expression is increased in tissues in a number of chronic fibrotic diseases in humans and experimental animals.

ECM Deposition and Scar Formation

As repair continues, the number of proliferating endothelial cells and fibroblasts decreases. Fibroblasts progressively deposit increased amounts of ECM. Fibrillar collagens form a major portion of the connective tissue in repair sites and are important for the development of strength in healing wounds. As described later in the discussion of cutaneous wound healing, collagen synthesis by fibroblasts begins within 3 to 5 days after injury and continues for several weeks, depending on the size of wound. Many of the same growth factors that regulate fibroblast proliferation also stimulate ECM synthesis (see Table 3-4 (Table Not Available)). For example, collagen synthesis is enhanced by several factors, including growth factors (PDGF, FGF, TGF-β) and cytokines (IL-1, IL-13), which are secreted by leukocytes and fibroblasts in healing wounds. *Net collagen accumulation, however, depends not only on increased collagen synthesis but also on decreased degradation.* Ultimately, the granulation tissue scaffolding is converted into a scar composed of spindle-shaped fibroblasts, dense collagen, fragments of elastic tissue, and other ECM components. As the scar matures, vascular regression continues, eventually transforming the richly vascularized granulation tissue into a pale, avascular scar.

Tissue Remodeling

The replacement of granulation tissue with a scar involves transitions in the composition of the ECM. Some of the growth factors that stimulate synthesis of collagen and other connective tissue molecules also modulate the synthesis and activation of metalloproteinases, enzymes that degrade these ECM components. The balance between ECM synthesis and degradation results in *remodeling* of the connective tissue framework—an important feature of both chronic inflammation and wound repair.

Degradation of collagen and other ECM proteins is achieved by a family of matrix metalloproteinases (MMPs), which are dependent on zinc ions for their activity[109] [110] (Fig. 3-19). This

Figure 3-19 Matrix metalloproteinase regulation. Four mechanisms are shown: (1) regulation of synthesis by growth factors or cytokines, (2) inhibition of synthesis by corticosteroids or TGF-β, (3) regulation of the activation of the secreted but inactive precursors, and (4) blockage of the enzymes by specific tissue inhibitors of metalloproteinase (TIMPs). *(Modified from Matrisian LM: Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet 6:122, 1990, with permission from Elsevier Science.)*

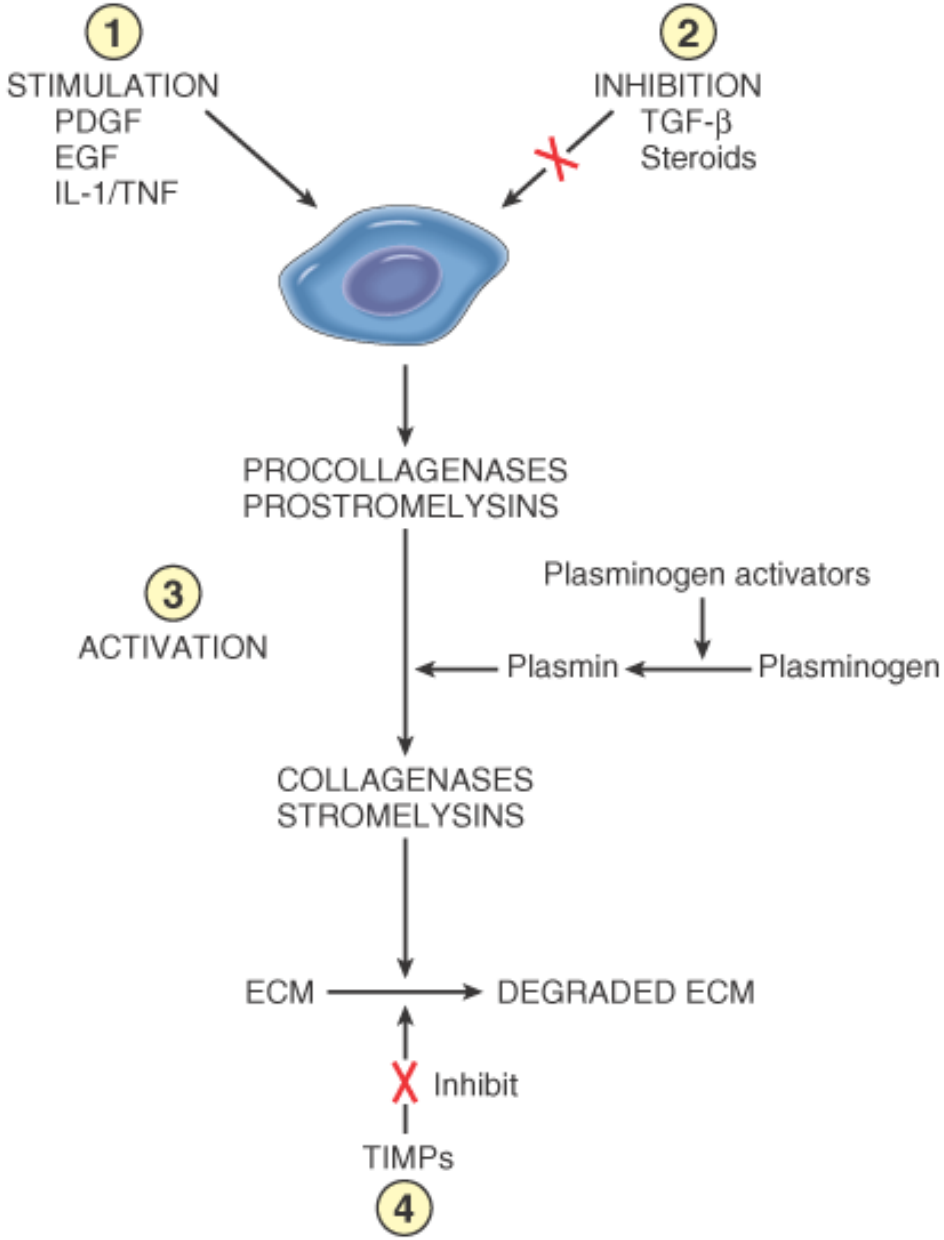


Figure 3-20 Phases of wound healing. *(Modified from Clark RAF: Wound repair. In Clark RAF (ed): The molecular and cellular biology of wound repair, 2nd ed, New York, Plenum Press, 1996, p. 3.)*

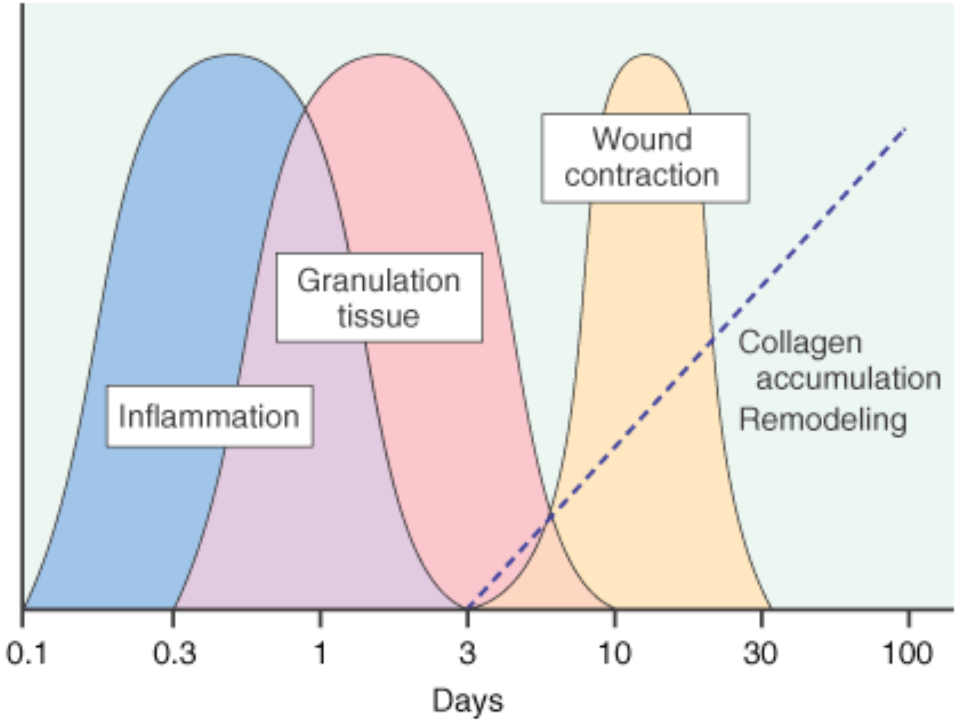
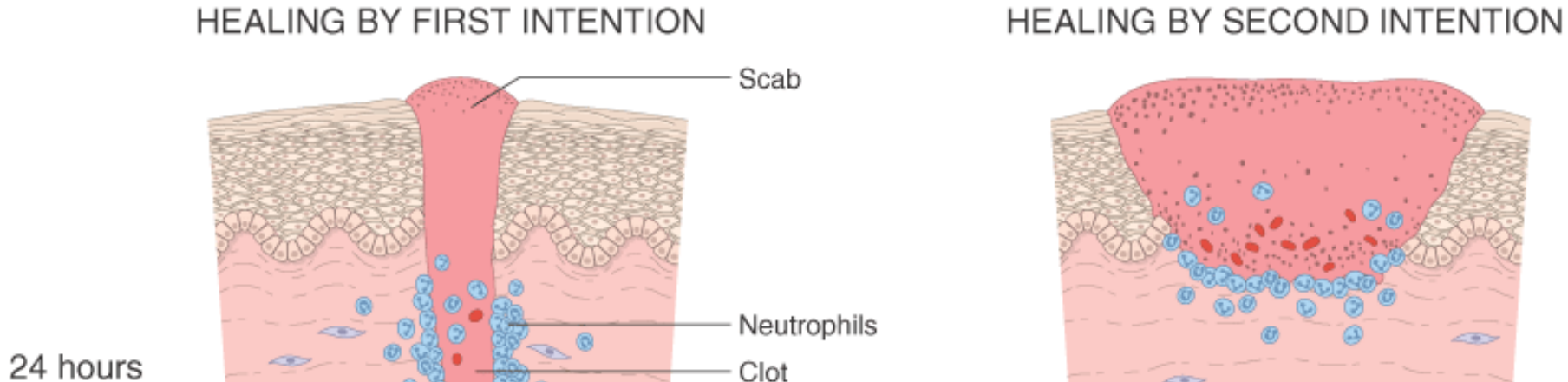


TABLE 3-4 -- Growth Factors and Cytokines Affecting Various Steps in Wound Healing

(Not Available)

Figure 3-21 Steps in wound healing by first intention *(left)* and second intention *(right)*. Note large amounts of granulation tissue and wound contraction in healing by second intention.



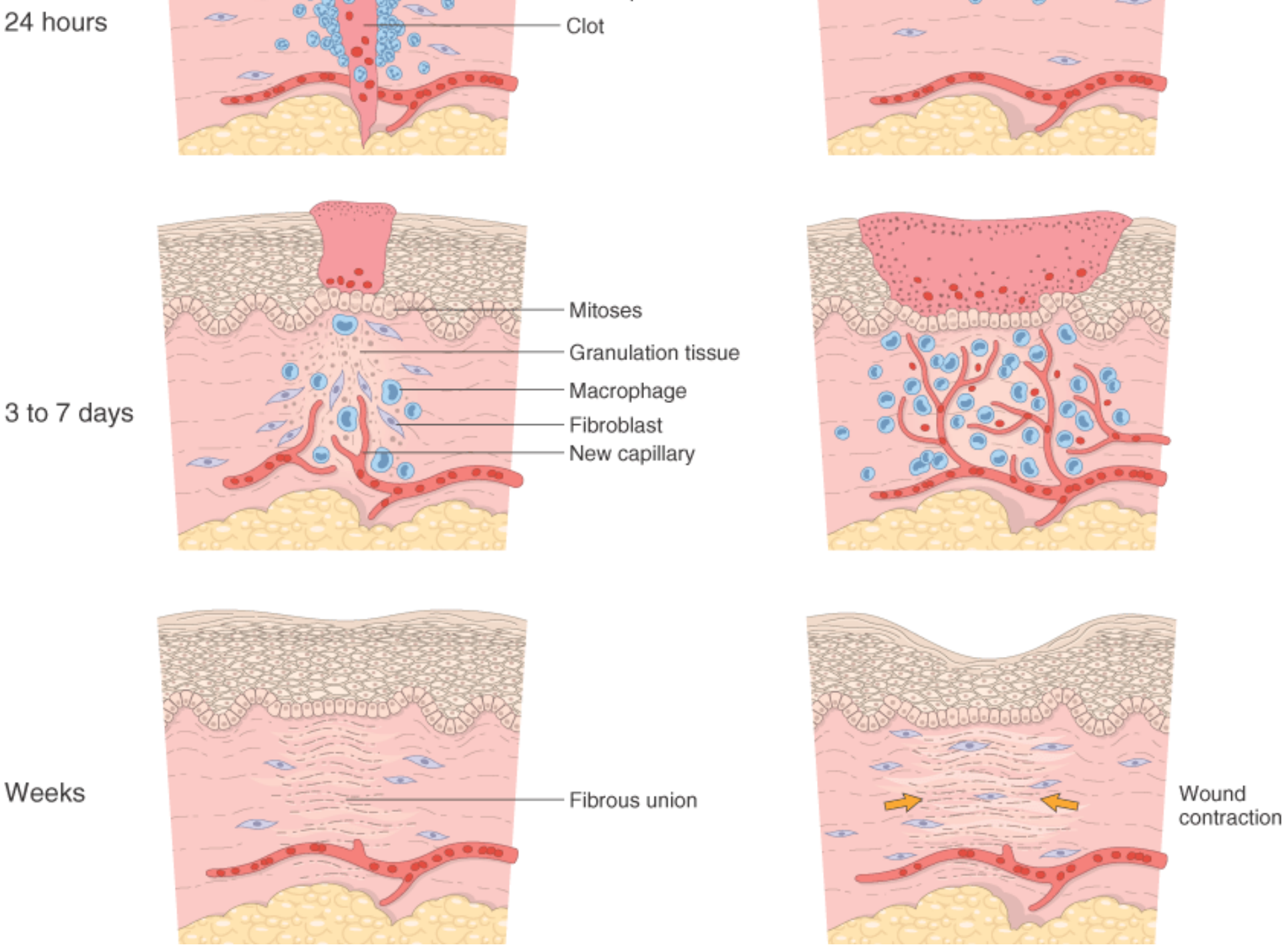


Figure 3-22 Healing of skin ulcers. *A*, Pressure ulcer of the skin, commonly found in diabetic patients. The histology slides show *B*, a skin ulcer with a large gap between the edges of the lesion; *C*, a thin layer of epidermal reepithelialization and extensive granulation tissue formation in the dermis; and *D*, continuing reepithelialization of the epidermis and wound contraction. *(Courtesy of Z. Argenyi, M.D., University of Washington.)*

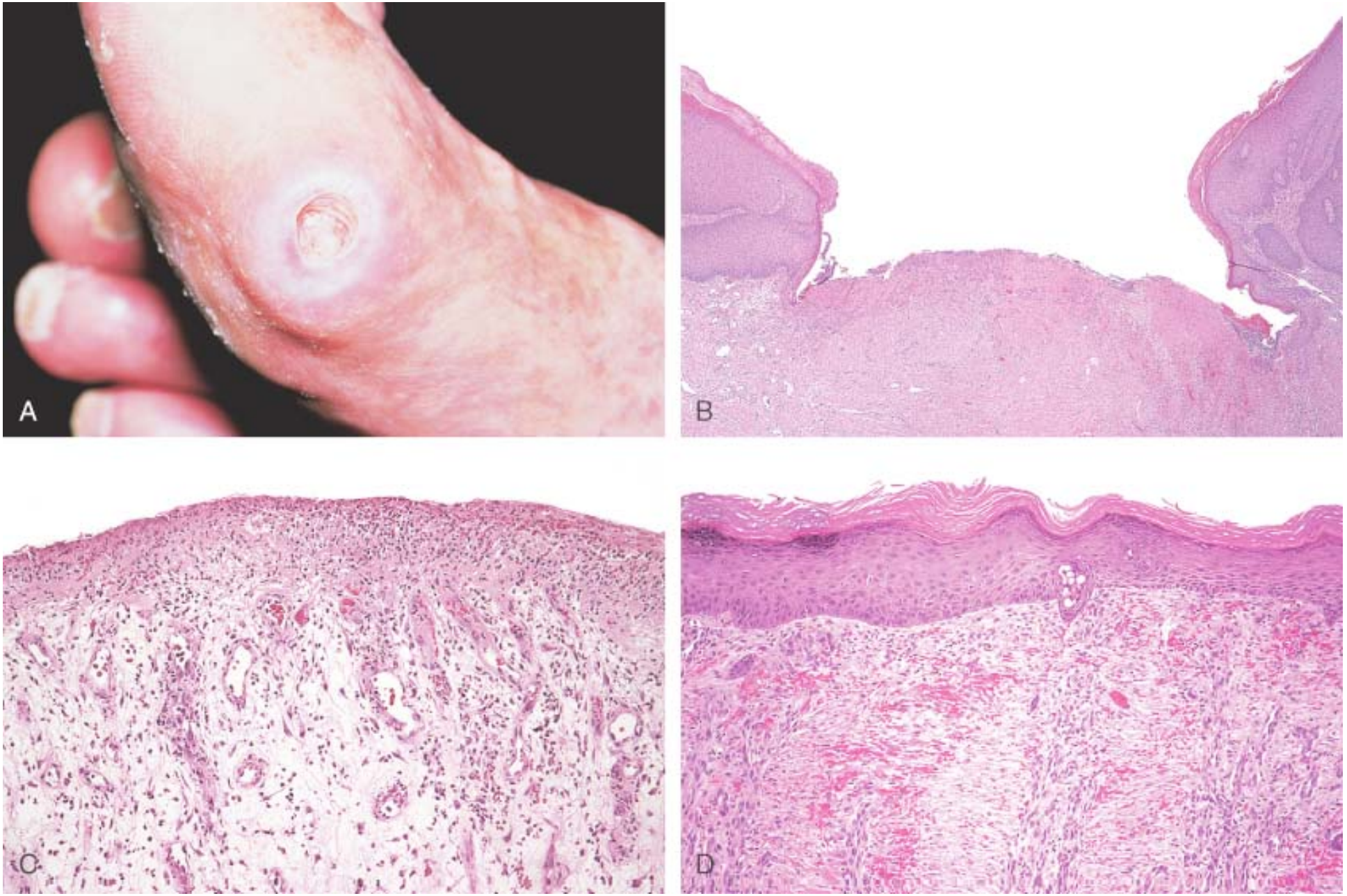


TABLE 3-5 -- Factors That Retard Wound Healing

Local Factors	
Blood supply	Mechanical stress
Denervation	Necrotic tissue

Local infection	Protection (dressings)
Foreign body	Surgical techniques
Hematoma	Type of tissue
Systemic Factors	
Age	Malnutrition
Anemia	Obesity
Drugs (steroids, cytotoxic medications, intensive antibiotic therapy)	Systemic infection
	Temperature
	Trauma, hypovolemia, and hypoxia
Genetic disorders (osteogenesis imperfecta, Ehlers-Danlos syndromes, Marfan syndrome)	
	Uremia
	Vitamin deficiency (vitamin C)
Hormones	Trace metal deficiency (zinc, copper)
Diabetes	
Malignant disease	
Adapted from Schwartz SI: Principles of Surgery. New York, McGraw Hill, 1999.	

- *Infection* is the single most important cause of delay in healing because it results in persistent tissue injury and inflammation.
- *Mechanical factors*, such as early motion of wounds, can delay healing, by compressing blood vessels and separating the edges of the wound.
- *Foreign bodies*, such as unnecessary sutures or fragments of steel, glass, or even bone, constitute impediments to healing.
- *Size, location, and type of wound influence healing.* Wounds in richly vascularized areas, such as the face, heal faster than those in poorly vascularized ones, such as the foot. As we have discussed, small incisional injuries heal faster and with less scar formation than large excisional wounds or wounds caused by blunt trauma.

SUMMARY OF CUTANEOUS WOUND HEALING

Various stages in the healing of an ulcer of the skin are shown in Figure 3-22 . The healing wound, as a prototype of tissue repair, is a dynamic and changing process. The early phase is one of inflammation, followed by formation of granulation tissue and subsequent tissue remodeling and scarring. Simple cutaneous incisional wounds heal by first intention. Large cutaneous wounds heal by second intention, generating a significant amount of scar tissue. Different mechanisms occurring at different times trigger the release of chemical signals that modulate the orderly migration, proliferation, and differentiation of cells and the synthesis and degradation of ECM proteins. These proteins, in turn, directly affect cellular events and modulate cell responsiveness to soluble growth factors. The magic behind the precise orchestration of these events under normal conditions remains beyond our grasp. It almost certainly lies in the regulation of specific soluble and membrane-anchored mediators and their receptors on particular cells, cell-matrix interactions, and the effect of physical factors, including ECM remodeling forces generated by changes in cell shape.

COMPLICATIONS IN CUTANEOUS WOUND HEALING

Complications in wound healing can arise from abnormalities in any of the basic components of the repair process. These aberrations can be grouped into three general categories: (1)

deficient scar formation, (2) *excessive formation of the repair components*, and (3) *formation of contractures*.

Inadequate formation of granulation tissue or assembly of a scar can lead to two types of complications: *wound dehiscence* and *ulceration*. Dehiscence or rupture of a wound is most common after abdominal surgery and is due to increased abdominal pressure. This mechanical stress on the abdominal wound can be generated by vomiting, coughing, or ileus. Wounds can ulcerate because of inadequate vascularization during healing. For example, lower extremity wounds in individuals with atherosclerotic peripheral vascular disease typically ulcerate (Chapter 11). Nonhealing wounds also form in areas devoid of sensation. These neuropathic ulcers are occasionally seen in patients with diabetic peripheral neuropathy (Chapter 24 and Chapter 27).

Excessive formation of the components of the repair process can also complicate wound healing. Aberrations of growth may occur even in what may begin initially as normal wound healing. The accumulation of excessive amounts of collagen may give rise to a raised scar known as a *hypertrophic scar*; if the scar tissue grows beyond the boundaries of the original wound and does not regress, it is called a *keloid* (Fig. 3-23). Keloid formation appears to be an individual predisposition, and for unknown reasons this aberration is somewhat more common in African-Americans. The mechanisms of keloid formation are still unknown. Another deviation in wound healing is the formation of excessive amounts of granulation tissue, which protrudes above the level of the surrounding skin and blocks re-epithelialization. This has been called *exuberant granulation* (or, with more literary fervor, *proud flesh*). Excessive granulation must be removed by cautery or surgical excision to permit restoration of the continuity of the epithelium. Finally (fortunately rarely), incisional scars or traumatic injuries may be followed by exuberant proliferation of fibroblasts and other connective tissue elements that may, in fact, recur after excision. Called *desmoids*, or *aggressive fibromatoses*, these lie in the interface between benign proliferations and malignant (though low-grade) tumors. The line between the benign hyperplasias characteristic of repair and neoplasia is frequently finely drawn (Chapter 7).

Contraction in the size of a wound is an important part of the normal healing process. An exaggeration of this process is called a *contracture* and results in deformities of the wound and the surrounding tissues. Contractures are particularly prone to develop on the palms, the soles, and the anterior aspect of the thorax. Contractures are commonly seen after serious burns and can compromise the movement of joints. Impaired wound contraction occurs in stromelysin-1 (MMP3)—deficient mice, suggesting that proteolysis by this metalloproteinase is required for the assembly of fibroblasts containing actin filaments, needed for the contraction of early wounds. [120]

Figure 3-23 *A*, Keloid. Excess collagen deposition in the skin forming a raised scar known as keloid. *(From Murphy GF, Herzberg AJ: Atlas of Dermatopathology. Philadelphia, Saunders, W.B. 1996, p. 219.) B*, Note the thick connective tissue deposition in the dermis. *(Slide courtesy of Z. Argenyi, M.D., University of Washington, Seattle, WA.)*

Figure 3-24 Development of fibrosis in chronic inflammation. The persistent stimulus of chronic inflammation activates macrophages and lymphocytes, leading to the production of growth factors and cytokines, which increase the synthesis of collagen. Deposition of collagen is enhanced by decreased activity of metalloproteinases.

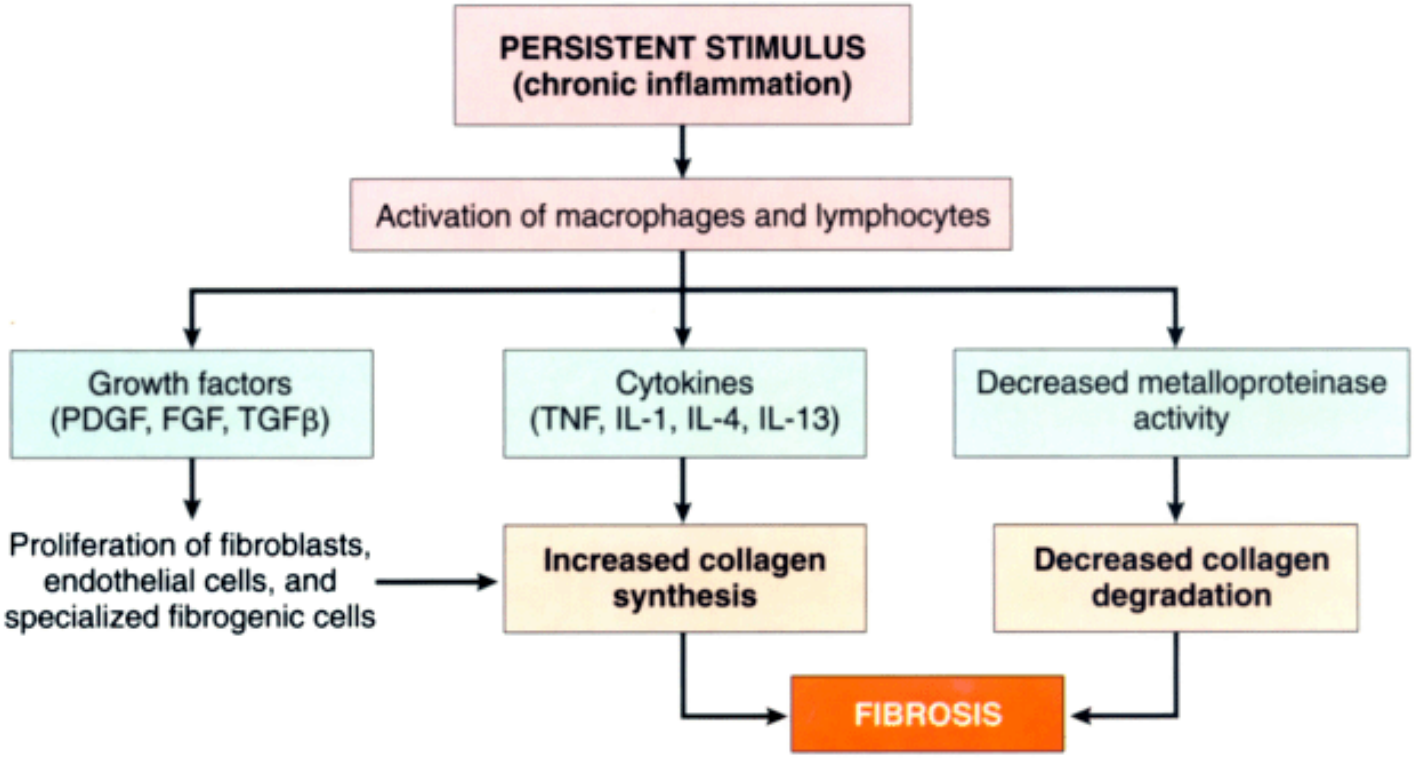
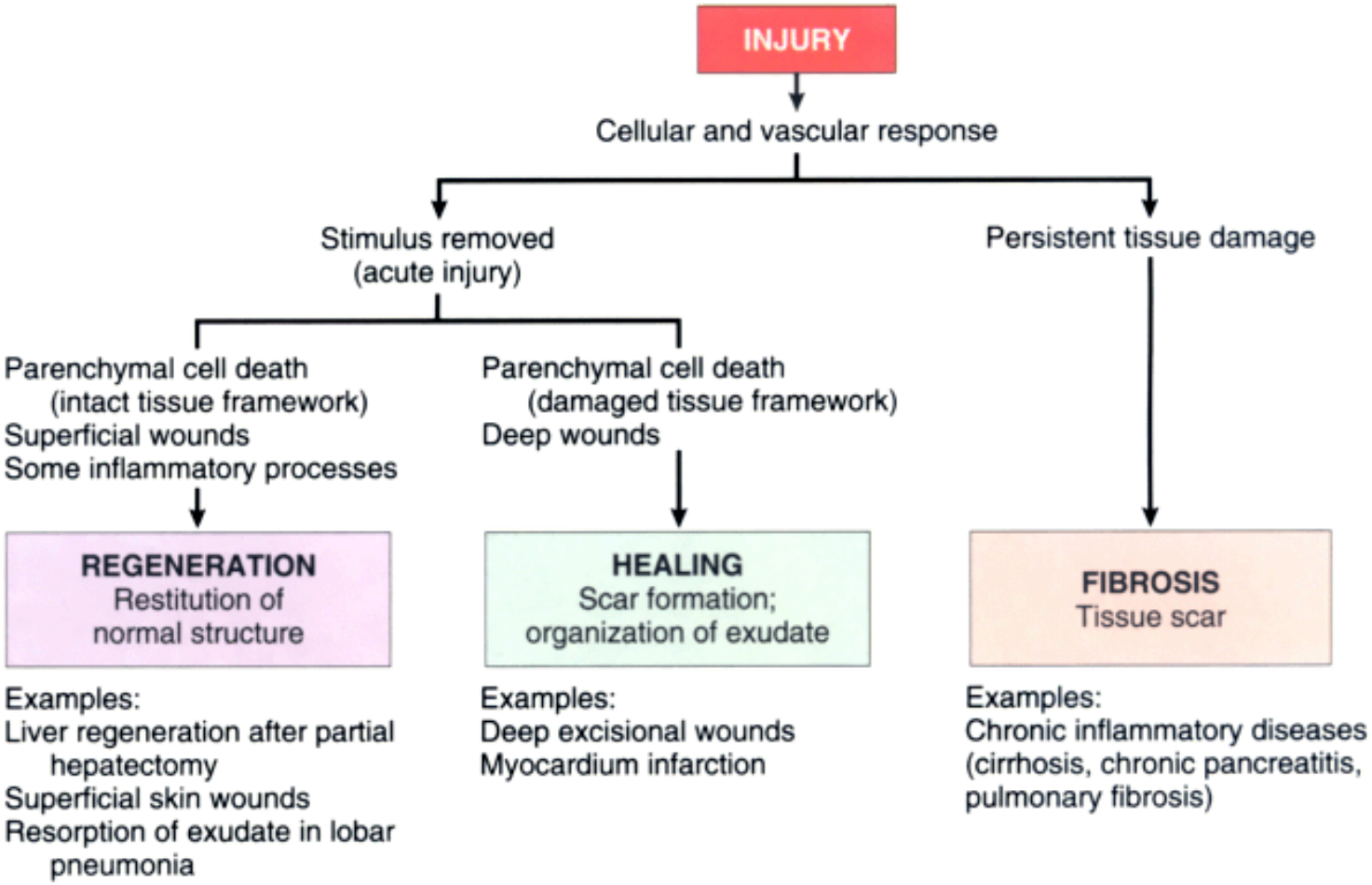


Figure 3-25 Repair responses after injury and inflammation. Repair after acute injury has several outcomes, including normal tissue restitution and healing with scar formation. Healing in chronic injury involves scar formation and fibrosis (see text).



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Chapter 4 - Hemodynamic Disorders, Thromboembolic Disease, and Shock

Richard N. Mitchell MD, PhD *

The health of cells and organs critically depends on an unbroken circulation to deliver oxygen and nutrients and to remove wastes. However, the well-being of tissues also requires normal fluid balance; abnormalities in vascular permeability or hemostasis can result in injury even in the setting of an intact blood supply. This chapter will describe major disturbances involving hemodynamics and the maintenance of blood flow, including edema, hemorrhage, thrombosis, embolism, infarction, and shock. *Normal fluid homeostasis encompasses maintenance of vessel wall integrity as well as intravascular pressure and osmolarity within certain physiologic ranges.* Changes in vascular volume, pressure, or protein content, or alterations in endothelial function, all affect the net movement of water across the vascular wall. Such water extravasation into the interstitial spaces is called *edema* and has different manifestations depending on its location. In the lower extremities, edema mainly causes swelling; in the lungs, edema causes water to fill alveoli, leading to difficulty in breathing. *Normal fluid homeostasis also means maintaining blood as a liquid until such time as injury necessitates clot formation.* Clotting at inappropriate sites *(thrombosis)* or migration of clots *(embolism)* obstructs blood flow to tissues and leads to cell death *(infarction)*. Conversely, inability to clot after vascular injury results in *hemorrhage*; local bleeding can compromise regional tissue perfusion, while more extensive hemorrhage can result in hypotension *(shock)* and death.

Some of the failures of fluid homeostasis reflect a *primary* pathology in a discrete vascular bed (e.g., hemorrhage due to local trauma) or in systemic coagulation (thrombosis due to hypercoagulability disorders); others may represent a

*The contributions of the late Dr. Ramzi Cotran to this chapter in previous editions are gratefully acknowledged.

secondary manifestation of some other disease process. Thus, pulmonary edema due to increased hydrostatic pressure may be a terminal complication of ischemic or valvular heart disease. Similarly, shock may be the fatal sequela of infection. Overall, disturbances in normal blood flow are major sources of human morbidity and mortality; thrombosis, embolism, and infarction underlie three of the most important causes of pathology in Western society—myocardial infarction, pulmonary embolism, and cerebrovascular accident (stroke). Thus, the hemodynamic disorders described in this chapter are important in a wide spectrum of human disease.

Edema

Approximately 60% of lean body weight is water; two thirds of this water is intracellular, and the remainder is found in the extracellular space, mostly as interstitial fluid (only about 5% of total body water is in blood plasma). The term *edema* signifies increased fluid in the interstitial tissue spaces. In addition, depending on the site, fluid collections in the different body cavities are variously designated *hydrothorax, hydropericardium,* and *hydroperitoneum* (the last is more commonly called *ascites*). *Anasarca* is a severe and generalized edema with profound subcutaneous tissue swelling.

Table 4-1 lists the pathophysiologic categories of edema. The mechanisms of inflammatory edema are largely related to

TABLE 4-1 -- Pathophysiologic Categories of Edema

Increased Hydrostatic Pressure
Impaired venous return
••Congestive heart failure
••Constrictive pericarditis
••Ascites (liver cirrhosis)
••Venous obstruction or compression
••••Thrombosis
••••External pressure (e.g., mass)
••••Lower extremity inactivity with prolonged dependency
Arteriolar dilation
••Heat
••Neurohumoral dysregulation
Reduced Plasma Osmotic Pressure (Hypoproteinemia)
Protein-losing glomerulopathies (nephrotic syndrome)
Liver cirrhosis (ascites)
Malnutrition
Protein-losing gastroenteropathy
Lymphatic Obstruction
Inflammatory
Neoplastic
Postsurgical
Postirradiation
Sodium Retention
Excessive salt intake with renal insufficiency
Increased tubular reabsorption of sodium
Renal hypoperfusion
Increased renin-angiotensin-aldosterone secretion
Inflammation
Acute inflammation
Chronic inflammation

Angiogenesis

Modified from Leaf A, Cotran RS: Renal Pathophysiology, 3rd ed., New York, Oxford University Press, 1985, p 146. Used by permission of Oxford Press, Inc.

local increases in vascular permeability and are discussed in Chapter 2 . The *noninflammatory causes of edema* are described in further detail below. Because of increased vascular permeability, inflammatory edema is a protein-rich *exudate*, with a specific gravity usually over 1.020. Conversely, the edema fluid occurring in hydrodynamic derangements is typically a protein-poor *transudate*, with a specific gravity below 1.012.

In general, the opposing effects of vascular hydrostatic pressure and plasma colloid osmotic pressure are the major factors that govern movement of fluid between vascular and interstitial spaces. Normally the exit of fluid into the interstitium from the arteriolar end of the microcirculation is nearly balanced by inflow at the venular end; a small residuum of excess interstitial fluid is drained by the lymphatics. *Either increased capillary pressure or diminished colloid osmotic pressure can result in increased interstitial fluid* (Fig. 4-1). As extravascular fluid accumulates, the increased tissue hydrostatic pressure and plasma colloid osmotic pressure eventually achieve a new equilibrium, and water reenters the venules. Any excess interstitial edema fluid is typically removed by lymphatic drainage, ultimately returning to the bloodstream via the thoracic duct (see Fig. 4-1); clearly, lymphatic obstruction (e.g., due to scarring or tumor) will also impair fluid drainage and result in edema. Finally, a primary retention of sodium (and its obligatory associated water) in renal disease also leads to edema.

Increased Hydrostatic Pressure.

Local increases in hydrostatic pressure may result from impaired venous outflow. For example, *deep venous thrombosis* in the lower extremities leads to edema, which is restricted to the affected leg. *Generalized increases* in venous pressure, with resulting systemic edema, occur most commonly in *congestive heart failure* (Chapter 12) affecting right ventricular cardiac function.

Figure 4-1 Factors affecting fluid balance across capillary walls. Capillary hydrostatic and osmotic forces are normally balanced so that there is no *net* loss or gain of fluid across the capillary bed. However, *increased* hydrostatic pressure or *diminished* plasma osmotic pressure leads to a net accumulation of extravascular fluid *(edema)*. As the interstitial fluid pressure increases, tissue lymphatics remove much of the excess volume, eventually returning it to the circulation via the thoracic duct. If the ability of the lymphatics to drain tissue is exceeded, persistent tissue edema results.

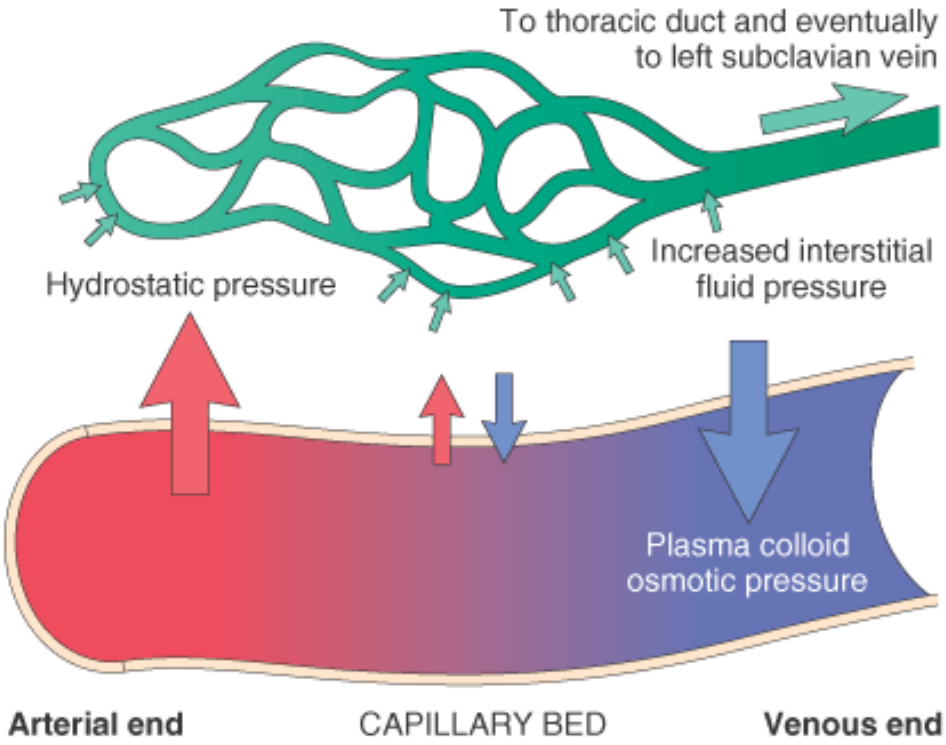


Figure 4-2 Sequence of events leading to systemic edema due to primary heart failure, primary renal failure, or reduced plasma osmotic pressure (as in malnutrition, diminished hepatic protein synthesis, or loss of protein owing to the nephrotic syndrome). ADH, antidiuretic hormone; GFR, glomerular filtration rate.

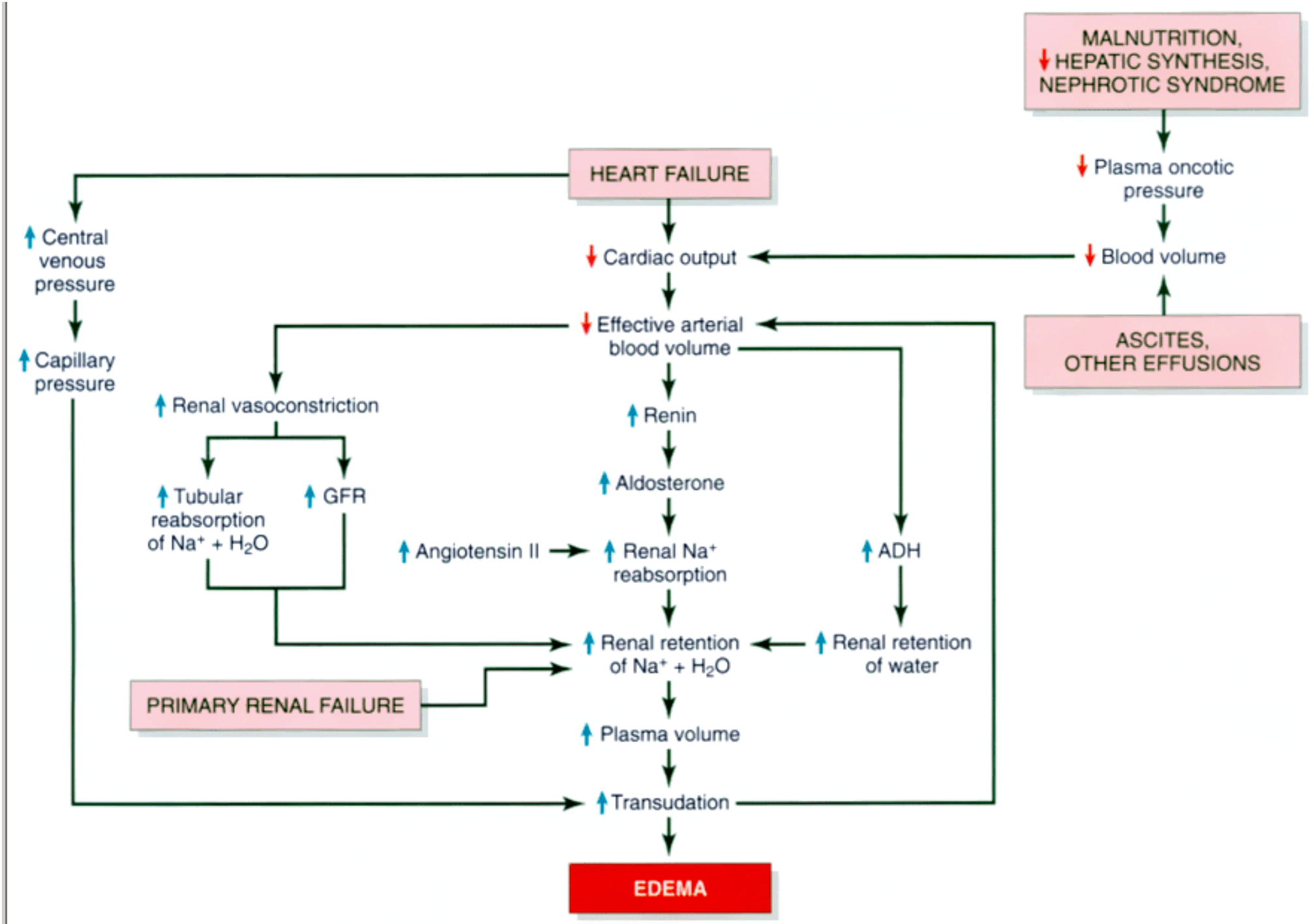


Figure 4-3 Hyperemia versus congestion. In both cases there is an increased volume and pressure of blood in a given tissue with associated capillary dilation and a potential for fluid extravasation. In hyperemia, increased inflow leads to engorgement with oxygenated blood, resulting in *erythema*. In congestion, diminished outflow leads to a capillary bed swollen with

deoxygenated venous blood and resulting in *cyanosis*.

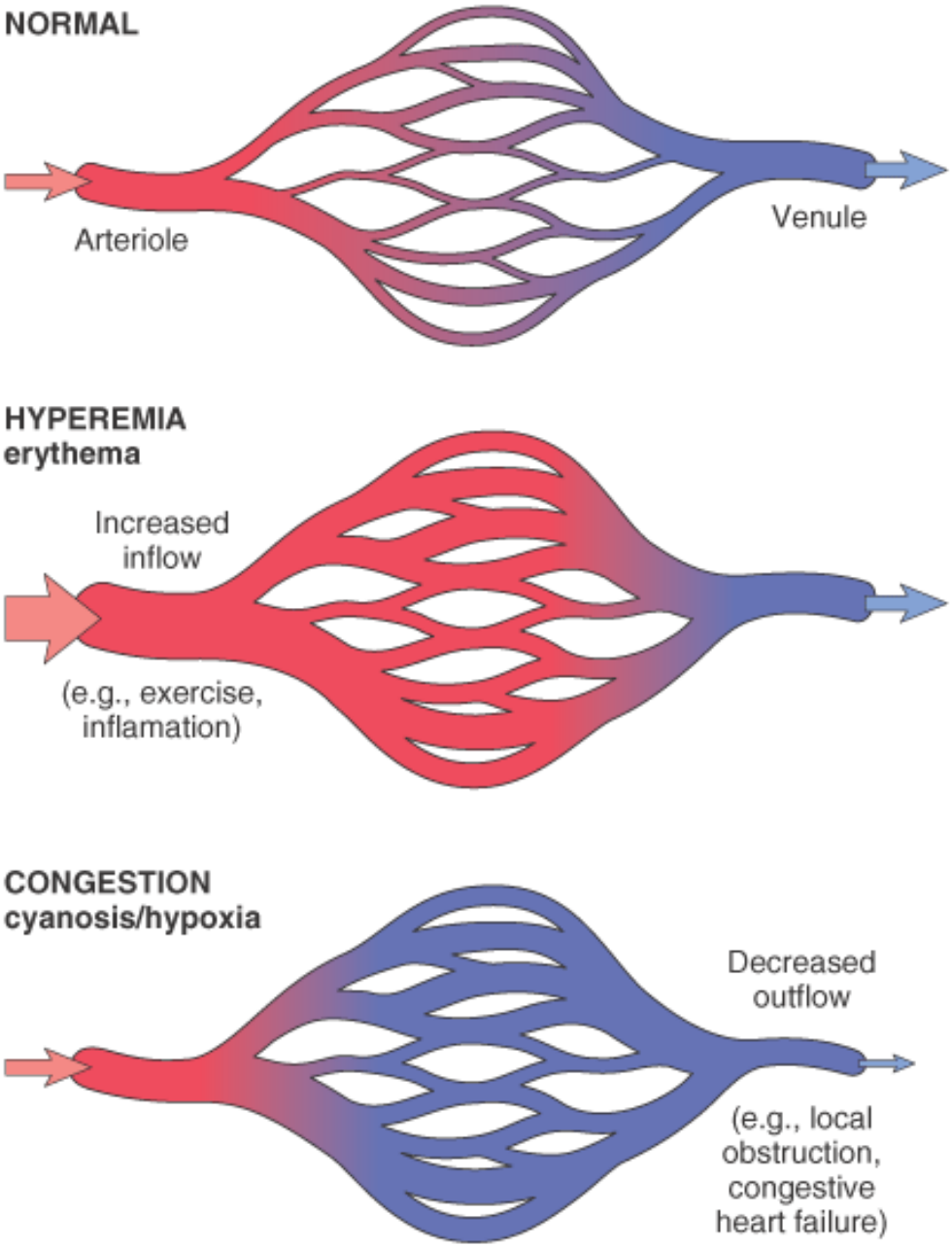


Figure 4-4 Liver with chronic passive congestion and hemorrhagic necrosis. *A*, Central areas are red and slightly depressed compared with the surrounding tan viable parenchyma, forming the so-called "nutmeg liver" pattern. *B*, Centrilobular necrosis with degenerating hepatocytes, hemorrhage, and sparse acute inflammation. *(Courtesy of Dr. James Crawford, Department of Pathology, University of Florida, Gainesville, FL.)*

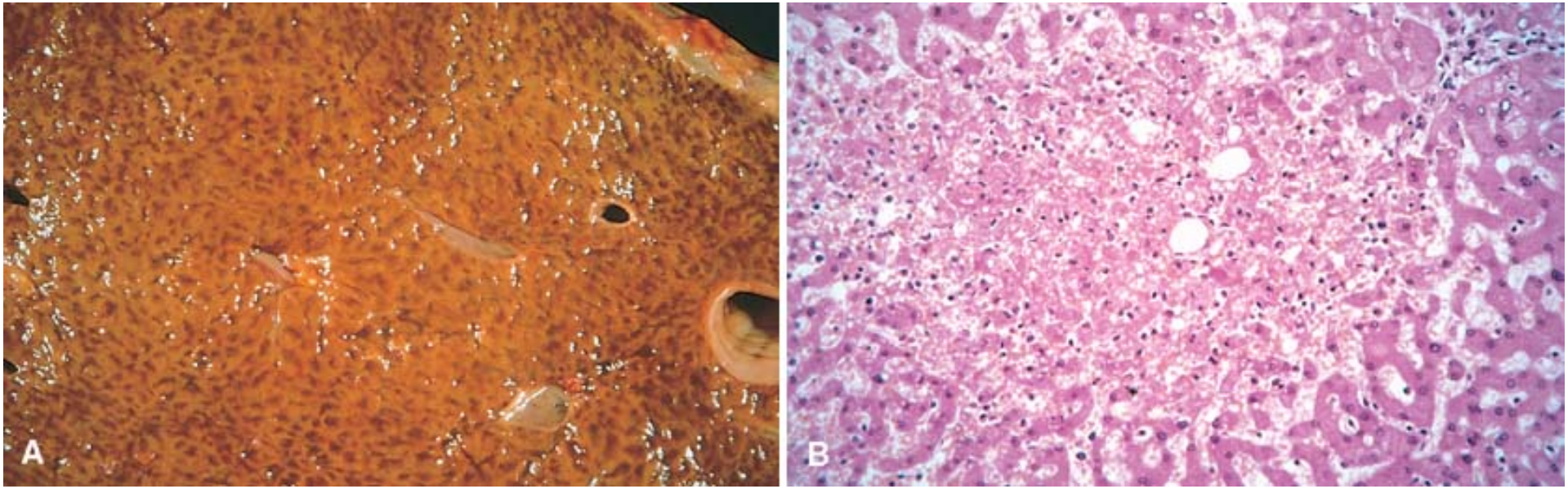


Figure 4-5 *A*, Punctate petechial hemorrhages of the colonic mucosa, seen here as a consequence of thrombocytopenia. *B*, Fatal intracerebral bleed. Even relatively inconsequential volumes of hemorrhage in a critical location, or into a closed space (such as the cranium), can have fatal outcomes.

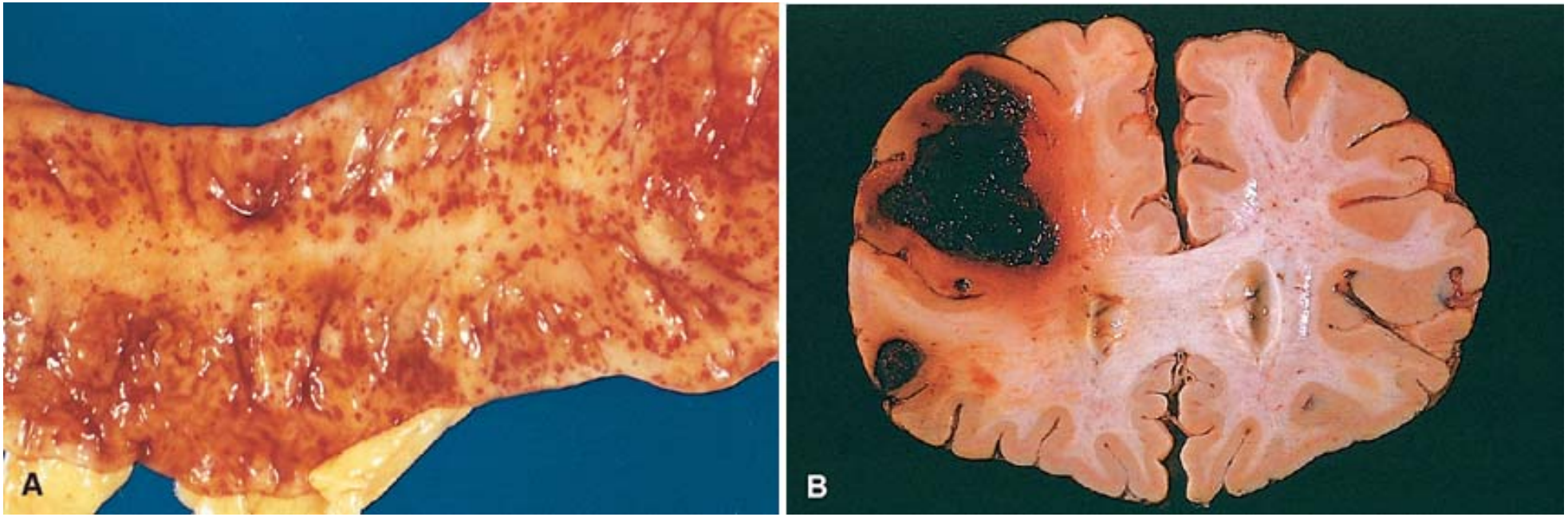
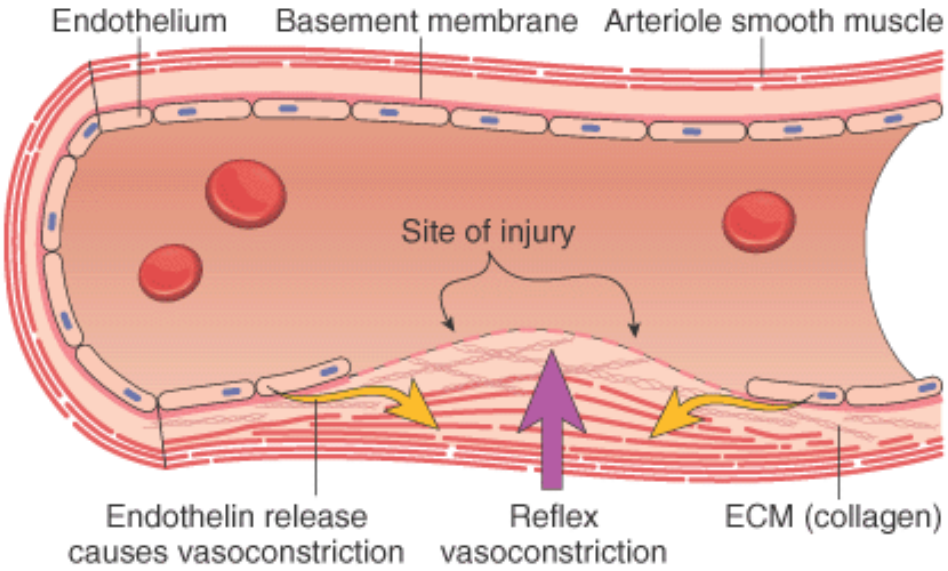


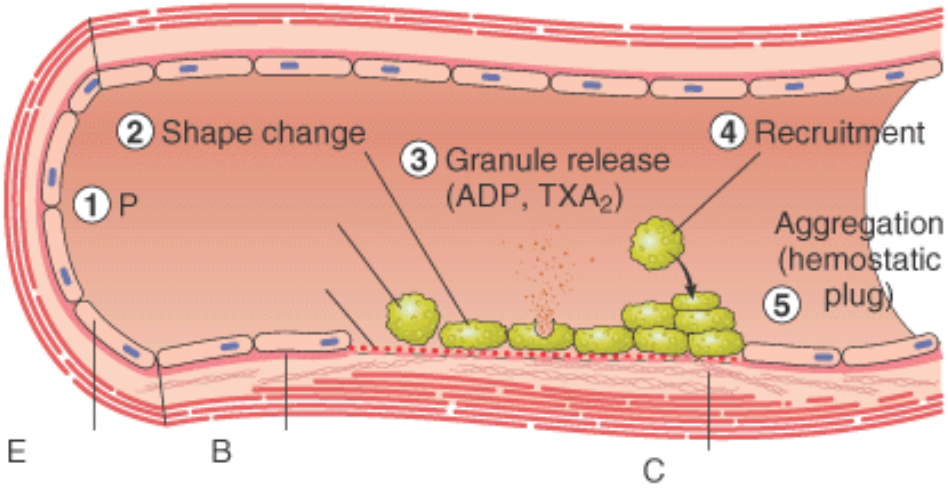
Figure 4-6 Diagrammatic representation of the normal hemostatic process. *A*, After vascular injury, local neurohumoral factors induce a transient vasoconstriction. *B*, Platelets adhere to exposed extracellular matrix (ECM) via von Willebrand factor (vWF) and are activated, undergoing a shape change and granule release; released adenosine diphosphate (ADP) and thromboxane A_2 (TxA_2) lead to further platelet aggregation to form the primary hemostatic plug. *C*, Local activation of the coagulation cascade (involving tissue factor and platelet phospholipids) results in fibrin polymerization, "cementing" the platelets into a definitive secondary hemostatic plug. *D*, Counter-regulatory mechanisms, such as release of tissue type

plasminogen activator (t-PA) (fibrinolytic) and thrombomodulin (interfering with the coagulation cascade), limit the hemostatic process to the site of injury.

A. VASOCONSTRICTION

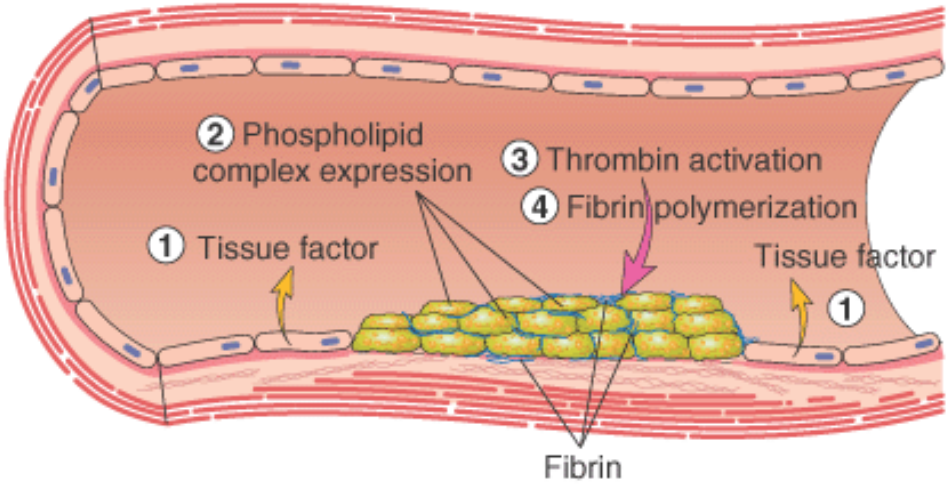


B. PRIMARY HEMOSTASIS



C. SECONDARY HEMOSTASIS

C. SECONDARY HEMOSTASIS



D. THROMBUS AND ANTITHROMBOTIC EVENTS

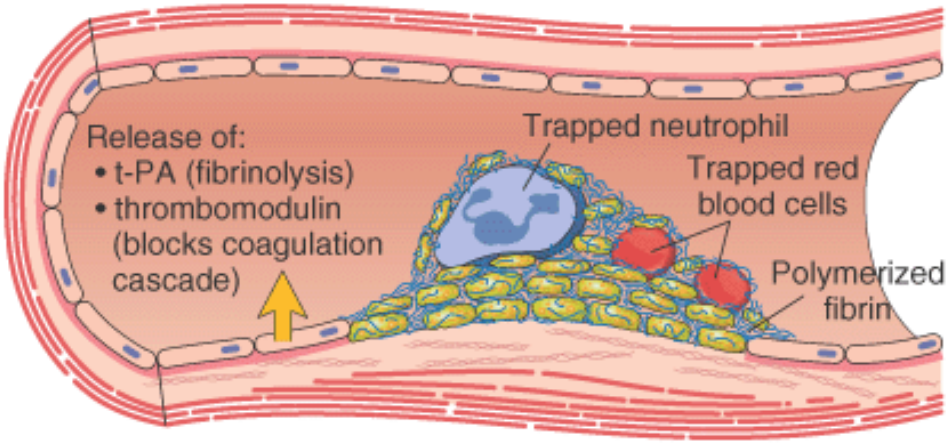


Figure 4-7 Schematic illustration of some of the pro- and anticoagulant activities of endothelial cells. Not shown are the pro- and antifibrinolytic properties. vWF, von Willebrand factor; PGI_2 , prostacyclin; NO, nitric oxide; t-PA, tissue plasminogen activator. Thrombin receptor is referred to as protease activated receptor (PAR; see text).

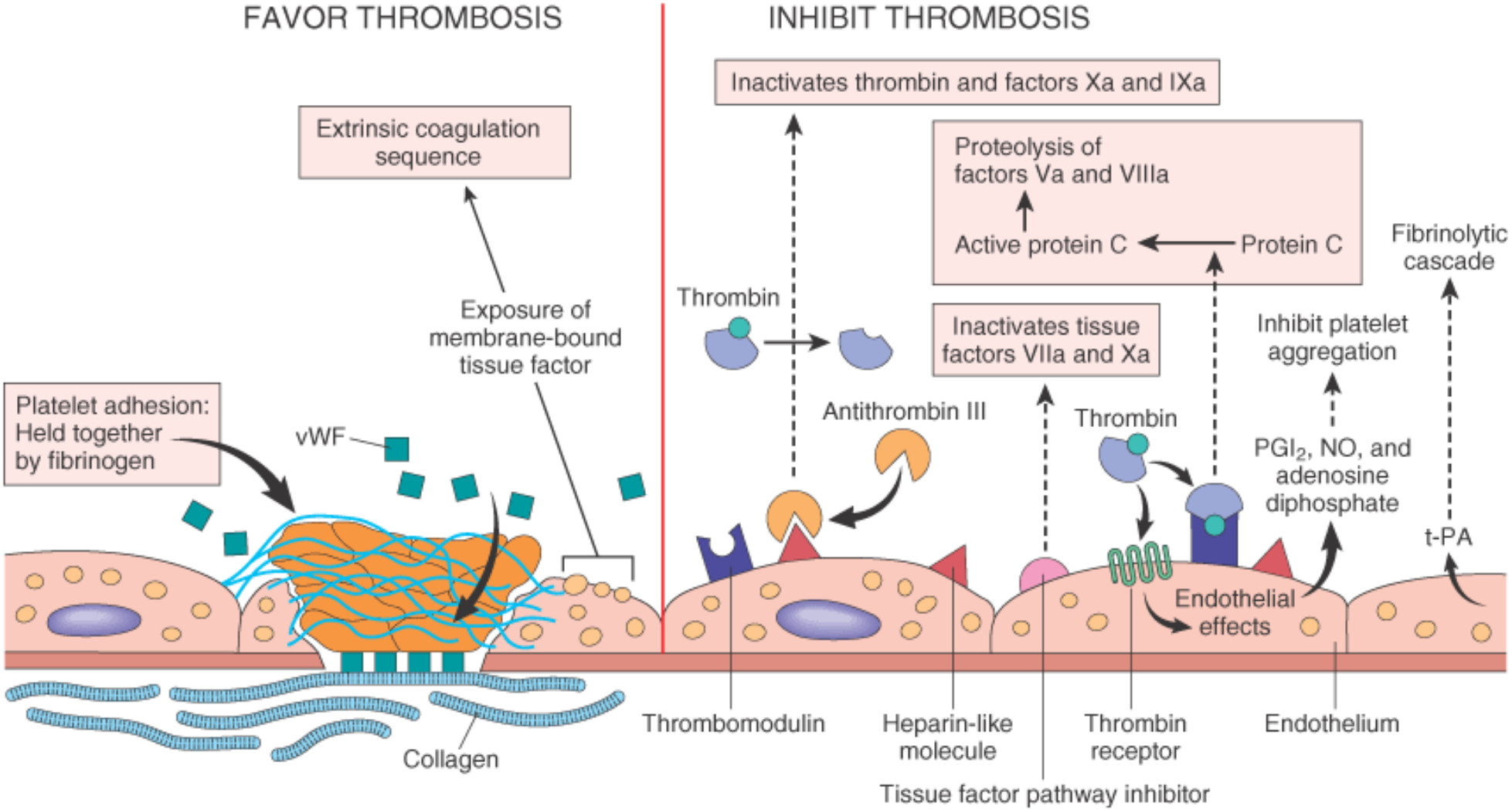


Figure 4-8 Platelet adhesion and aggregation. von Willebrand factor functions as an adhesion bridge between subendothelial collagen and the GpIb platelet receptor complex (the functional complex is composed of GpIb in association with factors V and IX). Aggregation involves linking platelets via fibrinogen bridges bound to the platelet GpIIb-IIIa receptors.

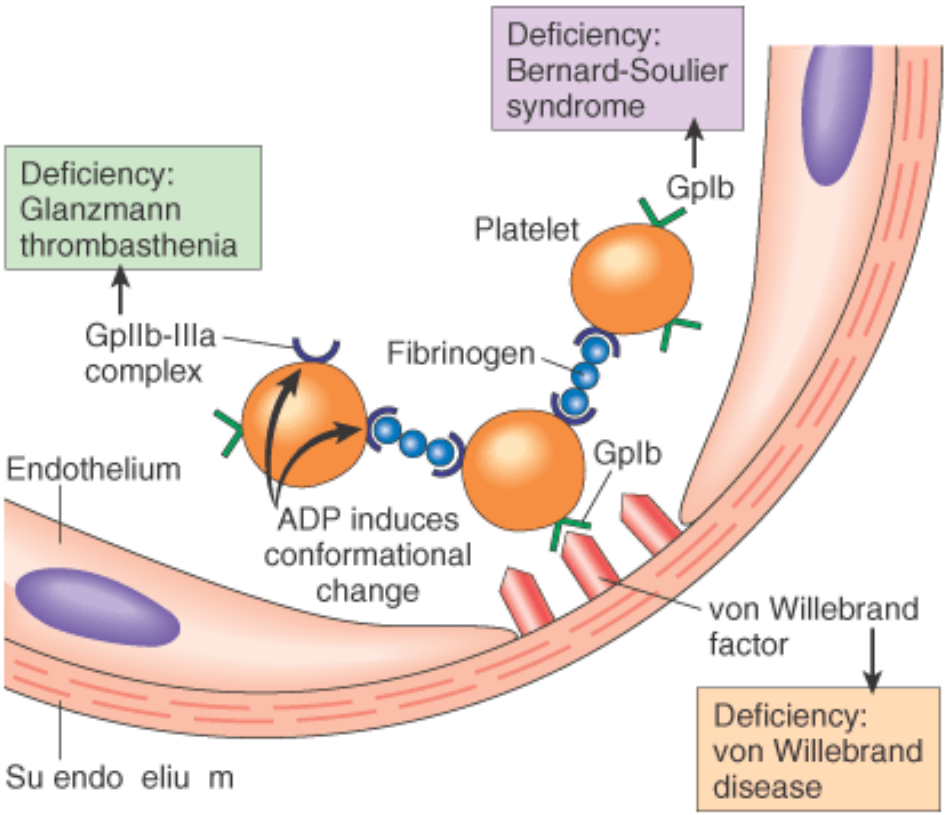
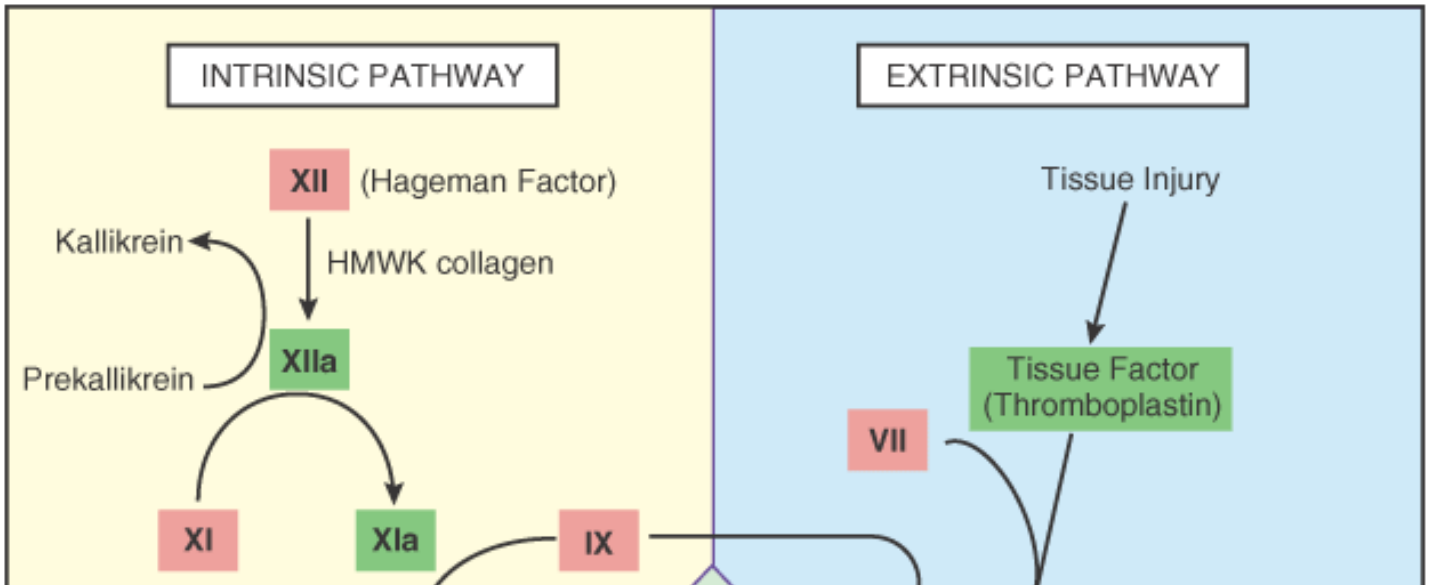


Figure 4-9 The coagulation cascade. Note the common link between the intrinsic and extrinsic pathways at the level of factor IX activation. Factors in red boxes represent inactive molecules; activated factors are indicated with a lower case "a" and a green box. PL, phospholipid surface; HMWK, high-molecular-weight kininogen. Not shown are the anticoagulant inhibitory pathways (see Fig. 4-7 and Fig. 4-12).



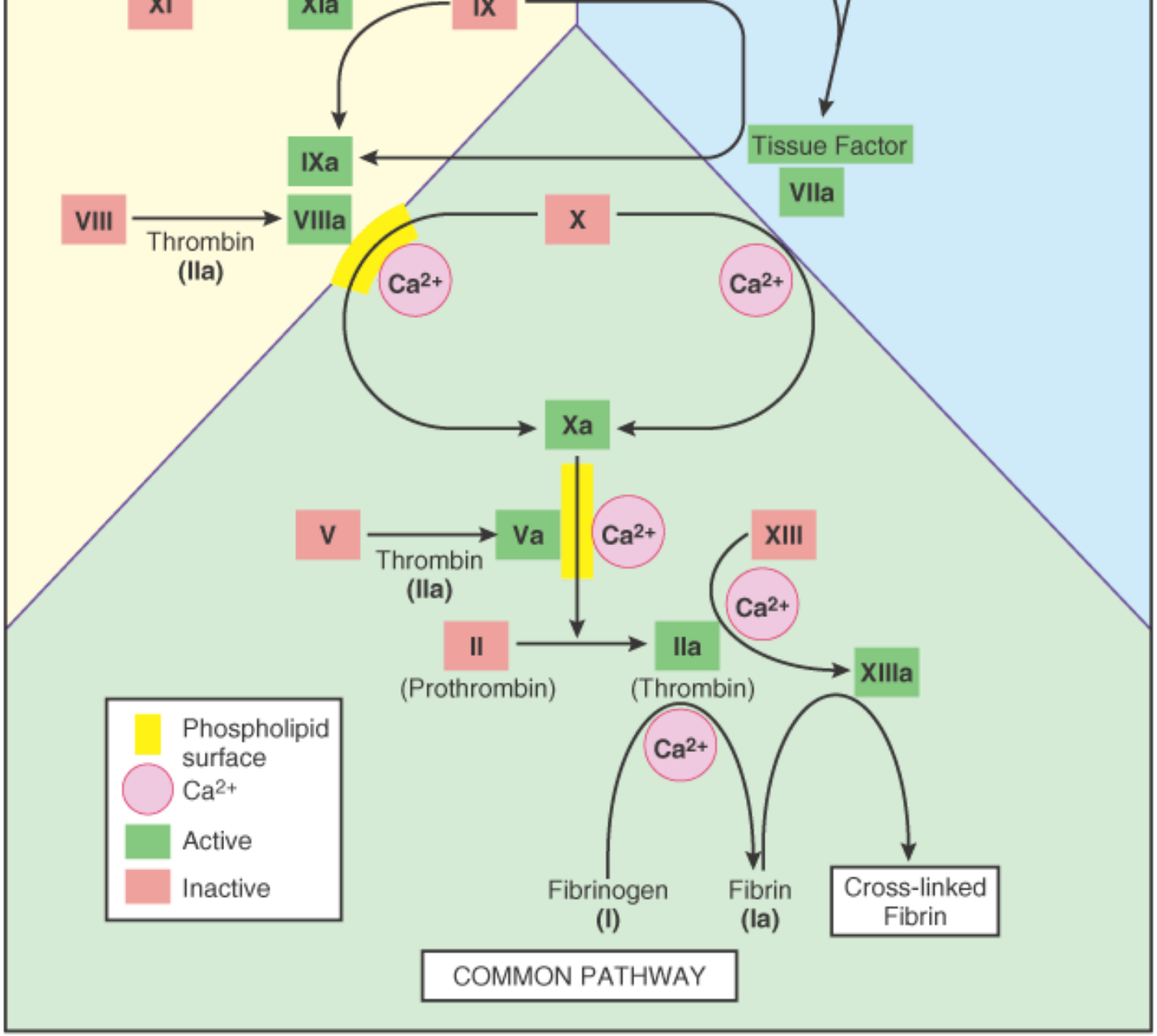


Figure 4-10 Schematic illustration of the conversion of factor X to factor Xa, which in turn converts factor II (prothrombin) to factor IIa (thrombin). The initial reaction complex consists of an enzyme (factor IXa), a substrate (factor X), and a reaction accelerator (factor VIIIa), which are assembled on the phospholipid surface of platelets. Calcium ions hold the assembled components together and are essential for reaction. Activated factor Xa then becomes the enzyme part of the second adjacent complex in the coagulation cascade, converting the prothrombin substrate (II) to thrombin (IIa), with the cooperation of the reaction accelerator factor Va. *(Modified from Mann KG: Clin Lab Med 4:217, 1984.)*

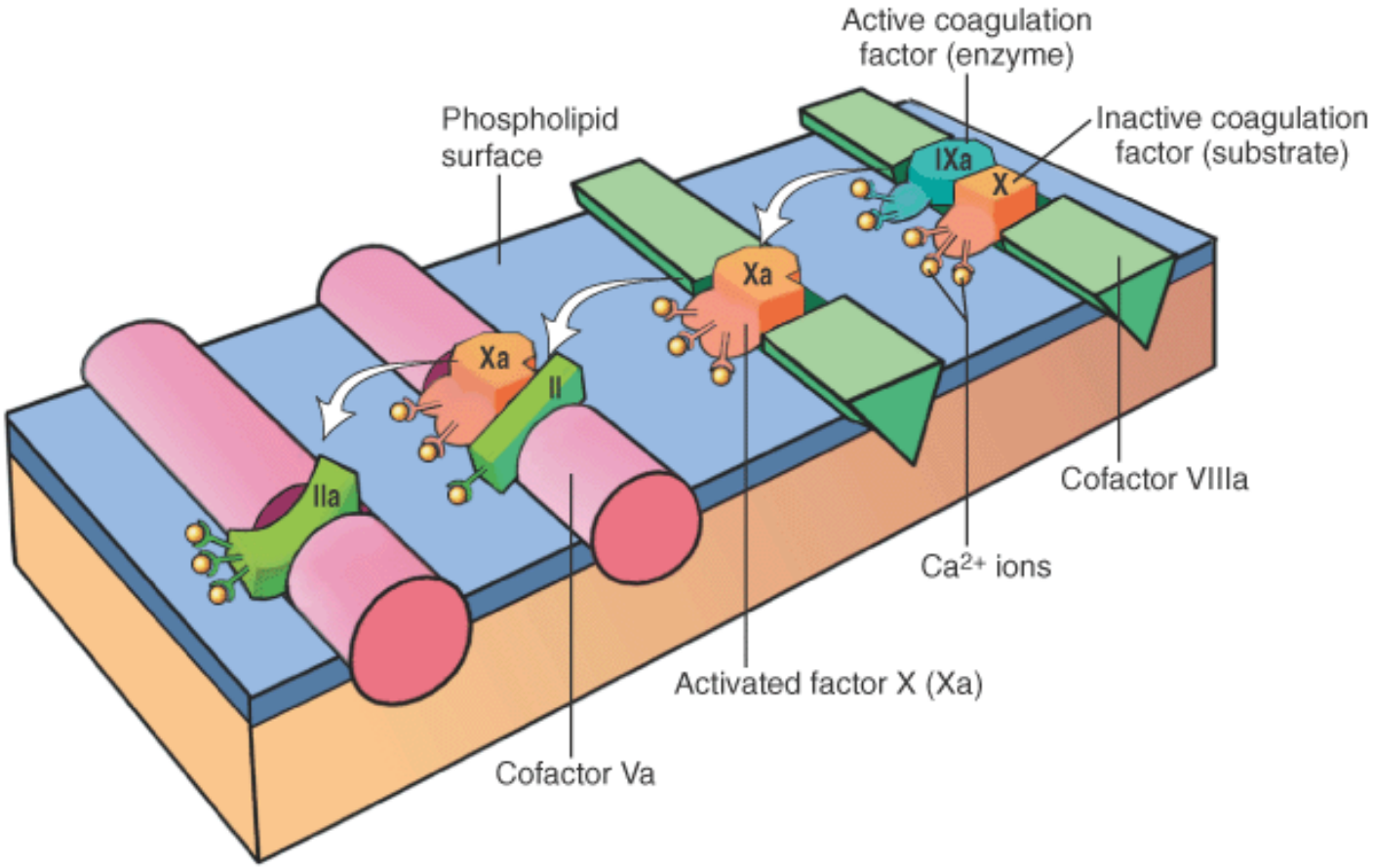


Figure 4-11 The central roles of thrombin in hemostasis and cellular activation. In addition to a critical function in generating cross-linked fibrin (via cleavage of fibrinogen to fibrin and activation of factor XIII), thrombin also directly induces platelet aggregation and secretion (e.g., of TxA_2). Thrombin also activates endothelium to generate leukocyte adhesion molecules and a variety of fibrinolytic (t-PA), vasoactive (NO, PGI_2), or cytokine (PDGF) mediators. Likewise, mononuclear inflammatory cells may be activated by the direct actions of thrombin. ECM, extracellular matrix; NO, nitric oxide; PDGF, platelet-derived growth factor; PGI_2 , prostacyclin; TxA_2 , thromboxane A_2 ; t-PA, tissue type plasminogen activator. See Fig. 4-7 for additional anticoagulant modulators of thrombin activity, such as antithrombin III and thrombomodulin. *(Modified with permission from Shaun Coughlin, MD, PhD, Cardiovascular Research Institute, University of California at San Francisco.)*

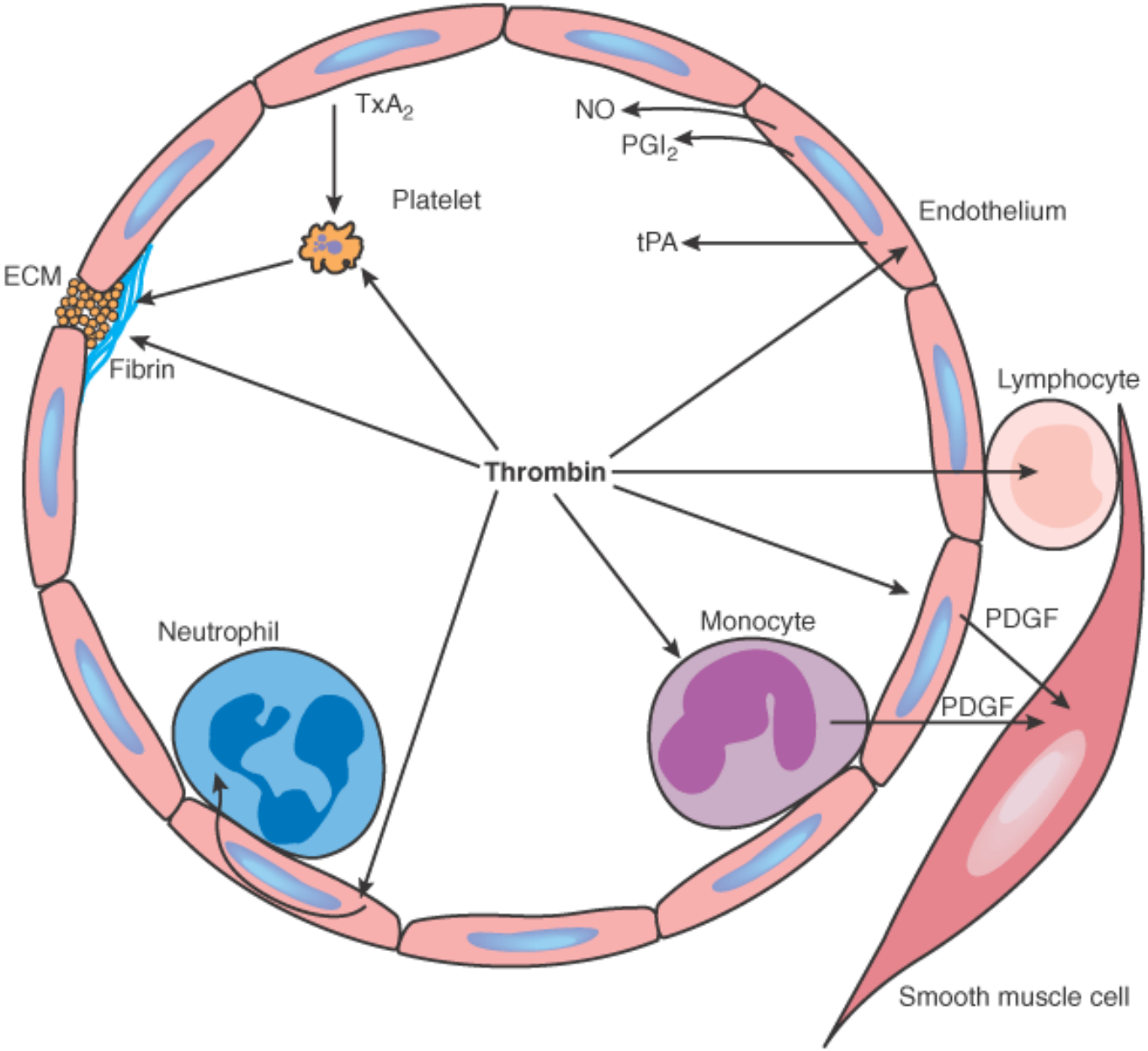


Figure 4-12 The fibrinolytic system, illustrating the plasminogen activators and inhibitors.

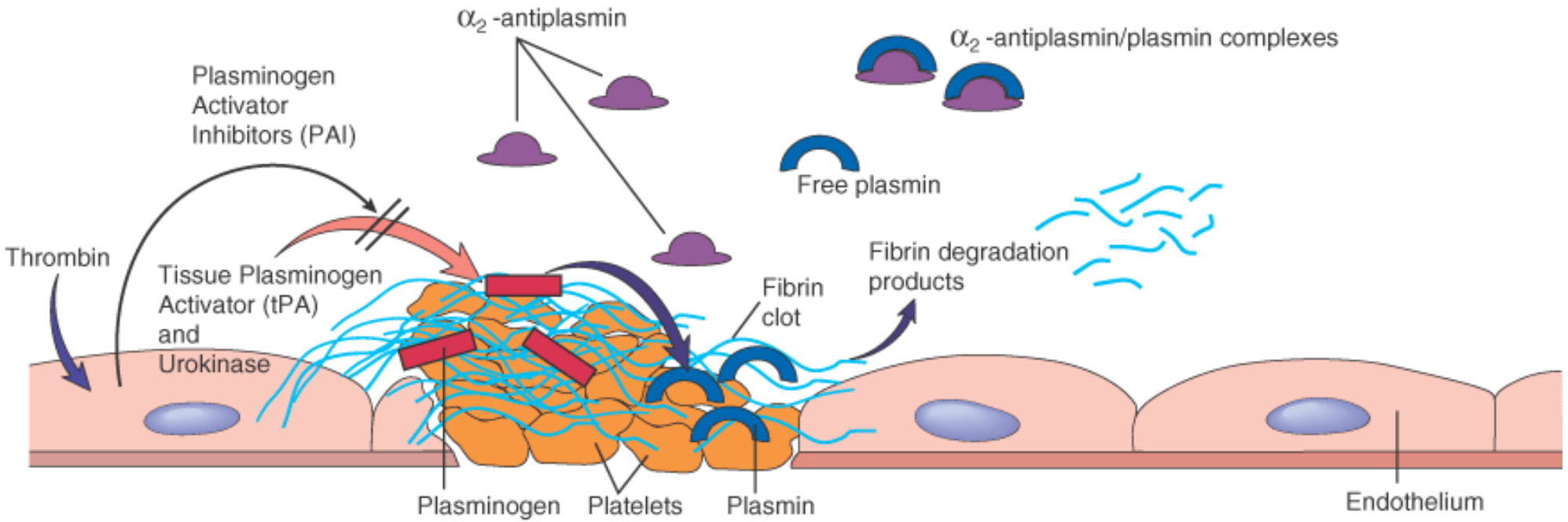


Figure 4-13 Virchow triad in thrombosis. Endothelial integrity is the single most important factor. Note that injury to endothelial cells can affect local blood flow and/or coagulability; abnormal blood flow (stasis or turbulence) can, in turn, cause endothelial injury. The elements of the triad may act independently or may combine to cause thrombus formation.

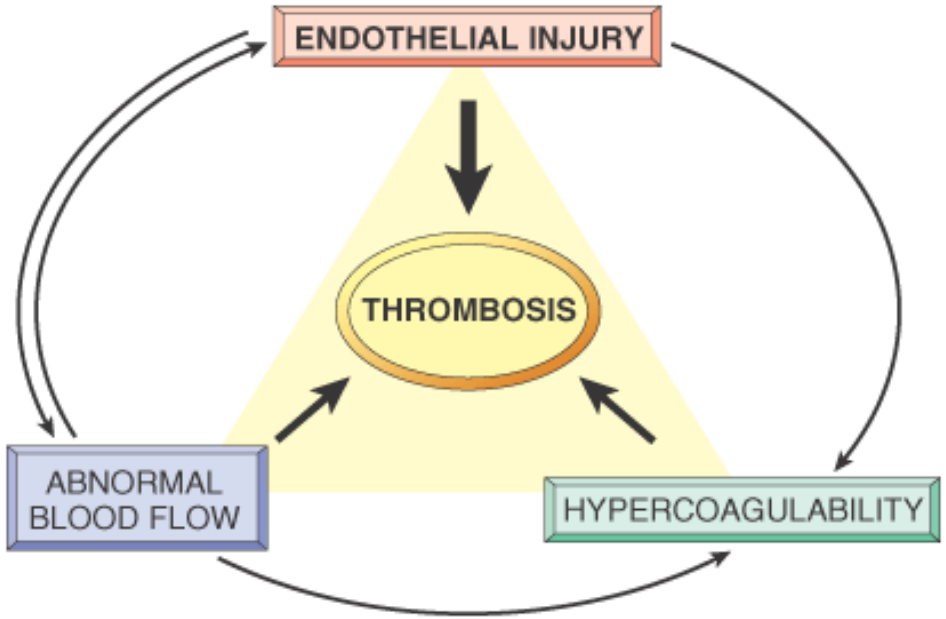


TABLE 4-2 -- Hypercoagulable States

Primary (Genetic)
Common
••Mutation in factor V gene (factor V Leiden)
••Mutation in prothrombin gene
••Mutation in methyltetrahydrofolate gene
Rare
••Antithrombin III deficiency
••Protein C deficiency
••Protein S deficiency
Very rare
••Fibrinolysis defects
Secondary (Acquired)
High risk for thrombosis
••Prolonged bed rest or immobilization
••Myocardial infarction
••Atrial fibrillation
••Tissue damage (surgery, fracture, burns)
••Cancer
••Prosthetic cardiac valves
••Disseminated intravascular coagulation
••Heparin-induced thrombocytopenia
••Antiphospholipid antibody syndrome (lupus anticoagulant syndrome)
Lower risk for thrombosis
••Cardiomyopathy
••Nephrotic syndrome
••Hyperestrogenic states (pregnancy)
••Oral contraceptive use
••Sickle cell anemia
••Smoking

to increased susceptibility to platelet aggregation and reduced PGI_2 release by endothelium. Smoking and obesity promote hypercoagulability by unknown mechanisms.

Among the acquired causes of thrombotic diatheses, the so-called *heparin-induced thrombocytopenia syndrome* and *antiphospholipid antibody syndrome* (previously called the *lupus anticoagulant syndrome*) deserve special mention.

Heparin-induced thrombocytopenia syndrome. [33] [34] Seen in upward of 5% of the population, this syndrome occurs when administration of unfractionated heparin (for purposes of therapeutic anticoagulation) induces formation of antibodies that bind to molecular complexes of heparin and platelet factor 4 membrane protein. This antibody can also bind to similar complexes present on platelet and endothelial surfaces; the result is platelet activation, endothelial injury, and a prothrombotic state. To reduce this problem, specially manufactured low-molecular-weight heparin preparations—which retain anticoagulant activity but do not interact with platelets—are used. These have the additional benefit of a prolonged serum half-life.

Antiphospholipid antibody syndrome. [35] [36] This syndrome has protean clinical presentations, including multiple thromboses; the clinical manifestations are associated with high titers of circulating antibodies directed against anionic phospholipids (e.g., cardiolipin) or, more accurately, against plasma protein epitopes that are unveiled by binding to such phospholipids (e.g., prothrombin). Patients with anticardiolipin antibodies also have a false-positive serologic test for syphilis because the antigen in the standard tests is embedded in cardiolipin. In vitro these antibodies interfere with the assembly of phospholipid complexes and thus inhibit coagulation. However, in vivo, the antibodies induce a *hypercoagulable* state.

Patients with antiphospholipid antibody syndrome fall into two categories. Many have a well-defined autoimmune disease, such as systemic lupus erythematosus (Chapter 6) and have *secondary antiphospholipid syndrome* (such patients previously carried the designation of *lupus anticoagulant syndrome*). The remainder show no evidence of other autoimmune disorder and exhibit only the manifestations of a hypercoagulable state *(primary antiphospholipid syndrome)*. Occasionally the syndrome can occur in association with certain drugs or infections. How antiphospholipid antibodies lead to hypercoagulability is not clear, but possible explanations include direct platelet activation, inhibition of PGI_2 production by endothelial cells, or interference with protein C synthesis or activity. Although antiphospholipid antibodies are associated with thrombotic diatheses, they have also been identified in 5% to 15% of apparently normal individuals and may therefore be necessary but not sufficient to cause full-blown antiphospholipid antibody syndrome.

Individuals with the antiphospholipid antibody syndrome present with an extreme variety of clinical manifestations; these are typically characterized by *recurrent venous* or *arterial thrombi* but also include *repeated miscarriages, cardiac valvular vegetations*, or *thrombocytopenia*. [37] Venous thromboses occur most commonly in deep leg veins, but renal, hepatic, and retinal veins are also susceptible. Arterial thromboses typically occur in the cerebral circulation, but coronary, mesenteric, and renal arterial occlusions have also been described. Depending on the vascular bed involved, the clinical presentations can vary from pulmonary embolism (due to a lower extremity venous thrombus), to pulmonary hypertension (from recurrent subclinical pulmonary emboli), to stroke, bowel infarction, or renovascular hypertension. Fetal loss is attributable to antibody-mediated inhibition of t-PA activity necessary for trophoblastic invasion of the uterus. Antiphospholipid antibody syndrome is also a cause of renal microangiopathy, resulting in renal failure owing to multiple capillary and arterial thromboses (Chapter 20). Patients with antiphospholipid antibody syndrome are at increased risk of a fatal event (upward of 7% in one series of patients with lupus erythematosus, particularly with arterial thromboses or thrombocytopenia). Current treatment includes anticoagulation therapy (aspirin, heparin, and warfarin) and immunosuppression in refractory cases. [35] [37] [38]

Morphology.

Thrombi may develop anywhere in the cardiovascular system: within the cardiac chambers; on valve cusps; or in arteries, veins, or capillaries. They are of variable size and shape, depending on the site of origin and the circumstances leading to their development. Arterial or cardiac thrombi usually begin at a site of endothelial injury (e.g., atherosclerotic plaque) or turbulence (vessel bifurcation); venous thrombi characteristically occur in sites of stasis. An area of attachment to the underlying vessel or heart wall, frequently firmest at the point of origin, is characteristic of all thromboses. Arterial thrombi tend to grow in a retrograde direction from the point of attachment, whereas venous thrombi extend in the direction of blood flow (i.e., toward the heart). The propagating tail may not be well attached and, particularly in veins, is prone to fragmentation, creating an **embolus**.

When formed in the heart or aorta, thrombi may have grossly (and microscopically) apparent laminations, called **lines of Zahn**; these are produced by alternating pale layers of platelets admixed with some fibrin and darker layers containing more red cells. Lines of Zahn are significant only in that they imply thrombosis at a site of blood flow; in veins or in smaller arteries, the laminations are typically not as apparent, and, in fact, thrombi formed in the sluggish flow of venous blood usually resemble statically coagulated blood (similar to blood clotted in a test tube). Nevertheless, careful evaluation generally reveals irregular, somewhat ill-defined laminations.

When arterial thrombi arise in heart chambers or in the aortic lumen, they usually adhere to the wall of the underlying structure and are termed **mural thrombi**. Abnormal myocardial contraction (arrhythmias, dilated cardiomyopathy, or myocardial infarction) leads to cardiac mural thrombi (Fig. 4-14*A*), while ulcerated atherosclerotic plaque and aneurysmal dilation are the precursors of aortic thrombus formation (Fig. 4-14*B*).

Arterial thrombi are usually **occlusive**; the most common sites, in descending order, are coronary, cerebral, and femoral arteries. The thrombus is usually superimposed on an atherosclerotic plaque, although other forms of vascular injury (vasculitis, trauma) may be involved. The thrombi are typically firmly adherent to the injured arterial wall and are gray-white and friable, composed of a tangled mesh of platelets, fibrin, erythrocytes, and degenerating leukocytes.

Venous thrombosis, or **phlebothrombosis**, is almost invariably occlusive; the thrombus often creates a long cast of the vein lumen. Because these thrombi form in a relatively static environment, they tend to contain more enmeshed erythrocytes and are therefore known as **red**, or **stasis**, **thrombi**. Phlebothrombosis most commonly affects the veins of the lower extremities (90% of cases). Less commonly, venous thrombi may develop in the upper extremities, periprostatic plexus, or the ovarian and periuterine veins; under special circumstances, they may be found in the dural sinuses, the portal vein, or the hepatic vein. At autopsy, postmortem clots may be

Figure 4-14 Mural thrombi. *A*, Thrombus in the left and right ventricular apices, overlying a white fibrous scar. *B*, Laminated thrombus in a dilated abdominal aortic aneurysm.

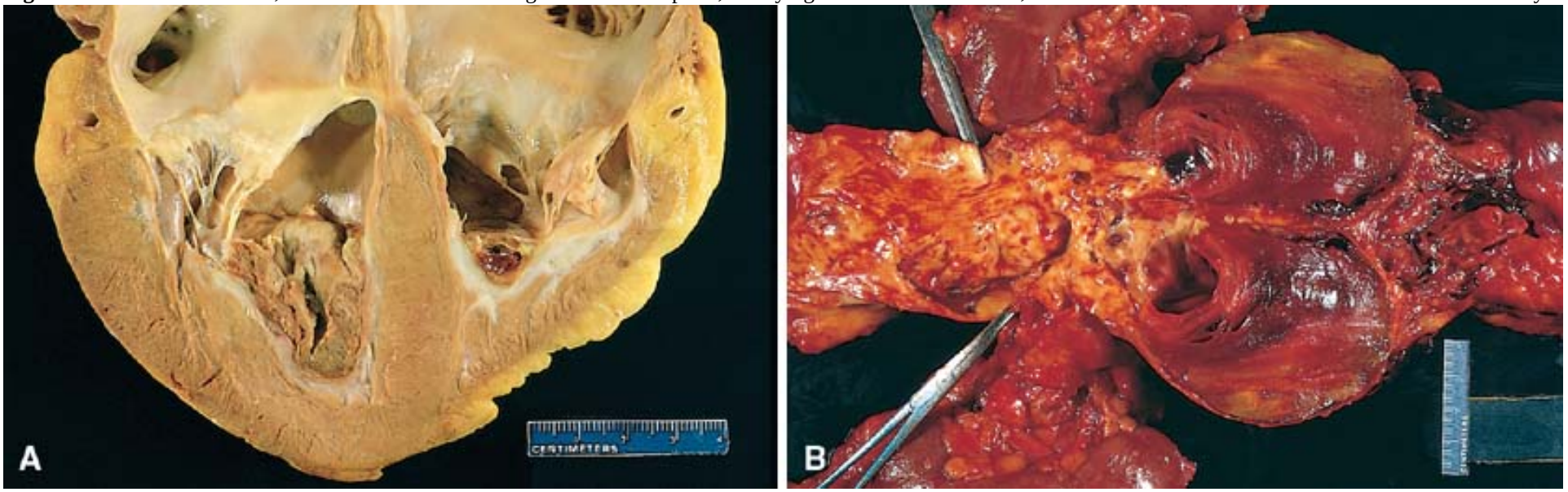


Figure 4-15 Potential outcomes of venous thrombosis.

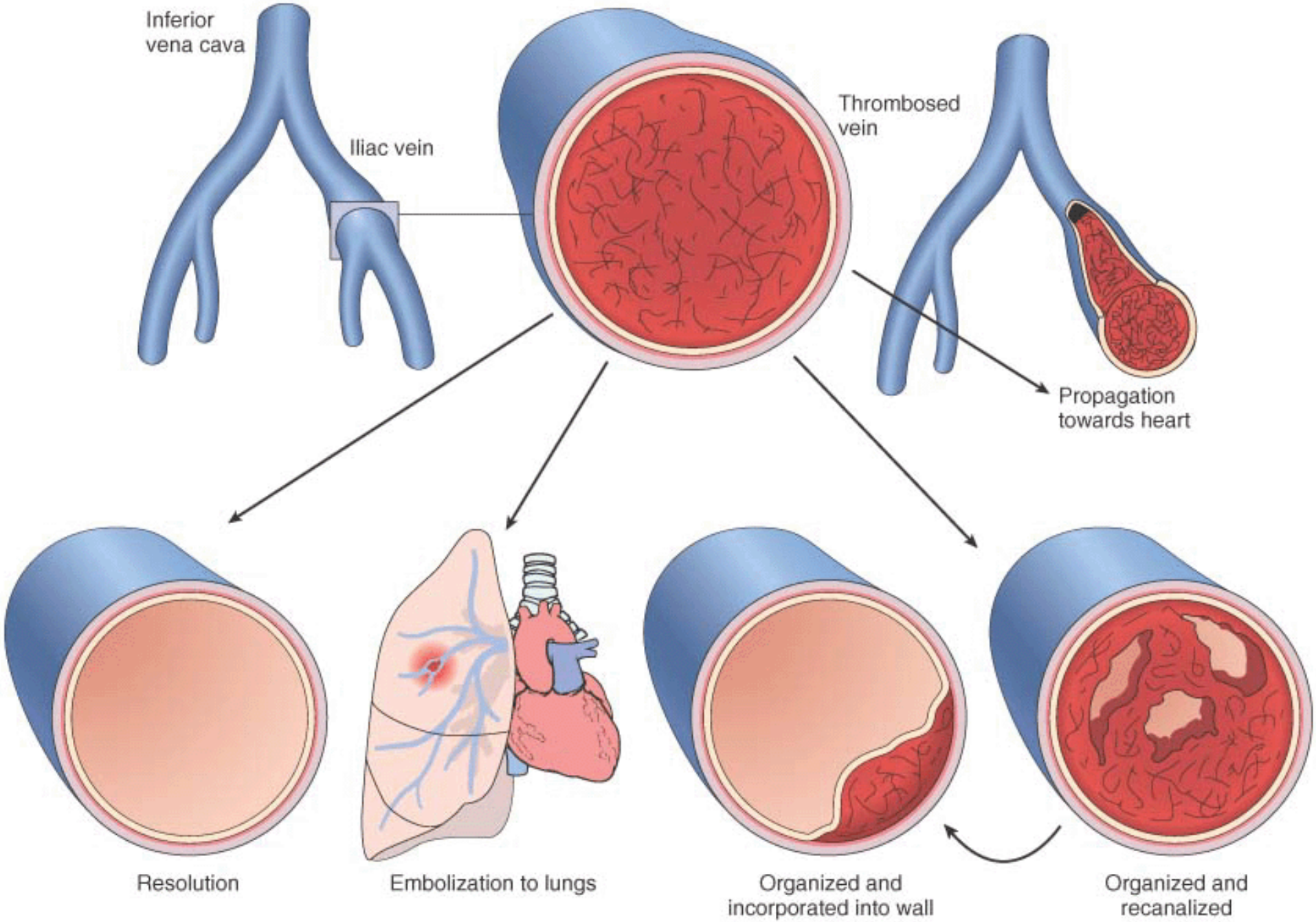


Figure 4-16 Low-power view of a thrombosed artery. *A*, H&E-stained section. *B*, Stain for elastic tissue. The original lumen is delineated by the internal elastic lamina *(arrows)* and is totally filled with organized thrombus, now punctuated by a number of small recanalized channels.

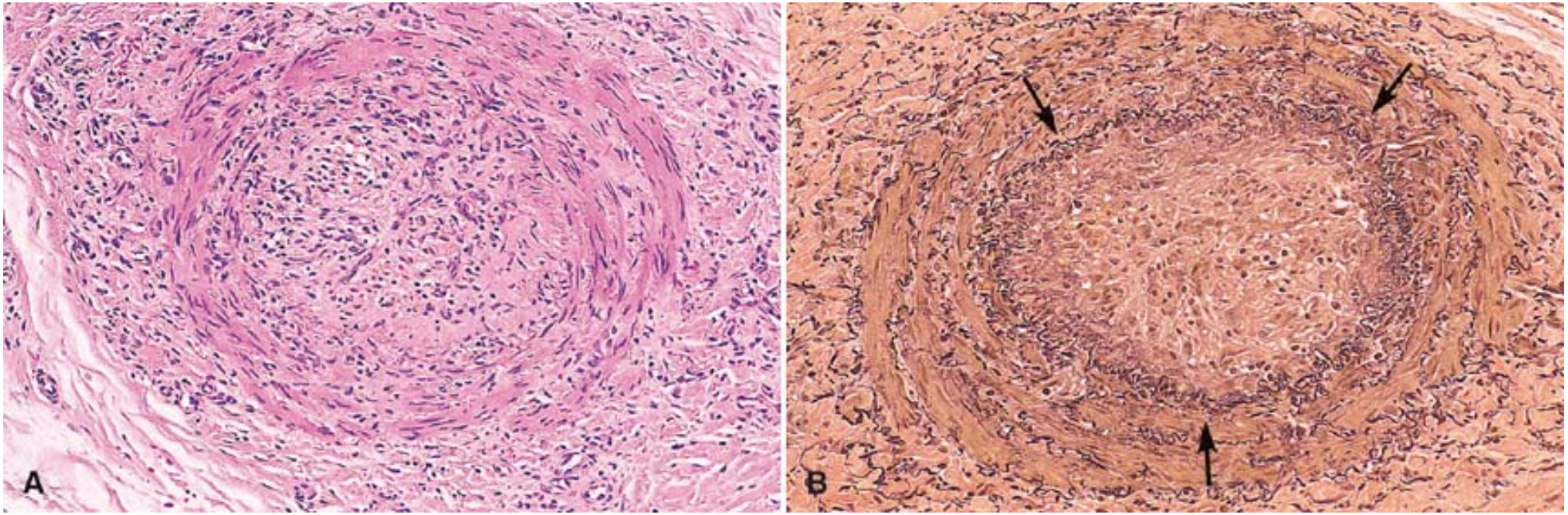


Figure 4-17 Large embolus derived from a lower extremity deep venous thrombosis and now impacted in a pulmonary artery branch.

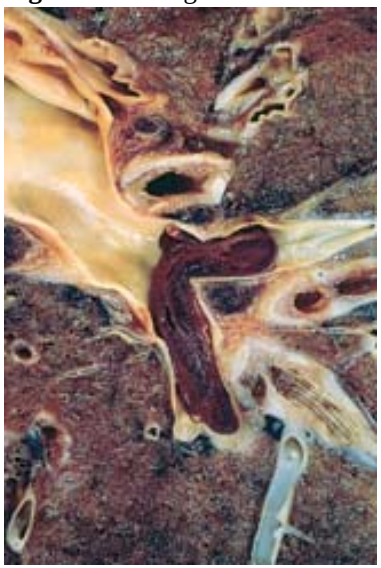


Figure 4-18 Bone marrow embolus in the pulmonary circulation. The cleared vacuoles represent marrow fat that is now impacted in a distal vessel along with the cellular hematopoietic precursors.

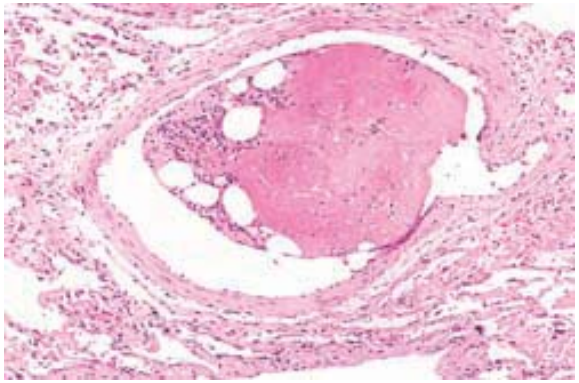


Figure 4-19 Examples of infarcts. *A*, Hemorrhagic, roughly wedge-shaped pulmonary infarct. *B*, Sharply demarcated white infarct in the spleen.

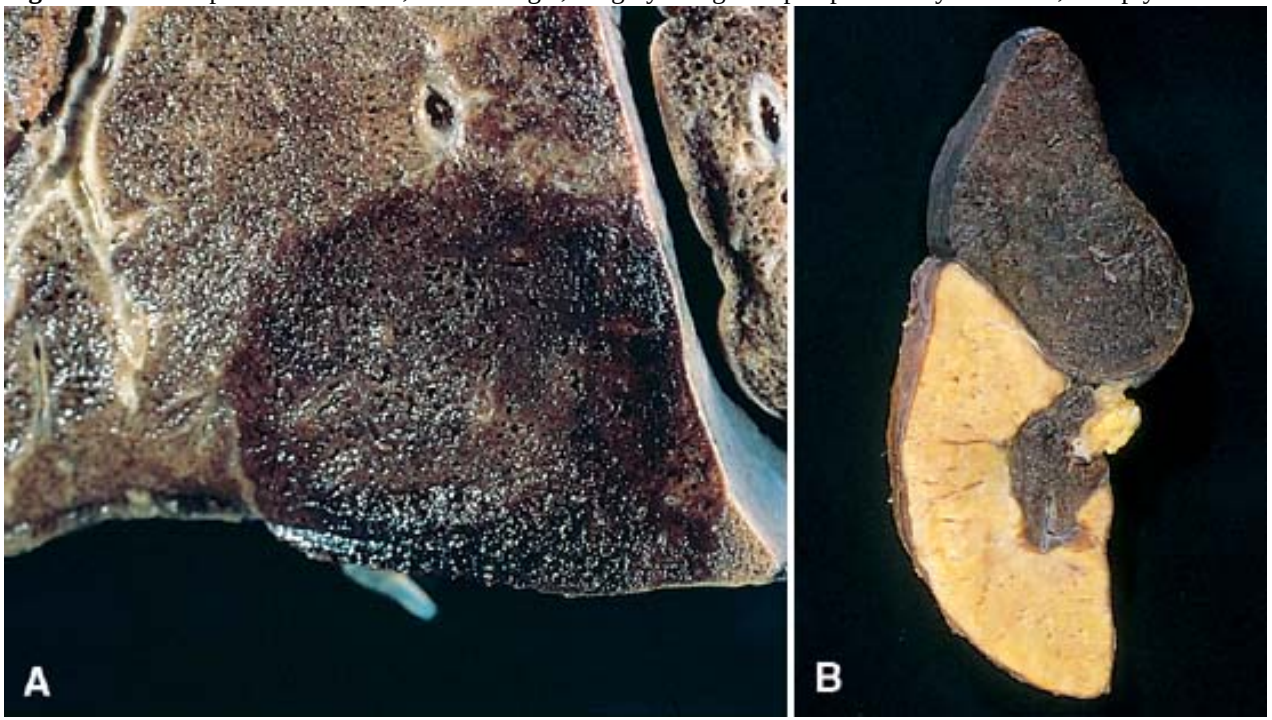


Figure 4-20 Remote kidney infarct, now replaced by a large fibrotic cortical scar.

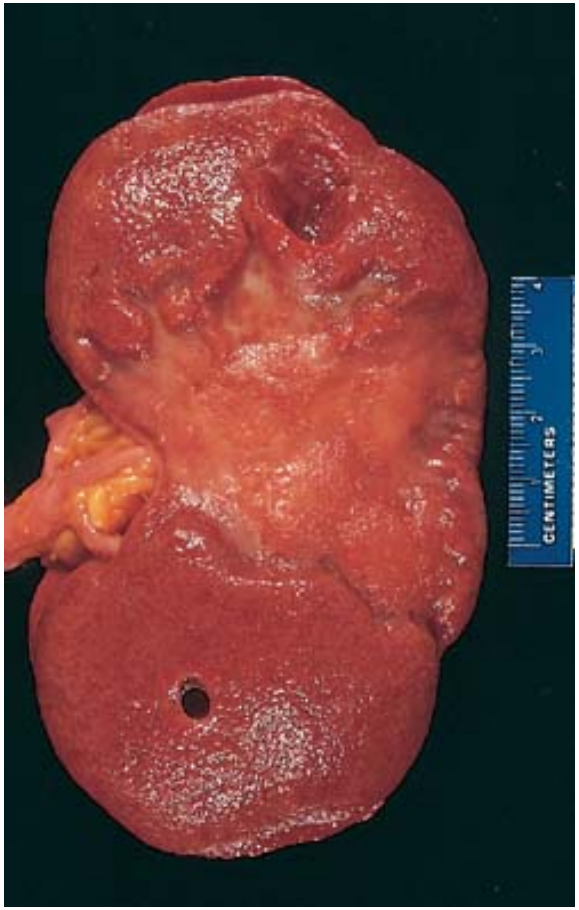


TABLE 4-3 -- Three Major Types of Shock

Type of Shock	Clinical Examples	Principal Mechanisms
Cardiogenic		
	Myocardial infarction	Failure of myocardial pump owing to intrinsic myocardial damage, extrinsic pressure, or obstruction to outflow
	Ventricular rupture	
	Arrhythmia	
	Cardiac tamponade	
	Pulmonary embolism	
Hypovolemic		
	Hemorrhage	Inadequate blood or plasma volume

	Fluid loss, e.g., vomiting, diarrhea, burns, or trauma	
Septic		
	Overwhelming microbial infections	Peripheral vasodilation and pooling of blood; endothelial activation/injury; leukocyte-induced damage; disseminated intravascular coagulation; activation of cytokine cascades
	Endotoxic shock	
	Gram-positive septicemia	
	Fungal sepsis	
	Superantigens	

estimated to account for over 200,000 deaths annually in the United States.[53] Moreover, the reported incidence of sepsis syndromes has increased dramatically in the past two decades, owing to improved life support for high-risk patients, increasing use of invasive procedures, and growing numbers of immunocompromised hosts (secondary to chemotherapy, immunosuppression, or human immunodeficiency virus infection). Septic shock results from spread and expansion of an initially localized infection (e.g., abscess, peritonitis, pneumonia) into the bloodstream.

Most cases of septic shock (approximately 70%) are caused by endotoxin-producing gram-negative bacilli (Chapter 8), hence the term *endotoxic shock*. Endotoxins are bacterial wall lipopolysaccharides (LPSs) that are released when the cell walls are degraded (e.g., in an inflammatory response). LPS consists of a toxic fatty acid *(lipid A)* core and a complex polysaccharide coat (including O antigens) unique to each bacterial species. Analogous molecules in the walls of gram-positive bacteria and fungi can also elicit septic shock.

All of the cellular and resultant hemodynamic effects of septic shock may be reproduced by injection of LPS alone. Free LPS attaches to a circulating LPS-binding protein, and the complex then binds to a cell-surface receptor (called CD14), followed by binding of the LPS to a signal-transducing protein called *mammalian Toll-like receptor protein 4* (TLR-4). (Toll is a *Drosophila* protein involved in fly development; a variety of molecules with homology to Toll [i.e., "Toll-like"] participate in innate immune responses to different microbial components; see Box 6-1 , Chapter 6.) Signals from TLR-4 can then directly activate vascular wall cells and leukocytes or initiate a cascade of cytokine mediators, which propagates the pathologic state. [54] [55] Engagement of TLR-4 on endothelial cells can lead directly to down-regulation of natural anticoagulation mechanisms, including diminished synthesis of tissue factor pathway inhibitor (TFPI) and thrombomodulin. Engagement of the receptor on monocytes and macrophages (even at doses of LPS as minute as 10 picograms/ml) causes profound mononuclear cell activation with the subsequent production of potent effector cytokines such as IL-1 and TNF (Chapter 6). Presumably, this series of responses helps to isolate organisms and to trigger elements of the innate immune system to efficiently eradicate invading microbes. Unfortunately, depending on the dosage and numbers of macrophages that are activated, the secondary effects of LPS release can also cause severe pathologic changes, including fatal shock.

- At low doses, LPS predominantly serves to activate monocytes and macrophages, with effects intended to enhance their ability to eliminate invading bacteria. LPS can also directly activate complement, which likewise contributes to local bacterial eradication. The mononuclear phagocytes respond to LPS by producing cytokines, mainly TNF, IL-1, IL-6, and chemokines. TNF and IL-1 both act on endothelial cells to stimulate the expression of adhesion molecules (Chapter 2 ; Fig. 4-21) and the production of other cytokines and chemokines. Thus, the initial release of LPS results in a circumscribed cytokine cascade doubtless intended to enhance the *local* acute inflammatory response and improve clearance of the infection.
- With moderately severe infections, and therefore with higher levels of LPS (and a consequent augmentation of the cytokine cascade), cytokine-induced secondary effectors (e.g., nitric oxide; Chapter 2) become significant. In addition, systemic effects of the cytokines such as TNF and IL-1 may begin to be seen; these include fever and increased synthesis of acute phase reactants (Chapter 2 ; Fig. 4-21). LPS at higher doses also results in diminished endothelial cell production of thrombomodulin and TFPI, tipping the coagulation cascade toward thrombosis.
- Finally, at still higher levels of LPS, the syndrome of septic shock supervenes (Fig. 4-22); the same cytokines and secondary mediators, now at high levels, result in:
- Systemic vasodilation (hypotension)
- Diminished myocardial contractility

- Widespread endothelial injury and activation, causing systemic leukocyte adhesion and pulmonary alveolar capillary damage (*acute respiratory distress syndrome*; Chapter 15)
- Activation of the coagulation system, culminating in DIC

The hypoperfusion resulting from the combined effects of widespread vasodilation, myocardial pump failure, and DIC induces *multiorgan system failure* affecting the liver, kidneys,

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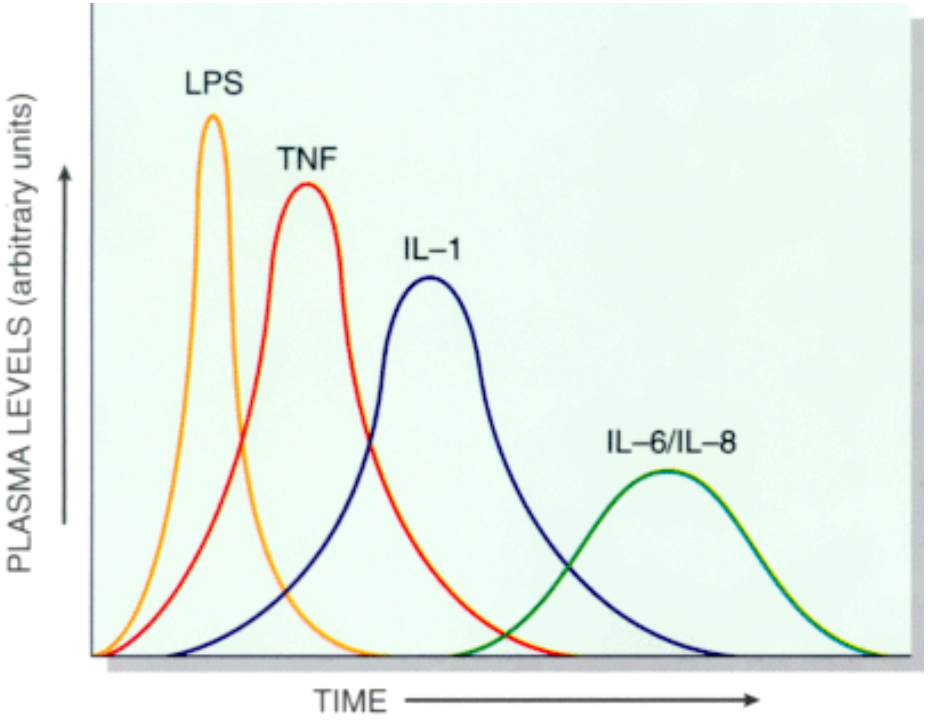
Figure 4-21 Cytokine cascade in sepsis. After release of lipopolysaccharide (LPS) from invading gram-negative microorganisms, there are successive waves of tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6 secretion. *(Modified from Abbas AK, et al: Cellular and Molecular Immunology, 4th ed. Philadelphia, WB Saunders, 2000.)*



A *progressive stage* characterized by tissue hypoperfusion and onset of worsening circulatory and metabolic imbalances, including acidosis

An *irreversible stage* that sets in after the body has incurred cellular and tissue injury so severe that even if the hemodynamic defects are corrected, survival is not possible.

In the early nonprogressive phase of shock, a variety of *neurohumoral mechanisms* help maintain cardiac output and blood pressure. These include baroreceptor reflexes, release of catecholamines, activation of the renin-angiotensin axis, antidiuretic hormone release, and generalized sympathetic stimulation. The net effect is *tachycardia, peripheral vasoconstriction, and renal conservation of fluid*. Cutaneous vasoconstriction, for example, is responsible for the characteristic coolness and pallor of skin in well-developed shock (although septic shock may initially cause cutaneous *vasodilation* and thus present with warm, flushed skin). Coronary and cerebral vessels are less sensitive to this compensatory sympathetic response and thus maintain relatively normal caliber, blood flow, and oxygen delivery to their respective vital organs.

If the underlying causes are not corrected, shock passes imperceptibly to the progressive phase, during which there is widespread tissue hypoxia. In the setting of persistent oxygen deficit, intracellular aerobic respiration is replaced by anaerobic glycolysis with excessive production of lactic acid. The resultant metabolic *lactic acidosis lowers the tissue pH and blunts the vasomotor response*; arterioles dilate, and blood begins to pool in the microcirculation. Peripheral pooling not only worsens the cardiac output, but also puts endothelial cells at risk for developing anoxic injury with subsequent DIC. With widespread tissue hypoxia, vital organs are affected and begin to fail; *clinically the patient may become confused, and the urine output declines*.

Unless there is intervention, the process eventually enters an irreversible stage. Widespread cell injury is reflected in lysosomal enzyme leakage, further aggravating the shock state. Myocardial contractile function worsens in part because of nitric oxide synthesis. If ischemic bowel allows intestinal flora to enter the circulation, endotoxic shock may be superimposed. At this point, the patient has complete renal shutdown owing to acute tubular necrosis (Chapter 20), and despite heroic measures, the downward clinical spiral almost inevitably culminates in death.

Morphology.

The cellular and tissue changes induced by shock are essentially those of hypoxic injury (Chapter 1); since shock is characterized by **failure of multiple organ systems**, the cellular changes may appear in any tissue. Nevertheless, they are particularly evident in brain, heart, lungs, kidneys, adrenals, and gastrointestinal tract.

The **brain** may develop so-called ischemic encephalopathy, discussed in Chapter 28 . The **heart** may undergo focal or widespread coagulation necrosis or

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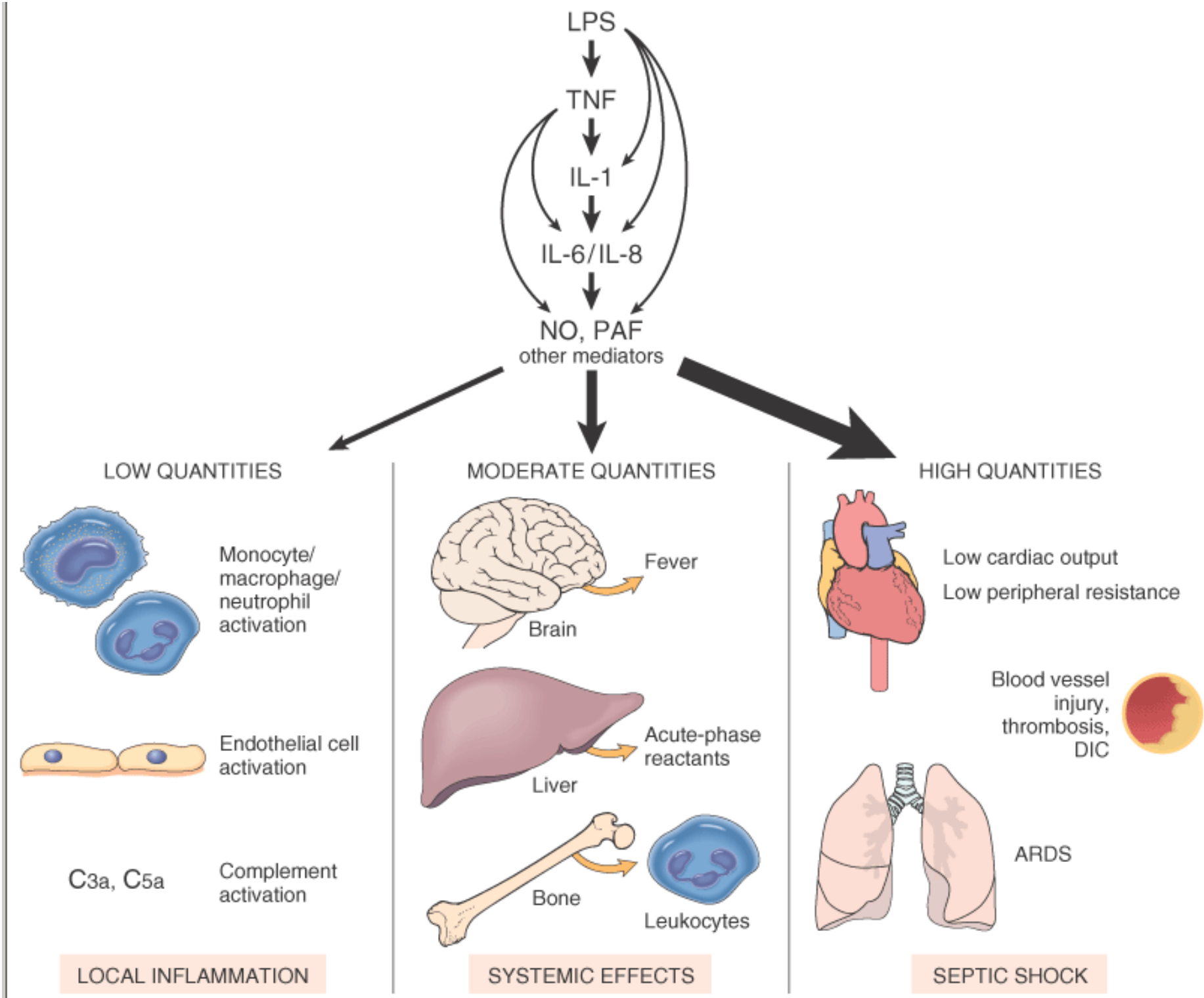
Figure 4-22 Effects of lipopolysaccharide (LPS) and secondarily induced effector molecules. LPS initiates the cytokine cascade described in Figure 4-21 ; in addition, LPS and the various factors can directly stimulate downstream cytokine production, as indicated. Secondary effectors that become important include nitric oxide (NO) and platelet-activating factor (PAF). At low levels, only local inflammatory effects are seen. With moderate levels, more systemic events occur in addition to the local vascular effects. At high concentrations, the syndrome of septic shock is seen. DIC, disseminated intravascular coagulation; ARDS, adult respiratory distress syndrome. *(Modified from Abbas AK, et al: Cellular and Molecular Immunology, 4th ed. Philadelphia, WB Saunders, 2000.)*


LPS
TNF
IL-1
IL-6/IL-8
NO, PAF
other mediators
LOW QUANTITIES
MODERATE QUANTITIES
HIGH QUANTITIES
Monocyte/ macrophage/ neutrophil activation
Endothelial cell activation
C3a, C5a
Complement activation
Fever
Brain
Acute-phase reactants
Liver
Bone
Leukocytes
Low cardiac output
Low peripheral resistance
Blood vessel injury, thrombosis, DIC
ARDS
LOCAL INFLAMMATION
SYSTEMIC EFFECTS
SEPTIC SHOCK

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Chapter 5 - Genetic Disorders

Genetic disorders are far more common than is widely appreciated. The lifetime frequency of genetic diseases is estimated to be 670 per 1000.[1] Included in this figure are not only the "classic" genetic disorders but also cancer and cardiovascular diseases, the two most common causes of death in the Western world. Both of these have major genetic components. Cardiovascular diseases, such as atherosclerosis and hypertension, result from complex interactions of genes and environment, and most cancers are now known to result from an accumulation of mutations in somatic cells (Chapter 7).

The genetic diseases encountered in medical practice represent only the tip of the iceberg, that is, those with less extreme genotypic errors permitting full embryonic development and live birth. It is estimated that 50% of spontaneous abortuses during the early months of gestation have a demonstrable chromosomal abnormality; there are, in addition, numerous smaller detectable errors and many others still beyond our range of identification. About 1% of all newborn infants possess a gross chromosomal abnormality, and approximately 5% of individuals under age 25 develop a serious disease with a significant genetic component. How many more mutations remain hidden?

The draft sequence of the human genome is complete and much has been learned about the "genetic architecture" of humans. Some of what has been revealed was quite unexpected.[2] For example, we now know that less than 2% of the human genome codes for proteins, whereas more than one half represents blocks of repetitive nucleotide codes whose functions remain mysterious. What was totally unexpected was that humans have a mere 30,000 genes rather than the 100,000 predicted only recently. Quite remarkably, this figure is not much greater than that of the mustard plant, with 26,000 genes! However, it is also known that by alternative splicing, 30,000 genes can give rise to greater than 100,000 proteins. In addition, very recent studies indicate that fully formed proteins can be sliced and stitched together to give rise to peptides that could not have been predicted from the structure of the gene.[2A] Humans are not so poor, after all. With the completion of the human genome project, a new term, called *genomics*, has been added to the medical vocabulary. Whereas genetics is the study of single or a few genes and their phenotypic effects, genomics is the study of all the genes in the genome and their interactions. DNA microarray analysis of tumors (Chapter 7) is an excellent example of genomics in current clinical use.[3] However, the most important contribution of genomics to human health will be in the unraveling of complex multifactorial diseases (discussed later) that arise from the interaction of multiple genes with environmental factors.[4]

Another surprising revelation from the recent progress in genomics is that, on average, any two individuals share 99.9% of their DNA sequences. Thus, the remarkable diversity of humans is encoded in about 0.1% of our DNA. The secrets to disease predisposition and response to environmental agents and drugs must therefore reside within these variable regions. Although small as compared to the total nucleotide sequences, this 0.1% represents about 3 million base pairs. The most common form of DNA variations in the human genome is the single nucleotide polymorphism (SNP). Typically, the SNPs are biallelic (i.e., only two choices exist at a given site within the population), and they may occur anywhere in the genome—within exons, introns, or intergenic regions. Less than 1% of SNPs occur in coding regions. These could of course alter the gene product and give rise to a disease. Much more commonly, however, the SNP is just a marker that is co-inherited with a disease-causing gene, due to physical proximity. Another way of expressing this is to say that the SNP and the genetic factor are in linkage disequilibrium. Much effort is ongoing to make SNP maps of the human genome so that we can decipher genetic determinants of disease.[5] Just as genomics involves the study of all the DNA sequences, *proteomics* concerns itself with the measurement of all proteins expressed in a cell or tissue. Currently, progress in proteomics is lagging behind genomics, because the methodology to identify hundreds of distinct proteins simultaneously is not fully developed, but much effort continues.

Although genomics and proteomics are revealing a treasure-trove of information, our ability to organize and mine such a vast array of data is not yet fully developed. To simultaneously analyze patterns of expression involving thousands of genes and proteins has required the parallel development of computer-based techniques that can manage vast collections of data. In response to this, an exciting new discipline called *bioinformatics* has sprouted. This has involved biologists, computer scientists, physicists, and mathematicians, a true example of a multidisciplinary approach in modern medical practice.[6]

Much of the progress in medical genetics has resulted from the spectacular advances in molecular biology, involving recombinant DNA technology. The details of these techniques are well known and are not repeated here. Some examples, however, of the impact of recombinant DNA technology on medicine are worthy of attention.

- *Molecular basis of human disease:* Two general strategies have been used to isolate and characterize involved genes (Fig. 5-1). The *functional cloning*, or *classic*, approach has been successfully used to study a variety of inborn errors of metabolism, such as phenylketonuria and disorders of hemoglobin synthesis. Common to these genetic diseases is knowledge of the abnormal gene product and the corresponding protein. When the affected protein is known, a variety of methods can be employed to isolate the normal gene, to

clone it, and ultimately to determine the molecular changes that affect the gene in patients with the disorder. Because in many common single-gene disorders, such as cystic fibrosis, there was no clue to the nature of the defective gene product, an alternative strategy called *positional cloning*, or the "candidate gene," approach had to be employed. This strategy initially ignores the biochemical clues from the phenotype and relies instead on mapping the disease phenotype to a particular chromosome location. This mapping is accomplished if the disease is associated with a distinctive cytogenetic change (e.g., fragile-X syndrome) or by linkage analysis. In the latter, the approximate location of the gene is determined by linkage to known "marker genes" or SNPs that are in close proximity to the disease locus. Once the region in which the mutant gene lies has been localized within reasonably narrow limits, the next step is to clone several pieces of DNA from the relevant segment of the genome. Expression of the cloned DNA in vitro, followed by identification of the protein products, can then be used to identify the aberrant protein encoded by the mutant genes. This approach has been used successfully in several diseases, such as cystic fibrosis, neurofibromatosis, Duchenne muscular dystrophy (a hereditary disorder characterized by progressive muscle weakness), polycystic

kidney disease, and Huntington disease. In addition to this step-by-step approach to cloning single genes, cDNA microarray analysis allows simultaneous detection of thousands of genes and their RNA products. When normal and diseased tissues are analyzed in this fashion, changes in the expression levels of multiple genes can be detected, thus providing a more comprehensive profile of genetic alterations in diseased tissues.

- *Production of human biologically active agents:* An array of ultrapure biologically active agents can now be produced in virtually unlimited quantities by inserting the requisite gene into bacteria or other suitable cells in tissue culture. Some examples of genetically engineered products already in clinical use include soluble TNF receptor for blocking TNF in treatment of rheumatoid arthritis, tissue plasminogen activator for the treatment of thrombotic states, growth hormone for the treatment of deficiency states, erythropoietin to reverse several types of anemia, and myeloid growth and differentiation factors (granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor) to enhance production of monocytes and neutrophils in states of poor marrow function.
- *Gene therapy:* The goal of treating genetic diseases by transfer of somatic cells transfected with the normal gene, although simple in concept, has yet to succeed on a large scale. Problems include designing appropriate vectors to carry the gene and unexpected complications resulting from random insertion of the normal gene in the host genome. In recent well-publicized cases, gene therapy in patients with x-linked SCID (severe combined immunodeficiency, Chapter 6) who lack the common γ chain of cytokine receptors had to be put on hold because the transduced gene inserted next to a host gene that controls proliferation of cells. The resulting dysregulation gave rise to T-cell leukemia in the patient.
- *Disease diagnosis:* Molecular probes are proving to be extremely useful in the diagnosis of both genetic and non-genetic (e.g., infectious) diseases. The diagnostic applications of recombinant DNA technology are detailed at the end of this chapter.

Figure 5-1 Schematic illustration of the strategies employed in functional and positional cloning. Functional cloning begins with relating the clinical phenotype to biochemical-protein abnormalities, followed by isolation of the mutant gene. Positional cloning, also called candidate gene approach, begins by mapping and cloning the disease gene by linkage analysis, without any knowledge of the gene product. Identification of the gene product and the mechanism by which it produces the disease follow the isolation of the mutant gene.

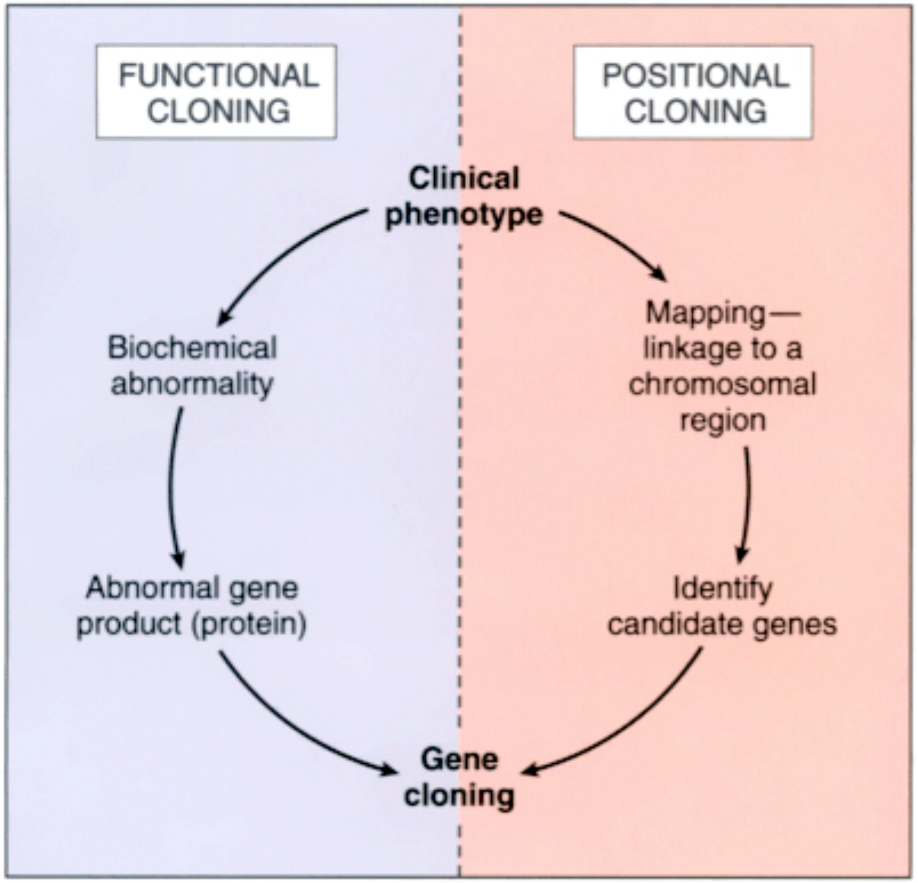

Figure 5-2 Schematic illustration of a point mutation resulting from a single base pair change in the DNA. In the example shown, a CTC to CAC change alters the meaning of the genetic code (GAG to GUG in the opposite strand), leading to replacement of glutamic acid by valine in the polypeptide chain. This change, affecting the sixth amino acid of the normal β-globin (β_A) chain, converts it to sickle β-globin (β_S).

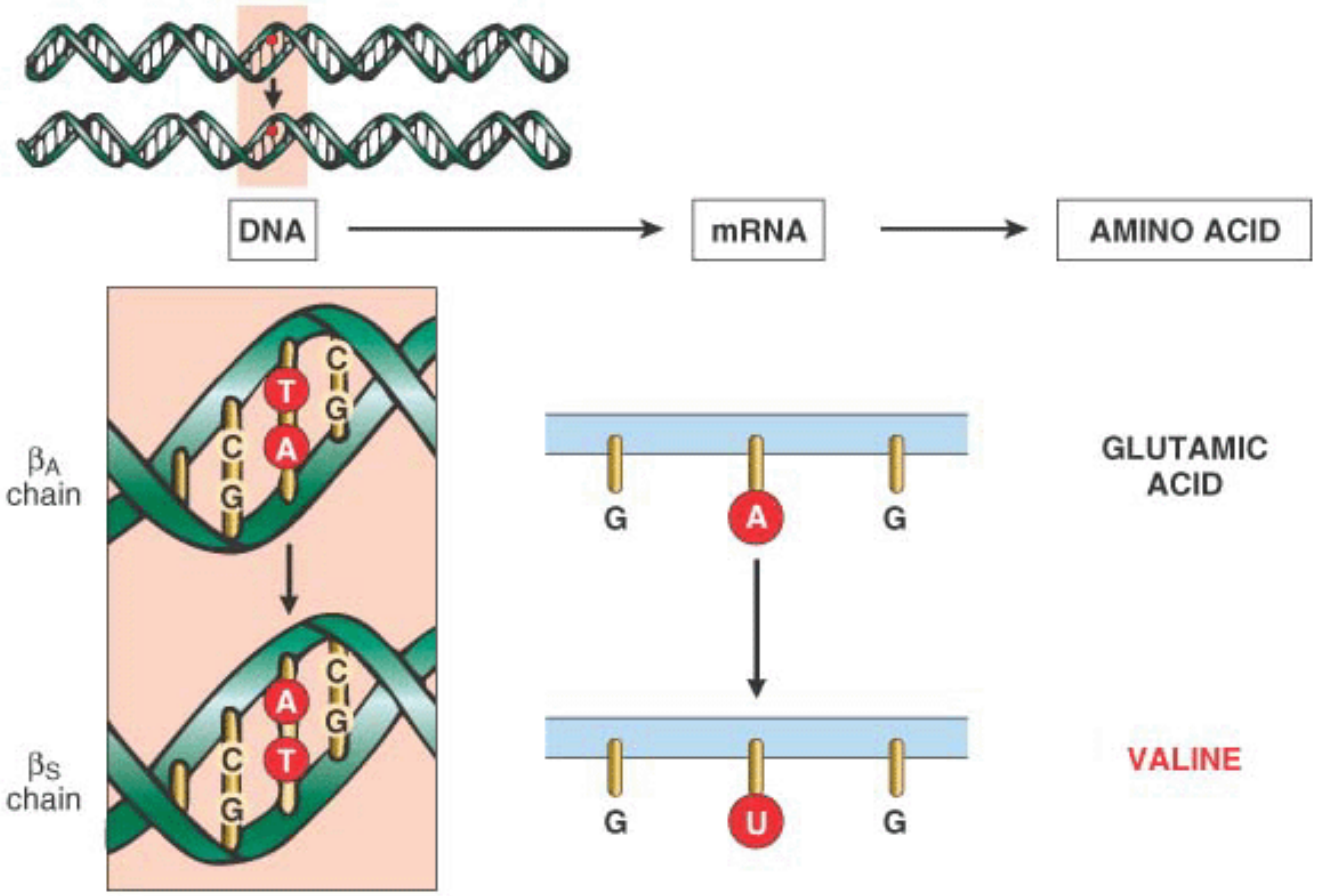

Figure 5-3 Single-base deletion at the ABO (glycosyltransferase) locus, leading to a frameshift mutation responsible for the O allele. *(From Thompson MW, et al: Thompson and Thompson Genetics in Medicine, 5th ed. Philadelphia, WB Saunders, 1991, p 134.)*

ABO A allele

. . . Leu – Val – Val – Thr – Pro . . .

. . . CTC GTG GTG ACC CCT T . . .

ABO O allele

. . . CTC GTG GT– ACC CCT T . . .

. . . Leu – Val – **Val – Pro – Leu** . . .

altered reading frame →

Figure 5-4 Four-base insertion in the hexosaminidase A gene in Tay-Sachs disease, leading to a frameshift mutation. This mutation is the major cause of Tay-Sachs disease in Ashkenazi Jews. *(From Nussbaum, RL, et al: Thompson and Thompson Genetics in Medicine, 6th ed. Philadelphia, WB Saunders, 2001, p. 212.)*

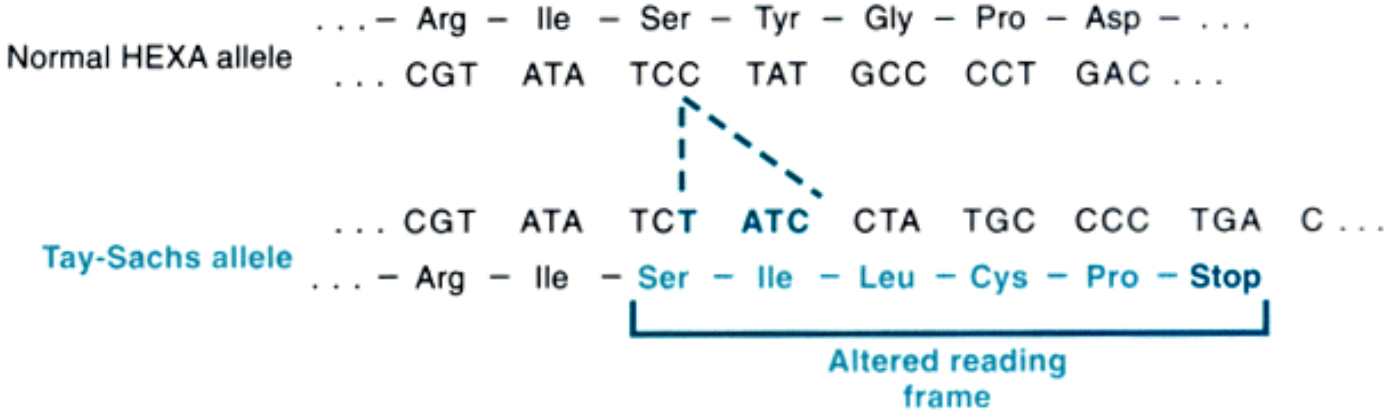

Figure 5-5 Point mutation leading to premature chain termination. Partial mRNA sequence of the β-globin chain of hemoglobin showing codons for amino acids 38 to 40. A point mutation (C→U) in codon 39 changes glutamine (Gln) codon to a stop codon, and hence protein synthesis stops at the 38th amino acid.

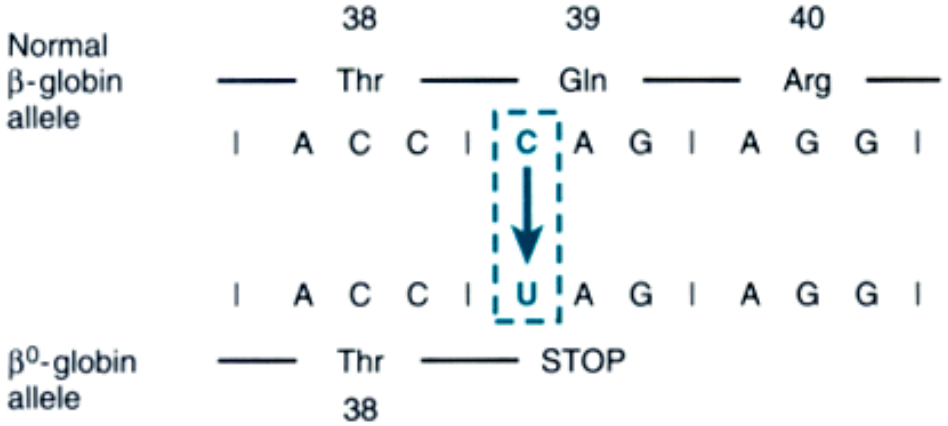

Figure 5-6 Three-base deletion in the common cystic fibrosis (CF) allele results in synthesis of a protein that is missing amino acid 508 (phenylalanine). Because the deletion is a multiple of three, this is not a frameshift mutation. *(From Thompson MW, et al: Thompson and Thompson Genetics in Medicine, 5th ed. Philadelphia, WB Saunders, 1991, p. 135.)*

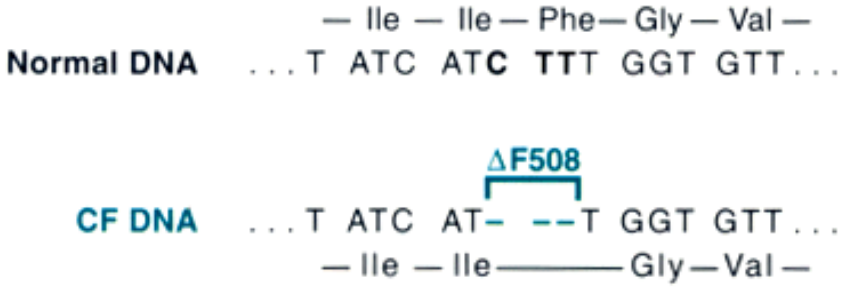

TABLE 5-1 -- Autosomal Dominant Disorders

System	Disorder
Nervous	Huntington disease
	Neurofibromatosis *
	Myotonic dystrophy
	Tuberous sclerosis
Urinary	Polycystic kidney disease
Gastrointestinal	Familial polyposis coli
Hematopoietic	Hereditary spherocytosis
	von Willebrand disease
Skeletal	Marfan syndrome *
	Ehlers-Danlos syndrome (some variants) *
	Osteogenesis imperfecta
	Achondroplasia
Metabolic	Familial hypercholesterolemia *
	Acute intermittent porphyria

*Discussed in this chapter. Other disorders listed are discussed in appropriate chapters of this book.

Autosomal recessive disorders include almost all inborn errors of metabolism. The various consequences of enzyme deficiencies are discussed later. The more common of these conditions are listed in Table 5-2 . Most are presented elsewhere; a few prototypes are discussed later in this chapter.

TABLE 5-2 -- Autosomal Recessive Disorders

System	Disorder
Metabolic	Cystic fibrosis
	Phenylketonuria
	Galactosemia
	Homocystinuria
	Lysosomal storage diseases *
	α_1 -Antitrypsin deficiency

	Wilson disease
	Hemochromatosis
	Glycogen storage diseases *
Hematopoietic	Sickle cell anemia
	Thalassemias
Endocrine	Congenital adrenal hyperplasia
Skeletal	Ehlers-Danlos syndrome (some variants) *
	Alkaptonuria *
Nervous	Neurogenic muscular atrophies
	Friedreich ataxia
	Spinal muscular atrophy

*Discussed in this chapter. Many others are discussed elsewhere in the text.

X-Linked Disorders

All sex-linked disorders are X-linked, almost all X-linked recessive. Several genes are encoded in the "male-specific region of Y"; all of these are related to spermatogenesis.[9] Males with mutations affecting the Y-linked genes are usually infertile, and hence there is no Y-linked inheritance. As discussed later, a few additional genes with homologues on the X chromosome have been mapped to the Y chromosome, but no disorders resulting from mutations in such genes have been described.

X-linked recessive inheritance accounts for a small number of well-defined clinical conditions. The Y chromosome, for the most part, is not homologous to the X, and so mutant genes on the X are not paired with alleles on the Y. Thus, the male is said to be *hemizygous* for X-linked mutant genes, so these disorders are expressed in the male. Other features that characterize these disorders are as follows:

- An affected male does not transmit the disorder to his sons, but all daughters are carriers. Sons of heterozygous women have, of course, one chance in two of receiving the mutant gene.
- The heterozygous female usually does not express the full phenotypic change because of the paired normal allele. Because of the random inactivation of one of the X chromosomes in the female, however, females have a variable proportion of cells in which the mutant X chromosome is active. Thus, it is remotely possible for the normal allele to be inactivated in most cells, permitting full expression of heterozygous X-linked conditions in the female. Much more commonly, the normal allele is inactivated in only some of the cells, and thus the heterozygous female expresses the disorder partially. An illustrative condition is *glucose-6-phosphate dehydrogenase (G6PD) deficiency*. Transmitted on the X chromosome, this enzyme deficiency, which predisposes to red cell hemolysis in patients receiving certain types of drugs (Chapter 13), is expressed principally in males. In the female, a proportion of the red cells may be derived from marrow cells with inactivation of the normal allele. Such red cells are at the same risk for undergoing hemolysis as are the red cells in the

hemizygous male. Thus, the female is not only a carrier of this trait, but also is susceptible to drug-induced hemolytic reactions. Because the proportion of defective red cells in heterozygous females depends on the random inactivation of one of the X chromosomes, however, the severity of the hemolytic reaction is almost always less in heterozygous women than in hemizygous men. Most of the X-linked conditions listed in Table 5-3 are covered elsewhere in the text.

TABLE 5-3 -- X-Linked Recessive Disorders

System	Disease
Musculoskeletal	Duchenne muscular dystrophy
Blood	Hemophilia A and B
	Chronic granulomatous disease
	Glucose-6-phosphate dehydrogenase deficiency
Immune	Agammaglobulinemia
	Wiskott-Aldrich syndrome
Metabolic	Diabetes insipidus
	Lesch-Nyhan syndrome
Nervous	Fragile-X syndrome *

*Discussed in this chapter. Others discussed in appropriate chapters in the book.

There are only a few *X-linked dominant* conditions. They are caused by dominant disease alleles on the X chromosome. These disorders are transmitted by an affected heterozygous female to half her sons and half her daughters and by an affected male parent to all his daughters but none of his sons, if the female parent is unaffected. Vitamin D-resistant rickets is an example of this type of inheritance.

BIOCHEMICAL AND MOLECULAR BASIS OF SINGLE-GENE (MENDELIAN) DISORDERS

Mendelian disorders result from alterations involving single genes. The genetic defect may lead to the formation of an abnormal protein or a reduction in the output of the gene product. As mentioned earlier, mutations may affect protein synthesis by affecting transcription, mRNA processing, or translation. The phenotypic effects of a mutation may result directly, from abnormalities in the protein encoded by the mutant gene, or indirectly, owing to interactions of the mutant protein with other normal proteins. For example, all forms of Ehlers-Danlos syndrome (EDS) are associated with abnormalities of collagen. In some forms (e.g., vascular type), there is a mutation in one of the collagen genes, whereas in others (e.g., kyphoscoliosis type), the collagen genes are normal, but there is a mutation in the gene that encodes lysyl hydroxylase, an enzyme essential for the cross-linking of collagen. In these patients, collagen weakness is secondary to a deficiency of lysyl hydroxylase.

Virtually any type of protein may be affected in single-gene disorders and by a variety of mechanisms (Table 5-4). To some extent, the pattern of inheritance of the disease is related to the kind of protein affected by the mutation, as was discussed earlier and is reiterated subsequently. For the purposes of this discussion, the mechanisms involved in single-gene disorders can be classified into four categories: (1) *enzyme defects and their consequences*; (2) *defects in membrane receptors and transport systems*; (3) *alterations in the structure, function, or quantity of nonenzyme proteins*; and (4) *mutations resulting in unusual reactions to drugs*.

Mutations may result in the synthesis of a defective enzyme with reduced activity or in a reduced amount of a normal enzyme. In either case, the consequence is a metabolic block. Figure 5-7 provides an example of an enzyme reaction in which the substrate is converted by intracellular enzymes, denoted as 1, 2, and 3, into an end product through intermediates 1 and 2. In this model, the final product exerts feedback control on enzyme 1. A minor pathway producing small quantities of M_1 and M_2 also exists. The biochemical consequences of an enzyme defect in such a reaction may lead to three major consequences:

1. *Accumulation of the substrate*, depending on the site of block, may be accompanied by accumulation of one or both intermediates. Moreover, an increased concentration of intermediate 2 may stimulate the minor pathway and thus

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lead to an excess of $M_1$and M_2. Under these conditions, tissue injury may result if the precursor, the intermediates, or the products of alternative minor pathways are toxic in high concentrations. For example, in galactosemia, the deficiency of galactose-1-phosphate uridyltransferase (Chapter 10) leads to the accumulation of galactose and consequent tissue damage. In phenylketonuria, a deficiency of phenylalanine hydroxylase (Chapter 10) results in the accumulation of phenylalanine. Excessive accumulation of complex substrates within the lysosomes as a result of deficiency of degradative enzymes is responsible for a group of diseases generally referred to as *lysosomal storage diseases*.

2. *An enzyme defect can lead to a metabolic block and a decreased amount of end product* that may be necessary for normal function. For example, a deficiency of melanin may result from lack of tyrosinase, which is necessary for the biosynthesis of melanin from its precursor, tyrosine. This results in the clinical condition called *albinism*. If the end product is a feedback inhibitor of the enzymes involved in the early reactions (in Fig. 5-7 , it is shown that the product inhibits enzyme 1), the deficiency of the end product may permit overproduction of intermediates and their catabolic products, some of which may be injurious at high concentrations. A prime example of a disease with such an underlying mechanism is the Lesch-Nyhan syndrome (Chapter 26).
3. *Failure to inactivate a tissue-damaging substrate* is best exemplified by α_1 -antitrypsin (α_1 -AT) deficiency. Patients who have an inherited deficiency of serum α_1 -AT are unable to inactivate neutrophil elastase in their lungs. Unchecked activity of this protease leads to destruction of elastin in the walls of lung alveoli, leading eventually to pulmonary emphysema (Chapter 15).

TABLE 5-4 -- Biochemical and Molecular Basis of Some Mendelian Disorders

Protein Type/Function	Example	Molecular Lesion	Disease
Enzyme	Phenylalanine hydroxylase	Splice site mutation: reduced amount	Phenylketonuria
	Hexosaminidase	Splice site mutation or frameshift mutation with stop codon: reduced amount	Tay-Sachs disease
	Adenosine deaminase	Point mutations: abnormal protein with reduced activity	Severe combined immunodeficiency
Enzyme Inhibitor	α_1 -Antitrypsin	Missense mutations: impaired secretion from liver to serum	Emphysema and liver disease
Receptor	Low-density lipoprotein receptor	Deletions, point mutations: reduction of synthesis, transport to cell surface, or binding to low-density lipoprotein	Familial hypercholesterolemia
	Vitamin D receptor	Point mutations: failure of normal signaling	Vitamin D-resistant rickets
Transport			

Oxygen	Hemoglobin	Deletions: reduced amount	α-Thalassemia
		Defective mRNA processing: reduced amount	β-Thalassemia
		Point mutations: abnormal structure	Sickle cell anemia
Ions	Cystic fibrosis transmembrane conductance regulator	Deletions and other mutations	Cystic fibrosis
Structural			
Extracellular	Collagen	Deletions or point mutations cause reduced amount of normal collagen or normal amounts of mutant collagen	Osteogenesis imperfecta; Ehlers-Danlos syndromes
	Fibrillin	Missense mutations	Marfan syndrome
Cell membrane	Dystrophin	Deletion with reduced synthesis	Duchenne/Becker muscular dystrophy
	Spectrin, ankyrin, or protein 4.1	Heterogeneous	Hereditary spherocytosis
Hemostasis	Factor VIII	Deletions, insertions, nonsense mutations, and others: reduced synthesis or abnormal factor VIII	Hemophilia A
Growth Regulation	Rb protein	Deletions	Hereditary retinoblastoma
	Neurofibromin	Heterogeneous	Neurofibromatosis type 1

Figure 5-7 Scheme of a possible metabolic pathway in which a substrate is converted to an end product by a series of enzyme reactions. M1, M2, products of a minor pathway.

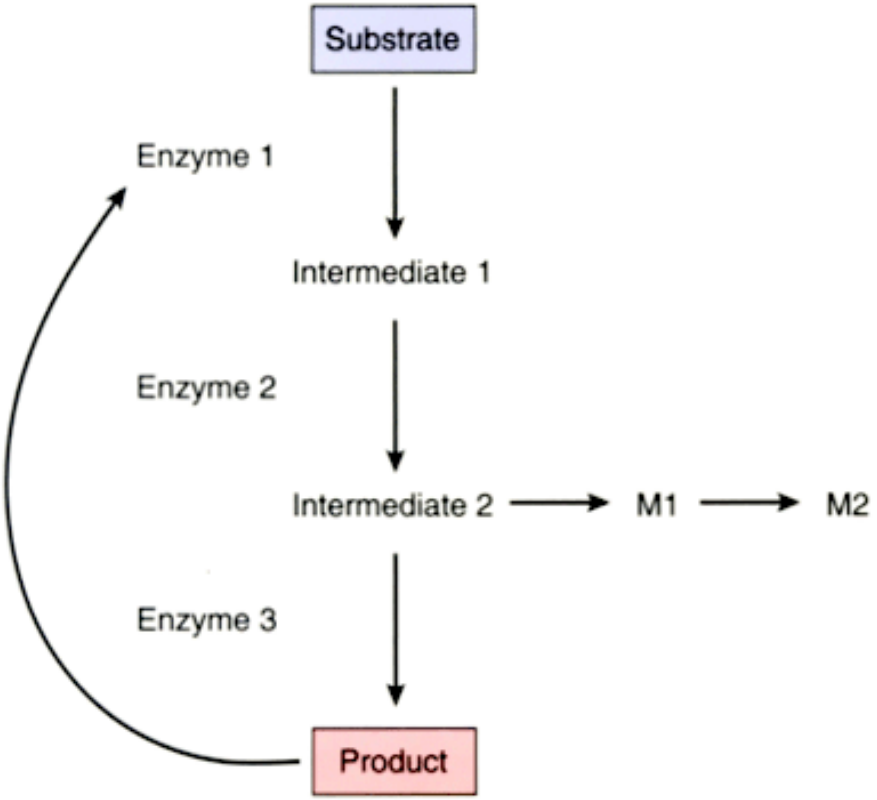


TABLE 5-5 -- Classification of Ehlers-Danlos Syndromes (EDS)

EDS Type *	Clinical Findings	Inheritance	Gene Defects
Classical (I/II)	Skin and joint hypermobility, atrophic scars, easy bruising	Autosomal dominant	COL5A1, COL5A2
Hypermobility (III)	Joint hypermobility, pain, dislocations	Autosomal dominant	Unknown
Vascular (IV)	Thin skin, arterial or uterine rupture, bruising, small joint hyperextensibility	Autosomal dominant	COL3A1
Kyphoscoliosis (VI)	Hypotonia, joint laxity, congenital scoliosis, ocular fragility	Autosomal recessive	Lysyl-hydroxylase
Arthrochalasia (VIIa,b)	Severe joint hypermobility, skin changes mild, scoliosis, bruising	Autosomal dominant	COL1A1, COL1A2
Dermatosparaxsis (VIIc)	Severe skin fragility, cutis laxa, bruising	Autosomal recessive	Procollagen *N*-peptidase

*EDS were previously classified by Roman numerals. Parentheses show previous numerical equivalents.

deficiency of lysyl hydroxylase results in the synthesis of collagen that lacks normal structural stability.

The *vascular type of EDS results from abnormalities of type III collagen*. This form is genetically heterogeneous because at least three distinct types of mutations affecting the *COL3A1* gene for collagen type III can give rise to this variant. Some affect the rate of synthesis of pro α1 (III) chains, others affect the secretion of type III procollagen, and still others lead to the synthesis of structurally abnormal type III collagen. Some mutant alleles behave as dominant negatives (see discussion under autosomal dominant disorders) and thus produce severe phenotypic effects. These molecular studies provide a rational basis for the pattern of transmission and clinical features that are characteristic of this variant. First, because vascular type EDS results from mutations involving a structural protein (rather than an enzyme protein), an autosomal dominant pattern of inheritance would be expected. Second, because blood vessels and intestines are known to be rich in collagen type III, an abnormality of this collagen is consistent with severe defects (e.g., spontaneous rupture) in these organs.

In two forms of EDS—arthrochalasia type and dermatosparaxis type—the fundamental defect is in the conversion of type I procollagen to collagen. This step in collagen synthesis involves cleavage of noncollagen peptides at the N-terminal and C-terminal of the procollagen molecule. This is accomplished by N-terminal-specific and C-terminal-specific peptidases. *The defect in the conversion of procollagen to collagen in the arthrocalasic type has been traced to mutations that affect one of the two type I collagen genes, COL1A1* and *COL1A2*. As a result, structurally abnormal pro α1 (I) or pro α2 (I) chains that resist cleavage of N-terminal peptides are formed. In patients with a single mutant allele, only 50% of the type I collagen chains are abnormal, but because these chains interfere with the formation of normal collagen helices, heterozygotes manifest the disease. By contrast, the related dermatosparaxis type is caused by mutations in the procollagen-N-peptidase genes, essential for the cleavage of collagens. In this case, the enzyme deficiency leads to an autosomal recessive form of inheritance.

Finally, the *classical type of EDS* is worthy of brief mention, since molecular analysis of the variant suggests that genes other than collagen genes may be involved in the pathogenesis of EDS. In 30% to 50% of these cases, mutations in the genes for type V collagen *(COL5A1* and *COL5A1)* have been detected. Surprisingly, despite a phenotype typical of EDS, no other collagen gene abnormalities have been found in these cases. This has led to the speculation that other proteins in the extracellular matrix, such as tenascin-X, may also be involved in regulating collagen synthesis.

To summarize, the common thread in EDS is some abnormality of collagen. These disorders, however, are extremely heterogeneous. At the molecular level, a variety of defects, varying from mutations involving structural genes for collagen to those involving enzymes that are responsible for post-transcriptional modifications of mRNA, have been detected. Such molecular heterogeneity results in the expression of EDS as a clinically heterogeneous disorder with several patterns of inheritance.

DISORDERS ASSOCIATED WITH DEFECTS IN RECEPTOR PROTEINS

Familial Hypercholesterolemia

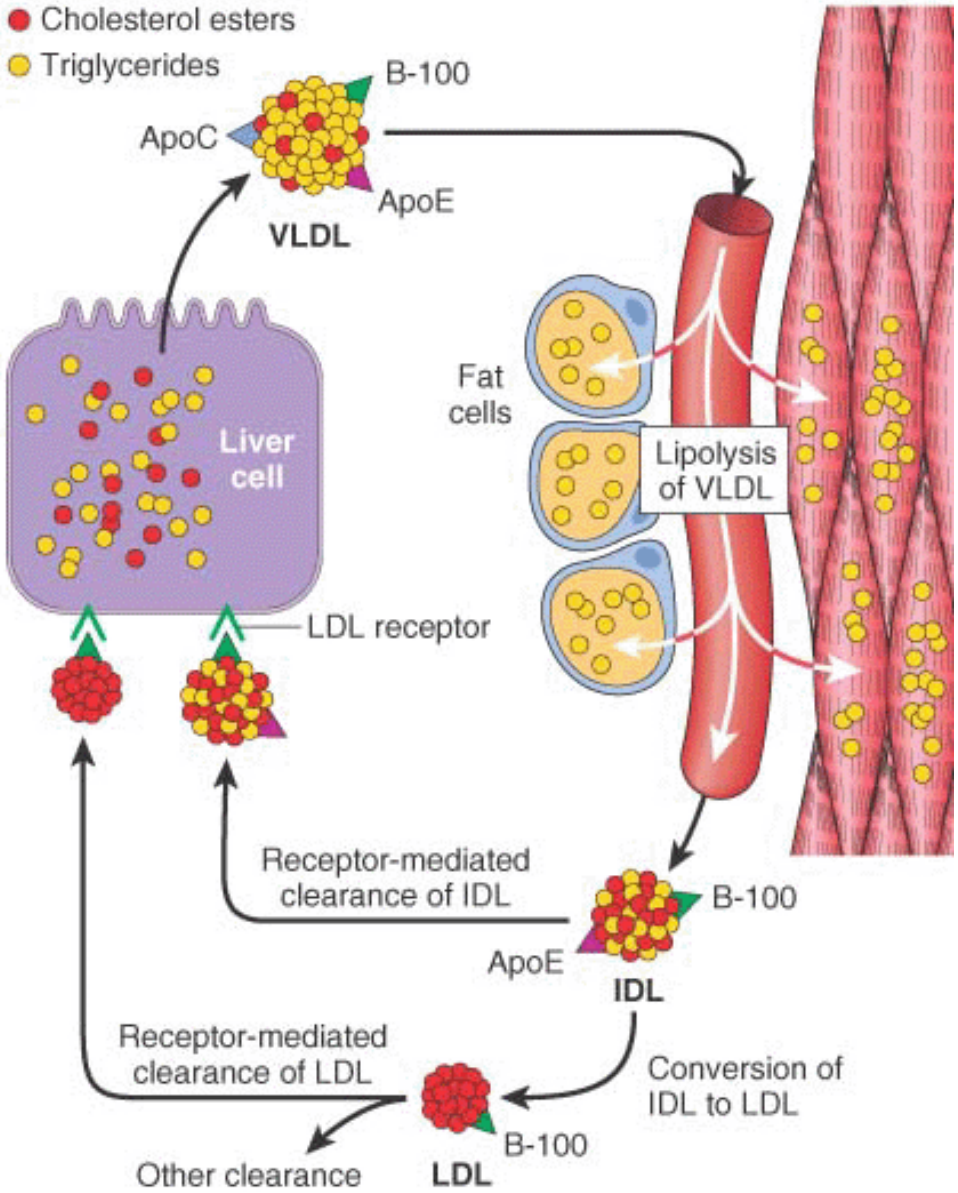
Familial hypercholesterolemia is a "receptor disease" that is the consequence of a *mutation in the gene encoding the receptor for low density lipoprotein (LDL), which is involved in the transport and metabolism of cholesterol*. As a consequence of receptor abnormalities, there is a loss of feedback control and elevated levels of cholesterol that induce premature atherosclerosis, leading to a greatly increased risk of myocardial infarction.[19] [20]

Familial hypercholesterolemia is possibly the most frequent mendelian disorder. Heterozygotes with one mutant gene, representing about 1 in 500 individuals, have from birth a twofold to threefold elevation of plasma cholesterol level, leading to tendinous xanthomas and premature atherosclerosis in adult life (Chapter 11). Homozygotes, having a double dose of the mutant gene, are much more severely affected and may have fivefold to sixfold elevations in plasma cholesterol levels. These individuals develop skin xanthomas and coronary, cerebral, and peripheral vascular atherosclerosis at an early age. Myocardial infarction may develop before age 20. Large-scale studies have found that familial hypercholesterolemia is present in 3% to 6% of survivors of myocardial infarction.

An understanding of this disorder requires that we briefly review the normal process of cholesterol metabolism and transport. Approximately 7% of the body's cholesterol circulates in the plasma, predominantly in the form of LDL. As might be expected, the level of plasma cholesterol is influenced by its synthesis and catabolism and the liver plays a crucial role in both these

processes (Fig. 5-8). The first step in this complex sequence is the secretion of very-low-density lipoproteins (VLDL) by the liver into the bloodstream. VLDL particles are rich in triglycerides, although they do contain lesser amounts of cholesteryl esters. When a VLDL particle reaches the capillaries of adipose tissue or muscle, it is cleaved by lipoprotein lipase, a process that extracts most of the triglycerides. The resulting molecule, called *intermediate-density lipoprotein (IDL)*, is reduced in triglyceride content and enriched in cholesteryl esters, but it retains two of the three apoproteins (B-100 and E) present in the parent VLDL particle (see Fig. 5-8). After release from the capillary endothelium, the IDL particles have one of two fates. Approximately 50% of newly formed IDL is rapidly taken up by the liver through a receptor-mediated transport. The receptor responsible for the binding of IDL to liver cell membrane recognizes both apoprotein B-100 and apoprotein E. It is called the *LDL receptor*, however, because it is also involved in the hepatic clearance of LDL, as described later. In the liver cells,

Figure 5-8 Schematic illustration of low-density lipoprotein (LDL) metabolism and the role of the liver in its synthesis and clearance. Lipolysis of very-low-density lipoprotein (VLDL) by lipoprotein lipase in the capillaries releases triglycerides, which are then stored in fat cells and used as a source of energy in skeletal muscles.



Other clearance LDL

Figure 5-9 The LDL receptor pathway and regulation of cholesterol metabolism.

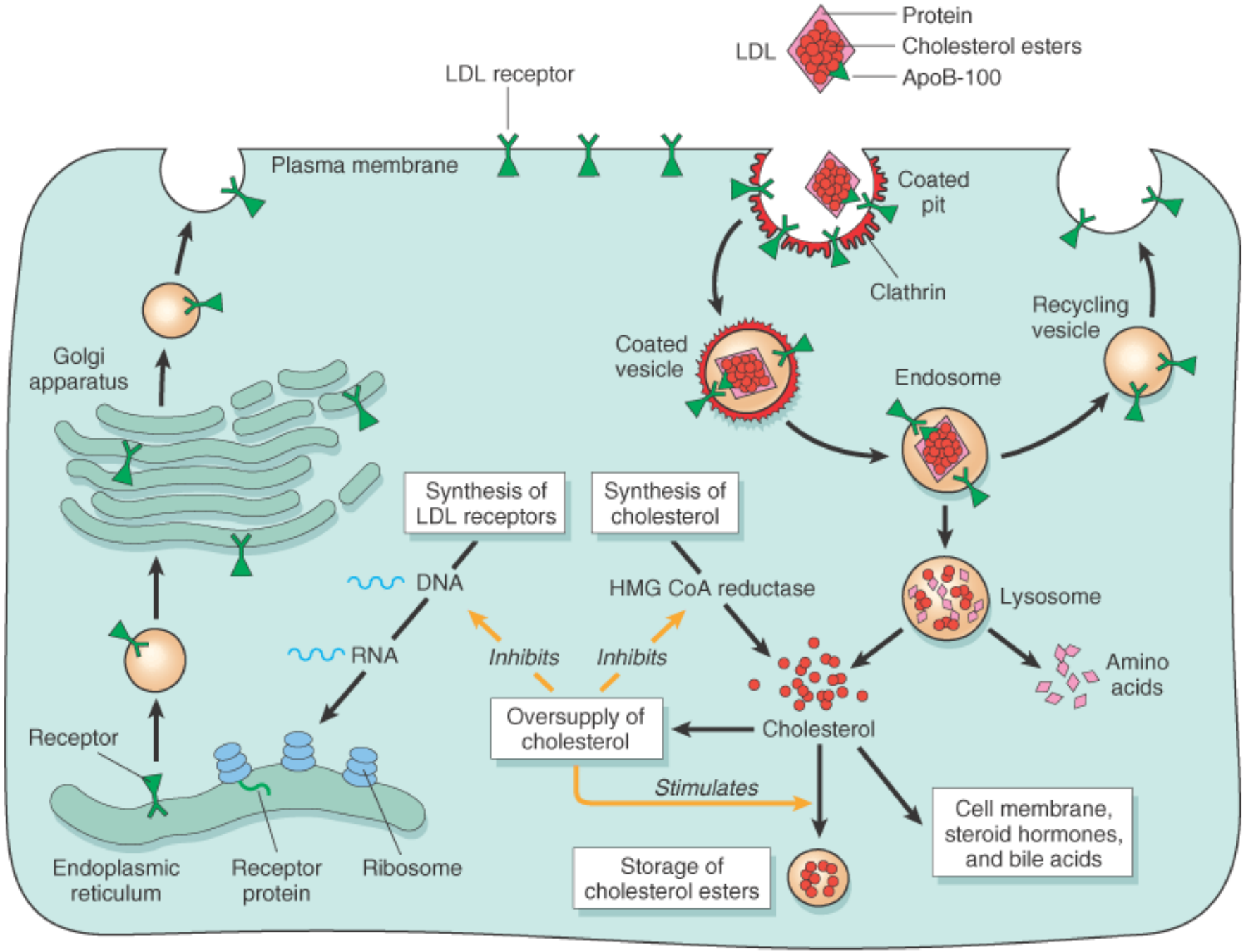
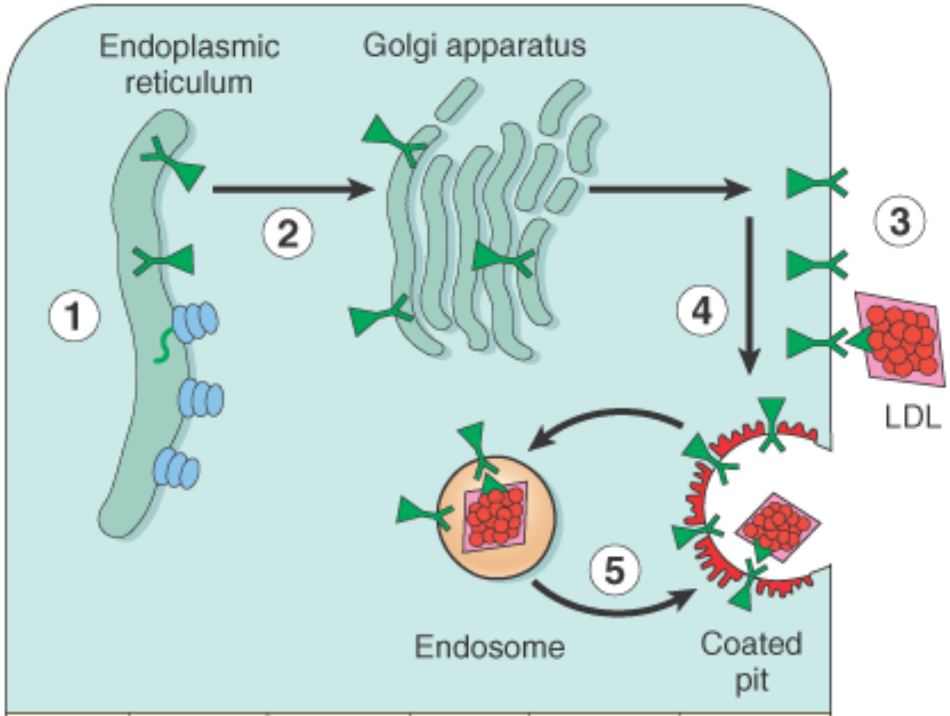


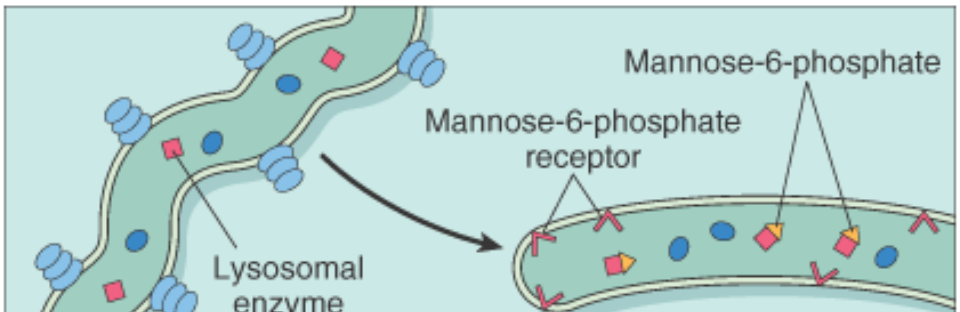
Figure 5-10 Classification of LDL receptor mutations based on abnormal function of the mutant protein. These mutations disrupt the receptor's synthesis in the endoplasmic reticulum,

transport to the Golgi complex, binding of apoprotein ligands, clustering in coated pits, and recycling in endosomes. Each class is heterogeneous at the DNA level. *(Modified with permission from Hobbs HH, et al: The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. Annu Rev Genet 24:133–170, 1990. © 1990 by Annual Reviews.)*



Mutation class	Synthesis	Transport	Binding	Clustering	Recycling
I	X				
II	→	X			
III	→	→	X		
IV	→	→	→	X	
V	→	→	→	→	X

Figure 5-11 Synthesis and intracellular transport of lysosomal enzymes.



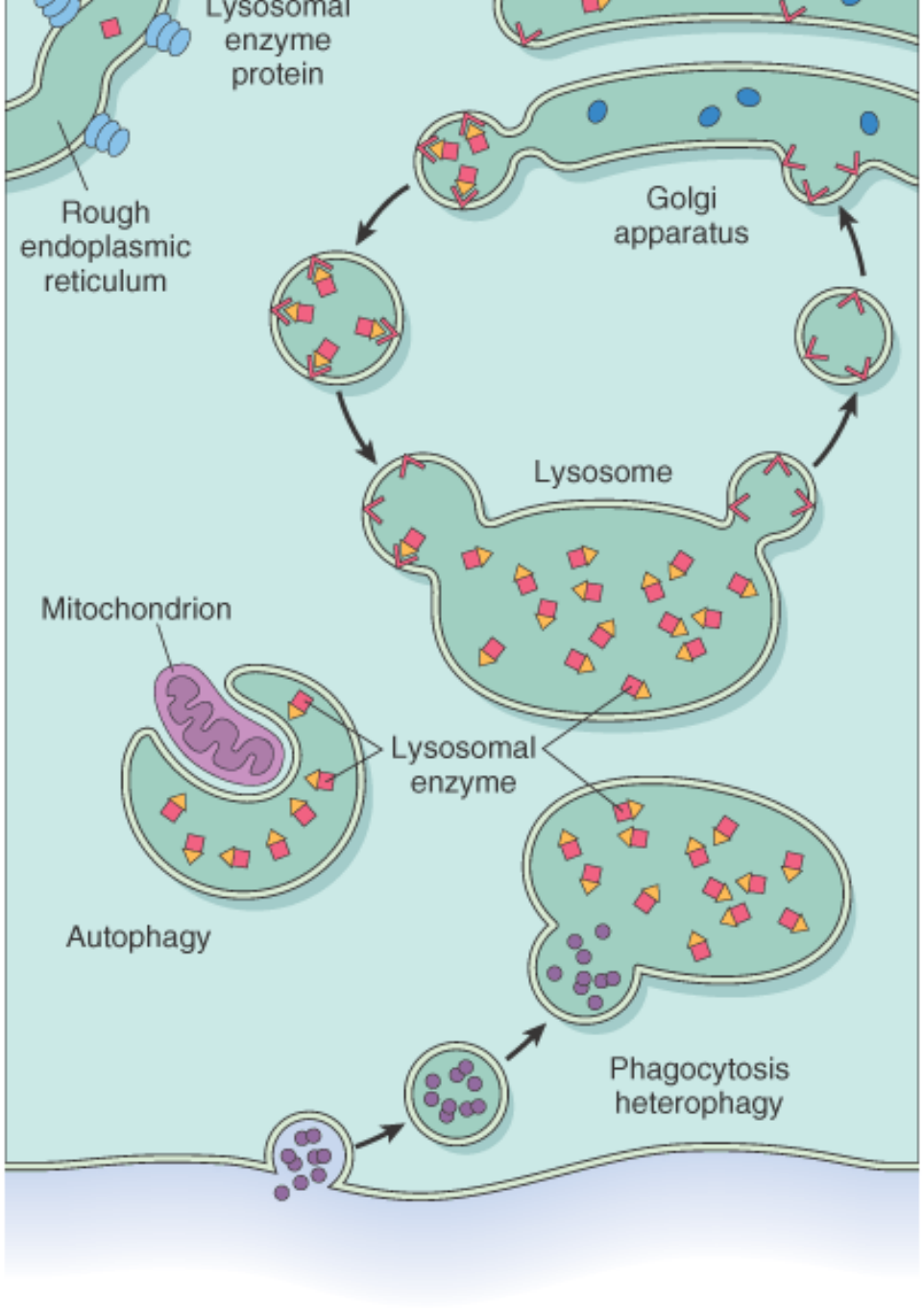


Figure 5-12 Schematic diagram illustrating the pathogenesis of lysosomal storage diseases. In the example shown, a complex substrate is normally degraded by a series of lysosomal enzymes (A, B, and C) into soluble end products. If there is a deficiency or malfunction of one of the enzymes (e.g., B), catabolism is incomplete and insoluble intermediates accumulate in the lysosomes.

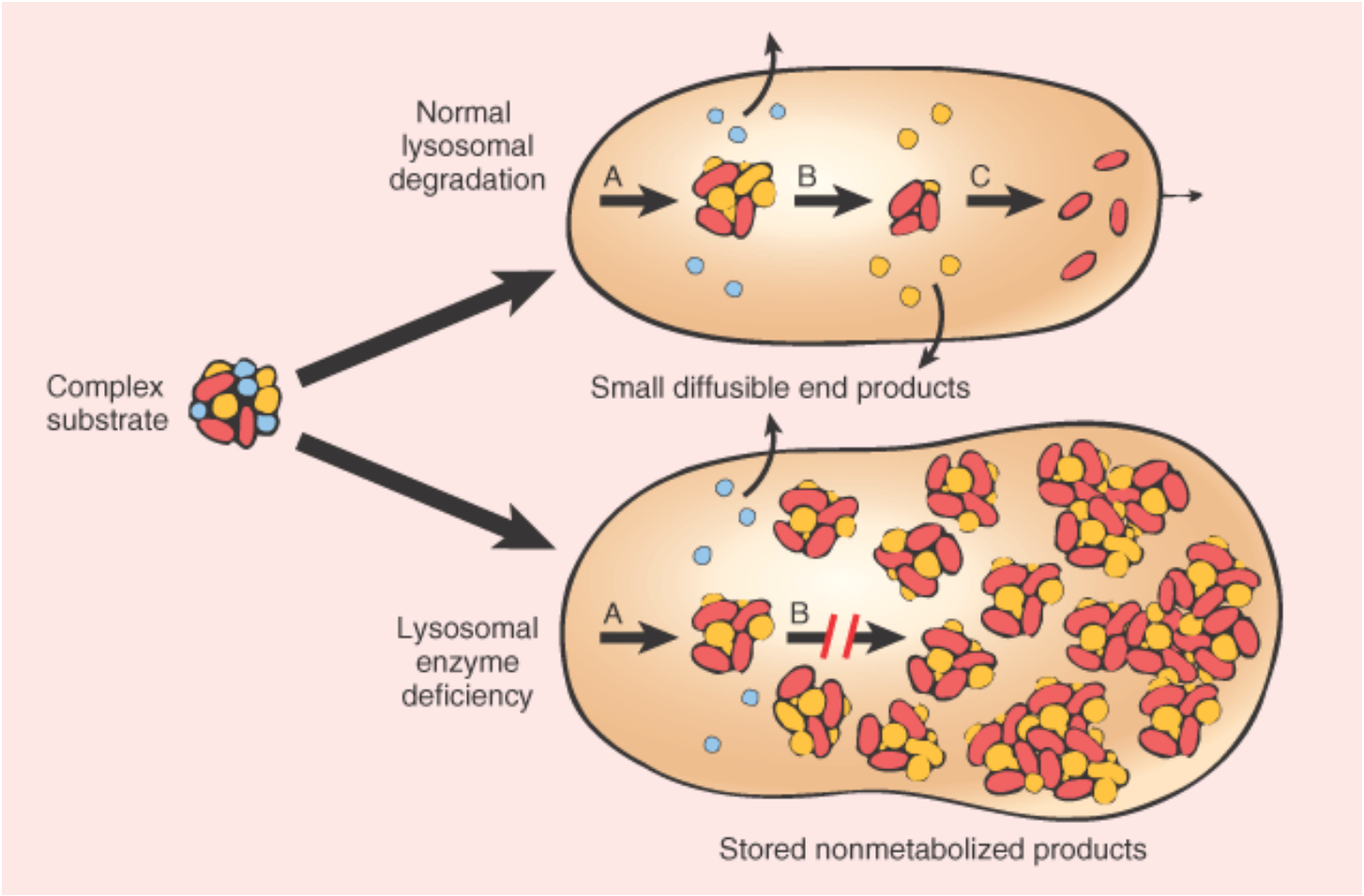


TABLE 5-6 -- Lysosomal Storage Diseases

Disease	Enzyme Deficiency	Major Accumulating Metabolites
Glycogenosis		
Type 2—Pompe disease	α-1,4-Glucosidase (lysosomal glucosidase)	Glycogen
Sphingolipidoses		
GM_1 gangliosidosis	GM_1 ganglioside β-galactosidase	GM_1 ganglioside, galactose-containing oligosaccharides
••Type 1—infantile, generalized		
••Type 2—juvenile		
GM_2 gangliosidosis		

••Tay-Sachs disease	Hexosaminidase-α subunit	GM_2 ganglioside
••Sandhoff disease	Hexosaminidase-β subunit	GM_2 ganglioside, globoside
••GM_2 gangliosidosis, variant AB	Ganglioside activator protein	GM_2 ganglioside
Sulfatidoses		
Metachromatic leukodystrophy	Arylsulfatase A	Sulfatide
Multiple sulfatase deficiency	Arylsulfatases A, B, C; steroid sulfatase; iduronate sulfatase; heparan N-sulfatase	Sulfatide, steroid sulfate, heparan sulfate, dermatan sulfate
Krabbe disease	Galactosylceramidase	Galactocerebroside
Fabry disease	α-Galactosidase A	Ceramide trihexoside
Gaucher disease	Glucocerebrosidase	Glucocerebroside
Niemann-Pick disease: types A and B	Sphingomyelinase	Sphingomyelin
Mucopolysaccharidoses (MPS)		
MPS I H (Hurler)	α-L-Iduronidase	Dermatan sulfate, heparan sulfate
MPS II (Hunter)	L-Iduronosulfate sulfatase	
Mucolipidoses (ML)		
I-cell disease (ML II) and pseudo-Hurler polydystrophy	Deficiency of phosphorylating enzymes essential for the formation of mannose-6-phosphate recognition marker; acid hydrolases lacking the recognition marker cannot be targeted to the lysosomes but are secreted extracellularly	Mucopolysaccharide, glycolipid
Other Diseases of Complex Carbohydrates		
Fucosidosis	α-Fucosidase	Fucose-containing sphingolipids and glycoprotein fragments
Mannosidosis	α-Mannosidase	Mannose-containing oligosaccharides
Aspartylglycosaminuria	Aspartylglycosamine amide hydrolase	Aspartyl-2-deoxy-2-acetamido-glycosylamine
Other Lysosomal Storage Diseases		
Wolman disease	Acid lipase	Cholesterol esters, triglycerides
Acid phosphate deficiency	Lysosomal acid phosphatase	Phosphate esters

of cytoplasmic inclusions can be visualized, the most prominent being whorled configurations within lysosomes composed of onion-skin layers of membranes (Fig. 5-14*B*). In time, there is progressive destruction of neurons, proliferation of microglia, and accumulation of complex lipids in phagocytes within the brain substance. A similar process occurs in the cerebellum as well as in neurons throughout the basal ganglia, brain stem, spinal cord, and dorsal root ganglia and in the neurons of the autonomic nervous system. The ganglion cells in the retina are similarly swollen with GM_2 ganglioside, particularly at the margins of the macula. A **cherry-red spot** thus appears in the macula, representing accentuation of the normal color of the macular choroid

contrasted with the pallor produced by the swollen ganglion cells in the remainder of the retina (Chapter 29). This finding is characteristic of Tay-Sachs disease and other storage disorders affecting the neurons.

Many alleles have been identified at the α-subunit locus, each associated with a variable degree of enzyme deficiency and hence with variable clinical manifestations. The affected infants appear normal at birth but begin to manifest signs and symptoms at about age 6 months. There is relentless motor and mental deterioration, beginning with motor incoordination, mental obtundation leading to muscular flaccidity, blindness, and increasing dementia. Sometime during the early course of the disease, the characteristic, but not pathognomonic, cherry-red spot appears in the macula of the eye grounds in almost all patients. Over the span of 1 or 2 years, a complete, pathetic vegetative state is reached, followed by death at age 2 to 3 years.

Antenatal diagnosis and carrier detection are possible by enzyme assays and DNA-based analysis.[25] The clinical features of the two other forms of GM_2 gangliosidosis (see Fig. 5-13), Sandhoff disease, resulting from β-subunit defect, and GM_2 activator deficiency, are similar to those of Tay-Sachs disease.

Figure 5-13 The three-gene system required for hexosaminidase A activity and the diseases that result from defects in each of the genes. The function of the activator protein is to bind the ganglioside substrate and present it to the enzyme. *(Modified from Sandhoff K, et al: The GM_2 gangliosidoses. In Scriver CR, et al [eds]: The Metabolic Basis of Inherited Disease, 6th ed. New York, McGraw-Hill, 1989, p. 1824.)*

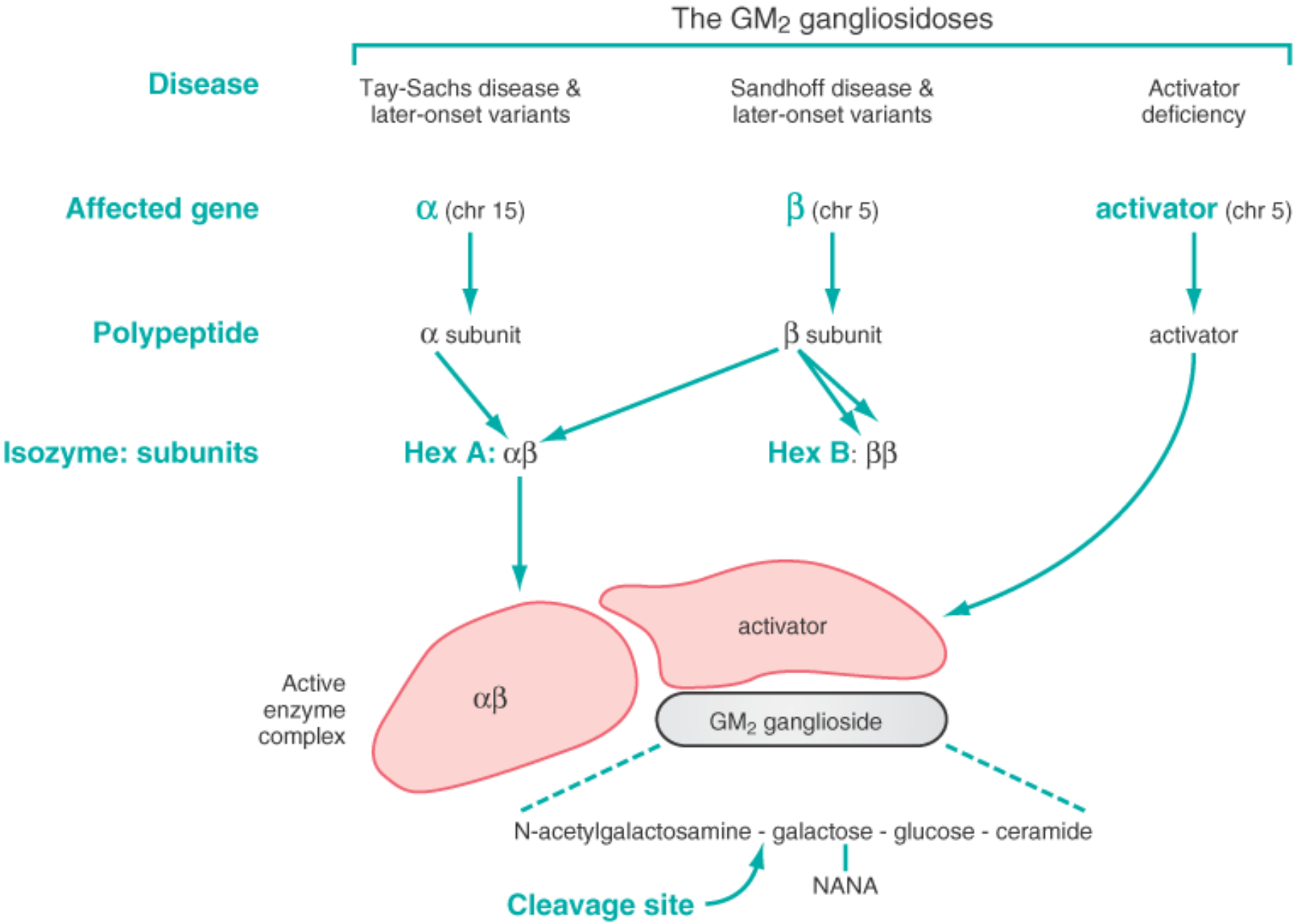


Figure 5-14 Ganglion cells in Tay-Sachs disease. *A*, Under the light microscope, a large neuron has obvious lipid vacuolation. *(Courtesy of Dr. Arthur Weinberg, Department of Pathology, University of Texas Southwestern Medical Center, Dallas.) B*, A portion of a neuron under the electron microscope shows prominent lysosomes with whorled configurations. Part of the nucleus is shown above. *(Electron micrograph courtesy of Dr. Joe Rutledge, University of Texas Southwestern Medical Center, Dallas, TX.)*

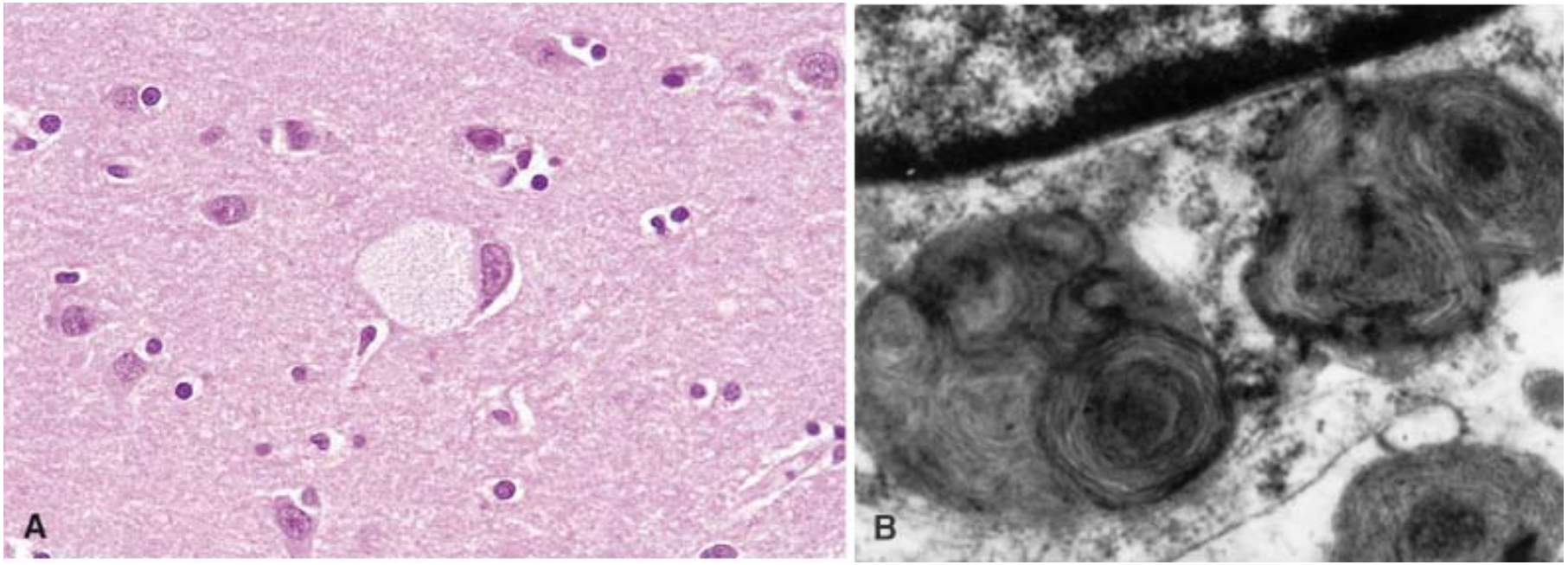


Figure 5-15 Niemann-Pick disease in liver. The hepatocytes and Kupffer cells have a foamy, vacuolated appearance owing to deposition of lipids. *(Courtesy of Dr. Arthur Weinberg, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

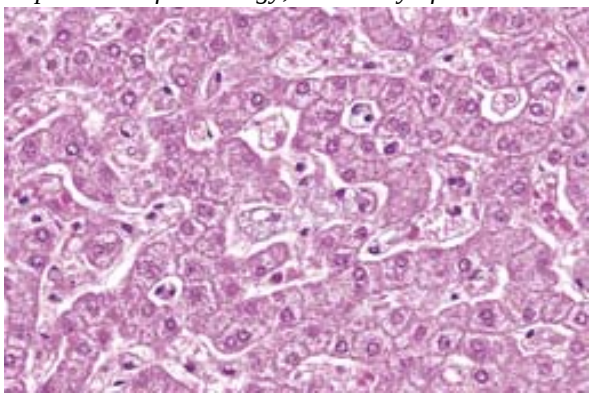


Figure 5-16 Gaucher disease involving the bone marrow. *A*, Gaucher cells with abundant lipid-laden granular cytoplasm. *B*, Electron micrograph of Gaucher cells with elongated distended lysosomes. *(Courtesy of Dr. Matthew Fries, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

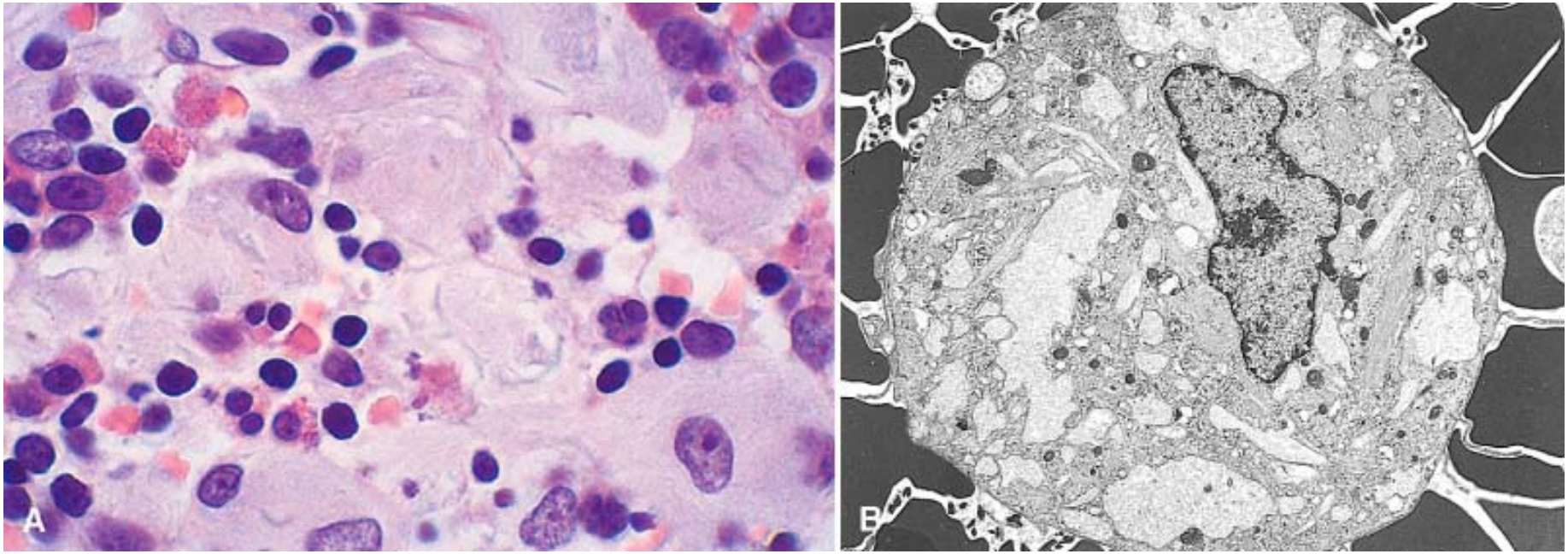
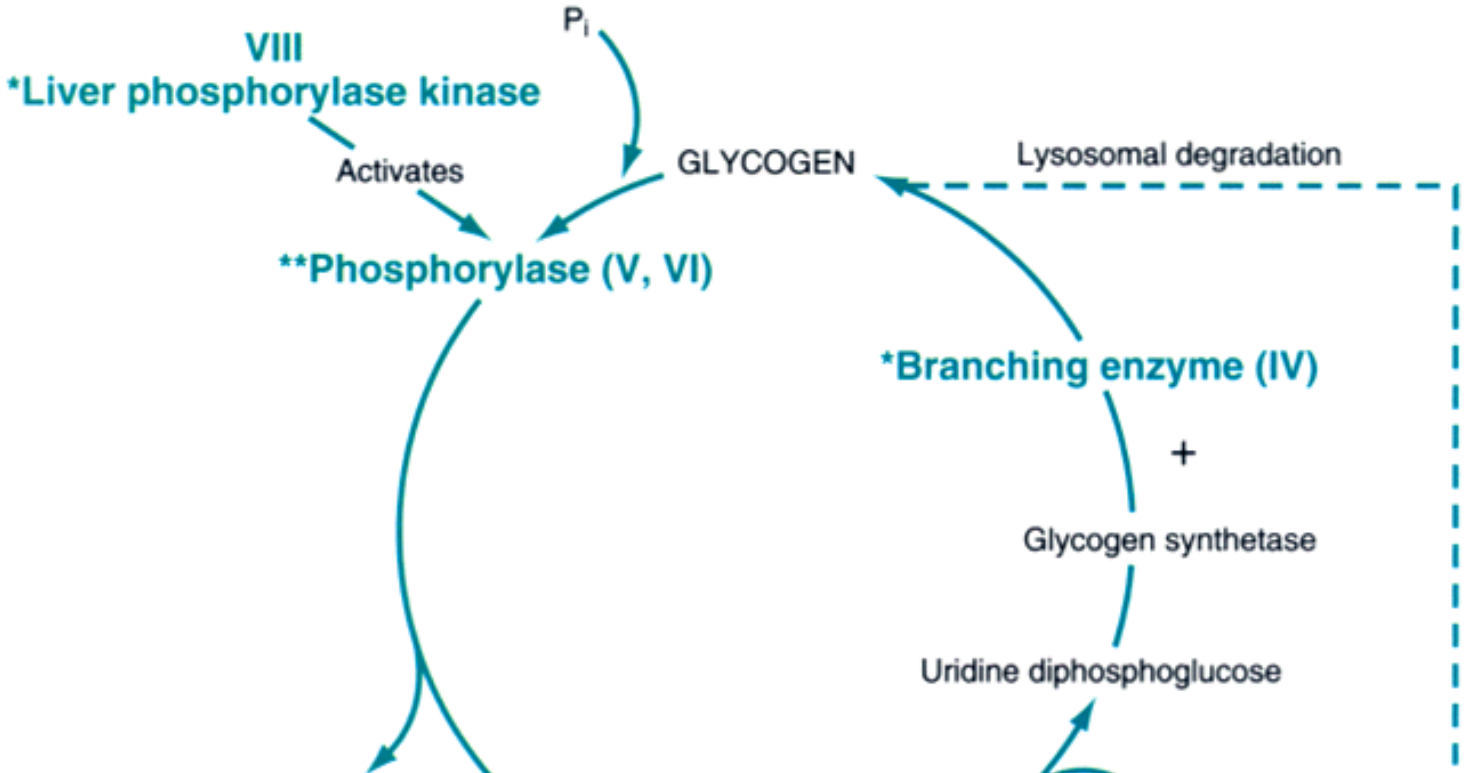


Figure 5-17 Pathways of glycogen metabolism. Asterisks mark the enzyme deficiencies associated with glycogen storage diseases. Roman numerals indicate the type of glycogen storage disease associated with the given enzyme deficiency. Types V and VI result from deficiencies of muscle and liver phosphorylases, respectively. *(Modified from Hers H, et al: Glycogen storage diseases. In Scriver CR, et al [eds]: The Metabolic Basis of Inherited Disease, 6th ed. New York, McGraw-Hill, 1989, p. 425.)*



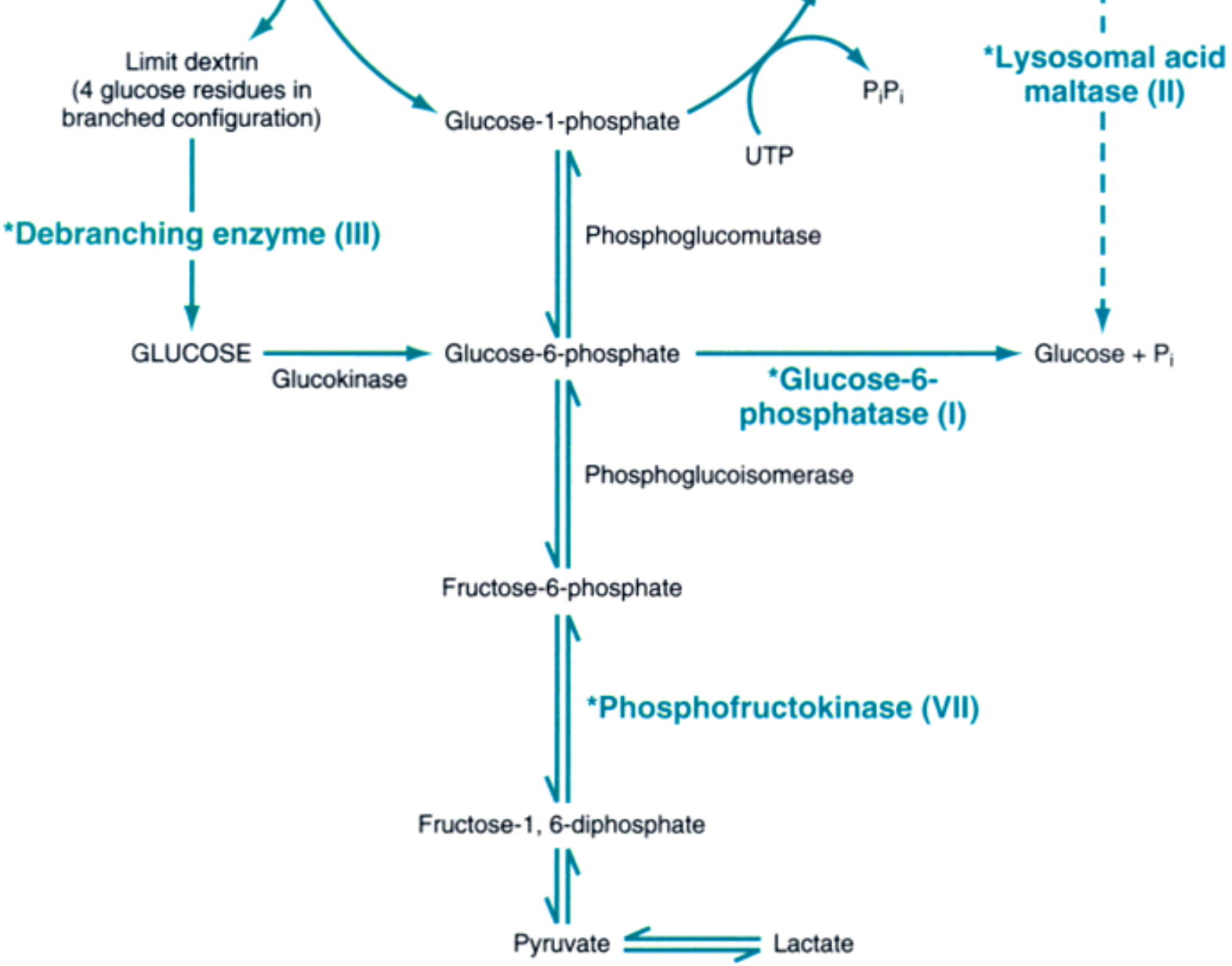


Figure 5-18 *Top*, Simplified schema of normal glycogen metabolism in the liver and skeletal muscles. *Middle*, Effects of an inherited deficiency of hepatic enzymes involved in glycogen metabolism. *Bottom*, Consequences of a genetic deficiency in the enzymes that metabolize glycogen in skeletal muscles.

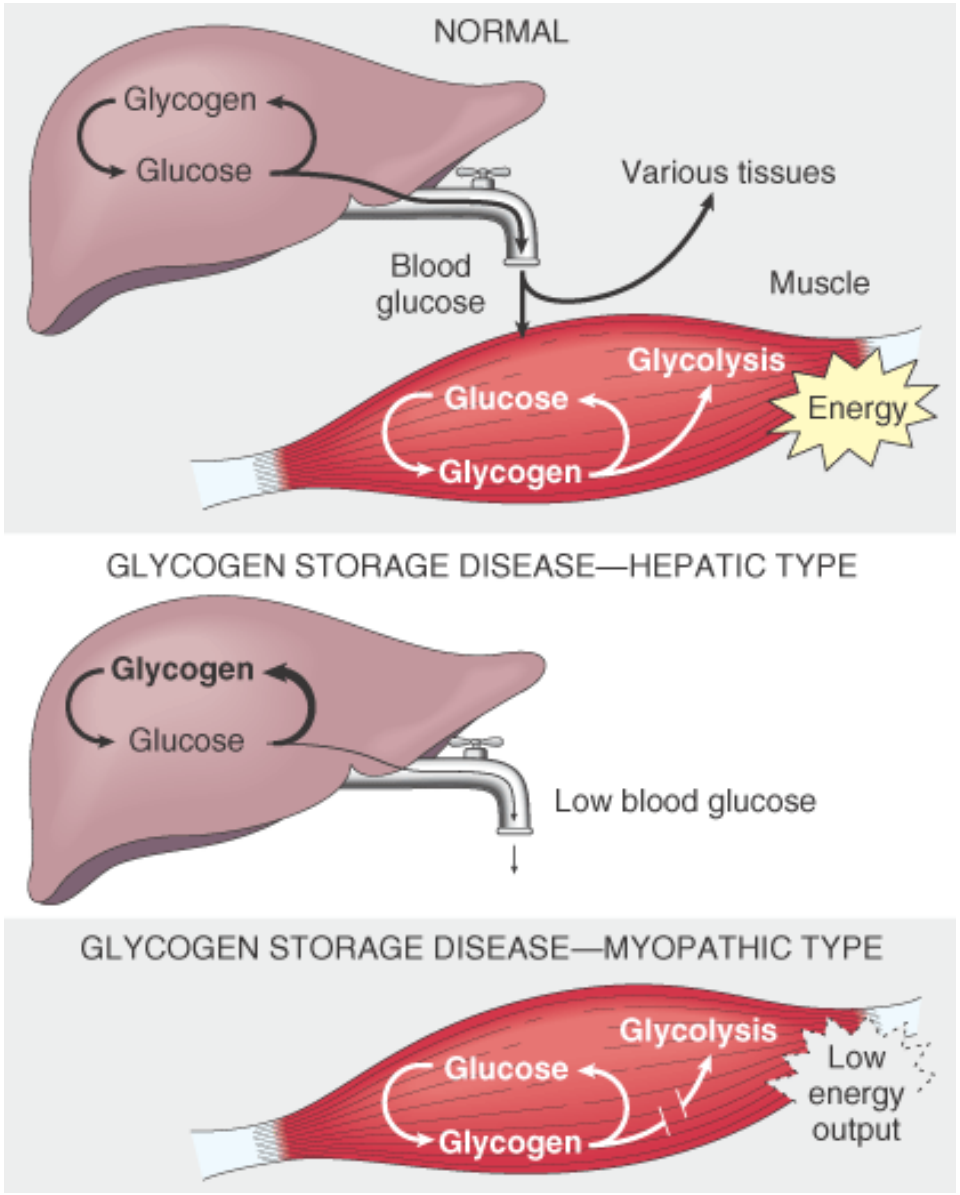


Figure 5-19 Pompe disease (glycogen storage disease type II). *A*, Normal myocardium with abundant eosinophilic cytoplasm. *B*, Patient with Pompe disease (same magnification) showing the myocardial fibers full of glycogen seen as clear spaces. *(Courtesy of Dr. Trace Worrell, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

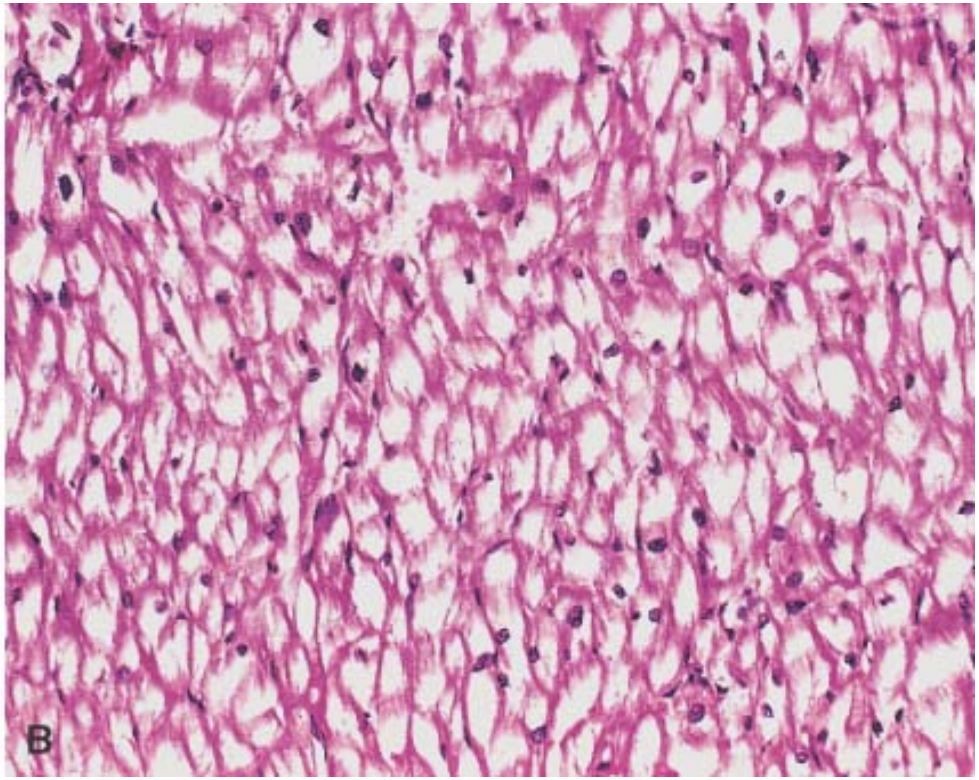


TABLE 5-7 -- Principal Subgroups of Glycogenoses

Clinicopathologic Category	Specific Type	Enzyme Deficiency	Morphologic Changes	Clinical Features
Hepatic Type	Hepatorenal—von Gierke disease (type I)	Glucose-6-phosphatase	Hepatomegaly—intracytoplasmic accumulations of glycogen and small amounts of lipid; intranuclear glycogen	In untreated patients: failure to thrive, stunted growth, hepatomegaly, and renomegaly. Hypoglycemia due to failure of glucose mobilization, often leading to convulsions. Hyperlipidemia and hyperuricemia resulting from deranged glucose metabolism; many patients develop gout and skin xanthomas. Bleeding tendency due to platelet dysfunction. With treatment most survive and develop late complications, e.g., hepatic adenomas
			Renomegaly—intracytoplasmic accumulations of glycogen in cortical tubular epithelial cells	
Myopathic Type	McArdle syndrome (type V)	Muscle phosphorylase	Skeletal muscle only—accumulations of glycogen predominant in subsarcolemmal location	Painful cramps associated with strenuous exercise. Myoglobinuria occurs in 50% of cases. Onset in adulthood (>20 years). Muscular exercise fails to raise lactate level in venous blood. Serum creatine kinase always elevated. Compatible with normal longevity
Miscellaneous Types	Generalized glycogenosis —Pompe disease (type II)	Lysosomal glucosidase (acid maltase)	Mild hepatomegaly—ballooning of lysosomes with glycogen, creating lacy cytoplasmic pattern	Massive cardiomegaly, muscle hypotonia, and cardiorespiratory failure within 2 years. A milder adult form with only skeletal muscle involvement, presenting

			Cardiomegaly—glycogen within sarcoplasm as well as membrane-bound	with chronic myopathy
			Skeletal muscle—similar to changes in heart	

the urine if allowed to stand and undergo oxidation.[38] The gene encoding homogentisic oxidase, mapped to 3q21, was cloned in 1996,[39] 64 years after the initial description of the disease by Garrod.

Morphology.

The retained homogentisic acid selectively binds to collagen in connective tissues, tendons, and cartilage, imparting to these tissues a blue-black pigmentation **(ochronosis)** most evident in the ears, nose, and cheeks. **The most serious consequences of ochronosis, however, stem from deposits of the pigment in the articular cartilages of the joints.** In some obscure manner, the pigmentation causes the cartilage to lose its normal resiliency and become brittle and fibrillated.[40] Wear-and-tear erosion of this abnormal cartilage leads to denudation of the subchondral bone, and often tiny fragments of the fibrillated cartilage are driven into the underlying bone, worsening the damage. The vertebral column, particularly the intervertebral disc, is the prime site of attack, but later the knees, shoulders, and hips may be affected. The small joints of the hands and feet are usually spared.

The metabolic defect is present from birth, but the degenerative arthropathy develops slowly and usually does not become clinically evident until the thirties. Although it is not life-threatening, it may be severely crippling. The disability may be as extreme as that encountered in the severe forms of osteoarthritis (Chapter 26) of the elderly, but in alkaptonuria the arthropathy occurs at a much earlier age.

DISORDERS ASSOCIATED WITH DEFECTS IN PROTEINS THAT REGULATE CELL GROWTH

Normal growth and differentiation of cells is regulated by two classes of genes: proto-oncogenes and tumor-suppressor genes, whose products promote or restrain cell growth (Chapter 7). It is now well established that mutations in these two classes of genes are important in the pathogenesis of tumors. In the vast majority of cases, cancer-causing mutations affect somatic cells and hence are not passed in the germ line. In approximately 5% of all cancers, however, mutations transmitted through the germ line contribute to the development of cancer. Most familial cancers are inherited in an autosomal dominant fashion, but a few recessive disorders have also been described. This subject is discussed in greater detail in Chapter 7 . Here we provide an example of two common familial neoplasms.

Neurofibromatosis: Types 1 and 2

Neurofibromatoses comprise two autosomal dominant disorders, affecting approximately 100,000 people in the United States. They are referred to as *neurofibromatosis type 1* (previously called *von Recklinghausen disease*) and *neurofibromatosis type 2* (previously called *acoustic neurofibromatosis*). Although there is some overlap in clinical features, these two entities are genetically distinct.[41]

Neurofibromatosis type 1 is a relatively common disorder, with a frequency of almost 1 in 3000. Although approximately 50% of the patients have a definite family history consistent

with autosomal dominant transmission, the remainder appear to represent new mutations. In familial cases, the expressivity of the disorder is extremely variable, but the penetrance is 100%.

Neurofibromatosis type 1 has three major features: (1) *multiple neural tumors (neurofibromas) dispersed anywhere on or in the body*; (2) *numerous pigmented skin lesions, some of which are café au lait spots*; and (3) *pigmented iris hamartomas, also called Lisch nodules*. A bewildering assortment of other abnormalities (cited later) may accompany these cardinal manifestations.

Morphology.

The neurofibromas arise within or are attached to nerve trunks anywhere in the skin, including the palms and soles, as well as in every conceivable internal site, including the cranial nerves. Three types of neurofibromas are found in individuals with neurofibromatosis type 1: cutaneous, subcutaneous, and plexiform. **Cutaneous**, or dermal, neurofibromas are soft, sessile, or pedunculated lesions that vary in number from a few to many hundreds. **Subcutaneous neurofibromas** grow just beneath the skin; they are firm, round masses that are often painful. The cutaneous and subcutaneous neurofibromas may be less than 1 cm in diameter; moderate-sized pedunculated lesions; or huge, multilobar pendulous masses, 20 cm or more in greatest diameter. The third variant, referred to as **plexiform neurofibroma**, diffusely involves subcutaneous tissue and contains numerous tortuous, thickened nerves; the overlying skin is frequently hyperpigmented. These may grow to massive proportions, causing striking enlargement of a limb or some other body part. Similar tumors may occur internally, and in general the deeply situated lesions tend to be large. Microscopically, neurofibromas reveal proliferation of all the elements in the peripheral nerve, including neurites, Schwann cells, and fibroblasts. Typically, these components are dispersed in a loose, disorderly pattern, often in a loose, myxoid stroma. Elongated, serpentine Schwann cells predominate, with their slender, spindle-shaped nuclei. The loose and disorderly architecture helps differentiate these neural tumors from schwannomas. The latter, composed entirely of Schwann cells, virtually never undergo malignant transformation, whereas plexiform neurofibromas become malignant in about 5% of patients with neurofibromatosis type 1.[42] Malignant transformation is most common in the large plexiform tumors attached to major nerve trunks of the neck or extremities. The superficial lesions, despite their size, rarely become malignant.

The cutaneous pigmentations, the second major component of this syndrome, are present in more than 90% of patients. Most commonly, they appear as light brown **café au lait** macules, with generally smooth borders, often located over nerve trunks. They are usually round to ovoid, with their long axes parallel to the underlying cutaneous nerve. Although normal individuals may have a few café au lait spots, it is a clinical maxim that when six or more spots greater than 1.5 cm in diameter are present in an adult, the patient is likely to have neurofibromatosis type 1.

Lisch nodules (pigmented hamartomas in the iris) are present in more than 94% of patients age 6 years or older. They do not produce any symptoms but are helpful in establishing the diagnosis.

A wide range of associated abnormalities has been reported in these patients. Perhaps most common (seen in 30% to 50% of patients) are skeletal lesions, which take a variety of forms, including (1) erosive defects owing to contiguity of neurofibromas to bone, (2) scoliosis, (3) intraosseous cystic lesions, (4) subperiosteal bone cysts, and (5) pseudoarthrosis of the tibia. Patients with neurofibromatosis type 1 have a twofold to fourfold greater risk of developing other tumors, especially Wilms tumors, rhabdomyosarcomas, meningiomas, optic gliomas, and pheochromocytomas. Affected children are at increased risk of developing chronic myeloid leukemia.

Although some patients with this condition have normal intelligence quotients (IQs), there is an unmistakable tendency for reduced intelligence. When neurofibromas arise within the gastrointestinal tract, intestinal obstruction or gastrointestinal bleeding may occur. Narrowing of a renal artery by a tumor may induce hypertension. Owing to variable expression of the gene, the range of clinical presentations is almost limitless, but ultimately the diagnosis rests on the concurrence of multiple café au lait spots and multiple skin tumors. The neurofibromatosis type 1 *(NF-1)* gene has been mapped to chromosome 17q11.2. It encodes a protein called *neurofibromin*, which down-regulates the function of the $p21^{Ras}$ oncoprotein (see section on oncogenes, Chapter 7). *NF-1* therefore belongs to the family of tumor-suppressor genes.

Neurofibromatosis type 2 is an autosomal dominant disorder in which patients develop a range of tumors, most commonly bilateral acoustic schwannomas and multiple meningiomas. Gliomas, typically ependymomas of the spinal cord, also occur in these patients. Many individuals with neurofibromatosis type 2 also have non-neoplastic lesions, which include nodular ingrowth of Schwann cells into the spinal cord (schwannosis), meningioangiomatosis (a proliferation of meningeal cells and blood vessels that grows into the brain), and glial hamartia (microscopic nodular collections of glial cells at abnormal locations, often in the superficial and deep layers of the cerebral cortex). Café au lait spots are present, but Lisch nodules in the iris are not found. This disorder is much less common than neurofibromatosis type 1, having a frequency of 1 in 40,000 to 50,000.

The *NF-2* gene, located on chromosome 22q12, is also a tumor-suppressor gene. As further discussed in Chapter 7 , the product of the *NF-2* gene, called *merlin*, shows structural similarity to the ezrin, radixin, moesin (ERM) family of proteins. These cytoskeletal proteins interact with actin on the one hand and membrane proteins on the cell surface on the other hand. It is thought

that *merlin* regulates contact inhibition and proliferation of Schwann cells.[43]

Disorders with Multifactorial Inheritance

As pointed out earlier, the multifactorial disorders result from the combined actions of environmental influences and two or more mutant genes having additive effects. The genetic component exerts a dosage effect—the greater the number of inherited deleterious genes, the more severe the expression of the disease. Because environmental factors significantly influence

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the expression of these genetic disorders, the term polygenic inheritance should not be used.

A number of normal phenotypic characteristics are governed by multifactorial inheritance, such as hair color, eye color, skin color, height, and intelligence. These characteristics exhibit a continuous variation in population groups, producing the standard bell-shaped curve of distribution. Environmental influences, however, significantly modify the phenotypic expression of multifactorial traits. For example, type II diabetes mellitus has many of the features of a multifactorial disorder. It is well recognized clinically that individuals often first manifest this disease after weight gain. Thus, obesity as well as other environmental influences unmasks the diabetic genetic trait. Nutritional influences may cause even monozygous twins to achieve different heights. The culturally deprived child cannot achieve his or her full intellectual capacity.

The following features characterize multifactorial inheritance. These have been established for the multifactorial inheritance of congenital malformations and, in all likelihood, obtain for other multifactorial diseases.[44]

- The risk of expressing a multifactorial disorder is conditioned by the number of mutant genes inherited. Thus, the risk is greater in siblings of patients having severe expressions of the disorder. For example, the risk of cleft lip in the siblings of an index case is 2.5% if the cleft lip is unilateral but 6% if it is bilateral. Similarly, the greater the number of affected relatives, the higher is the risk for other relatives.
- The rate of recurrence of the disorder (in the range of 2% to 7%) is the same for all first-degree relatives (i.e., parents, siblings, and offspring) of the affected individual. Thus, if parents have had one affected child, the risk that the next child will be affected is between 2% and 7%. Similarly, there is the same chance that one of the parents will be affected.
- The likelihood that both identical twins will be affected is significantly less than 100% but is much greater than the chance that both nonidentical twins will be affected. Experience has proven, for example, that the frequency of concordance for identical twins is in the range of 20% to 40%.
- The risk of recurrence of the phenotypic abnormality in subsequent pregnancies depends on the outcome in previous pregnancies. When one child is affected, there is up to a 7% chance that the next child will be affected, but after two affected siblings, the risk rises to about 9%.
- Expression of a multifactorial trait may be continuous (lack a distinct phenotype, e.g., height) or discontinuous (with a distinct phenotype, e.g., diabetes mellitus). In the latter, disease is expressed only when the combined influences of the genes and environment cross a certain threshold. In the case of diabetes, for example, the risk of phenotypic expression increases when the blood glucose levels go above a certain level.

Assigning a disease to this mode of inheritance must be done with caution. It depends on many factors but first on familial clustering and the exclusion of mendelian and chromosomal modes of transmission. A range of levels of severity of a disease is suggestive of multifactorial inheritance, but, as pointed out earlier, variable expressivity and reduced penetrance of single mutant genes may also account for this

TABLE 5-8 -- Multifactorial Disorders

Disorder	Chapter

Cleft lip or cleft palate (or both)	Chapter 10
Congenital heart disease	Chapter 12
Coronary heart disease	Chapter 12
Hypertension	Chapter 11
Gout	Chapter 27
Diabetes mellitus	Chapter 24
Pyloric stenosis	Chapter 17

phenomenon. Because of these problems, sometimes it is difficult to distinguish between mendelian and multifactorial inheritance.

In contrast to the mendelian disorders, many of which are uncommon, the multifactorial group includes some of the most common ailments to which humans are heir (Table 5-8). Most of these disorders are described in appropriate chapters elsewhere in this book.

Normal Karyotype

As is well known, human somatic cells contain 46 chromosomes; these comprise 22 homologous pairs of autosomes and two sex chromosomes, XX in the female and XY in the male. The study of chromosomes—*karyotyping*—is the basic tool of the cytogeneticist. The usual procedure of producing a chromosome spread is to arrest mitosis in dividing cells in metaphase by the use of mitotic spindle inhibitors (e.g., colcemid) and then to stain the chromosomes. In a metaphase spread, the individual chromosomes take the form of two chromatids connected at the centromere. A karyotype is a standard arrangement of a photographed or imaged stained metaphase spread in which chromosome pairs are arranged in order of decreasing length.

A variety of staining methods that allow identification of each individual chromosome on the basis of a distinctive and reliable pattern of alternating light and dark bands along the length of the chromosome have been developed. The one most commonly employed uses a Giemsa stain and is hence called *G banding*. A normal male karyotype with G banding is illustrated in Figure 5-20 . With G banding, approximately 400 to 800 bands per haploid set can be detected. The resolution obtained by banding techniques can be dramatically improved by obtaining the cells in prophase. The individual chromosomes appear markedly elongated, and up to 1500 bands per karyotype may be recognized. The use of these banding techniques permits certain identification of each chromosome as well as delineation of precise breakpoints and other subtle alterations, to be described later.

Before this discussion of the normal karyotype is concluded, reference must be made to commonly used cytogenetic terminology. Karyotypes are usually described using a shorthand system of notations. The following order is used: Total number of chromosomes is given first, followed by the sex chromosome complement, and finally the description of abnormalities in ascending numerical order. For example, a

Figure 5-20 Normal male karyotype with G banding. *(Courtesy of Dr. Nancy Schneider, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

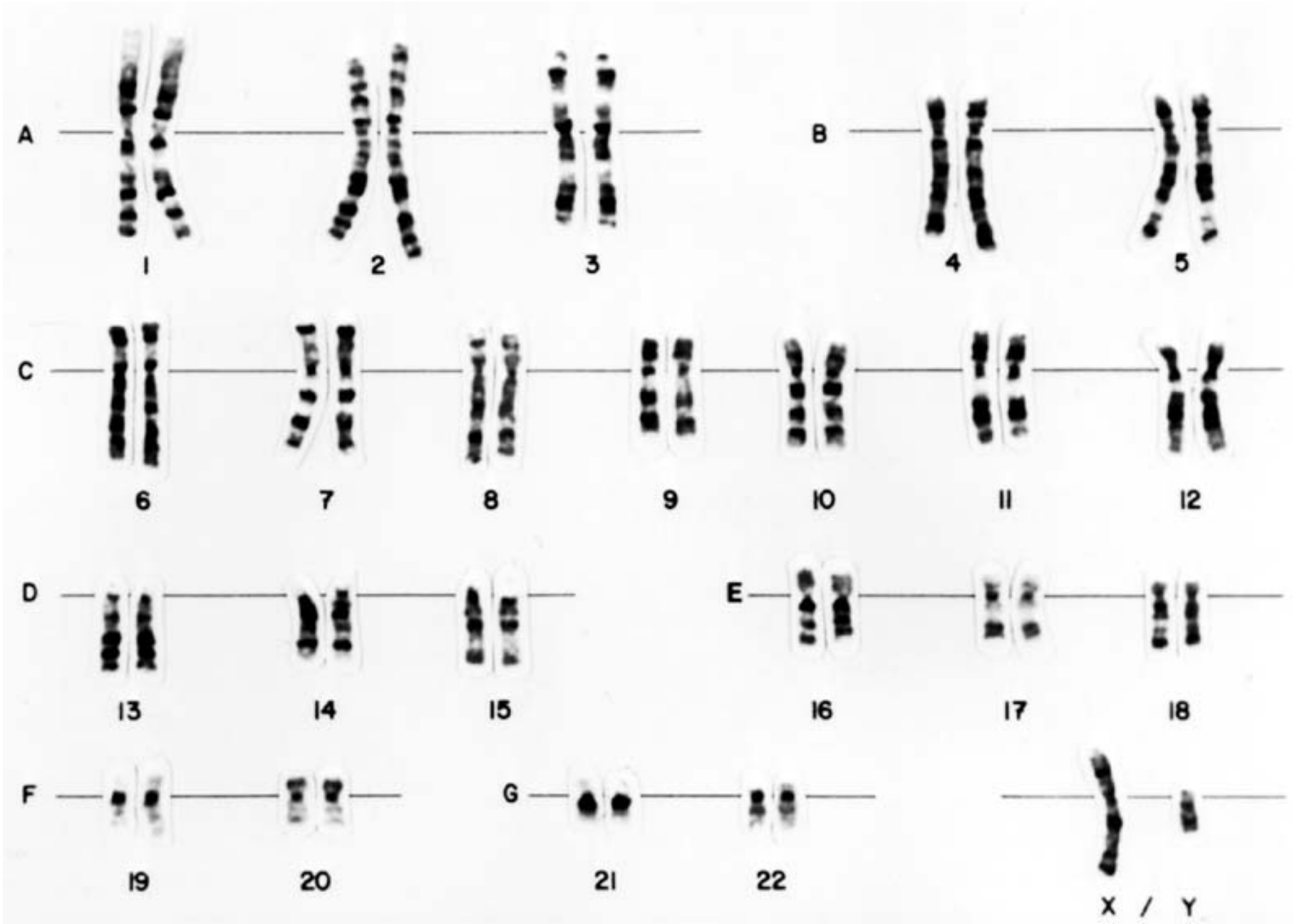


Figure 5-21 Details of banding pattern of the X chromosome (also called "idiogram"). Note the nomenclature of arms, regions, bands, and sub-bands. On the right side, the approximate locations of some genes that cause disease are indicated.

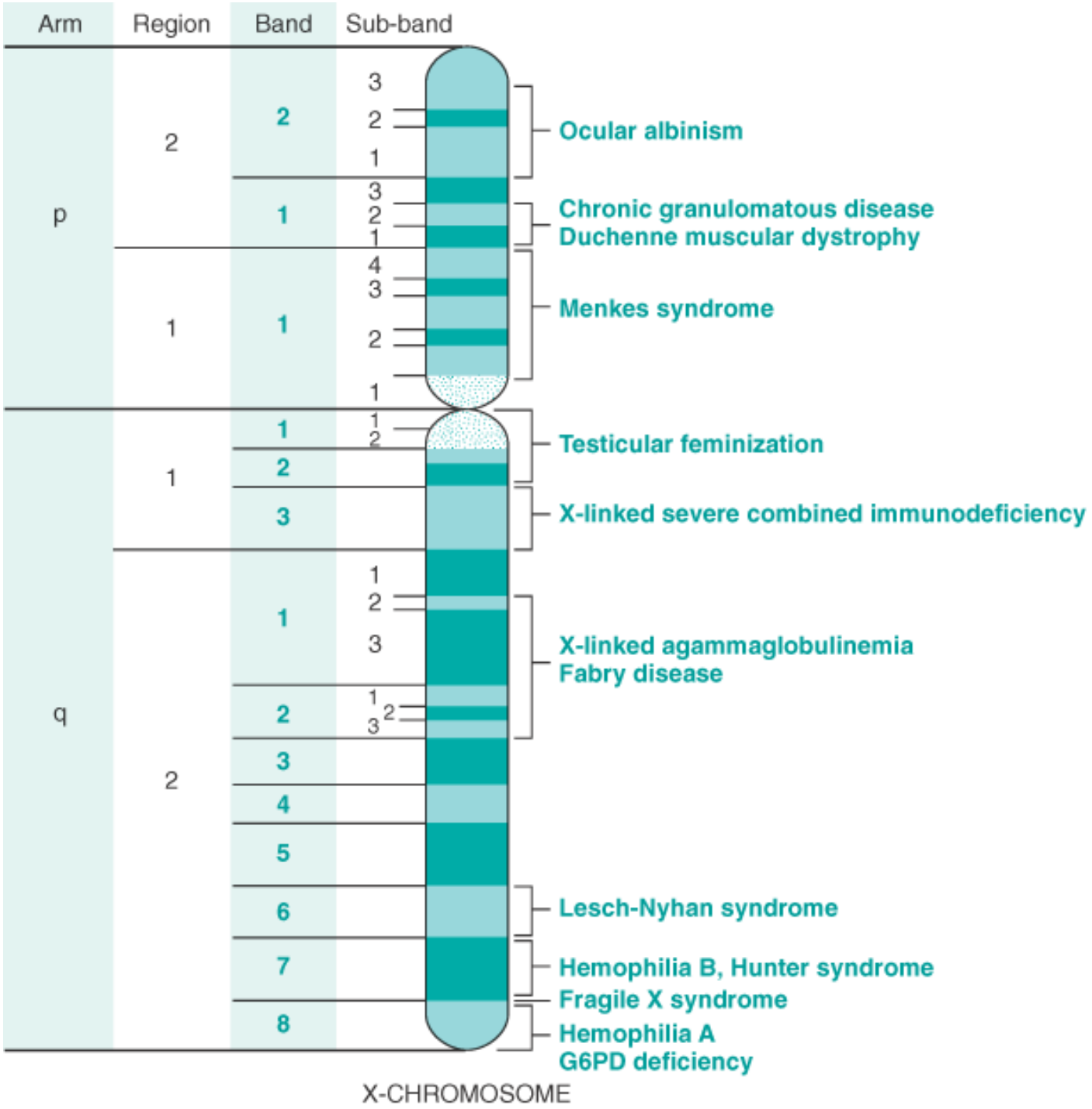


Figure 5-22 Fluorescence in situ hybridization (FISH). Interphase nuclei of a childhood hepatic cancer (hepatoblastoma) stained with a fluorescent DNA probe that hybridizes to chromosome 20. Under ultraviolet light, each nucleus reveals three bright yellow fluorescent dots, representing three copies of chromosome 20. Normal diploid cells *(not shown)* have two

fluorescent dots. *(Courtesy of Dr. Vijay Tonk, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

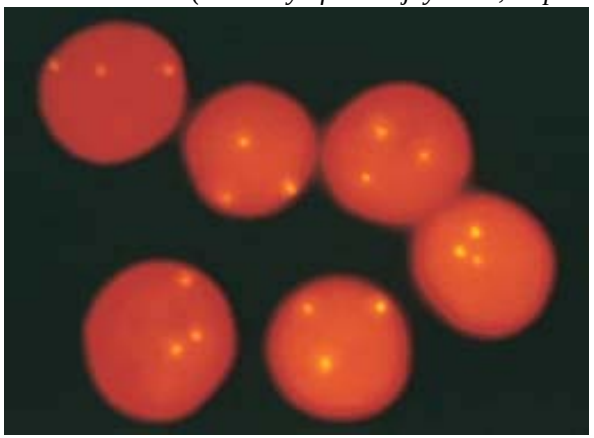


Figure 5-23 FISH. A metaphase spread in which two fluorescent probes, one for the terminal ends of chromosome 22 and the other for the D22S75 locus, which maps to chromosome 22, have been used. The terminal ends of the two chromosomes 22 have been labeled. One of the two chromosomes does not stain with the probe for the D22S75 locus, indicating a microdeletion in this region. This deletion gives rise to the 22q11.2 deletion syndrome. *(Courtesy of Dr. Nancy Schneider, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

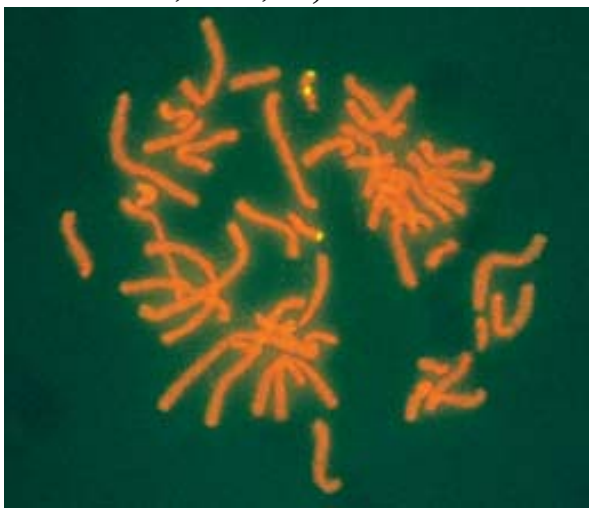


Figure 5-24 Chromosome painting with a library of chromosome 22-specific DNA probes. The presence of three fluorescent chromosomes indicates that the patient has trisomy 22. *(Courtesy of Dr. Charleen M. Moore, The University of Texas Health Science Center at San Antonio, TX.)*

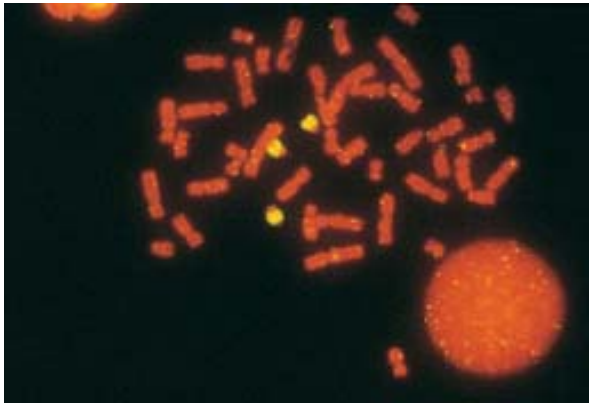


Figure 5-25 Spectral karyotype. *(Courtesy of Dr. Janet D. Rowley, University of Chicago Pritzker Medical School, Chicago, IL.)*

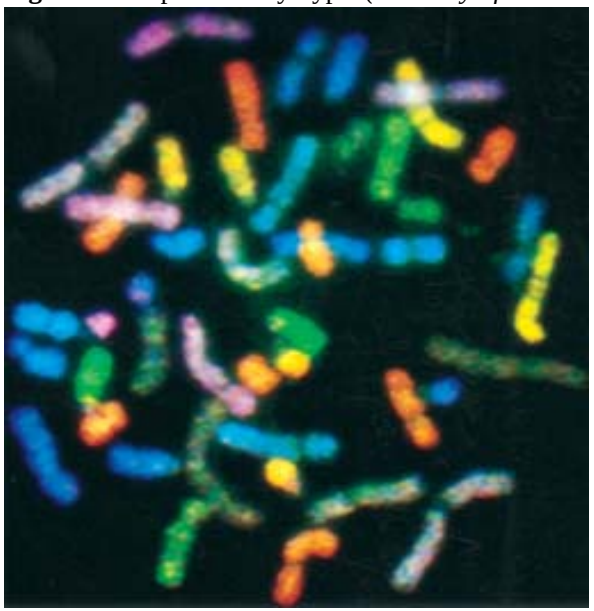


Figure 5-26 Types of chromosomal rearrangements.

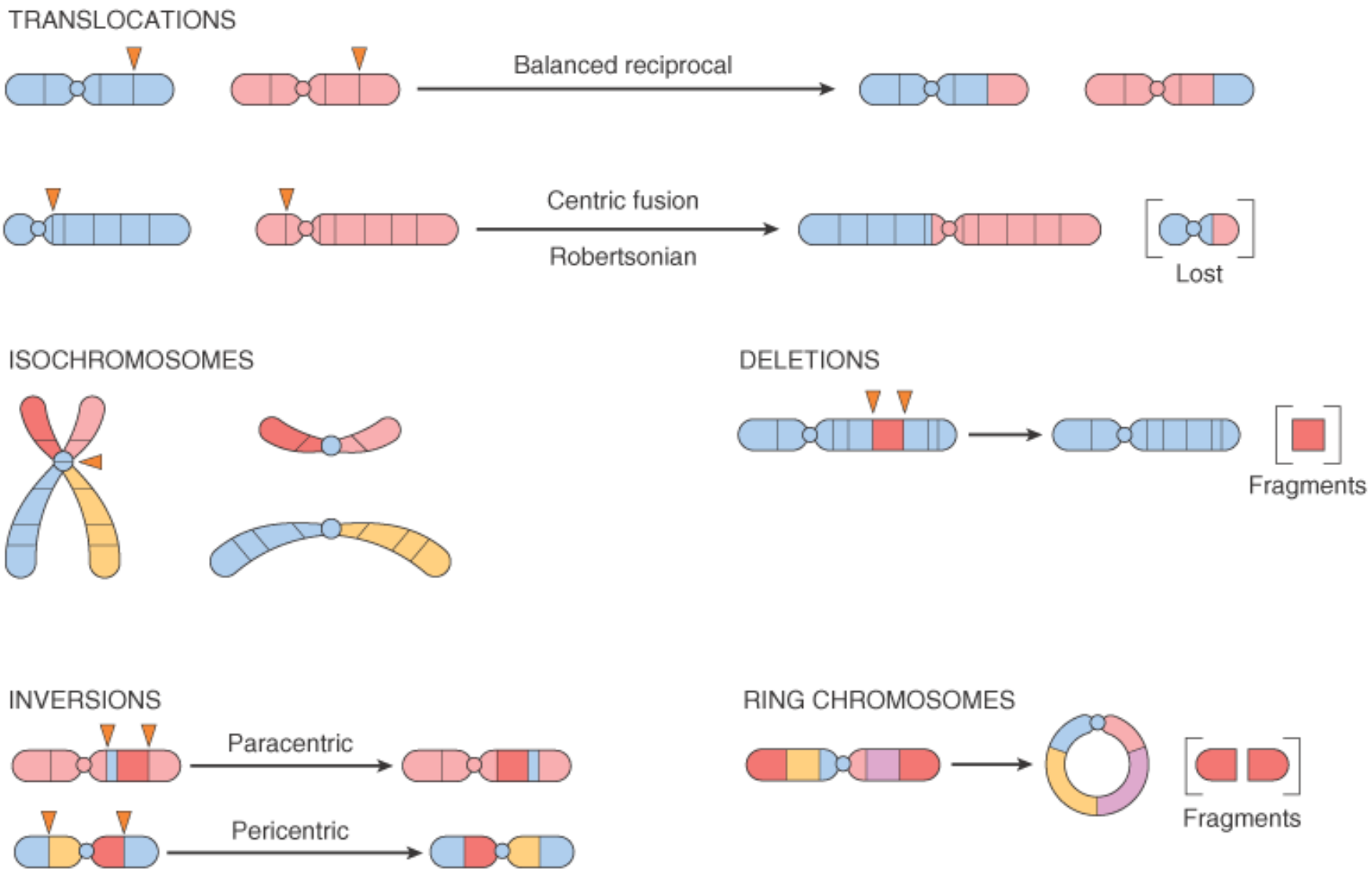


Figure 5-27 G banded karyotype of a male with trisomy 21. *(Courtesy of Dr. Nancy Schneider, University of Texas Southwestern Medical Center, Dallas, TX.)*

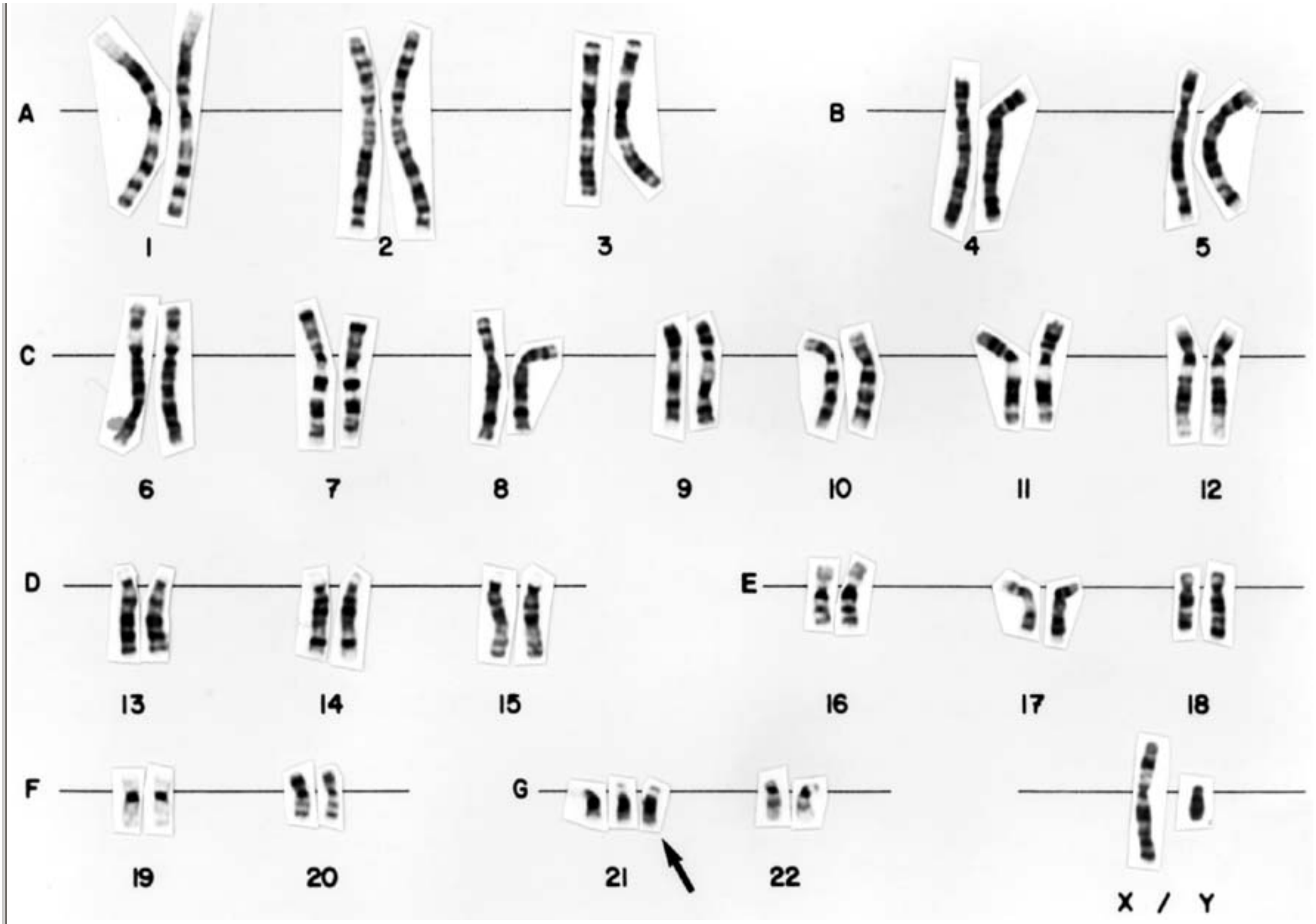
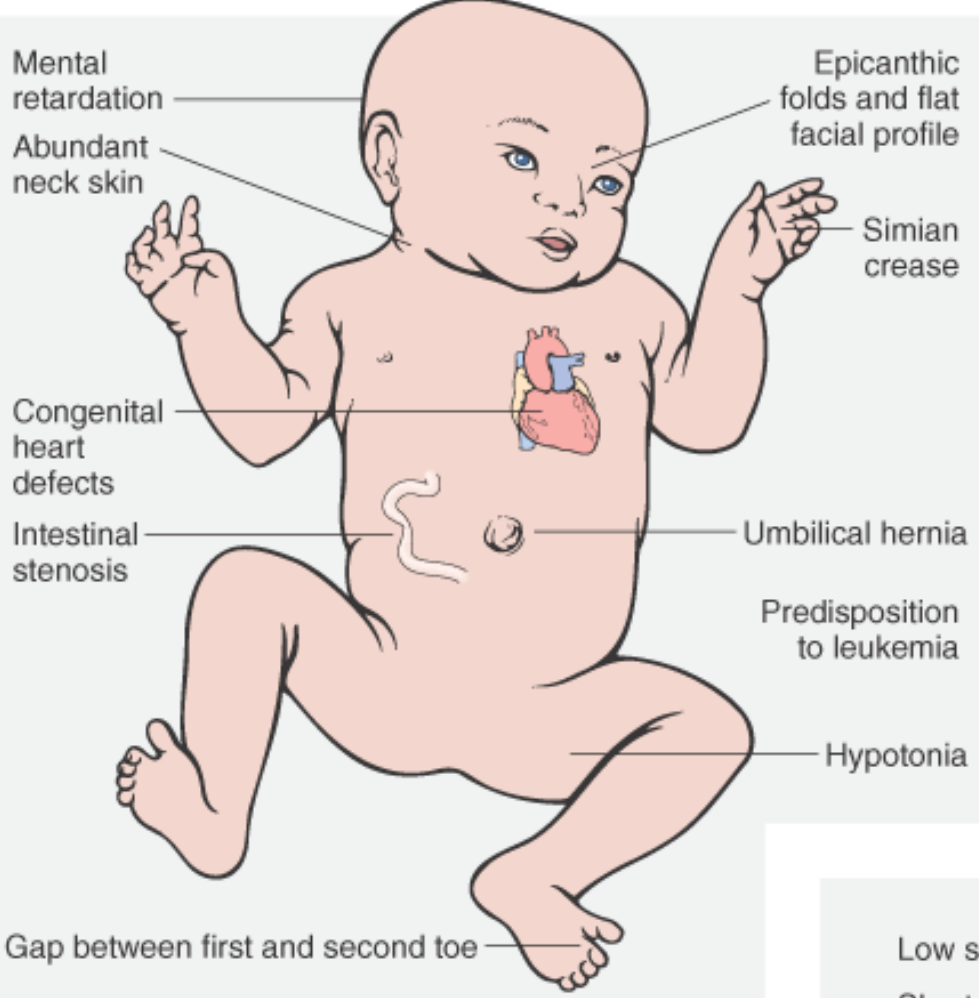


Figure 5-28 Clinical features and karyotypes of selected autosomal trisomies.

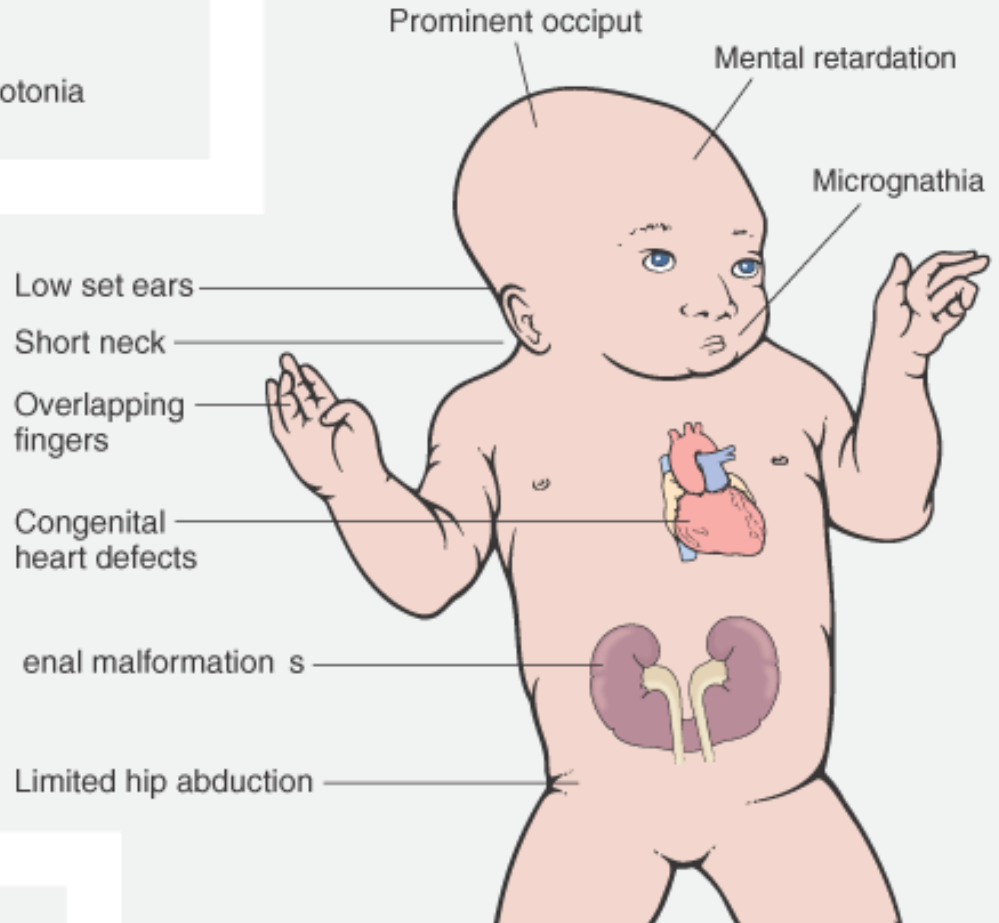


TRISOMY 21: DOWN SYNDROME

Incidence: 1 in 700 births

Karyotypes:

Trisomy 21 type:	47,XX, +21
Translocation type:	46,XX,der(14;21)(q10;q10),+21
Mosaic type:	46,XX/47,XX, +21



TRISOMY 18: EDWARDS SYNDROME

Incidence: 1 in 8000 births

Karyotypes:

Trisomy 18 type:	47,XX, +18
Mosaic type:	46,XX/47,XX, +18

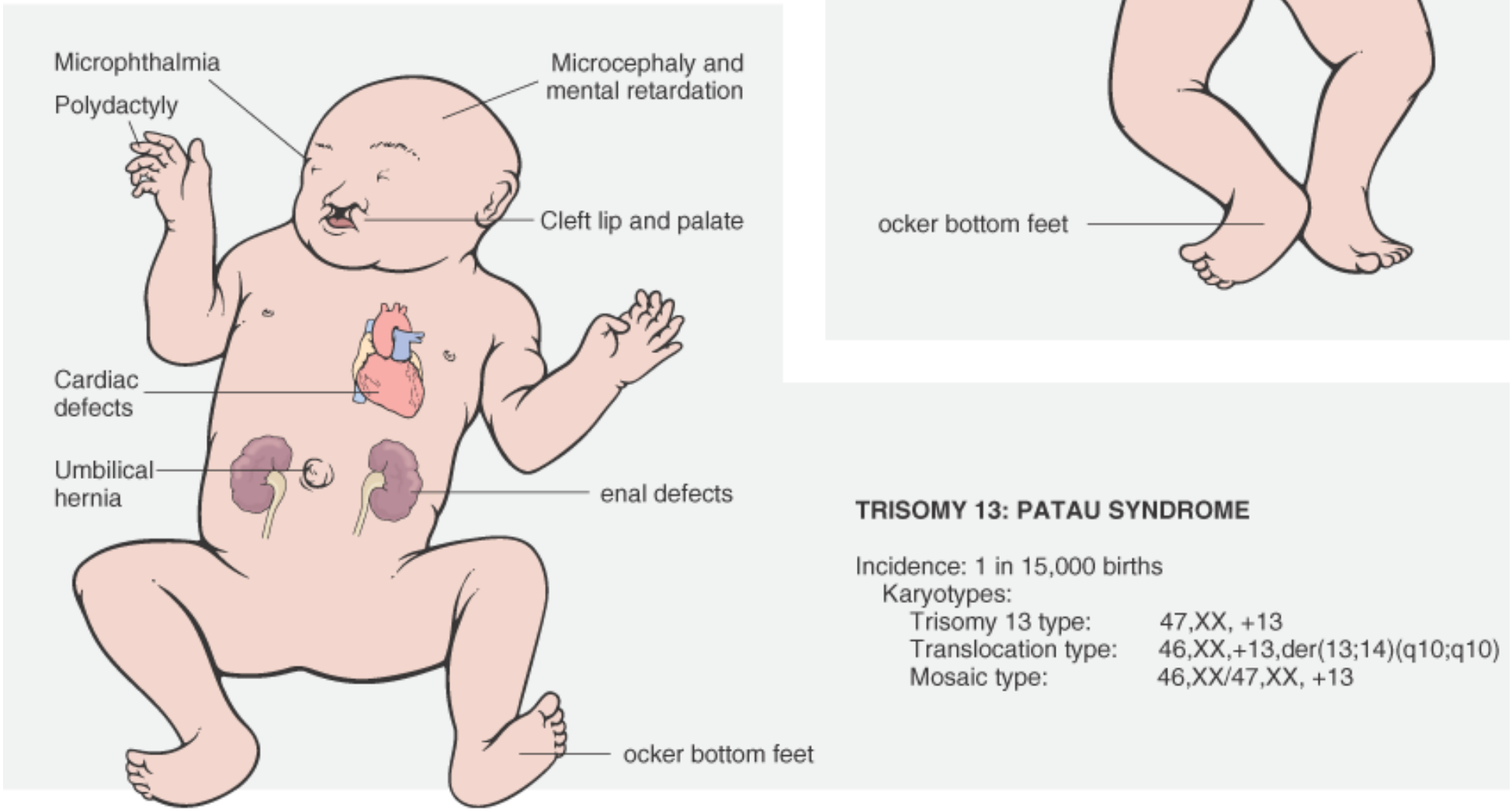


Figure 5-29 Clinical features and karyotypes of Turner syndrome.

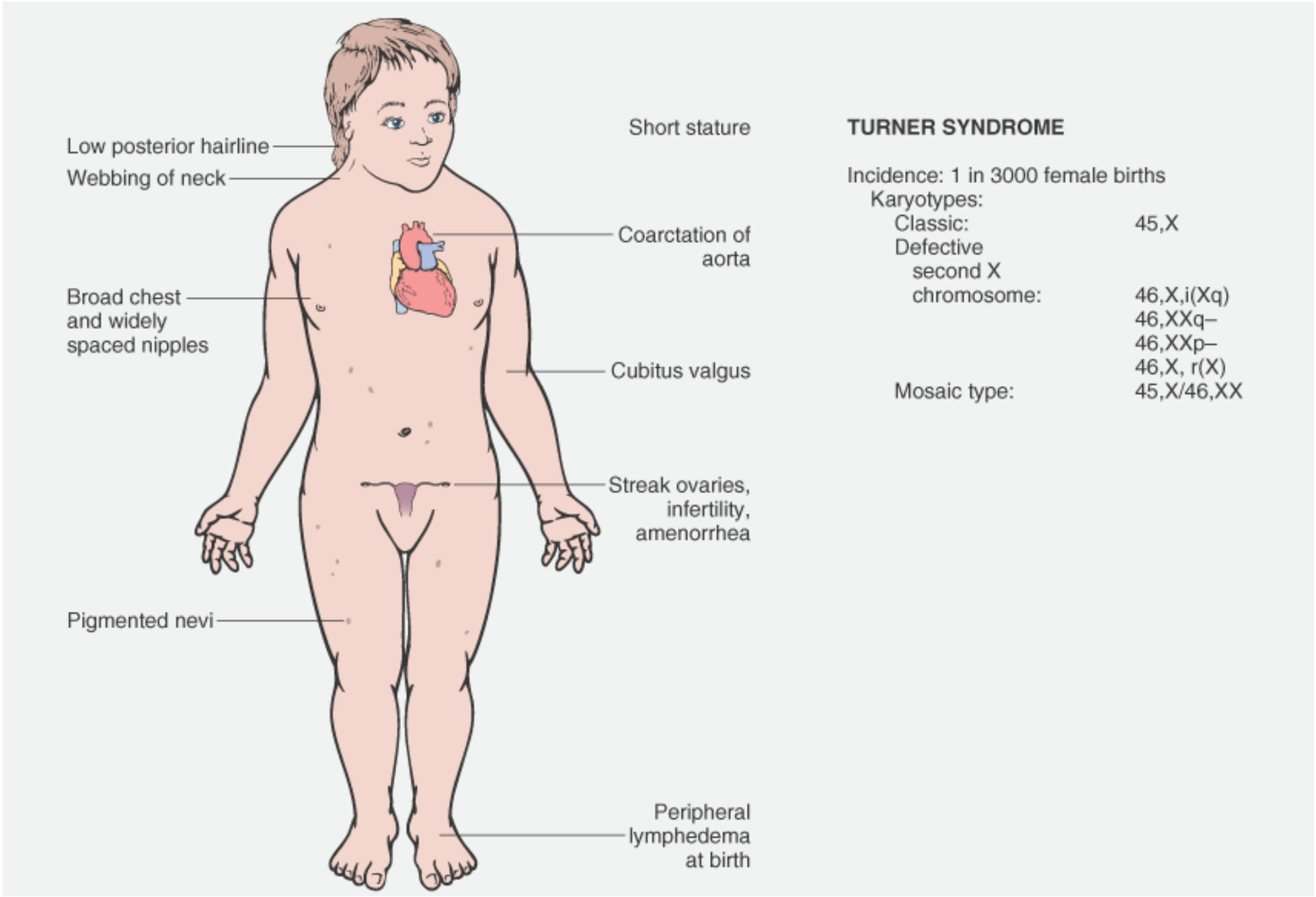


Figure 5-30 Turner syndrome critical regions and (candidate) genes. *SHOX*, short homeobox gene; *EIF1AX*, eukaryotic initiation factor 1A; *ZFX*, zinc finger X (transcription factor); *USP9X*, homologue of *Drosophila* gene involved in öogenesis; *DBX*, dead box polypeptide 3,X, a spermatogenesis gene; *UTX*, ubiquitously transcribed tetratricopeptide repeat gene, X chromosome; *SMCX*, homologue of the Y-encoded male antigen HY; *RPS4X* isoform of ribosomal protein S4 involved in lymphatic development. *(Courtesy of Dr. Andrew Zinn, University of Texas Southwestern Medical School, Dallas, TX.)*

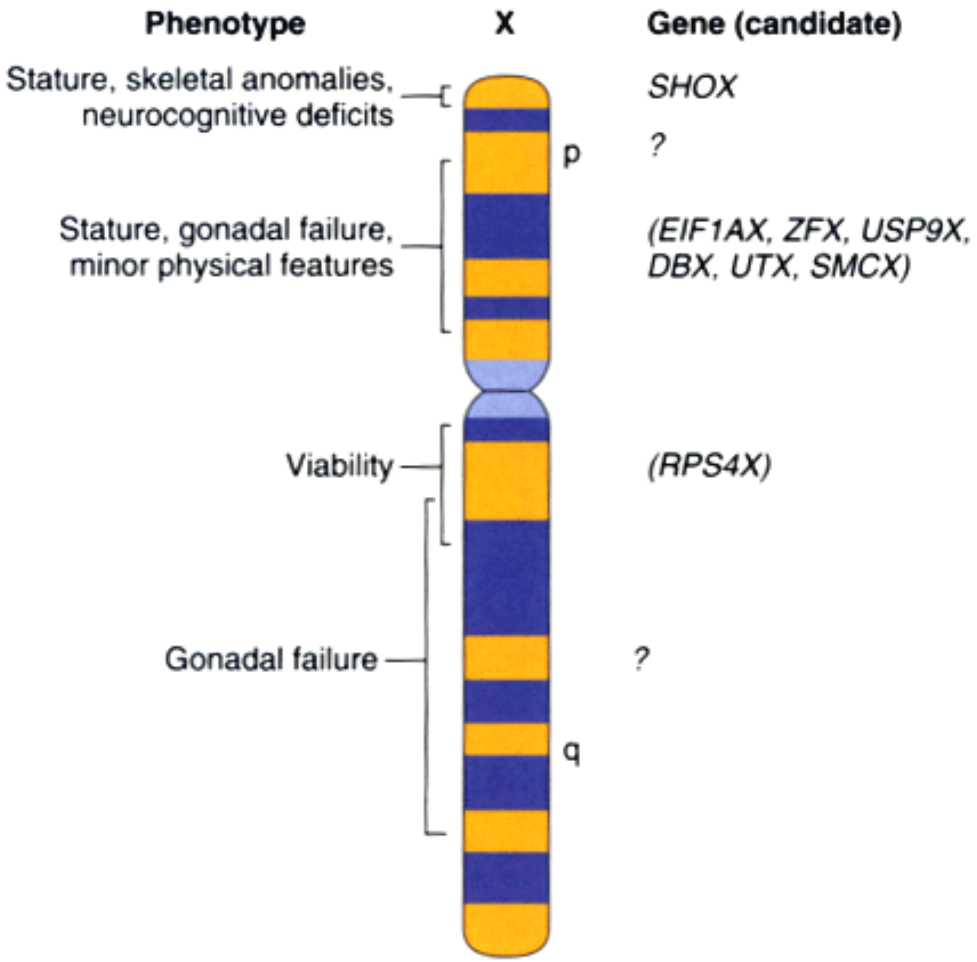


Figure 5-31 Fragile-X, seen as discontinuity of staining. *(Courtesy of Dr. Patricia Howard-Peebles, University of Texas Southwestern Medical Center, Dallas, TX.)*

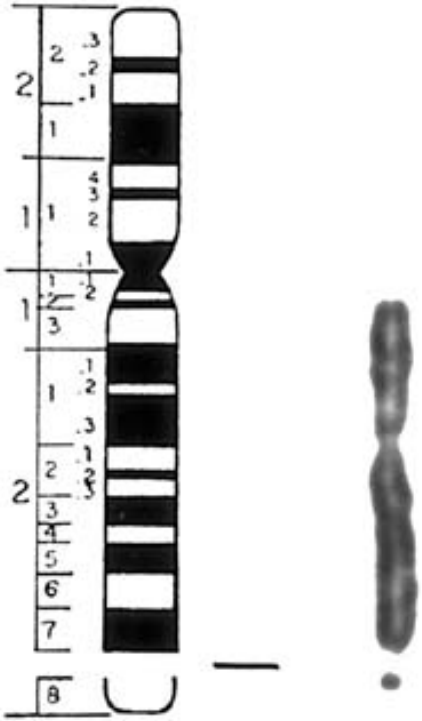


Figure 5-32 Fragile-X pedigree. Note that in the first generation all sons are normal and all females are carriers. During oogenesis in the carrier female, premutation expands to full mutation; hence in the next generation, all males who inherit the X with full mutation are affected. However, only 50% of females who inherit the full mutation are affected, and only mildly. *(Courtesy of Dr. Nancy Schneider, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

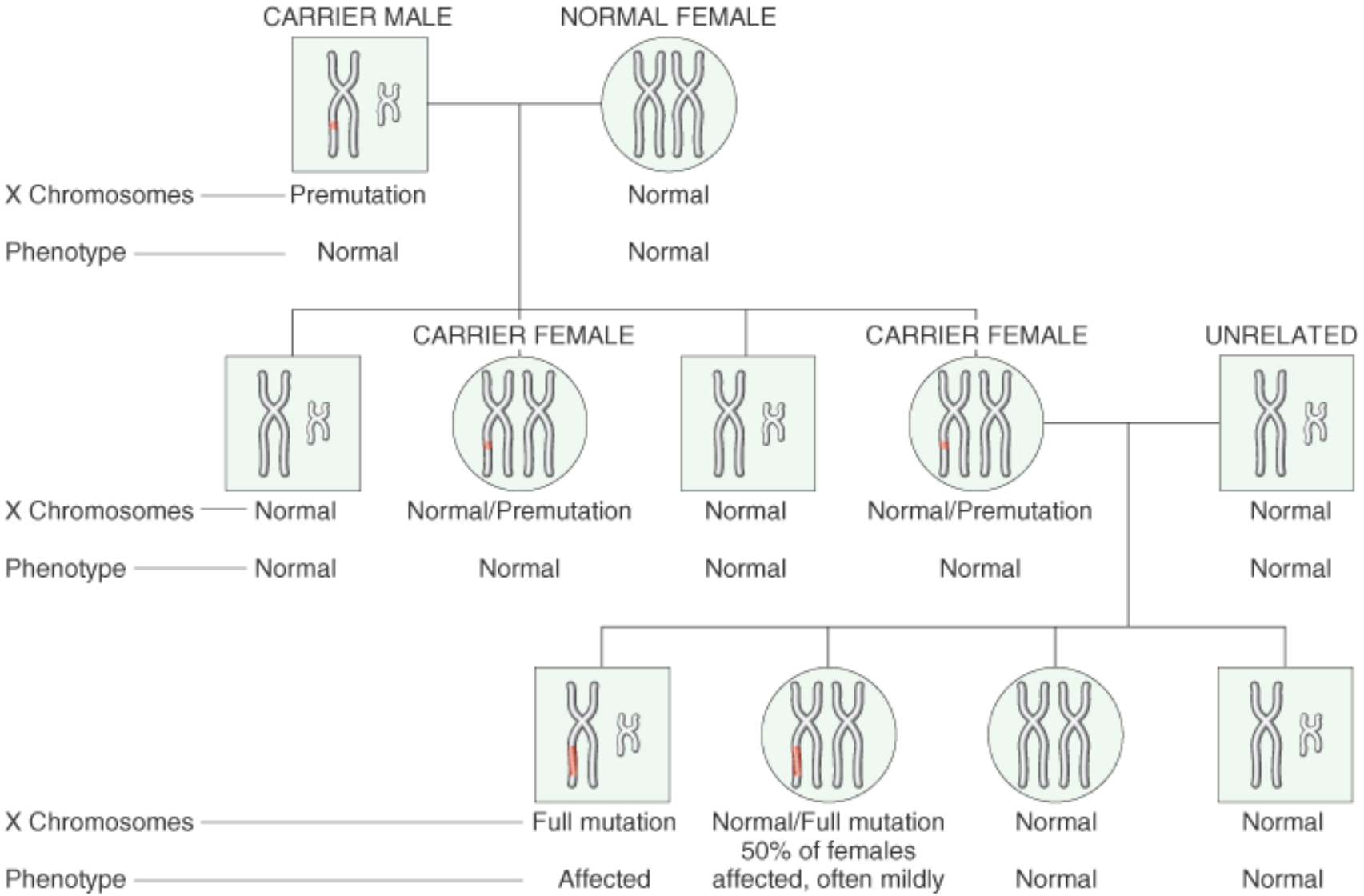


Figure 5-33 A model for the action of familial mental retardation protein (FMRP) in neurons. *(Adapted from Hin P, Warren ST: New insights into fragile-X syndrome: from molecules to neurobehavior. Trends Biochem Sci 28:152, 2003.)*

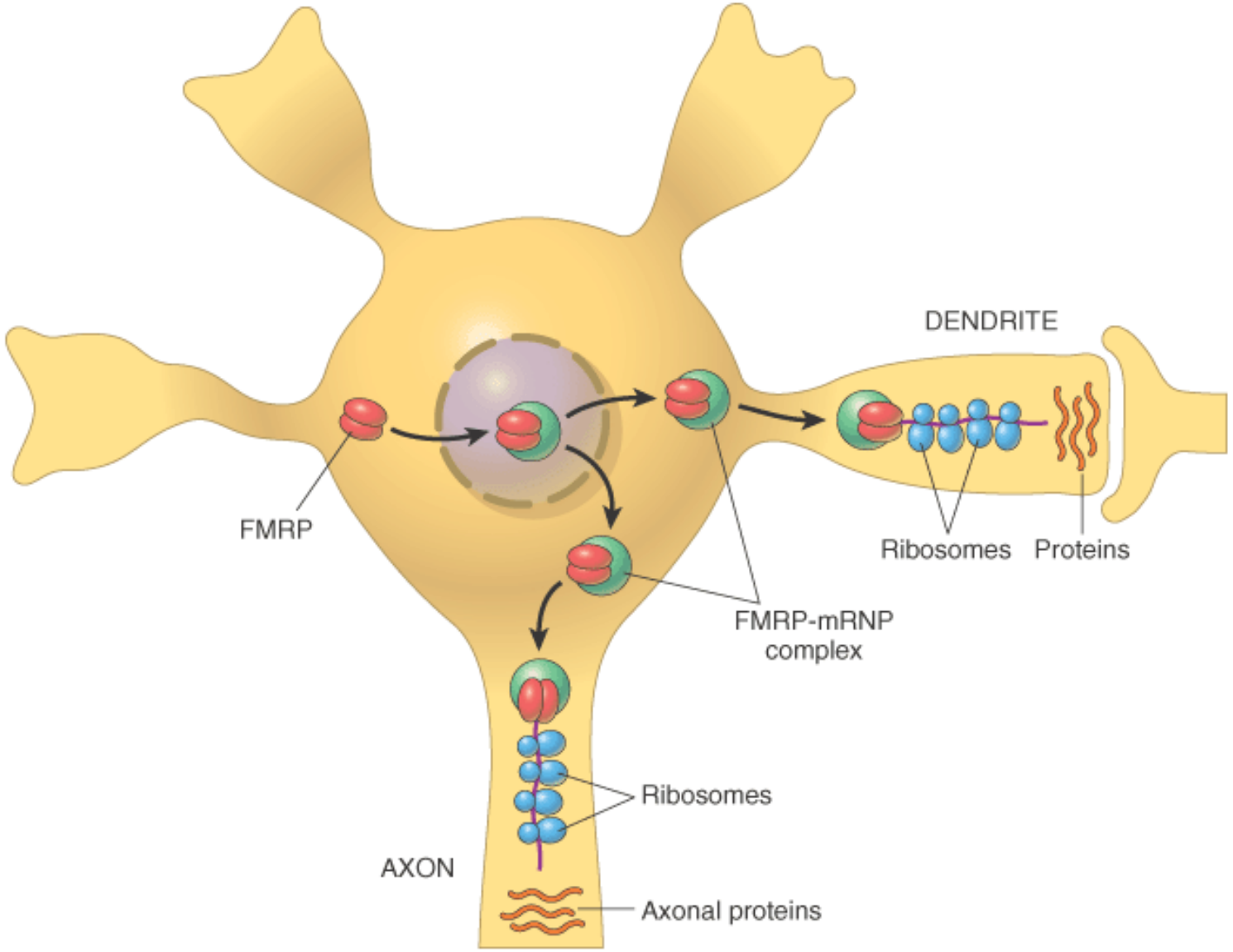


TABLE 5-9 -- Summary of Trinucleotide Repeat Disorders

Disease	Gene	Locus	Protein	Repeat	Normal	Disease
Expansions Affecting Noncoding Regions						
Fragile-X syndrome	*FMRI(FRAXA)*	Xq27.3	FMR-1 protein (FMRP)	CGG	6–53	60–200 (pre) >230 (full)
Friedreich ataxia	*X25*	9q13-21.1	Frataxin	GAA	7–34	34–80 (pre) >100 (full)

Myotonic dystrophy	*DMPK*	19q13	Myotonic dystrophy protein kinase (DMPK)	CTG	5–37	50-thousands
Expansions Affecting Coding Regions						
Spinobulbar muscular atrophy (Kennedy disease)	*AR*	Xq13-21	Androgen receptor (AR)	CAG	9–36	38–62
Huntington disease	*HD*	4p16.3	Huntingtin	CAG	6–35	36–121
Dentatorubral-pallidoluysian atrophy (Haw River syndrome)	*DRPLA*	12p13.31	Atrophin-1	CAG	6–35	49–88
Spinocerebellar ataxia type 1	*SCA1*	6p23	Ataxin-1	CAG	6–44	39–82
Spinocerebellar ataxia type 2	*SCA2*	12q24.1	Ataxin-2	CAG	15–31	36–63
Spinocerebellar ataxia type 3 (Machado-Joseph disease)	*SCA3 (MJD1)*	14q32.1	Ataxin-3	CAG	12–40	55–84
Spinocerebellar ataxia type 6	*SCA6*	19p13	α_{1A} -Voltage-dependent calcium channel subunit	CAG	4–18	21–33
Spinocerebellar ataxia type 7	*SCA7*	3p12-13	Ataxin-7	CAG	4–35	37–306

Figure 5-34 Sites of expansion and the affected sequence in selected diseases caused by nucleotide repeat mutations. UTR, untranslated region. *Although not strictly a trinucleotide repeat disease, progressive myoclonus epilepsy is caused, like others in this group, by a heritable DNA expansion. The expanded segment is in the promoter region of the gene.

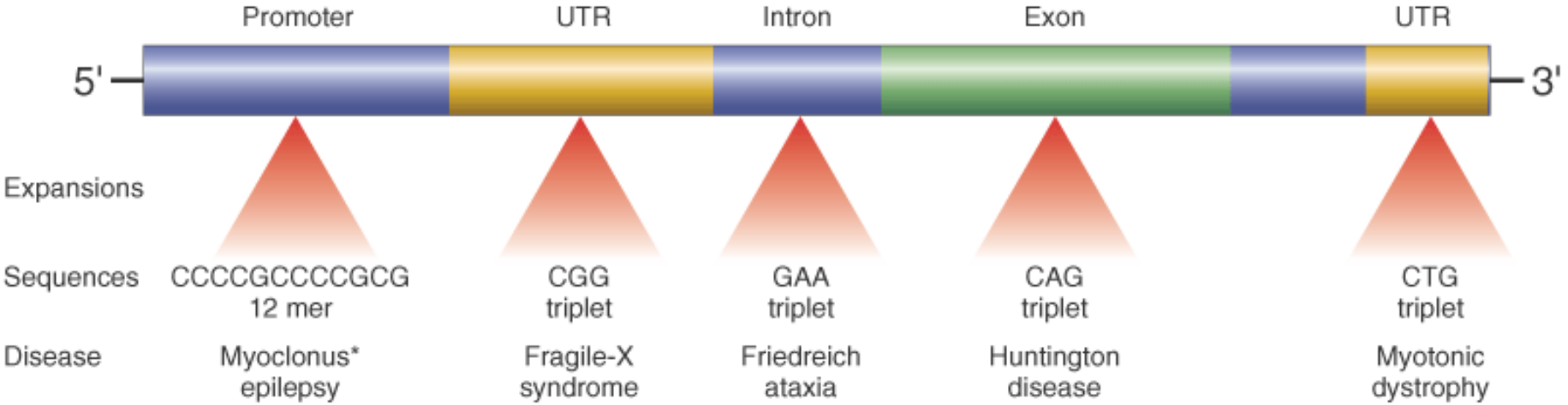


Figure 5-35 Pedigree of Leber hereditary optic neuropathy, a disorder caused by mutation in mitochondrial DNA. Note that all progeny of an affected male are normal, but all children, male and female, of the affected female manifest disease.

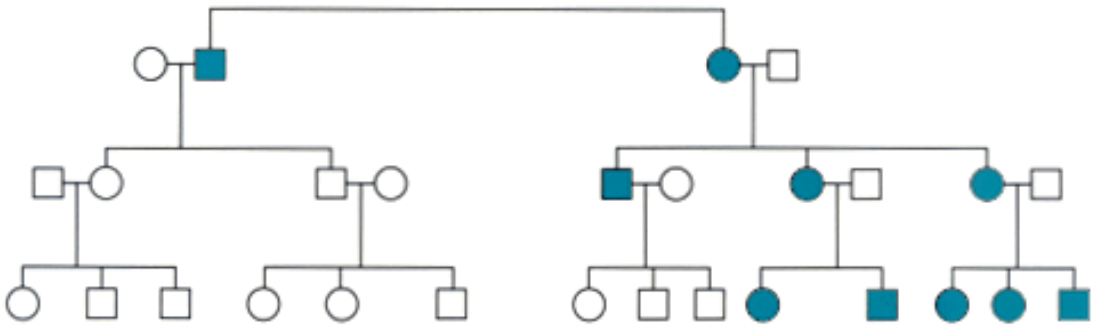
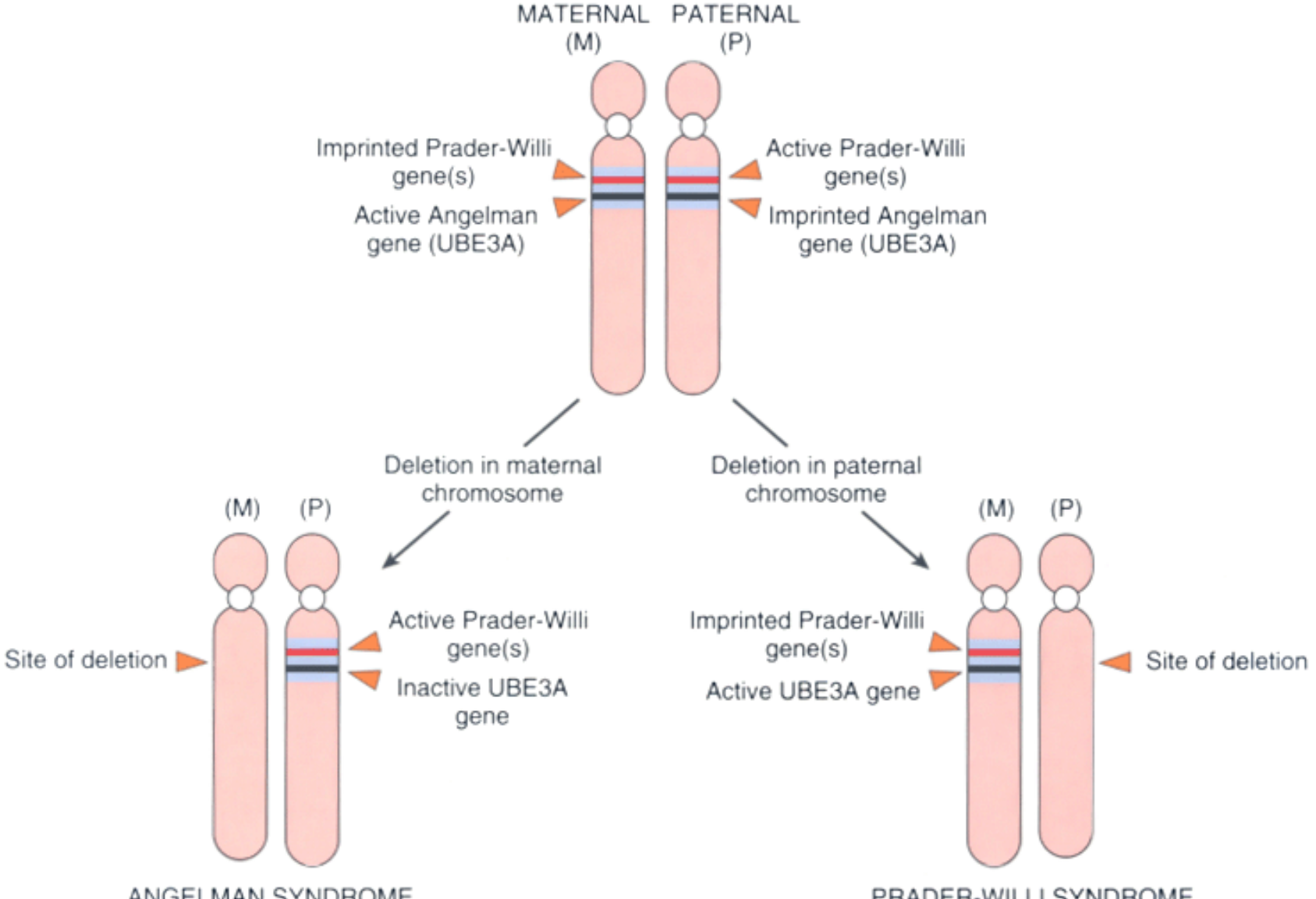


Figure 5-36 Diagrammatic representation of Prader-Willi and Angelman syndromes.



ANGELMAN SYNDROME

PRADER-WILLI SYNDROME

Figure 5-37 Direct gene diagnosis: detection of coagulation factor V mutation by polymerase chain reaction (PCR) analysis. A G→A substitution in an exon destroys one of the two Mnl1 restriction sites. The mutant allele therefore gives rise to two, rather than three, fragments by PCR analysis.

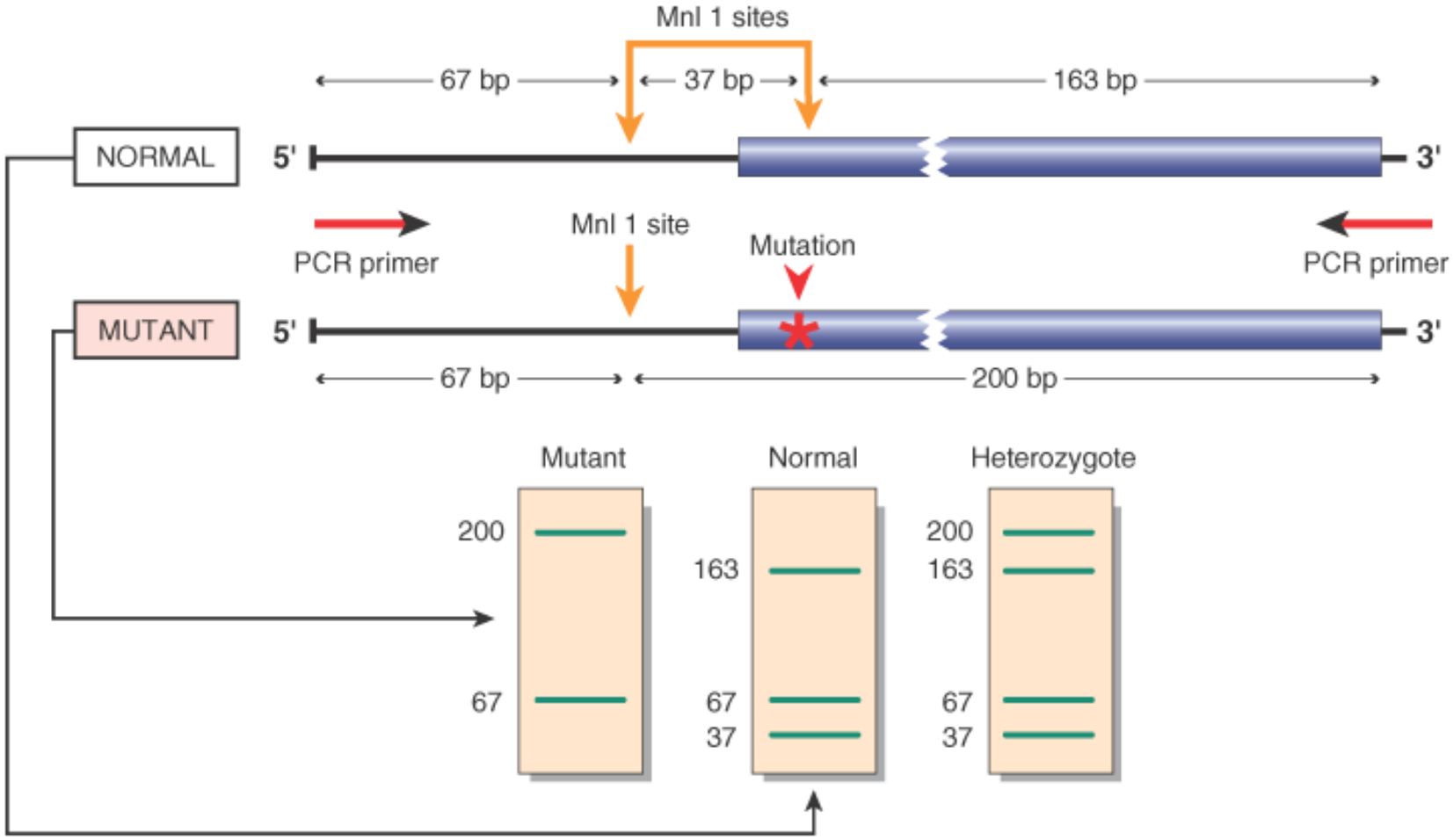
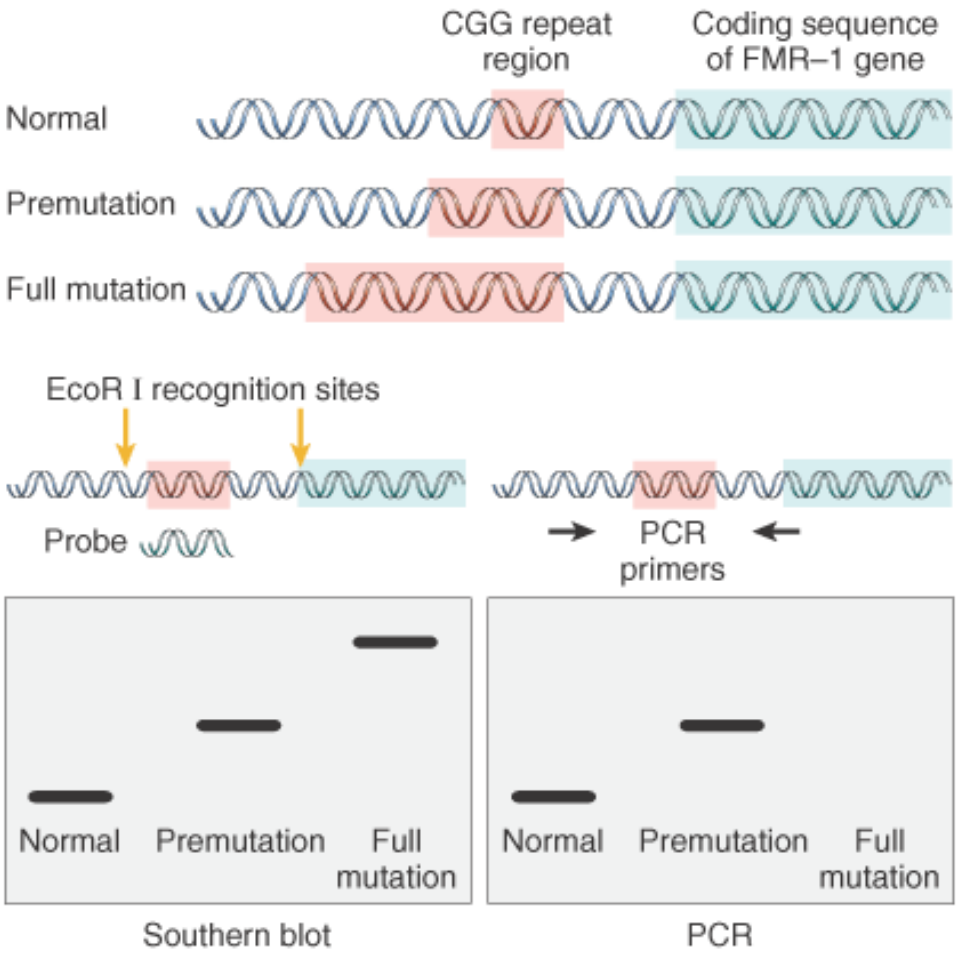


Figure 5-38 Diagnostic application of PCR and Southern blot analysis in fragile-X syndrome. With PCR, the differences in the size of CGG repeat between normal and premutation give rise to products of different sizes and mobility. With a full mutation, the region between the primers is too large to be amplified by conventional PCR. In Southern blot analysis the DNA is cut by enzymes that flank the CGG repeat region, and is then probed with a complementary DNA that binds to the affected part of the gene. A single small band is seen in normal males, a higher-molecular-weight band in males with premutation, and a very large (usually diffuse) band in those with the full mutation.



With this background, we can discuss how RFLPs can be used in gene tracking. Figure 5-39 illustrates the principle of RFLP analysis. In this example of an autosomal recessive disease, both of the parents are heterozygote carriers and the children are normal, are carriers, or are affected. In the illustrated example, the normal chromosome (A) has two restriction sites, 7.6 kb apart, whereas chromosome B, which carries the mutant gene, has a DNA sequence polymorphism resulting in the creation of an additional (third) restriction site for the same enzyme. Note that the additional restriction site has not resulted from the mutation but from a naturally occurring polymorphism. When DNA from such an individual is digested with the appropriate restriction enzyme and probed with a cloned DNA fragment that hybridizes with a stretch of sequences between

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the restriction sites, the normal chromosome yields a 7.6 kb band, whereas the other chromosome (carrying the mutant gene) produces a smaller, 6.8 kb, band. Thus, on Southern blot analysis, two bands are noted. It is possible by this technique to distinguish family members who have inherited both normal chromosomes from those who are heterozygous or homozygous for the mutant gene. PCR followed by digestion with the appropriate restriction enzyme and gel electrophoresis can also be used to detect RFLPs if the target DNA is of the size that can be amplified by conventional PCR.

• *Length polymorphisms:* Human DNA contains short repetitive sequences of noncoding DNA. Because the number of repeats affecting such sequences varies greatly between different individuals, the resulting length polymorphisms are quite useful for linkage analysis. These polymorphisms are often subdivided on the basis of their length into microsatellite

repeats and minisatellite repeats. Microsatellites are usually less than 1 kb and are characterized by a repeat size of 2 to 6 base pairs. Minisatellite repeats, by comparison, are larger (1 to 3 kb), and the repeat motif is usually 15 to 70 base pairs. It is important to note that the number of repeats, both in microsatellites and minisatellites, is extremely variable within a given population, and hence these stretches of DNA can be used quite effectively to distinguish different chromosomes (Fig. 5-40*A*). Figure 5-40*B* illustrates how microsatellite polymorphisms can be used to track the inheritance of autosomal dominant polycystic kidney disease (PKD). In this case, allele C, which produces a larger PCR product than allele A or B, carries the disease-related gene. Hence all individuals who carry the C allele are affected. Microsatellites have assumed great importance in linkage studies and hence in the development of the human genome map. Currently, linkage to all human chromosomes can be identified by microsatellite polymorphisms.[86]

Figure 5-39 Schematic illustration of the principles underlying restriction fragment length polymorphism analysis in the diagnosis of genetic diseases.

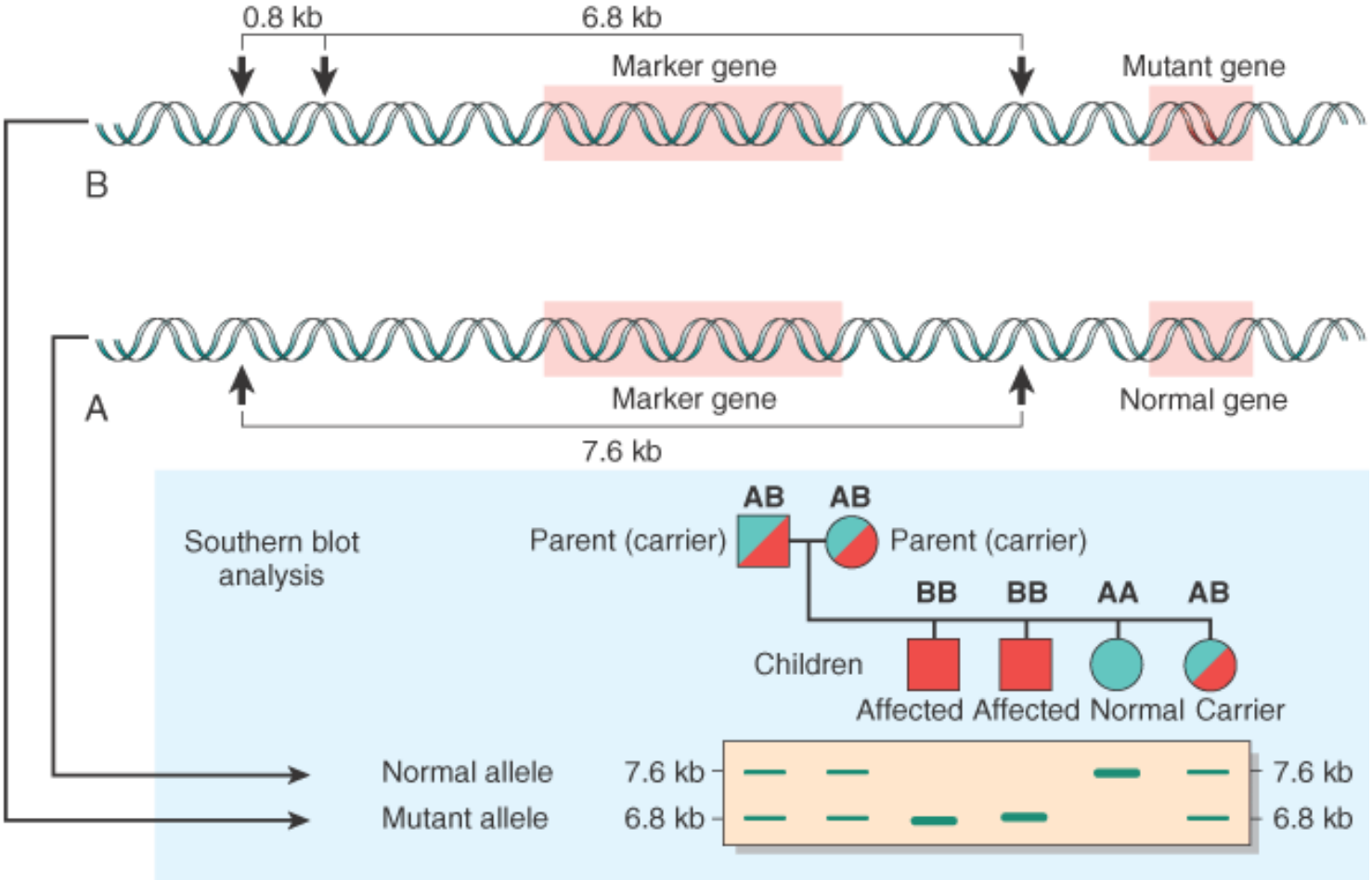
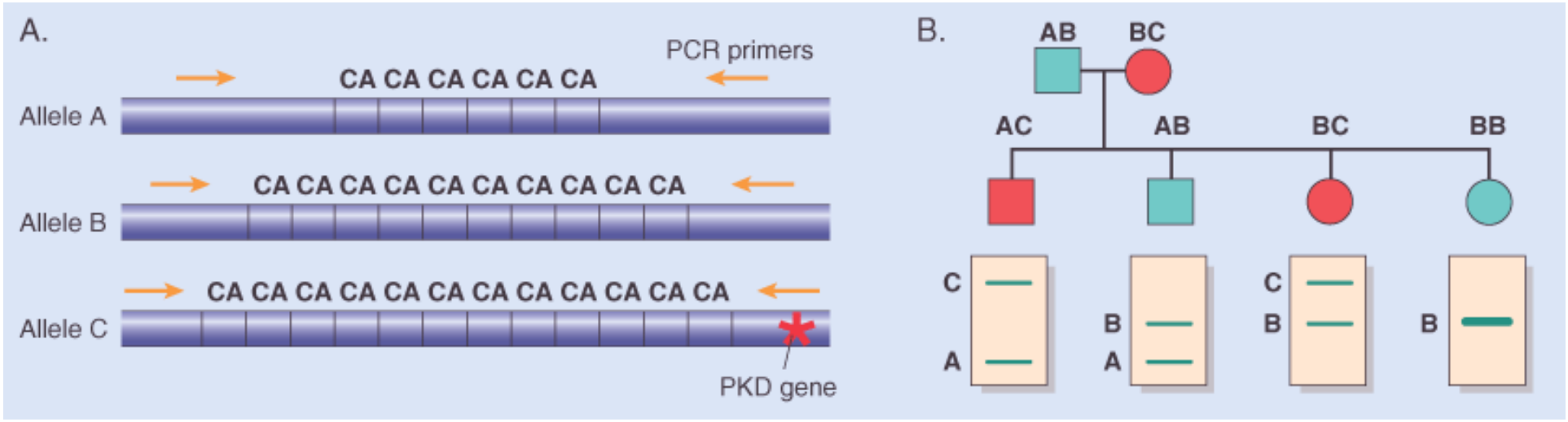


Figure 5-40 Schematic diagram of DNA polymorphisms resulting from a variable number of CA repeats. The three alleles produce PCR products of different sizes, thus identifying their origins from specific chromosomes. In the example depicted, allele C is linked to a mutation responsible for autosomal dominant polycystic kidney disease (PKD). Application of this to detect progeny carrying the disease gene is illustrated in one hypothetical pedigree.



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Chapter 6 - Diseases of Immunity

Abul K. Abbas MD

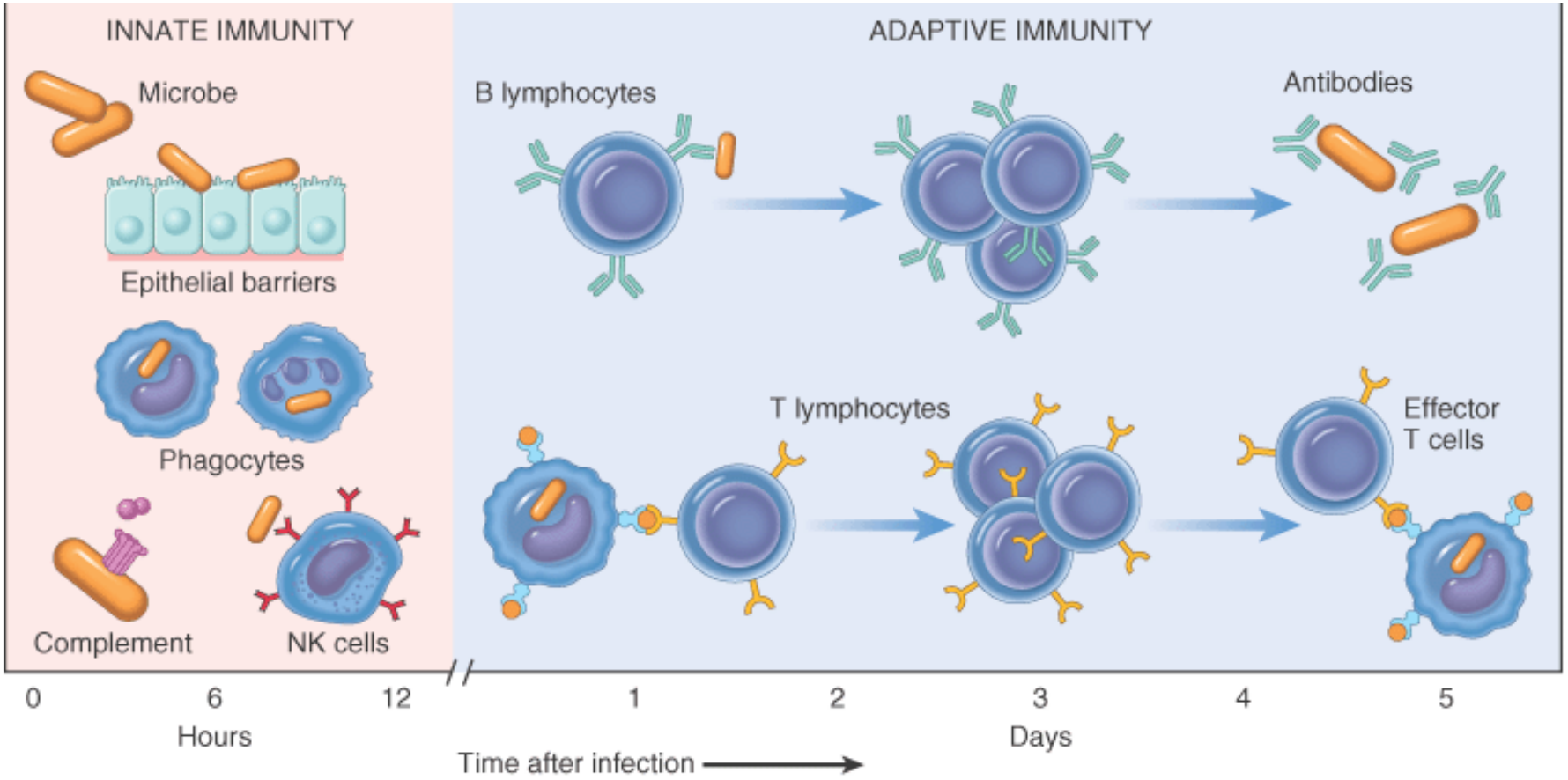
General Features of the Immune System

Although vital to survival, the immune system is similar to the proverbial two-edged sword. On the one hand, immunodeficiency states render humans easy prey to infections and possibly tumors; on the other hand, a hyperactive immune system may cause fatal disease, as in the case of an overwhelming allergic reaction to the sting of a bee. In yet another series of derangements, the immune system may lose its normal capacity to distinguish self from non-self, resulting in immune reactions against one's own tissues and cells (*autoimmunity*). This chapter considers diseases caused by too little immunity as well as those resulting from too much immunologic reactivity. We also consider amyloidosis, a disease in which an abnormal protein, derived in some cases from fragments of immunoglobulins, is deposited in tissues. First, we review some advances in the understanding of innate and adaptive immunity and lymphocyte biology, then give a brief description of the histocompatibility genes because their products are relevant to several immunologically mediated diseases and to the rejection of transplants.

INNATE AND ADAPTIVE IMMUNITY

The physiologic function of the immune system is to protect individuals from infectious pathogens. The mechanisms that are responsible for this protection fall into two broad categories (Fig. 6-1). *Innate immunity* (also called natural, or native, immunity) refers to defense mechanisms that are present even before infection and have evolved to specifically recognize microbes and protect multicellular organisms against infections. *Adaptive immunity* (also called acquired, or specific, immunity) consists of mechanisms that are stimulated by (adapt to) microbes and are capable of also recognizing nonmicrobial substances, called *antigens*. Innate immunity is the first line of defense, because it is always ready

Figure 6-1 Innate and adaptive immunity. The principal mechanisms of innate immunity and adaptive immunity are shown.



Box 6-1. Toll-like Receptors

The Toll-like receptors (TLRs) are membrane proteins that recognize a variety of microbe-derived molecules and stimulate innate immune responses against the microbes. The first protein to be identified in this family was the *Drosophila* Toll protein, which is involved in establishing the dorsal-ventral axis during embryogenesis of the fly, as well as mediating antimicrobial responses. Ten different mammalian TLRs have been identified based on sequence homology to *Drosophila* Toll, and they are named TLR1-10. All these receptors contain leucine-rich repeats flanked by characteristic cysteine-rich motifs in their extracellular regions, and a conserved signaling domain in their cytoplasmic region that is also found in the cytoplasmic tails of the IL-1 and IL-18 receptors and is called the Toll/IL-1 receptor (TIR) domain. The TLRs are expressed on many different cell types that participate in innate immune responses, including macrophages, dendritic cells, neutrophils, NK cells, mucosal epithelial cells, and endothelial cells.

Mammalian TLRs are involved in responses to widely divergent types of molecules that are commonly expressed by microbial but not mammalian cells (see Figure). Some of the microbial products that stimulate TLRs include Gram-negative bacterial lipopolysaccharide (LPS), Gram-positive bacterial peptidoglycan, bacterial lipoproteins, the bacterial flagellar protein flagellin, heat shock protein 60, unmethylated CpG DNA motifs (found in many bacteria), and double-stranded RNA (found in RNA viruses). The specificity of TLRs for microbial products is dependent on associations between different TLRs and non-TLR adapter molecules. For instance, LPS first binds to soluble LPS-binding protein (LBP) in the blood or extracellular fluid, and this complex serves to facilitate LPS binding to CD14, which exists as both a soluble plasma protein and a glycophosphatidylinositol-linked membrane protein on most cells. Once LPS binds to CD14, LBP dissociates, and the LPS-CD14 complex physically associates with TLR4. An additional extracellular accessory protein, called MD2, also binds to the complex with CD14. LPS, CD14, and MD2 are all required for efficient LPS-induced signaling, but it is not yet clear if direct physical interaction of LPS with TLR4 is necessary.

Signaling by TLRs results in the activation of transcription factors, notably NF-κB (see Figure). Ligand binding to the TLR at the cell surface leads to recruitment of cytoplasmic signaling molecules, the first of which is the adapter protein MyD88. A kinase called IL-1 receptor associated kinase (IRAK) is recruited into the signaling complex. IRAK undergoes autophosphorylation, dissociates from MyD88, and activates another signaling molecule, called TNF-receptor (TNF-R) associated factor-6 (TRAF-6). TRAF-6 then activates the I-κB kinase cascade, leading to activation of the NF-κB transcription factor. In some cell types certain TLRs also engage other signaling pathways, such as the MAP kinase cascade, leading to activation of the AP-1 transcription factor. Some TLRs may use adapter proteins other than MyD88. The relative importance of these various pathways of TLR signaling, and the way the "choice" of pathways is made, are not well understood.

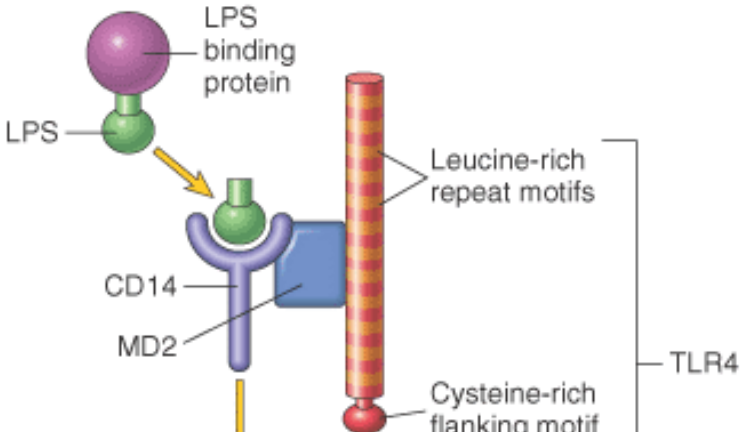
The genes that are expressed in response to TLR signaling encode proteins important in many different components of innate immune responses. These include inflammatory cytokines (TNF, IL-1, and IL-12), endothelial adhesion molecules (E-selectin), and proteins involved in microbial killing mechanisms (inducible nitric oxide synthase). The particular genes expressed will depend on the responding cell type.

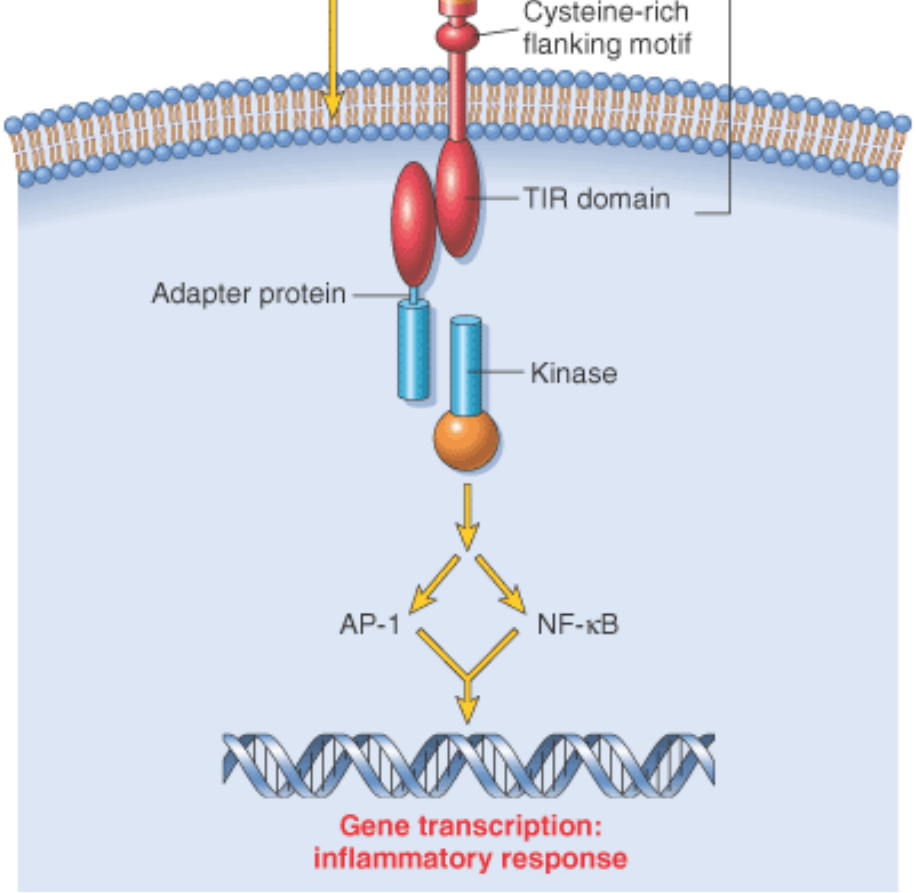
Figure 6- *A*, Different TLRs are involved in responses to different microbial products. *B*, Signaling by a prototypic TLR, TLR4, in response to bacterial LPS. An adapter protein links the TLR to a kinase, which activates transcription factors such as NF-κB and AP-1. TIR, Toll/IL-1 receptor domain.

A

TLR	Ligand	Microbial source
TLR2	Lipoproteins Peptidoglycan Zymosan LPS GPI anchor Lipoarabinomannan Phosphatidylinositol dimannoside	Bacteria Gram positive bacteria Fungi Leptospira Trypanosomes Mycobacteria Mycobacteria
TLR3	Double-stranded RNA	Viruses
TLR4	LPS HSF00	Gram negative bacteria Chlamydia
TLR5	Flagellin	Various bacteria
TLR6	CpG DNA	Bacteria, protozoans

B





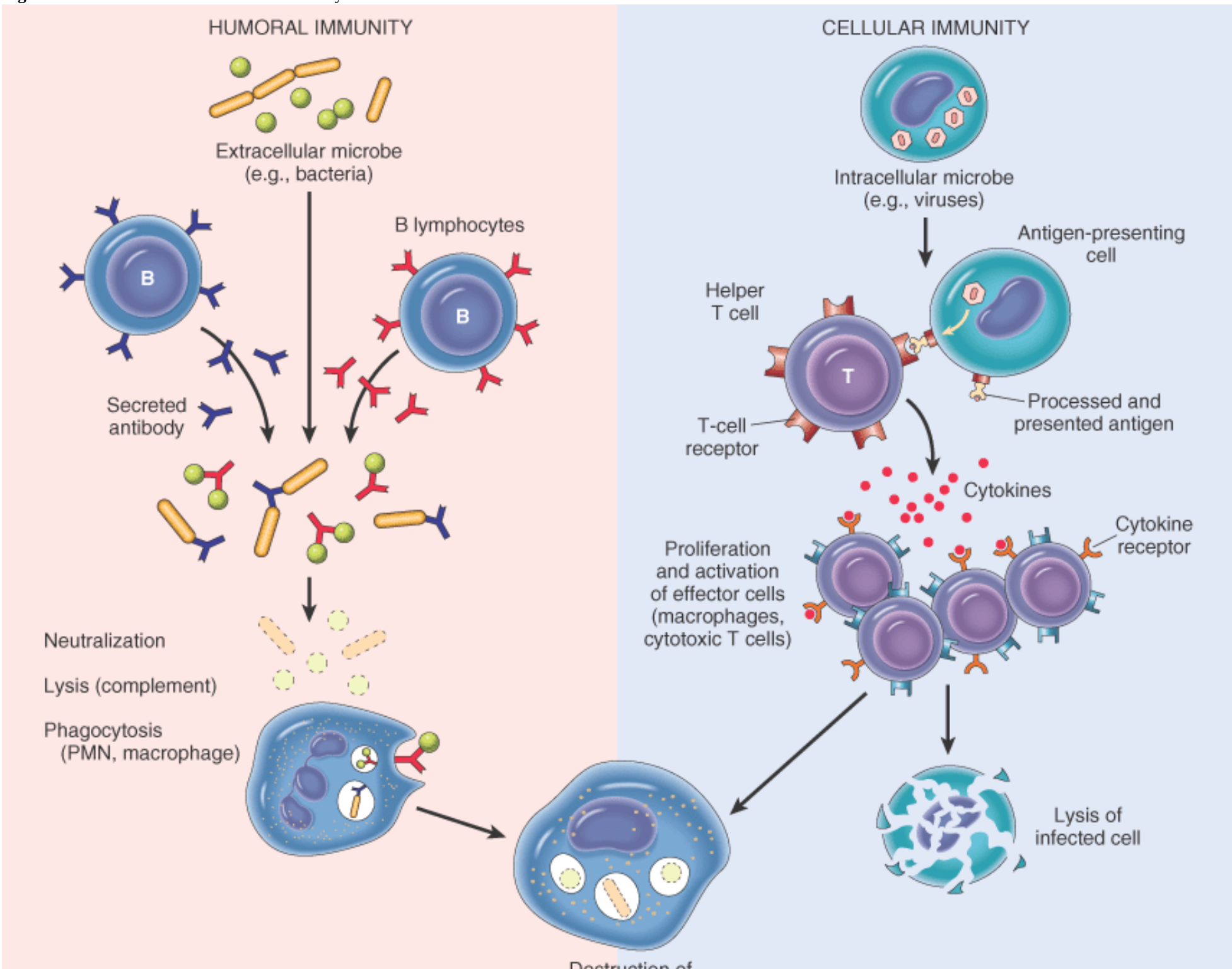
of innate immunity, providing protection against inhaled microbes.

The adaptive immune system consists of lymphocytes and their products, including antibodies. The receptors of lymphocytes are much more diverse than those of the innate immune system, but lymphocytes are not inherently specific for microbes, and they are capable of recognizing a vast array of foreign substances. In the remainder of this introductory section we focus on lymphocytes and the reactions of the adaptive immune system.

CELLS AND TISSUES OF THE IMMUNE SYSTEM

There are two main types of adaptive immunity—cell-mediated (or cellular) immunity, which is responsible for defense against intracellular microbes, and humoral immunity, which protects against extracellular microbes and their toxins (Fig. 6-2). Cellular immunity is mediated by T (thymus-derived) lymphocytes, and humoral immunity is mediated by B (bone marrow-derived) lymphocytes and their secreted products,

Figure 6-2 Humoral and cell-mediated immunity.



Destruction of
phagocytosed microbes

Figure 6-3 Histology of a lymph node. *A,* The organization of the lymph node, with an outer cortex containing follicles and an inner medulla. *B,* The location of B cells (stained green, using the immunofluorescence technique) and T cells (stained red) in a lymph node. *C,* A germinal center.

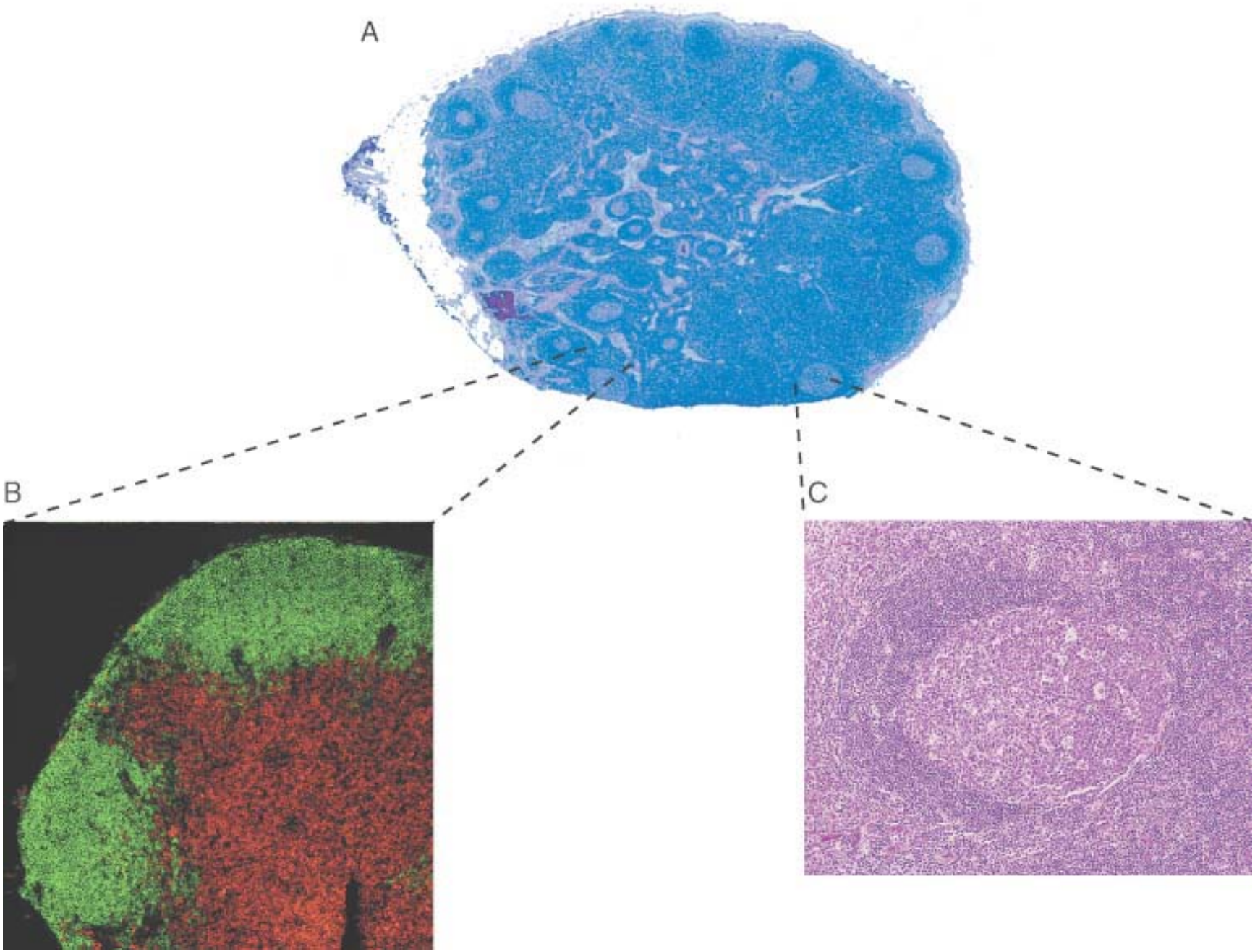
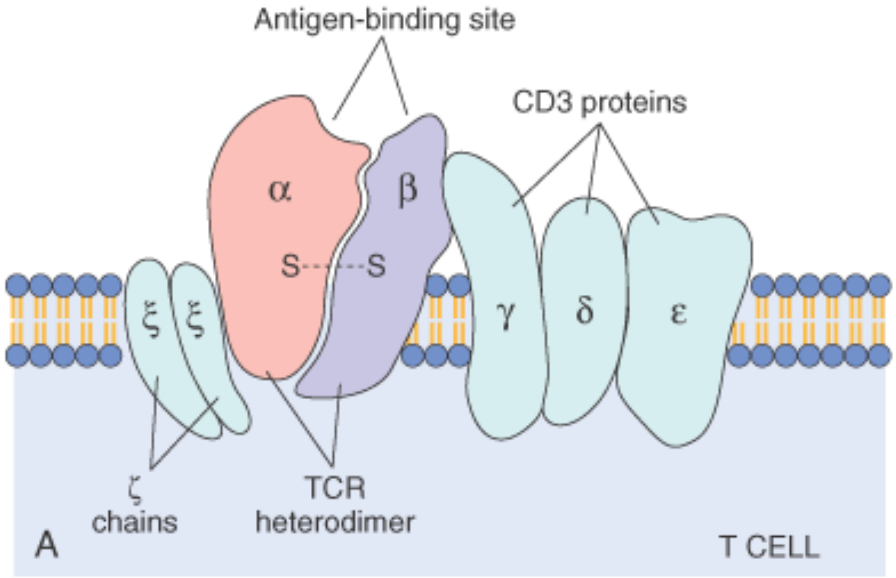
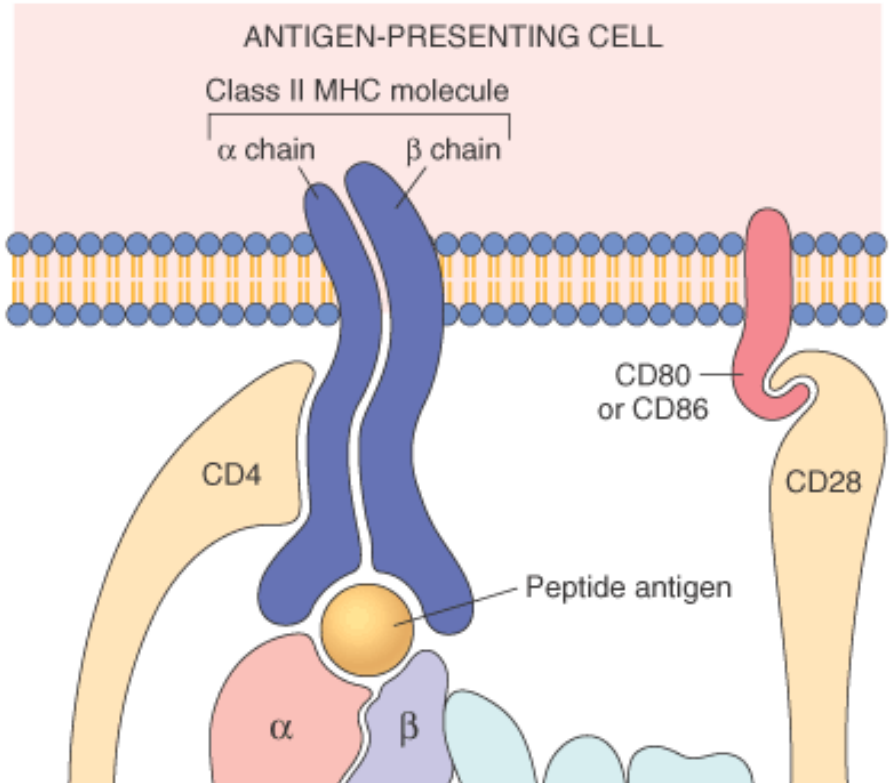


Figure 6-4 The T-cell receptor (TCR) complex. *A,* Schematic illustration of TCRα and TCRβ chains linked to the CD3 complex. *B,* Recognition of MHC-associated peptide displayed on an antigen-presenting cell (top) by the TCR. Note that the TCR-associated ζ chains and CD3 complex deliver signals (signal 1) upon antigen recognition, and CD28 delivers signals (signal 2) upon recognition of costimulators (B7 molecules).





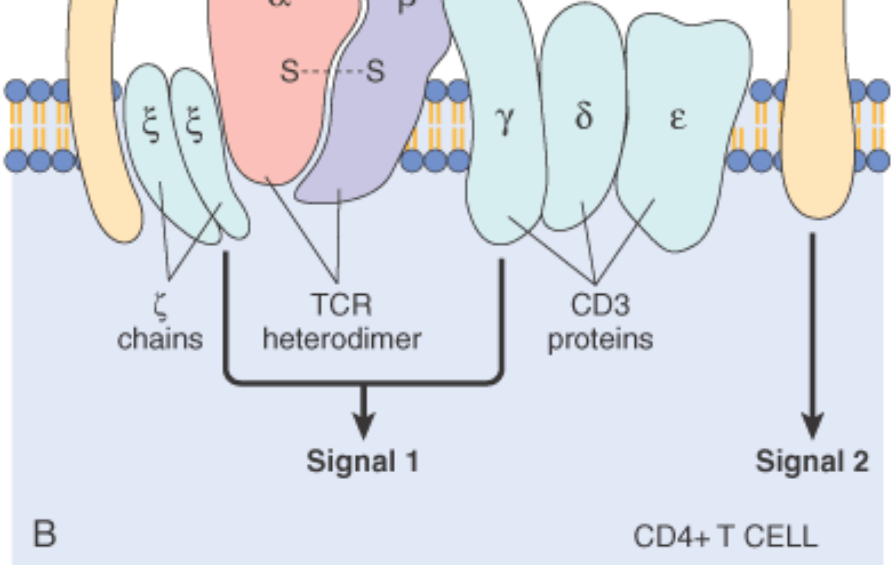
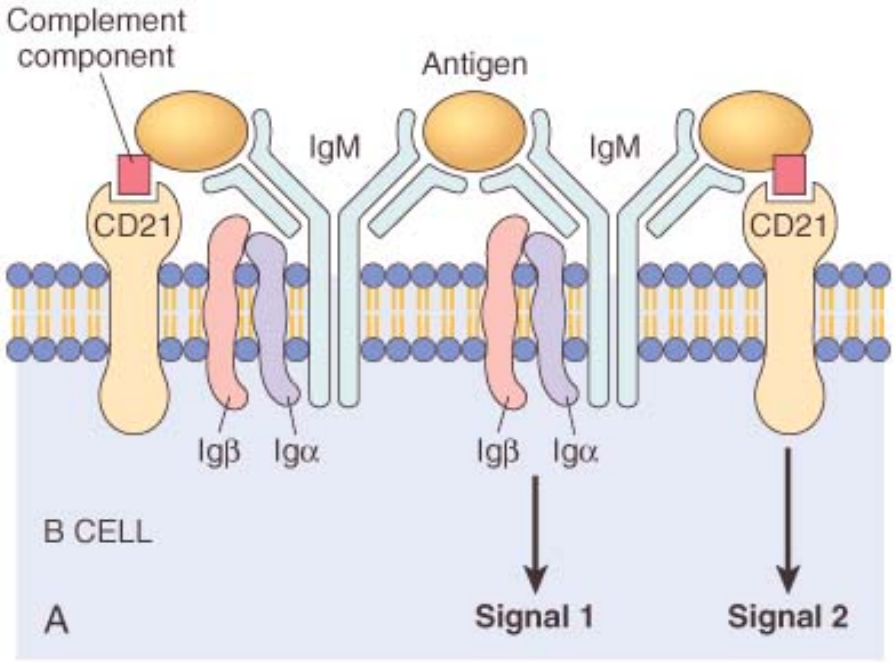


Figure 6-5 Structure of antibodies and the B-cell antigen receptor. *A*, The B-cell receptor complex composed of membrane IgM (or IgD, not shown) and the associated signaling proteins Igα and Igβ. CD21 is a receptor for a complement component that also promotes B-cell activation. *B*, Crystal structure of a secreted IgG molecule, showing the arrangement of the variable (V) and constant (C) regions of the heavy (H) and light (L) chains. *(Courtesy of Dr. Alex McPherson, University of California, Irvine, CA.)*



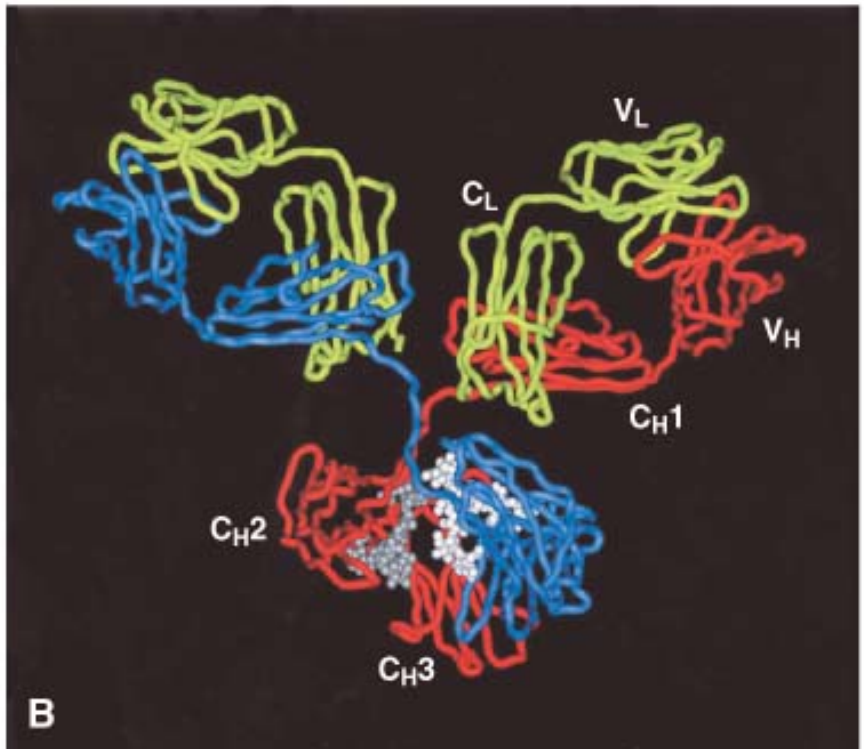
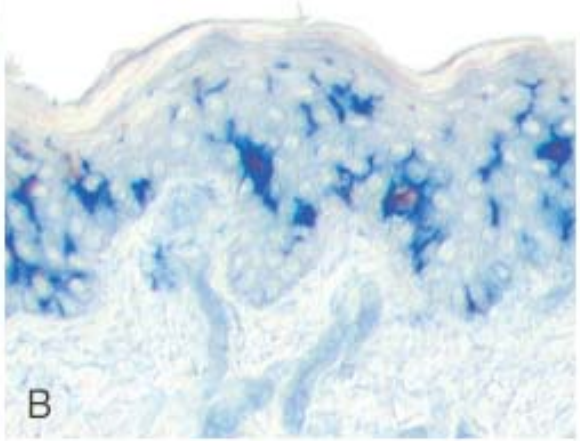
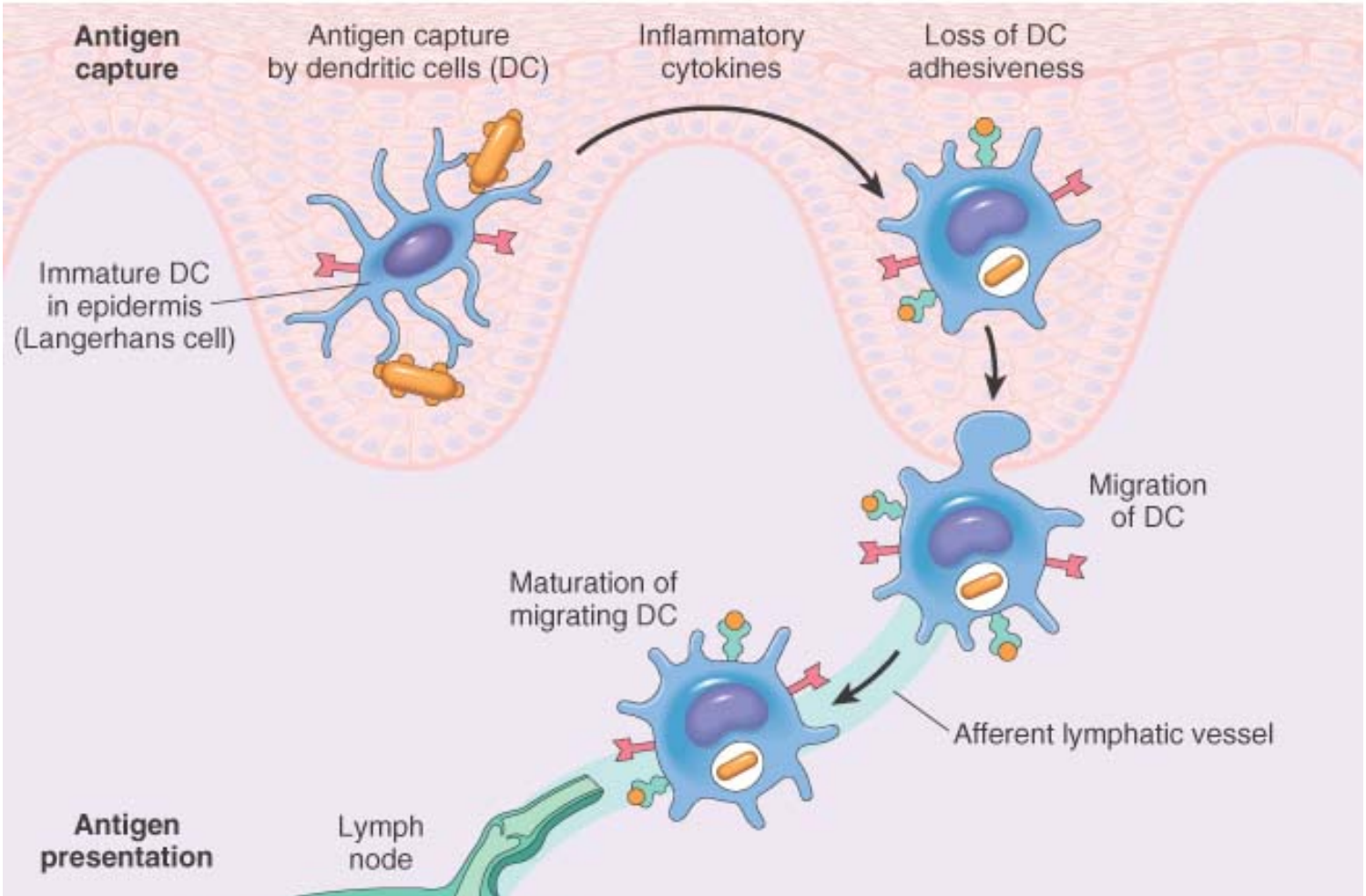


Figure 6-6 The morphology and functions of dendritic cells (DC). *A,* The morphology of cultured dendritic cells. *(Courtesy of Dr. Y-J. Liu, M. D. Anderson Cancer Center, Houston.) B,*

The location of dendritic cells (Langerhans cells) in the epidermis. *(Courtesy of Dr. Y-J. Liu, M. D. Anderson Cancer Center, Houston.) C*, The role of dendritic cells in capturing microbial antigens from epithelia and transporting them to regional lymph nodes.





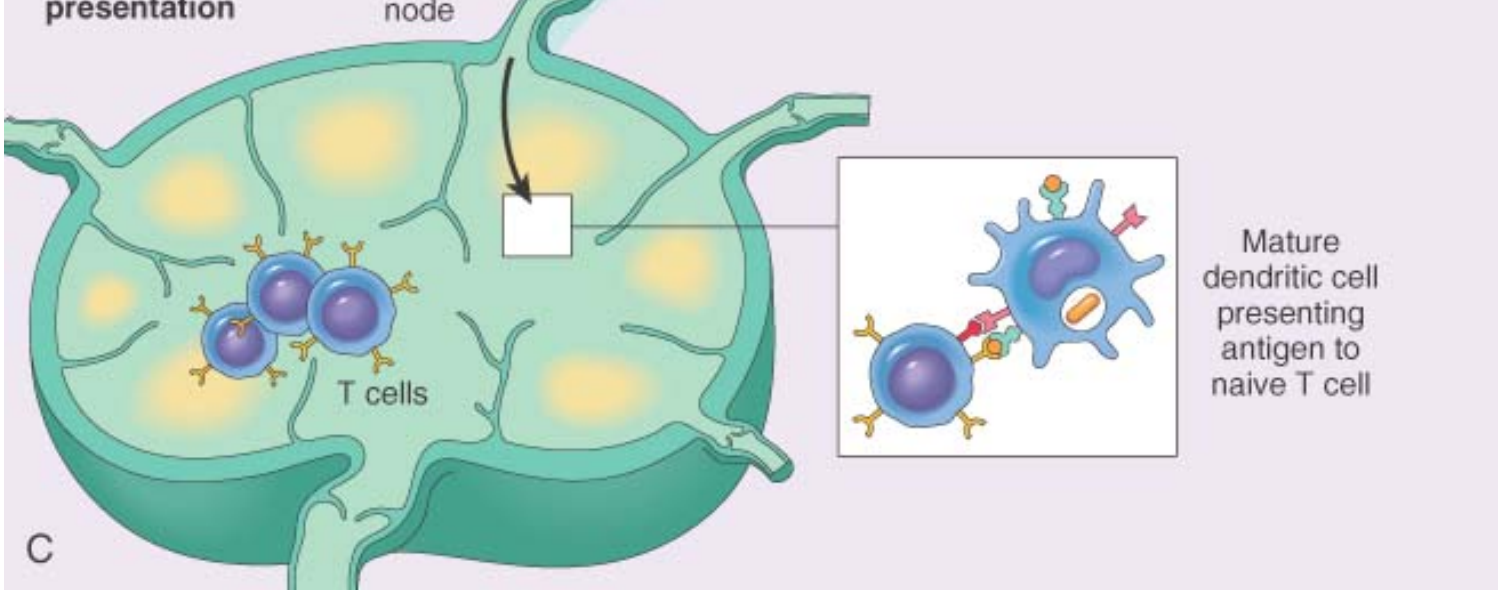


Figure 6-7 A highly activated natural killer cell with abundant cytoplasmic granules. *(Courtesy of Dr. Noelle Williams, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

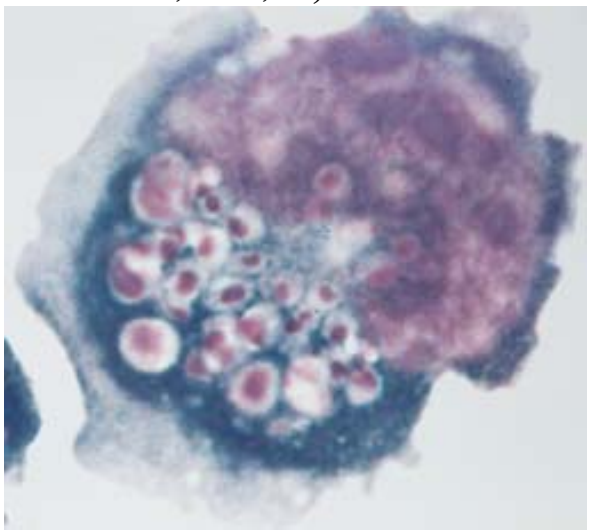
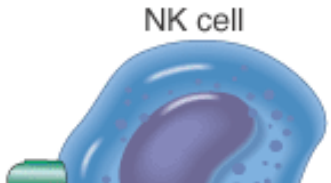


Figure 6-8 Schematic representation of NK-cell receptors and killing. NK cells express activating and inhibitory receptors; some examples of each are indicated. Normal cells are not killed because inhibitory signals from normal MHC class I molecules override activating signals. In tumor cells or virus-infected cells, there is increased expression of ligands for activating receptors, and reduced expression or alteration of MHC molecules, which interrupts the inhibitory signals, allowing activation of NK cells and lysis of target cells. KIR, killer cell Ig-like recepors.



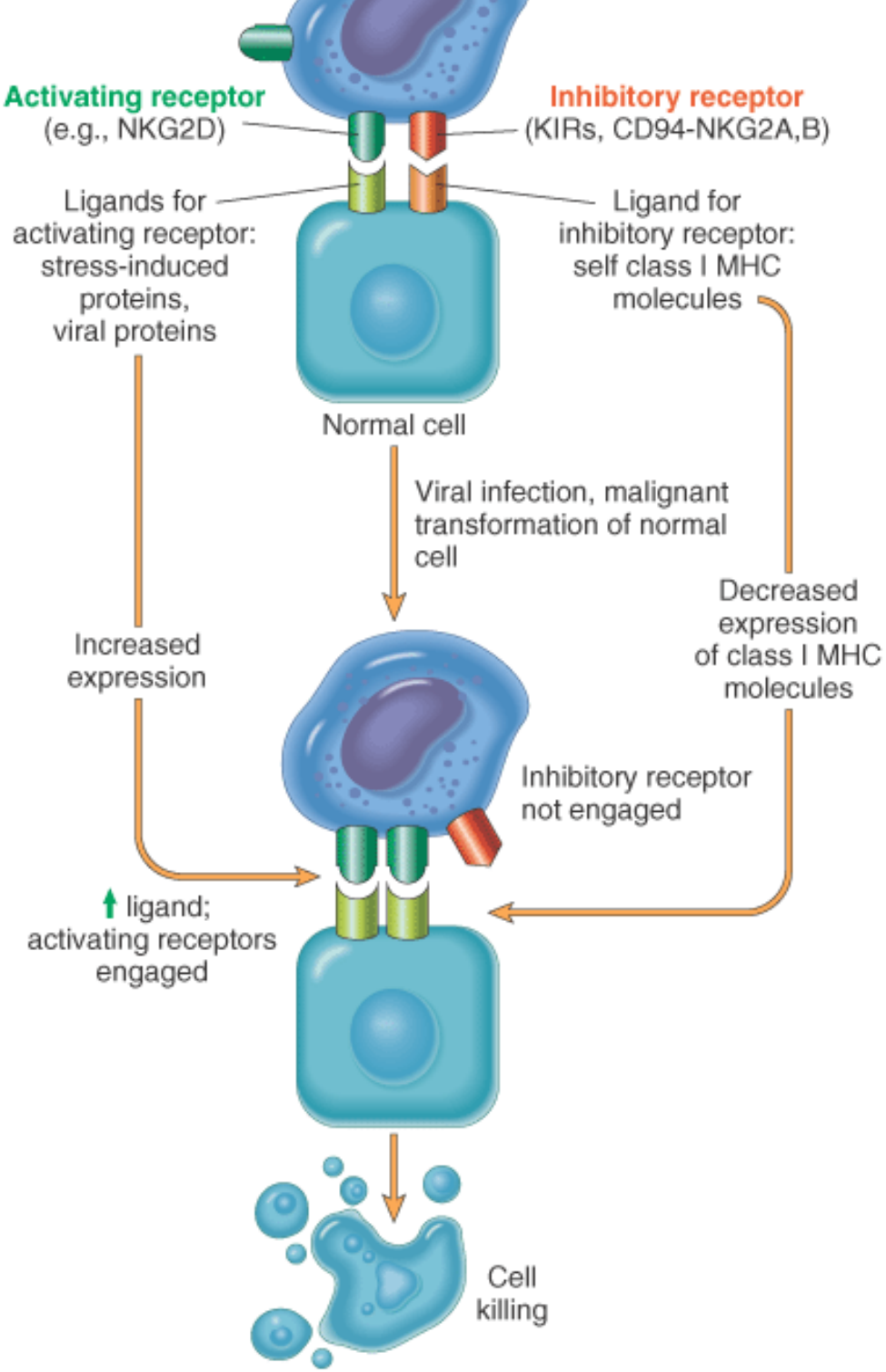

Activating receptor
(e.g., NKG2D)
Inhibitory receptor
(KIRs, CD94-NKG2A,B)
Ligands for activating receptor: stress-induced proteins, viral proteins
Ligand for inhibitory receptor: self class I MHC molecules
Normal cell
Viral infection, malignant transformation of normal cell
Increased expression
Decreased expression of class I MHC molecules
Inhibitory receptor not engaged
↑ ligand; activating receptors engaged
Cell killing

Figure 6-9 The HLA complex and the structure of HLA molecules. *A,* The location of genes in the HLA complex is shown. The sizes and distances between genes are not to scale. *B,* Schematic diagrams and crystal structures of class I and class II HLA molecules. *(Crystal structures are courtesy of Dr. P. Bjorkman, California Institute of Technology, Pasadena, CA.)*

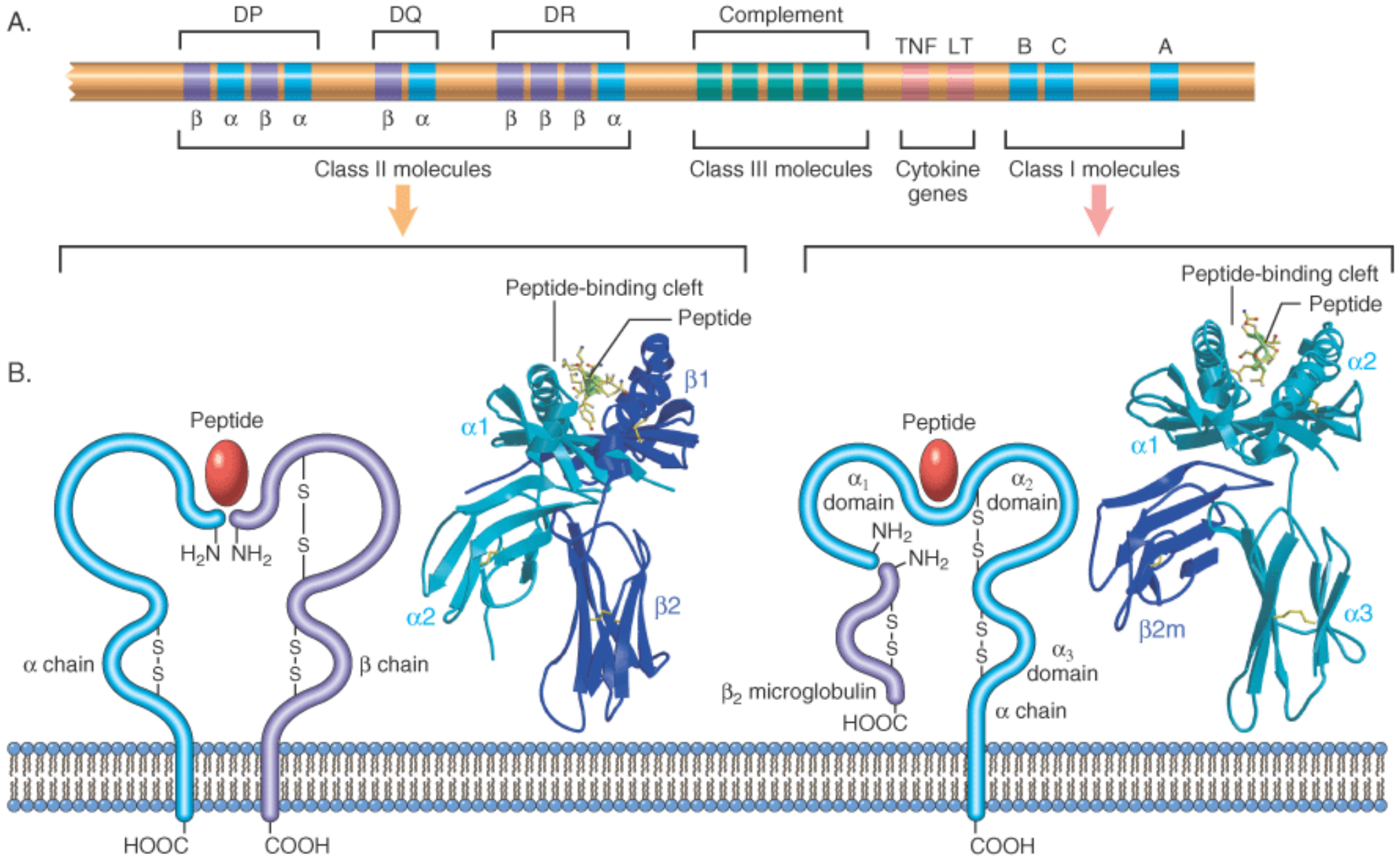
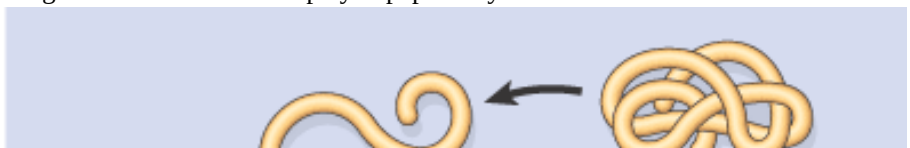


Figure 6-10 Antigen processing and recognition. The sequence of events in the processing of a cytoplasmic protein antigen and its display by class I MHC molecules are shown at the top. The recognition of this MHC-displayed peptide by a CD8+ T cell is shown at the bottom.



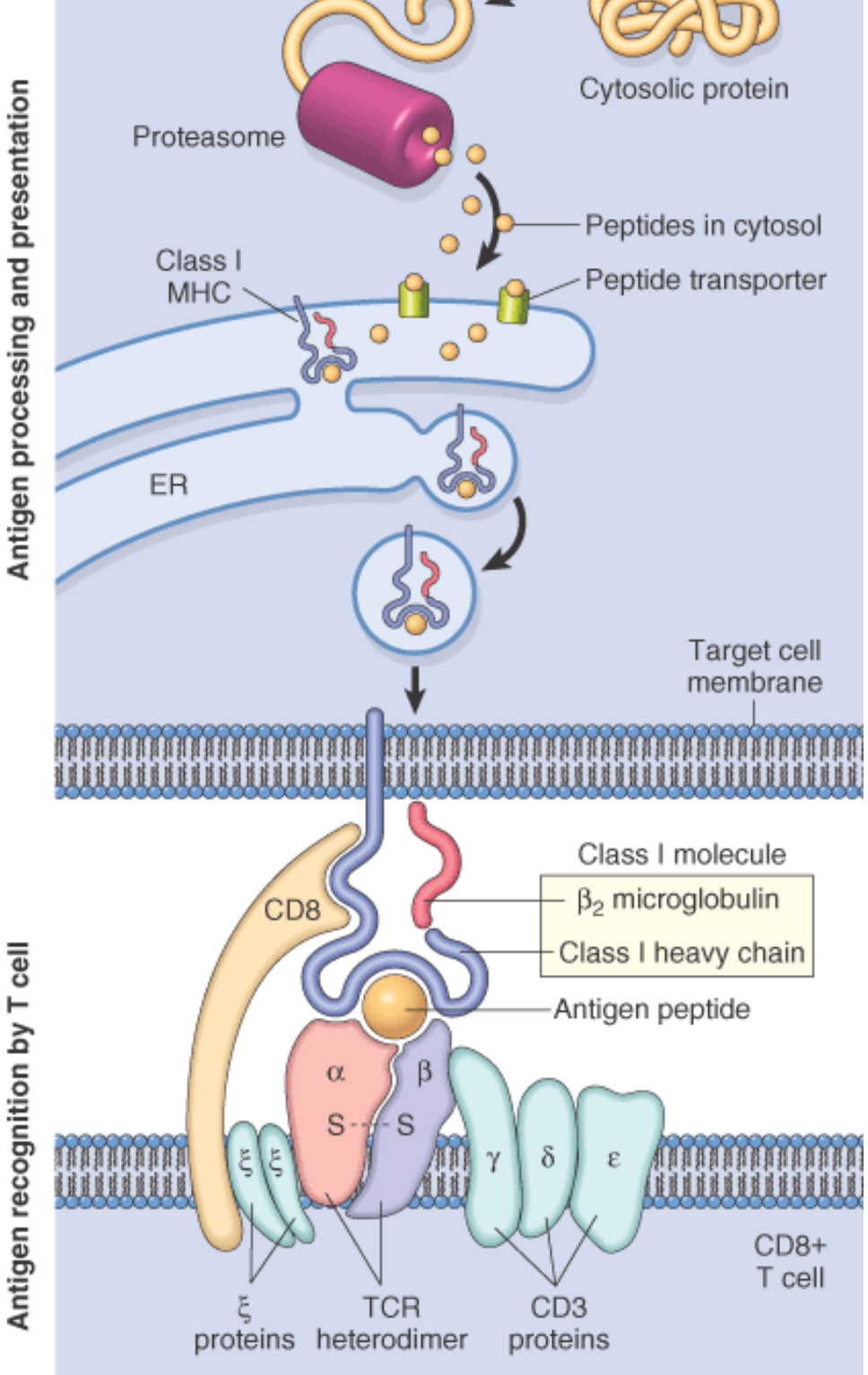

Antigen processing and presentation
Cytosolic protein
Proteasome
Peptides in cytosol
Class I
MHC
Peptide transporter
ER
Target cell
membrane
Antigen recognition by T cell
Class I molecule
CD8
β2 microglobulin
Class I heavy chain
Antigen peptide
α
β
S
S
ξ
ξ
γ
δ
ε
CD8+
T cell
ξ
proteins
TCR
heterodimer
CD3
proteins

TABLE 6-1 -- Association of HLA with Disease

Disease	HLA Allele	Relative Risk
Ankylosing spondylitis	B27	90
Postgonococcal arthritis	B27	14
Acute anterior uveitis	B27	14
Rheumatoid arthritis	DR4	•4
Chronic active hepatitis	DR3	13
Primary Sjögren syndrome	DR3	•9
Type-1 diabetes	DR3	•5
	DR4	•6
	DR3/DR4	20

MECHANISMS OF HYPERSENSITIVITY REACTIONS

Humans live in an environment teeming with substances capable of producing immunologic responses. Contact with antigen leads not only to induction of a protective immune response, but also to reactions that can be damaging to tissues. Exogenous antigens occur in dust, pollens, foods, drugs, microbiologic agents, chemicals, and many blood products used in clinical practice. The immune responses that may result from such exogenous antigens take a variety of forms, ranging from annoying but trivial discomforts, such as itching of the skin, to potentially fatal diseases, such as bronchial asthma. The various reactions produced are called *hypersensitivity reactions*, and tissue injury in these reactions may be caused by humoral or cell-mediated immune mechanisms.

Injurious immune reactions may be evoked not only by exogenous environmental antigens, but also by endogenous tissue antigens. Some of these immune reactions are triggered by homologous antigens that differ among individuals with different genetic backgrounds. Transfusion reactions and graft rejection are examples of immunologic disorders evoked by homologous antigens. Another category of disorders, those incited by self-, or autologous, antigens, constitutes the important group of autoimmune diseases (discussed later). These diseases arise because of the emergence of immune responses against self-antigens.

Hypersensitivity diseases can be classified on the basis of the immunologic mechanism that mediates the disease (Table 6-2). This classification is of value in distinguishing the manner in which the immune response ultimately causes tissue injury and disease, and the accompanying pathologic alterations. Prototypes of each of these immune mechanisms are presented in the subsequent sections.

- In *immediate hypersensitivity (type I hypersensitivity)*, the immune response releases vasoactive and spasmogenic substances that act on vessels and smooth muscle and pro-inflammatory cytokines that recruit inflammatory cells.
- In *antibody-mediated disorders (type II hypersensitivity)*, secreted antibodies participate directly in injury to cells by promoting their phagocytosis or lysis and injury to tissues by inducing inflammation. Antibodies may also interfere with cellular functions and cause disease without tissue injury.
- In *immune complex-mediated disorders (type III hypersensitivity)*, antibodies bind antigens and then induce inflammation directly or by activating complement. The leukocytes that are recruited (neutrophils and monocytes) produce tissue damage by release of lysosomal enzymes and generation of toxic free radicals.
- In *cell-mediated immune disorders (type IV hypersensitivity)*, sensitized T lymphocytes are the cause of the cellular and tissue injury.

Most hypersensitivity diseases show a genetic predisposition. Modern methods of mapping disease-associated susceptibility

TABLE 6-2 -- Mechanisms of Immunologically Mediated Diseases

Type	Prototype Disorder	Immune Mechanisms	Pathologic Lesions
Immediate (type I) hypersensitivity	Anaphylaxis; allergies; bronchial asthma (atopic forms)	Production of IgE antibody → immediate release of vasoactive amines and other mediators from mast cells; recruitment of inflammatory cells (late-phase reaction)	Vascular dilation, edema, smooth muscle contraction, mucus production, inflammation
Antibody-mediated (type II) hypersensitivity	Autoimmune hemolytic anemia; Goodpasture syndrome	Production of IgG, IgM → binds to antigen on target cell or tissue → phagocytosis or lysis of target cell by activated complement or Fc receptors; recruitment of leukocytes	Cell lysis; inflammation
Immune complex-mediated (type III) hypersensitivity	Systemic lupus erythematosus; some forms of glomerulonephritis; serum sickness; Arthus reaction	Deposition of antigen-antibody complexes → complement activation → recruitment of leukocytes by complement products and Fc receptors → release of enzymes and other toxic molecules	Necrotizing vasculitis (fibrinoid necrosis); inflammation
Cell-mediated (type IV) hypersensitivity	Contact dermatitis; multiple sclerosis; type I, diabetes; transplant rejection; tuberculosis	Activated T lymphocytes → i) release of cytokines and macrophage activation; ii) T cell-mediated cytotoxicity	Perivascular cellular infiltrates; edema; cell destruction; granuloma formation

genes are revealing the complex nature of these genetic influences. Many susceptibility loci have been identified in different diseases. Among the genes known to be associated with hypersensitivity diseases are MHC genes, but many non-MHC genes also play a role.

Immediate (Type I) Hypersensitivity

Immediate, or type I, hypersensitivity is a rapidly developing immunologic reaction occurring within minutes after the combination of an antigen with antibody bound to mast cells in individuals previously sensitized to the antigen.[20] [21] These reactions are often called allergy, and the antigens that elicit them are allergens. Immediate hypersensitivity may occur as a systemic disorder or as a local reaction. The systemic reaction usually follows injection of an antigen to which the host has become sensitized. Often within minutes, a state of shock is produced, which is sometimes fatal. The nature of local reactions varies depending on the portal of entry of the allergen and may take the form of localized cutaneous swellings (skin allergy, hives), nasal and conjunctival discharge (allergic rhinitis and conjunctivitis), hay fever, bronchial asthma, or allergic gastroenteritis (food allergy). Many local type I hypersensitivity reactions have two well-defined phases (Fig. 6-11). The *immediate, or initial, response* is characterized by vasodilation, vascular leakage, and depending on the location, smooth muscle spasm or glandular secretions. These changes usually become evident within 5 to 30 minutes after exposure to an allergen and tend to subside in 60 minutes. In many instances (e.g., allergic rhinitis and bronchial asthma), a *second, late-phase* reaction sets in 2 to 24 hours later without additional exposure to antigen and may last for several days. This late-phase reaction is characterized by infiltration of tissues with eosinophils, neutrophils, basophils, monocytes, and CD4+ T cells as well as tissue destruction, typically in the form of mucosal epithelial cell damage.

Because mast cells are central to the development of immediate hypersensitivity, we first review some of their salient characteristics and then discuss the immune mechanisms that underlie this form of hypersensitivity.[22] *Mast cells* are bone

Figure 6-11 Immediate hypersensitivity. *A,* Kinetics of the immediate and late-phase reactions. The immediate vascular and smooth muscle reaction to allergen develops within minutes after challenge (allergen exposure in a previously sensitized individual), and the late-phase reaction develops 2 to 24 hours later. *B, C,* Morphology: The immediate reaction (*B*) is characterized by vasodilation, congestion, and edema, and the late phase reaction (*C*) is characterized by an inflammatory infiltrate rich in eosinophils, neutrophils, and T cells. *(Courtesy of Dr. Daniel Friend, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

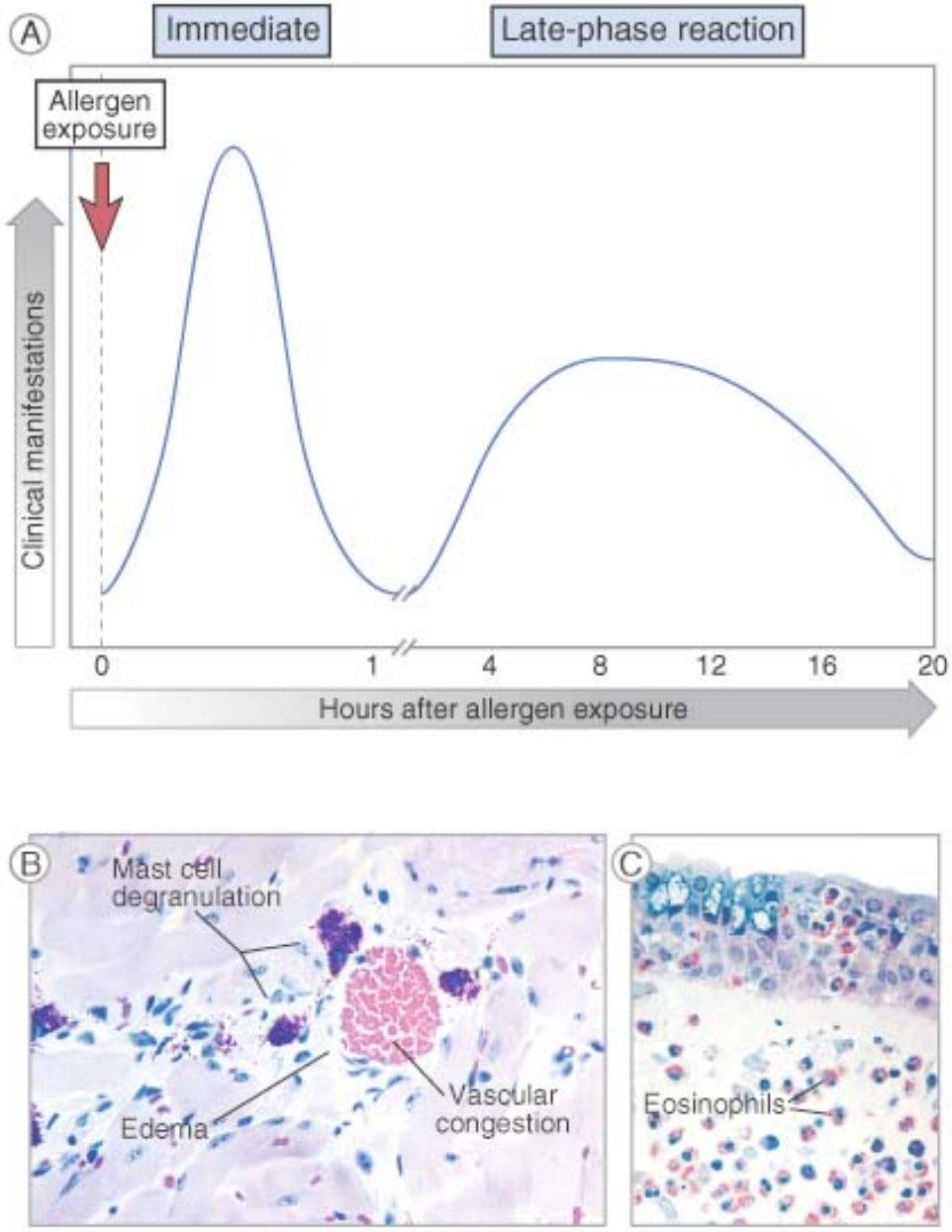
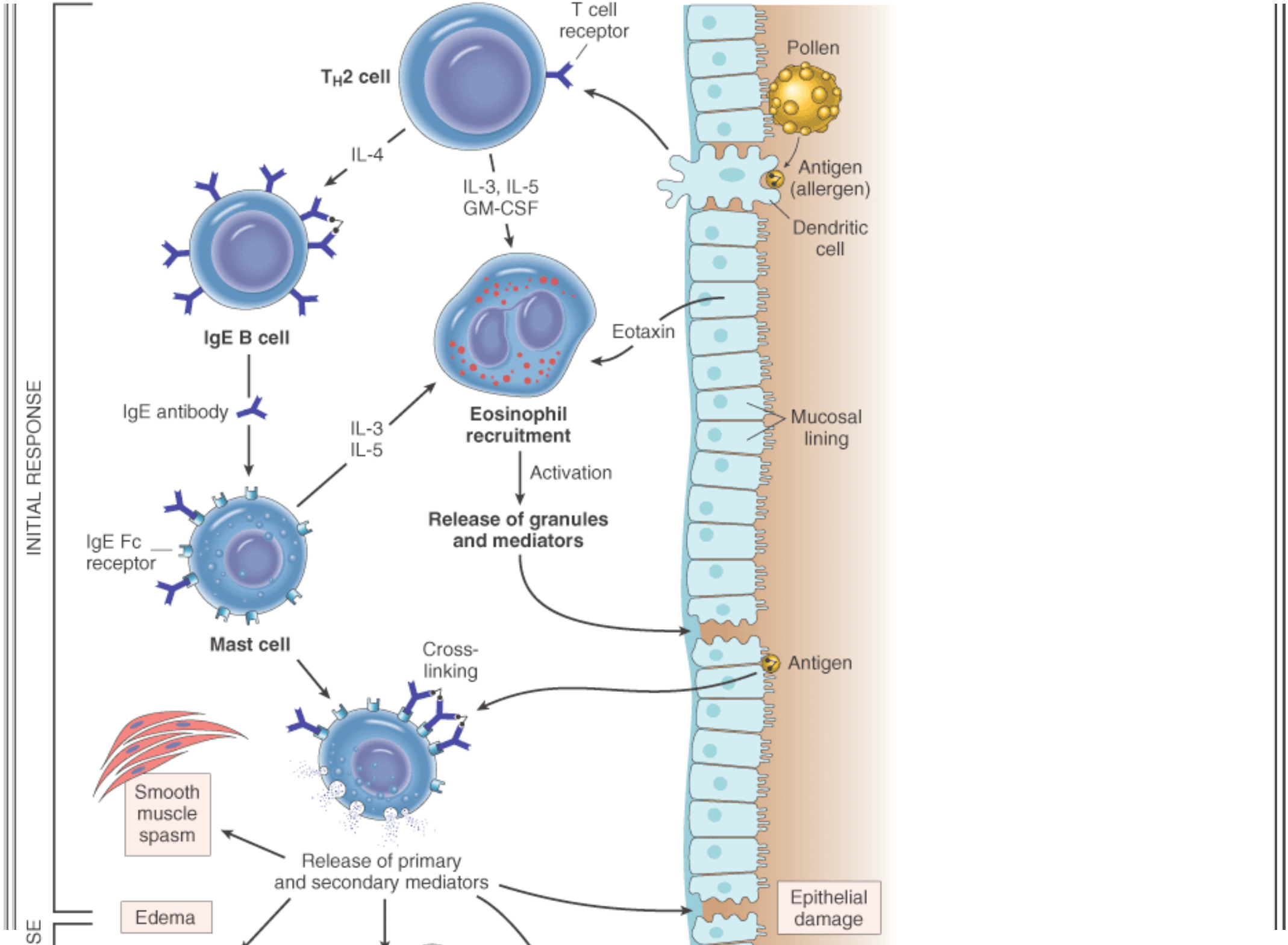


Figure 6-12 Pathogenesis of immediate (type I) hypersensitivity reaction. The late-phase reaction is dominated by leukocyte infiltration and tissue injury. T_H 2, T-helper type 2 CD4 cells.


INITIAL RESPONSE
T cell receptor
T_H2 cell
Pollen
IL-4
IL-3, IL-5
GM-CSF
Antigen (allergen)
Dendritic cell
IgE B cell
Eotaxin
IgE antibody
IL-3
IL-5
Eosinophil recruitment
Mucosal lining
Activation
IgE Fc receptor
Release of granules and mediators
Mast cell
Cross-linking
Antigen
Smooth muscle spasm
Release of primary and secondary mediators
Edema
Epithelial damage

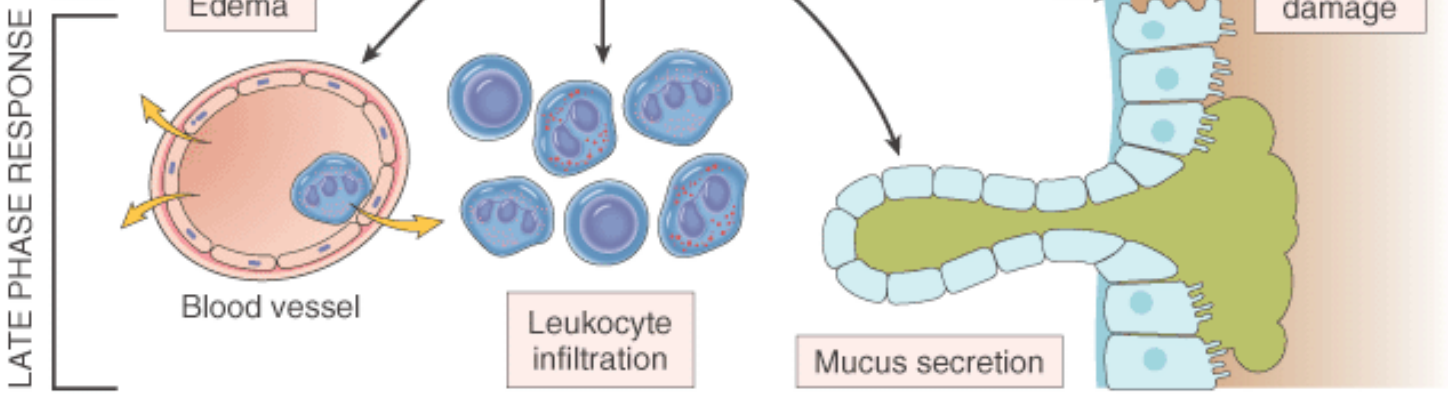
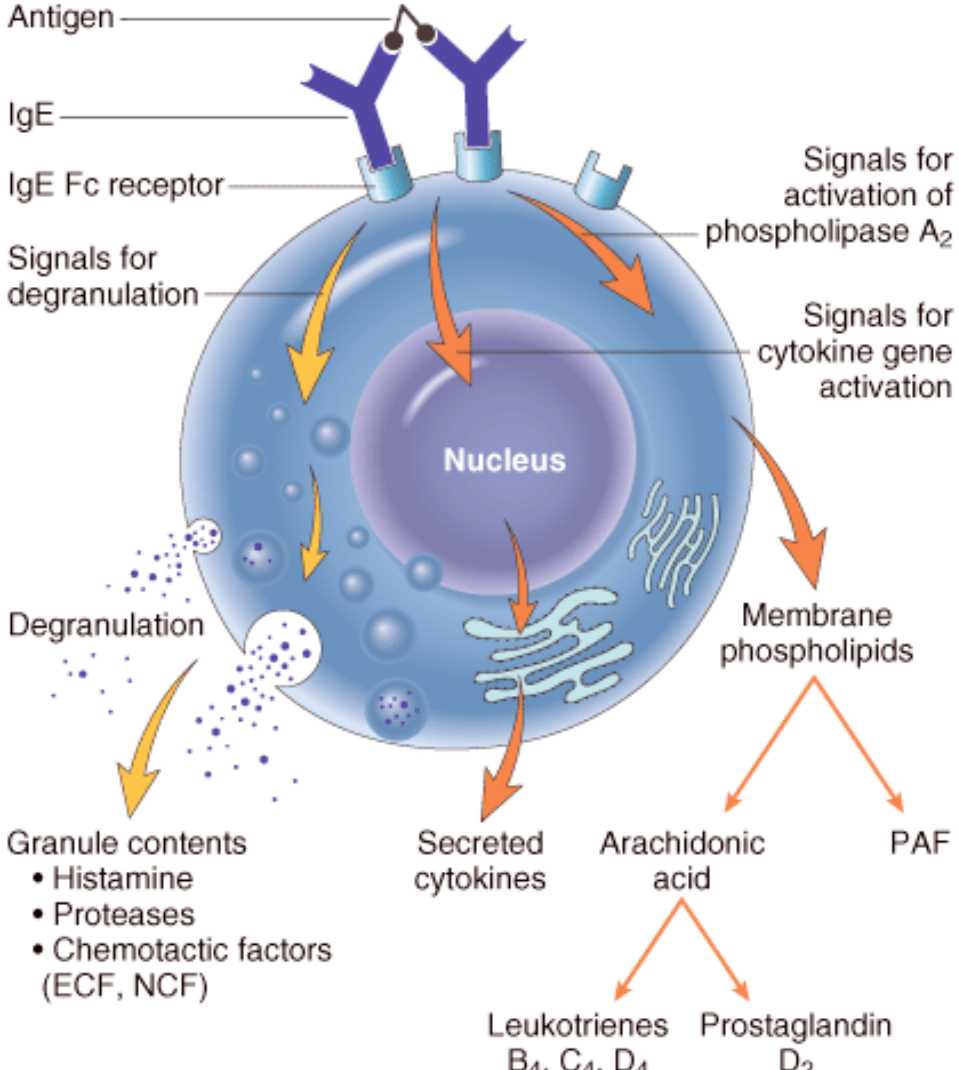


Figure 6-13 Activation of mast cells in immediate hypersensitivity and release of their mediators. ECF, eosinophil chemotactic factor; NCF, neutrophil chemotactic factor; PAF, platelet-activating factor.



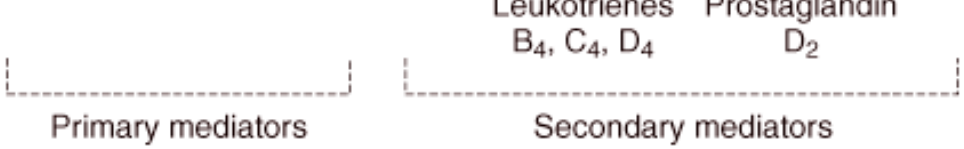


TABLE 6-3 -- Summary of the Action of Mast Cell Mediators in Immediate (Type I) Hypersensitivity

Action	Mediator
Vasodilation, increased vascular permeability	Histamine
	PAF
	Leukotrienes C_4 , D_4 , E_4
	Neutral proteases that activate complement and kinins
	Prostaglandin D_2
Smooth muscle spasm	Leukotrienes C_4 , D_4 , E_4
	Histamine
	Prostaglandins
	PAF
Cellular infiltration	Cytokines, e.g., TNF
	Leukotriene B_4
	Eosinophil and neutrophil chemotactic factors (not defined biochemically)
	PAF

PAF, platelet-activating factor; TNF, tumor necrosis factor.

produce leukotriene C_4 and PAF and directly activate mast cells to release mediators. Thus, *the recruited cells amplify and sustain the inflammatory response without additional exposure to the triggering antigen*. It is now believed that this late-phase inflammatory response is a major cause of symptoms in some type I hypersensitivity disorders, such as allergic asthma. Therefore, treatment of these diseases requires the use of broad-spectrum anti-inflammatory drugs, such as steroids.

A final point that should be mentioned in this general discussion of immediate hypersensitivity is that *susceptibility to these reactions is genetically determined*. The term *atopy* refers to a predisposition to develop localized immediate hypersensitivity reactions to a variety of inhaled and ingested allergens. Atopic individuals tend to have higher serum IgE levels, and more IL-4-producing T_H 2 cells, compared with the general population. A positive family history of allergy is found in 50% of atopic individuals. The basis of familial predisposition is not clear, but studies in patients with asthma reveal linkage to several gene loci.[27] Candidate genes have been mapped to 5q31, where genes for the cytokines IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF are located, consistent with the idea that these cytokines are involved in the reactions. Linkage has also been noted to 6p, close to the HLA complex, suggesting that the inheritance of certain HLA alleles permits reactivity to certain allergens. Another asthma-associated locus is on chromosome 11q13, the location of the gene encoding the β chain of the high-affinity IgE receptor, but many studies have failed to establish a linkage of atopy with the FcepsilonRI β chain chain or even this chromosomal region.

To summarize, immediate (type I) hypersensitivity is a complex disorder resulting from an IgE-mediated triggering of mast cells and subsequent accumulation of inflammatory cells at sites of antigen deposition. These events are regulated in large part by the induction of T_H 2-type helper T cells that promote synthesis of IgE and accumulation of inflammatory cells, particularly eosinophils. The clinical features result from release of mast-cell mediators as well as the accumulation of an eosinophilrich inflammatory exudate. With this consideration of the basic mechanisms of type I hypersensitivity, we turn to some conditions that are important examples of IgE-mediated disease.

Systemic Anaphylaxis

Systemic anaphylaxis is characterized by vascular shock, widespread edema, and difficulty in breathing. In humans, systemic anaphylaxis may occur after administration of foreign proteins (e.g., antisera), hormones, enzymes, polysaccharides, and drugs (such as the antibiotic penicillin).[28] The severity of the disorder varies with the level of sensitization. Extremely small doses of antigen may trigger anaphylaxis, for example, the tiny amounts used in ordinary skin testing for various forms of allergies. Within minutes after exposure, itching, hives, and skin erythema appear, followed shortly thereafter by a striking contraction of respiratory bronchioles and respiratory distress. Laryngeal edema results in hoarseness. Vomiting, abdominal cramps, diarrhea, and laryngeal obstruction follow, and the patient may go into shock and even die within the hour. The risk of anaphylaxis must be borne in mind when certain therapeutic agents are administered. Although patients at risk can generally be identified by a previous history of some form of allergy, the absence of such

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a history does not preclude the possibility of an anaphylactic reaction.

Local Immediate Hypersensitivity Reactions

Local immediate hypersensitivity, or allergic, reactions are exemplified by so-called atopic allergy. About 10% of the population suffers from allergies involving localized reactions to common environmental allergens, such as pollen, animal dander, house dust, foods, and the like. Specific diseases include urticaria, angioedema, allergic rhinitis (hay fever), and some forms of asthma, all discussed elsewhere in this book. The familial predisposition to the development of this type of allergy has been mentioned earlier.

Antibody-Mediated (Type II) Hypersensitivity

Type II hypersensitivity is mediated by antibodies directed toward antigens present on cell surfaces or extracellular matrix. The antigenic determinants may be intrinsic to the cell membrane or matrix, or they may take the form of an exogenous antigen, such as a drug metabolite, that is adsorbed on a cell surface or matrix. In either case, the hypersensitivity reaction results from the binding of antibodies to normal or altered cell-surface antigens. Three different antibody-dependent mechanisms involved in this type of reaction are depicted in Figure 6-14 and described next. Most of these reactions involve the effector mechanisms that are used by antibodies, namely the complement system and phagocytes.

Opsonization and Complement- and Fc Receptor-Mediated Phagocytosis

The depletion of cells targeted by antibodies is, to a large extent, because the cells are coated (opsonized) with molecules that make them attractive for phagocytes. When antibodies are deposited on the surfaces of cells, they may activate the complement system (if the antibodies are of the IgM or IgG class). Complement activation generates byproducts, mainly C3b and C4b, which are deposited on the surfaces of the cells and recognized by phagocytes that express receptors for these proteins. In addition, cells opsonized by IgG antibodies are recognized by phagocyte Fc receptors, which are specific for the Fc portions of some IgG subclasses. The net result is the phagocytosis of the opsonized cells and their destruction (Fig. 6-14*A*). Complement activation on cells also leads to the formation of the membrane attack complex, which disrupts membrane integrity by "drilling holes" through the lipid bilayer, thereby causing osmotic lysis of the cells.

Antibody-mediated destruction of cells may occur by another process called *antibody-dependent cellular cytotoxicity (ADCC).* This form of antibody-mediated cell injury does not involve

fixation of complement but instead requires the cooperation of leukocytes. Cells that are coated with low concentrations of IgG antibody are killed by a variety of effector cells, which bind to the target by their receptors for the Fc fragment of IgG, and cell lysis proceeds without phagocytosis. ADCC may be mediated by monocytes, neutrophils, eosinophils, and NK cells. Although, in most instances, IgG antibodies are involved in ADCC, in certain cases (e.g., eosinophil-mediated cytotoxicity against parasites), IgE antibodies are used. The role of ADCC in hypersensitivity diseases is uncertain.

Clinically, antibody-mediated cell destruction and phagocytosis occur in the following situations: (1) *transfusion reactions*, in which cells from an incompatible donor react with and are opsonized by preformed antibody in the host; (2) *erythroblastosis fetalis*, in which there is an antigenic difference between the mother and the fetus, and antibodies (of the IgG class) from the mother cross the placenta and cause destruction of fetal red cells; (3) *autoimmune hemolytic anemia*, *agranulocytosis*, and *thrombocytopenia*, in which individuals produce antibodies to their own blood cells, which are then destroyed; and (4) *certain drug reactions*, in which antibodies are produced that react with the drug, which may be attached to the surface of erythrocytes or other cells.

Complement- and Fc Receptor-Mediated Inflammation

When antibodies deposit in extracellular tissues, such as basement membranes and matrix, the resultant injury is because of inflammation and not because of phagocytosis or lysis of cells. The deposited antibodies activate complement, generating byproducts, such as C5a (and to a lesser extent C4a and C3a), that recruit neutrophils and monocytes. The same cells also bind to the deposited antibodies via their Fc receptors. The leukocytes are activated, they release injurious substances, such as enzymes and reactive oxygen intermediates, and the result is damage to the tissues (Fig. 6-14*B*). It was once thought that complement was the major mediator of antibody-induced inflammation, but knockout mice lacking Fc receptors also show striking reduction in these reactions. It is now believed that inflammation in antibody-mediated (and immune complex-mediated) diseases is because of both complement and Fc receptor-dependent reactions.[29]

Antibody-mediated inflammation is the mechanism responsible for tissue injury in some forms of *glomerulonephritis*, *vascular rejection* in organ grafts, and other diseases (Table 6-4). As we shall discuss in more detail below, the same reaction is involved in immune complex-mediated diseases.

Antibody-Mediated Cellular Dysfunction

In some cases, antibodies directed against cell-surface receptors impair or dysregulate function without causing cell injury or inflammation. For example, in *myasthenia gravis*, antibodies reactive with acetylcholine receptors in the motor end-plates of skeletal muscles impair neuromuscular transmission and therefore cause muscle weakness (Fig. 6-14*C*). In *pemphigus vulgaris*, antibodies against desmosomes disrupt intercellular junctions in epidermis, leading to the formation of skin vesicles. The converse (i.e., antibody-mediated stimulation of cell function) is noted in *Graves disease*. In this disorder, antibodies against the thyroid-stimulating hormone receptor on thyroid epithelial cells stimulate the cells, resulting in hyperthyroidism.

Immune Complex-Mediated (Type III) Hypersensitivity

Antigen-antibody complexes produce tissue damage mainly by eliciting inflammation at the sites of deposition. The toxic

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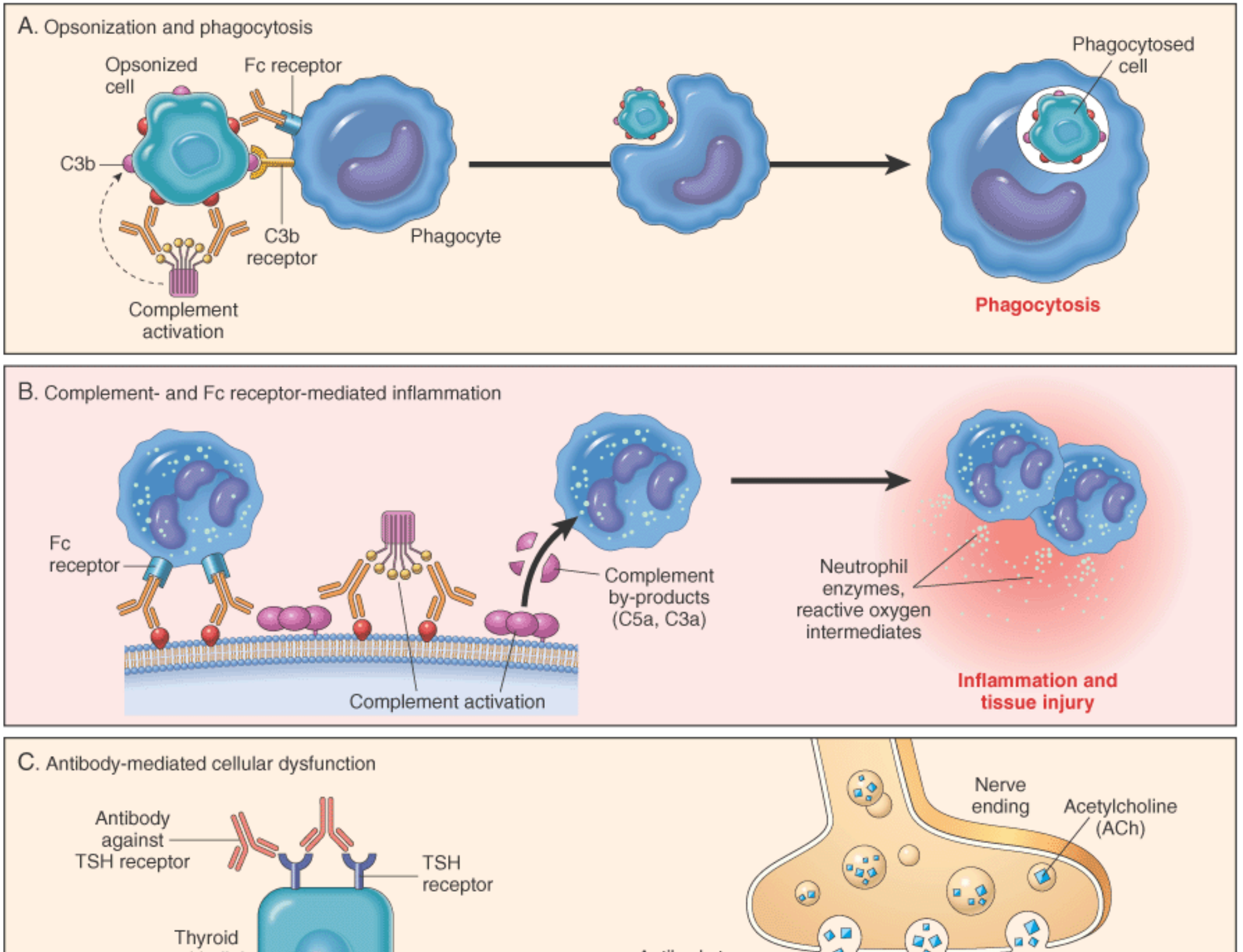
Figure 6-14 Schematic illustration of the three major mechanisms of antibody-mediated injury. *A*, Opsonization of cells by antibodies and complement components and ingestion by phagocytes. *B*, Inflammation induced by antibody binding to Fc receptors of leukocytes and by complement breakdown products. *C*, Antireceptor antibodies disturb the normal function of receptors. In these examples, antibodies against the thyroid stimulating hormone (TSH) receptor activate thyroid cells in Graves disease, and acetylcholine (ACh) receptor antibodies impair neuromuscular transmission in myasthenia gravis.


A. Opsonization and phagocytosis
Opsonized cell
Fc receptor
C3b
C3b receptor
Phagocyte
Complement activation
Phagocytosed cell
Phagocytosis
B. Complement- and Fc receptor-mediated inflammation
Fc receptor
Complement activation
Complement by-products (C5a, C3a)
Neutrophil enzymes, reactive oxygen intermediates
Inflammation and tissue injury
C. Antibody-mediated cellular dysfunction
Antibody against TSH receptor
TSH receptor
Thyroid
Nerve ending
Acetylcholine (ACh)

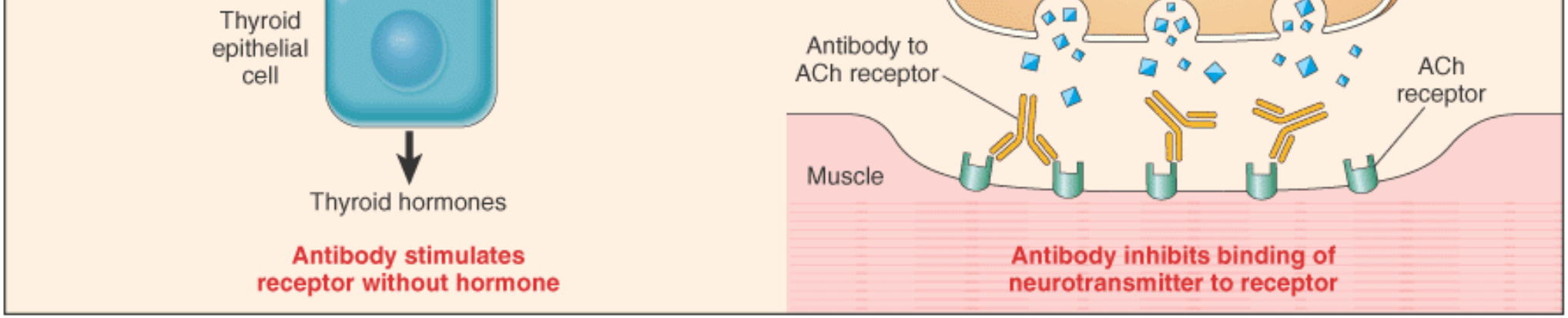


TABLE 6-4 -- Examples of Antibody-Mediated Diseases (Type II Hypersensitivity)

Disease	Target Antigen	Mechanisms of Disease	Clinicopathologic Manifestations
Autoimmune hemolytic anemia	Erythrocyte membrane proteins (Rh blood group antigens, I antigen)	Opsonization and phagocytosis of erythrocytes	Hemolysis, anemia
Autoimmune thrombocytopenic purpura	Platelet membrane proteins (gpllb:Illa intergrin)	Opsonization and phagocytosis of platelets	Bleeding
Pemphigus vulgaris	Proteins in intercellular junctions of epidermal cells (epidermal cadherin)	Antibody-mediated activation of proteases, disruption of intercellular adhesions	Skin vesicles (bullae)
Vasculitis caused by ANCA	Neutrophil granule proteins, presumably released from activated neutrophils	Neutrophil degranulation and inflammation	Vasculitis
Goodpasture syndrome	Noncollagenous protein in basement membranes of kidney glomeruli and lung alveoli	Complement- and Fc receptor-mediated inflammation	Nephritis, lung hemorrhage
Acute rheumatic fever	Streptococcal cell wall antigen; antibody cross-reacts with myocardial antigen	Inflammation, macrophage activation	Myocarditis, arthritis
Myasthenia gravis	Acetylcholine receptor	Antibody inhibits acetylcholine binding, down-modulates receptors	Muscle weakness, paralysis
Graves disease (hyperthyroidism)	TSH receptor	Antibody-mediated stimulation of TSH receptors	Hyperthyroidism
Insulin-resistant diabetes	Insulin receptor	Antibody inhibits binding of insulin	Hyperglycemia, ketoacidosis
Pernicious anemia	Intrinsic factor of gastric parietal cells	Neutralization of intrinsic factor, decreased absorption of vitamin B_{12}	Abnormal erythropoiesis, anemia

ANCA, antineutrophil cytoplasmic antibodies; TSH, thyroid-stimulating hormone.

From Abbas AK, Lichtman H: Cellular and Molecular Immunology. 5th edition. WB Saunders Company, Philadelphia, 2003.

Examples of immune complex disorders and the antigens involved are listed in Table 6-5 . Immune complex-mediated diseases can be *generalized*, if immune complexes are formed in the

circulation and are deposited in many organs, or *localized* to particular organs, such as the kidney (glomerulonephritis), joints (arthritis), or the small blood vessels of the skin if the complexes are formed and deposited locally. These two patterns are considered separately.

Systemic Immune Complex Disease

Acute serum sickness is the prototype of a systemic immune complex disease; it was at one time a frequent sequela to the administration of large amounts of foreign serum (e.g., immune serum from horses used for passive immunization.) The

TABLE 6-5 -- Examples of Immune Complex-Mediated Diseases

Disease	Antigen Involved	Clinicopathologic Manifestations
Systemic lupus erythematosus	DNA, nucleoproteins, others	Nephritis, arthritis, vasculitis
Polyarteritis nodosa	Hepatitis B virus surface antigen (in some cases)	Vasculitis
Poststreptococcal glomerulonephritis	Streptococcal cell wall antigen(s); may be "planted" in glomerular basement membrane	Nephritis
Acute glomerulonephritis	Bacterial antigens *(Treponema)*; parasite antigens (malaria, schistosomes); tumor antigens	Nephritis
Reactive arthritis	Bacterial antigens *(Yersinia)*	Acute arthritis
Arthus reaction	Various foreign proteins	Cutaneous vasculitis
Serum sickness	Various proteins, e.g., foreign serum (anti-thymocyte globulin)	Arthritis, vasculitis, nephritis

occurrence of diseases caused by immune complexes was suspected in the early 1900s by a physician named Clemens von Pirquet. Patients with diphtheria infection were being treated with serum from horses immunized with the diphtheria toxin. Von Pirquet noted that some of these patients developed arthritis, skin rash, and fever, and the symptoms appeared more rapidly with repeated injection of the serum. Von Pirquet concluded that the treated patients made antibodies to horse serum proteins, these antibodies formed complexes with the injected proteins, and the disease was due to the antibodies or immune complexes. He called this disease "serum disease"; it is now known as serum sickness. In modern times the disease is infrequent, but it is an informative model that has taught us a great deal about systemic immune complex disorders.

For the sake of discussion, the pathogenesis of systemic immune complex disease can be divided into three phases: (1) formation of antigen-antibody complexes in the circulation; (2) deposition of the immune complexes in various tissues, thus initiating; and (3) an inflammatory reaction at the sites of immune complex deposition (Fig. 6-15). The *first phase* is initiated by the introduction of antigen, usually a protein, and its interaction with immunocompetent cells, resulting in the formation of antibodies approximately a week after the injection of the protein. These antibodies are secreted into the blood, where they react with the antigen still present in the circulation to form antigen-antibody complexes. In the *second phase*, the circulating antigen-antibody complexes are deposited in various tissues.

The factors that determine whether immune complex formation will lead to tissue deposition and disease are not fully understood, but two possible influences are the size of the immune complexes and the functional status of the mononuclear phagocyte system:

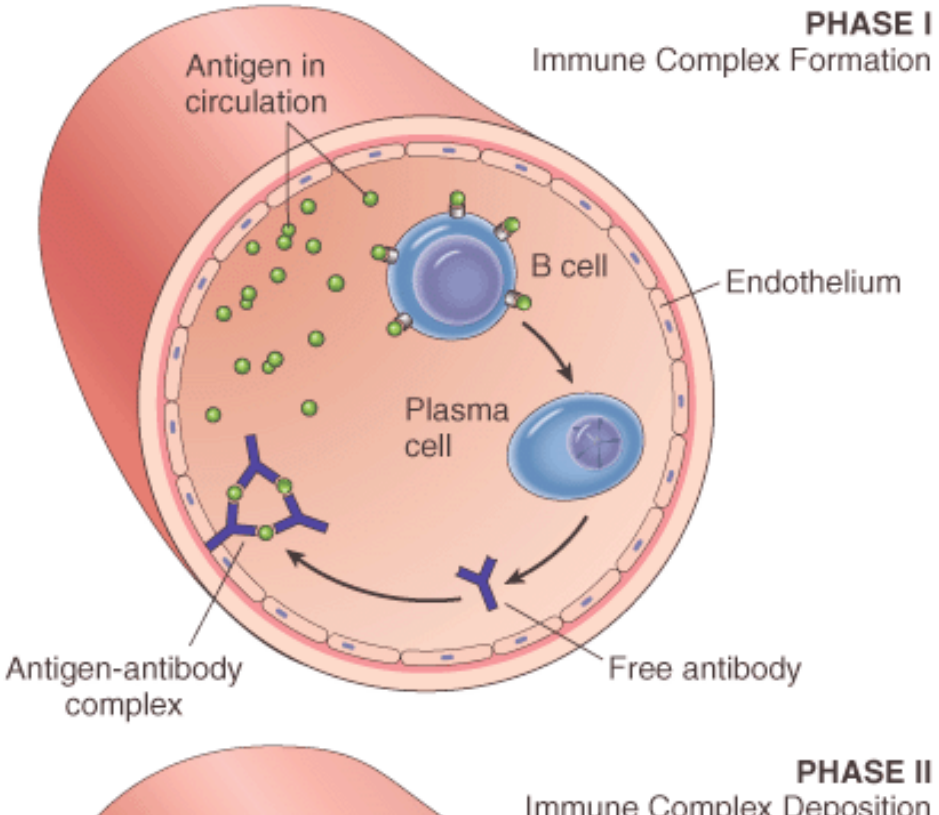
• Large complexes formed in great antibody excess are rapidly removed from the circulation by the mononuclear phagocyte system and are therefore relatively harmless. The most pathogenic complexes are of small or intermediate size (formed in slight antigen excess), which bind less avidly to phagocytic cells and therefore circulate longer.
• Because the mononuclear phagocyte system normally filters out the circulating immune complexes, its overload or intrinsic dysfunction increases the probability of persistence of immune complexes in circulation and tissue deposition.

In addition, several other factors, such as charge of the immune complexes (anionic versus cationic), valency of the antigen, avidity of the antibody, affinity of the antigen to various tissue components, three-dimensional (lattice) structure of the complexes, and hemodynamic factors, influence the tissue deposition of complexes. Because most of these influences have been investigated with reference to deposition of immune complexes in the glomeruli, they are discussed further in Chapter 20 . In addition to the renal glomeruli, the favored sites of immune complex deposition are joints, skin, heart, serosal surfaces, and small blood vessels. For complexes to leave the microcirculation and deposit in the vessel wall, an increase in vascular permeability must occur. This is believed to occur when immune complexes bind to inflammatory cells through their Fc or C3b receptors and trigger release of vasoactive mediators as well as permeability-enhancing cytokines. Mast cells may also be involved in this phase of the reaction.

Once complexes are deposited in the tissues, they initiate an acute inflammatory reaction *(third phase)*. During this phase (approximately 10 days after antigen administration), clinical features such as fever, urticaria, arthralgias, lymph node enlargement, and proteinuria appear.

Wherever complexes deposit, the tissue damage is similar. Two mechanisms are believed to cause *inflammation* at the sites of deposition (Fig. 6-16): (1) activation of the complement cascade, and (2) activation of neutrophils and macrophages through their Fc receptors. As discussed in Chapter 2 , *complement activation* promotes inflammation mainly by production of chemotactic factors, which direct the migration of polymorphonuclear leukocytes and monocytes

Figure 6-15 Schematic illustration of the three sequential phases in the induction of systemic immune complex-mediated disease (type III hypersensitivity).



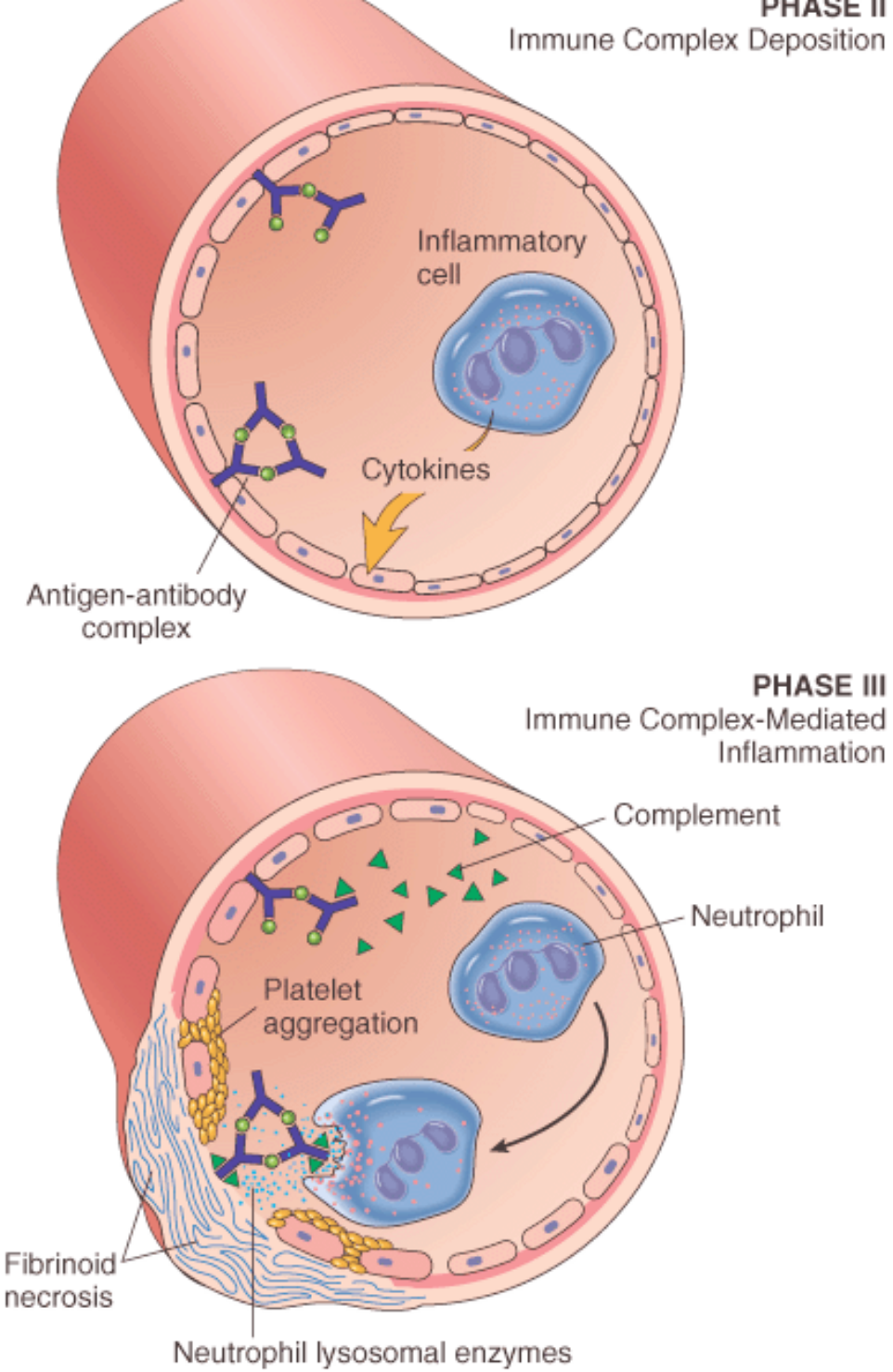


Figure 6-16 Pathogenesis of immune complex-mediated tissue injury. The morphologic consequences are depicted as boxed areas.

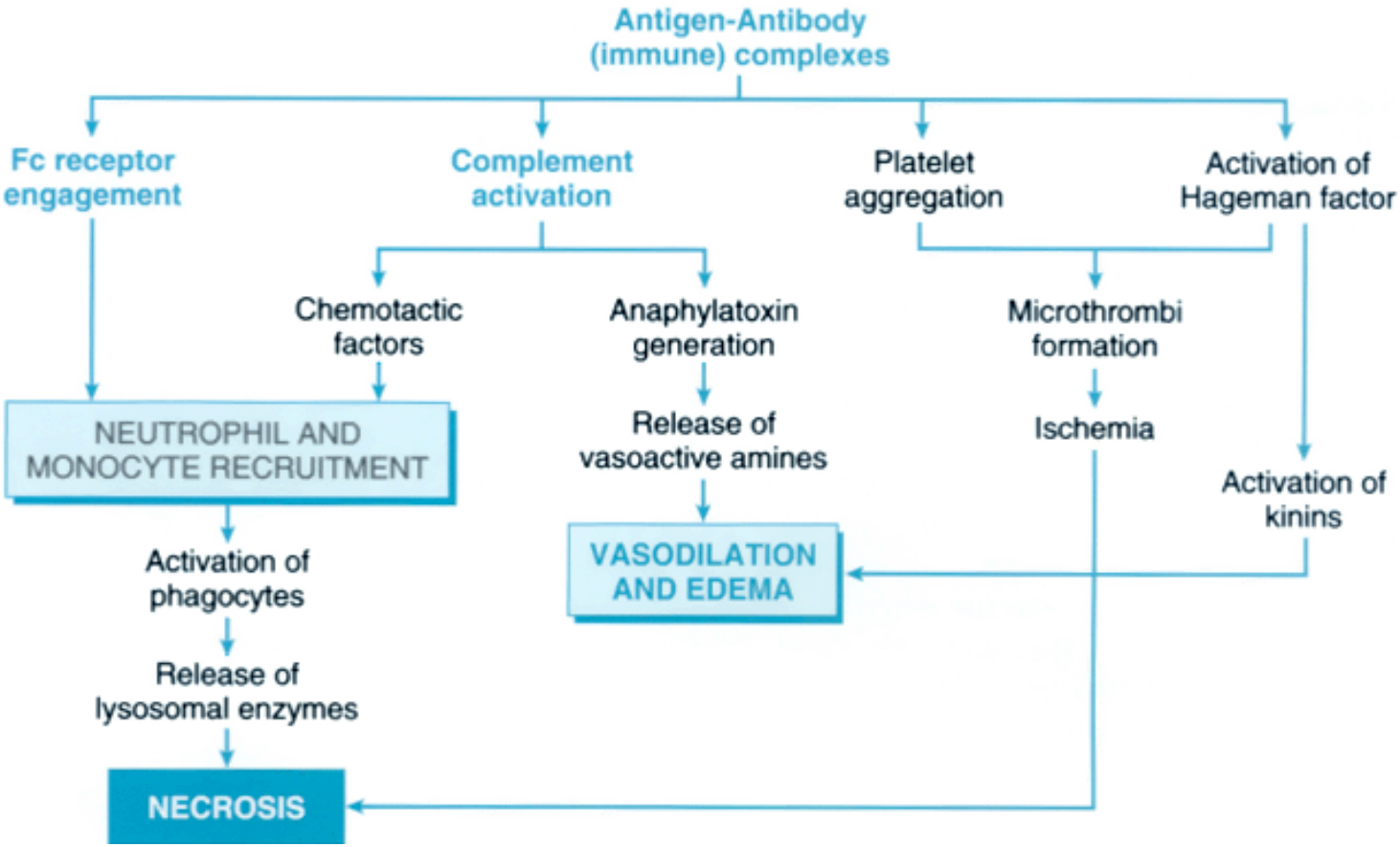


Figure 6-17 Immune complex vasculitis. The necrotic vessel wall is replaced by smudgy, pink "fibrinoid" material. *(Courtesy of Dr. Trace Worrell, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

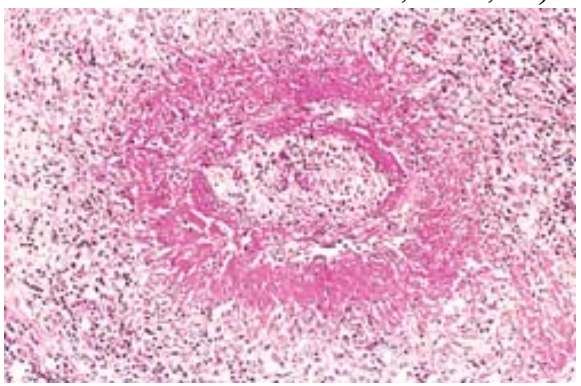


Figure 6-18 Mechanisms of T cell-mediated (type IV) hypersensitivity reactions. *A*, In delayed type hypersensitivity reactions, CD4+ T cells (and sometimes CD8+ cells) respond to tissue antigens by secreting cytokines that stimulate inflammation and activate phagocytes, leading to tissue injury. *B*, In some diseases, CD8+ cytolytic T lymphocytes (CTLs) directly kill tissue cells. APC, antigenpresenting cell.

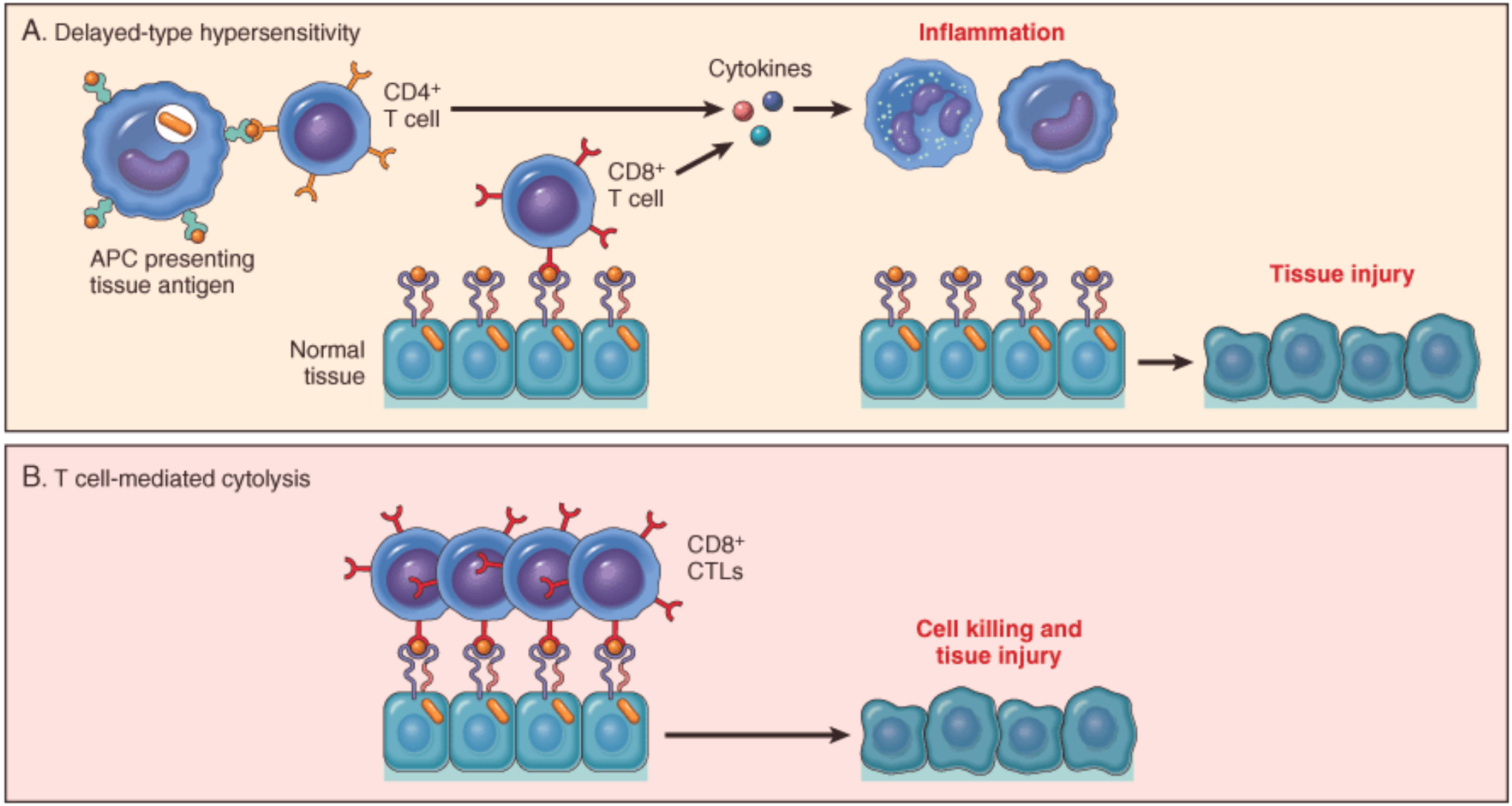


TABLE 6-6 -- Examples of T Cell-Mediated (Type IV) Hypersensitivity

Disease	Specificity of Pathogenic T Cells	Clinicopathologic Manifestations
Type 1 diabetes mellitus	Antigens of pancreatic islet β cells (insulin, glutamic acid decarboxylase, others)	Insulitis (chronic inflammation in islets), destruction of β cells; diabetes
Multiple sclerosis	Protein antigens in central nervous system myelin (myelin basic protein, proteolipid protein)	Demyelination in CNS with perivascular inflammation; paralysis, ocular lesions
Rheumatoid arthritis	Unknown antigen in joint synovium (type II collagen?); role of antibodies?	Chronic arthritis with inflammation, destruction of articular cartilage and bone

Peripheral neuropathy; Guillain-Barré syndrome?	Protein antigens of peripheral nerve myelin	Neuritis, paralysis

and inflammation (Fig. 6-17). Thrombi are formed in the vessels, resulting in local ischemic injury.

Cell-Mediated (Type IV) Hypersensitivity

The cell-mediated type of hypersensitivity is initiated by antigen-activated (sensitized) T lymphocytes. It includes the *delayed type hypersensitivity reactions* mediated by CD4+ T cells, and *direct cell cytotoxicity* mediated by CD8+ T cells (Fig. 6-18). It is the principal pattern of immunologic response not only to a variety of intracellular microbiologic agents, such as *Mycobacterium tuberculosis*, but also to many viruses, fungi, protozoa, and parasites. So-called contact skin sensitivity to chemical agents and graft rejection are other instances of cell-mediated reactions. In addition, many autoimmune diseases are now known to be caused by T cell-mediated reactions (Table 6-6). The two forms of T cell-mediated hypersensitivity are described next.

Figure 6-19 Delayed hypersensitivity in the skin. *A*, Perivascular infiltration by T cells and mononuclear phagocytes. *B*, Immunoperoxidase staining reveals a predominantly perivascular cellular infiltrate that marks positively with anti-CD4 antibodies. *(Courtesy of Dr. Louis Picker, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

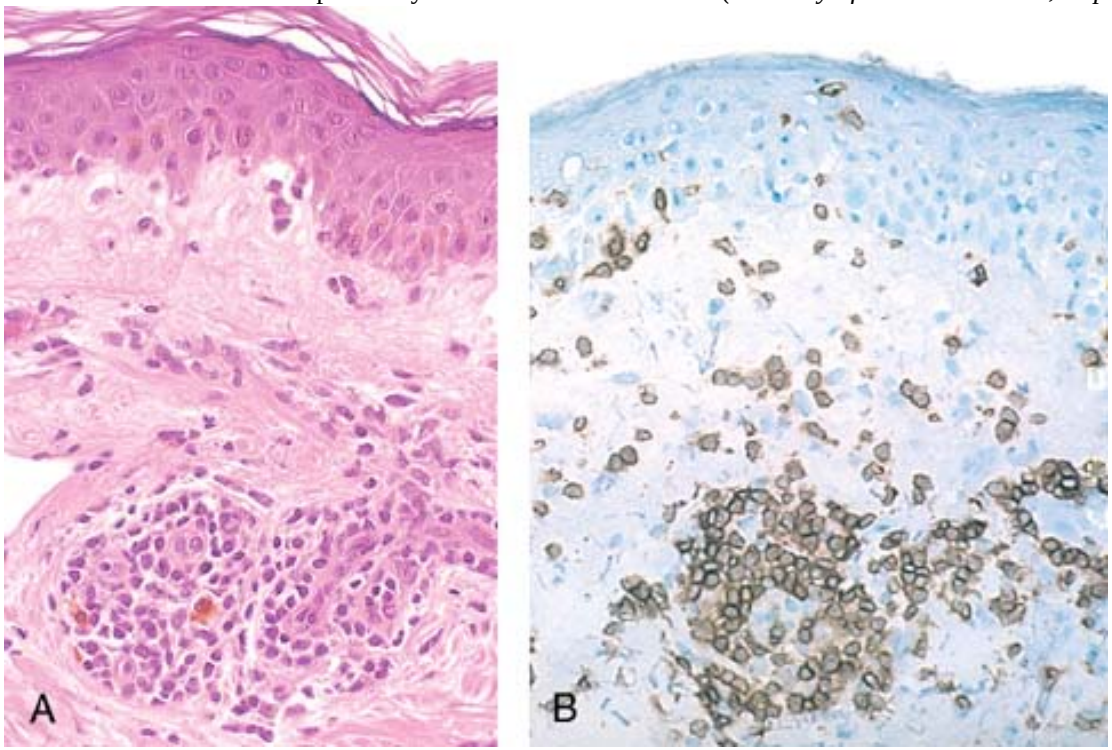


Figure 6-20 A section of a lymph node shows several granulomas, each made up of an aggregate of epithelioid cells and surrounded by lymphocytes. The granuloma in the center shows

several multinucleate giant cells. *(Courtesy of Dr. Trace Worrell, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

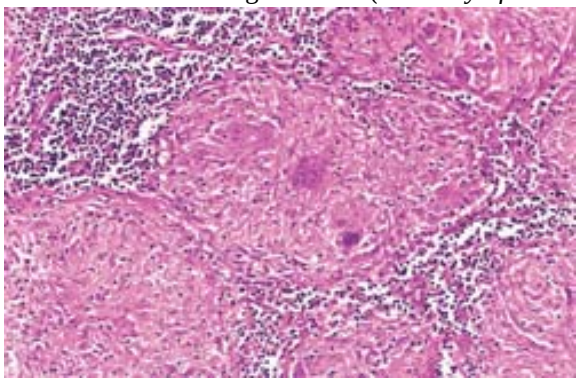


Figure 6-21 Schematic illustration of the events that give rise to the formation of granulomas in cell-mediated (type IV) hypersensitivity reactions. Note the role played by T cell-derived cytokines.

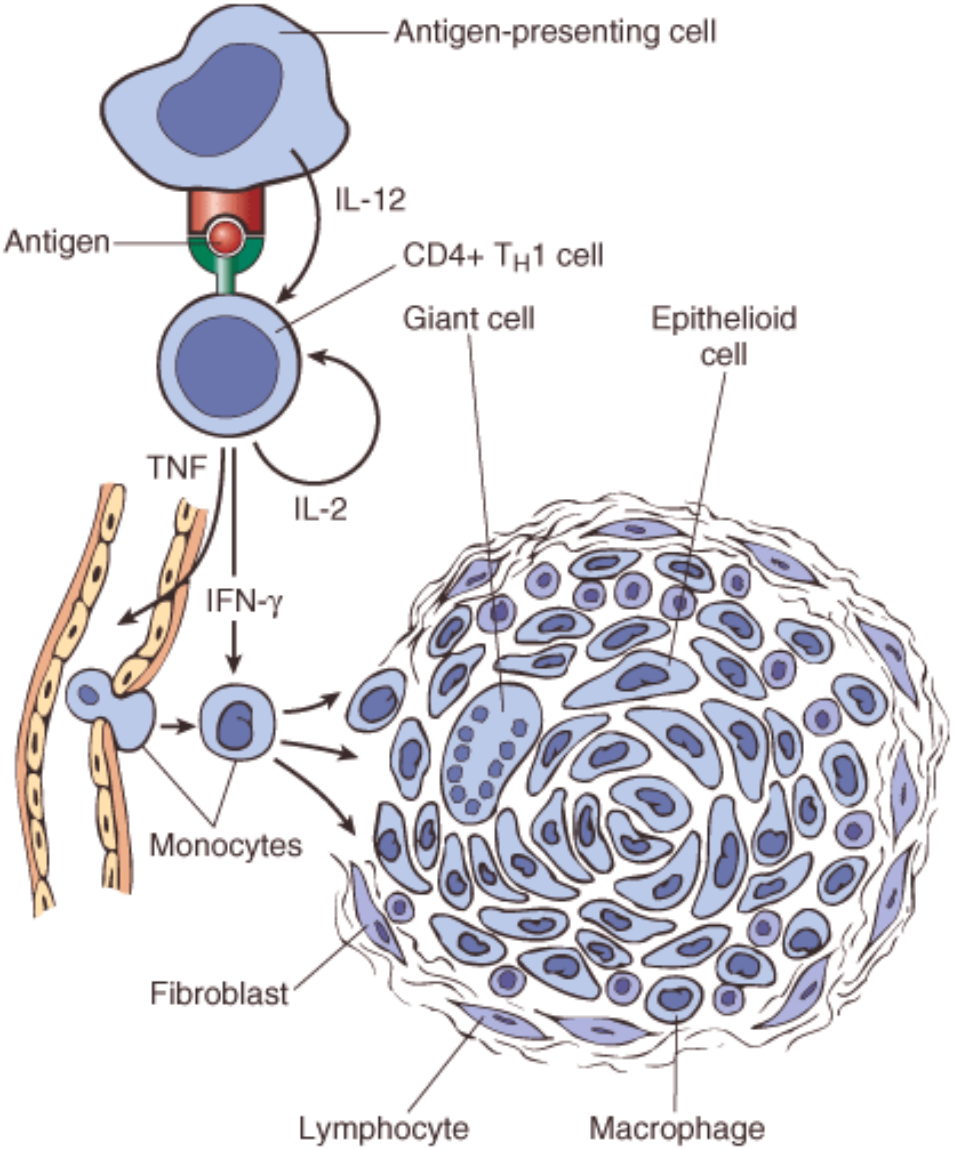


Figure 6-22 Contact dermatitis showing an epidermal blister (vesicle) with dermal and epidermal mononuclear infiltrates. *(Courtesy of Dr. Louis Picker, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

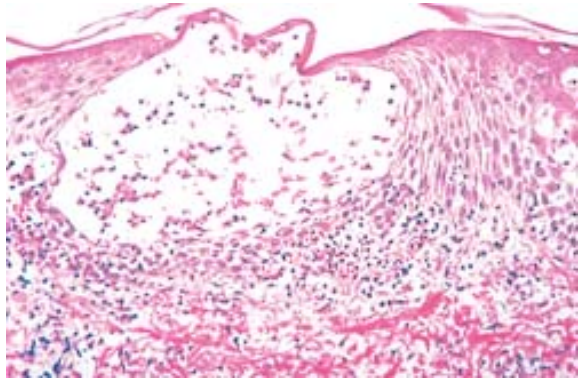
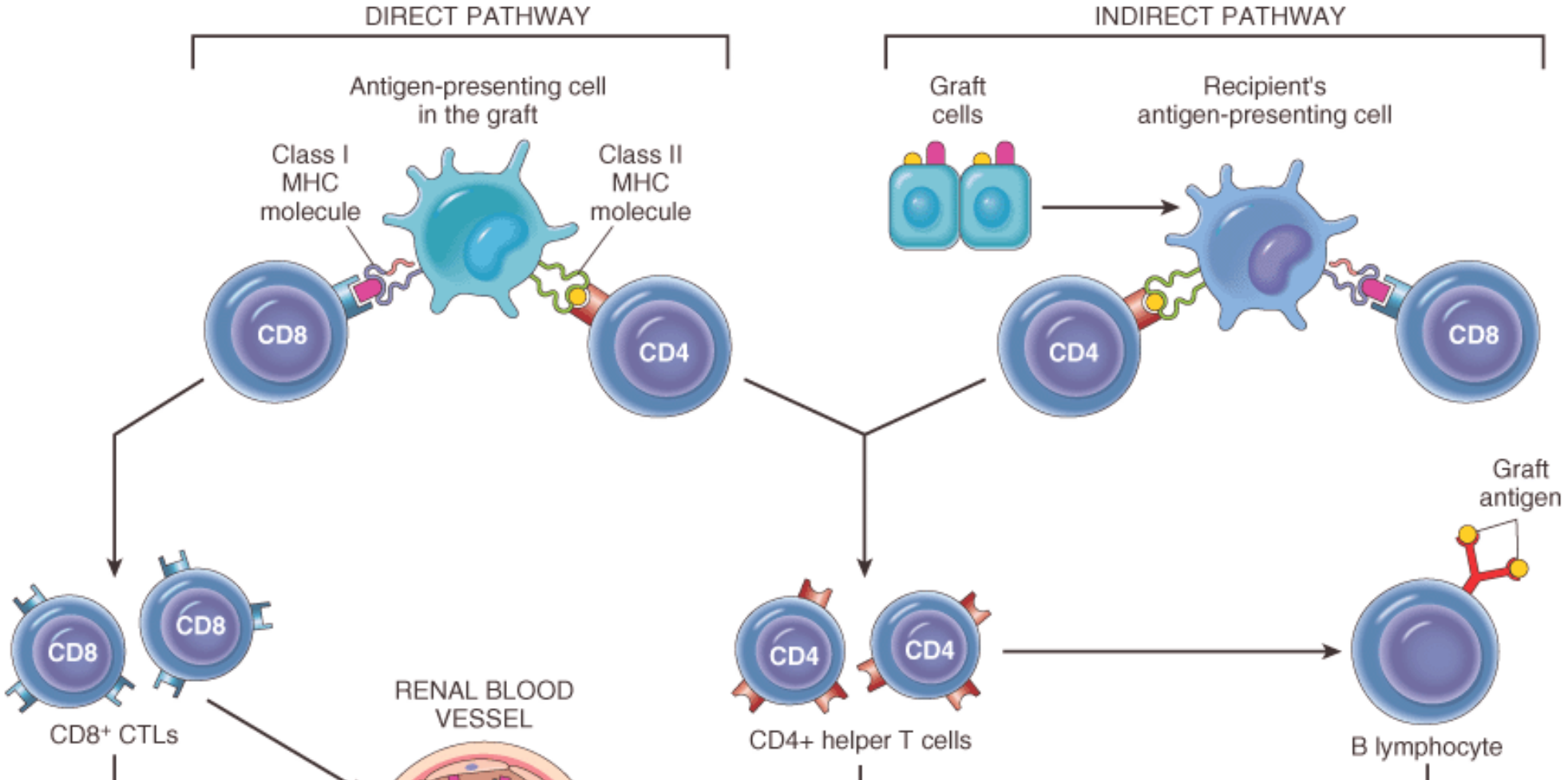


Figure 6-23 Schematic representation of the events that lead to the destruction of histoincompatible grafts. In the direct pathway, donor class I and class II antigens on antigen-presenting cells in the graft (along with B7 molecules, not shown) are recognized by CD8+ cytotoxic T cells and CD4+ helper T cells, respectively, of the host. CD4+ cells proliferate and produce cytokines that induce tissue damage by a local delayed hypersensitivity reaction and stimulate B cells and CD8+ T cells. CD8+ T cells responding to graft antigens differentiate into cytotoxic T lymphocytes that kill graft cells. In the indirect pathway, graft antigens are displayed by host APCs and activate CD4+ cells, which damage the graft by a local delayed hypersensitivity reaction. The example shown is of a kidney allograft.



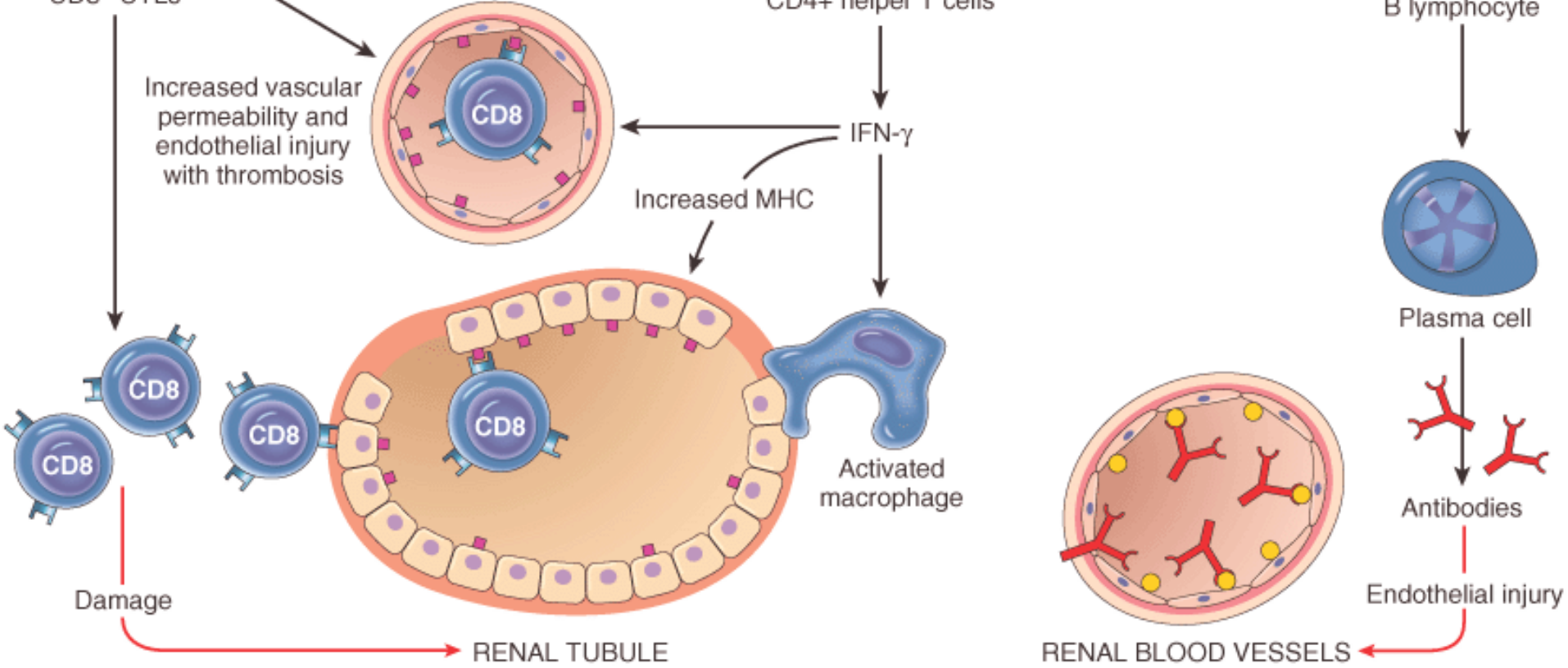


Figure 6-24 Acute cellular rejection of a renal allograft. *A,* An intense mononuclear cell infiltrate occupies the space between the tubules. *B,* T cells (stained brown by the immunoperoxidase technique) are abundant in the interstitium and infiltrating a tubule. *(Courtesy of Dr. Robert Colvin, Department of Pathology, Massachusetts General Hospital, Boston, MA.)*

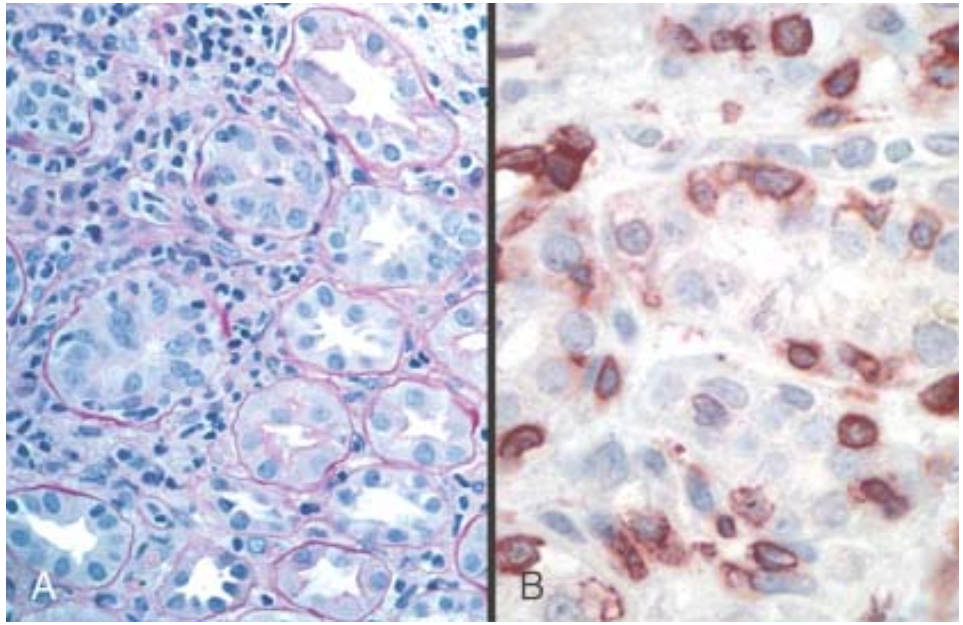


Figure 6-25 Antibody-mediated damage to the blood vessel in a renal allograft. The blood vessel is markedly thickened, and the lumen is obstructed by proliferating fibroblasts and foamy macrophages. *(Courtesy of Dr. Ihsan Housini, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

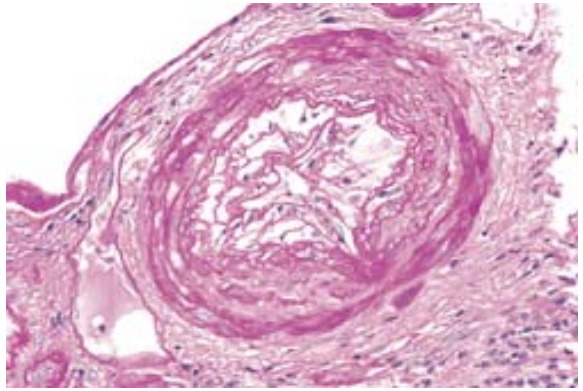


Figure 6-26 Chronic rejection in a kidney allograft. *A,* Changes in the kidney in chronic rejection. *B,* Graft arteriosclerosis. The vascular lumen is replaced by an accumulation of smooth muscle cells and connective tissue in the vessel intima. *(Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.)*

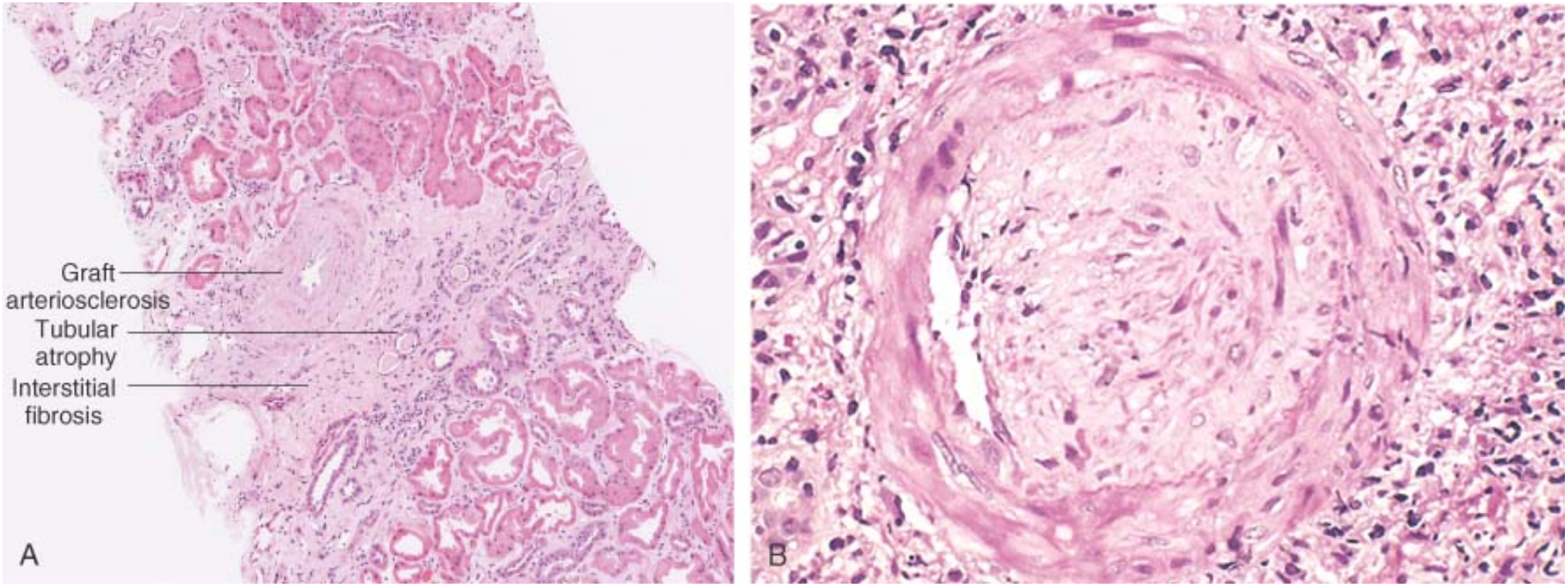


TABLE 6-7 -- Autoimmune Diseases

Organ-Specific	**Systemic**
Hashimoto thyroiditis	Systemic lupus erythematosus
Autoimmune hemolytic anemia	Rheumatoid arthritis
Autoimmune atrophic gastritis of pernicious anemia	Sjögren syndrome
Multiple sclerosis	Reiter syndrome
Autoimmune orchitis	Inflammatory myopathies *
Goodpasture syndrome	Systemic sclerosis (scleroderma) *
Autoimmune thrombocytopenia	Polyarteritis nodosa *
Insulin-dependent diabetes mellitus	
Myasthenia gravis	
Graves disease	
Primary biliary cirrhosis *	

Autoimmune (chronic active) hepatitis *	
Ulcerative colitis *	

*The evidence supporting an autoimmune basis of these disorders is not strong.

autoimmunity are type I diabetes mellitus, in which the autoreactive T cells and antibodies are specific for β cells of the pancreatic islets, and multiple sclerosis, in which autoreactive T cells react against central nervous system myelin. An example of systemic autoimmune disease is SLE, in which a diversity of antibodies directed against DNA, platelets, red cells, and protein-phospholipid complexes result in widespread lesions throughout the body. In the middle of the spectrum falls Goodpasture syndrome, in which antibodies to basement membranes of lung and kidney induce lesions in these organs.

It is obvious that autoimmunity results from the loss of self-tolerance, and the question arises as to how this happens. Before we look for answers to this question, we review the mechanisms of immunologic tolerance to self-antigens.

Immunologic Tolerance

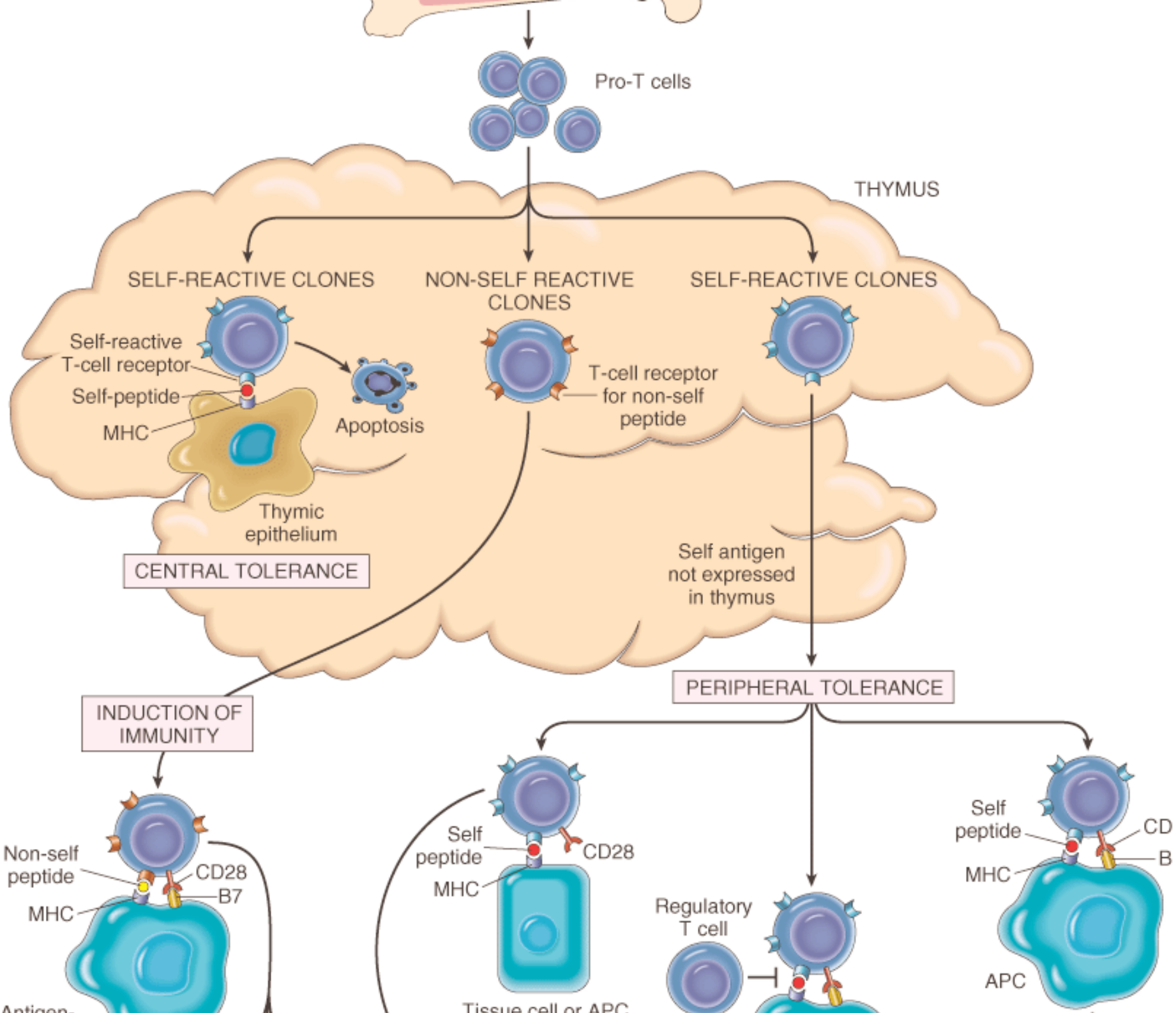
Immunologic tolerance is a state in which the individual is incapable of developing an immune response to a specific antigen. Self-tolerance refers to lack of responsiveness to an individual's own antigens, and it underlies our ability to live in harmony with our cells and tissues. Several mechanisms, albeit not well understood, have been postulated to explain the tolerant state. They can be broadly classified into two groups: *central tolerance* and *peripheral tolerance*.[37] [38] [39] [40] Each of these is considered briefly.

Central Tolerance.

This refers to death (deletion) of self-reactive T- and B-lymphocyte clones during their maturation in the central lymphoid organs (the thymus for T cells and the bone marrow for B cells). Deletion of developing intrathymic T cells has been extensively investigated. Experiments with transgenic mice provide abundant evidence that T lymphocytes that bear receptors for self-antigens undergo apoptosis within the thymus during the process of T-cell maturation. It

is proposed that many autologous protein antigens, including antigens thought to be restricted to peripheral tissues, are processed and presented by thymic antigen-presenting cells in association with self-MHC molecules.[37] A protein called AIRE (autoimmune regulator) is thought to stimulate expression of many "peripheral" self-antigens in the thymus and is thus critical for deletion of immature self-reactive T cells.[38] Mutations in the *AIRE* gene (either spontaneous in humans or created in knockout mice) are the cause of an autoimmune polyendocrinopathy (Chapter 24). The

Figure 6-27 Schematic illustration of the mechanisms involved in central and peripheral tolerance. The principal mechanisms of tolerance in CD4+ T cells are shown. APC, antigen-presenting cell.


Pro-T cells
THYMUS
SELF-REACTIVE CLONES
NON-SELF REACTIVE CLONES
SELF-REACTIVE CLONES
Self-reactive T-cell receptor
Self-peptide
MHC
Apoptosis
T-cell receptor for non-self peptide
Thymic epithelium
CENTRAL TOLERANCE
Self antigen not expressed in thymus
PERIPHERAL TOLERANCE
INDUCTION OF IMMUNITY
Non-self peptide
CD28
B7
MHC
Self peptide
CD28
MHC
Regulatory T cell
Self peptide
CD
B
MHC
APC
Tissue cell or APC

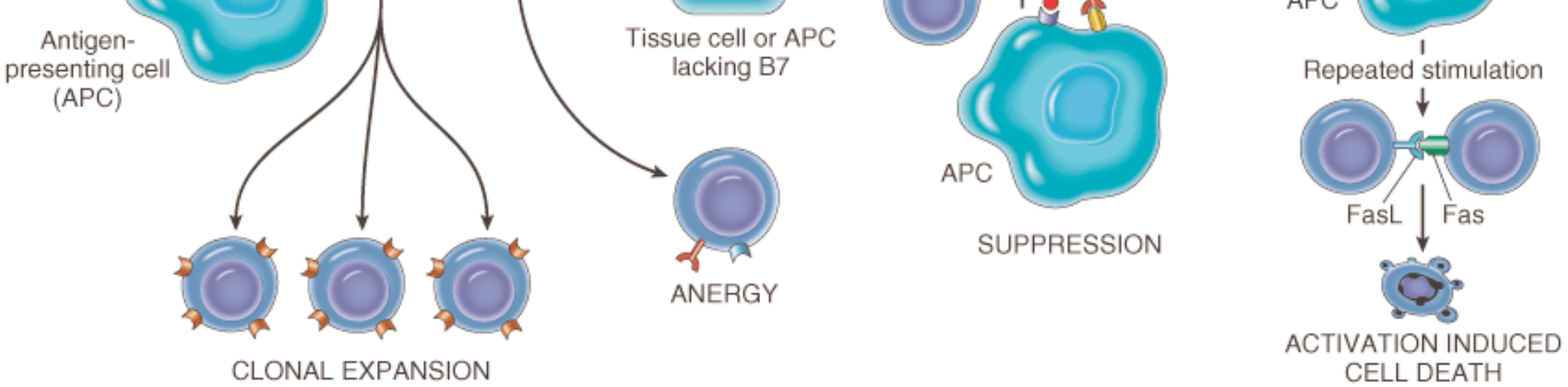


Figure 6-28 Pathogenesis of autoimmunity. Autoimmunity results from multiple factors, including susceptibility genes that may interfere with self-tolerance and environmental triggers (inflammation, other inflammatory stimuli) that promote lymphocyte entry into tissues, activation of lymphocytes, and tissue injury.

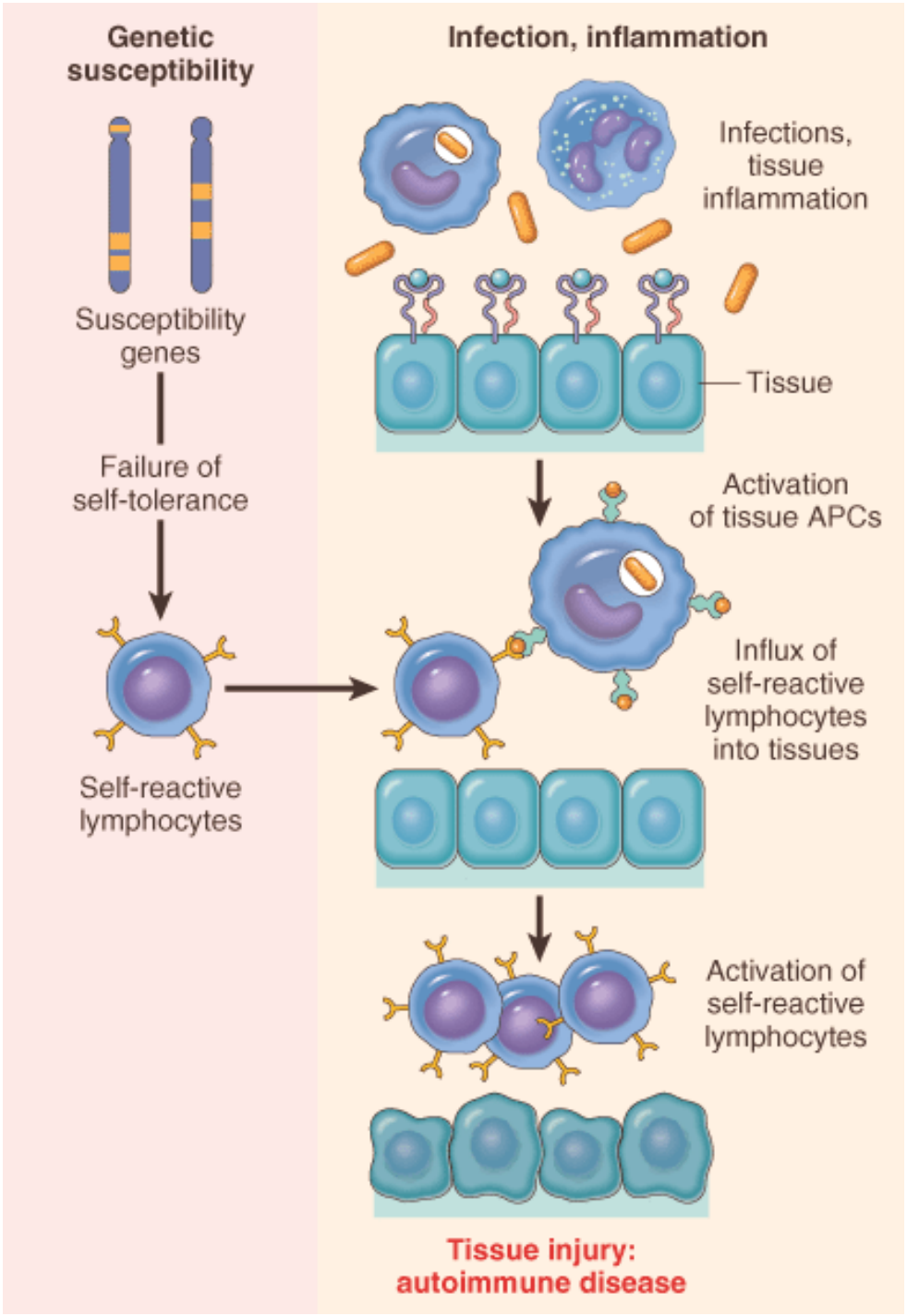


Figure 6-29 Role of infections in autoimmunity. Infections may promote activation of self-reactive lymphocytes by inducing the expression of costimulators (*A*), or microbial antigens may mimic self-antigens and activate self-reactive lymphocytes as a cross-reaction (*B*).

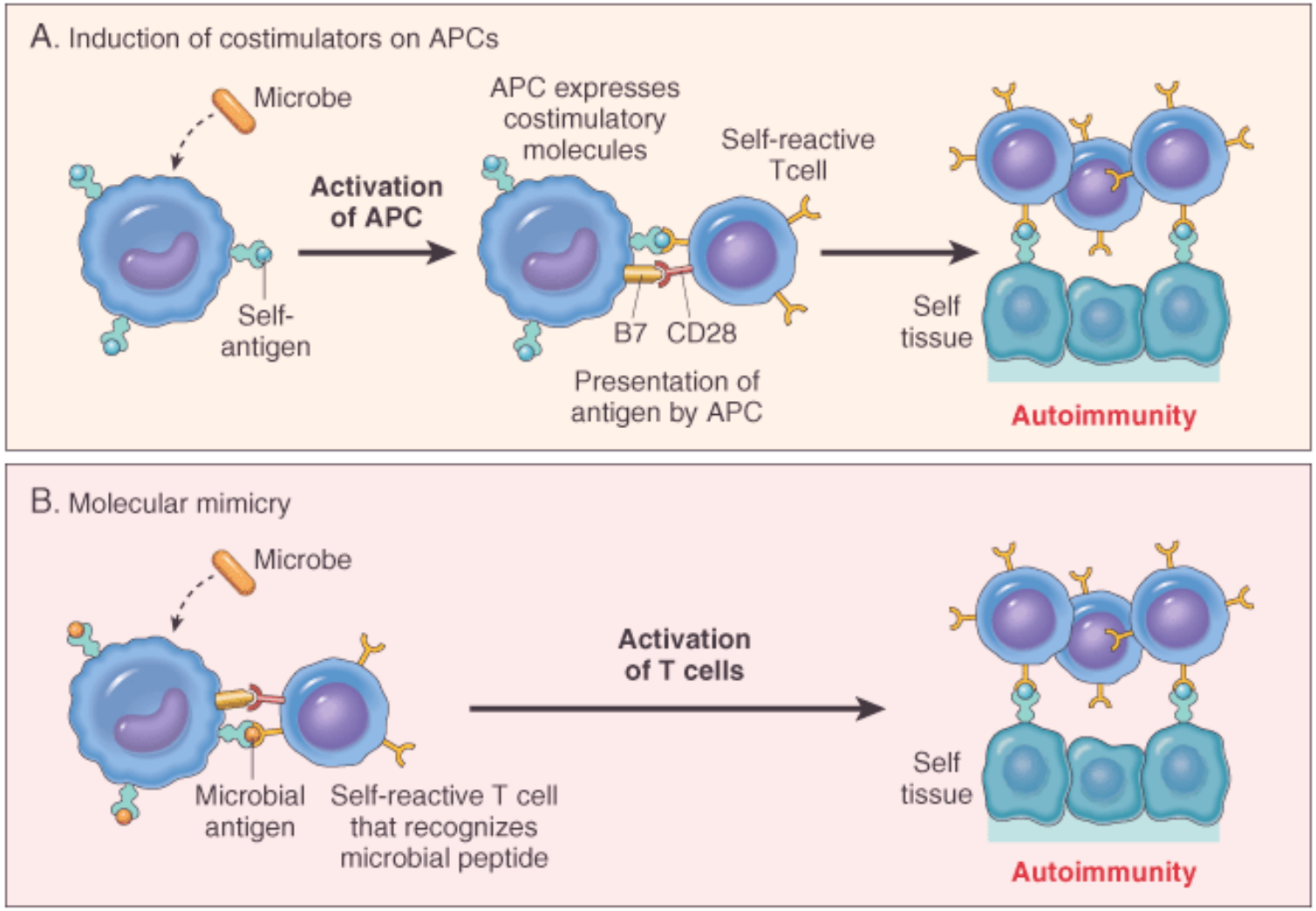


TABLE 6-8 -- 1997 Revised Criteria for Classification of Systemic Lupus Erythematosus

(Not Available)

Data from Tan EM, et al: The revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 25:1271, 1982; and Hochberg, MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 40:1725, 1997.

mechanisms that maintain self-tolerance. Antibodies have been identified against an array of nuclear and cytoplasmic components of the cell that are neither organ nor species specific. In addition, a third group of antibodies is directed against cell-surface antigens of blood cells. Apart from their value in the diagnosis and management of patients with SLE, these antibodies are of major pathogenetic significance, as, for example, in the immune complex-mediated glomerulonephritis so typical of this disease.[56]

ANAs are directed against several nuclear antigens and can be grouped into four categories:[56] (1) antibodies to DNA, (2) antibodies to histones, (3) antibodies to nonhistone proteins bound to RNA, and (4) antibodies to nucleolar antigens. Table 6-9 lists several ANAs and their association with SLE as well as with other autoimmune diseases to be discussed later.

Several techniques are used to detect ANAs. Clinically the most commonly used method is indirect immunofluorescence, which detects a variety of nuclear antigens, including DNA, RNA, and proteins (collectively called *generic ANAs*). The pattern of nuclear fluorescence suggests the type of antibody present in the patient's serum. Four basic patterns are recognized:

- *Homogeneous or diffuse nuclear staining* usually reflects antibodies to chromatin, histones and, occasionally, double-stranded DNA.
- *Rim or peripheral staining* patterns are most commonly indicative of antibodies to double-stranded DNA.
- *Speckled pattern* refers to the presence of uniform or variable-sized speckles. This is one of the most commonly observed patterns of fluorescence and therefore the least specific. It reflects the presence of antibodies to non-DNA nuclear constituents. Examples include Sm antigen, ribonucleoprotein, and SS-A and SS-B reactive antigens (Table 6-9).
- *Nucleolar pattern* refers to the presence of a few discrete spots of fluorescence within the nucleus and represents antibodies to nucleolar RNA. This pattern is reported most often in patients with systemic sclerosis.

The fluorescence patterns are not absolutely specific for the type of antibody, and because many autoantibodies may be present, combinations of patterns are frequent. *The immunofluorescence test for ANA is positive in virtually every patient with SLE; hence this test is sensitive, but it is not specific because patients with other autoimmune diseases also frequently score positive* (see Table 6-9). *Furthermore, approximately 5% to 15% of normal individuals have low titers of these antibodies.* The incidence increases with age.

Detection of antibodies to specific nuclear antigens requires specialized techniques. Of the numerous nuclear antigen-antibody systems,[57] some that are clinically useful are listed in Table 6-9 . *Antibodies to double-stranded DNA and the so-called Smith (Sm) antigen are virtually diagnostic of SLE.*

There is some, albeit imperfect, correlation between the presence or absence of certain ANAs and clinical manifestations. For example, high titers of double-stranded DNA antibodies are usually associated with active renal disease. Conversely the risk of nephritis is low if anti-SS-B antibodies are present.[56]

TABLE 6-9 -- Antinuclear Antibodies in Various Autoimmune Diseases

		Disease, % Positive					
Nature of Antigen	**Antibody System**	***SLE***	***Drug-Induced LE***	***Systemic Sclerosis—Diffuse***	***Limited Scleroderma—CREST***	***Sjögren Syndrome***	***Inflammatory Myopathies***
Many nuclear antigens (DNA, RNA, proteins)	Generic ANA (indirect IF)	>95	>95	70–90	70–90	50–80	40–60
Native DNA	Anti-double-stranded DNA	40–60	<5	<5	<5	<5	<5
Histones	Antihistone	50–70	>95	<5	<5	<5	<5

Core proteins of small nuclear ribonucleoprotein particles (Smith antigen)	Anti-Sm	20–30	<5	<5	<5	<5	<5
Ribonucleoprotein (U1RNP)	Nuclear RNP	30–40	<5	15	10	<5	<5
RNP	SS-A(Ro)	30–50	<5	<5	<5	70–95	10
RNP	SS-B(La)	10–15	<5	<5	<5	60–90	<5
DNA topoisomerase I	Scl-70	<5	<5	28–70	10–18	<5	<5
Centromeric proteins	Anticentromere	<5	<5	22–36	90	<5	<5
Histidyl-t-RNA synthetase	Jo-1	<5	<5	<5	<5	<5	25
Boxed entries indicate high correlation.							
SLE, systemic lupus erythematosus; LE, lupus erythematosus; ANA, antinuclear antibodies; RNP, ribonucleoprotein.							

In addition to ANAs, lupus patients have a host of other autoantibodies. Some are directed against elements of the blood, such as red cells, platelets, and lymphocytes; others are directed against proteins complexed to phospholipids. In recent years, there has been much interest in these so-called antiphospholipid antibodies. [58] They are present in 40% to 50% of lupus patients. Although initially believed to be directed against anionic phospholipids, they are actually directed against epitopes of plasma proteins that are revealed when the proteins are complexed to phospholipids. A variety of protein substrates have been implicated, including prothrombin, annexin V, β_2 -glycoprotein I, protein S, and protein C.[59] *Antibodies against the phospholipid-β_2 -glycoprotein complex also bind to cardiolipin antigen, used in syphilis serology, and therefore lupus patients may have a false-positive test result for syphilis*. Some of these antibodies interfere with in vitro clotting tests, such as partial thromboplastin time. Therefore, these antibodies are sometimes referred to as *lupus anticoagulant*. Despite having a circulating anticoagulant that delays clotting in vitro, these patients have complications associated with a *hypercoagulable state*.[60] They have venous and arterial thromboses, which may be associated with recurrent spontaneous miscarriages and focal cerebral or ocular ischemia. This constellation of clinical features, in association with lupus, is referred to as the *secondary antiphospholipid antibody syndrome*. The pathogenesis of thrombosis in these patients is unknown; possible mechanisms are discussed in Chapter 4 . Some patients develop these autoantibodies and the clinical syndrome without associated SLE. They are said to have the primary antiphospholipid syndrome (Chapter 4).

Given the presence of all these autoantibodies, we still know little about the mechanism of their emergence. Three converging lines of investigation hold center stage today: genetic predisposition, some nongenetic (environmental) factors, and a fundamental abnormality in the immune system.

Genetic Factors.

SLE is a complex genetic trait with contribution from MHC and multiple non-MHC genes. Many lines of evidence support a genetic predisposition. [51] [61]

- Family members of patients have an increased risk of developing SLE. Up to 20% of clinically unaffected first-degree relatives of SLE patients reveal autoantibodies and other immunoregulatory abnormalities.
- There is a higher rate of concordance (>20%) in monozygotic twins when compared with dizygotic twins (1% to 3%). Monozygotic twins who are discordant for SLE have similar patterns and titers of autoantibodies.[62] These data suggest that the genetic makeup regulates the formation of autoantibodies, but the expression of the disease (i.e., tissue injury) is influenced by non-genetic (possibly environmental) factors.
- Studies of HLA associations further support the concept that MHC genes regulate production of specific autoantibodies, rather than conferring a generalized predisposition to SLE. Specific alleles of the HLA-DQ locus have been linked to the production of anti-double-stranded DNA, anti-Sm, and antiphospholipid antibodies.
- Some lupus patients (approximately 6%) have inherited deficiencies of early complement components, such as C2, C4, or C1q. Lack of complement may impair removal of circulating immune complexes by the mononuclear phagocyte system, thus favoring tissue deposition. Knockout mice lacking C4 or certain complement receptors are also prone to develop lupus-like autoimmunity. Various mechanisms have been invoked, including failure to clear immune complexes and loss of B-cell self-tolerance. It has also been proposed that deficiency of C1q results in failure of phagocytic

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clearance of apoptotic cells.[63]Such cells are produced normally, and if they are not cleared their nuclear components may elicit immune responses.

- In animal models of SLE, several non-MHC susceptibility loci have been identified. The best-known animal model is the (NZBxNZW)F1 mouse strain. In different versions of this strain, up to 20 loci are believed to be associated with the disease. [51]

Environmental Factors.

There are many indications that, in addition to genetic factors, several *environmental* or non-genetic factors must be involved in the pathogenesis of SLE. The clearest example comes from the observation that *drugs* such as hydralazine, procainamide, and D-penicillamine can induce an SLE-like response in humans.[64] Exposure to *ultraviolet light* is another environmental factor that exacerbates the disease in many individuals. How ultraviolet light acts is not entirely clear, but it is suspected of modulating the immune response. For example, it induces keratinocytes to produce IL-1, a factor known to influence the immune response. In addition, UV irradiation may induce apoptosis in cells, and alter the DNA in such a way that it becomes immunogenic. [65] *Sex hormones* seem to exert an important influence on the occurrence and manifestations of SLE. During the reproductive years, the frequency of SLE is 10 times greater in women than in men, and exacerbation has been noted during normal menses and pregnancy.

Immunologic Factors.

With all the immunologic findings in SLE patients, there can be little doubt that some fundamental derangement of the immune system is involved in the pathogenesis of SLE. Although a variety of immunologic abnormalities affecting both T cells and B cells have been detected in patients with SLE, it has been difficult to relate any one of them to the causation of this disease. For years, it had been thought that an intrinsic B-cell hyperactivity is fundamental to the pathogenesis of SLE. Polyclonal B-cell activation can be readily demonstrated in patients with SLE and in murine models of this disease. Molecular analyses of anti-double-stranded DNA antibodies, however, strongly suggest that pathogenic autoantibodies are not derived from polyclonally activated B cells. Instead, it appears that the production of tissue-damaging antibodies is driven by self-antigens and results from an antigen-specific helper T cell-dependent B-cell response with many characteristics of responses to foreign antigens.[66] These observations have shifted the onus of driving the autoimmune response squarely on helper T cells.[66A] Based on these findings, a model for the pathogenesis of SLE has been proposed (Fig. 6-30). Other contributing factors include defective clearance of apoptotic cells, mentioned above, and dysregulation of cytokines, notably interferons.[61] SLE is a heterogeneous disease, however, and as mentioned earlier, the production of different autoantibodies is regulated by

distinct genetic factors. Hence, there may well be distinct immunoregulatory disturbances in patients with different genetic backgrounds and autoantibody profiles.[67]

Regardless of the exact sequence by which autoantibodies are formed, they are clearly the mediators of tissue injury. *Most of the visceral lesions are mediated by immune complexes (type III hypersensitivity).* DNA-anti-DNA complexes can be detected in the glomeruli and small blood vessels. Low levels of serum complement and granular deposits of complement

Figure 6-30 Model for the pathogenesis of systemic lupus erythematosus. *(Modified from Kotzin BL: Systemic lupus erythematosus. Cell 65:303, 1996. Copyright 1996, Cell Press.)*

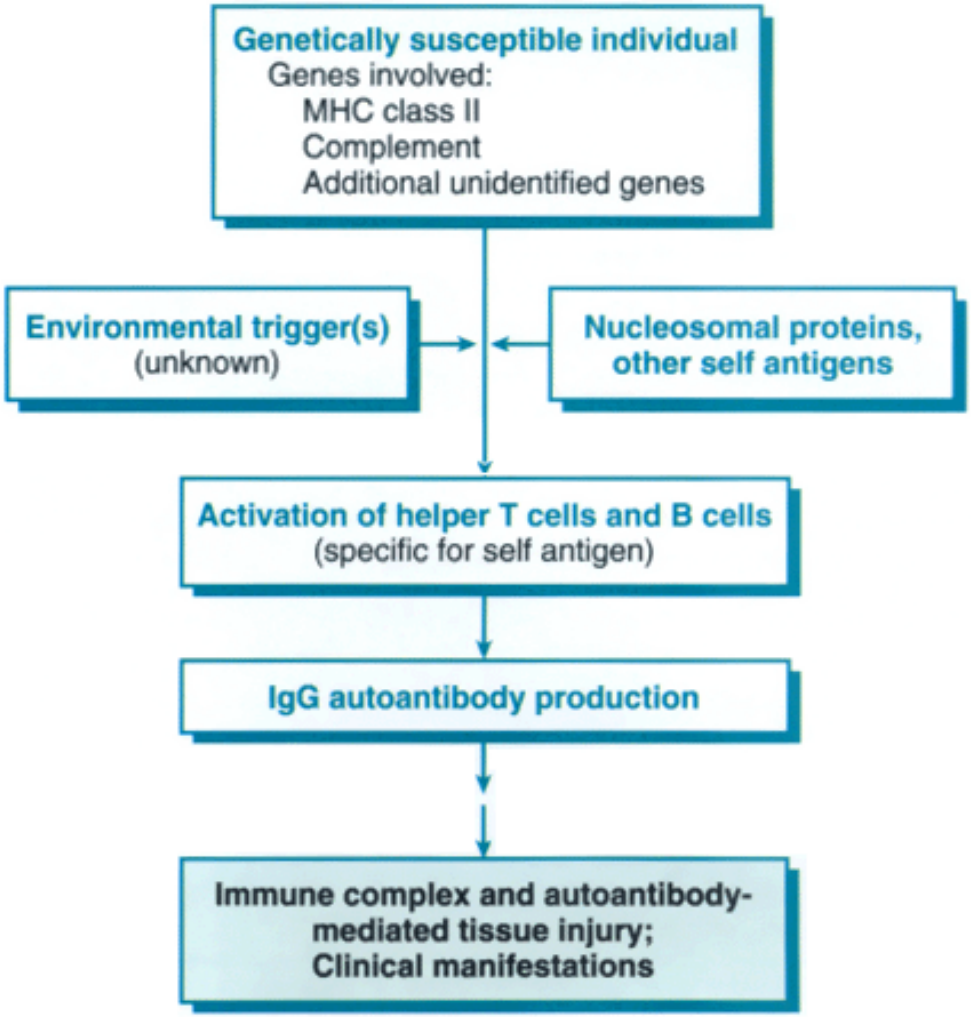


TABLE 6-10 -- Clinical and Pathologic Manifestations of Systemic Lupus Erythematosus

Clinical Manifestation	Prevalence in Patients, %
Hematologic	100
Arthritis	•90

Skin	•85
Fever	•83
Fatigue	•81
Weight loss	•63
Renal	•50
Central nervous system	•50
Pleuritis	•46
Myalgia	•33
Pericarditis	•25
Gastrointestinal	•21
Paynaud phenomenon	•20
Ocular	•15
Peripheral neuropathy	•14

lesions result from the deposition of immune complexes and are found in the blood vessels, kidneys, connective tissue, and skin.

An acute necrotizing vasculitis involving small arteries and arterioles may be present in any tissue.[68] The arteritis is characterized by fibrinoid deposits in the vessel walls. In chronic stages, vessels undergo fibrous thickening with luminal narrowing.

Kidney.

The kidney is a frequent target of injury in SLE. The principal mechanism of injury is immune complex deposition in renal structures, including glomeruli, tubular and peritubular capillary basement membranes, and larger blood vessels. Other forms of injury may include a thrombotic process involving the glomerular capillaries and extraglomerular vasculature, thought to be caused by antiphospholipid antibodies.

A morphologic classification of the patterns of immune complex-mediated glomerular injury in SLE has proven to be clinically useful.[69] There are several versions of the World Health Organization (WHO) classification of lupus nephritis, but in all, five patterns are recognized: (1) minimal or no detectable abnormalities (class I), which is rare, seen in renal biopsies from less than 5% of SLE patients; (2) mesangial lupus glomerulonephritis (class II); (3) focal proliferative glomerulonephritis (class III); (4) diffuse proliferative glomerulonephritis (class IV); and (5) membranous glomerulonephritis (class V). None of these patterns is specific for lupus.

Mesangial lupus glomerulonephritis is characterized by mesangial cell proliferation and lack of involvement of glomerular capillary walls. It is seen in 10% to 25% of patients, most of whom have minimal clinical manifestations, such as mild hematuria or transient proteinuria. There is a slight to moderate increase in the intercapillary mesangial matrix as well as in the number of mesangial cells. Despite the mild histologic changes, **granular mesangial deposits of immunoglobulin and complement are always present**. Such deposits presumably reflect the earliest change because filtered immune complexes accumulate primarily in the mesangium. The other changes to be described are usually superimposed on the mesangial changes.

Focal proliferative glomerulonephritis is seen in 20% to 35% of patients. It is a focal lesion, affecting fewer than 50% of the glomeruli and generally only portions of each glomerulus.

Typically, one or two tufts in an otherwise normal glomerulus exhibit swelling and proliferation of endothelial and mesangial cells, infiltration with neutrophils, and sometimes fibrinoid deposits and intracapillary thrombi (Fig. 6-31). Occasionally, affected glomeruli exhibit global injury. Focal lesions are associated with hematuria and proteinuria. In some patients, the nephritis progresses to diffuse proliferative disease.

Diffuse proliferative glomerulonephritis is the most serious of the renal lesions in SLE, occurring in 35% to 60% of patients who undergo biopsy. Anatomic changes are dominated by proliferation of endothelial, mesangial and, sometimes, epithelial cells (Fig. 6-32), producing in some cases epithelial crescents that fill the Bowman space (Chapter 20). The presence of fibrinoid necrosis, crescents, prominent infiltration by leukocytes, cell death as indicated by apoptotic bodies, and hyaline thrombi indicates active disease. Most or all glomeruli are involved in both kidneys, and the entire glomerulus is frequently affected. Patients with diffuse lesions are usually overtly symptomatic, showing microscopic or gross hematuria as well as proteinuria that is severe enough to cause the nephrotic syndrome in more than 50% of patients. Hypertension and mild to severe renal insufficiency are also common.

Membranous glomerulonephritis is a designation given to glomerular disease in which the principal histologic change consists of widespread thickening of the capillary walls. The lesions are similar to those encountered in idiopathic membranous glomerulonephritis, described more fully in Chapter 20 . This type of lesion is seen in 10% to 15% of patients with

Figure 6-31 Lupus nephritis. There are two focal necrotizing lesions in the glomerulus *(arrowheads)*. *(Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

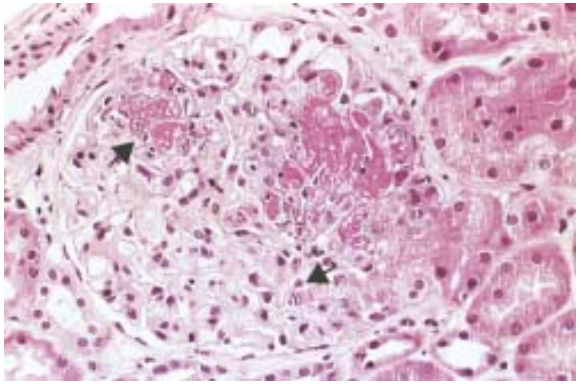


Figure 6-32 Lupus nephritis, diffuse proliferative type. Note the marked increase in cellularity throughout the glomerulus. *(Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

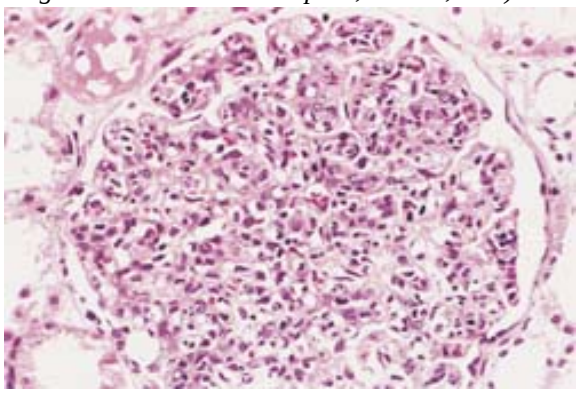


Figure 6-33 Immunofluorescence micrograph stained with fluorescent anti-IgG from a patient with diffuse proliferative lupus nephritis. One complete glomerulus and part of another one are seen. Note the mesangial and capillary wall deposits of IgG. *(Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

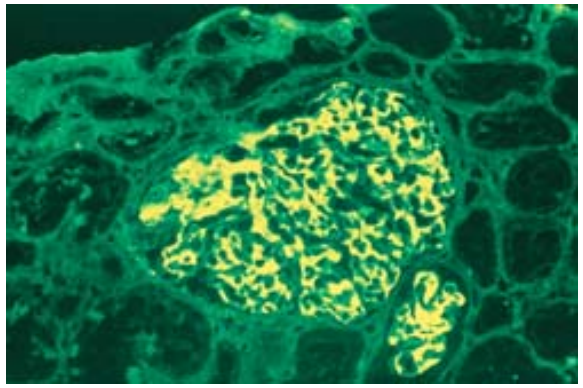


Figure 6-34 Electron micrograph of a renal glomerular capillary loop from a patient with systemic lupus erythematosus nephritis. Subendothelial dense deposits correspond to "wire loops" seen by light microscopy. Deposits are also present in the mesangium. *(Courtesy of Dr. Jean Olson, Department of Pathology, University of California San Francisco, San Francisco, CA.)*

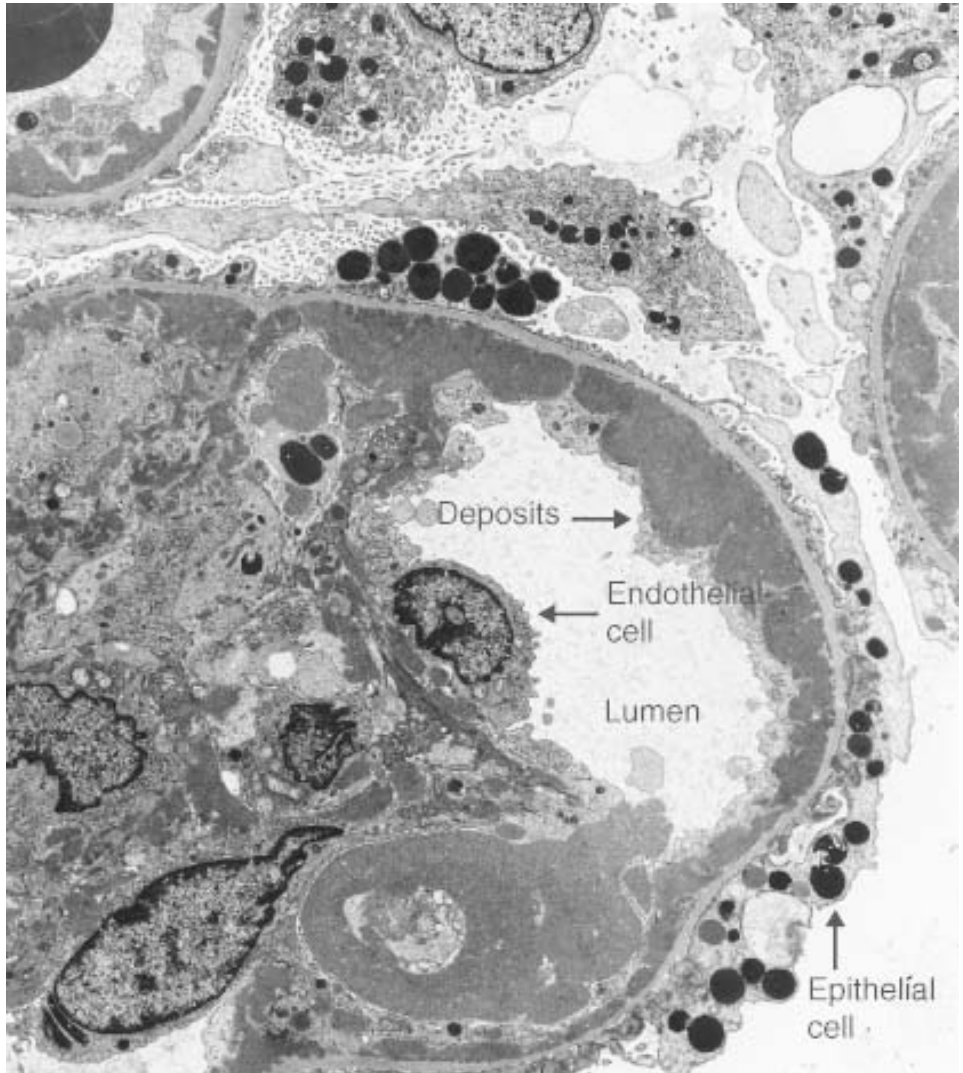


Figure 6-35 Lupus nephritis showing a glomerulus with several "wire loop" lesions representing extensive subendothelial deposits of immune complexes. (Periodic acid-Schiff [PAS] stain.) *(Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

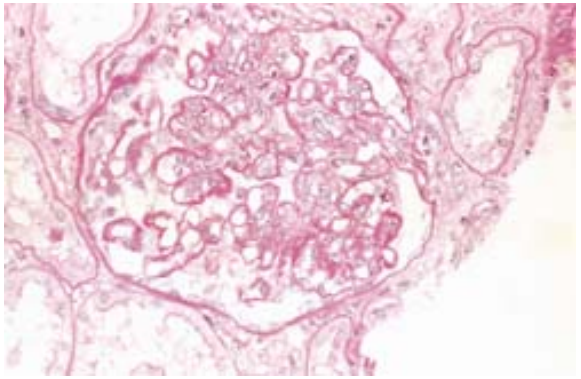


Figure 6-36 Systemic lupus erythematosus involving the skin. *A,* An H&E-stained section shows liquefactive degeneration of the basal layer of the epidermis and edema at the dermoepidermal junction. *(Courtesy of Dr. Jag Bhawan, Boston University School of Medicine, Boston, MA.) B,* An immunofluorescence micrograph stained for IgG reveals deposits of immunoglobulin along the dermal-epidermal junction. *(Courtesy of Dr. Richard Sontheimer, Department of Dermatology, University of Texas Southwestern Medical School, Dallas, TX.)*

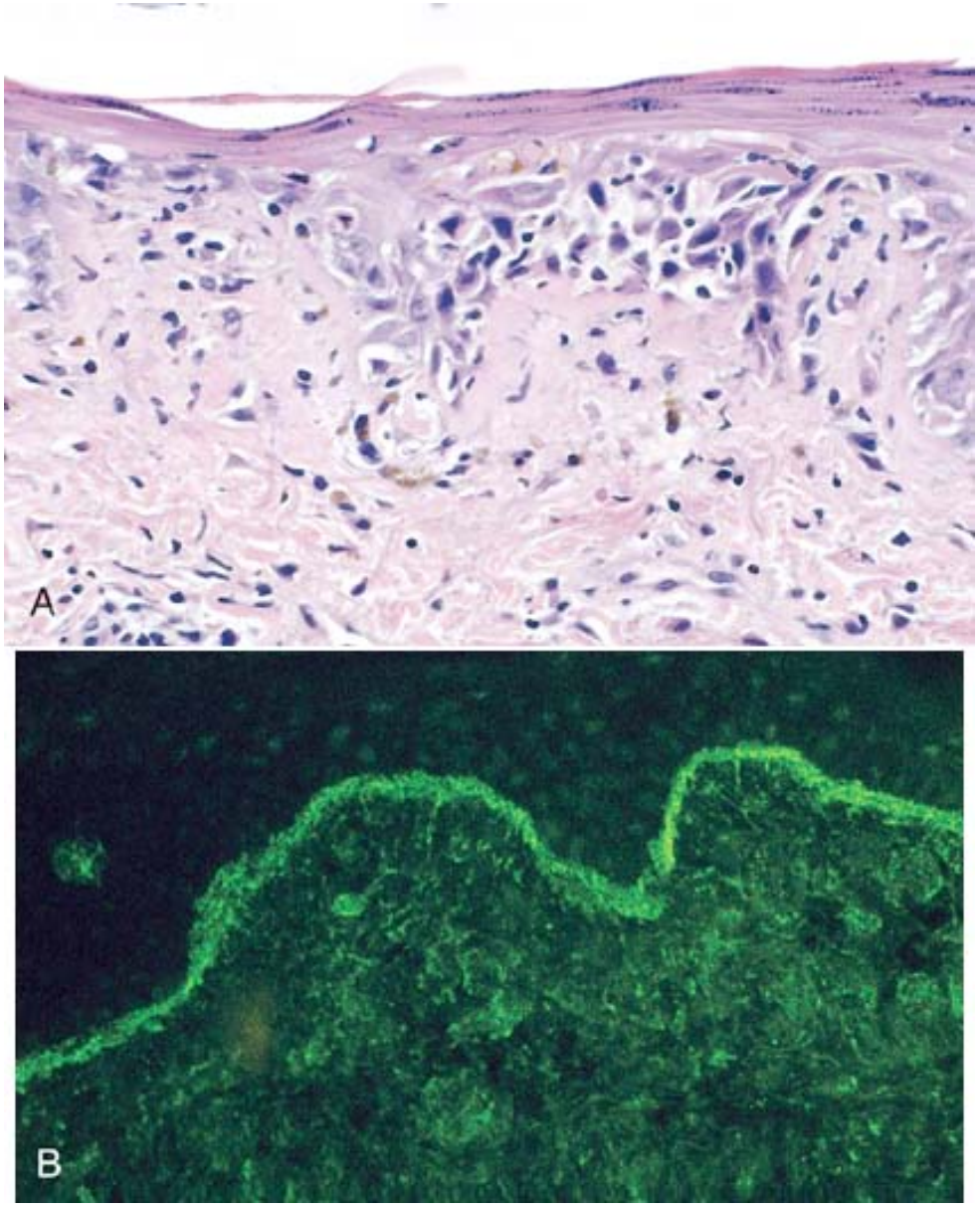


Figure 6-37 Libman-Sacks endocarditis of the mitral valve in lupus erythematosus. The vegetations attached to the margin of the thickened valve leaflet are indicated by *arrows*. *(Courtesy of Dr. Fred Schoen, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

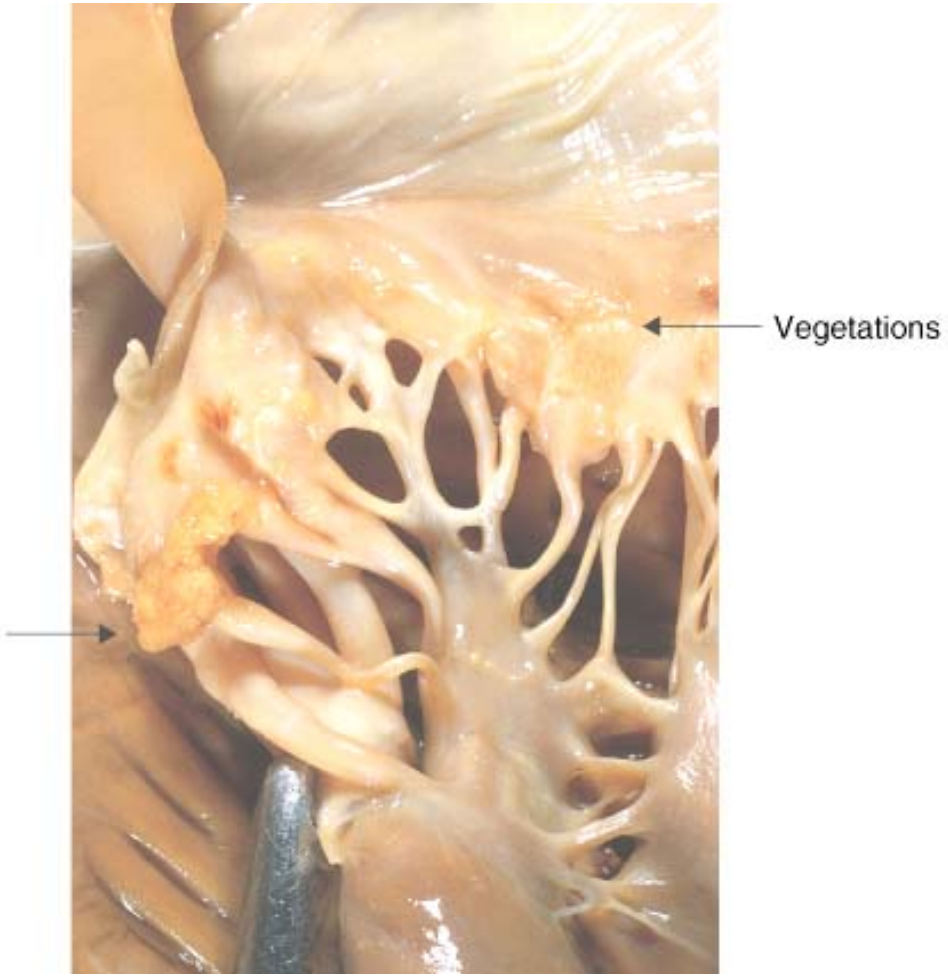


Figure 6-38 Sjögren syndrome. *A*, Enlargement of the salivary gland. *(Courtesy of Dr. Richard Sontheimer, Department of Dermatology, University of Texas Southwestern Medical School, Dallas, TX.) B*, Intense lymphocytic and plasma cell infiltration with ductal epithelial hyperplasia in a salivary gland. *(Courtesy of Dr. Dennis Burns, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

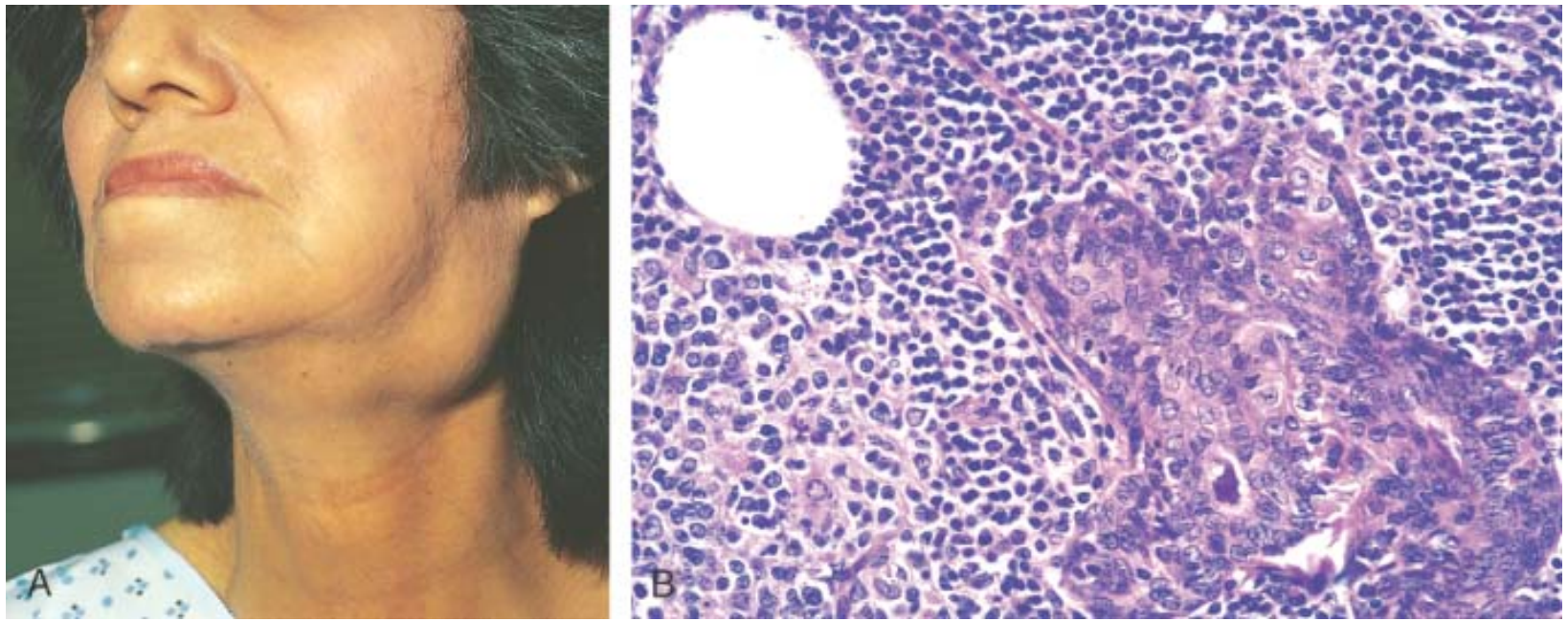


Figure 6-39 Schematic illustration of the possible mechanisms leading to systemic sclerosis.

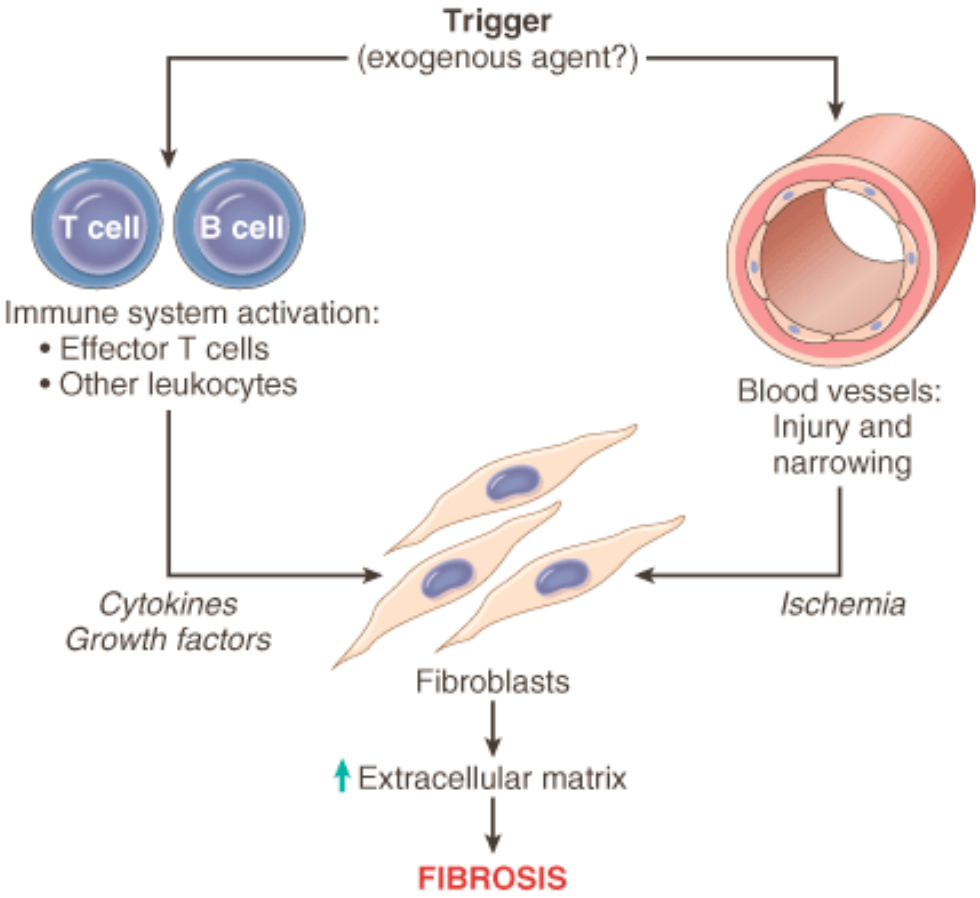


Figure 6-40 Systemic sclerosis. *A*, Normal skin. *B*, Skin biopsy from a patient with systemic sclerosis. Note the extensive deposition of dense collagen in the dermis with virtual absence of appendages (e.g. hair follicles) and foci of inflammation *(arrow)*.

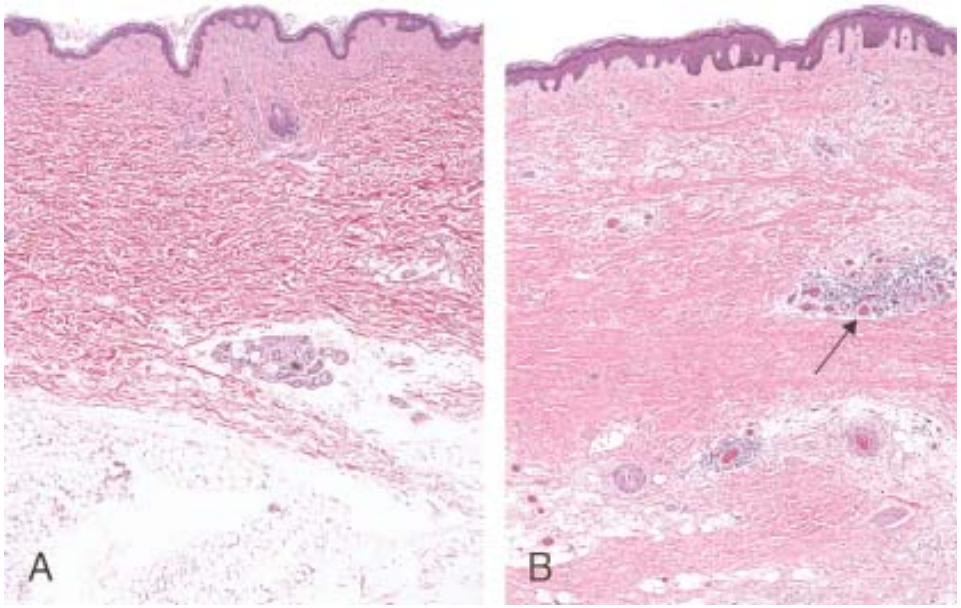


Figure 6-41 Advanced systemic sclerosis. The extensive subcutaneous fibrosis has virtually immobilized the fingers, creating a clawlike flexion deformity. Loss of blood supply has led to cutaneous ulcerations. *(Courtesy of Dr. Richard Sontheimer, Department of Dermatology, University of Texas Southwestern Medical School, Dallas, TX.)*

TABLE 6-11 -- Examples of Infections in Immunodeficiencies

Pathogen Type	T-Cell-Defect	B-Cell Defect	Granulocyte Defect	Complement Defect
Bacteria	Bacterial sepsis	Streptococci, staphylococci, *Haemophilus*	Staphylococci, *Pseudomonas*	Neisserial infections, other pyogenic bacterial infections
Viruses	Cytomegalovirus, Epstein-Barr virus, severe varicella, chronic infections with respiratory and intestinal viruses	Enteroviral encephalitis		
Fungi and parasites	*Candida, Pneumocystis carinii*	Severe intestinal giardiasis	*Candida, Nocardia, Aspergillus*	

Special features	Aggressive disease with opportunistic pathogens, failure to clear infections	Recurrent sinopulmonary infections, sepsis, chronic meningitis		

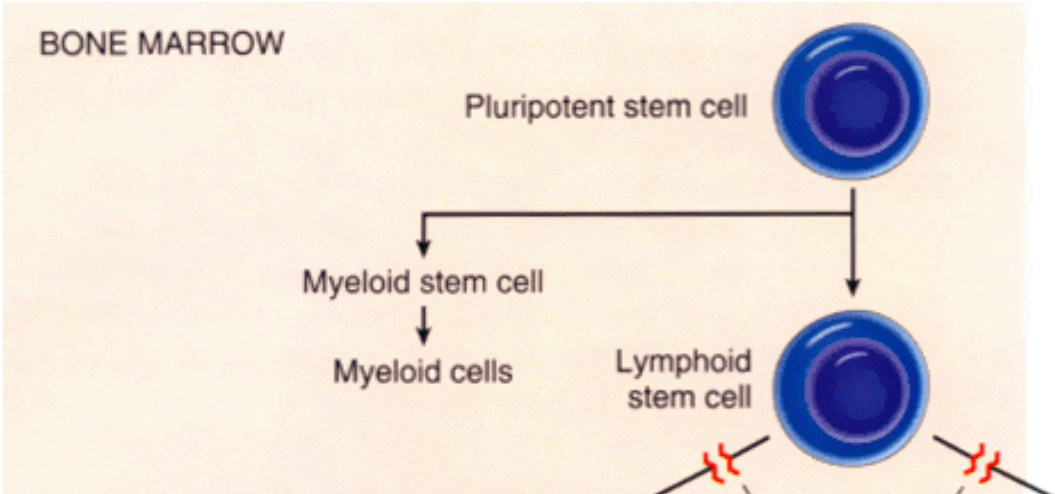
From Puck JM: Primary immunodeficiency diseases. JAMA 278:1835, 1997. Copyright 1997, American Medical Association.

of T cells are often indistinguishable clinically from combined deficiencies of T and B cells. Although originally thought to be quite rare, some forms, such as IgA deficiency, are common, and collectively they are a significant health problem, especially in children. Most primary immunodeficiencies manifest themselves in infancy, between 6 months and 2 years of life, and they are detected because the affected infants are susceptible to recurrent infections. The nature of infecting organisms depends to some extent on the nature of the underlying defect, as summarized in Table 6-11 . Detailed classification of the primary immunodeficiencies according to the suggested cellular defect may be found in the WHO report on immunodeficiency.[92] Defects of phagocytes were discussed in Chapter 2 . Here we present selected examples of other immunodeficiencies. We begin with isolated defects in B cells, followed by a discussion of combined immunodeficiencies and defects in complement proteins. Finally, Wiskott-Aldrich syndrome, a complex disorder affecting lymphocytes as well as platelets, is presented. With rapid advances in genetic analyses, in the past ten years the mutations responsible for many primary immunodeficiencies have been identified.[93]

X-Linked Agammaglobulinemia of Bruton

X-linked agammaglobulinemia is one of the more common forms of primary immunodeficiency.[94] It is *characterized by the failure of B-cell precursors (pro-B cells and pre-B cells) to mature into B cells*. During normal B-cell maturation in the bone marrow, the immunoglobulin heavy-chain genes are rearranged first, followed by rearrangement of the light chain genes. In X-linked agammaglobulinemia, B-cell maturation stops after the rearrangement of heavy chain genes. Because light chains are not produced, the complete immunoglobulin molecule (which contains heavy and light chains) cannot be assembled and transported to the cell membrane. Free heavy chains can be found in the cytoplasm. This block in differentiation is due to mutations in a cytoplasmic tyrosine kinase, called *B-cell tyrosine kinase (Btk)*.[95] Btk is a protein tyrosine kinase associated with the antigen receptor complex of pre-B and mature B cells. It is needed to transduce signals from the antigen receptor that are critical for driving maturation. When it is mutated, the pre-B cell receptor cannot deliver signals, and maturation stops at this stage. The *BTK* gene maps to the long arm of the X chromosome at Xq21.22.

Figure 6-42 Scheme of lymphocyte development and sites of block in primary immunodeficiency diseases. The affected genes are indicated in parentheses for some of the disorders. ADA, adenosine deaminase; CD40L, CD40 ligand; SCID, severe combined immunodeficiency.



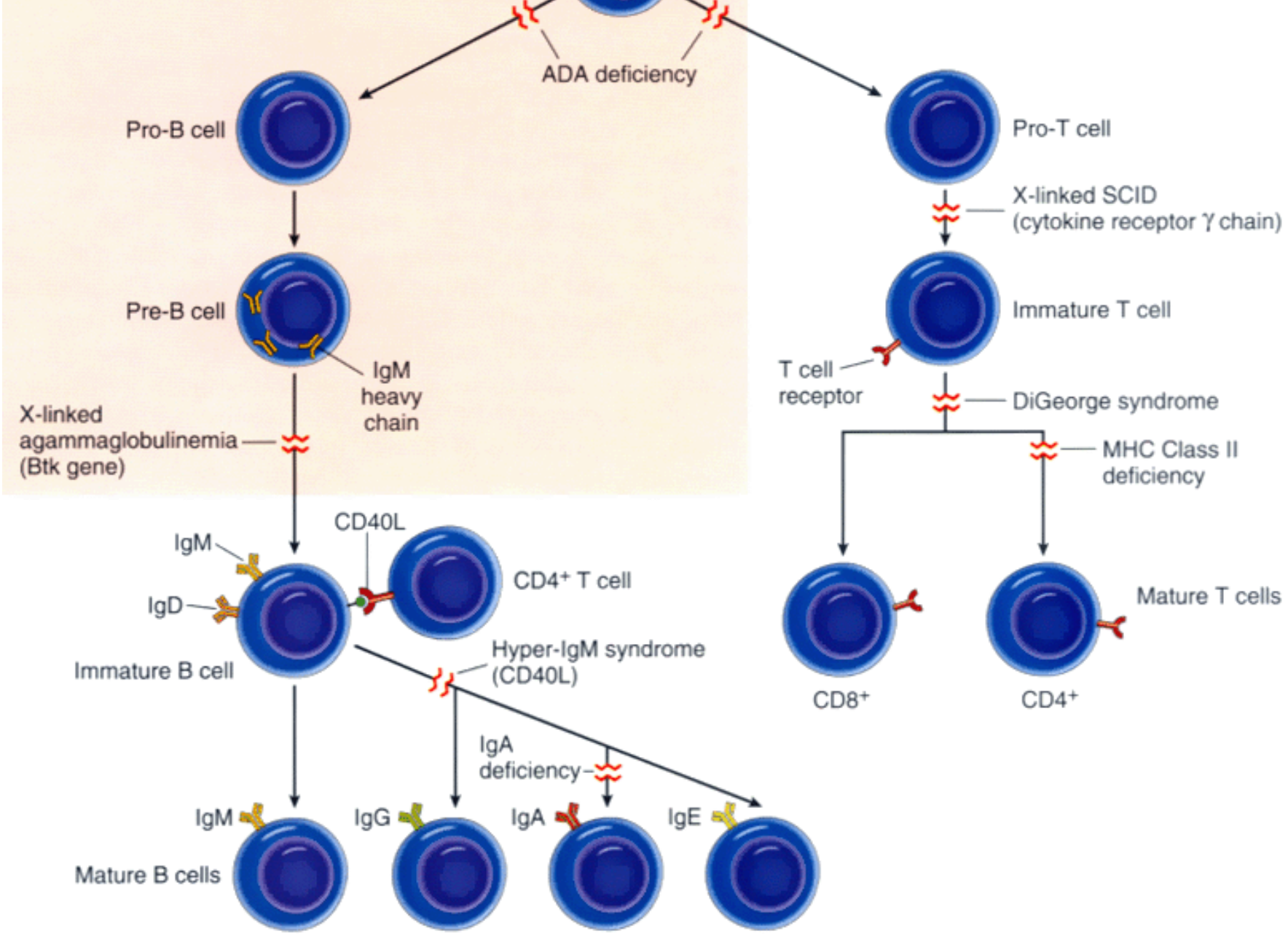


Figure 6-43 Schematic illustration of an HIV-1 virion. The viral particle is covered by a lipid bilayer that is derived from the host cell.

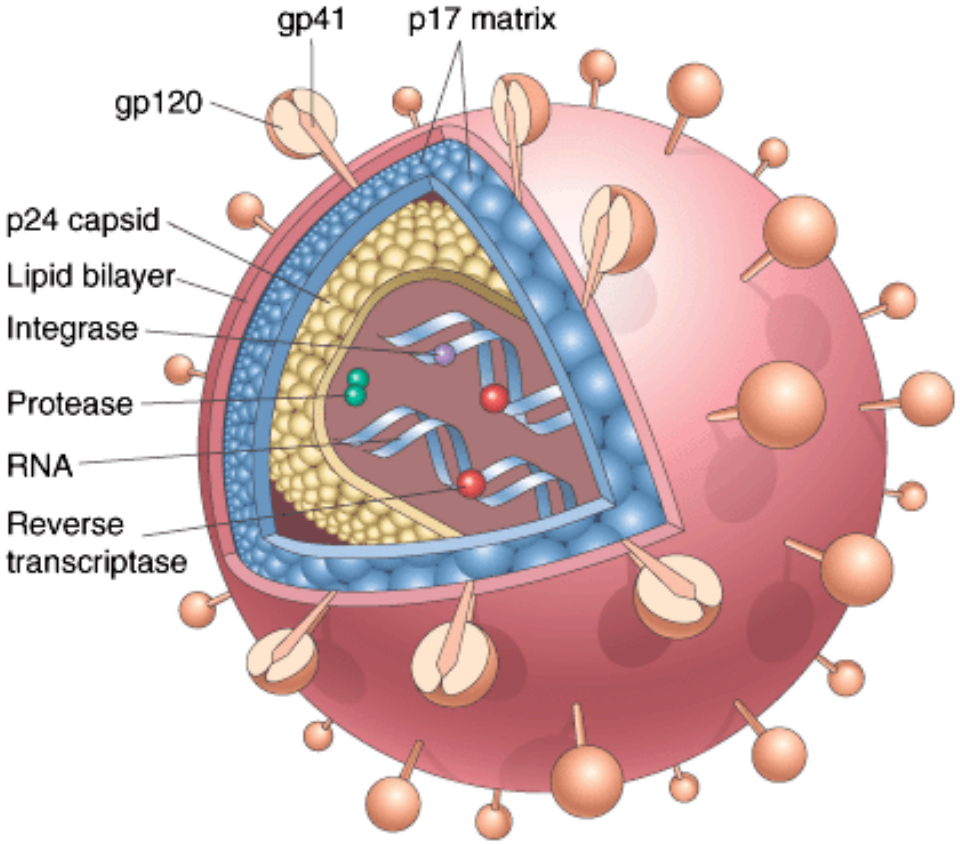


Figure 6-44 HIV proviral genome. Several viral genes and their corresponding functions are illustrated. The genes outlined in red are unique to HIV; others are shared by all retroviruses.

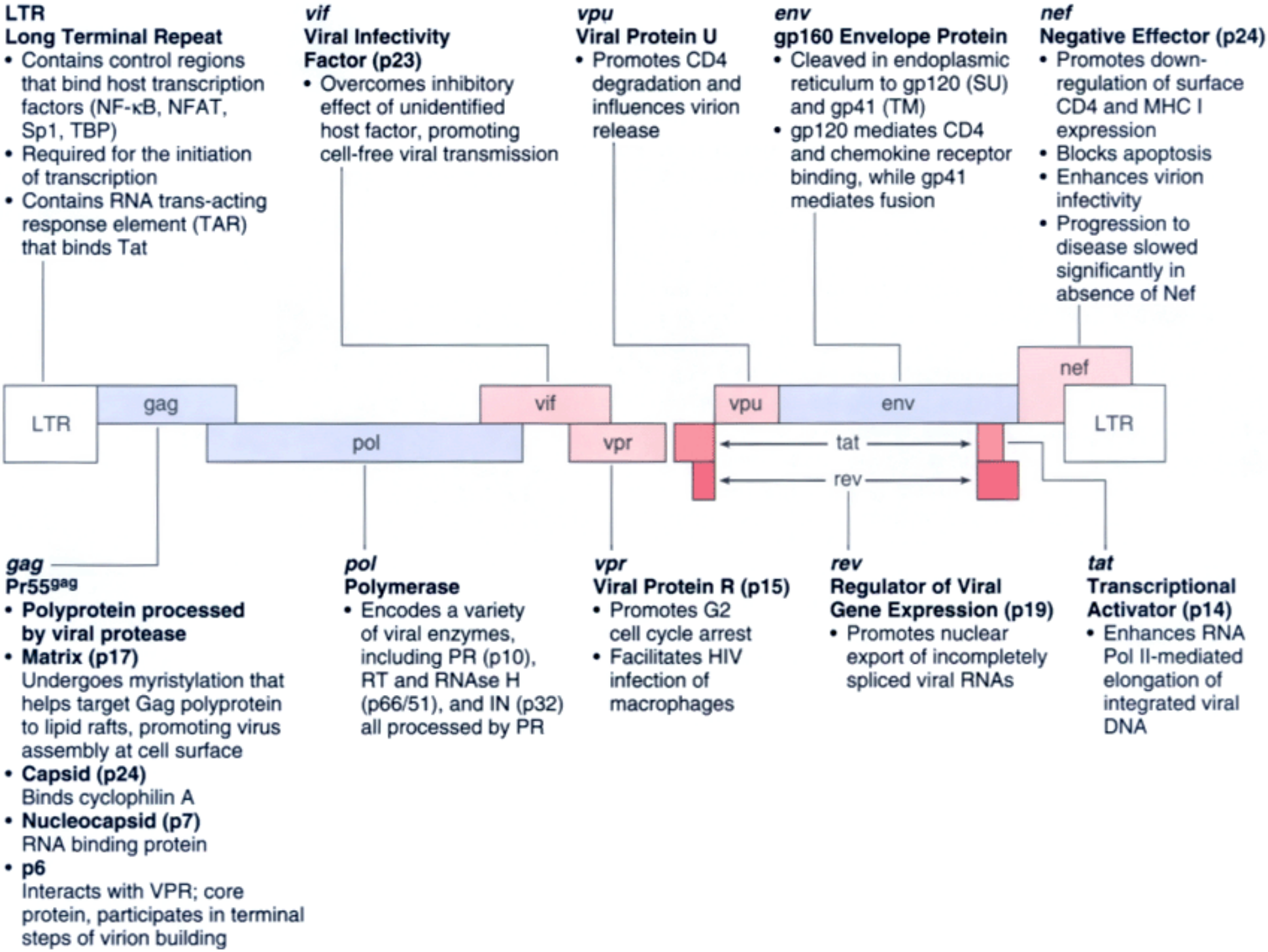
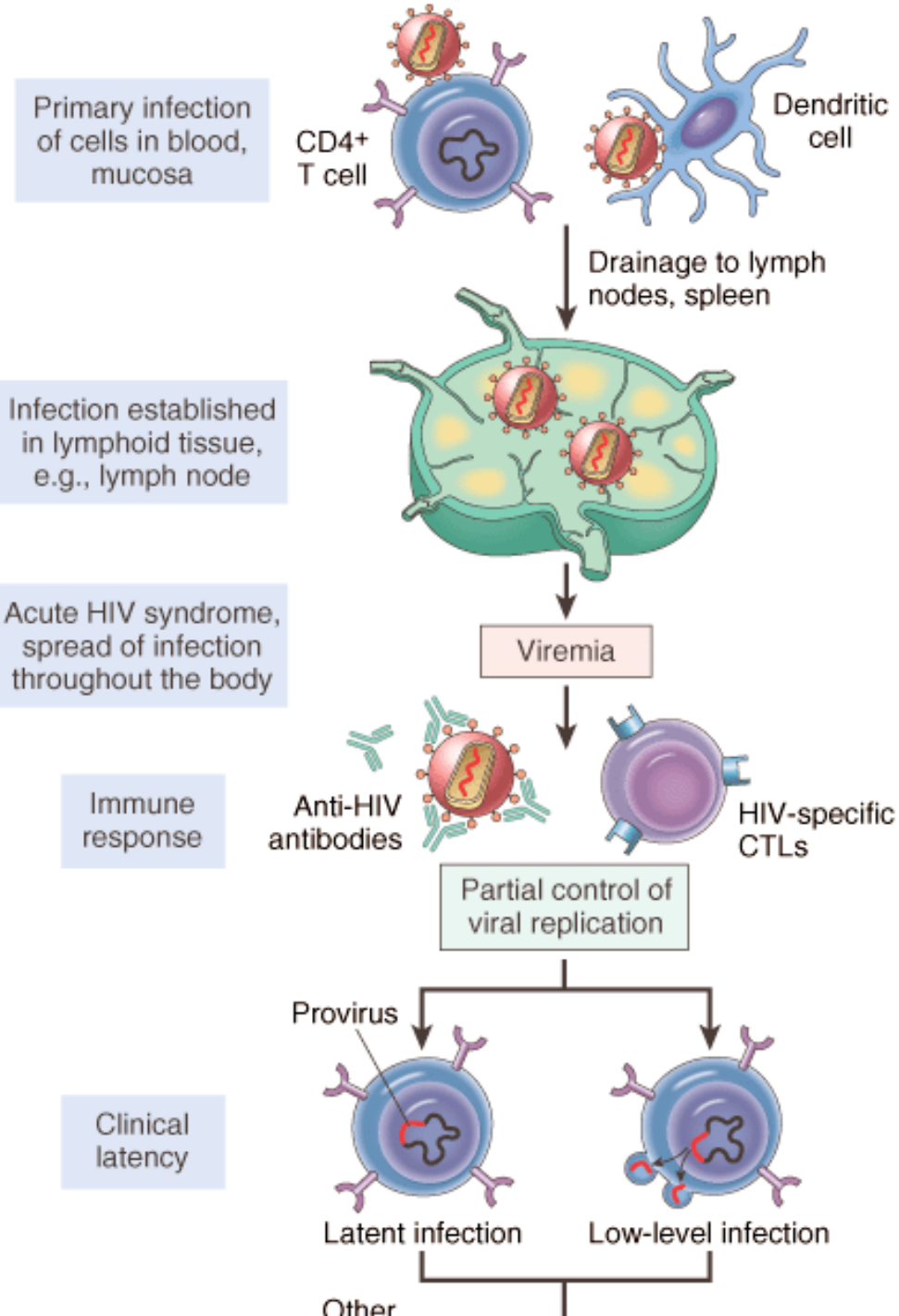


Figure 6-45 Pathogenesis of HIV-1 infection. Initially, HIV-1 infects T cells and macrophages directly or is carried to these cells by Langerhans cells. Viral replication in the regional

lymph nodes leads to viremia and widespread seeding of lymphoid tissue. The viremia is controlled by the host immune response *(not shown)*, and the patient then enters a phase of clinical latency. During this phase, viral replication in both T cells and macrophages continues unabated, but there is some immune containment of virus *(not illustrated)*. There continues a gradual erosion of CD4+ cells by productive infection (or other mechanisms, *not shown*). Ultimately, CD4+ cell numbers decline, and the patient develops clinical symptoms of full-blown AIDS. Macrophages are also parasitized by the virus early; they are not lysed by HIV-1, and they may transport the virus to tissues, particularly the brain.



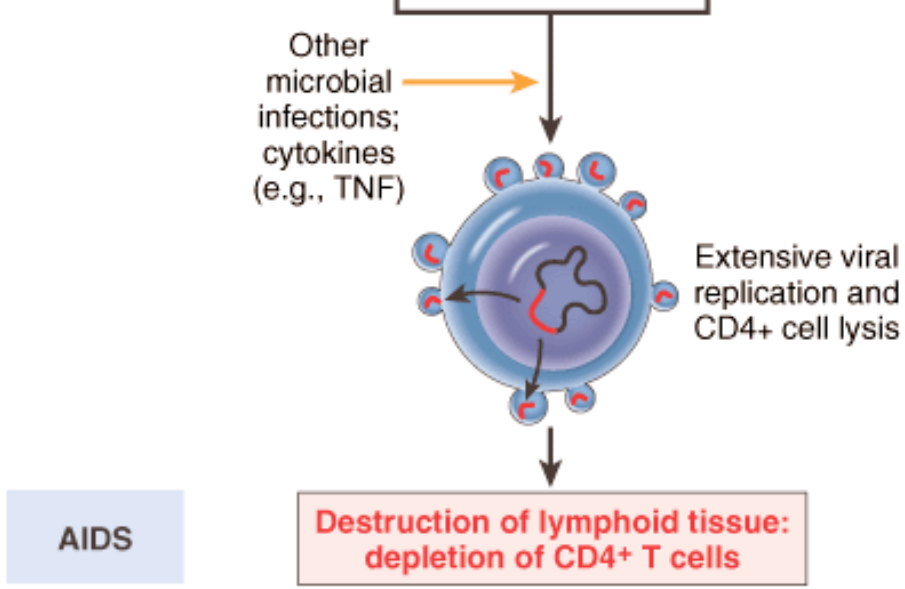


Figure 6-46 Mechanism of HIV entry into host cells. Interactions with CD4 and CCR5 coreceptor are illustrated. *(Adapted with permission from Wain-Hobson S: HIV. One on one meets two. Nature 384:117, 1996. Copyright 1996, Macmillam Magazines Limited.)*

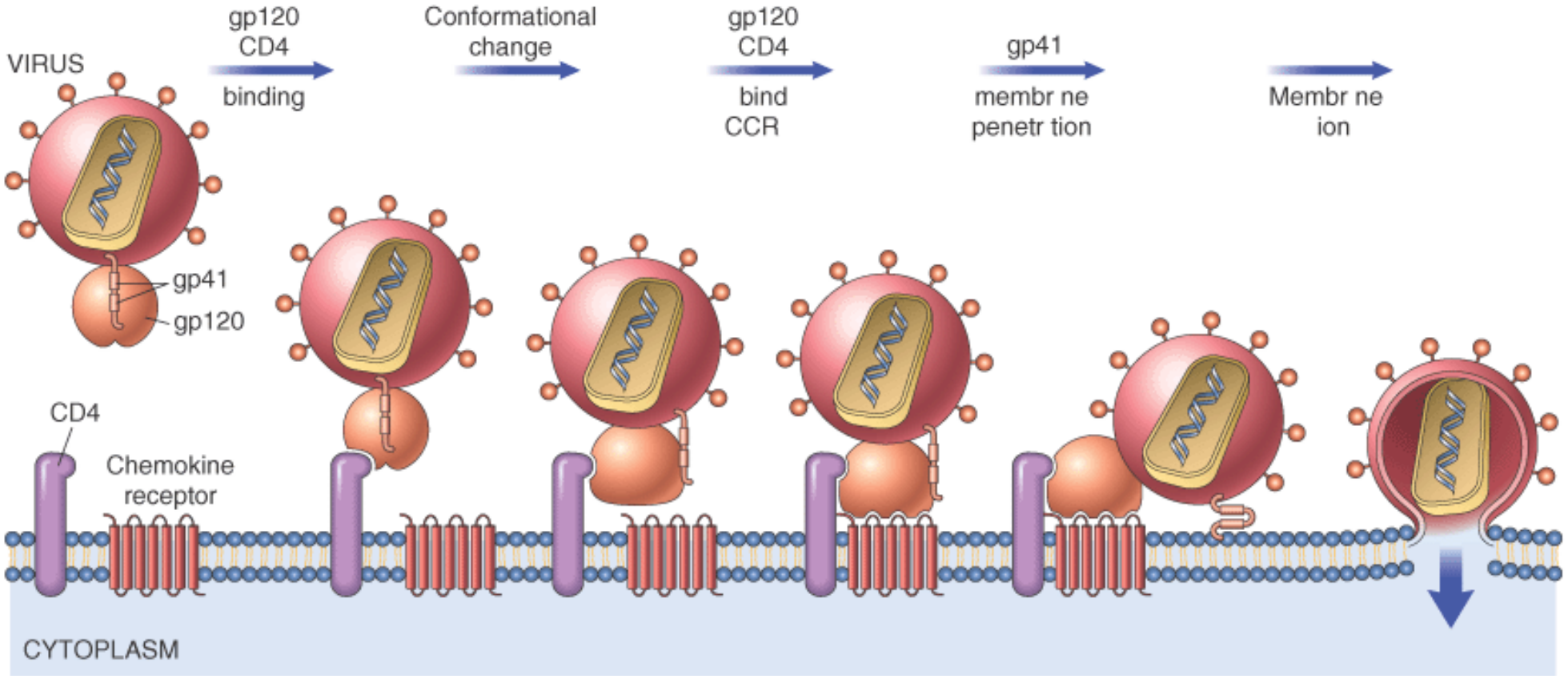


Figure 6-47 The life cycle of HIV. The steps from viral entry to production of infectious virions are illustrated.

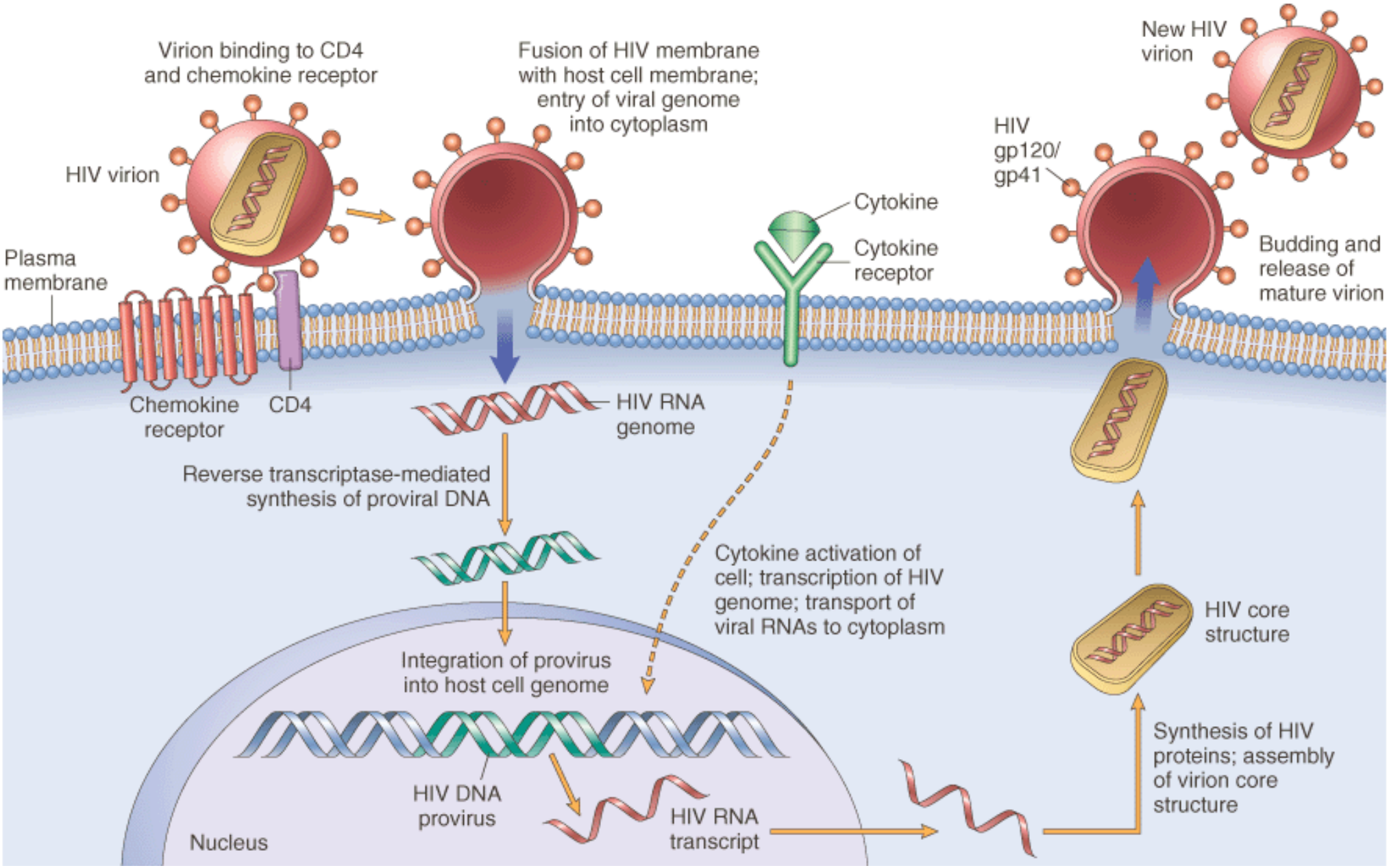


Figure 6-48 Mechanisms of CD4 cell loss in HIV infection.

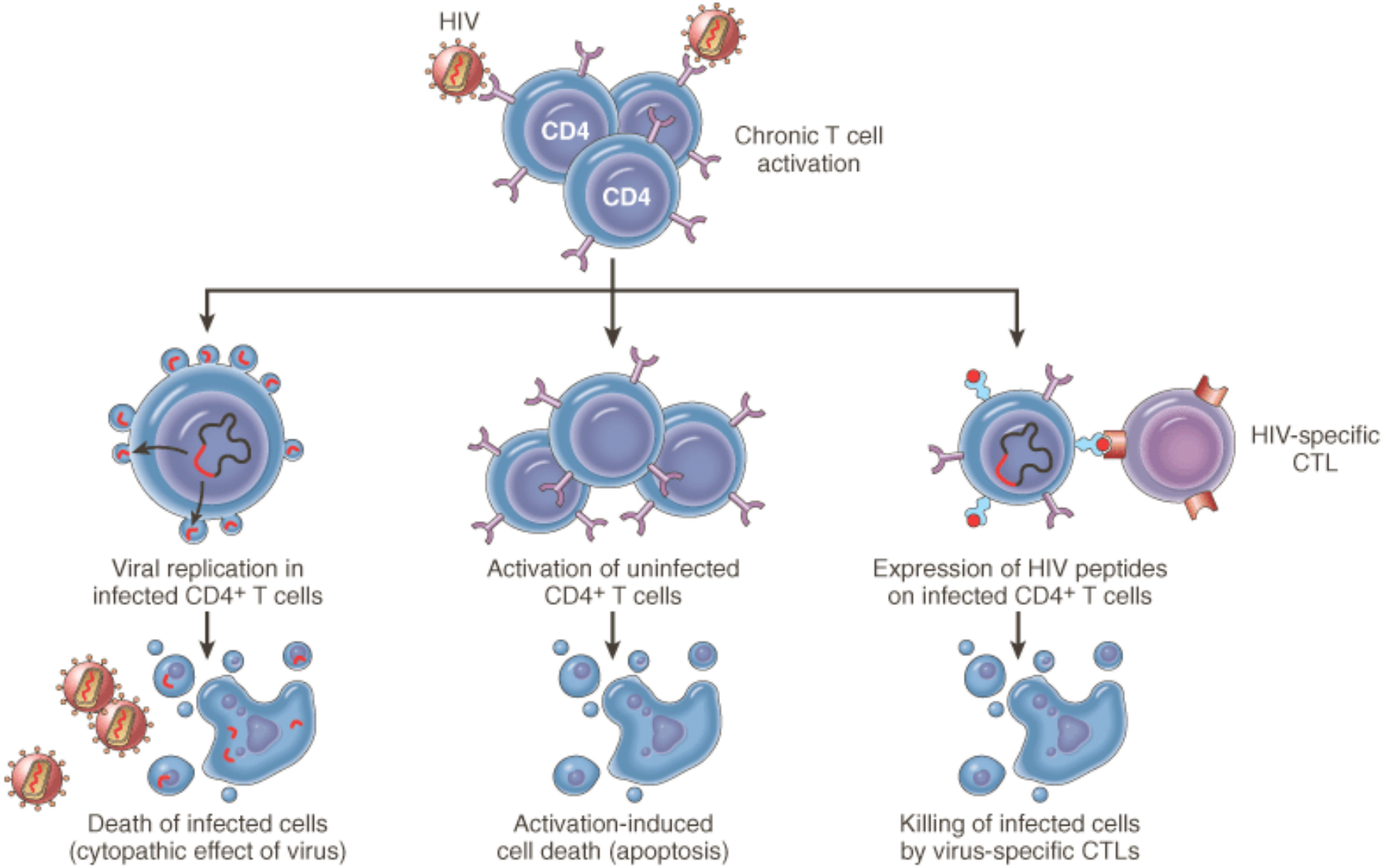


Figure 6-49 HIV infection showing the formation of giant cells in the brain. *(Courtesy of Dr. Dennis Burns, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

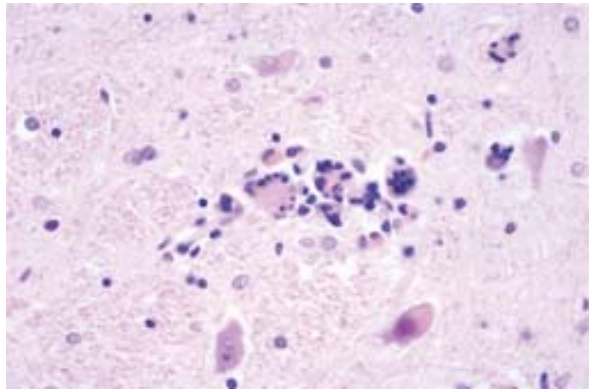


TABLE 6-12 -- Major Abnormalities of Immune Function in AIDS

Lymphopenia
Predominantly due to selective loss of the CD4+ helper-inducer T-cell subset; inversion of CD4:CD8 ratio
Decreased T-Cell Function In Vivo
Preferential loss of memory T cells
Susceptibility to opportunistic infections
Susceptibility to neoplasms
Decreased delayed-type hypersensitivity
Altered T-Cell Function In Vitro
Decreased proliferative response to mitogens, alloantigens, and soluble antigens
Decreased specific cytotoxicity
Decreased helper function for pokeweed mitogen-induced B-cell immunoglobulin production
Decreased IL-2 and TFN-γ production
Polyclonal B-Cell Activation
Hypergammaglobulinemia and circulating immune complexes
Inability to mount de novo antibody response to a new antigen or vaccine
Refractoriness to the normal signals for B-cell activation in vitro
Altered Monocyte or Macrophage Functions
Decreased chemotaxis and phagocytosis
Decreased HLA class II antigen expression
Diminished capacity to present antigen to T cells

Increased spontaneous secretion of IL-1, TNF, IL-6

HIV infection of macrophages has three important implications. First, monocytes and macrophages represent a veritable virus factory and reservoir, whose output remains largely protected from host defenses. Second, macrophages provide a safe vehicle for HIV to be transported to various parts of the body, including the nervous system. Third, in late stages of HIV infection, when the CD4+ T-cell numbers decline greatly, macrophages may be an important site of continued viral replication.[135]

In contrast to tissue macrophages, the number of monocytes in circulation infected by HIV is low, yet there are unexplained functional defects that have important consequences for host defense. These defects include impaired microbicidal activity, decreased chemotaxis, decreased secretion of IL-1, inappropriate secretion of TNF, and, most important, poor capacity to present antigens to T cells.

Studies have documented that, in addition to macrophages, two types of *dendritic cells* are also important targets for the initiation and maintenance of HIV infection: mucosal and follicular dendritic cells. It is thought that *mucosal dendritic cells are infected by the virus and transport it to regional lymph*

---

nodes, where CD4+ T cells are infected.[136] Dendritic cells also express a lectin-like receptor that specifically binds HIV and displays it in an intact, infectious form to T cells, thus promoting infection of the T cells.[137] *Follicular dendritic cells in the germinal centers of lymph nodes are, similar to macrophages, important reservoirs of HIV.*[138] Although some follicular dendritic cells may be susceptible to HIV infection, most virus particles are found on the surface of their dendritic processes. Follicular dendritic cells have receptors for the Fc portion of immunoglobulins, and hence they trap HIV virions coated with anti-HIV antibodies. The antibody-coated virions localized to follicular dendritic cells retain the ability to infect CD4+ T cells as they traverse the intricate meshwork formed by the dendritic processes of the follicular dendritic cells. *To summarize, CD4+ T cells, macrophages, and follicular dendritic cells contained in the lymphoid tissues are the major sites of HIV infection and persistence.*

Although much attention has been focused on T cells, macrophages, and dendritic cells because they can be infected by HIV, patients with AIDS also display profound abnormalities of B-cell function. Paradoxically, these patients have hypergammaglobulinemia and circulating immune complexes owing to polyclonal B-cell activation. This may result from multiple interacting factors: reactivation of or reinfection with cytomegalovirus and EBV, both of which are polyclonal B-cell activators, can occur; gp41 itself can promote B-cell growth and differentiation; and HIV-infected macrophages produce increased amounts of IL-6, which stimulates proliferation of B cells. *Despite the presence of spontaneously activated B cells, patients with AIDS are unable to mount antibody responses to new antigens.* This could be due, in part, to lack of T-cell help, but antibody responses against T-independent antigens are also suppressed, and hence there may be other defects in B cells as well. Impaired humoral immunity renders these patients prey to disseminated infections caused by encapsulated bacteria, such as *S. pneumoniae* and *H. influenzae*, both of which require antibodies for effective opsonization and clearance.

Pathogenesis of Central Nervous System Involvement.

The pathogenesis of neurologic manifestations deserves special mention because, in addition to the lymphoid system, the nervous system is a major target of HIV infection.[139] [140] Macrophages and microglia, cells in the central nervous system that belong to the monocyte and macrophage lineage, are the predominant cell types in the brain that are infected with HIV. It is widely believed that HIV is carried into the brain by infected monocytes. In keeping with this, the HIV isolates from the brain are almost exclusively M-tropic. The mechanism of HIV-induced damage of the brain, however, remains obscure. Because neurons are not infected by HIV, and the extent of neuropathologic changes is often less than might be expected from the severity of neurologic symptoms, most workers believe that neurologic deficit is caused indirectly by viral products and by soluble factors produced by infected microglia. Included among the soluble factors are the usual culprits, such as IL-1, TNF, and IL-6. In addition, nitric oxide induced in neuronal cells by gp41 has been implicated. Direct damage of neurons by soluble HIV gp120 has also been postulated. According to some investigators, these diverse soluble neurotoxins act by triggering excessive entry of Ca^{2+} into the neurons through their action on glutamate-activated ion channels that regulate intracellular calcium.

Natural History of HIV Infection

The course of HIV infection can be best understood in terms of an interplay between HIV and the immune system. Three phases reflecting the dynamics of virus-host interaction can be recognized: (1) an acute retroviral syndrome; (2) a middle, chronic phase; and (3) full-blown AIDS (see Fig. 6-45 ; also Fig. 6-50). [141] We first present the cardinal features of the phases of HIV infection and their associated clinical syndromes then recount the sequential virologic and immunologic findings during the course of HIV infection.

The *acute retroviral syndrome* represents the initial or primary response of an immunocompetent adult to HIV infection. [142] It is characterized initially by a high level of virus production, viremia, and *widespread seeding of the lymphoid tissues*. The initial infection, however, is readily controlled by the development of an antiviral immune response. It is estimated that 40% to 90% of individuals who acquire a primary infection develop the viral syndrome 3 to 6 weeks after infection, and this resolves spontaneously in 2 to 4 weeks. Clinically, this phase is associated with a self-limited acute illness with nonspecific symptoms, including sore throat, myalgias, fever, rash, weight loss, and fatigue, resembling a flulike syndrome. Other clinical features, such as rash, cervical adenopathy, diarrhea, and vomiting, may also occur.

The middle *chronic phase* represents a stage of relative containment of the virus, associated with a period of clinical latency. The immune system is largely intact, but *there is continuous HIV replication, predominantly in the lymphoid tissues, which may last for several years*. Patients are either asymptomatic or develop persistent generalized lymphadenopathy. In addition, many patients have minor opportunistic infections, such as thrush and herpes zoster. Thrombocytopenia may also be noted (Chapter 13). Persistent lymphadenopathy with significant constitutional symptoms (fever, rash, fatigue) reflects the onset of immune system decompensation, escalation of viral replication, and onset of the *crisis* phase.

The final phase is *progression to AIDS*. It is characterized by a breakdown of host defense, a dramatic increase in plasma virus, and clinical disease. Typically the patient presents with long-lasting fever (>1 month), fatigue, weight loss, and diarrhea. After a variable period, serious opportunistic infections, secondary neoplasms, or clinical neurologic disease (grouped under the rubric *AIDS indicator diseases*, discussed below) supervene, and the patient is said to have developed AIDS.

In the absence of treatment, most but not all patients with HIV infection progress to AIDS after a chronic phase lasting from 7 to 10 years. Exceptions to this typical course are exemplified by long-term nonprogressors and by rapid progressors. Nonprogressors are defined as untreated HIV-1-infected individuals who remain asymptomatic for 10 years or more, with stable CD4 + counts and low levels of plasma viremia. In rapid progressors, the middle, chronic phase is telescoped to 2 to 3 years after primary infection. The possible basis for these variant outcomes is discussed later.

With this overview of the phases of HIV disease, we can consider some details of host-parasite relationships during the course of a typical HIV infection. The initial entry of the virus may be through a mucosal surface, as in sexual intercourse (via rectal or cervical mucosa) or via blood exposure (e.g., after intravenous drug use). From the mucosal portal, the virus is carried to the regional lymph nodes by dendritic cells.

Figure 6-50 Typical course of HIV infection. *A*, During the early period after primary infection, there is widespread dissemination of virus and a sharp decrease in the number of CD4+ T cells in peripheral blood. An immune response to HIV ensues, with a decrease in viremia followed by a prolonged period of clinical latency. During this period, viral replication continues. The CD4+ T-cell count gradually decreases during the following years, until it reaches a critical level below which there is a substantial risk of opportunistic diseases. *(Redrawn from Fauci AS, Lane HC: Human immunodeficiency virus disease: AIDS and related conditions. In Fauci AS, et al (eds): Harrison's Principles of Internal Medicine, 14th ed. New York, McGraw-Hill, 1997, p 1791.) B*, Immune response to HIV infection. A cytolytic T lymphocyte (CTL) response to HIV is detectable by 2 to 3 weeks after the initial infection and peaks by 9 to 12 weeks. Marked expansion of virus-specific CD8+ T cell clones occurs during this time, and up to 10% of a patient's CTLs may be HIV specific at 12 weeks. The humoral immune response to HIV peaks at about 12 weeks.

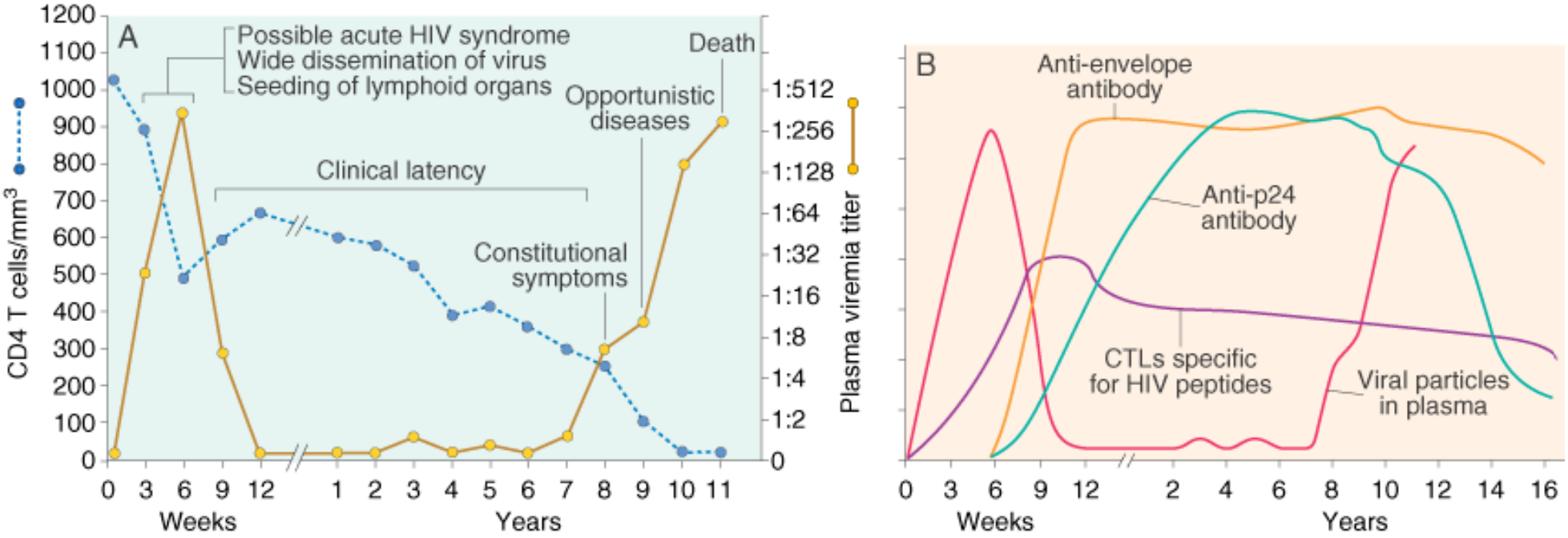


TABLE 6-13 -- CDC Classification Categories of HIV Infection

	CD4+ T-Cell Categories		
Clinical Categories	**1. ≥500/µL**	**2. 200–499/µL**	**3. ≤200/µL**
A. Asymptomatic, acute (primary) HIV, or persistent generalized lymphadenopathy	A1	A2	A3
B. Symptomatic, not A or C conditions	B1	B2	B3
C. AIDS indicator conditions: including constitutional disease, neurologic disease, or secondary infection or neoplasm			

Data from CDC. Centers for Disease Control and Prevention: 1993 revised classification system and expanded surveillance definition for AIDS among adolescents and adults. MMWR 41 (RR-17): 1, 1992.

CD4+ cell count and the development of AIDS, there is extensive turnover of the virus. In other words, *HIV infection lacks a phase of true microbiologic latency*, that is, a phase during which *all* the HIV is in the form of proviral DNA, and no cell is productively infected.

Before this discussion of the virus-host relationships is ended, some comments on those patients who are considered long-term nonprogressors are in order. Individuals in this group remain asymptomatic for long periods of time (10 years or more), have low levels of viremia, and have stable CD4+ cell counts. People with such an uncommon clinical course have attracted great attention in the hope that studying them may shed light on host and viral factors that influence disease progression. Studies to date suggest that this group is heterogeneous with respect to the factors that influence the course of the disease. In a small subset of nonprogressors, the infecting HIV had deletions or mutations in the *nef* gene, suggesting that Nef proteins are critical to disease progression. In most cases, the viral isolates do not show any qualitative abnormalities. In all cases, there is evidence of a vigorous anti-HIV immune response, but the immune correlates of protection are still unknown. Some of these patients have high levels of HIV-specific CD8+ cells, and these levels are maintained over the course of infection. It is not clear whether the robust CD8+ cell response is the cause or consequence of the slow progression. Further studies, it is hoped, will provide the answers to this and other questions critical to

disease progression.

Clinical Features of AIDS

The clinical manifestations of HIV infection can be readily surmised from the foregoing discussion. They range from a mild acute illness to severe disease. Because the salient clinical features of the acute early and chronic middle phases of HIV infection were described earlier, here we summarize the clinical manifestations of the terminal phase, AIDS. At the outset it should be pointed out that the clinical manifestations and opportunistic infections associated with HIV infection may differ in different parts of the world. Typically, HIV-infected individuals in Africa show a more rapid progression of the disease and a shorter survival time than in other geographic areas. Importantly, the clinical course of the disease has been greatly modified by new anti-retroviral therapies, and many complications that were once devestating are now infrequent.

In the United States, the typical adult patient with AIDS presents with fever, weight loss, diarrhea, generalized lymphadenopathy, multiple opportunistic infections, neurologic disease and, in many cases, secondary neoplasms. The infections and neoplasms listed in Table 6-14 are included in the surveillance definition of AIDS.[149]

Opportunistic infections account for the majority of deaths in patients with AIDS. The actual frequency of infections varies in different regions of the world, and has been greatly reduced by the advent of HAART.[150] A brief summary of selected opportunistic infections is provided here. Extensive reviews on the subject are available.[151] [152]

Approximately 15% to 30% of HIV-infected people develop pneumonia caused by the opportunistic fungus *P. carinii* (representing reactivation of a prior latent infection), despite prophylaxis. Prior to HAART, this infection was the presenting feature in about 20% of cases, but the incidence is much less in patients who respond to HAART. The risk of developing this infection is extremely high in individuals with fewer than 200 CD4+ cells/µL. Even in these patients there has been a substantial decline in the incidence of this infection because of effective prophylaxis.

An increasing number of patients present with an opportunistic infection other than *P. carinii* pneumonia. Among the most common pathogens are *Candida*, cytomegalovirus,

TABLE 6-14 -- AIDS-Defining Opportunistic Infections and Neoplasms Found in Patients with HIV Infection

INFECTIONS
Protozoal and Helminthic Infections
Cryptosporidiosis or isosporidiosis (enteritis)
Pneumocytosis (pneumonia or disseminated infection)
Toxoplasmosis (pneumonia or CNS infection)
Fungal Infections
Candidiasis (esophageal, tracheal, or pulmonary)
Cryptococcosis (CNS infection)
Coccidioidomycosis (disseminated)
Histoplasmosis (disseminated)
Bacterial Infections
Mycobacteriosis (atypical, e.g., *M. avium-intracellulare*, disseminated or extrapulmonary; *M. tuberculosis*, pulmonary or extrapulmonary)

Nocardiosis (pneumonia, meningitis, disseminated)
Salmonella infections, disseminated
Viral Infections
Cytomegalovirus (pulmonary, intestinal, retinitis, or CNS infections)
Herpes simplex virus (localized or disseminated)
Varicella-zoster virus (localized or disseminated)
Progressive multifocal leukoencephalopathy)
NEOPLASMS
Kaposi sarcoma
B-cell non-Hodgkin lymphomas
Primary lymphoma of the brain
Invasive cancer of uterine cervix
CNS, central nervous system.

atypical and typical mycobacteria, *Cryptococcus neoformans*, *Toxoplasma gondii*, *Cryptosporidium*, herpes simplex virus, papovaviruses, and *Histoplasma capsulatum*.

Candidiasis is the most common fungal infection in patients with AIDS. *Candida* infection of the oral cavity (thrush) and esophagus are the two most common clinical manifestations of candidiasis in HIV-infected patients. In asymptomatic HIV-infected individuals, oral candidiasis is a sign of immunologic decompensation, and it often heralds the transition to AIDS. Invasive candidiasis is not common in patients with AIDS, and it usually occurs when there is drug-induced neutropenia or use of indwelling catheters. *Cytomegalovirus* may cause disseminated disease, although, more commonly, it affects the eye and gastrointestinal tract. Chorioretinitis was seen in approximately 25% of patients pre-HAART, but this has decreased by over 50% after the intiation of HAART. Cytomegalovirus retinitis occurs almost exclusively in patients with CD4+ cell counts below 50/µl. Gastrointestinal disease, seen in 5% to 10% of cases, manifests as esophagitis and colitis, the latter associated with multiple mucosal ulcerations. Disseminated bacterial infection with *atypical mycobacteria* (mainly *M. avium-intracellulare*) also occurs late, in the setting of severe immunosuppression. Coincident with the AIDS epidemic, the incidence of tuberculosis has risen dramatically. [153] Worldwide, almost a third of all deaths in AIDS patients are attributable to tuberculosis; in the United states, about 5% of patients with AIDS develop active tuberculosis. Patients with AIDS have reactivation of latent pulmonary disease as well as outbreaks of primary infection. In contrast to infection with atypical mycobacteria, *M. tuberculosis* manifests itself early in the course of AIDS. As with tuberculosis in other settings, the infection may be confined to lungs or may involve multiple organs. The pattern of expression depends on the degree of immunosuppression; dissemination is more common in patients with very low CD4+ cell counts. Most worrisome are reports indicating that a growing number of isolates are resistant to multiple drugs.

Cryptococcosis occurs in about 10% of AIDS patients. Among fungal infections that prey on HIV-infected individuals, it is second only to candidiasis. As in other settings with immunosuppression, meningitis is the major clinical manifestation of cryptococcosis. In contrast to *Cryptococcus, T. gondii*, another frequent invader of the central nervous system in AIDS, causes encephalitis and is responsible for 50% of all mass lesions in the central nervous system. JC virus, a human papovavirus, is another important cause of central nervous system infections in HIV-infected patients. It causes progressive multifocal leukoencephalopathy (Chapter 28). *Herpes simplex virus infection* is manifested by mucocutaneous ulcerations involving the mouth, esophagus, external genitalia, and perianal region. *Persistent diarrhea*, so common in patients with AIDS, is often caused by infections with protozoans such as

Cryptosporidium, Isospora belli, or microsporidia. These patients have chronic, profuse, watery diarrhea with massive fluid loss. Diarrhea may also result from infection with enteric bacteria, such as *Salmonella* and *Shigella*, as well as *M. avium-intracellulare*. Depressed humoral immunity renders AIDS patients susceptible to severe, recurrent bacterial pneumonias.

Patients with AIDS have a high incidence of certain tumors, especially *Kaposi sarcoma (KS)*, non-Hodgkin B-cell lymphoma, cervical cancer in women, and anal cancer in men.[154] [155] It is estimated that 25% to 40% of HIV-infected individuals will eventually develop a malignancy. A common feature of these tumors is that they are all believed to be caused by oncogenic DNA viruses, that is, Kaposi sarcoma herpesvirus (Kaposi sarcoma), EBV (B-cell lymphoma), human papillomavirus (cervical and anal carcinoma). The increased risk of malignancy is thus mainly a consequence of increased susceptibility to infections by these viruses and decreased immunity against the tumors.

KS, a vascular tumor that is otherwise rare in the United States, is the most common neoplasm in patients with AIDS. The morphology of KS and its occurrence in patients not infected with HIV are discussed in Chapter 11 . At the onset of the AIDS epidemic, up to 30% of infected homosexual or bisexual men had KS, but in recent years, with use of HAART there has been a marked decline in its incidence, from 15 cases per 1000 person years to less than 5 cases.

The lesions of KS are characterized by the proliferation of spindle-shaped cells that express markers of both endothelial (vascular or lymphatic) and smooth muscle lineages. There is also a profusion of slit-like vascular spaces, suggesting that the lesions may arise from primitive mesenchymal precursors of vascular channels. In addition, KS lesions display chronic inflammatory cell infiltrates. There is still some debate about whether the lesions represent an exuberant hyperplasia or a malignant neoplasm, but the weight of evidence favors the former. For instance, spindle cells in many KS lesions are polyclonal or oligoclonal, although more advanced lesions occasionally show monoclonality.[156] Moreover, spindle cells in many KS lesions are diploid, dependent on growth factors for their proliferation, and do not form tumors in immunodeficient mice.[157] When KS cells are implanted subcutaneously in such mice, they transiently induce slit-like new blood vessels and inflammatory infiltrates in the surrounding tissue; these elements recall features of human KS, but interestingly are of murine origin. When the human KS cells involute, these elements also regress. These observations suggest that KS pathogenesis involves a complex web of paracrine signaling interactions among different types of cells, no one of which is fully autonomous. One popular view envisions that spindle cells produce pro-inflammatory and angiogenic factors, recruiting the inflammatory and neovascular components of the lesion, while the latter components supply signals that aid in spindle cell survival or growth[157] (Fig. 6-51).

But what initiates this cycle of events? Clues to this came from the observation that not all HIV patients are at equal risk for KS development. AIDS-related KS is twenty times more frequent in individuals who acquire HIV by sexual routes compared to those who acquire it parenterally. This observation suggested that a sexually transmitted agent other than HIV might be implicated in KS etiology and prompted a search for new viruses in KS. This search yielded a novel herpesvirus, aptly labeled *KS herpesvirus* (KSHV), or *human herpesvirus 8*.[158] Epidemiologic studies strongly link KSHV to KS development. Infection is uncommon in the general population and strikingly increased in prevalence in groups in which KS is common. In individual patients, KSHV infection precedes KS development and is highly correlated with increased KS risk. KSHV DNA is found in virtually all KS lesions, including those that occur in HIV-negative populations. In the lesions, KSHV is strikingly localized to the spindle cells, which display predominantly latent infection.[159] Thus,

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Figure 6-51 Proposed role of HIV, KSHV (HHV8), and cytokines in the pathogenesis of Kaposi sarcoma. Cytokines are produced by the mesenchymal cells infected by KSHV, or by HIV-infected CD4+ cells. B cells may also be infected by KSHV; their role in the disease is unclear.

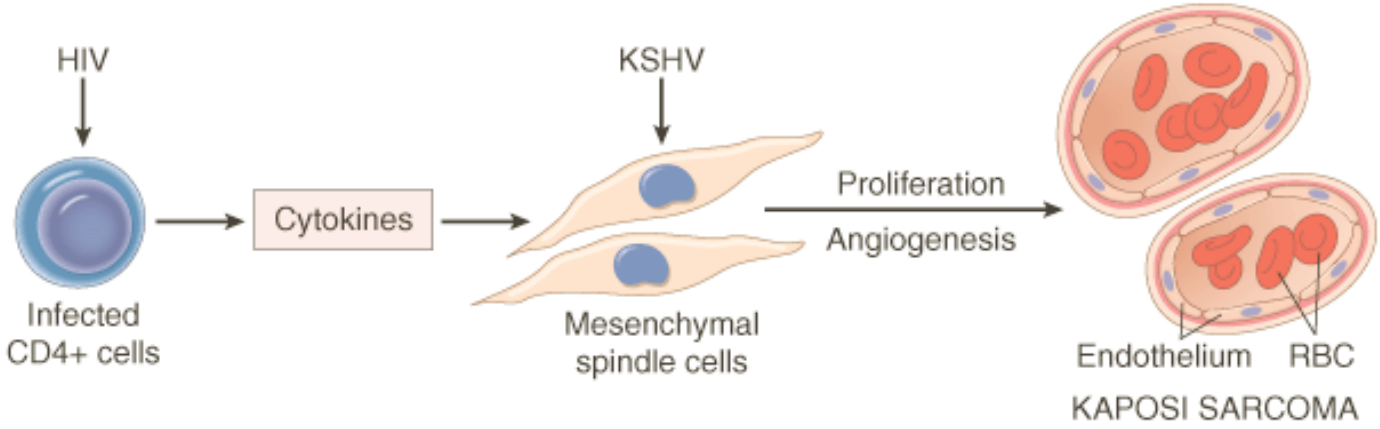

Figure 6-52 Amyloidosis. *A*, A section of the liver stained with Congo red reveals pink-red deposits of amyloid in the walls of blood vessels and along sinusoids. *B*, Note the yellow-green birefringence of the deposits when observed by polarizing microscope. *(Courtesy of Dr. Trace Worrell and Sandy Hinton, Department of Pathology, University of Texas Southwestern Medical School, Dallas TX.)*

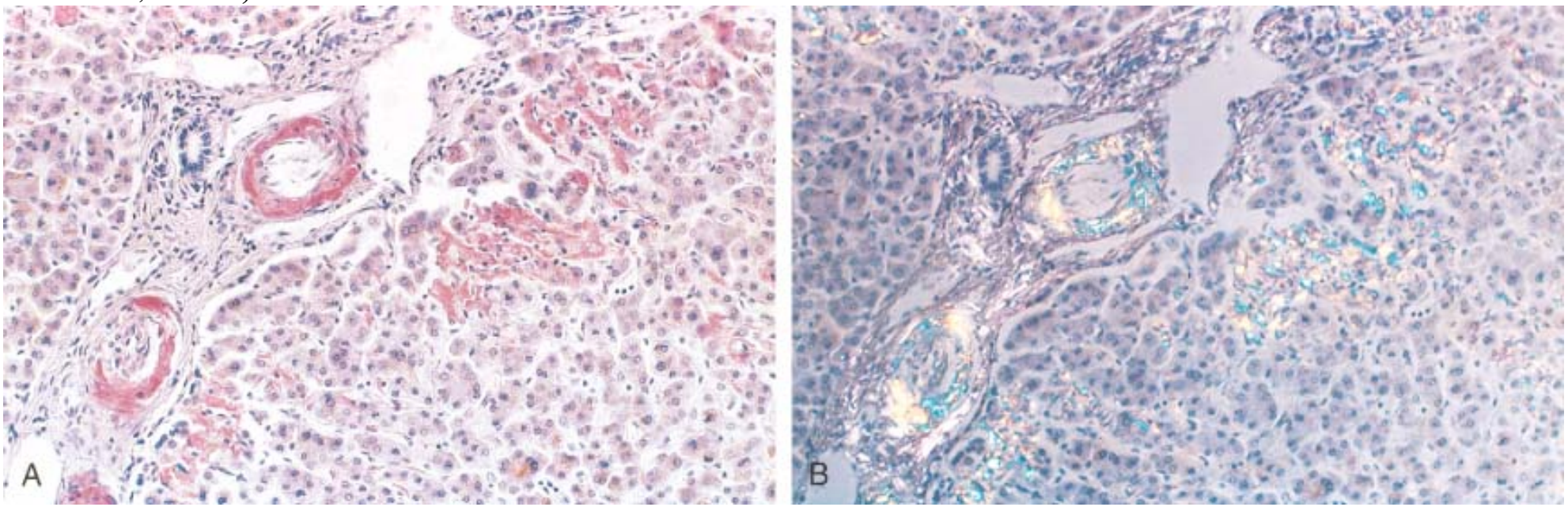

Figure 6-53 Structure of an amyloid fibril, depicting the β-pleated sheet structure and binding sites for the Congo red dye, which is used for diagnosis of amyloidosis. *(Modified from Glenner GG: Amyloid deposit and amyloidosis. The β-fibrilloses. N Engl J Med 52:148, 1980. By permission of The New England Journal of Medicine.)*

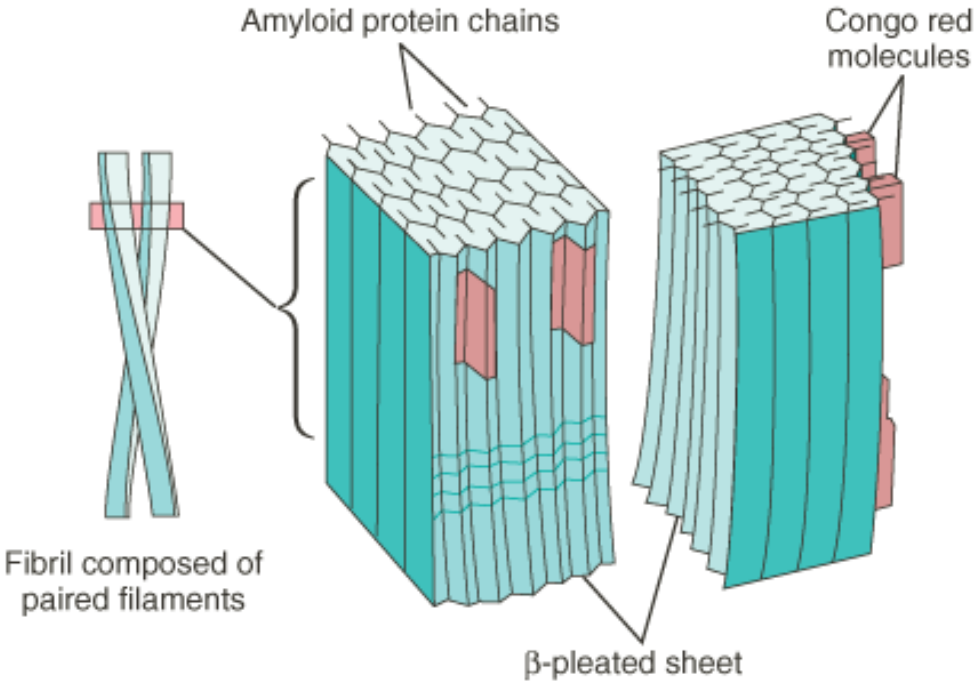

TABLE 6-15 -- Classification of Amyloidosis

Clinicopathologic Category	Associated Diseases	Major Fibril Protein	Chemically Related Precursor Protein
Systemic (Generalized) Amyloidosis			
Immunocyte dyscrasias with amyloidosis (primary amyloidosis)	Multiple myeloma and other monoclonal B-cell proliferations	AL	Immunoglobulin light chains, chiefly λ type
Reactive systemic amyloidosis (secondary amyloidosis)	Chronic inflammatory conditions	AA	SAA
Hemodialysis-associated amyloidosis	Chronic renal failure	$A\beta_2$ m	β_2 -microglobulin
Hereditary amyloidosis			
Familial Mediterranean fever	—	AA	SAA
Familial amyloidotic neuropathies (several types)	—	ATTR	Transthyretin
Systemic senile amyloidosis	—	ATTR	Transthyretin
Localized Amyloidosis			
Senile cerebral	Alzheimer disease	Aβ	APP
Endocrine			

••Medullary carcinoma of thyroid	—	A Cal	Calcitonin
••Islet of Langerhans	Type II diabetes	AIAPP	Islet amyloid peptide
Isolated atrial amyloidosis	—	AANF	Atrial natriuretic factor
Prion diseases	Various prion diseases of the CNS	Misfolded prion protein (PrP^{SC})	Normal prion protein PrP

In addition, other minor components are always present in amyloid. These include serum amyloid P component, proteoglycans, and highly sulfated glycosaminoglycans. Serum amyloid P protein may contribute to amyloid deposition by stabilizing the fibrils and decreasing their clearance.

Classification of Amyloidosis.

According to devoted "amyloidologists," who congregate every few years to discuss their favorite protein, amyloid should be classified based on its constituent chemical fibrils into categories such as AL, AA, and ATTR and not based on clinical syndromes.[170] Because a given biochemical form of amyloid (e.g., AA) may be associated with amyloid deposition in diverse clinical settings, we follow a combined biochemical-clinical classification for our discussion (Table 6-15). Amyloid may be *systemic* (generalized), involving several organ systems, or it may be *localized*, when deposits are limited to a single organ, such as the heart. As should become evident, several different biochemical forms of amyloid are encompassed by such segregation.

On clinical grounds, the systemic, or generalized, pattern is subclassified into *primary amyloidosis*, when associated with some immunocyte dyscrasia, or *secondary amyloidosis*, when it occurs as a complication of an underlying chronic inflammatory or tissue destructive process. *Hereditary* or *familial amyloidosis* constitutes a separate, albeit heterogeneous group, with several distinctive patterns of organ involvement.

Immunocyte Dyscrasias with Amyloidosis (Primary Amyloidosis).

Amyloid in this category is usually systemic in distribution

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and is of the AL type. With approximately 1275 to 3200 new cases every year in the United States, this is the most common form of amyloidosis. In many of these cases, the patients have some form of plasma cell dyscrasia. Best defined is the occurrence of systemic amyloidosis in 5% to 15% of patients with multiple myeloma, a plasma-cell tumor characterized by multiple osteolytic lesions throughout the skeletal system (Chapter 14). The malignant B cells characteristically synthesize abnormal amounts of a single specific immunoglobulin (monoclonal gammopathy), producing an M (myeloma) protein spike on serum electrophoresis. In addition to the synthesis of whole immunoglobulin molecules, only the light chains (referred to as *Bence Jones protein*) of either the λ or the κ variety may be elaborated and found in the serum. By virtue of the small molecular size of the Bence Jones protein, it is frequently excreted in the urine. The amyloid deposits contain the same light chain protein. Almost all the patients with myeloma who develop amyloidosis have Bence Jones proteins in the serum or urine, or both, but a great majority of myeloma patients who have free light chains do not develop amyloidosis. Clearly, therefore, *the presence of Bence Jones proteins, although necessary, is by itself not enough to produce amyloidosis*. We discuss later the other factors, such as the type of light chain produced (*amyloidogenic potential*) and the subsequent handling (possibly degradation) that may have a bearing on whether Bence Jones proteins are deposited as amyloid.

The great majority of patients with AL amyloid do not have classic multiple myeloma or any other overt B-cell neoplasm; such cases have been traditionally classified as primary amyloidosis because their clinical features derive from the effects of amyloid deposition without any other associated disease. In virtually all such cases, however, monoclonal immunoglobulins or free light chains, or both, can be found in the serum or urine. Most of these patients also have a modest increase in the number of plasma cells in the bone marrow,

which presumably secrete the precursors of AL protein. Clearly, these patients have an underlying B-cell dyscrasia in which production of an abnormal protein, rather than production of tumor masses, is the predominant manifestation. Recent studies have revealed chromosomal translocations in many of these patients, suggesting the presence of neoplastic clones.[171] Whether most of these clones would evolve into myeloma if the patients lived long enough can only be a matter for speculation.

Reactive Systemic Amyloidosis.

The amyloid deposits in this pattern are systemic in distribution and are composed of AA protein. This category was previously referred to as *secondary amyloidosis* because it is secondary to an associated inflammatory condition. The feature common to most of the conditions associated with reactive systemic amyloidosis is protracted breakdown of cells resulting from a wide variety of infectious and noninfectious chronic inflammatory conditions. At one time, tuberculosis, bronchiectasis, and chronic osteomyelitis were the most important underlying conditions, but with the advent of effective antimicrobial chemotherapy, the importance of these conditions has diminished. More commonly now, reactive systemic amyloidosis complicates rheumatoid arthritis, other connective tissue disorders such as ankylosing spondylitis, and inflammatory bowel disease, particularly Crohn disease and ulcerative colitis. Among these, the most frequent associated condition is rheumatoid arthritis. Amyloidosis is reported to occur in approximately 3% of patients with rheumatoid arthritis and is clinically significant in one half of those affected. Heroine abusers who inject the drug subcutaneously also have a high occurrence rate of generalized AA amyloidosis. The chronic skin infections associated with "skin-popping" of narcotics seem to be responsible for amyloidosis in this group of patients. Reactive systemic amyloidosis may also occur in association with non-immunocyte-derived tumors, the two most common being renal cell carcinoma and Hodgkin disease.

Hemodialysis-Associated Amyloidosis.

Patients on long-term hemodialysis for renal failure develop amyloidosis owing to deposition of β_2 -microglobulin. This protein is present in high concentrations in the serum of patients with renal disease and is retained in circulation because it cannot be filtered through the cuprophane dialysis membranes. In some series, as many as 60% to 80% of the patients on long-term dialysis developed amyloid deposits in the synovium, joints, and tendon sheaths.

Heredofamilial Amyloidosis.

A variety of familial forms of amyloidosis have been described. Most of them are rare and occur in limited geographic areas. The most common and best studied is an autosomal recessive condition called *familial Mediterranean fever*.[172] This is a febrile disorder of unknown cause characterized by attacks of fever accompanied by inflammation of serosal surfaces, including peritoneum, pleura, and synovial membrane. This disorder is encountered largely in individuals of Armenian, Sephardic Jewish, and Arabic origins. It is associated with widespread tissue involvement indistinguishable from reactive systemic amyloidosis. The amyloid fibril proteins are made up of AA proteins, suggesting that this form of amyloidosis is related to the recurrent bouts of inflammation that characterize this disease. The gene for familial Mediterranean fever has been cloned, and its product is called *pyrin* (for its relation to fever). Although its exact function is not known, it has been suggested that pyrin is responsible for regulating acute inflammation, presumably by inhibiting the function of neutrophils.[173] The relationship of this mutation to the disease is not understood.

In contrast to familial Mediterranean fever, a group of autosomal dominant familial disorders is characterized by deposition of amyloid predominantly in the nerves—peripheral and autonomic. These familial amyloidotic polyneuropathies have been described in different parts of the world. As mentioned previously, in all of these genetic disorders, the fibrils are made up of mutant transthyretins (ATTR).

Localized Amyloidosis.

Sometimes, amyloid deposits are limited to a single organ or tissue without involvement of any other site in the body. The deposits may produce grossly detectable nodular masses or be evident only on microscopic examination. Nodular (tumor-forming) deposits of amyloid are most often encountered in the lung, larynx, skin, urinary bladder, tongue, and the region about the eye. Frequently, there are infiltrates of lymphocytes and plasma cells in the periphery of these amyloid masses, raising the question of whether the mononuclear infiltrate is a response to the deposition of amyloid or instead is responsible for it. At least in some cases, the amyloid consists of AL protein and may therefore represent a localized form of immunocyte-derived amyloid.

Endocrine Amyloid.

Microscopic deposits of localized amyloid may be found in certain endocrine tumors, such as

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medullary carcinoma of the thyroid gland, islet tumors of the pancreas, pheochromocytomas, and undifferentiated carcinomas of the stomach, and in the islets of Langerhans in patients with type II diabetes mellitus. In these settings, the amyloidogenic proteins seem to be derived either from polypeptide hormones (e.g., medullary carcinoma) or from unique proteins (e.g., islet amyloid polypeptide).

Amyloid of Aging.

Several well-documented forms of amyloid deposition occur with aging.[174] *Senile systemic amyloidosis* refers to the systemic deposition of amyloid in elderly patients (usually in their seventies and eighties). Because of the dominant involvement and related dysfunction of the heart, this form was previously called *senile cardiac amyloidosis*. Those who are symptomatic present with a restrictive cardiomyopathy and arrhythmias. The amyloid in this form is composed of the normal TTR molecule. In addition to the sporadic senile systemic amyloidosis, another form, affecting predominantly the heart, that results from the deposition of a mutant form of TTR has also been recognized. Approximately 4% of the black population in the United States is a carrier of the mutant allele, and cardiomyopathy has been identified in both homozygous and heterozygous patients. The precise prevalance of patients with this mutation who develop clinically manifest cardiac disease is not known.

Pathogenesis.

Amyloidosis results from abnormal folding of proteins, which are deposited as fibrils in extracellular tissues and disrupt normal function. Misfolded proteins are often unstable and self-associate, ultimately leading to the formation of oligomers and fibrils that are deposited in tissues. The reason diverse conditions are associated with amyloidosis may be that each of these conditions results in excessive production of proteins that are prone to misfolding. The proteins that form amyloid fall into two general categories: (1) normal proteins that have an inherent tendency to fold improperly, associate and form fibrils, and do so when they are produced in

Figure 6-54 Proposed schema of the pathogenesis of the major forms of amyloid fibrils.

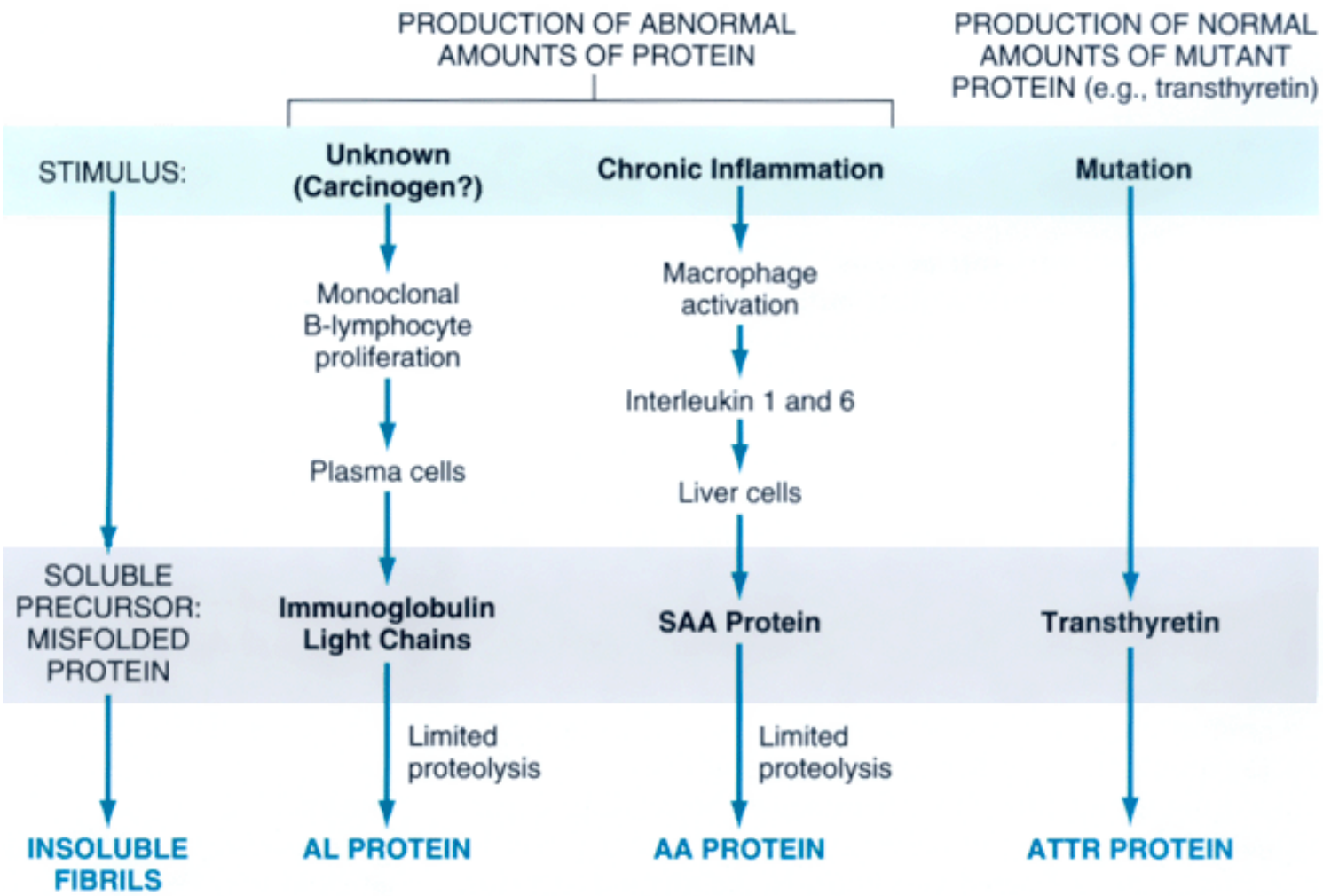


Figure 6-55 Amyloidosis of the kidney. The glomerular architecture is almost totally obliterated by the massive accumulation of amyloid.

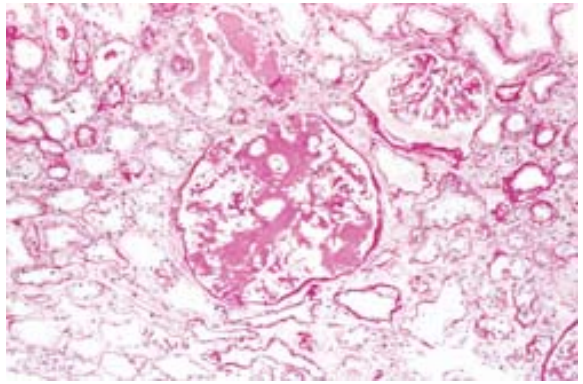
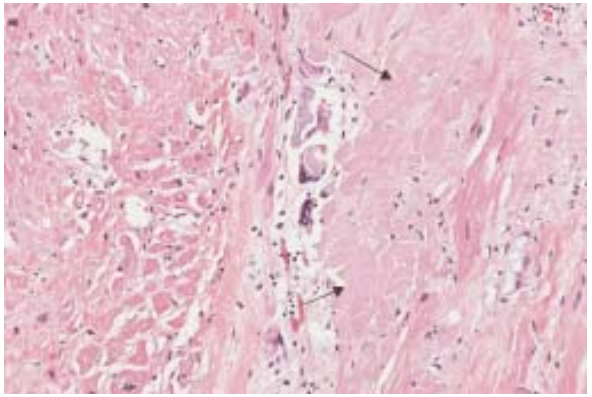


Figure 6-56 Cardiac amyloidosis. The atrophic myocardial fibers are separated by structureless, pink-staining amyloid *(arrows)*.



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Chapter 7 - Neoplasia

In the year 2000, there were 10 million new cases of cancer and 6 million cancer deaths worldwide.[1] [2] In the United States each year, almost 1.5 million individuals learn for the first time that they have some type of cancer. Not included in these figures are more than 1 million new cases of the most common types of nonpigmented skin cancers and incipient, noninvasive cancers. Not only these noninvasive lesions but many invasive tumors as well can be cured. Nonetheless, according to American Cancer Society estimates, cancer caused approximately 556,000 deaths in 2003, corresponding to 1500 cancer deaths per day, accounting for about 23% of all deaths in the United States.[3] Some good news, however, has emerged: cancer mortality for both men and women in the United States declined during the last decade of the 20th century.[4] Thus, there has been progress, but the problem is still overwhelming. The discussion that follows deals with both benign tumors and cancers; the latter receive more attention. The focus is on the basic morphologic and biologic properties of tumors and on the present understanding of the molecular basis of carcinogenesis. We also discuss the interactions of the tumor with the host and the host response to tumors. Although the discussion of therapy is beyond the scope of this chapter, there are now dramatic improvements in therapeutic responses and 5-year survival rates with many forms of malignancy, notably the leukemias and lymphomas. A greater proportion of cancers is being cured or arrested today than ever before.

Definitions

Neoplasia literally means the process of "new growth," and a new growth is called a *neoplasm*. The term *tumor* was originally applied to the swelling caused by inflammation. Neoplasms also may induce swellings, but by long precedent, the non-neoplastic usage of *tumor* has passed into limbo; thus, the term is now equated with neoplasm. *Oncology* (Greek *oncos* = tumor) is the study of tumors or neoplasms. *Cancer is the common term for all malignant tumors.* Although the ancient origins of this term are somewhat uncertain, it probably derives from the Latin for crab, *cancer*—presumably because a cancer "adheres to any part that it seizes upon in an obstinate manner like the crab."

Although all physicians know what they mean when they use the term *neoplasm*, it has been surprisingly difficult to develop an accurate definition. The eminent British oncologist Willis[5] has come closest: "A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change." We know that the persistence of tumors, even after the inciting stimulus is gone, results from *heritable genetic alterations that are passed down to the progeny of the tumor cells. These genetic changes allow excessive and unregulated proliferation that becomes autonomous (independent of physiologic growth stimuli),* although tumors generally remain dependent on the host for their nutrition and blood supply. As we shall discuss later, the entire population of cells within a tumor arises from a single cell that has incurred genetic change, and hence tumors are said to be *clonal*.

Nomenclature

All tumors, benign and malignant, have two basic components: (1) proliferating neoplastic cells that constitute their *parenchyma* and (2) supportive *stroma* made up of connective tissue and blood vessels. Although parenchymal cells represent the proliferating "cutting edge" of neoplasms and so determine their behavior and pathologic consequences, the growth and evolution of neoplasms are critically dependent on their stroma. An adequate stromal blood supply is requisite, and the stromal connective tissue provides the framework for the parenchyma. In addition, there is cross-talk between tumor cells and stromal cells that appears to directly influence the growth of tumors. In some tumors, the stromal support is scant and so the neoplasm is soft and fleshy. Sometimes the parenchymal cells stimulate the formation of an abundant collagenous stroma, referred to as *desmoplasia*. Some tumors—for example, some cancers of the female breast—are stony hard or *scirrhous*. The nomenclature of tumors is, however, based on the parenchymal component.

Benign Tumors.

In general, benign tumors are designated by attaching the suffix *-oma* to the cell of origin. Tumors of mesenchymal cells generally follow this rule. For example, a benign tumor arising from fibroblastic cells is called a *fibroma*, a cartilaginous tumor is a *chondroma*, and a tumor of osteoblasts is an *osteoma*. In contrast, nomenclature of benign epithelial tumors is more complex. They are variously classified, some based on their cells of origin, others on microscopic architecture, and still others on their macroscopic patterns.

Adenoma is the term applied to a benign epithelial neoplasm that forms glandular patterns as well as to tumors derived from glands but not necessarily reproducing glandular patterns. On this basis, a benign epithelial neoplasm that arises from renal tubular cells growing in the form of numerous tightly clustered small glands would be termed an *adenoma*, as would a heterogeneous mass of adrenal cortical cells growing in no distinctive pattern. Benign epithelial neoplasms producing microscopically or macroscopically visible finger-like or warty projections from epithelial surfaces are referred to as *papillomas* (Fig. 7-1). Those that form large cystic masses, as in the ovary, are referred to as *cystadenomas*.

Figure 7-1 Papilloma of the colon with finger-like projections into the lumen. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

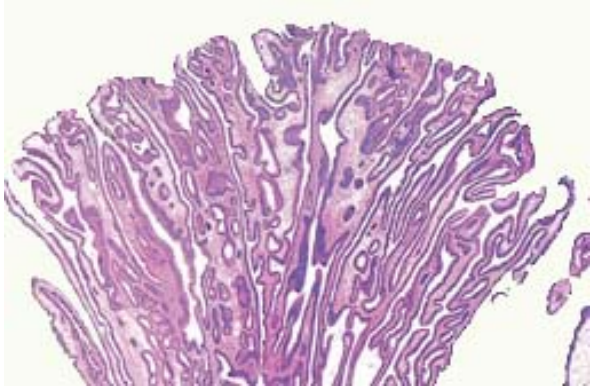

Figure 7-2 Colonic polyp. *A*, This benign glandular tumor (adenoma) is projecting into the colonic lumen and is attached to the mucosa by a distinct stalk. *B*, Gross appearance of several colonic polyps.

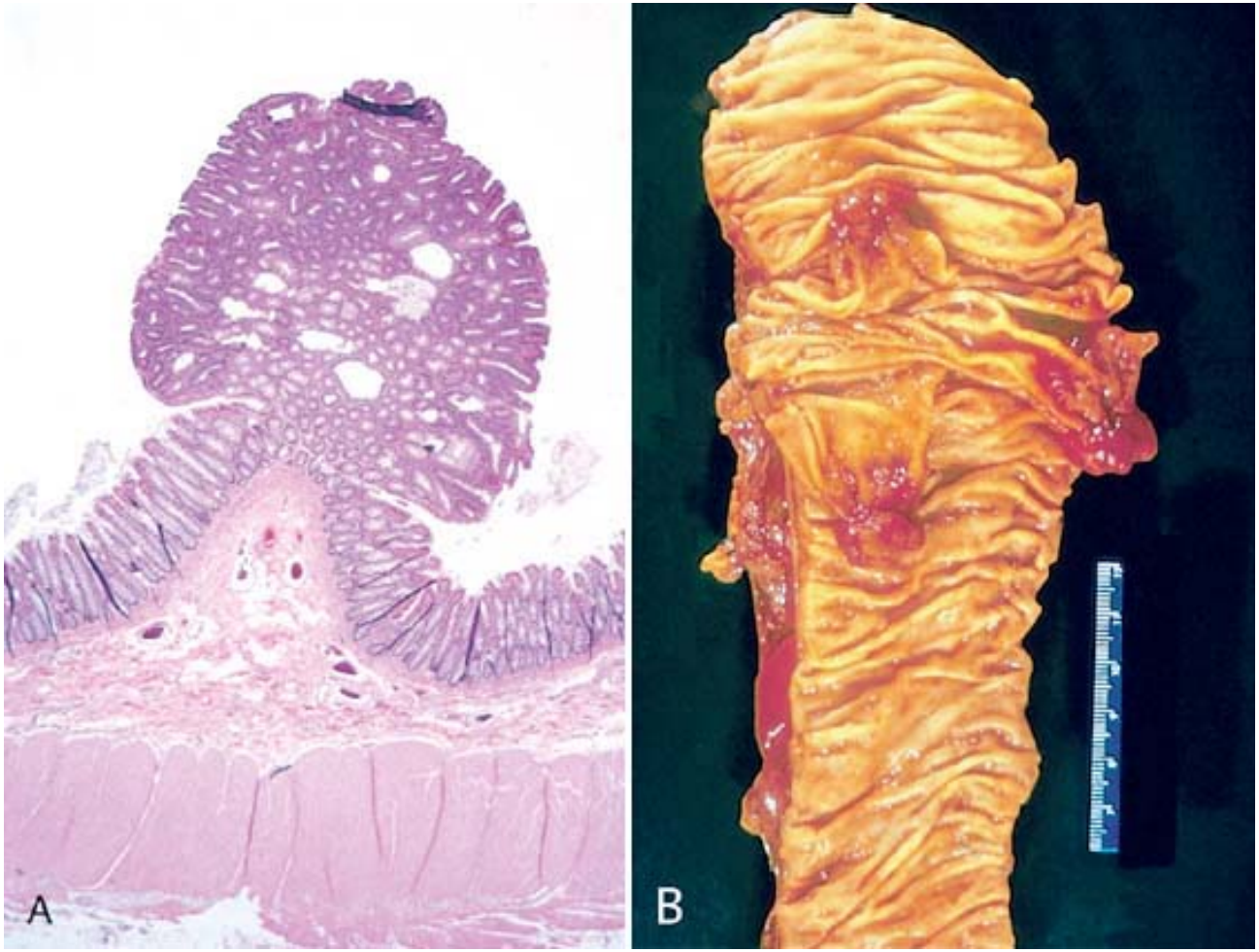

Figure 7-3 This mixed tumor of the parotid gland contains epithelial cells forming ducts and myxoid stroma that resembles cartilage. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

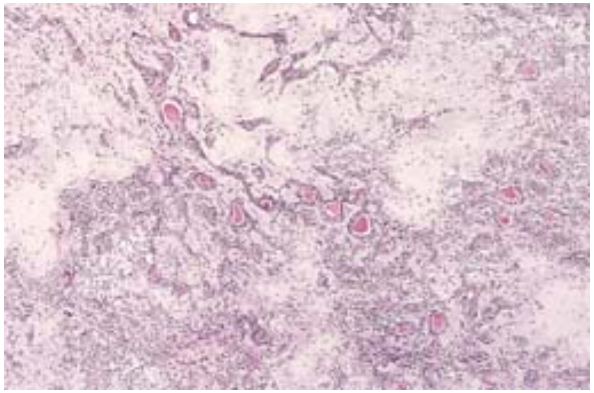

Figure 7-4 *A,* Gross appearance of an opened cystic teratoma of the ovary. Note the presence of hair, sebaceous material, and tooth. *B,* A microscopic view of a similar tumor shows skin, sebaceous glands, fat cells, and a tract of neural tissue *(arrow).*

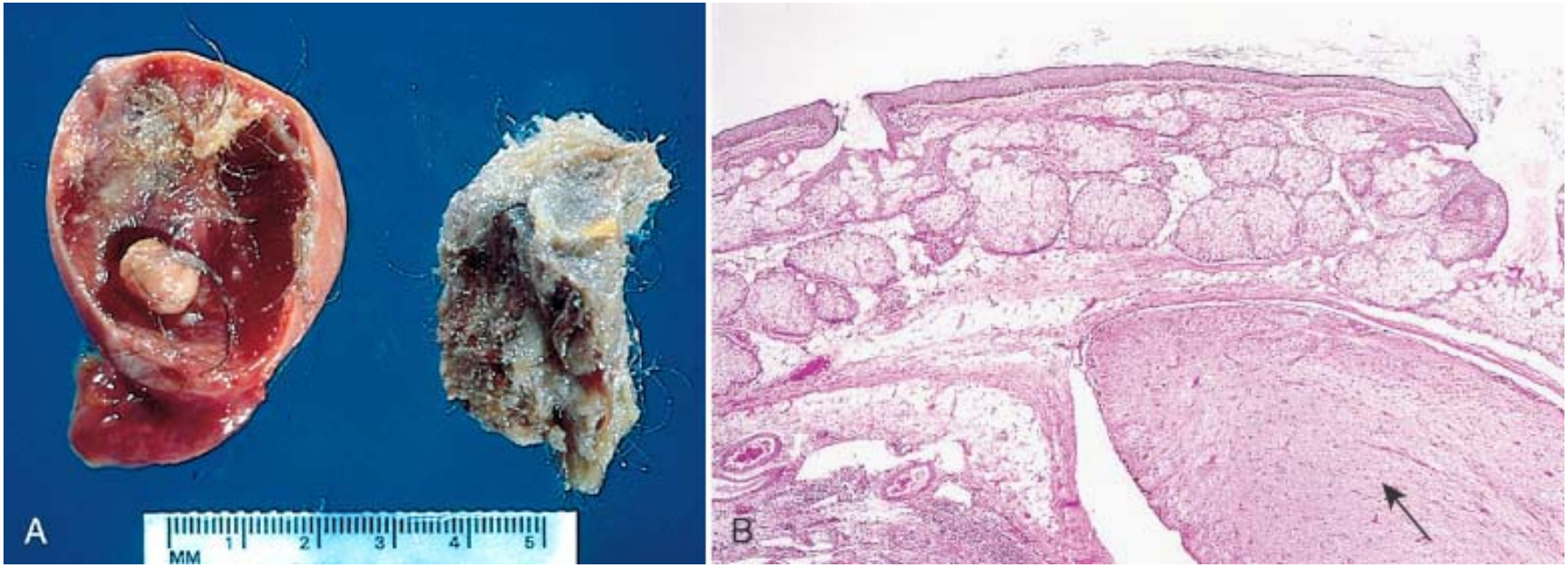

TABLE 7-1 -- Nomenclature of Tumors

Tissue of Origin	Benign	Malignant
Composed of One Parenchymal Cell Type		
Tumors of mesenchymal origin		
••Connective tissue and derivatives	Fibroma	Fibrosarcoma
	Lipoma	Liposarcoma
	Chondroma	Chondrosarcoma
	Osteoma	Osteogenic sarcoma
Endothelial and related tissues		
••Blood vessels	Hemangioma	Angiosarcoma
••Lymph vessels	Lymphangioma	Lymphangiosarcoma
••Synovium		Synovial sarcoma
••Mesothelium		Mesothelioma
••Brain coverings	Meningioma	Invasive meningioma
Blood cells and related cells		

••Hematopoietic cells		Leukemias
••Lymphoid tissue		Lymphomas
Muscle		
••Smooth	Leiomyoma	Leiomyosarcoma
••Striated	Rhabdomyoma	Rhabdomyosarcoma
Tumors of epithelial origin		
••Stratified squamous	Squamous cell papilloma	Squamous cell or epidermoid carcinoma
••Basal cells of skin or adnexa		Basal cell carcinoma
••Epithelial lining of glands or ducts	Adenoma	Adenocarcinoma
	Papilloma	Papillary carcinomas
	Cystadenoma	Cystadenocarcinoma
••Respiratory passages	Bronchial adenoma	Bronchogenic carcinoma
••Renal epithelium	Renal tubular adenoma	Renal cell carcinoma
••Liver cells	Liver cell adenoma	Hepatocellular carcinoma
••Urinary tract epithelium (transitional)	Transitional cell papilloma	Transitional cell carcinoma
••Placental epithelium	Hydatidiform mole	Choriocarcinoma
••Testicular epithelium (germ cells)		Seminoma
		Embryonal carcinoma
Tumors of melanocytes	Nevus	Malignant melanoma
More Than One Neoplastic Cell Type—Mixed Tumors, Usually Derived from One Germ Cell Layer		
Salivary glands	Pleomorphic adenoma (mixed tumor of salivary origin)	Malignant mixed tumor of salivary gland origin
Renal anlage		Wilms tumor
More Than One Neoplastic Cell Type Derived from More Than One Germ Cell Layer—Teratogenous		
Totipotential cells in gonads or in embryonic rests	Mature teratoma, dermoid cyst	Immature teratoma, teratocarcinoma

have primitive-appearing, unspecialized cells. In general, benign tumors are well differentiated (Fig. 7-6). The neoplastic cell in a benign smooth muscle tumor—a leiomyoma—so closely resembles the normal cell that it may be impossible to recognize it as a tumor by microscopic examination of individual cells. Only the massing of these cells into a nodule discloses the neoplastic nature of the lesion. One may get so close to the tree that one loses sight of the forest.

Malignant neoplasms, in contrast, range from well differentiated to undifferentiated. Malignant neoplasms composed of undifferentiated cells are said to be *anaplastic*. Lack of

differentiation, or *anaplasia,* is considered a hallmark of malignant transformation. Anaplasia literally means "to form backward," implying a reversion from a high level of differentiation to a lower level. There is substantial evidence, however, that most cancers do not represent "reverse differentiation" of mature normal cells but, in fact, arise from stem cells that are present in all specialized tissues. The well-differentiated cancer (Fig. 7-7) evolves from maturation or specialization of undifferentiated cells as they proliferate, whereas the undifferentiated malignant tumor derives from proliferation without complete maturation of the transformed cells.

Lack of differentiation, or anaplasia, is marked by a number of morphologic changes.

• *Pleomorphism.* Both the cells and the nuclei characteristically display *pleomorphism*—variation in size and shape (Fig. 7-8). Cells may be found that are many times larger than their neighbors, and other cells may be extremely small and primitive appearing.

• *Abnormal nuclear morphology.* Characteristically the nuclei contain an abundance of DNA and are extremely dark staining *(hyperchromatic).* The nuclei are disproportionately large for the cell, and the nucleus-to-cytoplasm ratio may approach 1:1 instead of the normal 1:4 or 1:6. The nuclear shape is very variable, and the chromatin is often coarsely clumped and distributed along the nuclear membrane. Large nucleoli are usually present in these nuclei.
• *Mitoses.* As compared with benign tumors and some well-differentiated malignant neoplasms, undifferentiated tumors usually possess large numbers of mitoses, reflecting the higher proliferative activity of the parenchymal cells. *The presence of mitoses, however, does not necessarily indicate that a tumor is malignant or that the tissue is neoplastic.* Many normal tissues exhibiting rapid turnover, such as bone marrow, have numerous mitoses, and non-neoplastic proliferations such as hyperplasias contain many cells in mitosis. More important as a morphologic feature of malignant neoplasia are atypical, bizarre mitotic figures, sometimes producing tripolar, quadripolar, or multipolar spindles (Fig. 7-9).
• *Loss of polarity.* In addition to the cytologic abnormalities, the *orientation of anaplastic cells is markedly disturbed (i.e., they lose normal polarity).* Sheets or large masses of tumor cells grow in an anarchic, disorganized fashion.
• *Other changes.* Another feature of anaplasia is the formation of *tumor giant cells,* some possessing only a single huge polymorphic nucleus and others having two or more nuclei. These giant cells are not to be confused with inflammatory Langhans or foreign body giant cells, which are derived from macrophages and contain many small, normal-appearing nuclei. In the cancer giant cell, the nuclei are hyperchromatic and large in relation to the cell. Although growing tumor cells obviously require a blood

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supply, often the vascular stroma is scant, and in many anaplastic tumors, large central areas undergo ischemic *necrosis.*

Figure 7-5 Leiomyoma of the uterus. This benign, well-differentiated tumor contains interlacing bundles of neoplastic smooth muscle cells that are virtually identical in appearance to normal smooth muscle cells in the myometrium.

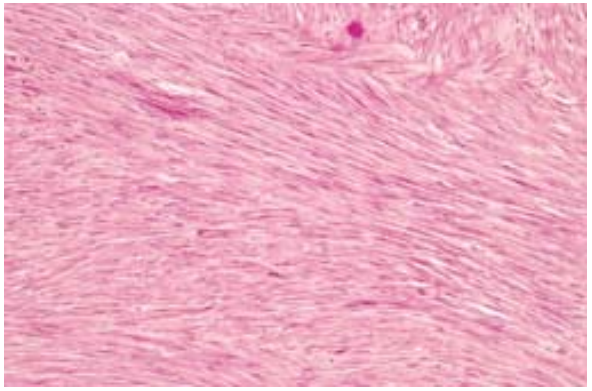

Figure 7-6 Benign tumor (adenoma) of the thyroid. Note the normal-looking (well-differentiated), colloid-filled thyroid follicles. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

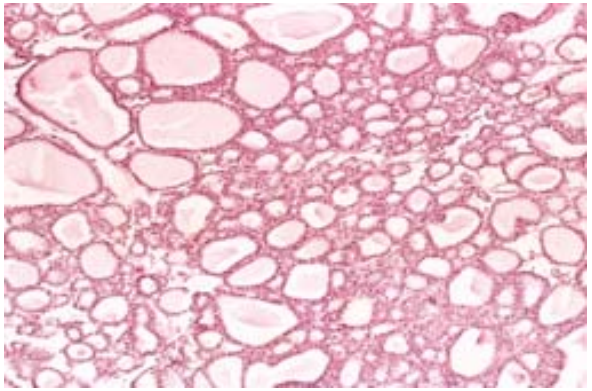

Figure 7-7 Malignant tumor (adenocarcinoma) of the colon. Note that compared with the well-formed and normal-looking glands characteristic of a benign tumor (see Fig. 7-6), the cancerous glands are irregular in shape and size and do not resemble the normal colonic glands. This tumor is considered differentiated because gland formation can be seen. The malignant glands have invaded the muscular layer of the colon. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

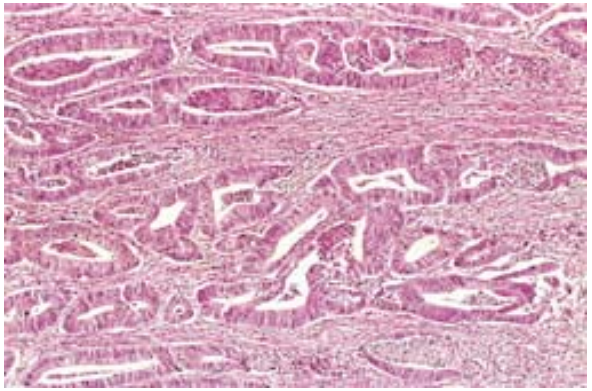

Figure 7-8 Anaplastic tumor of the skeletal muscle (rhabdomyosarcoma). Note the marked cellular and nuclear pleomorphism, hyperchromatic nuclei, and tumor giant cells. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

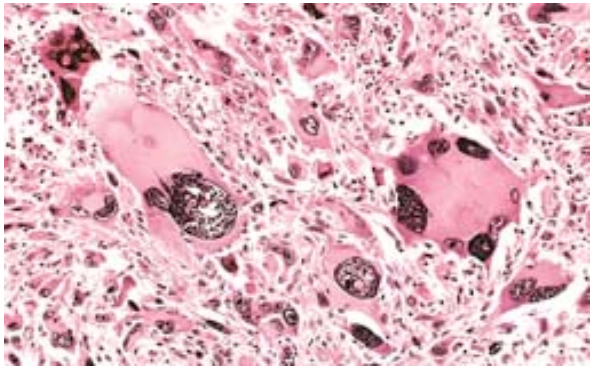

Figure 7-9 Anaplastic tumor showing cellular and nuclear variation in size and shape. The prominent cell in the center field has an abnormal tripolar spindle.

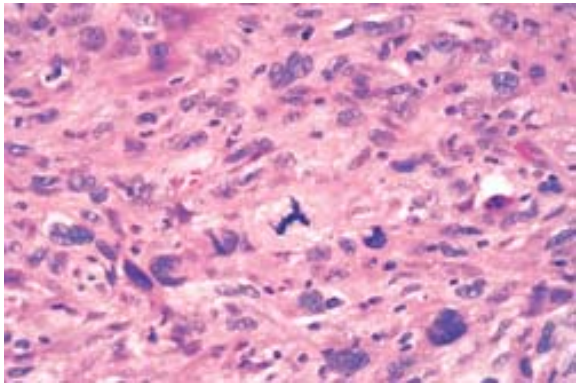

Figure 7-10 Well-differentiated squamous cell carcinoma of the skin. The tumor cells are strikingly similar to normal squamous epithelial cells, with intercellular bridges and nests of keratin pearls *(arrow). (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

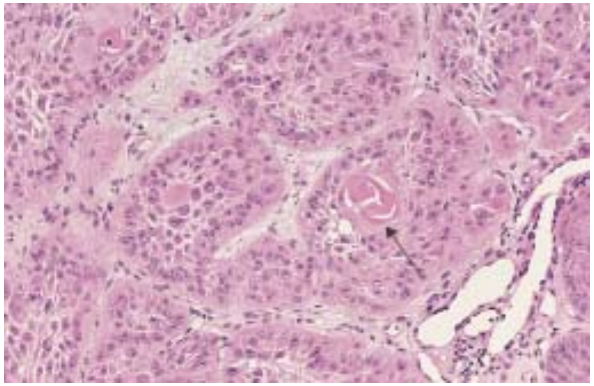

Figure 7-11 *A,* Carcinoma in situ. This low-power view shows that the entire thickness of the epithelium is replaced by atypical dysplastic cells. There is no orderly differentiation of squamous cells. The basement membrane is intact and there is no tumor in the subepithelial stroma. *B,* A high-power view of another region shows failure of normal differentiation, marked nuclear and cellular pleomorphism, and numerous mitotic figures extending toward the surface. The basement membrane *(below)* is not seen in this section.

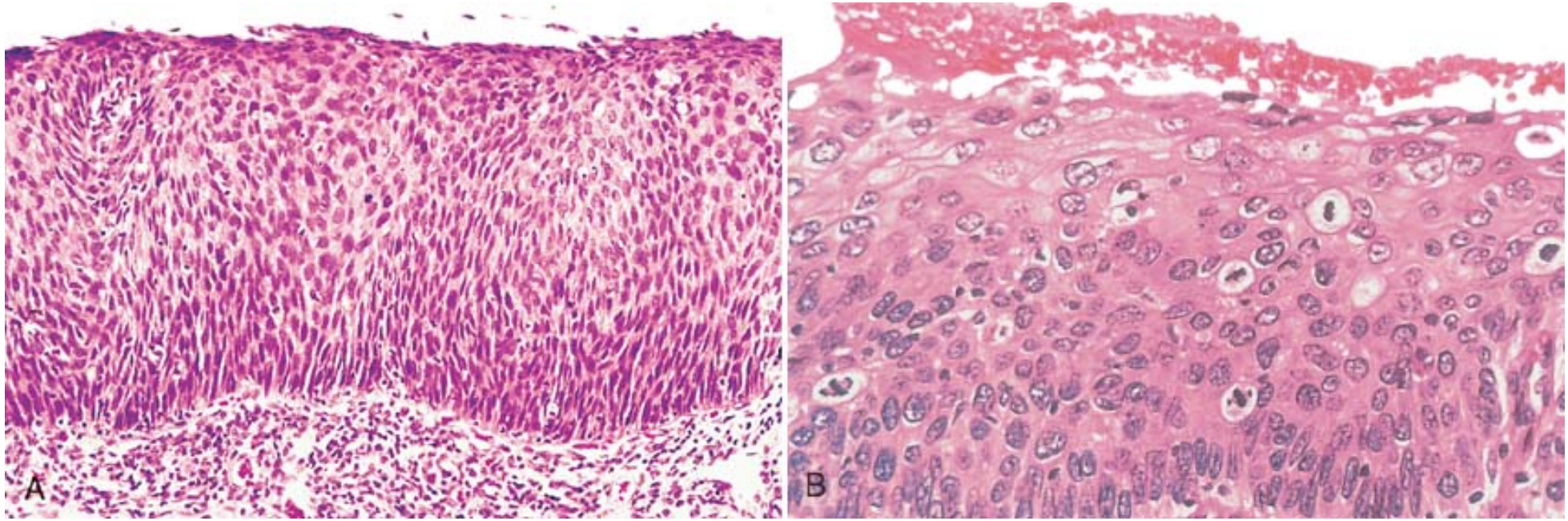

Figure 7-12 Biology of tumor growth. The left panel depicts minimal estimates of tumor cell doublings that precede the formation of a clinically detectable tumor mass. It is evident that by the time a solid tumor is detected, it has already completed a major portion of its life cycle as measured by cell doublings. The right panel illustrates clonal evolution of tumors and generation of tumor cell heterogeneity. New subclones arise from the descendants of the original transformed cell, and with progressive growth the tumor mass becomes enriched for those variants that are more adept at evading host defenses and are likely to be more aggressive. *(Adapted from Tannock IF: Biology of tumor growth. Hosp Pract 18:81, 1983.)*

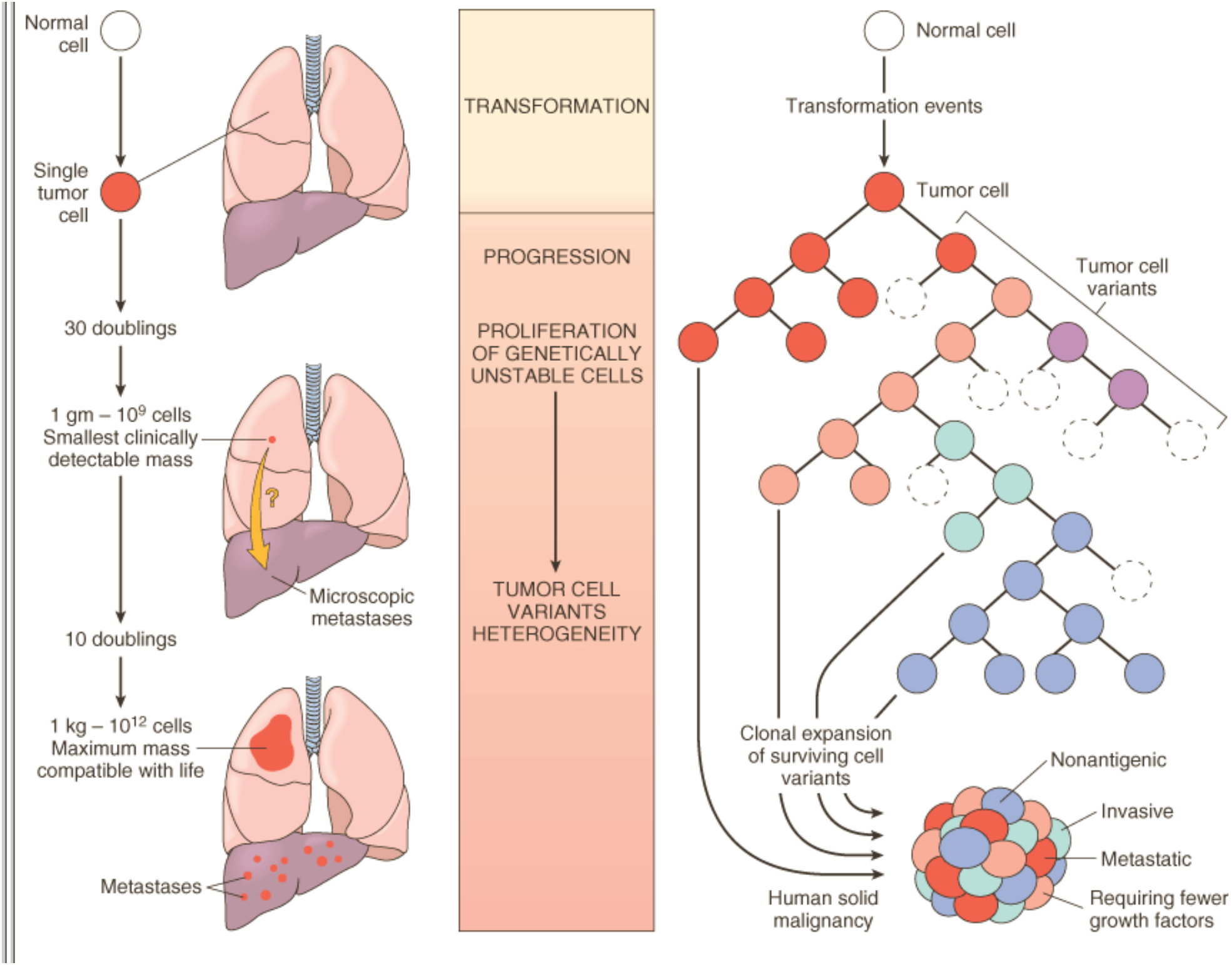
Normal cell
Single tumor cell
30 doublings
1 gm – 10^9 cells
Smallest clinically detectable mass
?
Microscopic metastases
10 doublings
1 kg – 10^{12} cells
Maximum mass compatible with life
Metastases
TRANSFORMATION
PROGRESSION
PROLIFERATION OF GENETICALLY UNSTABLE CELLS
TUMOR CELL VARIANTS HETEROGENEITY
Normal cell
Transformation events
Tumor cell
Tumor cell variants
Clonal expansion of surviving cell variants
Human solid malignancy
Nonantigenic
Invasive
Metastatic
Requiring fewer growth factors

Figure 7-13 Schematic representation of tumor growth. As the cell population expands, a progressively higher percentage of tumor cells leaves the replicative pool by reversion to G_0 , differentiation, and death.

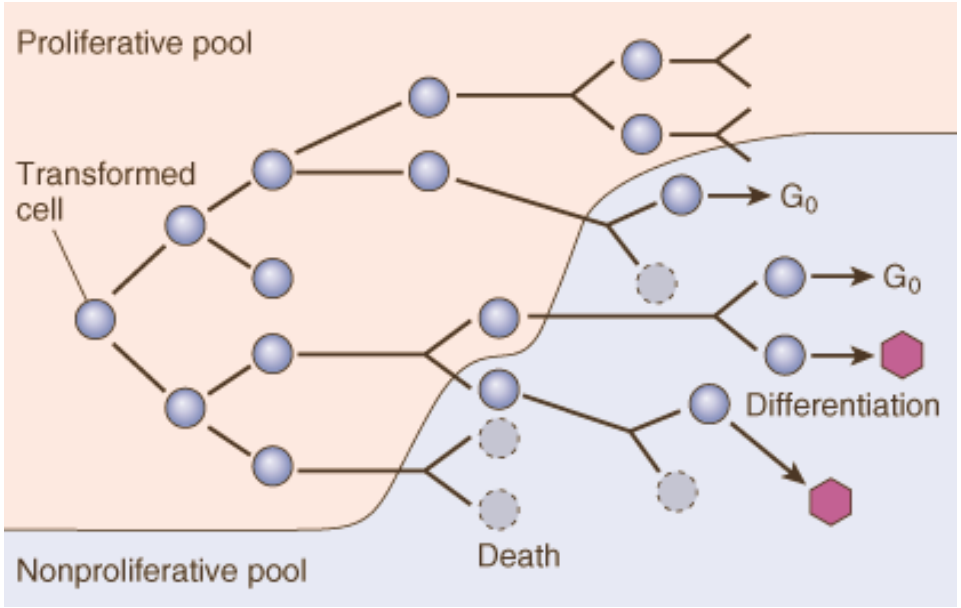

Figure 7-14 Fibroadenoma of the breast. The tan-colored, encapsulated small tumor is sharply demarcated from the whiter breast tissue.

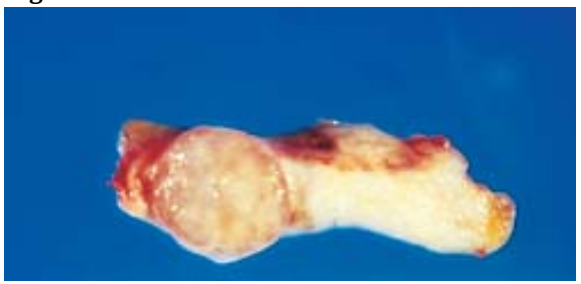

Figure 7-15 Microscopic view of fibroadenoma of the breast seen in Figure 7-14 . The fibrous capsule (*right*) delimits the tumor from the surrounding tissue. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

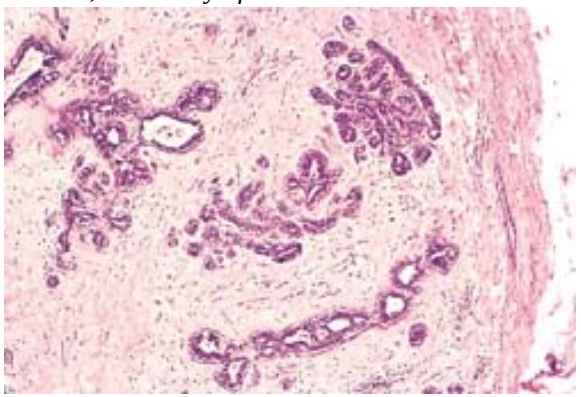

Figure 7-16 Cut section of an invasive ductal carcinoma of the breast. The lesion is retracted, infiltrating the surrounding breast substance, and would be stony hard on palpation.

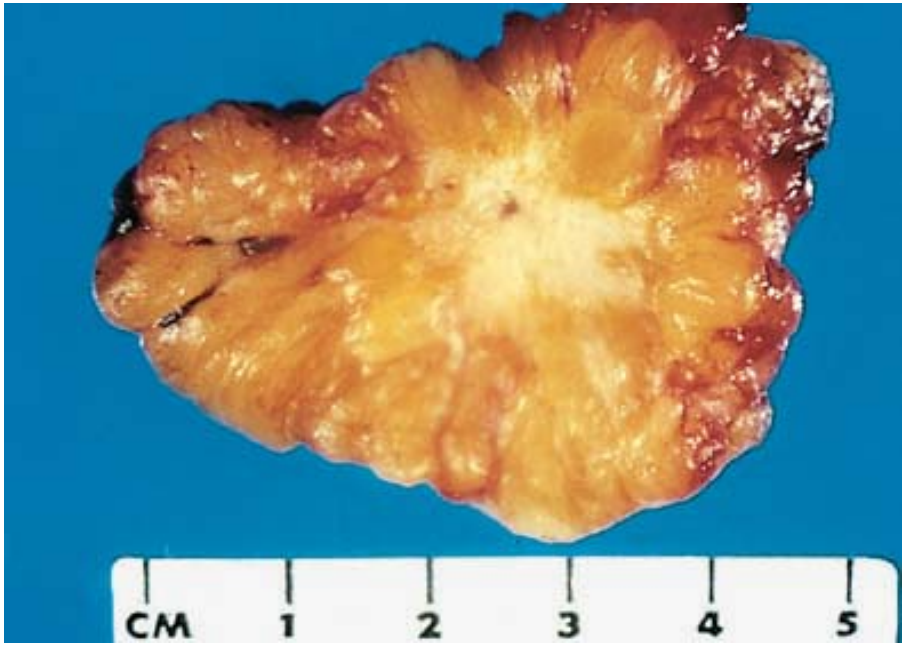

Figure 7-17 The microscopic view of the breast carcinoma seen in Figure 7-16 illustrates the invasion of breast stroma and fat by nests and cords of tumor cells (compare with fibroadenoma shown in Fig. 7-15). The absence of a well-defined capsule should be noted. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

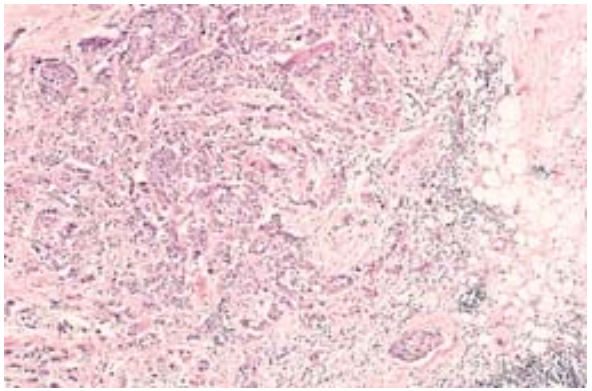

Figure 7-18 Colon carcinoma invading pericolonic adipose tissue. *(Courtesy of Dr. Melissa Upton, University of Washington, Seattle, WA.)*

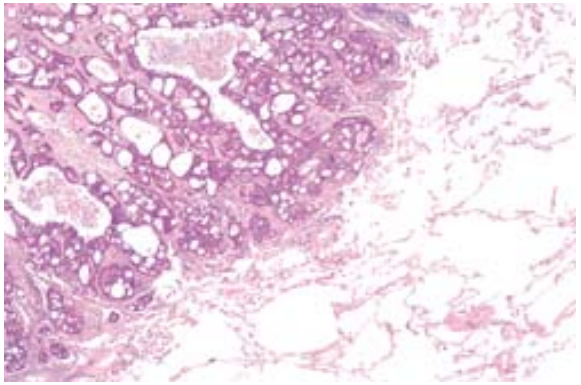

Figure 7-19 Axillary lymph node with metastatic breast carcinoma. The subcapsular sinus *(top)* is distended with tumor cells. Nests of tumor cells have also invaded the subcapsular cortex. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

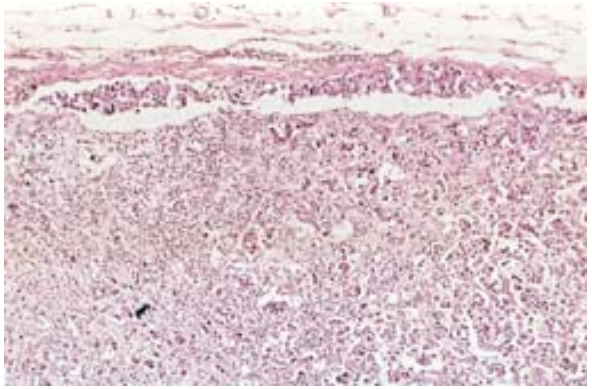

Figure 7-20 A liver studded with metastatic cancer.

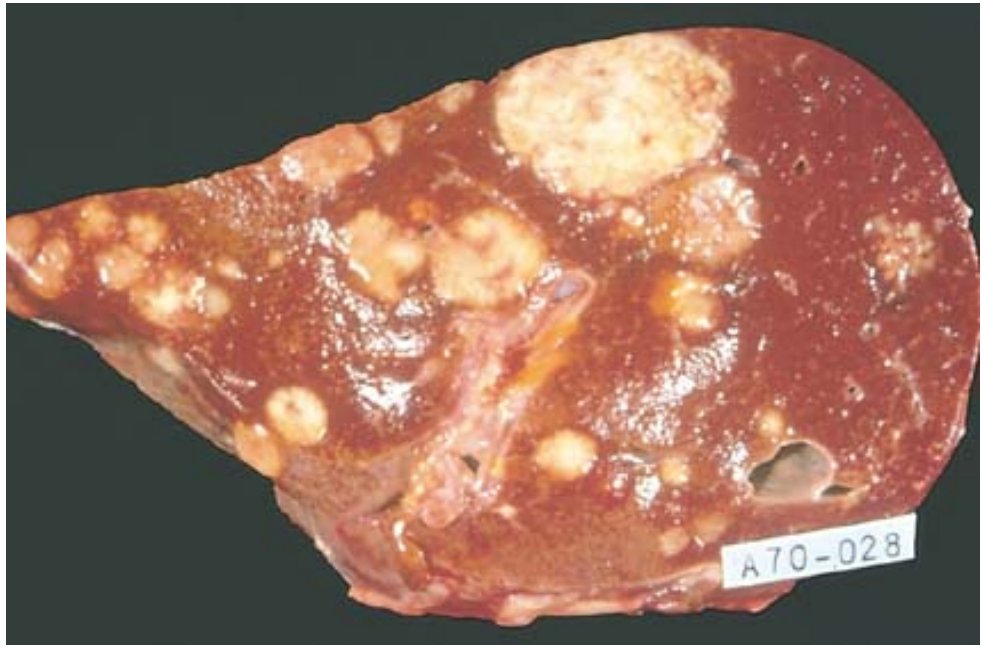

Figure 7-21 Microscopic view of liver metastasis. A pancreatic adenocarcinoma has formed a metastatic nodule in the liver. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallax, TX.)*

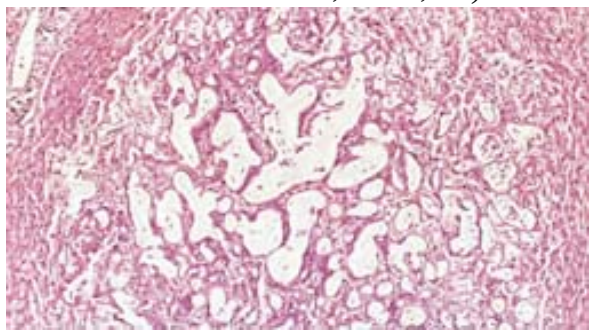

Figure 7-22 Comparison between a benign tumor of the myometrium (leiomyoma) and a malignant tumor of similar origin (leiomyosarcoma).

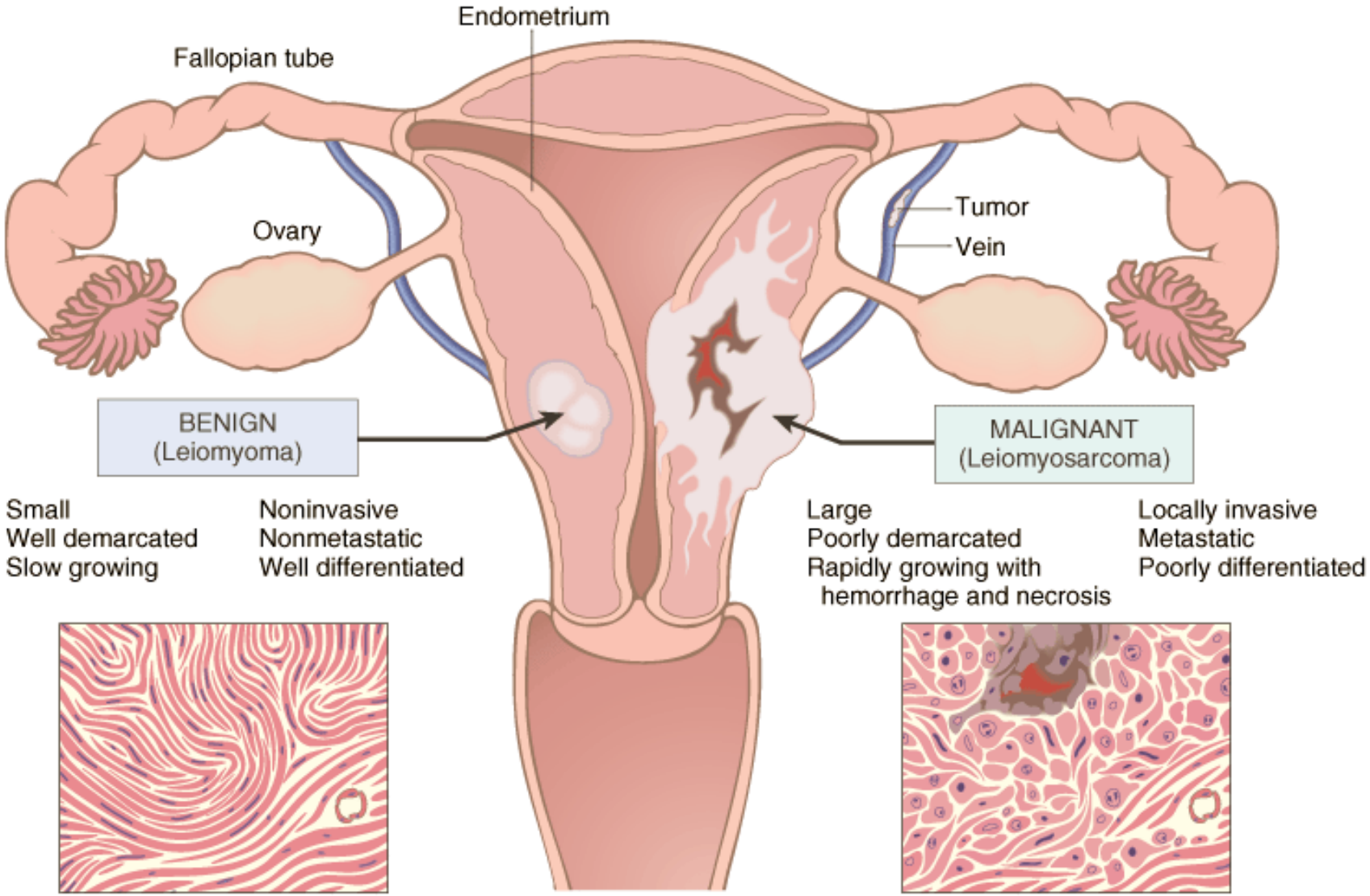

TABLE 7-2 -- Comparisons Between Benign and Malignant Tumors

Characteristics	Benign	Malignant
Differentiation/anaplasia	Well differentiated; structure may be typical of tissue of origin	Some lack of differentiation with anaplasia; structure is often atypical
Rate of growth	Usually progressive and slow; may come to a standstill or regress; mitotic figures are rare and normal	Erratic and may be slow to rapid; mitotic figures may be numerous and abnormal
Local invasion	Usually cohesive and expansile well-demarcated masses that do not invade or infiltrate surrounding normal tissues	Locally invasive, infiltrating the surrounding normal tissues; sometimes may be seemingly cohesive and expansile

Metastasis	Absent	Frequently present; the larger and more undifferentiated the primary, the more likely are metastases

reasons discussed later, they do not indicate the inevitable development of metastases.

The distinguishing features of benign and malignant tumors discussed in this overview are summarized in Table 7-2 and Figure 7-22 . With this background on the structure and behavior of neoplasms, we now discuss the origin of tumors, starting with insights gained from the epidemiology of cancer and followed by the molecular basis of carcinogenesis.

Epidemiology

Because cancer is a disorder of cell growth and behavior, its ultimate cause has to be defined at the cellular and subcellular levels. Study of cancer patterns in populations, however,

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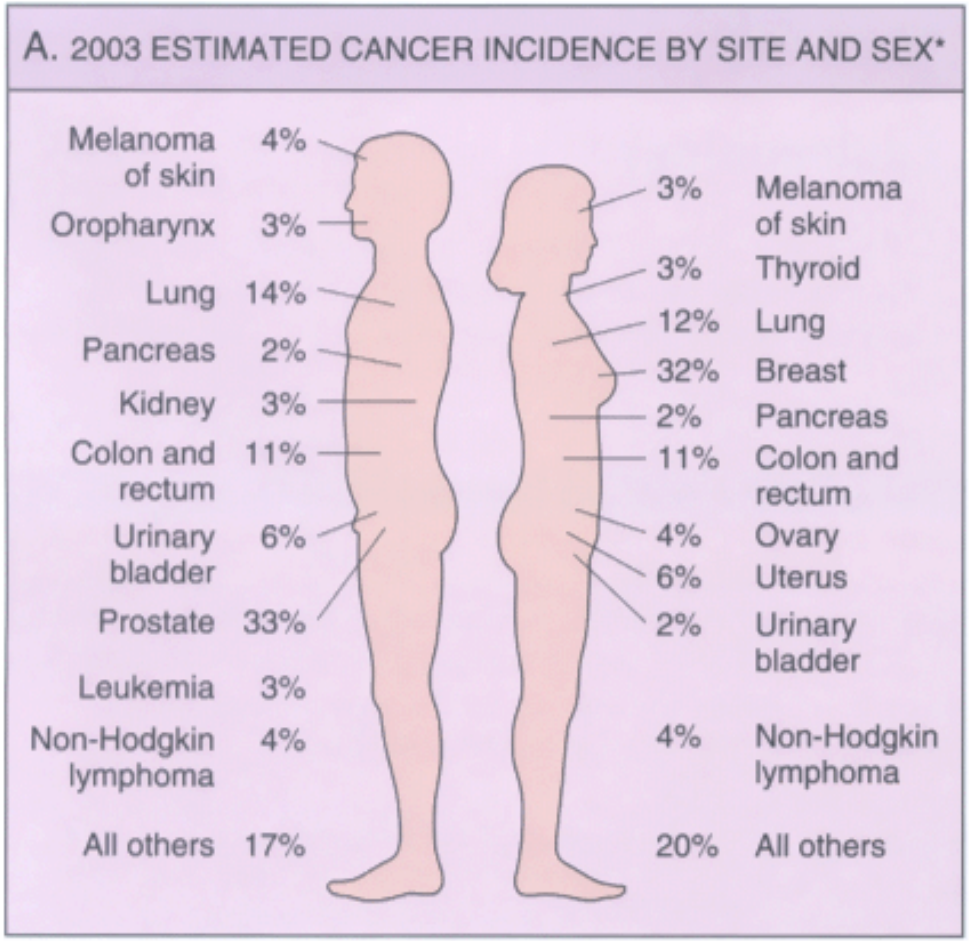
can contribute substantially to knowledge about the origins of cancer. For example, the concept that chemicals can cause cancer arose from the astute observations of Sir Percival Pott, who related the increased incidence of scrotal cancer in chimney sweeps to chronic exposure to soot. Thus, major insights into the cause of cancer can be obtained by epidemiologic studies that relate particular environmental, hereditary, and cultural influences to the occurrence of malignant neoplasms. In addition, certain diseases associated with an increased risk of developing cancer can provide insights into the pathogenesis of malignancy. Therefore, in the following discussion, we first summarize the overall incidence of cancer to provide an insight into the magnitude of the cancer problem, and then review a number of factors relating to both the patient and the environment that influence predisposition to cancer.

CANCER INCIDENCE

In some measure, an individual's likelihood of developing a cancer is expressed by national incidence and mortality rates. For example, residents of the United States have about a one in five chance of dying of cancer. There were, it is estimated, about 556,000 deaths from cancer in 2003, representing 23% of all mortality,[3] a frequency surpassed only by deaths caused by cardiovascular diseases. These data do not include an additional 1 million, for the most part readily curable, non-melanoma cancers of the skin and 100,000 cases of carcinoma in situ, largely of the uterine cervix but also of the breast. The major organ sites affected and the estimated frequency of cancer deaths are shown in Figure 7-23 . The most common tumors in men are prostate, lung, and colorectal cancers. In women, cancers of the breast, lung, and colon and rectum are the most frequent. Cancers of the lung, female breast, prostate, and colon/rectum constitute more than 50% of cancer diagnoses and cancer deaths in the U.S. population.[15]

Figure 7-23 Cancer incidence and mortality by site and sex. Excludes basal cell and squamous cell skin cancers and in situ carcinomas, except urinary bladder. *(Adapted from Jemal A, et al: Cancer statistics, 2003. CA Cancer J Clin 53:5, 2003.)*



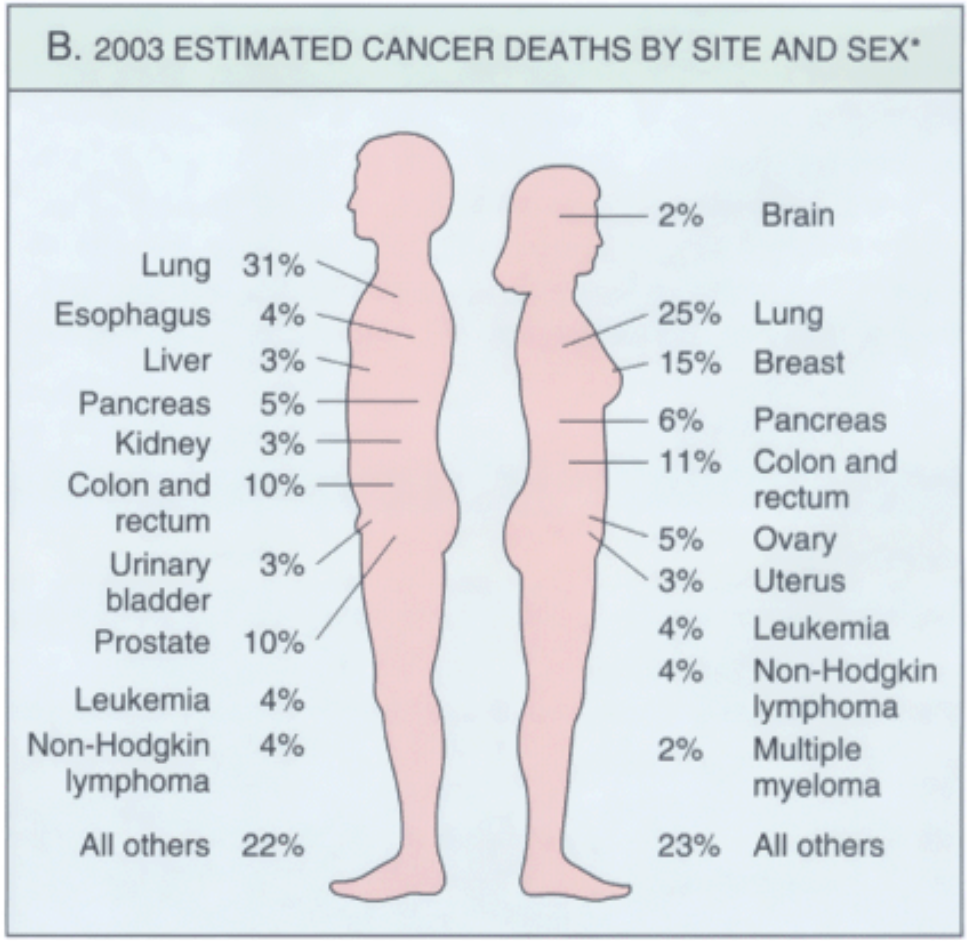
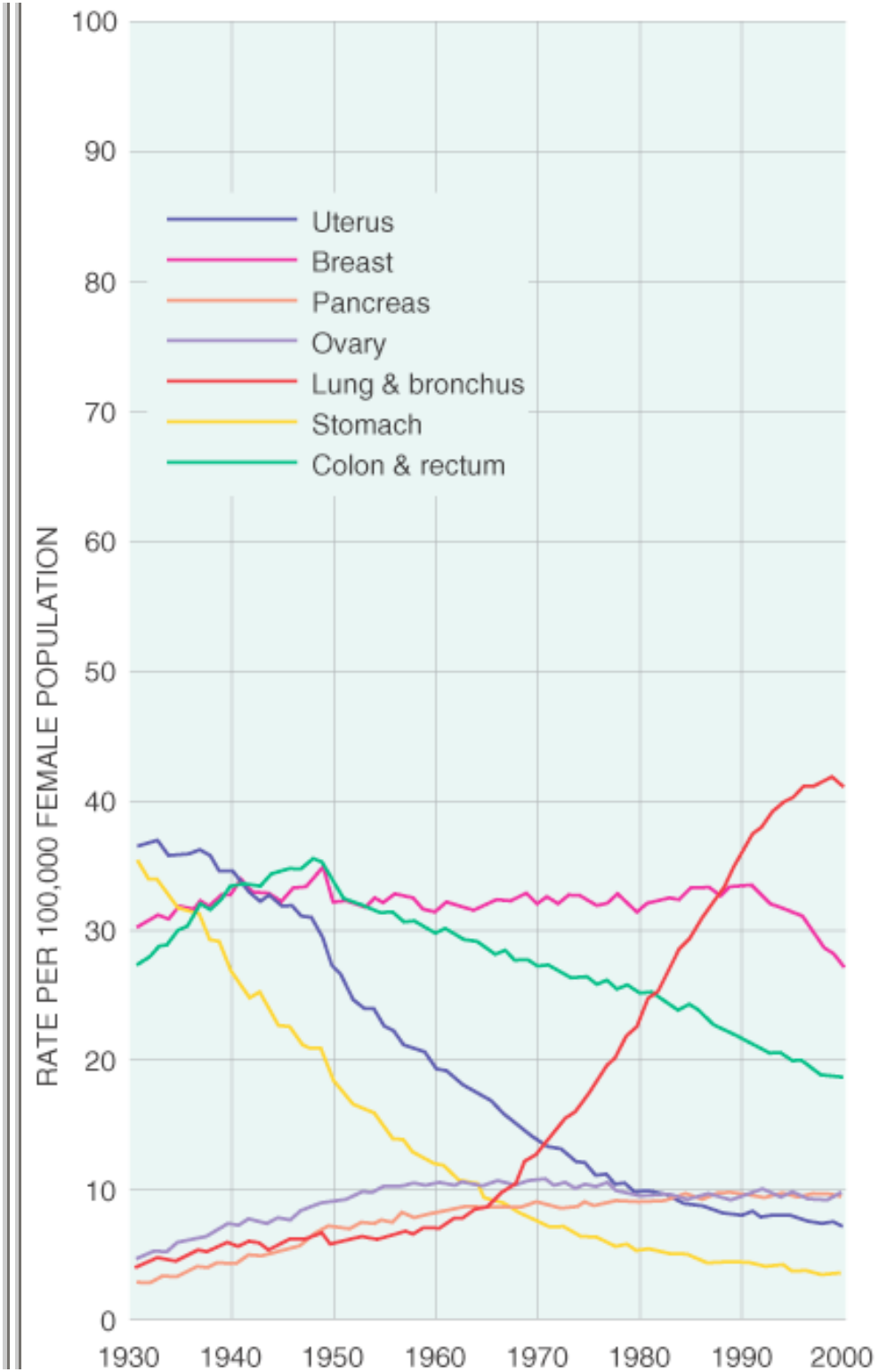
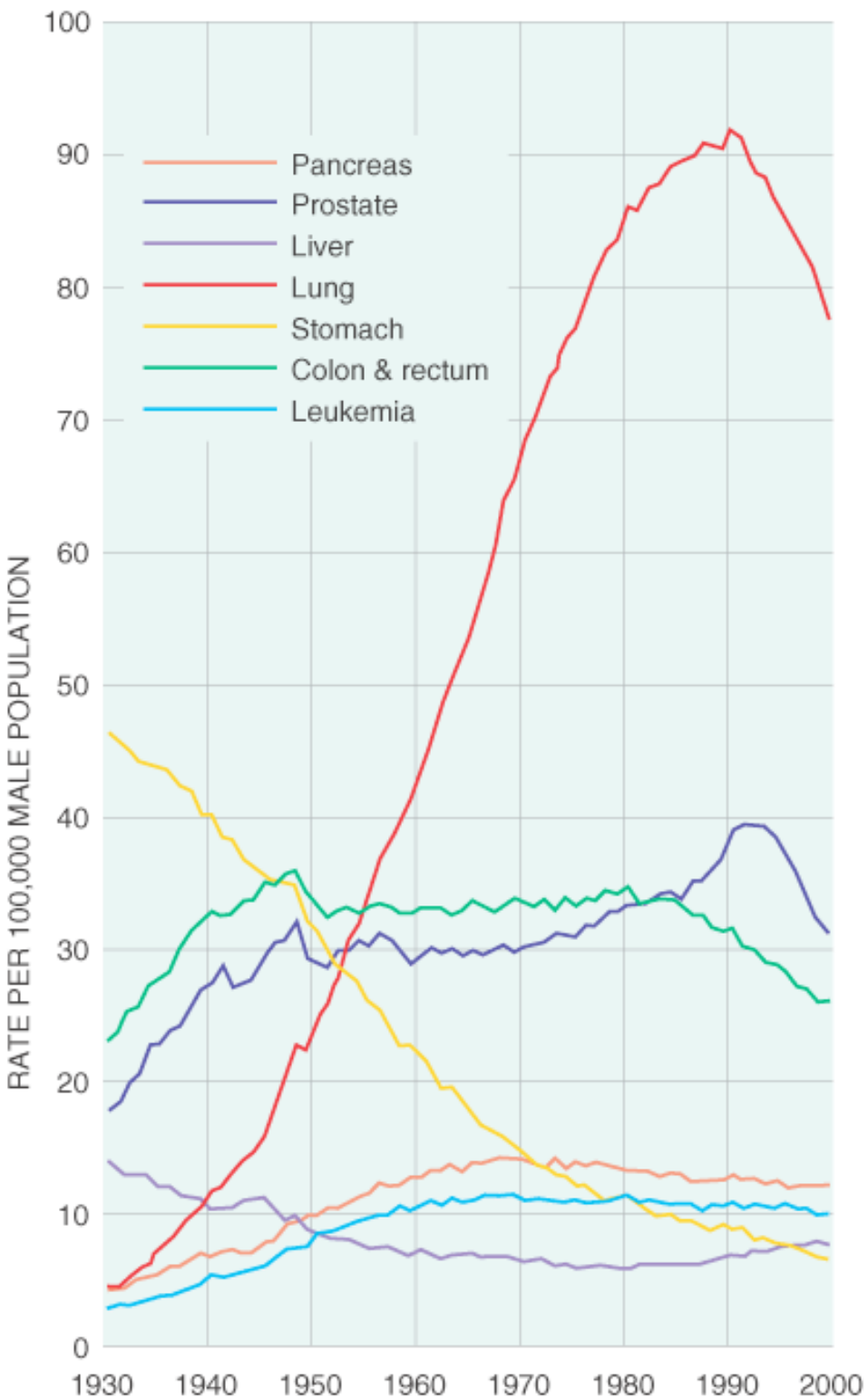


Figure 7-24 Age-adjusted cancer death rates for selected sites in the United States, adjusted for the 2000 U.S. population. *(Adapted from Jemal A, et al: Cancer statistics, 2003. CA Cancer J Clin 53:5, 2003.)*


RATE PER 100,000 FEMALE POPULATION
100
90
80
70
60
50
40
30
20
10
0
1930
1940
1950
1960
1970
1980
1990
2000
Uterus
Breast
Pancreas
Ovary
Lung & bronchus
Stomach
Colon & rectum


RATE PER 100,000 MALE POPULATION
100
90
80
70
60
50
40
30
20
10
0
1930
1940
1950
1960
1970
1980
1990
2000
Pancreas
Prostate
Liver
Lung
Stomach
Colon & rectum
Leukemia

1930 1940 1950 1960 1970 1980 1990 2000 1930 1940 1950 1960 1970 1980 1990 2000

Figure 7-25 The change in incidence of various cancers with migration from Japan to the United States provides evidence that the occurrence of cancers is related to components of the environment that differ in the two countries. The incidence of each kind of cancer is expressed as the ratio of the death rate in the population being considered to that in a hypothetical population of California whites with the same age distribution; the death rates for whites are thus defined as 1. The death rates among immigrants and immigrants' sons tend consistently toward California norms. *(From Cairns J: The cancer problem. In Readings from Scientific American—Cancer Biology. New York, WH Freeman, 1986, p. 13.)*

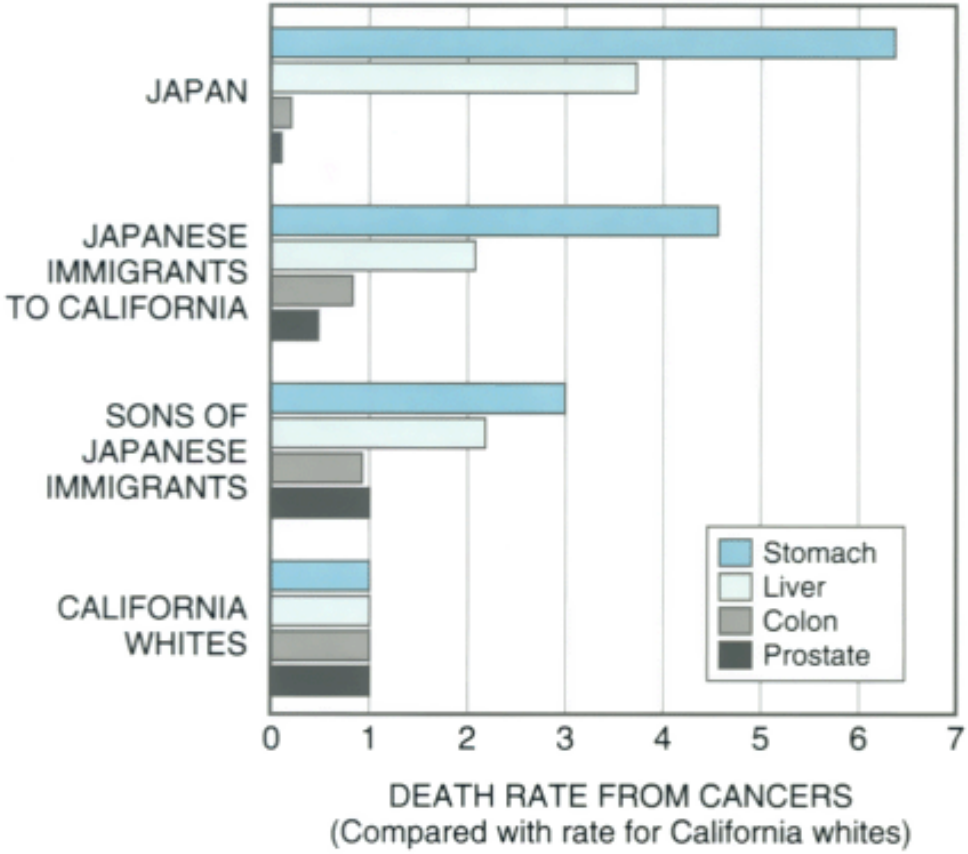


TABLE 7-3 -- Occupational Cancers

Agents or Groups of Agents	Human Cancer Site for Which Reasonable Evidence Is Available	Typical Use or Occurrence
Arsenic and arsenic compounds	Lung, skin, hemangiosarcoma	Byproduct of metal smelting. Component of alloys, electrical and semiconductor devices, medications and herbicides, fungicides, and animal dips
Asbestos	Lung, mesothelioma; gastrointestinal tract (esophagus, stomach, large intestine)	Formerly used for many applications because of fire, heat, and friction resistance; still found in existing construction as well as fire-resistant textiles, friction materials (i.e., brake linings), underlayment and roofing papers, and floor tiles

Benzene	Leukemia, Hodgkin lymphoma	Principal component of light oil. Although use as solvent is discouraged, many applications exist in printing and lithography, paint, rubber, dry cleaning, adhesives and coatings, and detergents. Formerly widely used as solvent and fumigant
Beryllium and beryllium compounds	Lung	Missile fuel and space vehicles. Hardener for lightweight metal alloys, particularly in aerospace applications and nuclear reactors
Cadmium and cadmium compounds	Prostate	Uses include yellow pigments and phosphors. Found in solders. Used in batteries and as alloy and in mental platings and coatings
Chromium compounds	Lung	Component of metal alloys, paints, pigments, and preservatives
Ethylene oxide	Leukemia	Ripening agent for fruits and nuts. Used in rocket propellant and chemical synthesis, in fumigants for foodstuffs and textiles, and in sterilants for hospital equipment
Nickel compounds	Nose, lung	Nickel plating. Component of ferrous alloys, ceramics, and batteries. Byproduct of stainless steel arc welding
Radon and its decay products	Lung	From decay of minerals containing uranium. Can be serious hazard in quarries and underground mines
Vinyl chloride	Angiosarcoma, liver	Refrigerant. Monomer for vinyl polymers. Adhesive for plastics. Formerly inert aerosol propellant in pressurized containers
Modified from Stellman JM, Stellman SD: Cancer and workplace. CA Cancer J Clin 46:70, 1996.		

of the colon and in virtually 100% of cases are fated to develop a carcinoma of the colon by age 50. Other autosomal dominant cancer syndromes are the Li-Fraumeni syndrome resulting from germ line mutations of the *p53* gene, multiple endocrine neoplasia types 1 and 2 (MEN-1 and MEN-2), and hereditary nonpolyposis colon cancer (*HNPCC)*, a condition caused by inactivation of a mismatch repair gene (also listed below among repair defects).

There are several features that characterize inherited cancer syndromes:

- In each syndrome, tumors involve specific sites and tissues, although they may involve more than one site. For example, in MEN-2, caused by a mutation of the *RET* protooncogene, thyroid, parathyroid, and adrenals are involved. There is no increase in predisposition to cancers in general.
- Tumors within this group are often associated with a specific marker phenotype. For example, there may be multiple benign tumors in the affected tissue, as occurs in familial polyposis of the colon and in MEN. Sometimes, there are abnormalities in tissue that are not the target of transformation (e.g., Lisch nodules and café-au-lait spots in neurofibromatosis type 1; see Chapter 5).
- As in other autosomal dominant conditions, both incomplete penetrance and variable expressivity occur.

Defective DNA Repair Syndromes.

Besides the dominantly inherited precancerous conditions, a group of cancerpredisposing conditions is collectively characterized by defects in DNA repair and resultant DNA instability. These conditions generally have an autosomal recessive pattern of inheritance. Included in this group are xeroderma pigmentosum, ataxiatelangectasia, and Bloom syndrome, all rare diseases characterized by genetic instability resulting from defects in DNA repair genes. Also included here is hereditary nonpolypoid colon cancer (HNPCC), an autosomal dominant condition caused by inactivation of a DNA mismatch repair gene.[24] HNPCC is the most common cancer predisposition syndrome, increasing the susceptibility to cancer in the colon and also in some other organs such as the small intestine, endometrium, and ovary (Chapter 17).

Familial Cancers.

Besides the inherited syndromes of cancer susceptibility, cancer may occur at higher frequency in certain families without a clearly defined pattern of transmission. Virtually all the common types of cancers that occur sporadically have also been reported to occur in familial forms. Examples include carcinomas of colon, breast, ovary, and

TABLE 7-4 -- Reported Deaths for the Five Leading Cancer Types for Males by Age, US, 2000

All Ages	Under Age 20	Age 20–39	Age 40–59	Age 60–70	Age 80+
All cancers	***All cancers***	***All cancers***	***All cancers***	***All cancers***	***All cancers***
286,082	1298	4832	50,069	158,990	70,883
Lung and bronchus	Leukemia	Brain and ONS *	Lung and bronchus	Lung and bronchus	Lung and bronchus
90,415	430	626	15,827	57,470	16,626
Prostate	Brain and ONS *	Leukemia	Colon and rectum	Colon and rectum	Prostate
31,078	307	611	4801	15,420	15,630
Colon and rectum	Bones and joints	Lung and bronchus	Pancreas	Prostate	Colon and rectum
28,484	105	481	2929	14,428	7821
Pancreas	Endocrine system	Non-Hodgkin lymphoma	Esophagus	Pancreas	Urinary Bladder
14,238	104	444	2345	8179	3222
Non-Hodgkin lymphoma	Non-Hodgkin lymphoma	Colon and rectum	Liver	Non-Hodgkin lymphoma	Leukemia
11,812	79	431	2308	6107	3187

"All Cancers" excludes in situ carcinomas except urinary bladder.

Source: US Mortality Public Use Data Tape, 2000, National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD 2002.

*ONS = other nervous system.

brain, as well as melanomas. *Features that characterize familial cancers include early age at onset, tumors arising in two or more close relatives of the index case, and sometimes, multiple or bilateral tumors.* Familial cancers are not associated with specific marker phenotypes. For example, in contrast to the familial adenomatous polyp syndrome, familial colonic cancers do not arise in pre-existing benign polyps. The transmission pattern of familial cancers is not clear. In general, siblings have a relative risk between two and three (two to three times greater than unrelated individuals). Segregation analyses of large families usually show that predisposition to the tumors is dominant, but multifactorial inheritance cannot be easily ruled out. It is likely that familial susceptibility to cancer may depend on multiple low-penetrance alleles, each contributing to only a small increase in the risk of tumor development. It has been

estimated that 10% to 20% of patients with breast or ovarian cancer have a first- or second-degree relative with one of these tumors. Although two breast cancer susceptibility genes, named *BRCA1* and *BRCA2*, have been identified, mutation of these genes occurs in no more than 3% of breast cancers. Thus, mutations in *BRCA1* and *BRCA2* cannot account for the large proportion of familial breast cancers.[25] Changes in other genes, probably in low-penetrance susceptibility alleles, appear to be necessary for the development of these tumors. A similar situation occurs in familial melanomas, in which a mutation of the *p16INK4a* tumor suppressor gene has been identified. However, mutation in this gene accounts for only about 20% of familial melanoma kindreds, suggesting that other factors are involved in the familial predisposition.[26]

Interactions Between Genetic and Non-Genetic Factors.

What can be said about the influence of heredity on the majority of malignant neoplasms? It could be argued that they are largely of environmental origin, but lack of family history does not preclude an inherited component. It is generally difficult to sort out the hereditary and acquired basis of a tumor because these factors often interact closely. The interaction between genetic and non-genetic factors is particularly complex when tumor development depends on the action of multiple contributory genes. Even in tumors with a well-defined inherited component, the risk of developing the tumor can be greatly influenced by non-genetic factors. For instance, breast cancer risk in female carriers of BRCA-1 or BRCA-2 mutations is almost three-fold higher for women born after 1940, compared to the risks for women born before that year.[27] [28] Furthermore, the genotype can significantly influence the likelihood of developing environmentally induced cancers. Inherited variations (polymorphisms) of enzymes that metabolize procarcinogens to their active carcinogenic forms (see "Initiation of Carcinogenesis") can influence the susceptibility to cancer. Of interest in this regard are genes that encode the cytochrome P-450 enzymes. As discussed later under "Chemical Carcinogenesis," polymorphism at one of the P-450 loci confers inherited susceptibility to lung cancers in cigarette smokers. More such correlations are likely to be found.

NONHEREDITARY PREDISPOSING CONDITIONS

The only certain way of avoiding cancer is not to be born; to live is to incur the risk. The risk is greater than average, however, under many circumstances, as is evident from the predisposing influences discussed earlier. Certain clinical conditions are also important. Because cell replication is involved in neoplastic transformation, regenerative, hyperplastic, and dysplastic proliferations are fertile soil for the origin of a malignant tumor. There is a well-defined association between certain forms of endometrial hyperplasia and endometrial carcinoma and between cervical dysplasia and cervical carcinoma (Chapter 22). The bronchial mucosal metaplasia and dysplasia of habitual cigarette smokers are ominous

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TABLE 7-5 -- Reported Deaths for the Five Leading Cancer Types for Females by Age, US, 2000

All Ages	Under Age 20	Age 20–39	Age 40–59	Age 60–70	Age 80+
All cancers	***All cancers***	***All cancers***	***All cancers***	***All cancers***	***All cancers***
267,009	973	5617	47,850	131,871	80,697
Lung and bronchus	Leukemia	Breast	Breast	Lung and bronchus	Lung and bronchus
65,016	302	1444	11,937	39,311	14,693
Breast	Brain and ONS *	Uterine cervix	Lung and bronchus	Breast	Colon and rectum
41,872	238	538	10,613	17,842	12,379
Colon and rectum	Endocrine system	Leukemia	Colon and rectum	Colon and rectum	Breast

28,950	82	452	3619	12,612	10,648
Pancreas	Bones and joints	Lung and bronchus	Ovary	Pancreas	Pancreas
15,094	76	397	3033	7825	5319
Ovary	Soft tissue	Brain and ONS *	Pancreas	Ovary	Non-Hodgkin lymphoma
14,060	69	354	1871	7217	4039
"All Cancers" excludes in situ carcinomas except urinary bladder.					
Source: US Mortality Public Use Data Tape, 2000, National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD, 2002.					

*ONS = other nervous system.

antecedents of bronchogenic carcinoma. About 80% of hepatocellular carcinomas arise in cirrhotic livers, which are characterized by active parenchymal regeneration (Chapter 18).

Chronic Inflammation and Cancer.

In 1863 Virchow proposed that *cancer develops at sites of chronic inflammation* and the potential relationships between cancer and inflammation have been studied since then.[29] This is exemplified by the increased risk of cancer development in patients affected by a

TABLE 7-6 -- Inherited Predisposition to Cancer

Inherited Cancer Syndromes (Autosomal Dominant)	
Gene	***Inherited Predisposition***
••*RB*	Retinoblastoma
••*p53*	Li-Fraumeni syndrome (various tumors)
••*p16INK4A*	Melanoma
••*APC*	Familial adenomatous polyposis/colon cancer
••*NF1, NF2*	Neurofibromatosis 1 and 2
••*BRCA1, BRCA2*	Breast and ovarian tumors
••*MEN1, RET*	Multiple endocrine neoplasia 1 and 2
••*MSH2, MLH1, MSH6*	Hereditary nonpolyposis colon cancer
••*PATCH*	Nevoid basal cell carcinoma syndrome
Familial Cancers	
Familial clustering of cases, but role of inherited predisposition not clear for each individual	

••Breast cancer
••Ovarian cancer
••Pancreatic cancer
Inherited Autosomal Recessive Syndromes of Defective DNA Repair
••Xeroderma pigmentosum
••Ataxia-telangiectasia
••Bloom syndrome
••Fanconi anemia

variety of chronic inflammatory diseases of the gastrointestinal tract. These include ulcerative colitis, Crohn disease, *Helicobacter pylori* gastritis, viral hepatitis, and chronic pancreatitis. The precise mechanisms that link inflammation and cancer development have not been established.[30] Chronic inflammatory reactions may result in the production of cytokines, which stimulate the growth of transformed cells. In some cases, chronic inflammation may increase the pool of tissue stem cells, which become subject to the effect of mutagens. Interestingly, chronic inflammation may also directly promote genomic instability in cells through the production of reactive oxygen species (ROS), thus predisposing to malignant transformation. Whatever the precise mechanism, such a link may have practical implications. For instance, expression of the enzyme *cyclooxygenase-2 (COX-2),* which converts arachidonic acid into prostaglandins (Chapter 2), is induced by inflammatory stimuli and is increased in colon cancers and other tumors.[31] The development of COX-2 inhibitors for cancer treatment is an active and promising area of research.[32]

Precancerous Conditions.

Certain non-neoplastic disorders—*the chronic atrophic gastritis of pernicious anemia, solar keratosis of the skin, chronic ulcerative colitis, and leukoplakia of the oral cavity, vulva, and penis*—have such a well-defined association with cancer that they have been termed *precancerous conditions*. This designation is somewhat unfortunate because in the great majority of these lesions no malignant neoplasm emerges. Nonetheless, the term persists because it calls attention to the increased risk. Certain forms of benign neoplasia also constitute precancerous conditions. The villous adenoma of the colon, as it increases in size, develops cancerous change in up to 50% of cases. It might be asked: Is there not a risk with all benign neoplasms? Although some risk may be inherent, a large cumulative experience indicates that most benign neoplasms do not become cancerous. Nonetheless, numerous examples could be offered of cancers arising, albeit rarely, in benign tumors; for example, a leiomyosarcoma beginning in a leiomyoma, and carcinoma appearing in longstanding pleomorphic adenomas. Generalization is impossible

because each type of benign neoplasm is associated with a particular level of risk ranging from virtually never to frequently. Only follow-up studies of large series of each neoplasm can establish the level of risk, and always the question remains: Did the cancer arise from a non-malignant cell in the benign tumor or did the benign tumor contain, from the outset, a silent or indolent malignant focus?

Molecular Basis of Cancer

The literature on the molecular basis of cancer continues to proliferate at such a rapid pace that it is easy to get lost in the growing forest of information. We list some fundamental principles before delving into the details of the molecular basis of cancer.

• *Nonlethal genetic damage lies at the heart of carcinogenesis.* Such genetic damage (or mutation) may be acquired by the action of environmental agents, such as chemicals, radiation, or viruses, or it may be inherited in the germ line. The term "environmental," used in this context, involves any acquired defect caused by exogenous agents or endogenous products of cell metabolism. Not all mutations, however, are "environmentally" induced. Some may be spontaneous and stochastic.
• *A tumor is formed by the clonal expansion of a single precursor cell that has incurred the genetic damage (i.e., tumors are monoclonal).* Clonality of tumors can be assessed in women who are heterozygous for polymorphic X-linked markers, such as the enzymes glucose-6-phosphate dehydrogenase (G6PD), iduronate-2-sulfatase and phosphoglycerate kinase. The principle underlying such an analysis is illustrated in Figure 7-26 . The most commonly used method to determine tumor clonality involves the analysis of methylation patterns adjacent to the highly polymorphic locus of the human androgen receptor gene *(HUMARA)*.[33] The frequency of *HUMARA* polymorphism in the general population is more than 90%, so it is easy to establish clonality by showing that all the cells in a tumor express the same allele. For tumors with a specific translocation, such as in myeloid leukemias, the presence of the translocation can be used to assess clonality. Immunoglobulin receptor and T-cell receptor gene rearrangements serve as markers of clonality in B- and T-cell lymphomas, respectively.
• *Four classes of normal regulatory genes—the growth-promoting protooncogenes, the growth-inhibiting tumor suppressor genes, genes that regulate programmed cell death (apoptosis), and genes involved in DNA repair—are the principal targets of genetic damage.* Mutant alleles of protooncogenes are considered dominant because they transform cells despite the presence of a normal counterpart. In contrast, both normal alleles of the tumor suppressor genes must be damaged for transformation to occur, so this family of genes is sometimes referred to as *recessive oncogenes*. However, there are exceptions to this rule, and some tumor suppressor genes lose their suppressor activity when a single allele is lost or inactivated.[34] This loss of function of a recessive gene caused by damage of a single allele is called *haploinsufficiency*. Genes that regulate apoptosis may be dominant, as are protooncogenes, or they may behave as tumor suppressor genes.
• *DNA repair genes affect cell proliferation or survival indirectly by influencing the ability of the organism to repair nonlethal damage in other genes, including protooncogenes, tumor suppressor genes, and genes that regulate apoptosis.* A disability in the DNA repair genes can predispose to mutations in the genome and *hence to neoplastic transformation*. Such propensity to mutations is called a *mutator phenotype*. [35] With some exceptions, both alleles of DNA repair genes must be inactivated to induce such genomic instability; in this sense, DNA repair genes may also be considered as tumor suppressor genes.
• *Carcinogenesis is a multistep process at both the phenotypic and the genetic levels.* A malignant neoplasm has several phenotypic attributes, such as excessive growth, local invasiveness, and the ability to form distant metastases. These characteristics are acquired in a stepwise fashion, a phenomenon called *tumor progression*. At the molecular level, progression results from accumulation of genetic

lesions that in some instances are favored by defects in DNA repair.

Figure 7-26 Diagram depicting the use of X-linked isoenzyme cell markers as evidence of the monoclonality of neoplasms. Because of random X inactivation, all females are mosaics with two cell populations (with G6PD isoenzyme A or B in this case). When neoplasms that arise in women who are heterozygous for X-linked markers are analyzed, they are made up of cells that contain the active maternal (X_A) or the paternal (X_B) X chromosome but not both.

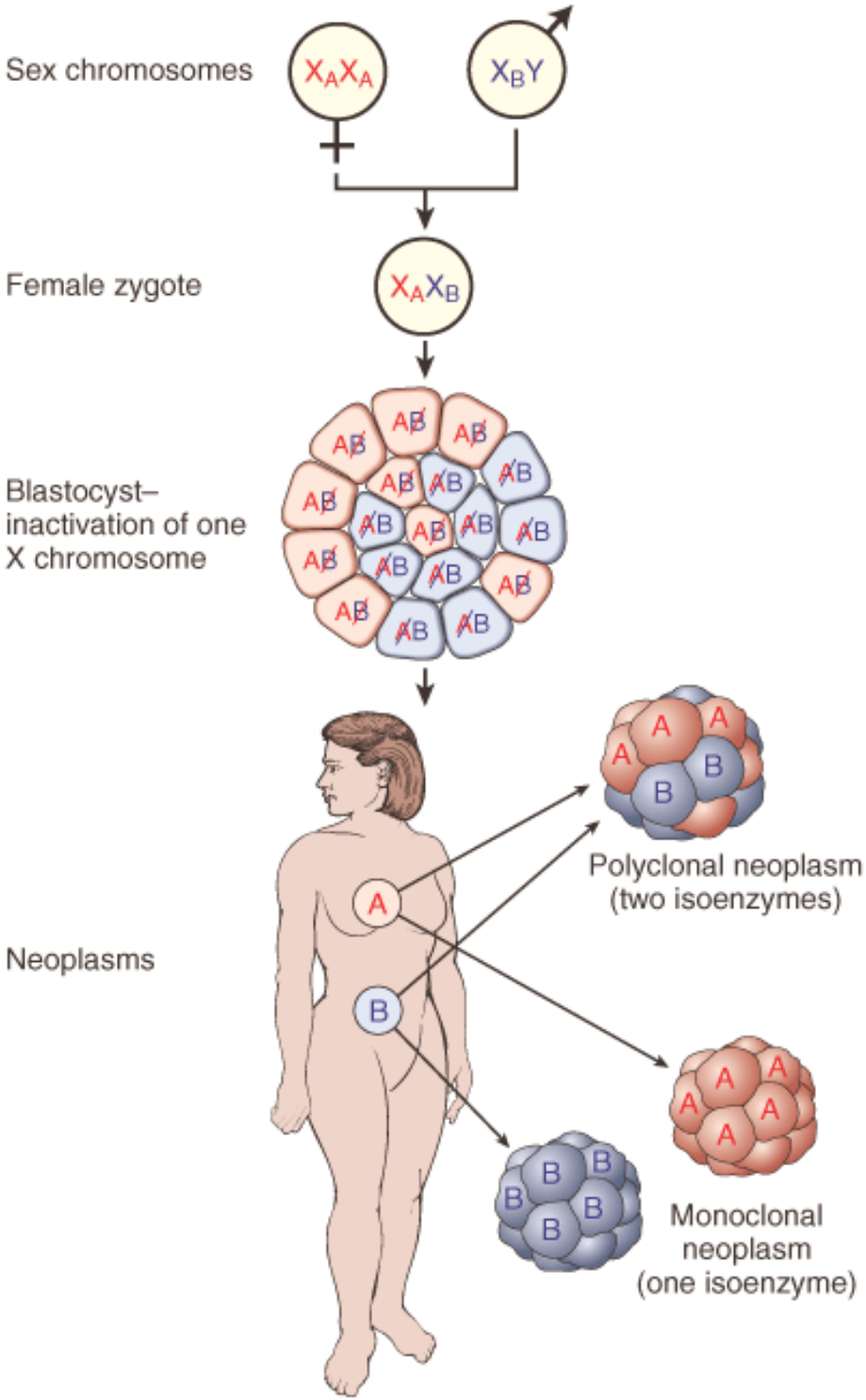
Sex chromosomes
X_AX_A
X_BY
Female zygote
X_AX_B
Blastocyst–
inactivation of one
X chromosome
Neoplasms
A
B
Polyclonal neoplasm
(two isoenzymes)
Monoclonal
neoplasm
(one isoenzyme)

Figure 7-27 Flow chart depicting a simplified scheme of the molecular basis of cancer.

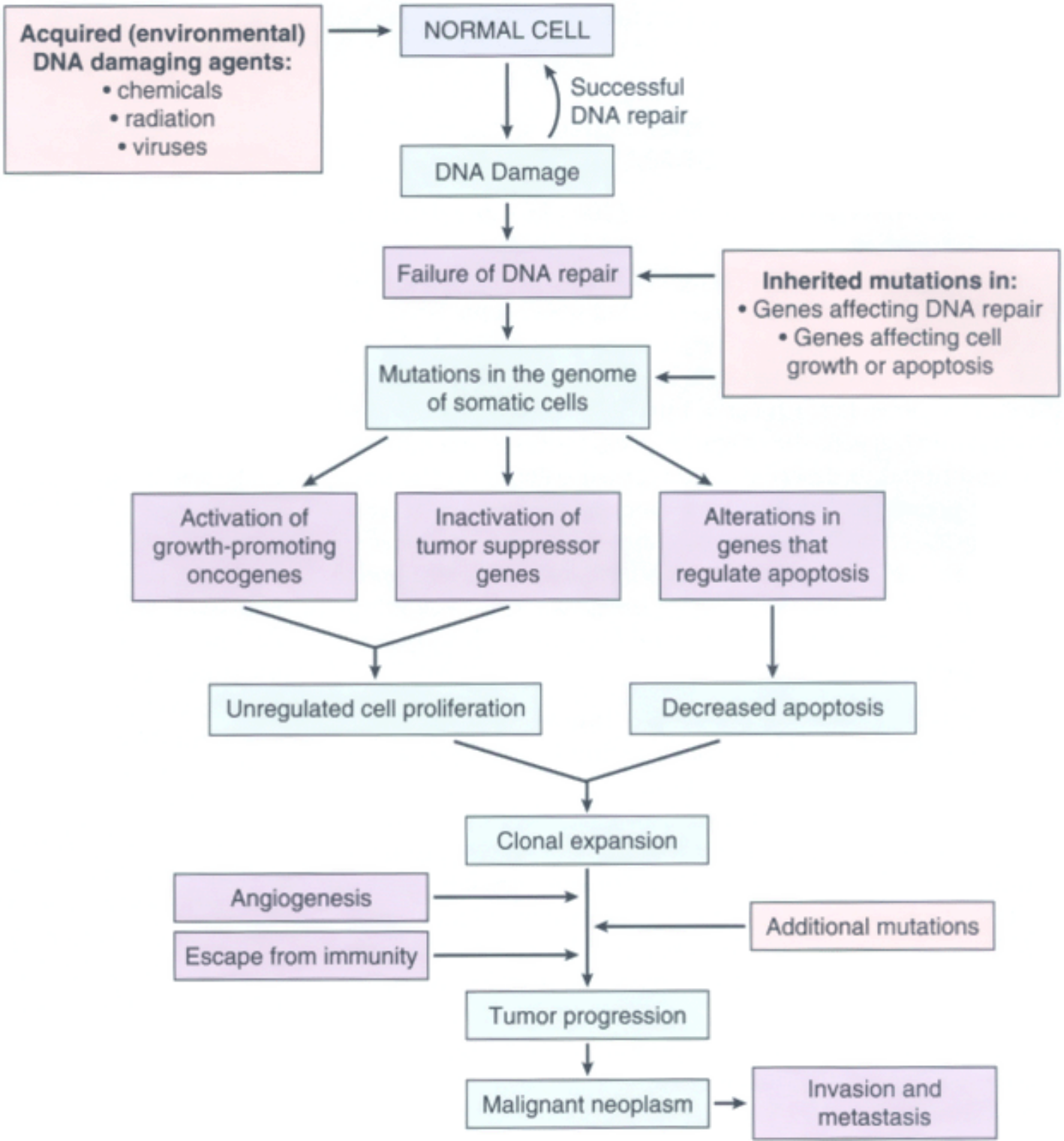

Figure 7-28 Expression of cyclin-cyclin-dependent kinase (CDK) complexes during the cell cycle. The phases of the cycle are indicated inside the arrows. *(Modified from Pollard TD, Earnshaw WC: Cell Biology. Philadelphia, WB Saunders, 2002.)*

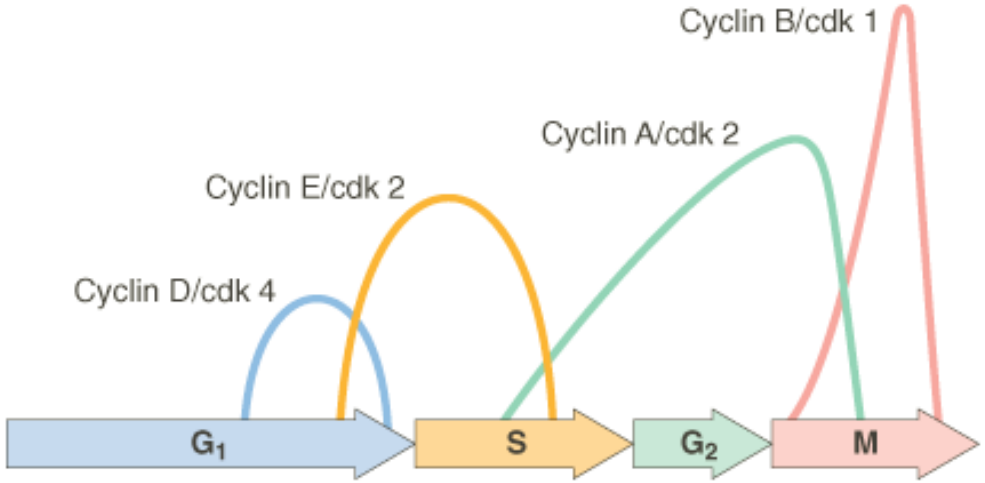

Figure 7-29 Schematic illustration of the role of cyclins, CDKs, and cyclin-dependent kinase inhibitors in regulating the G_1 /S cell-cycle transition. External signals activate multiple signal transduction pathways, including those involving the *MYC* and *RAS* genes, which lead to synthesis and stabilization of cyclin D (there are several D cyclins, but, for simplification, we refer to them as "cyclin D"). Cyclin D binds to CDK4, forming a complex with enzymatic activity (cyclin D can also bind to CDK6, which appears to have a similar role as CDK4). The cyclin D-CDK4 complex phosphorylates RB, located in the E2F/DP1/RB complex in the nucleus, activating the transcriptional activity of E2F (E2F is a family of transcription factors, which we refer to as "E2F"), which leads to transcription of cyclin E, cyclin A and other proteins needed for the cell to go through the late G_1 restriction point. The cell cycle can be blocked by the Cip/Kip inhibitors p21 and p27 *(red boxes)* and the INK4A/ARF inhibitors p16INK4A and p14ARF *(green boxes)*. Cell-cycle arrest in response to DNA damage and other cellular stresses is mediated through p53. The levels of p53 are under negative regulation by MDM2, through a feedback loop that is inhibited by p14ARF.

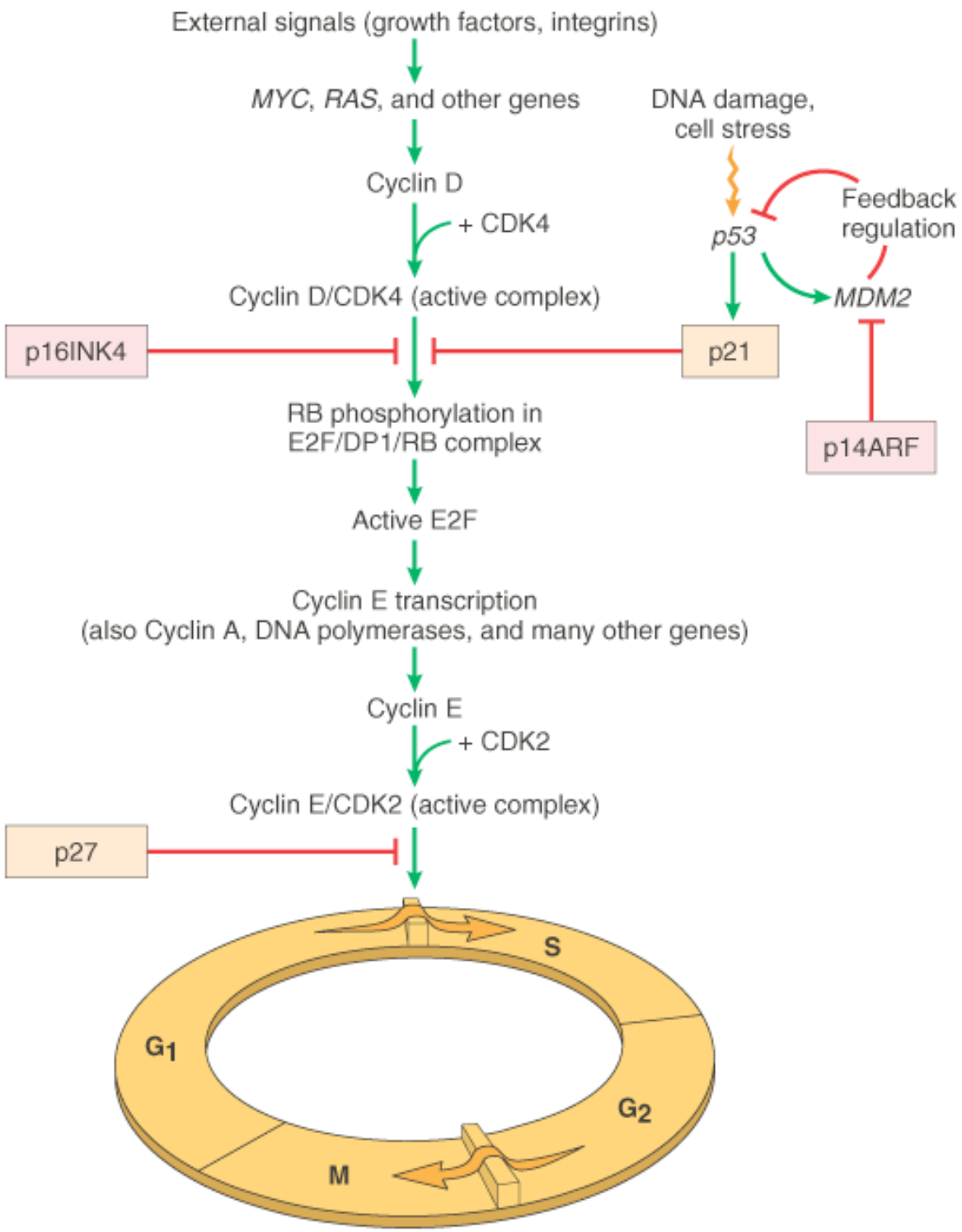
External signals (growth factors, integrins)
MYC, RAS, and other genes
DNA damage, cell stress
Cyclin D
+ CDK4
p53
Feedback regulation
Cyclin D/CDK4 (active complex)
MDM2
p16INK4
p21
RB phosphorylation in E2F/DP1/RB complex
p14ARF
Active E2F
Cyclin E transcription (also Cyclin A, DNA polymerases, and many other genes)
Cyclin E
+ CDK2
Cyclin E/CDK2 (active complex)
p27
S
G1
G2
M

Figure 7-30 Mechanism of cell-cycle regulation by RB. In a resting cell, RB is a component of the E2F/DP1/RB complex, which represses gene transcription through the recruitment of histone deacetylase, an enzyme that alters the conformation of chromatin, making it more compact. Phosphorylation of RB by cyclin D-CDK4 removes histone deacetylase from chromatin, allowing the activation of E2F transcriptional activity (RB can also be phosphorylated by cyclin E-CDK2). E2F-mediated transcription of cyclins E and A, and of genes required for DNA replication, permit the passage through the G_1 restriction point. *(Adapted from Pollard TD, Earnshaw WC: Cell Biology. Philadelphia, WB Saunders, 2002, p. 689.)*

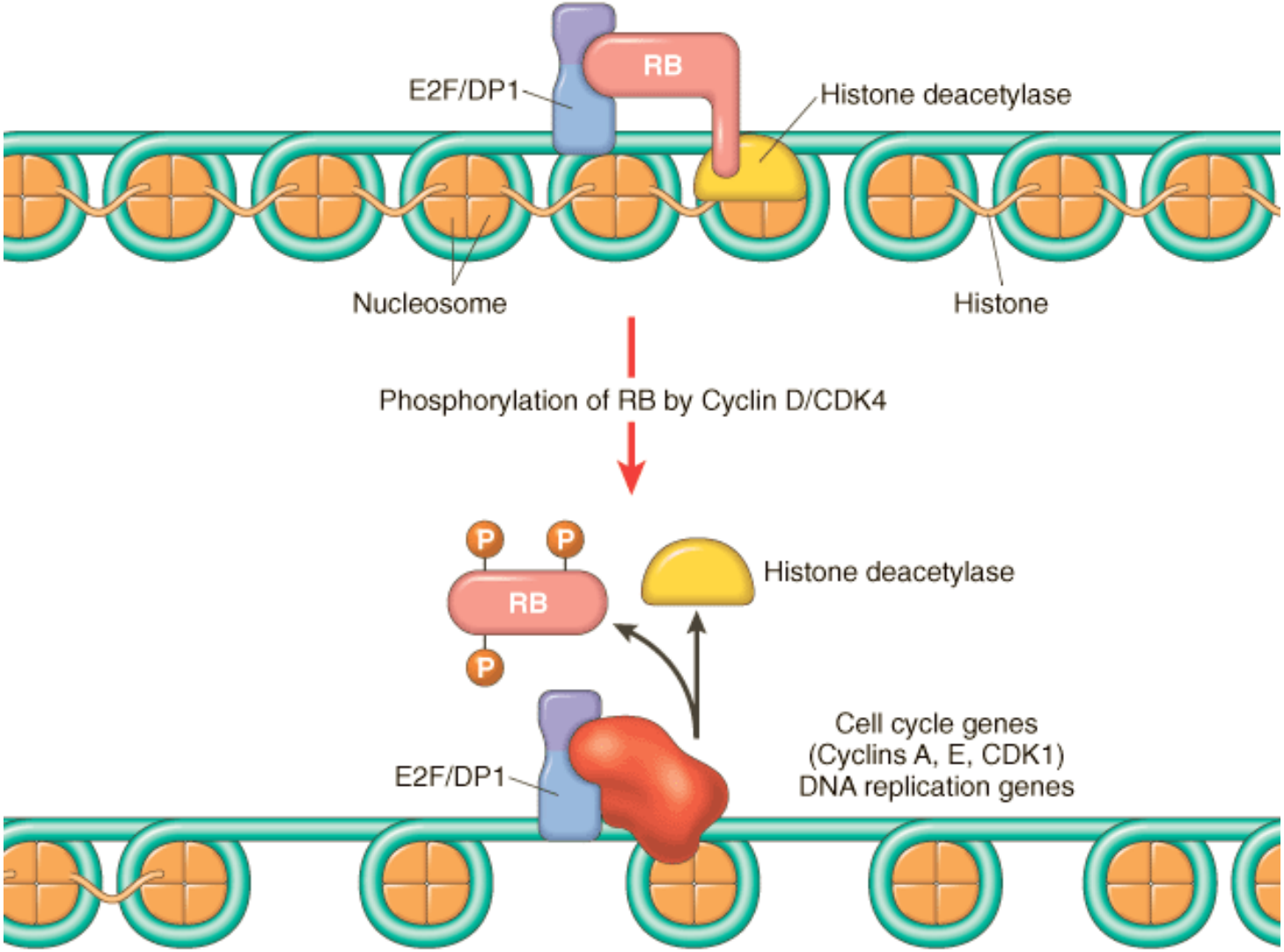

TABLE 7-7 -- Main Cell-Cycle Components and Their Inhibitors

Cell-Cycle Component	Main Function
Cyclin-Dependent Kinases	
• CDK4	Forms a complex with cyclin D. The complex phosphorylates RB, allowing the cell to progress through the G_1 restriction point.

• CDK2	Forms a complex with cyclin E in late G_1 , which is involved in the G_1 /S transition. Forms a complex with cyclin A at the S phase that facilitates the G_2 /M transition.
• CDK1	Forms a complex with cyclin B, which acts on the G_2 /M transition.
Inhibitors	
• Cip/Kip family: p21, p27	Block the cell cycle by binding to cyclin-CDK complexes. p21 is induced by the tumor suppressor *p53*. p27 responds to growth suppressors such as transforming growth factor-β.
• 1NK4/ARF family: p16INK4A, p14ARF	p16INK4a binds to cyclin D-CDK4 and promotes the inhibitory effects of RB. p14ARF increases p53 levels by inhibiting MDM2 activity.
Checkpoint Components	
• p53	Tumor suppressor altered in the majority of cancers; causes cell-cycle arrest and apoptosis. Acts mainly through p21 to cause cell-cycle arrest. Causes apoptosis by inducing the transcription of pro-apoptotic genes such as *BAX*. Levels of p53 are negatively regulated by MDM2 through a feedback loop. p53 is required for the G_1 /S checkpoint and is a main component of the G_2 /M checkpoint.
• Ataxia-telangiectasia mutated (*ATM*)	Activated by mechanisms that sense double stranded DNA breaks. Transmits signals to arrest the cell cycle after DNA damage. Acts through p53 in the G_1 /S checkpoint. At the G_2 /M checkpoint, it acts both through p53-dependent mechanisms and through the inactivation of CDC25 phosphatase, which disrupts the cyclin B-CDK1 complex. Component of a network of genes that include *BRCA1* and *BRCA2*, which link DNA damage with cell-cycle arrest and apoptosis.

three components, p21, p27, and p57, which bind to and inactivate the complexes formed between cyclins and CDKs. Transcriptional activation of p21 is under the control of *p53, a tumor suppressor gene that is mutated in a large proportion of human cancers*. The main role of p53 in the cell cycle is one of surveillance, triggering checkpoint controls that slow down or stop cell-cycle progression of damaged cells, or causes apoptosis. The human *INK4a/ARF* locus (a notation for "*in*hibitor of *k*inase *4/a*lternative *r*eading *f*rame") encodes two proteins, p16INK4a and p14ARF, which block the cell cycle and act as tumor suppressors. p16INK4a competes with cyclin D for binding to CDK4 and inhibits the ability of the cyclin D-CDK4 complex to phosphorylate RB, thus causing cell-cycle arrest at late G_1 . It is frequently mutated or inactivated by hypermethylation (discussed later) in human cancers. The *INK4a* locus encodes a second gene product, p14ARF (p19ARF in mice), which acts on p53. p14ARF arises from an alternative reading of the *INK4a* gene, providing for an "economical" way to utilize gene-coding sequences.[42] Although both p16INK4a and p14ARF block the cell cycle, their targets are different; p16INK4a acts on cyclin D-CDK4, whereas p14ARF prevents p53 degradation.

Cell-Cycle Checkpoints.

The cell cycle has its own internal controls, called *checkpoints*. There are two main checkpoints, one at the G_1 /S transition and another at G_2 /M.[43] [44] The S phase is the point of no return in the cell cycle, and before a cell makes the final commitment to replicate, the G_1 /S checkpoint checks for DNA damage. If DNA damage is present, the DNA repair machinery and mechanisms that arrest the cell cycle are put in motion. The delay in cell-cycle progression provides the time needed for DNA repair; if the damage is not repairable, apoptotic pathways are activated to kill the cell. Thus, the G_1 /S checkpoint prevents the replication of cells that have defects in DNA, which would be perpetuated as mutations or chromosomal breaks in the progeny of the cell. DNA damaged after its replication can still be repaired as long as the chromatids have not separated. The G_2 /M checkpoint monitors the completion of DNA replication and checks whether the cell can safely initiate mitosis and separate sister chromatids. This checkpoint is particularly important in cells exposed to ionizing radiation. Cells damaged by ionizing radiation activate the G_2 /M checkpoint and arrest in G_2 ; defects in this checkpoint give rise to chromosomal abnormalities. To function properly, cell-cycle checkpoints require sensors of DNA damage, signal transducers, and effector molecules. [44] The sensors and transducers of DNA damage appear to be similar for the G_1 /S and G_2 /M checkpoints. They include, as sensors, proteins of the RAD family and ataxia telangiectasia mutated (ATM) and as transducers, the CHK kinase families. The checkpoint effector

molecules differ, depending on the cell-cycle stage at which they act. In the G_1 /S checkpoint, cell-cycle arrest is mostly mediated through p53, which induces the cell-cycle inhibitor p21. Arrest of the cell cycle by the G_2 /M checkpoint involves both p53-dependent and independent mechanisms. *Defect in cell-cycle checkpoint components is a major cause of genetic instability in cancer cells.*

With this background on the cell cycle and its control, we now proceed to discuss the genes that determine the malignant phenotype. This discussion will take place in the context of the seven fundamental changes in cell physiology (listed earlier) that are the hallmarks of malignant cells.

SELF-SUFFICIENCY IN GROWTH SIGNALS: ONCOGENES

Genes that promote autonomous cell growth in cancer cells are called *oncogenes*, and their normal cellular counterparts are called *protooncogenes*. Protooncogenes are physiologic regulators of cell proliferation and differentiation; oncogenes are characterized by the ability to promote cell growth in the

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absence of normal mitogenic signals. Their products, called *oncoproteins*, resemble the normal products of protooncogenes with the exception that oncoproteins are devoid of important regulatory elements. Their production in the transformed cells becomes constitutive, that is, not dependent on growth factors or other external signals. To aid in the understanding of the nature and functions of oncoproteins, and their role in cancer, it is necessary to briefly mention the sequential steps that characterize normal cell proliferation. Under physiologic conditions, cell proliferation can be readily resolved into the following steps:

- The binding of a growth factor to its specific receptor generally located on the cell membrane
- Transient and limited activation of the growth factor receptor, which, in turn, activates several signal-transducing proteins on the inner leaflet of the plasma membrane
- Transmission of the transduced signal across the cytosol to the nucleus via second messengers or by signal transduction molecules that directly activate transcription
- Induction and activation of nuclear regulatory factors that initiate DNA transcription
- Entry and progression of the cell into the cell cycle, ultimately resulting in cell division

With this background, we can readily identify the strategies used by cancer cells to acquire self-sufficiency in growth signals. They can be grouped on the basis of their role in growth factor-mediated signal transduction cascades and cell-cycle regulation. We start with a description of oncogenes and their protein products, and how these were discovered.

Protooncogenes, Oncogenes, and Oncoproteins

As often happens in science, the discovery of protooncogenes was not straightforward. These cellular genes were first discovered in their mutated or "oncogenic" forms as "passengers" within the genome of *acute transforming retroviruses* by the 1989 Nobel laureates Harold Varmus and Michael Bishop. These retroviruses cause rapid induction of tumors in animals and can also transform animal cells in vitro. Molecular dissection of their genomes revealed the presence of unique transforming sequences (viral oncogenes [v-*onc*]) not found in the genomes of nontransforming retroviruses. Most surprisingly, molecular hybridization revealed that the v-*onc* sequences were almost identical to sequences found in normal cellular DNA. From this evolved the concept that during evolution, cellular oncogenes were *transduced* (captured) by the virus through a chance recombination with the DNA of a (normal) host cell that had been infected by the virus. Because they were discovered initially as *viral genes*, these protooncogenes were named after their viral homologues. Each v-*onc* is designated by a three-letter word that relates the oncogene to the virus from which it was isolated. Thus, the v-*onc* contained in *fe*line sarcoma virus is referred to as v-*FES*, whereas the oncogene in *si*mian sarcoma virus is called v-*SIS*. The corresponding protooncogenes are referred to as *FES* and *SIS*, dropping the prefix.

The viral oncogenes are not present in several cancer-causing RNA viruses. One such example is a group of so-called slow transforming viruses that cause leukemias in rodents after a long latent period. The mechanism by which they cause neoplastic transformation implicates protooncogenes. Molecular dissection of the cells transformed by these leukemia viruses revealed

that the proviral DNA is always integrated (inserted) near a protooncogene. One consequence of proviral insertion near a protooncogene is to induce a structural change in the cellular gene, thus converting it into a cellular oncogene (c-*onc*, or *onc*). This mode of protooncogene activation is called *insertional mutagenesis*. Alternatively, strong retroviral promoters inserted in the vicinity of the protooncogenes lead to dysregulated expression of the cellular gene.

Although the study of transforming animal retroviruses provided the first glimpse of protooncogenes, these investigations did not explain the origin of human tumors, which (with rare exceptions) are not caused by infection with retroviruses. Hence the question was raised: Do nonviral tumors contain oncogenic DNA sequences? The answer was provided by experiments involving DNA-mediated gene transfer (DNA transfection). When DNA extracted from several different human tumors was transfected into mouse fibroblast cell lines in vitro, the recipient cells acquired some properties of neoplastic cells. The conclusion from such experiments was inescapable: DNA of spontaneously arising cancers contains oncogenic sequences, or oncogenes. One of the first oncogenic sequences detected in cancers was a mutated form of the *RAS* protooncogene. This protooncogene is the forbear of v-*oncs* contained in Harvey (H) and Kirsten (K) sarcoma viruses.

A large number of protooncogenes have been identified during the past 20 years, most of which do not have a viral counterpart. Protooncogenes have multiple roles, participating in cellular functions related to growth and proliferation. Proteins encoded by protooncogenes may function as growth factor ligands and receptors, signal transducers, transcription factors, and cell-cycle components (Fig. 7-31). Oncoproteins encoded by oncogenes generally serve similar functions as their normal counterparts (Table 7-8). However, because they are constitutively expressed, *oncoproteins endow the cell with self-sufficiency in growth*.[45]

To summarize, protooncogenes may be converted into cellular oncogenes (c-oncs) that are involved in tumor development. Two questions follow: (1) What are the functions of oncogene products, the oncoproteins? (2) How do the normally "civilized" protooncogenes turn into "enemies within"? These issues are discussed below.

Growth Factors.

Many cancer cells develop growth self-sufficiency by acquiring the ability to synthesize the same growth factors to which they are responsive. The protooncogene *SIS*, which encodes the β chain of platelet-derived growth factor (PDGF), is overproduced in many tumors, especially low-grade astrocytomas and osteosarcomas. Furthermore, it appears that the same tumors also express receptors for PDGF and are hence responsive to autocrine stimulation. Although an autocrine loop is considered to be an important element in the pathogenesis of several tumors, in most instances the growth factor gene itself is not altered or mutated. More commonly, products of other oncogenes such as *RAS* (that lie along many signal transduction pathways) cause overexpression of growth factor genes, thus forcing the cells to secrete large amounts of growth factors, such as transforming growth factor-α (TGF-α). This growth factor is related to epidermal growth factor (EGF) and induces proliferation by binding to the EGF receptor. TGF-α is often

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Figure 7-31 Subcellular localization and functions of major classes of cancer-associated genes. The protooncogenes are colored red, cancer suppressor genes blue, DNA repair genes green, and genes that regulate apoptosis purple.

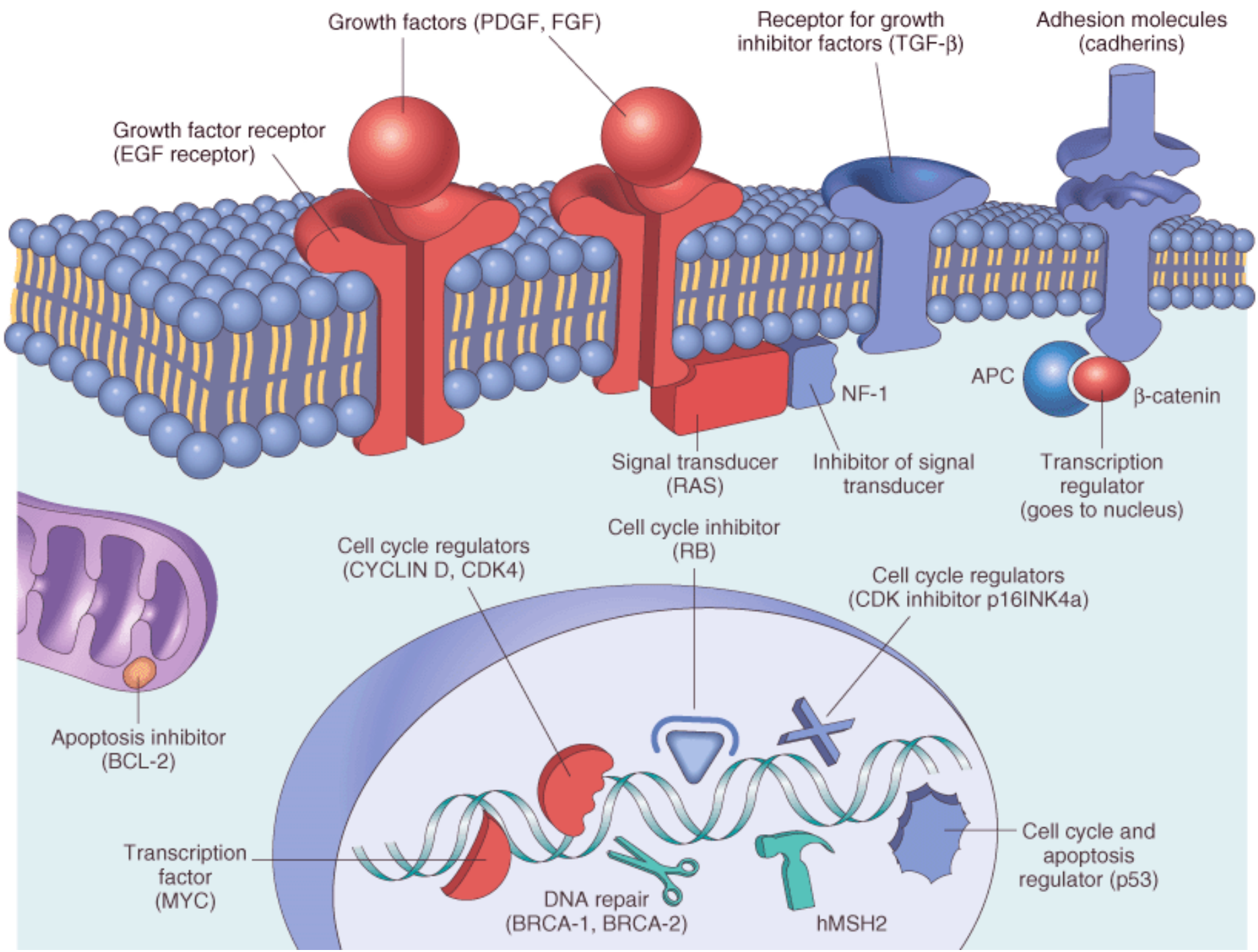

TABLE 7-8 -- Selected Oncogenes, Their Mode of Activation, and Associated Human Tumors

Category	Protooncogene	Mode of Activation	Associated Human Tumor
Growth Factors			
PDGF-β chain	*SIS*	Overexpression	Astrocytome
			Osteosarcoma
Fibroblast growth factors	*HST-1*	Overexpression	Stomach cancer
	INT-2	Amplification	Bladder cancer
			Breast cancer
			Melanoma
TGFα	TGFα	Overexpression	Astrocytomas
			Hepatocellular carcinomas
HGF	HGF	Overexpression	Thyroid cancer
Growth Factor Receptors			
EGF-receptor family	*ERB-B1 (ECFR)*	Overexpression	Squamous cell carcinomas of lung, gliomas
	ERB-B2	Amplification	Breast and ovarian cancers
CSF-1 receptor	*FMS*	Point mutation	Leukemia
Receptor for neurotrophic factors	*RET*	Point mutation	Multiple endocrine neoplasia 2A and B, familial medullary thyroid carcinomas
PDGF receptor	*PDGF-R*	Overexpression	Gliomas
Receptor for stem cell (steel) factor	*KIT*	Point mutation	Gastrointestinal stromal tumors and other soft tissue tumors
Proteins Involved in Signal Transduction			
GTP-binding	*K-RAS*	Point mutation	Colon, lung, and pancreatic tumors
	H-RAS	Point mutation	Bladder and kidney tumors
	N-RAS	Point mutation	Melanomas, hematologic malignancies
Nonreceptor tyrosine kinase	*ABL*	Translocation	Chronic myeloid leukemia
			Acute lymphoblastic leukemia
RAS signal transduction	*BRAF*	Point mutation	Melanomas
WNT signal transduction	β-*catenin*	Point mutation	Hepatoblastomas, hepatocellular carcinoma
		Overexpression	
Nuclear Regulatory Proteins			
Transcriptional activators	*C-MYC*	Translocation	Burkitt lymphoma

	N-MYC	Amplification	Neuroblastoma, small cell carcinoma of lung
	L-MYC	Amplification	Small cell carcinoma of lung
Cell-Cycle Regulators			
Cyclins	*CYCLIN D*	Translocation	Mantle cell lymphoma
		Amplification	Breast and esophageal cancers
	CYCLIN E	Overexpression	Breast cancer
Cyclin-dependent kinase	*CDK4*	Amplification or point mutation	Glioblastoma, melanoma, sarcoma

domain alter the substrate specificity of the tyrosine kinase and lead to thyroid and adrenal tumors but no involvement of the parathyroid. Complete loss of RET function results in Hirschsprung disease (Chapter 17), in which there is lack of development of intestinal nerve plexuses. In all these familial conditions, the affected individuals inherit the *RET* mutation in the germ line. Sporadic medullary carcinomas of the thyroid are associated with somatic rearrangements of the *RET* gene, generally similar to those found in MEN 2B.[46] [47]

Oncogenic conversions by mutations and rearrangements have been found in other growth factor receptor genes. Point mutations that activate c-*FMS*, the gene encoding the colony-stimulating factor 1 (CSF-1) receptor, have been detected in myeloid leukemias. In certain chronic myelomonocytic leukemias with the t(12;9) translocation, the entire cytoplasmic domain of the PDGF receptor is fused with a segment of the ETS family transcription factor, resulting in permanent dimerization of the PDGF receptor.

Far more common than mutations of these protooncogenes is overexpression of normal forms of growth factor receptors. In sporadic papillary thyroid carcinomas, c-MET is overexpressed in almost every case.[48] In these tumors, increased expression of c-MET is not caused by gene mutation but results from enhanced transcription of the gene. In some tumors, increased receptor expression results from gene amplification, but in many cases, the molecular basis of

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increased receptor expression is not fully known. Two members of the EGF receptor family are most commonly involved. The normal form of *ERB B1*, the EGF receptor gene, usually referred to as *EGFR*, is overexpressed in up to 80% of squamous cell carcinomas of the lung, in 50% or more of high-grade astrocytomas called *glioblastomas* (Chapter 28), in 80% to 100% of head and neck tumors, and less commonly, in carcinomas of the urinary bladder and the gastrointestinal tract.[49] [50] In contrast, the *ERB B2* gene (also called *HER 2/Neu*), the second member of the EGF receptor family, is amplified in approximately 25% of breast cancers and in human adenocarcinomas arising within the ovary, lung, stomach, and salivary glands.[51] Because the molecular alteration in *ERB B2* is specific for the cancer cells, new therapeutic agents consisting of monoclonal antibodies against ERB B2 have been developed and are currently in use clinically.[49] [51] This type of therapy, directed to a specific alteration in the cancer cell, is called *targeted therapy*. [52] Another example of very successful targeted cancer therapy is the blockage of receptor tyrosine kinase activity of c-KIT in stromal tumors of the gastrointestinal tract.[53] In these tumors, a mutation in c-*KIT*, the gene encoding the receptor for stem cell factor (also known as *steel factor*), constitutively activates the receptor tyrosine kinase, independent of ligand binding.

Signal-Transducing Proteins.

Several examples of oncoproteins that mimic the function of normal cytoplasmic signal-transducing proteins have been found. Most such proteins are strategically located on the inner leaflet of the plasma membrane, where they receive signals from outside the cell (e.g., by activation of growth factor receptors) and transmit them to the cell's nucleus. Biochemically, the signal-transducing proteins are heterogeneous. *The best and most well studied example of a signal-transducing oncoprotein is the RAS family of guanine triphosphate (GTP)-binding proteins (G proteins).*

The RAS Oncogene.

The RAS proteins were discovered as products of viral oncogenes. *Point mutation of RAS family genes is the single most common abnormality of dominant oncogenes in human tumors.* Approximately 15% to 20% of all human tumors contain mutated versions of RAS proteins.[54] Several distinct mutations of *RAS* have been identified in cancer cells, all of which dramatically reduce the GTPase activity of the RAS proteins. The mutations generally involve codons 12, 59, or 61 of *HRAS, KRAS,* and *NRAS*. The frequency of such mutations varies with different tumors, but in some types it is very high. For example, 90% of pancreatic adenocarcinomas and cholangiocarcinomas contain a *RAS* point mutation, as do about 50% of colon, endometrial, and thyroid cancers and 30% of lung adenocarcinomas and myeloid leukemias.[55] [56] [57] In general, carcinomas (particularly from colon and pancreas) have mutations of *KRAS*, bladder tumors have *HRAS* mutations, and hematopoietic tumors bear *NRAS* mutations. *RAS* mutations are infrequent in certain other cancers, particularly those arising in the uterine cervix or breast.

Several studies indicate that RAS plays an important role in mitogenesis induced by growth factors. For example, blockade of RAS function by microinjection of specific antibodies blocks the proliferative response to EGF, PDGF, and CSF-1. Normal RAS proteins are tethered to the cytoplasmic aspect of the plasma membrane, and they flip back and forth between an activated, signal-transmitting form and an inactive, quiescent state. Recently it was found that these proteins may also be found in the endoplasmic reticulum and Golgi membranes, where they can be activated by growth factor binding to the plasma membrane, through a still-uncertain mechanism. [58] In the inactive state, RAS proteins bind guanosine diphosphate (GDP); when cells are stimulated by growth factors or other receptor-ligand interactions, RAS becomes activated by exchanging GDP for GTP (Fig. 7-32). Activated RAS, in turn, acts on the MAP kinase pathway by recruiting the cytosolic protein RAF-1. The MAP kinases so activated target nuclear transcription factors and thus promote mitogenesis. In normal cells, the activated signal-transmitting stage of the RAS protein is transient because its intrinsic GTPase activity hydrolyzes GTP to GDP, thereby returning RAS to its quiescent ground state (described below).

The orderly cycling of the RAS protein depends on two reactions: (1) nucleotide exchange (GDP by GTP), which activates RAS protein, and (2) GTP hydrolysis, which converts the GTP-bound, active RAS to the GDP-bound, inactive form. Both these processes are enzymatically regulated. The removal of GDP and its replacement by GTP during RAS activation are catalyzed by a family of guanine nucleotide-releasing proteins that are recruited to the cytosolic domain of activated growth factor receptors by adapter proteins. More importantly, the GTPase activity intrinsic to normal RAS proteins is dramatically accelerated by *GTPase-activating proteins (GAPs).* These widely distributed proteins bind to the active RAS and augment its GTPase activity by more than 1000-fold, leading to rapid hydrolysis of GTP to GDP and termination of signal transduction. Thus, GAPs function as "brakes" that prevent uncontrolled RAS activity. The response to this braking action of GAPs seems to falter when mutations affect the *RAS* gene. *Mutant RAS proteins bind GAP, but their GTPase activity fails to be augmented.* Hence the mutant proteins are "trapped" in their excited GTP-bound form, causing, in turn, a pathologic activation of the mitogenic signaling pathway. The importance of GTPase activation in normal growth control is underscored by the fact that a disabling mutation of neurofibromin *(NF-1),* a GTPase-activating protein, is also associated with neoplasia (see discussion of tumor suppressor genes below).

In addition to RAS, other members of the RAS signaling cascade (RAS/RAF/MAP kinase) may also be altered in cancer cells. Thus, mutations in *BRAF*, one of the members of the *RAF* family, have been detected in more than 60% of melanomas and in more than 80% of benign nevi.[59] [60] This suggests that dysregulation of the RAS/RAF/MAP kinase pathway may be one of the initiating events in the development of melanomas, although it is not sufficient by itself to cause tumorigenesis.

Recent studies have revealed that, in addition to its role in transducing growth factor signals, RAS is also involved in regulation of the cell cycle. As described above, the passage of cells from G_1 to the S phase is modulated by cyclins and CDKs. RAS proteins can indirectly regulate the levels of cyclins by activating the MAP kinase pathway and the AP-1 transcription factor.

Because *RAS* is so frequently mutated in human cancers, much effort has been spent to develop anti-RAS modalities of targeted therapy. Several such strategies for cancer treatment are being evaluated. The specific targets include blockade of

Figure 7-32 Model for action of *RAS* genes. When a normal cell is stimulated through a growth factor receptor, inactive (GDP-bound) RAS is activated to a GTP-bound state. Activated RAS recruits RAF and stimulates the MAP-kinase pathway to transmit growth-promoting signals to the nucleus. The mutant RAS protein is permanently activated because of inability to hydrolyze GTP, leading to continuous stimulation of cells without any external trigger. The anchoring of RAS to the cell membrane by the farnesyl moiety is essential for its action.

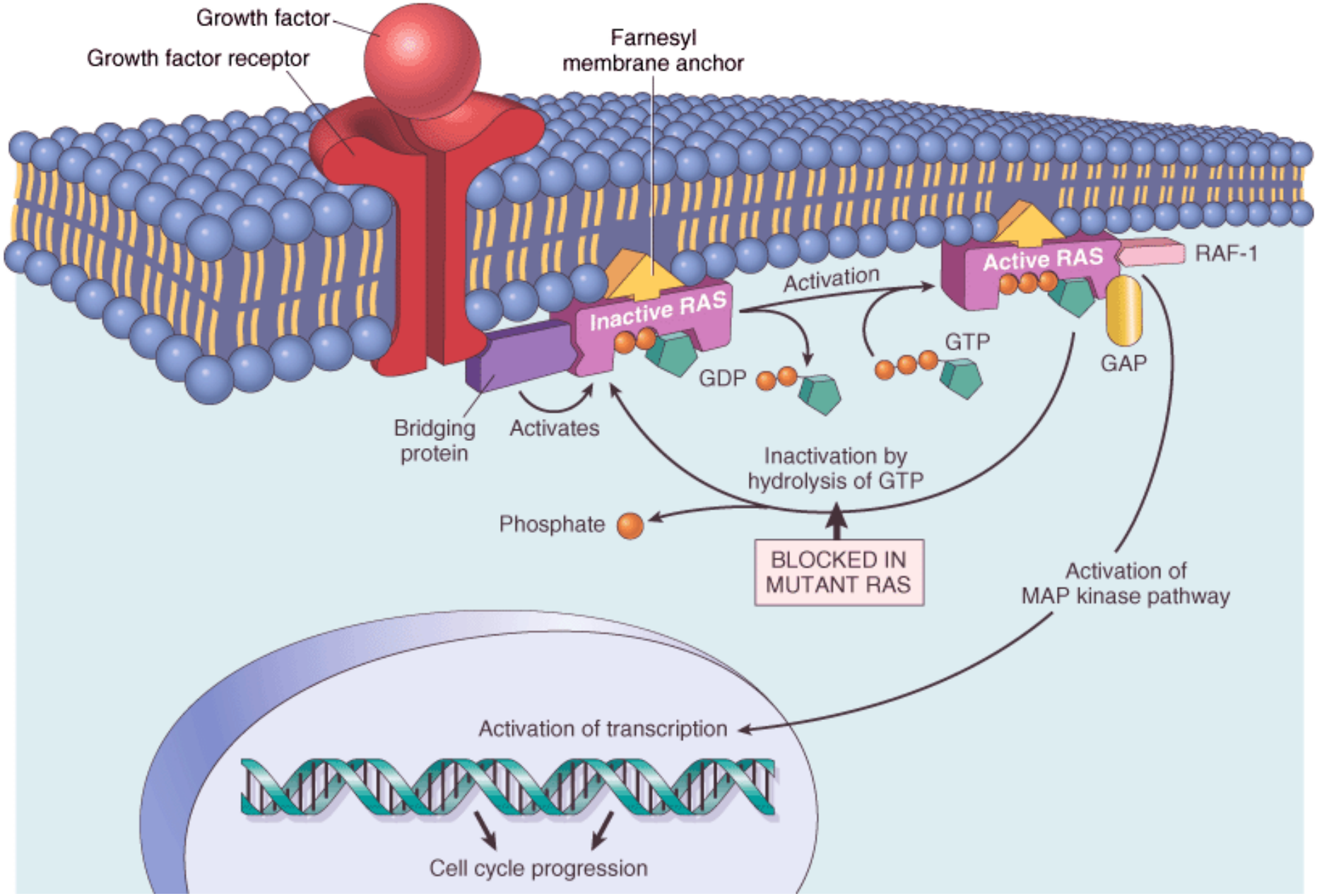
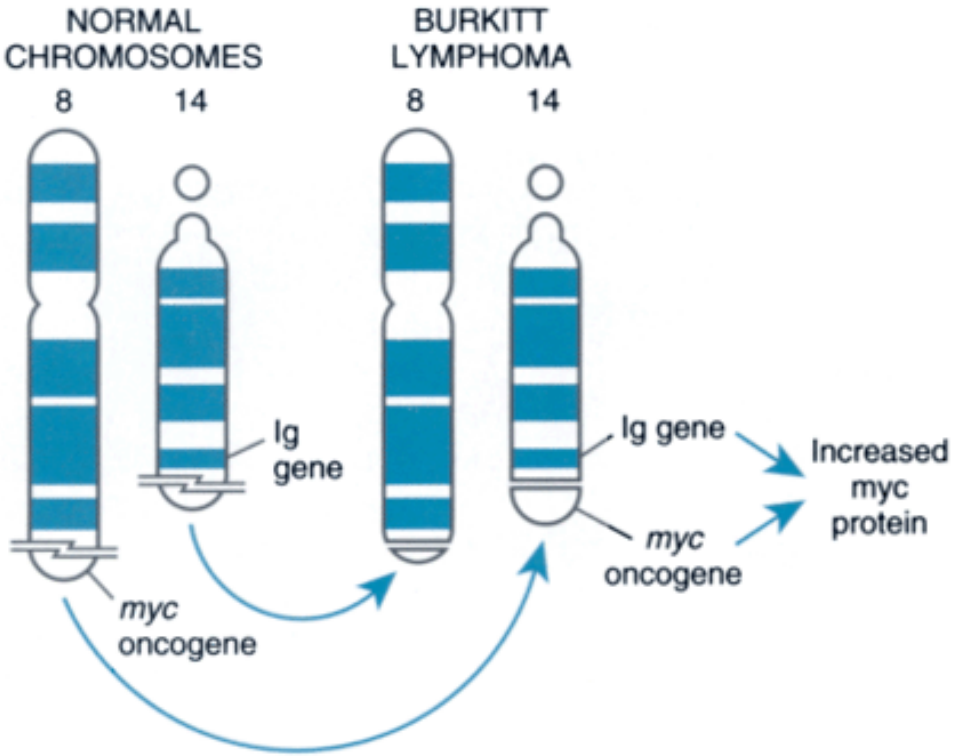


Figure 7-33 The chromosomal translocation and associated oncogenes in Burkitt lymphoma and chronic myelogenous leukemia.



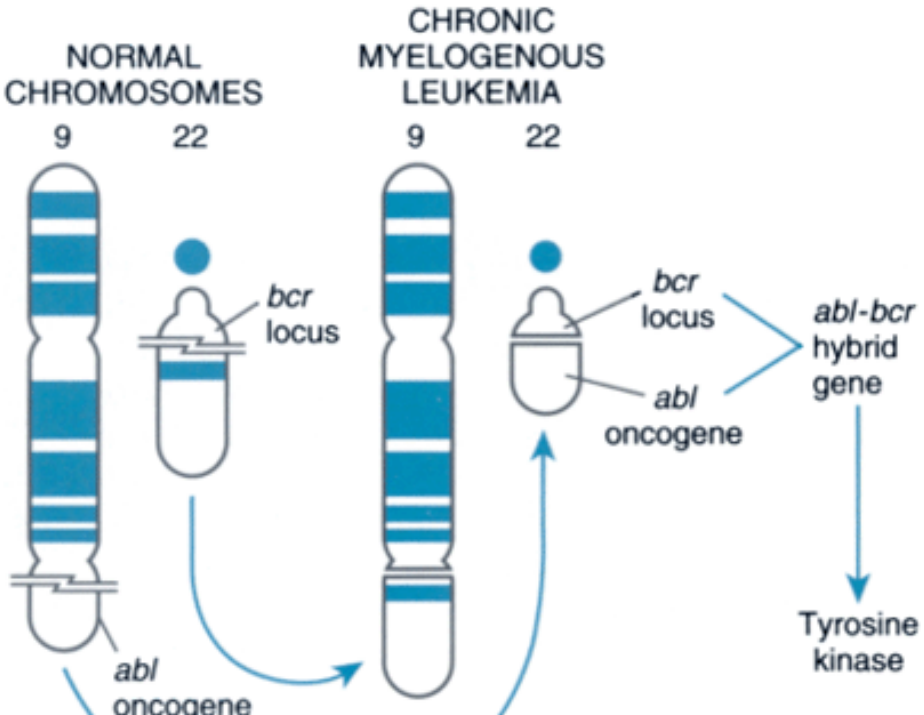


Figure 7-34 Amplification of the N-*MYC* gene in human neuroblastomas. The N-*MYC* gene, normally present on chromosome 2p, becomes amplified and is seen either as extra chromosomal double minutes or as a chromosomally integrated, homogeneous staining region. The integration involves other autosomes, such as 4, 9, or 13. *(Modified from Brodeur GM: Molecular correlates of cytogenetic abnormalities in human cancer cells: implications for oncogene activation. In Brown EB (ed): Progress in Hematology, Vol 14. Orlando, FL, Grune & Stratton, 1986, pp. 229–256.)*

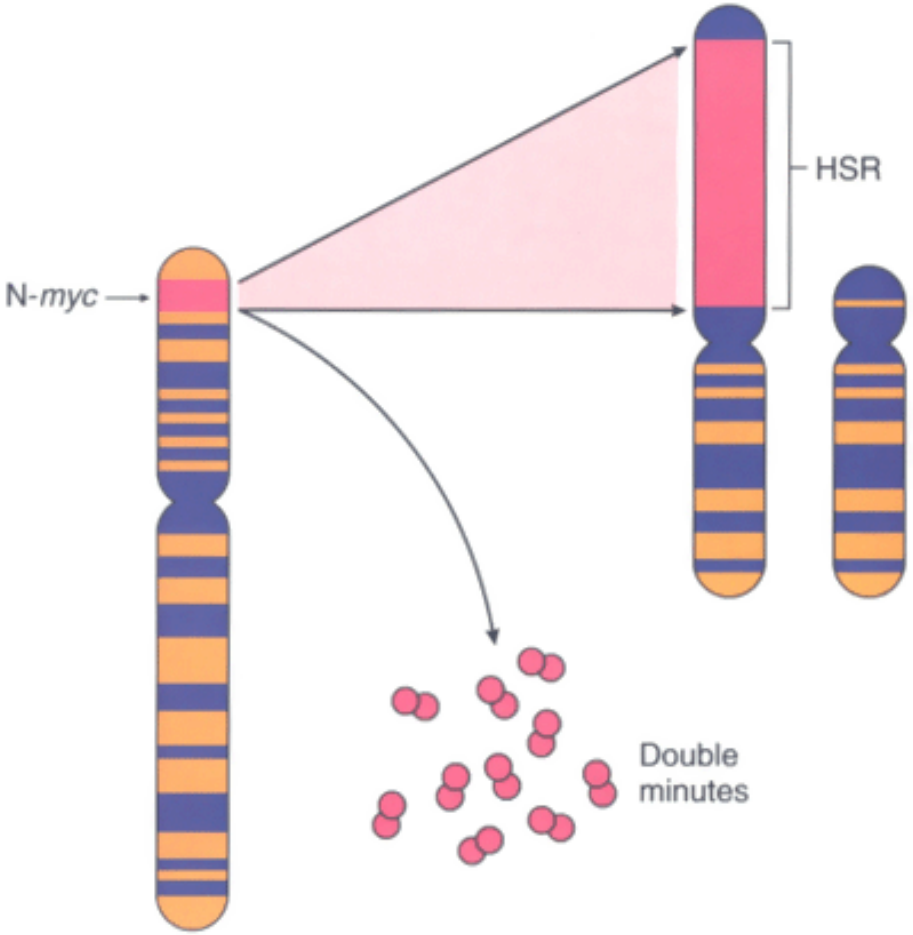


TABLE 7-9 -- Selected Tumor Suppressor Genes Involved in Human Neoplasms

Subcellular Location	Gene	Function	Tumors Associated with Somatic Mutations	Tumors Associated with Inherited Mutations
Cell surface	*TGF-β receptor*	Growth inhibition	Carcinomas of colon	Unknown

	E-cadherin	Cell adhesion	Carcinoma of stomach	Familial gastric cancer
Inner aspect of plasma membrane	*NF-1*	Inhibition of RAS signal transduction and of p21 cell-cycle inhibitor	Neuroblastomas	Neurofibromatosis type 1 and sarcomas
Cytoskeleton	*NF-2*	Cytoskeletal stability	Schwannomas and meningiomas	Neurofibromatosis type 2, acoustic schwannomas and meningiomas
Cytosol	*APC/β-catenin*	Inhibition of signal transduction	Carcinomas of stomach, colon, pancreas; melanoma	Familial adenomatous polyposis coli/ colon cancer
	PTEN	PI-3 kinase signal transduction	Endometrial and prostate cancers	Unknown
	SMAD 2 and *SMAD 4*	TGF-β signal transduction	Colon, pancreas tumors	Unknown
Nucleus	*RB*	Regulation of cell cycle	Retinoblastoma; osteosarcoma carcinomas of breast, colon, lung	Retinoblastomas, osteosarcoma
	p53	Cell-cycle arrest and apoptosis in response to DNA damage	Most human cancers	Li-Fraumeni syndrome; multiple carcinomas and sarcomas
	WT-1	Nuclear transcription	Wilms tumor	Wilms tumor
	p16 (INK4a)	Regulation of cell cycle by inhibition of cyclin-dependent kinases	Pancreatic, breast, and esophageal cancers	Malignant melanoma
	BRCA-1 and *BRCA-2*	DNA repair	Unknown	Carcinomas of female breast and ovary; carcinomas of male breast
	KLF6	Transcription factor	Prostate	Unknown

(Fig. 7-36). When cells enter the S phase, they can continue to cell division independent of growth factors. It should be obvious from this discussion that if RB is absent (owing to gene deletions) or its ability to regulate E2F transcription factors is derailed, the molecular brakes on the cell cycle are released, and the cells move into the S phase followed by cell replication. The mutations of *RB* genes found in tumors are localized to a region of the *RB* protein, called the "*RB* pocket," that is involved in binding to E2F.

It was mentioned previously that germ-line loss or mutations of the *RB* gene predispose to occurrence of retinoblastomas and to a lesser extent osteosarcomas. Furthermore, somatically acquired mutations have been described in glioblastomas, small cell carcinomas of lung, breast cancers, and bladder carcinomas. Given the presence of RB in every cell and its importance in cell-cycle control, two questions arise: (1) Why do patients with germ line mutation of the *RB* locus develop mainly retinoblastomas? (2) Why are inactivating mutations of *RB* not much more common in human cancer? The basis for the occurrence of tumors restricted to the retina in patients who inherit one defective allele of *RB* is not fully understood, but some possible explanations have emerged from the study of mice with targeted disruption of the *RB* locus. For instance, *RB* mutation may be a critical initiating event for retinoblastomas but may be only an accessory factor for malignancies at other sites.

With respect to the second question (i.e., why the loss of *RB* is not more common in human tumors), the answer is much simpler: Mutations in other genes that control RB phosphorylation can mimic the effect of *RB* loss, and such genes are mutated in many cancers that may have normal *RB* genes. Thus, for example, mutational activation of cyclin D or CDK4 would favor cell proliferation by facilitating RB phosphorylation. As previously discussed, cyclin D is overexpressed in many tumors because of gene amplification or translocation. Mutational inactivation of CDK inhibitors would also drive the cell cycle by unregulated activation of cyclins and CDKs. Thus, *the emerging paradigm is that loss of normal cell-cycle control is central to malignant transformation and that at least one of four key regulators of the cell cycle (p16INK4a, CYCLIN D, CDK4, RB) is dysregulated in the vast majority of human cancers*.[38] In cells that harbor mutations in any one of these other genes, the function of RB is disrupted even if the *RB* gene itself is not mutated.[45]

Several other pathways of cell growth regulation, some to be discussed in more detail later, also converge on RB (Fig. 7-36):

• TGF-β induces inhibition of cellular proliferation. This effect of TGF-β is mediated, at least in part, by up-regulation of the CDK inhibitor p27.

• The transforming proteins of several oncogenic animal and human DNA viruses seem to act, in part, by neutralizing the growth inhibitory activities of RB. In these cases, RB protein is functionally deleted by the binding of a viral protein and no longer acts as a cell-cycle inhibitor. Simian virus 40 and polyomavirus large T antigens, adenoviruses EIA protein, and human papillomavirus (HPV) E7 protein, all bind to the hypophosphorylated form of RB. The binding occurs in the same RB pocket that normally sequesters E2F transcription factors; in the case of HPV, the binding is particularly strong for viral types, such as HPV 16, which confer high risk for the development of

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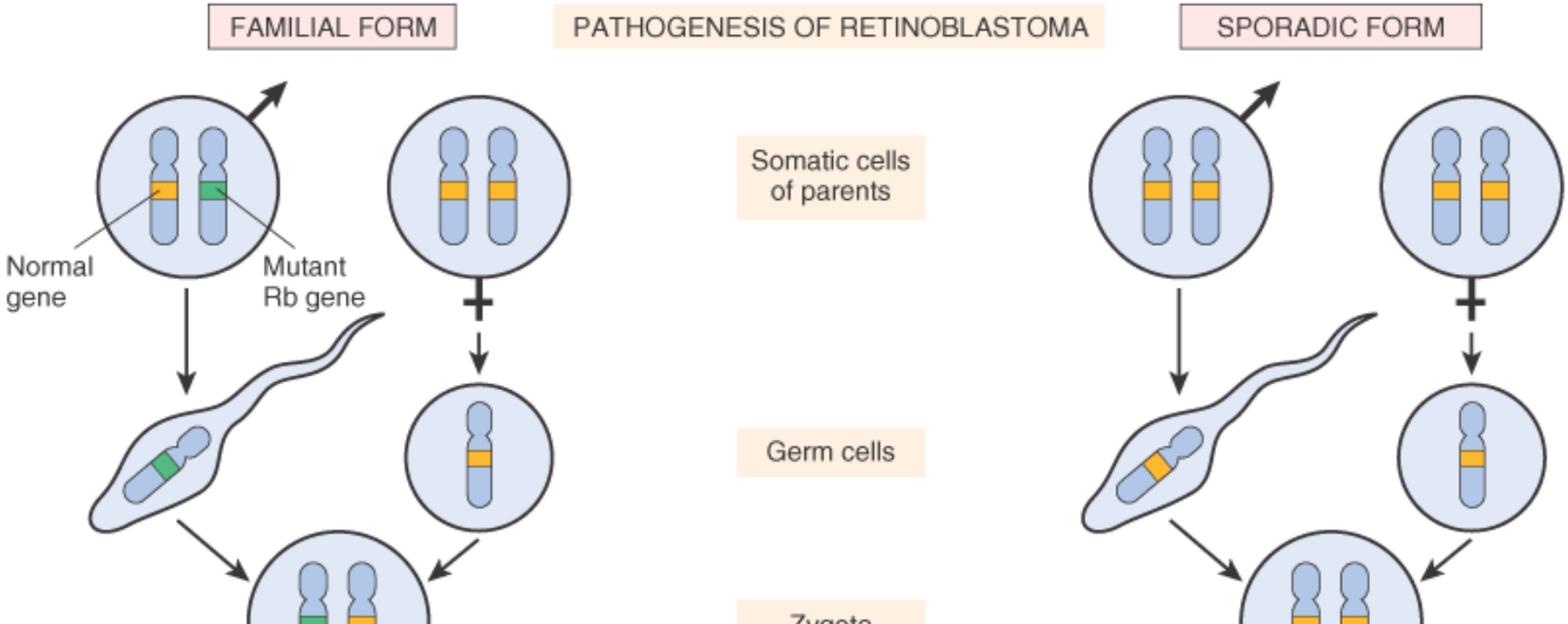
cervical carcinomas. Thus, the RB protein, unable to bind the E2F transcription factors, is functionally deleted, and the transcription factors are free to cause cell-cycle progression.

• The *p53* tumor suppressor gene exerts its growth-inhibiting effects at least in part by up-regulating the synthesis of the CDK inhibitor p21 (see Fig. 7-29 and Fig. 7-36).

Figure 7-35 Pathogenesis of retinoblastoma. Two mutations of the *RB* locus on chromosome 13q14 lead to neoplastic proliferation of the retinal cells. In the familial form, all somatic cells inherit one mutant *RB* gene from a carrier parent. The second mutation affects the *Rb* locus in one of the retinal cells after birth. In the sporadic form, on the other hand, both mutations at the *RB* locus are acquired by the retinal cells after birth.



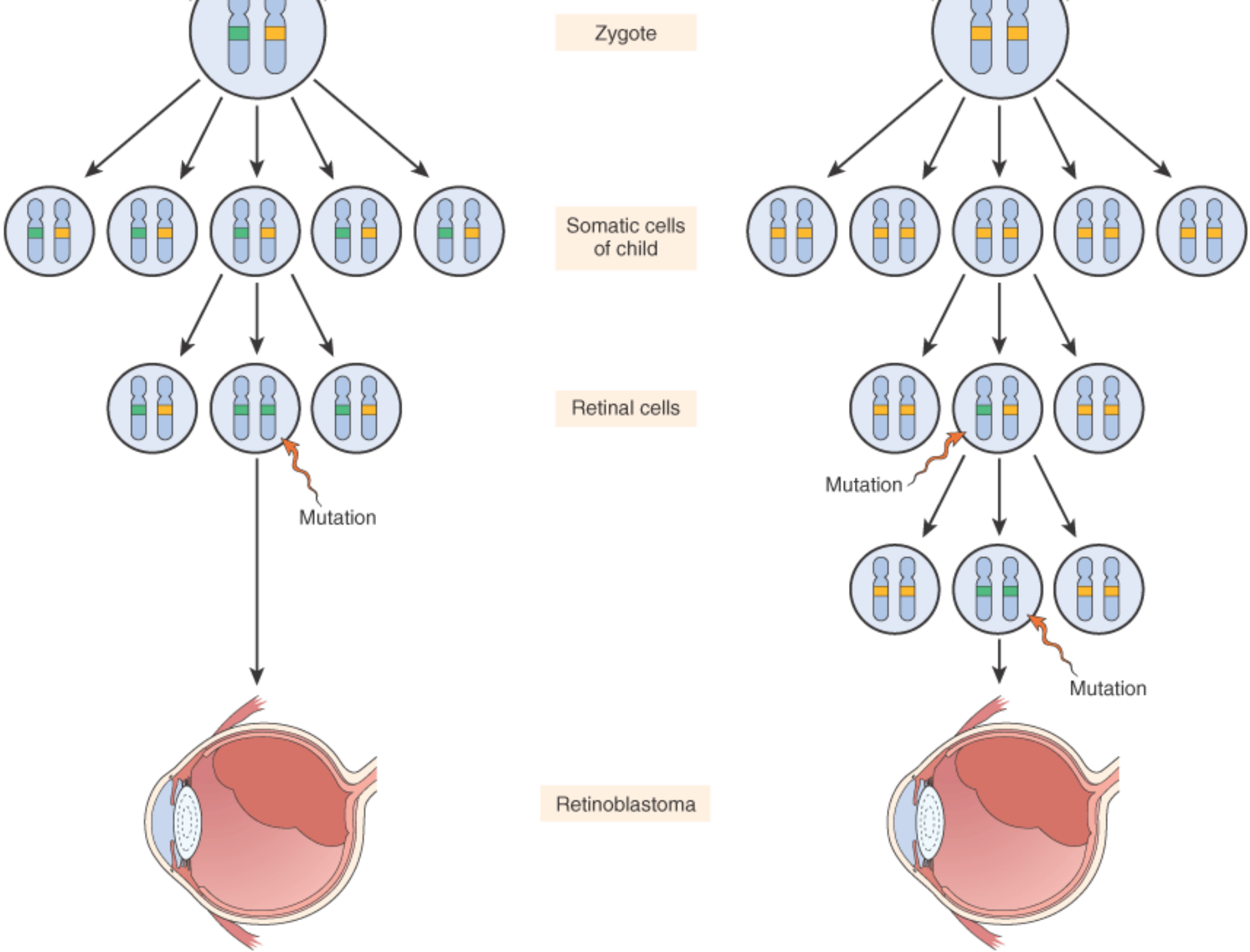

Zygote
Somatic cells of child
Retinal cells
Mutation
Retinoblastoma
Mutation
Mutation

Figure 7-36 Role of RB as a cell-cycle regulator. Various growth factors promote the formation of the cyclin D-CDK4 complex. This complex (and to some extent cyclin E-CDK2) phosphorylates RB, changing it from an active (hypophosphorylated) to an inactive state (hyperphosphorylation). RB inactivation allows the cell to pass the G_1 /S restriction point. Growth inhibitors such as TGF-β and *p53* and the Cip/Kip (e.g., p21, p57) and INK4a (p161NK4a and p19ARF) cell-cycle inhibitors prevent RB activation. Transforming proteins of oncogenic viruses bind hypophosphorylated RB and cause its functional inactivation. Virtually all cancers show dysregulation of the cell cycle by affecting the four genes marked by an asterisk.

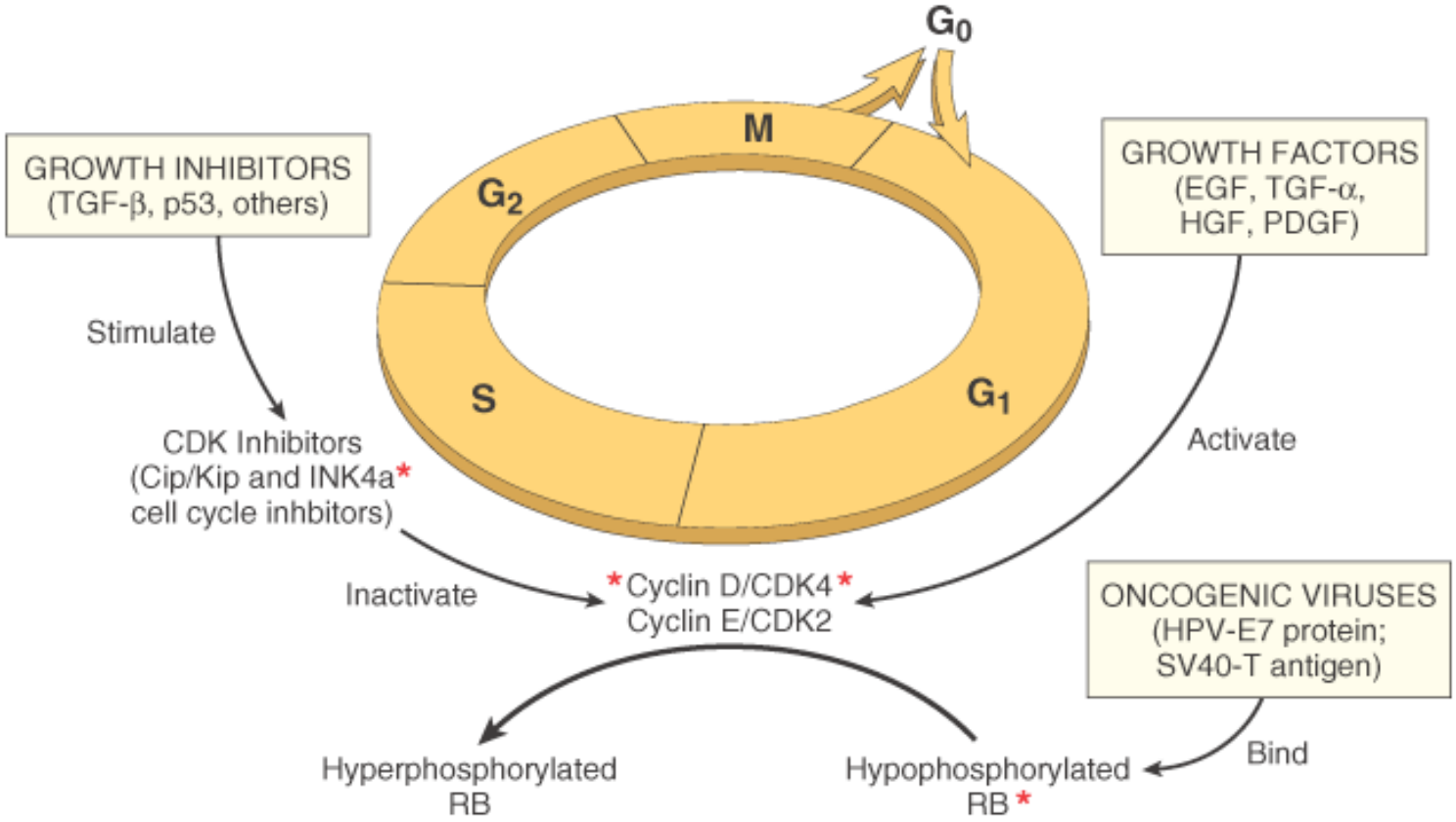
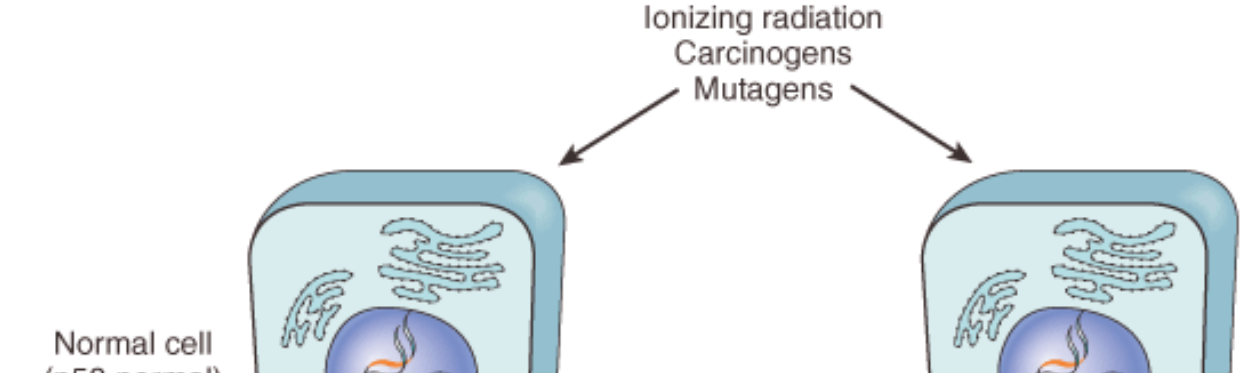


Figure 7-37 The role of *p53* in maintaining the integrity of the genome. Activation of normal *p53* by DNA-damaging agents or by hypoxia leads to cell-cycle arrest in G_1 and induction of DNA repair, by transcriptional up-regulation of the cyclin-dependent kinase inhibitor *p21*, and the *GADD45* genes, respectively. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, *p53*-induced activation of the *BAX* gene promotes apoptosis. In cells with loss or mutations of *p53*, DNA damage does not induce cell-cycle arrest or DNA repair, and hence genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.



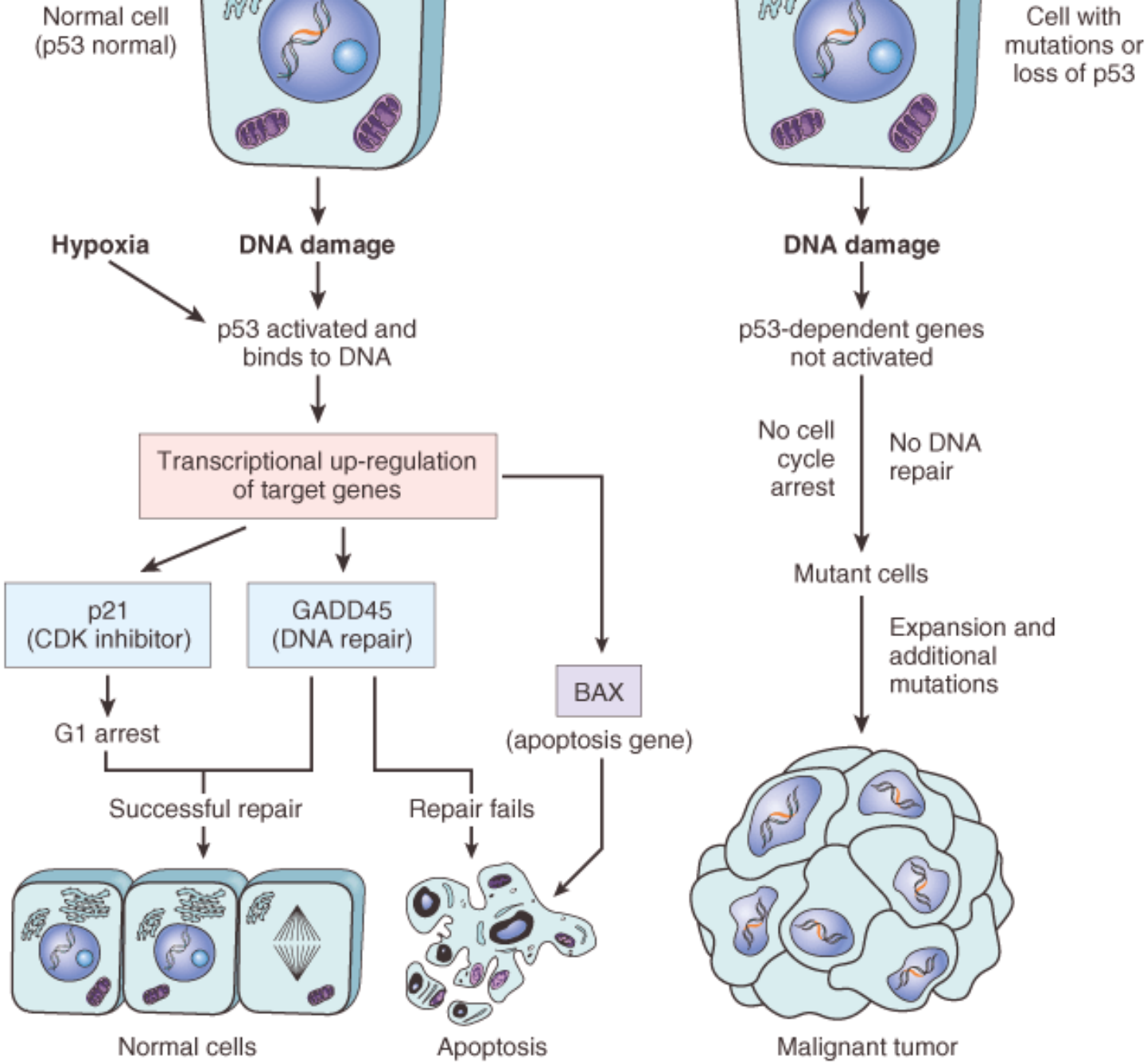


Figure 7-38 *A,* The role of APC in regulating the stability and function of β-catenin. APC and β-catenin are components of the WNT signaling pathway. In resting cells (not exposed to WNT), β-catenin forms a macromolecular complex containing the APC protein. This complex leads to the destruction of β-catenin, and intracellular levels of β-catenin are low. *B,* When cells are stimulated by secreted WNT molecules, the *destruction complex* is deactivated, β-catenin degradation does not occur, and cytoplasmic levels increase. β-catenin translocates to the nucleus, where it binds to TCF, a transcription factor that activates several genes involved in the cell cycle. *C,* When *APC* is mutated or absent, the destruction of β-catenin cannot occur. β-Catenin translocates to the nucleus and coactivates genes that promote the cell cycle, and cells behave as if they are under constant stimulation by the WNT pathway.

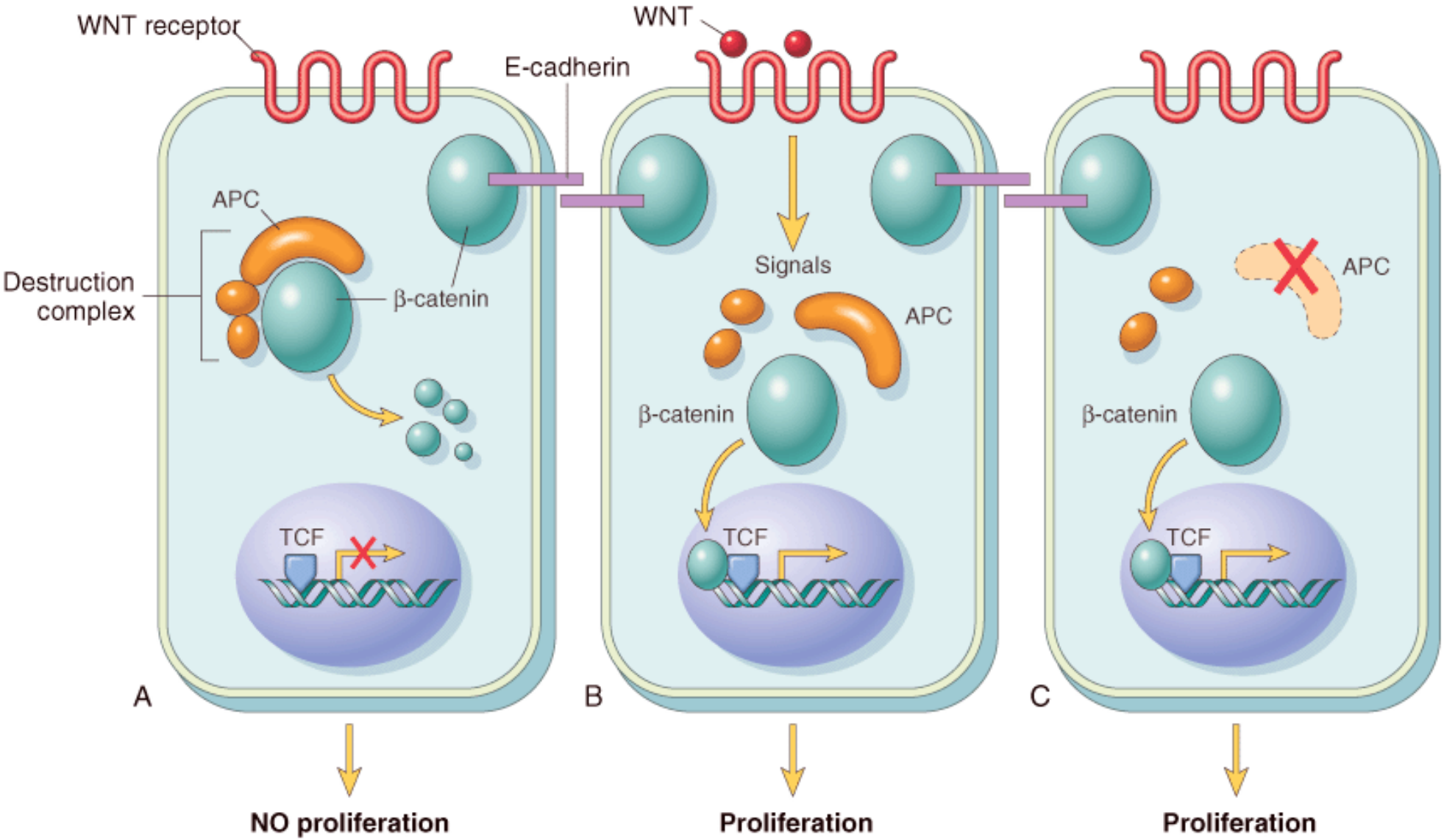


Figure 7-39 Interaction between cancer susceptibility genes and DNA repair. *ATM* (ataxia-telangiectasia mutated) senses a double-strand break in DNA, induced by agents such as ionizing radiation. *ATM* and *CHEK2* phosphorylate *BRCA1*, promoting its migration to the break site. The Fanconi's anemia protein complex (proteins A, C, E, F, G) triggers the ubiquitination and colocalization of the Fanconi protein D2 with *BRCA1* at the break site. *BRCA2* carries RAD51, an enzyme involved in DNA recombination repair, to the same site. BRCA1, BRCA2, and RAD51 repair the DNA break by an error-free recombination mechanism. RAD51 is a component of cell cycle check points. *(Redrawn from Venkitaraman AR: A growing network of cancer-susceptibility genes. N Engl J Med 348:1917, 2003.)*

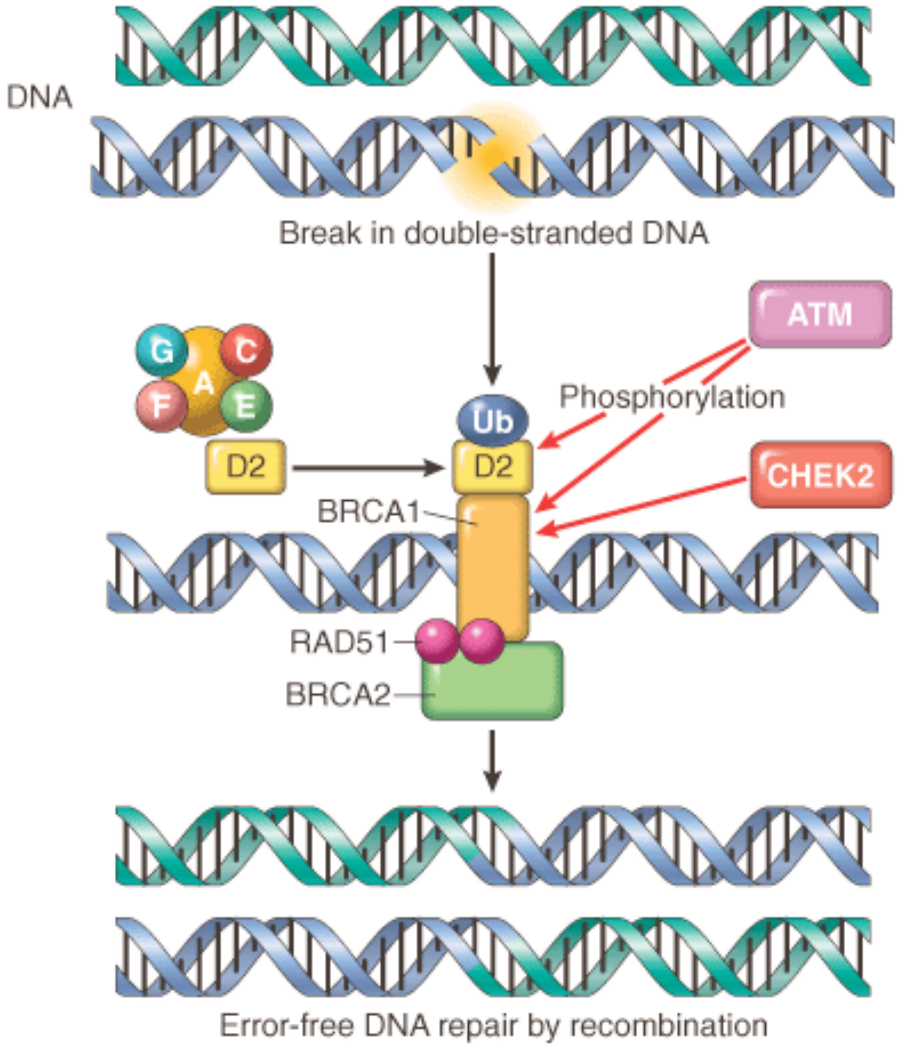


Figure 7-40 Cellular responses to telomere shortening. The figures show the responses of normal cells, which have intact cell-cycle checkpoints and of cells with checkpoint defects. *(From Wong JMY, Collins K: Telomere maintenance and disease. Lancet 362:983, 2003.)*

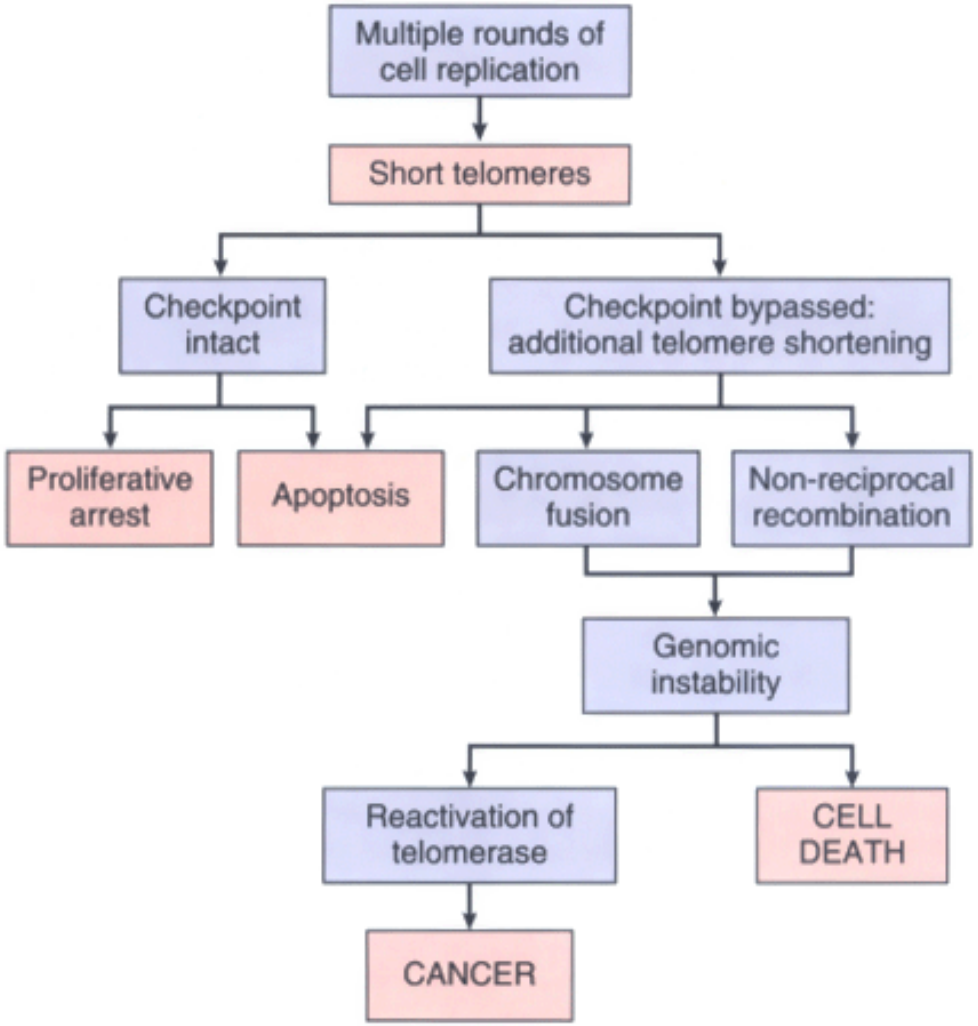
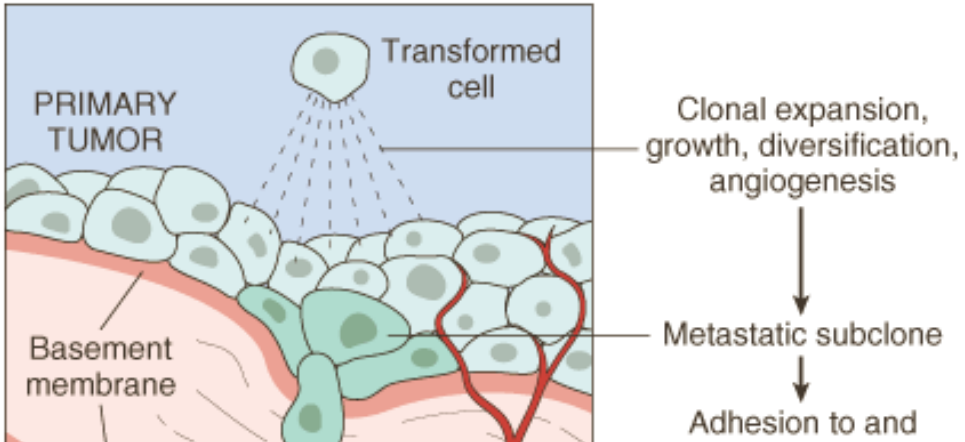
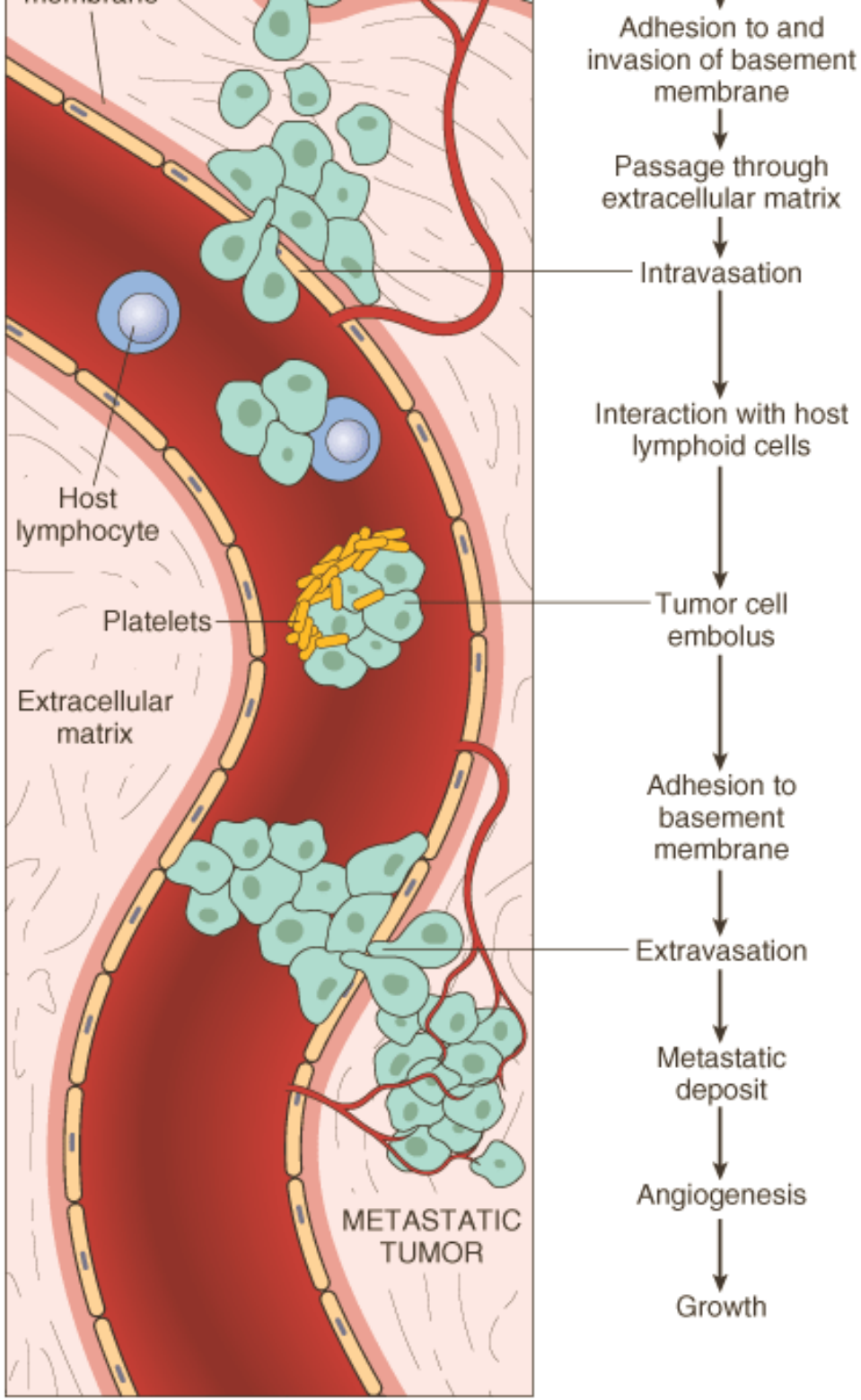


Figure 7-42 The metastatic cascade. Schematic illustration of the sequential steps involved in the hematogenous spread of a tumor.





Adhesion to and invasion of basement membrane

↓

Passage through extracellular matrix

↓

Intravasation

↓

Interaction with host lymphoid cells

↓

Tumor cell embolus

↓

Adhesion to basement membrane

↓

Extravasation

↓

Metastatic deposit

↓

Angiogenesis

↓

Growth

Figure 7-43 Mechanisms of metastasis development within a primary tumor. A nonmetastatic primary tumor is shown *(light blue)* on the left side of all diagrams. Four models are presented: *A*, Metastasis is caused by rare variant clones that develop in the primary tumor; *B*, Metastasis is caused by the gene expression pattern of most cells of the primary tumor, referred to as a metastatic signature; *C*, A combination of *A* and *B*, in which metastatic variants appear in a tumor with a metastatic gene signature; *D*, Metastasis development is greatly influenced by the tumor stroma, which may regulate angiogenesis, local invasiveness and resistance to immune elimination, allowing cells of the primary tumor, as in *C*, to become metastatic.

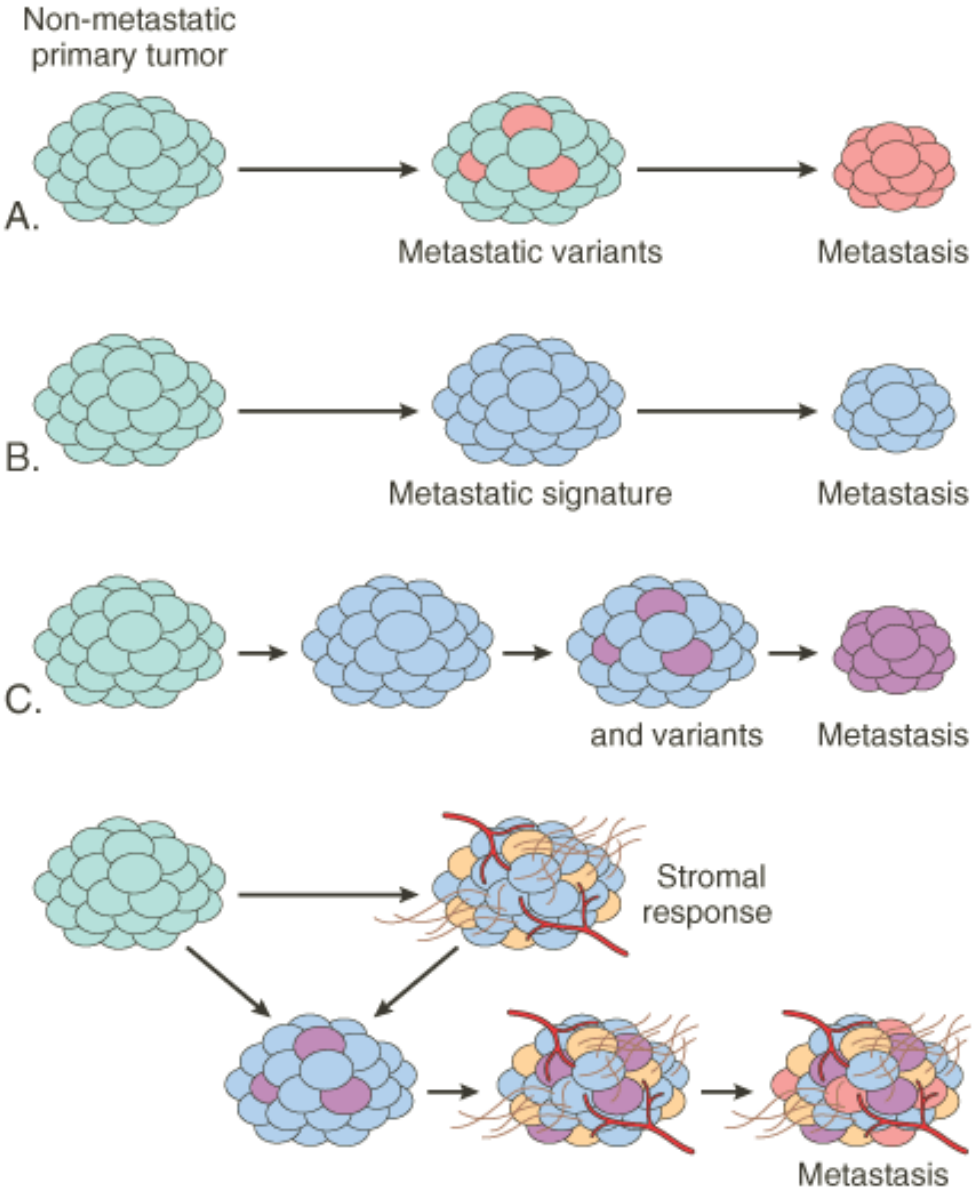
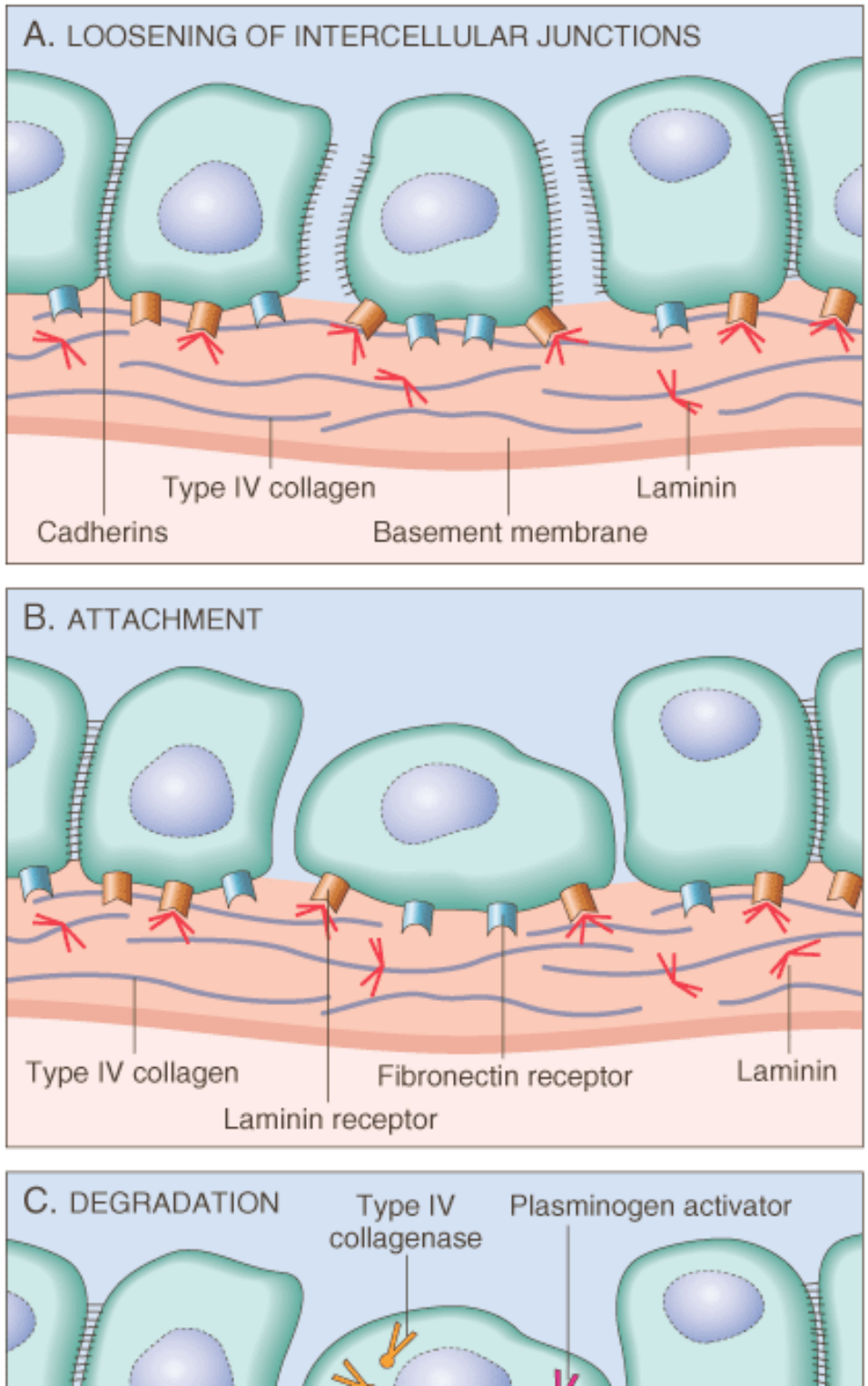
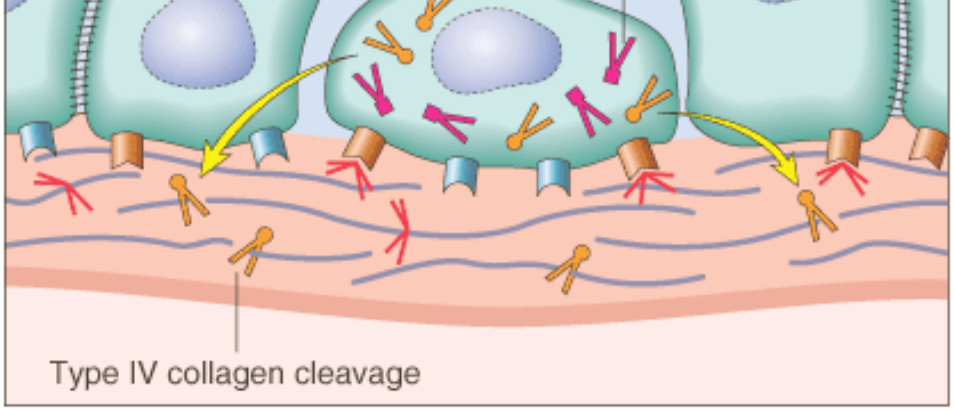


Figure 7-44 *A–D*, Schematic illustration of the sequence of events in the invasion of epithelial basement membranes by tumor cells. Tumor cells detach from each other because of reduced adhesiveness, and cells then attach to the basement membrane via the laminin receptors and secrete proteolytic enzymes, including type IV collagenase and plasminogen activator.

Degradation of the basement membrane and tumor cell migration follow.





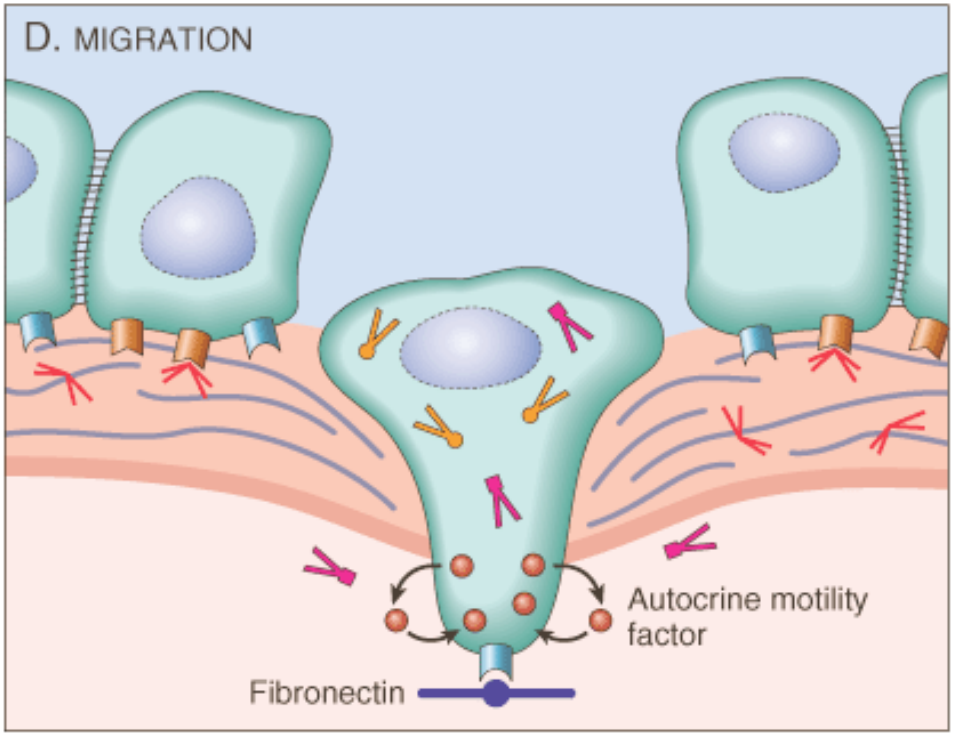


TABLE 7-10 -- Selected Examples of Oncogenes Activated by Translocation

Malignancy	Translocation	Affected Genes
Chronic myeloid leukemia	(9;22)(q34;q11)	Ab1 9q34
		bcr 22q11
Acute leukemias (AML and ALL)	(4;11)(q21;q23)	AF4 4q21
		MLL 11q23

	(6;11)(q27;q23)	AF6 6q27
		MLL 11q23
Burkitt lymphoma	(8;14)(q24;q32)	c-*myc* 8q24
		IgH 14q32
Mantle cell lymphoma	(11;14)(q13;q32)	Cyclin D 11q13
		IgH 14q32
Follicular lymphoma	(14;18)(q32;q21)	IgH 14q32
		bcl-2 18q21
T-cell acute lymphoblastic leukemia	(8;14)(q24;q11)	*c-myc* 8q24
		TCR-α 14q11
	(10;14)(q24;q11)	*Hox* 11 10q24
		TCR-α 14q11
Ewing sarcoma	(11;22)(q24;q12)	Fl-1 11q24
		EWS 22q12
Underlined genes are involved in multiple translocations.		
AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia.		

course. The translocations associated with the *ABL* oncogene in chronic myeloid leukemia and with c-*MYC* in Burkitt lymphoma have been mentioned earlier, in conjunction with the discussion of molecular defects in cancer cells (see Fig. 7-32). Several other karyotype alterations in cancer cells are presented in the discussion of specific forms of neoplasia.

Two types of chromosomal rearrangements can activate protooncogenes—translocations and inversions. Chromosomal translocations are much more common (Table 7-10) and are discussed here. Translocations can activate protooncogenes in two ways:

- In lymphoid tumors, specific translocations result in overexpression of protooncogenes by removing them from their regulatory elements.
- In many hematopoietic tumors, the translocations allow normally unrelated sequences from two different chromosomes to recombine and form hybrid genes that encode growth-promoting chimeric proteins.

Overexpression of a protooncogene caused by translocation is best exemplified by Burkitt lymphoma. All such tumors carry one of three translocations, each involving chromosome 8q24, where the *MYC* gene has been mapped, as well as one of the three immunoglobulin gene-carrying chromosomes. At its normal locus, the expression of the *MYC* gene is tightly controlled; it is expressed only during certain stages of the cell cycle. In Burkitt lymphoma, the most common form of translocation results in the movement of the *MYC*-containing segment of chromosome 8 to chromosome 14q band 32 (Fig. 7-33), placing it close to the immunoglobulin heavy-chain (IgH) gene. The genetic notation for the translocation is t(8:14)(q24;q32). The molecular mechanisms of the translocation-associated activation of *MYC* are variable, as are the precise breakpoints within the gene. In most cases, the translocation causes mutations or loss of the regulatory sequences of the *MYC* gene. As the coding sequences remain intact, the gene is constitutively expressed at high levels. The gene may be translocated to the antigen receptor loci simply because these loci are accessible (i.e. in "open" chromatin) and active in developing lymphocytes. The invariable presence of the translocated *MYC* gene in Burkitt lymphomas attests to the importance of *MYC* overexpression in the pathogenesis of this tumor.

There are other examples of oncogenes translocated to antigen receptor loci in lymphoid tumors. As mentioned earlier, in mantle cell lymphoma, the *CYCLIN D1* gene on chromosome 11q13 is overexpressed by juxtaposition to the IgH locus on 14q32. In follicular lymphomas, a t(14;18)(q32;q21) translocation, the most common translocation in lymphoid malignancies, causes activation of the *BCL-2* gene. Not unexpectedly, all these tumors in which the immunoglobulin gene is involved are of B-cell origin. In an analogous situation, overexpression of several protooncogenes in T-cell tumors results from translocations of oncogenes into the T-cell antigen receptor locus. The affected oncogenes are diverse, but in most cases, as with *MYC*, they encode nuclear transcription factors.

The Philadelphia chromosome, characteristic of chronic myeloid leukemia and a subset of acute lymphoblastic leukemias, provides the prototypic example of an oncogene formed by *fusion of two separate genes*. In these cases, a reciprocal translocation between chromosomes 9 and 22 relocates

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a truncated portion of the protooncogene c-*ABL* (from chromosome 9) to the *BCR (b*reak point *c*luster *r*egion) on chromosome 22 (Fig. 7-33). The hybrid fusion gene *BCR-ABL* encodes a chimeric protein that has constitutive tyrosine kinase activity. As mentioned, BCR-ABL tyrosine kinase has served as a target for leukemia therapy, with remarkable success so far. Although the translocations are cytogenetically identical in chronic myeloid leukemia and acute lymphoblastic leukemias, they differ at the molecular level. In chronic myeloid leukemia, the chimeric protein has a molecular weight of 210 kD, whereas in the more aggressive acute leukemias, a 190-kD BCR-ABL fusion protein is formed.[62] [63] The molecular pathways activated by the BCR-ABL protein are complex and not completely understood. It inhibits apoptosis, decreases the requirement for growth factors, binds to cytoskeleton components, decreases cell adhesion, and activates multiple pathways, including those of RAS, PI-3 kinase, and STATs (Chapter 3). BCR-ABL also acts on DNA repair and may cause genomic instability that contributes to the progression of the disease.

Transcription factors are often the partners in gene fusions occurring in cancer cells. For instance, the *MLL* (myeloid, lymphoid leukemia) gene on 11q23 is known to be involved in 25 different translocations with several different partner genes, some of which encode transcription factors (see Table 7-10). The *Ew*ing *S*arcoma (*EWS*) gene at 22q12 was first described in the t(11;22)(q24;12) reciprocal translocation present in Ewing sarcoma (a highly malignant tumor of children; Chapter 26) but may be translocated in other types of sarcomas. *EWS* is itself a transcription factor, and all of its partner genes analyzed so far also encode a transcription factor. In Ewing tumor, for example, the *EWS* gene fuses with the *FLI 1* gene; the resultant chimeric EWS-FLI 1 protein is a member of the ETS transcription factor family, which has transforming ability.

Gene Amplification

Activation of protooncogenes associated with overexpression of their products may result from reduplication and *amplification* of their DNA sequences. Such amplification may produce several hundred copies of the protooncogene in the tumor cell.[137] The amplified genes can be readily detected by molecular hybridization with appropriate DNA probes. In some cases, the amplified genes produce chromosomal changes that can be identified microscopically. Two mutually exclusive patterns are seen: multiple small, chromosome-like structures called *double minutes* (dms), and *homogeneous staining regions* (HSRs). The latter derive from the assembly of amplified genes into new chromosomes; because the regions containing amplified genes lack a normal banding pattern, they appear homogeneous in a G-banded karyotype (see Fig. 7-34). The most interesting cases of amplification involve N-*MYC* in neuroblastoma and *ERB B2* in breast cancers. N-*MYC* is amplified in 25% to 30% of neuroblastomas, and the amplification is associated with poor prognosis. In neuroblastomas with N-*MYC* amplification, the gene is present both in dms and HSRs. *ERB B2* amplification occurs in about 20% of breast cancers and may represent a distinct tumor phenotype. Amplification of C-*MYC*, L-*MYC*, and N-*MYC* correlates with disease progression in small cell cancer of the lung. Another gene frequently amplified is *CYCLIN D1* (breast carcinomas, head and neck carcinomas, and other squamous cell carcinomas).

Epigenetic Changes

It has become evident during the past few years that certain tumor suppressor genes may be inactivated not because of structural changes but because the gene *is silenced by hypermethylation of promoter sequences without a change in DNA base sequence*.[138] Such changes appear to be stably maintained through multiple rounds of cell division. Methylation

takes place in CpG islands in DNA, but de novo methylation rarely occurs in normal tissues. However, methylation has been detected in various tumor suppressor genes in human cancers. They include *p14ARF* in colon and stomach cancers, *p16INK4a* in various types of cancers, *BRCA1* in breast cancer, *VHL* in renal cell carcinomas, and the *MLH1* mismatch repair gene in colorectal cancer.[139] Methylation also participates in the phenomenon called *genomic imprinting,* in which the maternal or paternal allele of a gene or chromosome is modified by methylation and is inactivated. The reverse phenomenon, that is, demethylation of an imprinted gene leading to its biallelic expression (loss of imprinting) can also occur in tumor cells.[140] Although the discussion of whether methylation of tumor suppressor genes has a causal role in cancer development continues, there has been great interest in developing potential therapeutic agents that act to demethylate DNA sequences in tumor suppressor genes. Recent data demonstrating that genomic hypomethylation causes chromosomal instability and induces tumors in mice greatly strengthens the notion that epigenetic changes may directly contribute to tumor development.[141]

Molecular Profiles of Cancer Cells

A new era in cancer research was initiated with the development of methods to measure the expression of thousands of genes in tumors and normal tissues. Among these new methods, the determination of RNA levels by microarray analysis has found wide application (Box 7-1). Currently, this method can measure RNA expression from virtually all known genes (Fig. 7-45). The expression profiles obtained from DNA microarray analysis are known as *gene expression signatures or molecular profiles*. The application of this technique to the study of breast cancers and leukemias has been particularly rewarding (see Box 7-1). It was recently found that there are breast cancer subtypes that can be identified by their molecular profiles and that the molecular signatures of some of these subtypes can help predict the course of the disease (see Box 23-1 , Chapter 23).[142] Analysis of acute lymphoblastic leukemia by DNA microarrays has established the molecular signatures of prognostic subtypes and uncovered novel markers associated with these subtypes.[143]

Molecular Basis of Multistep Carcinogenesis

The notion that malignant tumors arise from a protracted sequence of events is supported by epidemiologic, experimental, and molecular studies. Many eons ago, before

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Box 7-1. Gene Expression Profiles of Human Cancers. Microarrays and Proteomics

Until recently, studies of gene expression in tumors involved the analysis of individual genes. These studies have been revolutionized by the introduction of methods that can measure the expression of thousands of genes simultaneously. [208] [209] The most common method for large-scale analysis of gene expression in use today is based on DNA microarray technology. In this method, DNA fragments, either cDNAs or oligonucleotides, are spotted on a glass slide or on some other solid support. As the techniques used for the spotting are similar to those employed to produce semiconductor chips for electronic products, the arrays are known as "*gene chips*." Chips can be purchased from commercial suppliers or produced in-house, and can contain more than 20,000 gene fragments. The fragments are typically obtained from complementary DNA (cDNA) libraries or sets of nucleotides from known and uncharacterized genes. The gene chip is then hybridized to "probes" prepared from tumor and control samples (the probes are usually cDNA copies of RNAs extracted from tumor and uninvolved tissues). Before hybridization to the chip, the probes are labeled with fluorochromes that emit different colors (e.g. red color for tumor RNA and green color for control RNA). After hybridization the chip is read using a laser scanner (Fig. 7-45); each spot on the array will be red (increased expression of a gene in the tumor), green (decreased expression in the tumor) or, if there is no difference in gene expression between the tumor and control sample, the spots will be either black or yellow (depending on the type of fluorescent scanning). Sophisticated software has been developed to measure the intensity of the fluorescence for each spot and produce data sets in which genes with similar expression patterns are clustered. [210] This method of analysis, called *hierarchical clustering,* groups together genes according to the similarity of their gene expression patterns. The software can be linked to large sequencing and array databases available through the Web. This allows appropriate gene identification and comparison between expression profiles from various

sources. A major problem in the analysis of gene expression in tumors is the heterogeneity of the tissue. In addition to the heterogeneity between tumor cells, samples may contain variable amounts of stromal connective tissue, inflammatory infiltrates, and normal tissue cells. One way to overcome this problem is to obtain nearly pure tumor cells or small tumors free from associated tissues using *laser capture microdissection*. In this technique, the dissection of the tumor or cells is made under a microscope through a focused laser. The dissected material is then captured or "catapulted" into a small cap and processed for RNA and DNA isolation.

Gene expression profiling of tumors has multiple uses, and the number of publications using this technique has grown enormously during the past few years. Much of the work performed is not directed toward proving or disproving a proposed hypothesis. Gene expression analysis can be used to classify tumors; to predict metastatic potential, prognosis, and response to therapy; to reveal gene expression patterns that are dependent on the mutation of a single oncogene; and to analyze the effects of hormones and environmental agents on cancer development. [209] The applications of this technology keep expanding and being refined, but much has already been accomplished.[141] [209] [210] [211] We mention only a few interesting examples. Profiling of cells from adult and pediatric T-cell acute lymphoblastic leukemia has identified the patterns of gene expression in leukemic blast cells and has accurately classified each prognostic subtype.[211] The work that has received the highest publicity involves gene expression profiling of breast cancers. In addition to identifying new subtypes of breast cancers, a 70-gene prognosis profile was established. Using this type of profile, it has been reported that: (1) the profile was a powerful predictor of disease prognosis for young patients; (2) it was particularly accurate for predicting metastasis during the first 5 years after diagnosis; and (3) prognosis determined by gene expression profiles correlated highly with histologic grade and estrogen receptor status but not with lymphatic spread of the tumor.[211] A more recent analysis has pooled together data gathered by different laboratories and has confirmed the identification of distinct subtypes of breast cancer.[142] Given all of these remarkable results, it is time to ask whether this technology is "ready for prime time"; that is, ready for day to day clinical applications. Things are moving very fast in this area, but before clinical applications are considered, many issues need to settled. Not only do larger trials need to be conducted to prove the reliability and accuracy of the analysis but also, just as important, the procedures for handling samples, performing the analyses, and reporting the data need to be standardized, so that data obtained in various laboratories can be compared.

Next on the horizon of molecular techniques for the global analysis of gene expression in cancers is *proteomics*, a technique used to obtain expression profiles of proteins contained in tissues, serum, or other body fluids. The original method consisted of the separation of proteins by 2-dimensional gel electrophoresis, followed by identification of individual proteins by mass spectrometry. A more recent technique, called *ICAT* (*i*sotope-*c*oding *a*ffinity *t*ags) does not rely on electrophoresis for protein separation. In ICAT, proteins in the test and control samples are labeled with light or heavy isotopes. The differentially labeled proteins are then identified and quantified by mass spectrometry. A variation of proteomic analysis has been used to obtain protein profiles in the blood of cancer patients without identification of individual proteins.[215]

The excitement created by the development of new techniques for the global molecular analysis of tumors has led some scientists to predict that the end of histopathology is in sight, and to consider existing approaches to tumor diagnosis as the equivalent of magical methods of divination. Indeed, it is hard to escape the excitement generated by the development of entirely new and powerful methods of molecular analysis. However, what lies ahead is not the replacement of one set of techniques by another. On the contrary, the most accurate diagnosis and prognosis of cancer will be arrived at by a combination of morphologic and molecular techniques. [208]

Figure 7-45 Schematic representation of the steps required for the analysis of global gene expression by DNA microarray. RNA is extracted from tumor and normal tissue. cDNA synthesized from each preparation is labeled with fluorescent dyes (in the example shown, normal tissue cDNA is labeled with a green dye; tumor cDNA is labeled with a red dye). The array consists of a solid support in which DNA fragments from many thousands of genes are spotted. The labeled cDNAs from tumor and normal tissue are combined and hybridized to the genes contained in the array. Hybridization signals are detected using a confocal laser scanner and downloaded to a computer for analysis (*red squares*, expression of the gene is higher in tumor; *green square*, expression of the gene is higher in normal tissue; *black squares*, no difference in the expression of the gene between tumor and normal tissue). In the display, the horizontal rows correspond to each gene contained in the array; each ventrical row corresponds to single samples.

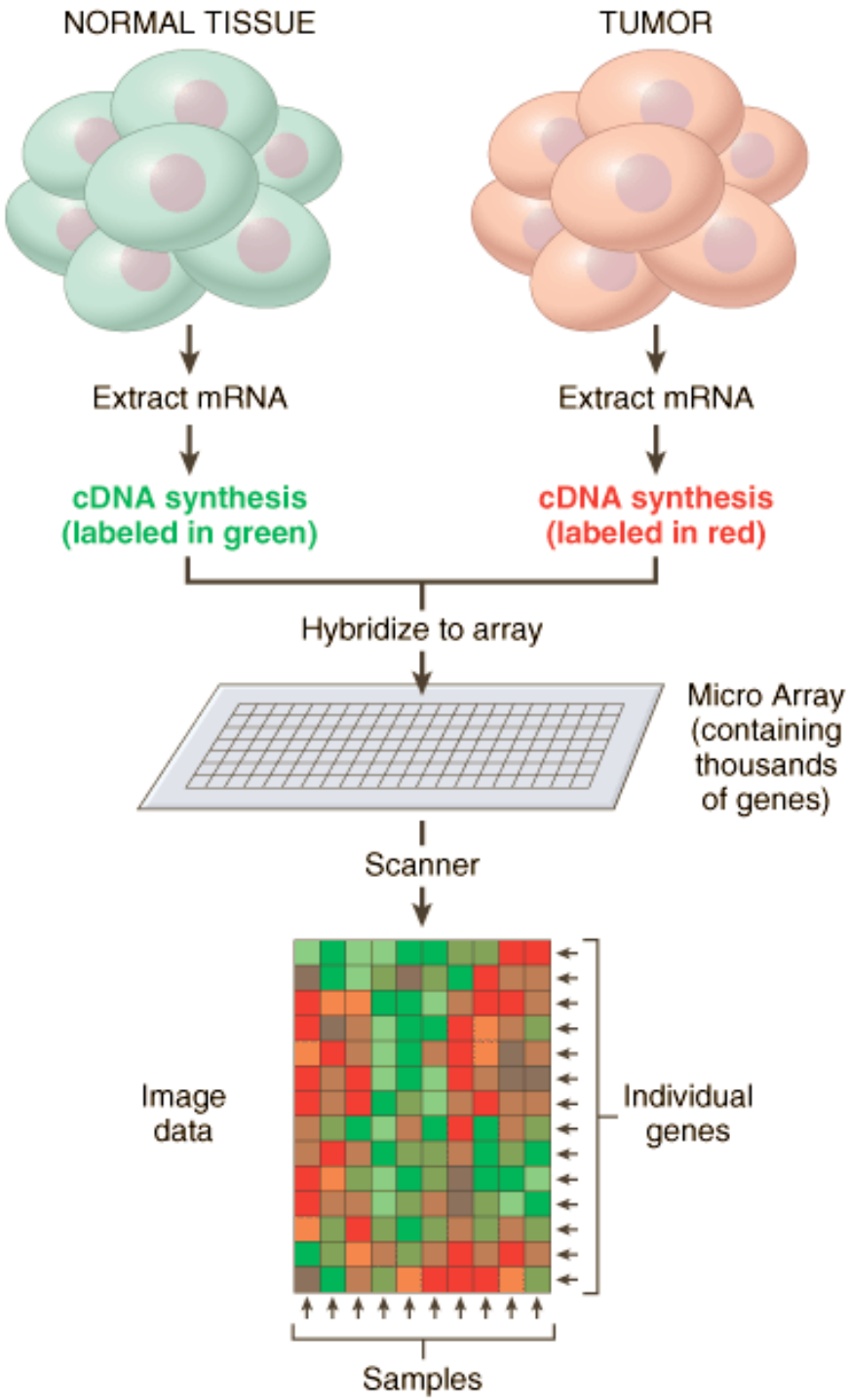

Figure 7-47 Schematic illustration of the pathways of malignancy initated by mutation of the gatekeeper genes (e.g., *APC*, *NF-1*, *RB*) or caretaker genes (e.g., *hMSH2*, *BRCA-1*, *BRCA-2*).

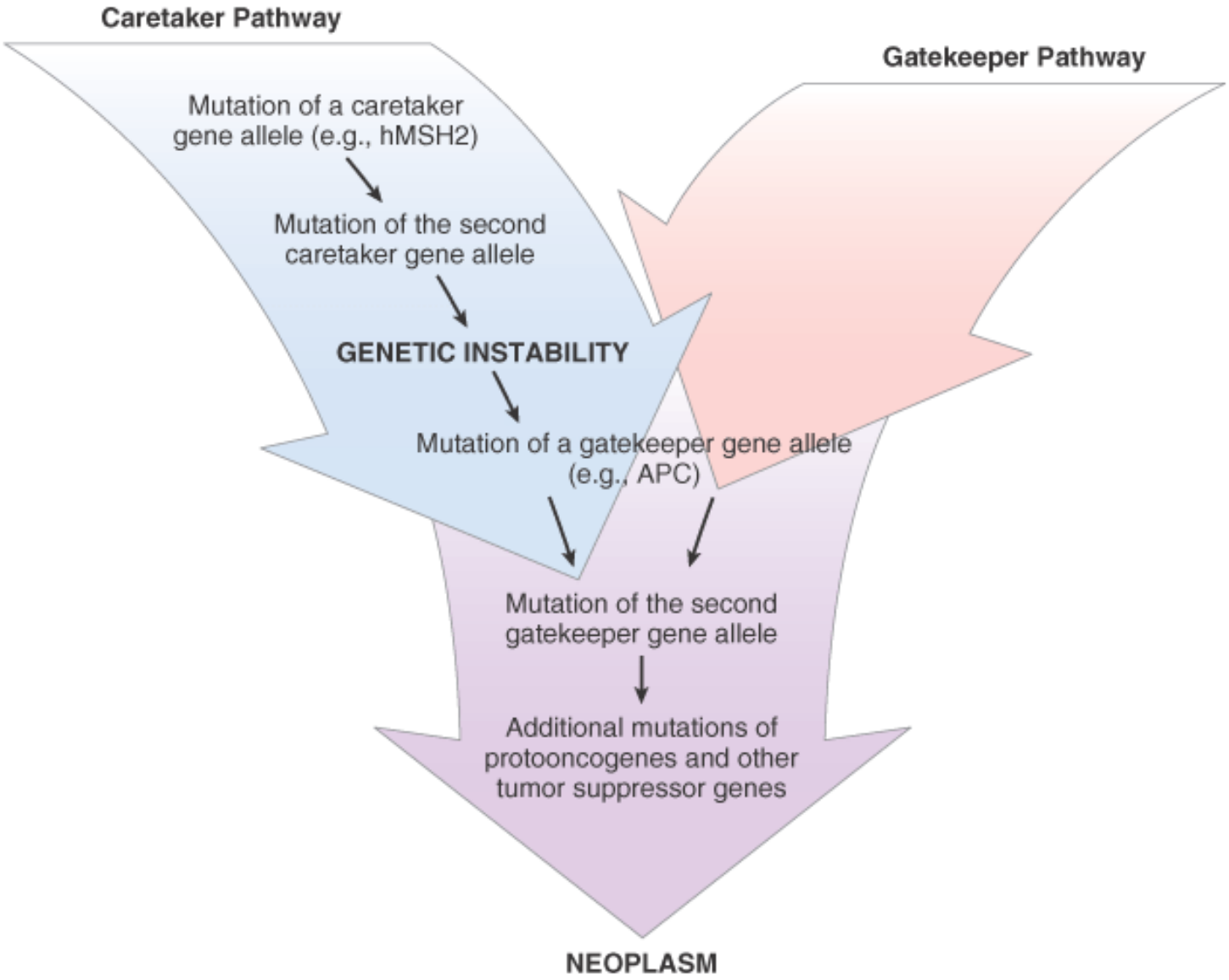

Figure 7-48 Experiments demonstrating the initiation and promotion phases of carcinogenesis in mice. Group 2: application of promoter repeated at twice-weekly intervals for several months. Group 3: application of promoter delayed for several months and then applied twice weekly. Group 6: promoter applied at monthly intervals.

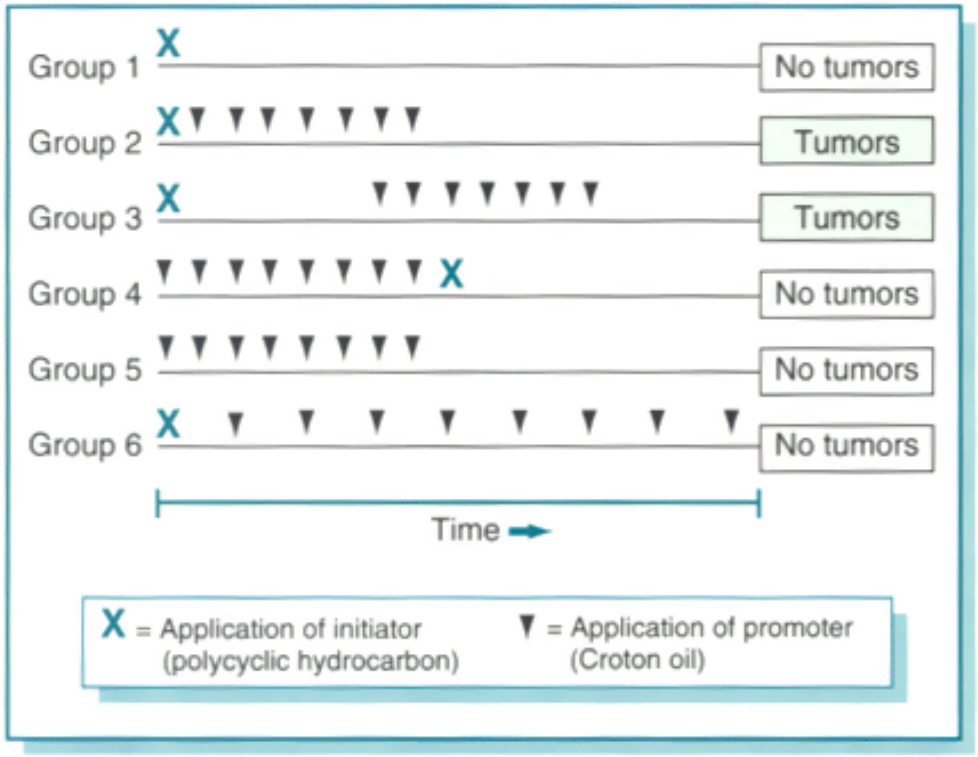

Figure 7-49 General schema of events in chemical carcinogenesis. Note that promoters cause clonal expansion of the initiated cell, thus producing a preneoplastic clone. Further proliferation induced by the promoter or other factors causes accumulation of additional mutations and emergence of a malignant tumor.

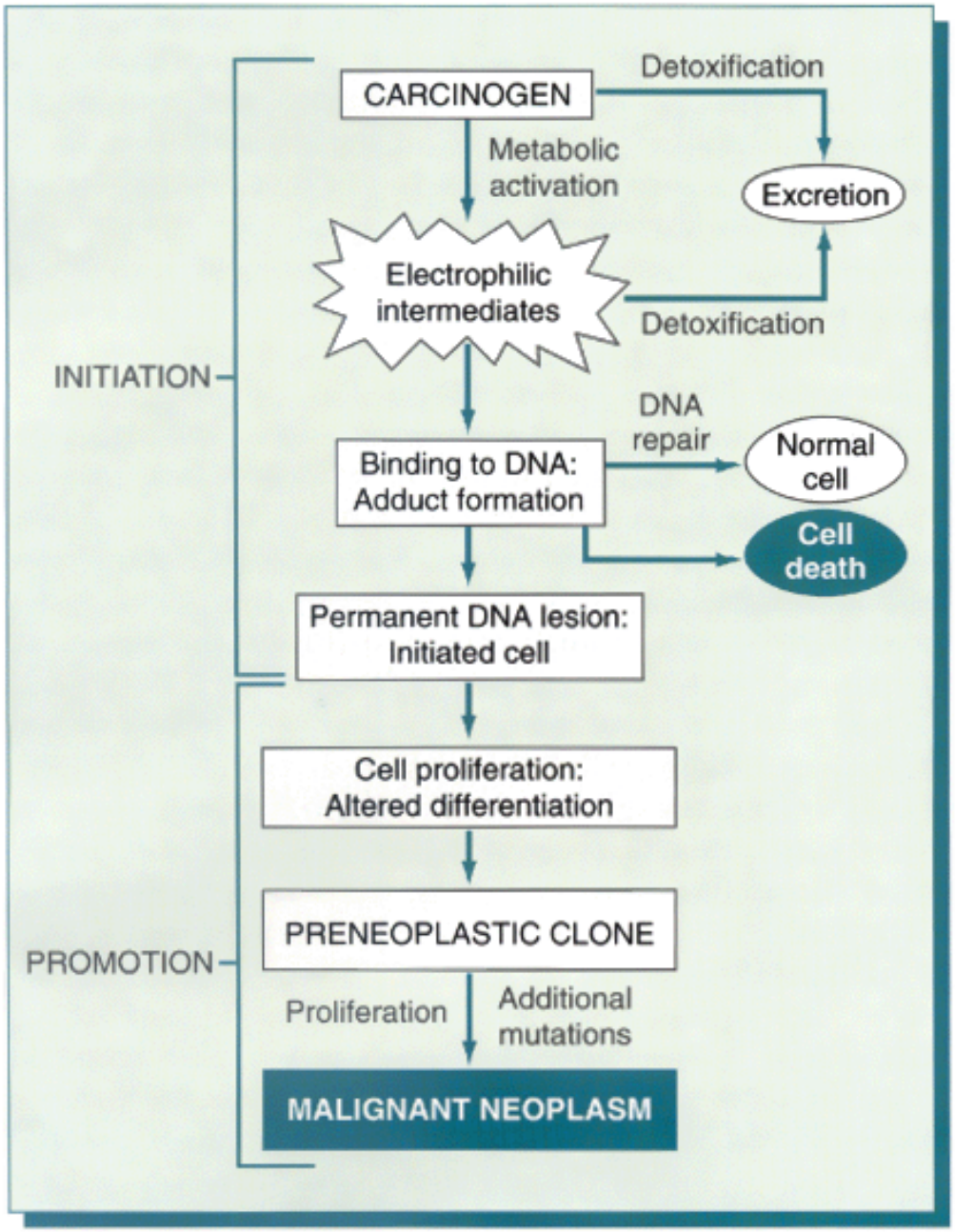

TABLE 7-11 -- Major Chemical Carcinogens

Direct-Acting Carcinogens
Alkylating Agents

β-Propiolactone
Dimethyl sulfate
Diepoxybutane
Anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, and others)
Acylating Agents
1-Acetyl-imidazole
Dimethylcarbamyl chloride
Procarcinogens That Require Metabolic Activation
Polycyclic and Heterocyclic Aromatic Hydrocarbons
Benz(a)anthracene
Benzo(a)pyrene
Dibenz(a,h)anthracene
3-Methylcholanthrene
7,12-Dimethylbenz(a)anthracene
Aromatic Amines, Amides, Azo Dyes
2-Naphthylamine (β-naphthylamine)
Benzidine
2-Acetylaminofluorene
Dimethylaminoazobenzene (butter yellow)
Natural Plant and Microbial Products
Aflatoxin B_1
Griseofulvin
Cycasin
Safrole
Betel nuts
Others
Nitrosamine and amides
Vinyl chloride, nickel, chromium
Insecticides, fungicides

of promoters leads to proliferation and clonal expansion of initiated (mutated) cells. Initiated cells respond differently to promoters than do normal cells and hence expand selectively. Such cells (especially after *RAS* activation) have reduced growth factor requirements and may also be less responsive to growth inhibitory signals in their extracellular milieu. Forced to proliferate, the initiated clone of cells suffers additional mutations, developing eventually into a malignant tumor. Thus, the process of tumor promotion includes multiple steps: Proliferation of preneoplastic cells, malignant conversion, and eventually tumor progression, which depends on changes in tumor cells and the tumor stroma.

The initiation-promotion sequence of chemical carcinogenesis raises an important question: *Since promoters are not mutagenic, how do they contribute to tumorigenesis?* Although the effects of tumor promoters are pleiotropic, induction of cell proliferation is a sine qua non of tumor promotion. TPA (tetradecanoyl phorbol-13 acetate), a phorbol ester tumor promoter, is a powerful activator of protein kinase C (see Chapter 3), an enzyme that phosphorylates several substrates involved in signal transduction pathways, including those activated by growth factors. The promoting effect of phenobarbital in liver carcinogenesis has been linked to stimulation of cell proliferation associated with blockage of the TGF-β pathway.[154]

The concept that sustained cell proliferation increases the risk of mutagenesis and hence neoplastic transformation is also applicable to human carcinogenesis. For example, pathologic hyperplasia of the endometrium (Chapter 22) and increased regenerative activity that accompanies chronic liver cell injury (Chapter 18) are associated with the development of cancer in these organs.

Carcinogenic Chemicals

Before closing this discussion of chemical carcinogenesis, we briefly describe some initiators (see Table 7-11) and promoters of chemical carcinogenesis, with special emphasis on those that have been linked to cancer development in humans.[155]

Direct-Acting Alkylating Agents.

These agents are activation independent, and in general they are weak carcinogens. Nonetheless, they are important because many therapeutic agents (e.g., cyclophosphamide, chlorambucil, busulfan, and melphalan) fall into this category. These are used as anticancer drugs but have been documented to induce lymphoid neoplasms, leukemia, and other forms of cancer. Some alkylating agents, such as cyclophosphamide, are also powerful immunosuppressive agents and are therefore used in treatment of immunologic disorders, including rheumatoid arthritis and Wegener granulomatosis. Although the risk of induced cancer with these agents is low, judicious use of them is indicated. Alkylating agents appear to exert their therapeutic effects by interacting with and damaging DNA, but it is precisely these actions that render them carcinogenic.

Polycyclic Aromatic Hydrocarbons.

These agents represent some of the most potent carcinogens known. They require metabolic activation and can induce tumors in a wide variety of tissues and species. Painted on the skin, they cause skin cancers; injected subcutaneously, they induce sarcomas; introduced into a specific organ, they cause cancers locally. The polycyclic hydrocarbons are of particular interest as carcinogens because they are produced in the combustion of tobacco, particularly with cigarette smoking, and are thought to contribute to the causation of lung and bladder cancers.[156] The various components of cigarette smoke that may be associated with carcinogenicity are listed in Chapter 9 . Polycyclic aromatic hydrocarbons are also produced from animal fats in the process of broiling meats and are present in smoked meats and fish.

Aromatic Amines and Azo Dyes.

The carcinogenicity of most aromatic amines and azo dyes is exerted mainly in the liver, where the "ultimate carcinogen" is formed by the action of the cytochrome P-450 oxygenase systems. Thus, fed to rats, acetylaminofluorene and the azo dyes induce hepatocellular carcinomas (but not cancers of the gastrointestinal tract). An agent implicated in human cancers, β-naphthylamine, is an exception. In the past, it was responsible for a 50-fold increased incidence of bladder cancer in heavily exposed workers in aniline dye and rubber industries.[157] After absorption, it is hydroxylated into an active form, then detoxified by conjugation with glucuronic acid. When excreted in the urine, the nontoxic conjugate is split by the urinary enzyme glucuronidase to release the electrophilic reactant again, thus inducing bladder cancer. Regrettably, humans are one of the few species to possess urinary glucuronidase. Some of the azo dyes were developed as food coloring (e.g., butter yellow to give margarine the appearance of butter and scarlet red to impart the seductive coloration of certain foods such as maraschino cherries). These dyes are now federally regulated in the United States because of the fear that they may be dangerous to humans.

Naturally Occurring Carcinogens.

Among the several known chemical carcinogens produced by plants and microorganisms, the potent hepatic carcinogen aflatoxin B1 is particularly important. This mycotoxin is produced by some strains of the fungus *Aspergillus flavus* that thrive on improperly stored corn, rice, and peanuts. A strong correlation has been found between the dietary level of this hepatocarcinogen and the incidence of hepatocellular carcinoma in some parts of Africa and China. As discussed earlier, the aflatoxin and HBV collaborate in the production of this form of neoplasia.

Nitrosamines and Amides.

These carcinogens are of interest because of the possibility that they are formed in the gastrointestinal tract of humans and so may contribute to the induction of some forms of cancer, particularly gastric carcinoma. They are derived in the stomach from the reaction of nitrostable amines and nitrate used as a preservative, which is converted to nitrites by bacteria. Concerns about these agents have led many to shun processed food containing nitrate preservatives.

Miscellaneous Agents.

Scores of other chemicals have been indicted as carcinogens. Only a few that represent important industrial hazards are listed in Table 7-3 and are briefly mentioned here. Occupational exposure to *asbestos* has been associated with an increased incidence of bronchogenic carcinomas, mesotheliomas, and gastrointestinal cancers, as discussed in Chapter 15 . Concomitant cigarette smoking heightens the risk of bronchogenic carcinoma manyfold. *Vinyl chloride,* the monomer from which the polymer polyvinyl chloride is fabricated, was first identified as a carcinogen in animals, but investigations soon disclosed a scattered incidence

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of the extremely rare hemangiosarcoma of the liver among workers exposed to this chemical. *Chromium, nickel,* and other metals, when volatilized and inhaled in industrial environments, have caused cancer of the lung. Skin cancer associated with arsenic is also well established. Similarly, there is reasonable evidence that many insecticides, such as aldrin, dieldrin, and chlordane and the polychlorinated biphenyls, are carcinogenic in animals (Chapter 9).

Promoters of Chemical Carcinogenesis.

Certain promoters may contribute to cancers in humans. It has been argued that promoters are at least as important as initiating chemicals in carcinogenesis because cells initiated by exposure to environmental carcinogens are innocuous unless subjected to repeated assault by promoters. Tumor promotion may occur after exposure to exogenous agents, such as cigarette smoke or viral infections, that cause tissue damage and reactive hyperplasia. Perhaps more serious, because they are difficult to control, are endogenous promoters such as hormones and bile salts. Hormones such as estrogens serve in animals as promoters of liver tumors. The prolonged use of diethylstilbestrol is implicated in the production of postmenopausal endometrial

carcinoma and in the causation of vaginal cancer in offspring exposed in utero (Chapter 22). Intake of high levels of dietary fat has been associated with increased risk of colon cancer. This may be related to an increase in synthesis of bile acids, which have been shown to act as promoters in experimental models of colon cancer. Alcohol consumption increases the risk of development of cancers of the mouth, pharynx, and larynx by more than tenfold, probably by acting as a promoting agent (Chapter 9).

RADIATION CARCINOGENESIS

Radiant energy, whether in the form of the UV rays of sunlight or as ionizing electromagnetic and particulate radiation, can transform virtually all cell types in vitro and induce neoplasms in vivo in both humans and experimental animals. UV light is clearly implicated in the causation of skin cancers, and ionizing radiation exposure from medical or occupational exposure, nuclear plant accidents, and atomic bomb detonations have produced a variety of forms of malignant neoplasia. Although the contribution of radiation to the total human burden of cancer is probably small, the well-known latency of radiant energy and its cumulative effect require extremely long periods of observation and make it difficult to ascertain its full significance. An increased incidence of breast cancer has become apparent decades later among women exposed during childhood to the atomic bomb. The incidence peaked during 1988–1992 and then declined during the period 1993–1997.[158] Moreover, radiation's possible additive or synergistic effects with other potential carcinogenic influences add another dimension to the picture. The effects of UV light on DNA differ from those of ionizing radiation. The cellular and molecular effects of ionizing radiation are discussed in Chapter 9 .

Ultraviolet Rays

There is ample evidence from epidemiologic studies that *UV rays* derived from the sun induce an increased incidence of squamous cell carcinoma, basal cell carcinoma, and possibly malignant melanoma of the skin.[159] The degree of risk depends on the type of UV rays, the intensity of exposure, and the quantity of light-absorbing "protective mantle" of melanin in the skin. Persons of European origin who have fair skin that repeatedly gets sunburned but stalwartly refuses to tan and who live in locales receiving a great deal of sunlight (e.g., Queensland, Australia, close to the equator) have among the highest incidence of skin cancers in the world. The UV portion of the solar spectrum can be divided into three wavelength ranges: UVA (320 to 400 nm), UVB (280 to 320 nm), and UVC (200 to 280 nm). Of these, UVB is believed to be responsible for the induction of cutaneous cancers. UVC, although a potent mutagen, is not considered significant because it is filtered out by the ozone shield around the earth (hence the concern about ozone depletion).

UV rays have a number of effects on cells, including inhibition of cell division, inactivation of enzymes, induction of mutations and, in sufficient dosage, death of cells. *The carcinogenicity of UVB light is attributed to its formation of pyrimidine dimers in DNA.* This type of DNA damage is repaired by the nucleotide excision repair (NER) pathway. There are five steps in NER: (1) recognition of the DNA lesion, (2) incision of the damaged strand on both sites of the lesion, (3) removal of the damaged nucleotide, (4) synthesis of a nucleotide patch, and (5) its ligation. In mammalian cells, the process may involve 30 or more proteins. It is postulated that with excessive sun exposure, the capacity of the NER pathway is overwhelmed; hence, some DNA damage remains unrepaired. This leads to large transcriptional errors and, in some instances, cancer. The importance of the NER pathway of DNA repair is most graphically illustrated by a study of patients with the hereditary disorder *xeroderma pigmentosum*. This autosomal recessive disorder is characterized by extreme photosensitivity, a 2000-fold increased risk of skin cancer in sun-exposed skin and, in some cases, neurologic abnormalities. The molecular basis of the degenerative changes in sun-exposed skin and occurrence of cutaneous tumors rests on an inherited inability to repair UV-induced DNA damage. Xeroderma pigmentosum is a genetically heterogeneous condition, with at least seven different variants. Each of these is caused by a mutation in one of several genes involved in NER.[160]

As with other carcinogens, UVB also causes mutations in oncogenes and tumor suppressor genes. In particular, mutant forms of the *RAS* and *p53* genes have been detected both in human skin cancers and in UVB-induced cancers in mice. These mutations occur mainly at dipyrimidine sequences within the DNA, thus implicating UVB-induced genetic damage in the causation of skin cancers. In animal models, *p53* mutations occur early after exposure to UVB, before the appearance of tumors.

Ionizing Radiation

Electromagnetic (x-rays, γ rays) and particulate (α particles, β particles, protons, neutrons) radiations are all carcinogenic. The evidence is so voluminous that a few examples suffice. Many of the pioneers in the development of X-rays developed skin cancers. Miners of radioactive elements in central Europe and the Rocky Mountain region of the United States have a tenfold increased incidence of lung cancers. Most telling is the follow-up of survivors of the atomic bombs dropped on

Hiroshima and Nagasaki. Initially, there was a marked increase in the incidence of leukemias—principally acute and chronic myelocytic leukemia—after an average latent period of about 7 years. Subsequently the incidence of many solid tumors with longer latent periods (e.g., breast, colon, thyroid, and lung) increased.

Residents of the Marshall Islands were exposed on one occasion to accidental fallout from a hydrogen bomb test that contained thyroid-seeking radioactive iodines. As many as 90% of the children under age 10 years on Rongelap Island developed thyroid nodules within 15 years, and about 5% of these nodules proved to be thyroid carcinomas. A marked increase in the incidence of thyroid cancer has also been noted in areas exposed to the fallout from the nuclear power plant accident in Chernobyl in 1986. In addition to approximately 30 deaths that occurred at the time of the accident, more than 2000 cases of thyroid cancers have been recorded in children living in the area.[161] Cytogenetic studies have detected an elevated frequency of chromosomal alterations in persons who did cleanup work at the power plant after the accident.[162] It is evident that radiant energy—whether absorbed in the pleasant form of sunlight, through the best intentions of a physician, or by tragic exposure to an atomic bomb blast or radiation released by nuclear plant accidents—has awesome carcinogenic potential. Even therapeutic irradiation has been documented to be carcinogenic. Thyroid cancers have developed in approximately 9% of those exposed during infancy and childhood to head and neck radiation. The previous practice of treating ankylosing spondylitis with therapeutic irradiation yielded a 10- to 12-fold increase in the incidence of leukemia years later.

In humans, there is a hierarchy of vulnerability of different tissues to radiation-induced cancers. Most frequent are the leukemias, except for chronic lymphocytic leukemia, which, for unknown reasons, almost never develops after radiation. Cancer of the thyroid follows closely but only in the young. In the intermediate category are cancers of the breast, lungs, and salivary glands. In contrast, skin, bone, and the gastrointestinal tract are relatively resistant to radiation-induced neoplasia, even though the gastrointestinal epithelial cells are vulnerable to the acute cell-killing effects of radiation, and the skin is in the pathway of all external radiation. Nonetheless, the physician dare not forget: practically *any* cell can be transformed into a cancer cell by sufficient exposure to radiant energy.

MICROBIAL CARCINOGENESIS

A large number of DNA and RNA viruses have proved to be oncogenic in a wide variety of animals, ranging from amphibia to primates, and the evidence grows stronger that certain forms of human cancer are of viral origin. In the following discussion, the better-characterized and most intensively studied human oncogenic viruses are presented first. This is followed by a brief account of the association between infection by the bacterium *Helicobacter pylori* and gastric tumors.

Oncogenic DNA Viruses

Several DNA viruses have been associated with the causation of cancer in animals.[163] Some, such as adenoviruses, cause tumors only in laboratory animals, whereas others, such as the bovine papillomaviruses, cause benign as well as malignant neoplasms in their natural hosts. Of the various human DNA viruses, four (papillomaviruses [HPV], Epstein-Barr virus [EBV], hepatitis B virus [HBV], and Kaposi sarcoma herpesvirus [KSHV]) are of particular interest because they have been implicated in the causation of human cancer. KSHV is discussed in Chapter 6 and Chapter 11 . Although not a DNA virus, hepatitis C virus (HCV) is also associated with cancer. Before we discuss the role of these viruses in carcinogenesis, a few general comments relating to transformation by DNA viruses are offered:

- The genomes of oncogenic DNA viruses integrate into and form stable associations with the host cell genome. The virus is unable to complete its replicative cycle because the viral genes essential for completion of replication are interrupted during integration of viral DNA. Thus, the virus can remain in a latent state for years.
- Those viral genes that are transcribed early in the viral life cycle (early genes) are important for transformation, and are expressed in transformed cells.

Human Papillomavirus.

Approximately 70 genetically distinct types of HPV have been identified. Some types (e.g., 1, 2, 4, and 7) cause benign squamous papillomas (warts) in humans. Human papillomaviruses

have been implicated in the genesis of several cancers, particularly squamous cell carcinoma of the cervix and anogenital region, and in some cases, to the causation of oral and laryngeal cancers (Chapter 16).[164]

Epidemiologic studies suggest that carcinoma of the cervix is caused by a sexually transmitted agent, and HPV is the culprit. DNA sequences of HPV 16 and 18 and, less commonly, HPV 31, 33, 35, and 51 are found in approximately 85% of invasive squamous cell cancers and their presumed precursors (severe dysplasias and carcinoma in situ). In contrast to cervical cancers, genital warts with low malignant potential are associated with distinct HPV types, predominantly HPV 6 and HPV 11 ("low-risk" types).

Molecular analyses of HPV-associated carcinomas and benign genital warts reveal differences that may be pertinent to the transforming activity of these viruses. In benign warts and in preneoplastic lesions, the HPV genome is maintained in an episomal (nonintegrated) form, whereas in cancers, the viral DNA is usually integrated into the host cell genome. This suggests that integration of viral DNA is important in malignant transformation. Although the site of viral integration in host chromosomes is random (the viral DNA is found at different locations in different cancers), the pattern of integration is clonal; that is, the site of integration is identical within all cells of a given cancer. This would not occur if HPV were merely a passenger that infects cells after transformation. Furthermore, the viral DNA is interrupted at a fairly constant site in the process of integration: It is almost always within the E1/E2 open reading frame of the viral genome. Because the E2 region of the viral DNA normally represses the transcription of the E6 and E7 early viral genes, its interruption causes over-expression of the E6 and E7 proteins of HPV 16 and HPV 18. The oncogenic potential of HPV 16 and HPV 18 can be related to these two early viral gene products, which act in conjunction to immortalize and transform cells.[162] [165] The replication of DNA viruses is dependent on the replication machinery of

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the host cells, and E6 and E7 act to overcome the activity of cell-cycle inhibitors (Fig. 7-50).[166] E6 binds to p53 and E7 binds to RB, inducing the degradation of these proteins. In addition, E7 can interfere with *p53* transcriptional activity and also inactivate *p21*. Thus, E6 and E7 block *p53* and *RB* cell cycle suppression pathways. The affinity of these viral proteins for the products of tumor suppressor genes differs depending on the oncogenic potential of HPV. E6 proteins derived from high-risk HPV (HPV 16, 18, and 31) inactivate p53 by enhancing its degradation through ubiquitin-dependent proteolysis.[167] E6 proteins of low-risk HPV (HPV 6 and 11) bind p53 with low affinity and have no effect on p53 stability. E7 proteins from high-risk HPV strongly bind to RB, disrupting the E2F/RB complex and promoting the degradation of RB. By contrast, E7 proteins from low-risk HPV have lower affinity for RB and have a weak capacity to transform cells. Thus, *E6 and E7 proteins of high-risk HPV disable two important tumor suppressor proteins that regulate the cell cycle*. In HPV-induced tumors, *p53* mutations are extremely uncommon, presumably because loss of *p53* function is accomplished by binding to the E6 oncoprotein. This binding not only blocks the inhibitory effect of p53 on the cell cycle but also interferes with p53 activation after DNA damage, a mechanism that allows DNA repair or elimination of cells with genomic damage. Moreover, E6 may have other effects independent of its binding of p53, such as the activation of telomerase and tyrosine kinases.[164]

The E6-p53 interaction may also offer some clues regarding risk factors for cervical cancer development in infected persons. Human *p53* is polymorphic at amino acid 72, encoding either a proline or arginine residue at that position. It turns out that the arginine-containing p53 at position 72 is much more susceptible to degradation by E6. The arginine form is found more frequently in infected individuals with cervical

Figure 7-50 Effect of HPV proteins E6 and E7 on the cell cycle. E6 and E7 enhance p53 degradation, causing a block in apoptosis and decreased activity of the p21 cell cycle inhibitor. E7 associates with p21 and prevents its inhibition of the Cyclin/CDK4 complex; E7 can bind to RB, removing cell cycle restriction. The net effect of HPV E6 and E7 proteins is to block apoptosis and remove the restrains to cell proliferation (see Fig. 7-29). *(Modified from Münger K, Howley PM: Human papillomavirus immortalization and transformation functions. Virus Research 89:213–228, 2002.)*

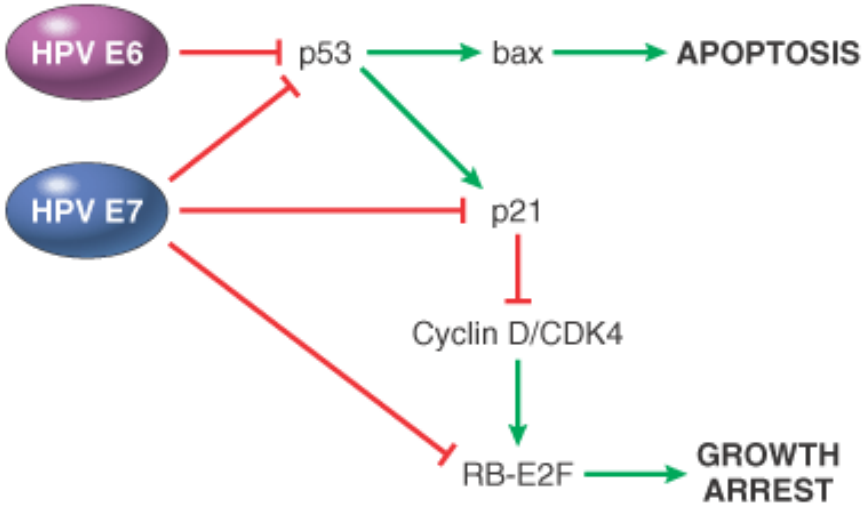


Figure 7-51 Schema depicting the possible evolution of Epstein-Barr virus (EBV)-induced Burkitt lymphoma.

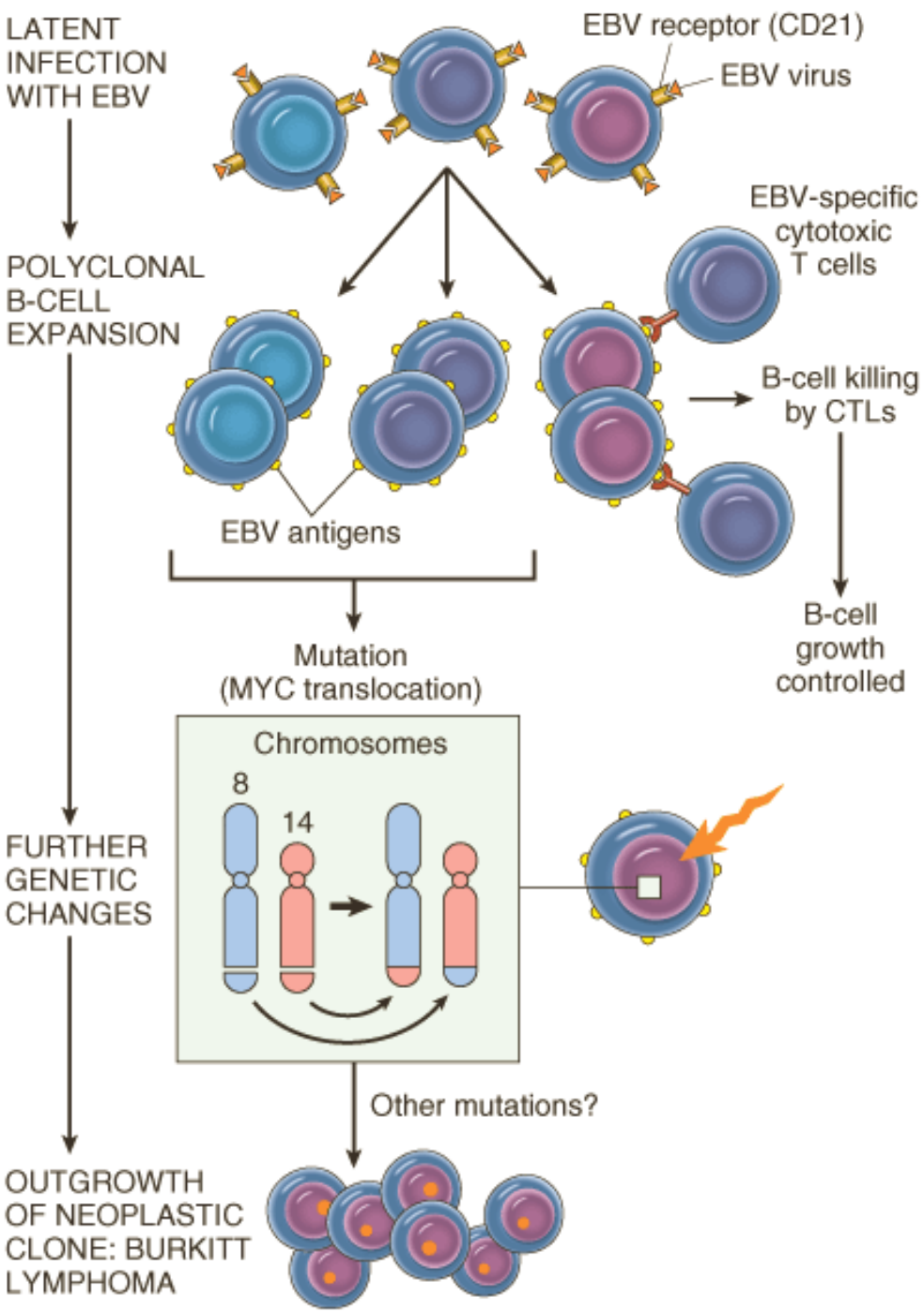


Figure 7-52 Tumor antigens recognized by CD8+ T cells. *(Modified from Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed. Philadelphia, WB Saunders, 2003.)*

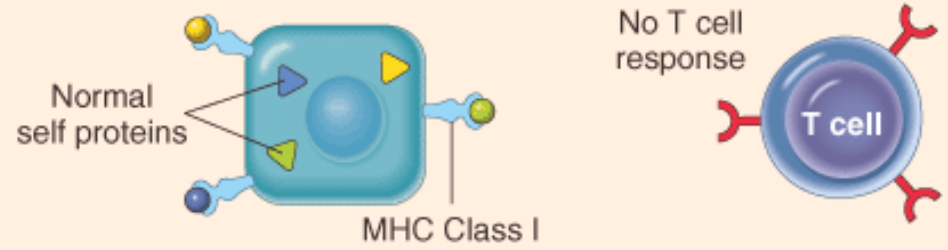
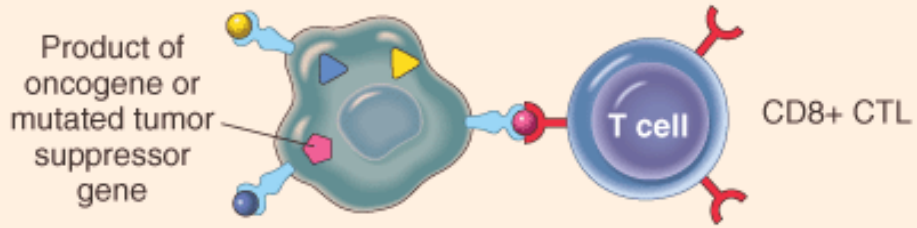
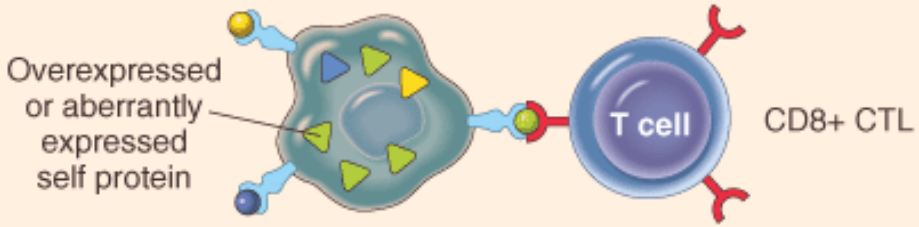
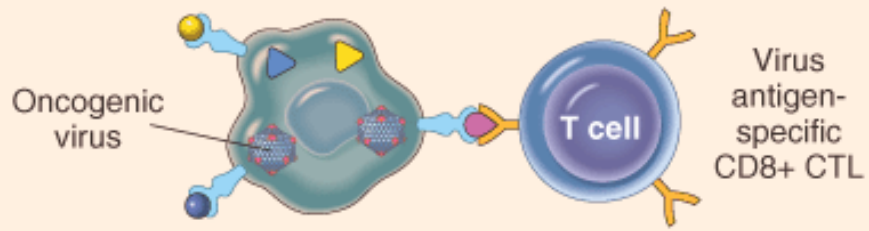
Normal host cell displaying multiple MHC-associated self antigens		EXAMPLES
Tumor cells expressing different types of tumor antigens		Oncogene products: mutated RAS, Bcr/Abl fusion proteins Tumor suppressor gene products: mutated p53 protein
		Various mutant proteins in carcinogen, or radiation, induced animal tumors; various mutated proteins in melanomas
		Overexpressed: tyrosinase, gp100, MART in melanomas Aberrantly expressed: cancer-testis antigens (MAGE, BAGE)
		Human papilloma virus E6, E7 proteins in cervical carcinoma; EBNA proteins in EBV induced lymphoma

Figure 7-53 Mechanisms by which tumors evade the immune system. *(Reprinted from Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed. Philadelphia, WB Saunders, 2003.)*

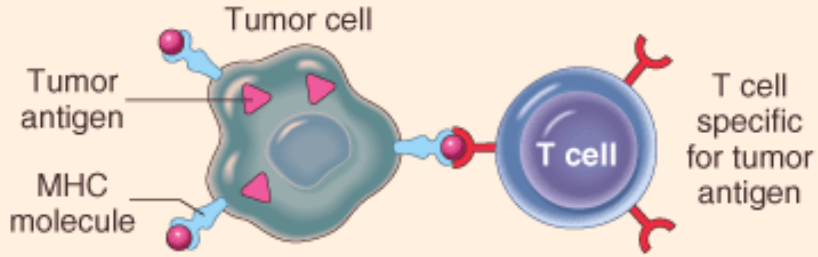
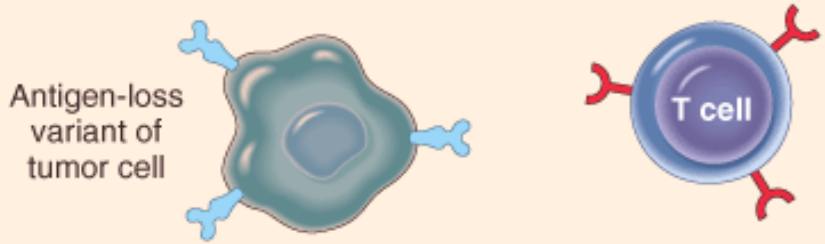
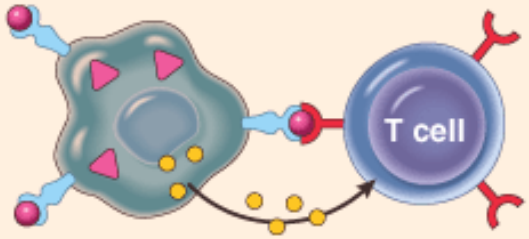
Anti-tumor immunity		T cell recognition of tumor antigen leading to T cell activation
Immune evasion by tumors	**Failure to produce tumor antigen** 	Lack of T cell recognition of tumor
	Mutations in MHC genes or genes needed for antigen processing 	Lack of T cell recognition of tumor
	Production of immuno-suppressive proteins 	Inhibition of T cell activation

TABLE 7-12 -- Paraneoplastic Syndromes

Clinical Syndromes	Major Forms of Underlying Cancer	Causal Mechanism
Endocrinopathies		
Cushing syndrome	Small cell carcinoma of lung	ACTH or ACTH-like substance
	Pancreatic carcinoma	
	Neural tumors	
Syndrome of inappropriate antidiuretic hormone secretion	Small cell carcinoma of lung; intracranial neoplasms	Antidiuretic hormone or atrial natriuretic hormones
Hypercalcemia	Squamous cell carcinoma of lung	Parathyroid hormone-related protein (PTHRP), TGF-α, TNF, IL-1
	Breast carcinoma	
	Renal carcinoma	
	Adult T-cell leukemia/lymphoma	
	Ovarian carcinoma	
Hypoglycemia	Fibrosarcoma	Insulin or insulin-like substance
	Other mesenchymal sarcomas	
	Hepatocellular carcinoma	
Carcinoid syndrome	Bronchial adenoma (carcinoid)	Serotonin, bradykinin
	Pancreatic carcinoma	
	Gastric carcinoma	
Polycythemia	Renal carcinoma	Erythropoietin
	Cerebellar hemangioma	
	Hepatocellular carcinoma	
Nerve and Muscle Syndromes		
Myasthenia	Bronchogenic carcinoma	Immunologic
Disorders of the central and peripheral nervous systems	Breast carcinoma	
Dermatologic Disorders		
Acanthosis nigricans	Gastric carcinoma	Immunologic; secretion of epidermal growth factor
	Lung carcinoma	
	Uterine carcinoma	
Dermatomyositis	Bronchogenic, breast carcinoma	Immunologic
Osseous, Articular, and Soft Tissue Changes		

Hypertrophic osteoarthropathy and clubbing of the fingers	Bronchogenic carcinoma	Unknown
Vascular and Hematologic Changes		
Venous thrombosis (Trousseau phenomenon)	Pancreatic carcinoma	Tumor products (mucins that activate clotting)
	Bronchogenic carcinoma	
	Other cancers	
Nonbacterial thrombotic endocarditis	Advanced cancers	Hypercoagulability
Anemia	Thymic neoplasms	Unknown
Others		
Nephrotic syndrome	Various cancers	Tumor antigens, immune complexes

ACTH, adrenocorticotropic hormone; TGF, transforming growth factor; TNF, tumor necrosis factor; IL, interleukin.

are small. It is thought that PTHRP regulates calcium transport in the lactating breast and across the placenta. Tumors most often associated with paraneoplastic hypercalcemia are carcinomas of the breast, lung, kidney, and ovary. In breast cancers, PTHRP production is associated with osteolytic bone disease, bone metastasis, and humoral hypercalcemia. The most common lung neoplasm associated with hypercalcemia is the squamous cell bronchogenic carcinoma, rather than small cell cancer of the lung (more often associated with endocrinopathies). In addition to PTHRP, several other factors, such as IL-1, TGF-α, TNF, and dihydroxyvitamin D, have also been implicated in causing the hypercalcemia of malignancy.

The *neuromyopathic paraneoplastic syndromes* take diverse forms, such as peripheral neuropathies, cortical cerebellar degeneration, a polymyopathy resembling polymyositis, and a myasthenic syndrome similar to *myasthenia gravis*. The cause of these syndromes is poorly understood. In some cases, antibodies,

presumably induced against tumor cells that cross-react with neuronal cells, have been detected. It is postulated that some neural antigens are ectopically expressed by visceral cancers. For some unknown reason, the immune system recognizes these antigens as foreign and mounts an immune response.

Acanthosis nigricans is characterized by gray-black patches of verrucous hyperkeratosis on the skin. This disorder occurs rarely as a genetically determined disease in juveniles or adults (Chapter 25). In addition, in about 50% of the cases, particularly in those over age 40, the appearance of such lesions is associated with some form of cancer. Sometimes the skin changes appear before discovery of the cancer.

Hypertrophic osteoarthropathy is encountered in 1% to 10% of patients with bronchogenic carcinomas. Rarely, other forms of cancer are involved. This disorder is characterized by (1) periosteal new bone formation, primarily at the distal ends of long bones, metatarsals, metacarpals, and proximal phalanges; (2) arthritis of the adjacent joints; and (3) clubbing of the digits. Although the osteoarthropathy is seldom seen in non-cancer patients, clubbing of the fingertips may be encountered in liver diseases, diffuse lung disease, congenital cyanotic heart disease, ulcerative colitis, and other disorders. The cause of hypertrophic osteoarthropathy is unknown.

Several *vascular and hematologic manifestations* may appear in association with a variety of forms of cancer. As mentioned in the discussion of thrombosis (Chapter 4), *migratory thrombophlebitis* (Trousseau syndrome) may be encountered in association with deep-seated cancers, most often carcinomas of the pancreas or lung. Disseminated intravascular

coagulation may complicate a diversity of clinical disorders (Chapter 13). Acute disseminated intravascular coagulation is most commonly associated with acute promyelocytic leukemia and prostatic adenocarcinoma. Bland, small, nonbacterial fibrinous vegetations sometimes form on the cardiac valve leaflets (more often on left-sided valves), particularly in patients with advanced mucin-secreting adenocarcinomas. These lesions, called *nonbacterial thrombotic endocarditis,* are described further in Chapter 12 . The vegetations are potential sources of emboli that can further complicate the course of cancer.

GRADING AND STAGING OF TUMORS

Prognosis of the course of the disease and the determination of efficacy of various forms of cancer treatment require a high degree of similarity among the tumors being considered. Systems have been developed to express, at least in semiquantitative terms, the level of differentiation, or *grade,* and extent of spread of a cancer within the patient, or *stage,* as parameters of the clinical gravity of the disease.

Grading of a cancer is based on the degree of differentiation of the tumor cells and the number of mitoses within the tumor as presumed correlates of the neoplasm's aggressiveness. Thus, cancers are classified as grades I to IV with increasing anaplasia. Criteria for the individual grades vary with each form of neoplasia and so are not detailed here, but all attempt, in essence, to judge the extent to which the tumor cells resemble or fail to resemble their normal counterparts. Although histologic grading is useful, the correlation between histologic appearance and biologic behavior is less than perfect. In recognition of this problem and to avoid spurious quantification, it is common practice to characterize a particular neoplasm in descriptive terms, for example, well-differentiated, mucin-secreting adenocarcinoma of the stomach, or highly undifferentiated, retroperitoneal malignant tumor—probably sarcoma. In general, with a few exceptions, such as soft tissue sarcomas, grading of cancers has proved of less clinical value than has staging.

The staging of cancers is based on the size of the primary lesion, its extent of spread to regional lymph nodes, and the presence or absence of blood-borne metastases. Two major staging systems are currently in use, one developed by the Union Internationale Contre Cancer (UICC) and the other by the American Joint Committee (AJC) on Cancer Staging. The UICC employs a classification called the *TNM system—T* for primary tumor, *N* for regional lymph node involvement, and *M* for metastases. The TNM staging varies for each specific form of cancer, but there are general principles. With increasing size, the primary lesion is characterized as T1 to T4. T0 is added to indicate an in situ lesion. N0 would mean no nodal involvement, whereas N1 to N3 would denote involvement of an increasing number and range of nodes. M0 signifies no distant metastases, whereas M1 or sometimes M2 indicates the presence of blood-borne metastases and some judgment as to their number.

The AJC employs a somewhat different nomenclature and divides all cancers into stages 0 to IV, incorporating within each of these stages the size of the primary lesions as well as the presence of nodal spread and distant metastases. The staging systems and additional details are mentioned in appropriate chapters, in conjunction with the discussion of specific tumors. It merits emphasis here, however, that staging of neoplastic disease has assumed great importance in the selection of the best form of therapy for the patient. It bears repeating that *staging has proved to be of greater clinical value than grading*. In some cases, such as for lung cancers, staging has been greatly aided by imaging techniques such as *positron emission tomography*.[55]

LABORATORY DIAGNOSIS OF CANCER

Every year the approach to laboratory diagnosis of cancer becomes more complex, more sophisticated, and more specialized. For virtually every neoplasm mentioned in this text, the experts have characterized a number of subcategories; we must walk, however, before we can run. Each of the following sections attempts to present the state of the art, avoiding details of method.

Histologic and Cytologic Methods.

The laboratory diagnosis of cancer is, in most instances, not difficult. The two ends of the benign-malignant spectrum pose no problems; however, in the middle lies a gray zone where one should tread cautiously. The focus here is on the roles of the clinician (often a surgeon) and the pathologist in facilitating the correct diagnosis.

Clinical data are invaluable for optimal pathologic diagnosis, but often clinicians tend to underestimate the value of the clinical data. Radiation changes in the skin or mucosa can be similar

to those associated with cancer. Sections taken from a healing fracture can mimic an osteosarcoma. Moreover the

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laboratory evaluation of a lesion can be only as good as the specimen made available for examination. It must be adequate, representative, and properly preserved. Several sampling approaches are available: (1) excision or biopsy, (2) needle aspiration, and (3) cytologic smears. When excision of a small lesion is not possible, selection of an appropriate site for biopsy of a large mass requires awareness that the margins may not be representative and the center largely necrotic. Appropriate preservation of the specimen is obvious, yet it involves such actions as prompt immersion in a usual fixative (commonly formalin solution, but other fluids can be used), preservation of a portion in a special fixative (e.g., glutaraldehyde) for electron microscopy, or prompt refrigeration to permit optimal hormone, receptor, or other types of molecular analysis. Requesting "quick-frozen section" diagnosis is sometimes desirable, for example, in determining the nature of a mass lesion or in evaluating the margins of an excised cancer to ascertain that the entire neoplasm has been removed. This method permits histologic evaluation within minutes. In experienced, competent hands, frozen-section diagnosis is highly accurate, but there are particular instances in which the better histologic detail provided by the more time-consuming routine methods is needed—for example, when extremely radical surgery, such as the amputation of an extremity, may be indicated. Better to wait a day or two despite the drawbacks, than to perform inadequate or unnecessary surgery.

Fine-needle aspiration of tumors is another approach that is widely used. The procedure involves aspirating cells and attendant fluid with a small-bore needle, followed by cytologic examination of the stained smear. This method is used most commonly for the assessment of readily palpable lesions in sites such as the breast, thyroid, and lymph nodes. Modern imaging techniques enable the method to be extended to lesions in deep-seated structures, such as pelvic lymph nodes and pancreas. Fine-needle aspiration is less invasive and more rapidly performed than are needle biopsies. In experienced hands, it is an extremely reliable, rapid, and useful technique.

Cytologic (Pap) smears provide yet another method for the detection of cancer (Chapter 22). This approach is widely used

Figure 7-54 A normal cervicovaginal smear shows large, flattened squamous cells and groups of metaplastic cells; interspersed are some neutrophils. There are no malignant cells. *(Courtesy of Dr. P.K. Gupta, University of Pennsylvania, Philadelphia, PA.)*

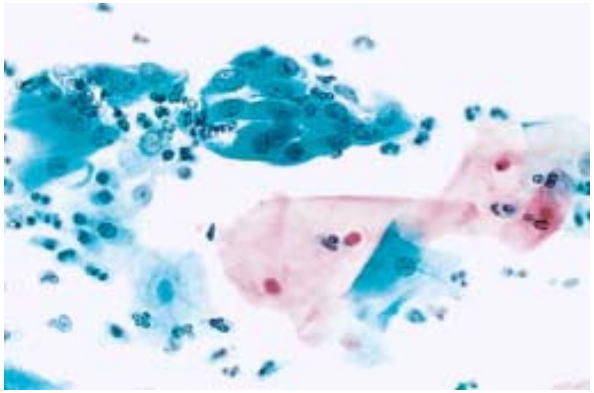

Figure 7-55 An abnormal cervicovaginal smear shows numerous malignant cells that have pleomorphic, hyperchromatic nuclei; interspersed are some normal polymorphonuclear leukocytes. *(Courtesy of Dr. P.K. Gupta, University of Pennsylvania, Philadelphia, PA.)*

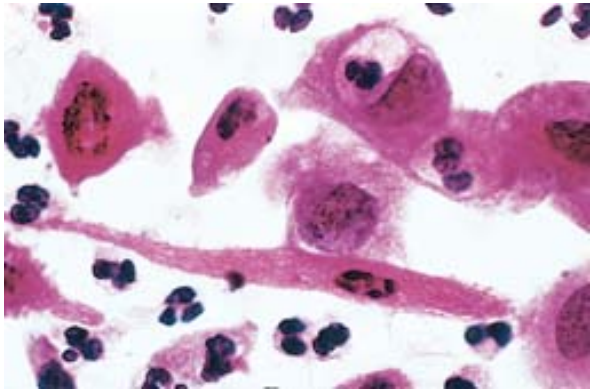

Figure 7-56 Anticytokeratin immunoperoxidase stain of a tumor of epithelial origin (carcinoma). *(Courtesy of Dr. Melissa Upton, University of Washington, Seattle, WA.)*

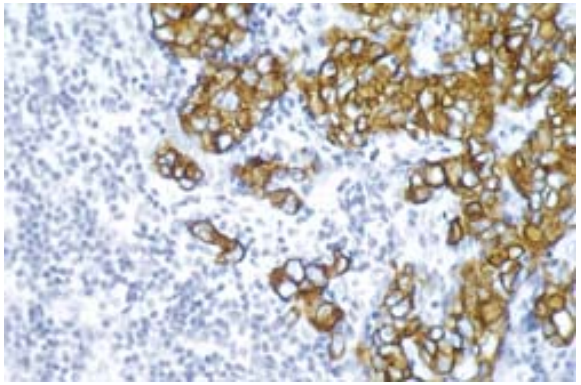

TABLE 7-13 -- Selected Tumor Markers

Markers	**Associated Cancers**
Hormones	
Human chorionic gonadotropin	Trophoblastic tumors, nonseminomatous testicular tumors
Calcitonin	Medullary carcinoma of thyroid
Catecholamine and metabolites	Pheochromocytoma and related tumors
Ectopic hormones	See Paraneoplastic Syndromes in Table 7-12
Oncofetal Antigens	
α-Fetoprotein	Liver cell cancer, nonseminomatous germ cell tumors of testis
Carcinoembryonic antigen	Carcinomas of the colon, pancreas, lung, stomach, and heart
Isoenzymes	
Prostatic acid phosphatase	Prostate cancer

Neuron-specific enolase	Small cell cancer of lung, neuroblastoma
Specific Proteins	
Immunoglobulins	Multiple myeloma and other gammopathies
Prostate-specific antigen and prostate-specific membrane antigen	Prostate cancer
Mucins and Other Glycoproteins	
CA-125	Ovarian cancer
CA-19-9	Colon cancer, pancreatic cancer
CA-15-3	Breast cancer
New Molecular Markers	
p53, *APC*, *RAS* mutations in stool and serum	Colon cancer
p53 and *RAS* mutations in stool and serum	Pancreatic cancer
p53 and *RAS* mutations in sputum and serum	Lung cancer
p53 mutations in urine	Bladder cancer

development of tests to detect cancer markers in blood and body fluids is an active area of research. Some of the markers being evaluated include the detection of mutated *APC, p53,* and *RAS* in the stool of patients with colorectal carcinomas; the presence of mutated *p53* and of hypermethylated genes in the sputum of patients with lung cancer and in the saliva of patients with head and neck cancers; and the detection of mutated *p53* in the urine of patients with bladder cancer.[207]

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Chapter 8 - Infectious Diseases

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General Principles of Microbial Pathogenesis

Despite the availability and use of effective vaccines and antibiotics, infectious diseases remain an important cause of death in the United States and worldwide. In the United States, two of the top 10 leading causes of death are infectious diseases (pneumonia and influenza, and septicemia).[1] Infectious diseases are particularly important causes of death among the elderly and people with acquired immunodeficiency syndrome (AIDS), those with chronic diseases, and those receiving immunosuppressive drugs. In developing countries, unsanitary living conditions and malnutrition contribute to a massive burden of infectious diseases that kills more than 10 million people each year. Most of these deaths are among children, especially from respiratory and diarrheal infections.[2]

HISTORY

The history of infectious disease pathology is intertwined with that of microbiology. Some of the major historical events in these fields are briefly described here to provide a perspective for the concepts of pathogenesis to be discussed later. Some important experiments that were performed in the past would not be ethically acceptable today.

Louis Pasteur and Robert Koch were pioneers in establishing the microbiologic etiology of infectious diseases. Pasteur is credited with proving that microorganisms can cause disease (the germ theory of disease). Pasteur also created the first attenuated vaccines, including a rabies vaccine for humans in 1885. In 1882, Koch championed criteria for linking a specific microorganism to a disease. Koch's postulates require that (1) the organism is found in the lesions of the disease, (2) the organism can be isolated as single colonies on solid media, (3) inoculation of the organism causes lesions in experimental animals, and (4) the organism can be recovered from the experimental animal. Koch also isolated the bacteria that cause tuberculosis *(Mycobacterium tuberculosis)* and anthrax *(Bacillus anthracis)*.

Ronald Ross, an English military physician posted in India, demonstrated in 1897 that mosquitoes carry malaria. At the time, it was believed that malaria was caused by breathing the air near swamps ("malaria" comes from the Italian for "bad air"). Ross's demonstration that *Anopheles* mosquitoes transmit malaria led to public health efforts to reduce malaria through control of mosquitoes. This was successful in the United States, but malaria continues to be a major health problem in many parts of the world.

Walter Reed, an American military physician, led a team of investigators in Cuba in 1900 who demonstrated that yellow fever, like malaria, is transmitted by the bite of mosquitoes. Military volunteers allowed themselves to be bitten by mosquitoes that had previously bitten people sick with yellow fever. Following Reed's result, Dr. James Carroll showed in 1901 that yellow fever was caused by a virus. This was the first demonstration that a virus causes disease in humans.

F. Peyton Rous found the first evidence for an infectious cause of cancer in 1909. In 1911, Rous demonstrated that a virus causes sarcoma in chickens. Although a viral cause has not been found for most human cancers, we now know that viruses can contribute to the development of some; such associations include human papillomaviruses and cervical cancer.

The dawn of modern microbiology, which is based on molecular genetics, came in 1944, when Oswald Avery demonstrated that transfer of DNA from virulent to avirulent *Streptococcus pneumoniae* transformed the latter into a virulent phenotype. This showed that DNA is the genetic material, leading to an explosion of research in molecular genetics. Today, the entire genomic sequences of many species, including microbes and humans, are known, and this holds great promise for future research into the pathogenesis, diagnosis, and treatment of infectious diseases. Knowledge of the genomes of the host and pathogens promise to produce a much richer description of the host response to infectious

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agents than the morphologic descriptions of antimicrobial responses in this chapter.

NEW AND EMERGING INFECTIOUS DISEASES

Although infectious diseases such as leprosy have been known since biblical times and parasitic schistosomes and mycobacteria have been demonstrated in Egyptian mummies, a surprising number of new infectious agents continue to be discovered (Table 8-1). The infectious causes of some diseases with significant morbidity and mortality (e.g., *Helicobacter pylori* gastritis, hepatitis B and hepatitis C, human metapneumovirus respiratory disease, and Legionnaire's pneumonia) were previously unrecognized because the infectious agents are difficult to culture. Some infectious agents are genuinely new to humans, e.g., human immunodeficiency virus (HIV), which causes the acquired immunodeficiency syndrome (AIDS); *Borrelia burgdorferi*, which causes Lyme disease; and the coronavirus that may cause severe acute respiratory syndrome (SARS) (Chapter 15). Other infections are much more

TABLE 8-1 -- Some Recently Recognized Infectious Agents and Manifestations

1977	Ebola virus	Epidemic hemorrhagic fever
	Hantaan virus	Hemorrhagic fever with renal disease
	Legionella pneumophila	Legionnaire's disease
	Campylobacter jejuni	Enteritis
1980	HTLV-I	T-cell lymphoma or leukemia
1981	*Staphylococcus aureus*	Toxic shock syndrome
1982	HTLV-II	Hairy cell leukemia
	Escherichia coli O 157:H7	Hemolytic-uremic syndrome
	Borrelia burgdorferi	Lyme disease
1983	HIV	AIDS
	Helicobacter pylori	Gastric ulcers
1985	*Enterocytozoon bieneusi*	Chronic diarrhea
1988	HHV-6	Roseola subitum

	Hepatitis E	Enterically transmitted hepatitis
1989	Hepatitis C	Hepatitis C
	Ehrlichia chaffeensis	Human monocytic ehrlichiosis
1992	*Vibrio cholerae* O 139	New epidemic cholera strain
	Bartonella henselae	Cat-scratch disease
1993	*Encephalitozoon cuniculi*	Opportunistic infections
1994	*Anaplasma phagocytophilium*	Human granulocytic ehrlichiosis (anaplasmosis)
1995	KSHV (HHV-8)	Kaposi sarcoma in AIDS
2001	Human metapneumovirus	Respiratory infections
2002	West Nile virus	Acute flaccid paralysis
2003	SARS coronavirus	Severe acute respiratory syndrome

Adapted from Lederberg J: Infectious disease as an evolutionary paradigm. Emerg Infect Dis 3:417, 1997.

commonly seen because of immunosuppression caused by AIDS (e.g., cytomegalovirus [CMV], Kaposi sarcoma herpesvirus, *Mycobacterium avium-intracellulare*, *Pneumocystis jiroveci* (*carinii*), and *Cryptosporidium parvum*).[3] [4] Finally, infectious diseases that are common in one area may be introduced into a new area. West Nile virus was common in Europe, Asia, and Africa when it was first described in the United States in 1999.

Human demographics and behavior are among the many factors that contribute to the emergence of infectious diseases. AIDS has been predominantly (but not exclusively) a disease of homosexuals and drug abusers in the United States and Western countries, while in Africa, AIDS is predominantly a heterosexual disease that is much more frequent in areas where men remain uncircumcised.[5] Changes in the environment occasionally drive rates of infectious diseases. Reforestation of the eastern United States has led to massive increases in the populations of deer and mice, which carry the ticks that transmit Lyme disease, babesiosis, and ehrlichiosis. [6] Failure of DDT to control the mosquitoes that transmit malaria and the development of drug-resistant parasites have dramatically increased the morbidity and mortality of *Plasmodium falciparum* in Asia, Africa, and Latin America. Microbial adaptation to widespread antibiotic use contributed to the development of new drug-resistant strains of *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, and *Enterococcus faecium*.

AGENTS OF BIOTERRORISM

Sadly, the anthrax attacks in the United States in 2001 transformed the theoretical threat of bioterrorism into reality. The Centers for Disease Control and Prevention (CDC) have evaluated the microorganisms that pose the greatest danger as weapons on the basis of how efficiently disease can be transmitted, how hard the microorganisms are to produce and distribute, how well they can be defended against, and how likely they are to alarm the public and produce widespread fear. The CDC has ranked bioweapons into three categories, A, B, and C, based on these criteria. These agents are listed in Table 8-2 . [7]

Category A agents are the highest-risk agents and can be readily disseminated or transmitted from person to person, can cause high mortality with potential for major public health impact, might cause public panic and social disruption, and require special action for public health preparedness. For example, smallpox is a category A agent owing to its high transmissibility in any climate or season, case mortality rate of 30% or greater, and lack of effective antiviral therapy. This agent can be easily disseminated because of the stability of the virus in aerosol form and the very small dose needed for infection. Smallpox naturally spreads from person to person mainly by respiratory aerosol or by direct contact with virus in skin lesions or contaminated clothing or bedding. Symptoms appear after 7 to 17 days. Initially, there is high fever, headache, and backache, followed by the appearance of the rash, which first appears on

the mucosa of the mouth and pharynx, face, and forearms and later spreads to the trunk and legs and becomes vesicular and later pustular. Because people are infectious during the incubation period, this virus has the potential to continue to spread throughout an unprotected population. Since vaccination ended in the United States in 1972 and vaccination immunity has waned, the population is

TABLE 8-2 -- Potential Agents of Bioterrorism *

Category A Diseases/Agents	Category B Diseases/Agents	Category C Diseases/Agents
• Anthrax *(Bacillus anthracis)*	• Brucellosis (*Brucella* species)	• Emerging infectious disease threats such as Nipah virus and Hantavirus
• Botulism *(Clostridium botulinum* toxin*)*	• Epsilon toxin of *Clostridium perfringens*	
• Plague *(Yersinia pestis)*	• Food safety threats (e.g., *Salmonella* species, *Escherichia coli* 0157: H7, *Shigella*)	
• Smallpox *(Variola major virus)*		
• Tularemia *(Francisella tularensis)*	• Glanders *(Burkholderia mallei)*	
• Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg], arenaviruses [Lassa fever virus and New World arenaviruses], bunyaviruses [e.g. Crimean-Congo hemorrhagic fever and Rift Valley Fever viruses]	• Melioidosis *(Burkholderia pseudomallei)*	
	• Psittacosis *(Chlamydia psittaci)*	
	• Q fever *(Coxiella burnetti)*	
	• Ricin toxin from *Ricinus communis* (castor beans)	
	• Staphylococcal enterotoxin B	
	• Typhus fever *(Rickettsia prowazekii)*	
	• Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis])	
	• Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*)	

**Adapted from Centers for Disease Control Information.*

highly susceptible to smallpox. Recent concern that smallpox could be used for bioterrorism has led to a return of vaccination for selected groups in the U.S. and Israel.

Category B agents are moderately easy to disseminate, produce moderate morbidity but low mortality, and require specific diagnostic and disease surveillance. Many of these agents are foodborne or waterborne. Category C agents include emerging pathogens that could be engineered for mass dissemination because of availability, ease of production and dissemination, potential for high morbidity and mortality, and great impact on health.

CATEGORIES OF INFECTIOUS AGENTS

Infectious agents belong to a wide range of classes and vary in size from the ~27-kD nucleic acid-free prion to 20-nm poliovirus to 10-m tapeworms (Table 8-3).

TABLE 8-3 -- Classes of Human Pathogens and Their Habitats

Taxonomic	Size	Site of Propagation	Sample Species	Disease
Viruses	20–300 nm	Obligate intracellular	Poliovirus	Poliomyelitis
Chlamydiae	200–1000 nm	Obligate intracellular	*Chlamydia trachomatis*	Trachoma, urethritis
Rickettsiae	300–1200 nm	Obligate intracellular	*Rickettsia prowazekii*	Typhus fever
Mycoplasmas	125–350 nm	Extracellular	*Mycoplasma pneumoniae*	Atypical pneumonia
Bacteria	0.8–15 µm	Cutaneous	*Staphylococcus aureus*	Wound
		Mucosal	*Vibrio cholerae*	Cholera
		Extracellular	*Streptococcus pneumoniae*	Pneumonia
		Facultative intracellular	*Mycobacterium tuberculosis*	Tuberculosis
Fungi	2–200 µm	Cutaneous	*Trichophyton* sp.	Tinea pedis (athlete's foot)
		Mucosal	*Candida albicans*	Thrush
		Extracellular	*Sporothrix schenckii*	Sporotrichosis
		Facultative intracellular	*Histoplasma capsulatum*	Histoplasmosis
Protozoa	1–50 µm	Mucosal	*Giardia lamblia*	Giardiasis
		Extracellular	*Trypanosoma gambiense*	Sleeping sickness
		Facultative intracellular	*Trypanosoma cruzi*	Chagas disease
		Obligate intracellular	*Leishmania donovani*	Kala-azar
Helminths	3 mm–10 m	Mucosal	*Enterobius vermicularis*	Enterobiasis
		Extracellular	*Wuchereria bancrofti*	Filariasis
		Intracellular	*Trichinella spiralis*	Trichinosis

Prions

Prions are apparently composed of abnormal forms of a host protein, termed prion protein (PrP).[8] These agents cause transmissible spongiform encephalopathies, including kuru (associated with human cannibalism), Creutzfeldt-Jakob disease (CJD; associated with corneal transplants), bovine spongiform encephalopathy (BSE; better known as mad cow disease), and variant Creutzfeldt-Jakob disease (vCJD; likely transmitted to humans from BSE-infected cattle). [9] PrP is normally found in neurons. Diseases occur when the prion protein undergoes a conformational change that confers resistance to proteases. The protease-resistant PrP promotes conversion of the normal protease-sensitive PrP to the abnormal form, explaining the infectious nature of these diseases. Accumulation of abnormal PrP leads to neuronal damage and distinctive spongiform pathologic changes in the brain.

Spontaneous or inherited mutations in PrP, which make PrP protease resistant, have been observed in the sporadic and familial forms of CJD, respectively. These diseases are discussed in detail in Chapter 28 .

Viruses

Viruses are obligate intracellular parasites that depend on the host cell's metabolic machinery for their replication. They consist of a nucleic acid genome surrounded by a protein coat (called a capsid) that is sometimes encased in a lipid membrane. Viruses are classified by their nucleic acid genome (DNA or RNA but not both), the shape of the capsid (icosahedral or helical), the presence or absence of a lipid

TABLE 8-4 -- Selected Human Viral Diseases and Their Pathogens

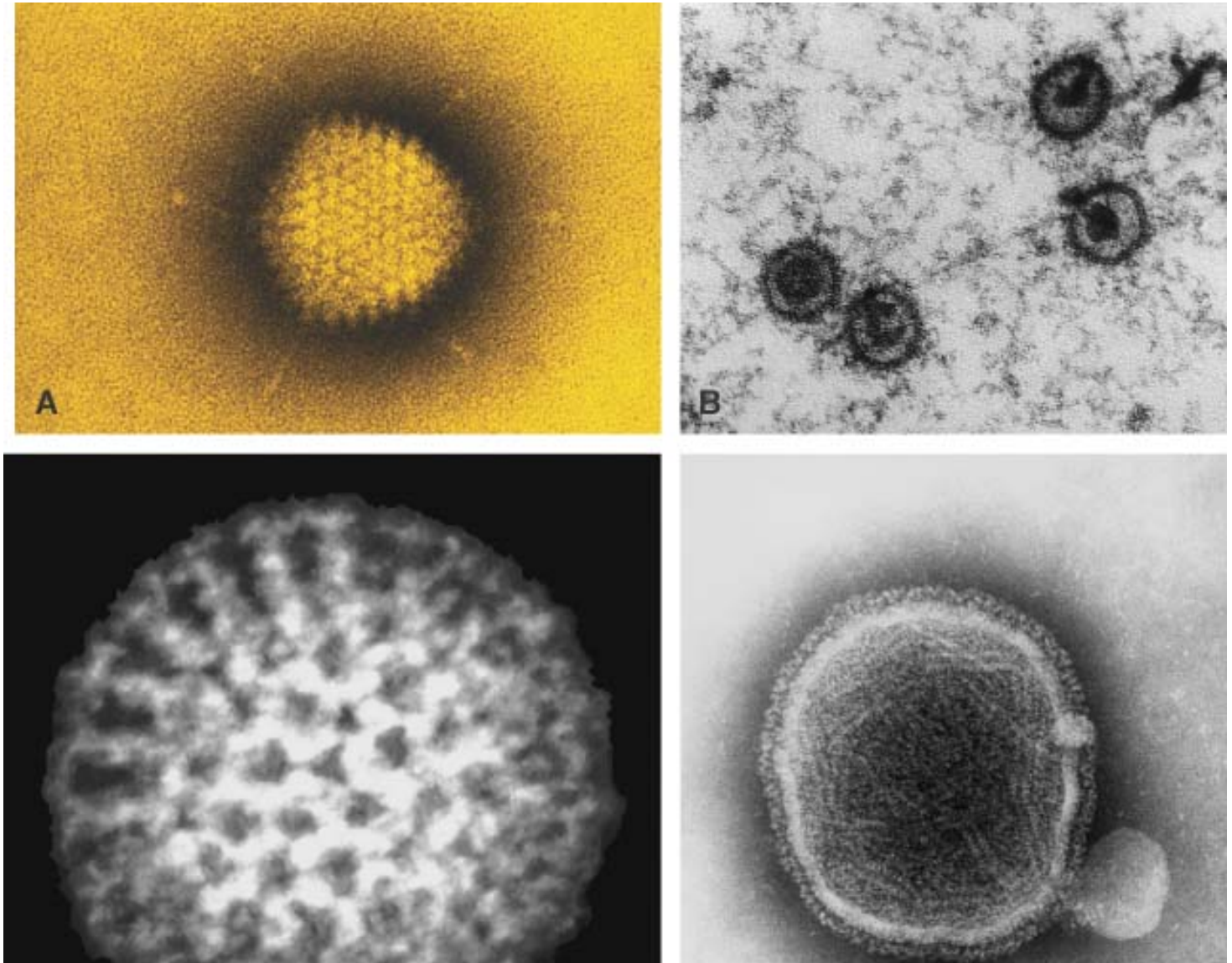
Viral Pathogen	Virus Family	Genomic Type	Disease Expression
Respiratory			
Adenovirus	Adenoviridae	DS DNA	Upper and lower respiratory tract infections, conjunctivitis, diarrhea
Rhinovirus	Picornaviridae	SS RNA	Upper respiratory tract infection
Coxsackievirus	Picornaviridae	SS RNA	Pleurodynia, herpangina, hand-foot-and-mouth disease, SARS
Coronavirus	Coronaviridae	SS RNA	Upper respiratory tract infection
Influenza viruses A, B	Orthomyxoviridae	SS RNA	Influenza
Respiratory syncytial virus	Paramyxoviridae	SS RNA	Bronchiolitis, pneumonia
Digestive			
Mumps virus	Paramyxoviridae	SS RNA	Mumps, pancreatitis, orchitis
Rotavirus	Reoviridae	DS RNA	Childhood diarrhea
Norwalk agent	Caliciviridae	SS RNA	Gastroenteritis
Hepatitis A virus	Picornaviridae	SS RNA	Acute viral hepatitis
Hepatitis B virus	Hepadnaviridae	DS DNA	Acute or chronic hepatitis

Hepatitis D virus	Viroid-like	SS RNA	With HBV, acute or chronic hepatitis
Hepatitis C virus	Flaviviridae	SS RNA	Acute or chronic hepatitis
Hepatitis E virus	Norwalk-like	SS RNA	Enterically transmitted hepatitis
Systemic with Skin Eruptions			
Measles virus	Paramyxoviridae	SS RNA	Measles (rubeola)
Rubella virus	Togaviridae	SS RNA	German measles (rubella)
Parvovirus	Parvoviridae	SS DNA	Erythema infectiosum, aplastic anemia
Vaccinia virus	Poxviridae	DS DNA	Smallpox vaccine
Varicella-zoster virus	Herpesviridae	DS DNA	Chickenpox, shingles
Herpes simplex virus 1	Herpesviridae	DS DNA	"Cold sore"
Herpes simplex virus 2	Herpesviridae	DS DNA	Genital herpes
Systemic with Hematopoietic Disorders			
Cytomegalovirus	Herpesviridae	DS DNA	Cytomegalic inclusion disease
Epstein-Barr virus	Herpesviridae	DS DNA	Infectious mononucleosis
HTLV-I	Retroviridae	SS RNA	Adult T-cell leukemia; tropical spastic paraparesis
HIV-1 and HIV-2	Retroviridae	SS RNA	AIDS
Arboviral and Hemorrhagic Fevers			
Dengue virus 1–4	Togaviridae	SS RNA	Dengue, hemorrhagic fever
Yellow fever virus	Togaviridae	SS RNA	Yellow fever
Regional hemorrhagic fever viruses	Filoviridae	SS RNA	Ebola, Marburg disease
	Hantavirus	SS RNA	Korean, U.S. pneumonia
Warty Growths			
Papillomavirus	Papovaviridae	DS DNA	Condyloma; cervical carcinoma
Central Nervous System			
Poliovirus	Picornaviridae	SS RNA	Poliomyelitis
JC virus	Papovaviridae	DS DNA	Progressive multifocal leukoencephalopathy (opportunistic)
Arboviral encephalitis viruses	Togaviridae	SS RNA	Eastern, Western, Venezuelan, St. Louis,
DS, double-stranded; SS, single-stranded.			

envelope, their mode of replication, the preferred cell type for replication (called tropism), or the type of pathology (Table 8-4). Because viruses are only 20 to 300 nm in size, they are best visualized with the electron microscope (Fig. 8-1). However, some viral particles aggregate within the cells they infect and form characteristic inclusion bodies, which may be seen

with the light microscope and are useful for diagnosis. For example, cytomegalovirus (CMV)-infected cells are enlarged and show a large eosinophilic nuclear inclusion and smaller basophilic cytoplasmic inclusions; herpesviruses form a large nuclear inclusion surrounded by a clear halo; and both smallpox and rabies viruses form characteristic cytoplasmic inclusions. Many viruses do not give rise to inclusions (e.g., Epstein Barr virus [EBV]).

Figure 8-1 The variety of viral structures, as seen by electron microscopy. A, Adenovirus, an icosahedral nonenveloped DNA virus with fibers. B, Epstein Barr virus, an icosahedral enveloped DNA virus. C, Rotavirus, a nonenveloped, wheel-like, RNA virus. D, Paramyxovirus, a spherical enveloped RNA virus. RNA is seen spilling out of the disrupted virus. *(Photos courtesy of Science Source; © Photo Researchers, Inc., New York, New York.)*



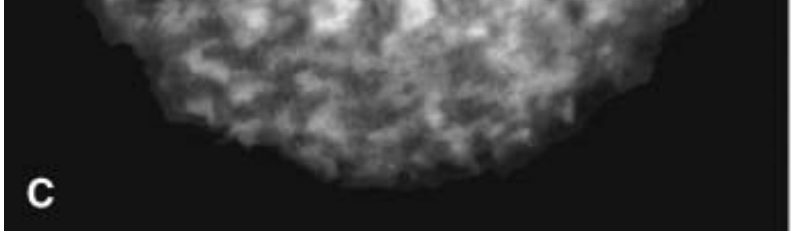


TABLE 8-5 -- Examples of Bacterial, Spirochetal, and Mycobacterial Diseases

Clinical or Microbiologic Category	Species	Frequent Disease Presentations
Infections by pyogenic cocci	*Staphylococcus aureus, S. epidermidis*	Abscess, cellulitis, pneumonia, septicemia
	Streptococcus pyogenes, β-hemolytic	Upper respiratory tract infection, erysipelas, scarlet fever, septicemia
	Streptococcus pneumoniae (pneumoccoccus)	Lobar pneumonia, meningitis
	Neisseria meningitidis (meningococcus)	Cerebrospinal meningitis
	Neisseria gonorrhoeae (gonococcus)	Gonorrhea
Gram-negative infections, common	* *Escherichia coli*	Urinary tract infection, wound infection, abscess, pneumonia, septicemia, endotoxemia, endocarditis
	* *Klebsiella pneumoniae*	
	* *Enterobacter (Aerobacter) aerogenes*	
	* *Proteus* spp. *(P. mirabilis, P. morgagni)*	
	* *Serratia marcescens*	
	* *Pseudomonas* spp. *(P. aeruginosa)*	
	Bacteroides spp. *(B. fragilis)*	Anaerobic infection
	Legionella spp. *(L. pneumophila)*	Legionnaires disease
Contagious childhood bacterial diseases	*Haemophilus influenzae*	Meningitis, upper and lower respiratory tract infections
	Bordetella pertussis	Whooping cough
	Corynebacterium diphtheriae	Diphtheria
Enteropathic infections	Enteropathogenic *E. coli*	Invasive or noninvasive gastroenterocolitis, some with septicemia
	Shigella spp.	
	Vibrio cholerae	
	Campylobacter fetus, C. jejuni	
	Yersinia enterocolitica	

	Salmonella spp. (1000 strains)	
	Salmonella typhi	Typhoid fever
Clostridial infections	*Clostridium tetani*	Tetanus (lockjaw)
	Clostridium botulinum	Botulism (paralytic food poisoning)
	Clostridium perfringens, C. septicum	Gas gangrene, necrotizing cellulitis
	* *Clostridium difficile*	Pseudomembranous colitis
Zoonotic bacterial infections	*Bacillus anthracis*	Anthrax (malignant pustule)
	* *Listeria monocytogenes*	*Listeria* meningitis, listeriosis
	Yersinia pestis	Bubonic plague
	Francisella tularensis	Tularemia
	Brucella melitensis, B. suis, B. abortus	Brucellosis (undulant fever)
	Burkholderia mallei, B. pseudomallei	Glanders, melioidosis
	Leptospira spp. (many groups)	Leptospirosis, Weil disease
	Borrelia recurrentis	Relapsing fever
	Borrelia burgdorferi	Lyme borreliosis
	Bartonella henselae	Cat-scratch disease; bacillary angiomatosis
	Spirillum minus, Streptobacillus moniliformis	Rat-bite fever
Human treponemal infections	*Treponema pallidum*	Venereal, endemic syphilis (bejel)
	Treponema pertenue	Yaws (frambesia)
	Treponema carateum (T. herrejoni)	Pinta (carate, mal del pinto)
Mycobacterial infections	* *Mycobacterium tuberculosis, M. bovis* (Koch bacillus)	Tuberculosis
	M. leprae (Hansen bacillus)	Leprosy
	* *M. kansasii, M. avium, M. intracellulare*	Atypical mycobacterial infections
	M. ulcerans	Buruli ulcer
Actinomycetaceae	* *Nocardia asteroides*	Nocardiosis
	Actinomyces israelii	Actinomycosis

*Important opportunistic infections.

include *Staphylococcus epidermidis* and *Propionibacterium acnes*, the cause of acne. Aerobic and anaerobic bacteria in the mouth, particularly *Streptococcus mutans*, contribute to dental plaque, a major cause of tooth decay. In the colon, 99.9% of bacteria are anaerobic, including *Bacteroides* species. Many bacteria remain extracellular when they invade the body, while others can survive and replicate either outside or inside of host cells (*facultative intracellular* bacteria) and some grow only inside host cells (*obligate intracellular* bacteria).

Chlamydiae, Rickettsiae, Mycoplasmas

These microbes are grouped together because, like other bacteria, they divide by binary fission and are sensitive to antibiotics, but they lack certain structures (e.g., *Mycoplasma* lack a cell wall) or metabolic capabilities (e.g., *Chlamydia* cannot synthesize adenosine triphosphate [ATP]). *Chlamydia* and *Rickettsiae* are obligate intracellular organisms that replicate in membrane-bound vacuoles in epithelial cells and the

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Figure 8-2 Molecules on the surface of Gram-negative and Gram-positive bacteria involved in pathogenesis. Not shown is the type 3 secretory apparatus of Gram-negative bacteria (see text).

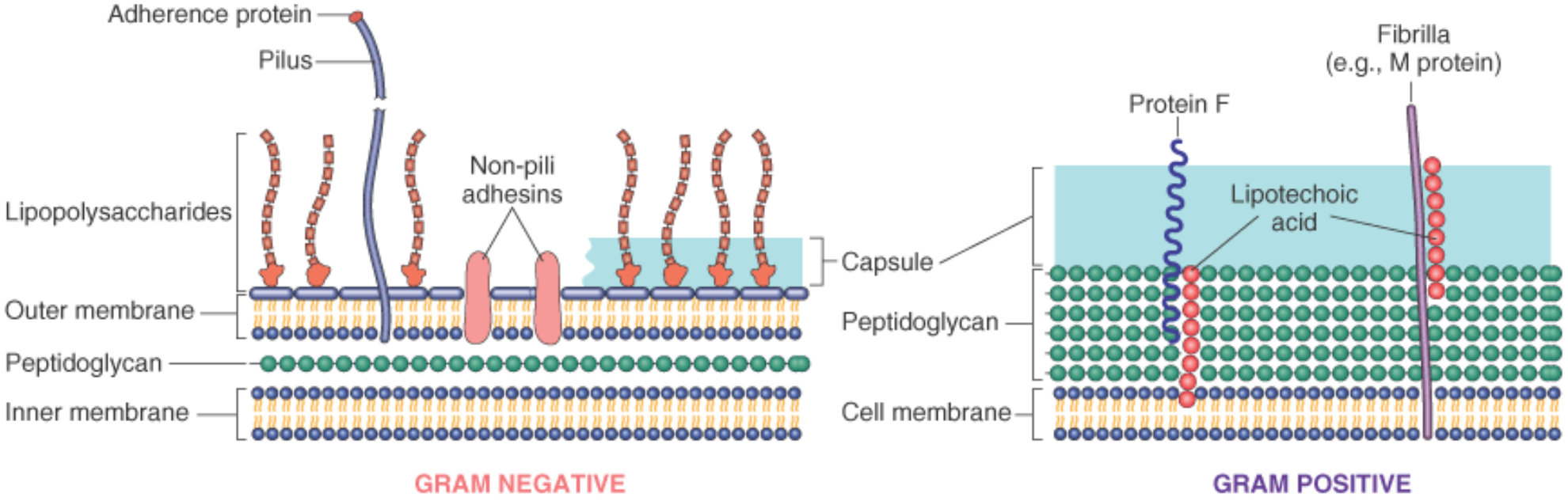

Figure 8-3 The variety of bacterial morphology. *A*, Gram stain of sputum from patient with pneumonia. There are Gram-positive cocci in clusters *(Staphylococcus aureus)* with degenerating neutrophils. *B*, Gram stain of sputum from a patient with pneumonia. Gram-positive, elongated cocci in pairs and short chains *(Streptococcus pneumoniae)* and a neutrophil is seen. *C*, Gram stain of *Clostridium sordellii* grown in culture. A mixture of Gram-positive and Gram-negative rods, many of which have subterminal spores (clear areas), are present. *Clostridia* species often stain as both Gram-positive and negative, although they are true Gram-positive bacteria. *D*, Gram stain of a bronchoalveolar lavage specimen showing Gram-negative intracellular rods typical of Enterobacteriaceae such as *Klebsiella pneumoniae* or *Escherichia coli*. *E*, Gram stain of urethral discharge from a patient with gonorrhea. Many Gram-negative diplococci *(Neisseria gonorrhoeae)* are present within a neutrophil. *F*, Silver stain of brain tissue from a patient with Lyme disease meningoencephalitis. Two helical spirochetes *(Borrelia burgdorferi)* are indicated by arrows. The panels are at different magnifications. *(D, Courtesy of Dr. Karen Krisher, Clinical Microbiology Institute, Wilsonville, OR. All other panels courtesy of Dr. Kenneth Van Horn.)*

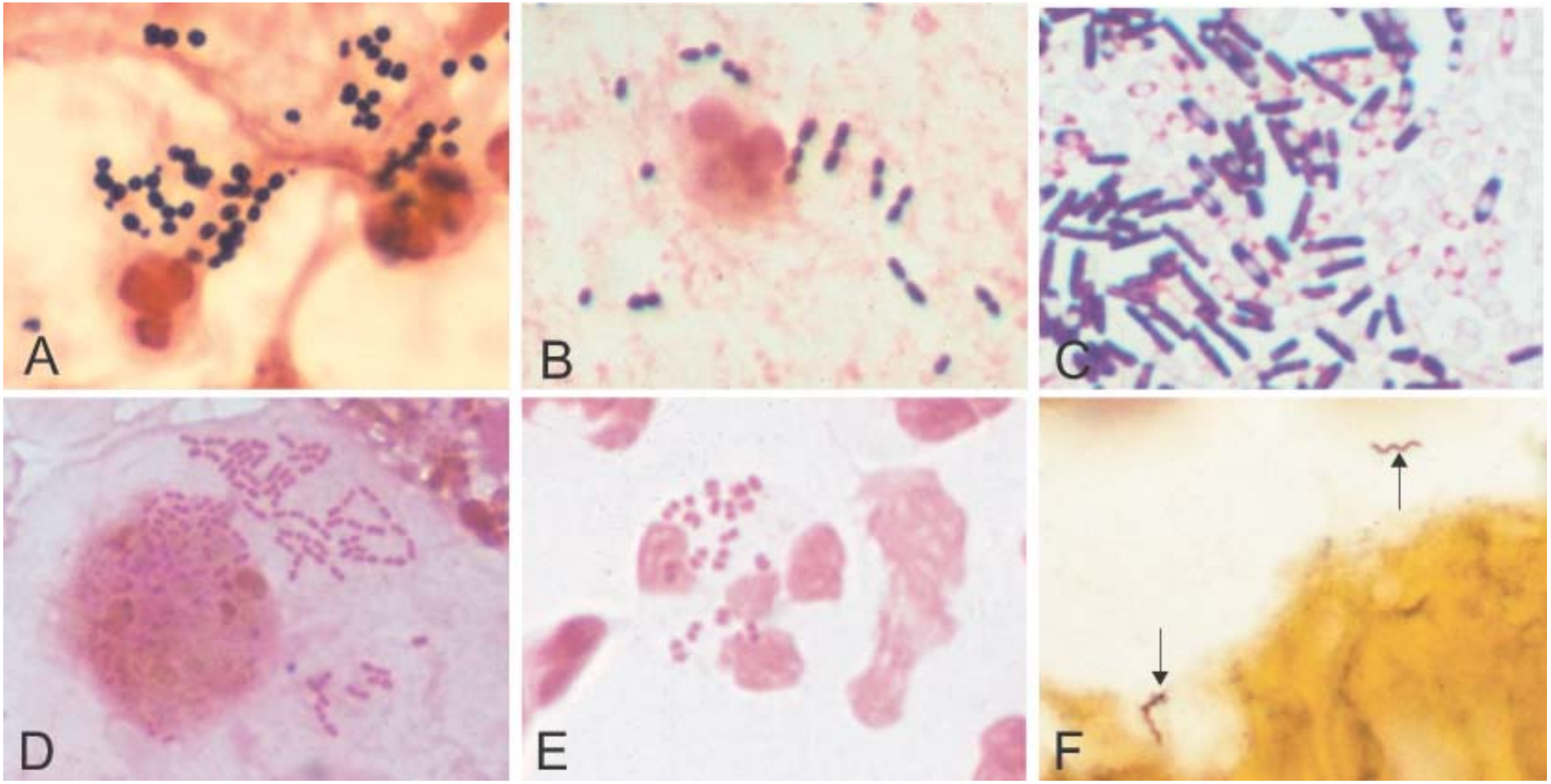

TABLE 8-6 -- Protozoa Pathogenic for Humans

Species	Order	Form, Size	Disease
Luminal or Epithelial			
Entamoeba histolytica	Amebae	Trophozoite 15–20 µm	Amebic dysentery; liver abscess
Balantidium coli	Ciliates	Trophozoite 50–100 µm	Colitis
Naegleria fowleri	Ameboflagellates	Trophozoite 10–20 µm	Meningoencephalitis
Acanthamoeba sp.	Ameboflagellates	Trophozoite 15–30 µm	Meningoencephalitis or ophthalmitis
Giardia lamblia	Mastigophora	Trophozoite 11–18 µm	Diarrheal disease, malabsorption
Isospora belli	Coccidia	Oocyst 10–20 µm	Chronic enterocolitis or malabsorption or both
Cryptosporidium sp.	Coccidia	Oocyst 5–6 µm	

Trichomonas vaginalis	Mastigophora	Trophozoite 10–30 µm	Urethritis, vaginitis
Bloodstream			
Plasmodium species	Hemosporidia	Trophozoites, schizonts, gametes (all small and inside red cells)	Malaria
Babesia microti, B. bovis	Hemosporidia	Trophozoites inside red cells	Babesiosis
Trypanosoma species	Hemoflagellates	Trypomastigote 14–33 µm	African sleeping sickness
Intracellular			
Trypanosoma cruzi	Hemoflagellates	Trypomastigote 20 µm	Chagas disease
Leishmania donovani	Hemoflagellates	Amastigote 2 µm	Kala-azar
Leishmania species	Hemoflagellates	Amastigote 2 µm	Cutaneous and mucocutaneous leishmaniasis
Toxoplasma gondii	Coccidia	Tachyzoite 4–6 µm (cyst larger)	Toxoplasmosis

immunosuppressed individuals do opportunistic fungi give rise to life-threatening infections characterized by tissue necrosis, hemorrhage, and vascular occlusion, with minimal to no inflammatory response. In addition, AIDS patients are victims of the opportunistic fungus *Pneumocystis jiroveci (carinii)*.

Protozoa

Parasitic protozoa are single-celled eukaryotes that are major causes of disease and death in developing countries (Table 8-6). Protozoa can replicate intracellularly within a variety of cells (e.g., *Plasmodium* in red blood cells, *Leishmania* in macrophages) or extracellularly in the urogenital system, intestine, or blood. *Trichomonas vaginalis* are flagellated protozoal parasites that are sexually transmitted and can colonize the vagina and male urethra. The most prevalent intestinal protozoans, *Entamoeba histolytica* and *Giardia lamblia*, have two forms: (1) motile trophozoites that attach to the intestinal epithelial wall and may invade and (2) immobile cysts that are resistant to stomach acids and are infectious when ingested. Blood-borne protozoa (e.g., *Plasmodium*, *Trypanosoma*, and *Leishmania*) are transmitted by insect vectors, in which they replicate before being passed to new human hosts. *Toxoplasma gondii* is acquired either by contact with oocyst-shedding kittens or by eating cyst-ridden, undercooked meat.

Helminths

Parasitic worms are highly differentiated multicellular organisms. Their life cycles are complex; most alternate between sexual reproduction in the definitive host and asexual multiplication in an intermediary host or vector. Thus, depending on parasite species, humans may harbor either adult worms (e.g., *Ascarus lumbricoides*) or immature stages (e.g., *Toxocara canis*) or asexual larval forms (e.g., *Echinococcus* species). Once adult worms take up residence in humans,

they do not multiply but generate eggs or larvae destined for the next phase of the cycle. An exception is *Strongyloides stercoralis*, the larvae of which can become infectious in the gut and cause overwhelming autoinfection in immunosuppressed persons. There are two important consequences of the lack of replication of adult worms: (1) Disease is often caused by inflammatory responses to the eggs or larvae rather than to the adults (e.g., schistosomiasis), and (2) disease is in proportion to the number of organisms that have infected the individual (e.g., 10 hookworms cause little disease, whereas 1000 hookworms cause severe anemia by consuming 100 mL of blood per day).

Ectoparasites

Ectoparasites are insects (lice, bedbugs, fleas) or arachnids (mites, ticks, spiders) that attach to and live on or in the skin. Arthropods may produce disease directly by damaging the human host or indirectly by serving as the vectors for transmission of an infectious agent into a human host. Some arthropods may cause itching and excoriations (e.g., pediculosis caused by lice attached to hair shafts, or scabies caused by mites burrowing into the stratum corneum). At the site of the bite, mouthparts may be found associated with a mixed infiltrate of lymphocytes, macrophages, and eosinophils. In addition, attached arthropods can be vectors for other pathogens. For example, deer ticks transmit the Lyme disease spirochete *Borrelia burgdorferi*.

TRANSMISSION AND DISSEMINATION OF MICROBES

Host Barriers to Infection

The outcome of infection is determined by the ability of the microbe to infect, colonize, and damage host tissues and the ability of host defense mechanisms to eradicate the infection. *Host barriers to infection prevent microbes from entering the body and consist of innate and adaptive immune defenses* [11] (see Fig. 6-1 , Chapter 6). Innate immune defense mechanisms exist before infection and respond rapidly to microbes. These mechanisms include physical barriers to infection, phagocytic cells and natural killer cells, and plasma proteins, including the complement system proteins and other mediators of inflammatory responses (cytokines, collectins, acute phase reactants). Adaptive immune responses are stimulated by exposure to microbes and increase in magnitude, speed, and effectiveness with successive exposures to microbes. Adaptive immunity is mediated by T and B lymphocytes and their products (Chapter 6).

Microbes can enter the host by inhalation, ingestion, sexual transmission, insect or animal bites, or injection. The first barriers to infection are intact host skin and mucosal surfaces and their secretory products. In general, respiratory, gastrointestinal, or genitourinary tract infections occur in healthy persons and are caused by relatively virulent microorganisms that are capable of damaging or penetrating intact epithelial barriers. In contrast, most skin infections in healthy persons are caused by less virulent organisms entering the skin through damaged sites (cuts and burns).

Skin.

The dense, keratinized outer layer of skin is a natural barrier to infection, and the low pH of the skin (about 5.5) and the presence of fatty acids inhibit growth of microorganisms other than residents of the normal flora. Human skin is normally inhabited by a variety of bacterial and fungal species, including some potential opportunists, such as *Staphyloccus epidermidis* and *Canadida albicans*. Although skin is usually an effective barrier, certain types of fungi (dermatophytes) can infect the stratum corneum, hair, and nails, and a few microorganisms are able to traverse the unbroken skin. For example, *Schistosoma* larvae released from freshwater snails penetrate swimmers' skin by releasing collagenase, elastase, and other enzymes that dissolve the extracellular matrix. Most microorganisms, however, penetrate through breaks in the skin, including superficial pricks (fungal infections), wounds (staphylococci), burns *(Pseudomonas aeruginosa)*, and diabetic and pressure-related foot sores (multibacterial infections). Intravenous catheters in hospitalized patients can produce local or systemic infection (bacteremia). Needle sticks can expose the recipient to potentially infected blood and may transmit HBV, HCV, or HIV. Some pathogens penetrate the skin via an insect or animal bite. For instance, bites by fleas, ticks, mosquitoes, mites, and lice break the skin and transmit arboviruses (causes of yellow fever and encephalitis), rickettsiae (Rocky Mountain spotted fever), bacteria (plague, Lyme disease), protozoa (malaria, leishmaniasis), and helminths (filariasis). Animal bites can lead to infections with bacteria or with rabies virus.

Gastrointestinal Tract.

Most gastrointestinal pathogens are transmitted by food or drink contaminated with fecal material. Where hygiene fails, diarrheal disease becomes rampant.

Acidic gastric secretions are important defenses within the gastrointestinal tract and are lethal for many gastrointestinal pathogens.[11] Healthy volunteers do not become infected by *Vibrio cholerae* unless they are fed $10^{[11]}$ organisms, whereas volunteers given *Vibrio cholerae* and sodium bicarbonate have a 10,000-fold increase in susceptibility to cholera. In contrast, some ingested agents, such as *Shigella* and *Giardia* cysts, are relatively resistant to gastric acid; hence, as few as 100 organisms of each are sufficient to cause illness.

Other normal defenses within the gastrointestinal tract include (1) the viscous mucous layer covering the gut, (2) lytic pancreatic enzymes and bile detergents, (3) mucosal antimicrobial peptides called defensins, (4) normal flora, and (5) secreted IgA antibodies. IgA antibodies are made by B cells located in mucosa-associated lymphoid tissues (MALT). These lymphoid aggregates are covered by a single layer of specialized epithelial cells called M cells. M cells are important for transport of antigens to MALT and for binding and uptake of numerous gut pathogens, including poliovirus, enteropathic *Escherichia coli, Vibrio cholerae, Salmonella typhi*, and *Shigella flexneri*. [12]

Infections via the gastrointestinal tract occur when local defenses are weakened or the organisms develop strategies to overcome these defenses. Host defenses are weakened by low gastric acidity, by antibiotics that unbalance the normal bacterial flora (e.g., in pseudomembranous colitis), or when there is stalled peristalsis or mechanical obstruction (e.g., in blind loop syndrome). Most enveloped viruses are killed by the bile and digestive enzymes, but nonenveloped viruses may be resistant (e.g., the hepatitis A virus, rotaviruses, reoviruses, and Norwalk agents).

Enteropathogenic bacteria elicit gastrointestinal disease by a variety of mechanisms:

- While growing on contaminated food, certain staphylococcal strains release powerful enterotoxins that cause food poisoning symptoms without any bacterial multiplication in the gut.
- *V. cholerae* and toxigenic *E. coli* multiply inside the mucous layer overlying the gut epithelium and release exotoxins that cause the gut epithelium to secrete high volumes of watery diarrhea.
- *Shigella, Salmonella*, and *Campylobacter* invade and damage the intestinal mucosa and lamina propria and so cause ulceration, inflammation, and hemorrhage, clinically manifested as dysentery.[13]
- *S. typhi* passes from the damaged mucosa through Peyer patches and mesenteric lymph nodes and into the bloodstream, resulting in a systemic infection.

Fungal infection of the gastrointestinal tract occurs mainly in immunologically compromised patients. *Candida*, part of the normal gastrointestinal flora, shows a predilection for stratified squamous epithelium, causing oral thrush or membranous esophagitis, but may also disseminate to the stomach, lower gastrointestinal tract, and systemic organs.

The cyst forms of intestinal protozoa are essential for their transmission because cysts resist stomach acid. In the gut, cysts convert to motile trophozoites and attach to sugars on the intestinal epithelia through surface lectins. Thereafter, there is wide species variation. *Giardia lamblia* attaches to the epithelial brush border, whereas cryptosporidia are taken up by enterocytes, in which they form gametes and spores. *Entamoeba histolytica* causes contact-mediated cytolysis through a channel-forming pore protein and thereby ulcerates and invades the colonic mucosa. Intestinal helminths, as a rule, cause disease only when they are present in large numbers or in ectopic sites, for example, by obstructing the gut or invading and damaging the bile ducts *(Ascaris lumbricoides)*. Hookworms may cause iron deficiency anemia by chronic loss of blood sucked from intestinal villi; the fish tapeworm *Diphyllobothrium latum* can deplete its host of vitamin B_{12} , giving rise to an illness resembling pernicious anemia. Finally, the larvae of several helminth parasites pass through the gut briefly on their way toward another organ habitat; for example, *Trichinella spiralis* larvae preferentially encyst in muscle, *Echinococcus* species larvae in the liver or lung.

Respiratory Tract.

Some 10,000 microorganisms, including viruses, bacteria, and fungi, are inhaled daily by every city inhabitant. The distance these microorganisms travel into the respiratory system is inversely proportional to their size.[11] Large microbes are trapped in the mucociliary blanket that lines the nose and the upper respiratory tract. Microorganisms are trapped in the mucus secreted by goblet cells and are then transported by ciliary action to the back of the throat, where they are swallowed and cleared. Organisms smaller than 5 µm travel directly to the alveoli, where they are phagocytosed by alveolar macrophages or by neutrophils recruited to the lung by cytokines.

Damage to the mucociliary defense results from repeated insults in smokers and patients with cystic fibrosis, while acute injury occurs in intubated patients and in those who aspirate gastric acid. Successful respiratory microbes evade the mucociliary defenses in part by attaching to epithelial cells in the lower respiratory tract and pharynx. For example, influenza viruses possess hemagglutinin proteins that project from the surface of the virus and bind to sialic acid on the surface of epithelial cells. This attachment induces the host cell to engulf the virus, leading to viral entry and replication within the host cell. However, sialic acid binding prevents newly synthesized viruses from leaving the host cell. Influenza viruses have another cell surface protein, neuraminidase, which cleaves sialic acid and allows virus to release from the host cell. Neuraminidase also lowers the viscosity of mucus and facilitates viral transit within the respiratory tract. Interestingly, some anti-influenza drugs are sialic acid analogs that inhibit neuraminidase and prevent viral release from host cells.

Certain respiratory bacterial pathogens can impair ciliary activity. For instance, *Haemophilus influenza* and *Bordetella pertussis* elaborate toxins that paralyze mucosal cilia; *Pseudomonas aeruginosa*, a cause of severe respiratory infection in persons with cystic fibrosis, and *Mycoplasma pneumoniae* produce ciliostatic substances. Some bacteria such as *Streptococcus pneumoniae* or *Staphylococcus* species lack specific adherence factors and often gain access after viral infection causes loss of ciliated epithelium, making individuals who have had viral respiratory infection more susceptible to secondary bacterial respiratory infection. *Mycobacterium tuberculosis*, in contrast, gains its foothold in normal alveoli because it is able to escape phagocytic killing by macrophages. Growth requirements for microorganisms can determine their site of infection in the respiratory tract. For example, rhinoviruses, which cause the common cold, grow optimally at 33°C, the temperature of the nasal mucosa, but grow poorly at 37°C, the temperature of the lower respiratory tract. Finally, opportunistic fungi infect the lungs when cellular immunity is depressed or when leukocytes are reduced in number (e.g., *P. jiroveci* [*carinii*] in AIDS patients and *Aspergillus* species in chemotherapy patients).

Urogenital Tract.

The urinary tract is almost always invaded from the exterior via the urethra.[11] The regular flushing of the urinary tract with urine serves as a defense against invading microorganisms. Urine in the bladder is normally sterile, and successful pathogens (e.g., gonococci, *E. coli*) adhere to the urinary epithelium. Anatomy is an important factor for infection. Women have more than 10 times as many urinary tract infections (UTIs) as men, because the distance between the urinary bladder and skin (i.e., the length of the urethra) is 5 cm, in contrast to 20 cm in men. Obstruction of urinary flow and/or reflux can compromise normal defenses and increase susceptibility to UTIs. UTIs can spread retrogradely from the bladder to the kidney and cause acute and chronic pyelonephritis, which is the major preventable cause of renal failure.

From puberty until menopause, the vagina is protected from pathogens by a low pH resulting from catabolism of glycogen in the normal epithelium by lactobacilli. Antibiotics can kill the lactobacilli and make the vagina susceptible to infection. To be successful as pathogens, microorganisms have developed specific mechanisms for attaching to vaginal or cervical mucosa or enter via local breaks in the mucosa during sex (genital warts, syphilis).

Spread and Dissemination of Microbes

Some microorganisms proliferate locally, at the site of infection, whereas others penetrate the epithelial barrier and spread to other sites via the lymphatics, the blood, or nerves[11] (Fig. 8-4). Some of the superficial pathogens stay confined to the lumen of hollow viscera (e.g., cholera); others adhere to or proliferate exclusively in or on epithelial cells (e.g., papillomaviruses, dermatophytes). A variety of pathogenic bacteria, fungi, and helminths are invasive by virtue of their motility or ability to secrete lytic enzymes (e.g., streptococci and staphylococci secrete hyaluronidase, which degrades the extracellular matrix between host cells). Microbial spread initially follows tissue planes of least resistance and regional lymphatic and vascular anatomy. For example, staphylococcal infections may progress from a localized abscess or furuncle to regional lymphadenitis that sometimes leads to bacteremia and colonization of distant organs (heart, liver, brain, kidney, bone). Within the blood, microorganisms may be transported free or within host cells. Some viruses (e.g., poliovirus and HBV), most bacteria and fungi, some protozoa (e.g., African trypanosomes), and all helminths are transported free in the plasma. Leukocytes can carry herpesviruses, HIV, mycobacteria, and *Leishmania* and *Toxoplasma* organisms. Certain viruses (e.g., Colorado tick fever virus) and parasites (*Plasmodium* and *Babesia*) are carried by red blood cells. Viruses also may propagate

Figure 8-4 Routes of entry, dissemination, and release of microbes from the body. *(Adapted from Mims CA: The Pathogenesis of Infectious Disease, 4th ed. San Diego, CA, Academic Press, 1996.)*

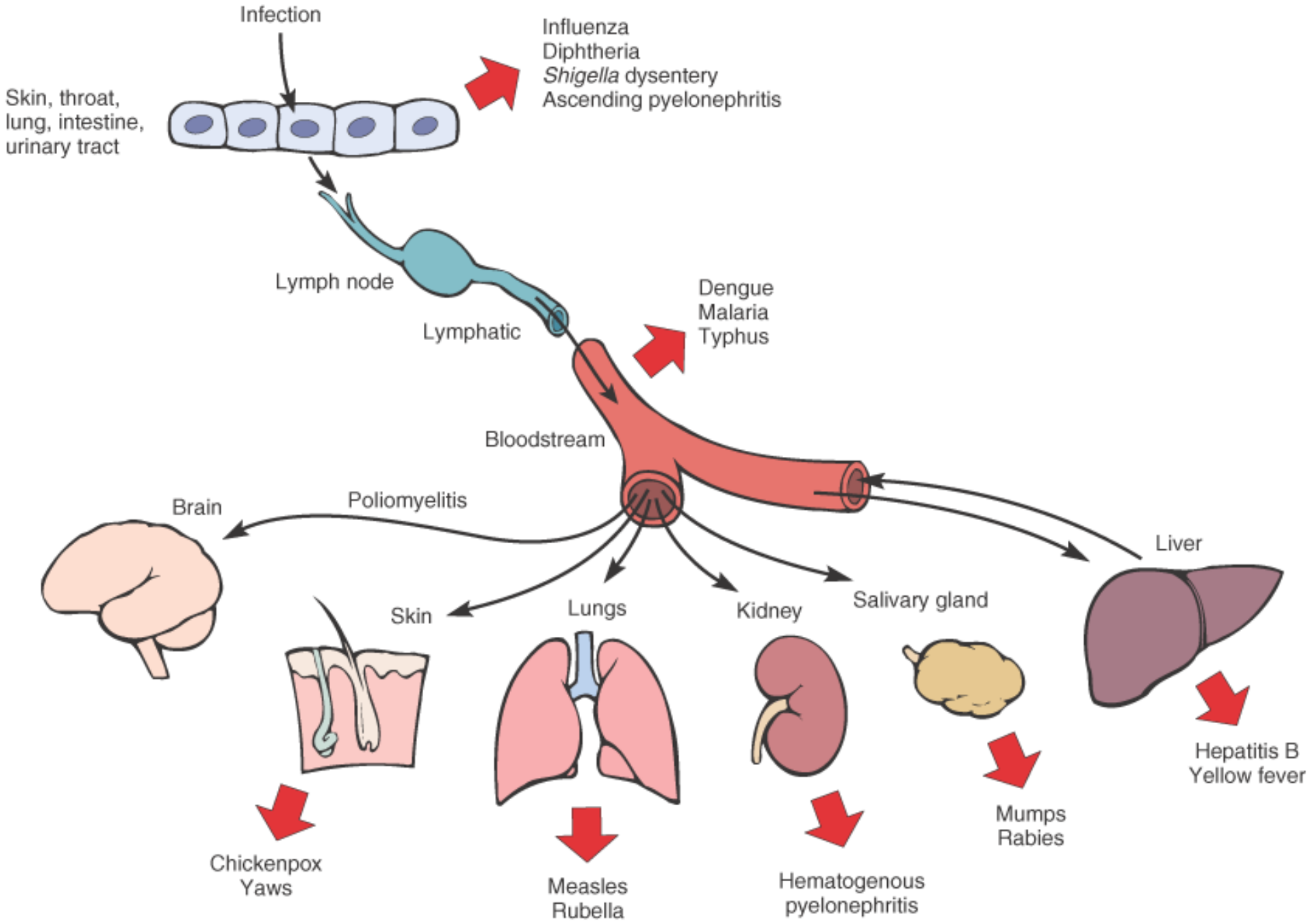

TABLE 8-7 -- Classification of Important Sexually Transmitted Diseases

	Disease or Syndrome and Population Principally Affected		
Pathogens	***Males***	***Both***	***Females***
Viruses			
••Herpes simplex virus		Primary and recurrent herpes, neonatal herpes	
••Hepatitis B virus		Hepatitis	
••Human papillomavirus	Cancer of penis (some cases)	Condyloma acuminatum	Cervical dysplasia and cancer, vulvar cancer
••Human immunodeficiency virus		Acquired immunodeficiency syndrome	
Chlamydiae			
••*Chlamydia trachomatis*	Urethritis, epididymitis, proctitis	Lymphogranuloma venereum	Urethral syndrome, cervicitis, bartholinitis, salpingitis and sequelae
Mycoplasmas			
••*Ureaplasma urealyticum*	Urethritis		
Bacteria			
••*Neisseria gonorrhoeae*	Epididymitis, prostatitis, urethral stricture	Urethritis, proctitis, pharyngitis, disseminated gonococcal infection	Cervicitis, endometritis, bartholinitis, salpingitis, and sequelae (infertility, ectopic pregnancy, recurrent salpingitis)
Treponema pallidum		Syphilis	
Haemophilus ducreyi		Chancroid	
Calymmatobacterium granulomatis		Granuloma inguinale (donovanosis)	
Shigella	* Enterocolitis		
Campylobacter	* Enterocolitis		
Protozoa			Vaginitis
••*Trichomonas vaginalis*	Urethritis, balanitis		
••*Entamoeba histolytica*	* Amebiasis		
••*Giardia lamba*	* Giardiasis		
Modified and updated from Krieger JN: Biology of sexually transmitted diseases. Urol Clin North Am 11:15, 1984.			

*Most important in homosexual populations.

Syphilis is discussed later in this chapter, and other STIs are described in Chapter 21 and Chapter 22 .

HOW MICROORGANISMS CAUSE DISEASE

Infectious agents establish infection and damage tissues in three ways:

- They can contact or enter host cells and directly cause cell death.
- They may release toxins that kill cells at a distance, release enzymes that degrade tissue components, or damage blood vessels and cause ischemic necrosis.
- They can induce host cellular responses that, although directed against the invader, cause additional tissue damage, usually by immune-mediated mechanisms. Thus, as we discussed in Chapter 2 and Chapter 6 , the defensive responses of the host are a two-edged sword: They are necessary to overcome the infection but at the same time may directly contribute to tissue damage.

Here we describe some of the mechanisms whereby viruses and bacteria damage host tissues.

Mechanisms of Viral Injury

Viruses can directly damage host cells by entering them and replicating at the host's expense. The predilection for viruses to infect certain cells and not others is called tissue tropism and is determined by several factors, including (1) host cell receptors for the virus, (2) cellular transcription factors that recognize viral enhancer and promoter sequences, (3) anatomic barriers, and (4) local temperature, pH, and host defenses.[15] Each of these is described briefly.

A major determinant of tissue tropism is the presence of viral receptors on host cells. Viruses possess specific cell-surface proteins that bind to particular host cell-surface proteins. Many viruses use normal cellular receptors of the host to enter cells. For example, HIV gp120 binds to CD4 on T cells and to the chemokine receptors CXCR4 (mainly on T cells) and CCR5 (mainly on macrophages). Rhinoviruses bind to the same site on ICAM-1 as LFA-1, an integrin on the surface of lymphocytes that is an important adhesion molecule for lymphocyte activation and migration.[16] In some cases, host proteases are needed to enable binding of virus to host cells; for instance, a host protease cleaves and activates the influenza virus hemagglutinin.

Another determinant of viral tropism is the ability of the virus to replicate inside some cells but not in others, and this is related to the presence of cell-type—specific transcription factors. For example, the JC virus, which causes leukoencephalopathy (Chapter 28), is restricted to oligodendroglia in the central nervous system because the promoter and enhancer DNA sequences upstream from the viral genes are active in glial cells but not in neurons or endothelial cells. Physical barriers also can contribute to tissue tropism. For example, enteroviruses replicate in the intestine in part because they can resist inactivation by acids, bile, and digestive enzymes. Rhinoviruses replicate only within the upper respiratory tract because they survive optimally at the lower temperature of the upper respiratory tract.

Once viruses are inside host cells, they can kill the cells and/or cause tissue damage in a number of ways (Fig. 8-5):

- Viruses may inhibit host cell DNA, RNA, or protein synthesis. For example, poliovirus inactivates cap-binding protein, which is essential for translation of host cell mRNAs, but leaves translation of poliovirus mRNAs unaffected.
- Viral proteins may insert into the host cell's plasma membrane and directly damage its integrity or promote cell fusion (HIV, measles virus, and herpesviruses).
- Viruses may lyse host cells. For example, respiratory epithelial cells are killed by influenza virus replication, liver cells by yellow fever virus, and neurons by poliovirus and rabies virus.
- Viruses may manipulate programmed cell death (apoptosis). Some virus-encoded proteins (including TAT and gp120 of HIV, adenovirus E1A) can induce cell death. In contrast,

some viruses encode one or more genes that inhibit apoptosis (e.g., homologues of the cellular *bcl-2* gene), suggesting that apoptotic cell death may be a protective host response to eliminate virus-infected cells. It has been hypothesized that viral antiapoptotic strategies may enhance viral replication, promote persistent viral infections, or promote virus-induced cancers.[17]

• Viral proteins on the surface of the host cells may be recognized by the immune system, and the host lymphocytes may attack the virus-infected cells. Acute liver failure during hepatitis B infection may be accelerated by cytotoxic T lymphocyte (CTL)-mediated destruction of infected hepatocytes (a normal response to clear the infection). FAS ligand on CTLs, which bind to FAS receptors on the surface of hepatocytes, also can induce apoptosis in target cells.[18]

• Viruses may damage cells involved in host antimicrobial defense, leading to secondary infections. For example, viral damage to respiratory epithelium predisposes to the subsequent development of pneumonia by *Streptococcus pneumoniae* and *Haemophilus influenzae*. HIV depletes CD4+ helper lymphocytes and thereby causes opportunistic infections.

• Viral killing of one cell type may cause the death of other cells that depend on them. For example, denervation by the attack of poliovirus on motor neurons causes atrophy and sometimes death of distal skeletal muscle supplied by such neurons.

• Some viruses can cause *cell proliferation and transformation* (e.g., EBV, HBV, human papillomavirus, or HTLV-1), resulting in cancer. The mechanisms of viral transformation are numerous and are discussed in Chapter 7 .

Figure 8-5 Mechanisms by which viruses cause injury to cells.

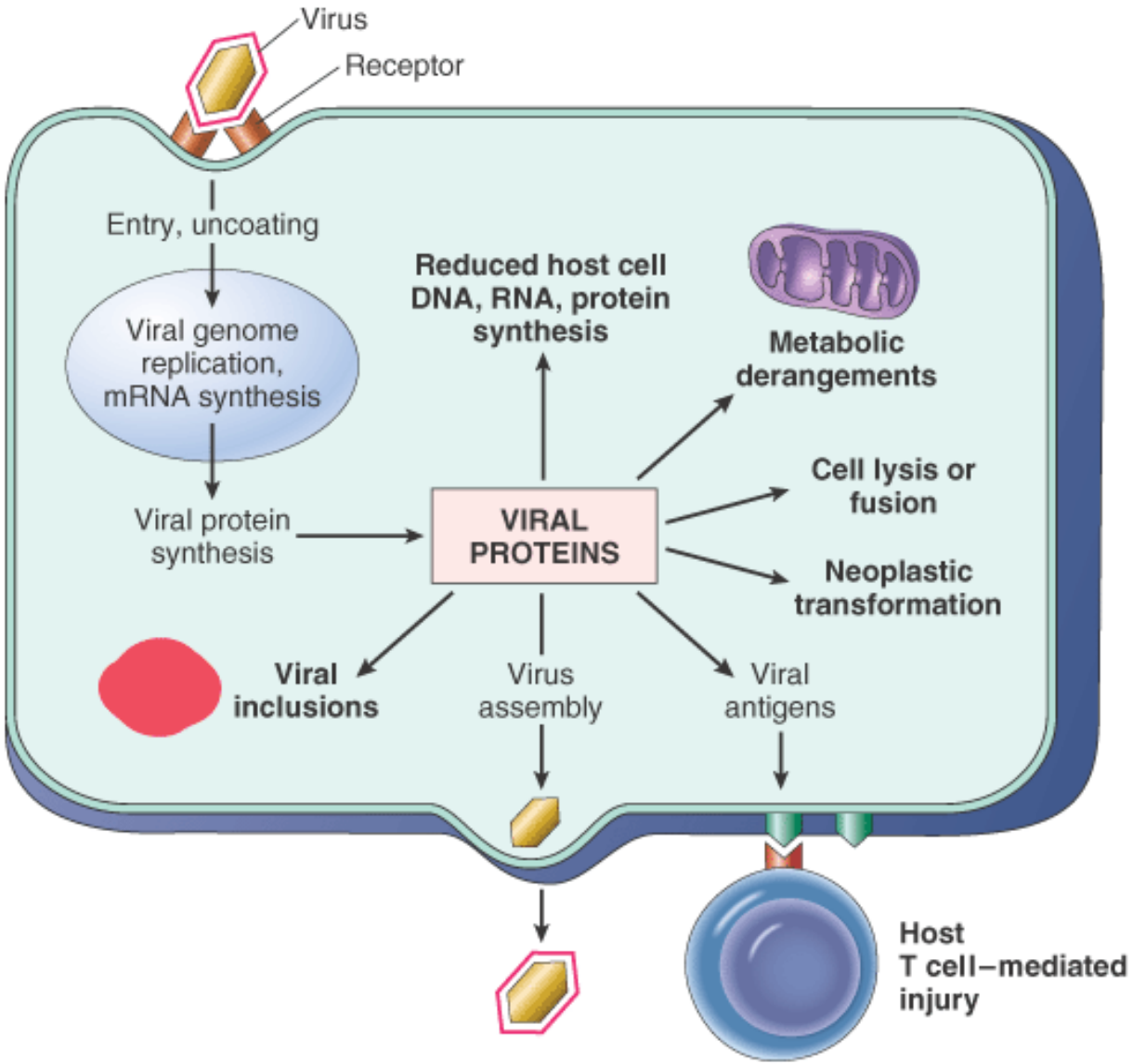

TABLE 8-8 -- Pathogens with Significant Antigenic Variation

Rhinoviruses	Colds
Influenza virus	Influenza
Neisseria gonorrhoeae	Gonorrhea
Borrelia hermsii	Relapsing fever
Borrelia burgdorferi	Lyme disease

Trypanosoma brucei	African sleeping sickness
Giardia lamblia	Giardiasis
Plasmodium falciparum	Severe malaria

neutrophils and macrophages.[33] The carbohydrate capsule on the surface of all the major bacteria that cause pneumonia or meningitis (pneumococcus, meningococcus, *Haemophilus influenzae*) makes them more virulent by shielding bacterial antigens and by preventing phagocytosis of the organisms by neutrophils. For example, *E. coli* with the sialic acid-containing K1 capsule causes meningitis in newborns. Sialic acid will not bind C3b, which is critical for activation of the alternative complement pathway, so the bacteria escape from complement-mediated lysis and opsonization-directed phagocytosis. Many bacteria make toxic proteins that kill phagocytes, prevent their migration, or diminish their oxidative burst. Bacteria also can circumvent immune defenses by covering themselves with host proteins. *S. aureus* are covered by protein A molecules that bind the Fc portion of antibodies and so inhibit phagocytosis. *Neisseria*, *Haemophilus*, and *Streptococcus* all secrete proteases that degrade antibodies. Another successful strategy for circumventing phagocytic defense mechanisms is to replicate within phagocytic cells. A number of viruses, rickettsias, some intracellular bacteria (including mycobacteria, Listeria, and Legionella), fungi (e.g., *Cryptococcus neoformans*), and protozoa (e.g., leishmania, trypanosomes, toxoplasmas) can multiply within phagocytes.

Viruses can produce molecules that inhibit innate immunity.[15] [35] [36] Some viruses (e.g., herpesviruses and poxviruses) produce proteins that block complement activation. Viruses have developed a large number of strategies to combat interferons (IFN), an early host defense against viruses. Some viruses produce soluble homologues of IFN-α/β or IFN-γ receptors that inhibit actions of extracellular IFNs, or produce proteins that inhibit intracellular JAK/STAT signaling downstream of IFN receptors or inactivate or inhibit dsRNA-dependent protein kinase (PKR), a key mediator of the antiviral effects of IFN. Viruses also can produce homologues of chemokines or chemokine receptors, and these can function as antagonists and inhibit recruitment of inflammatory cells to favor survival of viruses. Viruses also can produce soluble cytokine mimics (e.g., EBV produces a homologue of the immunosuppressive cytokine IL-10) or soluble cytokine receptor homologues.

Some microbes can decrease recognition of infected cells by CD4+ helper T cells and CD8+ cytotoxic T cells. For example, several DNA viruses (e.g., herpesviruses, including HSV, HCMV, and EBV) can bind to or alter localization of MHC class I proteins, impairing peptide presentation to CD8+ T cells[36] [37] (Fig. 8-6). Downregulation of MHC class I molecules might make it likely that virus-infected cells would be targets for NK cells. However, herpesviruses also express MHC class I homologues that act as effective inhibitors of NK cells by engaging killer inhibitory receptors (Chapter 6). Similarly, herpesviruses can target MHC class II molecules for degradation, impairing antigen presentation to CD4+ T helper cells. Viruses also can infect lymphocytes and directly compromise their function. HIV infects CD4+ T cells, macrophages, and dendritic cells, and EBV infects B lymphocytes.

INFECTIONS IN IMMUNOSUPPRESSED HOSTS

Different types of immunosuppression affect different cells of the immune system. The opportunistic infections that an immunosuppressed person contracts depend on the types of

Figure 8-6 Inhibition of MHC expression by viruses. The steps at which different viruses inhibit the class I MHC antigen presentation pathway are shown. *(Modified with permission from Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed., Philadelphia, Saunders, 2003.)*

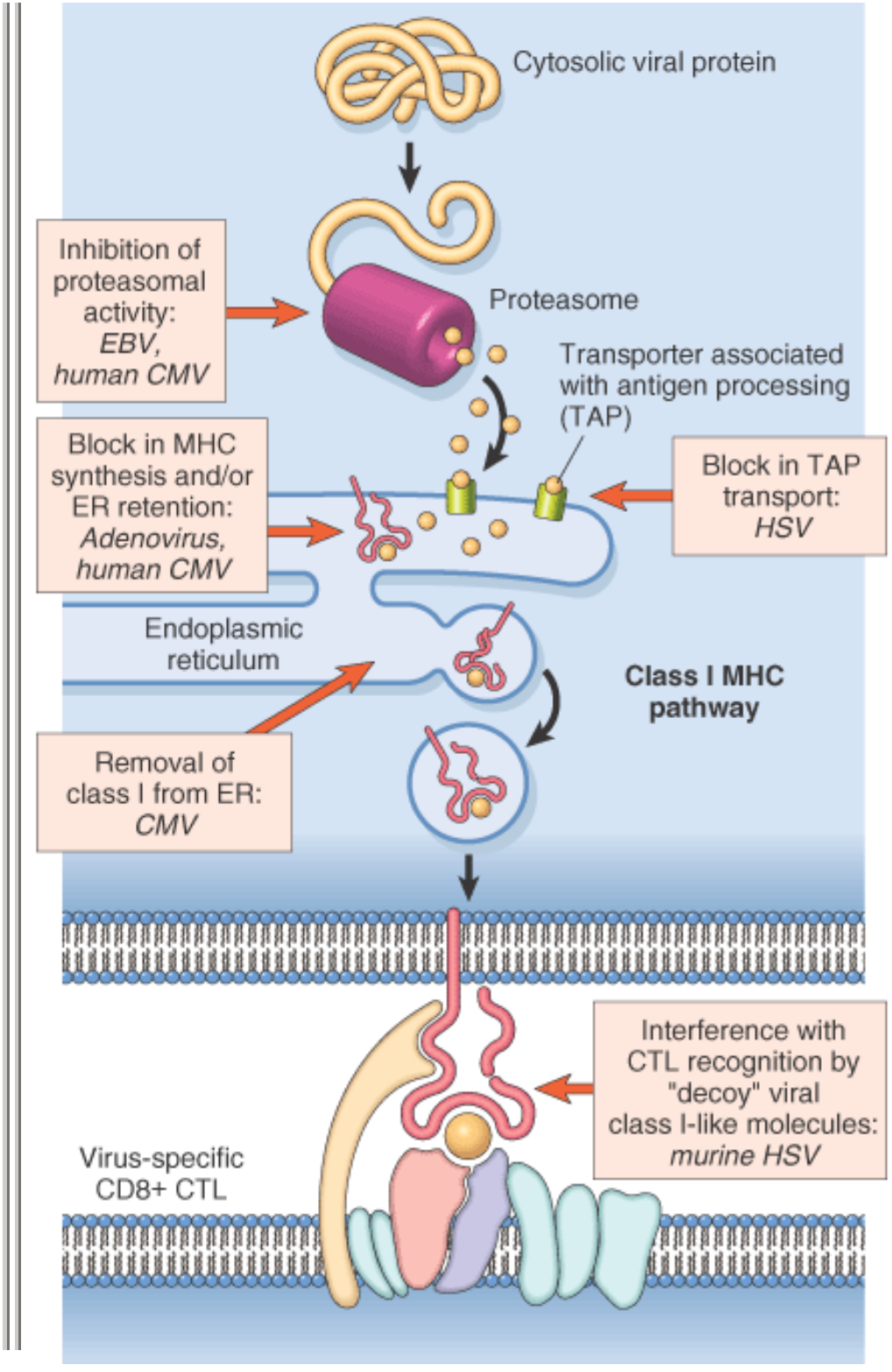
Cytosolic viral protein
Inhibition of proteasomal activity: *EBV, human CMV*
Proteasome
Transporter associated with antigen processing (TAP)
Block in MHC synthesis and/or ER retention: *Adenovirus, human CMV*
Block in TAP transport: *HSV*
Endoplasmic reticulum
Class I MHC pathway
Removal of class I from ER: *CMV*
Interference with CTL recognition by "decoy" viral class I-like molecules: *murine HSV*
Virus-specific CD8+ CTL

TABLE 8-9 -- Special Techniques for Diagnosing Infectious Agents

Gram stain	Most bacteria
Acid-fast stain	Mycobacteria, nocardiae (modified)
Silver stains	Fungi, legionellae, pneumocystis
Periodic acid-Schiff	Fungi, amebae
Mucicarmine	Cryptococci
Giemsa	Campylobacteria, leishmaniae, malaria parasites
Antibody probes	Viruses, rickettsiae
Culture	All classes
DNA probes	Viruses, bacteria, protozoa

of a lesion rather than at its center, particularly if there is necrosis.

Nucleic acid-based tests have become routine methods for detecting or quantifying several pathogens. Molecular diagnostics have become particularly important in the care of people infected with HIV.[40] Quantification of the viral RNA is an important guide to antiretroviral therapy. The management of hepatitis B and C infections is similarly guided by nucleic acid-based viral quantification or typing to predict resistance to antiviral drugs.

Nucleic acid amplification tests (NAATs), such as polymerase chain reaction (PCR) and transcription-mediated amplification, have become routine for diagnosis of gonorrhea, chlamydia, tuberculosis, and herpes encephalitis. In some cases, molecular assays are much more sensitive than conventional testing.[41] [42] PCR testing of cerebrospinal fluid (CSF) for herpes simplex virus encephalitis has a sensitivity of about 80%, while viral culture of CSF has a sensitivity of less than 10%. Similarly, NAATs for genital chlamydia detect 10% to 30% more infections than does conventional chlamydia culture. In other cases, such as gonorrhea, the sensitivity of NAAT testing is similar to that of culture.

SPECTRUM OF INFLAMMATORY RESPONSES TO INFECTION

In contrast to the vast molecular diversity of microbes, the morphologic patterns of tissue responses to microbes are limited, as are the mechanisms directing these responses. At the microscopic level, therefore, many pathogens produce identical reaction patterns, and few features are unique or pathognomonic for a particular microorganism. Moreover, it is the interaction between the microorganism and the host that determines the histologic features of the inflammatory response. Thus, pyogenic bacteria, which normally evoke vigorous leukocyte responses, may cause rapid tissue necrosis with little leukocyte exudation in a profoundly neutropenic host. Similarly, in a normal patient, *M. tuberculosis* causes well-formed granulomas with few mycobacteria present, whereas in an AIDS patient, the same mycobacteria multiply profusely in macrophages, which fail to coalesce into granulomas.

There are five major histologic patterns of tissue reaction in infections.

Suppurative (Polymorphonuclear) Inflammation

This pattern is the reaction to acute tissue damage, described in Chapter 2 , characterized by increased vascular permeability and leukocytic infiltration, predominantly of neutrophils (Fig. 8-7). The neutrophils are attracted to the site of infection by release of chemoattractants from the "pyogenic" bacteria that evoke this response, mostly extracellular Gram-positive cocci and Gram-negative rods. Massing of neutrophils forms pus. The sizes of exudative lesions vary from tiny microabscesses formed in multiple organs during bacterial sepsis secondary to a

colonized heart valve to diffuse involvement of entire lobes of the lung during pneumonia. How destructive the lesions are depends on their location and the organism involved. For example, pneumococci usually spare alveolar walls and cause lobar pneumonia that resolves

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Figure 8-7 Pneumococcal pneumonia. Note the intra-alveolar polymorphonuclear exudate and intact alveolar septa.

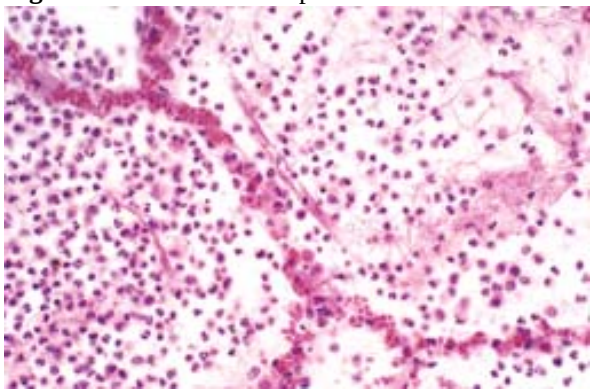


Figure 8-8 Secondary syphilis in the dermis with perivascular lymphoplasmacytic infiltrate and endothelial proliferation.

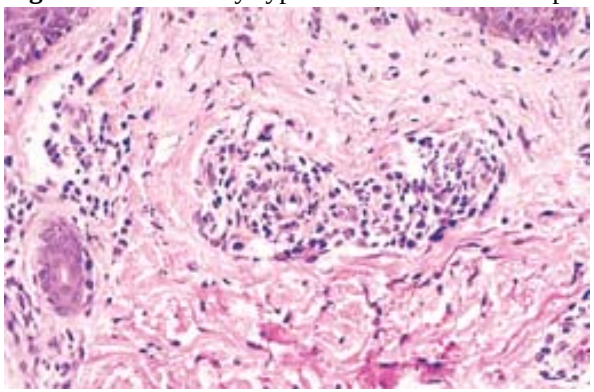


Figure 8-9 Herpesvirus blister in mucosa. See Figure 8-13 for viral inclusions.

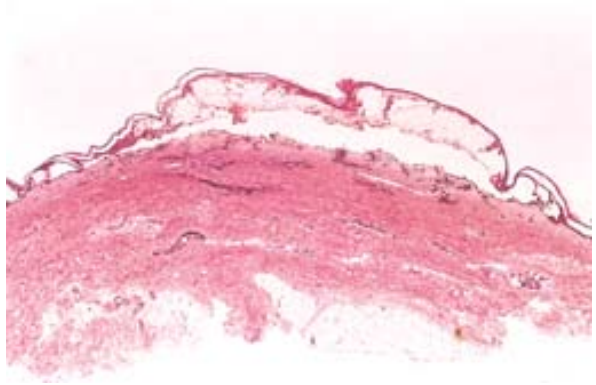


Figure 8-10 *Schistosoma haematobium* infection of the bladder with numerous calcified eggs and extensive scarring.

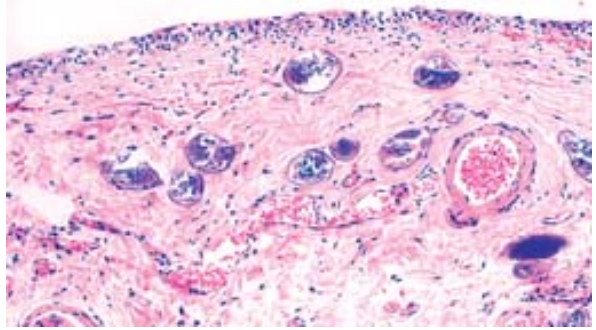


Figure 8-11 Measles giant cells in the lung. Note the glassy eosinophilic intranuclear inclusions.

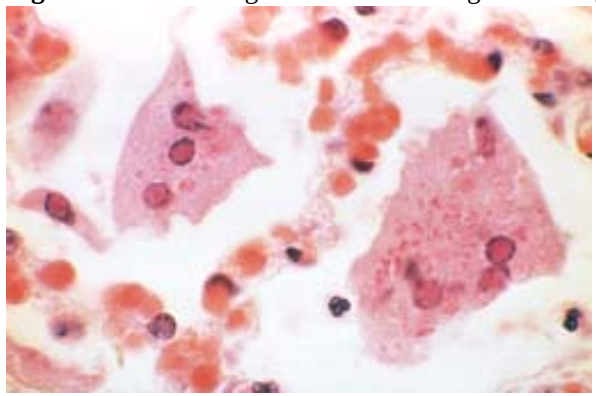


Figure 8-12 High-power view of cells from the blister in Figure 8-9 showing glassy intranuclear herpes simplex inclusion bodies.

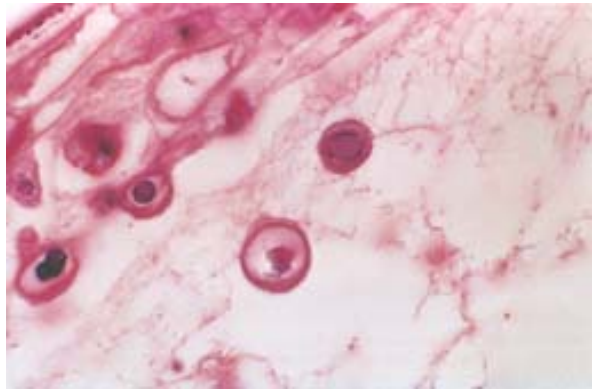


Figure 8-13 Cytomegalovirus: distinct nuclear and ill-defined cytoplasmic inclusions in the lung.

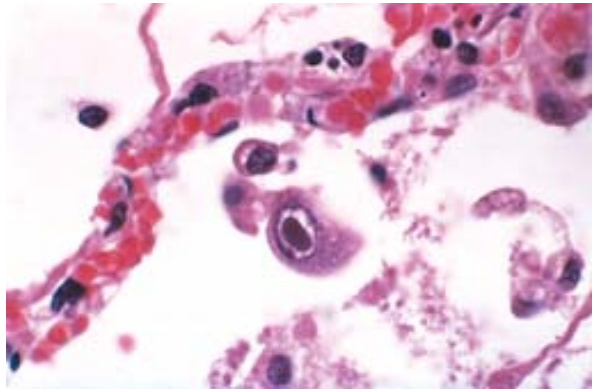


Figure 8-14 Skin lesion of chickenpox (varicella zoster virus) with intraepithelial vesicle.

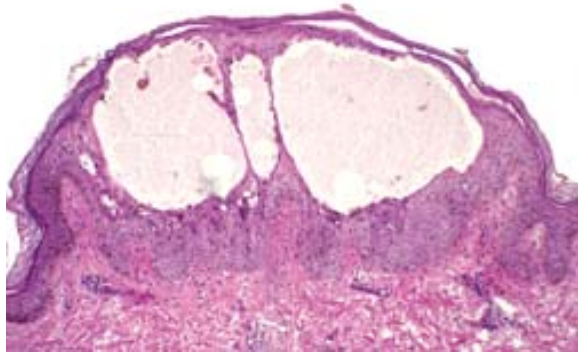


Figure 8-15 Dorsal root ganglion with varicella zoster virus infection. Note the ganglion cell necrosis and associated inflammation. *(Courtesy of Dr. James Morris, Radcliffe Infirmary, Oxford, England.)*

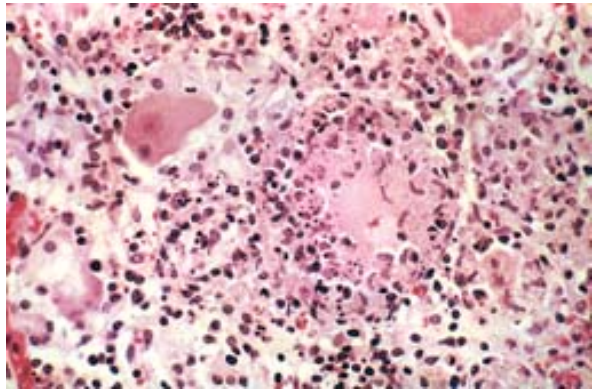


Figure 8-16 Pathways of transmission of the Epstein-Barr virus. In an individual with normal immune function, infection leads to mononucleosis. In the setting of cellular immunodeficiency, proliferation of infected B cells is uncontrolled and may cause B-cell neoplasms. One secondary genetic event that collaborates with Epstein-Barr virus (EBV) to cause B-cell transformation is a balanced 8;14 chromosomal translocation, which is seen in Burkitt lymphoma. EBV has also been implicated in the pathogenesis of nasopharyngeal carcinoma, Hodgkin disease, and certain other rare non-Hodgkin lymphomas.

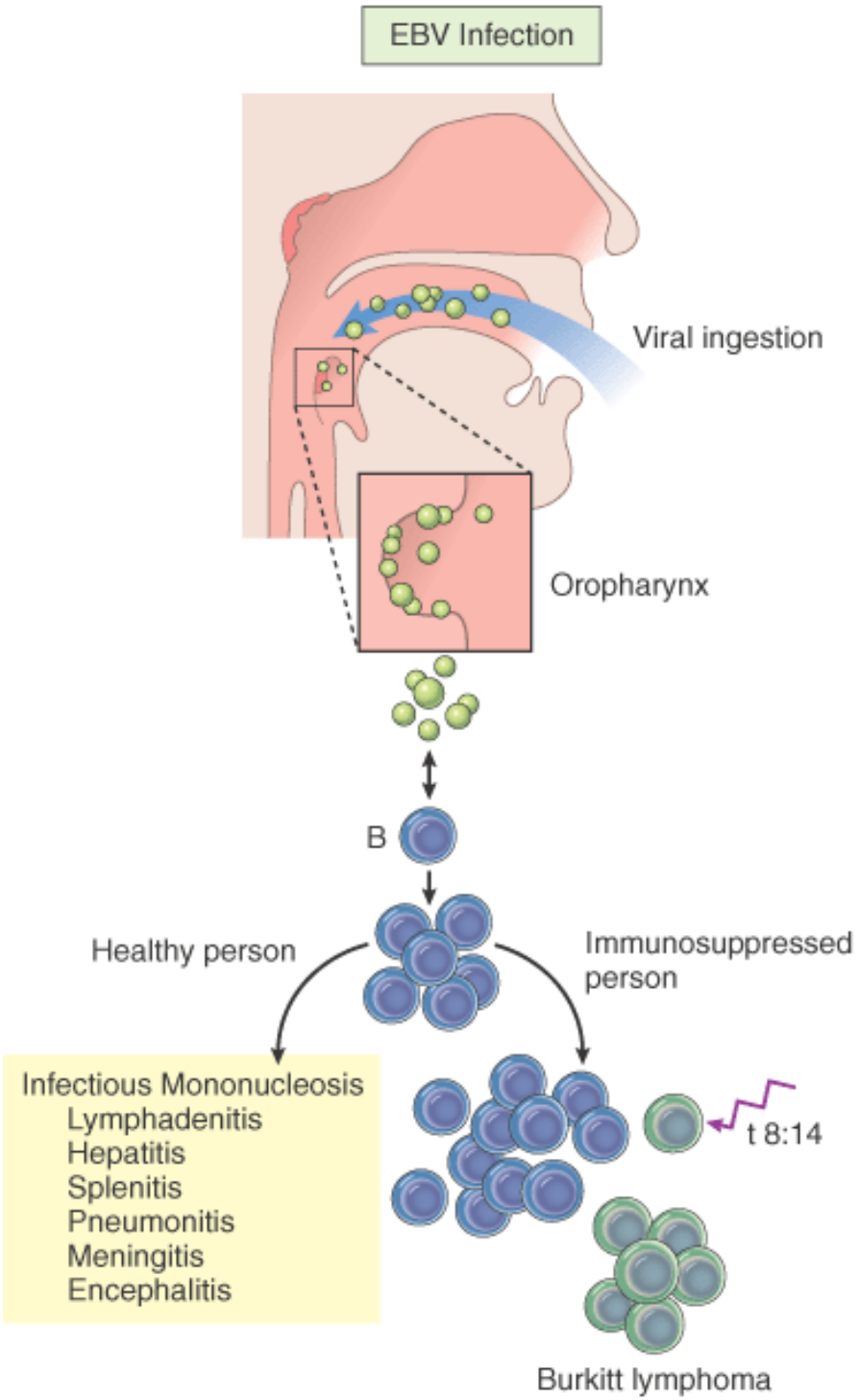


Figure 8-17 Atypical lymphocytes in infectious mononucleosis.

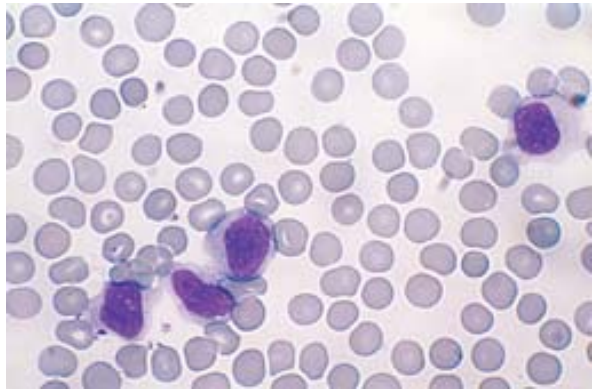
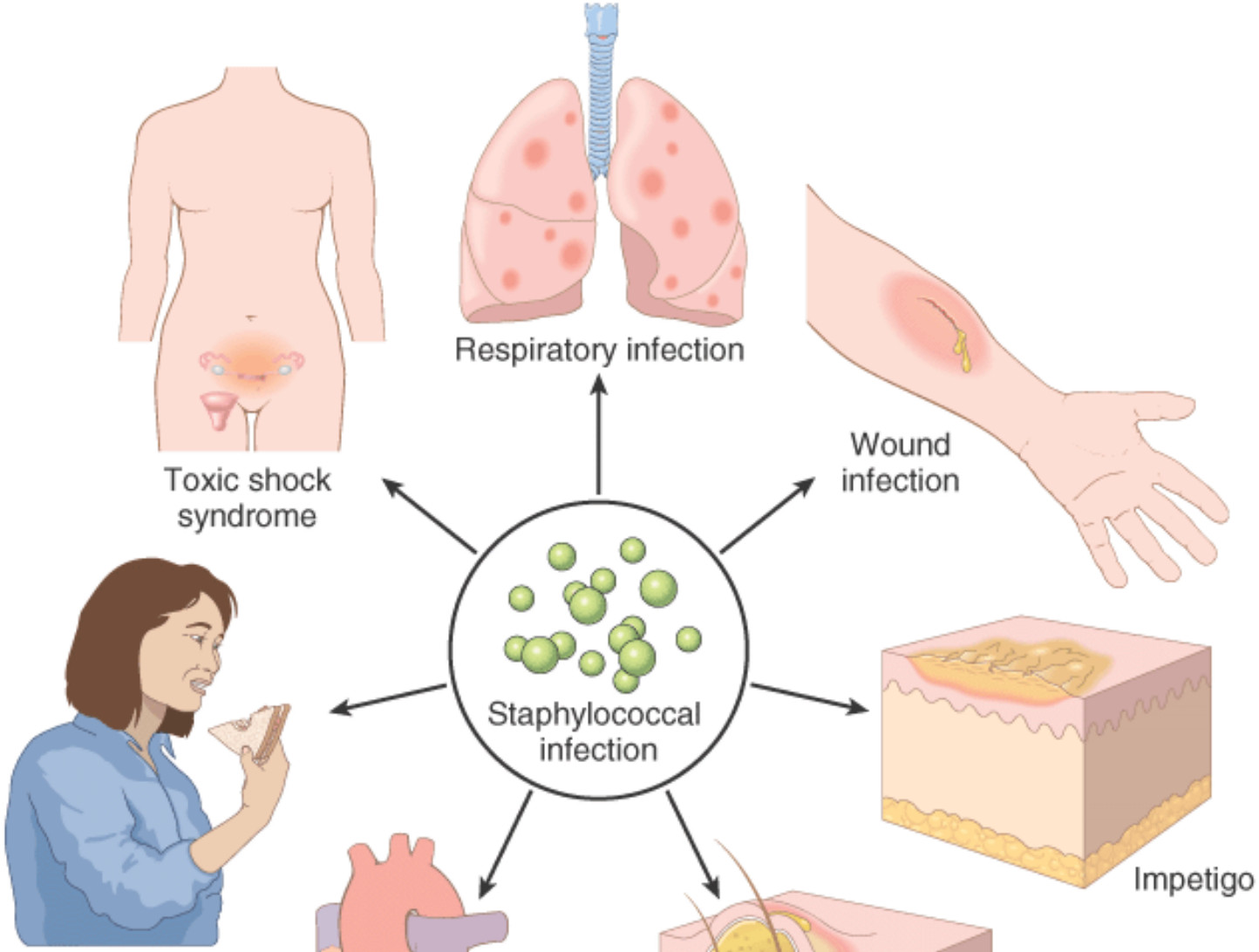


Figure 8-18 The many consequences of staphylococcal infection.



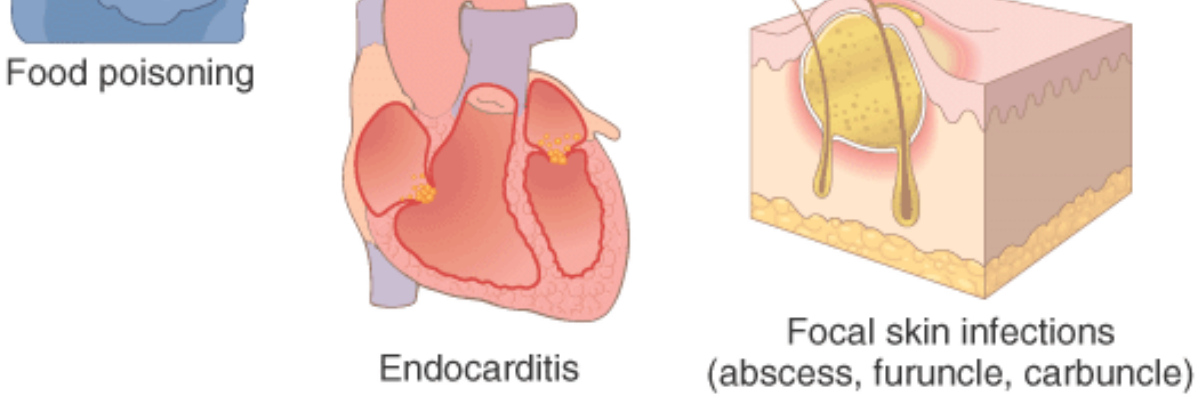


Figure 8-19 Staphylococcal abscess of the lung with extensive neutrophilic infiltrate and destruction of the alveoli (contrast with Figure 8-8).

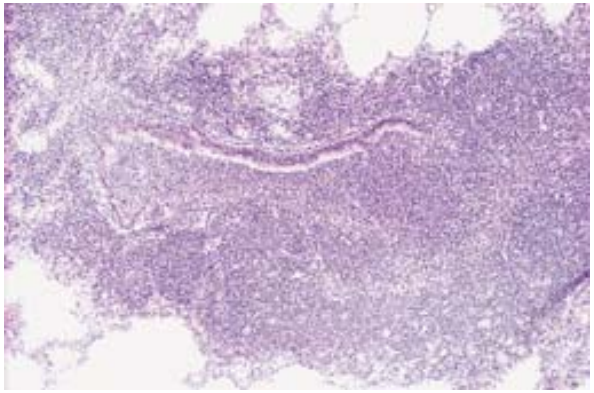


Figure 8-20 Streptococcal erysipelas.

Figure 8-21 Membrane of diphtheria lying within a transverse bronchus (*A*) and forming a perfect cast (removed from the lung) of the branching respiratory tree (*B*).

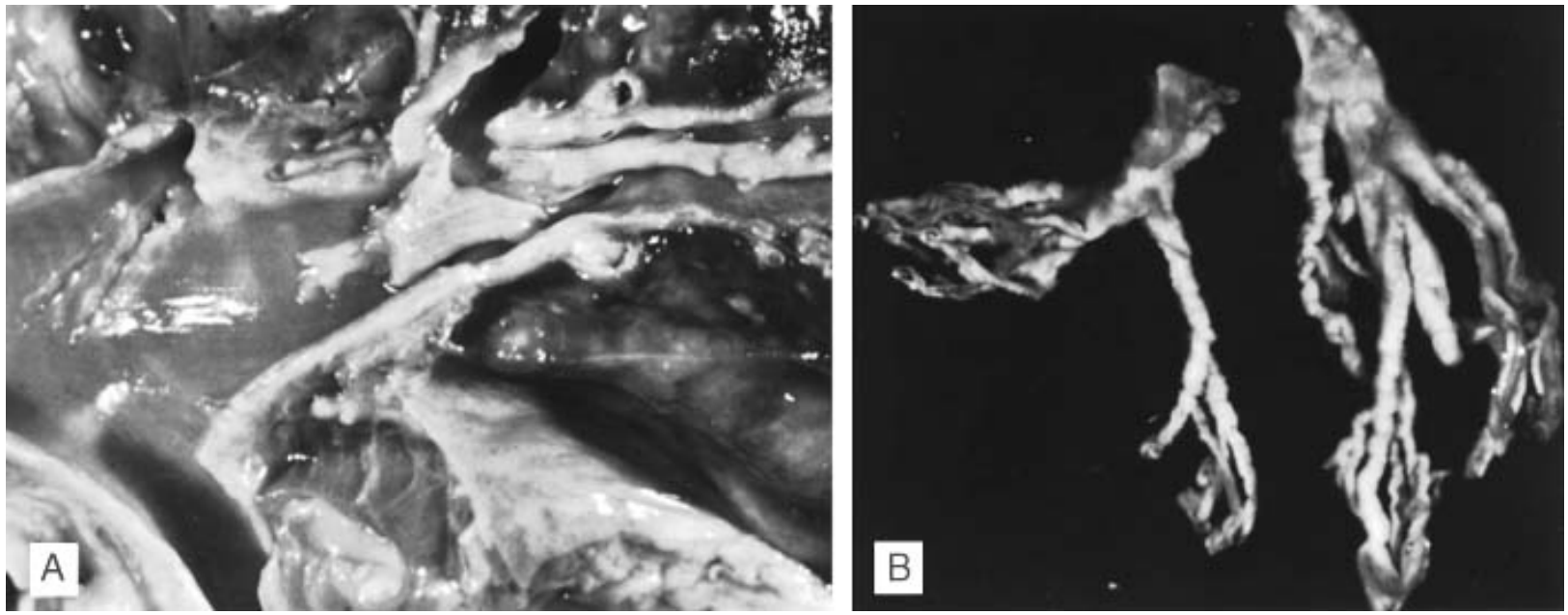


Figure 8-22 Mechanism of action of anthrax toxins. *(Adapted from Mourez et al: 2001: a year of major advances in anthrax toxin research. Trends Microbiol 10(6):287, 2002.)*

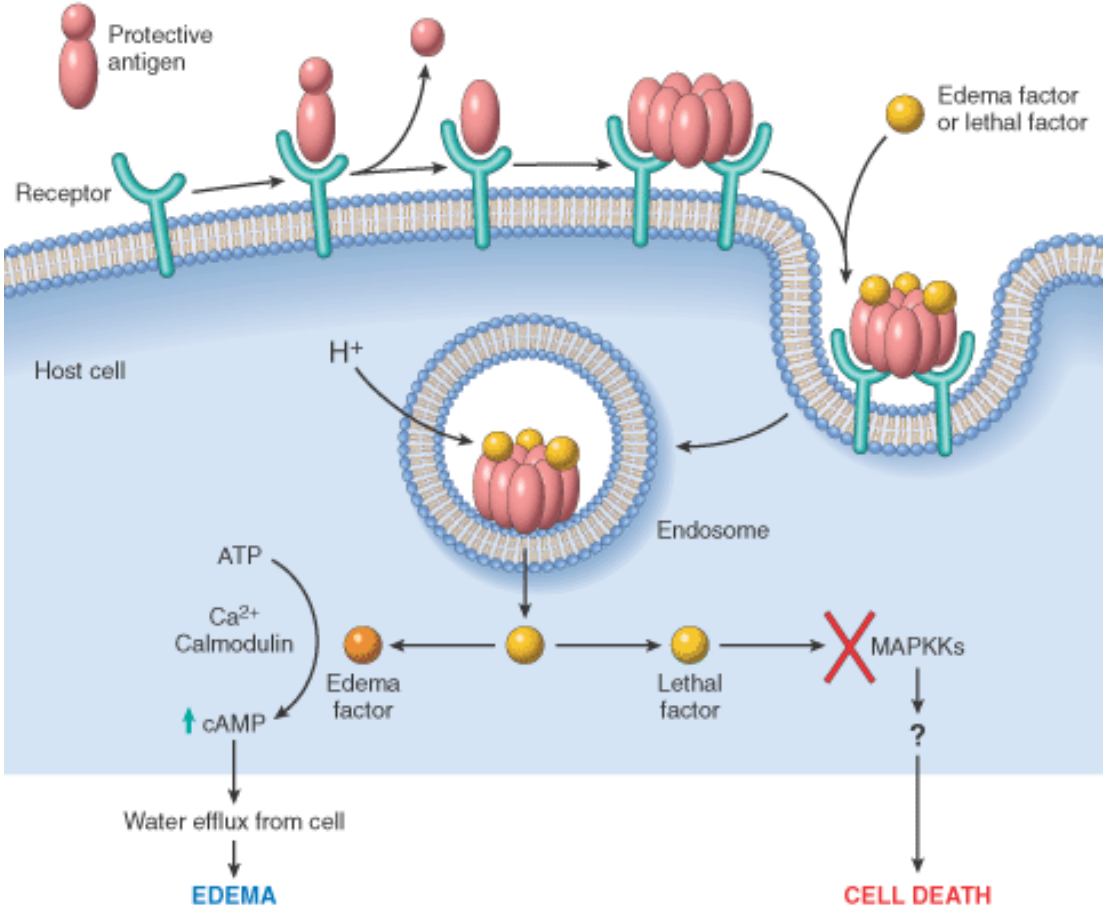


Figure 8-23 *B. anthracis* in the subcapsular sinus of a hilar lymph node of a patient who died of inhalational anthrax. *(Courtesy of Dr. Lev Grinberg, Department of Pathology, Hospital 40, Ekaterinburg, Russia and Dr. David Walker, UTMB Center for Biodefense and Emerging Infectious Diseases, Galveston, TX.)*

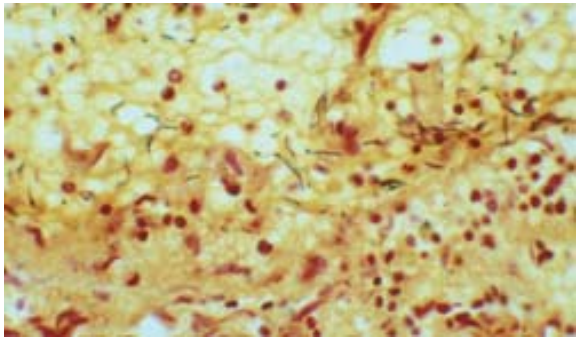


Figure 8-24 *Nocardia asteroides* in a Gram-stained sputum sample. Note the beaded, branched Gram-positive organisms and leukocytes. *(Courtesy of Dr. Ellen Jo Baron, Stanford University Medical Center, Stanford, CA.)*

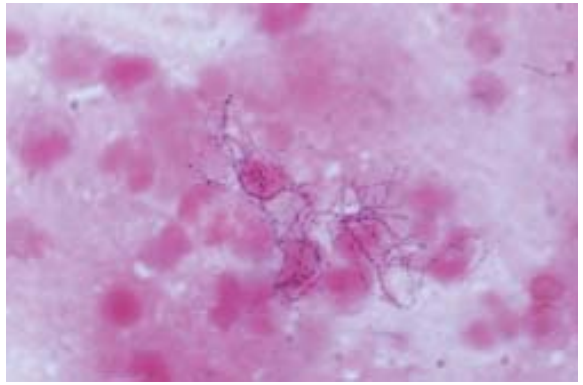


Figure 8-25 Gonococcal culture showing pili, as seen by scanning microscopy (*A*), and in clusters, as seen by transmission electron microscopy (*B*). *(Courtesy of Dr. John Swanson, Rocky Mountain Laboratories, Hamilton, MT.)*

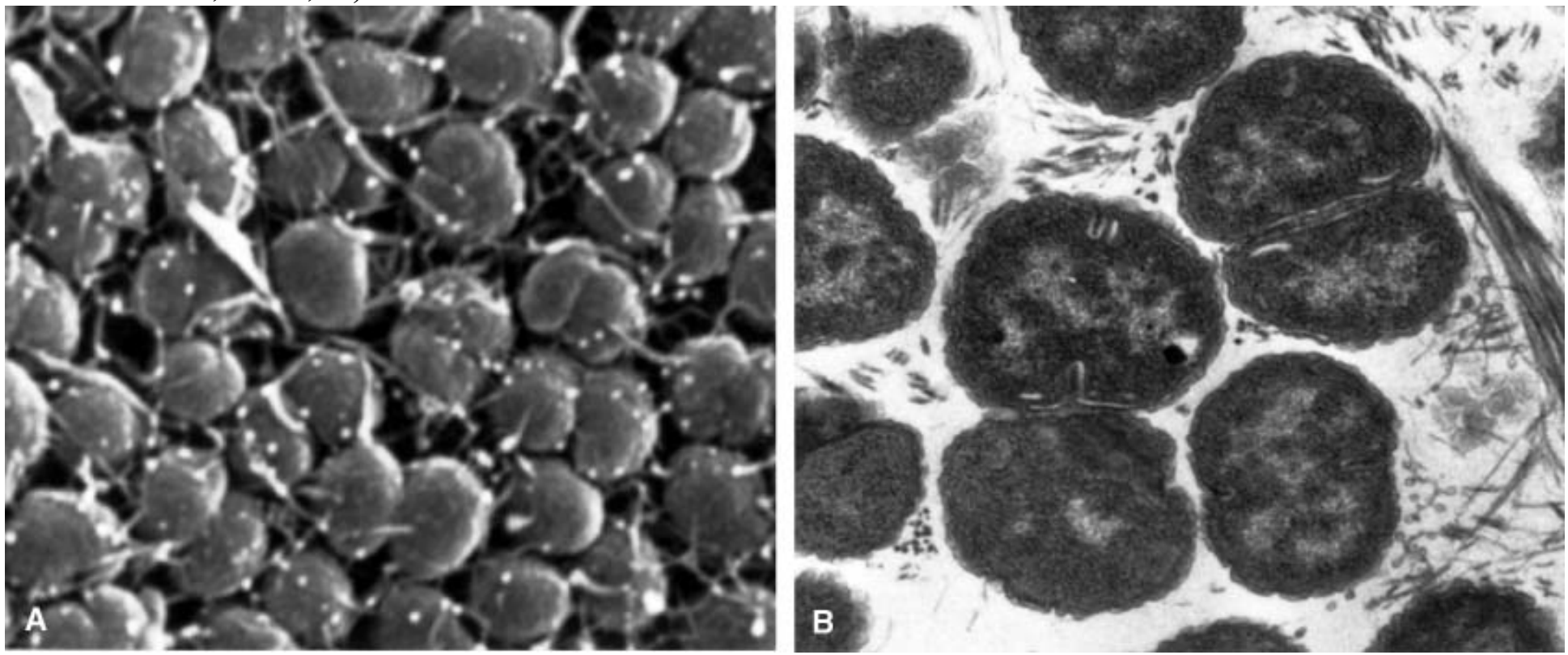


Figure 8-26 Whooping cough showing a haze of bacilli *(arrows)* etangled with the cilia of bronchial epithelial cells.

Figure 8-27 *Pseudomonas* vasculitis in which masses of organisms form a perivascular blue haze.

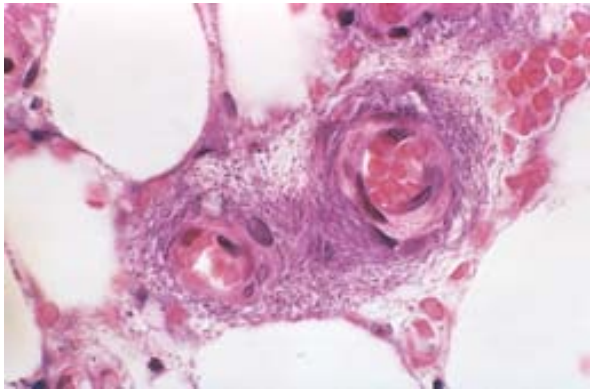


Figure 8-28 The sequence of events in primary pulmonary tuberculosis, commencing with inhalation of virulent *M. tuberculosis* and culminating with the development of cell-mediated immunity to the organism. *A*, Events occurring in the first 3 weeks after exposure. *B*, events thereafter. The development of resistance to the organism is accompanied by the appearance of a positive tuberculin test. Cells and bacteria are not drawn to scale. iNOS, inducible nitric oxide synthase; MHC, major histocompatibility complex; MTB, *M. tuberculosis*; NRAMP1, natural resistance-associated macrophage protein.

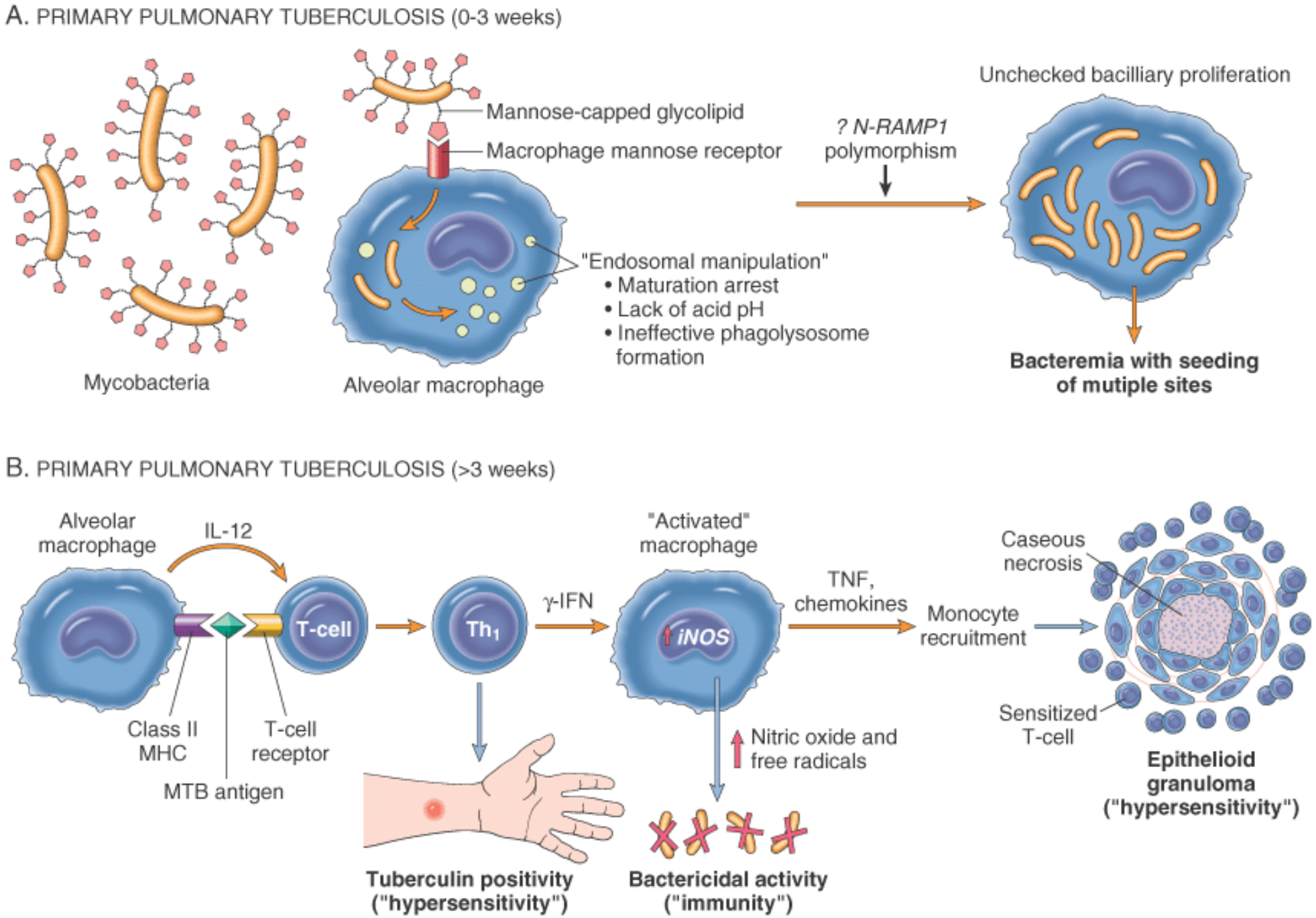


Figure 8-29 The natural history and spectrum of tuberculosis. *(Adapted from a sketch provided by Dr. R. K. Kumar, The University of New South Wales, School of Pathology, Sydney, Australia.)*

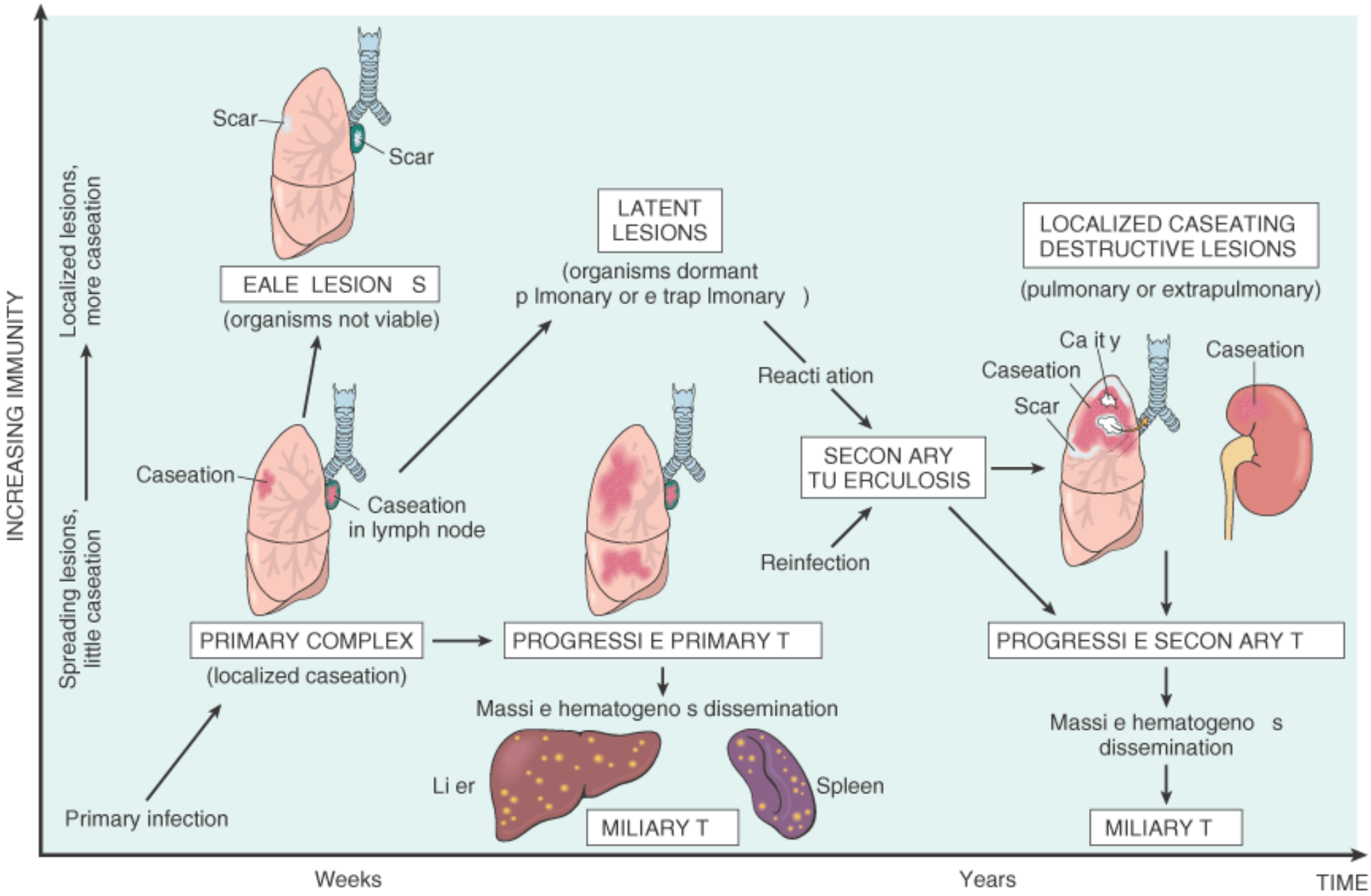


Figure 8-30 Primary pulmonary tuberculosis, Ghon complex. The gray-white parenchymal focus is under the pleura in the lower part of the upper lobe. Hilar lymph nodes with caseation are seen on the left.

Figure 8-31 The morphologic spectrum of tuberculosis. A characteristic tubercle at low magnification (*A*) and in detail (*B*) illustrates central caseation surrounded by epithelioid and multinucleated giant cells. This is the usual response seen in patients who have developed cell mediated immunity to the organism. Occasionally, even in immunocompetent individuals, tubercular granulomas might not show central caseation (*C*); hence, irrespective of the presence or absence of caseous necrosis, special stains for acid-fast organisms need to be performed when granulomas are present in histologic section. In immunosuppressed individuals, tuberculosis may not elicit a granulomatous response ("nonreactive tuberculosis"); instead, sheets of foamy histiocytes are seen, packed with mycobacteria that are demonstrable with acid-fast stains (*D*). *(D, Courtesy of Dr. Dominick Cavuoti, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

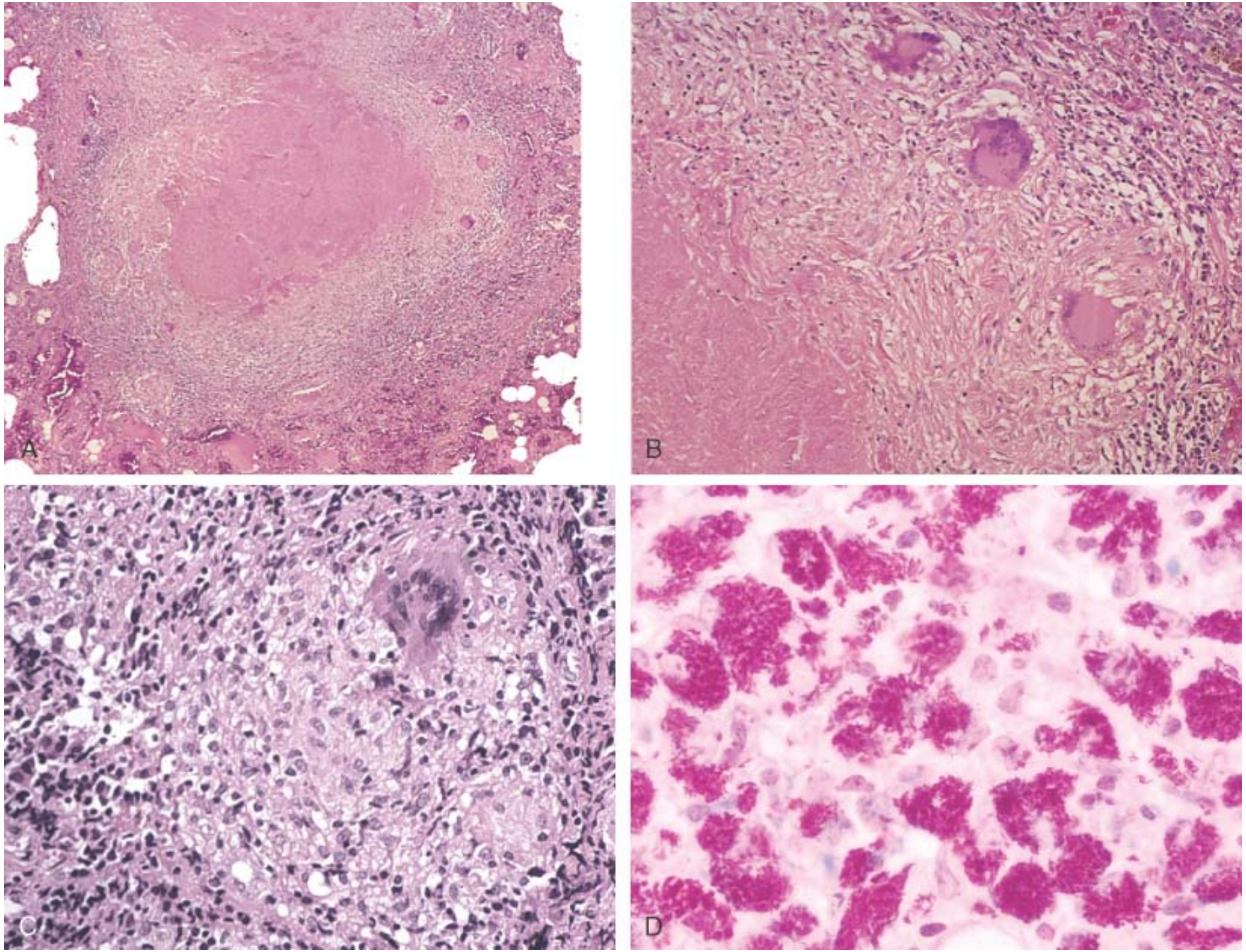


Figure 8-32 Secondary pulmonary tuberculosis. The upper parts of both lungs are riddled with gray-white areas of caseation and multiple areas of softening and cavitation.

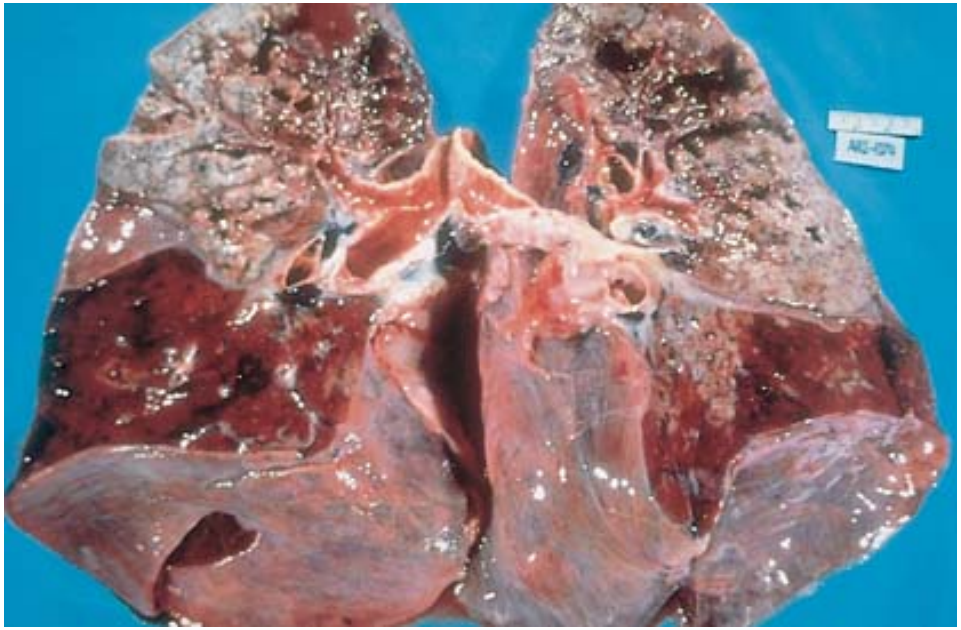


Figure 8-33 Miliary tuberculosis of the spleen. The cut surface shows numerous gray-white granulomas.

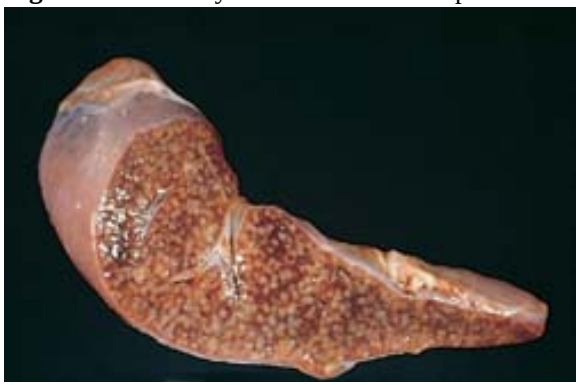


Figure 8-34 *Mycobacterium avium* infection in a patient with AIDS, showing massive infection with acid-fast organisms.

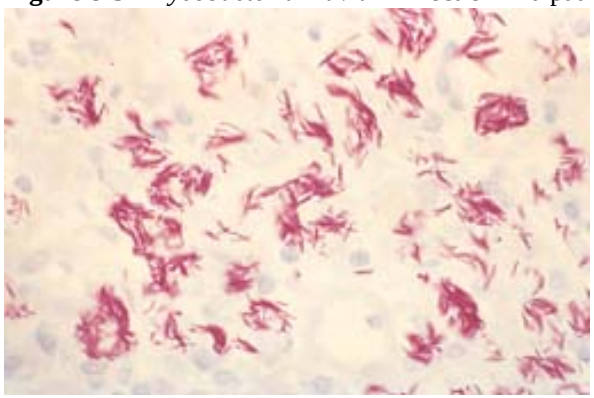


Figure 8-35 Leprosy. *A*, Peripheral nerve. Note the inflammatory cell infiltrates in the endoneural and epineural compartments. *B*, Cells within the endoneurium contain acid-fast positive lepra bacilli. *(Courtesy of E.P. Richardson, Jr. and U. De Girolami, Harvard Medical School.)*

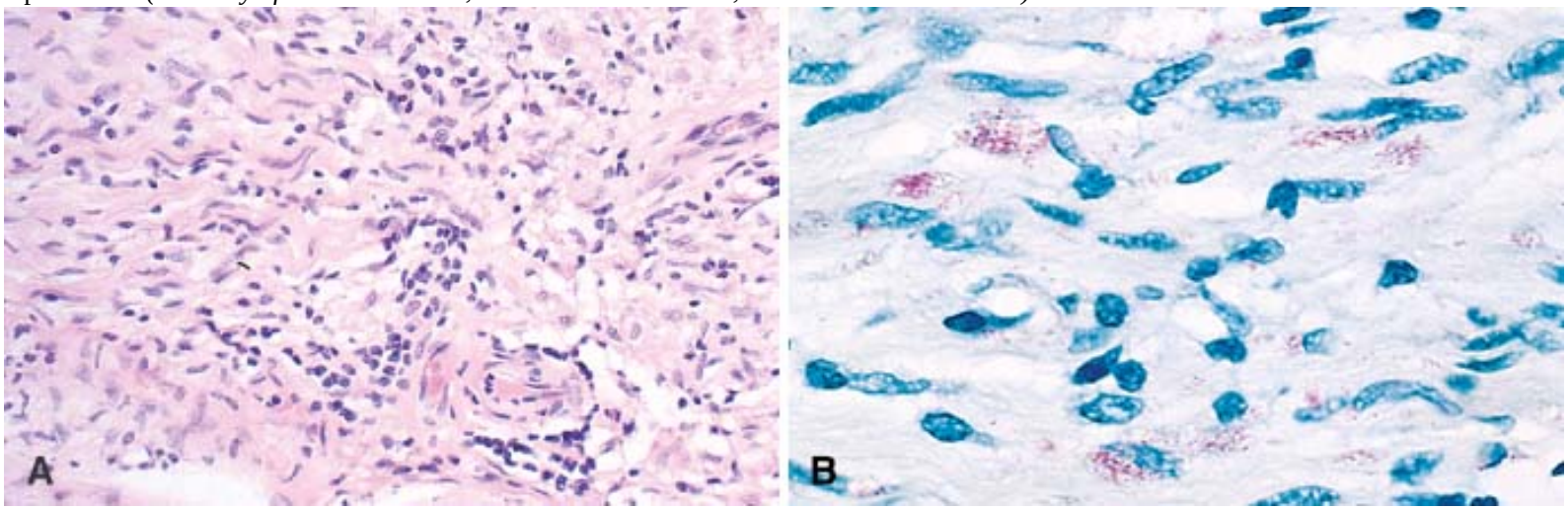


Figure 8-36 Lepromatous leprosy. Acid-fast bacilli ("red snappers") within macrophages.

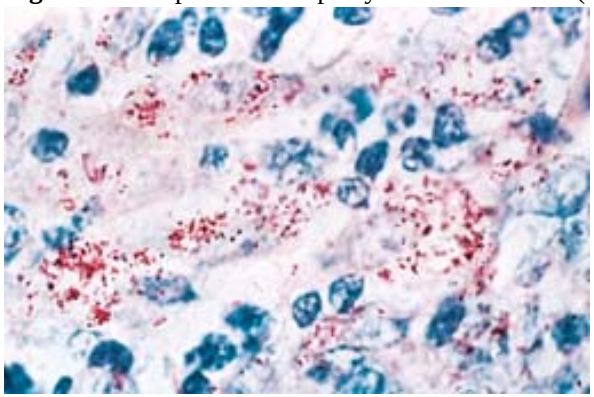


Figure 8-37 *Treponema pallidum* (dark-field microscopy) showing several spirochetes in scrapings from the base of a chancre. *(Courtesy of Dr. Paul Southern, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

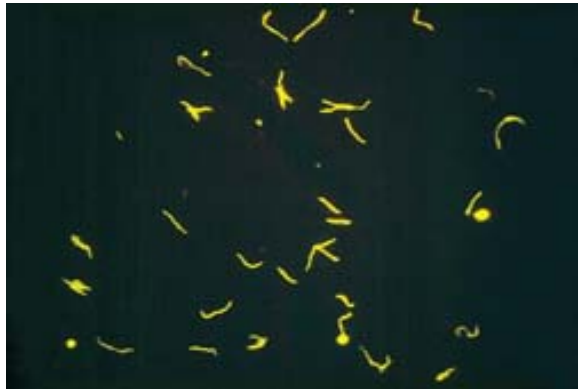


Figure 8-38 Protean manifestations of syphilis.

STAGE	PATHOLOGY	
Primary	Chancre	
Secondary	Palmar, rash	
	Lymphadenopathy	
	Condyloma latum	
Tertiary	Neurosyphilis:	Meningovascular
		Tabes dorsalis
		General paresis
	Aortitis:	Aneurysms
		Aortic regurgitation
	Gummas:	Hepar lobatum
		Skin, bone, others
Congenital	Late abortion or stillbirth	
	Infantile:	Rash
		Osteochondritis
		Periostitis
		Liver and lung fibrosis
	Childhood:	Interstitial keratitis
		Hutchinson teeth
		Eighth nerve deafness

Hutchinson teeth
Eighth nerve deafness

Figure 8-39 Syphilitic chancre in the scrotum (see Figure 8-8 for the histopathology of syphilis). *(Courtesy of Dr. Richard Johnson, Beth Israel-Deaconess Hospital, Boston, MA.)*

Figure 8-40 Trichrome stain of liver shows liver gumma (scar), stained blue, which is caused by tertiary syphilis (also known as hepar lobatum). Compare with nodules of alcoholic cirrhosis (Chapter 18).

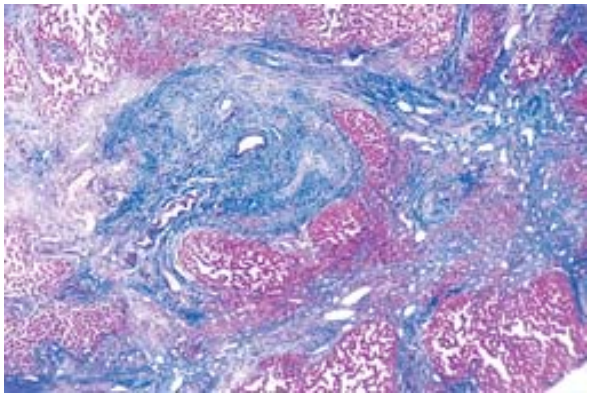


Figure 8-41 Tiny deer tick *(bottom)*, which transmits Lyme disease and Babesia and Ehrlichia organisms, contrasted with a larger dog tick *(top)*, which is not thought to transmit human infections. *(Courtesy of Dr. F.R. Matuschka, Free University of Berlin, Germany.)*

Figure 8-42 Clinical stages of Lyme disease.

STAGE 1

Acute illness (weeks)

- Tick bite—erythematous papule
- Erythema chronicum migrans
- Lymphadenitis

STAGE 2

Dissemination (weeks to months)

- CNS:
  - Meningoencephalitis
  - Cranial neuritis
- Cardiac:
  - Heart block
  - Pericarditis
  - Myocarditis

STAGE 3

Late chronic form

- Destructive chronic arthritis
- Acrodermatitis atrophicans
- Neuropathy

Figure 8-43 Boxcar-shaped Gram-positive *Clostridium perfringens* in gangrenous tissue.

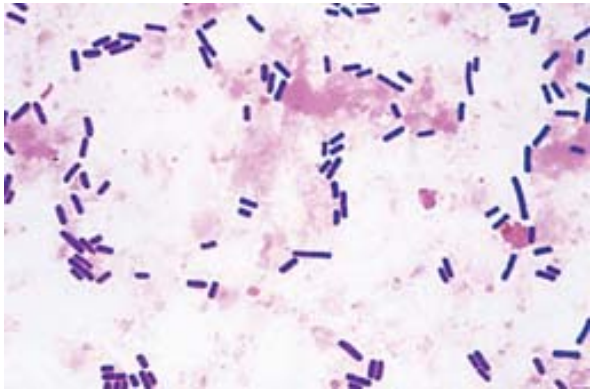


Figure 8-44 Peripheral blood granulocyte (band neutrophil) containing an Ehrlichia inclusion (*arrow*). *(Courtesy of Dr. Stephen Dumler, Johns Hopkins Medical Institutions, Baltimore, MD.)*

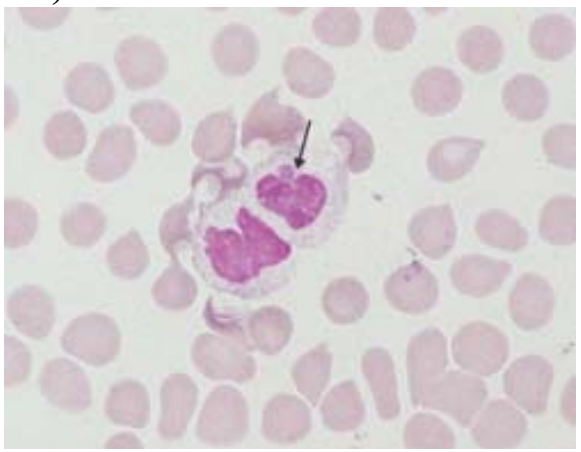


TABLE 8-10 -- Rickettsial Diseases and Pathogens

Typhus Group (No Eschar)				
Organism	***Disease***	***Geography***	***Transmission***	***Distinctive Features***
R. prowazekii	Epidemic typhus Brill-Zinsser disease	Worldwide (war, famine)	Louse feces	Endothelial infection; centrifugal rash; reactivation with mild disease
R. typhi	Murine typhus	Worldwide (rat related)	Rat flea feces	Similar to epidemic typhus, but mortality is lower
Spotted Fever Group				
Organism	***Disease***	***Geography***	***Transmission***	***Distinctive Features***
R. rickettsii	Rocky Mountain spotted fever	North and South America	Tick bite	Endothelia and vascular smooth muscle infected; centripetal rash, eschar rare
R. conorii	Boutonneuse fever	Africa, Southern Europe, India	Tick bite	Prominent eschar, tache noire

R. africae	Africa tick fever	Africa, Caribbean	Tick bite	Multiple eschars
R. sibirica	North Asia tick typhus	Eurasia	Tick bite	Typical spotted fever with eschar
R. japonica	Japanese spotted fever	Japan	Tick bite	Typical spotted fever with eschar
R. australis	Queensland tick typhus	Eastern Australia	Tick bite	Typical spotted fever with eschar
R. akari	Rickettsialpox	United States, Ukraine, Korea, Croatia	Mite bite	Mild spotted fever with eschar
R. felis	Similar to murine typhus	United States	Opossum flea	Similar to murine typhus
Orientia tsutsugamushi	Scrub typhus	Eastern Asia and Western Pacific region	Chigger bite	Eschar common, insects present in scrub vegetation
Ehrlichiosis Group				
Organism	***Disease***	***Geography***	***Transmission***	***Distinctive Features***
Ehrlichia chaffeensis	Monocytic ehrlichiosis	United States, Europe	Tick bite	Fever, lymphadenopathy, no eschar, rash in 40%
Anaplasma phagocytophilum and *E. ewingii*	Granulocytic ehrlichiosis	United States, Europe	Tick bite	Fever, lymphadenopathy, no eschar or rash

The innate immune response to rickettsial infection is mounted by natural killer cells, which produce γ-interferon, reducing bacterial proliferation. Cytotoxic T-lymphocyte responses are critical for elimination of rickettsial infections. IFN-γ and TNF, from activated natural killer cells, CD4+, and CD8+ T lymphocytes, stimulate the production of bactericidal nitric oxide. Cytotoxic T lymphocytes lyse infected cells, reducing bacterial proliferation. Rickettsial infections are diagnosed by immunostaining of organisms or by detection of antirickettsial antibodies in the serum.

Morphology

Typhus Fever.

In mild cases, the gross changes are limited to a rash and small hemorrhages due to the vascular lesions. In more severe cases, there may be areas of necrosis of the skin with gangrene of the tips of the fingers, nose, earlobes, scrotum, penis, and vulva. In such cases, irregular ecchymotic hemorrhages may be found internally, principally in the brain, heart muscle, testes, serosal membrane, lungs, and kidneys.

The most prominent microscopic changes are the small-vessel lesions that underlie the rash and the focal areas of hemorrhage and inflammation in the various organs and tissues affected. Endothelial swelling in the capillaries, arterioles, and venules may narrow the lumina of these vessels. A cuff of mononuclear inflammatory cells usually surrounds the affected vessel. The vascular lumina are sometimes thrombosed, but necrosis of the vessel wall is unusual in typhus compared with RMSF. Vascular thromboses lead to the gangrenous necroses of the skin and other structures in a minority of cases. In the brain, characteristic typhus nodules are composed of focal microglial proliferations with an infiltrate of mixed T lymphocytes and macrophages (Fig. 8-45).

Scrub typhus, or mite-borne infection, is usually a milder version of typhus fever. The rash is usually transitory or might not appear. Vascular necrosis or thrombosis is rare, but there may be a prominent inflammatory lymphadenopathy.

Rocky Mountain spotted fever.

A hemorrhagic rash that extends over the entire body, including the palms of the hands and soles of the feet, is the hallmark

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Figure 8-45 Typhus nodule in the brain.

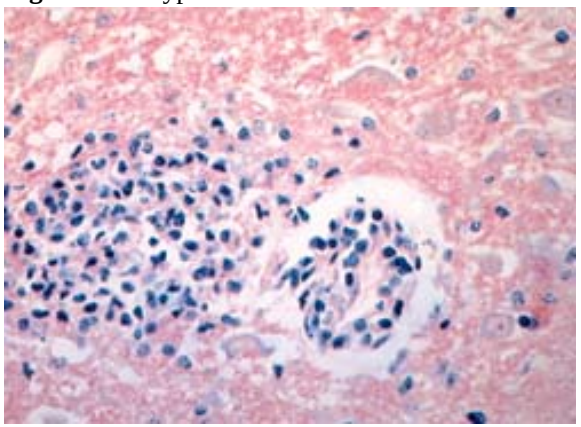


Figure 8-46 Rocky Mountain spotted fever with a thrombosed vessel and vasculitis.

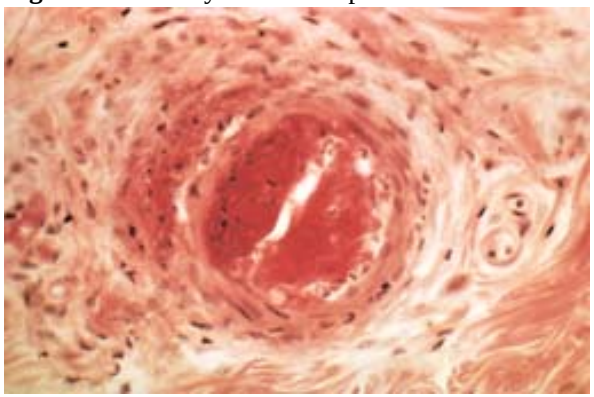


Figure 8-47 The morphology of *Candida* infections. *A*, Severe candidiasis of the distal esophagus. *B*, Silver stain of esophageal candidiasis reveals the dense mat of *Candida*. *C*, Characteristic pseudohyphae and blastoconidia (budding yeast) of *Candida*. *(C, Courtesy of Dr. Dominick Cuvuoti, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

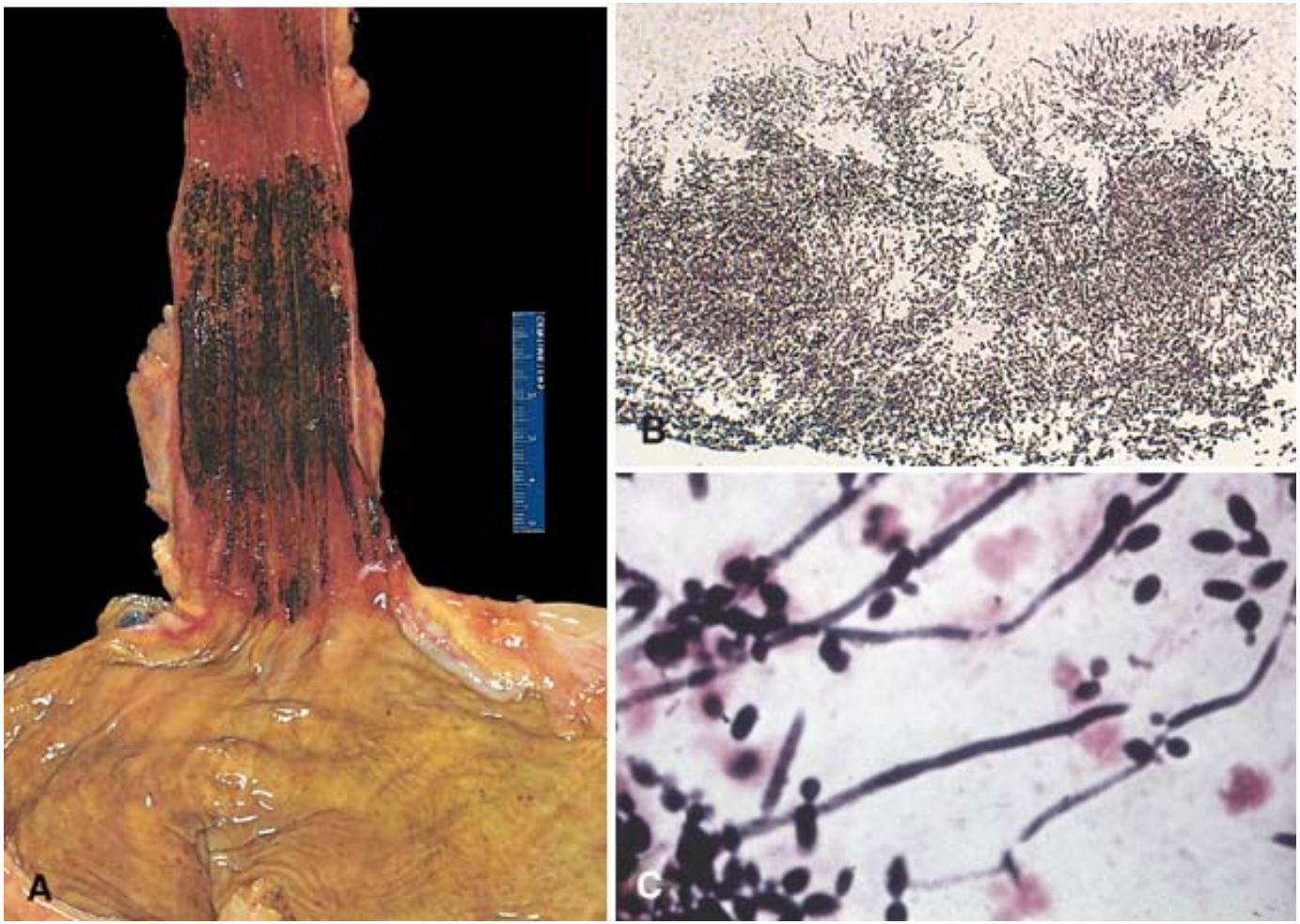


Figure 8-48 Mucicarmine stain of cryptococci (staining red) in a Virchow-Robin perivascular space of the brain (soap-bubble lesion).

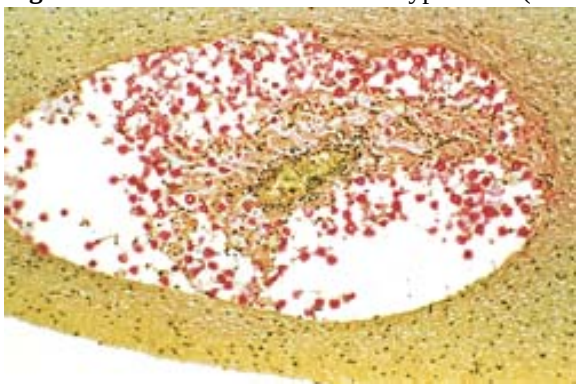


Figure 8-49 *Aspergillus* morphology. *A*, Invasive aspergillosis of the lung in a bone marrow transplant patient. *B*, Histologic sections from this case, stained with Gomori methenamine-silver (GMS) stain, show septate hyphae with acute-angle branching, features consistent with *Aspergillus*. Occasionally, *Aspergillus* may demonstrate fruiting bodies (inset) when it grows

in areas that are well aerated (such as the upper respiratory tract).

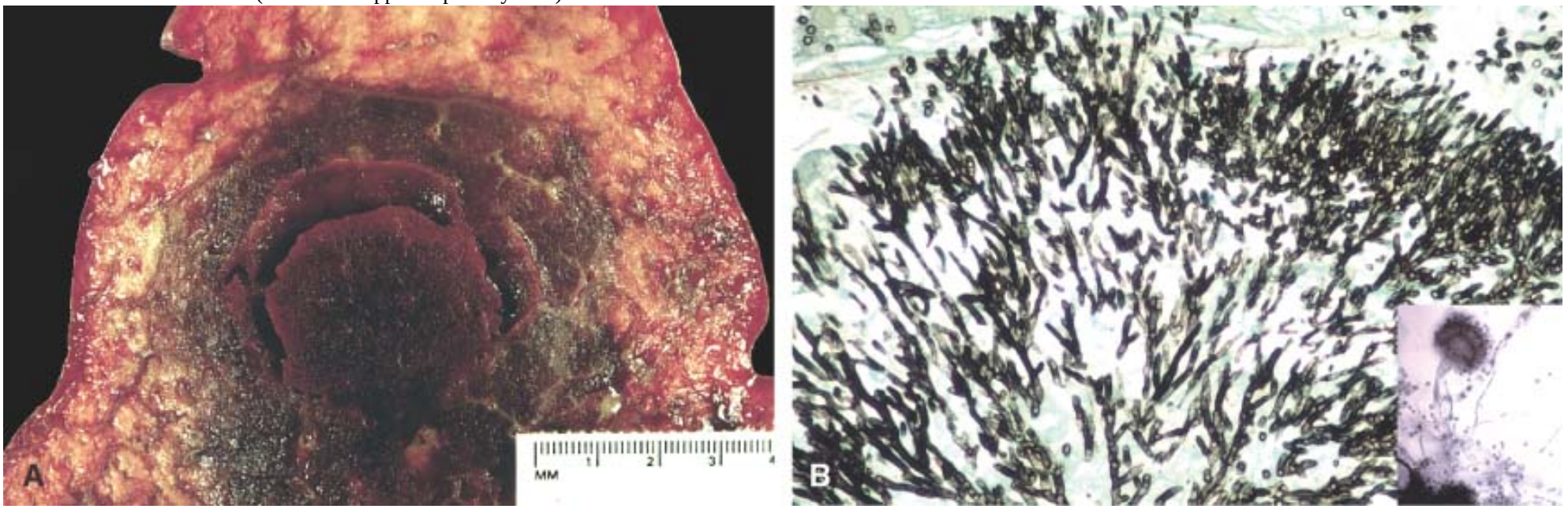


Figure 8-50 PAS stain of mucormycosis showing hyphae, which have an irregular width and right-angle branching, invading an artery wall.

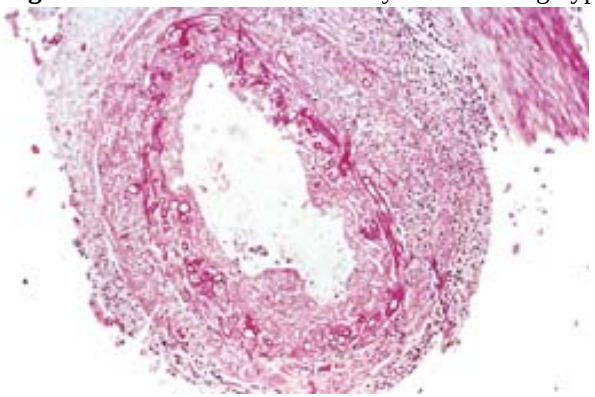


Figure 8-51 Life cycle of *Plasmodium falciparum*. *(Drawn by Dr. Jeffrey Joseph, Beth Israel-Deaconess Hospital, Boston, MA.)*

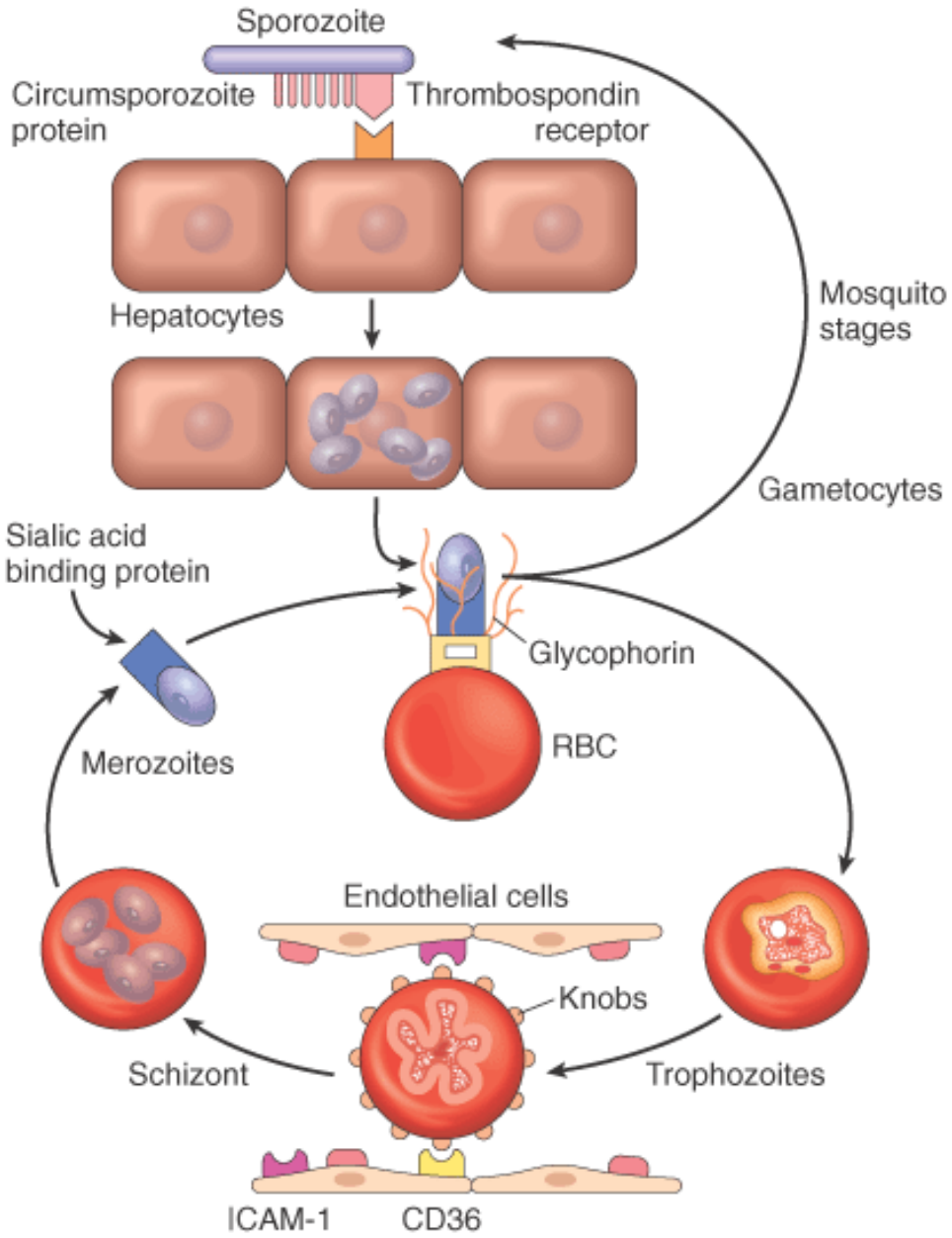


Figure 8-52 *P. falciparum*-infected red cells marginating within a vein in cerebral malaria.

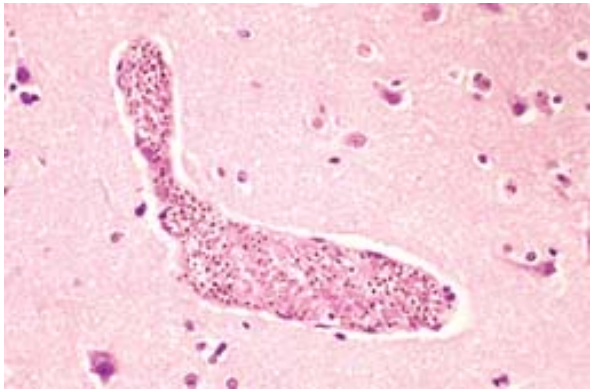


Figure 8-53 Erythrocytes with *Babesia*, including the distinctive Maltese cross form. *(Courtesy of Lynne Garcia, LSG and Associates, Santa Monica, CA.)*

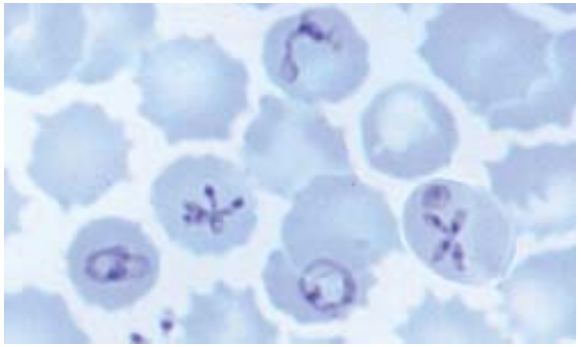


Figure 8-54 *Leishmania donovani* parasites within the macrophages of a lymph node in visceral leishmaniasis (kala-azar).

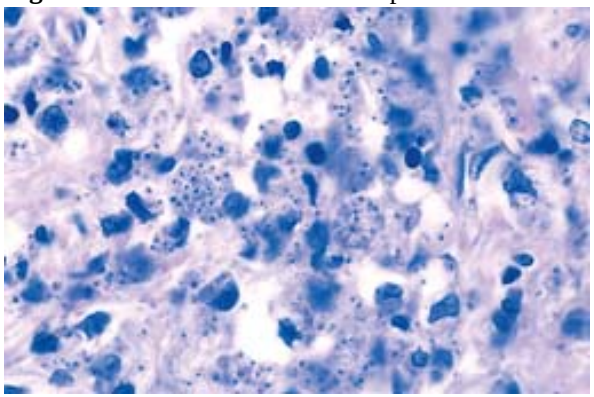


Figure 8-55 Slender bloodstream parasites of African trypanosomiasis.

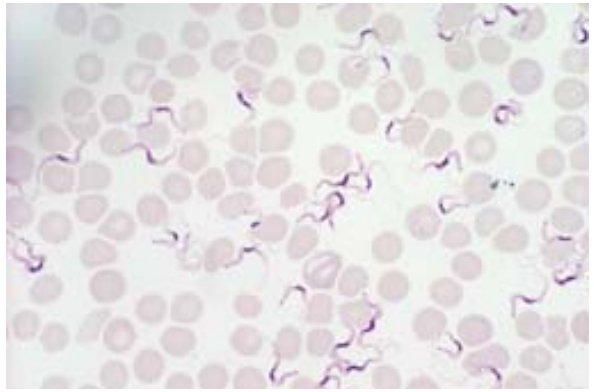


Figure 8-56 Strongyloides hyperinfection in a patient treated with high-dose cortisone. A female, her eggs and rhabditoid larvae are in the duodenal crypts; filariform larvae are entering the blood vessels and muscularis mucosa. *(Courtesy of Dr. Franz C. Von Lichtenberg, Brigham and Women's Hospital, Boston, MA.)*

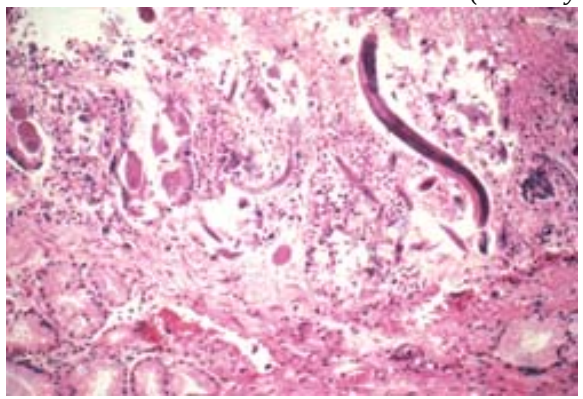


Figure 8-57 Portion of a cysticercus cyst.

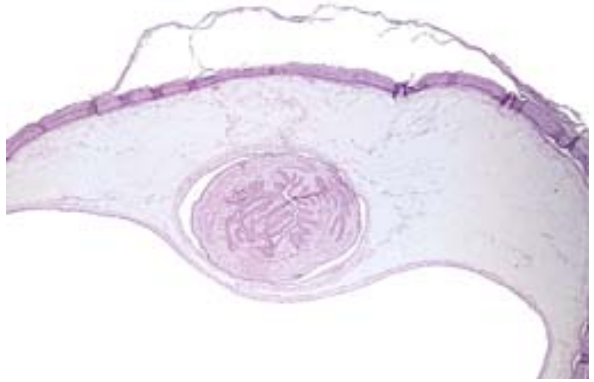


Figure 8-58 Coiled *Trichinella spiralis* larva within a skeletal muscle cell.

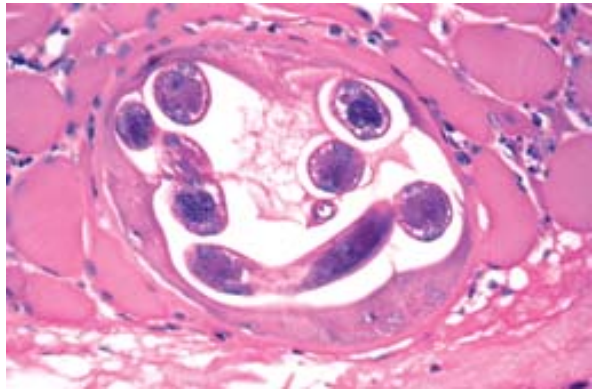


Figure 8-59 Schistosome life cycle.

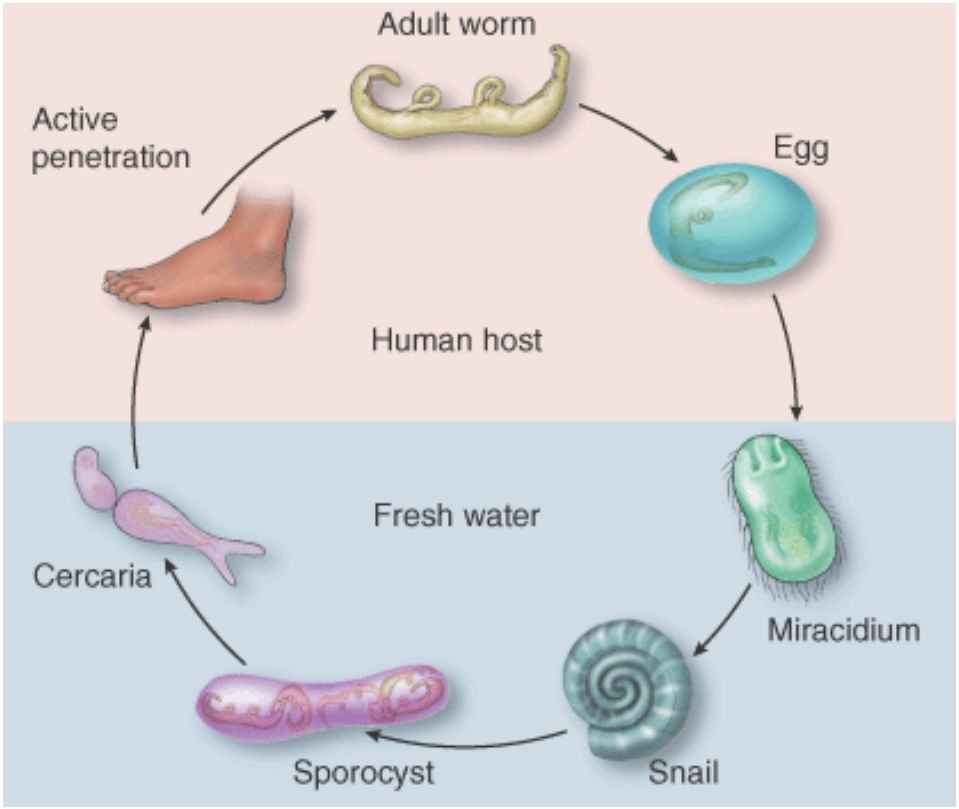


Figure 8-60 *Schistosoma mansoni* granuloma with a miracidium-containing egg *(center)* and numerous, adjacent, scattered eosinophils.

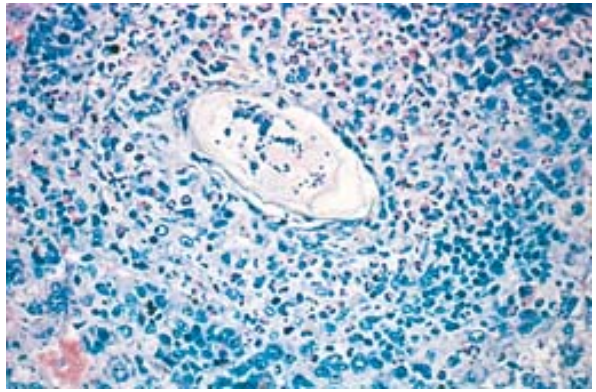
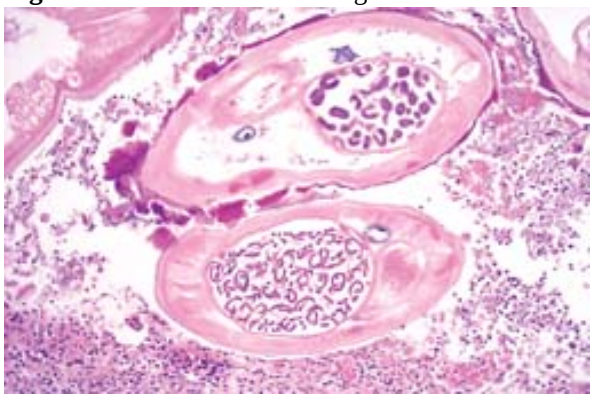


Figure 8-61 Pipe-stem fibrosis of the liver due to chronic *Schistosoma japonicum* infection.

Figure 8-62 Massive edema and elephantiasis caused by filariasis of the leg. *(Courtesy of Dr. Willy Piessens, Harvard School of Public Health, Boston, MA.)*

Figure 8-63 Microfilaria-laden gravid female of *Onchocerca volvulus* in a subcutaneous fibrous nodule.



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Chapter 9 - Environmental and Nutritional Pathology

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Environment and Disease

Environmental and occupational health encompasses the diagnosis, treatment, and prevention of injuries and illnesses resulting from exposure to exogenous chemical or physical agents. Such exposure may occur in the workplace, or people may voluntarily expose themselves to these hazards, for example, by abusing drugs or ethanol and smoking cigarettes. These personal habits may lead to involuntary exposure of fetuses and infants to drugs, ethanol, or environmental tobacco smoke.

People are often confused about the magnitude of the adverse health effects of exogenous physical and chemical agents. There is widespread concern about the potential chronic or delayed effects of exposure to low levels of contaminants in air, water, and food, and hence patients frequently seek advice and information from their health care

professionals about the risk of disease associated with specific environmental and occupational exposures. This chapter provides a basic foundation in the most important diseases associated with environmental and occupational exposures, emphasizing the mechanisms leading to these diseases. This framework will help physicians to recognize and treat injuries and

illness resulting from environmental and occupational exposures and to educate their patients about the risks of these exposures.[1]

RECOGNITION OF OCCUPATIONAL AND ENVIRONMENTAL DISEASES

Accidents, illness, and premature deaths threaten the health of 130 million workers in the United States. Occupational health risks are even greater in developing countries, where children and women constitute a larger proportion of the work force. In the United States, the annual rate of occupational injuries is 7400 per 100,000 workers. The overall fatality rate is 4.8 per 100,000 workers; the highest rates occur in the mining, agricultural, construction, transportation, and public utility industries. In addition to physical injury, occupational exposures contribute to a wide range of illnesses that may lead to premature death (Table 9-1). The magnitude of occupational diseases is most likely underestimated because workers and their employers fear economic or legal pressures, physicians may not recognize that an illness is work related, and there may be a long latent period between exposure and the development of clinical illness. Nevertheless, occupational diseases are preventable if there is adequate surveillance by state and federal governments, responsible leadership in industry, and access to health professionals trained in occupational safety and health.[1]

The magnitude and extent of illness related to environmental exposures are difficult to ascertain. The Environmental Protection Agency estimates that more than 80,000 chemicals are currently used in the United States; approximately 1500 are pesticides and 5500 are food additives that affect our water and food supplies. Although only 600 of these chemicals have been tested, 10% have produced cancer in at least one rodent species.[2] Industrial chemicals, production

TABLE 9-1 -- Reported Occupational Diseases in the United States in 1997

Disease	Number of Workers	Percentage
Repeated trauma	276,600	64
Skin disorders	57,900	13
Lung conditions due to toxic exposures	20,300	5
Physical injury	16,600	4
Poisoning	5100	1
Lung disease due to dusts	2900	1
All other illnesses	50,600	12
Total	430,000	100

Data from Levy BS, Wegman DH: Occupational health — an overview. In Levy BS, et al. (eds): Occupational Health. Recognizing and Preventing Work-Related Disease and Injury, fourth ed. Philadelphia, Lippincott Williams & Wilkins, 2000, p. 3; and Bureau of Labor Statistics, U.S. Department of Labor, www.hls.gov.

byproducts, and metals are commonly detected at hazardous waste sites (Table 9-2). There are currently 11,300 Superfund-designated waste sites in the United States. The potential human health hazards associated with exposure to chemical mixtures is a major concern.[2]

There is considerable difference in the magnitudes of exposure in the occupational and environmental settings. Occupational exposures affect a defined cohort of workers who are exposed to chemicals in the range of parts per million (ppm); by contrast, environmental exposures to these same chemicals in the air, water, or hazardous waste sites may be in the parts per billion (ppb) or parts per trillion (ppt) range. The health effects of such chronic, low-level exposures are unknown.

In the United States, four regulatory agencies determine exposure limits for environmental and occupational hazards: the Environmental Protection Agency, the Food and Drug

Administration (FDA), the Occupational Safety and Health Administration, and the Consumer Products Safety Commission. The Environmental Protection Agency regulates exposure to pesticides, toxic chemicals, water and air pollutants, and hazardous wastes. The FDA regulates drugs, medical devices, food additives, and cosmetics. The Occupational Safety and Health Administration mandates that employers (including hospitals and physicians) provide safe working conditions for employees. All other products sold for use in homes, schools, or recreation are regulated by the Consumer Products Safety Commission.

Physicians should be familiar with current approaches used by regulatory agencies in the United States and be prepared to explain the strengths and limitations of the scientific evidence in nontechnical terms. Health care providers must be prepared to counsel patients about the primary prevention of disease related to occupational and environmental exposures, taking into account potential synergistic effects of mixed exposures and individual genetic susceptibility. Prevention of tobacco smoking would prevent 80% to 90% of lung cancers; however, this objective has been difficult to achieve, especially in teenagers. Strategies for secondary prevention of lung cancer in former or current smokers (e.g., chemoprevention) have been disappointing so far.[3] Prevention of occupationally

TABLE 9-2 -- Common Chemicals at Hazardous Waste Sites

Acetone	DDT, DDE, DDD
Aldrin/Dieldrin	1,1 and 1,2-Dichloroethane
Arsenic	Lead
Barium	Mercury
Benzene	Methylene chloride
2-Butanone	Nickel
Cadmium	Pentachlorophenol
Carbon tetrachloride	Polychlorinated biphenyls
Chlordane	Tri- and Tetrachloroethylene
Chloroform	Toluene
Chromium	Vinyl Chloride
Cyanide	Zinc
Data from U.S. Environmental Protection Agency, www.epa.gov/superfund/resources/chemicals.htm.	

related diseases rests on defining and enforcing safe exposure levels, developing new technologies to reduce industrial exposures, and identifying less toxic substitutes for industrial and chemical agents. These strategies require a basic understanding of biochemical and molecular mechanisms of toxicity.

MECHANISMS OF TOXICITY

Toxicology is the scientific discipline that studies the detection, effects, and mechanisms of action of poisons and toxic chemicals. *Toxicity* is a relative phenomenon that depends on the inherent structure and properties of a chemical and on its dose. Dose-response curves are typically generated in laboratory animals exposed to various amounts of a chemical. A typical

dose-response curve for acute toxicity is illustrated in Figure 9-1 . In this example, a measurable response occurs at a dose of 0.1 mg/kg; this is defined as the *threshold dose*. To the left of this dose, at subthreshold levels, there is no measurable response. For this chemical, this is the *no observed effect level* and can be considered a safe dose. This information is used to establish a daily or annual *threshhold limit value* or *permissible exposure level* for occupational exposures. Frequently, a plateau is reached at higher doses; this is defined as the *ceiling effect*. It is uncertain whether carcinogens show a threshold effect or whether the dose-response curve should be extrapolated linearly to zero.[4]

Despite the inherent limitations of toxicity testing in animals, several important toxicologic principles have been established by this experimental approach. Exogenous chemicals are absorbed after ingestion, inhalation, or skin contact, and then distributed to various organs (Fig. 9-2). Chemicals are frequently metabolized, often by multiple enzymatic pathways, to products that may be more toxic or less toxic than the parent chemical. One or more of these products then interacts with the target macromolecule, resulting in a toxic effect.[5] The site of toxicity is frequently the site where metabolism or excretion of toxic metabolites occurs. The dose administered (external dose) may not be the same as the *biologic effective dose* delivered to the target organ and target macromolecule.

Figure 9-1 The dose-response curve for acute chemical toxicity. Th, threshold dose; STh, subthreshold levels. *(Data from Hughes WW: Essentials of Environmental Toxicology: The Effects of Environmentally Hazardous Substances on Human Health. Washington, DC, Taylor & Francis, 1996, p. 33.)*

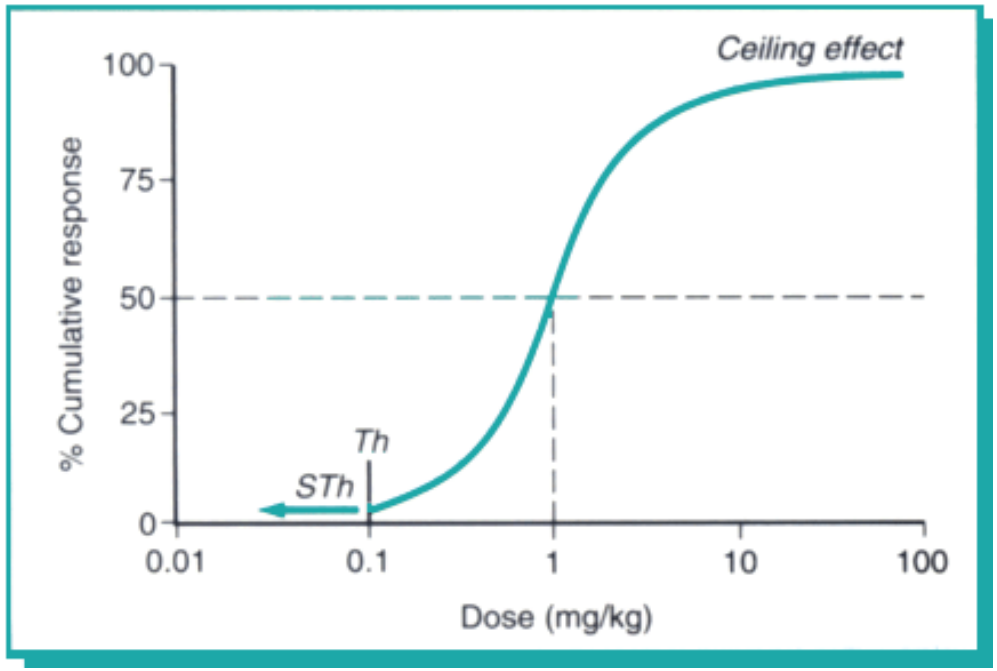

Figure 9-2 Absorption and distribution of toxicants. *(From Hodgson E, Levi PE: Absorption and distribution of toxicants. In Hodgson E, Levi PE [eds]: A Textbook of Modern Toxicology. Stamford, CT, Appleton & Lange, 1997, p. 52.)*

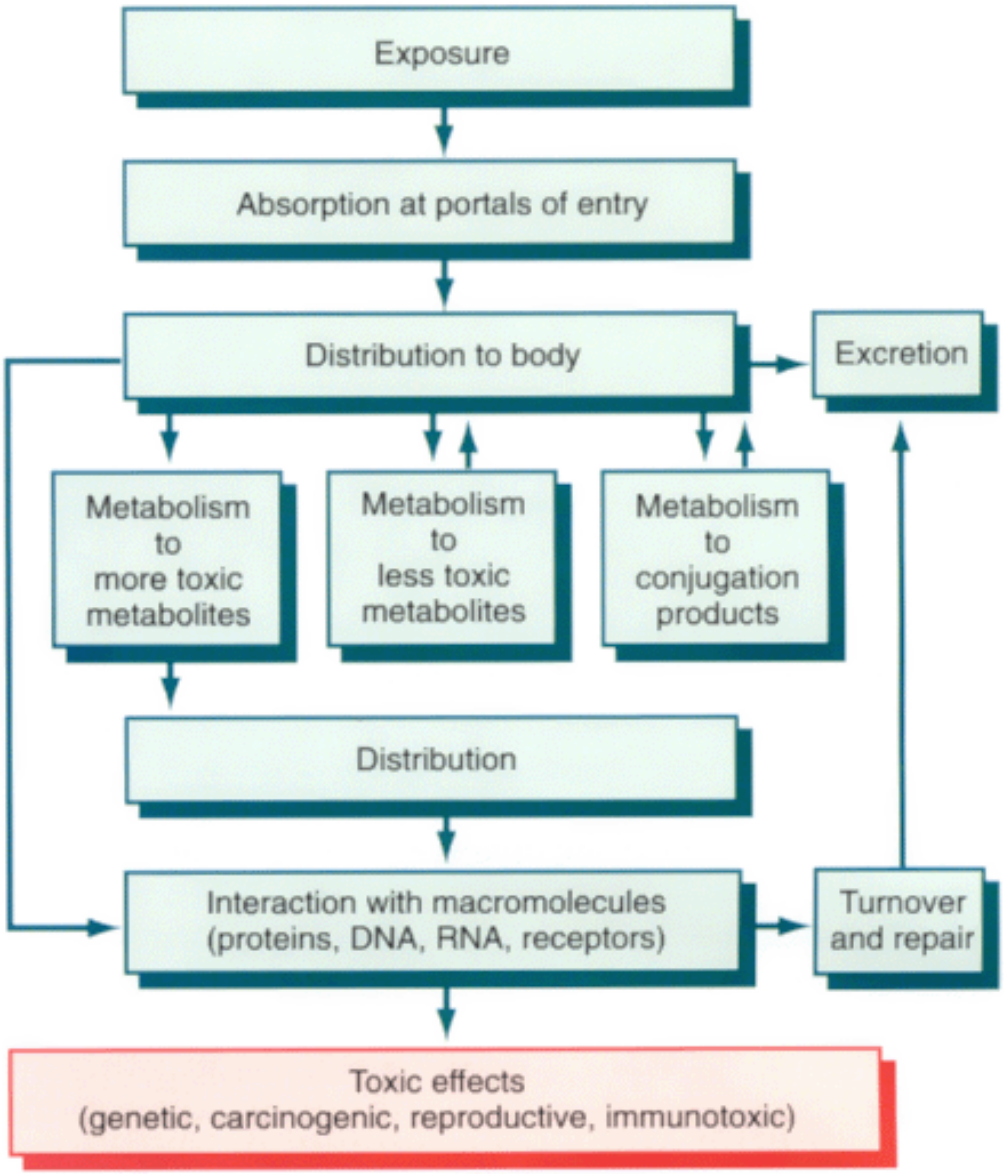

Figure 9-3 Biotransformation of lipophilic toxicants to hydrophilic metabolites. *(Adapted from Hodgson E: Metabolism of toxicants. In Hodgson E, Levi PE [eds]: A Textbook of Modern Toxicology. Stamford, CT, Appleton & Lange, 1997, p. 57.)*

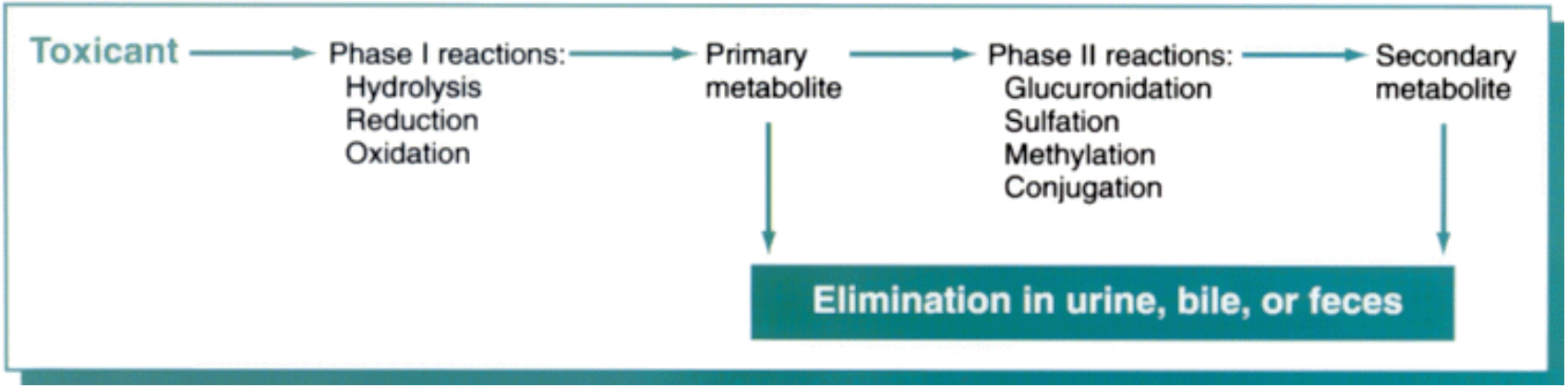

TABLE 9-3 -- Organ-Specific Carcinogens in Tobacco Smoke

Organ	Carcinogen
Lung, larynx	Polycyclic aromatic hydrocarbons
	4-(Methylnitrosoamino)-1-(3-pyridyl)-1-buta-none (NNK)
	Polonium 210
Esophagus	*N'*-Nitrosonornicotine (NNN)
Pancreas	NNK (?)
Bladder	4-Aminobiphenyl, 2-naphthylamine
Oral cavity (smoking)	Polycyclic aromatic hydrocarbons, NNK, NNN
Oral cavity (snuff)	NNK, NNN, polonium 210
Data from Szczesny LB, Holbrook JH: Cigarette smoking. In Rom WH (ed): Environmental and Occupational Medicine, 2nd ed. Boston, Little, Brown, 1992, p. 1211.	

such synergism is the increase in risk of lung cancer in cigarette smokers exposed to asbestos.[13]

Mainstream cigarette smoke inhaled by the smoker is composed of a particulate phase and a gas phase; tar is the total particulate phase without water or nicotine. There are 0.3 to 3.3 billion particles per milliliter of mainstream smoke and more than 4000 constituents, including 43 known carcinogens. Examples of the organ-specific carcinogens found in tobacco smoke and snuff are listed in Table 9-3 . In addition to these chemical carcinogens, cigarette smoke contains carcinogenic metals such as arsenic, nickel, cadmium, and chromium; potential promoters such as acetaldehyde and phenol; irritants such as nitrogen dioxide and formaldehyde; cilia toxins such as hydrogen cyanide; and carbon monoxide. Carbon monoxide is a colorless, odorless gas produced during incomplete combustion of fossil fuels or tobacco. It has 200 times higher affinity for hemoglobin than oxygen does and it impairs release of oxygen from hemoglobin. Thus, carbon monoxide exposure decreases the delivery of oxygen to peripheral tissues. Carbon monoxide also binds to other heme-containing proteins such as myoglobin and cytochrome oxidase. Nicotine is an important constituent of cigarette smoke. It is an alkaloid that readily crosses the blood-brain barrier and stimulates nicotine receptors in the brain. It is also responsible for the acute pharmacologic effects associated with tobacco use that are most likely mediated by catecholamines: increased heart rate and blood pressure, increased coronary artery blood flow, increased contractility and cardiac output, and mobilization of free fatty acids. Nicotine is responsible for tobacco addiction.

The inhaled agents in cigarette smoke may act directly on the mucous membranes, may be swallowed in saliva, or may be absorbed into the bloodstream from the abundant alveolar

capillary bed. By various routes of delivery, the constituents of cigarette smoke act on distant target organs and cause a variety of systemic diseases, listed in Table 9-4 . The greatest numbers of deaths attributable to cigarette smoking are due to lung cancer, ischemic heart disease, and chronic obstructive lung disease. Lung cancer is caused by multiple carcinogens and promoters in cigarette smoke. As described in Chapter 15 , specific preneoplastic changes are found in the tracheobronchial lining of cigarette smokers. These cellular changes

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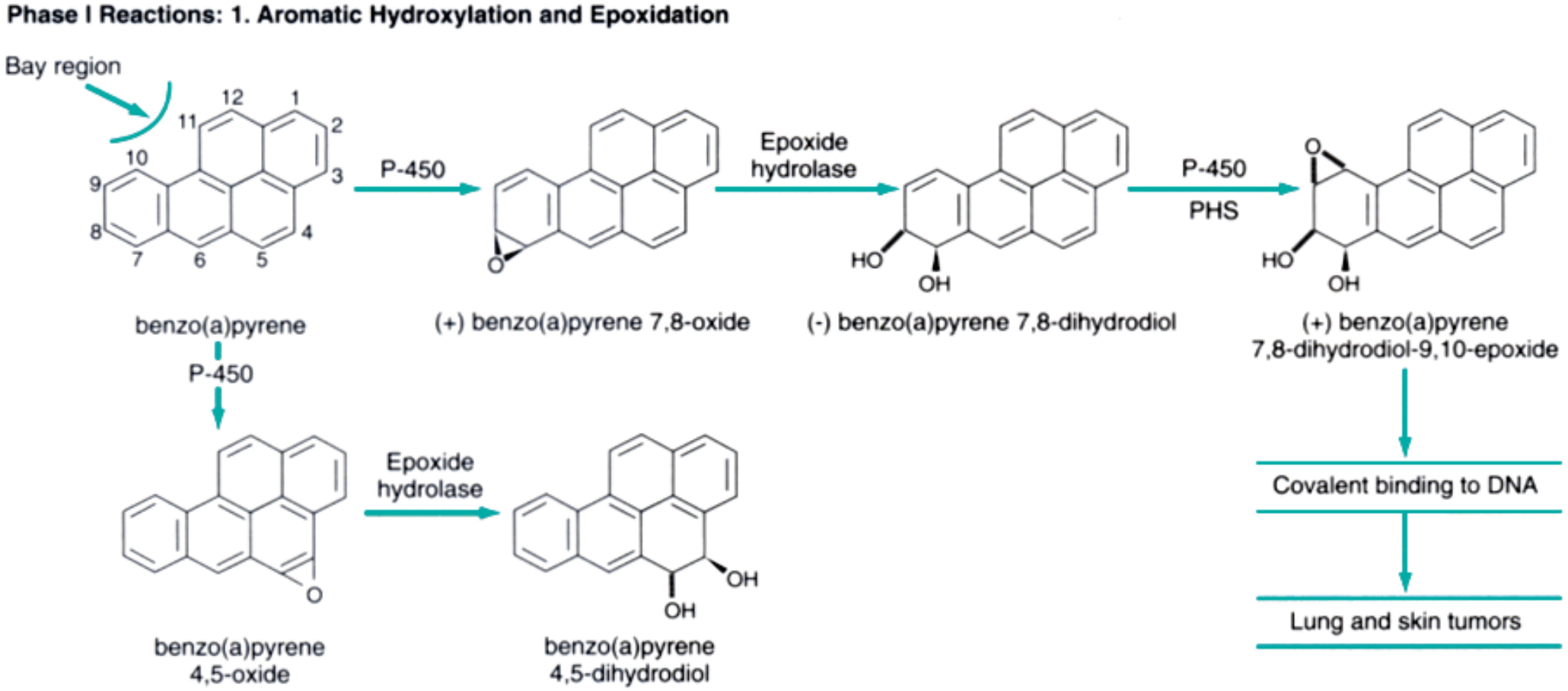
Figure 9-4a *A,* Xenobiotic metabolism: phase I reactions. FMO, flavin-containing monooxygenase; PHS, prostaglandin-H synthases.



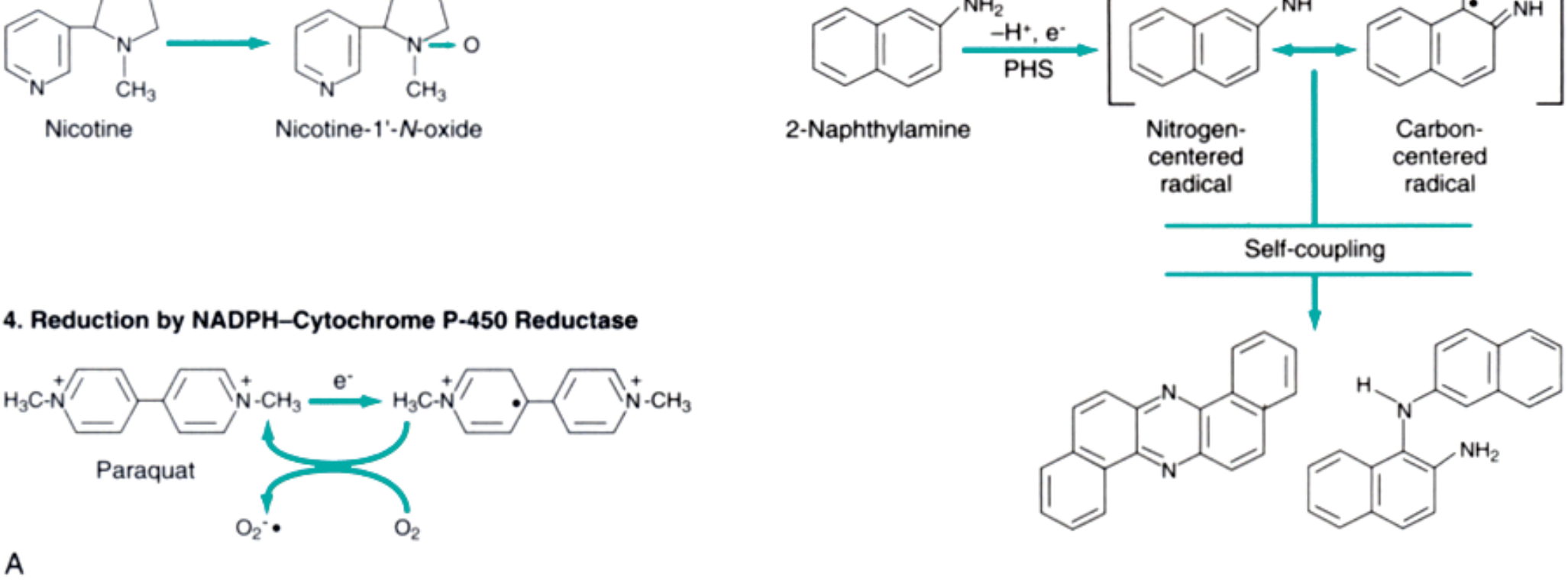


Figure 9-4b *B,* Xenobiotic metabolism: phase II reactions (see text for details). *(Adapted from Parkinson A: Biotransformation of xenobiotics. In Klaasen CD [ed]: Casarett and Doull's Toxicology: The Basic Science of Poisons, 5th ed. New York, McGraw-Hill, 1996, pp. 113–186; and Hodgson E, Levi PE [(eds]: A Textbook of Modern Toxicology. Stamford, CT, Appleton & Lange, 1997, pp. 57, 95.)*

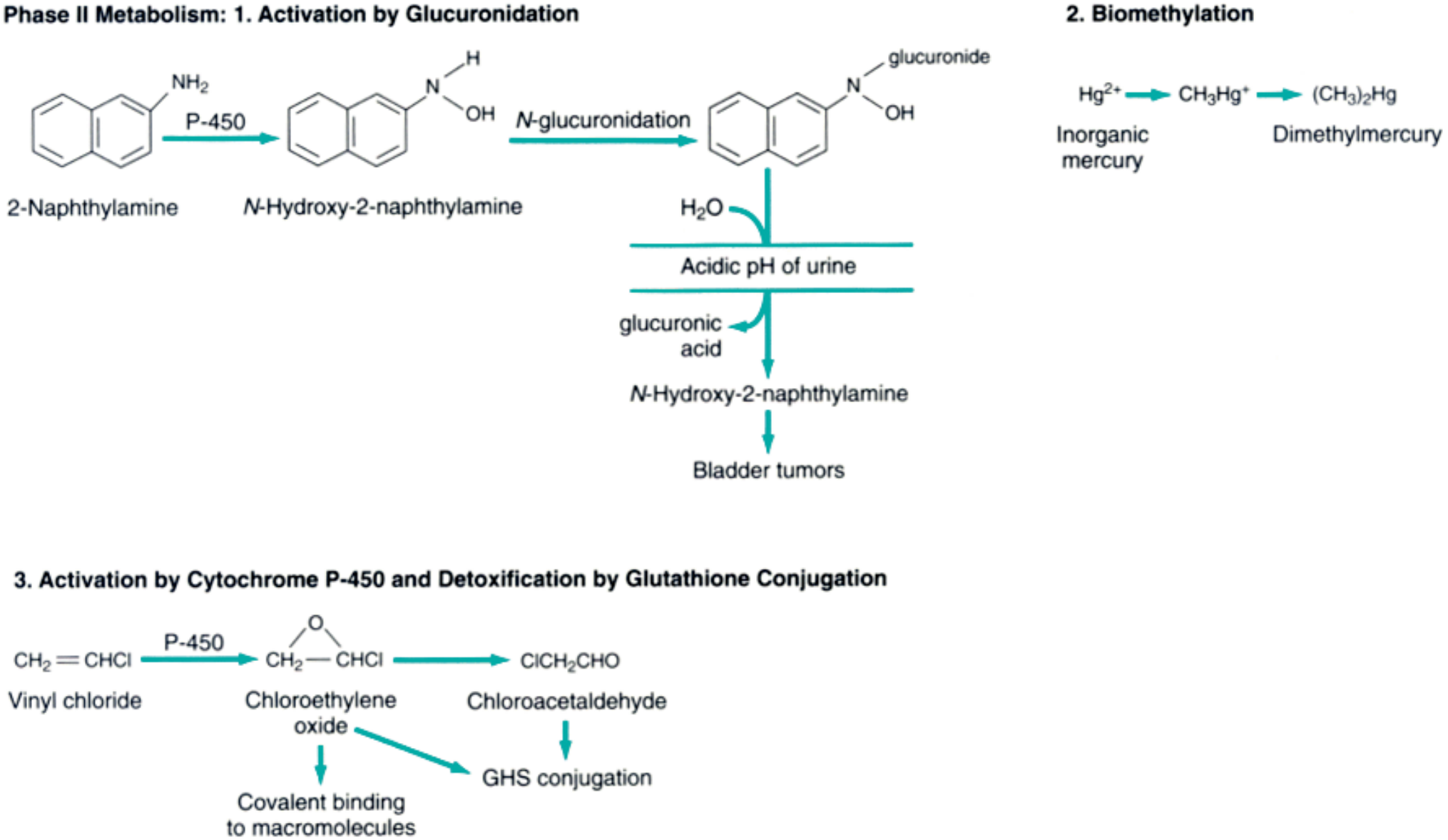


TABLE 9-4 -- Deaths per Year Attributable to Cigarette Smoking in the United States

	Number of Deaths	
Cause of Death	***Men***	***Women***
Cancer	102,812	54,664
Cardiovascular disease	90,906	57,699

Respiratory disease	53,713	44,429
Residential fires	589	377
Perinatal deaths	598	407
Lung cancer and heart disease attributable to passive smoking	15,517	22,536
Total	264,135	80,112

Data from CDC. Annual smoking-attributable mortality, years of potential life lost, and economic costs—United States, 1995–1999. MMWR 51:300, 2002.

exposure to silica, coal dust, grain dust, cotton dust, and welding fumes.

Tobacco use also increases the prevalence of peptic ulcers; smoking impairs healing of ulcers and increases the likelihood of recurrence. Smoking may also increase pyloric reflux and decrease bicarbonate secretion from the pancreas.

In addition to the health hazards of mainstream tobacco smoke, there are risks associated with exposure to sidestream smoke, also called passive smoking or environmental tobacco smoke (ETS). In 1986, two reports issued by the National Research Council and the Surgeon General concluded that ETS increases the risk of lung cancer, ischemic heart disease, and acute myocardial infarction.[14] The Environmental Protection Agency classified ETS as a known human carcinogen in 1992. ETS is especially hazardous for infants and young children. Maternal smoking increases the incidence of sudden infant death syndrome. Young children in households of cigarette smokers suffer from an increased incidence of respiratory and ear infections and exacerbation of asthma.

Alcohol Abuse

Ethanol is the most widely used and abused agent throughout the world. There are 15 to 20 million alcoholics in the United States; approximately 100,000 deaths in the United States are attributed to alcohol abuse per year, with an economic cost of $100 to $130 billion.[15] Ethanol is ingested in alcoholic beverages such as beer, wine, and distilled spirits. A blood alcohol concentration of 80 to 100 mg/dL is the legal

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definition for driving under the influence of alcohol in many states. Approximately 3 ounces (44 ml) of ethanol are required to produce this blood alcohol level in a 70-kg person. This is equivalent to 12 ounces of fortified wine, 8 bottles of beer (12 ounces each), or 6 ounces of 100-proof whiskey. In occasional drinkers, a blood alcohol level of 200 mg/dL produces inebriation, with coma, death, and respiratory arrest at 300 to 400 mg/dL. Habitual drinkers can tolerate blood alcohol levels up to 700 mg/dL. This metabolic tolerance is partially explained by a fivefold to tenfold induction of the cytochrome P-450 xenobiotic-metabolizing enzyme CYP2E1. Such induction increases the metabolism of ethanol as well as that of other drugs and chemicals, including cocaine and acetaminophen. Although no specific receptor for ethanol has been identified, chronic use results in psychologic and physical dependence. The biologic basis for ethanol addiction is unknown, although genetic factors may be involved.

Ethanol is metabolized to acetaldehyde by alcohol dehydrogenase in the gastric mucosa and liver, and by cytochrome P-450 (CYP2E1) and catalase in the liver (Fig. 9-5). Acetaldehyde is converted to acetic acid by aldehyde dehydrogenase. There are genetic polymorphisms in aldehyde dehydrogenase that affect ethanol metabolism; approximately 50% of Chinese, Vietnamese, and Japanese people have reduced activity of this enzyme due to a point mutation that converts glutamine to lysine at amino acid 487. These ethnic groups also rapidly convert ethanol to acetaldehyde, which builds up and triggers a facial flushing syndrome. Women have lower levels of gastric alcohol dehydrogenase activity than men do; therefore, they may develop higher blood alcohol levels than men after drinking the same quantity of ethanol.[15]

The metabolism of ethanol is directly responsible for most of its toxic effects. In addition to its acute action as a

Figure 9-5 Metabolism of ethanol. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase. *(From Parkinson A: Biotransformation of xenobiotics. In Klassen CD [ed]: Casarett and Doull's Toxicology: The Basic Science of Poisons, 5th ed. New York, McGraw-Hill, 1996, p. 128.)*

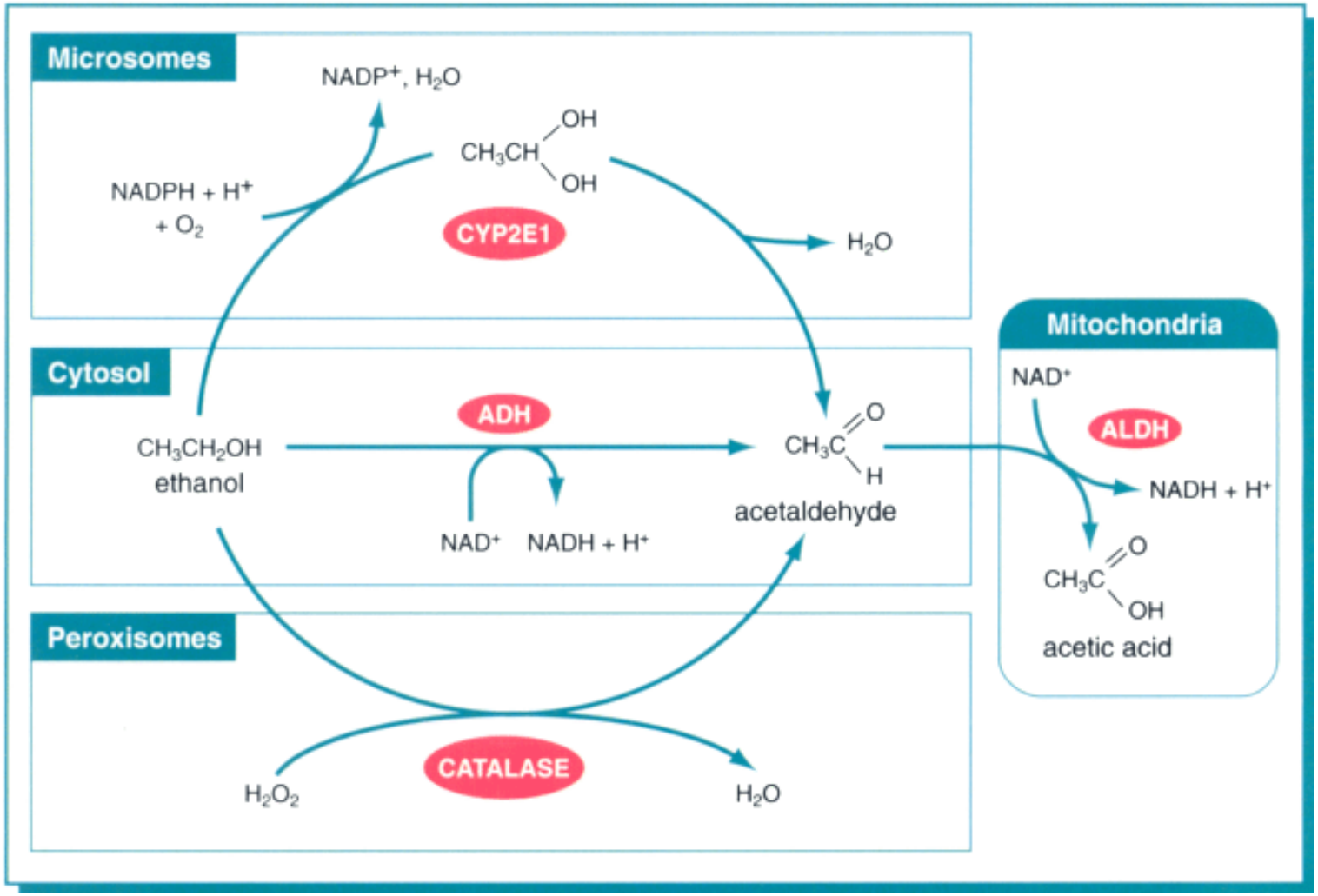

TABLE 9-5 -- Mechanisms of Disease Caused by Ethanol Abuse

Organ System	Lesion	Mechanism
Liver	Fatty change	Toxicity
	Acute hepatitis	
	Alcoholic cirrhosis	

Nervous system	Wernicke syndrome	Thiamine deficiency
	Korsakoff syndrome	Toxicity and thiamine deficiency
	Cerebellar degeneration	Nutritional deficiency
	Peripheral neuropathy	Thiamine deficiency
Cardiovascular system	Cardiomyopathy	Toxicity
	Hypertension	Vasopressor
Gastrointestinal tract	Gastritis	Toxicity
	Pancreatitis	Toxicity
Skeletal muscle	Rhabdomyolysis	Toxicity
Reproductive system	Testicular atrophy	?
	Spontaneous abortion	?
Fetal alcohol syndrome	Growth retardation	Toxicity
	Mental retardation	
	Birth defects	

Data from Rubin E: Alcohol abuse. In Craighead JE (ed): Pathology of Environmental and Occupational Disease. St. Louis, Mosby-Year Book, 1996, p. 249; and Lewis DD, Woods SE: Fetal alcohol syndrome. Am Fam Physician 50:1025, 1994.

central nervous system depressant, chronic ethanol use can cause a wide range of systemic effects (Table 9-5). Some of these chronic effects can be attributed to specific vitamin deficiencies; for example, damage to the peripheral and central nervous systems is related to thiamine deficiency, whereas

other systemic effects result from direct toxicity. The effects of ethanol on various organ systems are discussed next.

Liver.

Ethanol can cause fatty change, acute alcoholic hepatitis, and cirrhosis. *Fatty change* is an acute, reversible manifestation of ethanol ingestion. In chronic alcoholism, fat accumulation can cause massive enlargement of the liver. The biochemical mechanisms responsible for fat accumulation in hepatocytes are the following:

- Catabolism of fat by peripheral tissues is increased, and there is increased delivery of free fatty acids to the liver.
- Metabolism of ethanol in the cytosol and of its derivative, acetaldehyde, in the mitochondria converts the oxidized form of nicotinamide adenine dinucleotide (NAD^+) to the reduced form (NADH); an excess of NADH over NAD stimulates lipid biosynthesis.
- Oxidation of fatty acids by mitochondria is decreased.
- Acetaldehyde forms adducts with tubulin and impairs function of microtubules, resulting in decreased transport of lipoproteins from the liver.

Acute alcoholic hepatitis is another potentially reversible form of liver injury (Chapter 18). Although fatty change is asymptomatic except for liver enlargement, alcoholic hepatitis can produce fever, liver tenderness, and jaundice. On histologic examination, there are focal areas of hepatocyte necrosis and cell injury manifest by fat accumulation and alcoholic hyalin, or Mallory bodies. Neutrophils accumulate around foci of necrosis (Fig. 9-6). Ethanol and its metabolites are directly toxic to hepatocytes; this toxicity is believed to be mediated by glutathione depletion, mitochondrial injury, altered metabolism of methionine, and cytokine release from Kupffer cells.[16] Hepatocellular necrosis, as well as fibrosis, begins around the central vein, suggesting that hypoxia may contribute to this injury. With chronic ethanol use, 10% to 15% of alcoholics develop irreversible liver damage, or *alcoholic cirrhosis*. This is characterized by a hard, shrunken liver with formation of micronodules of regenerating hepatocytes surrounded by dense bands of collagen (Fig. 9-7). Alcoholic cirrhosis is a serious, potentially fatal disease accompanied by weakness, muscle wasting, ascites, gastrointestinal hemorrhage, and coma. Perisinusoidal fibrosis occurs initially, with

Figure 9-6 Acute alcoholic hepatitis. The liver cells show cytoplasmic accumulation of fat and hyalin *(arrow)*. A scattered inflammatory infiltrate is present. *(MEDCOM © 1976.)*

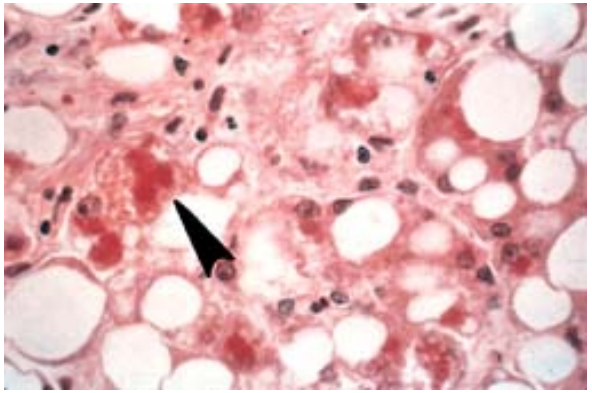

Figure 9-7 Micronodular cirrhosis is a late complication of chronic alcoholism. The liver architecture is distorted by regenerating nodules of hepatocytes surrounded by dense bands of fibrous tissue that stain blue (Masson trichrome stain). *(Courtesy of Dr. Steve Kroft, Department of Pathology, Southwestern Medical School, Dallas, TX.)*

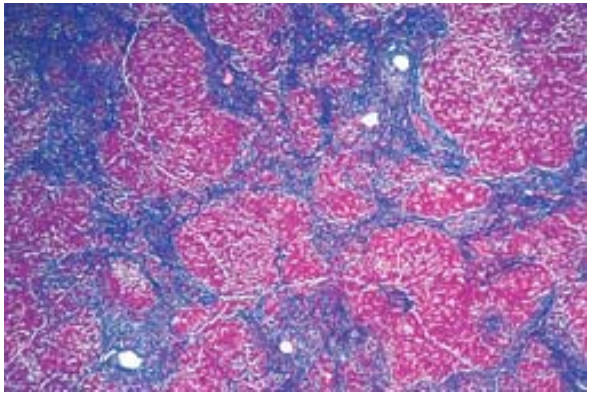

TABLE 9-6 -- Common Drugs of Abuse

Class	Molecular Target	Example
Opioid narcotics	Mu opioid receptor (agonist)	Heroin, hydromorphone (Dilaudid)
		Oxycodone (Percodan, Percocet, Oxycontin)
		Methadone (Dolophine)

		Meperidine (Demerol)
Sedative-hypnotics	$GABA_A$ receptor (agonist)	Barbiturates
		Ethanol
		Methaqualone (Quaalude)
		Glutethimide (Doriden)
		Ethchlorvynol (Placidyl)
Psychomotor stimulants	Dopamine transporter (antagonist)	Cocaine
		Amphetamine
	Serotonin receptors (toxicity)	3,4-methylenedioxymethamphetamine (MDMA, ecstasy)
Phencyclidine-like drugs	NMDA glutamate receptor channel (antagonist)	Phencyclidine (PCP, angel dust)
		Ketamine
Cannabinoids	CBI cannabinoid receptors (agonist)	Marijuana
		Hashish
Nicotine	Nicotine acetylcholine receptor (agonist)	Tobacco products
Hallucinogens	Serotonin 5-HT_2 receptors (agonist)	Lysergic acid diethylamide (LSD)
		Mescaline
		Psilocybin
Data from Hyman SE: A 28-year-old man addicted to cocaine. JAMA 286:2586, 2001.		

more slowly than ethanol, resulting in initial symptoms of intoxication, followed by toxic effects after several hours or days. *Methanol* is metabolized to formaldehyde and formic acid, resulting in metabolic acidosis, dizziness, vomiting, blurred vision or blindness, and respiratory depression. Methanol has been proposed as a gasoline additive or substitute, but there is concern that chronic inhalation of methanol-containing fumes may cause central nervous system depression. The lethal dose of *ethylene glycol* is only 1.4 mL/kg; it is metabolized by alcohol dehydrogenase to aldehydes, glycolate, oxalate, and lactate. If a person survives the initial toxicity, acute renal failure may occur several days later because of obstruction of the kidney tubules by calcium oxalate crystals. Acute methanol or ethylene glycol poisoning is treated by administration of ethanol, which slows the production of toxic metabolites.

Drug Abuse

Drug abuse, addiction, and overdose are serious public health problems. In a recent survey, 8% to 23% of teenagers reported marijuana use, and 2% reported cocaine use during the previous month. A National Comorbidity Survey conducted in 1995 discovered that 7.5% of US residents 15 to 54 years old had a history of drug dependence. Risk factors for drug use include family history, male sex, psychiatric disorders, ethanol abuse, easy access to drugs, and peer pressure.[21] The molecular targets of many commonly abused drugs have recently been identified, as summarized in Table 9-6 . Identification of specific neurotransmitter pathways that may activate reward circuits in the brain, as diagrammed in Figure 9-8 , may lead to more effective therapies for drug abuse and addiction. [19]

Sedative-Hypnotics.

Ethanol is the most widely abused central nervous system depressant, as discussed before. Barbiturates are circulated illegally and are known as downers. They

Figure 9-8 Effects of cocaine on neurotransmitters. Cocaine inhibits the reuptake of the neurotransmitters dopamine and norepinephrine in the central and peripheral nervous systems.

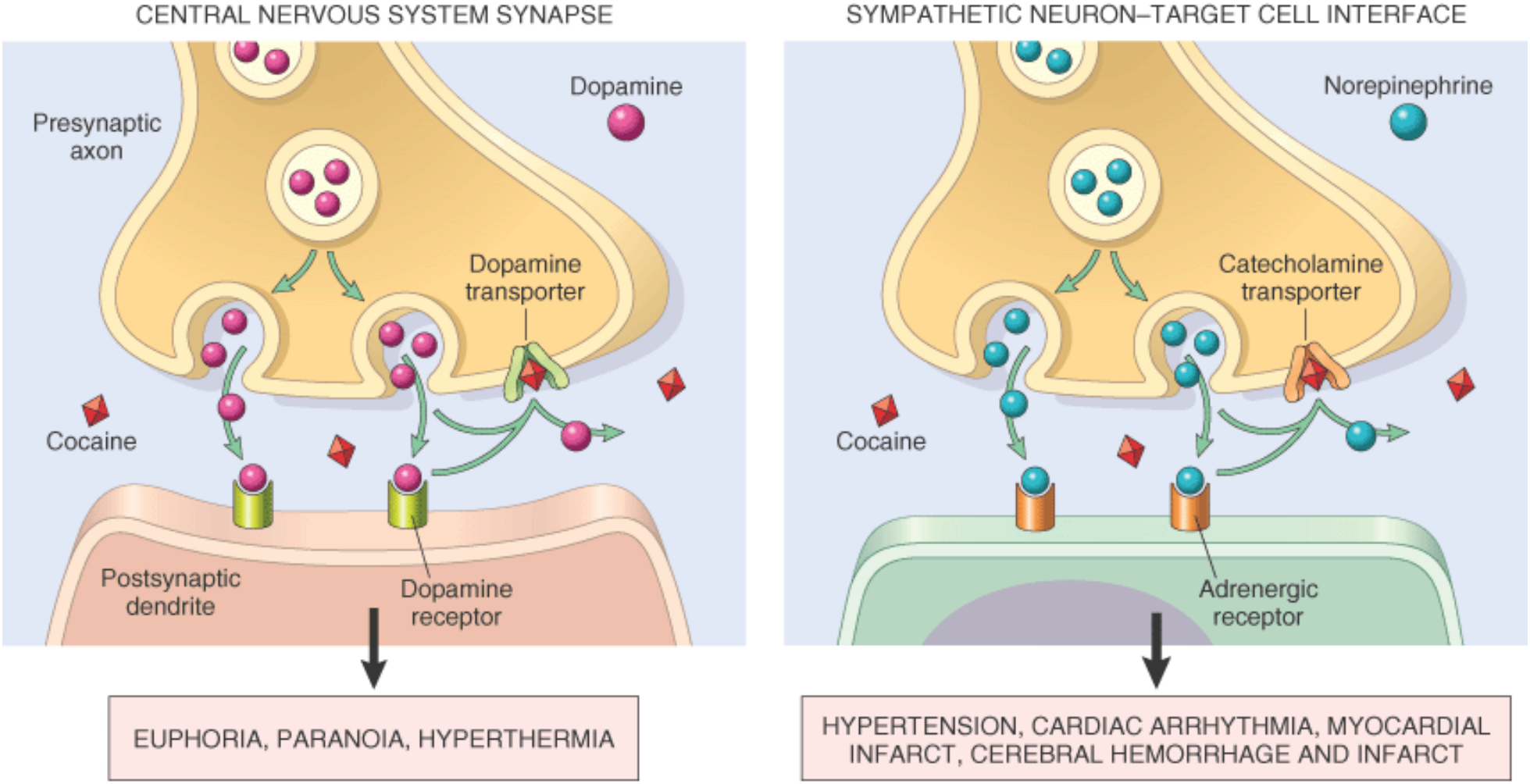

TABLE 9-7 -- Mechanisms of Adverse Drug Reactions

Mechanism	Example	Adverse Effect
Toxicity due to overdose	Acetaminophen	Liver necrosis and failure

Predictable reaction based on pharmacologic mechanism	Nonselective, nonsteroidal anti-inflammatory drugs	Peptic ulcer
Altered drug metabolism related to:		
••Thiopurine S-methyltransferase deficiency	Azathioprine	Bone marrow failure
••Cytochrome P-450 CYP2C9 variants	Oral anticoagulants	Bleeding
••Cytochrome P-450 CYP2D6 variants	Some antipsychotic drugs	Excessive sedation; parkinsonism
••N-acetyltransferase, slow acetylator phenotype	Hydralazine	Lupus
Idiopathic	Chloramphenicol	Aplastic anemia

and numbness. PCP characteristically induces nystagmus. High doses can induce coma lasting a few hours up to 10 days. Lysergic acid diethylamide (LSD) is a potent synthetic drug usually taken orally. It is absorbed rapidly and produces psychic effects, visual illusions, and altered perception for up to 12 hours. In high doses, LSD can cause death.

THERAPEUTIC DRUGS

An adverse drug reaction is defined as a toxic or undesired response to a drug used at therapeutic doses to prevent, diagnose, or treat disease. It is estimated that approximately 2 million hospitalized patients suffered from serious adverse drug reactions in 1994, resulting in 106,000 deaths. These estimates are conservative because they do not include errors in drug dosage or administration or patient noncompliance.[24] In Table 9-7 , adverse drug reactions are classified on the basis of their underlying mechanisms. Predictable reactions are based on the known toxicity or mechanism of action of a drug; these reactions are usually related to dose. Individual variations or polymorphisms in drug-metabolizing enzymes contribute to variable responses to drug therapy and an increased incidence of side effects. At least 5% of commonly prescribed drugs are metabolized by the cytochrome P-450 CYP1A2 pathway; approximately 12% of Caucasians carry variant alleles that reduce drug metabolism by this pathway. [25] *Pharmacogenomics* is a new field that uses genotyping to predict and prevent adverse drug reactions; for example, children with leukemia are screened for thiopurine methyltransferase variants to determine the optimal dose of azathiopurine,[26] and genotyping for the cytochrome P-450 CYP2D6 enzyme will help individualize doses of antipsychotic drugs to reduce side effects.[27] In contrast to these predictable types of adverse drug reactions, idiopathic or idiosyncratic reactions are rare and unpredictable, although the consequences may be severe or even fatal.

Herbal medicines are widely used in the United States and throughout the world; although many of these preparations have been shown to be effective in short-term trials, there is lack of quality control in this industry and few long-term studies of effectiveness and safety. As summarized in Table 9-8 , the most commonly used herbal medicines in the United States can produce adverse effects, including allergic or hypersensitivity reactions, and potentially serious interactions with prescription drugs.[28]

Oral Contraceptives and Hormone Replacement Therapy

Estrogens, alone or in combination with progestin, have been widely used for over 35 years as oral contraceptives or as hormone replacement therapy by perimenopausal and postmenopausal women. Most oral contraceptives combine synthetic ethinyl estradiol or mestranol with a progestin or use progestin alone; hormone replacement therapy uses natural estrogens alone or in combination with progesterone. Recent epidemiologic evidence has clarified the potential benefits and risks of these widely used drugs.

Oral Contraceptives.

There has been considerable concern and controversy about the safety of oral contraceptives, especially in relation to breast cancer. Two population-based, case-control studies, the Cancer and Steroid Hormone Study published in 1986 and the Women's Contraceptive and Reproductive Experiences Study published in 2002, explored the association between past or current use of oral contraceptives and breast cancer. [29] The most recent study included women between ages 35 and 64 diagnosed with breast cancer between 1994 and 1998. Potential effects of duration, formulations containing a high dose of estrogen, and family history of breast cancer were compared in women diagnosed with breast cancer or in control women without cancer. Past or current use of oral contraceptives was not found to be associated with an increased risk of breast cancer in white or black women in the United States.[30] Previous studies of other hormone-responsive cancers have also shown no increased risk of cancer; in fact, oral contraceptive use was found to decrease the risk of endometrial and ovarian cancers. In contrast, women infected with human papillomavirus have an increased risk of developing cervical cancer if they use oral contraceptives, although this risk may be related to other lifestyle factors (Chapter 22).

Uncommon adverse effects of oral contraceptives include:

- *Venous thrombosis and pulmonary embolism.* Oral contraceptives increase the risk of thrombosis; this risk is higher in carriers of mutations in factor V or prothrombin,[26] as described in Chapter 4 . The older, high-dose preparations incurred a greater risk, but a smaller risk persists even with the low-estrogen-containing oral contraceptives. The newer, third-generation oral contraceptives that combine low-dose estrogen with synthetic progestins confer an even higher risk.
- *Cardiovascular disease.* Estrogens and progestins have opposing effects on high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels. The overall effect on lipoproteins depends on the preparations used, especially the dose of progestin in the formulation. Recent epidemiologic evidence suggests that nonsmoking healthy women younger than age 45 who use the newer low-estrogen formulations do not have an increased risk of atherosclerosis or myocardial infarction. However, the risk of myocardial infarction is increased in women older than age 35 who smoke. The risk of ischemic stroke is also increased, regardless of age or smoking history.
- *Liver tumors.* Benign hepatic adenomas may occur, especially in older women who have used oral contraceptives for prolonged periods. These tumors may rupture and cause intra-abdominal bleeding.

TABLE 9-8 -- Adverse Effects of Herbal Medicines

Example	Adverse Effects	Drug Interactions
Echinacea	Allergic reactions	None described
Ginkgo	Headache, nausea	Potentiates anticoagulants
Ginseng	Headache, insomnia, euphoria, diarrhea	Interacts with monamine oxidase inhibitors, hypoglycemic drugs, anticoagulants
Saw palmetto	Constipation, decreased libido, urine retention	None described
St. John's wort	Allergic reactions, nausea, photosensitivity	Accelerated drug metabolism (oral contraceptives, anticoagulants)

Data from Ernst E: The risk-benefit profile of commonly used herbal therapies: ginkgo, St. John's wort, ginseng, echinacea, saw palmetto, and kava. Ann Intern Med 136:42, 2002.

Hormone Replacement Therapy.

In the United States, approximately one third of perimenopausal and postmenopausal women use hormone replacement therapy (HRT), either estrogen in combination with a progestin or a natural estrogen alone. There has been recent controversy about the risks and benefits of HRT. Short-term benefits include reduction in symptoms that accompany menopause, including hot flashes, vaginal dryness, and sleep disturbances. Long-term benefits include maintenance of bone mineral density and prevention of osteoporotic fractures. The major controversies surrounding HRT are the potential increased risk of cancer versus the potential benefits associated with prevention of ischemic heart disease and dementia. Recent results from the Women's Health Initiative and the Heart and Estrogen/Progestin Replacement Study have provided new information about the risks and benefits of HRT:[31]

• *Cancer*. Unopposed estrogen therapy greatly increases the risk of endometrial hyperplasia and cancer; therefore, most postmenopausal women now use estrogen in combination with a progestin. This combination drastically reduces or eliminates the risk of endometrial cancer. The risk of colon cancer was reduced in women who used HRT in some studies, but not in the Heart and Estrogen/Progestin Replacement Study. Recent results from the Women's Health Initiative indicate an increased risk of breast cancer in women who used HRT combined therapy for 5 years.
• *Venous thrombosis and pulmonary embolism.* The risk of thromboembolic events, including deep vein thrombosis,

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pulmonary embolism, stroke, and retinal thrombosis, is elevated approximated twofold in HRT users, especially within the first 2 years.
• *Cardiovascular disease.* The recent Women's Health Initiative reported an approximate 29% increased risk of myocardial infarction, especially during the first year of combined HRT use. This is in contrast to earlier studies in which either no effect or slight protection against cardiovascular diseases was reported. Methodologic differences probably underlie these divergent results. [32]
• *Cholecystitis.* There is an increased risk of gallbladder disease in HRT users that increases with time.
• *Dementia.* The current studies are not adequate to evaluate whether HRT use prevents dementia.

Overall, the risks and benefits associated with the use of oral contraceptives and HRT must be evaluated for each individual patient in the context of her overall health, individual risk factors, and family history.

Acetaminophen

When taken in large doses, this widely used nonprescription analgesic and antipyretic causes *hepatic necrosis*. The window between the usual therapeutic dose (0.5 gm) and the toxic dose (15 to 25 gm) is large, however, and the drug is ordinarily safe in adults. Doses should be reduced for infants and children, especially in the setting of fever, reduced food intake, or dehydration, since these conditions may predispose to liver injury.[33] Toxicity begins with nausea, vomiting, diarrhea, and sometimes shock, followed in a few days by evidence of jaundice; with serious overdosage, liver failure ensues, with centrilobular necrosis that may extend to the entire lobule. Some patients show evidence of concurrent renal and myocardial damage.

Aspirin (Acetylsalicylic Acid)

Overdose may result from accidental ingestion by young children; in adults, overdose is frequently suicidal. The major untoward consequences are metabolic with few morphologic changes. At first respiratory alkalosis develops, followed by metabolic acidosis that often proves fatal before anatomic changes can appear. Ingestion of as little as 2 to 4 gm by children or 10 to 30 gm by adults may be fatal, but survival has been reported after doses five times larger.

TABLE 9-9 -- National Ambient Air Quality Standards: Sources and Number of People at Risk

Pollutant	Primary Standard	Tons Emitted (Millions)	People at Risk (Millions)
Ozone	0.08 ppm 8 hr average	Not applicable	143••
Nitrogen oxides	0.053 ppm annual arithmetic mean	25	Not available
Sulfur dioxide	0.03 ppm annual arithmetic mean	19	0.3
Particulates (PM_{10})	50 µg/µL annual arithmetic mean	24	8.7

Carbon monoxide	9 ppm 8 hr average	97	31••
Lead	1.5 µg/µL quarterly average	30	2.5

Data from U.S. Environmental Protection Agency: epa.gov/oar/oaqps, www.scorecard.org/env-releases, the American Lung Association: www.lungusa.org/air, and Goldman LR: Environmental health and its relationship to occupational health. In Levy BS, et al. (eds): Occupational Health. Recognizing and Preventing Work-Related Disease and Injury, fourth ed. Philadelphia, Lippincott Williams & Wilkins, 2000, p. 51.

Chronic aspirin toxicity (salicylism) may develop in persons who take 3 gm or more daily, the dose required to treat chronic inflammatory conditions. Chronic salicylism is manifested by headache, dizziness, ringing in the ears (tinnitus), difficulty in hearing, mental confusion, drowsiness, nausea, vomiting, and diarrhea. The central nervous system changes may progress to convulsions and coma. The morphologic consequences of chronic salicylism are varied. Most often there is an acute erosive gastritis (Chapter 17), which may produce overt or covert gastrointestinal bleeding and lead to gastric ulceration. A bleeding tendency may appear concurrently with chronic toxicity, because aspirin acetylates platelet cyclooxygenase and blocks the ability to make thromboxane A_2 , an activator of platelet aggregation. Petechial hemorrhages may appear in the skin and internal viscera, and bleeding from gastric ulcerations may be exaggerated.

Proprietary analgesic mixtures of aspirin and phenacetin or its active metabolite, acetaminophen, when taken for a span of years, have caused renal papillary necrosis, referred to as *analgesic nephropathy* (Chapter 20).

OUTDOOR AIR POLLUTION

Air pollution is a serious problem in the United States and many other industrialized countries. In the United States, the Environmental Protection Agency is charged with identification and regulation of pollutants in the ambient air that may cause adverse health effects. The current National Ambient Air Quality Standards for the six major pollutants are listed in Table 9-9 . Despite federal and state regulations, many cities and regions in the United States currently do not meet these primary standards. Epidemiologic research, human clinical studies, and animal toxicologic studies continue to provide evidence for adverse health effects of ambient air pollutants, even at exposure levels below the current standards. The major sources of ambient air pollutants are:

- *Combustion of fossil fuels.* These are divided into mobile sources such as motor vehicles, stationary sources such as power plants and factories, and other sources such as barbecues and fireplaces. Tailpipe emissions from motor vehicles are a complex mixture of carbon monoxide, oxides of nitrogen, hydrocarbons, diesel exhaust particles, and other particulates including lead oxide from tetraethyl lead contained in leaded gasoline.

- *Photochemical reactions.* Oxides of nitrogen and volatile hydrocarbons interact in the atmosphere to produce ozone (O_3) as a secondary pollutant.
- *Power plants.* These release sulfur dioxide (SO_2) and particulates into the atmosphere. Coal and oil contain sulfur, leading to atmospheric formation of sulfates. Automobiles release oxides of nitrogen, leading to atmospheric formation of nitrates. Aerosolized acid sulfates contribute to acid rain.
- *Waste incinerators, industry, smelters.* These point sources release acid aerosols, metals, mercury vapor, and organic compounds that may be hazardous for human health. One example of the numerous hazardous chemicals emitted by these sources is methyl isocyanate that was accidentally released at Bhopal in India in 1984, resulting in 3000 deaths due to pulmonary edema. Some of the air toxins, such as polycyclic aromatic hydrocarbons, are known carcinogens.[34]

Lungs are the major target of common outdoor air pollutants; especially vulnerable are children, asthmatics, and people with chronic lung or heart disease, as summarized in Table 9-10 . The serious toxicity associated with lead exposure is discussed subsequently under Industrial Exposures. The major air pollutants and the mechanisms responsible for their adverse health

effects are summarized briefly.[34]

Ozone.

Ozone is a major component of smog that accompanies summer heat waves over much of the United States. Exposure of exercising children and adults to as little as 0.08 ppm produces cough, chest discomfort, and inflammation in the lungs. Asthmatics are especially sensitive and require more frequent visits to emergency rooms and more hospitalizations during smog episodes. It is not known whether these acute changes lead to chronic, irreversible lung injury. Ozone is highly reactive and oxidizes polyunsaturated lipids to hydrogen peroxide and lipid aldehydes. These products act as irritants and induce release of inflammatory mediators,

TABLE 9-10 -- Health Effects of Outdoor Air Pollutants

Pollutant	Populations at Risk	Effects
Ozone	Healthy adults and children	Decreased lung function
		Increased airway reactivity
		Lung inflammation
	Athletes, outdoor workers	Decreased exercise capacity
	Asthmatics	Increased hospitalizations
Nitrogen dioxide	Healthy adults	Increased airway reactivity
	Asthmatics	Decreased lung function
	Children	Increased respiratory infections
Sulfur dioxide	Healthy adults	Increased respiratory symptoms
	Patients with chronic lung disease	Increased mortality
		Increased hospitalization
	Asthmatics	Decreased lung function
Acid aerosols	Healthy adults	Altered mucociliary clearance
	Children	Increased respiratory infections
	Asthmatics	Decreased lung function
		Increased hospitalizations
Particulates	Children	Increased respiratory infections
		Decreased lung function
	Patients with chronic lung or heart disease	Excess mortality
	Asthmatics	Increased attacks

Data from Bascom R, et al: Health effects of outdoor air pollution, Am J Respir Crit Care Med 153:3, 477, 1996.

cause increased epithelial permeability and reactivity of the airways, and decrease ciliary clearance. The highest inhaled dose is delivered at the bronchoalveolar junction; however, ozone also causes inflammation of the upper respiratory tract.

Nitrogen Dioxide.

Oxides of nitrogen include NO and NO_2 . These have lower reactivity than ozone. Nitrogen dioxide dissolves in water in the airways to form nitric and nitrous acids, which damage the airway epithelial lining. Children and patients with asthma have increased susceptibility to nitrogen dioxide; there is a wide variation in individual responses to this pollutant.

Sulfur Dioxide.

This pollutant is highly soluble in water; it is absorbed in the upper and lower airways, where it releases H^+ , HSO_3^- (bisulfite), and SO_3^- (sulfite), which cause local irritation.

Acid Aerosols.

Primary combustion products of fossil fuels are emitted by tall smoke stacks at high altitudes and are transported by air. In the atmosphere, sulfur and nitrogen dioxide are oxidized to sulfuric acid and nitric acid, respectively, which are dissolved in water droplets or adsorbed to particulates. These acid aerosols are irritants to the airway epithelium and alter mucociliary clearance. Asthmatics have decreased lung function and increased hospitalizations when exposed to acid aerosols, although there is a wide variation in airway responses.

Particulates.

As discussed in Chapter 15 , the deposition and clearance of particulates inhaled into the lungs depend on their size. Ambient particulates are highly heterogeneous in size and in chemical composition. It is uncertain which characteristics of ambient particulates contribute to their adverse health effects. Recent epidemiologic and toxicologic studies suggest that ultrafine particles (less than 0.1 μm in aerodynamic diameter) are more hazardous. They contribute to increased morbidity and mortality, especially among infants, the elderly, and people with chronic cardiopulmonary disease. The mechanisms responsible for these adverse health effects are suspected to involve: (1) systemic cytokine release

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associated with pulmonary inflammation; (2) increased blood viscosity; and (3) autonomic changes associated with variable heart rates and arrhythmias.[35]

INDOOR AIR POLLUTION

Rising energy costs during the past 30 years have led to increased insulation and decreased ventilation of homes, which elevates the level of indoor air pollutants. The health hazards of environmental tobacco smoke have already been discussed. Other sources of indoor air pollutants are gas cooking stoves and furnaces, wood stoves, construction materials, furniture, radon, allergens associated with pets, dust mites, and fungal spores and bacteria. The major categories of indoor air pollutants and their health effects are summarized in Table 9-11 and discussed briefly next.[36]

Carbon Monoxide.

This odorless, colorless gas is a byproduct of combustion produced from burning gasoline, oil, coal, wood, and natural gas. It is also a major pollutant in tobacco smoke, and its untoward

effects were discussed earlier along with cigarette smoking. Here we should note that carbon monoxide levels in ambient air should not exceed 9 ppm; however, indoor levels of 2 to 4 ppm have been measured in homes during the winter. Such carbon monoxide pollution of indoor air can reduce exercise capacity and aggravate myocardial ischemia. Higher levels can cause poisoning manifested as headaches, dizziness, loss of motor control, and coma. Approximately 900 accidental deaths due to asphyxia are caused by indoor carbon monoxide pollution each year in the United States.

Nitrogen Dioxide.

Gas stoves and kerosene space heaters can raise indoor levels of nitrogen dioxide to 20 to 40 ppm in homes; this is several orders of magnitude higher than outdoor air levels. Children are more susceptible to the untoward

TABLE 9-11 -- Health Effects of Indoor Air Pollutants

Pollutant	Populations at Risk	Effects
Carbon monoxide	Adults and children	Acute poisoning
Nitrogen dioxide	Children	Increased respiratory infections
Wood smoke	Children	Increased respiratory infections
Formaldehyde	Adults and children	Eye and nose irritation, asthma
Radon	Adults and children	Lung cancer
Asbestos fibers	Maintenance and abatement workers	Lung cancer, mesothelioma
Manufactured mineral fibers	Maintenance and construction workers	Skin and airway irritation
Bioaerosols	Adults and children	Allergic rhinitis, asthma

Data from Lambert WE, Samet JM: Indoor air pollution. In Harber P, et al (eds): Occupational and Environmental Respiratory Disease. St. Louis, Mosby-Year Book, 1996, p. 784; and Menzies D, Bourbeau J: Building-related illnesses. N Engl J Med 337:1524, 1997.

effects of nitrogen dioxide. It impairs lung defenses and is hence associated with increased respiratory infections.

Wood Smoke.

This is a complex mixture of nitrogen oxides, particulates, and polycyclic aromatic hydrocarbons. High concentrations of wood smoke in poorly ventilated homes can increase the incidence of respiratory infections in children.

Formaldehyde.

This highly soluble, volatile chemical has been used in the manufacture of many consumer products, including textiles, pressed wood, furniture, and urea formaldehyde foam insulation. Although indoor levels are usually less than 1 ppm, it can cause acute irritation of the eyes and upper respiratory tract and exacerbation of asthma. Formaldehyde is frequently emitted with acrolein and acetaldehyde, which may have additive or synergistic irritant effects. Additional volatile organic compounds that may be present at low levels in indoor air include benzene, tetrachloroethylene, polycyclic aromatic hydrocarbons, and chloroform. The potential for toxicity or carcinogenicity at these exposure levels is low, although occupational exposure to these volatile compounds can be hazardous. Formaldehyde at high doses (6 to 14 ppm) has produced nasal tumors in rats.[37]

Radon.

Radon, a radioactive gas, is a decay product of uranium widely distributed in the soil. Radon gas emanating from the earth is prevalent in homes. Indoor levels of radon average around 1.5 pCi/L; approximately 4% of homes have an annual average level greater than 4 pCi/L. Radon gas is inhaled into the lungs; its decay products emit alpha radiation, which has been associated with lung cancer in miners. According to some estimates, the low levels found in indoor air account for 10,000 lung cancers per year in the United States.[38]

Asbestos Fibers.

Homes and public buildings built before the 1970s in the United States contain asbestos insulation, pipe covers, ceiling tiles, and flooring. If these materials are non-friable and undisturbed, low levels of fibers can be measured in indoor air. Maintenance and abatement workers who repair or remove asbestos-containing materials are at risk for lung cancer and mesothelioma if they do not use respirators. [39] [40]

Manufactured Mineral Fibers.

Fiberglass has been widely used as an asbestos substitute for home insulation. Low levels of these fibers can be measured in indoor air. Maintenance and construction workers can develop skin and lung irritation when using these materials. [41]

Bioaerosols.

Aerosolization of bacteria responsible for *Legionella* pneumonia has been associated with contaminated heating and cooling systems in public buildings (Chapter 8). More common hazards in indoor air are allergens associated with pets, dust mites, cockroaches, fungi, and molds. These allergens cause allergic rhinitis and exacerbate asthma. [41]

The etiology of the so-called *sick building syndrome*, or *multiple chemical sensitivity syndrome*, is less clear. In some cases, high levels of one or more of these indoor air pollutants may be responsible. In most cases, poor ventilation is at fault.[41]

INDUSTRIAL EXPOSURES

For centuries, physicians have recognized that occupational exposures contribute to human disease. The spectrum of human diseases associated with occupational exposures is summarized in Table 9-12 . Almost all organ systems can be affected, resulting in acute toxicity or irritation, hypersensitivity

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reactions, chronic toxicity, fibrosis, and cancer. The chronic effects of occupational exposures are complex; they include degenerative changes in the nervous system, reproductive dysfunction, lung fibrosis, and cancer. The mechanisms responsible for these effects are not well understood. Some examples of acute and chronic diseases resulting from occupational exposures and potential hazards of environmental exposures are discussed in the following sections.

Volatile Organic Compounds

Large volumes of organic solvents and vapors are used in industry and in homes. These chemicals are known as volatile organic compounds (VOCs). They are used in manufacturing, degreasing, and dry cleaning and as components of paint removers and aerosol sprays. VOCs and petroleum products such as kerosene, mineral oil, and turpentine are stored in

underground tanks. Surface spills and leakage from storage tanks can cause contamination of underground water supplies. In general, high levels of exposure encountered in industry cause headache, dizziness, and liver or kidney toxicity. At lower levels of exposure, there is concern about potential carcinogenicity and adverse reproductive effects. Some VOCs and their adverse effects are described next.

Aliphatic Hydrocarbons.

These compounds are the most widely used industrial solvents and dry-cleaning agents. All of these chemicals are readily absorbed through the lungs, skin, and gastrointestinal tract. In addition to acute central nervous system depression, they can cause liver and kidney toxicity. Common examples of these chemicals are chloroform and carbon tetrachloride; both are carcinogenic in rodents. Methylene chloride, another such chemical, is used in paint

TABLE 9-12 -- Human Diseases Associated with Occupational Exposures

Organ	Effect	Toxicant
Cardiovascular system	Heart disease	Carbon monoxide, lead, solvents, cobalt, cadmium
Respiratory system	Nasal cancer	Isopropyl alcohol, wood dust
	Lung cancer	Radon, asbestos, silica, bis(chloromethyl)ether, nickel, arsenic, chromium, mustard gas
	Chronic obstructive lung disease	Grain dust, coal dust, cadmium
	Hypersensitivity	Beryllium, isocyanates
	Irritation	Ammonia, sulfur oxides, formaldehyde
	Fibrosis	Silica, asbestos, cobalt
Nervous system	Peripheral neuropathies	Solvents, acrylamide, methyl chloride, mercury, lead, arsenic, DDT
	Ataxic gait	Chlordane, toluene, acrylamide, mercury
	Central nervous system depression	Alcohols, ketones, aldehydes, solvents
	Cataracts	Ultraviolet radiation
Urinary system	Toxicity	Mercury, lead, glycol ethers, solvents
	Bladder cancer	Naphthylamines, 4-aminobiphenyl, benzidine, rubber products
Reproductive system	Male infertility	Lead, phthalate plasticizers
	Female infertility	Cadmium, lead
	Teratogenesis	Mercury, polychlorinated biphenyls
Hematopoietic system	Leukemia	Benzene, radon, uranium
Skin	Folliculitis and acneiform dermatosis	Polychlorinated biphenyls, dioxins, herbicides
	Cancer	Ultraviolet radiation
Gastrointestinal tract	Liver angiosarcoma	Vinyl chloride

Data from Leigh JP, et al: Occupational injury and illness in the United States. Estimates of costs, morbidity, and mortality. Arch Intern Med 157:1557, 1997; Mitchell FL: Hazardous waste. In Rom WN (ed): Environmental and Occupational Medicine, 2nd ed. Boston, Little, Brown, 1992, p. 1275; and Levi PE: Classes of toxic chemicals. In Hodgson E, Levi PE (eds): A Textbook of Modern Toxicology, Stamford, CT, Appleton & Lange, 1997, p. 229.

removers and aerosols. In enclosed areas, high concentrations of methylene chloride can be reached because it is highly volatile. Methylene chloride is metabolized by cytochrome P-450 to carbon dioxide and carbon monoxide. Carbon monoxide can form carboxyhemoglobin, causing respiratory depression and death. Perchloroethylene and related compounds are widely used in the dry-cleaning industry. Acute exposure causes central nervous system depression, confusion, dizziness, impaired gait, and nausea. Repeated exposures may cause dermatitis. Perchloroethylene is a potential human carcinogen.

Petroleum Products.

Gasoline, kerosene, mineral oil, and turpentine are highly volatile and are a common cause of poisoning in children. Inhalation of these vapors causes dizziness, incoordination, and central nervous system depression.

Aromatic Hydrocarbons.

Benzene, toluene, and xylene are widely used solvents in the rubber and shoe industries and in printing and paper-coating. Although toluene and xylene are not carcinogenic, inhalation of benzene is hazardous because it can cause bone marrow toxicity, aplastic anemia, and acute leukemia. Benzene is metabolized by the cytochrome P-450 system in liver, producing benzoquinone and muconaldehyde. These metabolic products are believed to cause bone marrow toxicity.

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons are among the most potent chemical carcinogens (Chapter 7). The carcinogenicity of these compounds was recognized in 1775, with the description of scrotal cancer in English chimney sweepers exposed to soot. A variety of polycyclic aromatic hydrocarbons characterized

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by three or more fused benzene rings are produced by combustion of fossil fuels; high-temperature processing of coke, coal, and crude oil; and iron and steel foundries. Benzo[a]pyrene is the prototype of polycyclic aromatic hydrocarbons. As described earlier (see Fig. 9-4*A*), it is metabolized by cytochrome P-450, prostaglandin H synthetase, and epoxide hydrolase, an inducible microsomal enzyme in the liver. Activated epoxide intermediates bind to DNA; these adducts have been used as markers of polycyclic aromatic hydrocarbon exposure. Occupational exposure to polycyclic aromatic hydrocarbons is associated with an increased risk of lung and bladder cancers.[42] Cigarette smoking is another important source of benzo[a] pyrene. Mutations in the *p53* tumor-suppressor gene found in lung cancers associated with cigarette smoking are most commonly G:C→T:A transversions. This mutational spectrum is consistent with metabolism of benzo[a]pyrene to reactive intermediates that attack deoxyguanines on the nontranscribed DNA strand.[7]

Plastics, Rubber, and Polymers

Millions of tons of synthetic plastics, rubber, and polymers are produced throughout the world. These products are then fabricated into latex fabrics, pipe, cables, flooring, home and recreational products, medical products, and containers. In 1974, occupational exposure to vinyl chloride monomers used to produce polyvinyl chloride resins was found to be associated with angiosarcoma of the liver. Vinyl chloride is a colorless gas that is flammable and explosive. Before the polymerization step in the manufacturing of polyvinyl chloride, it can be absorbed through the skin or lungs. Vinyl chloride is metabolized by the cytochrome P-450 system in the liver to chloroacetaldehyde. This metabolite covalently binds to DNA and is mutagenic. Exposure of rubber workers to 1,3-butadiene

TABLE 9-13 -- Toxic and Carcinogenic Metals

Metal	Disease	Occupation
Lead	Renal toxicity	Battery and ammunition workers, foundry workers, spray painting, radiator repair
	Anemia, colic	
	Peripheral neuropathy	
	Insomnia, fatigue	
	Cognitive deficits	
Mercury	Renal toxicity	Chlorine-alkali industry
	Muscle tremors, dementia	
	Cerebral palsy	
	Mental retardation	
Arsenic	Cancer of skin, lung, liver	Miners, smelters, oil refinery workers, farm workers
Beryllium	Acute lung irritant	Beryllium refining, aerospace manufacturing, ceramics
	Chronic lung hypersensitivity	
	? Lung cancer	
Cobalt and tungsten carbide	Lung fibrosis	Toolmakers, grinders, diamond polishers
	Asthma	
Cadmium	Renal toxicity	Battery workers, smelters, welders, soldering
	? Prostate cancer	
Chromium	Cancer of lung and nasal cavity	Pigment workers, smelters, steel workers
Nickel	Cancer of lung and nasal sinuses	Smelters, steel workers, electroplating

Data from Levi PE: Classes of toxic chemicals. In Hodgson E, Levi PE (eds): A Textbook of Modern Toxicology. Stamford, CT, Appleton & Lange, 1997, p. 229; and Sprince NL: Hard metal disease. In Rom WN (eds): Environmental and Occupational Medicine, 2nd ed. Boston, Little, Brown, 1992, p. 791.

has been shown to be associated with an increased risk of leukemia. Plastics are widely used in consumer products, including food and beverage containers. Public exposure to plasticizers, such as phthalate esters, and to additives such as bisphenol-A raises concern about potential adverse reproductive effects of these synthetic chemicals. Phthalate esters have been shown to induce testicular injury in rats, and bisphenol-A mimics the proliferative effects of estrogen.

Metals

Occupational exposure to metals in mining and manufacturing is associated with acute and chronic toxicity, as well as carcinogenicity, as summarized in Table 9-13 .[43] Occupational as

well as environmental exposure to lead continues to be a serious public health problem. Agricultural exposure to arsenic-containing pesticides is discussed subsequently. The pulmonary effects of beryllium are described in Chapter 15 . The health effects of inorganic and organic mercury were discussed earlier in this chapter under "Mechanisms of Toxicity." The untoward effects of some of the remaining metals listed in Table 9-13 are described here.

Lead.

More than 4 million tons of lead are produced each year for use in batteries, alloys, exterior red lead paint, and ammunition. Workers employed in these industries as well as in mining, smelting, spray painting, recycling, and radiator repair are exposed to lead. In some countries, tetraethyl lead is still used as a gasoline additive, thus polluting the air. *Inhalation is the most important route of occupational exposure.* Environmental sources of lead are urban air due to use of leaded gasoline, soil contaminated with exterior lead paint, the water supply due to lead plumbing, and house dust in homes with interior lead paint. Consumers may be exposed to lead-glazed ceramics, lead solder in food and soft drink cans, and illegally

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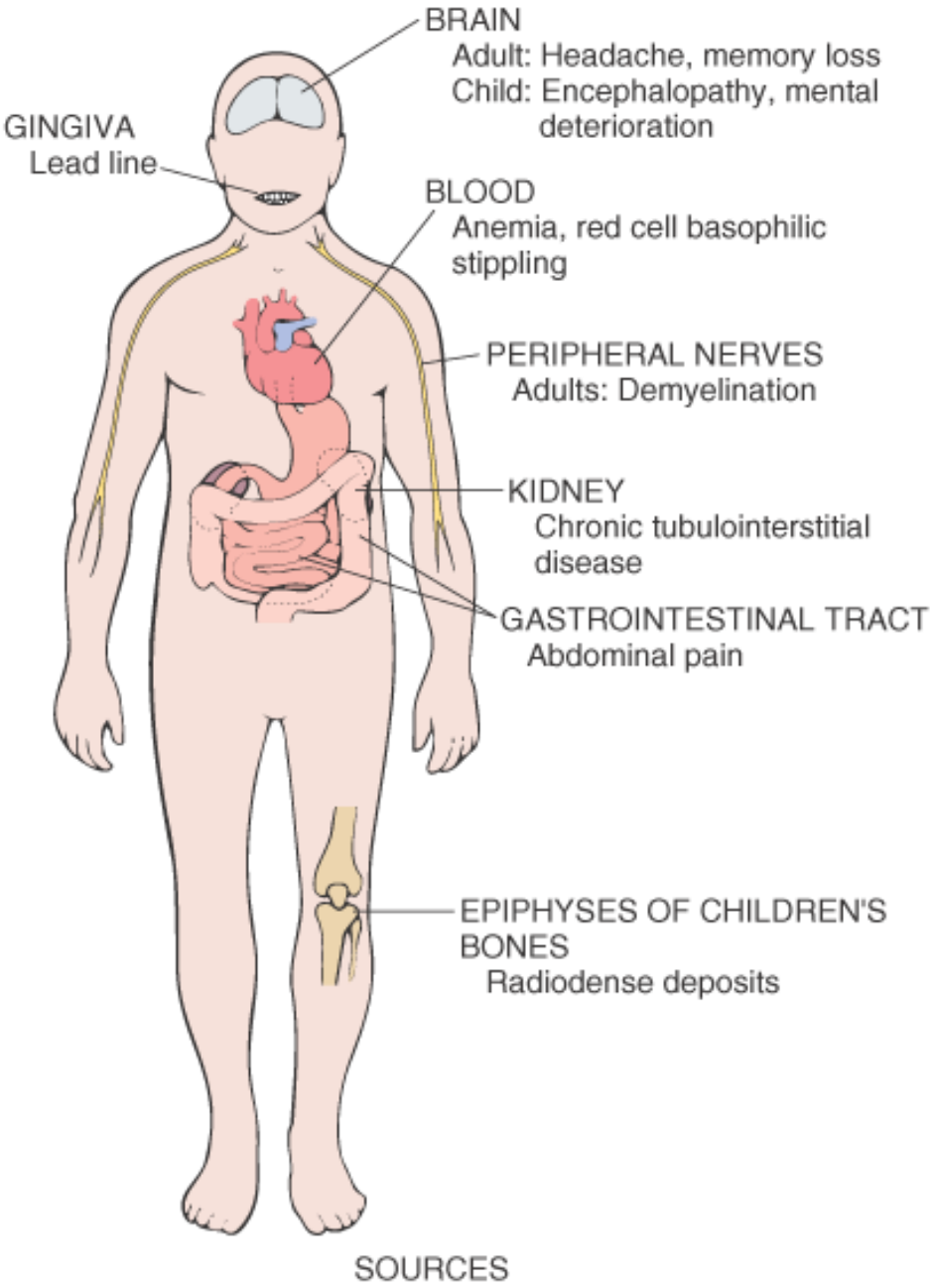
produced alcoholic beverages (moonshine). Lead ingested in this manner is absorbed through the gastrointestinal tract. Intestinal absorption of lead is enhanced by calcium, iron, or zinc deficiency; compared with adults, the absorption is greater in children and infants and hence they are particularly vulnerable to lead toxicity. Absorbed lead is mainly (80% to 85%) taken up by bone and developing teeth in children; the blood accumulates 5% to 10%, and the remainder is distributed throughout the soft tissues. Lead clears rapidly from blood, but that deposited in bones has a half-life of 30 years. Thus, the presence of lead in blood indicates recent exposure, and it does not allow the determination of total body burden. The toxicity of lead is related to its multiple biochemical effects:

- *High affinity for sulfhydryl groups.* The most important enzymes inhibited by lead due to this mechanism are involved in heme biosynthesis: δ-aminolevulinic acid dehydratase and ferroketolase. These enzymes catalyze the incorporation of iron into the heme molecule, and hence patients develop hypochromic anemia.
- *Competition with calcium ions.* As a divalent cation, lead competes with calcium and is stored in bone. It also interferes with nerve transmission and brain development.
- *Inhibition of membrane-associated enzymes.* Lead inhibits 5'-nucleotidase activity and sodium-potassium ion pumps, leading to decreased survival of red blood cells (hemolysis), renal damage, and hypertension.
- *Impaired production* of 1,25-dihydroxyvitamin D, the active metabolite of vitamin D.

Lead contributes to multiple chronic health effects, illustrated in Figure 9-9 . *Injury to the central and peripheral nervous systems* causes headache, dizziness, memory deficits, and decreased nerve conduction velocity. *Blood changes* occur early and are characteristic. Because lead interferes with heme biosynthesis, it causes a microcytic hypochromic anemia; punctate basophilic stippling of erythrocytes is characteristic. There is also an element of hemolysis because lead inhibits membrane-associated red cell enzymes. Because lead inhibits incorporation of iron into heme, the iron is displaced, and zinc protoporphyrin is formed. Thus, an elevated blood level of zinc protoporphyrin or its product, free erythrocyte protoporphyrin, is an important indicator of lead poisoning. *Gastrointestinal symptoms* include colic and anorexia. The kidneys are a major route of excretion of lead. Acutely, there is *damage to the proximal tubules*, with intranuclear lead inclusions and clinical evidence of renal tubule dysfunction. Chronically, lead can cause diffuse interstitial fibrosis, gout, and renal failure. Even in the absence of overt clinical symptoms of kidney damage, lead causes hypertension. Lead can cause infertility in men due to testicular injury; failure of implantation of the fertilized ovum can occur in women.[44]

Infants and children are especially vulnerable to lead toxicity. It is estimated by the CDC that in the year 2000 approximately 454,000 children in the United States had blood lead levels greater than 10 µg/dL. A recent study indicates that even below this level there is an inverse correlation between blood lead concentration and IQ scores. Very slightly elevated blood levels (~3 µg/dL) in young females have also been reported to delay puberty.[45] Thus, lead toxicity continues to be a matter of concern. Lead may be mobilized from the maternal skeleton during pregnancy and it readily crosses the placental barrier. Hence

Figure 9-9 Consequences of lead exposure.



Battery burning
Urban soil
Newsprint
Automotive exhaust

TABLE 9-14 -- Health Effects of Agricultural Pesticides

Category	Example	Effects and Disease Associations
Insecticides	Organochlorines	Neurotoxicity; hepatotoxicity
	••DDT	
	••Chlordane	
	••Lindane	
	••Methoxychlor	
	Organophosphates	Neurotoxicity; delayed neuropathy
	••Parathion	
	••Diazinon	
	••Malathion	
	Carbamates	Neurotoxicity (reversible)
	••Aldicarb	
	••Carbaryl	
	Botanical agents	Paresthesia; lung irritant; allergic dermatitis
	••Nicotine	
	••Pyrethrins	
	••Rotenone	
Herbicides	Arsenic compounds	Hyperpigmentation; gangrene; anemia; sensory neuropathy; cancer
	Dinitrophenols	Hyperthermia; sweating
	Chlorophenoxy herbicides	
	••2,4-D and 2,4,5-T	? Lymphoma; sarcoma
	••TCDD	Fetotoxicity; immunotoxicity; cancer
	Paraquat	Acute lung injury
	Atrazine	? Cancer
	Alachlor	? Cancer
Fungicides	Captan	? Reproductive toxicity
	Maneb	

	Benomyl	
Rodenticides	Fluoroacetate	Cardiac and respiratory failure
	Warfarin	Hemorrhage
	Strychnine	Respiratory failure
Fumigants	Carbon disulfide	Cardiac toxicity
	Ethylene dibromide	Neurotoxicity
	Phosphine	Lung edema; brain damage
	Chloropicrin	Eye irritation; lung edema; arrhythmias

Data from Hodgson E: Introduction to toxicology. In Hodgson E, Levi PE (eds): A Textbook of Modern Toxicology. Stamford, CT, Appleton & Lange, 1997, p. 1; and Levi PE: Classes of toxic chemicals. In Hodgson E, Levi PE (eds): A Textbook of Modern Toxicology. Stamford, CT, Appleton & Lange, 1997, p. 229.

soil and water supplies. Environmental contamination is a threat to wildlife; some pesticides undergo bioaccumulation and persist in wildlife and humans for decades. Bioaccumulation and biopersistence are characteristic of organochlorines, such as DDT (dichlorodiphenyltrichloroethane), and dioxins, such as TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). There is considerable controversy about the adverse health effects of these persistent pesticides and their metabolites, especially concerning their relationship to breast cancer,[48] reproductive abnormalities,[49] and cognitive deficits.[50]

Agricultural pesticides are divided into five categories, depending on the target pest: insecticides, herbicides, fungicides, rodenticides, and fumigants (Table 9-14). All pesticides are toxic to some plant or rodent species; at higher doses, they can also be toxic to farm animals, pets, and humans. In general, herbicides used to control weeds have low acute toxicity for mammals; fungicides are characterized as moderately toxic. Acute toxicity of insecticides for mammals ranges from low to high. For example, DDT was widely used as an insecticide in the 1940s and 1950s because it has low acute toxicity for humans. However, DDT persists in the environment and accumulates in the food chain. Birds that ingested DDT-contaminated insects and fish suffered reproductive defects. DDT and its major metabolite, DDE

(1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene), accumulate in fat tissue and have been detected in human milk. Organochlorines, as well as industrial chemicals such as polychlorinated biphenyls (PCBs), are weakly estrogenic. Some of these chemicals are carcinogenic in rodents and cause reproductive dysfunction in amphibians, birds, and fish.[48] Although several epidemiologic studies have not found increased levels of DDE or PCBs in women with breast cancer compared with matched control subjects, there is still concern that these persistent organochlorines, other potentially estrogenic pesticides, and natural phytoestrogens in plants such as soybeans may have adverse reproductive effects in humans. The mechanisms of action of these xenoestrogens, alone or in combination, in the development of cancer and in reproductive dysfunction are unknown.[48] [49]

The major health effects of the most common agricultural pesticides are summarized in Table 9-14 . Selected examples are discussed here.

• *Organochlorines*, such as DDT, have low acute toxicity for humans; however, they bioaccumulate and persist in the environment and in fat tissue. These chemicals are absorbed through the skin, gastrointestinal tract, and lungs. As alluded to earlier, the role of DDT and its metabolites as an endocrine-disrupting agent is controversial. *Chlordane* is representative of cyclodienes that are used to control termites and other soil insects. Acute toxicity causes hypothermia, tremor, and convulsions. Chlordane also causes immune dysfunction and may act as a nongenotoxic carcinogen. These effects may contribute to the increased incidence of lymphoma observed in some farm workers. *Lindane* is an isomer

of benzene hexachloride that is used to control lice and scabies, as a wood preservative, and as a household fumigant. It has been reported to cause immune dysfunction and reproductive problems in women.

• *Organophosphates* are irreversible inhibitors of cholinesterases resulting in abnormal transmission at peripheral and central nerve endings. These chemicals are absorbed through the skin, gastrointestinal tract, and lungs. Up to 40% of farm workers in the United States show measurable inhibition of red blood cell or plasma cholinesterase activity; fatalities have been reported from organophosphate exposure. *Carbamates* are reversible inhibitors of cholinesterase that produce acute neurotoxic effects similar to those of organophosphate insecticides. Carbaryl (Sevin) is potentially mutagenic and teratogenic because it poisons the mitotic spindle.

• *Herbicides* like the dioxin TCDD has received much attention. During the Vietnam War, the defoliant Agent Orange was contaminated with TCDD. A chemical factory explosion in Seveso, Italy, in 1976 caused local environmental contamination and human exposure to TCDD, resulting in chloracne and an increased incidence of leukemia, lymphoma, and sarcomas. TCDD and structurally similar dioxins are also produced in the paper pulp industry using chlorine bleach and by waste incinerators. Low doses of dioxin are present in our food, soil, and water. In some laboratory animals, TCDD is highly toxic, immunosuppressive, teratogenic, and carcinogenic. The sensitivity of some strains of laboratory mice to dioxin is linked to the aryl hydrocarbon hydroxylase receptor. TCDD can induce liver cytochrome P-450 enzyme activity, increase estrogen metabolism, and interfere with development of the male reproductive tract. TCDD also decreases thyroxine levels in adult rats. Extrapolation of these multiple adverse effects observed in laboratory animals to low-dose exposure of humans is difficult.[51]

• *Rodenticides* are highly toxic chemicals with restricted use. The major health threat is death from suicidal or accidental ingestion.

TABLE 9-15 -- Natural Toxins

Category	Example	Source	Effects and Associated Diseases
Mycotoxins	Ergot alkaloids	*Claviceps* fungi	Gangrene, convulsions, abortion
	Aflatoxins	*Aspergillus flavus*	Liver cancer
	Tricothecenes	*Fusarium, Trichoderma*	Diarrhea, ataxia
Phytotoxins	Cycasin	Cycad flour	Amyotrophic lateral sclerosis
	Monocrotaline	*Senecio* plants	Hepatitis
	Safrole	Black pepper; oil of Sassafras	Cancer
	Solanine	Solanaceae plants (potato)	Neurotoxin
Animal toxins	Venoms	Snakes	Cardiotoxin, neurotoxin
		Bees	Direct toxicity, cardiotoxin
	Saxitoxin	Dinoflagellates	Neurotoxin, paralysis
	Ciguatoxin	Dinoflagellates	Paresthesia, paresis, vomiting, diarrhea
	Tetrodotoxin	Puffer fish	Neurotoxin, shock

Data from Hodgson E: Introduction to toxicology. In Hodgson E, Levi PE (eds): A Textbook of Modern Toxicology. Stamford, CT, Appleton & Lange, 1997, p. 1.

NATURAL TOXINS

In addition to manufactured pesticides, potent toxins and carcinogens are present in the natural environment, as summarized in Table 9-15 . These mycotoxins and phytotoxins may contaminate foods. For example, cycad flour is used in arid climates. This plant contains the toxin cycasin (methylazoxymethanol β-glucoside). If the plant and seeds are cut into small pieces, soaked in water, and dried, the toxin is leached. However, if these precautions are not followed, a degenerative neurologic disorder (amyotrophic lateral sclerosis) is produced by

ingestion of cycasin. Animal toxins can be ingested by eating fish, snails, or mollusks. The most common poisoning results from eating tropical fish and snails that have ingested dinoflagellates containing ciguatoxin. Ciguatera

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poisoning can be severe and occurs in the South Pacific and the Caribbean. Paralytic shellfish poisoning occurs in North America after eating mollusks that have ingested dinoflagellates that contain saxitoxin. Aflatoxin B_1 is produced by fungi that contaminate peanuts, corn, and cottonseed. It is a potent carcinogen that contributes to the high incidence of liver cancer in some regions of Africa and the Far East (Chapter 7 and Chapter 18).

RADIATION INJURY

Radiation is energy distributed across the electromagnetic spectrum as waves (long wavelengths, low frequency) or particles (short wavelengths, high frequency). The types, frequencies, and biologic effects of electromagnetic radiation are summarized in Table 9-16 . Approximately 80% of radiation is derived from natural sources, including cosmic radiation, ultraviolet light, and natural radioisotopes, especially radon gas. The remaining 20% is derived from manufactured sources that include instruments used in medicine and dentistry, consumer products that emit radio waves or microwaves, and nuclear power plants. The potentially catastrophic effects of radiation are most vividly illustrated by the effects of nuclear explosions. The atomic bombs dropped on Hiroshima and Nagasaki in 1945 not only caused acute injury and death but also increased incidence of various cancers among the survivors. Numerous historical incidents document the deleterious effects of therapeutic radiation. For example, early in the 20th century, American radiologists experienced an increased incidence of aplastic anemia and neoplasms of the skin, brain, and hematopoietic system. Children who were treated with radiation for an enlarged thymus or benign skin lesions between 1910 and 1959 suffered from an increased incidence of thyroid abnormalities, thyroid tumors, and leukemias and lymphomas. Exposure of the fetus to radiation can produce mental retardation, congenital anomalies, leukemia, and solid tumors. Investigation of these deliberate or accidental exposures to radiation led to an understanding of the relationship between the dose and timing of radiation and the acute and chronic health effects. However, in general, these historical exposures were higher than radiation currently received by the general population from natural and manufactured sources,

TABLE 9-16 -- Ionizing and Nonionizing Electromagnetic Radiation

Frequency (Hz)	Radiation	Biologic Effects
1–50	Electric power	?
10^6 –10^{11}	Radio waves and radar	Thermal effects, cataracts
10^9 –10^{10}	Microwaves	Lens opacities
10^{11} –10^{14}	Infrared	Cataracts
10^{15}	Visible light	Retinal burns (lasers)
10^{15} –10^{18}	Ultraviolet light	Skin burns, cancer
10^{18} –10^{20}	X-rays and gamma rays	Acute and delayed injury; cancer
10^{27}	Cosmic radiation	?

by patients undergoing diagnostic procedures such as mammography or chest radiography, and by nuclear power plant workers. Unfortunately, fear of widespread radiation exposure following a terrorist attack reinforces the importance of understanding the mechanisms and clinical manifestations of radiation injury.[52] Despite our understanding of the health effects of

high doses of radiation, the potential adverse effects of low doses are controversial. Furthermore, accidents at nuclear power plants in Windscale, England, in 1957, at Three Mile Island in Pennsylvania in 1979, and at Chernobyl in the former Soviet Union in 1986 perpetuate public anxiety about excess cancers associated with the medical, commercial, and military uses of radioactivity.[53]

Electromagnetic radiation characterized by long wavelengths and low frequencies is described as *nonionizing radiation*. Electric power, radio waves and microwaves, infrared, and ultraviolet light are examples of nonionizing radiation. They produce vibration and rotation of atoms in biologic molecules. Radiation energy of short wavelengths and high frequency can ionize biologic target molecules and eject electrons. X-rays, gamma rays, and cosmic rays are forms of *ionizing radiation*. Ionizing radiation can be in the form of electromagnetic waves, such as x-rays produced by a roentgen tube or gamma rays emitted from natural sources, or particles that are released by natural decay of radioisotopes or by artificial acceleration of subatomic particles. *Particulate radiation* is classified by the type of particles emitted: alpha particles, beta particles or electrons, protons, neutrons, mesons, or deuterons. The energy of these particles is measured in million electron volts (MeV). Radioisotopes decay by emission of alpha or beta particles or by capture of electrons. In the case of radon gas, unstable daughter nuclei are produced that subsequently disintegrate, releasing alpha particles. *Alpha particles* consist of two neutrons and two protons; they have strong ionizing power but low penetration because of their large size. In contrast, *beta particles* are electrons emitted from the nucleus of an atom; these have weaker ionizing power but higher penetration than alpha particles. The decay of radioisotopes is expressed by the *curie* (Ci), 3.7×10^{10} disintegrations per second, or the *becquerel* (Bq), 1 disintegration per second. The rate of decay of radioisotopes is usually expressed as the half-life ($t_{1/2}$) and ranges from a few seconds to centuries. Internal deposition of radioisotopes with long half-lives is especially dangerous because it results in continuous release of radioactive particles and gamma rays. For example, radium was used to paint watch dials and treat cancer in the first half of the 20th century; its long half-life of 1638 years and ability to be concentrated in the skeleton result in delayed appearance of bone tumors.

Ionizing Radiation

The dose of ionizing radiation is measured in several units:

- *roentgen:* unit of charge produced by x-rays or gamma rays that ionize a specific volume of air
- *rad:* the dose of radiation that will produce absorption of 100 ergs of energy per gram of tissue; 1 gm of tissue exposed to 1 roentgen of gamma rays is equal to 93 ergs
- *gray* (Gy): the dose of radiation that will produce absorption of 1 joule of energy per kilogram of tissue; 1 Gy corresponds to 100 rad

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- *rem:* the dose of radiation that causes a biologic effect equivalent to 1 rad of x-rays or gamma rays
- *sievert* (Sv): the dose of radiation that causes a biologic effect equivalent to 1 Gy of x-rays or gamma rays; 1 Sv corresponds to 100 rem.[53]

These measurements do not directly quantify energy transferred per unit of tissue and therefore do not predict the biologic effects of radiation. The following terms provide a better approximation of such information.

- *Linear energy transfer* (LET) expresses energy loss per unit of distance traveled as electron volts per micrometer. This value depends on the type of ionizing radiation. LET is high for alpha particles, less so for beta particles, and even less for gamma rays and x-rays. Thus, alpha and beta particles penetrate short distances and interact with many molecules within that short distance. Gamma rays and x-rays penetrate deeply but interact with relatively few molecules per unit distance. It should be evident that if equivalent amounts of energy entered the body in the form of alpha and gamma radiation, the alpha particles would induce heavy damage in a restricted area, whereas gamma rays would dissipate energy over a longer course and produce considerably less damage per unit of tissue.
- *Relative biologic effectiveness* (RBE) is simply a ratio that represents the relationship of the LETs of various forms of irradiation to cobalt gamma rays and megavolt x-rays, both of which have an RBE of unity (1).

In addition to the physical properties of the radioactive material and the dose, the biologic effects of ionizing radiation depend on several factors:

• Dose rate: a single dose can cause greater injury than divided or fractionated doses that allow time for cellular repair.
• Since DNA is the most important subcellular target of ionizing radiation, rapidly dividing cells are more radiosensitive than are quiescent cells. Hematopoietic cells, germ cells, gastrointestinal epithelium, squamous epithelium, endothelial cells, and lymphocytes are highly susceptible to radiation injury; bone, cartilage, muscle, and peripheral nerves are more resistant.
• A single dose of external radiation administered to the whole body is more lethal than regional doses with shielding. For example, the median lethal dose (LD_{50}) of ionizing radiation is 2.5 to 4.0 Gy (250 to 400 rad), whereas doses of 40 to 70 Gy (4000 to 7000 rad) can be delivered in a fractionated manner during several weeks for cancer therapy.
• Cells in the G_2 and mitotic phases of the cell cycle are most sensitive to ionizing radiation.
• Different cell types differ in the extent of their adaptive and reparative responses.
• Since ionizing radiation produces oxygen-derived radicals from the radiolytic cleavage of water (Chapter 1), cell injury induced by x-rays and gamma rays is enhanced by hyperbaric oxygen. Halogenated pyrimidines can also increase radiosensitivity to tumor cells. Conversely, free radical scavengers and antioxidants protect against radiation injury.

Cellular Mechanisms of Radiation Injury.

The acute effects of ionizing radiation range from overt necrosis at high doses (>10 Gy), killing of proliferating cells at intermediate doses (1 to 2 Gy), and no histopathologic effect at doses less than 0.5 Gy. Subcellular damage does occur at these lower doses, primarily targeting DNA; however, most cells show adaptive and reparative responses to low doses of ionizing radiation. If cells undergo extensive DNA damage or if they are unable to repair this damage, they undergo apoptosis (Chapter 7). Surviving cells may show delayed effects of radiation injury: mutations, chromosome aberrations, and genetic instability. These genetically damaged cells may become malignant; tissues with rapidly proliferating cell populations are especially susceptible to the carcinogenic effects of ionizing radiation. Most cancers induced by ionizing radiation have occurred after doses greater than 0.5 Gy. Acute cell death, especially of vascular endothelial cells, can cause delayed organ dysfunction several months or years after radiation exposure. In general, this delayed injury is caused by a combination of atrophy of parenchymal cells, ischemia due to vascular damage, and fibrosis.[53] Acute and delayed effects of ionizing radiation are listed in Table 9-17 , and their mechanisms are described next.

Acute Effects.

Ionizing radiation can produce a variety of lesions in DNA, including DNA-protein cross-links, cross-linking of DNA strands, oxidation and degradation of bases, cleavage of sugar-phosphate bonds, and single-stranded or double-stranded DNA breaks. This damage may be produced directly by particulate radiation, x-rays, or gamma rays or indirectly by oxygen-derived free radicals or soluble products derived from peroxidized lipids.[54] Even relatively low doses of ionizing radiation (less than 0.5 Gy) induce alterations in gene expression in some target cell populations. Free radicals generated directly or indirectly by exposure to ionizing radiation may produce oxidant stress that activates transcription factors (such as NF-κB) that increase gene expression.[55] DNA damage itself stimulates the expression of several genes involved in DNA repair, cell-cycle arrest, and apoptosis. As discussed in Chapter 7 , the tumor-suppressor gene *p53* is activated after many different forms of DNA damage. The end-points resulting from activation of this p53-mediated DNA damage response are discussed in Chapter 7 . Briefly, activation of p53 induces cell-cycle arrest, DNA repair and, in some cases, apoptosis. Apoptosis of microvascular endothelial cells may be the primary target of acute radiation in the GI tract, resulting in secondary damage to intestinal crypt stem cells[56] and the GI syndrome (see Table 9-18).

Fibrosis.

An important delayed complication of ionizing radiation, usually at doses used for cancer therapy, is replacement of normal parenchymal tissue by fibrosis, resulting in scarring and loss of function. These fibrotic changes may be secondary to ischemic injury caused by vascular damage, death of parenchymal cells, or deletion of stem cells.[57] The mechanisms responsible for fibrosis have been explored in a murine model of radiation-induced pulmonary fibrosis using microarray analysis of gene expression. Up-regulation of chemokines that recruit inflammatory cells to the lungs as well as cytokines and growth factors involved in fibroblast activation and collagen deposition are central components of radiation-induced fibrosis.[58] As described in Chapter 3 , these chemokines, cytokines, and growth factors also play important roles in wound healing.

TABLE 9-17 -- Acute Injury and Delayed Complications Caused by Ionizing Radiation

Organ	Acute Injury	Delayed Complications
Bone marrow	Atrophy	Hypoplasia, leukemia
Skin	Erythema	Atrophy of epidermis and fibrosis of dermis; cancer
Heart	—	Interstitial fibrosis
Lung	Edema, endothelial and epithelial cell death	Interstitial and intra-alveolar fibrosis; cancer
Gastrointestinal tract	Edema, mucosal ulcers	Ulcers; fibrosis; strictures; adhesions; cancer
Liver	Veno-occlusive disease	Cirrhosis; liver tumors
Kidney	Vasodilation	Cortical atrophy, interstitial fibrosis
Urinary bladder	Mucosal erosion	Submucosal fibrosis; cancer
Brain	Edema, necrosis	Necrosis of white matter, gliosis; brain cancer
Testis	Necrosis	Tubular atrophy
Ovary	Atresia of follicles	Stromal fibrosis
Thyroid	—	Hypothyroidism; cancer
Breast	—	Fibrosis; cancer
Thymus, lymph nodes	Atrophy	Lymphoma

Carcinogenesis.

Occupational or accidental exposures to ionizing radiation produce an increased incidence of various types of cancer, including skin cancers, leukemia, osteogenic sarcomas, and lung cancer. There is usually a latent period of 10 to 20 years before appearance of these cancers. In survivors of the atomic blasts at Hiroshima and Nagasaki, all types of leukemias were especially common, with the exception of chronic lymphocytic leukemia. Exposure of children to irradiation causes an increased incidence of breast and thyroid cancers as well as gastrointestinal and urinary tract tumors. The nuclear power accident at Chernobyl in 1986 caused more than 50 deaths, with estimated exposures of 50 to 300 rad. More than 20,000 people were exposed to up to 40 rem. As early as 1990, an increased incidence of thyroid cancer was seen in exposed children. Approximately 2 million people living near Three Mile Island were exposed to low doses of 100 mrem in 1979; no adverse effects have yet been reported. Workers in the nuclear energy industry and in health care and research are exposed annually to doses ranging from 1 to 9 mSv. The annual maximal permissible exposure level for

TABLE 9-18 -- Clinical Features of the Acute Radiation Syndrome

Category	Whole-Body Dose (rem)	Symptoms	Prognosis

Subclinical	••<200	Mild nausea and vomiting	100% survival
		Lymphocytes <1500/µL	
Hematopoietic	200–600	Intermittent nausea and vomiting	Infections
		Petechiae, hemorrhage	May require bone marrow transplant
		Maximum neutrophil and platelet depression in 2 wk	
		Lymphocytes <1000/µL	
Gastrointestinal	600–1000	Nausea, vomiting, diarrhea	Shock and death in 10–14 days even with replacement therapy
		Hemorrhage and infection in 1–3 wk	
		Severe neutrophil and platelet depression	
		Lymphocytes <500/µL	
Central nervous system	••>1000	Intractable nausea and vomiting	Death in 14–36 hr
		Confusion, somnolence, convulsions	
		Coma in 15 min–3 hr	
		Lymphocytes absent	

these workers is 50 mSv or 1 rem. There is uncertainty about the potential carcinogenic risk at these low exposures because the shape of the dose-response curve is unknown.

The mechanisms responsible for the delayed carcinogenic effects of ionizing radiation are not completely understood. The latent period between acute exposure to ionizing radiation and the delayed appearance of cancer may be due to a phenomenon called *induced genetic instability*. Quantitative analysis of mutation rates in irradiated cells in culture shows that mutations continue to be expressed in surviving cells after several generations. Accumulation of these delayed mutations may be the result of persistent DNA lesions that are not repaired or due to an epigenetic mechanism, such as altered methylation at CpG sites or shortening of telomeres. Delayed chromosome aberrations are also observed after exposure to ionizing radiation, especially in human lymphocytes.[59] These mechanisms may be responsible for induction of secondary cancers, especially leukemias, in cancer patients treated with radiation therapy.

Clinical Manifestations of Exposure to Ionizing Radiation

The clinical effects of ionizing radiation depend on the dose, duration, and mode of exposure. These are described next.

Acute, Whole-Body Exposure.

Whole-body irradiation is potentially lethal; the clinical manifestations are dose dependent and described as the *acute radiation syndrome* or *radiation sickness*. On the basis of calculated doses delivered in nuclear reactor accidents or the atomic bombing of Japan, the LD_{50} at 60 days for humans exposed to a single dose of x-rays or gamma radiation is 2.5 to 4.0 Gy (250 to

400 rad). Depending on the dose, four clinical syndromes are produced: a subclinical or prodromal syndrome, hematopoietic syndrome, gastrointestinal syndrome, or central nervous system syndrome. These are summarized in Table 9-18 . The acute symptoms are manifestations of the high sensitivity of rapidly proliferating tissues, such as the lymphohematopoietic cells and gastrointestinal epithelium, to acute radiation-induced necrosis or apoptosis (Fig. 9-10). If the patient survives the acute radiation syndrome, sublethally injured cells may repair the radiation damage, and the necrotic or apoptotic cells may be replaced by the progeny of more radioresistant stem cells.

Effects of Radiation Therapy.

External radiation is delivered to malignant neoplasms at fractionated doses up to 40 to 70 Gy (4000 to 7000 rad), with shielding of adjacent normal tissues. Radiation therapy, especially when it is delivered to the chest or abdomen, can cause acute radiation sickness and neutrophil and platelet depression. These patients may experience transient fatigue, vomiting, and anorexia that may require reduction of the dose. Acutely, radiation therapy may shrink the tumor mass and relieve pain or compression of adjacent tissues. Unfortunately, cancer patients treated with radiation therapy may develop sterility, a secondary malignant neoplasm, or delayed radiation injury (described later).[53]

Effects on Growth and Development.

The developing fetus and young children are highly sensitive to growth and developmental abnormalities induced by ionizing radiation. Four susceptible phases can be defined:

Figure 9-10 Atrophy of the thymus gland after exposure to ionizing radiation. The right panel shows a normal thymus with deeply staining cortex and pale staining medulla; the left panel shows depletion of lymphocytes with preservation of (pink, concentric) Hassall corpuscles. *(American Registry of Pathology © 1990.)*

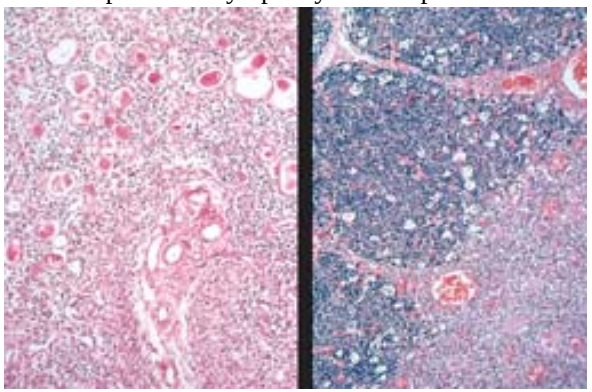

Figure 9-11 Acute vascular injury with fibrinoid necrosis and edema after exposure to ionizing radiation. *(American Registry of Pathology © 1990.)*

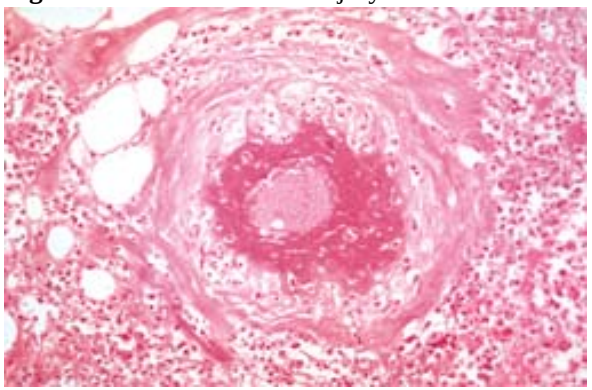

Figure 9-12 Chronic vascular injury with subintimal fibrosis occluding the lumen. *(American Registry of Pathology © 1990.)*

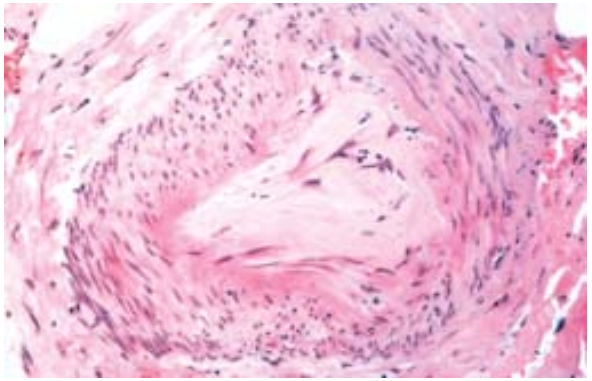

Figure 9-13 Chronic radiation dermatitis with atrophy of the epidermis, dermal fibrosis, and telangiectasia of the subcutaneous blood vessels. *(American Registry of Pathology © 1990.)*

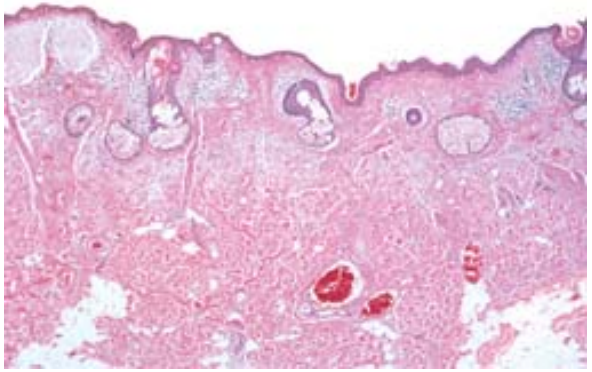

Figure 9-14 Extensive mediastinal fibrosis after radiotherapy for carcinoma of the lung. Note the markedly thickened pericardium. *(From the teaching collection of the Department of Pathology, Southwestern Medical School, Dallas, TX.)*

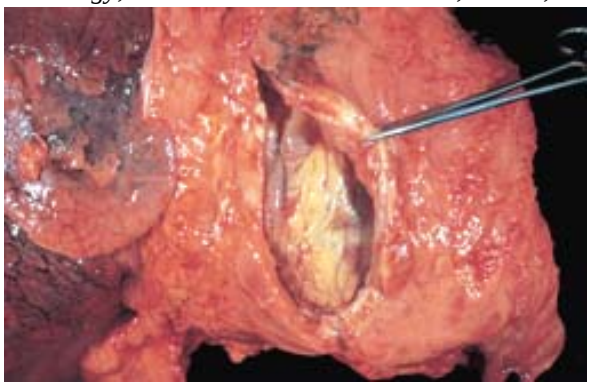

Figure 9-15 Radiation fibrosis of the breast stroma after radiotherapy for infiltrating ductal carcinoma. The nests of remaining tumor cells are pleomorphic and multinucleated. *(American Registry of Pathology © 1990.)*

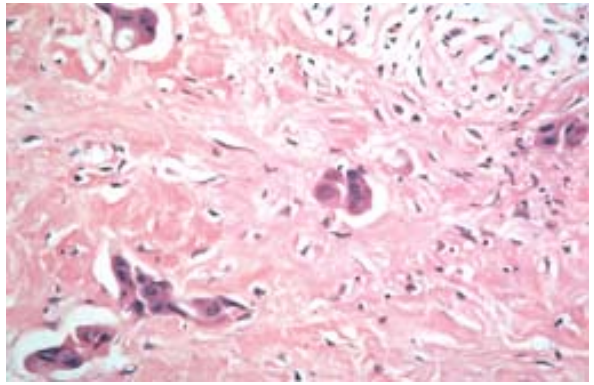

TABLE 9-19 -- Acute and Delayed Effects of Ultraviolet Radiation

Radiation	Wavelength (nm)	Acute Effects	Delayed Effects
UVA	320–400	Erythema 8–48 hr	Tanning
		Depletion of Langerhans cells	? Skin cancer
		Pigment darkening	
		Dermal inflammation	
UVB	290–320	Erythema 3–24 hr	Tanning
		Apoptosis of keratinocytes	Solar elastosis
		Depletion of Langerhans cells	Premature aging
			Actinic keratosis
			Skin cancer
UVC	200–290		? Skin cancer

Data from Rosen CF: Ultraviolet radiation. In Craighead JE (ed): Pathology of Environmental and Occupational Disease. St. Louis, Mosby-Year Book, 1996, p. 193.

Ultraviolet Radiation

Solar radiation spans the spectrum of wavelengths between 200 and 4000 nm, including ultraviolet, visible, and infrared radiation. Ultraviolet radiation is divided into ultraviolet A (UVA), ultraviolet B (UVB), and ultraviolet C (UVC); 3% to 5% of the total solar radiation that penetrates the earth's surface is ultraviolet radiation. Ozone in the atmosphere is an important protective agent against ultraviolet radiation because it completely absorbs all UVC and partially absorbs UVB. Chlorofluorocarbons, used commercially as propellants, as solvents, and in refrigerators and air conditioners, interact with and deplete ozone. Such depletion is predicted to contribute to an increase in UVB and possibly UVC exposure, thus triggering a 2% to 4% increase in the incidence of skin cancers. Some protection from the effects of UV light is afforded by window glasses: they absorb UVB radiation, but they transmit UVA radiation. Sunblocks and sunscreens offer greater protection because they absorb or block UVB and UVA to variable degrees. There are two major health effects of ultraviolet radiation: premature aging of the skin and skin cancer (Table 9-19). The carcinogenic effects of ultraviolet light are discussed in Chapter 7 . Here we focus on other effects of ultraviolet radiation.

The acute effects of UVA and UVB are short-lived and reversible. They include erythema, pigmentation, and injury to Langerhans cells and keratinocytes in the epidermis. The kinetics and chemical mediators of these reactions differ in response to UVA and UVB. Depending on the intensity and length of exposure, erythema, edema, and acute inflammation are mediated

by release of histamine from mast cells in the dermis, synthesis of arachidonic acid metabolites, and the production of pro-inflammatory cytokines like IL-1. UVA produces oxidation of melanin with transient, immediate

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darkening, especially in individuals with darker skin. Tanning induced by UVA and UVB is due to a delayed increase in the number of melanocytes, elongation and extension of dendritic processes, and transfer of melanin to keratinocytes. Tanning induced by UVB is protective against subsequent exposures; tanning induced by UVA provides limited protection. Both UVA and UVB deplete Langerhans cells and thus reduce the processing of antigens introduced through the epidermis. UVB causes apoptosis of keratinocytes in the epidermis, resulting in dyskeratotic, sunburn cells.

Repeated exposures to ultraviolet radiation give rise to changes in the skin that are characteristic of premature aging (e.g., wrinkling, solar elastosis, and irregularities in pigmentation). In contrast to ionizing radiation that increases deposition of collagen in the dermis, ultraviolet radiation causes degenerative changes in elastin and collagen, leading to wrinkling, increased laxity, and a leathery appearance. These connective tissue alterations accumulate over time and are largely irreversible. They are caused by increased expression of the elastin gene, increased expression of matrix metalloproteinases that degrade collagen, and induction of a tissue inhibitor of matrix metalloproteinase. The end result of these changes in connective tissue enzymes is degradation of type I collagen fibrils and disorganization and degeneration of the dermal connective tissue[61] (Fig. 9-16).

Skin damage induced by UVB is believed to be caused by the generation of reactive oxygen species and by damage to endogenous chromophores such as melanin. Ultraviolet radiation also damages DNA, resulting in the formation of pyrimidine dimers between adjacent pyrimidines on the same DNA strand. Other forms of DNA damage, for example, formation of pyrimidine-pyrimidone (6-4) photoproducts, single-stranded breaks, and DNA-protein cross-links, are also noted.[61] A unique spectrum of mutations has been identified in premalignant and malignant skin lesions in humans,

Figure 9-16 Solar elastosis with basophilic degeneration of the connective tissue in the upper layer of the dermis. *(American Registry of Pathology © 1990.)*

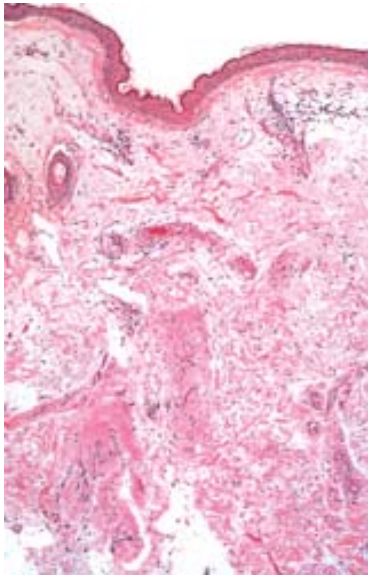


TABLE 9-20 -- Adult Mortality Rates in the United States, Ages 25–44, in 1998

	Rate per 100,000 population		
Cause	***Hispanic***	***Black***	***White***
Unintentional injuries	33.4	40.1	31.6
Cancer	16.8	38.0	25.3
Homicide	13.1	36.2	4.7
Human immunodeficiency virus	12.1	43.3	4.8
Heart disease	10.3	43.5	18.3
Suicide	7.8	—	17.0
Total	130.2	303.7	139.4

Data from CDC Fact Book, 2000/2001, Department of Health and Human Services, Centers for Disease Control and Prevention.

abuse, is a major concern in the United States. Hence, firearm safety and access are important matters of public health.[66]

Injuries caused by the physical environment, resulting from human activities as well as from external forces, can be divided into four categories: mechanical force, heat and cold, electrical injuries, and high altitudes.

Mechanical Force

Mechanical force may inflict soft-tissue injuries, bone injuries, and head injuries. Injuries of the bones and of the head are considered in Chapter 28 . Soft-tissue injuries can be superficial, involving mainly the skin, or deep, associated with visceral damage. The skin injuries can be further described as follows.

Abrasion.

This type of skin injury represents basically a scrape, in which the superficial epidermis is torn off by friction or force (Fig. 9-17). Regeneration without scarring usually occurs promptly unless infection complicates the process.

Figure 9-17 Abrasion. Note the superficial tears in the epidermis. There is bleeding under the skin as well. *(From the teaching collection of the Department of Pathology, Southwestern Medical School, Dallas, TX.)*

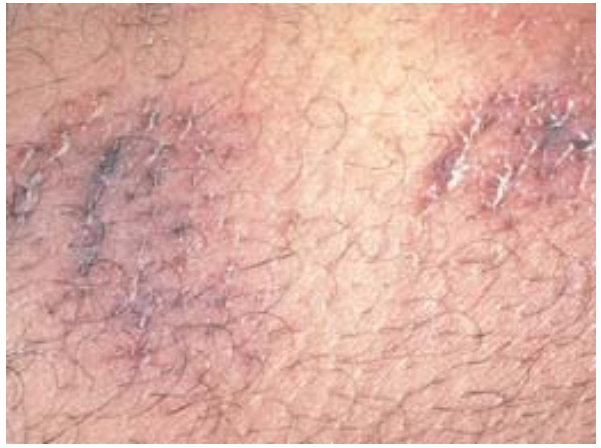


Figure 9-18 Laceration of the scalp. The bridging strands of the fibrous tissue are evident. *(From the teaching collection of the Department of Pathology, Southwestern Medical School, Dallas, TX.)*

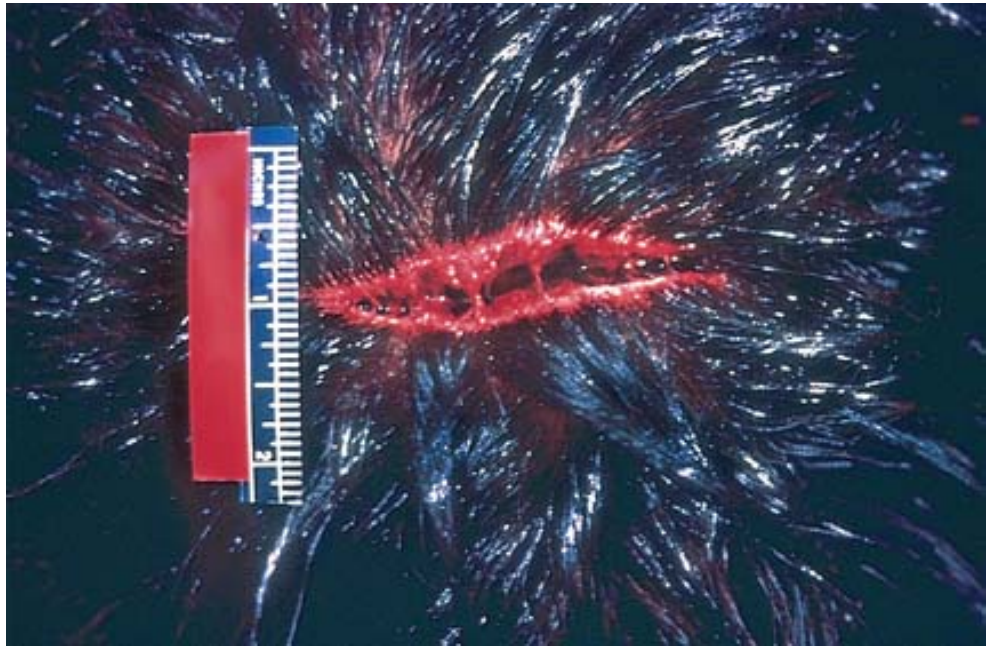


Figure 9-19 Contusion resulting from blunt trauma. The skin is intact, but there is hemorrhage in subcutaneous vessels, producing extensive discoloration. *(From the teaching collection of the Department of Pathology, Southwestern Medical School, Dallas, TX.)*

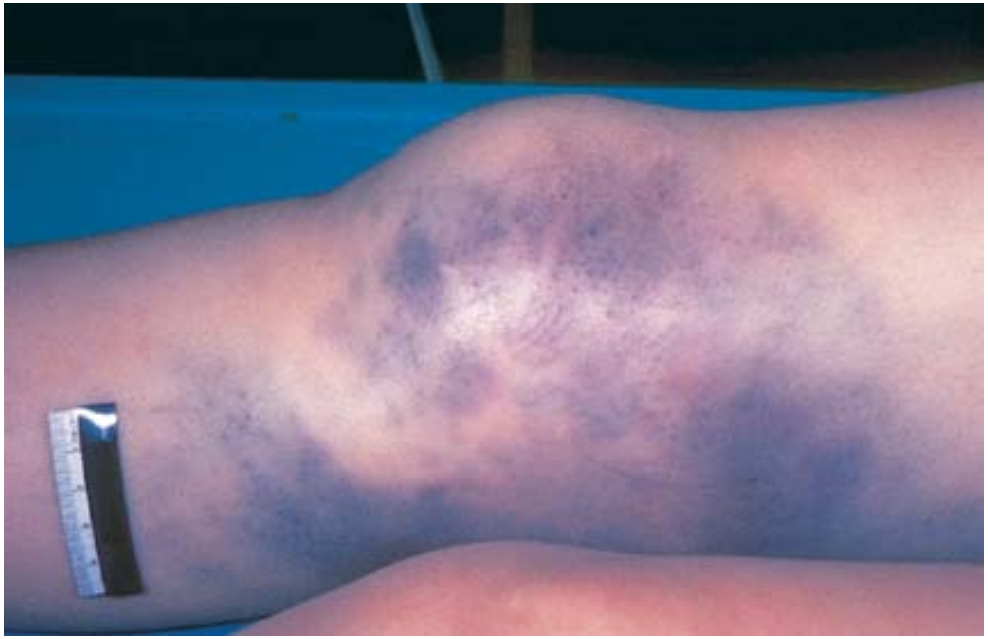


Figure 9-20 *A,* Gunshot wound of entry from a long distance. *(From the teaching collection of the Department of Pathology, Southwestern Medical School, Dallas, TX.) B,* An entry gunshot wound at close range revealing the prominent black discoloration produced by unburned powder, heat, and smoke as well as the more peripheral stippling resulting from larger particles of unburned powder. *(Courtesy of George Katsas, MD, Forensic Pathologist, Boston, MA.)*

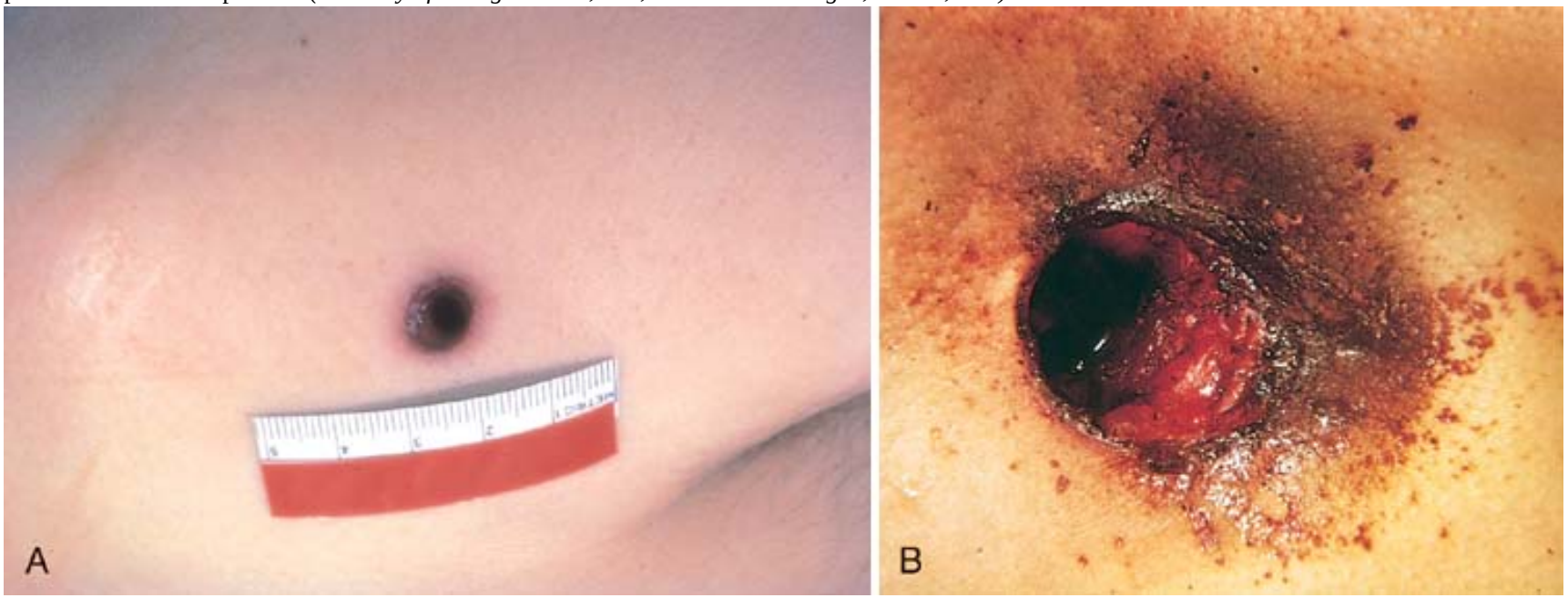


Figure 9-21 Kwashiorkor. The infant shows generalized edema, seen in the form of puffiness of the face, arms, and legs.

TABLE 9-21 -- Comparison of Severe Marasmus-Like and Kwashiorkor-Like Secondary Protein-Energy Malnutrition

Syndrome	Clinical Setting	Time Course	Clinical Features	Laboratory Findings	Prognosis
Marasmus-like protein-energy malnutrition	Chronic illness (e.g., chronic lung disease, cancer)	Months	History of weight loss	Normal or mildly reduced serum proteins	Variable; depends on underlying disease
			Muscle wasting		
			Absent subcutaneous fat		
Kwashiorkor-like protein-energy malnutrition	Acute, catabolic illness (e.g., severe trauma, burns, sepsis)	Weeks	Normal fat and muscle	Serum albumin <2.8 gm/dL	Poor
			Edema		
			Easily pluckable hair		

Data from Bennett JC, Plum F (eds): Cecil Textbook of Medicine, 20th ed. Philadelphia, WB Saunders, 1996, p. 1156.

weak and bedridden may show physical signs of protein and energy malnutrition: (1) depletion of subcutaneous fat in the arms, chest wall, shoulders, or metacarpal regions; (2) wasting of the quadriceps femoris and deltoid muscles; and (3) ankle or sacral edema.

Bedridden or hospitalized malnourished patients have an increased risk of infection, sepsis, impaired wound healing, and death after surgery.[68] The biochemical mechanisms responsible for secondary PEM in patients with cachexia are complex. In contrast to patients with anorexia nervosa, described next, patients with cachexia show loss of fat as well as muscle mass, which may occur before a decrease in appetite. Cachectic patients show increased expenditure of resting energy; in contrast, in chronic starvation, the basal metabolic rate is decreased. Cytokines produced by the host during sepsis, for example, or by tumors have been postulated to be involved in cachexia: tumor necrosis factor, interleukin-1, interleukin-6, and interferon-γ. In addition, as discussed in Chapter 7 , lipid- and protein-mobilizing factors have been isolated from animals and people with cancer cachexia.[69]

Morphology.

The central anatomic changes in PEM are (1) growth failure; (2) peripheral edema in kwashiorkor; and (3) loss of body fat and atrophy of muscle, more marked in marasmus.

The **liver** in kwashiorkor, but not in marasmus, is enlarged and fatty; superimposed cirrhosis is rare.

In kwashiorkor (rarely in marasmus), the **small bowel** shows a decrease in the mitotic index in the crypts of the glands, associated with mucosal atrophy and loss of villi and microvilli. In such cases, concurrent loss of small intestinal enzymes occurs, most often manifested as disaccharidase deficiency. Hence, infants with kwashiorkor initially may not respond well to a full-strength, milk-based diet. With treatment, the mucosal changes are reversible.

The **bone marrow** in both kwashiorkor and marasmus may be hypoplastic, mainly because of decreased numbers of red cell precursors. How much of this derangement is due to a deficiency of protein and folates or to reduced synthesis of transferrin and ceruloplasmin is uncertain. Thus, anemia is usually present, most often hypochromic microcytic anemia, but a concurrent deficiency of folates may lead to a mixed microcytic-macrocytic anemia.

The **brain** in infants who are born to malnourished mothers and who suffer PEM during the first 1 or 2 years of life has been reported by some observers to show cerebral atrophy, a reduced number of neurons, and impaired myelinization of the white matter, but there is no universal agreement on the validity of these findings.

Many **other changes** may be present, including (1) thymic and lymphoid atrophy (more marked in kwashiorkor than in marasmus); (2) anatomic alterations induced by intercurrent infections, particularly with all manner of endemic worms and other parasites; and (3) deficiencies of other required nutrients, such as iodine and vitamins.

Anorexia Nervosa and Bulimia

Anorexia nervosa is self-induced starvation, resulting in marked weight loss; bulimia is a condition in which the patient binges on food and then induces vomiting. These eating disorders occur primarily in previously healthy young women who have developed an obsession with attaining thinness.

The clinical findings in anorexia nervosa are generally similar to those in severe PEM. In addition, effects on the endocrine system are prominent. *Amenorrhea*, resulting from decreased secretion of gonadotropin-releasing hormone (and subsequent decreased secretion of luteinizing hormone and follicle-stimulating hormone), is so common that its presence is a diagnostic feature for the disorder. Other common findings, related to decreased thyroid hormone release, include cold intolerance, bradycardia, constipation, and changes in the skin and hair. The skin becomes dry and scaly and may be yellow because of excess carotene in the blood. Body hair may be increased but is usually fine and pale (lanugo). Bone density is decreased, most likely owing to low estrogen levels, which mimic the postmenopausal acceleration of osteoporosis. As expected with severe PEM, anemia, lymphopenia, and hypoalbuminemia may be present. A major complication of anorexia nervosa is an increased susceptibility to cardiac arrhythmia and sudden death, resulting in all likelihood from hypokalemia.

In bulimia, binge eating is the norm. Huge amounts of food, principally carbohydrates, are ingested, only to be followed by induced vomiting. Although menstrual irregularities are

common, amenorrhea occurs in less than 50% of bulimia patients, probably because weight and gonadotropin levels are maintained near normal. The major medical complications relate to continual induced vomiting and include (1) electrolyte imbalances (hypokalemia), which predispose the patient to cardiac arrhythmias; (2) pulmonary aspiration of gastric contents; and (3) esophageal and cardiac rupture.

Vitamin Deficiencies

Thirteen vitamins are necessary for health; four—A, D, E, and K—are fat-soluble, and the remainder are water-soluble. The distinction between fat- and water-soluble vitamins is important, because although fat-soluble vitamins are more readily stored in the body, they are likely to be poorly absorbed in gastrointestinal disorders of fat malabsorption (Chapter 17). Small amounts of some vitamins can be synthesized endogenously—vitamin D from precursor steroids; vitamin K and biotin by the intestinal microflora; and niacin from tryptophan, an essential amino acid—but the rest must be supplied in the diet. A deficiency of vitamins may be primary (dietary in origin) or secondary (because of disturbances in intestinal absorption, transport in the blood, tissue storage, or metabolic conversion). In the following sections, the major vitamins, together with their well-defined deficiency states, are discussed individually (with the exception of vitamin B_{12} and folate, which are discussed in Chapter 13) beginning with the fat-soluble vitamins. However, deficiencies of a single vitamin are uncommon, and the expression of a deficiency of a combination of vitamins may be submerged in concurrent PEM. A summary of all the essential vitamins, along with their functions and deficiency syndromes, is presented in Table 9-22 .

Vitamin A.

Vitamin A is actually a group of related natural and synthetic chemicals that exert a hormone-like activity or function. The relationship of some important members of this

TABLE 9-22 -- Vitamins: Major Functions and Deficiency Syndromes

Vitamin	Functions	Deficiency Syndromes
Fat-Soluble		
Vitamin A	A component of visual pigment	Night blindness, xerophthalmia, blindness
	Maintenance of specialized epithelia	Squamous metaplasia
	Maintenance of resistance to infection	Vulnerability to infection, particularly measles
Vitamin D	Facilitates intestinal absorption of calcium and phosphorus and mineralization of bone	Rickets in children
		Osteomalacia in adults
Vitamin E	Major antioxidant; scavenges free radicals	Spinocerebellar degeneration
Vitamin K	Cofactor in hepatic carboxylation of procoagulants—factors II (prothrombin), VII, IX, and X; and protein C and protein S	Bleeding diathesis
Water-Soluble		
Vitamin B_1 (thiamine)	As pyrophosphate, is coenzyme in decarboxylation reactions	Dry and wet beriberi, Wernicke syndrome, ?Korsakoff syndrome

Vitamin B_2 (riboflavin)	Converted to coenzymes flavin mononucleotide and flavin adenine dinucleotide, cofactors for many enzymes in intermediary metabolism	Ariboflavinosis, cheilosis, stomatitis, glossitis, dermatitis, corneal vascularization
Niacin	Incorporated into nicotinamide adenine dinucleotide (NAD) and NAD phosphate, involved in a variety of redox reactions	Pellagra—three "D's": dementia, dermatitis, diarrhea
Vitamin B_6 (pyridoxine)	Derivatives serve as coenzymes in many intermediary reactions	Cheilosis, glossitis, dermatitis, peripheral neuropathy
Vitamin B_{12}	Required for normal folate metabolism and DNA synthesis	Combined system disease (megaloblastic pernicious anemia and degeneration of posterolateral spinal cord tracts)
	Maintenance of myelinization of spinal cord tracts	
Vitamin C	Serves in many oxidation-reduction (redox) reactions and hydroxylation of collagen	Scurvy
Folate	Essential for transfer and use of 1-carbon units in DNA synthesis	Megaloblastic anemia, neural tube defects
Pantothenic acid	Incorporated in coenzyme A	No nonexperimental syndrome recognized
Biotin	Cofactor in carboxylation reactions	No clearly defined clinical syndrome

group is presented in Figure 9-22 . *Retinol*, perhaps the most important form of vitamin A, is the transport form and, as the retinol ester, also the storage form. It is oxidized in vivo to the aldehyde *retinal* (the form used in visual pigment) and the acid *retinoic acid*. Important dietary sources of vitamin A are animal derived (e.g., liver, fish, eggs, milk, butter). Yellow and leafy green vegetables such as carrots, squash, and spinach supply large amounts of carotenoids, many of which are provitamins that can be metabolized to active vitamin A in vivo; the most important of these is beta-carotene. A widely used term, *retinoids*, refers to both natural and synthetic chemicals that are structurally related to vitamin A but do not necessarily have vitamin A activity.

As with all fats, the digestion and absorption of carotenes and retinoids require bile, pancreatic enzymes, and some level of antioxidant activity in the food. *Retinol*, whether derived from ingested esters or from beta-carotene (through an intermediate oxidation step involving retinal), is transported in chylomicrons to the liver for esterification and storage. More than 90% of the body's vitamin A reserves are stored in the liver, predominantly in the perisinusoidal stellate (Ito) cells. In normal persons who consume an adequate diet, these reserves are sufficient for at least 6 months' deprivation. *Retinoic acid*, on the other hand, can be absorbed unchanged; it represents a small fraction of vitamin A in the blood and is active in epithelial differentiation and growth but not in the maintenance of vision.

Figure 9-22 Interrelationships of retinoids and their major functions.

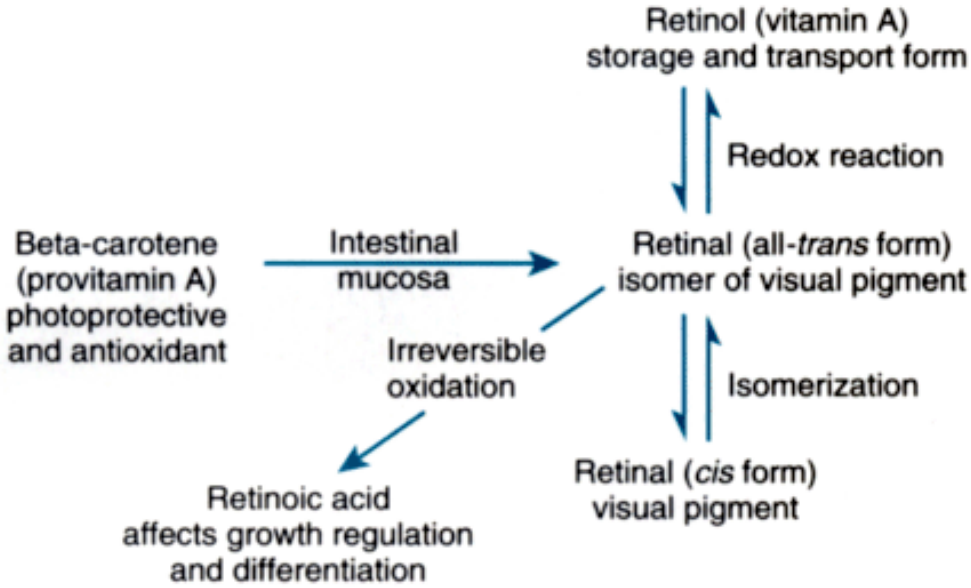


Figure 9-23 Vitamin A deficiency: its major consequences in the eye and in the production of keratinizing metaplasia of specialized epithelial surfaces, and its possible role in potentiating neoplasia.

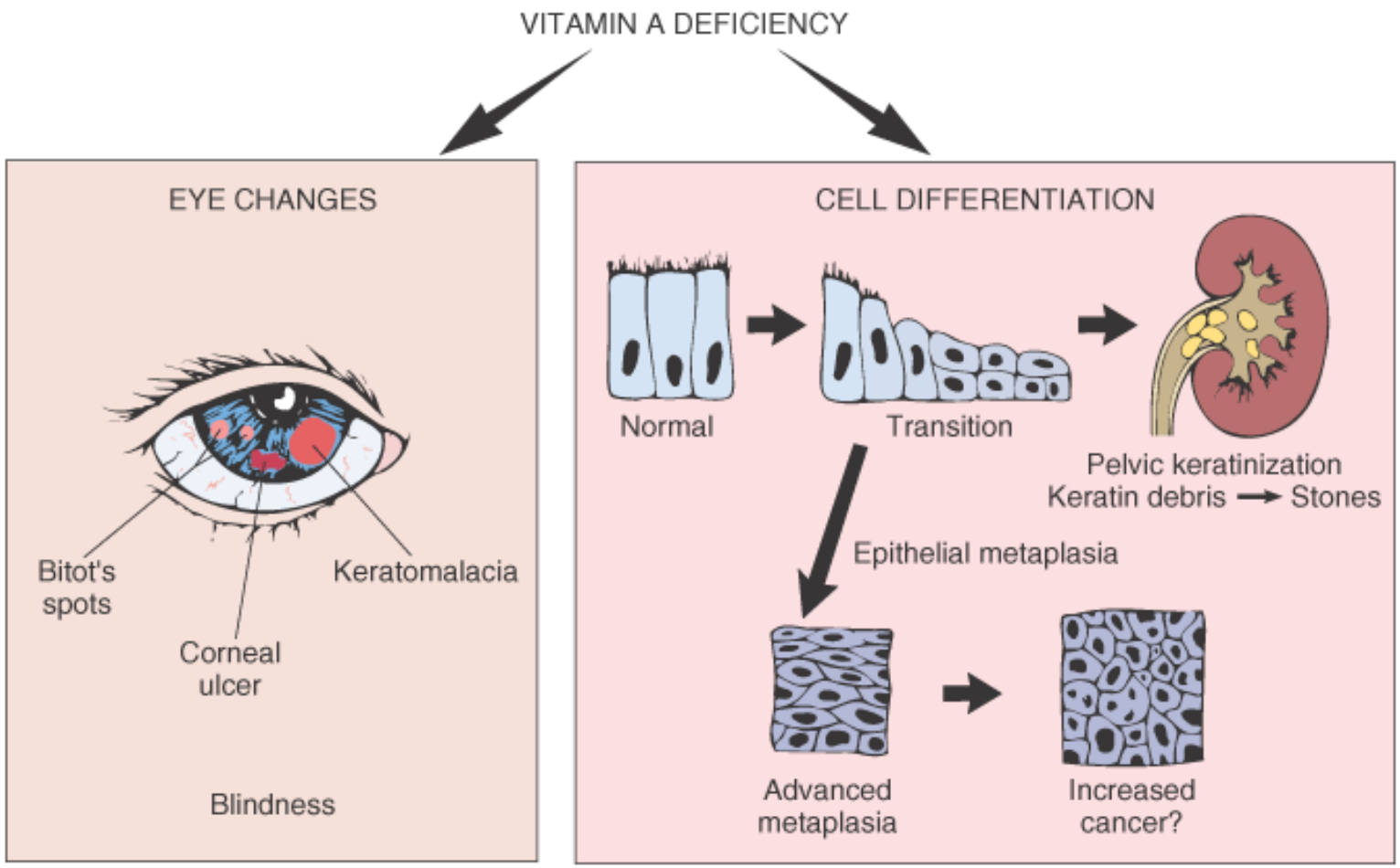
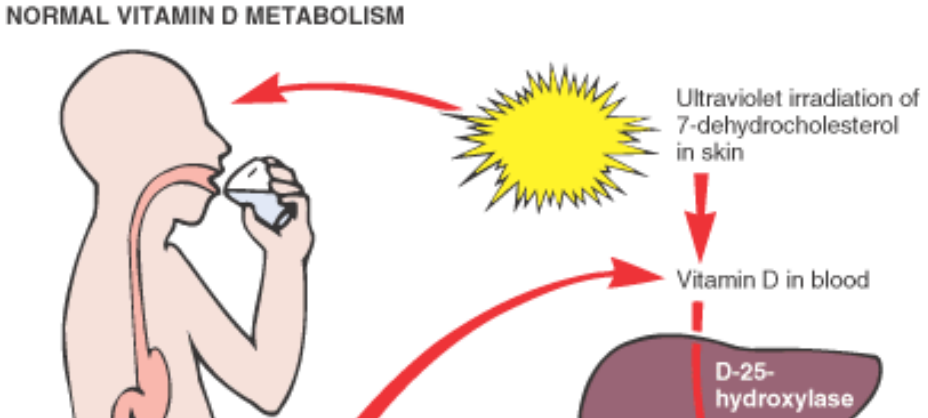
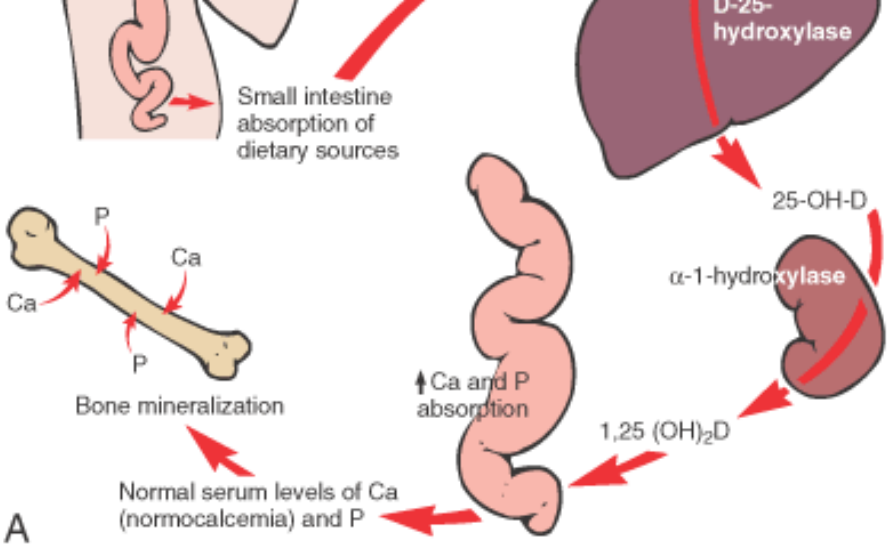


Figure 9-24 *A,* Schema of normal vitamin D metabolism. *B,* Vitamin D deficiency. There is inadequate substrate for the renal hydroxylase (1), yielding a deficiency of $1,25(OH)_2$ D (2), and deficient absorption of calcium and phosphorus from the gut (3), with consequent depressed serum levels of both (4). The hypocalcemia activates the parathyroid glands (5), causing mobilization of calcium and phosphorus from bone (6a). Simultaneously, the parathyroid hormone (PTH) induces wasting of phosphate in the urine (6b) and calcium retention. Consequently, the serum levels of calcium are normal or nearly normal, but the phosphate is low; hence, mineralization is impaired (7).





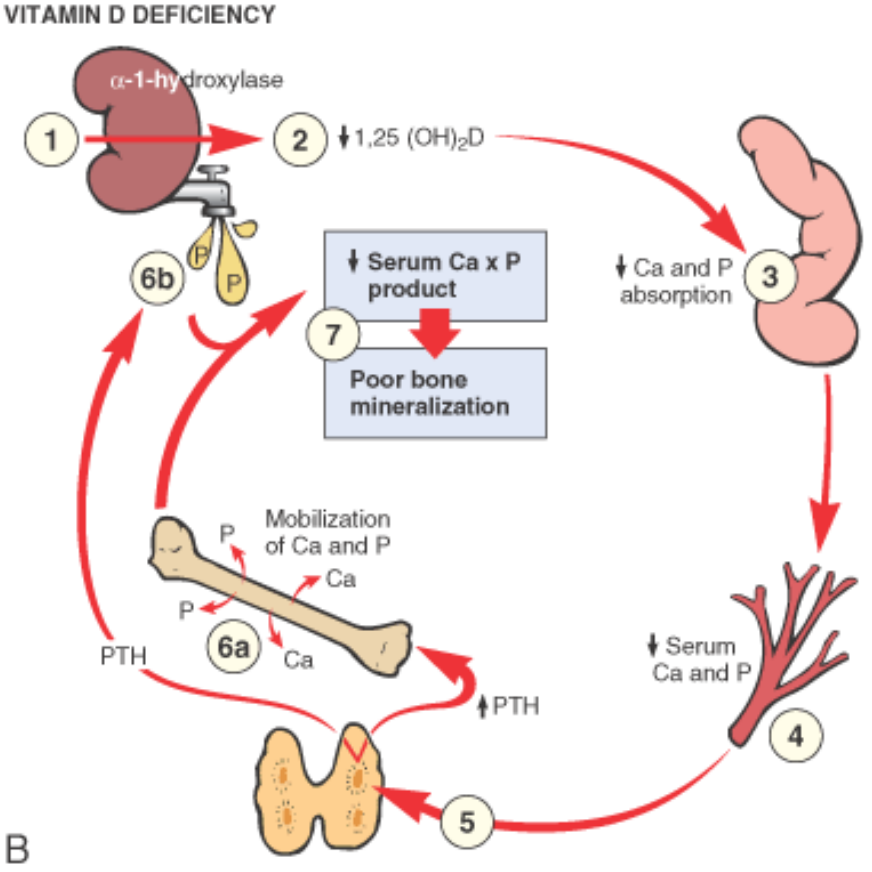


TABLE 9-23 -- Predisposing Conditions for Rickets or Osteomalacia

Inadequate Synthesis or Dietary Deficiency of Vitamin D
Inadequate exposure to sunlight
Limited dietary intake of fortified foods
Poor maternal nutrition
Dark skin pigmentation
Decreased Absorption of Fat-Soluble Vitamin D
Cholestatic liver disease
Pancreatic insufficiency
Biliary tract obstruction
Celiac sprue
Extensive small-bowel disease
Derangements in Vitamin D Metabolism
Increased degradation of vitamin D and 25(OH)D
••Induction of cytochrome P-450 enzymes (phenytoin, phenobarbital, rifampin)
Impaired synthesis of 25(OH)D
••Diffuse liver disease
Decreased synthesis of $1,25(OH)_2$ D
••Advanced renal disease
••Inherited deficiency of renal α_1 -hydroxylase (vitamin D-dependent rickets type I)
End-Organ Resistance to $1,25(OH)_2$ D
Inherited absence of or defective receptors for acute metabolite of vitamin D (vitamin D-dependent rickets type II)
Phosphate Depletion
Poor absorption of phosphate due to chronic use of antacids—binding by aluminum hydroxide
Excess renal tubule excretion of phosphate (X-linked hypophosphatemic rickets)

Figure 9-25 *A,* Detail of a rachitic costochondral junction. The palisade of cartilage is lost. Some of the trabeculae are old, well-formed bone, but the paler ones consist of uncalcified osteoid. *B,* For comparison, normal costochondral function from a young child demonstrates the orderly transition from cartilage to new bone formation.

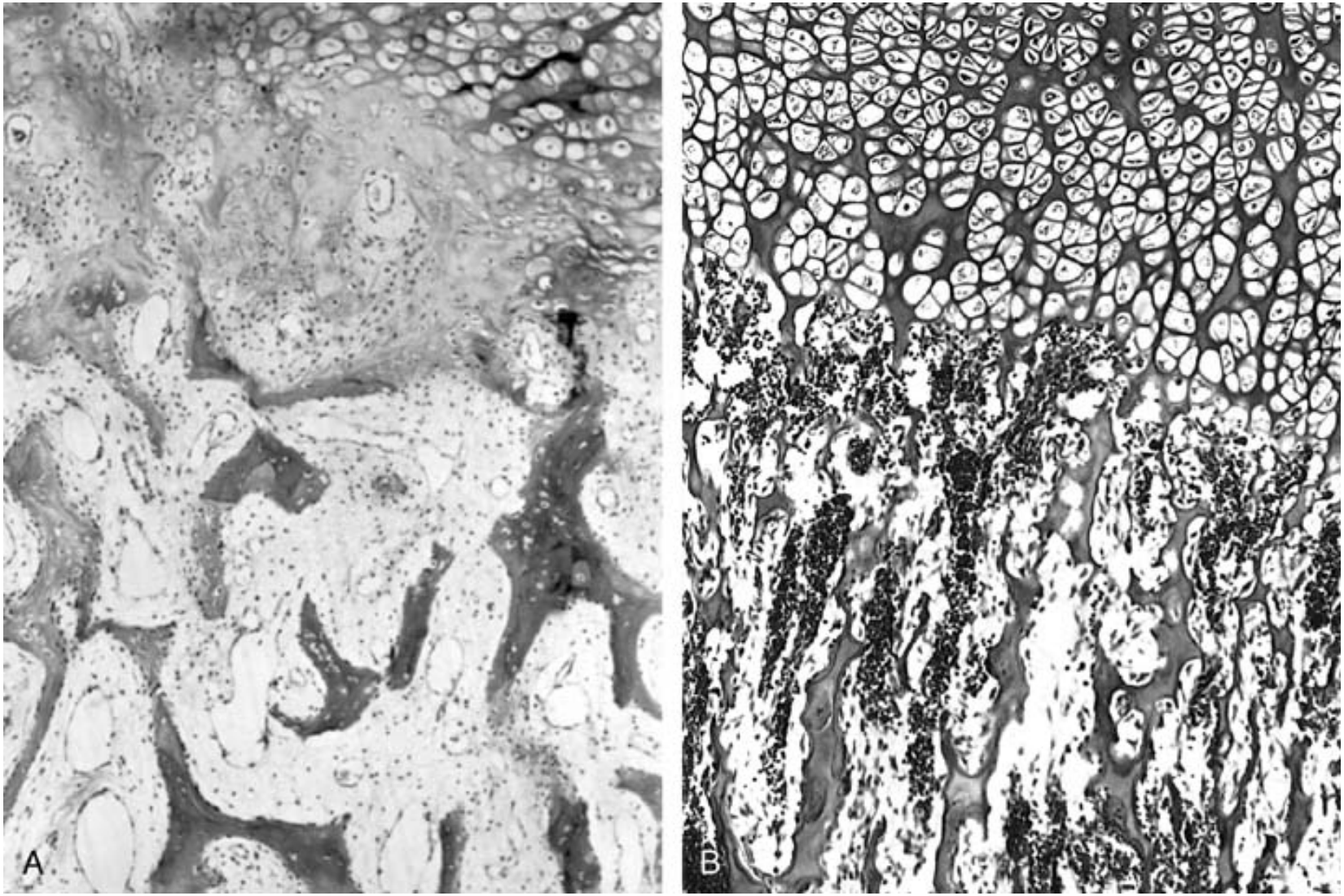


Figure 9-26 Rickets. The bowing of legs in a toddler due to the formation of poorly mineralized bones is evident.

Figure 9-27 *A,* The flabby, four-chambered, dilated heart of wet beriberi. *B,* The peripheral neuropathy with myelin degeneration leading to footdrop, wristdrop, and sensory changes in dry beriberi. *C,* Hemorrhages into the mamillary bodies in the Wernicke-Korsakoff syndrome.

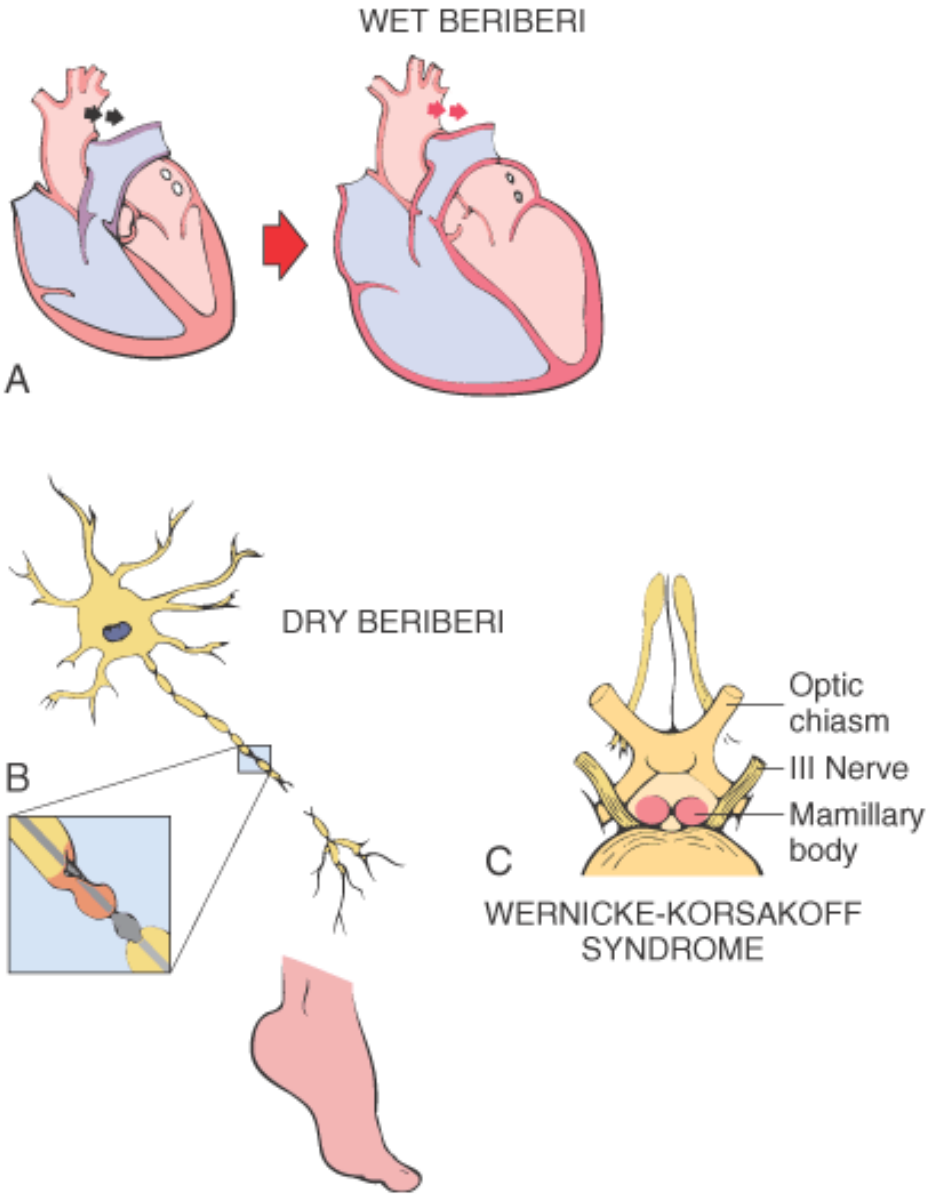
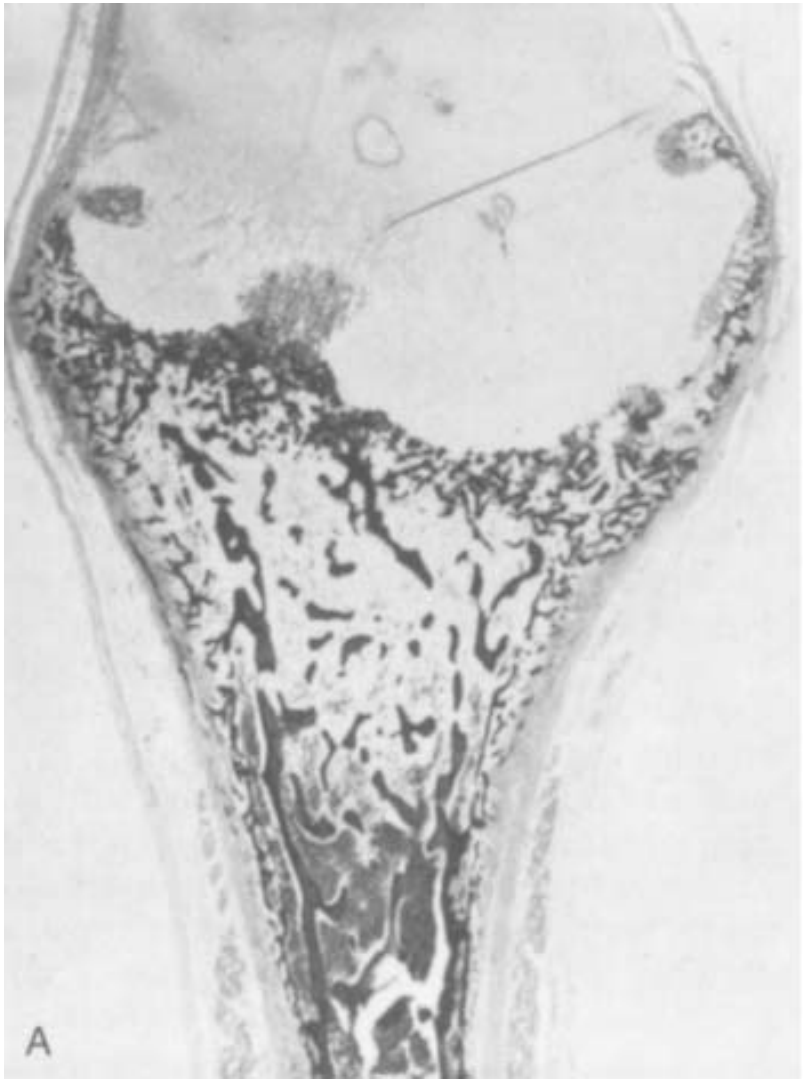


Figure 9-28 The sharply demarcated, characteristic scaling dermatitis of pellagra.

Figure 9-29 *A,* Longitudinal section of a scorbutic costochondral junction with widening of the epiphyseal cartilage and projection of masses of cartilage into the adjacent bone. *B,* Detail of a scorbutic costochondral junction. The orderly palisade is totally destroyed. There is dense mineralization of the spicules but no evidence of newly formed osteoid.



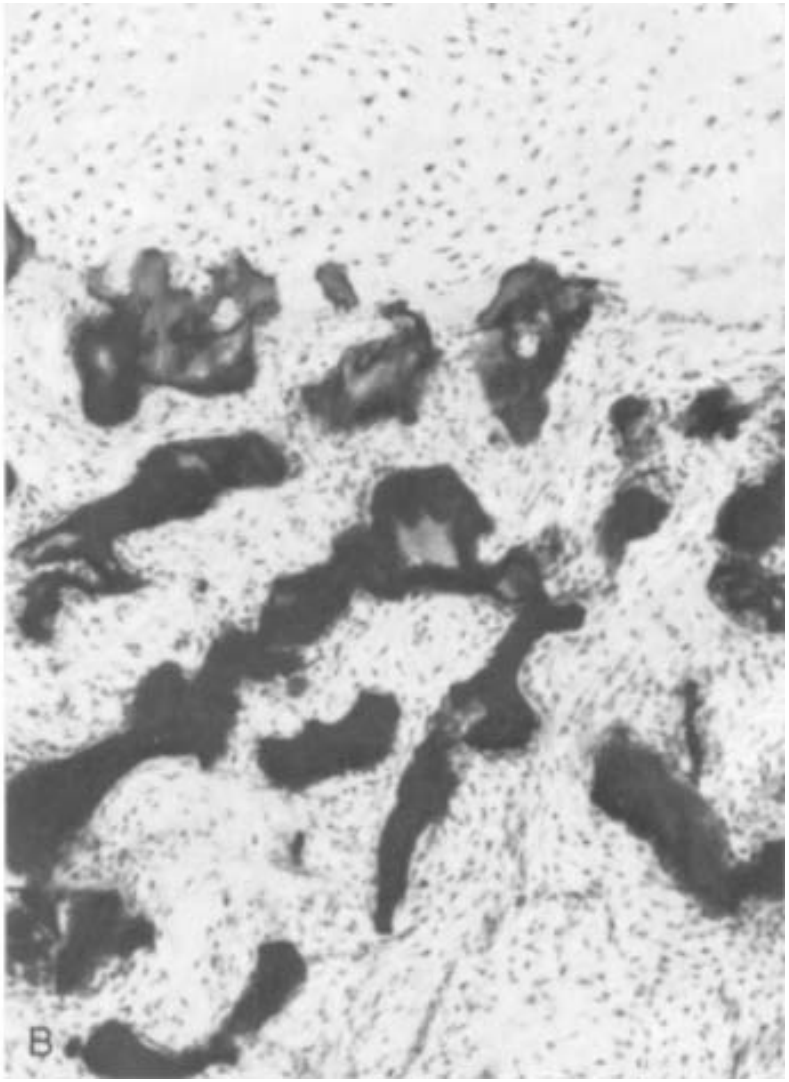


Figure 9-30 The major consequences of vitamin C deficiency.

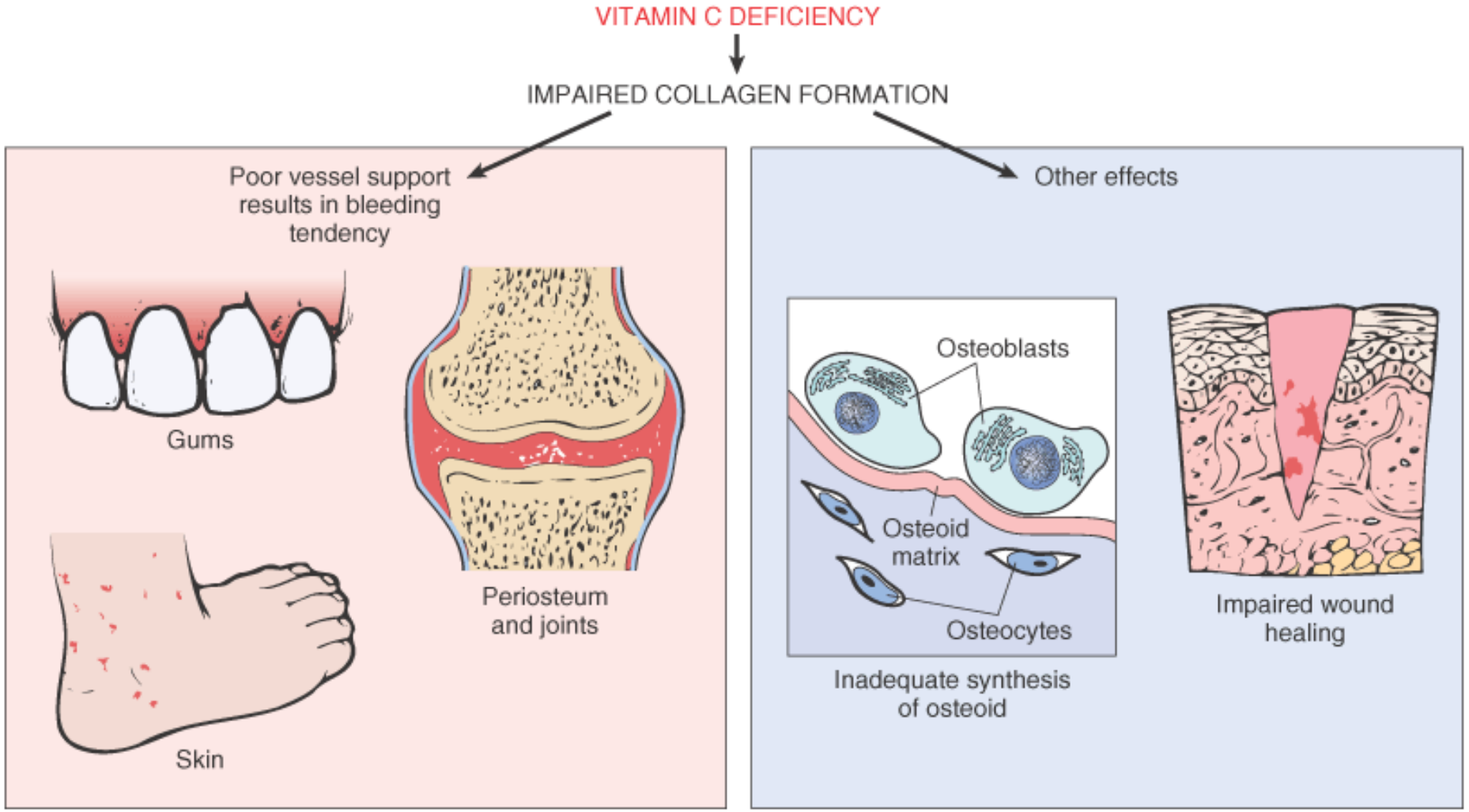


TABLE 9-24 -- Functions of Trace Metals and Deficiency Syndromes

Nutrient	Functions	Deficiency Syndromes
Iron	Essential component of hemoglobin as well as a number of iron-containing metalloenzymes	Hypochromic microcytic anemia
Zinc	Component of enzymes, principally oxidases	Acrodermatitis enteropathica, growth retardation, infertility
Iodine	Component of thyroid hormone	Goiter and hypothyroidism
Selenium	Component of glutathione peroxidase	Myopathy, rarely cardiomyopathy
Copper	Component of cytochrome *c* oxidase, dopamine β-hydroxylase, tyrosinase, lysyl oxidase, and unknown enzyme involved in cross-linking keratin	Muscle weakness, neurologic defects, hypopigmentation, abnormal collagen cross-linking

Manganese	Component of metalloenzymes, including oxidoreductases, hydrolases, and lipases	No well-defined deficiency syndrome
Fluoride	Mechanism unknown	Dental caries

Figure 9-31 Zinc deficiency with hemorrhagic dermatitis around the mouth and eyes.

TABLE 9-25 -- Body Mass Index Associated Disease Risk

Obesity Class		**BMI (kg/m^2)**	**Risk**
Underweight		<18.5	Increased
Normal		18.5–24.9	Normal
Overweight		25.0–29.9	Increased
Obesity	I	30.0–34.9	High
	II	35.0–39.9	Very high
Extreme Obesity	III	≥40.0	Extremely high

Data from National Institutes of Health, National Heart, Lung, and Blood Institute. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults —The evidence report. Obes Res 6 (suppl 2):515, 1998.

These three components are described next.[80] Not shown in Figure 9-32 is that energy expenditure occurs through a variety of hormonal (e.g., thyrotropin-releasing hormone) and autonomic intermediaries.

Among the afferent signals, insulin and leptin exert long-term control over the energy cycle by activating catabolic circuits and inhibiting anabolic pathways, as discussed in greater detail below. By contrast, ghrelin is predominately a short-term mediator. Produced in the stomach, ghrelin levels rise sharply before every meal and fall promptly when the stomach is "filled." In fact, it is thought that the success of gastric bypass surgery in massively obese individuals may relate more to the associated suppression of ghrelin levels than to an anatomic reduction in stomach capacity.

Whereas both insulin and leptin influence the energy cycle, available data suggest that leptin has a more important role than insulin in the central nervous system control of energy homeostasis.[81] [81A] Hence, our discussion will be focused on leptin, recognizing that leptin and insulin share some of their actions.

It is now established that adipocytes communicate with the hypothalamic centers that control appetite and energy expenditure by secreting leptin, a member of the cytokine family. When there is an abundance of stored energy in the form of adipose tissue, the resultant high levels of leptin cross the blood-brain barrier, binding to leptin receptors. Leptin receptor signaling has two effects: it inhibits anabolic circuits that normally promote food intake and inhibit energy expenditure, and, through a distinct set of neurons, leptin triggers catabolic circuits (Fig. 9-32). *The net effect of leptin, therefore, is to reduce food intake and promote energy expenditure.* Hence, over a period of time, energy stores (adipocytes) are reduced, and weight is lost. This in turn reduces the circulating levels of leptin, and a new equilibrium is reached. This cycle is reversed when adipose tissue is lost and leptin levels are reduced below a threshold. Equilibrium is again reached, since with low leptin levels, the anabolic circuits are relieved of inhibition and catabolic circuits are not activated, resulting in net gain of weight.

The molecular basis of leptin action is extremely complex and not yet fully unraveled. For the most part, leptin exerts its function through a series of integrated neural pathways referred to as the *leptin-melanocortin circuit*, described in Box 9-1 and illustrated in Figure 9-33 . The understanding of this circuitry is important since obesity is a serious public health problem, and development of antiobesity drugs will depend on a full understanding of these pathways.

Obesity, particularly *central obesity, increases the risk for a number of conditions*,[79] including diabetes, hypertension, osteoarthritis, pancreatitis, and many others, listed in Table 9-26 . Only some of these complications are discussed here. The mechanisms underlying these associations are complex and likely to be interrelated. Obesity, for instance, is associated with *insulin resistance* and hyperinsulinemia, important features of non-insulin-dependent, or type II, diabetes, and weight loss is associated with improvement. It has been speculated that excess insulin, in turn, may play a role in the retention of sodium, expansion of blood volume, production of

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Figure 9-32 A simplified schema of the circuitry that regulates energy balance. When sufficient energy is stored in adipose tissue and the individual is well fed, afferent adiposity signals (insulin, leptin, ghrelin) are delivered to the central neuronal processing units, in the hypothalamus. Here the adiposity signals inhibit anabolic circuits and activate catabolic circuits. The effector arms of these central circuits then impact on energy balance by inhibiting food intake and promoting energy expenditure. This in turn reduces the energy stores and the adiposity signals are obtunded. Conversely, when energy stores are low, the available anabolic circuits take over at the expense of catabolic circuits to generate energy stores in the form of adipose tissue, thus generating an equilibrium.

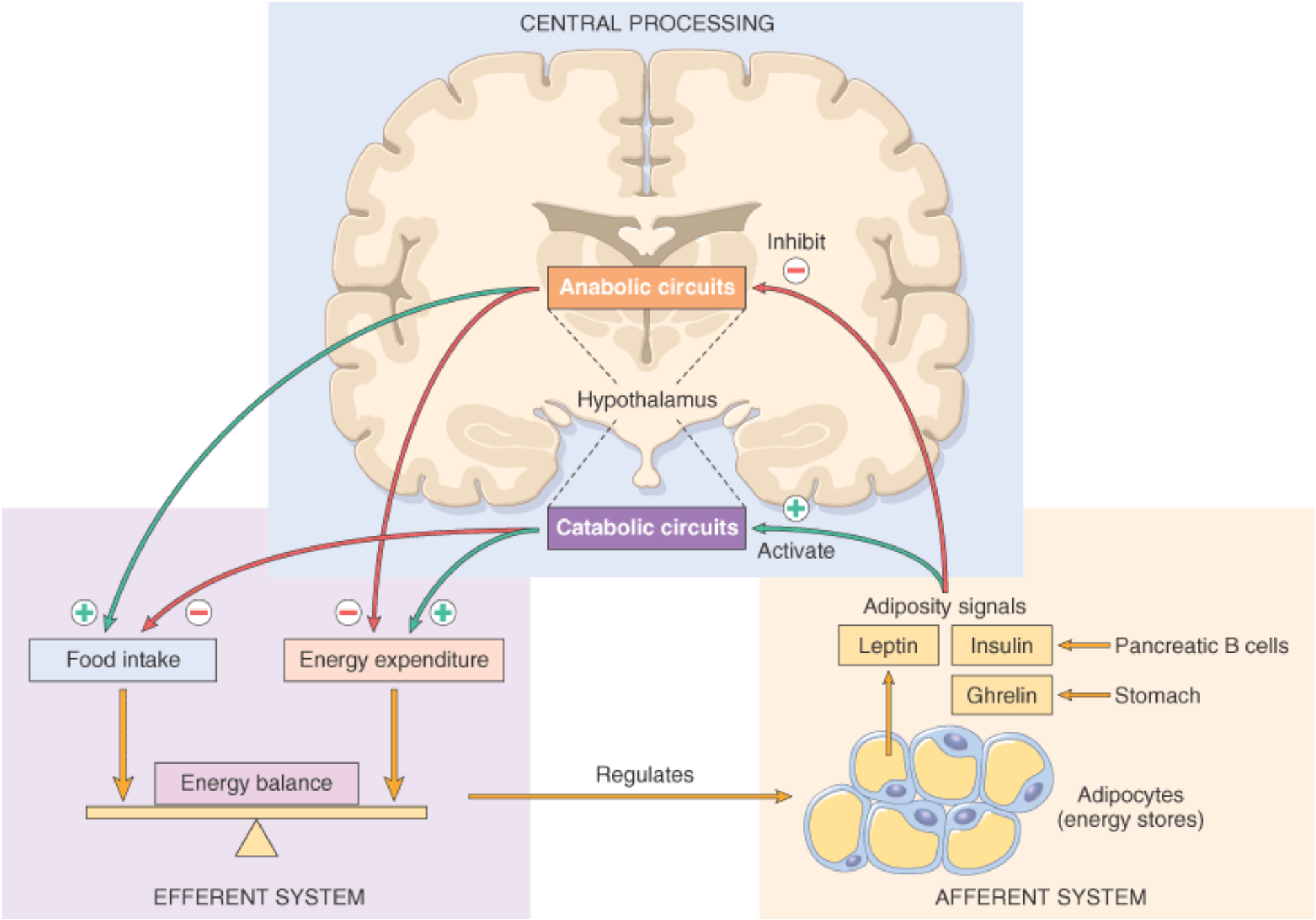
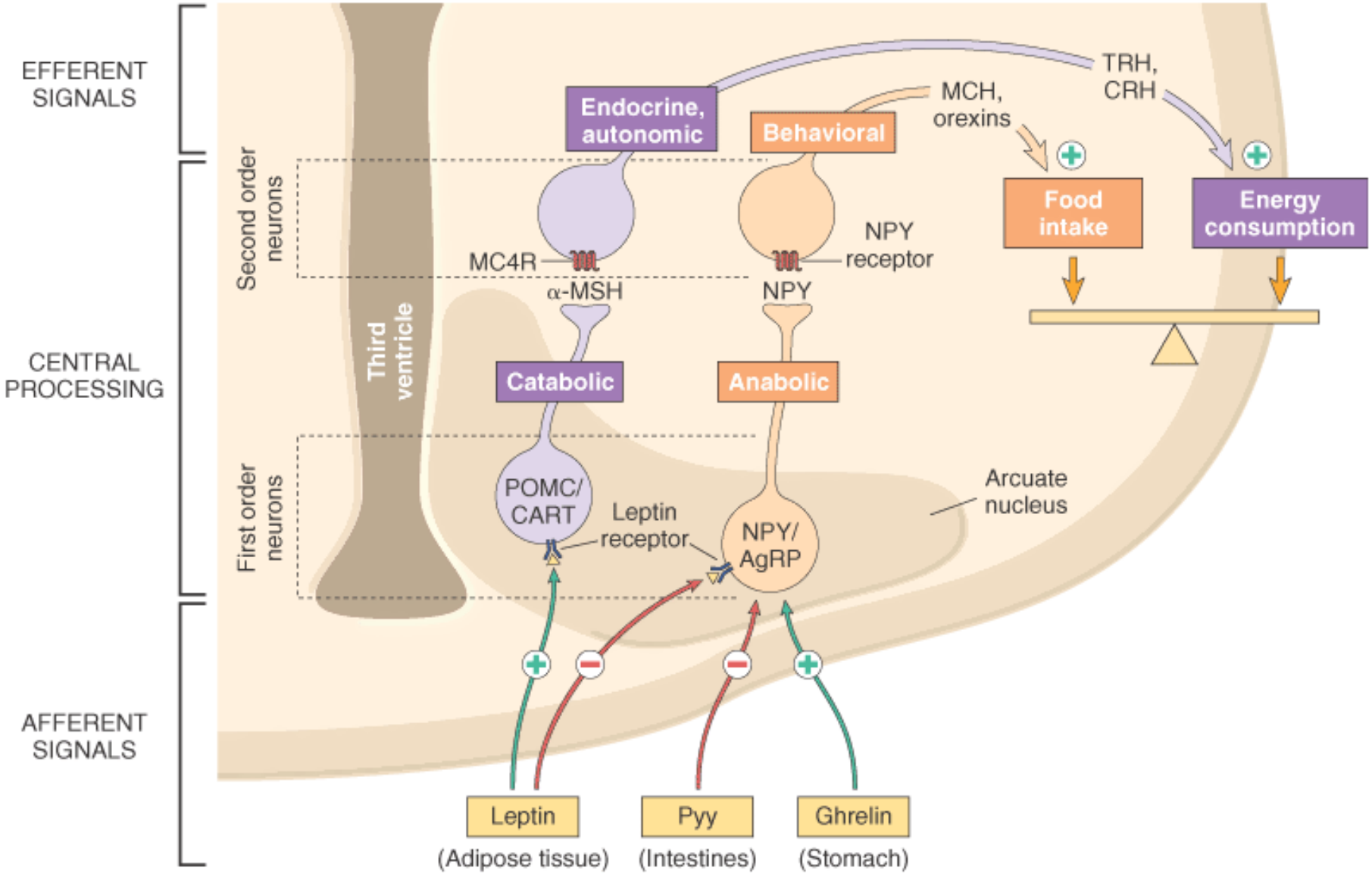


Figure 9-33 The neurohumoral circuits in the hypothalamus that regulate energy balance. Details are in the text.


EFFERENT SIGNALS
CENTRAL PROCESSING
AFFERENT SIGNALS
Second order neurons
First order neurons
Third ventricle
Endocrine, autonomic
Behavioral
TRH, CRH
MCH, orexins
Food intake
Energy consumption
MC4R
α-MSH
NPY receptor
NPY
Catabolic
Anabolic
POMC/ CART
Leptin receptor
NPY/ AgRP
Arcuate nucleus
Leptin
(Adipose tissue)
Pyy
(Intestines)
Ghrelin
(Stomach)

Box 9-1. Genetics of Obesity

Obesity is a disorder with a multifactorial etiology. Only rarely does it result from single gene disorders. Evidence supporting an important role for genes in weight control includes familial clustering of obesity and higher concordance of body mass index (BMI) among monozygotic twins (74%) versus dizygotic twins (32%) living in the same environment. Although monogenic forms of obesity in humans are rare, studies of these genetic forms of obesity and their murine counterparts have significantly advanced our understanding of the molecular basis of obesity. Some of these are discussed below.

In recent years many "obesity" genes have been identified. As might be expected, they encode the molecular components of the neuroendocrine system that regulates energy balance. Leptin, the key player in energy homeostasis, is the product of the *OB* gene. Its role as an antiobesity factor is buttressed by the observation that mice homozygous for mutations in the leptin gene (*OB/OB*) do not secrete leptin, are massively obese, and are "cured" by the administration of exogenous leptin. Mice with mutations in the leptin receptor (*db/db*) are also obese, but, unlike the case with *ob/ob* mice, their obesity cannot be ameliorated by the administration of leptin. In these mice, obesity occurs because the leptin-mediated afferent signals impinging on the hypothalamus fail to regulate appetite and energy expenditure.

Although leptin receptors are expressed at several sites in the brain, those most critical for regulation of the leptin-melanocortin circuit are expressed in the arcuate nucleus of the hypothalamus. There are two major types of neurons in this locale that bear leptin receptors: one set (*oraxogenic*) produces appetite-stimulating neurotransmitters called neuropeptide Y (NPY) and agouti-related peptide (AgRP). These are appropriately called NPY/AgRP neurons (see Fig. 9-33). As can be surmised from the discussion in the text, leptin reduces the expression of NPY and AgRP. The other set of leptin-sensitive neurons, the so-called POMC/CART neurons, transcribe two *anorexigenic* neuropeptides—α-melanocyte-stimulating hormone (α-MSH) and cocaine and amphetamine-related transcript (CART). Both of these peptides are products of proopiomelanocortin (POMC). When the POMC/CART neurons are activated by leptin signals, they exert catabolic effects mainly through the secretion of α-MSH. As indicated in Figure 9-33 , the NPY/AgRP and POMC/CART neurons are referred to as first-order neurons of the leptin-melanocortin circuit, since they are the initial targets of leptin action. The neurotransmitters produced by them (NPY, AgRP, and α-MSH) then interact through their own specific receptors with second-order neurons that trigger the efferent systems with peripheral actions. The effects of these neurotransmitters are described next.

In the anabolic pathway, the first-order NPY/AgRP neurons make monosynaptic connections to second-order neurons, which express oraxogenic peptides melanin-concentrating hormone (MCH) and oraxins A and B. As illustrated in Figure 9-33 , NPY released from first-order neurons binds to its receptor on second-order neurons and thus transmits feeding signals. Such signals are attenuated when leptin is in excess and are activated by low levels of leptin. AgRP, like NPY, exerts anabolic effects but by a somewhat distinct mechanism.

α-MSH produced by the POMC/CART neurons exerts its catabolic effects by binding to a set of second-order neurons (in the paraventricular nucleus) that express the melanocortin 4 receptor (MC4R). Catabolic output from the MC4R neurons is relayed to the periphery via the endocrine and autonomic systems. This reduces feeding and increases energy expenditure. The energy-consuming actions of MC4R neurons are mediated in part by the release of thyrotropin-releasing hormone (TRH), which activates the thyroxine axis through the anterior pituitary; TRH not only increases thermogenesis via secretion of thyroxine, but it is also an appetite suppressant. Corticotropin-releasing hormone (CRH) is another product of MC4R neurons. It induces anorexia and also activates the sympathetic nervous system. A subset of MC4R neurons projects to sympathetic motor output areas. Fibers from these areas innervate brown adipose tissue, rich in β_3 -adrenergic receptors. When these receptors are stimulated, they cause fatty acid hydrolysis and also uncouple energy production from storage. Thus, the fats are literally burned, and energy so produced is dissipated as heat.

It is noteworthy that each of the six single gene defects that give rise to human obesity involves proteins in the leptin-melanocortin pathway. Four of these are autosomal recessive and affect the leptin receptor, POMC, and PC1. (The last mentioned is a prohormone convertase that cleaves POMC). In all these cases, there is profound hyperphagia and childhood-onset massive obesity. While these four forms of genetic obesity are quite rare, those caused by mutations in the melanocortin receptor, MC4R, are by comparison quite common. In a recent study, 5% to 8% of a cohort of 500 obese individuals had functionally important mutations in the MC4R gene.[83] In these patients, despite abundant fat stores and leptin, energy consumption cannot be stimulated. The sixth monogenic form of human obesity results from mutation in a transcription factor (SIM1) that is essential for the formation of second-order leptin neurons.

Despite the remarkable advances in our understanding of genetic control of pathways that regulate energy balance, the genetic basis of the most common forms of human obesity

remains mysterious. As a multifactorial disorder, one might expect mutations or polymorphisms in several genes of small effect that give rise to obesity in concert with environmental factors. It is interesting to note that blood leptin levels are elevated in most humans with obesity. Clearly, the high levels of leptin are unable to down-regulate the anabolic pathways or activate the catabolic pathways. The basis of such leptin resistance is unclear but it may be contributed to by a decrease in the ability of leptin to cross the blood-brain barrier, possibly due to defective transport across endothelial cells. The fact that in some obese individuals leptin levels in the cerebrospinal fluid are lower than in the plasma supports this hypothesis.

TABLE 9-26 -- Medical Complications Associated with Obesity

Gastrointestinal	Gallstones, pancreatitis, abdominal hernia, NAFLD (steatosis, steatohepatitis, and cirrhosis), and possibly GERD
Endocrine/metabolic	Metabolic syndrome, insulin resistance, impaired glucose tolerance, type II diabetes mellitus, dyslipidemia, polycystic ovary syndrome
Cardiovascular	Hypertension, coronary artery disease, congestive heart failure, arrhythmias, pulmonary hypertension, ischemic stroke, venous stasis, deep vein thrombosis, pulmonary embolus
Respiratory	Abnormal pulmonary function, obstructive sleep apnea, obesity hypoventilation syndrome
Musculoskeletal	Osteoarthritis, gout, low back pain
Gynecologic	Abnormal menses, infertility
Genitourinary	Urinary stress incontinence
Ophthalmologic	Cataracts
Neurologic	Idiopathic intracranial hypertension (pseudotumor cerebri)
Cancer	Esophagus, colon, gallbladder, prostate, breast, uterus, cervix, kidney
Postoperative events	Atelectasis, pneumonia, deep vein thrombosis, pulmonary embolus

Data from Klein S, Wadden T, Sugerman HJ: AGA technical review on obesity. Gastroenterol 123:882, 2002. NAFLD, non-alcoholic fatty liver disease; GERD, gastroesophageal reflux disease.

excess norepinephrine, and smooth muscle proliferation that are the hallmarks of hypertension. Regardless of whether these pathogenic mechanisms are actually operative, *the risk of developing hypertension among previously normotensive persons increases proportionately with weight*. Obesity is also associated with a somewhat distinctive metabolic syndrome, the so-called *syndrome X*, which is characterized by abdominal obesity, insulin resistance, hypertriglyceridemia, low serum HDL, hypertension, and increased risk for coronary artery disease.[82]

Obese persons are likely to have hypertriglyceridemia and a low HDL cholesterol value, and these factors may increase the risk of *coronary artery disease*. The association between obesity and heart disease is not straightforward, and the linkage may be related to the associated diabetes and hypertension rather than to weight. Nevertheless, the American Heart Association has recently added obesity to its list of major risk factors.[79]

Nonalcoholic steatohepatitis occurs in adolescents and adults who are obese and have type II diabetes. Fatty change accompanied by liver cell injury and inflammation may progress to fibrosis or regress following weight loss.

Cholelithiasis (gallstones) is six times more common in obese than in lean subjects. The mechanism is mainly an increase in total body cholesterol, increased cholesterol turnover, and augmented biliary excretion of cholesterol in the bile, which in turn predisposes to the formation of cholesterol-rich gallstones (Chapter 18).

Hypoventilation syndrome is a constellation of respiratory abnormalities in very obese persons. It has been called the *pickwickian syndrome,* after the fat lad who was constantly falling asleep in Charles Dickens' *Pickwick Papers*. Hypersomnolence, both at night and during the day, is characteristic and is often associated with apneic pauses during sleep, polycythemia, and eventual right-sided heart failure.

Marked adiposity predisposes to the development of degenerative joint disease (*osteoarthritis*). This form of arthritis, which typically appears in older persons, is attributed in large part to the cumulative effects of wear and tear on joints. It is reasonable to assume that the greater the body burden of fat, the greater the trauma to joints with passage of time.

Obesity increases the risk of *ischemic stroke* in both men and women. Abdominal obesity is associated with increased risk of *venous thrombosis*.

Somewhat controversial is the association between obesity and cancer. A recent large prospective study has revealed an association between increasing BMI and mortality from many forms of cancer, including cancers of the esophagus, colon, rectum, liver, and non-Hodgkin lymphoma.[84] The basis of this association is difficult to discern. With hormone-dependent cancers, such as those arising in the endometrium, the blame can be placed on hormonal imbalance since obesity is known to raise estrogen levels, but for others we remain in the dark.

DIET AND SYSTEMIC DISEASES

The problems of undernutrition and overnutrition, as well as specific nutrient deficiencies, have been discussed; however, the composition of the diet, even in the absence of any of these problems, may make a significant contribution to the causation and progression of a number of diseases. A few examples suffice here.

Currently one of the most important and controversial issues is the contribution of diet to atherogenesis. The central question is, Can dietary modification prevent or retard the development of atherosclerosis (most importantly, coronary artery disease)? The average adult in the United States consumes an inordinate amount of fat and cholesterol daily, with a ratio of saturated fatty acids to polyunsaturated fatty acids of about 3:1. Vegetable oils (e.g., corn and safflower oils) and fish oils contain polyunsaturated fatty acids and are good sources of cholesterol-lowering lipids. Fish oil fatty acids belonging to the omega-3, or n-3, family have more double bonds than do the omega-6, or n-6, fatty acids found in vegetable oils. A recent meta-analysis of 11 studies with over 16,000 patients revealed that a diet enriched in omega-3 fatty acids (vs. placebo) significantly reduced the incidence of fatal myocardial infarction and sudden cardiac death.[85]

There are other examples of the effect of diet on disease:

- Hypertension is beneficially affected by restricting sodium intake.
- Dietary fiber, or roughage, resulting in increased fecal bulk, has a preventive effect against diverticulosis of the colon.
- People who consume diets that contain abundant fresh fruits and vegetables with limited intake of meats and processed foods have a lower risk of myocardial infarction. One mechanism that may explain these epidemiologic observations is the association of hyperhomocysteinemia with increased intake of meats and decreased intake of vitamin B_6 , vitamin B_{12} , and folate. Excess levels of homocysteine are hypothesized to contribute to atherosclerosis (Chapter 11).
- Calorie restriction has been convincingly demonstrated to increase life span in experimental animals. The basis of this striking observation is not entirely clear (Chapter 1).

• Even lowly garlic has been touted to protect against heart disease (and also, alas, kisses), although research has yet to prove the effect on heart disease unequivocally.

CHEMOPREVENTION OF CANCER

Epidemiologic studies have provided evidence that populations who consume large quantities of fruits and vegetables in their diets have a lower risk of cancer. It is hypothesized that carotenoids that are converted to vitamin A in the liver and intestine may be important in the primary chemoprevention of cancer. [86] The following mechanisms are proposed for the anticarcinogenic effects of carotenoids and retinoids:

- Retinoic acid promotes differentiation of mucus-secreting epithelial tissues. Supplementation of the diet with beta-carotene and retinol is hypothesized to reverse squamous metaplasia and preneoplastic lesions in the respiratory tract of cigarette smokers and workers exposed to asbestos.
- Fruits and vegetables provide antioxidants such as betacarotene, vitamins C and E, and selenium that prevent oxidative damage to DNA.
- Vitamin A can enhance immune responses; other retinoids may modulate inflammatory reactions that are potential sources of reactive oxygen and nitrogen intermediates.

Notwithstanding such theoretical considerations, clinical studies on the role of vitamin A supplementation and cancer risk have failed to provide clear answers. Clinical trials using beta-carotene and retinyl palmitate as primary preventive agents against lung cancer were terminated because the participants showed an excess of lung cancers and increased mortality. On the other hand, 13-*cis*-retinoic acid was effective in prevention of secondary squamous cell carcinomas of the head and neck region. These apparently conflicting results are not easily explained; however, there are multiple chemical forms of retinoids that alter gene expression, cell proliferation, differentiation, and apoptosis by binding to six different nuclear receptors. Some retinoids are associated with significant toxicity, including dry skin, conjunctivitis, and hypertriglyceridemia. Until the biochemical and molecular mechanisms of action of individual retinoids and other antioxidants are understood, it is unwise to recommend dietary supplements for the primary chemoprevention of cancer. However, a diet rich in fruits, vegetables, and unprocessed grains that is low in fat and animal protein has been associated with a decreased risk of cardiovascular disease and some types of cancer.[84]

High animal fat intake combined with low fiber intake has been implicated in the causation of colon cancer. The most convincing explanation for these associations is as follows: high fat intake increases the level of bile acids in the gut, which in turn modifies intestinal flora, favoring the growth of microaerophilic bacteria. The bile acids or bile acid metabolites produced by these bacteria might serve as carcinogens or promoters. The protective effect of a high-fiber diet might relate to (1) increased stool bulk and decreased transit time, which decrease the exposure of mucosa to putative offenders, and (2) the capacity of certain fibers to bind carcinogens and thereby protect the mucosa.

Attempts to document these theories in clinical and experimental studies have, on the whole, led to contradictory results.

Thus, we must conclude that, despite many tantalizing trends and proclamations by "diet gurus," to date there is no definite proof that diet can cause or protect against cancer. Nonetheless, concern persists that carcinogens lurk in things as pleasurable as a juicy steak and rich ice cream.

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Chapter 10 - Diseases of Infancy and Childhood *

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Children are not merely little adults, and the diseases they get are not merely variants of adult diseases. Many childhood conditions are unique to, or at least take distinctive forms in, this stage of life and so are discussed separately in this chapter. Diseases originating in the perinatal period are important in that they account for significant morbidity and mortality. As would be expected, the chances for survival of live-born infants improve with each passing week. This differential represents, at least in part, a triumph of improved medical care. Better prenatal care, more effective methods of monitoring the condition of the fetus, and judicious resort to cesarean section before term when there is evidence of fetal distress all contribute to bringing into this "mortal coil" live-born infants who in past years might have been stillborn. These infants represent an increased number of *high-risk* infants. Nonetheless, the infant mortality rate in the United States has shown a decline from a level of 20.0 deaths per 1000 live births in 1970 to about 6.9 deaths in 2000.[1] Although the death rate has continued

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* The contributions of Dr. Deborah Scofield to this chapter in earlier editions are gratefully acknowledged.

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to decline for all infants, American blacks continue to have an infant mortality rate more than twice (13.9 deaths per 1000 live births) that of American whites (6.0 deaths). Worldwide, the infant mortality rates vary widely, from as low as 3 deaths per 1,000 live births in Sweden, to as high as 82 deaths in the Indian subcontinent.

Each stage of development of the infant and child is prey to a somewhat different group of disorders. The data available permit a survey of four time spans: (1) the neonatal period (the first 4 weeks of life), (2) infancy (the first year of life), (3) age 1 to 4 years, and (4) age 5 to 14 years.

The major causes of death in infancy and childhood are cited in Table 10-1 . Congenital anomalies, disorders relating to short gestation (prematurity) and low birth weight, and sudden infant death syndrome (SIDS) represent the leading causes of death in the first 12 months of life. Once the infant survives the first year of life, the outlook brightens measurably. In the next two age groups—1 to 4 years and 5 to 14 years—injuries resulting from accidents have become the leading cause of death (see Table 10-1). Among the natural diseases, in order of importance, congenital anomalies and malignant neoplasms assume major significance. It would appear then that, in a sense, life is an obstacle course. For the great majority, the obstacles are surmounted or, even better, bypassed. We now take a closer look at the specific conditions encountered during the various stages of infant and child development.

Congenital Anomalies

Congenital anomalies are morphologic defects that are present at birth, but some, such as cardiac defects and renal anomalies, may not become clinically apparent until years later. The term *congenital* does not imply or exclude a genetic basis for the birth defect. It is estimated that about 3% of newborns have a *major anomaly*, defined as an anomaly having either cosmetic or functional significance. As indicated in Table 10-1 , they are the most common cause of mortality in the first year of life and contribute significantly to morbidity and mortality throughout the early years of life. In a real sense, anomalies found in live-born infants represent the less serious developmental failures in embryogenesis that are compatible with live birth. Perhaps 20% of fertilized ova are so anomalous that they are blighted from the outset. Others may be compatible with early fetal development, only to lead to spontaneous abortion. Less severe anomalies allow more prolonged intrauterine survival, with some disorders terminating in still-birth and those still less significant permitting live birth despite the handicaps imposed.

DEFINITIONS

Before proceeding, we define some of the terms used for various kinds of errors in morphogenesis—*malformations, disruptions, deformations, sequences*, and *syndromes*.

• *Malformations* represent primary errors of morphogenesis, in other words there is an *intrinsically abnormal developmental process* (Fig. 10-1). They are usually multifactorial rather than the result of a single gene or chromosomal defect. Malformations may present in several patterns. Some, such as congenital heart defects and anencephaly (absence of brain), involve single body systems, whereas in other cases multiple malformations involving many organs may coexist.

• *Disruptions* result from secondary destruction of an organ or body region that was previously normal in development; thus, in contrast to malformations, disruptions arise from an *extrinsic disturbance in morphogenesis. Amniotic*

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bands, denoting rupture of amnion with resultant formation of "bands"that encircle, compress, or attach to parts of the developing fetus, are the classic example of a disruption (Fig. 10-2). A variety of environmental agents may cause disruptions (see below). Understandably, disruptions are not heritable and hence are not associated with risk of recurrence in subsequent pregnancies.

• *Deformations*, like disruptions, also represent an *extrinsic disturbance of development* rather than an intrinsic error of morphogenesis. Deformations are common problems, affecting approximately 2% of newborn infants to varying degrees. Fundamental to the pathogenesis of deformations is localized or generalized compression of the growing fetus by *abnormal biomechanical forces*, leading eventually to a variety of structural abnormalities. The most common underlying factor responsible for deformations is *uterine constraint*. Between the 35th and 38th weeks of gestation, rapid increase in the size of the fetus outpaces the growth of the uterus, and the relative amount of amniotic fluid (which normally acts as a cushion) also decreases. Thus, even the normal fetus is subjected to some form of uterine constraint. Several factors increase the likelihood of excessive compression of the fetus resulting in deformations. *Maternal factors* include first pregnancy, small uterus, malformed (bicornuate) uterus, and leiomyomas. *Fetal or placental factors* include oligohydramnios, multiple fetuses, and abnormal fetal presentation. An example of a deformation is clubfeet, often a component of Potter sequence, described later.

• A *sequence* is a pattern of cascade anomalies. Approximately half the time, congenital anomalies occur singly; in the remaining cases, multiple congenital anomalies are recognized. In some instances, the constellation of anomalies may be explained by a single, localized aberration in organogenesis (malformation, disruption, or deformation) leading to secondary effects in other organs. A good example of a sequence is the *oligohydramnios* (or *Potter*) *sequence* (Fig. 10-3). Oligohydramnios (decreased amniotic fluid) may be caused by a variety of unrelated maternal, placental, or fetal abnormalities. Chronic leakage of amniotic fluid because of rupture of the amnion, uteroplacental

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insufficiency resulting from maternal hypertension or severe toxemia, and renal agenesis in the fetus (as fetal urine is a major constituent of amniotic fluid) are all causes of oligohydramnios. The fetal compression associated with significant oligohydramnios, in turn, results in a classic phenotype in the newborn infant, including flattened facies and positional abnormalities of the hands and feet (Fig. 10-4). The hips may be dislocated. Growth of the chest wall and the contained lungs is also compromised so that the lungs are frequently hypoplastic, occasionally to the degree that they are the cause of fetal demise. Nodules in the amnion *(amnion nodosum)*are frequently present.

• A *syndrome* is a constellation of congenital anomalies, believed to be pathologically related, that, in contrast to a sequence, *cannot* be explained on the basis of a single, localized, initiating defect. Syndromes are most often caused by a single etiologic agent, such as a viral infection or specific chromosomal abnormality, which simultaneously affects several tissues.

TABLE 10-1 -- Cause of Death Related with Age

Causes *	**Rate** †
Under 1 Year: All Causes	**727.4**
Congenital malformations, deformations, and chromosomal anomalies	
Disorders related to short gestation and low birth weight	
Sudden infant death syndrome (SIDS)	
Newborn affected by maternal complications of pregnancy	

Newborn affected by complications of placenta, cord, and membranes	
Respiratory distress of newborn	
Accidents (unintentional injuries)	
Bacterial sepsis of newborn	
Intrauterine hypoxia and birth asphyxia	
Diseases of the circulatory system	
1–4 Years: All Causes	**32.6**
Accidents and adverse effects	
Congenital malformations, deformations, and chromosomal abnormalities	
Malignant neoplasms	
Homicide and legal intervention	
Diseases of the heart ‡	
Influenza and pneumonia	
5–14 Years: All Causes	**18.5**
Accidents and adverse effects	
Malignant neoplasms	
Homicide and legal intervention	
Congenital malformations, deformations, and chromosomal abnormalities	
Suicide	
Diseases of the heart	
15–24 Years: All Causes	**80.7**
Accidents and adverse effects	
Homicide	
Suicide	
Malignant neoplasms	
Diseases of the heart	

*Causes are listed in decreasing order of frequency. All causes and rates are preliminary 2000 statistics. *(Minino AM, Smith BL. Deaths: Preliminary data for 2000. National Vital Statistics Report, 49:12, 2001).*

†Rates are expressed per 100,000 population.

‡Excludes congenital heart disease.

Figure 10-1 Malformations. Human malformations can range in severity from the incidental to the lethal. *Polydactyly* (one or more extra digits) and *syndactyly* (fusion of digits), both of which are illustrated in *A*, have little functional consequence when they occur in isolation. Similarly, *cleft lip (B)*, with or without associated *cleft palate*, is compatible with life when it occurs as an isolated anomaly; in the present case, however, this child had an underlying *malformation syndrome* (trisomy 13) and expired because of severe cardiac defects. The stillbirth illustrated in *C* represents a severe and essentially lethal malformation, where the midface structures are fused or ill-formed; in almost all cases, this degree of external dysmorphogenesis is associated with severe internal anomalies such as maldevelopment of the brain and cardiac defects. *(Pictures A and C courtesy of Dr. Reade Quinton, and B courtesy of Dr. Beverly Rogers, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

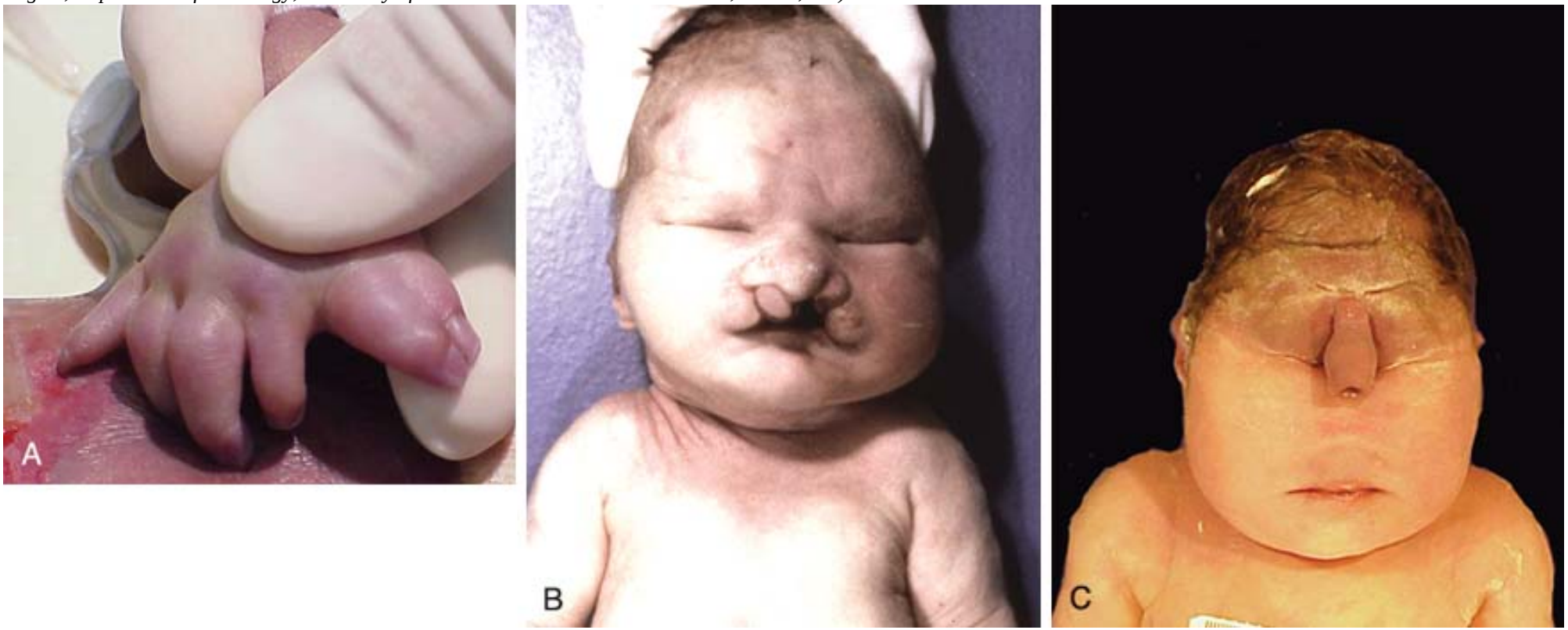

Figure 10-2 Disruption. Disruptions occur in a normally developing organ because of an extrinsic abnormality that interferes with normal morphogenesis. *Amniotic bands* are a frequent cause of disruptions. In the illustrated example, note the placenta at the right of the diagram and the band of amnion extending from the top portion of the amniotic sac to encircle the leg of the fetus. *(Courtesy of Dr. Theonia Boyd, Children's Hospital of Boston, MA.)*

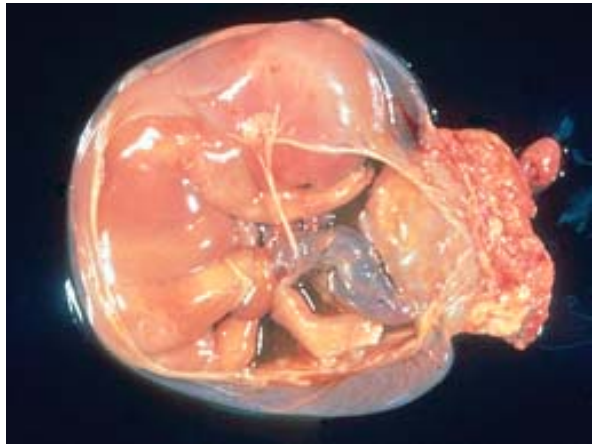

Figure 10-3 Schematic diagram of the pathogenesis of the oligohydramnios sequence.

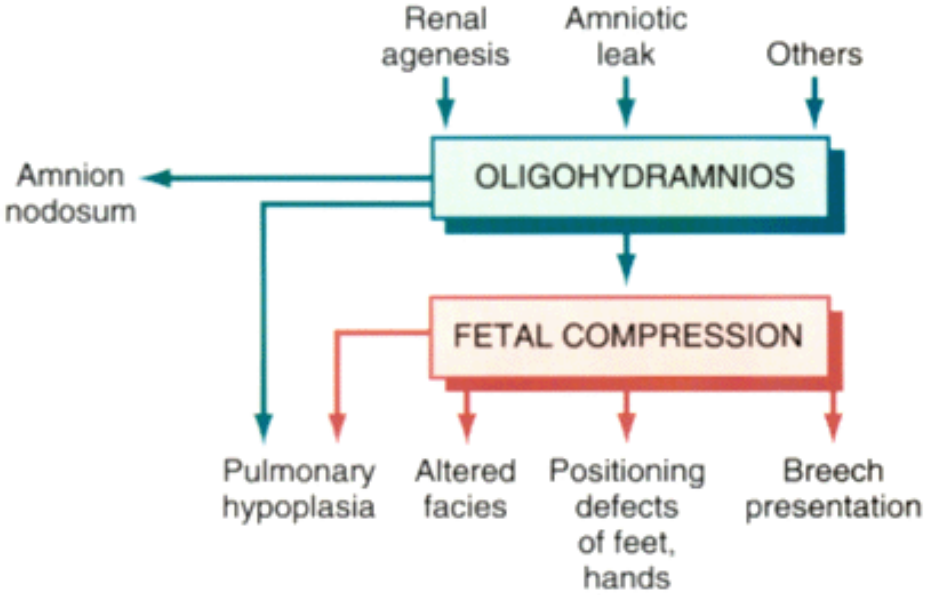

Figure 10-4 Infant with oligohydramnios sequence. Note the flattened facial features and deformed right foot (talipes equinovarus).

TABLE 10-2 -- Causes of Congenital Anomalies in Humans

Cause	Frequency (%)
Genetic	
Chromosomal aberrations	10–15
Mendelian inheritance	•2–10
Environmental	
Maternal/placental infections	•2–3
••Rubella	

••Toxoplasmosis	
••Syphilis	
••Cytomegalovirus	
••Human immunodeficiency virus (HIV)	
Maternal disease states	•6–8
••Diabetes	
••Phenylketonuria	
••Endocrinopathies	
Drugs and chemicals	•1
••Alcohol	
••Folic acid antagonists	
••Androgens	
••Phenytoin	
••Thalidomide	
••Warfarin	
••13-*cis*-retinoic acid	
••Others	
Irradiations	•1
Multifactorial (Multiple Genes ? Environment)	**20–25**
Unknown	**40–60**
Adapted from Stevenson RE, et al (eds): Human Malformations and Related Anomalies. New York, Oxford University Press, 1993, p. 115.	

Single gene mutations of large effect may underlie major congenital anomalies, which, as expected, follow mendelian patterns of inheritance.[2] Of these, approximately 90% are inherited in an autosomal dominant or recessive pattern, while the remainder segregates in an X-linked pattern. Not surprisingly, many of the mutations that give rise to birth defects involve abrogation of function of genes involved in normal organogenesis and development. For example, holoprosencephaly is the most common developmental defect of the forebrain and midface in humans (see Chapter 28); mutations of *sonic hedgehog*, a gene involved in developmental patterning (see below), have been reported in a subset of patients with holoprosencephaly.[3] Similarly, mutations of a downstream target of sonic hedgehog signaling, *GLI3*, have been reported in patients with anomalies of digits, either conjoined digits *(syndactyly)* or supernumerary digits *(polydactyly)*.

Environmental Causes

Environmental influences, such as viral infections, drugs, and irradiation, to which the mother was exposed during pregnancy may cause fetal malformations (the appellation of "malformation" is loosely used in this context, since technically, these anomalies represent *disruptions*).

Viruses.

Many viruses have been implicated in causing malformations, including the agents responsible for rubella, cytomegalic inclusion disease, herpes simplex, varicella-zoster infection, influenza, mumps, human immunodeficiency virus (HIV), and enterovirus infections. Among these, the rubella virus and cytomegalovirus are the most extensively investigated. With all viruses, the gestational age at which the infection occurs in the mother is critically important. *The at-risk period for rubella infection extends from shortly before conception to the 16th week of gestation*, the hazard being greater in the first 8 weeks than in the second 8 weeks.[4] The incidence of malformations is reduced from 50% to 20% to 7% if infection occurs in the first, second, or third month of gestation. The fetal defects are varied, but the major tetrad comprises cataracts, heart defects (persistent ductus arteriosus, pulmonary artery hypoplasia or stenosis, ventricular septal defect, tetralogy of Fallot), deafness, and mental retardation, referred to as *rubella embryopathy*.

Intrauterine infection with cytomegalovirus, mostly asymptomatic, is the most common fetal viral infection. This viral disease is considered in detail in Chapter 8 ; *the highest at-risk period is the second trimester of pregnancy*. Because organogenesis is largely completed by the end of the first trimester, congenital malformations occur less frequently than in rubella; nevertheless, the effects of virus-induced injury on the formed organs are often severe. Involvement of the central nervous system is a major feature, and the most prominent clinical changes are mental retardation, microcephaly, deafness, and hepatosplenomegaly.

Drugs and Other Chemicals.

A variety of drugs and chemicals have been suspected to be teratogenic, but perhaps less than 1% of congenital malformations are caused by these agents. The list includes thalidomide, folate antagonists, androgenic hormones, alcohol, anticonvulsants, warfarin (oral anticoagulant), and 13-*cis*-retinoic acid used in the treatment of severe acne.[5] For example, *thalidomide*, once used as a tranquilizer in Europe, caused an extremely high frequency (50% to 80%) of limb abnormalities in exposed fetuses.[6] *Alcohol*, perhaps the most widely used agent today, is a teratogen. Affected infants show growth retardation, microcephaly, atrial septal defect, short palpebral fissures, maxillary hypoplasia, and several other minor anomalies. These together are labeled the *fetal alcohol syndrome*.[7] While cigarette smoke-derived nicotine has not been convincingly demonstrated to be a teratogen, there is a high incidence of spontaneous abortions, premature labor, and placental abnormalities in pregnant smokers; babies born to smoking mothers often have a low birth weight and may be prone to sudden infant death syndrome (see later). *In light of these findings, it is best to avoid nicotine exposure altogether during pregnancy.*

Radiation.

In addition to being mutagenic and carcinogenic, radiation is teratogenic. Exposure to heavy doses of radiation during the period of organogenesis leads to malformations, such as microcephaly, blindness, skull defects, spina bifida, and other deformities. Such exposure occurred in the past when radiation was used to treat cervical cancer.

Maternal Diabetes.

Among maternal conditions listed in Table 10-2 , diabetes mellitus is a common entity, and despite advances in antenatal obstetric monitoring and glucose

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control, the incidence of major malformations in infants of diabetic mothers stands between 6% and 10% in most series. Maternal hyperglycemia-induced fetal hyperinsulinemia results in increased body fat, muscle mass, and organomegaly *(fetal macrosomia)*; cardiac anomalies, neural tube defects, and other central nervous system malformations are some of the major anomalies seen in *diabetic embryopathy*.[8]

Multifactorial Causes

The genetic and environmental factors just discussed account for no more than half of human congenital anomalies. The causes of the vast majority of birth defects, including some relatively common disorders such as cleft lip and cleft palate, remain unknown. In these anomalies, it would appear that inheritance of a certain number of mutant genes and their interaction with the environment is required before the disorder is expressed. In the case of congenital dislocation of the hip, for example, depth of the acetabular socket and laxity of the ligaments are believed to be genetically determined, whereas a significant environmental factor is believed to be frank breech position in utero, with hips flexed and knees extended. The importance of environmental contribution to multifactorial inheritance is underscored by a dramatic reduction in the incidence of neural tube defects by periconceptional intake of folic acid in the diet.[9] [10] The approximate frequency of some common congenital anomalies in the United States is presented in Table 10-3 . Both temporal and regional variability are common in the reporting of many malformations. For example, between 1979 and 1989, there was a mean annual percent decrease in the incidence of anencephaly of 6.4 and a mean annual increase in the incidence of atrial septal defect of 22.0.[11]

PATHOGENESIS OF CONGENITAL ANOMALIES

The pathogenesis of congenital anomalies is complex and still poorly understood, but certain general principles of

TABLE 10-3 -- Approximate Frequency of the More Common Congenital Malformations in the United States

Malformation	Frequency per 10,000 Total Births
Clubfoot without central nervous system anomalies	25.7
Patent ductus arteriosus	16.9
Ventricular septal defect	10.9
Cleft lip with or without cleft palate	•9.1
Spina bifida without anencephalus	•5.5
Congenital hydrocephalus without anencephalus	•4.8
Anencephalus	•3.9
Reduction deformity (musculoskeletal)	•3.5
Rectal and intestinal atresia	•3.4

Adapted from James LM: Maps of birth defects occurrence in the U.S., birth defects monitoring program (BDMP)/CPHA, 1970–1987. Teratology 48:551, 1993.

developmental pathology are relevant regardless of the etiologic agent.

The timing of the prenatal teratogenic insult has an important impact on the occurrence and the type of anomaly produced (Fig. 10-5). The intrauterine development of humans can be divided into two phases: (1) the embryonic period occupying the first 9 weeks of pregnancy and (2) the fetal period terminating at birth.

In the *early embryonic period* (first 3 weeks after fertilization), an injurious agent damages either enough cells to cause death and abortion or only a few cells, presumably allowing the embryo to recover without developing defects. *Between the third and the ninth weeks, the embryo is extremely susceptible to teratogenesis,* and the peak sensitivity during this period occurs between the fourth and the fifth weeks. During this period, organs are being crafted out of the germ cell layers. The *fetal period* that follows organogenesis is marked chiefly by the further growth and maturation of the organs, with greatly reduced susceptibility to teratogenic agents. Instead the fetus is susceptible to growth retardation or injury to already formed

organs. It is therefore possible for a given agent to produce different anomalies if exposure occurs at different times of gestation.

Teratogens and genetic defects may act at several steps involved in normal morphogenesis. These include the following: [12]

- Proper *cell migration* to predetermined locations that influence the development of other structures
- *Cell proliferation*, which determines the size and form of embryonic organs
- *Cellular interactions* among tissues derived from different structures (e.g., ectoderm, mesoderm), which affect the differentiation of one or both of these tissues
- *Cell-matrix associations*, which affect growth and differentiation
- *Programmed cell death (apoptosis)*, which, as we have seen, allows orderly organization of tissues and organs during embryogenesis (Chapter 1)
- *Hormonal influences* and *mechanical forces*, which affect morphogenesis at many levels.

The complex interplay between environmental teratogens and intrinsic genetic defects is underscored by the fact that features of dysmorphogenesis caused by environmental insults can be recapitulated by certain genetic defects. This is exemplified in the relationship between the teratogen, retinoic acid (see below and Fig. 10-6), and two growth factors—transforming growth factor (TGF) and fibroblast growth factor (FGF)—both involved in morphogenesis. As discussed later, retinoic acid can induce defects in palatal development *(cleft lip and cleft palate)*, possibly by impacting on multiple targets associated with secondary palatal development. In experimental models of retinoic acid teratogenesis, abnormal expression of TGF and FGF has been reported in the developing palate.[13] [14] Not unexpectedly, therefore, rare single gene mutations in one or more of these growth factors or their receptors may also cause palatal abnormalities. There is an association, for example, between rare mutations of the *TGF-α* gene and nonsyndromic cleft lip or cleft palate in humans;[15] in addition, loss of function of the epidermal growth factor receptor, which acts as a receptor for TGF-α, can result in abnormal palatogenesis.[16] Disruption of TGF-β3 in mice also results in cleft palate.[17]

Figure 10-5 Critical periods of development for various organ systems and the resultant malformations. *(Modified and redrawn from Moore KL: The Developing Human, 5th ed. Philadelphia, WB Saunders, 1993, p. 156.)*

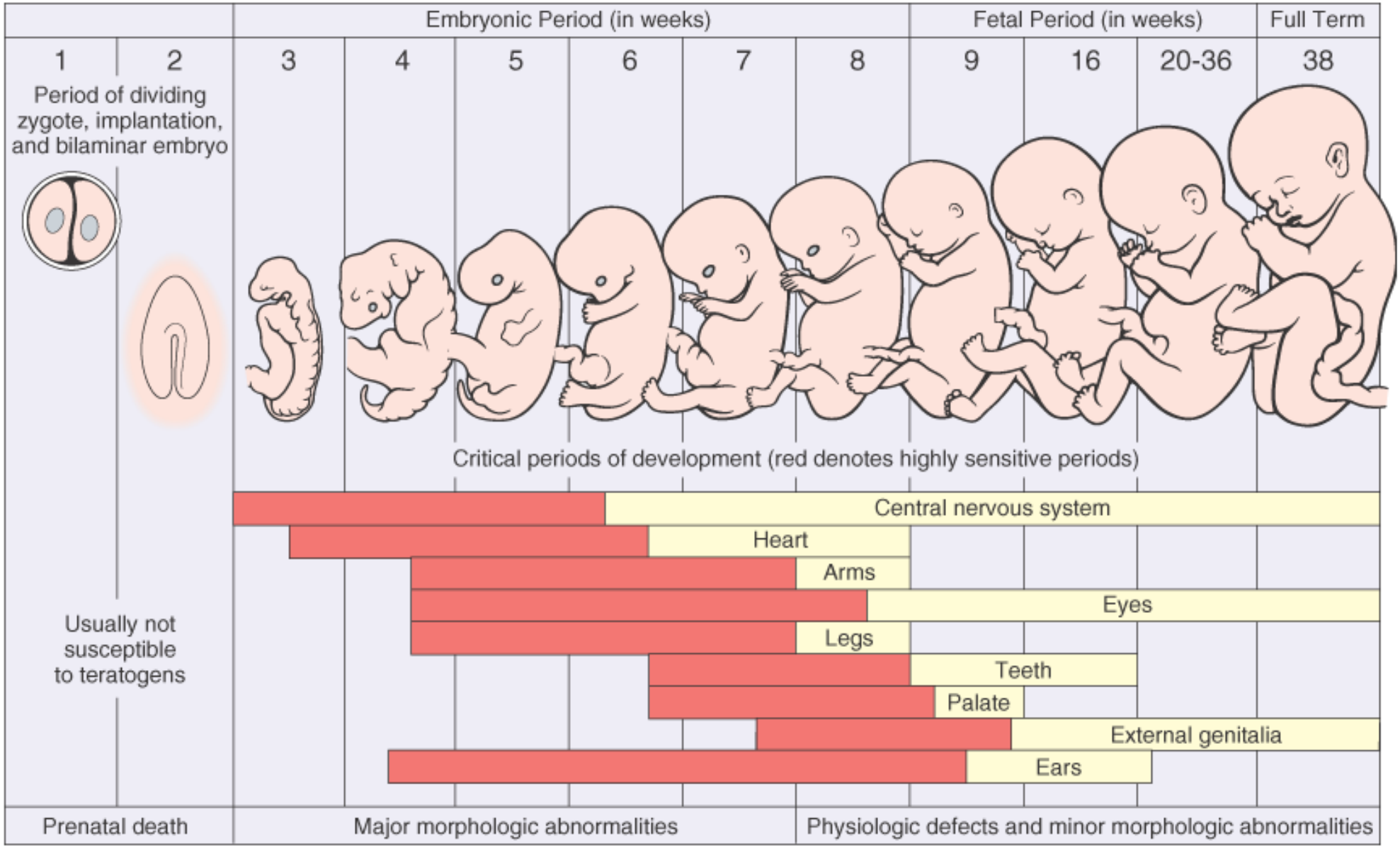


Figure 10-6 Schematic representation of the postulated role of retinoic acid in normal development, the general features of its deficiency (vitamin A deficiency) *(left)* and retinoic acid embryopathy *(right)*. *1*, Retinol in the maternal circulation is bound by retinol-binding protein (RBP), which is synthesized by the placenta and enters the fetal circulation. *2*, Once in fetal cells, retinol is bound by cytoplasmic retinol-binding protein (CRBP), which *(3)* regulates the conversion to retinoic acid and metabolites. The retinoic acid either remains in the cytoplasm (bound to cytoplasmic/cellular retinoic acid-binding protein *[CRABP])* or *(4)* enters the nucleus, where it is bound to nuclear retinoic acid receptors *(RAR, RXR)*. The retinoic acid-receptor complex acts as a transcriptional regulator of various patterning genes (e.g., *HOX*) that have the appropriate retinoic acid response element *(RARE)*. Expression of the binding proteins and receptors in various tissues and at various times during embryogenesis may be a mechanism of selectively modulating the action of retinoic acid. This differential expression may also explain the pattern of abnormalities seen in vitamin A deficiency and retinoic acid embryopathy.

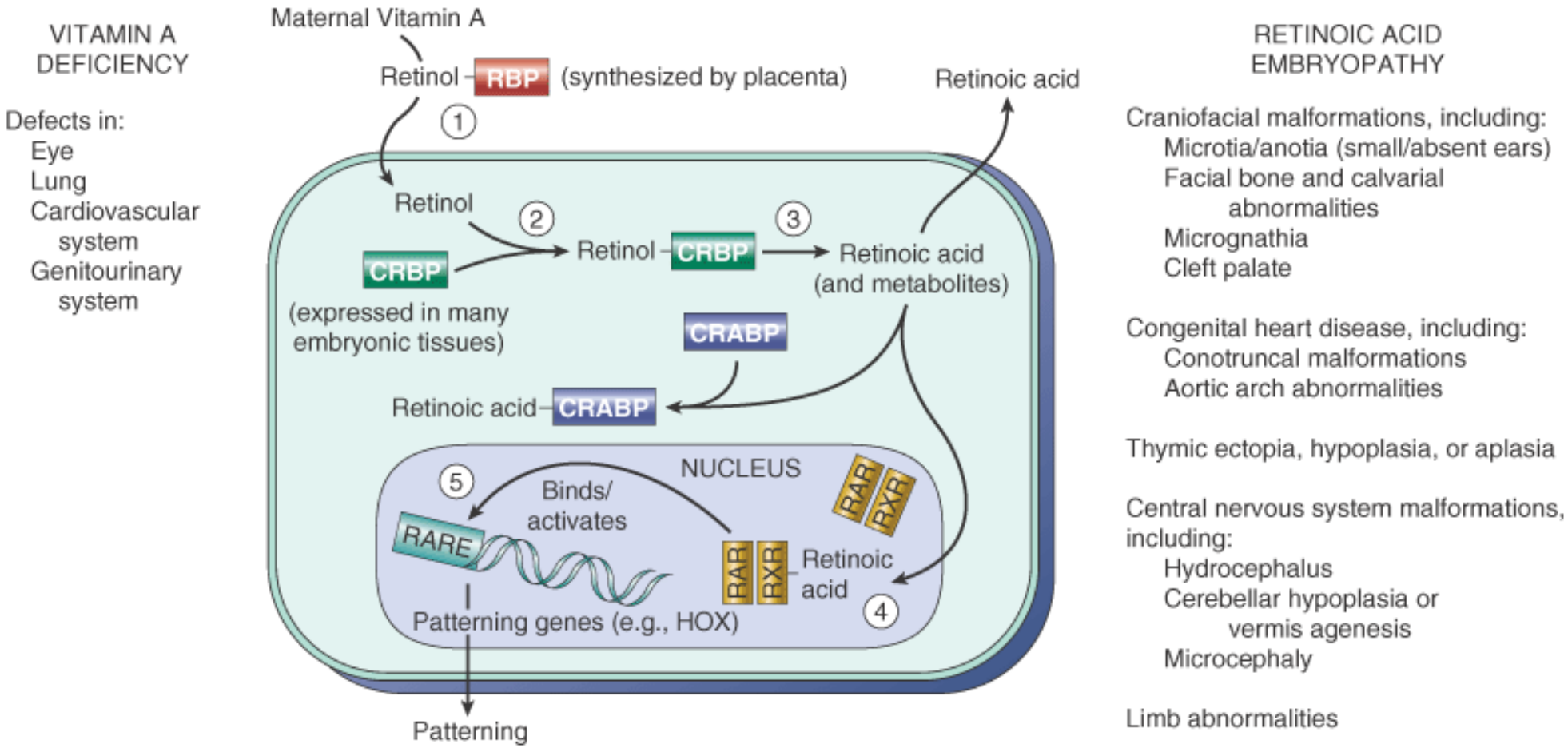


Figure 10-7 Diagrammatic representation of constitutional chromosomal mosaicism. *A*, Generalized. *B*, Confined to the placenta. *C*, Confined to the embryo. *(Modified and redrawn from Kalousek DK: Confined placental mosaicism and intrauterine development. Pediatr Pathol 10:69, 1990.)*

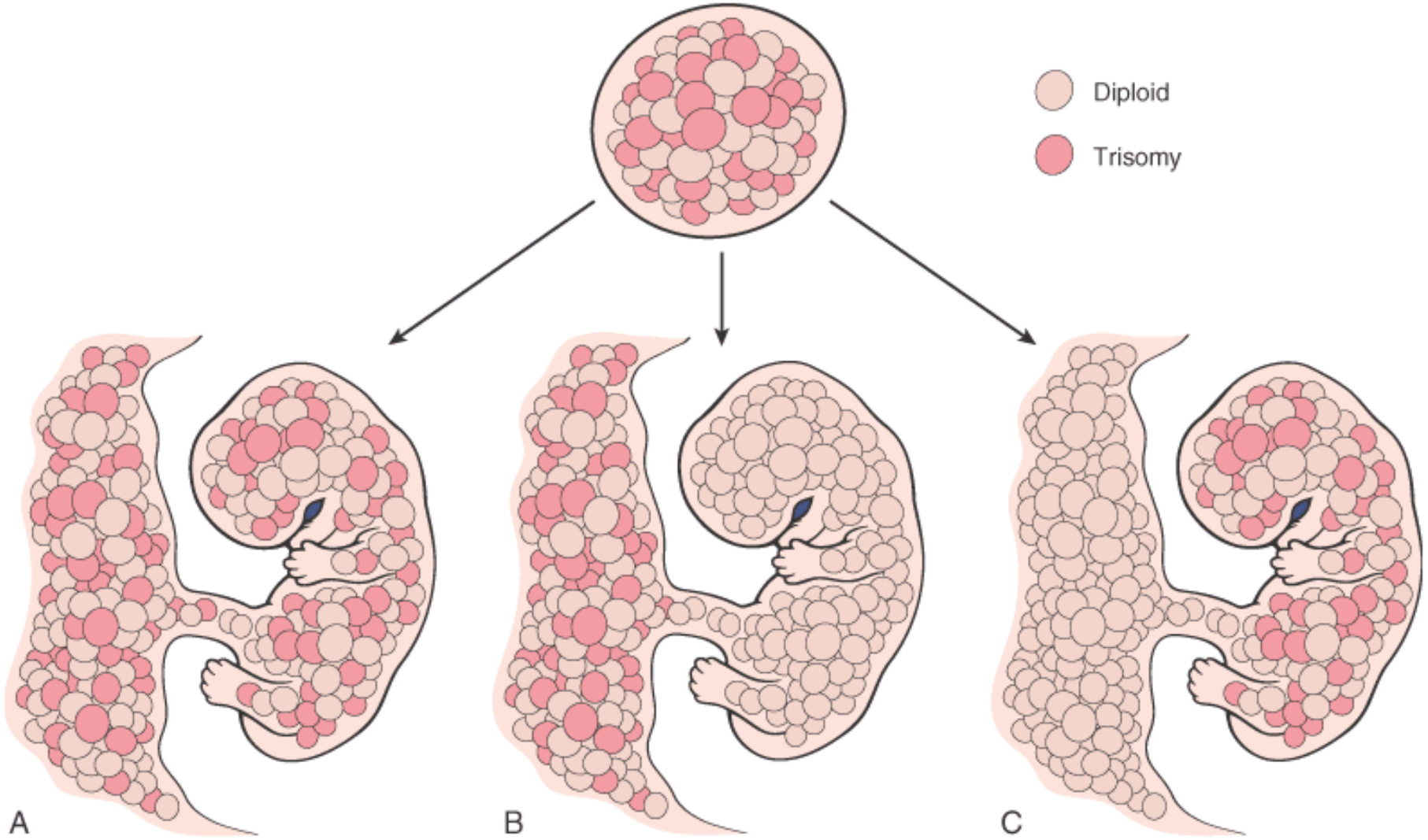
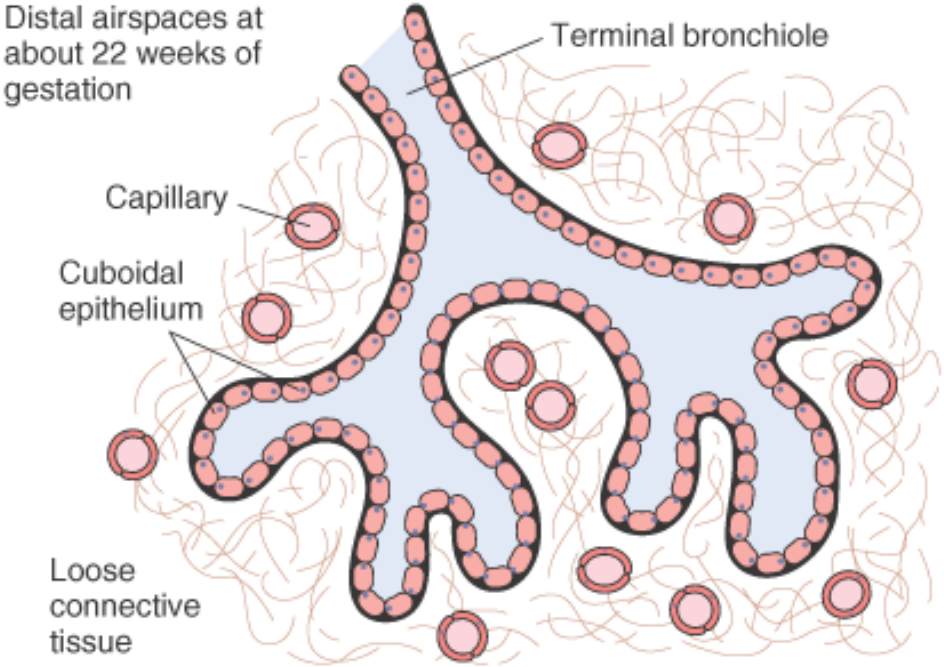


Figure 10-8 Schematic diagrams of fetal lung maturation.



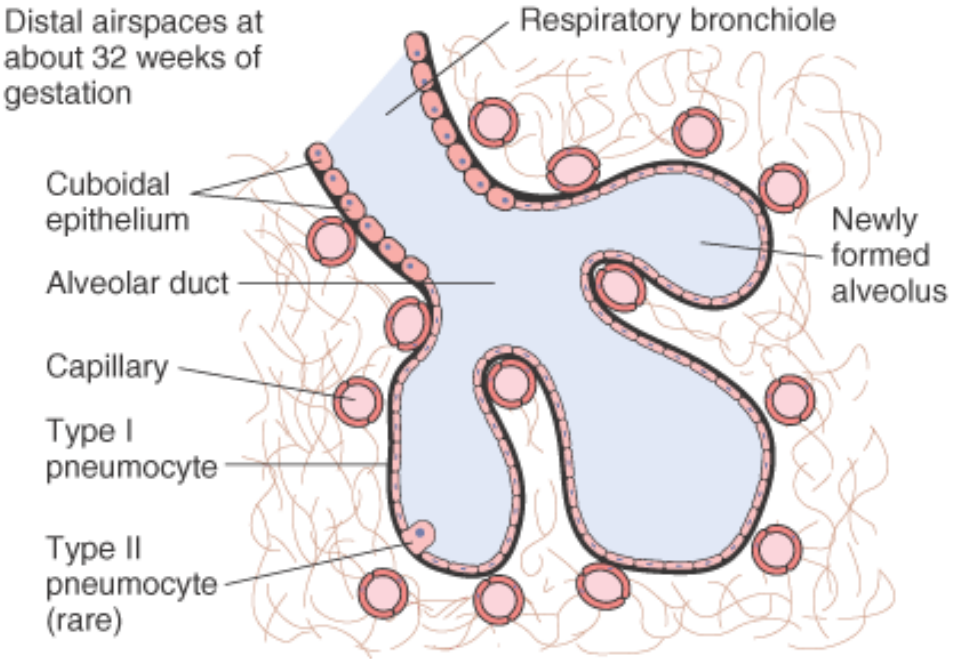


TABLE 10-4 -- Evaluation of the Newborn Infant *

Sign	0	1	2
Heart rate	Absent	Below 100	Over 100
Respiratory effort	Absent	Slow, irregular	Good, crying
Muscle tone	Limp	Some flexion of extremities	Active motion
Response to catheter in nostril (tested after oropharynx is clear)	No response	Grimace	Cough or sneeze
Color	Blue, pale	Body pink, extremities blue	Completely pink

Data from Apgar V: A proposal for a new method of evaluation of the newborn infant. Anesth Analg 32:260, 1953.

*Sixty seconds after the complete birth of the infant (disregarding removal of the cord and placenta), the five objective signs are evaluated and each is given a score of 0, 1, or 2. A total score of 10 indicates an infant in the best possible condition.

risk for birth injury, in particular those involving the skeletal system and peripheral nerves. We briefly discuss only injuries involving the head because they are the most ominous.

Intracranial hemorrhages are the most common important birth injury. These hemorrhages are generally related to excessive molding of the head or sudden pressure changes in its shape as it is subjected to the pressure of forceps or sudden precipitate expulsion. Prolonged labor, hypoxia, hemorrhagic disorders, or intracranial vascular anomalies are important predispositions. The hemorrhage may arise from tears in the dura or from rupture of vessels that traverse the brain. The substance of the brain may be torn or bruised, leading to intraventricular hemorrhages or bleeding into the brain substance. The consequences of intracranial hemorrhages are mentioned later under germinal matrix hemorrhage.

Caput succedaneum and *cephalhematoma* are so common, even in normal uncomplicated births, that they hardly merit the designation *birth injury*. The first refers to progressive accumulation of interstitial fluid in the soft tissues of the scalp, giving rise to a usually circular area of edema, congestion, and swelling at the site where the head begins to enter the lower uterine canal. Hemorrhage may occur into the scalp, producing a cephalhematoma. Both forms of injury are of little clinical significance and are important only insofar as they must be differentiated from skull fractures with attendant hemorrhage and edema. In approximately 25% of cephalhematomas, there is an underlying skull fracture. Such skull fractures may occur in cases of precipitate delivery, inappropriate use of forceps, or prolonged labor with disproportion between the size of the fetal head and birth canal.

Perinatal Infections

Infections of the embryo, fetus, and neonate are manifested in a variety of ways and are mentioned as etiologic factors in numerous other sections within this chapter. Here we discuss only the general routes and timing of infections. In general, fetal and perinatal infections are acquired via one of two primary routes—*transcervically* (also referred to as *ascending*) or *transplacentally (hematologic)*. Occasionally, infections occur by a combination of the two routes in that an ascending microorganism infects the endometrium and then the fetal bloodstream via the chorionic villi.

TRANSCERVICAL (ASCENDING) INFECTIONS

Most bacterial and a few viral (e.g., herpes simplex II) infections are acquired by the cervicovaginal route. Such infections may be acquired in utero or around the time of birth. In general,

the fetus acquires the infection either by inhaling infected amniotic fluid into the lungs shortly before birth or by passing through an infected birth canal during delivery. As previously stated, preterm birth is often an unfortunate consequence and may be related either to damage and rupture of the amniotic sac as a direct consequence of the inflammation or to the induction of labor associated with a release of prostaglandins by the infiltrating neutrophils. Chorioamnionitis of the placental membranes and funisitis are usually demonstrable, although the presence or absence and severity of chorioamnionitis do not necessarily correlate with the severity of the fetal infection. In the fetus infected via inhalation of amniotic fluid, pneumonia, sepsis, and meningitis are the most common sequelae.

TRANSPLACENTAL (HEMATOLOGIC) INFECTIONS

Most parasitic (e.g., toxoplasma, malaria) and viral infections and a few bacterial infections (i.e., *Listeria, Treponema*) gain access to the fetal bloodstream transplacentally via the chorionic villi. This hematogenous transmission may occur at any time during gestation or occasionally, as may be the case with hepatitis B and HIV, at the time of delivery via maternal-to-fetal transfusion. The clinical manifestations of these infections are highly variable, depending largely on the gestational timing and microorganism involved.

Some infections, such as those with *parvovirus B19* (which causes *fifth disease* in the mother), may induce spontaneous abortion, stillbirth, hydrops fetalis, and congenital anemia.[40] While the virus can bind to different cell types, replication occurs only in erythroid cells, and diagnostic viral cytopathic effect can be recognized in late erythroid progenitor cells of infected infants (Fig. 10-9).

The *TORCH group of infections* (see above) are grouped together because they may evoke similar clinical and pathologic manifestations, including *fever*, *encephalitis*, *chorioretinitis*, *hepatosplenomegaly*, *pneumonitis*, *myocarditis*, *hemolytic anemia*, *and vesicular or hemorrhagic skin lesions*. Such infections occurring early in gestation may also cause chronic sequelae in the child, including growth and mental retardation, cataracts, congenital cardiac anomalies, and bone defects.

ONSET OF SEPSIS

Perinatal infections can also be grouped clinically by whether they tend to result in *early-onset* (within the first 7 days of life) versus *late-onset* sepsis (from 7 days to 3 months). Most cases of early-onset sepsis are acquired at or shortly before birth and tend to result in clinical signs and symptoms of pneumonia, sepsis, and occasionally meningitis within 4 or 5 days of life. Group B streptococcus is the most common

Figure 10-9 Bone marrow from an infant infected with parvovirus B19. The arrows point to two erythroid precursors with large homogeneous intranuclear inclusions and a surrounding peripheral rim of residual chromatin.

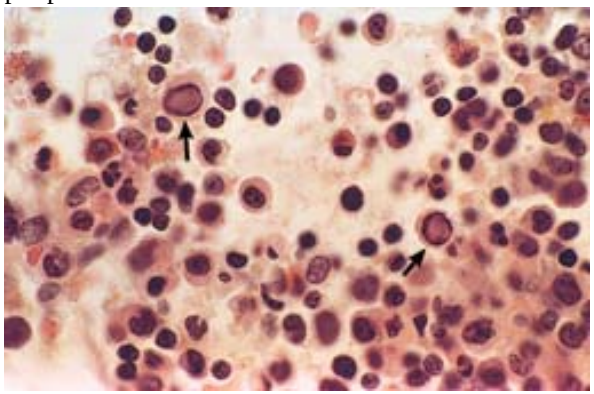


Figure 10-10 Schematic outline of the pathophysiology of the respiratory distress syndrome (see text).

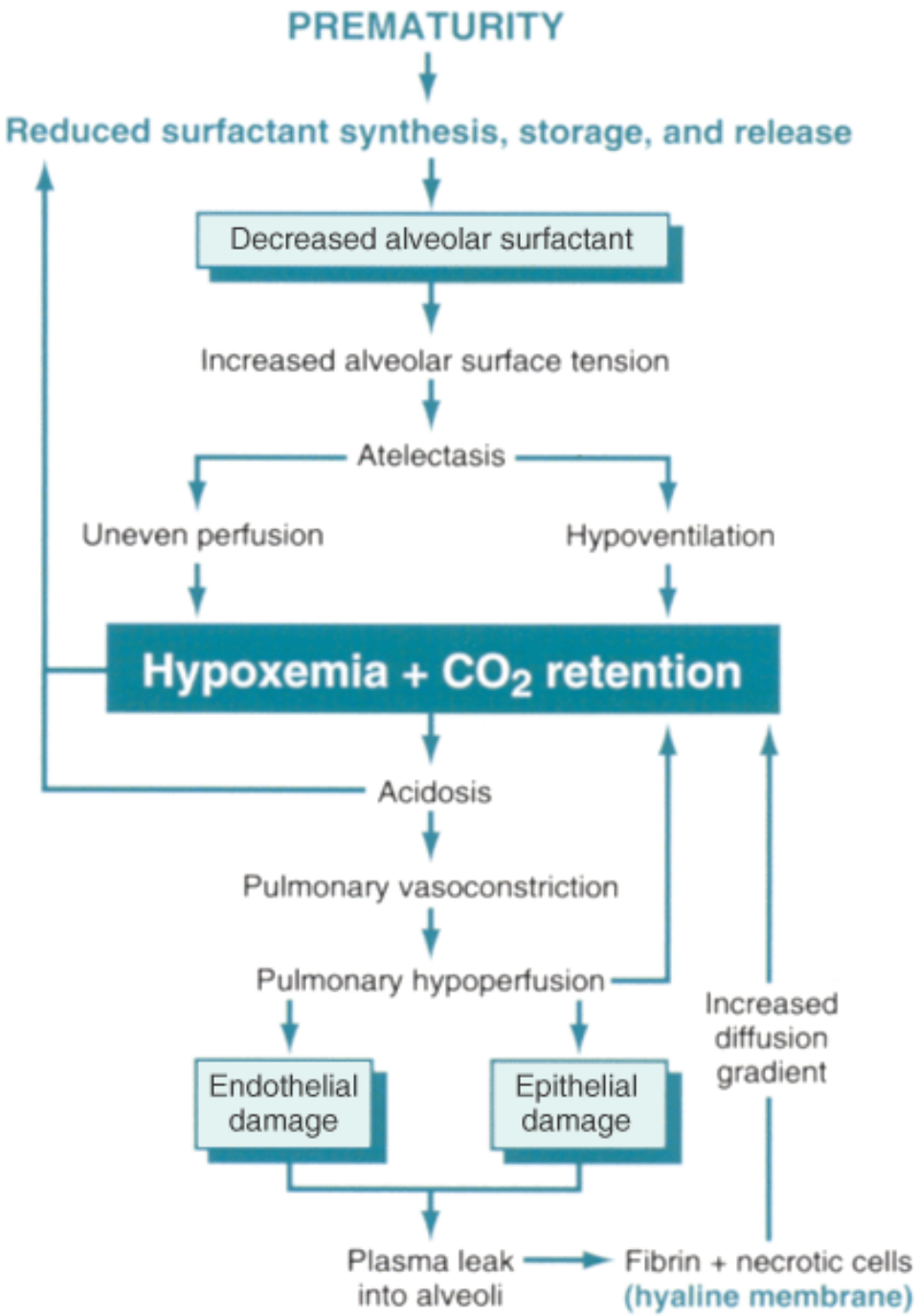


Figure 10-11 Hyaline membrane disease. There is alternating atelectasis and dilation of the alveoli. Note the eosinophilic thick hyaline membranes lining the dilated alveoli.

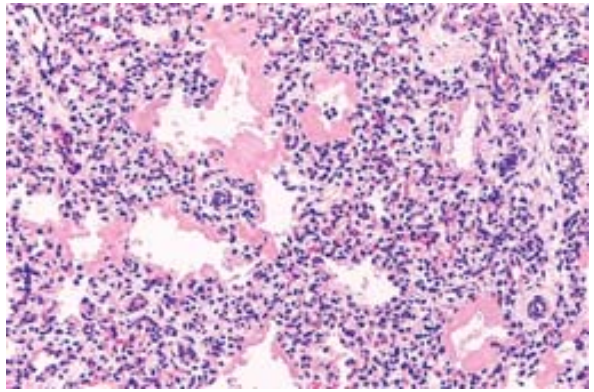


Figure 10-12 Necrotizing enterocolitis. *A,* Postmortem examination in a severe case of NEC shows the entire small bowel is markedly distended with a perilously thin wall (usually this implies impending perforation). *B,* The congested portion of the ileum corresponds to areas of hemorrhagic infarction and transmural necrosis microscopically. Submucosal gas bubbles *(pneumatosis intestinalis)* can be seen in several areas *(arrows).*

Figure 10-13 Hydrops fetalis. There is generalized accumulation of fluid in the fetus. In *B,* fluid accumulation is particularly prominent in the soft tissues of the neck, and this condition has been termed *cystic hygroma*. Cystic hygromas are characteristically seen, but not limited to, constitutional chromosomal anomalies such as 45,X0 karyotypes. *(Courtesy of Dr. Beverly Rogers, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

TABLE 10-5 -- Selected Causes of Hydrops Fetalis (in decreasing order of frequency)

Cardiovascular
Malformations
Tachyarrhythmia
High-output failure
Chromosomal
Turner syndrome
Trisomy 21, trisomy 18
Thoracic Causes
Cystic adenomatoid malformation

Diaphragmatic hernia
Fetal Anemia
Homozygous alpha-thalassemia
Parvovirus B19
Immune hydrops (Rh and ABO)
Twin Gestation
Twin-to-twin transfusion
Infection (excluding parvovirus)
Cytomegalovirus
Syphilis
Toxoplasmosis
Major Malformations
Tumors
Metabolic disorders
Note: The cause of fetal hydrops may be undetermined ("idiopathic") in up to 20% of cases. *Data from Machin GA: Hydrops, cystic hygroma, hydrothorax, pericardial effusions, and fetal ascites, In Gilbert-Barness E (ed): Potter's Pathology of Fetus and Infant. St. Louis, Mosby-Year Book, 1997.*

IMMUNE HYDROPS

Immune hydrops is defined as a hemolytic disease in the newborn caused by blood-group incompatibility between mother and child. When the fetus inherits red cell antigenic determinants from the father that are foreign to the mother, a maternal immune reaction may occur, leading to hemolytic disease in the infant. Any of the numerous red cell antigenic systems may theoretically be involved, but the major antigens known to induce clinically significant immunologic disease are the ABO and certain of the Rh antigens. The incidence of immune hydrops in urban populations has declined remarkably, owing largely to the current methods of preventing Rh immunization in at-risk mothers. Successful prophylaxis of this disorder has resulted directly from an understanding of its pathogenesis.

Etiology and Pathogenesis.

The underlying basis of immune hydrops is the immunization of the mother by blood group antigens on fetal red cells and the free passage of antibodies from the mother through the placenta to the fetus (Fig. 10-14). Fetal red cells may reach the maternal circulation during the last trimester of pregnancy, when the cytotrophoblast is no longer present as a barrier, or during childbirth itself. The mother thus becomes sensitized to the foreign antigen.

Of the numerous antigens included in the Rh system, only the D antigen is the major cause of Rh incompatibility. Several

Figure 10-14 Pathogenesis of immune hydrops fetalis (see text).

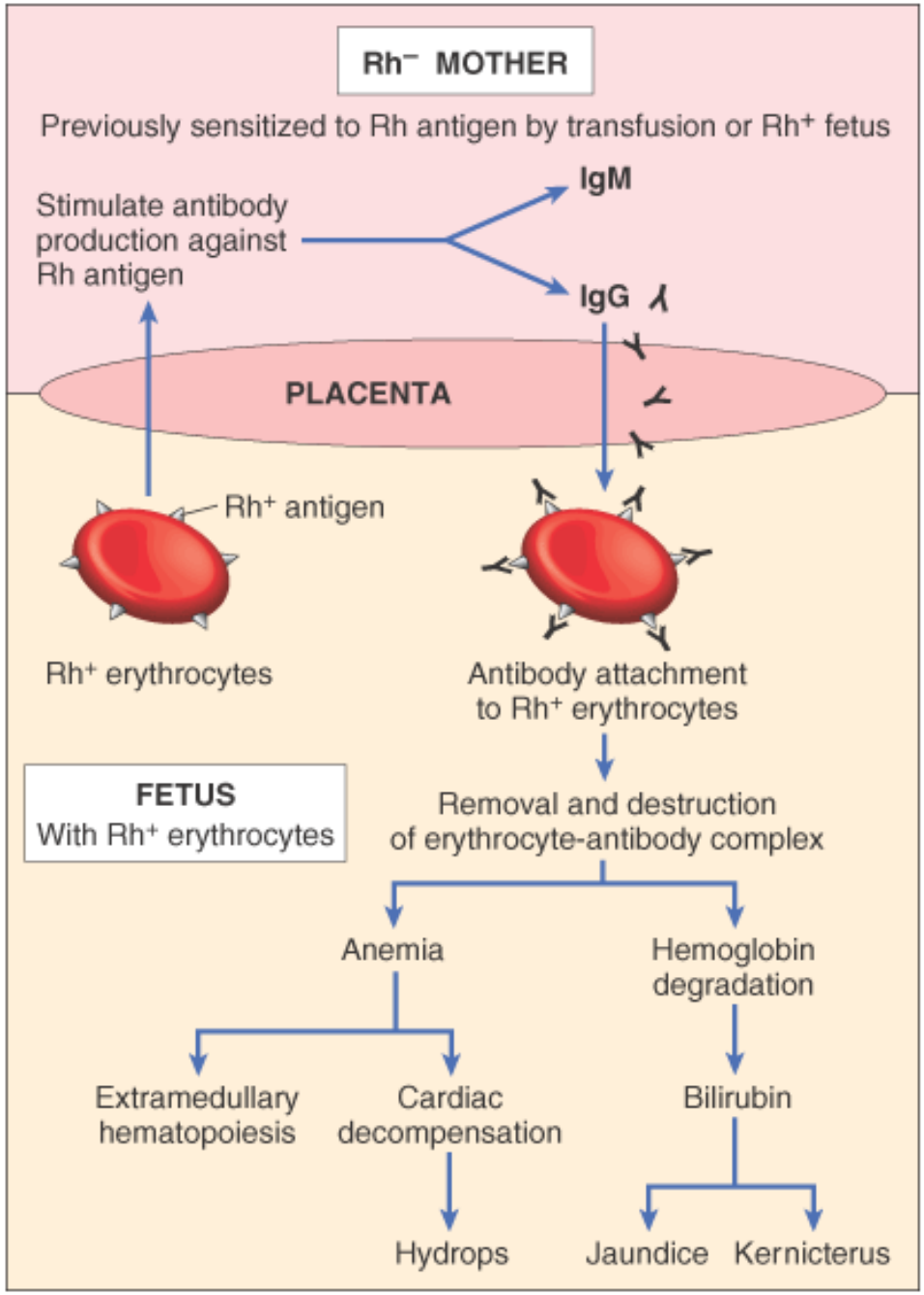


Figure 10-15 Numerous islands of extramedullary hematopoiesis (small blue cells) are scattered among mature hepatocytes in this infant with nonimmune hydrops fetalis.

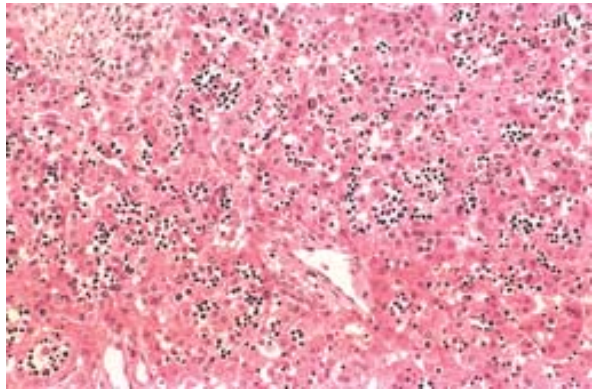


Figure 10-16 Kernicterus. Severe hyperbilirubinemia in the neonatal period, for example, secondary to immune hemolysis, results in deposition of bilirubin pigment in the brain parenchyma. This occurs because the blood-brain barrier is less well developed in the neonatal period than it is in adulthood. Infants who survive develop long-term neurologic sequelae.

TABLE 10-6 -- Abnormalities Suggesting Inborn Errors of Metabolism

General
Dysmorphic features

Deafness
Self-mutilation
Abnormal hair
Abnormal body or urine odor ("sweaty feet"; "mousy or musty"; "maple syrup")
Hepatosplenomegaly; cardiomegaly
Hydrops
Neurologic
Hypotonia or hypertonia
Coma
Persistent lethargy
Seizures
Gastrointestinal
Poor feeding
Recurrent vomiting
Jaundice
Eyes
Cataract
Cherry red macula
Dislocated lens
Glaucoma
Muscle, Joints
Myopathy
Abnormal mobility
Adapted from Barness LA and Gilbert-Barness E: Metabolic diseases, In Gilbert-Barness E (ed): Potter's Pathology of Fetus and Infant. St. Louis, Mosby-Year Book, 1997.

Homozygotes with this autosomal recessive disorder classically have a severe deficiency of phenylalanine hydroxylase, leading to hyperphenylalaninemia and its pathologic consequences. Affected infants are normal at birth but within a few weeks develop a rising plasma phenylalanine level, which in some way impairs brain development. Usually by 6 months of life *severe mental retardation* becomes evident; fewer than 4% of untreated PKU children have intelligence quotient values greater than 50 or 60. About one third of these children are never able to walk, and two thirds cannot talk. *Seizures*, other neurologic abnormalities, *decreased pigmentation of hair* and skin, and eczema often accompany the mental retardation in untreated children. Hyperphenylalaninemia and the resultant mental retardation can be avoided by restriction of phenylalanine intake early in life. Hence, a number of screening procedures are routinely used for detection of PKU in the immediate postnatal period.

Many clinically normal female PKU patients who are treated with dietary control early in life reach childbearing age. Most of them discontinue dietary treatment and have marked hyperphenylalaninemia. Between 75% and 90% of children born to such women are mentally retarded and

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microcephalic, and 15% have congenital heart disease, even though the infants themselves are heterozygotes. This syndrome, termed *maternal PKU*, results from the teratogenic effects of phenylalanine or its metabolites that cross the placenta and affect specific fetal organs during development.[62] The presence and severity of the fetal anomalies directly correlate with the maternal phenylalanine level, so *it is imperative that maternal dietary restriction of phenylalanine is initiated before conception and continues throughout the pregnancy*.

The biochemical abnormality in PKU is an inability to convert phenylalanine into tyrosine. In normal children, less than 50% of the dietary intake of phenylalanine is necessary for protein synthesis. The rest is irreversibly converted to tyrosine by a complex *hepatic phenylalanine hydroxylase system* (Fig. 10-17), which, in addition to the enzyme *phenylalanine hydroxylase*, has two other components: the cofactor *tetrahydrobiopterin (BH_4)* and the enzyme *dihydropteridine reductase*, which regenerates BH_4 . Although neonatal hyperphenylalaninemia can be caused by deficiencies in any of these components, 98% to 99% of cases are attributable to abnormalities in phenylalanine hydroxylase. With a block in phenylalanine metabolism owing to lack of phenylalanine hydroxylase, minor shunt pathways come into play, yielding phenylpyruvic acid, phenyllactic acid, phenylacetic acid, and *o*-hydroxyphenylacetic acid, which are excreted in large amounts in the urine in PKU. Some of these abnormal metabolites are excreted in the sweat, and phenylacetic acid in particular imparts a strong *musty* or *mousy odor* to affected infants. It is believed that excess phenylalanine or its metabolites contribute to the brain damage in PKU.

At the molecular level, several mutant alleles of the phenylalanine hydroxylase gene have been identified. Each mutation induces a particular alteration in the enzyme resulting in a corresponding quantitative effect on residual enzyme activity ranging from complete absence to 50% of normal values. The degree of hyperphenylalaninemia and clinical phenotype is inversely related to the amount of residual enzyme activity. Infants with mutations resulting in a lack of phenylalanine hydroxylase activity present with the classic features of PKU, while those with up to 6% residual activity present with milder disease. Moreover, some mutations result in only modest elevations of phenylalanine levels, and the affected children have no neurologic damage. This latter condition, referred to as *benign hyperphenylalaninemia*, or *mild PKU*, is important to recognize because the individuals may well test *positive* in screening tests but do not develop the stigmata of classic PKU.[63] Measurement of serum phenylalanine levels differentiates benign hyperphenylalaninemia and classic PKU.

Although dietary restriction of phenylalanine is relatively successful in reducing or preventing the mental retardation associated with PKU, there are problems with long-term compliance (resulting in a decline in mental or behavioral status)

Figure 10-17 The phenylalanine hydroxylase system.

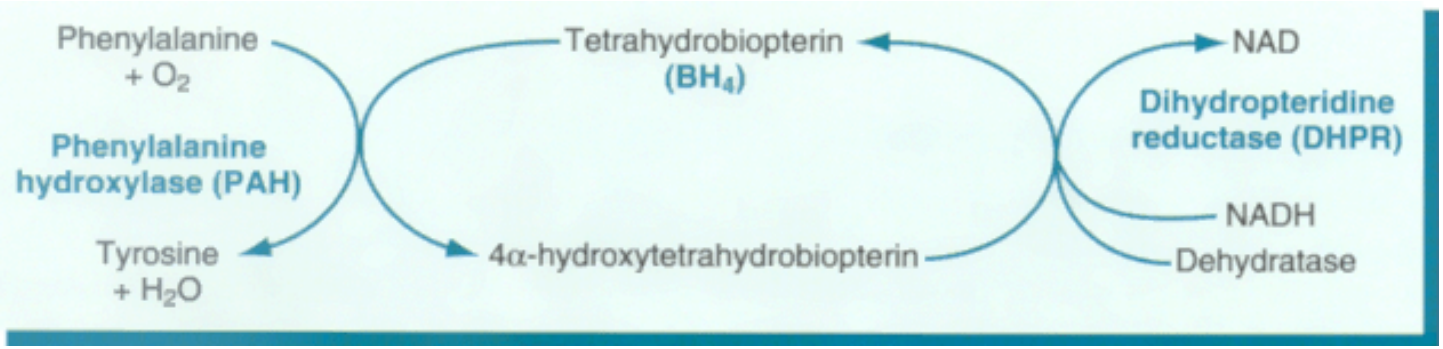

Figure 10-18 Pathways of galactose metabolism.

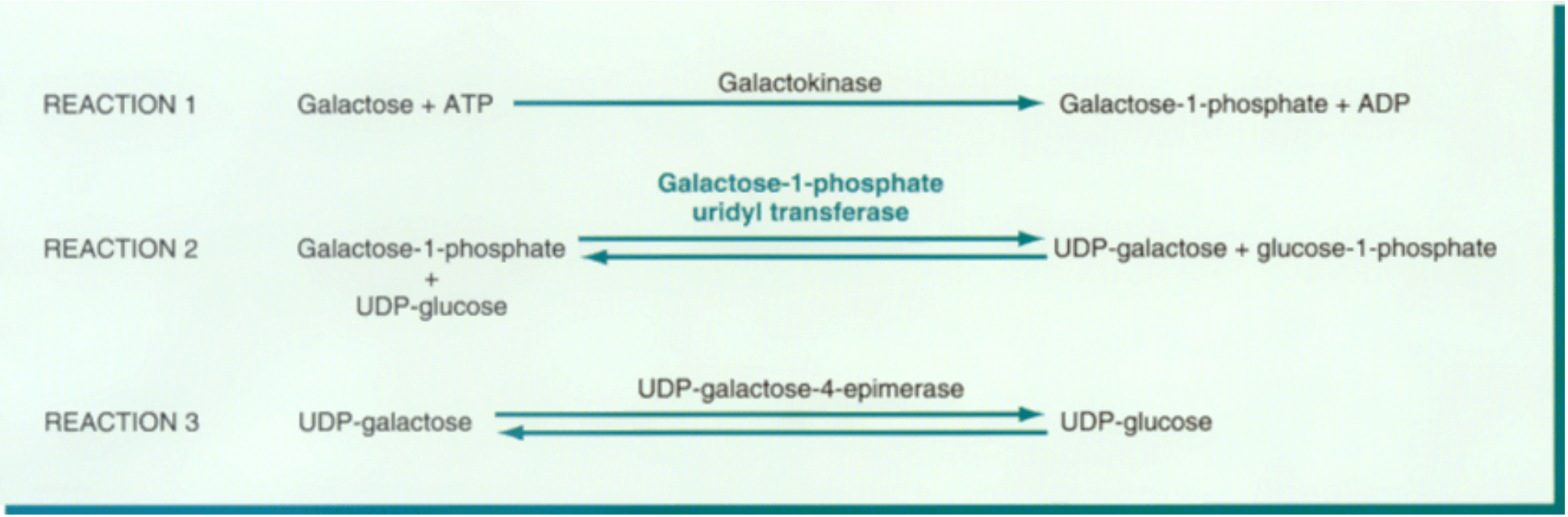

Figure 10-19 Galactosemia. The liver shows extensive fatty change and a delicate fibrosis. *(Courtesy of Dr. Wesley Tyson, The Children's Hospital, Denver, CO.)*

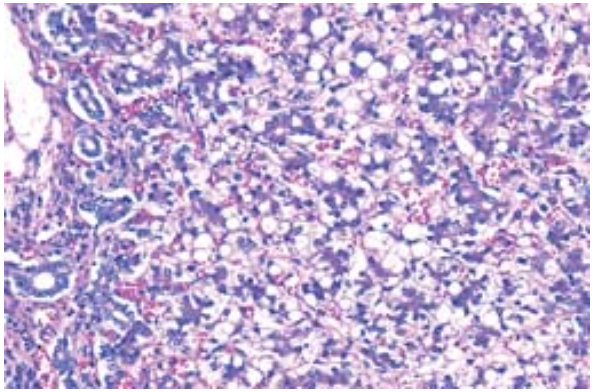

Figure 10-20 *Top*, Normal cystic fibrosis transmembrane conductance regulator (CFTR) structure and activation. CFTR consists of two transmembrane domains, two nucleotide-binding domains (NBD), and a regulatory R domain. Agonists (e.g., acetylcholine) bind to epithelial cells and increase cAMP, which activates protein kinase A, the latter phosphorylating the CFTR at the R domain, resulting in opening of the chloride channel. *Bottom*, CFTR from gene to protein. The most common mutation in the *CFTR* gene results in defective protein folding in the Golgi/ER and degradation of CFTR before it reaches the cell surface. Other mutations affect synthesis of CFTR, nucleotide-binding and R domains, and membrane-spanning domains.

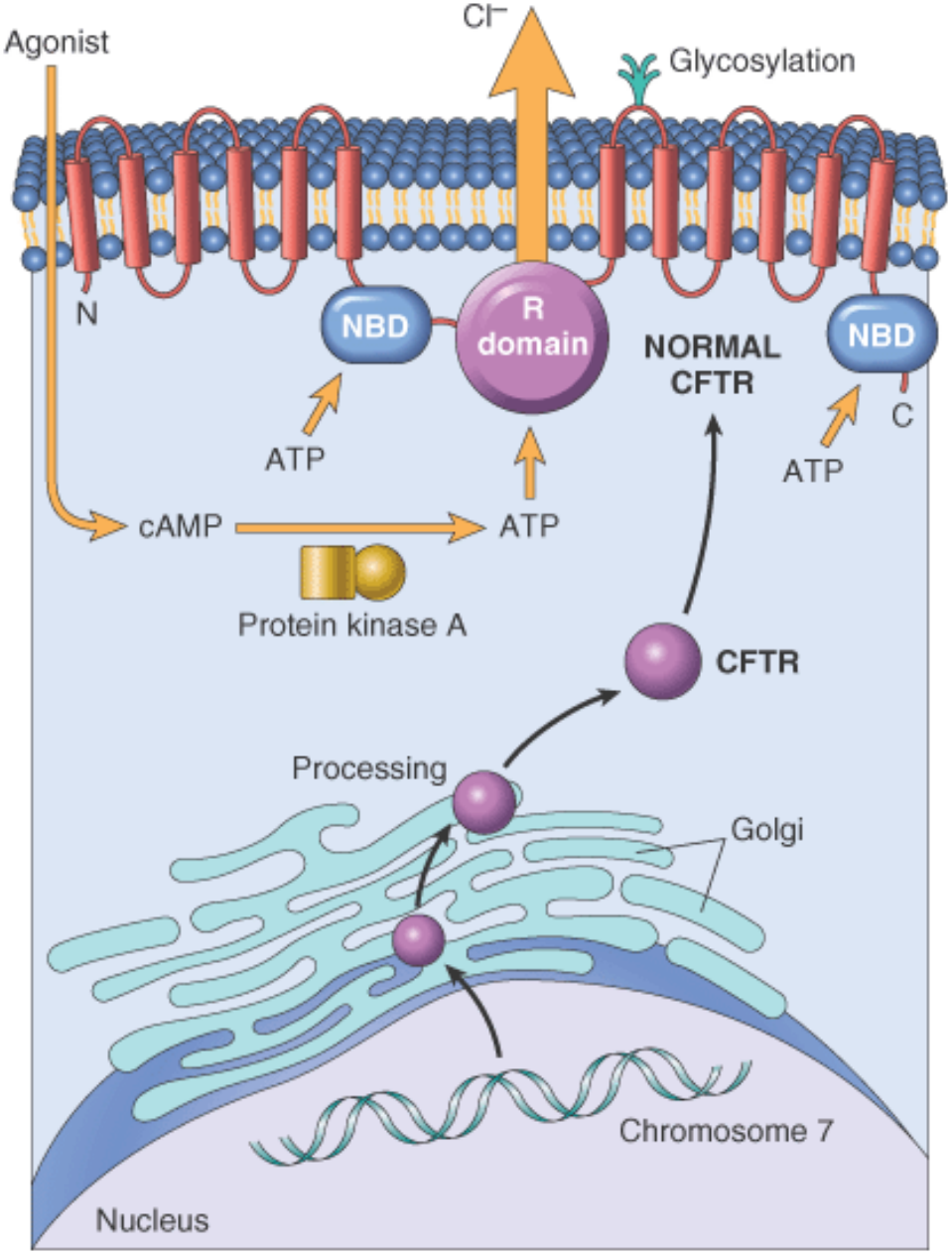

Figure 10-21 Chloride channel defect in the sweat duct *(top)* causes increased chloride and sodium concentration in sweat. In the airway *(bottom)*, cystic fibrosis patients have decreased chloride secretion and increased sodium and water reabsorption leading to dehydration of the mucus layer coating epithelial cells, defective mucociliary action, and mucus plugging of airways. CFTR, Cystic fibrosis transmembrane conductance regulator; EnaC, Epithelial sodium channel.

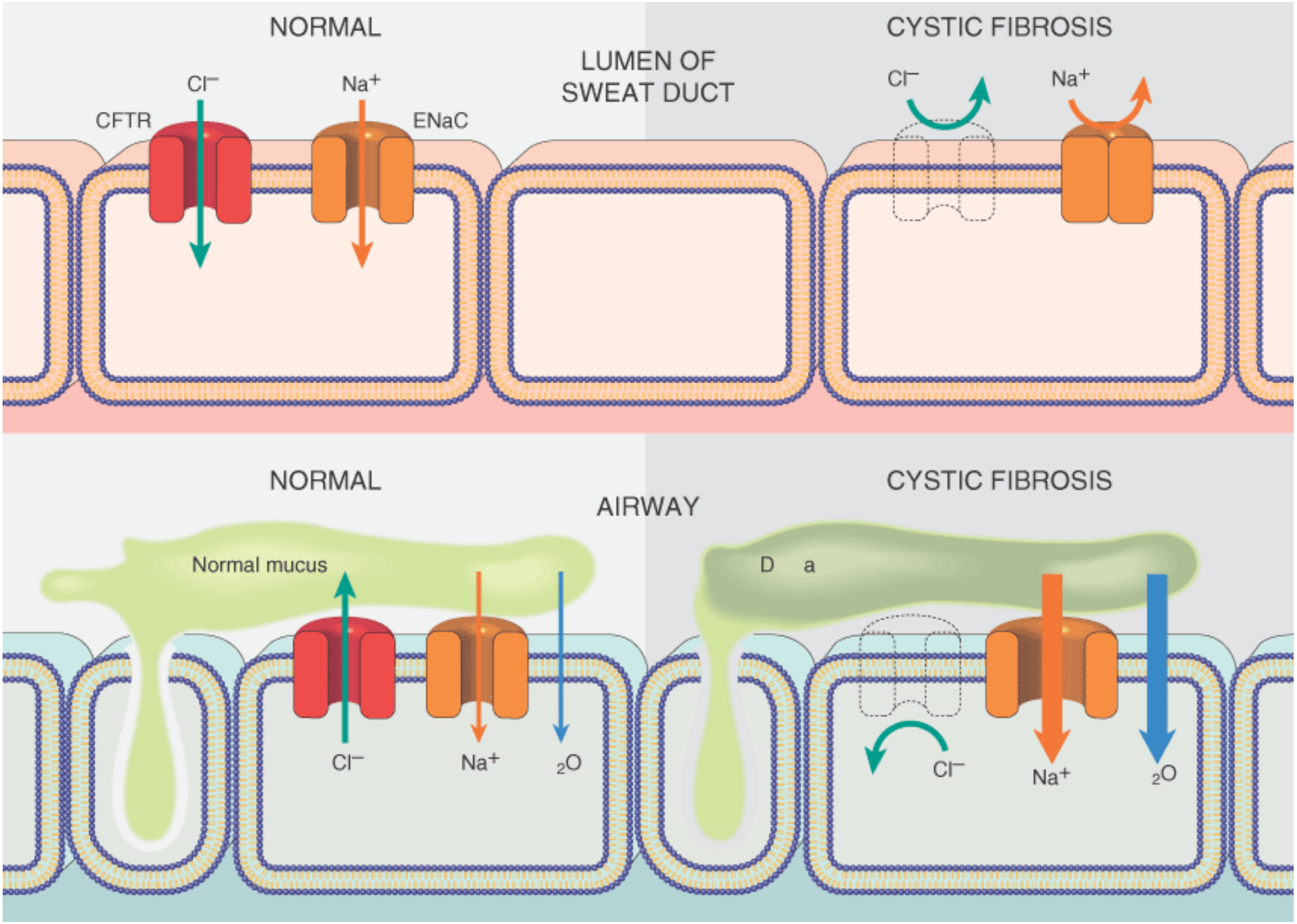

Figure 10-22 The many clinical manifestations of mutations in the cystic fibrosis gene, from most severe to asymptomatic. *(Redrawn from Wallis C: Diagnosing cystic fibrosis: blood, sweat, and tears. Arch Dis Child 76:85, 1997.)*

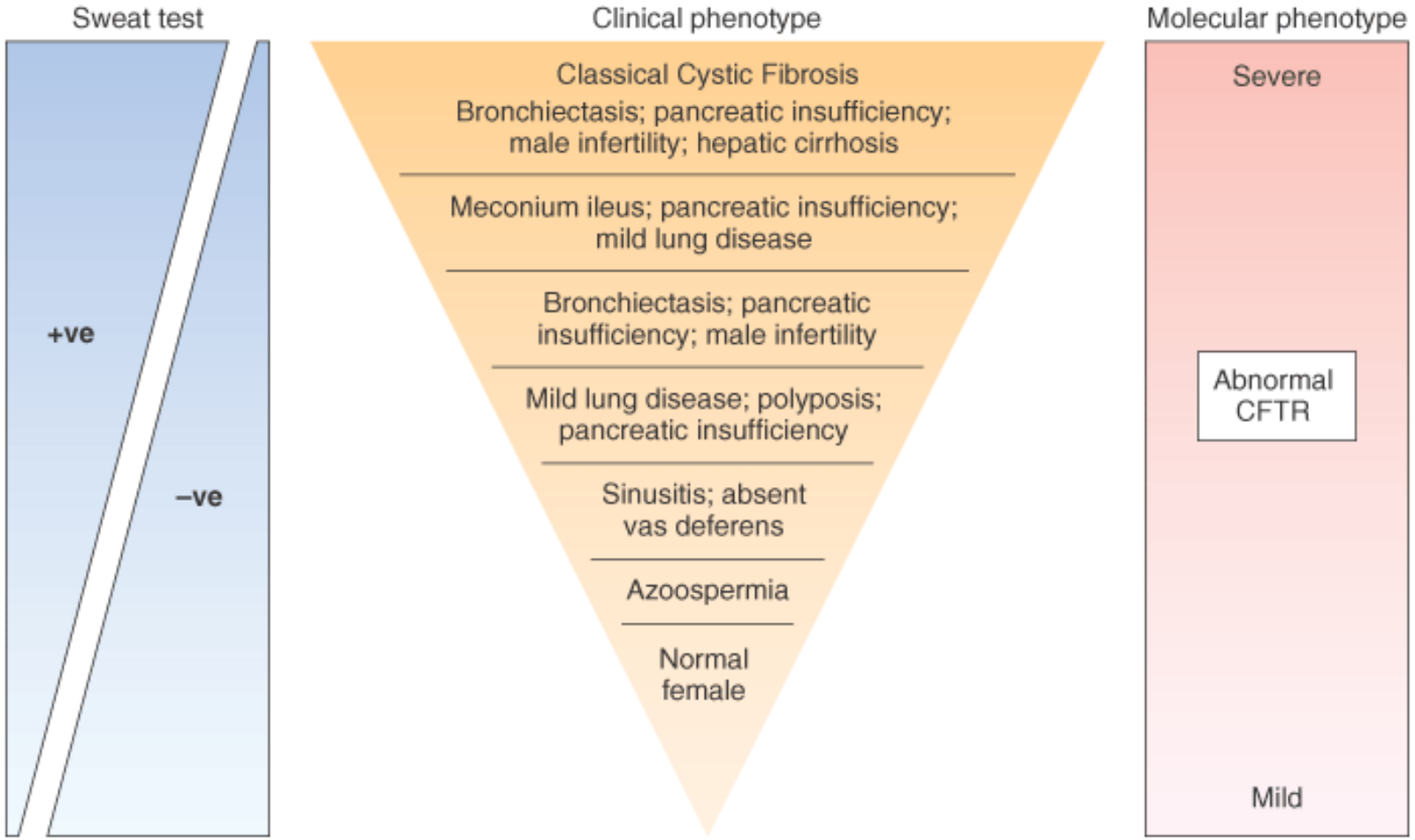

Figure 10-23 Lungs of a patient dying of cystic fibrosis. There is extensive mucus plugging and dilation of the tracheobronchial tree. The pulmonary parenchyma is consolidated by a combination of both secretions and pneumonia—the green color associated with *Pseudomonas* infections. *(Courtesy of Dr. Eduardo Yunis, Children's Hospital of Pittsburgh, Pittsburgh, PA.)*

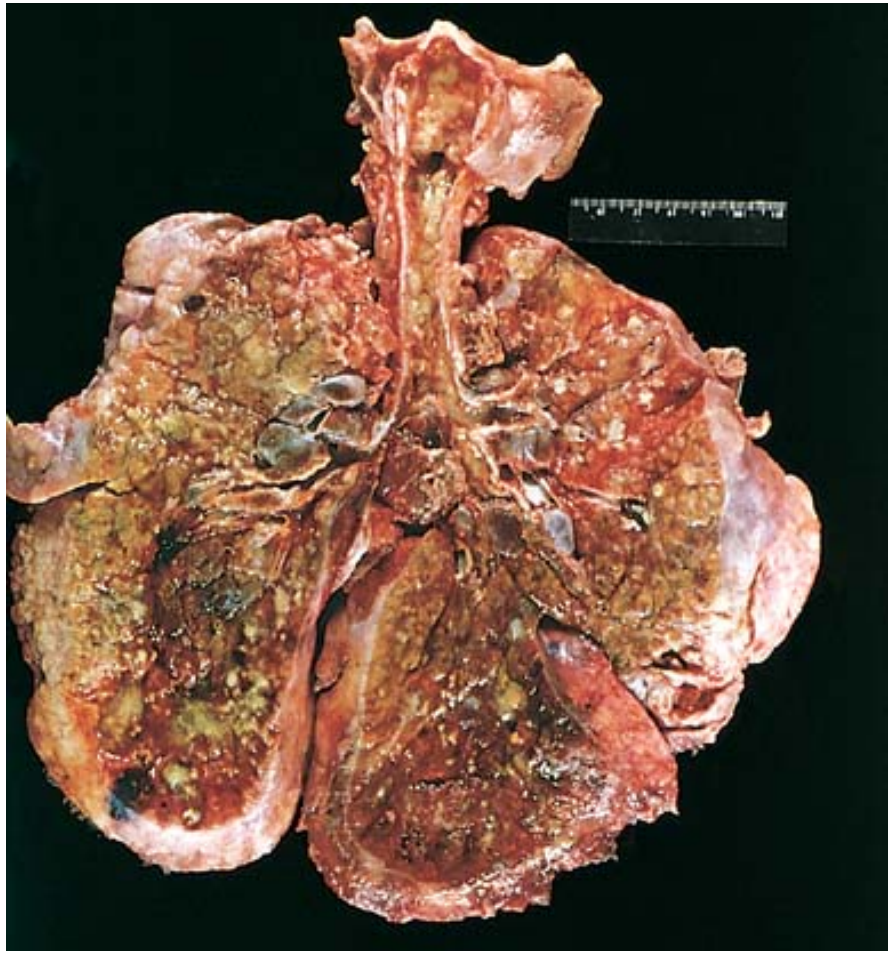

Figure 10-24 Mild to moderate cystic fibrosis changes in the pancreas. The ducts are dilated and plugged with eosinophilic mucin, and the parenchymal glands are atrophic and replaced by fibrous tissue.

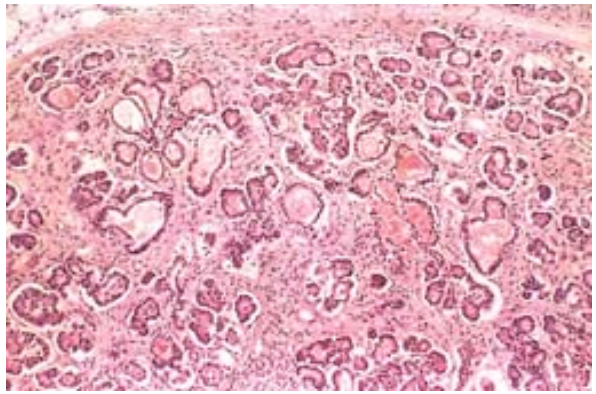

TABLE 10-7 -- Clinical Features and Diagnostic Criteria for Cystic Fibrosis

1. *Chronic sinopulmonary disease manifested by*
•••a. Persistent colonization/infection with typical cystic fibrosis pathogens, including *Staphylococcus aureus*, nontypeable *Hemophilus influenzae*, mucoid and nonmucoid *Pseudomonas aeruginosa*, *Burkholderia cepacia*
•••b. Chronic cough and sputum production
•••c. Persistent chest radiograph abnormalities (e.g., bronchiectasis, atelectasis, infiltrates, hyperinflation)
•••d. Airway obstruction manifested by wheezing and air trapping
•••e. Nasal polyps; radiographic or computed tomographic abnormalities of paranasal sinuses
•••f. Digital clubbing
2. *Gastrointestinal and nutritional abnormalities, including*
•••a. Intestinal: meconium ileus, distal intestinal obstruction syndrome, rectal prolapse
•••b. Pancreatic: pancreatic insufficiency, recurrent pancreatitis
•••c. Hepatic: chronic hepatic disease manifested by clinical or histologic evidence of focal biliary cirrhosis, or multilobular cirrhosis
•••d. Nutritional: failure to thrive (protein-calorie malnutrition), hypoproteinemia, edema, complications secondary to fat-soluble vitamin deficiency
3. *Salt-loss syndromes: acute salt depletion, chronic metabolic acidosis*
4. *Male urogenital abnormalities resulting in obstructive azoospermia (congenital bilateral absence of vas deferens)*
Criteria for Diagnosis of Cystic Fibrosis
One or more characteristic phenotypic features,
••OR a history of cystic fibrosis in a sibling,
••OR a positive newborn screening test result
AND
An increased sweat chloride concentration on two or more occasions
••OR identification of two cystic fibrosis mutations,
••OR demonstration of abnormal epithelial nasal ion transport
Adapted with permission from Rosenstein BJ, Cutting GR: The diagnosis of cystic fibrosis: a consensus statement. J Pediatrics 132:589;1998.

from onset at birth to onset years later, and from involvement of one organ system to involvement of many. Approximately 5% to 10% of the cases come to clinical attention at birth or soon after because of an attack of *meconium ileus*. Distal intestinal obstruction can also occur in older individuals, manifesting as recurrent episodes of right lower quadrant pain sometimes associated with a palpable mass in the right iliac fossa.

Exocrine pancreatic insufficiency occurs in the majority (85–90%) of patients with cystic fibrosis and is associated with "severe" *CFTR* mutations on *both* alleles (e.g., *ΔF508/ΔF508*), whereas 10% to 15% of patients with one "severe" and one "mild" *CFTR* mutation *(ΔF508/R117H)* or two "mild" *CFTR* mutations retain enough pancreatic exocrine function so as not to require enzyme supplementation (*pancreas sufficient* phenotype). [80] Pancreatic insufficiency is associated with protein and fat malabsorption and increased fecal loss. Manifestations of malabsorption (e.g., large, foul stools, abdominal distention, and poor weight gain) appear during the first year of life. The faulty fat absorption may induce deficiency of the fat-soluble

vitamins, resulting in manifestations of avitaminosis A, D, or K. Hypoproteinemia may be severe enough to cause generalized edema. Persistent diarrhea may result in rectal prolapse in up to 10% of children with cystic fibrosis. The *pancreas sufficient* phenotype is usually not associated with other gastrointestinal complications, and in general, these individuals demonstrate excellent growth and development. The diagnosis of an underlying *CFTR* mutation in individuals with pancreas sufficient cystic fibrosis is suspected because of abnormal or borderline sweat chloride levels, a positive family history, or because of concomitant infertility in a male patient. "*Idiopathic*" *chronic pancreatitis* occurs in a subset of patients with pancreas sufficient cystic fibrosis and is associated with recurrent abdominal pain with

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life-threatening complications.[85] These patients have other features of cystic fibrosis, such as pulmonary disease. By contrast, "idiopathic" chronic pancreatitis can also occur as an isolated late-onset finding in the absence of other stigmata of cystic fibrosis (Chapter 19); bi-allelic *CFTR* mutations (usually one "mild", one "severe") are demonstrable in the majority of these individuals, qualifying their inclusion as *nonclassic or atypical cystic fibrosis. Endocrine pancreatic insufficiency* (i.e., diabetes) is uncommon in cystic fibrosis, and usually accompanied by substantial destruction of pancreatic parenchyma.

Cardiorespiratory complications, such as persistent lung infections, obstructive pulmonary disease, and *cor pulmonale*, are the single most common cause of death (~80%) in patients in the United States. By age 18, 80% of patients with classic cystic fibrosis harbor *P. aeruginosa*, and 3.5% harbor *Burkholderia cepacia.* [86] With the indiscriminate use of antibiotic prophylaxis against *Staphylococcus*, there has been an unfortunate resurgence of resistant strains of *Pseudomonas* in many patients. Individuals who carry a "severe" *CFTR* mutation on one allele and a "mild" *CFTR* mutation on the other allele may exhibit late-onset mild pulmonary disease, another example of nonclassic or atypical cystic fibrosis.[81] Patients with mild pulmonary disease usually have mild or no pancreatic disease. *Idiopathic bronchiectasis*, a poorly defined entity in adults where no discernible cause for the bronchiectasis can be found, has been linked to *CFTR* mutations in a subset of cases. *Recurrent sinonasal polyps* can occur in up to 25% of patients with cystic fibrosis; hence, children who present with this finding should be tested for abnormalities of sweat chloride.

Significant *liver disease* occurs late in the natural history of cystic fibrosis and used to be foreshadowed by pulmonary and pancreatic involvement; however, with increasing life expectancies, liver disease has also received increasing attention. In fact, after cardiopulmonary and transplantation-related complications, liver disease is the most common cause of death in cystic fibrosis. Most studies suggest that symptomatic or biochemical liver disease in cystic fibrosis has its onset at or around puberty, with a prevalence of approximately 13% to 17%. [87] However, *asymptomatic hepatomegaly* may be present in up to a third of the individuals. Obstruction of the common bile duct may occur due to stones or sludge; it presents with abdominal pain and the acute onset of jaundice. As previously noted, *diffuse biliary cirrhosis* may develop in up to 5% of individuals with cystic fibrosis.

Approximately 95% of males with cystic fibrosis are *infertile*, as a result of obstructive azoospermia. As mentioned earlier, this is most commonly due to bilateral absence of vas deferens (also called CBAVD). CBAVD can occur as a consequence of several conditions, but bi-allelic *CFTR* mutations are the most common cause (present in 50% to 75% of cases).[88] [89]

In most cases, the diagnosis of cystic fibrosis is based on persistently elevated sweat electrolyte concentrations (often the mother makes the diagnosis because her infant tastes salty), characteristic clinical findings (sinopulmonary disease and gastrointestinal manifestations), or a family history. A minority of patients with cystic fibrosis, especially those with at least one "mild" *CFTR* mutation, may have a normal or near-normal sweat test (<60 mM/L). Measurement of nasal transepithelial potential difference in vivo can be a useful adjunct under these circumstances; individuals with cystic fibrosis demonstrate a significantly more negative baseline nasal potential difference than controls. Sequencing the *CFTR* gene is, of course, the "gold standard" for diagnosis of cystic fibrosis. Therefore, in patients with suggestive clinical findings or family history (or both), genetic analysis may be warranted. It is important to inform the molecular laboratory whether the individual has classic cystic fibrosis or conforms to one of the nonclassic or atypical variants (CBAVD, late-onset pulmonary disease, or idiopathic chronic pancreatitis), so the appropriate "mild" *CFTR* mutations are also analyzed. A recent study has demonstrated that a subset of patients with nonclassic or atypical cystic fibrosis may not reveal *CFTR* mutations in one or both alleles.[90] This indicates that other genetic loci (perhaps those that encode proteins interacting with CFTR) may also produce a partial phenotype resembling cystic fibrosis.

Advances in management of cystic fibrosis include both improved control of infections and bilateral lung (or lobar), heart-lung, liver, pancreas, or liver-pancreas transplantation. Children and adolescents undergoing bilateral lung transplantation have overall survival rates around 70%. These improvements in management mean that more patients are now surviving to adulthood; the median life expectancy is close to 30 years and continues to increase. In principle, cystic fibrosis, like other single gene disorders, should be amenable to gene therapy. In vitro, it has been possible to correct the chloride defect in epithelial cells of cystic fibrosis patients by both viral and nonviral vector-based transfer of the *CFTR* gene; even a single copy of the wild-type *CFTR* gene is able to revert the cystic fibrosis phenotype. Clinical trials with gene therapy in humans are still in their early stages but provide a source of hope for millions of cystic fibrosis patients worldwide.

Sudden Infant Death Syndrome (SIDS)

SIDS is a disease of unknown cause. The National Institute of Child Health and Human Development defines SIDS as "the sudden death of an infant under 1 year of age which remains unexplained after a thorough case investigation, *including performance of a complete autopsy, examination of the death scene, and review of the clinical history*."[91] An aspect of SIDS that is not stressed in the definition is that the infant usually dies while asleep, hence the pseudonyms of *crib death* or *cot death*.

Epidemiology.

As infantile deaths owing to nutritional problems and microbiologic infections have come under control in countries with higher standards of living, SIDS has assumed greater importance in many countries, including the United States. SIDS is the leading cause of death between age 1 month and 1 year in this country and the third leading cause of death overall in infancy, after congenital anomalies and diseases of prematurity and low birth weight. Due largely to nationwide SIDS awareness campaigns by organizations such as the American Academy of Pediatrics, there has been a significant drop in SIDS-related mortality in the past decade, from over 5000 annual deaths in 1990 to approximately 2600 deaths in 1999. Worldwide, in countries where unexpected infant deaths are diagnosed as SIDS only after postmortem examination, the death rates from SIDS (20 to 100/100,000 live births) are comparable to death rates in the United States (77/100,000 live births).

Approximately 90% of all SIDS deaths occur during the first 6 months of life, most between ages 2 and 4 months. This narrow window of peak susceptibility is a unique characteristic that is independent of other risk factors (to be described) and the geographic locale. Most infants who die of SIDS, die at home, usually during the night after a period of sleep. Only rarely is the catastrophic event observed, but even when seen, it is reported that the apparently healthy infant suddenly turns blue, stops breathing, and becomes limp without emitting a cry or struggling. Most infants have had minor manifestations of an upper respiratory infection preceding the fatal event. The term *apparent life-threatening event (ALTE)* has been applied to those infants who could be resuscitated after such an episode. [92] Infants with ALTE are often siblings of SIDS victims and harbor a range of physiologic abnormalities such as frequent or prolonged apnea, diminished chemoreceptor sensitivity to hypercarbia and hypoxia, and impaired control of heart, respiratory rate, and vagal tone.[92] Some of these infants later succumb to SIDS.

Morphology.

At autopsy, a variety of findings have been reported. They are usually subtle and of uncertain significance and are not present in all cases. **Multiple petechiae** are the most common finding in the typical SIDS autopsy (~80% of cases); these are usually present on the thymus, visceral and parietal pleura, and epicardium. Grossly, the lungs are usually congested, and **vascular engorgement** with or without **pulmonary edema** is demonstrable microscopically in the majority of cases. These changes possibly represent agonal events, since they are found with comparable frequencies in *explained* sudden deaths in infancy. Within the upper respiratory system (larynx and trachea), there may be some histologic evidence of recent infection (correlating with the clinical symptoms), although the changes are not sufficiently severe to account for death and should not detract from the diagnosis of SIDS. The central nervous system demonstrates **astrogliosis** of the brain stem and cerebellum. Sophisticated morphometric studies have revealed quantitative brainstem abnormalities such as **hypoplasia of the arcuate nucleus** or a subtle decrease in brain stem neuronal populations in several cases;[93] [94] these observations are not uniform, however, and not amenable to most "routine" autopsy

procedures. Nonspecific findings include frequent persistence of hepatic **extramedullary hematopoiesis** and **periadrenal brown fat**; it is tempting to speculate that these latter findings relate to chronic hypoxemia, retardation of normal development, and chronic stress. Thus, autopsy usually fails to provide a clear cause of death, and this may well be related to the etiologic heterogeneity of SIDS. The importance of a postmortem examination rests in identifying other causes of sudden unexpected death in infancy, such as unsuspected infection, congenital anomaly, or a genetic disorder (Table 10-8), the presence of any of which would *exclude* a diagnosis of SIDS, and in ruling out the unfortunate possibility of traumatic child abuse.

Pathogenesis.

The circumstances surrounding SIDS have been explored in great detail, and it is generally accepted that

TABLE 10-8 -- Risk Factors and Postmortem Findings Associated with Sudden Infant Death Syndrome

Parental
Young maternal age (age <20 years)
Maternal smoking during pregnancy
Drug abuse in *either* parent, specifically paternal marijuana and maternal opiate, cocaine use
Short intergestational intervals
Late or no prenatal care
Low socioeconomic group
African American and American Indian ethnicity (? socioeconomic factors)
Infant
Brain stem abnormalities, associated defective arousal, and cardiorespiratory control
Prematurity and/or low birth weight
Male sex
Product of a multiple birth
SIDS in a prior sibling
Antecedent respiratory infections
? Gastroesophageal reflux
Environment
Prone sleep position
Sleeping on a soft surface
Hyperthermia
Postnatal passive smoking

Postmortem Abnormalities Detected in Cases of Sudden Unexpected Infant Death *
Infections
• Viral myocarditis
• Bronchopneumonia
Unsuspected congenital anomaly
• Congenital aortic stenosis
• Anomalous origin of the left coronary artery from the pulmonary artery (ALCAPA)
Traumatic child abuse
• Intentional suffocation (filicide)
Genetic and metabolic defects
• Long QT syndrome (*SCN5A* and *KCNQ1* mutations)
• Fatty acid oxidation disorders (*MCAD*, *LCHAD*, *SCHAD* mutations)
• Histiocytoid cardiomyopathy (*MTCYB* mutations)
• Abnormal inflammatory responsiveness (partial deletions in *C4a* and *C4b*)

*SIDS is not the only cause of sudden unexpected death in infancy but rather is *a diagnosis of exclusion*. Therefore, performance of an autopsy may often reveal findings that would explain the cause of sudden unexpected death. These cases should *not*, strictly speaking, be labeled as "SIDS." SCN5A, sodium channel, voltage-gated, type V, alpha polypeptide; KCNQ1, potassium voltage-gated channel, KQT-like subfamily, member 1; MCAD, medium-chain acyl coenzyme A dehydrogenase; LCHAD, long-chain 3-hydroxyacyl coenzyme A dehydrogenase; SCHAD, short-chain 3-hydroxyacyl coenzyme A dehydrogenase; MTCYB, mitochondrial cytochrome b; C4, complement component 4.

it is a *multifactorial condition*, with a variable mixture of contributing factors. A "triple risk" model of SIDS has been proposed, which postulates the intersection of three overlapping factors: (1) *a vulnerable infant*, (2) *a critical developmental period in homeostatic control*, and (3) *an exogenous stressor(s)*. [95] According to this model, several factors make the infant vulnerable to sudden death during the critical developmental period (i.e., age 1 month to 1 year). These vulnerability factors may be attributable to the parents or the infant, while the exogenous stressor(s) is attributable to the environment (Table 10-8).

While numerous factors have been proposed to account for a vulnerable infant, *the most compelling hypothesis is that SIDS reflects a delayed development of arousal and cardiorespiratory control*.[96] Regions of the brain stem, particularly the *arcuate nucleus*, located in the ventral medullary surface, play a critical role in the body's "arousal" response to noxious stimuli such as hypercarbia, hypoxia, and thermal stress encountered during sleep. In addition, these areas regulate breathing, heart rate, and body temperature. In certain infants, for yet unexplained reasons, there may be a maldevelopment or delay in maturation of this region, compromising the arousal response to noxious stimuli. This physiologic impairment is compounded by other factors, such as sleeping position or infection (see below). Support of this hypothesis comes from postmortem studies in SIDS victims demonstrating both *quantitative* abnormalities (e.g., arcuate hypoplasia and decrease in neuronal density) as well as *qualitative* abnormalities (e.g., reduced serotonergic and muscarinic receptor binding) in the

brain stem.[93] [97] [98] Whether these changes are primary or merely the manifestation of a more "upstream" deficit remains to be elucidated. Recently, some candidate genes have been identified from experimental animal models, which may provide a genetic basis to abnormal neural regulation in the brainstem. For example, *Krox20*, a homeobox gene, appears to be required for hindbrain segmentation and myelination. Mouse models lacking *Krox20* function exhibit abnormally slow respiratory rhythm and prolonged apnea.[99] Similarly, brain-derived neurotrophic factor (BDNF) is required for normal development of the central respiratory rhythm, including the stabilization of central respiratory output that occurs after birth. Loss of one or both *BDNF* alleles results in an approximately 50% depression of central respiratory frequency compared with wild-type controls, while hypoxic ventilatory drive is deficient or absent.[100] Whether knowledge gleamed from these animal models of central respiratory dysfunction will be applicable to humans remains to be seen.

Epidemiologic studies of infant deaths have found additional risk factors for SIDS (Table 10-8). Infants who are born before term or who are low birth weight are at increased risk, and risk increases with decreasing gestational age or birth weight. Male sex is associated with a slightly greater incidence of SIDS. SIDS in a prior sibling is associated with a fivefold relative risk of recurrence, underscoring the importance of a genetic and/or shared environmental predisposition; *traumatic child abuse needs to be carefully excluded under these circumstances*. Most SIDS babies have an immediate prior history of a mild respiratory tract infection, but no single causative organism has been isolated. These infections may predispose an already vulnerable infant to even greater impairment of cardiorespiratory control and delayed arousal. In this context, laryngeal chemoreceptors have emerged as a putative "missing link" between upper respiratory tract infections, the prone position (see below), and SIDS. When stimulated, these laryngeal chemoreceptors elicit an apneic and bradycardic reflex.[101] Stimulation of the chemoreceptors is augmented by respiratory tract infections, which increase the volume of secretions, and by the prone position, which impairs swallowing and clearing of the airways even in healthy infants. In a previously vulnerable infant with impaired arousal, the apneic and bradycardic reflex may prove fatal.

Maternal smoking during pregnancy has consistently emerged as a risk factor in epidemiologic studies of SIDS, with children exposed to in utero nicotine having more than double the risk of SIDS compared to children born to nonsmokers. [102] Young maternal age, frequent childbirths, and inadequate prenatal care are all risk factors associated with increased incidence of SIDS in the offspring. African Americans and American Indians have significantly higher rates of SIDS deaths than Caucasians. It is not obvious whether these ethnic trends represent the effects of genetic make up or the effects of lower socioeconomic status, which by itself is a risk factor for SIDS.

Among the potential environmental factors, prone sleeping position, sleeping on soft surfaces, and thermal stress are possibly the most important modifiable risk factors for SIDS.[103] The prone position predisposes an infant to one or more recognized noxious stimuli (hypoxia, hypercarbia, and thermal stress) during sleep. In addition, the prone position is also associated with decreased arousal responsiveness compared to the supine position. Results of studies from Europe, Australia, New Zealand, and the United States showed clearly increased risk for SIDS in infants who sleep in a prone position, prompting the American Academy of Pediatrics to recommend placing *healthy infants on their back* when laying them down to sleep. This "Back To Sleep" campaign has resulted in substantial decreases in SIDS-related deaths since its inception in 1994.[104]

It should be noted that SIDS is not the only cause of sudden unexpected deaths in infancy. In fact, SIDS is a diagnosis of exclusion, requiring careful examination of the death scene and a complete postmortem examination. The latter can reveal an unsuspected cause of sudden death in up to 20% or more of "SIDS" babies (Table 10-8). Infections (e.g., viral myocarditis or bronchopneumonia) are the most common causes of sudden "unexpected" death, followed by an unsuspected congenital anomaly. In part due to advancements in molecular diagnostics and knowledge of the human genome, several genetic causes of sudden "unexpected" infant death have emerged. For example, fatty acid oxidation disorders, characterized by defects in mitochondrial fatty acid oxidative enzymes, may be responsible for up to 5% of sudden death in infancy; of these, a *deficiency in medium-chain acyl-coenzyme A dehydrogenase* is the most common.[105] Retrospective analyses of SIDS cases have also revealed mutations of cardiac sodium and potassium channels, which result in a form of cardiac arrhythmia characterized by prolonged QT intervals; these account for no more than 1% of SIDS deaths.[106] Other newly emerging genetic causes of *explained* sudden death are listed in Table 10-8 .

Only 2% of all malignant tumors occur in infancy and childhood; nonetheless, cancer (including leukemia) is a leading cause of death from disease in the United States in children over age 4 and up to age 14. Neoplastic disease accounts for approximately 9% of all deaths in this cohort; only accidents cause significantly more deaths. Benign tumors are even more common than cancers. Most benign tumors are of little concern, but on occasion they cause serious disease by virtue of their location or rapid increase in size.

It is sometimes difficult to segregate, on morphologic grounds, true tumors or neoplasms from tumor-like lesions in the infant and child. In this context, two special categories of tumor-like lesions should be distinguished from true tumors.

The term *heterotopia* (or *choristoma*) is applied to microscopically normal cells or tissues that are present in abnormal locations. Examples of heterotopias include a rest of pancreatic tissue found in the wall of the stomach or small intestine or a small mass of adrenal cells found in the kidney, lungs, ovaries, or elsewhere. The heterotopic rests are usually of little significance, but they can be confused clinically with neoplasms. Rarely, they are sites of origin of true neoplasms, producing the paradox of an adrenal carcinoma arising in the ovary.

The term *hamartoma* refers to an excessive but focal overgrowth of cells and tissues native to the organ in which it occurs. Although the cellular elements are mature and identical to those found in the remainder of the organ, they do not reproduce the normal architecture of the surrounding tissue. Hamartomas can be thought of as the linkage between malformations and neoplasms—the line of demarcation between a hamartoma and a benign neoplasm is frequently tenuous and is variously interpreted. Hemangiomas, lymphangiomas, rhabdomyomas of the heart, adenomas of the liver, and developmental cysts within the kidneys, lungs, or pancreas are interpreted by some as hamartomas and by others as true neoplasms. The frequency of these lesions in infancy and childhood and their clinical behavior give credence to the

Figure 10-25 Congenital capillary hemangioma at birth (*A*) and at age 2 years (*B*) after spontaneous regression. *(Courtesy of Dr. Eduardo Yunis, Children's Hospital of Pittsburgh, Pittsburgh, PA.)*

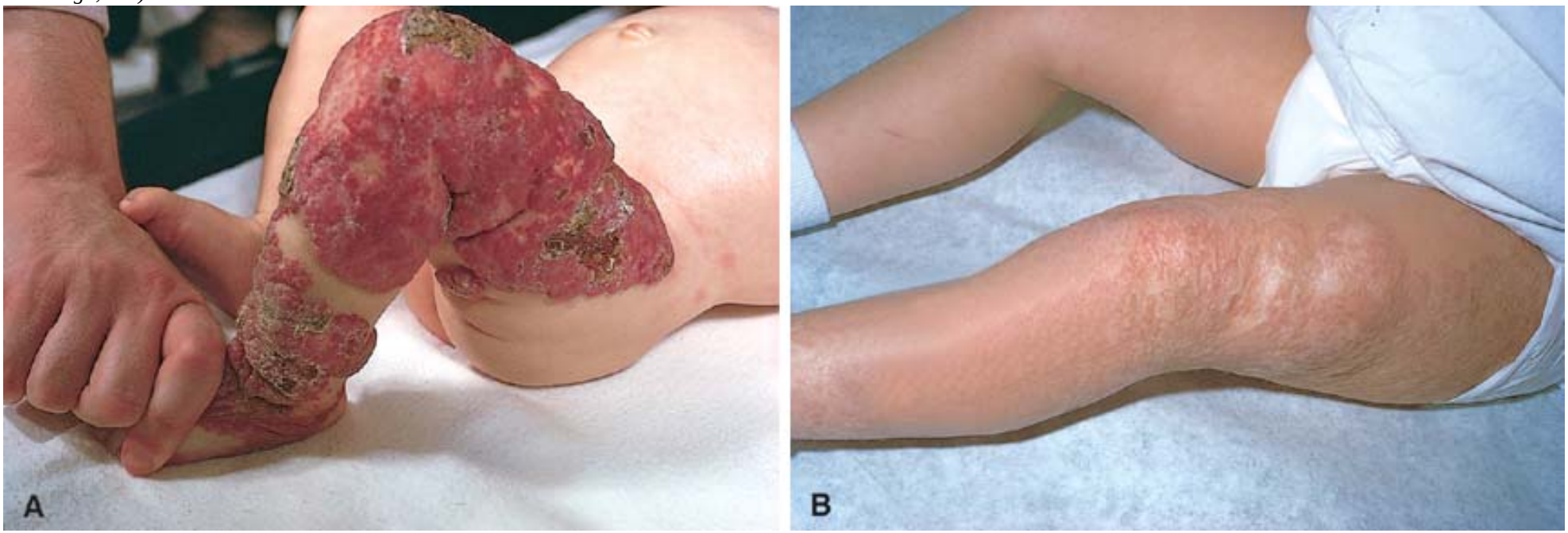


Figure 10-26 Sacrococcygeal teratoma. Note the size of the lesion compared with that of the infant.

TABLE 10-9 -- Common Malignant Neoplasms of Infancy and Childhood

0 to 4 Years	5 to 9 Years	10 to 14 Years
Leukemia	Leukemia	
Retinoblastoma	Retinoblastoma	
Neuroblastoma	Neuroblastoma	
Wilms tumor		
Hepatoblastoma	Hepatocarcinoma	Hepatocarcinoma
Soft tissue sarcoma (especially rhabdomyosarcoma)	Soft tissue sarcoma	Soft tissue sarcoma
Teratomas		
Central nervous system tumors	Central nervous system tumors	
	Ewing sarcoma	
	Lymphoma	Osteogenic sarcoma
		Thyroid carcinoma
		Hodgkin disease

Histologically, many of the malignant pediatric neoplasms are unique. In general, they tend to have a more primitive *(embryonal)* rather than pleomorphic-anaplastic microscopic appearance, are often characterized by sheets of cells with small, round nuclei, and frequently exhibit features of organogenesis specific to the site of tumor origin. Because of this

TABLE 10-10 -- Genetic and Other Useful Markers of Small Round Cell Tumors of Childhood

Tumor Type	Genetic Markers	Other Diagnostically Useful Features
Neuroblastoma	17q gain, * 1p deletion *	Clinical elevation in level of urinary catecholamines
	N-*myc* amplification *	Neurosecretory granules by electron microscopy
	DNA hyperdiploidy, near triploidy †	Neuron-specific enolase expression
	t(11;22), ‡ t(21;22), t(7;22)	*MIC2* (CD99) gene expression
	EWS-FLI1 or *EWS-ERG* fusion transcript	
Rhabdomyosarcoma	t(2;13), ‡ * t(1;13)—alveolar rhabdomyosarcoma (ARMS)	Myogenin and Myo D1 expression (all subtypes)
	11p15.5 deletion—embryonal rhabdomyosarcoma (ERMS)	Alternating thick and thin filaments by electron microscopy
	PAX3-FKHR and *PAX7-FKHR* fusion transcript (ARMS)	
Burkitt lymphoma	t(8;14), ‡ t(2;8), t (8;22)	B-cell phenotype expressing CD19, CD20, CD10, IgM
		Epstein-Barr virus latent infection (endemic cases)
Lymphoblastic lymphoma/ acute lymphoblastic leukemia	Hyperdiploidy (>50), † Hypodiploidy(<46) *	Terminal deoxynucleotidyl transferase (TdT)+
	B-lineage: various translocations, including t(12;21) (*TEL-AML1*), ‡ , † t (9;22) (*BCR-ABL*, Philadelphia chromosome), * t(4;11) (AF4-MLL) * , t (1;19) (*PBX-E2A*) T-lineage: 1p32 abnormalities (*TAL1* gene)	Various B- and T-lineage antigens
Wilms tumor	11p13 (*WT1*) deletion/mutation	
	11p15.5 abnormalities of imprinting (e.g., *IGF2*, *H19*, $p57^{KIP2}$)	
	16q, * 1p, * 7p deletion	
Retinoblastoma	13q14 (*RB*) deletion/mutation	Retinal S antigen expression
Medulloblastoma	17p deletion	Evidence of neuronal differentiation (synaptophysin expression) or glial differentiation (glial fibrillary acid protein [GFAP] expression)
	Isochromosome 17q	

PNET, peripheral neuroectodermal tumor.

*Generally associated with a poorer prognosis.
†Generally associated with a better prognosis.

‡Most common translocation.

latter characteristic, these tumors are frequently designated by the suffix *-blastoma*, for example, nephroblastoma (Wilms tumor), hepatoblastoma, and neuroblastoma. Owing to their primitive histologic appearance, many childhood tumors have been collectively referred to as *small round blue cell tumors*. The differential diagnosis of such tumors includes neuroblastoma, Wilms tumor, lymphoma, rhabdomyosarcoma, and Ewing sarcoma/primitive neuroectodermal tumor. Rendering a definitive diagnosis is usually possible on histologic examination alone, or in combination with chromosome analysis, immunoperoxidase stains, and electron microscopy. The diagnostic features associated with the more common childhood neoplasms are summarized in Table 10-10 . Two of these tumors are particularly illustrative and are discussed here: the neuroblastic tumors, specifically neuroblastoma, and Wilms tumor. The remaining tumors are discussed in their respective organ-specific chapters.

The Neuroblastic Tumors

The term "neuroblastic tumor" includes tumors of the sympathetic ganglia and adrenal medulla that are derived from primordial neural crest cells populating these sites. As a family, neuroblastic tumors demonstrate certain characteristic features such as *spontaneous or therapy-induced differentiation of primitive neuroblasts into mature elements, spontaneous tumor regression*, and a *wide range of clinical behavior and prognosis*, which often mirror the extent of histologic differentiation. Neuroblastoma is the most important member of this family. It is the second most common solid malignancy of childhood

after brain tumors, accounting for 7% to 10% of all pediatric neoplasms, and as many as 50% of malignancies diagnosed in infancy.[108] Approximately 650 new cases are diagnosed in the United States each year, accounting for an incidence of approximately 9.5 cases per million children. The median age at diagnosis is 22 months; a little more than a third of the cases are diagnosed in infancy. There is a higher incidence of neuroblastoma in Caucasian as compared to African American populations, and males are at a marginally greater risk than females. Alone, it accounts for at least 15% of all childhood cancer deaths, although the 5-year survival rate has improved from 25% in the early 1960s to almost 55% in the mid-1990s. As will be evident later, age and stage have a remarkable effect on prognosis, and, in general, infants tend to have a significantly better prognosis than older individuals. Most occur sporadically, but a few are familial with autosomal dominant transmission, and in such cases the neoplasms may involve both of the adrenals or multiple primary autonomic sites.

Morophology.

In childhood, about 40% of neuroblastomas arise in the adrenal medulla. The remainder occur anywhere along the sympathetic chain, with the most common locations being the paravertebral region of the abdomen (25%) and posterior mediastinum (15%). Tumors may arise in numerous other sites, including the pelvis and neck and within the brain (cerebral neuroblastomas).

Macroscopically, neuroblastomas range in size from minute nodules (the **in situ lesions**) to large masses more than 1 kg in weight (Fig. 10-27). In situ neuroblastomas are reported to be 40 times more frequent than overt tumors. The great majority of these silent lesions spontaneously regress, leaving only a focus of fibrosis or calcification in the adult. Some neuroblastomas

Figure 10-27 Adrenal neuroblastoma in a 6-month-old child. The hemorrhagic, partially encapsulated tumor has displaced the opened left kidney and is impinging on the aorta and left renal artery. *(Courtesy of Dr. Arthur Weinberg, University of Texas Southwestern Medical School, Dallas, TX.)*

Figure 10-28 Adrenal neuroblastoma. This tumor is composed of small cells embedded in a finely fibrillar matrix.

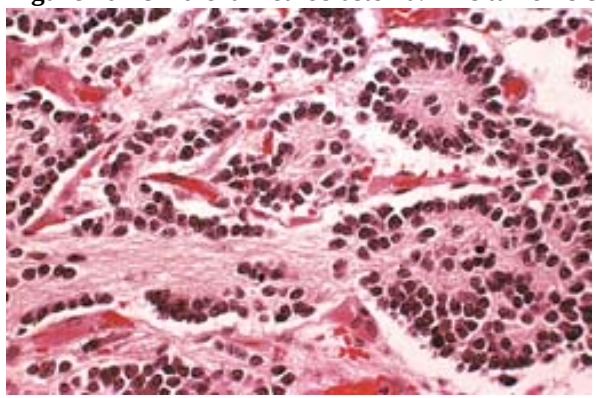
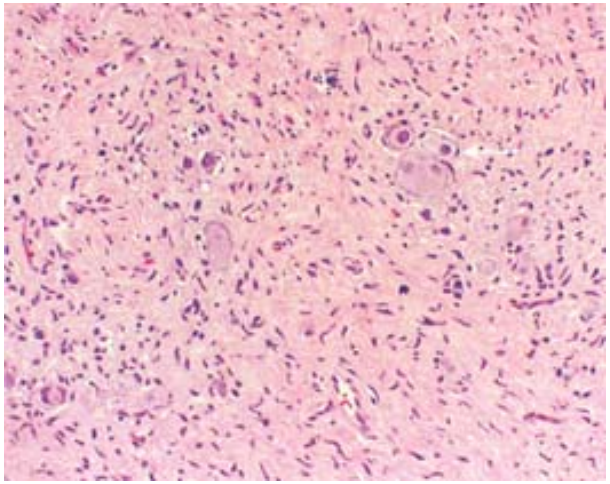


Figure 10-29 Ganglioneuromas, arising from spontaneous or therapy-induced maturation of neuroblastomas, are characterized by clusters of large cells with vesicular nuclei and abundant eosinophilic cytoplasm, representing neoplastic ganglion cells *(arrow)*. Spindle-shaped Schwann cells are present in the background stroma.



Stage 2A: Localized tumor with incomplete gross resection. Representative ipsilateral nonadherent lymph nodes negative for tumor microscopically.

Stage 2B: Localized tumor with or without complete gross excision, ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes, which are negative for tumor microscopically.

Stage 3: Unresectable unilateral tumor infiltrating across the midline with or without regional lymph node involvement; or localized unilateral tumor with contralateral regional lymph node involvement.

Stage 4: Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs *(except as defined for stage 4S)*.

Stage 4S ("S" = special): Localized primary tumor (as defined for Stages 1, 2A, or 2B) with dissemination limited to skin, liver, and/or bone marrow; *Stage 4S is limited to infants <1 yr*.

Unfortunately, most (60% to 80%) children present with Stage 3 or 4 tumors, and only 20% to 40% present with Stage 1, 2A, 2B, or 4S neuroblastomas. The staging system is of paramount importance in determining prognosis.

Clinical Course and Prognostic Features.

In young children, under age 2 years, neuroblastomas generally present with large abdominal masses, fever, and possibly weight loss. In older children, they may not come to attention until metastases produce manifestations, such as bone pain, respiratory symptoms, or gastrointestinal complaints. Neuroblastomas may metastasize widely through the hematogenous and lymphatic systems, particularly to liver, lungs, and bones, in addition to the bone marrow. Proptosis and ecchymosis may also be present because the periorbital region is a common metastatic site. Bladder and bowel dysfunction may be caused by paraspinal neuroblastomas that impinge on nerves. In neonates, disseminated neuroblastomas may present with multiple cutaneous metastases with deep blue discoloration to the skin (earning the rather unfortunate designation of "*blueberry muffin baby*"). *About 90% of neuroblastomas, regardless of location, produce catecholamines* (similar to the catecholamines associated with pheochromocytomas), which are an important diagnostic feature (i.e., elevated blood levels of catecholamines and elevated urine levels of metabolites, vanillylmandelic acid [VMA], and homovanillic acid [HVA]). Despite the elaboration of catecholamines, hypertension is much less frequent with these neoplasms than with pheochromocytomas (Chapter 24). Ganglioneuromas, unlike their malignant counterparts, tend to produce either asymptomatic mass lesions or symptoms related to compression.

The course of neuroblastomas is extremely variable. Several clinical, histopathologic, molecular, and biochemical factors have been identified in neuroblastomas that have a bearing on prognosis (see Table 10-11):

Age and stage are the most important determinants of outcome. Infants younger than age 1 year have an excellent prognosis regardless of the stage of the neoplasm. Most often in this age group, the neoplasms are Stage 1, 2A, or 2B, and therapy yields a greater than 90% 5-year survival.[114] At this early age, even when metastases are present, in about half the spread is limited to the liver, bone marrow, and skin (stage 4S), and such infants have at least an 80% 5-year survival with only minimal therapy. In fact, with Stage 4S disease, it is not uncommon for the primary or metastatic tumors to undergo spontaneous regression. [115] The biologic basis of this welcome behavior is not clear. Even when the dissemination is more widespread in the first year of life or the tumor is accompanied by unfavorable biologic characteristics such as N-*myc* amplification (see below), the survival is greater than 50%. Children between ages 1 and 5 years have an intermediate prognosis for low-stage tumors that have otherwise favorable

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TABLE 10-11 -- Prognostic Factors in Neuroblastomas

Variable	Favorable	Unfavorable
Stage *	Stage 1, 2A, 2B, 4S	Stage 3, 4
Age *	≤ 1 year	>1 year
Histology *		
••Evidence of schwannian stroma and gangliocytic differentiation [a]	Present	Absent
••Mitotic rate [b]	Low	High
••Mitosis-karyorrhexis index [c]	≤200/5000 cells	>200/5000 cells
••Intratumoral calcification	Present	Absent
DNA ploidy *	Hyperdiploid or near-triploid	Diploid, near-diploid, or near-tetraploid
N-myc *	Not amplified	Amplified
Chromosome 17q Gain	Absent	Present
Chromosome 1p Loss	Absent	Present
Trk-A Expression	Present	Absent
Telomerase Expression	Low or absent	Highly expressed
MRP Expression	Absent	Present
CD44 Expression	Present	Absent
Serum Biochemical Markers		

••Ferritin	Normal	Elevated
••Lactate Dehydrogenase	≤1500 U/mL	> 1500 U/mL

Trk-A, tyrosine kinase receptor A; *MRP*, multidrug resistance-associated protein.

*Corresponds to the most commonly used parameters in clinical practice for assessment of prognosis and risk stratification.

[a] It is not only the presence but also the *amount* of schwannian stroma that confers the designation of a favorable histology. At least *50% or more schwannian stroma* is required before a neoplasm can be classified as ganglioneuroblastoma or ganglioneuroma.

[b] Mitotic rate is classified as *low* (≤10 mitoses/10 high power fields) or *high* (>10 mitoses/10 high power fields).

[c] Mitotic karyorrhexis index (MKI) is defined as the number of mitotic or karyorrhectic cells per 5000 tumor cells in random foci.

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biologic characteristics (see below), while those with advanced stage disease have <20% 5-year survival, irrespective of other prognostic variables. In contrast, children older than age 5 years usually have extremely poor outcomes irrespective of stage.

Morphology is an independent prognostic variable in neuroblastic tumors.[112] An age-linked morphologic classification of neuroblastic tumors has recently been proposed that divides them into *favorable* and *unfavorable* histologic subtypes. The specific morphologic features that bear in prognosis are listed in Table 10-11 .

Ploidy of the tumor cells correlates with outcome. In general, *hyperdiploidy* and *near-triploidy* have a correlation with young age, low stage, and a good prognosis, whereas *diploidy*, *near-diploidy*, and *near-tetraploidy* are associated with an unfavorable outcome irrespective of age. For example, in infants and children younger than age 2 years who have advanced disease, the presence of hyperdiploidy or near-triploidy correlates with response to chemotherapy and long-term disease-free survival, while corresponding diploid tumors have a significantly worse prognosis (the beneficial prognostic effects of ploidy tend to be negated in older children with advanced disease).

Amplification of the N-myc oncogene in neuroblastomas is a molecular event that has possibly the most profound impact on prognosis. [116] N-*myc* is located on the distal short arm of chromosome 2 (2p23-24). Amplification of N-*myc* does not karyotypically manifest at the resident 2p23-24 site, but rather as extrachromosomal *double minute chromatin bodies* or *homogeneously staining regions* on other chromosomes (Fig. 10-30). N-*myc* amplification is present in about 25% to 30% of primary tumors, most in advanced-stage disease. Up to 300 copies of N-*myc* have been observed in some tumors; the greater the number of copies, the worse the prognosis. N-*myc* amplification is currently the most important genetic abnormality used in risk stratification of neuroblastic tumors (see below).

Partial gain of the distal long arm of chromosome 17 is the most common karyotypic abnormality in neuroblastomas, present in up to 50% of tumors.[117] The mechanism of 17q gain is via an *unbalanced translocation*, where a portion of 17q is translocated to a partner chromosome (most commonly the distal short arm of chromosome 1, or the distal long arm of chromosome 11). Partial gain of 17q demonstrates significant association with adverse outcome in neuroblastomas, independent of other prognostic variables.

Deletion of the distal short arm of chromosome 1 in the region of band p36 has been demonstrated in 25% to 35% of primary tumors.[118] In addition, constitutional deletions of 1p36 have been demonstrated in a subset of patients with neuroblastomas. The loss of genetic material implies that one or more putative tumor suppressor genes in this region may be important in the pathogenesis of neuroblastomas, but their identity remains elusive. At least two distinct loci of deletions on 1p36 have been identified. The first, more distal region appears to demonstrate preferential loss of the maternal allele in tumors,

Figure 10-30 Fluorescence in situ hybridization using a fluorescein-labeled cosmid probe for N-*myc* on a tissue section. Note the neuroblastoma cells on the upper half of the photo with large areas of staining *(yellow-green)*; this corresponds to amplified N-*myc* in the form of homogeneously staining regions. Renal tubular epithelial cells in the lower half of the photograph show no nuclear staining and background *(green)* cytoplasmic staining. *(Courtesy of Dr. Timothy Triche, Children's Hospital, Los Angeles, CA.)*

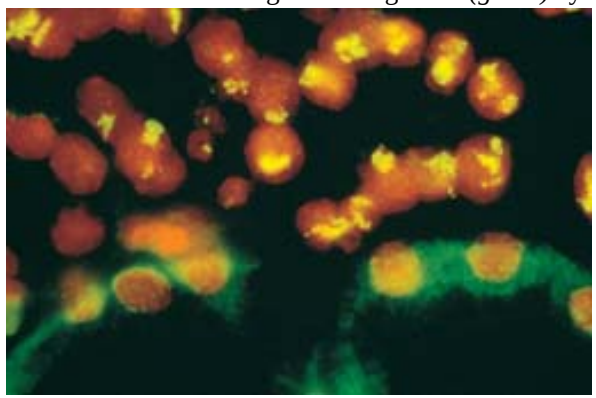
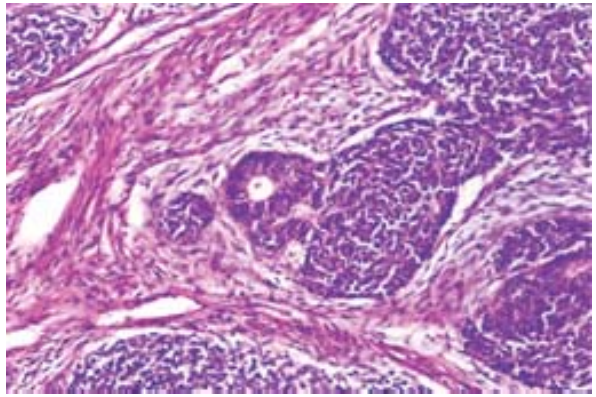


Figure 10-31 Wilms tumor in the lower pole of the kidney with the characteristic tan-to-gray color and well-circumscribed margins.

Figure 10-32 Triphasic histology of Wilms' tumor: the stromal component is comprised of spindle-shaped cells in the less cellular area on the left; the immature tubule in the center is an example of the epithelial component and the tightly packed blue cells, of the blastemal elements. *(Courtesy of Dr. Charles Timmons, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)* Anaplasia in Wilms' tumor is characterized by cells with large, hyperchromatic, pleomorphic nuclei and abnormal mitoses *(inset)*.



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Section II - Diseases of Organ Systems

Chapter 11 - Blood Vessels

Frederick J. Schoen MD, PhD

Diseases of arteries are responsible for more morbidity and mortality than any other type of human disease. Disorders of veins less commonly cause clinically significant problems. Vascular abnormalities cause clinical disease by two principal mechanisms:

- *Narrowing* or *completely obstructing* the lumens, either progressively (e.g., by atherosclerosis) or precipitously (e.g., by thrombosis or embolism).
- *Weakening* of the walls, leading to dilation or rupture.

To understand the diseases that affect blood vessels, we first consider some of the anatomic and functional characteristics of these highly specialized and dynamic tissues.

Normal

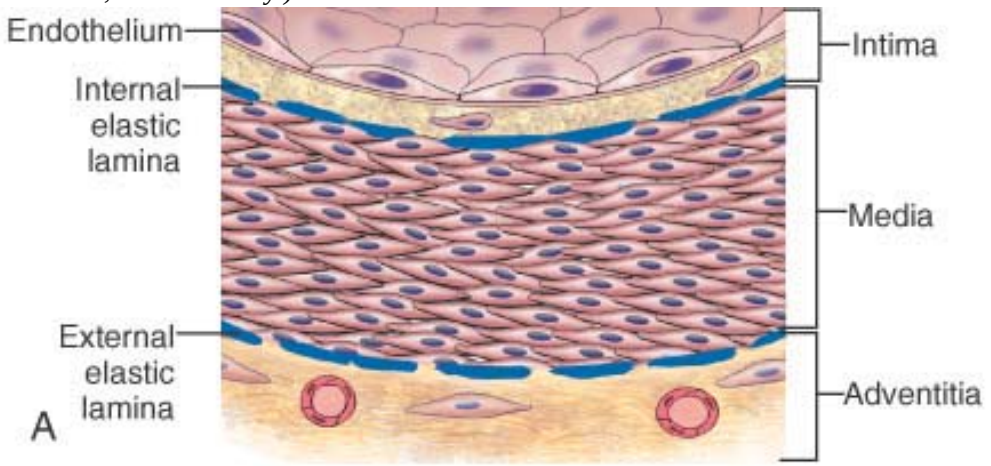
The general architecture and cellular composition of blood vessels are the same throughout the cardiovascular system. However, certain features of the vasculature vary with and reflect distinct functional requirements at different locations (see below). To withstand the pulsatile flow and higher blood pressures in arteries, arterial walls are generally thicker than the walls of veins. Arterial wall thickness gradually diminishes as the vessels become smaller, but the ratio of wall thickness to lumen diameter becomes greater.

The basic constituents of the walls of blood vessels are endothelial cells and smooth muscle cells, and extracellular matrix (ECM), including elastin, collagen, and glycosoaminoglycans. The three concentric layers—*intima*, *media*, and *adventitia*—are most clearly defined in the larger vessels, particularly arteries (Fig. 11-1). In normal arteries, the intima consists of a single layer of endothelial cells with minimal underlying subendothelial connective tissue. It is separated from the media by a dense elastic membrane called the *internal elastic lamina*. The smooth muscle cell layers of the media near the vessel lumen receive oxygen and nutrients by direct diffusion from the vessel lumen, facilitated by holes in the internal elastic membrane. However, diffusion from the lumen is inadequate for the outer portions of the media in large and medium-sized vessels, therefore these areas are nourished by small arterioles arising from outside the vessel (called *vasa vasorum*, literally "vessels of the vessels") coursing into the outer one half to two thirds of the media. The outer limit of the media of most arteries is a well-defined *external elastic lamina*. External to the media is the adventitia, consisting of connective tissue with nerve fibers and the vasa vasorum.

Based on their size and structural features, *arteries* are divided into three types: (1) large or *elastic arteries*, including the aorta, its large branches (particularly the innominate, subclavian, common carotid, and iliac), and pulmonary arteries; (2) medium-sized or *muscular arteries*, comprising other

Figure 11-1 The vascular wall. *A*, Graphic representation of the cross section of a small muscular artery (e.g., renal or coronary artery). *B*, Photomicrograph of histologic section containing a portion of an artery (A) and adjacent vein (V). Elastic membranes are stained black (internal elastic membrane of artery highlighted by *arrow*). Because it is exposed to higher pressures, the artery has a thicker wall that maintains an open, round lumen, even when blood is absent. Moreover, the elastin of the artery is more organized than in the corresponding vein. In contrast, the vein has a larger, but collapsed, lumen, and the elastin in its wall is diffusely distributed. *(B, Courtesy of Mark Flomenbaum, M.D., Ph.D., Office of the Chief Medical*

Examiner, New York City.)



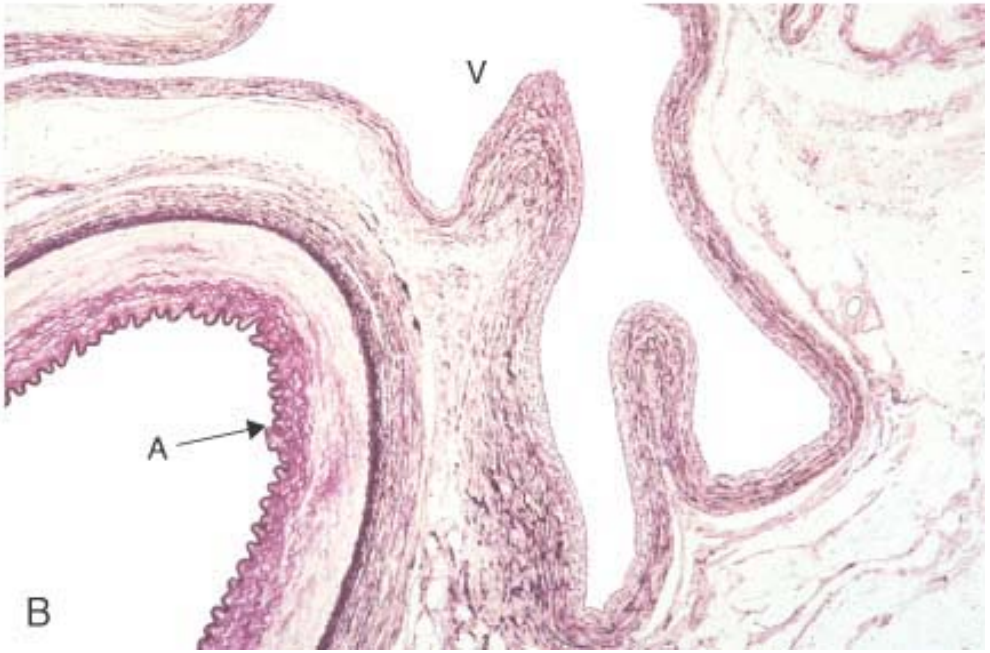


TABLE 11-1 -- Endothelial Cell Properties and Functions

Maintenance of Permeability Barrier
Elaboration of Anticoagulant, Antithrombotic, Fibrinolytic Regulators
Prostacyclin
Thrombomodulin
Heparin-like molecules

Plasminogen activator
Elaboration of Prothrombotic Molecules
Von Willebrand factor
Tissue factor
Plasminogen activator inhibitor
Extracellular Matrix Production (collagen, proteoglycans)
Modulation of Blood Flow and Vascular Reactivity
Vasconstrictors: endothelin, ACE
Vasodilators: NO, prostacyclin
Regulation of Inflammation and Immunity
IL-1, IL-6, chemokines
Adhesion molecules: VCAM-1, ICAM, E-selectin P-selectin
Histocompatibility antigens
Regulation of Cell Growth
Growth stimulators: PDGF, CSF, FGF
Growth inhibitors: heparin, TGF-β
Oxidation of LDL
ACE, angiotensin converting enzyme; NO, nitric oxide; IL, interleukin; PDGF, platelet-derived growth factor; CSF, colony-stimulating factor; FGF, fibroblast growth factor; TGF-β, transforming growth factor-beta; LDL, low-density lipoprotein.

Structurally intact ECs can respond to various pathophysiologic stimuli by adjusting their usual (constitutive) functions and by expressing newly acquired (inducible) properties—a process termed *endothelial activation* (Fig. 11-2). [4] [5] Inducers of endothelial activation include cytokines and bacterial products, which cause inflammation and septic shock (Chapter 2); hemodynamic stresses and lipid products, critical to the pathogenesis of atherosclerosis (see later); advanced glycosylation end products (important in diabetes, Chapter 24), as well as viruses, complement components, and hypoxia. Activated ECs, in turn, express adhesion molecules (Chapter 2), and produce other cytokines and chemokines, growth factors, vasoactive molecules that result either in vasoconstriction or in vasodilation, major histocompatibility complex molecules, procoagulant and anticoagulant moieties, and a variety of other biologically active products. ECs influence the vasoreactivity of the underlying smooth muscle cells through the production of both relaxing factors (e.g., nitric oxide [NO]) and contracting factors (e.g., endothelin). Normal endothelial function is characterized by a balance of these factors and the ability of the vessel to respond appropriately to various pharmacologic stimuli (e.g., vasorelaxation in response to acetylcholine).

Endothelial dysfunction, as defined by an altered phenotype that impairs vasoreactivity or induces a surface that is thrombogenic or abnormally adhesive to inflammatory cells, is responsible, at least in part, for the initiation of thrombus formation,

Figure 11-2 Endothelial cell response to environmental stimuli: causes (activators) and consequences (induced genes).

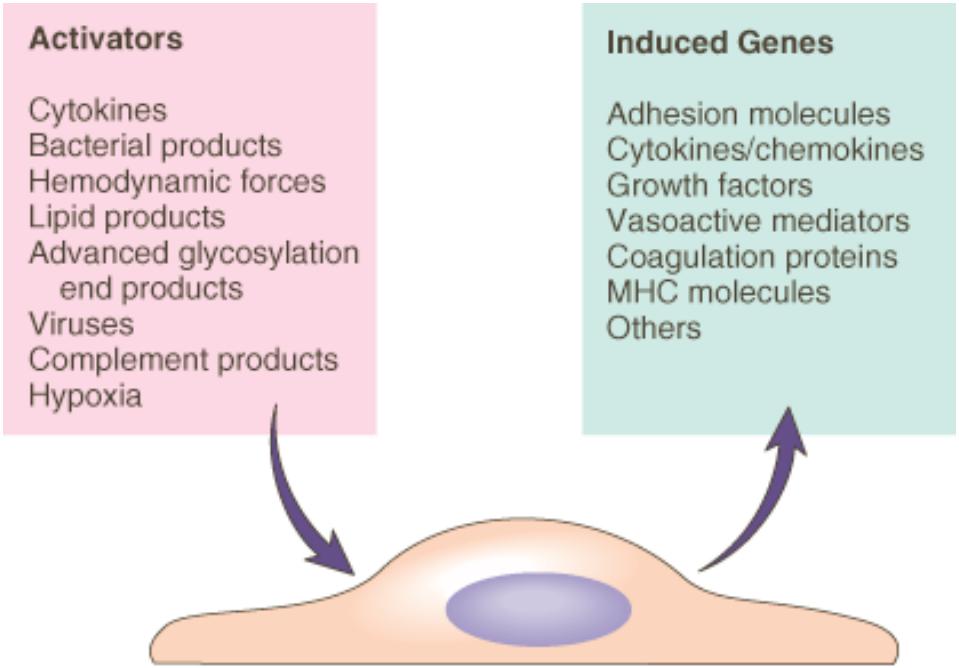


Figure 11-3 Schematic diagram of the mechanism of intimal thickening, emphasizing smooth muscle cell migration to, and proliferation and extracellular matrix elaboration in, the intima. *(Modified and redrawn from Schoen FJ: Interventional and Surgical Cardiovascular Pathology: Clinical Correlations and Basic Principles. Philadelphia, W.B. Saunders Co., 1989, p. 254.)*

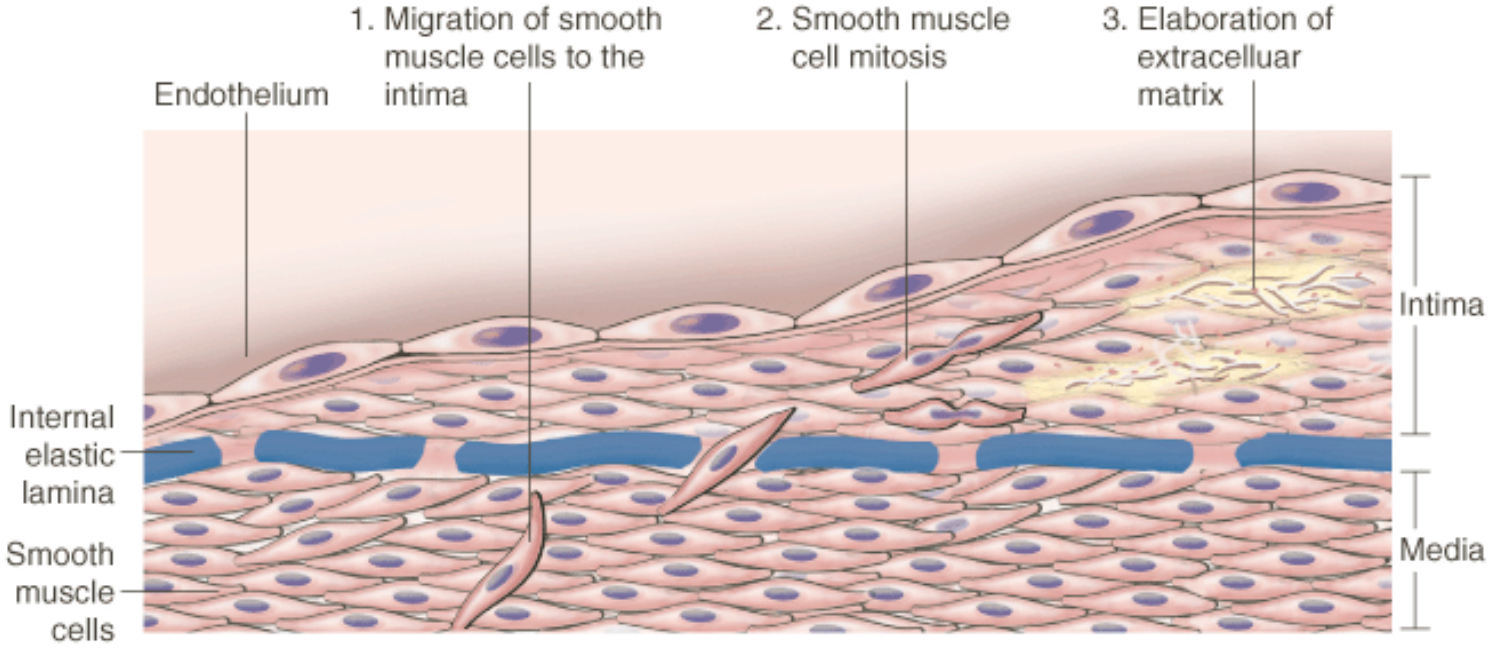


Figure 11-4 American Heart Association classification of human atherosclerotic lesions from the fatty dot (type I) to the complicated type VI lesion. The diagram also includes growth mechanisms and clinical correlations. *(Modified from Stary HC, et al: A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. Circulation 92:1355, 1995.)*

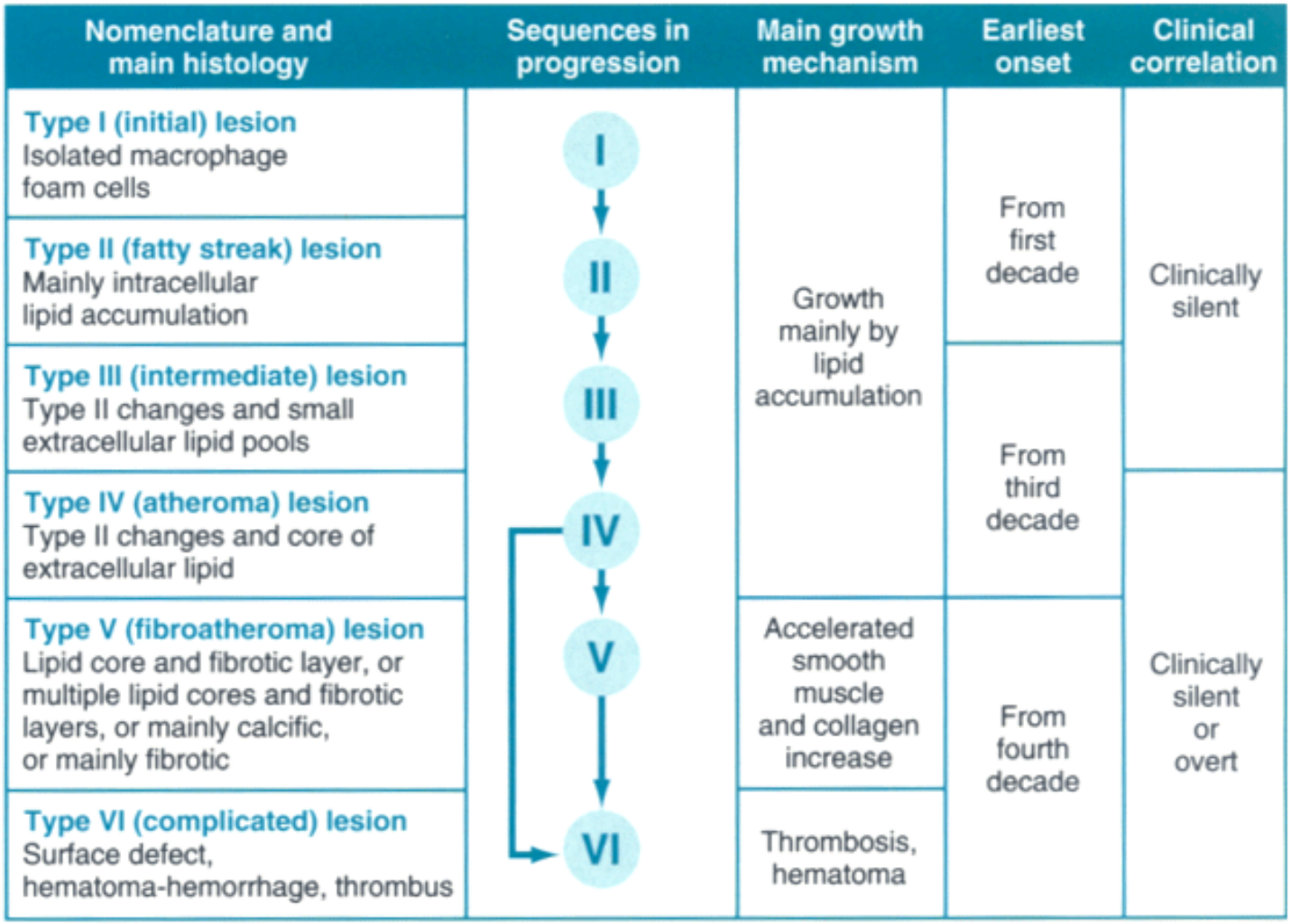


Figure 11-5 Schematic summary of the natural history, morphologic features, main pathogenetic events, and clinical complications of atherosclerosis in the coronary arteries.

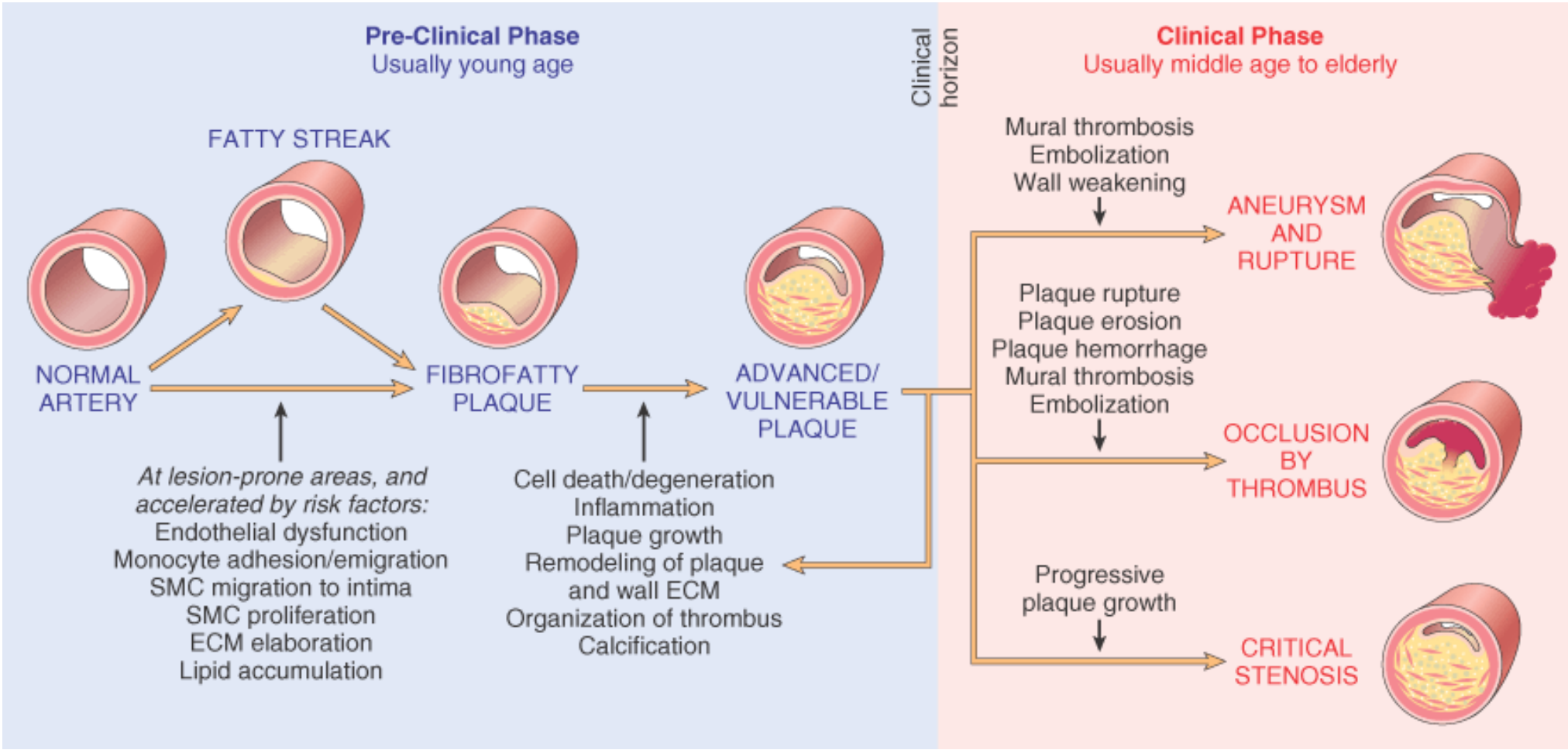


Figure 11-6 Fatty streak—a collection of foam cells in the intima. *A,* Aorta with fatty streaks (arrows), associated largely with the ostia of branch vessels. *B,* Close-up photograph of fatty streaks from aorta of experimental hypercholesterolemic rabbit shown following staining with Sudan red, a lipid-soluble dye, again illustrating the relationship of lesions to branch vessel ostia. *C,* Photomicrograph of fatty streak in experimental hypercholesterolemic rabbit, demonstrating intimal, macrophage-derived foam cells *(arrow). (B and C, Courtesy of Myron I. Cybulsky, M.D., University of Toronto, Canada).*

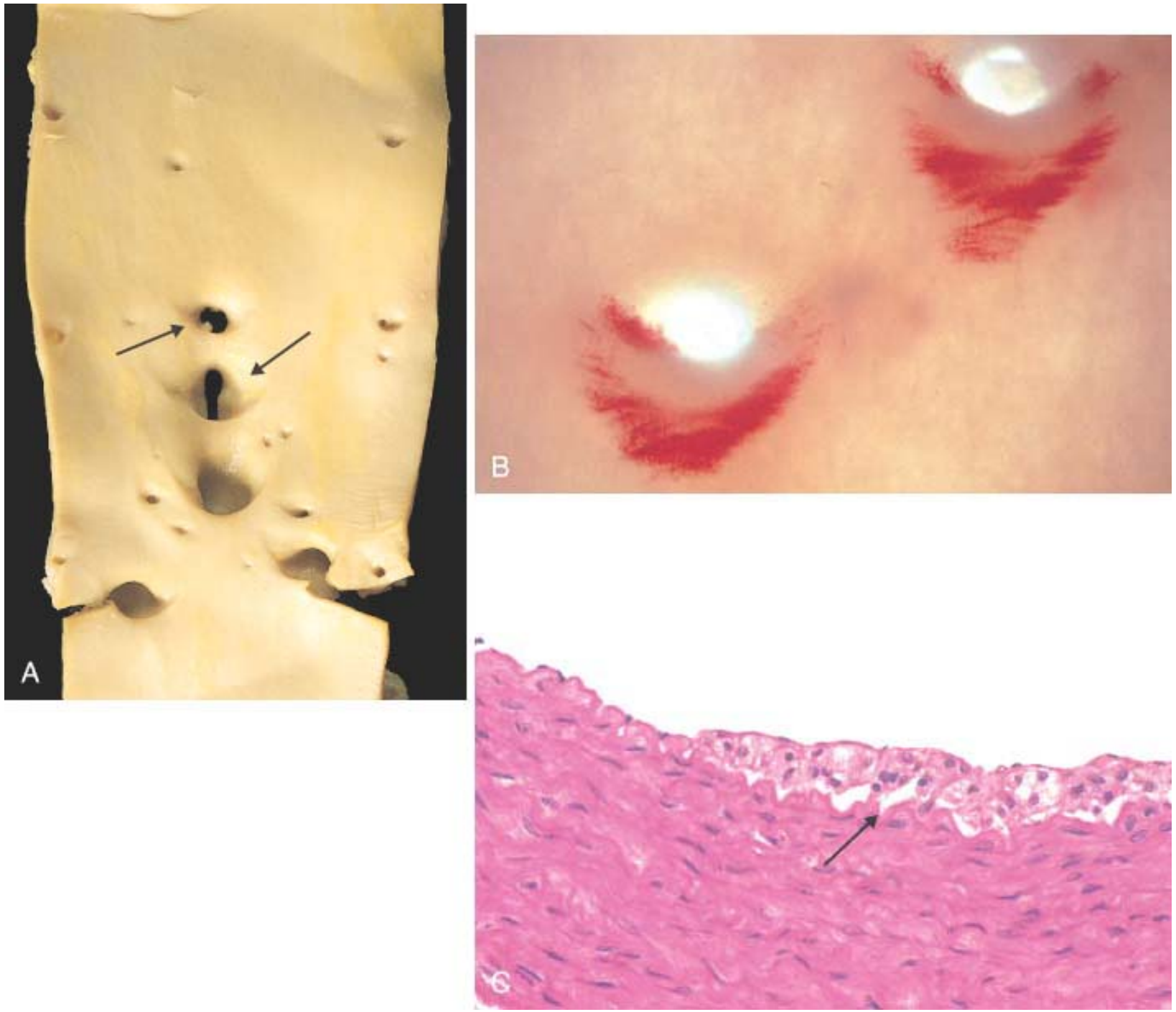


Figure 11-7 Schematic depiction of the major components of well-developed intimal atheromatous plaque overlying an intact media.

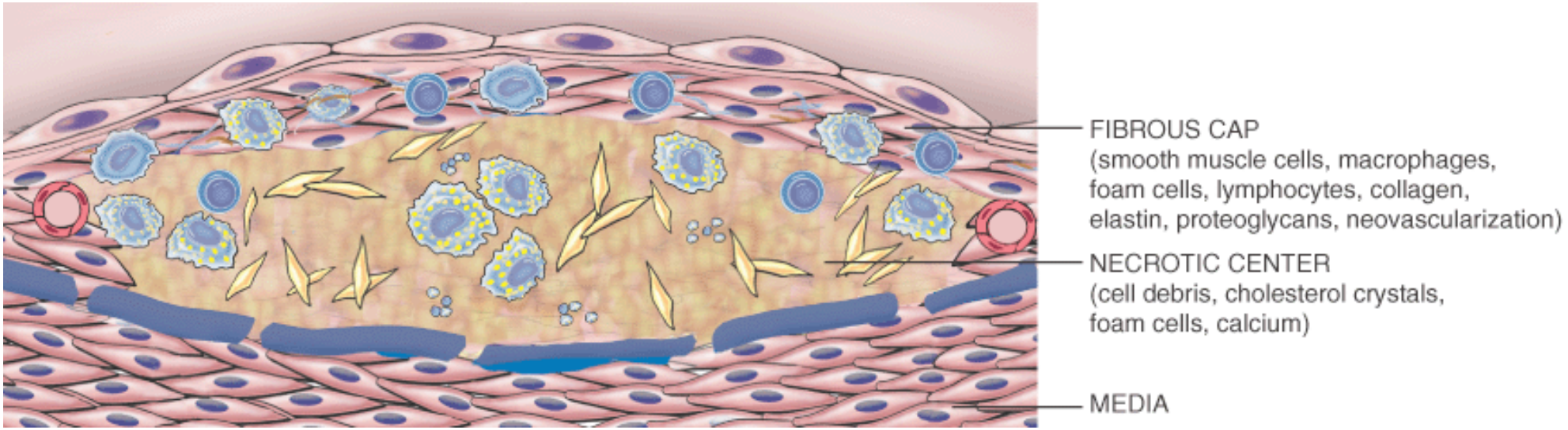


Figure 11-8 Gross views of atherosclerosis in the aorta. *A*, Mild atherosclerosis composed of fibrous plaques, one of which is denoted by the *arrow*. *B*, Severe disease with diffuse and complicated lesions.

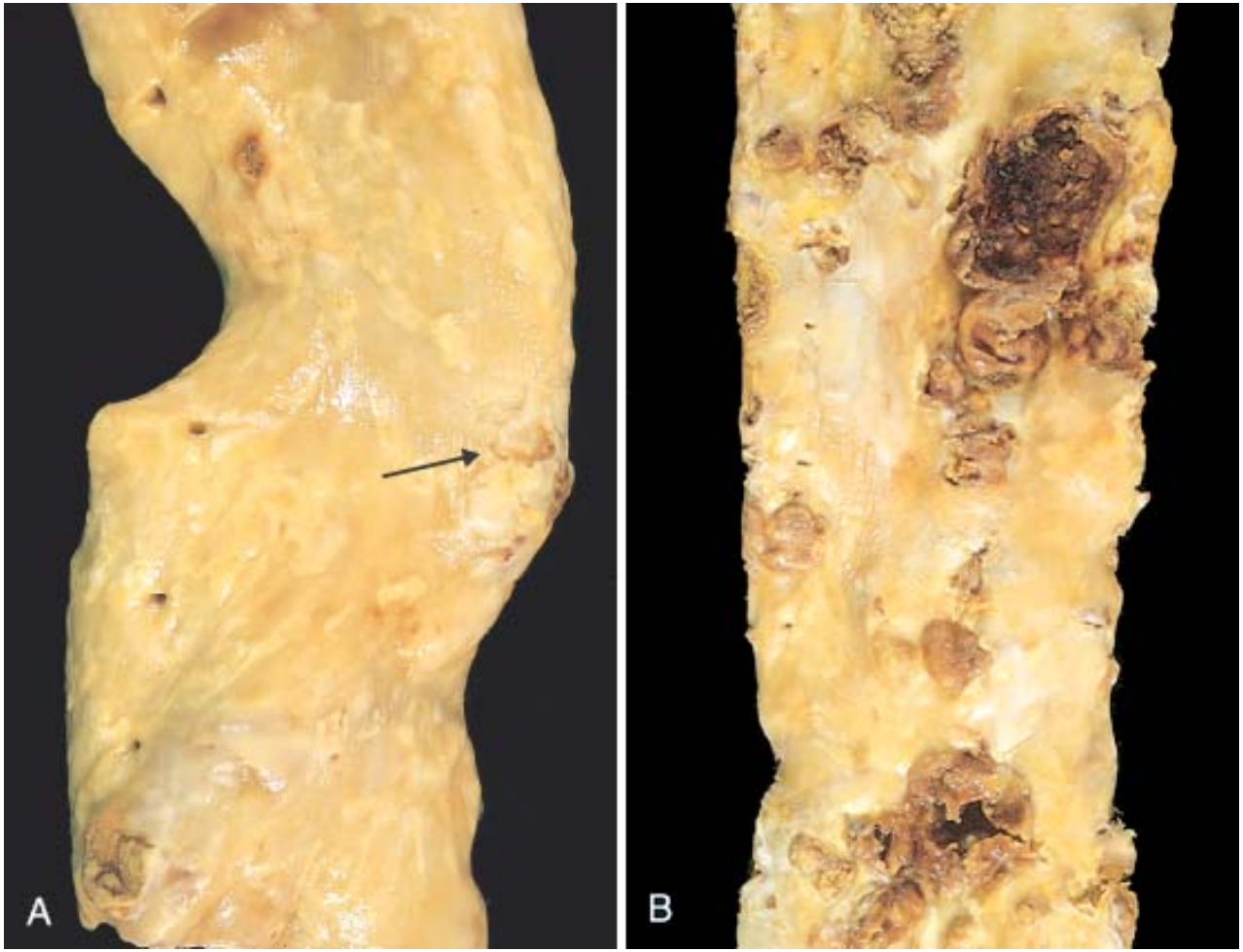


Figure 11-9 Histologic features of atheromatous plaque in the coronary artery. *A,* Overall architecture demonstrating fibrous cap (F) and a central necrotic (largely lipid) core (C). The lumen (L) has been moderately narrowed. Note that a segment of the wall is plaque free *(arrow).* In this section, collagen has been stained blue (Masson's trichrome stain). *B,* Higher-power photograph of a section of the plaque shown in *A,* stained for elastin (black), demonstrating that the internal and external elastic membranes are destroyed and the media of the artery is thinned under the most advanced plaque *(arrow). C,* Higher-magnification photomicrograph at the junction of the fibrous cap and core, showing scattered inflammatory cells, calcification *(broad arrow),* and neovascularization *(small arrows).*

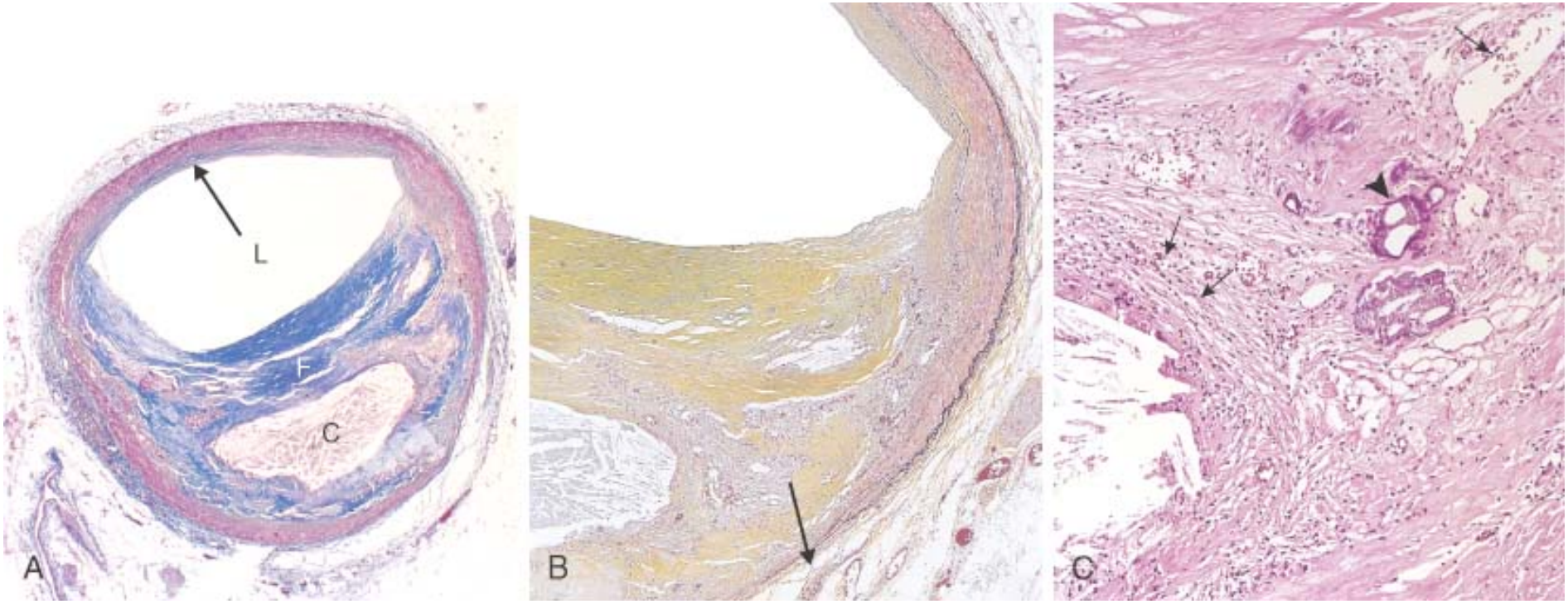


TABLE 11-2 -- Risk Factors for Atherosclerosis

Major	**Lesser, Uncertain, or Nonquantitated**
Nonmodifiable	
Increasing age	Obesity
Male gender	Physical inactivity
Family history	Stress ("type A" personality)
Genetic abnormalities	Postmenopausal estrogen deficiency
	High carbohydrate intake
Potentially Controllable	
Hyperlipidemia	Alcohol
Hypertension	Lipoprotein Lp(a)
Cigarette smoking	Hardened (trans)unsaturated fat intake
Diabetes	*Chlamydia pneumoniae*

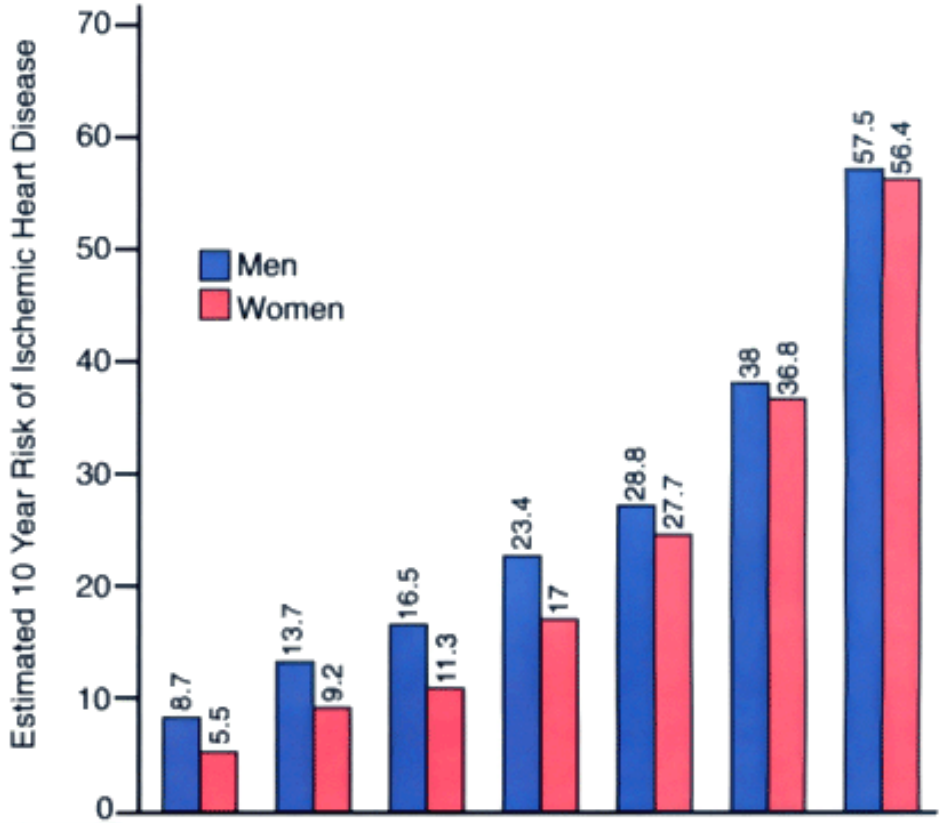
high blood lipid levels, such as familial hypercholesterolemia, which was discussed in Chapter 5 .

Other, nongenetic *risk factors, particularly diet, lifestyle, and personal habits, are to a large extent potentially reversible*. The four major risk factors potentially responsive to change are hyperlipidemia, hypertension, cigarette smoking, and diabetes.

Hyperlipidemia.

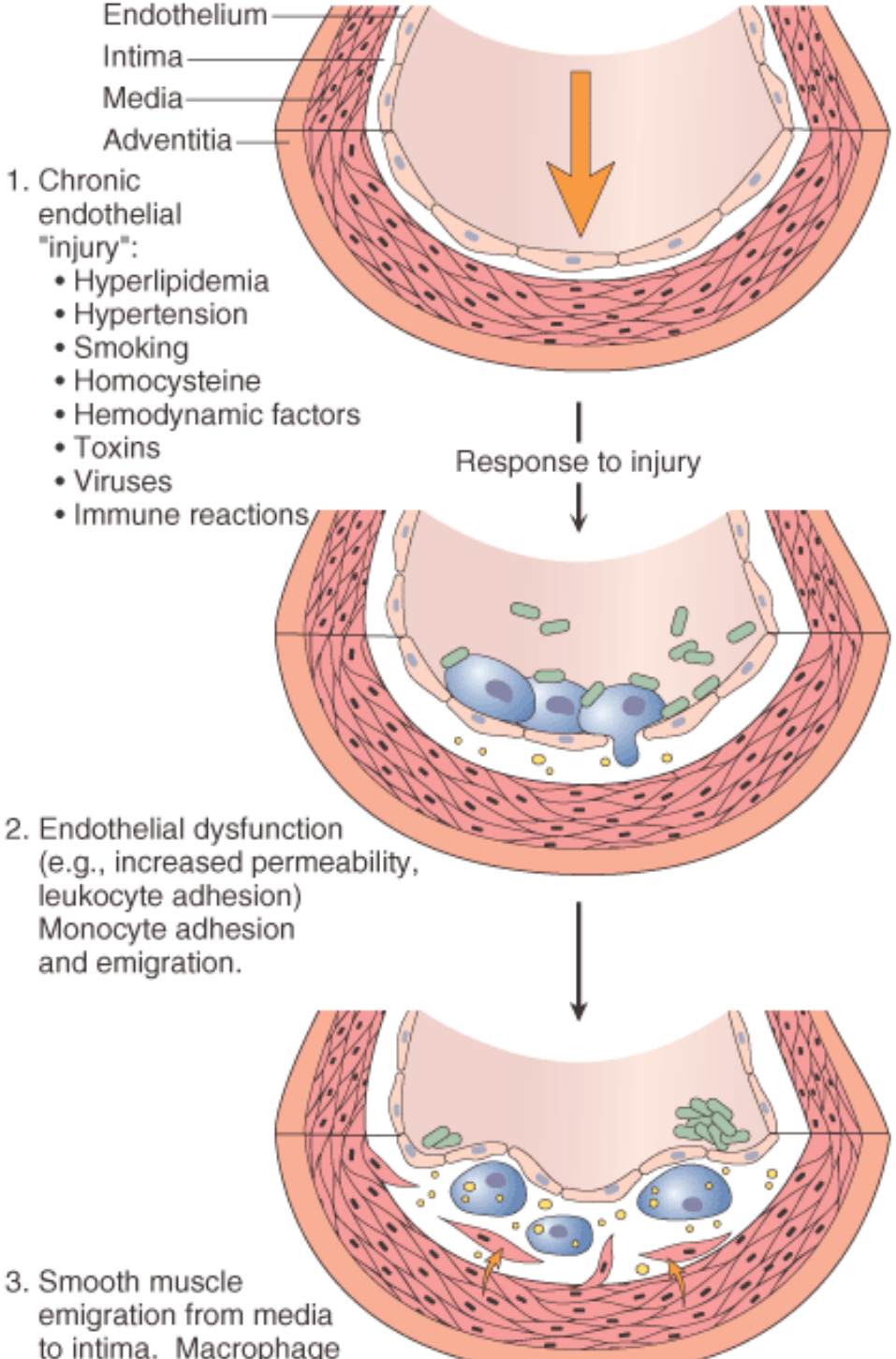
Hyperlipidemia is a major risk factor for atherosclerosis. Most of the evidence specifically implicates *hypercholesterolemia*. Elevated levels of serum cholesterol are sufficient to stimulate lesion development, even if other risk

Figure 11-10 Estimated 10-year risk of coronary artery disease according to various combinations of risk factor levels, expressed as the probability of an event in 10 years. HDL-C, high density lipoprotein cholesterol *(From Kannel WB, et al: An update on coronary risk factors. Med Clin North Am 79:951, 1995.)*



BP Systolic	120	160	160	160	160	160	160
Cholesterol	220	220	260	260	260	260	260
HDL-C	50	50	50	35	35	35	35
Diabetes	—	—	—	—	+	+	+
Cigarettes	—	—	—	—	—	+	+

Figure 11-11 Evolution of arterial wall changes in the response to injury hypothesis. *1*, Normal. *2*, Endothelial injury with adhesion of monocytes and platelets (the latter to denuded endothelium). *3*, Migration of monocytes (from the lumen) and smooth muscle cells (from the media) into the intima. *4*, Smooth muscle cell proliferation in the intima. *5*, Well-developed plaque (see Fig. 11-7 for details of mature plaque structure).



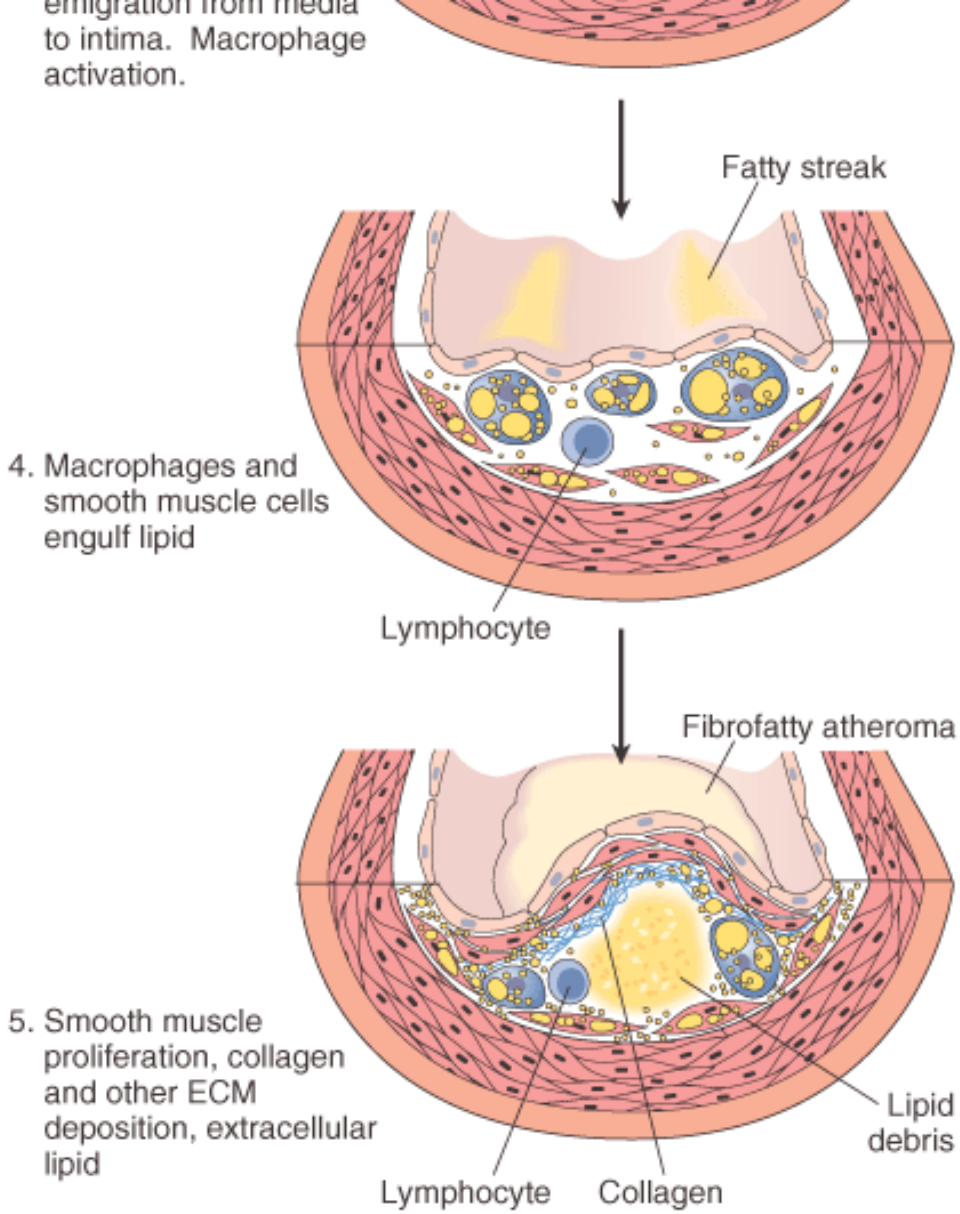


Figure 11-12 Schematic diagram of hypothetical sequence of cellular interactions in atherosclerosis. Hyperlipidemia and other risk factors are thought to cause endothelial injury, resulting in adhesion of platelets and monocytes and release of growth factors, including platelet-derived growth factor (PDGF), which lead to smooth muscle cell migration and proliferation. Foam cells of atheromatous plaques are derived from both macrophages and smooth muscle cells—from macrophages via the very-low-density lipoprotein (VLDL) receptor and low-density lipoprotein (LDL) modifications recognized by scavenger receptors (e.g., oxidized LDL), and from smooth muscle cells by less certain mechanisms. Extracellular lipid is derived from insudation from the vessel lumen, particularly in the presence of hypercholesterolemia, and also from degenerating foam cells. Cholesterol accumulation in the plaque reflects an imbalance between influx and efflux, and high-density lipoprotein (HDL) likely helps clear cholesterol from these accumulations. Smooth muscle cells migrate to the intima, proliferate, and produce extracellular matrix, including collagen and proteoglycans.

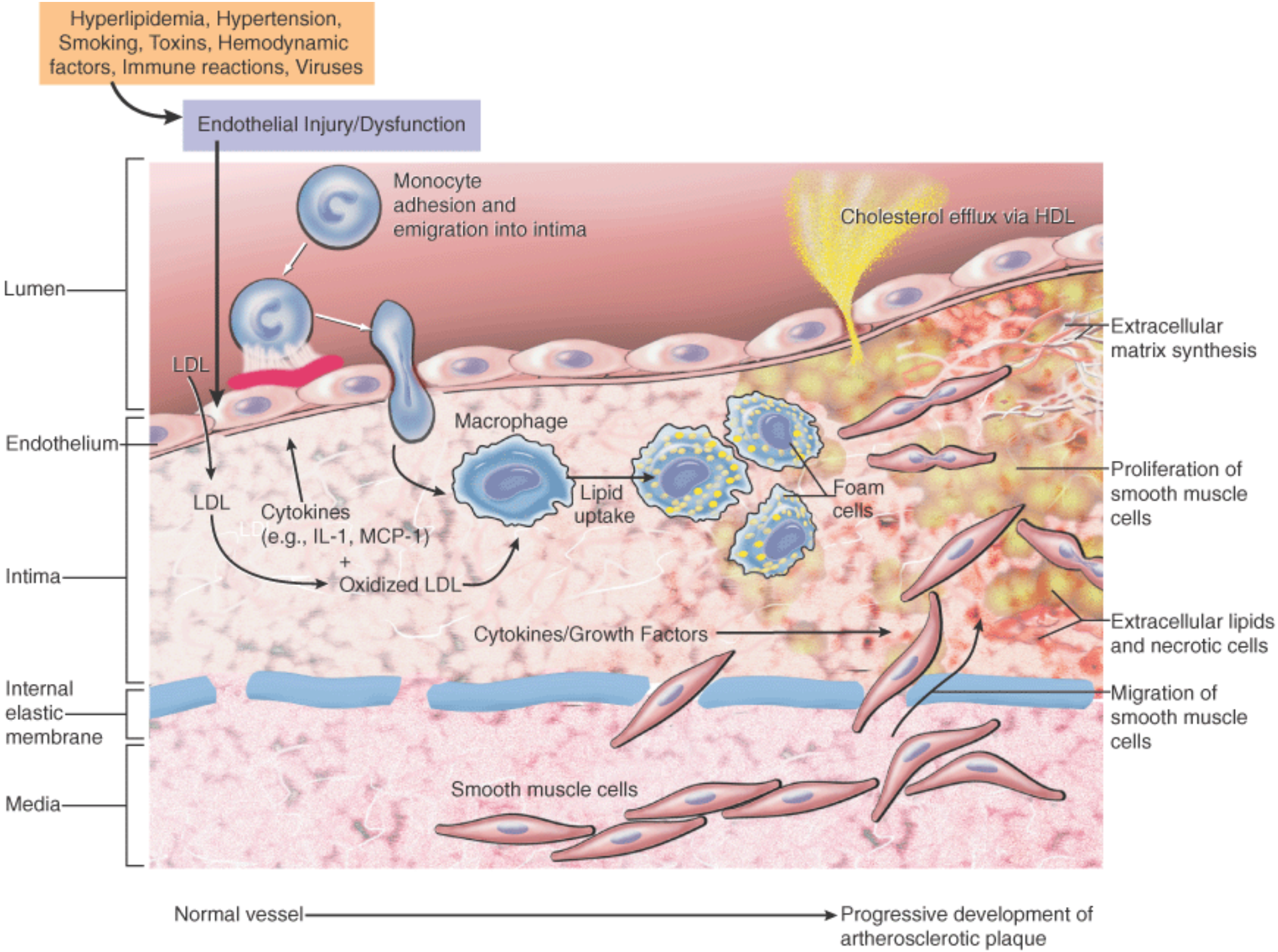

Hyperlipidemia, Hypertension, Smoking, Toxins, Hemodynamic factors, Immune reactions, Viruses
Endothelial Injury/Dysfunction
Monocyte adhesion and emigration into intima
Cholesterol efflux via HDL
Lumen
Endothelium
Intima
Internal elastic membrane
Media
LDL
LDL
Cytokines (e.g., IL-1, MCP-1) + Oxidized LDL
Macrophage
Lipid uptake
Foam cells
Extracellular matrix synthesis
Proliferation of smooth muscle cells
Extracellular lipids and necrotic cells
Migration of smooth muscle cells
Cytokines/Growth Factors
Smooth muscle cells
Normal vessel
Progressive development of artherosclerotic plaque

TABLE 11-3 -- Types and Causes of Hypertension

Essential Hypertension
Secondary Hypertension
••Renal
••••Acute glomerulonephritis
••••Chronic renal disease
••••Polycystic disease
••••Renal artery stenosis
••••Renal artery fibromuscular dysplasia
••••Renal vasculitis
••••Renin-producing tumors
••Endocrine
••••Adrenocortical hyperfunction (Cushing syndrome, primary aldosteronism, congenital adrenal hyperplasia, licorice ingestion)
••••Exogenous hormones (glucocorticoids, estrogen [including pregnancy-induced and oral contraceptives], sympathomimetics and tyramine-containing foods, monoamine oxidase inhibitors)
••••Pheochromocytoma
••••Acromegaly
••••Hypothyroidism (myxedema)
••••Hyperthyroidism (thyrotoxicosis)
••••Pregnancy-induced
••Cardiovascular
••••Coarctation of aorta
••••Polyarteritis nodosa (or other vasculitis)
••••Increased intravascular volume
••••Increased cardiac output
••••Rigidity of the aorta
••Neurologic
••••Psychogenic
••••Increased intracranial pressure
••••Sleep apnea

••••Acute stress, including surgery

Cushing syndrome, pheochromocytoma), narrowing of the renal artery, usually by an atheromatous plaque (renovascular hypertension) or other identifiable cause (secondary hypertension). *However, about 95% of hypertension is idiopathic (called essential hypertension). This form of hypertension generally does not cause short-term problems;* especially when controlled, is compatible with long life and is asymptomatic, unless a myocardial infarction, cerebrovascular accident, or other complication supervenes. Thus, this subgroup is often called *benign hypertension*.

A small percentage, perhaps 5%, of hypertensive persons show a rapidly rising blood pressure that if untreated, leads to death within a year or two. Called *accelerated* or *malignant hypertension*, the clinical syndrome is characterized by severe hypertension (i.e., systolic pressure over 200 mm Hg, diastolic pressure over 120 mm Hg), renal failure, and retinal hemorrhages and exudates, with or without papilledema. It may develop in previously normotensive persons but more often is superimposed on pre-existing benign hypertension, either essential or secondary.

Pathogenesis of Hypertension.

The multiple mechanisms of hypertension constitute aberrations of the normal physiologic regulation of blood pressure.[36] *Arterial hypertension occurs when the relationship between cardiac output and total peripheral resistance is altered.* For many of the secondary forms of hypertension, these factors are reasonably well understood. For example, in *renovascular hypertension*, renal artery stenosis causes decreased glomerular flow and pressure in the afferent arteriole of the glomerulus. This (1) induces renin secretion, initiating angiotensin II-mediated vasoconstriction and increased peripheral resistance, and (2) increases sodium reabsorption and therefore blood volume through the aldosterone mechanism. In pheochromocytoma, a tumor of the adrenal medulla (see Chapter 24), catecholamines produced by tumor cells cause episodic vasoconstriction and thus induce hypertension.

Regulation of Normal Blood Pressure.

Blood pressure is proportional to cardiac output and peripheral vascular resistance (Fig. 11-13). *Indeed, the blood pressure level is a complex trait that is determined by the interaction of multiple genetic, environmental, and demographic factors that influence cardiac output and vascular resistance.* The major factors that determine blood pressure variation within and between populations include age, gender, body mass index, and diet, principally sodium intake.

Cardiac output is highly dependent on blood volume, itself greatly influenced by the whole body sodium homeostasis. Peripheral vascular resistance is determined mainly at the level of the arterioles and is affected by neural and hormonal factors. Normal vascular tone reflects the balance between humoral vasoconstricting influences (including angiotensin II, catecholamines, and endothelin) and vasodilators (including kinins, prostaglandins, and NO). Resistance vessels also exhibit *autoregulation*, whereby increased blood flow induces vasoconstriction to protect against tissue hyperperfusion. Other local factors such as pH and hypoxia, and the α- and β-adrenergic systems, which influence heart rate, cardiac contraction, and vascular tone, may be important. The integrated function of these systems ensures adequate perfusion of all tissues, despite regional differences in demand.

The kidneys play an important role in blood pressure regulation as follows:

Figure 11-13 The critical roles of cardiac output and peripheral resistance in blood pressure regulation. NO, nitric oxide.

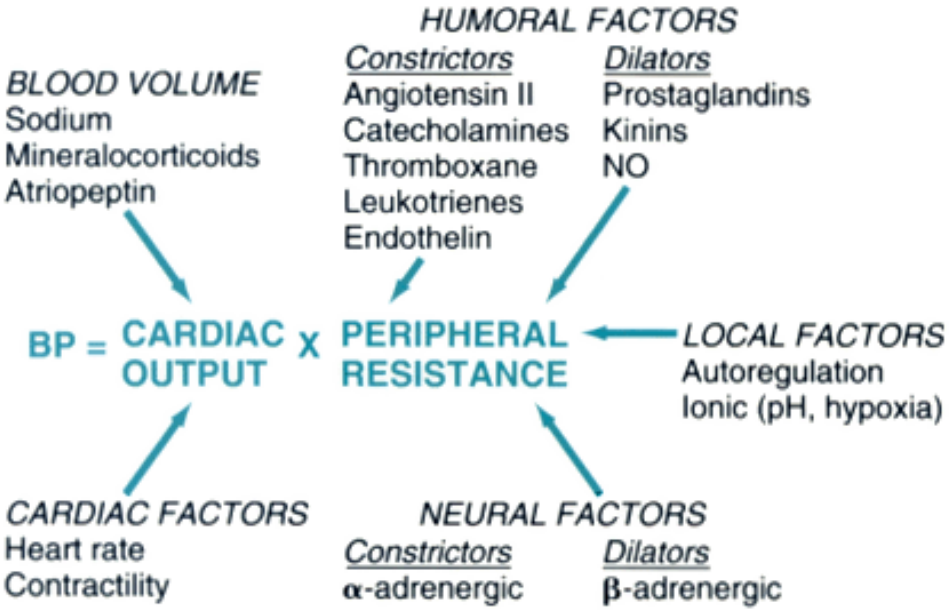


Figure 11-14 Blood pressure regulation by the renin-angiotensin system and the central roles of sodium metabolism in specific causes of inherited and acquired forms of hypertension. Components of the systemic renin-angiotensin system are shown in black. Genetic disorders that affect blood pressure by altering activity of this pathway are indicated in red; *arrows* indicate sites in the pathway altered by mutation. Genes that are mutated in these disorders are indicated in parentheses. Acquired disorders that alter blood pressure through effects on this pathway are indicated in blue. *(From Lifton RP, et al: Molecular genetics of human blood pressure variation. Science 272:676, 1996.)*

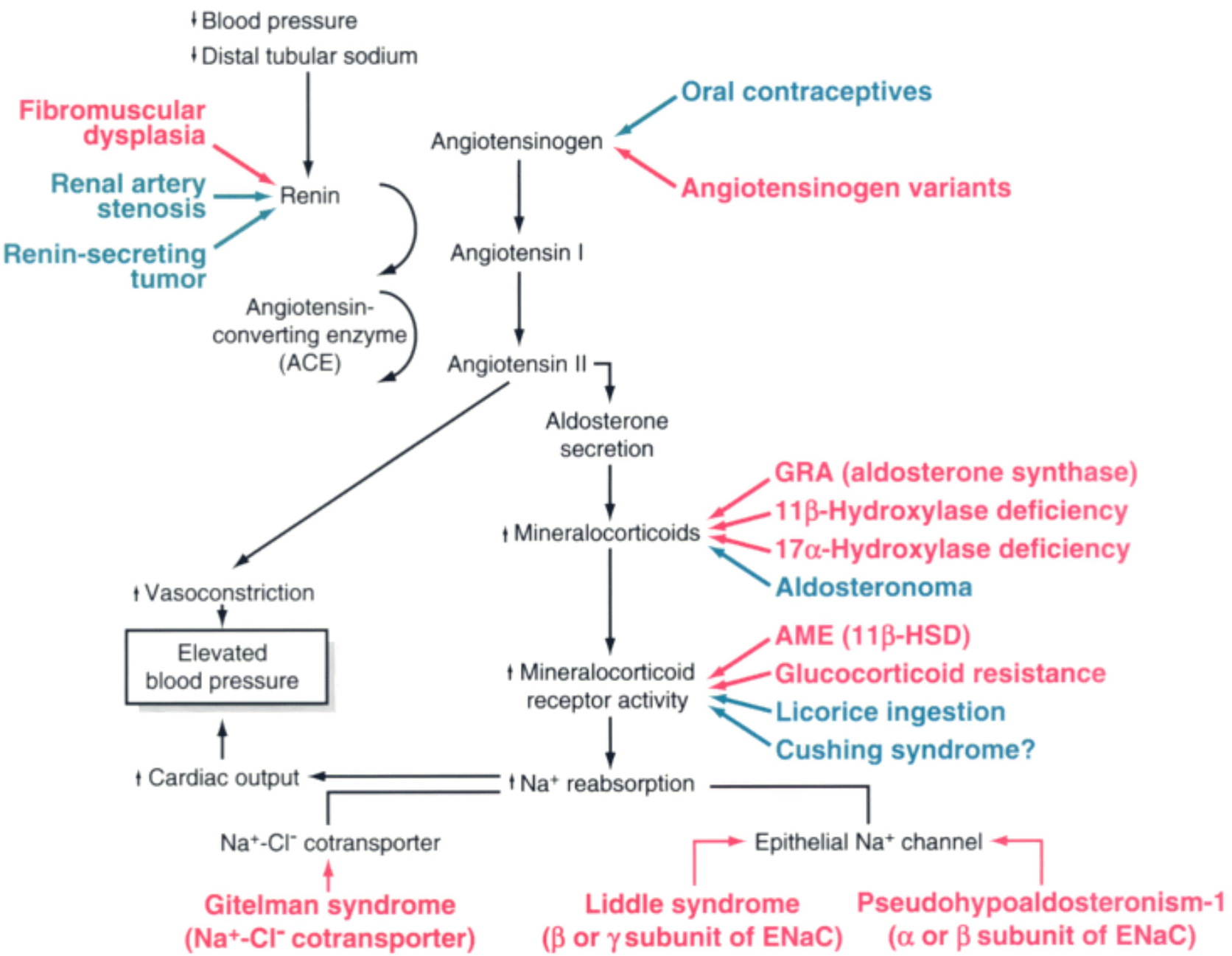


Figure 11-15 Hypothetical scheme for the pathogenesis of essential hypertension, implicating genetic defects in renal excretion of sodium, functional regulation of vascular tone, and structural regulation of vascular caliber. Environmental factors, especially increased salt intake, may potentiate the effects of genetic factors. The resultant increases in cardiac output and peripheral resistance contribute to hypertension. ECF, extracellular fluid.

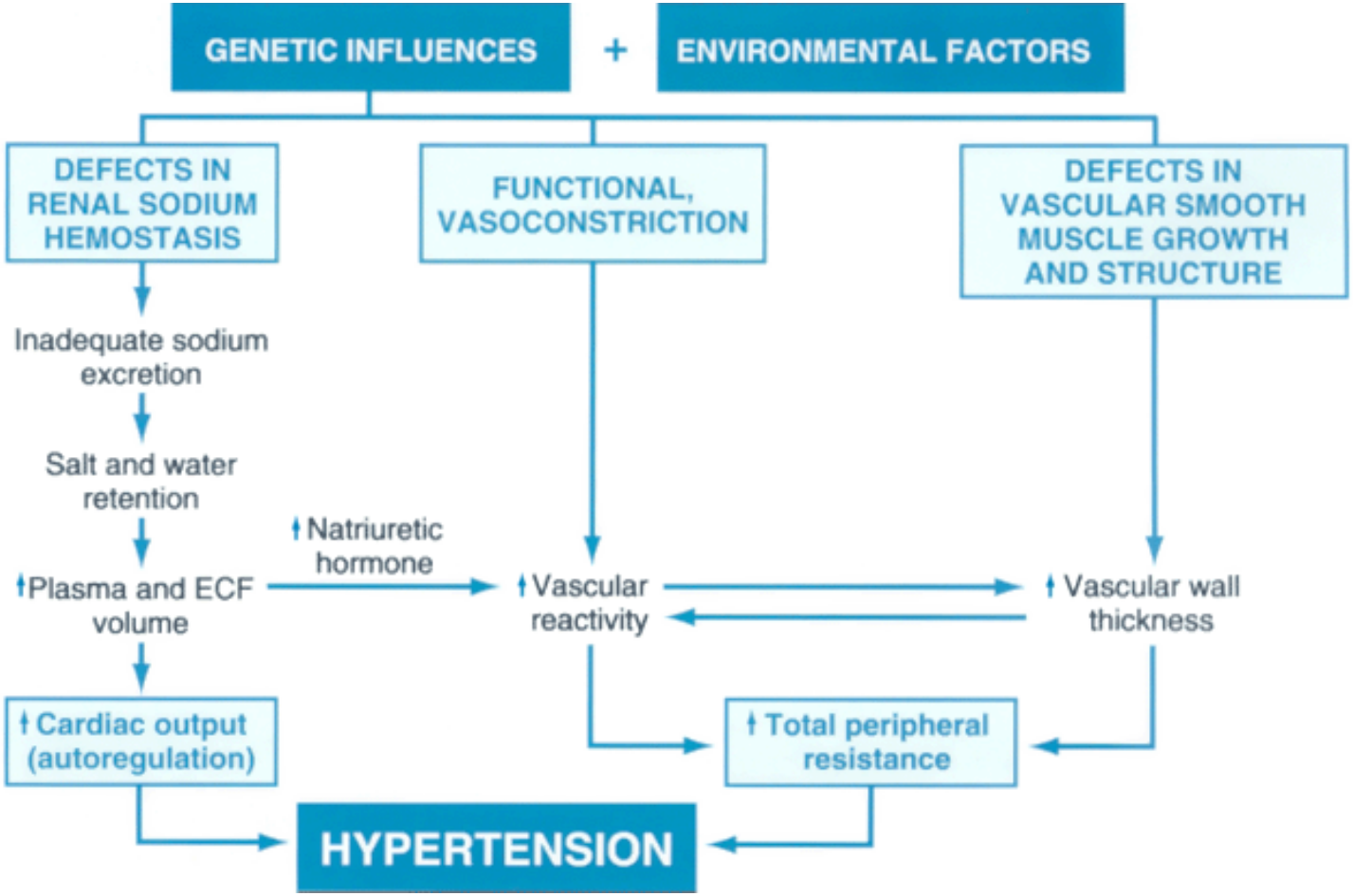


Figure 11-16 Mutations altering blood pressure in humans. A diagram of a nephron, the filtering unit of the kidney, is shown. The molecular pathways mediating NaCl reabsorption in individual renal cells in the thick ascending limb of the loop of Henle (TAL), distal convoluted tubule (DCT), and the cortical collecting tubule (CCT) are indicated, along with the pathway of the renin-angiotensin system, the major regulator of renal salt reabsorption. Single gene defects that manifest as inherited diseases affecting these pathways are indicated, with hypertensive disorders in red and hypotensive disorders in blue. Abbreviations: Al, angiotensin I; ACE, angiotensin converting enzyme; All, angiotensin II; MR, mineralocorticoid receptor; GRA, glucocorticoid-remediable aldosteronism; PHA1, pseudohypoaldosteronism, type 1; AME, apparent mineralocorticoid excess; 11β-HSD2, 11β-hydroxysteroid dehydrogenase-2; and DOC, deoxycorticosterone. *(From Lifton RP, et al: Molecular mechanisms of human hypertension. Cell 104:545, 2001.)*

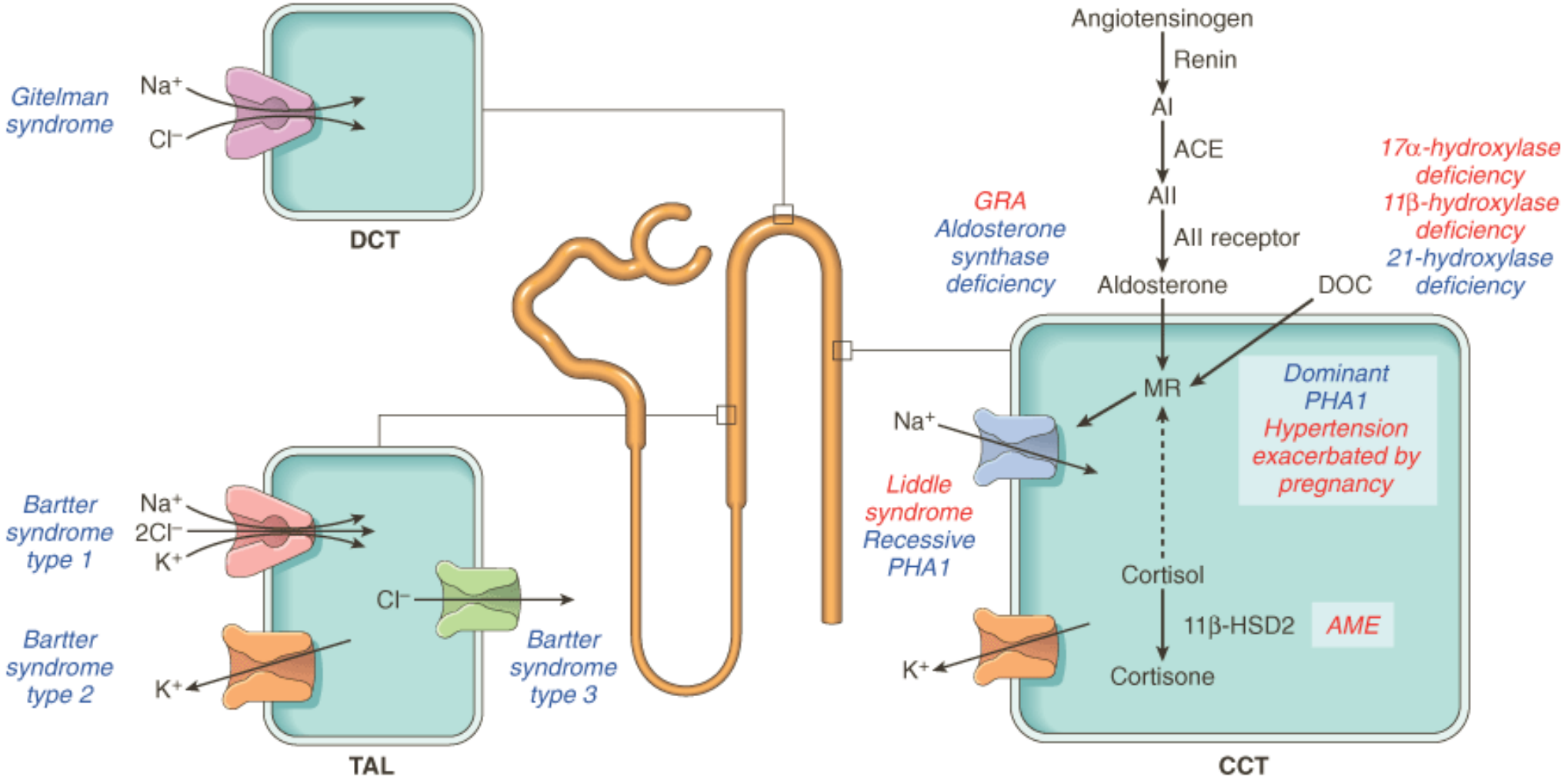


Figure 11-17 Vascular pathology in hypertension. *A,* Hyaline arteriolosclerosis. The arteriolar wall is hyalinized, and the lumen is markedly narrowed. *B,* Hyperplastic arteriolosclerosis (onionskinning) causing luminal obliteration *(arrow),* with secondary ischemic changes, manifested by wrinkling of the glomerular capillary vessels at the upper left (periodic acid-Schiff [PAS] stain). *(Courtesy of Helmut Rennke, M.D., Brigham and Women's Hospital, Boston, MA.)*

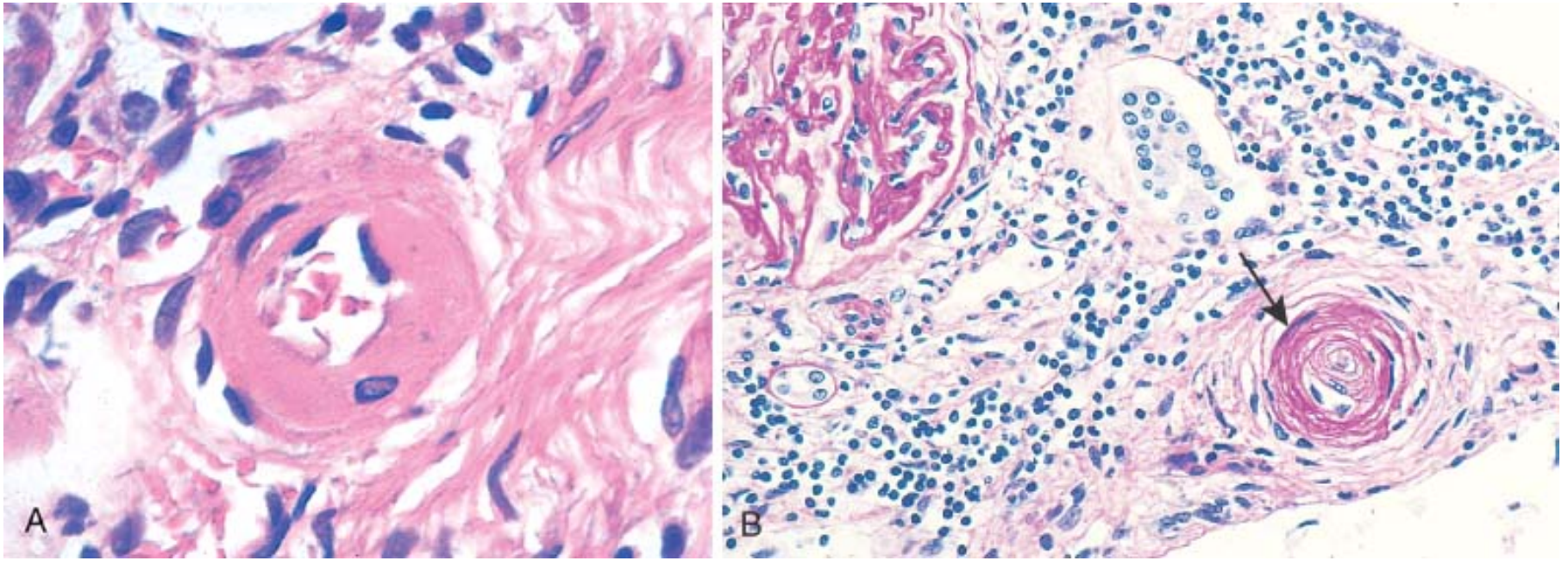


Figure 11-18 True and false aneurysms. *Center,* Normal vessel. *Left,* True aneurysm. The wall bulges outward and may be attenuated but is intact. *Right,* False aneurysm. The wall is ruptured, and there is a collection of blood (hematoma) that is bounded externally by adherent extravascular tissues.

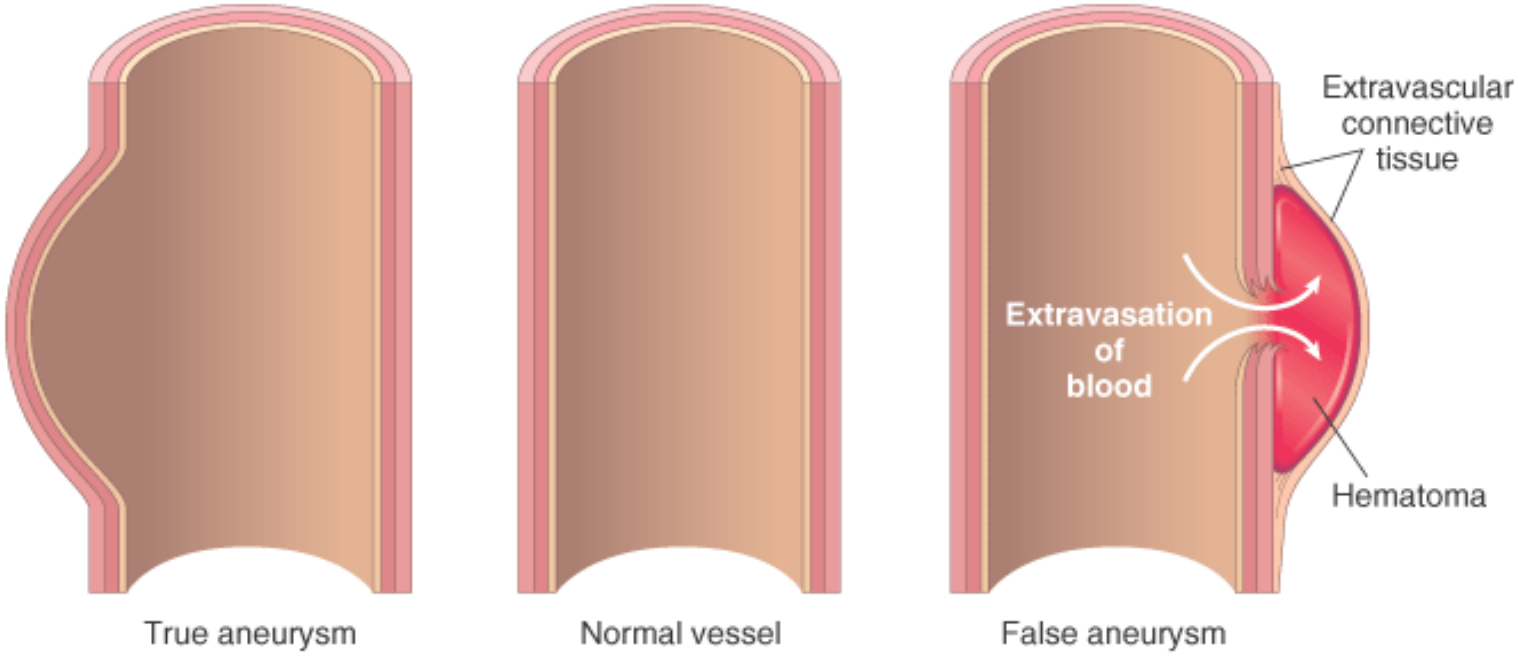


Figure 11-19 Abdominal aortic aneurysm. *A,* External view, gross photograph of a large aortic aneurysm that ruptured; the rupture site is indicated by the *arrow*. *B,* Opened view, with the location of the rupture tract indicated by a probe. The wall of the aneurysm is exceedingly thin, and the lumen is filled by a large quantity of layered but largely unorganized thrombus.

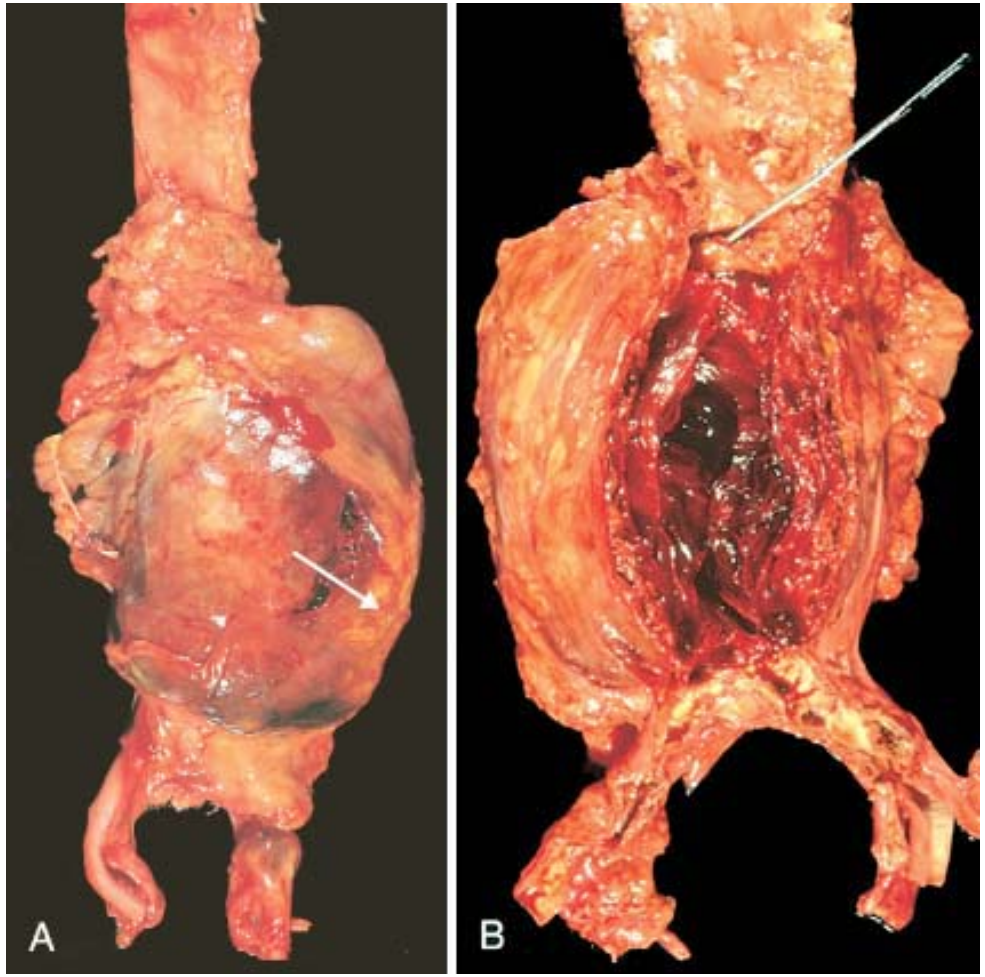


Figure 11-20 Aortic dissection. *A,* Gross photograph of opened aorta with proximal dissection, demonstrating a small, oblique intimal tear (demarcated by probe), allowing blood to enter the media, creating an intramural hematoma *(thin arrows)*. Note that the intimal tear has occurred in a region largely free from atherosclerotic plaque, and that propagation of the intramural hematoma was arrested at a site more distally, where atherosclerosis begins *(broad arrow). B,* Histologic view of the dissection demonstrating an aortic intramural hematoma *(asterisk)*. Aortic elastic layers are black, and blood is red in this section, stained with the Movat stain.

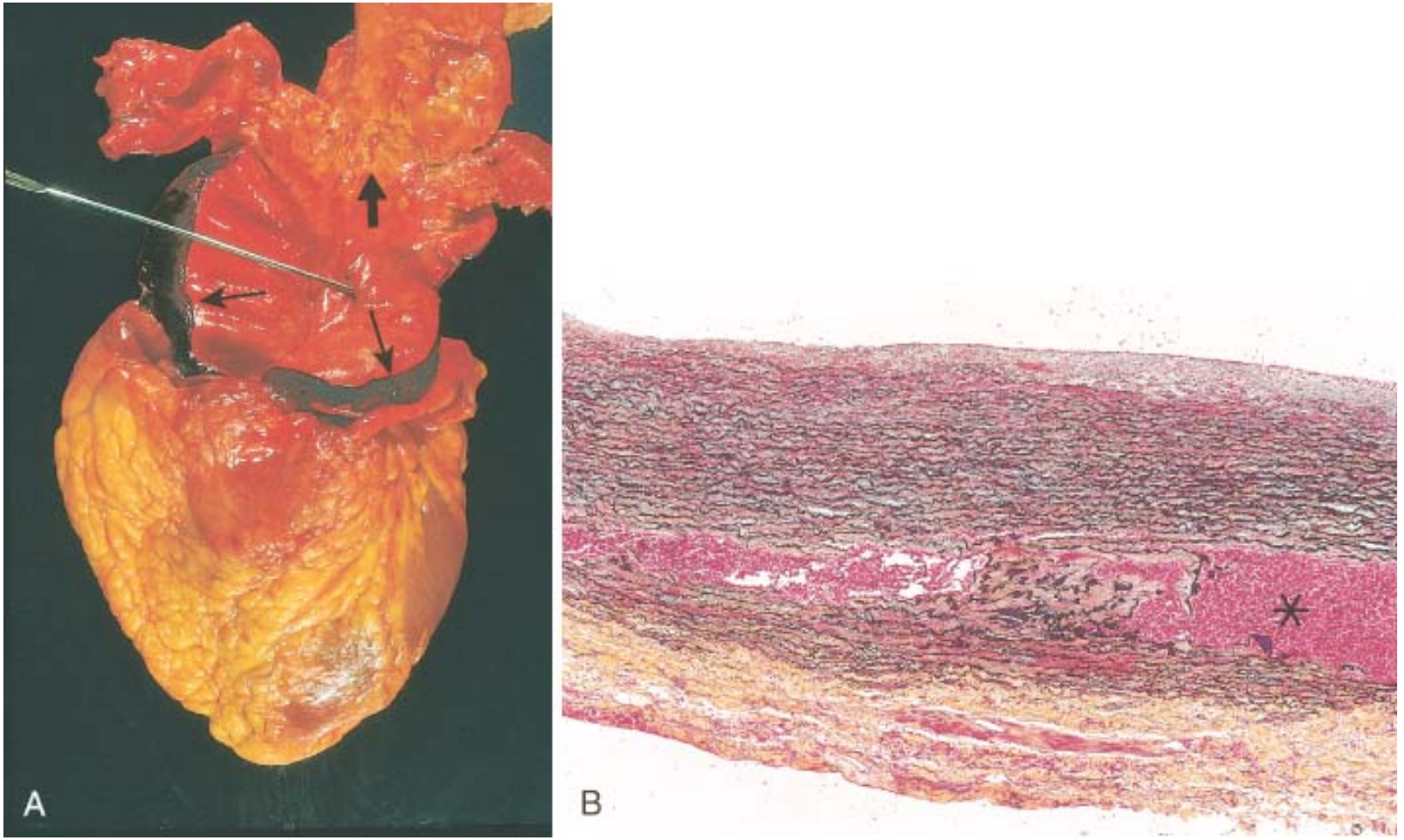


Figure 11-21 Medial degeneration. *A,* Cross-section of aortic media with marked elastin fragmentation and formation of areas devoid of elastin that resemble cystic spaces, from a patient with Marfan syndrome. *B,* Normal media for comparison, showing the regular layered pattern of elastic tissue. In both *A* and *B,* the tissue section is stained to highlight elastin as black.

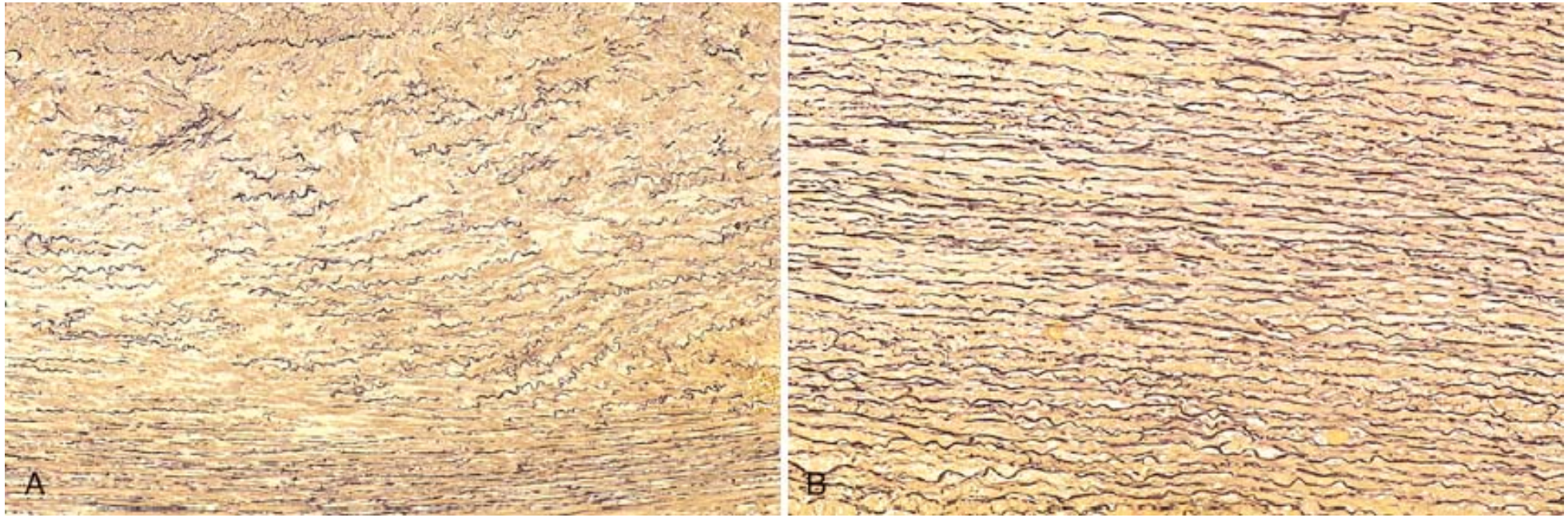


Figure 11-22 Classification of dissection into types A and B. Type A (proximal) involves the ascending aorta, whereas type B (distal) does not. The serious complications predominantly occur in the region from the aortic valve through the arch.

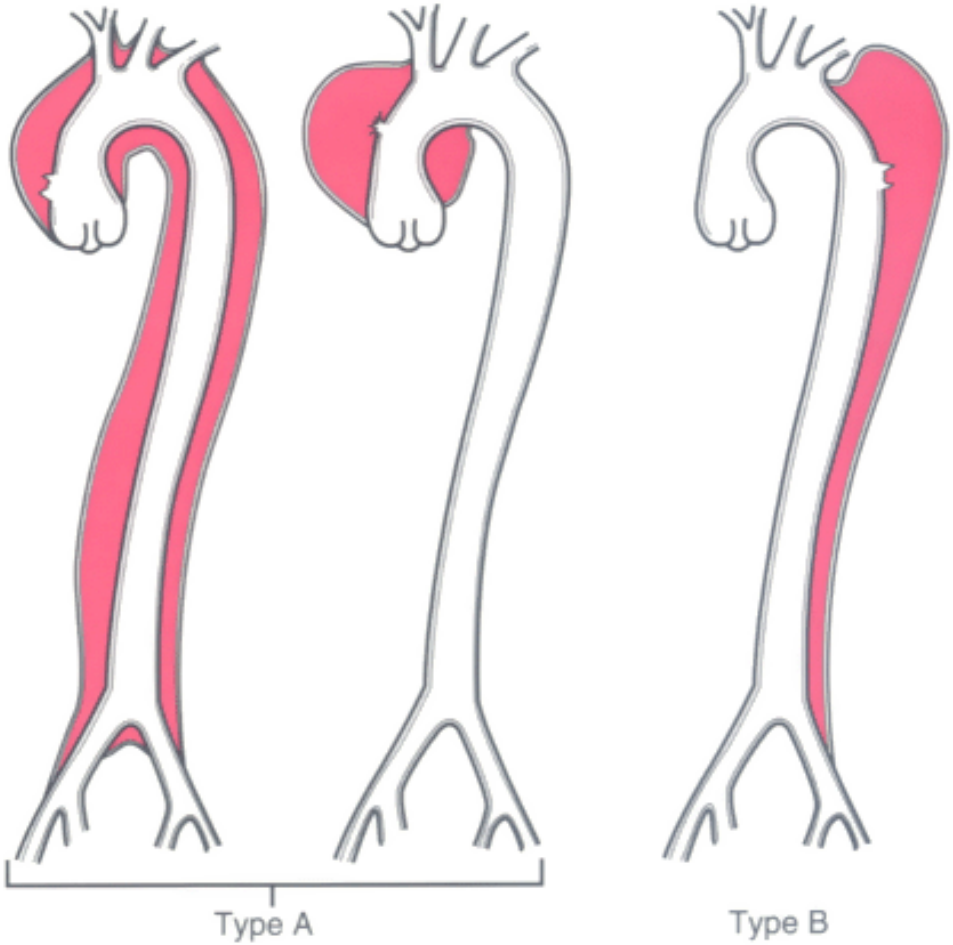


TABLE 11-4 -- Classification of Vasculitis Based on Pathogenesis

(Not Available)
Data from Jennette JC, Falk RJ: Update on the pathobiology of vasculitis. In Schoen FJ, Gimbrone MA (eds); Cardiovascular Pathology: Clinicopathologic Correlations and Pathogenetic Mechanisms. Baltimore, Williams & Wilkins, 1995, p 156.

chemical injury, such as irradiation, mechanical trauma, and toxins can also cause vascular damage.

Pathogenesis of Noninfectious Vasculitis.

The main immunologic mechanisms that initiate noninfectious vasculitis are: (1) immune complex deposition, (2) antineutrophil cytoplasmic antibodies, and (3) anti-endothelial cell antibodies.

Immune Complexes.

The evidence for involvement of immune complexes in vasculitides can be summarized as follows:

- The vascular lesions resemble those found in experimental immune complex-mediated conditions, such as the local Arthus phenomenon and serum sickness (Chapter 6). Immune reactants and complement can be detected in the serum or vessels of patients with vasculitis (e.g., DNA-anti-DNA complexes are present in the vascular lesions of systemic lupus erythematosus-associated vasculitis and IgG, IgM, and complement in cryoglobulinemic vasculitis).
- Hypersensitivity to drugs causes approximately 10% of vasculitic skin lesions, largely through vascular deposits of immune complexes. Some, such as penicillin, conjugate serum proteins; others, like streptokinase, are themselves foreign proteins. The manifestations vary and range from small-vessel hypersensitivity and leukocytoclastic vasculitis to polyarteritis nodosa, Wegener granulomatosis, and Churg-Strauss syndrome (see later for descriptions of these entities), and from mild and self-limiting to severe and even fatal. Identification of the disorder as a drug reaction is particularly important, as discontinuation of the offending agent is often followed by rapid improvement. [50]
- In vasculitis associated with viral infections, immune complexes can be found in the serum and in the vascular lesions of some patients, particularly in cases of polyarteritis nodosa (for example, HBsAg-anti-HbsAg in hepatitis-induced vasculitis).

Whether immune complexes deposit in vessel walls from the circulation, or are formed in situ, or both, is not known (see Chapter 6). However, many small vessel vasculitides show a paucity of vascular immune deposits and therefore other mechanisms have been sought for these so-called pauci-immune vasculitides.

Antineutrophil Cytoplasmic Antibodies.

Serum from many patients with vasculitis reacts with cytoplasmic antigens in neutrophils, indicating the presence of *antineutrophil cytoplasmic antibodies (ANCAs)*.[51] ANCAs are a heterogeneous group of autoantibodies directed against enzymes mainly found within the azurophil or primary granules in neutrophils, in the lysosomes of monocytes, and in ECs. The description of these autoantibodies is based on the immunofluorescent patterns of staining of ethanol-fixed neutrophils. Two main patterns are recognized: one shows cytoplasmic localization of the staining (c-ANCA), and the most common target antigen is proteinase-3 (PR3), a neutrophil granule constituent. The second shows perinuclear staining (p-ANCA) and is usually specific for myeloperoxidase (MPO). Either ANCA specificity may occur in a patient with ANCA-associated small-vessel vasculitis but c-ANCA is typically found in Wegener granulomatosis and p-ANCA is found in most cases of microscopic polyangiitis and Churg-Strauss syndrome. The disorders characterized by circulating ANCAs are called the *ANCA-associated vasculitides*.

ANCAs serve as useful quantitative diagnostic markers for these conditions, and their levels may reflect the degree of inflammatory activity.[52] ANCAs rise in episodes of recurrence, and thus are useful in management. In addition, the close association between ANCA titers and disease activity, particularly c-ANCA in Wegener granulomatosis, suggests that they may be important in the pathogenesis of this disease. Experimental data are consistent with a pathophysiologic mechanism for ANCA and/or ANCA antigen autoimmune responses in these diseases, but the precise mechanisms are unknown. However, there is yet no definitive proof that ANCAs play a causative role in the development of systemic vasculitis.

One plausible hypothesis for a causative role of ANCAs in vasculitis is summarized briefly as follows:[53] (1) An underlying disorder (e.g., an infection) elicits pro-inflammatory cytokines such as TNF, and granulocyte-macrophage colony-stimulating factor, and microbial products such as endotoxin, which together cause neutrophils and other inflammatory cells to express PR3 and MPO on their surfaces. (2) These stimulate the formation of ANCAs. (3) ANCAs react with circulating cytokine-primed neutrophils and cause them to degranulate (4) PMNs activated by ANCA cause endothelial

cell toxicity and other direct tissue injury. Interestingly, ANCAs directed against neutrophil constituents other than PR3 and MPO are also found in some patients with a wide range of inflammatory but nonvasculitic disorders such as inflammatory bowel disease, autoimmune liver disease, primary sclerosing cholangitis, and rheumatoid arthritis, and in some patients with malignancies and infections.

Anti-endothelial Cell Antibodies.

Antibodies to ECs, perhaps induced by defects in immune regulation, may predispose to certain vasculitides, such as those associated with SLE and Kawasaki disease.

Classification.

The systemic vasculitides are classified on the basis of the size and anatomic site of the involved blood vessels (Fig. 11-23), histologic characteristics of the lesion, and clinical manifestations. There is considerable clinical and pathologic overlap among these disorders summarized in Table 11-5 (Table Not Available) and discussed below.

GIANT CELL (TEMPORAL) ARTERITIS

Giant cell (temporal) arteritis, the most common form of systemic vasculitis in adults, is an acute and chronic, often granulomatous, inflammation of arteries of large to small size.[54] It affects principally the arteries in the head—especially the temporal arteries—but also the vertebral and ophthalmic arteries and the aorta, where it may cause thoracic aortic aneurysm. Ophthalmic arterial involvement may lead to permanent blindness. Therefore, visual loss caused by giant cell arteritis is a medical emergency that requires prompt recognition

Figure 11-23 Diagrammatic representation of the sites of the vasculature involved by the major forms of vasculitis. The widths of the trapezoids indicate the frequencies of involvement of various portions. LCA, leukocytoclastic angiitis. *(Reproduced from Jennette JC, and Falk RJ: Small-vessel vasculitis. New Engl J Med 337:1512, 1997.)*

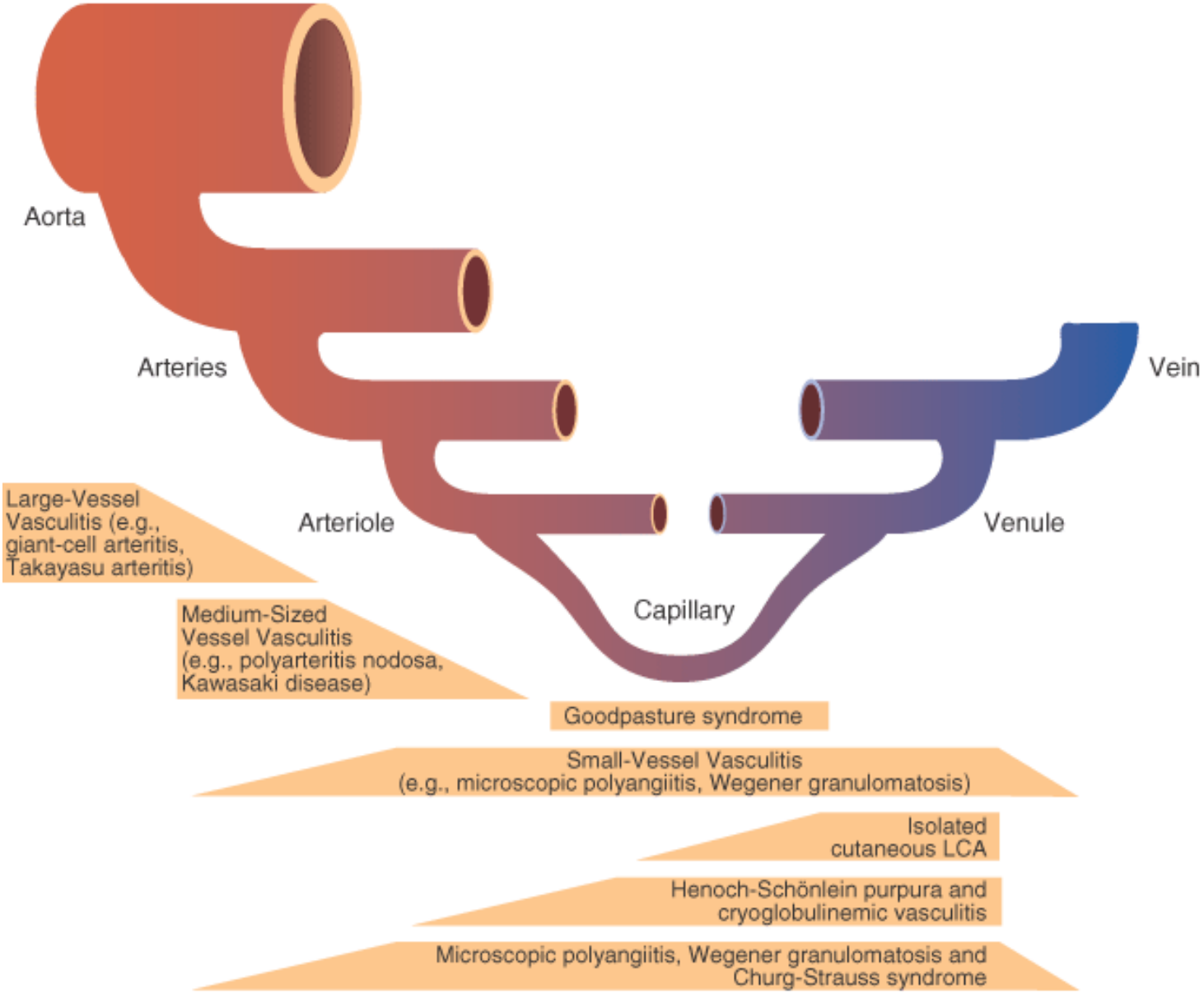


TABLE 11-5 -- Classification and Characteristics of Selected Vasculitis

(Not Available)
Modified from Jennette JC, et al: Nomenclature of systemic vasculitides: the proposal of an international consensus conference. Arthritis Rheum 37:187, 1994.

Figure 11-24 Temporal (giant cell) arteritis. *A*, H&E stain of section of temporal artery showing giant cells at the degenerated internal elastic membrane in active arteritis (*arrow*). *B,*

Elastic tissue stain demonstrating focal destruction of internal elastic membrane (*arrow*) and intimal thickening (IT) characteristic of long-standing or healed arteritis. *C,* Examination of the temporal artery of a patient with giant-cell arteritis shows a thickened, nodular, and tender segment of a vessel on the surface of head (*arrow*). *(C Reproduced from Salvarani C, et al. Polymyalgia rheumatica and giant-cell arteritis. N Engl J Med 347:261, 2002.)*

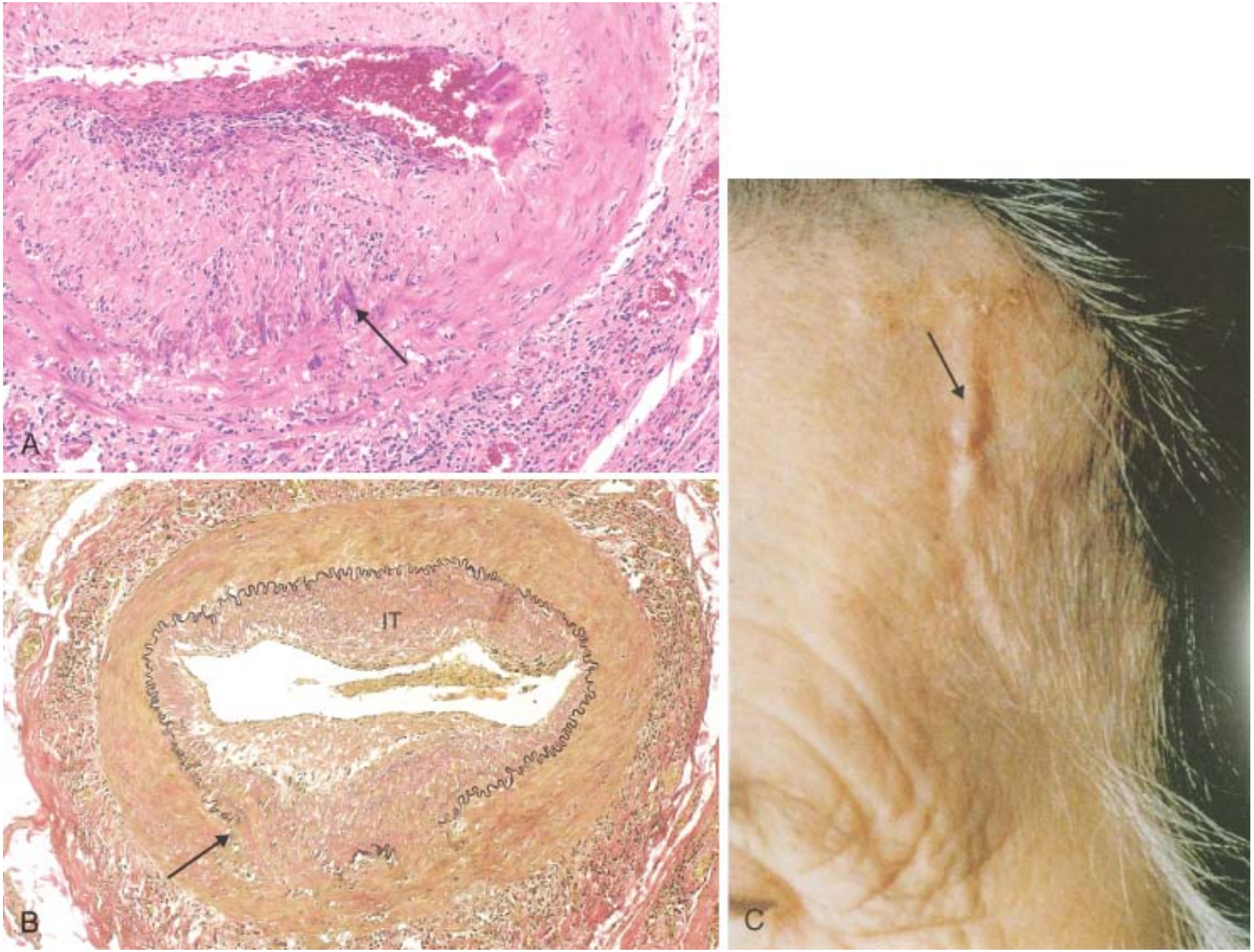
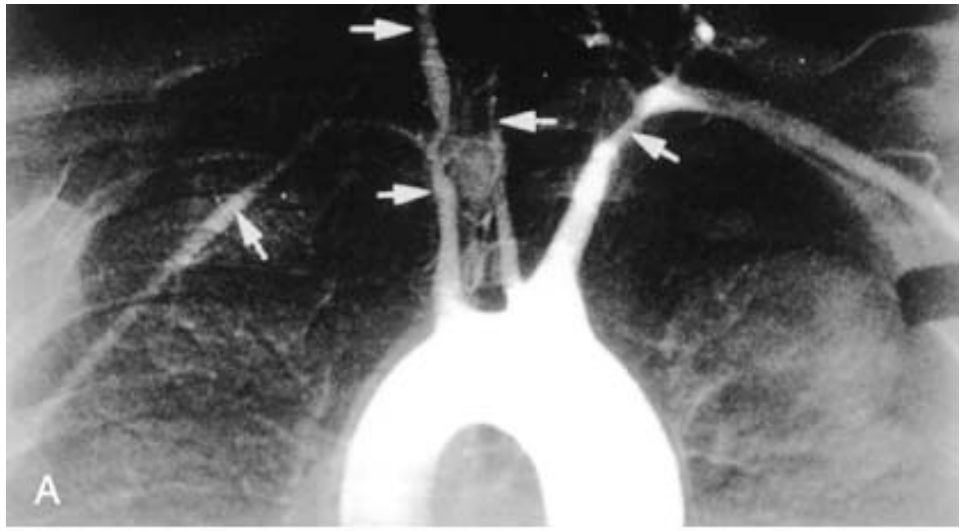


Figure 11-25 Takayasu arteritis. *A,* Aortic arch angiogram showing narrowing of brachiocephalic, carotid, and subclavian arteries *(arrows). B,* Gross photograph of two cross-sections of the right carotid artery taken at autopsy of the patient shown in *A,* demonstrating marked intimal thickening with minimal residual lumen. *C,* Histologic view of active Takayasu aortitis, illustrating destruction of the arterial media by mononuclear inflammation with giant cells.


A

B

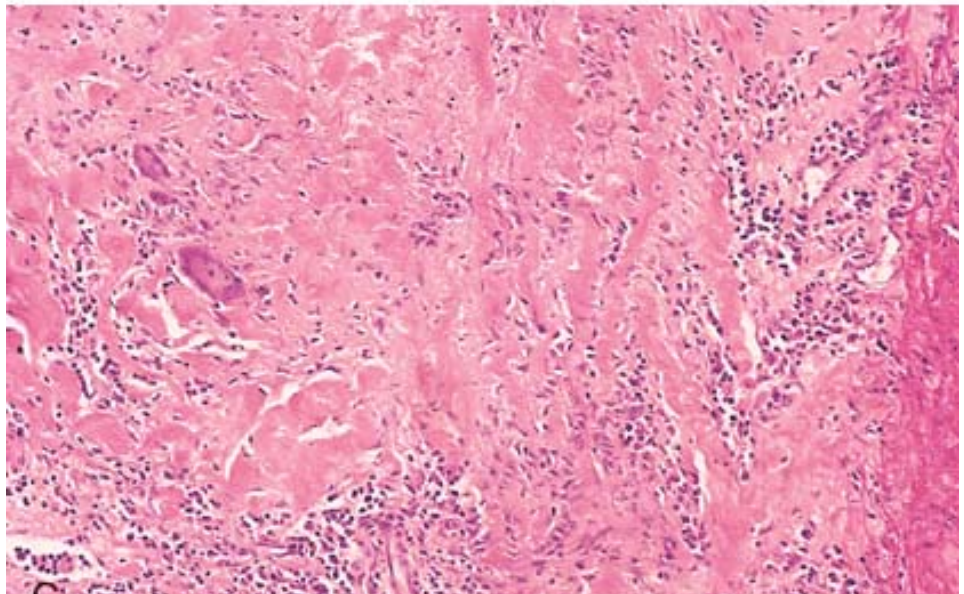
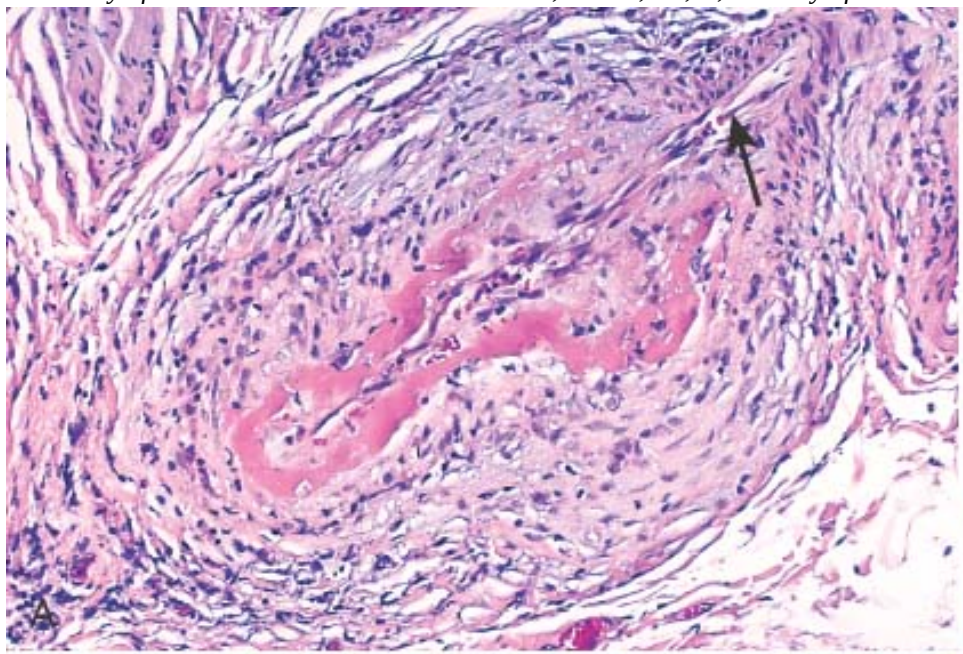
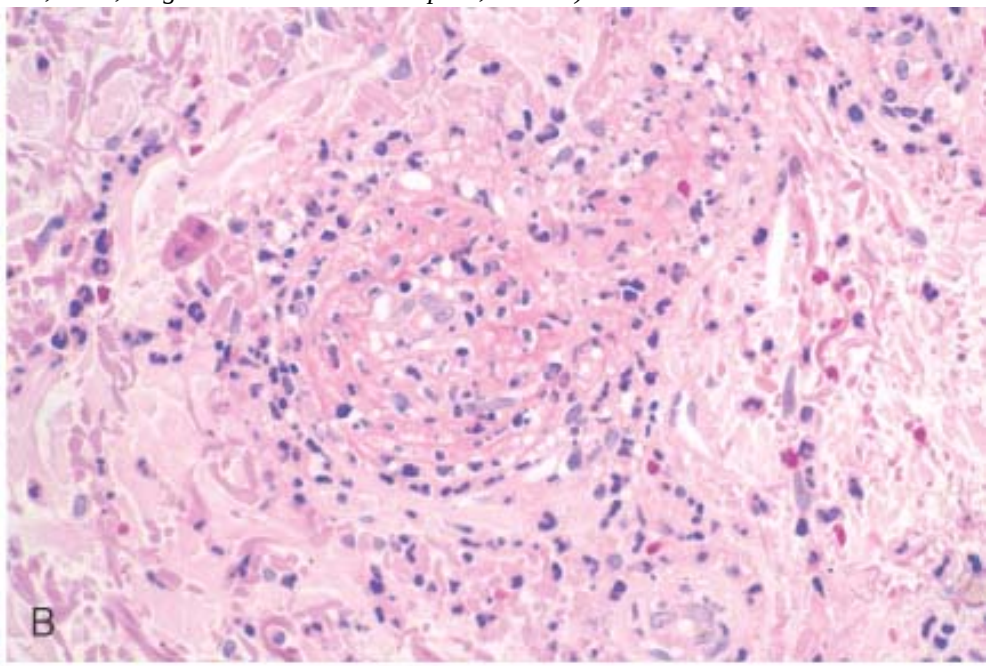
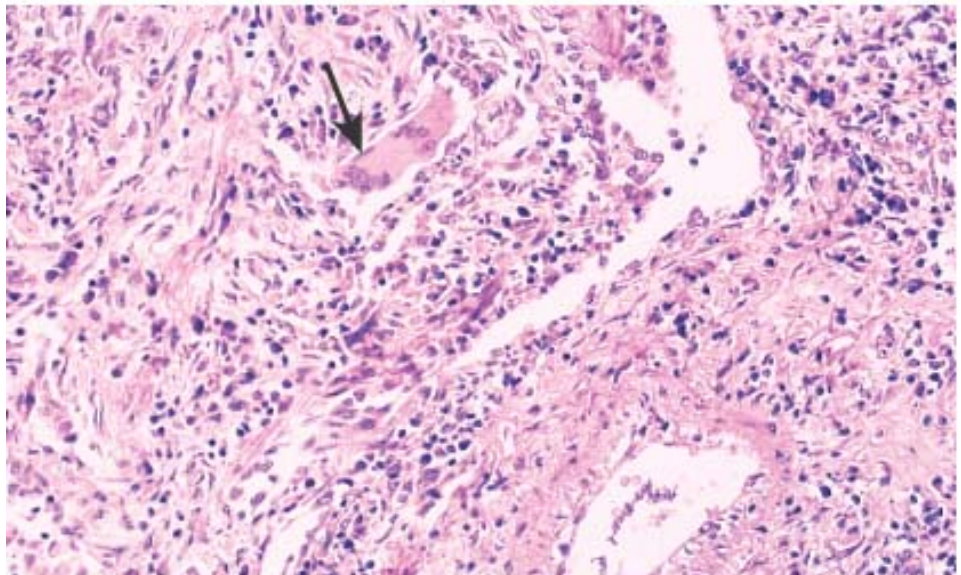
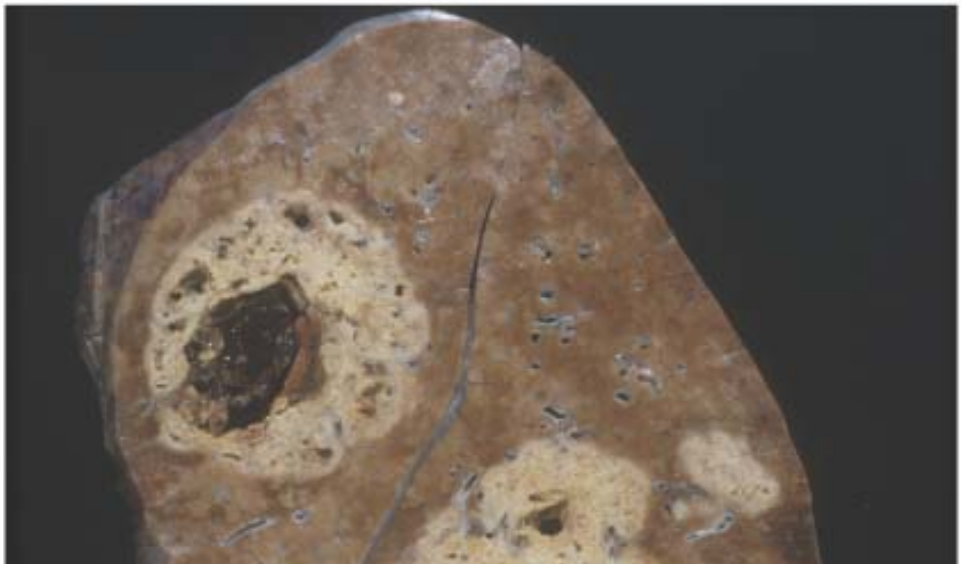


Figure 11-26 Representative forms of systemic medium-sized to small vessel vasculitis. *A,* Polyarteritis nodosa. *B,* Leukocytoclastic vasculitis. *C* and *D,* Wegener granulomatosis. *E,* Thromboangiitis obliterans (Buerger disease). In polyarteritis nodosa (*A*), there is segmental fibrinoid necrosis and thrombotic occlusion of the lumen of this small artery. Note that part of the vessel wall at the upper right *(arrow)* is uninvolved. In leukocytoclastic vasculitis (*B*), shown here from a skin biopsy, there is fragmentation of neutrophils in and around blood vessel walls. In Wegener granulomatosis (*C*), there is inflammation (vasculitis) of a small artery along with adjacent granulomatous inflammation, in which epithelioid cells and giant cells *(arrows)* are seen. *D,* Gross photo from the lung of a patient with fatal Wegener granulomatosis, demonstrating large nodular lesions. In a typical case of Buerger disease (*E*), the lumen is occluded by a thrombus containing two abscesses *(arrow)*. The vessel wall is infiltrated with leukocytes. *(A, and D, courtesy of Sidney Murphree, MD, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX; B, courtesy of Scott Granter, M.D., Brigham and Women's Hospital, Boston.)*









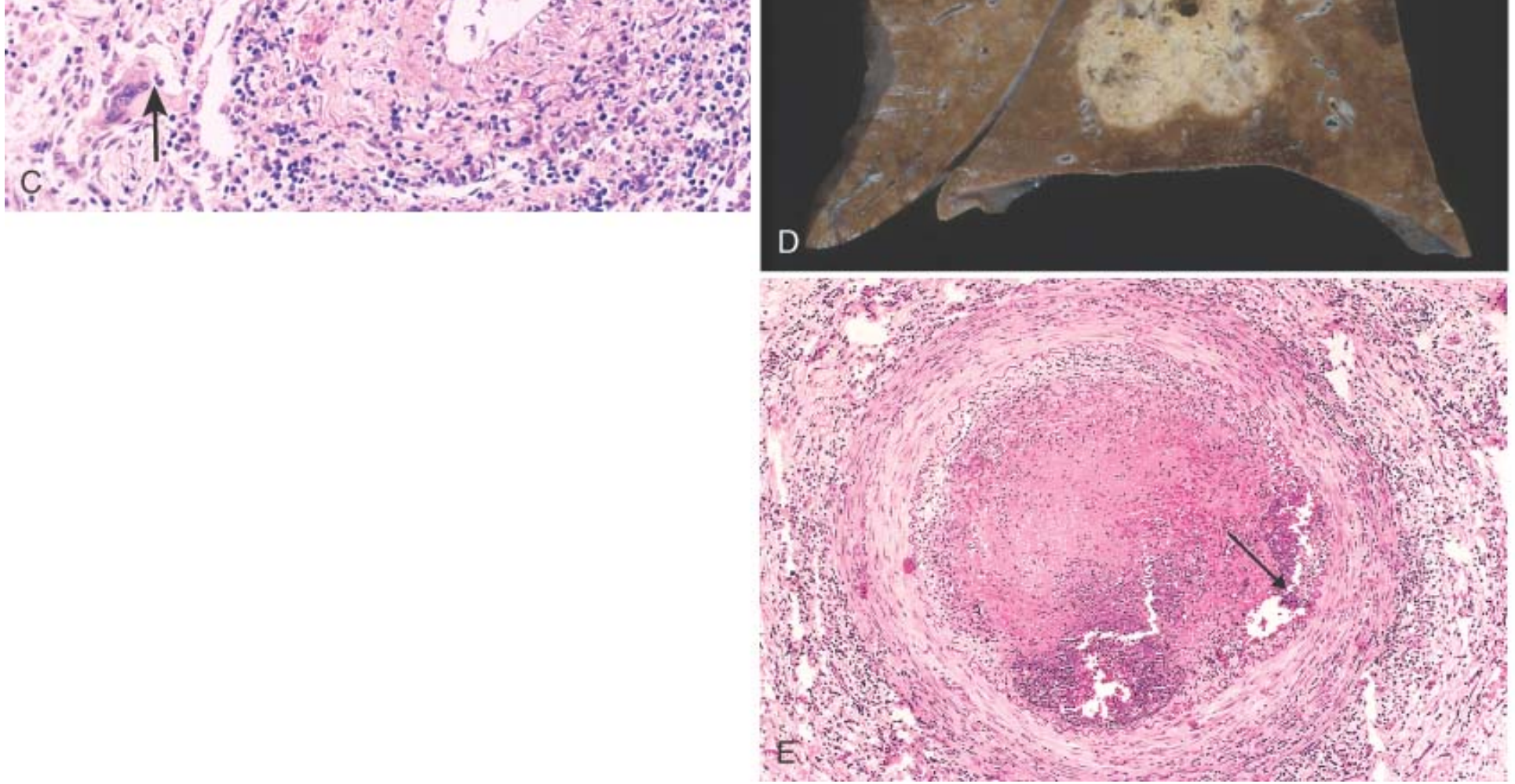


Figure 11-27 Vasculitis with fibrinoid necrosis in a patient with active systemic lupus erythematosus.

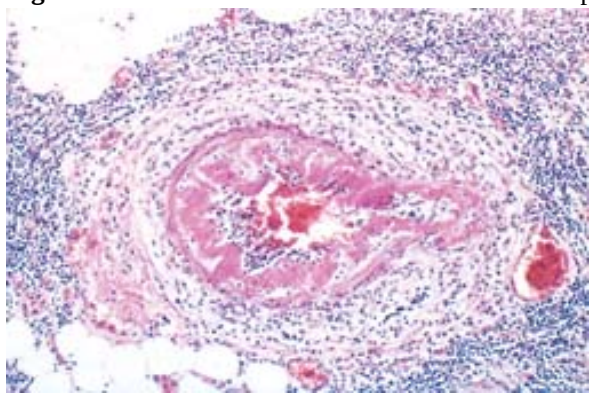


Figure 11-28 Raynaud phenomenon. *A*, Sharply demarcated pallor of the distal fingers resulting from the closure of digital arteries. *B*, Cyanosis of the fingertips. *(Reproduced from Salvarani C, et al.: Polymyalgia rheumatica and giant-cell arteritis. N Engl J Med 347:261, 2002.)*

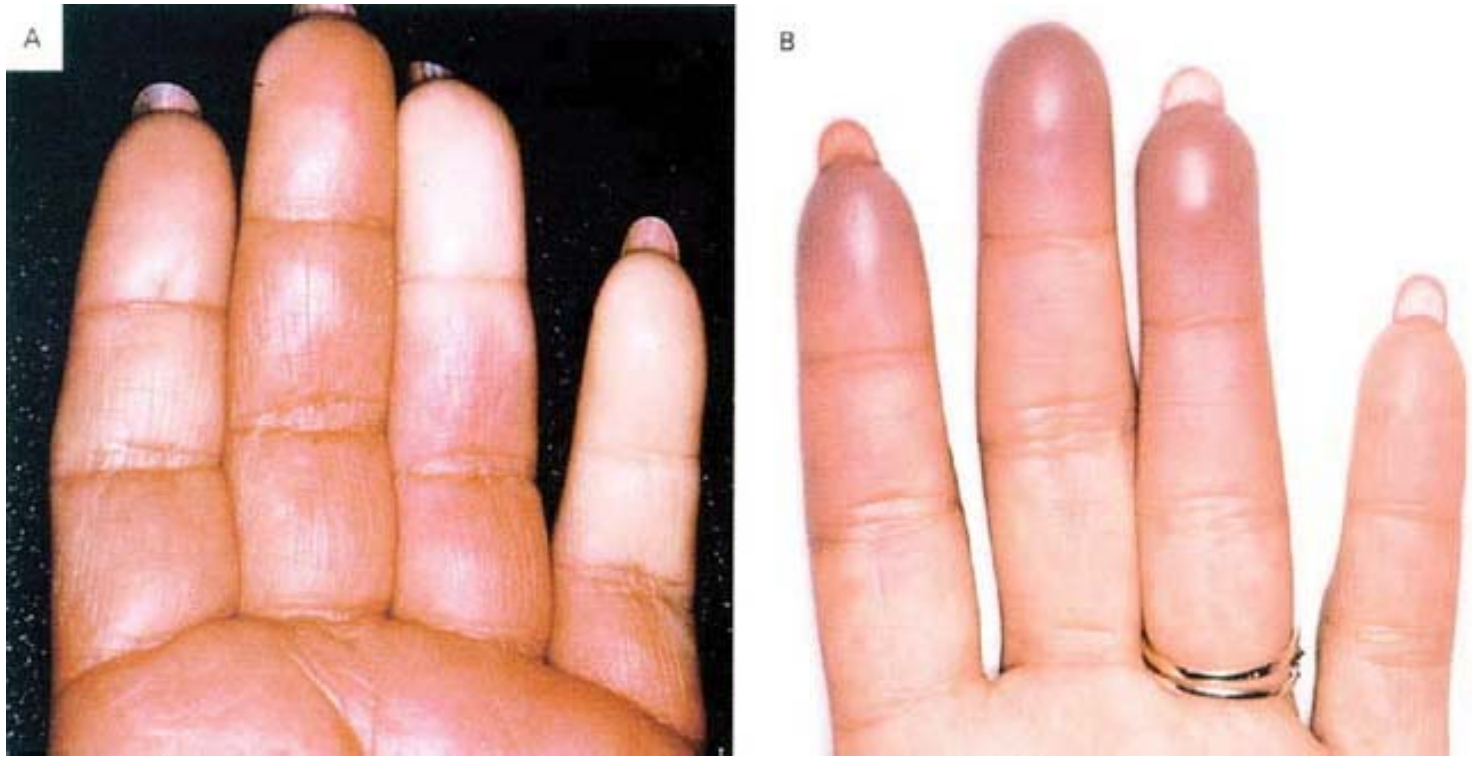


TABLE 11-6 -- Classification of Vascular Tumors and Tumor-Like Conditions

Benign Neoplasms, Developmental and Acquired Conditions
Hemangioma
••Capillary hemangioma
••Cavernous hemangioma
••Pyogenic granuloma (lobular capillary hemangioma)
Lymphangioma
••Simple (capillary) lymphangioma
••Cavernous lymphangioma (cystic lymphangioma)
Glomus tumor
Vascular ectasias
••Nevus flammeus
••Spider telangiectasia (arterial spider)
••Hereditary hemorrhagic telangiectasis (Osler-Weber-Rendu disease)

Reactive vascular proliferations
••Bacillary angiomatosis
Intermediate-Grade Neoplasms
Kaposi sarcoma
Hemangioendothelioma
Malignant Neoplasms
Angiosarcoma
Hemangiopericytoma

The endothelial derivation of neoplastic proliferations that do not form distinct vascular lumina can usually be confirmed by immunohistochemical demonstration of endothelium-specific markers such as CD31, CD34, or vWF. Because these lesions constitute abnormalities of unregulated vascular proliferation, the possibility of controlling such growth by agents that inhibit blood vessel formation (anti-angiogenic factors) is particularly exciting.

BENIGN TUMORS AND TUMOR-LIKE CONDITIONS

Hemangioma

Difficult to distinguish with certainty from malformations or hamartomas, *hemangiomas (angiomas)* are most commonly localized; however, some involve large segments of the body such as an entire extremity (called *angiomatosis*). The majority are superficial lesions, often of the head or neck, but they may occur internally, with nearly one third in the liver. Malignant transformation occurs rarely if at all (Fig. 11-30).

Hemangiomas constitute 7% of all benign tumors in infancy and childhood (Chapter 10). Most are present from birth and expand along with the growth of the child. Nevertheless, many of the capillary lesions regress spontaneously at or before puberty. There are several histologic and clinical variants.

Capillary Hemangioma.

Capillary hemangiomas, the largest single type of vascular tumor, are most common in the skin, subcutaneous tissues, and mucous membranes of the oral cavities and lips, but they may also occur in the liver, spleen, and kidneys. The "strawberry type" of capillary hemangioma *(juvenile hemangioma)* of the skin of newborns is extremely common (1 in 200 births), may be multiple, grows rapidly in the first few months, begins to fade when the

Figure 11-30 Hemangiomas. *A,* Hemangioma of the tongue. *B,* Histology of juvenile capillary hemangioma. *C,* Histology of cavernous hemangioma. *D,* Pyogenic granuloma of the lip. *(A and D, courtesy of John Sexton, M.D., Beth Israel Hospital, Boston; B, courtesy of Christopher D.M. Fletcher, M.D., Brigham and Women's Hospital, Boston; and C, courtesy of Thomas Rogers, M.D., University of Texas Southwestern Medical School, Dallas, TX.)*

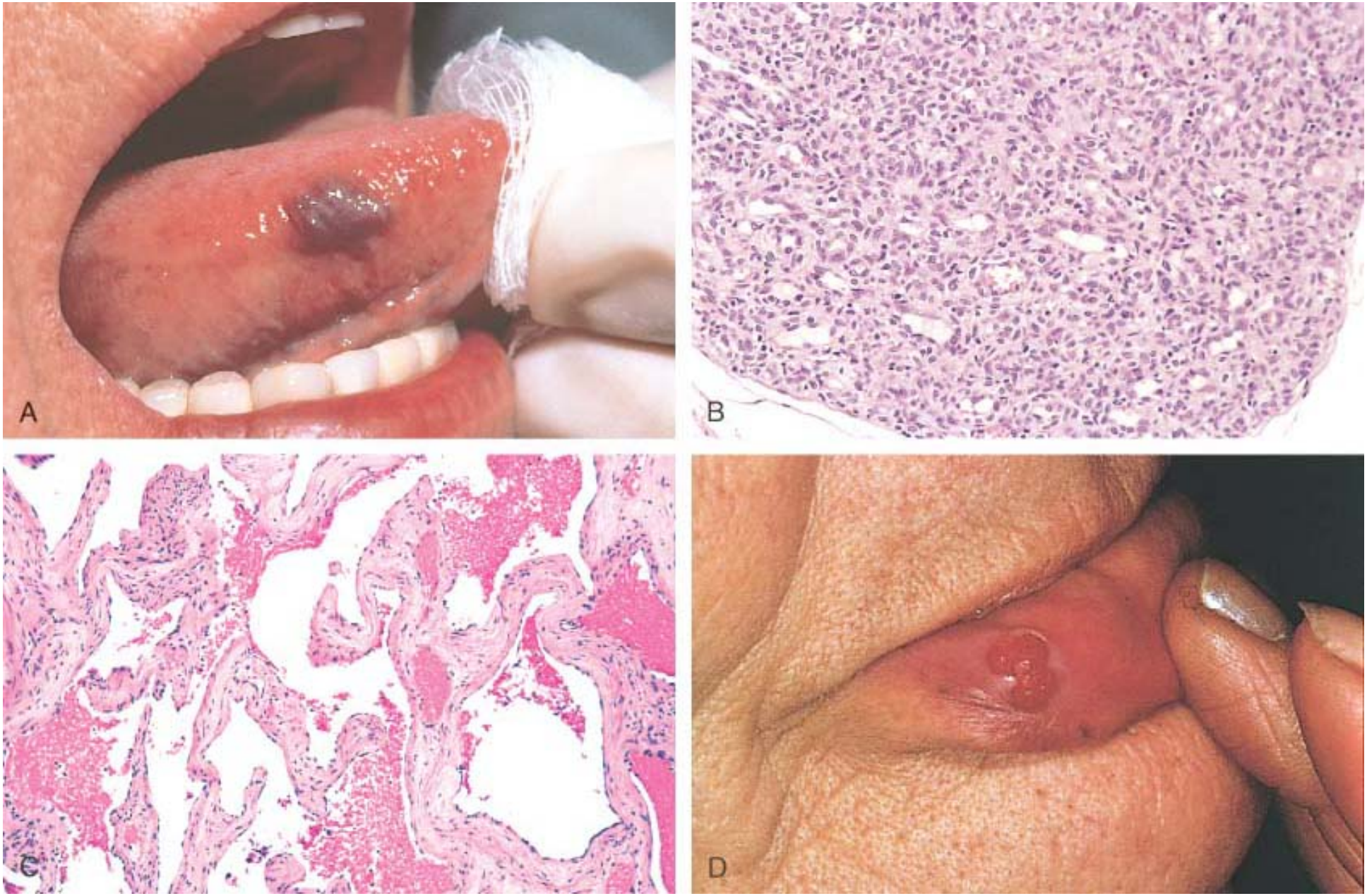


Figure 11-31 Bacillary angiomatosis. *A,* Photograph of a moist, erosive cutaneous lesion. *B,* Histologic appearance with acute neutrophilic inflammation and vascular (capillary) proliferation. *Inset,* demonstration by modified silver (Warthin-Starry) stain of clusters of tangled bacilli (black). *(A, courtesy of Richard Johnson, M.D. Beth Israel Deaconess Medical Center, Boston, MA; B and inset, courtesy of Scott Granter, M.D., Brigham and Women's Hospital, Boston, MA.)*

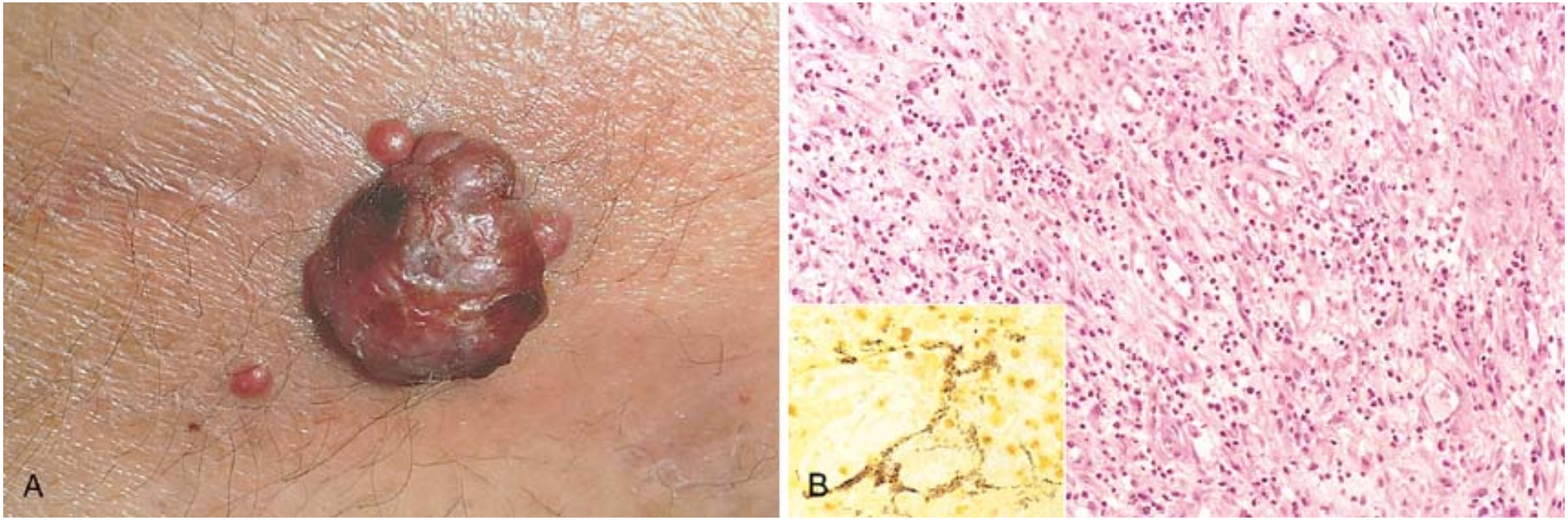


Figure 11-32 Kaposi sarcoma. *A,* Gross photograph, illustrating coalescent red-purple macules and plaques of the skin. *B,* Histology of nodular form, demonstrating sheets of plump, proliferating spindle cells. *(B, courtesy of Christopher D.M. Fletcher, M.D., Brigham and Women's Hospital, Boston, MA.)*

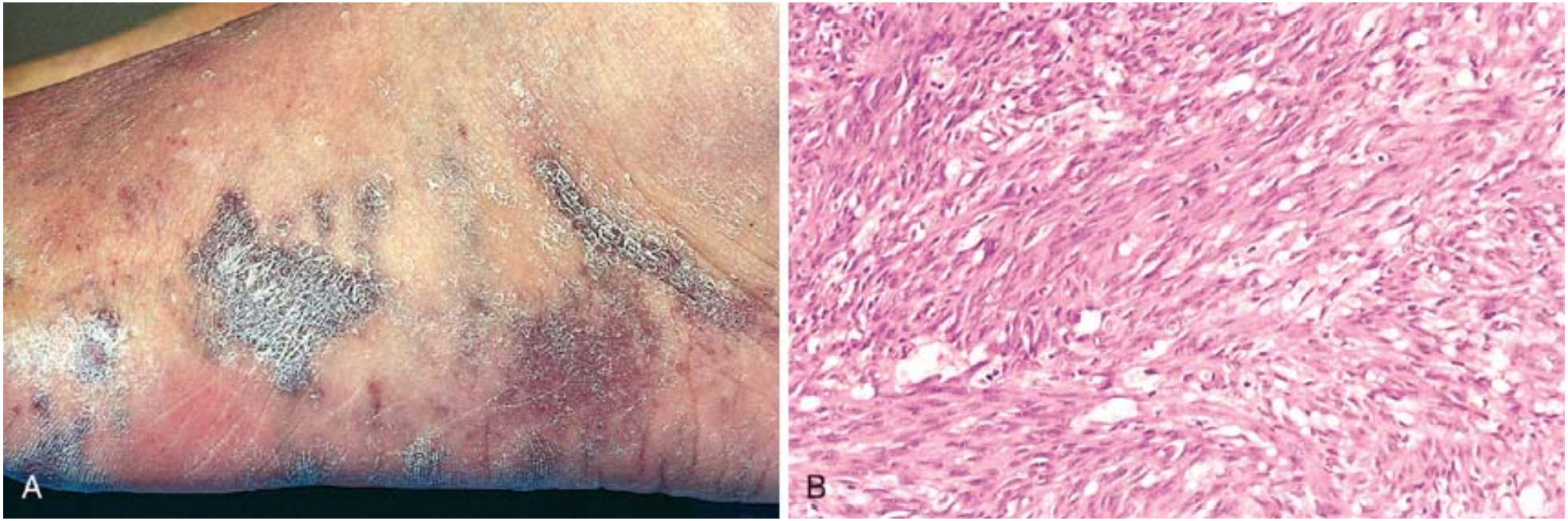


Figure 11-33 Angiosarcoma. *A,* Gross photograph of angiosarcoma of the heart (right ventricle). *B,* Photomicrograph of moderately well-differentiated angiosarcoma with dense clumps of irregular, moderate anaplastic cells and distinct vascular lumens. *C,* Positive immunohistochemical staining of angiosarcoma for the endothelial cell marker CD31, proving the endothelial nature of the tumor cells.

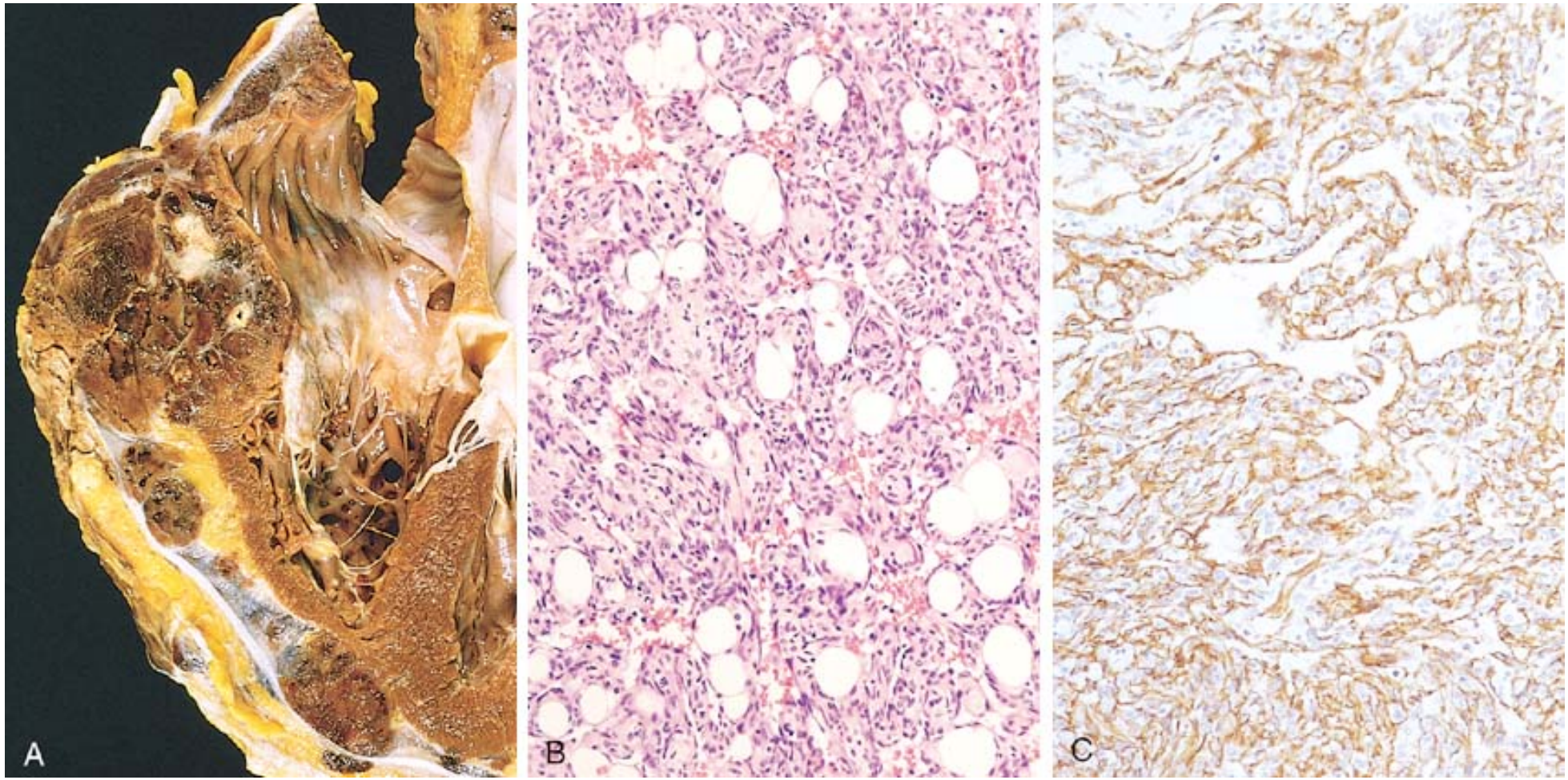


Figure 11-34 Balloon angioplasty and endovascular stents. *A*, Coronary artery with recent balloon angioplasty, in a low-power photomicrograph showing the split encompassing the intima and media *(arrow)* and partial circumferential dissection. *B*, Gross photograph of restenosis following balloon angioplasty, demonstrating residual atherosclerotic plaque *(left arrow)* and a new, glistening proliferative lesion *(right arrow). C*, Coronary arterial stent implanted long term, demonstrating thickened neointima separating the stent wires (black spot shown by *arrow*) from the lumen *(asterisk). (C, Reproduced from Schoen FJ, Edwards WD. Pathology of cardiovascular interventions, including endovascular therapies, revascularization, vascular replacement, cardiac assist/replacement, arrhythmia control, and repaired congenital heart disease. In Silver MD, Gotlieb AI, Schoen FJ (eds): Cardiovascular Pathology, 3rd ed. Philadelphia, Churchill Livingstone, 2001.)*

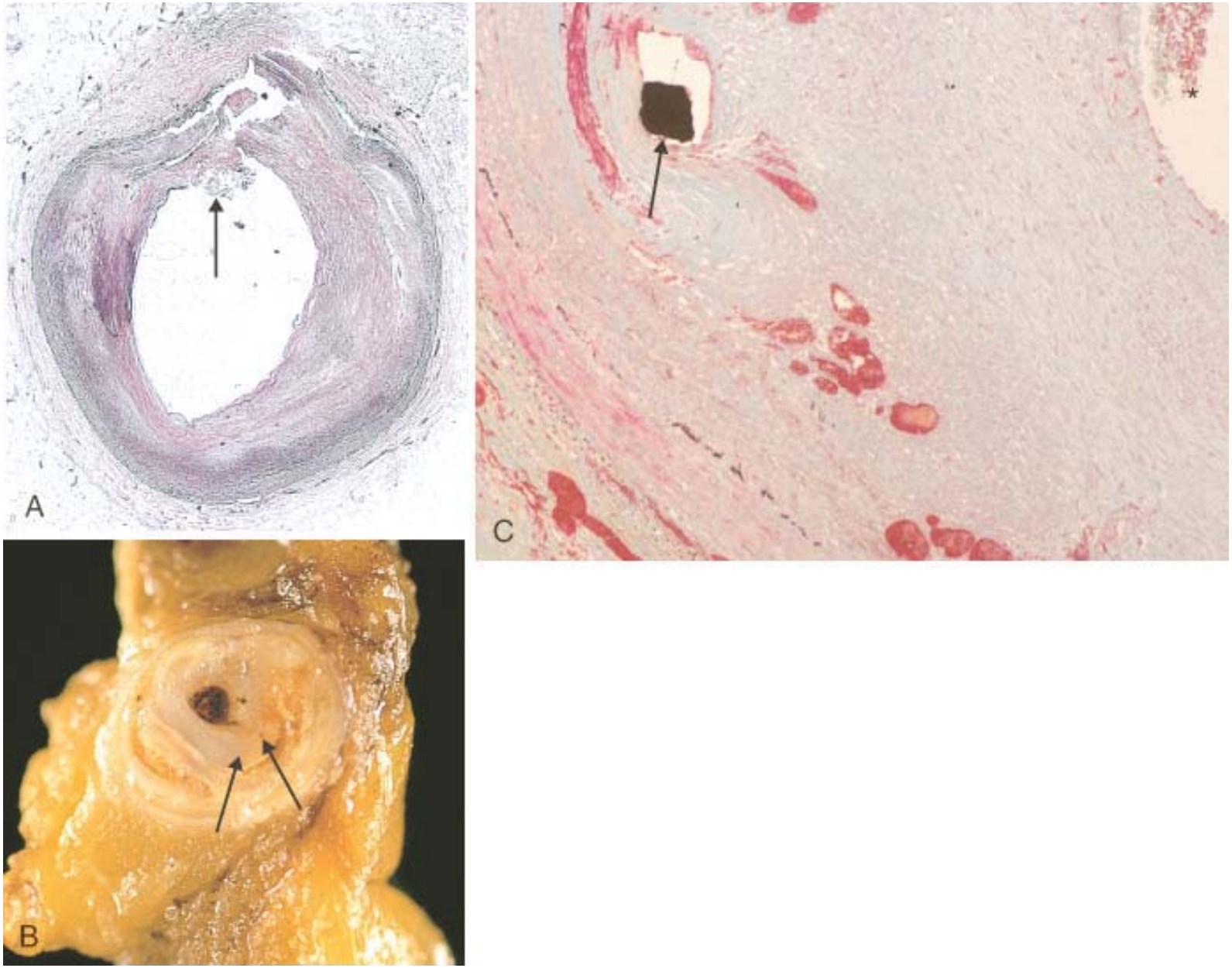
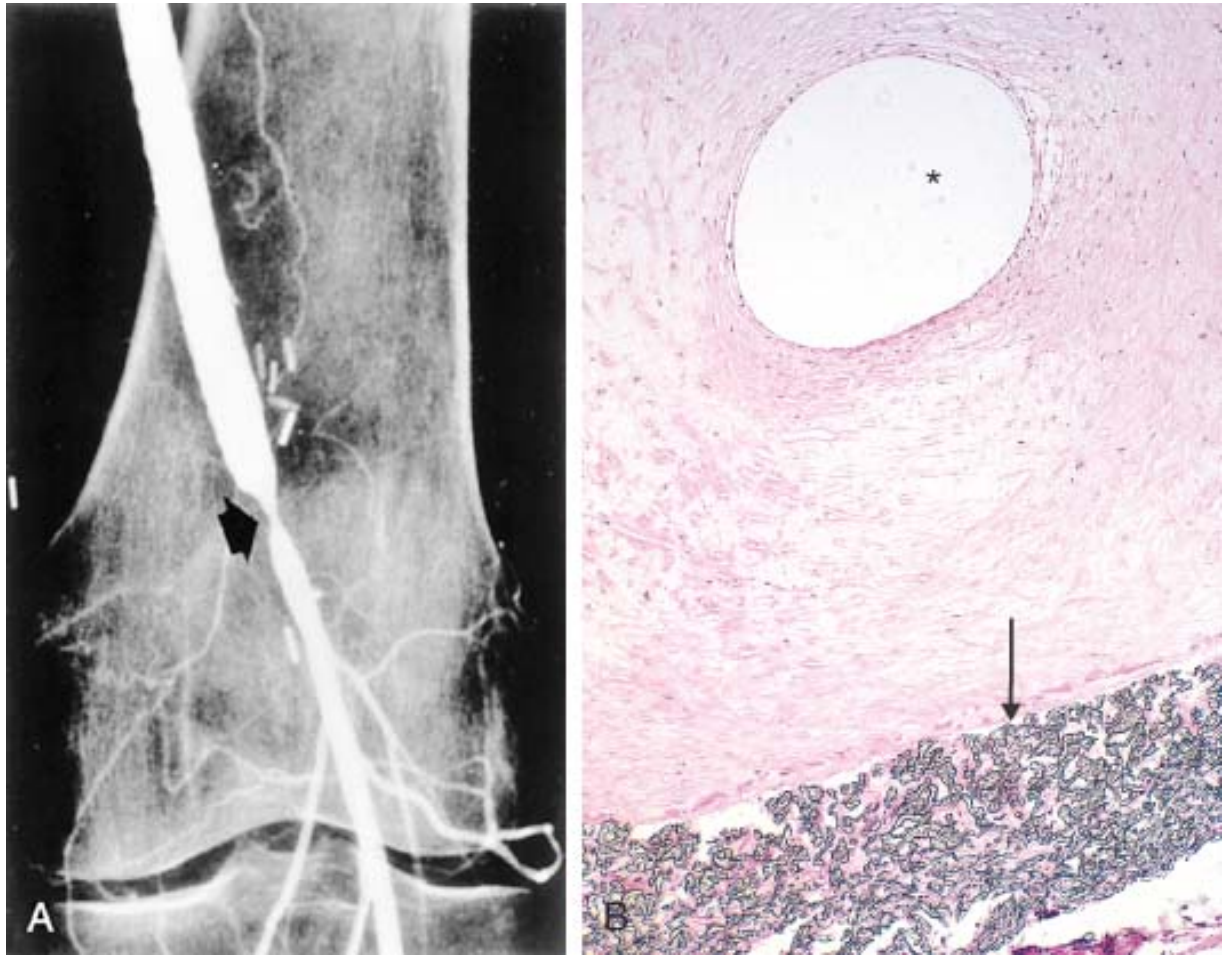


Figure 11-35 Anastomotic hyperplasia at the distal anastomosis of synthetic femoropopliteal graft. *A,* Angiogram demonstrating constriction *(arrow). B,* Photomicrograph demonstrating Gore-Tex graft *(arrow)* with prominent intimal proliferation and very small residual lumen (asterisk). *(A, courtesy of Anthony D. Whittemore, M.D., Brigham and Women's Hospital, Boston, MA.)*



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Chapter 12 - The Heart

The human heart is a remarkably efficient, durable, and reliable pump that propels over 6000 liters of blood through the body daily and beats more than 40 million times a year during an individual's lifetime, thereby providing the tissues with a steady supply of vital nutrients and facilitating the excretion of waste products. As might be anticipated, cardiac dysfunction can be associated with devastating physiologic consequences. Heart disease is the predominant cause of disability and death in industrialized nations. In the United States, it accounts for about 40% of all postnatal deaths, totaling about 750,000 individuals annually and nearly twice the number of deaths caused by all forms of cancer combined. The yearly economic burden of ischemic heart disease, the most prevalent subgroup, is estimated to be in excess of $100 billion. The major categories of cardiac diseases considered in this chapter include congenital heart abnormalities, ischemic heart disease, heart disease caused by systemic hypertension, heart disease caused by pulmonary diseases (cor pulmonale), diseases of the cardiac valves, and primary myocardial diseases. A few comments about pericardial diseases and cardiac neoplasms as well as cardiac transplantation are also offered. Before considering details of specific conditions, we will review salient features of normal anatomy and function as well as the principles of cardiac hypertrophy and failure, the common end points of many different types of heart disease.

Normal

The normal heart weight varies with body height and weight; it averages approximately 250 to 300 g in females and 300 to 350 g in males. The usual thickness of the free wall of the right ventricle is 0.3 to 0.5 cm and that of the left ventricle 1.3 to 1.5 cm. As will be seen, increases in cardiac size and weight accompany many forms of heart disease. Greater heart weight or ventricular thickness indicates *hypertrophy*, and an enlarged chamber size implies *dilation*. An increase in cardiac weight or size (owing to hypertrophy and/or dilation) is termed *cardiomegaly*.

Myocardium

Basic to the heart's function is the near-inexhaustible cardiac muscle, the *myocardium*, composed primarily of a collection of specialized muscle cells called *cardiac myocytes* (Fig. 12-1). They are arranged largely in a circumferential and

Figure 12-1 Myocardium (cardiac muscle). *A* The histology of myocardium is shown, emphasizing the centrally-placed nuclei of the cardiac myocytes (arrowhead), intercalated discs (representing specialized end-to-end junctions of adjoining cells; highlighted by a double arrow) and the sarcomeric structure visible as cross-striations within myocytes. A capillary endothelial cell is indicated by an arrow. *(Photomicrograph courtesy of Mark Flomenbaum, M.D., Ph.D., Office of the Chief Medical Examiner, New York City, NY.) B* Electron microscopy of myocardium, showing myofibrillar (my) and mitochondrial (mi) architecture and the sarcolemmal membrane (s). Z bands are indicated by arrows. Bar = 1 µm. *(Reproduced by permission from Vivaldi MT, et al. Triphenyltetrazolium staining of irreversible injury following coronary artery occlusion in rats. Am J Pathol 121:522, 1985. Copyright J.B. Lippincott, 1985.)*

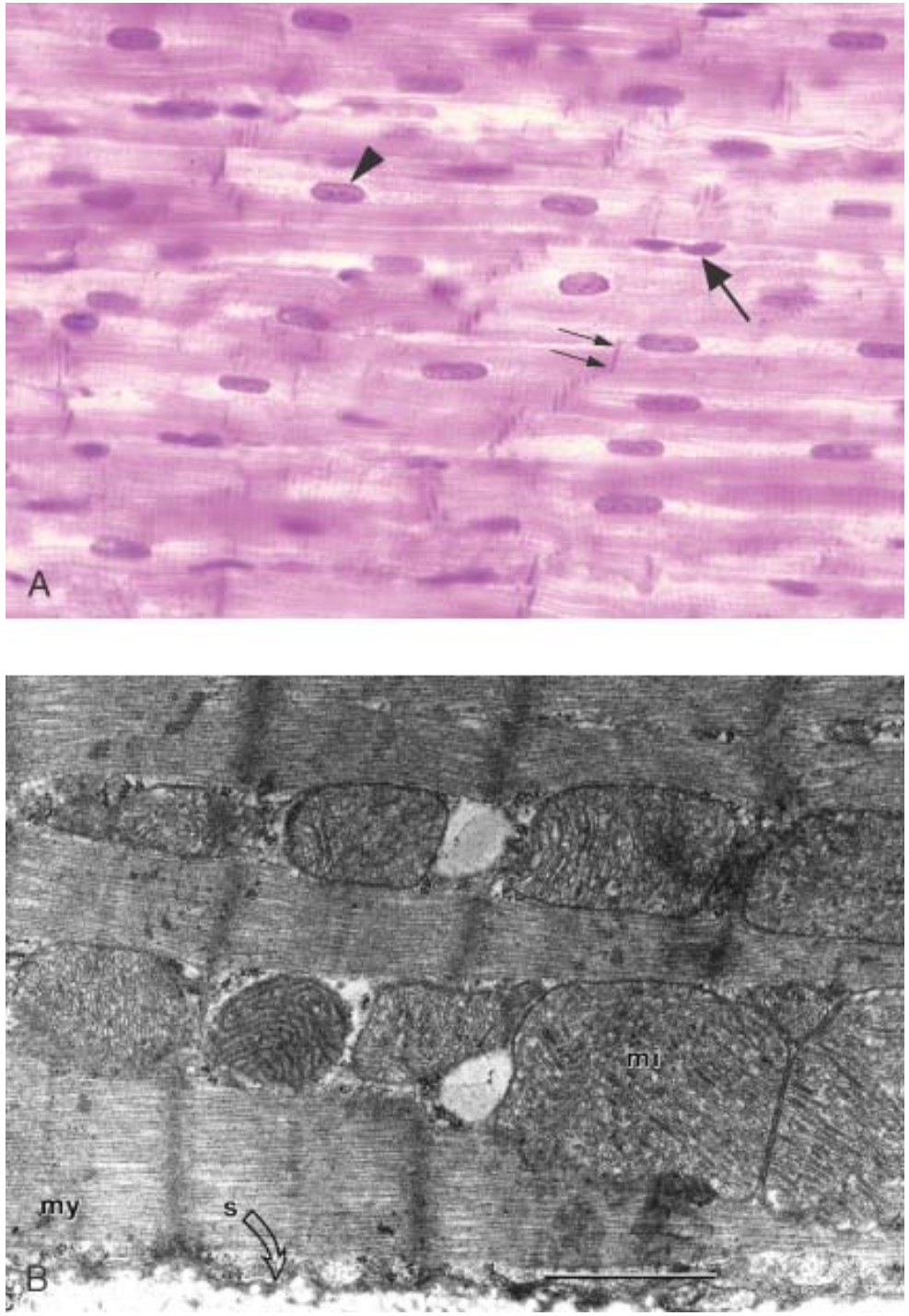

Figure 12-2 Aortic valve histology, shown as a low-magnification photomicrograph of cuspal cross-section in the systolic (nondistended) state, emphasizing three major layers (ventricularis [v], spongiosa [s], and fibrosa [f]). Superficial endothelial cells *(arrow)* and diffusely distributed deep interstitial cells are noted. The strength of the valve is predominantly

derived from the fibrosa, with its dense collagen *(yellow)*. This section highlights the dense, laminated elastic tissue in the ventricularis *(double arrow)*. The outflow surface is at top. *(Reproduced by permission from Schoen FJ: Aortic valve structure-function correlations: Role of elastic fibers no longer a stretch of the imagination. J Heart Valve Dis 6:1, 1997.)*

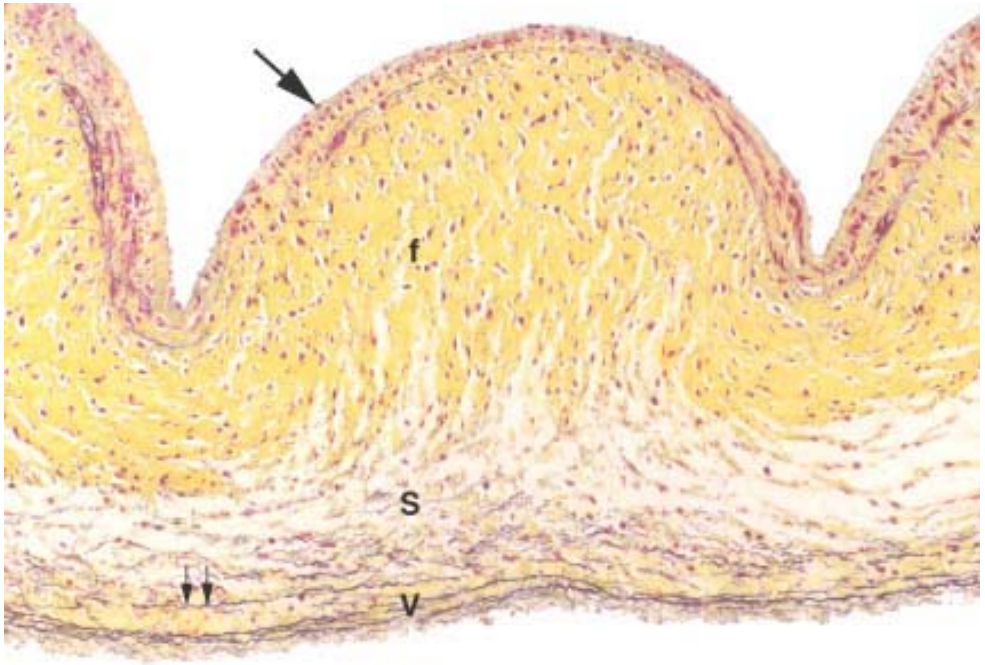

TABLE 12-1 -- Changes in the Aging Heart

Chambers
Increased left atrial cavity size
Decreased left ventricular cavity size
Sigmoid-shaped ventricular septum
Valves
Aortic valve calcific deposits
Mitral valve annular calcific deposits
Fibrous thickening of leaflets
Buckling of mitral leaflets toward the left atrium
Lambl excrescences
Epicardial Coronary Arteries
Tortuosity
Increased cross-sectional luminal area

Calcific deposits
Atherosclerotic plaque
Myocardium
Increased mass
Increased subepicardial fat
Brown atrophy
Lipofuscin deposition
Basophilic degeneration
Amyloid deposits
Aorta
Dilated ascending aorta with rightward shift
Elongated (tortuous) thoracic aorta
Sinotubular junction calcific deposits
Elastic fragmentation and collagen accumulation
Atherosclerotic plaque

With advancing age, the amount of epicardial fat increases, particularly over the anterior surface of the right ventricle and in the atrial septum. A reduction in the size of the left ventricular cavity, particularly in the base-to-apex dimension, is associated with increasing age and accentuated by systemic hypertension. Accompanied by a rightward shift and tortuosity of a dilated ascending aorta, this chamber alteration causes the basal ventricular septum to bend leftward, bulging into the left ventricular outflow tract (termed *sigmoid septum*). Such reduction in the size of the left ventricular cavity can simulate the obstruction to blood leaving the left ventricle that often occurs with hypertrophic cardiomyopathy, discussed later in this chapter.

Several changes of the valves are noted with aging, including calcification of the mitral annulus and aortic valve, the latter frequently leading to aortic stenosis. In addition, the valves can develop fibrous thickening, and the mitral leaflets tend to buckle back toward the left atrium during ventricular systole, simulating a prolapsing (myxomatous) mitral valve (see later). Moreover, many older persons develop small filiform processes *(Lambl excrescences)* on the closure lines of aortic and mitral valves, probably arising from the organization of small thrombi on the valve contact margins.

Compared with younger myocardium, "elderly" myocardium also has fewer myocytes, increased collagenized connective tissue and, in some individuals, deposition of amyloid. In the muscle cells, lipofuscin deposits (Chapter 1), and *basophilic degeneration*, an accumulation within cardiac myocytes of a gray-blue byproduct of glycogen metabolism, may be present. Extensive lipofuscin deposition in a small, atrophied heart is called *brown atrophy*; this change often accompanies cachectic weight loss, as seen in terminal cancer.

Although the morphologic changes described are common in elderly patients at necropsy, and they may mimic disease, in only a minority are they associated with clinical cardiac dysfunction.

Pathology

Although many diseases can involve the heart and blood vessels, [6] [7] cardiovascular dysfunction results from one or more of five principal mechanisms:

• *Failure of the pump.* In the most common circumstance, the cardiac muscle contracts weakly or inadequately, and the chambers cannot empty properly. In some conditions, however, the muscle cannot relax sufficiently to permit ventricular filling.
• *An obstruction to flow*, owing to a lesion preventing valve opening or otherwise causing increased ventricular chamber pressure (e.g., aortic valvular stenosis, systemic hypertension, or aortic coarctation). The increased pressure overworks the chamber that pumps against the obstruction.
• *Regurgitant flow* causes some of the output from each contraction to flow backward, adding a volume workload to each of the chambers, which must pump the extra blood (e.g., left ventricle in aortic regurgitation; left atrium and left ventricle in mitral regurgitation).
• *Disorders of cardiac conduction.* Heart block or arrhythmias owing to uncoordinated generation of impulses (e.g., atrial or ventricular fibrillation) lead to nonuniform and inefficient contractions of the muscular walls.
• *Disruption of the continuity of the circulatory system* that permits blood to escape (e.g., gunshot wound through the thoracic aorta).

Most cardiovascular disease arises from the interaction of environmental factors and genetic susceptibility. *The contemporary view holds that most clinical cardiovascular diseases result from a complex interplay of genetics and environmental factors that disrupt networks controlling morphogenesis, myocyte survival, biomechanical stress responses, contractility, and electrical conduction.* [8] For example, there is growing recognition that pathogenesis of congenital heart defects, in many cases, involves an underlying genetic abnormality whose expression is strongly modified by external (environmental or maternal) factors. Moreover, since a diverse group of cytoskeletal protein mutations have been linked with cardiac muscle cell dysfunction in the cardiomyopathies, perhaps subtle mutations or polymorphisms in these genes could confer an increased risk or more rapid onset of heart failure in response to acquired cardiac injury. In these and other examples, the clinical expression of cardiac disease represents the end result of multiple internal and external cues for growth, death, and survival of cardiac myocytes. These factors and pathways are shared with other normal tissues and pathological processes.[9]

Heart Failure

The abnormalities described above often culminate in heart failure, an extremely common result of many forms of heart disease. In heart failure, often called *congestive heart failure (CHF)*, the heart is unable to pump blood at a rate commensurate with the requirements of the metabolizing tissues or can do so only at an elevated filling pressure. Although usually caused by a slowly developing intrinsic deficit in myocardial contraction, a similar clinical syndrome is present in some patients with heart failure caused by conditions in which the normal heart is suddenly presented with a load that exceeds its capacity (e.g., fluid overload, acute myocardial infarction, acute valvular dysfunction) or in which ventricular filling is impaired (see below). CHF is a common and often recurrent condition with a poor prognosis. The magnitude of the problem is exemplified by the impact of CHF in the United States, where each year it affects nearly 5 million individuals, is the underlying or contributing cause of death of an estimated 300,000, and necessitates over 1 million hospitalizations.[10] Moreover, CHF is the leading discharge diagnosis in hospitalized patients over age 65 and has an associated annual cost of $18 billion. In many pathologic states, the onset of heart failure is preceded by *cardiac hypertrophy*, the compensatory response of the myocardium to increased mechanical work (see below).

The cardiovascular system maintains arterial pressure and perfusion of vital organs in the presence of excessive hemodynamic burden or disturbance in myocardial contractility by a number of mechanisms.[11] The most important are:

• *The Frank-Starling mechanism*, in which the increased preload of dilation (thereby increasing cross-bridges within the sarcomeres) helps to sustain cardiac performance by enhancing contractility
• *Myocardial structural changes, including augmented muscle mass (hypertrophy) with or without cardiac chamber dilation*, in which the mass of contractile tissue is augmented
• *Activation of neurohumoral systems*, especially (1) release of the neurotransmitter norepinephrine by adrenergic cardiac nerves (which increases heart rate and augments myocardial contractility and vascular resistance), (2) activation of the renin-angiotensin-aldosterone system, and (3) release of atrial natriuretic peptide.

These adaptive mechanisms may be adequate to maintain the overall pumping performance of the heart at relatively normal levels, but their capacity to sustain cardiac performance may ultimately be exceeded. Moreover, pathologic changes, such as apoptosis, cytoskeletal alterations, and extracellular matrix (particularly collagen) synthesis and remodeling, may also occur, causing structural and functional disturbances. Most instances of heart failure are the consequence of progressive deterioration of myocardial contractile function *(systolic dysfunction)*, as often occurs with ischemic injury, pressure or volume overload, or dilated cardiomyopathy. The most frequent specific causes are ischemic heart disease and hypertension. Sometimes, however, failure results from an inability of the heart chamber to relax, expand, and fill sufficiently during diastole to accommodate an adequate ventricular blood volume *(diastolic dysfunction)*, as can occur with massive left ventricular hypertrophy, myocardial fibrosis, deposition of amyloid, or constrictive pericarditis.[12] *Whatever its basis, CHF is characterized by diminished cardiac output (sometimes called forward failure) or damming back of blood in the venous system (so-called backward failure), or both.*

The molecular, cellular, and structural changes in the heart that occur as a response to injury, and cause changes in size, shape, and function, are often called *left ventricular remodeling*. Our discussion focuses on structural changes and considers heart failure to be a progressive disorder, which can culminate in a clinical syndrome characterized by impaired cardiac function and circulatory congestion. Nevertheless, we recognize that the modern treatment of chronic heart failure emphasizes the neurohumoral hypothesis, in which neuroendocrine activation is important in the progression of heart failure. Thus, inhibition of neurohormones may have long-term beneficial effects on morbidity and mortality.[13] In the future, patients with CHF may be helped by implanted mechanical cardiac assist devices, an area in which considerable progress has recently been made.[14]

CARDIAC HYPERTROPHY: PATHOPHYSIOLOGY AND PROGRESSION TO FAILURE

The cardiac myocyte is generally considered a terminally differentiated cell that has lost its ability to divide. Under normal circumstances, functionally useful augmentation of myocyte number *(hyperplasia)* cannot occur. Increased mechanical load causes an increase in the content of subcellular components and a consequent increase in cell size *(hypertrophy)*. Increased mechanical work owing to pressure or volume overload or trophic signals (e.g., hyperthyroidism through stimulation of beta-adrenergic receptors) increases the rate of protein synthesis, the amount of protein in each cell, the number of sarcomeres and mitochondria, the dimension and mass of myocytes and, consequently, the size of the heart. Nevertheless, the extent to which adult cardiac myocytes have some capacity to synthesize DNA and whether this leads to some degree of cell division is an area of considerable recent attention and debate.[15]

The extent of hypertrophy varies for different underlying causes. Heart weight usually ranges from 350 to 600 gm (up to approximately two times normal) in pulmonary hypertension and ischemic heart disease; from 400 to 800 gm (up to two to three times normal) in systemic hypertension, aortic stenosis, mitral regurgitation, or dilated cardiomyopathy; from 600 to 1000 gm (three or more times normal) in aortic regurgitation or hypertrophic cardiomyopathy. Hearts weighing more than 1000 gm are rare.

The pattern of hypertrophy reflects the nature of the stimulus (Fig. 12-3). Pressure-overloaded ventricles (e.g., in hypertension or aortic stenosis) develop *pressure-overload* (also called *concentric*) *hypertrophy* of the left ventricle, with an increased wall thickness. In the left ventricle the augmented muscle may reduce the cavity diameter. In pressure overload, the predominant deposition of sarcomeres is parallel to the long axes of cells; cross-sectional area of myocytes is expanded (but cell length is not). In contrast, volume overload stimulates deposition of new sarcomeres and cell length (as well as

width) is increased. Thus, *volume-overload hypertrophy* is characterized by dilation with increased ventricular diameter. In volume overload, muscle mass and wall thickness are increased approximately in proportion to chamber diameter. However, owing to dilation, wall thickness of a heart in which both hypertrophy and dilation have occurred is not necessarily increased, and it may be normal or less than normal. Thus, wall thickness is by itself not an adequate measure of volume-overload hypertrophy.

Cardiac hypertrophy is also accompanied by numerous transcriptional and morphologic changes. With prolonged hemodynamic overload, gene expression is altered, leading to re-expression of a pattern of protein synthesis analogous to that seen in fetal cardiac development; other changes are analogous to events that occur during mitosis of normally proliferating cells (Chapter 1). Early mediators of hypertrophy include the immediate-early genes (e.g., *c-fos, c-myc, c-jun* and *EGR1*). Selective up-regulation or re-expression of embryonic/fetal

forms of contractile and other proteins also occurs, including β-myosin heavy chain, ANP, and collagen (see Chapter 1). The increased myocyte size that occurs in cardiac hypertrophy is usually accompanied by decreased capillary density, increased intercapillary distance, and deposition of fibrous tissue. Nevertheless, the enlarged muscle mass has increased metabolic requirements and increased wall tension, both major determinants of the oxygen consumption of the heart. The other major factors in oxygen consumption are heart rate and contractility (inotropic state, or force of contraction), both of which are often increased in hypertrophic states.

Thus, the geometry, structure, and composition (cells and extracellular matrix) of the hypertrophied heart are not normal. Cardiac hypertrophy constitutes a tenuous balance between adaptive characteristics (including new sarcomeres) and potentially deleterious structural and biochemical/molecular alterations

Figure 12-3 Left ventricular hypertrophy. *A,* Pressure hypertrophy due to left ventricular outflow obstruction. The left ventricle is on the lower right in this apical four-chamber view of the heart. *B,* Altered cardiac configuration in left ventricular hypertrophy without and with dilation, viewed in transverse heart sections. Compared with a normal heart (*center*), the pressure-hypertrophied hearts (*left* and in *A*) have increased mass and a thick left ventricular wall, but the hypertrophied and dilated heart *(right)* has increased mass but a normal wall thickness. *(Reproduced by permission from Edwards WD: Cardiac anatomy and examination of cardiac specimens. In Emmanouilides GC, Riemenschneider TA, Allen HD, Gutgesell HP (eds): Moss and Adams Heart Disease in Infants, Children, and Adolescents: Including the Fetus and Young Adults, 5th ed. Philadelphia, Williams and Wilkins, 1995, p. 86.)*

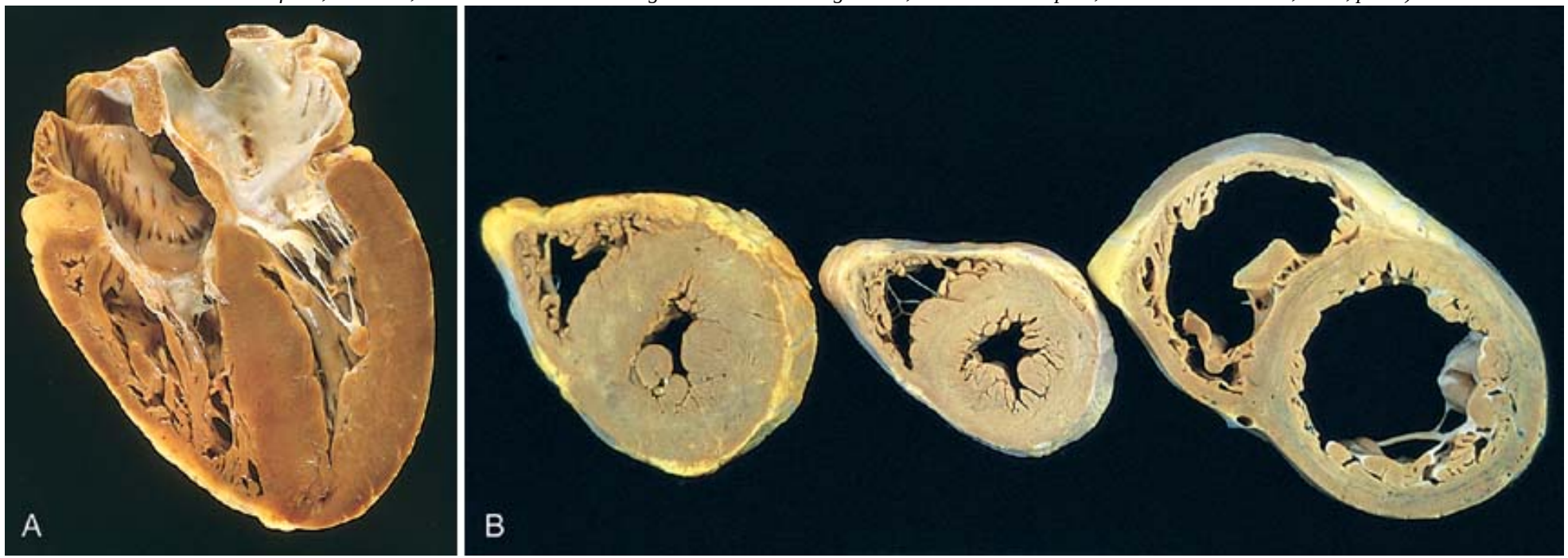

Figure 12-4 Schematic representation of the sequence of events in cardiac hypertrophy and its progression to heart failure, emphasizing cellular and extracellular changes.

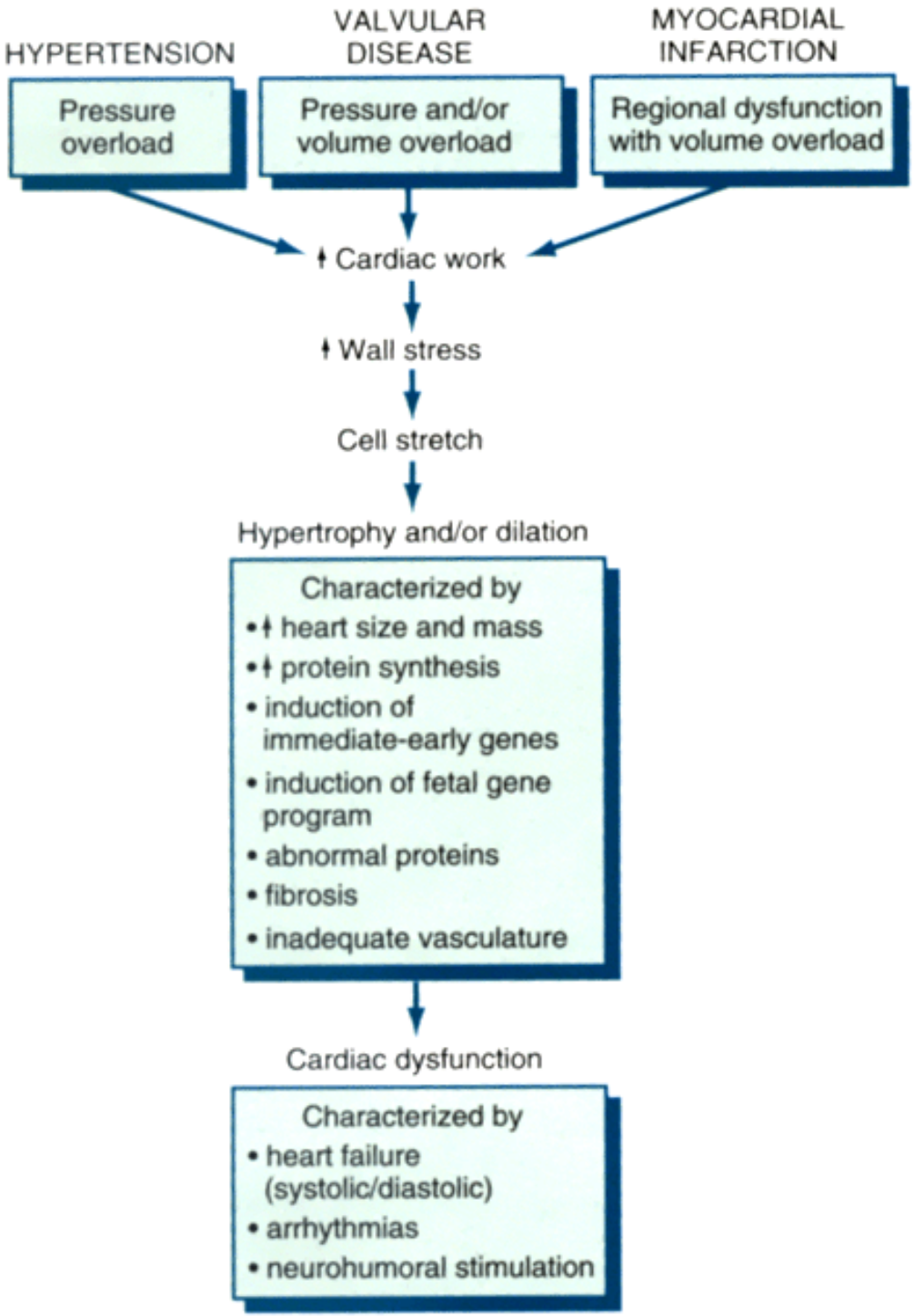

TABLE 12-2 -- Frequencies of Congenital Cardiac Malformations [*]

Malformation	Incidence per Million Live Births	%
Ventricular septal defect	4482	42
Atrial septal defect	1043	10
Pulmonary stenosis	•836	•8
Patent ductus arteriosus	•781	•7
Tetralogy of Fallot	•577	•5
Coarctation of aorta	•492	•5
Atrioventricular septal defect	•396	•4
Aortic stenosis	•388	•4
Transposition of great arteries	•388	•4
Truncus arteriosus	•136	•1
Total anomalous pulmonary venous connection	•120	•1
Tricuspid atresia	•118	•1
TOTAL	9757	

Source: Hoffman JIE, Kaplan S: The incidence of congenital heart disease. J Am Coll Cardiol 39:1890, 2002.

*Presented as upper quartile of 44 published studies. Percentages do not add to 100% owing to rounding.

echocardiography and magnetic resonance imaging). The enhanced resolving power of noninvasive methods should prove particularly useful in the study of familial structural defects, because apparently unaffected relatives can be evaluated for subclinical evidence of anomalies.

Etiology and Pathogenesis.

The etiology of congenital malformations in general was discussed in Chapter 10 . We therefore confine our remarks to factors of particular relevance to congenital cardiac malformations.

Congenital heart defects are caused by developmental abnormalities. However, the genes that may be involved in these defects have been identified in only a minority of conditions. In fact, well-defined genetic or environmental influences are identifiable in only about 10% of cases of congenital heart disease, but the understanding of probable genetic links is increasing. The obvious role of genetic factors in some cases is demonstrated by the occurrence of familial forms of congenital heart disease and by an association of congenital cardiac malformations with certain chromosomal abnormalities (e.g., trisomies 13, 15, 18, and 21, and the Turner syndrome). Indeed, a congenital heart defect in a parent or preceding sibling is the greatest risk factor for developing a cardiac malformation. Trisomy 21 (associated with Down syndrome) is the most common known genetic cause of congenital heart disease. *Environmental factors*, such as congenital rubella infection or teratogens, are responsible for some additional cases. Multifactorial genetic, environmental, and maternal factors probably account for the remaining majority of cases in which a cause is not apparent.

The growing understanding of the genetics of congenital heart disease has also led to the recognition that powerful disease modifiers must exist. There is wide variation in the

nature and severity of lesions in patients with identical genetic abnormalities. This suggests that altering key environmental or maternal factors could modify disease in high-risk individuals, whether or not the disease is caused by a distinct genetic abnormality. For instance, this type of strategy has resulted in marked reduction in neural tube defects by increasing maternal dietary folate.[25]

Genetics of Cardiac Development and Congenital Heart Disease.

Composed of diverse cell lineages, the heart is among the first organs to form and function in vertebrate embryos. Cardiac morphogenesis involves a myriad of genes and is tightly regulated to ensure an effective embryonic circulation. Key steps involve specification of cardiac cell fate, morphogenesis and looping of the heart tube, segmentation and growth of the cardiac chambers, cardiac valve formation, and connection of the great vessels to the heart.[26] The genetic regulation of heart formation has been widely studied in model organisms, including chick, frog, mouse, and zebrafish. In recent years, the zebrafish, an organism that is transparent and has external fertilization, a brief generation time, and no requirement of a functional cardiovascular system for survival during embryogenesis, has permitted detailed genetic analysis of both normal development and cardiac defects. [27] [28] The molecular pathways controlling cardiac development provide a foundation for understanding the basis of some congenital heart defects and can be used to reveal pathways and interactions important in human disease.[29]

Several congenital heart diseases are associated with mutations in transcription factors. For example, mutation of the gene that encodes the transcription factor, TBX5, has been shown to cause the ASD and VSD observed in the Holt-Oram syndrome, a rare hereditary condition associated with heart, arm, and hand defects.[30] Another gene, encoding the transcription factor NKX2.5, causes nonsyndromic (isolated) ASD in humans when one copy is missing. This gene is the human counterpart of the *tinman* gene of the fruit fly (so named because, like the Tin Man in *The Wizard of Oz*, fruit fly embryos lacking both copies of *tinman* have no hearts). Nevertheless, most ASDs do not have an identifiable genetic etiology, and the mechanisms by which mutated transcription factors cause clinically important heart defects are just beginning to be understood.[31]

Until recently, in most studies, defects were classified by their pathology; for example, all VSDs were considered as one group. A major advance has been to examine familial aggregation of defects based on presumed pathogenesis. Since some cardiac structures share developmental pathways, anatomically and clinically distinct lesions may be related by a common genetic defect. Thus, the occurrence of distinct defects in the same family remains consistent with a genetic model. Defects unrelated by pathogenesis would require a different interpretation.

Developmental errors in mesenchymal tissue migration exemplify the concept that distinct syndromes share a common pathogenesis. Included in this category is a wide range of anomalies of the outflow tract, some due to failure of fusion and others due to failure of septation. These lesions include isolated interruption of the aortic arch, persistent truncus arteriosus (failure of separation of aorta and pulmonary arteries), and tetralogy of Fallot (malalignment of aorta and pulmonary artery with the ventricles). Comprising 15% of congenital heart defects, outflow tract defects may be caused by the abnormal development of neural crest-derived cells, whose migration into the embryonic heart is required for formation of the outflow tracts of the heart (Fig. 12-5). Considerable progress has been made during the past few years in identifying a region of chromosome 22 that has a major role in development of the conotruncus, the branchial arches, and the face. Chromosome 22q11.2 deletions are seen in 15% to 50% of these disorders, rendering this abnormality a common genetic cause of congenital heart defects (see also Chapter 5). This condition includes developmental anomalies of the fourth branchial arch and derivatives of the third and fourth pharyngeal pouches. Hypoplasia of the thymus and parathyroids causes immune deficiency (Di George syndrome, Chapter 5) and hypocalcemia.

Other common mechanisms of congenital heart disease include extracellular matrix abnormalities and situs and looping defects. The endocardial cushions have received the most attention as an area where defects in cell-cell and cell-extracellular matrix interactions might produce malformations, as evidenced by a high frequency of endocardial cushion defects and atrioventricular septal defects in Down syndrome. Situs and looping defects may arise from single genes that have a major effect on determining laterality.

Clinical Features.

The varied structural anomalies in congenital heart disease fall primarily into three major categories:

- Malformations causing a *left-to-right shunt*
- Malformations causing a *right-to-left shunt*
- Malformations causing an *obstruction*.

A *shunt* is an abnormal communication between chambers or blood vessels. Abnormal channels permit the flow of blood from left to right or the reverse, depending on pressure relationships. When blood from the right side of the heart enters the left side *(right-to-left shunt)*, a dusky blueness of the skin and mucous membranes *(cyanosis)* results because there is diminished pulmonary blood flow, and poorly oxygenated blood enters the systemic circulation (called *cyanotic congenital heart disease*). The most important examples of right-to-left shunts are tetralogy of Fallot, transposition of the great arteries, persistent truncus arteriosus, tricuspid atresia, and total anomalous pulmonary venous connection. Moreover, with right-to-left shunts, bland or septic emboli arising in peripheral veins can bypass the normal filtration action of the lungs and thus directly enter the systemic circulation *(paradoxical embolism)*; brain infarction and abscess are potential consequences. Clinical findings frequently associated with severe, long-standing cyanosis include *clubbing of the tips of the fingers and toes* (hypertrophic osteoarthropathy) and polycythemia.

In contrast, *left-to-right shunts* (such as ASD, VSD, and patent ductus arteriosus [PDA]) increase pulmonary blood flow and are not initially associated with cyanosis. However, they expose the postnatal, low-pressure, low-resistance pulmonary circulation to increased pressure and/or volume, which can result in right ventricular hypertrophy and, potentially, failure. Shunts associated with increased pulmonary blood flow include ASDs; shunts associated with both increased pulmonary blood flow and pressure include VSDs and PDA. The muscular pulmonary arteries (<1 mm diameter) first respond to increased pressure by medial hypertrophy

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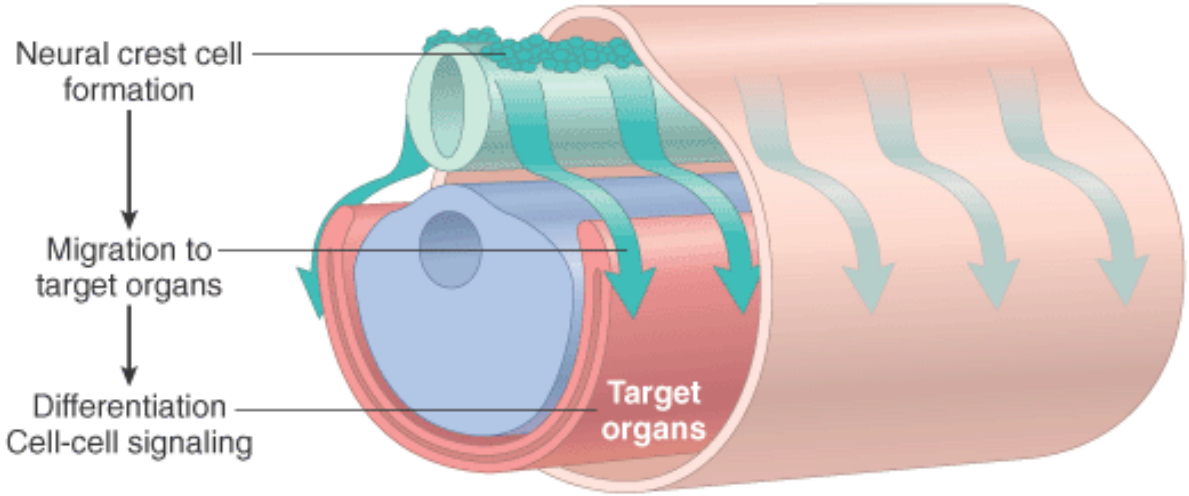
Figure 12-5 Cardiac defects related to neural crest abnormalities. *A*, Biologic pathways for cardiac neural crest-related defects. *B*, Disease phenotypes. DORV, double-outlet right ventricle; TGA, transposition of the great arteries. *(Reproduced by permission from Chien KR: Genomic circuits and the integrative biology of cardiac diseases. Nature 407:227, 2000.)*

A



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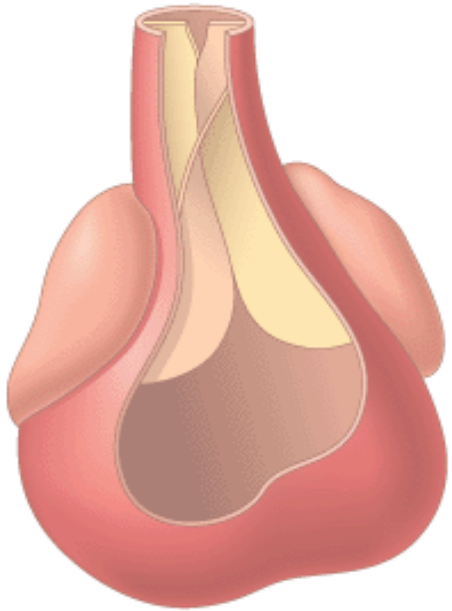
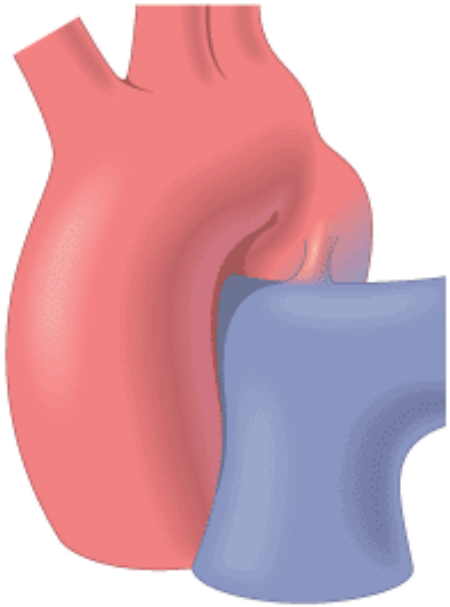


Cardiac outflow tract

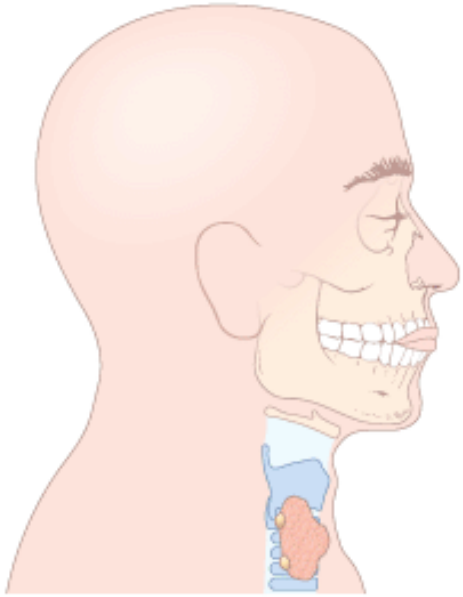
- Persistent truncus arteriosus
- DORV
- TGA?
- Hypoplastic left heart syndrome?



Aortic arch

↓

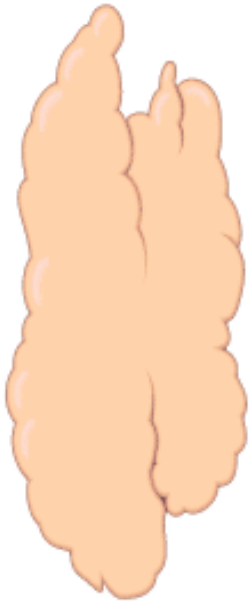
Interruption/ coarctation of aortic arch



Craniofacial structures

↓

Craniofacial defect
Hypothyroidism
Hypoparathyroidism



Thymus

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T cell deficiency

arteriosus syndrome? of aortic arch Hypoparathyroidism

Figure 12-6 Schematic diagram of congenital left-to-right shunts. *A,* Atrial septal defect (ASD). *B,* Ventricular septal defect (VSD). With VSD the shunt is left-to-right, and the pressures are the same in both ventricles. Pressure hypertrophy of the right ventricle and volume hypertrophy of the left ventricle are generally present. *C,* Patent ductus arteriosus (PDA). *D,* Atrioventricular septal defect (AVSD). *E,* Large VSD with irreversible pulmonary hypertension. The shunt is right-to-left (shunt reversal). Volume hypertrophy and pressure hypertrophy of the right ventricle are present. Arrow indicates the direction of blood flow. The right ventricular pressure is now sufficient to yield a right-to-left shunt (Ao, aorta; LA, left atrium; LV, left ventricle; PT, pulmonary trunk; RA, right atrium; RV, right ventricle.)

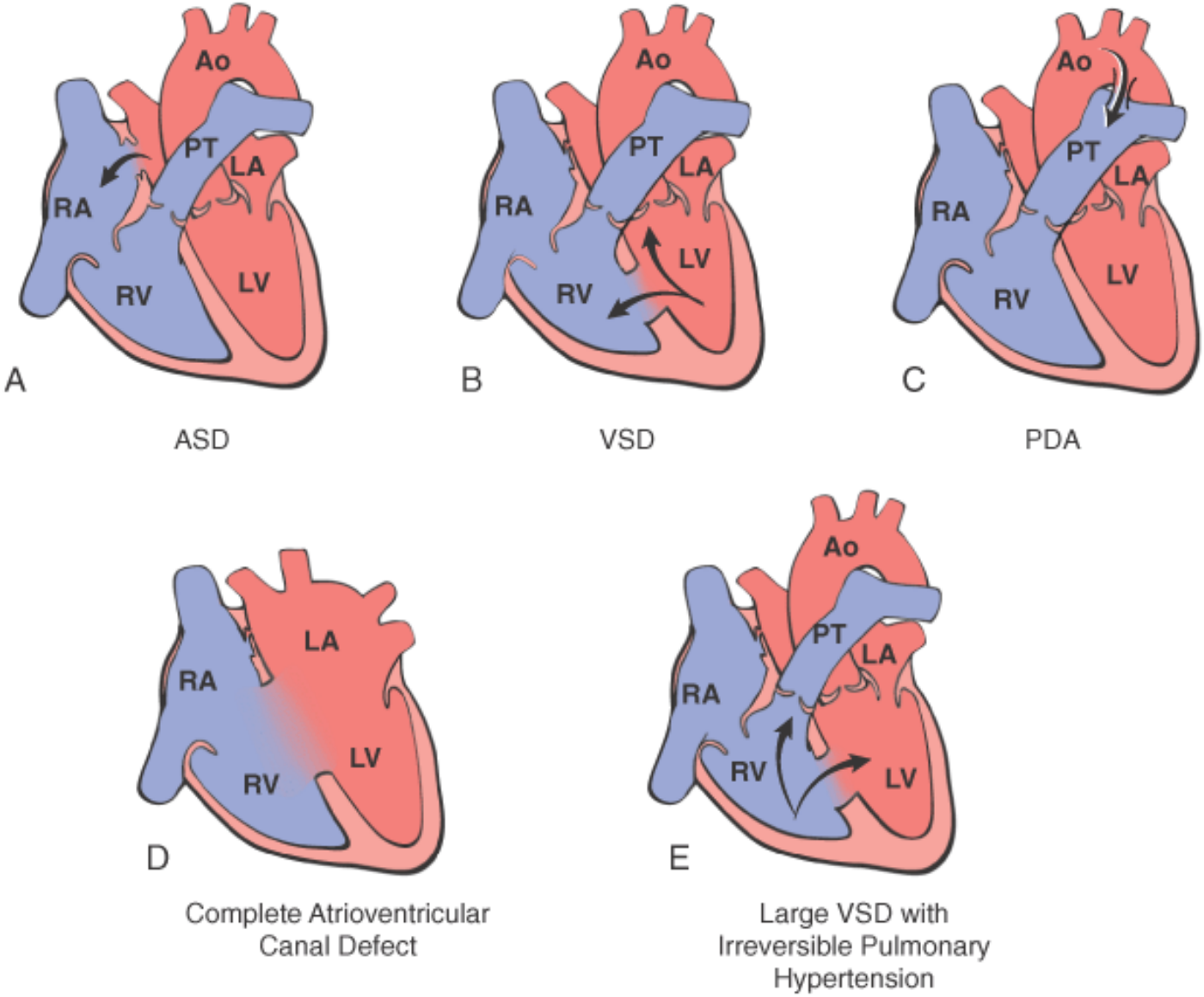

Figure 12-7 Gross photograph of a ventricular septal defect (membranous type); defect denoted by arrow. *(Courtesy of William D. Edwards, M.D., Mayo Clinic, Rochester, MN.)*

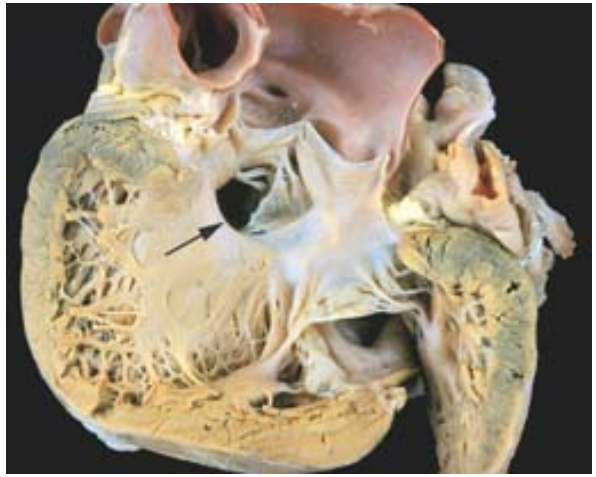

Figure 12-8 Schematic diagram of the most important right-to-left shunts *(cyanotic congenital heart disease)*. *A*, Tetralogy of Fallot. Diagrammatic representation of anatomic variants, indicating that the direction of shunting across the VSD depends on the severity of the subpulmonary stenosis. Arrows indicate the direction of the blood flow. *B*, Transposition of the great vessels with and without VSD. (Ao, aorta; LA, left atrium; LV, left ventricle; PT, pulmonary trunk; RA, right atrium; RV, right ventricle.) *(Courtesy of William D. Edwards, M.D., Mayo Clinic, Rochester, MN.)*

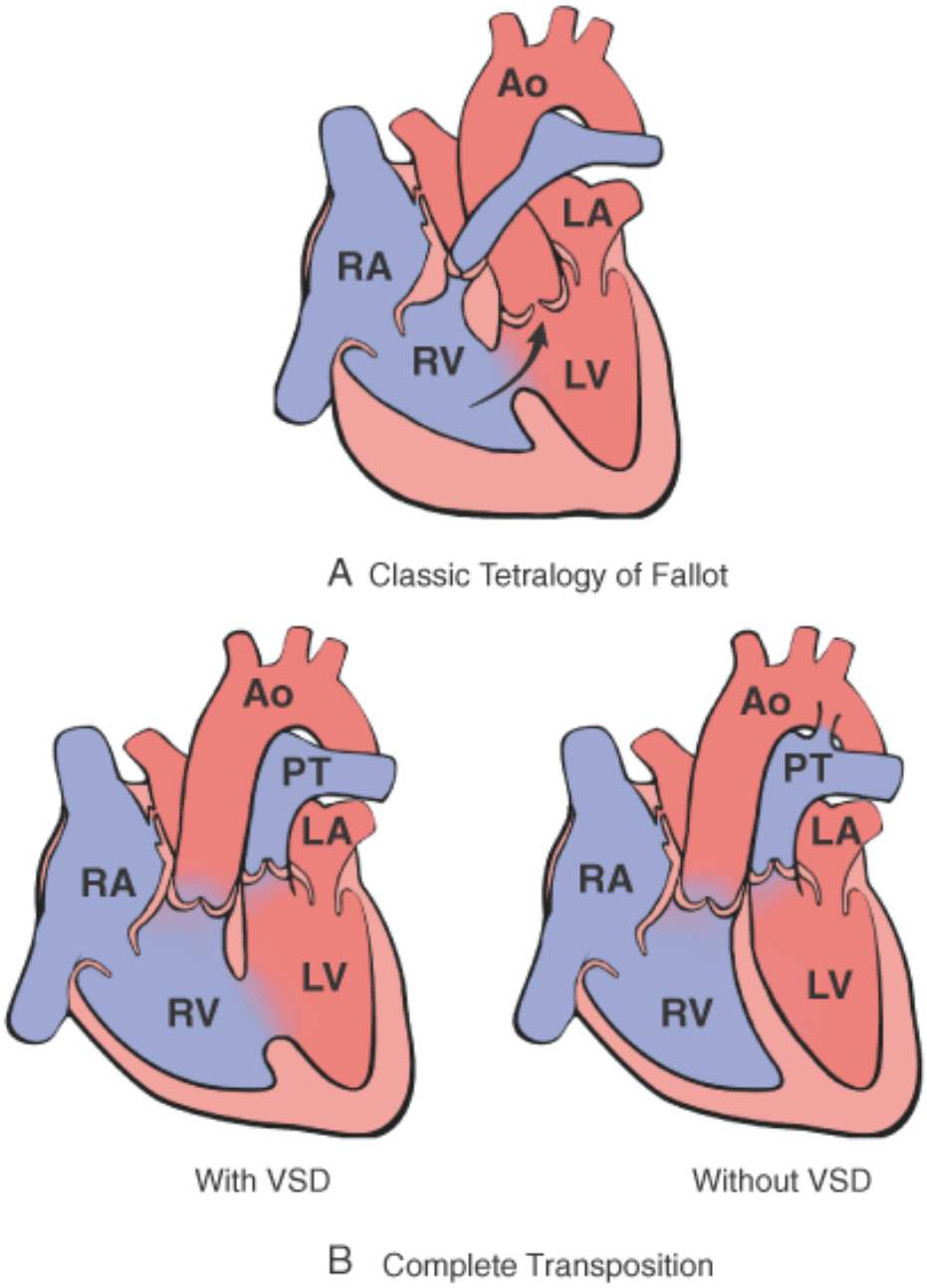

Figure 12-9 Transposition of the great arteries. *(Courtesy of William D. Edwards, M.D., Mayo Clinic, Rochester, MN.)*

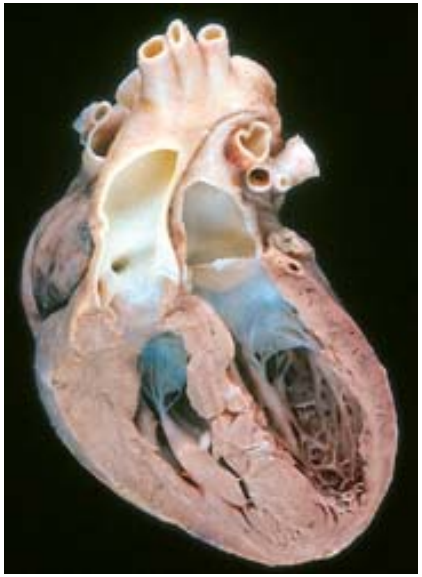

Figure 12-10 Diagram showing coarctation of the aorta with and without PDA. (Ao, aorta; LA, left atrium; LV, left ventricle; PT, pulmonary trunk; RA, right atrium; RV, right ventricle; PDA, persistent ductus arteriosus.) *(Courtesy of William D. Edwards, M.D., Mayo Clinic, Rochester, MN.)*

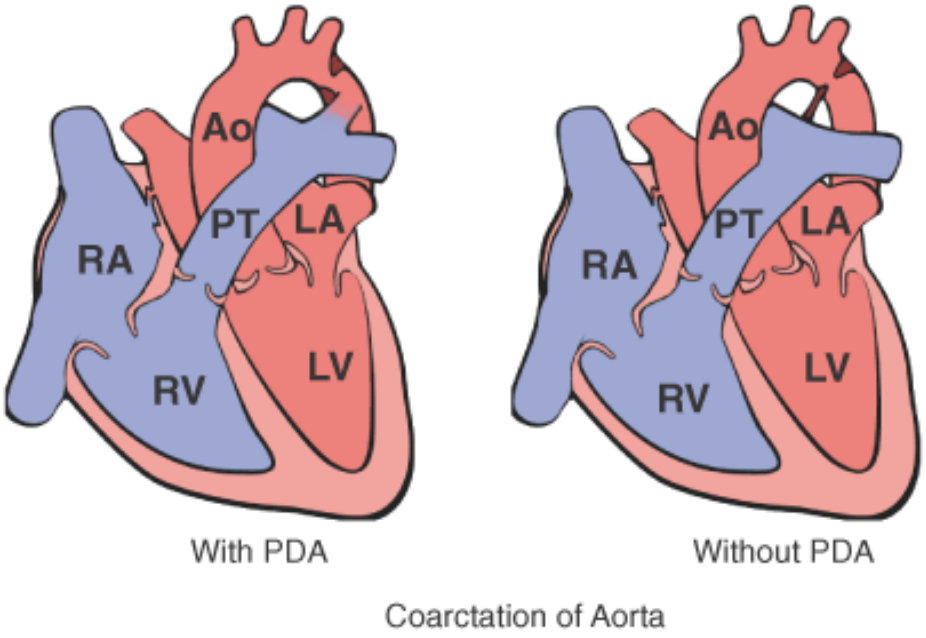

Figure 12-11 Atherosclerotic plaque rupture. *A,* Plaque rupture without superimposed thrombus, in patient who died suddenly. *B,* Acute coronary thrombosis superimposed on an atherosclerotic plaque with focal disruption of the fibrous cap, triggering fatal myocardial infarction. *C,* Massive plaque rupture with superimposed thrombus, also triggering a fatal myocardial infarction (special stain highlighting fibrin in red). In both *A* and *B,* an arrow points to the site of plaque rupture. *(B, reproduced from Schoen FJ: Interventional and Surgical Cardiovascular Pathology: Clinical Correlations and Basic Principles. Philadelphia, W.B. Saunders, 1989, p. 61.)*

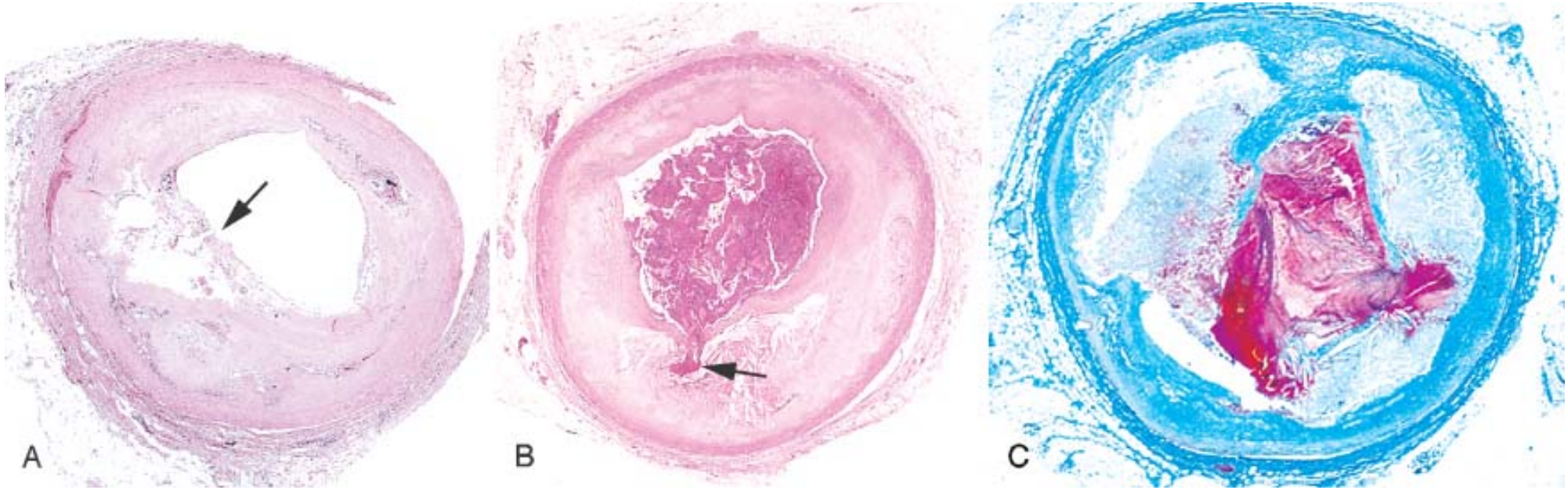

Figure 12-12 Schematic representation of sequential progression of coronary artery lesion morphology, beginning with stable chronic plaque responsible for typical angina and leading to the various acute coronary syndromes. *(Modified and redrawn from Schoen FJ: Interventional and Surgical Cardiovascular Pathology: Clinical Correlations and Basic Principles. Philadelphia, W.B. Saunders Co., 1989, p. 63.)*

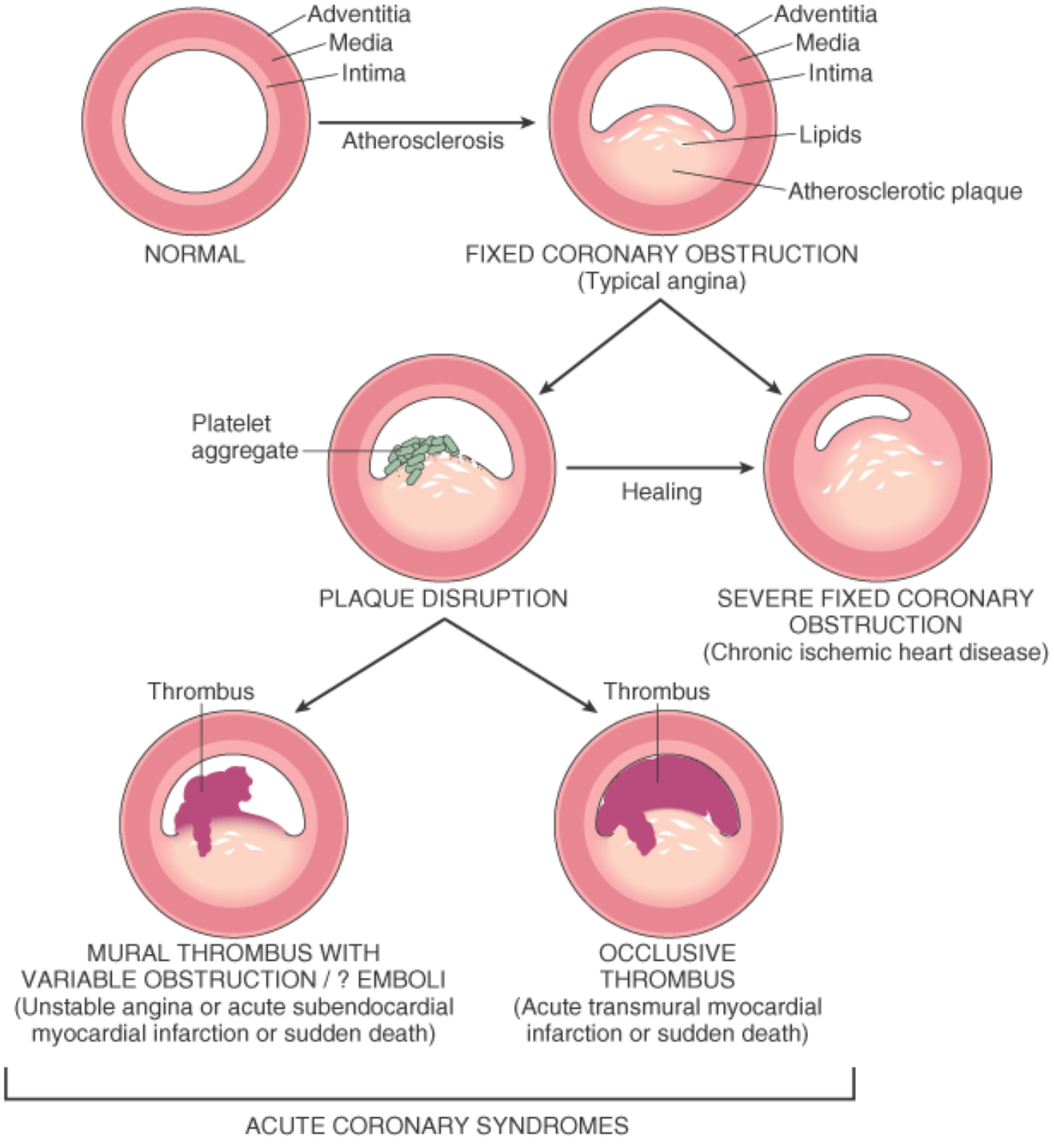
Adventitia
Media
Intima
Atherosclerosis
Adventitia
Media
Intima
Lipids
Atherosclerotic plaque
NORMAL
FIXED CORONARY OBSTRUCTION
(Typical angina)
Platelet
aggregate
Healing
PLAQUE DISRUPTION
SEVERE FIXED CORONARY
OBSTRUCTION
(Chronic ischemic heart disease)
Thrombus
Thrombus
MURAL THROMBUS WITH
VARIABLE OBSTRUCTION / ? EMBOLI
(Unstable angina or acute subendocardial
myocardial infarction or sudden death)
OCCLUSIVE
THROMBUS
(Acute transmural myocardial
infarction or sudden death)
ACUTE CORONARY SYNDROMES

TABLE 12-3 -- Coronary Artery Pathology in Ischemic Heart Disease

Syndrome	Stenoses	Plaque Disruption	Plaque-Associated Thrombus
Stable angina	>75%	No	No
Unstable angina	Variable	Frequent	Nonocclusive, often with thromboemboli
Transmural myocardial infarction	Variable	Frequent	Occlusive
Subendocardial myocardial infarction	Variable	Variable	Widely variable, may be absent, partial/complete, or lysed
Sudden death	Usually severe	Frequent	Often small platelet aggregates or thrombi and/or thromboemboli

ANGINA PECTORIS

Angina pectoris is a symptom complex of IHD characterized by paroxysmal and usually recurrent attacks of substernal or precordial chest discomfort (variously described as constricting, squeezing, choking, or knifelike) caused by transient (15 seconds to 15 minutes) myocardial ischemia that falls short of inducing the cellular necrosis that defines infarction. There are three overlapping patterns of angina pectoris: (1) stable or typical angina, (2) Prinzmetal or variant angina, and (3) unstable or crescendo angina. They are caused by varying combinations of increased myocardial demand and decreased myocardial perfusion, owing to fixed stenosing plaques, disrupted plaques, vasospasm, thrombosis, platelet aggregation, and embolization. Moreover, it is being increasingly recognized that not all ischemic events are perceived by patients, even though such events may have adverse prognostic implications *(silent ischemia).*

Stable angina, the most common form and therefore called *typical angina pectoris, appears to be caused by the reduction of coronary perfusion to a critical level by chronic stenosing coronary atherosclerosis;* this renders the heart vulnerable to further ischemia whenever there is increased demand, such as that produced by physical activity, emotional excitement, or any other cause of increased cardiac workload. Typical angina pectoris is usually relieved by rest (thereby decreasing demand) or nitroglycerin, a strong vasodilator. Although the coronary arteries are usually maximally dilated by intrinsic regulatory influences, nitroglycerin also decreases cardiac work by dilating the peripheral vasculature. In particular instances, local vasospasm may contribute to the imbalance between supply and demand.

Prinzmetal variant angina is an uncommon pattern of episodic angina that occurs at rest and is due to coronary artery spasm. Usually there is an elevated ST segment on the electrocardiogram (ECG), indicative of transmural ischemia. Although individuals with this form of angina may well have significant coronary atherosclerosis, the anginal attacks are unrelated to physical activity, heart rate, or blood pressure. Prinzmetal angina generally responds promptly to vasodilators, such as nitroglycerin and calcium channel blockers.

Unstable or *crescendo angina* refers to a pattern of pain that occurs with progressively increasing frequency, is precipitated with progressively less effort, often occurs at rest, and tends to be of more prolonged duration. *As discussed above, in most patients, unstable angina is induced by disruption of an atherosclerotic plaque with superimposed partial (mural) thrombosis and possibly embolization or vasospasm (or both).* Although the ischemia that occurs in unstable angina falls precariously close to inducing clinically detectable infarction, unstable angina is often the prodrome of subsequent acute MI. Thus this syndrome is sometimes referred to as *preinfarction angina*, and in the spectrum of IHD, unstable angina lies intermediate between stable angina on the one hand and MI on the other.

MYOCARDIAL INFARCTION (MI)

MI, also known as "heart attack," is the death of cardiac muscle resulting from ischemia. It is by far the most important form of IHD and alone is the leading cause of death in the United States and industrialized nations. About 1.5 million individuals in the United States suffer an acute MI annually and approximately one third of them die. At least 250,000 people a year die of a heart attack before they reach the hospital.

Transmural versus Subendocardial Infarction.

Most myocardial infarcts are *transmural*, in which the ischemic necrosis involves the full or nearly full thickness of the ventricular wall in the distribution of a single coronary artery. This pattern of infarction is usually associated with coronary atherosclerosis, acute plaque change, and superimposed thrombosis (as discussed previously). In contrast, a *subendocardial (nontransmural) infarct* constitutes an area of ischemic necrosis limited to the inner one third or at most one half of the ventricular wall; under some circumstances, it may extend laterally beyond the perfusion territory of a single coronary artery. As previously pointed out, the subendocardial zone is normally the least well-perfused region of myocardium and therefore is most vulnerable to any reduction in coronary flow. A subendocardial infarct can occur as a result of a plaque disruption followed by coronary thrombus that becomes lysed before myocardial necrosis extends across the major thickness of the wall; in this case the infarct will be limited to the distribution of one coronary artery with plaque change. However, subendocardial infarcts can also result from sufficiently prolonged and severe reduction in systemic blood pressure, as in shock, often superimposed on chronic, otherwise noncritical, coronary stenoses. In cases of global hypotension, resulting subendocardial infarcts are usually circumferential or nearly so, rather than limited to the distribution of a single major coronary artery.

Incidence and Risk Factors.

The risk factors for atherosclerosis, the major underlying cause of IHD in general, are discussed in Chapter 11 and are not reiterated here. Suffice it to say that *MI may occur at virtually any age, but the frequency rises progressively with increasing age and when predispositions*

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to atherosclerosis are present, such as hypertension, cigarette smoking, diabetes mellitus, genetic hypercholesterolemia, and other causes of hyperlipoproteinemia. Nearly 10% of myocardial infarcts occur in people under age 40, and 45% occur in people under age 65. Blacks and whites are equally affected. Throughout life, men are at significantly greater risk of MI than women; the differential progressively declines with advancing age. *Except for those having some predisposing atherogenic condition, women are remarkably protected against MI during the reproductive years.* Nevertheless, the decrease of estrogen following menopause can permit rapid development of coronary artery disease (CAD), and IHD is the overwhelming cause of death in elderly women. Moreover, recent epidemiologic evidence suggests that postmenopausal hormone replacement therapy does not protect women against MI.[53]

Pathogenesis.

We now consider the basis for and subsequent consequences of myocardial ischemia, particularly as they relate to the typical transmural myocardial infarct.

Coronary Arterial Occlusion.

As discussed above, transmural acute MI results from a dynamic interaction among several or all of the following—coronary atherosclerosis, acute atheromatous plaque change (such as rupture), superimposed platelet activation, thrombosis, and vasospasm—resulting in an occlusive intracoronary thrombus overlying a disrupted plaque. In addition, either increased myocardial demand (as with hypertrophy or tachycardia) or hemodynamic compromise (as with a drop in blood pressure) can worsen the situation. Recall also that collateral circulation may provide perfusion to ischemic zones from a relatively unobstructed branch of the coronary tree, bypassing the point of obstruction and protecting against the effects of an acute coronary occlusion.

In the typical case of MI, the following sequence of events can be proposed:

- *The initial event is a sudden change in the morphology of an atheromatous plaque*, that is, disruption—manifest as intraplaque hemorrhage, erosion or ulceration, or rupture or fissuring.
- Exposed to subendothelial collagen and necrotic plaque contents, *platelets undergo adhesion, aggregation, activation, and release of potent aggregators* including thromboxane

A_2 , serotonin, and platelet factors 3 and 4.

- Vasospasm is stimulated by platelet aggregation and the release of mediators.
- Other mediators activate the extrinsic pathway of coagulation, adding to the bulk of the thrombus.
- *Frequently within minutes, the thrombus evolves to completely occlude the lumen of the coronary vessel.*

The evidence for this sequence is compelling and derives from (1) autopsy studies of patients dying with acute MI, (2) angiographic studies demonstrating a high frequency of thrombotic occlusion early after MI, (3) the high success rate of therapeutic thrombolysis and primary angioplasty, and (4) the demonstration of residual disrupted atherosclerotic lesions by angiography after thrombolysis. Although coronary angiography performed within 4 hours of the onset of apparent MI shows a thrombosed coronary artery in almost 90% of cases, the observation of occlusion is seen in only about 60% when angiography is delayed until 12 to 24 hours after onset. [54] Thus with the passage of time, at least some occlusions appear to clear spontaneously owing to lysis of the thrombus or relaxation of spasm or both.

In approximately 10% of cases, transmural acute MI is not associated with atherosclerotic plaque thrombosis stimulated by disruption. In such situations, other mechanisms may be involved:

- *Vasospasm*: isolated, intense, and relatively prolonged, with or without coronary atherosclerosis, perhaps in association with platelet aggregation (sometimes related to cocaine abuse).
- *Emboli*: from the left atrium in association with atrial fibrillation, a left-sided mural thrombus or vegetative endocarditis; or *paradoxical emboli* from the right side of the heart or the peripheral veins which cross to the systemic circulation, through a patent foramen ovale, causing coronary occlusion.
- *Unexplained*: cases without detectable coronary atherosclerosis and thrombosis may be caused by diseases of small intramural coronary vessels such as vasculitis, hematologic abnormalities such as hemoglobinopathies, amyloid deposition in vascular walls, or other unusual disorders, such as vascular dissection and inadequate protection during cardiac surgery.

Myocardial Response.

The consequence of coronary arterial obstruction is the loss of critical blood supply to the myocardium (Fig. 12-13), *which induces profound functional, biochemical, and morphologic consequences*. Occlusion of a major coronary artery results in ischemia and, potentially, cell death throughout

Figure 12-13 Postmortem angiogram showing the posterior aspect of the heart of a patient who died during the evolution of acute myocardial infarction, demonstrating total occlusion of the distal right coronary artery by an acute thrombus *(arrow)* and a large zone of myocardial hypoperfusion involving the posterior left and right ventricles, as indicated by arrowheads, and having almost absent filling of capillaries, that is, less white. The heart has been fixed by coronary arterial perfusion with glutaraldehyde and cleared with methyl salicylate, followed by intracoronary injection of silicone polymer. *Photograph courtesy of Lewis L. Lainey. (Reproduced by permission from Schoen FJ: Interventional and Surgical Cardiovascular Pathology: Clinical Correlations and Basic Principles. Philadelphia, WB Saunders, 1989, p. 60.)*

TABLE 12-4 -- Approximate Time of Onset of Key Events in Ischemic Cardiac Myocytes

Feature	Time
Onset of ATP depletion	Seconds
Loss of contractility	<2 min
ATP reduced	
••to 50% of normal	10 min
••to 10% of normal	40 min
Irreversible cell injury	20–40 min
Microvascular injury	>1 hr

ATP, adenosine triphosphate.

leading cause of mortality in IHD patients, can be caused by massive cell injury with mechanical failure but is most often due to ventricular fibrillation caused by myocardial irritability induced by ischemia or infarction. Interestingly, studies of resuscitated survivors of "sudden death" show that the majority do not develop acute MI; in such cases, myocardial irritability induced by ischemia presumably led directly to the serious arrhythmia.

The progression of ischemic necrosis in the myocardium is summarized in Figure 12-14 . Irreversible injury of ischemic myocytes occurs first in the subendocardial zone. With more extended ischemia, a *wavefront* of cell death moves through the myocardium to involve progressively more of the transmural thickness of the ischemic zone. The precise location, size, and specific morphologic features of an acute myocardial infarct depend on:

- The location, severity, and rate of development of coronary atherosclerotic obstructions
- The size of the vascular bed perfused by the obstructed vessels
- The duration of the occlusion
- The metabolic/oxygen needs of the myocardium at risk
- The extent of collateral blood vessels

- The presence, site, and severity of coronary arterial spasm
- Other factors, such as alterations in blood pressure, heart rate, and cardiac rhythm.

The necrosis is largely complete within 6 hours in experimental models and humans, involving nearly all of the ischemic myocardial bed at risk supplied by the occluded coronary artery. Progression of necrosis, however, may follow a more protracted course in some patients (possibly over 6 to 12 hours or longer) in whom the coronary arterial collateral system, stimulated by chronic ischemia, is better developed and thereby more effective.

Morphology.

The evolution of the morphologic changes in acute MI and its healing are summarized in Table 12-5 .

Nearly all transmural infarcts involve at least a portion of the left ventricle (including the ventricular septum). About 15% to 30% of those that affect the posterior free wall and posterior portion of the septum transmurally extend into the adjacent right ventricular wall. Isolated infarction of the right ventricle, however, occurs in only 1% to 3% of cases. Associated infarction of atrial tissue accompanies a large posterior left ventricular infarct in some cases. Transmural infarcts usually encompass nearly the entire perfusion zone of the occluded coronary artery. Almost always there is a narrow rim (approximately 0.1 mm) of preserved subendocardial myocardium sustained by diffusion of oxygen and nutrients from the lumen.

The frequencies of critical narrowing (and thrombosis) of each of the three main arterial trunks and the corresponding sites of myocardial lesions resulting in infarction (in the typical right dominant heart) are as follows:

- Left anterior descending coronary artery (40% to 50%): infarct involves anterior wall of left ventricle

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near apex; anterior portion of ventricular septum; apex circumferentially
- Right coronary artery (30% to 40%): infarct involves inferior/posterior wall of left ventricle; posterior portion of ventricular septum; inferior/posterior right ventricular free wall in some cases
- Left circumflex coronary artery (15% to 20%): infarct involves lateral wall of left ventricle except at apex

Figure 12-14 Schematic representation of the progression of myocardial necrosis after coronary artery occlusion. Necrosis begins in a small zone of the myocardium beneath the endocardial surface in the center of the ischemic zone. This entire region of myocardium *(shaded)* depends on the occluded vessel for perfusion and is the area at risk. Note that a very narrow zone of myocardium immediately beneath the endocardium is spared from necrosis because it can be oxygenated by diffusion from the ventricle. The end result of the obstruction to blood flow is necrosis of the muscle that was dependent on perfusion from the coronary artery obstructed. Nearly the entire area at risk loses viability. The process is called *myocardial infarction*, and the region of necrotic muscle is a *myocardial infarct*.

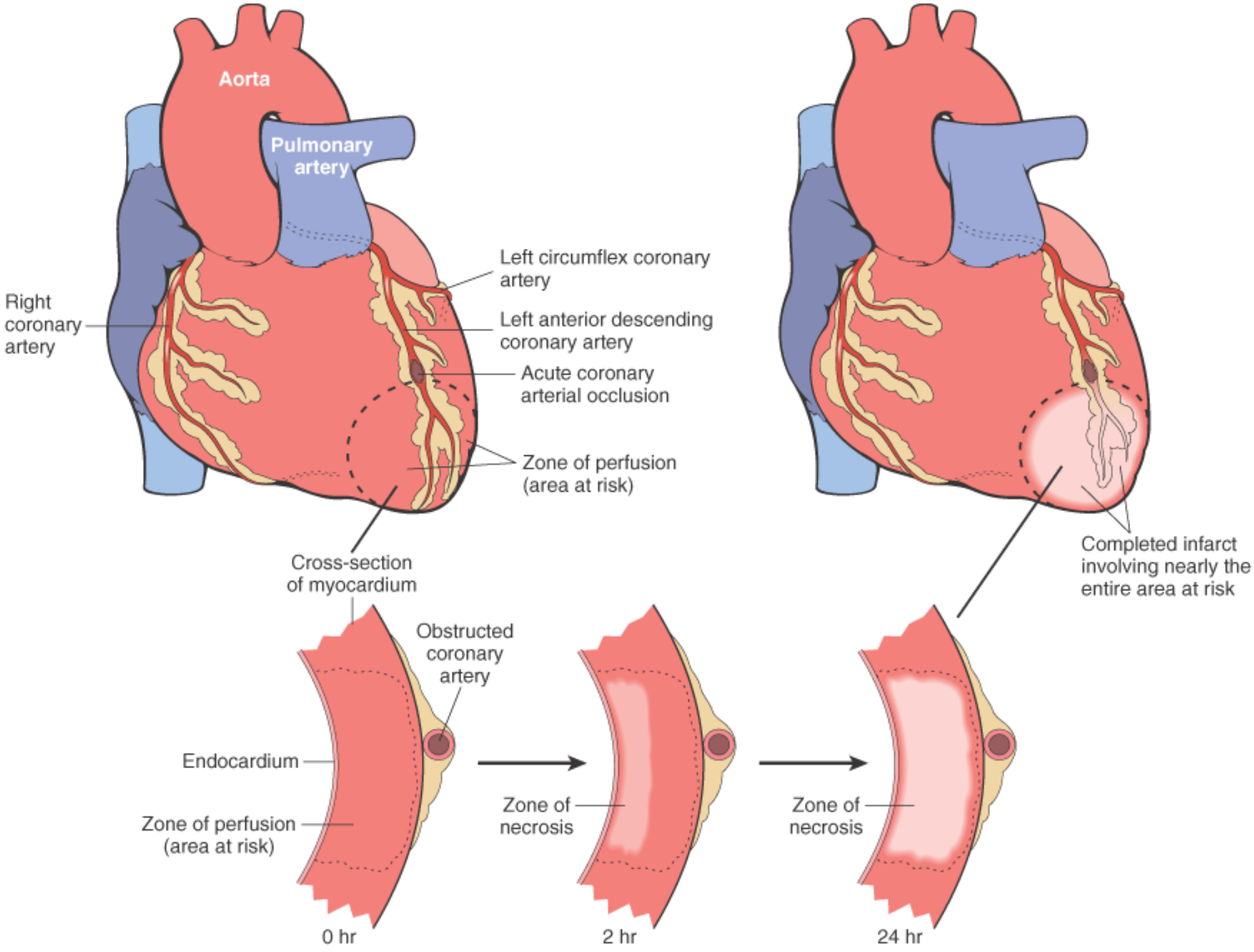

Aorta
Pulmonary artery
Left circumflex coronary artery
Right coronary artery
Left anterior descending coronary artery
Acute coronary arterial occlusion
Zone of perfusion (area at risk)
Completed infarct involving nearly the entire area at risk
Cross-section of myocardium
Obstructed coronary artery
Endocardium
Zone of perfusion (area at risk)
Zone of necrosis
Zone of necrosis
0 hr
2 hr
24 hr

TABLE 12-5 -- Evolution of Morphologic Changes in Myocardial Infarction

Time	Gross Features	Light Microscope	Electron Microscope
Reversible Injury			
0–½ hr	None	None	Relaxation of myofibrils; glycogen loss; mitochondrial swelling
Irreversible Injury			
½–4 hr	None	Usually none; variable waviness of fibers at border	Sarcolemmal disruption; mitochondrial amorphous densities
4–12 hr	Occasionally dark mottling	Beginning coagulation necrosis; edema; hemorrhage	
12–24 hr	Dark mottling	Ongoing coagulation necrosis; pyknosis of nuclei; myocyte hypereosinophilia; marginal contraction band necrosis; beginning neutrophilic infiltrate	
1–3 days	Mottling with yellow-tan infarct center	Coagulation necrosis, with loss of nuclei and striations; interstitial infiltrate of neutrophils	
3–7 days	Hyperemic border; central yellow-tan softening	Beginning disintegration of dead myofibers, with dying neutrophils; early phagocytosis of dead cells by macrophages at infarct border	
7–10 days	Maximally yellow-tan and soft, with depressed red-tan margins	Well-developed phagocytosis of dead cells; early formation of fibrovascular granulation tissue at margins	
10–14 days	Red-gray depressed infarct borders	Well-established granulation tissue with new blood vessels and collagen deposition	
2–8 wk	Gray-white scar, progressive from border toward core of infarct	Increased collagen deposition, with decreased cellularity	
>2 mo	Scarring complete	Dense collagenous scar	

solution of *triphenyltetrazolium chloride* (TTC). This histochemical stain imparts a brick-red color to intact, noninfarcted myocardium where the dehydrogenase enzymes are preserved. Because dehydrogenases are depleted in the area of ischemic necrosis (they leak out through the damaged cell membranes), an infarcted area is revealed as an unstained pale zone (while old scarred infarcts appear white and glistening) (Fig. 12-15). Subsequently, by 12 to 24 hours, an infarct can be identified in routinely fixed gross slices owing to a red-blue hue caused by stagnated, trapped blood. Progressively thereafter, the infarct becomes a more sharply defined, yellow-tan, somewhat softened area that by 10 days to 2 weeks is rimmed by a hyperemic zone of highly vascularized granulation tissue. Over the succeeding weeks, the injured region evolves to a fibrous scar.

The histopathologic changes also have a fairly predictable sequence (summarized in Table 12-5 and Figure 12-16). Using light microscopic examination of routinely stained tissue sections, the typical changes of coagulative necrosis become detectable variably in the first 4 to 12 hours. "Wavy fibers" may be present at the periphery of the infarct; these changes probably result from the forceful systolic tugs by the viable fibers immediately adjacent to the noncontractile dead fibers, thereby stretching and buckling them. An additional but sublethal ischemic change may be seen in the margins of infarcts: so-called vacuolar degeneration or **myocytolysis**, involving large vacuolar spaces within cells, probably containing water. This potentially reversible alteration is particularly frequent in the thin zone of viable subendocardial cells. Subendocardial

Figure 12-15 Acute myocardial infarct, predominantly of the posterolateral left ventricle, demonstrated histochemically by a lack of staining by the triphenyltetrazolium chloride (TTC) stain in areas of necrosis *(arrow)*. The staining defect is due to the enzyme leakage that follows cell death. Note the myocardial hemorrhage at one edge of the infarct that was associated with cardiac rupture, and the anterior scar *(arrowhead)*, indicative of old infarct. (Specimen the oriented with the posterior wall at the top.)

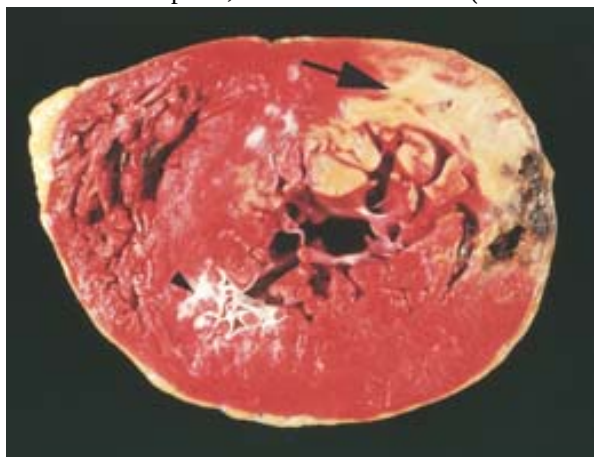
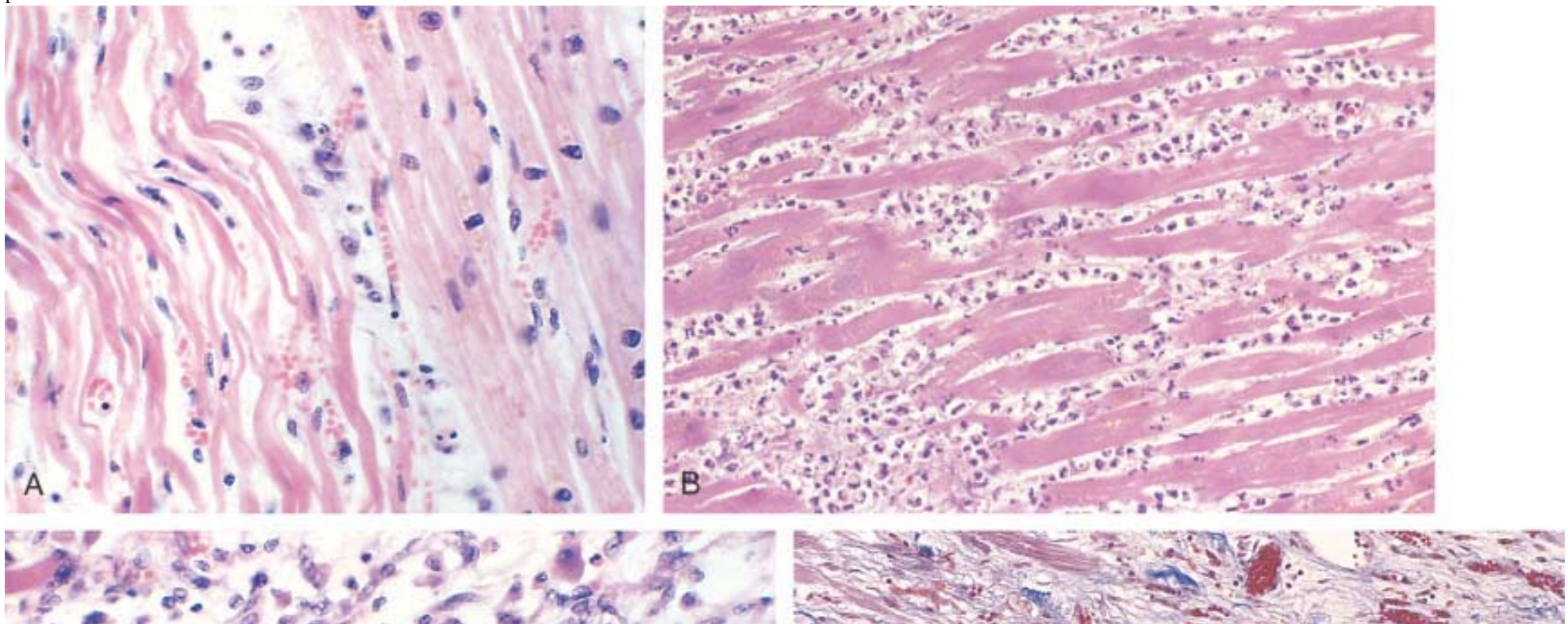
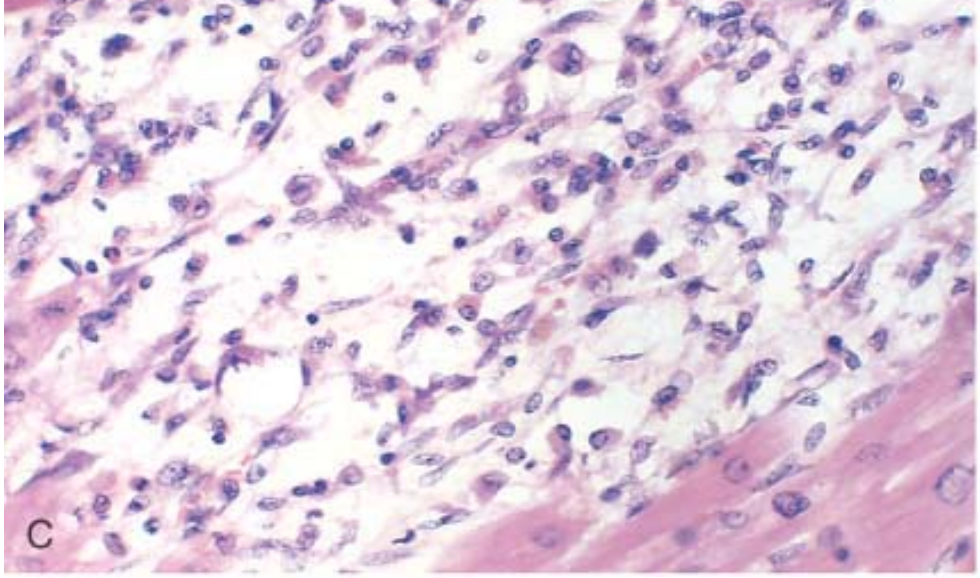
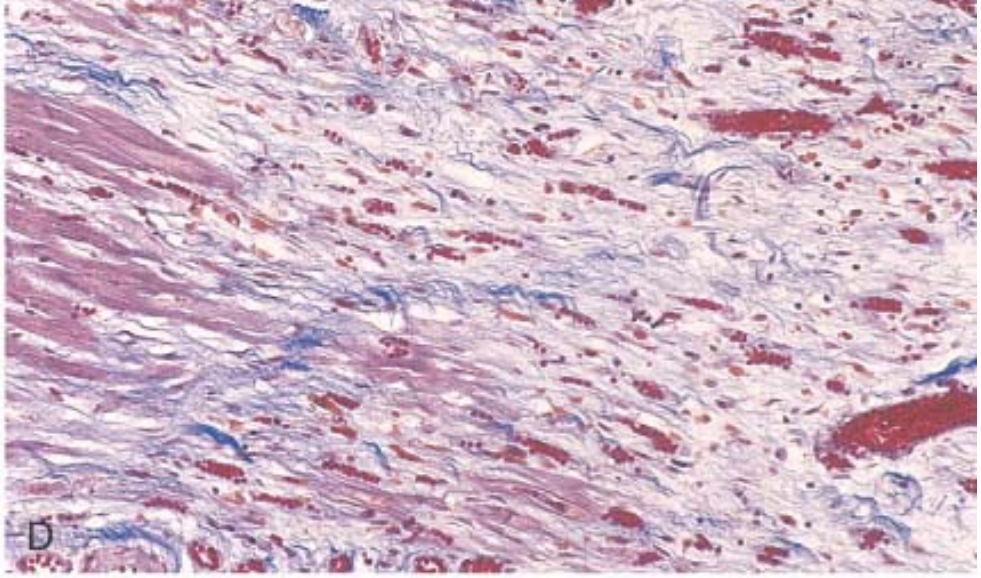


Figure 12-16 Microscopic features of myocardial infarction and its repair. *A*, One-day-old infarct showing coagulative necrosis along with wavy fibers (elongated and narrow), compared with adjacent normal fibers (at right). Widened spaces between the dead fibers contain edema fluid and scattered neutrophils. *B*, Dense polymorphonuclear leukocytic infiltrate in area of acute myocardial infarction of 3 to 4 days' duration. *C*, Nearly complete removal of necrotic myocytes by phagocytosis (approximately 7 to 10 days). *D*, Granulation tissue characterized by loose collagen and abundant capillaries. *E*, Well-healed myocardial infarct with replacement of the necrotic fibers by dense collagenous scar. A few residual cardiac muscle cells are present.







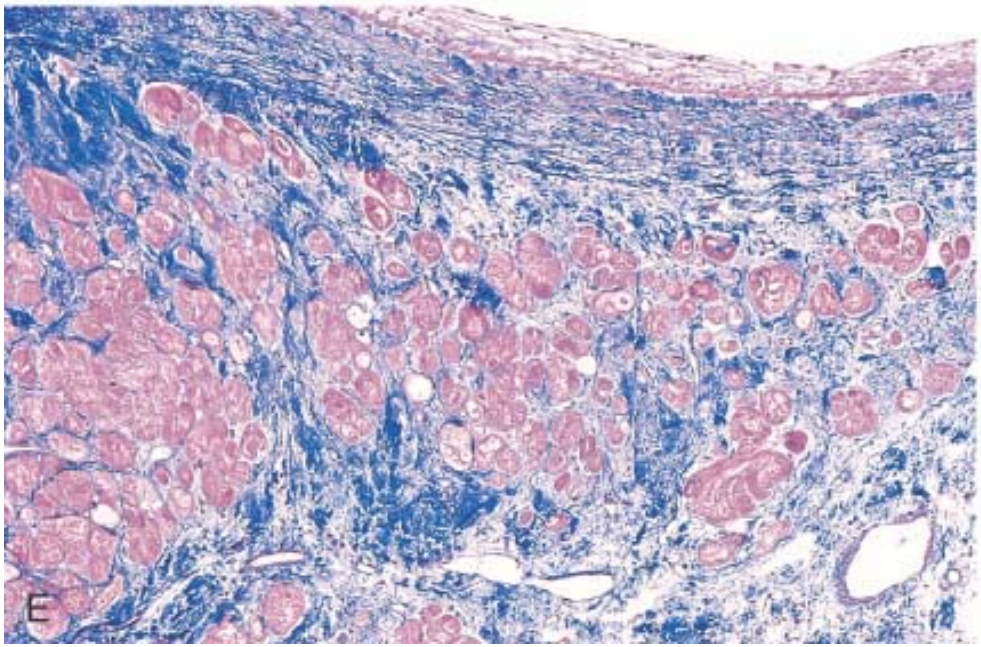
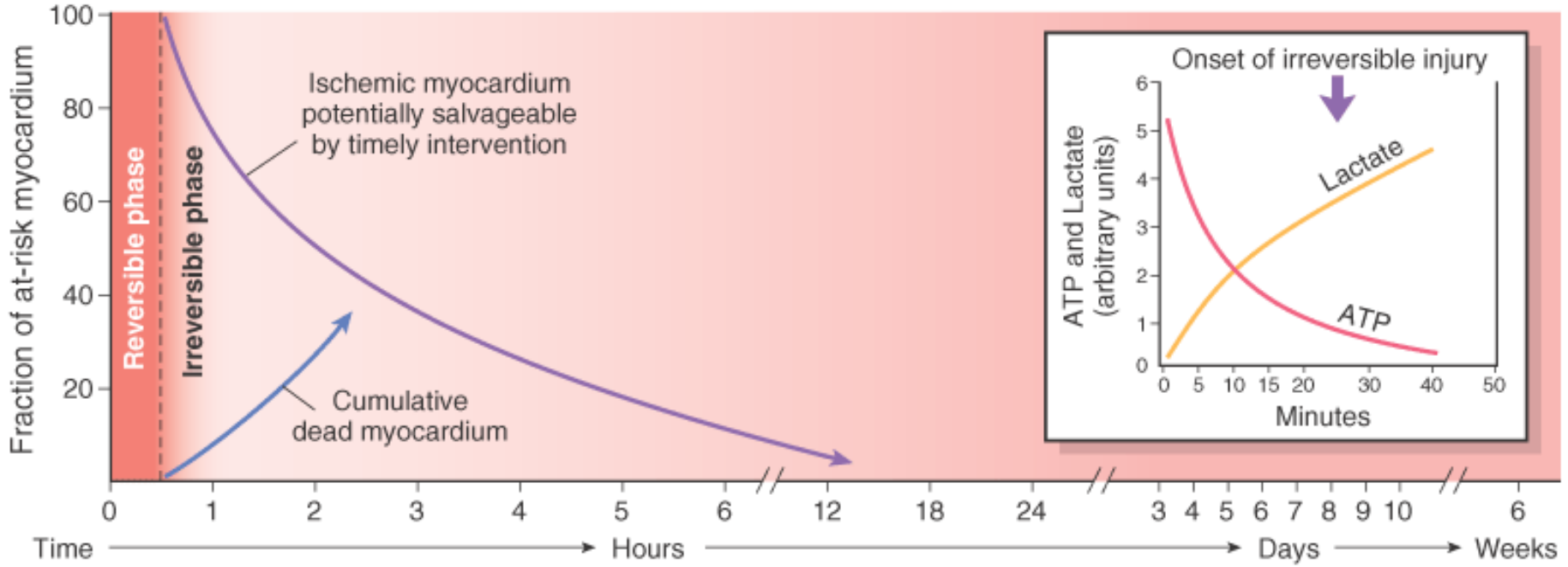


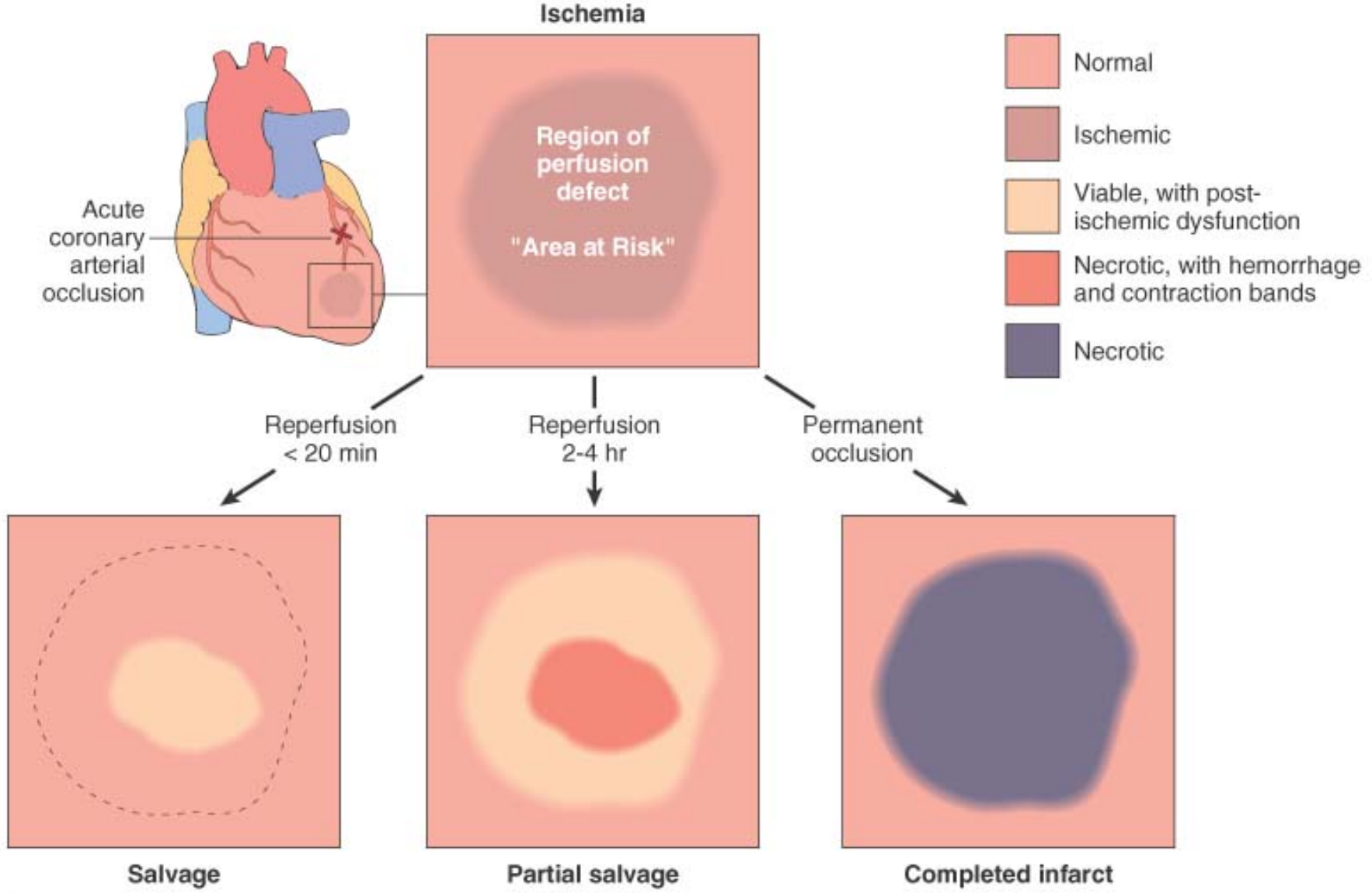
Figure 12-17 Temporal sequence of early biochemical, ultrastructural, histochemical, and histologic findings after onset of severe myocardial ischemia. For approximately 30 minutes after the onset of even the most severe ischemia, myocardial injury is potentially reversible. Thereafter, progressive loss of viability occurs that is complete by 6 to 12 hours. The benefits of reperfusion are greatest when it is achieved early, with progressively smaller benefit occurring as reperfusion is delayed. *(Modified with permission from Antman E: Acute myocardial infarction. In Braunwald E, Zipes DP, Libby P (eds): Heart Disease: A Textbook of Cardiovascular Medicine, 6th ed. Philadelphia, WB Saunders, 2001, pp. 1114–1231.)*



Electron microscopy	**Reversible phase:** Glycogen depletion; mitochondrial swelling and relaxation of myofibrils **Irreversible phase:** Sarcolemmal disruption; mitochondrial amorphous densities						
Histochemistry	TTC staining defect						
Light microscopy	Waviness of fibers at border	Beginning coagulation necrosis; edema; focal hemorrhage; beginning neutrophilic infiltrate	Continuing coagulation necrosis; pallor (shrunken nuclei and eosinophilic cytoplasm); myocyte contraction bands	Coagulation necrosis with loss of nuclei and striations; neutrophilic infiltrate	Disintegration of myofibers and phagocytosis by macrophages	Completion of phagocytosis; prominent granulation tissue with neovascularization and fibrovascular reaction	Mature fibrous scar
Gross changes			Pallor	Pallor, sometimes hyperemia; yellowing at periphery	Hyperemic border; central yellow-brown softening	Maximally yellow and soft vascularized edges; red-brown and depressed	

red-brown
and depressed

Figure 12-18 Consequences of myocardial ischemia followed by reperfusion. *A,* Schematic illustration of the progression of myocardial ischemic injury and its modification by restoration of flow (reperfusion). Hearts suffering brief periods of ischemia of <20 minutes followed by reperfusion do not develop necrosis (reversible injury). Brief ischemia followed by reperfusion results in stunning. If coronary occlusion is extended beyond 20 minutes' duration, a wavefront of necrosis progresses from subendocardium to subepicardium over time. Reperfusion before 3 to 6 hours of ischemia salvages ischemic but viable tissue. (This salvaged tissue may demonstrate stunning.) Reperfusion beyond 6 hours does not appreciably reduce myocardial infarct size. Late reperfusion may still have a beneficial effect on reducing or preventing myocardial infarct expansion and left ventricular remodeling. *B,* Gross and *C,* microscopic appearance of myocardium modified by reperfusion. *B,* Large, densely hemorrhagic, anterior wall acute myocardial infarction from patient with left anterior descending artery thrombus treated with streptokinase intracoronary thrombolysis (triphenyl tetrazolium chloride-stained heart slice). (Specimen oriented with posterior wall at top.) *C,* Myocardial necrosis with hemorrhage and contraction bands, visible as dark bands spanning some myofibers *(arrow).* This is the characteristic appearance of markedly ischemic myocardium that has been reperfused.



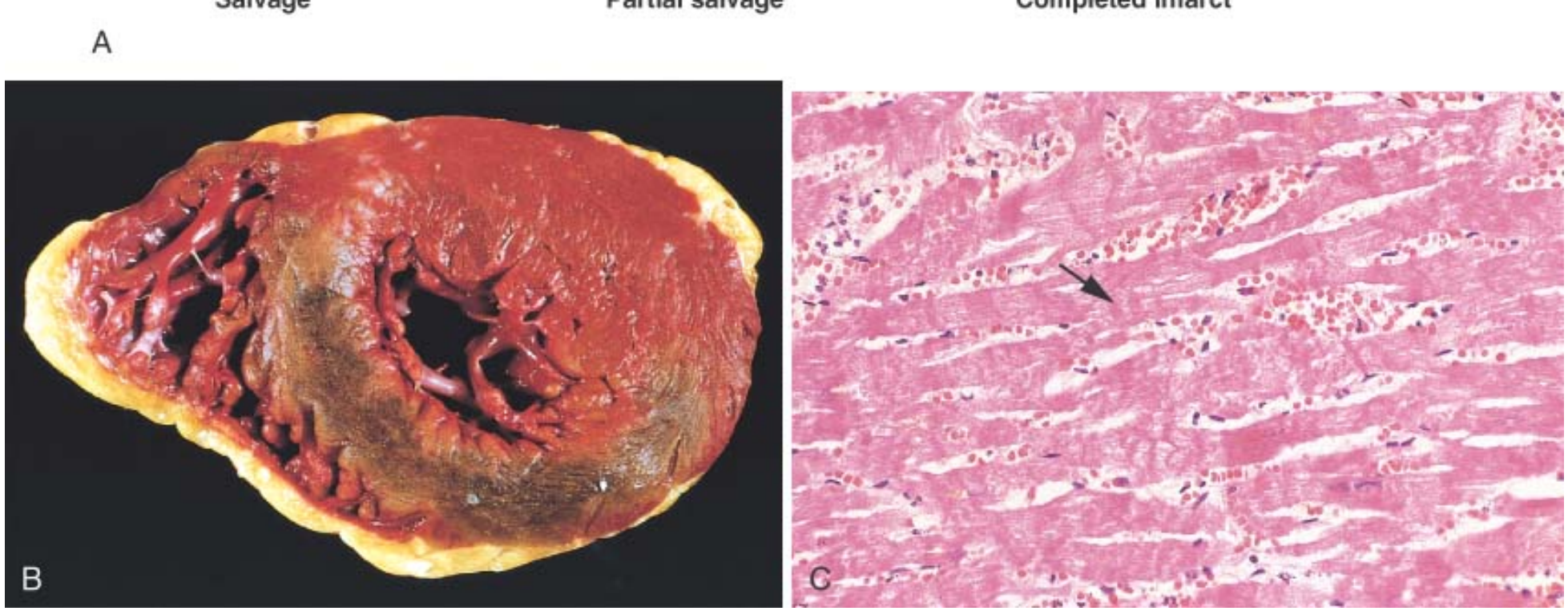
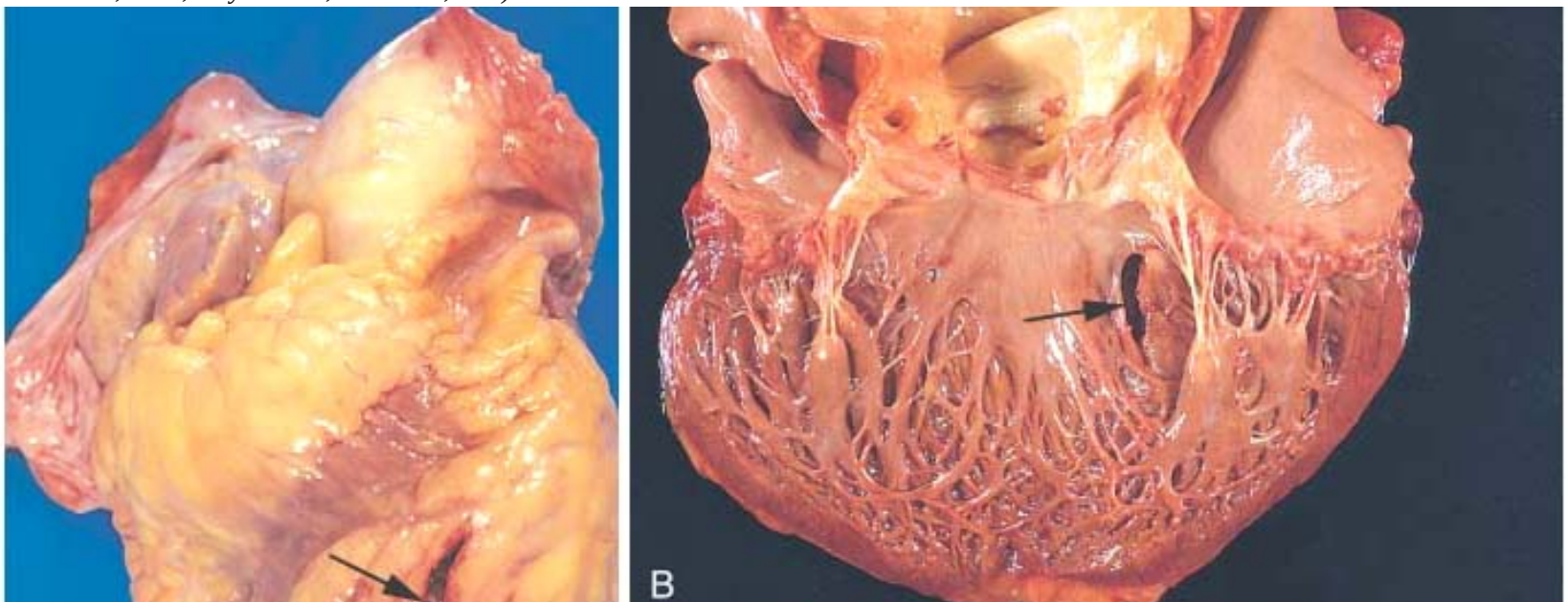


Figure 12-19 Complications of myocardial infarction. Cardiac rupture syndromes *(A, B,* and *C). A,* Anterior myocardial rupture in an acute infarct *(arrow). B,* Rupture of the ventricular septum *(arrow). C,* Complete rupture of a necrotic papillary muscle. *D,* Fibrinous pericarditis, showing a dark, roughened epicardial surface overlying an acute infarct. *E,* Early expansion of anteroapical infarct with wall thinning *(arrow)* and mural thrombus. *F,* Large apical left ventricular aneurysm. The left ventricle is on the right in this apical four-chamber view of the heart. *(A–E, Reproduced by permission from Schoen FJ: Interventional and Surgical Cardiovascular Pathology: Clinical Correlations and Basic Principles, Philadelphia, WB Saunders, 1989.) (F, Courtesy of William D. Edwards, M.D., Mayo Clinic, Rochester, MN.)*



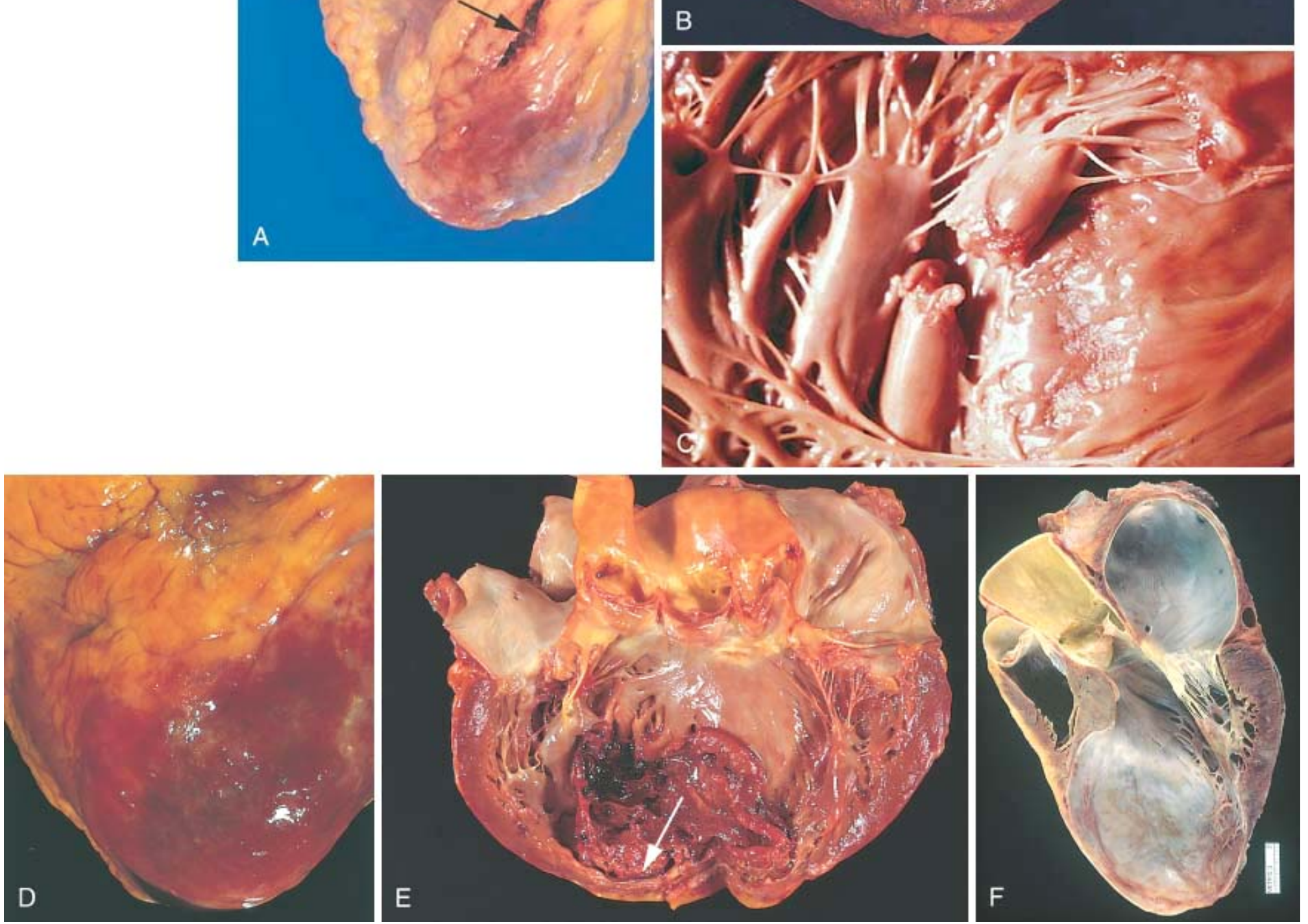


Figure 12-20 Hypertensive heart disease with marked concentric thickening of the left ventricular wall causing reduction in lumen size. The left ventricle is on the right in this apical four-chamber view of the heart. A pacemaker is incidentally present in the right ventricle *(arrow)*.

TABLE 12-6 -- Disorders Predisposing to Cor Pulmonale

Diseases of the Pulmonary Parenchyma
Chronic obstructive pulmonary disease
Diffuse pulmonary interstitial fibrosis
Pneumoconioses
Cystic fibrosis
Bronchiectasis
Diseases of the Pulmonary Vessels
Recurrent pulmonary thromboembolism
Primary pulmonary hypertension
Extensive pulmonary arteritis (e.g., Wegener granulomatosis)
Drug-, toxin-, or radiation-induced vascular obstruction
Extensive pulmonary tumor microembolism
Disorders Affecting Chest Movement
Kyphoscoliosis
Marked obesity (pickwickian syndrome)
Neuromuscular diseases
Disorders Inducing Pulmonary Arterial Constriction

Metabolic acidosis
Hypoxemia
Chronic altitude sickness
Obstruction to major airways
Idiopathic alveolar hypoventilation

Figure 12-21 Chronic cor pulmonale, characterized by a markedly dilated and hypertrophied right ventricle, with thickened free wall and hypertrophied trabeculae (apical four-chamber view of heart, right ventricle on left). The shape of the left ventricle (to the right) has been distorted by the right ventricular enlargement. Compare with Figure 12-20 .

TABLE 12-7 -- Major Etiologies of Acquired Heart Valve Disease

Mitral Valve Disease	**Aortic Valve Disease**
Mitral Stenosis	*Aortic Stenosis*
Postinflammatory scarring (rheumatic heart disease)	Postinflammatory scarring (rheumatic heart disease)
	Senile calcific aortic stenosis
	Calcification of congenitally deformed valve
Mitral Regurgitation	*Aortic Regurgitation*
Abnormalities of Leaflets and Commissures	***Intrinsic Valvular Disease***
Postinflammatory scarring	Postinflammatory scarring (rheumatic heart disease)
Infective endocarditis	Infective endocarditis
Mitral valve prolapse	

Fen-phen-induced valvular fibrosis	
Abnormalities of Tensor Apparatus	***Aortic Disease***
Rupture of papillary muscle	Degenerative aortic dilation
Papillary muscle dysfunction (fibrosis)	Syphilitic aortitis
Rupture of chordae tendineae	Ankylosing spondylitis
	Rheumatoid arthritis
	Marfan syndrome
Abnormalities of Left Ventricular Cavity and/or Annulus	
LV enlargement (myocarditis, dilated cardiomyopathy)	
Calcification of mitral ring	
LV, Left ventricular.	
Modified from Schoen, FJ: Surgical pathology of removed natural and prosthetic valves. Hum Pathol 18:558, 1987.	

valvular insufficiency, only a relatively few mechanisms produce acquired valvular stenosis. The most frequent causes of the major functional valvular lesions are as follows:

- Aortic stenosis: calcification of anatomically normal and congenitally bicuspid aortic valves
- Aortic insufficiency: dilation of the ascending aorta, related to hypertension and aging.
- Mitral stenosis: rheumatic heart disease
- Mitral insufficiency: myxomatous degeneration (mitral valve prolapse)

VALVULAR DEGENERATION CAUSED BY CALCIFICATION

The heart valves are subjected to high repetitive mechanical stresses, particularly at the hinge points of the cusps and leaflets owing to (1) 40 million or more cardiac cycles per year, (2) substantial tissue deformations at each cycle, and (3) transvalvular pressure gradients in the closed phase of approximately 120 mm for the mitral and 80 mm for the aortic valve. It is therefore not surprising that these normally delicate structures suffer cumulative damage complicated by formation of calcific deposits (composed of calcium phosphate mineral), which may lead to clinically important disease (see Chapter 1). The most frequent calcific valvular diseases, illustrated in Figure 12-22 , are calcific aortic stenosis, calcification of a congenitally bicuspid aortic valve, and mitral annular calcification. Each comprises primarily dystrophic calcification without significant lipid deposition or cellular proliferation, a process distinct from but with some features of atherosclerosis.

Figure 12-22 Calcific valvular degeneration. *A*, Calcific aortic stenosis of a previously normal valve having three cusps (viewed from aortic aspect). Nodular masses of calcium are heaped-up within the sinuses of Valsalva *(arrow)*. Note that the commissures are not fused, as in postrheumatic aortic valve stenosis (see Fig. 12-24*E*). *B*, Calcific aortic stenosis occurring on a congenitally bicuspid valve. One cusp has a partial fusion at its center, called a *raphe* (arrow). *C and D*, Mitral annular calcification, with calcific nodules at the base (attachment margin) of the anterior mitral leaflet *(arrows)*. *C*, Left atrial view. *D*, Cut section of myocardium.

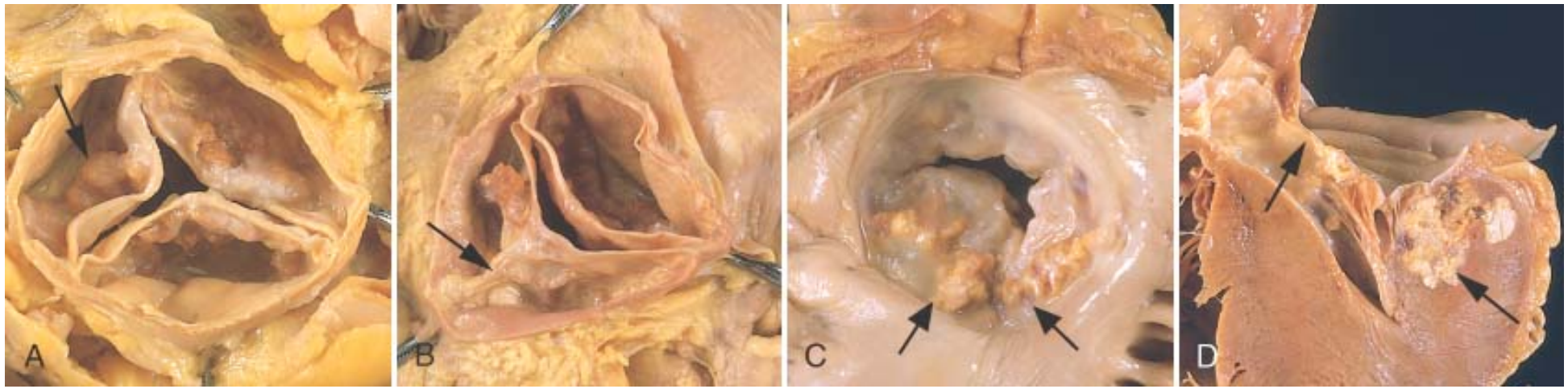
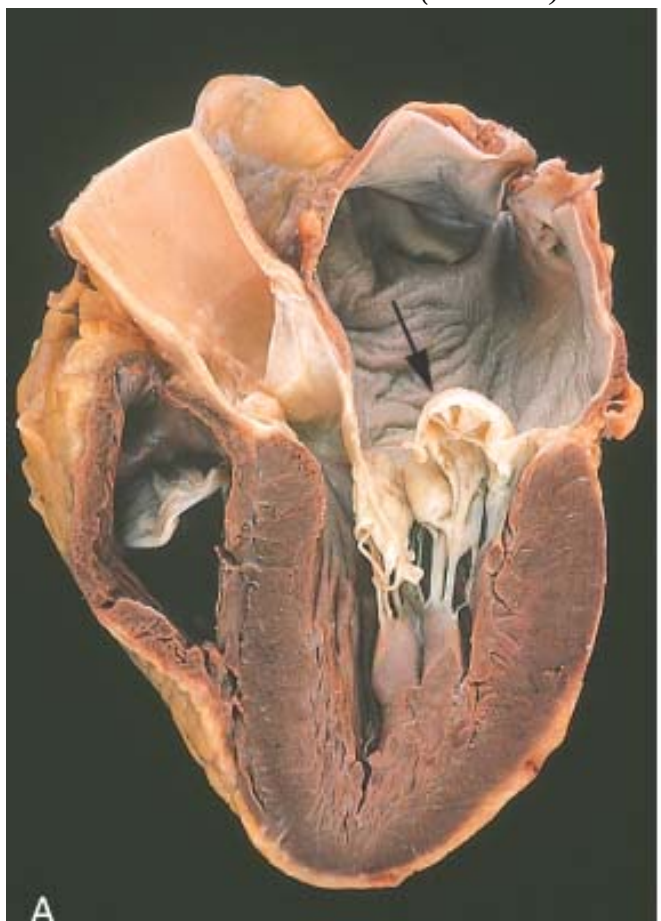


Figure 12-23 Myxomatous degeneration of the mitral valve. *A,* Long axis of left ventricle demonstrating hooding with prolapse of the posterior mitral leaflet into the left atrium *(arrow).* The left ventricle is on right in this apical four-chamber view. *(Courtesy of William D. Edwards, M.D., Mayo Clinic, Rochester, MN.) B,* Opened valve, showing pronounced hooding of the posterior mitral leaflet with thrombotic plaques at sites of leaflet-left atrium contact *(arrows). C,* Opened valve with pronounced hooding from patient who died suddenly *(double arrows).* Note also mitral annular calcification *(arrowhead).*



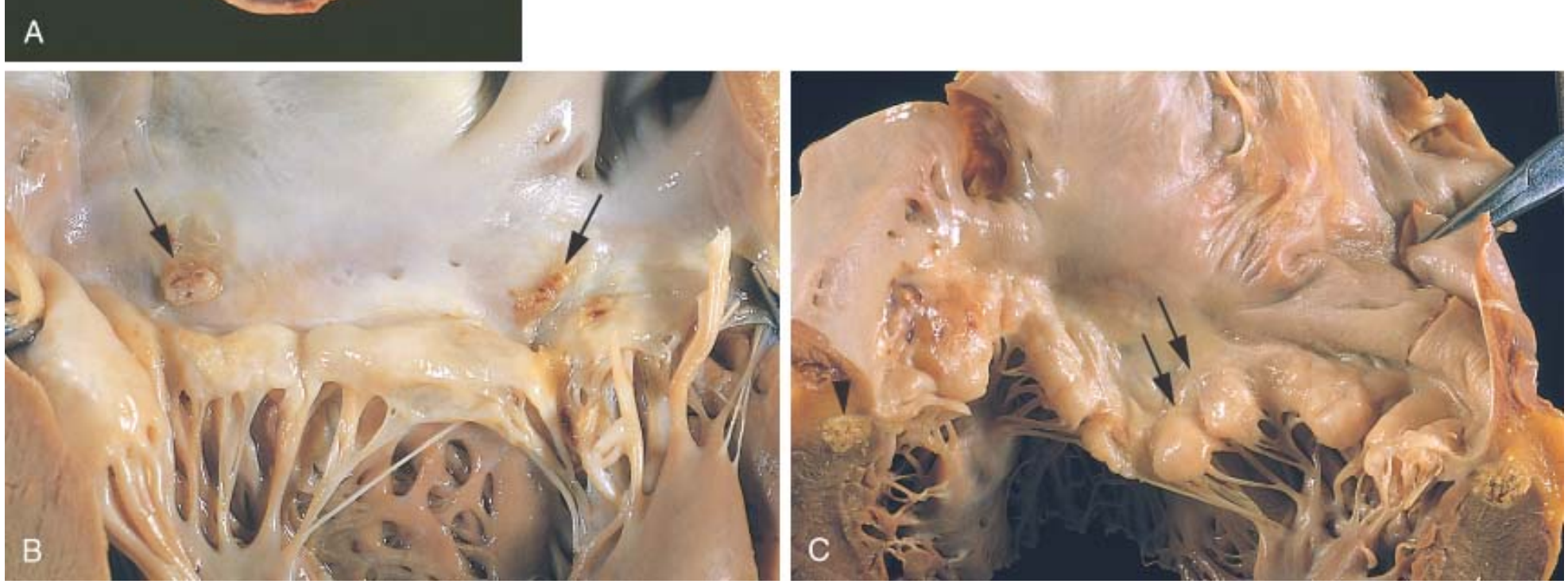
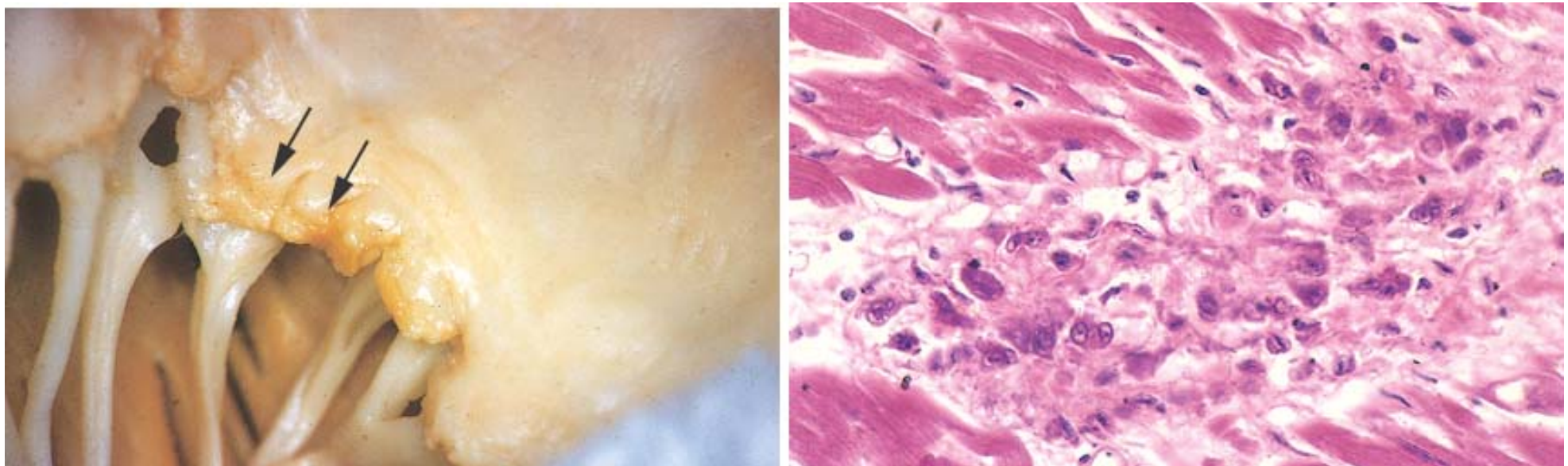


Figure 12-24 Acute and chronic rheumatic heart disease. *A,* Acute rheumatic mitral valvulitis superimposed on chronic rheumatic heart disease. Small vegetations (verrucae) are visible along the line of closure of the mitral valve leaflet *(arrows).* Previous episodes of rheumatic valvulitis have caused fibrous thickening and fusion of the chordae tendineae. *B,* Microscopic appearance of Aschoff body in a patient with acute rheumatic carditis. The myocardial interstitium has a circumscribed collection of mononuclear inflammatory cells, including some large histiocytes with prominent nucleoli and a prominent binuclear histiocyte, and central necrosis. *C and D,* Mitral stenosis with diffuse fibrous thickening and distortion of the valve leaflets, commissural fusion *(arrows),* and thickening and shortening of the chordae tendineae. Marked dilation of the left atrium is noted in the left atrial view *(C). D,* Opened valve. Note neovascularization of anterior mitral leaflet *(arrow). E,* Surgically removed specimen of rheumatic aortic stenosis, demonstrating thickening and distortion of the cusps with commissural fusion *(E, reproduced from Schoen FJ, St. John-Sutton M: Contemporary issues in the pathology of valvular heart disease. Human Pathol 18:568, 1967.)*



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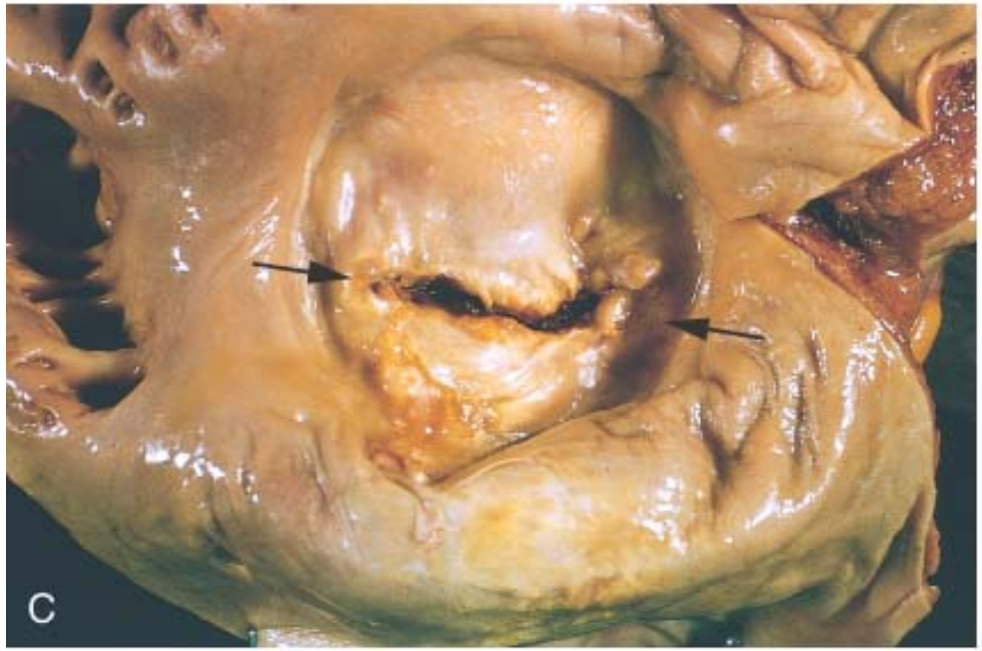
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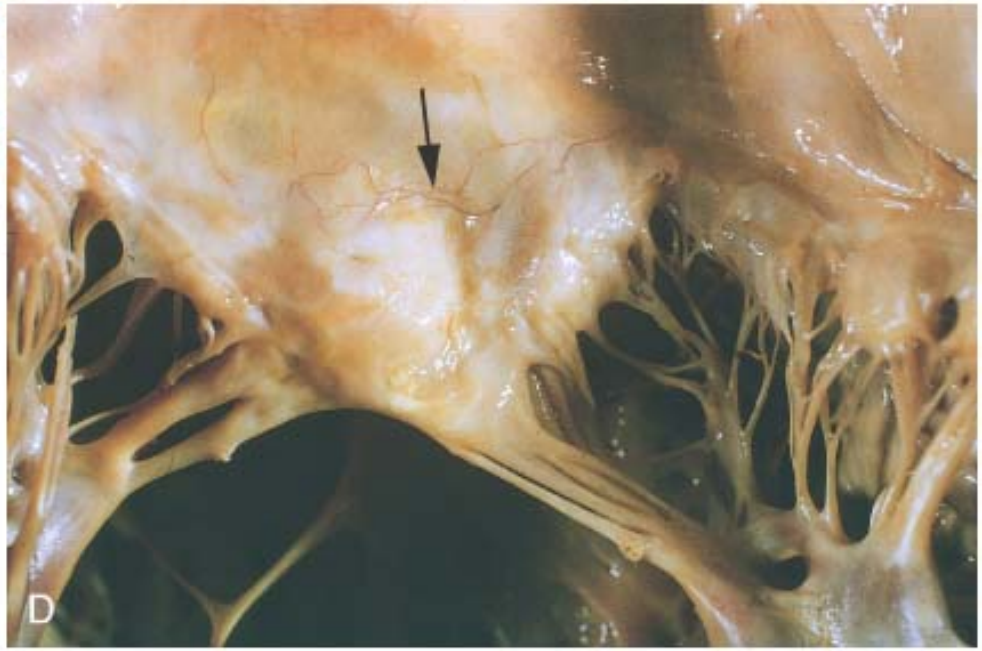
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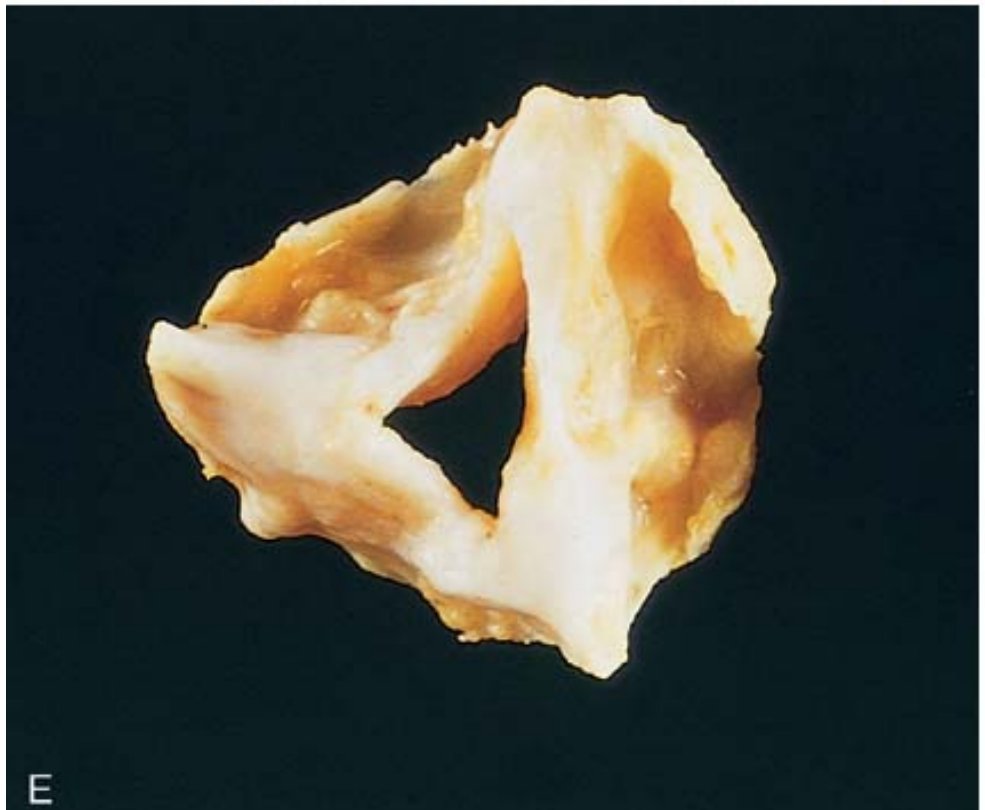
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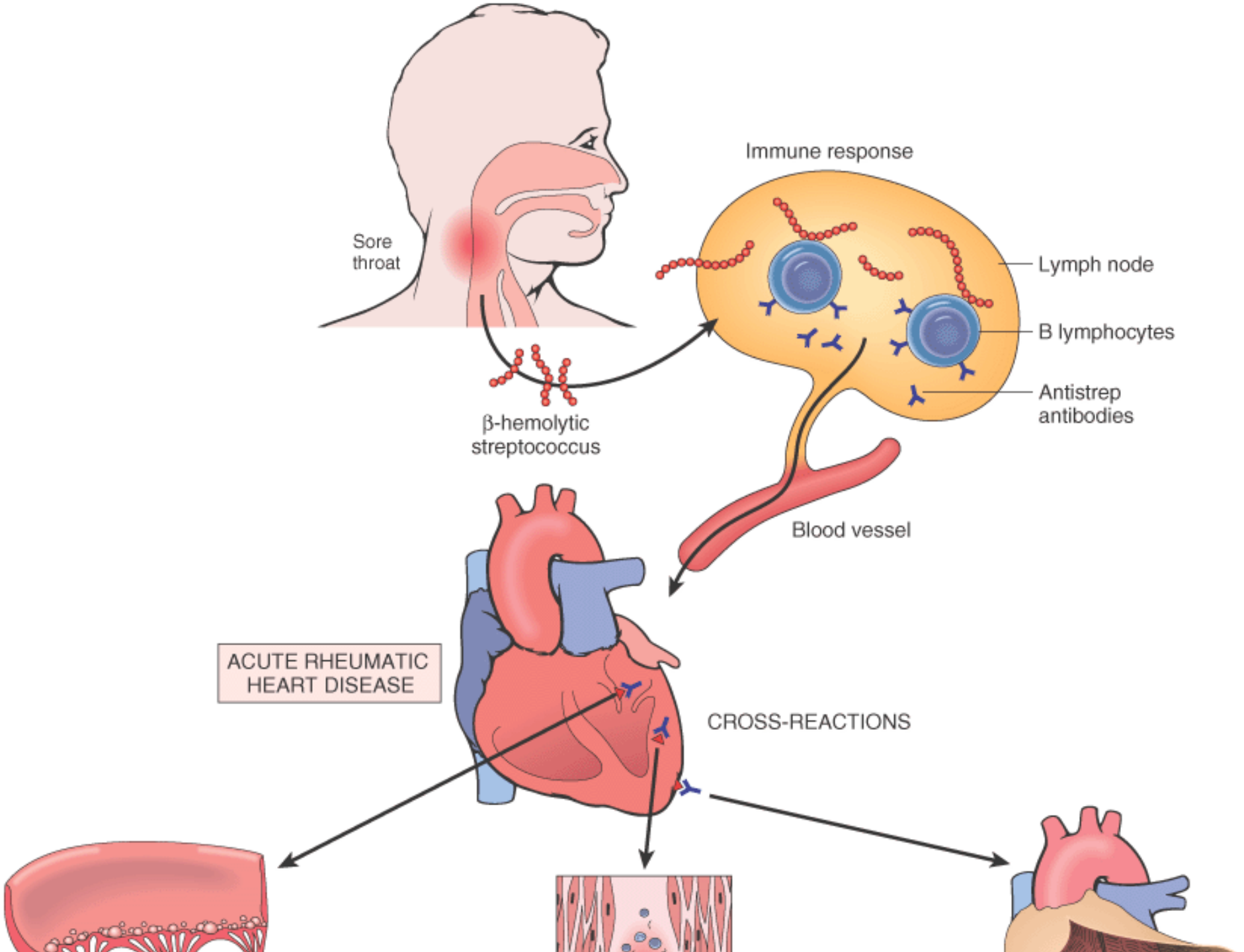
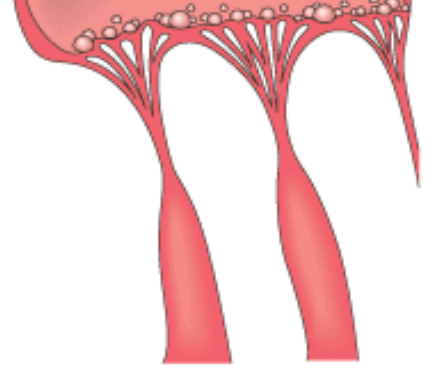
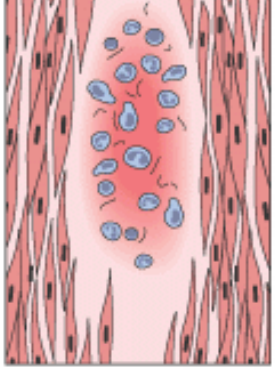
Figure 12-25 The pathogenetic sequence and key morphologic features of acute rheumatic heart disease.

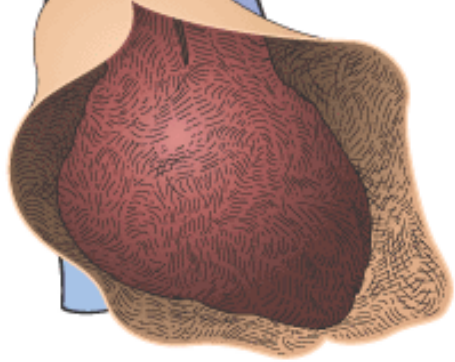




Vegetations



Myocardial Aschoff body



Fibrinous pericarditis

Figure 12-26 Infective (bacterial) endocarditis. *A,* Endocarditis of mitral valve (subacute, caused by *Strep. viridans*). The large, friable vegetations are denoted by arrows. *B,* Acute endocarditis of congenitally bicuspid aortic valve (caused by *Staph. aureus)* with extensive cuspal destruction and ring abscess *(arrow). C,* Histologic appearance of vegetation of endocarditis with extensive acute inflammatory cells and fibrin. Bacterial organisms were demonstrated by tissue Gram stain. *(C, reproduced from Schoen FJ: Surgical pathology of removed natural and prosthetic heart valves. Human Pathol 18:558, 1987.) D,* Healed endocarditis, demonstrating mitral valvular destruction but no active vegetations.

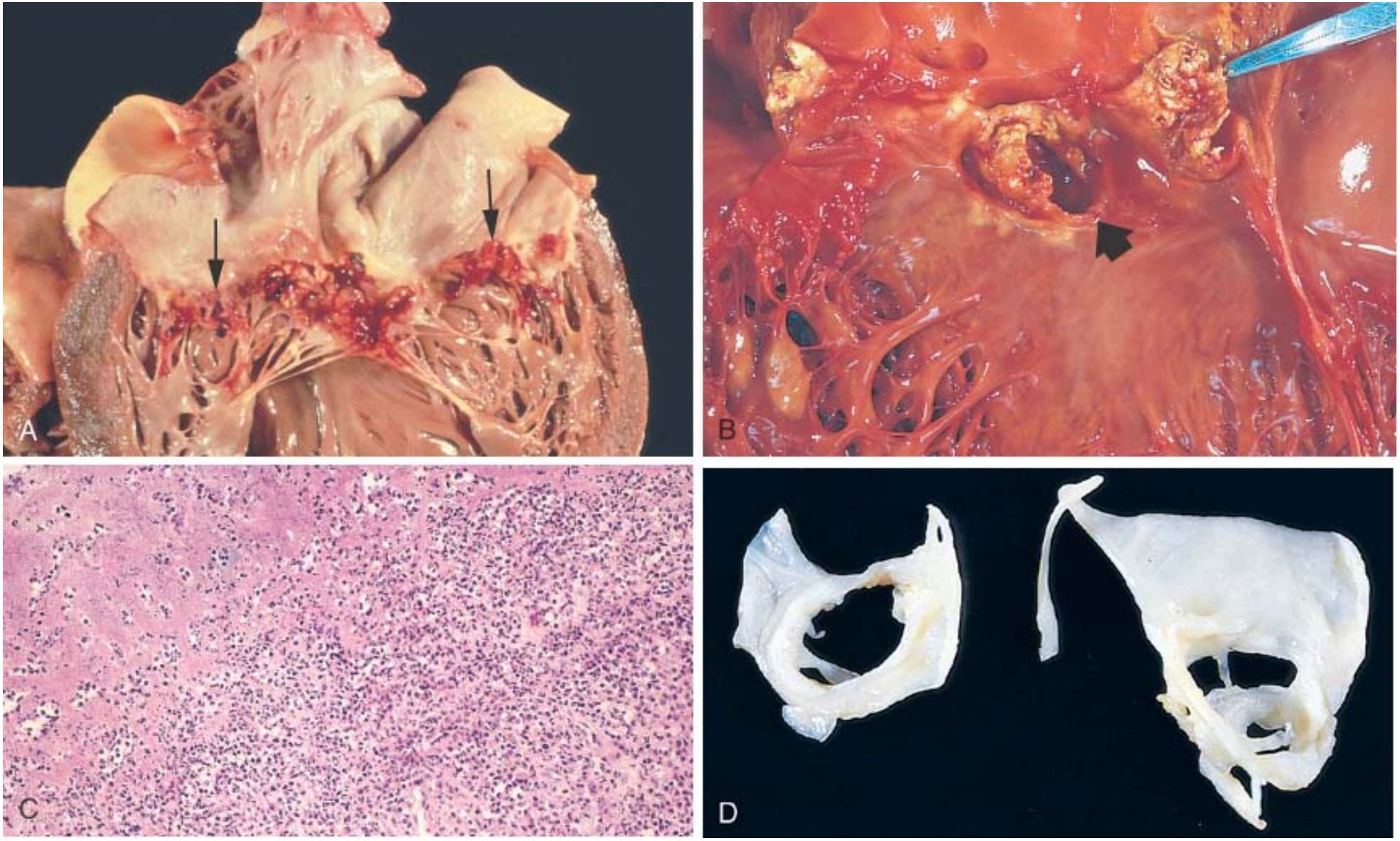


Figure 12-27 Diagrammatic comparison of the lesions in the four major forms of vegetative endocarditis. The rheumatic fever phase of RHD (rheumatic heart disease) is marked by a row of small, warty vegetations along the lines of closure of the valve leaflets. IE (infective endocarditis) is characterized by large, irregular masses on the valve cusps that can extend onto the chordae (see Fig. 12-26). NBTE (nonbacterial thrombotic endocarditis) typically exhibits small, bland vegetations, usually attached at the line of closure. One or many may be present (see Fig. 12-28). LSE (Libman-Sacks endocarditis) has small or medium-sized vegetations on either or both sides of the valve leaflets.

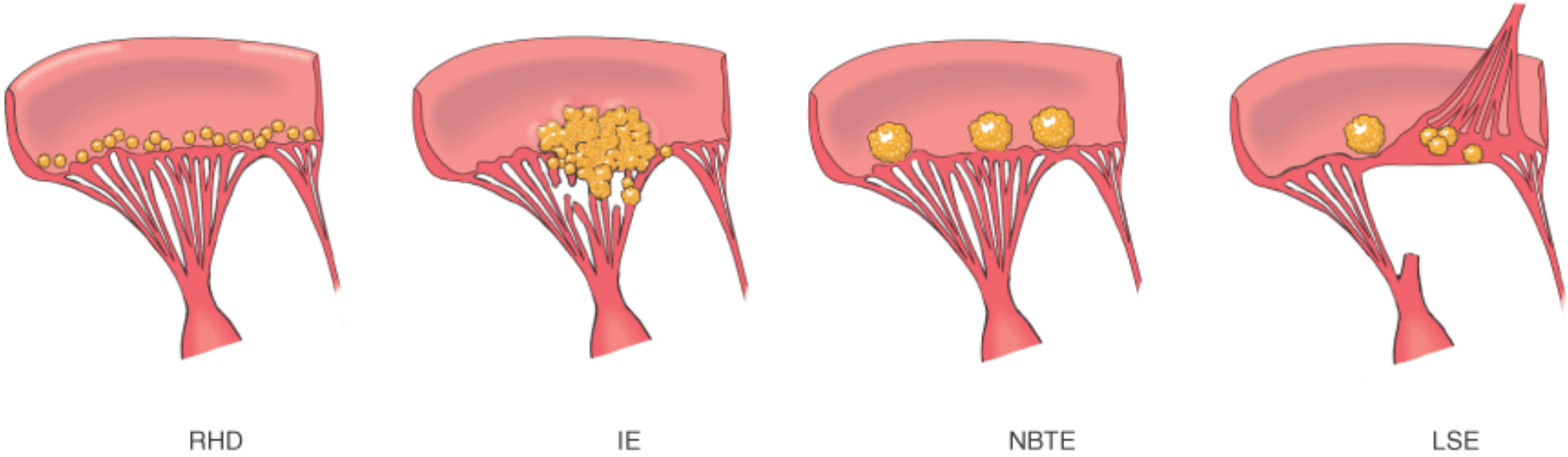


TABLE 12-8 -- Diagnostic Criteria for Infective Endocarditis [*]

Pathologic Criteria
Microorganisms, demonstrated by culture or histologic examination, in a vegetation, embolus from a vegetation, or intracardiac abscess
Histologic confirmation of active endocarditis in vegetation or intracardiac abscess
Clinical Criteria
Major
Positive blood culture(s) indicating characteristic organism or persistence of unusual organism
Echocardiographic findings, including valve-related or implant-related mass or abscess, or partial separation of artificial valve
New valvular regurgitation
Minor
Predisposing heart lesion or intravenous drug use
Fever
Vascular lesions, including arterial petechiae, subungual/splinter hemorrhages, emboli, septic infarcts, mycotic aneurysm, intracranial hemorrhage, Janeway lesions [†]
Immunologic phenomena, including glomerulonephritis, Osler nodes, [‡] Roth spots, [§] rheumatoid factor
Microbiologic evidence, including single culture showing uncharacteristic organism
Echocardiographic findings consistent with but not diagnostic of endocarditis, including new valvular regurgitation, pericarditis

*Diagnosis by these guidelines, often called the Duke Criteria, requires either pathologic or clinical criteria; if clinical criteria are used, 2 major, 1 major + 3 minor, or 5 minor criteria are required for diagnosis. *Modified from Durack DT, et al: Am J Med, 96:200, 1994 and Karchmer AW, In Braunwald E, Zipes DP, Libby P (eds): Heart Disease. A Textbook of*

Cardiovascular Medicine, 6th ed. Philadelphia, WB Saunders Co., 2001, p. 1723.
†Janeway lesions are small erythematous or hemorrhagic, macular, nontender lesions on the palms and soles and are the consequence of septic embolic events.
‡Osler nodes are small, tender subcutaneous nodules that develop in the pulp of the digits or occasionally more proximally in the fingers and persist for hours to several days.
§Roth spots are oval retinal hemorrhages with pale centers.

NONINFECTED VEGETATIONS

Nonbacterial Thrombotic Endocarditis (NBTE)

NBTE is characterized by the deposition of small masses of fibrin, platelets, and other blood components on the leaflets of the cardiac valves. In contrast to the vegetations of IE, discussed previously, *the valvular lesions of NBTE are sterile and do not contain microorganisms*. NBTE is often encountered in debilitated patients, such as those with cancer or sepsis—hence the previously used term *marantic endocarditis*. Although the local effect on the valves is usually unimportant, NBTE may achieve clinical significance by producing emboli and resultant infarcts in the brain, heart, or elsewhere.

Morphology.

In contrast to IE, the vegetations of NBTE are sterile, nondestructive, and small (1 to 5 mm), and occur singly or multiply along the line of closure of the leaflets or cusps (Fig. 12-28). Histologically, they are composed of bland thrombus without accompanying inflammatory reaction or induced valve damage. Should the patient survive the underlying disease, organization may occur, leaving delicate strands of fibrous tissue.

Pathogenesis.

NBTE frequently occurs concomitantly with venous thromboses or pulmonary embolism, suggesting *a common origin in a hypercoagulable state with systemic activation of blood coagulation such as disseminated intravascular coagulation* (Chapter 4). *This may be related to some underlying disease, such as a cancer*, and, in particular, mucinous adenocarcinomas of the pancreas. The striking association with mucinous adenocarcinomas in general may relate to the procoagulant effect of circulating mucin, and thus NBTE can be a part of the Trousseau syndrome (Chapter 7). Lesions of NBTE, however, are also seen occasionally in association with nonmucin-producing malignancy, such as acute promyelocytic leukemia, and in other debilitating diseases or conditions (e.g., hyperestrogenic states, extensive burns, or sepsis) promoting hypercoagulability. Endocardial trauma, as from an indwelling catheter, is also a well-recognized predisposing condition, and one frequently notes right-sided valvular and endocardial thrombotic lesions along the track of a Swan-Ganz pulmonary artery catheter.

Endocarditis of Systemic Lupus Erythematosus (Libman-Sacks Disease)

In SLE, mitral and tricuspid valvulitis with small, sterile vegetations, called *Libman-Sacks endocarditis* is occasionally encountered.

Morphology.

The lesions are small single or multiple, sterile, granular pink vegetations ranging from 1 to 4 mm in diameter. The lesions may be located on the undersurfaces of the atrioventricular valves, on the valvular endocardium, on the cords, or on the mural endocardium of atria or ventricles. Histologically the verrucae consist of a finely granular, fibrinous eosinophilic material that may contain hematoxylin bodies (the tissue equivalent of the lupus erythematosus cell of the blood and bone marrow, see Chapter 6). An intense valvulitis may be present, characterized by fibrinoid necrosis of the valve substance that is often contiguous with the vegetation. Leaflet vegetations can be difficult in some cases to distinguish from those of IE or NBTE (see Fig. 12-27). Subsequent fibrosis and serious deformity can result that resemble chronic RHD and require surgery.

Thrombotic heart valve lesions with sterile vegetations or rarely fibrous thickening commonly occur with the antiphospholipid

Figure 12-28 Nonbacterial thrombotic endocarditis (NBTE). *A,* Nearly complete row of thrombotic vegetations along the line of closure of the mitral valve leaflets *(arrows). B,* Photomicrograph of NBTE, showing bland thrombus, with virtually no inflammation in the valve cusp *(c)* or the thrombotic deposit *(t).* The thrombus is only loosely attached to the cusp *(arrow).*

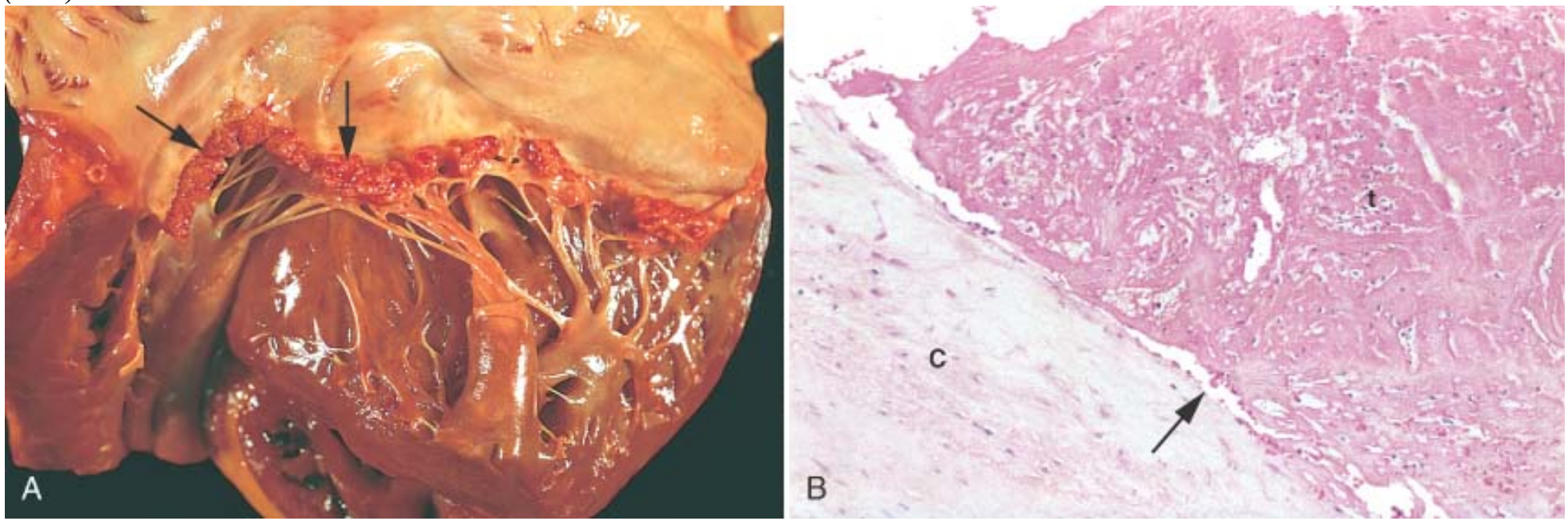


Figure 12-29 Carcinoid heart disease. *A,* Characteristic endocardial fibrotic lesion involving the right ventricle and tricuspid valve. *B,* Microscopic appearance of carcinoid heart disease with intimal thickening. Movat stain shows underlying myocardial elastic tissue black and acid mucopolysaccharides blue-green.

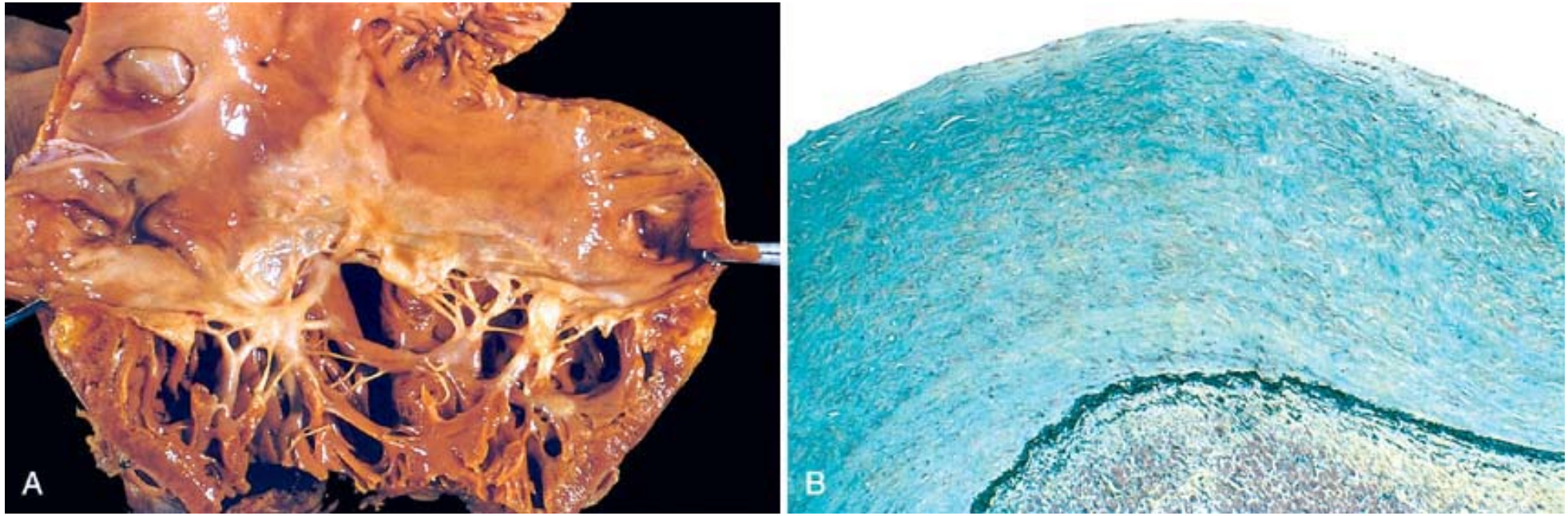
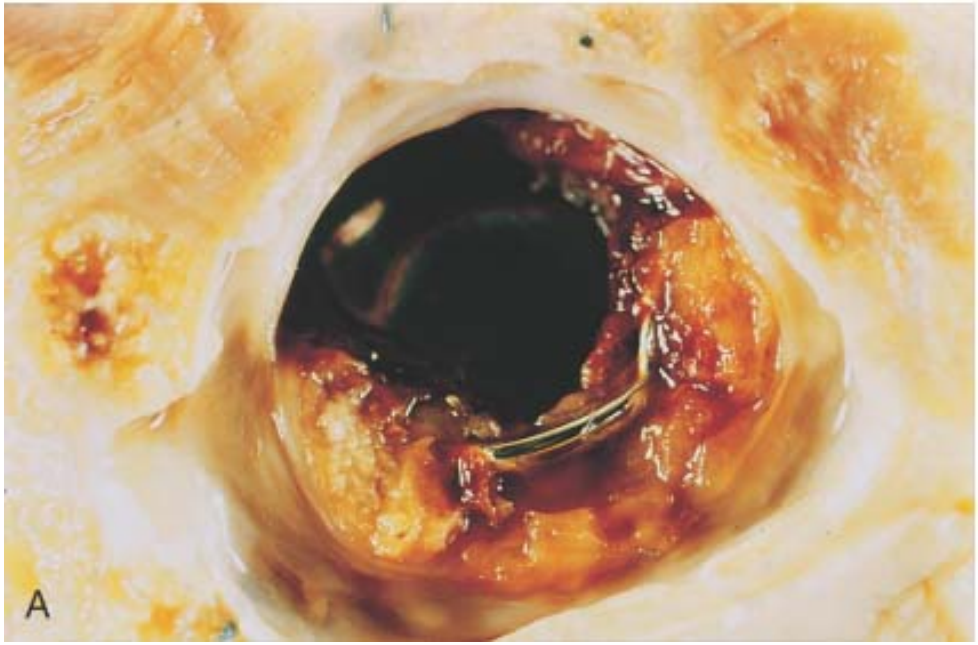


Figure 12-30 Complications of artificial heart valves. *A*, Thrombosis of a mechanical prosthetic valve. *B*, Calcification with secondary tearing of a porcine bioprosthetic heart valve, viewed from the inflow aspect.



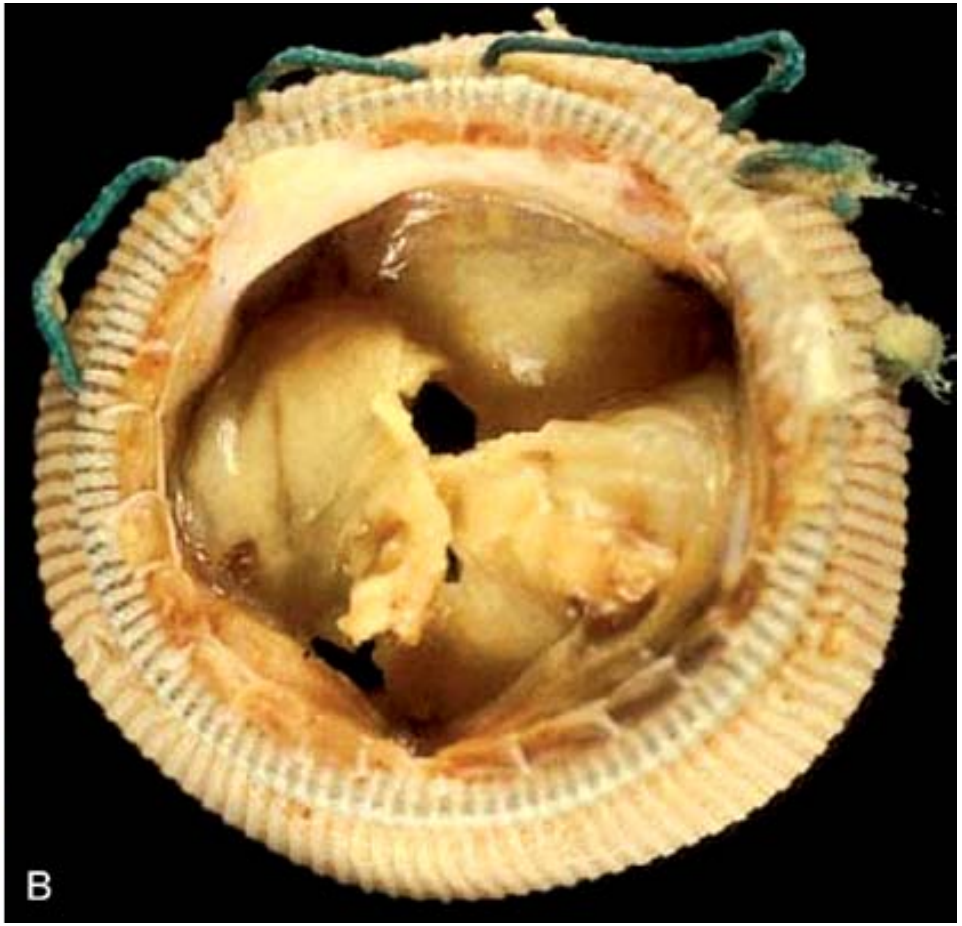


TABLE 12-9 -- Causes of Failure of Cardiac Valve Prostheses

Thrombosis/thromboembolism
Anticoagulant-related hemorrhage
Prosthetic valve endocarditis
Structural deterioration (intrinsic)
••Wear, fracture, poppet failure in ball valves, cuspal tear, calcification
Nonstructural dysfunction
••Granulation tissue, suture, tissue entrapment, paravalvular leak, disproportion, hemolytic anemia, noise

Cardiomyopathies

The previous sections emphasize that myocardial dysfunction occurs commonly but secondarily in a number of different conditions such as ischemic heart disease, hypertension, and

valvular heart disease. Far less frequently observed is disease whose cause is intrinsic to the myocardium. Myocardial diseases are a diverse group that includes inflammatory disorders *(myocarditis)*, immunologic diseases, systemic metabolic disorders, muscular dystrophies, genetic abnormalities in cardiac muscle cells, and an additional group of diseases of unknown etiology.

The term *cardiomyopathy* (literally, heart muscle disease) is used to describe *heart disease resulting from a primary abnormality in the myocardium*.[95] Although chronic myocardial dysfunction due to ischemia should be excluded from the cardiomyopathy rubric, the term *ischemic cardiomyopathy* has gained some popularity among clinicians to describe CHF caused by CAD (as discussed in the section "Chronic Ischemic Heart Disease").

In many cases cardiomyopathies are *idiopathic* (i.e., of unknown cause). However, a major advance in our understanding of myocardial diseases, previously considered idiopathic, has been the demonstration that specific genetic abnormalities in cardiac energy metabolism or structural and contractile proteins underlie myocardial dysfunction in many patients.[96] [97] [98] Thus, etiologic distinctions have become somewhat blurred in recent years. Moreover, myocardial disease of diverse and even unknown etiologies may have a similar morphologic appearance. Therefore, our discussion avoids the controversies associated with classification schemes and emphasizes clinicopathologic, etiologic, and mechanistic concepts.

Without additional data, the clinician encountering a patient with myocardial disease is usually unaware of the etiology. Hence the clinical approach is largely determined by one of the following three clinical, functional, and pathologic patterns (Fig. 12-31 and Table 12-10):

- Dilated cardiomyopathy
- Hypertrophic cardiomyopathy
- Restrictive cardiomyopathy

Among these three categories, the dilated form is most common (90% of cases), and the restrictive is least prevalent. Within the hemodynamic patterns of myocardial dysfunction, there is a spectrum of clinical severity, and overlap of clinical features often occurs between groups. Moreover, each of these patterns can be either idiopathic or due to a specific identifiable cause (Table 12-11) or secondary to primary extramyocardial disease.

Endomyocardial biopsies are used in the diagnosis and management of patients with myocardial disease and in cardiac transplant recipients. Endomyocardial biopsy involves inserting a device (called a *bioptome*) transvenously into the right side of the heart and snipping a small piece of septal myocardium in its jaws, which is then analyzed by a pathologist.[99]

DILATED CARDIOMYOPATHY

The term *dilated cardiomyopathy* (DCM) is applied to a form of cardiomyopathy characterized by *progressive cardiac dilation and contractile (systolic) dysfunction*, usually with concomitant hypertrophy. It is sometimes called congestive cardiomyopathy.

Figure 12-31 Graphic representation of the three distinctive and predominant clinical-pathologic-functional forms of myocardial disease.

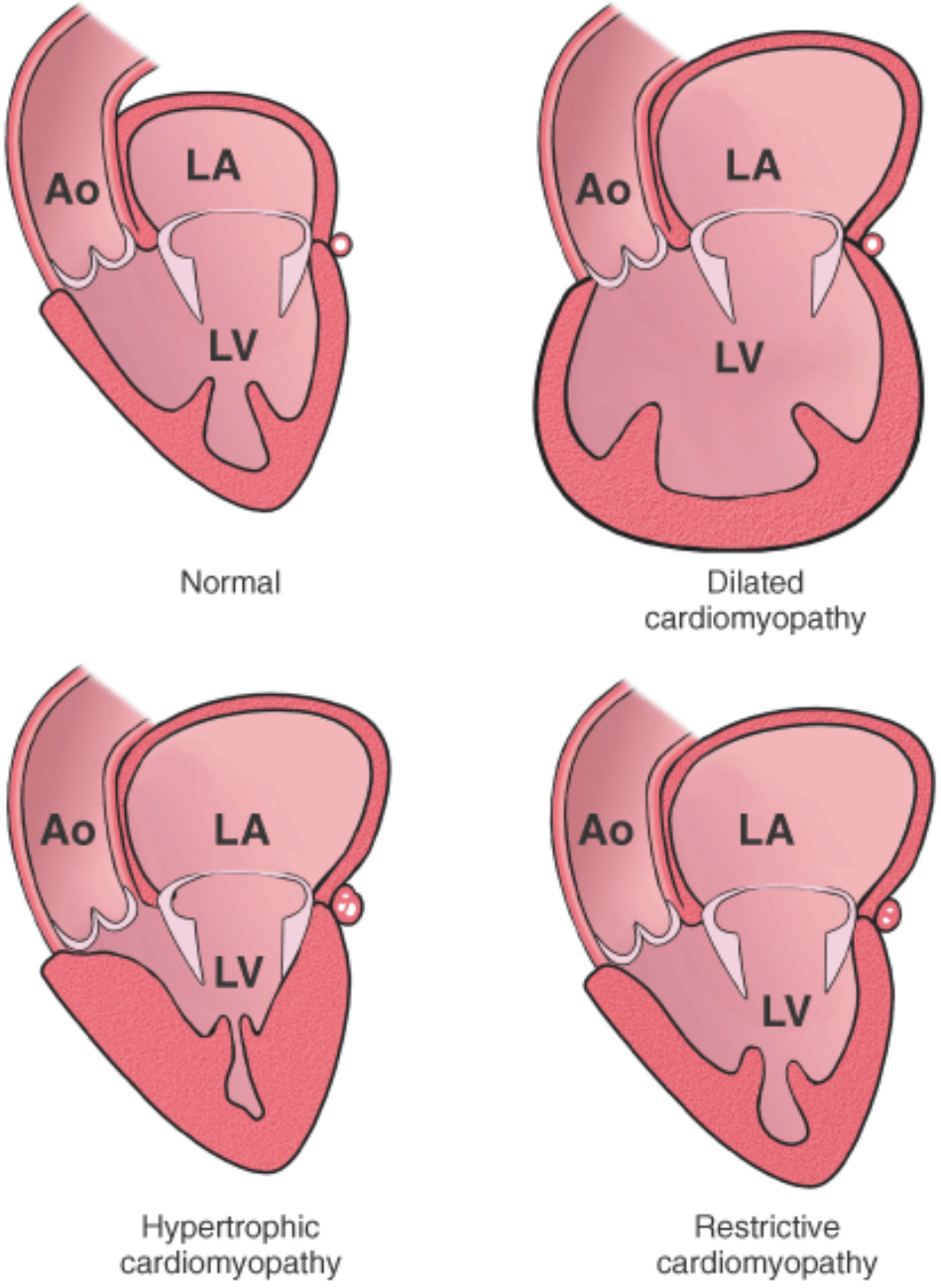


TABLE 12-10 -- Cardiomyopathy and Indirect Myocardial Dysfunction: Functional Patterns and Causes

Functional Pattern	Left Ventricular Ejection Fraction *	Mechanisms of Heart Failure	Causes	Indirect Myocardial Dysfunction (Not Cardiomyopathy)

Dilated	<40%	Impairment of contractility (systolic dysfunction)	Idiopathic; alcohol; peripartum; genetic; myocarditis; hemochromatosis; chronic anemia; doxorubicin (Adriamycin); sarcoidosis	Ischemic heart disease; valvular heart disease; hypertensive heart disease; congenital heart disease
Hypertrophic	50–80%	Impairment of compliance (diastolic dysfunction)	Genetic; Friedreich ataxia; storage diseases; infants of diabetic mothers	Hypertensive heart disease; aortic stenosis
Restrictive	45–90%	Impairment of compliance (diastolic dysfunction)	Idiopathic; amyloidosis; radiation-induced fibrosis	Pericardial constriction

*Normal, approximately 50–65%.

TABLE 12-11 -- Conditions Associated with Heart Muscle Diseases

Cardiac Infections
Viruses
Chlamydia
Rickettsia
Bacteria
Fungi
Protozoa
Toxins
Alcohol
Cobalt
Catecholamines
Carbon monoxide
Lithium
Hydrocarbons
Arsenic
Cyclophosphamide
Doxorubicin (Adriamycin) and daunorubicin
Metabolic
Hyperthroidism
Hypothyroidism

Hyperkalemia
Hypokalemia
Nutritional deficiency (protein, thiamine, other avitaminoses)
Hemochromatosis
Neuromuscular Disease
Friedreich ataxia
Muscular dystrophy
Congenital atrophies
Storage Disorders and Other Depositions
Hunter-Hurler syndrome
Glycogen storage disease
Fabry disease
Amyloidosis
Infiltrative
Leukemia
Carcinomatosis
Sarcoidosis
Radiation-induced fibrosis
Immunologic
Myocarditis (several forms)
Post-transplant rejection

Although it is recognized that approximately 25% to 35% of individuals with DCM have a familial (genetic) form, DCM can result from a number of acquired myocardial insults that ultimately yield a similar clinicopathologic pattern. These include toxicities (including chronic alcoholism, a history of which can be elicited in 10% to 20% of patients), myocarditis (an inflammatory disorder that precedes the development of cardiomyopathy in at least some cases, as documented by endomyocardial biopsy), and pregnancy-associated nutritional deficiency or immunologic reaction. In some patients, the cause of DCM is unknown; such cases are appropriately designated as *idiopathic dilated cardiomyopathy*.

Morphology.

In DCM, the heart is usually heavy, often weighing two to three times normal, and large and flabby, with dilation of all chambers (Fig. 12-32). Nevertheless, because of the wall thinning that accompanies dilation, the ventricular thickness may be less than, equal to, or greater than normal. Mural thrombi are common and may be a source of thromboemboli. There are no primary valvular alterations, and mitral or tricuspid regurgitation, when present, results from left ventricular chamber dilation (functional regurgitation). The coronary arteries are usually free of significant narrowing, but any coronary artery obstructions present are insufficient to explain the degree of cardiac dysfunction.

The histologic abnormalities in idiopathic DCM also are nonspecific and usually do not reflect a specific etiologic agent. Moreover, their severity does not necessarily reflect the degree of dysfunction or the patient's prognosis. Most muscle cells are hypertrophied with enlarged nuclei, but many are attenuated, stretched, and irregular. Interstitial and endocardial fibrosis of variable degree is present, and small subendocardial scars may replace individual cells or groups of cells, probably reflecting healing of previous secondary myocyte ischemic necrosis caused by hypertrophy-induced imbalance between perfusion, supply and demand.

Pathogenesis.

Historically, the etiologic associations in dilated cardiomyopathy have included myocardial inflammatory

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Figure 12-32 Dilated cardiomyopathy. *A,* Gross photograph. Four-chamber dilatation and hypertrophy are evident. There is granular mural thrombus at the apex of the left ventricle (on the right in this apical four-chamber view). The coronary arteries were unobstructed. *B,* Histology demonstrating variable myocyte hypertrophy and interstitial fibrosis (collagen is highlighted as blue in this Masson trichrome stain).

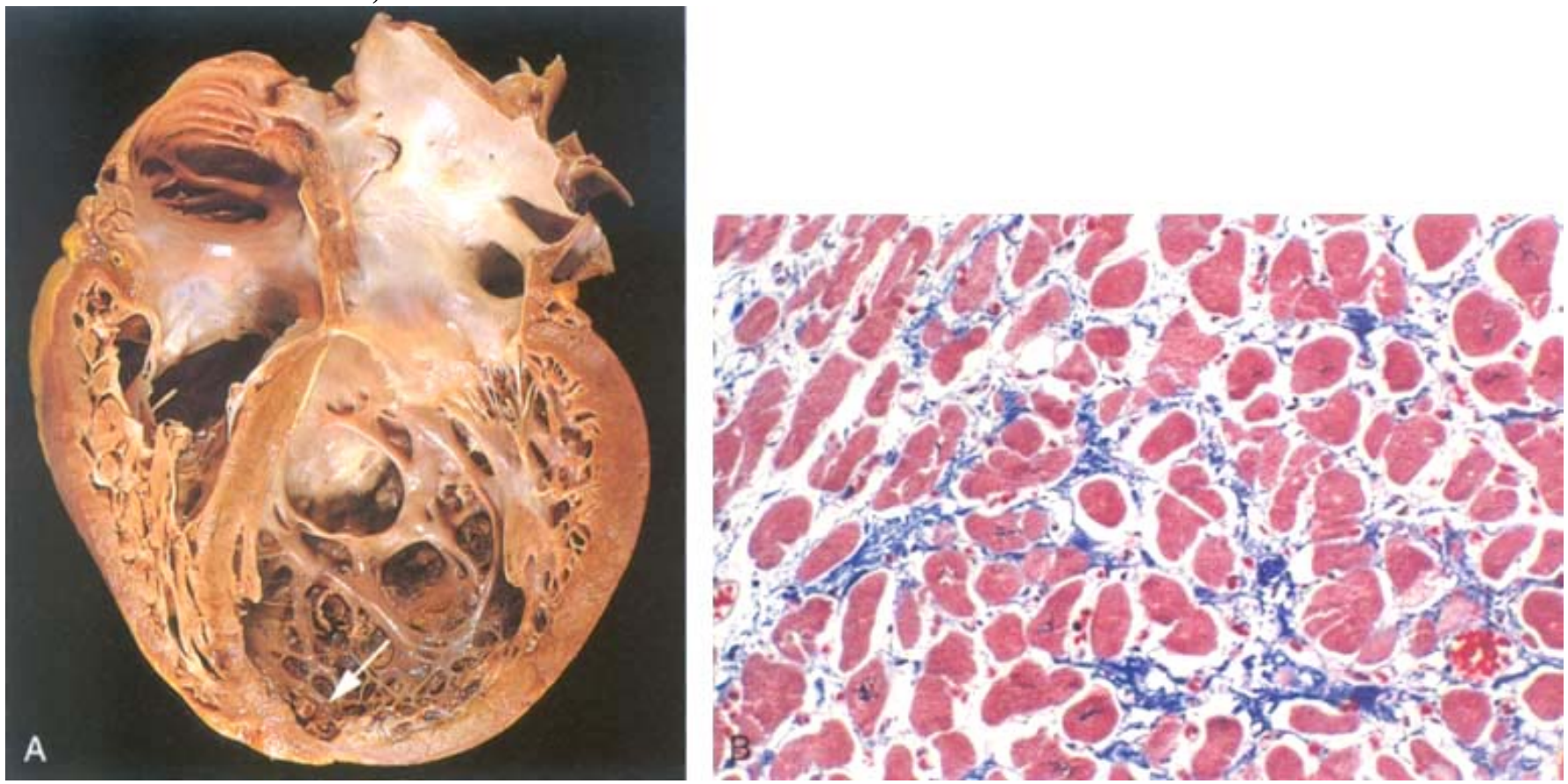


Figure 12-33 Arrythmogenic right ventricular cardiomyopathy. *A,* Gross photograph, showing dilation of the right ventricle and near transmural replacement of the right ventricular free-wall myocardium by fat and fibrosis. The left ventricle has a virtually normal configuration. *B,* Histologic section of the right ventricular free wall, demonstrating replacement of myocardium *(red)* by fibrosis *(blue, arrow)* and fat (collagen is blue in this Masson trichrome stain).

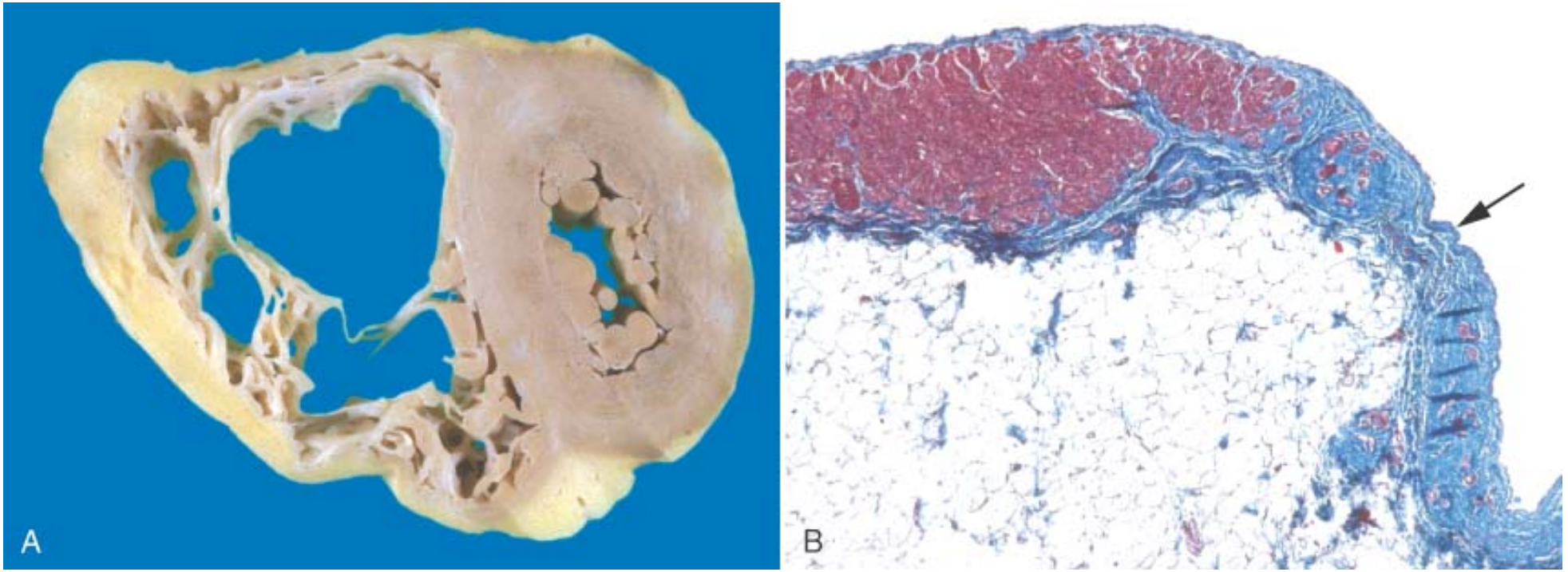
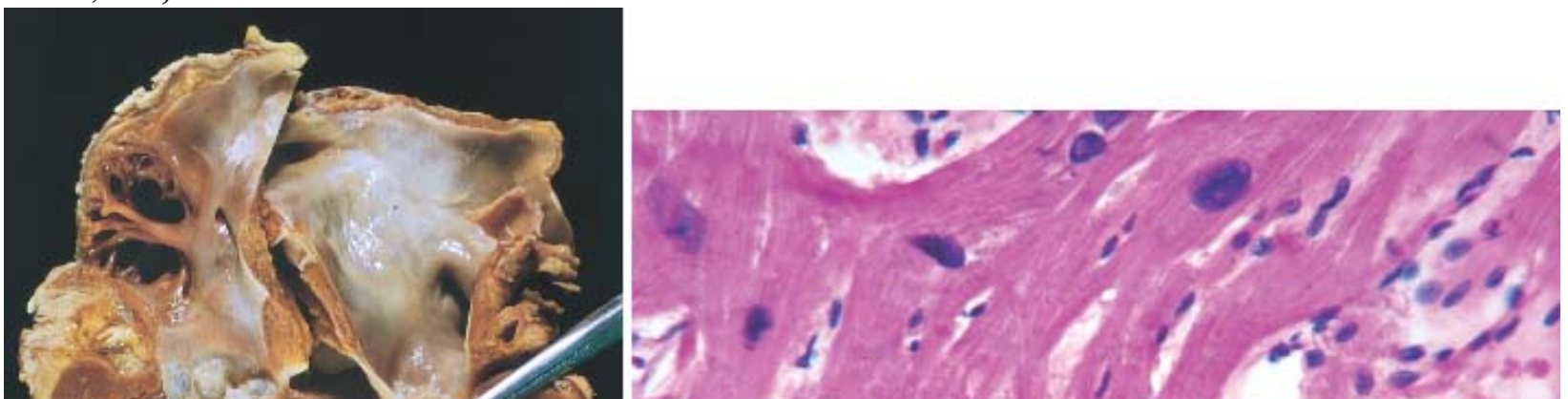


Figure 12-34 Hypertrophic cardiomyopathy with asymmetric septal hypertrophy. *A,* The septal muscle bulges into the left ventricular outflow tract, and the left atrium is enlarged. The anterior mitral leaflet has been moved away from the septum to reveal a fibrous endocardial plaque *(arrow)* (see text). *B,* Histologic appearance demonstrating disarray, extreme hypertrophy, and characteristic branching of myocytes as well as the interstitial fibrosis characteristic of hypertrophic cardiomyopathy (collagen is blue in this Masson trichrome stain). *C,* Schematic structure of the sarcomere of cardiac muscle, highlighting proteins in which mutations cause defective contraction, hypertrophy, and myocyte disarray in hypertrophic cardiomyopathy. The frequency of a particular gene mutation is indicated as a percentage of all cases of HCM; most common are mutations in β-myosin heavy chain. Normal contraction of the sarcomere involves myosin-actin interaction initiated by calcium binding to troponin C, I, and T and α-tropomyosin. Actin stimulates ATPase activity in the myosin head and produces force along the actin filaments. Myocyte-binding protein C modulates contraction. *(A, reproduced by permission from Schoen FJ: Interventional and Surgical Cardiovascular Pathology: Clinical Correlations and Basic Principles. Philadelphia, W.B. Saunders, 1989. C, from Spirito P, et al: The management of hypertrophic cardiomyopathy. N Engl J Med 336:775, 1997.)*



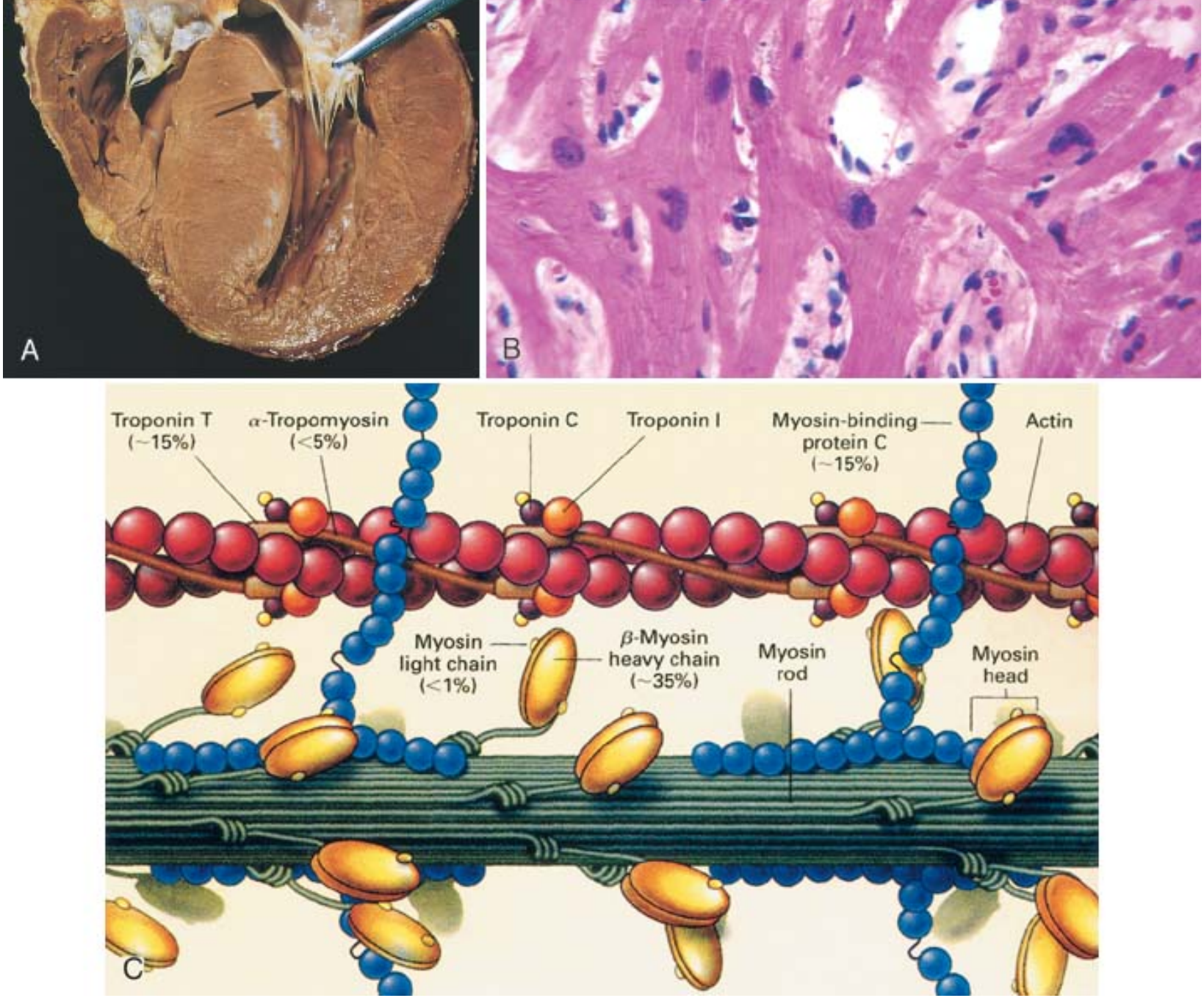


Figure 12-35 Pathways of dilated and hypertrophic cardiomyopathy, emphasizing several important concepts. Some forms of dilated cardiomyopathy (others are caused by myocarditis, alcohol, and other toxic injury or the peripartum state) and virtually all forms of hypertrophic cardiomyopathy are genetic in origin. The genetic causes of dilated cardiomyopathy involve mutations in any of a wide variety of proteins, predominantly of the cytoskeleton, but also the sarcomere, mitochondria, and nuclear envelope. In contrast, the mutated genes that cause hypertrophic cardiomyopathy encode proteins of the sarcomere. Although these two forms of cardiomyopathy differ greatly in subcellular basis and morphologic phenotypes, they share a common pathway of clinical complications.

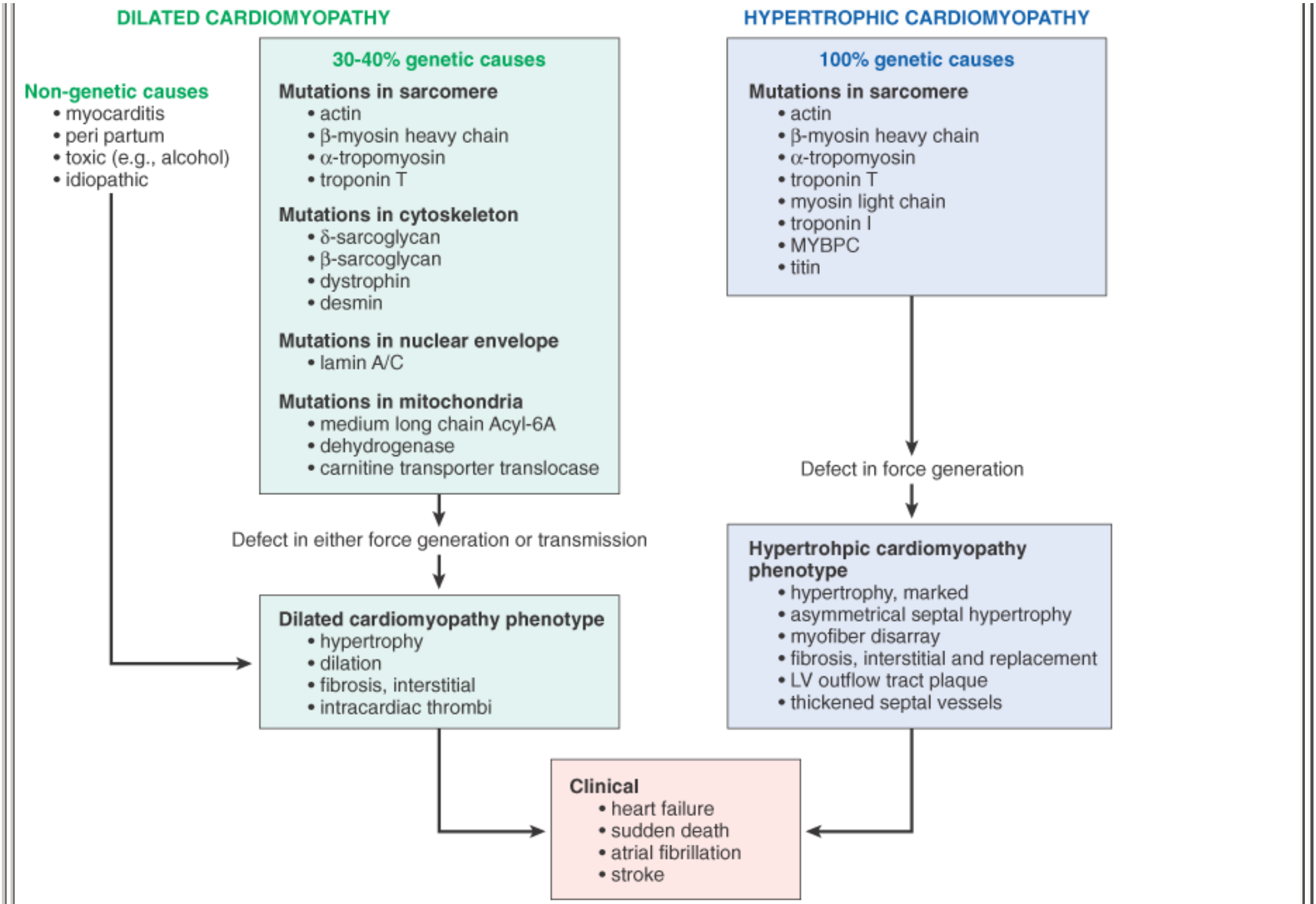


TABLE 12-12 -- Major Causes of Myocarditis

Infections
Viruses (e.g., coxsackievirus, ECHO, influenza, HIV, cytomegalovirus)
Chlamydiae (e.g., *C. psittaci*)
Rickettsiae (e.g., *R. typhi*, typhus fever)
Bacteria (e.g., *Corynebacterium diphtheriae, Neisseria meningococcus, Borrelia* (Lyme disease)
Fungi (e.g., *Candida*)
Protozoa (e.g., *Trypanosoma* Chagas disease, toxoplasmosis)
Helminths (e.g., trichinosis)
Immune-Mediated Reactions
Postviral
Poststreptococcal (rheumatic fever)
Systemic lupus erythematosus
Drug hypersensitivity (e.g., methyldopa, sulfonamides)
Transplant rejection
Unknown
Sarcoidosis
Giant cell myocarditis
HIV, human immunodeficiency virus.

inflammation and myocyte damage without a clear etiologic agent and (2) myocarditis caused directly by HIV or by an opportunistic pathogen.

There are also noninfectious causes of myocarditis. Myocarditis can be related to allergic reactions *(hypersensitivity myocarditis)*, often to a particular drug such as antibiotics, diuretics, and antihypertensive agents. Myocarditis can also be associated with systemic diseases of immune origin, such as RF, SLE, and polymyositis. Cardiac sarcoidosis and rejection of a transplanted heart are also considered forms of myocarditis.

Against this background we can turn to the anatomic changes seen in the major forms of myocarditis.

Morphology.

During the active phase of myocarditis, the heart may appear normal or dilated; some hypertrophy may be present. The lesions may be diffuse or patchy. The ventricular myocardium is typically flabby and often mottled by either pale foci or minute hemorrhagic lesions. Mural thrombi may be present in any chamber.

During active disease, myocarditis is most frequently characterized by an interstitial inflammatory infiltrate and focal necrosis of myocytes adjacent to the inflammatory cells (Fig. 12-36).[74] Myocarditis in which the infiltrate is mononuclear and predominantly lymphocytic is most common (see Fig. 12-36*A*). Although endomyocardial biopsies are diagnostic in some cases,

they can be spuriously negative because inflammatory involvement may be focal or patchy. If the patient survives the acute phase of myocarditis, the inflammatory lesions either resolve, leaving no residual changes, or heal by progressive fibrosis, as mentioned earlier.

Hypersensitivity myocarditis has interstitial infiltrates, principally perivascular, composed of lymphocytes, macrophages, and a high proportion of eosinophils (see Fig. 12-36*B*).

A morphologically distinctive form of myocarditis of uncertain cause, called **giant cell myocarditis**, is characterized by a widespread inflammatory cellular infiltrate containing multinucleate giant cells interspersed with lymphocytes, eosinophils, plasma cells, and macrophages and having at least focal but frequently extensive necrosis (Fig. 12-36*C*). The giant cells are of either macrophage or myocyte origin. This variant carries a poor prognosis.[117]

The myocarditis of **Chagas disease** is rendered distinctive by parasitization of scattered myofibers by trypanosomes accompanied by an inflammatory infiltrate of neutrophils, lymphocytes, macrophages, and occasional eosinophils (Fig. 12-36*D*).

Clinical Features.

The clinical spectrum of myocarditis is broad; at one end the disease is entirely asymptomatic, and such patients recover completely without sequelae. At the other extreme is the precipitous onset of heart failure or arrhythmias, occasionally with sudden death. A systolic murmur may appear, indicating functional mitral regurgitation related to dilation of the left ventricle. Between these extremes are the many levels of involvement associated with such symptoms as fatigue, dyspnea, palpitations, precordial discomfort, and fever. The clinical features of myocarditis can

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Figure 12-36 Myocarditis. *A,* Lymphocytic myocarditis, with mononuclear inflammatory cell infiltrate and associated myocyte injury. *B,* Hypersensitivity myocarditis, characterized by interstitial inflammatory infiltrate composed largely of eosinophils and mononuclear inflammatory cells, predominantly localized to perivascular and large interstitial spaces. This form of myocarditis is associated with drug hypersensitivity. *C,* Giant cell myocarditis, with mononuclear inflammatory infiltrate containing lymphocytes and macrophages, extensive loss of muscle, and multinucleated giant cells. *D,* The myocarditis of Chagas disease. A myofiber is distended with trypanosomes *(arrow).* There is a surrounding inflammatory reaction and individual myofiber necrosis.

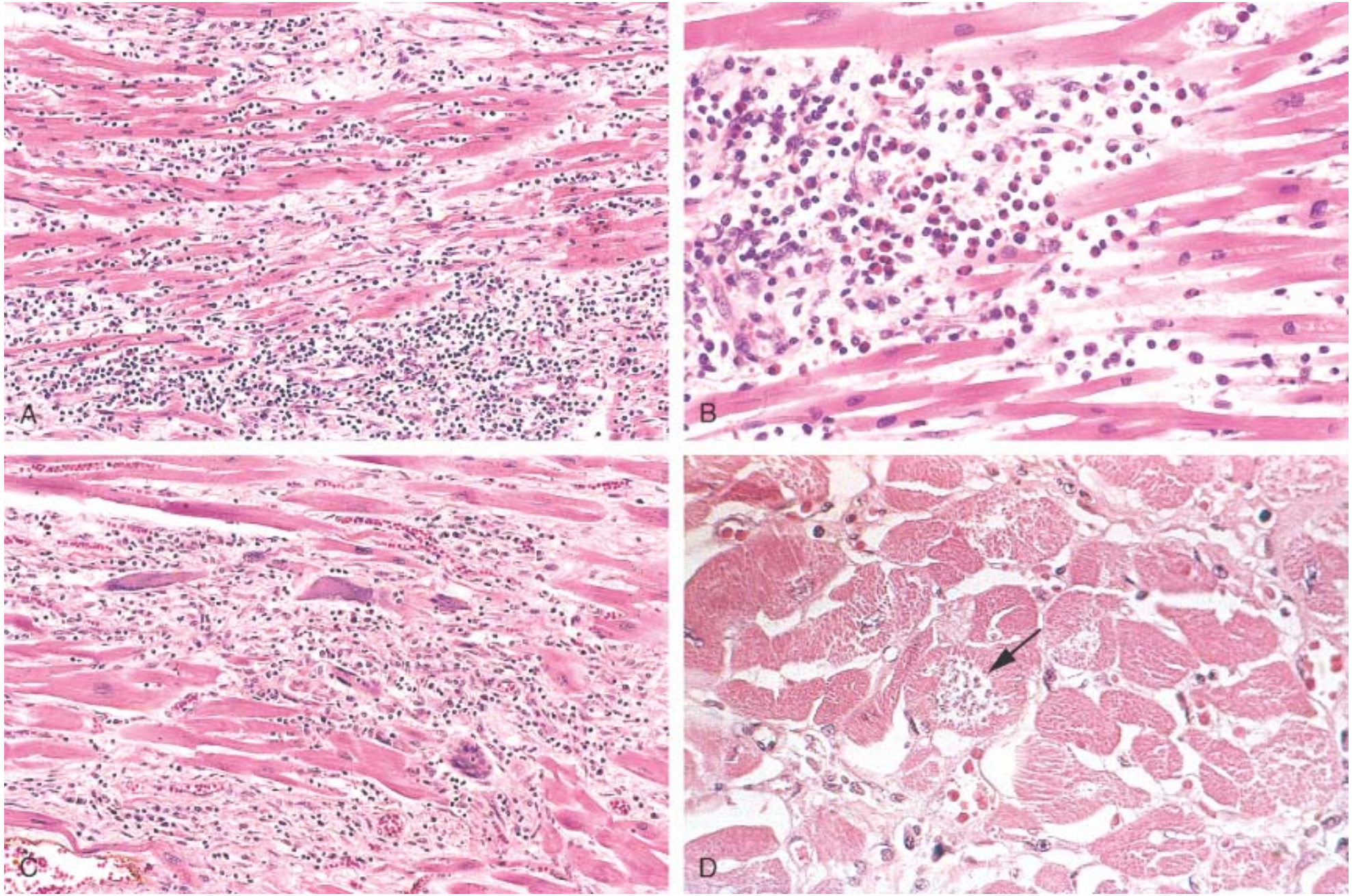


TABLE 12-13 -- Causes of Pericarditis

Infectious Agents
Viruses

Pyogenic bacteria
Tuberculosis
Fungi
Other parasites
Presumably Immunologically Mediated
Rheumatic fever
Systemic lupus erythematosus
Scleroderma
Postcardiotomy
Postmyocardial infarction (Dressler) syndrome
Drug hypersensitivity reaction
Miscellaneous
Myocardial infarction
Uremia
Following cardiac surgery
Neoplasia
Trauma
Radiation

in the tissues contiguous to the pericardium, for example, a bacterial pleuritis, may cause sufficient irritation of the parietal pericardial serosa to cause a sterile serous effusion that may progress to serofibrinous pericarditis and ultimately to a frank suppurative reaction. In some instances, a well-defined viral infection elsewhere—upper respiratory tract infection, pneumonia, parotitis—antedates the pericarditis and serves as the primary focus of infection. Infrequently, usually in young adults, a viral pericarditis occurs as an apparent primary involvement that may accompany myocarditis *(myopericarditis)*.

Morphology.

Whatever the cause, there is an inflammatory reaction in the epicardial and pericardial surfaces with scant numbers of polymorphonuclear leukocytes, lymphocytes, and macrophages. Usually the volume of fluid is not large (50 to 200 mL) and accumulates slowly. Dilation and increased permeability of the vessels due to inflammation produces a fluid of high specific gravity and rich protein content. A mild inflammatory infiltrate in the epipericardial fat consisting predominantly of lymphocytes is frequently termed chronic pericarditis. Organization into fibrous adhesions rarely occurs.

Fibrinous and Serofibrinous Pericarditis.

These two anatomic forms are *the most frequent type of pericarditis* and are composed of serous fluid mixed with a fibrinous exudate. Common causes include acute MI (recall Fig. 12-19D), the postinfarction (Dressler) syndrome (likely an autoimmune condition appearing several weeks after a MI), uremia, chest radiation, RF, SLE, and trauma. A fibrinous reaction also

follows routine cardiac surgery.

Morphology.

In fibrinous pericarditis, the surface is dry, with a fine granular roughening. In serofibrinous pericarditis, an increased inflammatory process induces more and thicker fluid, which is yellow and cloudy owing to leukocytes and erythrocytes (which may be sufficient to give a visibly bloody appearance), and often fibrin. As with all inflammatory exudates, **fibrin may be digested with resolution of the exudate** or **it may become organized** (see Chapter 3).

From the clinical standpoint, *the development of a loud pericardial friction rub is the most striking characteristic of fibrinous pericarditis*, and pain, systemic febrile reactions, and signs suggestive of cardiac failure may be present. However, a collection of serous fluid may obliterate the rub by separating the two layers of the pericardium.

Purulent or Suppurative Pericarditis.

This almost invariably denotes the invasion of the pericardial space by infective organisms, which may reach the pericardial cavity by several routes: (1) direct extension from neighboring inflammation, such as an empyema of the pleural cavity, lobar pneumonia, mediastinal infections, or extension of a ring abscess through the myocardium or aortic root in infective endocarditis; (2) seeding from the blood; (3) lymphatic extension; or (4) direct introduction during cardiotomy. Immunosuppression predisposes to infection by all of these pathways.

Morphology.

The exudate ranges from a thin to a creamy pus of up to 400 to 500 mL in volume. The serosal surfaces are reddened, granular, and coated with the exudate (Fig. 12-37). Microscopically there is an acute inflammatory reaction. Sometimes the inflammatory process extends into surrounding structures to induce a so-called **mediastinopericarditis**. Organization is the usual outcome; resolution is infrequent. Because of the great intensity of the inflammatory response, the organization frequently produces **constrictive pericarditis**, a serious consequence (see later).

The clinical findings in the active phase are essentially the same as those present in fibrinous pericarditis, but signs of systemic infection are usually marked: for example, spiking temperatures, chills, and fever.

Hemorrhagic Pericarditis.

An exudate composed of blood mixed with a fibrinous or suppurative effusion is most commonly caused by malignant neoplastic involvement of the pericardial space; in such cases, cytologic examination of fluid removed through a pericardial tap may yield neoplastic cells. Hemorrhagic pericarditis may also be found in bacterial infections, in patients with an underlying bleeding diathesis, and in tuberculosis. Hemorrhagic pericarditis often follows cardiac surgery and sometimes is responsible for significant blood loss or even tamponade, requiring a "second-look" operation. The clinical significance is similar to that of the spectrum of fibrinous or suppurative pericarditis.

Caseous Pericarditis.

Caseation within the pericardial sac is, until proved otherwise, tuberculous in origin; infrequently, fungal infections evoke a similar reaction. Pericardial involvement

Figure 12-37 Acute suppurative pericarditis as an extension from a pneumonia. Extensive purulent exudate is evident in this in situ photograph.

Figure 12-38 Left atrial myxoma. *A*, Gross photograph showing large pedunculated lesion arising from the region of the fossa ovalis and extending into the mitral valve orifice. *B*, Microscopic appearance, with abundant amorphous extracellular matrix in which are scattered collections of myxoma cells in various groupings, including abnormal vascular formations *(arrow)*.

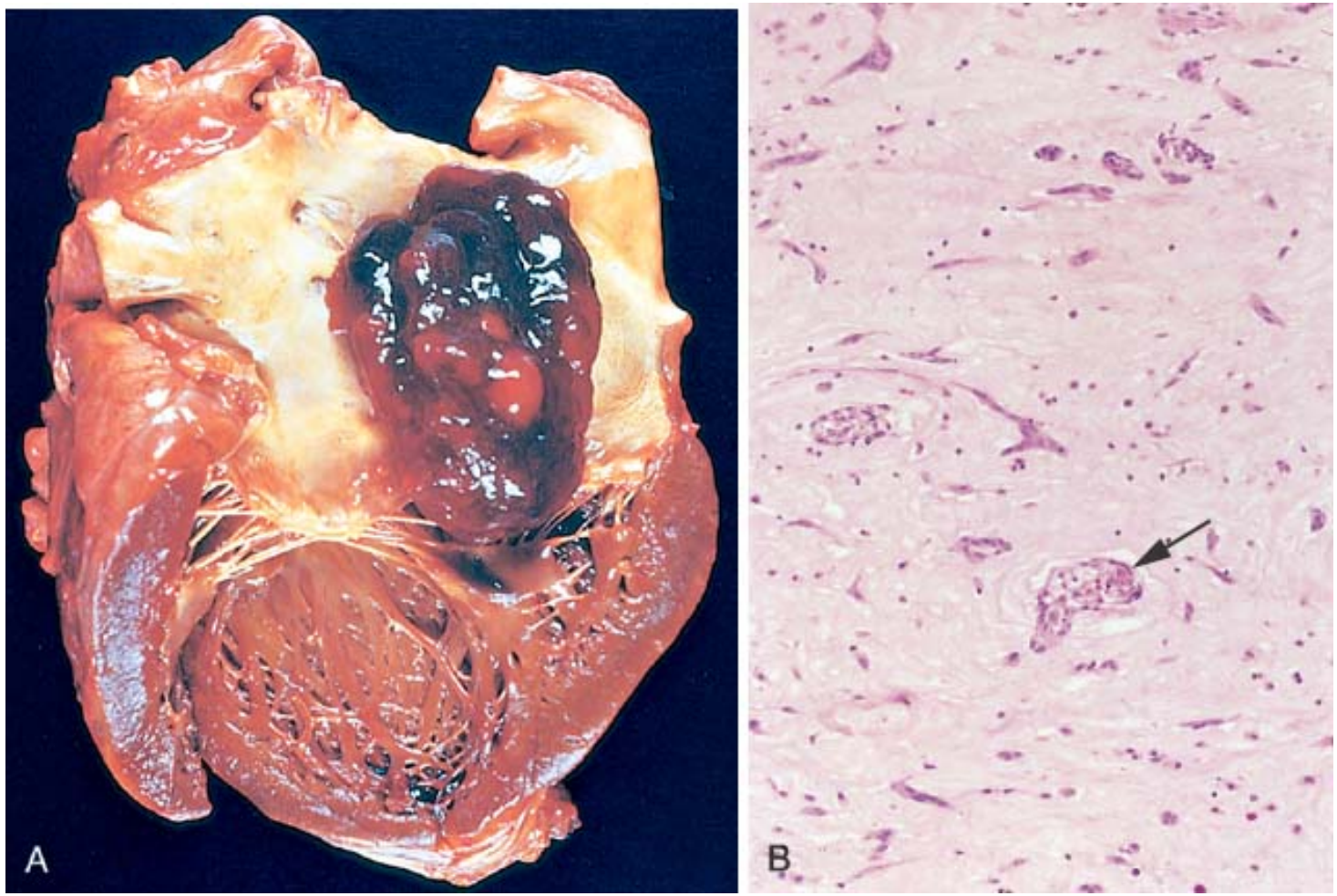


TABLE 12-14 -- Cardiovascular Effects of Noncardiac Neoplasms

Direct Consequences of Tumor
Pericardial and myocardial metastases
Large vessel obstruction
Pulmonary tumor emboli
Indirect Consequences of Tumor (Complications of Circulating Mediators)
Nonbacterial thrombotic endocarditis (NBTE)
Carcinoid heart disease
Pheochromocytoma-associated heart disease
Myeloma-associated amyloidosis

Effects of Tumor Therapy
Chemotherapy
Radiation therapy
Modified from Schoen, FJ, et al: Cardiac effects of non-cardiac neoplasms. Cardiol Clin 2:657, 1984.

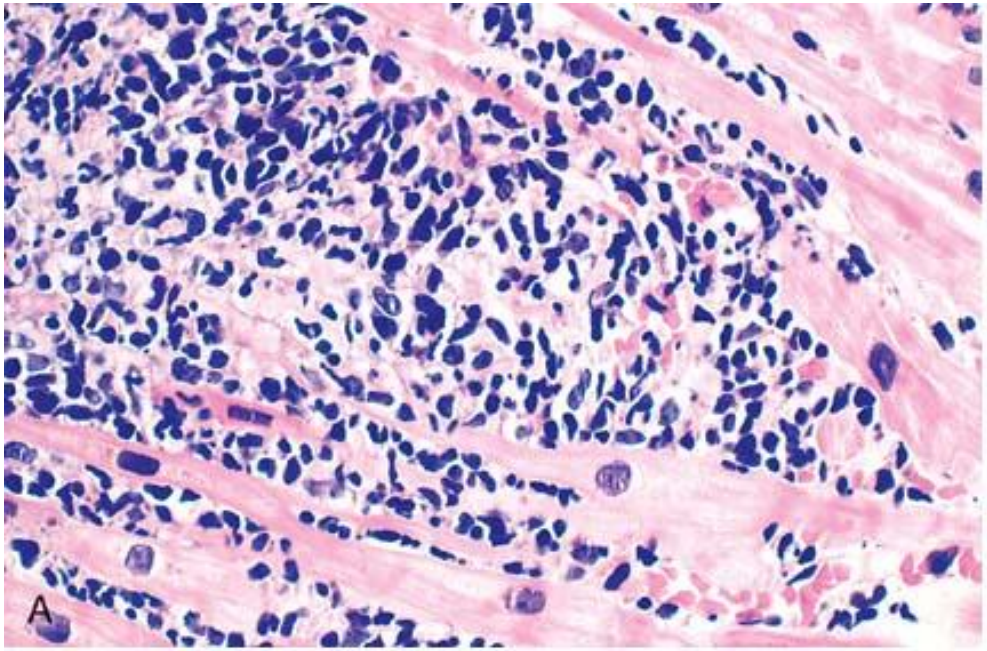
compliance. Bronchogenic carcinoma or malignant lymphoma may infiltrate the mediastinum extensively, causing encasement, compression, or invasion of the superior vena cava with resultant obstruction to blood coming from the head and upper extremities *(superior vena cava syndrome)*. Renal cell carcinoma, because of its high propensity to invade the renal vein, can grow in the lumen of the renal vein into and along the inferior vena cava and can, occasionally, extend into the right atrium, blocking venous return to the heart.

Noncardiac tumors cause indirect cardiac effects, sometimes via circulating tumor-derived substances (e.g., NBTE [see earlier], carcinoid heart disease, pheochromocytoma-associated myocardial damage, multiple myeloma-derived immunoglobulin-causing amyloidosis). Complications of chemotherapy were discussed earlier in this chapter. Radiation used to treat breast, lung, or mediastinal neoplasms can cause pericarditis, pericardial effusion, myocardial fibrosis, and chronic pericardial disorders. Other cardiac effects of radiotherapy include accelerated coronary artery disease and mural and valvular endocardial fibrosis.

Cardiac Transplantation

Transplantation of cardiac allografts is now frequently performed (approximately 3000 per year worldwide) for severe, intractable heart failure of diverse causes, the two most common of which are DCM and IHD. Three major factors contribute to the widespread success of cardiac transplantation since the first successful human to human transplant in 1967: (1) careful selection of candidates, (2) improved

Figure 12-39 Complications of heart transplantation. *A*, Cardiac allograft rejection typified by lymphocytic infiltrate, with associated damage to cardiac myocytes. *B*, Graft coronary arteriosclerosis, demonstrating severe diffuse concentric intimal thickening producing critical stenosis. The internal elastic lamina *(arrow)* and media are intact (Movat pentachrome stain, elastin black). *(B, reproduced by permission from Salomon RN, et al: Human coronary transplantation-associated arteriosclerosis. Evidence for chronic immune reaction to activated graft endothelial cells. Am J Pathol 138:791, 1991.)*



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Chapter 13 - Red Blood Cell and Bleeding Disorders

Jon C. Aster MD, PhD

The organs and tissues involved in hematopoiesis have been traditionally divided into *myeloid tissue*, which includes the bone marrow and the cells derived from it (e.g., erythrocytes, platelets, granulocytes, and monocytes), and *lymphoid tissue*, consisting of thymus, lymph nodes, and spleen. This subdivision is artificial with respect to both the normal physiology of hematopoietic cells and the diseases affecting them. For example, although bone marrow is not where most mature lymphoid cells are found, it is the source of lymphoid stem cells. Similarly, myeloid leukemias, neoplastic disorders of myeloid stem cells, originate in the bone marrow but secondarily involve the spleen and (to a lesser degree) lymph nodes. Some red cell disorders (hemolytic anemias) result from the formation of autoantibodies, signifying a primary disorder of lymphocytes. Thus, it is not possible to draw neat lines between diseases involving the myeloid and lymphoid tissues. Recognizing this difficulty, we somewhat arbitrarily divide diseases of the hematopoietic tissues into two chapters. In the first, we consider diseases of red cells and those affecting hemostasis. In the second, we discuss white cell diseases and disorders affecting primarily the spleen and thymus.

Normal

A complete discussion of normal hematopoiesis is beyond our scope, but certain features are helpful to an understanding of the diseases of blood.

Normal Development of Blood Cells

Blood cells first appear during the third week of fetal embryonic development in the yolk sac, but these cells are generated from a primitive stem cell population restricted to the production of myeloid cells. The origin of definitive hematopoietic stem cells that give rise to lymphoid and myeloid cells is still unsettled. Most studies suggest they arise in the mesoderm of the intraembryonic aorta/gonad/mesonephros (AGM) region,[1] but evidence also exists for an origin within a small subset of yolk sac-derived cells. By the third month of embryogenesis, stem cells derived from the AGM and/or yolk sac migrate to the liver, which is the chief site of blood cell formation until shortly before birth. Beginning in the fourth month of development, stem cells migrate to the bone marrow to commence hematopoiesis at this site. By birth, marrow throughout the skeleton is hematopoietically active and virtually the sole source of blood cells. In fullterm infants, hepatic hematopoiesis dwindles to a trickle, persisting only in widely scattered small foci that become inactive soon after birth. Up to the age of puberty, marrow throughout the skeleton remains red and hematopoietically active. By age 18 only the vertebrae, ribs, sternum, skull, pelvis, and proximal epiphyseal regions of the humerus and femur retain red marrow, the remaining marrow becoming yellow, fatty, and inactive. Thus, in adults, only about half of the marrow space is active in hematopoiesis.

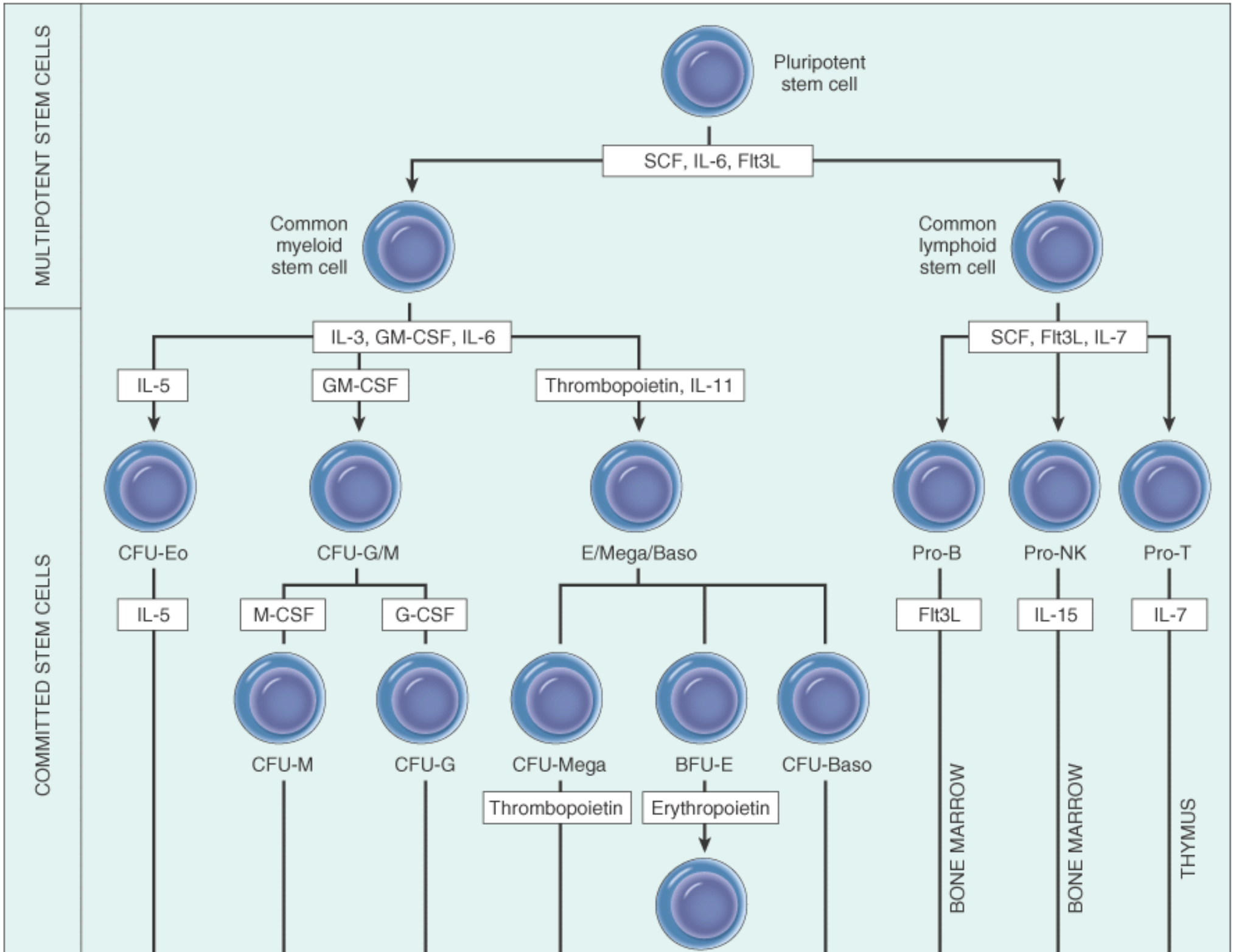
Several features of this normal sequence should be emphasized. By birth, the bone marrow is virtually the sole source of all forms of blood cells, including lymphocyte precursors. In the premature infant, foci of hematopoiesis are frequently evident in the liver and, rarely, in the spleen, lymph nodes, or thymus. Significant postembryonic extramedullary hematopoiesis is abnormal in the full-term infant. With an increased demand for blood cells in the adult, the fatty marrow can transform to red, active marrow. For example, in the face of red cell deficiency (anemia), the marrow can increase red cell production (erythropoiesis) as much as eight-fold. If the marrow stem cells and microenvironment are normal and the necessary nutrients are available (e.g., adequate amounts of iron, protein, requisite vitamins), premature loss of red cells (as occurs in hemolytic disorders) produces anemia only when marrow compensatory mechanisms are outstripped. Under these circumstances, extramedullary hematopoiesis can reappear within the spleen, liver, and even lymph nodes.

ORIGIN AND DIFFERENTIATION OF HEMATOPOIETIC CELLS

The formed elements of blood—red cells, granulocytes, monocytes, platelets, and lymphocytes—have a common origin from pluripotent hematopoietic stem cells sitting at the apex of a complex hierarchy of progenitors (Fig. 13-1). Most of the work supporting this scheme comes from studies conducted in mice, but it is believed hematopoiesis in man proceeds in a highly analogous fashion. The pluripotent stem cell gives rise to two types of multipotent progenitors, the common lymphoid and the common myeloid stem cell. The common lymphoid stem cell in turn gives rise to precursors of T cells (pro-T cells), B cells (pro-B cells), and natural killer cells.[2] The details of lymphoid differentiation are not discussed here, but it is worth pointing out that morphologic distinctions among lymphoid cells at various stages of differentiation are subtle at best. As a result, monoclonal antibodies recognizing differentiation-stage-specific antigens are used widely to define normal lymphocyte subsets (Chapter 14). From the common myeloid stem cell arise at least three types of *committed stem cells* capable of differentiating along the erythroid/megakaryocytic, eosinophilic, and granulocyte-macrophage pathways.[3] In functional assays the committed stem cells are called colony-forming units (CFU), because each can give rise to colonies of differentiated progeny in vitro (see Fig. 13-1). From the various committed stem cells are derived intermediate stages and ultimately the morphologically recognizable precursors of the differentiated cells, such as proerythroblasts, myeloblasts, megakaryoblasts, monoblasts, and eosinophiloblasts, which in turn give rise to mature progeny.

The specific characteristics of rare cells lying high up in the hierarchy shown in Figure 13-1 are still debated. What are agreed upon are certain overarching themes that apply to hematopoiesis. Since mature blood elements are terminally differentiated cells with finite life spans, their numbers must be replenished constantly. It follows that stem cells must not only differentiate, but also *self-renew, a critical property of stem cells*. Pluripotent stem cells have the greatest capacity for self-renewal, but normally most are not in cell cycle. As commitment to particular lines of differentiation proceeds, self-renewal becomes limited, but a greater fraction of committed cells divide actively. For example, few common myeloid stem cells are normally in cell cycle, but up to 50% of CFU-GM

Figure 13-1 Differentiation of hematopoietic cells. SCF, stem cell factor; Flt3L, Flt3 ligand; GM-CSF, granulocyte-macrophage colonys-timulating factor; M-CSF, macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor. *(Modified from Wyngaarden JB, et al [eds]: Cecil Textbook of Medicine, 19th ed. Philadelphia, WB Saunders, 1992, p. 820.)*


MULTIPOTENT STEM CELLS
COMMITTED STEM CELLS
Pluripotent stem cell
SCF, IL-6, Flt3L
Common myeloid stem cell
Common lymphoid stem cell
IL-3, GM-CSF, IL-6
SCF, Flt3L, IL-7
IL-5
GM-CSF
Thrombopoietin, IL-11
CFU-Eo
CFU-G/M
E/Mega/Baso
Pro-B
Pro-NK
Pro-T
IL-5
M-CSF
G-CSF
Flt3L
IL-15
IL-7
CFU-M
CFU-G
CFU-Mega
BFU-E
CFU-Baso
Thrombopoietin
Erythropoietin
BONE MARROW
BONE MARROW
THYMUS

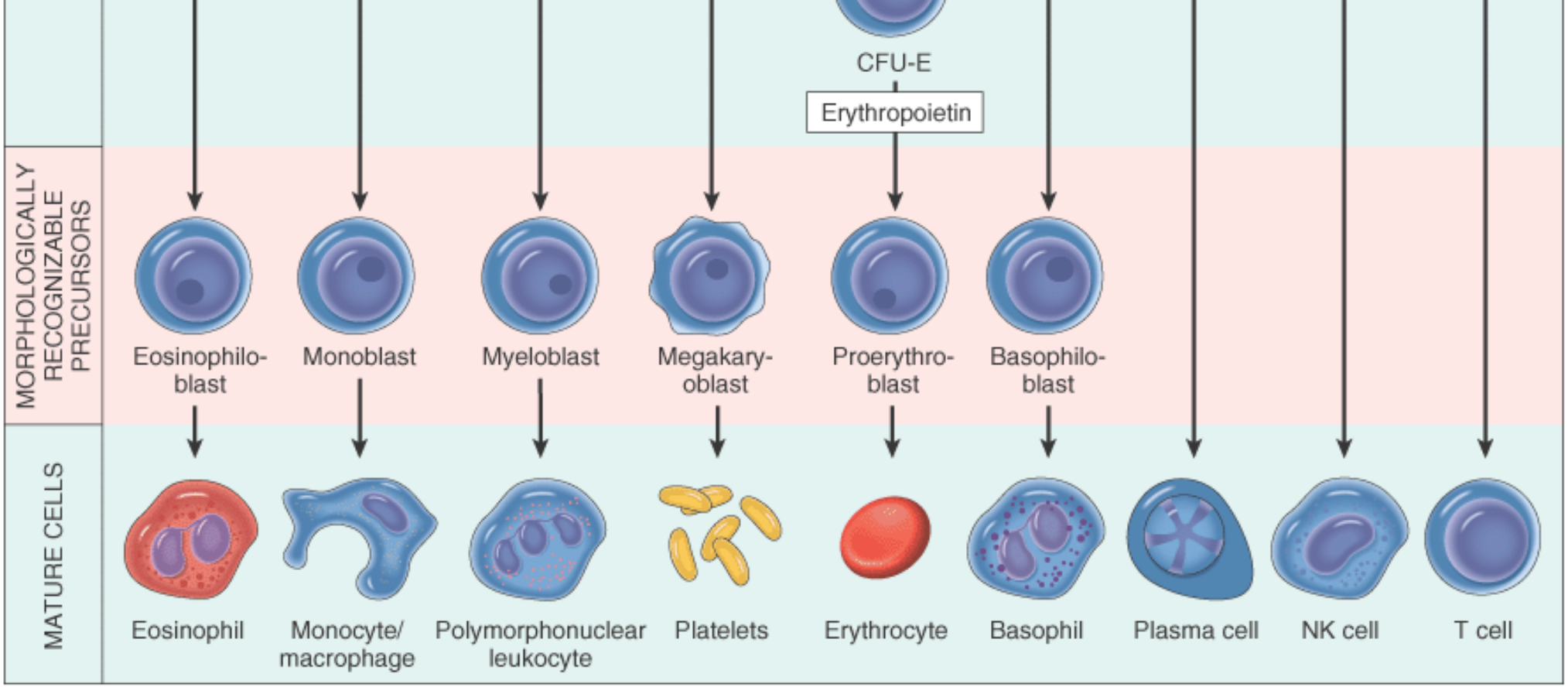


TABLE 13-1 -- Classification of Anemia According to Underlying Mechanism

Blood Loss
Acute: trauma
Chronic: lesions of gastrointestinal tract, gynecologic disturbances
Increased Rate of Destruction (Hemolytic Anemias)
Intrinsic (intracorpuscular) abnormalities of red cells
••*Hereditary*
••Red cell membrane disorders
••••Disorders of membrane cytoskeleton: spherocytosis, elliptocytosis
••••Disorders of lipid synthesis: selective increase in membrane lecithin
••Red cell enzyme deficiencies
••••Glycolytic enzymes: pyruvate kinase deficiency, hexokinase deficiency

••••Enzymes of hexose monophosphate shunt: G6PD, glutathione synthetase
••Disorders of hemoglobin synthesis
••••Deficient globin synthesis: thalassemia syndromes
••••Structurally abnormal globin synthesis (hemoglobinopathies): sickle cell anemia, unstable hemoglobins
••*Acquired*
••Membrane defect: paroxysmal nocturnal hemoglobinuria
Extrinsic (extracorpuscular) abnormalities
••Antibody mediated
••••Isohemagglutinins: transfusion reactions, erythroblastosis fetalis
••••Autoantibodies: idiopathic (primary), drug-associated, systemic lupus erythematosus, malignant neoplasms, mycoplasmal infection
••Mechanical trauma to red cells
••••Microangiopathic hemolytic anemias: thrombotic thrombocytopenic purpura, disseminated intravascular coagulation
••••Cardiac traumatic hemolytic anemia
••Infections: malaria, hookworm
••Chemical injury: lead poisoning
••Sequestration in mononuclear phagocyte system: hypersplenism
Impaired Red Cell Production
Disturbance of proliferation and differentiation of stem cells: aplastic anemia, pure red cell aplasia, anemia of renal failure, anemia of endocrine disorders
Disturbance of proliferation and maturation of erythroblasts
••Defective DNA synthesis: deficiency or impaired use of vitamin B_{12} and folic acid (megaloblastic anemias)
••Defective hemoglobin synthesis
••••Deficient heme synthesis: iron deficiency
••••Deficient globin synthesis: thalassemias
••Unknown or multiple mechanisms: sideroblastic anemia, anemia of chronic infections, myelophthisic anemias due to marrow infiltrations

(normocytic, microcytic, or macrocytic); degree of hemoglobinization, reflected in the color of red cells (normochromic or hypochromic); and other special features, such as shape. These red cell indices are often judged qualitatively by physicians, but precise quantitation is done in clinical laboratories using special instrumentation. The most useful red cell indices are as follows:

- *Mean cell volume:* the average volume of a red blood cell, expressed in femtoliters (cubic micrometers)
- *Mean cell hemoglobin:* the average content (mass) of hemoglobin per red blood cell, expressed in picograms
- *Mean cell hemoglobin concentration:* the average concentration of hemoglobin in a given volume of packed red blood cells, expressed in grams per deciliter

• *Red blood cell distribution width:* the coefficient of variation of red blood cell volume

Adult reference ranges for red cell indices are shown in Table 13-2 .

Whatever its cause, anemia leads to certain clinical features when sufficiently severe. Patients appear pale. Weakness, malaise, and easy fatigability are common complaints. The lowered oxygen content of the circulating blood leads to dyspnea on mild exertion. The nails can become brittle, lose their usual convexity, and assume a concave spoon shape (koilonychia). Anoxia can cause fatty change in the liver, myocardium, and kidney. If fatty changes in the myocardium are sufficiently severe, cardiac failure can develop and compound the respiratory difficulty caused by reduced oxygen transport. On occasion, the myocardial hypoxia manifests as angina pectoris, particularly when complicated by preexisting coronary artery disease. With acute blood loss and shock, oliguria and anuria can develop due to renal hypoperfusion. Central nervous system hypoxia can cause headache, dimness of vision, and faintness.

ANEMIAS OF BLOOD LOSS

Acute Blood Loss

The clinical and morphologic reactions to blood loss depend on the rate of hemorrhage and whether the bleeding

TABLE 13-2 -- Adult Reference Ranges for Red Blood Cells *

Measurement (units)	Men	Women
Hemoglobin (gm/dL)	13.6–17.2	12.0–15.0
Hematocrit (%)	39–49	33–43
Red cell count (10^6 /μL)	4.3–5.9	3.5–5.0
Reticulocyte count (%)	0.5–1.5	
Mean cell volume (μm^3)	82–96	
Mean corpuscular hemoglobin (pg)	27–33	
Mean corpuscular hemoglobin concentration (gm/dL)	33–37	
RBC distribution width	11.5–14.5	

RBC, red blood cell.

*Reference ranges vary among laboratories. The reference ranges for the laboratory providing the result should always be used in interpreting the test result.

is external or internal. The effects of acute blood loss are mainly due to the loss of intravascular volume, which can lead to cardiovascular collapse, shock, and death. If the patient survives, the blood volume is rapidly restored by shift of water from the interstitial fluid compartment. The resulting hemodilution lowers the hematocrit. Reduction in the oxygenation of renal

juxtaglomerular cells triggers increased production of erythropoietin, which stimulates the proliferation of committed erythroid stem cells (CFU-E) in the marrow. It takes about 5 days for the progeny of these CFU-Es to fully differentiate, an event marked by the appearance of increased numbers of newly released red cells (reticulocytes) in the peripheral blood. The iron in hemoglobin is recaptured if red cells are lost internally, as into the peritoneal cavity, but external bleeding leads to iron loss and possible iron deficiency, which can hamper restoration of normal red cell counts.

The earliest change in the peripheral blood immediately after acute blood loss is *leukocytosis,* due to the mobilization of granulocytes from marginal pools. Initially, red cells appear normal in size and color (normocytic, normochromic). However, as marrow production increases, *there is a striking increase in the reticulocyte count, reaching 10% to 15% after 7 days*. Reticulocytes are recognizable as polychromatophilic macrocytes in the usual blood smear. Early recovery from blood loss is often accompanied by *thrombocytosis,* which is caused by increased platelet production.

Chronic Blood Loss

Chronic blood loss induces anemia only when the rate of loss exceeds the regenerative capacity of the marrow or when iron reserves are depleted. Iron deficiency anemia, which has identical features regardless of underlying cause (e.g., bleeding, malnutrition, malabsorption states), will be discussed later.

HEMOLYTIC ANEMIAS

Hemolytic anemias share the following features:

- *A shortened red cell life span (normal = 120 days); that is, premature destruction of red cells*
- *Elevated erythropoietin levels and increased erythropoiesis in the marrow and other sites,* to compensate for the loss of red cells
- *Accumulation of the products of hemoglobin catabolism, due to an increased rate of red cell destruction*

The physiologic destruction of senescent red cells takes place within the mononuclear phagocytic cells of the spleen. In the great majority of hemolytic anemias, the premature destruction of red cells also occurs within the mononuclear phagocyte system (extravascular hemolysis), which undergoes a form of work-related hyperplasia marked by splenomegaly. Much less commonly, lysis of red cells within the vascular compartment (intravascular hemolysis) predominates.

Intravascular hemolysis of red cells is caused by mechanical injury, complement fixation, infection by intracellular parasites such as falciparum malaria (Chapter 8), or exogenous toxic factors. Mechanical injury caused by defective cardiac valves, thrombi within the microcirculation, or repetitive physical trauma (marathon running, bongo drum beating) can physically lyse red cells. Complement fixation can occur on antibody-coated cells during transfusion of mismatched blood. Toxic injury is exemplified by clostridial sepsis, which releases toxins that attack the red cell membrane.

Whatever the mechanism, *intravascular hemolysis is manifested by (1) hemoglobinemia, (2) hemoglobinuria, (3) jaundice*, and *(4) hemosiderinuria*. Free hemoglobin in plasma is promptly bound by an α_2 -globulin (haptoglobin), producing a complex that is rapidly cleared by the mononuclear phagocyte system, thus preventing excretion into the urine. *Decreased serum haptoglobin is characteristic of intravascular hemolysis.* When the haptoglobin is depleted, free hemoglobin is prone to oxidation to methemoglobin, which is brown in color. The renal proximal tubular cells reabsorb and catabolize much of the filtered hemoglobin and methemoglobin, but some passes out with the urine, imparting a red-brown color. Iron released from hemoglobin can accumulate within tubular cells, giving rise to renal hemosiderosis. Concomitantly, heme groups derived from the complexes are catabolized to bilirubin within the mononuclear phagocyte system, leading to jaundice. In hemolytic anemias, the serum bilirubin is unconjugated and the level of hyperbilirubinemia depends on the functional capacity of the liver and the rate of hemolysis. When the liver is normal, jaundice is rarely severe. Excessive bilirubin excreted by the liver into the gastrointestinal tract leads to increased formation and fecal excretion of urobilin (Chapter 18).

Extravascular hemolysis takes place whenever red cells are rendered "foreign" or become less deformable. Since extreme alterations in shape are required for red cells to navigate the splenic sinusoids successfully, reduced deformability makes the passage difficult and leads to sequestration within the cords, followed by phagocytosis (Fig. 13-2). This is an important

pathogenetic mechanism of extravascular hemolysis in a variety of hemolytic anemias. With extravascular hemolysis,

Figure 13-2 Schematic of splenic sinus (electron micrograph). A red cell is in the process of squeezing from the red pulp cords into the sinus lumen. Note the degree of deformability required for red cells to pass through the wall of the sinus.

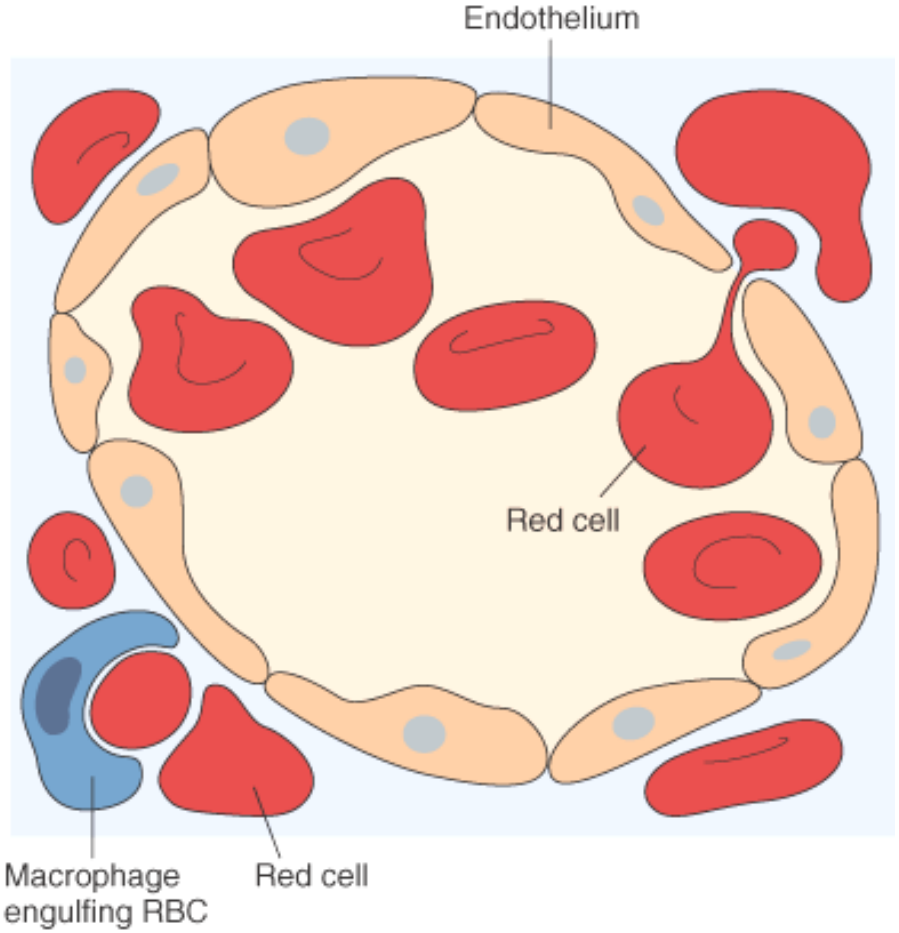


Figure 13-3 Marrow smear from a patient with hemolytic anemia. The marrow reveals greatly increased numbers of maturing erythroid progenitors (normoblasts). *(Courtesy of Dr. Steven Kroft, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

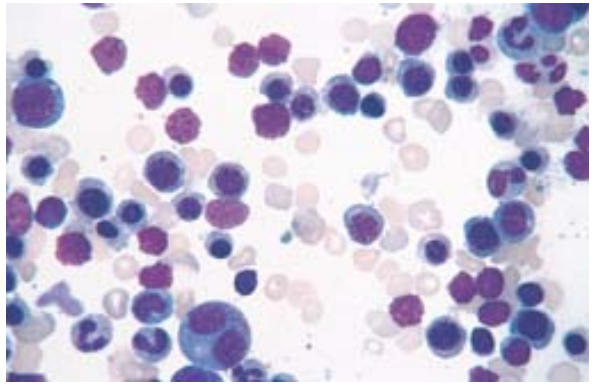


Figure 13-4 Schematic representation of the red cell membrane cytoskeleton and alterations leading to spherocytosis and hemolysis. Mutations weakening interactions involving α-spectrin, β-spectrin, ankyrin, band 4.2, or band 3 all cause the normal biconcave red cell to lose membrane fragments and adopt a spherical shape. Such spherocytic cells are less deformable than normal and therefore become trapped in the splenic cords, where they are phagocytosed by macrophages.

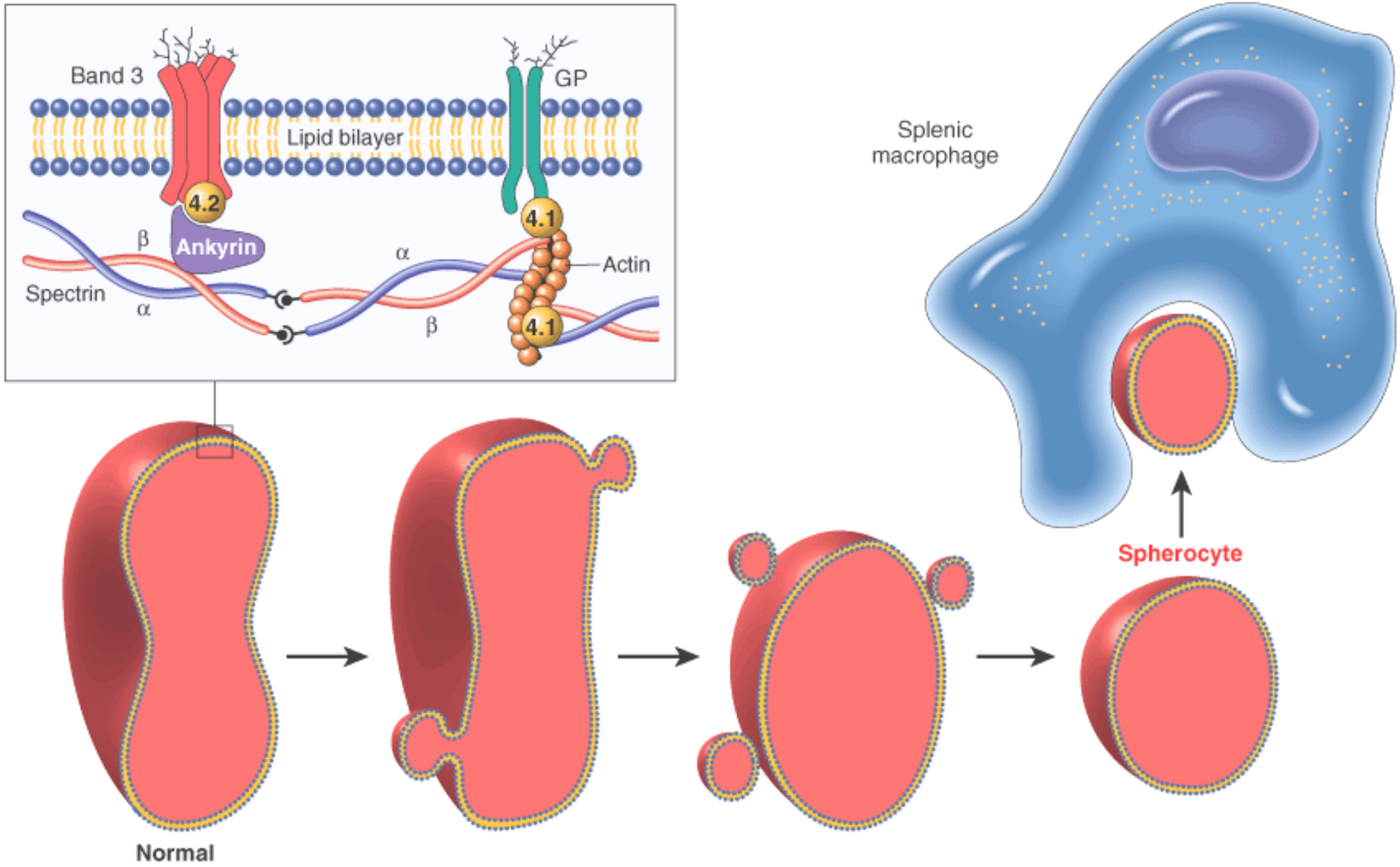


Figure 13-5 Model of the pathophysiology of hereditary spherocytosis. *(Adapted from Wyngaarden JB, et al [eds]: Cecil Textbook of Medicine, 19th ed. Philadelphia, WB Saunders, 1992, p. 859.)*

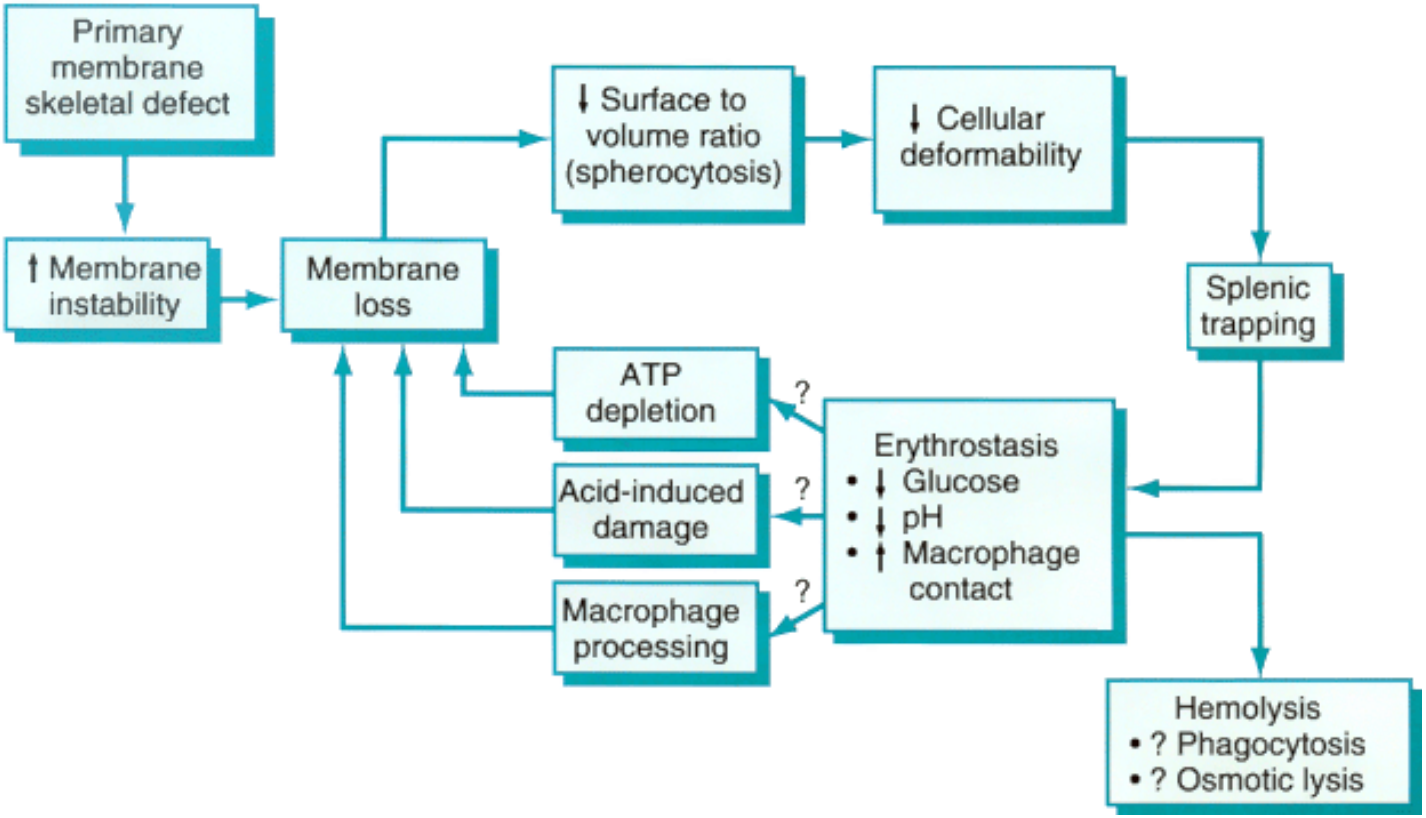


Figure 13-6 Hereditary spherocytosis (peripheral smear). Note the anisocytosis and several dark-appearing spherocytes with no central pallor. Howell-Jolly bodies (small dark nuclear remnants) are also present in red cells of this asplenic patient. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

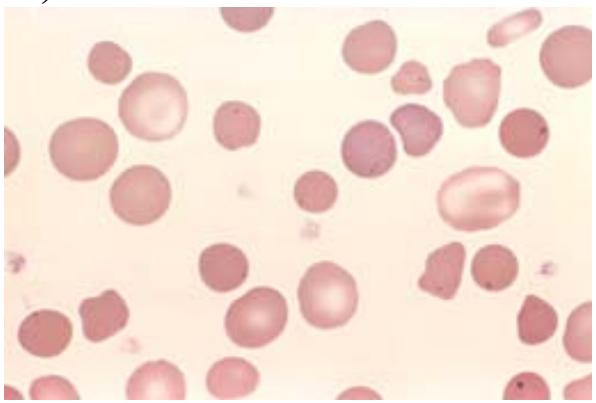


Figure 13-7 Role of glucose-6-phosphate dehydrogenase (G6PD) in defense against oxidant injury. The disposal of H_2O_2, a potential oxidant, is dependent on the adequacy of reduced glutathione (GSH), which is generated by the action of NADPH. The synthesis of NADPH is dependent on the activity of G6PD. GSSG, oxidized glutathione.

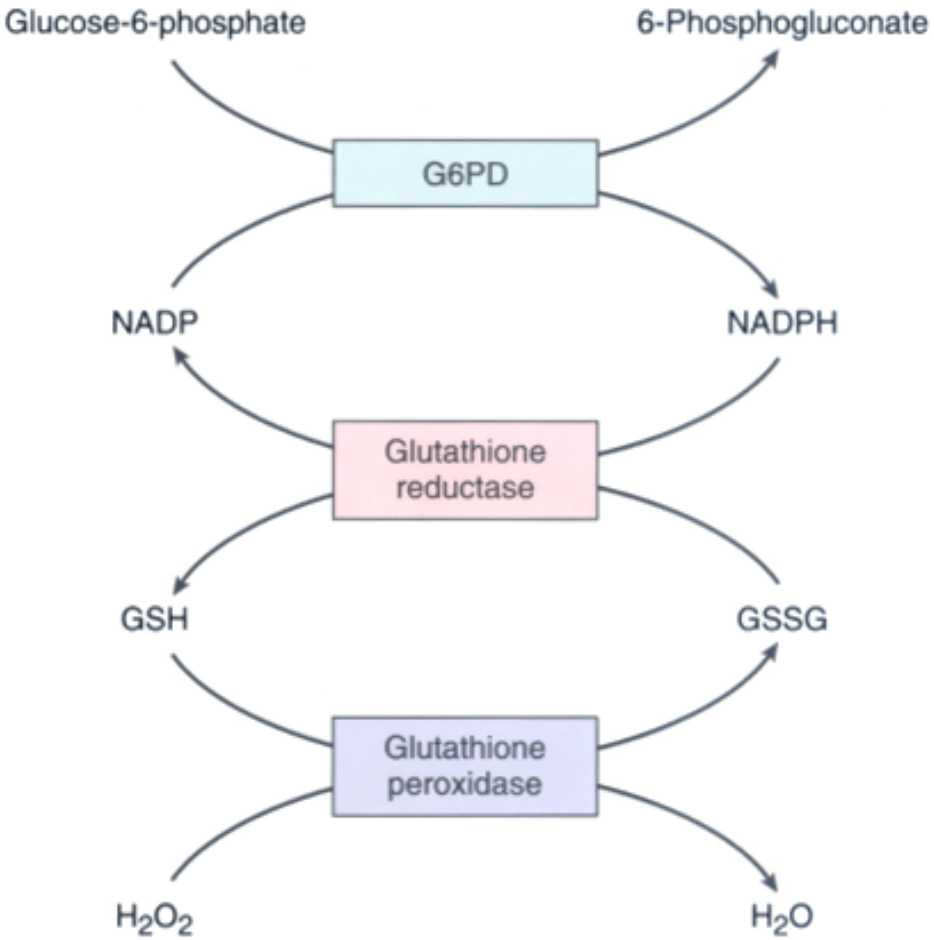


Figure 13-8 G6PD deficiency: effects of oxidant drug exposure (peripheral blood smear). *Inset*, Red cells with precipitates of denatured globin (Heinz bodies) revealed by supravital staining. As the splenic macrophages pluck out these inclusions, "bite cells" like the one in this smear are produced. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

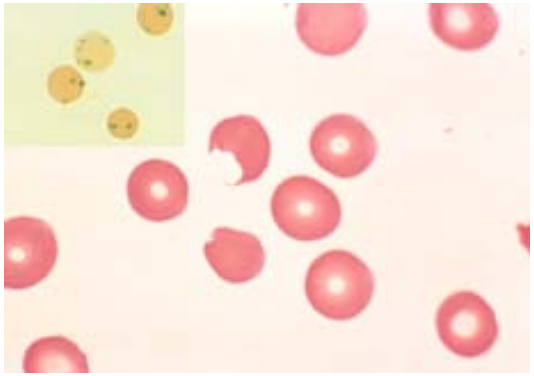


Figure 13-9 Sickle cell anemia (peripheral blood smear). *A*, Low magnification show sickle cells, anisocytosis, and poikilocytosis. *B*, Higher magnification shows an irreversibly sickled

cell in the center. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

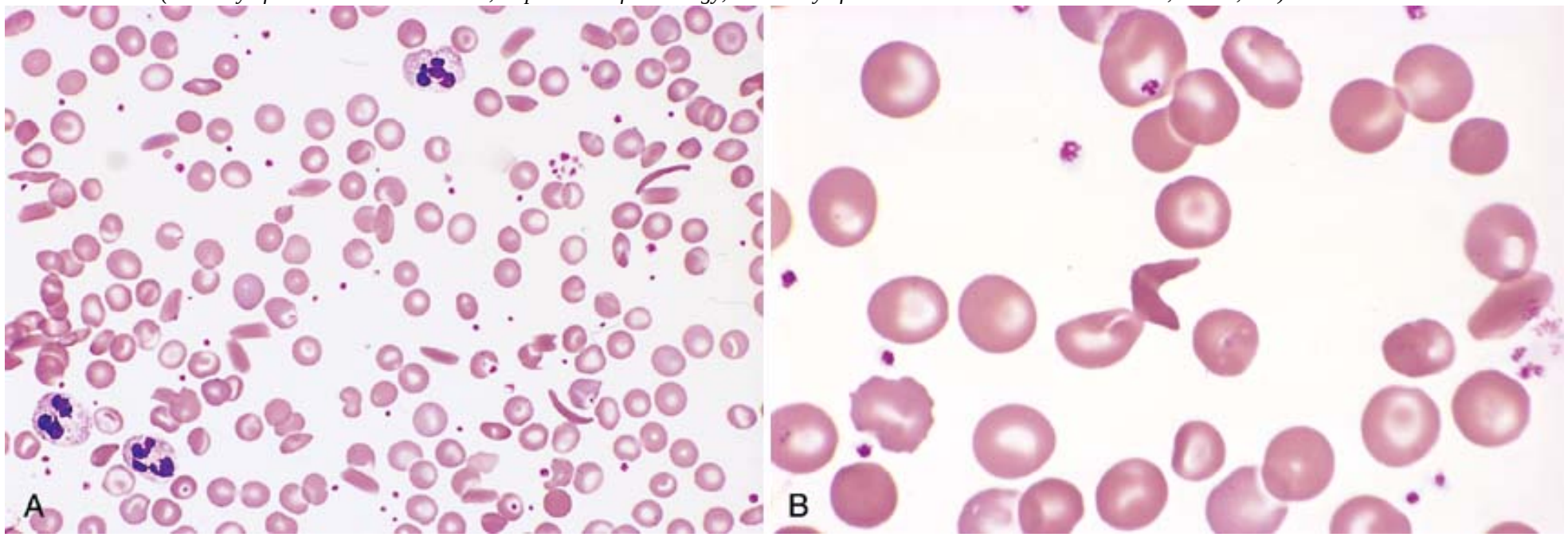


Figure 13-10 Pathophysiology of sickle cell anemia.

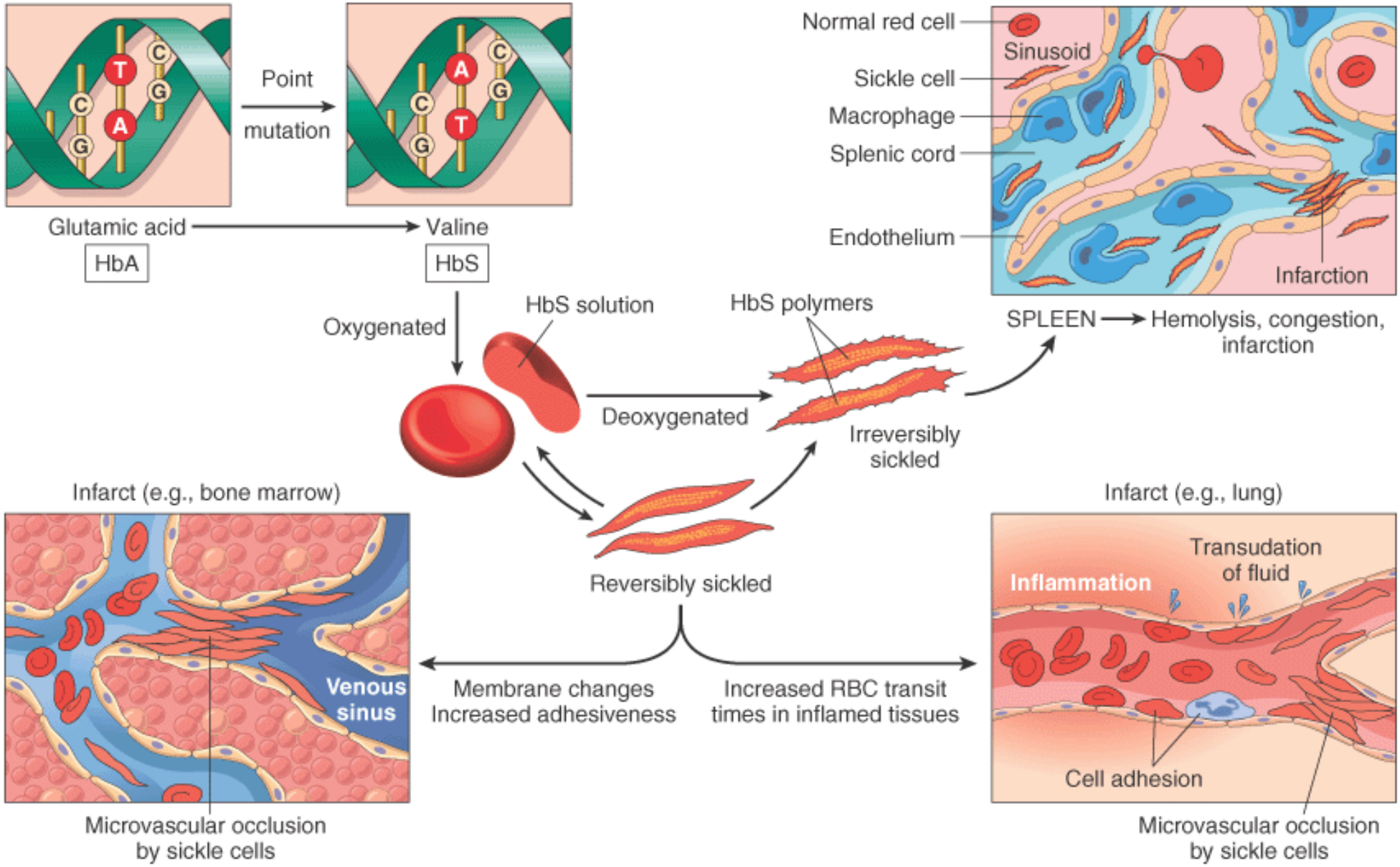


Figure 13-11 *A*, Spleen in sickle cell anemia (low power). Red pulp cords and sinusoids are markedly congested; between the congested areas, pale areas of fibrosis resulting from ischemic damage are evident. *B*, Under high power, splenic sinusoids are dilated and filled with sickled red cells. *(Courtesy of Dr. Darren Wirthwein, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

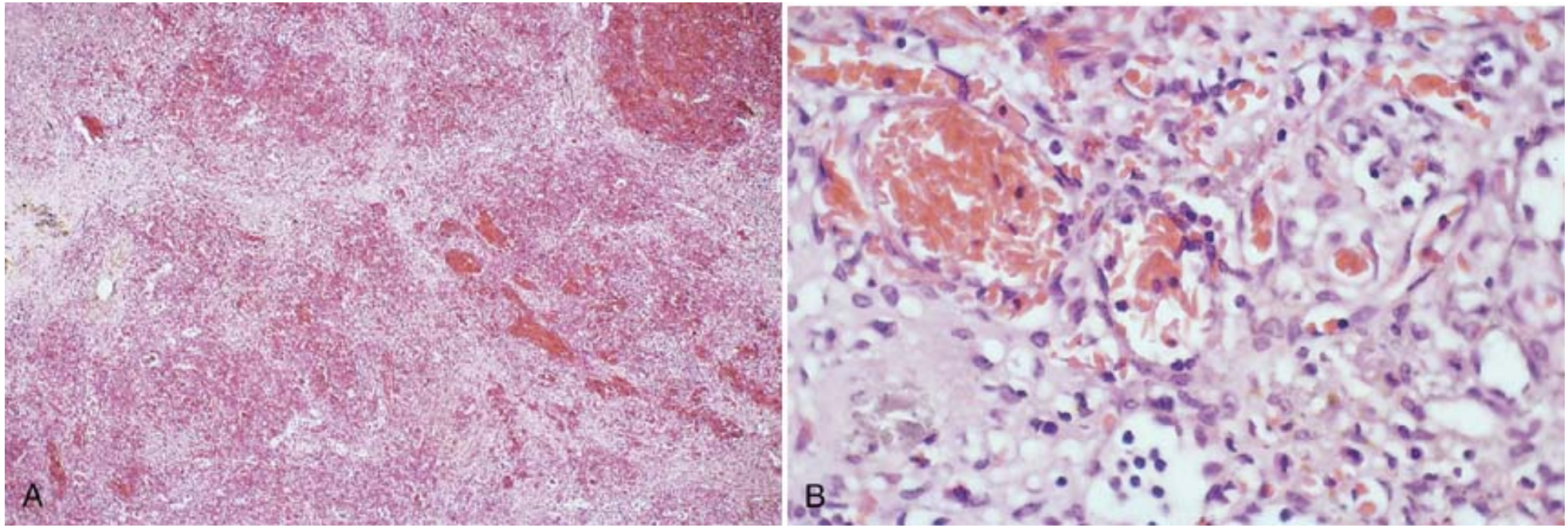


Figure 13-12 Splenic remnant in sickle cell anemia. *(Courtesy of Drs. Dennis Burns and Darren Wirthwein, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

Figure 13-13 Diagrammatic representation of the β-globin gene. Arrows denote sites where point mutations giving rise to thalassemia have been identified.

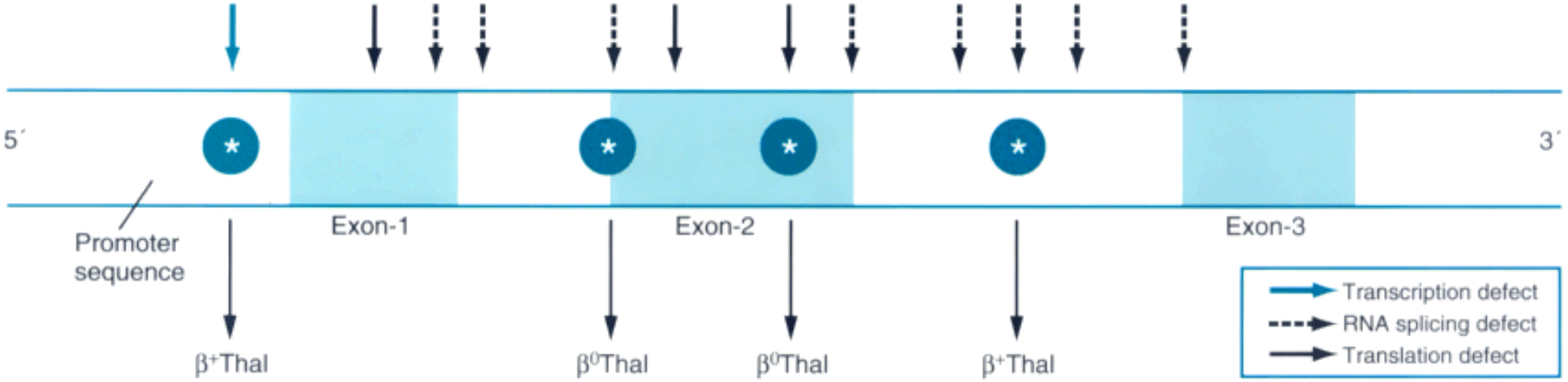
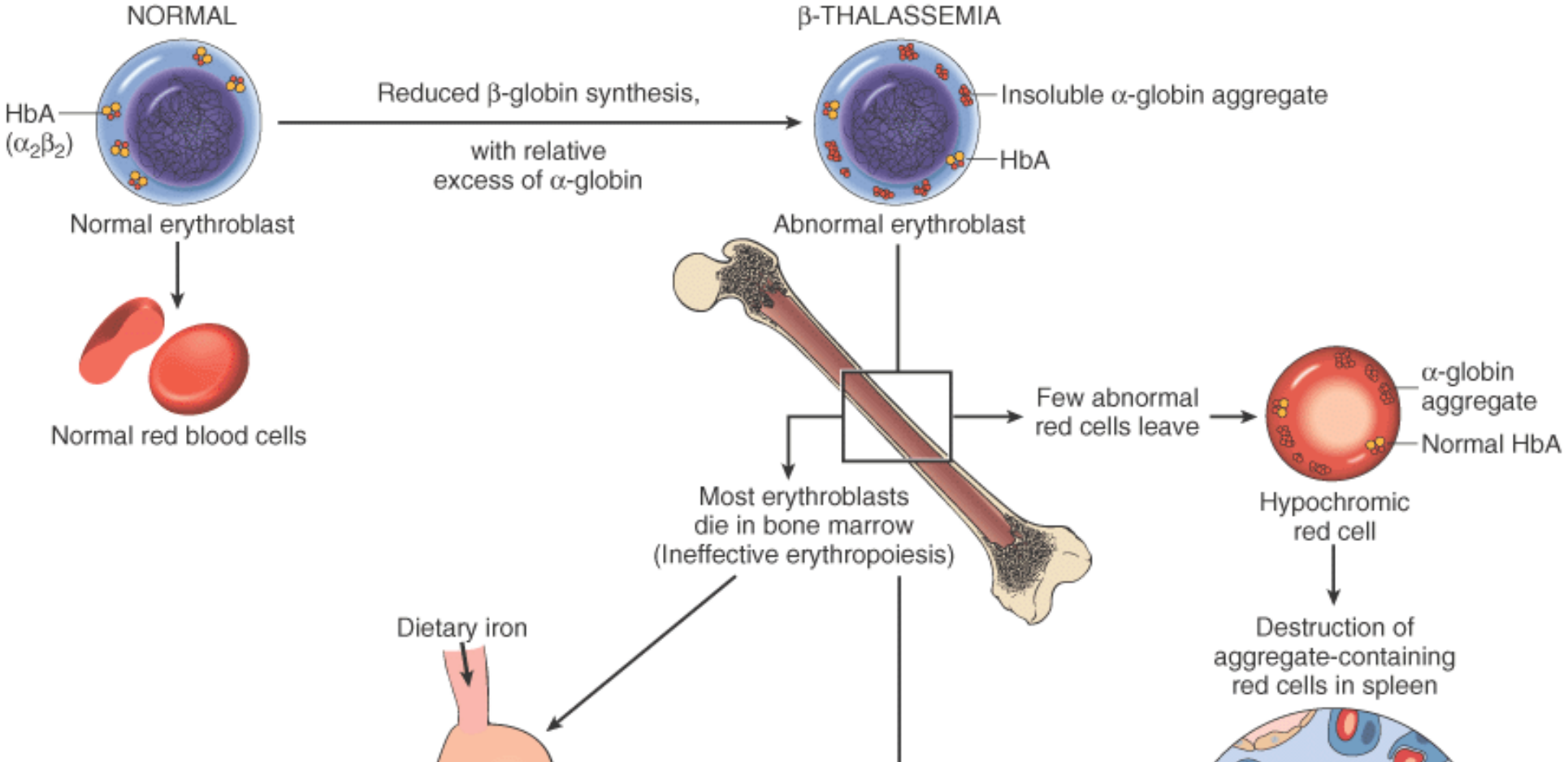


Figure 13-14 Pathogenesis of β-thalassemia major. Note that aggregates of unpaired α-globin chains are not visible in routinely stained blood smears. Blood transfusions are a double-edged sword, correcting the anemia and thereby reducing the stimulus for marrow expansion, but also adding to systemic iron overload.



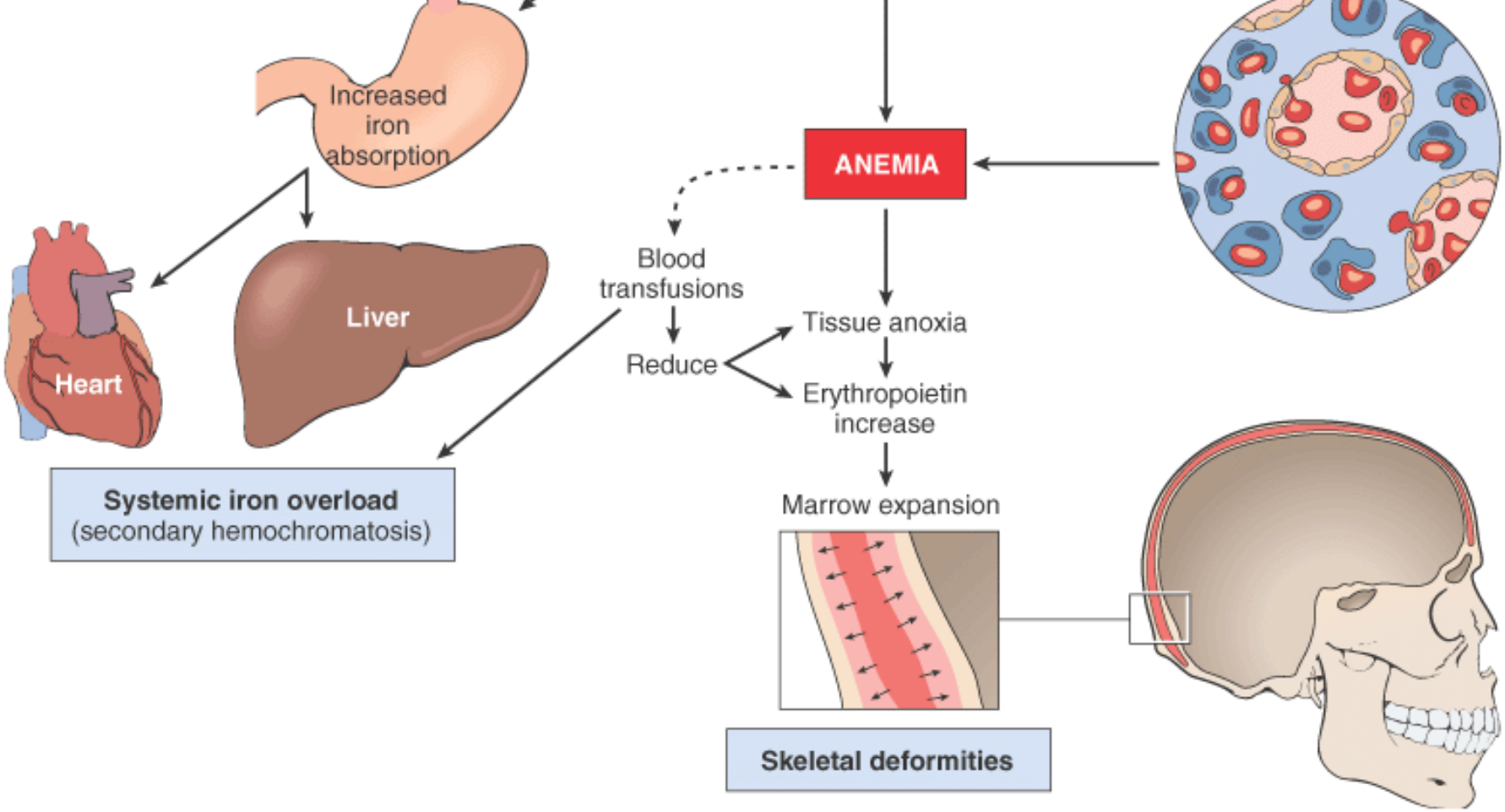


TABLE 13-3 -- Clinical and Genetic Classification of Thalassemias

Clinical Nomenclature	Genotype	Disease	Molecular Genetics
β-***Thalassemias***			
Thalassemia major	Homozygous β^0 -thalassemia (β^0/β^0)	Severe; requires blood transfusions	Rare gene deletions in β^0/β^0 Defects in transcription, processing, or translation of β-globin mRNA
	Homozygous β^+ -thalassemia (β^+/β^+)		
Thalassemia intermedia	β^0/β	Severe, but does not require regular blood transfusions	
	β^+/β^+		
Thalassemia minor	β^0/β	Asymptomatic with mild or absent anemia; red cell abnormalities seen	
	β^+/β		

α-*Thalassemias*			
Hydrops fetails	-/- -/-	Lethal in utero without transfusions	Mainly gene deletions
HbH disease	-/- -/α	Severe; resembles β-thalassemia intermedia	
α-Thalassemia trait	-/- α/α (Asian)	Asymptomatic, like β-thalassemia minor	
	-/α -/α (black African)		
Silent carrier	-/α α/α	Asymptomatic; no red cell abnormality	

the following discussions of thalassemia major and minor (Table 13-3).

Thalassemia Major.

β-Thalassemia is most common in Mediterranean countries and parts of Africa and Southeast Asia. In the United States, the incidence is highest in immigrants from these areas. As indicated in Table 13-3 , the genotype of affected patients can be β^+/β^+ , β^0/β^0 , or β^0/β^+ . With all these genotypes, the anemia manifests 6 to 9 months after birth, as hemoglobin synthesis switches from HbF to HbA. In untransfused patients, hemoglobin levels range between 3 and 6 gm/dL. The peripheral blood smear shows severe red cell morphologic abnormalities, including marked anisocytosis and poikilocytosis (variation in size and shape, respectively), microcytosis (small size), and hypochromia (poor hemoglobinization). Target cells (so called because hemoglobin collects in the center of the cells), basophilic stippling, and fragmented red cells are also common. Inclusions of aggregated α chains are efficiently removed by the spleen and not easily found in peripheral blood smears. The reticulocyte count is elevated, but because of ineffective erythropoiesis is lower than expected for the severity of anemia. Variable numbers of poorly hemoglobinized normoblasts are seen in the peripheral blood due to "stress" erythropoiesis and abnormal release of progenitors from sites of extramedullary hematopoiesis. The red cells can completely lack HbA (β^0/β^0 genotype) or contain small amounts (β^+/β^+ or β^0/β^+ genotypes). HbF is markedly increased and indeed constitutes the major red cell hemoglobin. HbA_2 levels may be normal, low, or high.

Morphology.

The major morphologic alterations, in addition to those found in all hemolytic anemias, involve the bone marrow and spleen. In the untransfused patient, there is striking expansion of hematopoietically active marrow, particularly in facial bones. This erodes existing cortical bone and induces new bone formation, giving rise to a "crew-cut" appearance on X-rays (Fig. 13-15). Both mononuclear phagocytic cell hyperplasia and extramedullary hematopoiesis contribute to enlargement of the spleen, which can weigh up to 1500 gm.

Figure 13-15 Thalassemia: x-ray film of the skull showing new bone formation on the outer table, producing perpendicular radiations resembling a crew-cut. *(Courtesy of Dr. Jack Reynolds, Department of Radiology, University of Texas Southwestern Medical School, Dallas, TX.)*

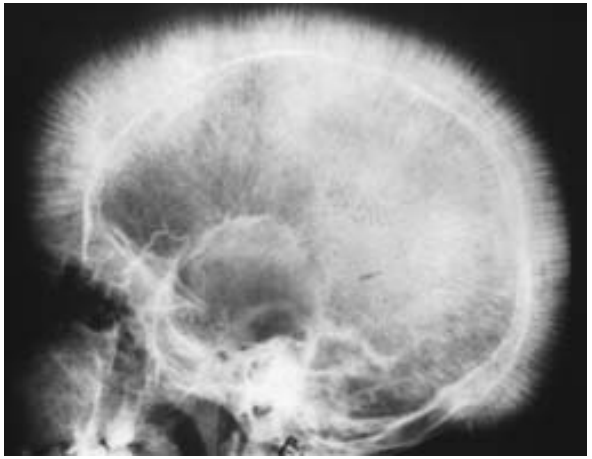


Figure 13-16 Two kinds of membrane proteins: transmembrane and glycosyl phosphatidyl inositol (GPI)-linked. The latter are anchored to cell membranes through a covalent attachment to a glycosyl phospatidyl inositol moiety. In PNH, GPI cannot be synthesized, leading to a global deficiency of GPI-linked membrane proteins.

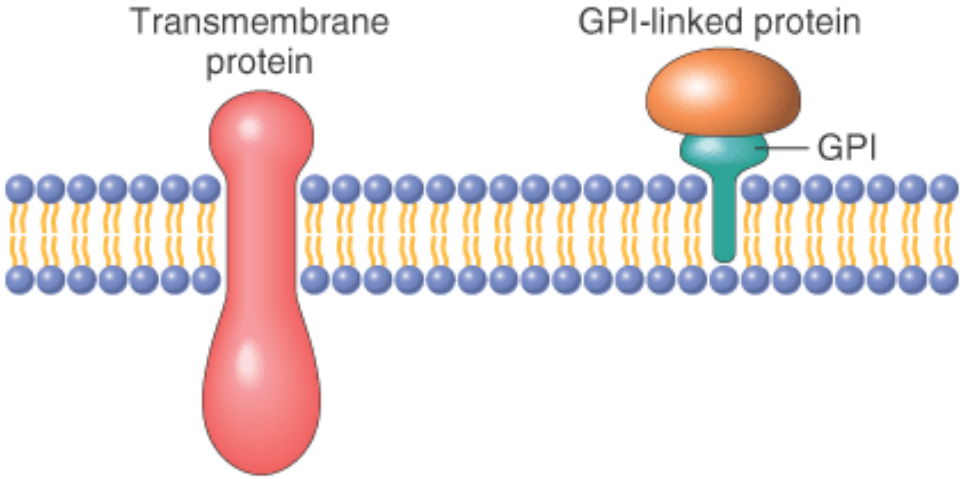


TABLE 13-4 -- Classification of Immunohemolytic Anemias

Warm Antibody Type
The antibody is of the IgG type, does not usually fix complement, and is active at 37°C.
Primary (idiopathic)
Secondary
••Lymphomas and leukemias
••Other neoplastic diseases
••Autoimmune disorder (particularly systemic lupus erythematosus)

••Drugs
Cold Agglutinin Type
The antibodies are IgM and most active in vitro at 0° to 4°C.
••Antibodies dissociate at 30°C or above; agglutination of cells by IgM and complement fixation occurs only in peripheral cool parts of the body (e.g., fingers, ears, and toes).
Acute (mycoplasmal infection, infectious mononucleosis)
Chronic
••Idiopathic
••Associated with lymphoma
Cold Hemolysins (Paroxysmal Cold Hemoglobinuria)
IgG antibodies bind red cells at low temperature, fix complement, and cause hemolysis when the temperature is raised above 30°C.

this test, patient red cells are mixed with heterologous antisera specific for human immunoglobulins or complement. If either is present, red cells are cross-linked by multivalent antibodies, causing clumping or agglutination. The indirect Coombs antiglobulin test, in which patient serum is tested for its ability to agglutinate defined test red cells, can then be used to characterize the target of the autoantibody. The temperature dependence of this reaction also helps to define the type of antibody responsible. Quantitative immunologic tests to measure such antibodies directly are also available.

Warm Antibody Immunohemolytic Anemia.

This is the most common form (48% to 70%) of immune hemolytic anemia. About 50% of cases are idiopathic (primary); the remainder arise secondarily in the setting of a predisposing condition (see Table 13-4) or drug exposure. Most causative antibodies are of the immunoglobulin G (IgG) class; only sometimes are IgA antibodies culpable. Most red cell destruction in this form of hemolytic disease is extravascular. IgG-coated red cells bind Fc receptors on monocytes and splenic macrophages, which results in loss of red cell membrane during "partial" phagocytosis. As in hereditary spherocytosis, *the loss of cell membrane converts the red cells to spherocytes, which are sequestered and removed in the spleen, the major site of red cell destruction in this disorder. Thus, moderate splenomegaly is characteristic of this form of anemia.*

As with other forms of autoimmunity, the cause of autoantibody formation is largely unknown. In many cases, the antibodies are directed against the Rh blood group antigens. The mechanisms of drug-induced hemolysis are better understood. Two predominant immunologic mechanisms have been implicated.[31]

- *Hapten model.* The drugs—exemplified by penicillin and cephalosporins—act as haptens by binding to the red cell membrane. Antibodies directed against the cell-bound drug result in the destructive sequence cited before. This form of hemolytic anemia is usually caused by large intravenous doses of the antibiotic and occurs 1 to 2 weeks after onset of therapy. Sometimes the antibodies bind only to the offending drug, as in penicillin-induced hemolytic anemia. In other cases, such as quinidine-induced hemolysis, the antibodies recognize a complex of the drug and a membrane protein. In drug-induced hemolytic anemias, the destruction of red cells can occur intravascularly after fixation of complement or extravascularly in the mononuclear phagocyte system.
- *Autoantibody model.* These drugs, of which the antihypertensive agent α-methyldopa is the prototype, in some manner initiate the production of antibodies directed against intrinsic red cell antigens, in particular the Rh blood group antigens. Approximately 10% of patients taking α-methyldopa develop autoantibodies, as assessed by the direct Coombs test. However, only 1% develops clinically significant hemolysis.

Cold Agglutinin Immunohemolytic Anemia.

This form of immunohemolytic anemia is caused by so-called *cold agglutinins,* IgM antibodies that bind and agglutinate red cells avidly at low temperatures (0° to 4°C).[32] It is less

common than warm antibody immunohemolytic anemia, accounting for 16% to 32% of cases of immunohemolytic anemia. Such antibodies appear *acutely* during the recovery phase of certain infectious disorders, such as mycoplasma pneumonia and infectious mononucleosis. In these settings, the disorder is self-limited and rarely induces clinical manifestations of hemolysis. Other infectious agents associated with this form of anemia include cytomegalovirus, influenza virus, and human immunodeficiency virus (HIV). *Chronic* cold agglutinin immunohemolytic anemias occur in association with certain lymphoid neoplasms or as an idiopathic condition. Clinical symptoms result from binding of IgM to red cells at sites such as exposed fingers, toes, and ears where the temperature is below 30°C. IgM binding agglutinates red cells and rapidly fixes complement on their surface. As the blood recirculates and warms, IgM is rapidly released, usually before complement-mediated hemolysis can occur. However, the transient interaction with IgM is sufficient to deposit sublytic quantities of C3b, an excellent opsonin, leading to rapid removal of affected red cells by mononuclear phagocytes in the liver and spleen. The hemolysis is of variable severity. Vascular obstruction caused by red cell agglutinates results in pallor, cyanosis of the body parts exposed to cold temperatures, and Raynaud phenomenon (Chapter 11).

Cold Hemolysin Hemolytic Anemia.

Cold hemolysins are autoantibodies responsible for an unusual entity known as *paroxysmal cold hemoglobinuria,* characterized by acute intermittent massive intravascular hemolysis, frequently with hemoglobinuria, after exposure to cold temperatures. This is the least common form of immunohemolytic anemia. Lysis is clearly complement dependent. The autoantibodies are IgGs that bind to the P blood group antigen on the red cell surface at low temperatures. Complement-mediated intravascular lysis does not occur until the cells recirculate to warm central regions, as the enzymes of the complement cascade function more efficiently at 37°C. The antibody, also known as the Donath-Landsteiner antibody, was first recognized in association with syphilis. Today, most cases of paroxysmal cold hemoglobinuria follow infections such as mycoplasma pneumonia,

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measles, mumps, and ill-defined viral and "flu" syndromes. The mechanisms responsible for production of such autoantibodies in these settings are unknown.

Hemolytic Anemia Resulting from Trauma to Red Cells

Red blood cells can be disrupted by physical trauma in a variety of circumstances. Of these, *hemolytic anemias caused by cardiac valve prostheses, or narrowing or obstruction of the microvasculature, are most important clinically*. Severe traumatic hemolytic anemia is more frequently associated with artificial mechanical valves than bioprosthetic porcine valves. Hemolysis in both instances stems from shear stresses produced by turbulent blood flow and abnormal pressure gradients. Microangiopathic hemolytic anemia, on the other hand, occurs when red cells are forced to squeeze through abnormally narrowed small vessels. Narrowing is most often caused by fibrin deposition in association with disseminated intravascular coagulation (discussed later in this chapter). Other causes of microangiopathic hemolytic anemia include malignant hypertension, systemic lupus erythematosus, thrombotic thrombocytopenic purpura (TTP), hemolytic-uremic syndrome (HUS), and disseminated cancer, most of which are discussed elsewhere in this book. The common feature among all these disorders is a microvascular lesion that causes mechanical injury to circulating red cells. This damage is evident in peripheral blood smears in the form of red cell fragments (schistocytes), "burr cells," "helmet cells," and "triangle cells" (Fig. 13-17). Except for TTP and HUS, hemolysis is not a major clinical problem in most instances.

Figure 13-17 Microangiopathic hemolytic anemia. A peripheral blood smear from a patient with hemolytic-uremic syndrome shows several fragmented red cells. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

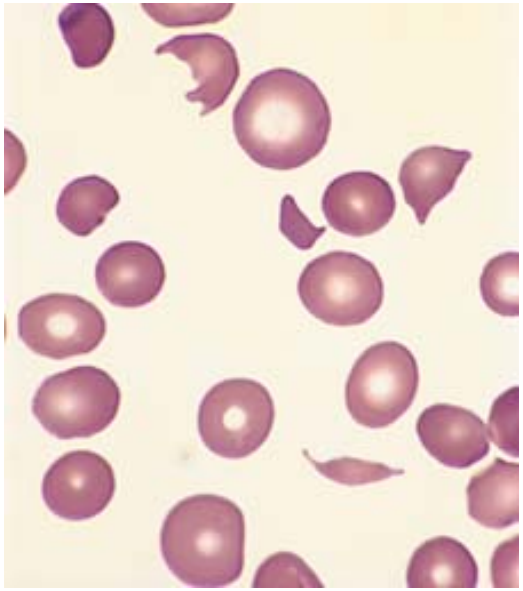


Figure 13-18 Megaloblastic anemia. A peripheral blood smear shows a hypersegmented neutrophil with a six-lobed nucleus. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

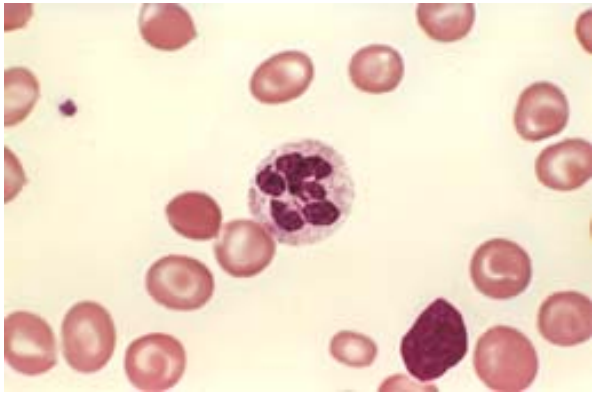


Figure 13-19 Megaloblastic anemia (bone marrow aspirate). *A* to *C*, Megaloblasts in various stages of differentiation. Note that the orthochromatic megaloblast *(B)* is hemoglobinized (as revealed by cytoplasmic color), but in contrast to normal orthochromatic normoblasts, the nucleus is not pyknotic. The granulocytic precursors are also large and have abnormally "immature" chromatin. *(Courtesy of Dr. Jose Hernandez, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

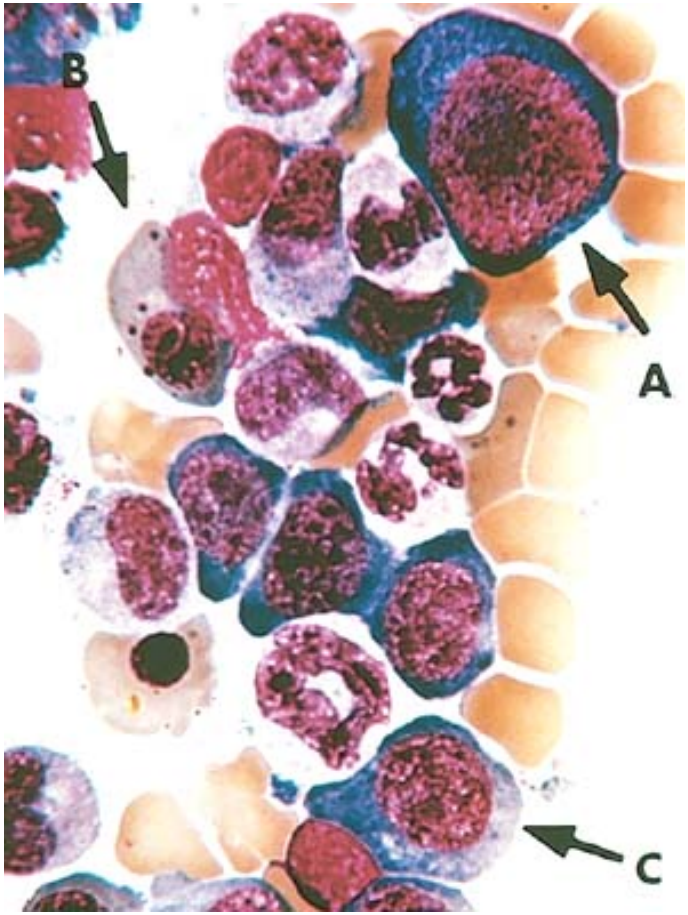


TABLE 13-5 -- Causes of Megaloblastic Anemia

Vitamin B_{12} Deficiency
Decreased intake
••Inadequate diet, vegetarianism
Impaired absorption
••Intrinsic factor deficiency
••••Pernicious anemia
••••Gastrectomy
••Malabsorption states
••Diffuse intestinal disease, e.g., lymphoma, systemic sclerosis
••Ileal resection, ileitis

••Competitive parasitic uptake
••••Fish tapeworm infestation
••Bacterial overgrowth in blind loops and diverticula of bowel
Increased requirement
••••Pregnancy, hyperthyroidism, disseminated cancer
Folic Acid Deficiency
Decreased intake
••Inadequate diet—alcoholism, infancy
Impaired absorption
••Malabsorption states
••Intrinsic intestinal disease
••Anticonvulsants, oral contraceptives
Increased loss
••Hemodialysis
Increased requirement
••Pregnancy, infancy, disseminated cancer, markedly increased hematopoiesis
Impaired use
••Folic acid antagonists
Unresponsive to Vitamin B_{12} or Folic Acid Therapy
Metabolic inhibitors of DNA synthesis and/or folate metabolism, e.g., methotrexate
Modified from Beck WS: Megaloblastic anemias. In Wyngaarden JB, Smith LH (eds): Cecil Textbook of Medicine, 18th ed. Philadelphia, WB Saunders, 1988, p. 900.

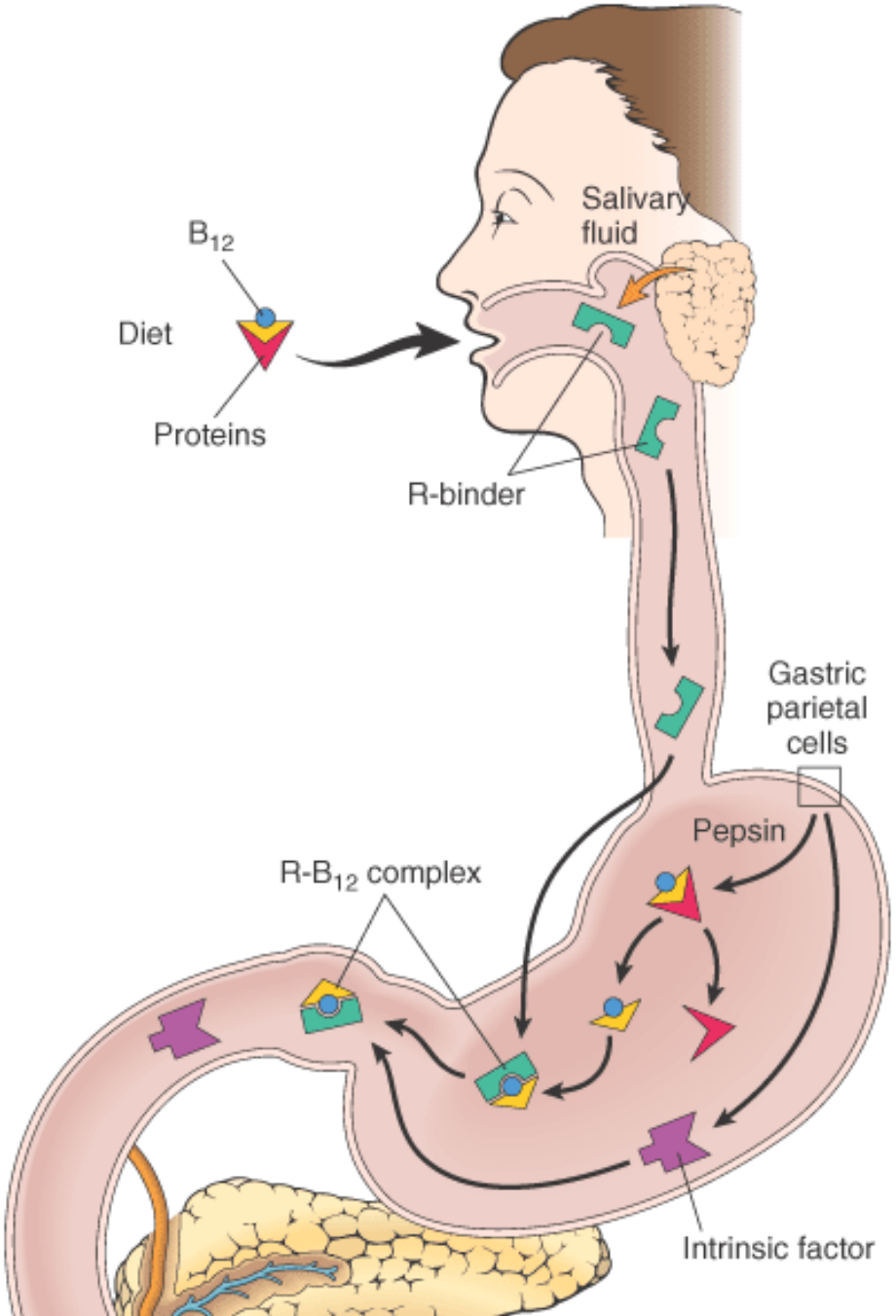
marrow and mucosal lining of the gastrointestinal tract. In addition to the intrinsic-factor dependent pathway, evidence also supports the existence of an alternative mechanism that is not dependent on availability of intrinsic factor or intact terminal ileum. The mechanism involved is not entirely clear but up to 1% of a large oral dose can be absorbed by this pathway, thus making it feasible to treat pernicious anemia by oral vitamin B_{12} therapy.[33]

Etiology of Vitamin B_{12} Deficiency.

With this background, we can consider the various causes of vitamin B_{12} deficiency (see Table 13-5). Inadequate diet is obvious but must be present for many years to deplete reserves. The absorption of vitamin B_{12} can be impaired by disruption of any one of the steps outlined earlier. With achlorhydria and loss of pepsin secretion (which occurs in some elderly individuals), vitamin B_{12} is not readily released from proteins in food. With gastrectomy and pernicious anemia, intrinsic factor is not available for transport to the ileum. With loss of exocrine pancreatic function, vitamin B_{12} cannot be released from R-binder-vitamin B_{12} complexes. Ileal resection or diffuse ileal disease can remove or damage the site of intrinsic factor-

vitamin B_{12} complex absorption. Tapeworm infestation, by competing for the nutrient, can induce a deficiency state. Under some circumstances, for example, pregnancy, hyperthyroidism, disseminated cancer, and chronic infections,

Figure 13-20 Schematic illustration of vitamin B_{12} absorption.



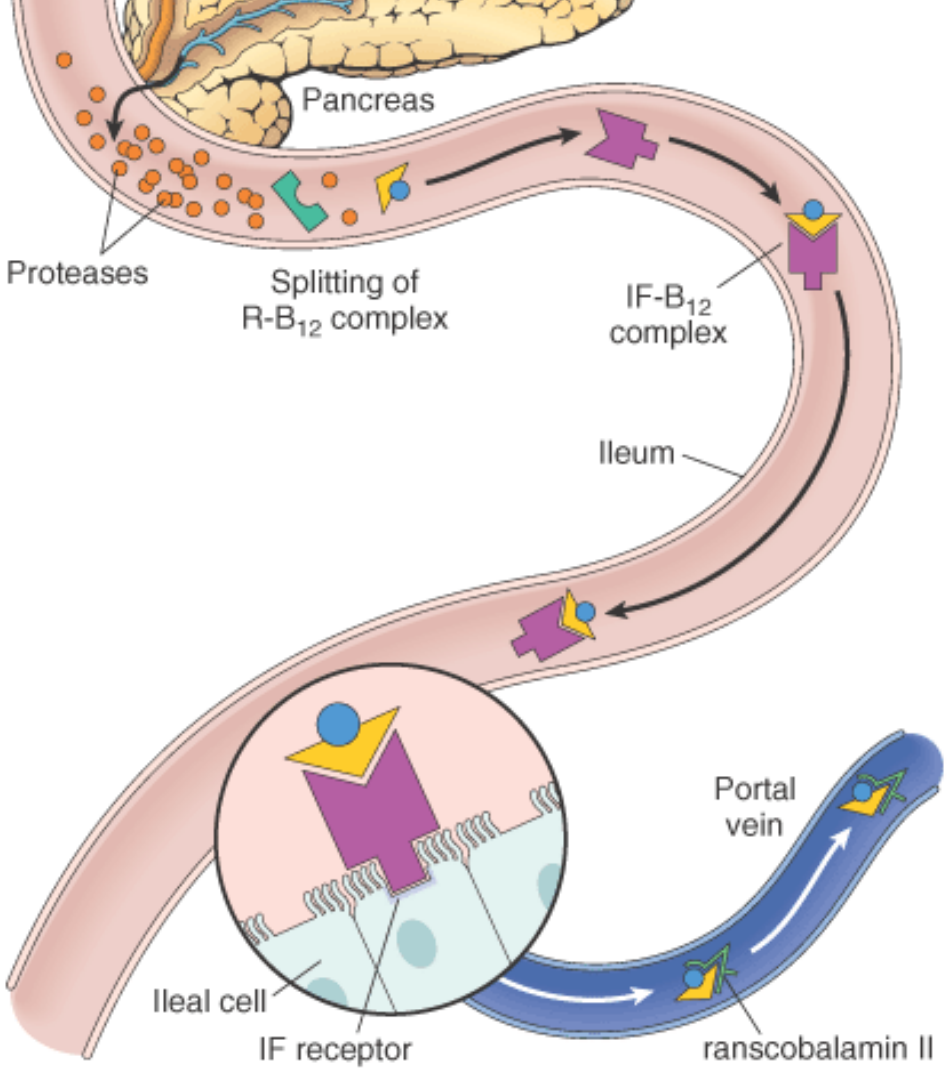


Figure 13-21 Relationship of N^5 -methyl FH_4 , methionine synthase, and thymidylate synthetase. In cobalamin deficiency, folate is sequestered as N^5 -methyl FH_4 . This ultimately deprives thymidylate synthetase of its folate coenzyme ($N^{5,10}$ -methylene FH_4), thereby impairing DNA synthesis.

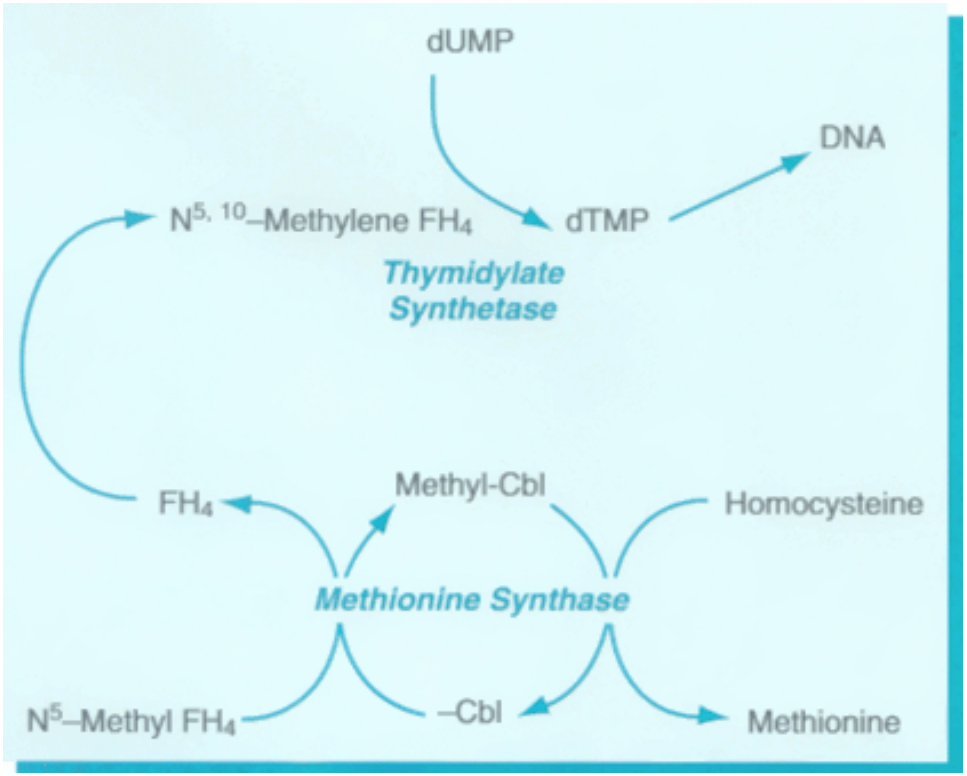


Figure 13-22 Role of folate derivatives in the transfer of one-carbon fragments for synthesis of biologic macromolecules. FH_4 , tetrahydrofolic acid; FH_2 , dihydrofolic acid; FIGlu, formiminoglutamate; dTMP, deoxythymidylate monophosphate. *Synthesis of methionine also requires vitamin B_{12} .

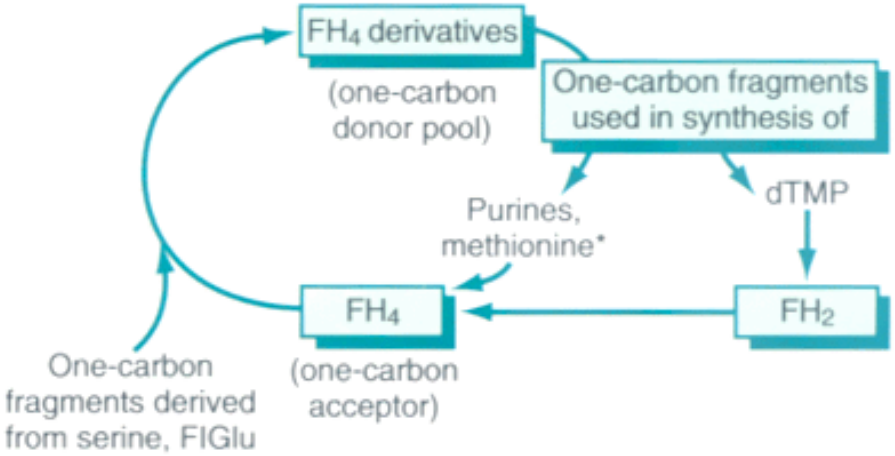


TABLE 13-6 -- Iron Distribution in Healthy Young Adults (mg)

Pool	Men	Women

Total	3450	2450
Functional		
••Hemoglobin	2100	1750
••Myoglobin	300	250
••Enzymes	50	50
Storage		
••Ferritin, hemosiderin	1000	400

Free iron is highly toxic, and the pool of storage iron is tightly bound to either ferritin or hemosiderin.[40] *Ferritin is a protein-iron complex* found in all tissues but particularly in liver, spleen, bone marrow, and skeletal muscles. In the liver, most ferritin is stored within the parenchymal cells; in other tissues, such as spleen and bone marrow, it is mainly in the mononuclear phagocytic cells. Hepatocytic iron is derived from plasma transferrin, whereas storage iron in the mononuclear phagocytic cells (Kupffer cells) is derived from the breakdown of red cells (Fig. 13-23). Intracellular ferritin is located in both the cytosol and lysosomes, in which partially degraded protein shells of ferritin aggregate into *hemosiderin* granules. With a hematoxylin and eosin stain, hemosiderin appears in cells as golden yellow granules. The iron in hemosiderin is chemically reactive and turns blue-black when exposed to potassium ferrocyanide, which is the basis for the Prussian blue stain. With normal iron stores, only trace amounts of hemosiderin are found in the body, principally in mononuclear phagocytic cells in the bone marrow, spleen, and liver. In iron-overloaded cells, most iron is stored in hemosiderin.

Very small amounts of ferritin normally circulate in the plasma. *Since plasma ferritin is derived largely from the storage pool of body iron, its levels correlate well with body iron stores.* In iron deficiency, serum ferritin is always below 12 µg/L, whereas in iron overload, high values approaching 5000 µg/L can be seen. Of physiologic importance, the storage iron pool can be readily mobilized if iron requirements increase, as may occur after loss of blood.

Iron is transported in plasma by an iron-binding glycoprotein called *transferrin* (see Fig. 13-23), which is synthesized in the liver. In normal individuals, transferrin is about 33% saturated with iron, yielding serum iron levels that average 120 µg/dL in men and 100 µg/dL in women. Thus, the total

Figure 13-23 The internal iron cycle. Plasma iron bound to transferrin is transported to the marrow, where it is transferred to developing red cells and incorporated into hemoglobin. Mature red blood cells are released into the circulation and, after 120 days, are ingested by macrophages in the reticuloendothelial system (RES). Here iron is extracted from hemoglobin and returned to the plasma, completing the cycle. *(From Wyngaarden JB, et al [eds]: Cecil Textbook of Medicine, 19th ed. Philadelphia, WB Saunders, 1992, p. 841.)*

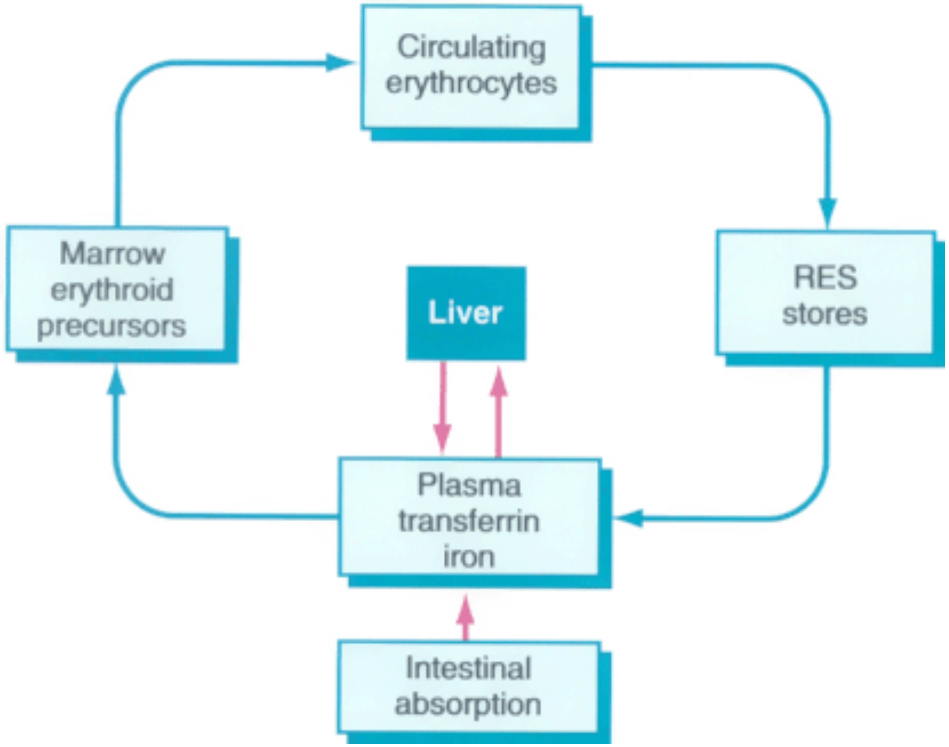


Figure 13-24 Diagrammatic representation of iron absorption. Mucosal uptake of heme and nonheme iron is depicted. When the storage sites of the body are replete with iron and erythropoietic activity is normal, most of the absorbed iron is lost into the gut by shedding of the epithelial cells. Conversely, when body iron needs increase or when erythropoiesis is stimulated, a greater fraction of the absorbed iron is transferred into plasma transferrin, with a concomitant decrease in iron loss through mucosal ferritin.

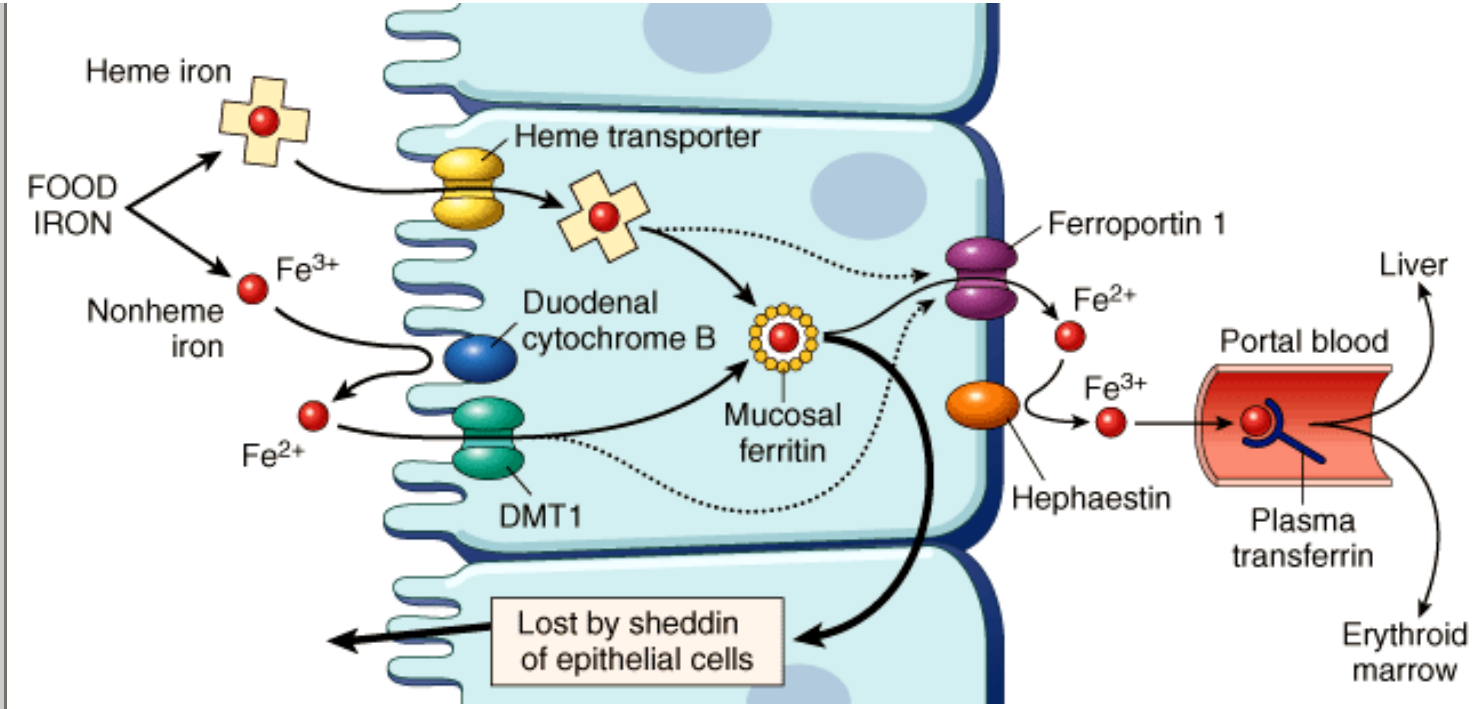


Figure 13-25 Hypochromic microcytic anemia of iron deficiency (peripheral blood smear). Note the small red cells containing a narrow rim of peripheral hemoglobin. Scattered fully hemoglobinized cells, present due to recent blood transfusion, stand in contrast. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

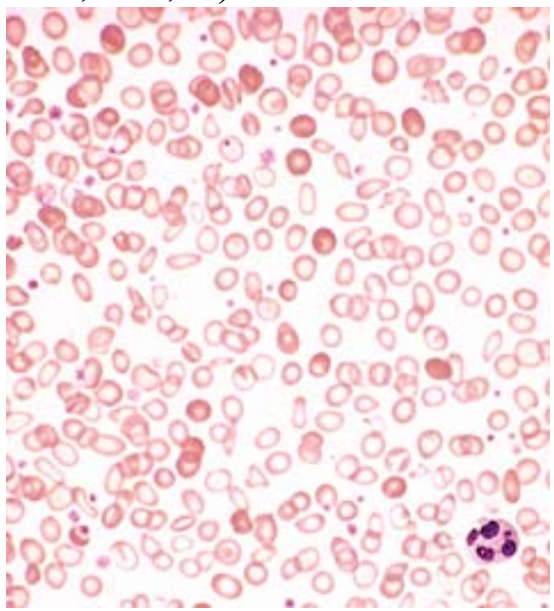


TABLE 13-7 -- Major Causes of Aplastic Anemia

Acquired
Idiopathic
••Primary stem cell defect
••Immune mediated
Chemical agents
••Dose related
••••Alkylating agents
••••Antimetabolites
••••Benzene
••••Chloramphenicol
••••Inorganic arsenicals
••Idiosyncratic
••••Chloramphenicol
••••Phenylbutazone
••••Organic arsenicals
••••Methylphenylethylhydantoin
••••Streptomycin
••••Chlorpromazine
••••Insecticides (e.g., DDT, parathion)
Physical agents (e.g., whole-body irradiation)
Viral infections
••Hepatitis (unknown virus)
••Cytomegalovirus infections
••Epstein-Barr virus infections
••Herpes varicella-zoster
Miscellaneous
••Infrequently, many other drugs and chemicals
Inherited
Fanconi anemia

required for DNA repair[45] (Chapter 7). Marrow hypofunction in Fanconi anemia becomes evident early in life and is accompanied by multiple congenital anomalies, such as hypoplasia of the kidney and spleen and hypoplastic anomalies of bone, often involving the thumbs or radii.

Despite all these possible causes, no provocative factor can be identified in fully 65% of the cases, which are lumped into the *idiopathic* category.

Pathogenesis.

The pathogenesis of aplastic anemia is not fully understood. Indeed, it is unlikely that a single mechanism underlies all cases. Two major etiologies have been invoked: an immunologically mediated suppression and an intrinsic abnormality of stem cells (Fig. 13-26).

Recent studies suggest that aplastic anemia results most commonly from suppression of stem cell function by activated T cells.[46] It is postulated that stem cells are first antigenically altered by exposure to drugs, infectious agents, or other unidentified environmental insults. This evokes a cellular immune response, during which activated T cells produce cytokines such as interferon-γ and TNF that prevent normal stem cell growth and development. This scenario is supported by several observations. Immunosuppressive therapy with antithymocyte globulin combined with drugs such as cyclosporine produces responses in 60% to 70% of patients, and successful bone marrow transplantation requires "conditioning" with high doses of myelotoxic drugs or radiation. In both instances, it is hypothesized these therapies work by suppressing or killing autoreactive T-cell clones. The target antigens for T-cell attack are not well defined. In some instances GPI-linked proteins may be the targets of sensitized T cells,

Figure 13-26 Pathophysiology of aplastic anemia. Damaged stem cells can produce progeny expressing neo-antigens that evoke an autoimmune reaction, or give rise to a clonal population with reduced proliferative capacity. Either pathway could lead to marrow aplasia.

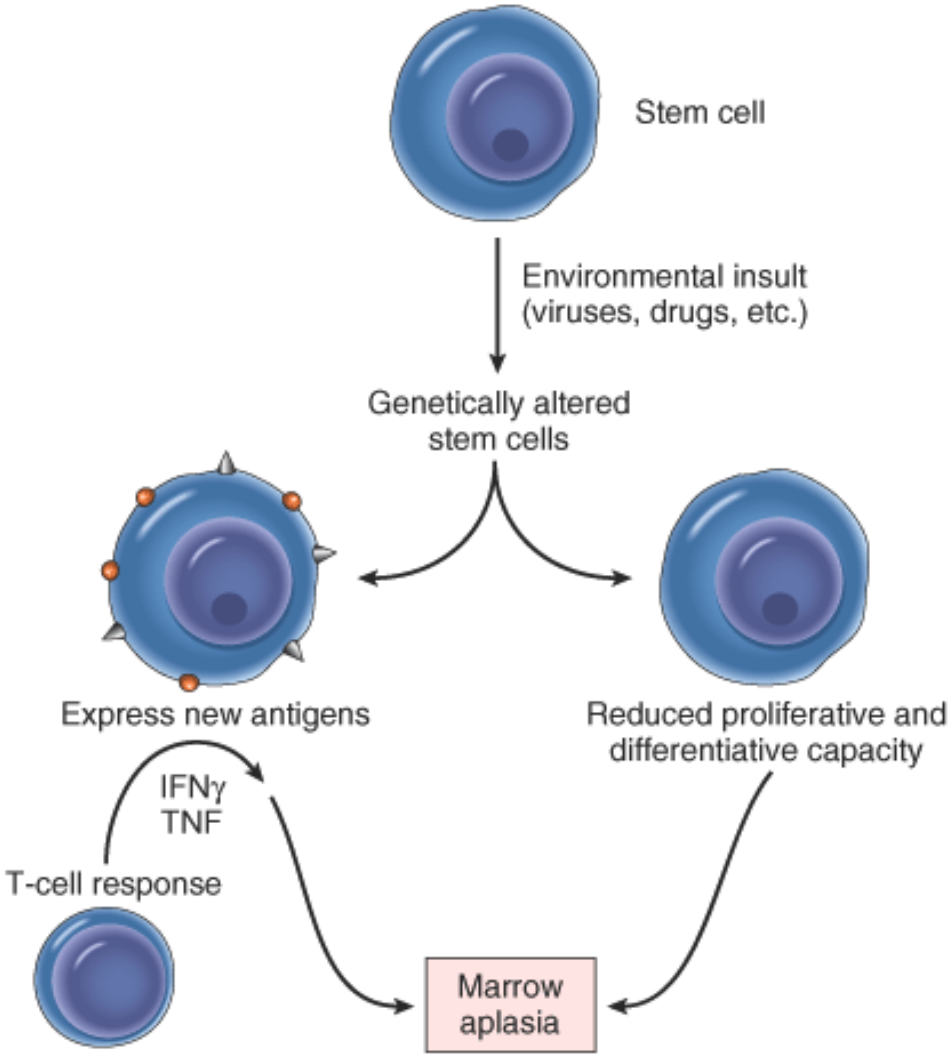


Figure 13-27 Aplastic anemia (bone marrow biopsy). Markedly hypocellular marrow contains mainly fat cells. *A*, Low power. *B*, High power. *(Courtesy of Dr. Steven Kroft, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

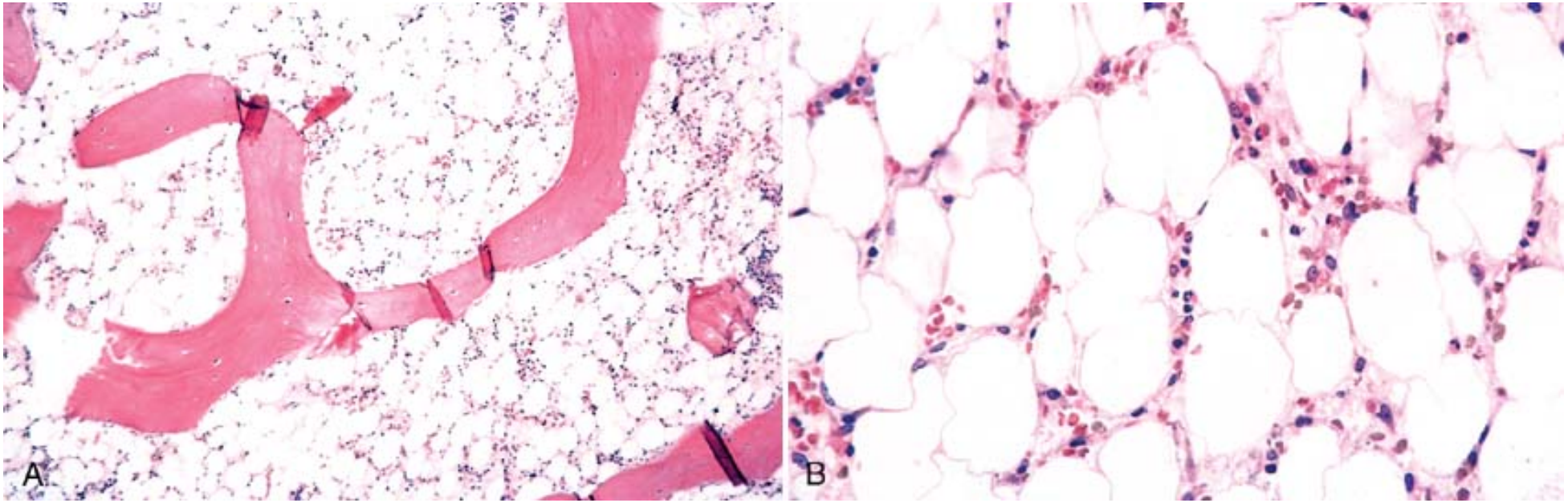


TABLE 13-8 -- Pathophysiologic Classification of Polycythemia

Relative	Reduced plasma volume (hemoconcentration)
Absolute	
Primary	Polycythemia vera, rare erythropoietin receptor mutations (low erythropoietin)
Secondary	High erythropoietin
	Appropriate: lung disease, high-altitude living, cyanotic heart disease
	Inappropriate: erythropoietin-secreting tumors (e.g., renal cell carcinoma, hepatocellular carcinoma, cerebellar hemangioblastoma)

More specialized tests are available to measure the levels of specific clotting factors, fibrinogen, fibrin split products, the presence of circulating anticoagulants, and platelet function. With this overview, we can turn to the various categories of bleeding disorders.

BLEEDING DISORDERS CAUSED BY VESSEL WALL ABNORMALITIES

Disorders within this category, sometimes called *nonthrombocytopenic purpuras,* are relatively common but do not usually cause serious bleeding problems. Most often, they induce small hemorrhages (petechiae and purpura) in the skin or mucous membranes, particularly the gingivae. On occasion, however, more significant hemorrhages can occur into joints, muscles, and subperiosteal locations or take the form of menorrhagia, nosebleeds, gastrointestinal bleeding, or hematuria. *The platelet count, bleeding time, and results of the coagulation tests (PT, PTT) are usually normal.*

The varied clinical conditions in which hemorrhages can be related to abnormalities in the vessel wall include the following:

- Many *infections* induce petechial and purpuric hemorrhages, but especially implicated are meningococcemia, other forms of septicemia, infective endocarditis, and several of the

rickettsioses. The involved mechanism is presumably microbial damage to the microvasculature (vasculitis) or disseminated intravascular coagulation (DIC). Failure to recognize meningococcemia as a cause of petechiae and purpura can be catastrophic for the patient.

• *Drug reactions* sometimes induce cutaneous petechiae and purpura without causing thrombocytopenia. In many instances, the vascular injury is mediated by drug-induced antibodies and deposition of immune complexes in the vessel walls, leading to hypersensitivity (leukocytoclastic) vasculitis (Chapter 11).

• *Scurvy and the Ehlers-Danlos syndrome* are associated with microvascular bleeding resulting from impaired formation of collagens needed for support of vessel walls. The same mechanism may account for spontaneous purpura commonly seen in the very elderly. The predisposition to skin hemorrhages in *Cushing syndrome,* in which the protein-wasting effects of excessive corticosteroid production cause loss of perivascular supporting tissue, has a similar etiology.

• *Henoch-Schönlein purpura* is a systemic hypersensitivity disease of unknown cause characterized by a purpuric rash, colicky abdominal pain (presumably due to focal hemorrhages into the gastrointestinal tract), polyarthralgia, and acute glomerulonephritis (Chapter 20). All these changes result from the deposition of circulating immune complexes within vessels throughout the body and within the glomerular mesangial regions.

• *Hereditary hemorrhagic telangiectasia* is an autosomal dominant disorder characterized by dilated, tortuous blood vessels with thin walls that bleed readily. Bleeding can occur anywhere in the body but is most common under the mucous membranes of the nose (epistaxis), tongue, mouth, and eyes and throughout the gastrointestinal tract.

• *Amyloid infiltration of blood vessels.* Systemic amyloidosis is associated with perivascular deposition of amyloid and consequent weakening of blood vessel wall. This is most commonly observed in plasma cell dyscrasias (Chapter 14) and is manifested as mucocutaneous petechiae.

Bleeding in these conditions is rarely life threatening with the exception of some cases of hereditary telangiectasia. Recognition of the presenting symptoms should prompt further studies to establish a specific diagnosis.

BLEEDING RELATED TO REDUCED PLATELET NUMBER: THROMBOCYTOPENIA

Reduction in platelet number constitutes an important cause of generalized bleeding. Normal platelet counts range from 150,000 to 300,000/μL. A count below 100,000/μL is generally considered to constitute thrombocytopenia. However, spontaneous bleeding does not become evident until the count falls below 20,000/μL. Platelet counts in the range of 20,000 to 50,000/μL can aggravate post-traumatic bleeding. Bleeding resulting from thrombocytopenia alone is associated with a prolonged bleeding time and normal PT and PTT.

The important role of platelets in hemostasis is discussed in Chapter 4 . It hardly needs reiteration that these cells are critical for hemostasis, as they form temporary plugs that quickly stop bleeding and promote key reactions in the clotting cascade. Spontaneous bleeding associated with thrombocytopenia most often involves small vessels. The common sites of such hemorrhage are the skin and the mucous membranes of the gastrointestinal and genitourinary tracts. Intracranial bleeding is a threat to any patient with a markedly depressed platelet count.

The many causes of thrombocytopenia can be classified into the four major categories listed in Table 13-9 .

• *Decreased production of platelets.* This can accompany generalized diseases of bone marrow such as aplastic anemia and leukemias or result from diseases that affect the megakaryocytes somewhat selectively. In vitamin B_{12} or folic acid deficiency, there is poor development and accelerated destruction of megakaryocytes within the bone marrow (ineffective megakaryopoiesis) because DNA synthesis is impaired.

• *Decreased platelet survival.* This important cause of thrombocytopenia can have an *immunologic* or *nonimmunologic* etiology. In the immune conditions, platelet destruction is caused by circulating antiplatelet antibodies or, less often, immune complexes. The antiplatelet antibodies can be directed against a self-antigen on the platelets (autoantibodies) or against platelet antigens that differ among different individuals (alloantibodies). Common antigenic targets of both autoantibodies and alloantibodies are the platelet membrane glycoprotein complexes IIb-IIIa and Ib-IX. Autoimmune thrombocytopenias include idiopathic thrombocytopenic purpura, certain drug-induced thrombocytopenias, and HIV-associated thrombocytopenias. All of these are discussed later. Alloimmune thrombocytopenias arise when an individual is exposed to platelets

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of another person, as may occur after blood transfusion or during pregnancy. In the latter case, neonatal or even fetal thrombocytopenia occurs by a mechanism analogous to

erythroblastosis fetalis.[52]

Nonimmunologic destruction of platelets may be caused by *mechanical injury*,in a manner analogous to red cell destruction in microangiopathic hemolytic anemia. The underlying conditions are also similar, including prosthetic heart valves and diffuse narrowing of the microvessels (e.g., malignant hypertension).

• *Sequestration.* Thrombocytopenia, usually moderate in severity, may develop in any patient with marked splenomegaly, a condition sometimes referred to as *hypersplenism* (Chapter 14). The spleen normally sequesters 30% to 40% of the body's platelets, which remain in equilibrium with the circulating pool. When necessary, hypersplenic thrombocytopenia can be ameliorated by splenectomy.

• *Dilutional.* Massive *transfusions* can produce a dilutional thrombocytopenia. Blood stored for longer than 24 hours contains virtually no viable platelets; thus, plasma volume and red cell mass are reconstituted by transfusion, but the number of circulating platelets is relatively reduced.

TABLE 13-9 -- Causes of Thrombocytopenia

Decreased production of platelets
Generalized diseases of bone marrow
••Aplastic anemia: congenital and acquired (see Table 13-7)
••Marrow infiltration: leukemia, disseminated cancer
Selective impairment of platelet production
••Drug-induced: alcohol, thiazides, cytotoxic drugs
••Infections: measles, human immunodeficiency virus (HIV)
Ineffective megakaryopoiesis
••Megaloblastic anemia
••Myelodysplastic syndromes
Decreased platelet survival
Immunologic destruction
••Autoimmune: idiopathic thrombocytopenic purpura, systemic lupus erythematosus
••Isoimmune: post-transfusion and neonatal
••Drug-associated: quinidine, heparin, sulfa compounds
••Infections: infectious mononucleosis, HIV infection, cytomegalovirus
Nonimmunologic destruction
••Disseminated intravascular coagulation
••Thrombotic thrombocytopenic purpura
••Giant hemangiomas
••Microangiopathic hemolytic anemias
Sequestration

Hypersplenism
Dilutional

Immune Thrombocytopenic Purpura (ITP)

ITP can occur in the setting of a variety of conditions and exposures (secondary ITP) or in the absence of any known risk factors (primary or idiopathic ITP). There are two clinical subtypes of primary ITP, acute and chronic; both are autoimmune disorders in which platelet destruction results from the formation of antiplatelet autoantibodies. We first discuss the more common chronic form of primary ITP; acute ITP, a self-limited disease of children, is discussed later.

Immunologically mediated destruction of platelets (immune thrombocytopenia) occurs in many different settings, including systemic lupus erythematosus, acquired immunodeficiency syndrome (AIDS), after viral infections, and as a complication of drug therapy. These *secondary forms of immune thrombocytopenia can sometimes mimic the idiopathic autoimmune variety*, and hence the diagnosis of this disorder should be made only after exclusion of other known causes of thrombocytopenia. Particularly important in this regard is systemic lupus erythematosus, a multisystem autoimmune disease (Chapter 6) that can present with thrombocytopenia.

Pathogenesis.

Chronic ITP is caused by the formation of autoantibodies against platelet membrane glycoproteins, most often IIb-IIIa or Ib-IX.[53] Antibodies reactive with these membrane glycoproteins can be demonstrated in the plasma as well as bound to the platelet surface (platelet-associated immunoglobulins) in approximately 80% of patients. In the overwhelming majority of cases, the antiplatelet antibodies are of the IgG class.

The mechanism of platelet destruction is similar to that seen in autoimmune hemolytic anemias. Opsonized platelets are rendered susceptible to phagocytosis by the cells of the mononuclear phagocyte system. About 75% to 80% of patients are remarkably improved after splenectomy, indicating that the spleen is the major site of removal of sensitized platelets. Since it is also an important site of autoantibody synthesis, the beneficial effects of splenectomy may in part derive from removal of the source of autoantibodies. Although destruction of sensitized platelets is the major mechanism responsible for thrombocytopenia, there is some evidence that megakaryocytes may be damaged by autoantibodies, leading to impairment of platelet production. In most cases, however, megakaryocyte injury is not significant enough to deplete their numbers.

Morphology.

The principal morphologic lesions of thrombocytopenic purpura are found in the spleen and bone marrow but they are not diagnostic. Secondary changes related to the bleeding diathesis may be found in any tissue or structure in the body.

The spleen is normal in size. On histologic examination, there is congestion of the sinusoids and hyperactivity and enlargement of the splenic follicles, manifested by the formation of prominent germinal centers. In many instances, scattered megakaryocytes are found within the sinuses and sinusoidal walls. This may represent a very mild form of extramedullary hematopoiesis driven by elevated levels of thrombopoietin. These splenic findings are not sufficiently distinctive to be considered diagnostic.

Bone marrow reveals a modestly increased number of megakaryocytes. Some are apparently immature, with large, nonlobulated, single nuclei. These findings are not specific for autoimmune thrombocytopenic purpura but merely reflect accelerated thrombopoiesis, being found in most forms of thrombocytopenia resulting from increased platelet destruction. The importance of bone marrow examination is to rule out thrombocytopenias resulting from bone marrow failure. A decrease in the number of megakaryocytes argues against the diagnosis of ITP. The secondary changes relate to the hemorrhages that are dispersed throughout the body.

Clinical Features.

Chronic ITP occurs most commonly in adult women younger than age 40 years. The female-to-male ratio is 3:1. This disorder is often insidious in onset and is characterized by bleeding into the skin and mucosal surfaces. Cutaneous bleeding is seen in the form of *pinpoint hemorrhages* (petechiae), especially prominent in the dependent areas where the capillary pressure is higher. Petechiae can become confluent, giving rise to *ecchymoses*. Often there is a history of easy bruising, nosebleeds, bleeding from the gums, and hemorrhages into soft tissues from relatively minor trauma. The disease may manifest first with melena, hematuria, or excessive menstrual flow. Subarachnoid hemorrhage and intracerebral hemorrhage are serious consequences of thrombocytopenic purpura but, fortunately, are rare in treated patients. Splenomegaly and lymphadenopathy are uncommon in primary ITP, and their presence should lead one to consider other possible diagnoses.

The clinical signs and symptoms associated with ITP are not specific for this condition but rather reflective of thrombocytopenia. Destruction of platelets as the cause of thrombocytopenia is supported by the findings of a low platelet count and normal or increased megakaryocytes in the bone marrow. Accelerated thrombopoiesis often leads to the formation of abnormally large platelets (megathrombocytes), detected easily in a blood smear. The bleeding time is prolonged, but PT and PTT are normal. Tests for platelet autoantibodies are not widely available. *Therefore, a diagnosis of ITP should be made only after other causes of platelet deficiencies, such as those listed in Table 13-9 , have been ruled out.*

Almost all patients respond to immunosuppressive doses of glucocorticoids, but many eventually relapse and come to splenectomy. Most maintain safe platelet counts postsplenectomy and require no further therapy. A significant minority, however, have refractory forms of ITP that can be very difficult to treat. Various immunosuppressive approaches may be effective in such patients.

Acute Immune Thrombocytopenic Purpura

Like chronic ITP, this condition is caused by antiplatelet autoantibodies, but its clinical features and course are distinct. Acute ITP is a disease of childhood occurring with equal frequency in both sexes. The onset of thrombocytopenia is abrupt and is preceded in many cases by a viral illness. The usual interval between the infection and onset of purpura is 2 weeks. Unlike the adult chronic form of ITP, the childhood disease is self-limited, usually resolving spontaneously within 6 months. Steroid therapy is indicated only if thrombocytopenia is severe. Approximately 20% of the children, usually those without a viral prodrome, have persistent low platelet counts beyond 6 months and appear to have chronic ITP similar in most respects to the adult disease.

Drug-Induced Thrombocytopenia: Heparin-Induced Thrombocytopenia

Like hemolytic anemia, thrombocytopenia can result from immunologically mediated destruction of platelets after drug ingestion.[54] The drugs most commonly involved are quinine, quinidine, sulfonamide antibiotics, and heparin. Heparin-induced thrombocytopenia (HIT) is of particular importance because this anticoagulant is used widely and failure to make a correct diagnosis can have severe consequences. Thrombocytopenia occurs in approximately 5% of patients receiving heparin. Most develop so-called type I thrombocytopenia, which occurs rapidly after onset of therapy, is modest in severity and clinically insignificant, and may resolve despite continuation of heparin therapy. It most likely results from a direct platelet-aggregating effect of heparin.

Type II thrombocytopenia is more severe. It occurs 5 to 14 days after commencement of therapy (or sometimes sooner if the patient has been previously sensitized to heparin) and can, paradoxically, lead to life-threatening venous and arterial thrombosis.[54] HIT is caused by an immune reaction directed against a complex of heparin and platelet factor 4, a normal component of platelet granules that binds tightly to heparin. It appears that heparin binding modifies the conformation of platelet factor 4, making it susceptible to immune recognition. [55] *Binding of antibody to platelet factor 4 produces immune complexes that activate platelets, promoting thrombosis even in the setting of marked thrombocytopenia.* The mechanism of platelet activation is not understood. Unless therapy is immediately discontinued, clots within large arteries may lead to vascular insufficiency and limb loss, and emboli from deep venous thrombosis can cause fatal pulmonary thromboembolism.

HIV-Associated Thrombocytopenia

Thrombocytopenia is perhaps the most common hematologic manifestation of HIV infection. Both impaired platelet production and increased destruction are responsible. CD4, the receptor for HIV on T cells, has also been demonstrated on megakaryocytes, making it possible for these cells to be infected by HIV.[56] Infected megakaryocytes are prone to apoptosis and are impaired in terms of platelet production. HIV infection also causes hyperplasia and dysregulation of B cells, which predispose to the development of immune-mediated thrombocytopenia. Antibodies directed against platelet membrane glycoprotein IIb-III complexes are detected in some patients' sera. These autoantibodies, which sometimes cross-react with HIV-associated gp120, are believed to act as opsonins, thus promoting the phagocytosis of platelets by splenic phagocytes. Some studies also implicate nonspecific deposition of immune complexes on platelets as a factor in their premature destruction by the mononuclear phagocyte system.

Thrombotic Microangiopathies: Thrombotic Thrombocytopenic Purpura (TTP) and Hemolytic-Uremic Syndrome (HUS)

The term *thrombotic microangiopathy* encompasses a spectrum of clinical syndromes that includes TTP and HUS. TTP, as originally defined, is associated with the pentad of fever, thrombocytopenia, microangiopathic hemolytic anemia, transient neurologic deficits, and renal failure. HUS is also associated with microangiopathic hemolytic anemia and thrombocytopenia but is distinguished from TTP by the absence of neurologic symptoms, the prominence of acute renal failure, and frequent affliction of children. Recent studies, however, have tended to blur these clinical distinctions. Many adult patients with "TTP" lack one or more of the five criteria, and some patients with "HUS" have fever and neurologic dysfunction. The common fundamental feature in both of these conditions is widespread formation of hyaline thrombi, comprised primarily of platelet aggregates, in the

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microcirculation. Consumption of platelets leads to *thrombocytopenia*, and the intravascular thrombi provide a likely mechanism for the *microangiopathic hemolytic anemia* and widespread organ dysfunction. It is believed the varied clinical manifestations of TTP and HUS are related to differing proclivities for thrombus formation in specific microvascular beds.

For many years, the pathogenesis of TTP was enigmatic, although treatment with plasma exchange (initiated in the early 1970s) changed an almost uniformly fatal condition into one that is successfully treated in more than 80% of cases. Recently, the underlying cause of many, but not all, cases of TTP has been elucidated. In brief, symptomatic patients are often deficient in an enzyme called ADAMTS 13. This enzyme is designated "vWF metalloprotease" and it normally degrades very high molecular weight multimers of von Willebrand factor (vWF).[57] (ADAMTS 13 is unrelated to the other tissue metalloproteases that cleave extracellular matrix.) In the absence of this enzyme, very high molecular weight multimers of vWF accumulate in plasma and, under some circumstances, promote platelet microaggregate formation throughout the microcirculation, leading to the symptoms of TTP. Superimposition of endothelial cell injury (caused by some other condition) may further predispose a patient to microaggregate formation, thus initiating or exacerbating clinically evident TTP.

The deficiency of ADAMTS 13 may be inherited or acquired.[58] In many patients an antibody that inhibits vWF metalloprotease is detected.[57] Much less commonly the patients have inherited an inactivating mutation in the gene encoding this enzyme. Despite these advances, it is clear that factors other than vWF metalloprotease deficiency must be involved in triggering full-blown TTP, because symptoms are episodic even in those with hereditary deficiency of vWF metalloprotease. It is important to consider the possibility of TTP in any patient presenting with thrombocytopenia and microangiopathic hemolytic anemia, as any delay in diagnosis and treatment can be fatal. Plasma exchange can be life saving by providing the missing enzyme.

In contrast to TTP, most patients with HUS have normal levels of vWF metalloprotease, indicating that HUS usually has a different pathogenesis. [59] One important cause of HUS in children and the elderly is infectious gastroenteritis caused by *E. coli* strain 0157:H7.[60] This strain elaborates a Shiga-like toxin that is absorbed from the inflamed gastrointestinal mucosa. It binds to and damages endothelial cells in the glomerulus and elsewhere, thus initiating platelet activation and aggregation. Affected children present with bloody diarrhea, and a few days later HUS makes its appearance. With appropriate supportive care, affected children often recover completely, but irreversible renal damage and death can occur in more severe cases. HUS can also be seen in adults following exposures that damage endothelial cells (e.g., certain drugs, radiation therapy). The prognosis of adults with HUS is guarded, as it is most often seen in the setting of other chronic, life-threatening conditions.

While DIC and thrombotic microangiopathies share features such as microvascular occlusion and microangiopathic hemolytic anemia, they are pathogenetically distinct. In TTP and HUS (unlike DIC), activation of the coagulation cascade is not of primary importance, and hence results of laboratory tests of coagulation, such as PT and PTT, are usually normal.

BLEEDING DISORDERS RELATED TO DEFECTIVE PLATELET FUNCTIONS

Qualitative defects of platelet function can be congenital or acquired. Several congenital disorders characterized by prolonged bleeding time and normal platelet count have been described. A brief discussion of these rare diseases is warranted by the fact that they provide excellent models for investigating the molecular mechanisms of platelet function.[61]

Congenital disorders of platelet function can be classified into three groups on the basis of the specific functional abnormality: (1) *defects of adhesion,* (2) *defects of aggregation,* and (3) *disorders of platelet secretion (release reaction).*

- Bleeding resulting from defective adhesion of platelets to subendothelial matrix is best illustrated by the autosomal recessive disorder *Bernard-Soulier syndrome*, which is caused by an inherited deficiency of the platelet membrane glycoprotein complex Ib-IX. This glycoprotein is a receptor for vWF and is essential for normal platelet adhesion to subendothelial matrix (Chapter 4).
- Bleeding due to *defective platelet aggregation* is exemplified by *Glanzmann's thrombasthenia,* which is also transmitted as an autosomal recessive trait. Thrombasthenic platelets fail to aggregate in response to adenosine diphosphate (ADP), collagen, epinephrine, or thrombin owing to deficiency or dysfunction of glycoprotein IIb-IIIa, a protein complex that participates in the formation of "bridges" between platelets by binding fibrinogen and vWF.
- *Disorders of platelet secretion* are characterized by normal initial aggregation with collagen or ADP, but subsequent responses, such as secretion of thromboxanes and release of granule-bound ADP, are impaired. The underlying biochemical defects of these so-called *storage pool disorders* are varied, complex, and beyond the scope of our discussion.

Among the *acquired defects* of platelet function, two are clinically significant.[62] The first is *ingestion of aspirin* and other nonsteroidal anti-inflammatory drugs, which significantly prolongs the bleeding time. Aspirin is a potent, irreversible inhibitor of the enzyme cyclooxygenase, which is required for the synthesis of thromboxane A_2 and prostaglandins (Chapter 2). These mediators play important roles in platelet aggregation and subsequent release reactions (Chapter 4). The antiplatelet effects of aspirin form the basis for its use in the prophylaxis of thrombosis (Chapter 12). *Uremia* (Chapter 20) is the second condition exemplifying an acquired defect in platelet function. Although the pathogenesis of bleeding in uremia is complex and not fully understood, several abnormalities of platelet function are found.

HEMORRHAGIC DIATHESES RELATED TO ABNORMALITIES IN CLOTTING FACTORS

A deficiency of every clotting factor has been reported to be the cause of a bleeding disorder, with the exception of factor XII deficiency, which does not cause bleeding. The bleeding in factor deficiencies differs from platelet deficiencies in that spontaneous petechiae or purpura are uncommon. Rather, *the bleeding is manifested by large post-traumatic ecchymoses or hematomas, or prolonged bleeding after a laceration or any form of surgical procedure*. Bleeding into the gastrointestinal and urinary tracts, and particularly into weight-bearing joints, is common. Typical stories include the patient who continues to

ooze for days after a tooth extraction or who develops a hemarthrosis after relatively trivial stress on a knee joint. The course of history may have been changed by a hereditary coagulation defect present in the intermarried royal families of Great Britain and other parts of Europe. Clotting abnormalities can also be acquired in many different conditions.

Acquired disorders are usually characterized by multiple clotting abnormalities. Vitamin K deficiency (Chapter 9) results in impaired synthesis of factors II, VII, IX, and X and protein C.

Since the liver makes virtually all the clotting factors, severe parenchymal liver disease can be associated with a hemorrhagic diathesis. Disseminated intravascular coagulation produces a deficiency of multiple coagulation factors.

Hereditary deficiencies have been identified for each of the clotting factors. Deficiencies of factor VIII (hemophilia A) and of factor IX (Christmas disease, or hemophilia B) are transmitted as sex-linked recessive disorders. Most others follow autosomal patterns of transmission. *These hereditary disorders typically involve a single clotting factor.*

Deficiencies of Factor VIII-vWF Complex

Hemophilia A and von Willebrand disease, two of the most common inherited disorders of bleeding, are caused by qualitative or quantitative defects involving the factor VIII-vWF complex. Before we can discuss these disorders, it is essential to review the structure and function of these proteins.[63] [64]

Plasma factor VIII-vWF is a complex made up of two separate proteins (factor VIII and vWF) that can be characterized according to functional, biochemical, and immunologic criteria. Factor VIII procoagulant protein, or factor VIII (Fig. 13-28 ;

Figure 13-28 Structure and function of factor VIII-von Willebrand factor (vWF) complex. Factor VIII is synthesized in the liver and kidney, and vWF is made in endothelial cells and megakaryocytes. The two associate to form a complex in the circulation. vWF is also present in the subendothelial matrix of normal blood vessels and the alpha granules of platelets. Following endothelial injury, exposure of subendothelial vWF causes adhesion of platelets, primarily via glycoprotein lb platelet receptor. Circulating vWF and vWF released from the alpha granules of activated platelets can bind exposed subendothelial matrix, further contributing to platelet adhesion and activation. Activated platelets form hemostatic aggregates; fibrinogen (and possibly vWF) participate in aggregation through bridging interactions with the platelet receptor GpIIb/III. Factor VIII takes part in the coagulation cascade as a cofactor in the activation of factor X on the surface of activated platelets.

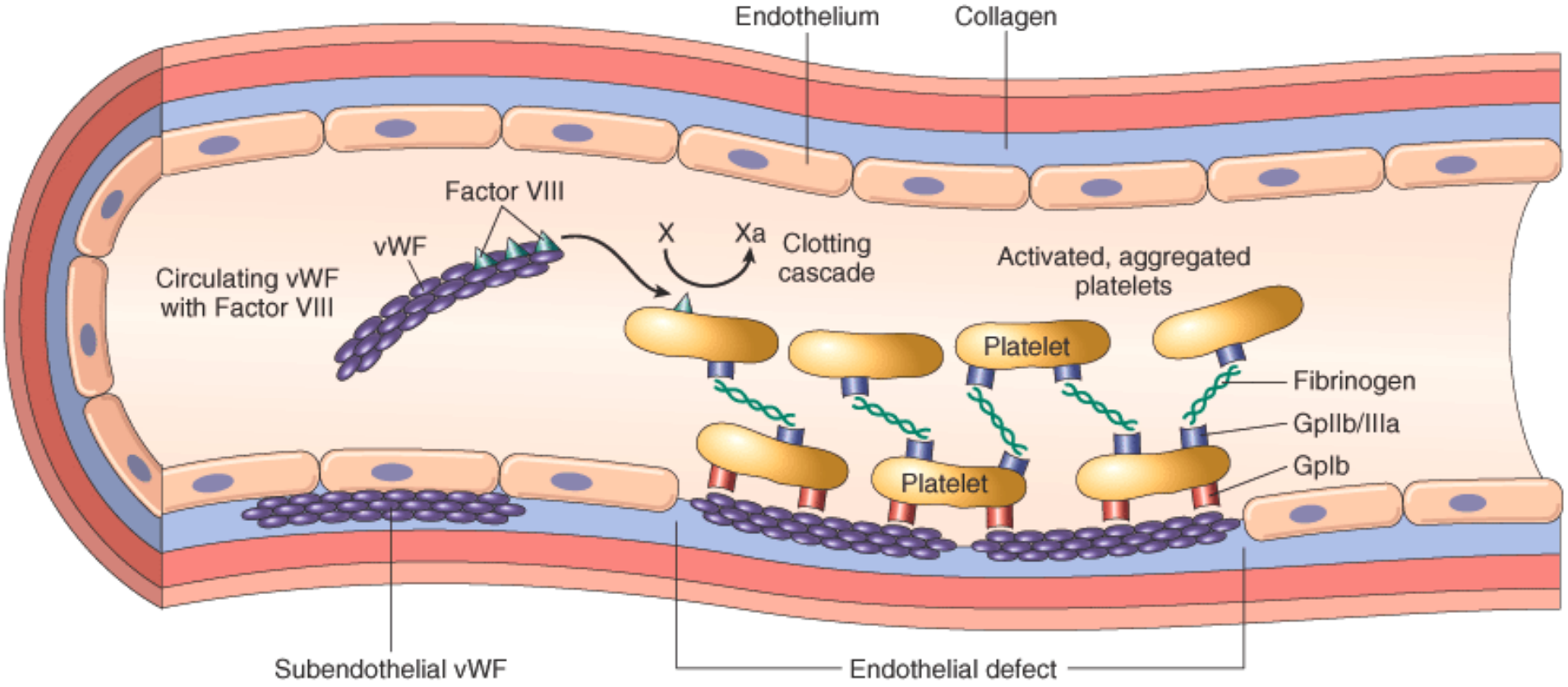

TABLE 13-10 -- Major Disorders Associated with Disseminated Intravascular Coagulation

Obstetric Complications
Abruptio placentae
Retained dead fetus
Septic abortion
Amniotic fluid embolism
Toxemia
Infections
Gram-negative sepsis
Meningococcemia
Rocky Mountain spotted fever
Histoplasmosis

Aspergillosis
Malaria
Neoplasms
Carcinomas of pancreas, prostate, lung, and stomach
Acute promyelocytic leukemia
Massive Tissue Injury
Traumatic
Burns
Extensive surgery
Miscellaneous
Acute intravascular hemolysis, snakebite, giant hemangioma, shock, heat stroke, vasculitis, aortic aneurysm, liver disease

increase the expression of tissue factor on endothelial cell membranes and simultaneously decrease the expression of thrombomodulin.[72] The net result is a shift in balance toward procoagulation.

Endothelial injury, the other major trigger, can initiate DIC by causing release of tissue factor, promoting platelet aggregation, and activating the intrinsic coagulation pathway. TNF is an extremely important mediator of endothelial cell inflammation and injury in septic shock. In addition to the effects previously mentioned, TNF up-regulates the expression of adhesion molecules on endothelial cells and thus favors adhesion of leukocytes, which in turn damage endothelial cells by releasing oxygen-derived free radicals and preformed proteases.[72] Even subtle endothelial injury can unleash procoagulant activity by enhancing membrane expression of tissue factor. Widespread endothelial injury may be produced by deposition of antigen-antibody complexes (e.g., systemic lupus erythematosus), temperature extremes (e.g., heat stroke, burns), or microorganisms (e.g., meningococci, rickettsiae).

Several disorders associated with DIC are listed in Table 13-10 . Of these, DIC is most likely to follow *obstetric complications, malignant neoplasia, sepsis,* and *major trauma*. The initiating factors in these conditions are often multiple and interrelated. For example, particularly in infections caused by gram-negative bacteria, released endotoxins can activate both the intrinsic and extrinsic pathways by producing endothelial cell injury and release of thromboplastins from inflammatory cells; furthermore, endotoxins inhibit the anticoagulant activity of protein C by suppressing thrombomodulin expression on endothelium. Endothelial cell damage can also be produced directly by meningococci, rickettsiae, and viruses. Antigen-antibody complexes formed during the infection can activate the classical complement pathway, and complement fragments can secondarily activate both platelets and granulocytes. Endotoxins as well as other bacterial products are also capable of directly activating factor XII. In *massive trauma, extensive surgery,* and *severe burns,* the major mechanism of DIC is believed to be the release of tissue thromboplastins. In *obstetric* conditions, thromboplastins derived from the placenta, dead retained fetus, or amniotic fluid may enter the circulation. However, hypoxia, acidosis, and shock, which often coexist with the surgical and obstetric conditions, also cause widespread endothelial injury. Supervening infection can complicate the problems further. Among cancers, acute promyelocytic leukemia and carcinomas of the lung, pancreas, colon, and stomach are most frequently associated with DIC. These tumors release of a variety of thromboplastic substances, including tissue factors, proteolytic enzymes, mucin, and other undefined tumor products.

The consequences of DIC are twofold. First, there is *widespread deposition of fibrin* within the microcirculation. This can lead to ischemia of the more severely affected or more vulnerable organs and to a *hemolytic anemia* resulting from fragmentation of red cells as they squeeze through the narrowed microvasculature (microangiopathic hemolytic anemia). Second, a *hemorrhagic diathesis* can dominate the clinical picture. This results from consumption of platelets and clotting factors as well as activation of plasminogen. Plasmin can not only cleave fibrin, but also digest factors V and VIII, thereby reducing their concentration further. In addition, fibrinolysis leads to the formation of fibrin degradation products, which inhibit platelet aggregation and fibrin polymerization and have antithrombin activity. All these influences lead to the hemostatic failure seen in DIC (Fig. 13-29).

Morphology.

In general, thrombi are found in the following sites in decreasing order of frequency: brain, heart, lungs, kidneys, adrenals, spleen, and liver. However, no tissue is spared, and thrombi are occasionally found in only one or several organs without affecting others. In giant hemangiomas, for example, thrombi are localized to the neoplasm, where they are believed to form due to local stasis and recurrent trauma to fragile blood vessels. The affected kidneys can reveal small thrombi in the glomeruli that may evoke only reactive swelling of endothelial cells or, in severe cases, microinfarcts or even bilateral renal cortical necrosis. Numerous fibrin thrombi may be found in alveolar capillaries, sometimes associated with pulmonary edema and fibrin exudation, creating "hyaline membranes" reminiscent of acute respiratory distress syndrome (Chapter 15). In the central nervous system, fibrin thrombi can cause microinfarcts, occasionally complicated by simultaneous hemorrhage. Such changes are the basis for the bizarre neurologic signs and symptoms sometimes observed in DIC. The manifestations of DIC in the endocrine glands are of considerable interest. In meningococcemia, fibrin thrombi within the microcirculation of the adrenal cortex are the likely basis for the massive adrenal hemorrhages seen in Waterhouse-Friderichsen syndrome (Chapter 24). Similarly, Sheehan postpartum pituitary necrosis (Chapter 24) is a form of DIC complicating labor and delivery. In toxemia of pregnancy (Chapter 22), the placenta exhibits widespread microthrombi, providing a plausible

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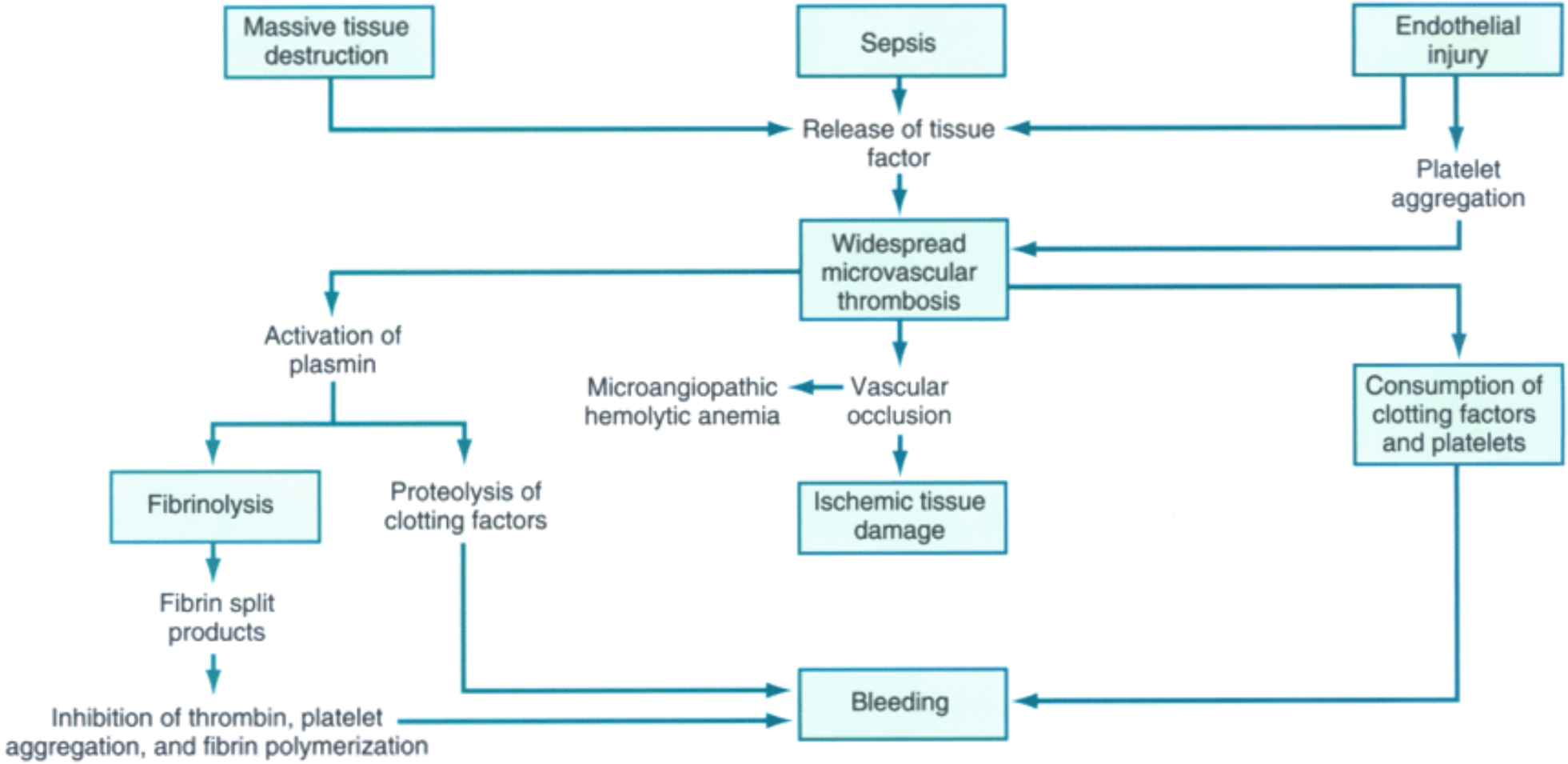
Figure 13-29 Pathophysiology of disseminated intravascular coagulation.



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Chapter 14 - Diseases of White Blood Cells, Lymph Nodes, Spleen, and Thymus

Jon C. Aster MD, PhD

White Blood Cells and Lymph Nodes

Normal

The origin and differentiation of white blood cells (granulocytes, monocytes, and lymphocytes) were briefly discussed in Chapter 13 . Lymphocytes and monocytes not only circulate in the blood and lymph but also accumulate in discrete, organized masses within lymph nodes, thymus, spleen, tonsils, adenoids, and Peyer patches. Less discrete collections of lymphoid cells occur in the bone marrow, lungs, gastrointestinal tract, and other tissues. Lymph nodes are the most widely distributed and easily accessible component of the lymphoid tissue and hence are frequently examined for diagnosis of lymphoreticular disorders. Before discussing these pathologic states, we will briefly review the normal morphology of lymph nodes (shown in Fig. 6-3 , Chapter 6).

Lymph nodes are discrete structures surrounded by a capsule composed of connective tissue and a few elastic fibrils. The capsule is perforated by multiple afferent lymphatics that empty into a fenestrated subcapsular peripheral sinus. Lymph extravasates from this sinus and slowly percolates through the node, eventually collecting in medullary sinusoids and exiting through a single efferent lymphatic vessel in the hilus, which is the point of penetration by a single small artery and vein. Situated in the cortex subjacent to the peripheral sinus are spherical or egg-shaped aggregates of small lymphocytes, the so-called primary *follicles*, which contain numerous immunologically naïve B cells. The paracortical region lying between primary follicles is populated by numerous evenly dispersed small T lymphocytes. Deep to the cortex lies the medulla, which contains variable numbers of plasma cells and relatively few lymphocytes.

This morphologic description reflects the static organization of a lymph node that is not responding to a foreign invader. As secondary lines of defense, lymph nodes constantly respond to stimuli, particularly infectious microbes, even in the absence of clinical disease. Within several days of antigenic stimulation, primary follicles enlarge and are transformed into palestaining *germinal centers*, highly dynamic structures in which B cells acquire the capacity to make high-affinity antibodies against specific antigens. Normal germinal centers are surrounded by a dark-staining mantle zone, which contains mainly small naïve B cells. In some reactive conditions, a rim of B cells with slightly more cytoplasm accumulates outside of the mantle zone; cells occupying this region are called *marginal zone B cells*. The paracortical T-cell zones also frequently undergo hyperplasia in immune reactions in which cellular immunity is particularly important, such as viral infections.

The degree and pattern of morphologic change are dependent on the inciting stimulus and the intensity of the immune response. Trivial injuries and infections induce subtle changes in lymph node histology, while more significant infections inevitably produce enlargement of nodes and sometimes leave residual scarring. For this reason, lymph nodes in adults are almost never "normal" or "resting," and it is often necessary to distinguish morphologic changes secondary to past experience from those related to present disease.

Pathology

Disorders of white blood cells can be classified into two broad categories: *proliferative disorders*, in which there is an expansion of leukocytes, and *leukopenias*, which are defined as a deficiency of leukocytes. Proliferations of white cells can be *reactive* or *neoplastic*. Since the major function of leukocytes is host defense, reactive proliferation in response to an underlying primary, often microbial, disease is fairly common. Neoplastic disorders, although less frequent, are much more important clinically. In the following discussion, we shall first

describe the leukopenic states and summarize the common reactive disorders and then consider in some detail malignant proliferations of white cells.

Leukopenia

The number of circulating white cells may be markedly decreased in a variety of disorders. An abnormally low white cell count *(leukopenia)* usually results from reduced numbers of neutrophils *(neutropenia, granulocytopenia). Lymphopenia* is less common; in addition to congenital immunodeficiency diseases (see Chapter 6), it is most commonly observed in specific settings, such as advanced HIV infection, following therapy with glucocorticoids or cytotoxic drugs, autoimmune disorders, malnutrition, and certain acute viral infections. Only the more common leukopenias involving granulocytes will be discussed further here.

NEUTROPENIA, AGRANULOCYTOSIS

Reduction in the number of granulocytes in the peripheral blood *(neutropenia)* can be seen in a wide variety of circumstances. A marked reduction in neutrophil count, referred to as *arganulocytosis*, has serious consequences by making individuals susceptible to infections.

Pathogenesis.

A reduction in circulating granulocytes will occur if there is (1) reduced or ineffective production of neutrophils or (2) accelerated removal of neutrophils from the circulating blood. *Inadequate or ineffective granulopoiesis* is observed in the setting of:

- Suppression of myeloid stem cells, as occurs in aplastic anemia (see Chapter 13) and a variety of infiltrative marrow disorders (tumors, granulomatous disease, etc.); in these conditions, granulocytopenia is accompanied by anemia and thrombocytopenia.
- Suppression of committed granulocytic precursors due to exposure to certain drugs, as discussed below.

- Disease states associated with ineffective granulopoiesis, such as megaloblastic anemias due to vitamin B_{12} or folate deficiency (see Chapter 13) and myelodysplastic syndromes, where defective precursors are susceptible to death in the marrow.
- Rare inherited conditions (such as Kostmann syndrome) in which genetic defects in specific genes result in impaired granulocytic differentiation.

Accelerated removal or destruction of neutrophils occurs with:

- Immunologically mediated injury to the neutrophils, which may be idiopathic, associated with a well-defined immunologic disorder (e.g., systemic lupus erythematosus), or produced by exposure to drugs.
- Splenic sequestration, in which excessive destruction occurs secondary to enlargement of the spleen, usually associated with increased destruction of red cells and platelets as well.
- Increased peripheral utilization, as may occur in overwhelming bacterial, fungal, or rickettsial infections.

Drugs are responsible for most of the significant neutropenias (agranulocytoses). Certain drugs, such as alkylating agents and antimetabolites used in cancer treatment, produce agranulocytosis in a predictable, dose-related fashion. Because such drugs cause a generalized suppression of the bone marrow, production of erythrocytes and platelets is also affected. Agranulocytosis can also occur as an idiosyncratic reaction to a large variety of agents. The roster of implicated drugs includes aminopyrine, chloramphenicol, sulfonamides, chlorpromazine, thiouracil, and phenylbutazone. The neutropenia induced by chlorpromazine and related phenothiazines may result from a toxic effect on granulocytic precursors in the bone marrow. In contrast, agranulocytosis following administration of aminopyrine, thiouracil, and certain sulfonamides likely stems from immunologically mediated destruction of mature

neutrophils through mechanisms similar to those involved in drug-induced hemolytic anemias (see Chapter 13).

In some patients with acquired idiopathic neutropenia, autoantibodies directed against neutrophil-specific antigens are detected.[1] Severe neutropenia can also occur in association with monoclonal proliferations of large granular lymphocytes (so-called LGL leukemia).[2] The mechanism of this neutropenia is not clear; suppression of marrow granulocytic progenitors is considered most likely.

Morphology.

The anatomic alterations in the bone marrow vary according to the underlying cause. When neutropenia is caused by excessive destruction of mature neutrophils, the marrow is usually hypercellular owing to the presence of increased numbers of granulocytic precursors. Hypercellularity is also the rule in neutropenias associated with ineffective granulopoiesis, as occurs in megaloblastic anemias and myelodysplastic syndromes. Agranulocytosis caused by agents that suppress or destroy granulocytic precursors is understandably associated with marrow hypocellularity.

Infections (most often bacterial or fungal) are a common consequence of agranulocytosis. Ulcerating necrotizing lesions of the gingiva, floor of the mouth, buccal mucosa, pharynx, or anywhere within the oral cavity (agranulocytic angina) are quite characteristic. These ulcers are typically deep, undermined, and covered by gray to green-black necrotic membranes from which numerous bacteria or fungi can be isolated. Less frequently, similar ulcerative lesions occur in the skin, vagina, anus, or gastrointestinal tract. Severe life-threatening invasive bacterial or fungal infections can occur in the lungs, urinary tract, and kidneys. The neutropenic patient is at particularly high risk for deep fungal infections caused by organisms such as *Candida* and *Aspergillus*. Sites of infection often show a massive growth of organisms with little leukocytic response. In the most dramatic instances, bacteria grow in colonies (botryomycosis) resembling those seen on nutrient media. The regional lymph nodes draining these infections are enlarged and inflamed.

Clinical Course.

The symptoms and signs of neutropenias are related to bacterial or fungal infections. They include malaise, chills, and fever, followed in sequence by marked weakness and fatigability. In severe agranulocytosis with virtual absence of neutrophils, these infections can be overwhelming and cause death within a few days.

A neutrophil count of less than 1000 cells per mm^3 of blood is worrisome, but most serious infections occur with counts below 500 per mm^3 . Because infections are often fulminant, broad-spectrum antibiotics are given expeditiously whenever signs or symptoms appear. In some instances, such as following myelosuppressive chemotherapy, neutropenia is treated with granulocyte colony-stimulating factor (G-CSF), a growth factor that stimulates the production of granulocytes from marrow precursors.

Reactive (Inflammatory) Proliferations of White Cells and Lymph Nodes

LEUKOCYTOSIS

Leukocytosis refers to an increase in the number of blood leukocytes. It is a common reaction to a variety of inflammatory states and is sometimes the first indication of neoplastic growth of leukocytes.

Pathogenesis.

The peripheral blood leukocyte count is influenced by several factors, including:

- The size of the myeloid (for granulocytes and monocytes) and lymphoid (for lymphocytes) precursor and storage cell pools in the bone marrow, circulation, and peripheral tissues
- The rate of release of cells from the storage pool into the circulation

- The proportion of cells that are adherent to blood vessel walls at any time (the marginating pool)
- The rate of extravasation of cells from the blood into tissues

As was discussed in Chapter 2 and Chapter 13 , leukocyte homeostasis is maintained by cytokines, growth factors, and adhesion molecules through their effects on the commitment, proliferation, differentiation, and extravasation of leukocytes and their progenitors. The mechanisms of leukocytosis vary

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Figure 14-1 (Figure Not Available) Mechanisms of neutrophilic leukocytosis. Neutrophils and their precursors are distributed in five pools: a bone marrow precursor pool; a bone marrow storage pool, consisting of mature and slightly immature neutrophils (band forms); a peripheral blood marginating pool; a peripheral blood circulating pool; and a tissue pool. Sampling of the peripheral blood assesses only the circulating pool, which can be enlarged by increased release of neutrophils and band forms from the marrow storage pool, decreased margination, diminished extravasation into tissues, or expansion of the marrow precursor cell pool. Diverse stimuli that increase the circulating pool through various mechanisms are listed. It should be noted that certain stimuli (e.g., acute infection) cause changes in flux between multiple pools simultaneously.

depending on the affected leukocyte pool and the particular factor. In acute infection, there is a rapid increase in the egress of mature granulocytes from the bone marrow pool, which is roughly 50 times the size of the peripheral blood marginal pool. The release of IL-1, TNF, and other inflammatory cytokines stimulates bone marrow stromal cells and T cells to produce increased amounts of colony-stimulating factors (CSFs), which enhance the proliferation and differentiation of committed granulocytic progenitors and, over several days, cause a sustained increase in neutrophil production. Figure 14-1 (Figure Not Available) summarizes the major mechanisms of neutrophilic leukocytosis and their causes.

TABLE 14-1 -- Causes of Leukocytosis

Neutrophilic leukocytosis	Acute bacterial infections, especially those caused by pyogenic organisms; sterile inflammation caused by, for example, tissue necrosis (myocardial infarction, burns)
Eosinophilic leukocytosis (eosinophilia)	Allergic disorders such as asthma, hay fever, allergic skin diseases (e.g., pemphigus, dermatitis herpetiformis); parasitic infestations; drug reactions; certain malignancies (e.g., Hodgkin disease and some non-Hodgkin lymphomas); collagen vascular disorders and some vasculitides; atheroembolic disease (transient)
Basophilic leukocytosis (basophilia)	Rare, often indicative of a myeloproliferative disease (e.g., chronic myelogenous leukemia)
Monocytosis	Chronic infections (e.g., tuberculosis), bacterial endocarditis, rickettsiosis and malaria; collagen vascular diseases (e.g., systemic lupus erythematosus) and inflammatory bowel diseases (e.g., ulcerative colitis)
Lymphocytosis	Accompanies monocytosis in many disorders associated with chronic immunologic stimulation (e.g., tuberculosis, brucellosis); viral infections (e.g., hepatitis A, cytomegalovirus, Epstein-Barr virus); *Bordetella pertussis* infection

Other growth factors preferentially stimulate other types of leukocytosis. For example, IL-5 causes eosinophilia by enhancing the growth, survival, and differentiation of eosinophils, while IL-7 plays a central role in lymphopoiesis. Such factors are differentially produced in response to various pathogenic stimuli, and as a result, the five principal types of leukocytosis (neutrophilic, eosinophilic, and basophilic leukocytosis, monocytosis, and lymphocytosis) each tend to be observed in particular clinical settings (summarized in Table 14-1).

In sepsis or severe inflammatory disorders (such as Kawasaki disease), leukocytosis is often accompanied by morphologic

changes in the neutrophils, such as toxic granulations, Döhle bodies, and cytoplasmic vacuoles (Fig. 14-2). *Toxic granules* are coarse and darker than the normal neutrophilic granules and are believed to represent abnormal azurophilic (primary) granules. *Döhle bodies* are patches of dilated endoplasmic reticulum that appear as sky-blue cytoplasmic "puddles" in smears stained with Wright-Giemsa stain.

In most instances, it is not difficult to distinguish reactive leukocytosis from leukocytosis caused by flooding of the peripheral blood by neoplastic white blood cells (leukemia), but uncertainties may arise in two settings. Particularly in children, acute viral infections can produce the appearance of activated lymphocytes in the peripheral blood and marrow that resemble neoplastic lymphoid cells. At other times, particularly in inflammatory states and severe chronic infections, many immature granulocytes appear in the blood, simulating a picture of myelogenous leukemia *(leukemoid reaction)*. Special laboratory studies (discussed later) are helpful in distinguishing reactive and neoplastic leukocytoses.

In addition to causing leukocytosis, infections and inflammatory stimuli often elicit immune reactions within lymph nodes. The infections that lead to lymphadenitis are numerous. Some that produce distinctive morphologic patterns are described in other chapters. Most, however, cause stereotypic patterns of lymph node reaction designated acute and chronic nonspecific lymphadenitis.

ACUTE NONSPECIFIC LYMPHADENITIS

Lymph nodes undergo reactive changes whenever they are challenged by microbiologic agents, cell debris, or foreign matter introduced into wounds or into the circulation. Acute lymphadenitis is most often seen in the cervical region due to microbial drainage from infections of the teeth or tonsils and in the axillary or inguinal regions secondary to infections in the extremities. Similarly, acute lymphadenitis often occurs in mesenteric lymph nodes draining acute appendicitis. Unfortunately, other self-limited infections can also cause mesenteric

Figure 14-2 Reactive changes in neutrophils. Neutrophils containing coarse purple cytoplasmic granules (toxic granulations) and blue cytoplasmic patches of dilated endoplasmic reticulum (Döhle bodies, *arrow*) are observed in this peripheral blood smear taken from a patient with bacterial sepsis.

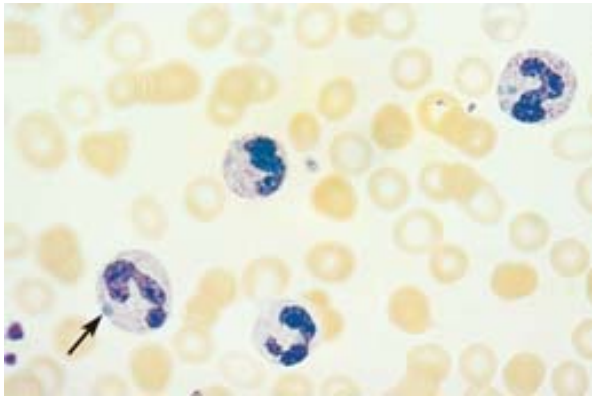

Figure 14-3 Follicular hyperplasia. *A*, Low-power view showing a reactive follicle and surrounding mantle zone. The dark-staining mantle zone is polarized, being much more prominent adjacent to the germinal center light zone in the left half of the follicle. The right half of the follicle consists of the dark zone. *B*, High-power view of the dark zone shows several mitotic figures and numerous macrophages containing phagocytosed apoptotic cells (tingible bodies).

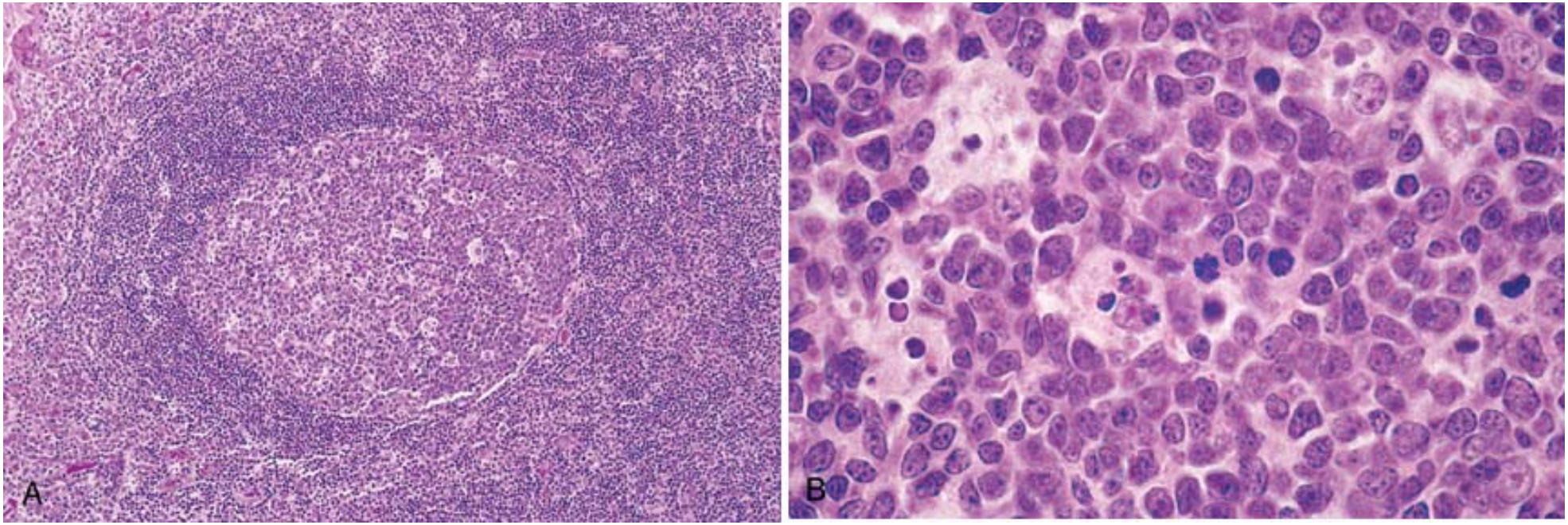

TABLE 14-2 -- The WHO Classification of the Lymphoid Neoplasms

I. Precursor B-Cell Neoplasms
Precursor-B lymphoblastic leukemia/lymphoma
II. Peripheral B-Cell Neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Lymphoplasmacytic lymphoma
Splenic and nodal marginal zone lymphomas
Extranodal marginal zone lymphoma
Mantle cell lymphoma
Follicular lymphoma
Marginal zone lymphoma
Hairy cell leukemia
Plasmacytoma/plasma cell myeloma
Diffuse large B-cell lymphoma
Burkitt lymphoma

III. Precursor T-Cell Neoplasms
Precursor-T lymphoblastic leukemia/lymphoma
IV. Peripheral T-Cell and NK-Cell Neoplasms
T-cell prolymphocytic leukemia
Large granular lymphocytic leukemia
Mycosis fungoides/Sézary syndrome
Peripheral T-cell lymphoma, unspecified
Anaplastic large cell lymphoma
Angioimmunoblastic T-cell lymphoma
Enteropathy-associated T-cell lymphoma
Panniculitis-like T-cell lymphoma
Hepatosplenic γδ T-cell lymphoma
Adult T-cell leukemia/lymphoma
NK/T-cell lymphoma, nasal type
NK-cell leukemia
V. Hodgkin Lymphoma
Classical subtypes
••Nodular sclerosis
••Mixed cellularity
••Lymphocyte-rich
••Lymphocyte depletion
Lymphocyte predominance

hematopathologists, oncologists, and molecular biologists came together to create the *Revised European-American Classification of Lymphoid Neoplasms* (REAL).[8] Of importance, this classification scheme incorporated objective criteria, such as immunophenotype and genetic aberrations, together with morphologic and clinical features, to define distinct clinicopathologic entities. Experience has shown that most entities in the REAL classification can be diagnosed reproducibly by experienced pathologists and stratify patients into good and bad prognosis groups.[9] [10] More recently, an international group of hematopathologists and oncologists convened by the World Health Organization (WHO) reviewed and updated the REAL classification, resulting in the inclusion of a number of additional rare entities.[11] Presented here is the WHO classification (Table 14-2), which sorts the lymphoid neoplasms into five broad categories, based on their cell of origin:

1. Precursor B-cell neoplasms (neoplasms of immature B cells)
2. Peripheral B-cell neoplasms (neoplasms of mature B cells)
3. Precursor T-cell neoplasms (neoplasms of immature T cells)

4. Peripheral T-cell and NK-cell neoplasms (neoplasms of mature T cells and natural killer cells)
5. Hodgkin lymphoma (neoplasms of Reed-Sternberg cells and variants).

Before we discuss the specific entities described in the WHO classification, some important principles relevant to the lymphoid neoplasms need to be emphasized.

• Lymphoid neoplasia can be suspected from the clinical features, but *histologic examination of lymph nodes or other involved tissues is required for diagnosis*.
• As will be recalled from Chapter 6 , antigen receptor genes rearrange during normal B- and T-cell differentiation through a mechanism that ensures that each developing lymphocyte makes a single, unique antigen receptor. *In most lymphoid neoplasms, antigen receptor gene rearrangement*

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precedes transformation; hence, the daughter cells derived from the malignant progenitor share the same antigen receptor gene configuration and sequence and synthesize identical antigen receptor proteins(either immunoglobulins or T-cell receptors). In contrast, normal immune responses are polyclonal and thus comprise populations of lymphocytes expressing many different antigen receptors. As a result, analyses of antigen receptor genes and/or their protein products can be used to distinguish reactive and malignant lymphoid proliferations. In addition, each antigen receptor gene rearrangement produces a unique DNA sequence that constitutes a highly specific clonal marker that can be used to detect small numbers of residual malignant cells after therapy.[12]

• *The vast majority of lymphoid neoplasms (80% to 85%) are of B-cell origin, most of the remainder being T-cell tumors; only rarely are tumors of NK origin encountered.* Most lymphoid neoplasms resemble some recognizable stage of B- or T-cell differentiation (Fig. 14-4), a feature that is used in their classification. Markers recognized by antibodies that are helpful in the characterization of lymphomas and leukemias are listed in Table 14-3 .

• *As tumors of the immune system, lymphoid neoplasms often disrupt normal architecture and function of the immune system, leading to immune abnormalities.* Both a loss of vigilance (as evidenced by susceptibility to infection) and breakdown of tolerance (manifested by autoimmunity) can be seen, sometimes in the same patient. In a further, ironic twist, patients with inherited or acquired immunodeficiency are themselves at high risk of developing certain lymphoid neoplasms, particularly those caused by oncogenic viruses (e.g., EBV).

• *Neoplastic B and T cells tend to recapitulate the behavior of their normal counterparts.* Like normal lymphocytes, transformed B and T cells tend to home to particular tissue sites, leading to characteristic patterns of involvement. For example, follicular lymphomas proliferate in the B-cell areas of the lymph node, producing a nodular or follicular pattern of growth, whereas T-cell lymphomas typically grow in paracortical T-cell zones. As is true of their normal

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counterparts, lymph node homing of neoplastic lymphocytes is likely regulated by expression of particular chemokine receptors. Variable numbers of neoplastic B and T lymphoid cells also recirculate periodically through the lymphatics and peripheral blood to distant sites. Sensitive molecular techniques have shown that most lymphoid tumors are widely disseminated at the time of diagnosis. The most notable exception to this rule is Hodgkin lymphoma, which is sometimes restricted to one group of lymph nodes.

• *Hodgkin lymphoma spreads in an orderly fashion, and as a result staging is of importance in determining therapy.* In contrast, the spread of NHL is less predictable, and as was noted above, most patients are assumed to have systemic disease at the time of diagnosis. Hence, staging in particular NHLs provides useful prognostic information but is generally not as important in guiding therapy as is the case in Hodgkin lymphoma.

Figure 14-4 Origin of lymphoid neoplasms. Stages of B- and T-cell differentiation from which specific lymphoid tumors emerge are shown. Key: CLP, common lymphoid precursor; BLB, pre-B lymphoblast; NBC, naive B cell; MC, mantle B cell; GC, germinal center B cell; MZ, marginal zone B cell; DN, CD4/CD8 double negative pre-T cell; DP, CD4/CD8 double positive pre-T cell; PTC, peripheral T cell.

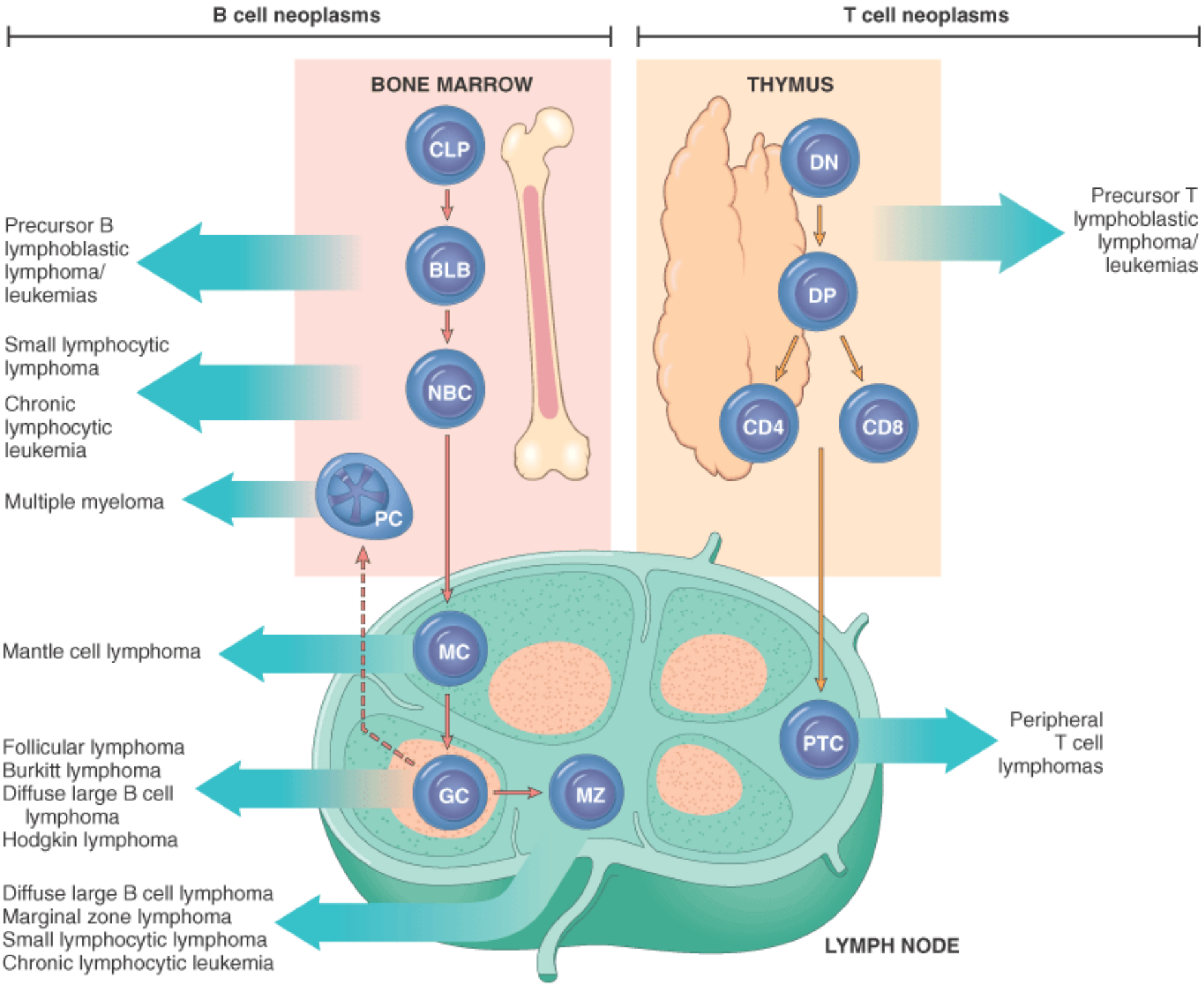

B cell neoplasms
T cell neoplasms
BONE MARROW
THYMUS
CLP
BLB
NBC
PC
DN
DP
CD4
CD8
Precursor B lymphoblastic lymphoma/ leukemias
Small lymphocytic lymphoma
Chronic lymphocytic leukemia
Multiple myeloma
Precursor T lymphoblastic lymphoma/ leukemias
Mantle cell lymphoma
Follicular lymphoma
Burkitt lymphoma
Diffuse large B cell lymphoma
Hodgkin lymphoma
Diffuse large B cell lymphoma
Marginal zone lymphoma
Small lymphocytic lymphoma
Chronic lymphocytic leukemia
MC
GC
MZ
PTC
Peripheral T cell lymphomas
LYMPH NODE

TABLE 14-3 -- Some Immune Cell Antigens Detected by Monoclonal Antibodies

Antigen Designation	Normal Cellular Distribution
Primarily T-Cell Associated	
CD1	Cortical thymocytes and Langerhans histiocytes
CD3	Thymocytes, peripheral T cells
CD4	Helper subset of peripheral T cells, single positive medullary thymocytes, and CD4/CD8 double positive thymocytes
CD5	T cells and a small subset of B cells
CD8	Cytotoxic subset of peripheral T cells, single positive medullary thymocytes, double positive cortical thymocytes, and some NK cells
Primarily B-Cell Associated	
CD10	Marrow pre-B cells and germinal center B cells; also called CALLA
CD19	Marrow pre-B cells and mature B cells but not plasma cells
CD20	Marrow pre-B cells after CD19 and mature B cells but not plasma cells
CD21	EBV receptor; present on mature B cells and follicular dendritic cells
CD23	Activated mature B cells
CD79a	Marrow pre-B cells and mature B cells.
Primarily Monocyte or Macrophage Associated	
CD11c	Granulocytes, monocytes, and macrophages; also expressed by hairy cell leukemias
CD13	Immature and mature monocytes and granulocytes
CD14	Monocytes
CD15	Granulocytes; also expressed by Reed-Sternberg cells and variants in classical Hodgkin lymphoma
CD33	Myeloid progenitors and monocytes
CD64	Mature myeloid cells
Primarily NK-Cell Associated	
CD16	NK cells and granulocytes
CD56	NK cells and a subset of T cells
Primarily Stem Cell and Progenitor Cell Associated	
CD34	Pluripotent hematopoietic stem cells and progenitor cells of many lineages
Activation Markers	
CD30	Activated B cells, T cells, and monocytes; also expressed by Reed-Sternberg cells and variants in classical Hodgkin lymphoma

Present on All Leukocytes	
CD45	All leukocytes; also known as leukocyte common antigen (LCA)
CD, cluster designation; NK, natural killer; CALLA, common acute lymphoblastic leukemia antigen; EBV, Epstein-Barr virus.	

We now turn to the specific entities of the WHO classification. In the discussion that follows, only the most salient immunophenotypic and karyotypic features are included; this information is summarized in Table 14-4 . We will begin with neoplasms of immature lymphoid cells and then move on to tumors of mature B cells, T cells, and NK cells. Within each immunophenotypic category, the most common (and thus most important) entities will be emphasized.

Precursor B- and T-Cell Neoplasms

Acute Lymphoblastic Leukemia/Lymphoma

Acute lymphoblastic leukemia/lymphoma (ALL) encompasses a group of neoplasms composed of immature, precursor B (pre-B) or T (pre-T) lymphocytes referred to as *lymphoblasts. The majority (~85%) of ALLs are precursor B-cell tumors that typically manifest as childhood acute "leukemias"* with extensive bone marrow and variable peripheral blood involvement. The less common *precursor T-cell ALLs tend to present in adolescent males as "lymphomas," often with thymic involvement*. It is worth noting, however, that there is considerable overlap in the clinical behavior of precursor B-cell and T-cell ALL; for example, pre-B cell tumors uncommonly present as "lymphomas," and many pre-T cell tumors evolve to a leukemic peripheral blood picture. Malignant pre-B and pre-T lymphoblasts are also morphologically indistinguishable, and subclassification of ALL is thus dependent on immunophenotyping. Because of their morphologic and clinical similarities, the various forms of ALL will be considered here together.

Approximately 2500 new cases of ALL are diagnosed each year in the United States, most cases occurring in individuals younger than 15 years of age. ALL is almost twice as common in whites as in nonwhites and is slightly more frequent in boys than in girls. The incidence of pre-B ALL is highest at about the age of 4, perhaps because the number of normal bone marrow pre-B lymphoblasts (the cell of origin) peaks in early childhood. Similarly, the peak incidence of pre-T ALL is in adolescence, the age when the thymus reaches its maximal size. Both pre-B and pre-T ALL occur in adults of all ages, but much less frequently than in children.

Morphology.

Because of different responses to chemotherapeutic agents, it is of great practical importance to distinguish ALL from acute myelogenous leukemia (AML), a neoplasm of immature myeloid cells that may cause identical signs and symptoms. Compared to myeloblasts, lymphoblasts have condensed chromatin, inconspicuous nucleoli, and scant agranular cytoplasm (Fig. 14-5*A*). However,

TABLE 14-4 -- Summary of Major Types of Lymphoid Neoplasms

Diagnosis	Cell of Origin	Genotype	Salient Clinical Features
Neoplasms of immature B and T cells			

Precursor B-cell acute lymphoblastic leukemia/ lymphoma	Bone marrow precursor B-cell expressing TdT and lacking surface Ig	Diverse chromosomal translocations; t(12;21) involving *CBFα* and *ETV6* most common rearrangement	Predominantly children with symptoms relating to pancytopenia secondary to marrow involvement; aggressive
Precursor T-cell acute lymphoblastic leukemia/ lymphoma	Precursor T-cell (often of thymic origin) expressing TdT	Diverse chromosomal translocations, many involving T-cell receptor loci; rearrangements of *TAL1* most common	Predominantly adolescent males with thymic masses; variable splenic, hepatic, and bone marrow involvement; aggressive
Neoplasms of mature B cells			
Burkitt lymphoma	Germinal center B-cell; CD10 expression usually seen	Translocations involving c-myc and Ig loci; usually t (8;14), but also t(2;8) or t(8;22). African (endemic) cases latently infected with EBV	Adolescents or young adults with jaw or extranodal abdominal masses; uncommonly presents as a "leukemia"; aggressive
Diffuse large B-cell lymphoma	Germinal center or postgerminal center B-cell	Diverse chromosomal aberrations; ~30% have rearrangements of *BCL6*; ~10% contain the t(14;18); c*REL* amplification in a subset	All ages, but most common in adults; often appears as a single rapidly growing mass; 30% extranodal; aggressive
Extranodal marginal zone lymphoma	Postgerminal center memory B-cell	Trisomy 18, t(11;18), t(1;14); latter create *MALT1-IAP2* and *BCL10-IgH* fusion genes, respectively	Arises at extranodal sites in adults with chronic inflammatory diseases; may remain localized; indolent
Follicular lymphoma	Germinal center B-cell; typically expresses CD10, BCL2, and BCL6	t(14;18) involving the *BCL2* gene	Older adults with generalized lymphadenopathy and marrow involvement; indolent
Hairy cell leukemia	Postgerminal center memory B-cell	No specific chromosomal abnormality	Older males with pancytopenia and splenomegaly; indolent
Mantle cell lymphoma	Naïve B-cell; expresses cyclin D1 and (usually) CD5	t(11;14) involving *BCL1* (cyclin D1) and *IgH*	Older males with disseminated disease; moderately aggressive
Multiple myeloma/solitary plasmacytoma	Plasma cell derived from a postgerminal center B-cell	Diverse rearrangements involving *IgH*	Myeloma: older adults with lytic bone lesions, pathologic fractures, hypercalcemia, renal failure, and primary amyloidosis. Plasmacytoma: isolated plasma cell masses in bone or soft tissue (e.g., oropharynx)
Small lymphocytic lymphoma/chronic lymphocytic leukemia	Naïve B-cell or postgerminal center memory B-cell; expresses CD5	Trisomy 12, deletions of 11q, 13q, and 17p	Older adults with bone marrow, lymph node, spleen and liver disease; most have peripheral blood involvement; autoimmune hemolysis and thrombocytopenia in a minority; indolent
Neoplasms of mature T-cells or NK-cells			
Adult T-cell leukemia/ lymphoma	Helper T-cell expressing CD25 (IL-2 receptor)	HTLV-1 provirus present in tumor cells	Adults with cutaneous lesions, marrow involvement, and hypercalcemia; Japan, West Africa, and the Caribbean; aggressive
Anaplastic large cell lymphoma	Cytotoxic T-cell	Rearrangements of *ALK*	Children and young adults, usually with lymph node and soft tissue disease; aggressive
Extranodal NK/T cell lymphoma	Natural killer cell (common) or cytotoxic T-cell (rare)	No specific chromosomal abnormality; uniformly EBV associated	Adults with destructive extranodal masses, most commonly sinonasal; often accompanied by hemophagocytic syndrome; aggressive

Mycosis fungoides/Sézary syndrome	Helper T-cell	No specific chromosomal abnormality	Adult patients with cutaneous patches, plaques, nodules, or generalized erythema; indolent
T-cell granular lymphocytic leukemia	Two types: (1) CD8+ T-cell, (2) NK-cell	No specific chromosomal abnormality	Adult patients with splenomegaly, neutropenia, and anemia, sometimes, accompanied by autoimmune disease
Hodgkin lymphoma			
Hodgkin lymphoma, lymphocyte-depletion subtype	Germinal center or postgerminal center B-cell	No specific chromosomal abnormality; >70% EBV associated	More common in the elderly and in HIV+ individuals; moderately aggressive
Hodgkin lymphoma, lymphocyte-predominance subtype	Germinal center B-cell	No specific chromosomal abnormality; not associated with EBV	Young to middle-aged males with cervical or axillary lymphadenophathy; indolent
Hodgkin lymphoma, lymphocyte-rich subtype	Germinal center or postgerminal center B-cell	No specific chromosomal abnormality; 40% EBV associated	More common in males, usually presents with lymphadenopathy; moderately aggressive
Hodgkin lymphoma, mixed cellularity subtype	Postgerminal center memory B-cell	No specific chromosomal abnormality; 70% EBV associated	More common in males, usually presents with lymphadenopathy; moderately aggressive
Hodgkin lymphoma, nodular sclerosing subtype	Germinal center or postgerminal center B-cell	No specific chromosomal abnormality; rarely EBV associated	Commonly presents as a mediastinal mass in young females; moderately aggressive

Figure 14-5 *A,* Acute lymphoblastic leukemia/lymphoma. Lymphoblasts with condensed nuclear chromatin, small nucleoli, and scant agranular cytoplasm. *B* and *C* represent the phenotype of the ALL shown in *A*, analyzed by flow cytometry. *B,* Note that the lymphoblasts represented by the red dots express TdT and the B-cell marker CD22. *C,* The same cells are positive for two other markers, CD10 and CD19, commonly expressed on pre-B lymphoblasts. Thus, this is a pre-B cell ALL. *(A, courtesy of Dr. Robert W. McKenna; B and C, courtesy of Dr. Louis Picker, Oregon Health Science Center, Portland, OR.)*

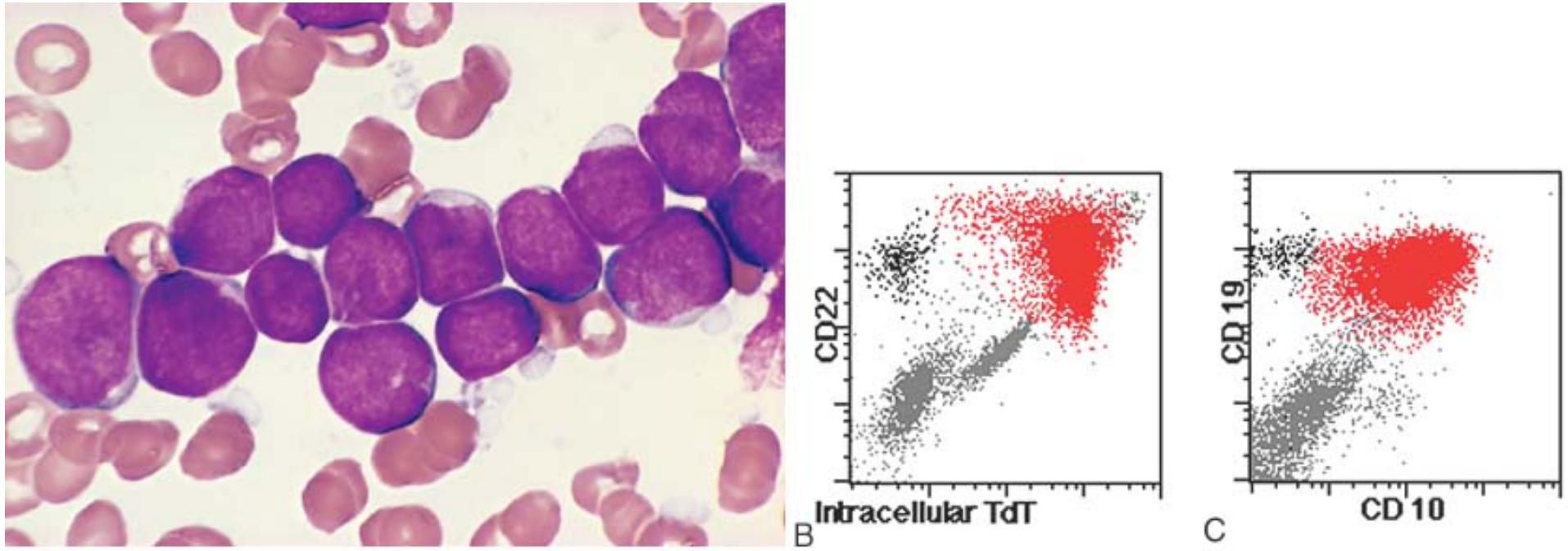


Figure 14-6 Small lymphocytic lymphoma/chronic lymphocytic leukemia (lymph node). *A,* Low-power view shows diffuse effacement of nodal architecture. *B,* At high power, the majority of the tumor cells are small round lymphocytes. A "pro-lymphocyte," a larger cell with a centrally placed nucleolus, is also present in this field (*arrow*). *(A, courtesy of Dr. José Hernandez, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

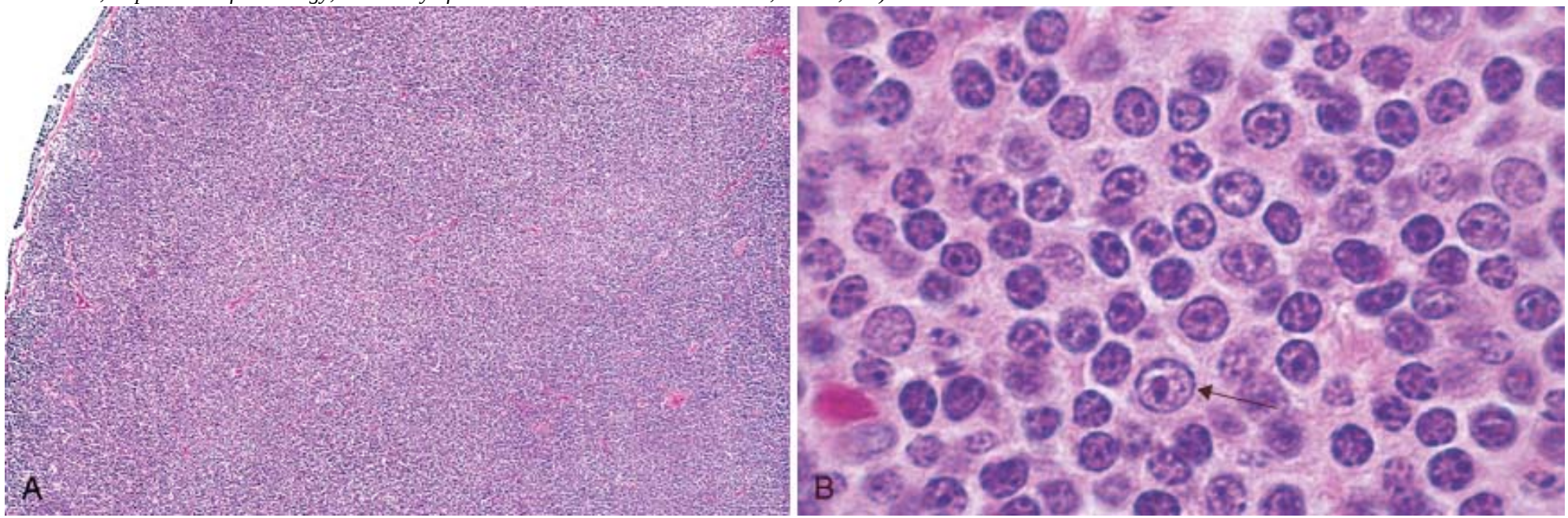


Figure 14-7 Chronic lymphocytic leukemia. This peripheral blood smear is flooded with small lymphocytes with condensed chromatin and scant cytoplasm. A characteristic finding is the

presence of disrupted tumor cells (smudge cells). A coexistent autoimmune hemolytic anemia (see Chapter 13) explains the presence of spherocytes (hyperchromatic, round erythrocytes). A nucleated erythroid cell is present in the lower left-hand corner of the field. In this setting, circulating nucleated red cells could stem from premature release of progenitors in the face of severe anemia, marrow infiltration by tumor (leukoerythroblastosis), or both. *(Courtesy of Dr. Jacqueline Mitus, Brigham and Women's Hospital, Boston, MA.)*

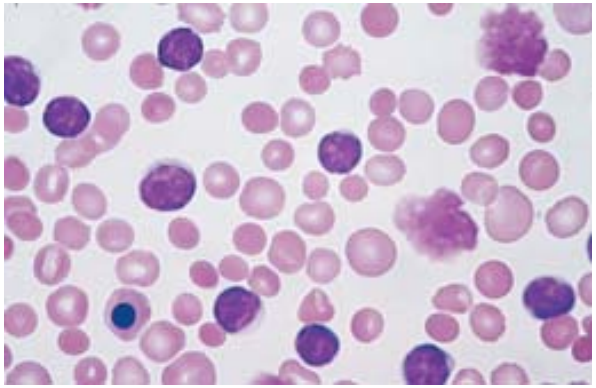


Figure 14-8 Small lymphocytic lymphoma/chronic lymphocytic leukemia (liver). Low-power view of a typical periportal lymphocytic infiltrate. *(Courtesy of Dr. Mark Fleming, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

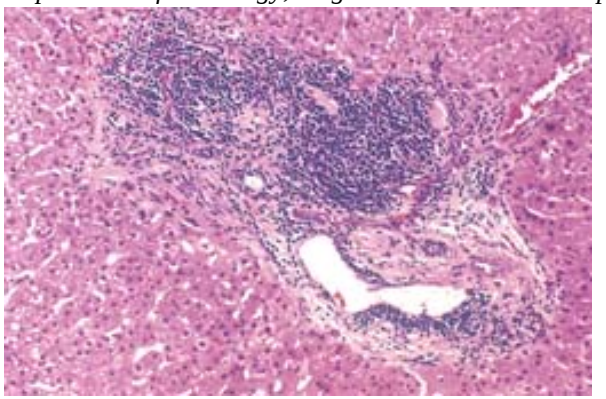


Figure 14-9 Follicular lymphoma (lymph node). *A*, Nodular aggregates of lymphoma cells are present throughout lymph node. *B*, At high magnification, small lymphoid cells with condensed chromatin and irregular or cleaved nuclear outlines (centrocytes) are mixed with a population of larger cells with nucleoli (centroblasts). *(A, courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

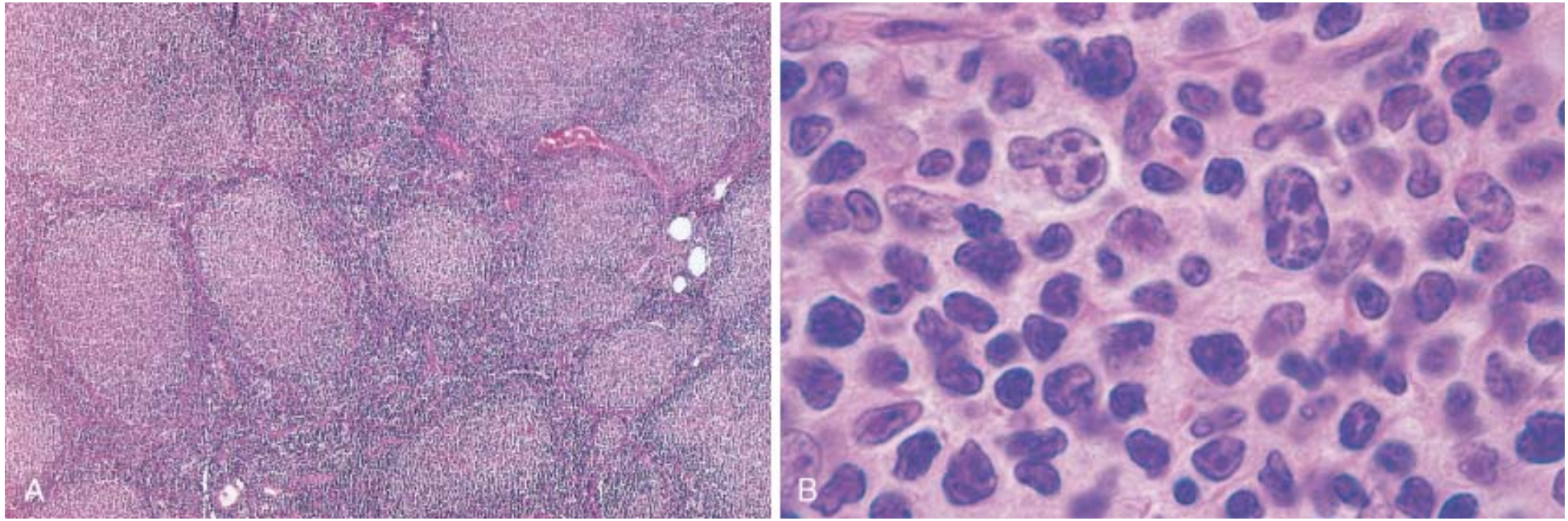


Figure 14-10 Follicular lymphoma (spleen). Prominent nodules represent white pulp follicles expanded by follicular lymphoma cells. Other indolent B-cell lymphomas (small lymphocytic lymphoma, mantle cell lymphoma, marginal zone lymphoma) can produce an identical pattern of involvement. *(Courtesy of Dr. Jeffrey Jorgenson, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

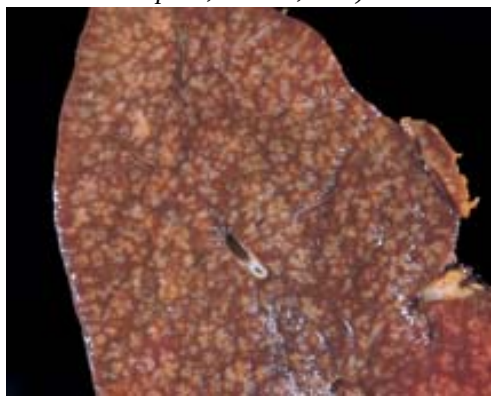


Figure 14-11 BCL2 expression in reactive and neoplastic follicles. BCL2 protein was detected by using an immunohistochemical technique that produces a brown stain. In reactive follicles (*A*), BCL2 is present in mantle zone cells but not follicular center B cells, whereas follicular lymphoma cells (*B*) exhibit strong BCL2 staining *(Courtesy of Dr. Jeffrey Jorgenson, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

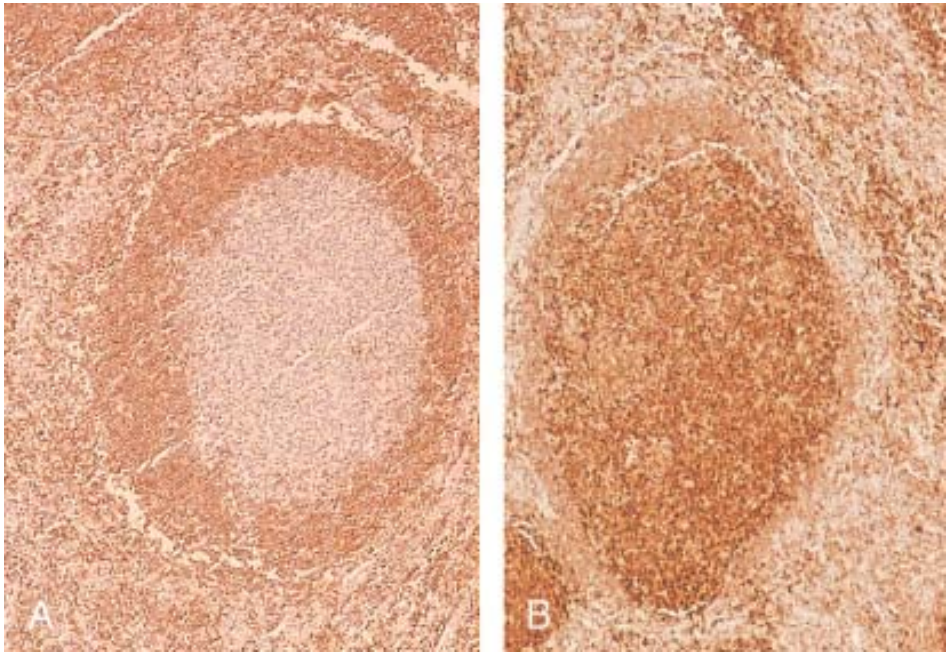


Figure 14-12 Diffuse large B-cell lymphoma. Tumor cells have large nuclei, open chromatin, and prominent nucleoli. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

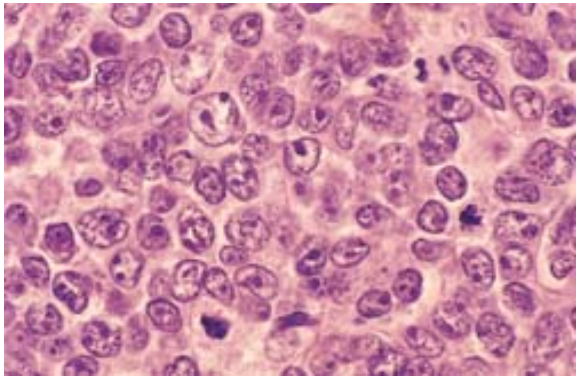


Figure 14-13 Diffuse large B-cell lymphoma (spleen). The presence of an isolated large mass is typical. In contrast, indolent B-cell lymphomas usually produce multifocal expansion of white pulp (see Fig. 14-10). *(Courtesy of Dr. Mark Fleming, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

Figure 14-14 Burkitt lymphoma. *A,* At low power, numerous pale tingible body macrophages are evident, producing a "starry sky" appearance. *B,* At high power, tumor cells have multiple small nucleoli and high mitotic index. The lack of significant variation in nuclear shape and size lends a monotonous appearance. *(B, courtesy of Dr. José Hernandez, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

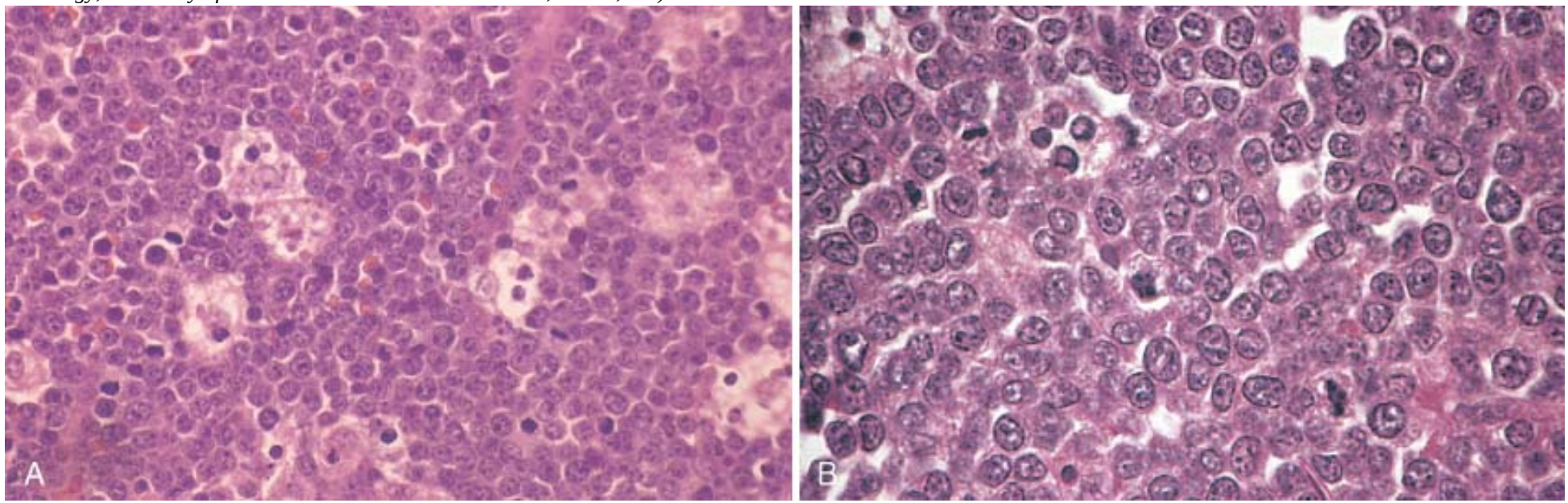


Figure 14-15 Multiple myeloma of the skull (radiograph, lateral view). The sharply punched-out bone lesions are most obvious in the calvarium.

Figure 14-16 Multiple myeloma (bone marrow aspirate). Normal marrow cells are largely replaced by plasma cells, including forms with multiple nuclei, prominent nucleoli, and cytoplasmic droplets containing immunoglobulin.

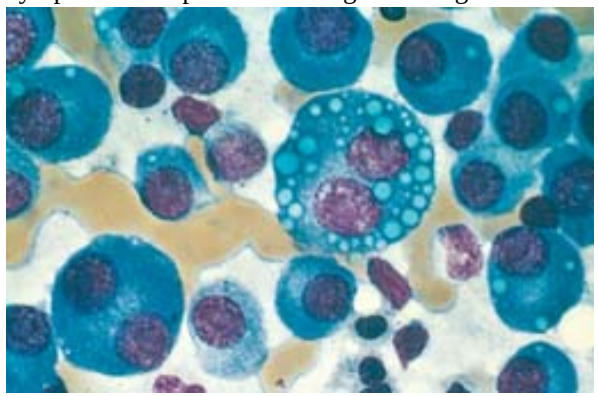


Figure 14-17 M protein detection, multiple myeloma. Serum protein electrophoresis (SP) is used to screen for a monoclonal immunoglobulin (M protein). Polyclonal IgG in normal serum (denoted by the *arrow*) appears as a broad band; in contrast, serum from a patient with multiple myeloma contains a single sharp protein band in this region of the electropherogram. The suspected monoclonal immunoglobulin is confirmed and characterized by immunofixation. In this procedure, proteins separated by electrophoresis within a gel are reacted with specific antisera. After extensive washing of the gel, only proteins that are cross-linked by antisera are retained. These are detected with a protein stain. Note the sharp band in the immunoglobulin region of the patient SP that is recognized by antisera against IgG heavy chain (G) and kappa light chain (κ), indicating the presence of a IgGκ M protein. Levels of polyclonal IgG, IgA (A), and lambda light chain (λ) are also decreased in the patient serum relative to normal, a common finding in multiple myeloma. *(Courtesy of Dr. David Sacks, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

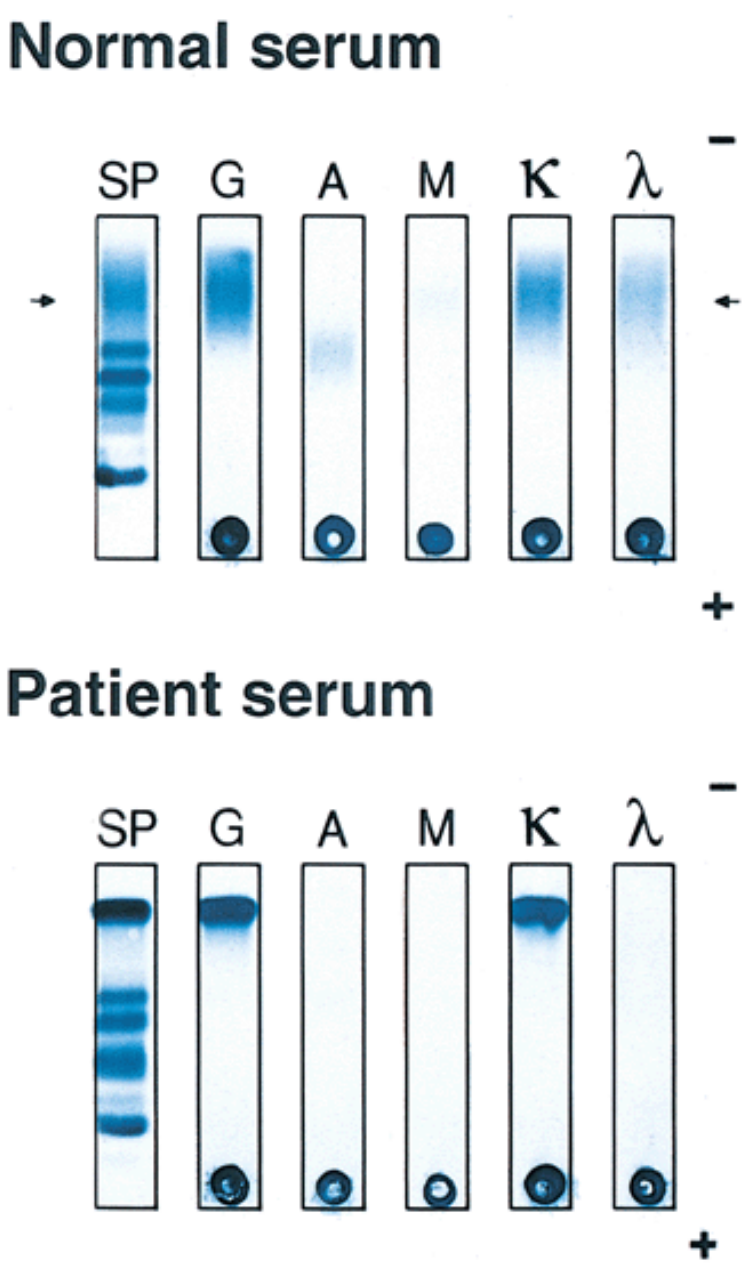


Figure 14-18 Lymphoplasmacytic lymphoma. Bone marrow biopsy shows a characteristic mixture of small lymphoid cells exhibiting various degrees of plasma cell differentiation. In addition, a mast cell with purplish-red cytoplasmic granules is present at the left-hand side of the field.

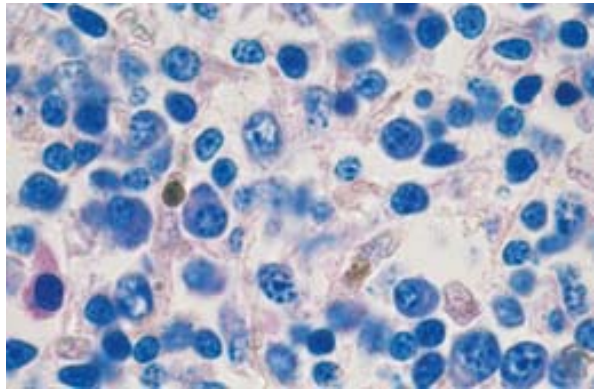


Figure 14-19 Mantle cell lymphoma. *A*, At low power, neoplastic lymphoid cells surround a small, atrophic germinal center, exhibiting a mantle zone pattern of growth. *B*, High-power view shows a homogeneous population of small lymphoid cells with somewhat irregular nuclear outlines, condensed chromatin, and scant cytoplasm. Large cells resembling prolymphocytes (seen in chronic lymphocytic leukemia) and centroblasts (seen in follicular lymphoma) are absent.

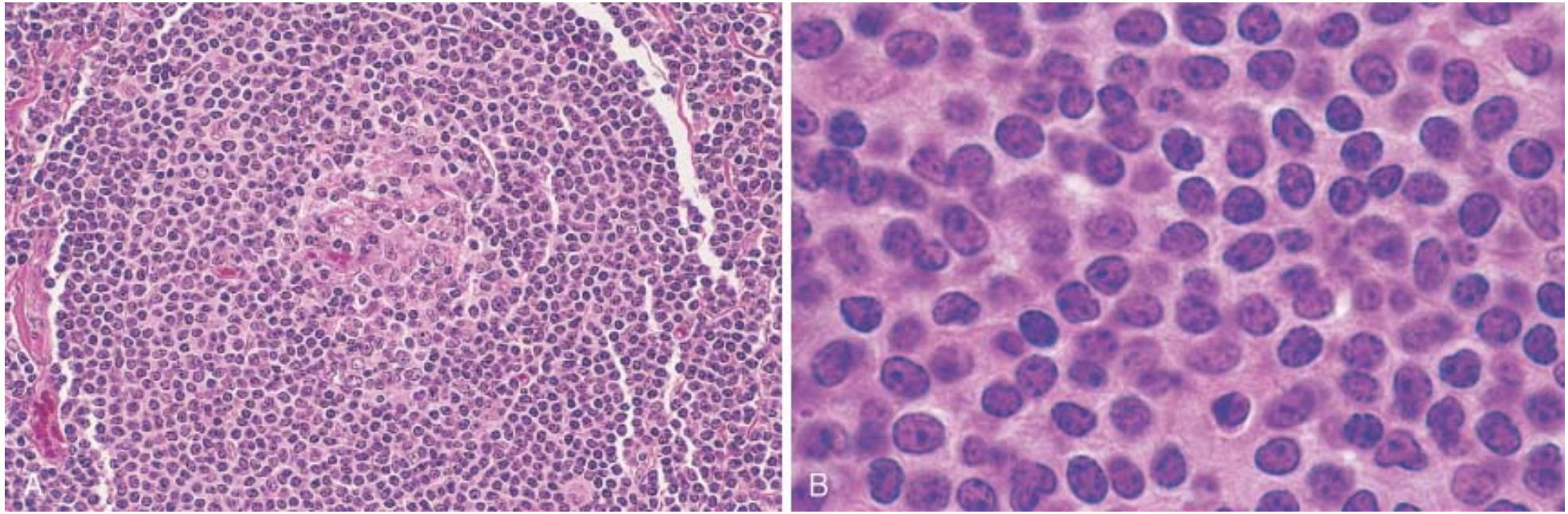


Figure 14-20 Hairy cell leukemia (peripheral blood smear). *A*, Phase-contrast microscopy shows tumor cells with fine hairlike cytoplasmic projections. *B*, In stained smears, these cells have round or folded nuclei and modest amounts of pale-blue, agranular cytoplasm. *(Courtesy of Dr. David Weinberg, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

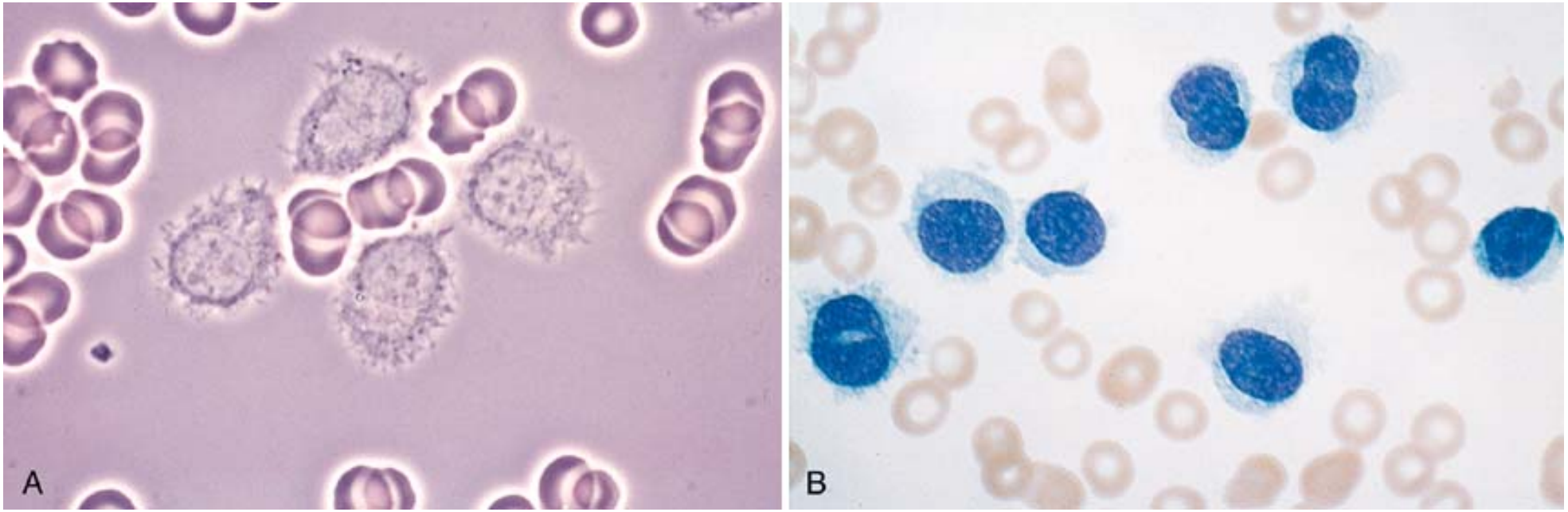


Figure 14-21 Peripheral T-cell lymphoma, unspecified (lymph node). A spectrum of small, intermediate, and large lymphoid cells, many with irregular nuclear contours, is seen.

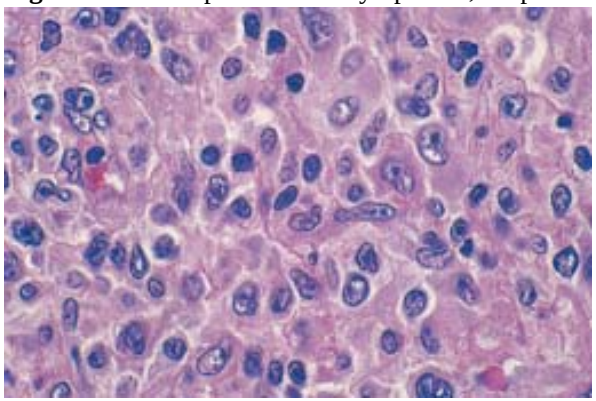


Figure 14-22 Anaplastic large cell lymphoma. *A,* Several "hallmark" cells with horseshoe-like or "embryo-like" nuclei and abundant cytoplasm lie near the center of the field. *B,* Immunohistochemical stain demonstrating expression of ALK protein. *(Courtesy of Dr. Jeffrey Kutok, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

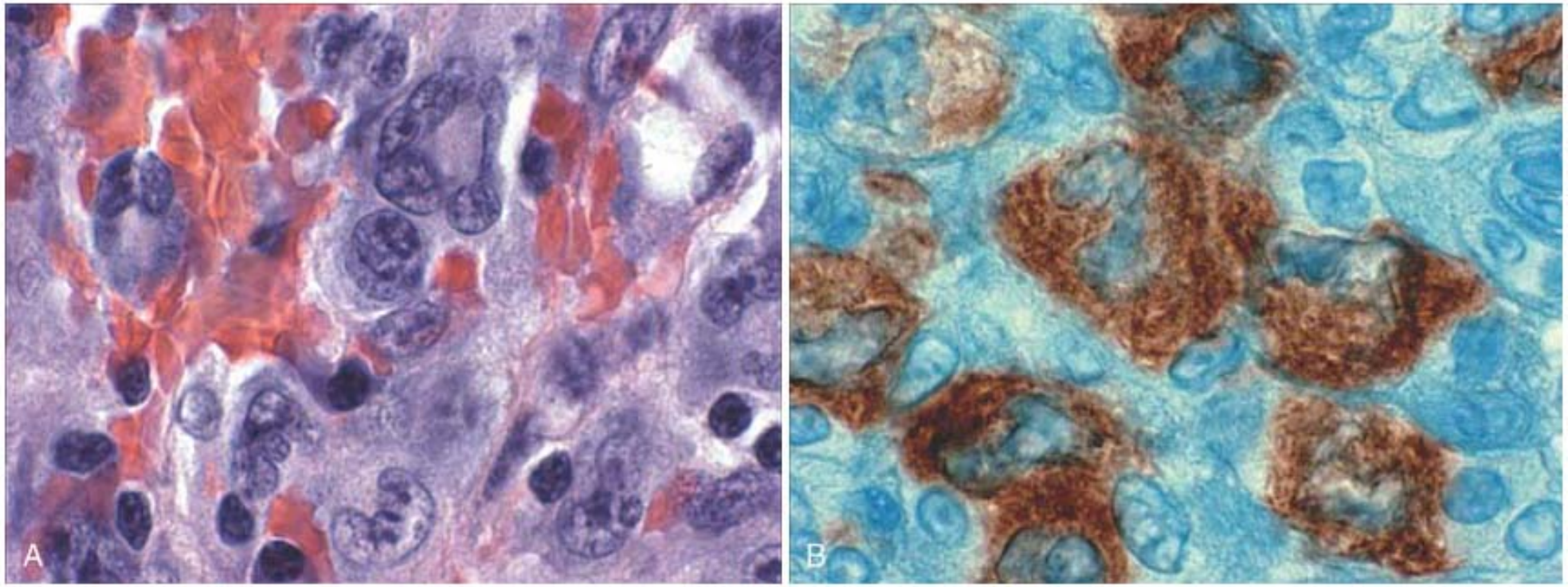


Figure 14-23 Reed-Sternberg cells and variants. *A*, Diagnostic Reed-Sternberg cell, with two nuclear lobes, large inclusion-like nucleoli, and abundant cytoplasm, surrounded by lymphocytes, macrophages, and an eosinophil. *B*, Reed-Sternberg cell, mononuclear variant. *C*, Reed-Sternberg cell, lacunar variant. This variant is characteristic of the nodular sclerosis subtype. It has a folded or multilobated nucleus lying within a clear space created by disruption of its cytoplasm during processing and cutting of the tissue. *D*, Reed-Sternberg cell, lymphohistiocytic (L&H) variant. Several such variants are present with complex nuclear irregularities, small nucleoli, fine chromatin, and abundant pale cytoplasm. *(A, courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

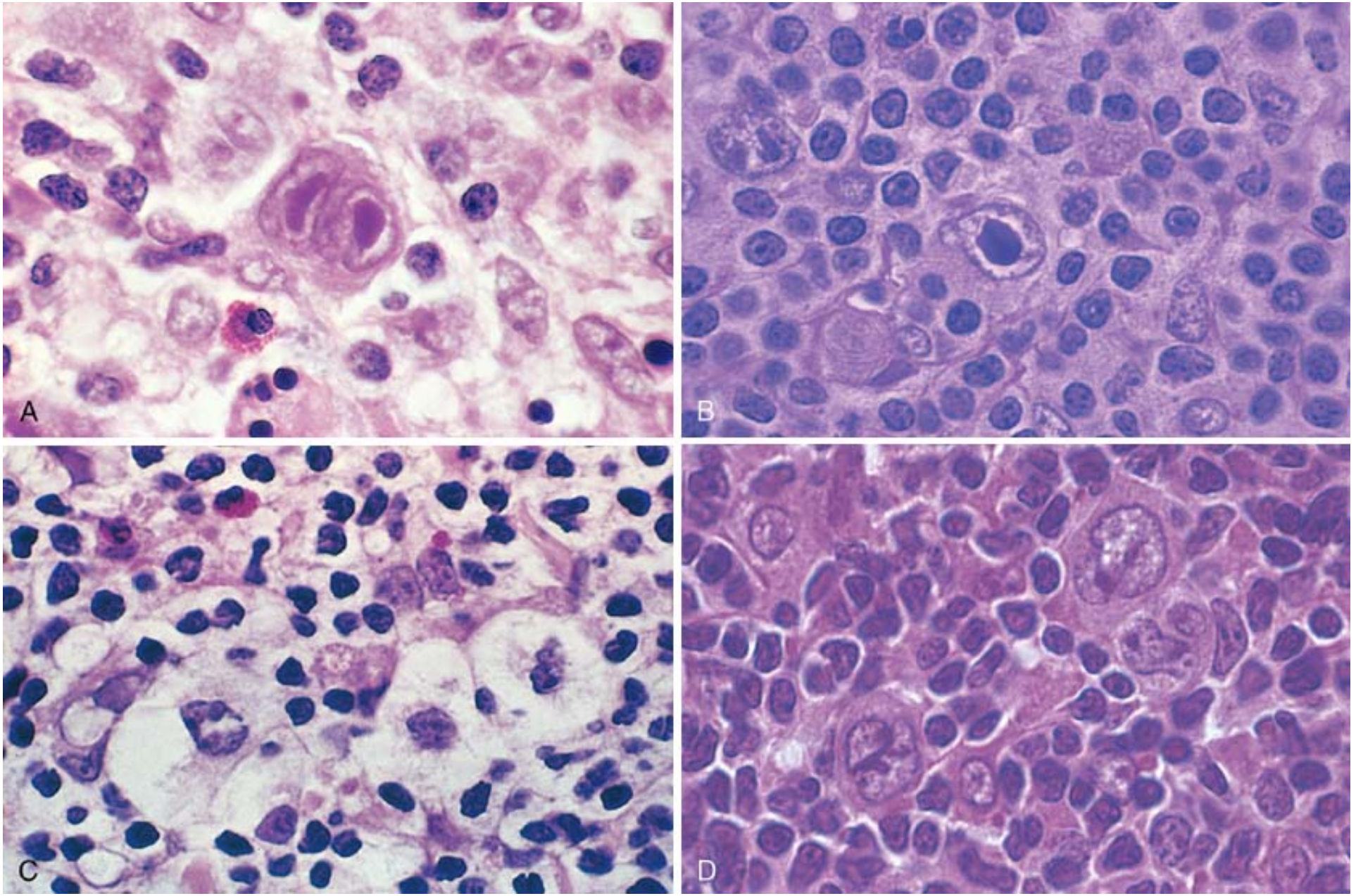


TABLE 14-5 -- Clinical Staging of Hodgkin and Non-Hodgkin Lymphomas (Ann Arbor Classification)

Stage	Distribution of Disease
I	Involvement of a single lymph node region (I) or involvement of a single extralymphatic organ or site (IE).

II	Involvement of two or more lymph node regions on the same side of the diaphragm alone (II) or with involvement of limited contiguous extralymphatic organ or tissue (IIE).
III	Involvement of lymph node regions on both sides of the diaphragm (III), which may include the spleen (IIIS) and/or limited contiguous extralymphatic organ or site (IIIE, IIIES).
IV	Multiple or disseminated foci of involvement of one or more extralymphatic organs or tissues with or without lymphatic involvement.
••All stages are further divided on the basis of the absence (A) or presence (B) of the following systemic symptoms: significant fever, night sweats, and/or unexplained weight loss of greater than 10% of normal body weight.	
Data from Carbone PT, et al: Symposium (Ann Arbor): Staging in Hodgkin's disease. Cancer Res 31:1707, 1971.	

eosinophils, plasma cells, and macrophages. Diagnostic Reed-Sternberg cells are less frequent than in the mixed cellularity and lymphocyte depletion types. The tumor cells have a characteristic immunophenotype: positive for CD15 and CD30 and negative for CD45 and B-cell and T-cell markers. As in other forms of HL, involvement of the spleen, liver, bone marrow,

TABLE 14-6 -- Classification of Hodgkin Lymphoma

Subtype	Morphology and Immunophenotype	Typical Clinical Features
Nodular sclerosis	Frequent lacunar cells and occasional diagnostic R-S cells; background infiltrate composed of T lymphocytes, eosinophils, macrophages and plasma cells; fibrous bands dividing cellular areas into nodules. R-S cells CD15+, CD30+; EBV-.	Stage 1 or 2 disease most common.
		Frequent mediastinal involvement.
		F = M, most patients young adults
Mixed cellularity	Frequent mononuclear and diagnostic R-S cells; background infiltrate rich in T lymphocytes, eosinophils, macrophages, plasma cells. R-S cells CD15+, CD30+; 70% EBV+.	More than 50% present as stage 3 or 4 disease. M > F. Biphasic incidence, peaking in young adults and again in adults older than 55.
Lymphocyte-rich	Frequent mononuclear and diagnostic R-S cells; background infiltrate rich in T lymphocytes. R-S cells CD15+, CD30+; 40% EBV+.	Uncommon. M > F. Tends to be seen in older adults.
Lymphocyte depletion	Reticular variant: Frequent diagnostic R-S cells and variants with a paucity of background reactive cells; diffuse fibrosis variant; hypocellular fibrillar background with scattered diagnostic R-S cells and variants and few reactive cells. R-S cells CD15+, CD30+; most EBV+.	Uncommon. More common in older males, HIV-infected individuals, and in developing countries. More likely to present with advanced disease.
Lymphocyte predominance	Frequent L&H (popcorn cell) variants in a background of follicular dendritic cells and reactive B cells. R-S cells CD20+, CD15-, C30-; EBV-.	Uncommon. Young males with cervical or axillary lymphadenopathy. Mediastinal.

and other organs and tissues can appear in due course and take the form of irregular tumor nodules resembling those present in the nodes.

The nodular sclerosis type occurs with equal frequency in males and females. It has a propensity to involve the lower cervical, supraclavicular, and mediastinal lymph nodes of adolescents or young adults and is only rarely associated with EBV. The prognosis is excellent.

Hodgkin Lymphoma, Mixed Cellularity Type.

This form of HL constitutes about 20% to 25% of cases. Lymph node involvement by the mixed cellularity type takes the form of **diffuse effacement** by a heterogeneous cellular infiltrate, which includes small lymphocytes, eosinophils, plasma cells, and benign macrophages admixed with the neoplastic cells (Fig. 14-25). **Diagnostic Reed-Sternberg cells and mononuclear variants are usually plentiful.** The immunophenotype is identical to that observed in the nodular sclerosis type. Small lymphocytes in the background are predominantly T cells, and early nodal disease preferentially involves paracortical T-cell zones.

Mixed cellularity HL is more common in males and strongly associated with EBV, as the Reed-Sternberg cells contain EBV genomes in at least 70% of cases. Compared to the lymphocyte predominance and nodular sclerosis subtypes, it is more likely to be associated with older age, systemic symptoms such as night sweats and weight loss, and advanced tumor stage. Nonetheless, the prognosis is very good.

Hodgkin Lymphoma, Lymphocyte-Rich Type.

This is an uncommon form of classical HL in which reactive

Figure 14-24 Hodgkin lymphoma, nodular sclerosis type. A low-power view shows well-defined bands of pink, acellular collagen that subdivide the tumor cells and associated reactive infiltrate into nodules. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

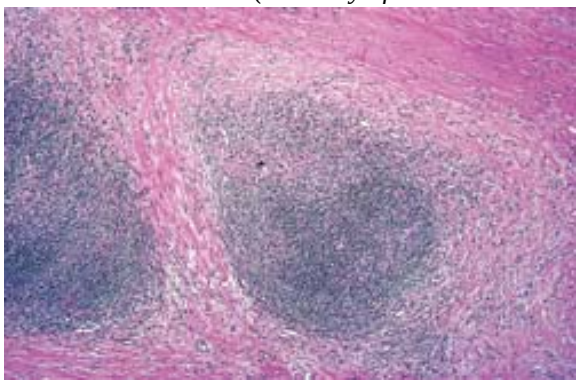


Figure 14-25 Hodgkin lymphoma, mixed cellularity type. A diagnostic, binucleate Reed-Sternberg cell is surrounded by reactive cells, including eosinophils (bright red cytoplasm), lymphocytes, and histiocytes. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

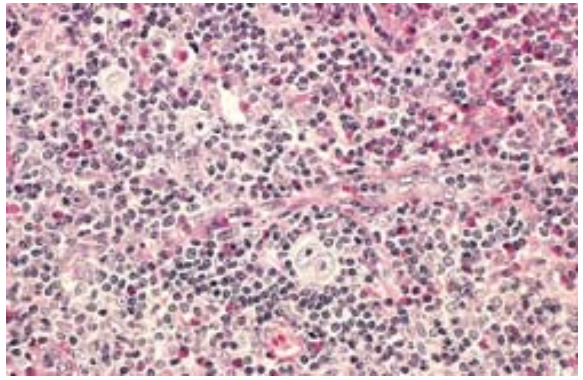


Figure 14-26 Hodgkin lymphoma, lymphocyte predominance type. Numerous mature-looking lymphocytes surround scattered, large, pale-staining L&H variants ("popcorn" cells). *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

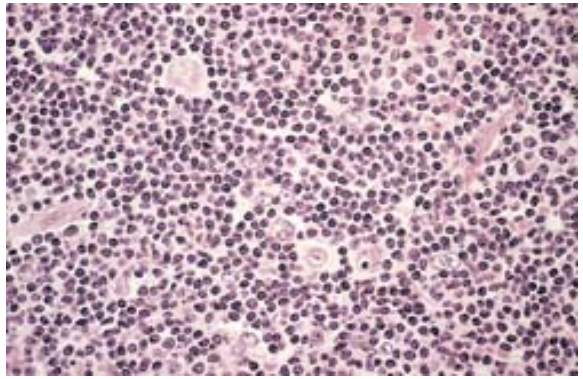


Figure 14-27 Proposed signals mediating "cross-talk" between Reed-Sternberg cells and surrounding normal cells in classical forms of Hodgkin lymphoma. bFGF, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGF, hepatocyte growth factor (binds to the C-MET receptor); TNFβ, tumor necrosis factor β (lymphotoxin); TGFβ, transforming growth factor β.

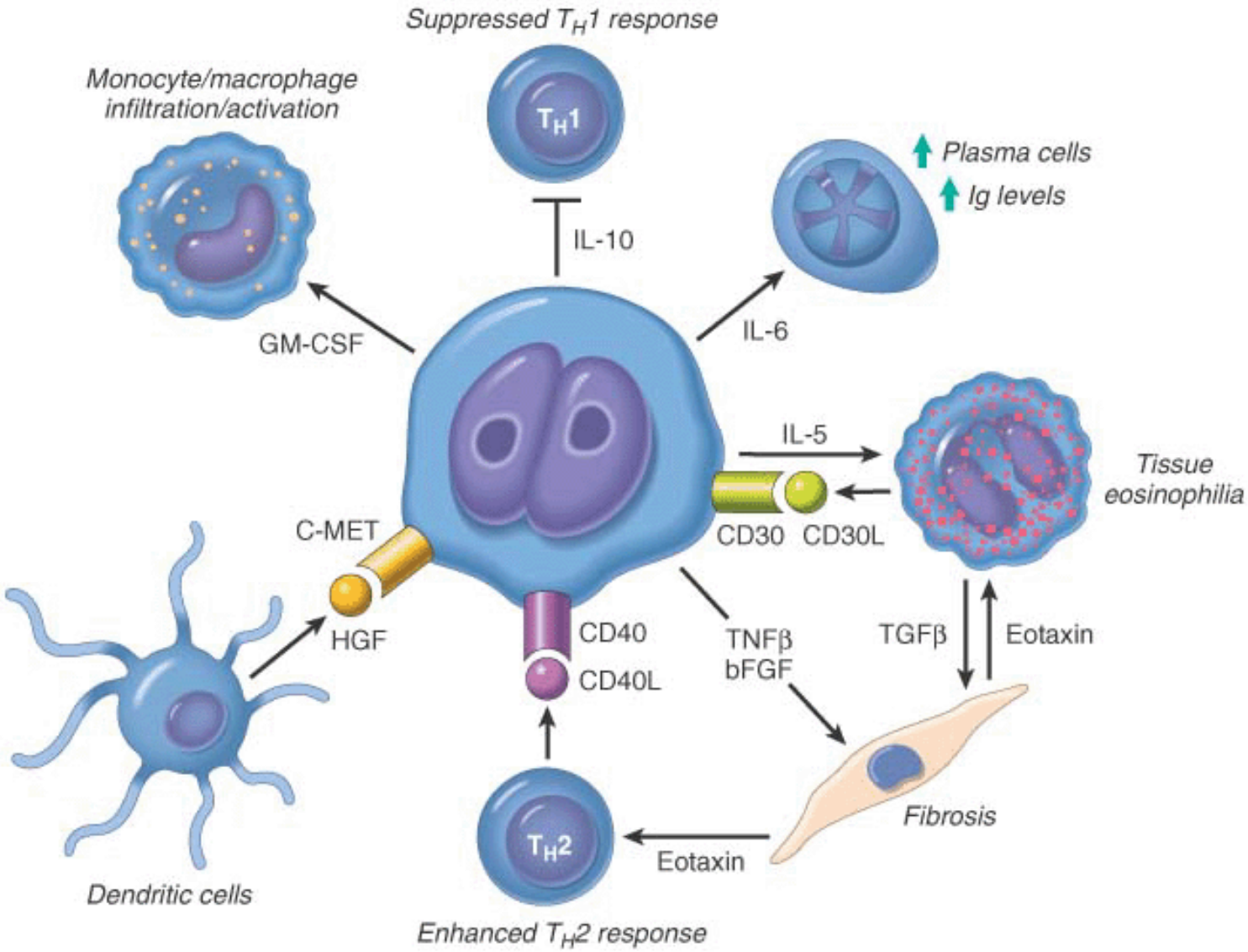


TABLE 14-7 -- Clinical Differences Between Hodgkin and Non-Hodgkin Lymphomas

Hodgkin Lymphoma	Non-Hodgkin Lymphoma
More often localized to a single axial group of nodes (cervical, mediastinal, para-aortic)	More frequent involvement of multiple peripheral nodes
Orderly spread by contiguity	Noncontiguous spread
Mesenteric nodes and Waldeyer ring rarely involved	Waldeyer ring and mesenteric nodes commonly involved
Extranodal involvement uncommon	Extranodal involvement common

myeloid series (erythrocytes, granulocytes, monocytes, and platelets). These diseases primarily involve the bone marrow and to a lesser degree the secondary hematopoietic organs (the spleen, liver, and lymph nodes) and present with altered hematopoiesis. Three broad categories of myeloid neoplasia exist:

- *Acute myelogenous leukemias*, characterized by the accumulation of immature myeloid forms in the bone marrow and the suppression of normal hematopoiesis
- *Myelodysplastic syndromes*, associated with ineffective hematopoiesis and associated cytopenias
- *Chronic myeloproliferative disorders*, usually associated with an increased production of terminally differentiated myeloid cells

The pathogenesis of myeloid neoplasms is best understood in the context of normal hematopoiesis, which (you will remember from Chapter 13) involves a hierarchy of hematopoietic progenitor cells. At the top of the hierarchy sits the pluripotent stem cell, which gives rise to multipotent progenitor cells committed to lymphoid or myeloid differentiation. The latter in turn produce more committed progenitors, which eventually give rise to terminally differentiated cells of a single type (e.g., erythrocyte, monocyte). In addition to giving rise to committed daughter cells, hematopoietic progenitor cells must also replicate themselves without differentiating (or else they would eventually disappear), a process known as self-renewal.

Normal hematopoiesis is finely tuned by homeostatic feedback mechanisms involving cytokines and growth factors that modulate the marrow output of red cells, granulocytes, and platelets. These mechanisms are deranged in marrows involved by myeloid neoplasms, which "escape" from normal homeostatic controls on growth and survival, and suppress the function of residual normal stem cells. The specific manifestations of the different myeloid neoplasms are further influenced by *(1) the position of the transformed cell within the hierarchy of progenitors* and *(2) the effect of the transforming events on differentiation programs, which may be blocked or preferentially shunted toward one lineage at the expense of others*. We will return to these themes as each type of myeloid neoplasm is discussed.

Given that all myeloid neoplasms originate from a transformed hematopoietic progenitor cell, it should come as no surprise that divisions between these neoplasms are sometimes blurred. Myeloid neoplasms, like other malignancies, tend to evolve over time to more aggressive forms of disease.

In particular, both the myelodysplastic syndromes and the chronic myeloproliferative disorders often "transform" to acute myelogenous leukemias. In the specific case of one of the myeloproliferative disorders, chronic myelogenous leukemia, transformation to acute lymphoblastic leukemia/lymphoma is also seen, indicating a likely origin from a transformed pluripotent stem cell.

Acute Myelogenous Leukemia

Acute myelogenous leukemias affect primarily adults, peaking in incidence between the ages of 15 and 39 years, but are also observed in older adults and children. AML is quite heterogeneous, reflecting the complexities of myeloid cell differentiation.

Pathophysiology.

Most AMLs are associated with acquired genetic alterations that inhibit terminal myeloid differentiation. As a result, normal marrow elements are replaced by relatively undifferentiated blasts exhibiting one or more types of early myeloid differentiation. The replication rate of these blasts is actually lower than that of normal myeloid progenitors, highlighting the pathogenic importance of blocked maturation and increased survival.

Specific recurrent chromosomal aberrations, including translocations, are seen in a high fraction of AMLs and tend to disrupt genes encoding transcription factors needed for normal myeloid differentiation. For example, the most common chromosomal rearrangements, t(8;21) and inv(16), involve genes that normally encode two subunits, CBF1α and CBF1β, of a single heterodimeric transcription factor. Both the t(8;21) and the inv(16) result in the formation of chimeric genes encoding fusion proteins with so-called dominant negative activity,

meaning they interfere with the function of the normal CBF1α/CBF1β heterodimer. Myeloid progenitors harboring such aberrations thus give rise to daughter cells exhibiting a partial or complete block in terminal differentiation. A deficit of CBF1α/CBF1β activity is not sufficient to cause leukemia, however, as "knockout" mice lacking either CBF1α or CBF1β, or "knock-in" mice expressing the fusion proteins created by the t(8;21) or inv(16),[66] succumb to hematopoietic failure. In such animals, the "blocked" progenitors die rather than undergoing transformation, indicating that other aberrations must collaborate with defects in critical transcription factors to produce AML.

An example of such a pathogenic collaboration underlies a form of AML, acute promyeloctyic leukemia, associated with a (15;17) chromosomal translocation. This translocation produces a fusion gene encoding a portion of a transcription factor, retinoic acid receptor-α (RARα), fused to a portion of another protein, PML. RARα normally activates transcription, but when fused to PML, it is converted to a repressor that turns off genes required for full and complete myeloid differentiation. In addition to the t(15;17), acute promyelocytic leukemia cells also frequently acquire point mutations in FLT3, a tyrosine kinase, that result in its constitutive activation.[67] As you will recall from Chapter 3 , tyrosine kinases produce signals that promote cellular proliferation and survival, activities that synergize with the block in differentiation produced by the RARα-PML fusion protein. This pathogenic collaboration has been proven in mouse models, in which coexpression of a RARα-PML fusion protein and activated forms of FLT3 produce the rapid onset of AML. It is believed that distinct, but pathogenically analogous, sets of synergistic genetic "hits" underlie other forms of AML.[68]

In all AMLs, the accumulation of proliferating neoplastic myeloid precursor cells in the marrow suppresses remaining normal hematopoietic progenitor cells by physical replacement as well as by other unknown mechanisms. The failure of normal hematopoiesis results in anemia, neutropenia, and thrombocytopenia, which cause most of the major clinical complications of AML. Therapeutically, the aim is to clear the bone marrow of the leukemic clone, thus permitting resumption of normal hematopoiesis. This can be accomplished by treatment with cytotoxic drugs or, in the specific case of acute promyelocytic leukemia, by overcoming the block in differentiation with pharmacologic doses of retinoic acid.

Classification.

In the most widely used system in current use, the revised FAB classification (Table 14-8*A*), AML is divided into eight (M0 to M7) categories.[69] This scheme takes into account both the degree of maturation (M0 to M3) and the lineage of the leukemic blasts (M4 to M7). Histochemical stains for peroxidase, specific esterase, and nonspecific esterase, and immunostains for myeloid specific antigens (see Table 14-8A) play important roles in defining the type of myeloid differentiation that blasts exhibit.

A recently proposed WHO classification for AML (Table 14-8*B*) retains the FAB categories M0 to M7 but also creates special categories for AMLs associated with particular chromosomal aberrations (e.g., the t(15;17), t(8;21), inv(16), or 11q23 rearrangements), which arise after prior chemotherapy or follow a myelodysplastic syndrome.[11] This classification thus attempts to define forms of AML according to molecular pathogenesis and outcome. Given the increasing role of cytogenetic and molecular features in directing therapy, a further shift toward molecular genetic classifications of AML seems inevitable and desirable.

Morphology.

The diagnosis of AML is based on finding that myeloid blasts make up more than 20% of the cells in the marrow. Several types of myeloid blasts are recognized, but more than one type of blast, or blasts with hybrid features, can be seen in individual patients. **Myeloblasts** have delicate nuclear chromatin, two to four nucleoli, and more voluminous cytoplasm than lymphoblasts (Fig. 14-28*A*). The cytoplasm often contains fine, azurophilic, peroxidase-positive granules. Distinctive red-staining peroxidase-positive structures called **Auer rods**, which represent abnormal azurophilic granules, are present in many cases and are particularly numerous in AML associated with the t(15;17) (acute promyelocytic leukemia) (Fig. 14-29*A*). The presence of Auer rods is taken to be definitive evidence of myeloid differentiation. **Monoblasts** (Fig. 14-29*B*) often have folded or lobulated nuclei, lack Auer rods, and are peroxidase negative and nonspecific esterase positive. In some AMLs, blasts exhibit megakaryocytic differentiation, which is often accompanied by marrow fibrosis caused by the release of fibrogenic cytokines. Rarely, the blasts of AML show evidence of erythroid differentiation (erythroblasts).

The number of leukemic cells in the peripheral blood is highly variable. Blast counts can be more than 100,000 cells per microliter but are under 10,000

TABLE 14-8A -- Revised FAB Classification of Acute Myelogenous Leukemias

Class	Incidence (% of AML)	Marrow Morphology/Comments
M0 Minimally differentiated AML	2–3%	Blasts lack definitive cytologic and cytochemical markers of myeloblasts (e.g., myeloperoxidase negative) but express myeloid lineage antigens and resemble myeloblasts ultrastructurally.
M1 AML without differentiation	20%	Very immature, but ≥3% of blasts are peroxidase positive; few granules or Auer rods and little maturation beyond the myeloblast stage.
M2 AML with maturation	30–40%	Full range of myeloid maturation through granulocytes; Auer rods present in most cases; often associated with the t (8;21).
M3 Acute promyelocytic leukemia	5–10%	Most cells are hypergranular promyelocytes, often with many Auer rods per cell; patients are younger (median age 35 to 40 years); high incidence of DIC; strong association with the t(15;17).
M4 Acute myelomonocytic leukemia	15–20%	Myelocytic and monocytic differentiation evident; myeloid elements show range of maturation; monoblasts are positive for nonspecific esterases; subset associated with the inv(16).
M5 Acute monocytic leukemia	10%	In M5a subtype, monoblasts (peroxidase-negative, nonspecific esterase-positive) and promonocytes predominate in marrow and blood; in M5b subtype, mature monocytes predominate in the peripheral blood; M5a and M5b occur in older patients; characterized by high incidence of organomegaly, lymphadenopathy, and tissue infiltration.
M6 Acute erythroleukemia	5%	Dysplastic erythroid precursors (some megaloblastoid, others with giant or multiple nuclei) predominate, and within the non-erythroid cells, >30% are myeloblasts; seen in advanced age; makes up 1% of de novo AML and 20% of therapy-related AML.
M7 Acute megakaryocytic leukemia	1%	Blasts of megakaryocytic lineage predominate; blasts react with platelet-specific antibodies directed against GPIIb/IIIa or vWF; myelofibrosis or increased marrow reticulin seen in most cases.

DIC, disseminated intravascular coagulation; vWF, von Willebrand factor.

TABLE 14-8B -- Proposed WHO Classification of Acute Myelogenous Leukemias

Class	Prognosis
I. AML with Recurrent Chromosomal Rearrangements	
AML with t(8;21)(q22;q22); *CBFα/ETO* fusion gene	Favorable
AML with inv(16)(p13;q22); *CBFβ/MYH11* fusion gene	Favorable
AML with t(15;17)(q22;11-12); *RARα/PML* fusion gene	Intermediate
AML with t(11q23;v); diverse *MML* fusion genes	Poor

II. AML with Multilineage Dysplasia	
With prior myelodysplastic syndrome	Very poor
Without prior myelodysplastic syndrome	Poor
III. AML, Therapy Related	
Alkylating agent related	Very poor
Epipodophyllotoxin related	Very poor
IV. AML, not Otherwise Specified	
Sub-classes defined by extent of differentiation and FAB classification (e.g., M0-M7)	Intermediate

cells per microliter in about 50% of the patients. **Occasionally, the peripheral smear might not contain any blasts** (aleukemic leukemia). For this reason, bone marrow examination is essential to exclude acute leukemia in pancytopenic patients.

Immunophenotype.

Because it is difficult to distinguish myeloblasts and lymphoblasts morphologically in some cases, the diagnosis of AML is typically confirmed by staining cells for myeloid-specific surface markers (Fig. 14-28*B*, *C*).

Chromosomal Abnormalities.

Special high-resolution banding techniques reveal chromosomal abnormalities in approximately 90% of all AML patients. In 50% to 70% of the cases, the karyotypic changes are detected by standard cytogenetic techniques.

Particular chromosomal abnormalities correlate with the clinical setting in which the tumor occurs. AML arising de novo in patients with no risk factors are often associated with balanced chromosomal translocations, particularly t(8;21), inv(16), and t(15;17). In contrast, AMLs following myelodysplastic syndromes or exposure to DNA-damaging agents (such as chemotherapy or radiation therapy) are commonly associated with deletions or monosomies involving chromosomes 5 and 7 and usually lack chromosomal translocations. The exception to this rule is AML occurring after treatment with topoisomerase II inhibitors, which is often associated with translocations involving the *MLL* gene on chromosome 11 at band q23.[70]

Clinical Features.

The clinical findings in AML are similar to those in acute lymphoblastic leukemia/lymphoma (ALL). *Most patients present within weeks or a few months of the onset of symptoms related to anemia, neutropenia, and thromobocytopenia*, most notably fatigue, fever, and spontaneous mucosal and cutaneous bleeding. Often, the bleeding diathesis caused by thrombocytopenia is the most striking clinical feature.

Figure 14-28 *A,* Acute myelogenous leukemia (FAB M1 subtype). Myeloblasts have delicate nuclear chromatin, prominent nucleoli, and fine azurophilic granules in the cytoplasm. *B,* In

the flow cytometric analysis shown, the myeloid blasts, represented by the red dots, express CD34, a marker of multipotent stem cells, but do not express CD64, a marker of mature myeloid cells. *C*, The same myeloid blasts express CD33, a marker of immature myeloid cells, and a subset express CD15, a marker of more mature myeloid cells. Thus, these blasts are minimally differentiated myeloid cells. *(A, courtesy of Dr. Robert W. McKenna Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX; B and C, courtesy of Dr. Louis Picker, Oregon Health Science Center, Portland, OR.)*

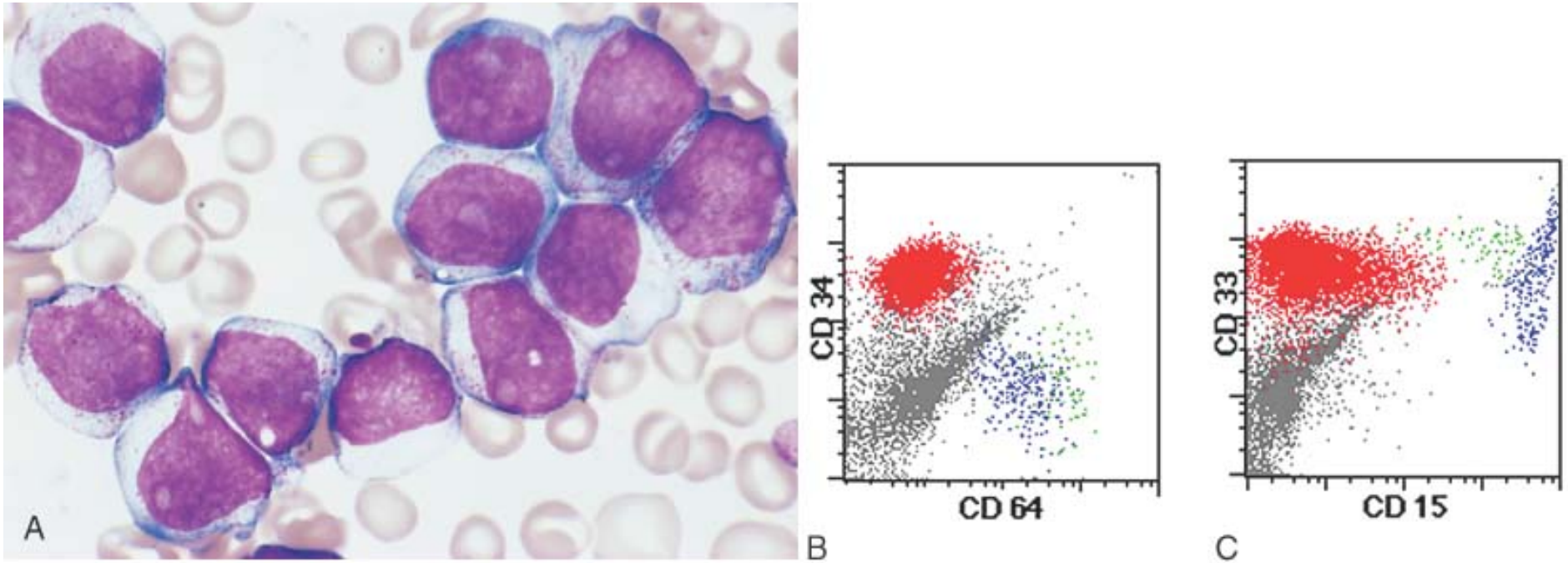


Figure 14-29 Acute myelogenous leukemia subtypes. *A*, Acute promyelocytic leukemia (FAB M3 subtype). Bone marrow aspirate shows neoplastic promyelocytes with abnormally coarse and numerous azurophilic granules. Other characteristic findings include the presence of several cells with bilobed nuclei and a cell in the center of the field that contains multiple needlelike Auer rods. *B*, Acute monocytic leukemia (FAB M5b subtype). Peripheral smear shows one monoblast and five promonocytes with folded nuclear membranes. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

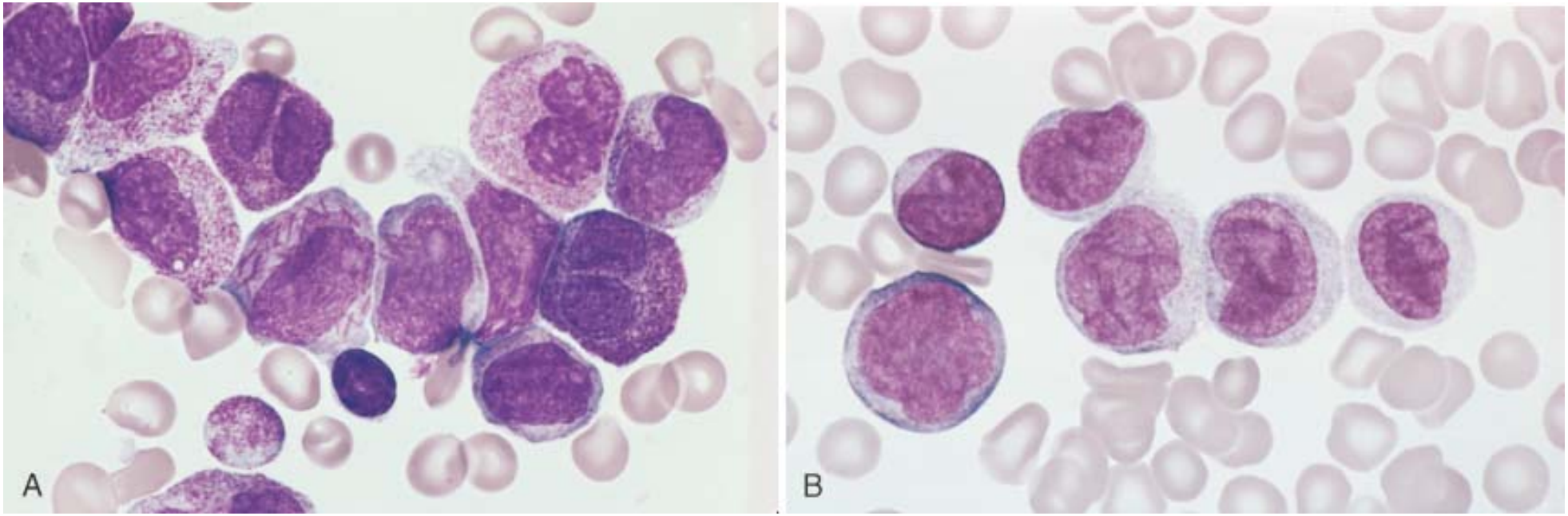


Figure 14-30 Myelodysplasia. Characteristic forms of dysplasia are shown. *A*, Nucleated red cell progenitors with multilobated or multiple nuclei. *B*, Ringed sideroblasts, erythroid progenitors with iron-laden mitochondria, seen as blue perinuclear granules (Prussian blue stain). *C*, Pseudo-Pelger-Hüet cells, neutrophils with only two nuclear lobes instead of the normal three to four, are observed at the top and bottom of this field. *D*, Megakaryocytes with multiple nuclei instead of the normal single multilobated nucleus. (*A*, *B*, *D*, marrow aspirates; *C*, peripheral blood smear.)

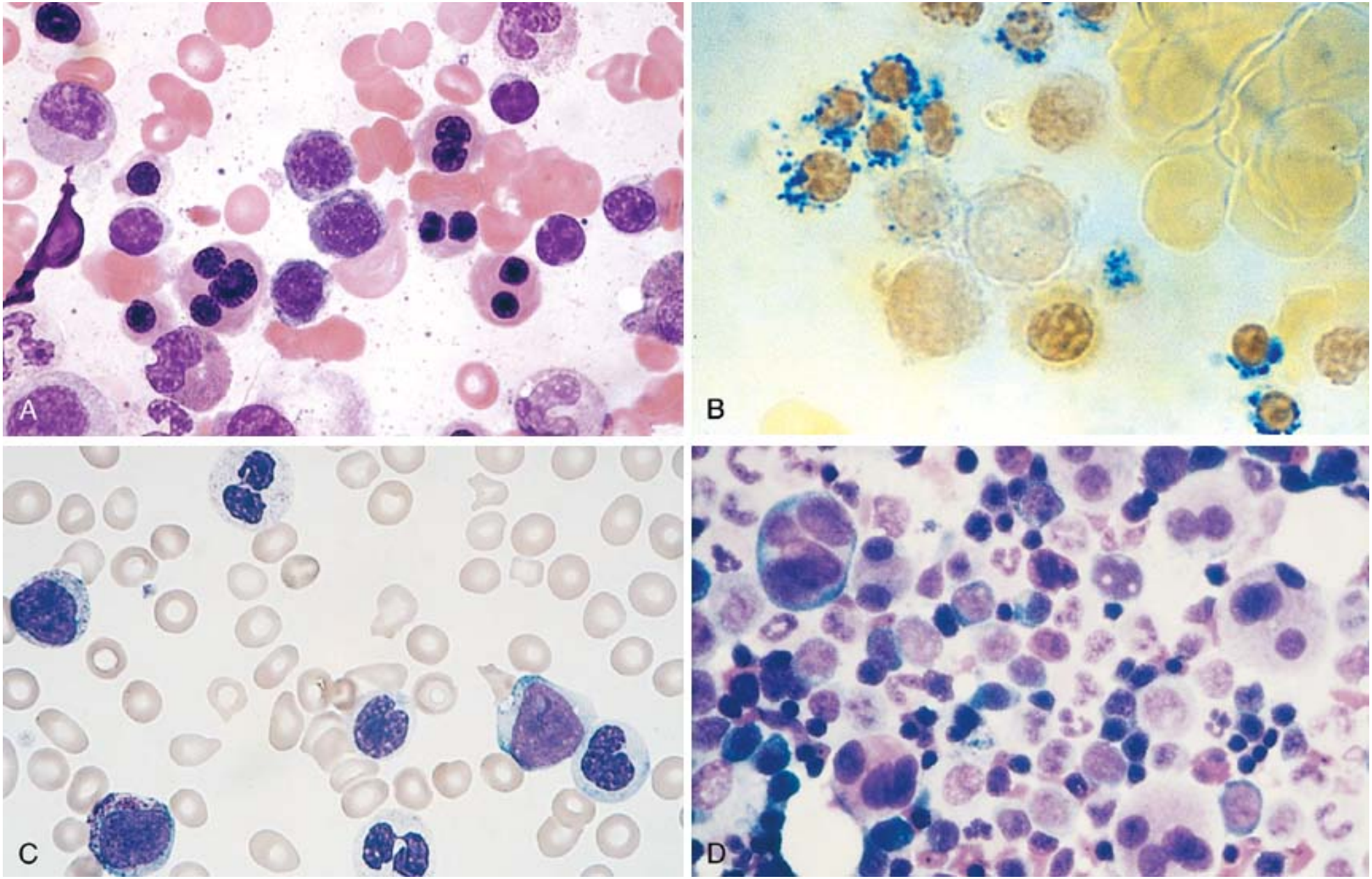
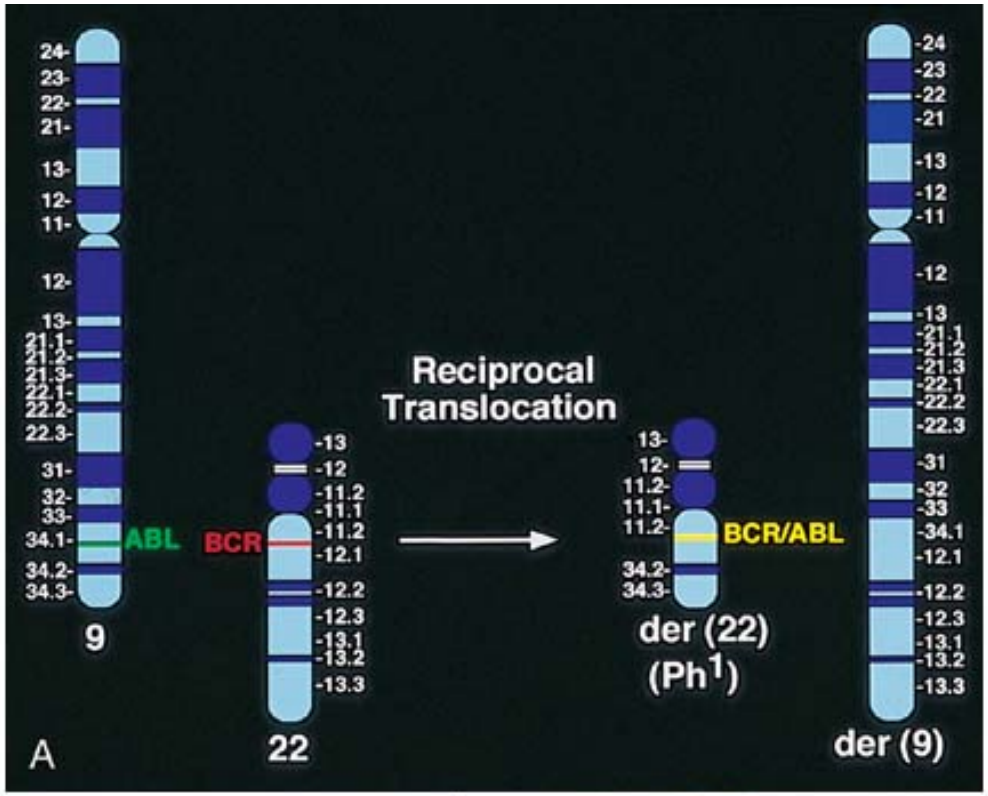
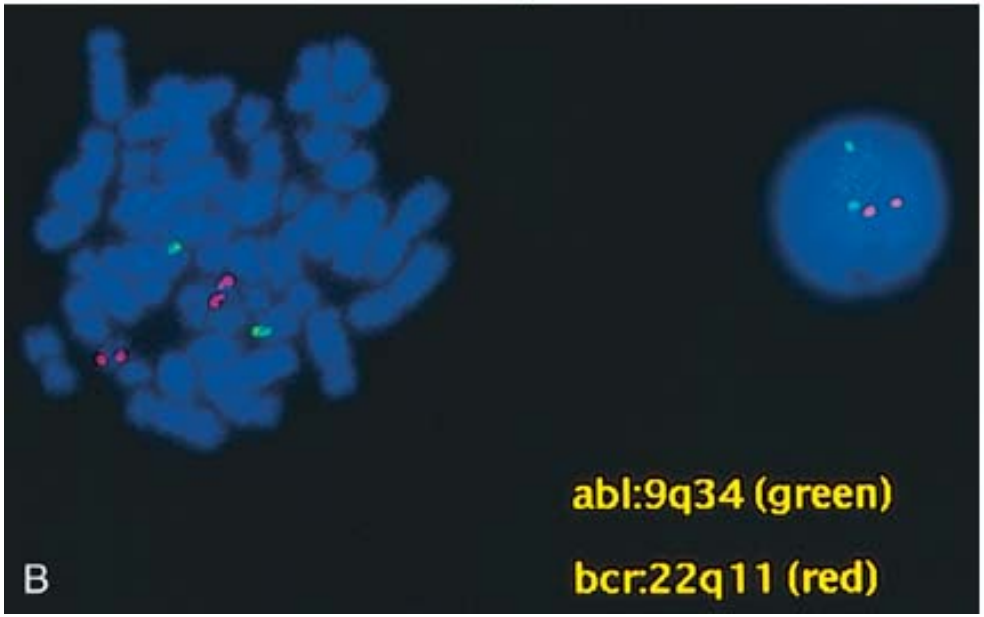


Figure 14-31 Detection of a *BCR-ABL* fusion gene by fluorescence in situ hybridization. *A,* An idiogram depicting chromosomes 9 and 22 and the position of the *ABL* and *BCR* genes. The Philadelphia chromosome (Ph) is created by a balanced chromosomal translocation that replaces the telomeric portion of 22q with the telomeric portion of 9q. At a molecular level, the breaking and rejoining of the DNA results in the formation of fusion gene on the Ph derived from the 5' end of *BCR* and the 3' end of *ABL* and hence brings *BCR* and *ABL* sequences that are normally far apart into close physical proximity. This abnormal colocalization of *BCR* and *ABL* can be detected by in situ hybridization with pairs of fluorescently tagged DNA probes complementary to genomic DNA sequences lying near the *BCR* and *ABL* breakpoints. *B,* A green *ABL* probe and a red *BCR* probe have been hybridized to metaphase chromosomes and interphase nuclei prepared from the peripheral blood cells of a normal individual. Because of the pairing of sister chromatids during mitosis, signals on metaphase chromosomes may be seen as a single dot or a pair of closely spaced dots. Two pairs of red signals and two green signals are seen on the metaphase chromosomes, while two red and two green signals are

present in the interphase nucleus, indicating the presence of normal, spatially distant copies of *ABL* and *BCR*, respectively. *C*, In contrast, metaphase chromosomes and an interphase nucleus prepared from the bone marrow cells of a patient with CML show one normal *ABL* signal, one normal *BCR* signal, and an abnormal yellow signal created by superimposition of one *BCR* and one *ABL* signal, a finding indicative of the presence of a *BCR-ABL* fusion gene. *(Courtesy of Dr. Cynthia Morton and Ms. Debbie Sandstrom, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*





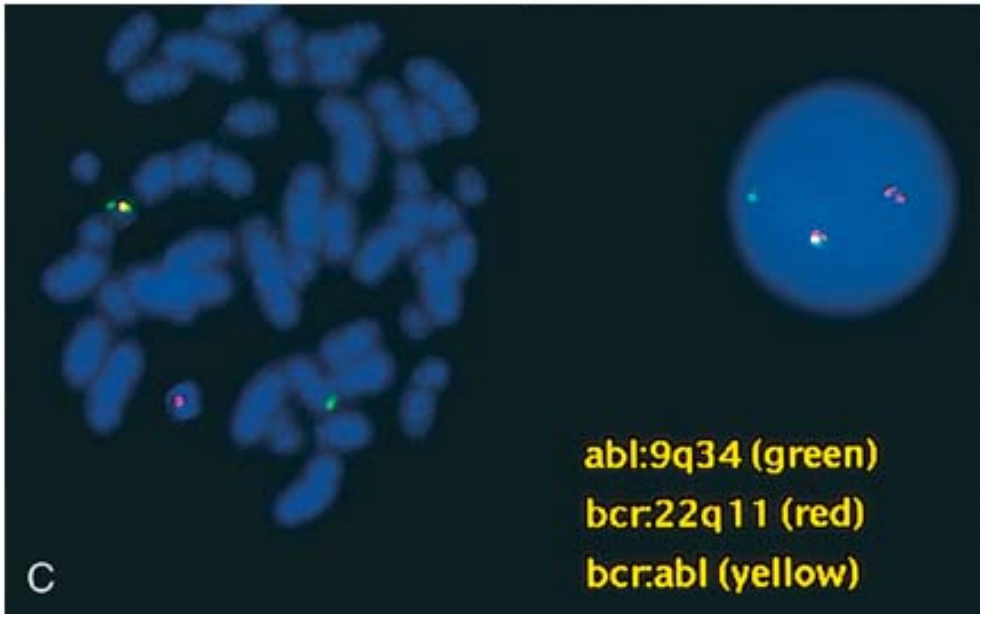


Figure 14-32 Chronic myelogenous leukemia. Peripheral blood smear shows many mature neutrophils, some metamyelocytes, and a myelocyte. *(Courtesy of Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

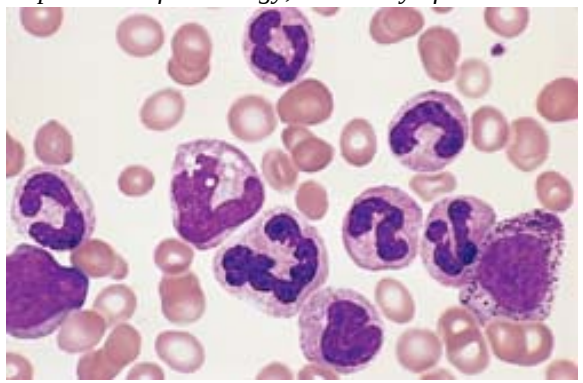


Figure 14-33 Chronic myelogenous leukemia (spleen). Enlarged spleen (2630 gm; normal: 150 to 200 gm) with greatly expanded red pulp stemming from neoplastic hematopoiesis. *(Courtesy of Dr. Daniel Jones, Department of Pathology, M.D. Anderson Cancer Center, Houston, TX.)*

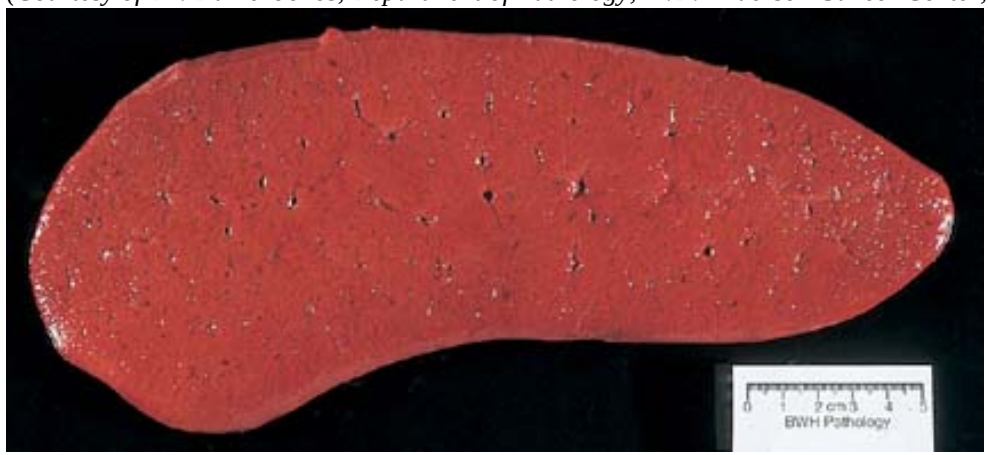


Figure 14-34 Polycythemia vera, spent phase. Massive splenomegaly (3020 gm; normal: 150 to 200 gm) largely owing to extramedullary hematopoiesis occurred in the setting of advanced marrow myelofibrosis. *(Courtesy of Dr. Mark Fleming, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

Figure 14-35 Essential thrombocytosis. Peripheral blood smear shows marked thrombocytosis, including giant platelets approximating the size of surrounding red cells. *(Courtesy of Dr. Jacqueline Mitus, Brigham and Women's Hospital, Boston, MA.)*

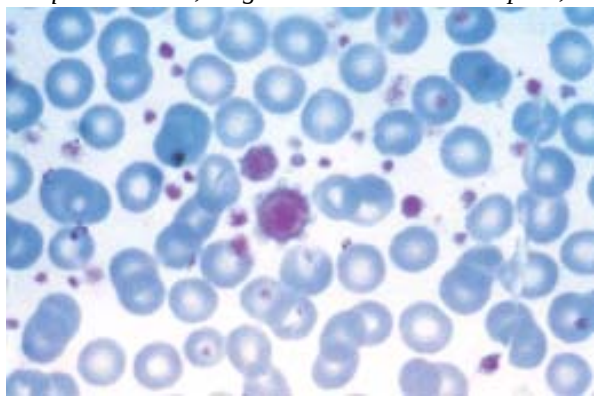


Figure 14-36 Primary myelofibrosis (peripheral blood smear). Two nucleated erythroid precursors and several teardrop-shaped red cells (dacryocytes) are evident. Immature myeloid cells were present in other fields. An identical picture can be seen in other diseases producing marrow distortion and fibrosis.

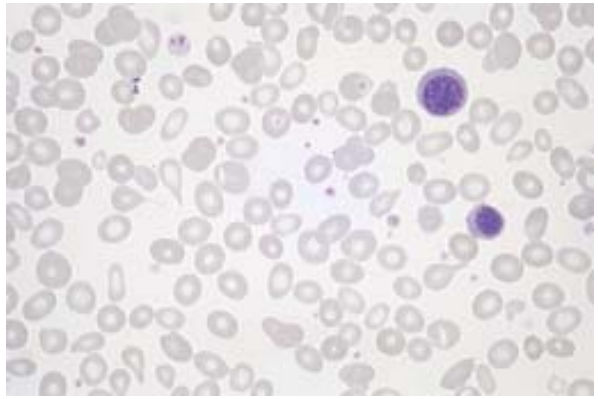


Figure 14-37 Langerhans cell histiocytosis. An electron micrograph shows rodlike Birbeck granules with characteristic periodicity and dilated terminal end. *(Courtesy of Dr. George Murphy, University of Pennsylvania School of Medicine, Philadelphia, PA.)*

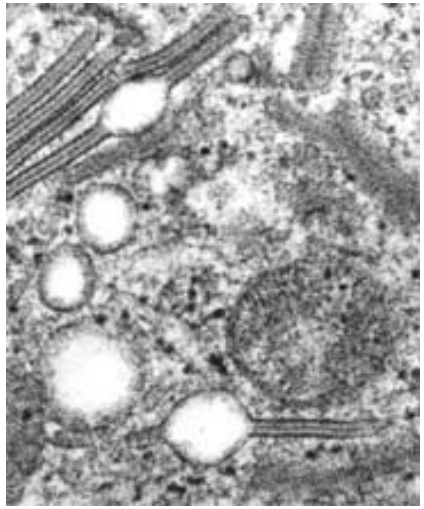


Figure 14-38 Normal splenic architecture. *(Modified from Faller DV: Diseases of the spleen. In Wyngaarden JB, Smith LH (eds): Cecil Textbook of Medicine, 18th ed. Philadelphia, WB Saunders, 1988, p. 1036.)*

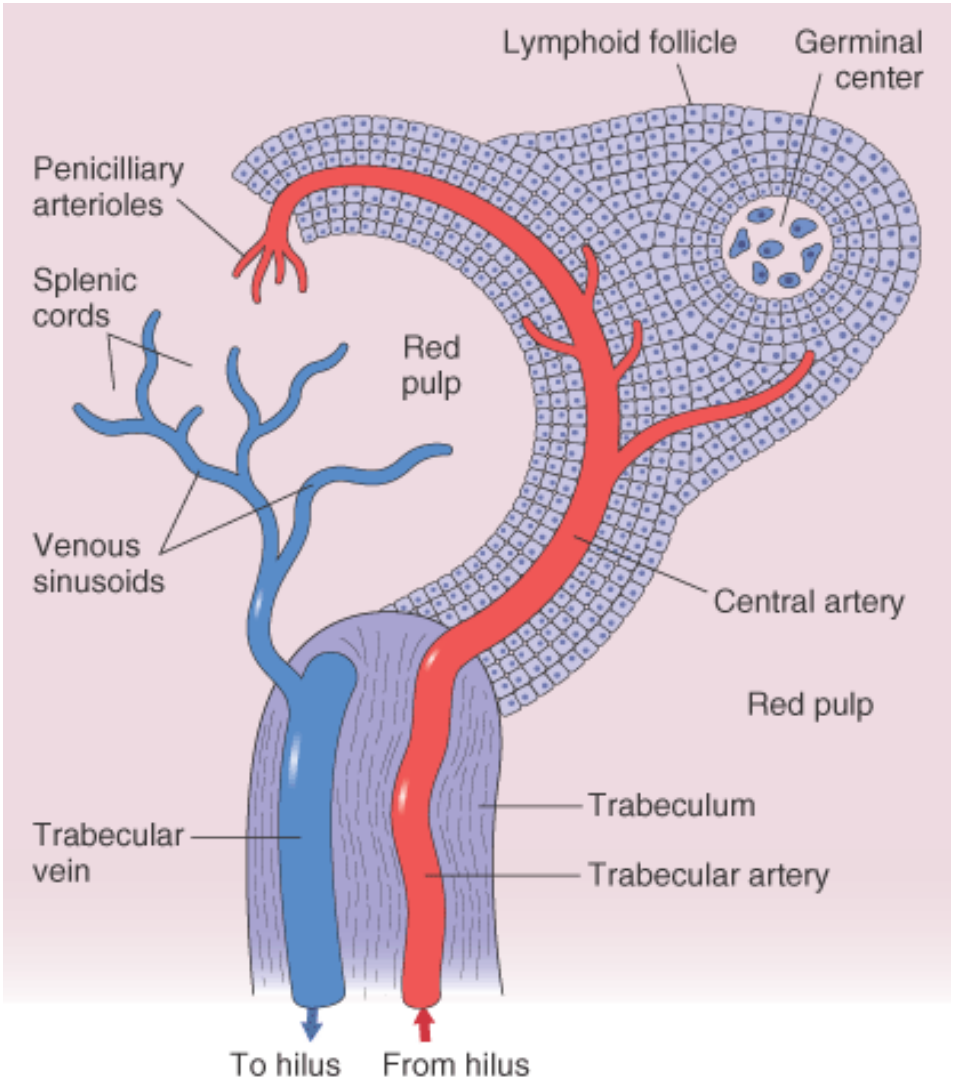


TABLE 14-9 -- Disorders Associated with Splenomegaly

I. Infections
Nonspecific splenitis of various blood-borne infections (particularly infective endocarditis)
Infectious mononucleosis
Tuberculosis
Typhoid fever
Brucellosis
Cytomegalovirus

Syphilis
Malaria
Histoplasmosis
Toxoplasmosis
Kala-azar
Trypanosomiasis
Schistosomiasis
Leishmaniasis
Echinococcosis
II. Congestive States Related to Portal Hypertension
Cirrhosis of the liver
Portal or splenic vein thrombosis
Cardiac failure
III. Lymphohematogenous Disorders
Hodgkin lymphoma
Non-Hodgkin lymphomas and lymphocytic leukemias
Multiple myeloma
Myeloproliferative disorders
Hemolytic anemias
Thromobocytopenic purpura
IV. Immunologic-Inflammatory Conditions
Rheumatoid arthritis
Systemic lupus erythematosus
V. Storage Diseases
Gaucher disease
Niemann-Pick disease
Mucopolysaccharidoses
VI. Miscellaneous
Amyloidosis
Primary neoplasms and cysts

Secondary neoplasms

NONSPECIFIC ACUTE SPLENITIS

Enlargement of the spleen occurs in any blood-borne infection. The nonspecific splenic reaction in these infections is caused both by the microbiologic agents themselves and by cytokines that are released as part of the immune response.

Morphology.

The spleen is enlarged (up to 200 to 400 gm) and soft. The splenic substance is often diffluent and can be so soft that it literally flows out from the cut surface. Microscopically, the major change is acute congestion of the red pulp, which can encroach on and sometimes virtually efface the lymphoid follicles. Neutrophils, plasma cells, and occasionally eosinophils are usually present throughout the white and red pulp. At times, there is acute necrosis of the centers of the splenic follicles, particularly when the causative agent is a hemolytic streptococcus. Rarely, abscess formation occurs.

CONGESTIVE SPLENOMEGALY

Chronic venous congestion can cause a form of splenic enlargement referred to as *congestive splenomegaly*. Venous congestion can be systemic in origin, caused by intrahepatic disorders that retard portal venous drainage, or may arise from extrahepatic disorders that directly obstruct the portal or splenic veins. All these disorders ultimately lead to portal or splenic vein hypertension. *Systemic, or central, venous congestion* is encountered in cardiac decompensation involving the right side of the heart, as can occur in tricuspid or pulmonic valvular disease, chronic cor pulmonale, or following left-sided heart failure. Systemic passive congestion produces only moderate enlargement of the spleen that rarely exceeds 500 gm in weight.

The only common causes of striking congestive splenomegaly are the various forms of cirrhosis of the liver. The "pipe-stem" hepatic fibrosis of schistosomiasis causes particularly severe congestive splenomegaly, while the diffuse fibrous scarring of alcoholic cirrhosis and pigment cirrhosis also evokes profound enlargements. Other forms of cirrhosis are less commonly implicated.

Congestive splenomegaly is also caused by obstruction of the extrahepatic portal vein or splenic vein. This can stem from *spontaneous portal vein thrombosis,* which is usually associated with some intrahepatic obstructive disease, or inflammation of the portal vein *(pylephlebitis),* such as follows intraperitoneal infections. Thrombosis of the splenic vein itself can be initiated by compression by tumors in neighboring organs, for example, carcinoma of the stomach or pancreas.

Morphology.

Long-standing congestion produces marked enlargement of the spleen (1000 gm or more); the organ is firm and becomes increasingly so the longer the congestion lasts. The weight can reach 5000 gm. The capsule is usually thickened and fibrous. The cut surface has a meaty appearance and varies from gray-red to deep red, depending on the amount of fibrosis. Often the white pulp is indistinct. Microscopically, the red pulp is congested in early chronic congestion but becomes increasingly more fibrous

and cellular with time. The increased portal venous pressure causes deposition of collagen in the basement membrane of the sinusoids, which appear dilated owing to the rigidity of their walls. The resultant slowing of blood flow from the cords to the sinusoids prolongs the exposure of the blood cells to the cordal macrophages, resulting in excessive destruction (hypersplenism). Foci of recent or old hemorrhage are often present. Organization of these focal hemorrhages gives rise to Gandy-Gamma nodules: foci of fibrosis containing iron and calcium salts deposited on connective tissue and elastic fibers.

SPLENIC INFARCTS

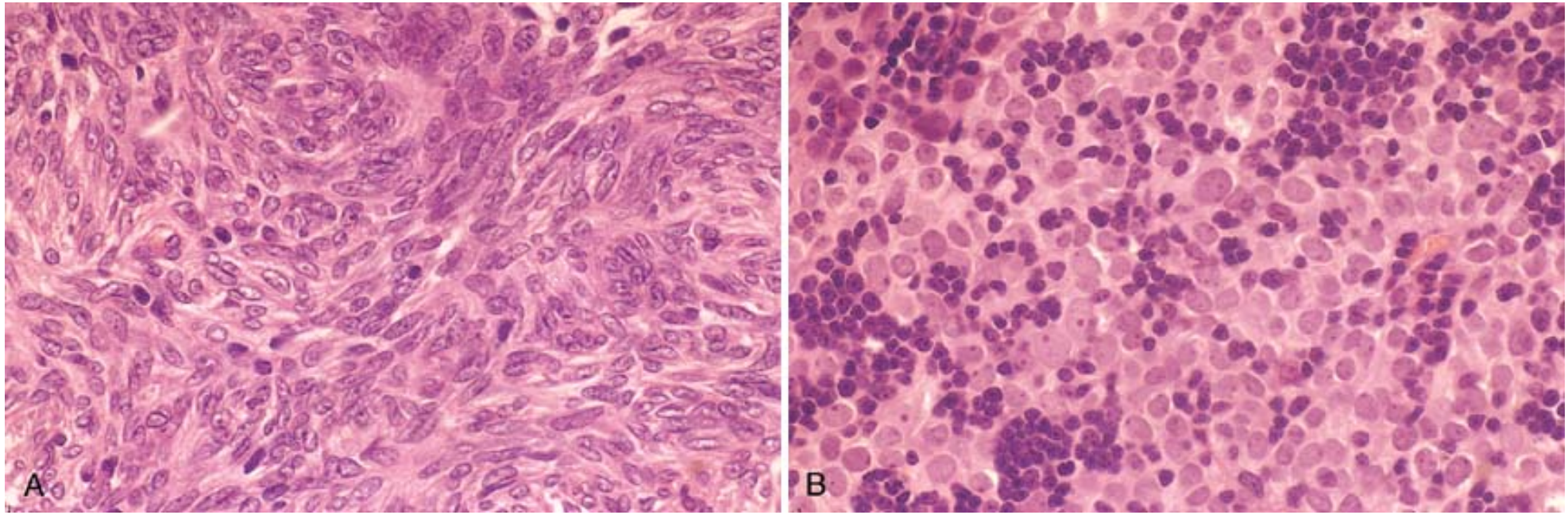
Splenic infarcts are common lesions. Caused by occlusion of the major splenic artery or any of its branches, in normal-sized spleens they are most often due to emboli that arise from thrombi in the heart. The spleen, along with kidneys and brain, ranks as one of the most frequent sites within which emboli lodge. The resulting infarcts can be small or large, single or multiple or can even involve the entire organ. They are usually bland but can be septic when associated with infectious endocarditis of mitral and aortic valves. Infarcts are also common in markedly enlarged spleens, presumably because the blood supply cannot keep up with the increased demands of the organ.

Morphology.

Bland infarcts are characteristically pale and wedge-shaped, with their bases at the periphery, where the overlying capsule is often covered with fibrin (Fig. 14-39). In septic infarcts, this appearance is modified by the development of suppurative necrosis. In the course of healing of splenic infarcts, large, depressed scars often develop.

Figure 14-39 Splenic infarcts. Multiple well-circumscribed infarcts are present in this spleen, which is massively enlarged (2820 gm; normal: 150 to 200) by extramedullary hematopoiesis secondary to a chronic myeloproliferative disorder (myelofibrosis). Recent infarcts are hemorrhagic, whereas older, more fibrotic infarcts are a pale yellow-gray color.

Figure 14-40 Thymoma. *A,* Benign thymoma (medullary type). The neoplastic epithelial cells are arranged in a swirling pattern and have bland, oval to elongated nuclei with inconspicuous nucleoli. Only a few small, reactive lymphoid cells are interspersed. *B,* Malignant thymoma, type I. The neoplastic epithelial cells are polygonal and have round to oval, bland nuclei with inconspicuous nucleoli. Numerous small, reactive lymphoid cells are interspersed. The morphologic appearance of this tumor is identical to that of benign thymomas of the cortical type. In this case, however, the tumor was locally aggressive, invading adjacent lung and pericardium.



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Chapter 15 - The Lung *

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Normal Lung

The lungs are ingeniously constructed to carry out their cardinal function: the exchange of gases between inspired air and blood. Developmentally, the respiratory system is an outgrowth from the ventral wall of the foregut. The midline trachea develops two lateral outpocketings, the lung buds. The right lung bud eventually divides into three branches—the main bronchi—and the left into two main bronchi, thus giving rise to three lobes on the right and two on the left. The lingula on the left is the middle lobe equivalent; however, the left lung is smaller than the right. The right main stem bronchus is more vertical and more directly in line with the trachea than is the left. Consequently, aspirated foreign material, such as vomitus, blood, and foreign bodies, tends to enter the right lung rather than the left. The main right and left bronchi branch dichotomously, giving rise to progressively smaller airways. Accompanying the branching airways is the double arterial supply to the lungs, that is, the pulmonary and bronchial arteries. In the absence of significant cardiac failure, the bronchial arteries of aortic origin can often sustain the vitality of the pulmonary parenchyma when pulmonary arterial supply is blocked, as by emboli.

Progressive branching of the bronchi forms *bronchioles*, which are distinguished from bronchi by the lack of cartilage and submucosal glands within their walls. Further branching of bronchioles leads to the *terminal bronchioles*, which are less than 2 mm in diameter. The part of the lung distal to the terminal bronchiole is called the *acinus*; it is approximately spherical, with a diameter of about 7 mm. As illustrated in Figure 15-5*A* , an acinus is composed of *respiratory bronchioles* (emanating from the terminal bronchiole), which give off several alveoli from their sides. These bronchioles then proceed into the *alveolar ducts*, which immediately branch into *alveolar sacs*, the blind ends of the respiratory passages, whose walls are formed entirely of alveoli, which are the site of gas exchange. The alveoli open into the ducts through large mouths. In the correct plane of section, therefore, all alveoli are open and have incomplete walls. A cluster of three to five terminal bronchioles, each with its appended acinus, is usually referred to as the pulmonary *lobule*. As will be seen subsequently, this lobular architecture assumes importance in distinguishing the major forms of emphysema.

From the microscopic standpoint, except for the vocal cords, which are covered by stratified squamous epithelium, the entire respiratory tree, including the larynx, trachea, and bronchioles, is lined by pseudostratified, tall, columnar, ciliated epithelial cells, heavily admixed in the cartilaginous airways with mucus-secreting goblet cells. The bronchial mucosa also contains neuroendocrine cells that exhibit neurosecretory-type granules and contain serotonin, calcitonin, and gastrin-releasing peptide (bombesin). Numerous submucosal, mucus-secreting glands are dispersed throughout the walls of the trachea and bronchi (but not the bronchioles).

The microscopic structure of the alveolar walls (or alveolar septa) consists, from blood to air, of the following (Fig. 15-1):

- The *capillary endothelium* lining the intertwining network of anastomosing capillaries.
- A *basement membrane and surrounding interstitial tissue* separating the endothelial cells from the alveolar lining

epithelial cells. In thin portions of the alveolar septum, the basement membranes of epithelium and endothelium are fused, whereas in thicker portions, they are separated by an interstitial space (*pulmonary interstitum*) containing fine elastic fibers, small bundles of collagen, a few fibroblast-like interstitial cells, smooth muscle cells, mast cells, and, rarely, lymphocytes and monocytes.

- *Alveolar epithelium*, which contains a continuous layer of two principal cell types: flattened, platelike *type I pneumocytes* (or membranous pneumocytes) covering 95% of the alveolar surface and rounded *type II pneumocytes*. Type II cells are important for at least two reasons: (1) They are the source of *pulmonary surfactant*, contained in osmiophilic *lamellar bodies* seen with electron microscopy, and (2) they are the main cell type involved in the repair of alveolar epithelium after destruction of type I cells.
- *Alveolar macrophages*, loosely attached to the epithelial cells or lying free within the alveolar spaces, derived from blood monocytes and belonging to the mononuclear phagocyte system. Often, they are filled with carbon particles and other phagocytosed materials.

Figure 15-1 Microscopic structure of the alveolar wall. Note that the basement membrane (*yellow*) is thin on one side and widened where it is continuous with the interstitial space. Portions of interstitial cells are shown.

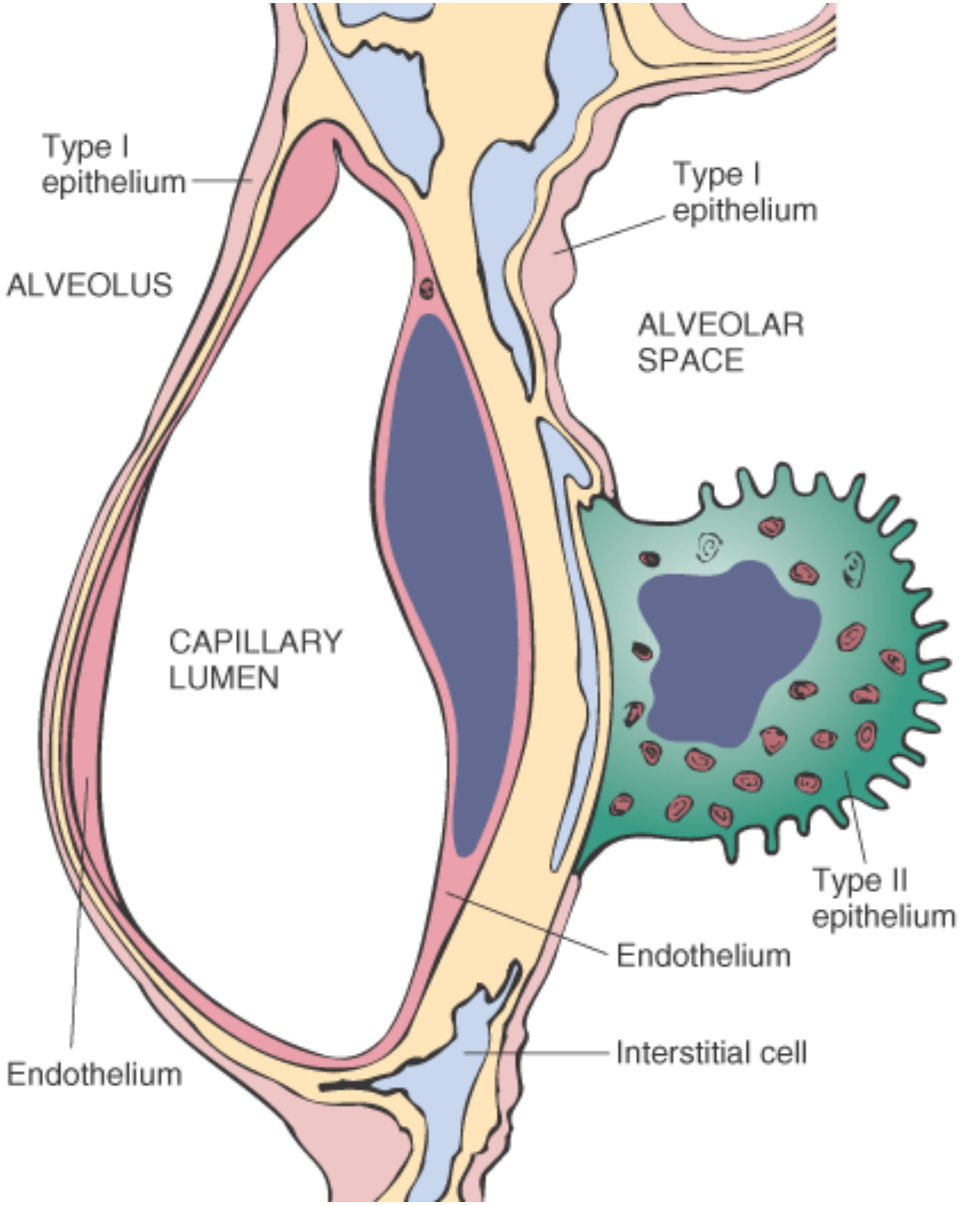
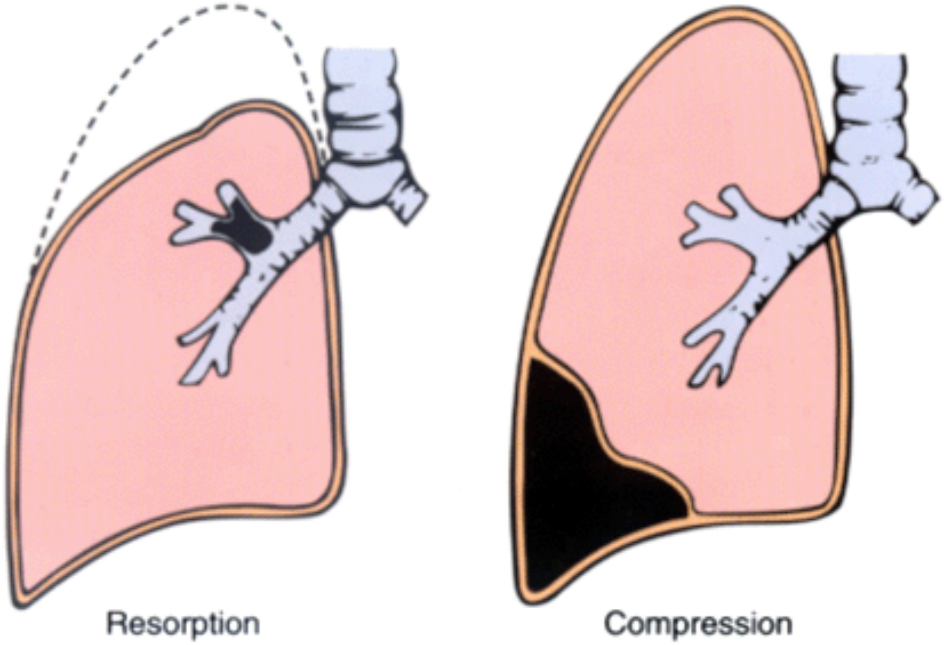


Figure 15-2 Various forms of atelectasis in adults.



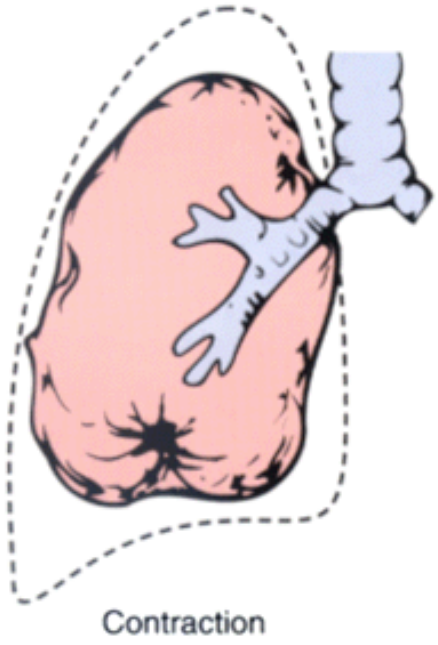


TABLE 15-1 -- Classification and Causes of Pulmonary Edema

Hemodynamic Edema

Increased hydrostatic pressure (increased pulmonary venous pressure)
••Left-sided heart failure (common)
••Volume overload
••Pulmonary vein obstruction
Decreased oncotic pressure (less common)
••Hypoalbuminemia
••Nephrotic syndrome
••Liver disease
••Protein-losing enteropathies
Lymphatic obstruction (rare)
Edema Due to Microvascular Injury (Alveolar Injury)
Infections: pneumonia, septicemia
Inhaled gases: oxygen, smoke
Liquid aspiration: gastric contents, near-drowning
Drugs and chemicals: chemotherapeutic agents (bleomycin), other medications (amphotericin B), heroin, kerosene, paraquat
Shock, trauma
Radiation
Transfusion related
Edema of Undetermined Origin
High altitude
Neurogenic (central nervous system trauma)

the clinical setting, pulmonary congestion and edema are characterized by heavy, wet lungs. Fluid accumulates initially in the basal regions of the lower lobes because hydrostatic pressure is greater in these sites (dependent edema). Histologically, the alveolar capillaries are engorged, and an intra-alveolar granular pink precipitate is seen. Alveolar microhemorrhages and hemosiderin-laden macrophages ("heart failure" cells) may be present. In long-standing cases of pulmonary congestion, such as those seen in mitral stenosis, hemosiderin-laden macrophages are abundant, and fibrosis and thickening of the alveolar walls cause the soggy lungs to become firm and brown (*brown induration*). These changes not only impair normal respiratory function, but also predispose to infection.

Edema Caused by Microvascular Injury

The second mechanism leading to pulmonary edema is *injury to the capillaries of the alveolar septa*. Here the pulmonary capillary hydrostatic pressure is usually not elevated, and hemodynamic factors play a secondary role. The edema results from primary injury to the vascular endothelium or damage to alveolar epithelial cells (with secondary microvascular injury). This results in leakage of fluids and proteins first into the interstitial space and, in more severe cases, into the alveoli. When the edema remains localized, as it does in most forms of pneumonia, it is overshadowed by the manifestations of infection. When diffuse, however, alveolar edema is an important contributor to a serious and often fatal condition, *acute respiratory distress syndrome*, discussed in the following section.

ACUTE RESPIRATORY DISTRESS SYNDROME (DIFFUSE ALVEOLAR DAMAGE)

Acute respiratory distress syndrome (ARDS) (synonyms include "shock lung," "diffuse alveolar damage," "acute alveolar injury," and "acute lung injury") is a clinical syndrome caused by diffuse alveolar capillary damage. It is characterized clinically by the rapid onset of severe life-threatening respiratory insufficiency, cyanosis, and severe arterial hypoxemia that is refractory to oxygen therapy and that may progress to extra-pulmonary multisystem organ failure. Chest radiographs show diffuse alveolar infiltration. Diffuse alveolar damage (DAD) is the histologic manifestation.

ARDS is a well-recognized complication of numerous and diverse conditions, including both direct injuries to the lungs and systemic disorders (Table 15-2). In many cases, a combination of predisposing conditions is present (e.g., shock, oxygen therapy, and sepsis).

Morphology.

In the acute stage, the lungs are heavy, firm, red, and boggy. They exhibit congestion, interstitial and intra-alveolar edema, inflammation, and fibrin deposition. The alveolar walls become lined with waxy **hyaline membranes** (Fig. 15-3) that are morphologically similar to those seen in hyaline membrane disease of neonates (Chapter 10). Alveolar hyaline membranes consist of fibrin-rich edema fluid mixed with the cytoplasmic and lipid remnants of necrotic epithelial cells. In the organizing stage, type

TABLE 15-2 -- Conditions Associated with Development of Acute Respiratory Distress Syndrome

Infection
Sepsis *
Diffuse pulmonary infections *
••Viral, *Mycoplasma*, and *Pneumocystis* pneumonia; miliary tuberculosis
Gastric aspiration *
Physical/Injury
Mechanical trauma, including head injuries *
Pulmonary contusions
Near-drowning
Fractures with fat embolism
Burns
Ionizing radiation
Inhaled Irritants

Oxygen toxicity
Smoke
Irritant gases and chemicals
Chemical Injury
Heroin or methadone overdose
Acetylsalicylic acid
Barbiturate overdose
Paraquat
Hematologic Conditions
Multiple transfusions
Disseminated intravascular coagulation
Pancreatitis
Uremia
Cardiopulmonary Bypass
Hypersensitivity Reactions
Organic solvents
Drugs

*More than 50% of cases of acute respiratory distress syndrome are associated with these four conditions.

Il epithelial cells undergo proliferation in an attempt to regenerate the alveolar lining. Resolution is unusual; more commonly, there is organization of the fibrin exudate, with resultant intra-alveolar fibrosis. Marked thickening of the alveolar septa ensues, caused by proliferation of interstitial cells and deposition of collagen. Fatal cases often have superimposed bronchopneumonia.

Pathogenesis.

ARDS and DAD are best viewed as the clinical and pathologic end results, respectively, of acute alveolar injury caused by a variety of insults and initiated by different mechanisms.[3] Central to the causation of ARDS is *diffuse damage to the alveolar capillary walls*; this is followed by a relatively nonspecific, often predictable series of morphologic

Figure 15-3 Diffuse alveolar damage (acute respiratory distress syndrome) shown in a photomicrograph. Some of the alveoli are collapsed; others are distended. Many contain dense proteinaceous debris, desquamated cells, and hyaline membranes (*arrows*).

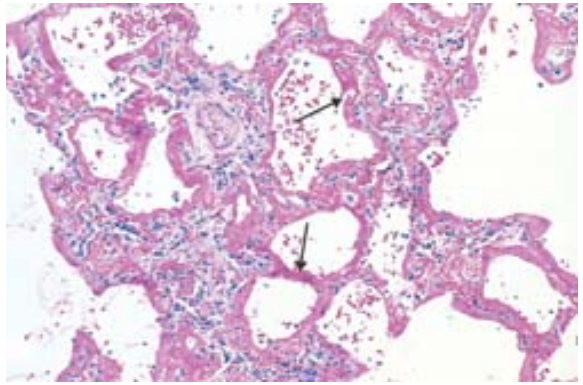


Figure 15-4 The normal alveolus (*left side*) compared with the injured alveolus in the early phase of acute lung injury and acute respiratory distress syndrome. Under the influence of proinflammatory cytokines such as interleukin 8 (IL-8), interleukin 1 (IL-1), and tumor necrosis factor (TNF) (released by macrophages), neutrophils initially undergo sequestration in the pulmonary microvasculature, followed by margination and egress into the alveolar space, where they undergo activation. Activated neutrophils release a variety of factors, such as leukotrienes, oxidants, proteases, and platelet-activating factor (PAF), which contribute to local tissue damage, accumulation of edema fluid in the airspaces, surfactant inactivation, and hyaline membrane formation. Macrophage inhibitory factor (MIF) released into the local milieu sustains the ongoing pro-inflammatory response. Subsequently, the release of macrophage-derived fibrogenic cytokines such as transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF) stimulate fibroblast growth and collagen deposition associated with the healing phase of injury. *(Modified with permission from Ware LB, Matthay MA: The acute respiratory distress syndrome. N Engl J Med 342:1334, 2000.)*

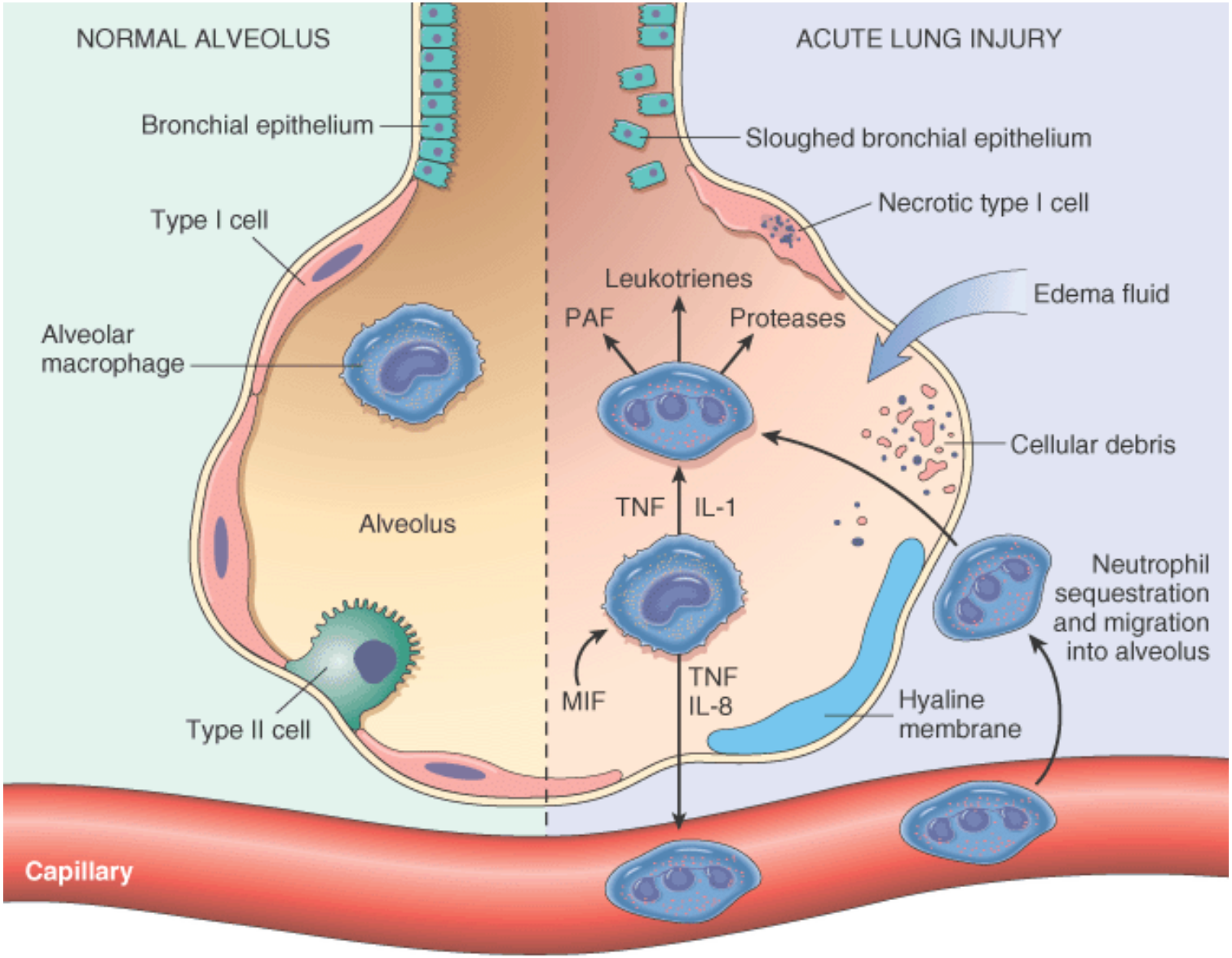


TABLE 15-3 -- Disorders Associated with Airflow Obstruction: The Spectrum of Chronic Obstructive Pulmonary Disease

Clinical Term	Anatomic Site	Major Pathologic Changes	Etiology	Signs/Symptoms
Chronic bronchitis	Bronchus	Mucous gland hyperplasia, hypersecretion	Tobacco smoke, air pollutants	Cough, sputum production
Bronchiectasis	Bronchus	Airway dilation and scarring	Persistent or severe infections	Cough, purulent sputum, fever

Asthma	Bronchus	Smooth muscle hyperplasia, excess mucus, inflammation	Immunologic or undefined causes	Episodic wheezing, cough, dyspnea
Emphysema	Acinus	Airspace enlargement; wall destruction	Tobacco smoke	Dyspnea
Small airway disease, * bronchiolitis	Bronchiole	Inflammatory scarring/obliteration	Tobacco smoke, air pollutants, miscellaneous	Cough, dyspnea

*A feature of chronic bronchitis (see text).

Of these, only the first two cause clinically significant airflow obstruction (Fig. 15-5). Centriacinar emphysema is far more common than the panacinar form, constituting more than 95% of cases. Clinical management does not rely on precise anatomic diagnosis and classification, which, however, do provide important clues to pathogenesis.

Centriacinar (Centrilobular) Emphysema.

The distinctive feature of this type of emphysema is the pattern of involvement of the lobules; *the central or proximal parts of the acini, formed by respiratory bronchioles, are affected, whereas distal alveoli are spared* (Fig. 15-6*B*). Thus, both emphysematous and normal airspaces exist within the same acinus and lobule. The lesions are more common and usually more severe in the

Figure 15-5 *A,* Diagram of normal structures within the acinus, the fundamental unit of the lung. A terminal bronchiole (*not shown*) is immediately proximal to the respiratory bronchiole. *B,* Centriacinar emphysema with dilation that initially affects the respiratory bronchioles. *C,* Panacinar emphysema with initial distention of the peripheral structures (i.e., the alveolus and alveolar duct); the disease later extends to affect the respiratory bronchioles.

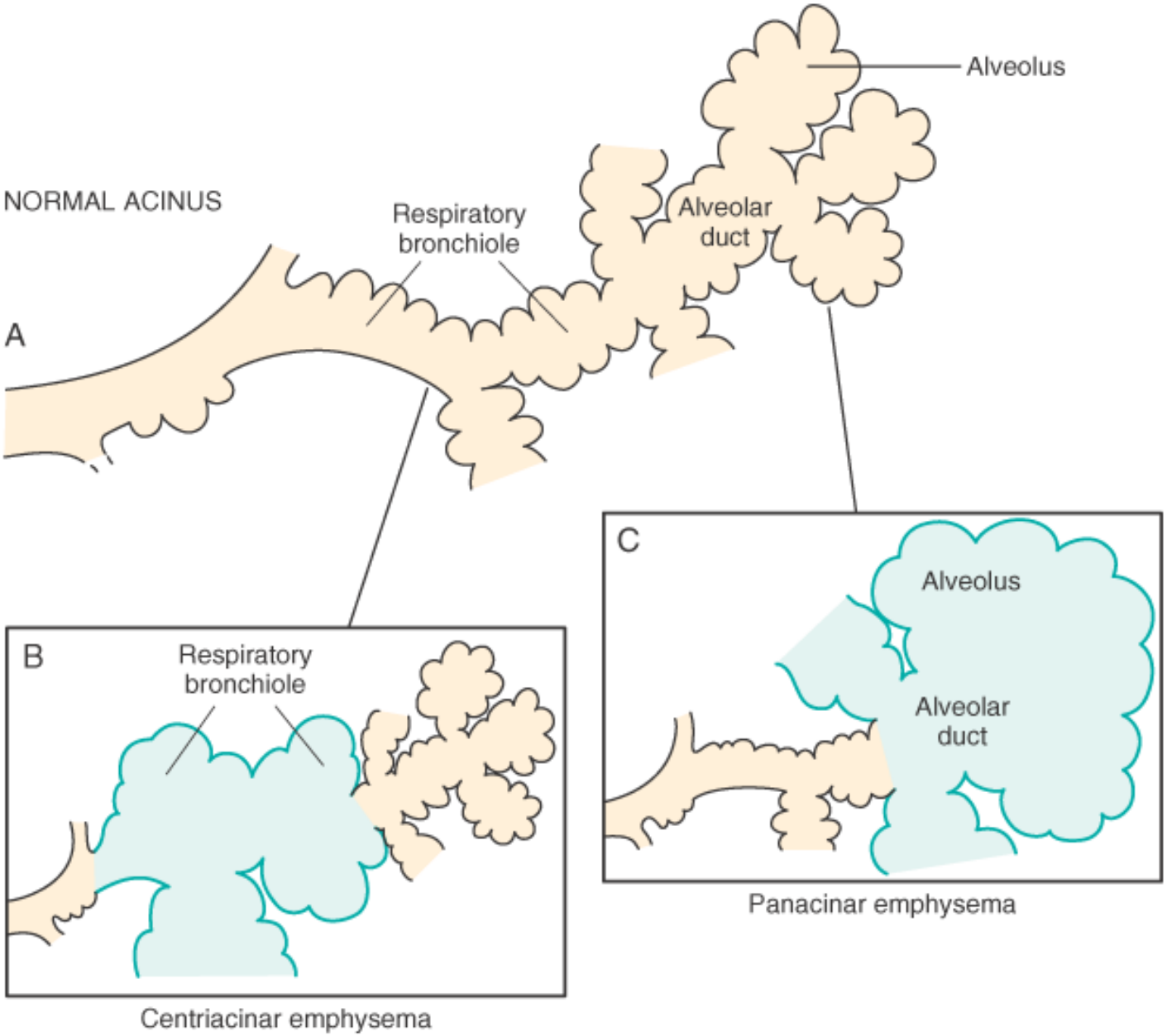


Figure 15-6 *A*, Centriacinar emphysema. Central areas show marked emphysematous damage (E), surrounded by relatively spared alveolar spaces. *B*, Panacinar emphysema involving the entire pulmonary architecture.

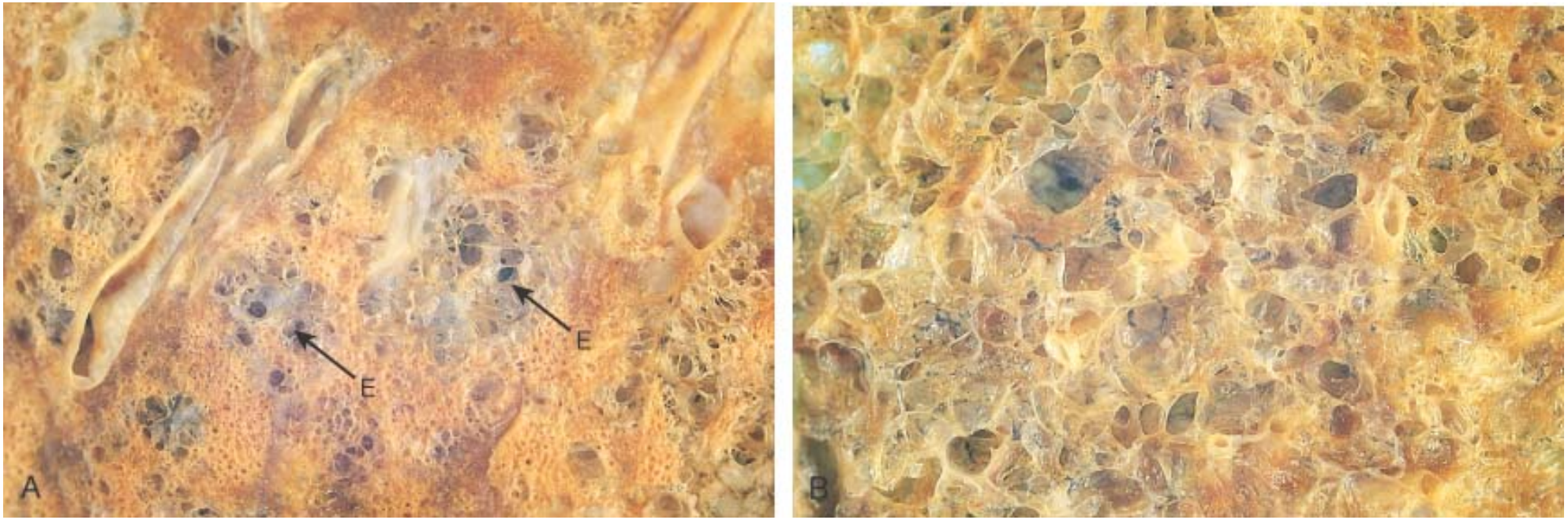


Figure 15-7 Pathogenesis of emphysema. The protease-antiprotease imbalance and oxidant-antioxidant imbalance are additive in their effects and contribute to tissue damage. α_1 -antitrypsin (α_1 -AT) deficiency can be either congenital or "functional" as a result of oxidative inactivation. See text for details. IL-8, interleukin 8; LTB_4 , leukotriene B_4 ; TNF, tumor necrosis factor.

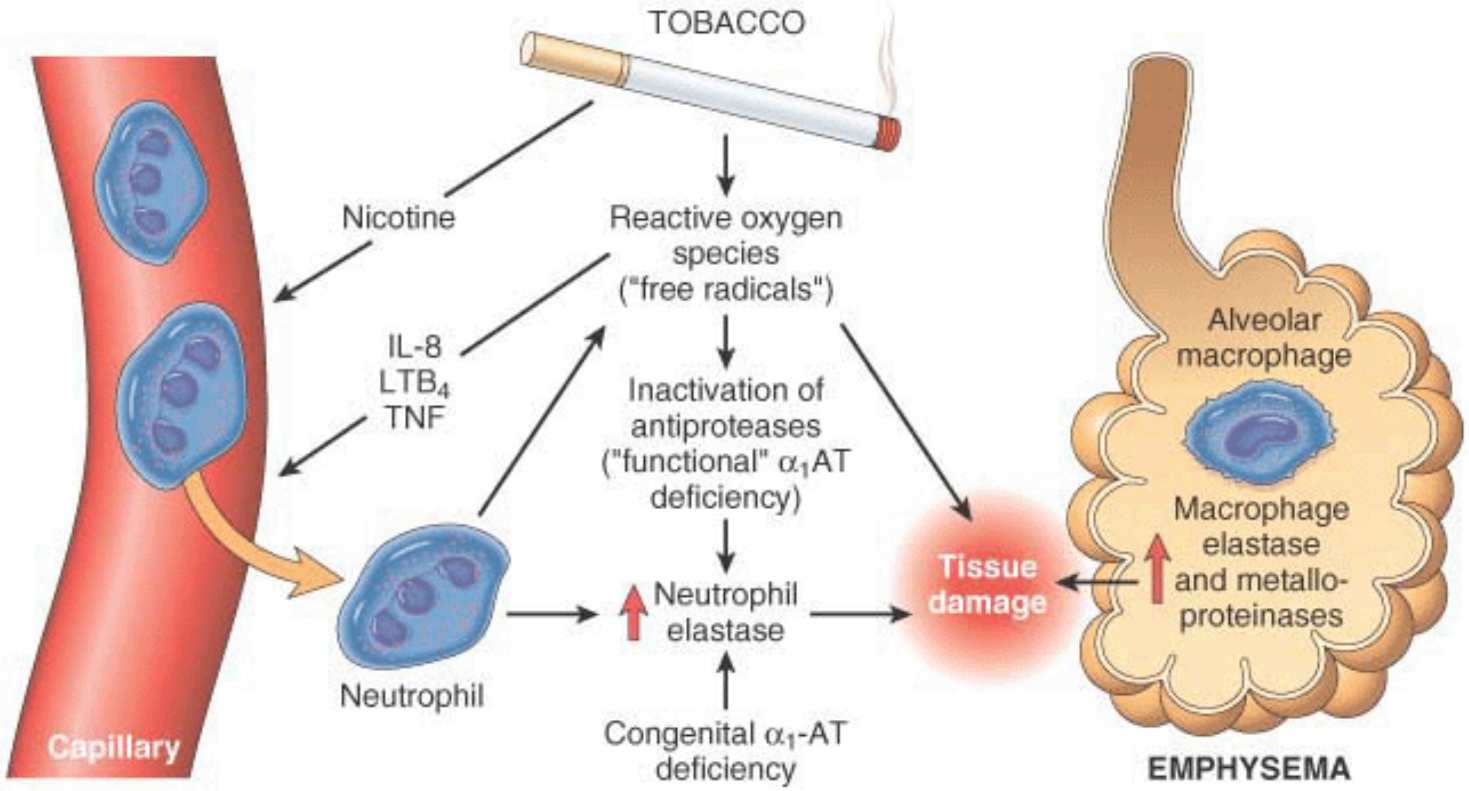


TABLE 15-4 -- Emphysema and Chronic Bronchitis

	Predominant Bronchitis	Predominant Emphysema
Age (yr)	40–45	50–75
Dyspnea	Mild; late	Severe; early
Cough	Early; copious sputum	Late; scanty sputum
Infections	Common	Occasional
Respiratory insufficiency	Repeated	Terminal
Cor pulmonale	Common	Rare; terminal
Airway resistance	Increased	Normal or slightly increased
Elastic recoil	Normal	Low
Chest radiograph	Prominent vessels; large heart	Hyperinflation; small heart
Appearance	*Blue bloater*	*Pink puffer*

Compensatory Hyperinflation (Emphysema).

The term *compensatory hyperinflation (emphysema)* is sometimes used to designate dilation of alveoli but not destruction of septal walls in response to loss of lung substance elsewhere. It is best exemplified by the hyperexpansion of the residual lung parenchyma that follows surgical removal of a diseased lung or lobe.

Obstructive Overinflation.

Obstructive overinflation refers to the condition in which the lung expands because air is trapped within it. A common cause is subtotal obstruction by a tumor or foreign object. A classic example is *congenital labor overinflation* in infants, probably resulting from hypoplasia of bronchial cartilage and sometimes associated with other congenital cardiac and lung abnormalities. Overinflation in obstructive lesions occurs either (1) because of a ball-valve action of the obstructive agent, so that air enters on inspiration but cannot leave on expiration, or (2) because the bronchus may be totally obstructed but ventilation through *collaterals* may bring in air from behind the obstruction. These collaterals are the *pores of Kohn* and other direct accessory *bronchioloalveolar connections* (the canals of Lambert). Obstructive overinflation can be a life-threatening emergency because the affected portion distends sufficiently to compress the remaining normal lung.

Bullous Emphysema.

Bullous emphysema refers merely to any form of emphysema that produces large subpleural blebs or bullae (spaces more than 1 cm in diameter in the distended state) (Fig. 15-8). They represent localized accentuations of one of the four forms of emphysema, are most often subpleural, and occur near the apex, sometimes in relation to old tuberculous scarring. On occasion, rupture of the bullae may give rise to pneumothorax.

Interstitial Emphysema

The entrance of air into the connective tissue stroma of the lung, mediastinum, or subcutaneous tissue is designated interstitial emphysema. In most instances, alveolar tears in pulmonary emphysema provide the avenue of entrance of air into the stroma of the lung, but rarely, a wound of the chest that allows air to be sucked in or a fractured rib that punctures the lung substance may underlie this disorder. Alveolar tears usually occur when there is a combination

Figure 15-8 Bullous emphysema with large subpleural bullae (*upper left*).

Figure 15-9 Schematic representation of evolution of chronic bronchitis (*left*) and emphysema (*right*). Although both can culminate in chronic bronchitis and emphysema, the pathways are different, and either one may predominate. The dashed arrows on the left indicate that in the natural history of chronic bronchitis, it is not known whether there is a predictable progression

from obstruction in small airways to chronic (obstructive) bronchitis. *(Redrawn from Fishman AP: The spectrum of chronic obstructive disease of the airways. In Fishman AP (ed): Pulmonary Diseases and Disorders, 2nd ed. New York, McGraw-Hill, 1988, p. 1164.)*

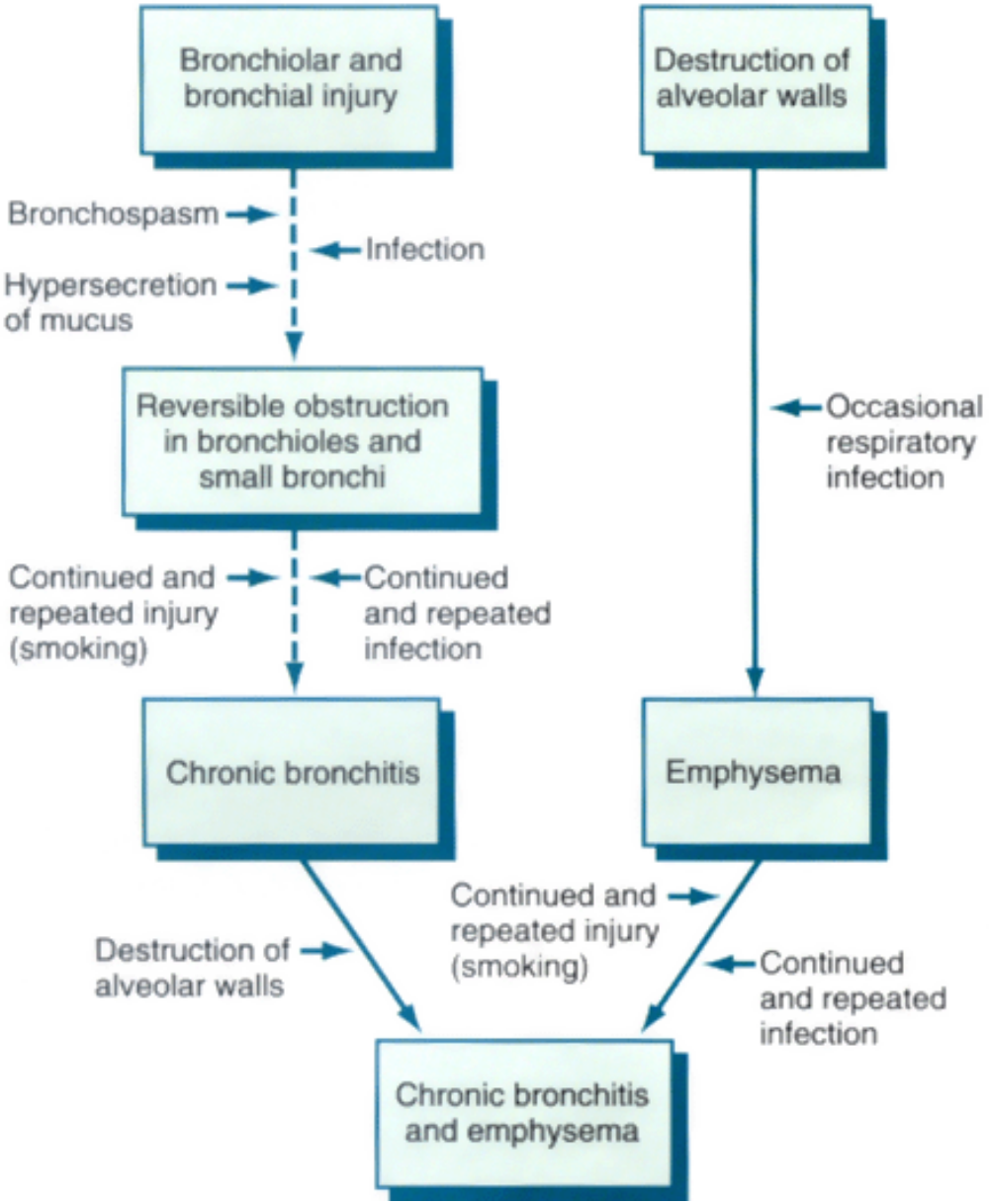


Figure 15-10 A simplified scheme of the system of type 1 helper T (T_H 1) and type 2 helper (T_H 2) cells. The differentiation of T_H 1 and T_H 2 cells depends on interleukin-12 and interleukin-4, cytokines produced by antigen-stimulated precursor CD4 T cells. In a regulatory loop, interferon-γ from T_H 1 cells inhibits T_H 2 cells and interleukin-4 from T_H 2 cells inhibits T_H 1 cells. An imbalance that favors T_H 2 cells may be important in asthma. Bronchial lymphocytes from patients with asthma have been found to lack T-bet, a transcription factor required for the production of interferon-γ (IFN-γ) by T_H 1 cells. *(From Schwartz RS: A new element in the mechanism of asthma. N Engl J Med 346(11):857, 2002. Permission requested.)*

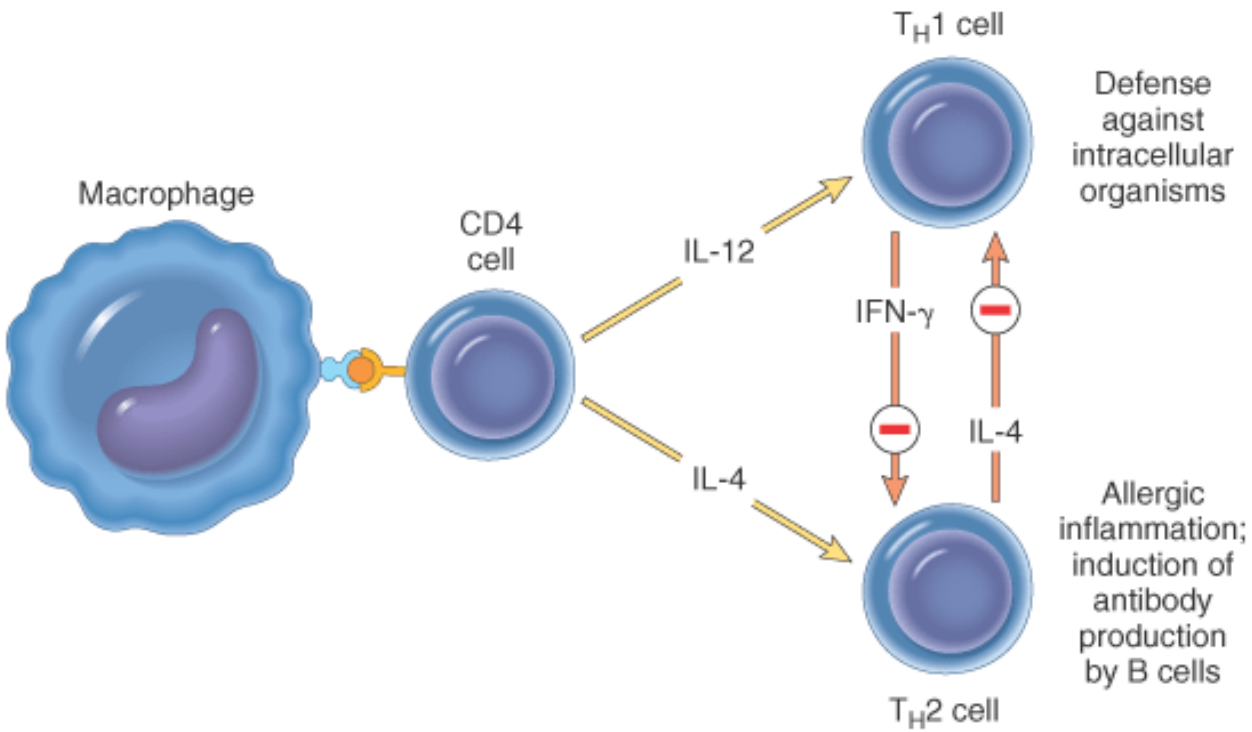
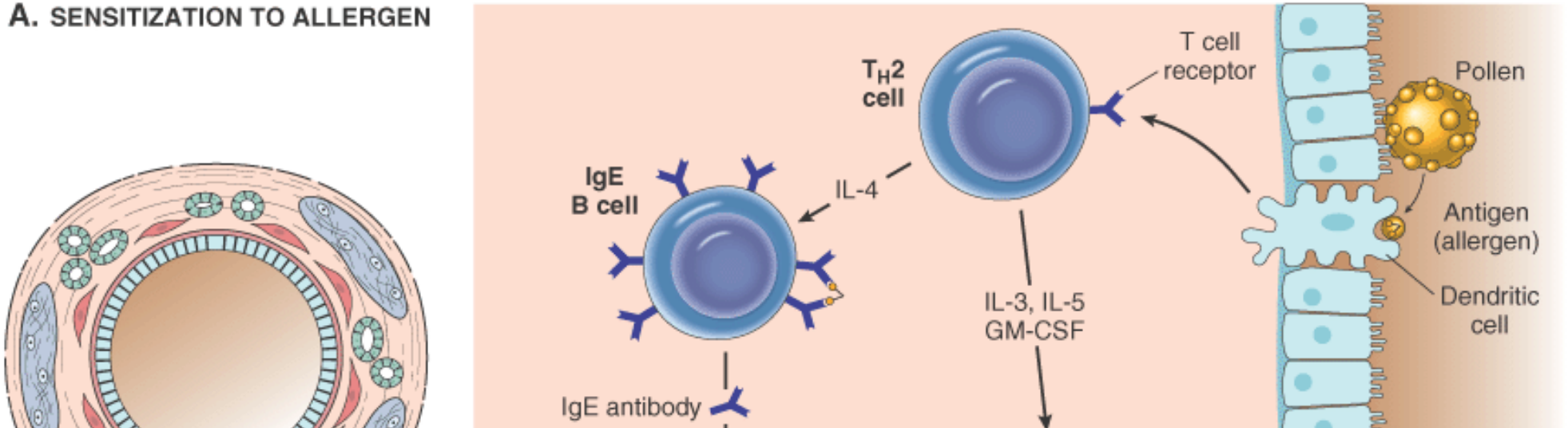
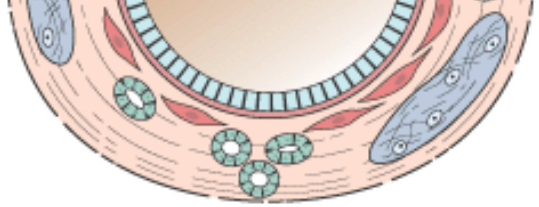
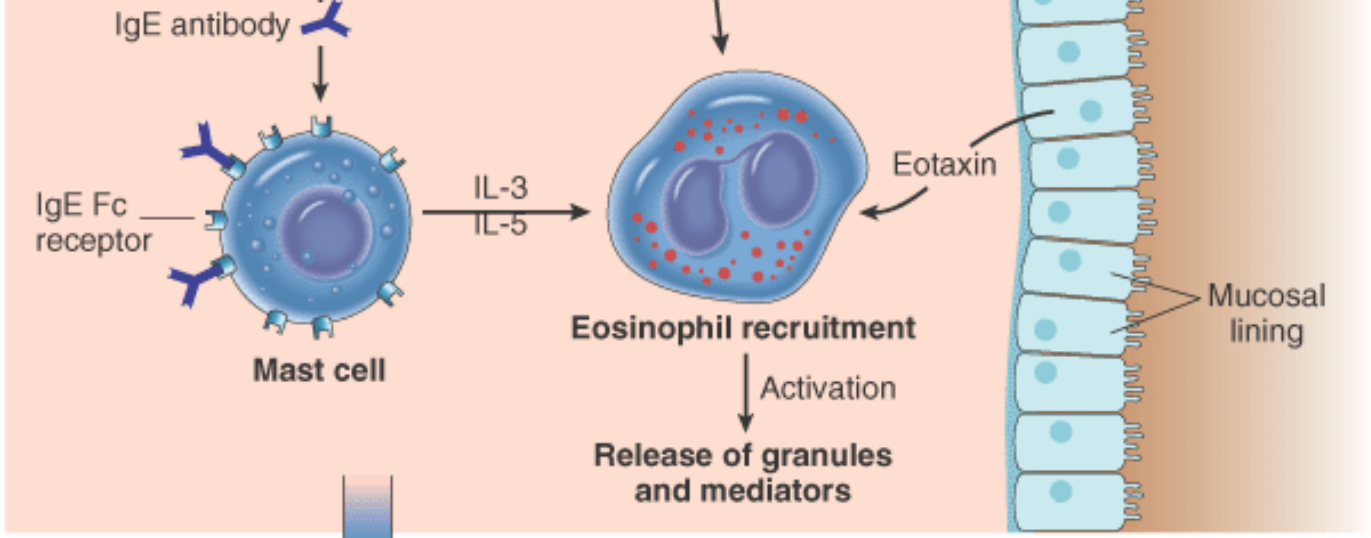


Figure 15-11 A model for allergic asthma. *A*, Inhaled allergens (antigen) elicit a T_H 2-dominated response favoring IgE production and eosinophil recruitment (priming or sensitization). *B*, On re-exposure to antigen (Ag), the immediate reaction is triggered by Ag-induced cross-linking of IgE bound to IgE receptors on mast cells in the airways. These cells release preformed mediators that open tight junctions between epithelial cells. Antigen can then enter the mucosa to activate mucosal mast cells and eosinophils, which in turn release additional mediators. Collectively, either directly or via neuronal reflexes, the mediators induce bronchospasm, increased vascular permeability, and mucus production and recruit additional mediator-releasing cells from the blood. *C*, The arrival of recruited leukocytes (neutrophils, eosinophils, and basophils; also lymphocytes and monocytes [*not shown*]) signals the initiation of the late phase of asthma and a fresh round of mediator release from leukocytes, endothelium, and epithelial cells. Factors, particularly from eosinophils (e.g., major basic protein, eosinophil cationic protein), also cause damage to the epithelium.

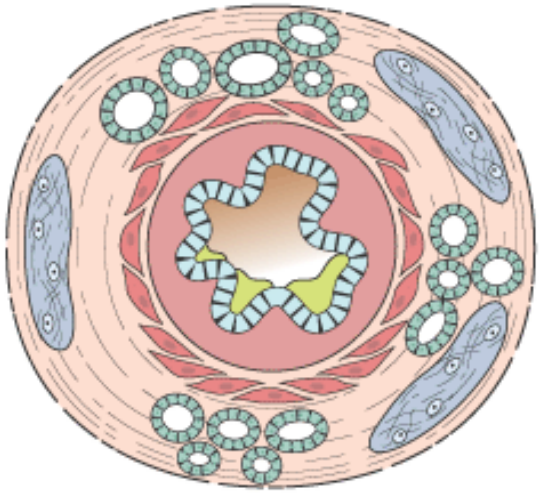




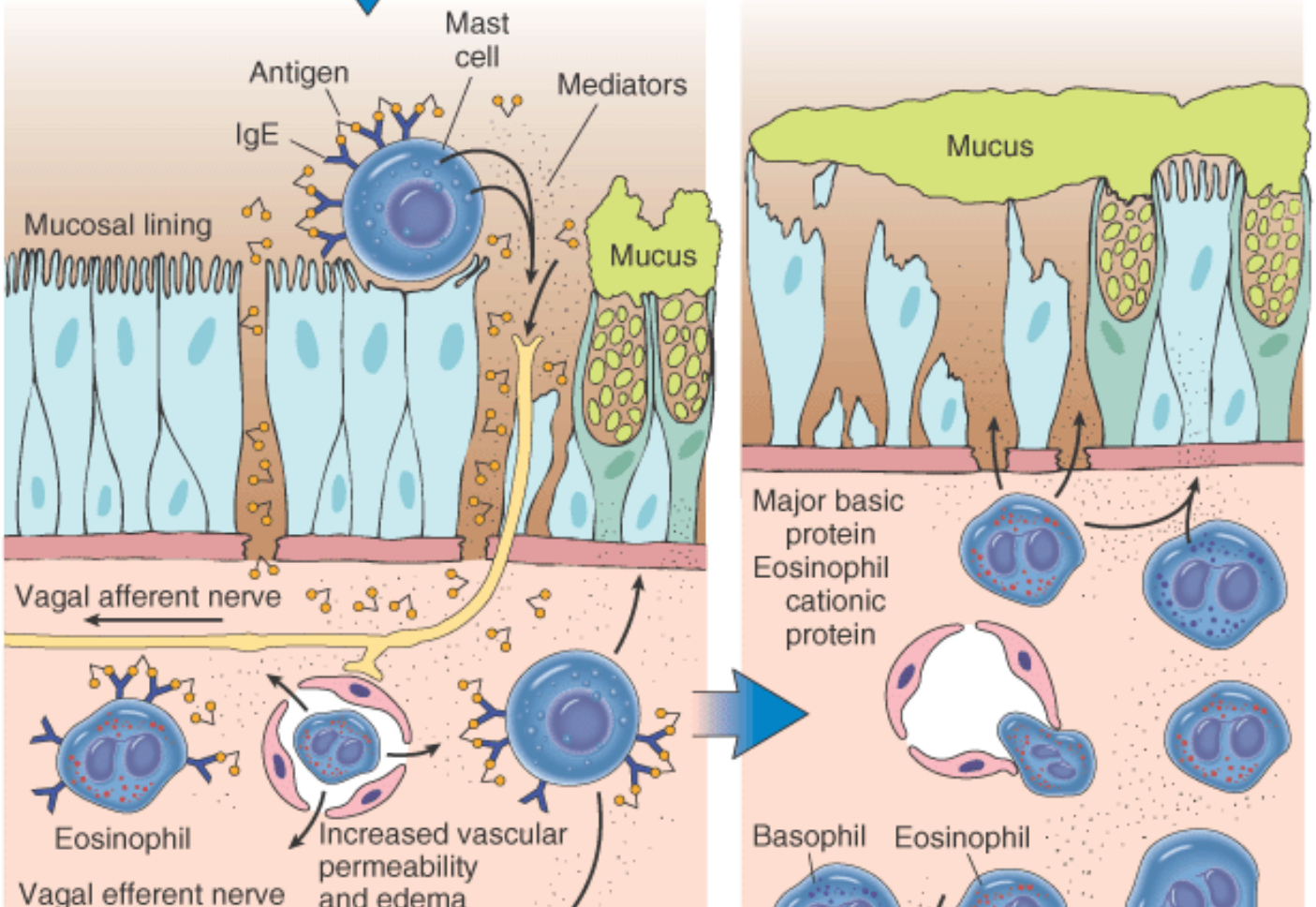
NORMAL AIRWAY



B. ALLERGEN-TRIGGERED ASTHMA



CONSTRICTED AIRWAY IN ASTHMA



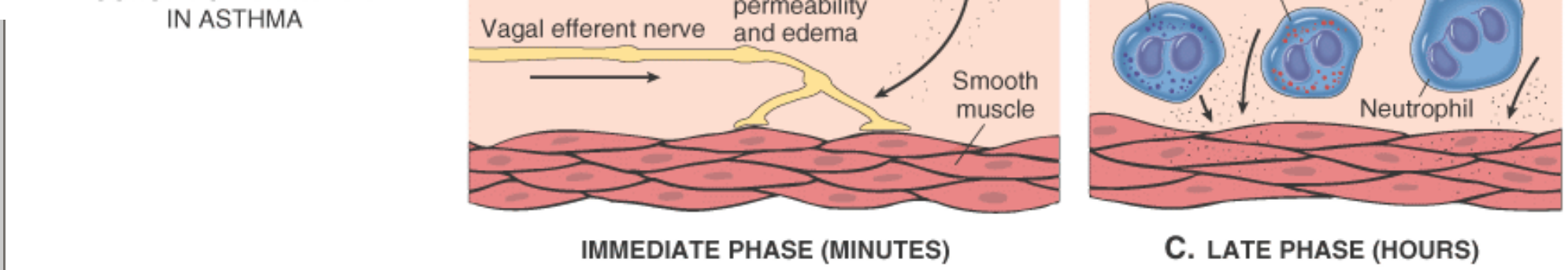


Figure 15-12 Comparison of a normal bronchiole with that in a patient with asthma. Note the accumulation of mucus in the bronchial lumen resulting from an increase in the number of mucus-secreting goblet cells in the mucosa and hypertrophy of submucosal mucus glands. In addition, there is intense chronic inflammation due to recruitment of eosinophils, macrophages, and other inflammatory cells. Basement membrane underlying the mucosal epithelium is thickened, and there is hypertrophy and hyperplasia of smooth muscle cells.

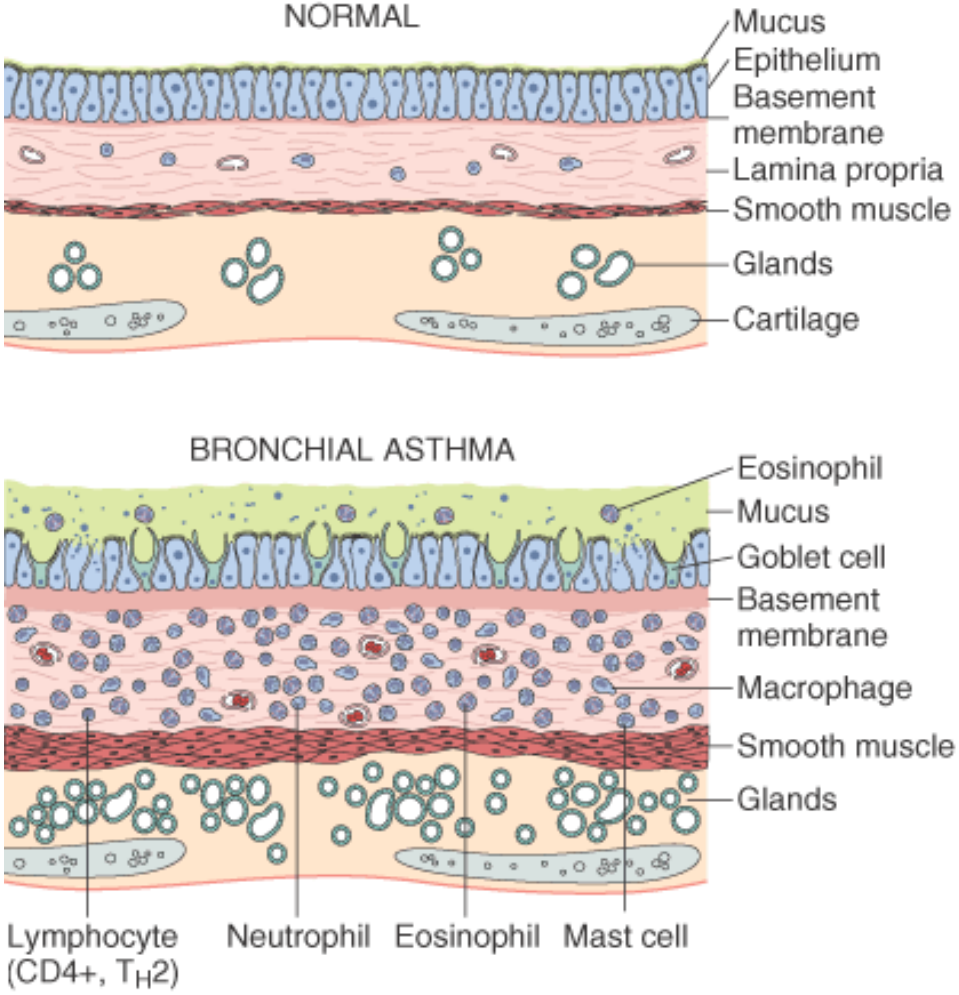


Figure 15-13 Bronchiectasis in a patient with cystic fibrosis, who underwent lung transplantation. Cut surface of lung shows markedly distended peripheral bronchi filled with mucopurulent secretions.

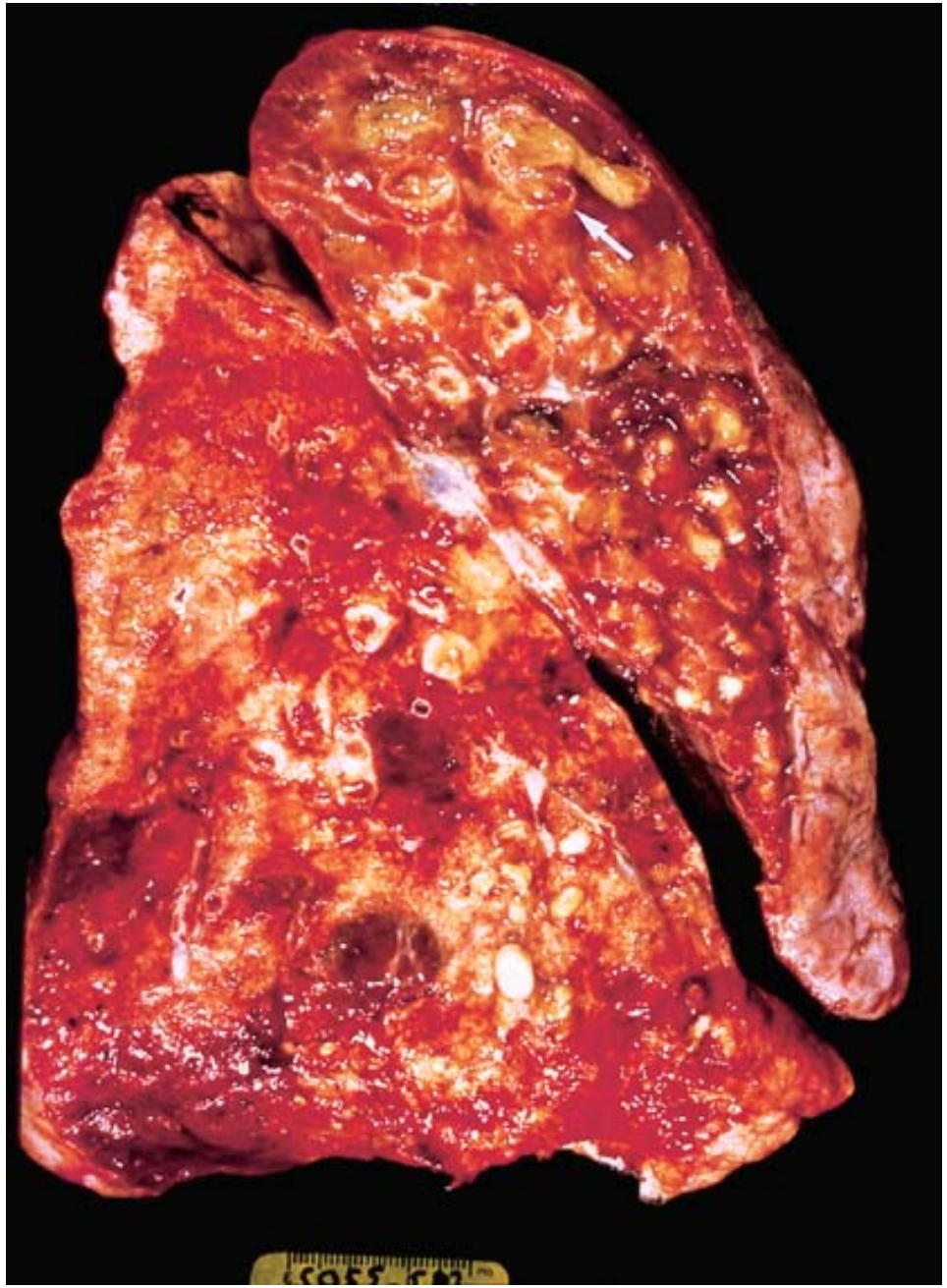


TABLE 15-5 -- Major Categories of Chronic Interstitial Lung Disease

Fibrosing

Usual interstitial pneumonia (idiopathic pulmonary fibrosis)
Nonspecific interstitial pneumonia
Cryptogenic organizing pneumonia
Associated with collagen vascular diseases
Pneumoconiosis
Drug reactions
Radiation pneumonitis
Granulomatous
Sarcoidosis
Hypersensitivity pneumonitis
Eosinophilic
Smoking-Related
Desquamative interstitial pneumonia
Respiratory bronchiolitis-associated interstitial lung disease
Other
Pulmonary alveolar proteinosis

toxic to endothelial cells, epithelial cells, or both. Beyond direct toxicity, a critical event is the *recruitment and activation of inflammatory and immune effector cells*. Neutrophil recruitment can be caused by complement activation in some disorders,[45] but in addition, the alveolar macrophages, which increase in number in all interstitial diseases, release *chemotactic factors* for neutrophils (e.g., IL-8,[46] leukotriene B_4 [47]). In diseases such as sarcoidosis, *cell-mediated immune reactions* result in the accumulation of monocytes and T lymphocytes and in the formation of granulomas (Chapter 6). It is thought that interactions among lymphocytes and macrophages and the release of lymphokines and monokines are responsible for the slowly progressive pulmonary fibrosis that ensues. The alveolar macrophage, in particular, plays a central role in the development of fibrosis, as reviewed in the discussion of chronic inflammation (Chapter 2).

FIBROSING DISEASES

Idiopathic Pulmonary Fibrosis

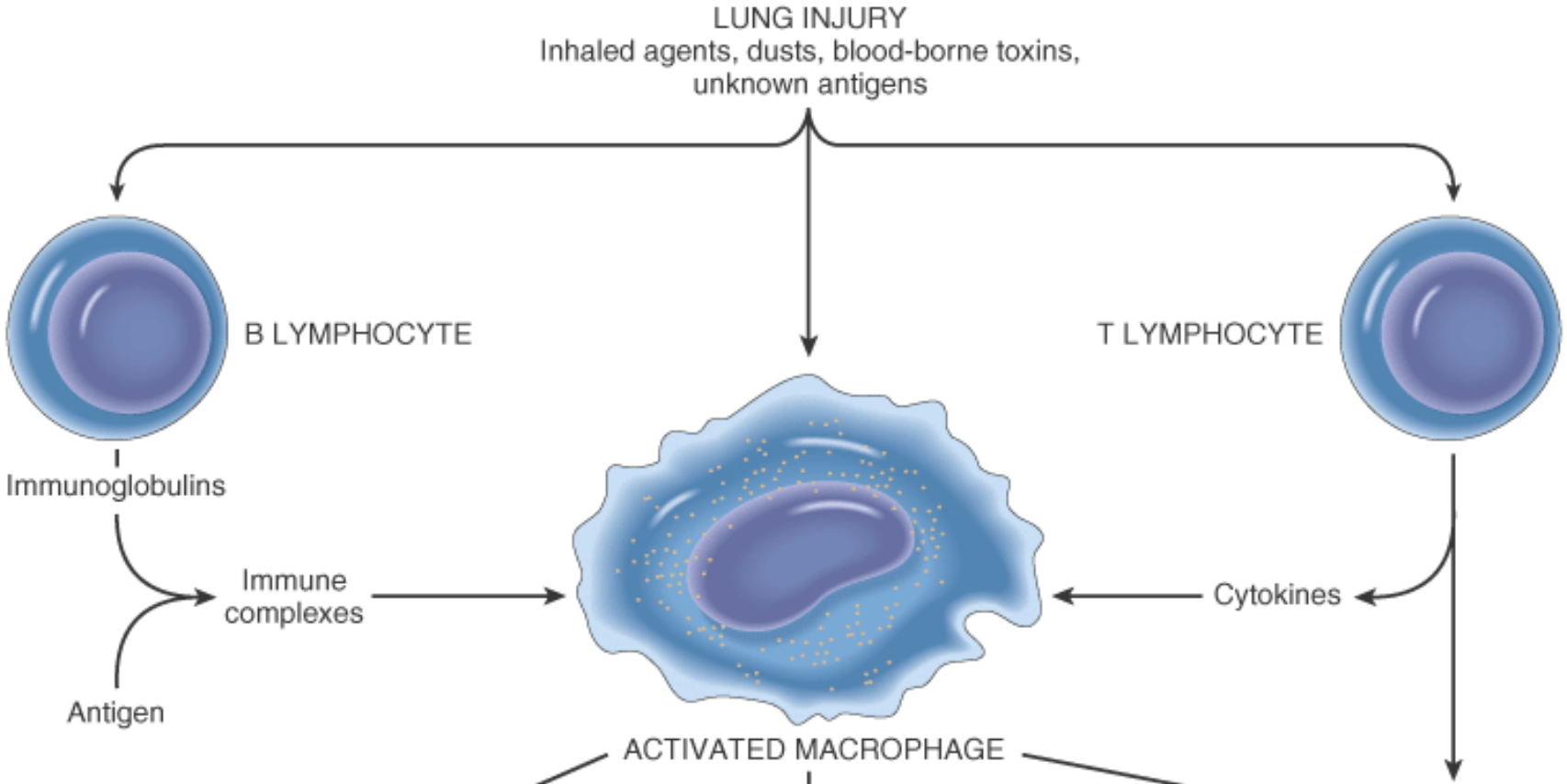
The term "idiopathic pulmonary fibrosis" (IPF) refers to a clinicopathologic syndrome with characteristic radiologic, pathologic, and clinical features. In Europe, the term "cryptogenic fibrosing alveolitis" is more popular. The histologic pattern of fibrosis is referred to as usual interstitial pneumonia (UIP), which is required for the diagnosis of IPF but can also be seen in other diseases, notably collagen vascular disorders and asbestosis. Desquamative interstitial pneumonia (DIP), previously considered an early form of IPF, has now been shown to be a smoking-related disorder. "Hamman-Rich syndrome" is a term that was used to describe a rapidly progressive type of IPF but is now considered a form of acute lung injury and is synonymous with acute interstitial pneumonia. The International Multidisciplinary Consensus Classification is an excellent reference for definitions and understanding of idiopathic interstitial pneumonias.[48] [49]

Pathogenesis.

While the causative agent(s) of IPF remain unknown, our concepts of pathogenesis have evolved over the past several years. The earlier view that IPF was initiated by an unidentified insult that gives rise to chronic inflammation resulting in fibrosis is probably not correct. According to this hypothesis, if the inflammatory response could be controlled before irreversible tissue injury occurs, fibrosis may be prevented. In clinical practice, however, anti-inflammatory therapy has failed to provide much benefit.[50] The current concept, therefore, is that IPF is caused by "repeated cycles" of acute lung injury (alveolitis) by some unidentified agent. "Wound healing" at these sites gives rise to exuberant fibroblastic proliferation, giving rise to the "fibroblastic foci" that are so characteristic of IPF. Mediators of wound healing such as TGF-β are expressed at these sites.

The inflammatory response in IPF may be modified by genetic or environmental factors and is thought to be of the T_H 2 type. Thus, eosinophils, mast cells, and IL-4 and IL-13 are found in the lesions. Repeated cycles of injury and wound healing ultimately lead to widespread fibrosis and loss of lung function. Hence, one effective therapeutic strategy might be to reduce or prevent fibroblast replication. It is interesting to note in this connection that there is an abnormal activation of the Wnt-β-catenin signaling pathways (Chapter 7) within the mesenchymal cells of the fibroproliferative lesions of IPF. [51]

Figure 15-14 A possible schema of the pathogenesis of idiopathic pulmonary fibrosis.



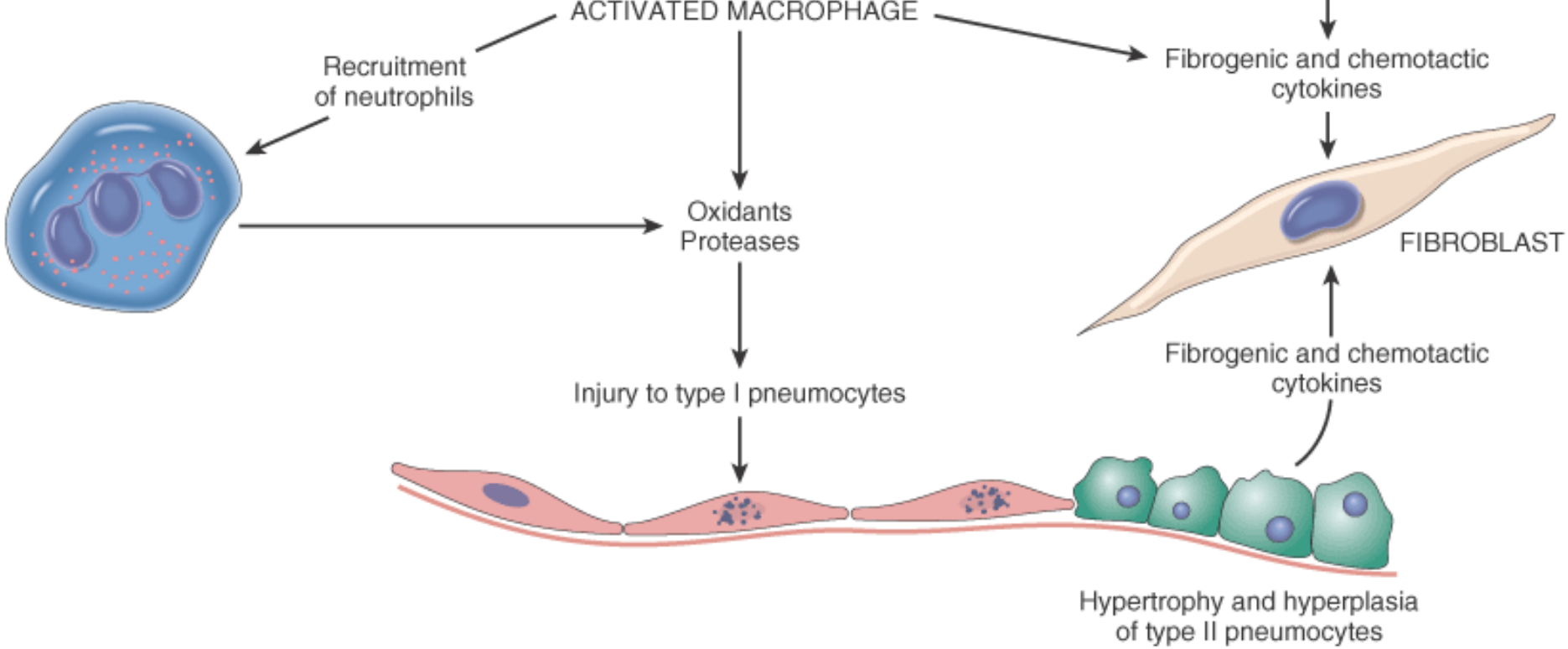


Figure 15-15 Usual interstitial pneumonia. The fibrosis, which varies in intensity, is more pronounced in the subpleural region.

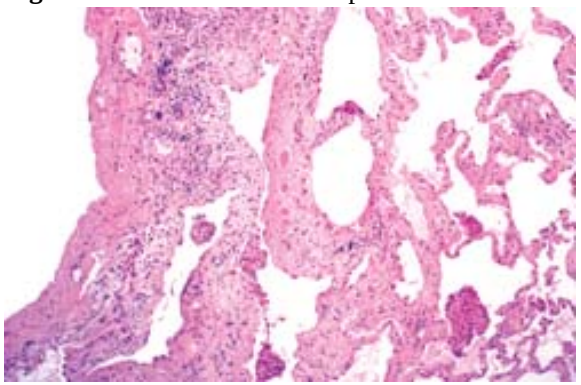


Figure 15-16 Usual interstitial pneumonia. Fibroblastic focus with fibers running parallel to surface and bluish myxoid extracellular matrix.

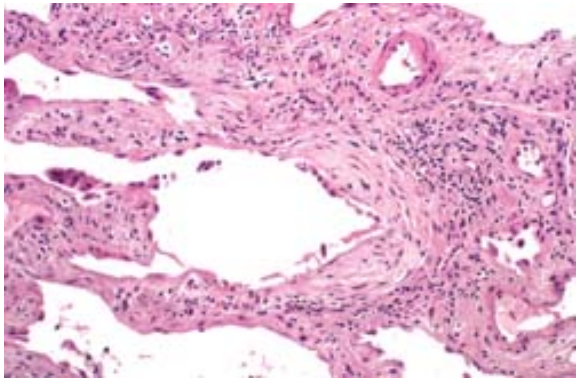


Figure 15-17 Cryptogenic organizing pneumonia (COP). Alveolar spaces are filled with balls of fibroblasts (Masson bodies), while the alveolar walls are relatively normal.

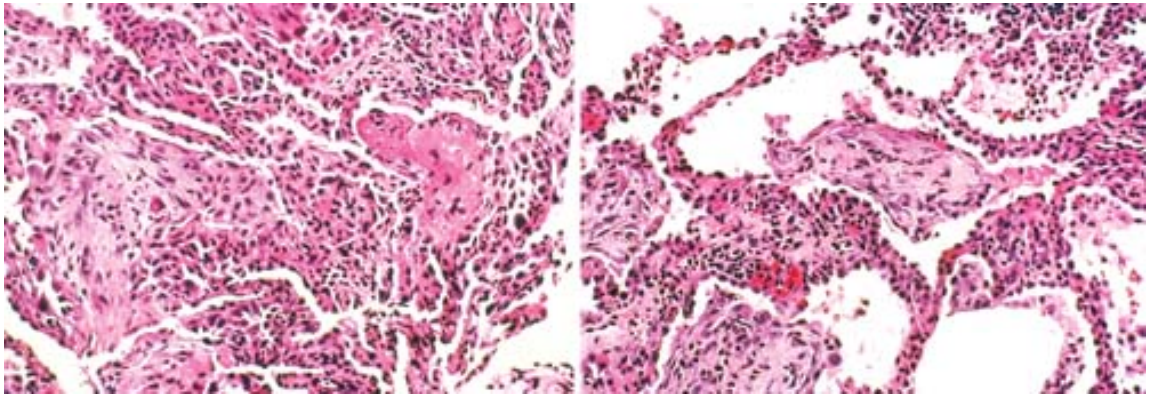


TABLE 15-6 -- Lung Diseases Caused by Air Pollutants

Agent	Disease	Exposure
Mineral Dusts		
Coal dust	Anthracosis	Coal mining (particularly hard coal)
	Macules	
	Progressive massive fibrosis	
	Caplan syndrome	
Silica	Silicosis	Foundry work, sandblasting, hardrock mining, stone cutting, others
	Caplan syndrome	
Asbestos	Asbestosis	Mining, milling, and fabrication; installation and removal of insulation
	Pleural plaques	

	Caplan syndrome	
	Mesothelioma	
	Carcinoma of the lung, larynx, stomach, colon	
Beryllium	Acute berylliosis	Mining, fabrication
	Beryllium granulomatosis	
	Bronchogenic carcinoma (?)	
Iron oxide	Siderosis	Welding
Barium sulfate	Baritosis	Mining
Tin oxide	Stannosis	Mining
Organic Dusts That Induce Hypersensitivity Pneumonitis		
Moldy hay	Farmer's lung	Farming
Bagasse	Bagassosis	Manufacturing wallboard, paper
Bird droppings	Bird-breeder's lung	Bird handling
Organic Dusts That Induce Asthma		
Cotton, flax, hemp	Byssinosis	Textile manufacturing
Red cedar dust	Asthma	Lumbering, carpentry
Chemical Fumes and Vapors		
Nitrous oxide, sulfur dioxide, ammonia, benzene, insecticides	Bronchitis, asthma	Occupational and accidental exposure
	Pulmonary edema	
	ARDS *	
	Mucosal injury	
	Fulminant poisoning	

*Acute respiratory distress syndrome.

In general, only a small percentage of exposed people develop occupational respiratory diseases. In one study, genetic variation of serum and erythrocytic proteins was shown to correlate with susceptibility to developing silicosis, chronic bronchitis, and occupational asthma. Such studies could be useful for assessment and forecast of individual risk of occupational diseases. [60] Many of the diseases listed in Table 15-6 are quite uncommon. Hence only a selected few that cause fibrosis of the lung are presented next.

COAL WORKERS' PNEUMOCONIOSIS (CWP).

Dust reduction measures in coal mines around the globe have drastically reduced the incidence of coal dust-induced disease. The spectrum of lung findings in coal workers is wide, varying from (1) asymptomatic anthracosis to (2) simple coal workers' pneumoconiosis (CWP) with little to no pulmonary dysfunction to (3) complicated CWP, or progressive massive fibrosis (PMF), in which lung function is compromised.[61] It should be noted that PMF is a generic term that applies to a confluent, fibrosing reaction in the lung that can be a complication of any pneumoconiosis, although it is more common in CWP and silicosis.

The pathogenesis of complicated CWP, particularly what causes the lesions of simple CWP to progress to PMF, is incompletely understood. Contaminating silica in the coal dust can favor progressive disease. In most cases, carbon dust itself is the major culprit, and studies have shown that complicated lesions contain considerably more dust than simple lesions do.

Morphology.

Anthracosis is the most innocuous coal-induced pulmonary lesion in coal miners and is commonly seen in all urban dwellers and tobacco smokers. Inhaled carbon pigment is engulfed by alveolar or interstitial macrophages, which then accumulate in the connective tissue along the lymphatics, including the pleural lymphatics, or in organized lymphoid tissue along the bronchi or in the lung hilus. At autopsy, linear streaks and aggregates of anthracotic pigment readily identify pulmonary lymphatics and mark the pulmonary lymph nodes.

Simple CWP is characterized by **coal macules** (1 to 2 mm in diameter) and the somewhat larger **coal nodules**. The coal macule consists of carbon-laden macrophages; the nodule also contains small amounts of a delicate network of collagen fibers. Although these lesions are scattered throughout the lung, the upper lobes and upper zones of the lower lobes are more heavily involved. They are located primarily adjacent to respiratory bronchioles, the site of initial dust accumulation. In due course, dilation

---

of adjacent alveoli occurs, a condition sometimes referred to as **centrilobular emphysema**.

Complicated CWP (PMF) occurs on a background of simple CWP and generally requires many years to develop. It is characterized by intensely blackened scars larger than 2 cm, sometimes up to 10 cm in greatest diameter. They are usually multiple (Fig. 15-18). Microscopically, the lesions consist of dense collagen and pigment. The center of the lesion is often necrotic, resulting most likely from local ischemia.

Clinical Course.

CWP is usually a benign disease that causes little decrement in lung function. Even mild forms of complicated CWP fail to demonstrate abnormalities of lung function. In a minority of cases (fewer than 10%), PMF develops, leading to increasing pulmonary dysfunction, pulmonary hypertension, and cor pulmonale. Once PMF develops, it may become progressive even if further exposure to dust is prevented. Unlike silicosis (discussed later), there is no convincing evidence that coal dust increases susceptibility to tuberculosis. There is some evidence that exposure to coal dust increases the incidence of chronic bronchitis and emphysema,

Figure 15-18 Progressive massive fibrosis superimposed on coal workers' pneumoconiosis. The large, blackened scars are located principally in the upper lobe. Note the extensions of scars into surrounding parenchyma and retraction of adjacent pleura. *(Courtesy of Dr. Werner Laquer, Dr. Jerome Kleinerman, and the National Institute of Occupational Safety and Health, Morgantown, WV.)*

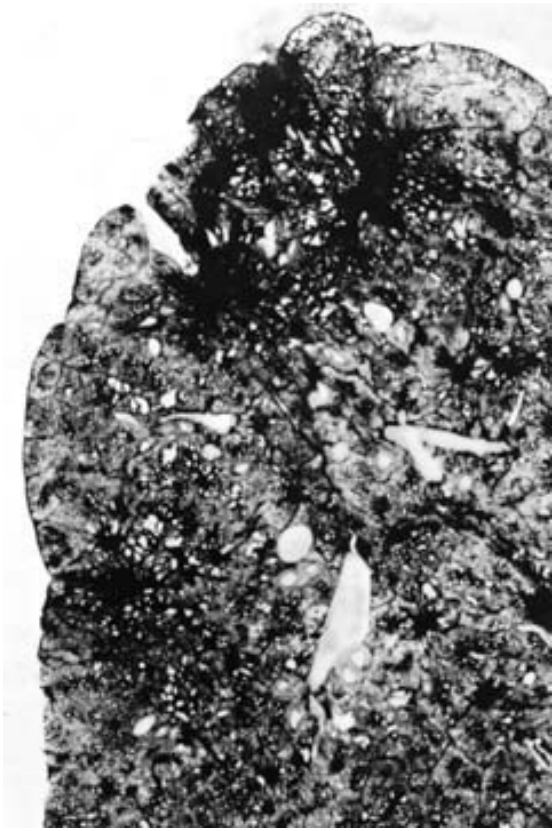


Figure 15-19 Advanced silicosis seen on transection of lung. Scarring has contracted the upper lobe into a small dark mass (*arrow*). Note the dense pleural thickening. *(Courtesy of Dr. John Godleski, Brigham and Women's Hospital, Boston, MA.)*

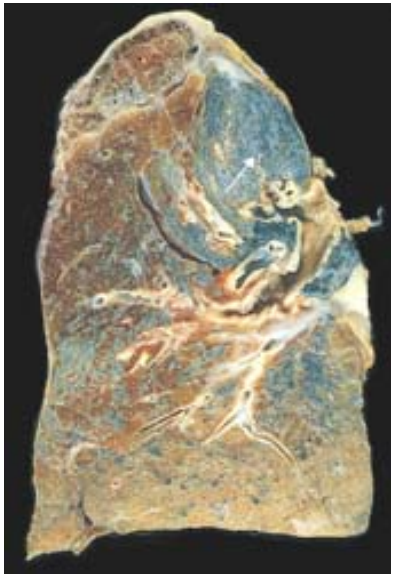


Figure 15-20 Several coalescent collagenous silicotic nodules. *(Courtesy of Dr. John Godleski, Brigham and Women's Hospital, Boston, MA.)*

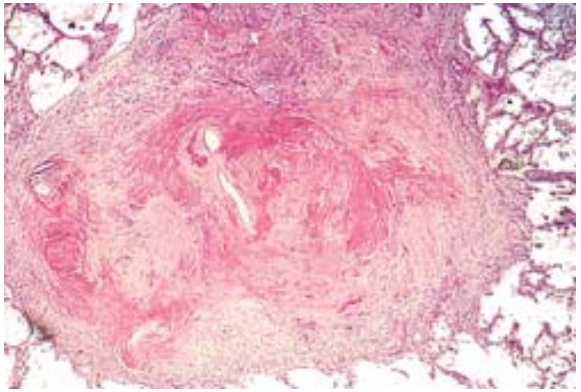


Figure 15-21 High-power detail of an asbestos body, revealing the typical beading and knobbed ends (*arrow*).

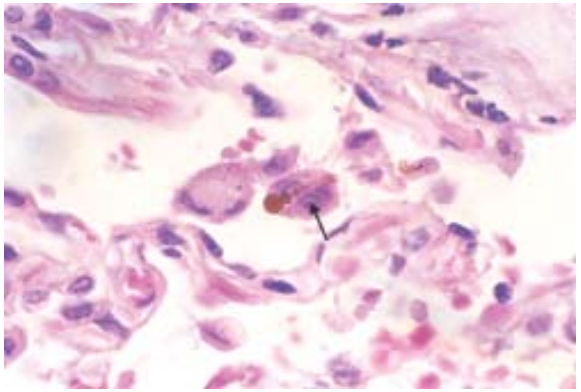


Figure 15-22 Asbestos exposure evidenced by severe, discrete, characteristic fibrocalcific plaques on the pleural surface of the diaphragm. *(Courtesy of Dr. John Godleski, Brigham and Women's Hospital, Boston, MA.)*

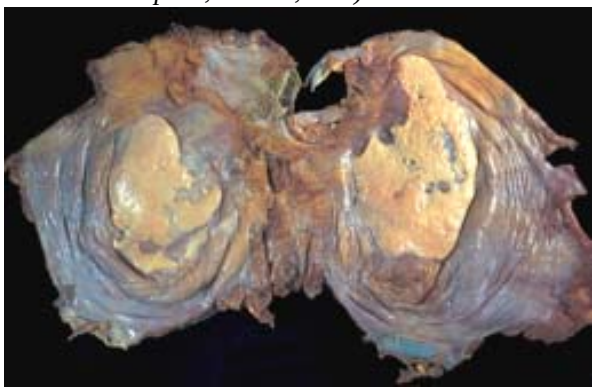


TABLE 15-7 -- Examples of Drug-Induced Pulmonary Disease

Drug	Pulmonary Disease

Cytotoxic drugs	
••Bleomycin	Pneumonitis and fibrosis
••Methotrexate	Hypersensitivity pneumonitis
Amiodarone	Pneumonitis and fibrosis
Nitrofurantoin	Hypersensitivity pneumonitis
Aspirin	Bronchospasm
β-Antagonists	Bronchospasm

changes are those of diffuse alveolar damage, including severe atypia of hyperplastic type II cells and fibroblasts. Epithelial cell atypia and foam cells within vessel walls are also characteristic of radiation damage.

GRANULOMATOUS DISEASES

Sarcoidosis

Sarcoidosis is a systemic disease of unknown cause characterized by noncaseating granulomas in many tissues and organs. Sarcoidosis presents many clinical patterns, but bilateral hilar lymphadenopathy or lung involvement is visible on chest radiographs in 90% of cases. Eye and skin lesions are next in frequency. Since other diseases, including mycobacterial or fungal infections and berylliosis, can also produce noncaseating (*hard*) granulomas, the histologic diagnosis of sarcoidosis is made by exclusion.

The prevalence of sarcoidosis is higher in women than in men but varies widely in different countries and populations. In the United States, the rates are highest in the Southeast; they are 10 times higher in American blacks than in whites. By contrast, among Chinese and Southeast Asians, the disease is rare.

Etiology and Pathogenesis.

Although the etiology of sarcoidosis remains unknown, several lines of evidence suggest that it is a disease of disordered immune regulation in genetically predisposed individuals exposed to certain environmental agents.[71] The role of each of these three contributory factors is summarized below.

Immunologic Factors.

There are several *immunologic abnormalities* in the local milieu of sarcoid granulomas that suggest the development of a cell-mediated response to an unidentified antigen. The process is driven by CD4+ helper T cells. These abnormalities include:[72]

- Intra-alveolar and interstitial accumulation of CD4+ T cells, resulting in CD4:CD8 T-cell ratios ranging from 5:1 to 15:1. There is oligoclonal expansion of T-cell subsets as determined by analysis of T-cell receptor rearrangement, suggesting an antigen-driven proliferation.
- Increased levels of T cell-derived T_H 1 cytokines such as IL-2 and interferon-γ (IFN-γ), resulting in T-cell expansion and macrophage activation, respectively.
- Increased levels of several cytokines in the local environment (IL-8, TNF, macrophage inflammatory protein 1α [MIP-1α]) that favor recruitment of additional T cells and monocytes and contribute to the formation of granulomas. TNF in particular is released at high levels by activated alveolar macrophages, and the TNF level in the bronchoalveolar fluid is a marker of disease activity.

Additionally, there are *systemic immunologic abnormalities* in patients with sarcoidosis:

- Anergy to common skin test antigens such as *Candida* or purified protein derivative (PPD)
- Polyclonal hypergammaglobulinemia, another manifestation of helper T-cell dysregulation

Genetic Factors.

Evidence of genetic influences can be seen:

- Familial and racial clustering of cases
- Association with certain HLA genotypes (e.g., class I HLA-A1 and HLA-B8)

Environmental Factors.

These are possibly the most tenuous of all the associations in the pathogenesis of sarcoidosis. Several putative microbes have been proposed as the inciting agent for sarcoidosis (e.g., mycobacteria, *Propionibacterium acnes*, and *Rickettsia* species).[73] To date, *there is no unequivocal evidence to suggest that sarcoidosis is caused by an infectious agent*.

Morphology.

Histologically, all involved tissues show the classic **noncaseating granulomas** (Fig. 15-23), each composed of an aggregate of tightly clustered epithelioid cells, often with Langhans or foreign body type giant cells. Central necrosis is unusual. With chronicity, the granulomas may become enclosed within fibrous rims or may eventually be replaced by hyaline fibrous scars. Two other microscopic features are often present in the granulomas: (1) laminated concretions composed of calcium and proteins known as Schaumann bodies and (2) stellate inclusions known as asteroid bodies enclosed within giant cells found in approximately 60% of the granulomas. Although characteristic, these microscopic features are not pathognomonic of sarcoidosis because asteroid and Schaumann bodies may be encountered in other granulomatous diseases (e.g., tuberculosis). Pathologic involvement of virtually every organ in the body has been cited at one time or another.

The **lungs** are common sites of involvement. [74] Macroscopically, there is usually no demonstrable alteration, although at times, the coalescence of granulomas may produce small nodules that are palpable or visible as 1- to 2-cm, noncaseating, noncavitated consolidations. Histologically, the lesions are distributed primarily along the lymphatics, around bronchi and blood vessels, although alveolar lesions are also seen. The relative frequency of granulomas in the bronchial submucosa accounts for the high diagnostic yield of bronchoscopic biopsies. A CD4/CD8 ratio >2.5 and the CD3/CD4 ratio <0.31 in bronchoalveolar lavage lymphocytes is commonly seen in sarcodosis.[75] There appears to be a strong tendency for

Figure 15-23 Characteristic sarcoid noncaseating granulomas in lung with many giant cells. *(Courtesy of Dr. Ramon Blanco, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

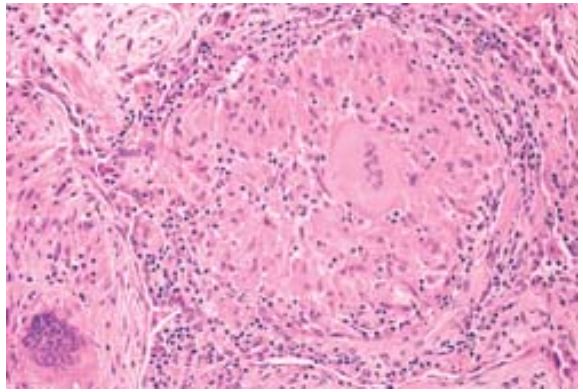


Figure 15-24 Hypersensitivity pneumonitis, histologic appearance. Loosely formed interstitial granulomas and chronic inflammation are characteristic.

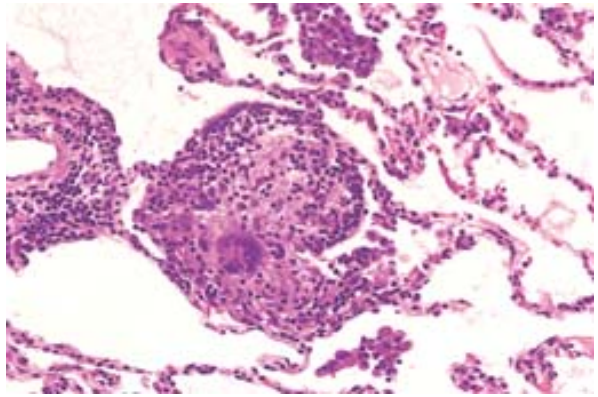


Figure 15-25 Desquamative interstitial pneumonia: medium-power detail of lung to demonstrate the accumulation of large numbers of mononuclear cells within the alveolar spaces with only mild fibrous thickening of the alveolar walls.

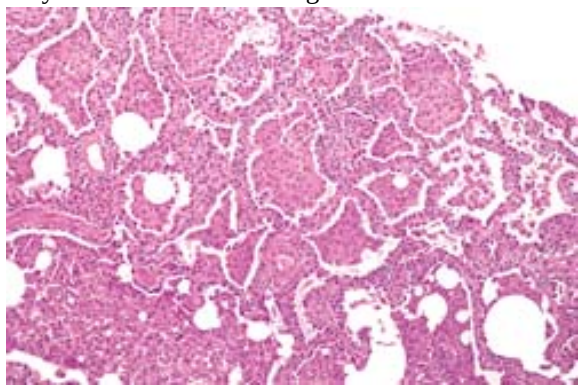


Figure 15-26 Pulmonary alveolar proteinosis, histologic appearance. The alveoli are filled with a dense, amorphous, protein-lipid granular precipitate, while the alveolar walls are normal.

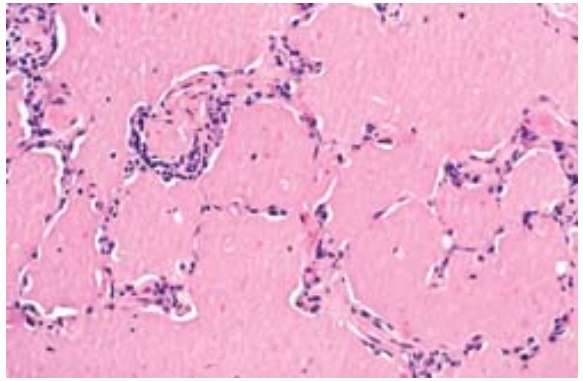


Figure 15-27 Large saddle embolus from the femoral vein lying astride the main left and right pulmonary arteries. *(From the teaching collection of the Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX.)*

Figure 15-28 Recent, small, roughly wedge-shaped hemorrhagic pulmonary infarct.

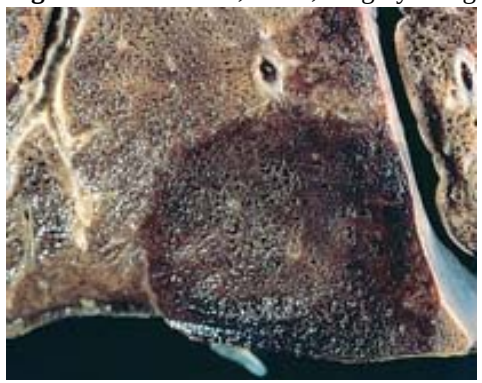


Figure 15-29 Pathogenesis of primary pulmonary hypertension.

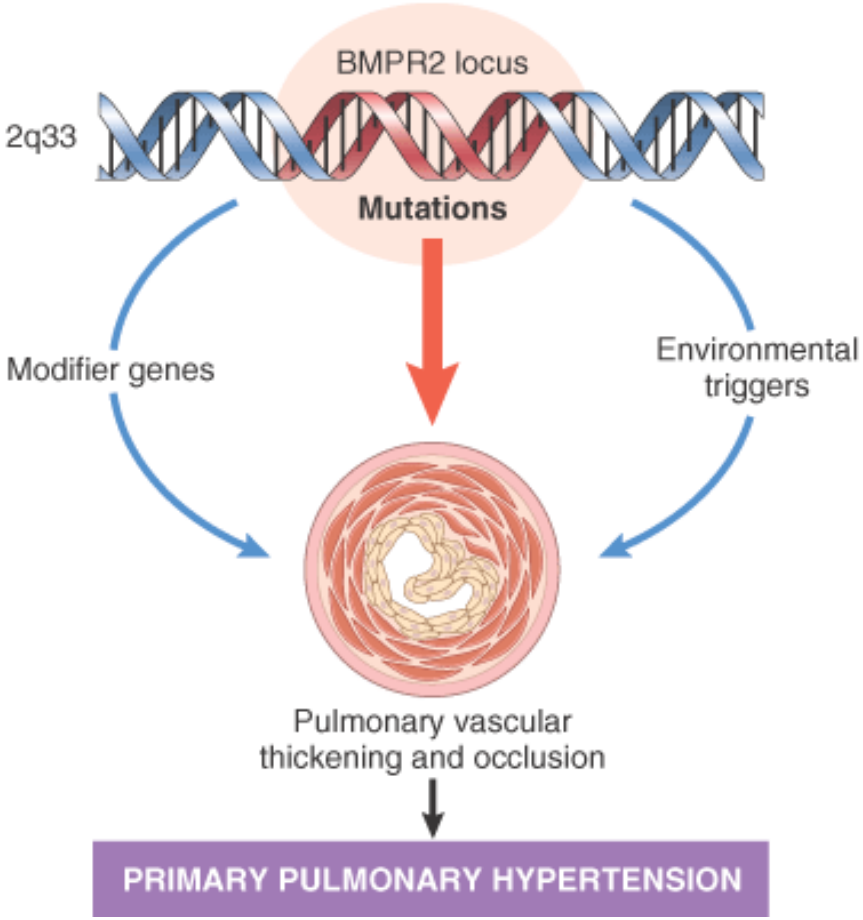


Figure 15-30 Vascular changes in pulmonary hypertension. *A*, Gross photograph of atheroma formation, a finding usually limited to large vessels. *B*, Marked medial hypertrophy. *C*, Plexogenic lesion characteristic of advanced pulmonary hypertension seen in small arteries.

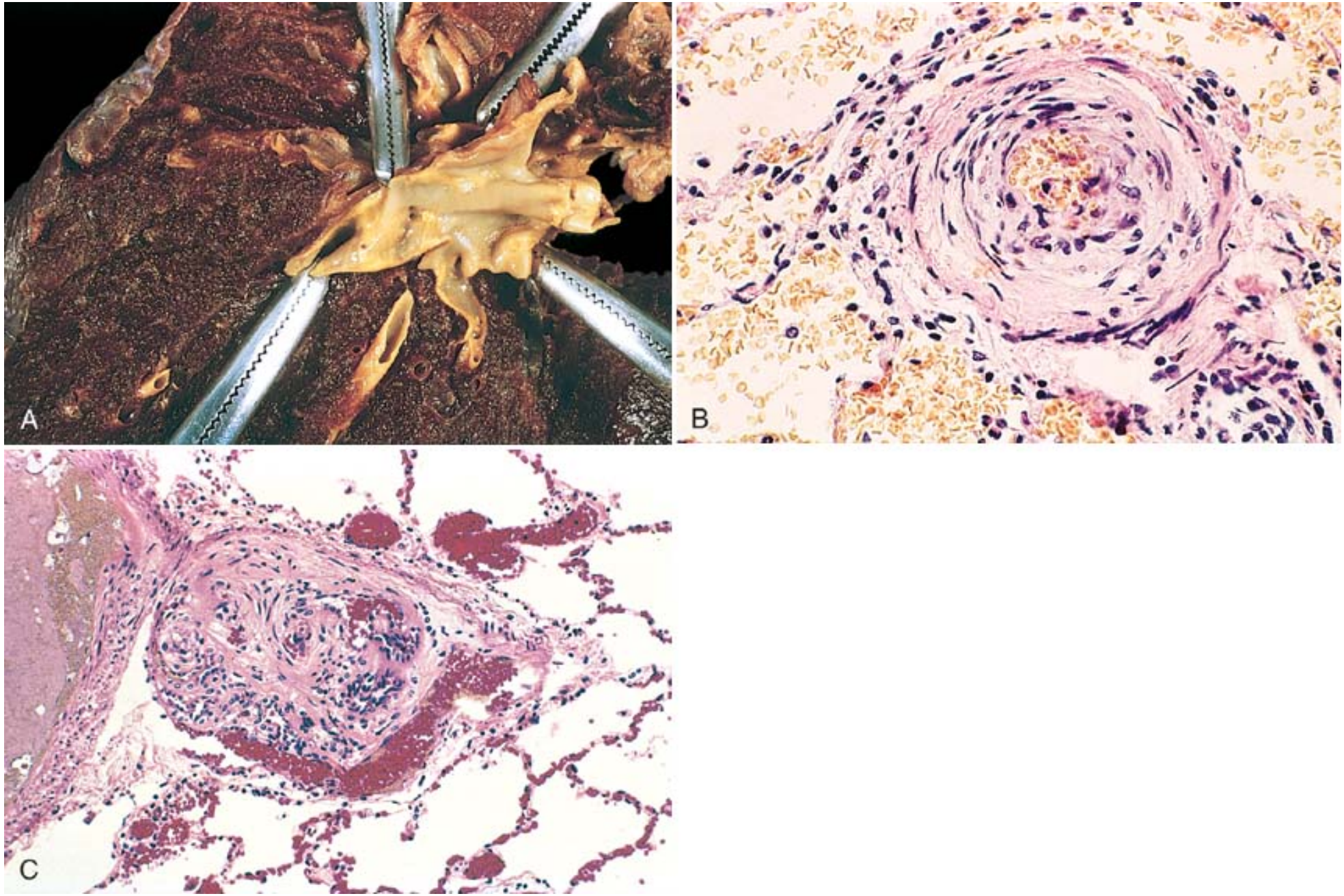


Figure 15-31 Acute intra-alveolar hemorrhage and hemosiderin-laden macrophages, reflecting previous hemorrhage, are common features of the diffuse pulmonary hemorrhage syndromes (Prussian blue stain for iron).

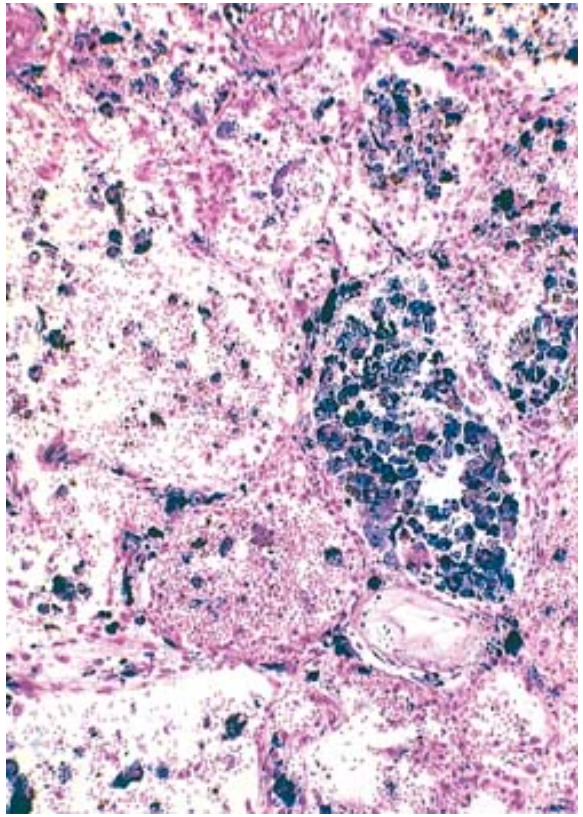


TABLE 15-8 -- The Pneumonia Syndromes

Community-Acquired Acute Pneumonia
Streptococcus pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Staphylococcus aureus
Legionella pneumophila
Enterobacteriaceae (*Klebsiella pneumoniae*) and *Pseudomonas* spp.
Community-Acquired Atypical Pneumonia
Mycoplasma pneumoniae
Chlamydia spp. (*C. pneumoniae, C. psittaci, C. trachomatis*)
Coxiella burnetti (Q fever)
Viruses: respiratory syncytial virus, parainfluenza virus (children); influenza A and B (adults); adenovirus (military recruits); SARS * virus

Nosocomial Pneumonia
Gram-negative rods belonging to Enterobacteriaceae (*Klebsiella* spp., *Serratia marcescens*, *Escherichia coli*) and *Pseudomonas* spp.
Staphylococcus aureus (usually penicillin-resistant)
Aspiration Pneumonia
Anaerobic oral flora (*Bacteroides, Prevotella, Fusobacterium, Peptostreptococcus*), admixed with aerobic bacteria *(Streptococcus pneumoniae, Staphylococcus aureus, Haemophilas influenzae*, and *Pseudomonas aeruginosa)*
Chronic Pneumonia
Nocardia
Actinomyces
Granulomatous: *Mycobacterium tuberculosis* and atypical mycobacteria, *Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis*
Necrotizing Pneumonia and Lung Abscess
Anaerobic bacteria (extremely common), with or without mixed aerobic infection
Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pyogenes, and type 3 pneumococcus (uncommon)
Pneumonia in the Immunocompromised Host
Cytomegalovirus
Pneumocystis carinii
Mycobacterium avium-intracellulare
Invasive aspergillosis
Invasive candidiasis
"Usual" bacterial, viral, and fungal organisms (listed above)

*SARS, Severe acute respiratory syndrome

COMMUNITY-ACQUIRED ACUTE PNEUMONIAS

Community-acquired pneumonias may be bacterial or viral. Here we discuss acute pneumonias caused by bacteria, viral pneumonias are considered later in the section on atypical pneumonias. Often, the bacterial infection follows an upper respiratory tract viral infection. Bacterial invasion of the lung parenchyma causes the alveoli to be filled with an inflammatory exudate, thus causing consolidation ("solidification") of the pulmonary tissue. Many variables, such as the specific etiologic agent, the host reaction, and the extent of involvement, determine the precise form of pneumonia. Predisposing conditions include extremes of age, chronic diseases (congestive heart failure, COPD, and diabetes), congenital or acquired immune deficiencies, and decreased or absent splenic function (sickle cell disease or post splenectomy, which puts the patient at risk for infection with encapsulated bacteria such as

pneumococcus). First we describe pneumonias caused by various organisms and then the morphologic and clinical features common to most pneumonias.

Streptococcus Pneumoniae

Streptococcus pneumoniae, or *pneumococcus*, is the most common cause of community-acquired acute pneumonia. Examination of Gram-stained sputum is an important step in the diagnosis of acute pneumonia. The presence of numerous neutrophils containing the typical Gram-positive, lancet-shaped diplococci supports the diagnosis of pneumococcal pneumonia, but it must be remembered that *S. pneumoniae* is a part of the endogenous flora in 20% of adults, and therefore false-positive results may be obtained. Isolation of pneumococci from blood cultures is more specific but less sensitive (in the early phase of illness, only 20% to 30% of patients have positive blood cultures). Pneumococcal pneumonias respond readily to penicillin treatment, but there are increasing numbers of penicillin-resistant strains of pneumococci, so whenever possible, antibiotic sensitivity should be determined. Pneumococcal vaccines containing capsular polysaccharides from the common serotypes are available for use in patients at high risk.

Haemophilus Influenzae

Haemophilus influenzae is a pleomorphic, Gram-negative organism that is a major cause of life-threatening acute lower respiratory tract infections and meningitis in young children. In adults it is a very common cause of community-acquired acute pneumonia.[98] This bacterium is a ubiquitous colonizer of the pharynx, where it exists in two forms: encapsulated (5%) and unencapsulated (95%). Typically, the encapsulated form dominates the unencapsulated forms by secreting an antibiotic called haemocin that kills the unencapsulated *H. influenzae*.[99] Although there are six serotypes of the encapsulated form (types a to f), type b, which has a polyribosephosphate capsule, used to be the most frequent cause of severe invasive disease. With routine use of *H. influenzae* conjugate vaccines, the incidence of disease caused by the b serotype has declined significantly. By contrast, infections with nonencapsulated forms are increasing. Also called nontypable forms, they spread along the surface of the upper respiratory tract and produce otitis media (infection of the middle ear), sinusitis, and bronchopneumonia.

Pili on the surface of *H. influenzae* mediate adherence of the organisms to the respiratory epithelium.[100] In addition, *H. influenzae* secretes a factor that disorganizes ciliary beating and a protease that degrades IgA, the major class of antibody secreted into the airways. Survival of *H. influenzae* in the bloodstream correlates with the presence of the capsule, which, like that of pneumococcus, prevents opsonization by complement and phagocytosis by host cells. Antibodies against the capsule protect the host from *H. influenzae* infection, hence the capsular polysaccharide b is incorporated in the vaccine for children against *H. influenzae*.

H. influenzae pneumonia, which may follow a viral respiratory infection, is a pediatric emergency and has a high mortality rate. Descending laryngotracheobronchitis results in airway obstruction as the smaller bronchi are plugged by dense, fibrin-rich exudate of polymorphonuclear cells, similar to that seen in pneumococcal pneumonias. Pulmonary consolidation is usually lobular and patchy but may be confluent and involve the entire lung lobe. Before a vaccine became widely available, *H. influenzae* was a common cause of suppurative meningitis in children up to 5 years of age. *H. influenzae* also causes an acute, purulent conjunctivitis (pinkeye) in children and, in predisposed older patients, may cause septicemia, endocarditis, pyelonephritis, cholecystitis, and suppurative arthritis. *H. influenzae* is the most common bacterial cause of acute exacerbation of COPD.

Moraxella Catarrhalis

Moraxella catarrhalis is being increasingly recognized as a cause of bacterial pneumonia, especially in the elderly. It is the second most common bacterial cause of acute exacerbation of COPD. Along with *S. pneumoniae* and *H. influenzae, M. catarrhalis* constitutes one of the three most common causes of otitis media in children.

Staphylococcus Aureus

Staphylcocus aureus is an important cause of secondary bacterial pneumonia in children and healthy adults following viral respiratory illnesses (e.g., measles in children and influenza in both children and adults). Staphylococcal pneumonia is associated with a high incidence of complications, such as lung abscess and empyema. *Intravenous drug abusers* are at high risk of developing staphylococcal pneumonia in association with endocarditis. It is also an important cause of nosocomial pneumonia, as will be discussed later.

Klebsiella Pneumoniae

Klebsiella pneumoniae is the most frequent cause of Gram-negative bacterial pneumonia. It commonly afflicts debilitated and malnourished people, particularly *chronic alcoholics*. Thick and gelatinous sputum is characteristic because the organism produces an abundant viscid capsular polysaccharide, which the patient may have difficulty coughing up.

Pseudomonas Aeruginosa

Although *Pseudomonas aeruginosa* most commonly causes nosocomial infections, it is mentioned here because of its

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occurrence in cystic fibrosis patients. It is common in patients who are neutropenic and it has a propensity to invade blood vessels with consequent extrapulmonary spread. *Pseudomonas* septicemia is a very fulminant disease.

Legionella Pneumophila

Legionella pneumophila is the agent of Legionnaires disease, an eponym for the epidemic and sporadic forms of pneumonia caused by this organism. Pontiac fever is a related self-limited upper respiratory tract infection caused by *L. pneumophila*, without pneumonic symptoms. This organism flourishes in artificial aquatic environments, such as water-cooling towers and within the tubing system of domestic (potable) water supplies. The mode of transmission is thought to be either inhalation of aerosolized organisms or aspiration of contaminated drinking water. *Legionella* pneumonia is common in individuals with some predisposing condition such as cardiac, renal, immunologic, or hematologic disease. *Organ transplant recipients are particularly susceptible.* It can be quite severe, frequently requiring hospitalization, and immunosuppressed patients may have fatality rates of up to 50%. Rapid diagnosis is facilitated by demonstration of *Legionella* antigens in the urine or by a positive fluorescent antibody test on sputum samples; culture remains the gold standard of diagnosis.

Morphology.

Bacterial pneumonia has two gross patterns of anatomic distribution: lobular bronchopneumonia and lobar pneumonia (Fig. 15-32). Patchy consolidation of the lung is the dominant characteristic of **bronchopneumonia** (Fig. 15-33). **Lobar pneumonia** is an acute bacterial infection resulting in fibrinosuppurative consolidation of a large portion of a lobe or of an entire lobe (Fig. 15-34). These anatomic but still classic categorizations are often difficult to apply in the individual case because patterns overlap. The patchy involvement may become confluent, producing virtually total lobar consolidation; in contrast, effective antibiotic therapy for any form of pneumonia may limit involvement to a subtotal consolidation. Moreover, the same organisms may produce bronchopneumonia

Figure 15-32 Comparison of bronchopneumonia and lobar pneumonia.

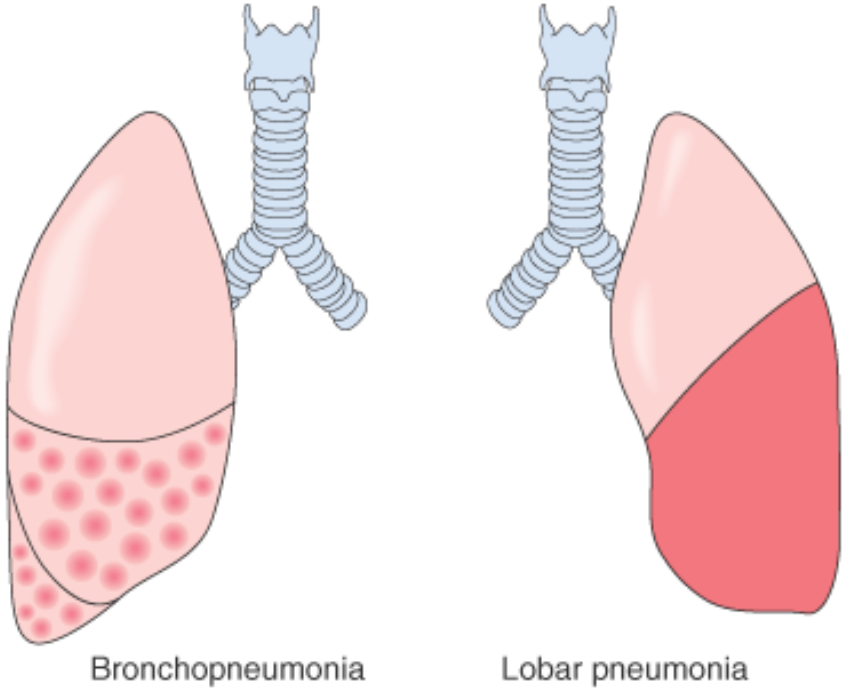

Figure 15-33 Bronchopneumonia. Gross section of lung showing patches of consolidation (*arrows*).

Figure 15-34 Lobar pneumonia—gray hepatization, gross photograph. The lower lobe is uniformly consolidated.

Figure 15-35 *A,* Acute pneumonia. The congested septal capillaries and extensive neutrophil exudation into alveoli corresponds to early red hepatization. Fibrin nets have not yet formed. *B,* Early organization of intra-alveolar exudate, seen in areas to be streaming through the pores of Kohn (*arrow*). *C,* Advanced organizing pneumonia (corresponding to gray hepatization), featuring transformation of exudates to fibromyxoid masses richly infiltrated by macrophages and fibroblasts.

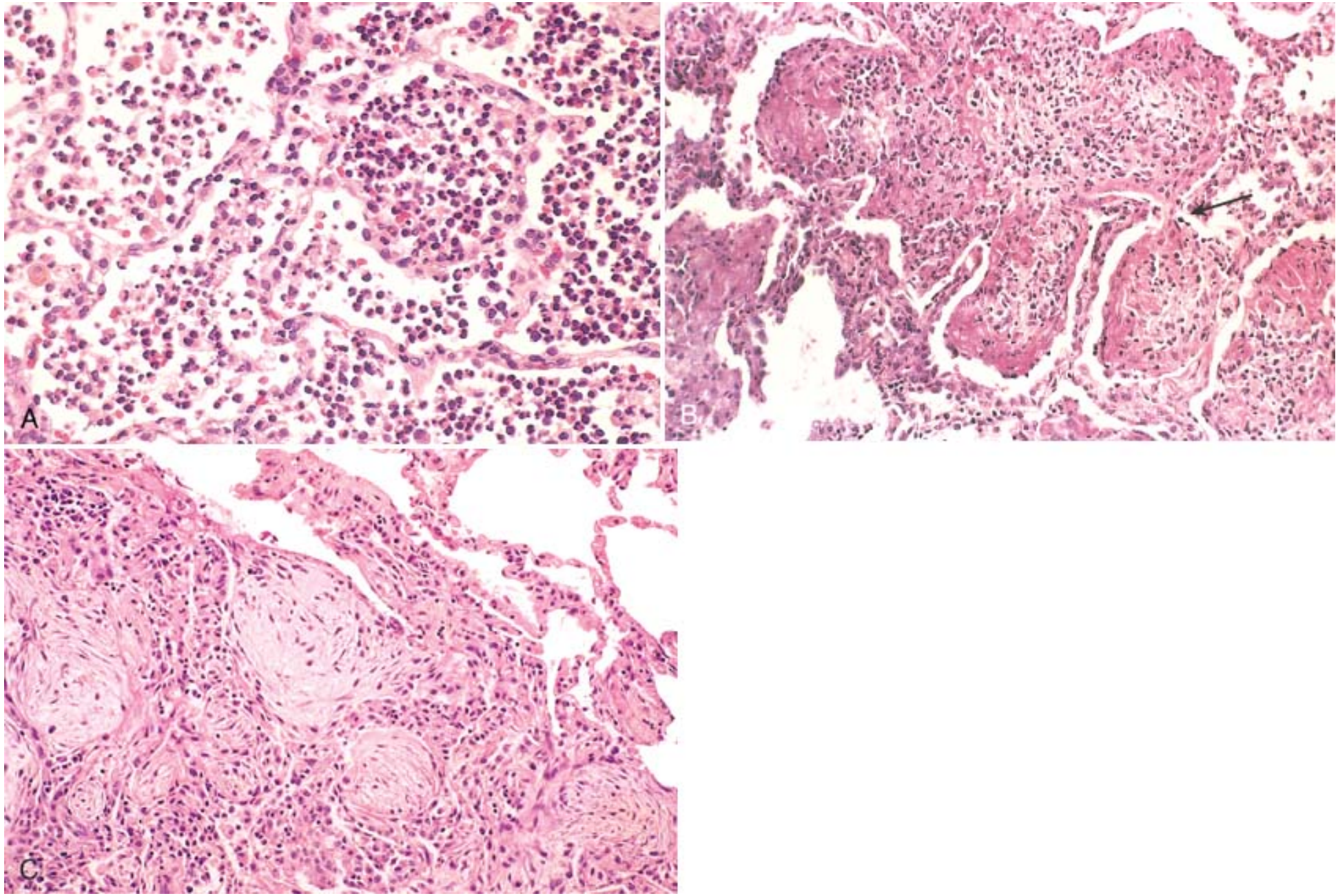

Figure 15-36 Pyemic lung abscess in the center of section with complete destruction of underlying parenchyma within the focus of involvement.

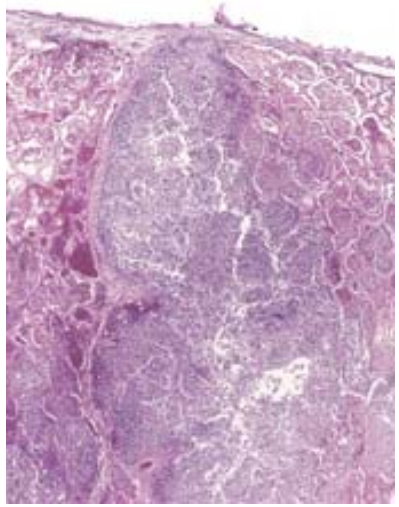

Figure 15-37 Laminated *Histoplasma* granuloma of the lung.

Figure 15-38 *Histoplasma capsulatum* yeast forms fill phagocytes in a lymph node of a patient with disseminated histoplasmosis.

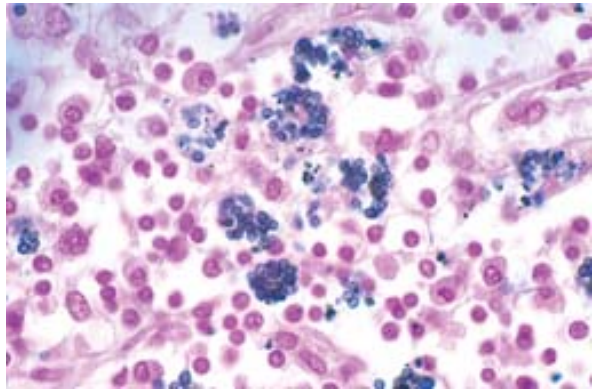

Figure 15-39 Blastomycosis. *A*, Rounded budding yeasts, larger than neutrophils, are present. Note the characteristic thick wall and nuclei (not seen in other fungi). *B*, Silver stain.

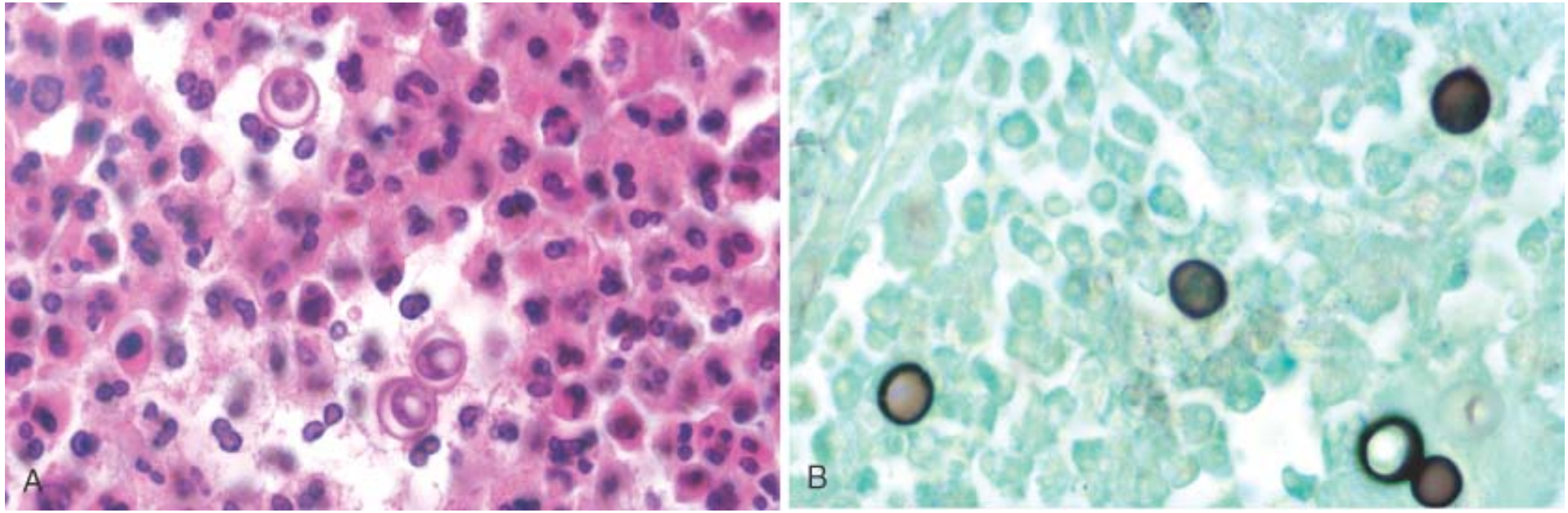

Figure 15-40 Coccidioidomycosis with intact spherules within multinucleated giant cells.

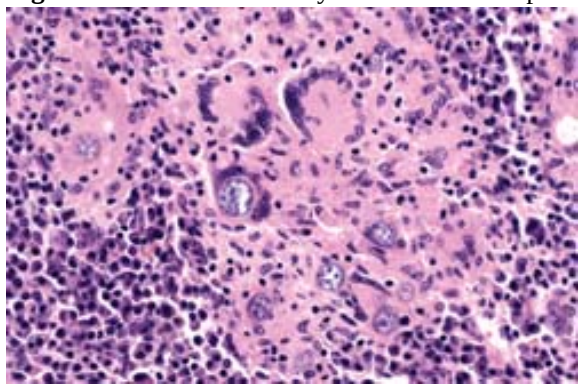

TABLE 15-9 -- Causes of Pulmonary Infiltrates in Immunocompromised Hosts

Diffuse Infiltrate	**Focal Infiltrate**
Common	***Common***
Cytomegalovirus	Gram-negative rods
Pneumocystis carinii	*Staphylococcus aureus*
Drug reaction	*Aspergillus*
	Candida
	Malignancy
Uncommon	***Uncommon***
Bacteria	*Cryptococcus*
Aspergillus	*Mucor*
Cryptococcus	*Pneumocystis carinii*
Malignancy	*Legionella pneumophila*

plethora of entities involved makes diagnosis and treatment a distinct challenge. Some of the individual microbial agents afflicting HIV-infected patients have already been discussed; this section will focus only on the general principles of HIV-associated pulmonary disease.

- Despite the emphasis on "opportunistic" infections, it must be remembered that bacterial lower respiratory tract infection caused by the "usual" pathogens is one of the most serious pulmonary disorders in HIV infection. The implicated organisms include *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, and Gram-negative rods. Bacterial pneumonias in HIV-infected patients are more common, more severe, and more often associated with bacteremia than in those without HIV infection.
- Not all pulmonary infiltrates in HIV-infected individuals are infectious in etiology. A host of noninfectious diseases, including Kaposi sarcoma (Chapter 6 and Chapter 11), pulmonary non-Hodgkin lymphoma (Chapter 14), and primary lung cancer, occur with increased frequency and need to be excluded.
- *The CD4+ T cell count can define the risk of infection with specific organisms.* As a rule of thumb, bacterial and tubercular infections are more likely at higher CD4+ counts (>200 cells/mm^3). Pneumocystis pneumonia usually strikes at CD4+ counts below 200 cells/mm^3 , while cytomegalovirus and *Mycobacterium avium* complex infections are uncommon until the very late stages of immunosuppression (CD4+ counts <50 cells/mm^3).

Finally, it is useful to remember that pulmonary disease in HIV-infected patients may result from more than one cause, and even common pathogens may present with atypical manifestations. Therefore, the diagnostic workup of these patients may be more extensive (and expensive) than would be mandated in an immunocompetent individual.

Lung Transplantation

Indications for transplantation may include almost all nonneoplastic terminal lung diseases, provided that the patient does not have any other serious disease, which would preclude lifelong immunosuppressive therapy. The most common indications are end-stage emphysema, idiopathic pulmonary fibrosis, cystic fibrosis, and primary pulmonary hypertension. While bilateral lung and heart-lung transplants are possible, in many cases a single lung transplant is performed, offering sufficient improvement in pulmonary function for each of two recipients from a single (and all too scarce) donor. When bilateral chronic infection is present (e.g., cystic fibrosis, bronchiectasis), both lungs of the recipient must be replaced to remove the reservoir of infection.

Morphology.

With improving surgical and organ preservation techniques, postoperative complications (e.g., anastomotic dehiscence, vascular thrombosis, primary graft dysfunction) are happily becoming rare. The transplanted lung is subject to two major complications: infection and rejection.

Pulmonary infections in lung transplant patients are essentially those of any immunocompromised host, discussed earlier. They include bacterial and viral (especially cytomegalovirus) pneumonias, *Pneumocystis carinii* pneumonia (PCP), and fungal infections. In the early posttransplant period (the first few weeks), bacterial infections are most common. With Gancyclovir prophylaxis and matching of donor-recipient CMV status, CMV pneumonia occurs less frequently and is less severe, although some resistant strains are emerging. Most cases occur in the third to twelfth month after transplant. PCP is rare, since almost all patients receive adequate prophylaxis (usually Bactrim™). Fungal infections are mostly due to *Candida* and *Aspergillus* species, and they involve the bronchial anastamotic site and/or the lung.

Acute rejection of the lung occurs to some degree in all patients despite routine immunosuppression postoperatively. It often occurs during the early weeks to months after surgery but may occur years later whenever immunosuppression is decreased. Patients present with fever, dyspnea, cough, and radiologic infiltrates. Since these are similar to the picture of infections, diagnosis often relies on transbronchial biopsy. The morphologic features of acute rejection are primarily those of inflammatory infiltrates (lymphocytes, plasma cells, and few neutrophils and eosinophils), either around small vessels, in the submucosa of airways, or both.[112]

Chronic rejection is a significant problem in at least half of all lung transplant patients by 3 to 5 years. It is manifested by cough, dyspnea, and an irreversible decrease in lung function tests. The major morphologic correlate of chronic rejection is **bronchiolitis obliterans,** the partial or complete occlusion of small airways by fibrosis, with or without active inflammation (Fig. 15-41). Bronchiolitis obliterans is patchy and therefore difficult to diagnose via transbronchial biopsy. Bronchiectasis may develop in long-standing cases.

The acute cellular airway rejection (the presumed forerunner of later, fibrous obliteration of these airways) is generally

Figure 15-41 Chronic rejection of lung allograft, with total occlusion of bronchiole (bronchiolitis obliterans). Adjacent pulmonary artery branch is normal. *(Courtesy of Dr. Thomas Krausz, Department of Pathology, The University of Chicago, Pritzker School of Medicine, Chicago, IL.)*

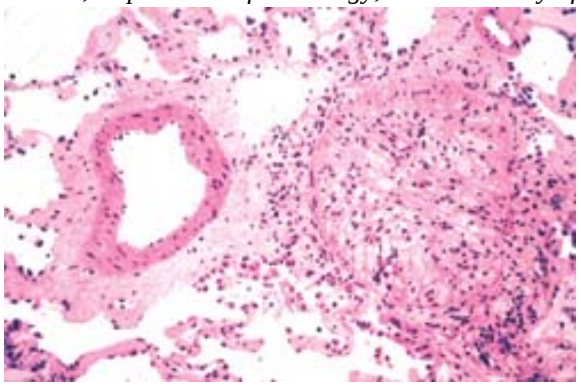

TABLE 15-10 -- Histologic Classification of Malignant Epithelial Lung Tumors

Squamous cell carcinoma
Small cell carcinoma
••Combined small cell carcinoma
Adenocarcinoma
••Acinar; papillary, bronchioloalveolar, solid, mixed subtypes
Large cell carcinoma
••Large cell neuroendocrine carcinoma
Adenosquamous carcinoma
Carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements
Carcinoid tumor
••Typical, atypical
Carcinomas of salivary gland type
Unclassified carcinoma

interesting postulate is that changes in cigarette type (filter tips, lower tar and nicotine) have caused smokers to inhale more deeply and thereby expose more peripheral airways and cells (with a predilection to adenocarcinoma) to carcinogens.[124] There may be mixtures of histologic patterns, even in the same cancer. Thus, combined types of squamous cell carcinoma and adenocarcinoma or of small cell and squamous cell carcinoma occur in about 10% of patients. For common clinical use, however, the various histologic types of lung cancer can be clustered into two groups on the basis of likelihood of metastases and response to available therapies: *small cell carcinomas* (most often metastatic, high initial response to chemotherapy) versus *non-small cell carcinomas* (less often metastatic, less responsive). The strongest relationship to smoking is with squamous cell and small cell carcinoma.

Morphology.

Lung carcinomas arise most often in and about the hilus of the lung. About three fourths of the lesions take their origin from first-order, second-order, and third-order bronchi. A small number of primary carcinomas of the lung arise in the periphery of the lung substance from the alveolar septal cells or terminal bronchioles. These are predominantly adenocarcinomas, including those of the bronchioloalveolar type, to be discussed separately.

Squamous cell carcinoma of the lung begins as an area of in situ cytologic dysplasia that, over an unknown interval of time, yields a small area of thickening or piling up of bronchial mucosa. With progression, this small focus, usually less than 1 cm^2 in area, assumes the appearance of an irregular, warty excrescence that elevates or erodes the lining epithelium. The tumor may then follow a variety of paths. It may continue to fungate into the bronchial lumen to produce an intraluminal mass. It can also rapidly penetrate the wall of the bronchus to infiltrate along the peribronchial tissue (Fig. 15-42) into the adjacent region of the carina or mediastinum. In other instances, the tumor grows along a broad front to produce a cauliflower-like intraparenchymal mass

Figure 15-42 Lung carcinoma. The gray-white tumor tissue is seen infiltrating the lung substance. Histologically, this large tumor mass was identified as a squamous cell carcinoma.

Figure 15-43 Cytologic diagnosis of lung cancer is often possible. *A*, A sputum specimen shows an orange-staining, keratinized squamous carcinoma cell with a prominent hyperchromatic nucleus (*arrow*). *B*, A fine-needle aspirate of an enlarged lymph node shows clusters of tumor cells from a small cell carcinoma, with molding and nuclear atypia characteristic of this tumor (see also Fig. 15-44*C*); note the size of the tumor cells compared with normal polymorphonuclear leukocytes in the left lower corner.

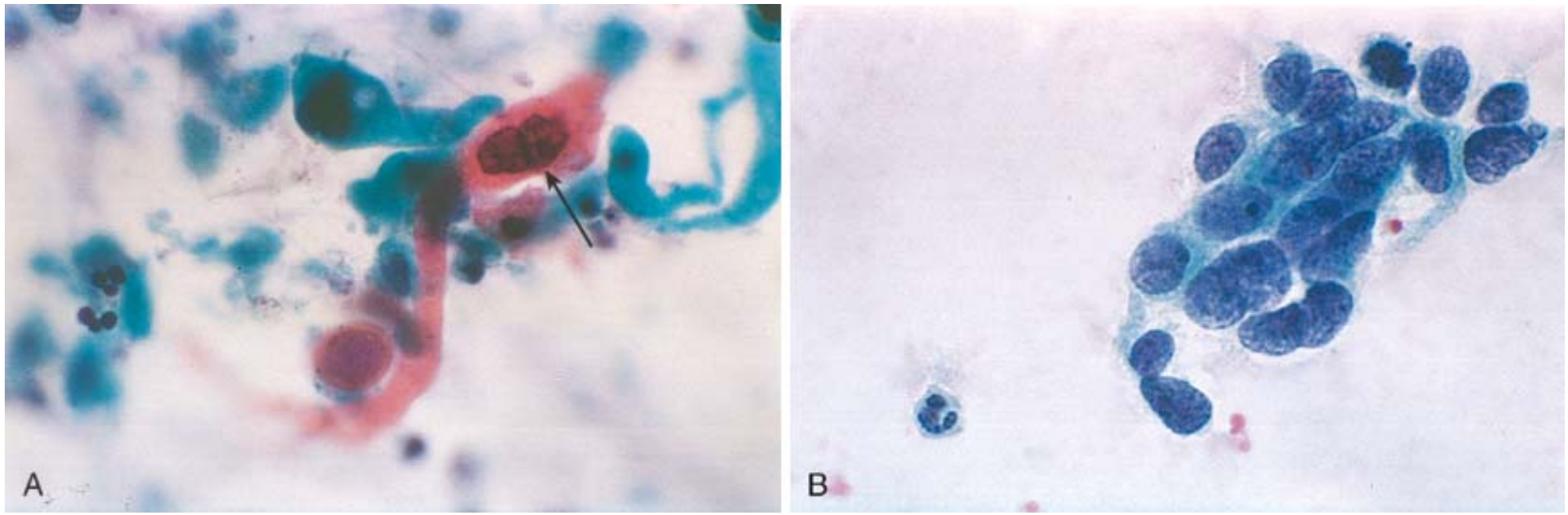

Figure 15-44 Histologic appearance of lung carcinoma. *A*, Well-differentiated squamous cell carcinoma showing keratinization. *B*, Gland-forming adenocarcinoma. *C*, Small cell

carcinoma with islands of small deeply basophilic cells and areas of necrosis. *D,* Large cell carcinoma, featuring pleomorphic, anaplastic tumor cells and absence of squamous or glandular differentiation.

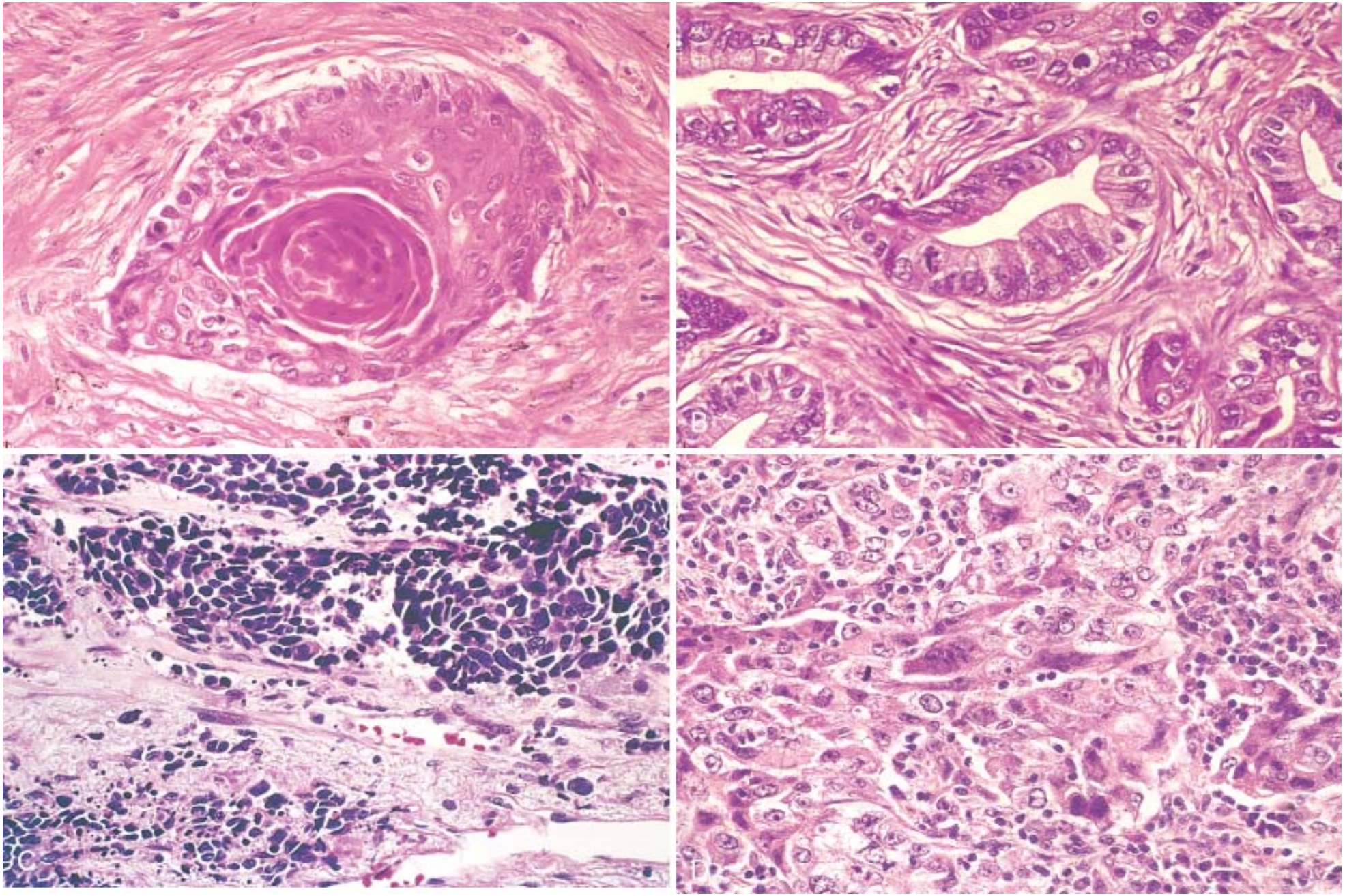

Figure 15-45 Bronchioloalveolar carcinoma with characteristic growth along pre-existing alveolar septa, without invasion. *(Courtesy of Dr. Jerome B. Taxy, Department of Pathology, The University of Chicago, Pritzker School of Medicine, Chicago, IL.)*

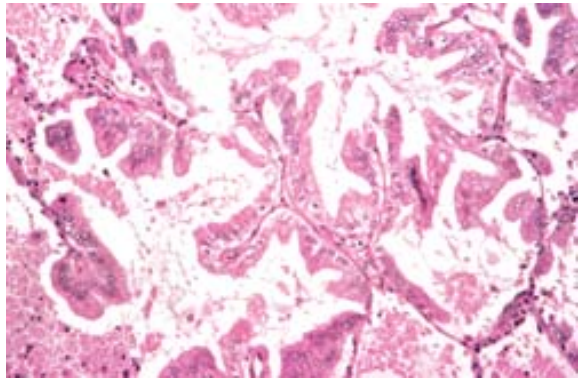

TABLE 15-11 -- New International Staging System for Lung Cancer

T1	Tumor <3 cm without pleural or main stem bronchus involvement
T2	Tumor >3 cm or involvement of main stem bronchus 2 cm from carina, visceral pleural involvement, or lobar atelectasis
T3	Tumor with involvement of chest wall (including superior sulcus tumors), diaphragm, mediastinal pleura, pericardium, main stem bronchus 2 cm from carina, or entire lung atelectasis
T4	Tumor with invasion of mediastinum, heart, great vessels, trachea, esophagus, vertebral body, or carina or with a malignant pleural effusion
N0	No demonstrable metastasis to regional lymph nodes
N1	Ipsilateral hilar or peribronchial nodal involvement
N2	Metastasis to ipsilateral mediastinal or subcarinal lymph nodes
N3	Metastasis to contralateral mediastinal or hilar lymph nodes, ipsilateral or contralateral scalene, or supraclavicular lymph nodes
M0	No (known) distant metastasis
M1	Distant metastasis present

	Stage Grouping		
Stage Ia	T1	N0	M0
Stage Ib	T2	N0	M0
Stage IIa	T1	N1	M0
Stage IIb	T2	N1	M0
	T3	N0	M0
Stage IIIa	T1–3	N2	M0
	T3	N1	M0
Stage IIIb	Any T	N3	M0
	T3	N2	M0

	T4	Any N	M0
Stage IV	Any T	Any N	M1
Adapted from Mountain C: Revisions in the International System for Staging Lung Cancer. Chest 111:1710, 1997.			

TABLE 15-12 -- Local Effects of Lung Tumor Spread

Clinical Feature	**Pathologic Basis**
Pneumonia, abscess, lobar collapse	Tumor obstruction of airway
Lipid pneumonia	Tumor obstruction; accumulation of cellular lipid in foamy macrophages
Pleural effusion	Tumor spread into pleura
Hoarseness	Recurrent laryngeal nerve invasion
Dysphagia	Esophageal invasion
Diaphragm paralysis	Phrenic nerve invasion
Rib destruction	Chest wall invasion
SVC syndrome	SVC compression by tumor
Horner syndrome	Sympathetic ganglia invasion
Pericarditis, tamponade	Pericardial involvement
SVC, superior vena cava.	

the overall 5-year survival rate is on the order of 15%. In many large clinics, not more than 20% to 30% of lung cancer patients have lesions sufficiently localized to permit even an attempt at resection. In general, the adenocarcinoma and squamous cell patterns tend to remain localized longer and have a slightly better prognosis than do the undifferentiated cancers, which usually are advanced lesions by the time they are discovered. The survival rate is 48% for cases detected when the disease is still localized. Only 15% of lung cancers are diagnosed at this early stage. Surgical resection for *small cell carcinoma* is so ineffective that the diagnosis essentially precludes surgery. Untreated, the survival time for patients with small cell cancer is 6 to 17 weeks. This cancer is particularly sensitive to radiation and chemotherapy, and potential cure rates of 15% to 25% for limited disease have been reported in some centers. Most patients have distant metastases on diagnosis. Thus, even with treatment, the mean survival after diagnosis is about 1 year.

Despite this discouraging outlook, some patients have been cured by lobectomy or pneumonectomy, emphasizing the continued need for early diagnosis and adequate prompt therapy.

Paraneoplastic Syndromes.

Lung carcinoma can be associated with a number of paraneoplastic syndromes[127] (Chapter 7), some of which may antedate the development of a gross pulmonary lesion. The hormones or hormone-like factors elaborated include

- *Antidiuretic hormone* (ADH), inducing hyponatremia owing to inappropriate ADH secretion
- *Adrenocorticotropic hormone* (ACTH), producing Cushing syndrome
- *Parathormone, parathyroid hormone-related peptide, prostaglandin E, and some cytokines*, all implicated in the hypercalcemia often seen with lung cancer
- *Calcitonin*, causing hypocalcemia

- *Gonadotropins*, causing gynecomastia
- *Serotonin and bradykinin*, associated with the carcinoid syndrome

The incidence of clinically significant syndromes related to these factors ranges from 1% to 10% of all lung cancer

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patients, although a much higher proportion of patients show elevated serum levels of these (and other) peptide hormones. Any one of the histologic types of tumors may occasionally produce any one of the hormones, but tumors that produce ACTH and ADH are predominantly small cell carcinomas, whereas those that produce hypercalcemia are mostly squamous cell tumors. The carcinoid syndrome is more common with the carcinoid tumor, described later, and is only rarely associated with small cell carcinoma. However, small cell carcinoma occurs much more commonly; therefore, one is much more likely to encounter carcinoid syndrome in these patients.

Other systemic manifestations of lung carcinoma include the *Lambert-Eaton myasthenic syndrome* (Chapter 27), in which muscle weakness is caused by auto-antibodies (possibly elicited by tumor ionic channels) directed to the neuronal calcium channel;[127] *peripheral neuropathy*, usually purely sensory; dermatologic abnormalities, including *acanthosis nigricans* (Chapter 25); hematologic abnormalities, such as *leukemoid reactions*; and finally, a peculiar abnormality of connective tissue called *hypertrophic pulmonary osteoarthropathy*, associated with clubbing of the fingers.

Apical lung cancers in the superior pulmonary sulcus tend to invade the neural structures around the trachea, including the cervical sympathetic plexus, and produce a group of clinical findings that includes severe pain in the distribution of the ulnar nerve and *Horner syndrome* (enophthalmos, ptosis, miosis, and anhidrosis) on the same side as the lesion. Such tumors are also referred to as *Pancoast tumors*.

NEUROENDOCRINE PROLIFERATIONS AND TUMORS

Neuroendocrine lesions share morphologic and biochemical features with cells of the *dispersed neuroendocrine cell system* (Chapter 24).[128] The normal lung contains neuroendocrine cells within the epithelium as single cells or as clusters, the neuroepithelial bodies. While virtually all pulmonary neuroendocrine cell hyperplasias are secondary to airway fibrosis and/or inflammation, a rare disorder called diffuse idiopathic pulmonary neuroendocrine cell hyperplasia appears to be a precursor to the development of multiple tumorlets and typical or atypical carcinoids.

Figure 15-46 *A*, Bronchial carcinoid growing as a spherical, pale mass (*arrow*) protruding into the lumen of the bronchus. *B*, Histologic appearance of bronchial carcinoid, demonstrating small, rounded, uniform cells.

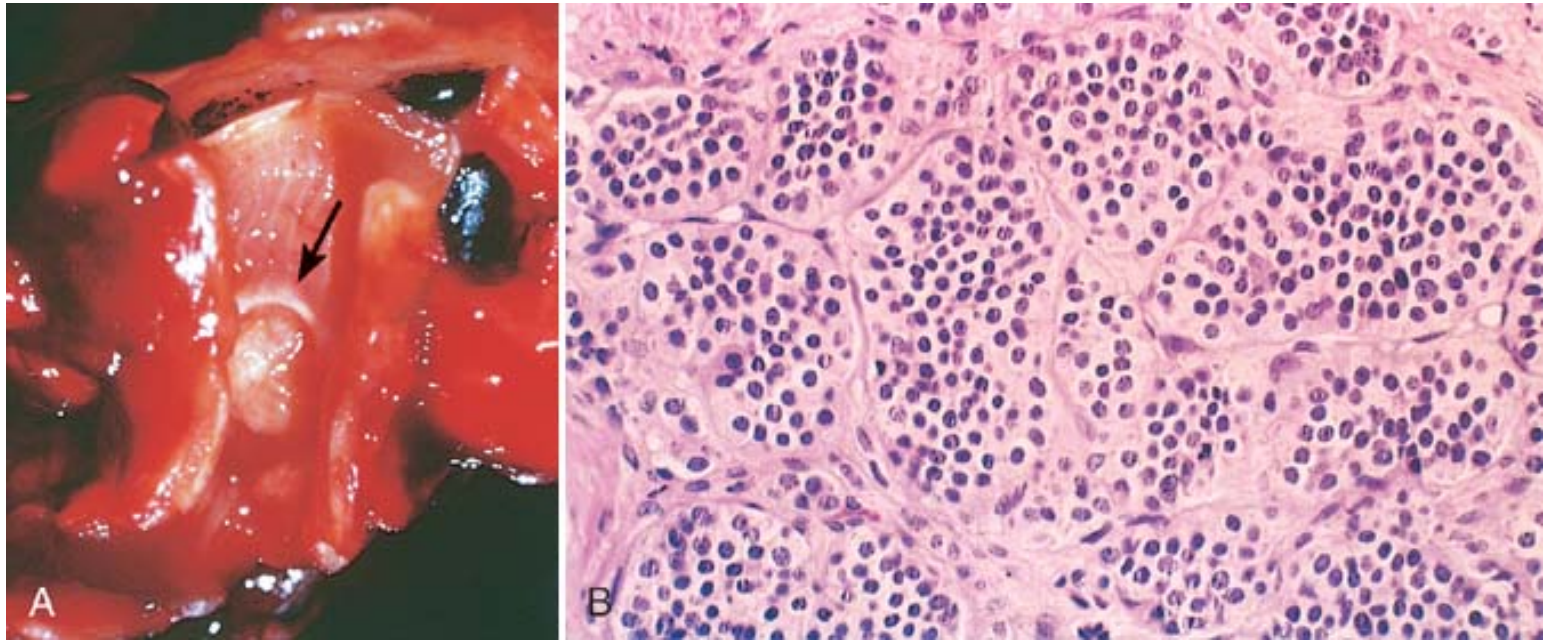


TABLE 15-13 -- Mediastinal Tumors and Other Masses

Superior Mediastinum
Lymphoma
Thymoma
Thyroid lesions
Metastatic carcinoma
Parathyroid tumors
Anterior Mediastinum
Thymoma
Teratoma
Lymphoma
Thyroid lesions
Parathyroid tumors
Posterior Mediastinum
Neurogenic tumors (schwannoma, neurofibroma)
Lymphoma

Gastroenteric hernia
Middle Mediastinum
Bronchogenic cyst
Pericardial cyst
Lymphoma

occurs most often with esophageal carcinomas and mediastinal lymphomas.

Morphology.

The pattern of metastatic growth within the lungs is quite variable. In the usual case, multiple discrete nodules (cannonball lesions) are scattered throughout all lobes (Fig. 15-47). These discrete lesions tend to occur in the periphery of the lung rather than in the central locations of the primary lung carcinoma. Other patterns include solitary nodule, endobronchial, pleural, pneumonic consolidation, and mixtures of the above. Foci of lepidic growth similar to bronchioloalveolar carcinoma are seen occasionally with metastatic carcinomas and may be associated with any of the patterns listed above.

Metastatic growth may be confined to peribronchiolar and perivascular tissue spaces, presumably when the tumor has extended to the lung through the lymphatics. In these cases, the lung septa and connective tissue are diffusely infiltrated with the gray-white tumor. The subpleural lymphatics may be outlined by the contained tumor, producing a gross appearance referred to as lymphangitis carcinomatosa. Least commonly, the metastatic tumor is not apparent on gross examination and becomes evident only on histologic section as a diffuse intralymphatic dissemination dispersed throughout the peribronchial and perivascular channels. In certain instances, microscopic tumor emboli fill the small pulmonary vessels and may result in life-threatening pulmonary hypertension or hemorrhage and hemoptysis.

Pleura

Pathologic involvement of the pleura is, with rare exceptions, a secondary complication of some underlying disease. Secondary infections and pleural adhesions are particularly common findings at autopsy. Occasionally, the secondary pleural disease assumes a dominant role in the clinical problem, as occurs in bacterial pneumonia with the development of empyema. Important primary disorders include (1) primary intrapleural bacterial infections that imply seeding of this space as an isolated focus in the course of a transient bacteremia and (2) a primary neoplasm of the pleura: mesothelioma (discussed later).

PLEURAL EFFUSION

Pleural effusion is a common manifestation of both primary and secondary pleural diseases. Normally, no more

TABLE 15-14 -- Pleural Space Fluid Accumulations

Condition	Type of Fluid	Common Associations
Inflammatory		

Serofibrinous pleuritis	Serofibrinous exudate	Inflammation in adjacent lung
		Collagen vascular disease
Suppurative pleuritis (empyema)	Pus	Suppurative infection in adjacent lung
Hemorrhagic pleuritis	Bloody exudate	Tumor
Noninflammatory		
Hydrothorax	Transudate	Congestive heart failure
Hemothorax	Blood	Ruptured aortic aneurysm, trauma
Chylothorax	Chyle (lymph)	Tumor obstruction of normal lymphatics

Figure 15-47 Numerous metastases from a renal cell carcinoma. *(Courtesy of Dr. Michelle Mantel, Brigham and Women's Hospital, Boston, MA.)*

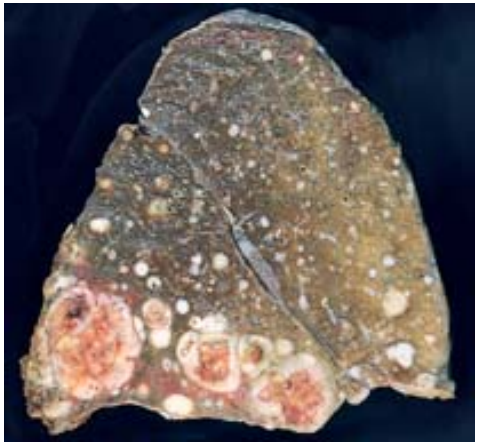


Figure 15-48 Malignant mesothelioma. Note the thick, firm, white pleural tumor tissue that ensheathes this bisected lung.

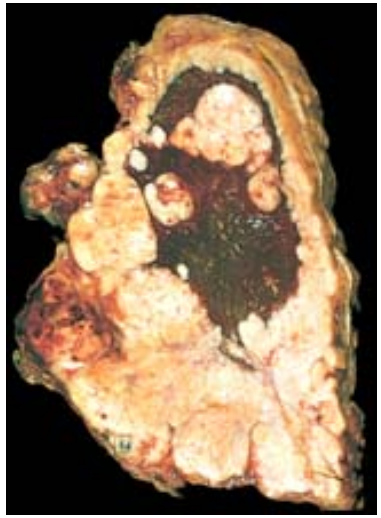


Figure 15-49 *A,* Malignant mesothelioma, epithelial type. *B,* Malignant mesothelioma, mixed type, stained for calretinin (immunoper-oxidase method). The epithelial component is strongly positive (*dark brown*), while the sarcomatoid component is less so. *(Courtesy of Dr. Thomas Krausz, Department of Pathology, The University of Chicago, Pritzker School of Medicine, Chicago, IL.)*

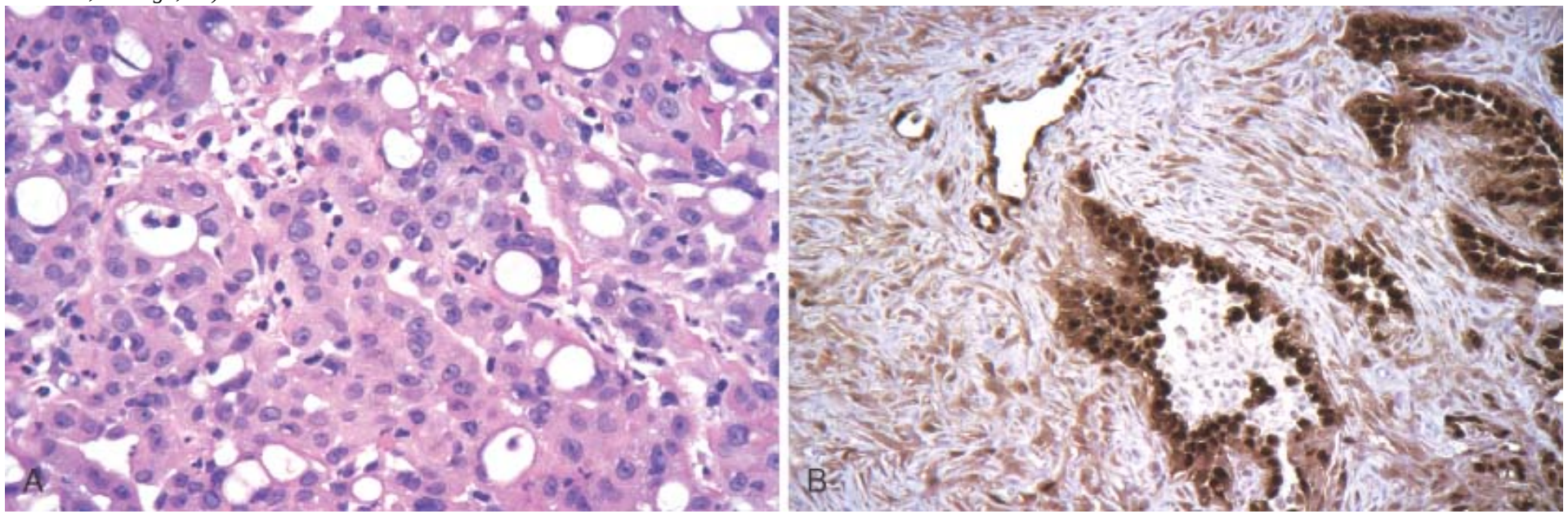
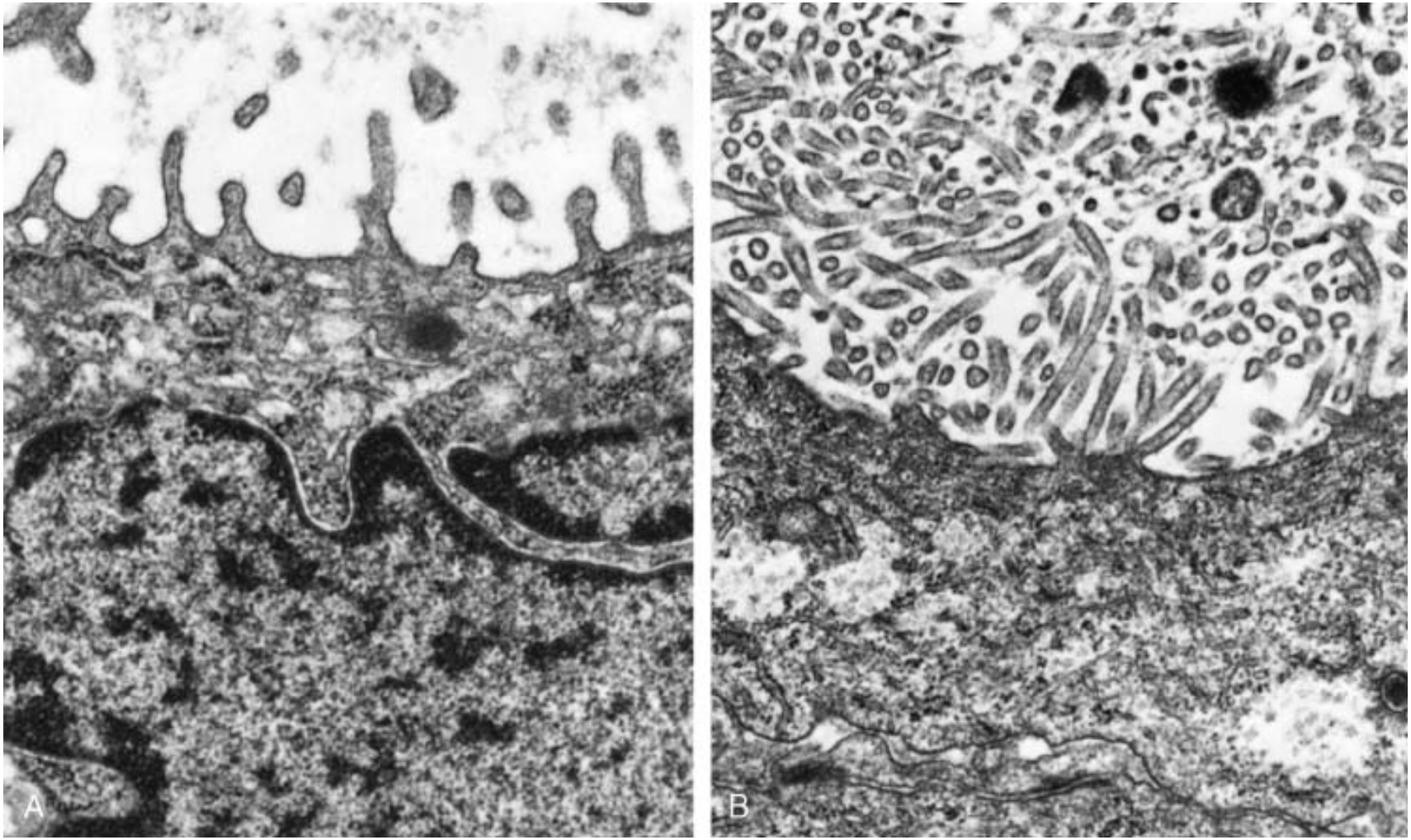


Figure 15-50 Ultrastructural features of pulmonary adenocarcinoma (*A*), characterized by short, plump microvilli, contrasted with those of mesothelioma (*B*), in which microvilli are numerous, long, and slender. *(Courtesy of Dr. Noel Weidner, University of California, San Francisco, School of Medicine, San Francisco, CA.)*



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Chapter 16 - Head and Neck

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Diseases of the head and neck range from the common cold to uncommon neoplasms of the nose. Those selected for discussion are assigned, sometimes arbitrarily, to one of the following anatomic sites: (1) oral cavity; (2) upper airways, including the nose, pharynx, larynx, and nasal sinuses; (3) ears; (4) neck; and (5) salivary glands.

Oral Cavity

The oral cavity is a fearsome orifice guarded by ranks of upper and lower "horns" (lamentably, quite subject to erosion), demanding constant gratification, and teeming with microorganisms, some of which are potentially harmful. Among the many disorders that affect its various parts, only the more important or frequent conditions involving the teeth and supporting structures, oral mucous membranes, lips, and tongue are considered.

Teeth and Supporting Structures

Teeth contribute to a number of important functions, including mastication and proper speech. It is useful to briefly review normal dental anatomy before we delve into the common pathologic conditions affecting teeth. As is well known, teeth are firmly implanted in the jaw and are surrounded by the gingival mucosa (Fig. 16-1). The anatomic crown of the tooth projects into the mouth and is covered by

Figure 16-1 Schematic representation of the normal dental anatomy and surrounding supporting tissues.

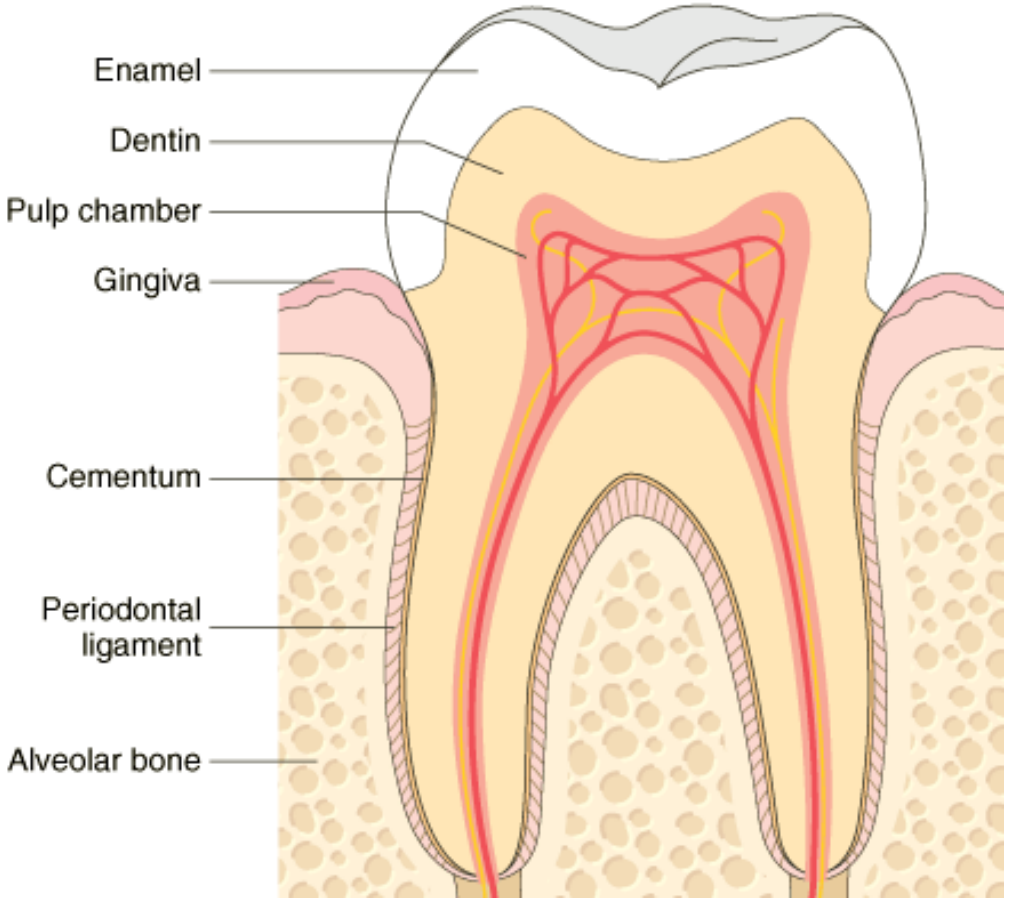

Figure 16-2 Fibroma. Smooth, pink, exophytic nodule on the buccal mucosa.

Figure 16-3 Pyogenic granuloma. Erythematous, hemorrhagic, and exophytic mass arising from the gingival mucosa.

Figure 16-4 Aphthous ulcer. Single ulceration with an erythematous halo surrounding a yellowish fibrinopurulent membrane.

TABLE 16-1 -- Oral Manifestations of Some Systemic Diseases

Infectious Diseases	
Scarlet fever	Fiery red tongue with prominent papillae (raspberry tongue); white coated tongue through which hyperemic papillae project (strawberry tongue)
Measles	A spotty enanthema in the oral cavity often precedes the rash; ulcerations on the buccal mucosa about Stensen duct produce Koplik spots
Infectious mononucleosis	An acute pharyngitis and tonsillitis that may cause coating with a gray-white exudative membrane; enlargement of lymph nodes in the neck
Diphtheria	A characteristic dirty white, fibrinosuppurative, tough, inflammatory membrane over the tonsils and retropharynx
Human immunodeficiency virus infection; AIDS	Predisposition to opportunistic oral infections, particularly with herpesvirus, *Candida*, and other fungi; sometimes oral lesions of Kaposi sarcoma and hairy leukoplakia (described in text)
Dermatologic Conditions *	
Lichen planus	Reticulate, lacelike, white keratotic lesions that rarely become bullous and ulcerated; seen in more than 50% of patients with cutaneous lichen planus; rarely, is the sole manifestation
Pemphigus	Usually vulgaris; vesicles and bullae prone to rupture, leaving hyperemic erosions covered with exudate
Bullous pemphigoid	Oral lesions resemble macroscopically those of pemphigus but can be differentiated histologically

Erythema multiforme	A maculopapular, vesiculobullous eruption that sometimes follows an infection elsewhere, ingestion of drugs, development of cancer, or a collagen vascular disease; when it involves the lips and oral mucosa, it is referred to as *Stevens-Johnson syndrome*
Hematologic Disorders	
Pancytopenia (agranulocytosis, aplastic anemia)	Severe oral infections in the form of gingivitis, pharyngitis, tonsillitis; may extend to cellulitis of the neck *(Ludwig angina)*
Leukemia	With depletion of functioning neutrophils, oral lesions may appear like those in pancytopenia
Monocytic leukemia	Leukemic infiltration and enlargement of the gingivae, often with accompanying periodontitis
Miscellaneous	
Melanotic pigmentation	May appear in Addison disease, hemochromatosis, fibrous dysplasia of bone (Albright syndrome), and Peutz-Jegher syndrome (gastrointestinal polyposis)
Phenytoin (Dilantin) ingestion	Striking fibrous enlargement of the gingivae
Pregnancy	A friable, red, pyogenic granuloma protruding from the gingiva ("pregnancy tumor")
Rendu-Osler-Weber syndrome	Autosomal dominant disorder with multiple aneurysmal telangiectasias from birth beneath the skin or mucosal surfaces of the oral cavity, lips, gastrointestinal tract, respiratory tract, and urinary tract as well as in internal viscera

*See Chapter 25 for details

most cells and is now accepted as the cause of the condition.[7] Sometimes there is superimposed candidal infection on the surface of the lesions, adding to the "hairiness." When the hairy leukoplakia is a harbinger of HIV infection, manifestations of AIDS generally appear within 2 or 3 years.

Tumors and Precancerous Lesions

A number of epithelial and soft tissue neoplasms can arise in the oral cavity. Many of these tumors (e.g., papillomas, hemangiomas, lymphomas) also occur elsewhere in the body and are described adequately in other chapters. Therefore, this discussion will consider only oral squamous cell carcinoma and its associated precancerous lesions.

LEUKOPLAKIA AND ERYTHROPLAKIA

As is discussed in more detail below, oral cancers are common worldwide, with a fairly high mortality. Screening and early detection in populations at risk have been proposed to decrease both the morbidity and mortality associated with oral cancer.[8] [9] However, the visual detection of definitive premalignant oral lesions is problematic. This is in stark contrast to skin lesions, where visual screening for melanomas of the skin has been shown to have sensitivity and specificity rates of 93% and 98%. [10] [11] One explanation for this discrepancy is that the early lesions frequently do not demonstrate any of the clinical characteristics observed in advanced oral cancer: ulceration, induration, pain, or associated cervical lymphadenopathy.[12] In addition, the clinical presentation of potentially premalignant lesions in the oral cavity is highly heterogeneous. We begin our discussion with two premalignant lesions—leukoplakia and erythroplakia.

The term *leukoplakia* is defined by the World Health Organization as "a white patch or plaque that cannot be scraped off and cannot be characterized clinically or pathologically as any other disease." Simply put, if a white lesion in the oral cavity can be given a specific diagnosis it is not a leukoplakia. This clinical term is reserved for lesions that are present in the oral cavity for no apparent reason. As such, white patches caused by entities such as lichen planus and candidiasis are not leukoplakias. Approximately 3% of the world's population have

leukoplakic lesions, and somewhere between 5% and 25% of these lesions are premalignant.[13] *Thus, until it is proved otherwise via histologic evaluation, all leukoplakias must be considered precancerous.*

Related to leukoplakia, but much less common and much more ominous, is *erythroplakia*. It represents a red, velvety, possibly eroded area within the oral cavity that usually remains level with or may be slightly depressed in relation to the surrounding mucosa (Fig. 16-5). The epithelium in such

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Figure 16-5 Erythroplakia. *A,* Lesion of the maxillary gingiva. *B,* Red lesion of the mandibular alveolar ridge. Biopsy of both lesions revealed carcinoma in situ.

Figure 16-6 Leukoplakia. Clinical appearance of leukoplakias is highly variable and can range from *A,* smooth and thin with well-demarcated borders. *B,* diffuse and thick. *C,* irregular with a granular surface. *D,* diffuse and corrugated. *(Courtesy of Drs. Neville, Damm, Allen, Bouquot [eds], Oral & Maxillofacial Pathology, Philadelphia, WB Saunders, 2002.)*

Figure 16-7 Clinical, histologic, and molecular progression of oral cancer. *A,* The typical clinical progression of oral cancer. *B,* The histologic progression of squamous epithelium from normal, to hyperkeratosis, to mild/moderate dysplasia, to severe dysplasia, to cancer. *C,* The sites of the most common genetic alterations identified as important for cancer development. *(Clinical photographs courtesy of Sol Silverman, M.D., from the text Silverman S: Oral Cancer. Hamilton, Ontario, BD Dekker, 2003.)*

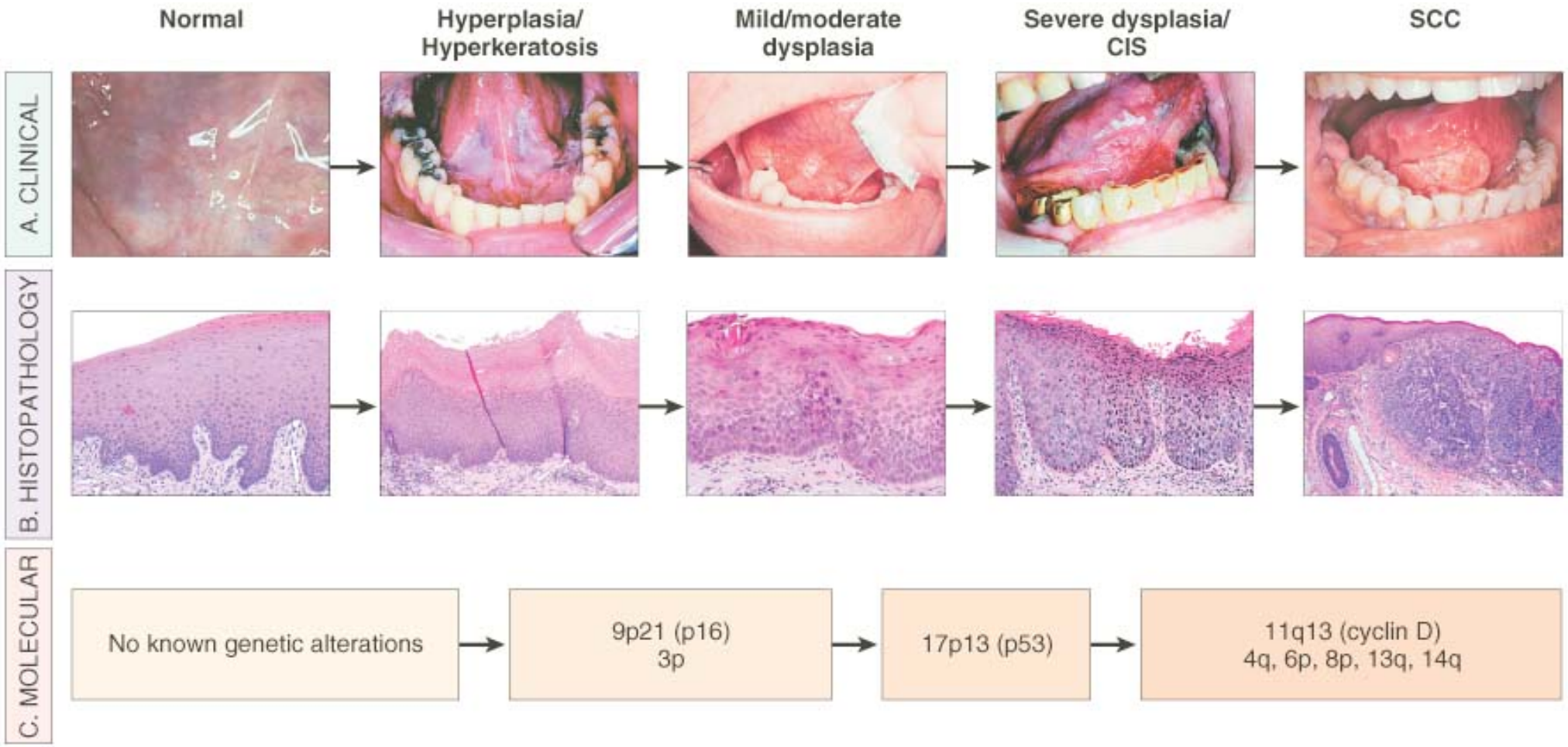

Figure 16-8 Schematic representation of the sites of origin of squamous cell carcinoma of the oral cavity, in numerical order of frequency.

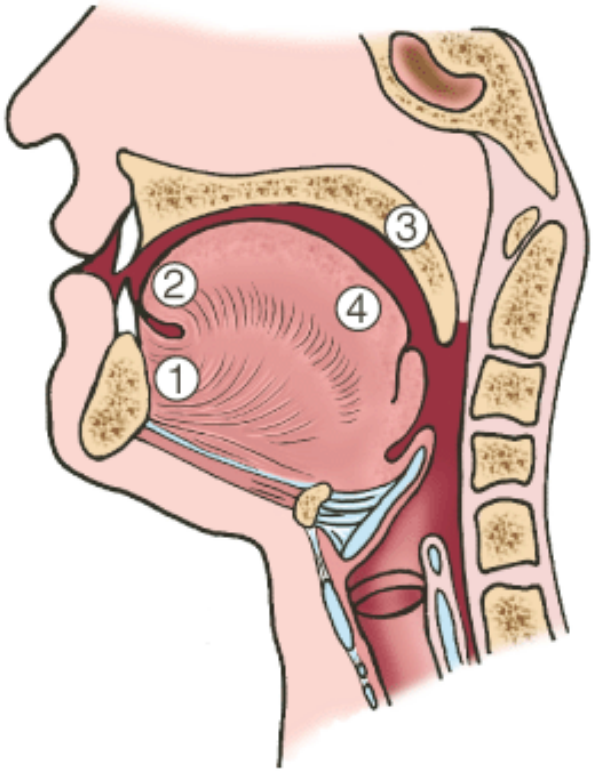

TABLE 16-2 -- Histologic Classification of Odontogenic Cysts

1. Inflammatory
•••a. Periapical cyst
•••b. Residual cyst
•••c. Paradental cyst
2. Developmental
•••a. Dentigerous cyst
•••b. Odontogenic keratocyst
•••c. Gingival cyst of newborn
•••d. Gingival cyst of adult
•••e. Eruption cyst
•••f. Lateral periodontal cyst
•••g. Glandular odontogenic cyst

•••h. Calcifying epithelial odontogenic cyst (Gorlin cyst)

which may be caused by advanced carious lesions or by trauma to the tooth in question. The inflammatory process may result in necrosis of the pulpal tissue, which can traverse the length of the root and exit the apex of the tooth into the surrounding alveolar bone giving rise to a periapical abscess. Over time, like any chronic inflammatory process, a lesion with granulation tissue (with or without an epithelial lining) may develop. While the term *granuloma* is not the most appropriate terminology (as the lesion does not show true granulomatous inflammation), old terminology, like bad habits, is difficult to shed. Periapical inflammatory lesions persist as a result of the continued presence of bacteria or other offensive agents in the area. Successful treatment therefore necessitates the complete removal of offending material and appropriate restoration of the tooth or extraction.

Odontogenic tumors are a complex group of lesions with diverse histology and clinical behavior.[33] Some are true neoplasms (both benign and malignant), while others are more likely hamartomas. Odontogenic tumors are derived from odontogenic epithelium, ectomesenchyme, or both (Table 16-3). The two most common and clinically significant tumors are:

- *Ameloblastoma,* which arises from odontogenic epithelium and shows *no* ectomesenchymal differentiation. It is commonly cystic, slow growing, and locally invasive but has a benign course in most cases.
- *Odontoma,* the most common type of odontogenic tumor, arises from epithelium but shows extensive depositions of enamel and dentin. Odontomas are probably hamartomas rather than true neoplasms and are cured by local excision.

TABLE 16-3 -- Histologic Classification of Odontogenic Tumors

1. Tumors of Odontogenic Epithelium
•••Benign
•••••a. Ameloblastoma
•••••b. Calcifying epithelial odontogenic tumor (Pindborg tumor)
•••••c. Squamous odontogenic tumor
•••Malignant
•••••a. Ameloblastic carcinoma
•••••b. Malignant ameloblastoma
•••••c. Clear cell odontogenic carcinoma
2. Tumors of Odontogenic Ectomesenchyme
•••a. Odontogenic fibroma
•••b. Odontogenic myxoma
•••c. Cementoblastoma
3. Tumors of Odontogenic Epithelium and Ectomesenchyme
•••Benign
•••••a. Ameloblastic fibroma
•••••b. Ameloblastic fibro-odontoma

•••••c. Ameloblastic fibrosarcoma
•••••d. Adenomatoid odontogenic tumor
•••••e. Odontoameloblastoma
•••••f. Complex odontoma
•••••g. Compound odontoma
•••Malignant
•••••a. Ameloblastic fibrosarcoma

Upper Airways

The term *upper airways* is used here to include the nose, pharynx, and larynx and their related parts. Disorders of these structures are among the most common afflictions of humans, but fortunately the overwhelming majority are more nuisances than threats.

Nose

Inflammatory diseases, mostly in the form of the common cold, as everyone knows, are the most common disorders of the nose and accessory air sinuses. Most of these inflammatory conditions are viral in origin, but they are often complicated by superimposed bacterial infections having considerably greater significance. Much less common are a few destructive inflammatory nasal diseases and tumors primary in the nasal cavity or paranasal sinuses.

INFLAMMATIONS

Infectious Rhinitis.

Infectious rhinitis, the more elegant way of saying "common cold," is in most instances caused by one or more viruses. Major offenders are adenoviruses, echoviruses, and rhinoviruses. They evoke a profuse catarrhal discharge that is familiar to all and the bane of the kindergarten teacher. During the initial acute stages, the nasal mucosa is thickened, edematous, and red; the nasal cavities are narrowed; and the turbinates are enlarged. These changes may extend, producing a concomitant pharyngotonsillitis. Secondary bacterial infection enhances the inflammatory reaction and produces an essentially mucopurulent or sometimes frankly suppurative exudate. But as everyone knows, these infections soon clear up—as the saying goes, in a week if treated but after 7 days if ignored.

Figure 16-9 *A*, Nasal polyps. Low-power magnification showing edematous masses lined by epithelium. *B*, High-power view showing edema and eosinophil-rich inflammatory infiltrate.

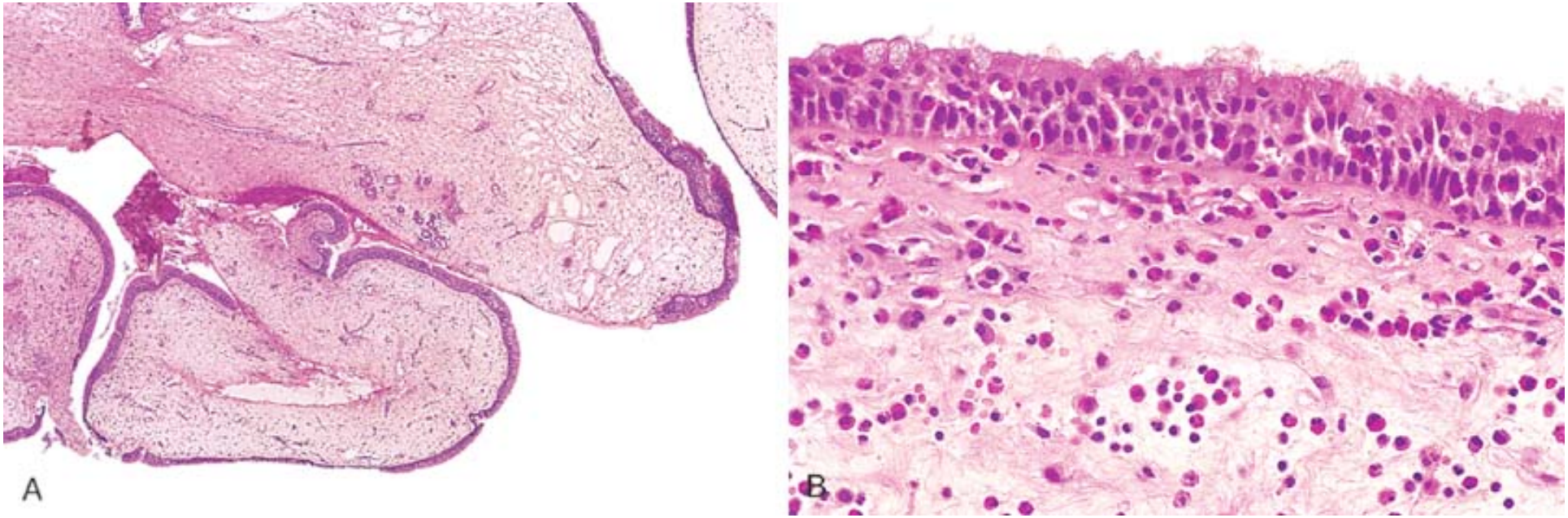

Figure 16-10 Inverted papilloma. The masses of squamous epithelium are growing inward; hence, the term inverted. *(Courtesy of Dr. James Gulizia, Brigham and Women's Hospital, Boston, MA.)*

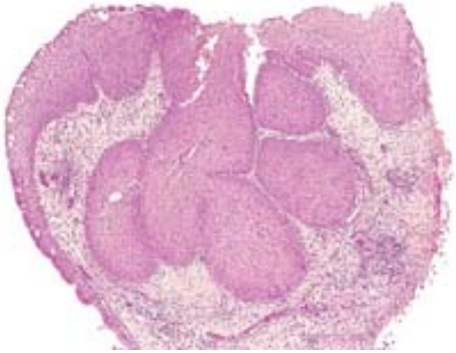

Figure 16-11 Nasopharyngeal carcinoma, lymphoepithelioma type. The syncytium-like nests of epithelium are surrounded by lymphocytes. *(Courtesy of Dr. James Gulizia, Brigham and Women's Hospital, Boston, MA.)*

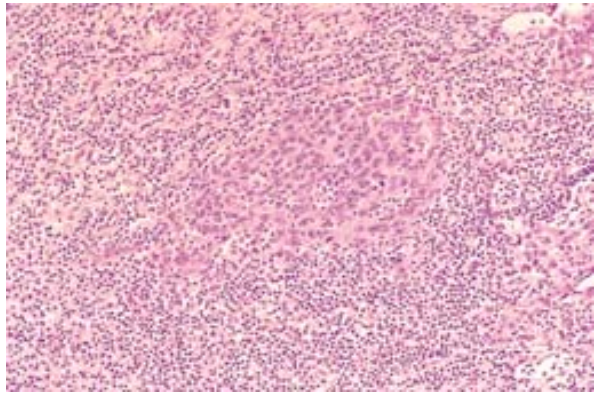

Figure 16-12 *A,* Laryngeal carcinoma. Note the large, ulcerated, fungating lesion involving the vocal cord and piriform sinus. *B,* Histologic appearance of laryngeal squamous cell carcinoma. Note the atypical lining epithelium and invasive keratinizing cancer cells in the submucosa.

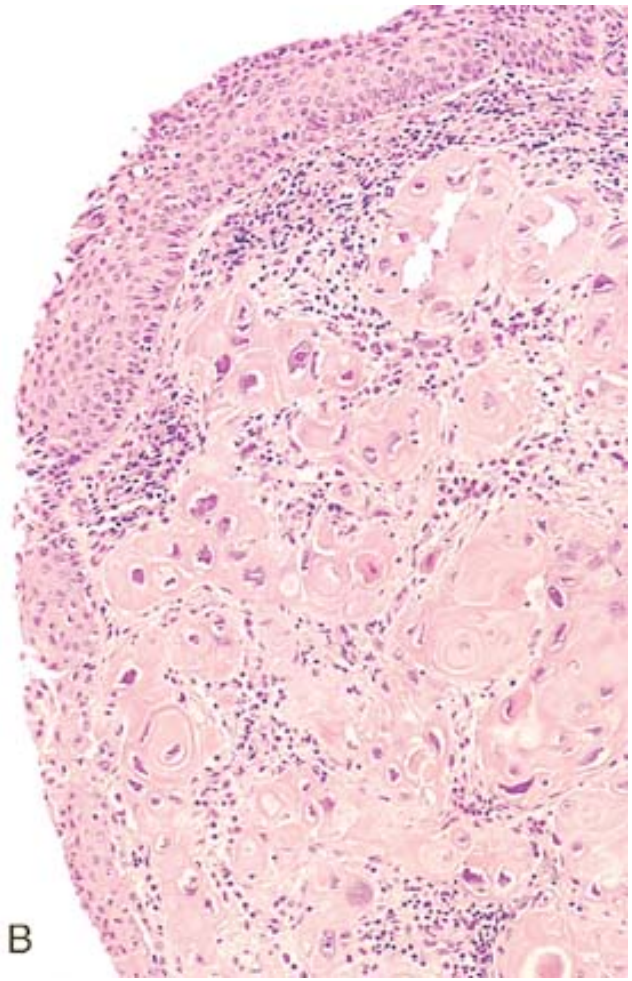

Figure 16-13 Diagrammatic comparison of a benign papilloma and an exophytic carcinoma of the larynx to highlight their quite different appearances.

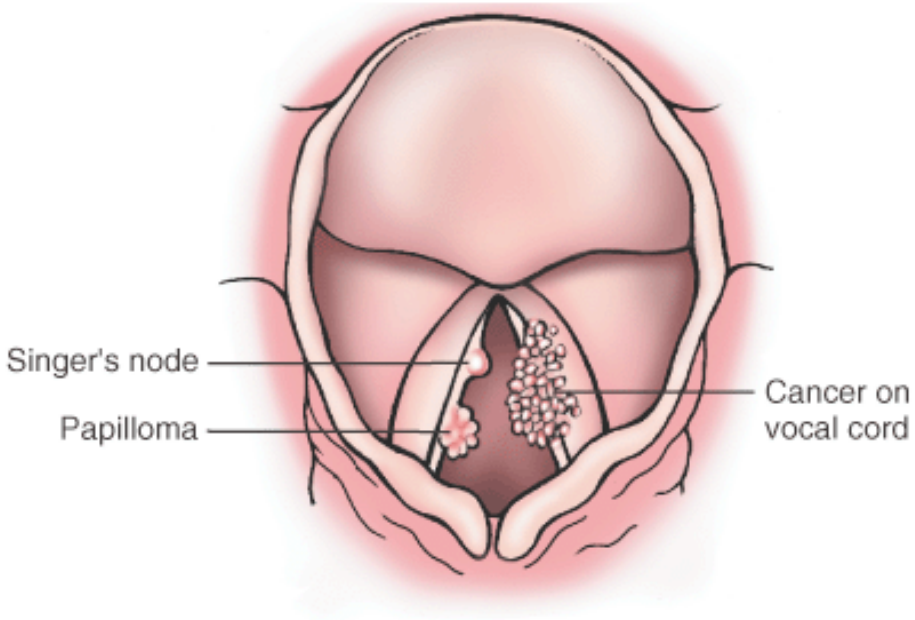

Figure 16-14 Carotid body tumor. *A,* Low-power view showing tumor clusters separated by septa (zellballen). *B,* High-power view of large, eosinophilic, slightly vacuolated tumor cells with elongated sustentacular cells in the septa.

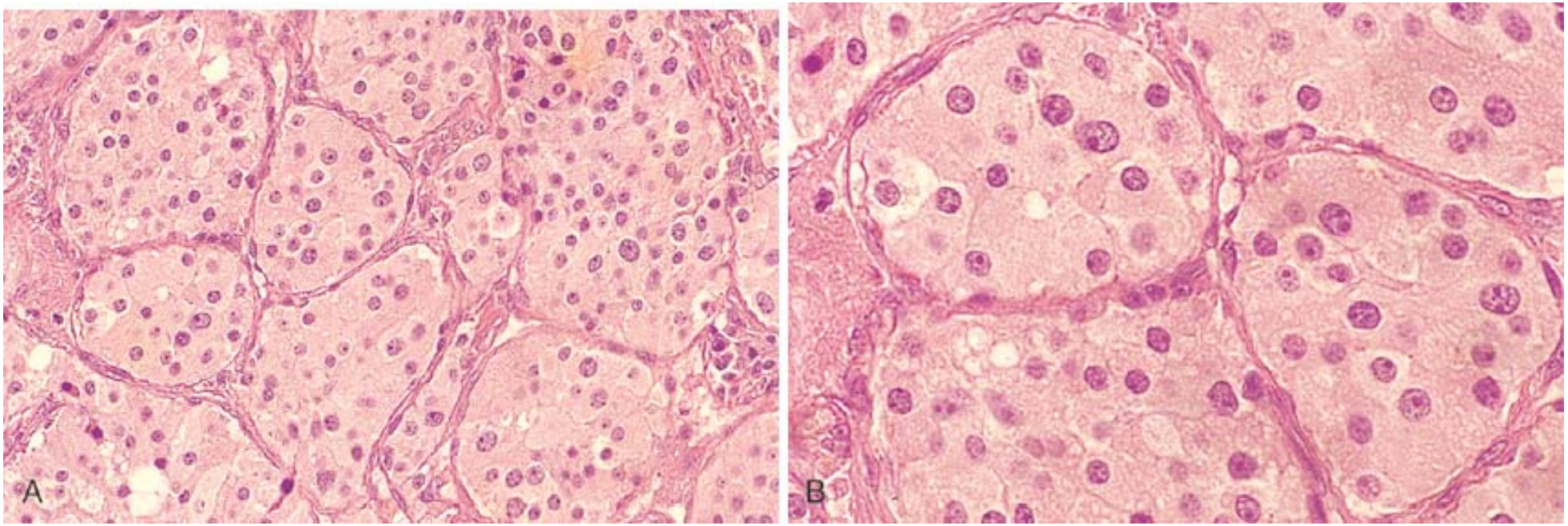

Figure 16-15 Mucocele. *A,* Fluctuant fluid-filled lesion on the lower lip subsequent to trauma. *B,* Cystlike cavity filled with mucinous material and lined by organizing granulation tissue.

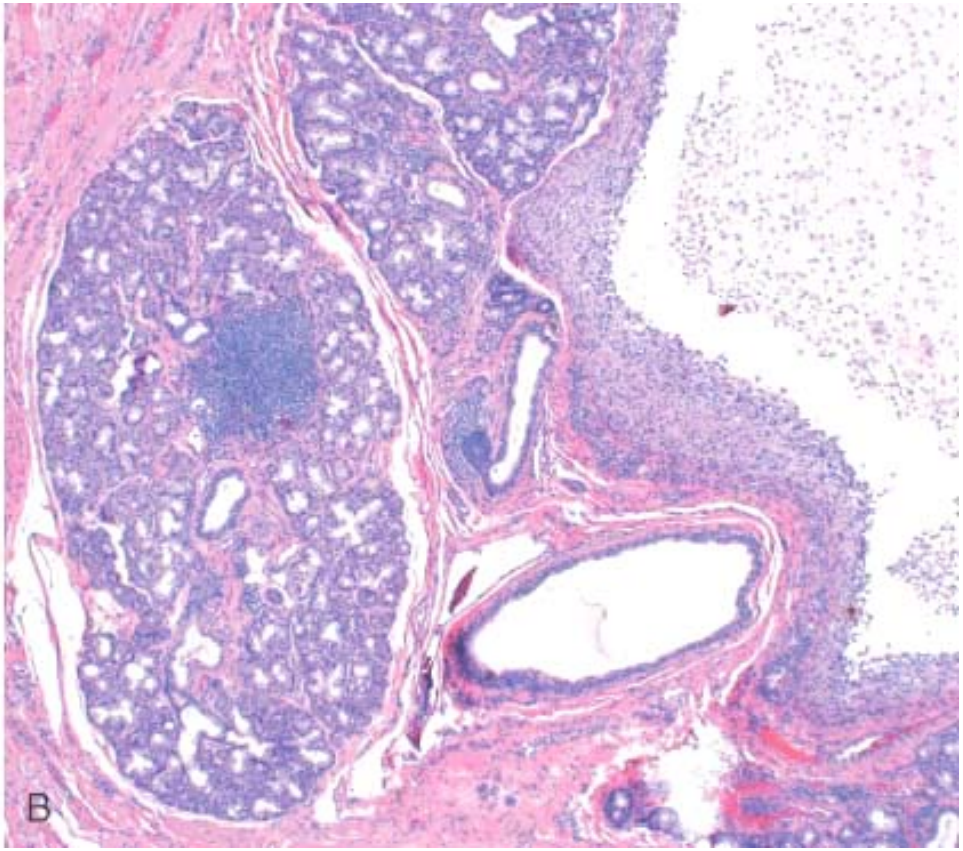

TABLE 16-4 -- Histologic Classification and Approximate Incidence of Benign and Malignant Tumors of the Salivary Glands

Benign	**Malignant**
Pleomorphic adenoma (50%) (mixed tumor)	Mucoepidermoid carcinoma (15%)
Warthin tumor (5%–10%)	Adenocarcinoma (NOS) (10%)
Oncocytoma (1%)	Acinic cell carcinoma (5%)
Other adenomas (5%–10%)	Adenoid cystic carcinoma (5%)
••Basal cell adenoma	Malignant mixed tumor (3%–5%)
••Canalicular adenoma	Squamous cell carcinoma (1%)
Ductal papillomas	Other carcinomas (2%)
NOS, not otherwise specified. *Data from Ellis GL, Auclair PL: Tumors of the Salivary Glands. Atlas of Tumor Pathology, Third Series. Washington, DC, Armed Forces Institute of Pathology, 1996.*	

ear. In general, when they are first diagnosed, both benign and malignant lesions range from 4 to 6 cm in diameter and are mobile on palpation except in the case of neglected malignant tumors. Although benign tumors are known to have been present usually for many months to several years before coming to clinical attention, cancers seem to demand attention more

promptly, probably because of their more rapid growth. Ultimately, however, there are no reliable criteria to differentiate, on clinical grounds, the benign from the malignant lesions, and morphologic evaluation is necessary.

PLEOMORPHIC ADENOMA

Because of their remarkable histologic diversity, these neoplasms have also been called *mixed tumors*. They represent about 60% of tumors in the parotid, are less common in the submandibular glands, and are relatively rare in the minor salivary glands. They are benign tumors that are derived from a mixture of ductal (epithelial) and myoepithelial cells, and therefore they show both epithelial and mesenchymal differentiation. They also reveal epithelial elements dispersed throughout a matrix along with varying degrees of myxoid, hyaline, chondroid (cartilaginous), and even osseous tissue. In some tumors, the epithelial elements predominate; in others, they are present only in widely dispersed foci.

Little is known about the origins of these neoplasms except that radiation exposure increases the risk. Equally uncertain is the histogenesis of the various components. A currently popular view is that all neoplastic elements, including those that appear mesenchymal, are of either myoepithelial or ductal reserve cell origin (hence the designation *pleomorphic adenoma*).

Morphology.

Most pleomorphic adenomas present as rounded, well-demarcated masses rarely exceeding 6 cm in greatest dimension (Fig. 16-16). Although they are encapsulated, in some locations (particularly the palate) the capsule is not fully developed, and expansile growth produces tonguelike protrusions

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Figure 16-16 Pleomorphic adenoma. *A,* Slowly enlarging neoplasm in the parotid gland of many years duration. *B,* The bisected, sharply circumscribed, yellow-white tumor can be seen surrounded by normal salivary gland tissue.

Figure 16-17 Pleomorphic adenoma. *A*, Low-power view showing a well-demarcated tumor with adjacent normal salivary gland parenchyma. *B*, High-power view showing epithelial cells as well as myoepithelial cells found within a chondroid matrix material.

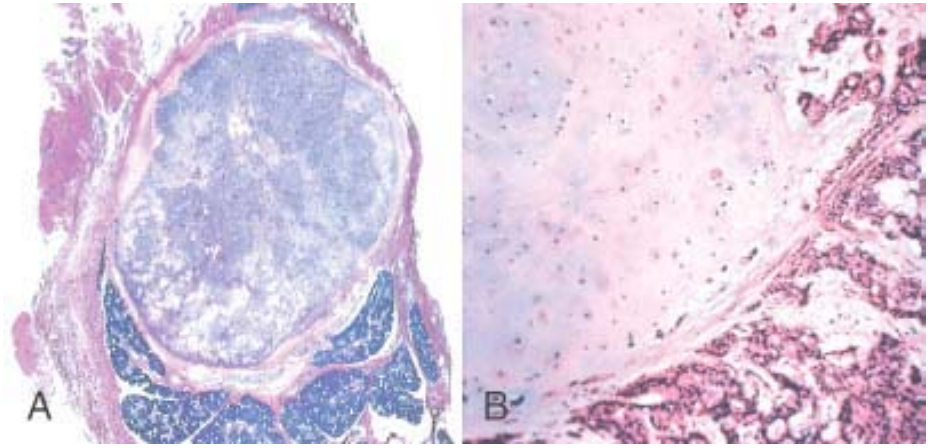


Figure 16-18 Warthin tumor. *A,* Low-power view showing epithelial and lymphoid elements. Note the follicular germinal center beneath the epithelium. *B,* Cystic spaces separate lobules of neoplastic epithelium consisting of a double layer of eosinophilic epithelial cells based on a reactive lymphoid stroma.

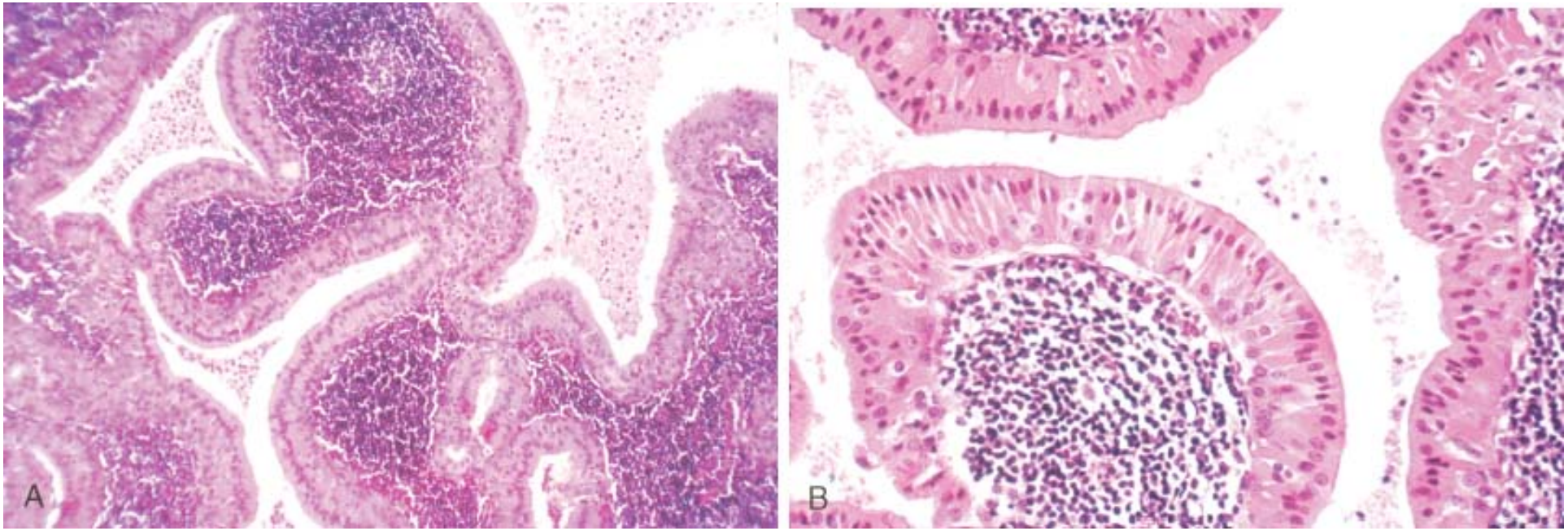


Figure 16-19 *A,* Mucoepidermoid carcinoma showing islands having squamous cells as well as clear cells containing mucin. *B,* Mucicarmine stains the mucin reddish-pink. *(Courtesy of Dr. James Gulizia, Brigham and Women's Hospital, Boston.)*

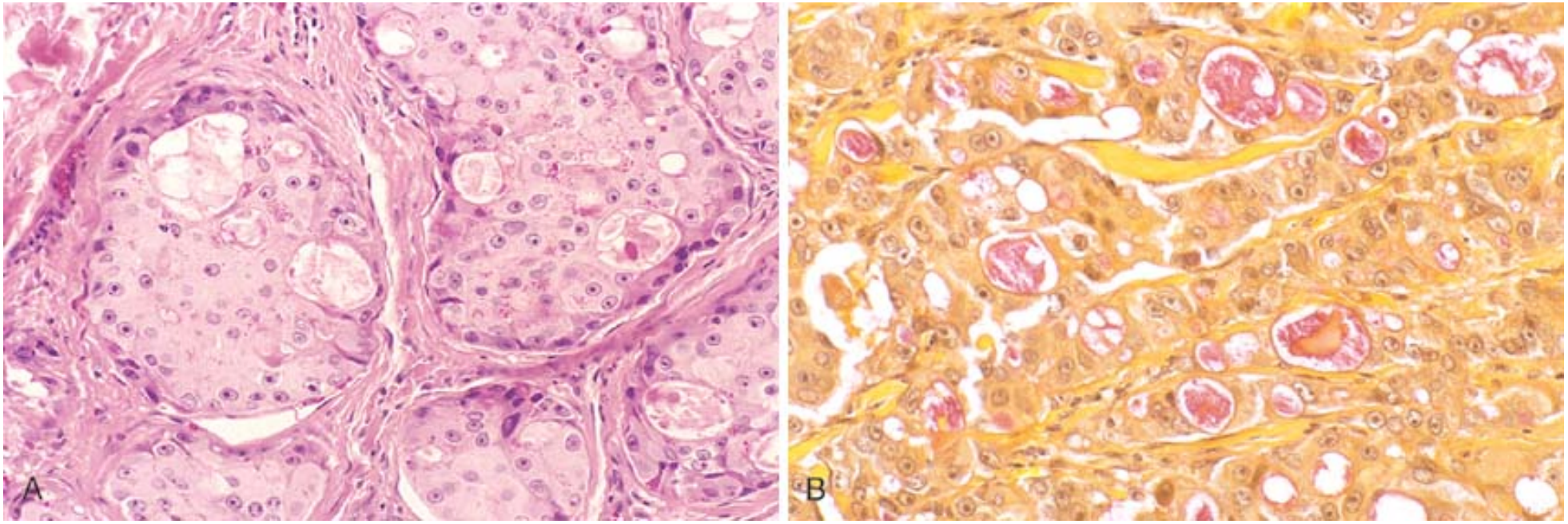
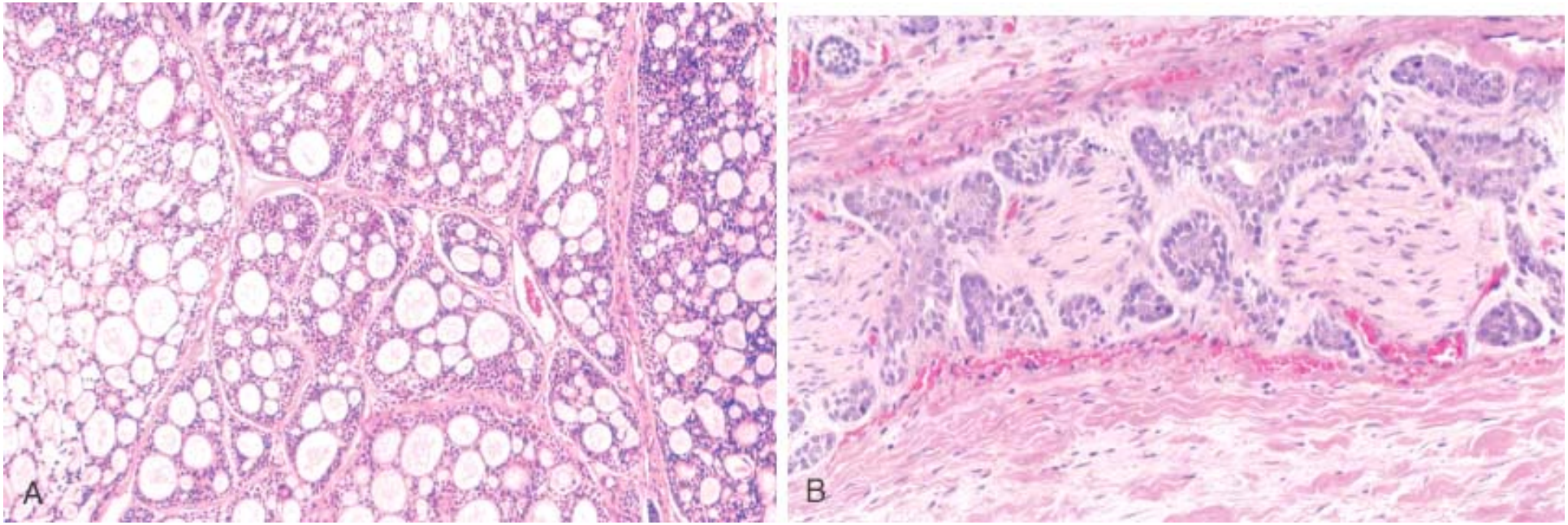


Figure 16-20 Adenoid cystic carcinoma in a salivary gland. *A*, Low-power view. The tumor cells have created a cribriform pattern enclosing secretions. *B*, Perineural invasion by tumor cells.



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Chapter 17 - The Gastrointestinal Tract

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Esophagus

Normal

The esophagus develops from the cranial portion of the foregut and is recognizable by the third week of gestation. The normal esophagus is a hollow, highly distensible muscular tube that extends from the epiglottis in the pharynx, at about the level of the C6 vertebra, to the gastroesophageal junction at the level of the T11 or T12 vertebra. Measuring between 10 and 11 cm in the newborn, it grows to a length of about 25 cm in the adult. For the endoscopist, the esophagus is recorded as the anatomic distance between 15 and 40 cm from the incisor teeth, with the gastroesophageal junction located at the 40-cm point. Several points of luminal narrowing can be identified along its course—proximally at the cricoid cartilage, midway in its course alongside the aortic arch and at the anterior crossing of the left main bronchus and left atrium, and distally where it pierces the diaphragm. Although the pressure in the esophageal lumen is negative compared with the atmosphere, manometric recordings of intraluminal pressures have identified two higher-pressure areas that remain relatively contracted in the resting phase. A 3-cm segment in the proximal esophagus at the level of the cricopharyngeus muscle is referred to as the upper esophageal sphincter (UES). The 2- to 4-cm segment just proximal to the

anatomic gastroesophageal junction, at the level of the diaphragm, is referred to as the lower esophageal sphincter (LES). Both "sphincters" are physiologic, in that there are no anatomic landmarks that delineate these higher-pressure regions from the intervening esophageal musculature.

The wall of the esophagus consists of a mucosa, submucosa, muscularis propria, and adventitia, reflecting the general structural organization of the gastrointestinal tract.[1] The

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mucosa has a smooth, glistening, and pink-tan surface. It has three components: a nonkeratinizing stratified squamous epithelial layer, lamina propria, and muscularis mucosa. The epithelial layer has mature squamous cells overlying basal cells. The basal cells, constituting 10% to 15 % of the mucosal thickness, are reserve cells with great proliferative potential. A small number of specialized cell types, such as melanocytes, endocrine cells, dendritic cells, and lymphocytes, are present in the deeper portion of the epithelial layer. The lamina propria is the nonepithelial portion of the mucosa, above the muscularis mucosae. It consists of areolar connective tissue and contains vascular structures and scattered leukocytes. Finger-like extensions of the lamina propria, called *papillae*, extend into the epithelial layer. The muscularis mucosae is a delicate layer of longitudinally oriented smooth-muscle bundles.

The *submucosa* consists of loose connective tissue containing blood vessels, a rich network of lymphatics, a sprinkling of leukocytes with occasional lymphoid follicles, nerve fibers (including the ganglia of the Meissner plexus), and submucosal glands. Submucosal glands connected to the lumen by squamous epithelium-lined ducts are scattered along the entire esophagus but are more concentrated in the upper and lower portions. Their mucin-containing fluid secretions help lubricate the esophagus.

As is true throughout the alimentary tract, the *muscularis propria* consists of an inner circular and an outer longitudinal coat of smooth muscle with an intervening, well-developed myenteric plexus (Auerbach plexus). The muscularis propria of the proximal 6 to 8 cm of the esophagus also contains striated muscle fibers from the cricopharyngeus muscle. Besides creating a unique histologic interplay of smooth muscle and skeletal muscle fibers, this feature explains why skeletal muscle disorders can cause upper esophageal dysfunction.

In sharp contrast to the rest of the gastrointestinal tract, the esophagus is mostly devoid of a serosal coat. Only small segments of the intra-abdominal esophagus are covered by serosa; the thoracic esophagus is surrounded by fascia that condenses around the esophagus to form a sheathlike structure. In the upper mediastinum, the esophagus is supported by this fascial tissue, which forms a similar sheath around adjacent structures, the great vessels and the tracheobronchial tree. This intimate anatomic proximity to important thoracic viscera is of significance in permitting the ready and widespread dissemination of infections and tumors of the esophagus into the posterior mediastinum. The rich network of mucosal and submucosal lymphatics that runs longitudinally along the esophagus further facilitates spread.

The main functions of the esophagus are to conduct food and fluids from the pharynx to the stomach, to prevent passive diffusion of substances from the food into the blood, and to prevent reflux of gastric contents into the esophagus. These functions require motor activity coordinated with swallowing, namely a wave of peristaltic contraction, relaxation of the LES in anticipation of the peristaltic wave, and closure of the LES after the swallowing reflex. The mechanisms governing this motor function are complex, involving both extrinsic and intrinsic innervation, humoral regulation, and properties of the muscle wall itself.

The control of the lower esophageal sphincter (LES) is critical to esophageal function.[2] Maintenance of sphincter tone is necessary to prevent reflux of gastric contents, which are under positive pressure relative to the esophagus. During deglutition, both active inhibition of the muscularis propria muscle fibers by inhibitory nonadrenergic/noncholinergic neurons and cessation of tonic excitation by cholinergic neurons enable the LES to relax. Many chemical agents (e.g., gastrin, acetylcholine, serotonin, prostaglandin $F_{2\alpha}$, motilin, substance P, histamine, and pancreatic polypeptide) increase LES tone, while some agents (nitric oxide, vasoactive intestinal peptide) decrease the tone. However, their precise roles in normal esophageal function remain unclear.

Pathology

Lesions of the esophagus run the gamut from highly lethal cancers to the merely annoying "heartburn" that has affected many a partaker of a large, spicy meal. Esophageal varices, the result of cirrhosis and portal hypertension, are of major importance, since their rupture is frequently followed by massive hematemesis (vomiting of blood) and even death by exsanguination. Esophagitis and hiatal hernias are far more frequent and rarely threaten life. Distressing to the physician is that all disorders of the esophagus tend to produce similar symptoms, namely heartburn, dysphagia, pain, and/or hematemesis.

Heartburn (retrosternal burning pain) usually reflects regurgitation of gastric contents into the lower esophagus. *Dysphagia* (difficulty in swallowing) is encountered both with deranged esophageal motor function and with diseases that narrow or obstruct the lumen. *Pain* and *hematemesis* are sometimes evoked by esophageal disease, particularly by those lesions associated with inflammation or ulceration of the esophageal mucosa. The clinical diagnosis of esophageal disorders often requires specialized procedures such as esophagoscopy, radiographic barium studies, and manometry.

Congenital Anomalies

Ectopic tissue rests are not uncommon in the esophagus. The most common is ectopic gastric mucosa in the upper third of the esophagus ("inlet patch"), occurring in up to 2% of individuals. Sebaceous glands or ectopic pancreatic tissue are much less frequent. The acid secretions of the ectopic gastric mucosa or pancreatic enzymatic secretions can produce localized inflammation and discomfort.

Embryologic formation of the foregut can also give rise to *congenital cysts*. These are usually duplication cysts, containing double smooth muscle layers and derived from the lower esophagus in 60% of cases. Rarely, bronchial or parenchymal pulmonary tissue may arise from the upper gut and is denoted *bronchogenic cyst* or *pulmonary sequestration*, respectively. These lesions usually present as masses. Lastly, impaired formation of the diaphragm may permit herniation of abdominal viscera into the thorax. When severe, this lesion is incompatible with life, since the lungs are severely hypoplastic at the time of birth. This condition is to be distinguished from hiatal hernias, to be discussed presently.

ATRESIA AND FISTULAS

Although developmental defects in the esophagus are uncommon, they must be corrected early because they are incompatible with life. Because they cause immediate regurgitation when feeding is attempted, they are usually discovered soon after birth. *Absence* (agenesis) of the esophagus is extremely rare; much more common are *atresia* and *fistula formation* (Fig. 17-1). In atresia, a segment of the esophagus is represented by only a thin, noncanalized cord, with a proximal blind pouch connected to the pharynx and a lower pouch leading to the stomach. Atresia is most commonly located at or near the tracheal bifurcation. It rarely occurs alone, but is usually associated with a fistula connecting the lower or upper pouch with a bronchus or the trachea. Associated anomalies include congenital heart disease, neurologic disease, genitourinary disease, and other gastrointestinal malformations. Atresia sometimes is associated with the presence of a single umbilical artery.[3] Aspiration and paroxysmal suffocation from food are obvious hazards; pneumonia and severe fluid and electrolyte imbalances may also occur.

WEBS, RINGS, AND STENOSIS

Esophageal mucosal webs are uncommon ledgelike protrusions of the mucosa into the esophageal lumen. These are semicircumferential, eccentric, and most common in the upper esophagus. Well-developed webs rarely protrude more than 5 mm into the lumen, with a thickness of 2 to 4 mm. The webs consist of squamous mucosa and a vascularized submucosal core. Webs can be congenital in origin, or they may arise in association with long-standing reflux esophagitis, chronic graft-versus-host disease (GVHD), or blistering skin diseases. When an upper esophageal web is accompanied by an iron-deficiency anemia, glossitis, and cheilosis, the condition is referred to as the Paterson-Brown-Kelly or Plummer-Vinson syndrome, with an attendant risk for postcricoid esophageal carcinoma.

Esophageal rings are concentric plates of tissue protruding into the lumen of the distal esophagus. One occurring above the squamocolumnar junction of the esophagus and stomach is

referred to as an *A ring*. One located at the squamocolumnar

Figure 17-1 Esophageal atresia and tracheoesophageal fistula. *A*, Blind upper and lower esophageal segments. *B*, Fistula between blind upper segment and trachea. *C*, Blind upper segment, fistula between blind lower segment and trachea. *D*, Blind upper segment only. E, Fistula between patent esophagus and trachea. Type C is the most common variety. *(Adapted from Morson BC, and Dawson IMP, eds., Gastrointestinal Pathology. Oxford, Blackwell Scientific Publications, 1972, p. 8.)*

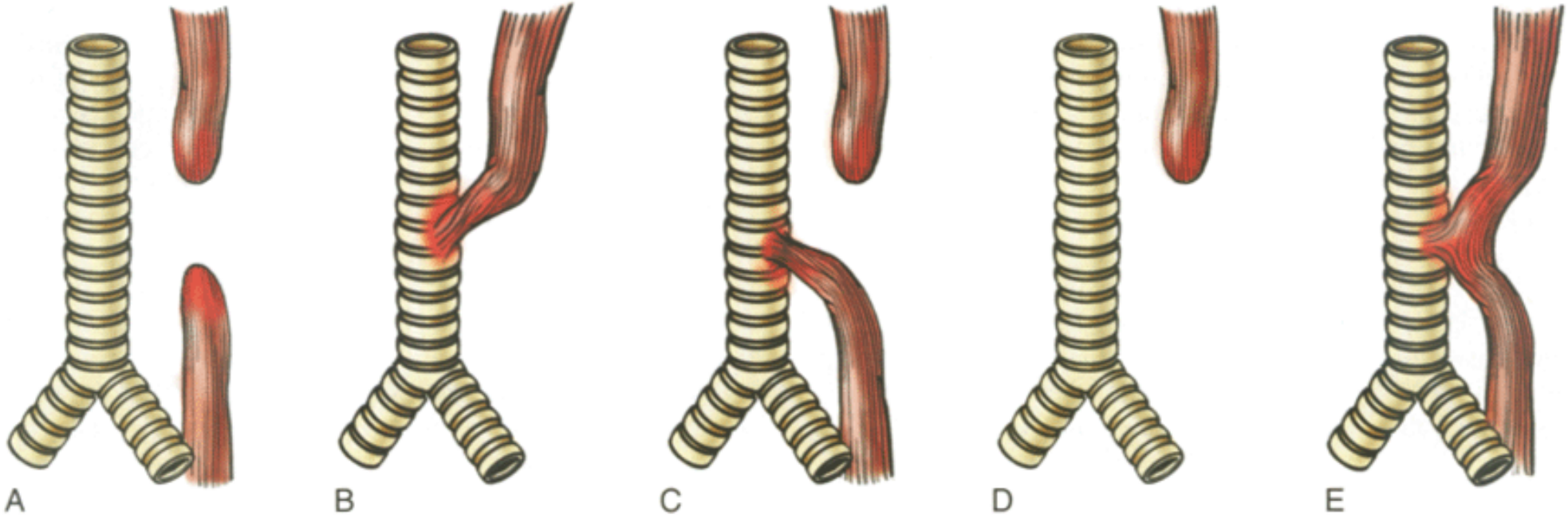


Figure 17-2 Major conditions associated with esophageal motor dysfunction.

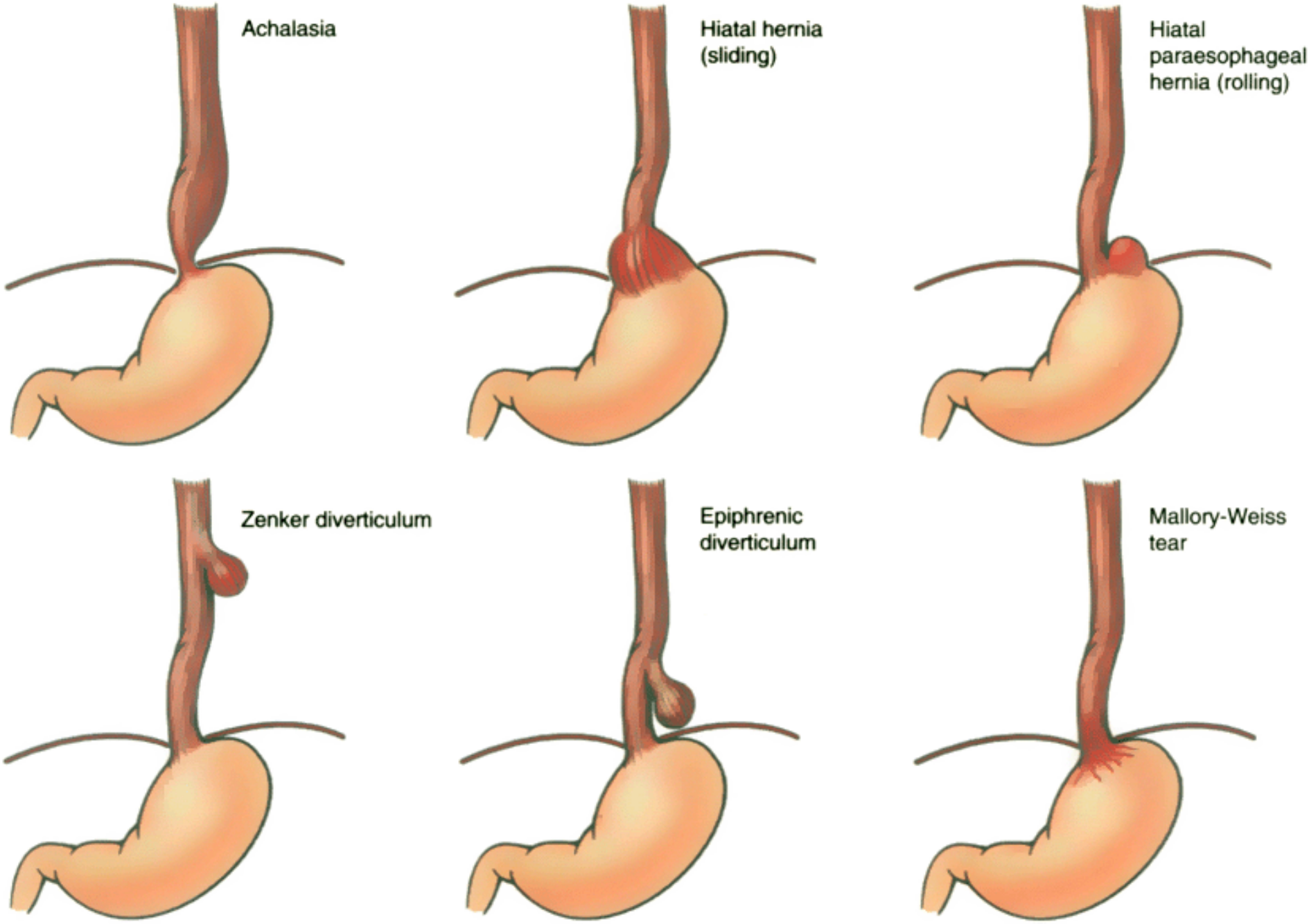


Figure 17-3 Esophageal laceration (Mallory-Weiss tears). Gross view demonstrating longitudinal lacerations extending from esophageal mucosa into stomach mucosa *(arrow)*. *(Courtesy of Dr. Richard Harruff, King County Medical Examiner's Office, Seattle, WA.)*

Figure 17-4 Esophageal varices. *A*, A view of the everted esophagus and gastroesophageal junction, showing dilated submucosal veins (varices). The blue-colored varices have collapsed in this postmortem specimen. *B*, Low-power cross-section of a dilated submucosal varix that has ruptured through the mucosa. A small amount of thrombus is present within the point of rupture. *C*, Hepatic venogram after injection of dye into portal veins (PV) to show a large tortuous gastroesophageal varix *(arrow)* extending superiorly from the patent main portal vein. *(C, courtesy of Dr. Emily Sedgwick, Brigham and Women's Hospital, Boston, MA.)*

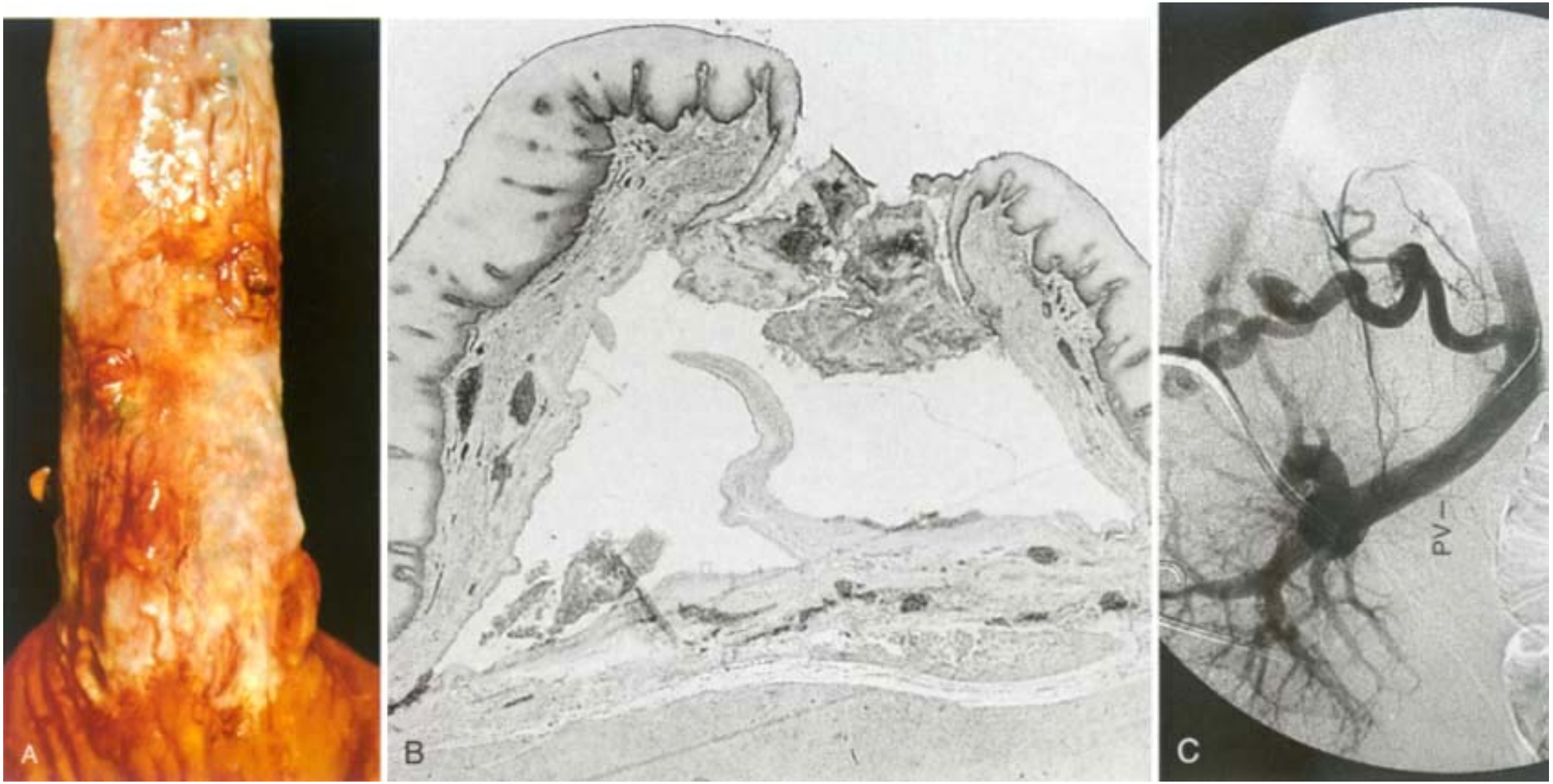


Figure 17-5 Reflux esophagitis. Low-power view of the superficial portion of the mucosa. Numerous eosinophils within the squamous epithelium, elongation of the lamina propria papillae, and basal zone hyperplasia are present.

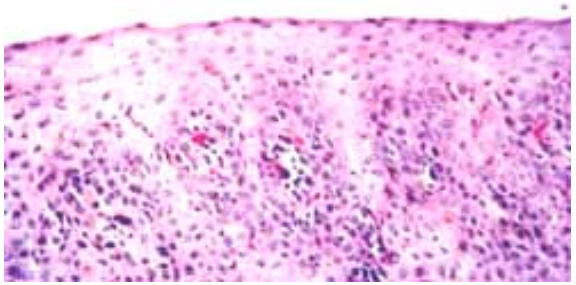


Figure 17-6 Barrett esophagus. *A, B,* Gross view of distal esophagus *(top)* and proximal stomach *(bottom),* showing *A,* the normal gastroesophageal junction *(arrow)* and *B,* the granular zone of Barrett esophagus *(arrow). C,* Endoscopic view of Barrett esophagus showing red velvety gastrointestinal mucosa extending from the gastroesophageal orifice. Note the paler squamous esophageal mucosa.

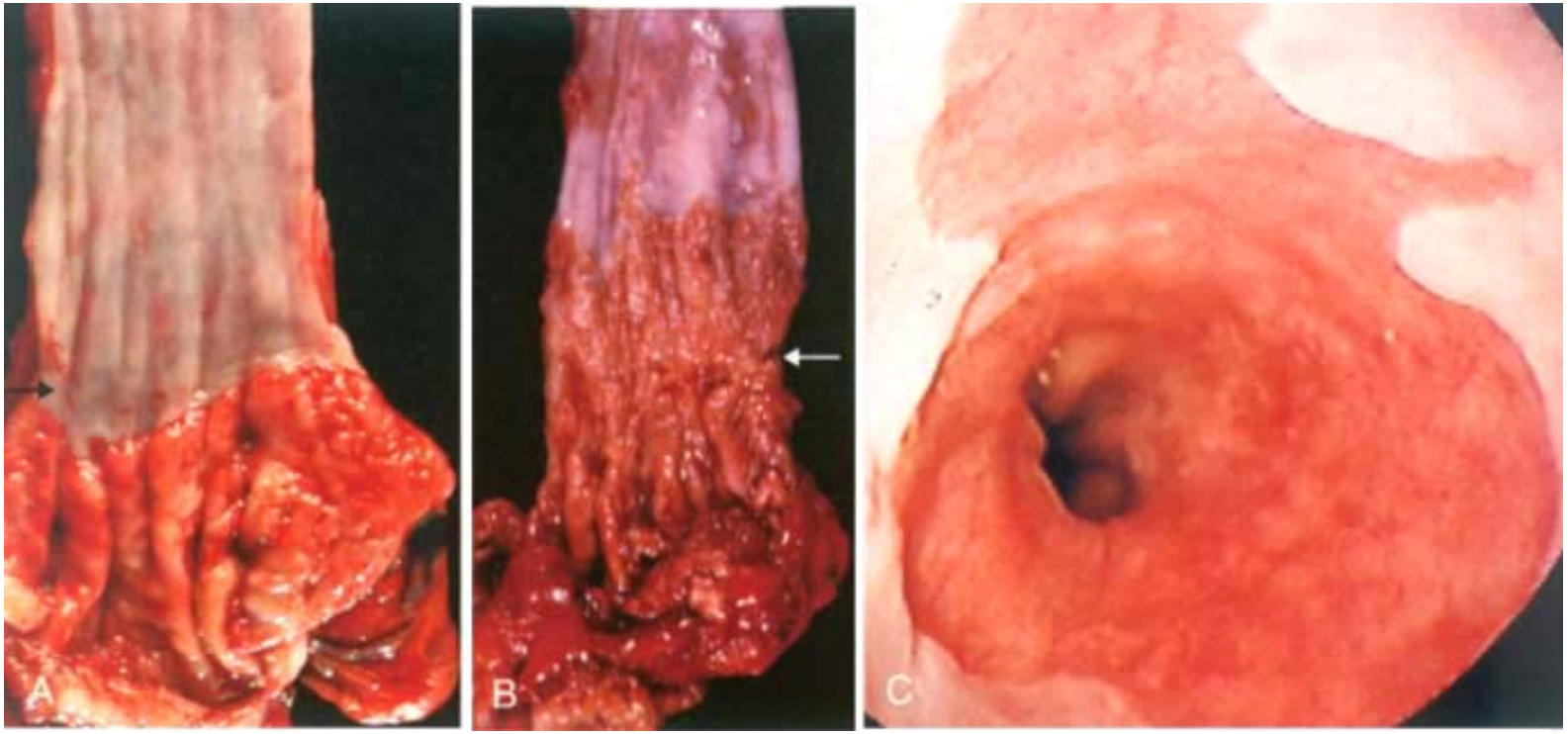


Figure 17-7 Barrett esophagus. Microscopic view showing squamous mucosa and intestinal-type columnar epithelial cells (goblet cells) in a glandular mucosa.

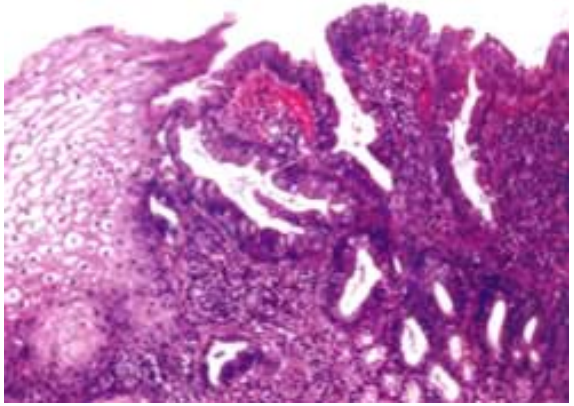


TABLE 17-1 -- Factors Associated with the Development of Squamous Cell Carcinoma of the Esophagus

Dietary
Deficiency of vitamins (A, C, riboflavin, thiamine, pyridoxine)
Deficiency of trace elements (zinc, molybdenum)
Fungal contamination of foodstuffs

High content of nitrites/nitrosamines
Betel chewing
Lifestyle
Burning-hot beverages or food
Alcohol consumption
Tobacco use
Urban environment
Esophageal Disorders
Long-standing esophagitis
Achalasia
Plummer-Vinson syndrome
Genetic Predisposition
Long-standing celiac disease
Ectodermal dysplasia
Epidermolysis bullosa
Racial disposition

States are attributable to alcohol and tobacco usage. Some alcoholic drinks contain significant amounts of such carcinogens as polycyclic hydrocarbons, fuel oils, and nitrosamines, along with other mutagenic compounds. Nutritional deficiencies associated with alcoholism may contribute to the process of carcinogenesis.

Alcohol and tobacco cannot be invoked as risk factors in many high-incidence regions of the world. The presence of carcinogens, such as fungus-contaminated and nitrosamine-containing foodstuffs in China, may play a significant role in the extraordinary high incidence of carcinoma in this region. Dietary deficiencies in vitamins and essential metals have been documented in China and South Africa. Human papillomavirus DNA is found frequently in esophageal squamous cell carcinomas from high-incidence regions, but is infrequent in cancer-bearing patients in North America. [13]

Based on the above considerations, dietary and environmental factors have been proposed to increase risk, with nutritional deficiencies acting as promoters or potentiators of the tumorigenic effects of environmental carcinogens. For example, methylating nitroso compounds in the diet and in tobacco smoke may be the reason for the broad spectrum of *p53* point mutations present in over half of esophageal cancers. Other genetic alterations, such as mutations in *p16INK4*, and amplification of *CYCLIN D1*, C-*MYC*, and epithelial growth factor receptor *(EGFR)*, are prevalent in these cancers as well. This is in keeping with the concept that stepwise acquisition and accumulation of genetic alterations ultimately give rise to cancer. [14] Notably rare in esophageal squamous cell carcinomas are K-*RAS* and adenomatous polyposis coli *(APC)* mutations.

Finally, the chronic esophagitis so commonly observed in persons living in areas of high incidence may itself be the result of sustained exposure to the carcinogens listed earlier. This chronic esophagitis results in an increased epithelial cell turnover, which, over a length of time in a continuously carcinogenic environment, progresses to dysplasia and eventually to carcinoma. The rate of progression along the chronic esophagitis-dysplasia-cancer sequence may well be modified or modulated by genetic or racial factors.

Morphology.

Like squamous cell carcinomas arising in other locations, those of the esophagus begin as apparent in situ lesions **(intraepithelial neoplasm or carcinoma in situ)**. When they become overt, about 20% of these tumors are located in the upper third, 50% in the middle third, and 30% in the lower third of the esophagus. Early lesions appear as small, gray-white, plaque-like thickenings or elevations of the mucosa. In months to years, these lesions become tumorous masses and may eventually encircle the lumen. Three morphologic patterns are described: (1) protruded (60%), a polypoid exophytic lesion that protrudes into the lumen; (2) flat (15%), a diffuse, infiltrative form that tends to spread within the wall of the esophagus, causing thickening, rigidity, and narrowing of the lumen; and (3) excavated (ulcerated, 25%; Fig. 17-8), a necrotic cancerous ulceration that excavates deeply into surrounding structures and may erode into the respiratory tree (with resultant fistula and pneumonia) or aorta (with catastrophic exsanguination) or may permeate the mediastinum and pericardium. The fortunate patient is found at the stage of superficial esophageal carcinoma, in which the malignant lesion is confined to the epithelial layer (in situ) or is superficially invading the lamina propria or submucosa (Fig. 17-9).

Most squamous cell carcinomas are moderately to well differentiated. Several histologic variants may be seen, such as verrucous squamous cell carcinoma, spindle cell carcinoma, and basaloid squamous cell carcinoma. Irrespective of their degree of differentiation, most symptomatic tumors are quite large by the time they are diagnosed and have already invaded the wall or beyond. The rich lymphatic network in the submucosa

Figure 17-8 Large ulcerated squamous cell carcinoma of the esophagus.

Figure 17-9 Squamous cell carcinoma of the esophagus: low-power microscopic view showing invasion into the submucosa.

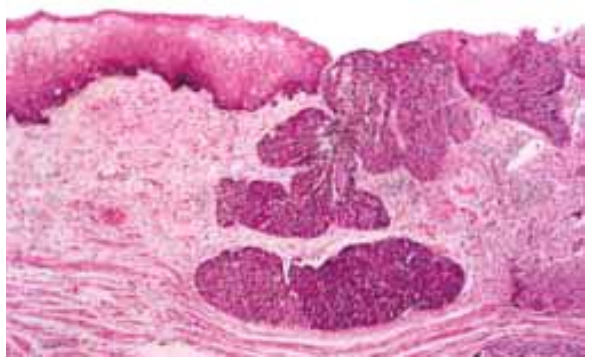


Figure 17-10 Transition from Barrett esophagus to adenocarcinoma.

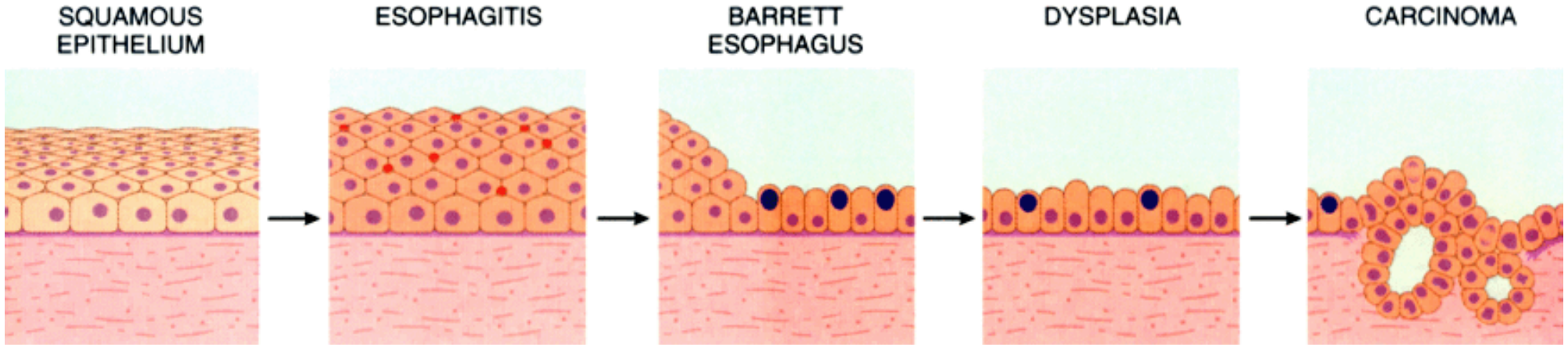
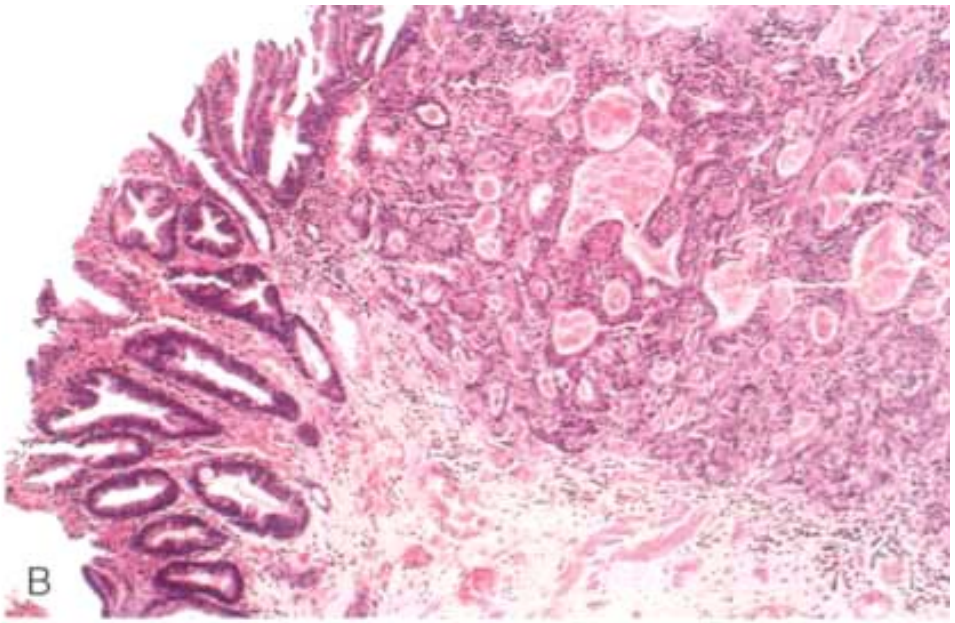


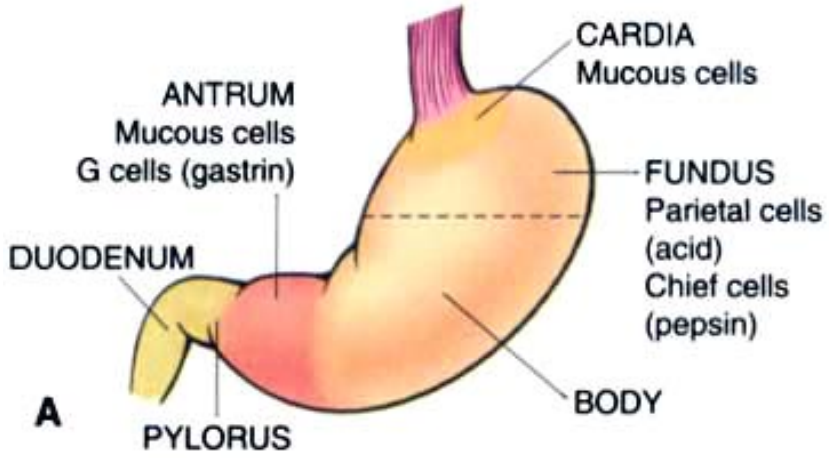
Figure 17-11 Adenocarcinoma of the esophagus. *A*, Gross view of an ulcerated, exophytic mass at the gastroesophageal junction, arising from the granular mucosa of Barrett esophagus. The gray-white esophageal mucosa is on the top, and the folds of gastric mucosa are below. *(A, courtesy of Dr. James Gulizia, Brigham and Women's Hospital, Boston, MA.) B,* Microscopic view of malignant intestinal-type glands in adenocarcinoma arising from Barrett esophagus.

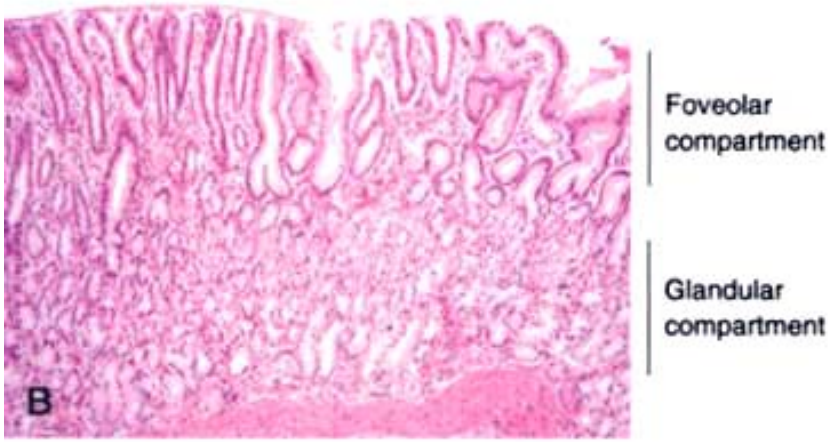
A



B

Figure 17-12 Anatomy and histology of the stomach. *A*, Gross anatomy. *B*, Microscopic view of antral mucosa. *C*, Microscopic view of fundic mucosa.





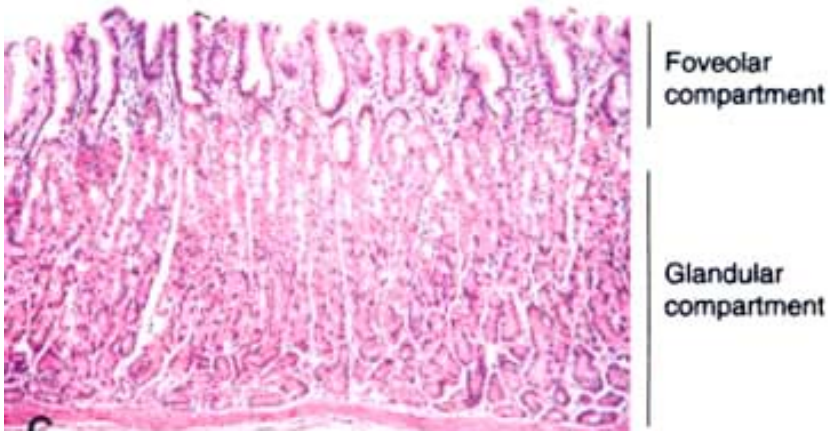
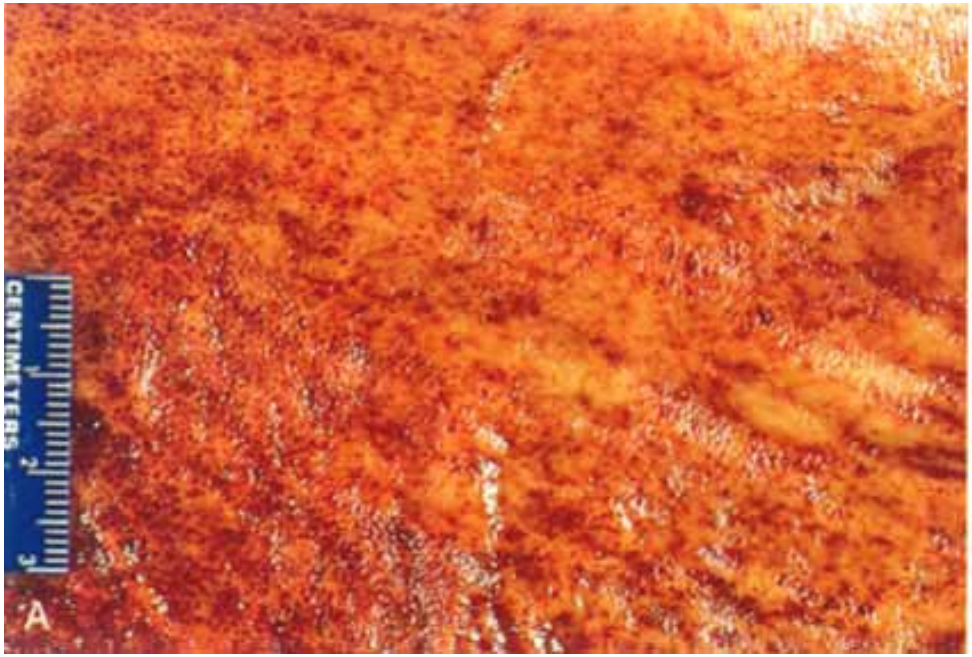


Figure 17-13 Acute gastritis. *A,* Gross view showing punctate erosions in an otherwise unremarkable mucosa; adherent blood is dark due to exposure to gastric acid. *B,* Low-power microscopic view of focal mucosal disruption with hemorrhage; the adjacent mucosa is normal.



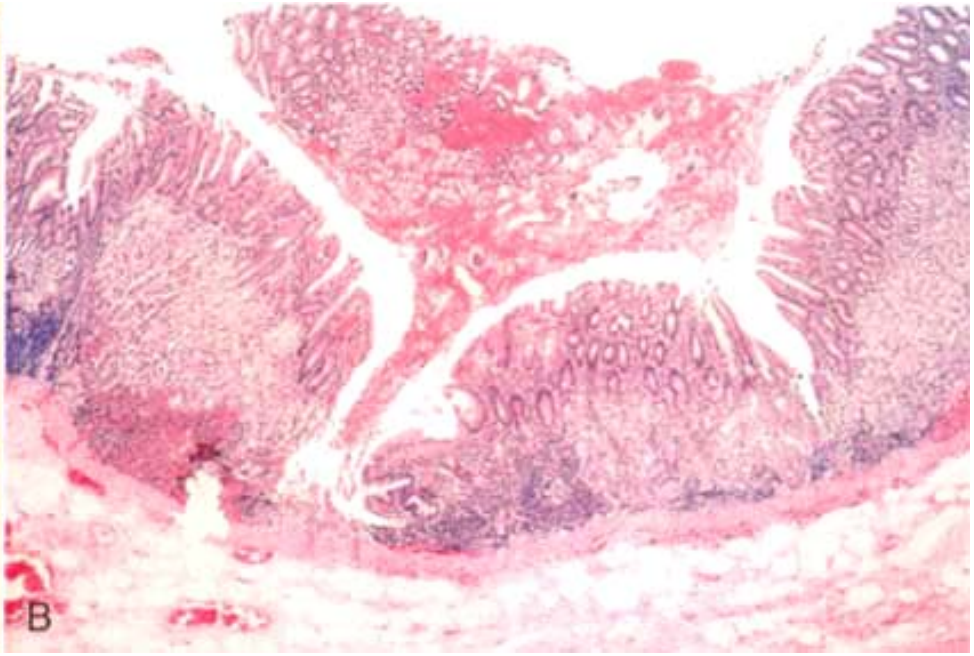


TABLE 17-2 -- Diseases Associated with *Helicobacter pylori* Infection

Disease	Association
Chronic gastritis	Strong causal association
Peptic ulcer disease	Strong causal association
Gastric carcinoma	Strong causal association
Gastric MALT lymphoma *	Definitive etiologic role

* MALT, mucosa-associated lymphoid tissue

areas where infection is endemic, the organism seems to be acquired in childhood and persists for decades. The mode of transmission of *H. pylori* has not been well defined, although oral-oral transmission, fecal-oral transmission, and environmental spread are among the possible routes. *Most infected persons also have the associated gastritis but are asymptomatic.* Nevertheless, infected persons are at increased risk for the development of peptic ulcer disease and possibly gastric cancer.

H. pylori is a nonsporing, curvilinear gram-negative rod measuring approximately 3.5 × 0.5 µm. *H. pylori* is part of a genus of bacteria that have adapted to the ecologic niche provided by gastric mucus, which is lethal to most bacteria. The specialized traits that allow it to flourish include:

- Motility (via flagella), allowing it to swim through viscous mucus
- Elaboration of a *urease*, which produces ammonia and carbon dioxide from endogenous urea, thereby buffering gastric acid in the immediate vicinity of the organism
- Expression of *bacterial adhesins*, such as BabA, which binds to the fucosylated Lewis B blood-group antigens, enhances binding to blood group O antigen bearing cells.[23]
- Expression of bacterial toxins, such as cytotoxin association gene A *(CagA)* and vacuolating cytotoxin gene A *(VacA)*.[24] These are discussed later under "Peptic Ulcer."

The *H. pylori* genome is 1.65 million base pairs and encodes approximately 1500 proteins. Extensive molecular studies suggest that the bacteria cause gastritis by stimulating production of pro-inflammatory cytokines and by directly injuring epithelial cells (discussed later).

After initial exposure to *H. pylori*, gastritis occurs in two patterns: a predominantly antral-type gastritis with high acid production and elevated risk for duodenal ulcer, and a pangastritis that is followed by multifocal atrophy (multifocal atrophic gastritis) with lower gastric acid secretion and higher risk for adenocarcinoma. The underlying mechanisms contributing to this difference are not completely clear, but host-microorganism interplay appears to be critical. IL-1β is a potent pro-inflammatory cytokine and a powerful gastric acid inhibitor. Patients who have higher IL-1β production in response to *H. pylori* infection tend to develop pangastritis, while patients who have lower IL-1β production exhibit antral-type gastritis. [25]

A number of diagnostic tests have been developed for the detection of *H. pylori*. Noninvasive tests include a serologic test for antibodies, fecal bacterial detection, and a urea breath test. The breath test is based on the generation of ammonia by bacterial urease. Invasive tests are based on the identification of *H. pylori* in gastric biopsy tissue. Detection methods in gastric tissue include visualization of the bacteria in histologic sections, bacterial culture, a rapid urease test, and bacterial DNA detection by the polymerase chain reaction.

Patients with chronic gastritis and *H. pylori* usually improve when treated with antibiotics. Relapses are associated with reappearance of the organism. The current treatment regimens include antibiotics and hydrogen pump inhibitors.[22] Prophylactic and therapeutic vaccine development is still in the early research stage, but it holds the promise to eradicate or at least greatly reduce the worldwide prevalence of *H. pylori* infection.

In addition to *H. pylori*, humans can also be infected by *Helicobacter heilmannii*, a spiral bacterium found in dogs, cats, and nonhuman primates.[26] This bacterium causes a relatively mild gastritis.

Autoimmune Gastritis.

This form of gastritis accounts for less than 10% of cases of chronic gastritis. It results from the presence of autoantibodies to components of gastric gland parietal cells, including antibodies against the acidproducing enzyme H^+ ,K^+ -ATPase,[27] gastrin receptor, and intrinsic factor. Gland destruction and mucosal atrophy lead to loss of acid production. In the most severe cases, production of intrinsic factor is lost, leading to pernicious anemia. This uncommon form of gastritis is seen in association with other autoimmune disorders such as Hashimoto thyroiditis, Addison disease, and type 1 diabetes. Patients with autoimmune gastritis have a significant risk for developing gastric carcinoma and endocrine tumors (carcinoid tumor).

Morphology.

Chronic gastritis may affect different regions of the stomach and exhibit varying degrees of mucosal damage.[19] Autoimmune gastritis is characterized by diffuse mucosal damage of the body-fundic mucosa, with less intense to absent antral damage, probably due to the autoantibodies against parietal cells. Gastritis in the setting of environmental etiologies (including infection by *H. pylori*) tends to affect antral mucosa or both antral and body-fundic mucosa (pangastritis). The mucosa is usually reddened and has a coarser texture than normal. The inflammatory infiltrate may create a mucosa with thickened rugal folds, mimicking early infiltrative lesions. Alternatively, with long-standing atrophic disease, the mucosa may become thinned and flattened. Irrespective of cause or location, the histologic changes are similar. An inflammatory infiltrate of lymphocytes and plasma cells is present within the lamina propria (Fig. 17-14). **"Active" inflammation is signified by the presence of neutrophils within the glandular and surface epithelial layer.** Active inflammation may be prominent or absent. Lymphoid aggregates, some with germinal centers, are frequently observed within the mucosa. Several additional histologic features are characteristic:

• ***Regenerative Change.*** A proliferative response to the epithelial injury is a constant feature of chronic gastritis. In the neck region of the gastric glands mitotic figures are increased. Epithelial cells of the

surface mucosa, and to a lesser extent the glands, exhibit enlarged, hyperchromatic nuclei and a higher nuclear-cytoplasmic ratio. Mucus vacuoles are diminished or absent in the superficial cells. When regenerative changes are severe, particularly with ongoing active inflammation, distinguishing regenerative change from dysplasia may be difficult.

• ***Metaplasia.*** The antral, body, and fundic mucosa may become partially replaced by metaplastic columnar absorptive cells and goblet cells of intestinal morphology **(intestinal metaplasia),** both along the surface epithelium and in rudimentary glands. Occasionally, villus-like projections may appear. Although small intestinal features predominate, in some instances, features of colonic epithelium may be present.

• ***Atrophy.*** Atrophic change is evident by marked loss in glandular structures. **Atrophy is quite frequently associated with autoimmune gastritis and pangastritis caused by *H. pylori***. Parietal cells, in particular, may be conspicuously absent in the autoimmune form. Persisting glands frequently undergo cystic dilatation. A particular feature of atrophic gastritis of autoimmune origin or chronic gastritis treated by inhibitors of acid secretion is hyperplasia of gastrin-producing G-cells in the antral mucosa. This is attributed to the hypochlorhydria or achlorhydria arising from severe parietal cell loss. The G-cell hyperplasia is responsible for the increased gastrinemia, which stimulates hyperplasia of enterochromaffin-like cells in the gastric body. As will be discussed later, the ECL cell hyperplasia is the frequent background for gastric carcinoid tumor formation.

• ***Dysplasia.*** With long-standing chronic gastritis, the epithelium develops cytologic alterations, including variation in size, shape, and orientation of epithelial cells, and nuclear enlargement and atypia. Intestinal metaplasia may precede the development of dysplasia. Dysplastic alterations may become so severe as to constitute in situ carcinoma. The development of dysplasia is thought to be a precursor lesion of gastric cancer in atrophic forms of gastritis, particularly in association with pernicious anemia (autoimmune gastritis) and *H. pylori*- associated chronic gastritis.

Figure 17-14 Chronic gastritis, showing partial replacement of the gastric mucosal epithelium by intestinal metaplasia (upper left) and inflammation of the lamina propria (right) containing lymphocytes and plasma cells.

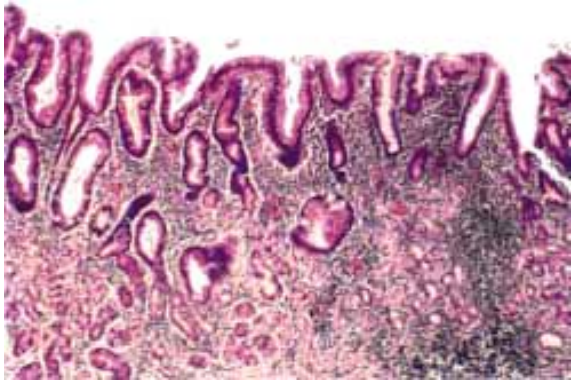


Figure 17-15 *Helicobacter pylori.* A Steiner silver stain demonstrates the numerous darkly stained *Helicobacter* organisms along the luminal surface of the gastric epithelial cells. Note that there is no tissue invasion by bacteria.

Figure 17-16 Reactive gastropathy. Gastric mucosa, showing hyperplasia of foveolar surface epithelial cells, glandular regenerative changes, and smooth muscle fibers extending into lamina propria.

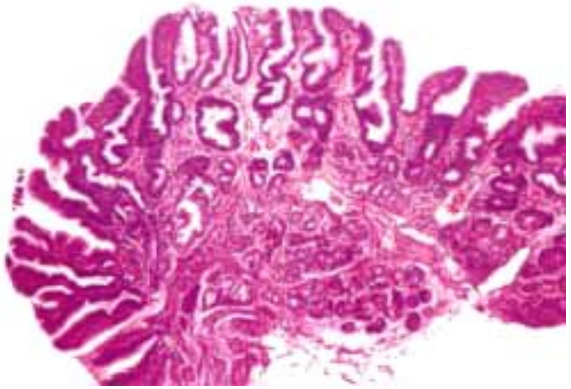


Figure 17-17 Diagram of causes of, and defense mechanisms against, peptic ulceration. Diagram of the base of a nonperforated peptic ulcer, demonstrating the layers of necrosis (N), inflammation (I), granulation tissue (G), and scar (S), moving from the luminal surface at the top to the muscle wall at the bottom.

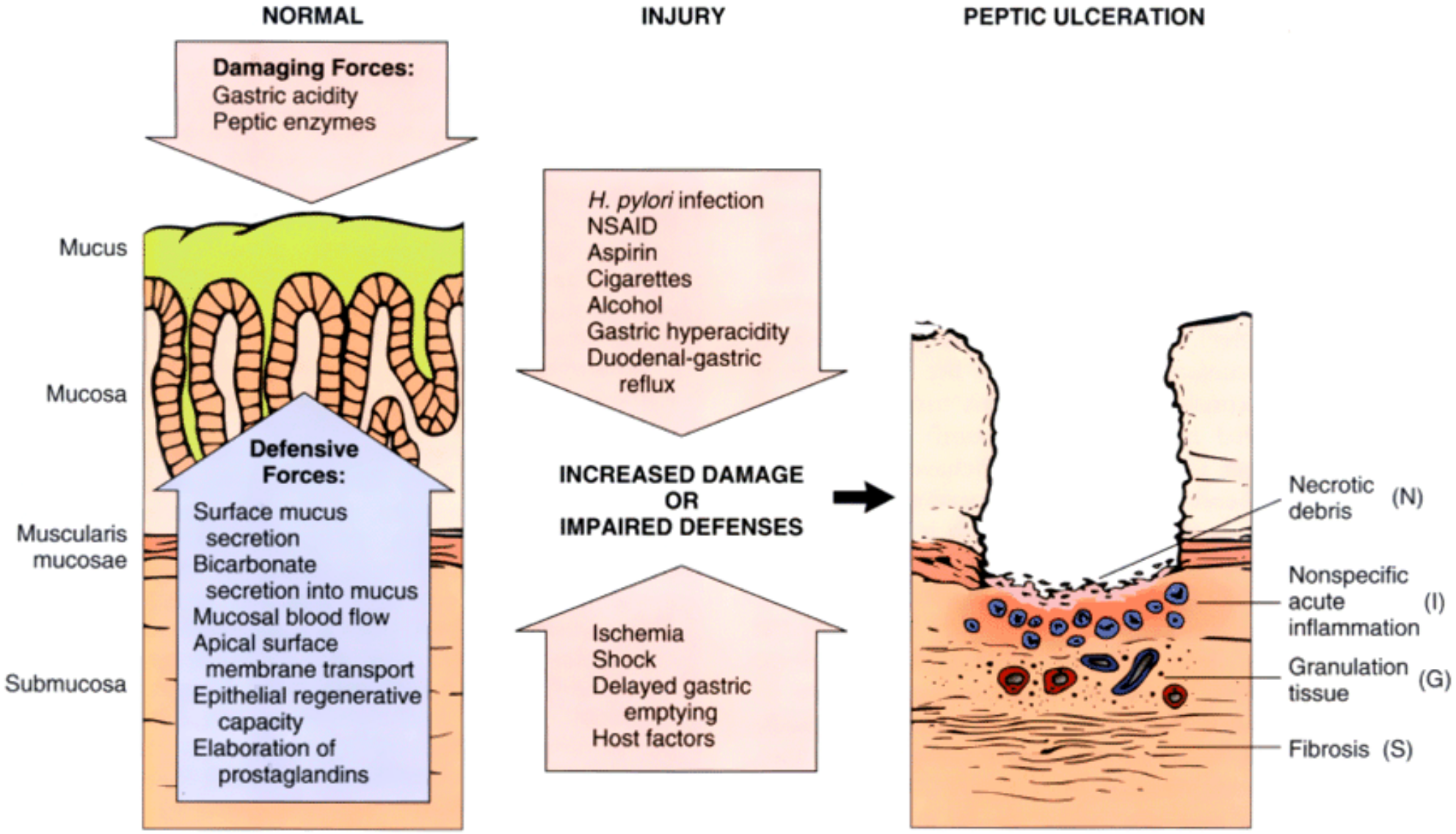


Figure 17-18 Peptic ulcer of the duodenum. Note that the ulcer is small (2 cm) with a sharply punched-out appearance. Unlike cancerous ulcers, the margins are not elevated. The ulcer base is clean. *(Courtesy of Robin Foss, University of Florida, Gainesville, FL.)*

TABLE 17-3 -- Complications of Peptic Ulcer Disease

Bleeding
• Occurs in 15% to 20% of patients
• Most frequent complication
• May be life-threatening
• Accounts for 25% of ulcer deaths
• May be the first indication of an ulcer
Perforation
• Occurs in about 5% of patients
• Accounts for two thirds of ulcer deaths
• Rarely, is the first indication of an ulcer
Obstruction from edema or scarring
• Occurs in about 2% of patients
• Most often due to pyloric channel ulcers
• May also occur with duodenal ulcers
• Causes incapacitating, crampy abdominal pain
• Rarely, may lead to total obstruction with intractable vomiting

tumors are designated *Cushing ulcers* and carry a high incidence of perforation.

The genesis of the acute mucosal defects in these varied clinical settings is poorly understood. No doubt, many factors are shared with acute gastritis, such as impaired oxygenation.

NSAID-induced ulcers are related to decreased prostaglandin production from the inhibition of cyclooxygenase. In the case of lesions associated with intracranial injury, the proposed mechanism involves the direct stimulation of vagal nuclei by increased intracranial pressure, leading to hypersecretion of gastric acid, which is common in these patients. Systemic acidosis, a frequent finding in these clinical settings, may contribute to mucosal injury by lowering the intracellular pH of mucosal cells. These cells are also hypoxic as a consequence of stress-induced splanchnic vasoconstriction.

Morphology.

Acute stress ulcers are usually less than 1 cm in diameter and are circular and small. The ulcer base is frequently stained a dark brown by the acid digestion of extruded blood (Fig. 17-19). Unlike chronic peptic ulcers, acute stress ulcers are found anywhere in the stomach, the gastric rugal pattern is essentially normal and the margins and base of the ulcers are not indurated. While they may occur singly, more often there are multiple stress ulcers throughout the stomach and duodenum. Microscopically, acute stress ulcers are abrupt lesions, with essentially unremarkable adjacent mucosa. Depending on the duration of the ulceration, there may be a suffusion of blood into the mucosa and submucosa and some inflammatory reaction. Conspicuously absent are scarring and thickening of blood vessels, as seen in chronic peptic ulcers. Healing with complete reepithelialization occurs after the causative factors are removed. The time required for complete healing varies from days to several weeks.

Clinical Features.

Most critically ill patients admitted to hospital intensive care units develop histologic evidence of gastric mucosal damage. Bleeding from superficial gastric erosions or ulcers sufficient to require transfusion develops in 1% to 4% of these patients. Although prophylactic H_2 -receptor antagonists and proton pump inhibitors may blunt the impact of stress ulceration, *the single most important determinant of*

Figure 17-19 Multiple stress ulcers of the stomach, highlighted by dark digested blood on their surfaces.

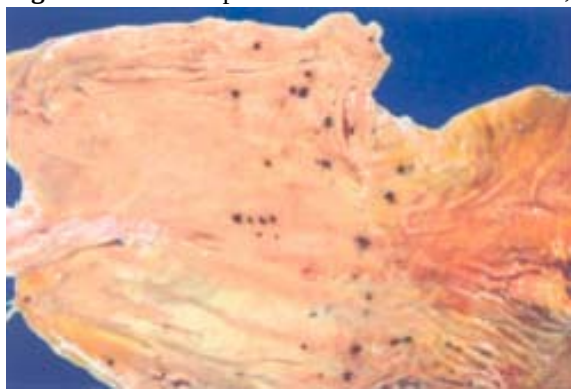


Figure 17-20 Trichobezoar, showing agglomeration of hair, food, and mucus that occurred within the gastric lumen.

Figure 17-21 Hypertrophic gastropathy, showing markedly thickened gastric folds.

Figure 17-22 Gastric hyperplastic polyp. Low-power microscopic view of the polyp showing hyperplastic foveolar epithelium and inflammation.

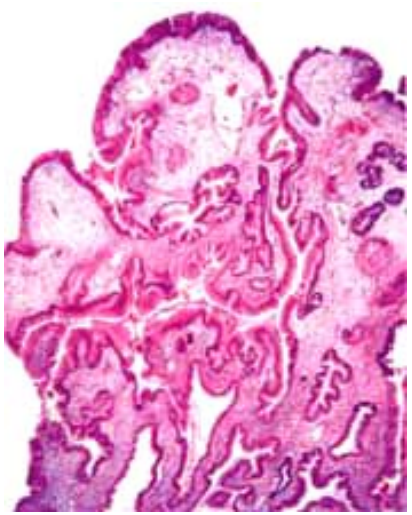


Figure 17-23 Gastric adenoma. Gross photograph showing a large polyp in the stomach.

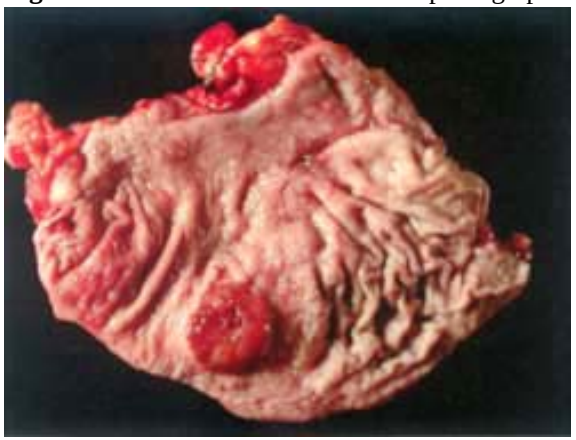


Figure 17-24 Inflammatory fibroid polyp; microscopic photograph showing submucosal growth of inflamed vascularized fibromuscular tissue with prominent eosinophilic infiltrate.

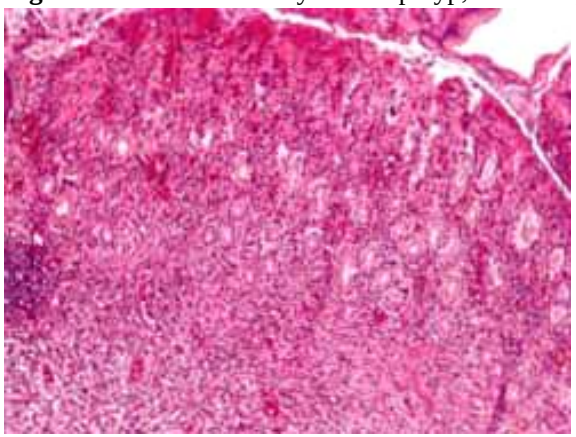


TABLE 17-4 -- WHO Histologic Classification of Gastric Tumors

Epithelial Tumors
Intraepithelial neoplasia: adenoma
Adenocarcinoma*
••• Papillary adenocarcinoma
••• Tubular adenocarcinoma
••• Mucinous adenocarcinoma
••• Signet-ring cell carcinoma
••• Undifferentiated carcinoma
••• Adenosquamous carcinoma
Small-cell carcinoma
Carcinoid tumor
Nonepithelial Tumors
Leiomyoma
Schwannoma
Granular cell tumor
Leiomyosarcoma
Gastrointestinal stromal tumor (GIST) (gradation from benign to malignant)
Kaposi sarcoma
Others
Malignant Lymphoma

* The Laurén classification subdivides adenocarcinomas into intestinal and diffuse types.

pathway by which intestinal-type gastric cancers develop. Adenomas containing mucosal dysplasia can also become malignant.

Environment.

Environmental influences may be critical in gastric carcinogenesis. [35] When families migrate from high-risk to low-risk areas (or the reverse), successive generations acquire the level of risk that prevails in the new locales. The *diet*

TABLE 17-5 -- Factors Associated with Increased Incidence of Gastric Carcinoma

Environmental Factors
Infection by *H. pylori*
• Present in most cases of intestinal-type carcinoma
Diet
• Nitrites derived from nitrates (water, preserved food)
• Smoked and salted foods, pickled vegetables, chili peppers
• Lack of fresh fruit and vegetables
Low socioeconomic status
Cigarette smoking
Host Factors
Chronic gastritis
• Hypochlorhydria: favors colonization with *H. pylori*
• Intestinal metaplasia is a precursor lesion
Partial gastrectomy
• Favors reflux of bilious, alkaline intestinal fluid
Gastric adenomas
• 40% harbor cancer at time of diagnosis
• 30% have adjacent cancer at time of diagnosis
Barrett esophagus
• Increased risk of gastroesophageal junction tumors
Genetic Factors
Slightly increased risk with blood group A
Family history of gastric cancer
Hereditary nonpolyposis colon cancer syndrome
Familial gastric carcinoma syndrome (E-cadherin mutation)

is suspected to be a primary factor, and adherence to certain culinary practices is associated with a high risk of gastric carcinoma. Lack of refrigeration; consumption of preserved, smoked,

cured, and salted foods; water contamination with nitrates; and lack of fresh fruit and vegetables are common themes in high-risk areas. The consumption of dietary carcinogens, such as N-nitroso compounds and benzopyrene, appears to be particularly important. Conversely, intake of green, leafy vegetables and citrus fruits, which contain antioxidants such as ascorbate (vitamin C), alpha-tocopherol (vitamin E), and beta-carotene, is negatively correlated with gastric cancer. A specific protective role for any one of these nutrients cannot be assumed, however, since intake of fresh food may simply displace consumption of preserved foods.

So far there is no conclusive evidence linking alcohol intake and cigarette smoking to the development of gastric cancer. Despite initial concern, *to date there appears to be no increased risk of stomach cancer from the use of antacid drug therapies*.

Host.

Autoimmune gastritis, like *H. pylori* infection, increases the risk of gastric cancer, presumably due to chronic inflammation and intestinal metaplasia. It has been noted that blood group A patients have higher risk but it is not yet clear whether this is related to the binding of *H. pylori* to Lewis B antigen, or to other mechanisms.

Within the United States, blacks, Native Americans, and Hawaiians have a higher risk of developing gastric cancer. But since only about 8% to 10% of patients with gastric cancer have a family history of this disease, genetic factors are unlikely to be a major influence. Environmental factors mentioned above, are likely to play a major role in the higher incidence of gastric cancer among these various groups. Genetic traits play a critical role in some familial cases of gastric cancer, including gastric carcinoma occurring in the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. Recently, E-cadherin gene *(CDH1)* germ-line mutations have also been identified as the underlying genetic basis for another familial gastric cancer syndrome that is characterized by early occurrence of diffuse type adenocarcinoma.[36] These patients are also at risk for developing lobular breast cancer.[37]

Other Risk Factors.

Peptic ulcer disease per se does not impart increased risk for development of gastric cancer. However,

Figure 17-25 Diagram of growth patterns and spread of gastric carcinoma. In early gastric carcinoma (*A*), the tumor is confined to the mucosa and submucosa and may exhibit an exophytic, flat or depressed, or excavated conformation. Advanced gastric carcinoma (*B*) extends into the muscularis propria and beyond. Linitis plastica is an extreme form of flat or depressed advanced gastric carcinoma.

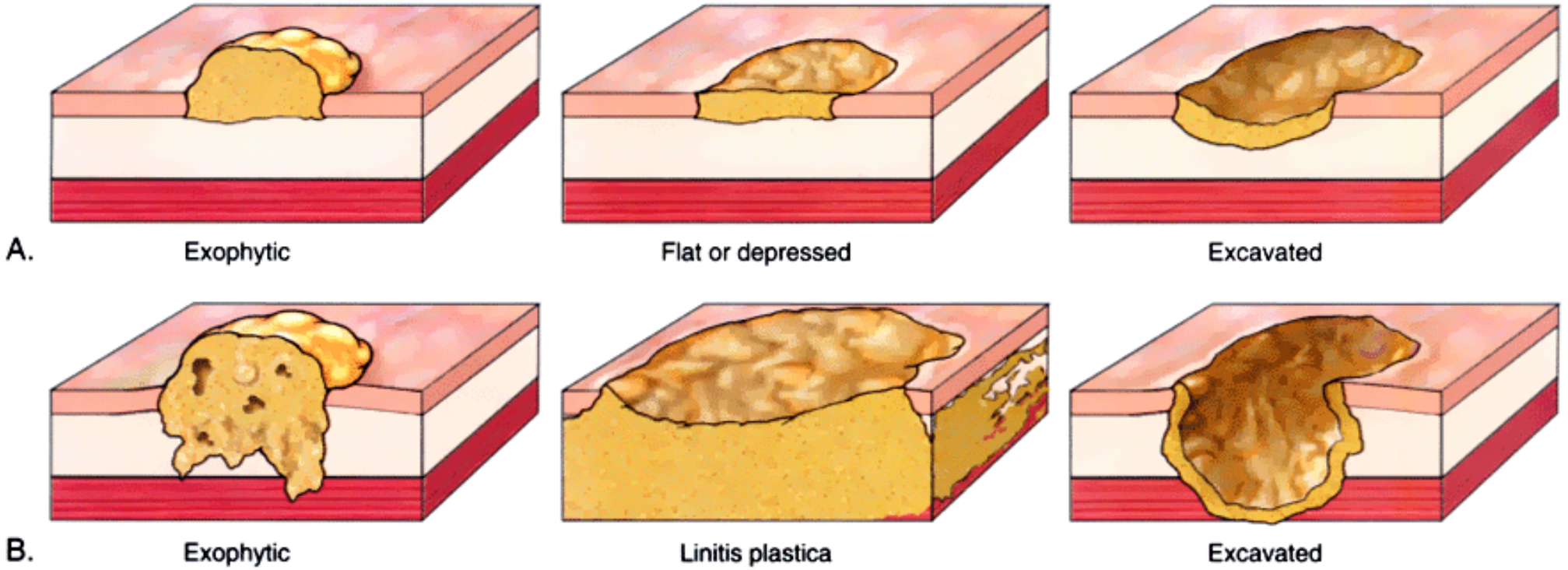


Figure 17-26 Gastric carcinoma. Gross photograph showing an ill-defined, excavated central ulcer surrounded by irregular, heaped-up borders.

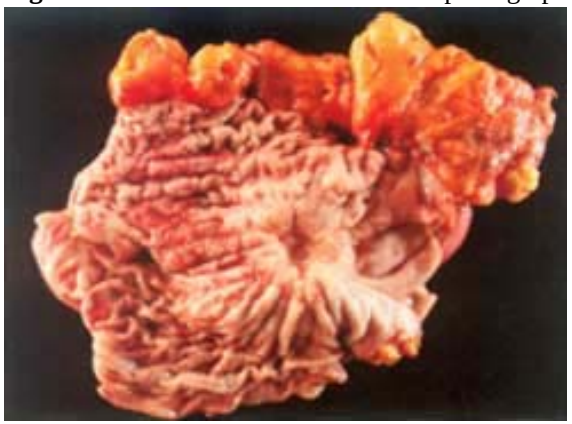


Figure 17-27 Gastric carcinoma. *A*, Intestinal type demonstrating gland formation by malignant cells, which are invading the muscular wall of the stomach. *B*, Diffuse type demonstrating signet-ring carcinoma cells.

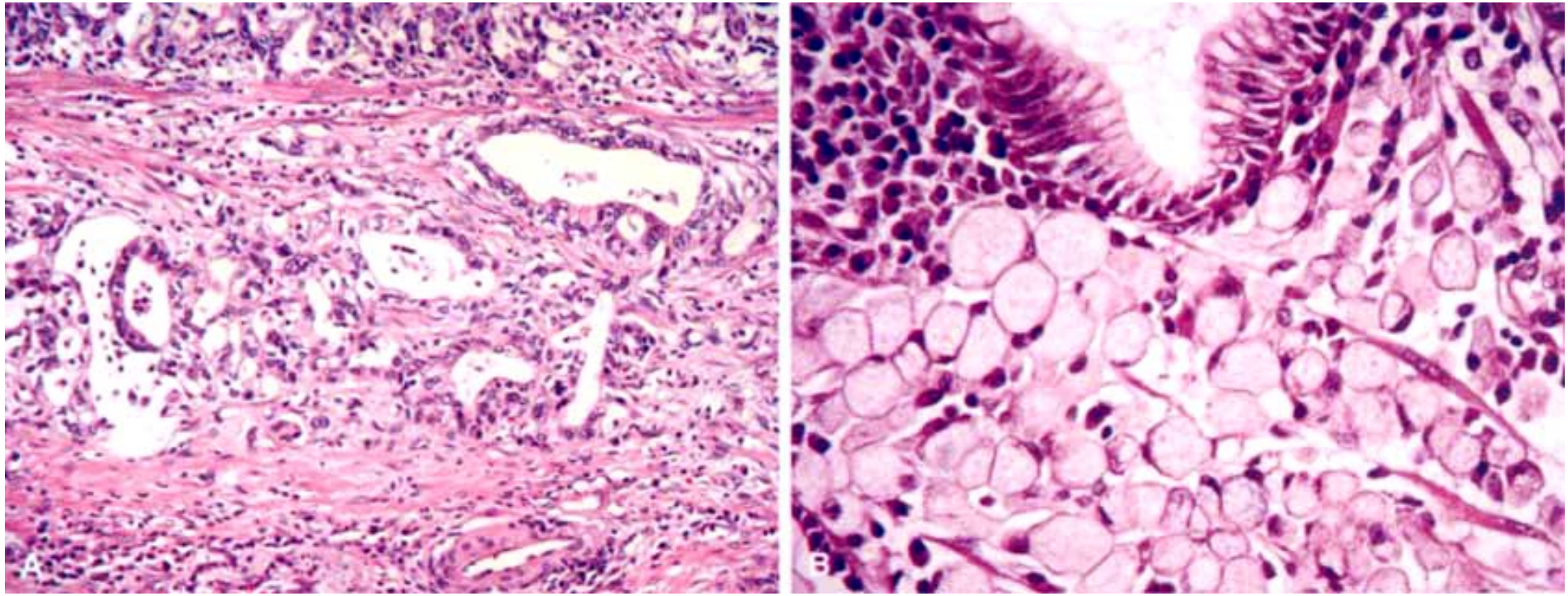


Figure 17-28 Gastric MALT lymphoma. Note the lymphoepithelial lesions *(arrows)*. *(Courtesy of Dr. Melissa Li, University of Florida, Gainesville, FL.)*

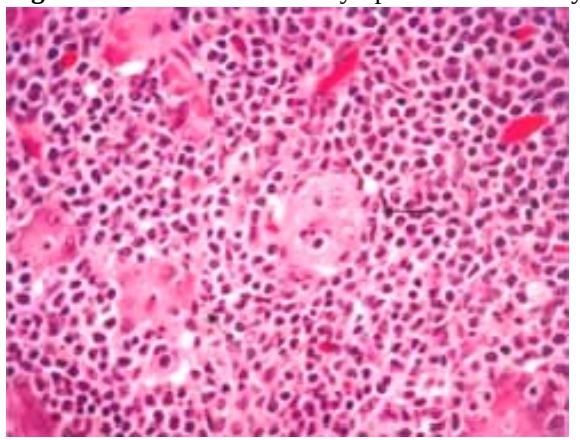


Figure 17-29 Gastrointestinal stromal tumor. *A,* Gross photograph of the tumor arising from the muscularis propria of the gastric wall. *B,* Microscopic view of the tumor showing spindle cell feature. *C,* Immunohistochemical stain showing the tumor cell c-*KIT* positivity.

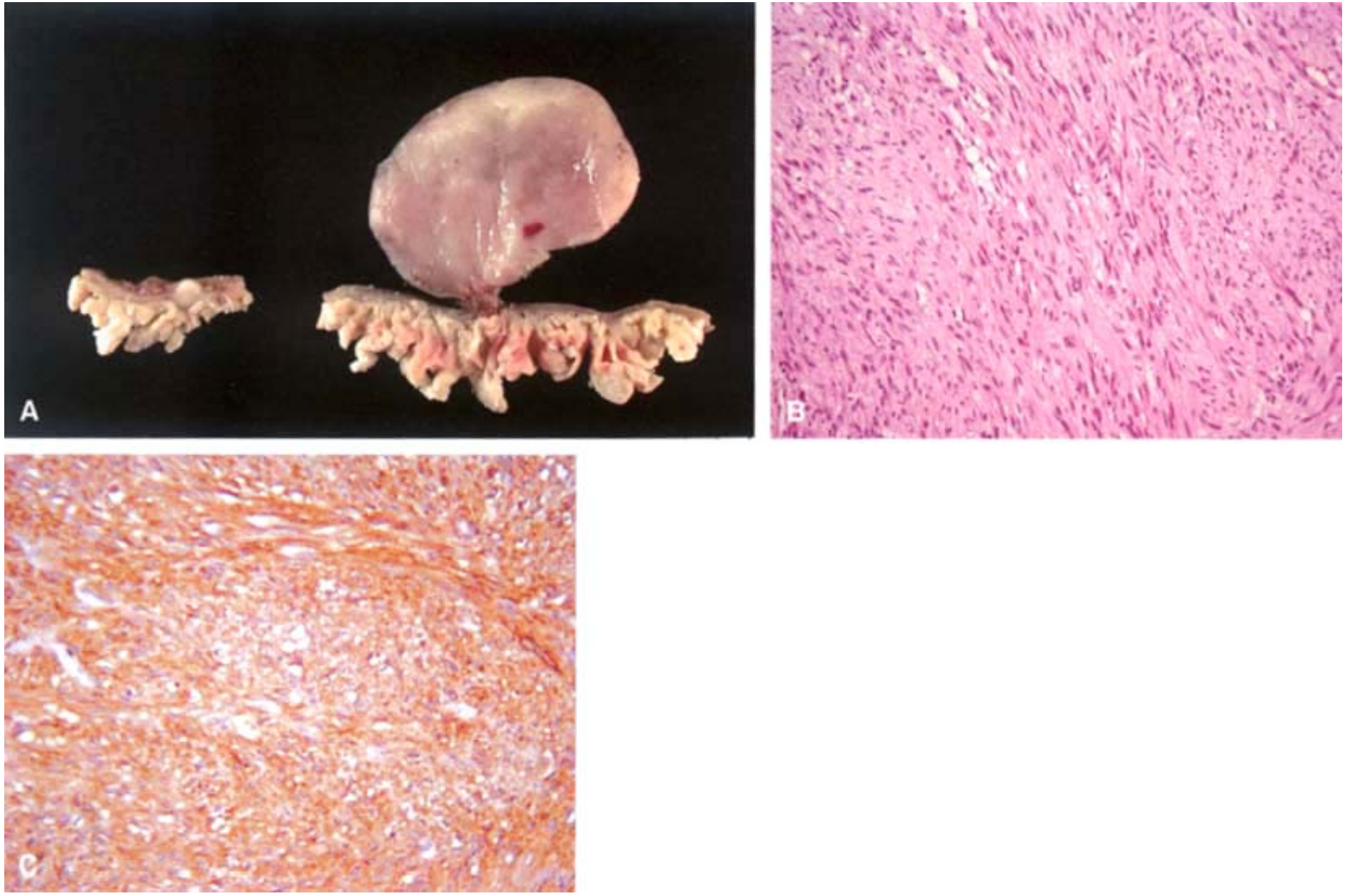


Figure 17-30 *A,* Normal small-bowel histology, showing mucosal villi and crypts, lined by columnar cells. *B,* Normal colon histology, showing flat mucosal surface and abundant vertically oriented crypts.

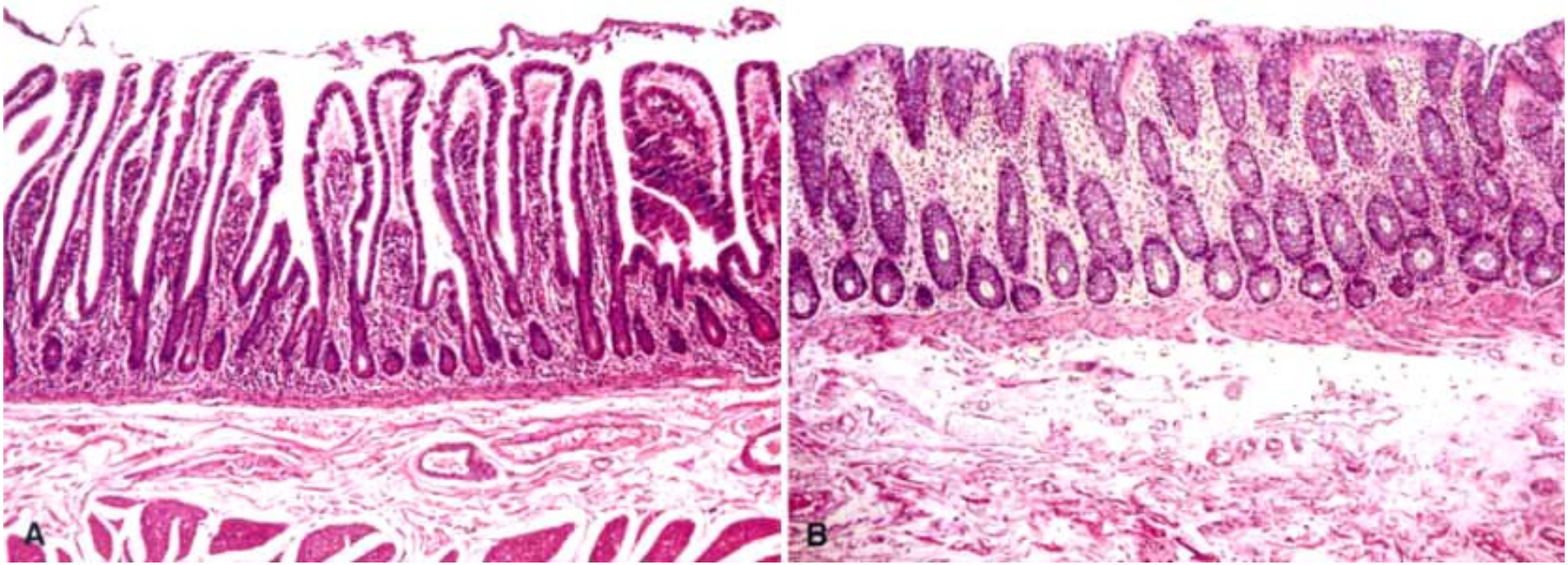


Figure 17-31 Meckel diverticulum. The blind pouch is located on the antimesenteric side of the small bowel.

TABLE 17-6 -- Major Causes of Diarrheal Illnesses

Secretory Diarrhea

Infectious: viral damage to mucosal epithelium
• Rotavirus
• Caliciviruses
• Enteric adenoviruses
• Astroviruses
Infectious: enterotoxin mediated
• *Vibrio cholerae*
• *Escherichia coli*
• *Bacillus cereus*
• *Clostridium perfringens*
Neoplastic
• Tumor elaboration of peptides, serotonin, prostaglandins
• Villous adenoma in distal colon (nonhormone mediated)
Excess laxative use
Osmotic Diarrhea
Disaccharidase (lactase) deficiencies
Lactulose therapy (for hepatic encephalopathy, constipation)
Prescribed gut lavage for diagnostic procedures
Antacids ($MgSO_4$ and other magnesium salts)
Primary bile acid malabsorption
Exudative Diseases
Infectious: bacterial damage to mucosal epithelium
• *Shigella*
• *Salmonella*
• *Campylobacter*
• *Entamoeba histolytica*
Idiopathic inflammatory bowel disease
Typhlitis (neutropenic colitis in the immunosuppressed)
Malabsorption

Defective intraluminal digestion
Primary mucosal cell abnormalities
Reduced small intestinal surface area
Lymphatic obstruction
Infectious: impaired mucosal cell absorption
• *Giardia lamblia* infection
Deranged Motility
Decreased intestinal transit time
• Surgical reduction of gut length
• Neural dysfunction, including irritable bowel syndrome
• Hyperthyroidism
• Diabetic neuropathy
• Carcinoid syndrome
Decreased motility (increased intestinal transit time)
• Small intestinal diverticula
• Surgical creation of a "blind" intestinal loop
• Bacterial overgrowth in the small intestine

- *Secretory diarrhea:* Net intestinal fluid secretion leads to the output of more than 500 mL of fluid stool per day, which is isotonic with plasma and persists during fasting.
- *Osmotic diarrhea:* Excessive osmotic forces exerted by luminal solutes lead to output of more than 500 mL of stool per day, which abates upon fasting. Stool exhibits an osmotic gap (stool osmolality exceeds plasma electrolyte concentration by ≥50 mOsm).
- *Exudative diseases:* Mucosal destruction leads to output of purulent, bloody stools that persist on fasting; stools are frequent but may be small or large volume.
- *Deranged motility:* Improper gut neuromuscular function may produce highly variable patterns of increased stool volume; other forms of diarrhea must be excluded.
- *Malabsorption:* Improper absorption of gut nutrients produces voluminous, bulky stools with increased osmolarity combined with excess stool fat (steatorrhea). The diarrhea usually abates on fasting.

INFECTIOUS ENTEROCOLITIS

Intestinal diseases of microbial origin are marked principally by diarrhea and sometimes ulcerative and inflammatory changes in the small and/or large intestine. *Infectious enterocolitis is a global problem of staggering proportions, causing more than 12,000 deaths per day among children in developing countries, and constituting one half of all deaths before age 5 worldwide.* [49] *Although far less prevalent in industrialized nations, these infections still have attack rates of one to two illnesses per person per year, second only to the common cold in frequency.* This results in an estimated 99 million acute cases of either vomiting or diarrhea per year in the United States, equivalent to 40% of the population. The infections are mainly associated with contaminated food and water.

Acute, self-limited infectious diarrhea, which is a major cause of morbidity among children, is most frequently caused by enteric viruses. In infants, infectious diarrhea may cause severe

dehydration and metabolic acidosis, which may result in hospitalization in developed countries and death in developing countries. Bacterial infections, such as enterotoxigenic *Escherichia coli*, are also common offenders. However, *many pathogens can cause diarrhea*; the major offenders vary with the age, nutrition, immune status of the host, environment (living conditions, public health measures), and special predispositions, such as hospitalization, wartime dislocation, or foreign travel. In 40% to 50% of cases, the specific agent cannot be isolated.

Viral Gastroenteritis

Symptomatic human infection is caused by several distinct groups of viruses (Table 17-7). *Rotavirus* accounts for an estimated 140 million cases and 1 million deaths worldwide per year. The target population is children age 6 to 24 months, but young infants and debilitated adults are susceptible to symptomatic infection. This virus accounts for 25% to 65% of severe diarrhea in infants and young children.[50] Rotavirus is an encapsulated virus with a segmented double-stranded RNA genome. *Rotavirus selectively infects and destroys mature enterocytes in the small intestine, without infecting crypt cells.* The surface epithelium of the villus is repopulated by immature secretory cells. With the loss of absorptive function and excess of secretory cells, there is net secretion of water and electrolytes, compounded by an osmotic diarrhea from incompletely absorbed nutrients. The minimal infective inoculum is approximately 10 viral particles, whereas an individual with rotavirus gastroenteritis typically sheds up to 10^{12} particles/mL stool. Thus, outbreaks among pediatric populations in hospitals and day-care centers are very common. The clinical syndrome has an incubation period of approximately 2 days, which is followed by vomiting and watery diarrhea for several days. Viral infection can induce protective immunity, but the protection for reinfection is often short-lived. Antirotavirus

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TABLE 17-7 -- Common Gastrointestinal Viruses

Virus	Genome	Size (nm)	% of U.S. Childhood Viral Enterocolitis	Host Age	Mode of Transmission	Prodrome/Duration of Illness
Rotavirus (Group A)	dsRNA	70	60	6–24 months	Person-to-person, food, water	2 days/3–5 days
Caliciviruses	ssRNA	35–40	20	Child or adult	Person-to-person, water, cold foods, raw shellfish	1–3 days/4 days
••Norwalk-like viruses						
••Sapporo-like viruses						
Enteric adenoviruses	dsDNA	80	8	Child <2 years	Person-to-person	3–10 days/7+ days
Astroviruses	ssRNA	28	4	Child	Person-to-person, water, raw shellfish	24–36 hours/1–4 days

Data from Goodgame RW: Viral causes of diarrhea. Gastroenterol Clin North Am 30:779,2001.

ds, double-stranded; ss, single stranded.

antibodies are present in mother's milk, so rotavirus infection is most frequent at the time of weaning.

Among the numerous types of *adenovirus*, the subtypes (enteric serotypes) Ad40, Ad41, and Ad31 appear to be responsible for enteric infections and are a common cause of diarrhea among infants. They can be distinguished from adenoviruses that cause respiratory disease by their failure to grow easily in culture. Adenoviruses cause a moderate gastroenteritis with diarrhea and vomiting, lasting for a week to 10 days after an incubation period of approximately 1 week. In the small intestine, adenoviral infection causes atrophy of the villi and

compensatory hyperplasia of the crypts similar to rotavirus, resulting in malabsorption and fluid loss. The virus can also cause colitis. Immunohistochemical stain of nuclear inclusions facilitates the diagnosis.

Caliciviruses include two major groups: the classic Caliciviruses (Sapporo-like viruses) and the Norwalk-like viruses (small round structured viruses). Sapporo-like viral infection is rare, while *Norwalk virus*, the prototype of Norwalk-like viruses, is responsible for the majority of cases of nonbacterial food-borne epidemic gastroenteritis in all age groups. Norwalk-like viruses are small icosahedral viruses containing a single-stranded RNA genome. They cause epidemic gastroenteritis with diarrhea, nausea, and vomiting among children. Outbreaks occur following exposure of multiple individuals to a common source. The clinical syndrome has an incubation period of 1 to 2 days, which is followed by 12 to 60 hours of nausea, vomiting, watery diarrhea, and abdominal pain.

Astrovirus is named after its starlike appearance. It primarily affects children, (it accounts for 4% of acute gastroenteritis in young children), and has a worldwide distribution. Those infected develop anorexia, headache, and fever. Other viruses such as enterotrophic coronaviruses and toroviruses are occasionally implicated in human diarrheal disease.

Despite the high incidence of viral gastroenteritis, insights into disease pathogenesis have been slow in coming.

Morphology.

Although the enteric viruses are genetically and morphologically different from each other, the lesions they cause in the intestinal tract are similar. The small intestinal mucosa usually exhibits modestly shortened villi and infiltration of the lamina propria by lymphocytes. Vacuolization and loss of the microvillus brush border in surface epithelial cells may be evident, and the crypts become hypertrophied. Viral particles may be visualized by electron microscopy within surface epithelial cells. In infants, rotavirus can produce a flat mucosa resembling celiac sprue (discussed later).

Bacterial Enterocolitis

Diarrheal illness may be caused by numerous bacteria (Table 17-8). There are several pathogenic mechanisms for bacterial enterocolitis (also termed *food poisoning*):

- *Ingestion of preformed toxin*, present in contaminated food. Major offenders are *Staphylococcus aureus*, *Vibrio*, and *Clostridium perfringens*. Symptoms develop within a matter of hours; explosive diarrhea and acute abdominal distress herald an illness that passes within a day or so. Ingested systemic neurotoxins, as from *Clostridium botulinum*, may produce rapid, and fatal, respiratory failure.
- *Infection by toxigenic organisms*, which proliferate within the gut lumen and elaborate an enterotoxin. An incubation period of several hours to days is followed by *diarrhea and dehydration* if the primary pathogenic mechanism is a secretory enterotoxin, or *dysentery* if the primary mechanism is a cytotoxin. *Traveler's diarrhea* (Montezuma's revenge, turista) usually occurs following ingestion of fecally contaminated food or water; it begins abruptly and subsides within 2 to 3 days. It affects 20% to 50% of the 35 million people who travel worldwide from industrialized countries to developing countries each year.
- *Infection by enteroinvasive organisms*, which proliferate, invade, and destroy mucosal epithelial cells, also leading to dysentery. As with ingestion of toxigenic organisms, the incubation period is several hours to days.

The main properties of bacteria that contribute to the pathogenesis of enterocolitis are: *(1) the ability to adhere to the mucosal epithelial cells and replicate, (2) the ability to elaborate enterotoxins, and (3) the capacity to invade.*

Bacterial Adhesion and Replication.

In order to produce disease, ingested organisms must adhere to the mucosa; otherwise they will be swept away by the fluid stream. Adherence of enterotoxigenic organisms such as *E. coli* and *Vibrio cholerae* is mediated by plasmid-encoded adhesins. These proteins are expressed on the surface of the organism, sometimes in the form of fimbriae or *pili*, which are rigid or wiry surface

TABLE 17-8 -- Major Causes of Bacterial Enterocolitis

Organism	Pathogenic Mechanism	Source	Clinical Features
Escherichia coli			Traveler's diarrhea, including:
• ETEC	Cholera-like toxin, no invasion	Food, water	Watery diarrhea
• EHEC	Shiga-like toxin, no invasion	Undercooked beef products	Hemorrhagic colitis, hemolytic-uremic syndrome
• EPEC	Attachment, enterocyte effacement, no invasion	Weaning foods, water	Watery diarrhea, infants and toddlers
• EIEC	Invasion, local spread	Cheese, water, person-to-person	Fever, pain, diarrhea, dysentery
Salmonella	Invasion, translocation, lymphoid inflammation, dissemination	Milk, beef, eggs, poultry	Fever, pain, diarrhea or dysentery, bacteremia, extraintestinal infection, common source outbreaks
Shigella	Invasion, local spread	Person-to-person, low-inoculum	Fever, pain, diarrhea, dysentery, epidemic spread
Campylobacter	Toxins, invasion	Milk, poultry, animal contact	Fever, pain, diarrhea, dysentery, food sources, animal reservoirs
Yersinia enterocolitica	Invasion, translocation, lymphoid inflammation, dissemination	Milk, pork	Fever, pain, diarrhea, mesenteric adenitis, extraintestinal infection, food sources
Vibrio cholerae, other *Vibrios*	Enterotoxin, no invasion	Water, shellfish, person-to-person spread	Watery diarrhea, cholera, pandemic spread
Clostridium difficile	Cytotoxin, local invasion	Nosocomial environmental spread	Fever, pain, bloody diarrhea, following antibiotic use, nosocomial acquisition
Clostridium perfringens	Enterotoxin, no invasion	Meat, poultry, fish	Watery diarrhea, food sources, "pigbel"
Mycobacterium tuberculosis	Invasion, mural inflammatory foci with necrosis and scarring	Contaminated milk, swallowing of coughed-up organisms	Chronic abdominal pain; complications of malabsorption, stricture, perforation, fistulae, hemorrhage

ETEC, enterotoxigenic *E. coli*; EHEC, enterohemorrhagic *E. coli*; EPEC, enteropathogenic *E. coli*; EIEC, enteroinvasive *E. coli*.

projections. Adherence of enteropathogenic and enterohemorrhagic organisms, including *E. coli* and *Shigella,* is also dependent on plasmid-encoded proteins, but the nature of these proteins is not known. Adherence causes effacement of the apical enterocyte membrane, with destruction of the microvillus brush border and changes in the underlying cell cytoplasm.[35] The factors regulating bacterial replication are not well understood, particularly since pathogenic organisms must compete with the normal bacterial flora to achieve a critical population density.

Bacterial Enterotoxins.

Bacterial enterotoxins are polypeptides that cause diarrhea. Some *enterotoxins* cause intestinal secretion of fluid and electrolytes without causing tissue damage; this is accomplished by binding of the toxin to the epithelial cell membrane, entry of a portion of the toxin into the cell, and massive activation of electrolyte secretion accompanied by water. *Cholera toxin, elaborated by Vibrio cholerae, is the prototype secretagogue toxin.* The toxin causes increased levels of intracellular calcium, resulting in dysfunction of the fluid and electrolyte transport, as discussed below under Cholera. *Strains of E. coli (enterotoxigenic E. coli) that produce heat-labile (LT) and heat-stable (ST) secretagogue toxins are the major cause of traveler's diarrhea.* The LT toxin is similar to cholera toxin, and the ST toxin induces cyclic guanosine monophosphate, resulting in increased fluid excretion. Leukocytes are absent from the stool of patients with traveler's diarrhea. A second group of enterotoxins are *cytotoxins*, exemplified by Shiga toxin produced by *Shigella dysenteriae* and Shiga-like toxins produced by enterohemorrhagic *E. coli* (e.g., *E. coli O157:H7*). These toxins cause direct tissue damage through epithelial cell necrosis. *Staphylococcal enterotoxins*, which are major causes of food poisoning, represent yet another group of enterotoxins; are proteins that bind to the antigen receptors of large numbers of T cells and activate the lymphocytes to secrete cytokines. The cytokines stimulate intestinal motility and fluid secretion.

Bacterial Invasion.

Both enteroinvasive *E. coli* and *Shigella* possess a large virulence plasmid that confers the capacity for epithelial cell invasion, apparently by microbe-stimulated endocytosis. This is followed by intracellular proliferation, cell lysis, and cell-to-cell spread. *Salmonella* quickly pass through intestinal epithelial cells via transcytosis with minimal epithelial damage; entry into the lamina propria leads to a 5% to 10% incidence of bacteremia, which can sometimes cause typhoid fever, meningitis, endocarditis, and osteomyelitis (commonly in the setting of sickle cell disease). *Yersinia enterocolitica* penetrates the ileal mucosa and multiplies within Peyer patches and regional lymph nodes. Bacteremia is rare and usually occurs in the setting of iron-overload disease, since iron is a growth factor for *Yersinia*.

Bacterial cytotoxins and invasion give rise to bacillary dysentery, which generates its own unique misery for its victims: abdominal cramping and tenesmus with loose stools containing blood, pus, and mucus. Bacillary dysentery, which results in as many as 500,000 deaths among children in developing countries each year, is caused by *Shigella dysenteriae, Shigella flexneri, Shigella boydii,* and *Shigella sonnei* as well as certain O type enterotoxic *E. coli*. (Amebic dysentery is caused by the protozoan parasite *Entamoeba histolytica,* discussed later in this chapter).

Shigella Bacillary Dysentery

Shigella species are gram-negative facultative anaerobes that infect only humans. *S. flexneri* is the major cause of endemic bacillary dysentery in locations of poor hygiene, including large regions of the developing world and institutions in the

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developed world. Epidemic shigellosis can occur when individuals consume uncooked foods at picnics or other events.

Pathogenesis.

Transmission is fecal-oral and is remarkable for the small number of organisms that may cause disease (10 ingested organisms cause illness in 10% of volunteers, and 500 organisms cause disease in 50% of volunteers). *Shigella* bacteria invade the intestinal mucosal cells but do not usually go beyond the lamina propria. Dysentery is caused when the bacteria escape the epithelial cell phagolysosome, multiply within the cytoplasm, and destroy host cells. Shiga toxin causes hemorrhagic colitis and hemolytic-uremic syndrome by damaging endothelial cells in the microvasculature of the colon and the glomeruli, respectively (Chapter 20). In addition, chronic arthritis secondary to *S. flexneri* infection, called *Reiter syndrome,* may be caused by a bacterial antigen; the occurrence of this syndrome is strongly linked to HLA-B27 genotype, but the immunologic basis of this reaction is not understood. [51]

Salmonellosis and Typhoid Fever

Salmonellae are flagellated, gram-negative bacteria that cause a self-limited food-borne and water-borne gastroenteritis (*S. enteritidis, S. typhimurium,* and others) or a life-threatening systemic illness, typhoid fever, marked by fever and systemic symptoms *(S. typhi)*. In the United States, *Salmonella* species cause approximately 500,000 reported cases of food poisoning, and many cases go unreported. Because *Salmonella* species other than *S. typhi* infect most commercially raised chickens and many cows, the major sources of *Salmonella* in the United States are feces-contaminated beef and chicken that are insufficiently washed and cooked. Stringent hygiene in the production plants and the home kitchen helps minimize risk of contamination. In contrast, humans are the only host of *S. typhi*, which is shed in the feces, urine, vomitus, and oral secretions by acutely ill persons and in the feces by chronic carriers without overt disease. Therefore, typhoid fever from *S. typhi* is a disease largely of developing countries, where sanitary conditions are insufficient to stop its spread. Typhoid fever is a protracted disease that is associated with bacteremia, fever, and chills during the first week; widespread mononuclear phagocyte involvement with rash, abdominal pain, and prostration in the second week; and ulceration of Peyer patches with intestinal bleeding and shock during the third week.

Pathogenesis.

Salmonella invades intestinal epithelial cells as well as tissue macrophages. Invasion of intestinal epithelial cells is controlled by invasion genes that are induced by the low oxygen tension found in the gut. These genes encode proteins involved in adhesion and in recruitment of host cytoskeletal proteins that internalize the bacterium. Similarly, intramacrophage growth is important in pathogenicity, and this seems to be mediated by bacterial genes that are induced by the acid pH within the macrophage phagolysosome. The enteric nervous system also is a critical regulator of fluid secretion in the normal gut. Neural reflex pathways increase epithelial fluid secretion in response to enteric pathogens such as *Salmonella* and *Clostridium difficile*. [52]

Campylobacter Enterocolitis

This comma-shaped, flagellated, gram-negative organism was once classified with the vibrios. When special culture conditions permitted its isolation in the 1970s, it became apparent that *Campylobacter* was an important cause of enterocolitis and septicemia in humans. In the United States, *Campylobacter jejuni* is responsible for twice the enteric disease of *Salmonella* and four times that of *Shigella*. Most infections with *Campylobacter* are sporadic and are associated with ingestion of improperly cooked chicken, which may be contaminated with *Campylobacter* and/or *Salmonella*. Sporadic infections may also be associated with contact with infected dogs. Outbreaks of *Campylobacter* are usually associated with unpasteurized milk or contaminated water.

Pathogenesis.

Invasiveness is strain dependent. Flagella of *Campylobacter,* which give the organism its comma shape and motility, are necessary for the bacterium to penetrate mucus covering epithelial surfaces. Three clinical outcomes of *Campylobacter* infection are possible: (1) diarrhea, which is independent of bacterial invasion; (2) dysentery with blood and mucus in the stool; and (3) enteric fever when bacteria proliferate within the lamina propria and mesenteric lymph nodes. Postinfectious complications of *Campylobacter* infections include reactive arthritis in HLA-B27 carriers (as with *Shigella* infection) and Guillain-Barré syndrome, a demyelinating disease of peripheral nerves due to autoantibodies against gangliosides G_{M1} and GQ1b, described in Chapter 27 . Recently, *C. jejuni* was found to be associated with the development of immunoproliferative small intestinal disease (discussed later).

Cholera

Vibrio cholerae are comma-shaped, gram-negative bacteria that have been the cause of seven great long-lasting epidemics (pandemics) of diarrheal disease. Many of these pandemics began in the Ganges Valley of India and Bangladesh, which is never free from cholera, and then moved east. Although there are 140 serotypes of *V. cholerae,* until recently only the 01 serotype was associated with severe diarrhea. Beginning in 1992, a new *V. cholerae* serotype (0139, also known as Bengal) has been associated with severe, watery diarrhea.[53]

Pathogenesis.

The vibrios never invade the epithelium but instead remain within the lumen and secrete an enterotoxin, which is encoded by a virulence phage. Flagellar proteins involved in motility and attachment are necessary for efficient bacterial colonization, as has been described for *Campylobacter*. (This is in contrast to *Shigella* species and certain *E. coli* strains, which are

nonmotile and yet invasive.) The *Vibrio* hemagglutinin, which is a metalloprotease, is important for detachment of *Vibrio* from epithelial cells.

The secretory diarrhea characteristic of the disease is caused by release of *cholera toxin* (Fig. 17-32). Cholera toxin is composed of five binding peptides B and a catalytic peptide A. The B peptides, serving as a "landing pad," bind to carbohydrates on G_{M1} ganglioside on the surface of epithelial cells of the small intestine, enabling calveolar-mediated endosomal entry of toxin subunit A into the cell. Reverse transport of the subunit A from the endosome into the cell cytoplasm is followed by cleavage of the disulfide bond linking the two fragments of peptide A (A1 and A2). Catalytic peptide A1 is generated, leading to the following sequence:

- A1 interacts with 20-kD cytosolic proteins called ADP-ribosylation factors (ARF).
- The ARF-A1 complex catalyzes ADP-ribosylation of a 49-kD G-protein (called $G_{s\alpha}$).[54]
- Binding of NAD and GTP generates an activated $G_{s\alpha}$, which in turn binds to and stimulates adenylate cyclase. ADP-ribosylated $G_{s\alpha}$ is permanently in an active GTP-bound

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state, resulting in persistent activation of adenylate cyclase.

- The activated adenylate cyclase generates high levels of intracellular cAMP from ATP.
- Cyclic AMP stimulates secretion of chloride and bicarbonate, with associated sodium and water secretion. Chloride and sodium resorption are also inhibited.

Figure 17-32 Mechanisms of cholera toxin action.

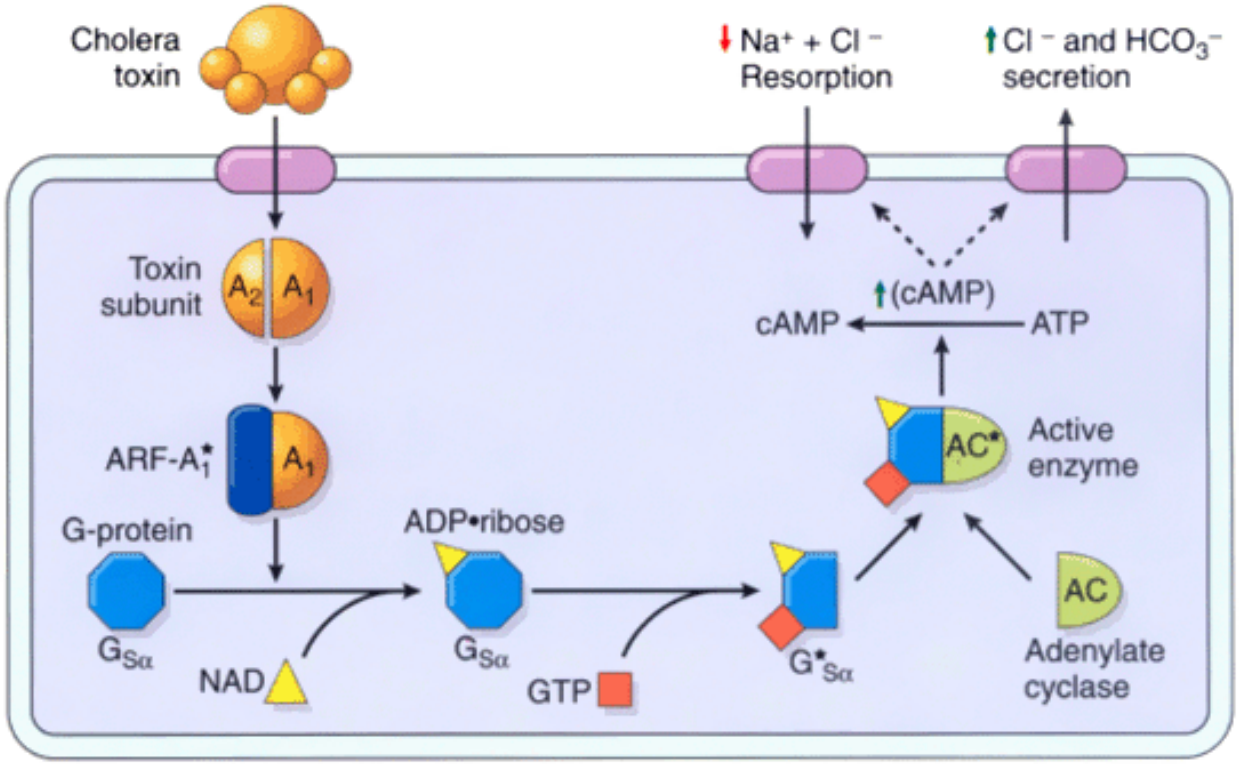


Figure 17-33 Shigella enterocolitis. Segment of colon showing pale, granular, inflamed mucosa with patches of coagulated exudate.

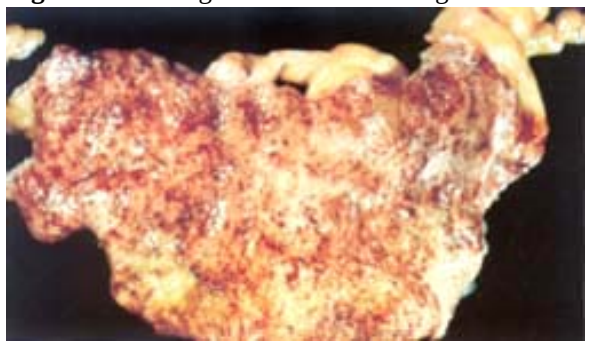


Figure 17-34 Pseudomembranous colitis from *C. difficile* infection. *A,* Gross photograph showing plaques of yellow fibrin and inflammatory debris adherent to a reddened colonic mucosa. *B,* Low-power micrograph showing superficial erosion of the mucosa and an adherent pseudomembrane of fibrin, mucus, and inflammatory debris.

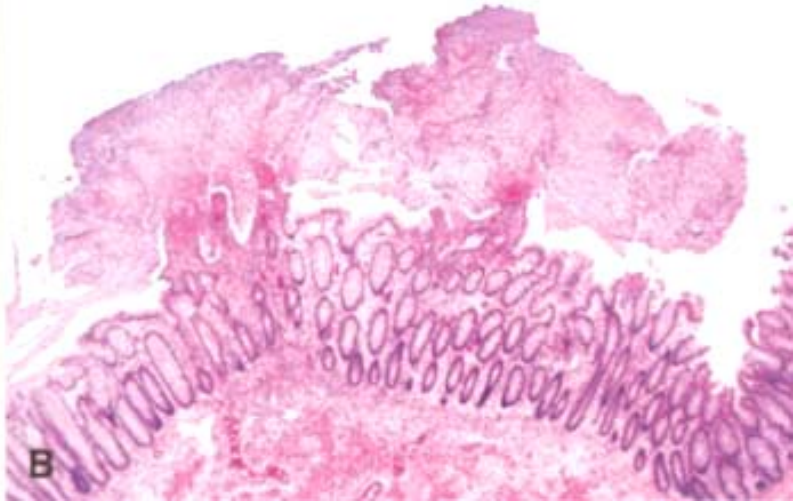


Figure 17-35 *Entamoeba histolytica* in colon. High-power view of the organisms. Note some of the organisms ingesting red blood cells.

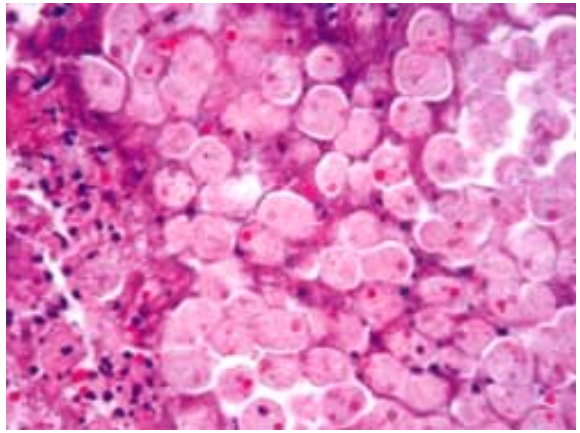


Figure 17-36 *Giardia lamblia.* Trophozoite *(arrow)* of the organism immediately adjacent to the duodenal surface epithelium.

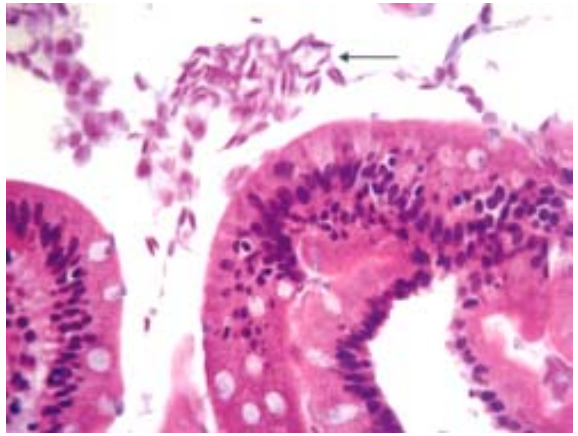


Figure 17-37 Graft-versus-host disease of the colon. Note the apoptotic cell in the crypt *(arrow)*.

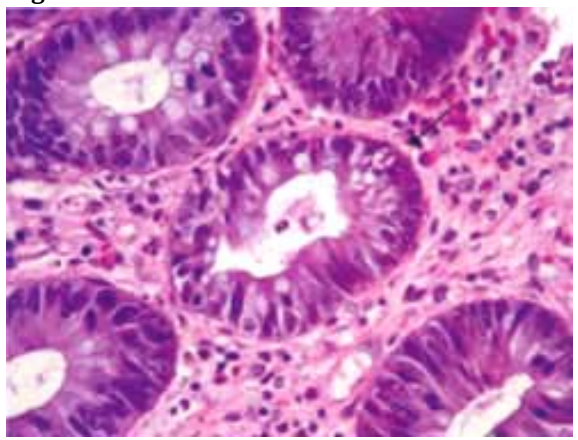


TABLE 17-9 -- Major Malabsorption Syndromes

Defective Intraluminal Digestion
Digestion of fats and proteins
• Pancreatic insufficiency, owing to pancreatitis or cystic fibrosis
• Zollinger-Ellison syndrome, with inactivation of pancreatic enzymes by excess gastric acid secretion
Solubilization of fat, owing to defective bile secretion
• Ileal dysfunction or resection, with decreased bile salt uptake
• Cessation of bile flow from obstruction, hepatic dysfunction
Nutrient preabsorption or modification by bacterial overgrowth
Primary Mucosal Cell Abnormalities
Defective terminal digestion
• Disaccharidase deficiency (lactose intolerance)
• Bacterial overgrowth, with brush border damage
Defective epithelial transport
• Abetalipoproteinemia
• Primary bile acid malabsorption owing to mutations in the ileal bile acid transporter
Reduced Small Intestinal Surface Area
Gluten-sensitive enteropathy (celiac disease)
Crohn disease
Lymphatic Obstruction
Lymphoma
Tuberculosis and tuberculous lymphadenitis
Infection
Acute infectious enteritis
Parasitic infestation
Tropical sprue
Whipple disease (*Tropheryma whippelii*)
Iatrogenic
Subtotal or total gastrectomy
Short-gut syndrome, following extensive surgical resection
Distal ileal resection or bypass

• *Alimentary tract:* diarrhea, both from nutrient malabsorption and excessive intestinal secretions, flatus, abdominal pain, weight loss, and mucositis resulting from vitamin deficiencies
• *Hematopoietic system:* anemia from iron, pyridoxine, folate, and/or vitamin B_{12} deficiency and bleeding from vitamin K deficiency
• *Musculoskeletal system:* osteopenia and tetany from calcium, magnesium, and vitamin D deficiency
• *Endocrine system:* amenorrhea, impotence, and infertility from generalized malnutrition; hyperparathyroidism from protracted calcium and vitamin D deficiency
• *Epidermis:* purpura and petechiae from vitamin K deficiency, edema from protein deficiency, dermatitis and hyperkeratosis from deficiencies of vitamin A, zinc, essential fatty acids and niacin
• *Nervous system:* peripheral neuropathy from vitamin A and B_{12} deficiencies.

The passage of abnormally bulky, frothy, greasy, yellow, or gray stools *(steatorrhea)* is a prominent feature of malabsorption, accompanied by weight loss, anorexia, abdominal distention, borborygmi, and muscle wasting. *The malabsorptive disorders most commonly encountered in the United States are celiac disease, pancreatic insufficiency, and Crohn disease.*

Pancreatic insufficiency, primarily from chronic pancreatitis or cystic fibrosis, is a major cause of *defective intraluminal digestion*. Typical features of defective intraluminal digestion are an osmotic diarrhea from undigested nutrients and steatorrhea. Excessive growth of normal bacteria within the proximal small intestine (*bacterial overgrowth,* discussed earlier) also impairs intraluminal digestion and can damage mucosal epithelial cells, causing impaired terminal digestion and epithelial absorption.

CELIAC DISEASE

Celiac disease (also referred to as celiac sprue, gluten-sensitive enteropathy) is a chronic disease, in which there is a characteristic mucosal lesion of the small intestine and impaired nutrient absorption, which improves on withdrawal of wheat gliadins and related grain proteins from the diet. [63] Celiac disease occurs largely in Caucasians and is rare or nonexistent among native Africans, Japanese, and Chinese. Its prevalence in the United States is somewhat difficult to define; in Europe the prevalence is in the range of 1:100 to 1:200.[64] The disease was first described more than a century ago, but its connection to gluten was not known until the 1940s, changing its clinical management.

Pathogenesis.

The fundamental disorder in celiac disease is a sensitivity to gluten, which is the alcohol-soluble, water-insoluble protein component (gliadin) of wheat and closely related grains (oat, barley, and rye). The hallmark of this disease is a T-cell mediated chronic inflammatory reaction with an autoimmune component, which most likely develops as a consequence of a loss of tolerance to gluten. Interplay between genetic predisposing factors, the host immune response, and environmental factors is central to disease pathogenesis. The small intestinal mucosa, when exposed to gluten, accumulates intraepithelial CD8+ T cells and large numbers of lamina propria CD4+ T cells, which are sensitized to gliadin. The recognized epitopes are confined to residues 57–75 of gliadin. [65]

It has been long known that family history is important in celiac disease. Almost all individuals with celiac disease share the major histocompatibility complex class II HLA-DQ2 or

Figure 17-38 Celiac disease (gluten-sensitive enteropathy). *A,* A peroral jejunal biopsy specimen of diseased mucosa shows diffuse severe atrophy and blunting of villi, with a chronic inflammatory infiltrate of the lamina propria. *B,* A normal mucosal biopsy.

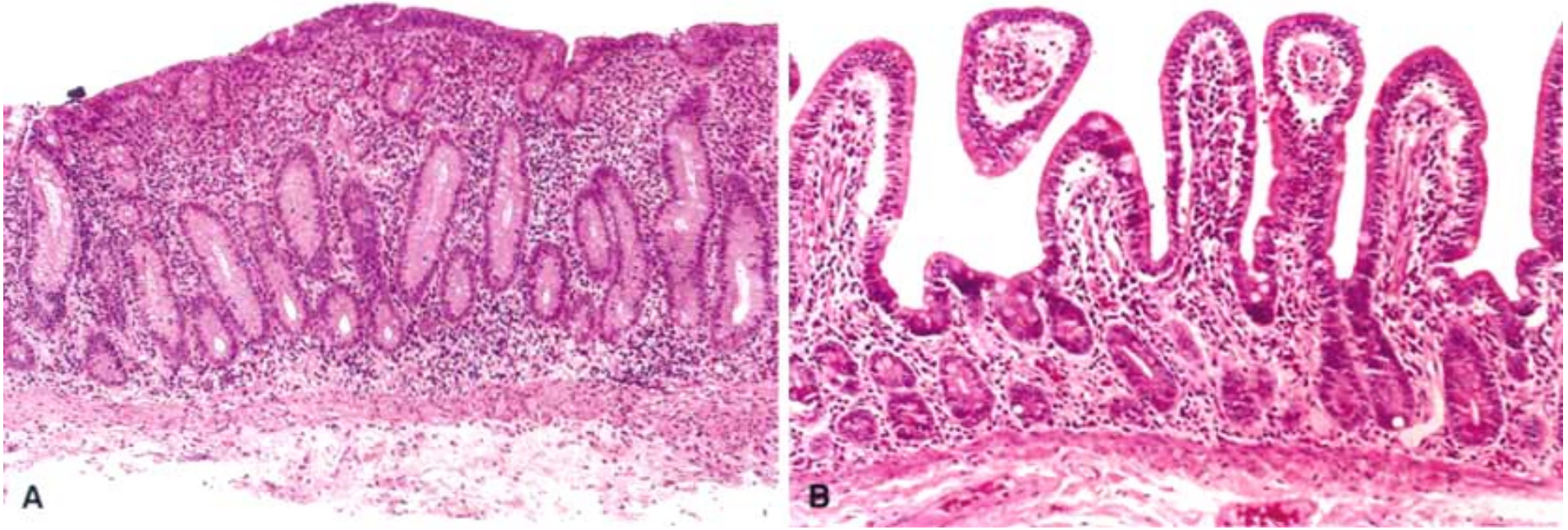
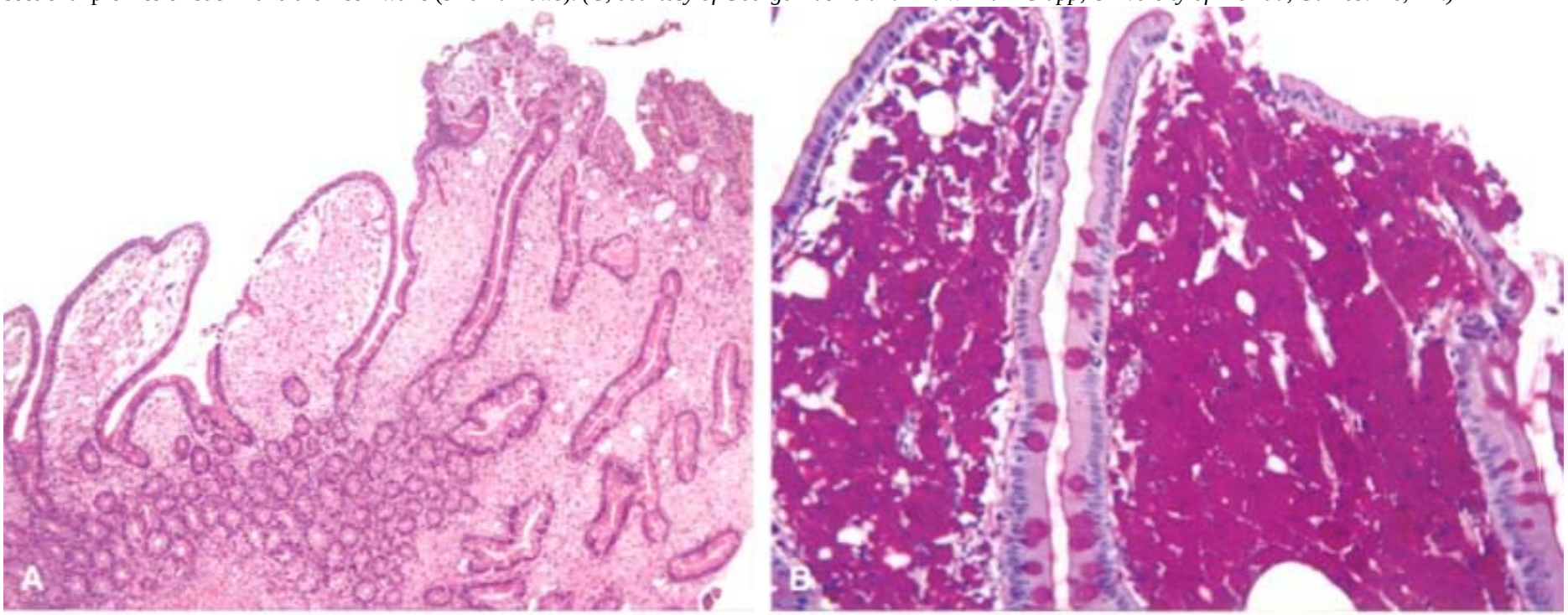


Figure 17-39 Whipple disease. *A,* Note foamy macrophages in the lamina propria. *B,* PAS stain showing the positive granules in the foamy macrophages. *C,* Electron micrograph of a lamina propria macrophage showing many bacilli within the cell (*arrow*) and in the extracellular space (*arrowhead*). *Inset,* Higher magnification of macrophage cytoplasm showing cross-sectional profiles of bacilli and their cell walls (small *arrows*). *(C, courtesy of George Kasnic and Dr. William Clapp, University of Florida, Gainesville, FL.)*



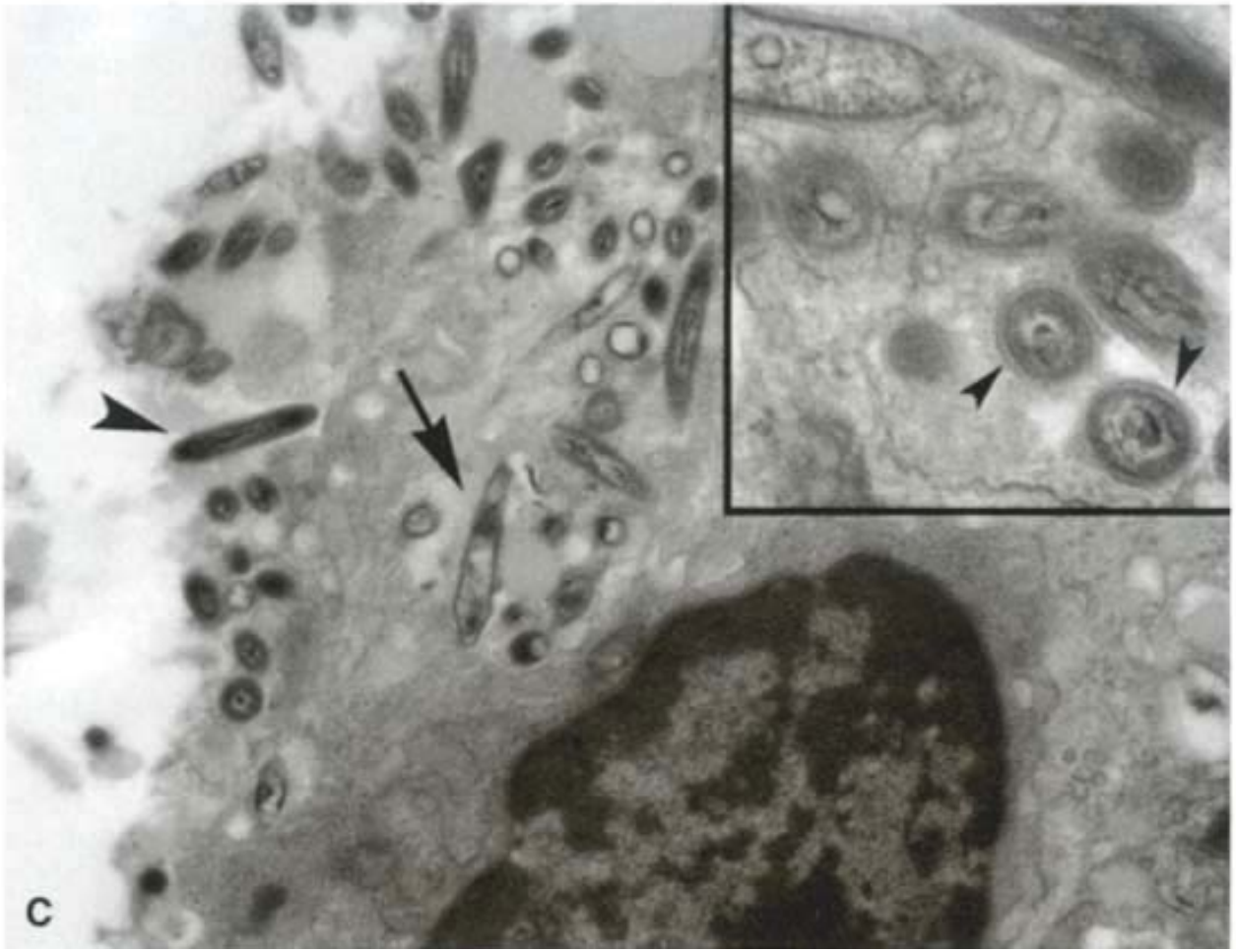


Figure 17-40 Crohn disease of ileum, showing narrowing of the lumen, bowel wall thickening, serosal extension of mesenteric fat ("creeping fat"), and linear ulceration of the mucosal surface *(arrowheads)*.

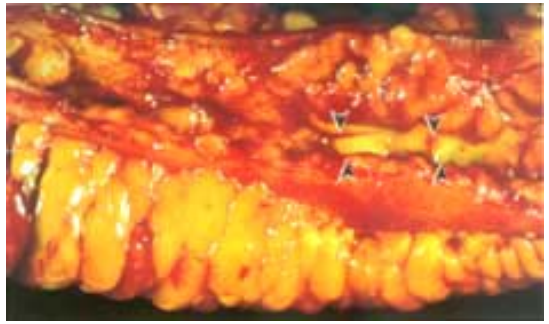


Figure 17-41 Crohn disease of the colon; a deep fissure extending into the muscle wall, a second, shallow ulcer (on the upper right), and relative preservation of the intervening mucosa. Abundant lymphocyte aggregates are present, evident as dense blue patches of cells at the interface between mucosa and submucosa.

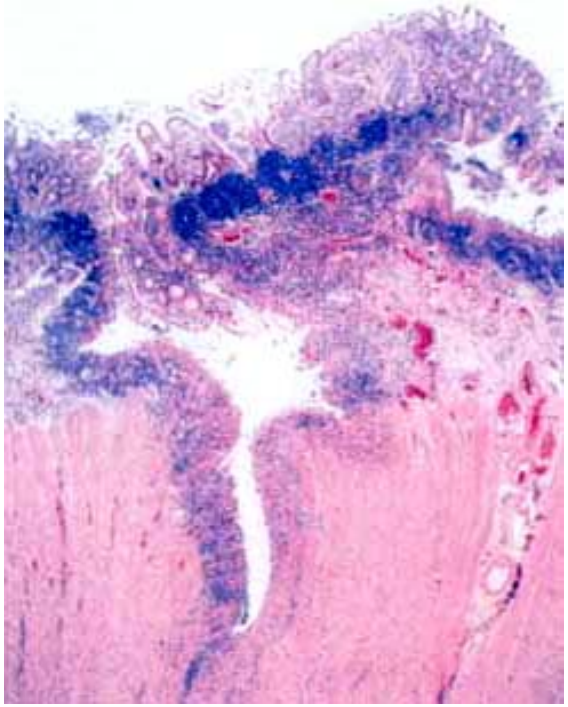


Figure 17-42 Crohn disease of the colon. A noncaseating granuloma is present in the lamina propria of an uninvolved region of colonic mucosa (*arrow*).

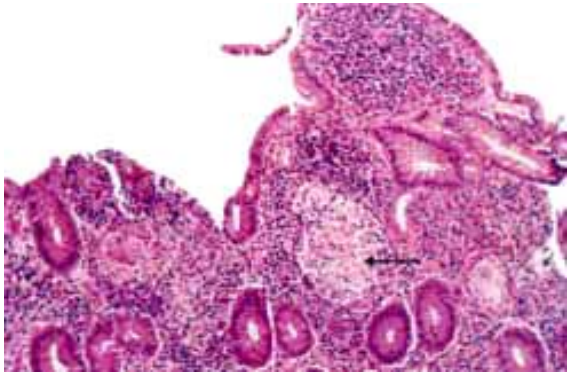


Figure 17-43 Comparison of the distribution patterns of Crohn disease and ulcerative colitis, as well as the different conformations of the ulcers and wall thickenings.

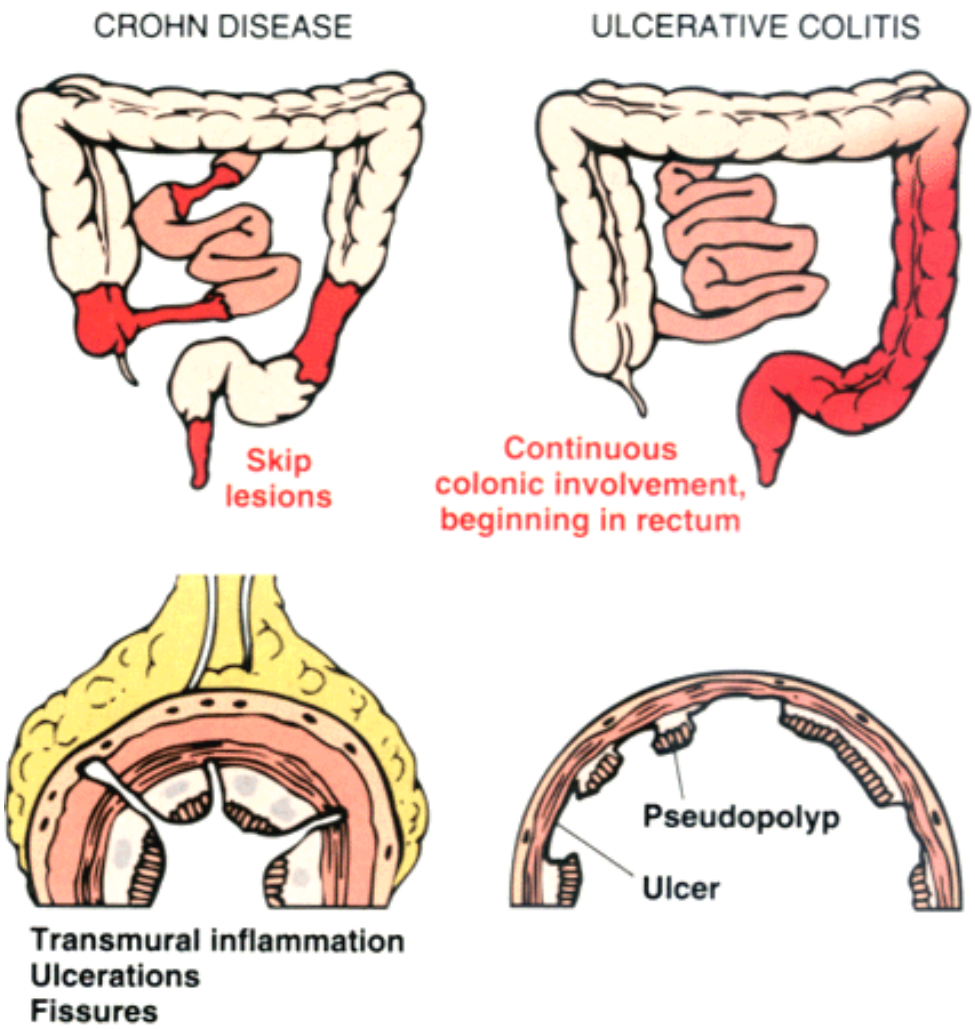


Figure 17-44 Ulcerative colitis. Ulcerated hemorrhagic surface with knobby pseudopolyps. *(Courtesy of Dr. Kim Bechard, Brigham and Women's Hospital, Boston, MA.)*

Figure 17-45 Ulcerative colitis. Low-power micrograph showing marked chronic inflammation of the mucosa with atrophy of colonic glands, moderate submucosal fibrosis, and a normal muscle wall.

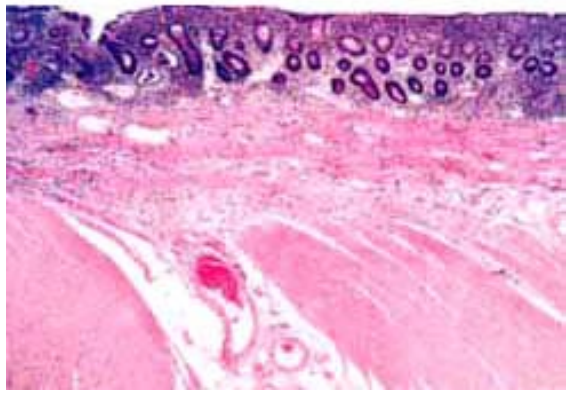


Figure 17-46 Toxic megacolon. Complete cessation of colon neuromuscular activity has led to massive dilatation of the colon and black-green discoloration signifying gangrene and impending rupture.

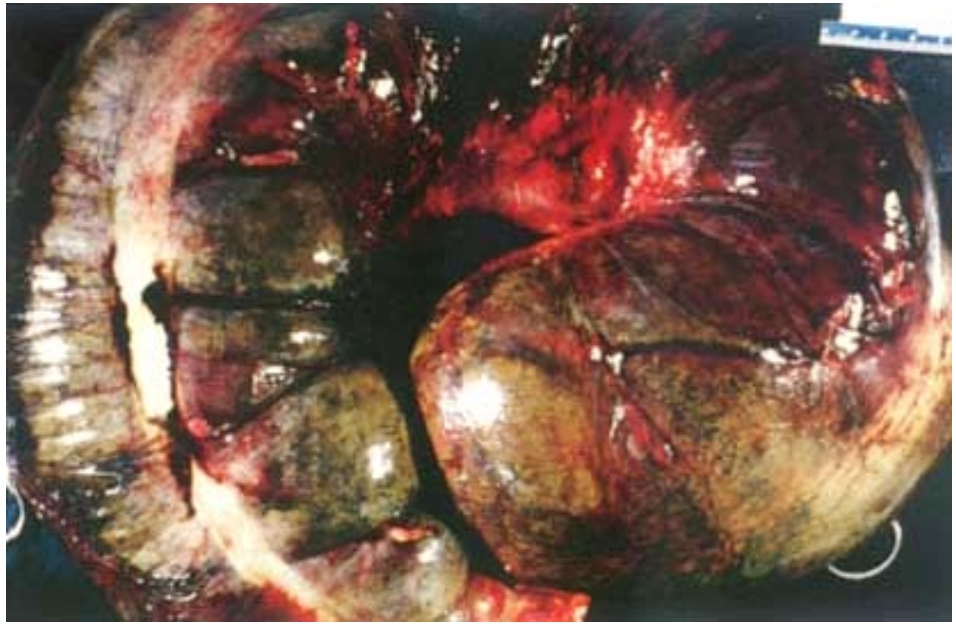


Figure 17-47 Ulcerative colitis. Microscopic view of the mucosa, showing diffuse active inflammation with crypt abscess and glandular architectural distortion.

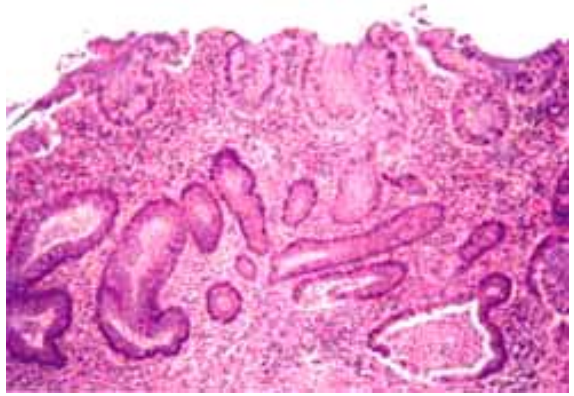


TABLE 17-10 -- Distinctive Features of Crohn Disease and Ulcerative Colitis *

Feature	Crohn Disease - SI	Crohn Disease - C	Ulcerative Colitis
Macroscopic			
Bowel region	Ileum ± colon	Colon ± ileum	Colon only
Distribution	Skip lesions	Skip lesions	Diffuse
Stricture	Early	Variable	Late/rare
Wall appearance	Thickened	Thin	Thin

Dilation	No	Yes	Yes
Microscopic			
Inflammation	Transmural	Transmural	Limited in mucosa
Pseudopolyps	No to slight	Marked	Marked
Ulcers	Deep, linear	Deep, linear	Superficial
Lymphoid reaction	Marked	Marked	Mild
Fibrosis	Marked	Moderate	Mild
Serositis	Marked	Variable	Mild to none
Granulomas	Yes (50%)	Yes (50%)	No
Fistulae/sinuses	Yes	Yes	No
Clinical			
Fat/vitamin malabsorption	Yes	Yes, if ileum	No
Malignant potential	Yes	Yes	Yes
Response to surgery	Poor	Fair	Good

*SI, Crohn disease of the small intestine; C, Crohn disease of the colon. Features are often not all present in a single case.

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the disease runs a fulminant course; unless medically or surgically controlled, this toxic form of the disease can lead to death soon after onset.

The most feared long-term complication of UC is cancer. There is a tendency for dysplasia to arise in multiple sites, and the underlying inflammatory disease may mask the symptoms and signs of carcinoma. UC is characterized by DNA damage with microsatellite instability in mucosal cells. More recently, genomic instability was detected in non-dysplastic areas of patients with UC, suggesting that these patients have DNA repair deficiency and genomic instability throughout the intestinal tract.[77] *The associated carcinomas are often infiltrative without obvious exophytic masses, further underscoring the importance of early diagnosis.* Historically, the risk of cancer is highest in patients with pancolitis of 10 or more years' duration, in whom it is 20- to 30-fold higher than in a control population.[78] However, recent screening programs of patients with UC now indicate that the rate of progression to dysplasia and carcinoma is in fact quite low, provided that initial examinations were negative for dysplasia. Since great cost is involved in mass screening, the debate over the cost-effectiveness of repeated colonoscopies in patients with long-term inactive disease continues; the modest improvement in patient outcome may be related to better patient care, rather than identification of dysplasia per se.

The features of CD and UC are compared in Table 17-10 .

Vascular Disorders

ISCHEMIC BOWEL DISEASE

Ischemic lesions may be restricted to the small or large intestine, or may affect both, depending on the particular vessel(s) affected. Acute occlusion of one of the three major supply trunks

of the intestines—celiac, superior mesenteric, and inferior mesenteric arteries—may lead to infarction of several meters of intestine. However, insidious loss of one

Figure 17-48 Acute ischemic bowel disease. Schematic of the three levels of severity, diagrammed for the small intestine.

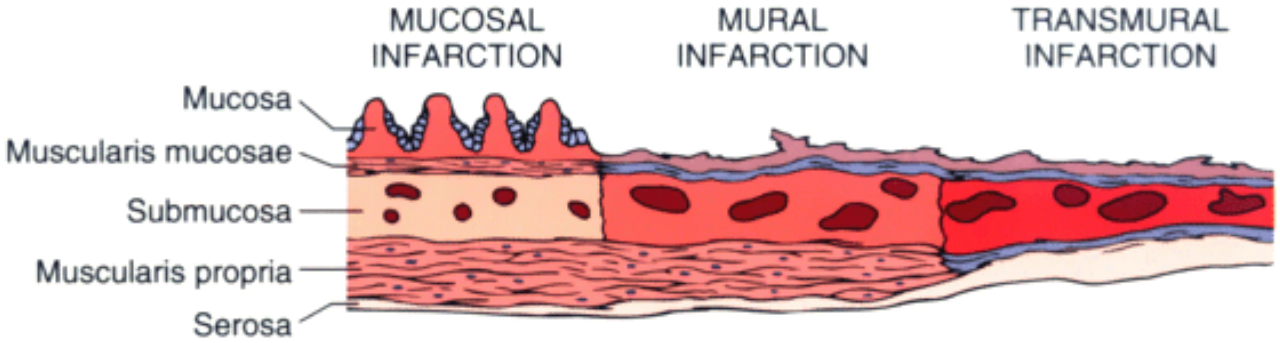

Figure 17-49 Infarcted small bowel, secondary to acute thrombotic occlusion of the superior mesenteric artery.

Figure 17-50 Mucosal infarction of the small bowel. The mucosa is hemorrhagic, and there is no epithelial layer. The remaining layers of the bowel are intact.

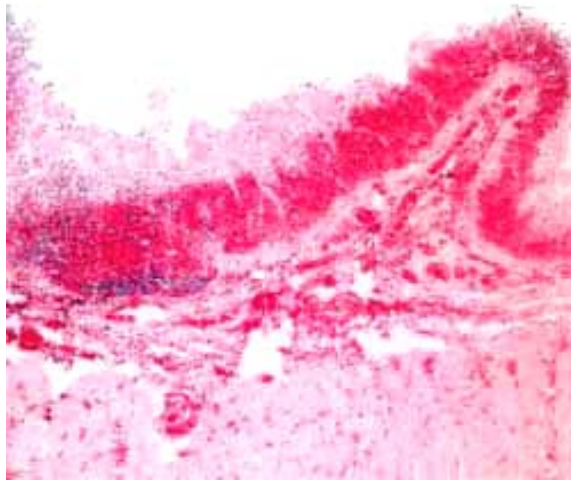

Figure 17-51 Chronic ischemia of the colon, resulting in chronic mucosal damage and a stricture.

Figure 17-52 Diverticulosis. *A,* Section through the sigmoid colon, showing multiple sac-like diverticula protruding through the muscle wall into the mesentery. The muscularis propria in between the diverticular protrusions is markedly thickened. *B,* Low-power photomicrograph of diverticulum of the colon, showing protrusion of mucosa and submucosa through the muscle wall. A dilated blood vessel at the base of the diverticulum was a source of bleeding; some blood clot is present within the diverticular lumen.

TABLE 17-11 -- Major Causes of Intestinal Obstruction

Mechanical Obstruction
Adhesions
Hernias, internal or external
Volvulus
Intussusception
Tumors
Inflammatory strictures
Obstructive gallstones, fecaliths, foreign bodies
Congenital strictures; atresias
Congenital bands
Meconium in mucoviscoidosis
Imperforate anus
Pseudo-obstruction
Paralytic ileus (e.g., postoperative)
Vascular—bowel infarction

Myopathies and neuropathies (e.g., Hirschsprung)

Figure 17-53 Schematic depicting the four major causes of intestinal obstruction: (1) Herniation of a segment in the umbilical or inguinal regions; (2) adhesion between loops of intestine; (3) intussusception; (4) volvulus formation.

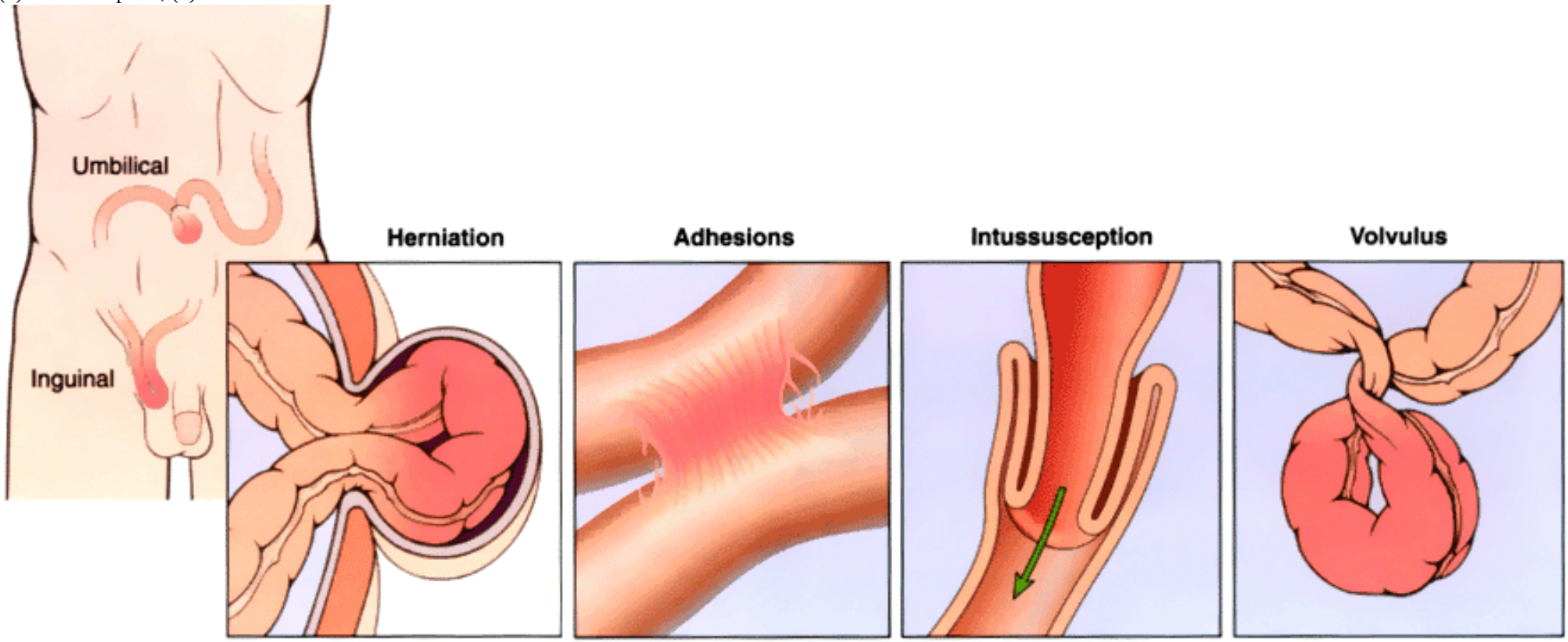

TABLE 17-12 -- Tumors of the Small Intestine and Colon

Non-neoplastic (Benign) Polyps
Hyperplastic polyps
Hamartomatous polyps
• Juvenile polyps
• Peutz-Jeghers polyps

Inflammatory polyps
Lymphoid polyps
Neoplastic Epithelial Lesions
Benign
• Adenoma *
Malignant
• Adenocarcinoma *
• Carcinoid tumor
• Anal zone carcinoma
Mesenchymal Lesions
Gastrointestinal stromal tumor (GIST) (gradation from benign to malignant)
Other benign lesions
• Lipoma
• Neuroma
• Angioma
Kaposi sarcoma
Lymphoma

* Benign and malignant counterparts of the most common neoplasms in the intestines; virtually all lesions are in the colon.

angiomas, and rare hamartomatous mucosal lesions comprise the remainder. One of the enigmas of medicine is the rarity of malignant tumors of the small intestine—annual U.S. death rate is under 1000, representing only about 1% of gastrointestinal malignancies. Small intestinal adenocarcinomas and carcinoids have roughly equal incidence, followed in order by lymphomas and sarcomas. As the latter three exhibit a broader distribution than the small intestine, they are discussed later.

Adenomas

Adenomas account for approximately 25% of benign small intestinal tumors, with benign mesenchymal tumors (especially leiomyomas), lipomas, and neuromatous lesions following in frequency. *Most adenomas occur in the region of the ampulla of Vater.* The usual presentation is that of a 30- to 60-year-old patient with occult blood loss, rarely with obstruction or intussusception; some are discovered incidentally during radiographic investigation. Patients with familial polyposis coli (discussed later) are particularly prone to developing periampullary adenomas. Macroscopically, the ampulla of Vater is enlarged and exhibits a velvety surface (Fig. 17-54). Microscopically, these adenomas resemble their counterparts in the colon (discussed later). Frequently, there is extension of adenomatous tissue into the ampullary orifice, rendering surgical excision difficult, short of a pancreatoduodenectomy to remove the entire ampullary region. Like its counterpart in the colon, the small intestinal adenoma is a premalignant lesion. The adenoma-carcinoma sequence has been demonstrated in small intestinal tumors.

Figure 17-54 Adenoma of the ampulla of Vater, showing exophytic tumor at the ampullary orifice.

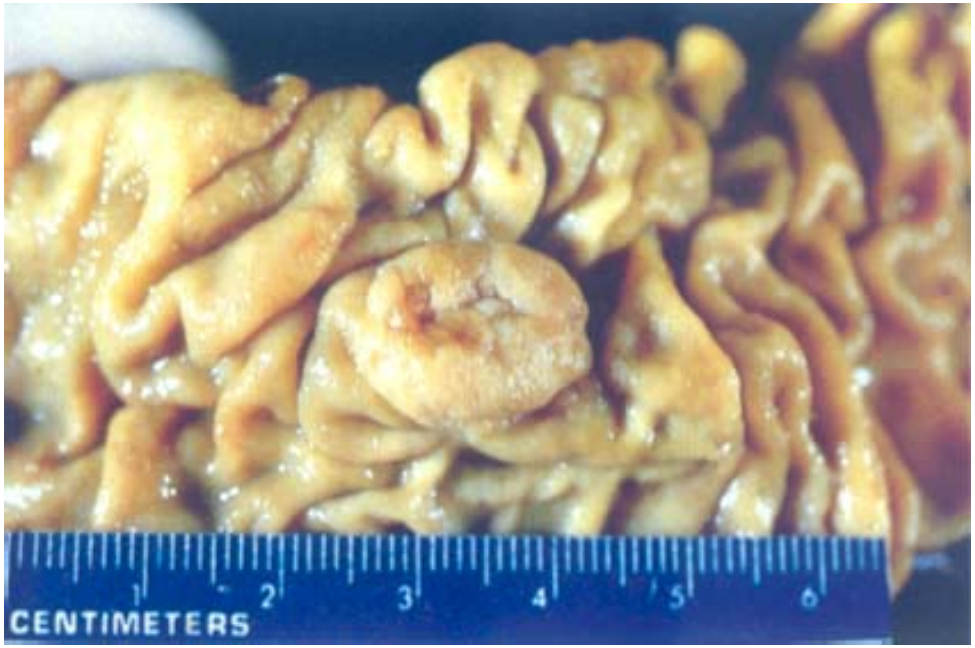

Figure 17-55 Diagrammatic representation of two forms of sessile polyp (hyperplastic polyp and adenoma) and of two types of adenoma (pedunculated and sessile). There is only a loose association between the tubular architecture for pedunculated adenomas and the villous architecture for sessile adenomas.

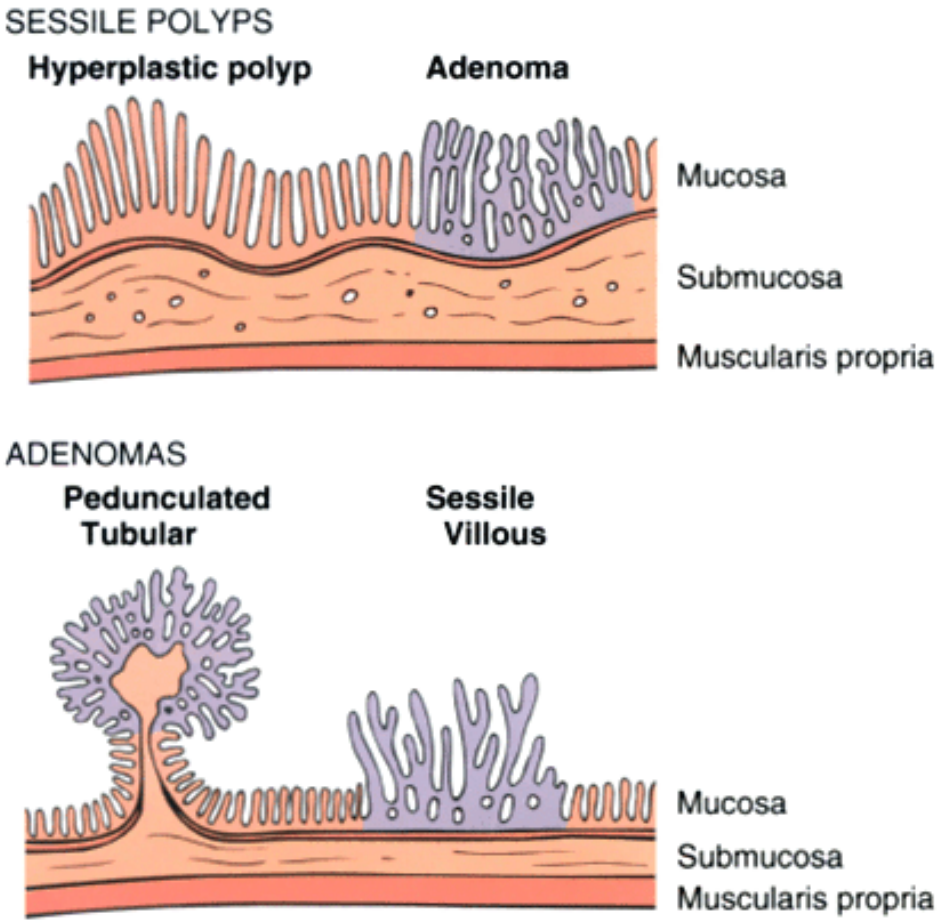

TABLE 17-13 -- Hereditary Syndromes Involving the Gastrointestinal Tract

Syndromes	Altered Gene	Pathology in GI Tract
Familial adenomatous polyposis (FAP)	*APC*	Multiple adenomatous polyps
• Classic FAP		
• Attenuated FAP		
• Gardner syndrome		
• Turcot syndrome		
Peutz-Jeghers syndrome	*STK11*	Hamartomatous polyps
Juvenile polyposis syndrome	*SMAD4*	Juvenile polyps
	BMPRIA	
Hereditary nonpolyposis colorectal carcinoma	Defects in mismatch DNA repair genes	Colon cancer

Tuberous sclerosis	*TSC1*	Inflammatory polyps
	TSC2	
Cowden disease	*PTEN*	Hamartomatous polyps

Non-Neoplastic Polyps

The overwhelming majority of intestinal polyps occur on a sporadic basis, particularly in the colon, and increase in frequency with age. Non-neoplastic polyps include the hyperplastic polyp, the hamartomatous polyp, the inflammatory polyp, and the lymphoid polyp. Hyperplastic polyps represent about 90% of all epithelial polyps in the large intestine. They may arise at any age but usually are discovered incidentally in the sixth and seventh decades. They are found in more than half of all persons age 60 and older. It is believed that the hyperplastic polyp results from decreased epithelial cell turnover and accumulation of mature cells on the surface. Harmatomatous polyps are malformations of the glands and the stroma. They can occur sporadically or occur in the setting of genetic syndromes (Table 17-13). Inflammatory polyps, also known as *pseudopolyps*, represent islands of inflamed regenerating mucosa surrounded by ulceration. These are seen primarily in patients with severe, active IBD. Lymphoid polyps are an essentially normal variant of the mucosal bumps containing intramucosal lymphoid tissue.

Morphology.

Hyperplastic Polyps.

These are small (usually <5 mm in diameter) epithelial polyps that appear as nipple-like, hemispheric, smooth, moist protrusions of the mucosa, usually positioned on the tops of mucosal folds. They may occur singly but more often are multiple, and over half are found in the rectosigmoid colon. Histologically, they are composed of well-formed glands and crypts lined by non-neoplastic epithelial cells, most of which show differentiation into mature goblet or absorptive cells. The delayed shedding of surface epithelial cells leads to infoldings of the crowded epithelial cells and fission of the crypts, creating a serrated epithelial profile and an irregular crypt architecture (Fig. 17-56*A*). Although large hyperplastic polyps may rarely coexist with foci of adenomatous change, **the usual small, hyperplastic polyp is considered to have virtually no malignant potential**. However, the hyperplastic polyps occurring in the setting of the rare hyperplastic polyposis syndrome can harbor epithelial cell dysplasia (adenoma), and hence are considered at risk for carcinoma. The

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underlying genetic basis for this syndrome is not known.

Hamartomatous Polyps.

Juvenile polyps represent focal hamartomatous malformations of the mucosal epithelium and lamina propria. For the most part they are sporadic lesions, with the vast majority occurring in children younger than age 5. Isolated hamartomatous polyps may be identified in the colon of adults; these incidental lesions are referred to as **retention polyps**. In both age groups, nearly 80% of the polyps occur in the rectum, but they may be scattered throughout the colon. Juvenile polyps tend to be large (1 to 3 cm in diameter), rounded, smooth or slightly lobulated lesions with stalks up to 2 cm in length; retention polyps tend to be smaller (<1 cm diameter). Histologically, lamina propria comprises the bulk of the polyp, enclosing abundant cystically dilated glands. Inflammation is common, and the surface may be congested or ulcerated. In general they occur singly and being hamartomatous lesions have no malignant potential. However, the rare autosomal dominant **juvenile polyposis syndrome,** in which there are multiple (50 to 100) juvenile polyps in the gastrointestinal tract, does carry a risk of adenomas and hence adenocarcinoma. Mutations in the *SMAD4/DPC4* gene (which encodes a TGF-β signaling intermediate) account for some cases of juvenile polyposis syndrome.[79]

Peutz-Jeghers polyps are hamartomatous polyps that involve the mucosal epithelium, lamina propria, and muscularis mucosa. These hamartomatous lesions may also occur singly or

multiply in the **Peutz-Jeghers syndrome**. This rare autosomal dominant syndrome is characterized by multiple hamartomatous polyps scattered throughout the entire gastrointestinal tract and melanotic mucosal and cutaneous pigmentation around the lips, oral mucosa, face, genitalia, and palmar surfaces of the hands. Patients with this syndrome are at risk for intussusception, which is a common cause of mortality. Peutz-Jeghers polyps tend to be large and pedunculated with a firm lobulated contour. Histologically, an arborizing network of connective tissue and well-developed smooth muscle extends into the polyp and surrounds normal abundant

Figure 17-56 Non-neoplastic colonic polyps. *A,* Hyperplastic polyp; high-power view showing the serrated profile of the epithelial layer. *B,* Peutz-Jeghers polyp; low-power view showing the splaying of smooth muscle into the superficial portion of the pedunculated polyp.

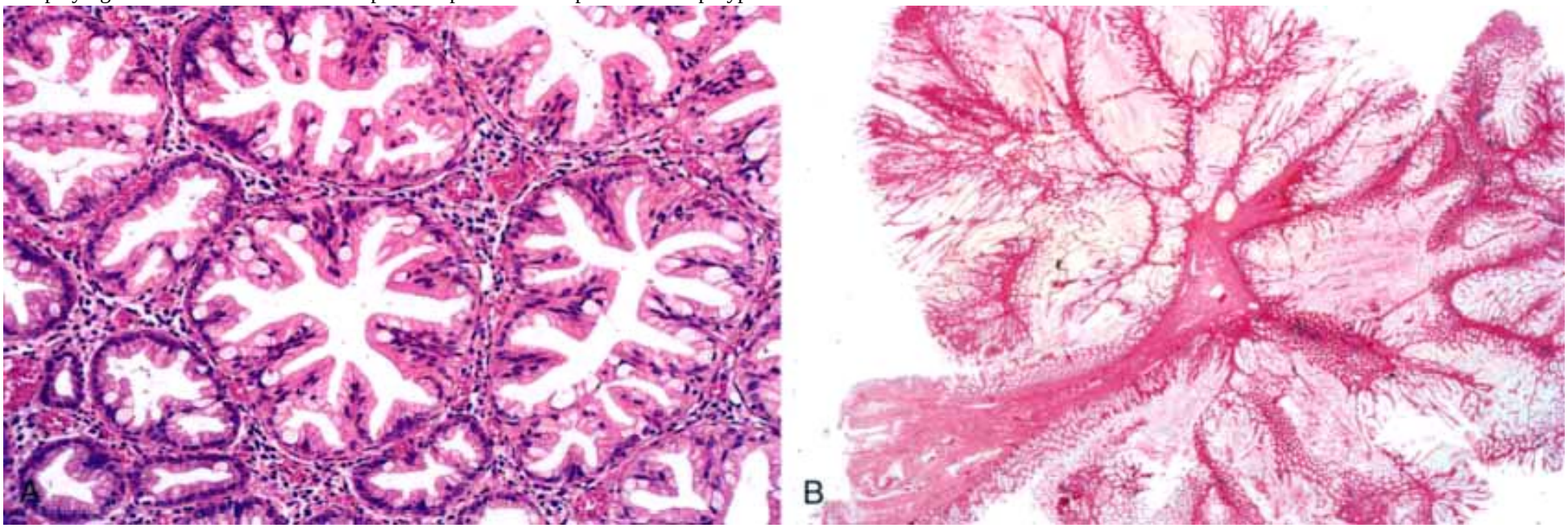


Figure 17-57 *A,* Pedunculated adenoma showing a fibrovascular stalk lined by normal colonic mucosa and a head that contains abundant dysplastic epithelial glands, hence the blue color with the H & E stain. *B,* A small focus of adenomatous epithelium in an otherwise normal (mucin-secreting, clear) colonic mucosa, showing how the dysplastic columnar epithelium (deeply stained) can populate a colonic crypt and create a tubular architecture.

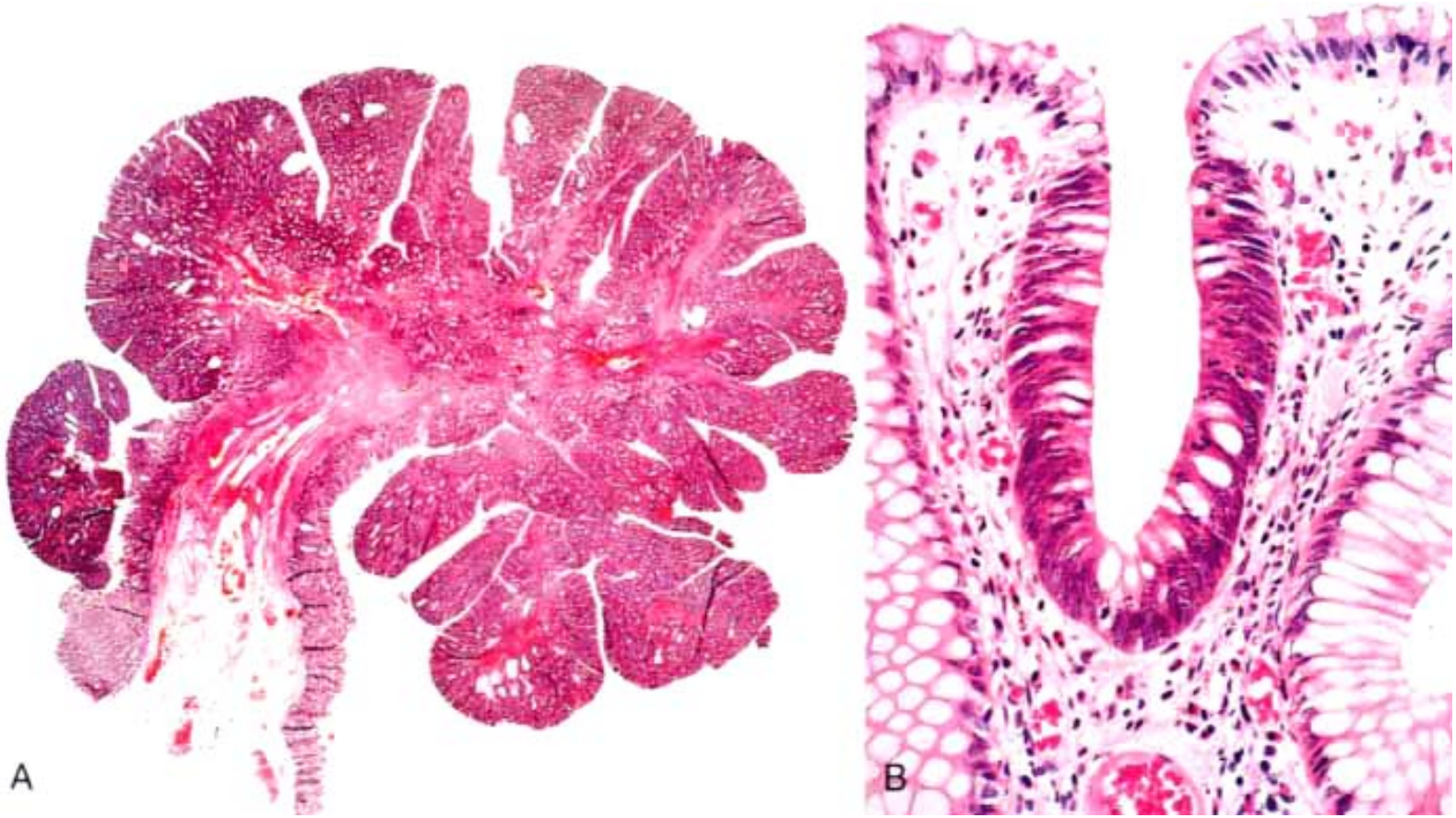


Figure 17-58 *A,* Sessile adenoma with villous architecture. Each frond is lined by dysplastic epithelium. *B,* Portion of a villous frond with dysplastic columnar epithelium on the left and normal colonic columnar epithelium on the right.

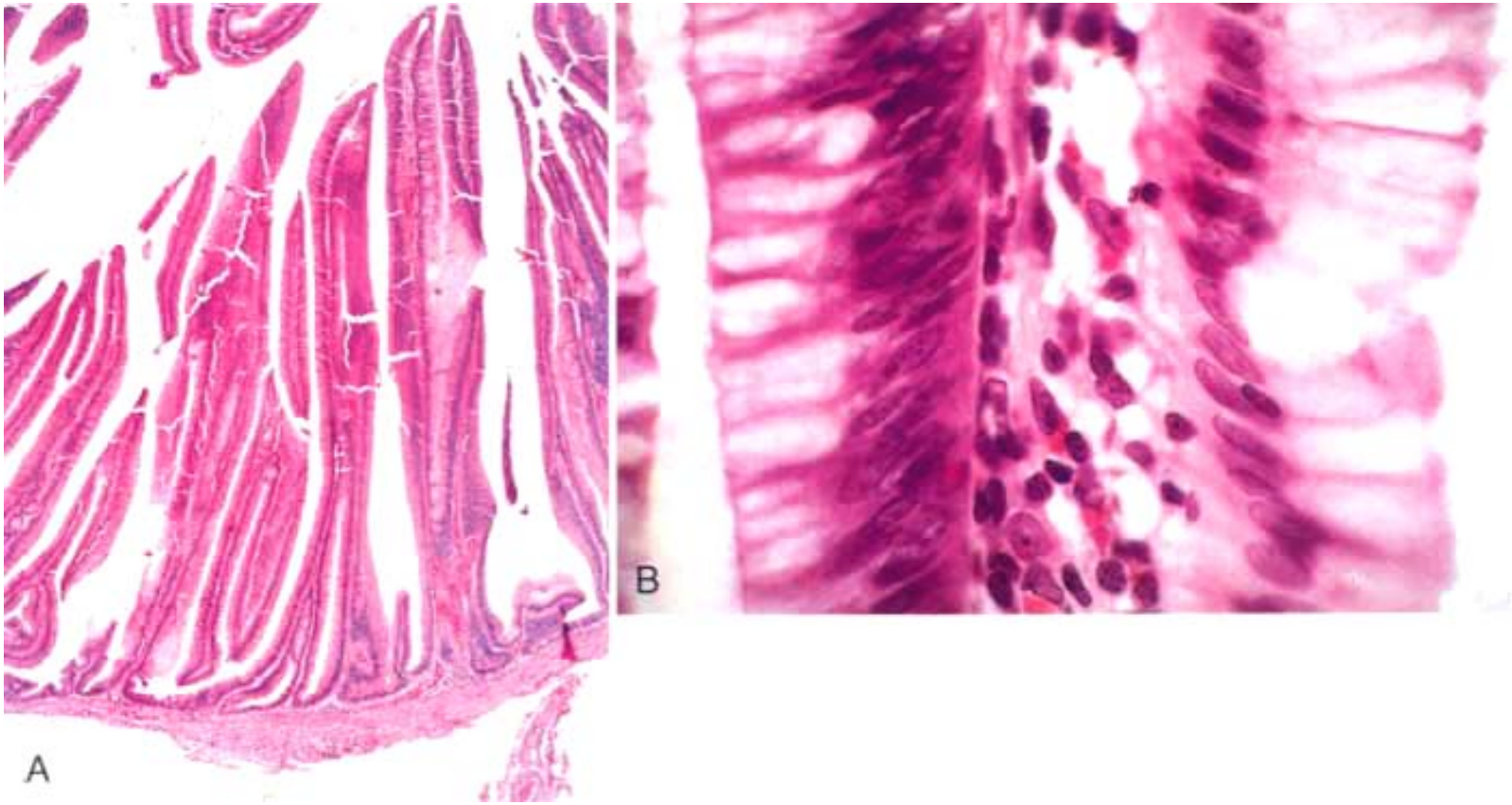


Figure 17-59 Familial adenomatous polyposis in an 18-year-old woman. The mucosal surface is carpeted by innumerable polypoid adenomas.

Figure 17-60 Schematic of the morphologic and molecular changes in the adenoma-carcinoma sequence. It is postulated that loss of one normal copy of the tumor suppressor gatekeeper gene *APC* occurs early. Indeed, individuals may be born with one mutant allele of APC, rendering them extremely likely to develop colon cancer. This is the "first hit," according to Knudson's hypothesis. The loss of the normal copy of the *APC* gene follows ("second hit"). Mutations of the oncogene *K-RAS* seem to occur next. Additional mutations or losses of

heterozygosity inactivate the tumor suppressor gene *p53* (on chromosome 17p) and *SMAD*2 and *SMAD*4 on chromosome 18q, leading finally to the emergence of carcinoma, in which additional mutations occur. It is important to note that while there seems to be a temporal sequence of changes, as shown, the accumulation of mutations, rather than their occurrence in a specific order, is more important.

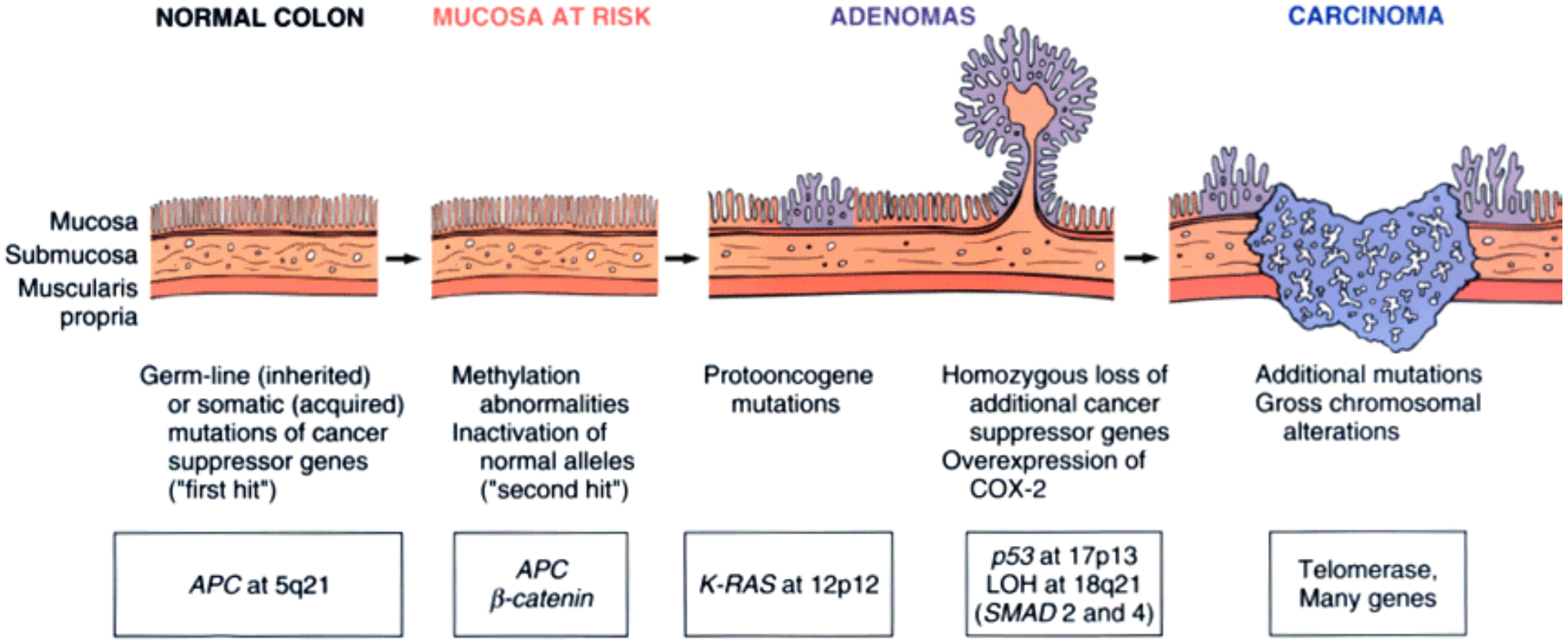


Figure 17-61 Carcinoma of the cecum. The fungating carcinoma projects into the lumen but has not caused obstruction.

Figure 17-62 Carcinoma of the descending colon. This circumferential tumor has heaped-up edges and an ulcerated central portion. The arrows identify separate mucosal polyps.

Figure 17-63 Invasive adenocarcinoma of colon, showing malignant glands infiltrating the muscle wall.

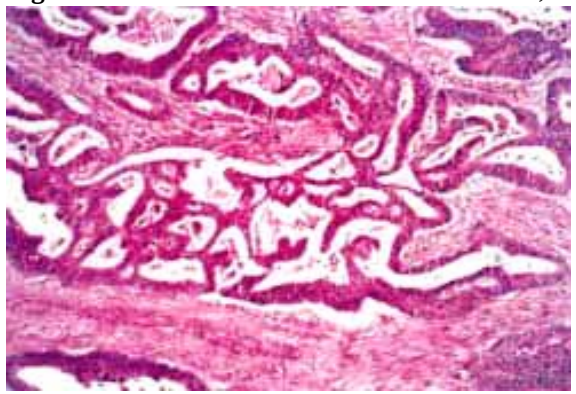


TABLE 17-14 -- TNM Classification of Carcinoma of the Colon and Rectum

Tumor Stage	Histologic Features of the Neoplasm
Tis	Carcinoma in situ (high-grade dysplasia) or intramucosal carcinoma (lamina propria invasion)
T1	Tumor invades submucosa
T2	Extending into the muscularis propria but not penetrating through it
T3	Penetrating through the muscularis propria into subserosa

T4	Tumor directly invades other organs or structures
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1 to 3 lymph nodes
N2	Metastasis in 4 or more lymph nodes
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Figure 17-64 Pathologic staging of colorectal cancer. Staging is based on the depth of tumor invasion.

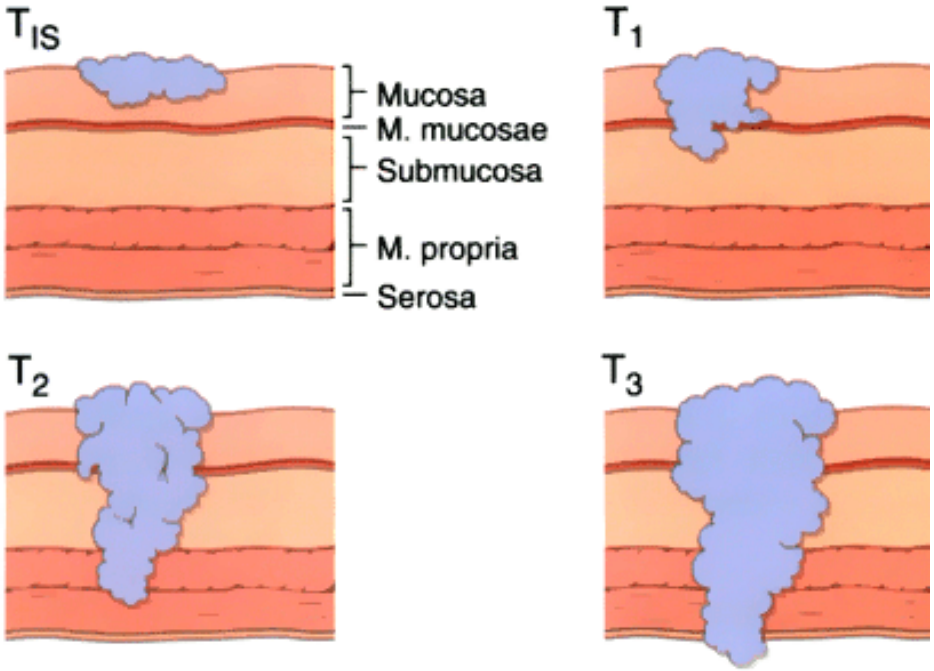
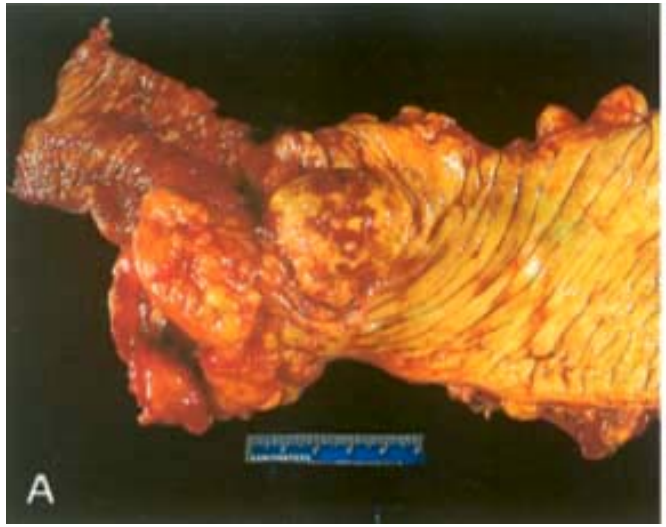
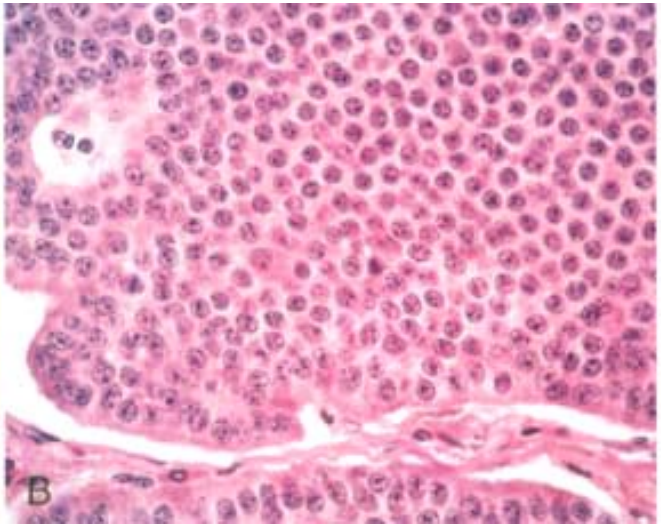


Figure 17-65 Carcinoid tumor. *A,* Multiple protruding tumors are present at the ileocecal junction. *B,* The tumor cells exhibit a monotonous morphology, with a delicate intervening fibrovascular stroma. *C,* Electron micrograph showing dense core bodies in the cytoplasm.





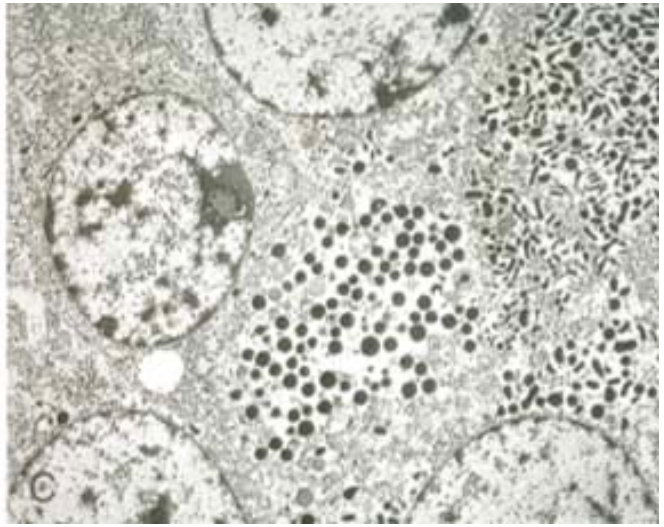


TABLE 17-15 -- Clinical Features of the Carcinoid Syndrome

• Vasomotor distubances
••Cutaneous flushes and apparent cyanosis (most patients)
• Intestinal hypermotility
••Diarrhea, Cramps, nausea, vomiting (most patients)
• Asthmatic bronchoconstrictive attacks
••Couth, wheezing, dyspnea (about one third of patients)
• Hepatomegaly
••Nodular liver owing to hepatic metastases (some patients)
• Systemic fibrosis (some patients)
••Cardiac involvement
••••Pulmonic and tricuspid valve thickening and stenosis
••••Endocardial fibrosis, principally in the right ventricle
••••(Bronchial carcinoids affect the left side)
••Retroperitoneal and pelvic fibrosis
••Collagenous pleural and intimal aortic plaques

metastases are usually not required for the production of a carcinoid syndrome by extraintestinal carcinoids (such as those arising in the lungs or ovaries), because active substances produced by the tumors are directly released into the systemic circulation. Other secretory products of carcinoids such as histamine, bradykinin, kallikrein, and prostaglandins may also contribute to the manifestations of the carcinoid syndrome.

The overall five-year survival rate for carcinoids (excluding appendiceal) is approximately 90%. Even with small-bowel tumors with hepatic metastases, it is better than 50%. However, widespread disease will usually cause death.

GASTROINTESTINAL LYMPHOMA

Any segment of the gastrointestinal tract may be secondarily involved by systemic dissemination of non-Hodgkin lymphomas. However, up to 40% of lymphomas arise in sites other than lymph nodes, and the gut is the most common location. Conversely, about 1% to 4% of all gastrointestinal malignancies are lymphomas. *By definition, primary gastrointestinal lymphomas exhibit no evidence of liver, spleen, mediastinal lymph node, or bone marrow involvement at the time of diagnosis*—regional lymph node involvement may be present. *Primary gastrointestinal lymphomas usually arise as sporadic neoplasms but also occur more frequently in certain patient populations: (1) Chronic gastritis caused by H. pylori, (2) chronic spruelike syndromes, (3) natives of the Mediterranean region, (4) congenital immunodeficiency states, (5) infection with human immunodeficiency virus, and (6) following organ transplantation with immunosuppression.*

Intestinal tract lymphomas can be classified into B-cell and T-cell lymphomas. The B-cell lymphoma can be subdivided into MALT lymphoma, immunoproliferative small-intestinal disease (IPSID), and Burkitt lymphoma.

1. *MALT lymphoma is a sporadic lymphoma, which* arises from the B cells of MALT (mucosa-associated lymphoid tissue, described under gastric lymphoma). *This type of lymphoma is the most common form in the Western hemisphere.* The biologic features of these lymphomas are different from node-based lymphomas in that (1) many behave as focal tumors in their early stages and are amenable to surgical resection; (2) relapse may occur exclusively in the gastrointestinal tract; (3) genotypic changes are different than those observed in nodal lymphomas: the t(11;18) translocation is relatively common in MALT lymphoma; and (4) the cells are usually CD5- and CD10-negative. This type of gastrointestinal lymphoma usually affects adults, has no gender predilection, and may arise anywhere in the gut: stomach (55% to 60% of cases); small intestine (25% to 30%), proximal colon (10% to 15%), and distal colon (up to 10%). The appendix and esophagus are only rarely involved.
   The pathogenesis of these lymphomas is under intense scrutiny. The concept has been advanced that lymphomas of MALT origin arise in the setting of mucosal lymphoid activation and that these lymphomas are the malignant counterparts of hypermutated, postgerminal-center memory B cells. As discussed earlier, *Helicobacter*-associated chronic gastritis, in particular, has been proposed as a driving force for the development of gastric MALT lymphoma, the result of antigen-driven somatic mutation of

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   gastric lymphoid tissue. However, the etiologic factors for intestinal lymphoma are still unknown, although history of IBD appears to increase the risk.
2. *IPSID is also referred to as Mediterranean lymphoma.* It is an unusual intestinal B-cell lymphoma arising in patients with Mediterranean ancestry, having a background of chronic diffuse mucosal plasmacytosis. The plasma cells synthesize an abnormal Igα heavy chain, in which the variable portion has been deleted. A high proportion of patients have malabsorption and weight loss preceding the development of the lymphoma. The diagnosis is made most commonly in children and young adults, and both sexes appear to be affected equally. The exact etiology of this type of lymphoma is not known, although infection appears to play a role.[95]
3. *The intestinal T-cell lymphoma* is usually associated with a long-standing malabsorption syndrome (such as celiac disease) that may not constitute a true gluten-sensitive enteropathy. This lymphoma occurs in relatively young individuals (age 30 to 40), often following a 10- to 20-year history of symptomatic malabsorption. Alternatively, a diffuse enteropathy with malabsorption may accompany the development of a lymphoma. Intestinal T-cell lymphoma arises most often in the proximal small bowel, and its overall prognosis is poor (reported 11% five-year survival rate).

Morphology.

Gastrointestinal lymphomas can assume a variety of gross appearances. Since all the gut lymphoid tissue is mucosal and submucosal, early lesions appear as plaque-like expansions of the mucosa and submucosa. Diffusely infiltrating lesions may produce full-thickness mural thickening, with effacement of the overlying mucosal folds and focal ulceration. Others may be polypoid, protruding into the lumen, or form large, fungating, ulcerated masses. Tumor infiltration into the muscularis propria splays the muscle fibers, gradually destroying them. Because of this feature, advanced lesions frequently cause motility problems with secondary obstruction. Large tumors sometimes perforate because of lack of stromal support; reduction in tumor

bulk during chemotherapy also may lead to perforation.

In the earliest histologic lesions, atypical lymphoid cells may be seen infiltrating the mucosa, with effacement and loss of glands and massive expansion of lymphoid tissue. Extreme numbers of atypical lymphoid cells may populate the superficial or glandular epithelium (lymphoepithelial lesion). With established lymphomas, the mucosa, submucosa, and even muscle wall are replaced by a monotonous infiltrate of malignant cells, consisting of a mixture of small lymphocytes and immunoblasts in varying proportions. Lymphoid follicles are occasionally formed. Most gut lymphomas are of B-cell type (over 95%) and are evenly split between low- and high-grade tumors. The small fraction of T-cell lymphomas occurring in the intestine are commonly high-grade lesions.

Clinical Features.

With the exception of T-cell lymphomas, primary gastrointestinal lymphomas generally have a better prognosis than do those arising in other sites. Ten-year survival for patients with localized mucosal or submucosal disease approaches 85%. Early discovery is key to survival; thus, gastric lymphomas generally have a better outcome than those of the small or large bowel. In general, the depth of local invasion, size of the tumor, the histologic grade of the tumor, and extension into adjacent viscera are important determinants of prognosis.

MESENCHYMAL TUMORS

Mesenchymal tumors may occur anywhere in the alimentary tract. The nomenclature for these tumors is largely based on the tumor cell phenotypes. Lipomas show a propensity for the submucosa of the small and large intestines, and lipomatous hypertrophy may occur in the ileocecal valve. A variety of spindle-cell lesions may arise in the muscle wall of any gut segment. The great majority of these tumors are of smooth muscle origin, and hence can be termed *leiomyomas* and *leiomyosarcomas*. Gastrointestinal stromal tumors (GISTs), are now considered to be a distinctive tumor type, characterized by c-KIT immunoreactivity, as discussed earlier (see "Gastric Tumors"). The small intestine is the second most common location for this tumor, (the stomach being the most common). Both benign and malignant versions of GIST may occur at any age and in either sex. Vascular tumors such as *Kaposi sarcomas* are considered elsewhere (see Chapter 11).

Morphology.

Lipomas are usually well-demarcated, firm nodules (almost always less than 4 cm in diameter) arising within the submucosa or muscularis propria. The overlying mucosa is stretched and attenuated. Rarely, they grow to larger size and produce hemispheric elevation of the mucosa with ulceration over the dome of the tumor. Malignant stromal tumors (primarily leiomyosarcoma) tend to produce large, bulky, intramural masses that eventually fungate and ulcerate into the lumen or project subserosally into the abdominal space. Histologically, lipomas, leiomyomas, and leiomyosarcomas resemble their counterparts encountered elsewhere (Chapter 26). In the case of the stromal tumors (e.g., leiomyomas and leiomyosarcomas), large size and a high mitotic rate are correlated with an aggressive course.

Clinical Features.

Most mesenchymal tumors are asymptomatic. In the stomach, larger lesions (benign or malignant) may produce symptoms resembling those of peptic ulcer, particularly bleeding that is sometimes massive. Intestinal lesions may present with bleeding, and for the small intestine, rare obstruction or intussusception. Benign lesions are easily resectable. Surgical removal is usually possible for the malignant lesions as well, since they tend to grow as cohesive masses. Five-year survival rate for leiomyosarcoma, for example, is 50% to 60%. Metastases, however, are present in about one third of cases.

TUMORS OF THE ANAL CANAL

The anal canal is the terminal portion of the large intestine. It is divided into three zones: the upper (covered with rectal mucosa), the middle (partially covered with a transitional mucosa), and the lower (covered by stratified squamous mucosa). The tumors located in this anatomic location are designated as carcinoma of the anal canal. Patterns of differentiation

include a basaloid pattern, squamous cell carcinoma, and adenocarcinoma.

Anal canal carcinoma with basaloid differentiation is a tumor populated by immature proliferative cells derived from the basal layer of a stratified squamous epithelium. These tumors may occur sporadically and be uniform in their histologic features. Alternatively, basaloid differentiation may be a component of a tumor that exhibits more genuine squamous cell differentiation and/or the mucin vacuole-containing features of adenocarcinoma. All such tumors remain classified as anal canal carcinoma.

Pure squamous cell carcinomas of the anal canal are closely associated with chronic HPV infection.[96] Some rare cases are also related to immunosuppression, as encountered in renal transplantation and in AIDS patients. As with the genital tract, chronic HPV infection of the anal canal often causes precursor lesions such as condyloma acuminatum, squamous epithelium dysplasia, and carcinoma in situ.

Pure adenocarcinoma of the anal canal is often the extension of rectal adenocarcinoma. Rarely, other tumors may arise from the anal canal, notably *Paget disease,* small-cell carcinoma, and melanoma.

Appendix

Normal

The appendix is an underdeveloped residuum of the otherwise voluminous cecum. The adult appendix averages 6 to 7 cm in length, is partially anchored by a mesenteric extension from the adjacent ileum, and has no known function. The appendix has the same four layers as the remainder of the gut and possesses a colonic-type mucosa. A distinguishing feature of this organ is the extremely rich lymphoid tissue of the mucosa and submucosa, which in young individuals forms an entire layer of germinal follicles and lymphoid pulp. This lymphoid tissue undergoes progressive atrophy during life to the point of complete disappearance in advanced age. In the elderly the appendix, particularly the distal portion, sometimes undergoes fibrous obliteration.

Pathology

Diseases of the appendix loom large in surgical practice; appendicitis is the most common acute abdominal condition the surgeon is called on to treat. Appendicitis is one of the best-known medical entities and yet may be one of the most difficult diagnostic problems to confront the emergency physician. A differential diagnosis must include virtually every acute process that can occur within the abdominal cavity, as well as some emergent conditions affecting organs of the thorax.

Acute Appendicitis

Inflammation in the right lower quadrant was considered a nonsurgical disease of the cecum (typhlitis or perityphlitis) until Fitz recognized acute appendicitis as a distinct entity in 1886. Appendiceal inflammation is associated with obstruction in 50% to 80% of cases, usually in the form of a fecalith and, less commonly, a gallstone, tumor, or ball of worms *(oxyuriasis vermicularis).* Continued secretion of mucinous fluid in the obstructed viscus presumably leads to a progressive increase in intraluminal pressure sufficient to cause eventual collapse of the draining veins. Ischemic injury then favors bacterial proliferation with additional inflammatory edema and exudation, further embarrassing the blood supply. Nevertheless, a significant minority of inflamed appendices have no demonstrable luminal obstruction, and the pathogenesis of the inflammation remains unknown.

Morphology.

At the earliest stages, only a scant neutrophilic exudate may be found throughout the mucosa, submucosa, and muscularis propria. Subserosal vessels are congested, and often there is a modest perivascular neutrophilic infiltrate. The inflammatory reaction transforms the normal glistening serosa into a dull, granular, red membrane; this transformation signifies **early acute appendicitis** for the operating surgeon. At a later stage, a prominent neutrophilic exudate generates a fibrinopurulent reaction over the serosa (Fig. 17-66). As the inflammatory process worsens, there is abscess formation within the wall, along with ulcerations and foci of suppurative necrosis in the mucosa. This state constitutes **acute suppurative appendicitis**. Further appendiceal compromise leads to large areas of hemorrhagic green ulceration of the mucosa and green-black gangrenous necrosis through the wall, extending to the serosa, creating **acute gangrenous appendicitis,** which is quickly followed by rupture and suppurative peritonitis.

The histologic criterion for the diagnosis of acute appendicitis is neutrophilic infiltration of the muscularis propria. Usually, neutrophils and ulcerations are also present within the mucosa. Since drainage of an exudate into the appendix from alimentary tract infection may also induce a mucosal neutrophilic infiltrate, evidence of muscular wall inflammation is requisite for the diagnosis.

Figure 17-66 Acute appendicitis. The inflamed appendix shown below is red, swollen, and covered with a fibrinous exudate. For comparison, a normal appendix is shown above.

Figure 17-67 Mucinous cystadenocarcinoma of the appendix, with spread into the immediate periappendiceal tissues.

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Chapter 18 - Liver and Biliary Tract

James M. Crawford MD, PhD

The Liver

Normal

The liver and biliary tree and the gallbladder occupy the right upper quadrant of the abdomen. The liver resides between the digestive tract and the rest of the body and functions as a way station between the splanchnic and systemic circulation. As the headwater of the biliary tree, the liver sits astride the enterohepatic circulation. The liver has the critical job of maintaining the body's metabolic homeostasis. This includes the processing of dietary amino acids, carbohydrates, lipids, and vitamins; removal of microbes and toxins in splanchnic blood en route to the systemic circulation; synthesis of many plasma proteins; and detoxification and excretion into bile of endogenous waste products and pollutant xenobiotics. Hepatic disorders, therefore, have far-reaching consequences.

The mature liver lies in the right hypochondrium under the rib cage and extends from the right fifth intercostal space at the midclavicular line to just below the costal margin. It projects slightly below the costal margin at the right intercostal line and under the xyphoid process in the midline. The conventional division of the liver into the right, left, caudate, and quadrate lobes is a topographic classification that does not correspond to the functional lobes or segments of the liver. The physiologic or functional right and left lobes are defined by the distribution of the right and left portal vein systems. The watershed between these two vascular beds corresponds to a plane that passes superiorly through the left side of the sulcus of the inferior vena cava to the middle of the gallbladder fossa inferiorly. The quadrate lobe and the greater part of the caudate lobe on the posterior aspect of the liver belong functionally to the left hemiliver. Of greater significance to the surgeon is the functional organization of the liver into eight segments, numbered I to VIII, the caudate lobe being segment I and the remainder, II to VIII, moving roughly from left to right across the liver. Each segment has its own independent vascular and biliary pedicle and venous drainage. *This anatomic arrangement facilitates limited segmental resections of the liver* as is sometimes performed for partial hepatectomy.

The normal adult liver weighs 1400 to 1600 gm, representing 2.5% of body weight. Incoming blood—approximately 25% of total cardiac output—arrives via the portal vein (60% to 70% of hepatic blood flow) and the hepatic artery (30% to 40%) through the hilum, the "gateway" of the liver (*porta hepatis*). The major bile ducts exit in this same region. The initial right and left branches of the portal vein, hepatic artery, and bile duct lie just outside the liver. The remaining branches travel in parallel within the liver in *portal tracts*, ramifying variably through 17 to 20 orders of branches. The vast expanse of hepatic parenchyma is serviced via approximately 450,000 terminal branches of the portal tract system. Portal vein blood enters the parenchyma via penetrating *septal venules*; hepatic arteriolar twigs supply the parenchyma, the major bile ducts, the vasa vasorum of the major portal veins and hepatic veins, and the hepatic capsule. Blood from all sources is collected into ramifications of the hepatic vein, which exits by the "back door" of the liver into the closely apposed inferior vena cava.

Microarchitecture.

Classically, the liver has been divided into 1- to 2-mm diameter hexagonal *lobules* oriented around the terminal tributaries of the hepatic vein (*terminal hepatic veins*), with portal tracts at the periphery of the lobule. Accordingly, the hepatocytes in the vicinity of the terminal hepatic vein are called "centrilobular" (or centrolobular); those near the portal tract are "periportal." However, since hepatocytes near the terminal hepatic veins are most remote from the blood supply, it has been argued that they are at the distal apices of roughly triangular *acini*, with the bases formed by penetrating septal venules from the portal vein extending out from the portal tracts (Fig. 18-1). [1] In the "acinus," the parenchyma is divided into three zones, zone 1 being closest to the vascular supply, zone 3 abutting the terminal hepatic venule, and zone 2 being intermediate. This zonation is of considerable metabolic consequence, since a lobular gradient of activity exists for many hepatic enzymes.[2] Moreover, many forms of hepatic injury exhibit a zonal distribution. While acinar architecture is of greater physiologic significance, the anatomic terminology of the liver remains anchored in the older lobular terminology.

The hepatic parenchyma is organized into cribiform, anastomosing sheets or "plates" of hepatocytes, seen in microscopic sections as cords of cells (Fig. 18-2). Hepatocytes immediately abutting the portal tract are referred to as the *limiting plate*, forming a discontinuous rim around the mesenchyme of the portal tract. There is a radial orientation of the hepatocyte cords around the terminal hepatic vein. Hepatocytes exhibit minimal variation in overall size, but nuclei may vary in size, number, and ploidy, particularly with advancing age. Uninucleate,

diploid cells tend to be the rule, but with increasing age, a significant fraction are binucleate, and the karyotype may range up to octaploidy.

Between the cords of hepatocytes are vascular sinusoids. Blood traverses the sinusoids and exits into the terminal hepatic vein through innumerable orifices in the vein wall. Hepatocytes are thus bathed on two sides by well-mixed portal venous and hepatic arterial blood, placing hepatocytes among the most richly perfused cells in the body. The sinusoids are lined by fenestrated and discontinuous endothelial cells, which demarcate an extrasinusoidal *space of Disse*, into which protrude abundant microvilli of hepatocytes. Scattered *Kupffer cells* of the mononuclear phagocyte system are attached to the luminal face of endothelial cells, and scattered fat-containing *perisinusoidal stellate cells* are found in the space of Disse. These stellate cells play a role in the storage and metabolism of vitamin A and are transformed into collagen-producing myofibroblasts when there is inflammation of the liver.

Between abutting hepatocytes are *bile canaliculi*, which are channels 1 to 2 μm in diameter, formed by grooves in the

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Figure 18-1 Microscopic anatomy of the liver. The portal tract carries branches of the portal vein, hepatic artery, and bile duct system. The portal vein gives rise to branching septal veins, which penetrate the hepatocellular parenchyma at regular intervals. Blood from the septal veins enters directly into the parenchymal sinusoids between hepatocytes. The hepatic artery gives off capillaries that supply the bile duct system; these capillaries usually dump into the portal vein but may deposit blood directly into sinusoids. Arterioles also occasionally convey blood directly to the sinusoids. The bile duct system gives off bile ductules, which traverse the mesenchyme of the portal tract to penetrate the parenchyma; at that point, they become hemicircular, abutting hepatocytes (not shown) to form the canals of Hering. Bile traveling through the bile canalicular system between hepatocytes enters into the biliary tree through these canals of Hering. Blood from the portal vein and hepatic artery travels through the sinusoids of the parenchyma toward the terminal hepatic vein, leaving the liver by this route. On the basis of blood flow, three zones can be defined, zone 1 being the closest to the blood supply and zone 3 being the farthest. Pathologists refer to the regions of the parenchyma as "periportal, midzonal, and centrilobular," the last term owing to the historical concept that the terminal hepatic vein was at the center of a "lobule."

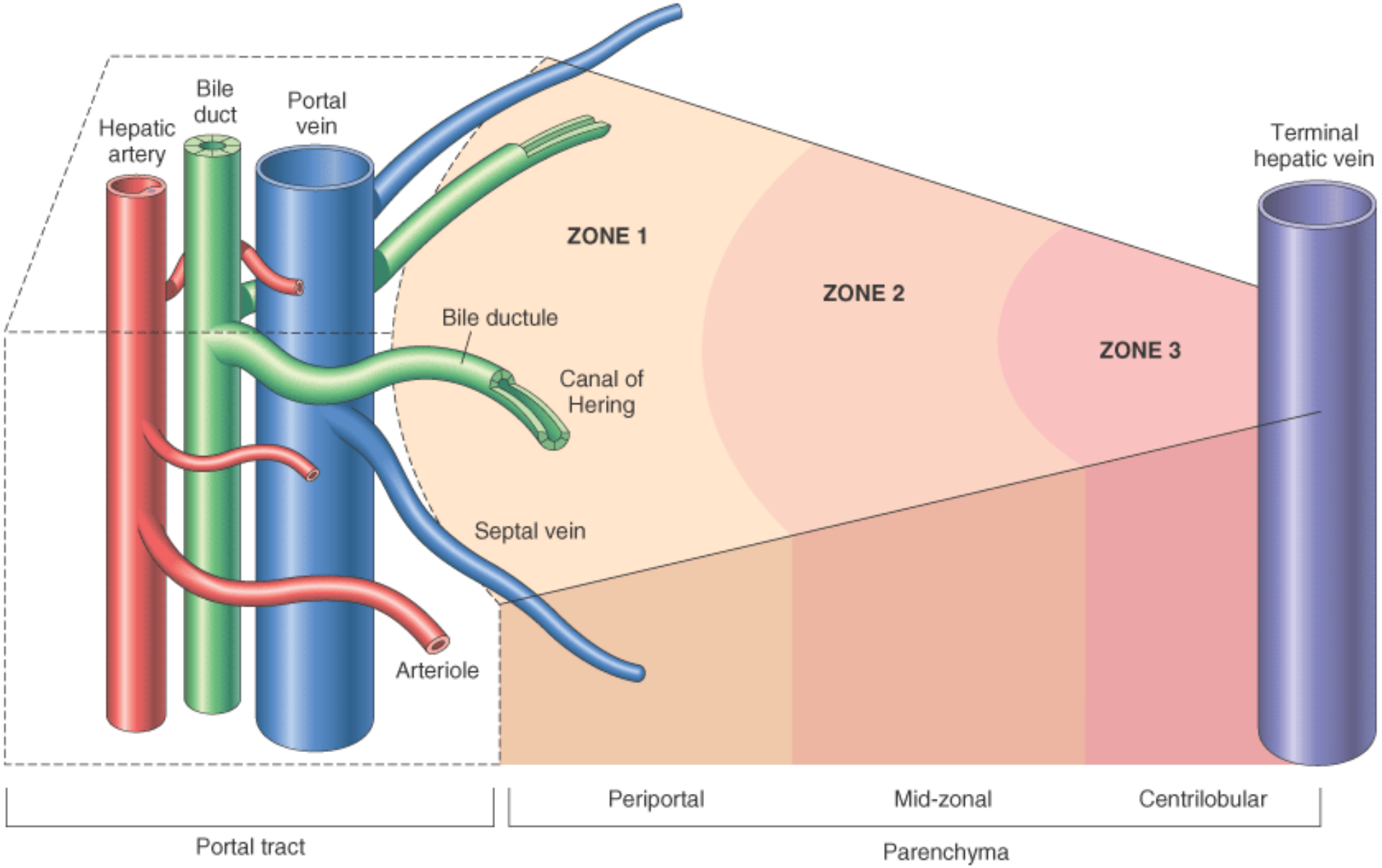
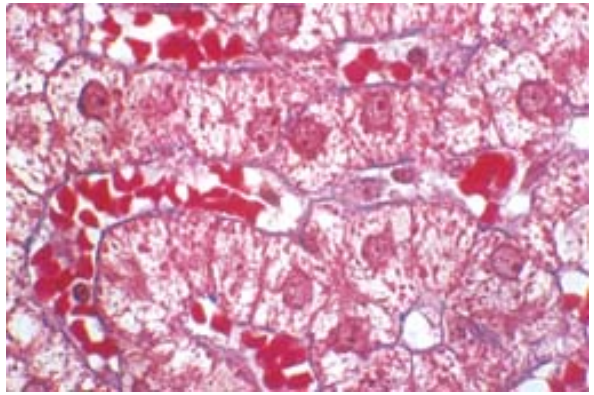


Figure 18-2 Photomicrograph of liver (trichrome stain). Note the blood-filled sinusoids and cords of hepatocytes; the delicate network of reticulin fibers in the subendothelial space of Disse stains light blue.



Necrosis frequently exhibits a zonal distribution. The most obvious is necrosis of hepatocytes immediately around the terminal hepatic vein (so-called **centrilobular necrosis,** using the historical terminology), an injury that is characteristic of ischemic injury and a number of drug and toxic reactions. Pure **midzonal** and **periportal necrosis** are rare; the latter may be seen in eclampsia. With most other causes of hepatic injury, a variable mixture of hepatocellular death through the parenchyma is encountered. The hepatocyte necrosis may be limited to scattered cells within hepatic lobules (**focal** or **spotty necrosis**) or to the interface between the periportal parenchyma and inflamed portal tracts (**interface hepatitis**). With more severe inflammatory injury, necrosis of contiguous hepatocytes may span adjacent lobules in a portal-to-portal, portal-to-central, or central-to-central fashion (**bridging necrosis**). Necrosis of entire lobules (**submassive necrosis**) or of most of the liver (**massive necrosis**) is usually accompanied by hepatic failure. With disseminated candidal or bacterial infection, macroscopic **abscesses** may occur.

• **Inflammation.** Injury to the liver associated with an influx of acute or chronic inflammatory cells is termed **hepatitis**. Direct toxic or ischemic hepatocyte necrosis incites an inflammatory reaction. With toxic damage, inflammation may also precede the onset of inflammation. Destruction of antigen-expressing liver cells by cytotoxic lymphocytes is a common mechanism of liver damage, especially during viral infection. In viral hepatitis, quiescent lymphocytes may collect in the portal tracts as a reflection of mild smoldering inflammation, spill over into the periportal parenchyma as activated lymphocytes (**interface hepatitis**) causing a moderately active hepatitis, or suffuse the entire parenchyma in severe hepatitis. Once killed, apoptotic hepatocytes do not incite an inflammatory reaction per se. However, scavenger macrophages (Kupffer cells and circulating monocytes recruited to the liver) engulf the apoptotic cell fragments within a few hours, generating clumps of inflammatory cells. Hence, identification of apoptotic hepatocytes is a sign of very recent hepatocyte destruction. Foreign bodies, organisms, and a variety of drugs may incite a granulomatous reaction.

• **Regeneration.** Hepatocytes have long life spans, and they proliferate in response to tissue resection or cell death (see Chapter 3). Regeneration occurs in all but the most fulminant hepatic diseases. Hepatocellular proliferation is marked by mitoses, thickening of the hepatocyte cords, and some disorganization of the parenchymal structure. The canal of Hering-bile ductule unit constitutes a reserve compartment for restitution of severe parenchymal injury; when it is activated, innumerable serpentine profiles resembling bile ductules appear —so-called **ductular reaction**. This compartment also proliferates during large bile duct obstruction. When hepatocellular necrosis occurs and

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leaves the connective tissue framework intact, almost perfect restitution of liver structure can occur, even when the necrosis is submassive or massive.

• **Fibrosis.** Fibrous tissue is formed in response to inflammation or direct toxic insult to the liver. Unlike other responses, which are reversible, **fibrosis points toward generally irreversible hepatic damage**. However, there is now considerable debate about the irreversibility of liver fibrosis and even cirrhosis (see below). Deposition of collagen has lasting consequences on patterns of hepatic blood flow and perfusion of hepatocytes. In the initial stages, fibrosis may develop around portal tracts or the terminal hepatic vein or may be deposited directly within the space of Disse. **With continuing fibrosis, the liver is subdivided into nodules of proliferating hepatocytes surrounded by scar tissue, termed "cirrhosis."** This end-stage form of liver disease is discussed later in this section.

The ebb and flow of hepatic injury may be imperceptible to the patient and detectable only by abnormal laboratory tests (Table 18-1). Alternatively, hepatic function may be so impaired as to be life threatening. The major clinical consequences of liver disease are listed in Table 18-2 and are discussed next.

HEPATIC FAILURE

The most severe clinical consequence of liver disease is *hepatic failure*. This may be the result of sudden and massive hepatic destruction, with about 2500 new cases per year in the United States. More often, it is the end point of progressive damage to the liver as part of chronic liver disease, either by insidious destruction of hepatocytes or by repetitive discrete

TABLE 18-1 -- Laboratory Evaluation of Liver Disease

Test Category	**Serum Measurement**
Hepatocyte integrity	Cytosolic hepatocellular enzymes
	••*Serum aspartate aminotransferase* (AST) *
	••*Serum alanine aminotransferase* (ALT) *
	••Serum lactate dehydrogenase (LDH) *
Biliary excretory function	Substances normally secreted in bile
	••*Serum bilirubin*
	••••*Total:* unconjugated plus conjugated *
	••••*Direct:* conjugated only *
	••••Delta: covalently linked to albumin *
	••Urine bilirubin *
	••Serum bile acids *
	Plasma membrane enzymes (from damage to bile canaliculus)
	••*Serum alkaline phosphatase* *
	••Serum γ-glutamyl transpeptidase *
	••Serum 5'-nucleotidase *
Hepatocyte function	Proteins secreted into the blood
	••*Serum albumin* †
	••*Prothrombin time* * (factors V, VII, X, prothrombin, fibrinogen)
	Hepatocyte metabolism
	••Serum ammonia *
	••Aminopyrine breath test (hepatic demethylation) †

	••Galactose elimination (intravenous injection) †
The most common tests are in italics.	

*An elevation implicates liver disease.
†A decrease implicates liver disease.

TABLE 18-2 -- Clinical Consequences of Liver Disease

Characteristic signs	Hepatic dysfunction:
	••Jaundice and cholestasis
	••Hypoalbuminemia
	••Hyperammonemia
	••Hypoglycemia
	••Fetor hepaticus
	••Palmar erythema
	••Spider angiomas
	••Hypogonadism
	••Gynecomastia
	••Weight loss
	••Muscle wasting
	Portal hypertension from cirrhosis:
	••Ascites
	••Splenomegaly
	••Hemorrhoids
	••Caput medusae—abdominal skin
Life-threatening complications	Hepatic failure
	••Multiple organ failure
	••Coagulopathy
	••Hepatic encephalopathy
	••Hepatorenal syndrome

	Portal hypertension from cirrhosis
	••Esophageal varices, risk of rupture
	Malignancy with chronic disease
	••Hepatocellular carcinoma

waves of parenchymal damage. Whatever the sequence, 80% to 90% of hepatic functional capacity must be eroded before hepatic failure ensues. In many cases, the balance is tipped toward decompensation by intercurrent diseases that place demands on the liver. These include gastrointestinal bleeding, systemic infection, electrolyte disturbances, and severe stress such as major surgery or heart failure. In most cases of severe hepatic dysfunction, liver transplantation is the only hope for survival. Overall, mortality from hepatic failure without liver transplantation is 70% to 95%.

The morphologic alterations that cause liver failure fall into three categories:

- *Massive hepatic necrosis.* This is most often drug- or toxin-induced, as from acetaminophen (38% of massive hepatic necrosis cases in the United States), halothane, antituberculosis drugs (rifampin, isoniazid), antidepressant monoamine oxidase inhibitors, industrial chemicals such as carbon tetrachloride, and mushroom poisoning (*Amanita phalloides*), collectively accounting for an additional 14% of cases. The mechanism may be direct toxic damage to hepatocytes (e.g., acetaminophen, carbon tetrachloride, mushroom toxins) but more often is a variable combination of toxicity and inflammation with immune-mediated hepatocyte destruction. Hepatitis A infection accounts for 4% of cases, hepatitis B infection accounts for 8%, and other causes (including unknown) account for 37%. Hepatitis C infection does not cause massive hepatic necrosis.
- *Chronic liver disease.* This is the most common route to hepatic failure and is the endpoint of relentless chronic hepatitis ending in cirrhosis. The many causes of cirrhosis will be discussed shortly.
- *Hepatic dysfunction without overt necrosis.* Hepatocytes may be viable but unable to perform normal metabolic function, as with Reye syndrome, tetracycline toxicity, and acute fatty liver of pregnancy.

Clinical Features.

Regardless of cause, the clinical signs of hepatic failure are much the same. *Jaundice* is an almost invariable finding. *Hypoalbuminemia*, which predisposes to peripheral edema, and *hyperammonemia*, which may play a role in cerebral dysfunction, are extremely worrisome developments. *Fetor hepaticus* is a characteristic body odor that is variously described as "musty" or "sweet and sour" and occurs occasionally. It is related to the formation of mercaptans by the action of gastrointestinal bacteria on the sulfur-containing amino acid methionine and shunting of splanchnic blood from the portal into the systemic circulation (portosystemic shunting). Impaired estrogen metabolism and consequent hyperestrogenemia are the putative causes of *palmar erythema* (a reflection of local vasodilatation) and *spider angiomas* of the skin. Each angioma is a central, pulsating, dilated arteriole from which small vessels radiate. In the male, hyperestrogenemia also leads to *hypogonadism* and *gynecomastia*.

Hepatic failure is life-threatening because *with severely impaired liver function, patients are highly susceptible to failure of multiple organ systems*. Thus, respiratory failure with pneumonia and sepsis combine with renal failure to claim the lives of many patients with hepatic failure. A *coagulopathy* develops, attributable to impaired hepatic synthesis of blood clotting factors II, VII, IX, and X. The resultant bleeding tendency can lead to massive gastrointestinal bleeding as well as petechial bleeding elsewhere. Intestinal absorption of blood places a metabolic load on the liver, which worsens the extent of hepatic failure. The outlook of full-blown hepatic failure is grave: A rapid downhill course is usual, death occurring within weeks to a few months in about 80% of cases. A fortunate few can endure an acute episode until hepatocellular regeneration restores adequate hepatic function. Alternatively, liver transplantation might save the patient.

Two particular complications merit separate consideration, as they herald the most grave stages of hepatic failure.

Hepatic encephalopathy is manifested by a spectrum of disturbances in consciousness, ranging from subtle behavioral abnormalities to marked confusion and stupor to deep coma and death. These changes may progress over hours or days in fulminant hepatic failure or more insidiously in a patient with marginal hepatic function from chronic liver disease. Associated fluctuating neurologic signs include rigidity, hyperreflexia, and particularly *asterixis*: nonrhythmic, rapid extension-flexion movements of the head and extremities, best seen when the arms are held in extension with dorsiflexed wrists. *Hepatic encephalopathy is regarded as a disorder of neurotransmission in the central nervous system and neuromuscular system*[5] and appears to be associated with elevated blood ammonia levels, which impair neuronal function and promote generalized brain edema. *In the great majority of instances, there are only minor morphologic changes in the brain, such as edema and an astrocytic reaction,* and the encephalopathy is reversible if the underlying hepatic condition can be corrected.

Hepatorenal syndrome refers to the appearance of renal failure in patients with severe chronic liver disease, in whom there are no intrinsic morphologic or functional causes for the renal failure. Sodium retention, impaired free-water excretion, and decreased renal perfusion and glomerular filtration rate are the main renal functional abnormalities.[6] Several factors are involved in its development, including a decreased renal perfusion pressure due to systemic vasodilation, activation of the renal sympathetic nervous system with vasoconstriction of the afferent renal arteriolae, and increased synthesis of renal vasoactive mediators, which further decrease glomerular filtration. Onset of this syndrome is typically heralded by a drop in urine output, associated with rising blood urea nitrogen and creatinine. *The ability to concentrate urine is retained, producing a hyperosmolar urine devoid of proteins and abnormal sediment, and surprisingly low in sodium* (unlike renal tubular necrosis). Rapid development of renal failure is usually associated with a precipitating stress factor such as infection, gastrointestinal hemorrhage, or a major surgical procedure. Insidious development of renal failure is the result of progressive destabilization of circulatory physiology, frequently in the setting of severe refractory ascites. The prognosis is poor, with a median survival of only 2 weeks in the rapid-onset form and 6 months with the insidious-onset form.

CIRRHOSIS

Cirrhosis is among the top 10 causes of death in the Western world. The chief worldwide contributors are alcohol abuse and viral hepatitis. Other causes include biliary disease, and iron overload. An example of the progression to cirrhosis is given under the subsequent discussion on alcohol. Cirrhosis as the end-stage of chronic liver disease is defined by three characteristics:

- Bridging *fibrous septae* in the form of delicate bands or broad scars linking portal tracts with one another and portal tracts with terminal hepatic veins
- *Parenchymal nodules* containing proliferating hepatocytes encircled by fibrosis, with diameters varying from very small (<3 mm, micronodules) to large (several centimeters, macronodules)
- *Disruption of the architecture of the entire liver*

Several features of cirrhosis should be underscored:

- *The parenchymal injury and consequent fibrosis are diffuse,* extending throughout the liver. Focal injury with scarring does not constitute cirrhosis, nor does diffuse nodular transformation without fibrosis.
- *Nodularity is part of the diagnosis* and reflects the balance between regenerative activity and constrictive scarring. It should be noted that rapid development of fibrosis, as in alcoholic hepatitis, may leave little time for the development of spherical nodules.
- *Vascular architecture is reorganized* by the parenchymal damage and scarring, with the formation of abnormal interconnections between vascular inflow and hepatic vein outflow channels. As a result, portal vein and arterial blood partially bypasses the functional hepatocyte mass through these abnormal channels.
- *Fibrosis is the key feature of progressive damage to the liver.* With cessation of the causal injury, slow regression of fibrosis may occur. Once cirrhosis has developed, reversal is thought to be rare. However, the liver contains abundant metalloproteinases and collagenases that are capable of degrading extracellular matrix. Collagen degradation is a slow process, since collagen I sustains extensive crosslinking after its deposition and hence becomes more resistant to collagenases over time. Nevertheless, there are a sufficient number of clinical reports of patients whose full-blown cirrhosis has subsided to a form of incomplete septation of the liver or apparent absence of fibrosis, to raise

hopes that even patients with cirrhosis may improve without resorting to liver transplantation.

The only satisfactory classification of cirrhosis is based on the presumed underlying etiology. The descriptive terms "micronodular" and "macronodular" should not be used as primary classifications. Many forms of cirrhosis (particularly alcoholic cirrhosis) are initially micronodular, but there is a tendency for nodules to increase in size with time, counterbalanced by the constraints imposed by fibrous scarring.

The etiology of cirrhosis varies both geographically and socially. The following is the approximate frequency of etiologic categories in the Western world, most of which are discussed in detail later:

Alcoholic liver disease	60% to 70%
Viral hepatitis	10%
Biliary diseases	5% to 10%
Primary hemochromatosis	5%
Wilson disease	Rare
α_1 -Antitrypsin deficiency	Rare
Cryptogenic cirrhosis	10% to 15%

Infrequent types of cirrhosis also include the cirrhosis developing in infants and children with galactosemia and tyrosinosis (Chapter 10), and drug-induced cirrhosis, as with α-methyldopa. Severe fibrosis can occur in the setting of cardiac disease (sometimes called "cardiac cirrhosis," discussed later). After all the categories of cirrhosis of known causation have been excluded, a substantial number of cases remain. Referred to as *cryptogenic cirrhosis*, the magnitude of this "wastebasket" category speaks eloquently to the difficulties in discerning the many origins of cirrhosis. A growing concern is that many of these cases are due to undiagnosed *nonalcoholic fatty liver disease*, to be discussed. *Once cirrhosis is established, it is usually impossible to establish an etiologic diagnosis on morphologic grounds alone.*

Pathogenesis.

The central pathogenetic processes in cirrhosis are progressive fibrosis and reorganization of the vascular microarchitecture of the liver.[7] In the normal liver, interstitial collagens (types I and III) are concentrated in portal tracts and around central veins, with occasional bundles in the space of Disse. The collagen (reticulin) coursing alongside hepatocytes is composed of delicate strands of type IV collagen in the space of Disse. In cirrhosis, types I and III collagen are deposited in the lobule, creating delicate or broad septal tracts. New vascular channels in the septae connect the vascular structures in the portal region (hepatic arteries and portal veins) and terminal hepatic veins, shunting blood around the parenchyma. Continued deposition of collagen in the space of Disse within preserved parenchyma is accompanied by the loss of fenestrations in the sinusoidal endothelial cells. In the process, the sinusoidal space comes to resemble a capillary rather than a channel for exchange of solutes between hepatocytes and plasma. In particular, hepatocellular secretion of proteins (e.g., albumin, clotting factors, lipoproteins) is greatly impaired.

The major source of excess collagen in cirrhosis is the perisinusoidal stellate cells, which lie in the space of Disse. Although normally functioning as vitamin A fat-storing cells, during the development of cirrhosis they become activated, a process that includes (1) robust mitotic activity in areas developing new parenchymal fibrosis, (2) a shift from the resting-state lipocyte phenotype to a transitional myofibroblast phenotype, and (3) increased capacity for synthesis and secretion of extracellular matrix. It is predominantly the cytokines secreted by activated Kupffer cells and other inflammatory cells that stimulate perisinusoidal stellate cells to divide and to produce large amounts of extracellular matrix. Moreover, the greatest activation of stellate cells is in areas of severe hepatocellular necrosis and inflammation. As depicted in Figure 18-3 , the stimuli for stellate cell activation may come from several sources:

- Chronic inflammation, with production of inflammatory cytokines such as tumor necrosis factor (TNF), lymphotoxin, and interleukin-1 (IL-1).
- Cytokine production by activated endogenous cells (Kupffer cells, endothelial cells, hepatocytes, and bile duct epithelial cells), including transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and lipid peroxidation products.
- Disruption of the extracellular matrix, as stellate cells are extraordinarily responsive to the status of their substrate.
- Direct stimulation of stellate cells by toxins.

Acquisition of myofibers by perisinusoidal stellate cells also increases vascular resistance within the liver parenchyma, since tonic contraction of these "myofibroblasts" constricts the sinusoidal vascular channels.

Throughout the process of liver damage and fibrosis, remaining hepatocytes are stimulated to regenerate and proliferate as spherical nodules within the confines of the fibrous septae. The net outcome is a fibrotic, nodular liver in which delivery of blood to hepatocytes is severely compromised, as is the ability of hepatocytes to secrete substances into plasma. Disruption of the interface between the parenchyma and portal tracts obliterates biliary channels as well. Thus, *the cirrhotic patient may develop jaundice and even hepatic failure, despite having a liver of normal mass.*

Clinical Features.

All forms of cirrhosis may be clinically silent. When symptomatic they lead to nonspecific clinical manifestations: anorexia, weight loss, weakness, osteoporosis, and, in advanced disease, frank debilitation. Incipient or overt hepatic failure may develop, usually precipitated by a superimposed metabolic load on the liver, as from systemic infection or a gastrointestinal hemorrhage. Imbalances of pulmonary blood flow, which are poorly understood, may lead to severely impaired oxygenation (*hepatopulmonary syndrome*), further stressing the patient. *The ultimate mechanism of most cirrhotic deaths is (1) progressive liver failure (discussed earlier), (2) a complication related to portal hypertension, or (3) the development of hepatocellular carcinoma.*

PORTAL HYPERTENSION

Increased resistance to portal blood flow may develop in a variety of circumstances, which can be divided into *prehepatic, intrahepatic, and posthepatic causes*. The major *prehepatic conditions* are obstructive thrombosis and narrowing of the portal vein before it ramifies within the liver. Massive splenomegaly may also shunt excessive blood into the splenic vein. The major *posthepatic causes* are severe right-sided heart failure, constrictive pericarditis, and hepatic vein outflow obstruction. *The dominant intrahepatic cause is cirrhosis, accounting for most cases of portal hypertension.* Far less frequent are schistosomiasis, massive fatty change, diffuse fibrosing granulomatous disease such as sarcoidosis and miliary

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Figure 18-3 Schematic of stellate cell activation and liver fibrosis in comparison to the normal liver. Kupffer cell activation leads to secretion of multiple cytokines; cytokines also may be released by endothelial cells, hepatocytes, and inflammatory cells entering the liver (not shown). These cytokines "activate" stellate cells, whereby they loose their lipid droplets (which are present in the quiescent state) and acquire a myofibroblastic state. Stellate cell proliferation is stimulated in particular by platelet-derived growth factor (PDGF); tumor necrosis factor (TNF) is a potent stimulant of the change to a myofibroblastic phenotype. Contraction of the activated stellate cells is stimulated by endothelin-1 (ET-1). Deposition of extracellular matrix (fibrogenesis) is stimulated especially by transforming growth factor β (TGF-β). Chemotaxis of activated stellate cells to areas of injury, such as where hepatocytes have undergone apoptosis, is promoted by PDGF and monocyte chemotactic protein-1 (MCP-1). Kupffer cells also are a major source of TNF released into the system circulation. *(Schematic based on concepts presented in Friedman SL: Molecular regulation of hepatic fibrosis: an integrated cellular response to tissue injury. J Biol Chem 275:2247–2250, 2000; and Crawford JM: Cellular and molecular biology of the liver. Curr Op Gastroenterol 13:175–185, 1997.)*

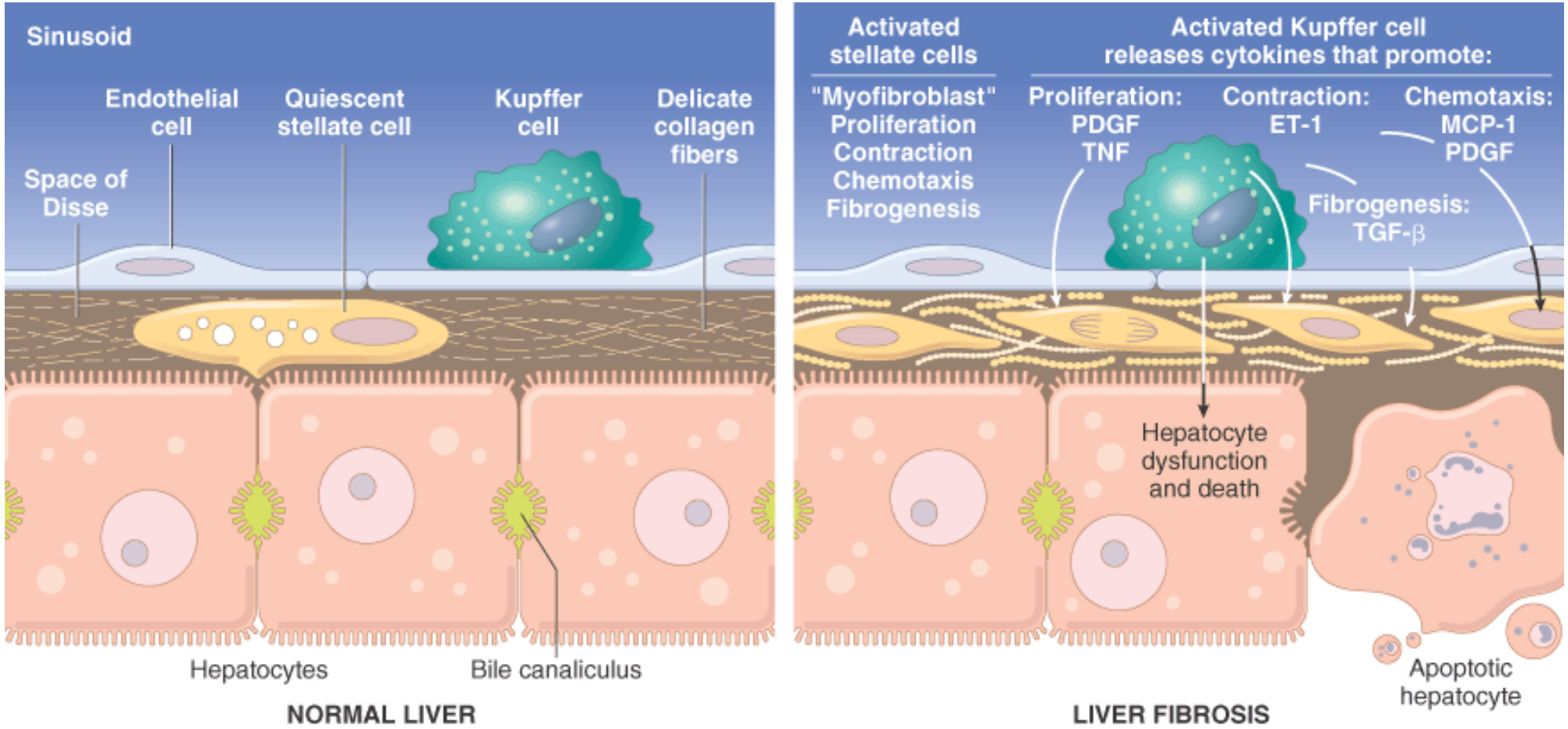


Figure 18-4 The major clinical consequences of portal hypertension in the setting of cirrhosis, shown for the male. In women, oligomenorrhea, amenorrhea, and sterility are frequent, owing to hypogonadism.

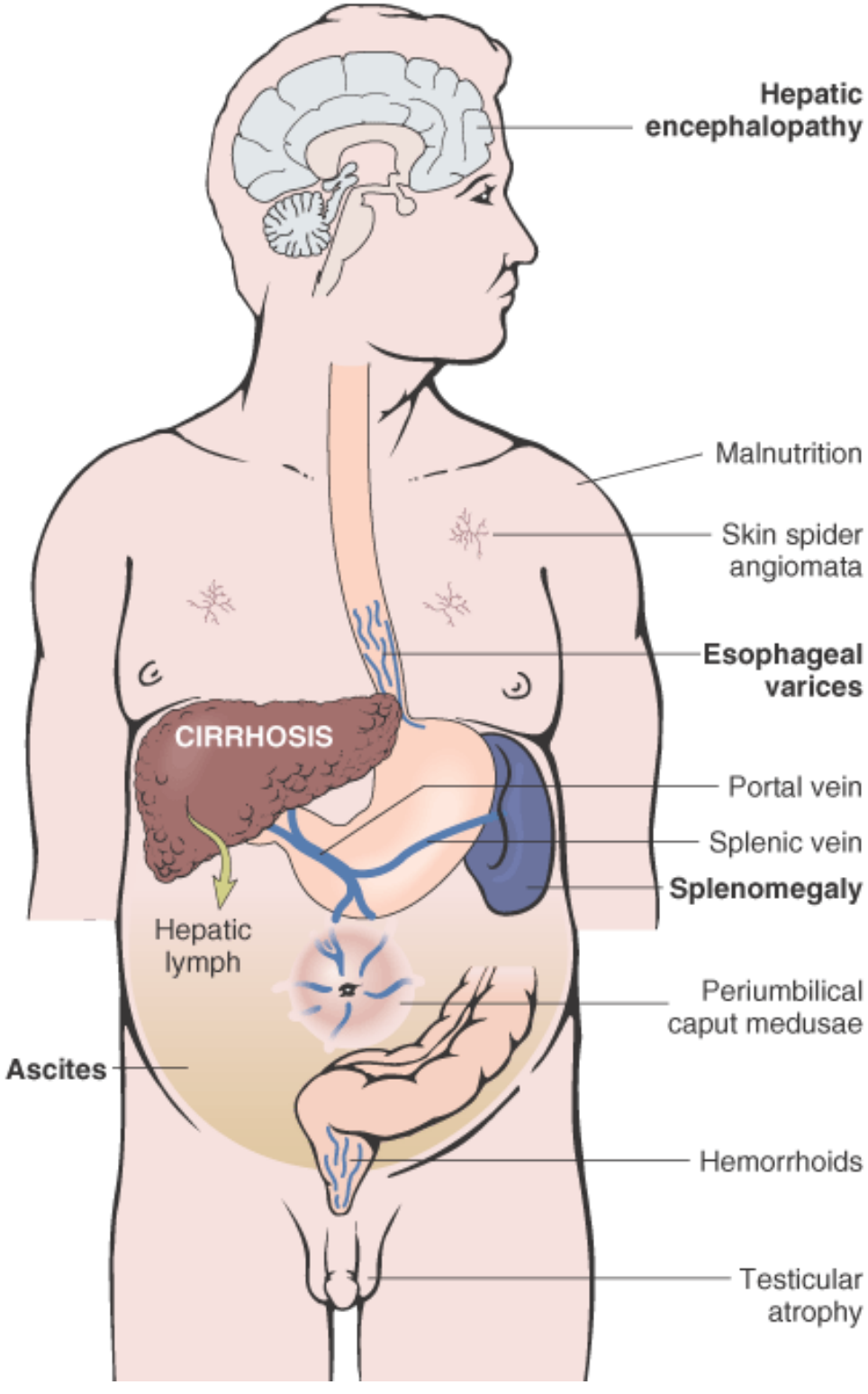
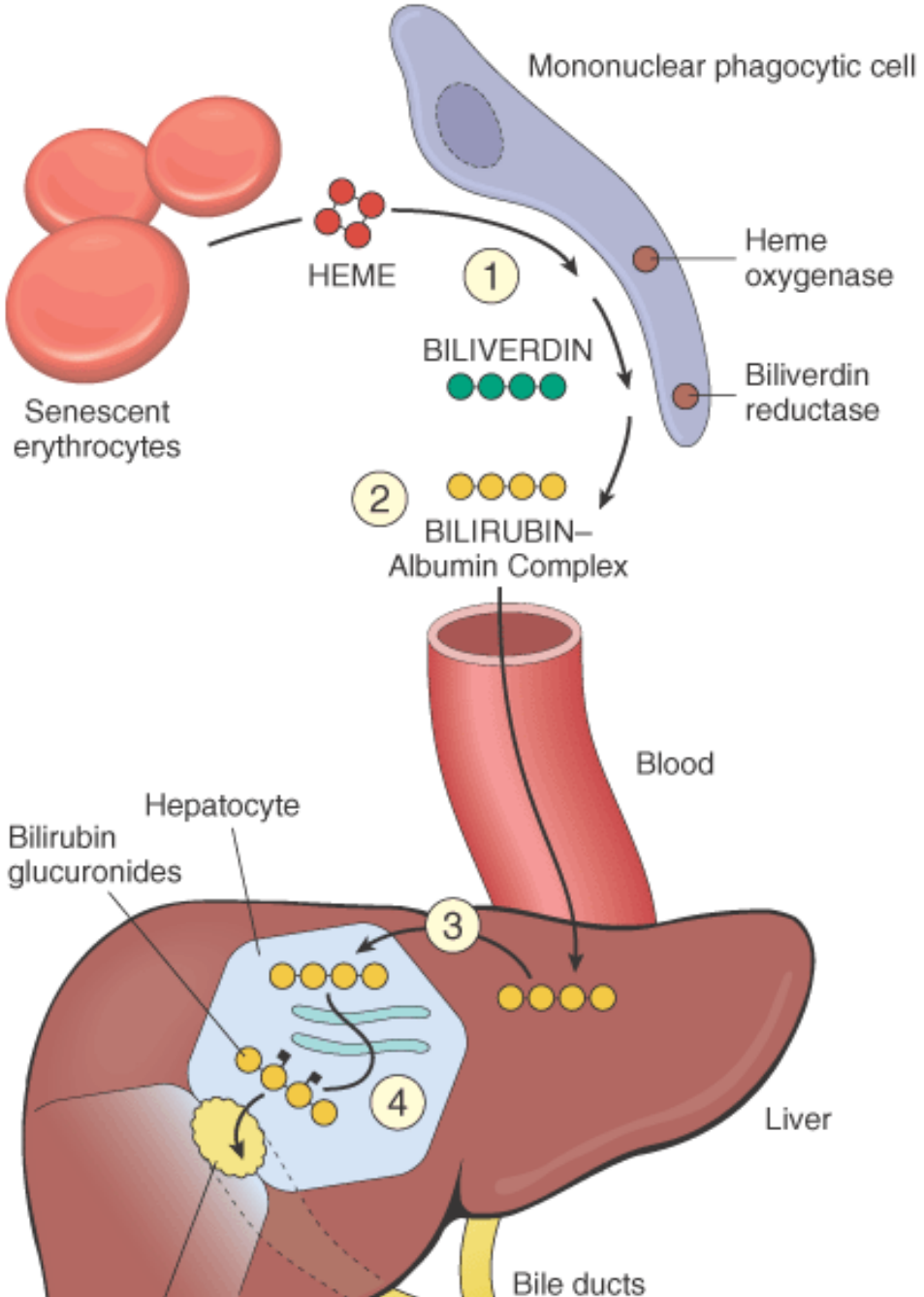

Hepatic encephalopathy
Malnutrition
Skin spider angiomata
Esophageal varices
CIRRHOSIS
Portal vein
Splenic vein
Splenomegaly
Hepatic lymph
Periumbilical caput medusae
Ascites
Hemorrhoids
Testicular atrophy

Figure 18-5 Bilirubin metabolism and elimination. 1, Normal bilirubin production from heme (0.2 to 0.3 gm/day) is derived primarily from the breakdown of senescent circulating erythrocytes, with a minor contribution from degradation of tissue heme-containing proteins. 2, Extrahepatic bilirubin is bound to serum albumin and delivered to the liver. 3, Hepatocellular uptake and (4) glucuronidation in the endoplasmic reticulum generate bilirubin monoglucuronides and diglucuronides, which are water soluble and readily excreted into bile. 5, Gut bacteria deconjugate the bilirubin and degrade it to colorless urobilinogens. The urobilinogens and the residue of intact pigments are excreted in the feces, with some reabsorption and excretion into urine.



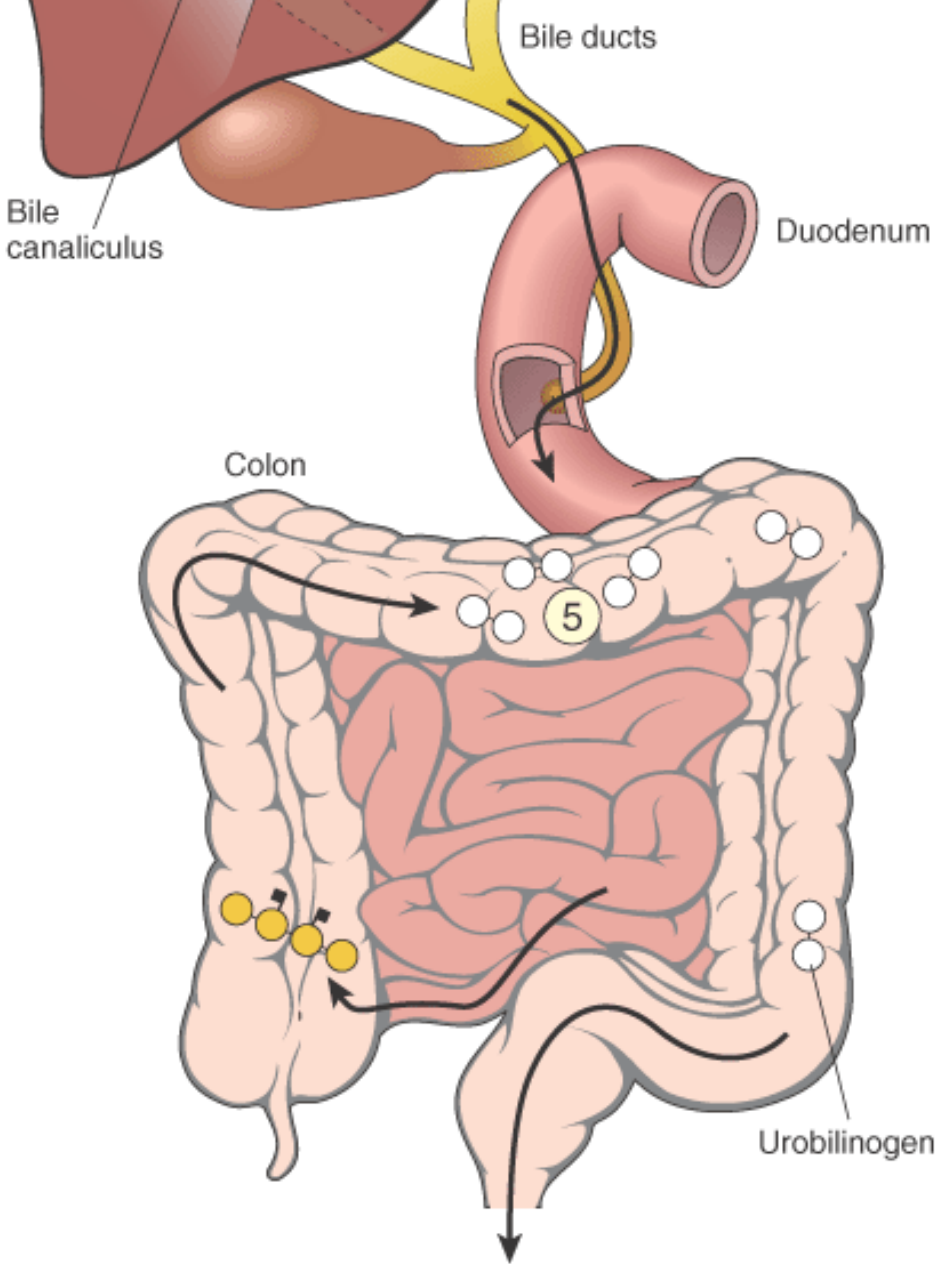


TABLE 18-3 -- Causes of Jaundice

Predominantly Unconjugated Hyperbilirubinemia
Excess production of bilirubin
••Hemolytic anemias

••Resorption of blood from internal hemorrhage (e.g., alimentary tract bleeding, hematomas)
••Ineffective erythropoiesis syndromes (e.g., pernicious anemia, thalassemia)
Reduced hepatic uptake
••Drug interference with membrane carrier systems
••Some cases of Gilbert syndrome
Impaired bilirubin conjugation
••Physiologic jaundice of the newborn (decreased UGT1A1 activity, decreased excretion)
••Breast milk jaundice (β-glucuronidases in milk)
••Genetic deficiency of UGT1A1 activity (Crigler-Najjar syndrome types I and II)
••Gilbert syndrome (mixed etiologies)
••Diffuse hepatocellular disease (e.g., viral or drug-induced hepatitis, cirrhosis)
Predominantly Conjugated Hyperbilirubinemia
Deficiency of canalicular membrane transporters (Dubin-Johnson syndrome, Rotor syndrome)
Impaired bile flow
UGT, uridine diphosphate-glucuronyltransferase.

Neonatal Jaundice.

Because the hepatic machinery for conjugating and excreting bilirubin does not fully mature until about 2 weeks of age, almost every newborn develops transient and mild unconjugated hyperbilirubinemia, termed neonatal jaundice or *physiologic jaundice of the newborn*. Breast-fed infants tend to exhibit jaundice with greater frequency, possibly the result of β-glucuronidases present in maternal milk. These enzymes deconjugate bilirubin glucuronides in the gut, increasing intestinal reabsorption of unconjugated bilirubin. Sustained jaundice in the newborn is indicative of a disease condition, discussed later under *neonatal hepatitis*.

Hereditary Hyperbilirubinemias.

In rare instances, there may be a genetic lack of UGT1A1 (Table 18-4). In *Crigler-Najjar syndrome type I*, the enzyme is completely absent. Multiple genetic defects in the locus coding for UGT1A1 may give rise to this disorder.[10] The liver is incapable of synthesizing a functional enzyme, and the colorless bile contains only trace amounts of unconjugated bilirubin. The liver is morphologically normal by light and electron microscopy. However, serum unconjugated bilirubin reaches very high levels, producing severe jaundice and icterus. Without liver transplantation, this condition is invariably fatal, causing death within 18 months of birth secondary to kernicterus.

Crigler-Najjar syndrome type II is a less severe, nonfatal disorder in which UGT1A1 enzyme activity is greatly reduced, and the enzyme is capable of forming only monoglucuronidated bilirubin. Unlike Crigler-Najjar syndrome type I, the only major consequence is extraordinarily yellow skin from moderate to high levels of circulating unconjugated bilirubin; phenobarbital treatment can improve bilirubin glucuronidation by inducing hypertrophy of the hepatocellular endoplasmic reticulum. Mutations either reduce the affinity of UGT1A1 toward bilirubin or reduce enzyme activity.[11] Almost

TABLE 18-4 -- Hereditary Hyperbilirubinemias

Disorder	Inheritance	Defects in Bilirubin Metabolism	Liver Pathology	Clinical Course
Unconjugated Hyperbilirubinemia				
Crigler-Najjar syndrome type I	Autosomal recessive	Absent UGT1A1 activity	Normal	Fatal in neonatal period
Crigler-Najjar syndrome type II	Autosomal dominant with variable penetrance	Decreased UGT1A1 activity	Normal	Generally mild, occasional kernicterus
Gilbert syndrome	Autosomal dominant?	Decreased UGT1A1 activity	Normal	Innocuous
Conjugated Hyperbilirubinemia				
Dubin-Johnson syndrome	Autosomal recessive	Impaired biliary excretion of bilirubin glucuronides due to mutation in canalicular multidrug resistance protein 2 (MRP2)	Pigmented cytoplasmic globules; ?epinephrine metabolites	Innocuous
Rotor syndrome	Autosomal recessive	Decreased hepatic uptake and storage?	Normal	Innocuous
		Decreased biliary excretion?		

UGT, uridine diphosphate-glucuronyltransferase.

all patients develop normally, but there is a risk for some neurologic damage from kernicterus.

Gilbert syndrome is a relatively common, benign, somewhat heterogeneous inherited condition presenting with mild, fluctuating hyperbilirubinemia. The primary cause is reduction in hepatic bilirubin glucuronidating activity to about 30% of normal levels. In most patients, two extra bases (TA) are found in the TATAA element of the 5' promoter region (creating an A $(TA)_7$ TAA element rather than the normal A$(TA)_6$ TAA, resulting in reduced expression of UGT1A1. Alternatively, patients may be heterozygous for missense mutations in the *UGT1A1* gene. Affecting some 6% of the population, the mild hyperbilirubinemia may go undiscovered for years and is not associated with functional derangements. When detected in adolescence or adult life, it is typically in association with stress, such as an intercurrent illness, strenuous exercise, or fasting. Gilbert syndrome has no clinical consequence except for the anxiety that a jaundiced sufferer might justifiably experience with this otherwise innocuous condition.

Dubin-Johnson syndrome results from a hereditary defect in hepatocellular excretion of bilirubin glucuronides across the canalicular membrane. The defect is due to absence of the canalicular protein, the *multidrug resistance protein 2* (MRP2; located on chromosome 10q24), that is responsible for transport of bilirubin glucuronides and related organic anions into bile.[12] The liver is darkly pigmented owing to coarse pigmented granules within the cytoplasm of hepatocytes (Fig. 18-6). Electron microscopy reveals that the pigment is located in lysosomes, and it appears to be composed of polymers of epinephrine metabolites, not bilirubin pigment. The liver is otherwise normal. Apart from chronic or recurrent jaundice of fluctuating intensity, most patients are asymptomatic and have a normal life expectancy.

Rotor syndrome is a rare form of asymptomatic conjugated hyperbilirubinemia with multiple defects in hepatocellular uptake and excretion of bilirubin pigments. The liver is not pigmented. As with Dubin-Johnson syndrome, patients with Rotor syndrome exhibit jaundice but otherwise live normal lives.

Cholestasis

Cholestatic conditions, which result from hepatocellular dysfunction or intrahepatic or extrahepatic biliary obstruction, also may present with jaundice. Alternatively, *pruritus* is a presenting symptom, related to the elevation in plasma bile acids and their deposition in peripheral tissues, particularly skin. *Skin xanthomas* (focal accumulations of cholesterol) sometimes appear, the result of hyperlipidemia and impaired excretion of cholesterol. *A characteristic laboratory finding is elevated serum alkaline phosphatase*, an enzyme present in bile duct epithelium and in the canalicular membrane of hepatocytes that is released into the circulation because of the detergent action of retained bile salts on hepatocyte membranes. An isozyme derived from posttranscriptional changes is normally present in many other tissues such as bone, so the increased levels must be verified as being hepatic in origin. Another canalicular ectoenzyme, *γ-glutamyl transpeptidase*, is also released into the circulation. The elevated levels of these

Figure 18-6 Dubin-Johnson syndrome, showing abundant pigment inclusions in otherwise normal hepatocytes (H&E).

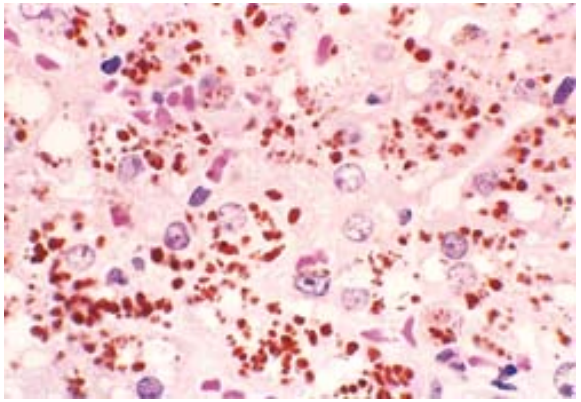
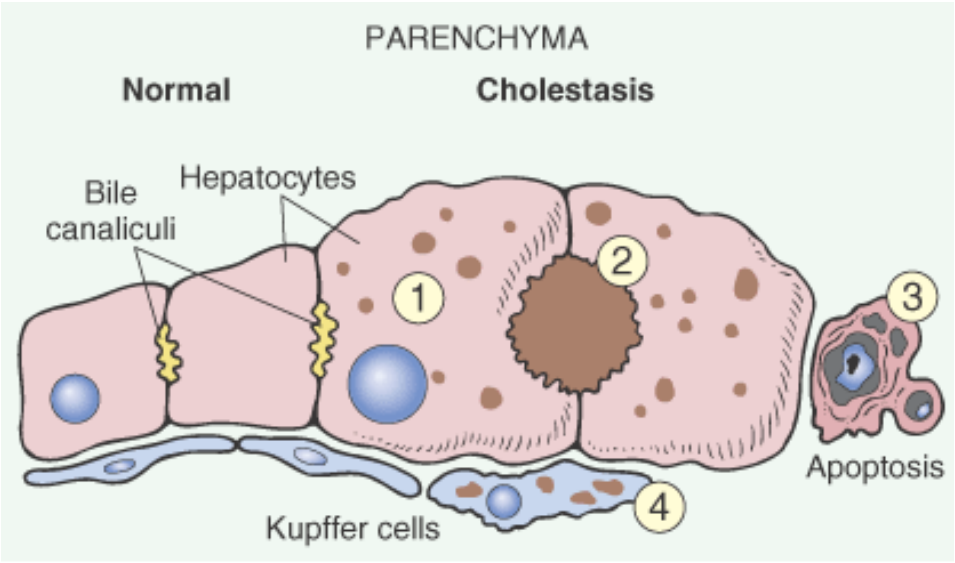


Figure 18-7 Illustration of the morphologic features of cholestasis (*right*) and comparison with normal liver (*left*). In the parenchyma (*upper panel*), cholestatic hepatocytes (1) are enlarged with dilated canalicular spaces (2). Apoptotic cells (3) may be seen, and Kupffer cells (4) frequently contain regurgitated bile pigments. In the portal tracts of obstructed liver (*lower panel*), there is also bile ductular proliferation (5), edema, bile pigment retention (6), and eventually neutrophilic inflammation (not shown). Surrounding hepatocytes (7) are swollen and undergoing degeneration.



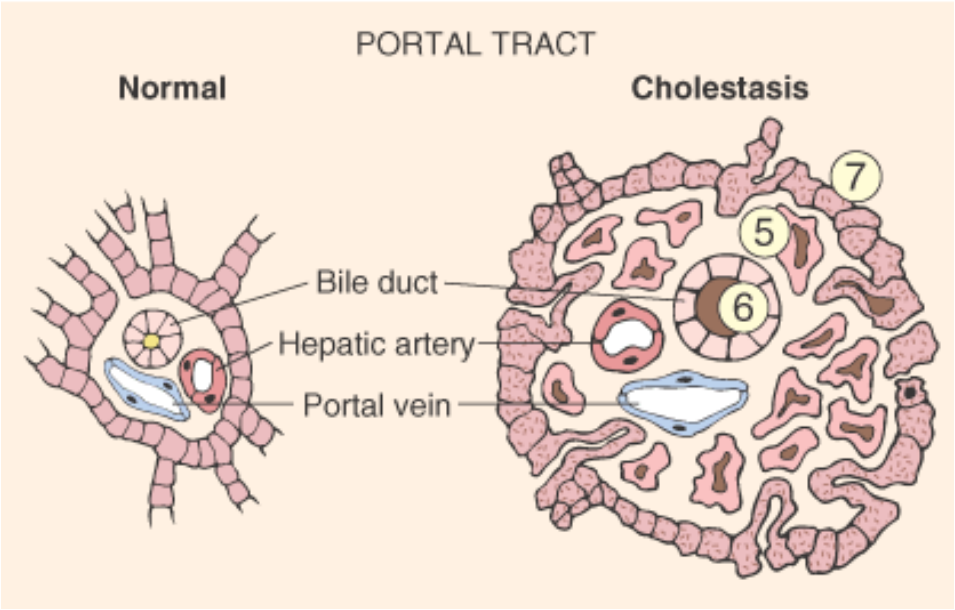


TABLE 18-5 -- Inherited Cholestatic Conditions

Common Names	Gene	Chromosome	Ligands	Location	Diseases
ABC1	ABCAI	9q22-q31	Lipids?	Many tissues	Tangier disease type 1
			Cholesterol?		
MDR3	ABCB4	7q21	Phosphatidylcholine	Hepatocyte apical (canalicular) membrane	PFIC-3

BSEP	ABCB11	2q24	Bile Salts	Hepatocyte apical (canalicular) membrane	PFIC-2
MRP2 (cMOAT)	ABCC2	10q24	Anionic conjugates with glutathione, sulfate, and glucuronate	Liver, intestine, kidney apical membranes	Dubin-Johnson Syndrome
CFTR	ABCC7	7q31-2	Organic anions? GSH?	Lung, intestine (crypt), cholangiocytes: apical membranes	Cystic Fibrosis
IBST	SLC10A2	13q33	Bile Salts	Cholangiocytes, intestine: apical membranes	PBAM
FIC1	ATP8B1	18q21-22	Aminophospholipid?	Cholangiocytes, hepatoctyes: apical membranes	PFIC-1, BRIC, Byler disease, Byler syndrome
ATP7B	ATP7B	13q14.3	Copper	Hepatocyte endoplasmic reticulum	Wilson disease

ABC, of the ABC transporter family; ATP, adenosine triphospatase; BRIC, benign recurrent intrahepatic cholestasis; BSEP, bile salt export pump; CFTR, cystic fibrosis transmembrane regulator; cMOAT, canalicular multiple organic anion transporter; PFIC, progressive familial intrahepatic cholestasis; IBST, intestinal bile salt transporter; MRP, multidrug resistant protein; PBAM, primary bile acid malabsorption; PFIC, progressive familial intrahepatic cholestasis.

(PFIC-3), due to mutations in the *ABCB4* gene on chromosome 7q21. The encoded protein, MDR3, is a canalicular transport protein that is responsible for flipping phosphatidylcholine from the internal to the external hemileaflet of the canalicular membrane. In patients with this disorder, the absence of secreted phosphatidylcholine in bile leaves the apical surfaces of the biliary tree epithelia subject to the full detergent action of secreted bile salts, with resultant toxic destruction of these epithelia and release of GGT into the circulation.

Children with severe cholestasis but with absence of elevated serum GGT and absence of pruritus may also have inherited defects in bile acid synthesis. The most common condition is a deficiency of *3β-hydroxysteroid dehydrogenase*, an enzyme located early in the pathway for bile acid synthesis from cholesterol.

Infectious Disorders

Inflammatory disorders of the liver dominate the clinical practice of hepatology. This is due in part to the fact that virtually any insult to the liver can kill hepatocytes and recruit inflammatory cells, but also because inflammatory diseases are frequently long-term chronic conditions that must be managed medically. Among inflammatory disorders, infection is by far the most frequent. The liver is almost inevitably involved in blood-borne infections, whether systemic or arising within the abdomen. The foremost hepatic infections are viral in origin. Other infections in which the hepatic lesion is prominent include miliary tuberculosis, malaria, staphylococcal bacteremia, the salmonelloses, candida, and amebiasis.

VIRAL HEPATITIS

Unless otherwise specified, the term "viral hepatitis" is reserved for infection of the liver caused by a group of viruses having a particular affinity for the liver (Table 18-6). [14] [15] Systemic viral infections that can involve the liver include (1) infectious mononucleosis (Epstein-Barr virus), which may cause a mild hepatitis during the acute phase; (2) cytomegalovirus, particularly in the newborn or immunosuppressed patient; and (3) yellow fever, which has been a major and serious cause of hepatitis in tropical countries. Infrequently, in children and immunosuppressed patients, the liver is affected in the course of rubella, adenovirus, herpesvirus, or enterovirus infections. Hepatotropic viruses cause overlapping patterns of disease. Each hepatotropic virus and the disease conditions it causes will be introduced before a general discussion of hepatitis.

Hepatitis A Virus

Hepatitis A virus (HAV), the scourge of military campaigns since antiquity, is a benign, self-limited disease with an incubation period of 2 to 6 weeks.[16] *HAV does not cause chronic hepatitis or a carrier state and only rarely causes fulminant hepatitis, so the fatality rate associated with HAV is about 0.1%.* The outcome of HAV infection may be more severe if it is superimposed on chronic hepatitis due to Hepatitis B virus (HBV), Hepatitis C virus (HCV), or alcohol. HAV occurs throughout the world and is endemic in countries with substandard

hygiene and sanitation, so populations there may have detectable anti-HAV by the age of 10 years. Clinical disease tends to be mild or asymptomatic and rare after childhood. In developed countries, the prevalence of seropositivity increases gradually with age, reaching 50% by age 50 years in the United States. In this population, acute HAV tends to be a sporadic febrile illness. Overall, HAV accounts for about 25% of clinically evident acute hepatitis worldwide and an estimated 270,000 new cases per year in the United States.[4]

HAV is a small, nonenveloped, single-stranded RNA picornavirus that occupies its own genus, *Hepatovirus*. Ultrastructurally, HAV is an icosahedral capsid 27 nm in diameter. HAV

TABLE 18-6 -- The Hepatitis Viruses

	Hepatitis A Virus	Hepatitis B Virus	Hepatitis C Virus	Hepatitis D Virus	Hepatitis E Virus	Hepatitis G Virus *
Agent	Icosahedral capsid, ssRNA	Enveloped dsDNA	Enveloped ssRNA	Enveloped ssRNA	Unenveloped ssRNA	ssRNA virus
Transmission	Fecal-oral	Parenteral; close contact	Parenteral; close contact	Parenteral; close contact	Waterborne	Parenteral
Incubation period	2–6 wk	4–26 wk	2–26 wk	4–7 wk	2–8 wk	Unknown
Carrier state	None	0.1–1.0% of blood donors in U.S. and Western world	0.2–1.0% of blood donors in U.S. and Western world	1–10% in drug addicts and hemophiliacs	Unknown	1–2% of blood donors in U.S.
Chronic hepatitis	None	5–10% of acute infections	>50%	<50% coinfection, 80% upon superinfection	None	None
Hepatocellular carcinoma	No	Yes	Yes	No increase above HBV	Unknown, but unlikely	None

*At present, hepatitis G virus is not considered pathogenic.

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is spread by ingestion of contaminated water and foods and is shed in the stool for 2 to 3 weeks before and 1 week after the onset of jaundice. Thus, close personal contact with an infected individual or fecal-oral contamination during this period accounts for most cases and explains the outbreaks in institutional settings such as schools and nurseries and the waterborne epidemics in places where people live in overcrowded, unsanitary conditions. HAV is not shed in any significant quantities in saliva, urine, or semen. In developed countries, sporadic infections may be contracted by the consumption of raw or steamed shellfish (oysters, mussels, clams), which concentrate the virus from seawater contaminated with human sewage. Infected workers in the food industry may also be the source of outbreaks. Outbreaks of the disease in September and November, 2003, in the United States involved more than 600 infected persons and caused at least three deaths. Consumption of raw green onions contaminated with HAV was the most likely cause of these outbreaks. *Because HAV viremia is transient, blood-borne transmission of HAV occurs only rarely; therefore, donated blood is not specifically screened for this virus.*

Serologic Diagnosis.

Specific antibody against HAV of the immunoglobulin (Ig) M type appears in blood at the onset of symptoms, constituting a reliable marker of acute infection (Fig. 18-8). Fecal shedding of the virus ends as the IgM titer rises. The IgM response usually begins to decline in a few months and is followed by the appearance of IgG anti-HAV. The latter persists for years,

perhaps for life, providing protective immunity against reinfection by all strains of HAV. Hence, the HAV vaccine is effective.

Hepatitis B Virus

Hepatitis B virus (HBV) can produce (1) acute hepatitis with resolution, (2) chronic hepatitis, which may evolve to cirrhosis, (3) fulminant hepatitis with massive liver necrosis, and (4) the backdrop for hepatitis D virus infection. Patients with chronic hepatitis represent carriers of actively replicating virus and hence are a source of infection to other individuals.[17] HBV also plays an important role in the development of hepatocellular carcinoma. The approximate frequencies of clinical outcomes of HBV infection are depicted in Figure 18-9 .

Liver disease due to HBV is an enormous problem globally, with an estimated worldwide carrier rate of 350 million. It is estimated that HBV has infected over 2 billion of the individuals alive today at some point in their lives. Seventy-five percent of all chronic carriers live in Asia and the Western Pacific rim. The global prevalence of chronic hepatitis B infection varies widely, from high (>8%) in Africa, Asia, and the Western Pacific to intermediate (2% to 7%) in Southern and Eastern Europe to low (<2%) in Western Europe, North America, and Australia. In the United States alone, there are

Figure 18-8 Sequence of serologic markers in acute hepatitis A viral hepatitis.

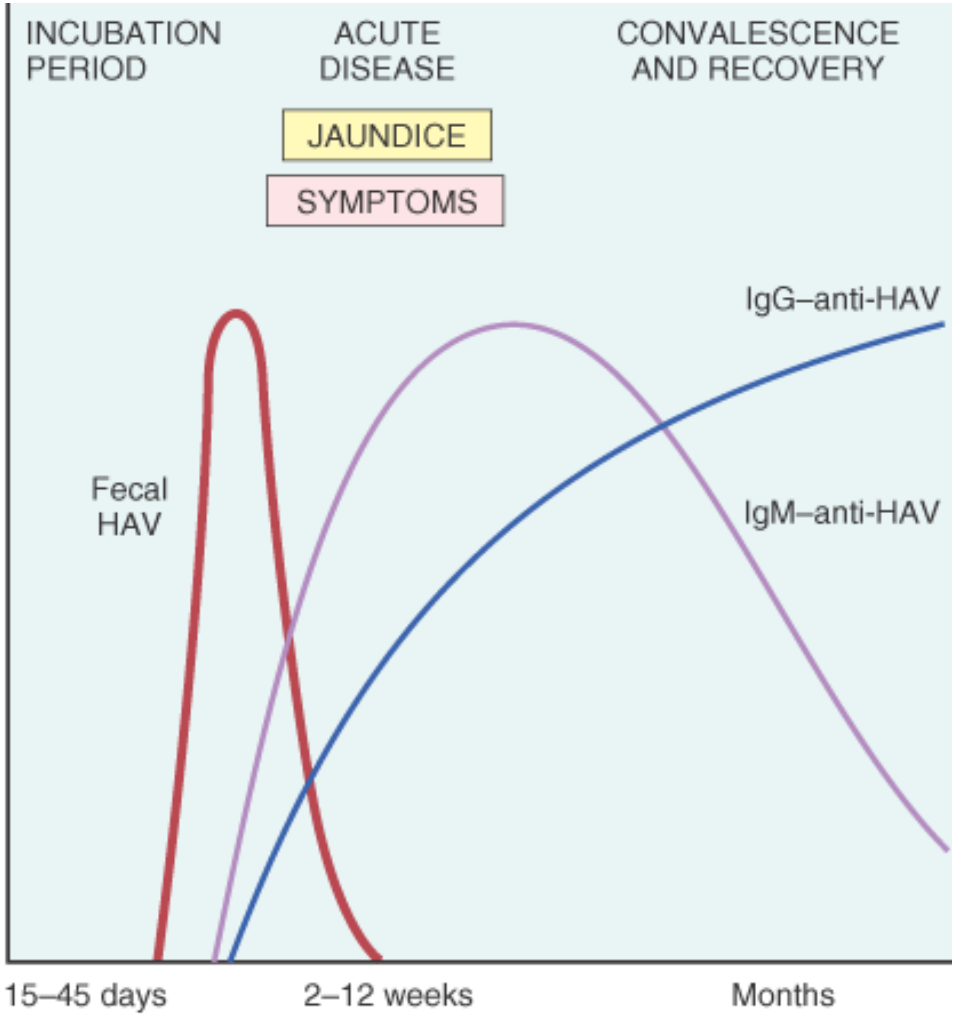

Figure 18-9 Schematic of the potential outcomes of hepatitis B infection in adults, with their approximate frequencies in the United States.

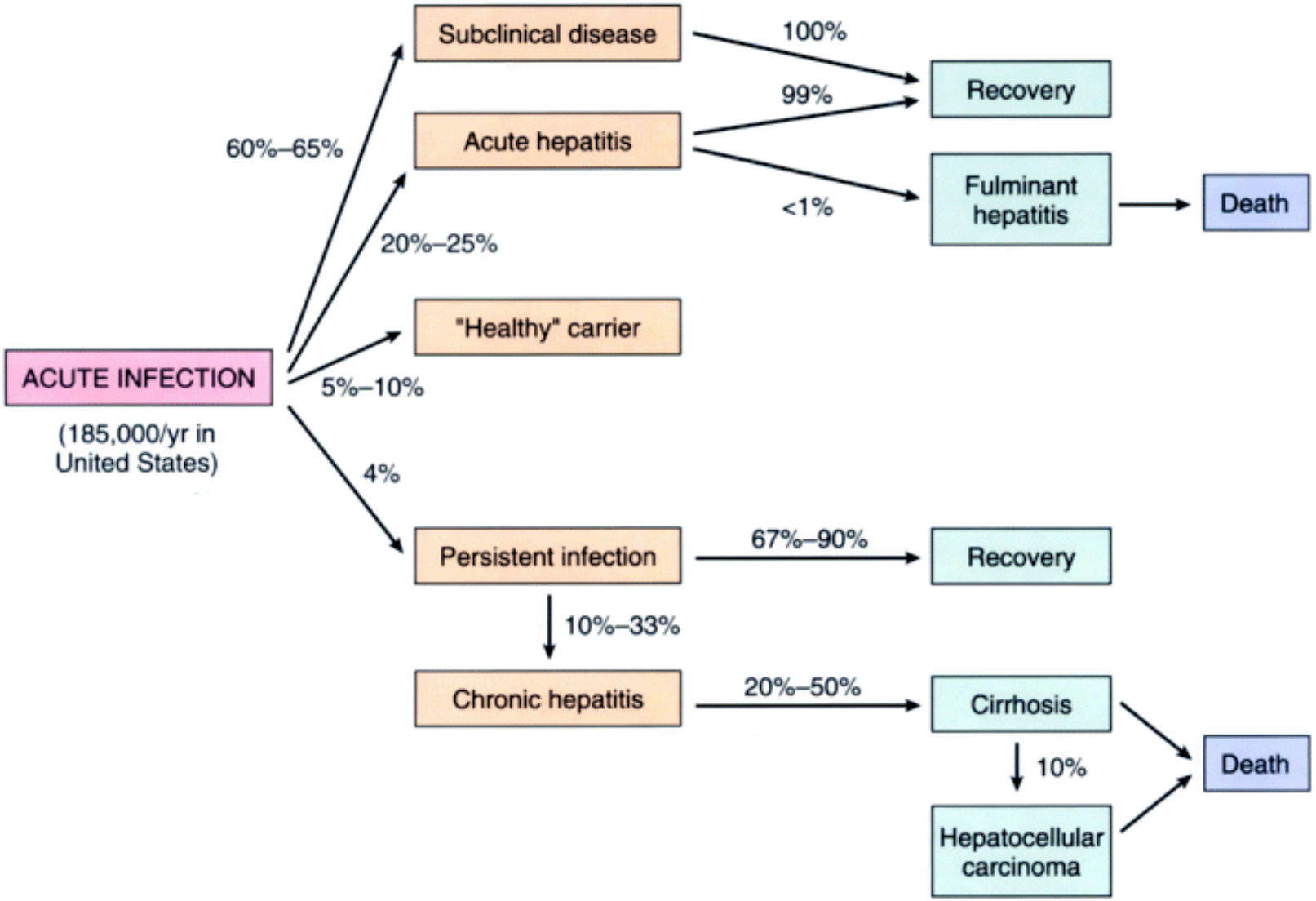

Figure 18-10 Diagrammatic representation of genomic structure and transcribed components of the hepatitis B virion. The innermost circles represent the DNA (+) strand and the DNA (-) strand of the virion. The thick bars labeled "P," "X," "pre-C," "C," "pre-S1," "pre-S2," and "S" denote the peptides derived from the virion. The outermost lines denote the mRNA transcripts of the virion. *(After Kidd-Ljunggren K, Myakawa Y, Kidd AH: J Gen Virol 83:1267–1280, 2002.)*

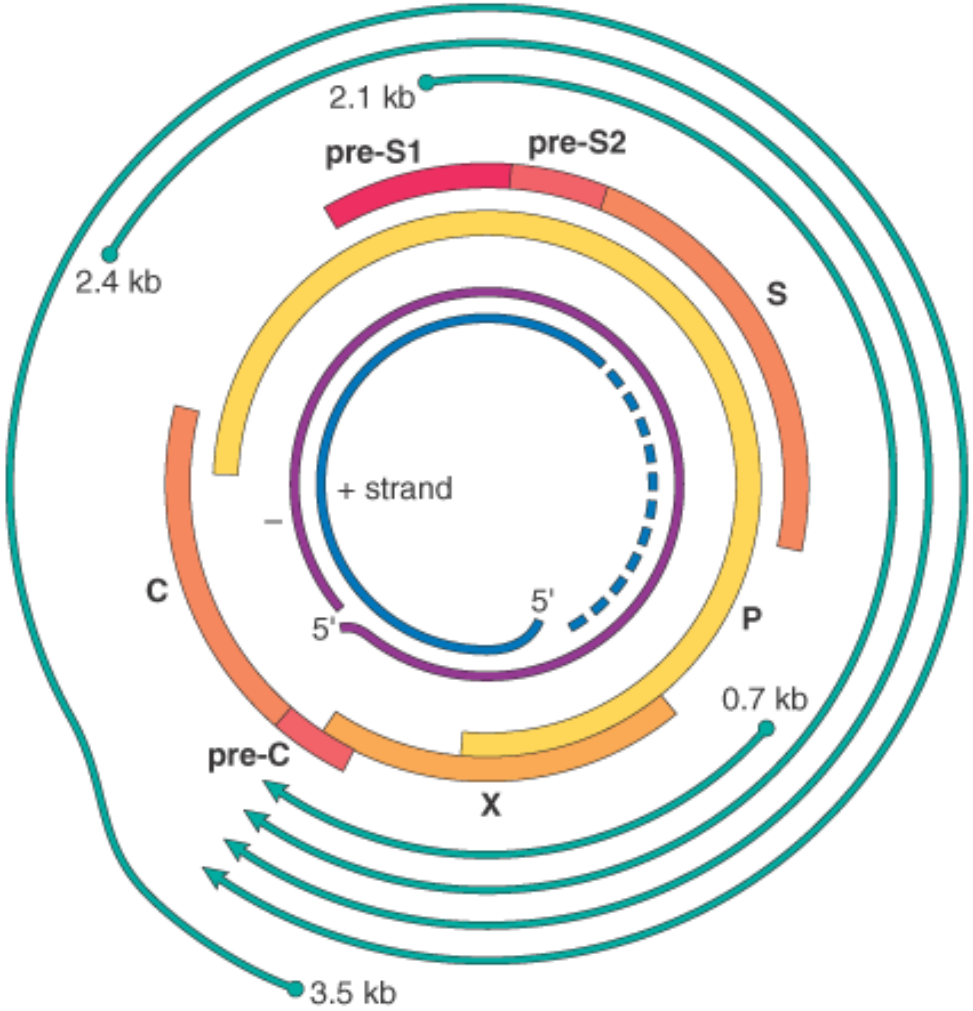

Figure 18-11 Sequence of serologic markers for hepatitis B viral hepatitis demonstrating *(A)* acute infection with resolution and *(B)* progression to chronic infection.

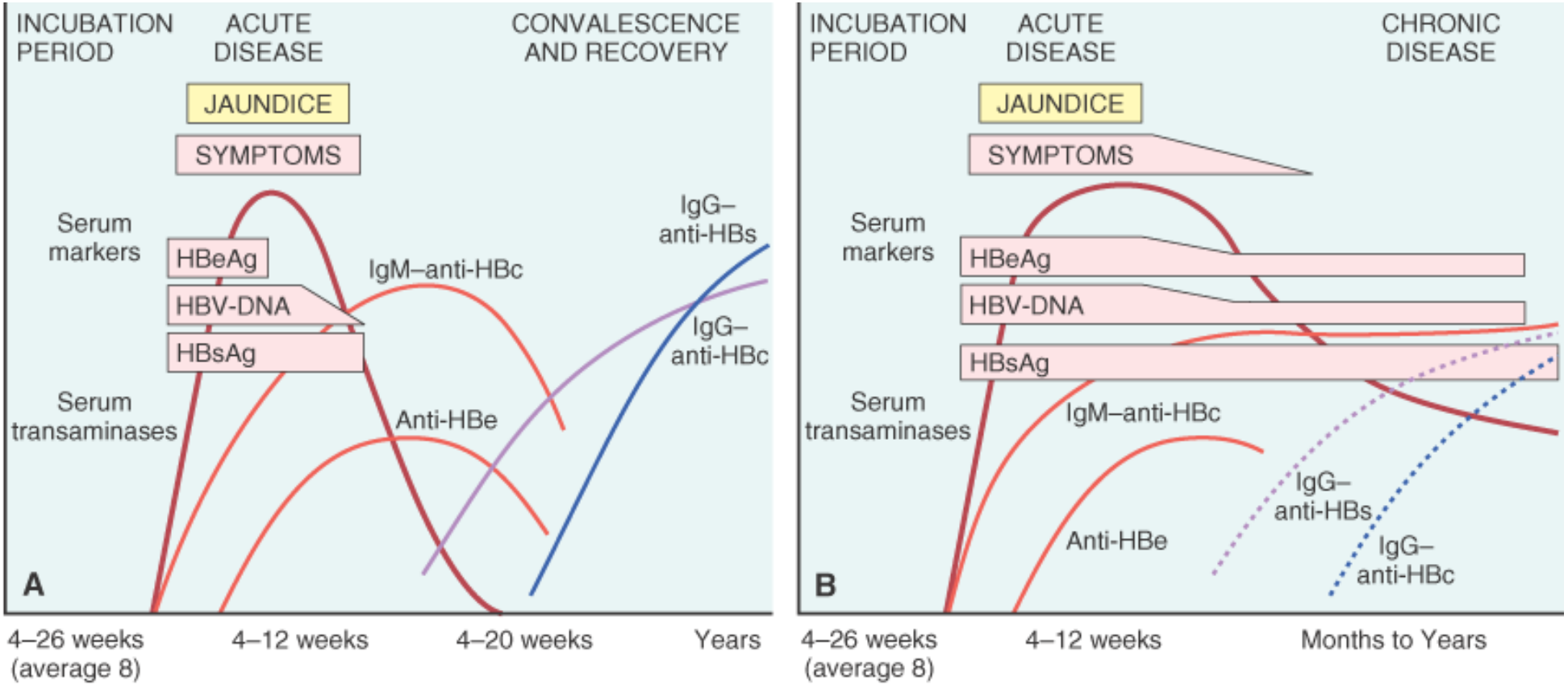

Figure 18-12 Schematic of the potential outcomes of hepatitis C infection in adults, with their approximate frequencies in the United States.

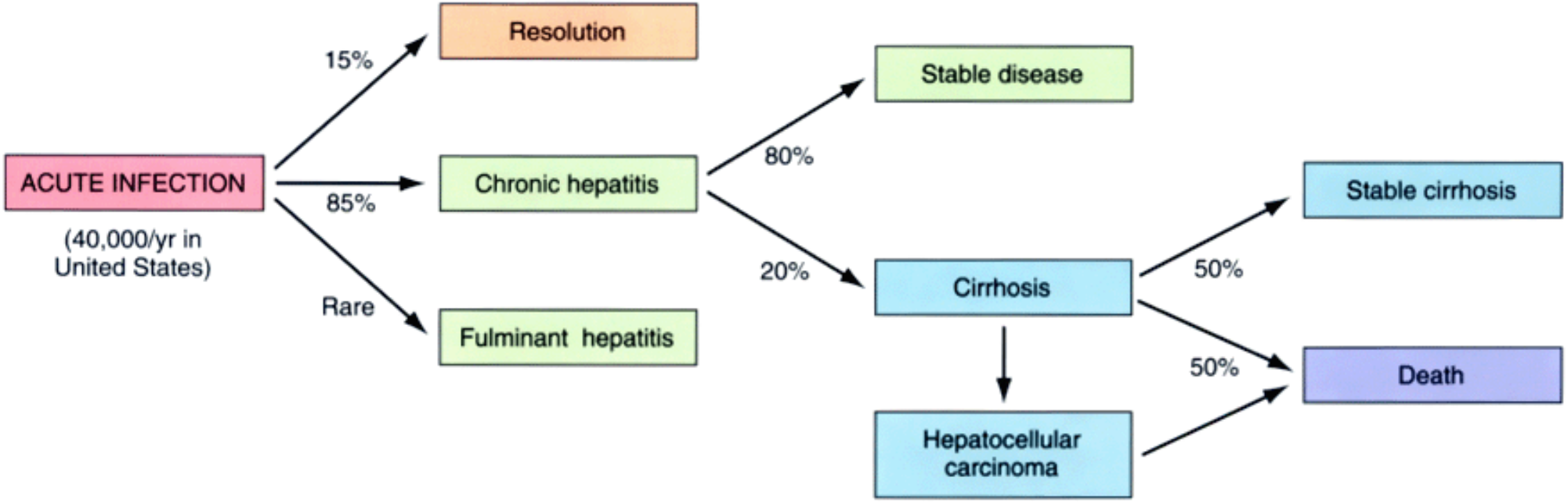

Figure 18-13 Diagrammatic representation of the genomic structure and transcribed components of the hepatitis C virion. The hepatitis C virion is transcribed in one single transcript, as depicted in the top line; 340 nucleotides at the 5' end and 128 nucleotides at the 3' end are not translated into protein. The protein products cleaved from the single translated peptide are shown in the bottom bar.

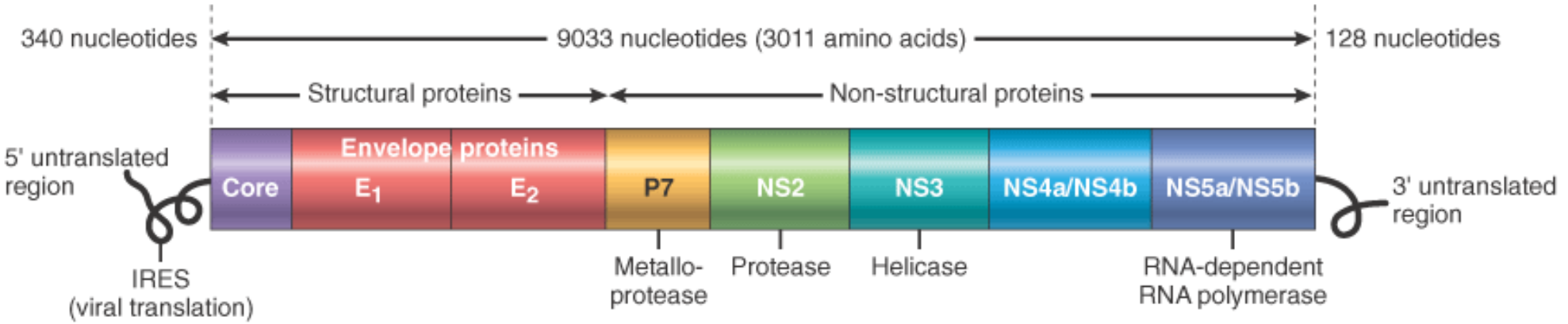

Figure 18-14 Sequence of serologic markers for hepatitis C viral hepatitis demonstrating (*A*) acute infection with resolution and (*B*) progression to chronic relapsing infection.

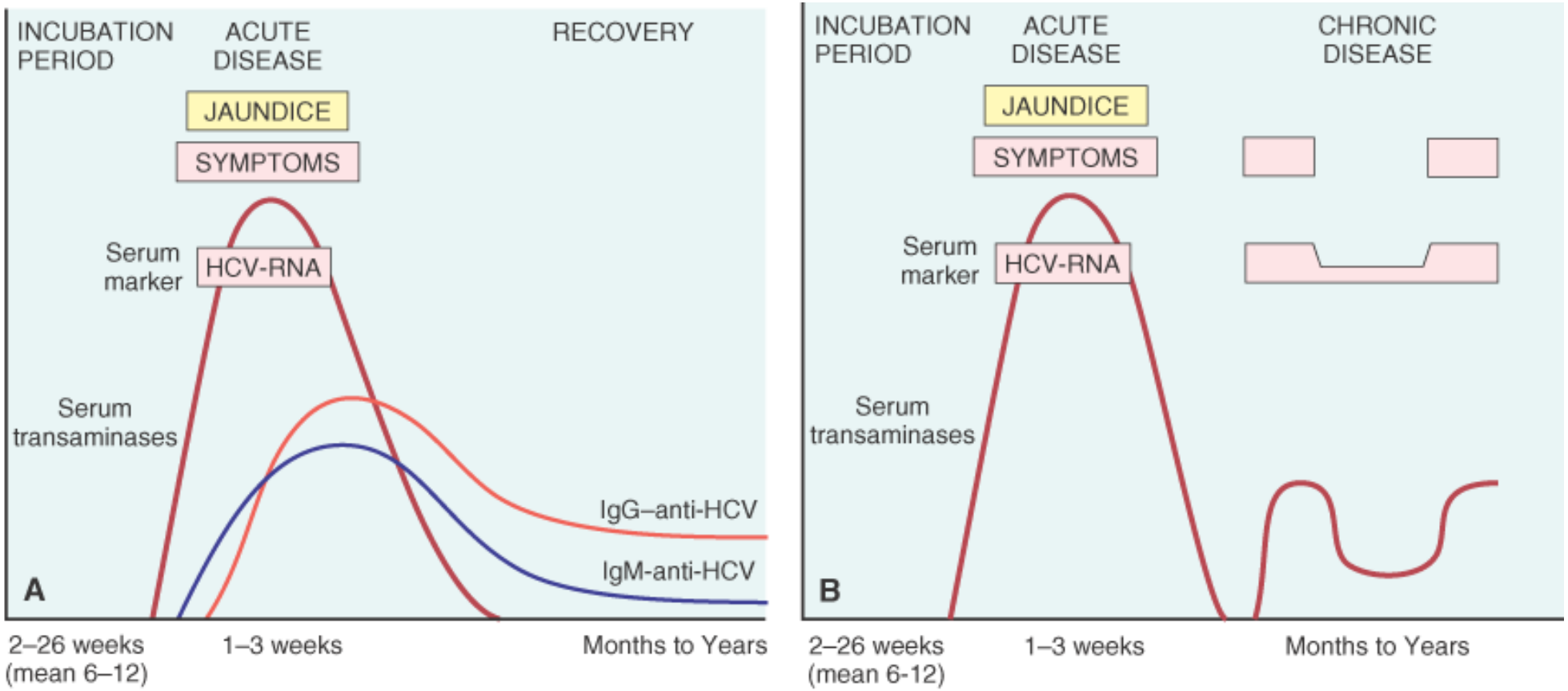

Figure 18-15 Differing clinical consequences of two patterns of combined hepatitis D virus and hepatitis B virus infection.

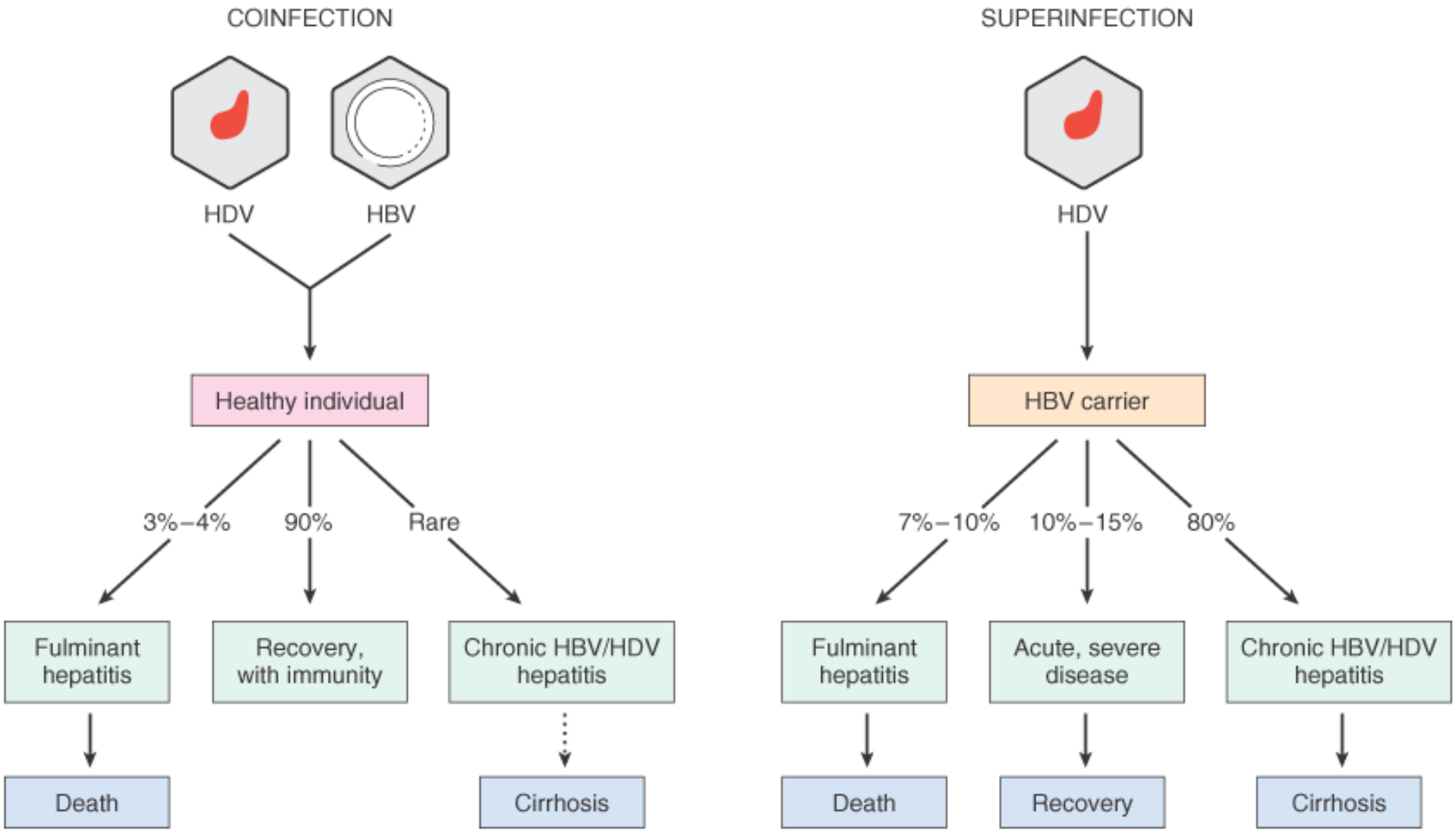

Figure 18-16 Sequence of serologic markers for hepatitis D viral hepatitis depicting (*A*) coinfection with hepatitis B virus (HBV) and (*B*) superinfection of an HBV carrier.

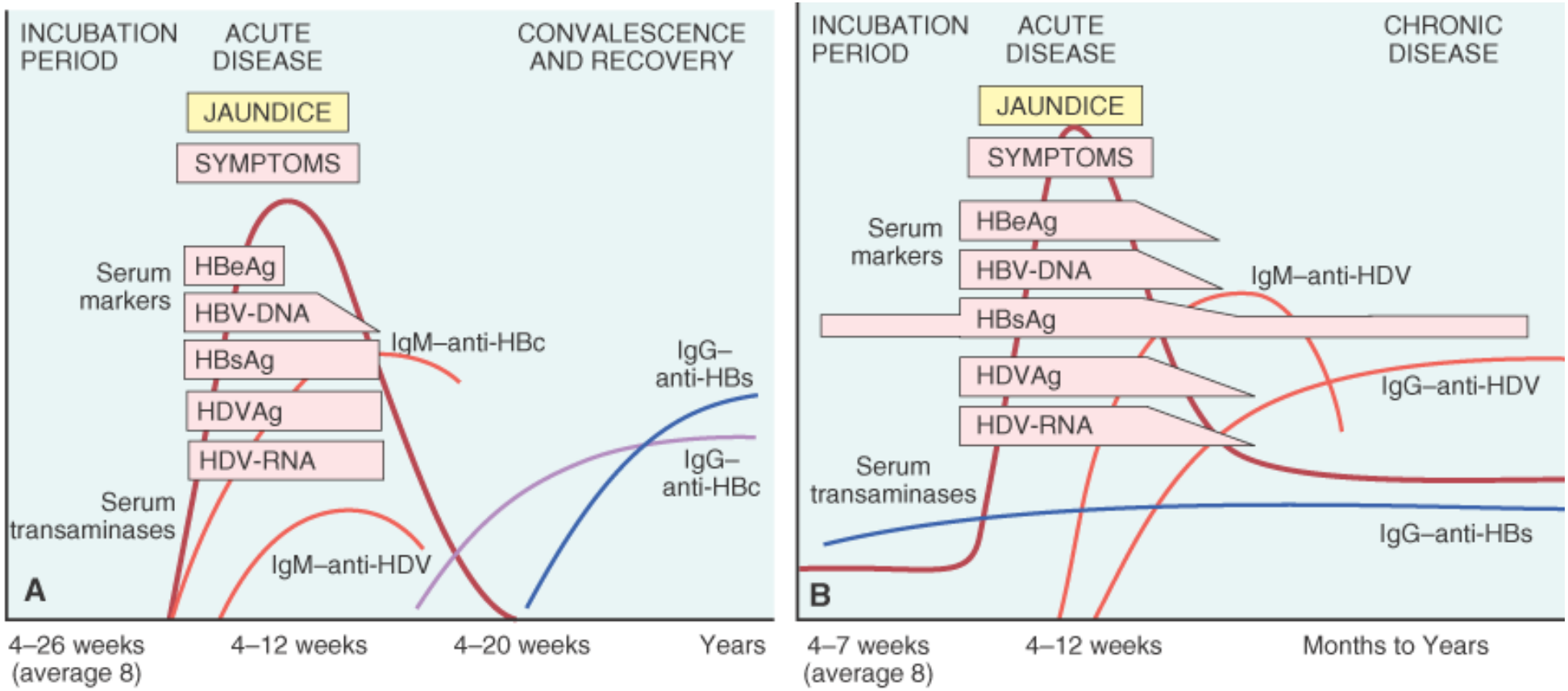

TABLE 18-7 -- Key Morphologic Features of Viral Hepatitis

Acute Hepatitis
Enlarged, reddened liver; greenish if cholestatic
Parenchymal changes:
••Hepatocyte injury: swelling (ballooning degeneration)
••••Cholestasis: canalicular bile plugs
••••HCV: mild focal fatty change of hepatocytes
••Hepatocyte necrosis: isolated cells or clusters
••••Cytolysis (rupture) or apoptosis (shrinkage)
••••If severe: bridging necrosis (portal-portal, central-central, portal-central)
••••Lobular disarray: loss of normal architecture
••Regenerative changes: hepatocyte proliferation

••Sinusoidal cell reactive changes:
••••Accumulation of phagocytosed cellular debris in Kupffer cells
••••Influx of mononuclear cells into sinusoids
••Portal tracts:
••••Inflammation: predominantly mononuclear
••••Inflammatory spillover into adjacent parenchyma, with hepatocyte necrosis
Chronic Hepatitis
Changes shared with acute hepatitis:
••Hepatocyte injury, necrosis, and regeneration
••Sinusoidal cell reactive changes
Portal tracts:
••Inflammation:
••••Confined to portal tracts, *or*
••••Spillover into adjacent parenchyma, with necrosis of hepatocytes ("interface hepatitis"), *or*
••••Bridging inflammation and necrosis
••Fibrosis:
••••Portal deposition, *or*
••••Portal and periportal deposition, *or*
••••Formation of bridging fibrous septa
HBV: "ground-glass" hepatocytes, "sanded" nuclei
HCV: bile duct epithelial cell proliferation, lymphoid aggregate formation
Cirrhosis: The end-stage outcome

The canalicular bile plugs result from cessation of the contractile activity of the hepatocyte pericanalicular actin microfilament web. Two patterns of hepatocyte cell death are seen. In the first, rupture of cell membranes leads to cytolysis and focal loss of hepatocytes. The sinusoidal collagen reticulin framework collapses where the cells have disappeared, and scavenger **macrophage aggregates** mark sites of hepatocyte loss. The second pattern of cell death, **apoptosis**, is more conspicuous. It is caused by anti-viral cytotoxic T cells. Apoptotic hepatocytes shrink, become intensely eosinophilic, and have fragmented nuclei; effector T cells may still be present in the immediate vicinity. Apoptotic cells also are phagocytosed within hours by macrophages and hence might be difficult to find despite a brisk rate of hepatocyte injury. In severe cases of acute hepatitis (not depicted in Fig. 18-17*A*), confluent necrosis of hepatocytes may lead to **bridging necrosis** connecting portal-to-portal, central-to-central, or portal-to-central regions of adjacent lobules. Hepatocyte swelling and regeneration compress sinusoids, and the more or less radial array of the parenchyma is lost.

Inflammation is a characteristic and usually prominent feature of acute hepatitis. **Kupffer cells undergo hypertrophy and hyperplasia** and are often laden with lipofuscin pigment due to phagocytosis of hepatocellular debris. **The portal tracts are usually infiltrated with a mixture of inflammatory cells.** The inflammatory infiltrate may spill over into the adjacent parenchyma to cause necrosis of periportal hepatocytes; this **"interface hepatitis"** can occur in both acute and chronic hepatitis. Finally, bile duct epithelia may become reactive and even

proliferate to form poorly defined ductular structures (ductular reaction), particularly in cases of HCV hepatitis.

Chronic Hepatitis.

The histologic features of chronic hepatitis (Fig. 18-17*B* and Fig. 18-20) range from exceedingly mild to severe. In the mildest forms, significant inflammation is limited to portal tracts and consists of lymphocytes, macrophages, occasional plasma cells, and rare neutrophils or eosinophils. Liver architecture is usually well preserved, but smoldering hepatocyte necrosis throughout the lobule may occur in all forms of chronic hepatitis. Even in mild chronic hepatitis due to HCV infection, common findings are **lymphoid aggregates** and **bile duct damage** in the portal tracts and focally mild to moderate macrovesicular **steatosis**. In all forms of chronic hepatitis, continued **interface hepatitis** and **bridging necrosis** are harbingers of progressive liver damage. **The hallmark of irreversible liver damage is the deposition of fibrous tissue.** At first, only portal tracts exhibit increased fibrosis, but with time, **periportal** septal **fibrosis** occurs, followed by linking of fibrous septa between lobules (**bridging fibrosis**).

Continued loss of hepatocytes and fibrosis results in cirrhosis, with fibrous septae and hepatocyte regenerative nodules. This pattern of cirrhosis is characterized by irregularly sized nodules separated by variable but mostly broad scars (Fig. 18-21). Historically, this pattern of cirrhosis has been termed **postnecrotic cirrhosis**, but it should be noted that the term "postnecrotic cirrhosis" has been applied to all forms of cirrhosis in which the liver shows large, irregular-sized nodules with broad scars, regardless of etiology. Autoimmune hepatitis, hepatotoxins (carbon tetrachloride, mushroom poisoning), pharmaceutical drugs (acetaminophen, α-methyldopa), and even alcohol (discussed later) may give rise to a cirrhotic liver with irregular-sized large nodules. In some cases that come to autopsy, the inciting cause of the so-called postnecrotic cirrhosis cannot be determined at all ("cryptogenic cirrhosis"). In essence, the morphology of the end-stage cirrhotic liver is neither helpful in determining the basis of the liver injury, nor can it be easily related to any specific set of clinical circumstances.

The clinical course of viral hepatitis is unpredictable. Patients may experience spontaneous remission or may have indolent disease without progression for many years. Conversely, some patients have rapidly progressive disease and develop cirrhosis within a few years. The major causes of death are cirrhosis, with liver failure and hepatic encephalopathy or massive hematemesis from esophageal varices, and hepatocellular carcinoma in those with long-standing HBV (particularly neonatal) or HCV infection.

Fulminant Hepatitis

When hepatic insufficiency progresses from onset of symptoms to hepatic encephalopathy within 2 to 3 weeks, it is

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Figure 18-17 Diagrammatic representations of the morphologic features of acute and chronic hepatitis. Bridging necrosis (and fibrosis) is shown only for chronic hepatitis; bridging necrosis may also occur in acute hepatitis (not shown).

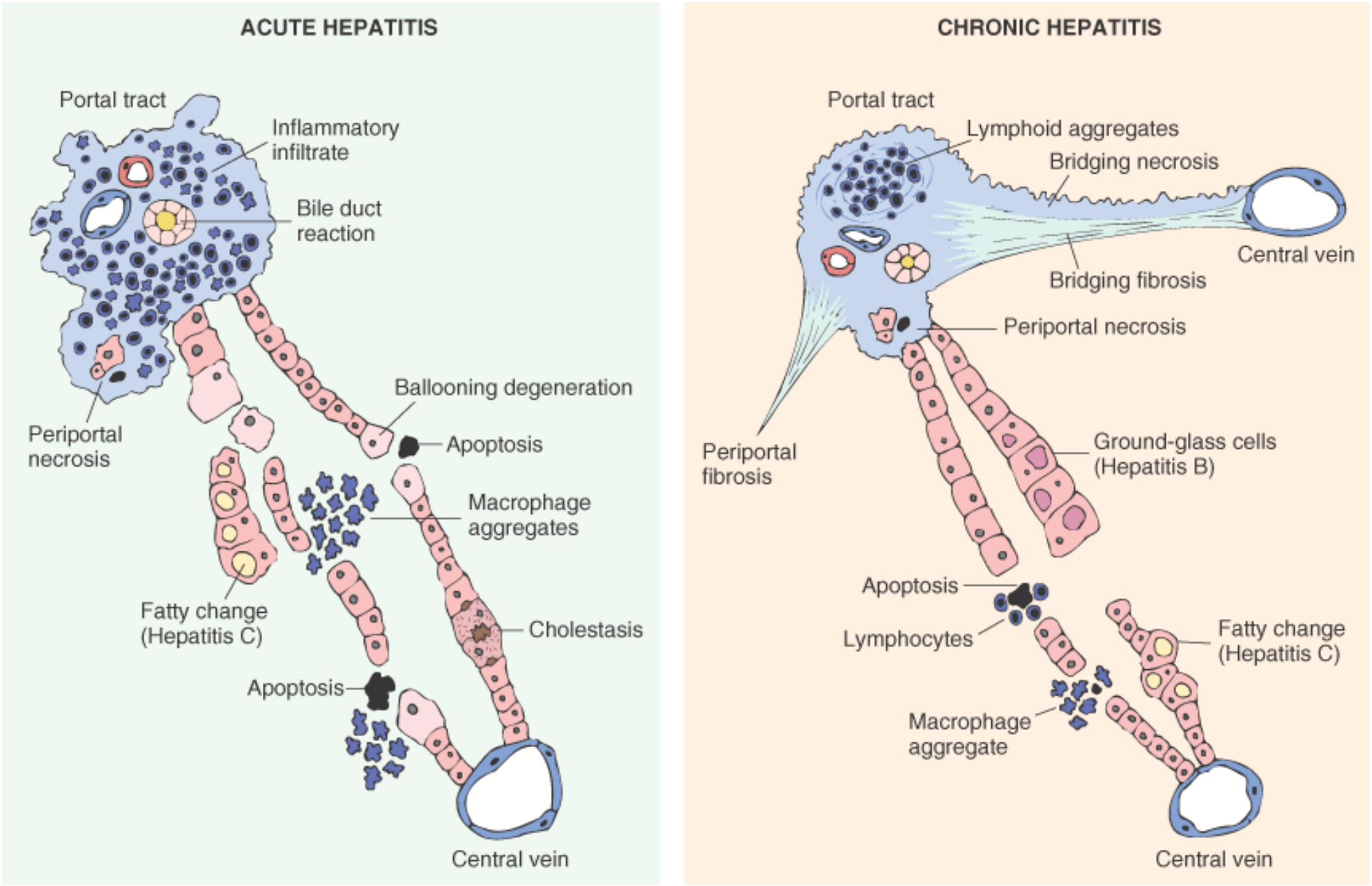


Figure 18-18 Hepatitis B viral infection. *A,* Liver parenchyma showing hepatocytes with diffuse granular cytoplasm, so-called ground glass hepatocytes. (H&E) *B,* Immunoperoxidase stain for HBsAg from the same case, showing cytoplasmic inclusions of viral particles.

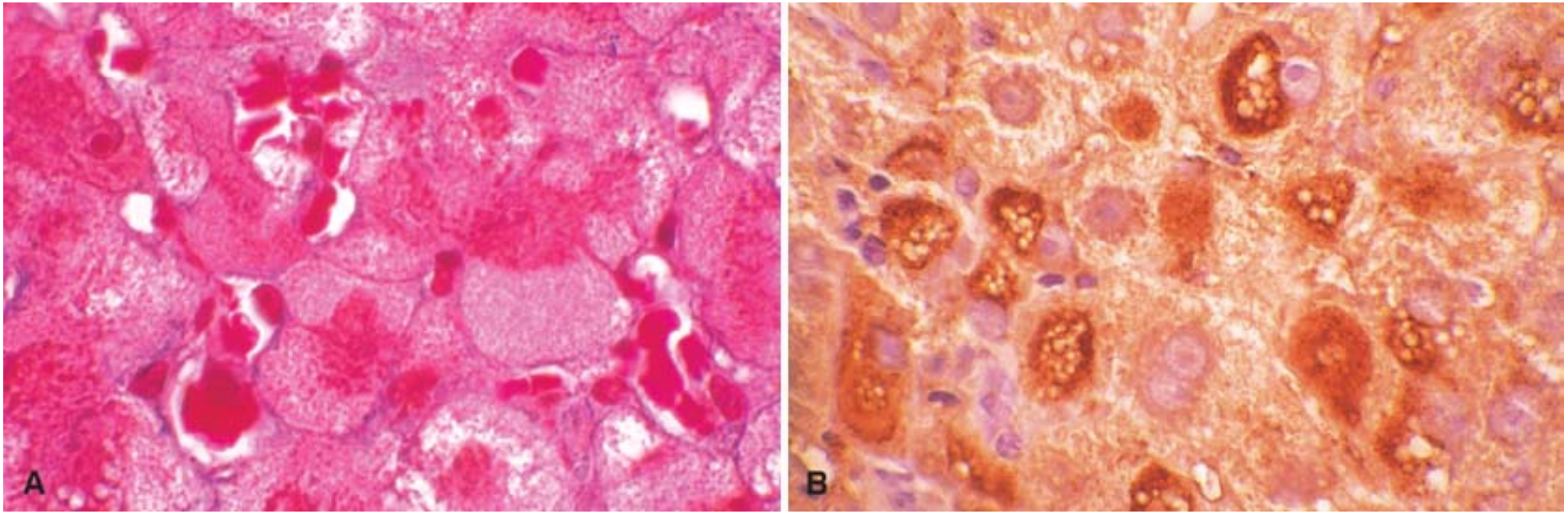


Figure 18-19 Acute viral hepatitis showing disruption of lobular architecture, inflammatory cells in the sinusoids, and hepatocellular apoptosis (*arrow*).

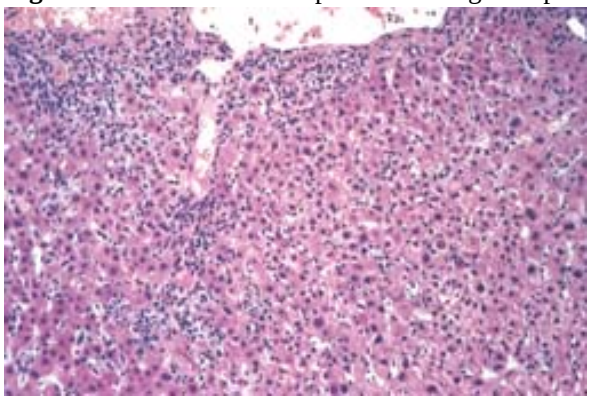


Figure 18-20 Chronic viral hepatitis due to hepatitis C virus, showing portal tract expansion with inflammatory cells and fibrous tissue and interface hepatitis with spillover of inflammation into the adjacent parenchyma. A lymphoid aggregate is present.

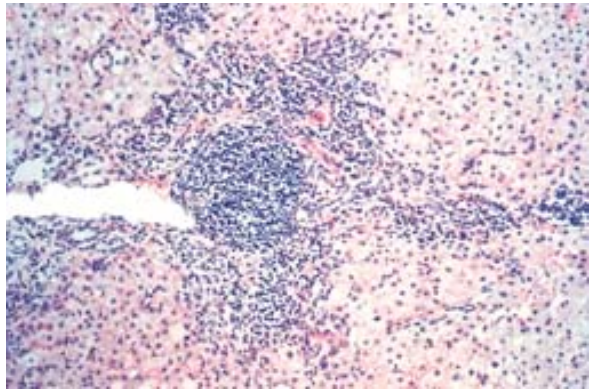
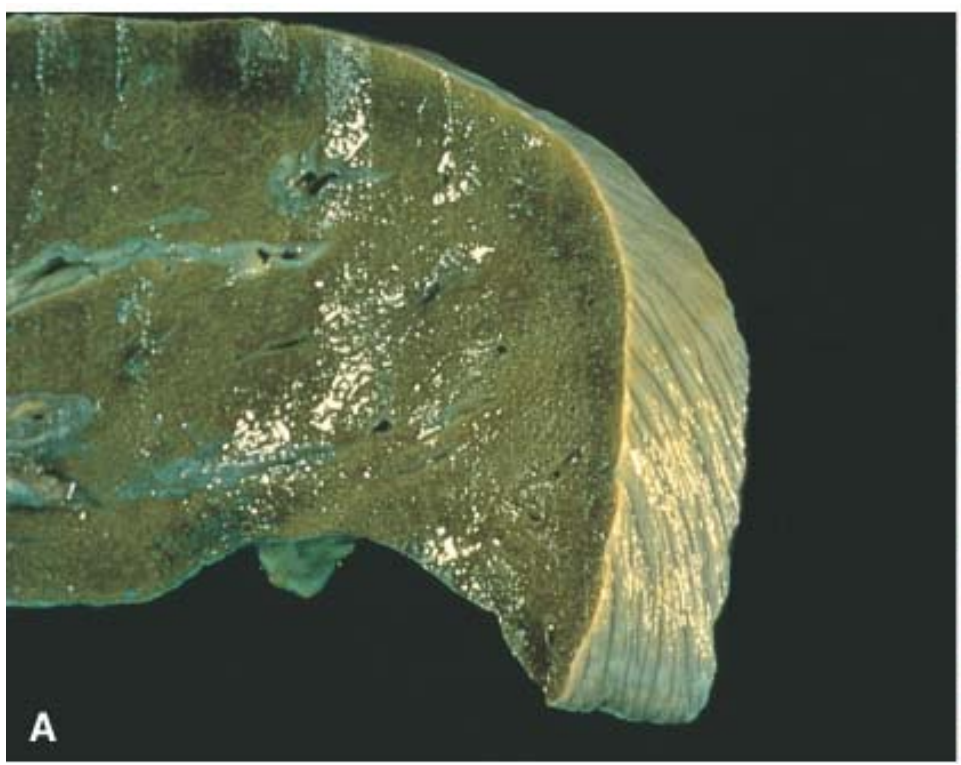


Figure 18-21 Cirrhosis resulting from chronic viral hepatitis. Note the broad scar and coarse nodular surface.

Figure 18-22 Massive necrosis. *A*, Cut section of liver. The liver is small (700 gm), bile-stained, and soft. The capsule is wrinkled. *B*, Microscopic section. Portal tracts and terminal hepatic veins are closer together than normal, owing to necrosis and collapse of the intervening parenchyma. The rudimentary ductal structures are the result of early ductular regeneration. An infiltrate of mononuclear inflammatory cells is present.



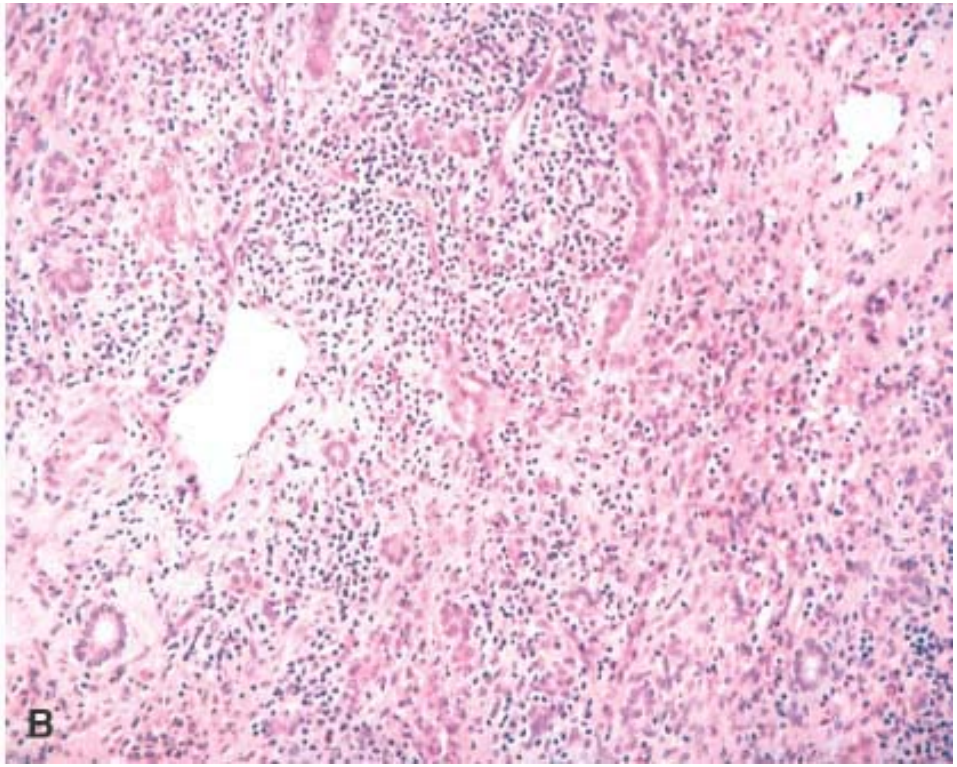


TABLE 18-8 -- Drug- and Toxin-Induced Hepatic Injury

Hepatocellular Damage	Examples
Microvesicular fatty change	• Tetracycline, salicylates, yellow phosphorus, ethanol
Macrovesicular fatty change	• Ethanol, methotrexate, amiodarone
Centrilobular necrosis	• Bromobenzene, CCl_4 , acetaminophen, halothane, rifampin
Diffuse or massive necrosis	• Halothane, isoniazid, acetaminophen, methyldopa, trinitrotoluene, *Amanita phalloides* (mushroom) toxin
Hepatitis, acute and chronic	• Methyldopa, isoniazid, nitrofurantoin, phenytoin, oxyphenisatin
Fibrosis-cirrhosis	• Ethanol, methotrexate, amiodarone, most drugs that cause chronic hepatitis
Granuloma formation	• Sulfonamides, methyldopa, quinidine, phenylbutazone, hydralazine, allopurinol
Cholestasis (with or without hepatocellular injury)	• Chlorpromazine, anabolic steroids, erythromycin estolate, oral contraceptives, organic arsenicals

Among the agents listed in Table 18-8 , hepatic injury is considered predictable from overdoses of acetaminophen (also called phenacetin or paracetamol) and exposure to *Amanita phalloides* toxin, carbon tetrachloride, and, to a certain extent, alcohol. However, individual genetic differences in the hepatic metabolism of xenobiotics through activating and detoxification pathways play a major role in individual susceptibility to "predictable" hepatotoxins. Many other xenobiotics, such as sulfonamides, α-methyldopa, and allopurinol, cause idiosyncratic reactions. *Reye syndrome*, a potentially fatal syndrome of mitochondrial dysfunction in liver, brain, and elsewhere, occurs predominantly in children who are given acetylsalicylic acid (aspirin) for the relief of virus-induced fever. This disease, which features extensive accumulation of fat droplets within hepatocytes (microvesicular steatosis), is

exceedingly rare. A causal relationship with use of salicylates was never established, but a national campaign in the 1970s and 1980s warning against the use of aspirin in children with febrile illness might have served to break the Reye syndrome epidemic.

Drug-induced liver disease is usually followed by recovery upon removal of the drug. *Exposure to a toxin or therapeutic agent should always be included in the differential diagnosis of liver disease.*

Figure 18-23 Alcoholic liver disease. The interrelationships among hepatic steatosis, hepatitis, and cirrhosis are shown, along with a depiction of key morphologic features at the morphologic level.

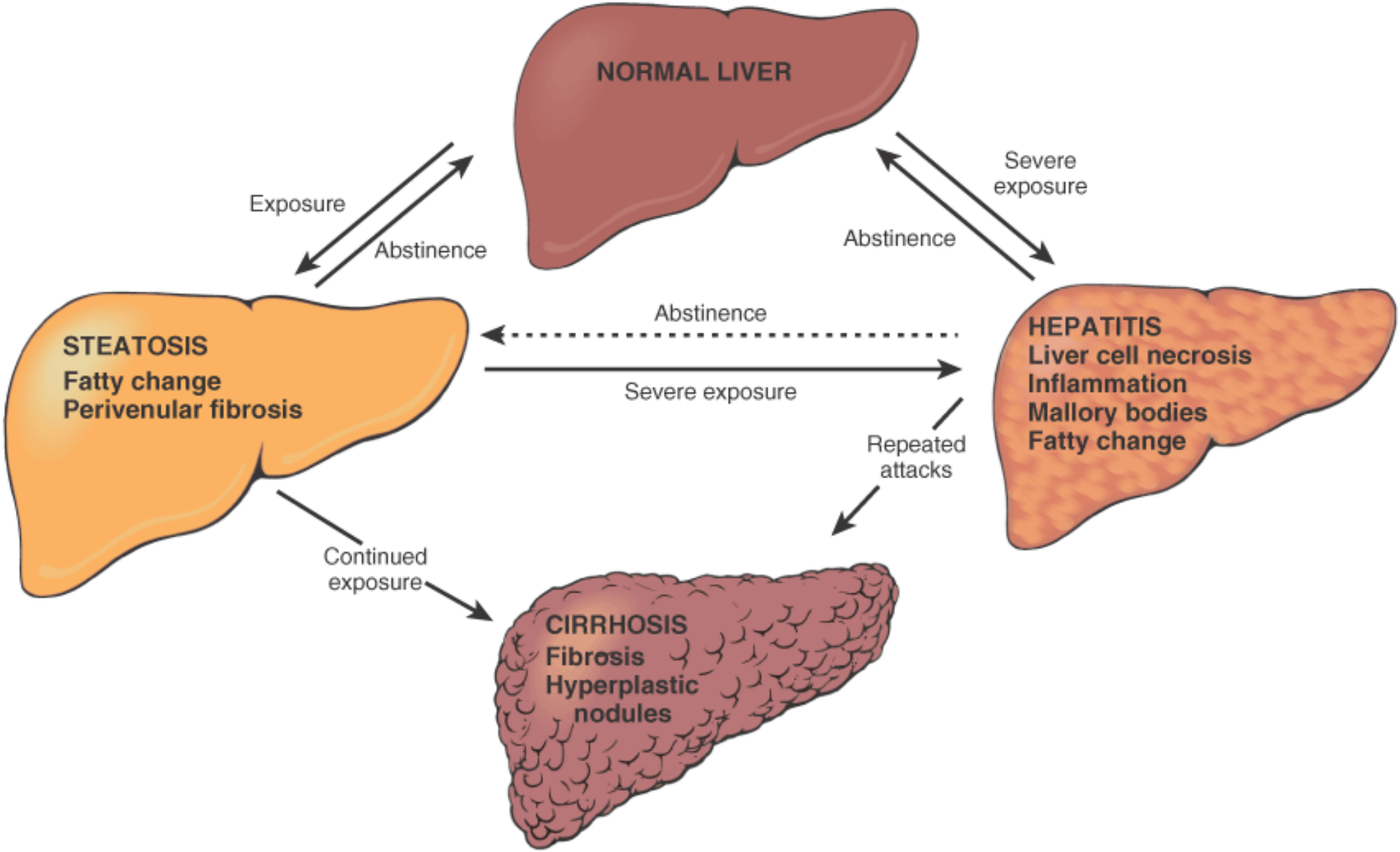


Figure 18-24 Alcoholic liver disease: macrovesicular steatosis, involving most regions of the hepatic lobule. The intracytoplasmic fat is seen as clear vacuoles. Some early fibrosis (stained blue) is present (Masson trichrome).

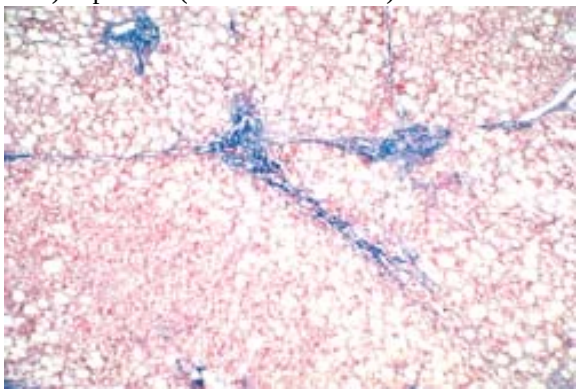


Figure 18-25 Alcoholic hepatitis. *A*, The cluster of inflammatory cells marks the site of a necrotic hepatocyte. A Mallory body is present in a second hepatocyte (*arrow*). *B*, Eosinophilic Mallory bodies are seen in hepatocytes, which are surrounded by fibrous tissue (H&E).

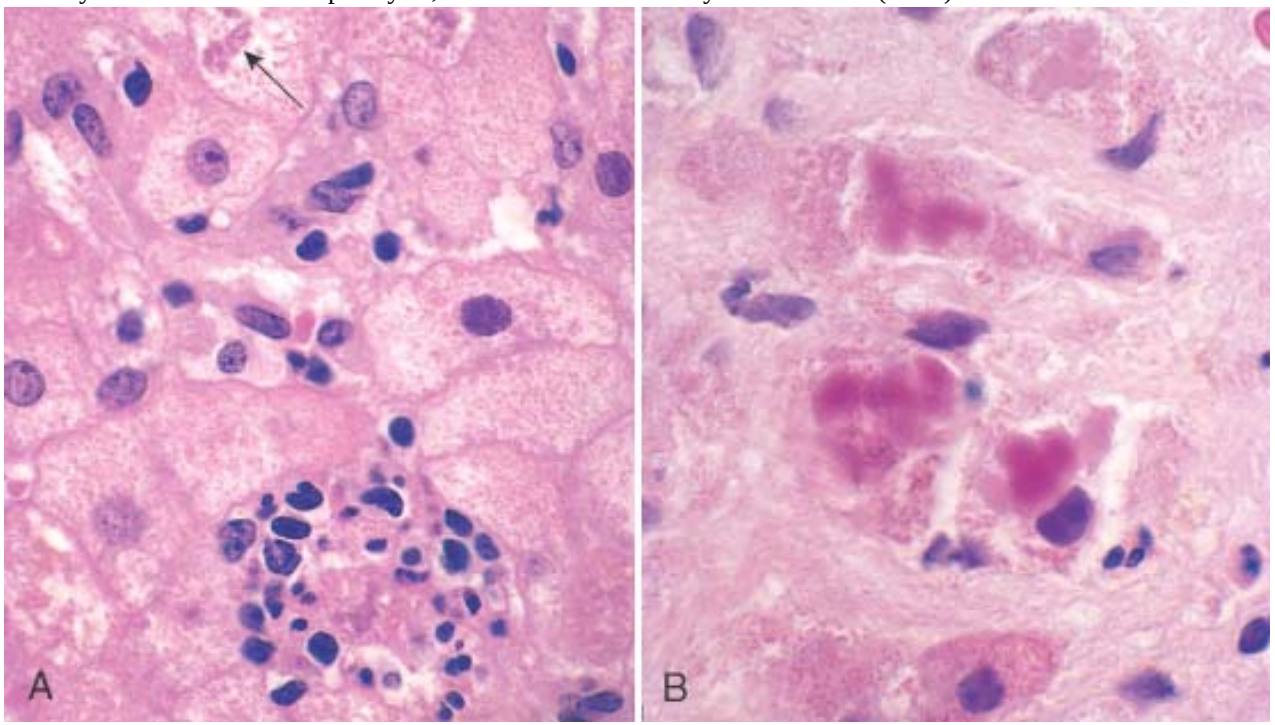
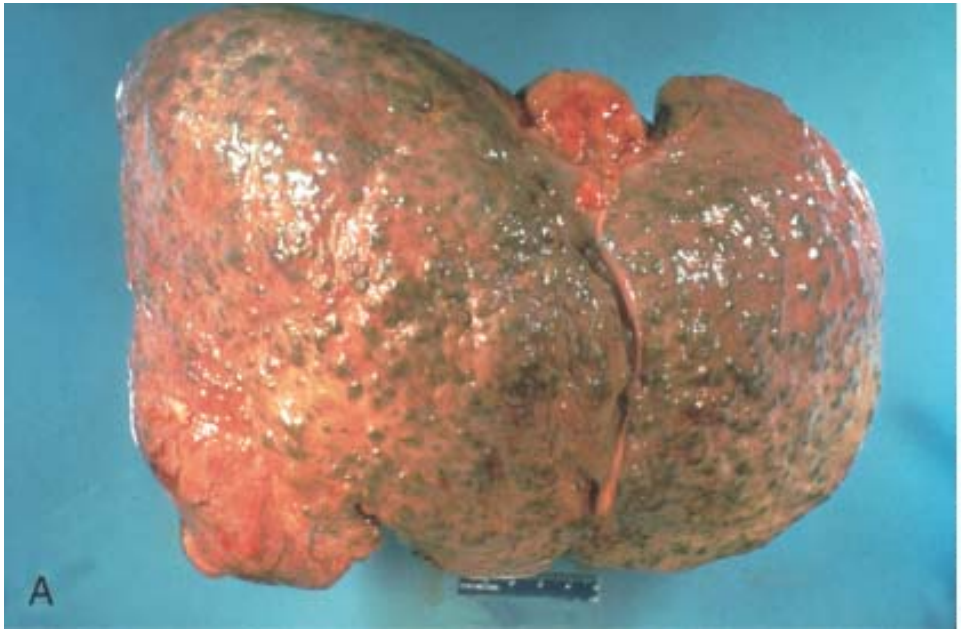


Figure 18-26 Alcoholic cirrhosis. *A*, The characteristic diffuse nodularity of the surface reflects the interplay between nodular regeneration and scarring. The greenish tint of some nodules is due to bile stasis. A hepatocellular carcinoma is present as a budding mass at the lower edge of the right lobe (lower left of figure). *B*, The microscopic view shows nodules of varying sizes entrapped in blue-staining fibrous tissue. The liver capsule is at the top (Masson trichrome).



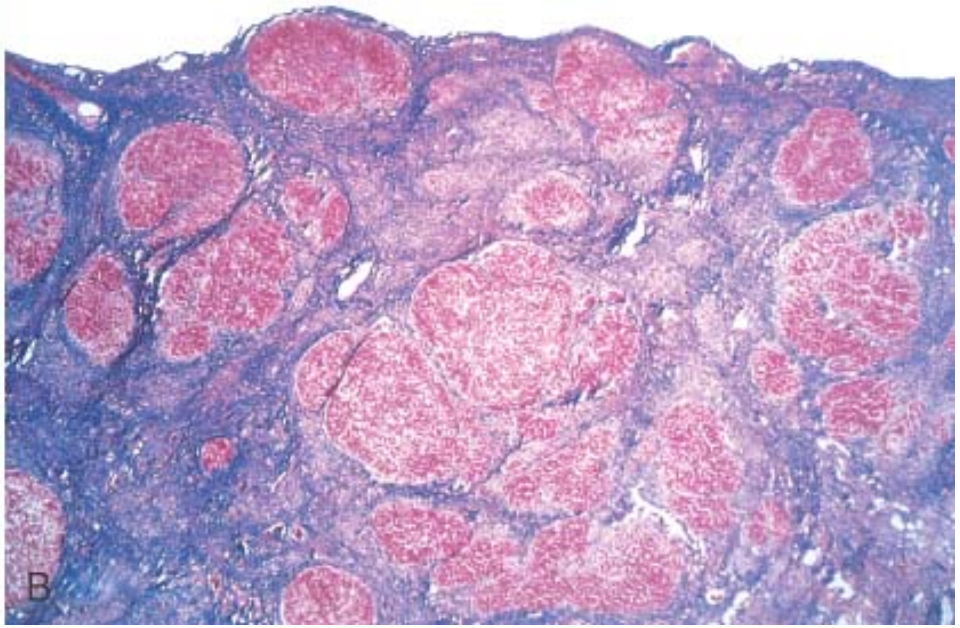


TABLE 18-9 -- Classification of Iron Overload

I. Hereditary Hemochromatosis
II. Secondary Hemochromatosis
••••A. Parenteral iron overload
•••••••Transfusions
••••••••••Long-term hemodialysis
••••••••••Aplastic anemia
••••••••••Sickle cell disease
••••••••••Myelodysplastic syndromes
••••••••••Leukemias
•••••••Iron-dextran injections
••••B. Ineffective erythropoiesis with increased erythroid activity
•••••••β-Thalassemia
•••••••Sideroblastic anemia
•••••••Pyruvate kinase deficiency
••••C. Increased oral intake of iron
•••••••African iron overload (Bantu siderosis)

••••D. Congenital atransferrinemia
••••E. Chronic liver disease
•••••••Chronic alcoholic liver disease
•••••••Porphyria cutanea tarda

In white populations of northern European extraction, the frequency of the C282Y mutation is estimated at 6.4% to 9.5%.[4] The frequency of homozygosity is 0.45% (1 of every 220 persons), and that for heterozygosity is 11% (1 of every 9 persons), making hereditary hemochromatosis one of the most common genetic disorders in humans. However, the penetrance of this disorder is only about 20% in patients with the homozygous C282Y mutation, so the genetic condition does not lead to disease in all individuals.

Pathogenesis.

It may be recalled that the total body content of iron is tightly regulated, as the limited daily losses of iron are matched by gastrointestinal absorption. *In hereditary hemochromatosis, regulation of intestinal absorption of dietary iron is lost, leading to net iron accumulation of 0.5 to 1.0 gm/year.* The disease manifests itself typically after 20 gm of storage iron have accumulated.

The critical site for HFE expression appears to be the basolateral surface of the small intestinal crypt epithelial cell, where it is prominently expressed. According to the current hypothesis (Fig. 18-27),[32] [33] HFE complexes with the transferrin receptor, TfR, enabling the binding of plasma transferrin and its bound iron. The TfR-Tf-iron complex is endocytosed into the crypt enterocyte; acidification of the endosome releases iron into the regulatory iron pool of the crypt cell. This is a sensing mechanism for the systemic iron balance, as increased levels of circulating iron bound to transferrin will lead to an increased iron regulatory pool in enterocytes. This pool "sets" the level of expression of apical iron uptake systems. *Crypt cells with mutant HFE lack the facilitating effect on TfR-dependent iron uptake, thus decreasing the regulatory iron pool in the crypt cell.* As small intestinal crypt cells are the progenitors of villus absorptive cells, these cells are preprogrammed to absorb dietary iron regardless of the systemic iron overload.

Excessive iron appears to be directly toxic to host tissues, by the following mechanisms: (1) lipid peroxidation via iron-catalyzed free radical reactions, (2) stimulation of collagen formation, and (3) interactions of reactive oxygen species and of iron itself with DNA, leading to lethal injury or predisposition to hepatocellular carcinoma. Whatever the actions of iron, they are reversible in cells that are not fatally injured, and removal of excess iron during therapy promotes recovery of tissue function.

The most common causes of secondary hemochromatosis are the hemolytic anemias associated with ineffective erythropoiesis, discussed in Chapter 13 . In these disorders, the excess iron may result not only from transfusions, but also from increased absorption. Transfusions alone, as in aplastic

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Figure 18-27 Schematic diagram of HFE function in the intestine. The *crypt epithelial cell* expresses HFE on its basolateral surface; complexing of HFE with β_2 -microglobulin is required for its expression on the cell surface. HFE-β_2 -microglobulin complexes with the transferrin receptor (TfR) to bind circulating transferring (Tf). Endocytosis ensues; on acidification of the recycling endosome, transferrin-bound iron (Fe(II)) is released and enters into the cytoplasm. High levels of cytoplasmic iron downregulate levels of the iron-regulatory proteins (IRP), a family of proteins with potent effects on nuclear transcription. With low levels of cytoplasmic iron, the IRP content of the cell remains high. IRPs upregulate nuclear transcription of the genes for several proteins required for intestinal absorption of dietary iron: Dcytb (duodenal cytochrome B), DMT1 (divalent metal transporter 1), ferritin (a cytoplasmic iron-binding protein), and FP1 (ferroportin 1). *A mutation in HFE prevents "sensing" of circulating iron levels by the crypt epithelial cell, leading to unregulated expression of these four proteins.* The

crypt epithelial cell is the precursor cell of the *mature absorptive enterocyte* on the tip of the villus, through migration up the villus axis. On the apical membrane of the absorptive enterocyte, Dcytb reduces dietary ferric iron (Fe(III)) to ferrous iron (Fe(II)). Fe(II) is then taken up by DMT1 into the enterocyte. Iron can be bound to ferritin (and hence sloughed back into the gut lumen) or transported across the basolateral plasma membrane by FP1 for binding to transferrin and entry into the systemic circulation. *In the patient with mutant HFE, the inability to downregulate expression of these four proteins leads to lifelong excessive absorption of dietary iron.*

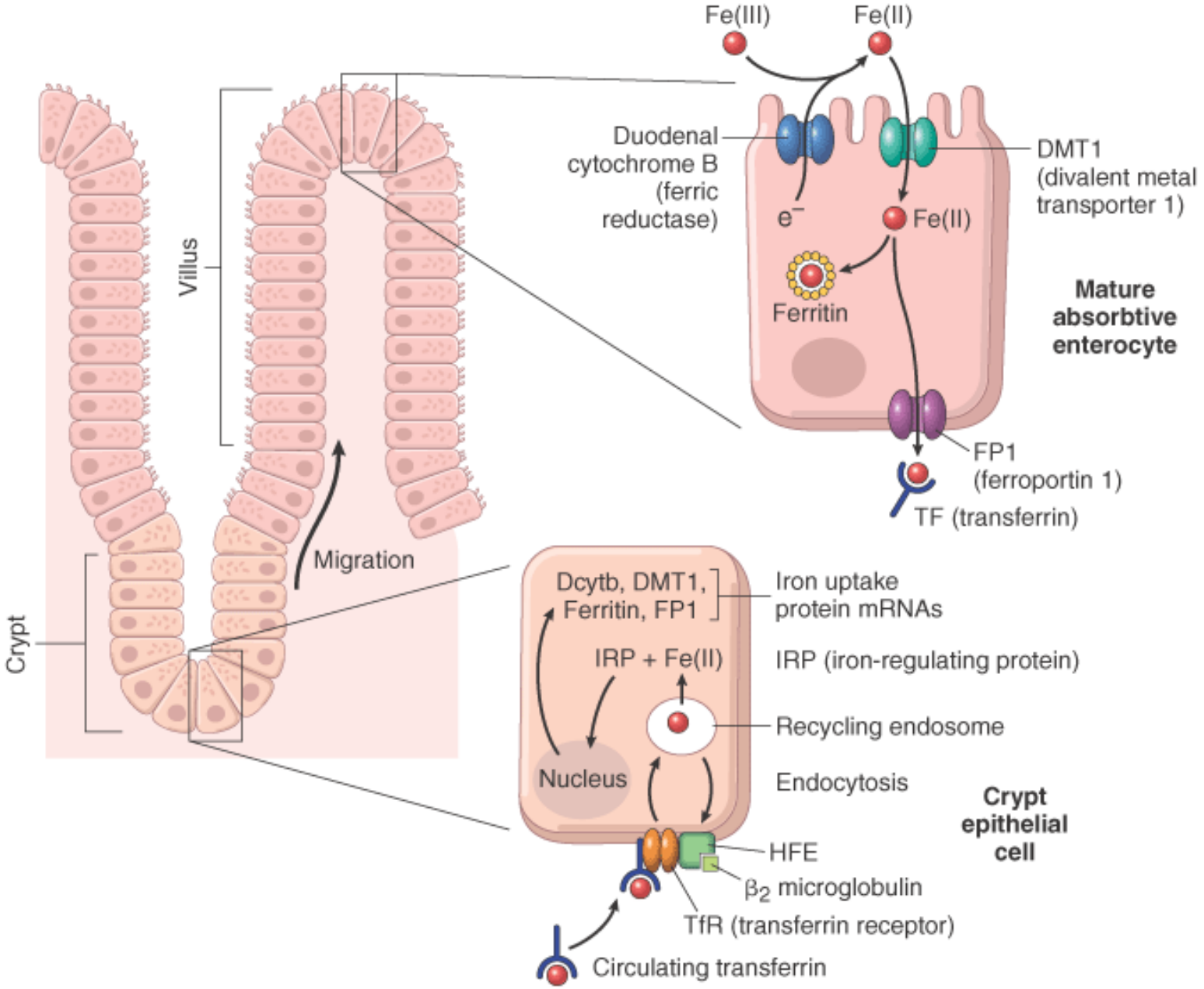

Figure 18-28 Hereditary hemochromatosis. Hepatocellular iron deposition is blue in this Prussian blue-stained section of an early stage of the disease, in which parenchymal architecture is normal.

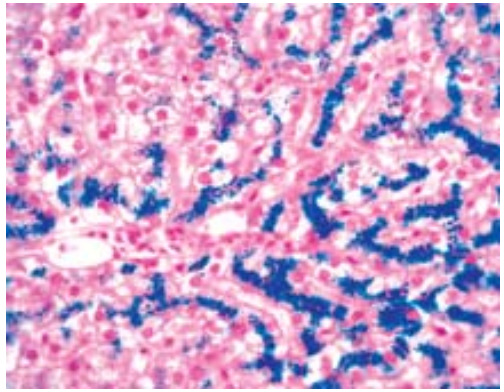

Figure 18-29 α_1 -Antitrypsin deficiency. Periodic acid-Schiff stain of the liver, highlighting the characteristic red cytoplasmic granules. *(Courtesy of Dr. I. Wanless, Toronto General Hospital, Toronto, Ontario, Canada.)*

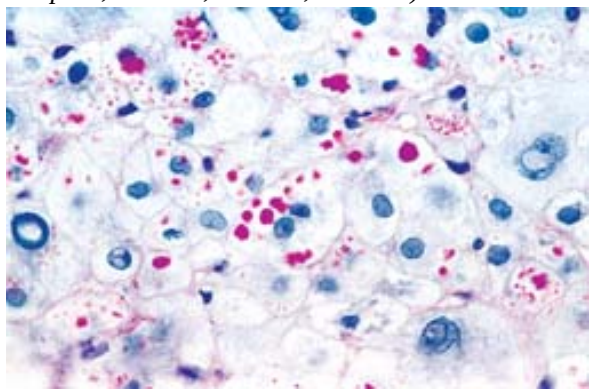

TABLE 18-10 -- Major Causes of Neonatal Cholestasis

Bile duct obstruction
••Extrahepatic biliary atresia
Neonatal infection
••Cytomegalovirus
••Bacterial sepsis
••Urinary tract infection
••Syphilis
Toxic
••Drugs
••Parenteral nutrition

Metabolic disease
••Tyrosinemia
••Niemann-Pick disease
••Galactosemia
••Defective bile acid synthetic pathways
••α_1 -Antitrypsin deficiency
••Cystic fibrosis
Miscellaneous
••Shock/hypoperfusion
••Indian childhood cirrhosis
••Alagille syndrome (paucity of bile ducts)
Idiopathic neonatal hepatitis

NEONATAL CHOLESTASIS

Prolonged conjugated hyperbilirubinemia in the neonate, termed *neonatal cholestasis*, affects approximately 1 in 2500 live births. The major conditions causing it are (1) cholangiopathies, primarily *biliary atresia* (discussed later) and (2) a variety of disorders causing conjugated hyperbilirubinemia in the neonate, collectively referred to as *neonatal hepatitis*. *Neonatal cholestasis and hepatitis are not specific entities, nor are the disorders necessarily inflammatory.* Instead, the finding of "neonatal cholestasis" should evoke a diligent search for recognizable toxic, metabolic, and infectious liver diseases, the more common of which are listed in Table 18-10 .[38] Once identifiable causes have been excluded, one is left with the syndrome of "idiopathic" neonatal hepatitis, which shows considerable clinical overlap with biliary atresia.

Affected infants have jaundice, dark urine, light or acholic stools, and hepatomegaly. Variable degrees of hepatic synthetic dysfunction may be identified, such as hypoprothrombinemia. Thus, liver biopsy is critical in distinguishing neonatal hepatitis from an identifiable cholangiopathy.

Morphology.

The morphologic features of neonatal hepatitis are:

- Lobular disarray with focal liver cell necrosis
- Panlobular giant cell transformation of hepatocytes and formation of hepatocyte "rosettes": radially arrayed hepatocytes
- Prominent hepatocellular and canalicular cholestasis
- Mild mononuclear infiltration of the portal areas
- Reactive changes in the Kupffer cells
- Extramedullary hematopoiesis

This predominantly parenchymal pattern of injury may blend imperceptibly into a ductal pattern of injury, with bile ductular proliferation and fibrosis of

portal tracts. Clear distinction from an obstructive cholangiopathy may therefore be impossible. Specific features that point toward a particular etiology include the inclusions of cytomegalovirus, or fatty change with cirrhosis in galactosemia and tyrosinemia. Electron microscopy may be helpful, for example, by showing phospholipid whorls in Neimann-Pick disease.

Despite the long list of disorders associated with neonatal cholestasis, most are quite rare. "Idiopathic" neonatal hepatitis represents up to 50% of cases, biliary atresia represents another 20%, and α_1 -antitrypsin deficiency represents 15%. Differentiation of biliary atresia from nonobstructive neonatal cholestasis assumes great importance, since definitive treatment of biliary atresia requires surgical intervention, whereas surgery may adversely affect the clinical course of a child with other disorders. Fortunately, discrimination can be made with clinical data, without or with liver biopsy, in about 90% of cases.

Intrahepatic Biliary Tract Disease

In this section, we discuss three disorders of intrahepatic bile ducts: secondary biliary cirrhosis, primary biliary cirrhosis, and primary sclerosing cholangitis, (summarized in Table 18-11). Secondary biliary cirrhosis is a condition resulting most often from uncorrected obstruction of the extrahepatic biliary tree. Primary biliary cirrhosis is a destructive disorder of the intrahepatic biliary tree. Primary sclerosing cholangitis involves both the extrahepatic and intrahepatic biliary tree. It should also be noted (although not discussed here) that intrahepatic bile ducts are frequently damaged as part of more general liver disease, as in drug toxicity, viral hepatitis, and transplantation—both orthotopic liver transplantation and graft-versus-host disease after bone marrow transplantation.

TABLE 18-11 -- Distinguishing Features of the Major Intrahepatic Bile Duct Disorders

	Secondary Biliary Cirrhosis	Primary Biliary Cirrhosis	Primary Sclerosing Cholangitis
Etiology	Extrahepatic bile duct obstruction: biliary atresia, gallstones, stricture, carcinoma of pancreatic head	Possibly autoimmune	Unknown, possibly autoimmune; 50–70% associated with inflammatory bowel disease
Sex predilection	None	Female to male: 6:1	Female to male: 1:2
Symptoms and signs	Pruritus, jaundice, malaise dark urine, light stools, hepatosplenomegaly	Same as secondary biliary cirrhosis; insidious onset	Same as secondary biliary cirrhosis; insidious onset
Laboratory findings	Conjugated hyperbilirubinemia, increased serum alkaline phosphatase, bile acids, cholesterol	Same as secondary biliary cirrhosis, plus elevated serum IgM autoantibodies (especially M2 form of antimitochondrial antibody-AMA)	Same as secondary biliary cirrhosis, plus elevated serum IgM, hypergammaglobulinemia
Important pathologic findings before cirrhosis develops	Prominent bile stasis in bile ducts, bile ductular proliferation with surrounding neutrophils, portal tract edema	Dense lymphocytic infiltrate in portal tracts with granulomatous destruction of bile ducts	Periductal portal tract fibrosis, segmental stenosis of extrahepatic and intrahepatic bile ducts

SECONDARY BILIARY CIRRHOSIS

Prolonged obstruction of the extrahepatic biliary tree results in profound alteration of the liver itself. The most common cause of obstruction in adults is extrahepatic cholelithiasis

(gallstones, described later), followed by malignancies of the biliary tree or head of the pancreas and strictures resulting from previous surgical procedures. Obstructive conditions in children include biliary atresia, cystic fibrosis, choledochal cysts (a cystic anomaly of the extrahepatic biliary tree, see later), and syndromes in which there are insufficient intrahepatic bile ducts (paucity of bile duct syndromes).[39] The initial morphologic features of *cholestasis* were described earlier and are entirely reversible with correction of the obstruction. However, secondary inflammation resulting from biliary obstruction initiates periportal fibrosis, which eventually leads to hepatic scarring and nodule formation, generating secondary biliary cirrhosis. Subtotal obstruction may promote secondary bacterial infection of the biliary tree (*ascending cholangitis*), which aggravates the inflammatory injury. Enteric organisms such as coliforms and enterococci are common culprits.

Morphology.

The end-stage obstructed liver exhibits extraordinary yellow-green pigmentation and is accompanied by marked icteric discoloration of body tissues and fluids. On cut surface, the liver is hard, with a finely granular appearance (Fig. 18-30). The histology is characterized by coarse fibrous septae that subdivide the liver in a jigsaw-like pattern. Embedded in the septa are distended small and large bile ducts, which frequently contain inspissated pigmented material. There is extensive proliferation of smaller bile ductules and edema, particularly at the interface between septa (formerly portal tracts) and the parenchyma. Cholestatic features in the parenchyma may be severe, with extensive feathery degeneration and formation of bile lakes. However, once regenerative nodules have formed, bile stasis

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Figure 18-30 Biliary cirrhosis. Sagittal section through the liver demonstrates the fine nodularity and bile staining of end-stage biliary cirrhosis.

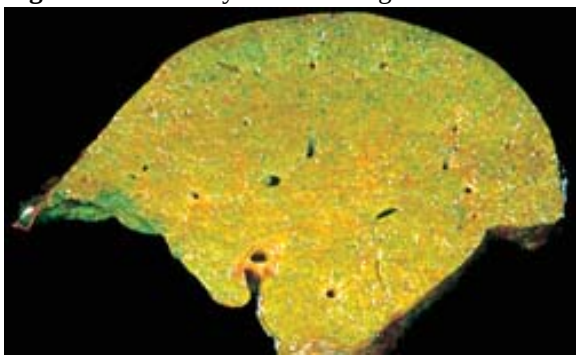


Figure 18-31 Primary biliary cirrhosis. A portal tract is markedly expanded by an infiltrate of lymphocytes and plasma cells. The granulomatous reaction to a bile duct undergoing destruction (florid duct lesion) is highlighted by the arrowheads.

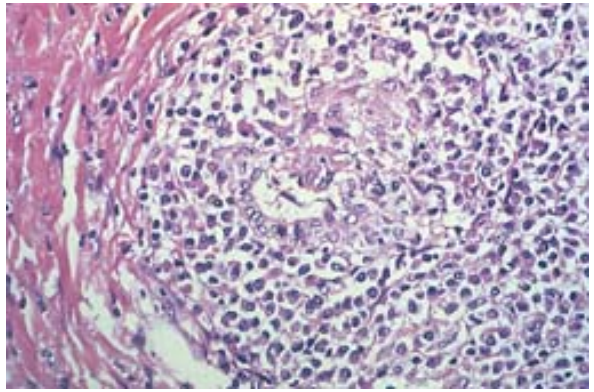


Figure 18-32 Primary sclerosing cholangitis. A bile duct under-going degeneration is entrapped in a dense, "onion-skin" concentric scar.

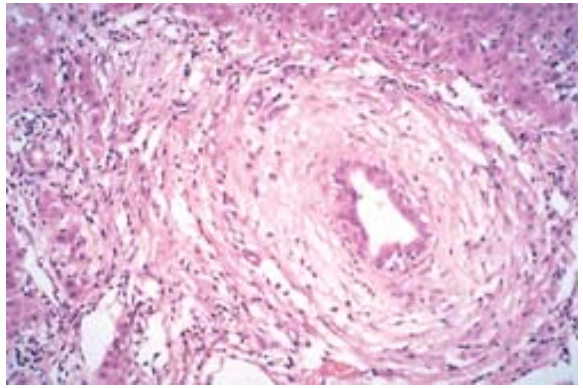
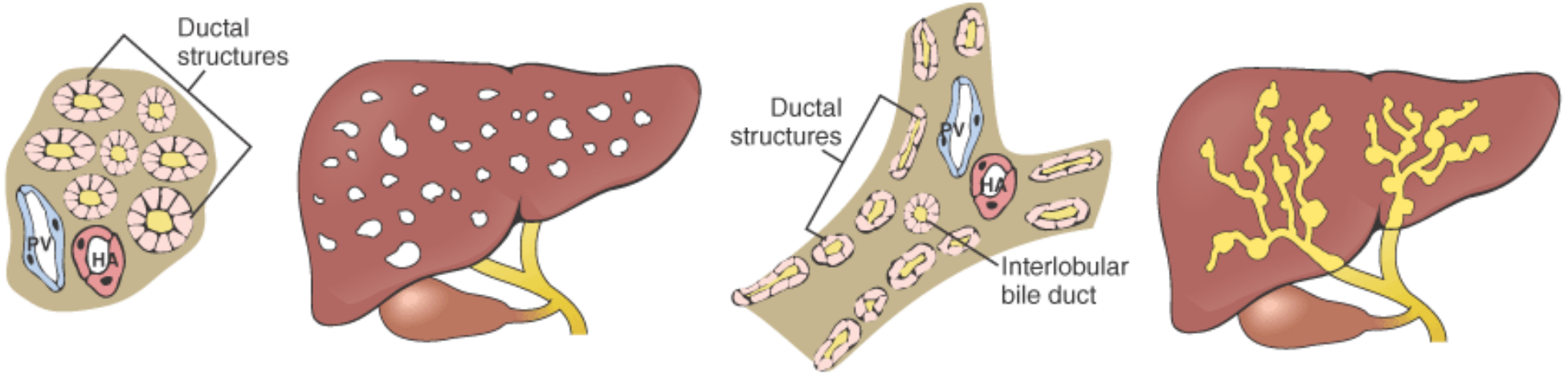


Figure 18-33 Bile duct anomalies. The morphologic features of the four major groups are diagrammed, along with apparent patterns of inheritance and associations with polycystic kidney disease. PV, portal vein. HA, hepatic artery.



	von Meyenburg Complex	Polycystic Liver Disease	Congenital Hepatic Fibrosis	Caroli Disease
INHERITANCE:	Sporadic	Autosomal Dominant	Autosomal Recessive	Autosomal Recessive
ASSOCIATION WITH POLYCYSTIC KIDNEY DISEASE (PKD)				
Autosomal dominant PKD:	+	+ + +	+	+
Autosomal recessive PKD:	-	-	+ + +	+

Figure 18-34 Hepatic circulatory disorders. The forms and clinical manifestations of impaired blood flow are contrasted.

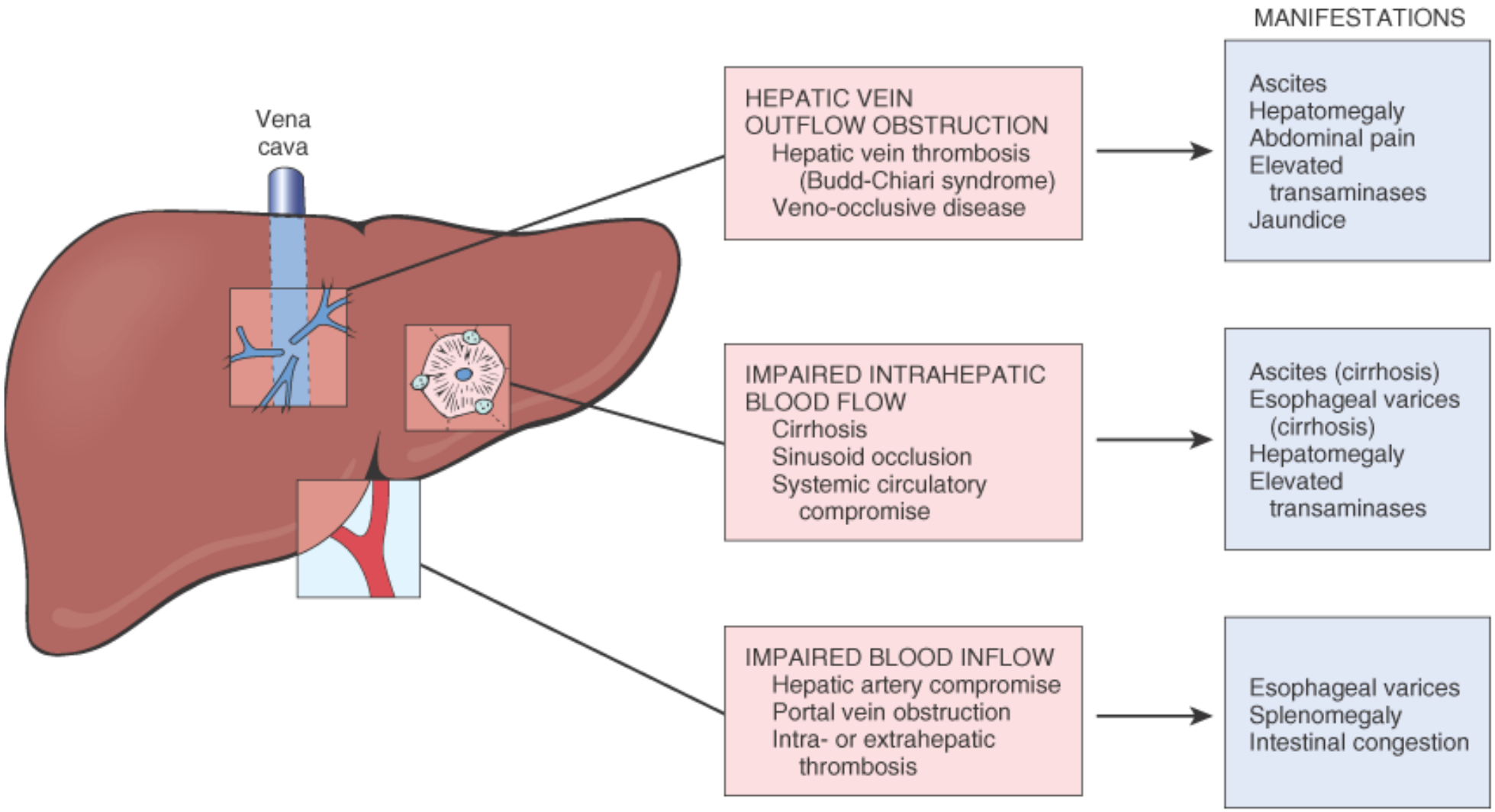


Figure 18-35 Liver infarct. A thrombus is lodged in a peripheral branch of the hepatic artery and compresses the adjacent portal vein; the distal hepatic tissue is pale, with a hemorrhagic margin.

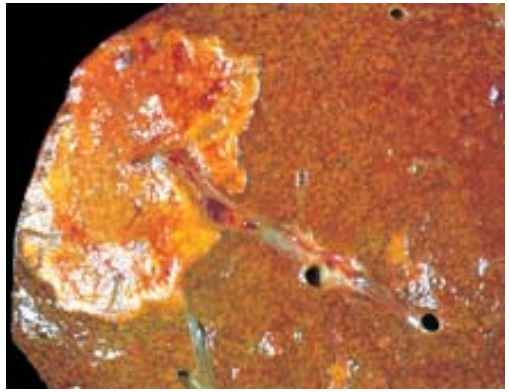


Figure 18-36 Centrolobular hemorrhagic necrosis. The cut liver section, in which major blood vessels are visible, is notable for a variegated, mottled, red appearance (nutmeg liver).

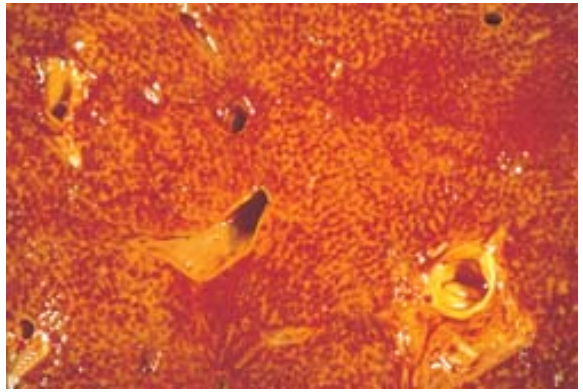


Figure 18-37 Budd-Chiari syndrome. Thrombosis of the major hepatic veins has caused extreme blood retention in the liver.

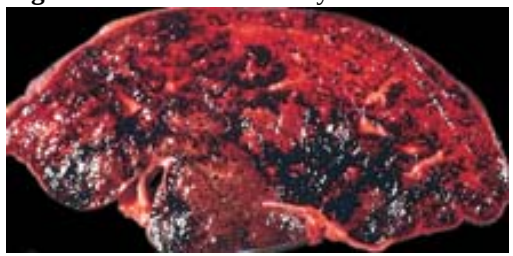


Figure 18-38 Veno-occlusive disease. A reticulin stain reveals the parenchyma framework of the lobule and the marked deposition of collagen within the lumen of the central vein.

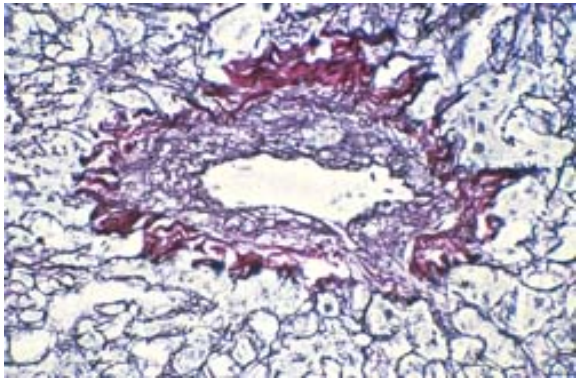


Figure 18-39 Eclampsia. Subcapsular hematoma dissecting under Glisson's capsule in a fatal case of eclampsia. *(Courtesy of Dr. Brian Blackbourne, Office of the Medical Examiner, San Diego, CA.)*

Figure 18-40 Focal nodular hyperplasia. *A,* Resected specimen showing lobulated contours and a central stellate scar. *B,* Low-power photomicrograph showing a broad fibrous scar with hepatic arterial and bile duct elements and chronic inflammation, present within hepatic parenchyma that lacks the normal sinusoidal plate architecture (H&E).

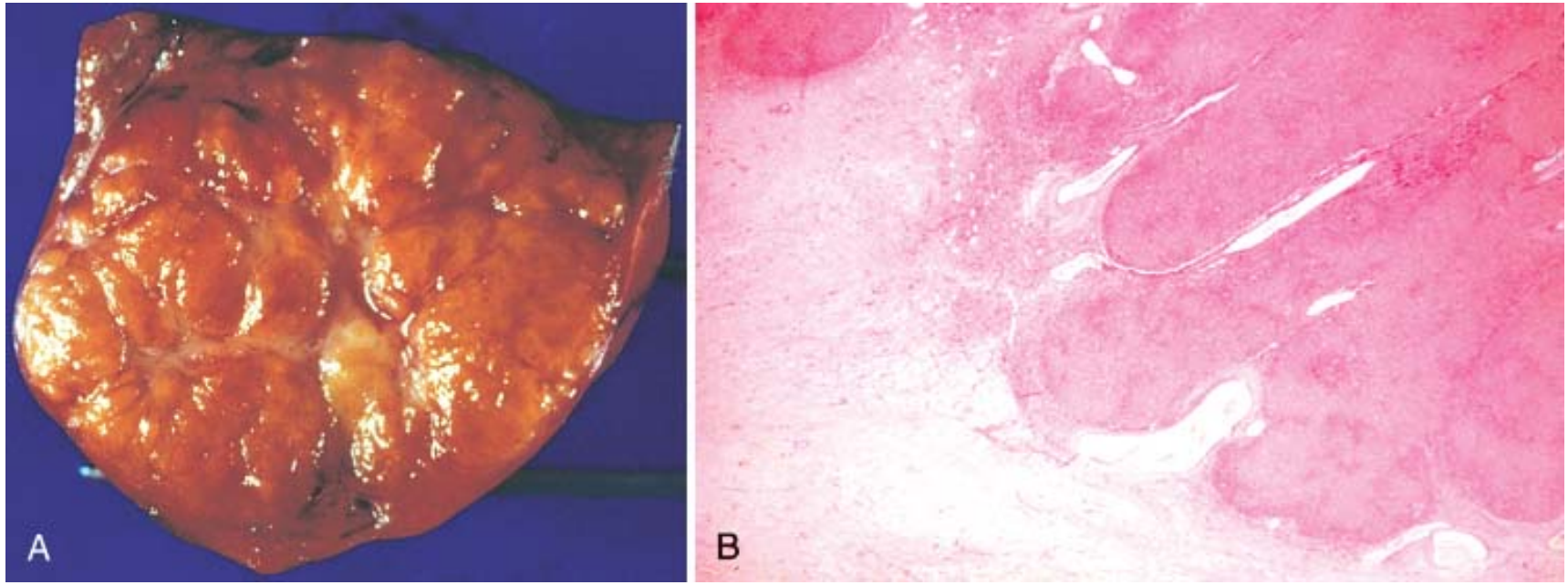


Figure 18-41 Nodular regenerative hyperplasia. Autopsied liver showing diffuse nodular transformation.

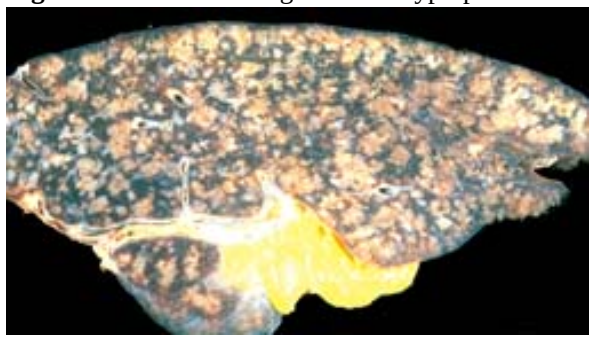


Figure 18-42 Liver cell adenoma. *A*, Resected specimen presenting as a pendulous mass arising from the liver. *B*, Microscopic view showing cords of hepatocytes, with an arterial vascular supply (*arrows*) and no portal tracts.

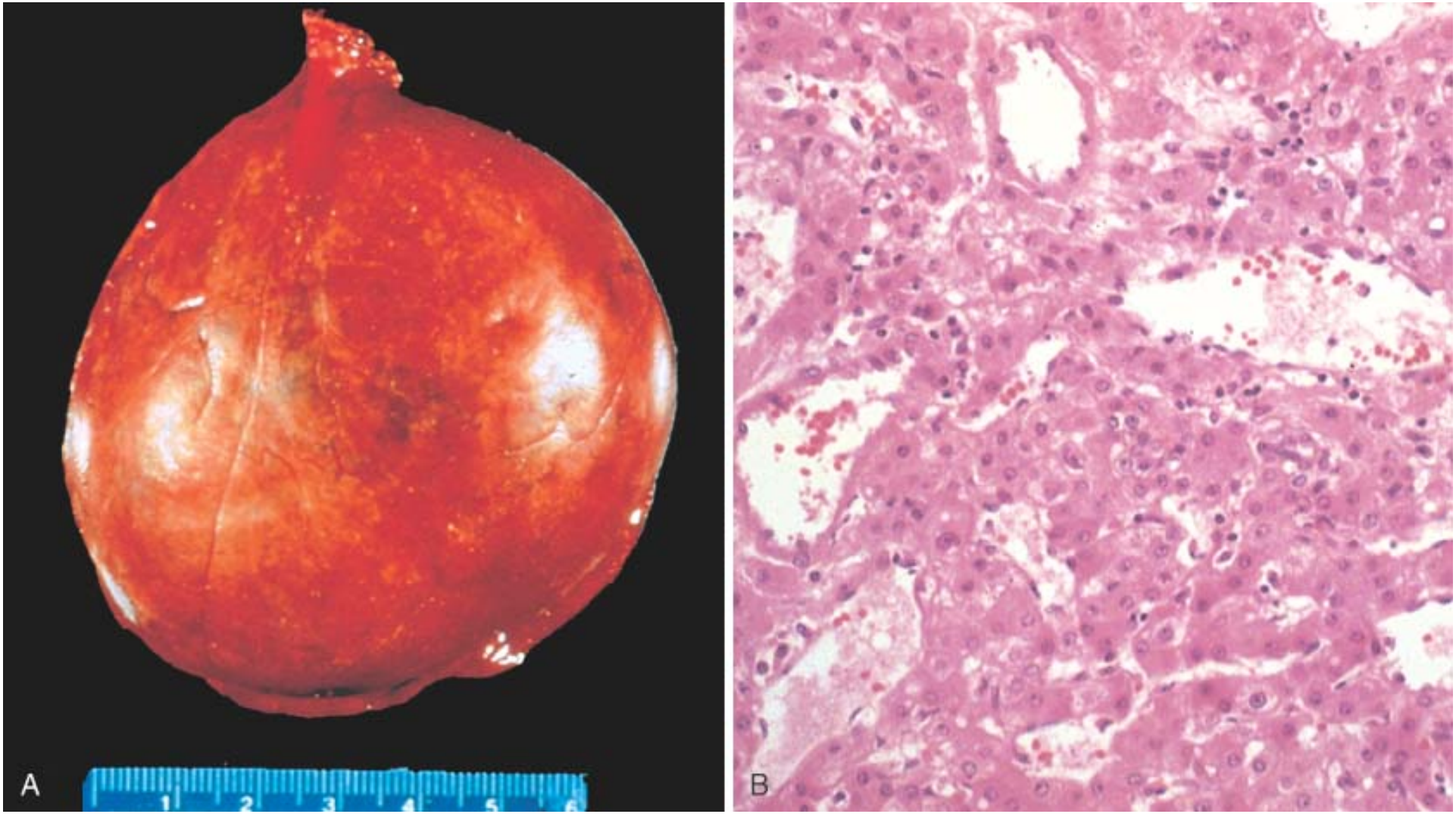


Figure 18-43 Hepatocellular carcinoma. *A,* Autopsied liver showing a unifocal, massive neoplasm replacing most of the right hepatic lobe in a noncirrhotic liver; a satellite tumor nodule is directly adjacent. *B,* In this microscopic view of a well-differentiated lesion, tumor cells are arranged in nests, sometimes with a central lumen, one of which contains bile (*arrow*). Other tumor cells contain intracellular bile pigment.

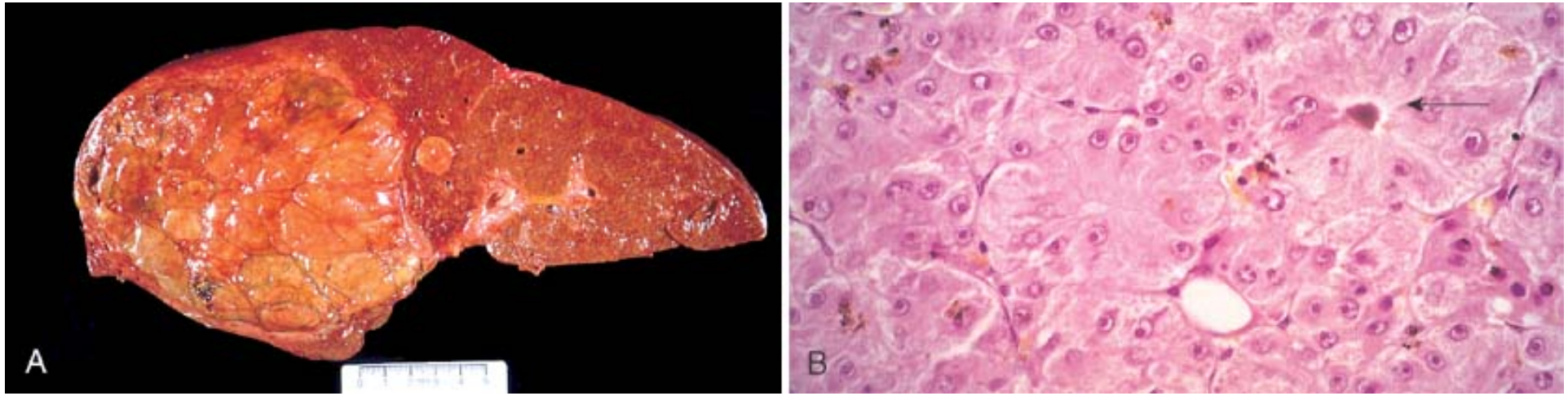


Figure 18-44 Fibrolamellar carcinoma. *A*, Resected specimen showing a demarcated nodule in an otherwise normal liver. *B*, Microscopic view showing nests and cords of malignant-appearing hepatocytes separated by dense bundles of collagen.

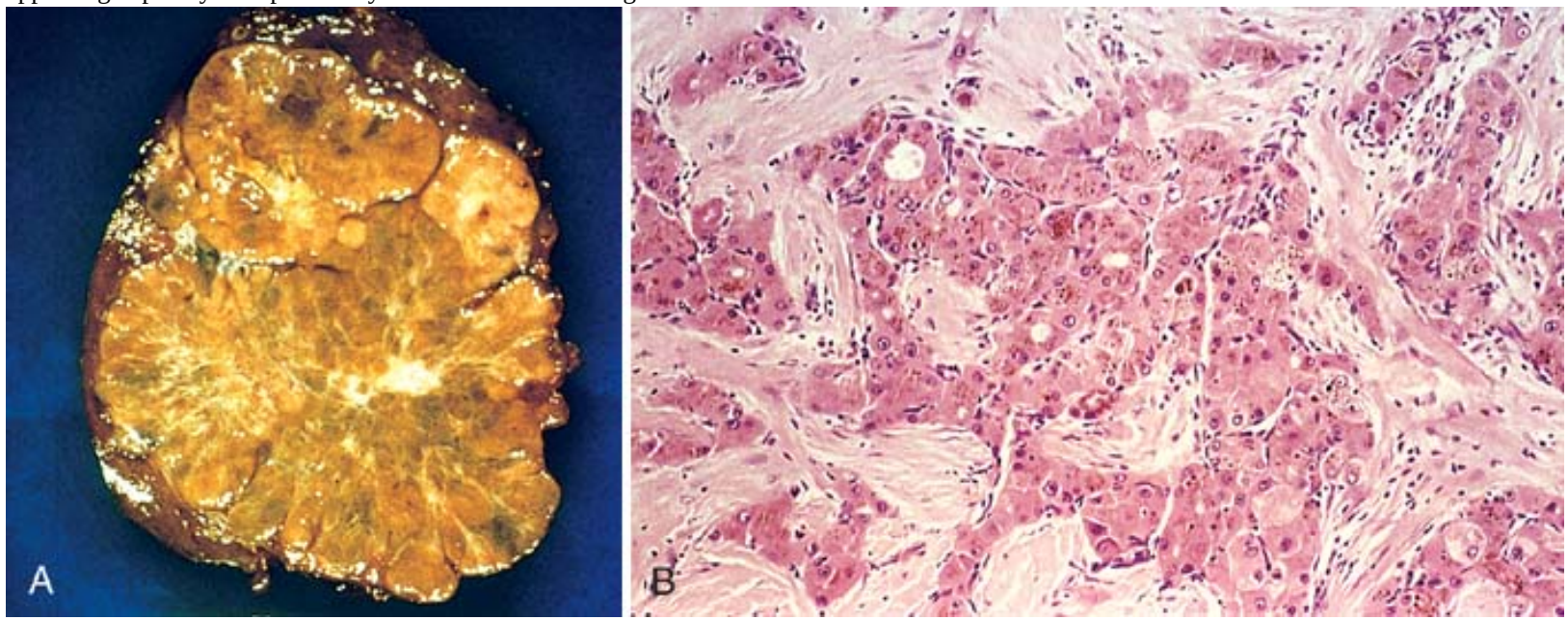


Figure 18-45 Cholangiocarcinoma. *A*, Autopsied liver showing a massive neoplasm in the right hepatic lobe and innumerable metastases permeating the entire liver. *B*, Microscopic view showing tubular glandular structures embedded in a dense sclerotic stroma.

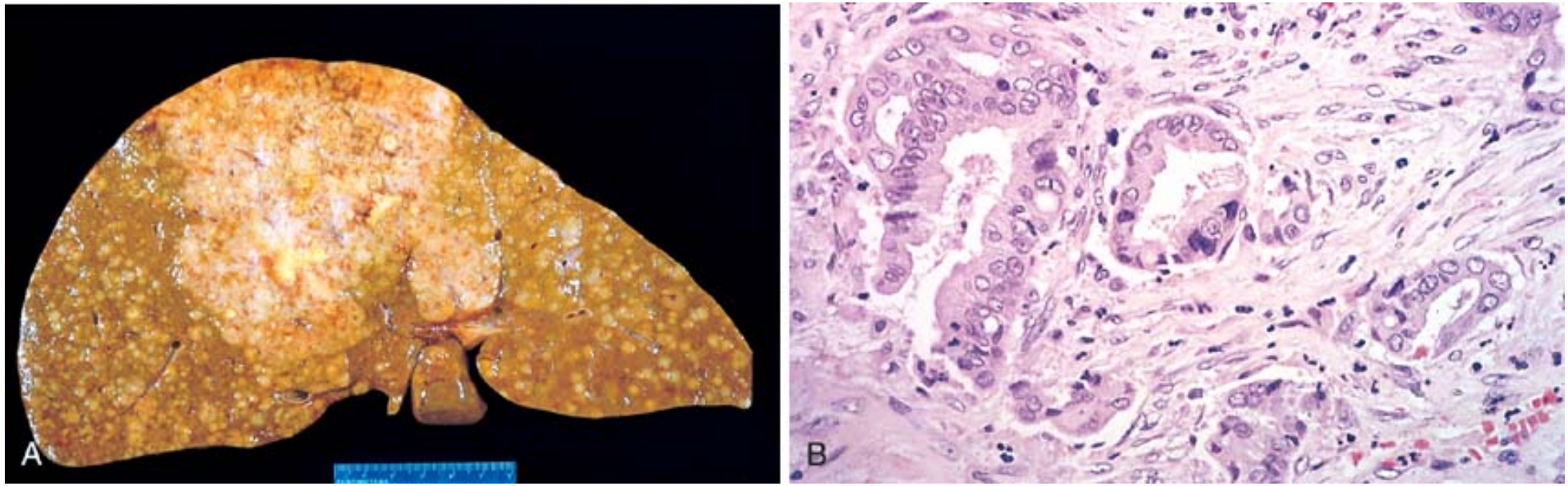


Figure 18-46 Multiple hepatic metastases from a primary colon adenocarcinoma.

Figure 18-47 Normal gallbladder histology. The undulating mucosal epithelium overlies a delicate lamina and only one smooth muscle layer. This is different from elsewhere in the gut, where two muscle layers exist (muscularis mucosa and muscularis propria).

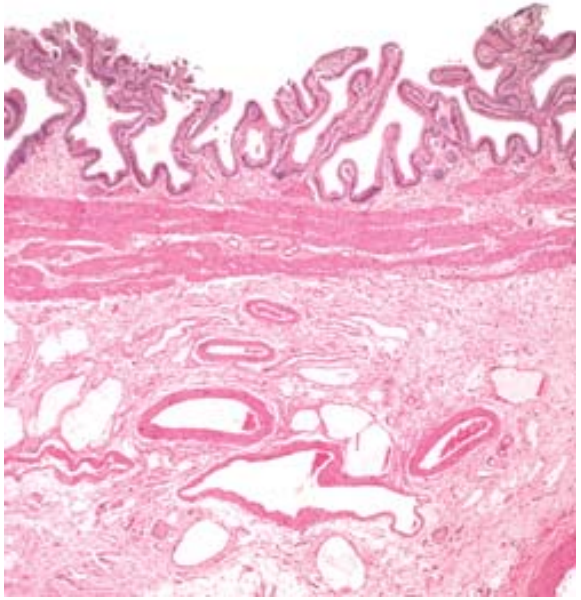


Figure 18-48 Phrygian cap of the gallbladder; the fundus is folded inward.

TABLE 18-12 -- Risk Factors for Gallstones

Cholesterol Stones
Demography: Northern Europe, North and South America, Native Americans, Mexican Americans
Advancing age
Female sex hormones
••Female gender
••Oral contraceptives

••Pregnancy
Obesity
Rapid weight reduction
Gallbladder stasis
Inborn disorders of bile acid metabolism
Hyperlipidemia syndromes
Pigment Stones
Demography: Asian more than Western, rural more than urban
Chronic hemolytic syndromes
Biliary infection
Gastrointestinal disorders: ileal disease (e.g., Crohn disease), ileal resection or bypass, cystic fibrosis with pancreatic insufficiency

Hereditary Factors.

In addition to ethnicity, family history alone imparts increased risk, as do a variety of inborn errors of metabolism that (1) lead to impaired bile salt synthesis and secretion or (2) generate increased serum and biliary levels of cholesterol, such as defects in lipoprotein receptors (hyperlipidemia syndromes), which engender marked increases in cholesterol biosynthesis. Animal studies strongly implicate specific genetic susceptibilities, many attributable to aberrant regulation of the transport proteins responsible for the secretion of biliary solutes into bile. [61]

Certain risk factors are well established for the development of pigment stones. Disorders that are associated with elevated levels of unconjugated bilirubin in bile include hemolytic syndromes, severe ileal dysfunction (or bypass), and bacterial contamination of the biliary tree.

Pathogenesis of Cholesterol Stones.

Cholesterol is rendered soluble in bile by aggregation with water-soluble bile salts and water-insoluble lecithins, both of which act as detergents. *When cholesterol concentrations exceed the solubilizing capacity of bile (supersaturation), cholesterol can no longer remain dispersed and nucleates into solid cholesterol monohydrate crystals. Cholesterol gallstone formation involves four simultaneous defects* (Fig. 18-49):

- Bile must be supersaturated with cholesterol.
- Gallbladder hypomotility promotes nucleation.
- Cholesterol nucleation in bile is accelerated.
- Mucus hypersecretion in the gallbladder traps the crystals, permitting their aggregation into stones.

Supersaturation of bile with cholesterol is the result of hepatocellular hypersecretion of cholesterol. This appears to be a primary defect, mediated by abnormal regulation of hepatic mechanisms for delivering cholesterol to bile.[62] The abundant free cholesterol is toxic to the gallbladder, penetrating the wall and exceeding the ability of the mucosa to detoxify it by esterification. *Gallbladder hypomotility ensues.* Muscular stasis appears to result both from intrinsic neuromuscular dysmotility and from diminished muscular responsiveness to cholecystokinin, the hormone secreted by the gut that promotes gallbladder contraction. The relative composition of trace proteins in bile also may be altered, such that the balance of antinucleating and pronucleating proteins shifts in favor of *accelerated nucleation of cholesterol crystals*. Nucleation is further promoted by the presence of microprecipitates of inorganic or organic calcium salts. As a result of these events, supersaturated bile is sequestered in a hypomotile gallbladder under favorable nucleating conditions. *Hypersecretion of gallbladder*

mucus completes the tetralogy, as the cholesterol crystals are trapped for sustained periods, enabling their growth into macroscopic concretions. Superimposed conditions exacerbate defective gallbladder emptying and the likelihood of forming cholesterol stones: prolonged fasting, pregnancy, rapid weight loss, total parenteral nutrition, and spinal cord injury.

Pathogenesis of Pigment Stones.

Pigment gallstones are complex mixtures of abnormal insoluble calcium salts of unconjugated bilirubin along with inorganic calcium salts.[63] Unconjugated bilirubin is normally a minor component of bile but increases when infection of the biliary tract leads to release of microbial β-glucuronidases, which hydrolyze bilirubin glucuronides. Thus, infection of the biliary tract, as with *Escherichia coli* or *Ascaris lumbricoides* or by the liver fluke *Opisthorchis sinensis*, increases the likelihood of pigment stone

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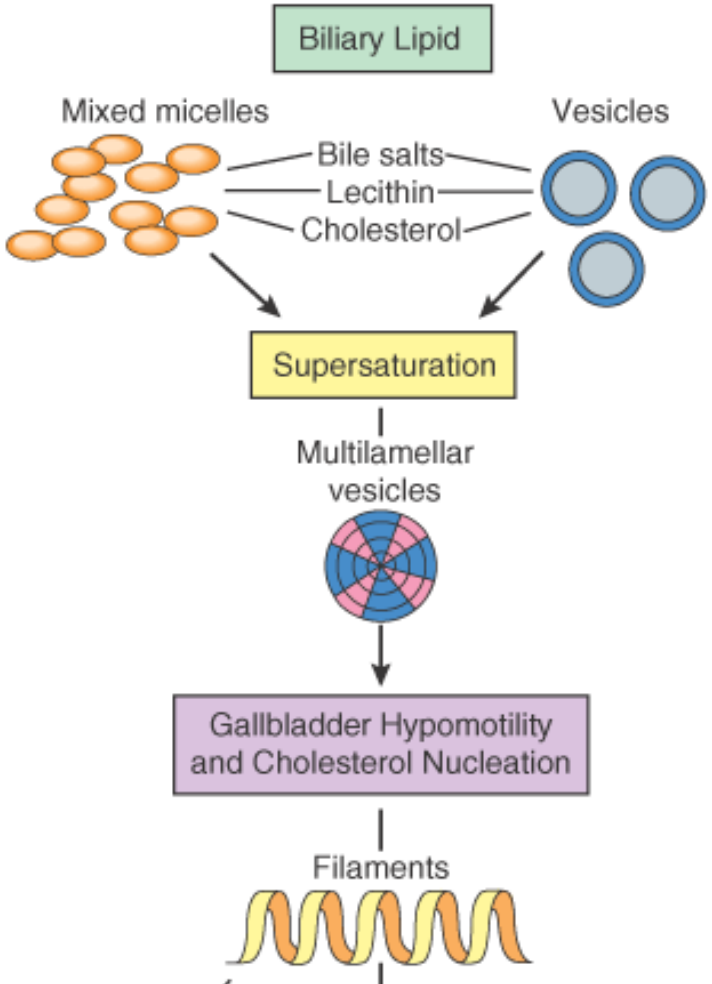
Figure 18-49 Schematic representation of the four contributing factors for cholelithiasis: supersaturation, gallbladder hypomotility, crystal nucleation, and accretion within the gallbladder mucous layer.



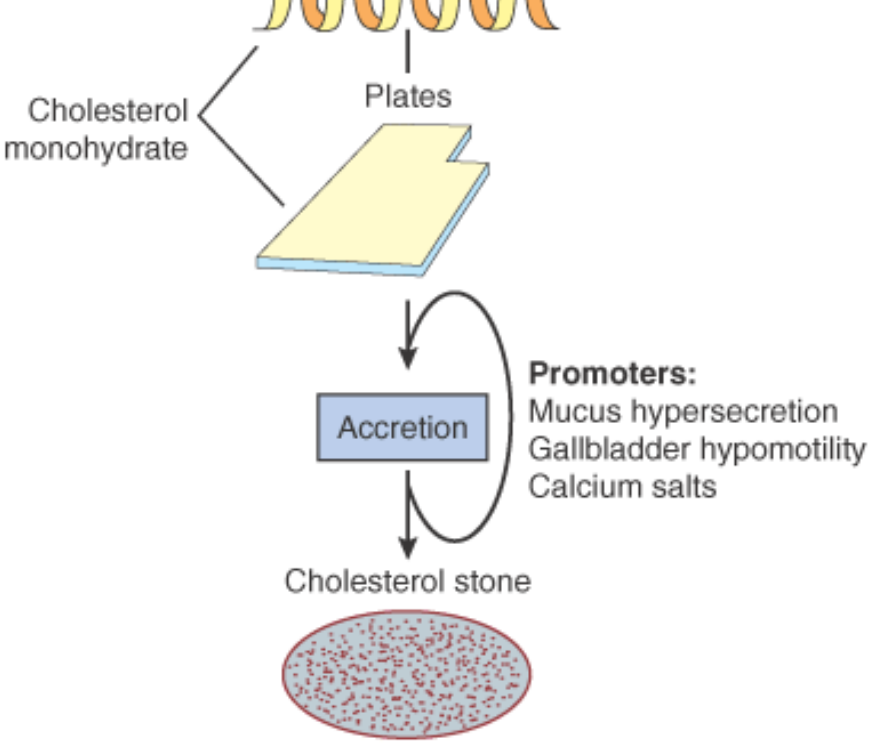


Figure 18-50 Cholesterol gallstones. Mechanical manipulation during laparoscopic cholecystectomy has caused fragmentation of several cholesterol gallstones, revealing interiors that are pigmented because of entrapped bile pigments. The gallbladder mucosa is reddened and irregular as a result of coexistent chronic cholecystitis.

Figure 18-51 Pigment gallstones. Several faceted black gallstones are present in this otherwise unremarkable gallbladder from a patient with a mechanical mitral valve prosthesis, leading

to chronic intravascular hemolysis.

Figure 18-52 Acute calculous cholecystitis; the stone was not photographed.

Figure 18-53 Chronic cholecystitis with cholesterol stones. The gallbladder wall is thickened and gray-white, owing to fibrosis and inflammation. The mucosa is effaced. Multiple faceted cholesterol gallstones are present within the lumen. The exterior of the specimen is black as a result of India ink application.

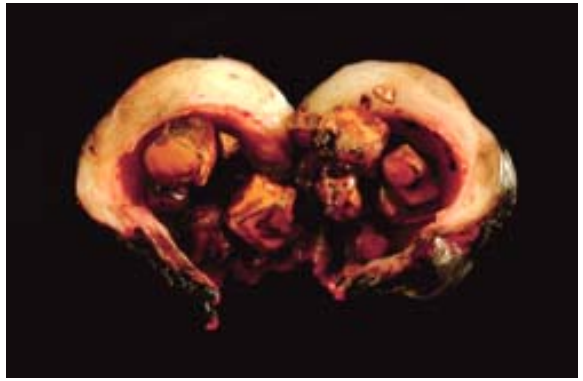


Figure 18-54 Biliary atresia, schematized to show the pattern of biliary tract injury.

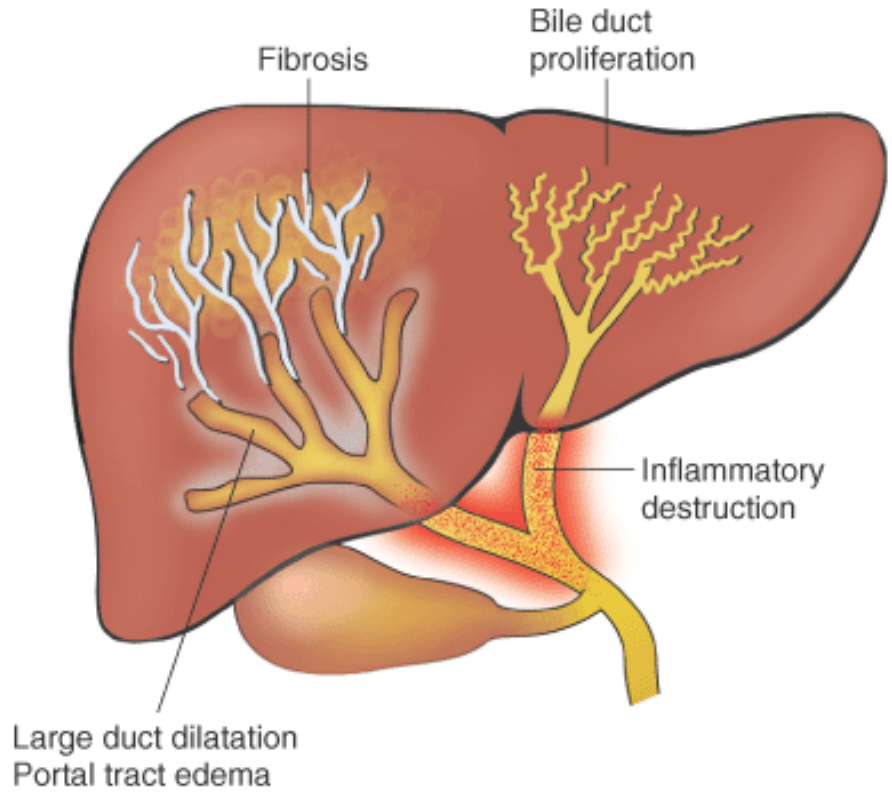
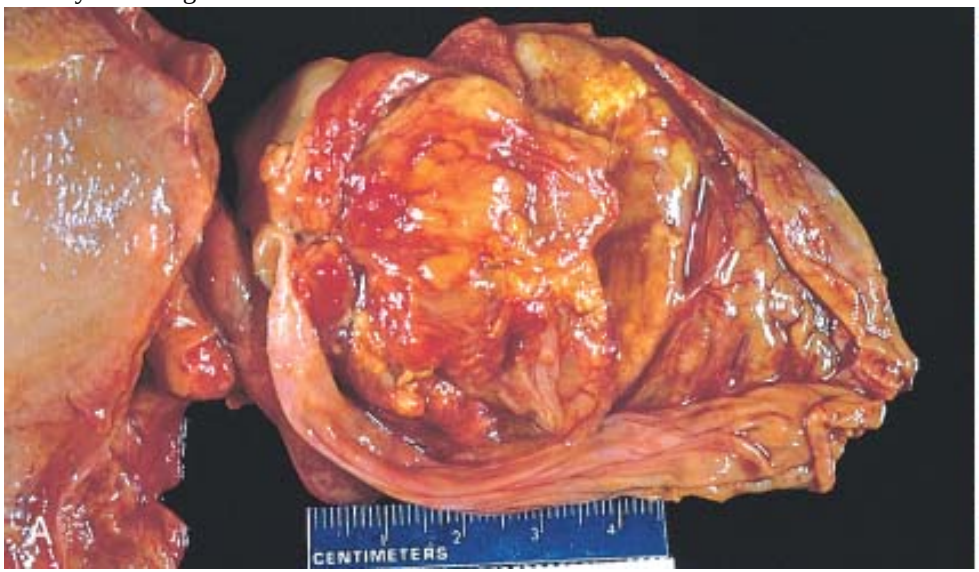
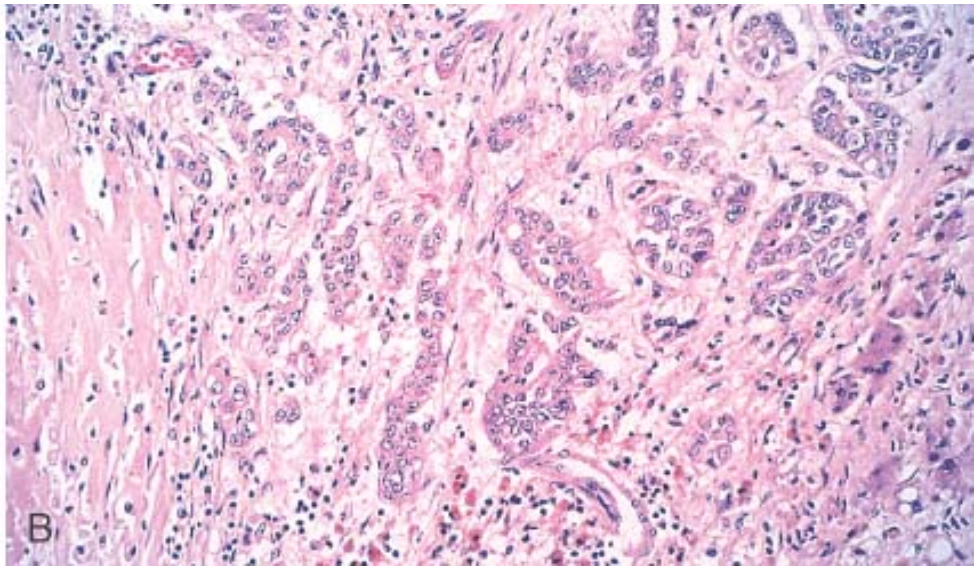


Figure 18-55 Gallbladder adenocarcinoma. *A*, The opened gallbladder contains a large, exophytic tumor that virtually fills the lumen. *B*, Malignant glandular structures are present within a densely fibrotic gallbladder wall.





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Chapter 19 - The Pancreas

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Normal

The pancreas has important endocrine and exocrine functions, and diseases of the pancreas cause significant morbidity and mortality. Despite the physiologic importance of the pancreas, the retroperitoneal location of the gland and the vague signs and symptoms associated with injury to the gland allow many diseases to progress relatively unnoticed for extended periods of time. Diseases of the pancreas thus remain a continuing source of frustration in modern medicine.

The adult pancreas is a transversely oriented retroperitoneal organ extending from the "C" loop of the duodenum to the hilum of the spleen (Fig. 19-1). On average, the pancreas measures 20 cm in length and weighs 90 gm in men and 85 gm in women.[1] Although the pancreas does not have well-defined anatomic subdivisions, the adjacent vasculature can be used to separate the pancreas into three parts: the head, body, and tail.

The pancreatic duct system is highly variable. The main pancreatic duct, also known as the duct of Wirsung, most commonly drains into the duodenum at the papilla of Vater, whereas the accessory pancreatic duct, also known as the duct of Santorini, most often drains into the duodenum through a separate minor papilla approximately 2 cm cephalad (proximal) to the major papilla of Vater (Fig. 19-2*A*). In many adults, the main pancreatic duct merges with the common bile duct proximal to the papilla of Vater, thus creating the ampulla of Vater, a common channel for biliary and pancreatic drainage. Owing to developmental variability, however, this ductal architecture can differ tremendously from patient to patient.

Embryologically, the pancreas arises from the fusion of dorsal and ventral outpouchings of the foregut.[1] During early embryonic development, the dorsal and ventral pancreatic primordia rotate and fuse at approximately the seventh week of gestation to form a single gland.[2] The majority of the gland, including the body, the tail, the superior/anterior aspect of the head, and the accessory duct of Santorini, is derived from the dorsal primordium. Although the ventral primordium gives rise only to the posterior/inferior part of the head of the pancreas, the ventral primordium is important because it drains into the papilla of Vater. Fusion of the dorsal and ventral duct systems allows the majority of the gland to drain through the larger Vaterian papilla.

Although the organ gets its name from the Greek *pankreas*, meaning "all flesh," the pancreas is, in fact, a complex lobulated organ with distinct exocrine and endocrine components. The exocrine portion of the gland, which produces digestive

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Figure 19-1 Anatomic relationships of the pancreas seen in a cross-section of the abdomen at the level of the upper lumbar vertebrae. *(From Go VW, et al (eds): The Pancreas: Biology, Pathobiology, and Disease, 2nd ed. New York, Raven Press, 1993.)*

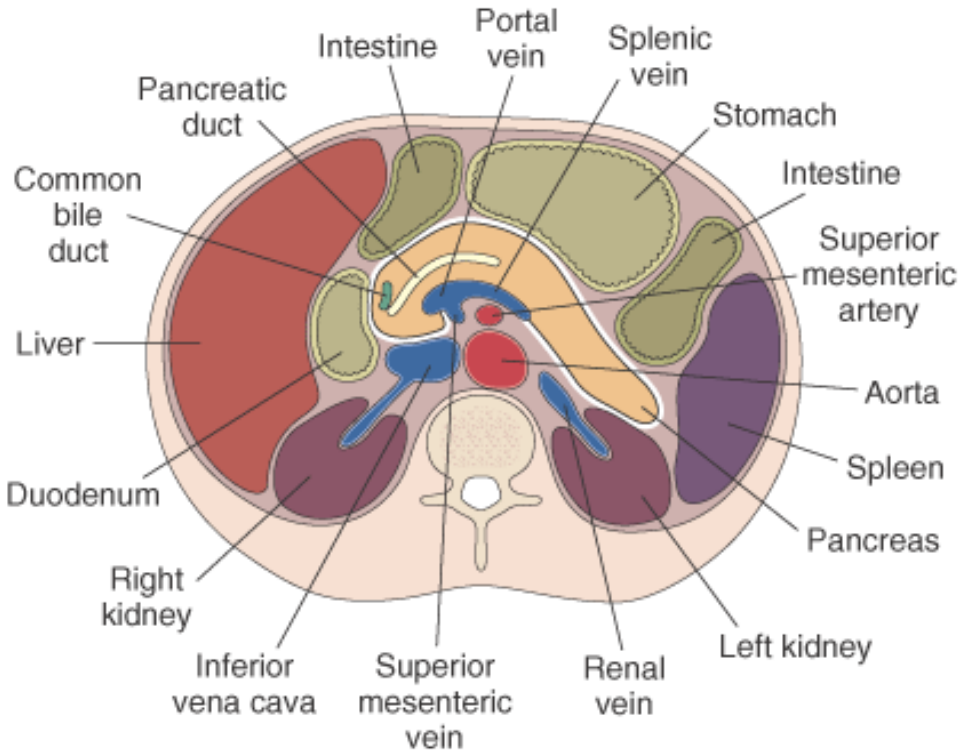

Figure 19-2 Pancreatic ductal anatomy. *A*, The normal ductal anatomy. *B*, The ductal anatomy in pancreatic divisum. *(Adapted from Gregg JA, Monaco AP, McDermott WV: Pancreas divisum: results of surgical intervention. Am J Surg 145:488–492, 1983.)*

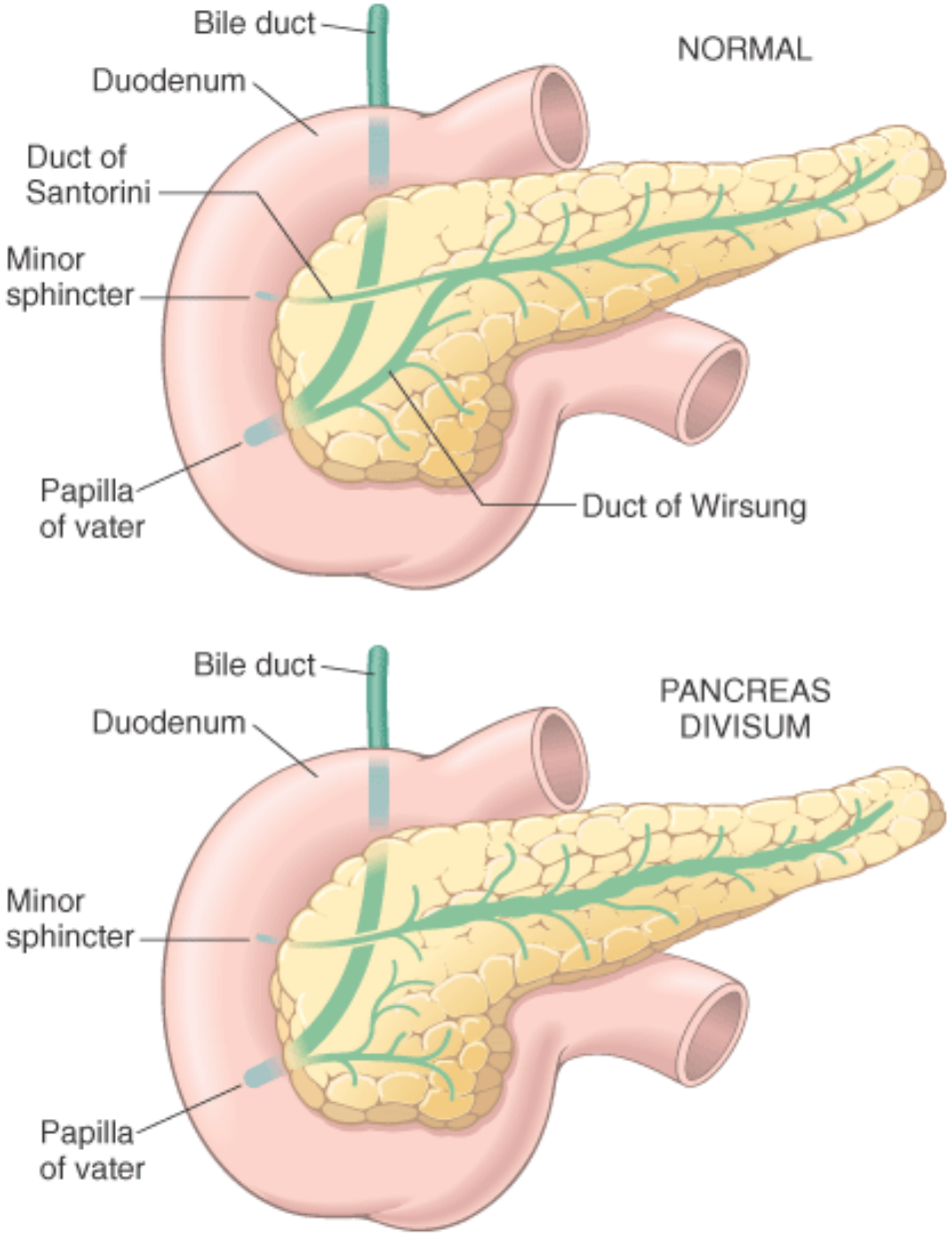

Figure 19-3 Pancreatic acini, showing the radial orientation of the pyramidal exocrine acinar cells. The cytoplasm is devoted to the synthesis and packaging of digestive enzymes for secretion into a central lumen.

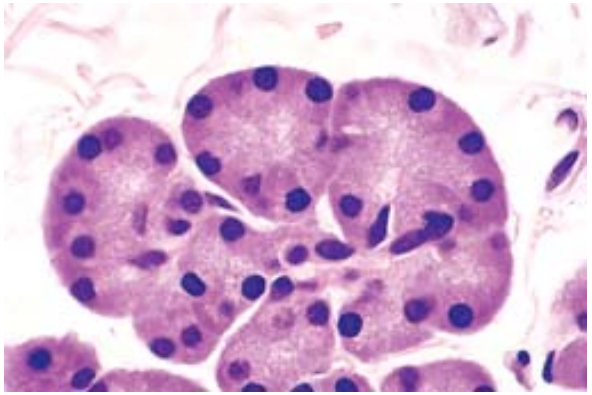

TABLE 19-1 -- Etiologic Factors in Acute Pancreatitis

Metabolic
Alcoholism
Hyperlipoproteinemia
Hypercalcemia
Drugs (e.g., thiazide diuretics)
Genetic
Mechanical
Trauma
Gallstones
Iatrogenic injury
••Perioperative injury
••Endoscopic procedures with dye injection
Vascular
Shock
Atheroembolism
Polyarteritis nodosa
Infectious
Mumps
Coxsackievirus
Mycoplasma pneumoniae

Of note, 10% to 20% of patients with acute pancreatitis have no known associated processes. Although this condition is currently termed *idiopathic*, a growing body of evidence suggests that many, in fact, have a genetic basis. The genetic alterations associated with the development of pancreatitis therefore deserve special note.[22]

Cationic Trypsinogen (PRSS1).

Hereditary pancreatitis is an autosomal-dominant disease with an 80% penetrance characterized by recurrent attacks of severe pancreatitis usually beginning in childhood.[19] This disorder is caused by germ line (inherited) mutations in the *cationic trypsinogen* gene (also known as *PRSS1*).[18] Most are point mutations, with G to A transitions, that result in an arginine (R) to histidine (H) substitution (called R122H).[19] This mutation abrogates a critical failsafe mechanism, by affecting a site on the cationic trypsinogen molecule that is essential for the cleavage (inactivation) of trypsin by trypsin itself.[23] When this site is mutated, trypsinogen and trypsin become resistant to inactivation, and the abnormally active trypsin activates other digestive proenzymes, resulting in the development of pancreatitis.

Serine Protease Inhibitor, Kazal Type 1 (SPINK1).

The *SPINK1* gene codes for a pancreatic secretory trypsin inhibitor that, as the name suggests, inhibits trypsin activity, helping to prevent the autodigestion of the pancreas by activated trypsin.[20] As one might suspect, inherited homozygous inactivating mutations in the *SPINK1* gene can also lead to the development of pancreatitis.

Morphology.

The morphology of acute pancreatitis ranges from trivial inflammation and edema to severe extensive necrosis and hemorrhage. The basic alterations are **(1) microvascular leakage causing edema, (2) necrosis of fat by lipolytic enzymes, (3) an acute inflammatory reaction, (4) proteolytic destruction of pancreatic parenchyma, and (5) destruction of blood vessels with subsequent interstitial hemorrhage.** The extent and predominance of each of these alterations depend on the duration and severity of the process.

In the milder form, acute interstitial pancreatitis, histologic alterations are limited to interstitial edema and focal areas of fat necrosis in the pancreatic substance and peripancreatic fat (Fig. 19-4). Fat necrosis, as we have seen, results from enzymatic destruction of fat cells. The released fatty acids combine with calcium to form insoluble salts that precipitate in situ (Chapter 1).

In the more severe form, **acute necrotizing pancreatitis**, necrosis of pancreatic tissue affects acinar and

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Figure 19-4 Acute pancreatitis. The microscopic field shows a region of fat necrosis on the right and focal pancreatic parenchymal necrosis (*center*).

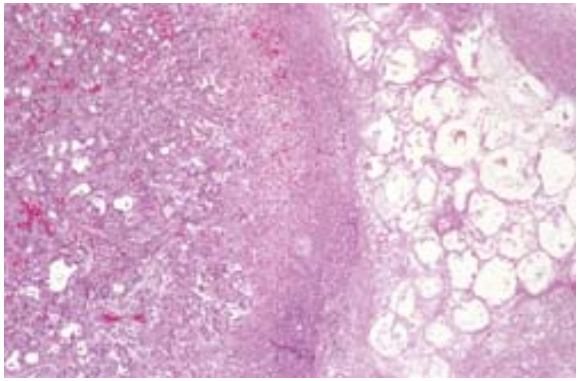

Figure 19-5 Acute pancreatitis. The pancreas has been sectioned across to reveal dark areas of hemorrhage in the head of the pancreas and a focal area of pale fat necrosis in the peripancreatic fat (*upper left*).

Figure 19-6 Three proposed pathways in the pathogenesis of acute pancreatitis.

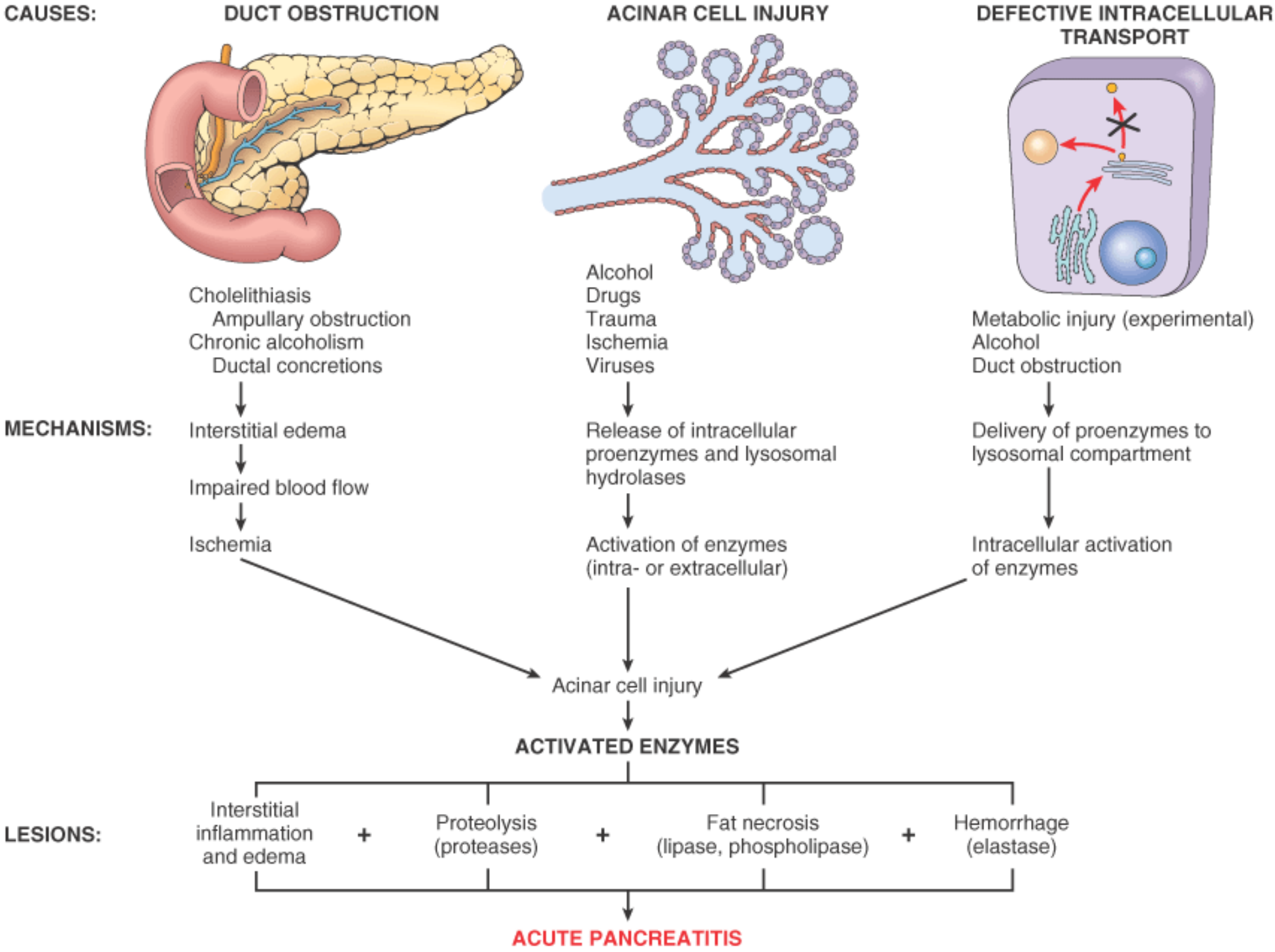

Figure 19-7 Comparison of the sequelae of acute and chronic pancreatitis. (ARDS = acute respiratory distress syndrome)

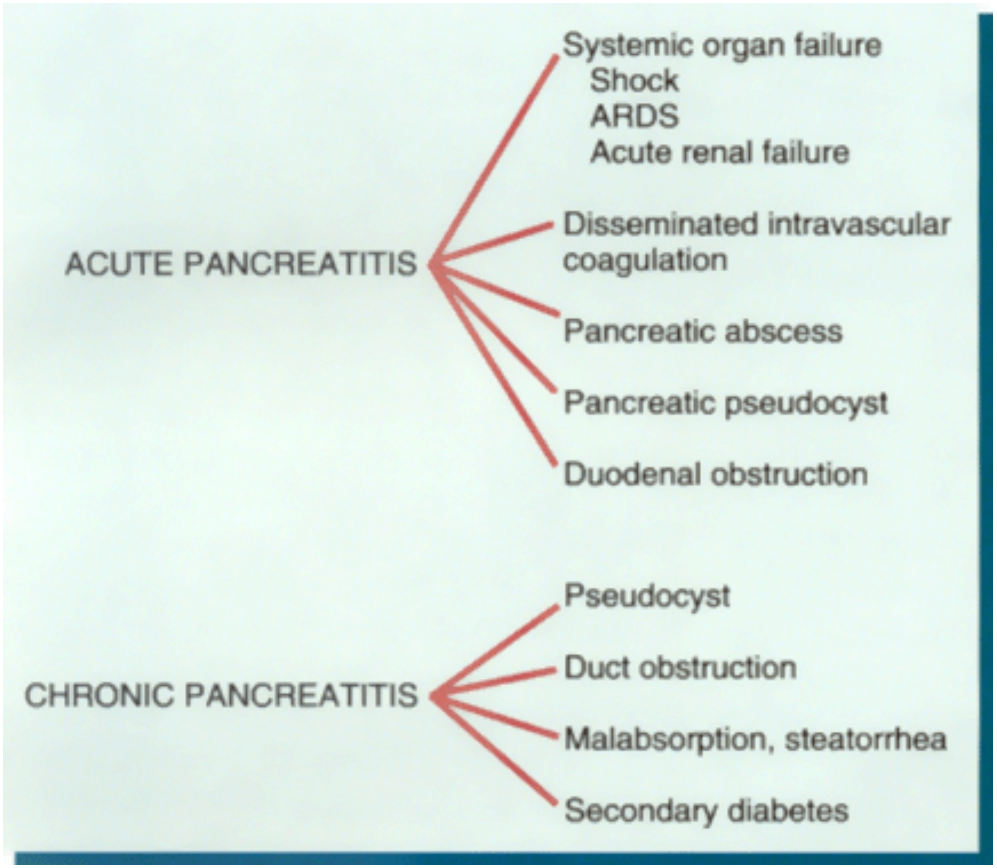

Figure 19-8 Chronic pancreatitis. *A*, Extensive fibrosis and atrophy has left only residual islets (*left*) and ducts (*right*), with a sprinkling of chronic inflammatory cells and acinar tissue. *B*, A higher-power view demonstrating dilated ducts with inspissated eosinophilic ductal concretions in a patient with alcoholic chronic pancreatitis.

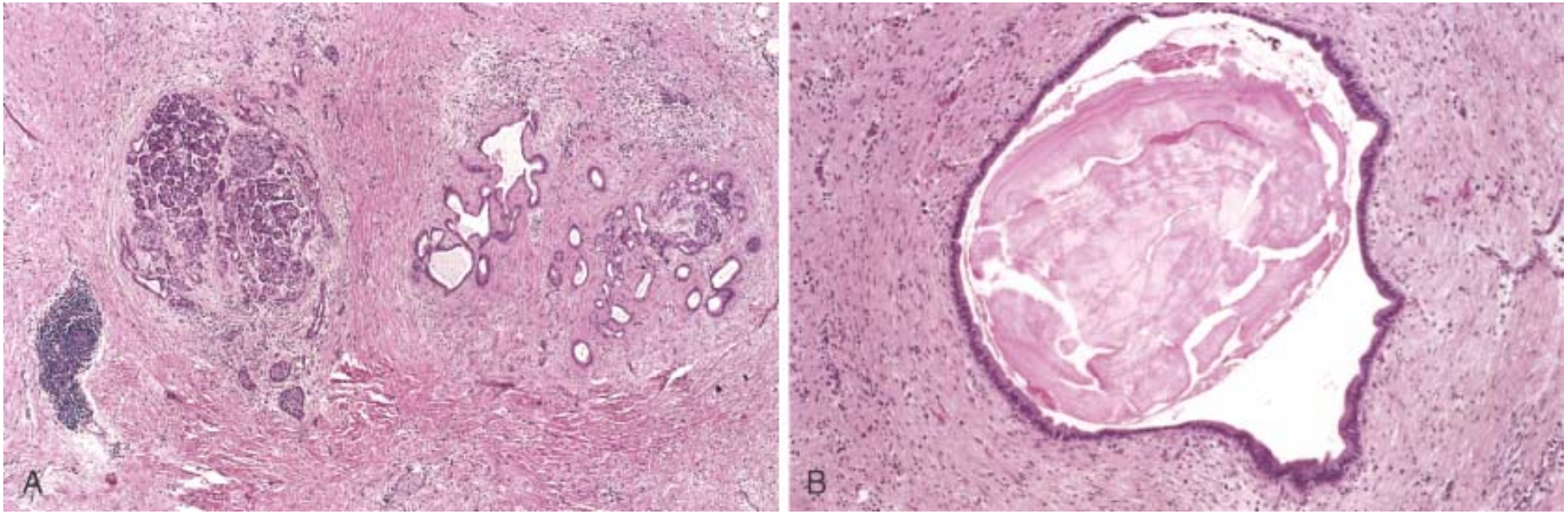

Figure 19-9 Pancreatic pseudocyst. *A,* Cross-section through this previously bisected lesion revealing a poorly defined cyst with a necrotic brown-black wall. *B,* Histologically, the cyst lacks a true epithelial lining and instead is lined by fibrin and granulation tissue.

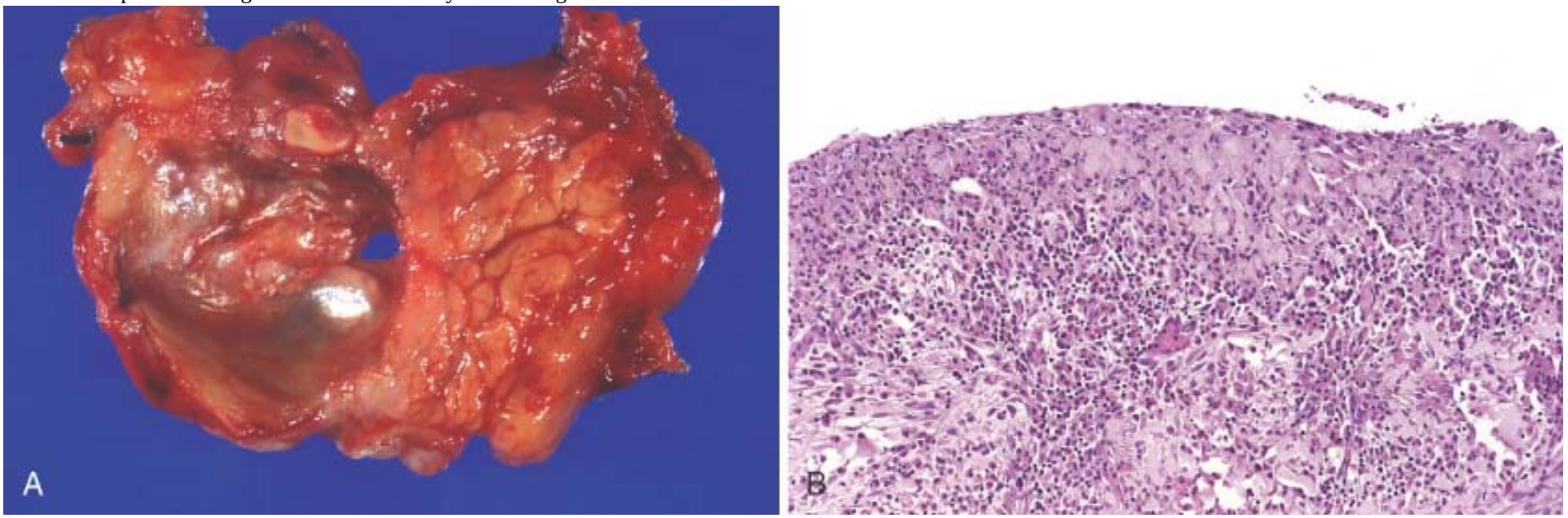

Figure 19-10 Serous cystadenoma. *A,* Cross-section through a serous cystadenoma. Only a thin rim of normal pancreatic parenchyma remains. The cysts are relatively small and contain clear, straw-colored fluid. *B,* The cysts are lined by cuboidal epithelium without atypia.

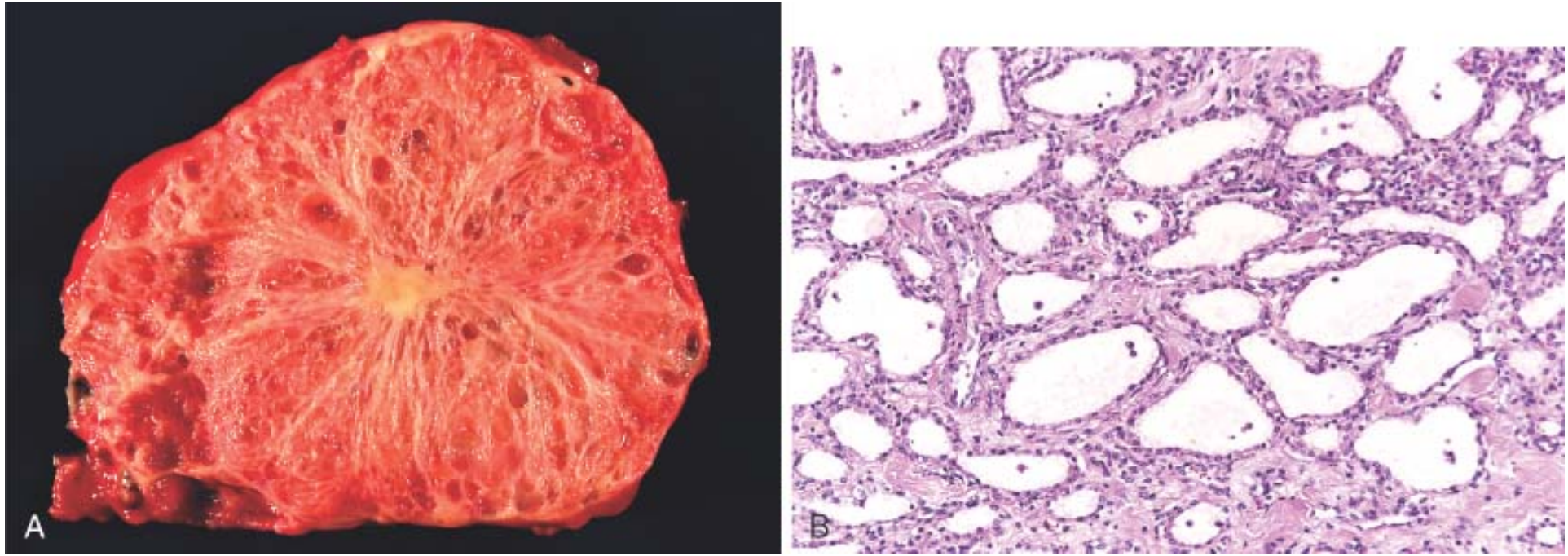

Figure 19-11 Pancreatic mucinous cystadenoma. *A,* Cross-section through a mucinous multiloculated cyst in the tail of the pancreas. The cysts are large and filled with tenacious mucin. *B,* The cysts are lined by columnar mucinous epithelium, and a dense "ovarian" stroma is noted.

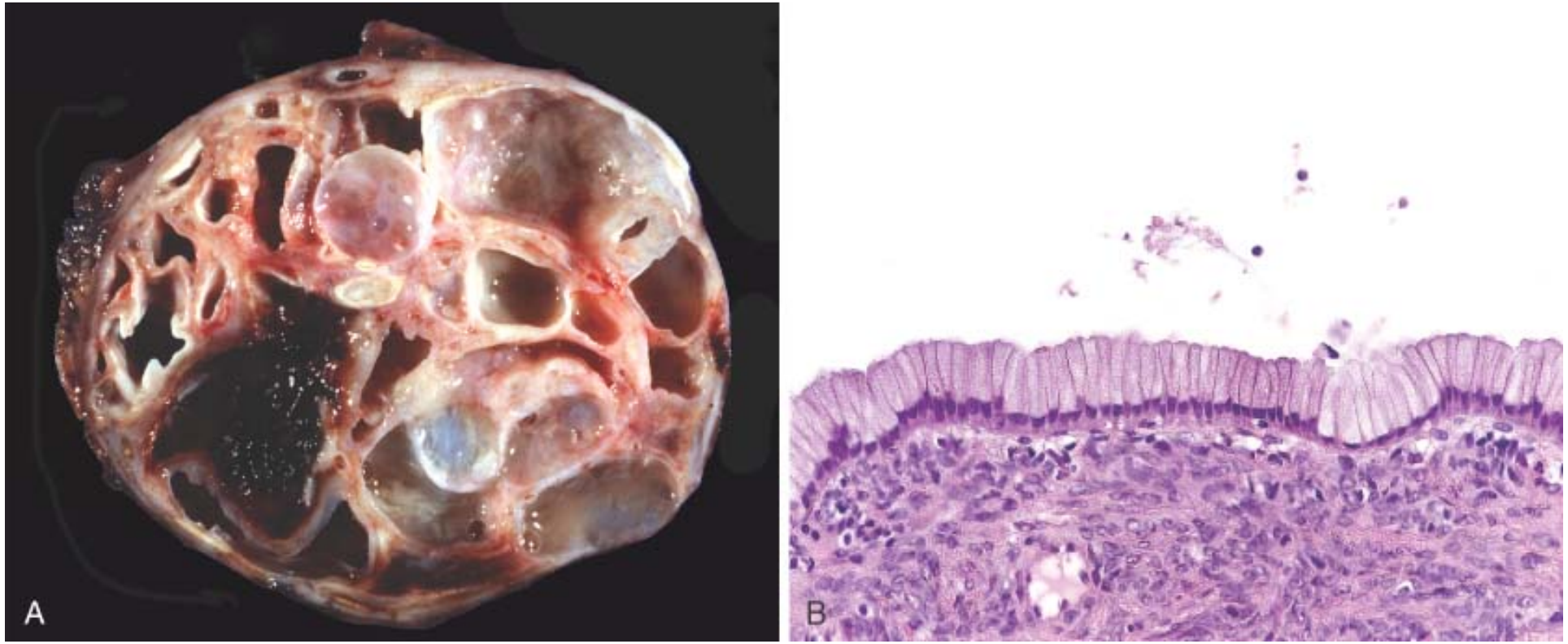

Figure 19-12 Intraductal papillary mucinous neoplasm. *A*, Cross-section through the head of the pancreas showing a prominent papillary neoplasm distending the main pancreatic duct. *B*, The papillary mucinous neoplasm involved the main pancreatic duct (*left*) and extending down into the smaller ducts and ductules (*right*).

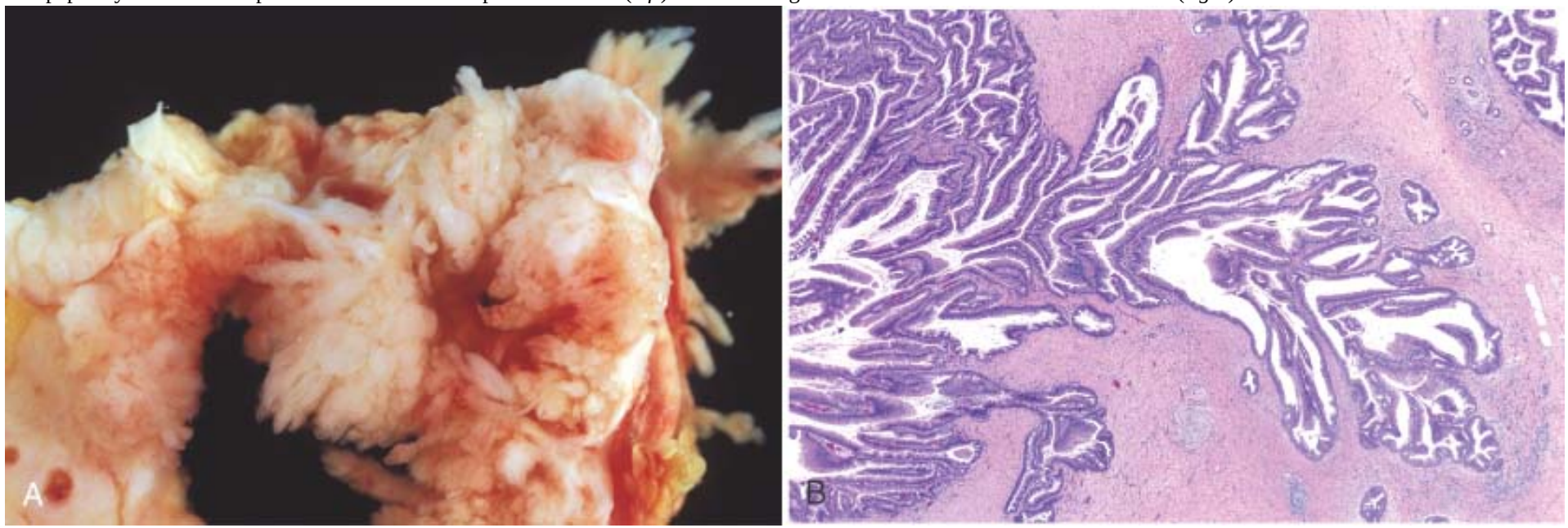

Figure 19-13 Progression model for the development of pancreatic cancer. It is postulated that telomere-shortening, and mutations of the oncogene *K-RAS* occur at early stages, that inactivation of the *p16* tumor suppressor gene occurs at intermediate stages, and the inactivation of the *p53*, *SMAD4* (*DPC4*), and *BRCA2* tumor suppressor genes occur at late stages. It is important to note that while there is a general temporal sequence of changes, the accumulation of multiple mutations is more important than their occurrence in a specific order. *(Adapted from Wilentz RE, Iacobuzio-Donahue CA, et al: Loss of expression of DPC4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. Cancer Res 2000; 60:2002.)*

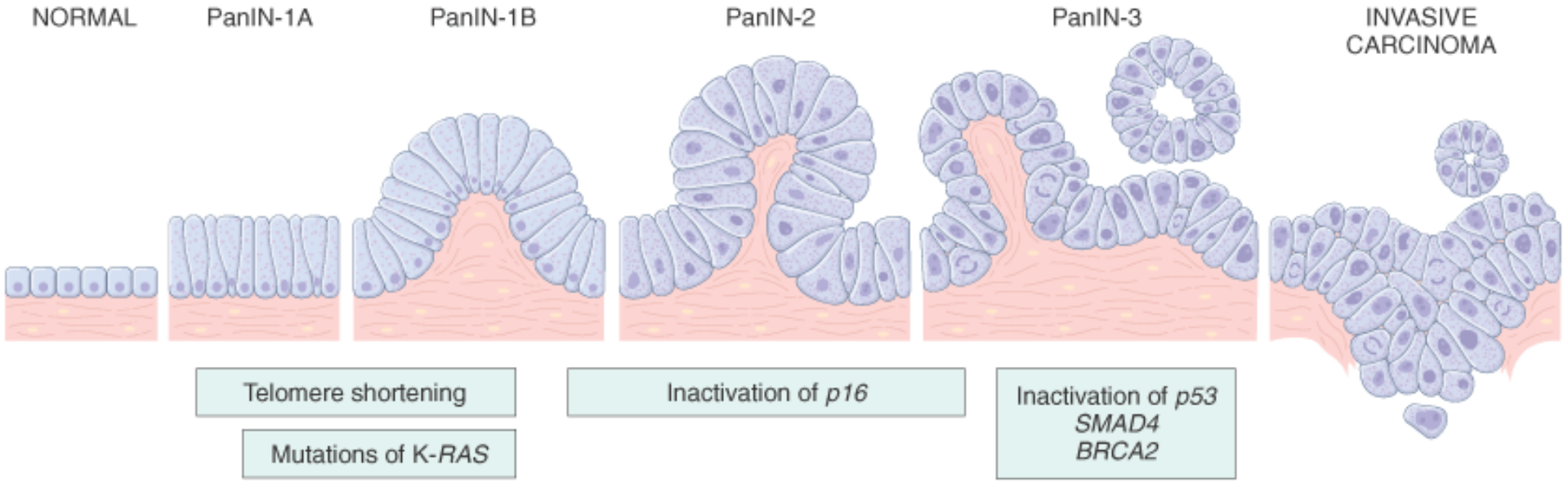

TABLE 19-2 -- Molecular Alterations in Invasive Pancreatic Adenocarcinoma

Gene (Chromosomal Region)	Percent of Tumors with Genetic Alteration
K-*ras* (12p)	>90%
p16 CDKN2A (9p)	>95%
p53 (17p)	50–70%
SMAD4 (18q)	55%
AKT2 (19q)	10–20%
MYB (6q)	10%
AIB1 (20q)	10%
BRCA2 (13q)	7–10%
LKB1/STK11 (19p)	<5%
MKK4 (17p)	<5%
TGFβ-R1 (9q) or *TGFβ-R2* (3p)	<5%

RB1 (13q)	<5%

K-RAS.

The *K-RAS* gene (chromosome 12p) is the most frequently altered oncogene in pancreatic cancer. This oncogene is activated by point mutation in 80% to 90% of pancreatic cancers. These point mutations impair the intrinsic GTPase activity of the K-*ras* gene product, resulting in a protein that is constitutively active. Ras in turn activates several intracellular signal transduction pathways that, among other effects, culminate in the activation of the transcription factors fos and jun.

p16.

The *p16* gene (chromosome 9p) is the most frequently inactivated tumor suppressor gene in pancreatic cancer.[62] It is inactivated in 95% of cases. The *p16* gene product, p16, plays a critical role in the control of the cell cycle, and inactivation of p16 abrogates an important cell cycle checkpoint.

SMAD4.

The *SMAD4* tumor suppressor gene (chromosome 18q) is inactivated in 55% of pancreatic cancers.[63] *SMAD4*, also known as *DPC4*, codes for a protein that plays an important role in signal transduction from the transforming growth factor-β (TGF-β) family of cell-surface receptors. The normal function of *SMAD4* is most likely to suppress growth and promote apoptosis. Loss of *SMAD4* therefore abrogates two important controls on cell population. *SMAD4* is only rarely inactivated in other cancer types.

p53.

Inactivation of the *p53* tumor suppressor gene (chromosome 17p) is seen in 50% to 70% of pancreatic cancers.[64] The *p53* gene product is a nuclear DNA-binding protein that acts both as a cell cycle checkpoint and as an inducer of cell death (apoptosis).

Other Genes.

A growing number of less common, but nonetheless important, genetic loci have been reported to be damaged in pancreatic cancer (Table 19-2). For example, the *AKT2* gene (chromosome 19q) is amplified in 10% to 20%, the *MYB* gene (6q) in 10%, and the *AIB1* gene (chromosome 20q) in 10%. The *BRCA2* (chromosome 13q), *LKB1/STK11* (chromosome 19p), *MKK4* (chromosome 17p), *TGFβ-R1* (chromosome 9q), *TGFβ-R2* (chromosome 3p), and *RB1* (chromosome 13q) tumor suppressor genes are inactivated in fewer than 10% of pancreatic cancers.

Methylation Abnormalities.

A number of methylation abnormalities also occur in pancreatic cancer. Hypermethylation of the promoter of a number of tumor suppressor genes is associated with transcriptional silencing of the genes.

Gene Expression.

In addition to DNA alterations, global analyses of gene expression have identified a number of genes that are highly overexpressed in pancreatic cancers. [65] [66] These overexpressed genes are potential targets for novel therapeutics and may form the basis of future screening tests. For example the hedgehog signaling pathway has recently been shown to be activated in pancreatic cancer. Inhibition of this pathway with the drug cyclopamine blocks growth of pancreatic cancers in experimental systems. [67]

Epidemiology, Etiology, and Pathogenesis.

Unlike other cancers of the alimentary tract, little is known about the cause of pancreatic cancer. Pancreatic cancer is primarily a disease in the elderly, 80% of cases occurring between the ages of 60 and 80.[68] It is more common in blacks than in whites, and it is slightly more common in individuals of Jewish decent.

The strongest environmental influence is *smoking*, which is believed to double the risk of pancreatic cancer.[68] Even though the magnitude of this increased risk is not great, the impact of smoking on pancreatic cancer is significant because of the large number of people who smoke. Consumption of a diet rich in fats has also been implicated but less consistently. Chronic pancreatitis and diabetes mellitus have both been associated with an increased risk of pancreatic cancer. Pancreatic cancer arises with greater frequency in patients with chronic pancreatitis, [50] but a causal role for pancreatitis, with the exception of hereditary pancreatitis, is not well established. Smoking and alcohol use in patients with chronic pancreatitis may underlie some of the association.[50] It is also hard to sort out whether chronic pancreatitis is the cause of pancreatic cancer or an effect of the disease, since small pancreatic cancers may block the pancreatic duct and produce chronic pancreatitis. A similar argument applies to the association of diabetes mellitus with pancreatic cancer, since diabetes may develop as a consequence of pancreatic cancer.

Familial clustering of pancreatic cancer has been reported, and a growing number of inherited genetic syndromes are now recognized that increase pancreatic cancer risk (Table 19-3). [69]

Morphology.

Approximately 60% of cancers of the pancreas arise in the head of the gland, 15% in the body, and 5% in the tail; in 20%, the neoplasm diffusely involves the entire gland. Carcinomas of the pancreas are usually hard, stellate, gray-white, poorly defined masses (Fig. 19-14*A*).

The vast majority of carcinomas are ductal adenocarcinomas that recapitulate to some degree the normal ductal epithelium by forming glands and secreting mucin. Two features are characteristic of pancreatic cancer: It is highly invasive (even "early" invasive pancreatic cancers extensively invade peripancreatic tissues), and it elicits an intense non-neoplastic host reaction composed of fibroblasts, lymphocytes, and extracellular matrix (called a "desmoplastic response").

Most carcinomas of the head of the pancreas obstruct the distal common bile duct as it courses

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TABLE 19-3 -- Familial Syndromes Predisposing to Pancreatic Cancer

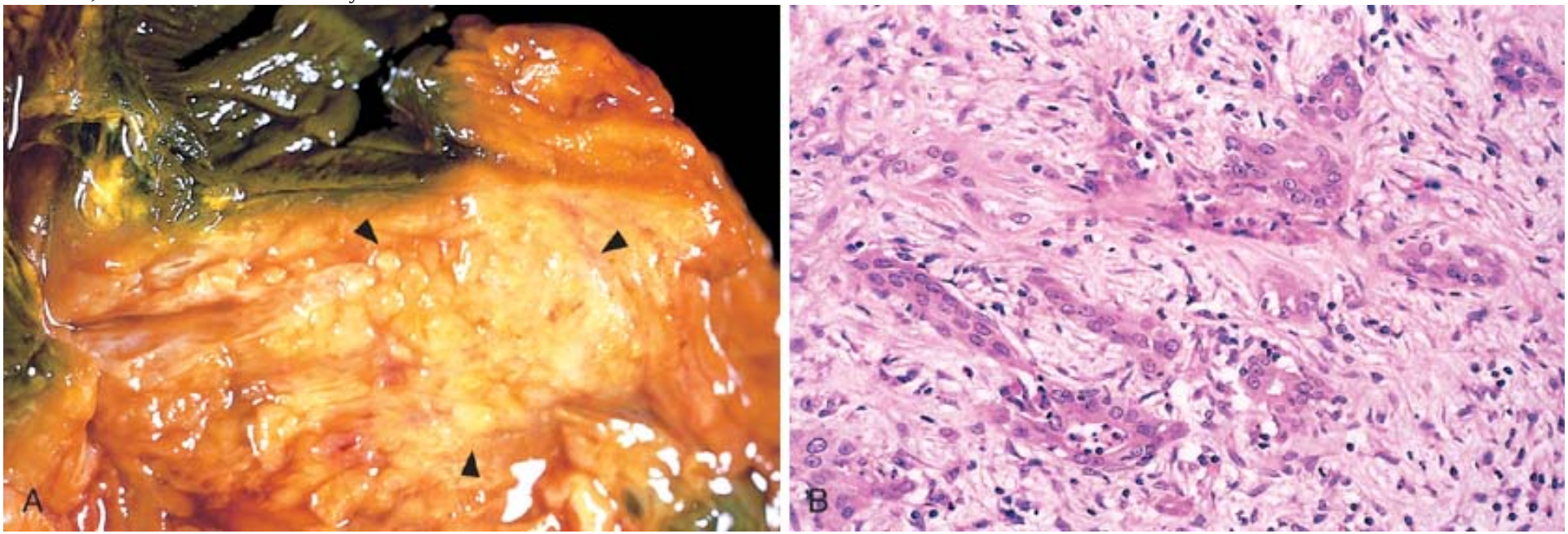
Disorder	Gene (Chromosome Location)	Increased Risk of Pancreatic Cancer
Hereditary nonpolyposis colorectal cancer (Lynch II variant)	*hMSH2* (2p22), *hMLH1* (3p21)	?
Hereditary breast and ovarian cancer	*BRCA2* (13q12-q13)	4–10X
Familial atypical multiple mole melanoma syndrome (FAMMM)	*p16* (9p21)	20–35X
Hereditary pancreatitis	*PRSS1* (7q35)	50–80X

Peutz-Jeghers syndrome	*STK11/LKB1* (19p13)	130X

through the head of the pancreas. As a consequence, there is marked distention of the biliary tree in about 50% of patients with carcinoma of the head of the pancreas, and most develop jaundice. In marked contrast, **carcinomas of the body and tail of the pancreas do not impinge on the biliary tract and hence remain silent for some time. They may be quite large and widely disseminated by the time they are discovered**. Pancreatic cancers often extend through the retroperitoneal space, entrapping adjacent nerves, and occasionally invade the spleen, adrenals, vertebral column, transverse colon, and stomach. Peripancreatic, gastric, mesenteric, omental, and portahepatic lymph nodes are frequently involved, and the liver is often enlarged owing to metastatic deposits. Distant metastases occur, principally to the lungs and bones.

Microscopically, there is no difference between carcinomas of the head of the pancreas and those of the body and tail of the pancreas. The appearance is usually that of a **moderately to poorly differentiated adenocarcinoma forming abortive tubular structures or cell clusters and exhibiting an aggressive, deeply infiltrative growth pattern** (Fig. 19-14*B*). Dense stromal fibrosis accompanies tumor invasion, and there is a proclivity for perineural invasion within and beyond the organ. Lymphatic invasion is also commonly seen. The malignant glands are atypical, irregular, small, and bizarre and are usually lined by

Figure 19-14 Carcinoma of the pancreas. *A,* A cross-section through the head of the pancreas and adjacent common bile duct showing both an ill-defined mass in the pancreatic substance (*arrowheads*) and the green discoloration of the duct resulting from total obstruction of bile flow. *B*, Poorly formed glands are present in densely fibrotic stroma within the pancreatic substance; there are some inflammatory cells.



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Chapter 20 - The Kidney

Charles E. Alpers MD

Normal

What is a human but an ingenious machine designed to turn, with "infinite artfulness, the red wine of Shiraz into urine"? So said the storyteller in Isak Dinesen's *Seven Gothic Tales*.[1] More accurately but less poetically, human kidneys serve to convert more than 1700 liters of blood per day into about 1 liter of a highly specialized concentrated fluid called urine. In so doing, the kidney excretes the waste products of metabolism, precisely regulates the body's concentration of water and salt, maintains the appropriate acid balance of plasma, and serves as an endocrine organ, secreting such hormones as erythropoietin, renin, and prostaglandins. The physiologic mechanisms that the kidney has evolved to carry out these functions require a high degree of structural complexity.

Each human adult kidney weighs about 150 gm. As the ureter enters the kidney at the hilum, it dilates into a funnel-shaped cavity, the *pelvis*, from which derive two or three main branches, the *major calyces*; each of these subdivides again into three or four *minor calyces*. There are about 12 minor calyces in the human kidney. On the cut surface, the kidney is made up of a *cortex* and a *medulla*, the former 1.2 to 1.5 cm in thickness. The medulla consists of *renal pyramids*, the apices of which are called *papillae*, each related to a calyx. Cortical tissue extends into spaces between adjacent pyramids as the *renal columns of Bertin*. From the standpoint of its diseases, the kidney can be divided into four components: blood vessels, glomeruli, tubules, and interstitium.

Blood Vessels.

The kidney is richly supplied by blood vessels, and although both kidneys make up only 0.5% of the total body weight, they receive about 25% of the cardiac output. The cortex is by far the most richly vascularized part of the kidney, receiving 90% of the total renal blood supply. The main renal artery divides into anterior and posterior sections at the hilum. From these, *interlobar arteries* emerge, course between lobes, and give rise to the *arcuate arteries*, which arch between cortex and medulla, in turn giving rise to the *interlobular arteries*. From the interlobular arteries, *afferent arterioles* enter the glomerular tuft, where they progressively subdivide into 20 to 40 capillary loops arranged in several units or lobules architecturally centered by a supporting mesangial stalk. Capillary loops merge to exit from the glomerulus as *efferent arterioles*. In general, efferent arterioles from superficial nephrons form a rich vascular network that encircles cortical tubules (*peritubular vascular network*), and deeper juxtamedullary glomeruli give rise to the *vasa recta*, which descend as straight vessels to supply the outer and inner medulla. These descending arterial vasa recta then make several loops in the inner medulla and ascend as the *venous vasa recta*.

The anatomy of renal vessels has several important implications. First, because the arteries are largely end-arteries, *occlusion of any branch usually results in infarction of the specific area it supplies*. Glomerular disease that interferes with blood flow through the glomerular capillaries has profound effects on the tubules, within both the cortex and the medulla, because *all tubular capillary beds are derived from the efferent arterioles*. The peculiarities of the blood supply to the renal medulla render them especially vulnerable to ischemia; *the medulla does not have its own arterial blood supply but is dependent on the blood emanating from the glomerular efferent arterioles*. The blood in the capillary loops in the medulla has a remarkably low level of oxygenation. Thus, minor interference with the blood supply of the medulla may result in medullary necrosis from ischemia.

Glomeruli.

The glomerulus consists of an anastomosing network of capillaries lined by fenestrated endothelium invested by two layers of epithelium (Fig. 20-1). The visceral epithelium is incorporated into and becomes an intrinsic part of the capillary wall, separated from endothelial cells by a basement membrane. The parietal epithelium, situated on Bowman's capsule, lines the urinary space, the cavity in which plasma filtrate first collects.

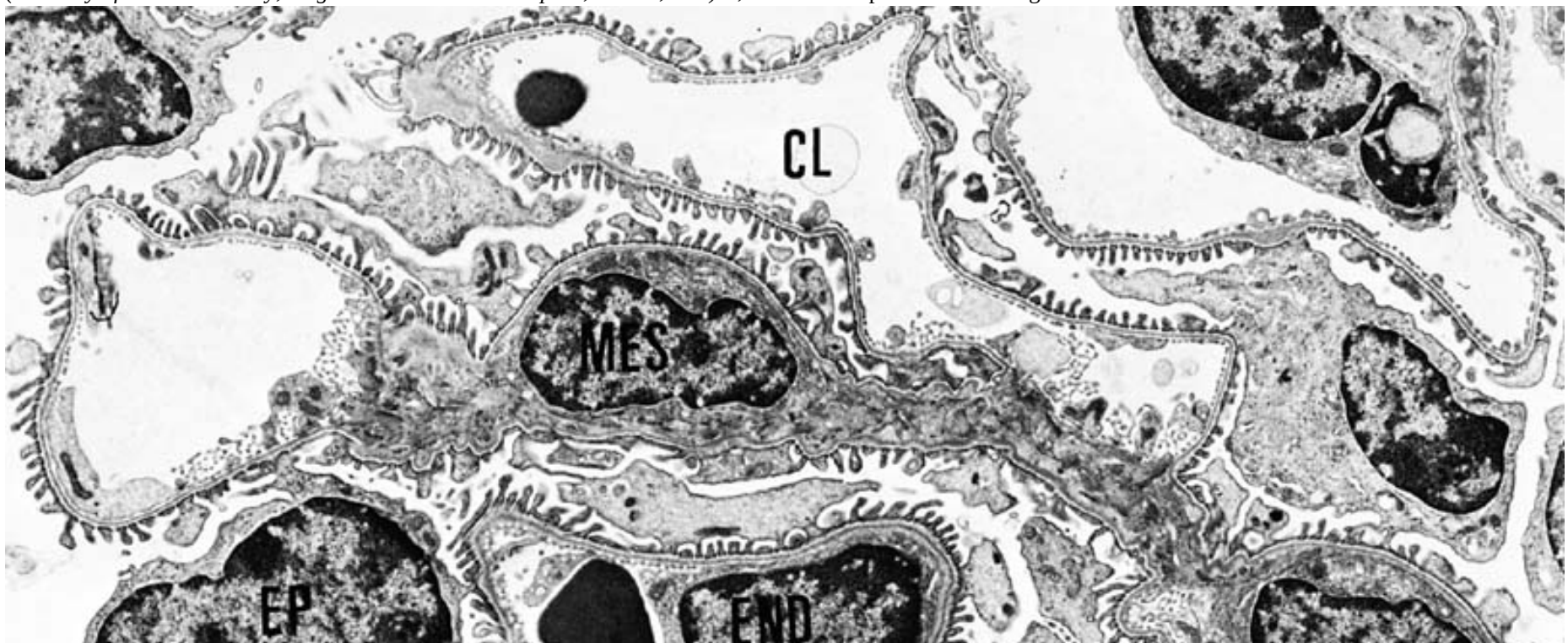
The glomerular capillary wall is the filtering membrane and consists of the following structures[2] (Fig. 20-2):

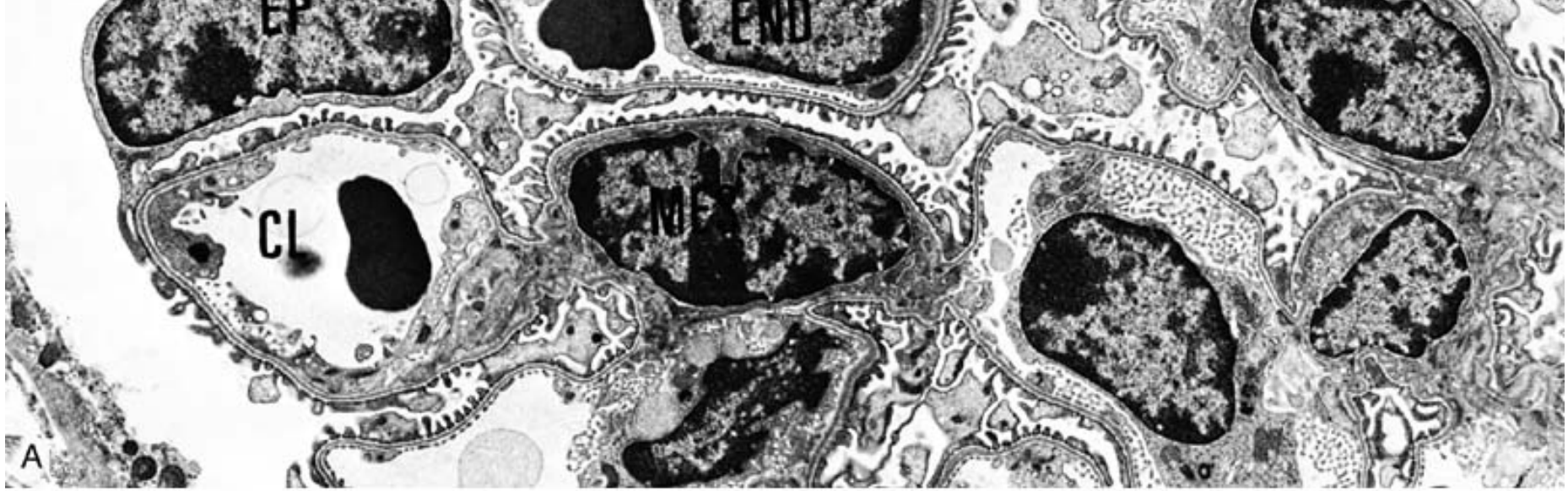
- A thin layer of fenestrated *endothelial cells*, each fenestrum being about 70 to 100 nm in diameter.
- A *glomerular basement membrane* (GBM) with a thick electron-dense central layer, the *lamina densa*, and thinner electron-lucent peripheral layers, the *lamina rara interna* and *lamina rara externa*. The GBM consists of collagen (mostly type IV), laminin, polyanionic proteoglycans (mostly heparan sulfate), fibronectin, entactin, and several other glycoproteins. Type IV collagen forms a network

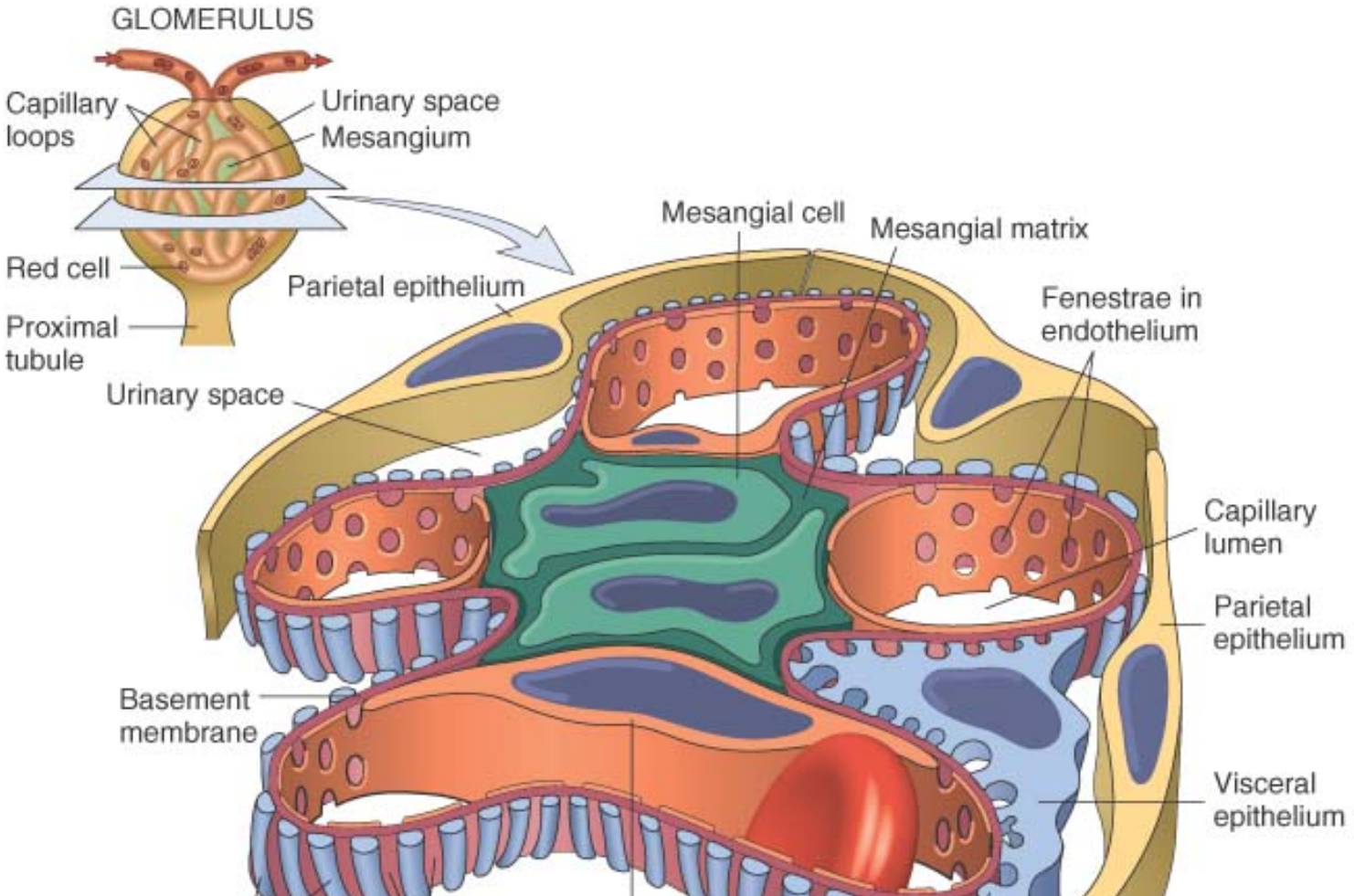
suprastructure to which other glycoproteins attach. The building block (monomer) of this network is a triple-helical molecule made up of three α-chains, composed of one or more of six types of α-chains ($\alpha_1$to $\alpha_6$or COL4A1 to COL4A6), the most common consisting of α_1, α_2, α_1(Fig. 20-3). [3]Each molecule consists of a 7S domain at the amino terminus, a triple-helical domain in the middle, and a globular noncollagenous domain (NC1) at the carboxyl terminus. The NC1 domain is important for helix formation and for assembly of collagen monomers into the basement membrane suprastructure. Glycoproteins (laminin, entactin) and acidic proteoglycans (heparan sulfate, perlecan) attach to the collagenous suprastructure[3][4][5](Fig. 20-4). *These biochemical determinants are critical to understanding glomerular diseases.*For example, as we shall see, the antigens in the NC1 domain are the targets of antibodies in anti-GBM nephritis; genetic defects in the α-chains underlie some forms of hereditary nephritis; and the acidic porous nature of the GBM determines its permeability characteristics.

- The *visceral epithelial cells* (podocytes), are structurally complex cells that possess interdigitating processes embedded in and adherent to the lamina rara externa of the basement membrane. Adjacent *foot processes* (pedicels) are separated by 20- to 30-nm-wide *filtration slits*, which are bridged by a thin diaphragm (see Fig. 20-2).
- The entire glomerular tuft is supported by *mesangial cells* lying between the capillaries. Basement membrane-like *mesangial matrix* forms a meshwork through which the mesangial cells are centered (Fig. 20-1). These cells, of mesenchymal origin, are contractile, phagocytic, and capable of proliferation, of laying down both matrix and collagen, and of secreting a number of biologically active mediators. Biologically, they are most akin to vascular smooth muscle cells and pericytes. They are, as we shall see, important players in many forms of human glomerulonephritis.

Figure 20-1 *A*, Low-power electron micrograph of renal glomerulus. CL, capillary lumen; MES, mesangium; END, endothelium; EP, visceral epithelial cells with foot processes. *(Courtesy of Dr. Vicki Kelley, Brigham and Women's Hospital, Boston, MA.) B*, Schematic representation of a glomerular lobe.




EP
END
CL
M
A


GLOMERULUS
Capillary loops
Urinary space
Mesangium
Red cell
Proximal tubule
Parietal epithelium
Urinary space
Mesangial cell
Mesangial matrix
Fenestrae in endothelium
Capillary lumen
Parietal epithelium
Basement membrane
Visceral epithelium

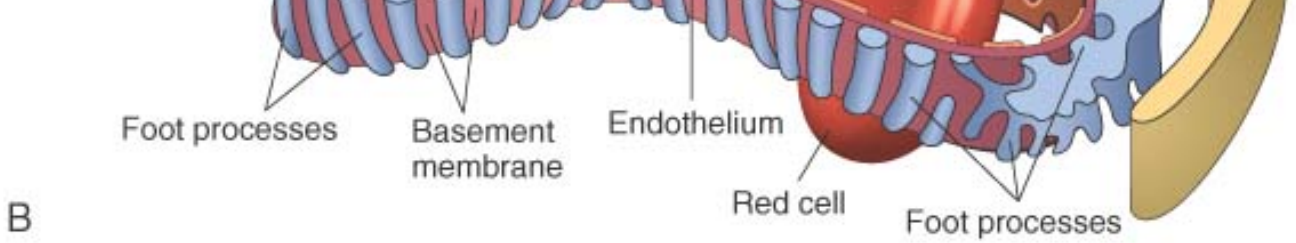


Figure 20-2 Glomerular filter consisting, from bottom to top, of fenestrated endothelium, basement membrane, and foot processes of epithelial cells. Note the filtration slits (*arrows*) and diaphragm. Note also that the basement membrane consists of a central lamina densa, sandwiched between two looser layers, the lamina rara interna and lamina rara externa. *(Courtesy of Dr. Helmut Rennke, Brigham and Women's Hospital, Boston, MA.)*

Figure 20-3 Schematic illustration of type IV collagen supramolecular network assembly. *A*, Six genetically distinct α-chains (α1 to α6) assemble into three distinct protomers. The protomers are characterized by a long central collagen triple helix, the 7S domain at the N terminus, and a globular NC1 trimer at the C terminus. *B*, NC1 domains provide specificity for chain association, alignment, registration, and propagation from the C- to N-terminal direction. This sequence of events, shown for the α1, α2 protomer, is true for other protomers also. *(Courtesy of Dr. Billy Hudson, Vanderbilt University, Nashville, TN, reprinted with permission.)*

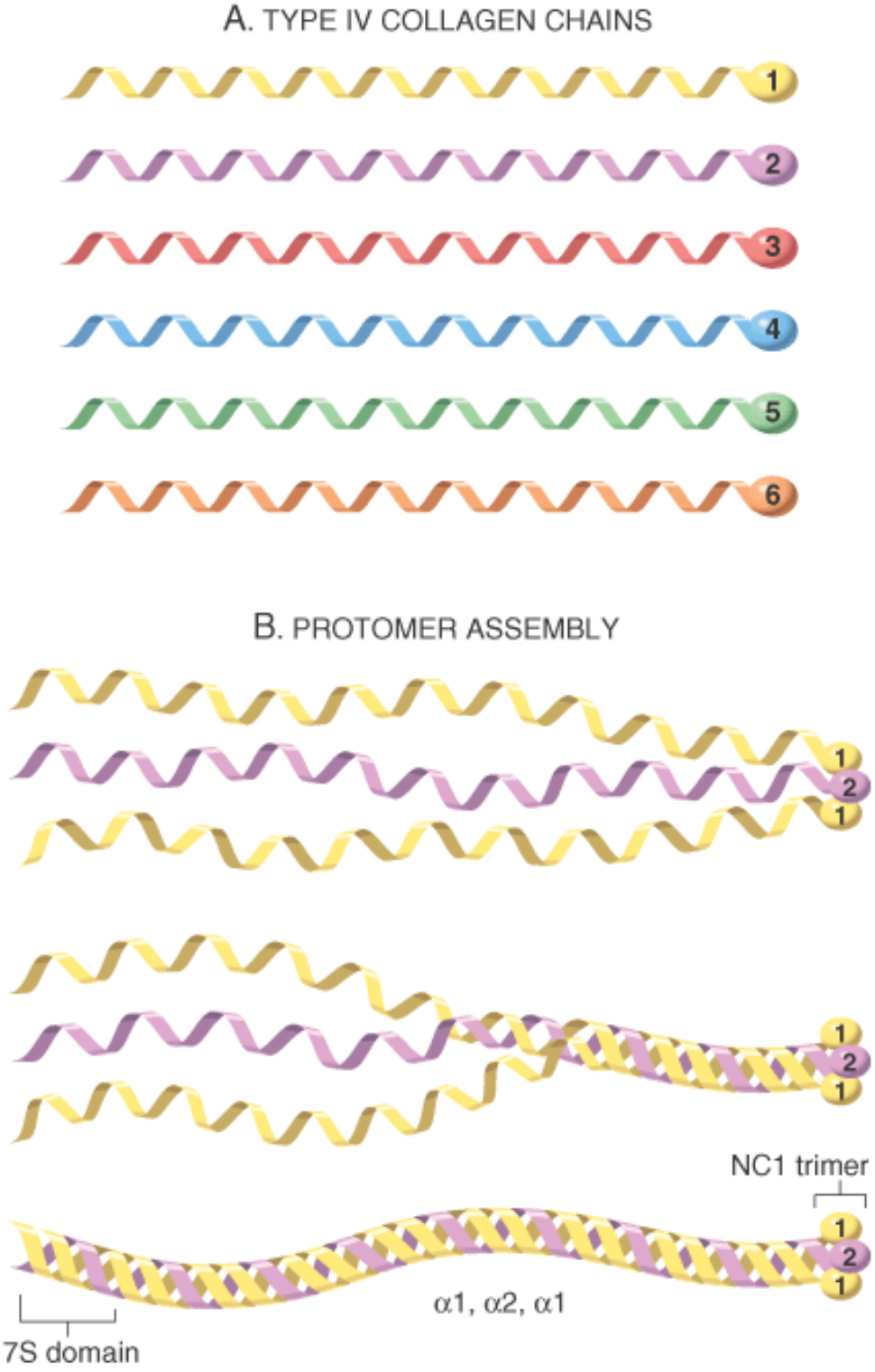

A. TYPE IV COLLAGEN CHAINS
1
2
3
4
5
6
B. PROTOMER ASSEMBLY
1
2
1
1
2
1
NC1 trimer
1
2
1
α1, α2, α1
7S domain

Figure 20-4 A proposed model of the GBM molecular architecture in which type IV collagen monomers (*gray*) form a stable network through their NC1 domains (dimeric interactions, gray spheres) and 7S domains (tetrameric interactions) and intertwine along the triple-helical domains. Laminin monomers (*red*) separately form a reversible meshwork. Entactin (*green*) connects laminin to the collagen network and binds to perlecan (*blue*), an anionic heparan sulfate proteoglycan. This anionic suprastructure determines the charged porous nature of the GBM. *(Courtesy of Dr. Peter Yurchenco, Robert W. Johnson Medical School, Piscataway, NJ.)*

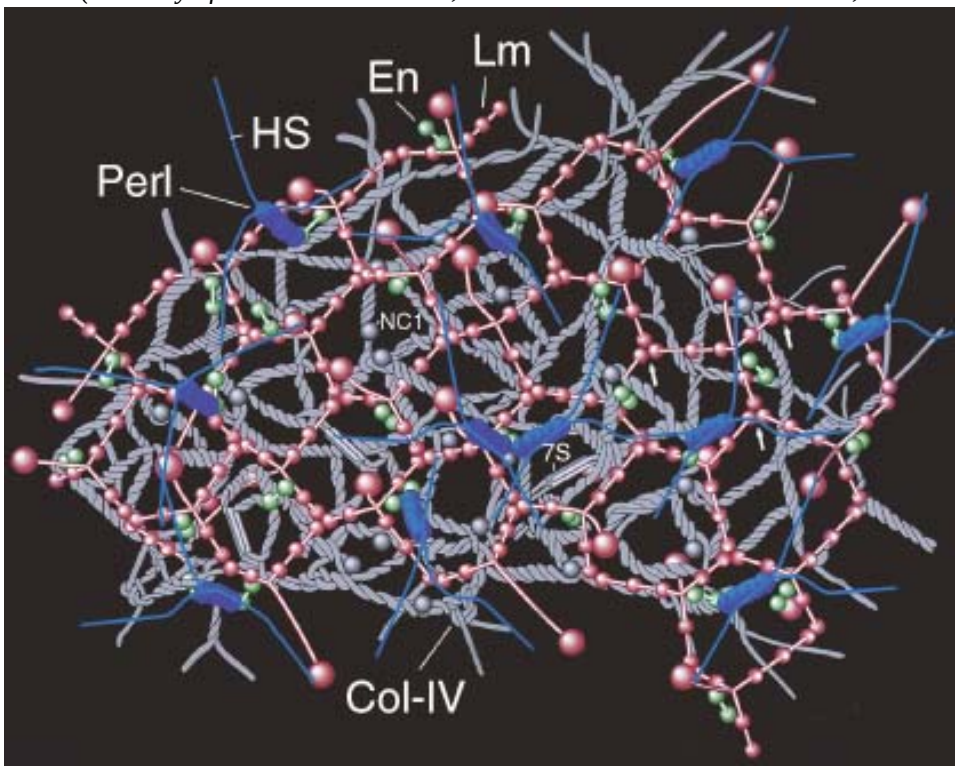


Figure 20-5 Schematic diagram of the proteins of the glomerular slit diaphragm. CD2AP, CD2-associated protein.

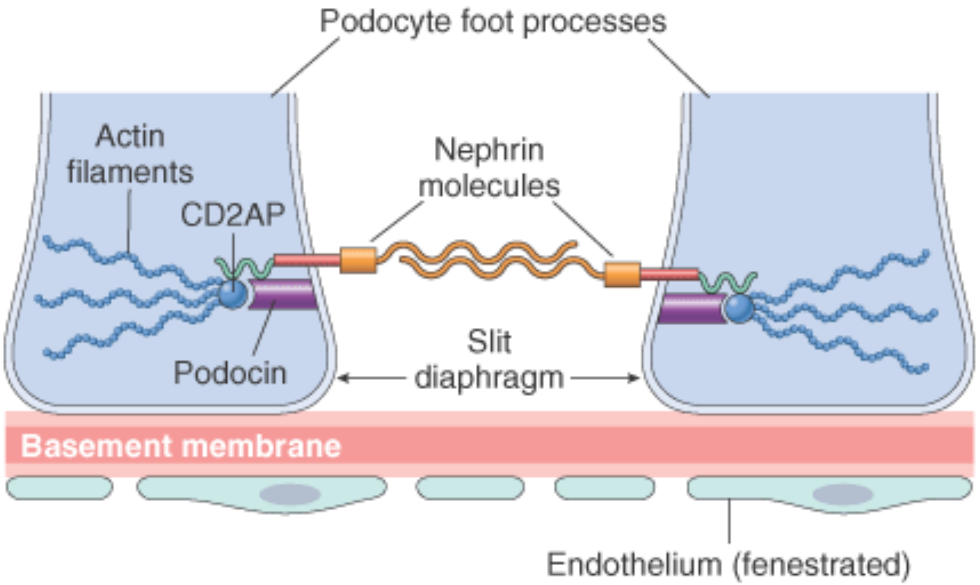


TABLE 20-1 -- Principal Systemic Manifestations of Chronic Renal Failure and Uremia

Fluid and Electrolytes
Dehydration
Edema
Hyperkalemia
Metabolic acidosis
Calcium Phosphate and Bone
Hyperphosphatemia
Hypocalcemia
Secondary hyperparathyroidism
Renal osteodystrophy
Hematologic
Anemia
Bleeding diathesis
Cardiopulmonary
Hypertension
Congestive heart failure

Pulmonary edema
Uremic pericarditis
Gastrointestinal
Nausea and vomiting
Bleeding
Esophagitis, gastritis, colitis
Neuromuscular
Myopathy
Peripheral neuropathy
Encephalopathy
Dermatologic
Sallow color
Pruritus
Dermatitis

Congenital Anomalies

About 10% of all people are born with potentially significant malformations of the urinary system. Renal dysplasias and hypoplasias account for 20% of chronic renal failure in children. Autosomal-dominant polycystic kidney disease, a congenital anomaly that becomes apparent in adults, is responsible for about 10% of chronic renal failure in humans.

Congenital renal disease can be hereditary but is most often the result of an acquired developmental defect that arises during gestation. As was discussed in Chapter 10 , defects in genes involved in development, including the Wilms tumor (WT1)-associated genes, cause urogenital anomalies. As a rule, developmental abnormalities involve structural components of the kidney and urinary tract. However, genetic abnormalities also cause enzymatic or metabolic defects in tubular transport, such as cystinuria and renal tubular acidosis. Here, we restrict the discussion to structural anomalies involving primarily the kidney. All except horseshoe kidney are uncommon. Anomalies of the lower urinary tract are discussed in Chapter 21 .

Agenesis of the Kidney.

Total bilateral agenesis, which is incompatible with life, is usually encountered in stillborn infants. It is often associated with many other congenital disorders (e.g., limb defects, hypoplastic lungs) and leads to early death. Unilateral agenesis is an uncommon anomaly that is compatible with normal life if no other abnormalities exist. The opposite kidney is usually enlarged as a result of compensatory hypertrophy. Some patients eventually develop progressive glomerular sclerosis in the remaining kidney as a result of the adaptive changes in hypertrophied nephrons, discussed later in the chapter, and in time, chronic renal failure ensues.

Hypoplasia.

Renal hypoplasia refers to failure of the kidneys to develop to a normal size. This anomaly may occur bilaterally, resulting in renal failure in early childhood, but it is more commonly encountered as a unilateral defect. True renal hypoplasia is extremely rare; most cases reported probably represent acquired scarring due to vascular, infectious, or other parenchymal diseases rather than an underlying developmental failure. Differentiation between congenital and acquired atrophic kidneys may be impossible, but *a truly hypoplastic kidney shows no*

scars and has a reduced number of renal lobes and pyramids, usually six or fewer. In one form of hypoplastic kidney, *oligomeganephronia*, the kidney is small but the nephrons are markedly hypertrophied.

Ectopic Kidneys.

The development of the definitive metanephros may occur in ectopic foci, usually at abnormally low levels. These kidneys lie either just above the pelvic brim or sometimes within the pelvis. They are usually normal or slightly small in size but otherwise are not remarkable. Because of their abnormal position, kinking or tortuosity of the ureters may cause some obstruction to urinary flow, which predisposes to bacterial infections.

Horseshoe Kidneys.

Fusion of the upper or lower poles of the kidneys produces a horseshoe-shaped structure that is continuous across the midline anterior to the great vessels. This anatomic anomaly is common and is found in about 1 in 500 to 1000 autopsies. Ninety per cent of such kidneys are fused at the lower pole, and 10% are fused at the upper pole.

Cystic Diseases of the Kidney

Although not all cysts of the kidney are congenital, all types of cysts are discussed here for convenience.

Cystic diseases of the kidney are a heterogeneous group comprising hereditary, developmental but nonhereditary, and acquired disorders. As a group, they are important for several reasons: (1) they are reasonably common and often represent diagnostic problems for clinicians, radiologists, and pathologists; (2) some forms, such as adult polycystic disease, are major causes of chronic renal failure; and (3) they can occasionally be confused with malignant tumors. A useful classification of renal cysts is as follows:[9]

1. Cystic renal dysplasia
2. Polycystic kidney disease
    a. Autosomal-dominant (adult) polycystic disease
    b. Autosomal-recessive (childhood) polycystic disease
3. Medullary cystic disease
    a. Medullary sponge kidney
    b. Nephronophthisis
4. Acquired (dialysis-associated) cystic disease
5. Localized (simple) renal cysts
6. Renal cysts in hereditary malformation syndromes (e.g., tuberous sclerosis)
7. Glomerulocystic disease
8. Extraparenchymal renal cysts (pyelocalyceal cysts, hilar lymphangitic cysts)

Only the more important of the cystic diseases are discussed below. Table 20-2 summarizes the characteristic features of the principal renal cystic diseases.

CYSTIC RENAL DYSPLASIA

This sporadic disorder is due to an abnormality in metanephric differentiation *characterized histologically by the*

TABLE 20-2 -- Summary of Renal Cystic Diseases

	Inheritance	Pathologic Features	Clinical Features or Complications	Typical Outcome	Diagrammatic Representation
Adult polycystic kidney disease	Autosomal dominant	Large multicystic kidneys, liver cysts, berry aneurysms	Hematuria, flank pain, urinary tract infection, renal stones, hypertension	Chronic renal failure beginning at age 40–60 yr	
Childhood polycystic kidney disease	Autosomal recessive	Enlarged, cystic kidneys at birth	Hepatic fibrosis	Variable, death in infancy or childhood	
Medullary sponge kidney	None	Medullary cysts on excretory urography	Hematuria, urinary tract infection, recurrent renal stones	Benign	
Familial juvenile nephronophthisis	Autosomal recessive	Corticomedullary cysts, shrunken kidneys	Salt wasting, polyuria, growth retardation, anemia	Progressive renal failure beginning in childhood	
Adult-onset medullary cystic disease	Autosomal dominant	Corticomedullary cysts, shrunken kidneys	Salt wasting, polyuria	Chronic renal failure beginning in adulthood	
Simple cysts	None	Single or multiple cysts in normal-sized kidneys	Microscopic hematuria	Benign	
Acquired renal cystic disease	None	Cystic degeneration in end-stage kidney disease	Hemorrhage, erythrocytosis, neoplasia	Dependence on dialysis	

persistence in the kidney of abnormal structures—cartilage, undifferentiated mesenchyme, and immature collecting ductules—and by abnormal lobar organization. Most cases are associated with ureteropelvic obstruction, ureteral agenesis or atresia, and other anomalies of the lower urinary tract.

Dysplasia can be unilateral or bilateral and is almost always cystic. In gross appearance, the kidney is usually enlarged, extremely irregular, and multicystic (Fig. 20-6*A*). The cysts vary in size from microscopic structures to some that are several centimeters in diameter. On histologic examination, they are lined by flattened epithelium. Although normal nephrons are present, many have immature ducts. The characteristic histologic feature is the *presence of islands of undifferentiated mesenchyme, often with cartilage, and immature collecting ducts* (Fig. 20-6*B*).

When unilateral, the dysplasia is discovered by the appearance of a flank mass that leads to surgical exploration and nephrectomy. The function of the opposite kidney is normal, and such patients have an excellent prognosis after surgical removal of the affected kidney. In bilateral renal dysplasia, renal failure may ultimately result.

AUTOSOMAL-DOMINANT (ADULT) POLYCYSTIC KIDNEY DISEASE

Autosomal-dominant (adult) polycystic kidney disease (ADPKD) is a hereditary disorder characterized by multiple expanding cysts of both kidneys that ultimately destroy the renal parenchyma and cause renal failure.[10] It is a common condition affecting roughly 1 of every 400 to 1000 live births and accounting for about 5% to 10% of cases of chronic renal failure requiring transplantation or dialysis. The pattern of inheritance is *autosomal dominant*, with high penetrance. The disease is universally bilateral; reported unilateral cases probably represent multicystic dysplasia. The cysts initially involve only portions of the nephrons, so renal function is retained until about the fourth or fifth decade of life. ADPKD is genetically

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Figure 20-6 Renal dysplasia. *A*, Gross appearance. *B*, Histologic section showing disorganized architecture, dilated tubules with cuffs of primitive stroma, and an island of cartilage (H&E stain). *(A, courtesy of Dr. D. Schofield, Children's Hospital, Los Angeles, CA; B, courtesy of Dr. Laura Finn, Children's Hospital, Seattle, WA.)*

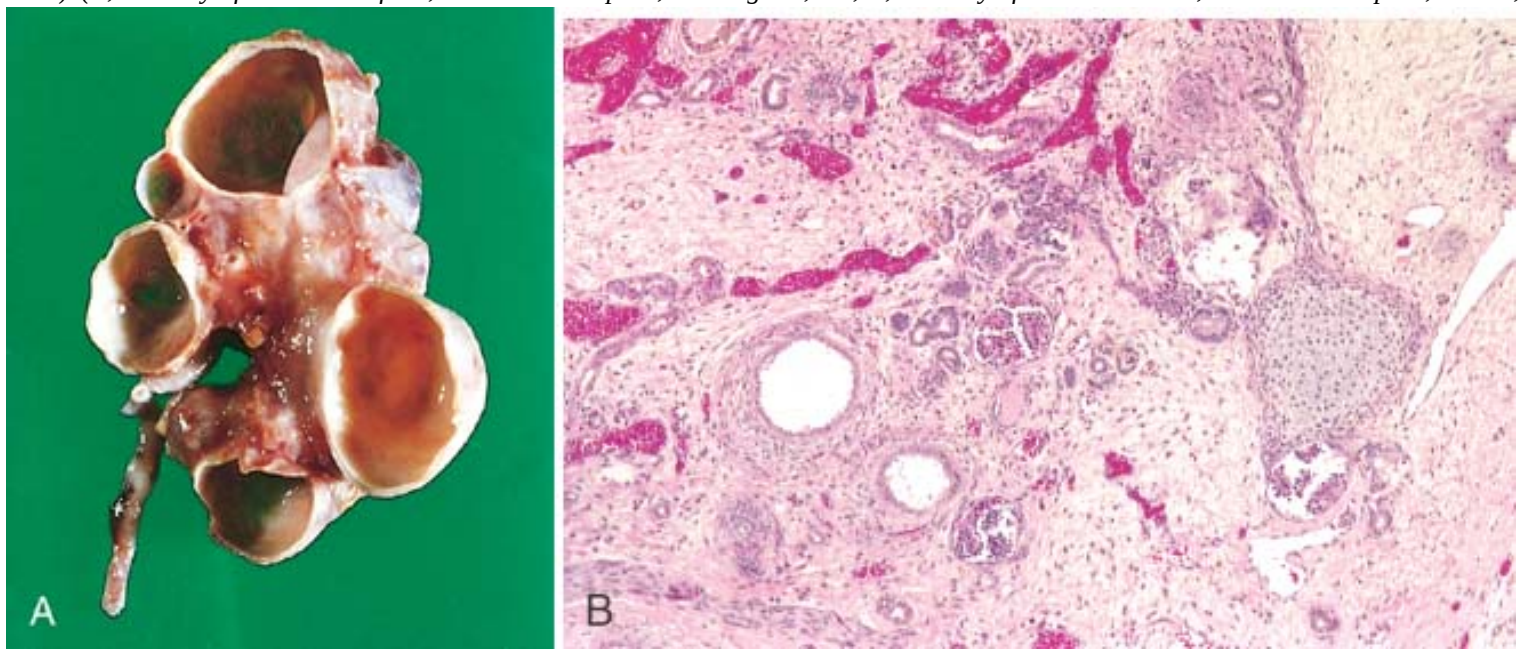

Figure 20-7 Possible mechanisms of cyst formation in polycystic kidney disease (see text).

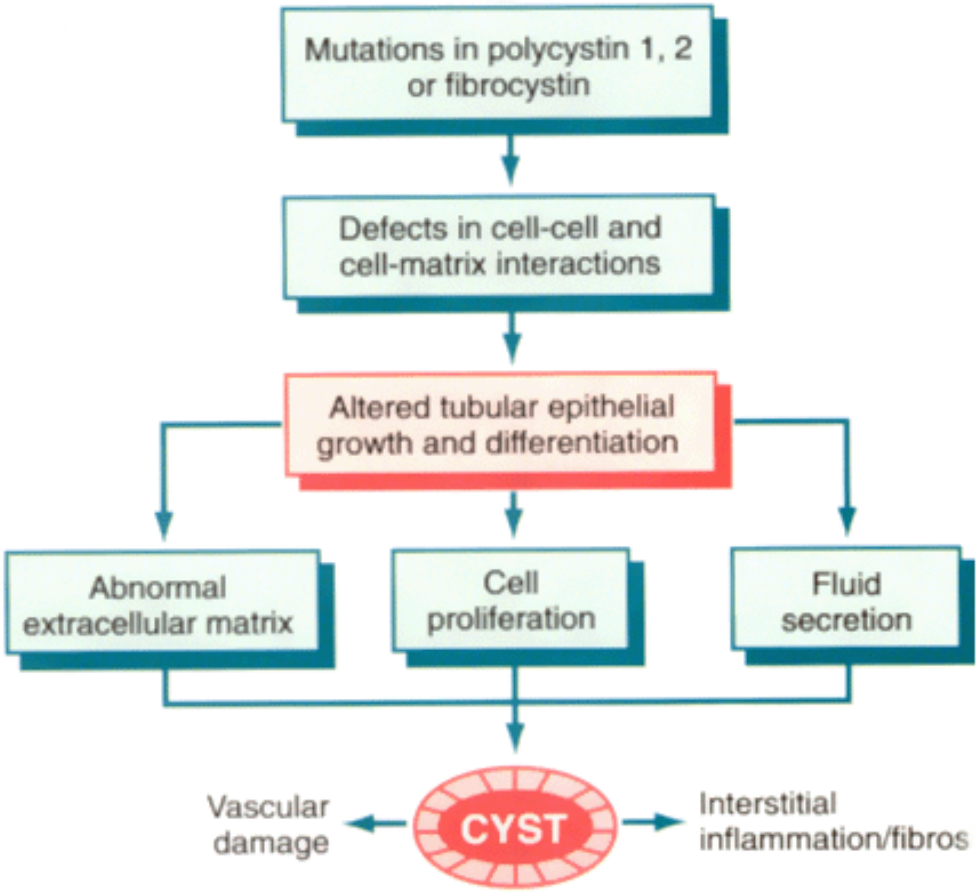

Figure 20-8 *A* and *B*, Autosomal-dominant adult polycystic kidney disease (ADPKD) viewed from the external surface and bisected. The kidney is markedly enlarged with numerous dilated cysts. *C*, Autosomal-recessive childhood polycystic kidney disease, showing smaller cysts and dilated channels at right angles to the cortical surface. *D*, Liver with cysts in adult PKD.

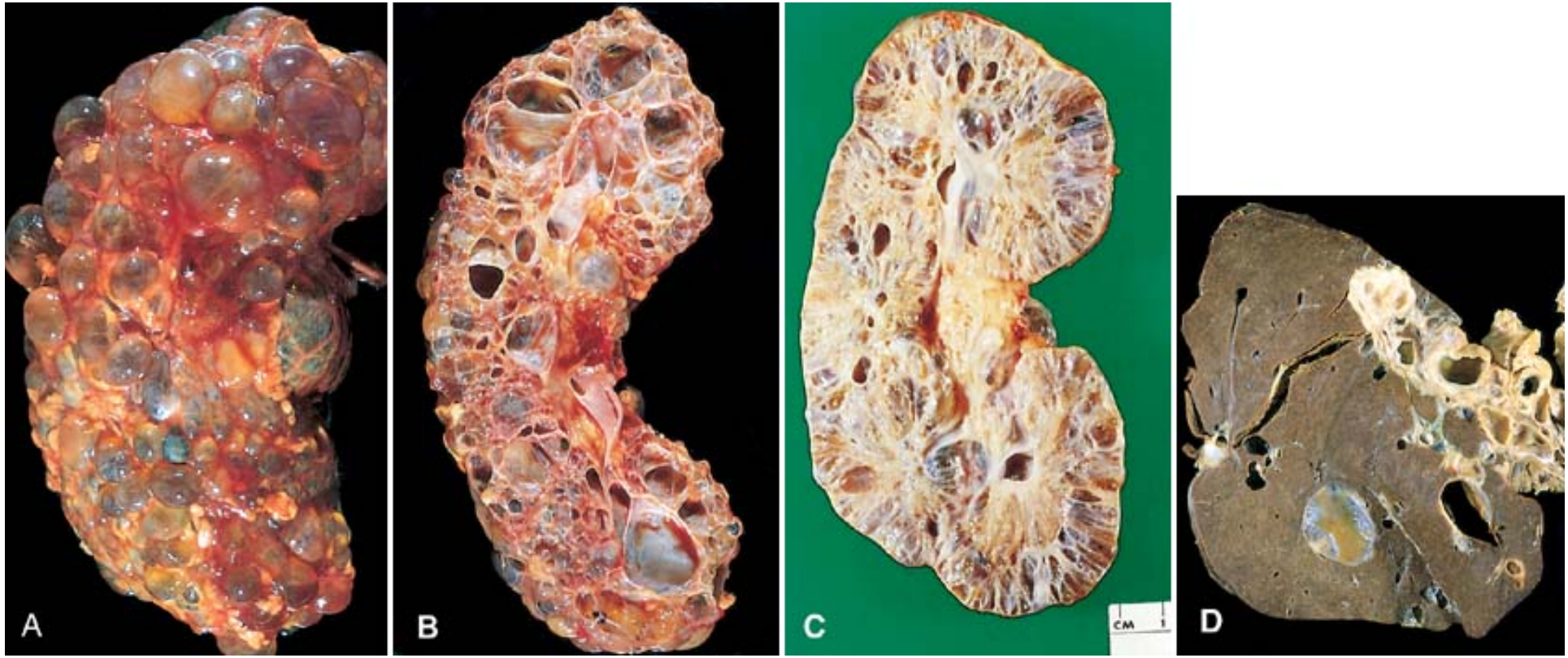

Figure 20-9 Uremic medullary cystic disease. Cut section of kidney showing cysts at the corticomedullary junction and in the medulla.

TABLE 20-3 -- Glomerular Diseases

Primary Glomerulopathies
Acute diffuse proliferative glomerulonephritis
••Poststreptococcal
••Non-poststreptococcal
Rapidly progressive (crescentic) glomerulonephritis
Membranous glomerulopathy
Minimal change disease
Focal segmental glomerulosclerosis
Membranoproliferative glomerulonephritis
IgA nephropathy
Chronic glomerulonephritis
Systemic Diseases with Glomerular Involvement
Systemic lupus erythematosus
Diabetes mellitus
Amyloidosis
Goodpasture syndrome
Microscopic polyarteritis/polyangiitis
Wegener granulomatosis
Henoch-Schönlein purpura
Bacterial endocarditis
Hereditary Disorders
Alport syndrome
Thin basement membrane disease
Fabry disease

TABLE 20-4 -- The Glomerular Syndromes

Acute nephritic syndrome	• Hematuria, azotemia, variable proteinuria, oliguria, edema, and hypertension
Rapidly progressive glomerulonephritis	• Acute nephritis, proteinuria, and acute renal failure
Nephrotic syndrome	• >3.5 gm proteinuria, hypoalbuminemia, hyperlipidemia, lipiduria

Chronic renal failure	• Azotemia → uremia progressing for years
Asymptomatic hematuria or proteinuria	• Glomerular hematuria; subnephrotic proteinuria

Basement Membrane Thickening.

By light microscopy, this change appears as thickening of the capillary walls, best seen in sections stained with periodic acid-Schiff (PAS). By electron microscopy, such thickening can be resolved as one of two alterations: (1) deposition of amorphous electrondense material, most often immune complexes, on the endothelial or epithelial side of the basement membrane or within the GBM itself. Fibrin, amyloid, cryoglobulins, and abnormal fibrillary proteins may also deposit in the GBM; or (2) thickening of the basement membrane proper, as occurs in diabetic glomerulosclerosis.

Hyalinization and Sclerosis.

Hyalinization, or hyalinosis, as applied to the glomerulus, denotes the accumulation of material that is homogeneous and eosinophilic by light microscopy. By electron microscopy, the hyalin is extracellular and consists of amorphous substance, made up of plasma proteins that have exuded from circulating plasma into glomerular structures. This change contributes to obliteration of capillary lumina of the glomerular tuft (a feature of sclerosis). Hyalinosis is usually a consequence of endothelial or capillary wall injury and typically is the end result of various forms of glomerular damage. Additional alterations include *intraglomerular thrombosis* or *accumulation of lipid* or other metabolic materials.

Because many of the primary glomerulonephritides are of unknown cause, they are often classified by their histology, as

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can be seen in Table 20-3 . The histologic changes can be further subdivided into *diffuse*, involving all glomeruli; *global*, involving the entire glomerulus; *focal*, involving only a proportion of the glomeruli; *segmental*, affecting a part of each glomerulus; and *mesangial*, affecting predominantly the mesangial region. These terms are sometimes appended to the histologic classifications.

PATHOGENESIS OF GLOMERULAR INJURY

Although we know little of etiologic agents and triggering events, it is clear that immune mechanisms underlie most forms of primary glomerulonephritis and many of the secondary glomerular disorders[25] [26] (Table 20-5). Glomerulonephritis can be readily induced experimentally by antigen-antibody reactions. Furthermore, glomerular deposits of immunoglobulins, often with various components of complement, are found in the majority of patients with glomerulonephritis. Cell-mediated immune reactions also clearly play a role, usually in concert with antibody-mediated events. We therefore begin this discussion with a review of antibody-instigated injury.

Two forms of antibody-associated injury have been established: (1) injury by *antibodies reacting in situ within the glomerulus, either with insoluble fixed (intrinsic) glomerular antigens or with molecules planted within the glomerulus*, and (2) injury resulting from *deposition of circulating antigen-antibody complexes* in the glomerulus. In addition, there is experimental evidence that *cytotoxic antibodies* directed against glomerular cell components may cause glomerular injury. These pathways are not mutually exclusive, and in humans, all may contribute to injury.

In Situ Immune Complex Deposition

In this form of injury, antibodies react directly with intrinsic tissue antigen, or antigens "planted" in the glomerulus from the circulation. There are two well-established experimental

TABLE 20-5 -- Immune Mechanisms of Glomerular Injury

Antibody-Mediated Injury
In Situ Immune Complex Deposition
Fixed intrinsic tissue antigens
••NC1 domain of collagen type IV antigen (anti-GBM nephritis)
••Heymann antigen (membranous glomerulopathy)
••Mesangial antigens
••Others
Planted antigens
••Exogenous (infectious agents, drugs)
••Endogenous (DNA, nuclear proteins, immunoglobulins, immune complexes, IgA)
Circulating Immune Complex Deposition
Endogenous antigens (e.g., DNA, tumor antigens)
Exogenous antigens (e.g., infectious products)
Cytotoxic Antibodies
Cell-Mediated Immune Injury
Activation of Alternative Complement Pathway

models for anti-tissue antibody-mediated glomerular injury, for which there are counterparts in human disease: antiglomerular basement membrane (anti-GBM) antibody-induced nephritis and Heymann nephritis.

Anti-GBM Antibody-Induced Nephritis

In this type of injury, *antibodies are directed against intrinsic fixed antigens that are normal components of the GBM proper*. It has its experimental counterpart in so-called Masugi or nephrotoxic nephritis, produced in rats by injections of anti-rat kidney antibodies prepared in rabbits by immunization with rat kidney tissue. The injected antibodies bind along the entire length of the GBM, *resulting in a diffuse linear pattern of staining for the antibodies by immunofluorescent techniques* (Figs. 20-10*B* and *E*). This is contrasted with the granular lumpy pattern of immunofluorescent staining seen in other in situ models, such as the Heymann model of membranous glomerulopathy, or after deposition of circulating immune complexes.

In the Masugi model, the injected anti-GBM antibody is rabbit immunoglobulin, which is foreign to the host and thus acts as an antigen eliciting anti-Ig antibody in the rat. The rat antibodies then react with the rabbit immunoglobulin deposited in the basement membrane, leading to further glomerular injury. Thus, experimental anti-GBM antibody-mediated glomerulonephritis consists of an initial *heterologous phase* caused by the injected anti-GBM antibody, and a subsequent, more injurious, *autologous phase* caused by host antibodies against the injected Ig. Often the anti-GBM antibodies cross-react with other basement membranes, especially those in the lung alveoli, resulting in simultaneous lung and kidney lesions (*Goodpasture syndrome*). *The GBM antigen that is responsible for classic anti-GBM antibody-induced nephritis and Goodpasture syndrome is a component of the non-collagenous domain*

(NC1) of the α_3 -chain of collagen type IV, which, as was discussed earlier (see Fig. 20-3), is critical for maintenance of GBM superstructure.[3] [4] [5] [27] Anti-GBM antibody-induced nephritis accounts for fewer than 5% of cases of human glomerulonephritis. It is solidly established as the cause of injury in Goodpasture syndrome, discussed later. Most instances of anti-GBM antibody-induced nephritis are characterized by severe crescentic glomerular damage and the clinical syndrome of rapidly progressive glomerulonephritis.

Heymann Nephritis

The Heymann model of rat glomerulonephritis is induced by immunizing animals with an antigen contained within preparations of proximal tubular brush border (Fig. 20-10*C*). The rats develop antibodies to this antigen, and a membranous glomerulopathy, resembling human membranous glomerulopathy, develops (discussed later; see also Fig. 20-19). On electron microscopy, the glomerulopathy is characterized by the presence of numerous electron-dense deposits (made up largely of immune reactants) along the *subepithelial aspect* of the basement membrane. The pattern of immune deposition by immunofluorescence microscopy is *granular* rather than linear (Fig. 20-10*C*). It is now clear that this type of disease results largely from the reaction of antibody with an antigen complex located on the basal surface of visceral epithelial cells and cross-reacting with the brush

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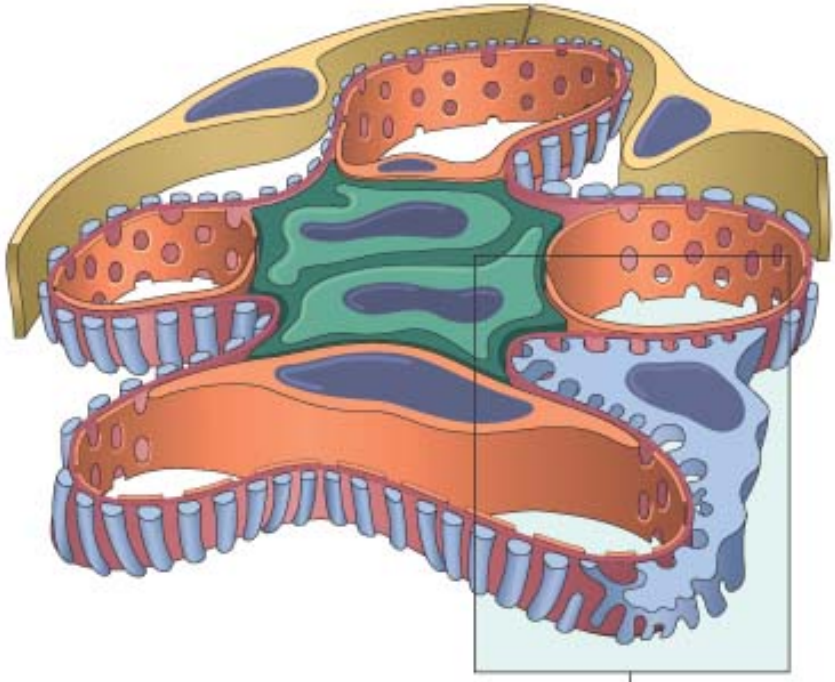
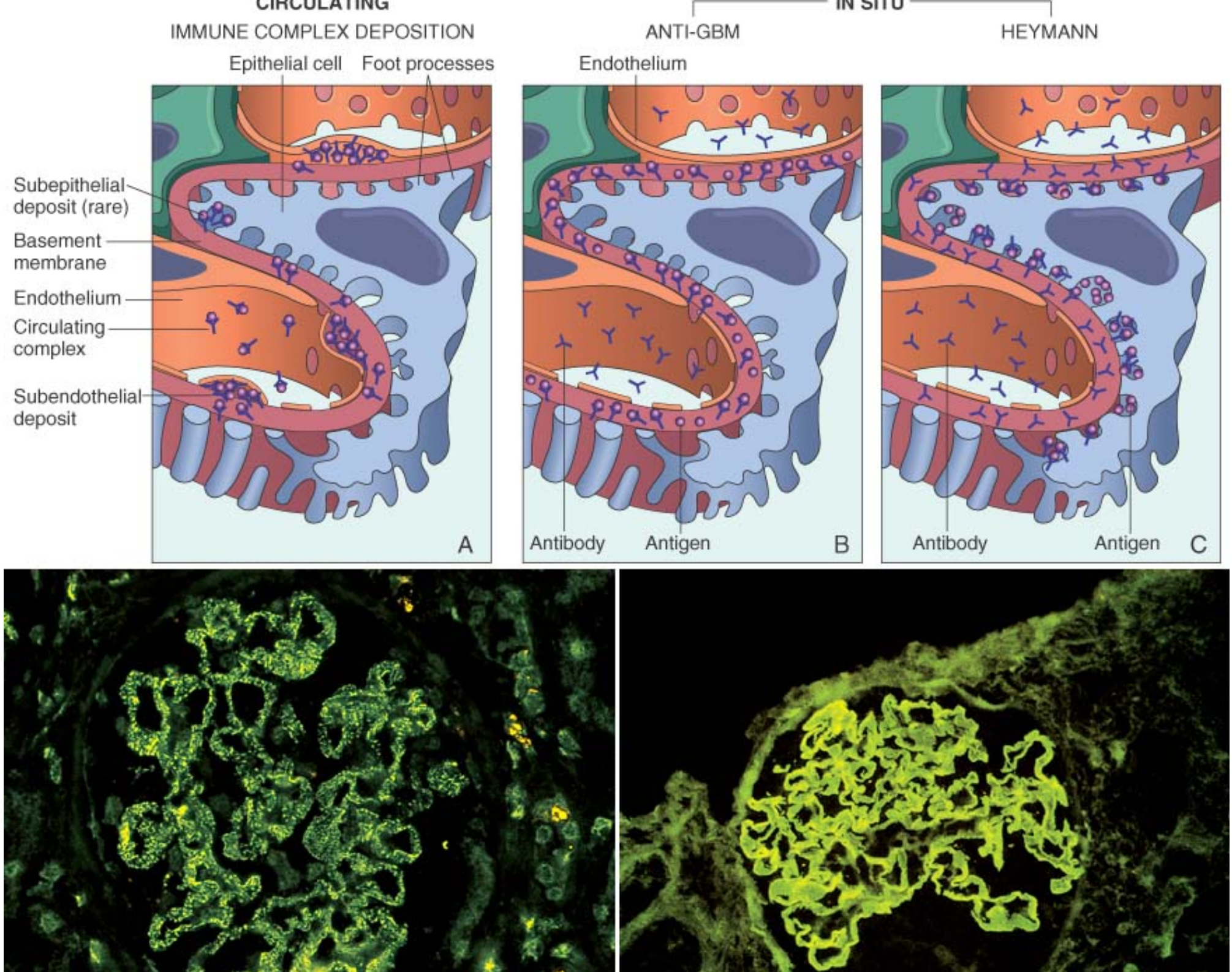
Figure 20-10 Antibody-mediated glomerular injury can result either from the deposition of circulating immune complexes (*A*) or, more commonly, from in situ formation of complexes exemplified by anti-GBM disease (*B*) or Heymann nephritis (*C*). *D* and *E*, Two patterns of deposition of immune complexes as seen by immunofluorescence microscopy: granular, characteristic of circulating and in situ immune complex nephritis (*D*) and linear, characteristic of classic anti-GBM disease (*E*).




CIRCULATING
IMMUNE COMPLEX DEPOSITION
IN SITU
ANTI-GBM
HEYMANN
Epithelial cell
Foot processes
Endothelium
Subepithelial deposit (rare)
Basement membrane
Endothelium
Circulating complex
Subendothelial deposit
Antibody
Antigen
A
B
Antibody
Antigen
C

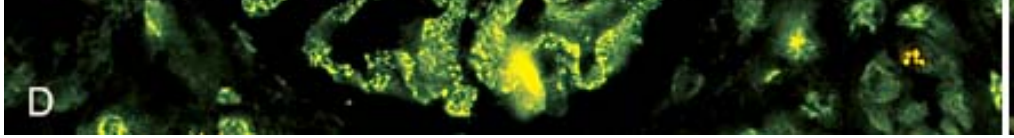


Figure 20-12 Epithelial cell injury. The postulated sequence is a consequence of antibodies against epithelial cell antigens, toxins, cytokines, or other factors causing injury with foot process effacement and sometimes detachment of epithelial cells and protein leakage through defective GBM and filtration slits.

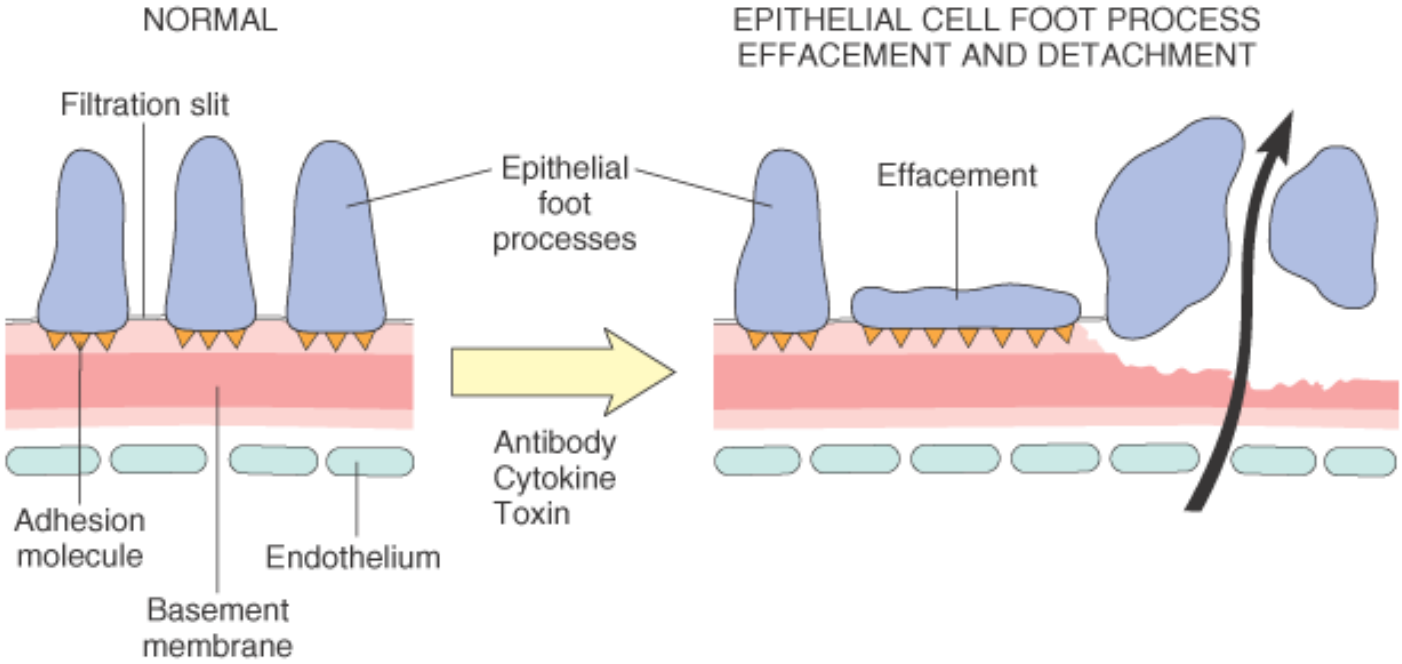


Figure 20-13 Mediators of immune glomerular injury including cells and soluble mediators (see text). *(Modified from Couser WG: Mediation of immune glomerular injury. J Am Soc Nephrol 1:13, 1990.)*

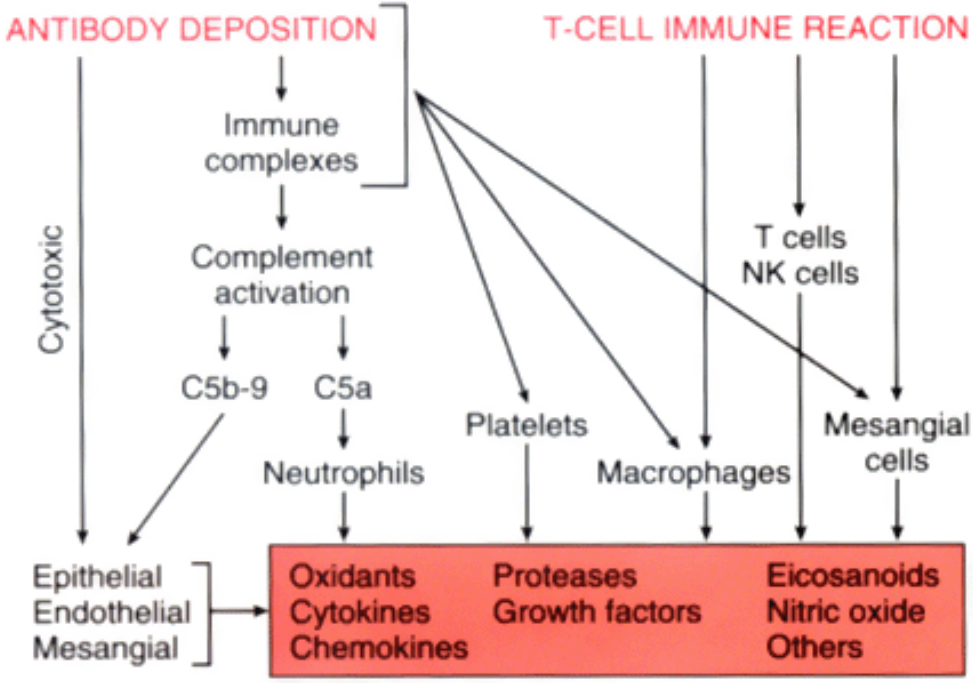


Figure 20-14 Renal ablation focal segmental glomerulosclerosis. The adaptive changes in glomeruli (hypertrophy and glomerular capillary hypertension), as well as systemic hypertension, cause epithelial and endothelial injury and resultant proteinuria. The mesangial response, involving mesangial cell proliferation and extracellular matrix (ECM) production together with intraglomerular coagulation, causes the glomerulosclerosis. This results in further loss of functioning nephrons and a vicious circle of progressive glomerulosclerosis.

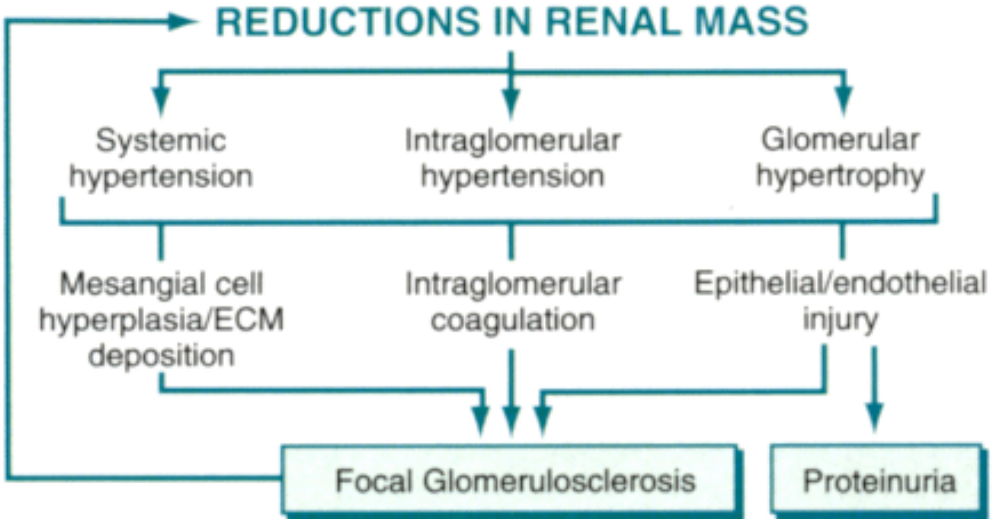


Figure 20-15 Mechanisms of chronic tubulointerstitial injury in glomerulonephritis (see text). Various components of the protein-rich filtrate and cytokines derived from leukocytes cause tubular cell activation and secretion of cytokines, growth factors, and other mediators. These, together with products of macrophages, incite interstitial inflammation and fibrosis. ET-1, endothelin-1, PAI-1, plasminogen activator inhibitor-1; TIMP-1, tissue inhibitor of metalloproteinases. *(Adapted and modified from Remuzzi G, Ruggenenti P, Benigni A: Understanding the nature of renal disease progression. Kidney Int 51:2, 1997; Schena FP, et al: Progression of renal damage in human glomerulonephritis. Kidney Int 52:1439, 1997; Fogo AB:*

Progression and potential regression of glomerulosclerosis. Kidney Int 59:804, 2001.)

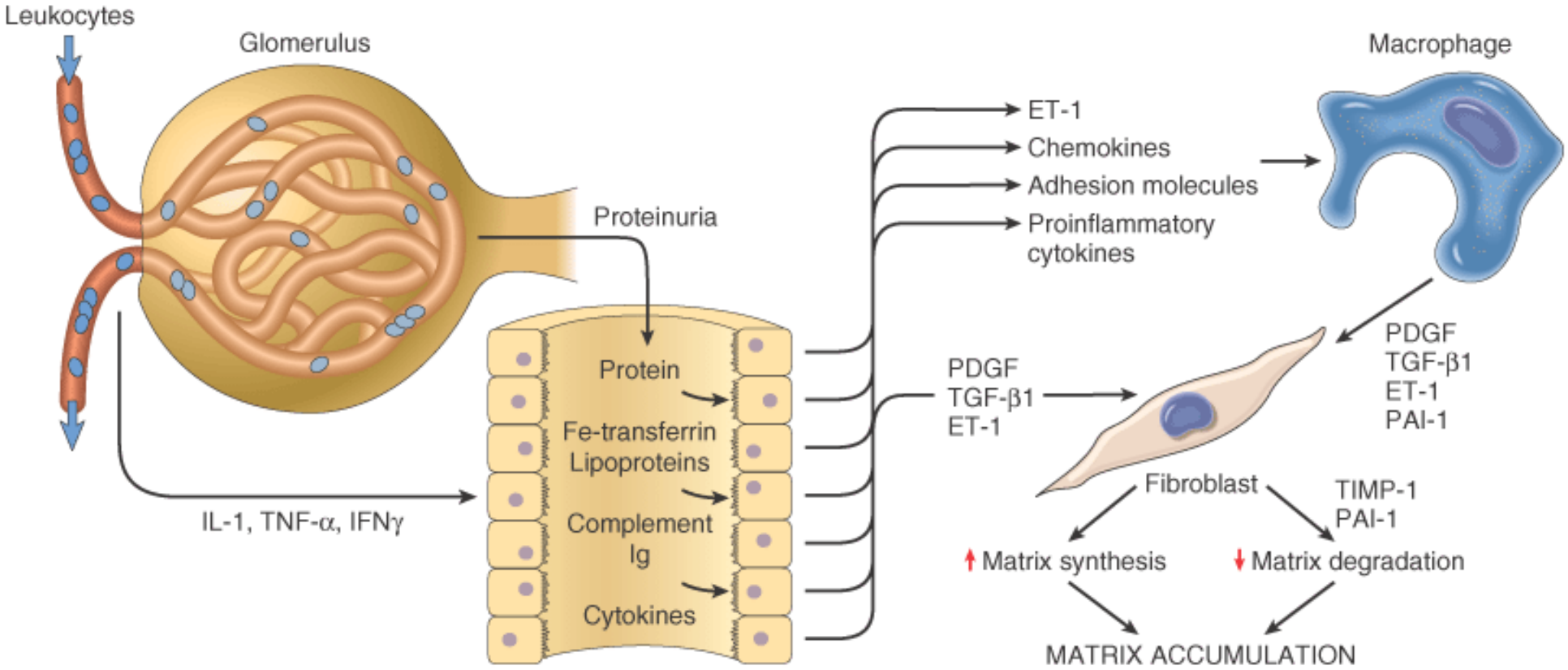


TABLE 20-6 -- Summary of Major Primary Glomerulonephritides

			Glomerular Pathology		
Disease	**Most Frequent Clinical Presentation**	**Pathogenesis**	***Light Microscopy***	***Fluorescence Microscopy***	***Electron Microscopy***
Poststreptococcal glomerulonephritis	Acute nephritis	Antibody mediated; circulating or planted antigen	Diffuse proliferation; leukocytic infiltration	Granular IgG and C3 in GBM and mesangium	Subepithelial humps
Goodpasture syndrome	Rapidly progressive glomerulonephritis	Anti-GBM COL4-A3 antigen	Proliferation; crescents	Linear IgG and C3; fibrin in crescents	No deposits; GBM disruptions; fibrin
Idiopathic RPGN	Rapidly progressive glomerulonephritis	Anti-GBM antibody	Proliferation; focal necrosis; crescents	Linear IgG and C3	No deposits
		Immune complex		Granular IgG or IgA or IgM	Deposits may be present
		ANCA-associated		Negative or equivocal	No deposits
Membranous glomerulopathy	Nephrotic syndrome	In situ antibody-mediated; antigen unknown	Diffuse capillary wall thickening	Granular IgG and C3; diffuse	Subepithelial deposits

Minimal change disease	Nephrotic syndrome	Unknown, loss of glomerular polyanion; podocyte injury	Normal; lipid in tubules	Negative	Loss of foot processes; no deposits
Focal segmental glomerulosclerosis	Nephrotic syndrome; non-nephrotic proteinuria	Unknown, Ablation nephropathy Plasma factor (?); podocyte injury	Focal and segmental sclerosis and hyalinosis	Focal; IgM and C3	Loss of foot processes; epithelial denudation
Membranoproliferative glomerulonephritis (MPGN) Type I	Nephrotic syndrome	(I) Immune complex		(I) IgG + C3; C1q + C4	(I) Subendothelial deposits
			Mesangial proliferation; basement membrane thickening; splitting		
Dense deposit disease (MPGN Type II)	Hematuria	(II) Autoantibody: alternative complement pathway		(II) C3 ± IgG; no C1q or C4	(II) Dense deposits
	Chronic renal failure				
IgA nephropathy	Recurrent hematuria or proteinuria	Unknown; see text	Focal proliferative glomerulonephritis; mesangial widening	IgA +/- IgG, IgM, and C3 in mesangium	Mesangial and paramesangial dense deposits
Chronic glomerulonephritis	Chronic renal failure	Variable	Hyalinized glomeruli	Granular or negative	
ANCA, antineutrophil cytoplasmic antibody; GBM, glomerular basement membrane; RPGN, rapidly progressive glomerulonephritis.					

all lobules of all glomeruli. There is also swelling of endothelial cells, and the combination of proliferation, swelling, and leukocyte infiltration obliterates the capillary lumens. There may be interstitial edema and inflammation, and the tubules often contain red cell casts.

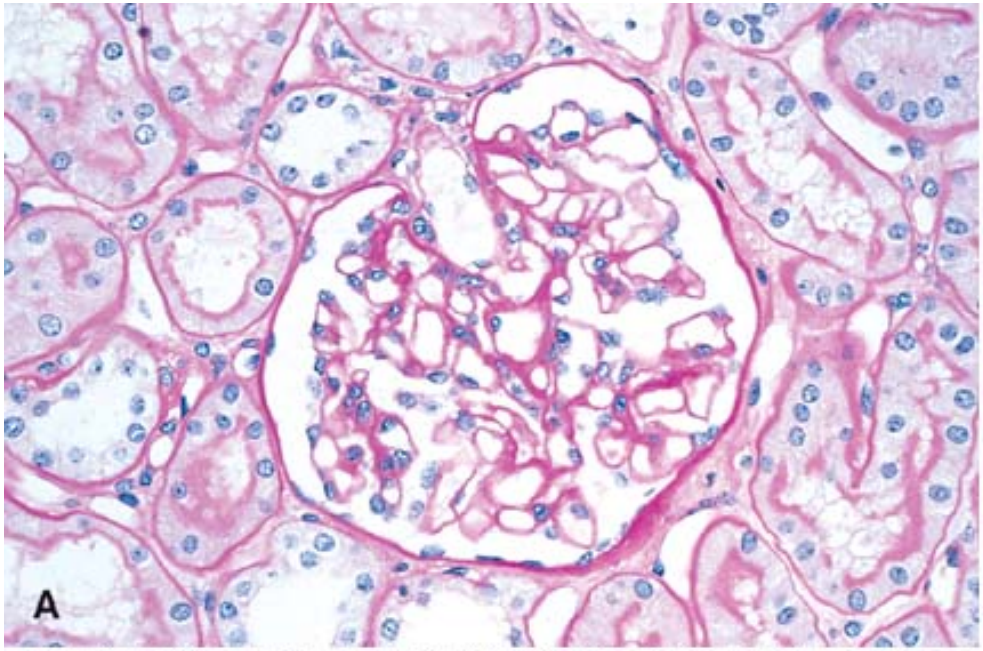
By **immunofluorescence microscopy,** there are granular deposits of IgG, IgM, and C3 in the mesangium and along the basement membrane. Although almost universally present, they are often focal and sparse. The characteristic **electron microscopic findings** are discrete, amorphous, electrondense deposits on the epithelial side of the membrane, often having the appearance of "humps" (Fig. 20-16*C)* , presumably representing the antigen-antibody complexes at the epithelial cell surface. Subendothelial and intramembranous deposits are also commonly seen, and mesangial deposits may be present. There is often swelling of endothelial and mesangial cells.

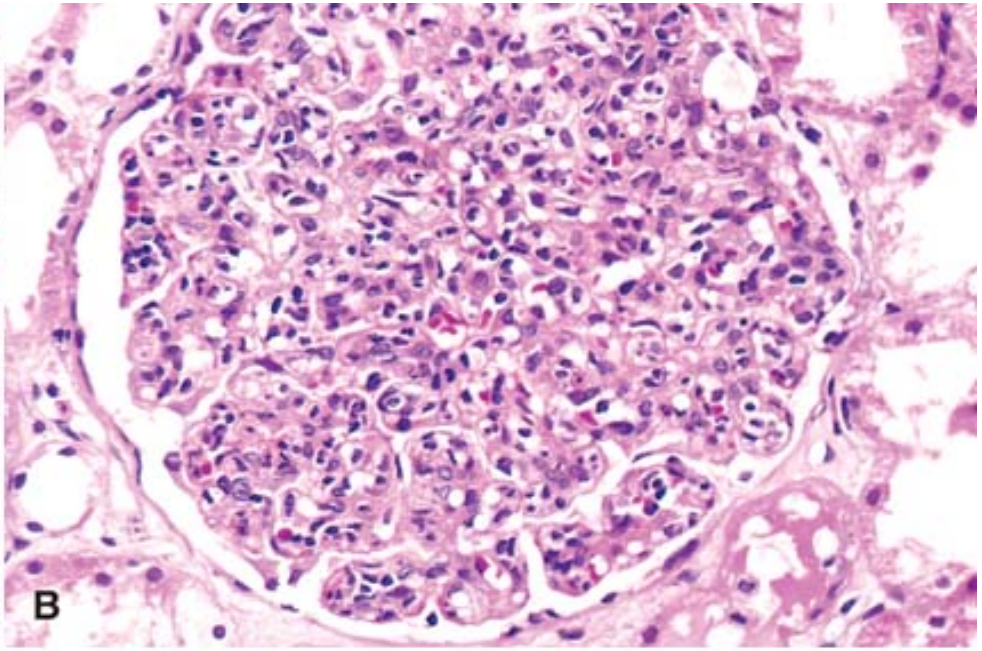
Clinical Course.

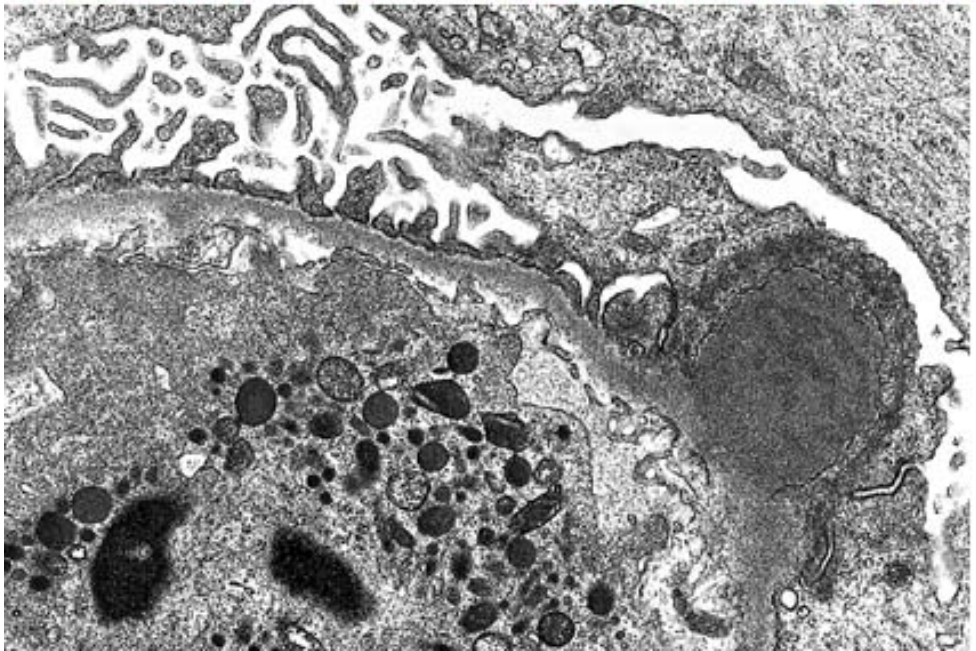
In the classic case, a young child abruptly develops malaise, fever, nausea, oliguria, and hematuria (smoky or cocoa-colored urine) 1 to 2 weeks after recovery from a sore throat. The patients exhibit red cell casts in the urine, mild proteinuria (usually less than 1 mg/day), periorbital edema, and mild to moderate hypertension. In adults, the onset is more likely to be atypical, with the sudden appearance of hypertension or edema, frequently with elevation of BUN. During epidemics caused by nephritogenic streptococcal infections, glomerulonephritis may be asymptomatic, discovered only on screening for microscopic hematuria. Important laboratory findings include elevations of antistreptococcal antibody (ASO) titers and a decline in the serum concentration of C3 and other components of the complement cascade and the presence of cryoglobulins in the serum.

More than 95% of affected children eventually recover totally with conservative therapy aimed at maintaining sodium and water balance. A small minority of children (perhaps less than 1%) do not improve, become severely oliguric, and develop a rapidly progressive form of glomerulonephritis

Figure 20-16 Acute proliferative glomerulonephritis. *A*, Normal glomerulus. *B*, Glomerular hypercellularity is due to intracapillary leukocytes and proliferation of intrinsic glomerular cells. *C*, Typical electron-dense subepithelial "hump" and a neutrophil in the lumen. *(Courtesy of Dr. H. Rennke, Brigham and Women's Hospital, Boston, MA.)*







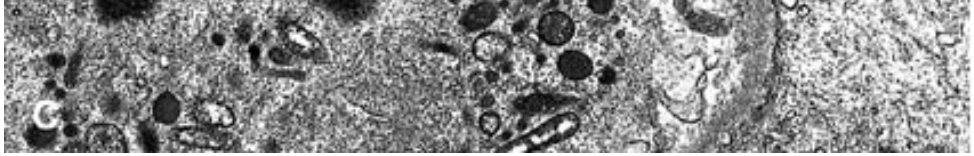


TABLE 20-7 -- Rapidly Progressive Glomerulonephritis (RPGN)

Type I RPGN (Anti-GBM Antibody)
Idiopathic
Goodpasture syndrome
Type II RPGN (Immune Complex)
Idiopathic
Postinfectious
Systemic lupus erythematosus
Henoch-Schönlein purpura (IgA)
Others
Type III RPGN (Pauci-Immune)
ANCA associated
Idiopathic
Wegener granulomatosis
Microscopic polyarteritis nodosa/microscopic polyangiitis

previously described.[48] In some of these patients, the anti-GBM antibodies cross-react with pulmonary alveolar basement membranes to produce the clinical picture of pulmonary hemorrhage associated with renal failure (*Goodpasture syndrome*). Plasmapheresis to remove the pathogenic circulating antibodies is usually part of the treatment, which also includes therapy to suppress the underlying immune response.

The Goodpasture antigen, as was noted earlier, is a peptide within the noncollagenous portion of the α_3 -chain of collagen type IV. [27] What triggers the formation of these antibodies is unclear in most patients. Exposure to viruses or hydrocarbon solvents (found in paints and dyes) has been implicated in some patients, as have various drugs and cancers. There is a high prevalence of certain HLA subtypes and haplotypes (e.g., HLA-DRB1) in affected patients, a finding consistent with the genetic predisposition to autoimmunity.[49]

The second type of *RPGN* is the result of *immune complex-mediated disease*. It can be a complication of any of the immune complex nephritides, including postinfectious glomerulonephritis, SLE, IgA nephropathy, and Henoch-Schönlein purpura. In all of these cases, immunofluorescence studies reveal the granular pattern of staining characteristic of immune complex deposition. These patients cannot usually be helped by plasmapheresis, and they require treatment for the underlying disease.

The third type of *RPGN*, also called *pauci-immune type*, is defined by the lack of anti-GBM antibodies or immune complexes by immunofluorescence and electron microscopy. Most patients with this type of RPGN have *antineutrophil cytoplasmic antibodies* (ANCA), of cytoplasmic (C) or perinuclear (P) patterns, in the serum, which, as we have seen (Chapter 11), play a role in some vasculitides. Hence, in some cases, this type of RPGN is a component of a systemic vasculitis such as Wegener granulomatosis or microscopic polyarteritis. In many

cases, however, pauci-immune crescentic glomerulonephritis is isolated and hence *idiopathic*. More than 90% of such idiopathic cases have c-ANCA or p-ANCA in the sera.[50] The presence of circulating ANCAs in both idiopathic RPGN and cases of RPGN that occur as a component of systemic vasculitis, and the similar pathologic features in either setting, have led to the idea that these disorders are pathogenetically related. According to this concept, all cases of RPGN of the pauci-immune type are manifestations of small vessel vasculitis or polyangiitis, which is limited to glomerular and perhaps peritubular capillaries in cases of idiopathic crescentic glomerulonephritis. [51] The clinical distinction between systemic vasculitis with pauci-immune renal involvement and idiopathic crescentic glomerulonephritis accordingly has become deemphasized, as these entities are viewed as part of a spectrum of vasculitic disease. ANCAs have proved to be invaluable as a highly sensitive diagnostic marker for pauci-immune RPGN, but proof of their role as a direct cause of RPGN has been elusive. Recent strong evidence of their pathogenic potential has been obtained by studies in which antibodies against myeloperoxidase (the target antigen of most p-ANCAs) are transferred into mice.[52]

To summarize, all three types of RPGN may be associated with a well-defined renal or extrarenal disease, but in many cases (approximately 50%), the disorder is idiopathic. Of the patients with this syndrome, about one fifth have anti-GBM antibody-induced disease without lung involvement; another one fourth have immune complex-mediated disease RPGN; and the remainder are of the pauci-immune type. *The common denominator in all types of RPGN is severe glomerular injury.*

Morphology.

The kidneys are enlarged and pale, often with petechial hemorrhages on the cortical surfaces. Depending on the underlying cause, the glomeruli may show focal necrosis, diffuse or focal endothelial proliferation, and mesangial proliferation. The histologic picture, however, is dominated by the formation of distinctive **crescents** (Fig. 20-17). Crescents are formed by proliferation of parietal cells and by migration of monocytes and macrophages into the urinary space. Neutrophils and lymphocytes may be present. The crescents eventually obliterate Bowman space and compress the glomerular tuft. **Fibrin strands are prominent between the cellular layers in the crescents;** indeed, as discussed earlier, the escape of fibrin into Bowman space is an important

Figure 20-17 Crescentic glomerulonephritis (PAS stain). Note the collapsed glomerular tufts and the crescent-shaped mass of proliferating cells and leukocytes internal to Bowman capsule. *(Courtesy of Dr. M.A. Venkatachalam, University of Texas Health Sciences Center, San Antonio, TX.)*

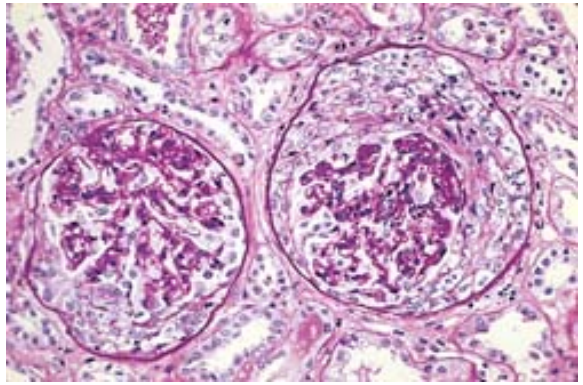


Figure 20-18 Rapidly progressive glomerulonephritis. Electron micrograph showing characteristic wrinkling of GBM with focal disruptions in its continuity (*arrows*).

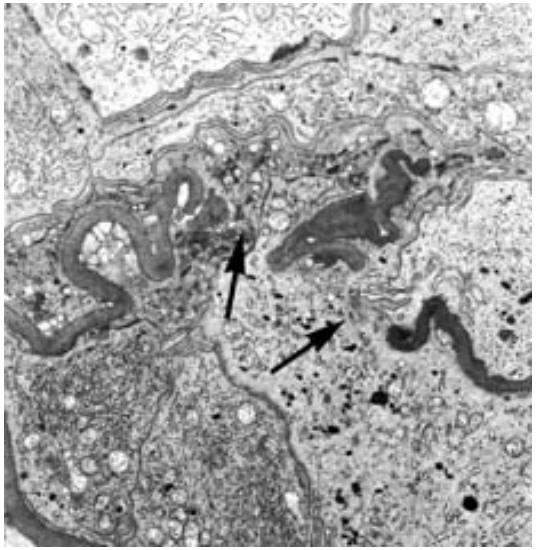


TABLE 20-8 -- Causes of Nephrotic Syndrome

	Prevalence (%) *	
	Children	***Adults***
Primary Glomerular Disease		
Membranous glomerulopathy	5	30
Minimal change disease	65	10
Focal segmental glomerulosclerosis	10	35
Membranoproliferative glomerulonephritides	10	10
Other proliferative glomerulonephritis (focal, "pure mesangial," IgA nephropathy)	10	15
Systemic Diseases		
Diabetes mellitus		
Amyloidosis		
Systemic lupus erythematosus		
Drugs (nonsteroidal anti-inflammatory, penicillamine, "street heroin")		
Infections (malaria, syphilis, hepatitis B and C, acquired immunodeficiency syndrome)		
Malignant disease (carcinoma, lymphoma)		
Miscellaneous (bee-sting allergy, hereditary nephritis)		

*Approximate prevalence of primary disease = 95% in children, 60% in adults. Approximate prevalence of systemic disease = 5% in children, 40% in adults.

MEMBRANOUS GLOMERULOPATHY (MEMBRANOUS NEPHROPATHY)

Membranous glomerulopathy is the most common cause of the nephrotic syndrome in adults. It is characterized by diffuse thickening of the glomerular capillary wall and the accumulation of electron-dense, immunoglobulin-containing deposits along the subepithelial side of the basement membrane.[54]

Membranous glomerulopathy occurring in association with other systemic diseases and a variety of identifiable etiologic agents is referred to as secondary membranous glomerulopathy. The most notable such associations are as follows:

- *Drugs* (penicillamine, captopril, gold, nonsteroidal anti-inflammatory drugs [NSAIDs]): 1% to 7% of patients with rheumatoid arthritis treated with penicillamine or gold (drugs now used infrequently for this purpose) develop membranous glomerulopathy. NSAIDs, as we shall see, also cause minimal change disease.
- *Underlying malignant tumors*, particularly carcinoma of the lung and colon and melanoma. According to some investigators, these are present in up to 5% to 10% of adults with membranous glomerulopathy.[55]
- *SLE.* About 15% of glomerulonephritis in SLE is of the membranous type.
- *Infections* (chronic hepatitis B, hepatitis C, syphilis, schistosomiasis, malaria)
- *Other autoimmune disorders*, such as thyroiditis

In about 85% of patients, no associated condition can be uncovered, and the disease is considered idiopathic.

Etiology and Pathogenesis.

Membranous glomerulopathy is a form of chronic immune complex-mediated disease. In secondary membranous glomerulopathy, particular antigens can sometimes be identified in the immune complexes. For example, membranous glomerulopathy in SLE is associated with deposition of autoantigen-antibody complexes. Exogenous (hepatitis B, *Treponema* antigens) or endogenous (thyroglobulin) antigens have been identified within deposits in some patients.

The lesions bear a striking resemblance to those of experimental Heymann nephritis, which, as you might recall, is induced by antibodies to a *megalin* antigenic complex. A similar but still unidentified antigen is presumed to be present in most cases of idiopathic membranous glomerulopathy in humans. Susceptibility to Heymann nephritis in rats and membranous glomerulopathy in humans is linked to the MHC locus, which influences the ability to produce antibodies to the nephritogenic antigen. Thus, idiopathic membranous glomerulopathy, like Heymann nephritis, is considered *an autoimmune disease linked to susceptibility genes and caused by antibodies to a renal autoantigen*.

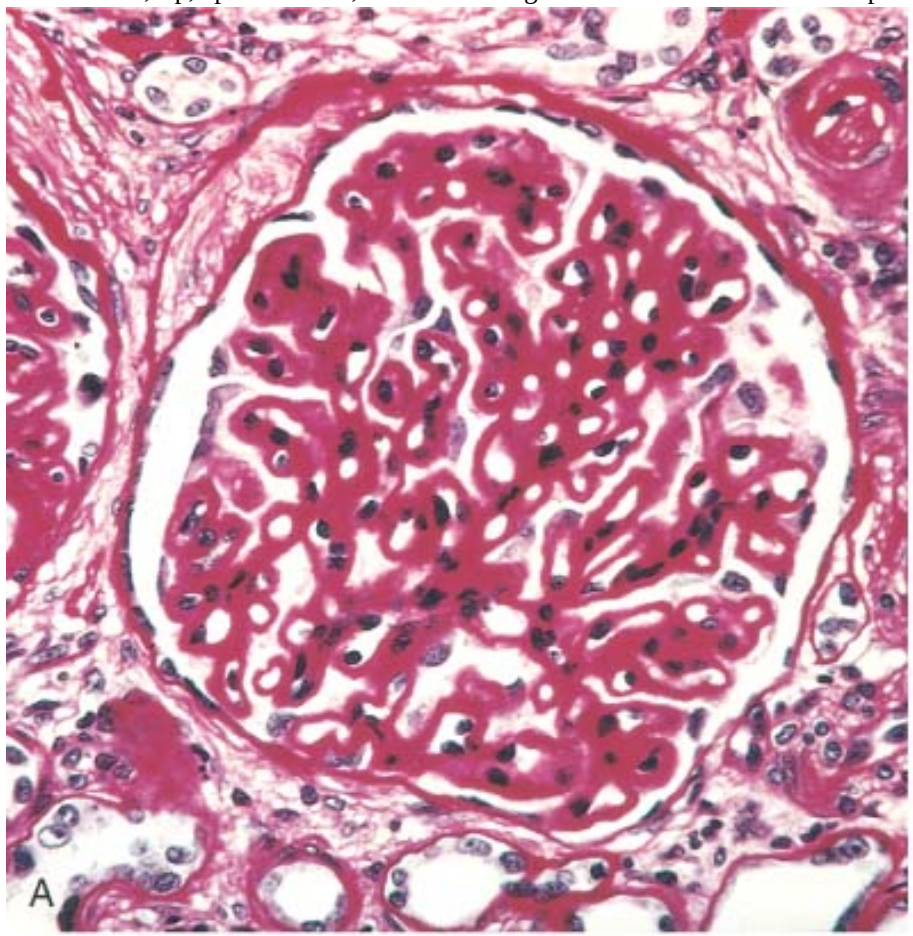
How does the glomerular capillary wall become leaky in membranous glomerulopathy? There is a paucity of neutrophils, monocytes, or platelets in glomeruli and the virtually uniform presence of complement, and experimental work suggests a direct action of C5b-C9, the pathway leading to the formation of the membrane attack complex. C5b-C9 causes activation of glomerular epithelial and mesangial cells, inducing them to liberate proteases and oxidants, which cause capillary wall injury and increased protein leakage.

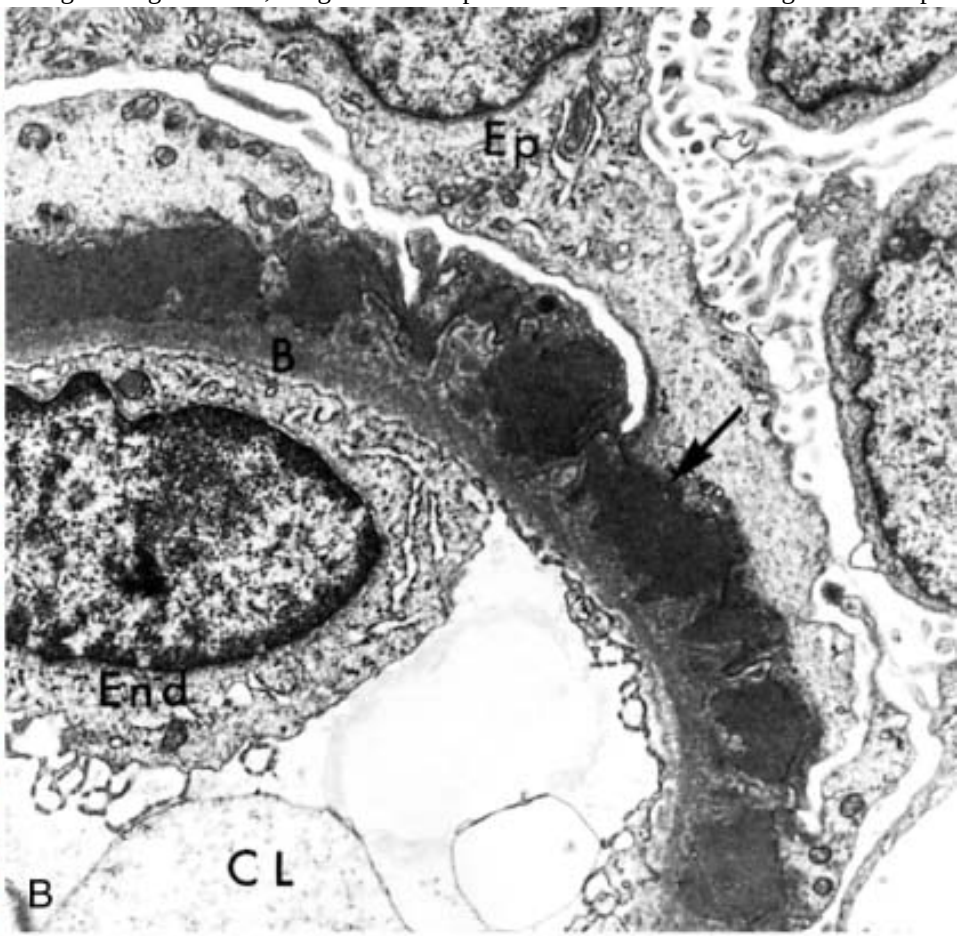
Morphology.

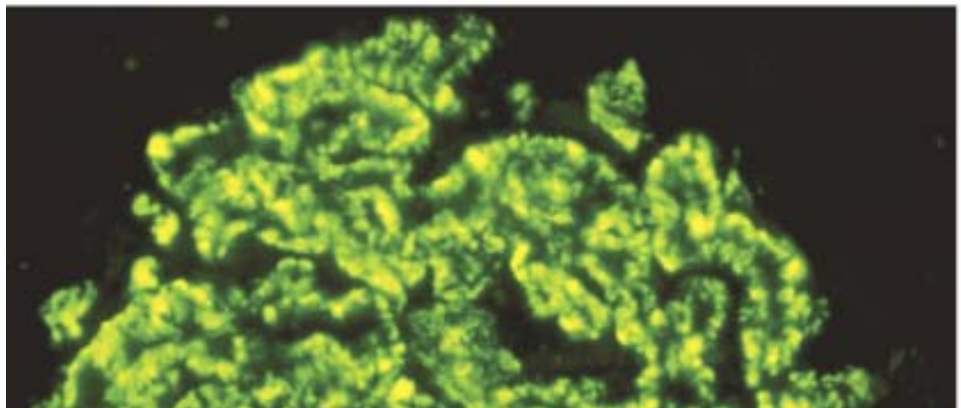
By light microscopy, the glomeruli either appear normal in the early stages of the disease or exhibit **uniform, diffuse thickening of the glomerular capillary wall** (Fig. 20-19*A*). By

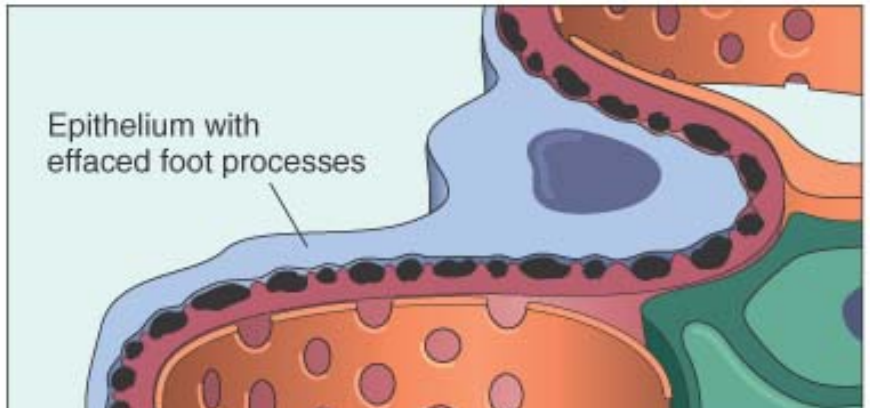
electron microscopy, the thickening is seen to be caused by irregular dense deposits between the basement membrane and the overlying epithelial cells, the latter

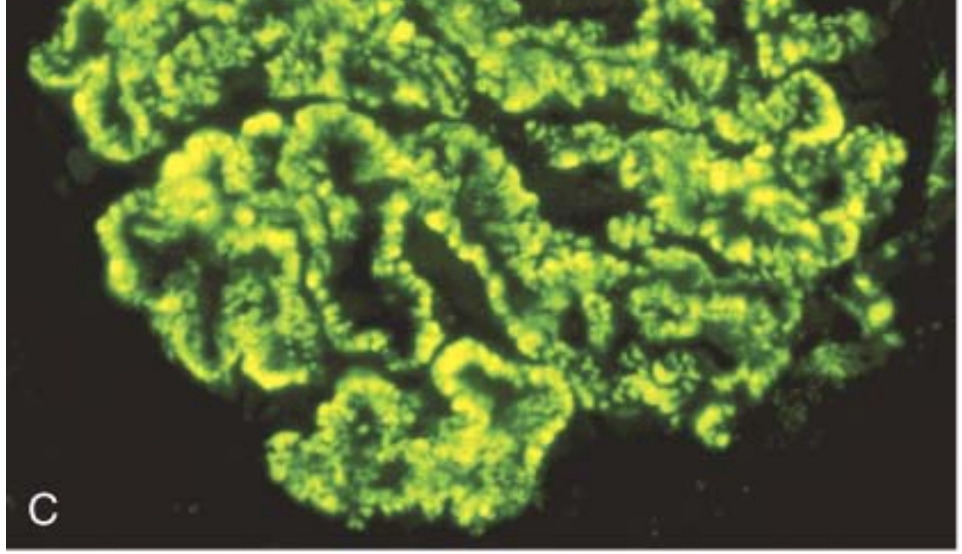
Figure 20-19 Membranous glomerulonephritis. *A*, PAS stain. Note the marked diffuse thickening of the capillary wall without an increase in the number of cells. *B*, Electron micrograph showing electron-dense deposits (*arrow*) along the epithelial side of the basement membrane (*B*). Note the obliteration of foot process overlying deposits. CL, capillary lumen; End, endothelium; Ep, epithelium. *C*, Characteristic granular immunofluorescent deposits of IgG along GBM. *D*, Diagrammatic representation of membranous glomerulonephritis.











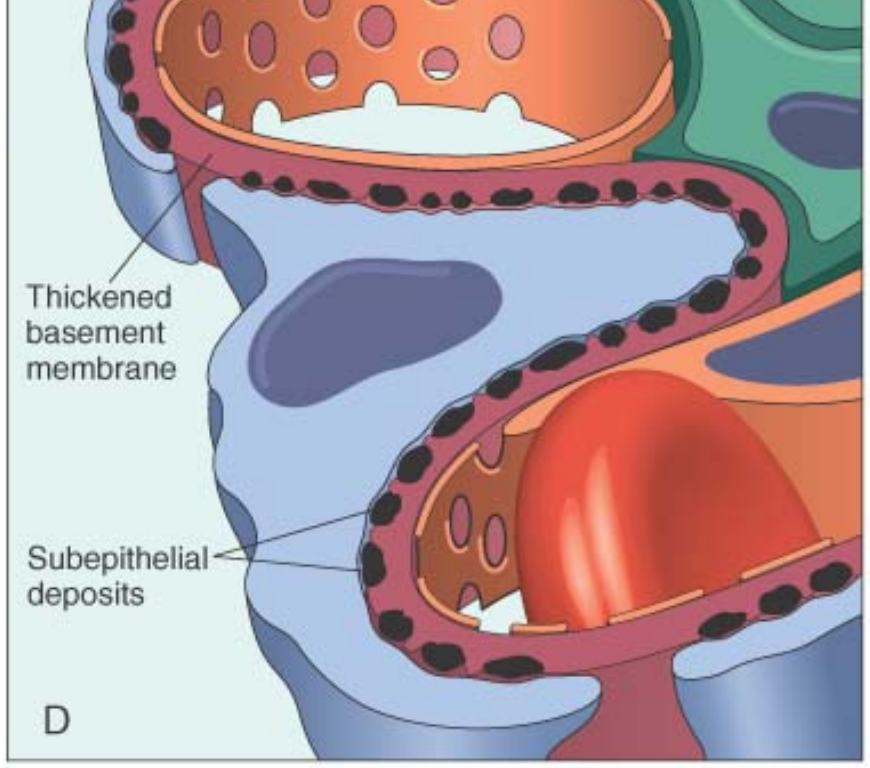


Figure 20-20 Minimal change disease. Glomerulus stained with PAS. Note normal basement membrane and absence of proliferation. Compare with membranous glomerulopathy in Figure 20-19*A* .

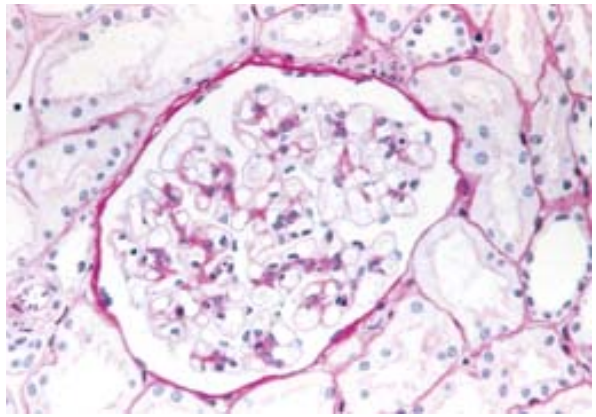


Figure 20-21 *A*, Ultrastructural characteristics of minimal change disease: effacement of foot processes (*double arrows*), absence of deposits, vacuoles (V), and microvilli in visceral epithelial cells (*single arrow*). *B*, Schematic representation of minimal change disease, showing diffuse effacement of foot processes.

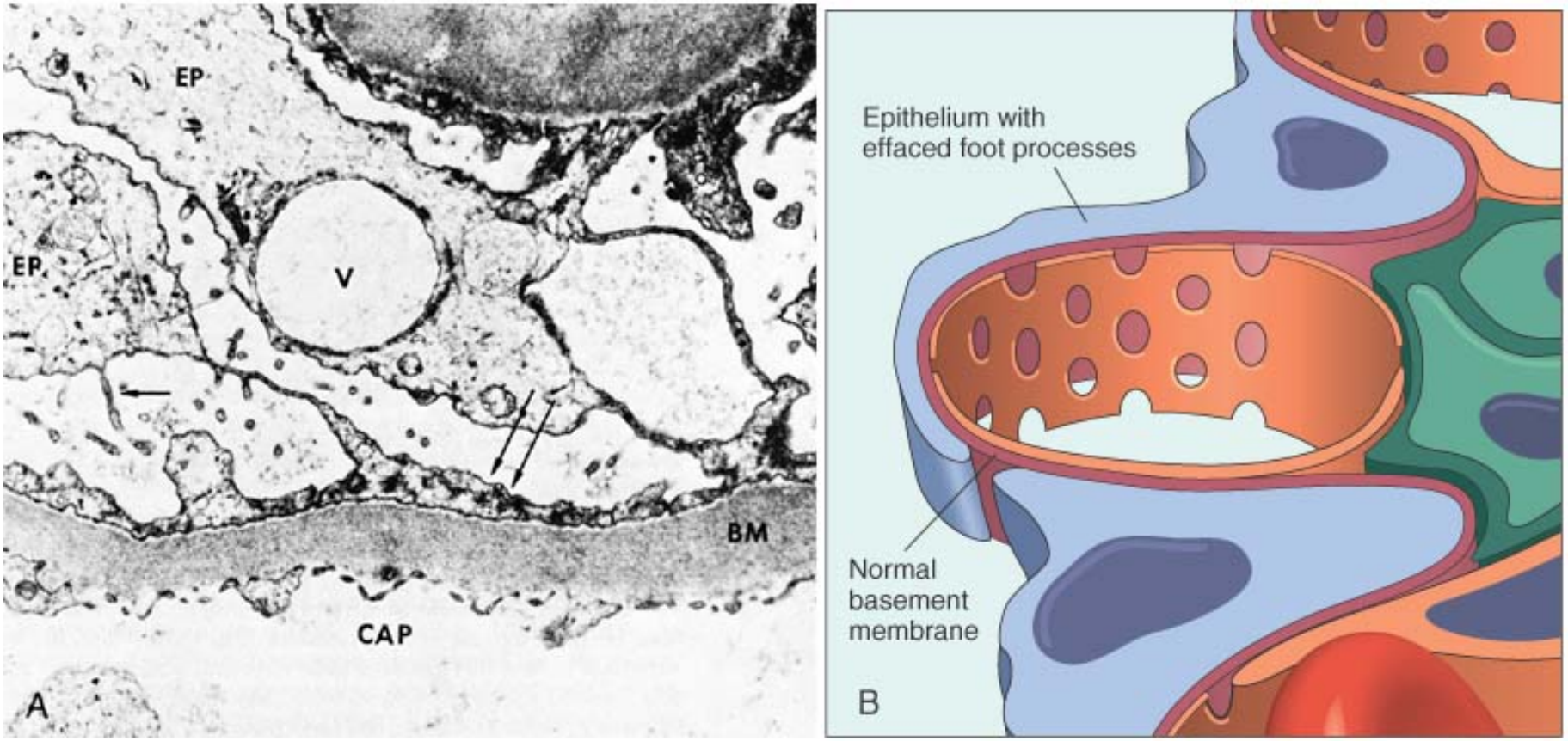


Figure 20-22 Focal segmental glomerulosclerosis, PAS stain. *A*, Low-power view showing segmental sclerosis in one of three glomeruli (at 3 o'clock). *B*, High-power view showing hyaline insudation and lipid (small vacuoles) in sclerotic area.

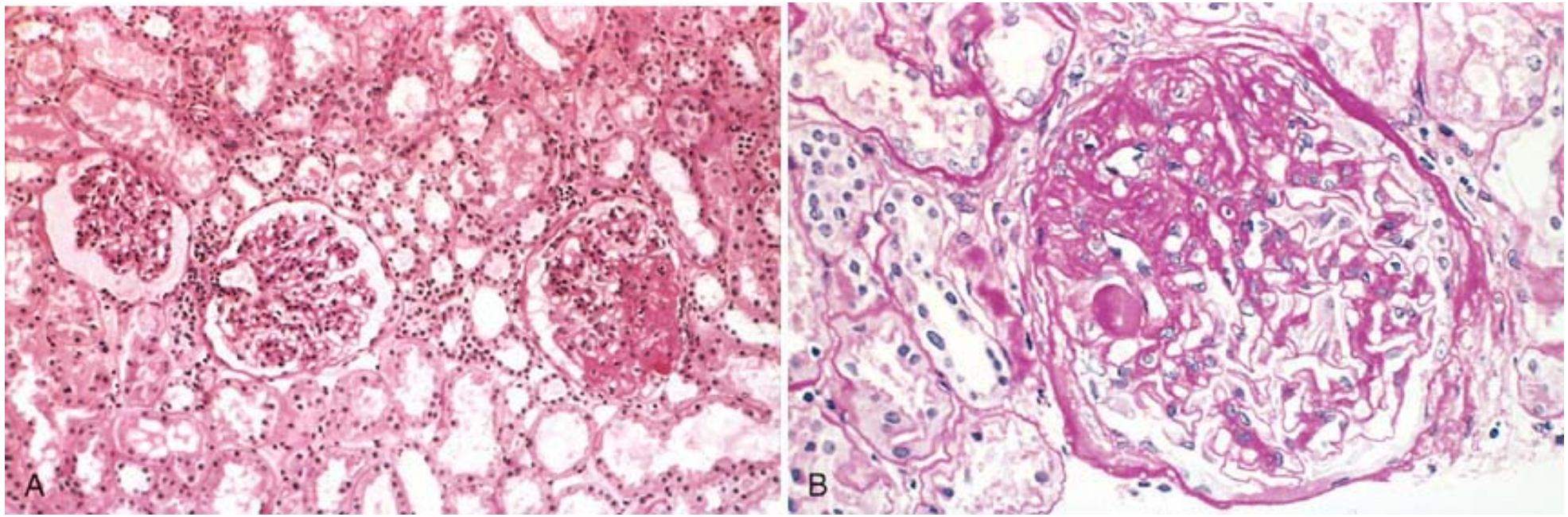


Figure 20-23 Membranoproliferative glomerulonephritis, showing mesangial cell proliferation, increased mesangial matrix (staining black with silver stain), basement membrane thickening and focal splitting, accentuation of lobular architecture, swelling of cells lining peripheral capillaries, and influx of leukocytes.

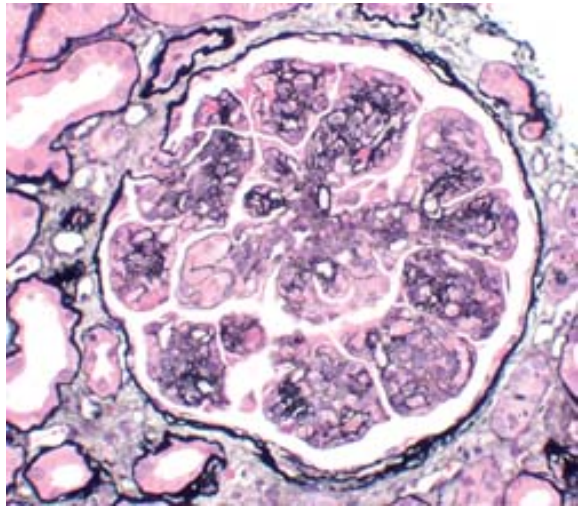
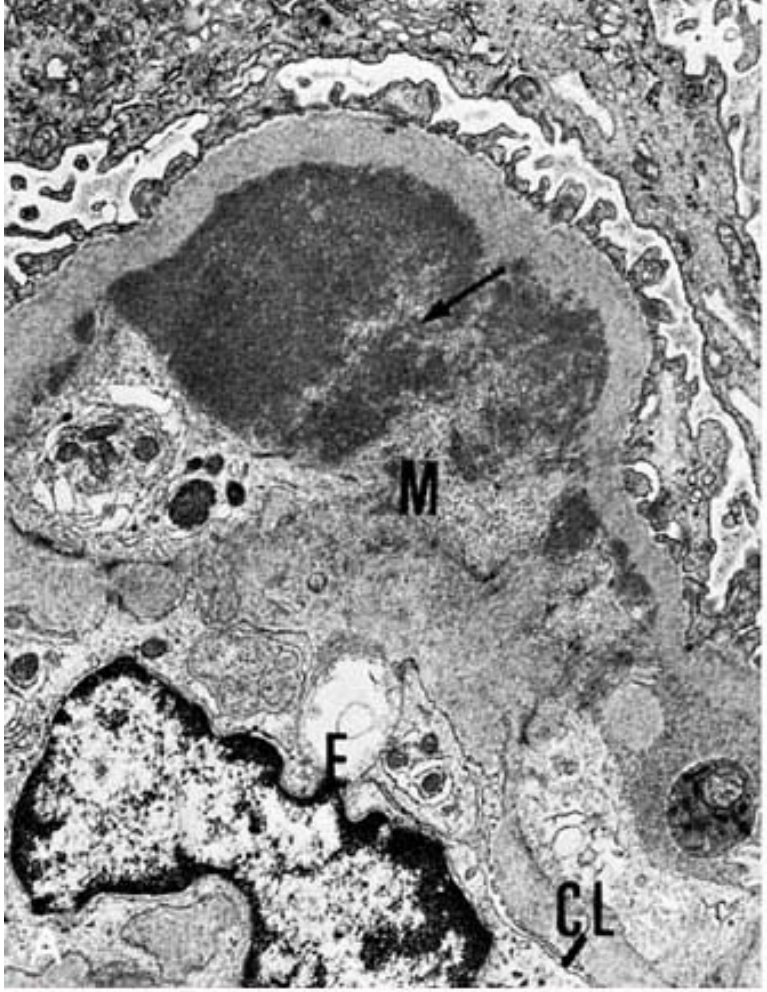
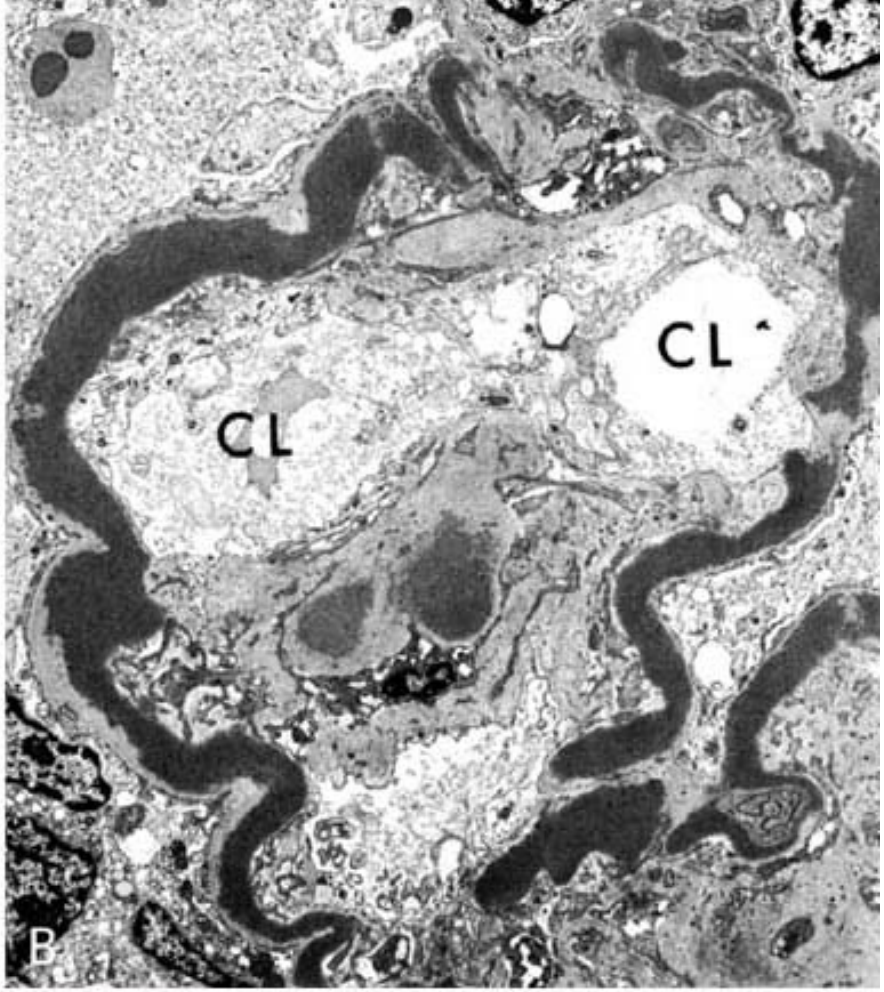
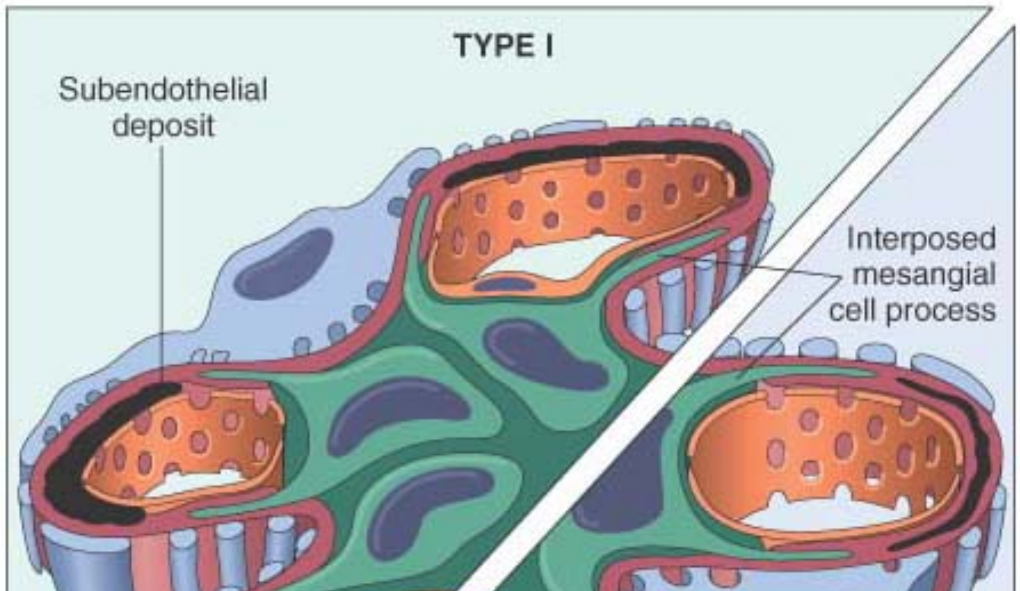


Figure 20-24 *A*, Membranoproliferative glomerulonephritis, type I. Note the large subendothelial deposit (*arrow*) incorporated into mesangial matrix (M). E, endothelium; EP, epithelium; CL, capillary lumen. *B*, Type II membranoproliferative glomerulonephritis, dense-deposit disease. There are markedly dense homogeneous deposits within the basement membrane proper. CL, capillary lumen. *C*, Schematic representation of patterns in the two types of membranoproliferative GN. In type I there are subendothelial deposits; type II is characterized by intramembranous dense deposits (dense-deposit disease). In both, mesangial interposition gives the appearance of split basement membranes when viewed in the light microscope.


M
E
CL


CL
CL
B


TYPE I
Subendothelial deposit
Interposed mesangial cell process

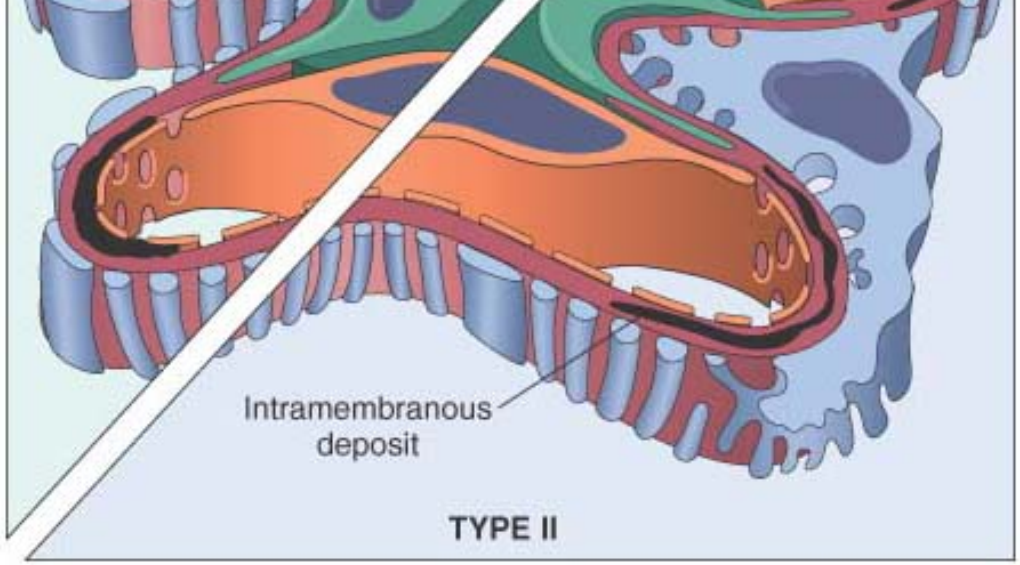


Figure 20-25 The alternative complement pathway. Note that C3NeF, present in the serum of patients with membranoproliferative glomerulonephritis, acts at the same step as properdin, serving to stabilize the alternative pathway C3 convertase, thus enhancing C3 breakdown and causing hypocomplementemia.

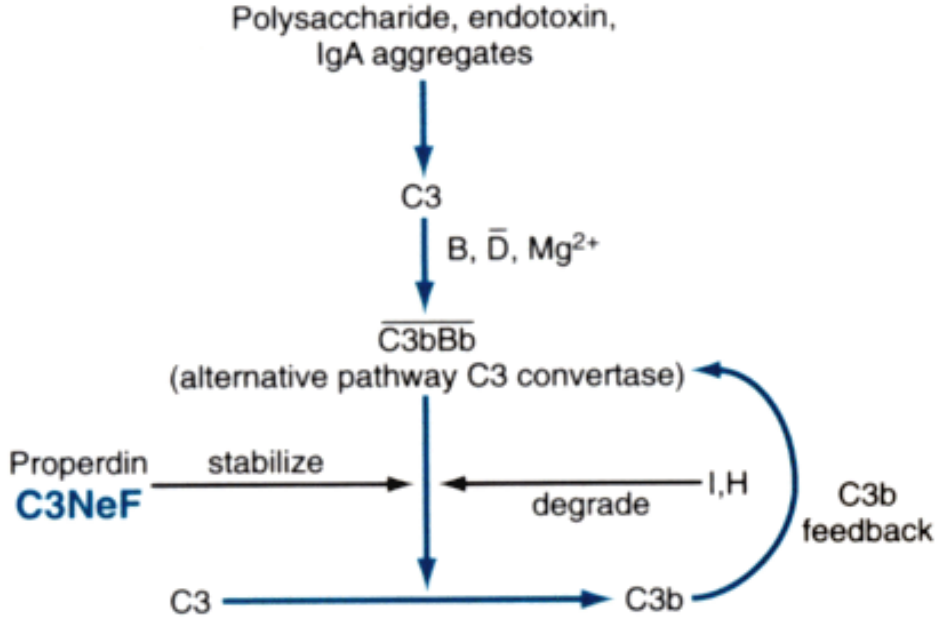


Figure 20-26 IgA nephropathy. *A*, Light microscopy showing mesangial proliferation and matrix increase. *B*, Characteristic deposition of IgA, principally in mesangial regions, detected by immunofluorescence.

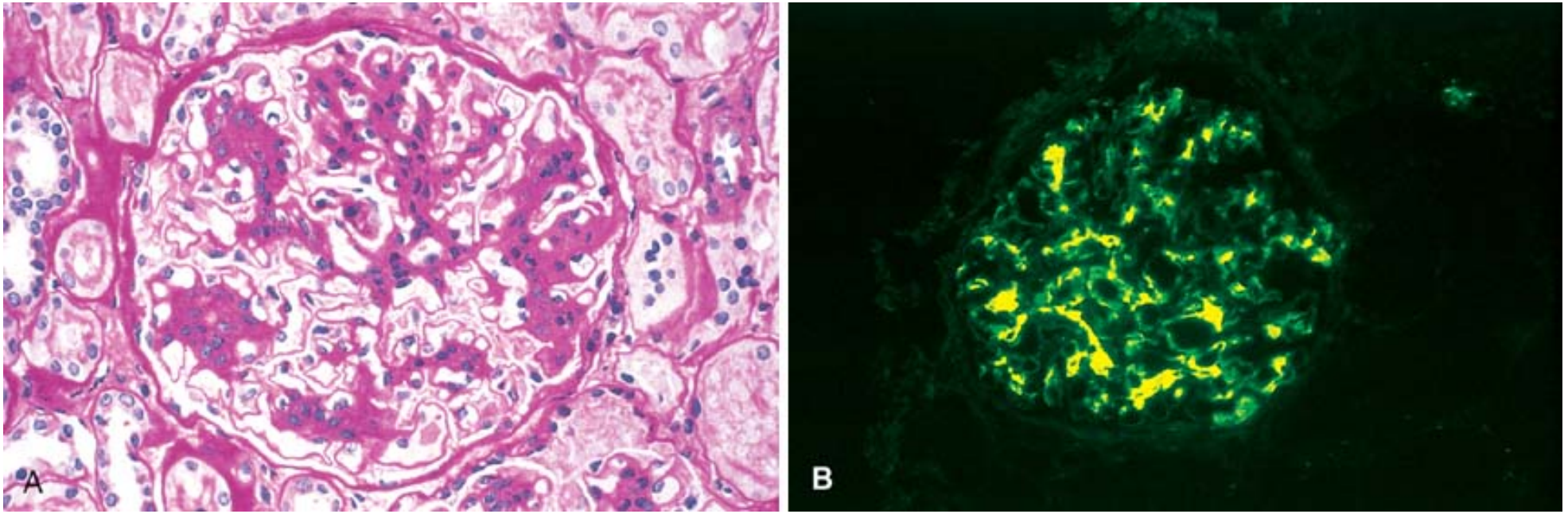


Figure 20-27 Hereditary nephritis. Electron micrograph of glomerulus with irregular thickening of the basement membrane, lamination of the lamina densa, and foci of rarefaction. Such changes may be present in other diseases but are most pronounced and widespread in hereditary nephritis. CL, capillary lumen; Ep, epithelium.

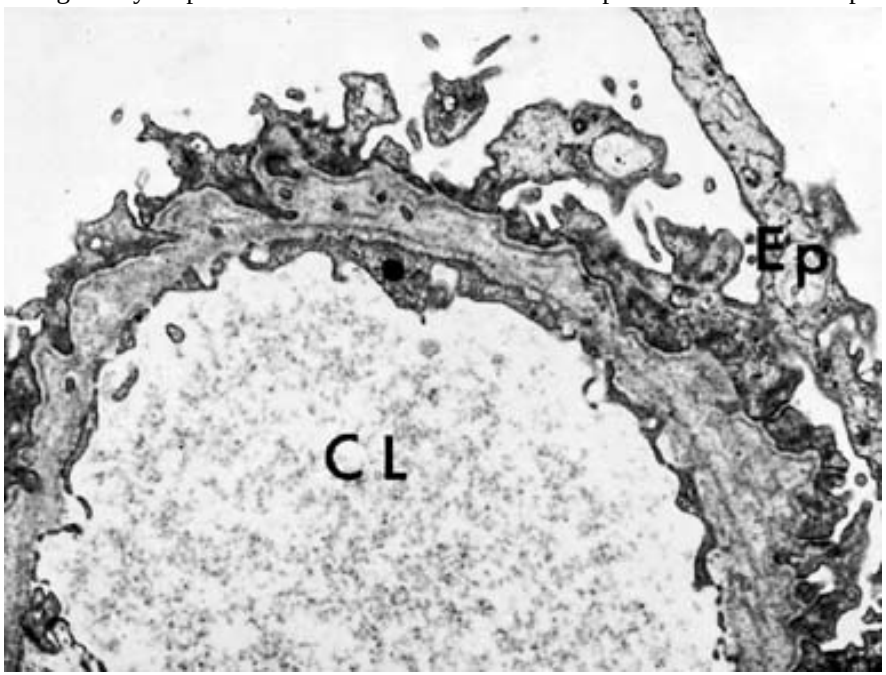


Figure 20-28 Primary glomerular diseases leading to chronic glomerulonephritis (GN). The thickness of the arrows reflects the approximate proportion of patients in each group who progress to chronic glomerulonephritis: poststreptococcal (1% to 2%); rapidly progressive (crescentic) (90%), membranous (30% to 50%), focal glomerulosclerosis (50% to 80%),

membranoproliferative glomerulonephritis (50%), IgA nephropathy (30% to 50%).

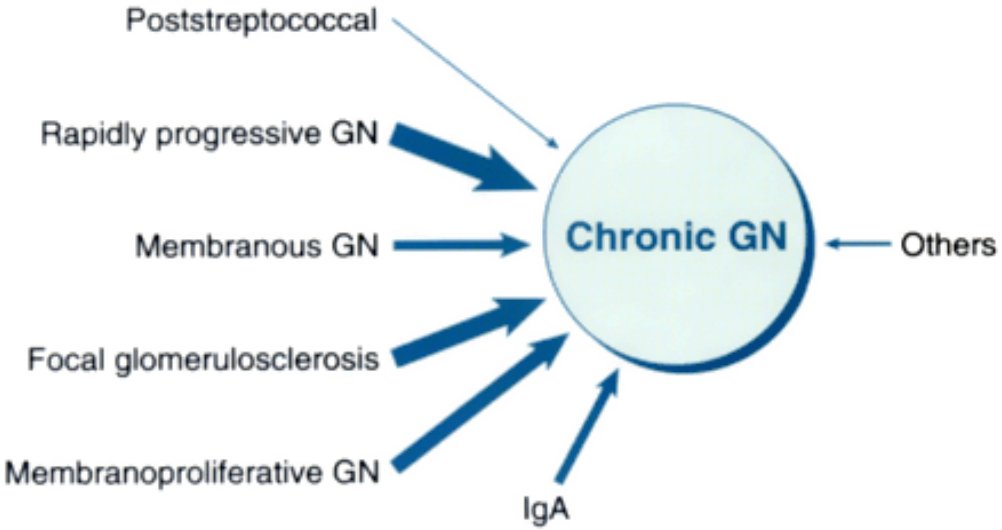


Figure 20-29 Chronic glomerulonephritis. A Masson trichrome preparation shows complete replacement of virtually all glomeruli by blue-staining collagen. *(Courtesy of Dr. M.A. Venkatachalam, Department of Pathology, University of Texas Health Sciences Center, San Antonio, TX.)*

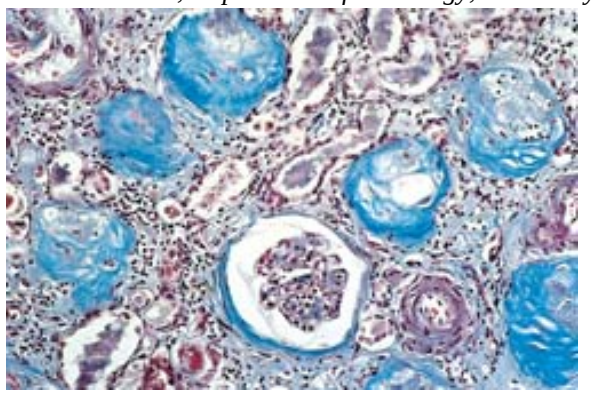


Figure 20-30 Electron micrograph of advanced diabetic glomerulosclerosis. Note the massive increase in mesangial matrix (Mes) encroaching on the glomerular capillary lumina (CL). The GBM and Bowman capsule (C) are markedly thickened. Ep, epithelium; E, endothelium.

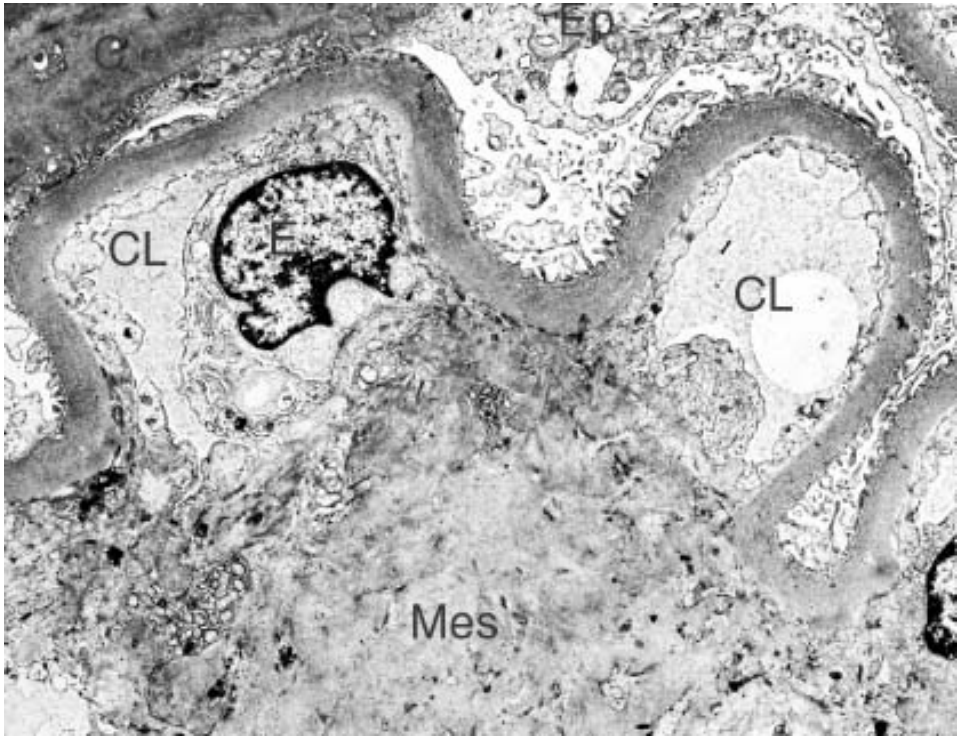
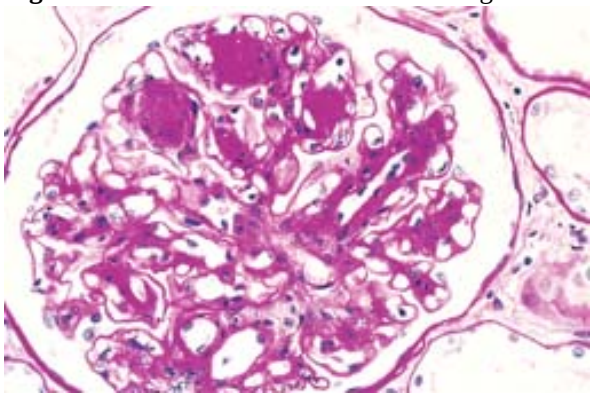


Figure 20-31 Diffuse and nodular diabetic glomerulosclerosis (PAS stain). Note the diffuse increase in mesangial matrix and characteristic acellular PAS-positive nodules.



Ischemia causes numerous structural and functional alterations in epithelial cells, as discussed in Chapter 1 . The structural changes include those of reversible injury (such as cellular swelling, loss of brush border, blebbing, loss of polarity, and cell detachment) and those associated with lethal injury (necrosis and apoptosis). Biochemically, there is depletion of adenosine triphosphate; accumulation of intracellular calcium; activation of proteases (e.g., calpain), which cause cytoskeletal disruption, and phospholipases, which damage membranes; generation of reactive oxygen species; and activation of caspases, which induce apoptotic cell death. One early reversible result of ischemia is *loss of*

cell polarity due to redistribution of membrane proteins (e.g., the enzyme Na^+ K^+ -ATPase) from the basolateral to the luminal surface of the tubular cells, resulting in abnormal ion transport across the cells, and *increased sodium delivery to distal tubules*. The latter incites vasoconstriction via *tubuloglomerular feedback*, which will be discussed below.[82] In addition, ischemic tubular cells express cytokines and adhesion molecules (such as ICAM-1), thus recruiting leukocytes that appear to participate in the subsequent injury.[83] In time, injured cells detach from the basement membranes and cause *luminal tubule obstruction*, increased intratubular pressure, and decreased GFR. In addition, fluid from the damaged tubules can leak into the interstitium, resulting in interstitial edema, increased interstitial pressure, and further damage to the tubule. All these effects, as shown in Figure 20-32 , contribute to the decreased GFR.

• *Disturbances in blood flow:* Ischemic renal injury is also characterized by *hemodynamic alterations* that cause reduced GFR. The major one is *intrarenal vasoconstriction*, which results in both reduced glomerular plasma flow and reduced oxygen delivery to the functionally important tubules in the outer medulla (thick ascending limb and straight segment of the proximal tubule). A number of vasoconstrictor pathways have been implicated, including the renin-angiotensin mechanism, stimulated by increased distal sodium delivery (via *tubuloglomerular feedback*), and *sublethal endothelial injury*, leading to increased release of the vasoconstrictor *endothelin* and decreased production of the vasodilators *nitric oxide* and PGI_2 . Finally, there is also some evidence of a direct effect of ischemia or toxins on the glomerulus, causing a reduced glomerular ultrafiltration coefficient, possibly due to mesangial contraction.

The patchiness of tubular necrosis and maintenance of the integrity of the basement membrane along many segments allow ready repair of the necrotic foci and recovery of function if the precipitating cause is removed. This repair is dependent on the capacity of reversibly injured epithelial cells to proliferate and differentiate. Re-epithelialization is mediated by a variety of growth factors and cytokines produced locally by the tubular cells themselves (autocrine stimulation) or by inflammatory cells in the vicinity of necrotic foci (paracrine stimulation).[84] Of these, epidermal growth factor (EGF), TGF-α, insulin-like growth factor type I, and hepatocyte growth factor have been shown to be particularly important in renal tubular repair. Growth factors, indeed, are being explored as possible therapeutic agents to enhance re-epithelialization in ATN.[84]

Figure 20-32 Possible pathogenetic mechanisms in ischemic acute renal failure (see text).

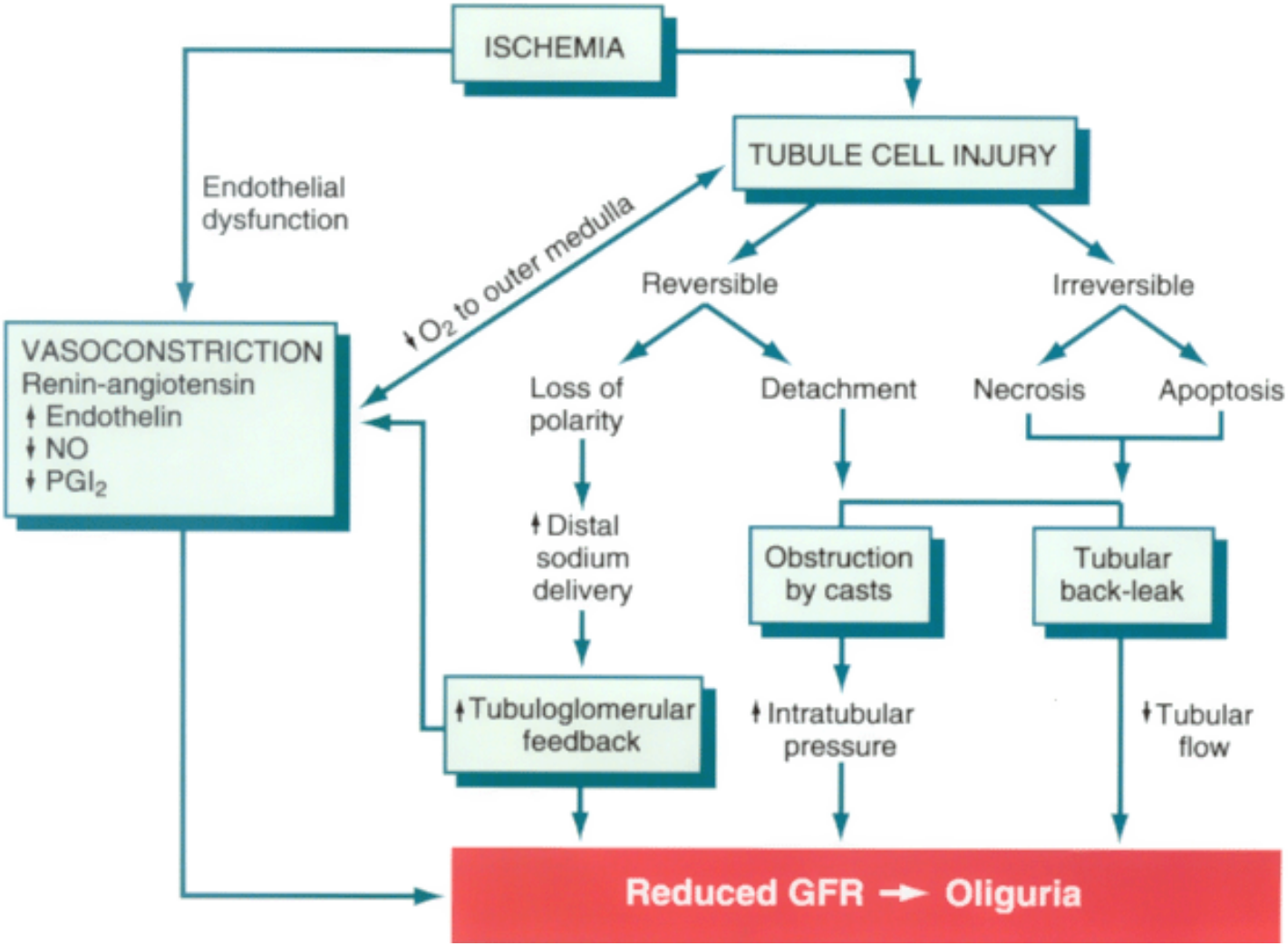


Figure 20-33 Patterns of tubular damage in ischemic and toxic acute tubular necrosis. In the ischemic type, tubular necrosis is patchy, relatively short lengths of tubules are affected, and straight segments of proximal tubules (PST) and ascending limbs of Henle's loop (HL) are most vulnerable. In toxic acute tubular necrosis, extensive necrosis is present along the proximal tubule segments (PCT) with many toxins (e.g., mercury), but necrosis of the distal tubule, particularly ascending Henle's loop, also occurs. In both types, lumens of the distal convoluted tubules (DCT) and collecting ducts (CD) contain casts.

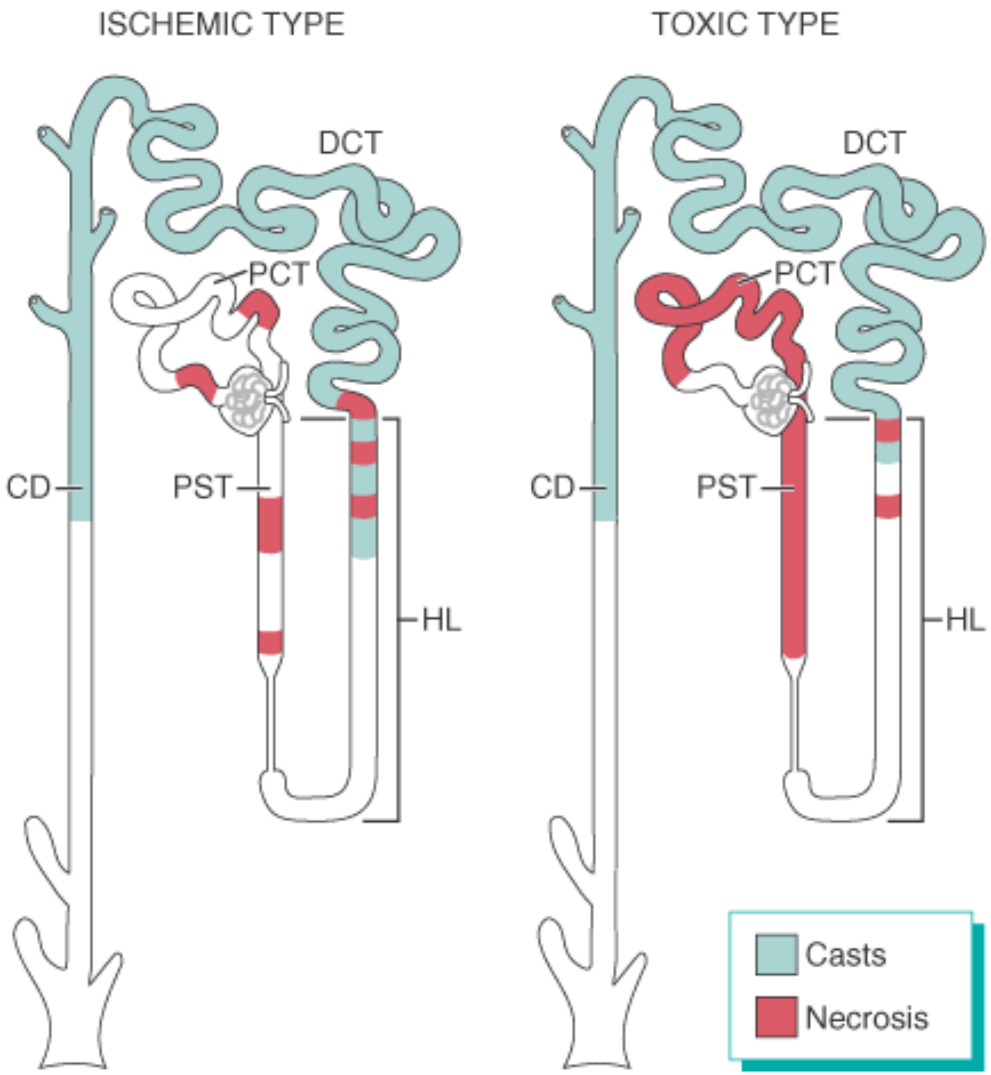


Figure 20-34 Acute tubular necrosis. Some of the tubular epithelial cells in the tubules are necrotic, and many have become detached (from their basement membranes) and been sloughed into the tubular lumina, whereas others are swollen, vacuolated, and regenerating. *(Courtesy of Dr. Agnes Fogo, Vanderbilt University, Nashville, TN.)*

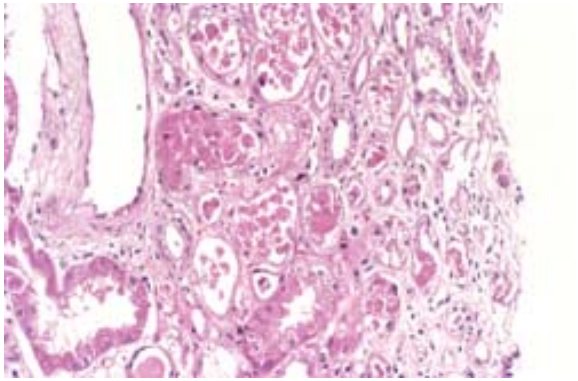


TABLE 20-9 -- Causes of Tubulointerstitial Nephritis

Infections
Acute bacterial pyelonephritis
Chronic pyelonephritis (including reflux nephropathy)
Other infections (e.g., viruses, parasites)
Toxins
Drugs
Acute hypersensitivity interstitial nephritis
Analgesic nephropathy
Heavy metals
Lead, cadmium
Metabolic Diseases
Urate nephropathy
Nephrocalcinosis (hypercalcemic nephropathy)
Hypokalemic nephropathy
Oxalate nephropathy
Physical Factors
Chronic urinary tract obstruction
Radiation nephropathy
Neoplasms
Multiple myeloma (cast nephropathy)

Immunologic Reactions
Transplant rejection
Sjögren syndrome
Sarcoidosis
Vascular Diseases
Miscellaneous
Balkan nephropathy
Nephronophthisis-medullary cystic disease complex
"Idiopathic" interstitial nephritis

are identified by cause or by associated disease (e.g., analgesic nephropathy, radiation nephropathy). Glomerular and vascular abnormalities may also be present but either are mild or occur only in advanced stages of these diseases.

Tubulointerstitial nephritis can be acute or chronic. Acute tubulointerstitial nephritis has a rapid clinical onset and is characterized histologically by interstitial edema, often accompanied by leukocytic infiltration of the interstitium and tubules, and focal tubular necrosis. In *chronic interstitial nephritis*, there is infiltration with predominantly mononuclear leukocytes, prominent interstitial fibrosis, and widespread tubular atrophy. Morphologic features that are helpful in separating acute from chronic tubulointerstitial nephritis include edema and, when present, eosinophils and neutrophils in the acute form, contrasted with fibrosis and tubular atrophy in the chronic form.

These conditions are distinguished clinically from the glomerular diseases by the absence, in early stages, of such hallmarks of glomerular injury as nephritic or nephrotic syndromes and by the presence of defects in tubular function. The latter may be subtle and include impaired ability to concentrate urine, evidenced clinically by polyuria or nocturia; salt wasting; diminished ability to excrete acids (metabolic acidosis); and isolated defects in tubular reabsorption or secretion. The advanced forms, however, may be difficult to distinguish clinically from other causes of renal insufficiency.

Some of the specific conditions listed in Table 20-9 are discussed elsewhere in this book. In this section, we deal principally with pyelonephritis and interstitial diseases induced by drugs.

Pyelonephritis and Urinary Tract Infection

Pyelonephritis is a renal disorder affecting the tubules, interstitium, and renal pelvis and is one of the most common diseases of the kidney. It occurs in two forms. *Acute pyelonephritis* is caused by bacterial infection and is the renal lesion associated with urinary tract infection. *Chronic pyelonephritis* is a more complex disorder: bacterial infection plays a dominant role, but other factors (vesicoureteral reflux, obstruction) are involved in its pathogenesis. Pyelonephritis is a serious complication of an extremely common clinical spectrum of *urinary tract infections* that affect the urinary bladder (cystitis), the kidneys and their collecting systems (pyelonephritis), or both. Bacterial infection of the lower urinary tract may be completely asymptomatic (asymptomatic bacteriuria) and most often remains localized to the bladder without the development of renal infection. However, lower urinary tract infection always carries the potential of spread to the kidney.

Etiology and Pathogenesis.

The dominant etiologic agents, accounting for more than 85% of cases of urinary tract infection, are the Gram-negative bacilli that are normal inhabitants of the intestinal tract.[86] By far the most common is *Escherichia coli*, followed by *Proteus*, *Klebsiella*, and *Enterobacter*. *Streptococcus faecalis*, also of enteric origin, staphylococci, and virtually every other bacterial and fungal agent can also cause lower urinary tract and renal infection. In immunocompromised patients, particularly those with transplanted organs, viruses such as polyoma virus,

cytomegalovirus, and adenovirus can also be a cause of renal infection.

In most patients with urinary tract infection, the infecting organisms are derived from the patient's own fecal flora. This is thus a form of *endogenous infection*. There are two routes by which bacteria can reach the kidneys: (1) through the blood-stream (hematogenous infection) and (2) from the lower urinary tract (ascending infection) (Fig. 20-35). Although the hematogenous route is the less common of the two, acute pyelonephritis does result from seeding of the kidneys by bacteria from distant foci in the course of septicemia or infective endocarditis. Hematogenous infection is more likely to occur in the presence of ureteral obstruction, in debilitated patients, in patients receiving immunosuppressive therapy, and with nonenteric organisms, such as staphylococci and certain fungi and viruses.

Ascending infection is the most common cause of clinical pyelonephritis. Normal human bladder and bladder urine are sterile; therefore, a number of steps must occur for renal infection to occur:

- The first step in the pathogenesis of ascending infection appears to be the *colonization of the distal urethra and introitus* (in the female) by coliform bacteria. This colonization is influenced by the ability of bacteria to adhere to urethral mucosal cells. Such bacterial adherence, as discussed in Chapter 8 , involves adhesive molecules (adhesins) on the P-fimbriae (pili) of bacteria that interact with receptors on the surface of uroepithelial cells. Specific adhesins (e.g., the *pap* variant) are associated with infection. In addition, certain types of fimbriae promote renal tropism, or persistence of infection, or an enhanced inflammatory response to bacteria.[87]
- *From the urethra to the bladder*, organisms gain entrance during urethral catheterization or other instrumentation. Long-term catheterization, in particular, carries a risk of infection. In the absence of instrumentation, *urinary infections are much more common in females*, and this has been variously ascribed to the shorter urethra in females, the absence of antibacterial properties such as are found in prostatic fluid, hormonal changes affecting adherence of bacteria to the mucosa, and urethral trauma during sexual intercourse or a combination of these factors.
- *Multiplication in the bladder.* Ordinarily, organisms introduced into the bladder are cleared by the continual flushing of voiding and by antibacterial mechanisms. However, outflow obstruction or bladder dysfunction results in incomplete emptying and increased residual volume of urine. In the presence of stasis, bacteria introduced into the bladder can multiply unhindered without being flushed out or destroyed in the bladder wall. Accordingly, urinary tract infection is particularly frequent among patients with lower urinary tract obstruction, such as may occur with benign prostatic hypertrophy, tumors, or calculi or with neurogenic bladder dysfunction caused by diabetes or spinal cord injury.
- *Vesicoureteral reflux.* Although obstruction is an important predisposing factor in the pathogenesis of ascending infection, *it is incompetence of the vesicoureteral valve* that allows bacteria to ascend the ureter into the renal pelvis. The normal ureteral insertion into the bladder is a competent one-way valve that prevents retrograde flow of urine, especially during micturition, when the intravesical pressure rises. An incompetent vesicoureteral orifice allows the reflux of bladder urine into the ureters (*vesicoureteral reflux*) (Fig. 20-36). Reflux is most often due to a congenital absence or shortening of the intravesical portion of the ureter (Fig. 20-37), such that the ureter is not compressed during micturition. In addition, bladder infection itself, probably as a result of the action of bacterial or inflammatory products on ureteral contractility, can cause or accentuate vesicoureteral reflux, particularly in children. *Acquired vesicoureteral reflux* in adults can result from persistent bladder atony caused by spinal cord injury. The effect of

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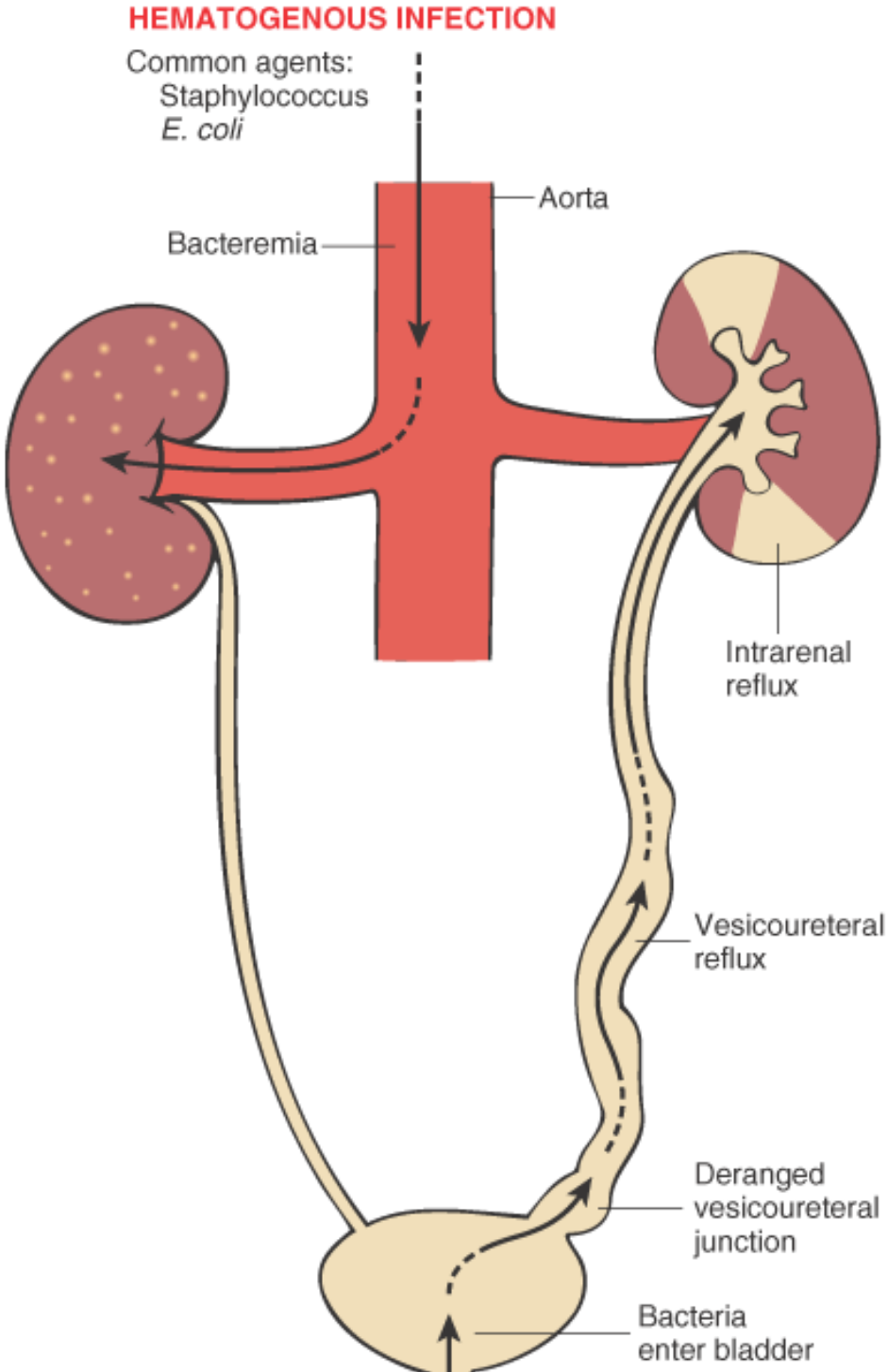
vesicoureteral reflux is similar to that of an obstruction in that after voiding, there is residual urine in the urinary tract, which favors bacterial growth.

- *Intrarenal reflux.* Vesicoureteral reflux also affords a ready mechanism by which the infected bladder urine can be propelled up to the renal pelvis and deep into the renal parenchyma through open ducts at the tips of the papillae (intrarenal reflux). Intrarenal reflux is most common in the upper and lower poles of the kidney, where papillae tend to have flattened or concave tips rather than the convex pointed type present in the midzones of the kidney (and depicted in most textbooks). Reflux can be demonstrated radiographically by a voiding cystourethrogram: The bladder is filled with a radio-opaque dye, and films are taken during micturition. Vesicoureteral reflux can be demonstrated in about 30% of infants and children with urinary tract infection (see Fig. 20-36).

Figure 20-35 Schematic representation of pathways of renal infection. Hematogenous infection results from bacteremic spread. More common is ascending infection, which results from a combination of urinary bladder infection, vesicoureteral reflux, and intrarenal reflux.



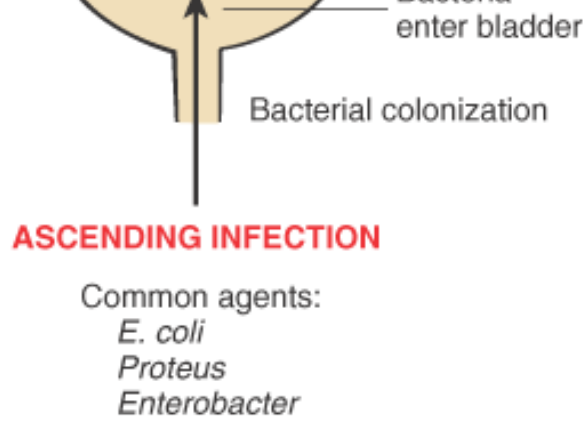


Figure 20-36 Vesicoureteral reflux demonstrated by a voiding cystourethrogram. Dye injected into the bladder refluxes into both dilated ureters, filling the pelvis and calyces.

Figure 20-37 The vesicoureteral junction. In normal individuals (*A*), the intravesical portion of the ureter is oblique, such that the ureter is closed by muscle contraction during micturition. The most common cause of reflux is congenital complete or partial absence of the intravesical ureter (*B*).

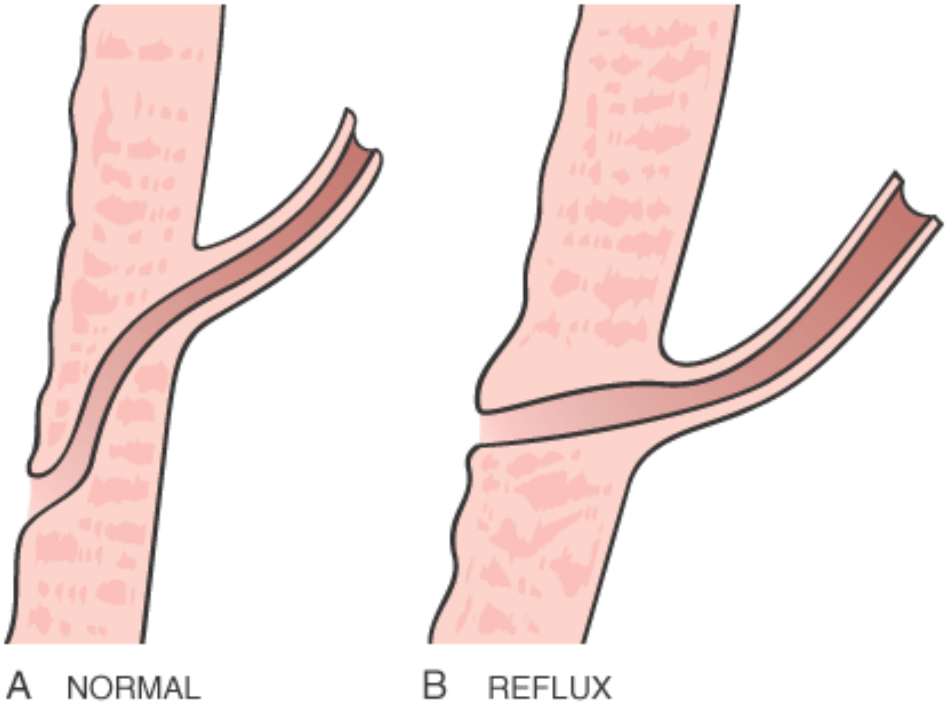


Figure 20-38 Acute pyelonephritis. Cortical surface exhibits grayish white areas of inflammation and abscess formation.

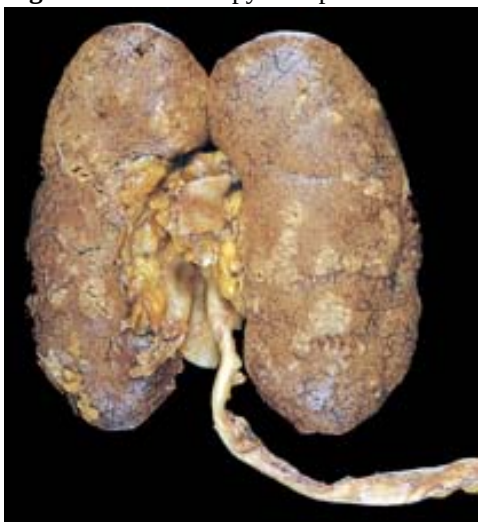


Figure 20-39 Acute pyelonephritis marked by an acute neutrophilic exudate within tubules and the renal substance.

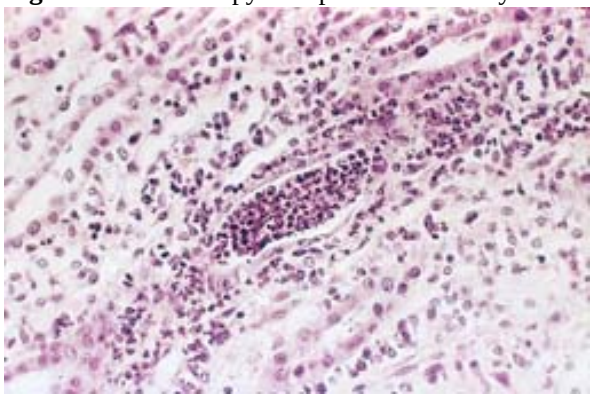


Figure 20-40 Papillary necrosis. Areas of pale gray necrosis are limited to the papillae.

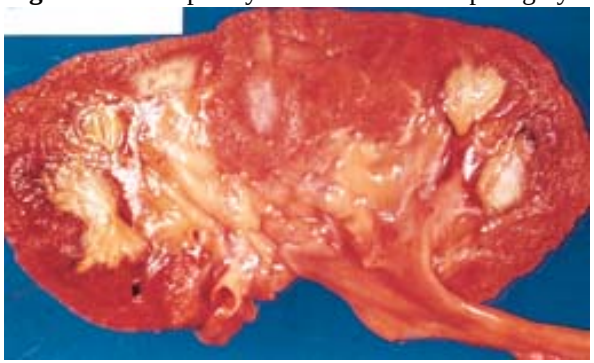


Figure 20-41 Polyoma virus nephropathy. *A*, The kidney shows enlarged tubular epithelial cells with nuclear inclusions (*arrow*) and interstitial inflammation (*arrowheads*). *B*, Intranuclear viral inclusions visualized by electron microscopy. *(Courtesy of Dr. Jean Olson, Department of Pathology, University of California San Francisco, San Francisco, CA.)*

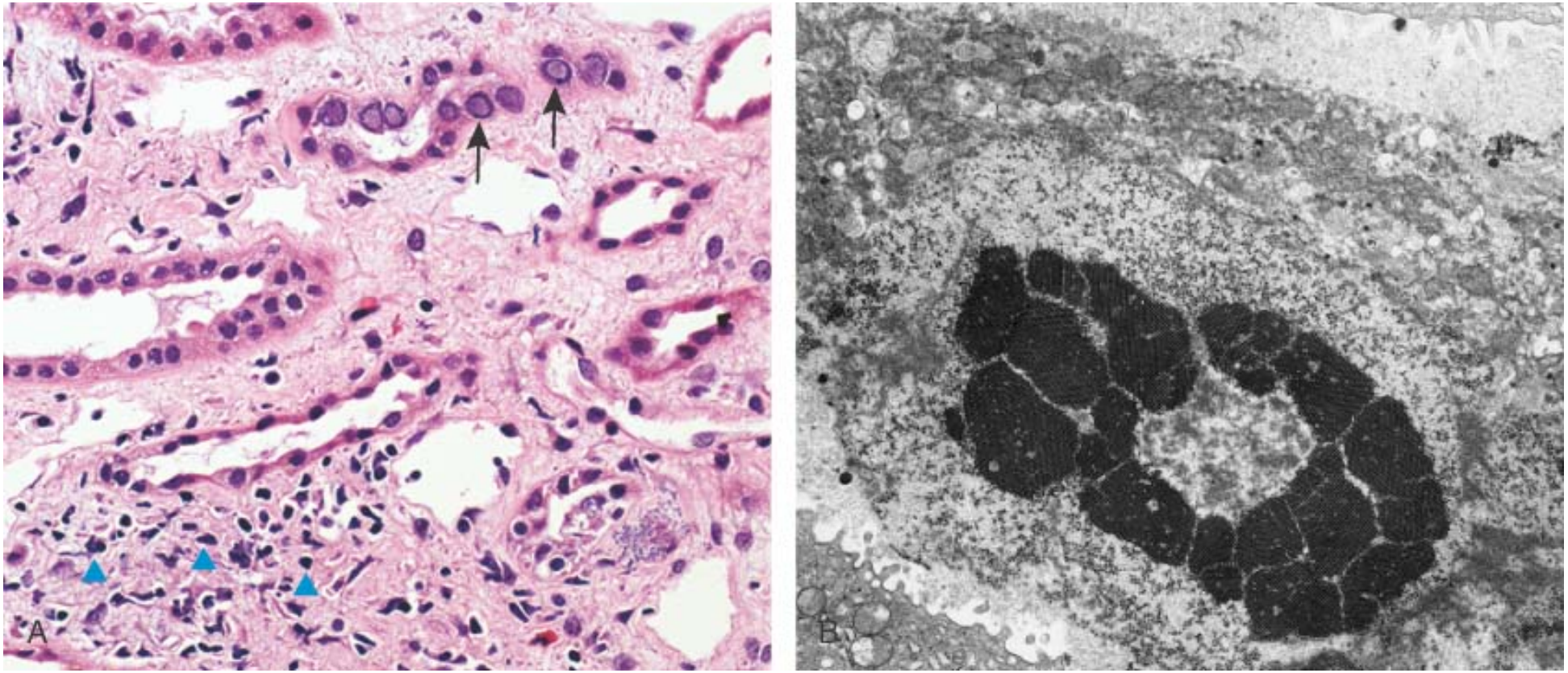


Figure 20-42 Typical coarse scars of chronic pyelonephritis associated with vesicoureteral reflux. The scars are usually polar and are associated with underlying blunted calyces.

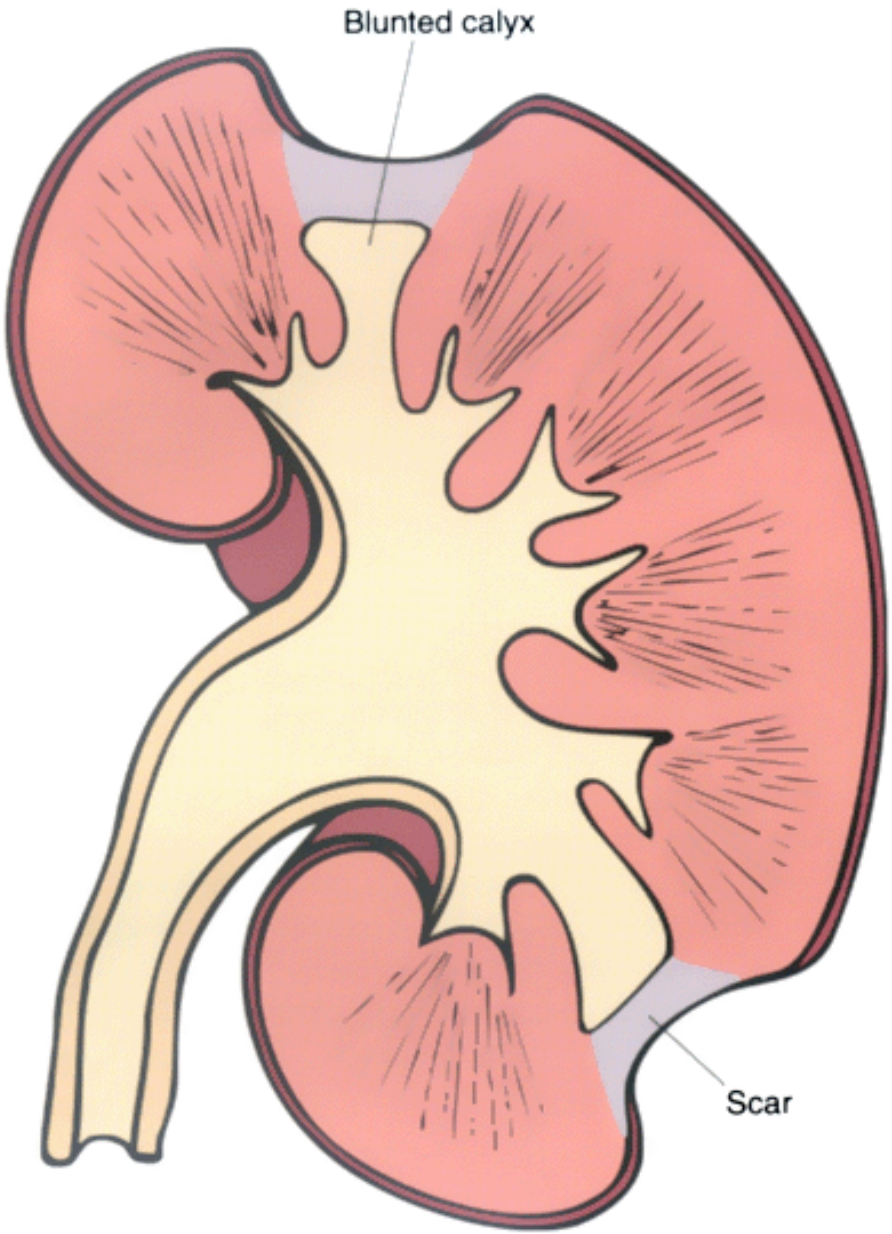


Figure 20-43 *A,* Chronic pyelonephritis. The surface (*left*) is irregularly scarred. The cut section (*right*) reveals characteristic dilation and blunting of calyces. The ureter is dilated and thickened, a finding that is consistent with chronic vesicoureteral reflux. *B,* Low-power view showing a corticomedullary renal scar with an underlying dilated deformed calyx. Note the thyroidization of tubules in the cortex.

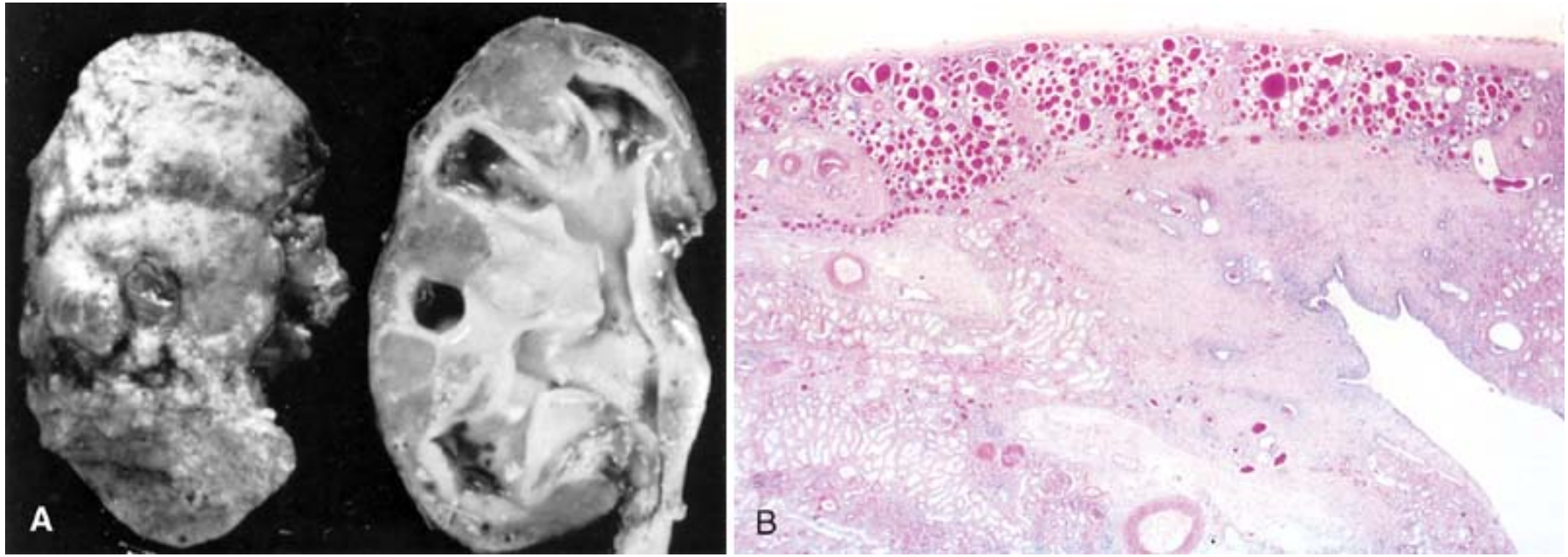


Figure 20-44 Drug-induced interstitial nephritis, with prominent eosinophilic and mononuclear cell infiltrate. *(Courtesy of Dr. H. Rennke, Brigham and Women's Hospital, Boston, MA.)*

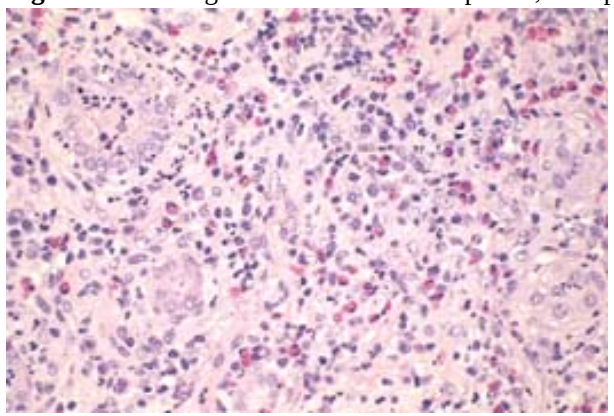
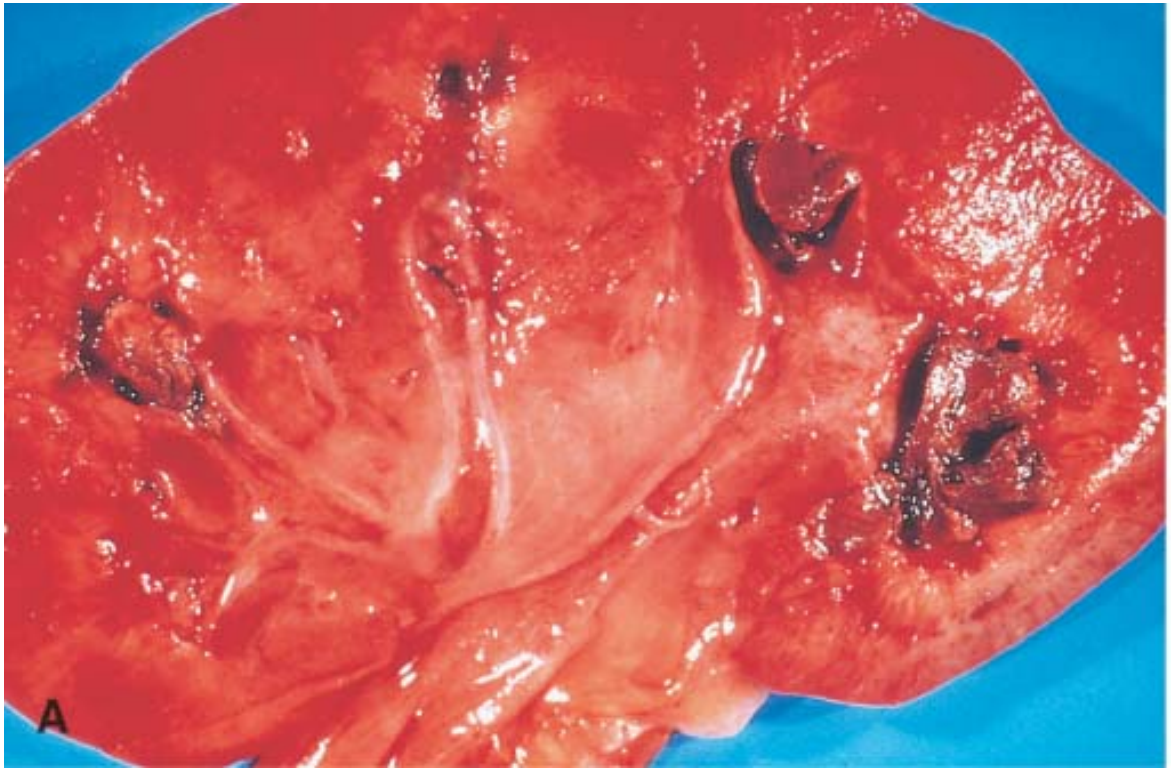


Figure 20-45 Analgesic nephropathy. *A*, The brownish necrotic papilla, transformed to a necrotic, structureless mass, fills the pelvis. *B*, Microscopic view. Note the fibrosis in the medulla. *(Courtesy of Dr. F.J. Gloor, Institut für Pathologie, Kantonsspital, St. Gallen, Switzerland.)*



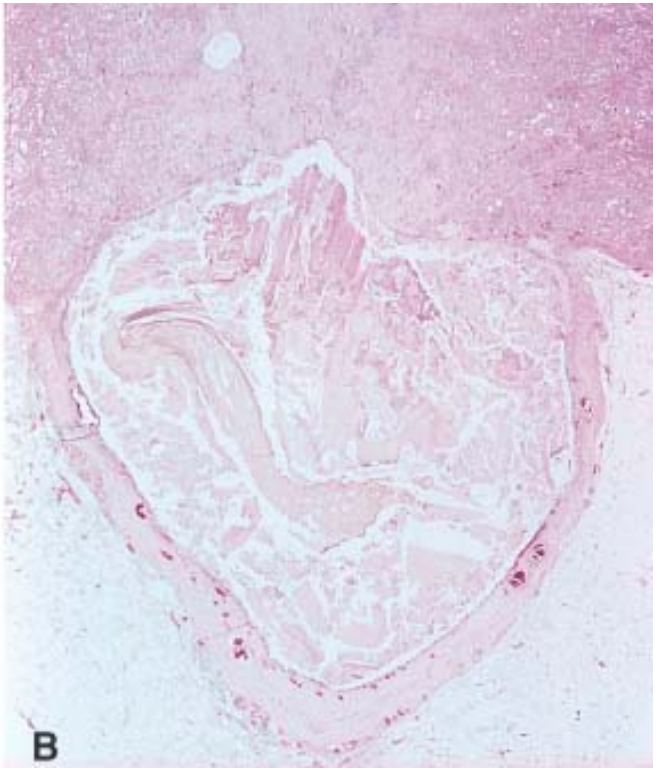


TABLE 20-10 -- Causes of Papillary Necrosis

	Diabetes Mellitus	Analgesic Nephropathy	Sickle Cell Disease	Obstruction
Male-to-female ratio	1:3	1:5	1:1	9:1
Time course	10 years	7 years of abuse	Variable	Variable
Infection	80%	25%	±	90%
Calcification	Rare	Frequent	Rare	Frequent
Number of papillae affected	Several; all of same stage	Almost all; different stages of necrosis	Few	Variable

Data from Seshan S, et al (eds): Classification and Atlas of Tubulointerstitial and Vascular Diseases. Baltimore, Williams & Wilkins, 1999.

Chinese Herbs Nephropathy

A syndrome of chronic tubulointerstitial nephritis caused by aristolochic acid, a supplement found in some formulations of herbal remedies, has been recognized recently. The drug causes a distinctive picture of renal failure with histopathologic features of interstitial fibrosis with a relative paucity of infiltrating interstitial leukocytes. As with analgesic nephropathy, there is an increased incidence of carcinoma in the kidney and urinary tract.

Other Tubulointerstitial Diseases

Urate Nephropathy

Three types of nephropathy can occur in patients with hyperuricemic disorders:

- *Acute uric acid nephropathy* is caused by the precipitation of uric acid crystals in the renal tubules, principally in collecting ducts, leading to obstruction of nephrons and the development of acute renal failure. This type is particularly likely to occur in patients with leukemias and lymphomas who are undergoing chemotherapy; the drugs increase the death of tumor cells, and uric acid is released as the nuclei of these cells disintegrate. Precipitation of uric acid is favored by the acidic pH in collecting tubules.
- *Chronic urate nephropathy*, or gouty nephropathy, occurs in patients with more protracted forms of hyperuricemia. The lesions are ascribed to the deposition of monosodium urate crystals in the acidic milieu of the distal tubules and collecting ducts as well as in the interstitium. *These deposits have a distinct histologic appearance and may form birefringent needle-like crystals either in the tubular lumina or in the interstitium* (Fig. 20-46). The urates induce a *tophus* consisting of foreign body giant cells, other

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mononuclear cells, and a fibrotic reaction (Chapter 26). Tubular obstruction by the urates causes cortical atrophy and scarring. Arterial and arteriolar thickening is common in these kidneys, owing to the relatively high frequency of hypertension in patients with gout. Clinically, urate nephropathy is a subtle disease associated with tubular defects that may progress slowly. Patients with gout who actually develop a chronic nephropathy commonly have evidence of increased exposure to lead, sometimes by way of drinking "moonshine"whiskey contaminated with lead.

- The third renal syndrome in hyperuricemia is *nephrolithiasis*; uric acid stones are present in 22% of patients with gout and 42% of those with secondary hyperuricemia (see later discussion of renal stones).

Hypercalcemia and Nephrocalcinosis

Disorders characterized by hypercalcemia, such as hyperparathyroidism, multiple myeloma, vitamin D intoxication, metastatic bone disease, or excess calcium intake (milk-alkali syndrome), may induce the formation of calcium stones and deposition of calcium in the kidney (nephrocalcinosis). Extensive degrees of calcinosis, under certain conditions, may lead to a form of chronic tubulointerstitial disease and renal insufficiency. The first damage induced by the hypercalcemia is at the *intracellular level*, in the tubular epithelial cells, resulting in mitochondrial distortion and evidence of cell injury. Subsequently, calcium deposits can be demonstrated within the mitochondria, cytoplasm, and basement membrane. Calcified cellular debris contributes to obstruction of tubular lumens and causes obstructive atrophy of nephrons with interstitial fibrosis and nonspecific chronic inflammation. Atrophy of entire cortical areas drained by calcified tubules may occur, and this accounts for the alternating areas of normal and scarred parenchyma seen in such kidneys.

Figure 20-46 Urate crystals in the renal medulla. Note the giant cells and fibrosis around the crystals.

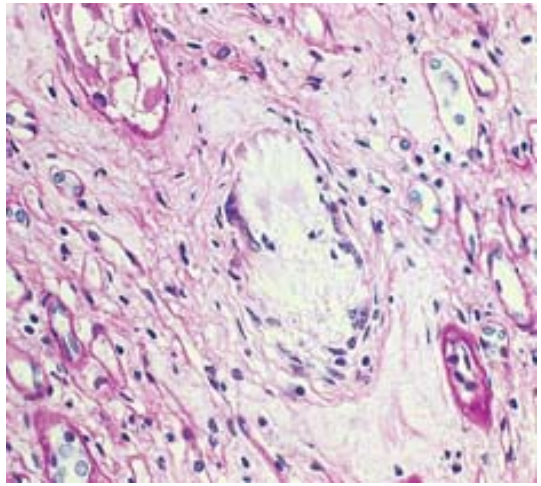


TABLE 20-11 -- Renal Involvement in Nonrenal Neoplasms

Direct tumor invasion of renal parenchyma
••Ureters (obstruction)
••Artery (renovascular hypertension)
Hypercalcemia
Hyperuricemia
Amyloidosis
Excretion of abnormal proteins (multiple myeloma)
Glomerulopathy
••Immune complex glomerulonephritis (carcinomas)
••Minimal change disease (Hodgkin disease)
••Membranoproliferative glomerulonephritis (leukemias and lymphomas)
Effects of radiotherapy, chemotherapy, secondary infection

Morphology.

The tubulointerstitial changes in multiple myeloma are fairly characteristic. The Bence Jones tubular casts appear as pink to blue amorphous masses, sometimes concentrically laminated, often with a fractured appearance, filling and distending the tubular lumens. Some of the casts are surrounded by multinucleate giant cells that are derived from mononuclear phagocytes (Fig. 20-47). The adjacent interstitial tissue usually shows a nonspecific inflammatory response and fibrosis. On occasion, the casts erode their way from the tubules into the interstitium and here evoke a granulomatous inflammatory reaction. The histologic features of amyloidosis, light-chain deposition disease and nephrocalcinosis and infection, may also be present.

Clinically, the renal manifestations are of several types. In the most common form, *chronic renal failure* develops insidiously and usually progresses slowly during a period of several months to years. Another form occurs suddenly and is manifested by *acute renal failure* with oliguria. Precipitating factors in these patients include dehydration, hypercalcemia, acute

infection, and treatment with nephrotoxic antibiotics. *Bence Jones proteinuria* occurs in 70% of patients with myeloma; the presence of significant non-light-chain proteinuria (e.g., albumin) suggests secondary amyloidosis or light-chain deposition disease.

Diseases of Blood Vessels

Nearly all diseases of the kidney involve the renal blood vessels secondarily. Systemic vascular diseases, such as various

Figure 20-47 Myeloma kidney. Note the angulated and tubular casts with macrophages, including multinucleate cells, engulfing them.

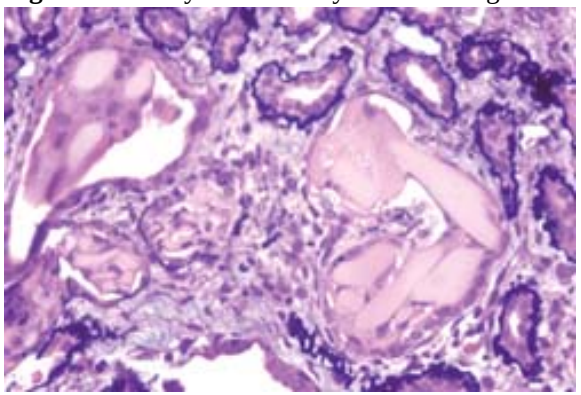


Figure 20-48 Close-up of the gross appearance of the cortical surface in benign nephrosclerosis illustrating the fine, leathery granularity of the surface.

Figure 20-49 Hyaline arteriolosclerosis. High-power view of two arterioles with hyaline deposition, marked thickening of the walls, and a narrowed lumen. *(Courtesy of Dr. M.A. Venkatachalam, Department of Pathology, University of Texas Health Sciences Center, San Antonio, TX.)*

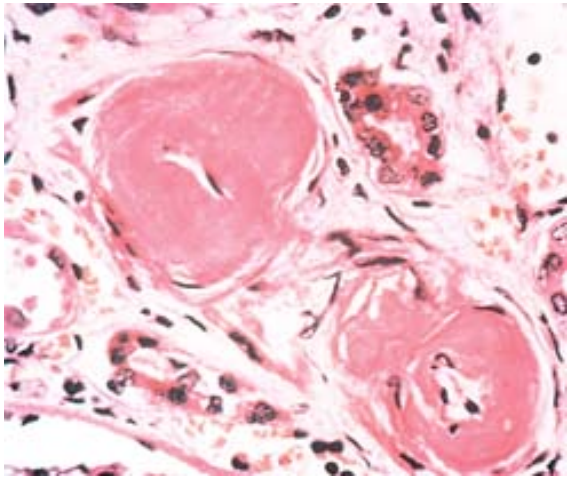


TABLE 20-12 -- Types of Hypertension

Primary or Essential Hypertension
Secondary Hypertension
Renal
••Acute glomerulonephritis
••Chronic renal disease
••Renal artery stenosis
••Renal vasculitis
••Renin-producing tumors
Endocrine
••Adrenocortical hyperfunction (Cushing syndrome)
••Oral contraceptives
••Pheochromocytoma
••Acromegaly
••Myxedema
••Thyrotoxicosis (systolic hypertension)
Vascular
••Coarctation of aorta
••Polyarteritis nodosa
••Aortic insufficiency (systolic hypertension)

Neurogenic
••Psychogenic
••Increased intracranial pressure
••Polyneuritis, bulbar poliomyelitis, others

hyperplastic arteriolosclerosis that is typical of malignant hypertension and further narrowing of the lumens. The kidneys become markedly ischemic. With severe involvement of the renal afferent arterioles, the renin-angiotensin system receives a powerful stimulus; indeed, *patients with malignant hypertension have markedly elevated levels of plasma renin*. This then sets up a self-perpetuating cycle in which angiotensin II causes intrarenal vasoconstriction, and the attendant renal ischemia perpetuates renin secretion. Other vasoconstrictors (e.g., endothelin) and loss of vasodilators (nitric oxide) may also contribute to vasoconstriction. Aldosterone levels are also elevated, and salt retention undoubtedly contributes to the elevation of blood pressure. The consequences of the markedly elevated blood pressure on the blood vessels throughout the body are known as *malignant arteriosclerosis*, and the renal disorder is malignant nephrosclerosis.

Morphology.

On gross inspection, the kidney size depends on the duration and severity of the hypertensive disease. Small, pinpoint petechial hemorrhages may appear on the cortical surface from rupture of arterioles or glomerular capillaries, giving the kidney a peculiar "flea-bitten" appearance.

Two histologic alterations characterize blood vessels in malignant hypertension (Fig. 20-50):

- **Fibrinoid necrosis of arterioles.** This appears as an eosinophilic granular change in the blood vessel wall, which stains positively for fibrin by histochemical or immunofluorescence techniques. This change represents an acute event, and it may be accompanied by limited inflammatory infiltrate within the wall. However, usually this pattern of necrosis is not accompanied by prominent inflammation.
- In the interlobular arteries and arterioles, there is intimal thickening caused by a proliferation of elongated, concentrically arranged smooth muscle cells, together with fine concentric layering of collagen and accumulation of pale staining material that likely represents accumulations of proteoglycans and plasma proteins. This alteration has been referred to as **onion-skinning** because of its concentric appearance. The lesion, also called hyperplastic arteriolitis, correlates well with renal failure in malignant hypertension. Sometimes the glomeruli become necrotic and infiltrated with neutrophils, and the glomerular capillaries may thrombose. The arteriolar and arterial lesions result in considerable narrowing of all vascular lumens, with ischemic atrophy and, at times, infarction distal to the abnormal vessels.

Figure 20-50 Malignant hypertension. *A*, Fibrinoid necrosis of afferent arteriole (PAS stain). *B*, Hyperplastic arteriolitis (onion-skin lesion). *(Courtesy of Dr. H. Rennke, Brigham and Women's Hospital, Boston, MA.)*

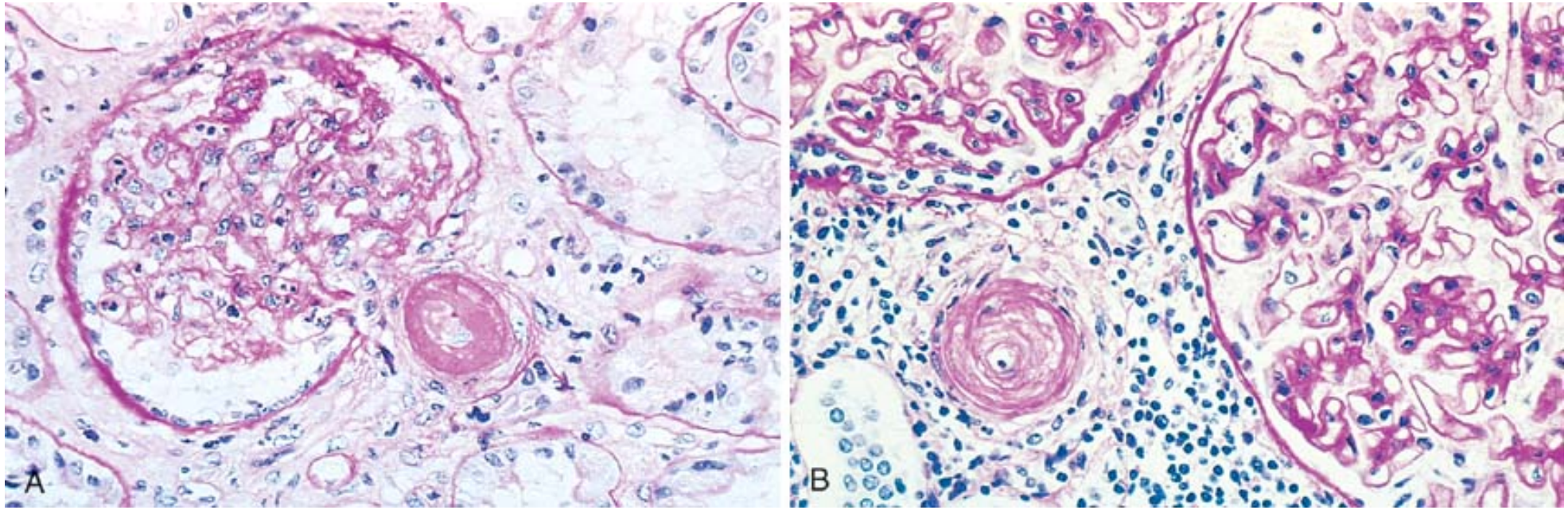


Figure 20-51 Fibromuscular dysplasia of the renal artery, medial type (elastic tissue stain). The medium shows marked fibrous thickening, and the lumen is stenotic. *(Courtesy of Dr. Seymour Rosen, Beth Israel Hospital, Boston, MA.)*

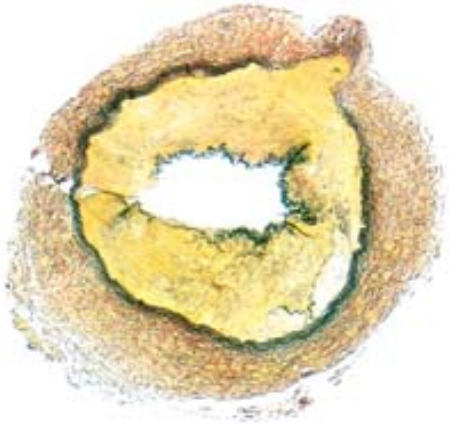


Figure 20-52 Fibrin stain showing platelet-fibrin thrombi (*red*) in the glomerular capillaries, characteristic of microangiopathic disorders.

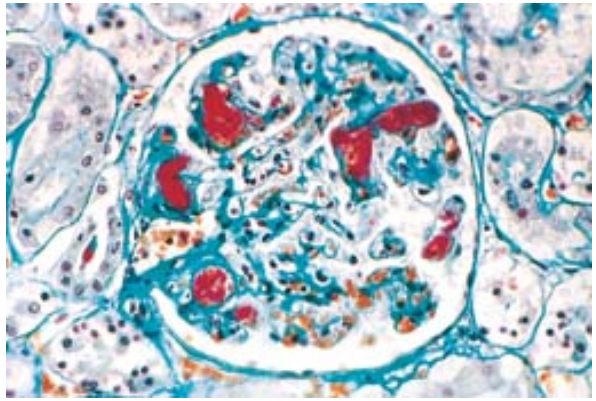


Figure 20-53 Atheroemboli with typical cholesterol clefts in an interlobar artery.

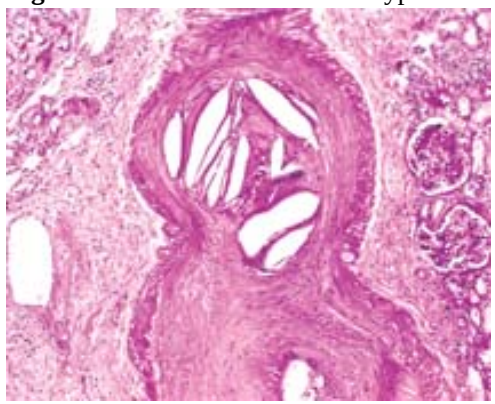


Figure 20-54 Diffuse cortical necrosis. The pale ischemic necrotic areas are confined to the cortex and columns of Bertin.

Figure 20-55 Obstructive lesions of the urinary tract.

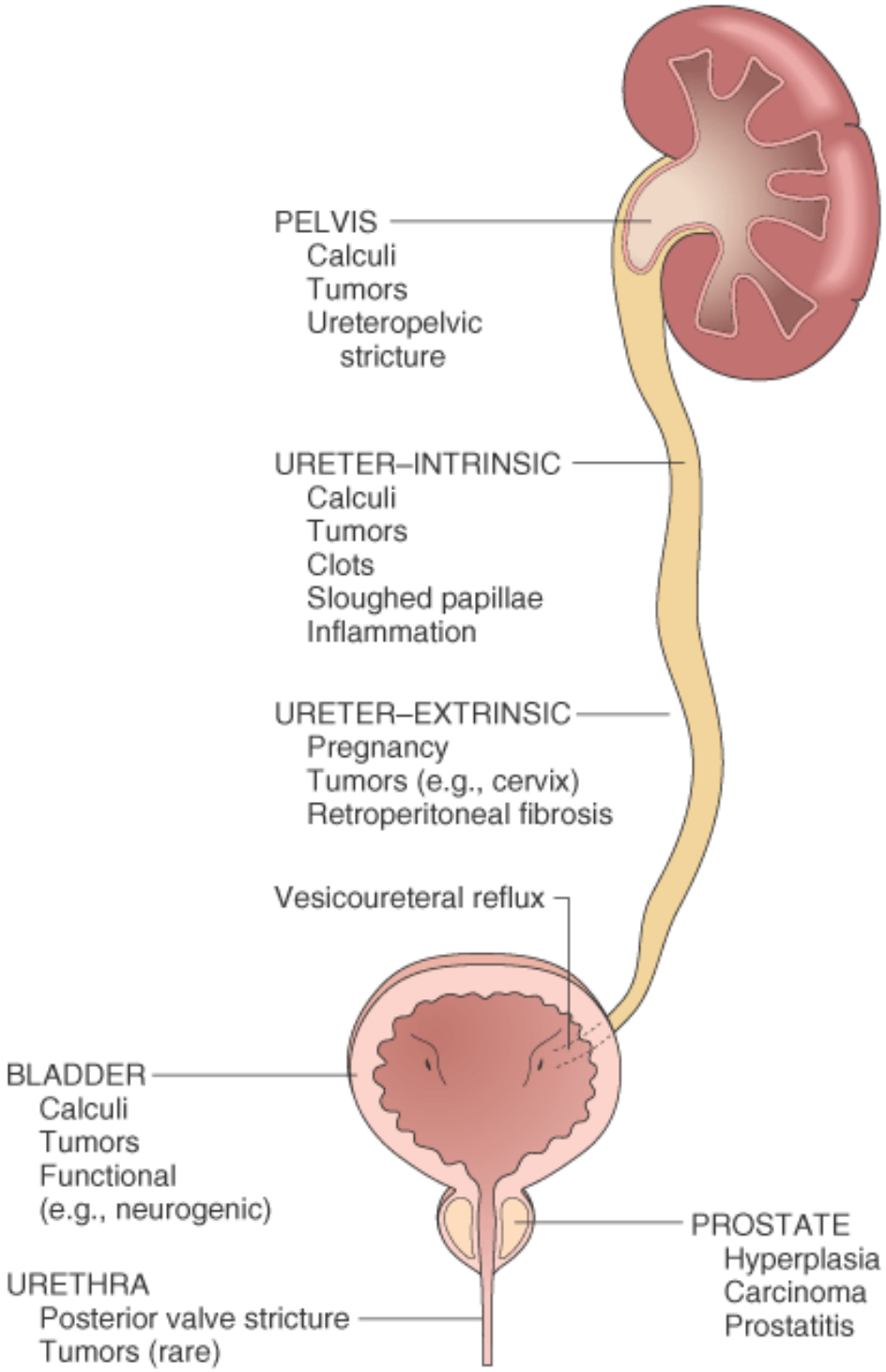

PELVIS
Calculi
Tumors
Ureteropelvic stricture
URETER–INTRINSIC
Calculi
Tumors
Clots
Sloughed papillae
Inflammation
URETER–EXTRINSIC
Pregnancy
Tumors (e.g., cervix)
Retroperitoneal fibrosis
Vesicoureteral reflux
BLADDER
Calculi
Tumors
Functional (e.g., neurogenic)
PROSTATE
Hyperplasia
Carcinoma
Prostatitis
URETHRA
Posterior valve stricture
Tumors (rare)

Figure 20-56 Hydronephrosis of the kidney, with marked dilation of the pelvis and calyces and thinning of the renal parenchyma.

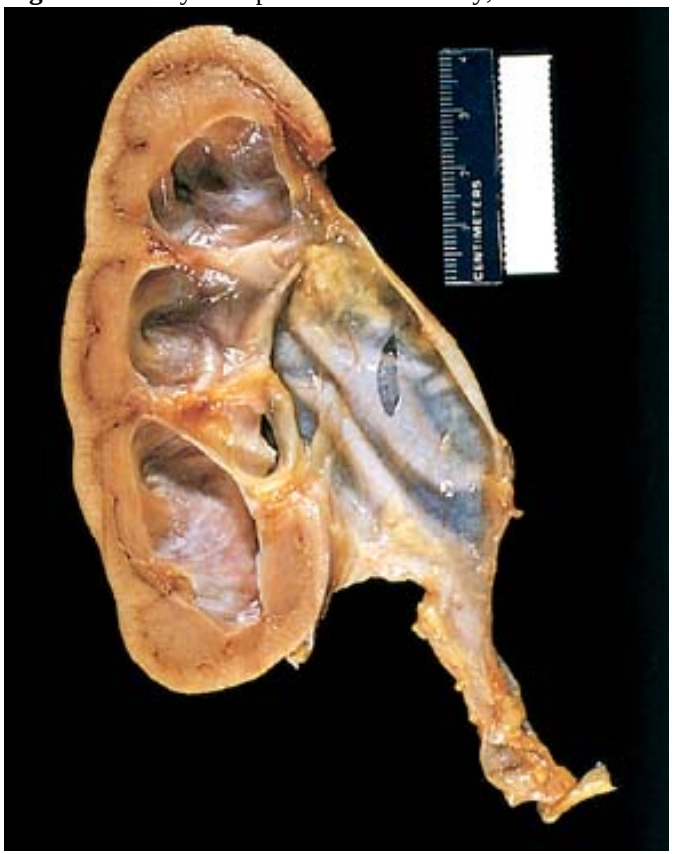


TABLE 20-13 -- Prevalence of Various Types of Renal Stones

	Percentage of All Stones
Calcium Oxalate and Phosphate	70
Idiopathic hypercalciuria (50%)	
Hypercalciuria and hypercalcemia (10%)	
Hyperoxaluria (5%)	
••Enteric (4.5%)	
••Primary (0.5%)	
Hyperuricosuria (20%)	
Hypocitraturia	
No known metabolic abnormality (15–20%)	
Magnesium Ammonium Phosphate (Struvite)	15–20

Uric Acid	5–10
Associated with hyperuricemia	
Associated with hyperuricosuria	
Idiopathic (50% of uric stones)	
Cystine	1–2
Others or Unknown	±5

magnesium ammonium phosphate; (3) *5% to 10% are uric acid stones*; and (4) *1% to 2% are made up of cystine*. An organic matrix of mucoprotein, making up 1% to 5% of the stone by weight, is present in all calculi. Although there are many causes for the initiation and propagation of stones, *the most important determinant is an increased urinary concentration of the stones' constituents, such that it exceeds their solubility in urine (supersaturation)*. *A low urine volume* in some metabolically normal patients may also favor supersaturation.

Calcium oxalate stones (Table 20-13) are associated in about 5% of patients with both *hypercalcemia* and *hypercalciuria*, caused by hyperparathyroidism, diffuse bone disease, sarcoidosis, and other hypercalcemic states. About 55% have *hypercalciuria without hypercalcemia*. This is caused by several factors, including hyperabsorption of calcium from the intestine (absorptive hypercalciuria), an intrinsic impairment in renal tubular reabsorption of calcium (renal hypercalciuria), or idiopathic fasting hypercalciuria with normal parathyroid function. As many as 20% of calcium oxalate stones are associated with increased uric acid secretion (*hyperuricosuric calcium nephrolithiasis*), with or without hypercalciuria. The mechanism of stone formation in this setting involves "nucleation" of calcium oxalate by uric acid crystals in the collecting ducts. Five per cent are associated with *hyperoxaluria*, either hereditary (primary oxaluria) or, more commonly, acquired by intestinal overabsorption in patients with enteric diseases. The latter, so-called enteric hyperoxaluria, also occurs in vegetarians, because much of their diet is rich in oxalates. *Hypocitraturia* associated with acidosis and chronic diarrhea of unknown cause may produce calcium stones. *In a variable proportion of patients with calcium stones*, no cause can be found (idiopathic calcium stone disease).

Magnesium ammonium phosphate stones are formed largely after infections by urea-splitting bacteria (e.g., *Proteus* and some staphylococci), which convert urea to ammonia. The resultant alkaline urine causes the precipitation of magnesium ammonium phosphate salts. These form some of the largest stones, as the amounts of urea excreted normally are huge. Indeed, so-called *staghorn calculi* occupying large portions of the renal pelvis are almost always a consequence of infection.

Uric acid stones are common in patients with hyperuricemia, such as gout, and diseases involving rapid cell turnover, such as the leukemias. However, *more than half of all patients with urate calculi have neither hyperuricemia nor increased urinary excretion of uric acid*. In this group, it is thought that an unexplained tendency to excrete urine of pH below 5.5 may predispose to uric acid stones, because uric acid is insoluble in relatively acidic urine. In contrast to the radio-opaque calcium stones, uric acid stones are radiolucent.

Cystine stones are caused by genetic defects in the renal reabsorption of amino acids, including cystine, leading to cystinuria. Stones form at low urinary pH.

It can therefore be appreciated that increased concentration of stone constituents, changes in urinary pH, decreased urine volume, and the presence of bacteria influence the formation of calculi. *However, many calculi occur in the absence of these factors; conversely, patients with hypercalciuria, hyperoxaluria, and hyperuricosuria often do not form stones.* It has therefore been postulated that stone formation is enhanced by a *deficiency in inhibitors of crystal formation in urine*. The list of such inhibitors is long, including pyrophosphate, diphosphonate,

citrate, glycosaminoglycans, osteopontin, and a glycoprotein called *nephrocalcin*.

Morphology.

Stones are unilateral in about 80% of patients. The favored sites for their formation are within the renal calyces and pelves (Fig. 20-57) and in the bladder. If formed in the renal pelvis, they tend to remain small, having an average diameter of 2 to 3 mm. These may have smooth contours or may take the form of an irregular, jagged mass of spicules. Often, many stones are found within one kidney. On occasion, progressive accretion of salts leads to the development of branching structures known as staghorn stones, which create a cast of the pelvic and calyceal system.

Clinical Course.

Stones are of importance when they obstruct urinary flow or produce ulceration and bleeding. They may be present without producing any symptoms or significant renal damage. In general, smaller stones are most hazardous, because they may pass into the ureters, producing pain referred to as colic (one of the most intense forms of pain) as well as ureteral obstruction. Larger stones cannot enter the ureters and are more likely to remain silent within the renal pelvis. Commonly, these larger stones first manifest themselves by hematuria. Stones also predispose to superimposed infection, both by their obstructive nature and by the trauma they produce.

Tumors of the Kidney

Both benign and malignant tumors occur in the kidney.[108] [109] With the exception of oncocytoma, the benign tumors rarely cause clinical problems. Malignant tumors, on the other hand, are of great importance clinically and deserve considerable emphasis. By far the most common of these malignant tumors is renal cell carcinoma, followed by Wilms tumor, which is found in children and is described in

Figure 20-57 Nephrolithiasis. A large stone impacted in the renal pelvis. *(Courtesy of Dr. E. Mosher, Brigham and Women's Hospital, Boston, MA.)*

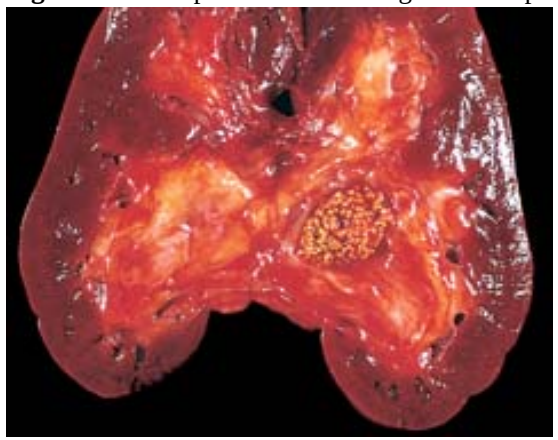


Figure 20-58 Cytogenetics (*blue*) and genetics (*red*) of clear cell versus papillary renal cell carcinoma. *(Courtesy of Dr. Keith Ligon, Brigham and Women's Hospital, Boston, MA.)*

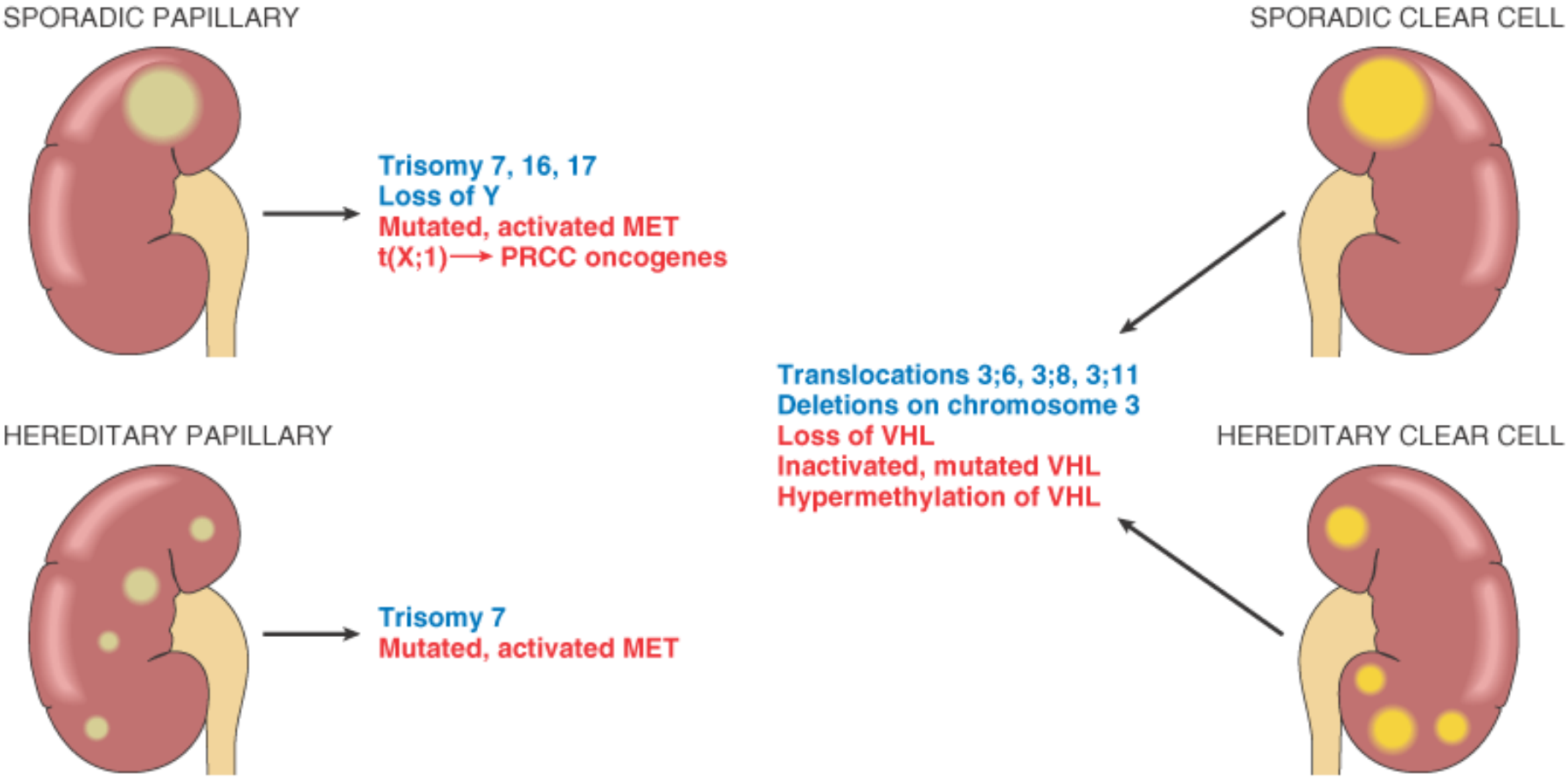


Figure 20-59 Renal cell carcinoma. Typical cross-section of yellowish, spherical neoplasm in one pole of the kidney. Note the tumor in the dilated thrombosed renal vein.

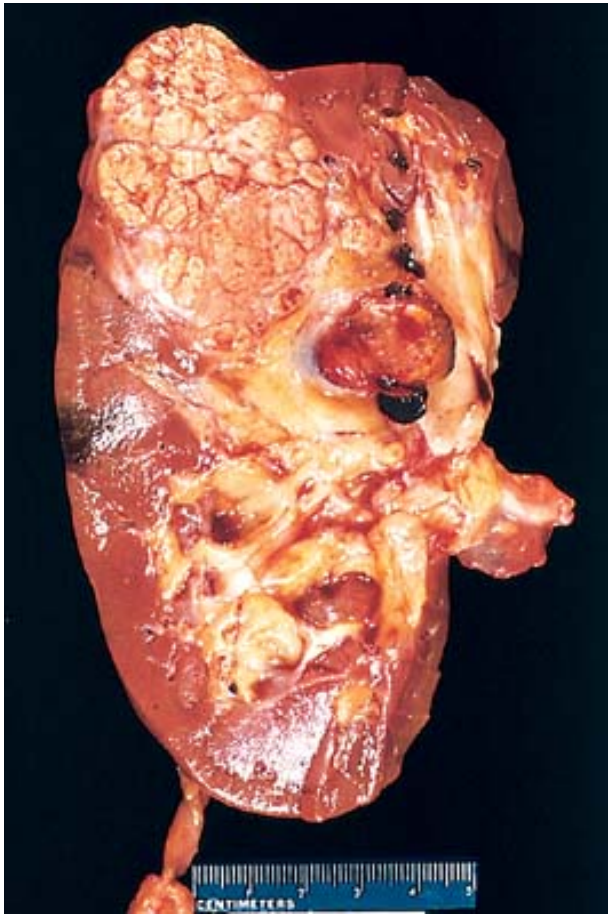


Figure 20-60 Renal cell carcinoma. *A*, Clear cell type, *B*, Papillary type. Note the papillae and foamy macrophages in the stalk. *C*, Chromophobe type. *(Courtesy of Dr. A. Renshaw, Brigham and Women's Hospital, Boston, MA.)*

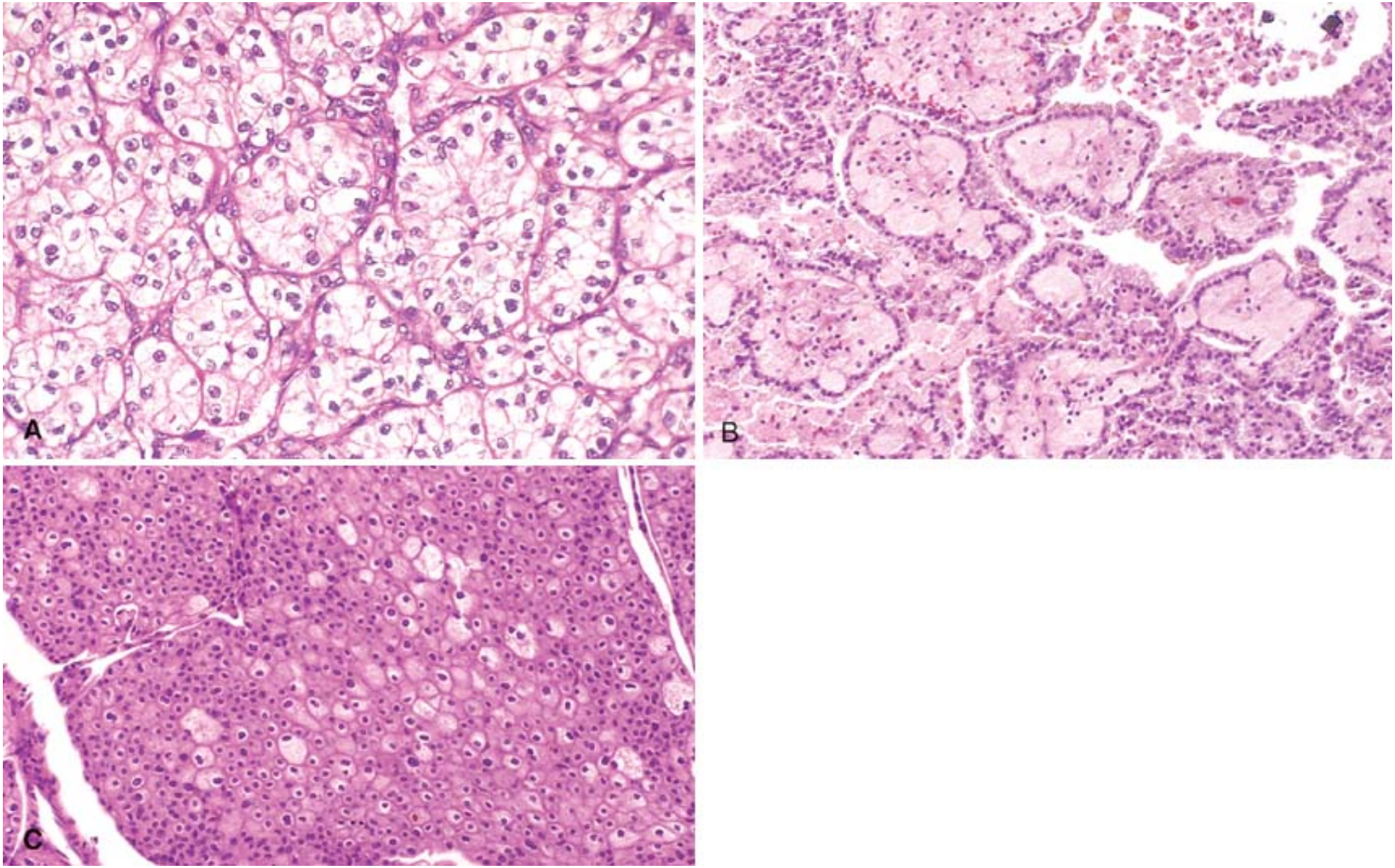
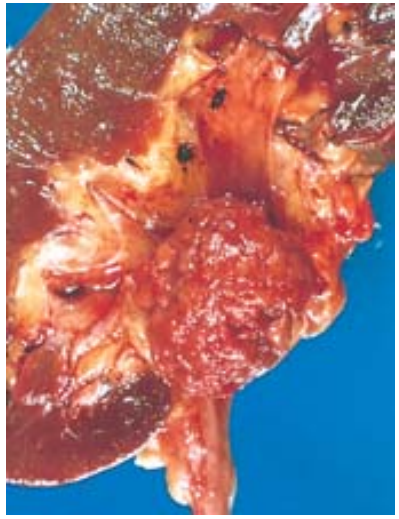


Figure 20-61 Urothelial carcinoma of the renal pelvis. The pelvis has been opened to expose the nodular irregular neoplasm, just proximal to the ureter.



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Chapter 21 - The Lower Urinary Tract and Male Genital System

Jonathan I. Epstein MD

The Lower Urinary Tract

NORMAL

Despite differing embryonic origins, the various components of the lower urinary tract come to have many morphologic similarities. The renal pelves, ureters, bladder, and urethra (except for its terminal portion) are lined by a special form of transitional epithelium (urothelium) that is two to three cells thick in the pelvis, three to five cells thick in the ureters, and three to seven cells thick in the bladder. The surface layer consists of large, flattened "umbrella cells" that cover several underlying cells. The umbrella cells have a trilaminar asymmetric unit membrane and possess apical plaques composed of specific proteins called *uroplakins*. Toward the basal layer, the cells become smaller or more cylindrical (particularly in contracted bladders), but they are capable of some flattening when the underlying wall is stretched. This epithelium rests on a well-developed basement membrane, beneath which there is a lamina

propria. The lamina propria in the urinary bladder contains wisps of smooth muscle that form a discontinuous muscularis mucosae. It is important to differentiate the muscularis mucosae from the deeper well-defined larger muscle bundles of the detrusor muscle (muscularis propria), since bladder cancers are staged on the basis of invasion of the latter. The bladder musculature is capable of great thickening if there is obstruction to the flow of urine.

Several variants of the normal epithelial patterns may be encountered. Nests of urothelium or inbudding of the surface epithelium may be found occasionally in the mucosa lamina propria; these are referred to as *Brunn nests*.

The ureters lie throughout their course in a retroperitoneal position. Retroperitoneal tumors or fibrosis may trap the ureters in neoplastic or dense, fibrous tissue, sometimes obstructing them. As ureters enter the pelvis, they pass anterior to either the common iliac or the external iliac artery. In the female pelvis, they lie close to the uterine arteries and are therefore vulnerable to injury in operations on the female genital tract. There are three points of slight narrowing: at the ureteropelvic junction, where they enter the bladder, and where they cross the iliac vessels, all providing loci where renal calculi may become impacted when they pass from the kidney to the bladder. As the ureters enter the bladder, they pursue an oblique course, terminating in a slitlike orifice. The obliquity of this intramural segment of the ureteral orifice permits the enclosing bladder musculature to act like a sphincteric valve, blocking the upward reflux of urine even in the presence of marked distention of the urinary bladder. As discussed in Chapter 20 , a defect in the intravesical portion of the ureter leads to vesicoureteral reflux. The orifices of the ureters demarcate an area at the base of the bladder known as the trigone. In women, the trigone is frequently covered by glycogenated squamous epithelium, a normal finding, not metaplasia resulting from injury.

The close relationship of the female genital tract to the bladder makes possible the spread of disease from one tract to the other. In middle-aged and elderly women, relaxation of pelvic support leads to prolapse (descent) of the uterus, pulling with it the floor of the bladder. In this fashion, the bladder protrudes into the vagina, creating a pouch (*cystocele*) that fails to empty readily with micturition. In men, the seminal vesicles and prostate have similar close relationships, being situated just posterior and inferior to the neck of the bladder. Thus, enlargement of the prostate, so common in middle to later life, constitutes an important cause of urinary tract obstruction. In the subsequent sections, we discuss the major pathologic lesions in the ureters, urinary bladder, and urethra separately.[1] [2]

Ureters

CONGENITAL ANOMALIES

Congenital anomalies of the ureters occur in about 2% or 3% of all autopsies. Although most have little clinical significance, certain anomalies may contribute to obstruction to the flow of urine and thus cause clinical disease. Anomalies of the ureterovesical junction, potentiating reflux, are discussed with pyelonephritis in Chapter 20 .

Double ureters (derived from a double or split ureteral bud) are almost invariably associated either with totally distinct double renal pelves or with the anomalous development of a large kidney having a partially bifid pelvis terminating in separate ureters. Double ureters may pursue separate courses to the bladder but commonly are joined within the bladder wall and drain through a single ureteral orifice. The majority of double ureters are unilateral and of no clinical significance.

Ureteropelvic junction obstruction, a congenital disorder, results in hydronephrosis. It usually presents in infants or children, much more commonly in boys, usually in the left ureter. However, it is bilateral in 20% of cases and may be associated with other congenital anomalies. *It is the most common cause of hydronephrosis in infants and children.* In adults, ureteropelvic junction obstruction is more common in women and is most often unilateral. There is agenesis of the kidney on the opposite side in a significant number of cases, probably resulting from obstructive lesions in utero.

Diverticula, saccular outpouchings of the ureteral wall, are uncommon lesions that are usually asymptomatic and found incidentally on imaging studies. They appear as congenital or acquired defects and are of importance as pockets of stasis and secondary infections. Dilation (*hydroureter*), elongation, and tortuosity of the ureters may occur as congenital anomalies or as acquired defects. Congenital hydroureter is thought to reflect some neurogenic defect in the innervation of the ureteral musculature. Massive enlargement of the ureter is known as *megaloureter* and is probably due to a functional defect of ureteral muscle. Hydronephrosis and decreased renal

function results if the lesion goes untreated. These anomalies are sometimes associated with some congenital defect of the kidney.

INFLAMMATIONS

Ureteritis may develop as one component of urinary tract infections. The morphologic changes are entirely nonspecific. Only infrequently does such ureteritis make a significant contribution to the clinical problem. Persistence of infection or repeated acute exacerbations may give rise to chronic inflammatory changes within the ureters.

Morphology.

In certain cases of long-standing chronic ureteritis, specialized reaction patterns are sometimes observed. The accumulation or aggregation of lymphocytes in the subepithelial region may cause slight elevations of the mucosa and produce a fine granular mucosal surface (**ureteritis follicularis**). At other times, the mucosa may become sprinkled with fine cysts varying in diameter from 1 to 5 mm (**ureteritis cystica**). These changes are also found in the bladder (described in greater detail later, in the section on the urinary bladder). The cysts may aggregate to form small, grapelike clusters (Fig. 21-1). Histologic sections through such cysts demonstrate a lining of modified transitional epithelium with some flattening of the superficial layer of cells.

TUMORS AND TUMOR-LIKE LESIONS

Primary neoplasia of the ureter is rare. Small *benign tumors* of the ureter are generally of mesenchymal origin. The two most common are fibroepithelial polyps and leiomyomas. The *fibroepithelial polyp* is a tumor-like lesion that grossly presents as a small mass projecting into the lumen. The lesion occurs more commonly in the ureters (left more often than right) but may also appear in the bladder, renal pelves, and urethra. The polyp presents as a loose, vascularized connective tissue mass lying beneath the mucosa.

Primary *malignant tumors* of the ureter follow patterns similar to those arising in the renal pelvis, calyces, and bladder, and the majority are transitional cell carcinomas (Fig. 21-2). They

Figure 21-1 Opened ureters showing ureteritis cystica. Note the smooth cysts projecting from the mucosa.

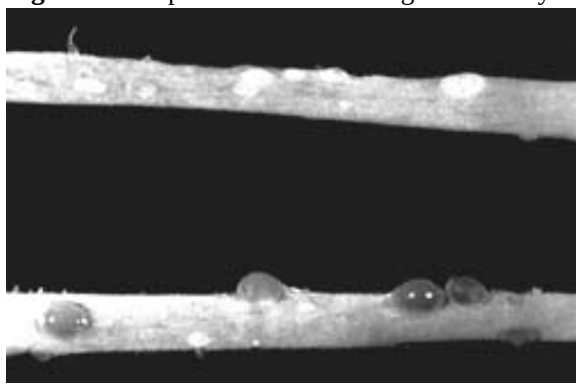


Figure 21-2 Papillary transitional cell carcinoma extensively involving the ureter. *(Courtesy of Dr. Cristina Magi-Galluzzi, The Johns Hopkins Hospital, Baltimore, MD.)*

TABLE 21-1 -- Major Causes of Ureteral Obstruction

Intrinsic	
Calculi	Of renal origin, rarely more than 5 mm in diameter
	Larger renal stones cannot enter ureters
	Impact at loci of ureteral narrowing—ureteropelvic junction, where ureters cross iliac vessels, and where they enter bladder—and cause excruciating "renal colic"
Strictures	Congenital or acquired (inflammations)
Tumors	Transitional cell carcinomas arising in ureters
	Rarely, benign tumors or fibroepithelial polyps
Blood clots	Massive hematuria from renal calculi, tumors, or papillary necrosis
Neurogenic	Interruption of the neural pathways to the bladder
Extrinsic	
Pregnancy	Physiologic relaxation of smooth muscle or pressure on ureters at pelvic brim from enlarging fundus
Periureteral inflammation	Salpingitis, diverticulitis, peritonitis, sclerosing retroperitoneal fibrosis
Endometriosis	With pelvic lesions, followed by scarring
Tumors	Cancers of the rectum, bladder, prostate, ovaries, uterus, cervix, lymphomas, sarcomas

typically results from proximal causes, whereas bilateral obstruction arises from distal causes, such as nodular hyperplasia of the prostate. Only sclerosing retroperitoneal fibrosis is discussed further.

Sclerosing Retroperitoneal Fibrosis.

This refers to an uncommon cause of ureteral narrowing or obstruction characterized by a *fibrous proliferative inflammatory process encasing the retroperitoneal structures and causing hydronephrosis*.[3] The disorder occurs in middle to late age. In some cases, specific causes can be identified, such as drugs (ergot derivatives, β-adrenergic blockers), adjacent inflammatory conditions (vasculitis, diverticulitis, Crohn disease), or malignant disease (lymphomas, urinary tract carcinomas). However, 70% of cases have no obvious cause and are considered primary or idiopathic (Ormond disease). Several cases have been reported with similar fibrotic changes in other sites (referred to as mediastinal fibrosis, sclerosing cholangitis, and Riedel fibrosing thyroiditis), suggesting that the disorder is systemic in distribution but preferentially involves the retroperitoneum. Thus, an autoimmune reaction, sometimes triggered by drugs, has been proposed.

On microscopic examination, the inflammatory fibrosis is marked by a prominent inflammatory infiltrate of lymphocytes, often with germinal centers, plasma cells, and eosinophils. Sometimes, foci of fat necrosis and granulomatous inflammation are seen in and about the fibrosis.

Urinary Bladder

Diseases of the bladder, particularly inflammation (cystitis), constitute an important source of clinical signs and symptoms. Usually, however, these disorders are more disabling than lethal. Cystitis is particularly common in young women of reproductive age and in older age groups of both sexes. Tumors of the bladder are an important source of both morbidity and mortality.

CONGENITAL ANOMALIES

Diverticula.

A bladder or vesical diverticulum consists of a pouchlike eversion or evagination of the bladder wall. Diverticula may arise as congenital defects but more commonly are acquired lesions from persistent urethral obstruction.

Congenital diverticula may be due to a focal failure of development of the normal musculature or to some urinary tract obstruction during fetal development. *Acquired diverticula* are most often seen with prostatic enlargement (hyperplasia or neoplasia), producing obstruction to urine outflow and marked muscle thickening of the bladder wall. The increased intravesical pressure causes outpouching of the bladder wall and the formation of diverticula. They are frequently multiple and have narrow necks located between the interweaving hypertrophied muscle bundles. In both the congenital and acquired forms, the diverticulum usually consists of a round to ovoid, saclike pouch that varies from less than 1 cm to 5 to 10 cm in diameter.

Although most diverticula are small and asymptomatic, they may be clinically significant, as they constitute sites of urinary stasis and predispose to infection and the formation of bladder calculi. They may also predispose to vesicoureteral reflux as a result of impingement on the ureter. Rarely, carcinomas may arise in bladder diverticuli. When invasive cancers arise in diverticula, they tend to be more advanced in stage as a result of diverticula's thin or absent muscle wall.

Exstrophy.

Exstrophy of the bladder implies the presence of a developmental failure in the anterior wall of the abdomen and in the bladder, so that the bladder either communicates directly through a large defect with the surface of the body or lies as an opened sac (Fig. 21-3). These lesions are amenable to surgical correction, and long-term survival is possible. The exposed bladder mucosa may undergo colonic glandular metaplasia and is subject to the development of infections that often spread to upper levels of the urinary system. In the course of persistent chronic infections, the mucosa often becomes converted into an ulcerated surface of granulation tissue, and the preserved marginal epithelium becomes transformed into a stratified squamous type. There is an increased tendency toward the development of carcinoma later in life, mostly adenocarcinoma of the colon.[4] Patients also have an increased risk of adenocarcinoma arising from the bladder remnant.

Miscellaneous Anomalies.

Vesicoureteral reflux is the most common and serious anomaly. As a major contributor to renal infection and scarring, it was discussed earlier in Chapter 20 in the consideration of pyelonephritis. Abnormal connections between the bladder and the vagina, rectum, or uterus may create *congenital fistulas*.

Rarely, the *urachus* may remain patent in part or in whole (persistent urachus). When it is totally patent, a fistulous urinary tract is created that connects the bladder with the umbilicus. At times, the umbilical end or the bladder end remains patent, while the central region is obliterated. A sequestered umbilical epithelial rest or bladder diverticulum is formed that may provide a site for the development of infection. At other times, only the central region of the urachus persists, giving rise to *urachal cysts*, lined by either transitional or metaplastic epithelium. *Carcinomas*, mostly glandular tumors resembling colonic adenocarcinomas, may arise in such cysts. These account for only a minority of all bladder cancers (0.1% to 0.3%) but 20% to 40% of bladder adenocarcinomas.[2]

Figure 21-3 Exstrophy of the bladder in a newborn boy. The tied umbilical cord is seen above the hyperemic mucosa of the everted bladder. Below is an incompletely formed penis with marked epispadias. *(Courtesy of Dr. John Gearhart, The Johns Hopkins Hospital, Baltimore, MD.)*

Figure 21-4 Cystitis with malacoplakia of bladder showing inflammatory exudate and broad, flat plaques.

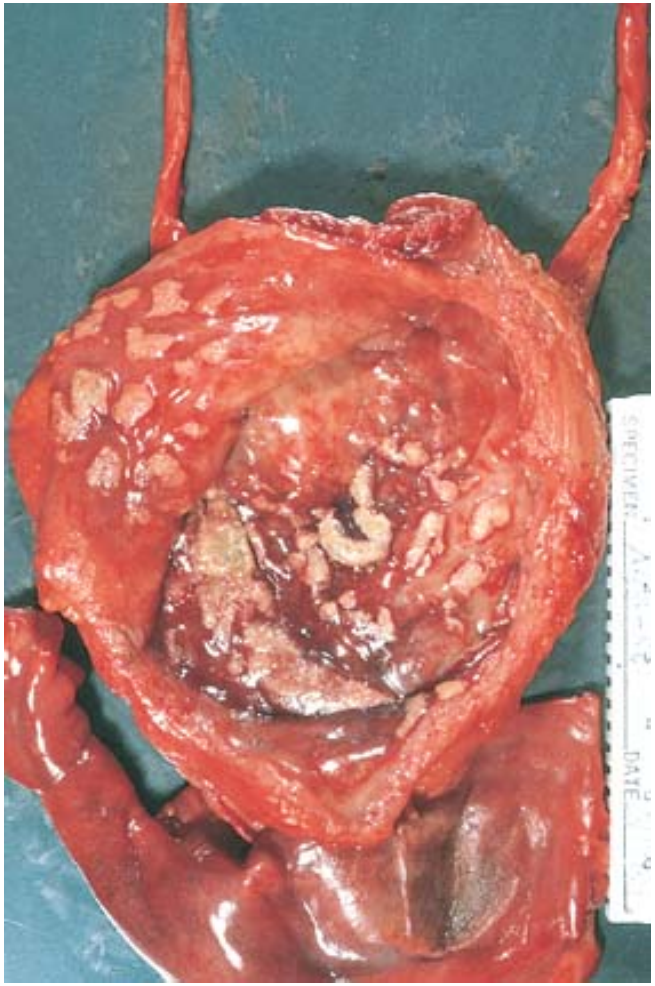


Figure 21-5 Malacoplakia, PAS stain. Note the large macrophages with granular PAS-positive cytoplasm and several dense, round Michaelis-Gutmann bodies surrounded by artifactual cleared holes in the upper middle field.

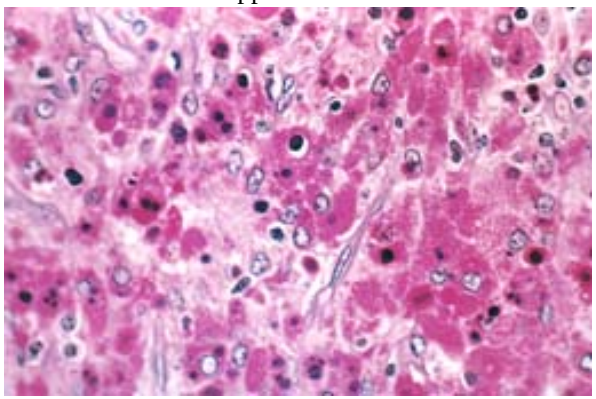


TABLE 21-2 -- Tumors of the Urinary Bladder

Urothelial (transitional cell) tumors
••Inverted papilloma
••Papilloma (exophytic)
••Urothelial tumors of low malignant potential
••Papillary urothelial carcinoma
••Carcinoma in situ
Squamous cell carcinoma
Mixed carcinoma
Adenocarcinoma
Small cell carcinoma
Sarcomas

described below may be seen at any site where there is urothelium, from the renal pelvis to the distal urethra.

There are two distinct precursor lesions to invasive urothelial carcinoma. The more common are noninvasive papillary tumors, which appear to arise from papillary urothelial hyperplasia.[14] These lesions demonstrate a range of atypia, and several grading systems exist to reflect their biologic behavior. The other precursor lesion is flat urothelial carcinoma, which is simply referred to as carcinoma in situ (CIS). This lesion is by definition high grade and hence not assigned a grade. In about half the patients with invasive bladder cancer, at the time of presentation the tumor has already invaded the bladder wall, and there is no associated precursor lesion. In these cases, it is presumed that the precursor lesion has been destroyed by the high-grade invasive component, which typically appears as a large mass that is often ulcerated. Although invasion into the lamina propria worsens the prognosis, the major decrease in survival is associated with tumor invading the muscularis propria (detrusor muscle). Once muscularis propria invasion occurs, there is a 50% 5-year mortality rate.

Table 21-3 lists two of many systems of grading these tumors.[15] [16] [17] [18] The World Health Organization (WHO) 1973 classification grades tumors into a rare totally benign papilloma and three grades of transitional cell carcinoma (grades I, II, and III). A more recent classification, based on a consensus

TABLE 21-3 -- Grading of Urothelial (Transitional Cell) Tumors

WHO/ISUP Grades *
Urothelial papilloma
Urothelial neoplasm of low malignant potential
Papillary urothelial carcinoma, low grade
Papillary urothelial carcinoma, high grade
WHO Grades †
Urothelial papilloma

Urothelial neoplasm of low malignant potential
Papillary urothelial carcinoma, Grade 1
Papillary urothelial carcinoma, Grade 2
Papillary urothelial carcinoma, Grade 3
WHO, World Health Organization; ISUP, International Society of Urological Pathology.

*Adopted as the WHO System in 2004.
†The 1973 WHO grades.

Figure 21-6 Four morphologic patterns of bladder tumors.

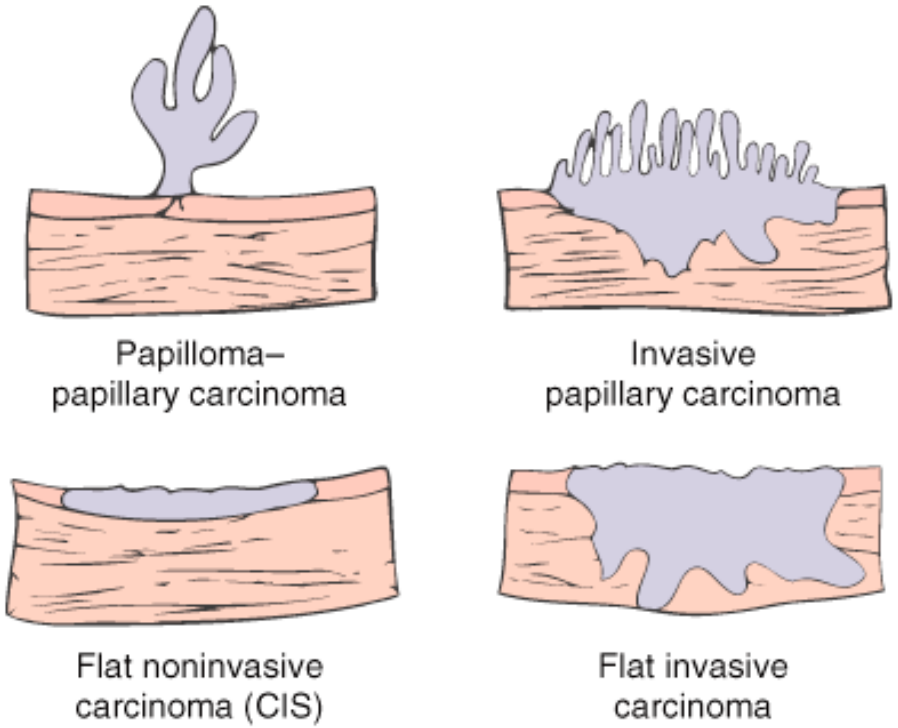


Figure 21-7 Cross-section of bladder with upper section showing a large papillary tumor. The lower section demonstrates multifocal smaller papillary neoplasms. *(Courtesy of Dr. Fred Gilkey, Sinai Hospital, Baltimore, MD.)*

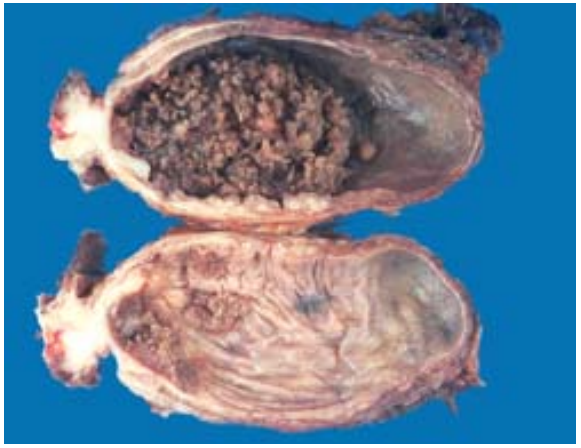


Figure 21-8 Papilloma consisting of small papillary fronds lined by normal-appearing urothelium.

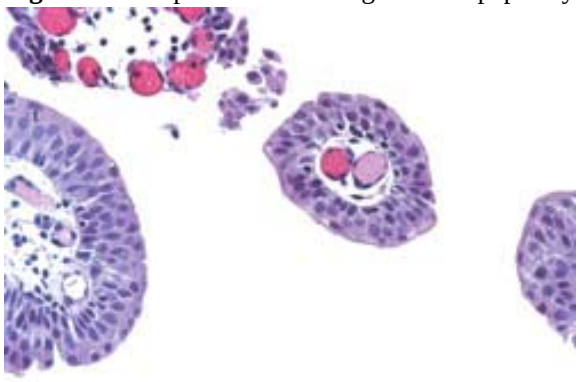


Figure 21-9 Low-grade papillary urothelial carcinoma with an overall orderly appearance, a thicker lining than papilloma, and scattered hyperchromatic nuclei and mitotic figures (*arrows*).

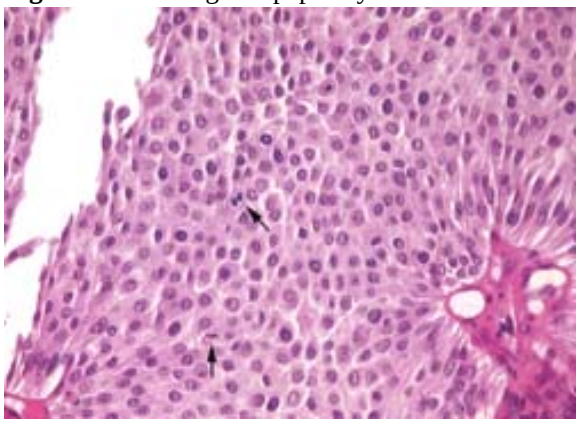


Figure 21-10 High-grade papillary urothelial carcinoma with marked cytologic atypia.

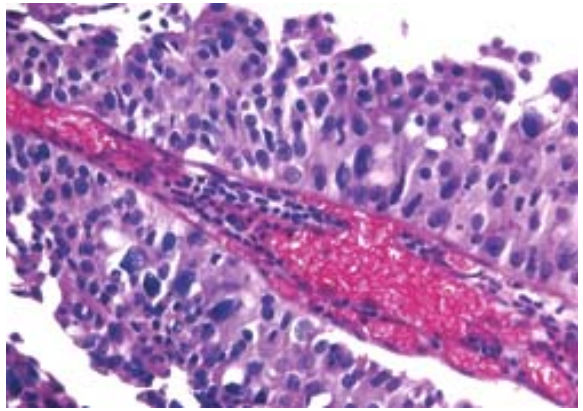


Figure 21-11 *A*, Normal urothelium with uniform nuclei and well-developed umbrella cell layer. *B*, Flat carcinoma in situ with numerous cells having enlarged and pleomorphic nuclei.

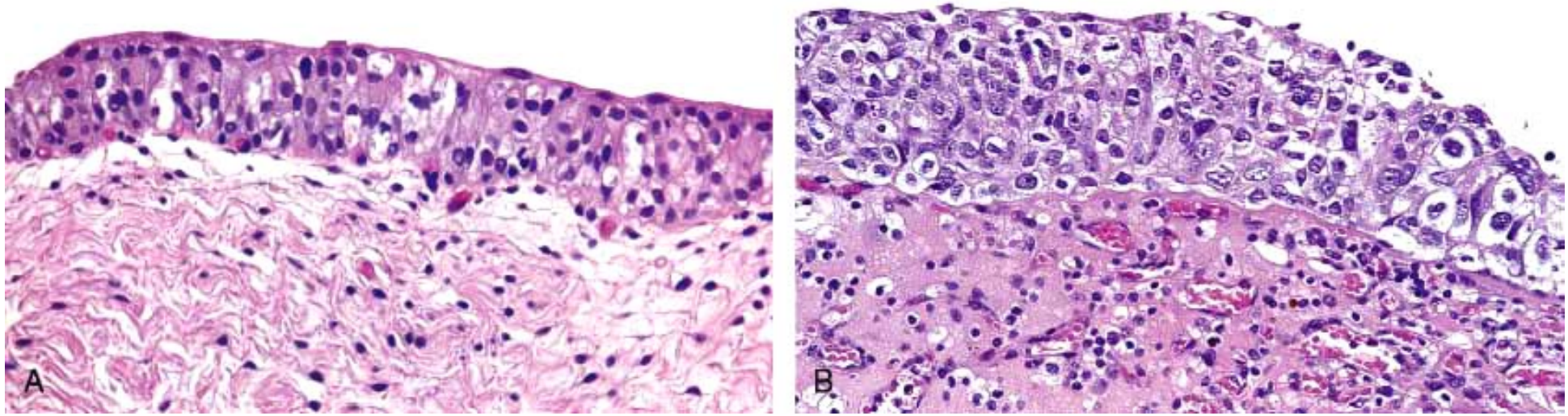


TABLE 21-4 -- Pathologic T (Primary Tumor) Staging of Bladder Carcinoma

AJCC/UICC	Depth of Invasion
Noninvasive, papillary	Ta
Carcinoma in situ (noninvasive, flat)	Tis
Lamina propria invasion	T1
Muscularis propria invasion	T2
Microscopic extravesicle invasion	T3a
Grossly apparent extravesicle invasion	T3b
Invades adjacent structures	T4

AJCC/UICC, American Joint Commission on Cancer/Union Internationale Contre le Cancer.

carcinomas with areas of squamous carcinoma are more frequent than pure squamous cell carcinomas. Most are invasive, fungating tumors or infiltrative and ulcerative. True papillary patterns are almost never seen. The level of cytologic differentiation varies widely, from the highly differentiated lesions producing abundant keratohyaline pearls to anaplastic giant

Figure 21-12 Opened bladder showing a high-grade invasive urothelial cell carcinoma at an advanced stage. The aggressive multinodular neoplasm has fungated into the bladder lumen and spread over a wide area. The yellow areas represent areas of ulceration and necrosis.

Figure 21-13 Hypertrophy and trabeculation of bladder wall secondary to polypoid hyperplasia of the prostate.

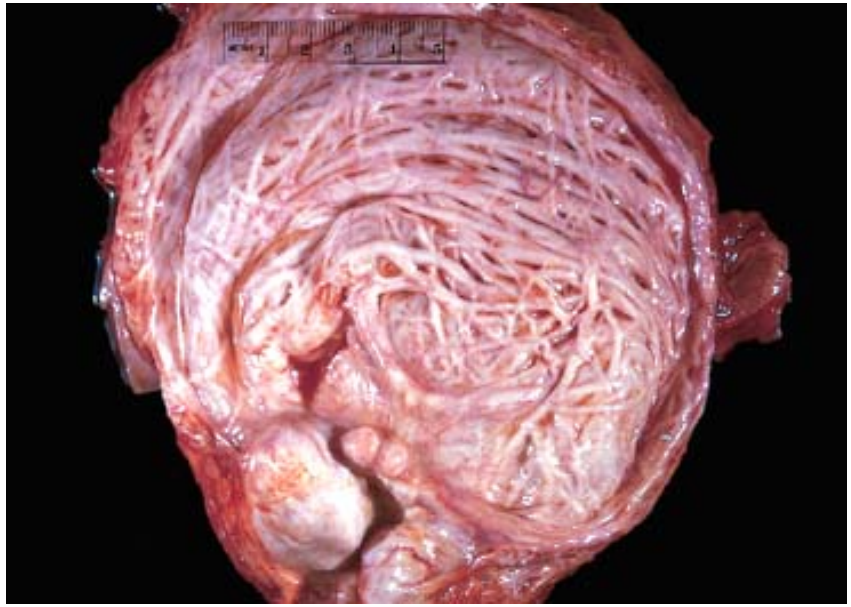


Figure 21-14 Carcinoma of urethra with typical fungating growth.

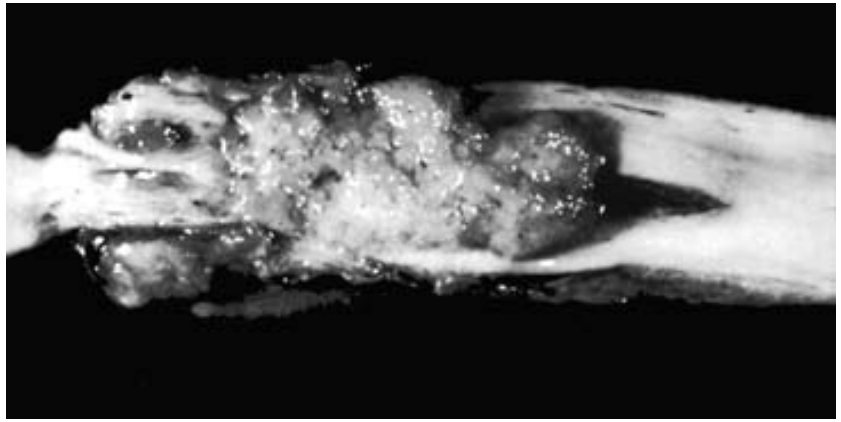


Figure 21-15 Condyloma acuminatum of the penis.

Figure 21-16 Condyloma acuminatum of the penis. Low magnification reveals the papillary (villous) architecture.

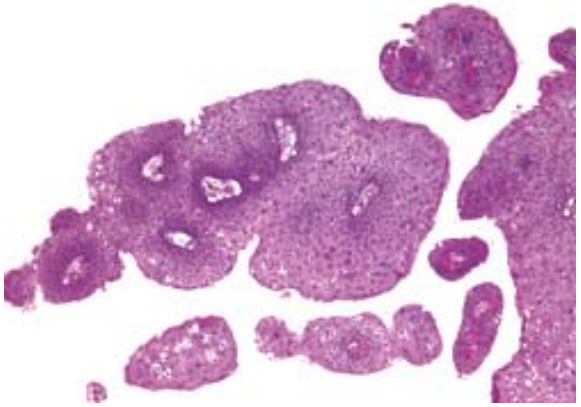


Figure 21-17 Condyloma acuminatum of the penis. The epithelium shows vacuolization (koilocytosis), characteristic of human papillomavirus (HPV) infection.

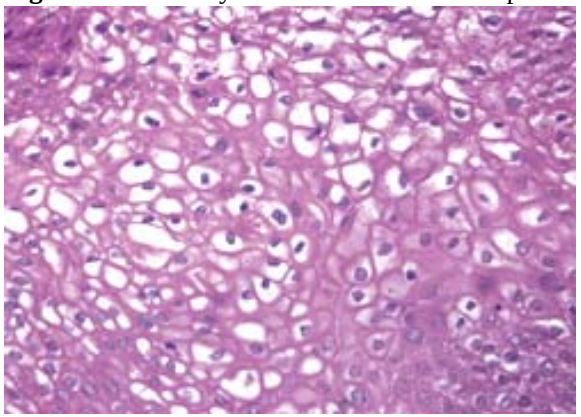


Figure 21-18 Bowen disease (carcinoma in situ) of the penis. The epithelium above the intact basement membrane (*not seen in this picture*) shows hyperchromatic, dysplastic dyskeratotic epithelial cells with scattered mitoses above the basal layer.

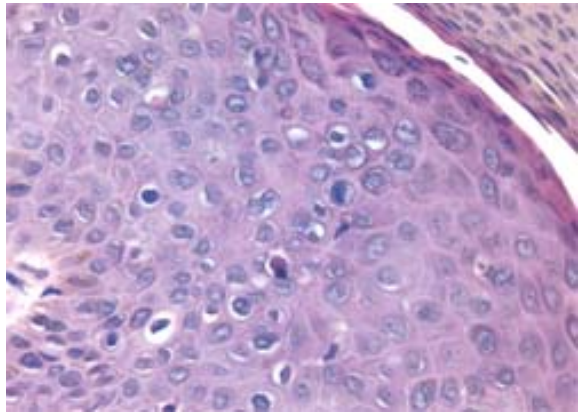


Figure 21-19 Carcinoma of the penis. The glans penis is deformed by a firm, ulcerated, infiltrative mass.

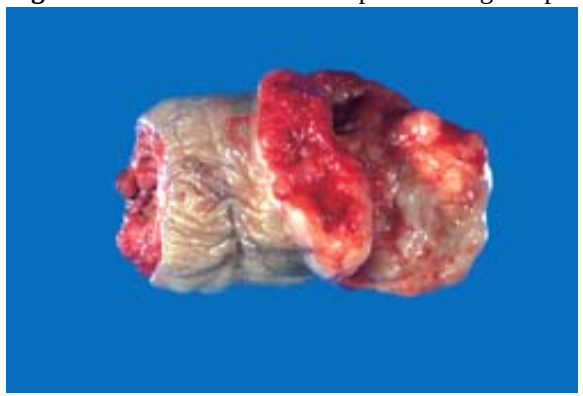


Figure 21-20 *A*, Normal testis shows tubules with active spermatogenesis. *B*, Testicular atrophy. The tubules show Sertoli cells but no spermatogenesis. There is thickening of basement membranes and an apparent increase in interstitial Leydig cells.

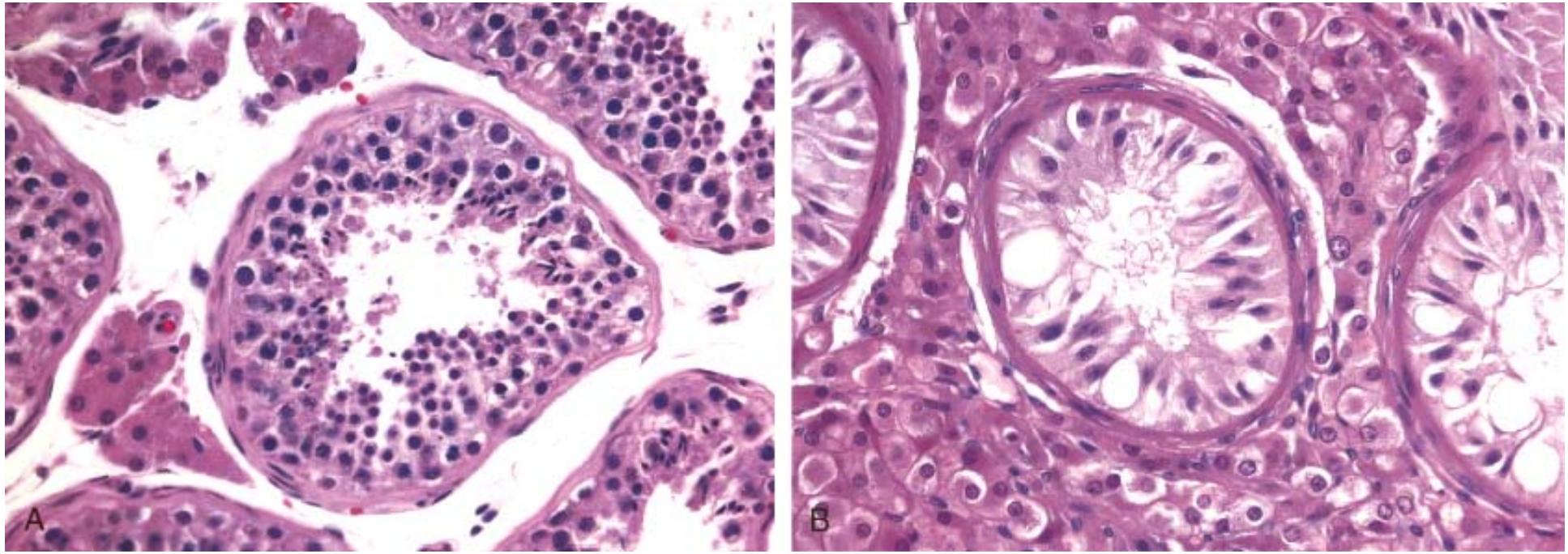


Figure 21-21 Acute epididymitis caused by gonococcal infection. The epididymis is replaced by an abscess. A normal testis is seen on the right.

Figure 21-22 Torsion of the testis.

TABLE 21-5 -- Pathologic Classification of Common Testicular Tumors

Germ Cell Tumors
Seminoma
Spermatocytic seminoma
Embryonal carcinoma
Yolk sac (endodermal sinus) tumor
Choriocarcinoma
Teratoma
Sex Cord-Stromal Tumors
Leydig cell tumor
Sertoli cell tumor

Most tumors in this group originate from intratubular germ cell neoplasia (ITGCN).[74] [75] ITGCN is seen adjacent to all germ cell tumors in adults except for spermatocytic seminoma and epidermoid and dermoid cysts. With rare exceptions, it is also not seen in pediatric tumors (teratomas, yolk sac tumors). ITCGN is encountered with a high frequency in the following conditions, listed in order of increasing risk: cryptorchidism, prior germ cell tumors, strong family history of germ cell tumor, androgen insensitivity syndrome, and gonadal dysgenesis syndrome. Untreated ITGCN progresses to invasive germ cell tumor in approximately 50% of cases over 5 years of follow-up. Thus its significance is similar to carcinoma in situ in other organs. If ITGCN is identified, it is treated by low-dose radiotherapy, which destroys the germ cells yet maintains the androgen production of the Leydig cells.

Neoplastic germ cells may differentiate along gonadal lines to give rise to *seminoma* or transform into a totipotential cell population that gives rise to *nonseminomatous tumors*. Such totipotential cells may remain largely undifferentiated to form embryonal carcinoma or may differentiate along extraembryonic lines to form *yolk sac tumors* or *choriocarcinomas*. Teratoma results from differentiation of the embryonic carcinoma cells along the lines of all three germ cell layers. Some studies suggest that seminomas are not end-stage neoplasms.

Similar to embryonal carcinomas, seminomas may also act as precursors from which other forms of testicular germ cell tumors originate. This view is supported by the fact that cells that form intratubular germ cell neoplasias (the presumed precursors of all types of germ cell tumors) share morphologic and molecular characteristics with tumor cells in seminomas. Despite the fascination of pathologists with the heterogeneity of testicular tumors, from a *clinical standpoint the most important distinction in germ cell tumors is between seminomas and nonseminomatous tumors*. As will be discussed later, this clinical distinction has important bearings on treatment and prognosis.

Pathogenesis

As with all neoplasms, little is known about the ultimate cause of germ cell tumors. Several predisposing influences, however, are important: (1) cryptorchidism, (2) testicular dysgenesis, and (3) genetic factors, all of which may contribute to a common denominator: germ cell maldevelopment. Reference has already been made to the increased incidence of neoplasms in *undescended testes*. In most large series of testicular tumors, approximately 10% are associated with cryptorchidism. The higher the location of the undescended testicle (intra-abdominal versus inguinal), the greater is the risk of developing cancer.

Patients with disorders of testicular development (testicular dysgenesis), including testicular feminization and Klinefelter syndrome, harbor an increased risk of developing germ cell tumors. The risk is highest in patients with testicular feminization. In cryptorchid and dysgenetic testes, foci of intratubular germ cell neoplasms can be detected at a high frequency before the development of invasive tumors.

Genetic predisposition also seems to be important, although no well-defined pattern of inheritance has been identified. In support, striking racial differences in the incidence of testicular tumors can be cited. Blacks in Africa have an extremely low incidence of these neoplasms, which is unaffected by migration to the United States. Familial clustering has been reported, and according to one study, sibs of affected individuals have a tenfold higher risk of developing testicular cancer than does the general population.

As with all tumors, genomic changes are undoubtedly important in the pathogenesis of testicular cancers. An *isochromosome of the short arm of chromosome 12*, i(*12p*), *is found in virtually all germ cell tumors*, regardless of their histologic type. In the approximately 10% of cases in which i(12p) is not detected, extra genetic material derived from 12p is found on other chromosomes. Obviously, dosage of genes located on 12p is critical for the pathogenesis of germ cell tumors, and several candidate genes have been identified, including a novel gene, called *DAD-R*, that prevents apoptosis.[76] [77] It is of interest that i(12p) is also noted in ovarian germ cell neoplasms, suggesting that the events leading to this alteration may be critical to the molecular pathogenesis of all germ cell neoplasms.

With this background of pathogenesis, we can discuss the morphologic patterns of germ cell tumors, followed by the clinical features that are common to most germinal tumors.

Seminoma

Seminomas are the most common type of germinal tumor (50%) and the type most likely to produce a uniform population of cells. They almost never occur in infants; they peak in the thirties. An identical tumor arises in the ovary, where it is called *dysgerminoma* (Chapter 22).

Morphology.

If not otherwise specified, the term "seminoma" refers to "classic" or "typical" seminoma. Spermatocytic seminoma, despite its nosologic similarity, is actually a distinct tumor; it has been segregated into a separate category and will be discussed later.

Seminomas produce bulky masses, sometimes 10 times the size of the normal testis. The typical seminoma has a homogeneous, gray-white, lobulated cut surface, usually devoid of hemorrhage or necrosis (Fig. 21-23). In more than half of cases, the entire testis is replaced. Generally, the tunica albuginea is not penetrated, but occasionally, extension to the epididymis, spermatic cord, or scrotal sac occurs.

Microscopically, the typical seminoma presents sheets of uniform cells divided into poorly demarcated lobules by delicate septa of fibrous tissue (Fig. 21-24*A*). **The classic seminoma**

cell is large and round to polyhedral and has a distinct cell membrane; a clear or watery-appearing cytoplasm; and a large,

---

Figure 21-23 Seminoma of the testis appears as a fairly well circumscribed, pale, fleshy, homogeneous mass.

Figure 21-24 Seminoma. *A*, Low magnification shows clear seminoma cells divided into poorly demarcated lobules by delicate septa. *B*, Microscopic examination reveals large cells with distinct cell borders, pale nuclei, prominent nucleoli, and a sparse lymphocytic infiltrate.

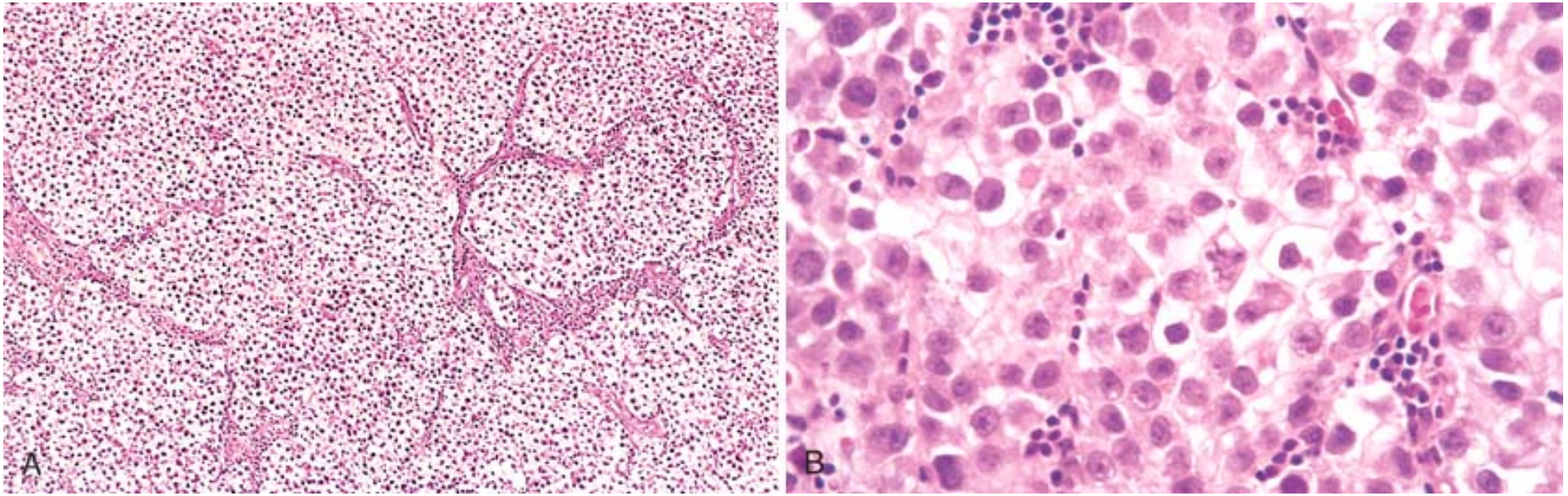


Figure 21-25 Embryonal carcinoma. In contrast to the seminoma illustrated in Figure 21-23 , the embryonal carcinoma is a hemorrhagic mass.

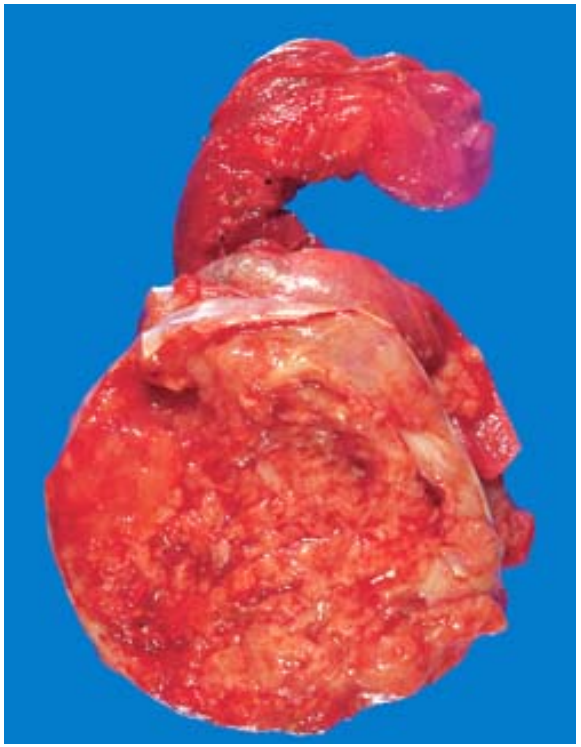


Figure 21-26 Embryonal carcinoma shows sheets of undifferentiated cells as well as primitive glandular differentiation. The nuclei are large and hyperchromatic.

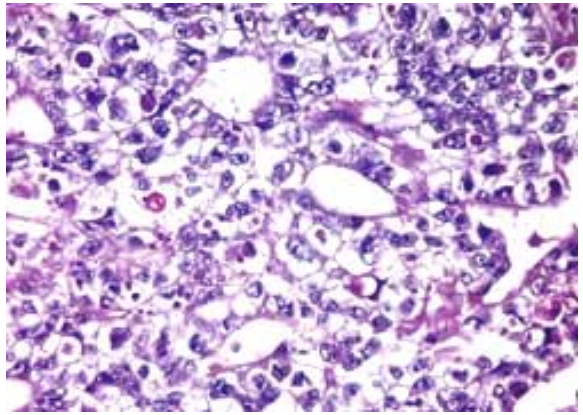


Figure 21-27 Choriocarcinoma shows clear cytotrophoblastic cells with central nuclei and syncytiotrophoblastic cells with multiple dark nuclei embedded in eosinophilic cytoplasm. Hemorrhage and necrosis are prominent.

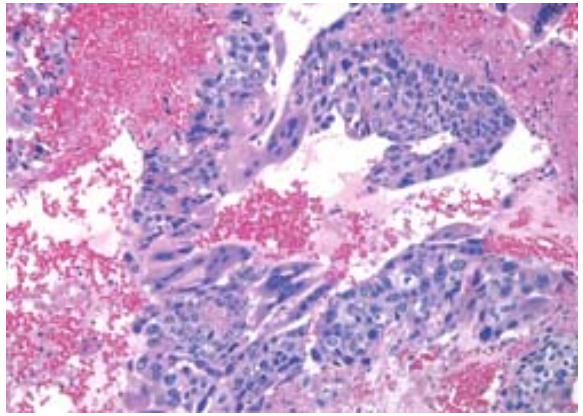


Figure 21-28 Teratoma of the testis. The variegated cut surface with cysts reflects the multiplicity of tissue found histologically.

Figure 21-29 Teratoma of the testis consisting of a disorganized collection of glands, cartilage, smooth muscle, and immature stroma.

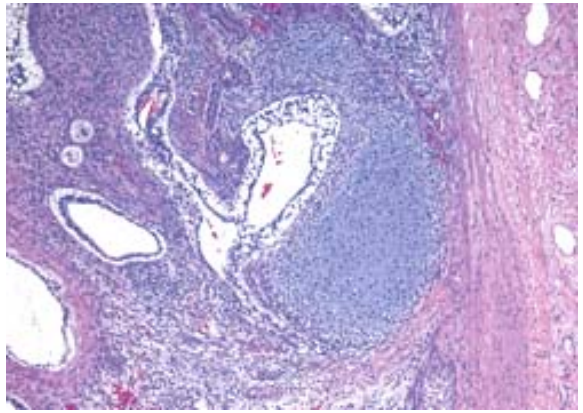


Figure 21-30 Adult prostate. The normal prostate contains several distinct regions, including a central zone (CZ), a peripheral zone (PZ), a transitional zone (TZ), and a periurethral zone. Most carcinomas arise from the peripheral glands of the organ and may be palpable during digital examination of the rectum. Nodular hyperplasia, in contrast, arises from more centrally situated glands and is more likely to produce urinary obstruction early on than is carcinoma.

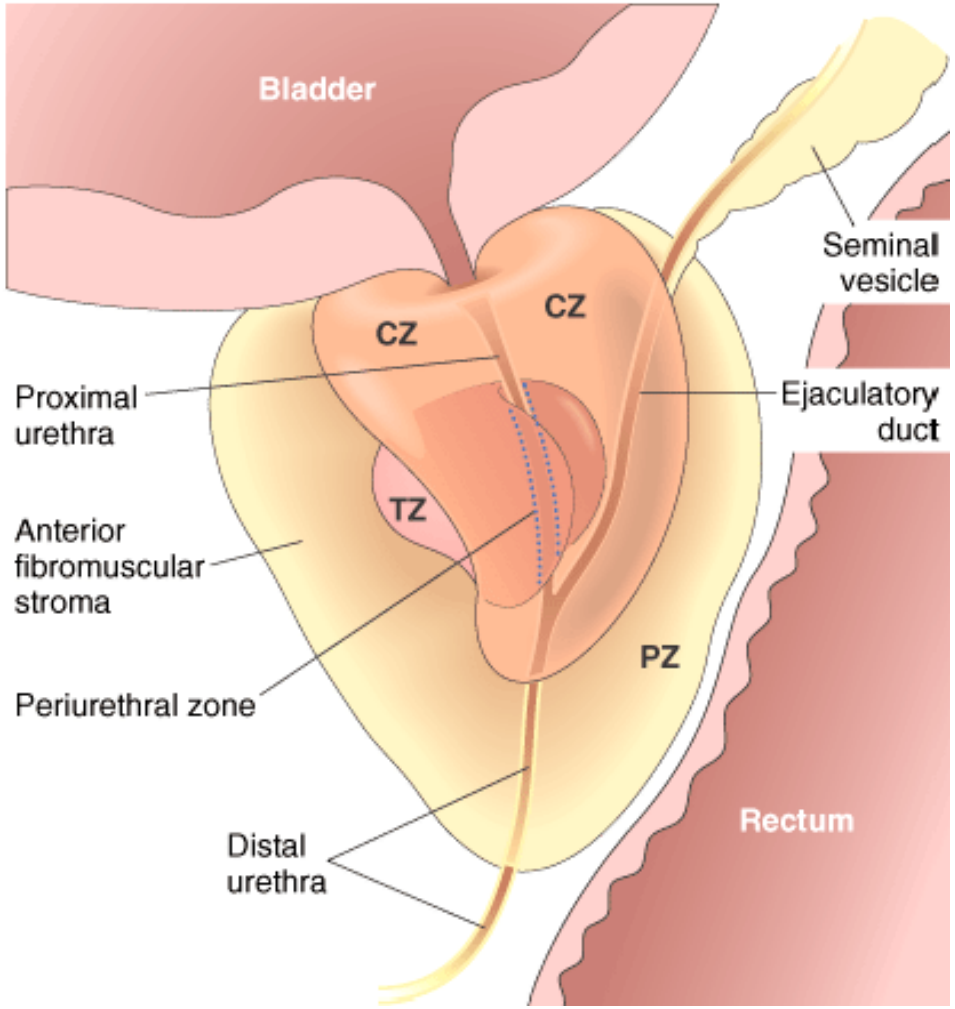


Figure 21-31 Benign prostate gland with basal cell and secretory cell layer.

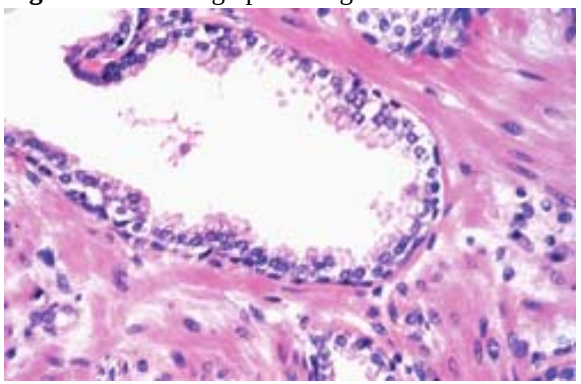


Figure 21-32 Simplified scheme of the pathogenesis of prostatic hyperplasia. The central role of the stromal cells in generating dihydrotestosterone should be noted.

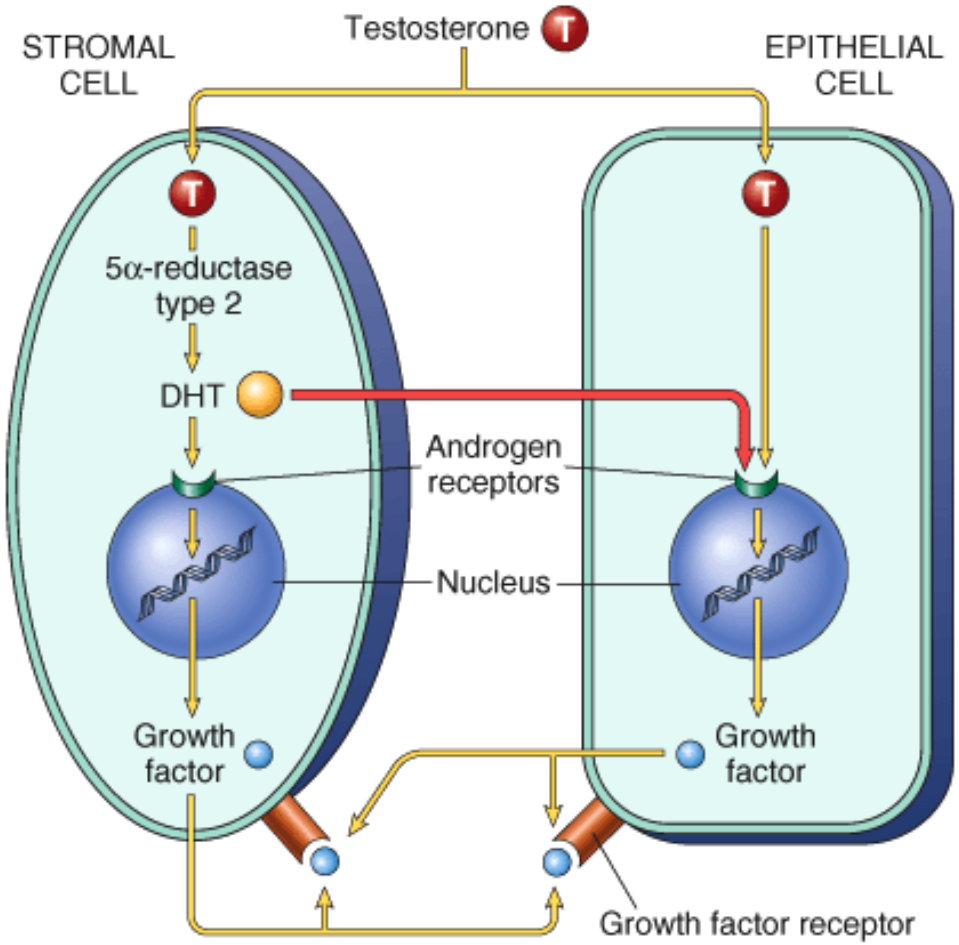


Figure 21-33 Nodular prostatic hyperplasia. *A*, Well-defined nodules of BPH compress the urethra into a slitlike lumen. *B*, A microscopic view of a whole mount of the prostate shows nodules of hyperplastic glands on both sides of the urethra.

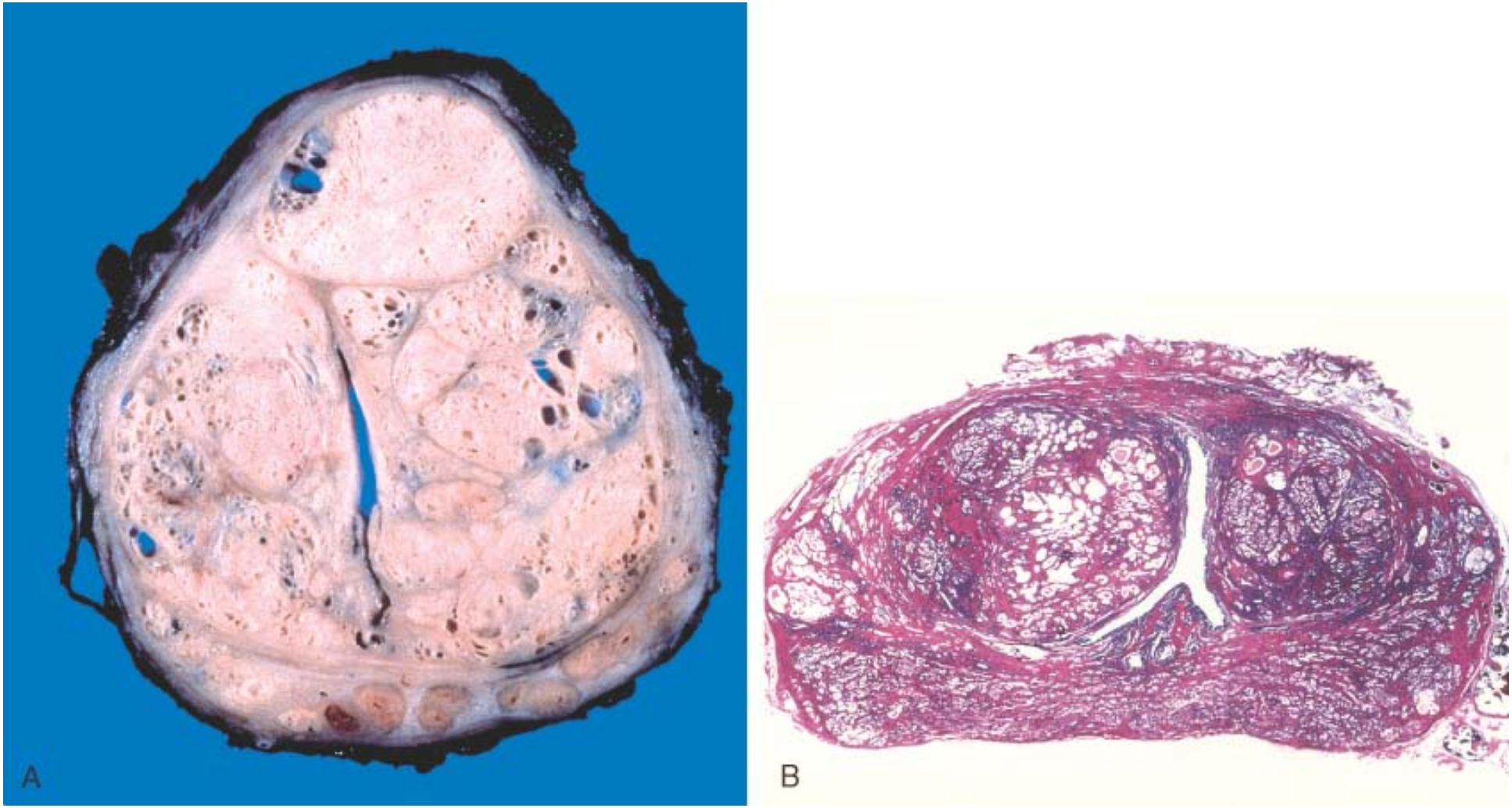


Figure 21-34 Adenocarcinoma of the prostate. Carcinomatous tissue is seen on the posterior aspect (*lower left*). Note the solid whiter tissue of cancer in contrast to the spongy appearance of the benign peripheral zone on the contralateral side.

Figure 21-36 *A*, Photomicrograph of a small focus of adenocarcinoma of the prostate demonstrating small glands crowded in between larger benign glands. *B*, Higher magnification shows several small malignant glands with enlarged nuclei, prominent nucleoli, and dark cytoplasm, compared to the larger benign gland (*top*).

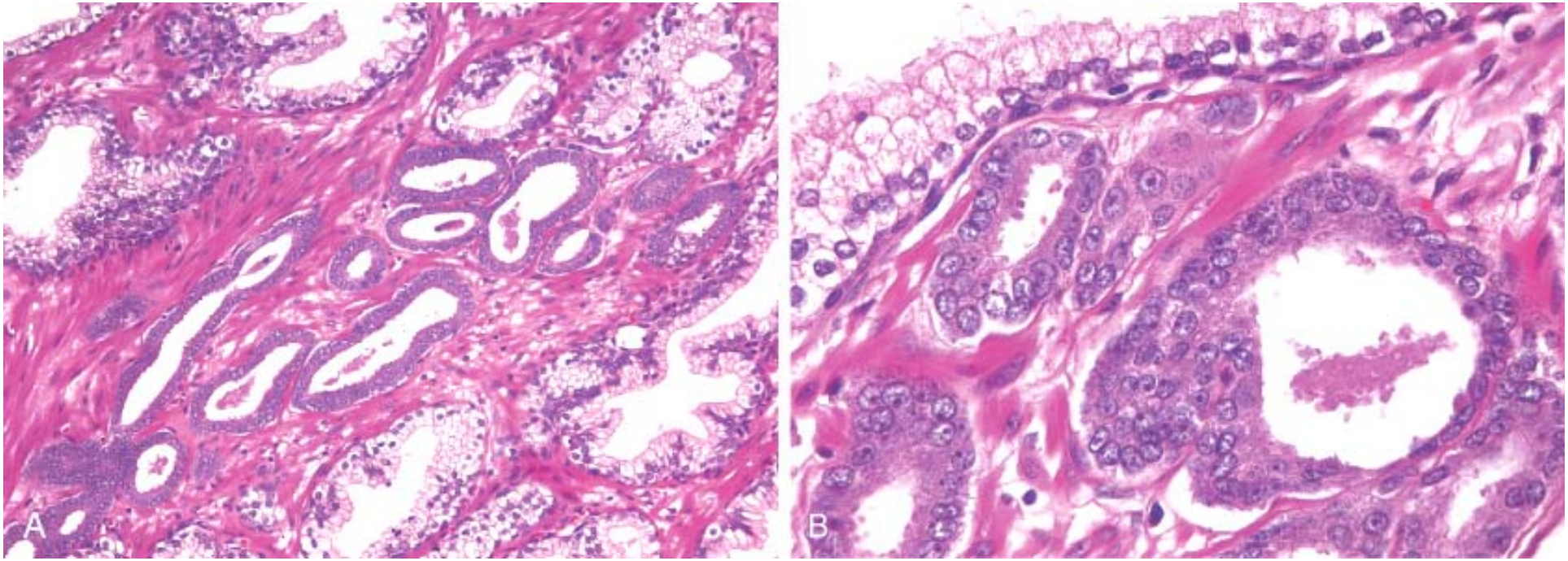


Figure 21-35 Metastatic osteoblastic prostatic carcinoma within vertebral bodies.

Figure 21-37 Carcinoma of the prostate showing perineural invasion by malignant glands. Compare to a benign gland (*left*).

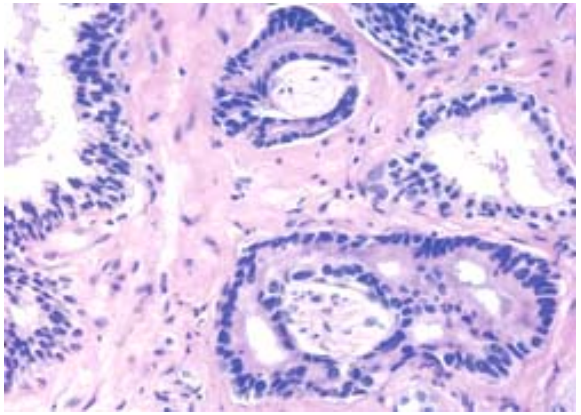
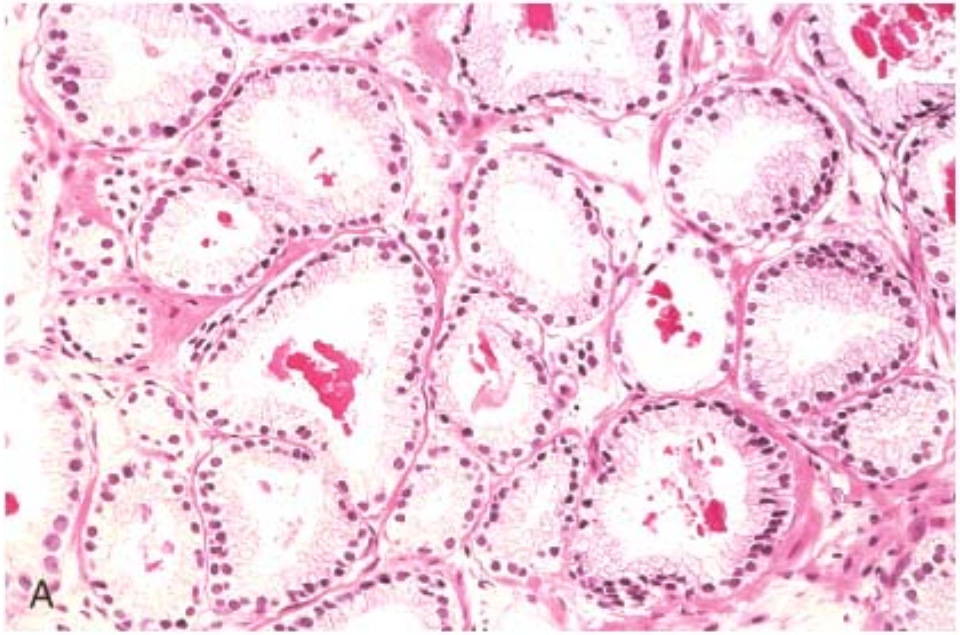
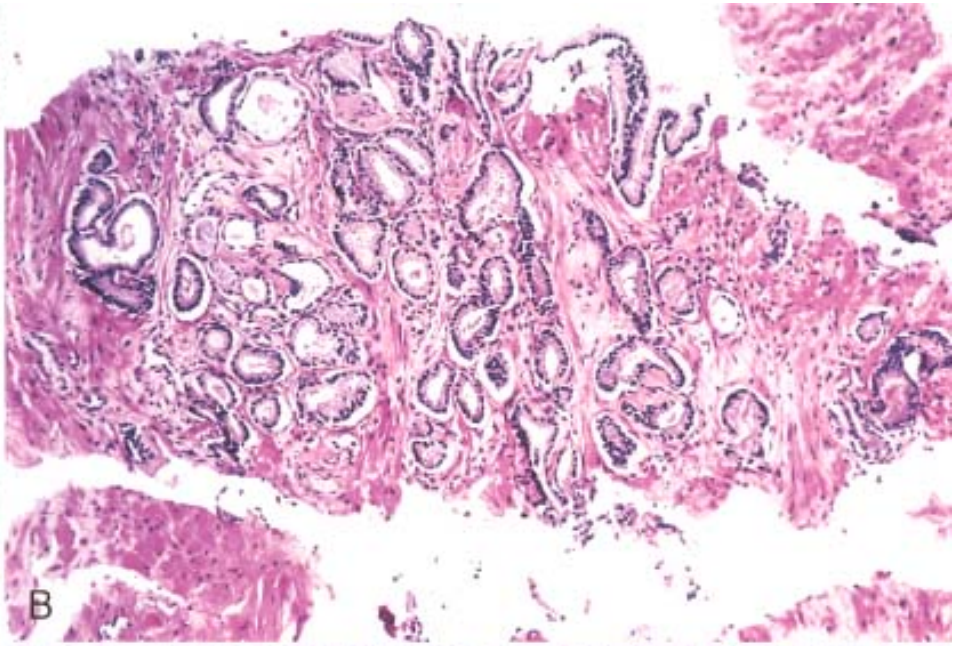
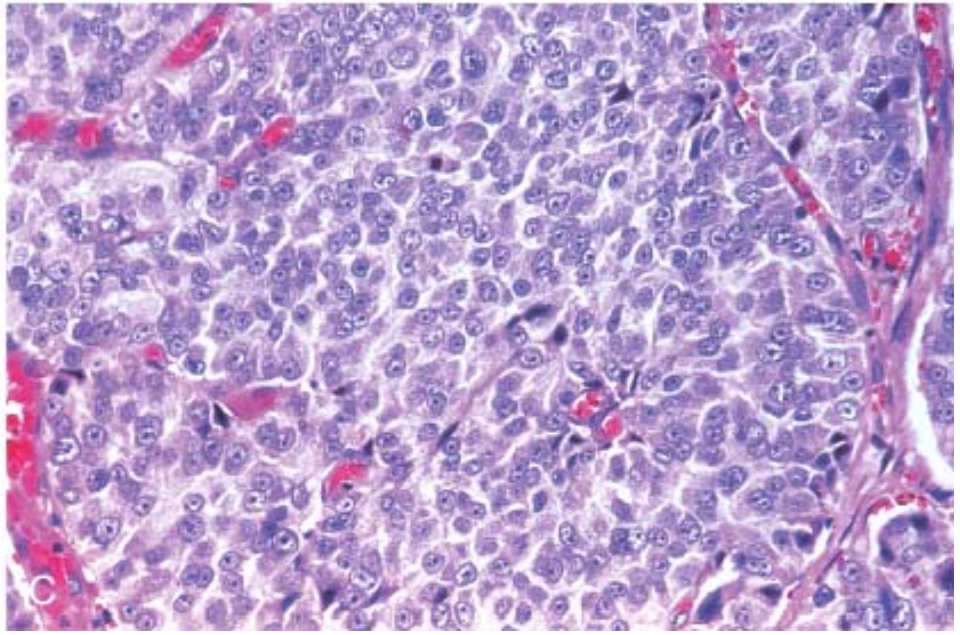


Figure 21-38 *A*, Low-grade (Gleason score 1 + 1 = 2) prostate cancer consisting of back to back, uniformly sized malignant glands. Glands contain eosinophilic intraluminal prostatic crystalloids, a feature that is more commonly seen in cancer than in benign glands and more frequently seen in lower grade than in higher grade prostate cancer. *B*, Needle biopsy of the prostate with variably sized, more widely dispersed glands of moderately differentiated (Gleason score 3 + 3 = 6) adenocarcinoma. *C*, Poorly differentiated Gleason score (5 + 5 = 10) adenocarcinoma composed of sheets of malignant cells.







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Chapter 22 - The Female Genital Tract

Christopher P. Crum MD

Normal

Embryology

The embryology of the female genital tract is relevant to both anomalies in this region and the histogenesis of various tumors. The primordial germ cells arise in the wall of the yolk sac by the fourth week of gestation; by the fifth or sixth week, they migrate into the urogenital ridge. The mesodermal epithelium of the urogenital ridge then proliferates, eventually to produce the epithelium and stroma of the gonad. The dividing germ cells—of endodermal origin—are incorporated into these proliferating epithelial cells to form the ovary.[1] Failure of germ cells to develop may result in either absence of ovaries or premature ovarian failure. Disruption of normal migration may account for extragonadal distribution of germ cell midline structures (retroperitoneum, mediastinum, and even pineal gland) and may rarely lead to tumors in these sites.

A second component of female genital development is the müllerian duct. At about the sixth week, invagination and subsequent fusion of the coelomic lining epithelium form the lateral müllerian (or paramesonephric) ducts. Müllerian ducts progressively grow caudally to enter the pelvis, where they swing medially to fuse with the urogenital sinus at the müllerian tubercle (Fig. 22-1A). Further caudal growth brings these fused ducts into contact with the urogenital sinus, formed when the cloaca is subdivided by the urorectal septum. The urogenital sinus

eventually becomes the vestibule of the external genitalia (Fig. 22-1*B*). Normally, the unfused portions mature into the fallopian tubes, the fused caudal portion developing into the uterus and upper vagina and the urogenital sinus forming the lower vagina and vestibule (Fig. 22-1*C*). Consequently, the entire lining of the uterus and tubes as well as the ovarian surface is ultimately derived from coelomic epithelium (mesothelium). This close embryologic relationship between the mesothelium and müllerian system may be reflected in adult life in the form of benign (endometriosis) and malignant (endometrioid and serous neoplasia) lesions, which may arise in both the surface mesothelium of the ovaries and the peritoneal surfaces.

The epithelium of the vagina, cervix, and urinary tract is formed by induction of basal cells from the underlying stroma, which undergo squamous and urothelial differentiation.[2] A portion of these cells remains uncommitted, forming the reserve cells of the cervix. The latter are capable of both squamous and columnar cell differentiation.[3]

In males, müllerian inhibitory substance[4] from the developing testis causes regression of the müllerian ducts, and the paired wolffian (or mesonephric) ducts form the epididymis and the vas deferens. Normally, the mesonephric duct regresses in the female, but remnants may persist into adult life as epithelial inclusions adjacent to the ovaries, tubes, and uterus. In the cervix and vagina, these rests may be cystic and are termed Gartner duct cysts. Many of the events in the

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Figure 22-1 Embryology and anatomy of the female genital tract. *A,* Early in development the mesonephric (*red*) and müllerian (*blue*) ducts merge at the urogenital sinus to form the müllerian tubercle. *B,* By birth the müllerian ducts have fused to form the fallopian tubes, uterus and endocervix (*blue*) merging with the vaginal squamous mucosa. The mesonephric ducts regress but may be found as a remnant in the ovary, adnexa and cervix (Gartner duct). *(Adapted from Langman J: Medical Embryology. Baltimore, Williams and Wilkins, 1981.) C,* Normal adult genital tract, with cervix, uterus, fallopian tubes, and ovaries. A small paratubal cyst is present on the right.

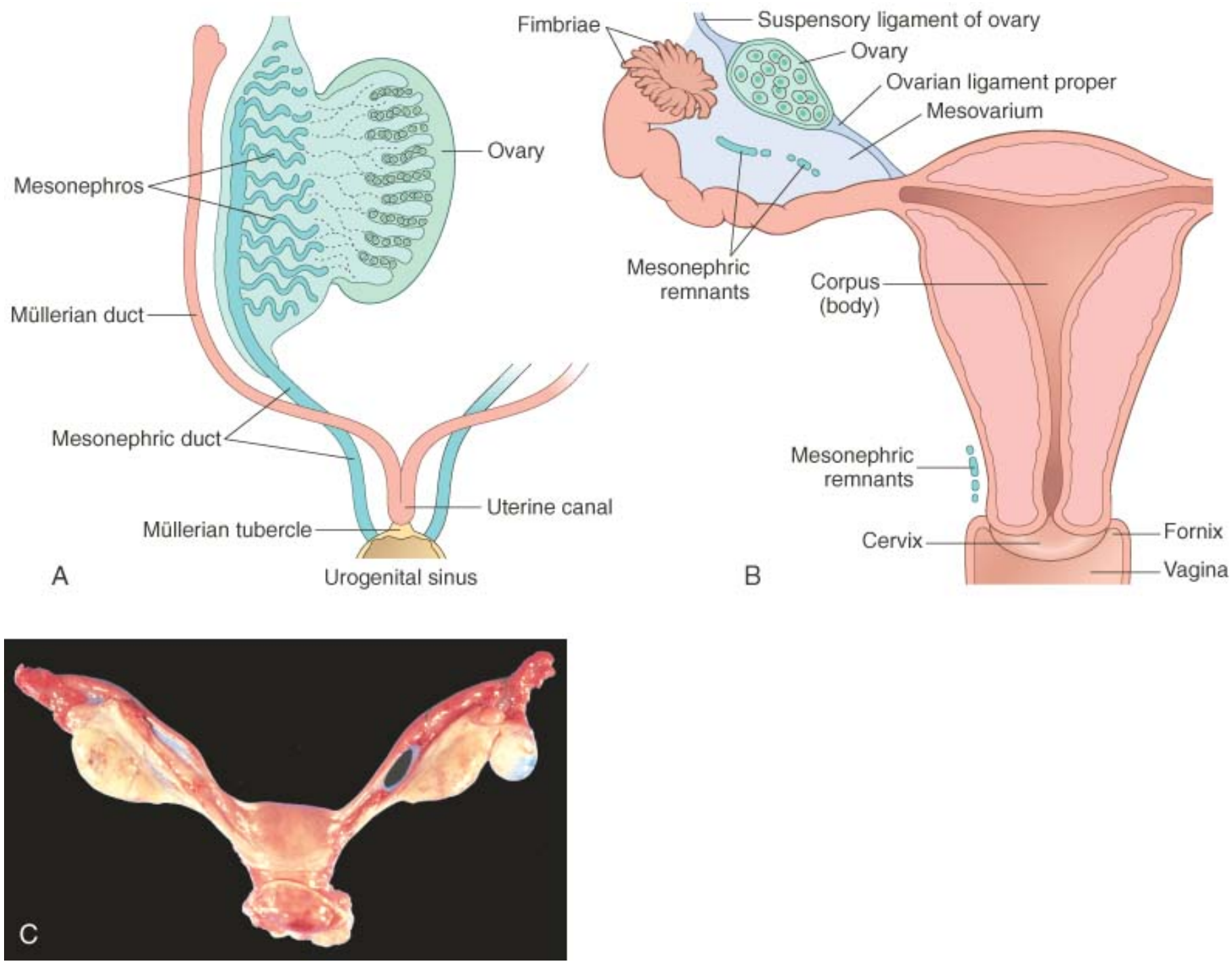
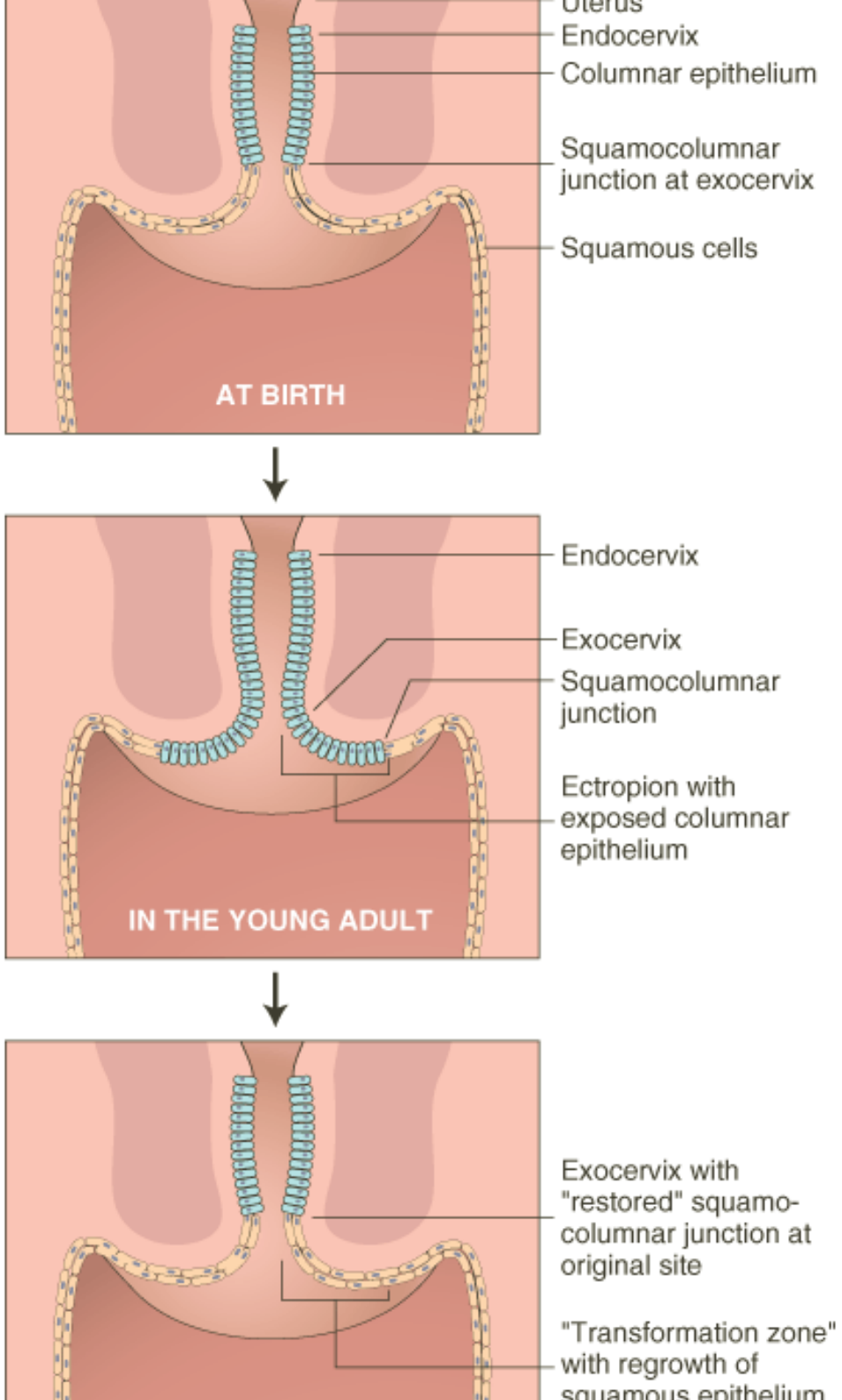


Figure 22-2 Schematic of the development of the cervical transformation zone.


Uterus
Endocervix
Columnar epithelium
Squamocolumnar junction at exocervix
Squamous cells
AT BIRTH
Endocervix
Exocervix
Squamocolumnar junction
Ectropion with exposed columnar epithelium
IN THE YOUNG ADULT
Exocervix with "restored" squamo-columnar junction at original site
"Transformation zone" with regrowth of squamous epithelium

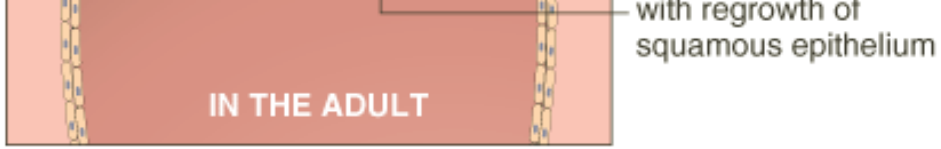


Figure 22-3 *A*, Colposcopic view of the cervix in a reproductive age woman. The portio epithelium (peripheral) merges with (at dotted boundary) and eventually replaces the endocervical columnar epithelium (red and grapelike) to form the transformation zone. The os is in the center. *B*, The postmenopausal cervix. The epithelial surface is smooth and completely covered by squamous epithelium. The squamocolumnar junction is not visible and is inside the endocervical canal. *(A and B, courtesy of Dr. Alex Ferenczy, McGill University, Montreal, Quebec.)*

TABLE 22-1 -- Anatomic Distribution of Common Female Genital Infections

		Location and Manifestations of Infection				
Organism	**Source**	***Vulva***	***Vagina***	***Cervix***	***Corpus***	***Adnexa***
Herpesvirus	STD	Herpetic ulcers				
Molluscum contagiosum	STD	Molluscum lesions				
HPV	STD	Genital warts, intrapeithelial neoplasia, invasive carcinoma				
Chlamydia trachomatis	STD		Follicular cervicitis, endometritis, salpingo-oophoritis			
Neisseria gonorrhoeae	STD	Skene gland adenitis	Vaginitis in children	Acute cervicitis	Acute endometritis and salpingitis	
Candida	Endogenous	Vulvovaginitis				

Trichomonas	STD		Cervicovaginitis			

HPV, human papillomavirus; STD, sexually transmitted disease.

risk is highest if the infection is active during delivery and particularly if it is a primary (initial) infection in the mother.[14]

Mycotic and *yeast (Candida) infections* are common; about 10% of women are thought to be carriers of vulvovaginal fungi. Diabetes mellitus, oral contraceptives, and pregnancy may enhance the development of infection, which manifests as small white surface patches similar to monilial lesions elsewhere. It is accompanied by leukorrhea and pruritus. The diagnosis is made by finding the organism in wet mounts of the lesions.

Trichomonas vaginalis is a large, flagellated ovoid protozoan that can be readily identified in wet mounts of vaginal discharge in infected patients (Fig. 22-4). Infections may occur at any age and are seen in about 15% of women in sexually transmitted disease clinics.[15] They are associated with a purulent vaginal discharge and discomfort; the underlying vaginal and cervical mucosa typically has a characteristic fiery red appearance, called strawberry cervix. On histologic examination, the inflammatory reaction is usually limited to the mucosa and immediately subjacent lamina propria.

Figure 22-4 Flagellated trophozoites of *Trichomonas vaginalis*.

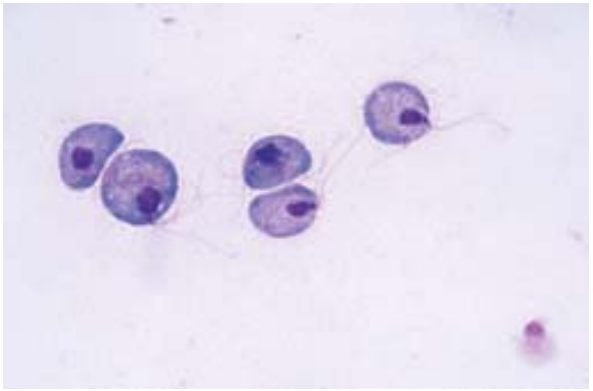


Figure 22-5 *A,* Acute salpingo-oophoritis, with tubo-ovarian abscess. The fallopian tube and ovaries have coalesced into an inflammatory mass adherent to the uterus. (Compare with Figure 22-1 .) *B,* Chronic salpingitis with fusion of the tubal plicae and inflammatory cell infiltrates.

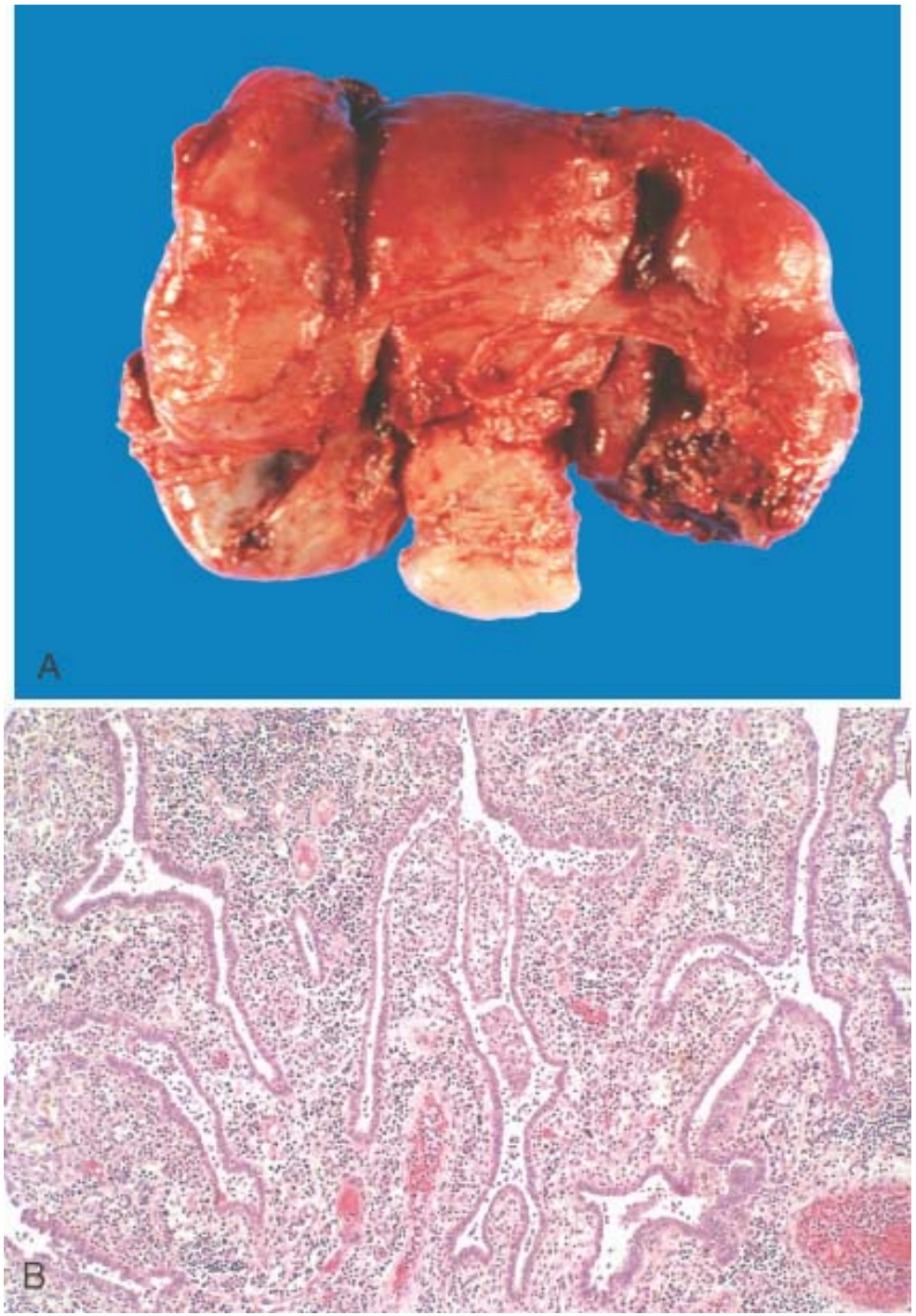


Figure 22-6 Inflammatory vulvar disorders. Lichen sclerosus (*upper panel*). Lichen simplex chronicus (*lower panel*). The main features of the lesions are indicated in the figures.

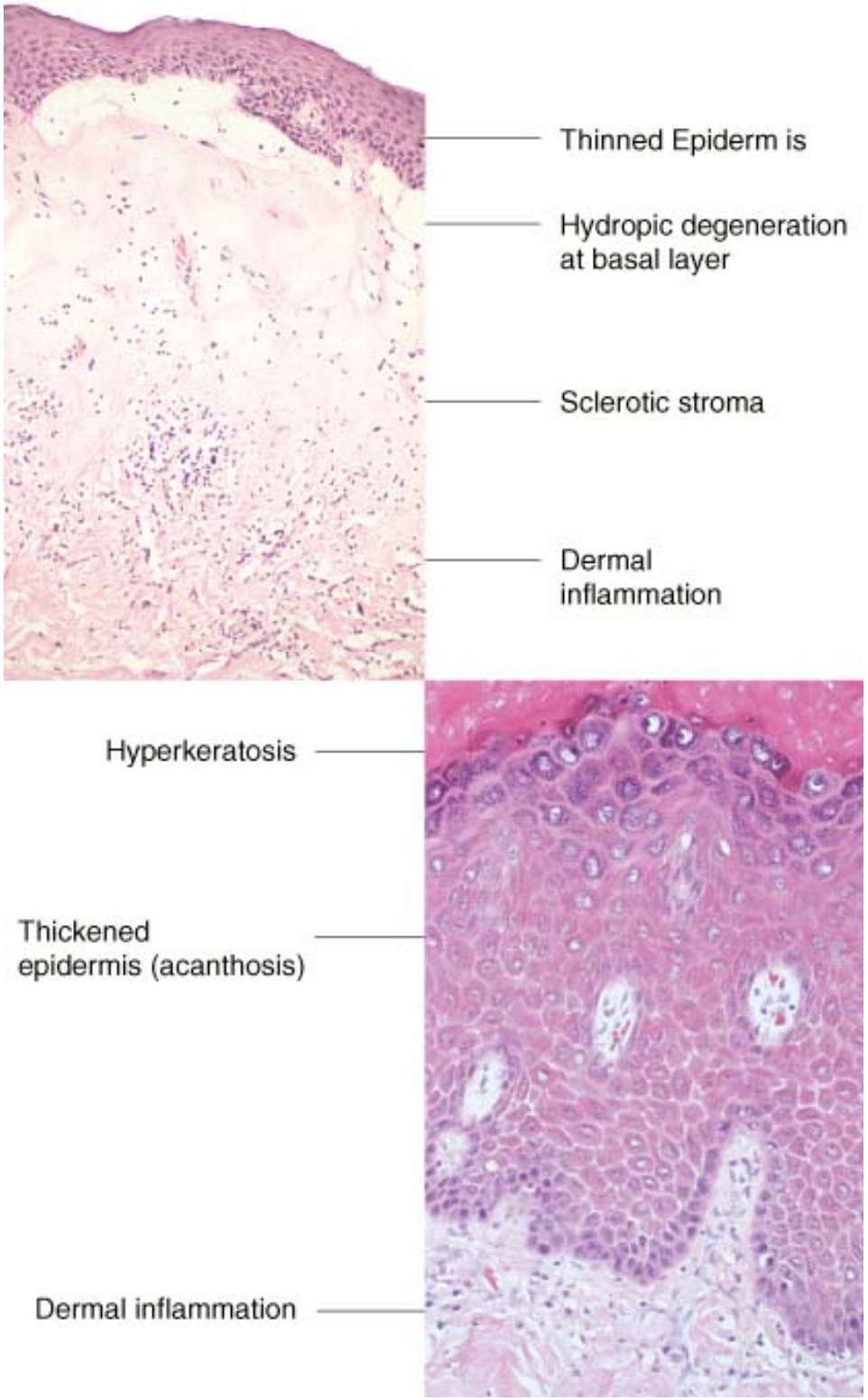

Thinned Epiderm is
Hydropic degeneration at basal layer
Sclerotic stroma
Dermal inflammation
Hyperkeratosis
Thickened epidermis (acanthosis)
Dermal inflammation

Figure 22-7 Lichen sclerosus, exhibiting the white parchment-like patches of the skin of the vulva and labial atrophy.

Figure 22-8 *A*, Numerous condylomas of the vulva encircling the introitus. *(Courtesy of Dr. Alex Ferenczy, McGill University, Montreal, Quebec.) B*, Histopathology of condyloma acuminatum showing acanthosis, hyperkeratosis, and cytoplasmic vacuolation (koilocytosis, *center*).

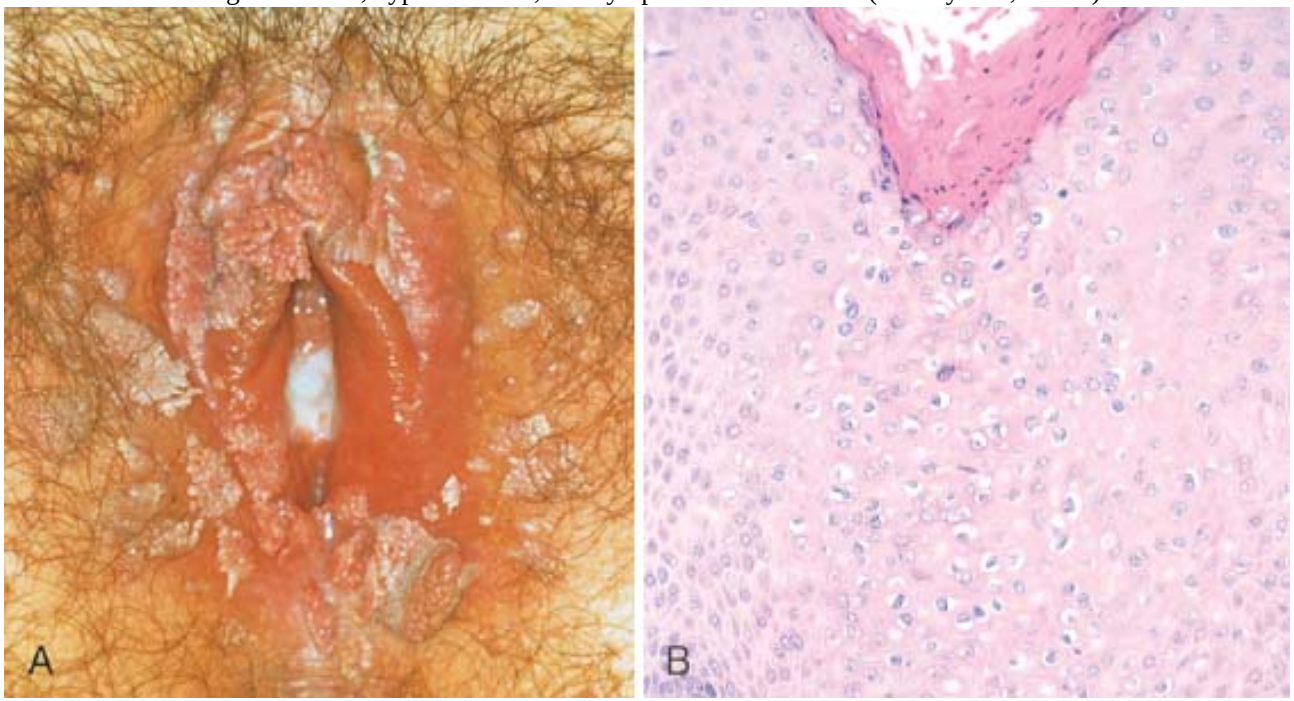


Figure 22-9 *A*, Histopathology of classic (HPV positive) vulvar intraepithelial neoplasia with diffuse cellular atypia, nuclear crowding, and increased mitotic index. *B*, differentiated (HPV negative) VIN, showing maturation, hyperkeratosis and basal cell atypia (*arrow*).

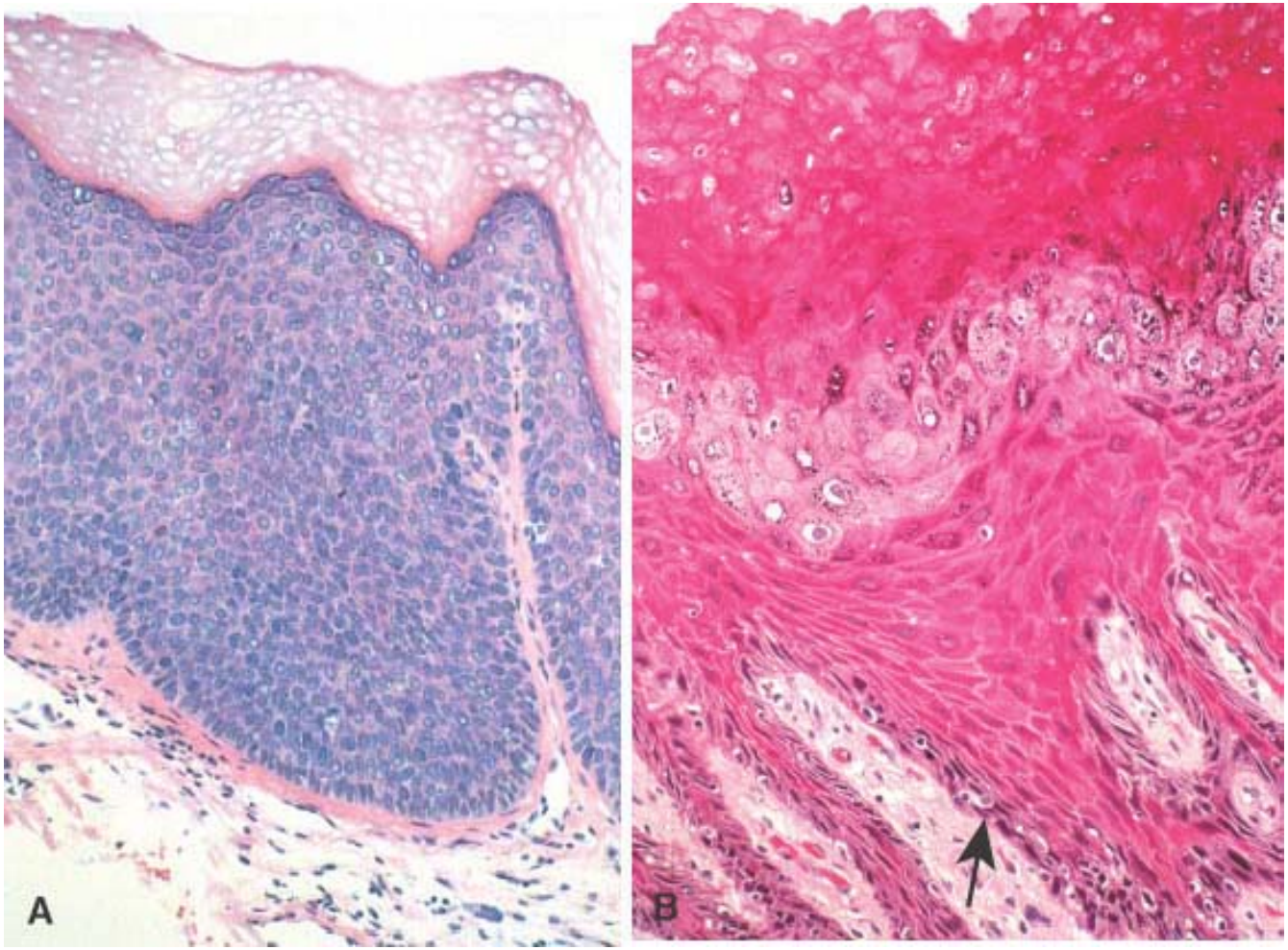


Figure 22-10 *A*, Poorly differentiated vulvar carcinoma associated with human papillomaviruses (HPV). *B*, Well-differentiated keratinizing vulvar carcinoma, typically HPV negative.

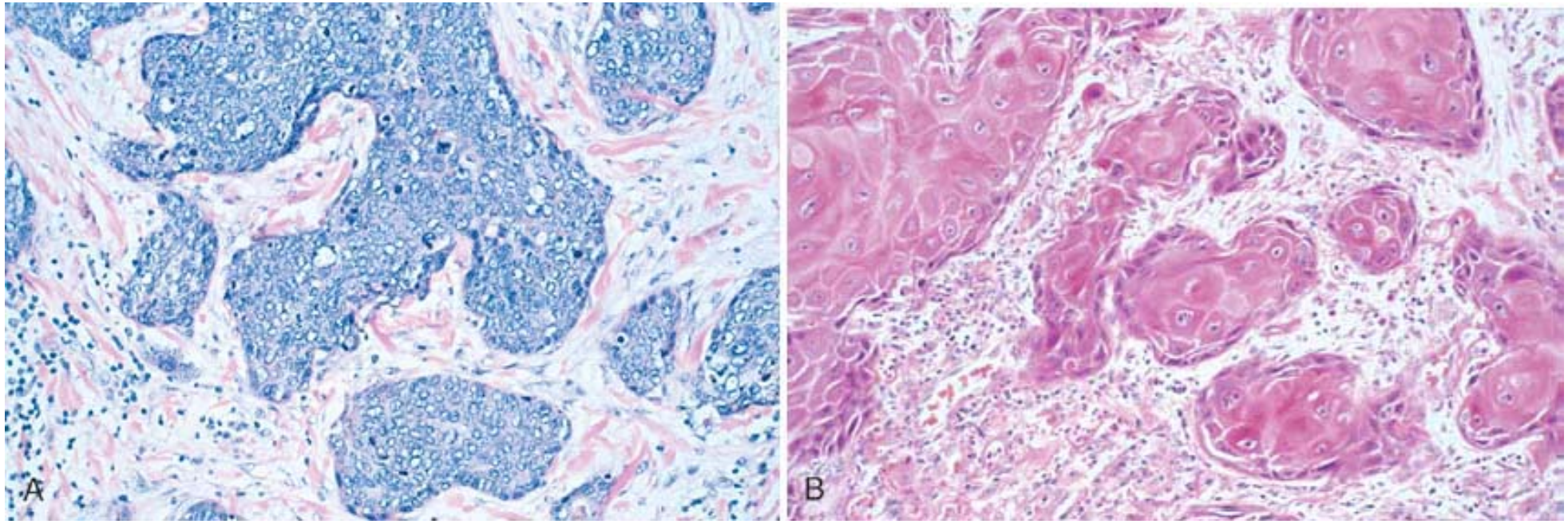


Figure 22-11 *A*, Verrucous carcinoma of the vulva. *B*, Basal cell carcinoma of the vulva.

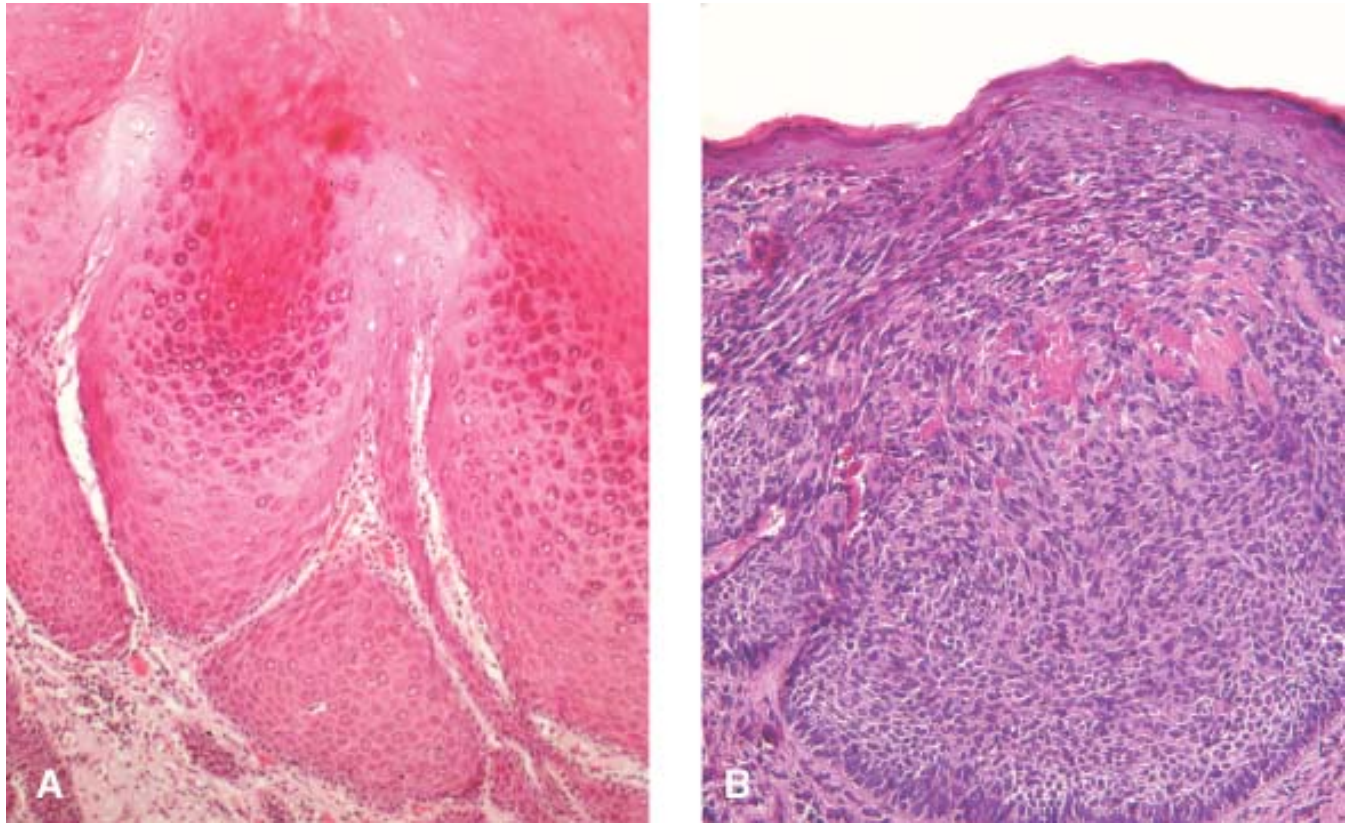


Figure 22-12 Paget disease of the vulva with a cluster of large clear tumor cells within the squamous epithelium.

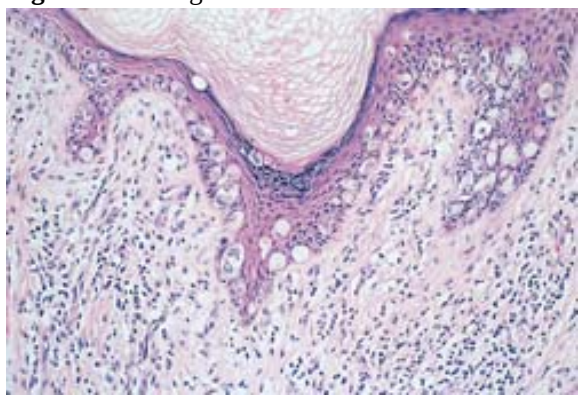


Figure 22-13 *A*, Malignant melanoma involving the vaginal introitus and labia minora. *B*, Histology of invasive melanoma with melanin production (*inset*).

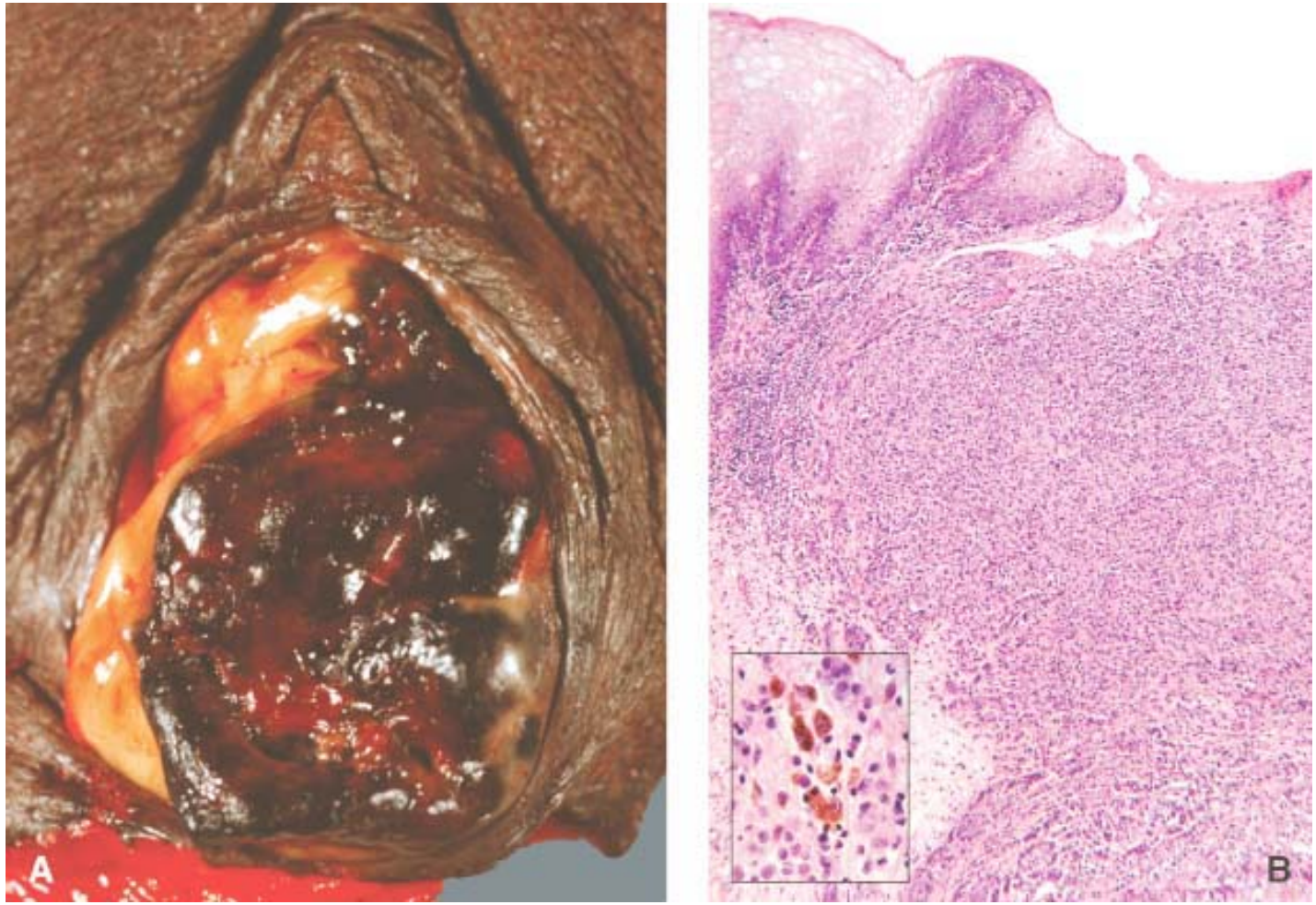


Figure 22-14 Clear cell adenocarcinoma of the vagina showing vacuolated tumor cells in clusters and glandlike structures.

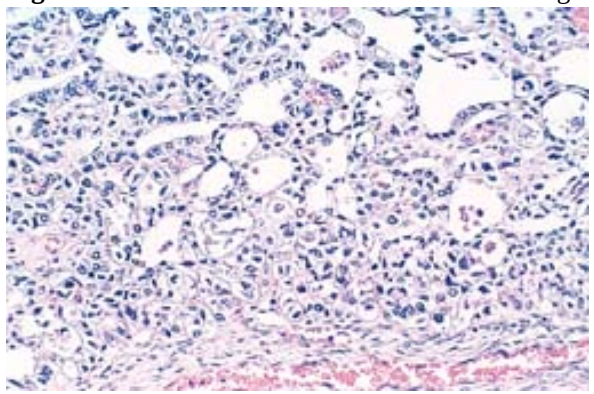


Figure 22-15 Sarcoma botryoides (embryonal rhabdomyosarcoma) of the vagina appearing as a polypoid mass protruding from the vagina. *(Courtesy of Dr. Michael Donovan, Children's*

Hospital, Boston, MA.)

Figure 22-16 In the diagram (*upper*), reserve cells in the transformation zone are continuous with the basal cells of the ectocervix (*right*) and may undergo columnar and squamous differentiation (metaplasia). Photomicrographs at bottom depict (*from left to right*) quiescent subcolumnar reserve cells, reserve cells undergoing columnar differentiation (*second from left*), reserve cells undergoing squamous metaplasia (*second from right*) and ectocervical squamous epithelium (*right*).

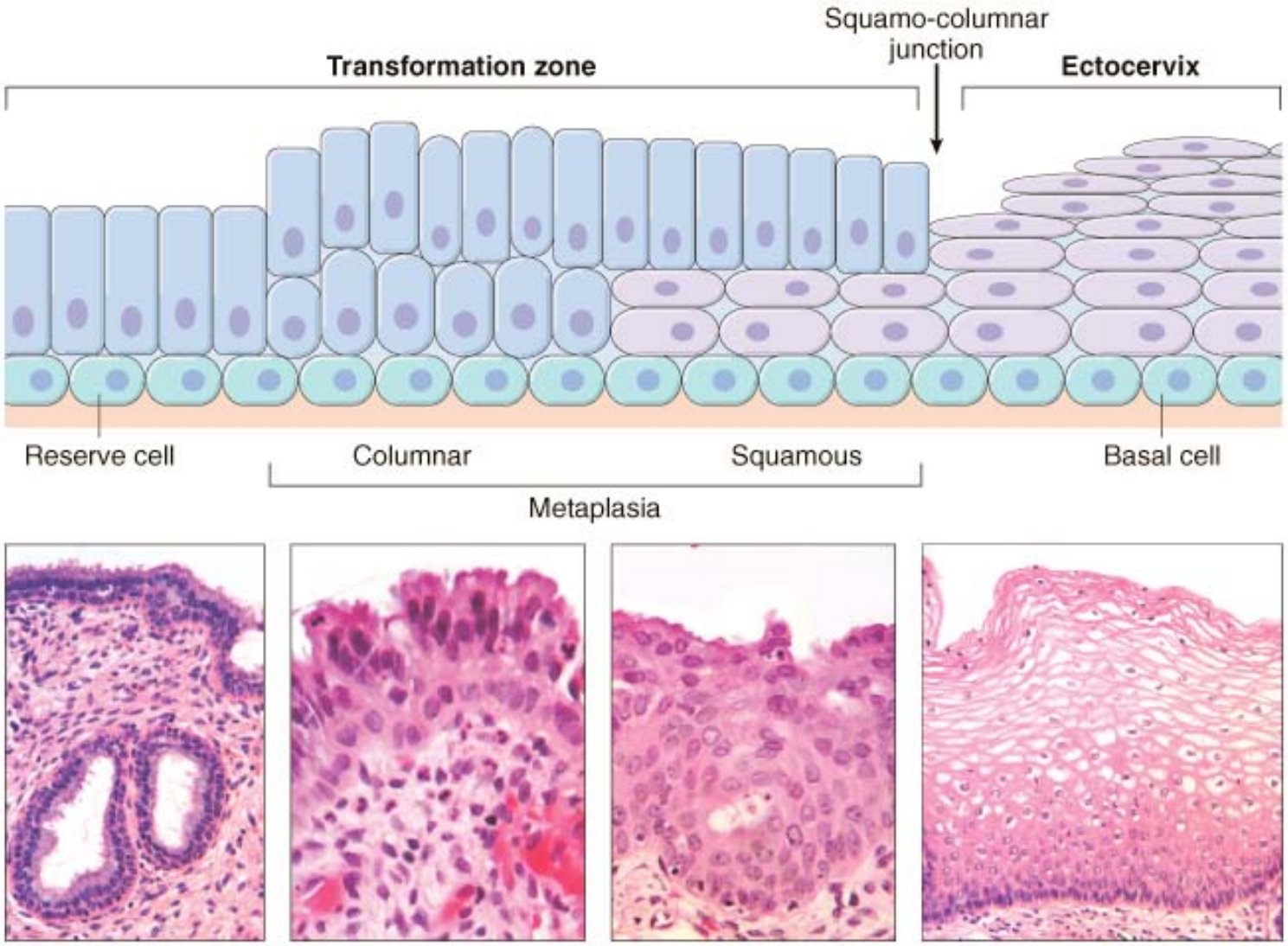


Figure 22-17 Endocervical polyp composed of a dense fibrous stroma covered with endocervical columnar epithelium.

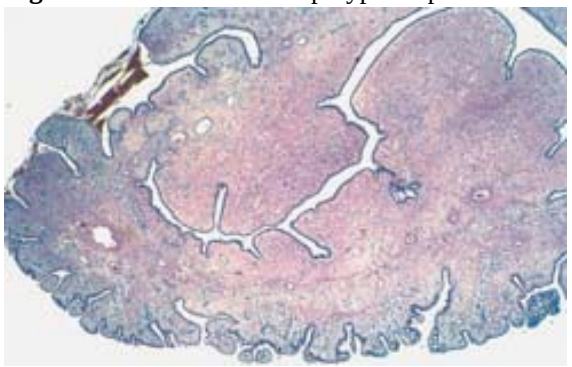


Figure 22-18 *A*, Postulated steps in the pathogenesis of cervical neoplasia. Conditions influencing progression are listed at the lower center of the diagram. *B*, Approximate lifetime risks of acquiring HPV infection (*left*) and dying of cervical cancer (*right*). The intermediate steps include risks of infection with high-risk HPV types, development of advanced cervical intraepithelial neoplasia (CIN), and progression to invasive carcinoma.

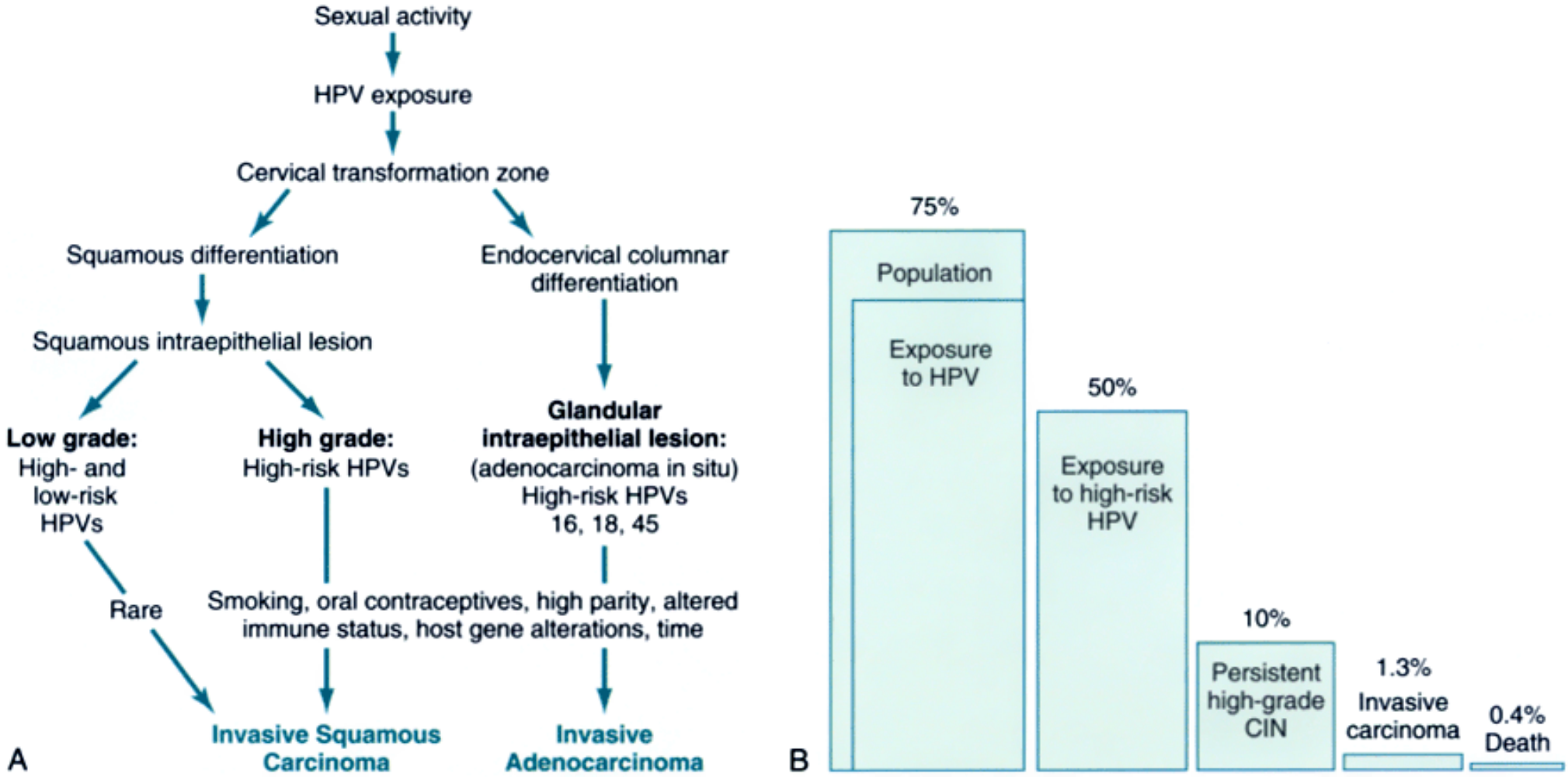


Figure 22-19 Spectrum of cervical intraepithelial neoplasia: normal squamous epithelium for comparison; CIN I with koilocytotic atypia; CIN II with progressive atypia in all layers of the epithelium; CIN III (carcinoma in situ) with diffuse atypia and loss of maturation.

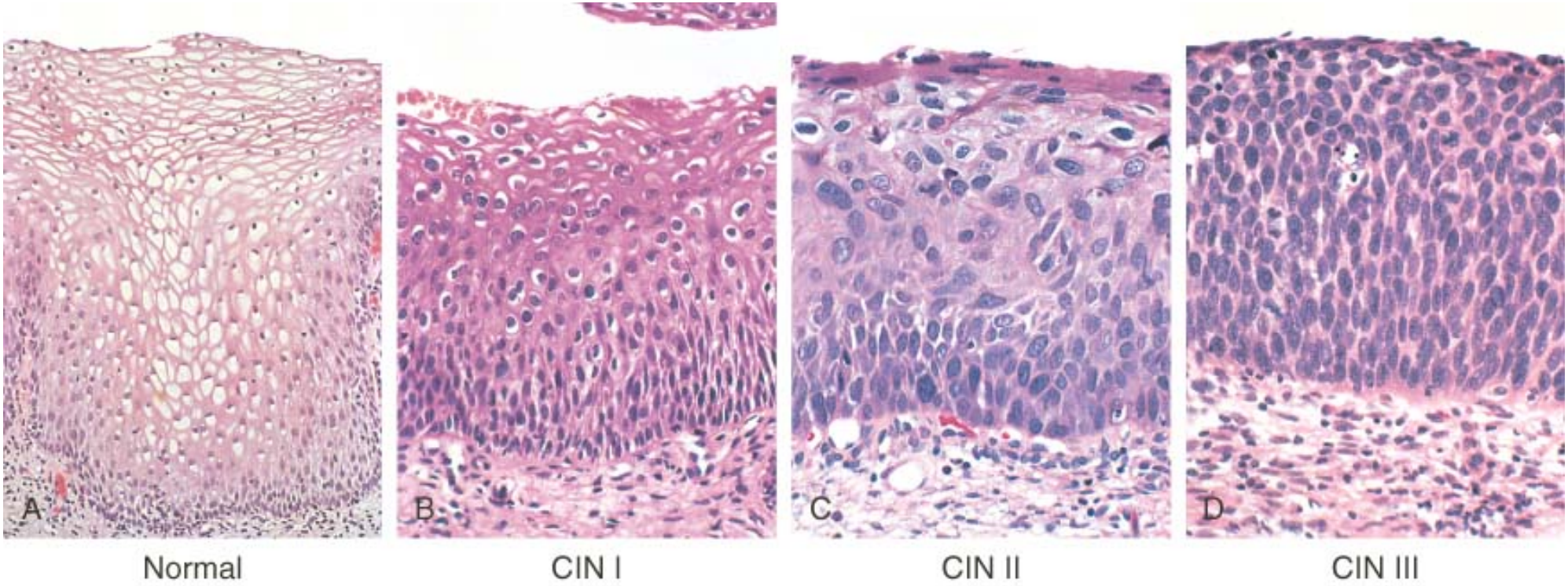


Figure 22-20 *A*, Histology of CIN I (flat condyloma), illustrating the prominent koilocytotic atypia in the upper epithelial cells, as evidenced by the prominent perinuclear halos. *B*, Nucleic acid in situ hybridization of the same lesion for HPV nucleic acids. The blue staining denotes HPV DNA, which is typically most abundant in the koilocytes. *C*, Diffuse immunostaining of CIN II for Ki-67, illustrating widespread deregulation of cell cycle controls. *D*, Up-regulation of p161NK4 (seen as intense immunostaining) characterizes high-risk HPV infections.

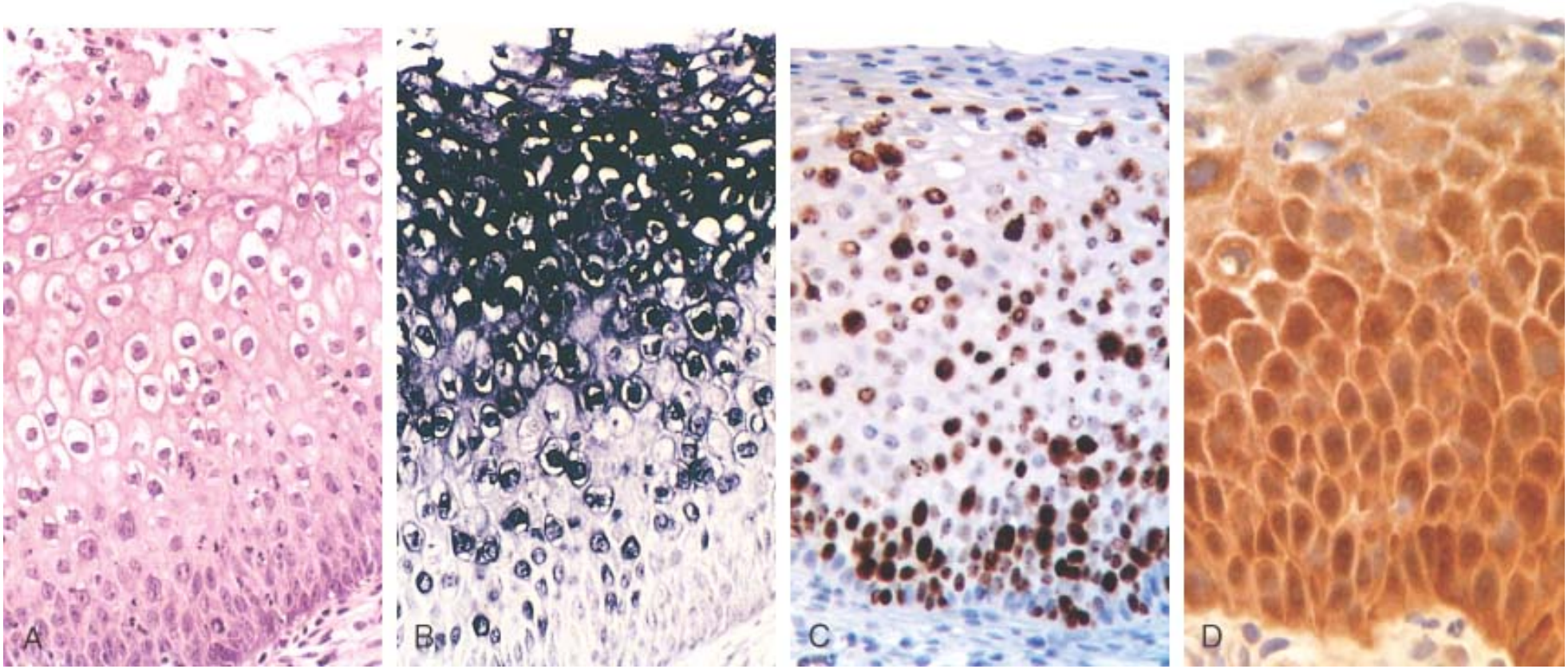
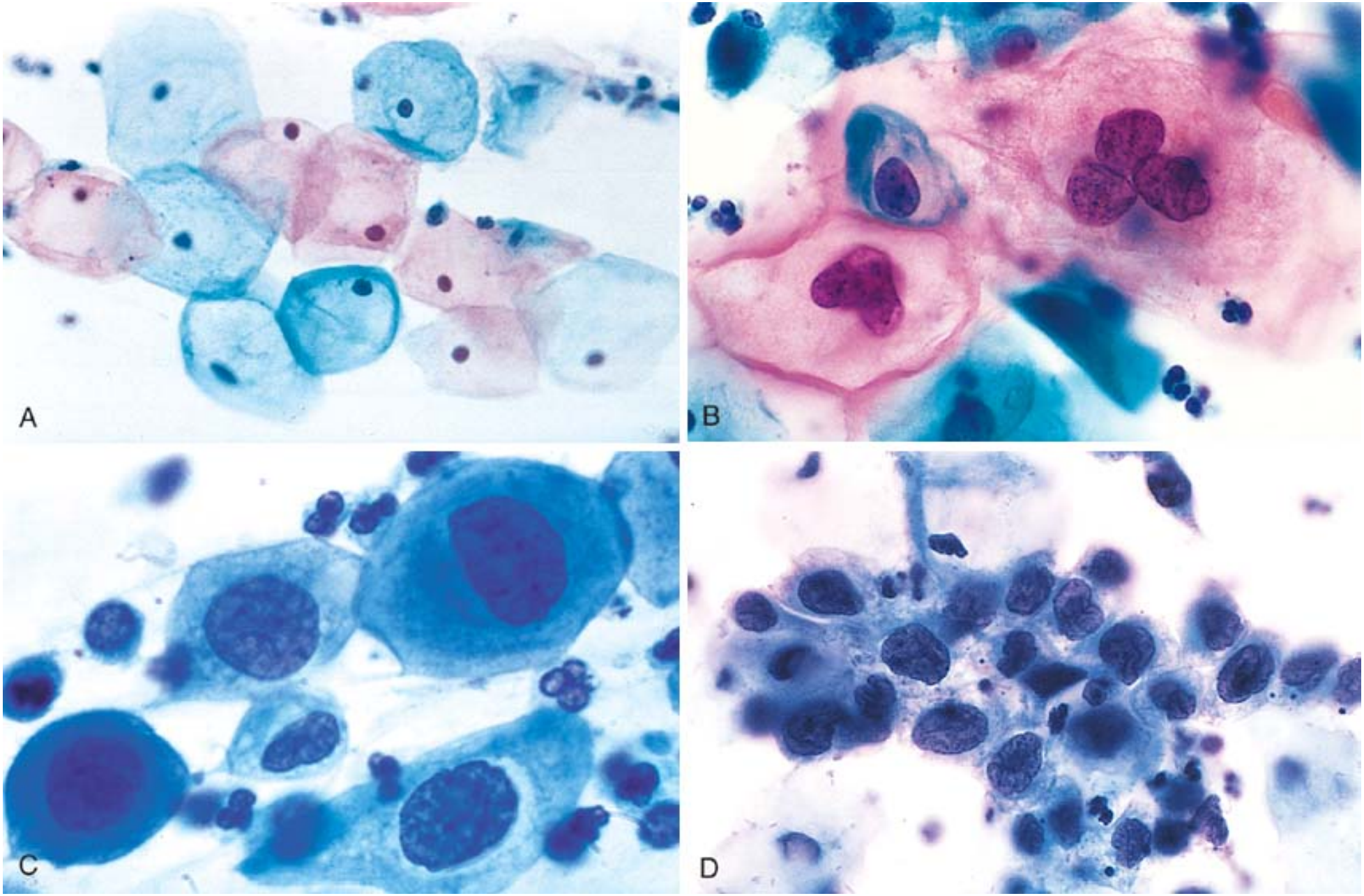


Figure 22-21 The cytology of cervical intraepithelial neoplasia as seen on the Papanicolaou smear. Cytoplasmic staining in superficial cells (*A&B*) may be either red or blue. *A*, Normal exfoliated superficial squamous epithelial cells. *B*, CIN I. *C*, CIN II. *D*, CIN III. Note the reduction in cytoplasm and the increase in the nucleus to cytoplasm ratio, which occurs as the grade of the lesion increases. This reflects the progressive loss of cellular differentiation on the surface of the lesions from which these cells are exfoliated (see Figure 22-19). *(Courtesy of Dr. Edmund S. Cibas, Brigham and Women's Hospital, Boston, MA.)*



Stage I. Carcinoma confined to the cervix

Ia. Preclinical carcinoma, that is, diagnosed only by microscopy

Ia1. Stromal invasion no greater than 3 mm and no wider than 7 mm (so-called **microinvasive carcinoma**) (Fig. 22-22*B*).

Ia2. Maximum depth of invasion of stroma greater than 3 mm and no greater than 5 mm taken from base of epithelium, either surface or glandular, from which it originates; horizontal invasion not more than 7 mm

Ib. Histologically invasive carcinoma confined to the cervix and greater than stage Ia2

Stage II. Carcinoma extends beyond the cervix but not onto the pelvic wall. Carcinoma involves the vagina but not the lower third.

Stage III. Carcinoma has extended onto pelvic wall. On rectal examination, there is no cancer-free space between the tumor and the pelvic wall. The tumor involves the lower third of the vagina.

Stage IV. Carcinoma has extended beyond the true pelvis or has involved the mucosa of the bladder or rectum. This stage obviously includes those with metastatic dissemination.

Ten per cent to 25% of cervical carcinomas are **adenocarcinomas, adenosquamous carcinomas,**

Figure 22-22 The spectrum of invasive cervical cancer. *A*, Carcinoma of the cervix, well advanced. *B*, Early stromal invasion occurring in a cervical intraepithelial neoplasm.

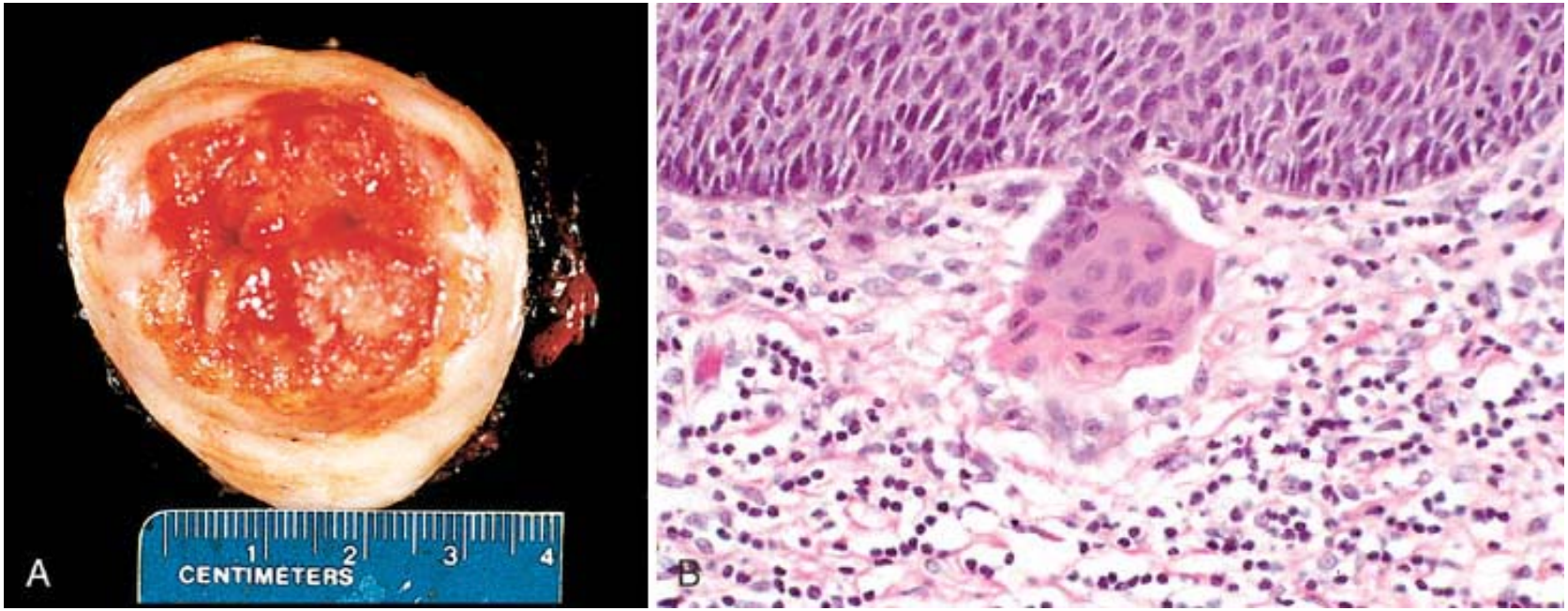


Figure 22-23 Morphology of cervical cancers. *A*, Squamous carcinoma. *B*, Adenocarcinoma in situ (*lower*), associated with CIN 3 (*upper*). *C*, Adenocarcinoma. *D*, Neuroendocrine carcinoma.

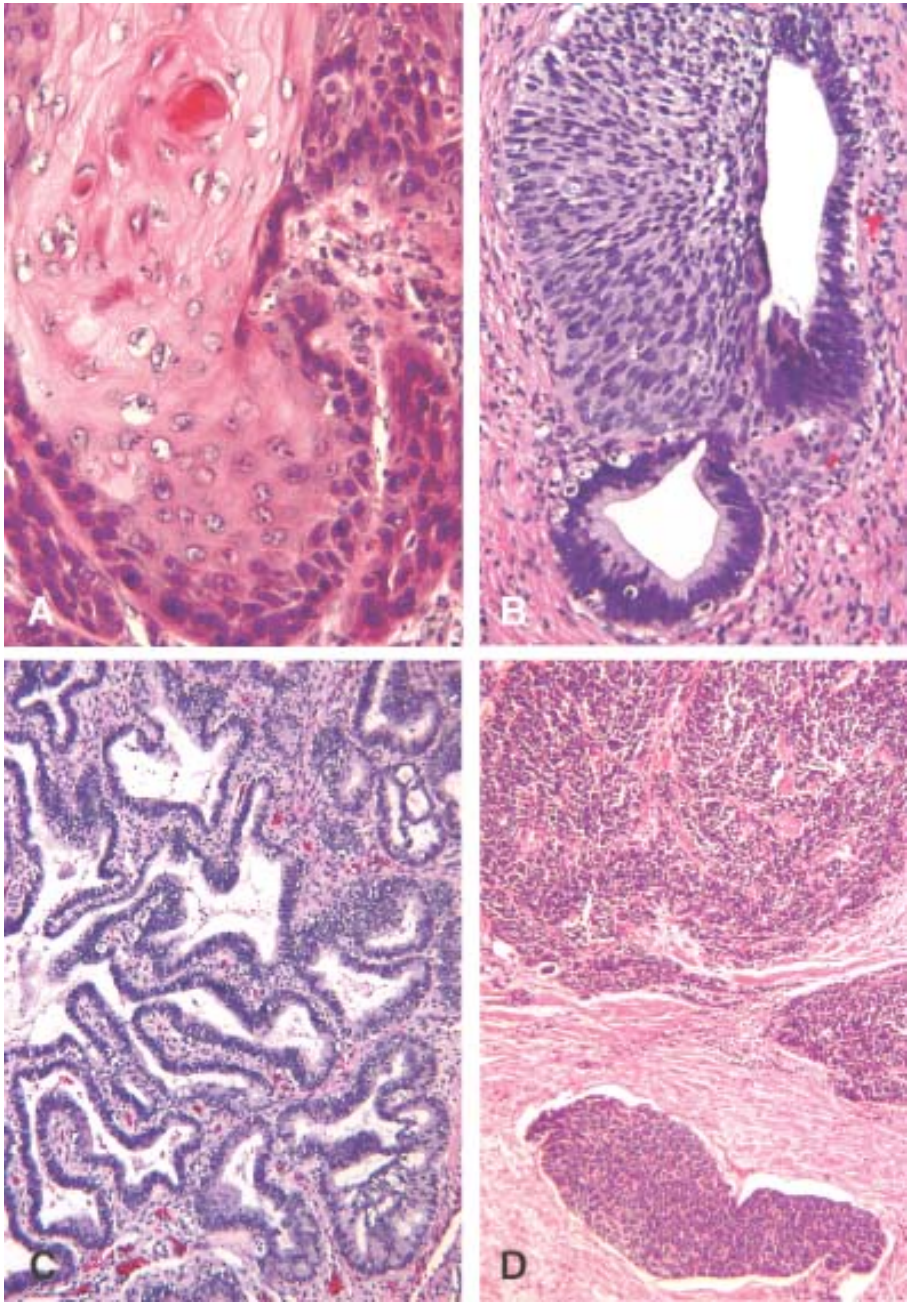


Figure 22-24 Electron micrograph of virus-like papillomavirus particles (VLPs) produced in eukaryotic cells by expression of the late region and used as vaccines. *(Courtesy of lan Frazer, MD, Princess Alexandra Hospital, University of Queensland, Australia.)*

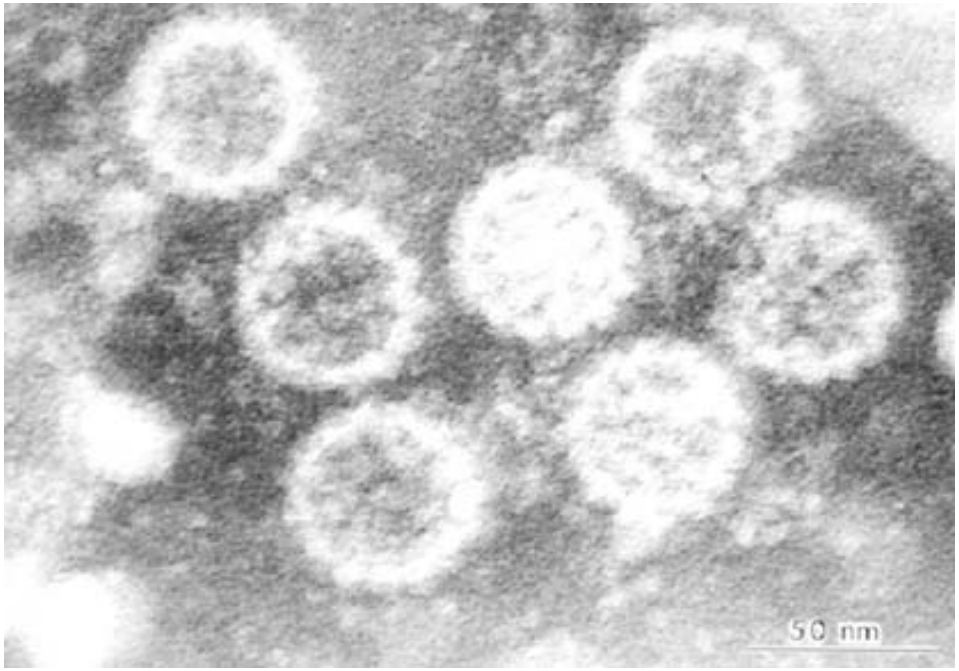
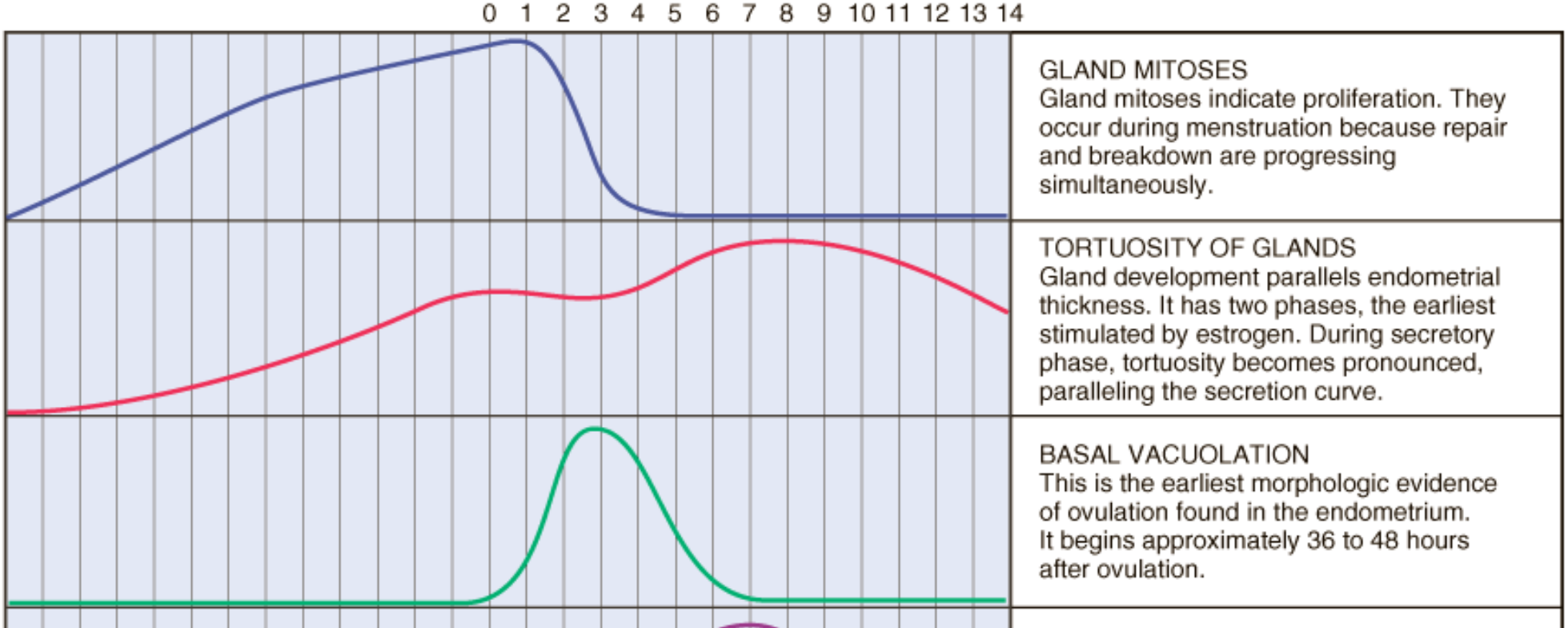


Figure 22-25 Approximate quantitative changes in seven morphologic criteria found to be most useful in dating human endometrium. *(Modified from Noyes RW: Normal phases of the endometrium. In Norris HJ, et al (eds): The Uterus. Baltimore, Williams & Wilkins, 1973.)*



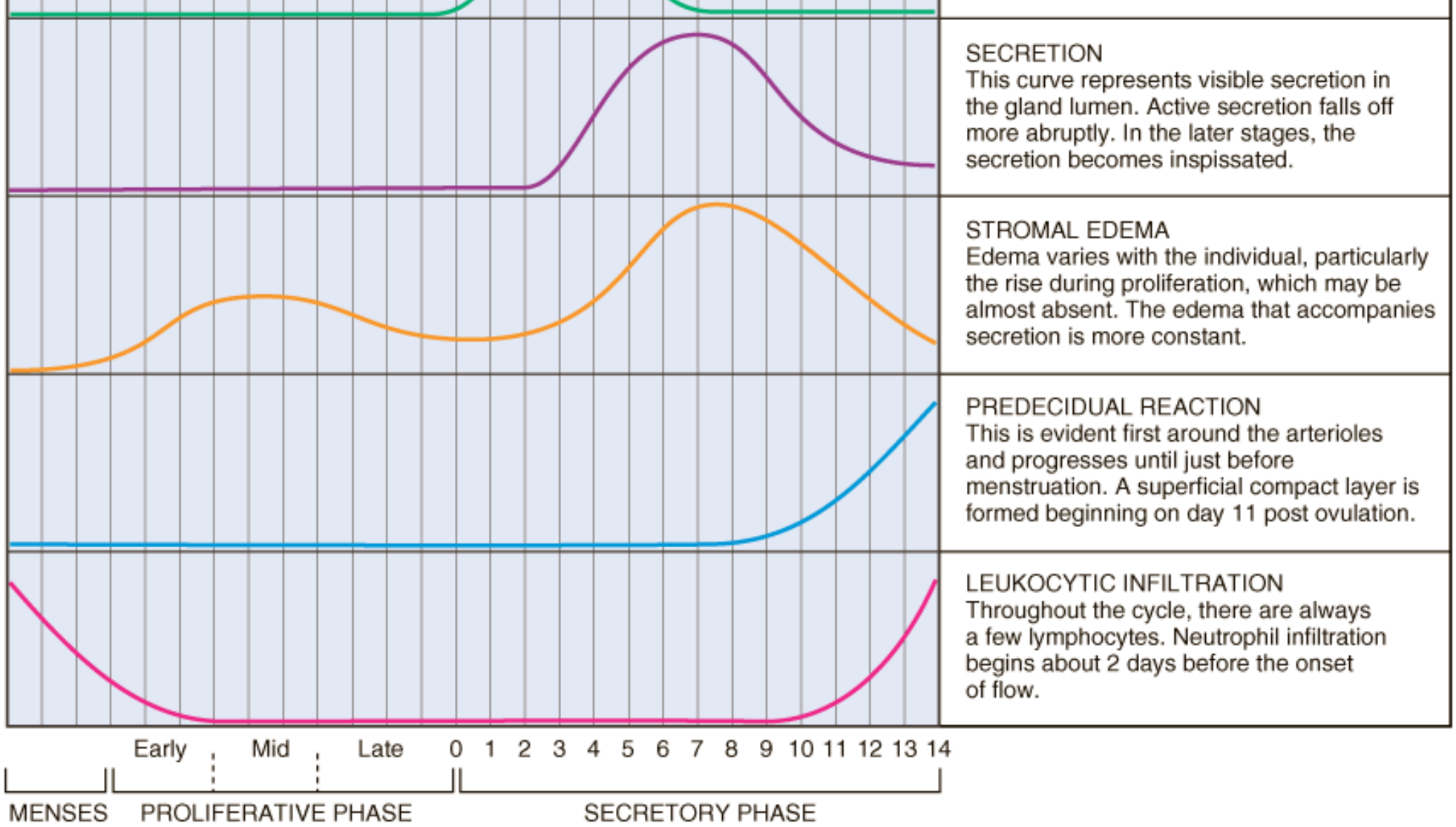


Figure 22-26 Histology of the menstrual cycle, including the proliferative phase with mitoses (*A*), the early secretory phase with subnuclear vacuoles (*B*) followed by secretory exhaustion (*C*), predecidual changes (*D*), stromal granulocytes (*E*), and stromal breakdown at the onset of menses (*F*) (see text).

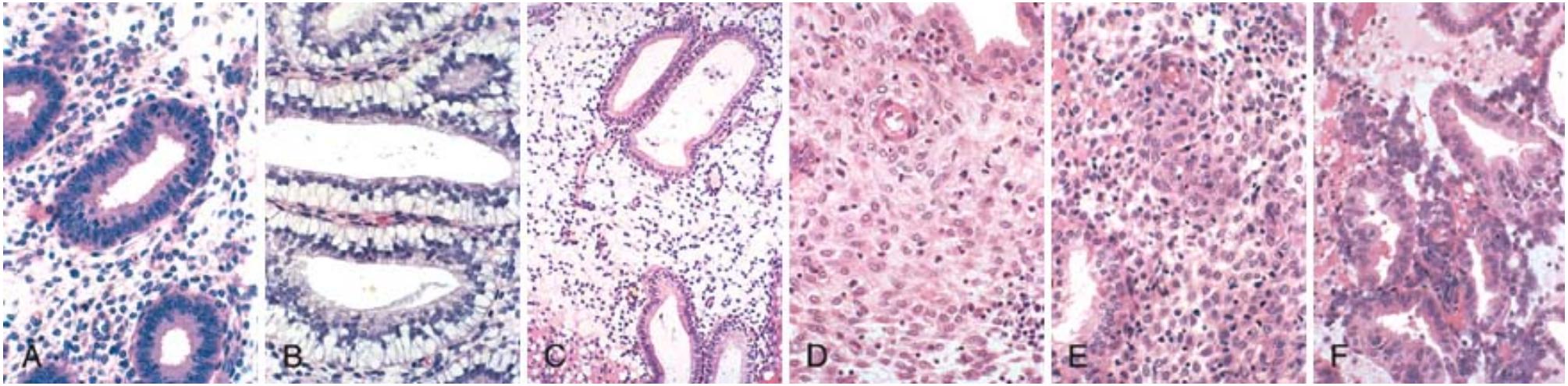


TABLE 22-2 -- Causes of Abnormal Uterine Bleeding by Age Group

Age Group	Causes
Prepuberty	Precocious puberty (hypothalamic, pituitary, or ovarian origin)
Adolescence	Anovulatory cycle, coagulation disorders
Reproductive age	Complications of pregnancy (abortion, trophoblastic disease, ectopic pregnancy)
	Organic lesions (leiomyoma, adenomyosis, polyps, endometrial hyperplasia, carcinoma)
	Anovulatory cycle
	Ovulatory dysfunctional bleeding (e.g., inadequate luteal phase)
Perimenopausal	Anovulatory cycle
	Irregular shedding
	Organic lesions (carcinoma, hyperplasia, polyps)
Postmenopausal	Organic lesions (carcinoma, hyperplasia, polyps)
	Endometrial atrophy

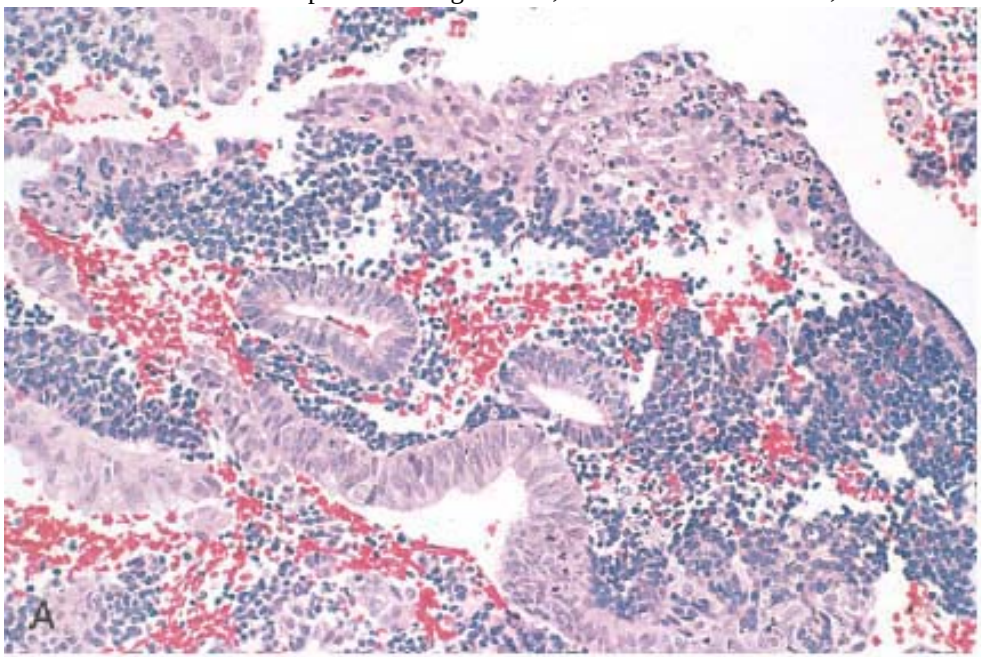
pituitary tumors; (2) a primary lesion of the ovary, such as a functioning ovarian tumor (granulose-theca cell tumors) or polycystic ovaries (see section on ovaries); or (3) a generalized metabolic disturbance, such as marked obesity, severe malnutrition, or any chronic systemic disease. In most patients, however, anovulatory cycles are unexplainable, probably occurring because of subtle hormonal imbalances. Anovulatory cycles are most common at menarche and the perimenopausal period.

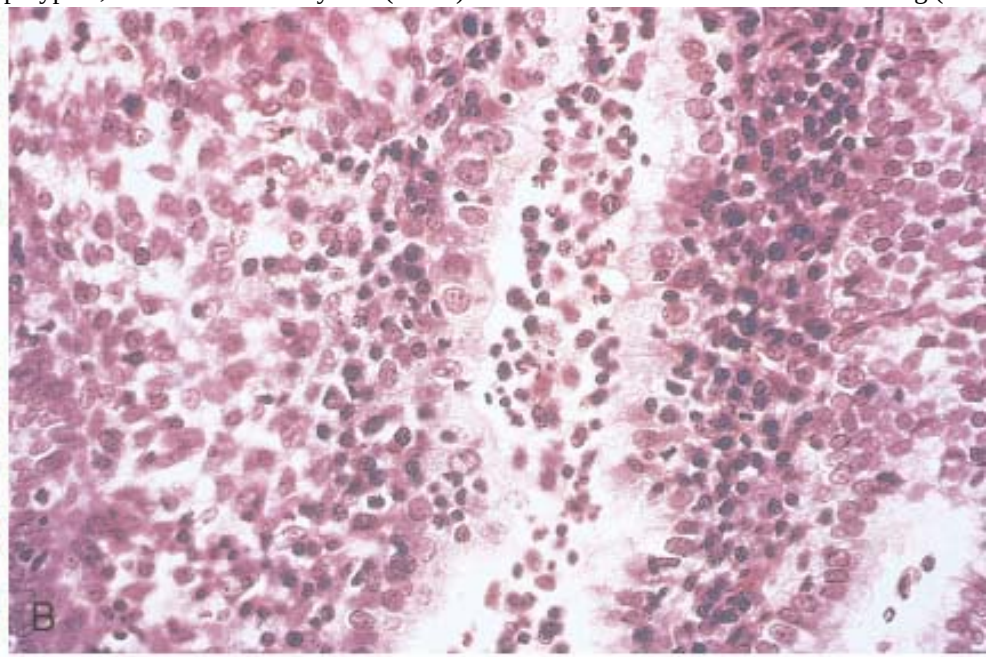
Failure of ovulation results in prolonged, excessive endometrial stimulation by estrogens. Under these circumstances, the endometrial glands undergo mild architectural changes, including cystic dilation (persistent proliferative endometrium). Unscheduled breakdown of the stroma may also occur ("anovulatory menstruation"), with no evidence of endometrial secretory activity (Fig. 22-27*A*). More severe consequences of anovulation are discussed under endometrial hyperplasia.

INADEQUATE LUTEAL PHASE

This term refers to the occurrence of inadequate corpus luteum function and low progesterone output, with an irregular ovulatory cycle. The condition often manifests clinically as infertility, with either increased bleeding or amenorrhea. Endometrial

Figure 22-27 Common causes of abnormal uterine bleeding. *A*, The most common is dysfunctional uterine bleeding, seen here as anovulatory endometrium with stromal breakdown. Note breakdown associated with proliferative glands. *B*, Chronic endometritis. *C*, Endometrial polyp. *D*, Submucosal leiomyoma (*lower*) with attenuation of the endometrial lining (*arrow*).





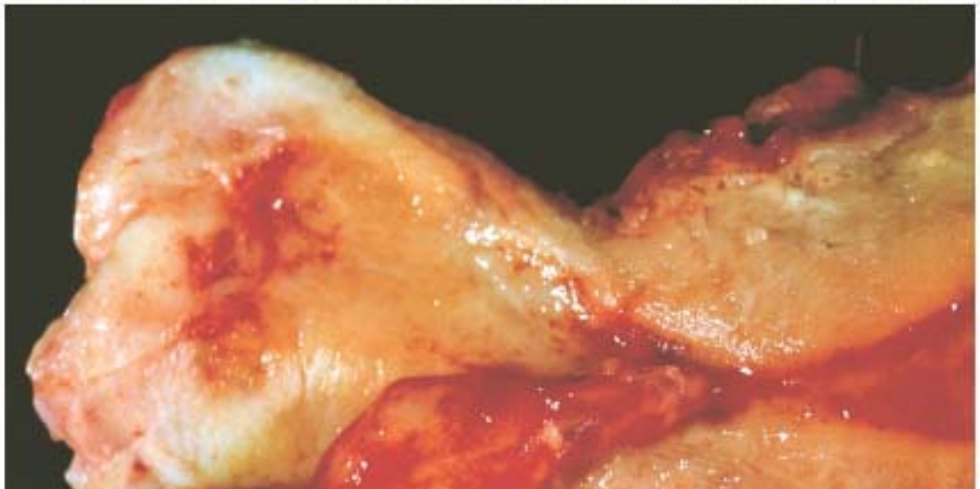


Figure 22-28 Adenomyosis. This disorder is characterized by functional endometrial nests within the myometrium, producing foci of hemorrhagic cysts within the uterine wall.

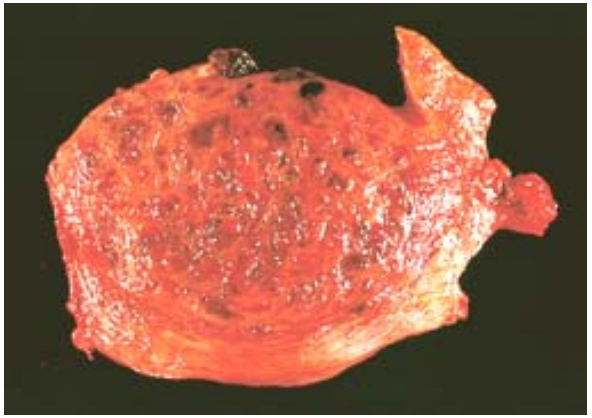


Figure 22-29 The potential origins of endometriosis.

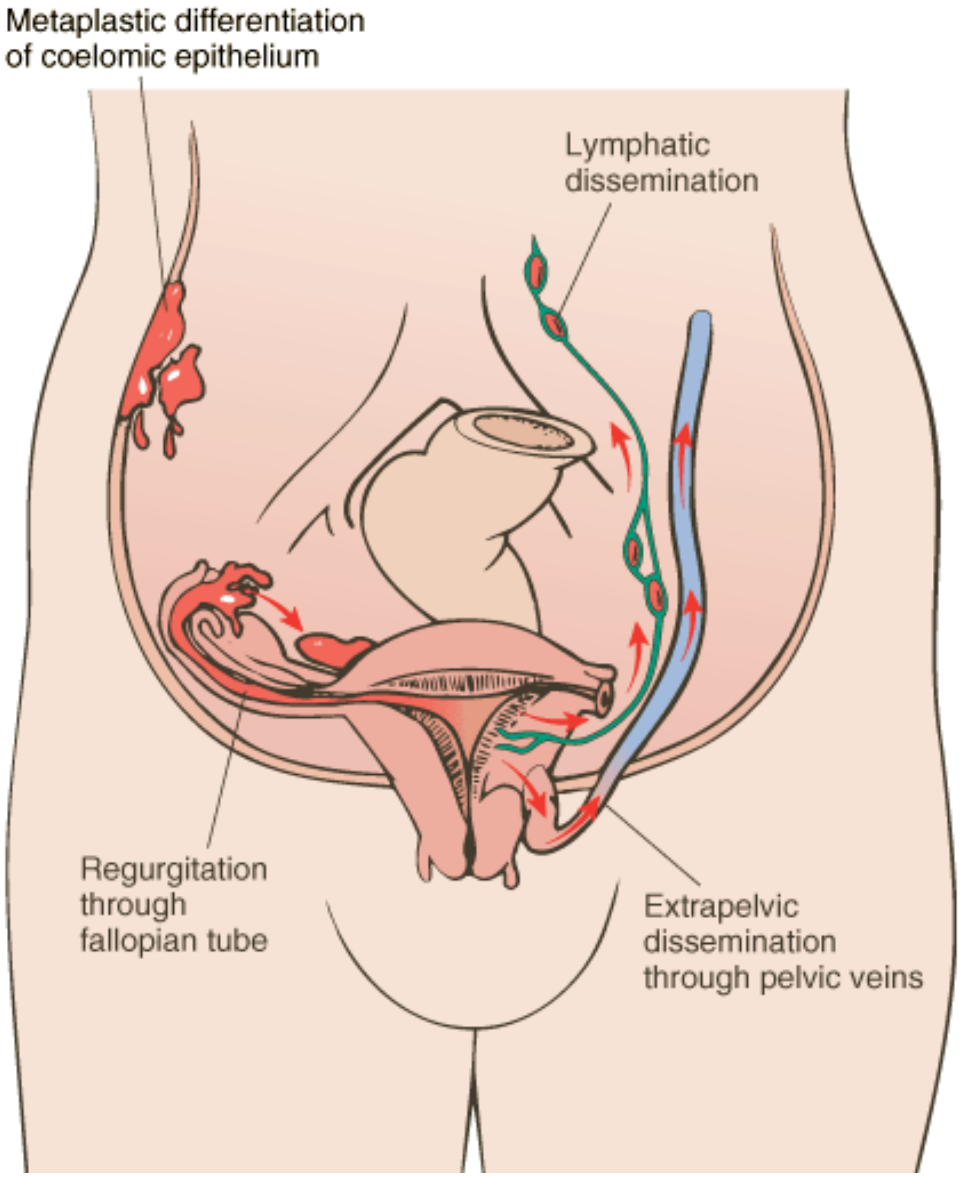


Figure 22-30 Endometriosis. *A*, This ovary has been sectioned to reveal a large endometriotic cyst containing necrotic brown material consisting of degenerated blood (chocolate cyst). *B*, Lining of an endometriotic cyst from a pregnant patient. On the right is an endometrial gland; on the left is endometrial stroma with plump stromal cells characteristic of decidual changes. In the center are numerous macrophages containing hemosiderin.

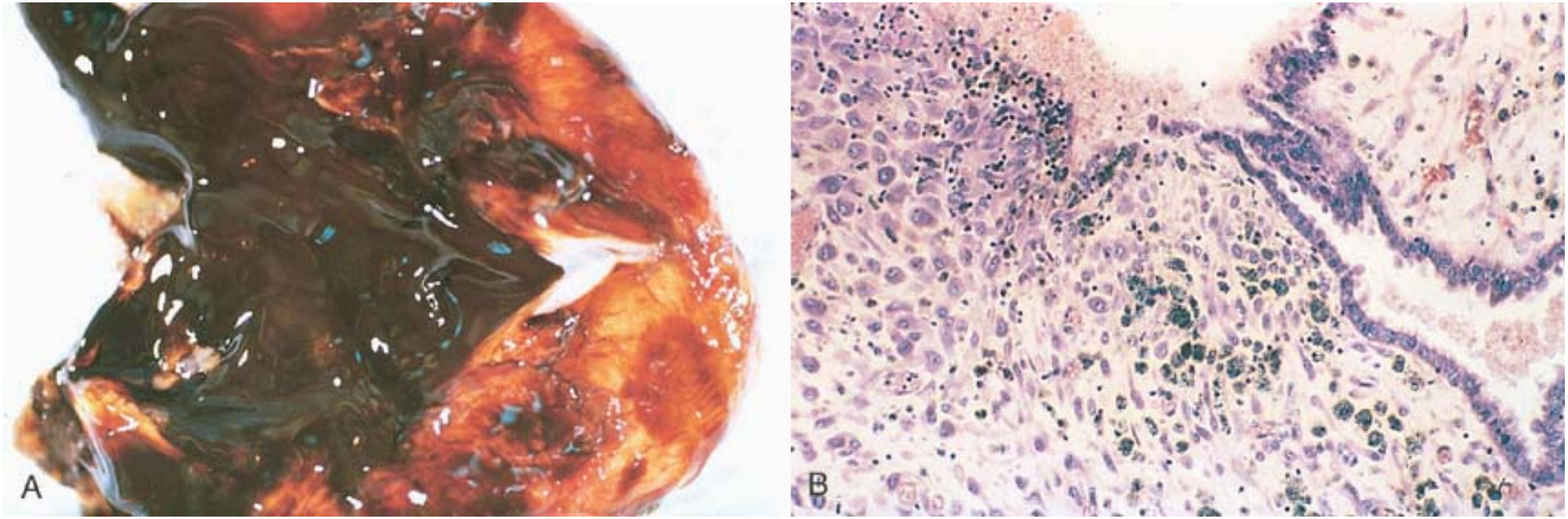


Figure 22-31 *A*, Lower-grade hyperplasias of the endometrium show principally architectural glandular changes with cystic glandular dilatation and are synonymous with anovulatory changes. *B*, Atypical hyperplasias (endometrial intraepithelial neoplasia) exhibit increased gland/stroma ratio (gland crowding) and epithelial stratification (*arrows*). *C*, Loss of *PTEN* gene expression in intraepithelial neoplasia, seen here as absence of staining. Compare to normal glands (*arrows*), which express the gene *(Courtesy of Dr. George L. Mutter, Brigham and Women's Hospital, Boston, MA.) D*, Endometrial hyperplasia with squamous metaplasia.

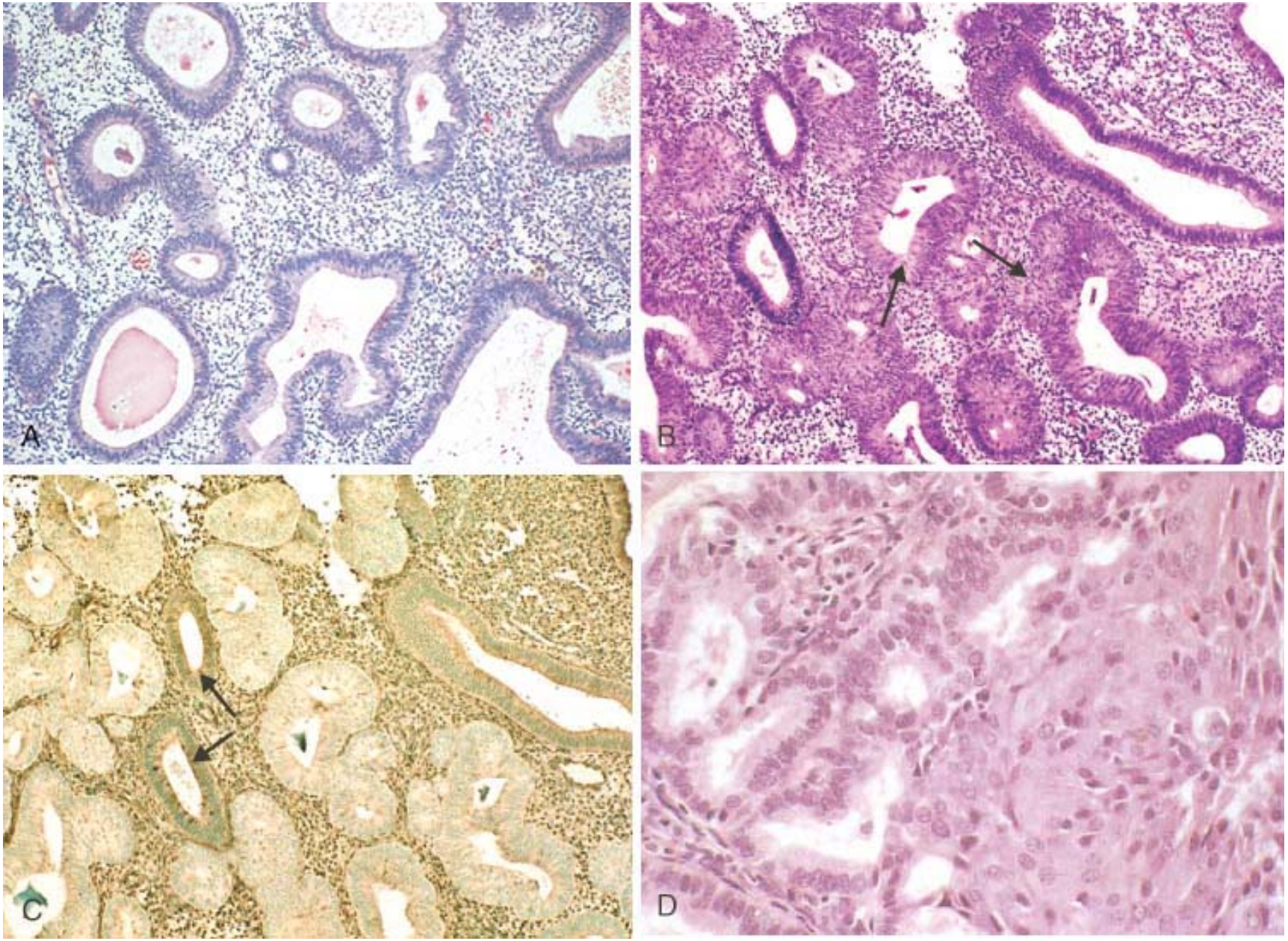
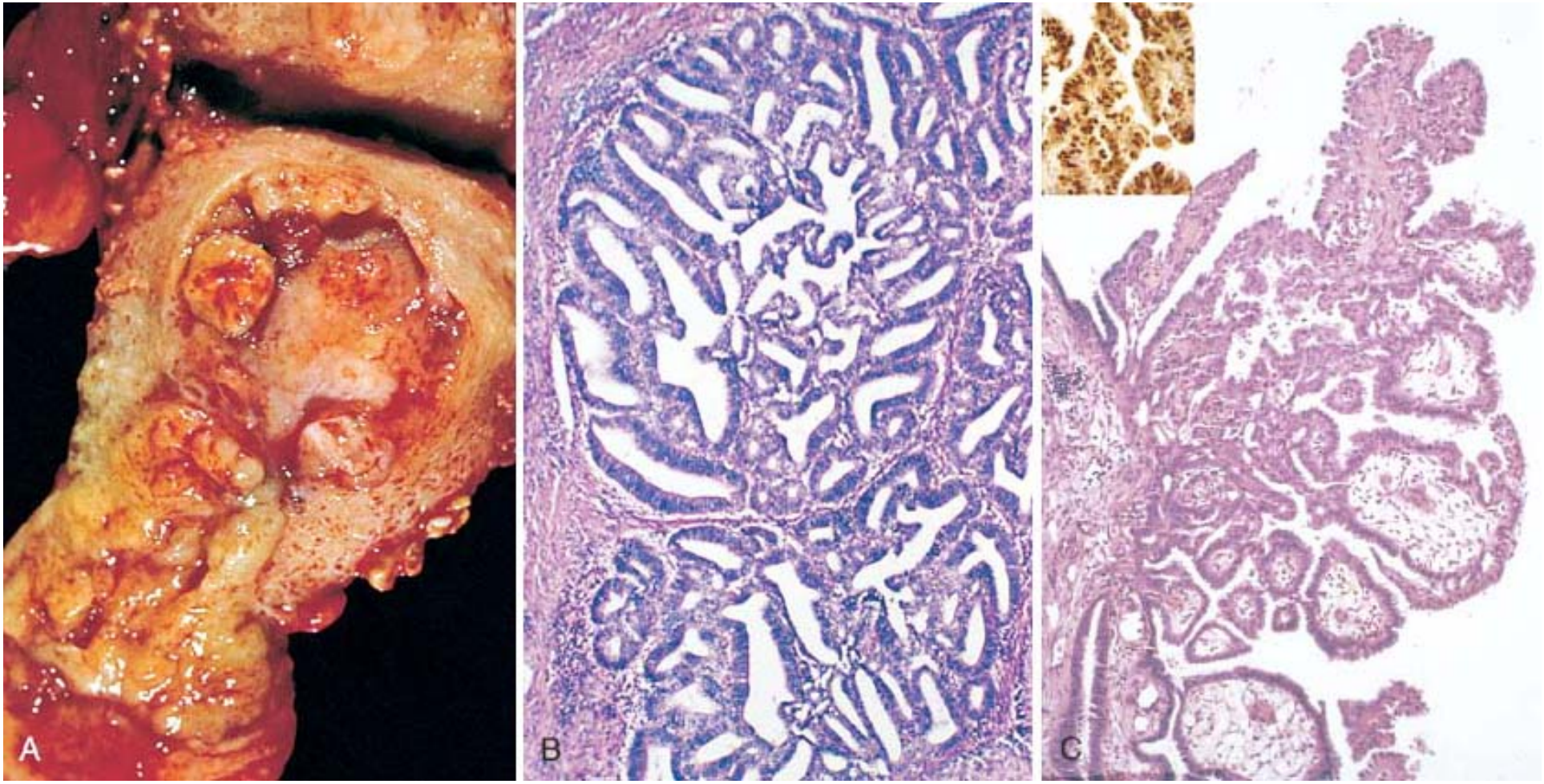


Figure 22-32 *A*, Endometrial adenocarcinoma presenting as a fungating mass in the fundus of the uterus. *B*, Well-differentiated endometrial adenocarcinoma. Glandular architecture is preserved but the tissue is confluent without intervening stroma, which distinguishes carcinoma from hyperplasia. *C*, Papillary serous carcinoma of the endometrium, with accumulation of nuclear p53 protein as seen by immunohistochemistry (*inset upper left*).



Stage II. Carcinoma has involved the corpus and the cervix.

Stage III. Carcinoma has extended outside the uterus but not outside the true pelvis.

Stage IV. Carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or the rectum.

Cases in various stages can also be subgrouped with reference to the three grades described above:

G1. Well-differentiated adenocarcinoma

G2. Differentiated adenocarcinoma with partly solid (less than 50%) areas

G3. Predominantly solid or entirely undifferentiated carcinoma. Serous and clear cell carcinomas are automatically classified as grade 3.

Clinical Course.

Carcinoma of the endometrium may be asymptomatic for periods of time but usually produces irregular vaginal bleeding with excessive leukorrhea. Uterine enlargement in the early stages may be deceptively absent. Cytologic detection on Papanicolaou smears is variable and most likely associated with serous carcinomas, which produce easily detached clusters of cells that are sampled in pap smears. Exclusion of a cervical adenocarcinoma can usually be based on cervix exam and the fact that older age groups are much more susceptible to primary endometrial (versus cervical) cancer. However, upper genital tract carcinomas (fallopian tube and ovary) may be associated with abnormal cytology. The diagnosis of endometrial cancer must ultimately be established by curettage and histologic examination of the tissue.

As would be anticipated, the prognosis depends heavily on the clinical stage of the disease when it is discovered, and its histologic grade and type. In the United States, most women (about 80%) have stage I disease clinically and have well-differentiated or moderately well-differentiated endometrioid carcinomas. Surgery, alone or in combination with irradiation, gives about 90% 5-year survival in stage I (grade 1 or 2) disease. This rate drops to approximately 75% for grade 3/stage I and to 50% or less for stage II and III endometrial carcinomas.

As mentioned, uterine papillary serous and clear cell carcinomas have a propensity for extrauterine (lymphatic or transtubal) spread, even when confined to the endometrium or its surface epithelium. Overall, fewer than 50% of patients with these tumors are alive 3 years after diagnosis and 35% after 5 years. If peritoneal cytology and adnexal histologic exam are negative, the five year survival of stage I disease is approximately 80% to 85%.[82] The additional advantage of prophylactic radiation or chemotherapy in early-stage disease is unclear.[83] [84]

Tumors of the Endometrium with Stromal Differentiation

A proportion of endometrial adenocarcinomas undergo stromal differentiation and are termed carcinosarcomas. A second group is composed of stromal neoplasias in association with benign glands (adenosarcomas). A third group consists of pure stromal neoplasms, ranging from benign (stromal nodule) to malignant (stromal sarcoma). Together, these tumors comprise less than 5% of endometrial cancers.

CARCINOSARCOMAS

Carcinosarcomas (formerly termed malignant mixed müllerian tumors) consist of endometrial adenocarcinomas in which malignant stromal differentiation takes place.[85] The stroma tends to differentiate into a variety of malignant mesodermal components, including muscle, cartilage, and even osteoid. The epithelial and stromal components are presumably derived from the same cell, a concept supported by the observation that the stromal cells often stain positive for epithelial cell markers. Carcinosarcomas occur in postmenopausal women and manifest, similarly to adenocarcinoma, with postmenopausal bleeding. Many affected patients give a history of previous radiation therapy.

Morphology.

In gross appearance, such tumors are somewhat more fleshy than adenocarcinomas, may be bulky and polypoid, and sometimes protrude through the cervical os. On histology, the tumors consist of adenocarcinoma mixed with the stromal (sarcoma) elements (Fig. 22-33*A*); alternatively, the tumor may contain two distinct and separate epithelial and mesenchymal components. Sarcomatous components may mimic extrauterine tissues (i.e., striated muscle cells, cartilage, adipose tissue, and bone).

Outcome is determined primarily by depth of invasion and stage. As with endometrial carcinomas, the prognosis may be influenced by the grade and type of the adenocarcinoma, being poorest with serous differentiation. It is noteworthy that carcinosarcomas usually metastasize as adenocarcinomas. The tumors are highly malignant, and patients have a 5-year survival rate of 25% to 30%.[85]

Figure 22-33 *A*, Carcinosarcoma, showing both epithelial (*upper right*) and stromal (*arrow*) differentiation. *B*, Endometrial stromal sarcoma infiltrating myometrium. *C*, Fluorescent in situ hybridization using two fluorescently labeled probes (*green and red*) that flank the breakpoint of the gene involved in the chromosomal translocation t(7;17) in endometrial stromal sarcoma. The yellow signal is indicative of the normal copy of chromosome 7. A separate green and red signal indicates that the gene on chromosome 7 has been redistributed. *(Courtesy of Dr. Marisa R. Nucci, Brigham and Women's Hospital, Boston, MA.)*

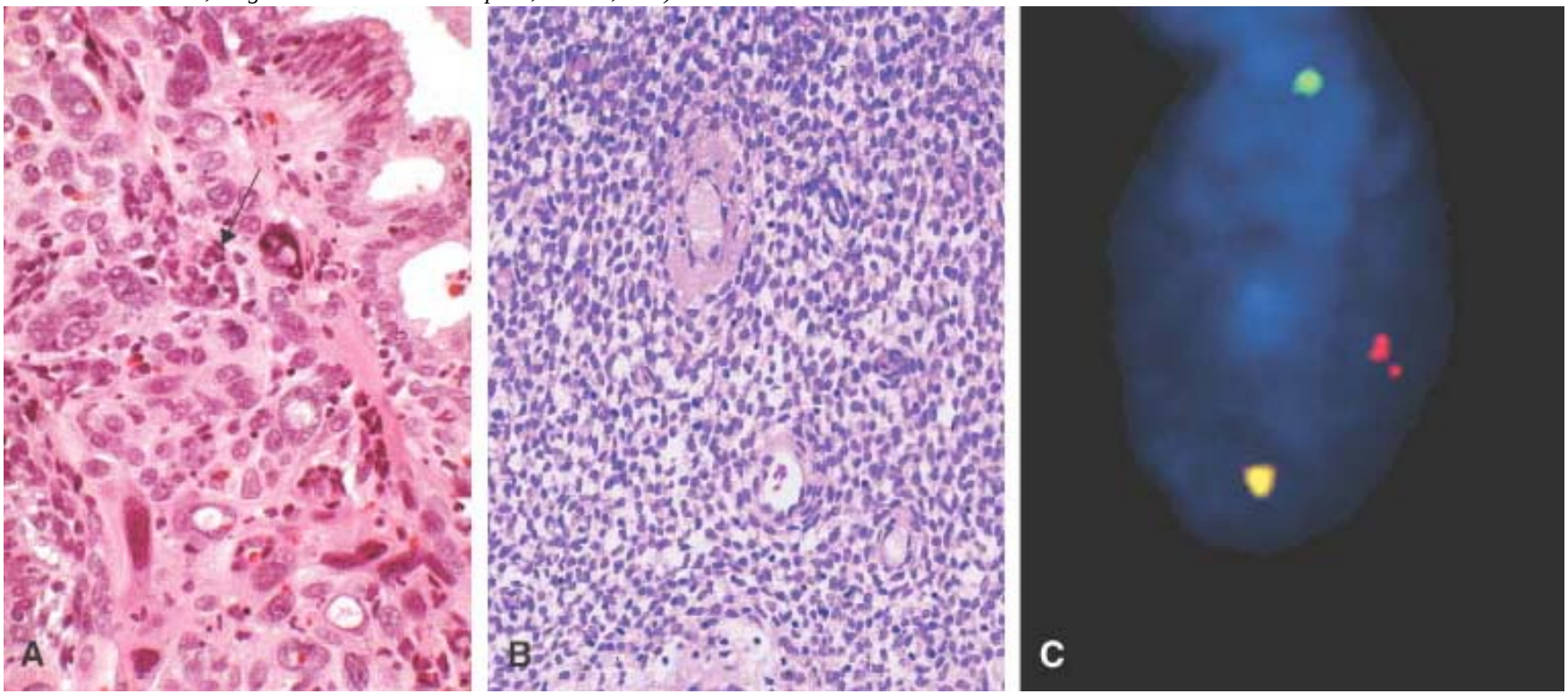


Figure 22-34 *A*, Leiomyomas of the myometrium. The uterus is opened to reveal the tumors bulging into the endometrial cavity and displaying a firm white appearance on sectioning. *B*, Leiomyoma showing well-differentiated, regular, spindle-shaped smooth muscle cells.

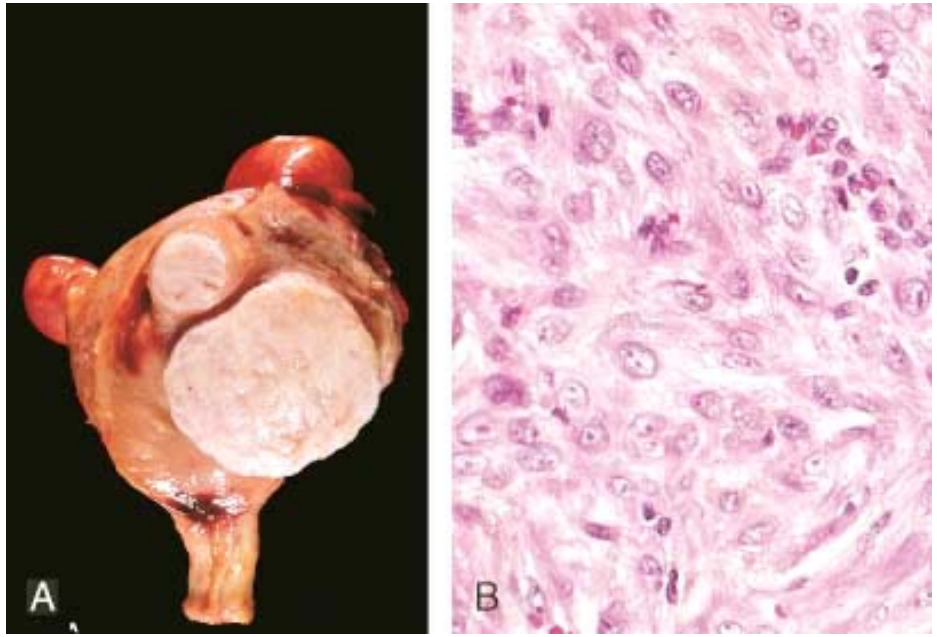
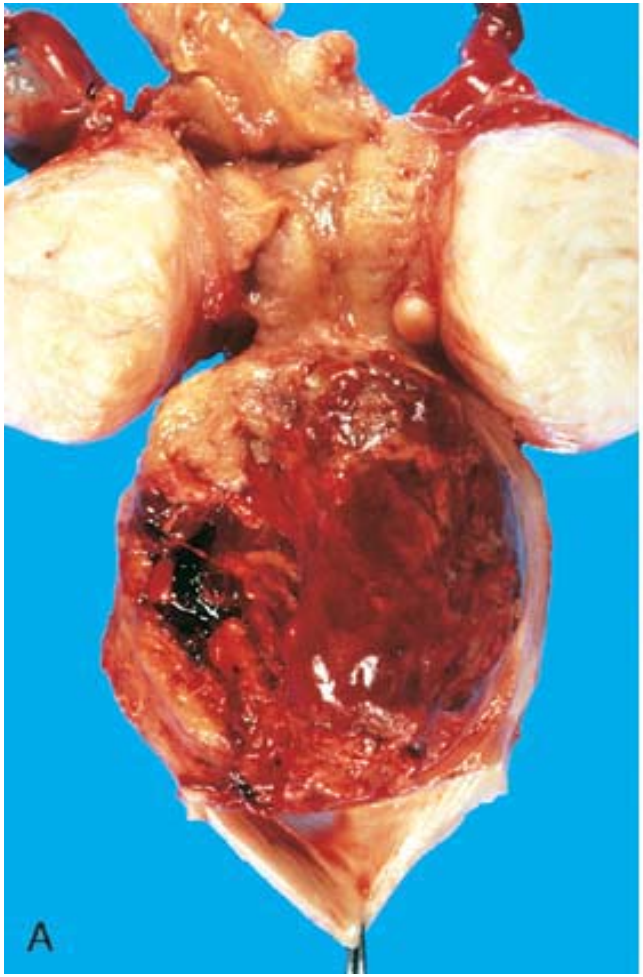


Figure 22-35 Leiomyosarcoma. *A,* A large hemorrhagic tumor mass distends the lower corpus and is flanked by two leiomyomas. *B,* The tumor cells are irregular in size and have hyperchromatic nuclei.



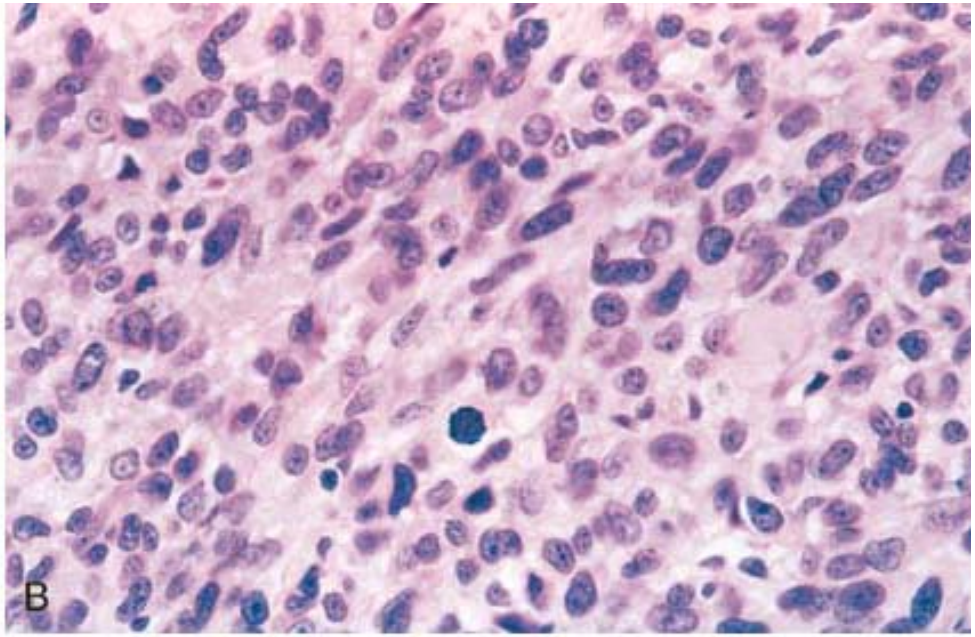


Figure 22-36 Polycystic ovarian disease and cortical stromal hyperplasia. *A,* The ovarian cortex reveals numerous clear cysts. *B,* Sectioning of the cortex reveals several subcortical cystic follicles. *C,* Cystic follicles seen in a low-power microphotograph. *D,* Cortical stromal hyperplasia manifests as diffuse stromal proliferation with symmetrical enlargement of the ovary.

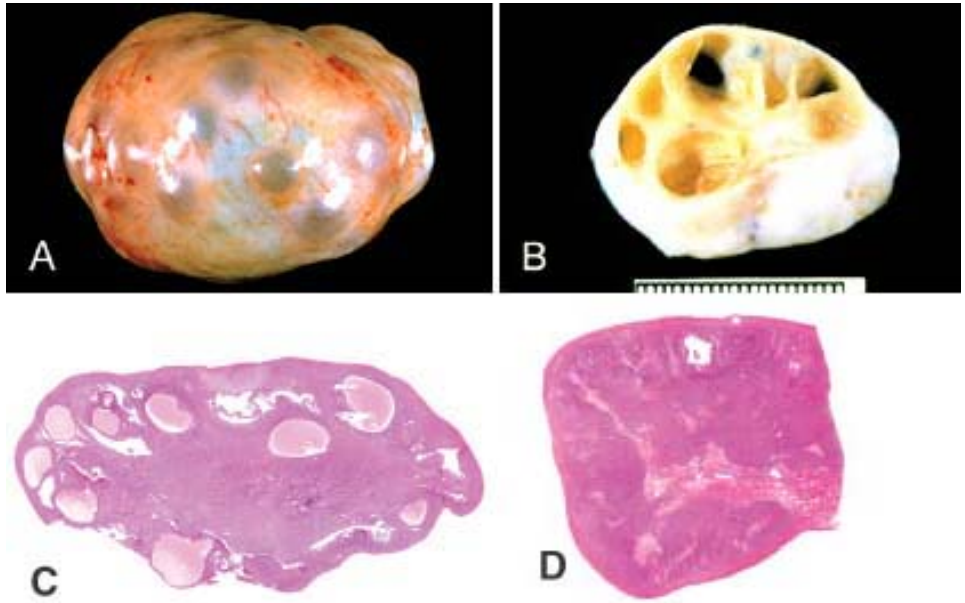


TABLE 22-3 -- Ovarian Neoplasms (1993 WHO Classification)

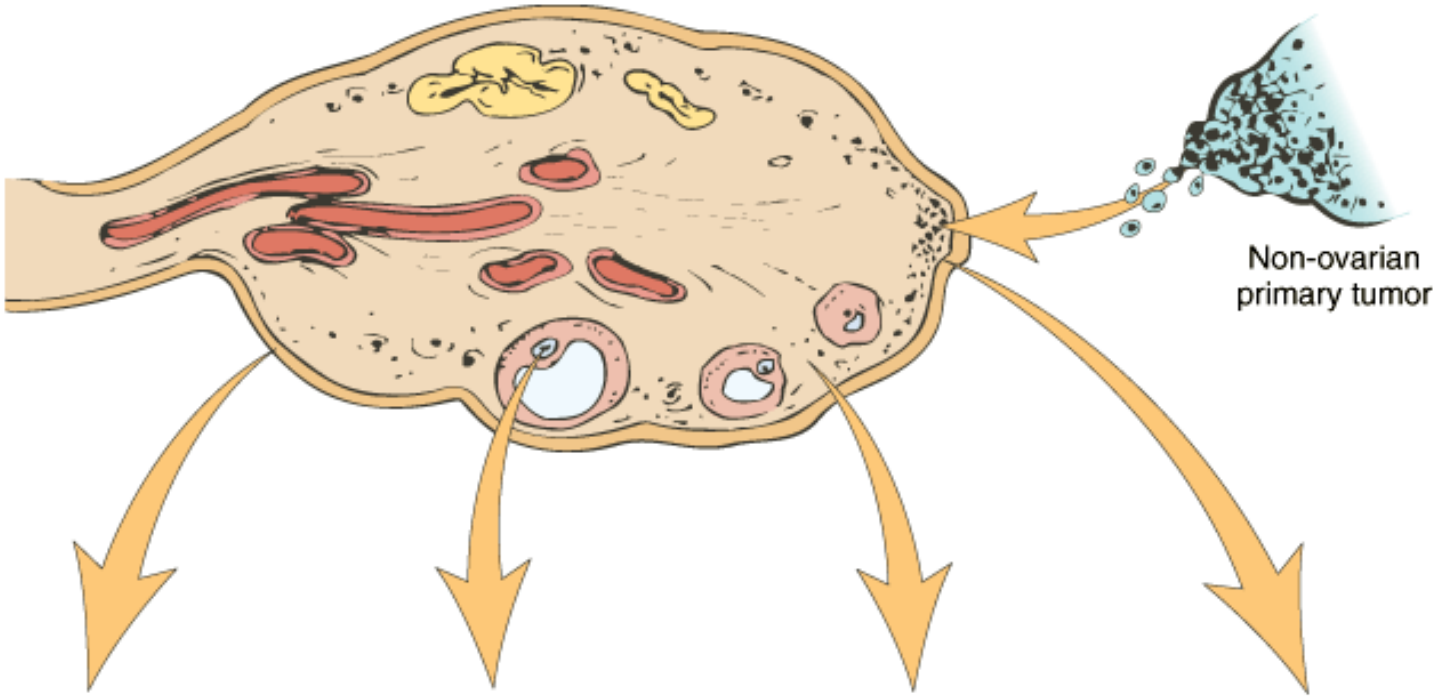
Surface Epithelial-Stromal Tumors
Serous tumors
••Benign (cystadenoma)
••Cystadenoma of borderline malignancy
••Malignant (serous cystadenocarcinoma)
Mucinous tumors, endocervical-like and intestinal type
••Benign
••Of borderline malignancy
••Malignant
Endometrioid tumors
••Benign
••Of borderline malignancy
••Malignant
••Epithelial-stromal
••••Adenosarcoma
••••Mesodermal (müllerian) mixed tumor

••Clear cell tumors
••••Benign
••••Of borderline malignancy
••••Malignant
••Transitional cell tumors
••••Brenner tumor
••••Brenner tumor of borderline malignancy
••••Malignant Brenner tumor
••••Transitional cell carcinoma (non-Brenner type)
Sex Cord-Stromal Tumors
Granulosa-stromal cell tumors
••Granulosa cell tumors
••Tumors of the thecoma-fibroma group
Sertoli-stromal cell tumors; androblastomas
Sex cord tumor with annular tubules
Gynandroblastoma
Steroid (lipid) cell tumors
Germ Cell Tumors
Teratoma
••Immature
••Mature (adult)
••••Solid
••••Cystic (dermoid cyst)
••Monodermal (e.g., struma ovarii, carcinoid)
Dysgerminoma
Yolk sac tumor (endodermal sinus tumor)
Mixed germ cell tumors
Malignant, Not Otherwise Specified
Metastatic Nonovarian Cancer (from Nonovarian Primary)
Data from the WHO Classification. (Courtesy Dr. Robert Scully, Massachusetts General Hospital, Boston, MA.)

being a common site of metastases from a variety of other cancers.

Although some of the specific tumors have distinctive features and are hormonally active, most are nonfunctional and tend to produce relatively mild symptoms until they have reached a large size. Malignant tumors have usually spread outside the ovary by the time a definitive diagnosis is made. Some of these tumors, principally epithelial tumors, tend to be bilateral. Table 22-4 lists these tumors and their subtypes and shows the frequency of bilateral occurrence. Abdominal pain and distention, urinary and gastrointestinal tract symptoms due to compression by tumor or cancer invasion, and abdominal and vaginal bleeding are the most common symptoms. The benign forms may be entirely asymptomatic and occasionally are unexpected findings on abdominal or pelvic examination or during surgery.

Figure 22-37 Derivation of various ovarian neoplasms and some data on their frequency and age distribution.



ORIGIN	SURFACE EPITHELIAL CELLS (Surface epithelial–stromal cell tumors)	GERM CELL	SEX CORD–STROMA	METASTASIS TO OVARIES
Overall frequency	65%-70%	15%-20%	5%-10%	5%
Proportion of malignant ovarian tumors	90%	3%-5%	2%-3%	5%
Age group affected	20+ years	0-25+ years	All ages	Variable
Types	• Serous tumor • Mucinous tumor • Endometrioid tumor • Clear cell tumor • Brenner tumor • Cystadenofibroma	• Teratoma • Dysgerminoma • Endodermal sinus tumor • Choriocarcinoma	• Fibroma • Granulosa–theca cell tumor • Sertoli–Leydig cell tumor	

TABLE 22-4 -- Frequency of Major Ovarian Tumors

Type	Percentage of Malignant Ovarian Tumors	Percentage That Are Bilateral

Serous	40	
••Benign (60%)		25
••Borderline (15%)		30
••Malignant (25%)		65
Mucinous	10	
••Benign (80%)		5
••Borderline (10%)		10
••Malignant (10%)		<5
Endometrioid carcinoma	20	40
Undifferentiated carcinoma	10	—
Clear cell carcinoma	6	40
Granulosa cell tumor	5	5
Teratoma		15
••Benign (96%)		
••Malignant (4%)	1	Rare
Metastatic	5	>50
Others	3	—

TUMORS OF MÜLLERIAN EPITHELIUM

Most primary neoplasms in the ovary fall within this category. There are three major types of such tumors: serous, endometrioid and mucinous tumors.[98] These neoplasms range in size and composition. Tumors may be small and grossly imperceptible or massive, filling the pelvis and even the abdominal cavity. Components of the tumors may include cystic areas (cystadenomas), cystic and fibrous areas (cystadenofibromas), and predominantly fibrous areas (adenofibromas). On gross examination, *the risk of malignancy increases as a function of the amount of discernible solid epithelial growth*, including papillary projections of soft tumor, thickened tumor lining the cyst spaces, or solid necrotic friable tissue depicting necrosis.

The most widely accepted theory for the derivation of müllerian epithelial tumors is through the transformation of coelomic mesothelium. This view is based on the embryologic pathway by which the müllerian *ducts are formed and evolve into serous (tubal), endometrioid (endometrium), and mucinous (cervix)* epithelia present in the normal female genital tract (see the section on anatomy). Such tumors occur predominantly in the ovary because coelomic epithelium is incorporated into the ovarian cortex to form mesothelial inclusion cysts. This incorporation occurs by the formation of surface adhesions, atrophy with epithelial infolding, and

repair of ovulation sites. The close association of ovarian carcinomas with either the ovarian surface mesothelium or inclusion cysts may explain the development of extraovarian

carcinomas of similar histology from similar coelomic epithelial rests (so-called endosalpingiosis) in the mesentery.[98] However, this is clearly an oversimplification of the pathogenesis of ovarian cancer. For example, endometrioid carcinomas may be derived from transplanted endometriosis, and some ovarian serous and mucinous (müllerian mucinous) tumors arise in association with endometriosis.[98] The origin of most mucinous tumors is unclear. Some arise within endometriosis; other proposed origins include teratomatous epithelium.

An intriguing question is the origin of the cortical müllerian inclusion cyst (CIC) and its relationship to serous carcinomas (Fig. 22-38). The continuity of pelvic mesothelium with these müllerian cysts suggests that the former gives rise to the latter. However, similarities between some of these cysts and endometrial or tubal epithelium raise the possibility of a uterine or tubal origin for the epithelium. Direct links between CICs and serous carcinoma are not common and studies of ovaries from women with *BRCA* mutations have not disclosed premalignant changes in these cysts.[100] Regardless of their specific origin(s), ovarian epithelial tumors composed of serous, mucinous, and endometrioid cell types are emblematic of the plasticity of müllerian epithelium and range from clearly benign, to tumors of borderline malignancy, to malignant tumors.[98]

Serous Tumors

These common cystic neoplasms are lined by tall, columnar, ciliated epithelial cells and are filled with clear serous fluid. Although the term serous appropriately describes the cyst fluid, it has become synonymous with the tubal-like epithelium in these tumors. Together the benign, borderline, and malignant types account for about 30% of all ovarian tumors. About 75% are benign or of borderline malignancy, and 25% are malignant. Serous cystadenocarcinomas account for approximately 40% of all cancers of the ovary and are the most common malignant ovarian tumors. Benign and borderline tumors are most common between the ages of 20 and 50 years. Cystadenocarcinomas occur later in life on average, although somewhat earlier in familial cases.

Figure 22-38 Cortical inclusion cysts of the ovary. These cysts appear to arise from the overlying mesothelium and are presumed to be the site of origin for many ovarian epithelial neoplasms. Another source of ovarian epithelium neoplasia is endometriosis (see Figure 22-29).

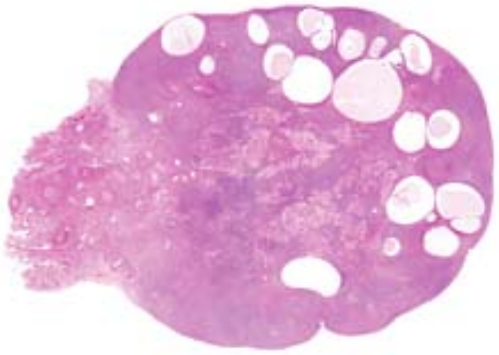


Figure 22-39 *A,* Borderline serous cystadenoma opened to display a cyst cavity lined by delicate papillary tumor growths. *B,* Cystadenocarcinoma. The cyst is opened to reveal a large, bulky tumor mass. *C,* Another borderline tumor growing on the ovarian surface (*lower*).

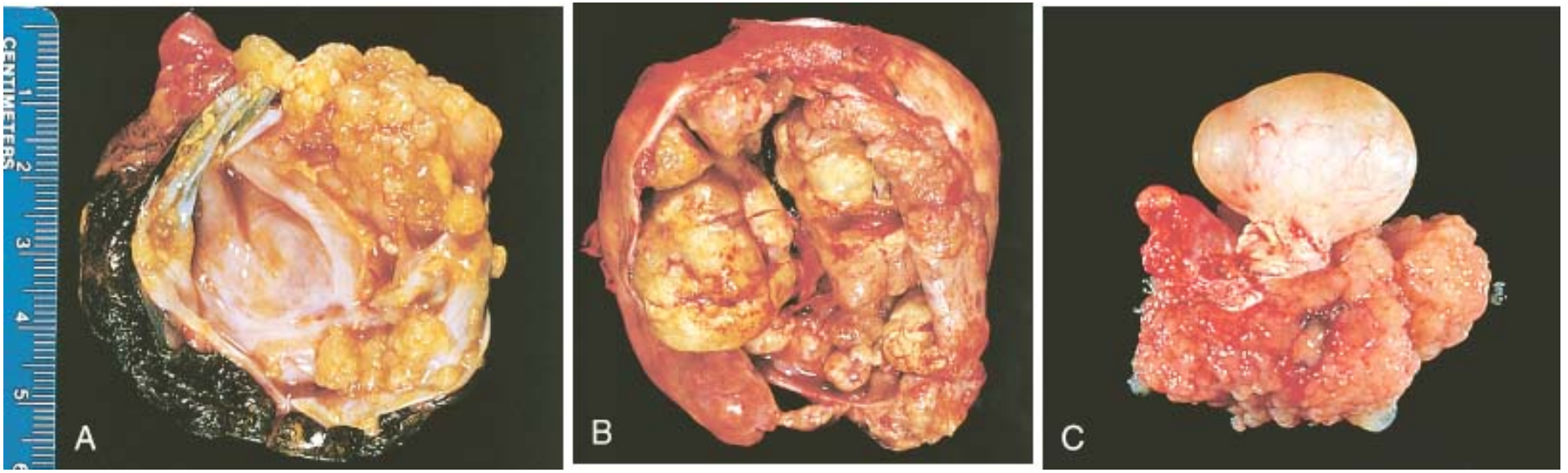


Figure 22-40 Papillary serous cystadenoma revealing stromal papillae with a columnar epithelium.

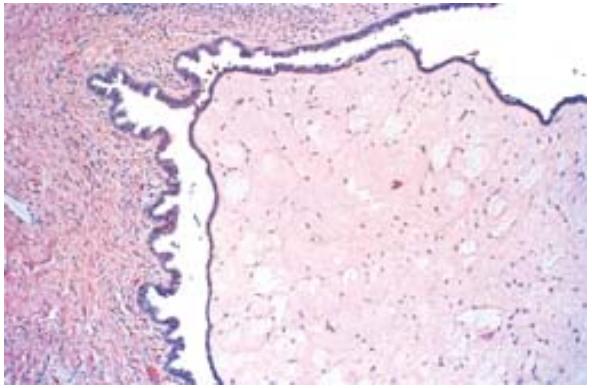


Figure 22-41 Borderline serous cystadenoma exhibiting increased architectural complexity and epithelial cell stratification.

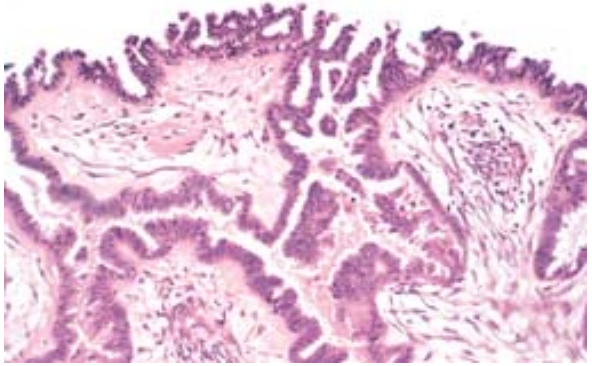


Figure 22-42 Papillary serous cystadenocarcinoma of the ovary with invasion of underlying stroma.

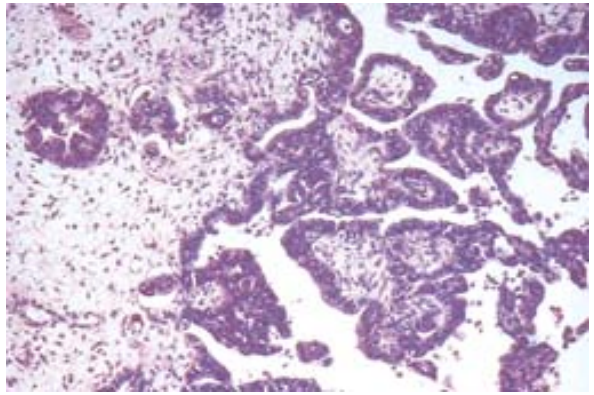


Figure 22-43 Complex micropapillary growth defines a low grade "micropapillary" serous carcinoma.

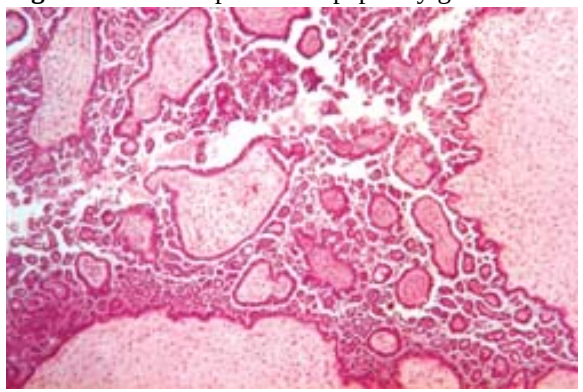


Figure 22-44 *A*, A mucinous cystadenoma with its multicystic appearance and delicate septa. Note the presence of glistening mucin within the cysts. *B*, Columnar cell lining of mucinous cystadenoma.

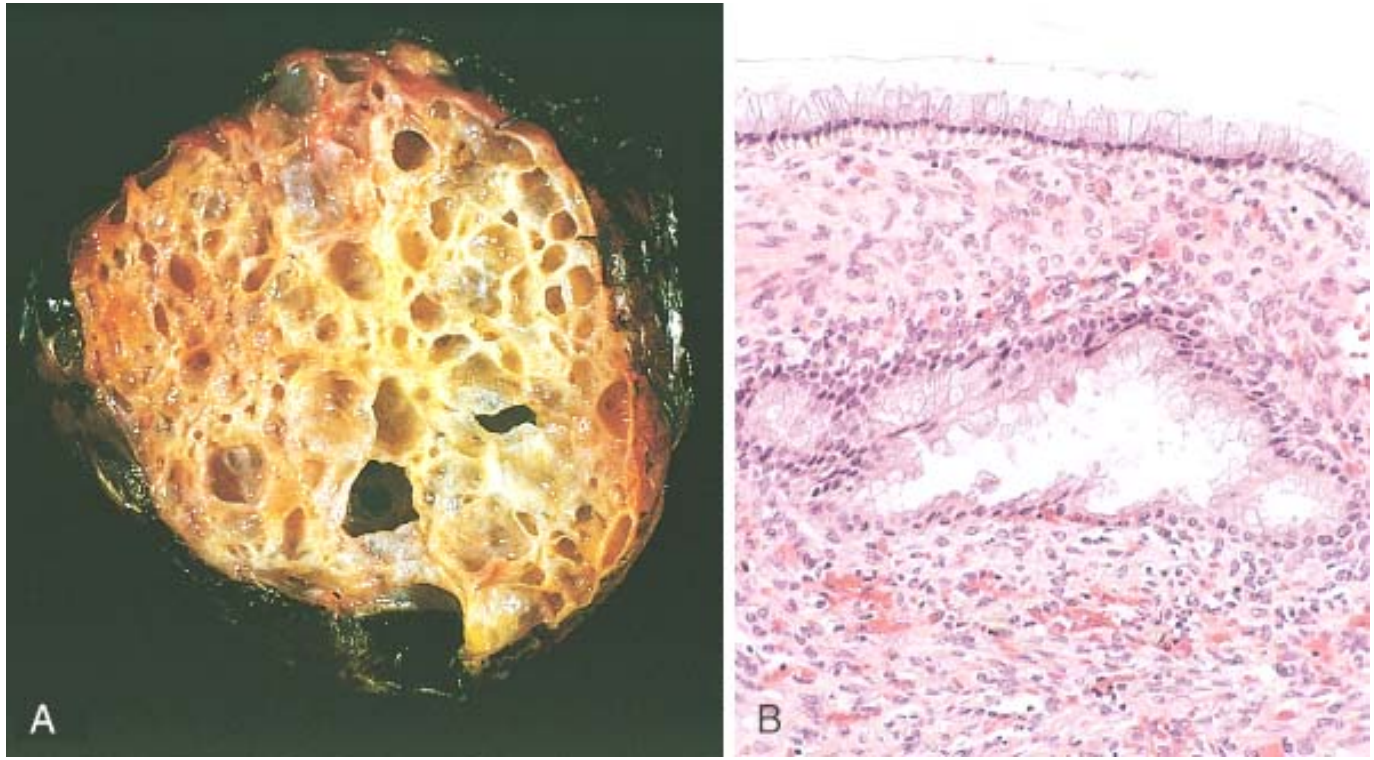


Figure 22-45 *A*, Pseudomyxoma peritonei viewed at laparotomy revealing massive overgrowth of a gelatinous metastatic tumor originating from the appendix. *(Courtesy of Dr. Paul H. Sugarbaker, Washington Hospital Cancer Center, Washington, DC.). B*, Histology of peritoneal implants from an appendiceal tumor, showing mucin-producing epithelium and free mucin (*arrows*).

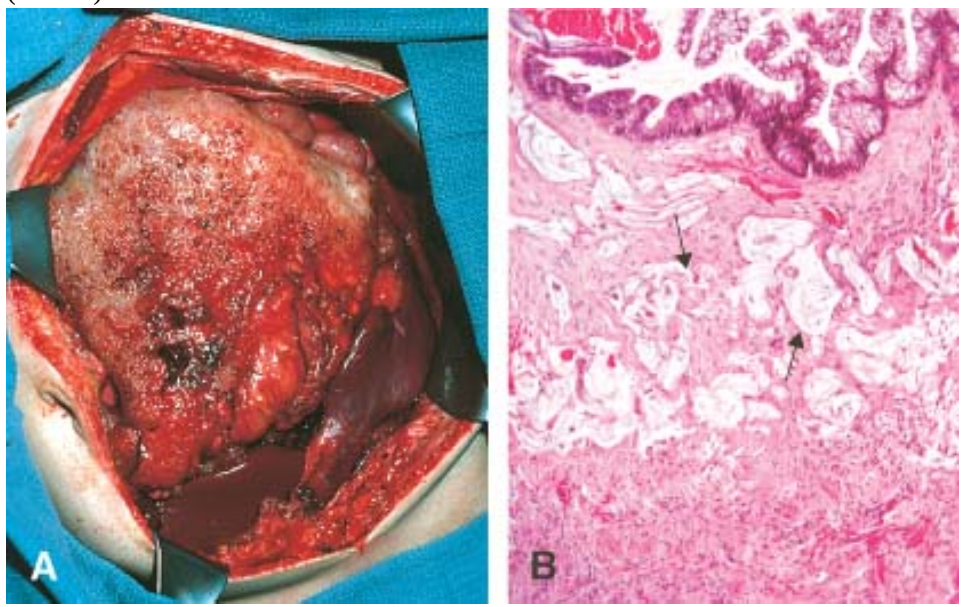


Figure 22-46 *A*, Brenner tumor (*right*) associated with a benign cystic teratoma (*left*). *B*, Histologic detail of characteristic epithelial nests within the ovarian stroma. *(Courtesy of Dr. M. Nucci, Brigham and Women's Hospital, Boston, MA.)*

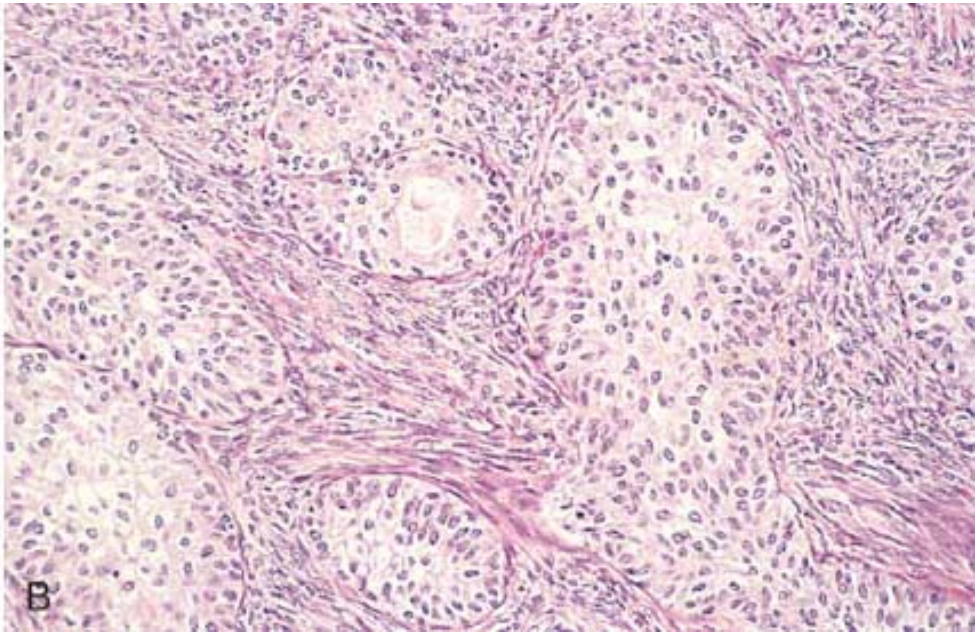


Figure 22-47 Histogenesis and interrelationships of tumors of germ cell origin.

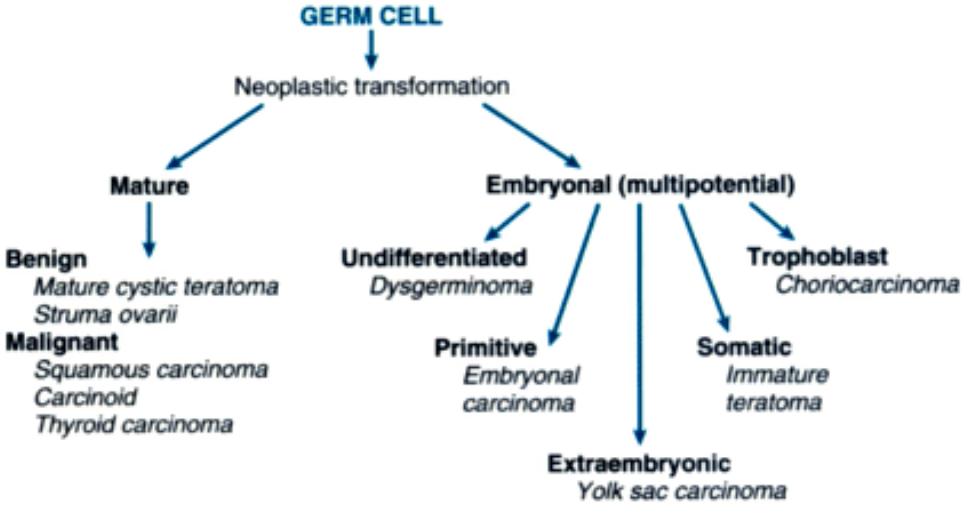


Figure 22-48 Opened mature cystic teratoma (dermoid cyst) of the ovary. Hair (*bottom*) and a mixture of tissues are evident.

Figure 22-49 Benign cystic teratoma. Low-power view of skin (*top*), beneath which there is brain tissue (*bottom*).

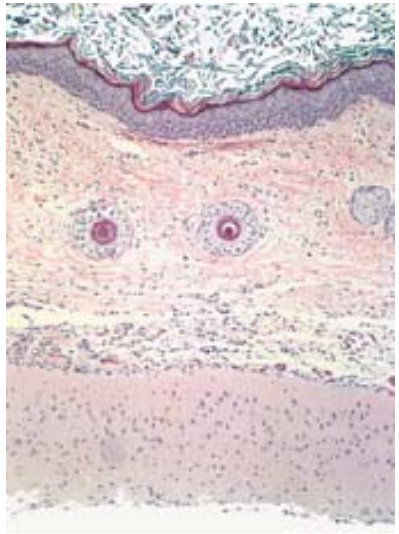


Figure 22-50 Immature teratoma of the ovary illustrating primitive neuroepithelium.

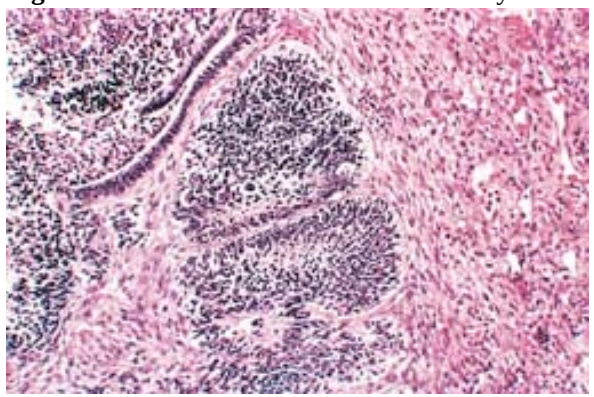


Figure 22-51 Dysgerminoma showing polyhedral tumor cells with round nuclei and adjacent inflammation.

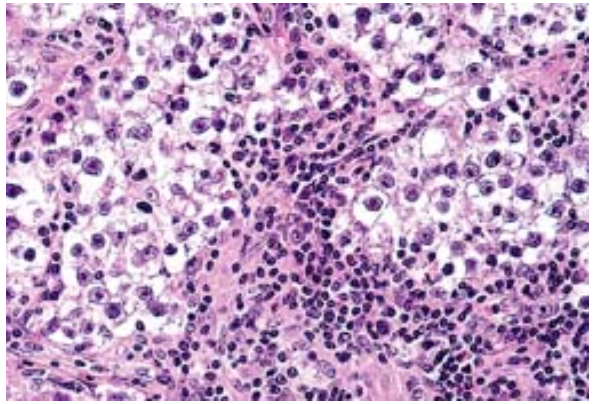


Figure 22-52 A Schiller-Duval body in yolk sac carcinoma.

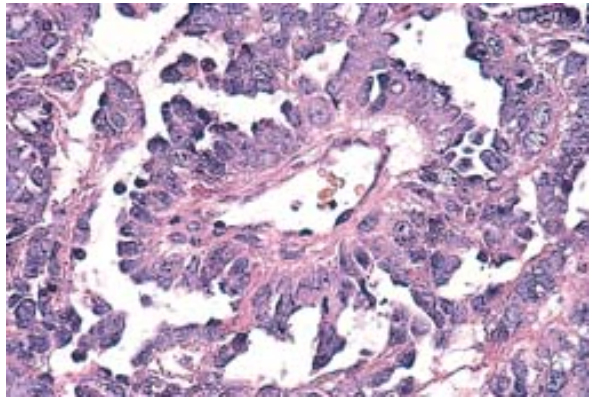


Figure 22-53 Granulosa cell tumor. *A*, The tumor cells are arranged in sheets punctuated by small follicle-like structures (Call-Exner bodies). *B*, Strong immunohistochemical positivity with an antibody to inhibin characterizes these tumors.

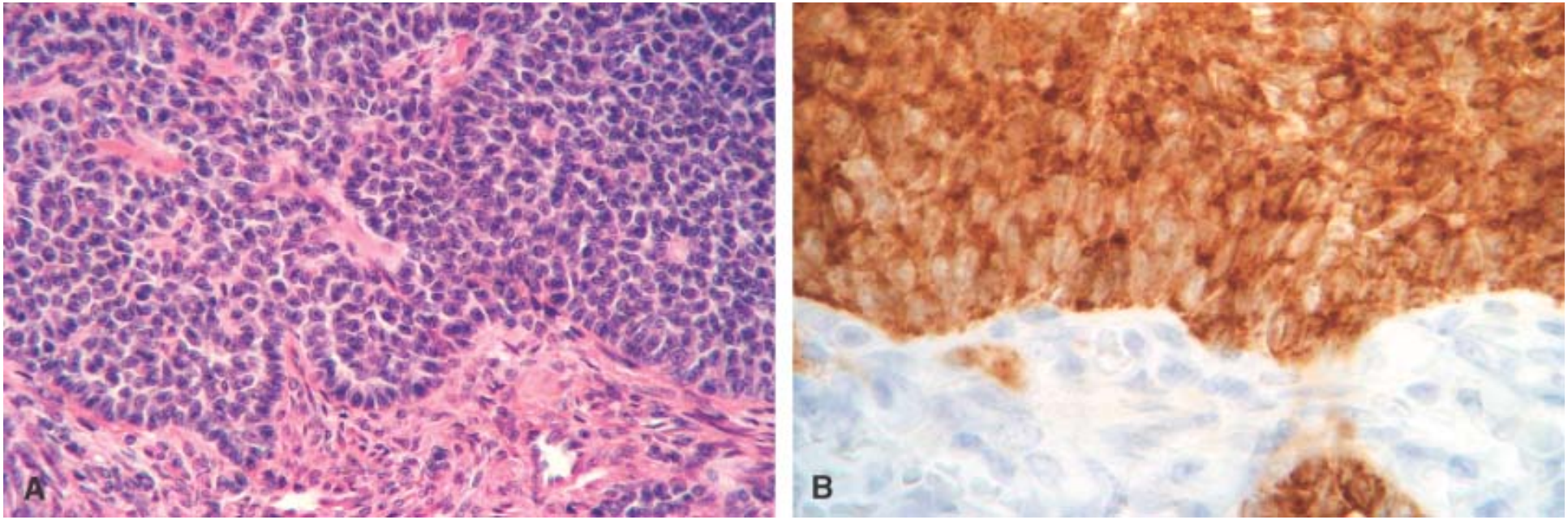


Figure 22-54 *A,* Thecoma-fibroma composed of plump, differentiated stromal cells with thecal appearance. *B,* Large bisected fibroma of the ovary apparent as a white, firm mass (*right*). The fallopian tube is attached.

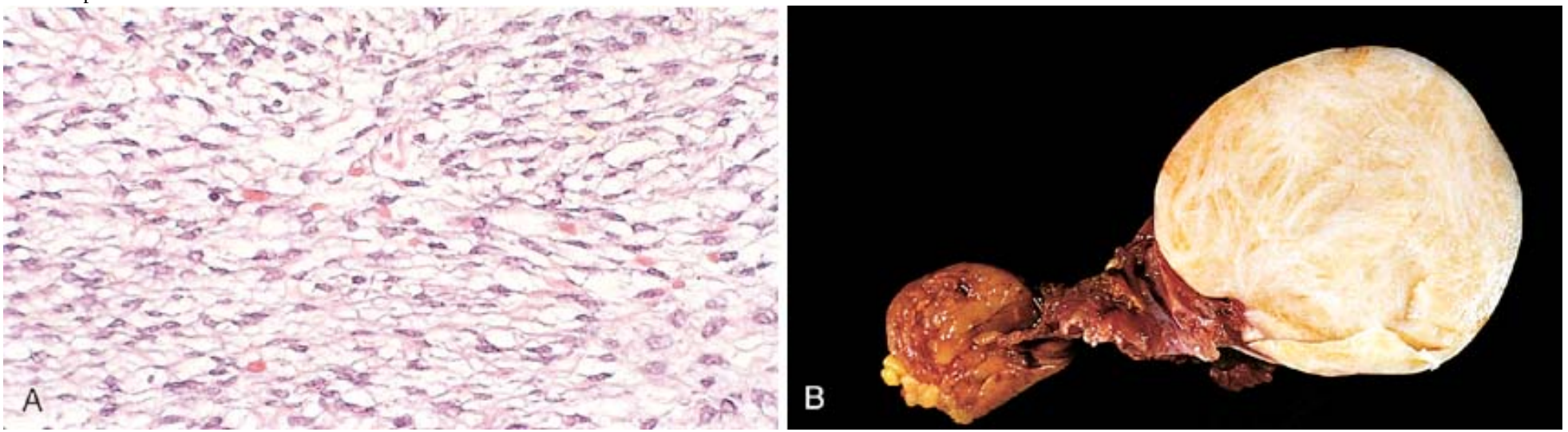


Figure 22-55 Sertoli cell tumor. *A,* Gross photograph illustrating characteristic golden yellow appearance of the tumor. *B,* Photomicrograph showing well-differentiated Sertoli cell tubules. *(Courtesy of Dr. William Welch, Brigham and Women's Hospital, Boston, MA.)*

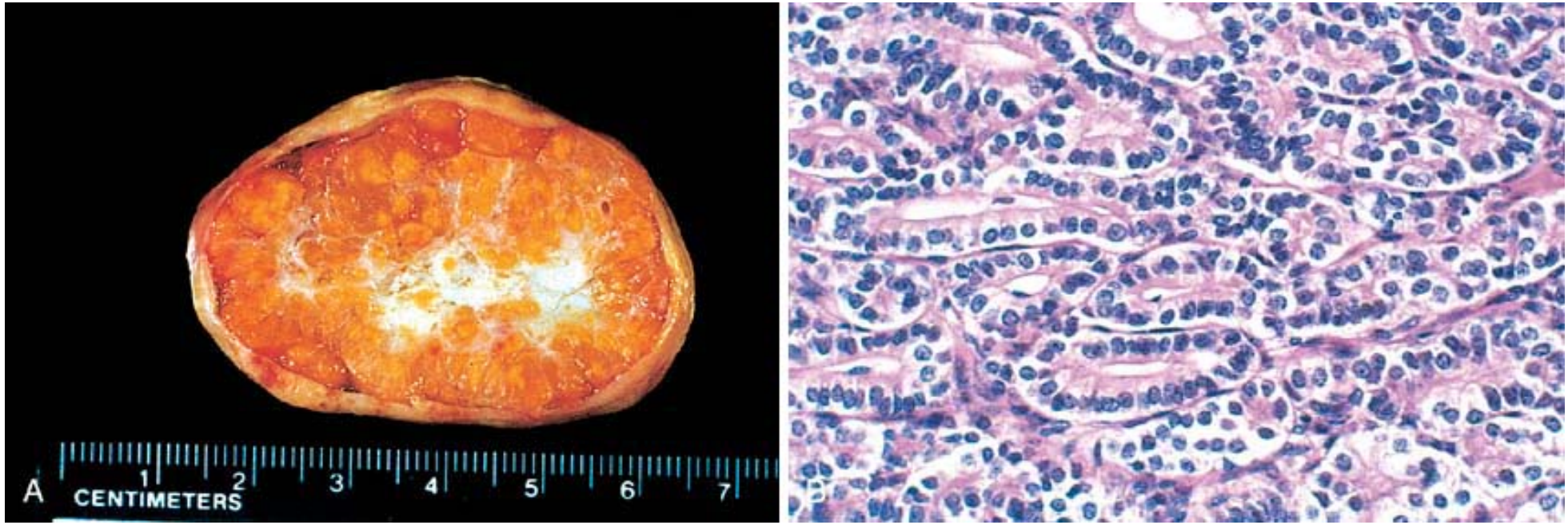


Figure 22-56 Potential sites for ectopic pregnancy, including the fallopian tube, ovary, cornu, and (rarely) abdominal viscera.

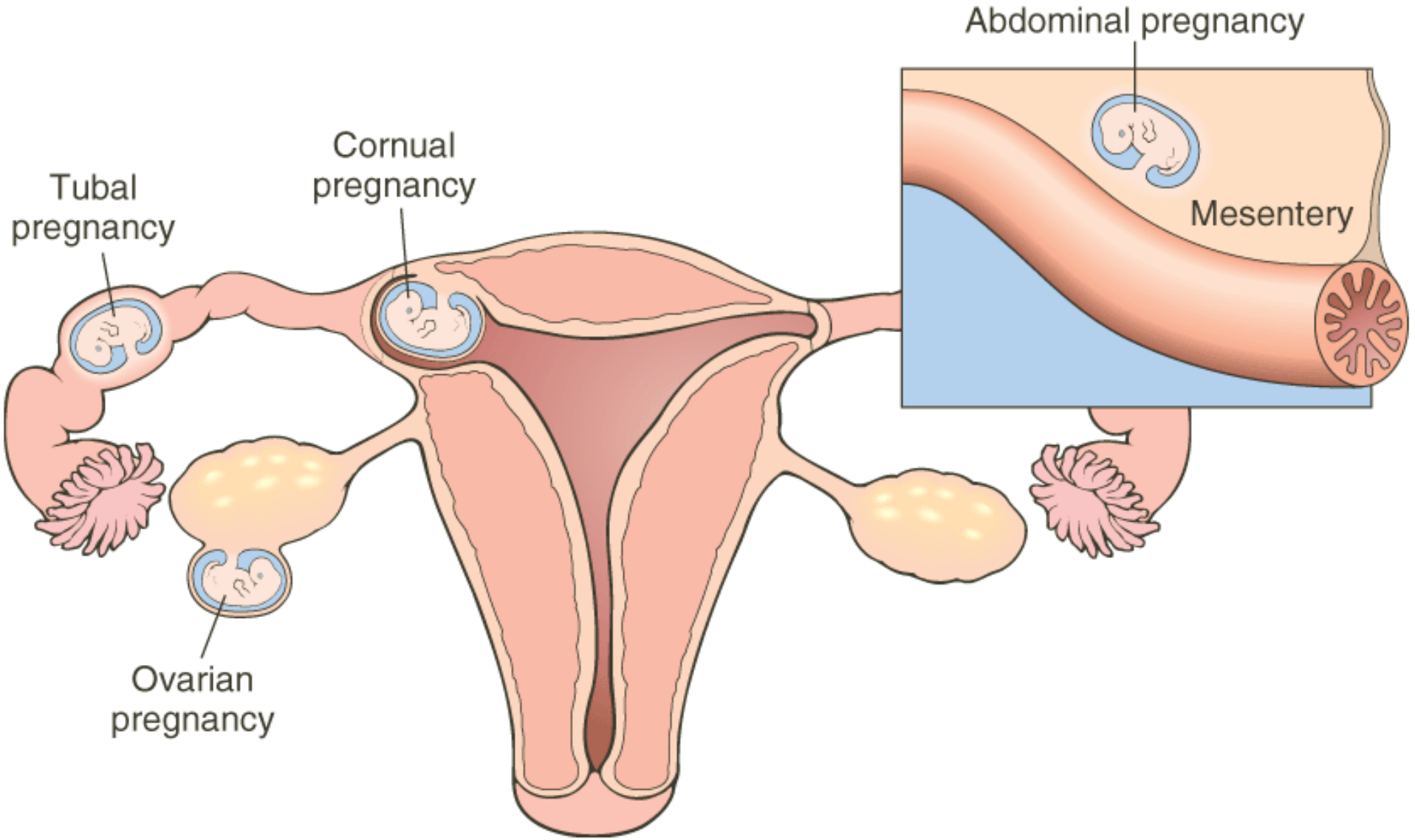
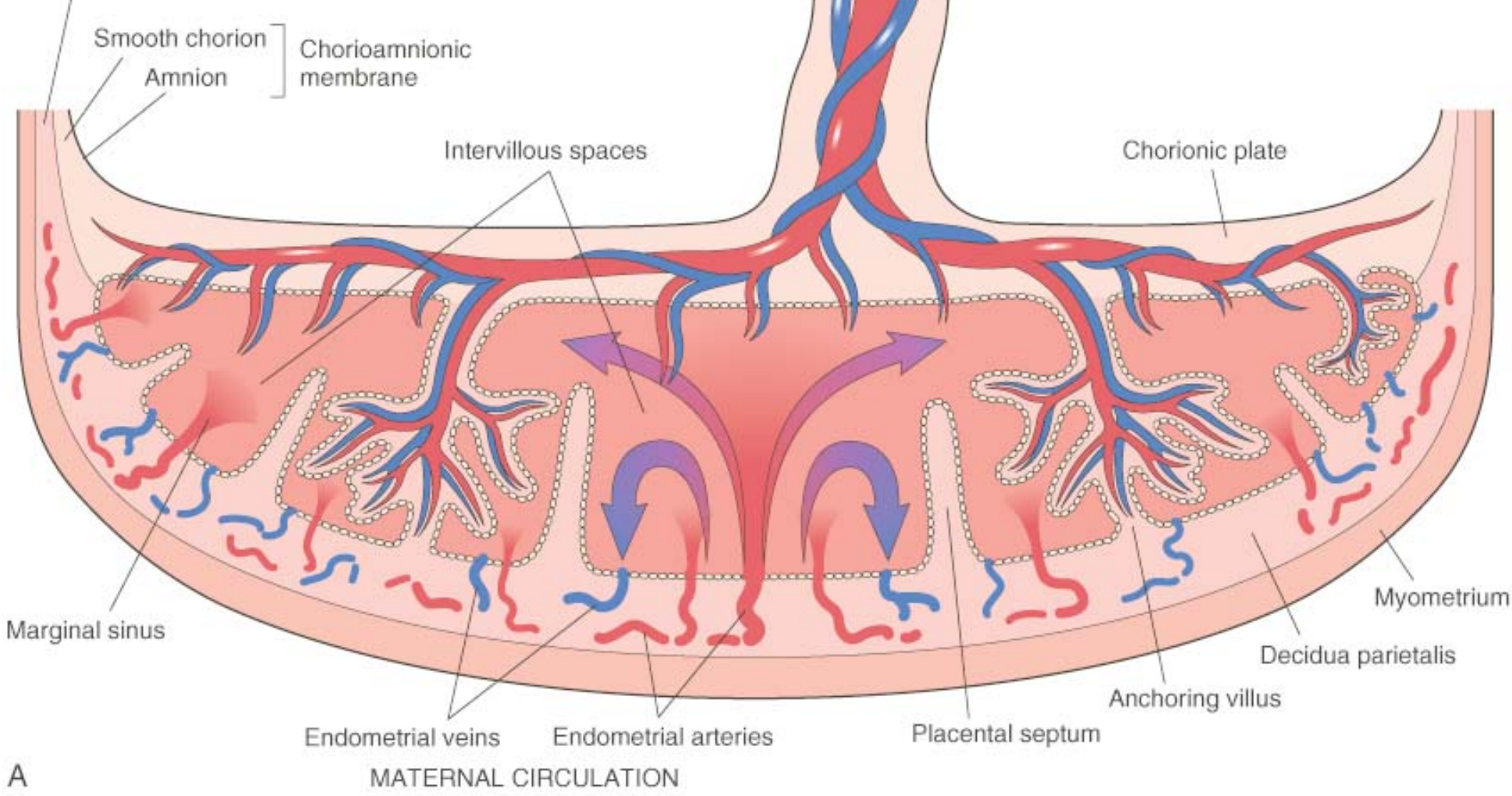


Figure 22-57 *A*, Diagram of placental anatomy. Within the outer boundary of myometrium is a layer of decidua, from which the maternal vessels originate and deliver blood to and from the intervillous spaces. Umbilical vessels branch and terminate in placental villi, where nutrient exchange takes place. *B*, Normal term placenta (fetal surface) with umbilical cord.


Smooth chorion
Amnion
Chorioamnionic membrane
Intervillous spaces
Chorionic plate
Marginal sinus
Myometrium
Decidua parietalis
Anchoring villus
Endometrial veins
Endometrial arteries
Placental septum
MATERNAL CIRCULATION

A

Figure 22-58 Diagrammatic representation of the various types of twin placentation and their membrane relationships. *(Adapted from Gersell D, et al: Diseases of the placenta. In Kurman, R (ed): Blaustein's Pathology of the Female Genital Tract. New York, Springer-Verlag, 1994.)*

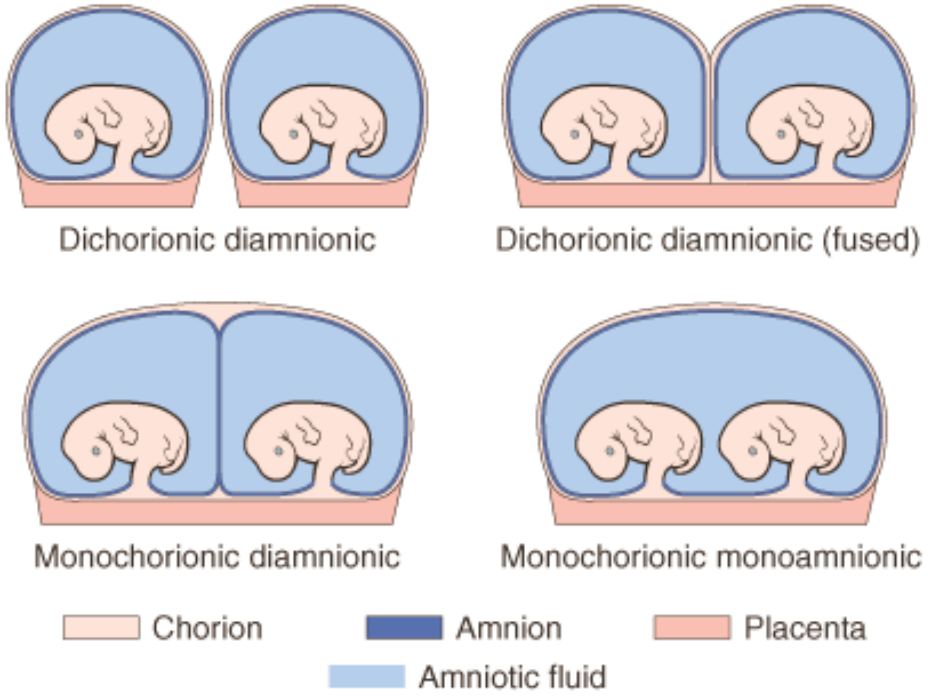
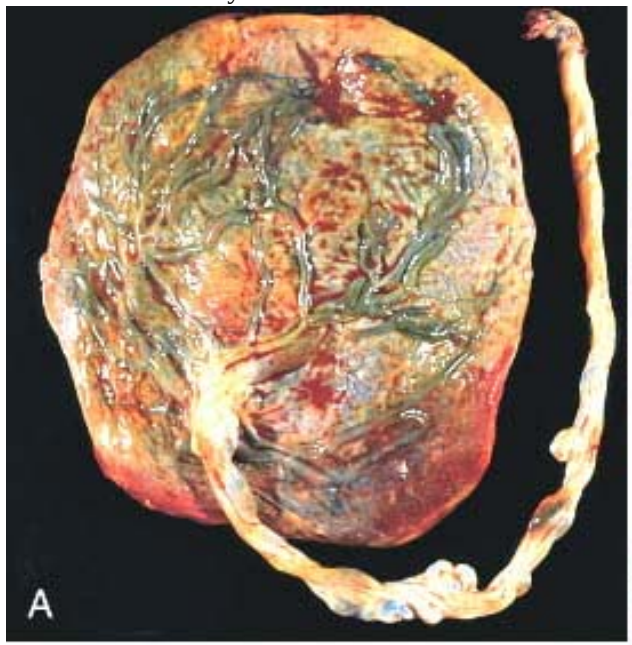
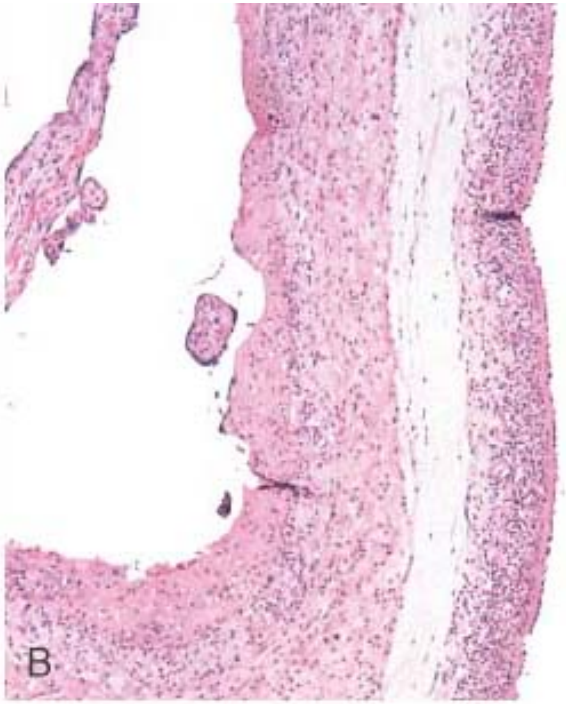


Figure 22-59 Twin-twin transfusion syndrome resulting in the death of both fetuses because of excessive (*left*) or deficient (*right*) blood volume.

Figure 22-60 Placental infections derived from ascending and blood-borne routes. Acute chorioamnionitis. *A,* On gross examination, the placenta contains greenish opaque membranes. Compare with Figure 22-55*B* . *B,* A photomicrograph illustrates a dense band-like inflammatory exudate on the amniotic surface (*top*). *C,* Acute necrotizing intervillositis, from a fetal-maternal infection by listeria.





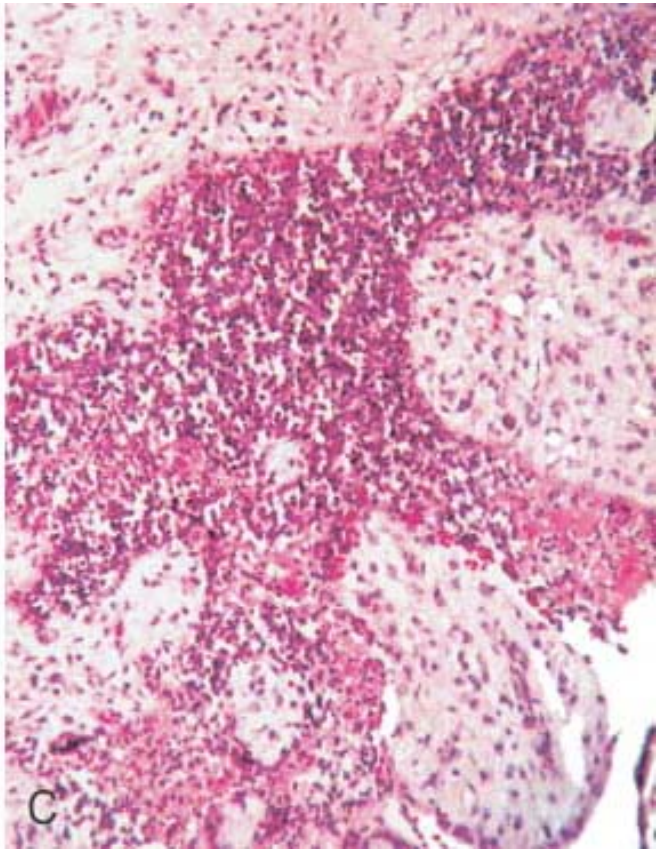


Figure 22-61 Proposed sequence of events in the pathogenesis of toxemia of pregnancy. The main features are (1) decreased uteroplacental perfusion; (2) increased vasoconstrictors and decreased vasodilators, resulting in local and systemic vasoconstriction; and (3) disseminated intravascular coagulation (DIC). *(Adapted from Friedman SA: Pre-eclampsia: a review of the role of prostaglandins. Obstet Gynecol 71:122, 1988. Reprinted by permission of the American College of Obstetricians and Gynecologists; and Khong TY, et al: Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small for gestational age infants. Br J Obstet Gynecol 93:1049, 1986.)*

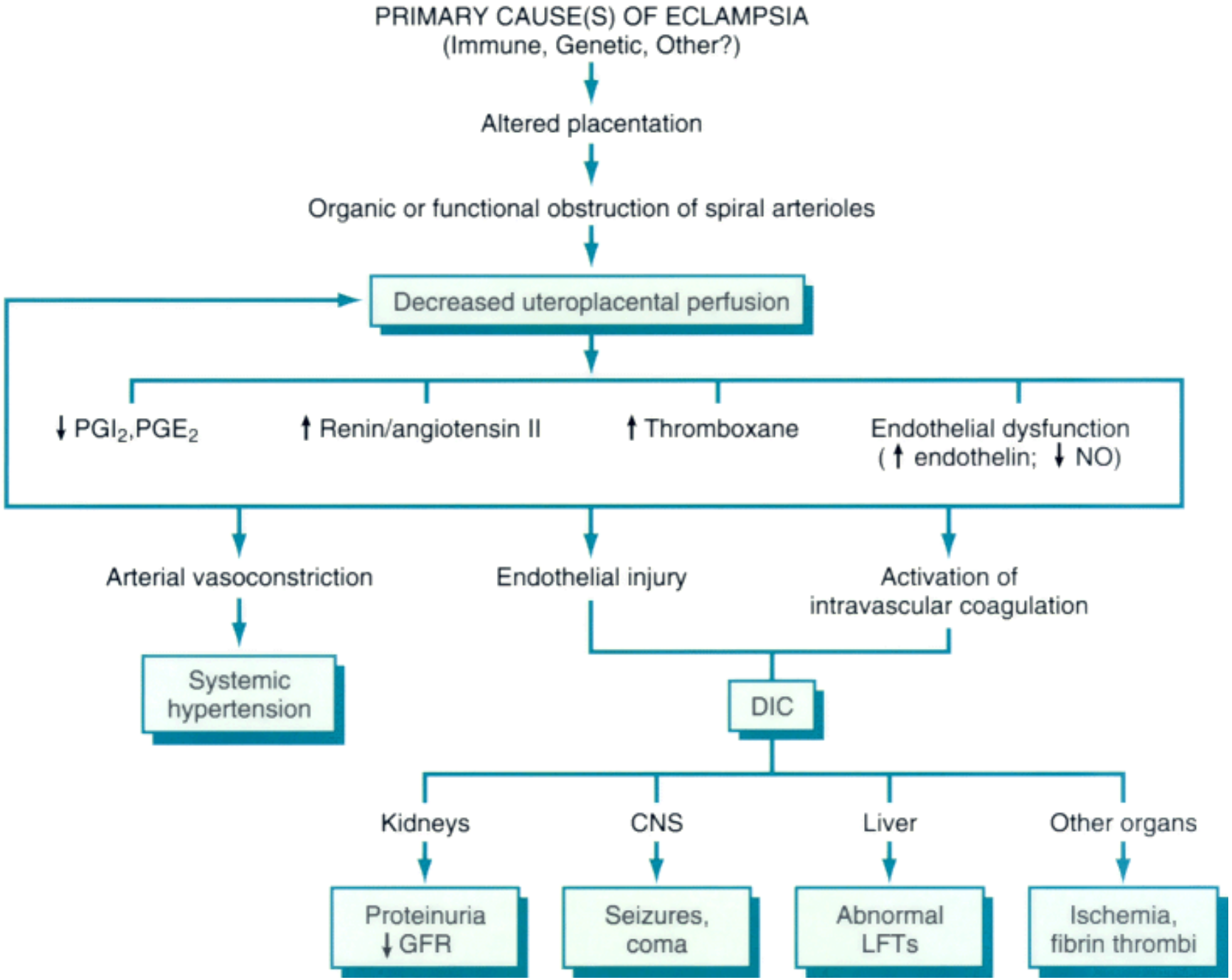


Figure 22-62 Acute atherosis of uterine vessels in eclampsia. Note fibrinoid necrosis of the vessel walls, subendothelial macrophages and perivascular lymphocytic infiltrate. *(Courtesy of Dr. Drucilla J. Roberts, Massachusetts General Hospital, Boston, MA.)*

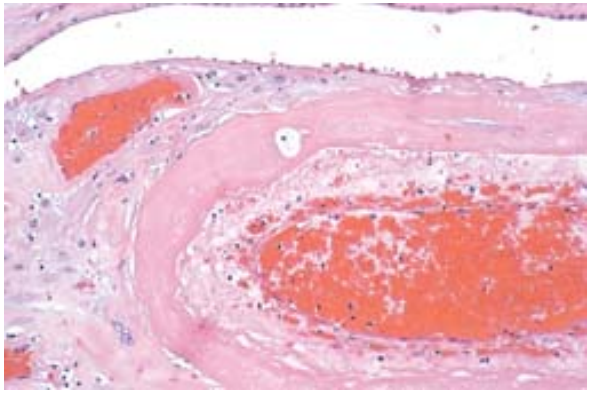


TABLE 22-5 -- Features of Complete Versus Partial Hydatidiform Mole

Feature	Complete Mole	Partial Mole
Karyotype	46,XX (46,XY)	Triploid
Villous edema	All villi	Some villi
Trophoblast proliferation	Diffuse; circumferential	Focal; slight
Atypia	Often present	Absent
Serum hCG	Elevated	Less elevated
HCG in tissue	++++	+
Behavior	2% choriocarcinoma	Rare choriocarcinoma

HCG, human chorionic gonadotropin.

Figure 22-63 Patterns of fertilization to account for chromosomal origin of complete (46,XX) and triploid partial moles (XXY). In a complete mole, one or two sperm fertilize an egg that has lost its chromosomes. Partial moles are due to fertilization of an egg by one diploid, or two haploid sperm, depicted in this example as one 23,X and one 23,Y.

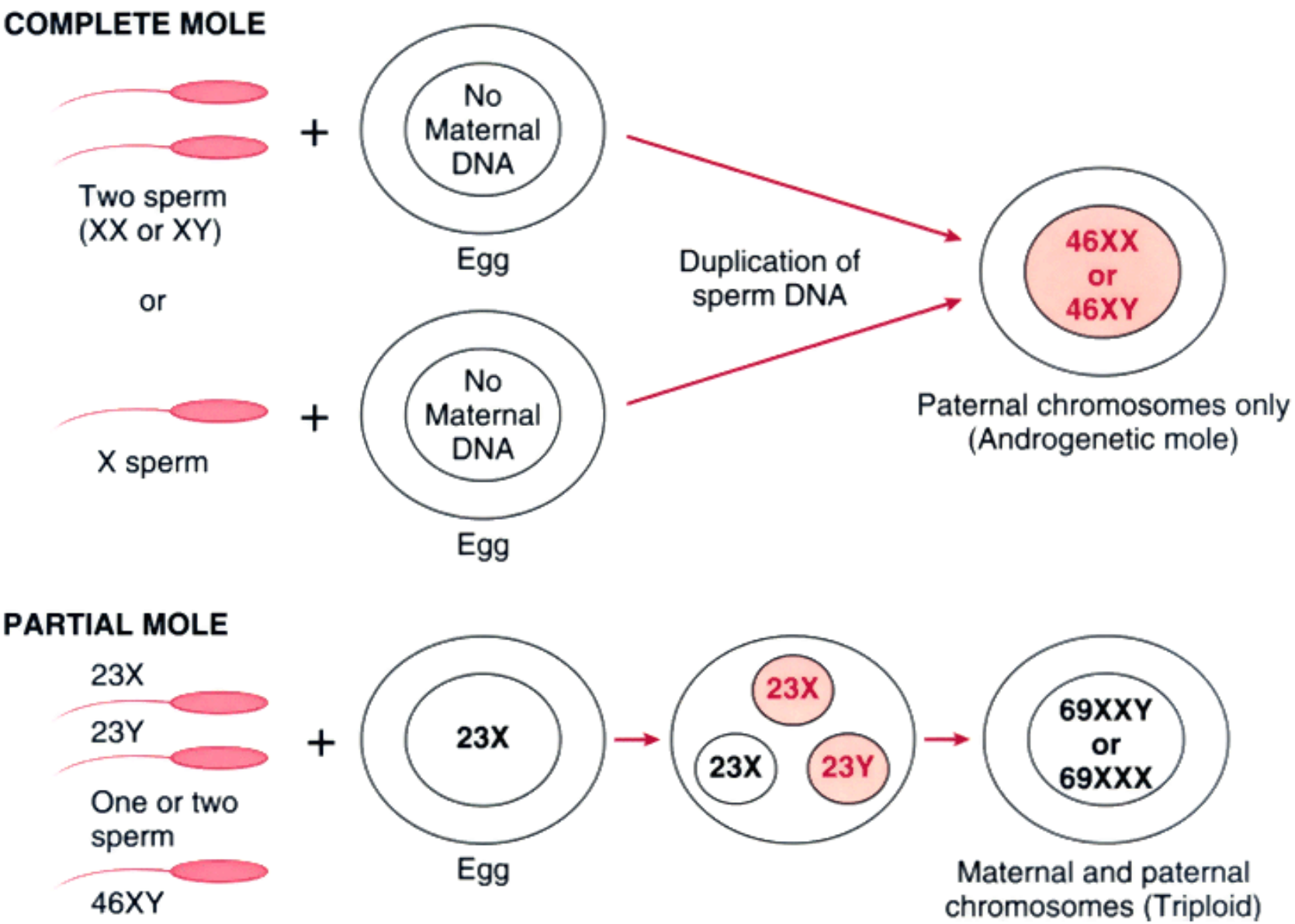


Figure 22-64 Complete hydatidiform mole suspended in saline showing numerous swollen (hydropic) villi.

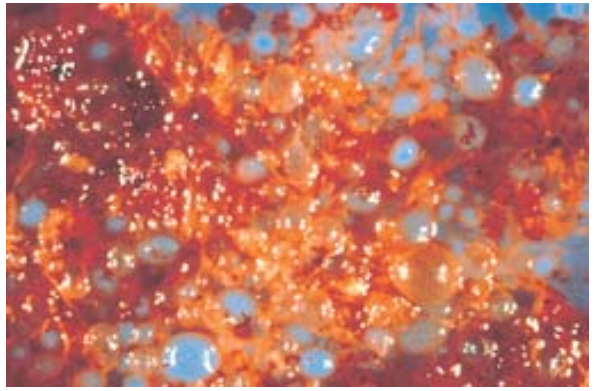


Figure 22-65 *A*, Photomicrograph of partial hydatidiform mole revealing swollen villi and slight hyperplasia of the surface trophoblast. *B*, Complete hydatidiform mole with extensive cytotrophoblastic hyperplasia (*lower field*) *(Courtesy of Dr. David R. Genest, Brigham and Women's Hospital, Boston, MA.). C*, Complete moles lack expression of *p57* in the cytotrophoblast (*arrowheads*) and villous stroma (*arrow*). *D*, Normal placenta immunostained for *p57* exhibits staining in both stromal (*arrows*) and cytotrophoblast (*arrowheads*) nuclei. *(Courtesy of Dr. Diego C. Castrillon, Brigham and Women's Hospital, Boston, MA.)*

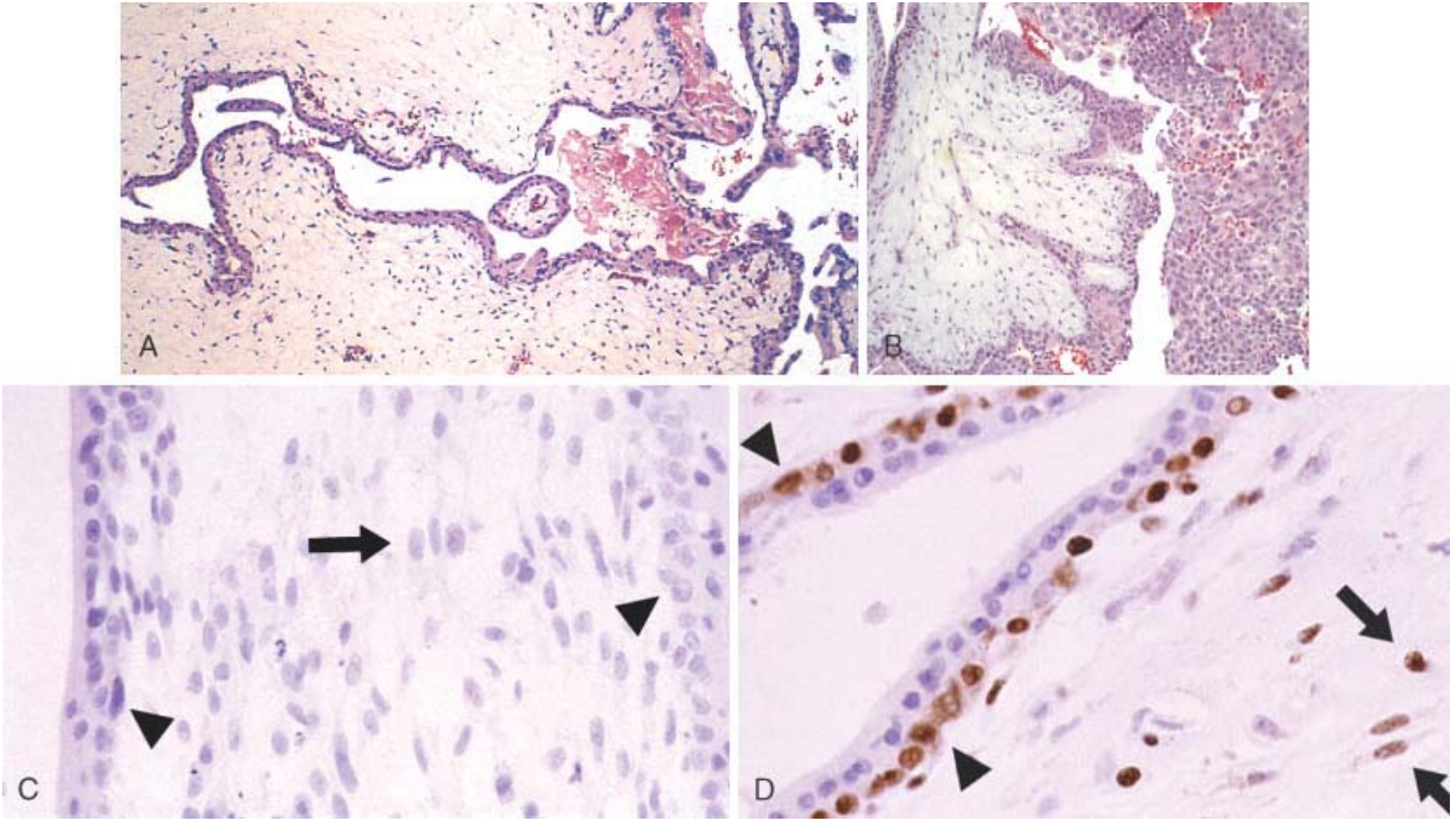


Figure 22-66 *A*, Invasive mole presenting as a hemorrhagic mass adherent to the uterine wall. *B*, On cross-section, the tumor invades into the myometrium. *(Courtesy of Dr. David R. Genest, Brigham and Women's Hospital, Boston, MA.)*

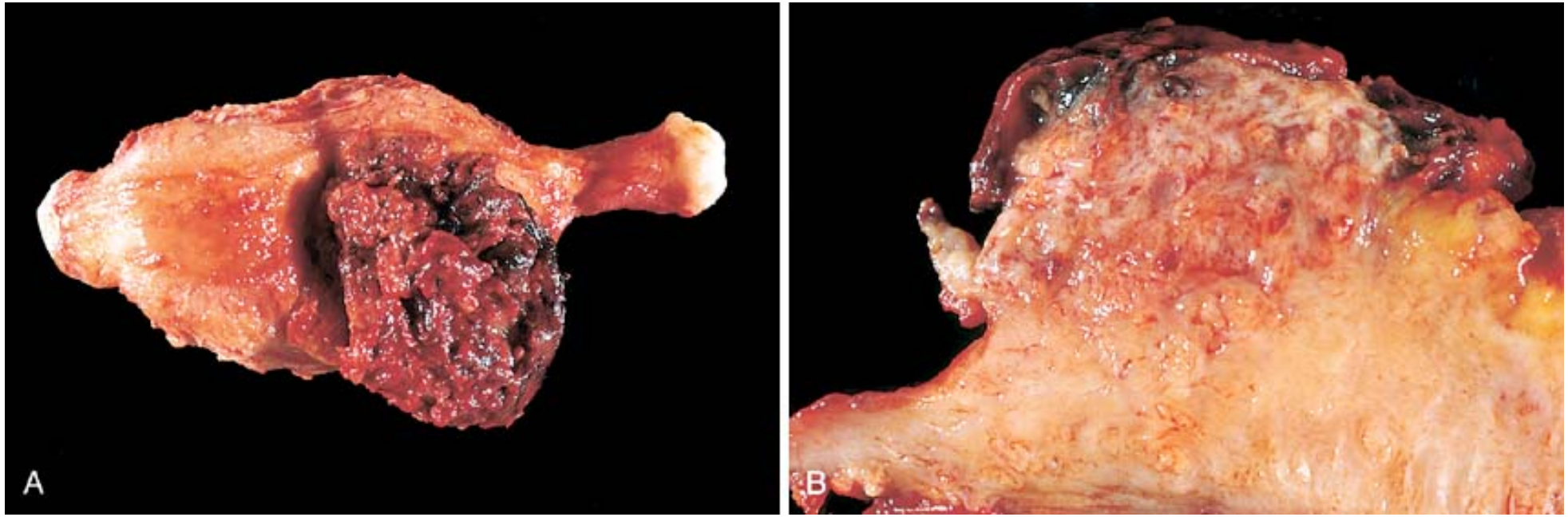


Figure 22-67 *A*, Choriocarcinoma presenting as a bulky hemorrhagic mass invading the uterine wall. *B*, Photomicrograph of choriocarcinoma illustrating both neoplastic cytotrophoblast and syncytiotrophoblast. *(Courtesy of Dr. David R. Genest, Brigham and Women's Hospital, Boston, MA.)*

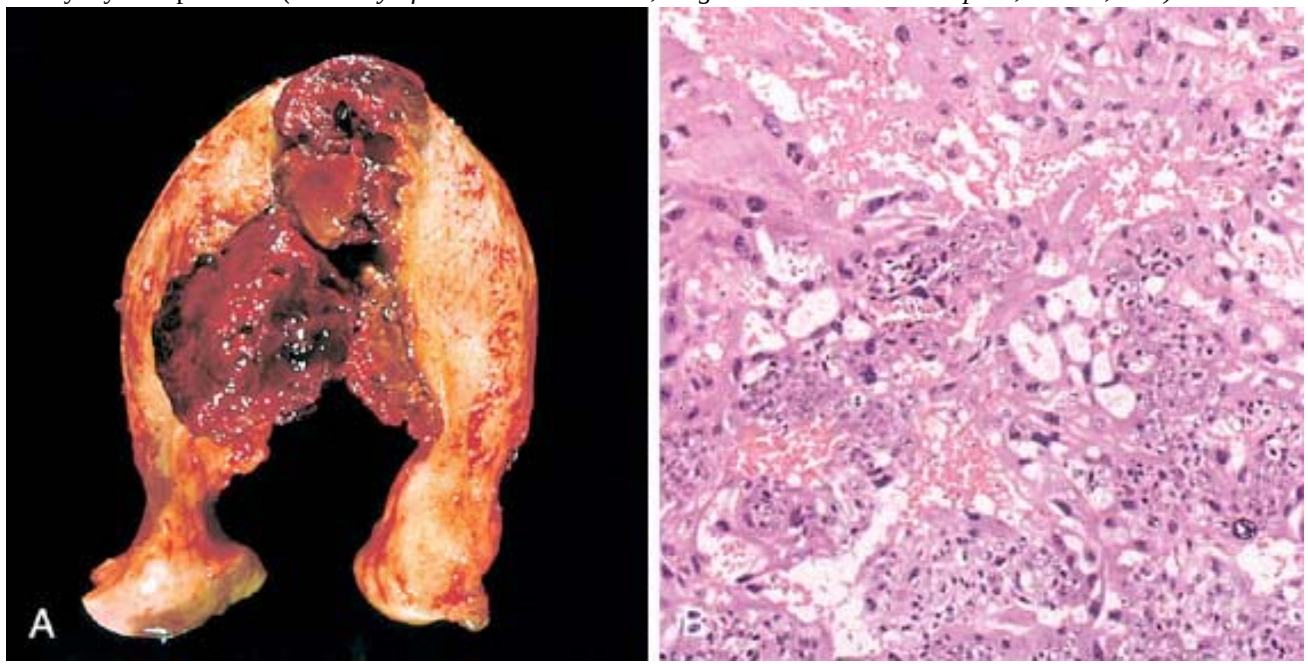
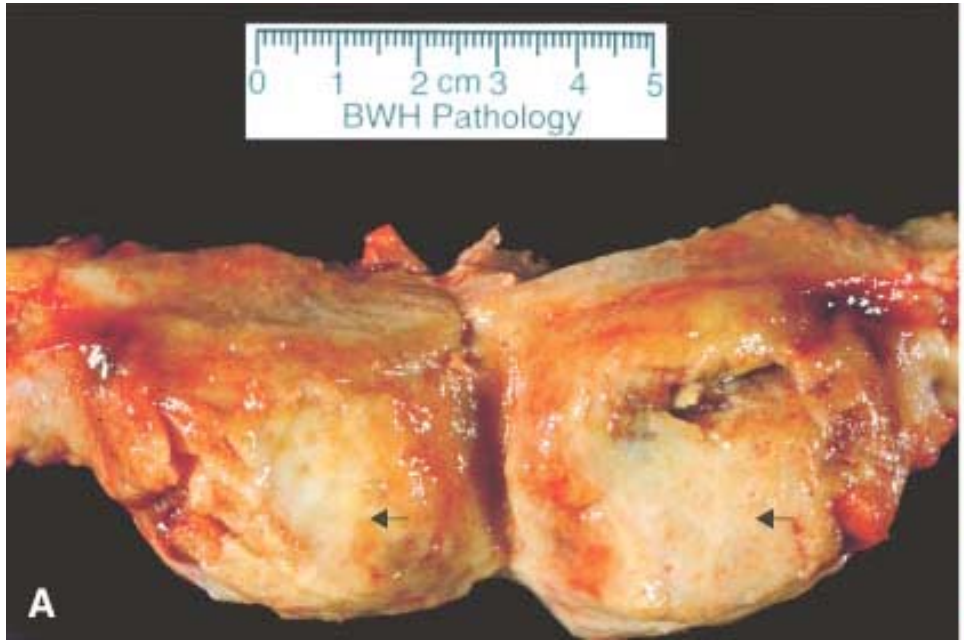
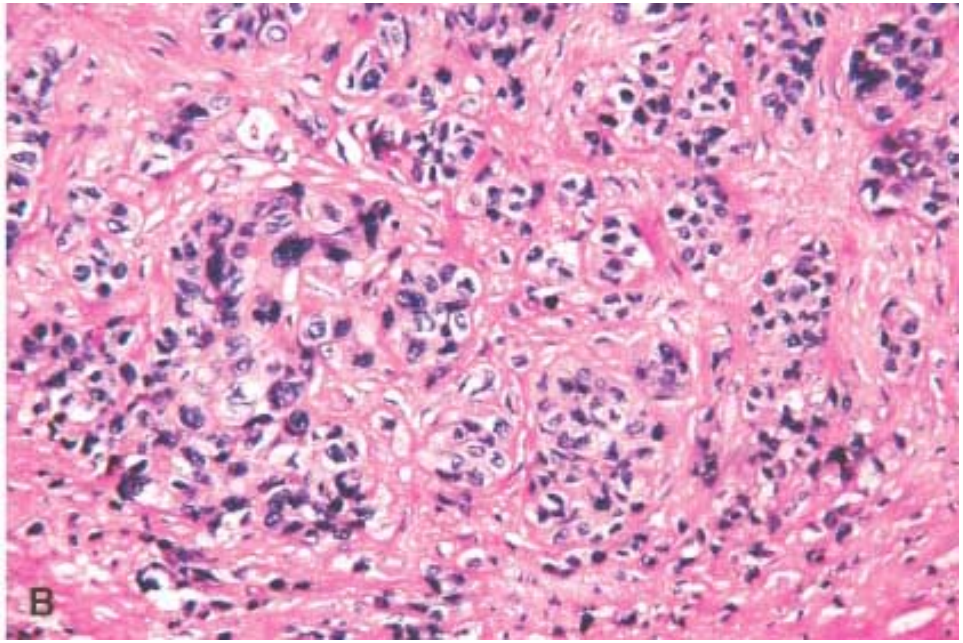


Figure 22-68 *A*, Placental site trophoblastic tumor, presenting as a discrete mass in the myometrium. *B*, Histology of PSTT. *(Courtesy of Dr. Bradley J. Quade, Brigham and Women's Hospital, Boston, MA.)*





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Chapter 23 - The Breast

Susan C. Lester MD, PhD

The Female Breast

Normal

The class Mammalia is remarkable for the evolution of modified skin appendages that provide complete nourishment and immunologic protection for the young. In humans, paired mammary glands rest on the pectoralis muscle on the upper chest wall. The breast is composed of specialized epithelium and stroma that give rise to both benign and malignant lesions specific to the organ (Fig. 23-1).

Six to ten major ductal systems originate at the nipple. The keratinizing squamous epithelium of the overlying skin continues into the ducts and then abruptly changes to a double-layered cuboidal epithelium. A small keratin plug is often found at the duct orifice. The surrounding areolar skin is pigmented and supported by smooth muscle.

Successive branching of the large ducts eventually leads to the terminal duct lobular unit (TDLU). In the adult woman, the terminal duct branches into a grapelike cluster of small acini to form a lobule (Fig. 23-1 and Fig. 23-2*B*). Each ductal system typically occupies over a quarter of the breast, and the systems extensively overlap each other. In some women, ducts extend into the subcutaneous tissue of the chest wall and into the axilla.

Figure 23-1 Normal breast anatomy and anatomical location of common breast lesions.

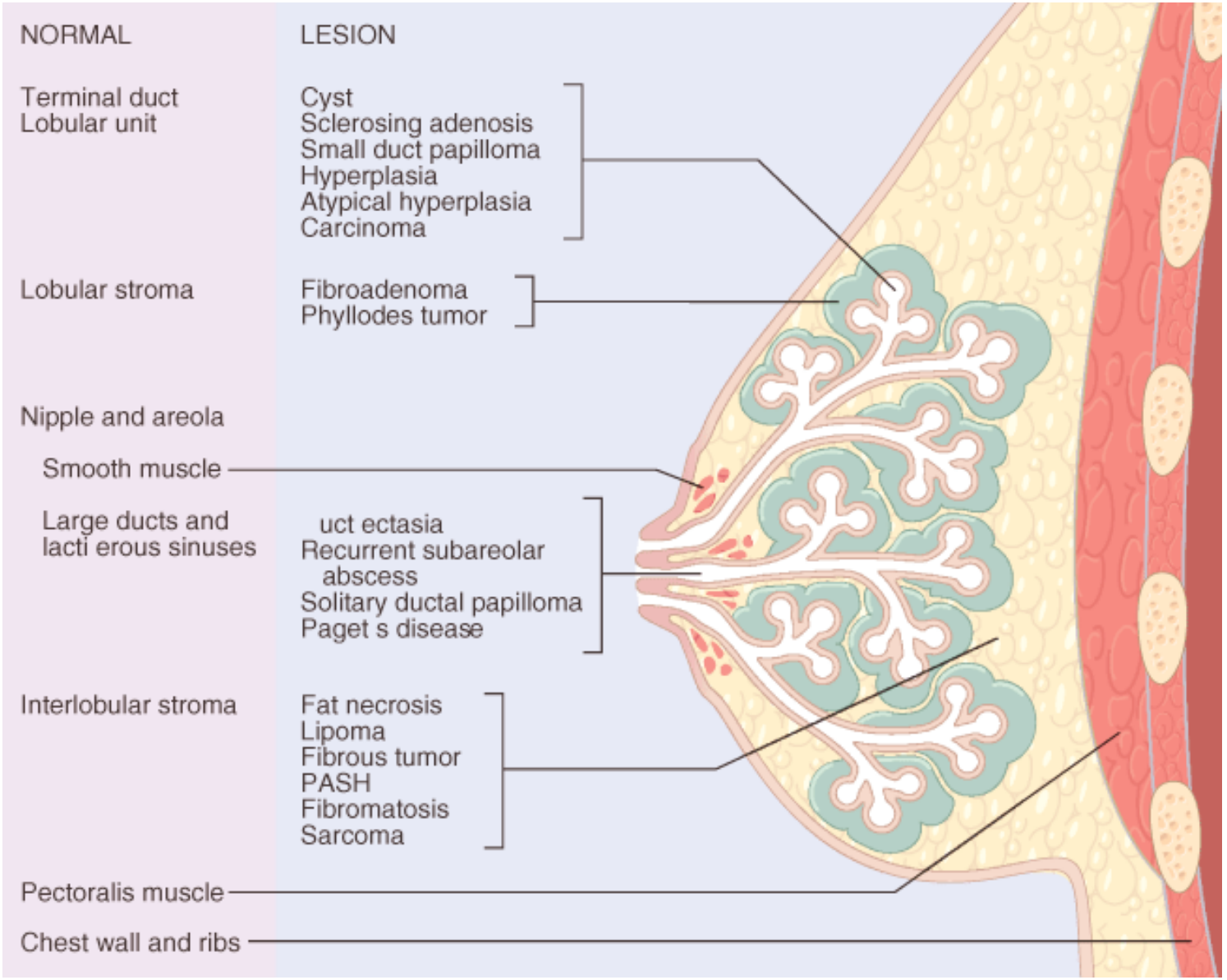

Figure 23-2 Lifecycle changes. *A*, Mammograms in young women are typically "dense" or white in appearance. In this setting, mass-forming lesions or calcifications can be difficult to detect. *(Courtesy of Dr. Darrell Smith, Brigham and Women's Hospital, Boston, MA.) B*, The density of a young woman's breast is due to the predominance of fibrous interlobular stroma and the paucity of adipose tissue (normally radiolucent or black). Prior to pregnancy, the terminal duct lobular units (TDLUs) are small and are invested by loose cellular intralobular stroma. Larger ducts interconnect the TDLUs. *C*, During pregnancy, branching of terminal ducts results in more numerous TDLUs, and the number of acini per TDLU increases. Luminal cells within TDLUs (but not the large duct system) undergo lactational change in preparation for milk production. *D*, With increasing age, the TDLUs decrease in size and number, and the interlobular stroma is replaced by adipose tissue. An older woman's breast typically consists of small ducts and atrophic lobules in adipose tissue. *E*, Mammograms become more radiolucent (darker) with age owing to the increase in adipose tissue. Radio-dense mass-forming lesions, and calcifications become easier to detect. *(Courtesy of Dr. Darrell Smith, Brigham and Women's Hospital, Boston, MA.)*

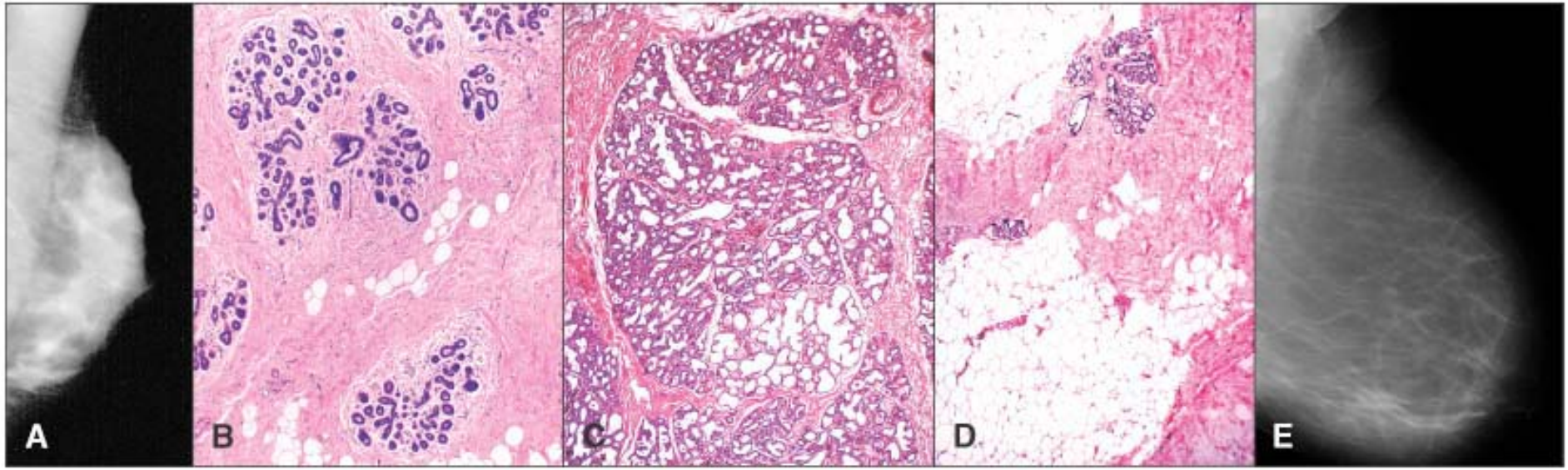

Figure 23-3 Common clinical presentations of breast disease. Over a 10-year period, 372 women over the age of 40 made 539 visits to a health maintenance organization for the listed breast symptoms.[9] Some women had more than one symptom and/or made more than one visit. In 10% of cases, the visit led to the performance of a biopsy.

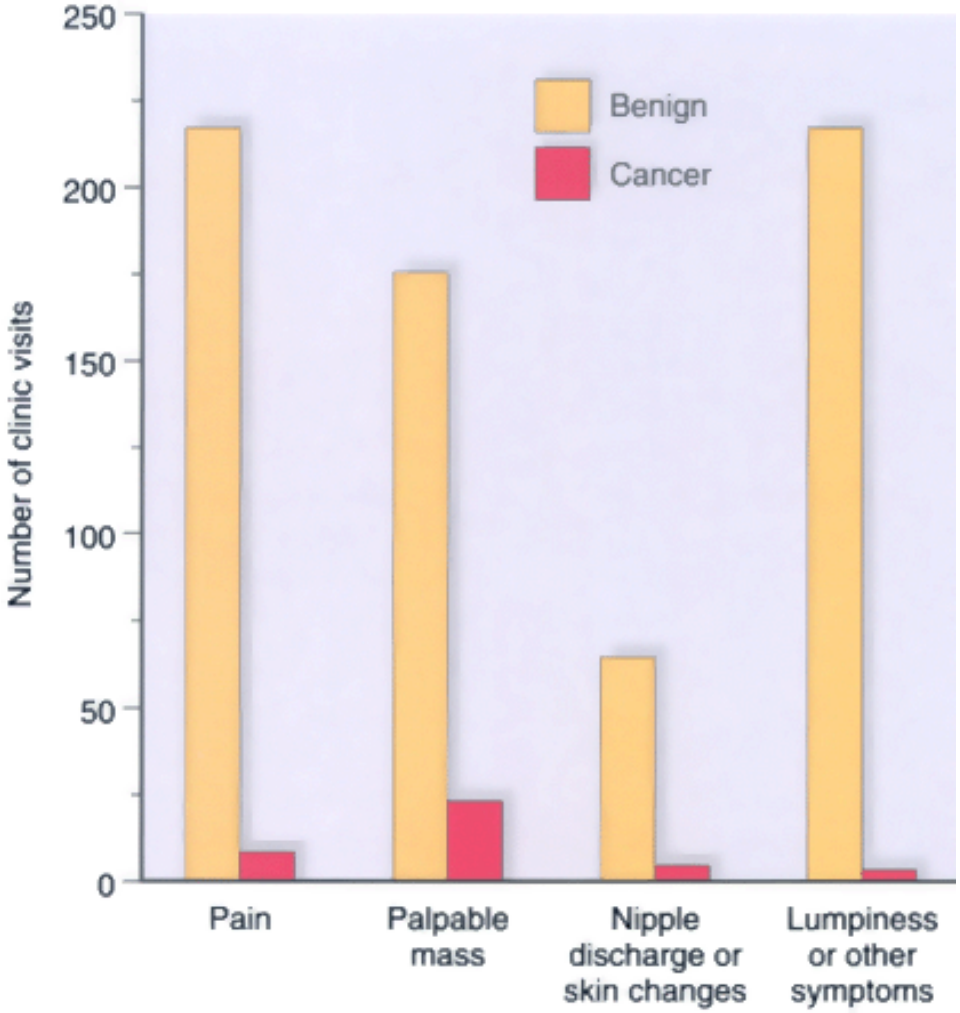

TABLE 23-1 -- Characteristics of Breast Carcinomas by Clinical Presentation

Clinical Presentation	Invasive Carcinoma (% of Carcinomas)	Average Size of Invasive Carcinomas	Carcinomas with Lymph Node Metastases	DCIS (% of Carcinomas)	LCIS (% of Carcinomas)
Palpable mass	94%	2.4 cm	58%	•2%	4%
Mammographic density	94%	1.1 cm	14%	•4%	2%
Mammographic calcifications	26%	0.6 cm	•6%	71%	3%
Based on the results of 235 carcinomas diagnosed in 914 women undergoing diagnostic biopsies at Brigham and Women's Hospital over a 6-month period in 2001.					
Mammographic lesions were nonpalpable.					
DCIS, ductal carcinoma in situ; LCIS, lobular carcinoma in situ.					

compared to 60% of masses in women over age 50 (Fig. 23-4). The most commonly encountered lesions are invasive carcinomas, fibroadenomas, and cysts. Approximately 50% of carcinomas arise in the upper outer quadrant, 10% in each of the remaining quadrants, and about 20% in the central or subareolar region.

Nipple discharge is a less common presenting symptom but is of concern when it is spontaneous and unilateral. A discharge produced by manipulating the breast is normal and unlikely to be associated with a pathologic lesion. A milky discharge (galactorrhea) is associated with increased production of prolactin (e.g., by a pituitary adenoma), hypothyroidism, or endocrine anovulatory syndromes. It can also occur in patients taking oral contraceptives, tricyclic antidepressants, methyldopa, or phenothiazines. Repeated nipple stimulation can also induce lactation (e.g., this method is sometimes used by women who wish to breast-feed adopted infants). Milky discharge has not been associated with malignancy. Bloody or serous discharges are most commonly associated with benign lesions but, rarely, can be due to a malignancy. A normal bloody discharge can also occur during pregnancy, possibly due to the rapid formation of new lobules. The risk of malignancy with discharge increases with age. Discharge is associated with carcinoma in 7% of women younger than 60 years and in 30% of women older than 60 years. The most common etiologies for discharge are a solitary large duct papilloma, cysts, or carcinoma (Fig. 23-4). Carcinomas presenting as nipple discharge not associated with a palpable mass are equally divided between invasive and in situ carcinomas.[10] There is considerable interest in developing the cytologic examination of induced nipple discharge into a screening test for breast cancer.

Mammographic screening was introduced in the 1980s as a means to detect small, nonpalpable breast carcinomas not associated with breast symptoms. The sensitivity and specificity of mammography increase with age. As the dense, fibrous interlobular tissue of the young woman is replaced by the fatty tissue of the older woman, it becomes easier to detect small masses and calcifications. Also, with increasing age, benign lesions become less frequent and malignant lesions become more frequent. Screening is generally recommended to start at age 40. Younger women usually undergo mammography only if they are at high risk for developing carcinoma, owing either to a prior palpable cancer or to a strong family history. Despite the screening of women at high risk of breast cancer, only 12% of mammographic lesions in women

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Figure 23-4 Frequency of benign and malignant breast lesions diagnosed after biopsy by clinical presentation and age. *(Based on 914 women who underwent diagnostic breast surgery at Brigham and Women's Hospital, Boston, from January to June 2001.)*

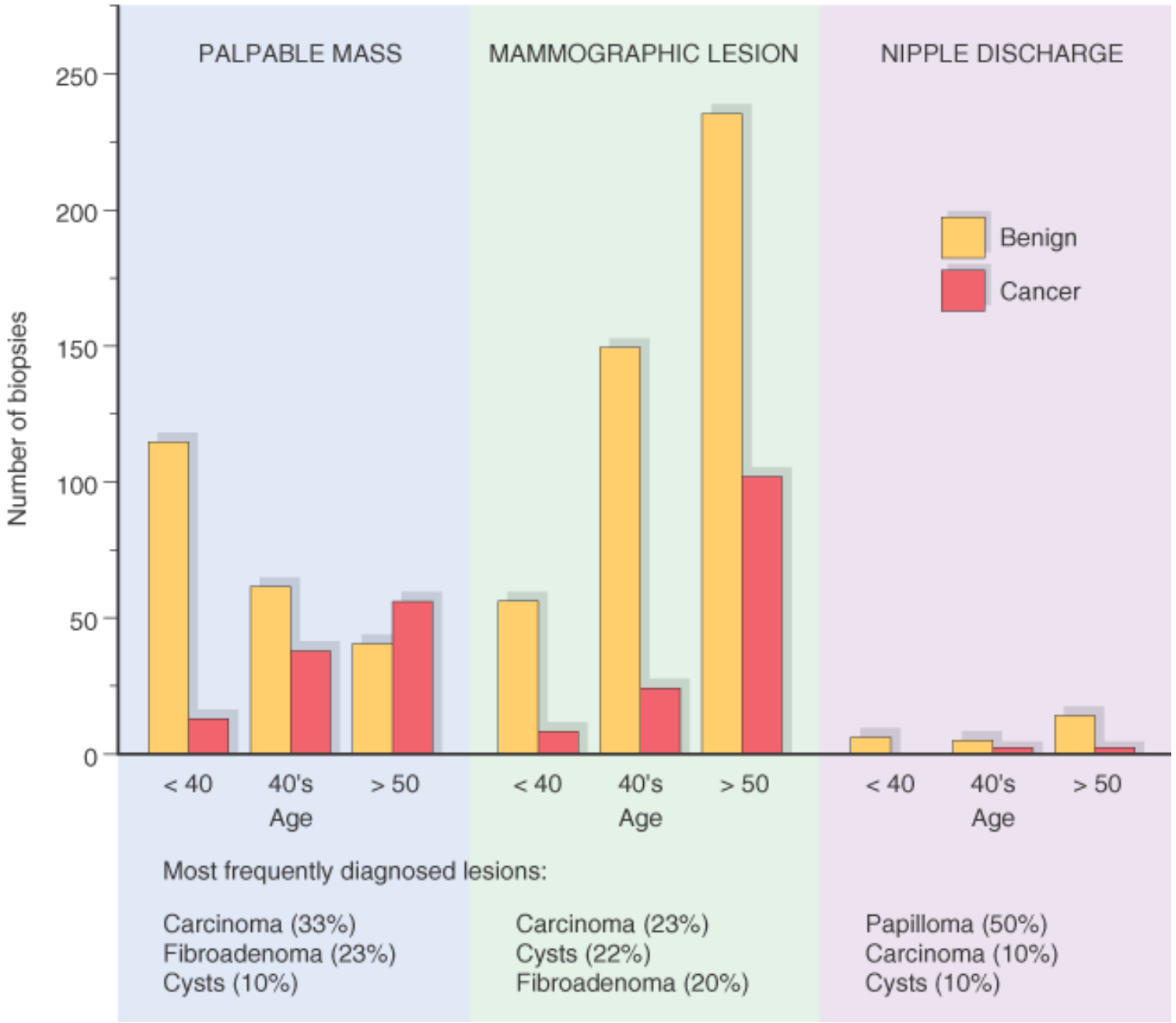

Figure 23-5 Recurrent subareolar abscess. When squamous metaplasia extends deep into a duct, keratin becomes trapped and accumulates. If the duct ruptures, the ensuing intense inflammatory response to keratin results in an erythematous painful mass. A fistula tract may burrow beneath the smooth muscle of the nipple to open at the edge of the areola.

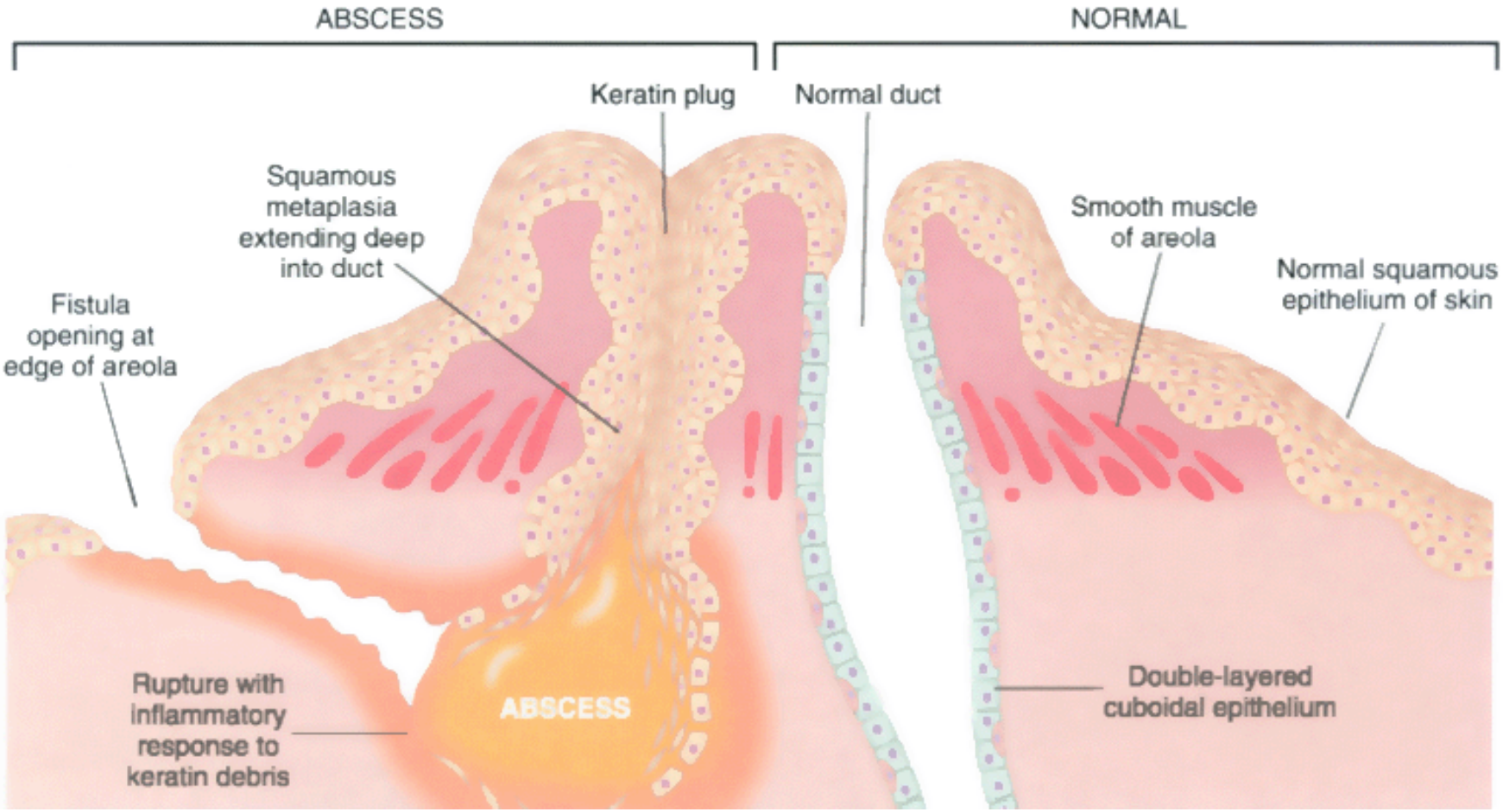

Figure 23-6 Mammary duct ectasia. Chronic inflammation and fibrosis surround an ectatic duct filled with inspissated debris. The fibrotic response can mimic the irregular shape of malignant carcinomas on palpation or mammogram.

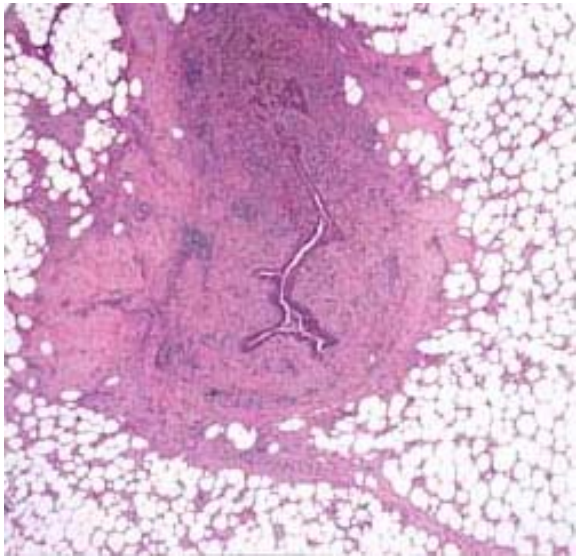

Figure 23-7 Apocrine cysts. Cells with round nuclei and abundant granular eosinophilic cytoplasm, resembling the cells of normal apocrine sweat glands, line the walls of a cluster of small cysts. Secretory debris, frequently with calcifications, is often present. Groups of cysts are common findings associated with clustered mammographic calcifications.

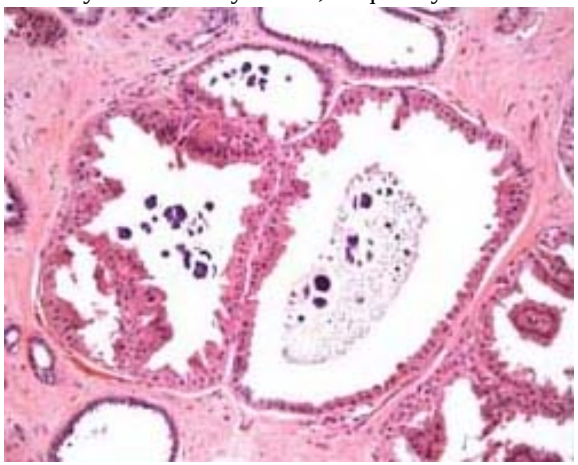

Figure 23-8 *A*, Normal. A normal duct or acinus has a single basally located myoepithelial cell layer (cells with dark, compact nuclei and scant cytoplasm) and a single luminal cell layer (cells with larger open nuclei, small nucleoli, and more abundant cytoplasm). *B*, Epithelial hyperplasia. The lumen is filled with a heterogeneous population of cells of different morphologies, often including both luminal and myoepithelial cell types. Irregular slitlike fenestrations are prominent at the periphary.

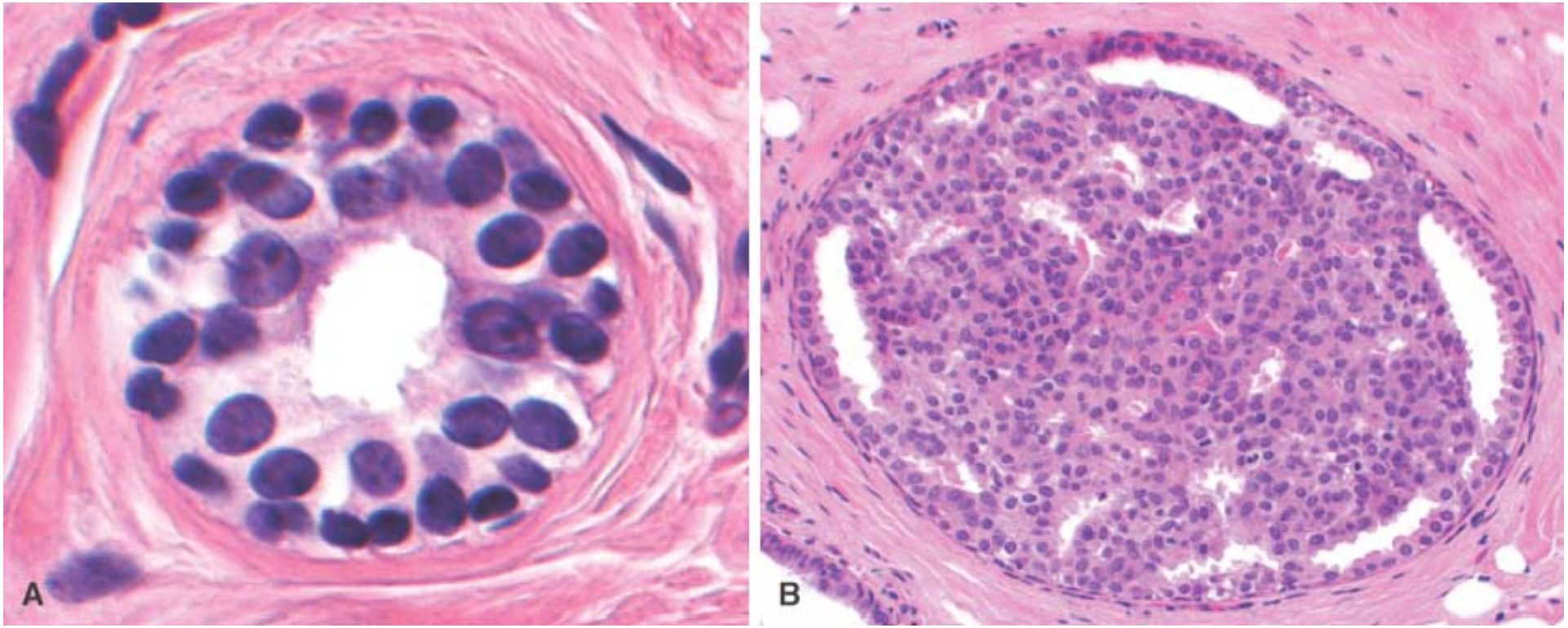

Figure 23-9 Sclerosing adenosis. The involved terminal duct lobular unit is enlarged, and the acini are compressed and distorted by the surrounding dense stroma. Calcifications are often present within the lumens. Although this lesion is frequently mistaken for an invasive carcinoma, unlike carcinomas, the acini are arranged in a swirling pattern, and the outer border is usually well circumscribed.

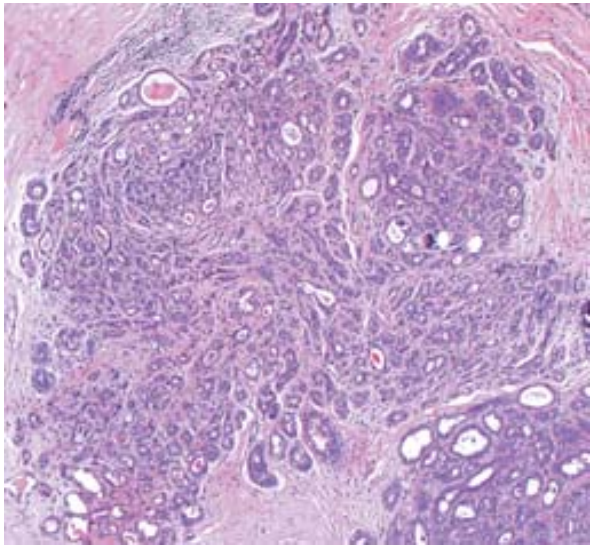

Figure 23-10 Complex sclerosing lesion (radial scar). There is a central nidus consisting of small tubules entrapped in a densely fibrotic stroma surrounded by radiating arms of epithelium

with varying degrees of cyst formation and hyperplasia. These lesions typically present as an irregular mammographic density and closely mimic an invasive carcinoma.

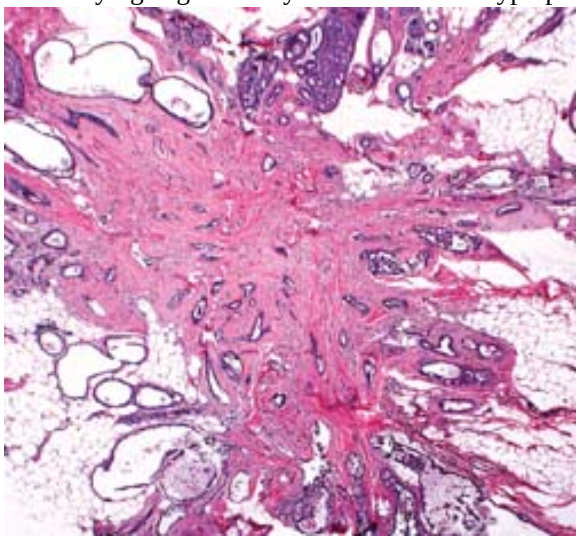

Figure 23-11 Intraductal papilloma. A central fibrovascular core extends from the wall of a duct. The papillae arborize within the lumen and are lined by myoepithelial and luminal cells.

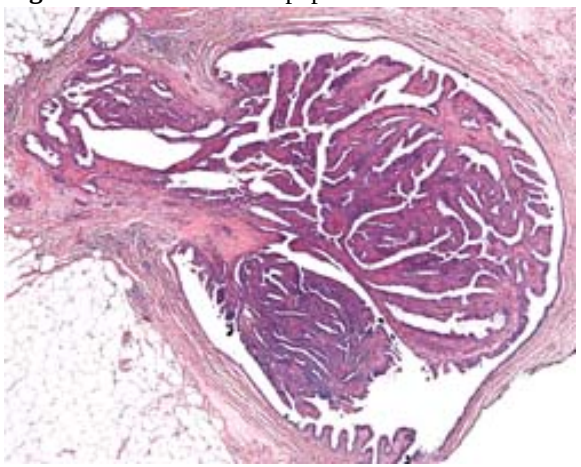

Figure 23-12 *A,* Atypical ductal hyperplasia. A duct is filled with a mixed population of cells consisting of oriented columnar cells at the periphery and more rounded cells within the central portion. Although some of the spaces are round and regular, the peripheral spaces are irregular and slitlike. These features are highly atypical but fall short of a diagnosis of DCIS. *B,* Atypical lobular hyperplasia. A population of monomorphic small, rounded, loosely cohesive cells partially fill a lobule. Some intracellular lumina can be seen. Although the cells are morphologically identical to the cells of LCIS, the extent of involvement is not sufficient for this diagnosis.

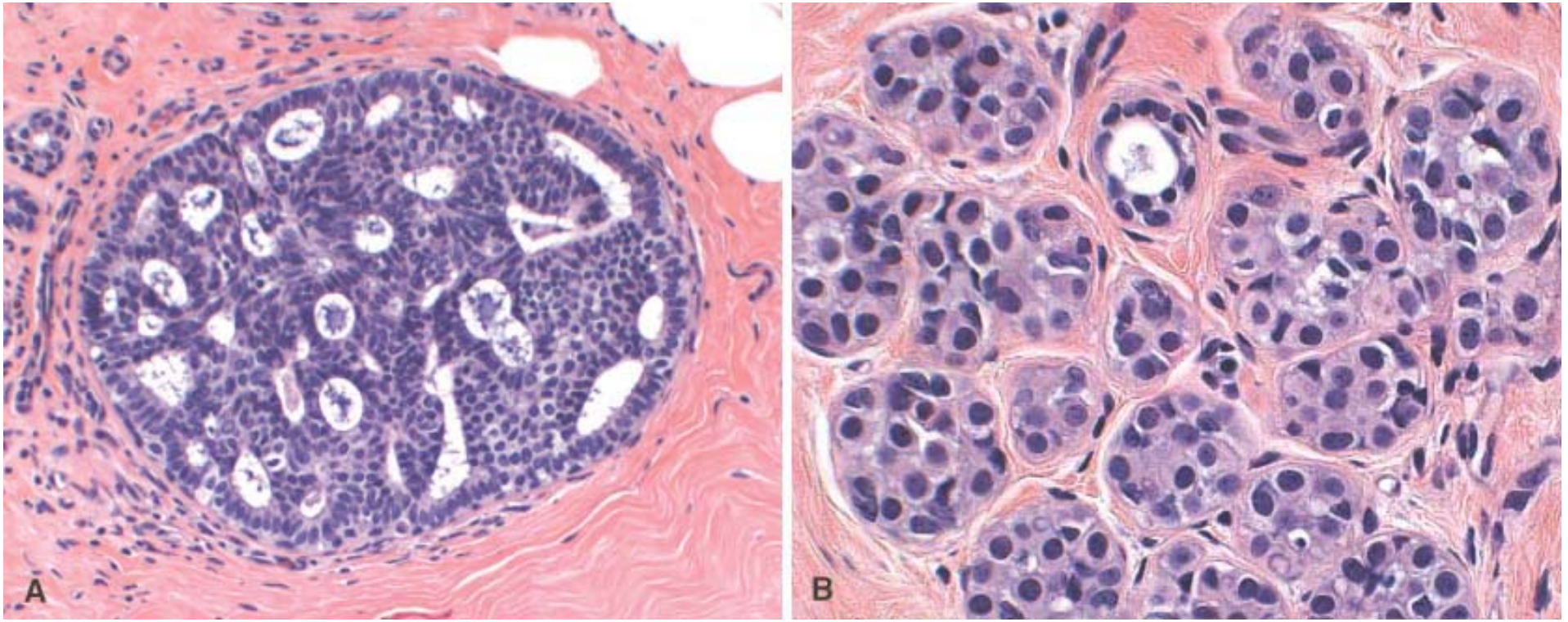

TABLE 23-2 -- Breast Lesions and Relative Risk of Developing Invasive Carcinoma

Pathologic Lesion	Relative Risk of Developing Invasive Carcinoma	Breast at Risk	Modifiers of Risk
Nonproliferative Breast Changes	1.0	Neither	
Duct ectasia			
Cysts			
Apocrine change			
Mild hyperplasia			
Adenosis			
Fibroadenoma without complex features			
Proliferative Disease Without Atypia	1.5–2.0	Both breasts	Increased risk if there is a family history of breast carcinoma
Moderate or florid hyperplasia			Decreased risk 10 years after biopsy
Sclerosing adenosis			
Papilloma			

Complex sclerosing lesion (radial scar)			
Fibroadenoma with complex features			
Proliferative Disease with Atypia	4.0–5.0	Both breasts	Increased risk if there is a family history of breast carcinoma
Atypical ductal hyperplasia			Increased risk if premenopausal
Atypical lobular hyperplasia			Decreased risk 10 years after biopsy for ALH
Carcinoma in Situ	8.0–10.0		
Lobular carcinoma in situ		Both breasts	Treatment (tamoxifen, bilateral mastectomy)
Ductal carcinoma in situ *		Ipsilateral breast	Treatment (tamoxifen, surgery to eradicate the lesion, radiation therapy)

*This risk applies to low-grade DCIS originally misdiagnosed as benign disease and followed without treatment. The risk for progression of high-grade DCIS is presumed to be greater than this.

INCIDENCE AND EPIDEMIOLOGY

After remaining constant for many years (except for a transient rise in 1974 attributed to increased awareness after the publicity surrounding Betty Ford and Happy Rockefeller developing breast cancer), the incidence of breast cancer

Figure 23-13 Breast cancer incidence and mortality rates for women over 50 years of age. Rates are per 100,000 women and are age-adjusted to the 2000 U.S. standard million population. *(SEER Cancer Statistics Review 1973–1999; http://seer.cancer.gov/.)*

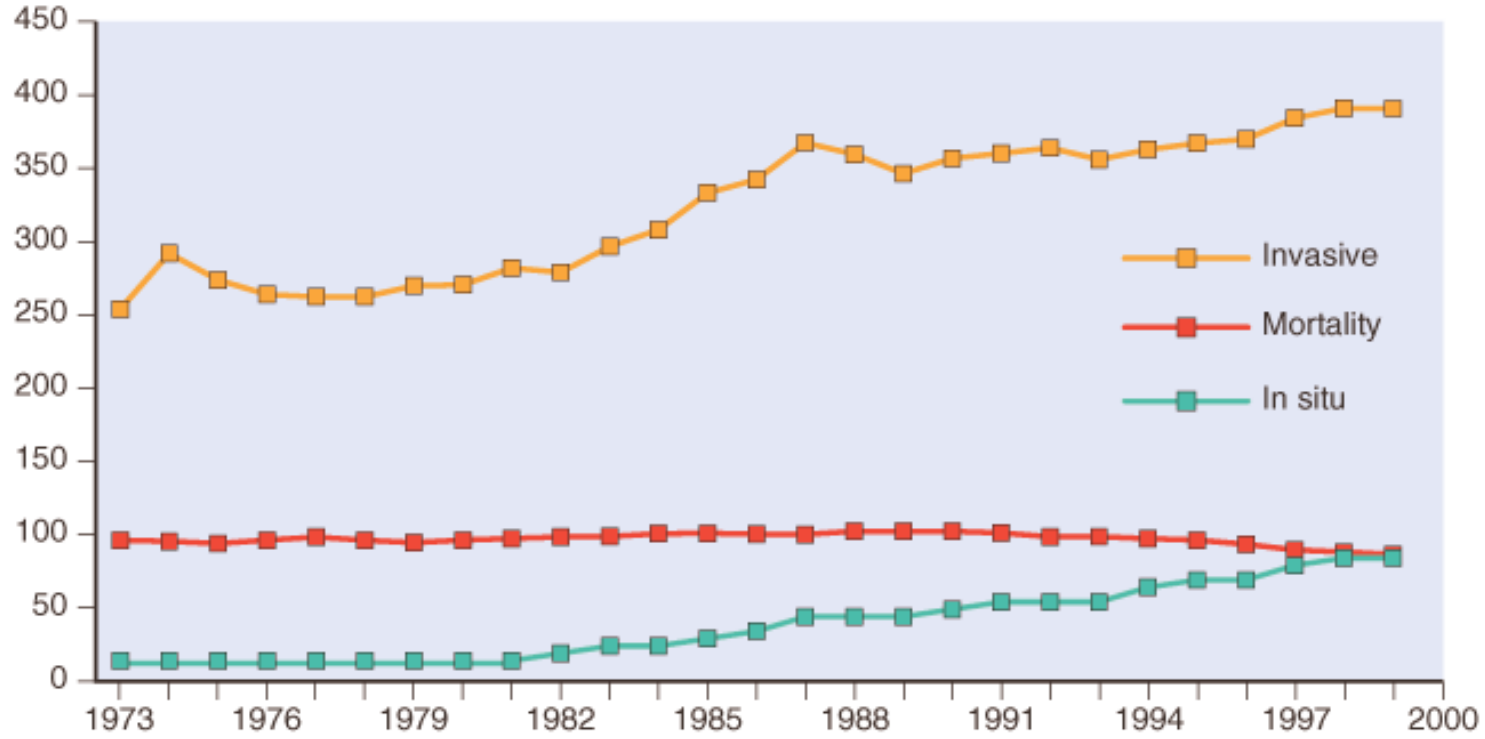

Figure 23-14 Change in stage of breast cancer at presentation from 1983 to 1996. *(SEER Cancer Statistics Review, http://seer.cancer.gov/.)*

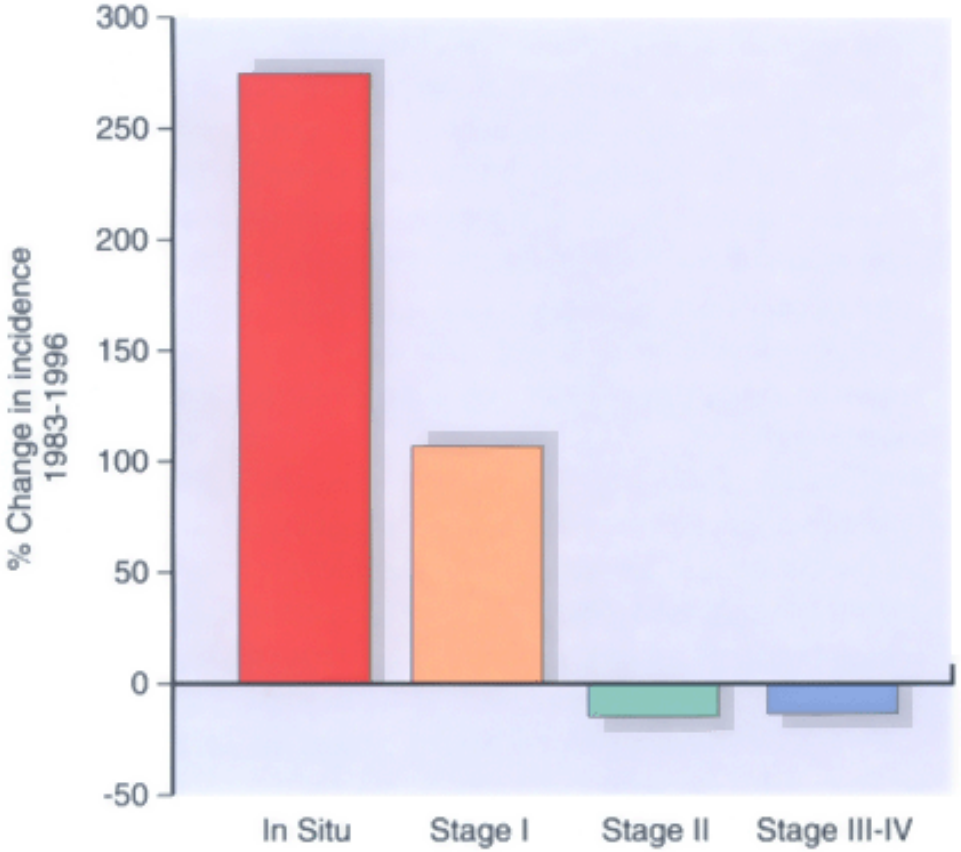

TABLE 23-3 -- *BRCA1* and *BRCA2*

	BRCA1	***BRCA2***
Chromosome	17q21	13q12.3
Gene size	81 kb	84 kb
Protein size	1863 amino acids	3418 amino acids
Function	Tumor suppressor	Tumor suppressor
	Transcriptional regulation	Transcriptional regulation
	Role in DNA repair	Role in DNA repair
Mutations	>500 identified	>300 identified
Mutations in population	about 0.1%	about 0.1%
Risk of breast cancer	60–80%	60–80%
Age at onset	Younger age (40s to 50s)	50 years

Families with breast cancer due to a single gene (%)	52%	32%
Families with breast and ovarian cancer (%)	81% (20–40% risk)	14% (10–20% risk)
Families with male and female breast cancer	<20%	76%
Risk of other tumors (varies with specific mutation)	Prostate, colon, pancreas	Prostate, pancreas, stomach, melanoma, colon
Mutations in sporadic breast cancer	Very rare (<5%)	Very rare (<5%)
Epidemiology	Specific mutations are found in certain ethnic groups	Specific mutations are found in certain ethnic groups
Pathology of breast cancers	Greater incidence of medullary carcinomas (13%), poorly differentiated carcinomas, ER-, PR-, and *Her2/neu*-negative carcinomas, carcinomas with *p53* mutations	Similar to sporadic breast cancers
Additional information about these genes can be found at http://www.ncbi.nlm.nih.gov/.		

cancer, and cataracts. Current clinical trials are attempting to identify other selective estrogen receptor modulators (SERMs) that have the same benefit but fewer side effects.

ETIOLOGY AND PATHOGENESIS

The major risk factors for the development of breast cancer are hormonal and genetic (family history). Breast carcinomas can, therefore, be divided into sporadic cases, possibly related to hormonal exposure, and hereditary cases, associated with family history or germ-line mutations. Hereditary carcinoma has received intense scrutiny in the hopes that the specific genetic mutations can be identified and that these alterations will illuminate the causes of all breast cancer. Recent studies have borne out these hopes. We begin our discussion with hereditary breast cancer and follow with sporadic breast cancer.

Hereditary Breast Cancer

A family history of breast cancer in a first-degree relative is reported in 13% of women with the disease.[21] However, only 1% of women have multiple affected relatives, a history suggestive of a highly penetrant germ-line mutation.

About 25% of familial cancers (or around 3% of all breast cancers) can be attributed to two highly penetrant autosomaldominant genes: BRCA1 and BRCA2 (Table 23-3). The probability of breast cancer associated with a mutation in these genes increases if there are multiple affected first-degree relatives, if individuals are affected before menopause and/or

have multiple cancers, if there is a case of male breast cancer, or if family members also develop ovarian cancer. The general lifetime breast cancer risk for female carriers is 60% to 85%, and the median age at diagnosis is about 20 years earlier compared to women without these mutations. The penetrance (i.e., the number of carriers who actually develop breast cancer) can vary with the specific type of mutation present. Mutated *BRCA1* also markedly increases the risk of developing ovarian carcinoma, which is as high as 20% to 40%. *BRCA2* confers a smaller risk for ovarian carcinoma (10% to 20%) but is associated more frequently with male breast cancer. *BRCA1* and *BRCA2* carriers are also susceptible to other cancers, such as colon, prostate, and pancreas, but to a lesser extent.

Although BRCA1 and BRCA2 do not show sequence homology, they function in similar pathways and interact with the same multiprotein complexes. Both act as tumor suppressors, as it is a loss of function that confers the risk of malignancy. A wide variety of functions have been suggested for these proteins, including transcriptional regulation, cell-cycle control, ubiquitin-mediated protein degradation pathways, and chromatin remodeling. A key function for both appears to be their role in protecting the genome from damage by halting the cell cycle and promoting DNA damage repair in a complex process that is not yet fully understood. BRCA1 is phosphorylated in response to damage and may transduce DNA damage signals from checkpoint kinases to effector proteins. BRCA1 is also bound with BRCA2 and RAD51 in a nuclear dot complex—presumably the site of DNA repair.[33] BRCA2 can bind directly to DNA and functions in homologous recombination for the error-free repair of double-strand DNA breaks.[34] Why loss of these functions specifically affects the breast is unclear. Perhaps the intermittent proliferation of breast epithelium (as opposed to the constitutive proliferation of other epithelia such as colon or skin) makes this organ more susceptible to the accumulation of genetic damage, or possibly, other cell types have additional mechanisms for DNA repair that the breast lacks. BRCA1, but not BRCA2, interacts with the ER and is involved in X chromosome inactivation—two features that may be related to its gender-specific risk.[35] Interestingly, male breast cancers are markedly increased only in families carrying *BRCA2* mutations.

Both genes have a total length of over 80 kb, and hundreds of different mutations distributed throughout the coding region have been reported for each one. The frequency of mutations is only 0.1% to 0.2% in the general population. Some mutations diminish the function of the genes and increase cancer risk, whereas others might be unimportant sequence variants. Genetic testing is difficult and often inconclusive unless several family members are affected or unless the individual belongs to an ethnic group with a known high incidence of specific mutations. [36] For example, people of Ashkenazi Jewish descent have a 2% to 3% risk of three specific mutations. Identification of carriers of clinically significant mutations is important, as prophylactic mastectomy and/or oophorectomy can reduce the risk of cancer mortality. [31] [37] [38]

In hereditary carcinomas, one mutant *BRCA* allele is inherited, and the second allele is inactivated by somatic mutation. Although *BRCA1* and *BRCA2* mutations are rarely found in sporadic tumors, about 50% of such tumors have decreased or absent expression of BRCA1. In most cases, this is accomplished by a combination of loss of heterozygosity (LOH) and methylation of the promoter to inactivate both alleles. [39] Hypermethylation of the promoter is detected in 13% of unselected carcinomas but is more common in medullary carcinomas (67% of tumors) and mucinous carcinomas (55% of tumors)—histologic subtypes that are more commonly found in *BRCA1* carriers. A similar mechanism has not yet been described for *BRCA2*.

BRCA1-associated breast cancers are more commonly poorly differentiated, have a syncytial growth pattern with pushing margins, have a lymphocytic response, and do not express hormone receptors or overexpress HER2/neu (an epidermal growth factor receptor that is commonly overexpressed in breast cancer, to be discussed later), as compared to sporadic breast carcinomas. *BRCA2*-associated breast carcinomas do not have a distinct morphologic appearance. Initial results using gene expression RNA profiling have revealed that *BRCA1*, *BRCA2*, and subtypes of sporadic cancers can be recognized by their gene expression patterns[40] [41] (Box 23-1). Sporadic carcinomas with an mRNA profile similar to *BRCA1* carcinomas have been termed "basal-like" carcinomas owing to the expression of genes that are characteristic of myoepithelial or possible breast progenitor cells. These results demonstrate that a subset of sporadic carcinomas have biologic similarities to hereditary carcinomas.

Genetic susceptibility due to other known genes is much less common, and together this group accounts for fewer than 10% of hereditary breast carcinomas. [42] Only five have been studied sufficiently to be worth noting. Mutations in the cell-cycle checkpoint kinase gene (*CHEK2*), which is an important component of the recognition and repair of DNA damage and which activates *BRCA1*, may account for 5% of familial cases. [43] The risk for a mutation carrier may be as low as 20%. Women with the Li-Fraumeni syndrome (due to a germ-line mutation in the *p53* gene) have an 18-fold higher risk of developing breast cancer before the age of 45. Mutations in *p53* also occur in 19% to 57% of sporadic breast carcinomas. Cowden syndrome ("multiple hamartoma syndrome" due to a mutation of the *PTEN* gene on chromosome 10q) confers a 25% to 50% lifetime risk of breast cancer in affected women. Mutations in the *PTEN* gene are rare in sporadic carcinomas, but LOH is found in 11% to 41%. Further studies will be necessary to determine whether the function of the other allele is altered (e.g., by methylation). Women with Peutz-Jeghers syndrome (caused by truncating mutations in the *LKBI* gene) are at increased risk for breast cancer. There is, as yet, no evidence that this gene plays a role in sporadic carcinoma. The role of the *ATM* gene in breast cancer susceptibility in ataxia telangiectasia carriers has been intensively studied owing to the high frequency of carriers in the population (approximately 7%) and the increased sensitivity to radiation exposure leading to concerns about screening mammography. Studies have had mixed results, some showing an increased risk and others not showing an association. The risk might be dependent on the type of germ-line mutation (e.g., truncating versus missense). Mutations in the *ATM* gene in sporadic carcinomas are rare.

All of these genes considered together still leave at least two-thirds of familial risk unexplained. The search for a putative "*BRCA3*" gene of high penetrance has, as yet, been unsuccessful, and such a gene might not exist.[44] A polygenic model

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in which many weakly penetrant genes (perhaps dozens or hundreds) act in combination to create a spectrum of risk could explain the majority of familial breast cancers, as well as risk in the general population.[45] [46] [47] This model suggests that most breast cancers arise in a minority of women carrying combinations of these susceptibility genes. The identification of these genes might allow better stratification of women into low-risk and high-risk groups, which would help to focus efforts toward prevention and early detection in these women. Yet to be determined are the number of genes that could be involved, the nature of interactions among these genes (e.g., additive or multiplicative), the interaction with environmental factors, and the possible role of protective alleles. Candidates for such genes have been identified by their ability to modify the expression of known genes such as *BRCA1*.

Genome-wide approaches (e.g., microarray technology; Box 23-1) could play an important role in identifying this potentially very large group of susceptibility genes. One current approach classifies hereditary cancers by mRNA profiling in the hopes that cancers arising due to the same germline mutation (or mutations) will have similar patterns, as has been demonstrated with *BRCA1* and *BRCA2*. [48] If true, this would simplify linkage analysis by identifying groups of families likely to carry similar mutations.

Many studies have confirmed that some of the genes involved in hereditary breast cancer (e.g., *BRCA1* and *p53*) are also important in many sporadic cancers. It is hoped that the continued investigation of the wide variety of naturally occurring mutations and combinations of mutations will provide important clues to breast cancer pathogenesis.

Sporadic Breast Cancer

The major risk factors for sporadic breast cancer are related to hormone exposure: gender, age at menarche and menopause, reproductive history, breast-feeding, and exogenous estrogens. The majority of these cancers occur in postmenopausal women and overexpress ER. Estrogen itself has at least two major roles in the development of breast cancer. Metabolites of estrogen can cause mutations or generate DNA-damaging free radicals.[49] Via its hormonal actions, estrogens drive the proliferation of premalignant lesions as well as cancers. However, other mechanisms also undoubtedly play a role, as a significant subset of breast carcinomas are ER-negative or occur in women without increased estrogen exposure.

Mechanisms of Carcinogenesis

The vast array of histologic appearances of proliferative and atypical breast disease, as well as carcinomas, are the outward manifestations of the dozens or hundreds of biologic changes taking place within these lesions and point to the complex and variable pathways to carcinogenesis. Indeed, not one common genetic or functional change can be found in every breast cancer. Most reported changes occur in only a subset of carcinomas and usually in highly variable combinations with other changes.

A general model for carcinogenesis postulates that a normal cell must achieve seven new capabilities, including genetic instability, to become malignant [50] [51] (see Chapter 7) (Fig. 23-15). In hereditary carcinoma, one or more of these alterations is facilitated by the inheritance of germ-line mutations. Each of the new capabilities can be achieved by a change in one of many genes. For example, changes in *ER, EGF-R, RAS,* or *HER2/neu* may result in self-sufficiency in growth signals. On the other hand, one cellular alteration (e.g., a change in a gene such as *p53* that has a central role in controlling the cell cycle, DNA repair, and apoptosis) can affect more than one of these capabilities.

The morphologic changes in the breast associated with the smallest increased risk of cancer are lesions with increased numbers of epithelial cells (proliferative changes). This suggests that these early changes are related to evasion of growth-inhibiting signals, evasion of apoptosis, and self-sufficiency in growth signals. There is evidence that even at this early stage, there is abnormal expression of hormone receptors and abnormal regulation of proliferation in association with hormone receptor positivity. [52]

Genetic instability, in the form of LOH, appears to be a later change, as it is rarely detected in proliferative changes but becomes more frequent in atypical hyperplasias and is almost universally present in carcinoma in situ. Frank aneuploidy, as observed by nuclear enlargement, irregularity, and hyperchromasia, or image analysis to measure DNA content, is seen only in high-grade DCIS and some invasive carcinomas. Limitless replicative potential is suggested by the ability of clonal populations of the cells of DCIS to completely fill a ductal system in the breast. Increased angiogenesis is evident surrounding the basement membrane of some ducts that are involved by some types of DCIS. This might be due to direct stimulation by the malignant cells, secondary stimulatory effects on stromal cells, or the loss of inhibition of angiogenesis by myoepithelial cells.

The morphologic and biologic features of carcinomas are usually established at the in situ stage, as in the majority of cases, the in situ lesion closely resembles the accompanying invasive carcinoma. For example, lobular carcinomas are associated with LCIS, well-differentiated carcinomas with low-grade DCIS, and high-grade carcinomas with high-grade DCIS. Recurrent carcinomas generally have the appearance of the original carcinoma. Breast carcinomas do not generally "dedifferentiate," or become more poorly differentiated over time.

This view of oncogenesis focuses on the malignant epithelial cell and does not take into account the other tissue components. The structure and function of the normal breast require complex interactions between luminal cells, myoepithelial cells, and stromal cells. The same functions that allow for normal formation of new ductal branch points and lobules during puberty and pregnancy—abrogation of the basement membrane, increased proliferation, escape from growth inhibition, angiogenesis, and invasion of stroma—can be co-opted during carcinogenesis by abnormal epithelial cells, stromal cells, or both.[53] While the changes described above are accumulating in the luminal cells (or, less commonly, myoepithelial cells), parallel changes also occur due to mutation or epigenetic changes (e.g., DNA methylation) or via abnormal signaling pathways in these other cell types, resulting in the loss of normal cellular interactions and tissue structure. [54] Loss of these normal functions also occurs with age, and this loss might contribute to the increased risk of breast cancer in older women.

The final step of carcinogenesis, the transition of carcinoma limited by the basement membrane to ducts and lobules (carcinoma

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Box 23-1. GENE EXPRESSION PORTRAITS OF BREAST CARCINOMAS

Until recently, changes occurring in cancer cells were studied one at a time or in small groups in small sets of tumors. New microarray technologies ("gene chips") have enabled investigators to simultaneously detect and quantify the expression of large numbers of genes (potentially all genes) in different tumors (see Box 7-1 , Chapter 7).

A major advantage of gene arrays is the ability to analyze a multitude of changes in cancer cells (i.e., a "molecular portrait") to discern overall patterns that would not be possible to detect by conventional techniques. An example of the type of data that may be generated from such assays is illustrated in a simplified form in the figure on the facing page. The results for 26 breast carcinomas (each corresponding to one column) for 28 genes (represented by each row) are displayed. A relative increased quantity of mRNA (relative to a reference standard) is shown by red, a relative decreased quantity by green, and an average amount by black. Also shown in the figure are the histology of the different tumor types and the expression of selected proteins detected by immunohistochemistry (ER, HER2, e-cadherin, and basal keratin).

Microarray studies, such as this one and others, have identified breast cancer subtypes previously identified by morphology (e.g., lobular carcinomas), by protein expression (e.g., ER-positive and HER2/neu-positive carcinomas), and by germ-line mutations (e.g., *BRCA1* and *BRCA2* carcinomas). In addition, new subtypes that were not previously well defined have been identified (e.g., the basal-like carcinomas). In the figure, results are not shown for many other tumor subtypes, such as tubular, mucinous, and medullary carcinomas, because these are relatively rare and too few cases have been examined to allow firm conclusions.

mRNA levels do not always correspond to changes in protein expression. The quantity of protein within a cell depends not only on the amount and rate of transcription and translation,

but also on protein degradation and the rate of transport out of the cell. Therefore, other assays are necessary to determine actual protein content. Immunohistochemistry (IHC) uses antibodies to detect proteins on tissue sections. Whereas tissue used for mRNA profiling may include both tumor and stromal cells, IHC has the advantage of being able to identify the cell type expressing the protein and the specific cellular location of the protein.

Estrogen Receptor-Positive Carcinomas. Seventy per cent to 80% of breast carcinomas express ER and are thought to arise from intrinsically ER-positive luminal cells. ER-positive ductal carcinomas ("no special type") are usually well to moderately differentiated and often show tubule formation. Most special types of breast cancer (i.e. lobular, tubular, mucinous, and papillary) are also ER-positive. In the microarray data illustrated, the group of ductal carcinomas, in general, show normal or overexpression of the ER-related gene cluster and luminal keratins, and exhibit low levels of mRNAs from the groups of genes characteristic of the other tumor types. In the lower part of the figure, IHC on one representative ductal carcinoma demonstrates that ER is present in the nucleus, e-cadherin on the cell membrane, and that HER2/neu and basal keratins are undetectable.

In contrast to traditional IHC assays that determine the expression of only ER and a single gene under its regulation, PR, mRNA profiling provides information about many other ER-regulated genes. Using this type of assay, it might be possible to identify the cancers that express ER but fail to respond to hormonal treatments due to disruption of the signaling pathway, resulting in low expression of other ER-regulated genes.

Lobular carcinomas can be identified by the distinctive morphologic pattern of infiltration as single cells or loosely cohesive cell clusters. This appearance has been linked to the loss of the normal cell adhesion molecule e-cadherin, which is retained in most other carcinomas within the ER-positive group. By expression profiling, the lobular carcinomas cluster together and are most closely related to the other ER-positive carcinomas. The absence of e-cadherin can be seen by both diminished mRNA and the absence of the protein by IHC.

Estrogen Receptor-Negative Carcinomas. These carcinomas may arise owing to loss of ER expression or from normally ER-negative cells. Expression profiling identifies two major types of ER-negative carcinomas.

HER2-*Positive Carcinomas.* This group of carcinomas was previously identified by overexpression of the HER2/neu protein. In the majority of carcinomas, the mechanism of over-expression is amplification of the gene resulting in increased transcription into mRNA and protein translation. Breast cancers are routinely assayed for *HER2/neu* gene and protein using FISH or IHC (Figs. 23-27*C* and *B* , respectively) in order to predict clinical responses to antibodies targeted to the protein. These carcinomas tend to be poorly differentiated.

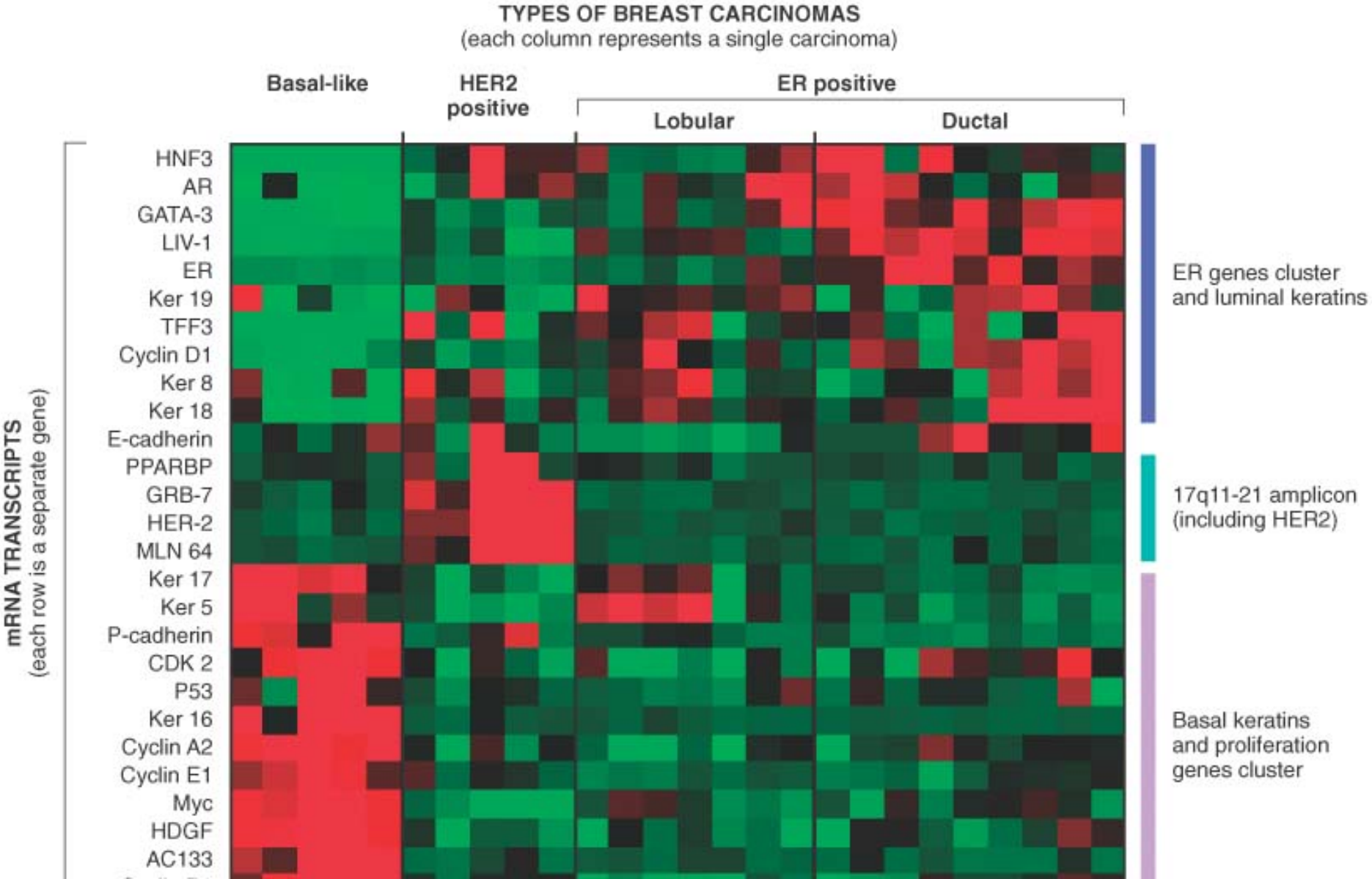
The expression profile reveals not only increased copies of *HER2/neu* mRNA, but also increased transcription of other adjacent genes that are amplified within this segment of DNA. These carcinomas do not overexpress the genes that are characteristic of the other subtypes of cancers in this array (e.g. ER and basal keratins), but do express e-cadherin.

Basal-like Carcinomas. This group of carcinomas is distinguished by the expression of keratins that are more typical of myoepithelial cells or potential breast progenitor cells; it has not been previously well characterized. Because the myoepithelial cell is located in the basal area of the lobules and ducts, in the absence of knowing the specific cell of origin, this group of carcinomas was termed *basal-like*. In addition to the expression of specific keratins, they also show expression of other genes in common with myoepithelial cells (e.g., p-cadherin) as well as numerous genes related to cell proliferation. This group of carcinomas does not express ER or ER-related genes or *HER2/neu*, as can be seen by the array data and by IHC.

Carcinomas arising in women with *BRCA1* mutations also cluster with this group. *BRCA1* carcinomas are similar to basal-like carcinomas in being poorly differentiated, lacking ER and HER2/neu expression, and expressing basal-like keratins. However, most women with basal-like carcinomas do not have germ-line *BRCA1* mutations.

Conclusions. mRNA expression profiling is a powerful tool for investigating breast carcinomas. Analogous arrays to analyze DNA and protein expression profiles are under development. In addition to identifying tumor types, as in this example, mRNA arrays have been used for predicting prognosis and response to therapy, examining tumor changes after therapy, and classifying hereditary carcinomas. Although it might not be feasible to perform transcriptional profiling on every clinical case of breast cancer, these studies will generate information that will lead to better diagnostic, prognostic, and therapeutic tests that are applicable to all patients.

Figure 23- Selected data from mRNA expression profiling (26 carcinomas and 28 genes) are shown in the top half of the figure. Each vertical column represents one carcinoma (and shows information acquired from one "gene chip") and each horizontal row represents the data for a gene (identified at the left). Red indicates an increase, green a decrease, and black no change in mRNA relative to a standard. Cluster analysis was used to group carcinomas with similar expression patterns and the groups are identified as basal-like, *HER2* positive, and the ER-positive lobular and ductal carcinomas. The most important gene clusters are identified on the right. These carcinomas have typical morphologic appearances as shown in the middle row of images (H&E). In the lower half of the figure, mRNA expression patterns are correlated with changes in protein expression by using antibodies to detect antigens within tissues. The presence of a protein is indicated by a brown reaction product within the tumor cells and can be localized to a subcellular site (estrogen receptor-nuclear; HER2/neu and e-cadherin-membrane; basal keratin-cytoplasmic). *The array data are courtesy of Dr. Andrea Richardson, Brigham and Women's Hospital, Boston, MA, as modified from Signoretti S, Di Marcotullio L, Richardson A, et al.: Oncogenic role of the ubiquitin ligase subunit Skp2 in human breast cancer, J Clin Invest 110:633–641, 2002.*



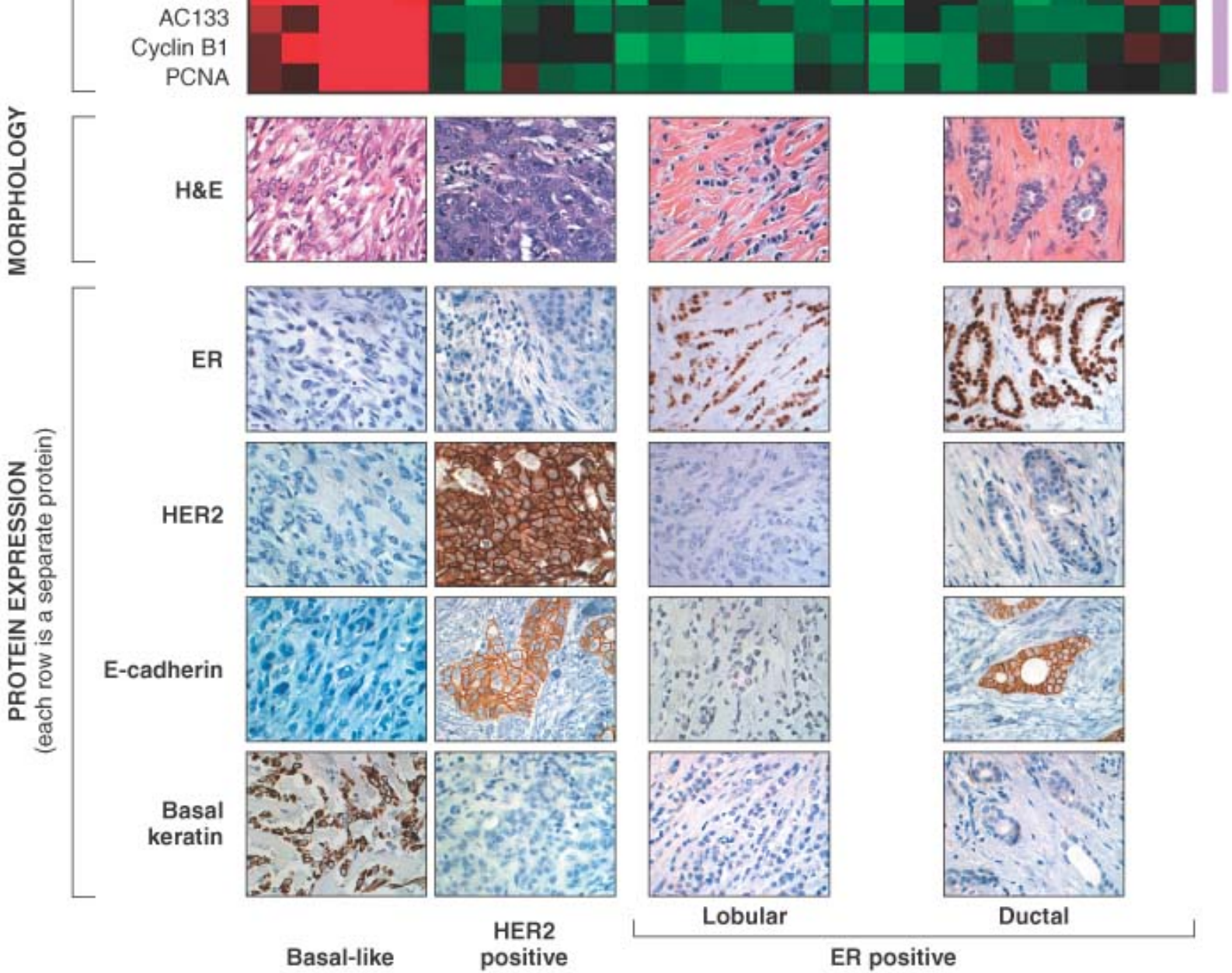


Figure 23-15 The normal breast is maintained by a complex set of interactions among luminal cells, myoepithelial cells, the basement membrane, and stromal cells (illustrated to the left of the figure). Morphologic changes are displayed according to the risk for subsequent invasive carcinoma (top row of pictures). The seven categories of changes in biologic functions

that must occur in successful malignant cells are shown in colored boxes. The changes need not occur in a specific order but accumulate until cells acquire malignant potential. The association of these changes with premalignant breast lesions suggests that the earliest events are related to evasion of growth-inhibiting signals, evasion of apoptosis, and self-sufficiency in growth signals. Hereditary carcinomas arise from cells that have germ line mutations that alter DNA repair and/or normal signals for apoptosis and therefore require fewer acquired changes. Luminal cells likely give rise to the majority of cancers, but myoepithelial cells can also undergo malignant transformation. Changes in the malignant cells are accompanied by alterations in the supporting myoepithelial and stromal cells due to a combination of genetic and epigenetic events and disruption of the normal intercellular signaling pathways. The final alteration, invasion of stroma, is the least well understood. It has been difficult to identify biologic changes that are specific to invasive carcinomas. It is possible that invasion is a result of the loss of the ability of myoepithelial and stromal cells to maintain the basement membrane rather than a gain of function by the malignant cells.

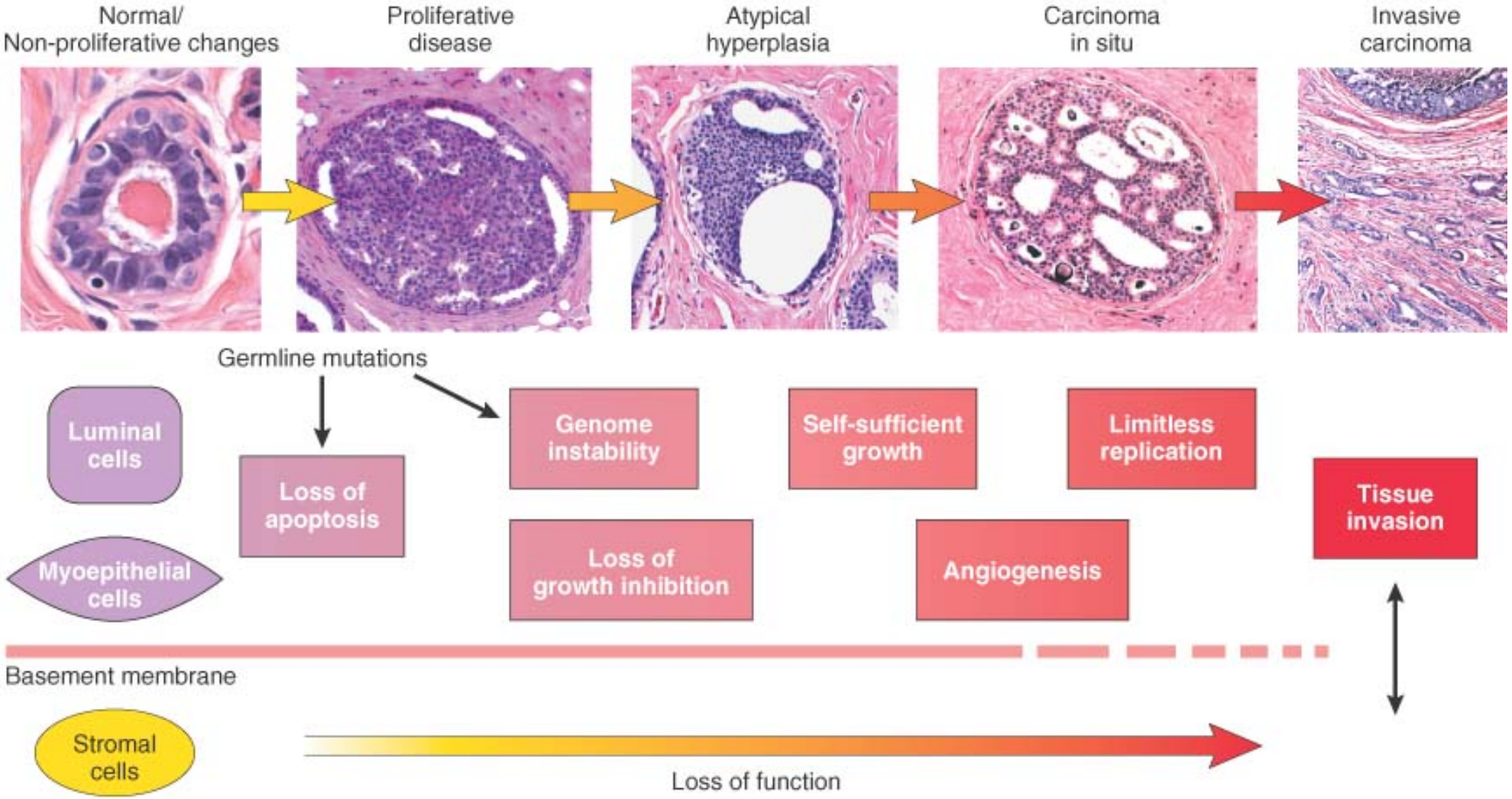


Figure 23-16 *A*, This mammogram reveals multiple clusters of small, irregular calcifications in a segmental distribution. Suspicious calcifications must be biopsied, as 20% to 30% will prove to be due to DCIS. *B*, Comedo DCIS fills several adjacent ducts (or completely replaced lobules) and is characterized by large central zones of necrosis with calcified debris. This type of DCIS is most frequently detected as radiologic calcifications. Less commonly, the surrounding desmoplastic response results in an ill-defined palpable mass or a mammographic density.

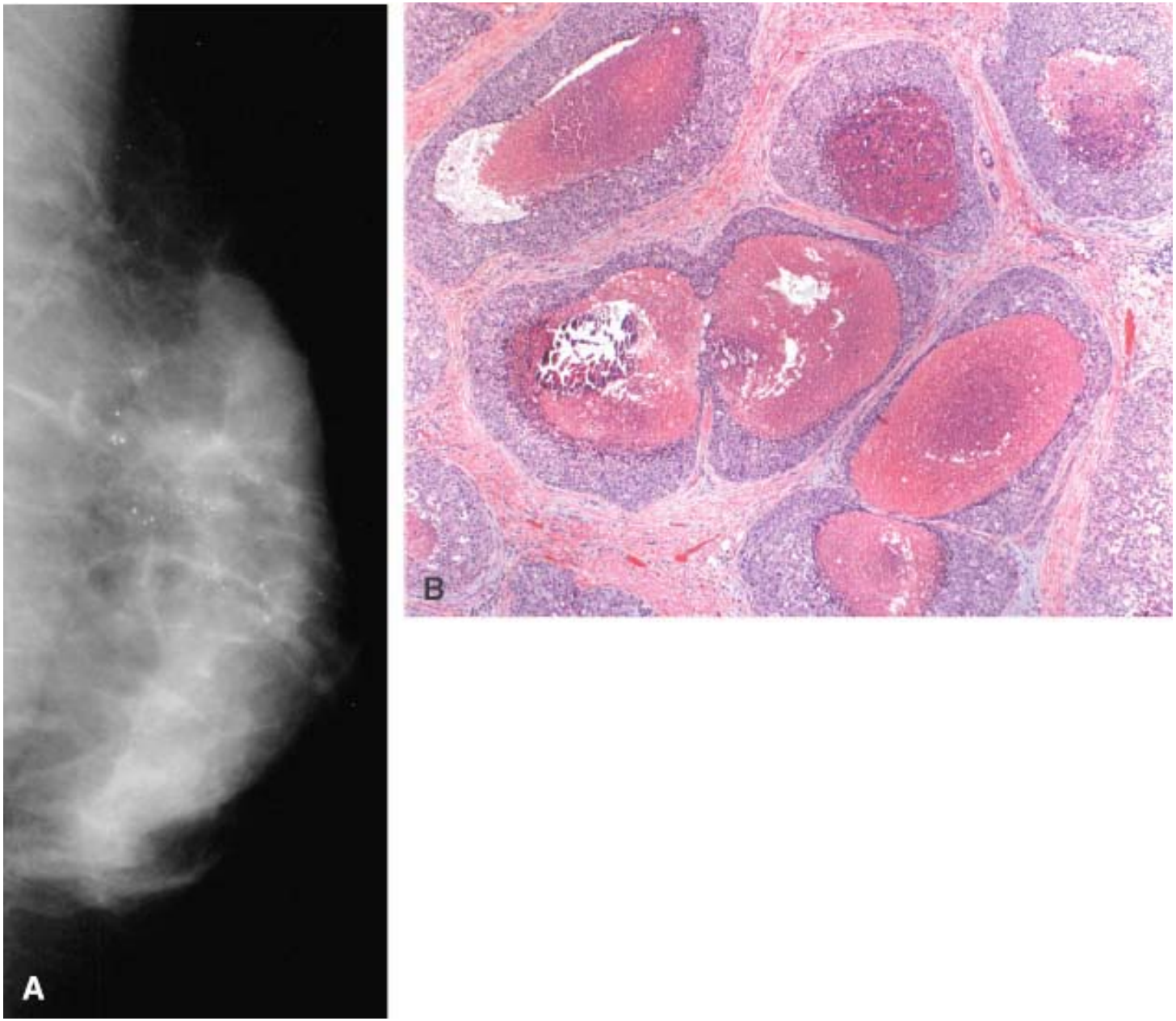


Figure 23-17 Noncomedo DCIS. *A*, Cribriform DCIS comprises cells forming round, regular ("cookie cutter") spaces. The lumens are often filled with calcifying secretory material. *B*, This solid DCIS has almost completely filled and distorted this lobule with only a few remaining luminal cells visible. This type of DCIS is not usually associated with calcifications and may be clinically occult.

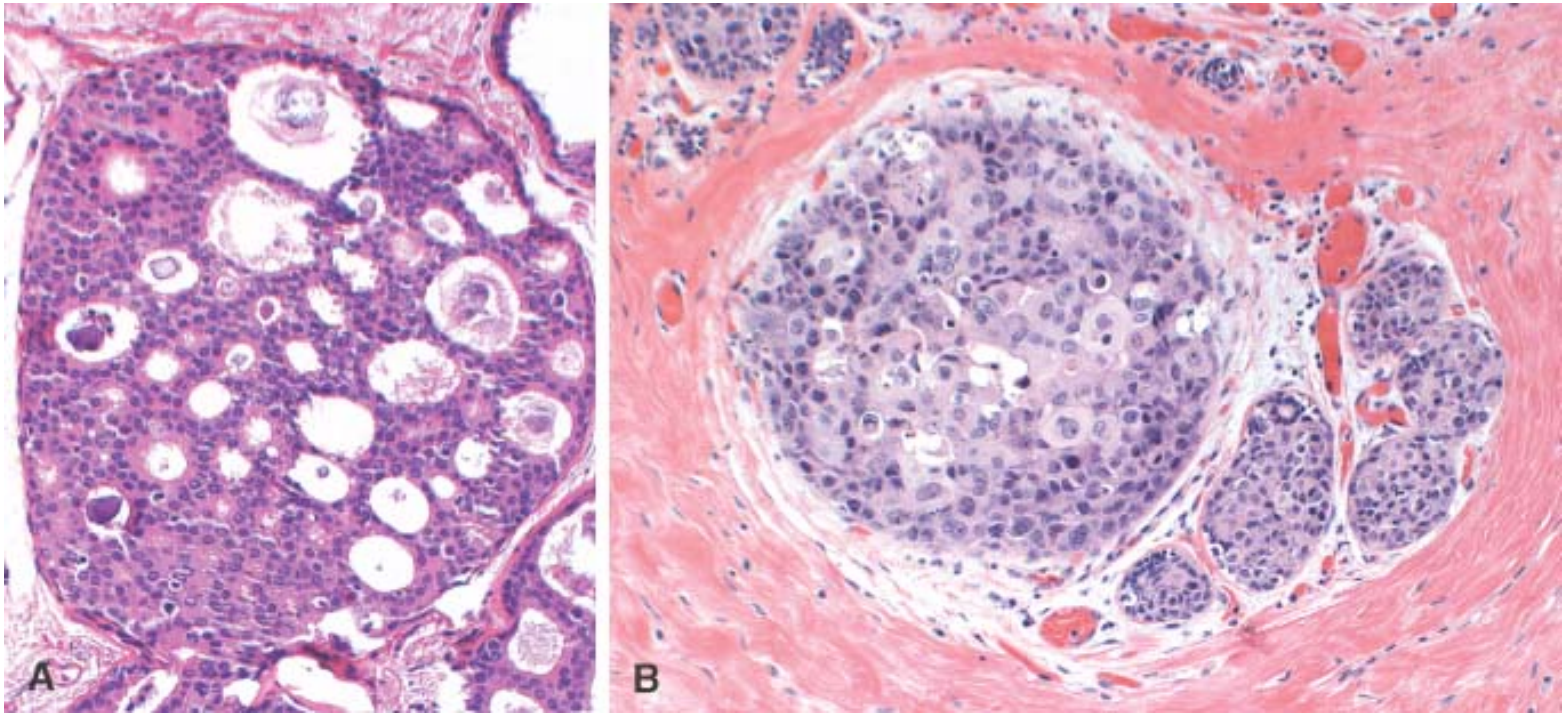


Figure 23-18 Noncomedo DCIS. *A*, Papillary DCIS. Delicate fibrovascular cores extend into a duct and are lined by a monomorphic population of tall columnar cells. Myoepithelial cells are absent. *B*, Micropapillary DCIS. The papillae are connected to the duct wall by a narrow base and often have bulbous or complex outgrowths. The papillae are solid and do not have fibrovascular cores.

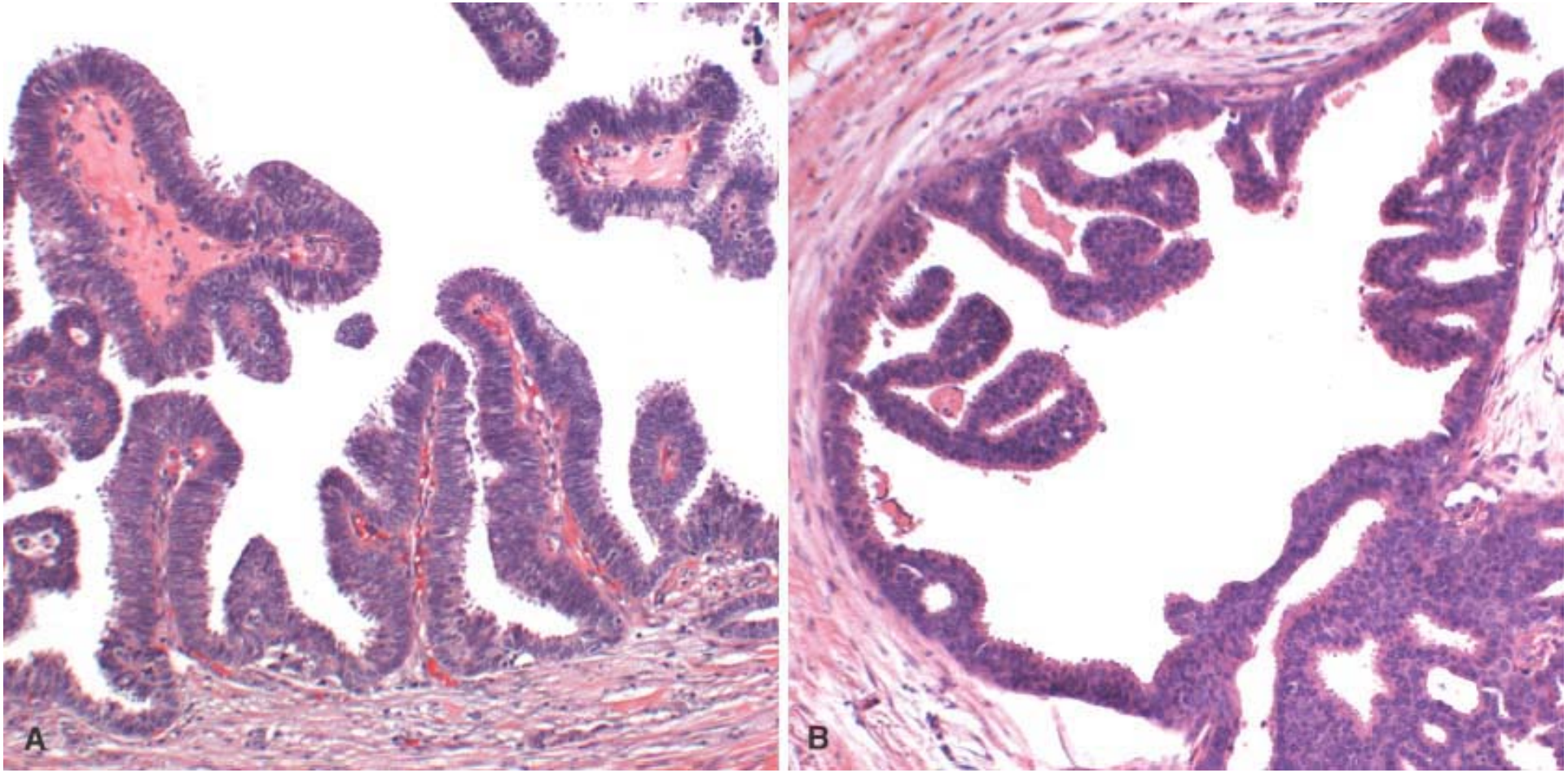


Figure 23-19 Paget disease of the nipple. DCIS arising within the ductal system of the breast can extend up the lactiferous ducts into nipple skin without crossing the basement membrane. The malignant cells disrupt the normally tight squamous epithelial cell barrier, allowing extracellular fluid to seep out and form an oozing scaly crust over the nipple skin.

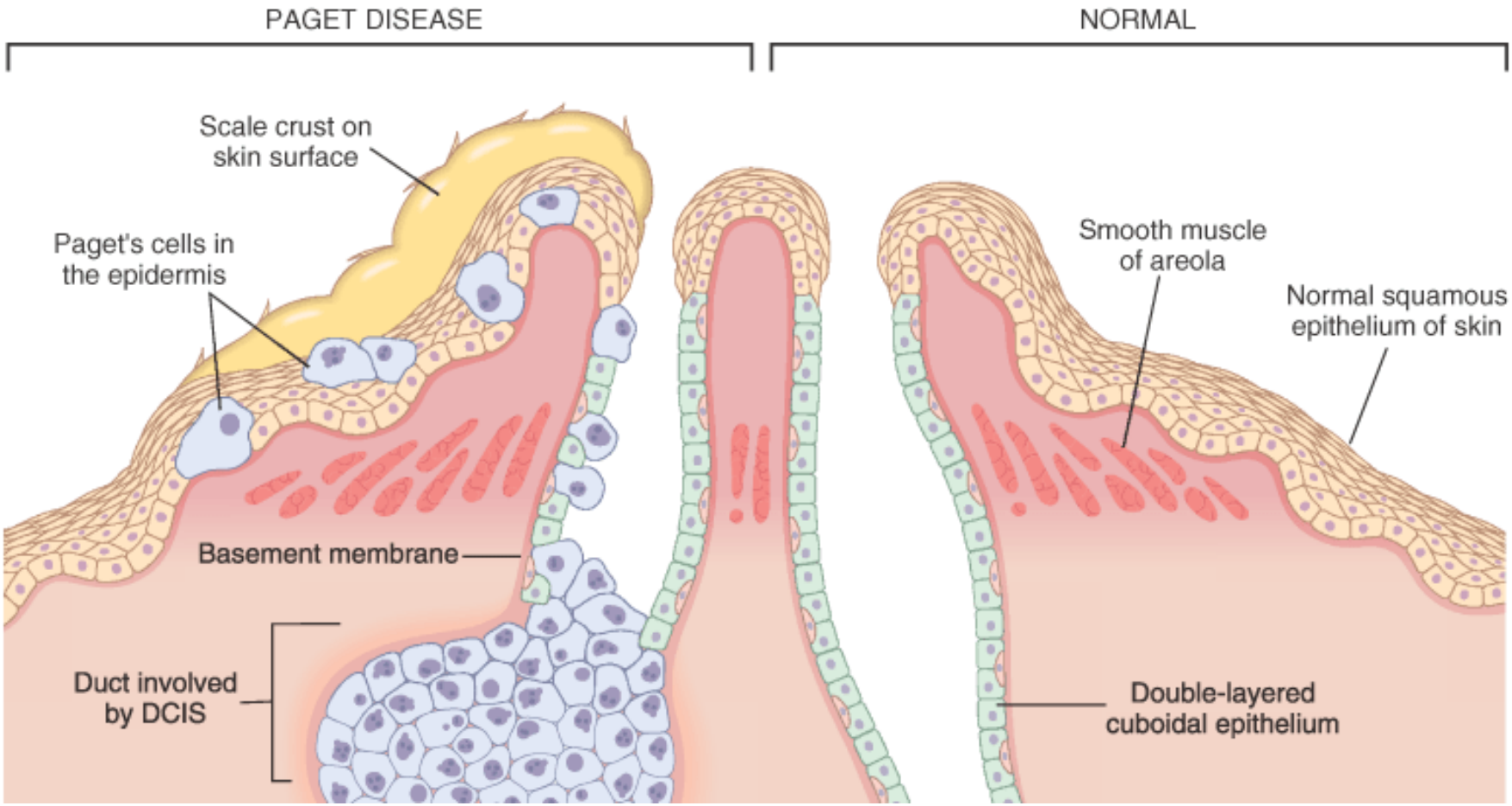


Figure 23-20 Lobular carcinoma in situ. A monomorphic population of small, rounded, loosely cohesive cells fills and expands the acini of a lobule. The underlying lobular architecture can still be recognized.

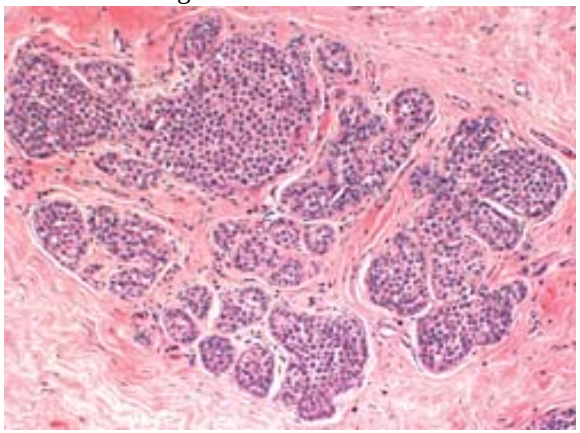


Figure 23-21 Invasive ductal carcinoma. *A*, This mammogram shows a density with an irregular border. There is a small, superimposed, incidental calcification. *(Courtesy of Dr. Jack Meyer, Brigham and Women's Hospital, Boston, MA.)* Over 90% of such masses will prove to be invasive carcinomas. Rarely, complex sclerosing lesions, prior surgical scars, and fibromatosis may present in this fashion. *B*, An irregular dense white mass is present within yellow adipose tissue. The pathologic gross differential diagnosis is the same as the radiologic differential diagnosis.

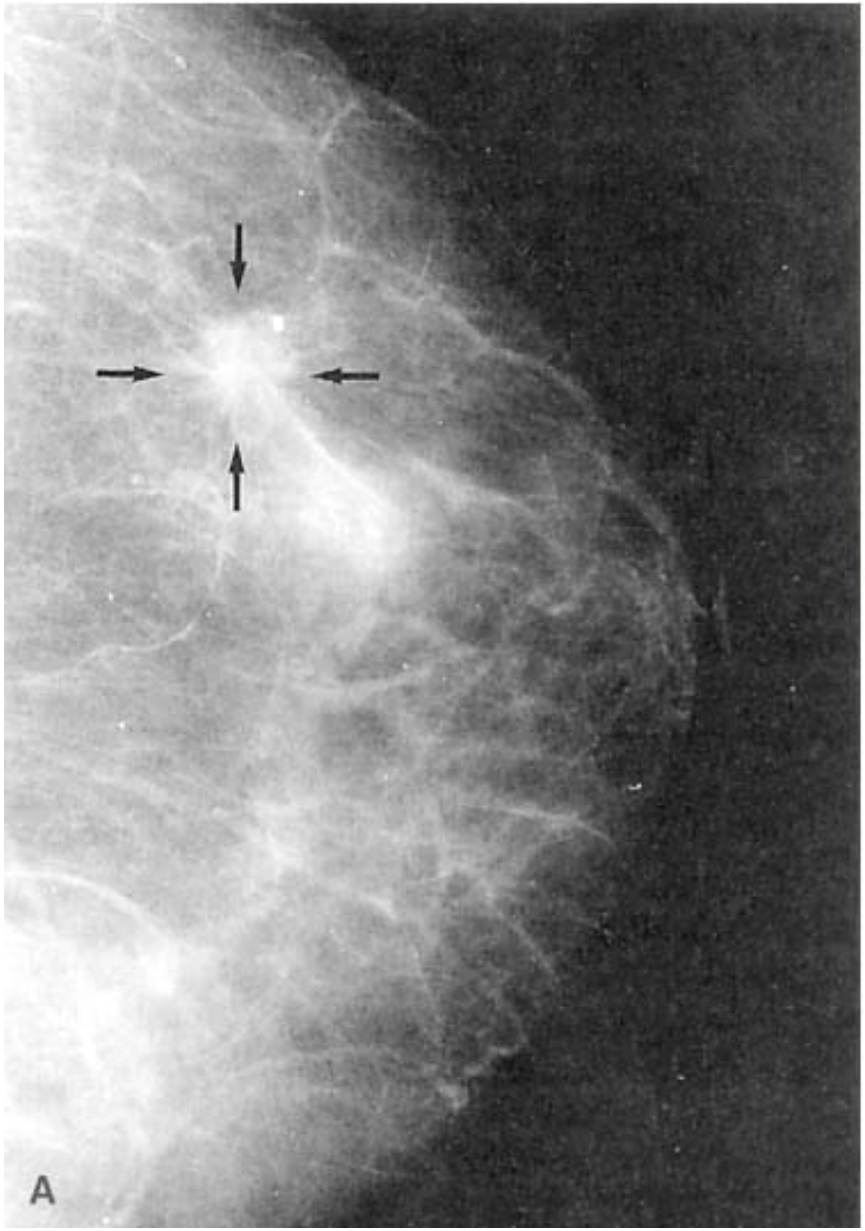


TABLE 23-4 -- Distribution of Histologic Types of Breast Cancer

Total Cancers	Per Cent

In Situ Carcinoma *	***15–30***
Ductal carcinoma in situ	80
Lobular carcinoma in situ	20
Invasive Carcinoma	***70–85***
No special type carcinoma ("ductal")	79
Lobular carcinoma	10
Tubular/cribriform carcinoma	6
Mucinous (colloid) carcinoma	2
Medullary carcinoma	2
Papillary carcinoma	1
Metaplastic carcinoma	<1
The data on invasive carcinomas are modified from Dixon JM, et al: Long-term survivors after breast cancer. Br J Surg 72:445, 1985.	

*The proportion of in situ carcinomas detected depends on the number of women undergoing mammographic screening and ranges from less than 5% in unscreened populations to almost 50% in patients with screen-detected cancers. Current observed numbers are between these two extremes.

typically express hormone receptors and do not overexpress *HER2/neu*. Others are composed of anastomosing sheets of pleomorphic cells (Fig. 23-22*B*) and are less likely to express hormone receptors and more likely to overexpress *HER2/neu*. The majority of invasive ductal carcinomas lie between these two extremes. Most carcinomas induce a marked increase in dense, fibrous desmoplastic stroma, giving the tumor a hard consistency on palpation and replace fat, resulting in a mammographic density (scirrhous carcinoma).

Carcinomas of NST are accompanied by varying amounts of DCIS. The grade of the DCIS usually correlates with the grade of the invasive carcinoma. For example, comedo DCIS is usually associated with poorly differentiated carcinomas, and low-grade DCIS is usually associated with well-differentiated carcinomas. Carcinomas associated with a large amount of DCIS require large excisions with wide margins to reduce local recurrences.

Morphologic analysis of this large group of carcinomas has not identified tumor types of significant clinical relevance beyond the specialized types to be described below. Recent studies using microarrays to analyze the transcriptional profile of these cancers have identified additional subgroups. (Box 23-1)[41] [56] [57] The challenge of future studies will be to show the clinical relevance of subtypes identified by gene expression profiling (e.g., with respect to etiology, presentation, prognosis, or response to treatment) and, if found, to determine whether these carcinomas can be recognized by more widely

Figure 23-22 *A*, Well-differentiated invasive carcinoma of no special type. Well-formed tubules and nests of cells with small monomorphic nuclei invade into the stroma with a surrounding desmoplastic response. *B*, Poorly differentiated invasive carcinoma of no special type. Ragged sheets of pleomorphic cells without tubule formation infiltrate into the

adjacent stroma.

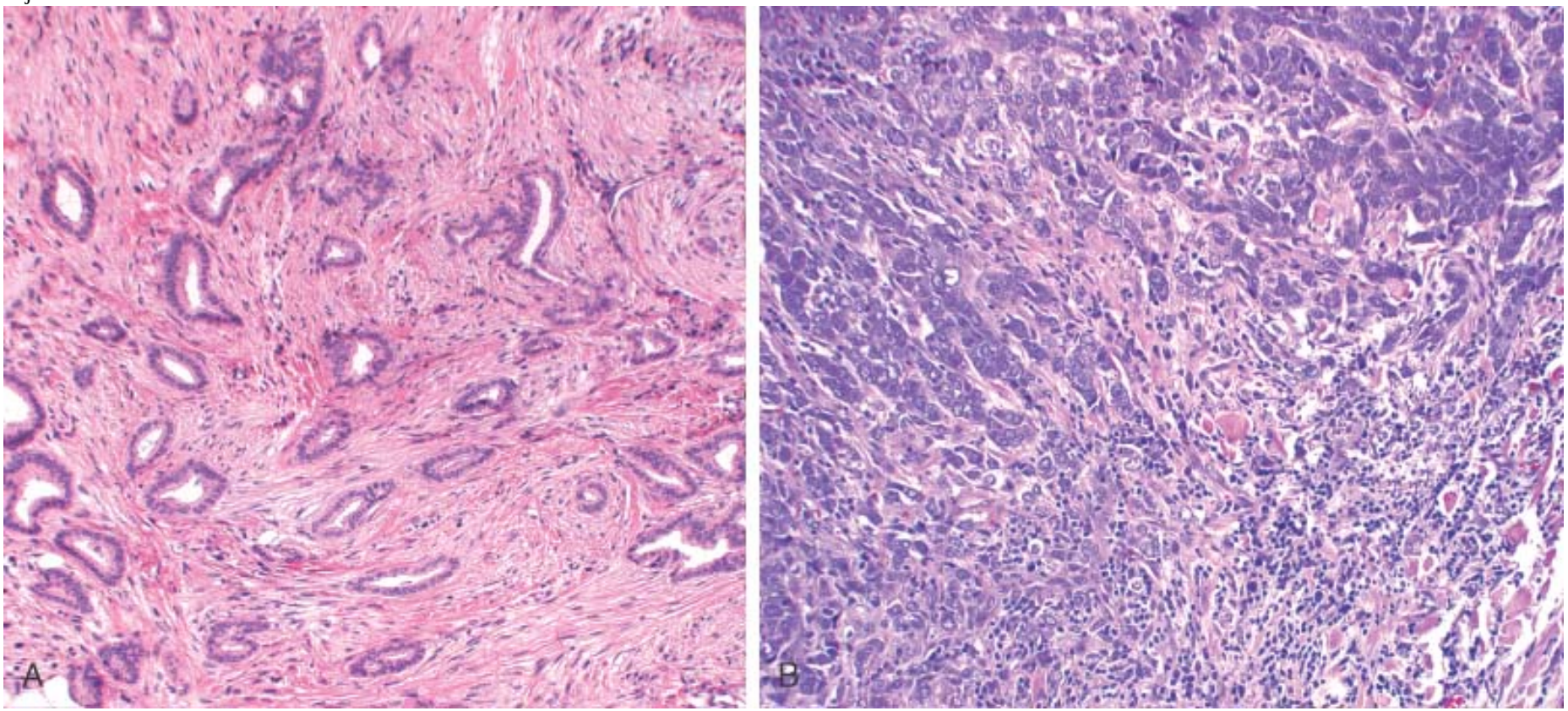


Figure 23-23 Invasive lobular carcinoma. Parallel arrays of small, regular cells with scant cytoplasm infiltrate singly in linear arrays or as small clusters of cells. There is often associated LCIS.

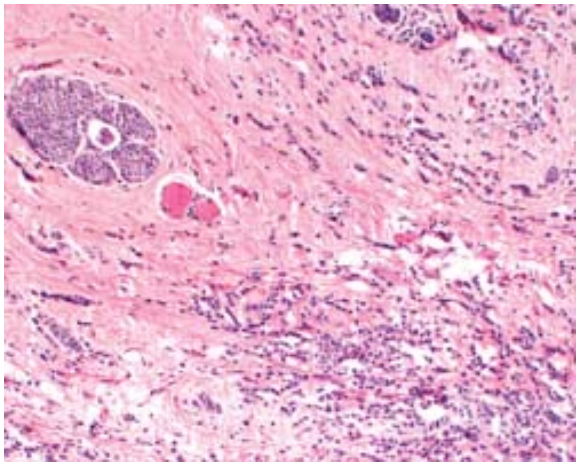


Figure 23-24 Medullary carcinoma. The cells are highly pleomorphic with frequent mitoses and grow as sheets of cohesive cells. A lymphoplasmacytic infiltrate is prominent.

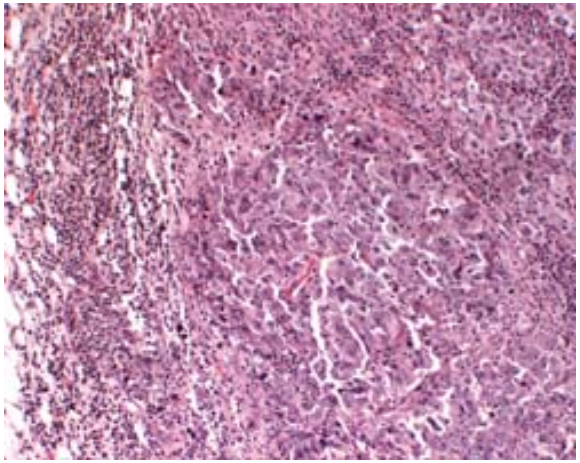


Figure 23-25 Mucinous (colloid) carcinoma. The tumor cells are present as small clusters within large pools of mucin. The borders are typically well circumscribed, and these cancers often mimic benign masses.

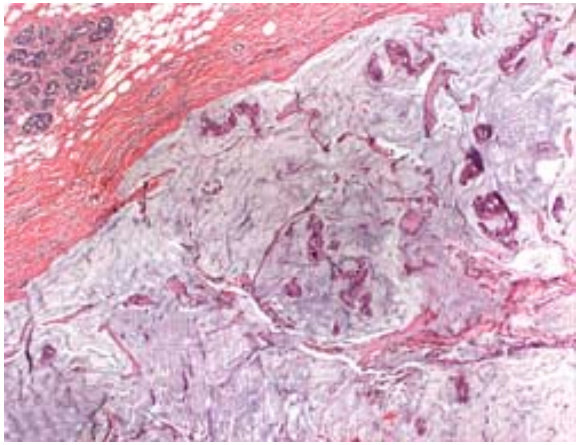
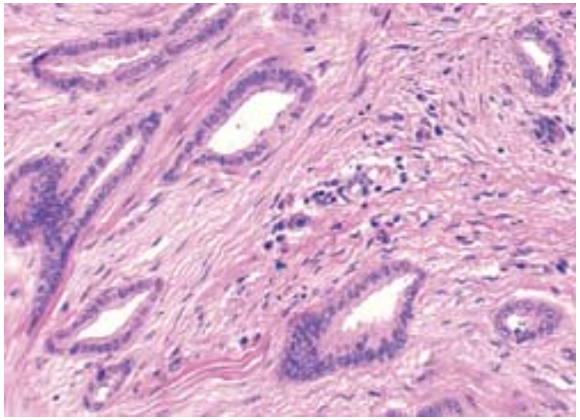


Figure 23-26 Tubular carcinoma. The carcinoma must be completely composed of well-formed tubules lined by a single layer of well-differentiated cells.



With no involvement, the 10-year disease-free survival rate is close to 70% to 80%; the rate falls to 35% to 40%

with one to three positive nodes and 10% to 15% in the presence of more than 10 positive nodes.
Most breast carcinomas drain to one or two *sentinel nodes* that can be identified by radiotracer, colored dye, or both. The sentinel node is highly predictive of the status of the remaining nodes. Sentinel node biopsy can spare women the increased morbidity of a complete axillary dissection. In some women, particularly those with medial tumors, the sentinel node may be an internal mammary node. These nodes are generally not biopsied owing to the morbidity associated with the procedure.
Macrometastases (>0.2 cm) are of proven prognostic importance. Because sentinel nodes often undergo more intense scrutiny with additional sections through the tissue, and immunohistochemistry or reverse transcriptase-polymerase chain reaction (RT-PCR) to detect rare tumor cells, increased numbers of women with minute metastatic deposits in lymph nodes are being identified. The clinical significance of small micrometastases is unclear and is being addressed by current clinical trials.

- **Tumor size.** The size of the carcinoma is the second most important prognostic factor and is independent from lymph node status. However, the risk of axillary lymph node metastases does increase with the size of the carcinoma. Women with node-negative carcinomas under 1 cm in diameter have a prognosis approaching that of women without breast cancer. The 10-year survival rate of such women without treatment is approximately 90%. On the other hand, over half of women with cancers over 2 cm in diameter present with lymph node metastases, and many of these women will eventually succumb to breast cancer.
- **Locally advanced disease.** Tumors invading into skin or skeletal muscle are frequently associated with concurrent or subsequent distant disease. With increased awareness of breast cancer detection, such cases have fortunately decreased in frequency and are now rare at initial presentation.
- **Inflammatory carcinoma.** Women presenting with the clinical appearance of breast swelling and skin thickening have a particularly poor prognosis with a 3-year survival rate of only 3% to 10%.

The major prognostic factors are used by the American Joint Committee on Cancer to divide breast carcinomas into clinical stages as follows:[70]

- Stage 0. DCIS or LCIS (5-year survival rate: 92%).
- Stage I. Invasive carcinoma 2 cm or less in diameter (including carcinoma in situ with microinvasion) without nodal involvement (or only metastases < 0.02 cm diameter) (5-year survival rate: 87%).
- Stage II. Invasive carcinoma 5 cm or less in diameter with up to three involved axillary nodes or invasive carcinoma greater than 5 cm without nodal involvement (5-year survival rate: 75%).
- Stage III. Invasive carcinoma 5 cm or less in diameter with four or more involved axillary nodes; invasive carcinoma greater than 5 cm in diameter with nodal involvement; invasive carcinoma with 10 or more involved axillary nodes; invasive carcinoma with involvement of the ipsilateral internal mammary lymph nodes; or invasive carcinoma with skin involvement (edema, ulceration, or satellite skin nodules), chest wall fixation, or clinical inflammatory carcinoma (5-year survival rate: 46%).
- Stage IV. Any breast cancer with distant metastases (5-year survival rate: 13%).

Minor Prognostic Factors.

Most women with nodal involvement and/or carcinomas over 1 cm in diameter will benefit from some form of systemic therapy. In this group, minor prognostic factors can be used to decide among chemotherapy regimens and/or hormonal therapies. For node-negative women with small carcinomas, minor prognostic factors are used to identify the women most likely to benefit from systemic therapy and those who might not need any additional treatment.[74] Three of these factors—estrogen receptor, progesterone receptor, and *HER2/neu*—are most useful as predictive factors for response to specific therapeutic agents.

1. **Histologic subtypes.** The 30-year survival rate of women with special types of invasive carcinomas (tubular, mucinous, medullary, lobular, and papillary) is greater than 60%, compared with less than 20% for women with cancers of no special type.[75]
2. **Tumor grade.** The most commonly used grading system to assess the degree of tumor differentiation (*Scarff Bloom Richardson*) combines nuclear grade, tubule formation, and mitotic rate. Eighty-five per cent of women with well-differentiated grade I tumors, 60% of women with moderately differentiated grade II tumors, and 15% of women with

poorly differentiated grade III tumors survive for 10 years.

3. **Estrogen and progesterone receptors.** Current assays use immunohistochemistry to detect the receptors in the nucleus (Fig. 23-27*A*). Fifty per cent to 85% of carcinomas express estrogen receptors, and such tumors are more common in postmenopausal women. Women with hormone receptor-positive cancers have a slightly better prognosis than do women with hormone receptor-negative carcinomas. The evaluation of hormone receptors is most valuable to predict response to therapy. Eighty per cent of tumors with estrogen receptors and progesterone receptors respond to hormonal manipulation, whereas only about 40% of those with only one type of receptor respond. Tumors with neither estrogen nor progesterone receptors have a less than 10% likelihood of responding.
4. *HER2/neu. HER2* (human epidermal growth factor receptor 2 or c-*erb B2* or *neu*) is a transmembrane glycoprotein involved in cell growth control.[76] [77] It does not appear to have a specific ligand but acts as a coreceptor for multiple growth factors. *HER2/neu* is overexpressed in 20% to 30% of breast carcinomas. In over 90% of cases, overexpression is associated with amplification of the gene on 17q21, and this can be determined either by evaluating protein content using immunohistochemistry or by determining gene copy number by using FISH (Figs. 23-27*B* and *C*). Although not all studies have come to the same conclusion, many have shown that overexpression of *HER2/neu* is associated with a poor prognosis. In addition, ongoing studies are addressing the possibility that *HER2/neu*-over-expressing tumors respond differently to hormonal or anthracycline chemotherapy regimens. However, evaluation of HER2/neu is most important to determine response to therapy targeted to this protein.

Trastuzumab (Herceptin) is a humanized monoclonal antibody to HER2/neu developed to specifically target tumor cells and, it is hoped, spare normal cells. In clinical trials, the combination of Trastuzumab with chemotherapy improved response in patients with carcinomas overexpressing *HER2/neu*. Unfortunately, cardiac toxicity, due to an unknown mechanism, could limit its usefulness. However, as the first gene-targeted therapeutic agent for a solid tumor, the results have been very promising.

5. **Lymphovascular invasion (LVI).** Tumor cells may be seen within vascular spaces (either lymphatics or small capillaries) surrounding tumors. This finding is strongly associated with the presence of lymph node metastases and is a poor prognostic factor in women without lymph node metastases. The presence of tumor cells in lymphatics of the dermis is strongly associated with the clinical appearance of inflammatory cancer and bodes a very poor prognosis. LVI must be strictly defined to have prognostic significance.
6. **Proliferative rate.** Proliferation can be measured by flow cytometry (as the S-phase fraction), by thymidine labeling index, by mitotic counts, or by immunohistochemical detection of cellular proteins (e.g., cyclins, Ki-67) produced during the cell cycle. Cyclin E content, when both full-length and low-molecular-weight isoforms are detected, is a very strong predictor of survival. [78] Tumors with high proliferation rates have a worse prognosis, but the most reliable method to assess proliferation has not yet been established. Mitotic counts are included as part of the standard grading system.
7. **DNA content.** The amount of DNA per tumor cell can be determined by flow cytometric analysis or by image analysis of tissue sections. Tumors with a DNA index of 1 have the same total amount of DNA as normal diploid cells, although marked karyotypic changes may be present. Aneuploid tumors are those with abnormal DNA indices and have a slightly worse prognosis.

Figure 23-27 Predictive markers. *A*, Estrogen receptor is detected in the nucleus by immunohistochemical studies. Progesterone receptor has the same appearance. *B, HER2/neu* overexpression is detected on the cell membrane by immunohistochemistry. *C,* Amplification of the *HER2/neu* gene can be detected by FISH analysis with a fluorescent probe for the gene. A normal cell has two copies of the gene. These tumor cells have over 25 signals, indicating amplification of the gene for *HER2/neu*. *(Courtesy of Dr. Jonathan Fletcher, Brigham and Women's Hospital, Boston, MA.)*

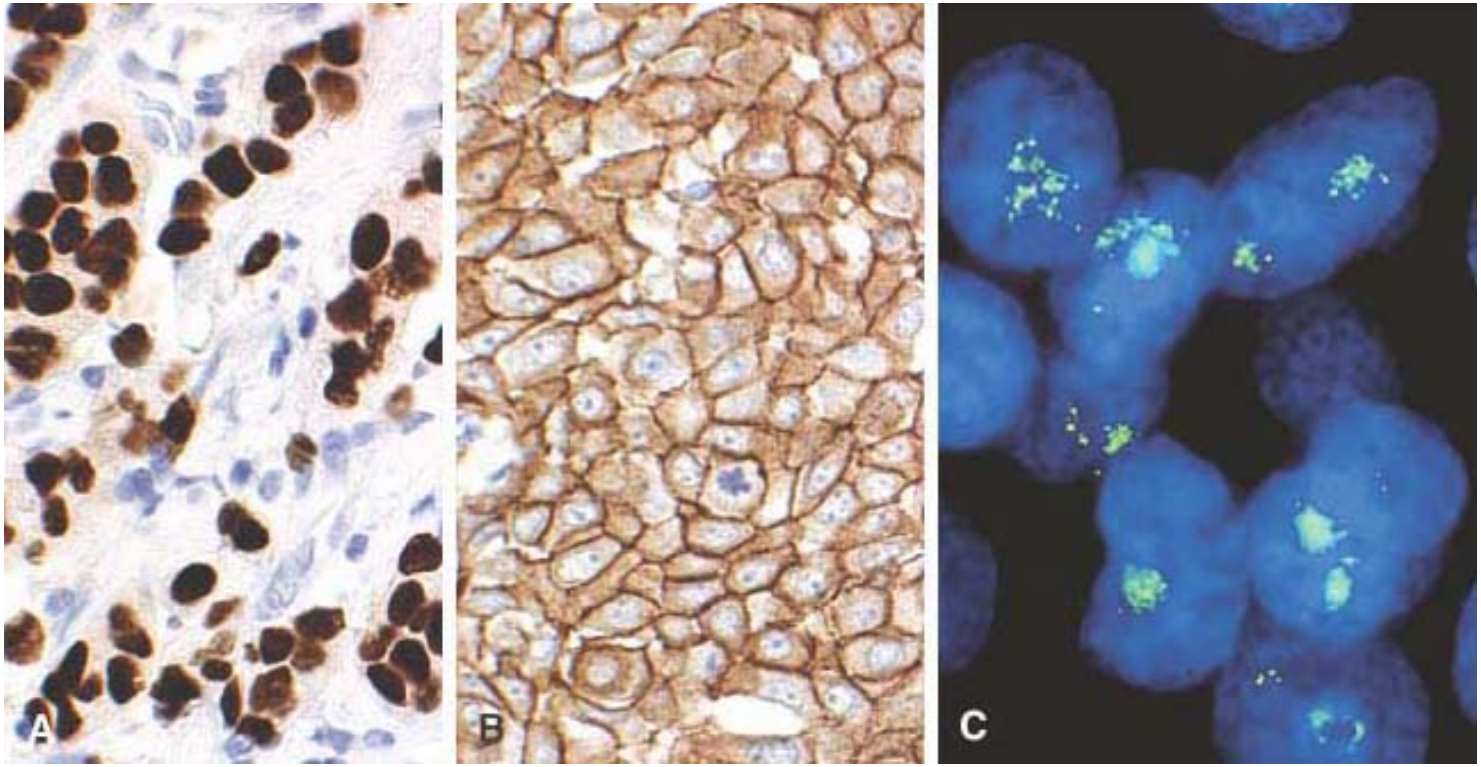


Figure 23-28 *A,* This mammogram shows a well-circumscribed mass. *(Courtesy of Dr. Jack Meyer, Brigham and Women's Hospital, Boston, MA.)* Although the most common lesion would be a fibroadenoma, other benign (e.g., fibrous lesions or PASH) and malignant (e.g., medullary or mucinous carcinomas) lesions can also have this appearance. *B,* Fibroadenoma. A rubbery, white, well-circumscribed mass is clearly demarcated from the surrounding yellow adipose tissue. The fibroadenoma does not contain adipose tissue and therefore appears denser than the surrounding normal tissue on mammogram.

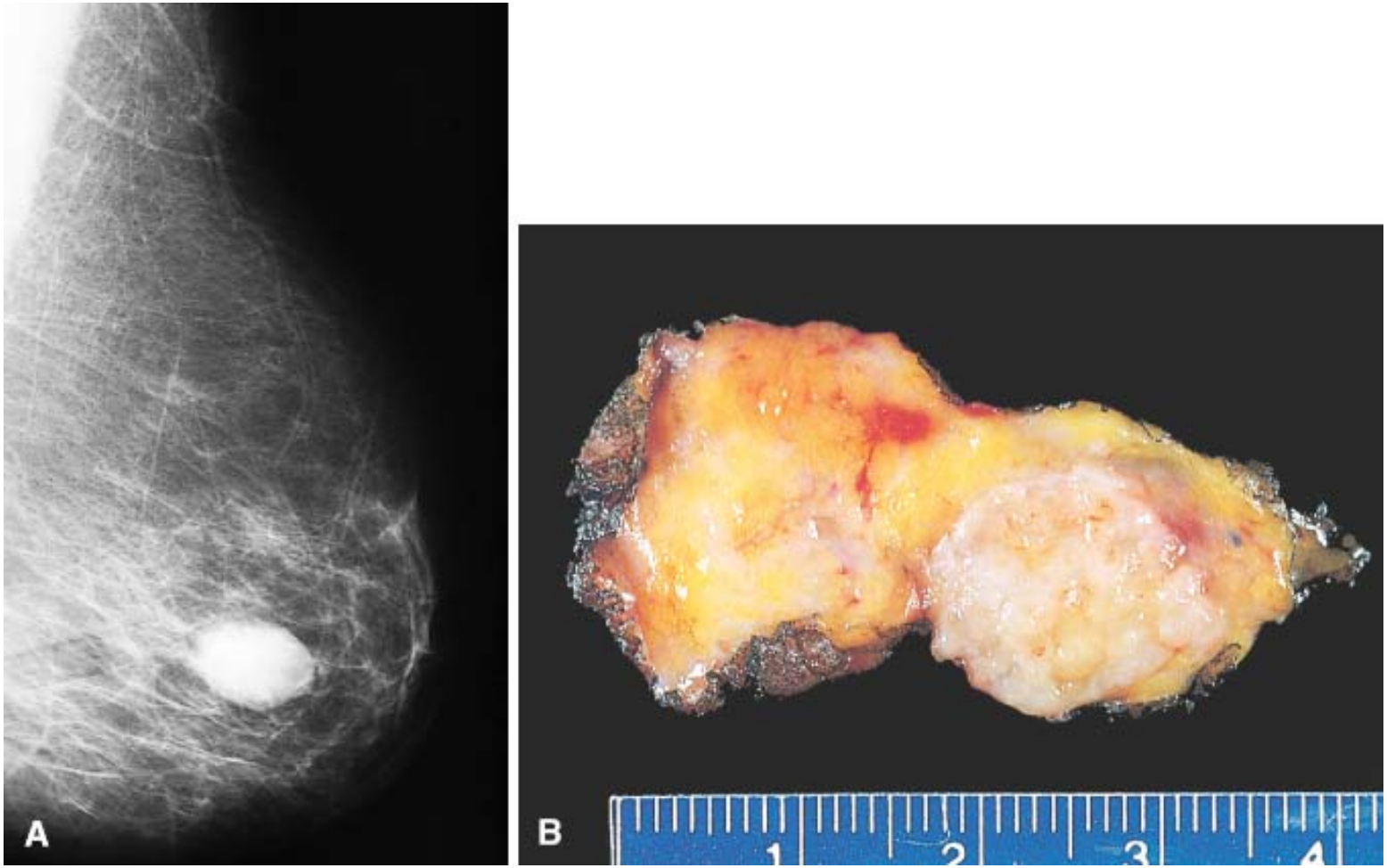


Figure 23-29 Fibroadenoma. The lesion consists of a proliferation of intralobular stroma surrounding and often pushing and distorting the associated epithelium. The border is sharply delimited from the surrounding tissue.

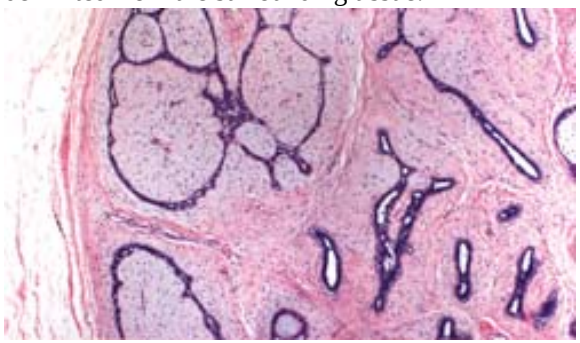


Figure 23-30 Phyllodes tumor. Compared to a fibroadenoma, there is increased stromal cellularity, cytologic atypia, and stromal overgrowth, giving rise to the typical leaflike architecture.

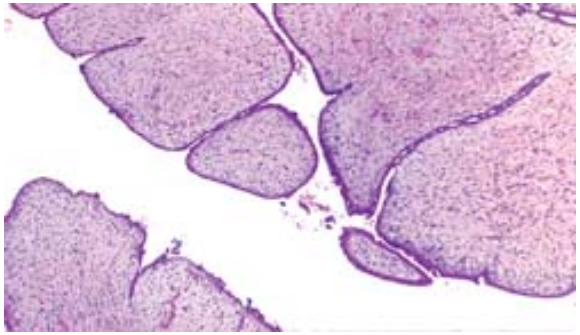
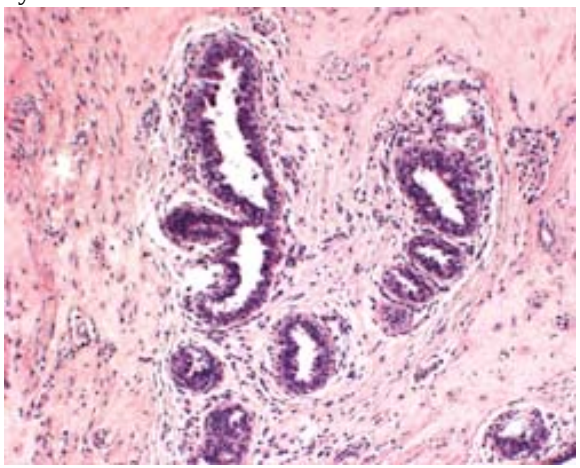


Figure 23-31 Gynecomastia. Terminal ducts (without lobule formation) are lined by a multilayered epithelium with small papillary tufts. There is typically surrounding periductal hyalinization and fibrosis.



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Chapter 24 - The Endocrine System

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The endocrine system contains a highly integrated and widely distributed group of organs that orchestrates a state of metabolic equilibrium, or homeostasis, among the various organs of the body. Signaling by extracellular secreted molecules can be classified into three types—autocrine, paracrine, or endocrine—on the basis of the distance over which the signal acts. In endocrine signaling, the secreted molecules, which are frequently called *hormones*, act on target cells that are distant from their site of synthesis. An endocrine hormone is frequently carried by the blood from its site of release to its target. Increased activity of the target tissue often down-regulates the activity of the gland that secretes the stimulating hormone, a process known as *feedback inhibition*.

Hormones can be classified into several broad categories on the basis of the nature of their receptors. Cellular receptors and signaling pathways were discussed in Chapter 3 , and only a few comments about signaling by hormone receptors follow:

- *Hormones that trigger biochemical signals upon interacting with cell-surface receptors:* This large class of compounds is composed of two groups: (1) peptide hormones, such as growth hormone and insulin, and (2) small molecules, such as epinephrine. Binding of these hormones to cell-surface receptors leads to an increase in intracellular signaling molecules, termed *second messengers*, such as cyclic adenosine monophosphate (cAMP); production of mediators from membrane phospholipids, such as inositol 1,4,5-trisphosphate or IP_3 ; and shifts in the intracellular levels of ionized calcium. The elevated levels of one or more of these can control proliferation, differentiation, survival, and functional activity of cells, mainly by regulating the expression of specific genes.
- *Hormones that diffuse across the plasma membrane and interact with intracellular receptors:* Many lipid-soluble hormones diffuse across the plasma membrane and interact with receptors in the cytosol or the nucleus. The resulting hormone-receptor complexes bind specifically to recognition elements in DNA, thereby affecting the expression of specific target genes. Hormones of this type include the steroids (e.g., estrogen, progesterone, and glucocorticoids), and thyroxine.

A number of processes can disturb the normal activity of the endocrine system, including impaired synthesis or release of hormones, abnormal interactions between hormones and their target tissues, and abnormal responses of target organs. Endocrine diseases can be generally classified as (1) diseases of *underproduction or overproduction* of hormones and their resulting biochemical and clinical consequences and (2) diseases associated with the development of *mass lesions*. Such lesions might be nonfunctional, or they might be associated with overproduction or underproduction of hormones. The study of endocrine diseases requires integration of morphologic findings with biochemical measurements of the levels of hormones, their regulators, and other metabolites.

Pituitary Gland

Normal

The pituitary is a small bean-shaped organ that measures about 1 cm in greatest diameter and weighs about 0.5 gm, although it enlarges during pregnancy. Its small size belies its great functional significance. It is located at the base of the brain, where it lies nestled within the confines of the sella turcica in close proximity to the optic chiasm and the cavernous sinuses. The pituitary is attached to the hypothalamus by the pituitary stalk, which passes out of the sella through an opening in the dura mater surrounding the brain. Along with the hypothalamus, the pituitary gland plays a critical role in

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Figure 24-1 Hormones released by the anterior pituitary. The adenohypophysis (anterior pituitary) releases five hormones that are in turn under the control of various stimulatory and inhibitory hypothalamic releasing factors. TSH, thyroid-stimulating hormone (thyrotropin); PRL, prolactin; ACTH, adrenocorticotrophic hormone (corticotropin); GH, growth hormone (somatotropin); FSH, follicle-stimulating hormone; LH, luteinizing hormone. The stimulatory releasing factors are TRH (thyrotropin-releasing factor), CRH (corticotropin-releasing factor), GHRH (growth hormone-releasing factor), GnRH (gonadotropin-releasing factor). The inhibitory hypothalamic influences are comprised of PIF (prolactin inhibitory factor or dopamine) and growth hormone inhibitory factor (GIH or somatostatin).

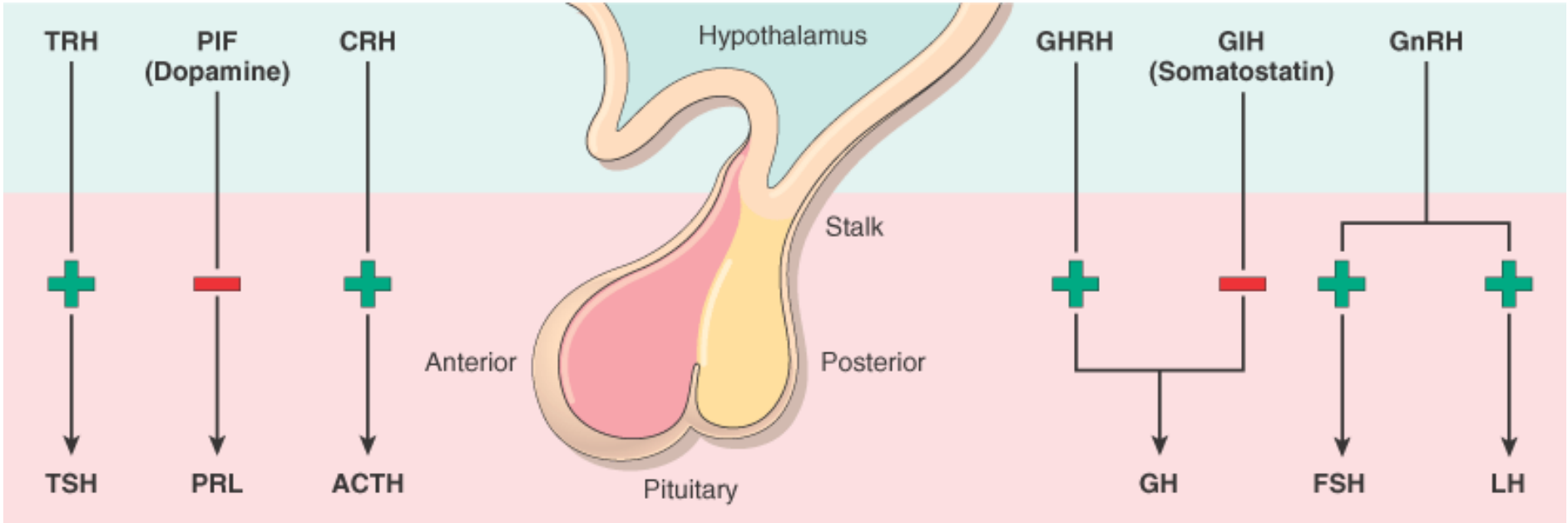

Figure 24-2 *A*, Photomicrograph of normal pituitary. The gland is populated by several distinct cell populations containing a variety of stimulating (trophic) hormones. *B*, Each of the hormones has different staining characteristics, resulting in a mixture of cell types in routine histologic preparations. Immunostain for human growth hormone.

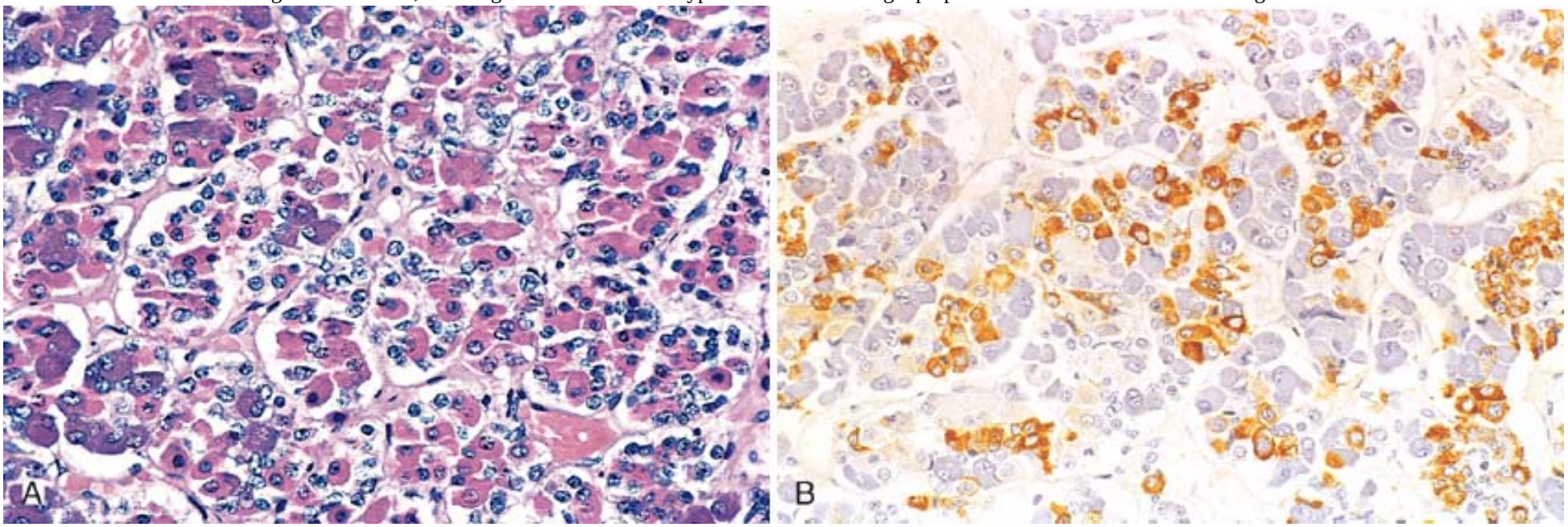

TABLE 24-1 -- Classification of Pituitary Adenomas

Prolactin cell (lactotroph) adenoma
Growth hormone cell (somatotroph) adenoma
••Densely granulated GH cell adenoma
••Sparsely granulated GH cell adenoma with fibrous bodies
Thyroid-stimulating hormone cell (thyrotroph) adenomas
ACTH cell (corticotroph) adenomas
Gonadotroph cell adenomas
••Silent gonadotroph adenomas include most so-called null cell and oncocytic adenomas
Mixed growth hormone-prolactin cell (mammosomatotroph) adenomas
Other plurihormonal adenomas
Hormone-negative adenomas

ACTH, adrenocorticotropic hormone.

demonstration of lineage-specific differentiation. Both silent and hormone-negative pituitary adenomas may cause hypopituitarism as they encroach on and destroy adjacent anterior pituitary parenchyma.

Clinically diagnosed pituitary adenomas are responsible for about 10% of intracranial neoplasms; they are discovered incidentally in up to 25% of routine autopsies. In fact, using high-resolution computed tomography or magnetic resonance imaging suggest that approximately 20% of "normal" adult pituitary glands harbor an incidental lesion measuring 3 mm or more in diameter, usually a silent adenoma. [1] Pituitary adenomas are usually found in adults, with a peak incidence from the thirties to the fifties. Most pituitary adenomas occur as isolated lesions. In about 3% of cases, however, adenomas are associated with *multiple endocrine neoplasia (MEN) type 1* (discussed later). Pituitary adenomas are designated, somewhat arbitrarily, *microadenomas* if they are less than 1 cm in diameter and *macroadenomas* if they exceed 1 cm in diameter. Silent and hormone-negative adenomas are likely to come to clinical attention at a later stage than those associated with endocrine abnormalities and are therefore more likely to be macroadenomas.

With recent advances in molecular techniques, substantial insight has been gained into *the genetic abnormalities associated with pituitary adenomas*:[2]

- The great majority of pituitary adenomas are monoclonal in origin, even those that are plurihormonal, suggesting that most arise from a single somatic cell. Some plurihormonal tumors may arise from clonal expansion of primitive stem cells, which then differentiate in several directions simultaneously.
- G-protein mutations are possibly the best-characterized molecular abnormalities in pituitary adenomas. G-proteins are described in Chapter 3 ; here we will review their function in the context of endocrine neoplasms. G-proteins play a critical role in signal transduction, transmitting signals from *cell-surface receptors* (e.g., GHRH receptor) to *intracellular effectors* (e.g., adenyl cyclase), which then generate *second messengers* (e.g., cyclic AMP, cAMP). These are heterotrimeric proteins, composed of a specific α-subunit that binds guanine nucleotide and interacts with both cell surface receptors and intracellular effectors (Fig. 24-3); the β- and γ-subunits are noncovalently bound to the specific α-subunit. G_s is a stimulatory G-protein that has a pivotal role in signal transduction in several endocrine organs, including the pituitary. The α-subunit of G_s (G_s α) is encoded by the *GNAS1* gene, located on chromosome 20q13. In the basal state, G_s exists as an inactive protein, with GDP bound to the guanine nucleotide-binding site of the α-subunit of G_s . On interaction with the ligand-bound cell-surface receptor, GDP dissociates, and GTP binds to G_s α, activating the G-protein. The activation of G_s α results in the generation of cAMP, which acts as a potent mitogenic stimulus for a variety of endocrine cell types (such as pituitary somatotrophs and corticotrophs, thyroid follicular cells, parathyroid cells), promoting cellular proliferation and hormone synthesis and secretion. The activation of G_s α, and resultant generation of cAMP, are *transient* because of an intrinsic GTPase activity in the α-subunit, which hydrolyzes GTP into GDP. *A mutation in the α-subunit that interferes with its intrinsic GTPase activity will therefore result in constitutive activation of G_s α, persistent generation of cAMP, and unchecked cellular proliferation* (Fig. 24-3). Approximately 40% of somatotroph cell adenomas bear *GNAS1* mutations that abrogate the GTPase activity of G_s α. The mutant form of *GNAS1* is also known as the *gsp oncogene* because of its effects on tumorigenesis. In addition, *GNAS1* mutations have also been described in a minority of corticotroph adenomas; in contrast, *GNAS1* mutations are absent in thyrotroph, lactotroph, and gonadotroph adenomas, since their respective hypothalamic release hormones do not mediate their action via cAMP-dependent pathways.
- Multiple endocrine neoplasia (MEN) syndrome (discussed in detail below) is a familial disorder associated with tumors and hyperplasias of multiple endocrine organs, including the pituitary. A subtype of MEN syndrome, known as MEN-1, is caused by germ line mutations of the gene *MEN1*, on chromosome 11q13. While *MEN1* mutations are, by definition, present in pituitary adenomas arising in context of the MEN-1 syndrome, they are uncommon in sporadic pituitary adenomas.
- Additional molecular abnormalities present in *aggressive or advanced pituitary adenomas* include activating mutations of the *RAS* oncogene and overexpression of the *c-MYC* oncogene, suggesting that these genetic events are linked to disease progression.[3]

Figure 24-3 The mechanism of G-protein mutations in endocrine neoplasia. Mutations in the G-protein-signaling pathway are seen in a variety of endocrine neoplasms, including pituitary, thyroid, and parathyroid adenomas. G-proteins play a critical role in signal transduction, transmitting signals from cell-surface receptors (GHRH, TSH, or PTH receptor) to intracellular effectors (e.g., adenyl cyclase), which then generate second messengers (cAMP).

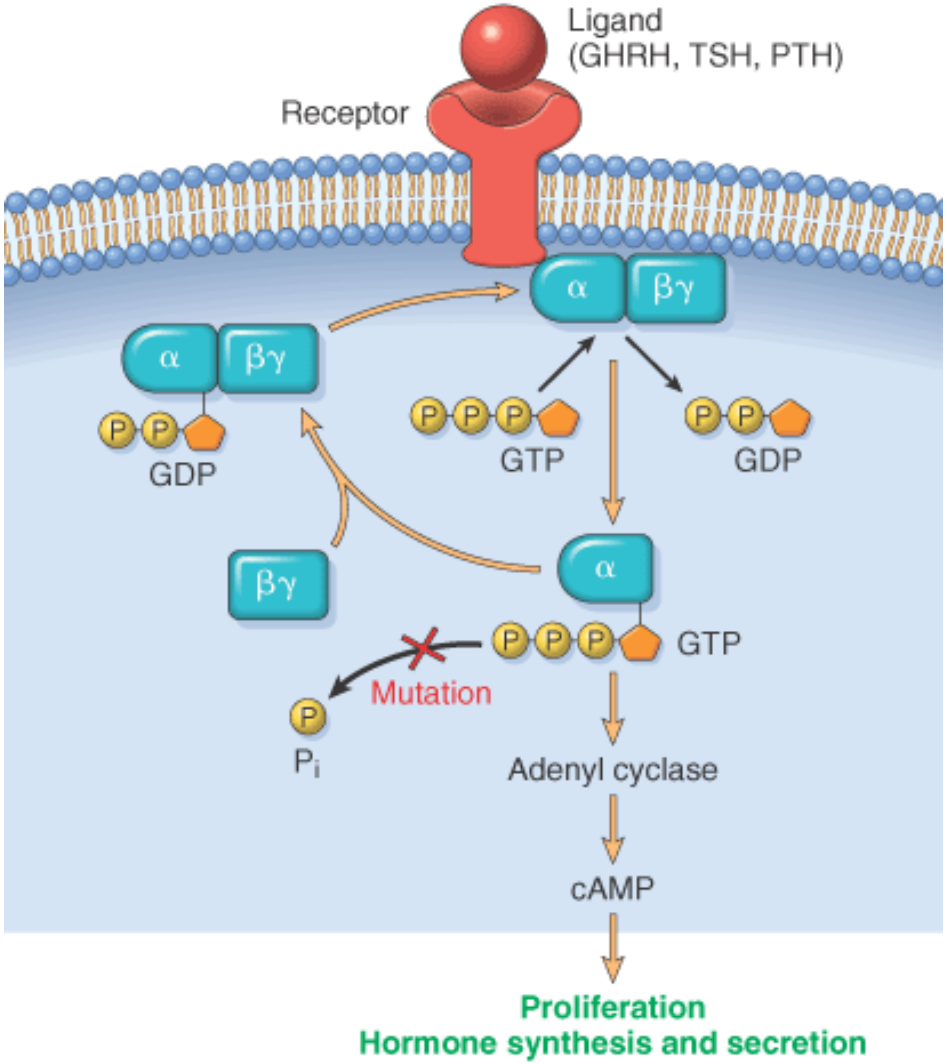

Figure 24-4 Pituitary adenoma. This massive, nonfunctional adenoma has grown far beyond the confines of the sella turcica and has distorted the overlying brain. Nonfunctional adenomas tend to be larger at the time of diagnosis than those that secrete a hormone.

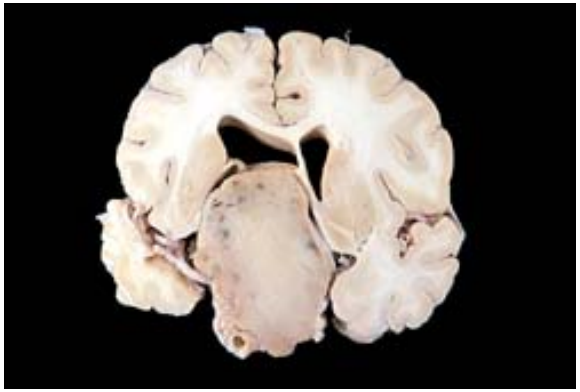

Figure 24-5 Pituitary adenoma. The monomorphism of these cells contrasts markedly with the mixture of cells seen in the normal anterior pituitary. Note also the absence of reticulin network.

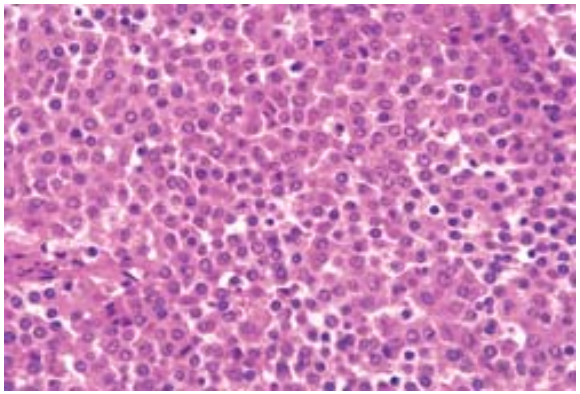

Figure 24-6 Ultrastructural features of prolactinomas. *A,* Electron micrograph of a sparsely granulated prolactinoma. The tumor cells contain abundant granular endoplasmic reticulum (indicative of active protein synthesis) and small numbers of secretory granules (6000X). *B,* Electron micrograph of densely granulated growth hormone-secreting adenoma. The tumor cells are filled with large, membrane-bound secretory granules (6000X). *(Courtesy of Dr. Eva Horvath, St. Michael's Hospital, Toronto, Ontario, Canada.)*

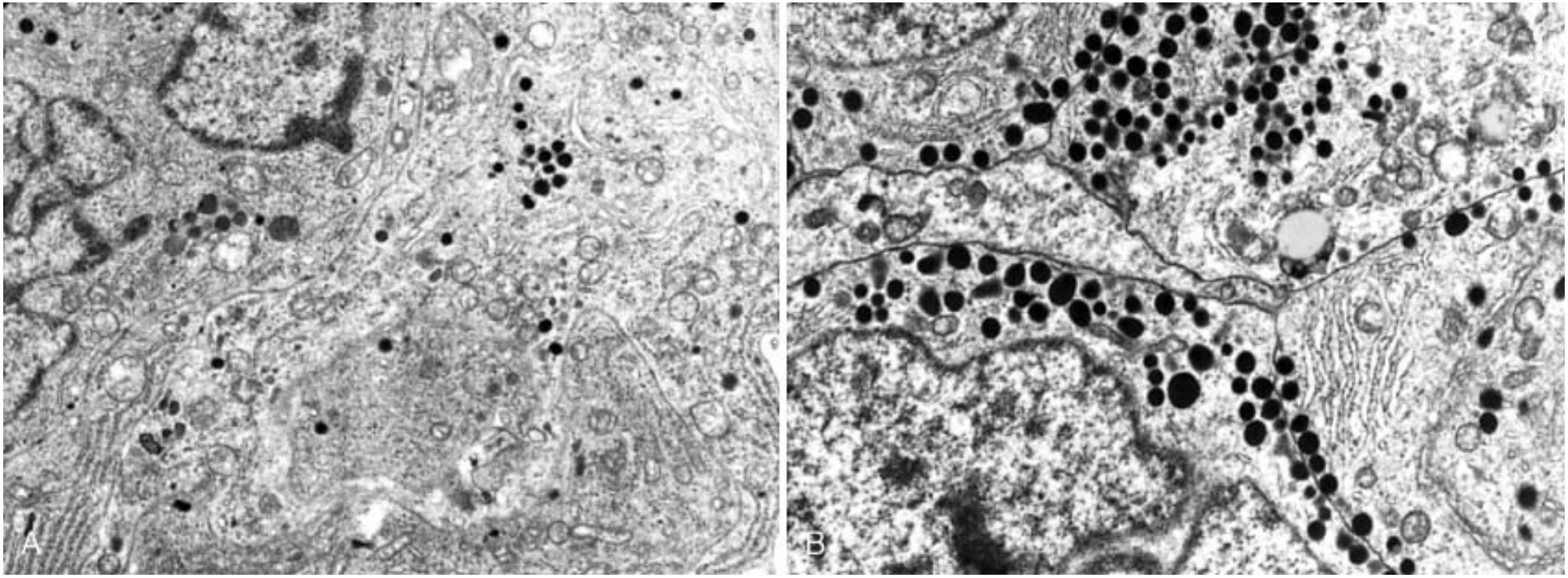

Figure 24-7 Homeostasis in the hypothalamus-pituitary-thyroid axis and mechanism of action of thyroid hormones. Secretion of thyroid hormones (T_3 and T_4) is controlled by trophic factors secreted by both the hypothalamus and the anterior pituitary. Decreased levels of T_3 and T_4 stimulate the release of thyrotropin-releasing hormone (TRH) from the hypothalamus and thyroid-stimulating hormone (TSH) from the anterior pituitary, causing T_3 and T_4 levels to rise. Elevated T_3 and T_4 levels, in turn, suppress the secretion of both TRH and TSH. This relationship is termed a negative-feedback loop. TSH binds to the TSH receptor on the thyroid follicular epithelium, which causes activation of G proteins, and cyclic AMP (cAMP)-mediated synthesis and release of thyroid hormones (T3 and T4). In the periphery, T_3 and T_4 interact with the thyroid hormone receptor (TR) to form a hormone-receptor complex that translocates to the nucleus and binds to so-called thyroid response elements (TREs) on target genes initiating transcription.

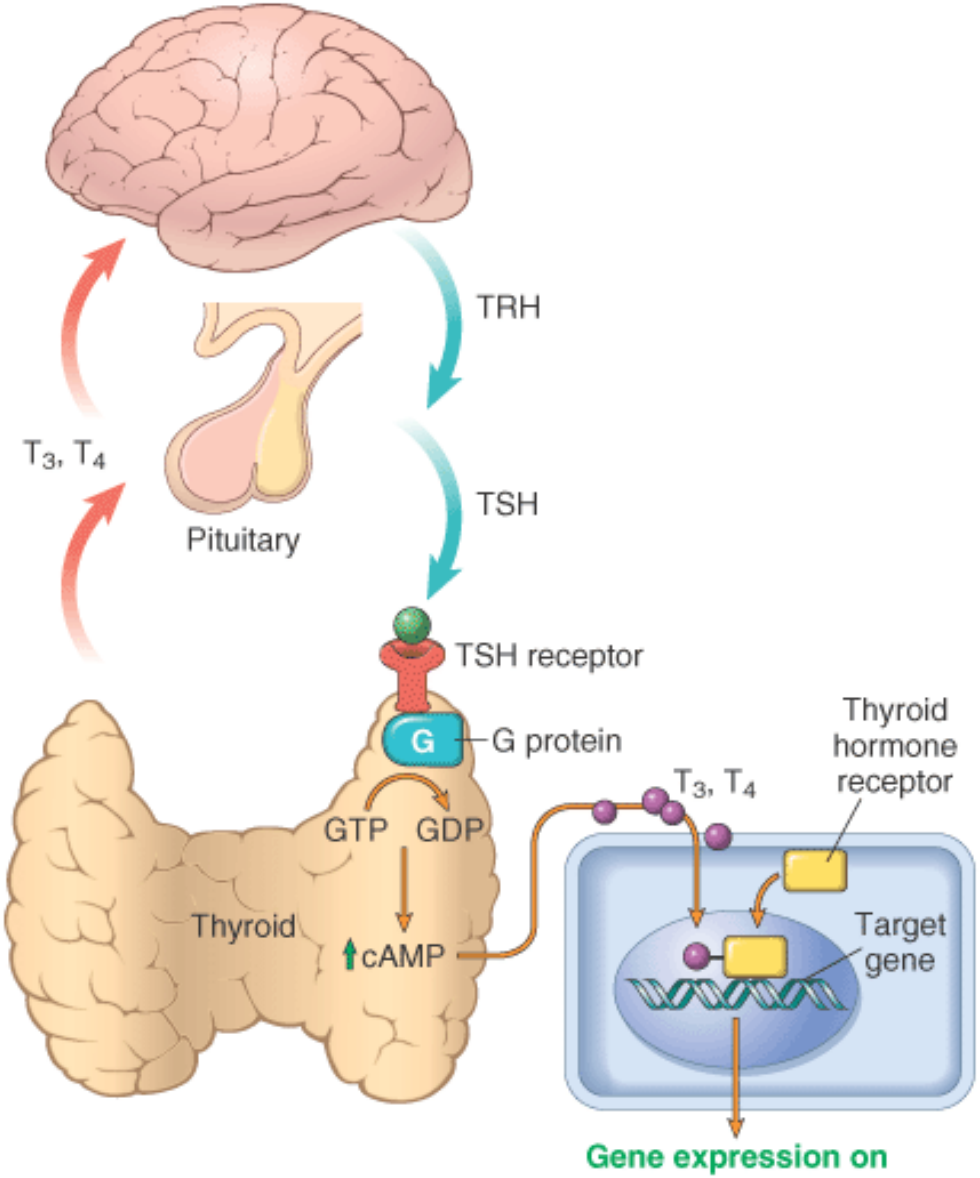

TABLE 24-2 -- Disorders Associated with Thyrotoxicosis

Associated with Hyperthyroidism
Primary
••Diffuse toxic hyperplasia (Graves disease)
••Hyperfunctioning ("toxic") multinodular goiter

••Hyperfunctioning ("toxic") adenoma
••Hyperfunctioning thyroid carcinoma
••Iodine-induced hyperthyroidism
••Neonatal thyrotoxicosis associated with maternal Graves disease
Secondary
••TSH-secreting pituitary adenoma (rare) *
Not Associated with Hyperthyroidism
Subacute granulomatous thyroiditis (*painful*)
Subacute lymphocytic thyroiditis (*painless*)
Struma ovarii (ovarian teratoma with ectopic thyroid)
Factitious thyrotoxicosis (exogenous thyroxine intake)

*Associated with increased TSH; all other causes of thyrotoxicosis associated with decreased TSH.

one (albeit the most common) cause of thyrotoxicosis. The terms *primary* and *secondary hyperthyroidism* are sometimes used to designate hyperthyroidism arising from an intrinsic thyroid abnormality and that arising from processes outside of the thyroid, such as a TSH-secreting pituitary tumor. With this disclaimer, we will follow the common practice of using the terms *thyrotoxicosis* and *hyperthyroidism* interchangeably. The three most common causes of thyrotoxicosis are also associated with hyperfunction of the gland and include the following:

- *Diffuse hyperplasia* of the thyroid associated with Graves disease (accounts for 85% of cases)
- Hyperfunctional *multinodular goiter*
- Hyperfunctional *adenoma* of the thyroid

Clinical Course.

The clinical manifestations of hyperthyroidism are protean and include changes referable to the *hypermetabolic state* induced by excess thyroid hormone as well as those related to overactivity of the *sympathetic nervous system* (i.e., an increase in the β-adrenergic "tone").

Excessive levels of thyroid hormone result in *an increase in the basal metabolic rate*. The *skin* of thyrotoxic patients tends to be soft, warm, and flushed because of increased blood flow and peripheral vasodilation to increase heat loss. *Heat intolerance* is common. Sweating is increased because of higher levels of calorigenesis. Increased basal metabolic rate also results in characteristic *weight loss despite increased appetite*.

Cardiac manifestations are among the earliest and most consistent features of hyperthyroidism. Patients with hyperthyroidism can have an increase in cardiac output, owing to both increased cardiac contractility and increased peripheral oxygen requirements. Tachycardia, palpitations, and cardiomegaly are common. Arrhythmias, particularly atrial fibrillation, occur frequently and are more common in older patients. Congestive heart failure may develop, particularly in elderly patients with pre-existing cardiac disease. Myocardial changes, such as foci of lymphocytic and eosinophilic infiltration, mild fibrosis in the interstitium, fatty changes in myofibers, and an increase in size and number of mitochondria, have been described. Some patients with thyrotoxicosis develop a reversible *diastolic dysfunction* and a "low-output" failure, so-called *thyrotoxic dilated cardiomyopathy*.

In the *neuromuscular system*, overactivity of the sympathetic nervous system produces tremor, hyperactivity, emotional lability, anxiety, inability to concentrate, and insomnia. Proximal muscle weakness is common with decreased muscle mass (*thyroid myopathy*).

Ocular changes often call attention to hyperthyroidism. A wide, staring gaze and lid lag are present because of sympathetic overstimulation of the levator palpebrae superioris (Fig. 24-8). However, true *thyroid ophthalmopathy* associated with proptosis is a feature seen only in Graves disease (see below).

In the *gastrointestinal system*, sympathetic hyperstimulation of the gut results in hypermotility, malabsorption, and diarrhea.

The *skeletal system* is also affected in hyperthyroidism. Thyroid hormone stimulates bone resorption, resulting in increased porosity of cortical bone and reduced volume of trabecular bone. The net effect is osteoporosis and an increased risk of fractures in patients with chronic hyperthyroidism.

Figure 24-8 A patient with hyperthyroidism. A wide-eyed, staring gaze, caused by overactivity of the sympathetic nervous system, is one of the features of this disorder. In Graves disease, one of the most important causes of hyperthyroidism, accumulation of loose connective tissue behind the eyeballs also adds to the protuberant appearance of the eyes.

TABLE 24-3 -- Causes of Hypothyroidism

Primary
Developmental (thyroid dysgenesis: *PAX-8, TTF-2, TSH-receptor* mutations)
Thyroid hormone resistance syndrome (TRβ mutations)
Postablative
••Surgery, radioiodine therapy, or external radiation
Autoimmune hypothyroidism

••Hashimoto thyroiditis *
Iodine deficiency *
Drugs (lithium, iodides, *p*-aminosalicylic acid) *
Congenital biosynthetic defect (dyshormonogenetic goiter) *
Secondary
Pituitary failure
Tertiary
Hypothalamic failure (rare)

*Associated with enlargement of thyroid ("goitrous hypothyroidism"). Hashimoto thyroiditis and postablative hypothroidism account for the majority of cases.

exogenous irradiation, such as external radiation therapy to the neck.

Autoimmune hypothyroidism is the most common cause of goitrous hypothyroidism in iodine-sufficient areas of the world. The vast majority of cases of autoimmune hypothyroidism are due to Hashimoto thyroiditis. Circulating autoantibodies, including *anti-TSH receptor autoantibodies*, are commonly found in Hashimoto thyroiditis. Some patients with hypothyroidism have circulating anti-TSH antibodies, but they usually do not have the goitrous enlargement or lymphocytic infiltrate characteristic of Hashimoto thyroiditis. In the past, many of these patients were classified as having primary "idiopathic" hypothyroidism, but the disease is now recognized as a type of autoimmune disorder of the thyroid, occurring either in isolation or in conjunction with other autoimmune endocrine manifestations.

Drugs given intentionally to decrease thyroid secretion (e.g., methimazole and propylthiouracil) can cause hypothyroidism, as can agents used to treat nonthyroid conditions (e.g., lithium, *p*-aminosalicylic acid).

Inborn errors of thyroid metabolism are an uncommon cause of goitrous hypothyroidism (*dyshormonogenetic goiter*). Any one of the multiple steps leading to thyroid hormone synthesis may be deficient: (1) iodide transport defect, (2) organification defect, (3) dehalogenase defect, and (4) iodotyrosine coupling defect. Organification of iodine involves binding of oxidized iodide with tyrosyl residues in thyroglobulin, and this process is deficient in patients with *Pendred syndrome*, wherein goitrous hypothyroidism is accompanied by sensorineural deafness.

Thyroid hormone resistance syndrome is a rare autosomal-dominant disorder caused by inherited mutations in the thyroid hormone receptor (TR), which abolish the ability of the receptor to bind thyroid hormones.[11] Patients demonstrate a generalized resistance to thyroid hormone, despite high circulating levels of T_3 and T_4 . Since the pituitary is also resistant to feedback from thyroid hormones, TSH levels tend to be high as well. In rare instances, there may be complete absence of thyroid parenchyma (*thyroid agenesis*), or the gland may be greatly reduced in size (*thyroid hypoplasia*). Mutations in the *TSH receptor* are a newly recognized cause of congenital hypothyroidism associated with a hypoplastic thyroid gland.[12] Recently, mutations in two transcription factors that are expressed in the developing thyroid and regulate follicular differentiation—*thyroid transcription factor-2* (*TTF-2*)[13] and *Paired*

Homeobox-8 (*PAX-8*) [14] —have been reported in patients with thyroid agenesis. Thyroid agenesis caused by TTF-2 mutations is usually associated with a cleft palate.

Secondary hypothyroidism is caused by TSH deficiency, and tertiary (central) hypothyroidism is caused by TRH deficiency. Secondary hypothyroidism can result from any of the causes of hypopituitarism. Frequently, the cause is a pituitary tumor; other causes include postpartum pituitary necrosis, trauma, and nonpituitary tumors, as was previously discussed. *Tertiary* (central) hypothyroidism can be caused by any disorder that damages the hypothalamus or interferes with hypothalamic-pituitary portal blood flow, thereby preventing delivery of TRH to the pituitary. This can result from hypothalamic damage from tumors, trauma, radiation therapy, or infiltrative diseases. Classic clinical manifestations of hypothyroidism include cretinism and myxedema.

CRETINISM

Cretinism refers to hypothyroidism that develops in infancy or early childhood. The term *cretin* was derived from the French *chrétien*, meaning Christian or Christlike, and was applied to these unfortunates because they were considered to be so mentally retarded as to be incapable of sinning. In the past, this disorder occurred fairly commonly in areas of the world where dietary iodine deficiency is endemic, such as the Himalayas, inland China, Africa, and other mountainous areas. It has become much less frequent in recent years, owing to the widespread supplementation of foods with iodine. On rare occasions, cretinism may also result from inborn errors in metabolism (e.g., enzyme deficiencies) that interfere with the biosynthesis of normal levels of thyroid hormone (*sporadic* cretinism).

Clinical features of cretinism include impaired development of the skeletal system and central nervous system, manifested by severe mental retardation, short stature, coarse facial features, a protruding tongue, and umbilical hernia. The severity of the mental impairment in cretinism appears to be related to the time at which thyroid deficiency occurs in utero. Normally, maternal hormones, including T_3 and T_4 , cross the placenta and are critical to fetal brain development. If there is maternal thyroid deficiency before the development of the fetal thyroid gland, mental retardation is severe. In contrast, reduction in maternal thyroid hormones later in pregnancy, after the fetal thyroid has developed, allows normal brain development.

MYXEDEMA

The term *myxedema* is applied to hypothyroidism developing in the older child or adult. Myxedema, or Gull disease, was first linked with thyroid dysfunction in 1873 by Sir William Gull in a paper addressing the development of a "cretinoid state" in adults. The clinical manifestations vary with the age of onset of the deficiency. The older child shows signs and symptoms intermediate between those of the cretin and those of the adult with hypothyroidism. In the adult, the condition appears insidiously and may take years to reach the level of clinical suspicion.

Clinical features of myxedema are characterized by a slowing of physical and mental activity. The initial symptoms include generalized fatigue, apathy, and mental sluggishness, which may mimic depression in the early stages of the disease. Speech and intellectual functions become slowed. Patients with myxedema are listless, cold-intolerant, and frequently overweight. Reduced cardiac output probably contributes to shortness of breath and decreased exercise capacity, two frequent complaints in patients with hypothyroidism. Decreased sympathetic activity results in constipation and decreased sweating. The skin in these patients is cool and pale because of decreased blood flow. Histologically, there is an accumulation of matrix substances, such as glycosaminoglycans and hyaluronic acid, in skin, subcutaneous tissue, and a number of visceral sites. This results in edema, a broadening and coarsening of facial features, enlargement of the tongue, and deepening of the voice.

Laboratory evaluation plays a vital role in the diagnosis of suspected hypothyroidism because of the nonspecific nature

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of symptoms. *Measurement of the serum TSH level is the most sensitive screening test for this disorder.* The TSH level is increased in primary hypothyroidism due to a loss of feedback

inhibition of TRH and TSH production by the hypothalamus and pituitary, respectively. The TSH level is not increased in patients with hypothyroidism due to primary hypothalamic or pituitary disease. *T_4 levels are decreased* in patients with hypothyroidism of any origin.

Thyroiditis

Thyroiditis, or inflammation of the thyroid gland, encompasses a diverse group of disorders characterized by some form of thyroid inflammation. These diseases include conditions that result in acute illness with severe thyroid pain (e.g., infectious thyroiditis, subacute granulomatous thyroiditis) and disorders in which there is relatively little inflammation and the illness is manifested primarily by thyroid dysfunction (subacute lymphocytic thyroiditis and fibrous [Reidel] thyroiditis).

Infectious thyroiditis may be either acute or chronic. Acute infections can reach the thyroid via hematogenous spread or through direct seeding of the gland, such as via a fistula from the piriform sinus adjacent to the larynx. Other infections of the thyroid, including mycobacterial, fungal, and *Pneumocystis* infections, are more chronic and frequently occur in immunocompromised patients. Whatever the cause, the inflammatory involvement may cause sudden onset of neck pain and tenderness in the area of the gland and is accompanied by fever, chills, and other signs of infection. Infectious thyroiditis can be self-limited or can be controlled with appropriate therapy. Thyroid function is usually not significantly affected, and there are few residual effects except for possible small foci of scarring. This section focuses on the more common and clinically significant types of thyroiditis: (1) Hashimoto thyroiditis (or chronic lymphocytic thyroiditis), (2) subacute granulomatous thyroiditis, and (3) subacute lymphocytic thyroiditis.

HASHIMOTO THYROIDITIS

Hashimoto thyroiditis (or chronic lymphocytic thyroiditis) is the most common cause of hypothyroidism in areas of the world where iodine levels are sufficient. It is characterized by gradual thyroid failure because of autoimmune destruction of the thyroid gland. The name *Hashimoto thyroiditis* is derived from the 1912 report by Hashimoto describing patients with goiter and intense lymphocytic infiltration of the thyroid (*struma lymphomatosa*). This disorder is most prevalent between 45 and 65 years of age and is more common in women than in men, with a female predominance of 10:1 to 20:1. Although it is primarily a disease of older women, it can occur in children and is a major cause of nonendemic goiter in children.

Epidemiologic studies have demonstrated a significant *genetic component* to Hashimoto thyroiditis, although, as in most other autoimmune disorders, the pattern of inheritance is non-Mendelian and likely to be influenced by subtle variations in the functions of multiple genes. The concordance rate in monozygotic twins is 30% to 60%, and up to 50% of asymptomatic first-degree relatives of Hashimoto patients demonstrate circulating antithyroid antibodies.[15] Several chromosomal abnormalities have been associated with thyroid autoimmunity. For example, adults with Turner syndrome (see Chapter 5) have a high prevalence of circulating antithyroid antibodies, and a substantial minority (~20%) develops subclinical or clinical hypothyroidism that is indistinguishable from Hashimoto thyroiditis. Similarly, adults with trisomy 21 (Down syndrome, see Chapter 5) are also at an increased risk for developing Hashimoto thyroiditis and hypothyroidism. There are reports that polymorphisms in the HLA locus, specifically the HLA-DR3 and HLA-DR5 alleles, are linked to Hashimoto thyroiditis, but the association is weak. Finally, genomewide linkage analyses in families with Hashimoto thyroiditis have provided evidence for several susceptibility loci, such as on chromosomes 6p and 12q, that may harbor genes predisposing to this disorder.[16]

Pathogenesis.

Hashimoto thyroiditis is an autoimmune disease in which the immune system reacts against a variety of thyroid antigens. The overriding feature of Hashimoto thyroiditis is progressive depletion of thyroid epithelial cells (thyrocytes), which are gradually replaced by mononuclear cell infiltration and fibrosis. Multiple immunologic mechanisms may contribute to the death of thyrocytes (Fig. 24-9).[17] [18] Sensitization of autoreactive CD4+ T-helper cells to thyroid antigens appears to be the initiating event. The effector mechanisms for thyrocyte death include the following:

- *CD8+ cytotoxic T cell-mediated cell death:* CD8+ cytotoxic T cells may cause thyrocyte destruction by one of two pathways: exocytosis of perforin/granzyme granules or engagement of death receptors, specifically CD95 (also known as Fas) on the target cell (Chapter 6).
- *Cytokine-mediated cell death:* CD4+ T cells produce inflammatory cytokines such as IFN-γ in the immediate thyrocyte milieu, with resultant recruitment and activation of

macrophages and damage to follicles.
• Binding of antithyroid antibodies (anti-TSH receptor antibodies, antithyroglobulin, and antithyroid peroxidase antibodies) followed by antibody-dependent cell-mediated cytotoxicity (ADCC) (Chapter 6).

Morphology.

The thyroid is often diffusely enlarged, although more localized enlargement may be seen in some cases. The capsule is intact, and the gland is well demarcated from adjacent structures. The cut surface is pale, yellow-tan, firm, and somewhat nodular. Microscopic examination reveals extensive infiltration of the parenchyma by a **mononuclear inflammatory infiltrate** containing small lymphocytes, plasma cells, and well-developed **germinal centers** (Fig. 24-10). The thyroid follicles are atrophic and are lined in many areas by epithelial cells distinguished by the presence of abundant eosinophilic, granular cytoplasm, termed **Hürthle** cells. This is a metaplastic response of the normally low cuboidal follicular epithelium to ongoing injury. In fine-needle aspiration biopsies, the presence of Hürthle cells in conjunction with a heterogeneous population of lymphocytes is characteristic of Hashimoto thyroiditis. In "classic" Hashimoto thyroiditis, interstitial connective tissue is increased and may be abundant. A **fibrous variant** is

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Figure 24-9 Pathogenesis of Hashimoto thyroiditis. Three proposed models for mechanism of thyrocyte destruction in Hashimoto disease. Sensitization of autoreactive CD4+ T cells to thyroid antigens appears to be the initiating event for all three mechanisms of thyroid cell death. See the text for details.

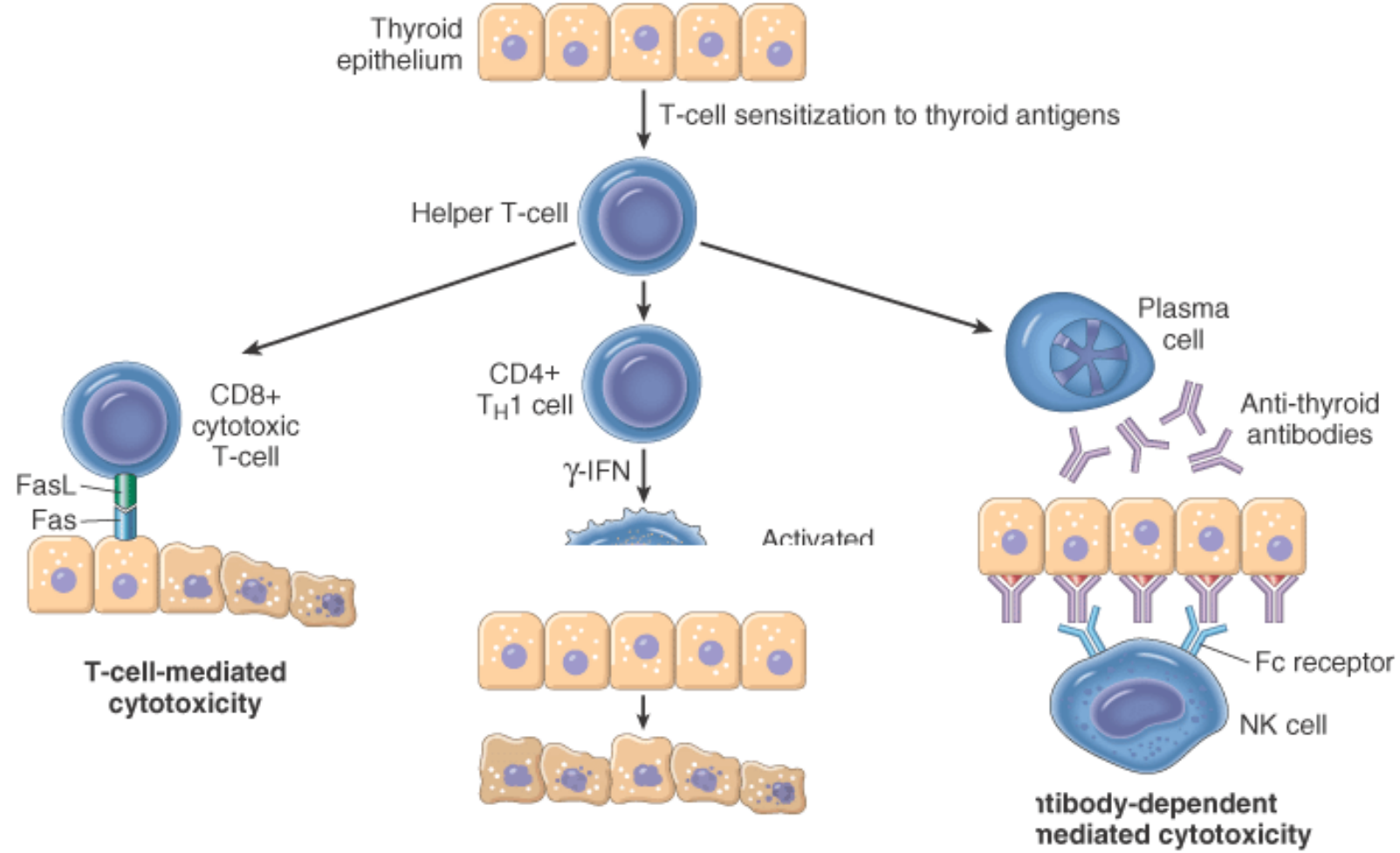


Figure 24-10 Hashimoto thyroiditis. The thyroid parenchyma contains a dense lymphocytic infiltrate with germinal centers. Residual thyroid follicles lined by deeply eosinophilic Hürthle cells are also seen.

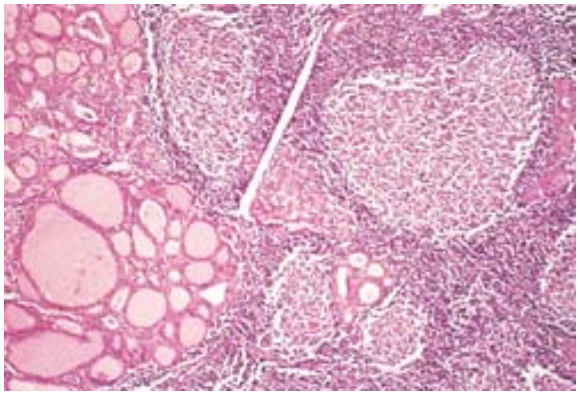


Figure 24-11 Subacute thyroiditis. The thyroid parenchyma contains a chronic inflammatory infiltrate with a multinucleate giant cell (*above left*) and a colloid follicle (*bottom right*).

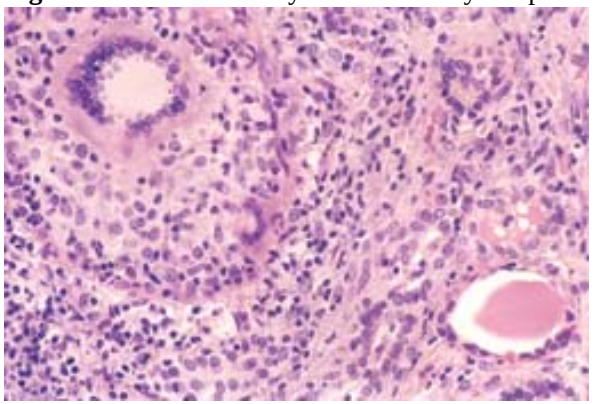


Figure 24-12 Diffusely hyperplastic thyroid in a case of Graves disease. The follicles are lined by tall, columnar epithelium. The crowded, enlarged epithelial cells project into the lumens of the follicles. These cells actively resorb the colloid in the centers of the follicles, resulting in the scalloped appearance of the edges of the colloid.

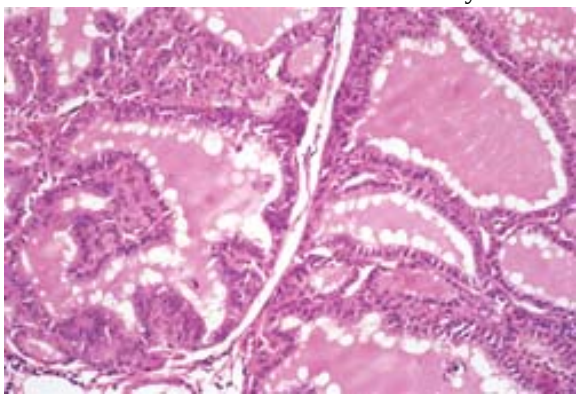


Figure 24-13 Nodular goiter. The gland is coarsely nodular and contains areas of fibrosis and cystic change.

Figure 24-14 Follicular adenoma of the thyroid. A solitary, well-circumscribed nodule is seen.

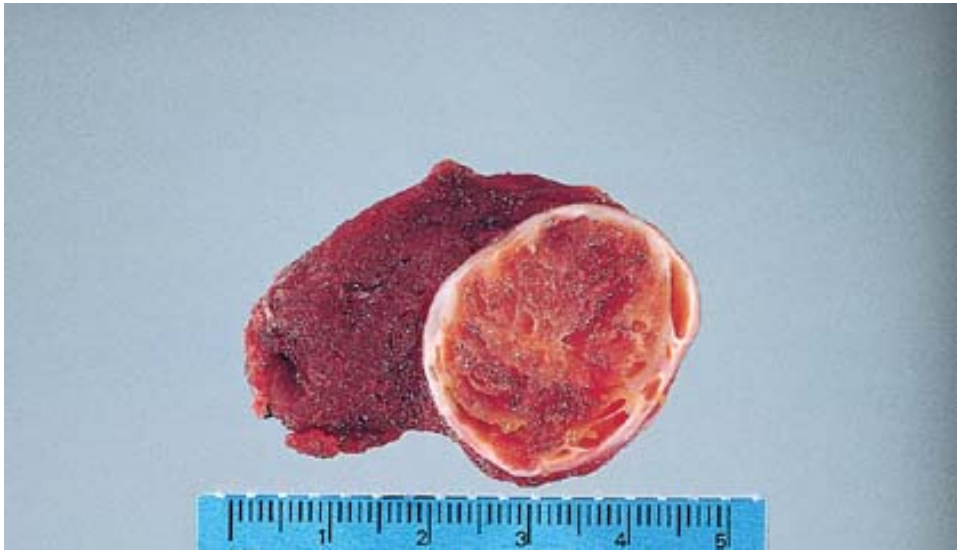


Figure 24-15 Follicular adenoma. The photomicrograph shows well-differentiated follicles resembling normal thyroid parenchyma.

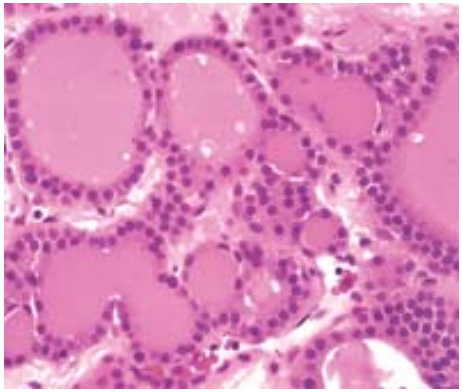


Figure 24-16 Hürthle cell tumor. A high-power view showing that the tumor is composed of cells with abundant eosinophilic cytoplasm and small regular nuclei. *(Courtesy of Dr. Mary Sunday, Brigham and Women's Hospital, Boston, MA.)*

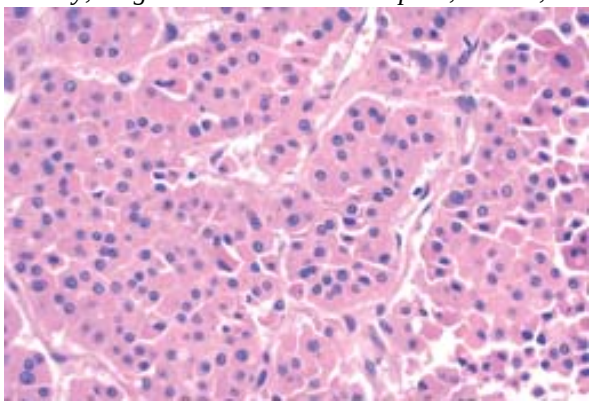
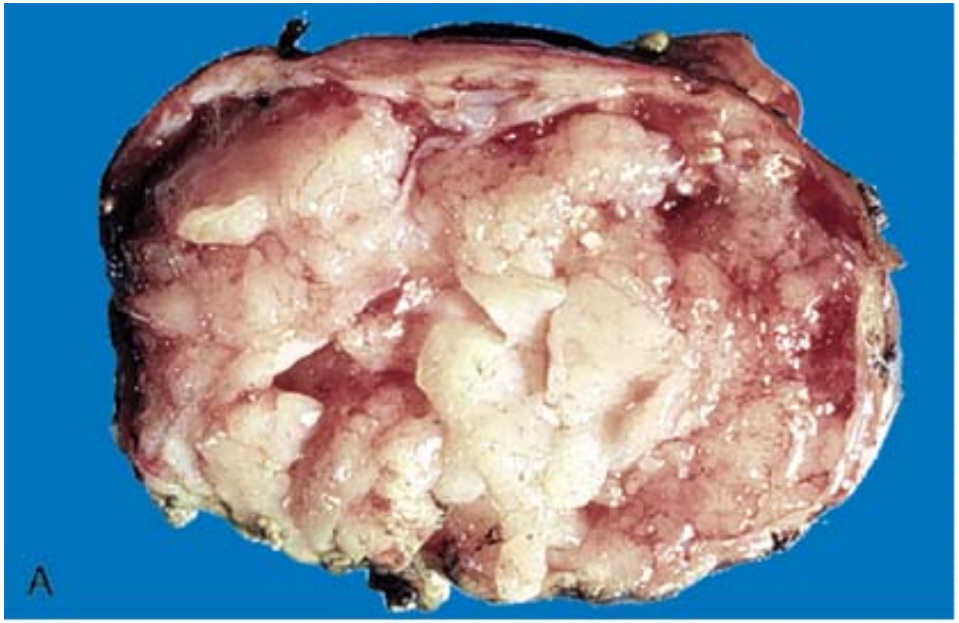
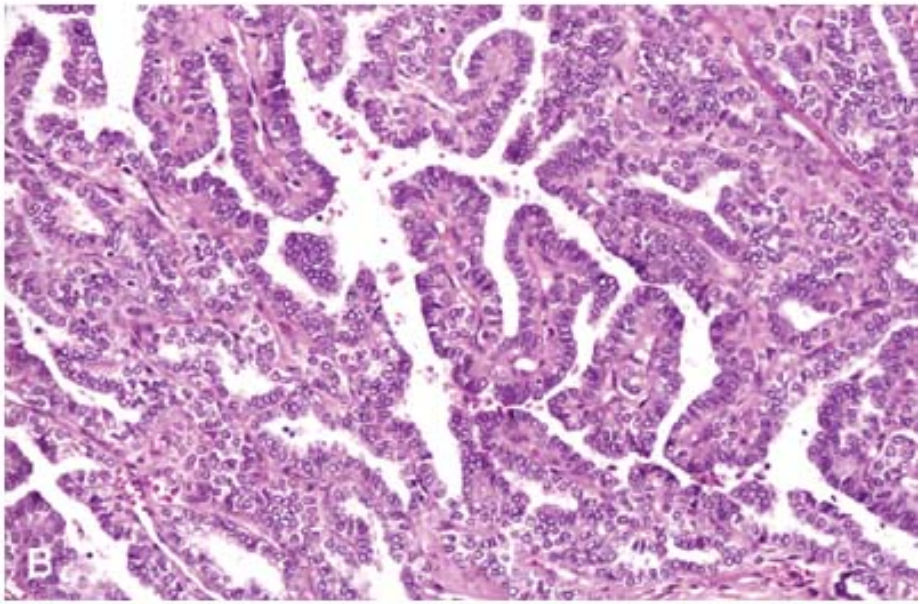
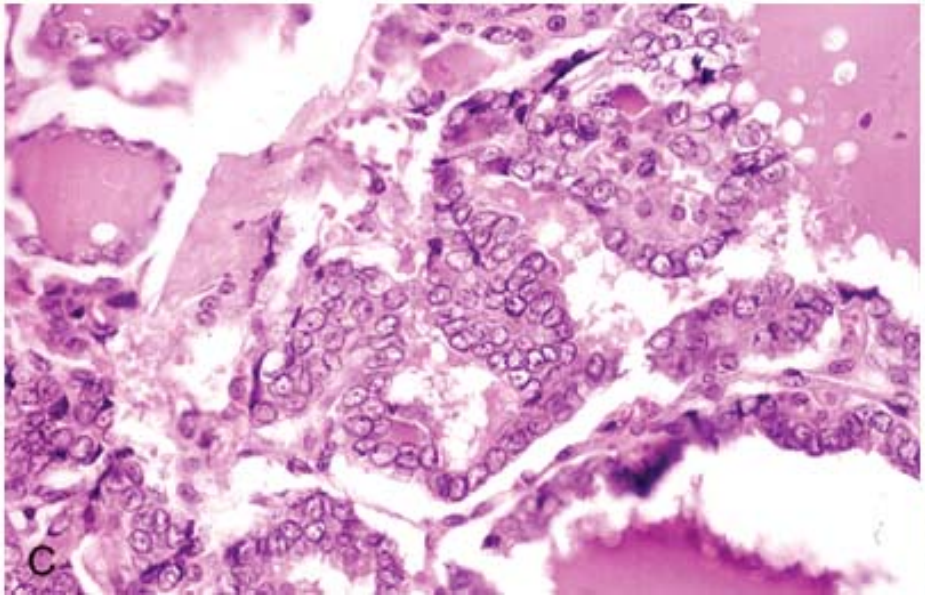


Figure 24-17 Papillary carcinoma of the thyroid. *A*, The macroscopic appearance of a papillary carcinoma with grossly discernible papillary structures. This particular example contains well-formed papillae (*B*), lined by cells with characteristic empty-appearing nuclei, sometimes termed "Orphan Annie eye" nuclei (*C*). *D*, Cells obtained by fine-needle aspiration of a papillary carcinoma. Characteristic intranuclear inclusions are visible in some of the aspirated cells.







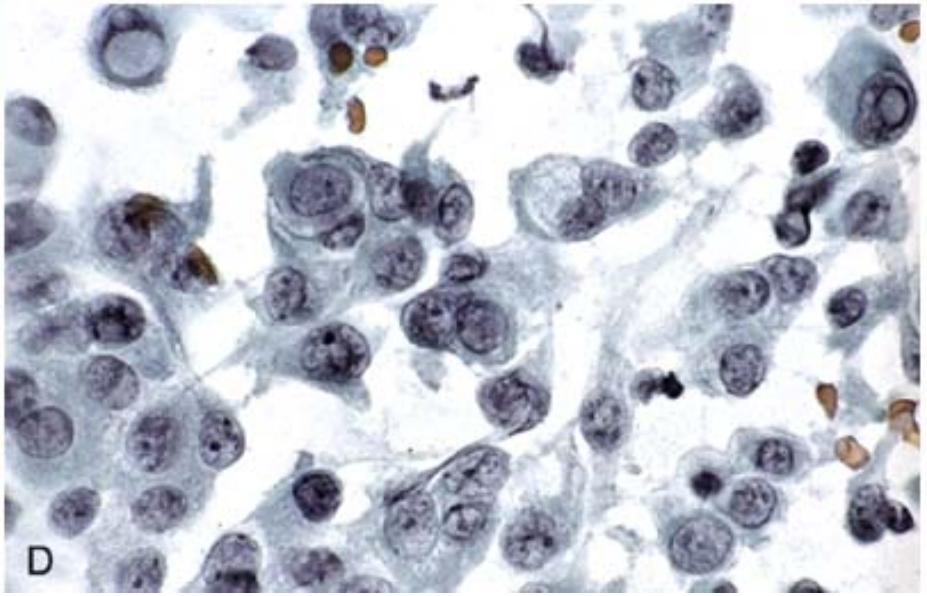


TABLE 24-4 -- Thyroid Lesions with a Follicular Architecture

Non-Neoplastic
Hyperplastic nodule in goiter
Neoplastic
Follicular adenoma *

Follicular carcinoma *
Follicular variant of papillary carcinoma †

*Differentiating follicular carcinoma from follicular adenoma requires histologic evidence of *capsular or blood vessel invasion*, or *documented metastasis*.
†The diagnosis of papillary carcinoma is rendered on the presence of *characteristic nuclear features*, irrespective of the presence or absence of papillae.

potential than the *ret/PTC* observed in usual papillary thyroid cancers. The presence of this genetic abnormality might result in more aggressive behavior.[42] [43]

An unusual **diffuse sclerosing variant** of papillary carcinoma occurs in younger individuals, including children. These tumors do not present with a mass, but rather with a bilateral goiter. There is a characteristic "gritty" sensation to the cut surface of the lesion due to the presence of abundant psammoma bodies. The tumor demonstrates a prominent papillary growth pattern, intermixed with solid areas containing nests of squamous cells (squamous morules). The neoplastic cells exhibit classic nuclear features of a papillary neoplasm. As the name suggests, there is extensive, diffuse fibrosis throughout the thyroid gland, often associated with a prominent lymphocytic infiltrate, simulating Hashimoto thyroiditis. The neoplastic cells have a peculiar propensity to invade intrathyroidal lymphatic channels; hence, nodal metastases are present in almost all cases.

Hyalinizing trabecular tumors, a group that includes both adenomas and carcinomas, have recently been reconsidered as a variant of papillary carcinomas, based on the presence of *ret/PTC* gene rearrangements in 30% to 60% of these tumors.[44] They are characterized by an "organoid" growth pattern, with nests and trabeculae of elongated tumor cells within a fibrovascular stroma; at first glance, the tumor may resemble an extra-adrenal paraganglioma (see below). Both intracellular and extracellular hyalinization are prominent and confer a pink hue on the tumor on low-power microscopic examination. The nuclear features resemble those seen in classic papillary carcinomas, and psammoma bodies may be present. Hyalinizing trabecular adenomas are well encapsulated, while carcinomas demonstrate capsular and/or vascular invasion.

Clinical Course.

Most papillary carcinomas present as asymptomatic thyroid nodules, but the first manifestation may be a mass in a cervical lymph node. Interestingly, the presence of isolated cervical nodal metastases does not appear to have a significant influence on the generally good prognosis of these lesions. The carcinoma, which is usually a single nodule, moves freely during swallowing and is not distinguishable from a benign nodule. Hoarseness, dysphagia, cough, or dyspnea suggests advanced disease. In a minority of patients, hematogenous metastases are present at the time of diagnosis, most commonly in the lung.

A variety of diagnostic tests have been employed to help separate benign from malignant thyroid nodules, including radionuclide scanning and fine-needle aspiration. Most papillary lesions are *cold* masses on scintiscans. Improvements in cytologic analysis have made fine-needle aspiration cytology a reliable test for distinguishing between benign and malignant nodules. The nuclear features are often nicely demonstrable in aspirated specimens.

Papillary thyroid cancers have an excellent prognosis, with a 10-year survival rate in excess of 95%. Five per cent to 20% of patients have local or regional recurrences, and 10% to 15% have distant metastases. The prognosis of a patient with papillary thyroid cancers is dependent on several factors including age (in general, the prognosis is less favorable among patients older than 40 years), the presence of extrathyroidal extension, and presence of distant metastases (stage).

Follicular Carcinoma

Follicular carcinomas are the second most common form of thyroid cancer, accounting for 10% to 20% of all thyroid cancers. They tend to present in women, and at an older age than do papillary carcinomas, with a peak incidence in the forties and fifties. The incidence of follicular carcinoma is increased in areas of dietary iodine deficiency, suggesting that in some cases, nodular goiter may predispose to the development of the neoplasm. The high frequency of *RAS* mutations in follicular adenomas and carcinomas suggests that the two may be related tumors.

Morphology.

Follicular carcinomas are single nodules that may be well circumscribed or widely infiltrative (Fig. 24-18). Sharply demarcated lesions may be exceedingly difficult to distinguish from follicular adenomas by gross examination. Larger lesions may penetrate the capsule and infiltrate well beyond the thyroid capsule into the adjacent neck. They are gray to tan to pink on cut section and, on occasion, are somewhat translucent when large, colloid-filled follicles are present. Degenerative changes, such as central fibrosis and foci of calcification, are sometimes present.

Microscopically, most follicular carcinomas are composed of fairly uniform cells forming small follicles containing colloid, quite reminiscent of normal thyroid (Fig. 24-19). In other cases, follicular differentiation may be less apparent, and there may be nests

Figure 24-18 Follicular carcinoma. Cut surface of a follicular carcinoma with substantial replacement of the lobe of the thyroid. The tumor has a light-tan appearance and contains small foci of hemorrhage.

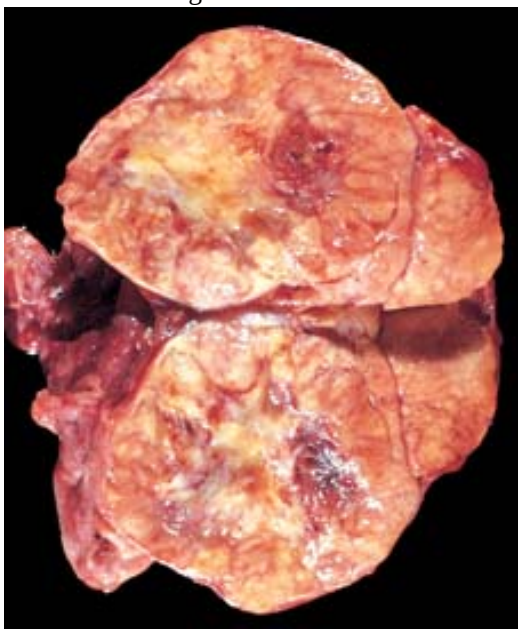


Figure 24-19 Follicular carcinoma of the thyroid. A few of the glandular lumens contain recognizable colloid.

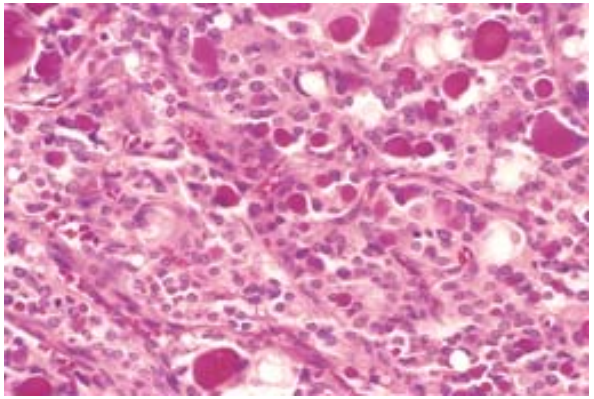


Figure 24-20 Capsular integrity in follicular neoplasms. Evaluating the integrity of the capsule is critical in distinguishing follicular adenomas from follicular carcinomas. In adenomas (*A*), a fibrous capsule, usually thin but occasionally more prominent, circumferentially surrounds the neoplastic follicles and no capsular invasion is seen (*arrowheads*); compressed normal thyroid parenchyma is usually present external to the capsule (*top of the panel*). In contrast, follicular carcinomas demonstrate capsular invasion (*B, arrow-heads*) that may be minimal, as in this case, or widespread with extension into local structures of the neck. The presence of vascular invasion is another feature of follicular carcinomas.

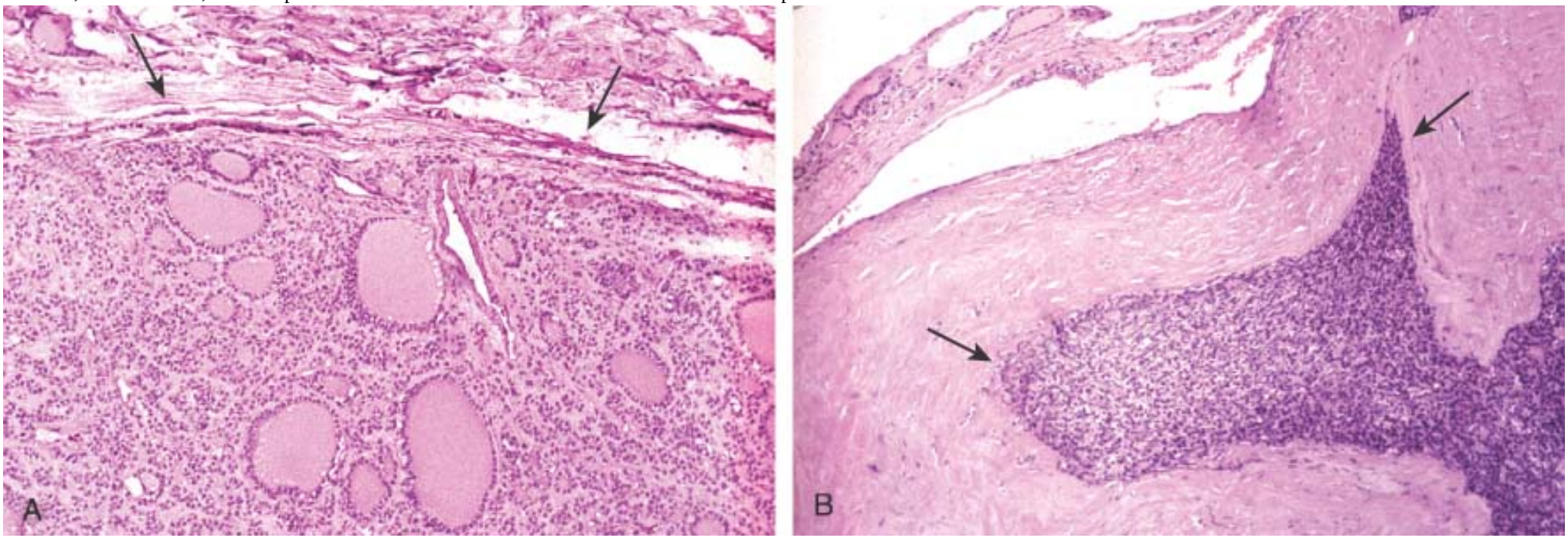


Figure 24-21 Medullary carcinoma of thyroid. These tumors typically show a solid pattern of growth and do not have connective tissue capsules. *(Courtesy of Dr. Joseph Corson, Brigham and Women's Hospital, Boston, MA.)*

Figure 24-22 Medullary carcinoma of the thyroid. These tumors typically contain amyloid, visible here as homogeneous extracellular material, derived from calcitonin molecules secreted by the neoplastic cells.

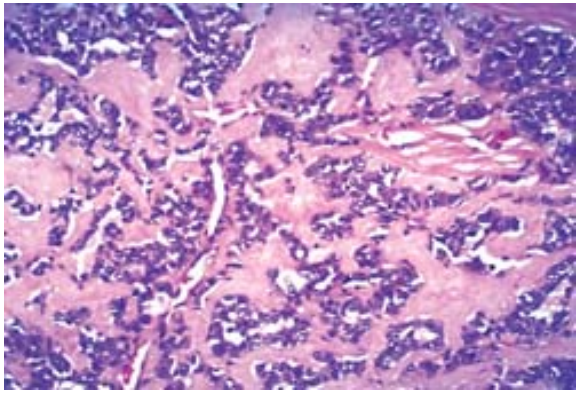


Figure 24-23 Electron micrograph of medullary thyroid carcinoma. These cells contain membrane-bound secretory granules that are the sites of storage of calcitonin and other peptides (30,000X).

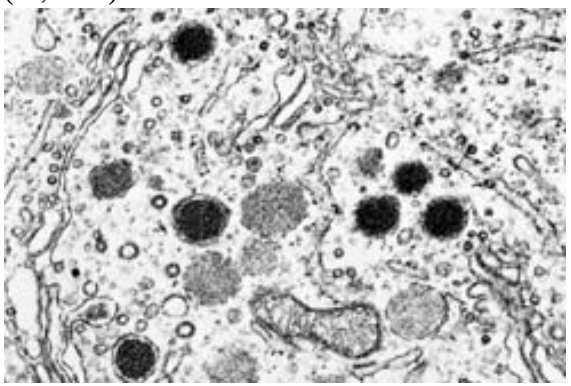


Figure 24-24 Parathyroid adenomas are almost always solitary lesions. Technetium-99m-sestamibi radionuclide scan demonstrates an area of increased uptake corresponding to the left

inferior parathyroid gland *(arrow)*. This patient had a parathyroid adenoma. Preoperative scintigraphy is useful in localizing and distinguishing adenomas from parathyroid hyperplasia, where more than one gland would demonstrate increased uptake.

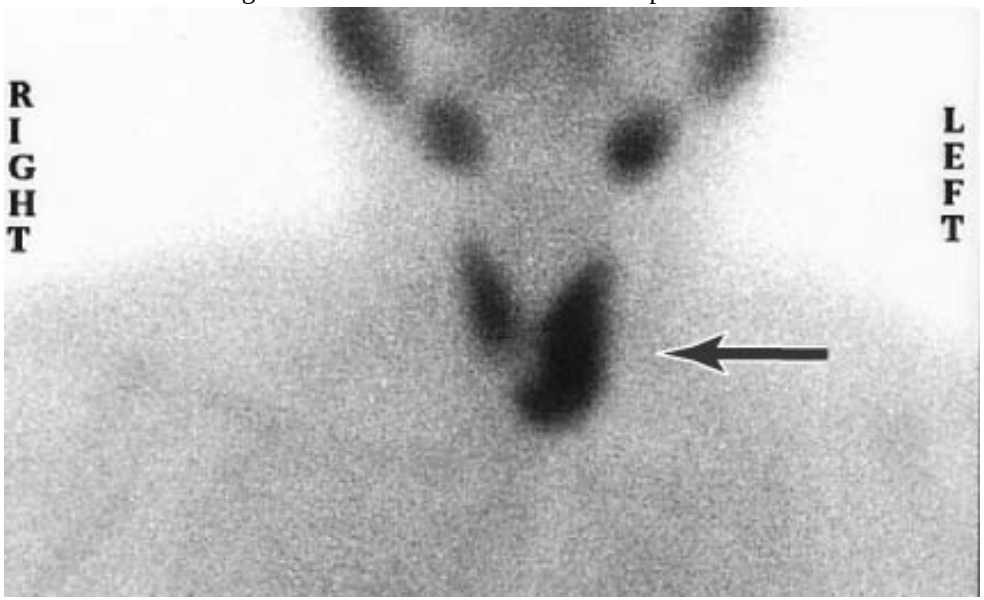


Figure 24-25 Parathyroid adenoma. *A*, Solitary chief cell parathyroid adenoma (low-power photomicrograph) revealing clear delineation from the residual gland below. *B*, High-power detail of a chief cell parathyroid adenoma. There is some slight variation in nuclear size but no anaplasia and some slight tendency to follicular formation.

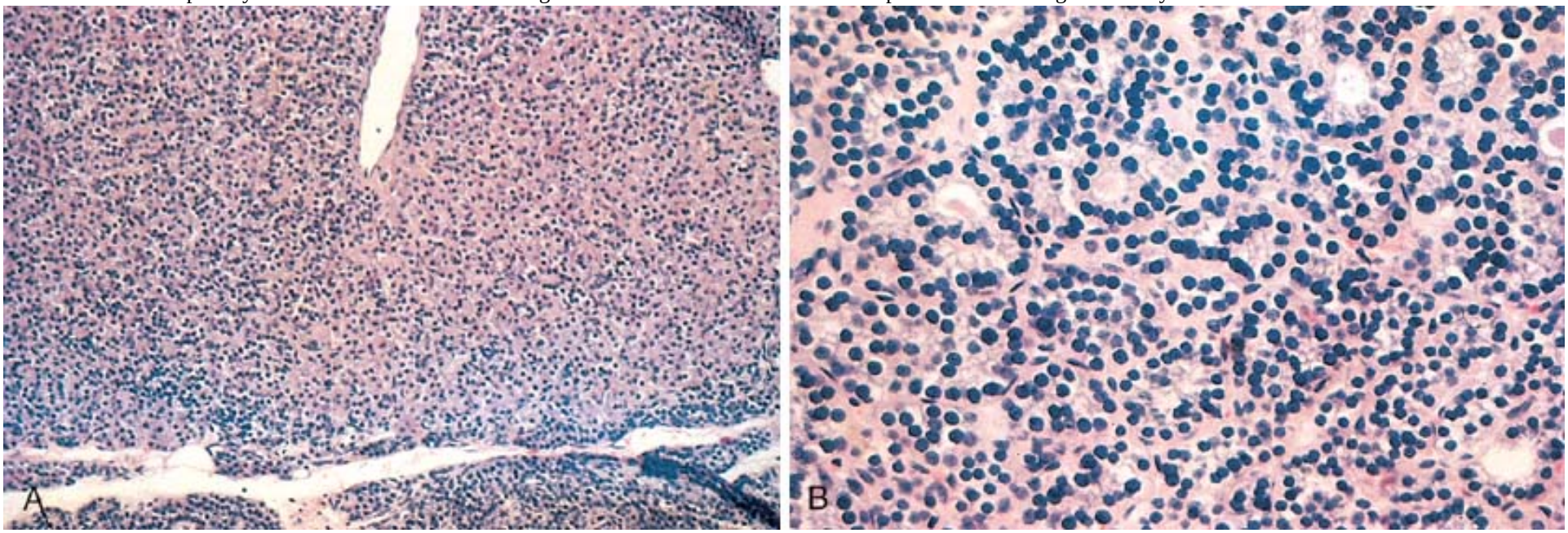


TABLE 24-5 -- Causes of Hypercalcemia

Raised PTH	Decreased PTH
Hyperparathyroidism	Hypercalcemia of malignancy
••Primary (adenoma > hyperplasia) *	••Osteolytic metastases (RANKL-mediated)
••Secondary †	••PTH-rP-mediated
••Tertiary †	Vitamin D toxicity
Familial hypocalciuric hypercalcemia	Immobilization
	Thiazide diuretics
	Granulomatous disease (sarcoidosis)

PTH-rP, Parathyroid hormone-related protein. RANKL, Receptor activator of nuclear factor κB ligand.

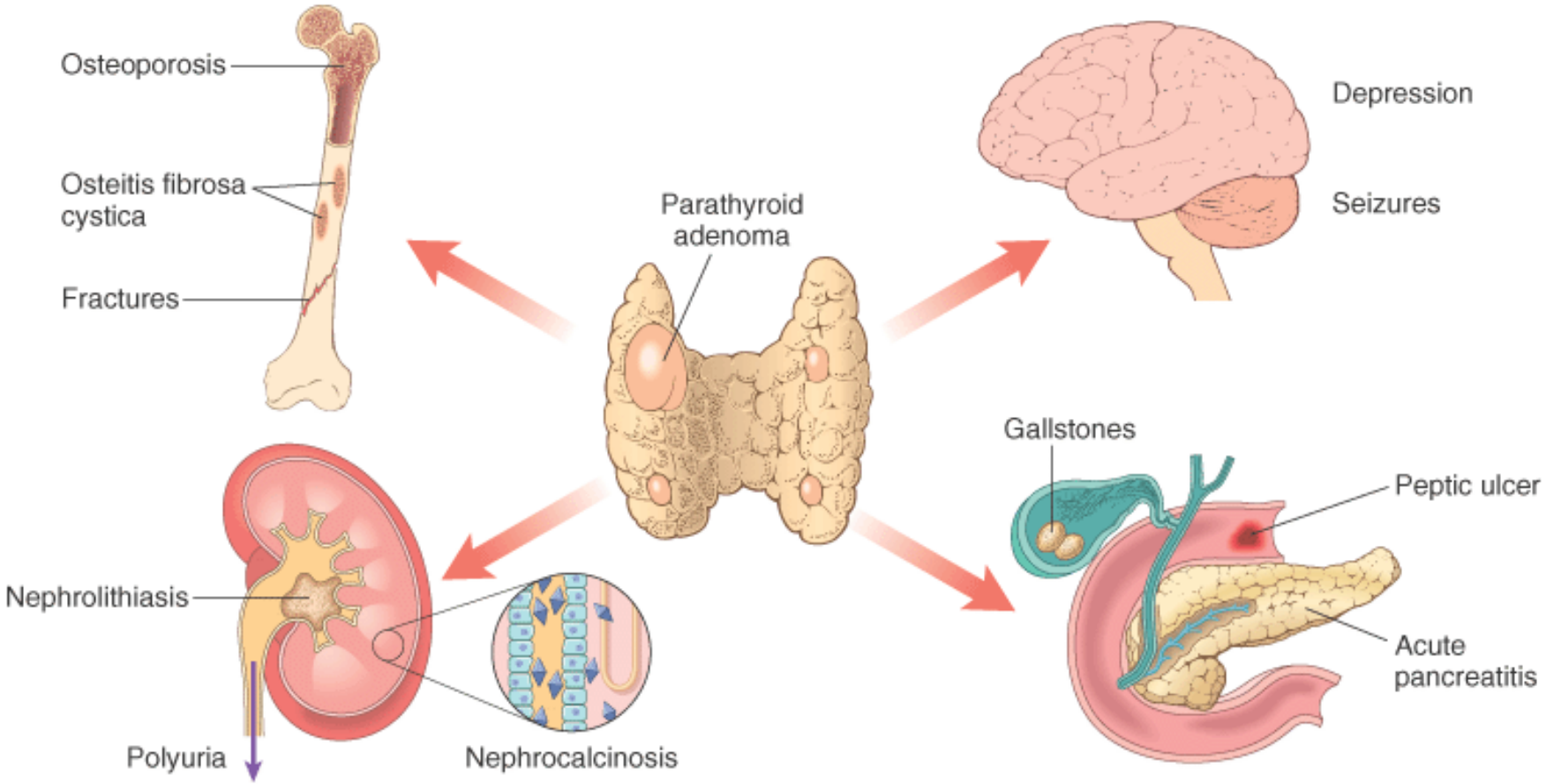
*Primary hyperparathyroidism is the most common cause of hypercalcemia overall. Malignancy is the most common cause of *symptomatic* hypercalcemia. Primary hyperparathyroidism and malignancy account for nearly 90% of cases of hypercalcemia.
†Secondary and tertiary hyperparathyroidism are most commonly associated with progressive renal failure.

In patients with primary hyperparathyroidism, serum PTH levels are inappropriately elevated for the level of serum calcium, whereas PTH levels are low to undetectable in hypercalcemia because of nonparathyroid disease (see Table 24-5). In patients with hypercalcemia caused by secretion of PTHrP by certain nonparathyroid tumors, radioimmunoassays specific for PTH and PTHrP can distinguish between the two molecules. Other laboratory alterations referable to PTH excess include hypophosphatemia and increased urinary excretion of both calcium and phosphate. Secondary renal disease may lead to phosphate retention with normalization of serum phosphates.

Symptomatic Primary Hyperparathyroidism.

The signs and symptoms of hyperparathyroidism reflect the combined effects of increased PTH secretion and hypercalcemia. Primary hyperparathyroidism has been traditionally associated

Figure 24-26 Cardinal features of hyperparathyroidism. With routine evaluation of calcium levels in most patients, primary hyperparathyroidism is often detected at a clinically silent stage. Hypercalcemia from any other cause can also give rise to the same symptoms.



Type 2 diabetes is caused by a combination of peripheral resistance to insulin action and an inadequate secretory response by the pancreatic β-cells ("relative insulin deficiency"). Approximately 80% to 90% of patients have type 2 diabetes.

A variety of monogenic and secondary causes are responsible for the remaining cases, and these will be discussed later. It should be stressed that while the major types of diabetes have different pathogenic mechanisms, *the long-term complications*

TABLE 24-6 -- Classification of Diabetes Mellitus

•**1. Type 1 diabetes** (β-cell destruction, leads to absolute insulin deficiency)
••••••Immune-mediated
••••••Idiopathic
•**2. Type 2 diabetes** (insulin resistance with relative insulin deficiency)
•**3. Genetic defects of β-cell function**
••••••Maturity-onset diabetes of the young (MODY), caused by mutations in:

•••••••Hepatocyte nuclear factor 4α [HNF-4α] (MODY1)
•••••••Glucokinase (MODY2)
•••••••Hepatocyte nuclear factor 1α [HNF-1α] (MODY3)
•••••••Insulin promoter factor [IPF-1] (MODY4)
•••••••Hepatocyte nuclear factor 1β [HNF-1β] (MODY5)
•••••••Neurogenic differentiation factor 1 [Neuro D1] (MODY6)
••••••Mitochondrial DNA mutations
•4. Genetic defects in insulin processing or insulin action
••••••Defects in proinsulin conversion
••••••Insulin gene mutations
••••••Insulin receptor mutations
•5. Exocrine pancreatic defects
••••••Chronic pancreatitis
••••••Pancreatectomy
••••••Neoplasia
••••••Cystic fibrosis
••••••Hemachromatosis
••••••Fibrocalculous pancreatopathy
•6. Endocrinopathies
••••••Acromegaly
••••••Cushing syndrome
••••••Hyperthyroidism
••••••Pheochromocytoma
••••••Glucagonoma
•7. Infections
••••••Cytomegalovirus
••••••Coxsackie virus B
•8. Drugs
••••••Glucocorticoids

••••••Thyroid hormone
••••••α-interferon
••••••Protease inhibitors
••••••β-adrenergic agonists
••••••Thiazides
••••••Nicotinic acid
••••••Phenytoin
•9. Genetic syndromes associated with diabetes
••••••Down syndrome
••••••Kleinfelter syndrome
••••••Turner syndrome
10. Gestational diabetes mellitus
Data from the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetic Care 25 (suppl. 1):S5–S20, 2002.

in kidneys, eyes, nerves, and blood vessels are the same, as are the principal causes of morbidity and death. The pathogenesis of the two major types is discussed separately, but first we briefly review normal insulin secretion and the mechanism of insulin signaling, since these aspects are critical to understanding the pathogenesis of diabetes.

NORMAL INSULIN PHYSIOLOGY

Normal glucose homeostasis is tightly regulated by three interrelated processes: glucose production in the liver; glucose uptake and utilization by peripheral tissues, chiefly skeletal

---

muscle; and actions of insulin and counter-regulatory hormones, including glucagon, on glucose.

Insulin and glucagon have opposing regulatory effects on glucose homeostasis. During fasting states, low insulin and high glucagon levels facilitate hepatic gluconeogenesis and glycogenolysis (glycogen breakdown) while decreasing glycogen synthesis, thereby preventing hypoglycemia. Thus, fasting plasma glucose levels are determined primarily by hepatic glucose output. Following a meal, insulin levels rise and glucagon levels fall in response to the large glucose load. Insulin promotes glucose uptake and utilization in tissues (discussed later). The skeletal muscle is the major insulin-responsive site for postprandial glucose utilization, and is critical for preventing hyperglycemia and maintaining glucose homeostasis.

Regulation of Insulin Release

The insulin gene is expressed in the β cells of the pancreatic islets (Fig. 24-27). Preproinsulin is synthesized in the rough endoplasmic reticulum from insulin mRNA and delivered to the Golgi apparatus. There, a series of proteolytic cleavage steps generate the mature insulin and a cleavage peptide, C-peptide. Both insulin and C-peptide are then stored in secretory granules and secreted in equimolar quantities after physiologic stimulation; increasingly, C-peptide

Figure 24-27 Hormone production in pancreatic islet cells. Immunoperoxidase staining shows a dark reaction product for insulin in β cells (*A*), glucagon in α cells (*B*), and somatostatin in δ cells (*C*). *D*, Electron micrograph of a β cell shows the characteristic membrane-bound granules, each containing a dense, often rectangular core and distinct halo. *E*, Portions of an α cell (*left*) and a δ cell (*right*) also exhibit granules, but with closely apportioned membranes. The α-cell granule exhibits a dense, round center. *(Electron micrographs courtesy of Dr. A. Like, University of Massachusetts Medical School, Worcester, MA.)*

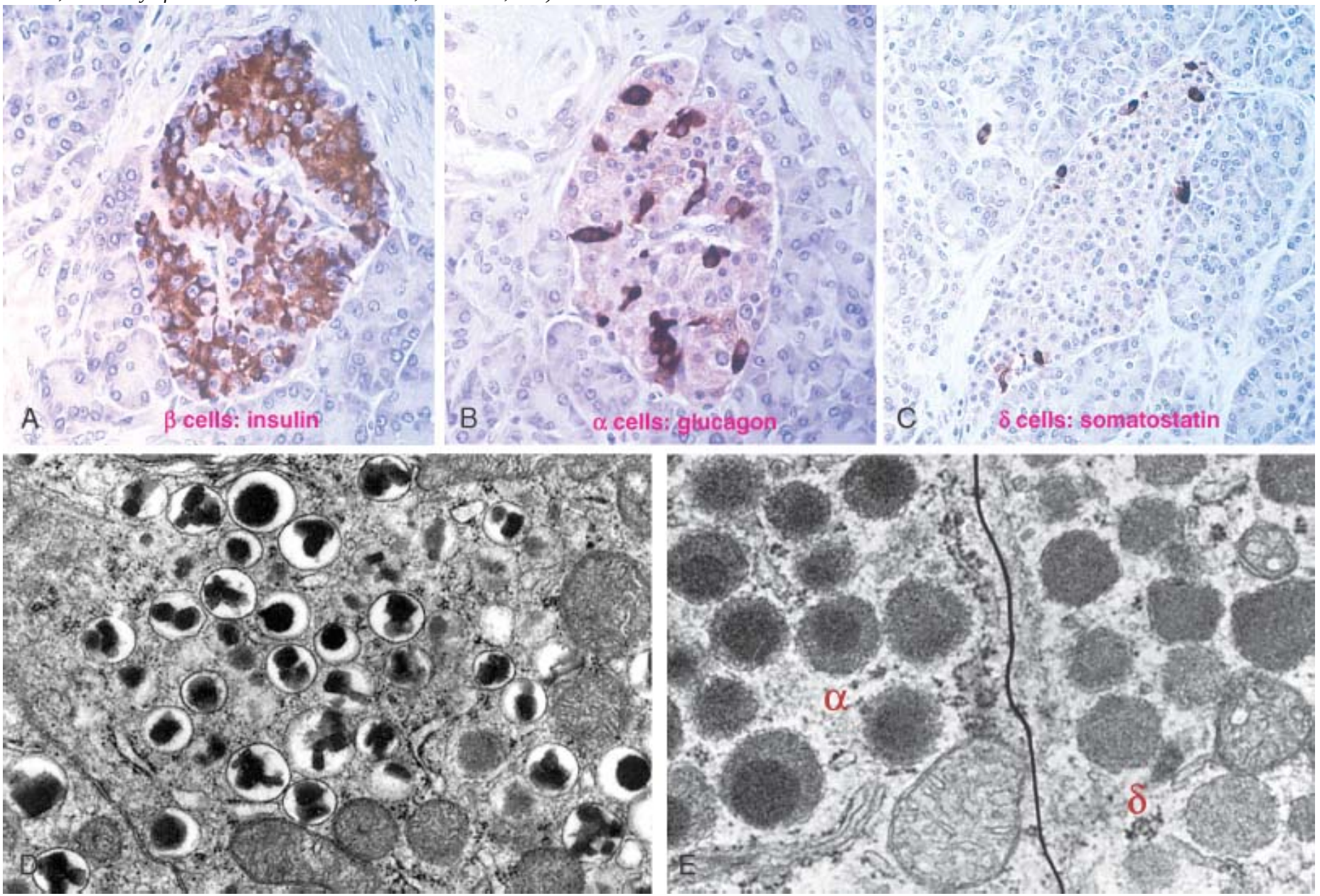


Figure 24-28 Insulin synthesis and secretion. Intracellular transport of glucose is mediated by GLUT-2, an insulin-independent glucose transporter in β cells. Glucose undergoes

oxidative metabolism in the β cell to yield ATP. ATP inhibits an inward rectifying potassium channel receptor on the β-cell surface; the receptor itself is a dimeric complex of the sulfonylurea receptor and a K^+ -channel protein. Inhibition of this receptor leads to membrane depolarization, influx of Ca^{2+} ions, and release of stored insulin from β cells.

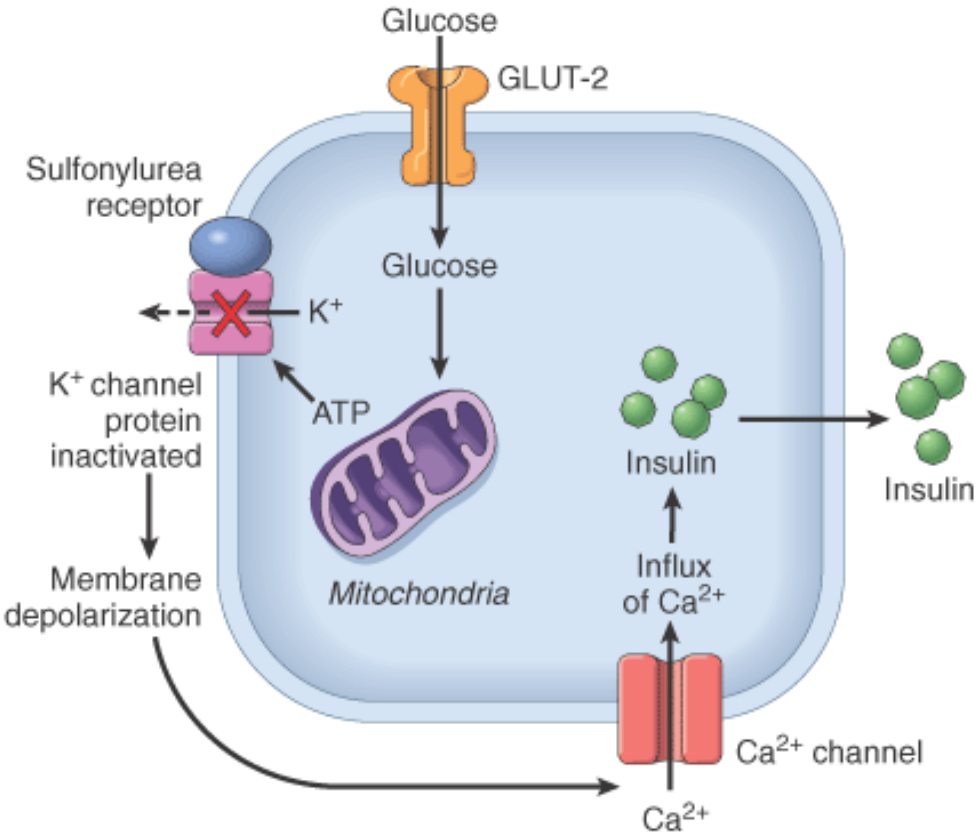


Figure 24-29 Metabolic actions of insulin in striated muscle, adipose tissue, and liver.

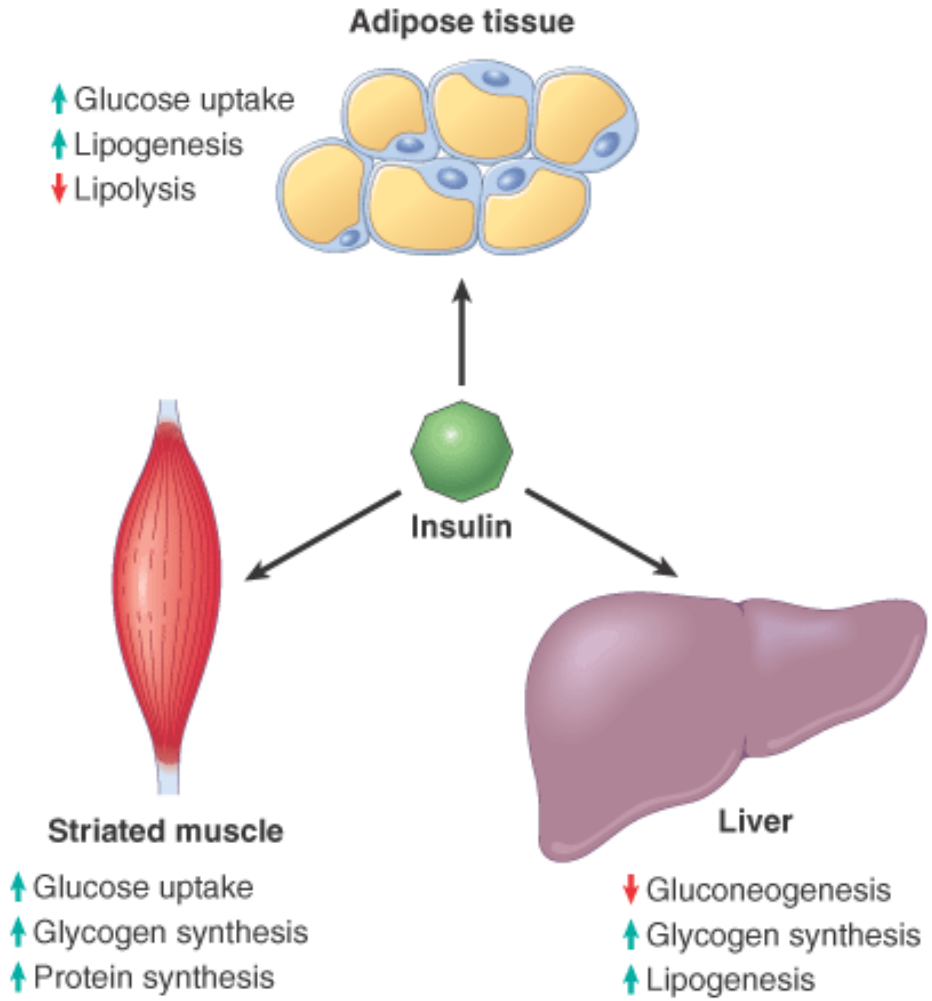


Figure 24-30 Insulin action on a target cell. Insulin binds to the α subunit of insulin receptor, leading to activation of the kinase activity in the β-subunit, and sets in motion a phosphorylation (i.e., activation) cascade of multiple downstream target proteins. The mitogenic functions of insulin (and the related insulin-like growth factors) are mediated via the mitogen-activated protein kinase (MAP kinase) pathway. The metabolic actions of insulin are mediated primarily by activation of the phosphatidylinositol-3-kinase (PI-3K) pathway. The PI-3K-signaling pathway is responsible for a variety of effects on target cells, including translocation of GLUT-4 containing vesicles to the surface; increasing GLUT-4 density on the membrane and rate of glucose influx; promoting glycogen synthesis via activation of glycogen synthase; and promoting protein synthesis and lipogenesis, while inhibiting lipolysis. The PI-3K pathway also promotes cell survival and proliferation.

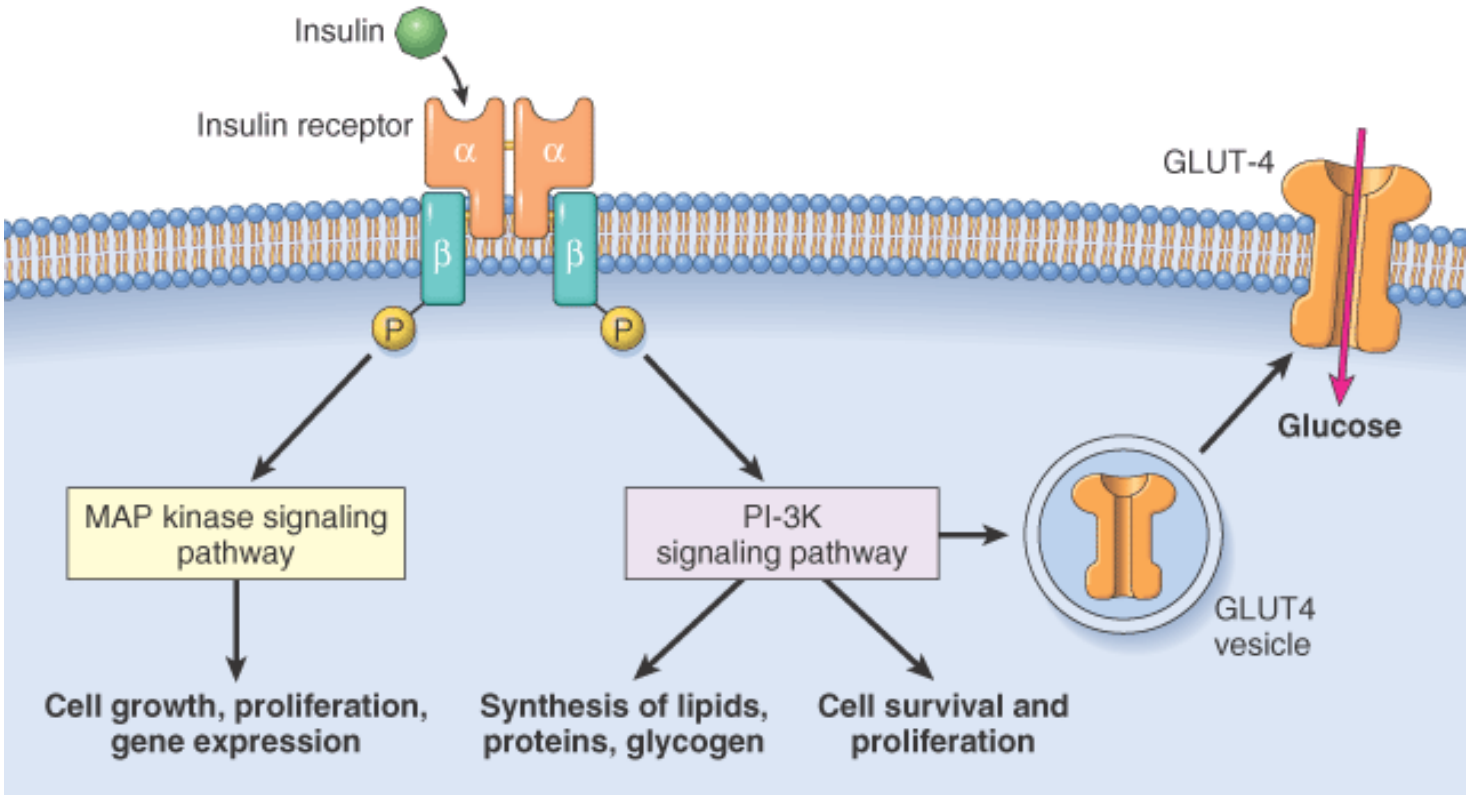


Figure 24-31 Stages in the development of type 1 diabetes mellitus. The stages are listed from left to right, and hypothetical β-cell mass is plotted against age. *(From Eisenbarth GE: Type 1 diabetes: a chronic autoimmune disease. N Engl J Med 314:1360, 1986. Copyright © 1986, Massachusetts Medical Society. All rights reserved.)*

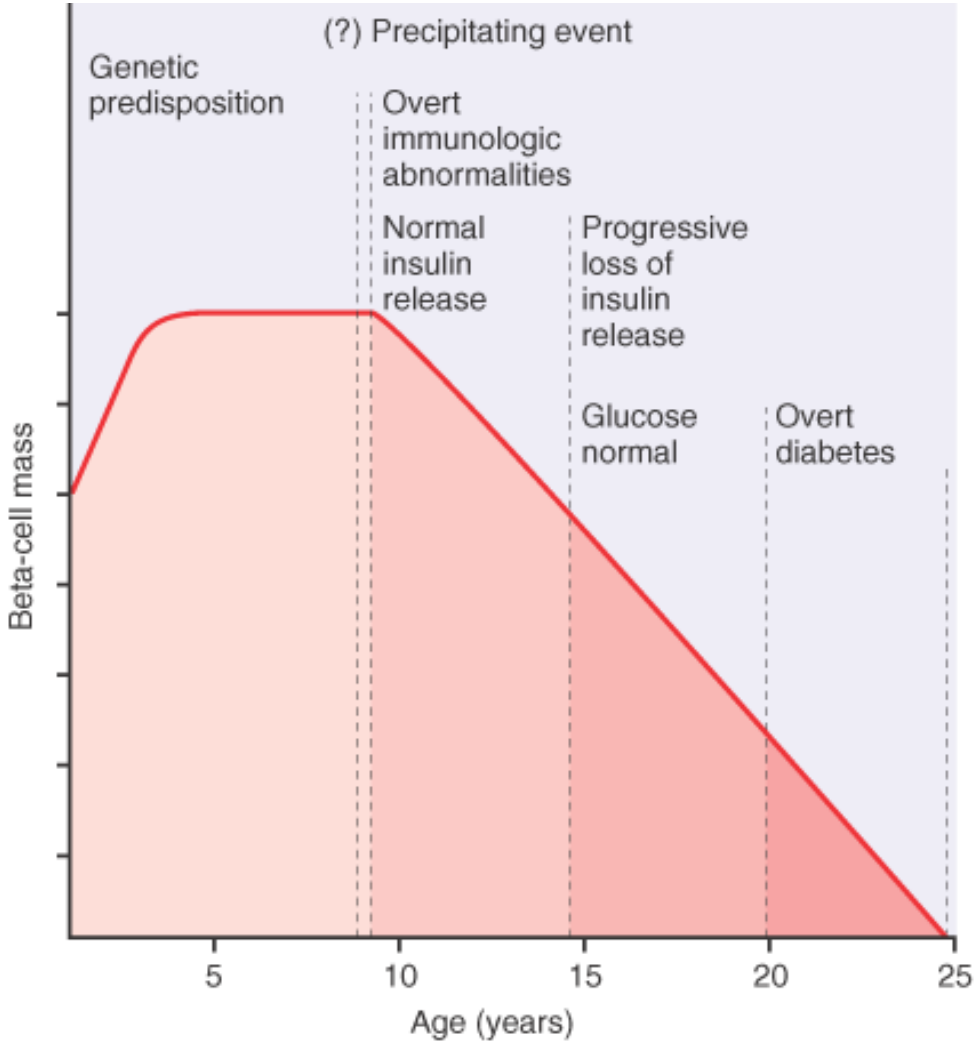


Figure 24-33 Obesity and insulin resistance: the missing links? Adipocytes release a variety of factors (free fatty acids and adipokines) that may play a role in modulating insulin resistance in peripheral tissues (illustrated here is striated muscle). Excess free fatty acids (FFAs) and resistin are associated with insulin resistance; in contrast, adiponectin, whose levels are decreased in obesity, is an insulin-sensitizing adipokine. Leptin is also an insulin-sensitizing agent, but it acts via central receptors (in the hypothalamus). The peroxisome proliferator-activated receptor gamma (PPARγ) is an adipocyte nuclear receptor that is activated by a class of insulin-sensitizing drugs called thiazolidinediones (TZDs). The mechanism of action of TZDs may eventually be mediated through modulation of adipokine and FFA levels that favor a state of insulin sensitivity.

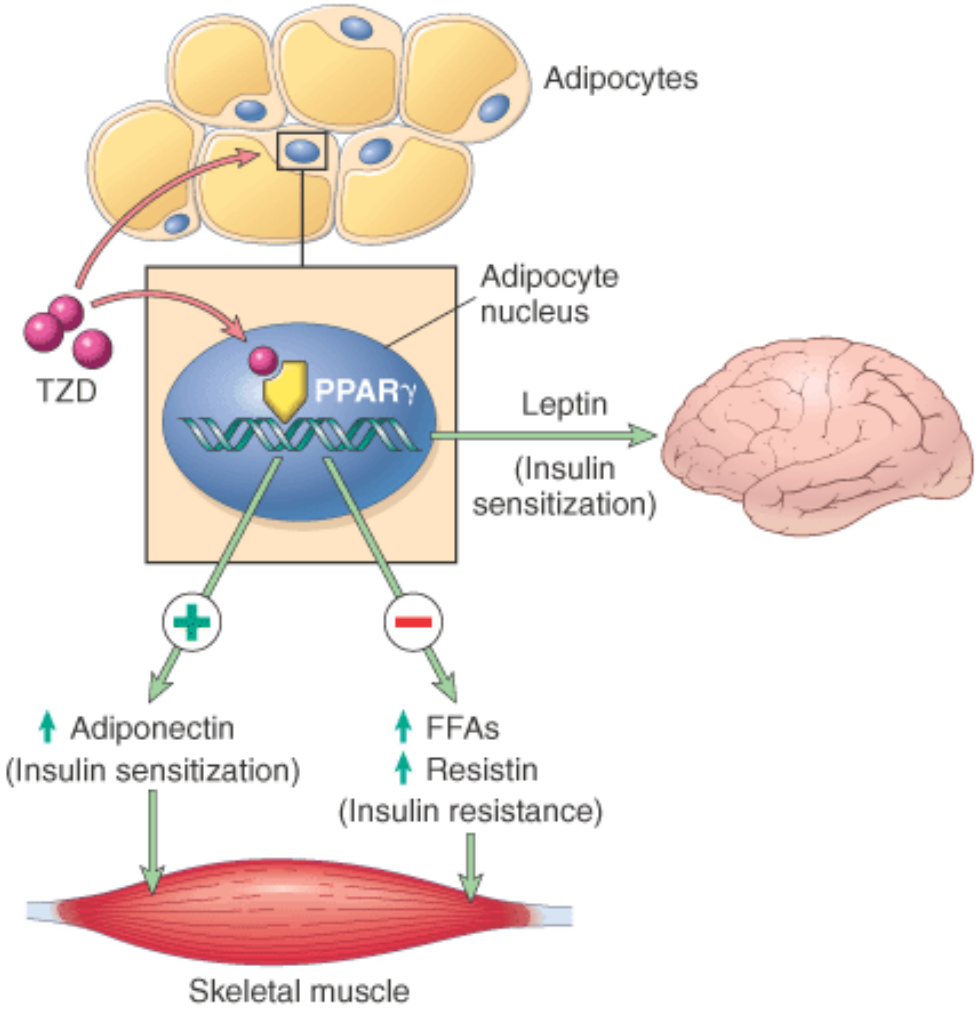


TABLE 24-7 -- Effects of Advanced Glycation End Products (AGEs)

Extracellular Matrix Components
Abnormal matrix-matrix and matrix-cell interactions
Corss-linking of polypeptides of same protein (e.g., collagen)
Trapping of nonglycated proteins (e.g., LDL, albumin)
Resistance to proteolytic digestion
Intracellular and Plasma Proteins
AGE receptor ligation leads to generation of reactive oxygen species and NF-κB activation
Target cells (endothelium, mesangial cells, macrophages) respond by:

••Cytokines and growth factor secretion
••Induction of procoagulant activity
Increased vascular permeability
••Enhanced ECM production
ECM, extracellular matrix; LDL, low-density lipoprotein.

You will recall from the discussion of atherosclerosis (Chapter 11) that *endothelial dysfunction*, particularly endothelial activation, is a critical process in vascular injury and atherogenesis. AGEs, by virtue of their ability to modify extracellular matrix components, as well as to activate NF-κB and its downstream targets in the vascular endothelium, are postulated to play a central role in the accelerated atherogenesis characteristic of diabetes. In addition to large vessel disease, AGEs also contribute to microvascular injury in diabetes. The AGE inhibitor aminoguanidine has recently been shown to retard the progression of nephropathy in type 1 diabetics.

Activation of Protein Kinase C.

Activation of intracellular protein kinase C (PKC) by calcium ions and the second messenger diacylglycerol (DAG) is an important signal transduction pathway in many cellular systems. Intracellular hyperglycemia can stimulate the de novo synthesis of DAG from glycolytic intermediates and hence cause activation of PKC. The downstream effects of PKC activation are numerous and include the following: [97]

- Production of the proangiogenic molecule vascular endothelial growth factor (VEGF), implicated in the neovascularization characterizing diabetic retinopathy (Chapter 29)
- Increased activity of the vasoconstrictor endothelin-1 and decreased activity of the vasodilator endothelial nitric oxide synthase (eNOS)
- Production of profibrogenic molecules like transforming growth factor-β (TGF-β), leading to increased deposition of extracellular matrix and basement membrane material
- Production of the procoagulant molecule plasminogen activator inhibitor-1 (PAI-1), leading to reduced fibrinolysis and possible vascular occlusive episodes
- Production of pro-inflammatory cytokines by the vascular endothelium.

It should be evident that some effects of AGEs and activated PKCs (e.g., activation of NF-κB) are overlapping. Not surprisingly, therefore, therapeutic inhibition of PKC can retard the progression of diabetic retinopathy. [98]

Intracellular Hyperglycemia with Disturbances in Polyol Pathways.

In some tissues that do not require insulin for glucose transport (e.g., nerves, lenses, kidneys, blood vessels), hyperglycemia leads to an increase in intracellular glucose that is then metabolized by the enzyme *aldose reductase* to sorbitol, a polyol, and eventually to fructose. In this process, intracellular NADPH is used as a cofactor. NADPH is also required as a cofactor by the enzyme glutathione reductase for regenerating reduced glutathione (GSH). You will recall that GSH is one of the important antioxidant mechanisms in the cell (Chapter 1), and a reduction in GSH levels increases cellular susceptibility to oxidative stress. [99] In the face of sustained hyperglycemia, progressive depletion of intracellular NADPH by aldol reductase leads to a compromise of

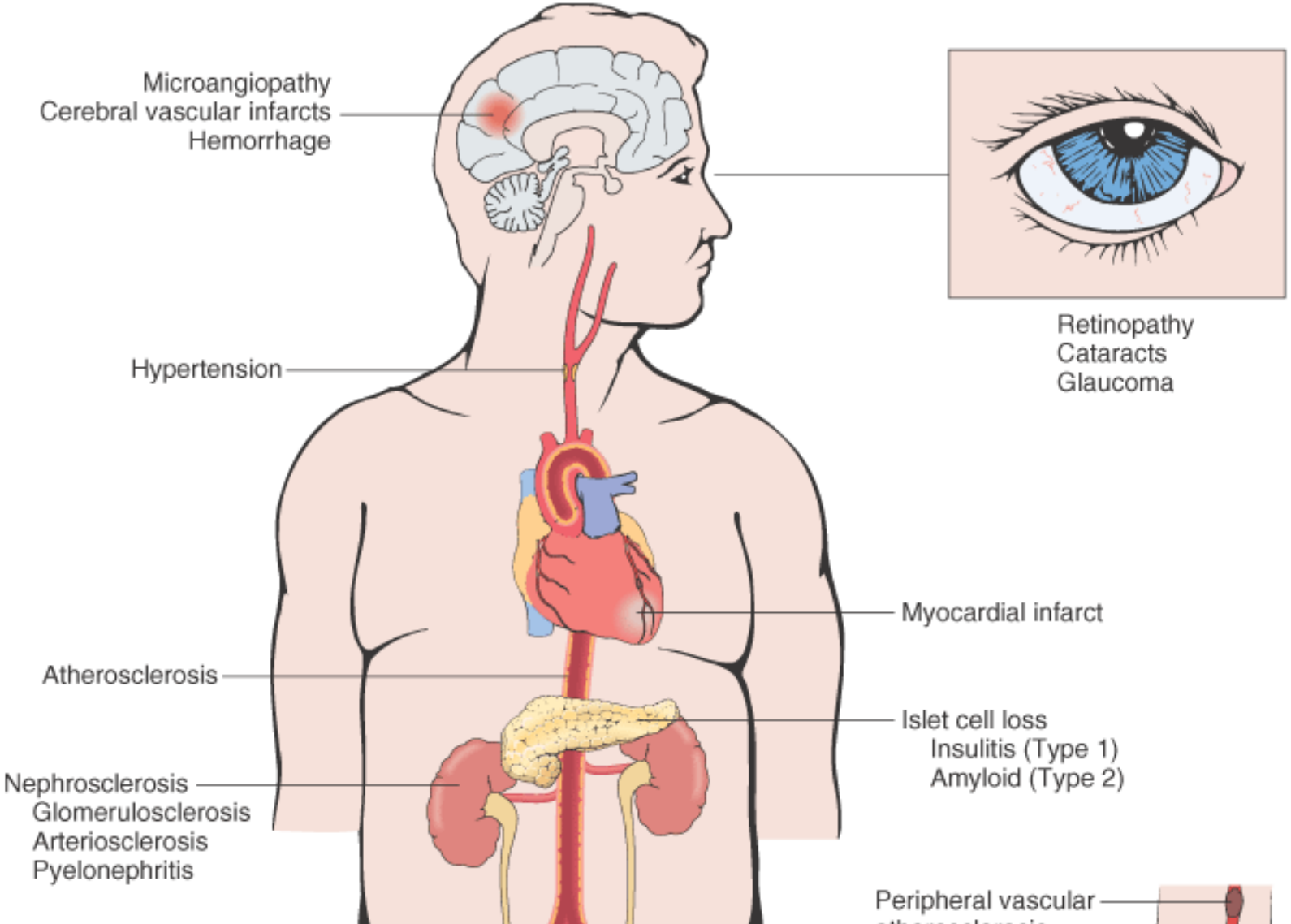
GSH regeneration. Thus, the deleterious consequences of the aldose reductase pathway arise primarily by increasing cellular susceptibility to oxidative stress. The importance of this

pathway in human diabetes was best exemplified in clinical trials using an aldose reductase inhibitor, which significantly ameliorated the development of diabetic neuropathy. Unfortunately, the effects of these inhibitors on other long-term complications have been less promising.

MORPHOLOGY OF DIABETES AND ITS LATE COMPLICATIONS

Pathologic findings in the pancreas are variable and not necessarily dramatic. The important morphologic changes are related to the many late systemic complications of diabetes. There is extreme variability among patients in the time of onset of these complications, their severity, and the particular organ or organs involved. In individuals with tight control of diabetes, the onset might be delayed. In most patients, however, morphologic changes are likely to be found in arteries (*macrovascular disease*), basement membranes of small vessels (*microangiopathy*), kidneys (*diabetic nephropathy*), retina (*retinopathy*), nerves (*neuropathy*), and other tissues. These

Figure 24-34 Long-term complications of diabetes.



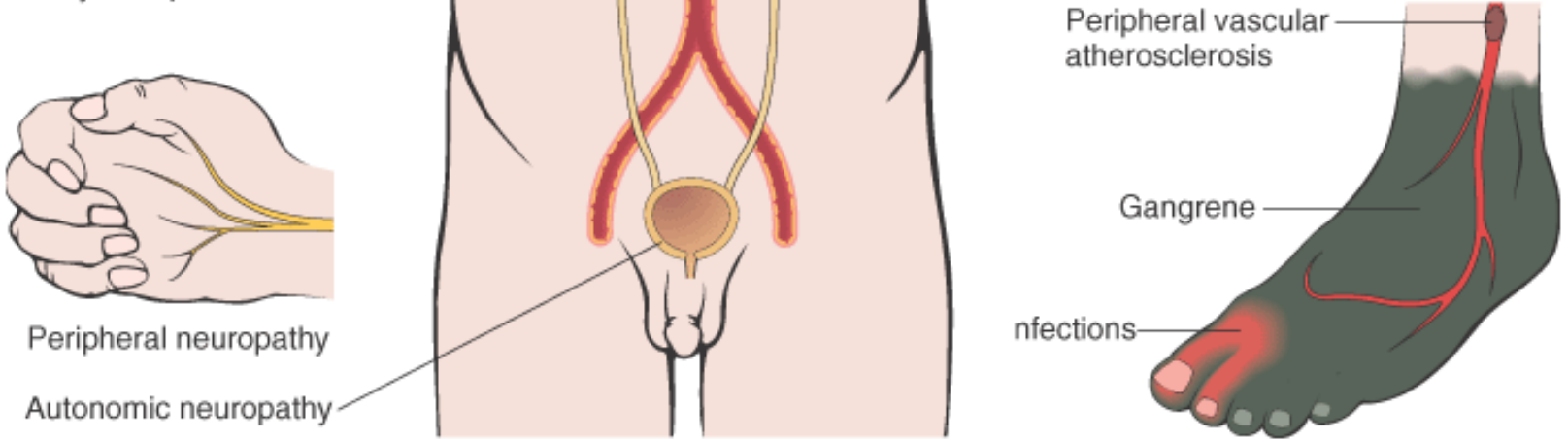


Figure 24-35 *A,* Insulitis, shown here from a rat (BB) model of autoimmune diabetes, also seen in type 1 human diabetes. *(Courtesy of Dr. Arthur Like, University of Massachusetts, Worchester, MA.) B,* Amyloidosis of a pancreatic islet in type 2 diabetes.

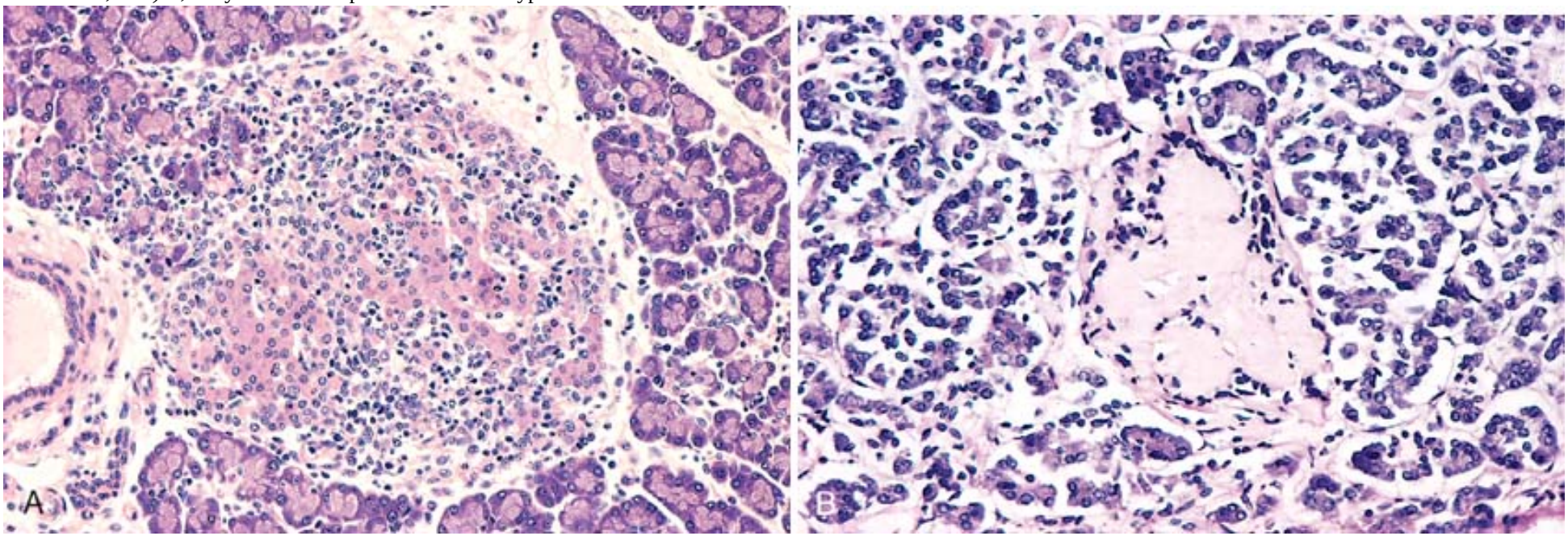


Figure 24-36 Severe renal hyaline arteriolosclerosis. Note a markedly thickened, tortuous afferent arteriole. The amorphous nature of the thickened vascular wall is evident. *(Periodic acid-Schiff [PAS] stain; courtesy of M.A. Venkatachalam, MD, Department of Pathology, University of Texas Health Science Center at San Antonio, TX.)*

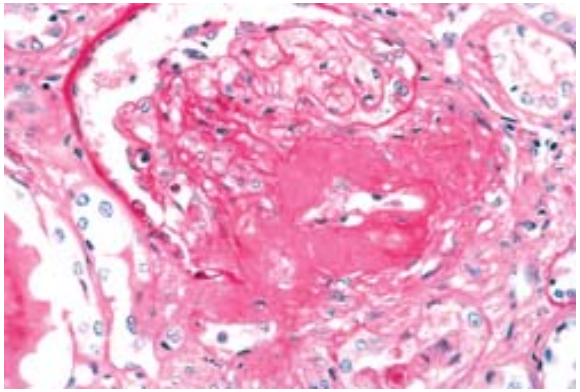


Figure 24-37 Renal cortex showing thickening of tubular basement membranes in a diabetic patient (PAS stain).

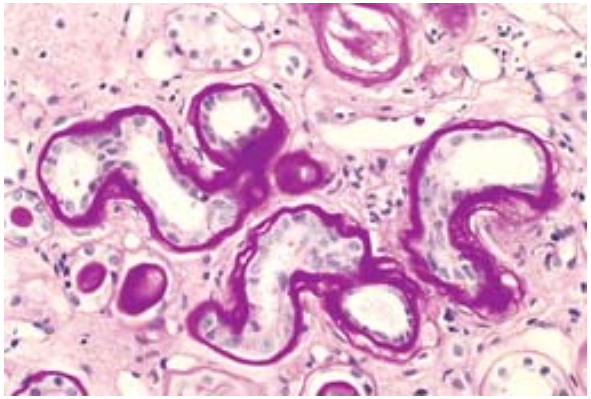


Figure 24-38 Electron micrograph of a renal glomerulus showing markedly thickened glomerular basement membrane (B) in a diabetic. L, glomerular capillary lumen; U, urinary space. *(Courtesy of Dr. Michael Kashgarian, Department of Pathology, Yale University School of Medicine, New Haven, CT.)*

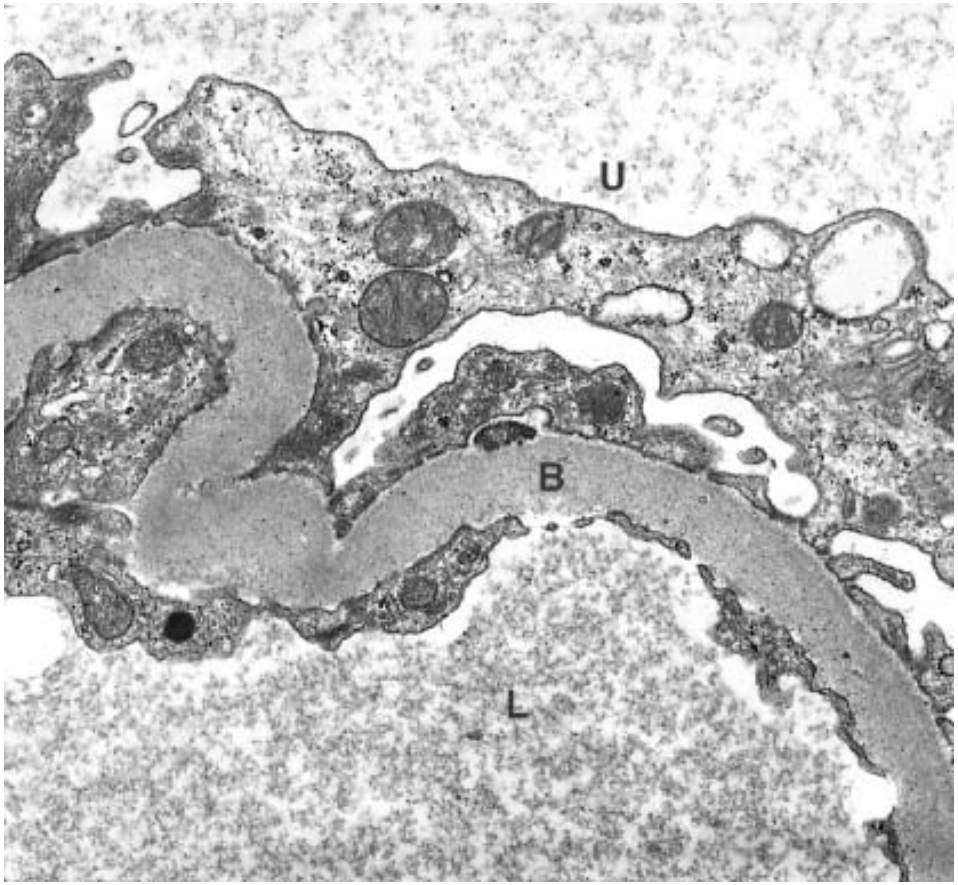
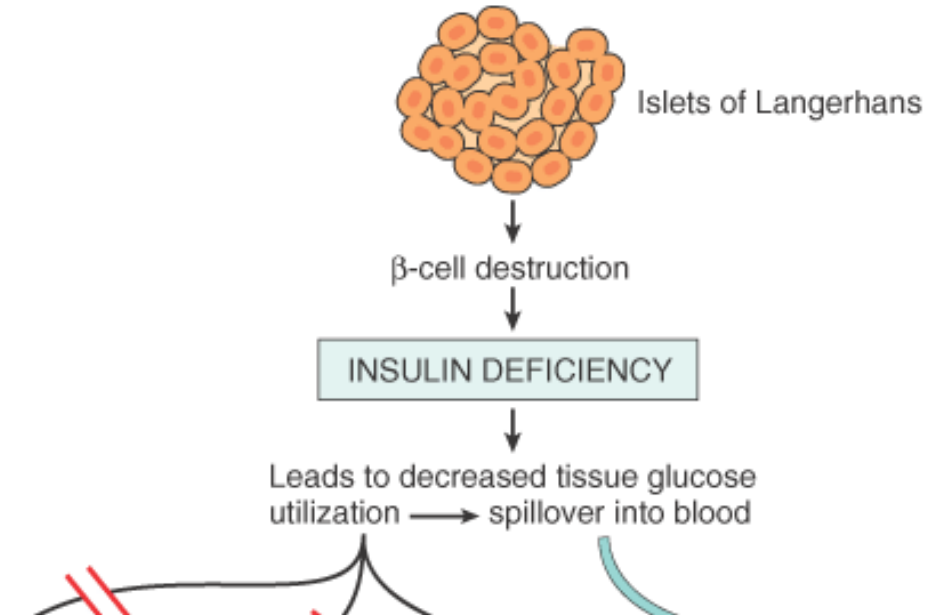
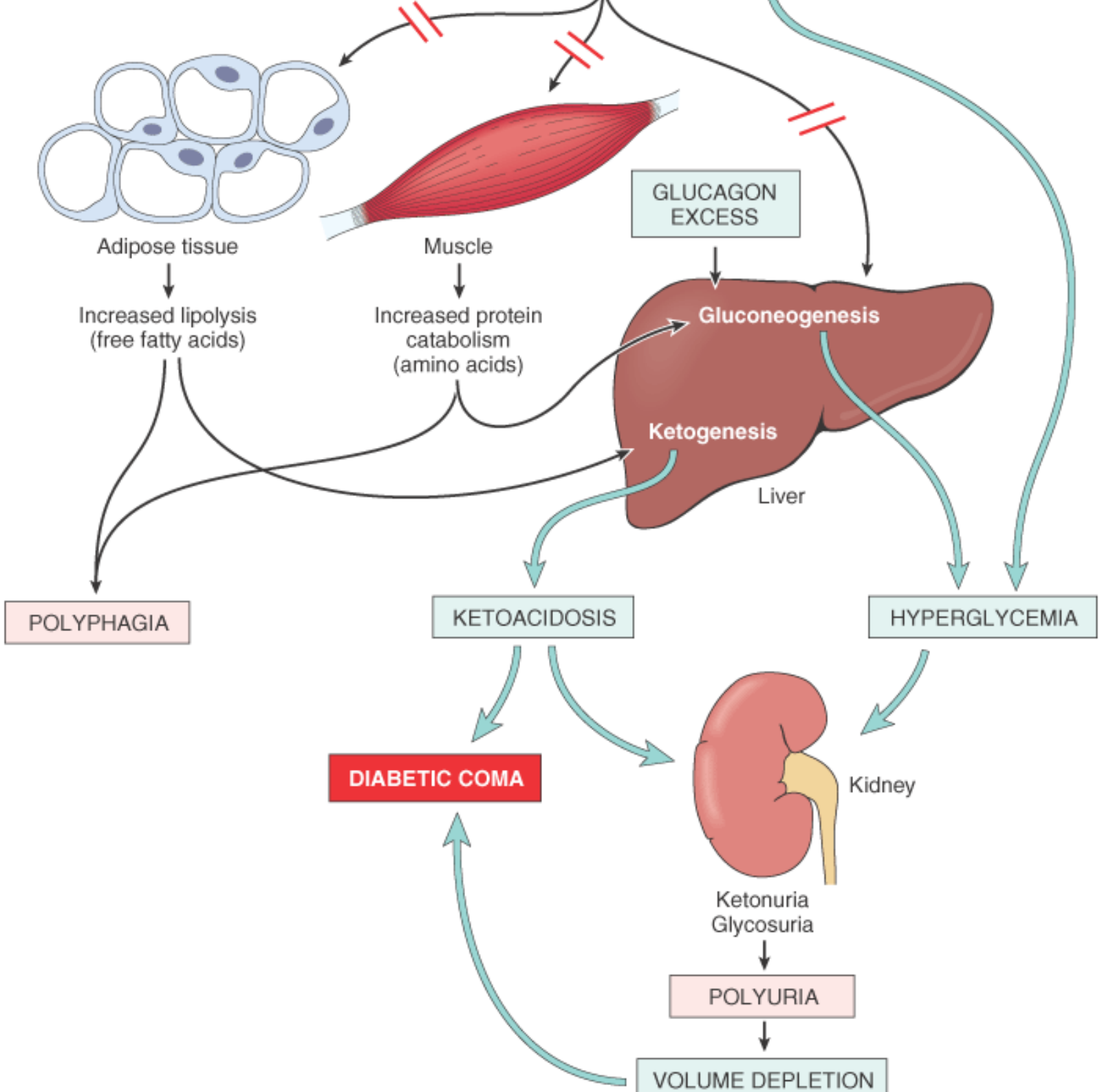


Figure 24-39 Nephrosclerosis in a patient with long-standing diabetes. The kidney has been bisected to demonstrate both diffuse granular transformation of the surface *(left)* and marked thinning of the cortical tissue *(right)*. Additional features include some irregular depressions, the result of pyelonephritis, and an incidental cortical cyst *(far right)*.

Figure 24-40 Sequence of metabolic derangements leading to diabetic coma in type 1 diabetes mellitus. An absolute insulin deficiency leads to a catabolic state, eventuating in ketoacidosis and severe volume depletion. These cause sufficient central nervous system compromise to lead to coma and eventual death if left untreated.




Adipose tissue
Muscle
GLUCAGON EXCESS
Increased lipolysis (free fatty acids)
Increased protein catabolism (amino acids)
Gluconeogenesis
Ketogenesis
Liver
POLYPHAGIA
KETOACIDOSIS
HYPERGLYCEMIA
DIABETIC COMA
Kidney
Ketonuria
Glycosuria
POLYURIA
VOLUME DEPLETION

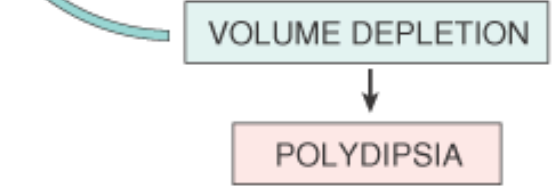


TABLE 24-8 -- Type 1 Versus Type 2 Diabetes Mellitus (DM)

	Type 1 DM	Type 2 DM
Clinical	Onset: <20 years	Onset: >30 years
	Normal weight	Obese
	Markedly decreased blood insulin	Increased blood insulin (early);normal to moderate decreased insulin (late)
	Anti-islet cell antibodies	No anti-islet cell antibodies
	Ketoacidosis common	Ketoacidosis rare; nonketotic hyperosmolar coma
Genetics	30–70% concordance in twins	50–90% concordance in twins
	Linkage to MHC Class II HLA genes	No HLA linkage
		Linkage to candidate diabetogenic genes (PPARγ, calpain 10)
Pathogenesis	Autoimmune destruction of β-cells mediated by T cells and humoral mediators (TNF, IL-1, NO)	Insulin resistance in skeletal muscle, adipose tissue and liver
		β-cell dysfunction and relative insulin deficiency
	Absolute insulin deficiency	
Islet cells	Insulitis early	No insulitis
	Marked atrophy and fibrosis	Focal atrophy and amyloid deposition
	β-cell depletion	Mild β-cell depletion

pertinent clinical, genetic, and histopathologic features that distinguish type 1 and type 2 diabetes.

In both forms, it is the long-term effects of diabetes, more than the acute metabolic complications, that are responsible for the overwhelming proportion of morbidity and mortality. In most instances, these complications appear approximately 15 to 20 years after the onset of hyperglycemia. *Cardiovascular events such as myocardial infarction, renal vascular insufficiency, and cerebrovascular accidents are the most common causes of mortality in long-standing diabetics.* The impact of cardiovascular disease can be gauged from the fact that it accounts for up to 80% of deaths in type 2 diabetes; in fact, diabetics have a 3 to 7.5 times greater incidence of death from cardiovascular causes compared to the nondiabetic population[100] (Fig. 24-41). The hallmark of cardiovascular disease is *accelerated atherosclerosis* of the large and medium-sized arteries (i.e., macrovascular disease). *The pathogenesis of accelerated atherosclerosis involves multiple factors.* We have previously

Figure 24-41 Incidence of death from cardiovascular causes in diabetic and nondiabetic individuals after a 7-year follow up. MI, myocardial infarction. *(Reproduced with permission from Haffner et al: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without myocardial infarction. N Engl J Med 339:229,*

1998.)

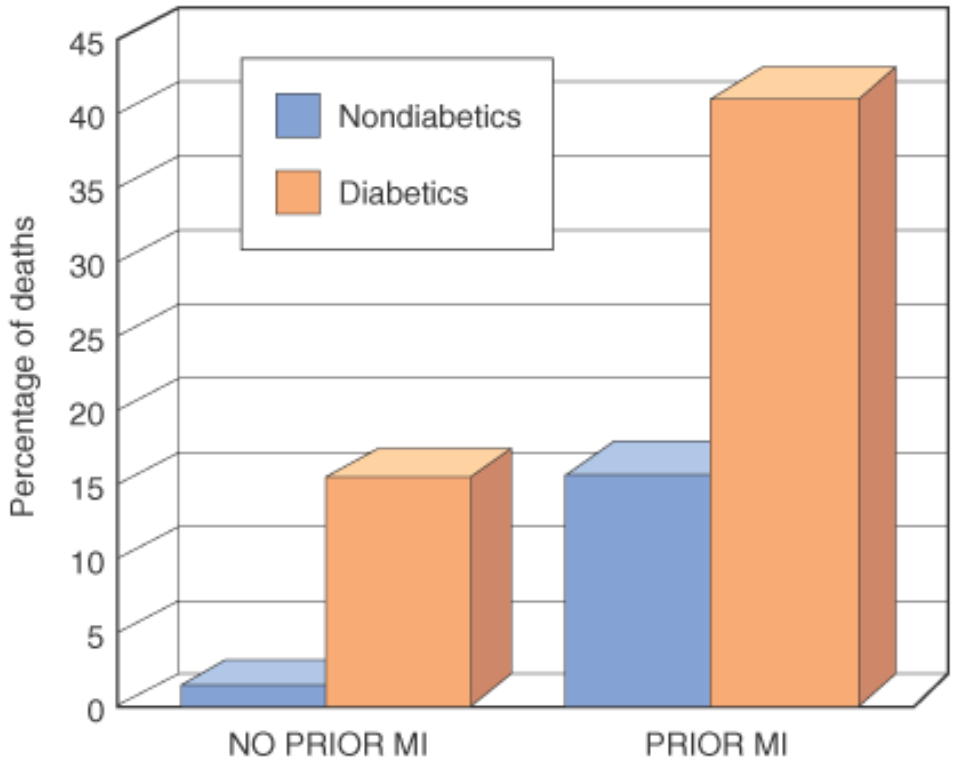


Figure 24-42 Pancreatic endocrine tumor ("islet cell tumor"). *A*, The neoplastic cells are monotonous and demonstrate minimal pleomorphism or mitotic activity (H & E stain). *B*, Immunoreactivity for insulin confirms the neoplasm is an insulinoma. Clinically, the patient had episodic hypoglycemia.

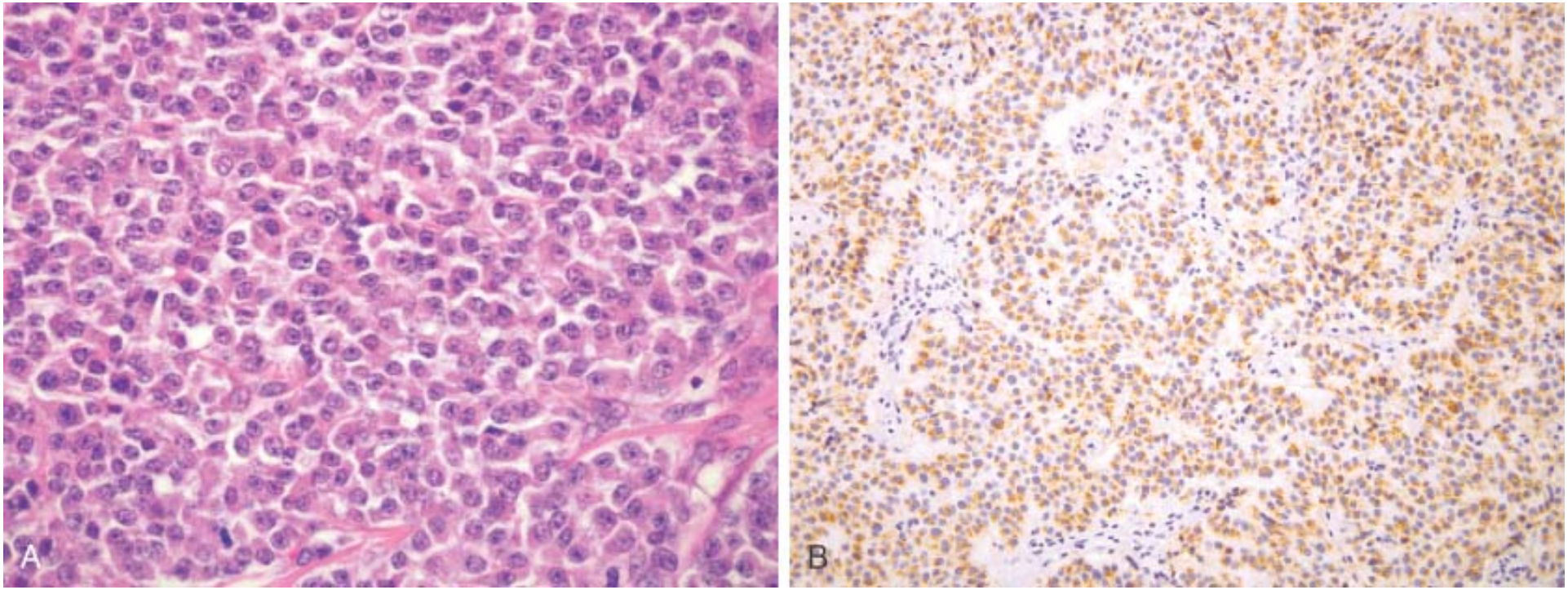


Figure 24-43 A schematic representation of the various forms of Cushing syndrome, illustrating the three endogenous forms as well as the more common exogenous (iatrogenic) form. ACTH, adrenocorticotropic hormone.

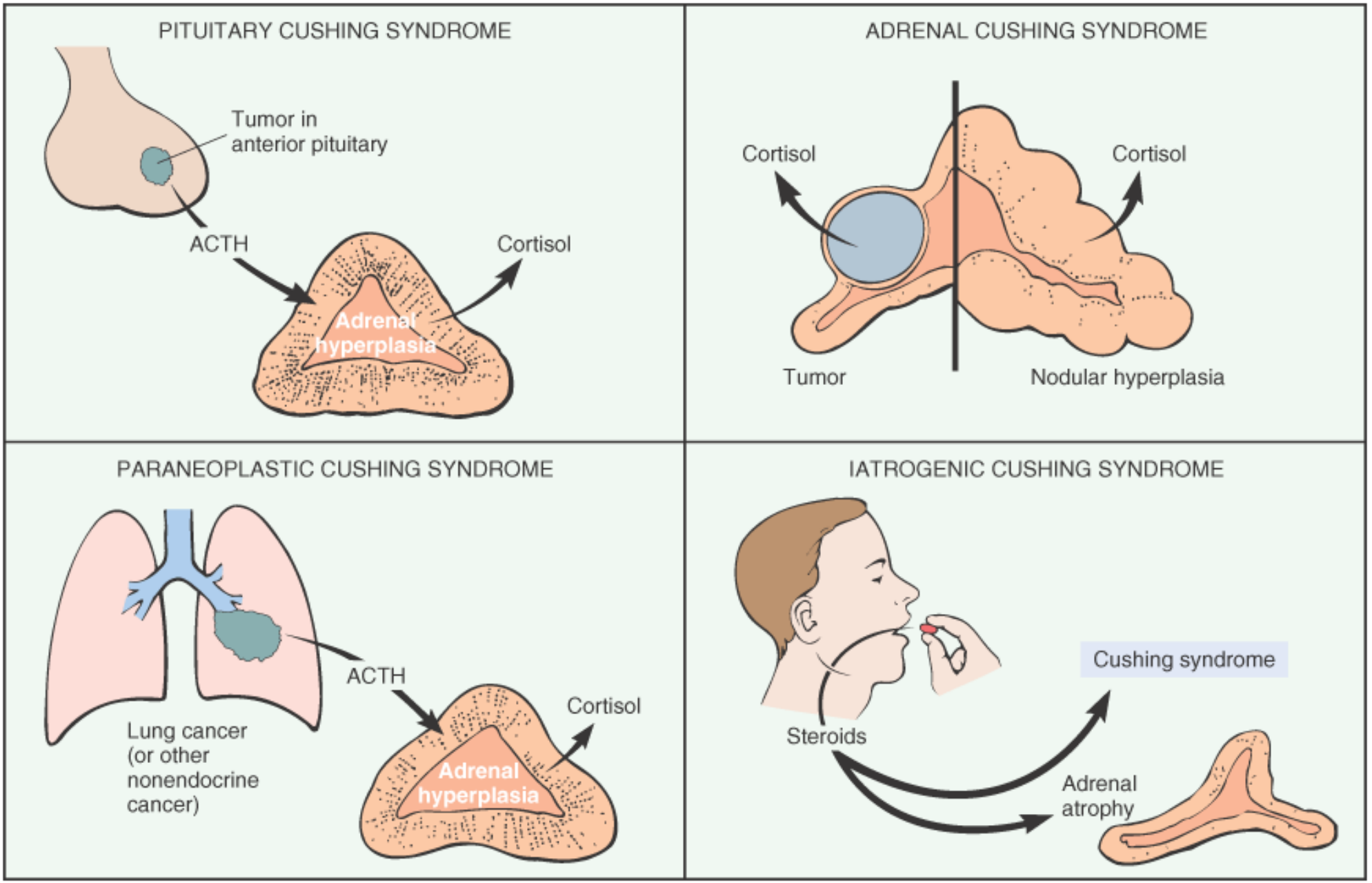


TABLE 24-9 -- Major Features of Cushing Syndrome with Approximate Frequency

Clinical Features	Percentages
Central obesity (about trunk and upper back)	85–90%

Moon facies	85%
Weakness and fatigability	85%
Hirsutism	75%
Hypertension	75%
Plethora	75%
Glucose intolerance/diabetes	75/20%
Osteoporosis	75%
Neuropsychiatric abnormalities	75–80%
Menstrual abnormalities	70%
Skin striae (sides of lower abdomen)	50%

because glucocorticoids suppress the immune response. Additional manifestations include a number of *mental disturbances*, including mood swings, depression, and frank psychosis, as well as *hirsutism* and *menstrual abnormalities*.

Cushing syndrome is diagnosed in the laboratory with the following: (1) the 24-hour urine free cortisol level, which is increased, and (2) loss of normal diurnal pattern of cortisol secretion. Determining the cause of Cushing syndrome depends on the level of serum ACTH and measurement of urinary steroid excretion after administration of dexamethasone. Three general patterns can be obtained:[111]

1. In pituitary Cushing syndrome, the most common form, ACTH levels are elevated and cannot be suppressed by the administration of a low dose of dexamethasone. Hence, there is no reduction in urinary excretion of 17-hydroxy-corticosteroids. After higher doses of injected dexamethasone, however, the pituitary responds by reducing ACTH secretion, which is reflected by suppression of urinary steroid secretion.
2. Ectopic ACTH secretion results in an elevated level of ACTH, but its secretion is completely insensitive to low or high doses of exogenous dexamethasone.
3. When Cushing syndrome is caused by an adrenal tumor, the ACTH level is quite low because of feedback inhibition of the pituitary. As with ectopic ACTH secretion, both low-dose and high-dose dexamethasone fail to suppress cortisol excretion.

Primary Hyperaldosteronism

Hyperaldosteronism is the generic term for a small group of closely related, uncommon syndromes, all characterized by chronic excess aldosterone secretion. *Excessive levels of aldosterone cause sodium retention and potassium excretion, with resultant hypertension and hypokalemia.* Hyperaldosteronism may be primary, or it may be a secondary event resulting from an extra-adrenal cause.

Primary hyperaldosteronism indicates an autonomous overproduction of aldosterone, with resultant suppression of the renin-angiotensin system and decreased plasma renin activity. Primary hyperaldosteronism is caused by one of three mechanisms[115] (Fig. 24-44):

• *Adrenocortical neoplasm*, either an aldosterone-producing adrenocortical adenoma (the most common cause) or, rarely, an adrenocortical carcinoma. In approximately 80% of cases, primary hyperaldosteronism is caused by a solitary aldosterone-secreting adenoma, a condition referred to as *Conn syndrome*. This syndrome occurs most frequently in adult middle life and is more common in women than in men (2:1). Multiple adenomas may be present in an occasional patient.
• *Primary adrenocortical hyperplasia (idiopathic hyperaldosteronism)*, characterized by bilateral nodular hyperplasia of the adrenal glands, highly reminiscent of those found in the nodular hyperplasia of Cushing syndrome. The genetic basis of idiopathic hyperaldosteronism is not clear, although it is possibly caused by an overactivity of the aldosterone synthase gene, *CYP11B2*. [116]
• *Glucocorticoid-remediable hyperaldosteronism* is an uncommon cause of primary hyperaldosteronism that is familial and genetic. In some families, it is caused by a chimeric gene resulting from fusion between *CYP11B1* (the 11β-hydroxylase gene) and *CYP11B2* (the aldosterone synthase gene).[117] This leads to a sustained production of hybrid steroids in addition to both cortisol and aldosterone. The activation of aldosterone secretion is under the influence of ACTH and hence is suppressible by exogenous administration of dexamethasone.

In *secondary hyperaldosteronism*, in contrast, aldosterone release occurs in response to activation of the renin-angiotensin system (Chapter 4). It is characterized by increased levels of plasma renin and is encountered in conditions such as the following:

• Decreased renal perfusion (arteriolar nephrosclerosis, renal artery stenosis)
• Arterial hypovolemia and edema (congestive heart failure, cirrhosis, nephrotic syndrome)
• Pregnancy (due to estrogen-induced increases in plasma renin substrate).

Morphology.

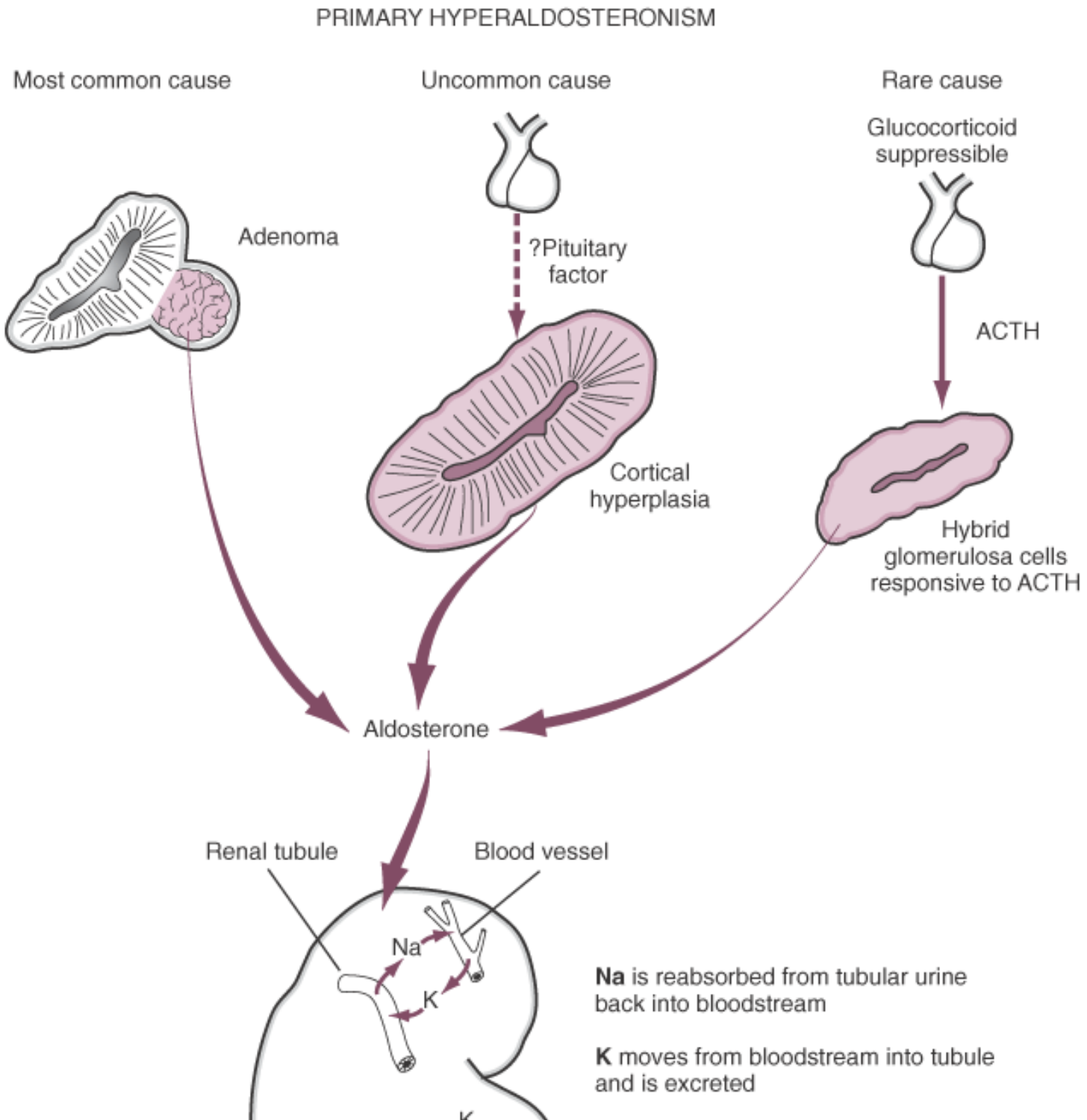
Aldosterone-producing adenomas are almost always solitary, small (<2 cm in diameter), well-circumscribed lesions, more often found on the left than on the right. They tend to occur in the thirties and forties, and in women more often than in men. These lesions are often buried within the gland and do not produce visible enlargement, a point to be remembered in interpreting sonographic or scanning images. They are bright yellow on cut section (Fig. 24-45) and, surprisingly, are composed of lipid-laden cortical cells that more closely resemble fasciculata cells than glomerulosa cells (the normal source of aldosterone). In general, the cells tend to be uniform in size and shape and resemble mature cortical cells; occasionally, there is some nuclear and cellular pleomorphism but no evidence of anaplasia (Fig. 24-46). A characteristic feature of aldesterone-producing adenomas is the presence of eosinophilic, laminated cytoplasmic inclusions, known as **spironolactone bodies**, found after treatment with the anti-hypertensive drug spironolactone. In contrast to cortical adenomas associated with Cushing syndrome, those associated with hyperaldosteronism do not usually suppress ACTH secretion. Therefore, the adjacent adrenal cortex and that of the contralateral gland are not atrophic.

Bilateral idiopathic hyperplasia (Fig. 24-47) is marked by diffuse and focal hyperplasia of cells resembling those of the normal zona glomerulosa. The hyperplasia is often wedge-shaped, extending from the periphery toward the center of the gland. Bilateral enlargement can be subtle in idiopathic hyperplasia, and as a rule, an adrenocortical adenoma should be carefully excluded as the cause for hyperaldosteronism.

Clinical Course.

The clinical manifestations of primary hyperaldosteronism are hypertension and hypokalemia. Serum renin, as was mentioned previously, is low. Hypokalemia results from renal potassium wasting and can cause a variety of neuromuscular manifestations, including weakness, paresthesias, visual disturbances, and occasionally frank tetany. Sodium retention increases the total body sodium and

Figure 24-44 The major causes of primary hyperaldosteronism and its principal effects on the kidney.



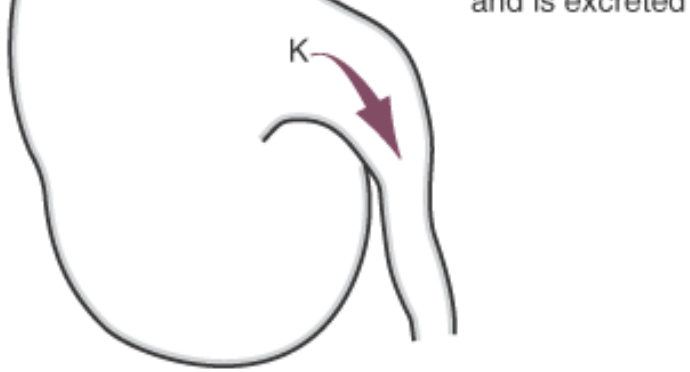


Figure 24-45 Adrenal cortical adenoma. The adenoma is distinguished from nodular hyperplasia by its solitary, circumscribed nature. The functional status of an adrenal cortical adenoma cannot be predicted from its gross or microscopic appearance.

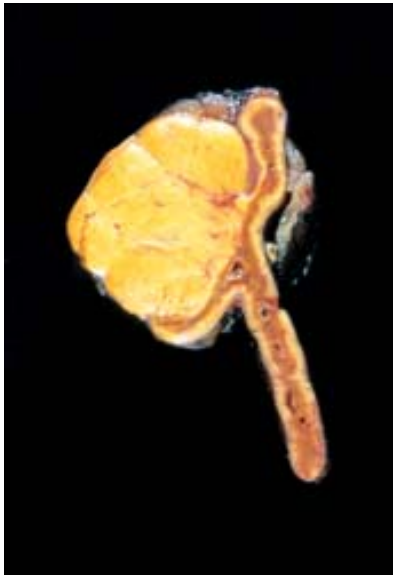


Figure 24-46 Histologic features of an adrenal cortical adenoma. The neoplastic cells are vacuolated because of the presence of intracytoplasmic lipid. There is mild nuclear pleomorphism. Mitotic activity and necrosis are not seen.

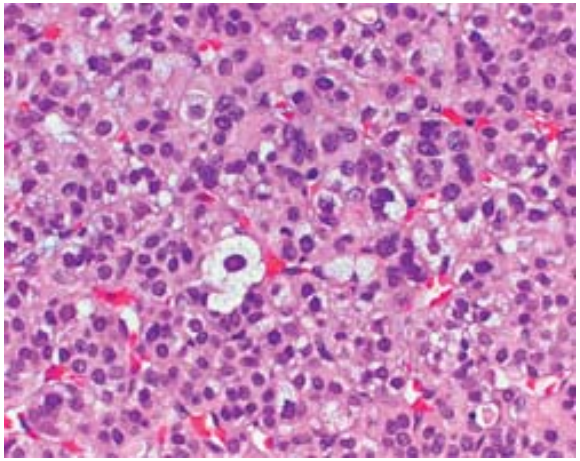


Figure 24-47 Nodular hyperplasia of the adrenal contrasted with normal adrenal gland. In cross-section, the adrenal cortex is yellow, thickened, and multinodular, owing to hypertrophy and hyperplasia of the lipid-rich zonae fasciculata and reticularis.

Figure 24-48 Consequences of C-21 hydroxylase deficiency. 21-Hydroxylase deficiency impairs the synthesis of both cortisol and aldosterone. The resultant decrease in feedback inhibition *(dashed line)* causes increased secretion of adrenocorticotropic hormone, resulting ultimately in adrenal hyperplasia and increased synthesis of testosterone. The sites of action of 11-, 17-, and 21-hydroxylase are shown by the numbers in circles.

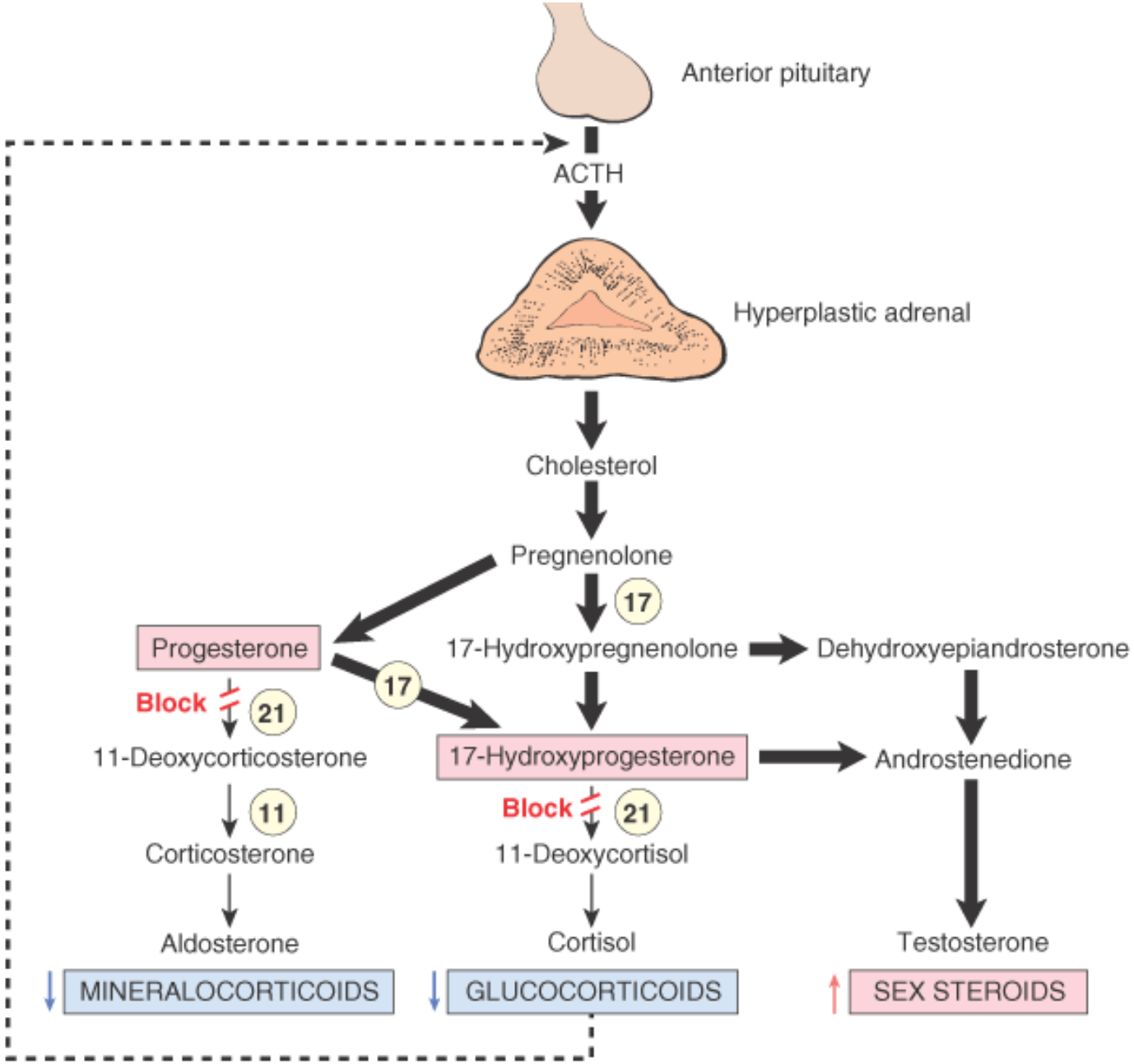


TABLE 24-10 -- Adrenocortical Insufficiency

Primary Insufficiency
Loss of cortex
••Congential adrenal *hypo*plasia
••••X-linked adrenal hypoplasia (*DAX-1* gene on Xp21)
••••"Miniature" type adrenal hypoplasia (unknown cause)
••Adrenoleukodystrophy (*ALD* gene on Xq28)
••Autoimmune adrenal insufficiency
••••Autoimmune polyendocrinopathy syndrome type 1 (*AIRE-1* gene on 21q22)
••••Autoimmune polyendocrinopathy syndrome type 2 (polygenic)
••••Isolated autoimmune adrenalitis (polygenic)
••Infection
••••Acquired immune deficiency syndrome
••••Tuberculosis
••••Fungi
••••Acute hemorrhagic necrosis (*Waterhouse-Friderichsen syndrome)*
••Amyloidosis, sarcoidosis, hemochromatosis
••Metastatic carcinoma
Metabolic failure in hormone production
••Congenital adrenal *hyper*plasia (cortisol and aldosterone deficiency with virlization)
••Drug- and steroid-induced inhibition of adrenocorticotropic hormone or cortical cell function
Secondary Insufficiency
Hypothalamic pituitary disease
••Neoplasm, inflammation (sarcoidosis, tuberculosis, pyogens, fungi)
Hypothalamic pituitary suppression
••Long-term steroid administration
••Steroid-producing neoplasms

Waterhouse-Friderichsen Syndrome

This uncommon but catastrophic syndrome is characterized by the following:

• An overwhelming bacterial infection, which is classically associated with *Neisseria meningitidis* septicemia but occasionally is caused by other highly virulent organisms, such as *Pseudomonas* species, pneumococci, *Haemophilus influenzae*, or staphylococci
• Rapidly progressive hypotension leading to shock
• Disseminated intravascular coagulation with widespread purpura, particularly of the skin

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• Rapidly developing adrenocortical insufficiency associated with massive bilateral adrenal hemorrhage

Figure 24-49 Waterhouse-Friderichsen syndrome in a child. The dark, hemorrhagic adrenal glands are distended with blood.

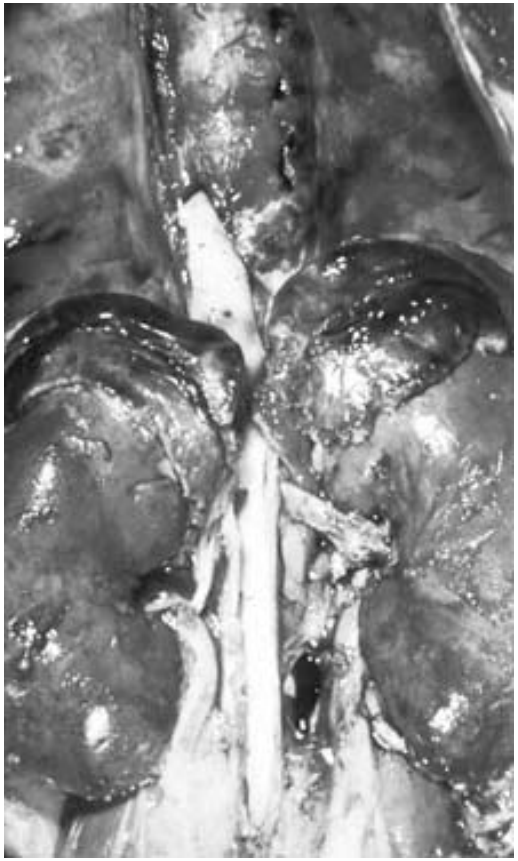

Figure 24-50 Waterhouse-Friderichsen syndrome. At autopsy, the adrenals were grossly hemorrhagic and shrunken; microscopically, little residual cortical architecture is discernible.

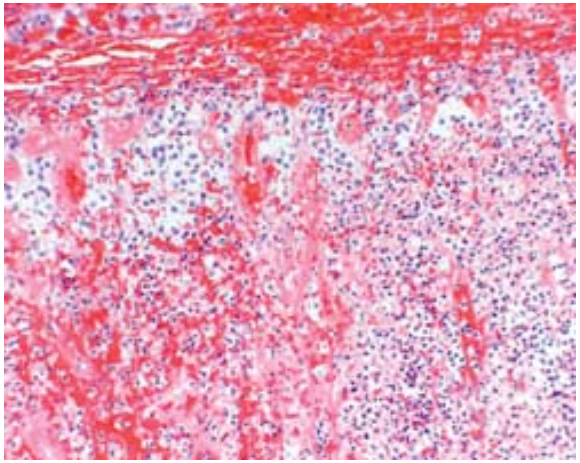

Figure 24-51 Autoimmune adrenalitis. In addition to loss of all but a subcapsular rim of cortical cells, there is an extensive mononuclear cell infiltrate.

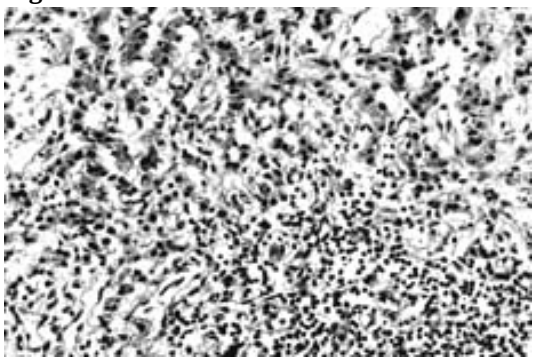

Figure 24-52 Adrenal carcinoma. The hemorrhagic and necrotic tumor dwarfs the kidney and compresses the upper pole.

Figure 24-53 Adrenal carcinoma *(A)* revealing marked anaplasia, contrasted with normal cortical cells (*B*).

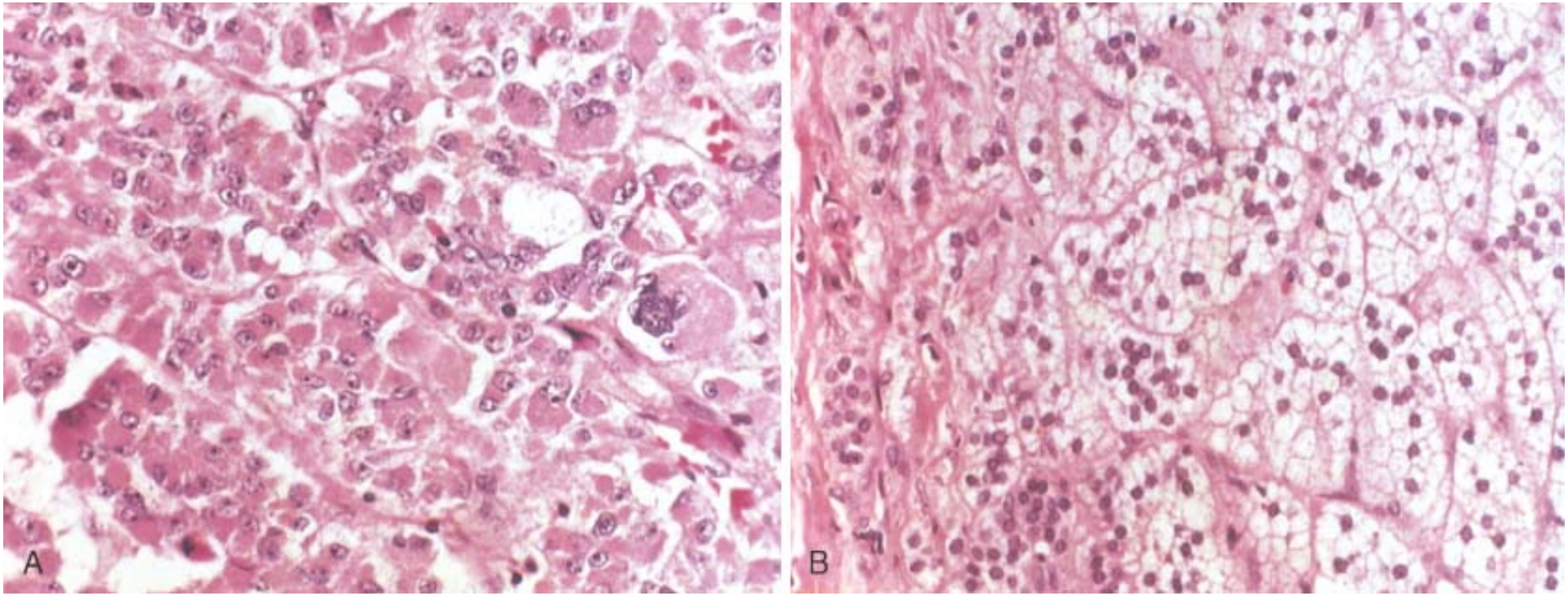

Figure 24-54 The paraganglion system. This schematic representation of the paraganglion system demonstrates sites of paraganglion cell nests, in which neoplasms may form. The extra-adrenal portion of the paraganglion system is grouped into three families based on anatomic distribution, innervation, and microscopic structure: (1) branchiomeric, (2) intravagal, and (3) aorticosympathetic. *(From Whalen RK, et al: Extra-adrenal pheochromocytoma. J Urol 147:1–10, 1992; copyright Williams & Wilkins, 1992.)*

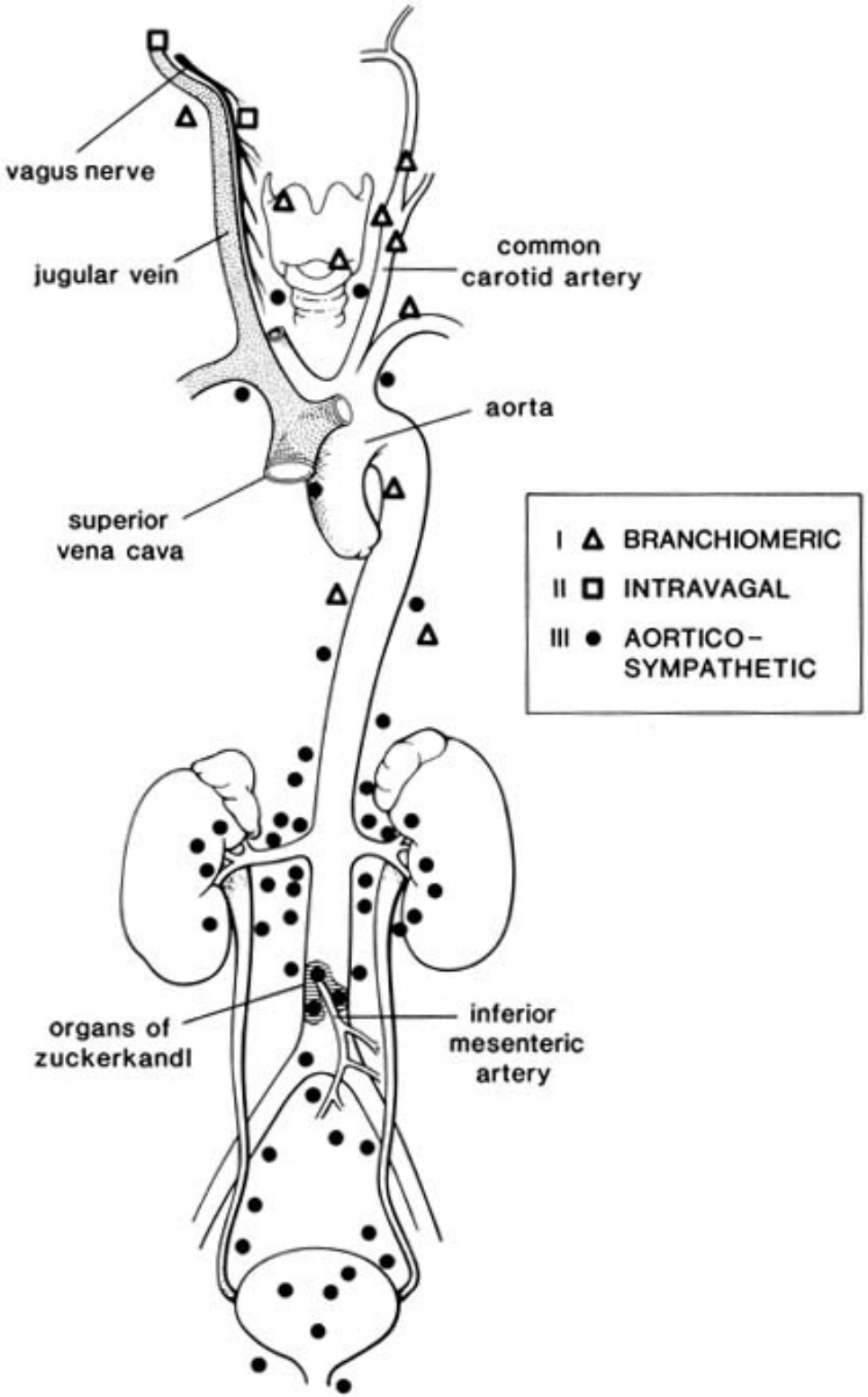
vagus nerve
jugular vein
common
carotid artery
aorta
superior
vena cava
I BRANCHIOMERIC
II INTRAVAGAL
III AORTICO-
SYMPATHETIC
organs of
zuckerkandl
inferior
mesenteric
artery

TABLE 24-11 -- Familial Syndromes Associated with Pheochromocytoma

Syndrome	Components
MEN, type 2A	Medullary thyroid carcinomas and C-cell hyperplasia
	Pheochromocytomas and adrenal medullary hyperplasia
	Parathyroid hyperplasia
MEN, type 2B	Medullary thyroid carcinomas and C-cell hyperplasia
	Pheochromocytomas and adrenal medullary hyperplasia
	Mucosal neuromas
	Marfanoid features
von Hippel-Lindau	Renal, hepatic, pancreatic, and epididymal cysts
	Renal cell carcinomas
	Pheochromocytomas
	Angiomatosis
	Cerebellar hemangioblastomas
von Recklinghausen	Neurofibromatosis
	Café au lait skin spots
	Schwannomas, meningiomas, gliomas
	Pheochromocytomas
Sturge-Weber	Cavernous hemangiomas of fifth cranial nerve distribution
	Pheochromocytomas
MEN, multiple endocrine neoplasia.	
Data from Silverman ML, Lee AK: Anatomy and pathology of the adrenal glands. Urol Clin North Am 16:417, 1989.	

Morphology.

Pheochromocytomas range from small, circumscribed lesions confined to the adrenal (Fig. 24-55) to large hemorrhagic masses weighing kilograms. The average weight of a pheochromocytoma is 100 gm, but variations from just over 1 gm to almost 4000 gm have been reported. The larger tumors are well demarcated by either connective tissue or compressed cortical or medullary tissue. Richly vascularized fibrous trabeculae pass into the tumor and produce a lobular pattern. In many tumors, remnants of the adrenal gland can be seen, stretched over the surface or attached at one pole. On section, the cut surfaces of smaller pheochromocytomas are yellow-tan. Larger lesions tend to be hemorrhagic, necrotic, and cystic and typically efface the adrenal gland. Incubation of fresh tissue with a potassium dichromate solution turns the tumor a dark brown color owing to oxidation of stored catecholamines, thus the term *chromaffin*.

The histologic pattern in pheochromocytoma is quite variable. The tumors are composed of polygonal to spindle-shaped chromaffin cells or chief cells, clustered with the sustentacular cells into small nests or alveoli (**zellballen**) by a rich vascular network (Fig. 24-56). Uncommonly, the dominant cell type is a spindle or small cell; various patterns can be found in any one tumor. The cytoplasm has a finely granular appearance, best demonstrated with silver stains, owing to the appearance of granules containing catecholamines. The nuclei are usually round to ovoid, with a stippled "salt and pepper" chromatin that is characteristic of most neuroendocrine tumors. Electron microscopy reveals variable numbers of membrane-bound, electron-dense granules, representing

Figure 24-55 Pheochromocytoma. The tumor is enclosed within an attenuated cortex and demonstrates areas of hemorrhage. The comma-shaped residual adrenal is seen below.

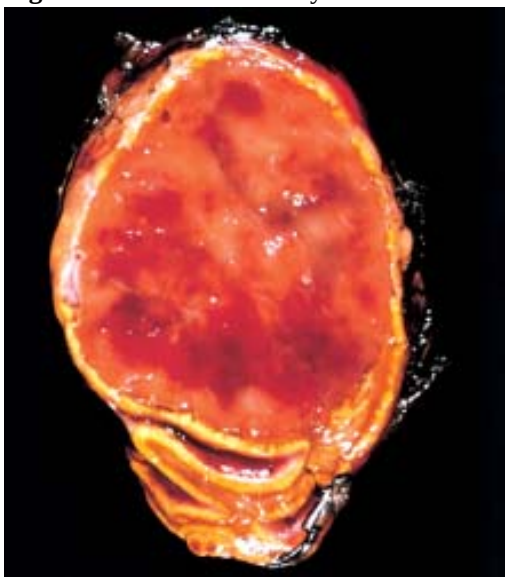

Figure 24-56 Pheochromocytoma demonstrating characteristic nests of cells ("zellballen") with abundant cytoplasm. Granules containing catecholamine are not visible in this preparation. It is not uncommon to find bizarre cells even in pheochromocytomas that are biologically benign, and this criterion by itself should not be used to diagnose malignancy.

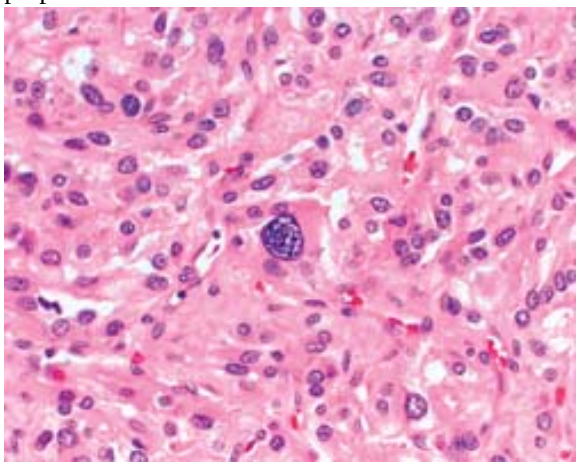

Figure 24-57 Electron micrograph of pheochromocytoma. This tumor contains membrane-bound secretory granules in which catecholamines are stored (30,000X).

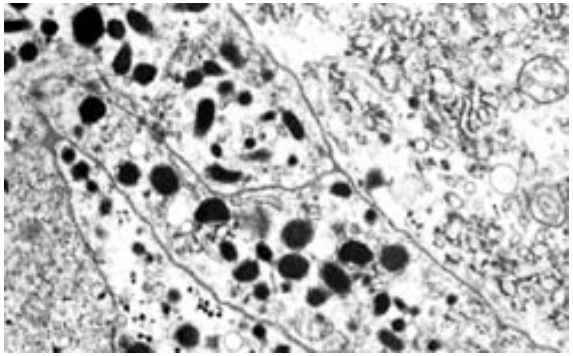

TABLE 24-12 -- Multiple Endocrine Neoplasia (MEN) Syndromes

	MEN-1	MEN-2A	MEN-2B
Pituitary	Adenomas		
Parathyroid	Hyperplasia +++	Hyperplasia +	
	Adenomas +		
Pancreatic islets	Hyperplasia ++		
	Adenomas ++		
	Carcinomas +++		
Adrenal	Cortical hyperplasia	Pheochromocytoma ++	Pheochromocytoma +++
Thyroid		C-cell hyperplasia +++	C-cell hyperplasia +++
		Medullary carcinoma +++	Medullary carcinoma +++
Extraendocrine changes			Mucocutaneous ganglioneuromas
			Marfanoid habitus
Mutant gene locus	*MEN1*	*RET*	*RET*
Relative frequency: +, uncommon; +++, common.			

MEN-1 syndrome is caused by germ-line mutations in the *MEN1* gene at 11q13. This gene encodes a 610-amino acid product known as menin, which localizes primarily to the nucleus. *MEN1* is a classic tumor suppressor gene (Chapter 7) in that both alleles are inactivated in the MEN-1-associated tumors.[131] The precise role of menin in tumor suppression remains elusive, although recent studies have shown that it may be important in regulating the cell cycle and transcription. [132]

The dominant clinical manifestations of MEN-1 are usually defined by the peptide hormones that are overproduced and include such abnormalities as recurrent hypoglycemia due to insulinomas, intractable peptic ulcers in patients with Zollinger-Ellison syndrome, nephrolithiasis caused by PTH-induced hypercalcemia, or symptoms of prolactin excess from a pituitary tumor. As expected, malignant behavior by one or more of the endocrine tumors arising in these patients is often the proximate cause of death.

MULTIPLE ENDOCRINE NEOPLASIA, TYPE 2

MEN-2 is subclassified into three distinct syndromes: MEN-2A, MEN-2B, and familial medullary thyroid cancer.

• *MEN-2A*, or *Sipple syndrome*, is characterized by *pheochromocytoma*, *medullary carcinoma*, and *parathyroid hyperplasia*. Medullary carcinomas of the thyroid occur in almost 100% of patients. They are usually multifocal and are virtually always associated with foci of C-cell hyperplasia in the adjacent thyroid. The medullary carcinomas may elaborate calcitonin and other active products and are usually clinically aggressive. Forty per cent to 50% of patients with MEN-2A have pheochromocytomas, which are often bilateral and may arise in extra-adrenal sites. As in the case of pheochromocytomas in general, they may be benign or malignant. Ten per cent to 20% of patients have parathyroid hyperplasia and evidence of hypercalcemia or renal stones. MEN-2A is clinically and genetically distinct from MEN-1 and has been linked to germ-line mutations in the *RET* (rearranged during transfection) protooncogene on chromosome 10q11.2. As was noted earlier, the *RET* protooncogene is a receptor tyrosine kinase that binds *glialderived neurotrophic factor* (GDNF) and other ligands in the GDNF family and transmits growth and differentiation signals (Chapter 7). *Loss of function* mutations in *RET* result in intestinal aganglionosis and Hirschsprung disease (Chapter 17). In contrast, in MEN-2A (as well as in MEN-2B), germ-line mutations constitutively activate the *RET* receptor, resulting in *gain of function*.[133] This scenario is different from most other inherited predispositions to neoplasia, which are due to heritable loss of function mutations that inactivate tumor-suppressor proteins (Chapter 7).

• *MEN-2B* has significant clinical overlap with MEN-2A. Patients develop medullary thyroid carcinomas, which are usually multifocal and more aggressive than in MEN-2A, and pheochromocytomas. However, unlike in MEN-2A, primary hyperparathyroidism is not present. In addition, MEN-2B is accompanied by *neuromas* or ganglioneuromas involving the skin, oral mucosa, eyes, respiratory tract, and gastrointestinal tract, and a *marfanoid habitus*, with long axial skeletal features and hyperextensible joints. A single amino acid change in *RET* (*RET*^{Met918Thr}), distinct from the

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mutational spectra that are seen in MEN-2A, appears to be responsible for virtually all cases of MEN-2B and affects a critical region of the tyrosine kinase catalytic domain of the protein.[134]

• *Familial medullary thyroid cancer* is a variant of MEN-2A, in which there is a strong predisposition to medullary thyroid cancer but not the other clinical manifestations of MEN-2A or MEN-2B. A substantial majority of cases of medullary thyroid cancer are sporadic, but as many as 20% may be familial. Familial medullary thyroid cancers develop at an older age than those occurring in the full-blown MEN-2 syndrome and follow a more indolent course.

In contrast to MEN-1, in which the long-term benefit of early diagnosis via genetic screening is not well established, diagnosis via screening of at-risk family members in MEN-2A kindred is important because medullary thyroid carcinoma is a life-threatening disease that can be prevented by early thyroidectomy. Prior to the advent of genetic testing, family members of patients with the MEN-2 syndrome were screened with annual biochemical tests, which often lacked sensitivity. Now, routine genetic testing identifies *RET* mutation carriers earlier and more reliably in MEN-2 kindred; *all individuals carrying germ-line RET mutations are advised to undergo prophylactic thyroidectomy to prevent the inevitable development of medullary carcinomas.*

Pineal Gland

Normal

The rarity of clinically significant lesions (virtually only tumors) justifies brevity in the consideration of the pineal gland. It is a minute, pinecone-shaped organ (hence its name), weighing 100 to 180 mg and lying between the superior colliculi at the base of the brain. It is composed of a loose, neuroglial stroma enclosing nests of epithelial-appearing *pineocytes*, cells with photosensory and neuroendocrine functions (hence the designation of the pineal gland as the "third eye"). Silver impregnation stains reveal that these cells have long, slender processes reminiscent of primitive neuronal precursors intermixed with the processes of astrocytic cells.

Pathology

All tumors involving the pineal are rare; most (50% to 70%) arise from sequestered embryonic germ cells. They most commonly take the form of so-called *germinomas*, resembling testicular seminoma (Chapter 21) or ovarian dysgerminoma (Chapter 22). Other lines of germ cell differentiation include embryonal carcinomas; choriocarcinomas; mixtures of germinoma, embryonal carcinoma, and choriocarcinoma; and, uncommonly, typical teratomas (usually benign). Whether to characterize these germ cell neoplasms as pinealomas is still a subject of debate, but most "pinealophiles" favor restricting the term *pinealoma* to neoplasma arising from the pineocytes.

PINEALOMAS

These neoplasms are divided into two categories, pineoblastomas and pineocytomas, based on their level of differentiation, which, in turn, correlates with their neoplastic aggressiveness. [135]

Morphology.

Pineoblastomas are encountered mostly in the first two decades of life and appear as soft, friable, gray masses punctuated with areas of hemorrhage and necrosis. They typically invade surrounding structures, such as the hypothalamus, midbrain, and lumen of the third ventricle. Histologically, they are composed of masses of pleomorphic cells two to four times the diameter of an erythrocyte. Large hyperchromatic nuclei appear to occupy almost the entire cell, and mitoses are frequent. The cytology is that of **primitive embryonal tumor** ("small blue cell neoplasm") similar to medulloblastoma (Chapter 28) or retinoblastoma (Chapter 29).

Pineoblastomas, like medulloblastomas, tend to spread via the cerebrospinal fluid. As might be expected, the enlarging mass may compress the aqueduct of Sylvius, giving rise to internal hydrocephalus and all its consequences. Survival beyond 1 or 2 years is rare.

In contrast, **pineocytomas** occur mostly in adults and are much slower-growing than pineoblastomas. They tend to be well-circumscribed, gray, or hemorrhagic masses that compress but do not infiltrate surrounding structures. **Histologically, the tumors may be pure pineocytomas or exhibit divergent glial, neuronal, and retinal differentiation.** The tumors are composed largely of pineocytes having darkly staining, round-to-oval, fairly regular nuclei. Necrosis is unusual, and mitoses are virtually absent. The neoplastic cells resemble normal pineocytes in their strong immunoreactivity for neuro-specific enolase and synaptophysin. Particularly distinctive are the **pineocytomatous pseudorosettes** rimmed by rows of pineocytes. The centers of these rosettes are filled with eosinophilic cytoplasmic material representing tumor cell processes. These cells are set against a background of thin, fibrovascular, anastomosing septa, which confer a lobular growth pattern to the tumor. Glial and retinal differentiation is detectable by immunoreactivity for glial fibrillary acidic protein and retinal S-antigen, respectively.

The clinical course of patients with pineocytomas is prolonged, averaging 7 years. The manifestations are the consequence of their pressure effects and consist of visual disturbances, headache, mental deterioration, and sometimes dementia-like behavior. The lesions being located where they are, it is understandable that successful excision is at best difficult.

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Chapter 25 - The Skin

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Normal

The Skin: More Than a Mechanical Barrier

Little more than 100 years ago, the noted pathologist Rudolph Virchow understood the skin as a protective covering for more delicate and functionally sophisticated internal viscera.[1] Then, and for the century that followed, the skin was considered primarily a passive barrier to fluid loss and mechanical injury. During the past three decades, however, enormously productive avenues of scientific inquiry have demonstrated the skin to be a complex organ in which precisely

Figure 25-1 *A,* The skin is composed of an epidermal layer (e) from which specialized adnexa (hair follicles, h; sweat glands, g; and sebaceous glands, s) descend into the underlying dermis (d). *B,* This projection of the epidermal layer (e) and underlying superficial dermis demonstrates the progressive upward maturation of basal cells (b) into cornified squamous epithelial cells of the stratum corneum (sc). Melanin-containing dendritic melanocytes (m) (and rare Merkel cells containing neurosecretory granules) and midepidermal dendritic Langerhans cells (lc) are also present. The underlying dermis contains small vessels (v), fibroblasts (f), perivascular mast cells (mc), and dendrocytes (dc), potentially important in dermal immunity and repair.

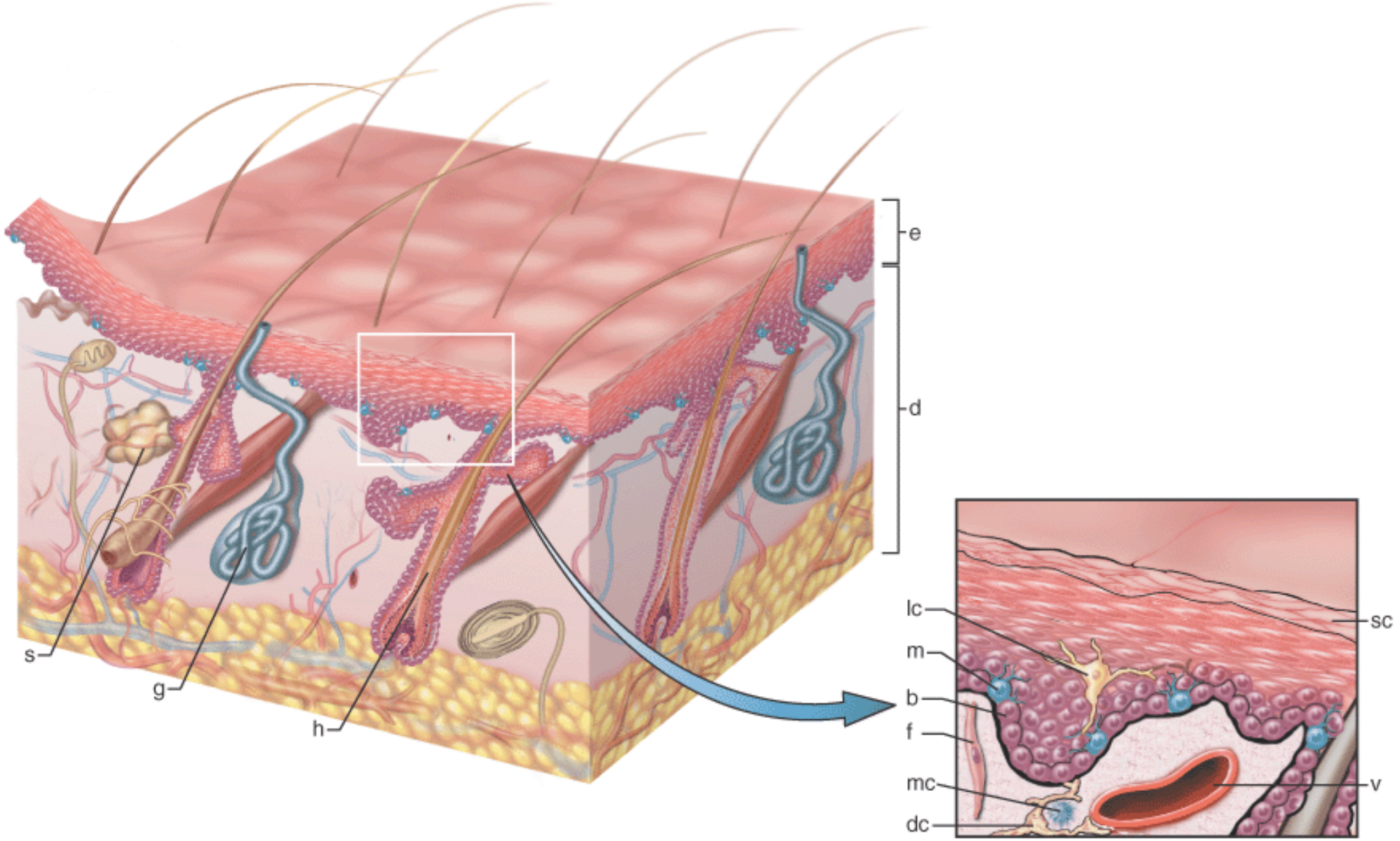
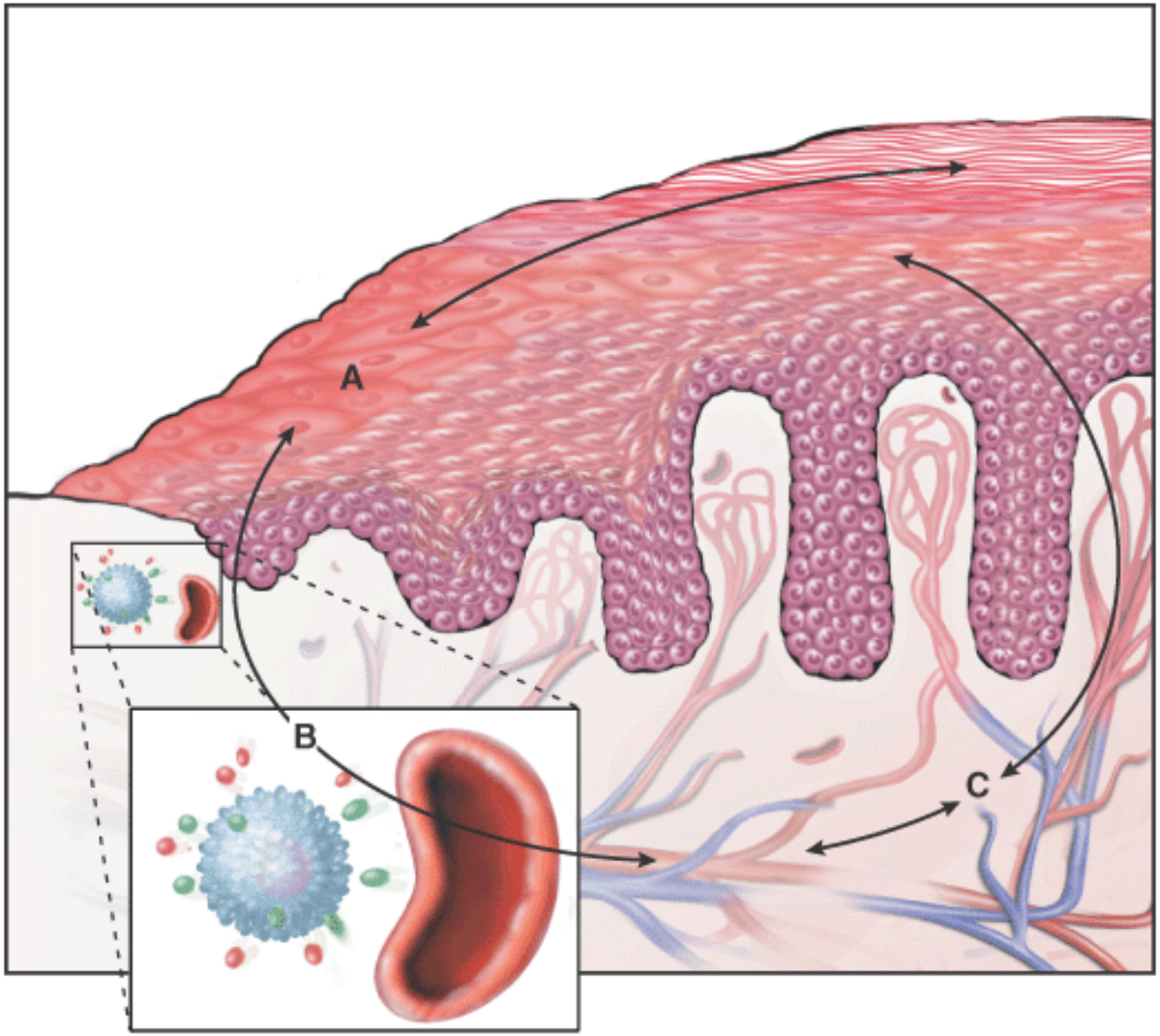


Figure 25-2 Schematic representation of dynamic interaction between the epidermal layer and the dermal layer. Keratinocytes at the edge of an ulcer *(A)* produce cytokines and factors that influence both keratinization and the function of underlying dermal cells *(B)*. In turn, dermal cells *(B)*, such as mast cells, also release cytokines *(green granules)* and proteases *(red granules)*, which may regulate both endothelial cells and overlying keratinocytes. Perturbations in these interactions between epidermal cells and dermal cells may contribute to pathologic processes, such as psoriasis *(C)*, in which both compartments become morphologically abnormal.



Scale
Dry, horny, platelike excrescence; usually the result of imperfect cornification.

Lichenification
Thickened and rough skin characterized by prominent skin markings; usually the result of repeated rubbing in susceptible persons.

Excoriation
Traumatic lesion characterized by breakage of the epidermis, causing a raw linear area (i.e., a deep scratch); often self-induced.

Onycholysis
Separation of nail plate from nail bed.

DEFINITIONS OF MICROSCOPIC TERMS

Hyperkeratosis
Thickening of the stratum corneum, often associated with a qualitative abnormality of the keratin.

Parakeratosis
Modes of keratinization characterized by the retention of the nuclei in the stratum corneum. On mucous membranes, parakeratosis is normal.

Hypergranulosis
Hyperplasia of the stratum granulosum, often due to intense rubbing.

Acanthosis
Diffuse epidermal hyperplasia.

Papillomatosis
Surface elevation caused by hyperplasia and enlargement of contiguous dermal papillae.

Dyskeratosis
Abnormal keratinization occurring prematurely within individual cells or groups of cells below the stratum granulosum.

Acantholysis
Loss of intercellular connections resulting in loss of cohesion between keratinocytes.

Spongiosis
Intercellular edema of the epidermis.

Hydropic swelling (ballooning)
Intracellular edema of keratinocytes, often seen in viral infections.

Exocytosis
Infiltration of the epidermis by inflammatory or circulating blood cells.

Erosion
Discontinuity of the skin exhibiting incomplete loss of the epidermis.

Ulceration
Discontinuity of the skin exhibiting complete loss of the epidermis and often of portions of the dermis and even subcutaneous fat.

Vacuolization
Formation of vacuoles within or adjacent to cells; often refers to basal cell-basement membrane zone area.

Lentiginous
Referring to a linear pattern of melanocyte proliferation within the epidermal basal cell layer. Lentiginous melanocytic hyperplasia can occur as a reactive change or as part of a neoplasm of melanocytes.

Disorders of Pigmentation and Melanocytes

Skin pigmentation has historically had major societal implications. Cosmetic desire for increased pigmentation (tanning) has resulted in many deleterious alterations that are described in the pages that follow. Focal or widespread loss of normal protective pigmentation not only renders individuals extraordinarily vulnerable to the harmful effects of sunlight (as in albinism), but has also resulted in severe emotional stress and, in some cultures, profound social and economic discrimination (as in vitiligo). Change in preexisting skin pigmentation may signify important primary events in the skin (e.g., malignant transformation of a mole) or disorders of internal viscera (e.g., in Addison disease, see Chapter 24).

VITILIGO

Vitiligo is a common disorder characterized by partial or complete loss of pigment-producing melanocytes within the epidermis. All ages and races are affected, but lesions are most noticeable in darkly pigmented individuals. Vitiligo may be entirely unapparent in lightly pigmented skin until tanning occurs in the surrounding normal skin. In darkly pigmented individuals with extensive involvement, residual zones of normal skin may at first appear to represent hyperpigmented lesions (Fig. 25-3*A*).

Clinical lesions are asymptomatic, flat, well-demarcated macules and patches of pigment loss; their size varies from few to many centimeters. Vitiligo often involves the hands and wrists; axillae; and perioral, periorbital, and anogenital skin. A curious phenomenon called *koebnerization* often occurs in vitiligo (as well as in certain other conditions; see lichen planus), where lesions develop primarily at sites of repeated trauma.

Morphology.

On histologic examination, vitiligo usually appears indistinguishable from normal skin. However, it is characterized by loss of melanocytes, as revealed by electron microscopy; it also may be diagnosed by immunohistochemistry for melanocyte-associated proteins (e.g., tyrosinase or Melan-A, or S-100; Fig. 25-3*B*). This is in contrast to some forms of **albinism,** in which melanocytes are present but melanin pigment is not produced because of a lack of or defect in tyrosinase. There are other causes of hypopigmentation that are unrelated to diminished expression of melanin or melanocytes (e.g., post-inflammatory hypopigmentation, which represents redistribution of existing pigment within skin possibly coupled with diminished transfer of pigment to keratinocytes).

Pathogenesis.

Why are melanocytes progressively lost or destroyed in vitiligo? Theories of pathogenesis include (1) autoimmunity, (2) neurohumoral factors toxic to melanocytes and released by nearby nerve endings, and (3) self-destruction of melanocytes by toxic intermediates of melanin synthesis. Most evidence supports autoimmune causation, focusing on the presence of circulating antibodies against melanocytes[7] and the association of vitiligo with other autoimmune disorders, such as pernicious anemia, Addison disease, and autoimmune thyroiditis. Abnormalities in macrophages,[8] and in T lymphocytes in skin[9] and in the peripheral blood have been described recently, suggesting that aberrations in cell-mediated immunity may also be operative in the pathogenesis of vitiligo. Another interesting facet of vitiligo is its response to therapy with UV light of the A wavelength coupled with use of the photosensitizing drug, psoralen (a therapy known as *PUVA*). Lesions so treated may regain pigment initially at the ostia of hair follicles, suggesting that melanocyte precursors harbored within the uppermost follicular epithelium are stimulated by this therapeutic approach.

Figure 25-3 *A*, Clinical appearance of vitiligo. Well-demarcated zones of pigment loss result from depletion of melanocytes that produce small melanin granules. Note small macules of normal pigment within the patches of vitiligo; Some of these appear to surround follicular ostia. *B*, Immunochemistry for S-100 protein revealing positively stained melanocytes within the basal cell layer of the epidermis; these cells are decreased or absent in vitiligo.

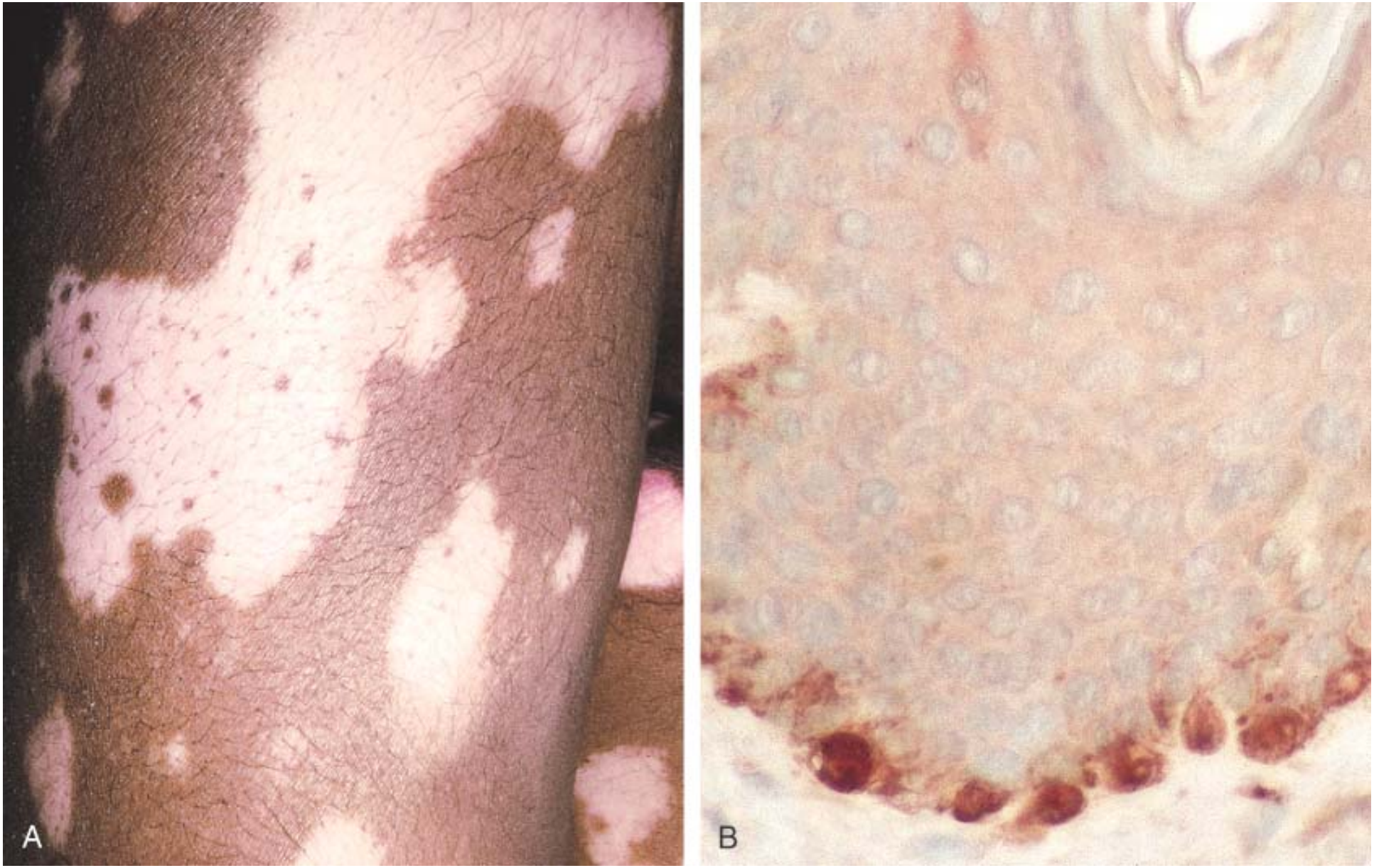


Figure 25-4 Nevocellular nevus, junctional type. *A*, In clinical appearance, lesions are small, relatively flat, symmetric, and uniform. *B*, On histologic examination, junctional nevi are characterized by rounded nests of nevus cells originating at the tips of rete ridges along the dermoepidermal junction.

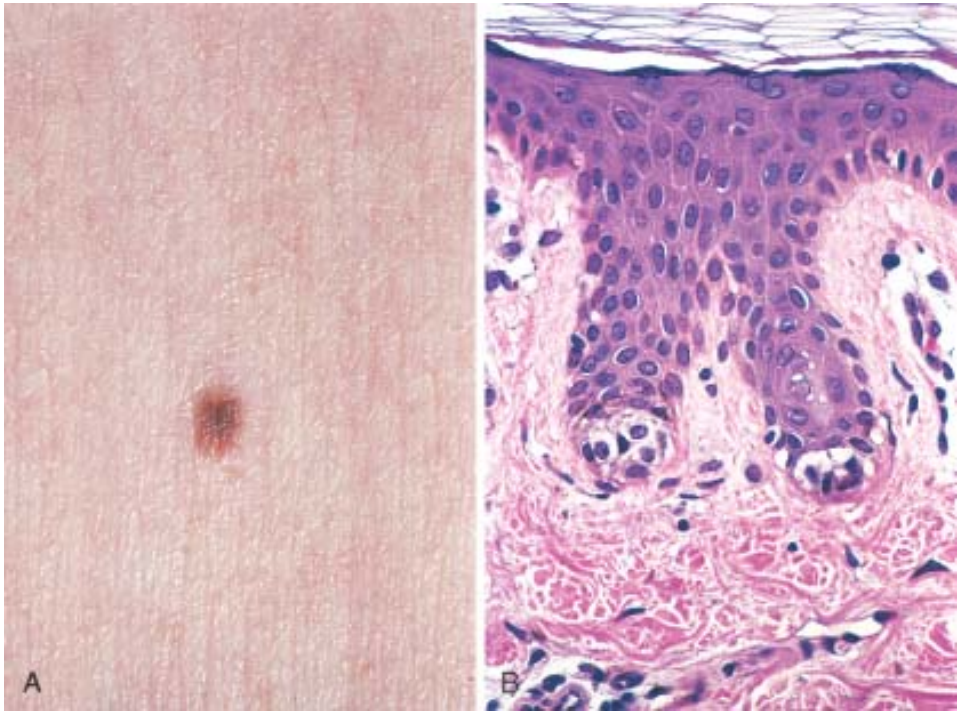


Figure 25-5 Nevocellular nevus, compound type. In contrast to the junctional nevus, the compound nevus *(A)* is more raised and dome shaped. The symmetry and uniform pigment distribution suggest a benign process. Histologically *(B)*, compound nevi combine the features of junctional nevi (intraepidermal nevus cell nests) with nests and cords of nevus cells in the underlying dermis.

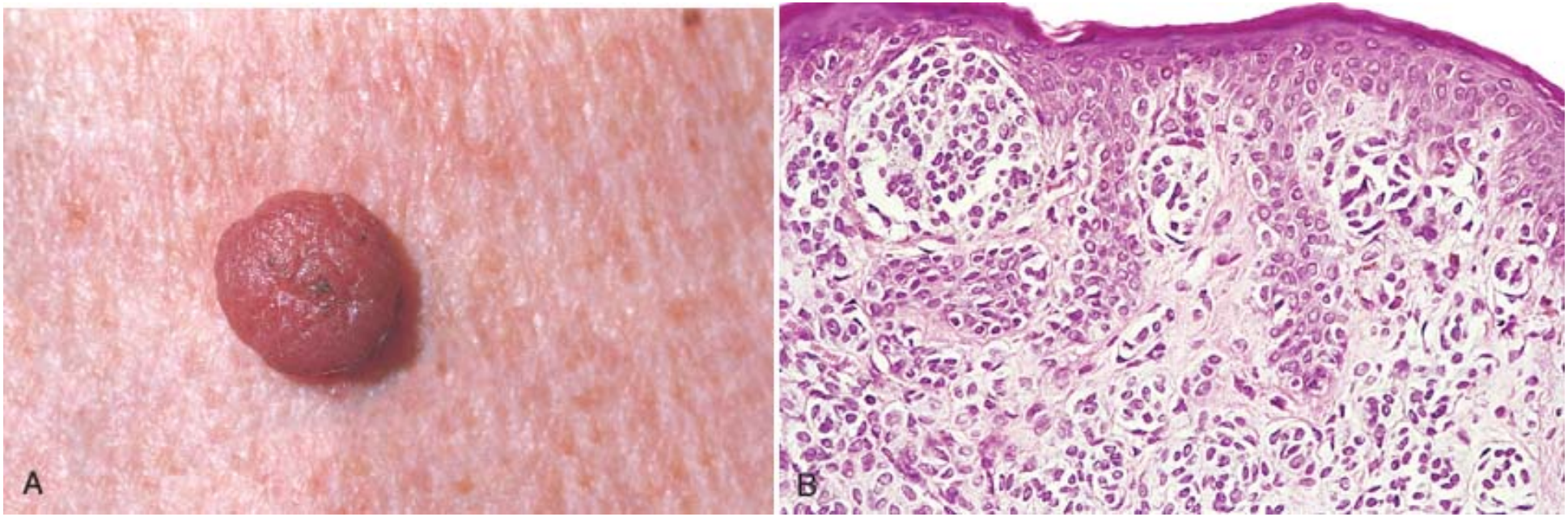


TABLE 25-1 -- Variant Forms of Nevocellular Nevi

Nevus Variant	Diagnostic Architectural Features	Diagnostic Cytologic Features	Clinical Significance
Congenital nevus	Deep dermal and sometimes subcutaneous growth around adnexa, neurovascular bundles, and blood vessel walls	Identical to ordinary acquired nevi	Present at birth; large variants have increased melanoma risk
Blue nevus	Non-nested dermal infiltration, often with associated fibrosis	Highly dendritic, heavily pigmented nevus cells	Black-blue nodule; often confused with melanoma clinically
Spindle and epithelioid cell nevus (Spitz nevus)	Fascicular growth	Large, plump cells with pink-blue cytoplasm; fusiform cells	Common in children; red-pink nodule; often confused with hemangioma clinically
Halo nevus	Lymphocytic infiltration surrounding nevus cells	Identical to ordinary acquired nevi	Host immune response against nevus cells and surrounding normal melanocytes
Dysplastic nevus	Large, coalescent intraepidermal nests	Cytologic atypia	Potential precursor of malignant melanoma

well as on sun-exposed body surfaces. Dysplastic nevi have been documented in multiple members of families prone to the development of malignant melanoma *(the heritable melanoma syndrome)*.[12] In these cases, genetic analyses have demonstrated the trait to be inherited as an autosomal dominant.[13] [14] (See discussion under Molecular Genetics of Skin Cancers.) Transitions from these lesions to early melanoma have actually been documented clinically and histologically within a period as short as several weeks. However, most dysplastic nevi are clinically stable lesions. Dysplastic nevi may also occur as isolated lesions not associated with the heritable melanoma syndrome, in which case the risk of malignant change appears to be low.

Morphology.

On histologic examination (see Fig. 25-6*A,B*), dysplastic nevi consist of compound nevi with both architectural and cytologic evidence of abnormal growth. **Nevus cell nests within the epidermis may be enlarged and exhibit abnormal fusion or coalescence with adjacent nests. As part of this**

Figure 25-6 Dysplastic nevus. *A*, The lesion often has a compound nevus component (right side of scanning field) and an asymmetric "shoulder" composed of a junctional nevus component (left side of scanning field). The former correlates clinically with the more pigmented and raised central zone and the latter with the less pigmented, flat peripheral rim *(inset)*. *B*, An important feature is the presence of cytologic atypia (irregularly shaped, dark-staining nuclei) at high magnification. The dermis underlying the atypical cells characteristically shows linear, or lamellar, fibrosis.

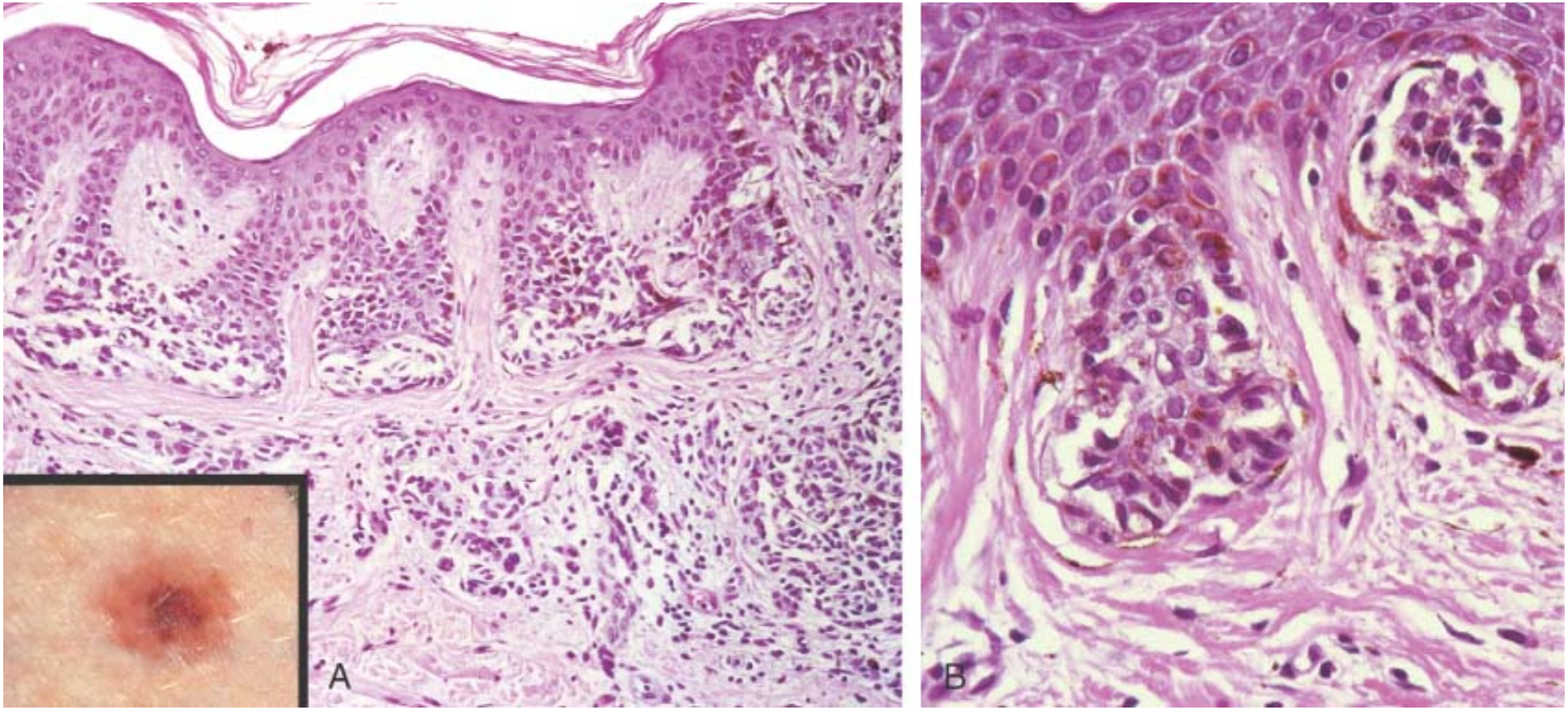


Figure 25-7 Steps of tumor progression in dysplastic nevi. *A*, Lentiginous melanocytic hyperplasia. *B*, Lentiginous junctional nevus. *C*, Lentiginous compound nevus with abnormal architectural and cytologic features (dysplastic nevus). *D*, Early melanoma, or radial growth phase melanoma (large dark cells in epidermis). *E*, Advanced melanoma (vertical growth phase) with malignant spread into the dermis and vessels.

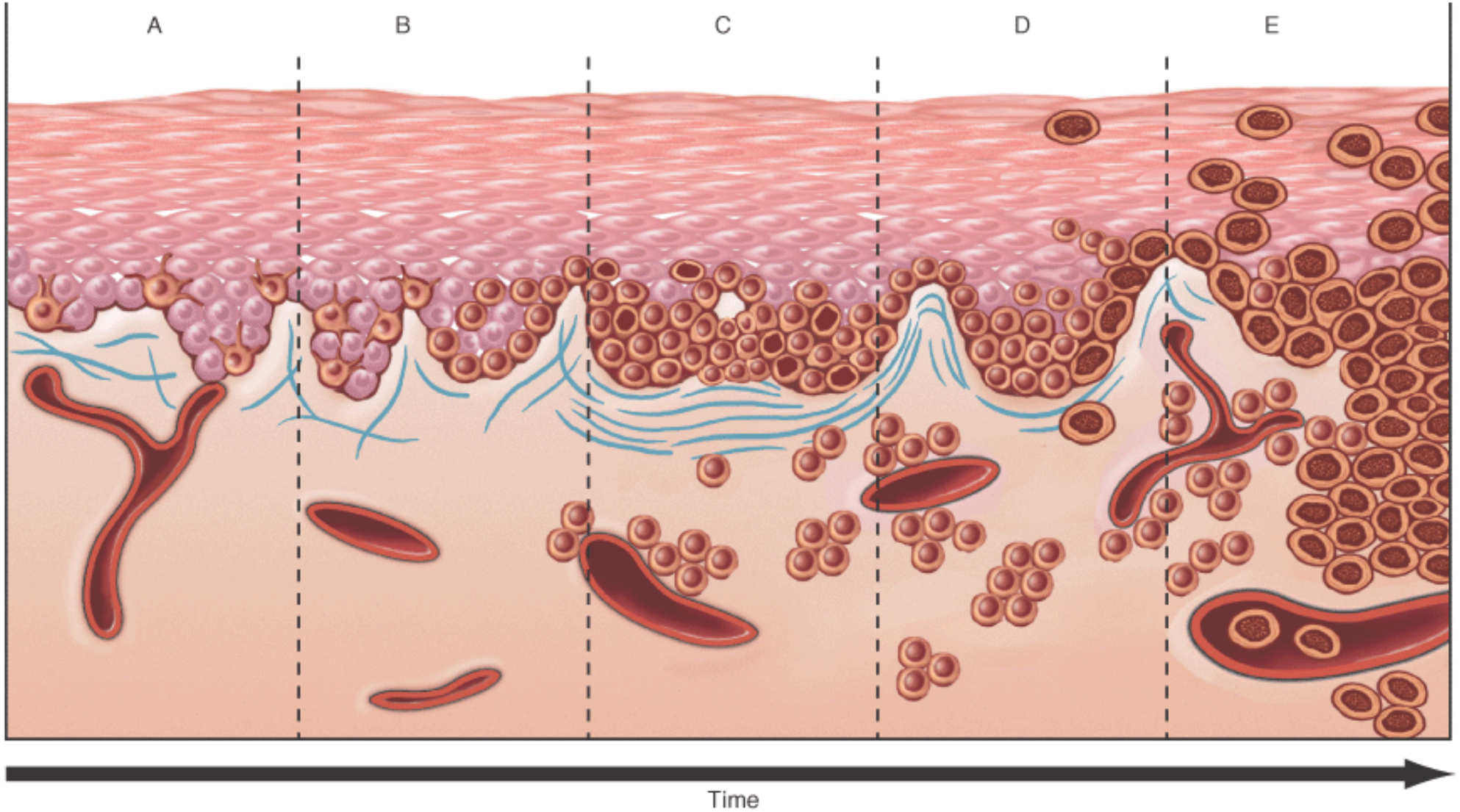


Figure 25-8 Malignant melanoma. *A*, In clinical appearance, lesions are irregular in contour and pigmentation. Macular areas correlate with the radial growth phase, while raised areas usually correspond to nodular aggregates of malignant cells in the vertical phase of growth. *B*, Radial growth phase of malignant melanoma, showing irregular nested and single-cell growth of melanoma cells within the epidermis and an underlying inflammatory response within the dermis. *C*, Photomicrograph of lesion in the vertical phase of growth, demonstrating nodular aggregates of infiltrating cells. *D*, High-power view of malignant melanoma cells.

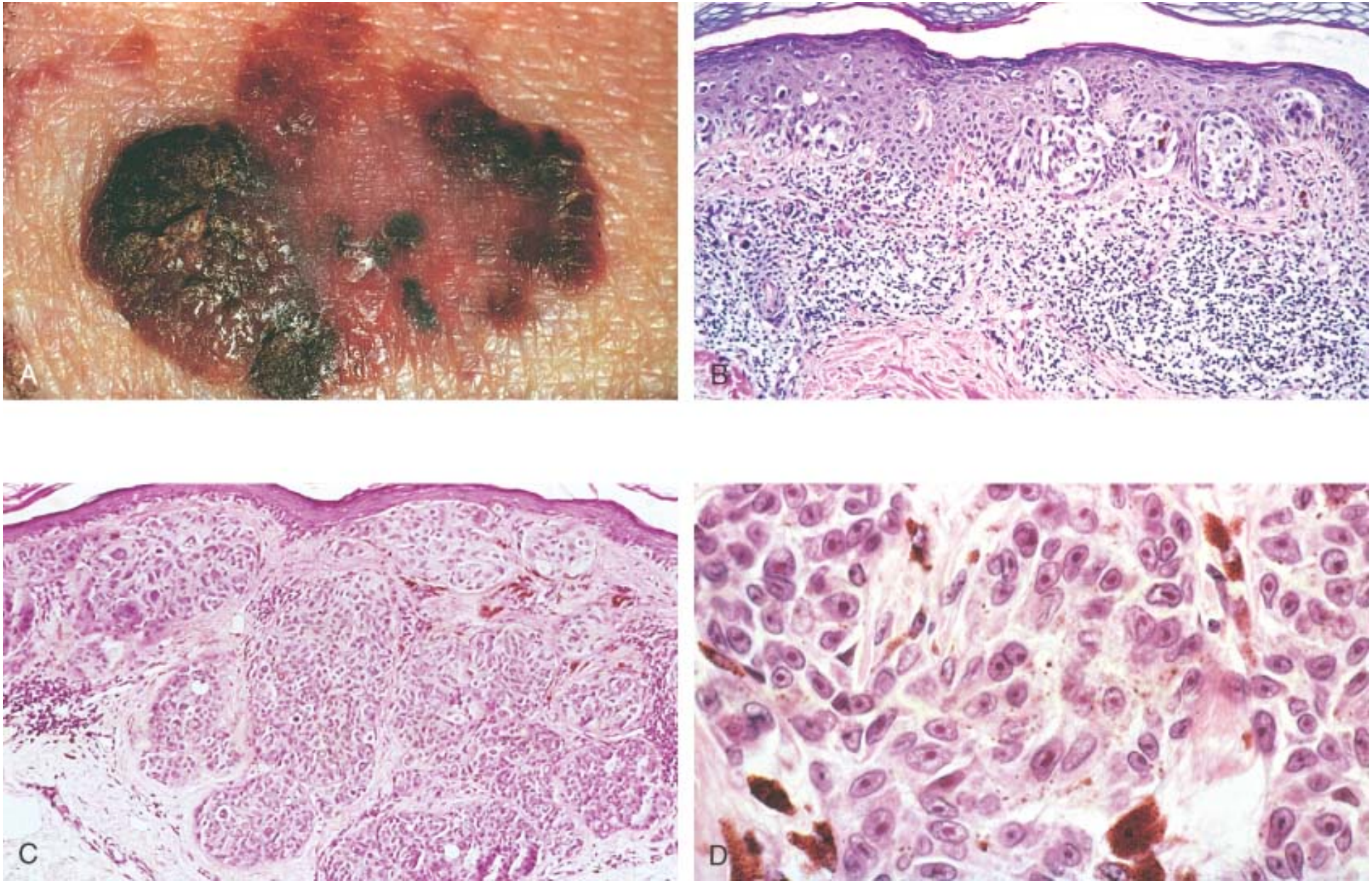


Figure 25-9 Seborrheic keratosis. *A,* A well-demarcated coinlike pigmented lesion containing dark keratin-filled surface plugs is composed histologically of proliferations of basaloid cells with formation of prominent keratin-filled "horn" cysts *(B),* some of which communicate with the surface (pseudo-horn cysts) and correlate with the plugs observed clinically.

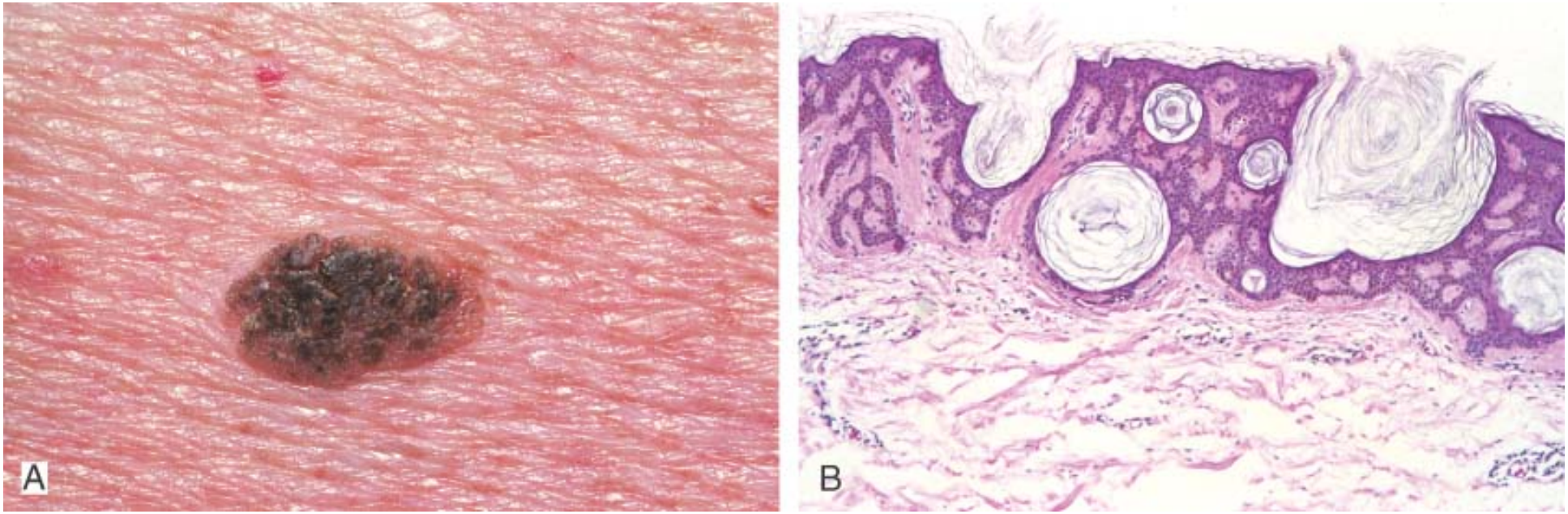


Figure 25-10 Adnexal tumors. The clinical appearance is often nondescript (*A* shows multiple cylindromas and *C* shows multiple trichoepitheliomas). *B*, On histologic examination, the cylindroma is composed of islands of basaloid cells containing occasional ducts and seemingly fitting together like pieces of a jigsaw puzzle. *D*, Trichoepithelioma is composed of buds of basaloid cells that resemble primitive hair follicles. Here the small ductlike structures are actually keratin-filled microcysts.

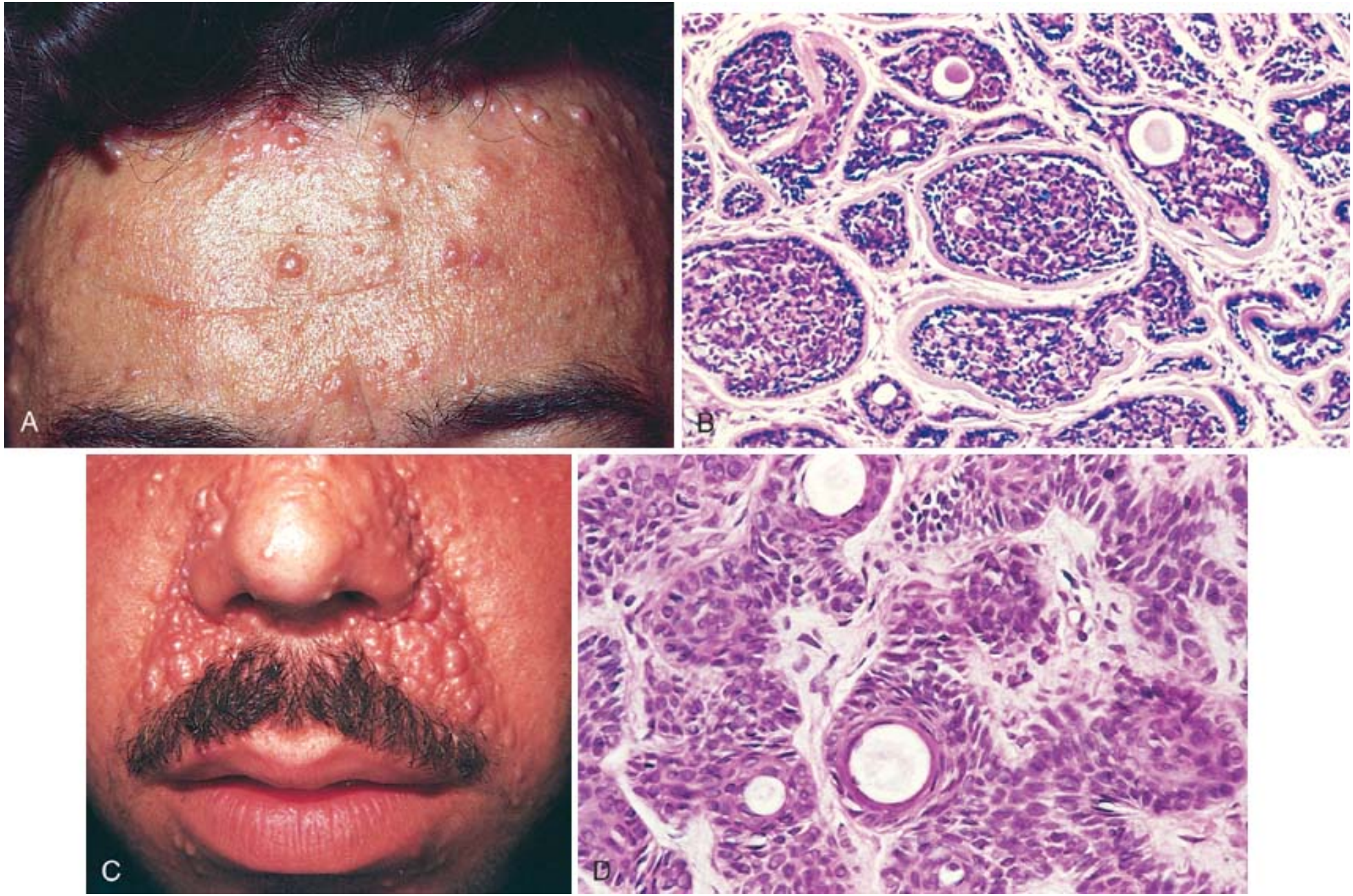


Figure 25-11 Adnexal tumors. *A*, Mixed tumor (chondroid syringoma). *B*, Trichilemmoma. *C*, Hidradenoma papilliferum.

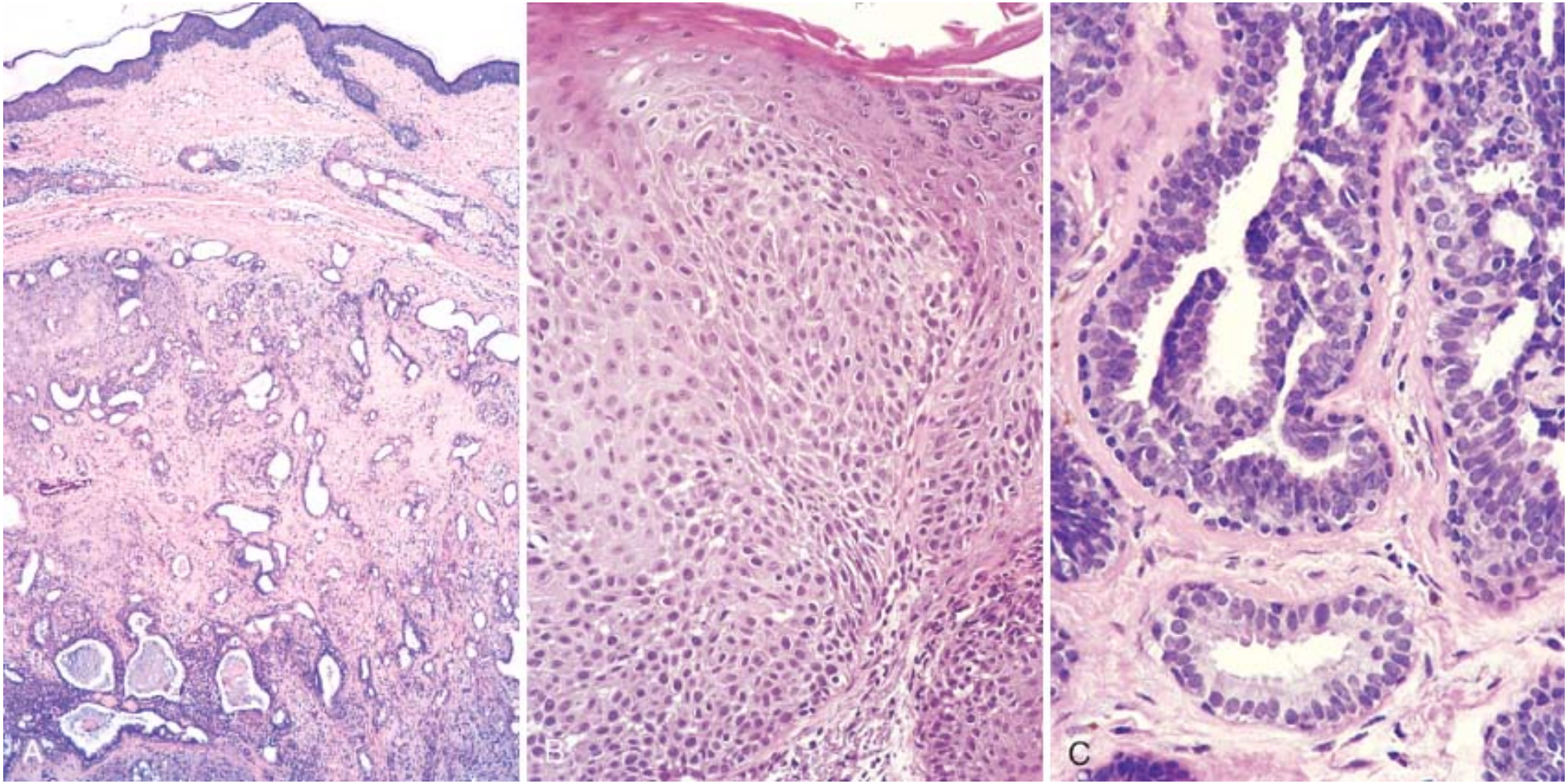


TABLE 25-2 -- Common Adnexal Tumors

Adnexal Tumors	Mature Counterpart	Histologic Features	Clinical Significance
Trichoepithelioma	Hair follicle	Hair matrix, outer root sheath differentiation	Multiple trichoepitheliomas, dominant inheritance
Trichofolliculoma			
Sebaceous adenoma	Sebaceous gland	Cytoplasmic lipid vacuoles	Association with internal malignancy
Sebaceous epithelioma			
Syringocystadenoma papilliferum	Apocrine gland	Apocrine type ("decapitation") secretion	May develop in mixed epidermal-adnexal hamartomas of face and scalp termed *nevus sebaceus*
Syringoma	Eccrine gland	Eccrine ducts lined by membranous eosinophilic cuticles; tadpole-like epithelial structures	May be confused with basal cell carcinoma clinically

Premalignant and Malignant Epidermal Tumors

ACTINIC KERATOSIS

Before the development of overt malignancy of the epidermis, a series of progressively dysplastic changes occur, a phenomenon analogous to the atypia that precedes carcinoma of the squamous mucosa of the uterine cervix (Chapter 22). Because this dysplasia is usually the result of chronic exposure to sunlight and is associated with build-up of excess keratin, these lesions are called *actinic keratoses*. As would be expected, they occur with particularly high incidence in lightly pigmented individuals. Exposure to ionizing radiation, hydrocarbons, and arsenicals may induce similar lesions.

Actinic keratoses are usually less than 1 cm in diameter; are tan-brown, red, or skin-colored; and have a rough, sandpaper-like consistency. Some lesions may produce so much keratin that a "cutaneous horn" develops (Fig. 25-13*A*). Such horns may become so prominent that they actually resemble the

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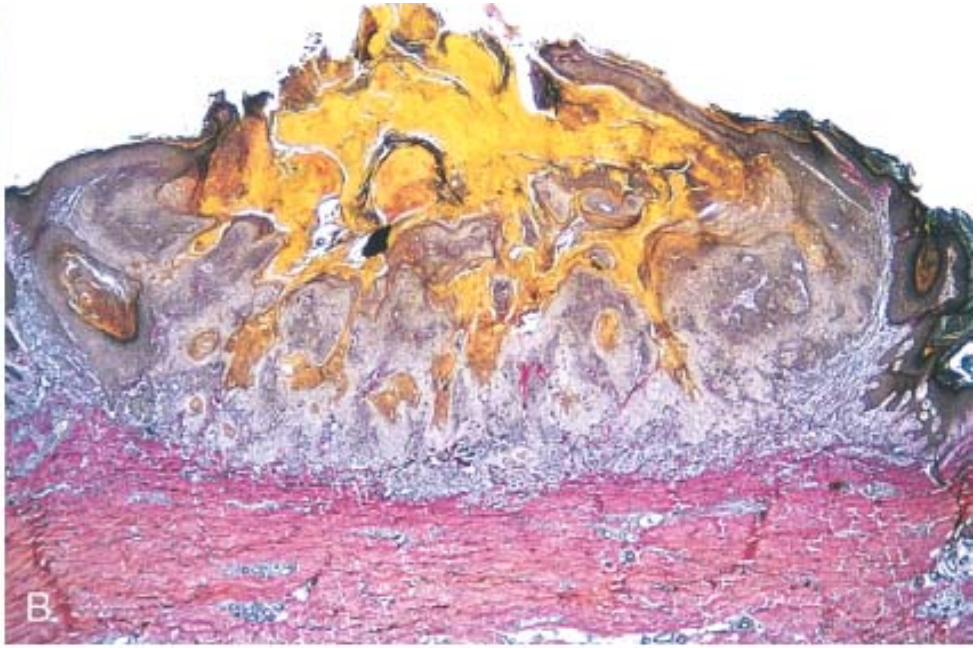
Figure 25-12 Keratoacanthoma. *A*, This symmetric crater-like nodule has a prominent central keratin plug. *B*, At low power, the crater-like architecture may be appreciated with an elastic tissue stain where the dermis is red, epithelial elements are gray, and the central keratin plug is yellow. *C*, Higher power view shows keratoacanthoma to be composed of large, glassy squamous cells and central islands of eosinophilic keratin.



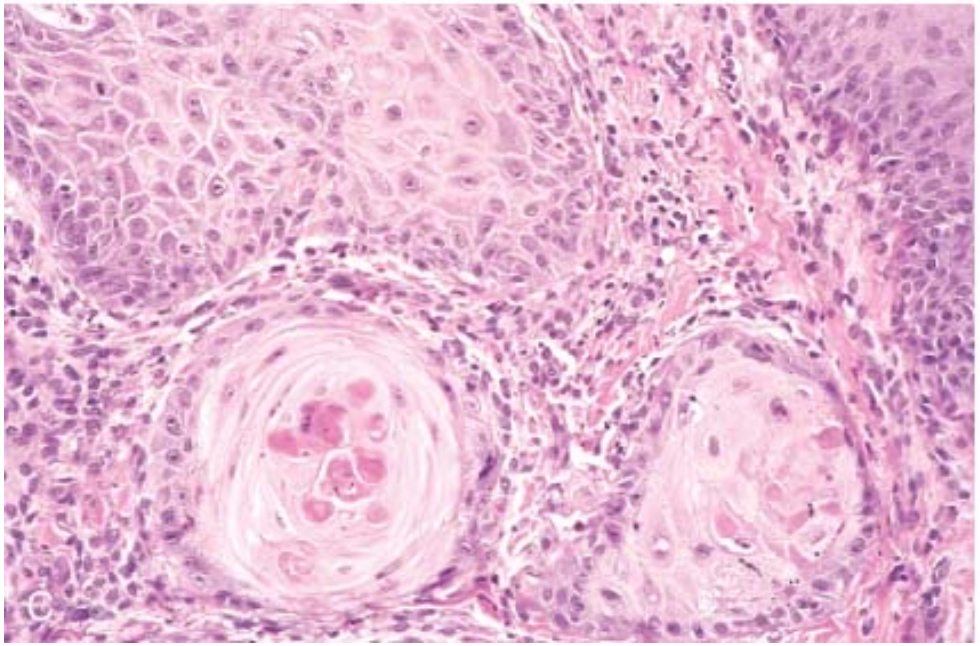


Figure 25-13 Actinic keratosis. *A*, Excessive scale formation in this lesion has produced a "cutaneous horn." *B*, Basal cell layer atypia is associated with marked hyperkeratosis and parakeratosis. *C*, Progression to full-thickness nuclear atypia, with or without the presence of superficial epidermal maturation, heralds the development of early squamous cell carcinoma in situ.

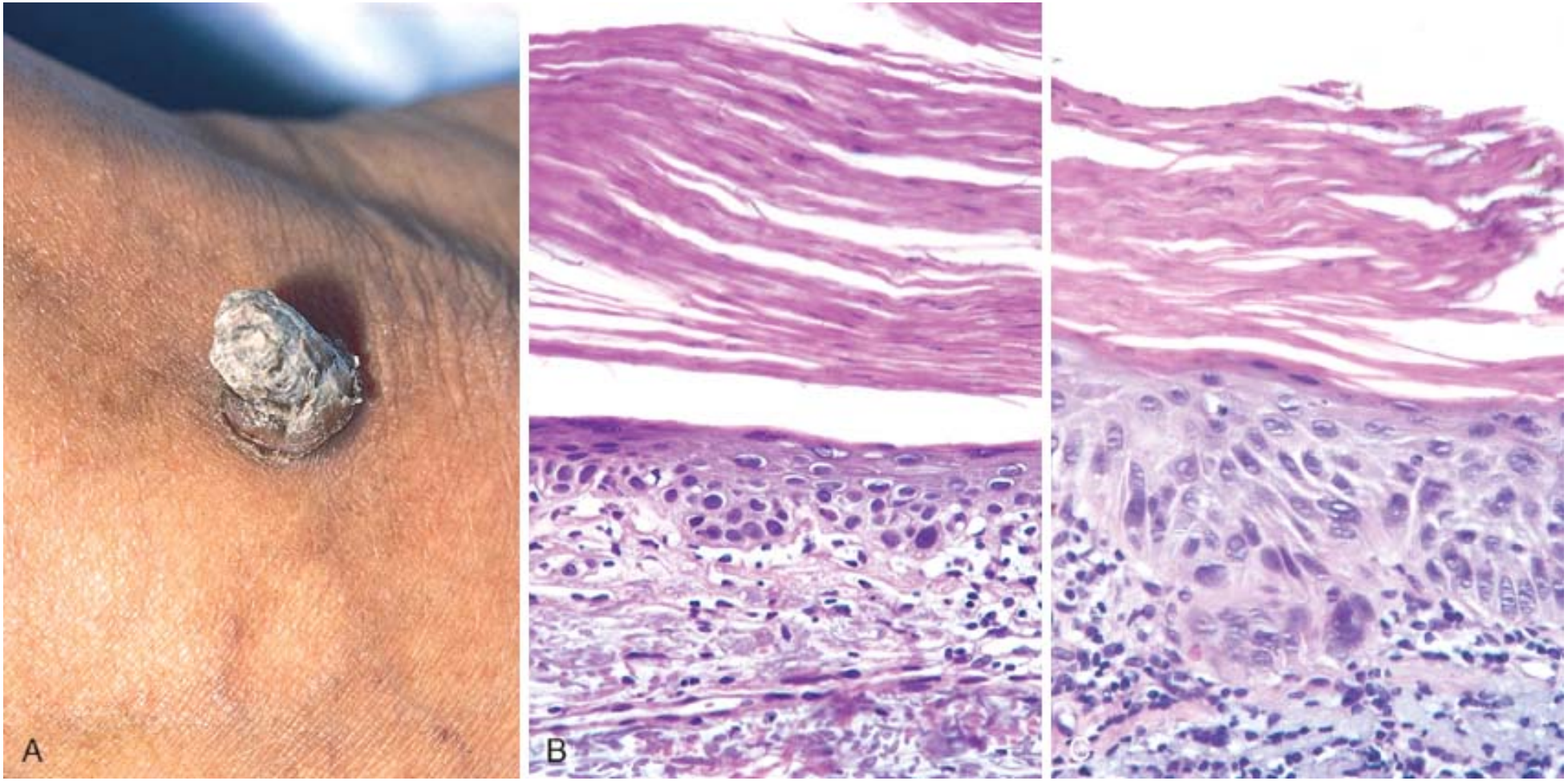


Figure 25-14 Invasive squamous cell carcinoma. *A,* Lesions are often nodular and ulcerated. *B,* Tongues of atypical squamous epithelium have transgressed the basement membrane, invading deeply into the dermis. *C,* Invasive tumor cells exhibit enlarged nuclei with angulated contours and prominent nucleoli.

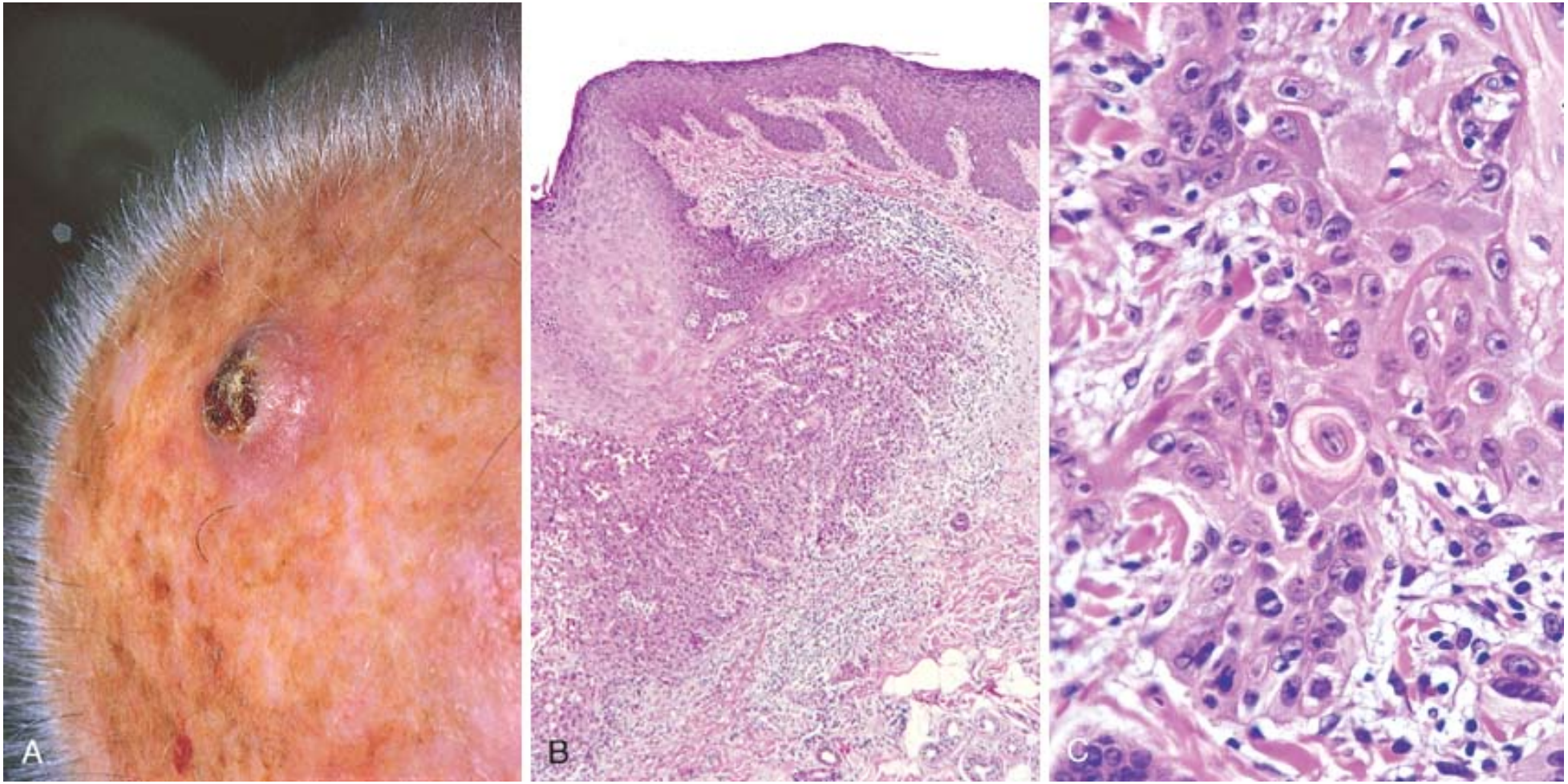


Figure 25-15 Basal cell carcinoma. Pearly, telangiectatic nodules *(A)* are composed of nests of basaloid cells within the dermis *(B)* that are often separated from the adjacent stroma by thin clefts *(C)*.

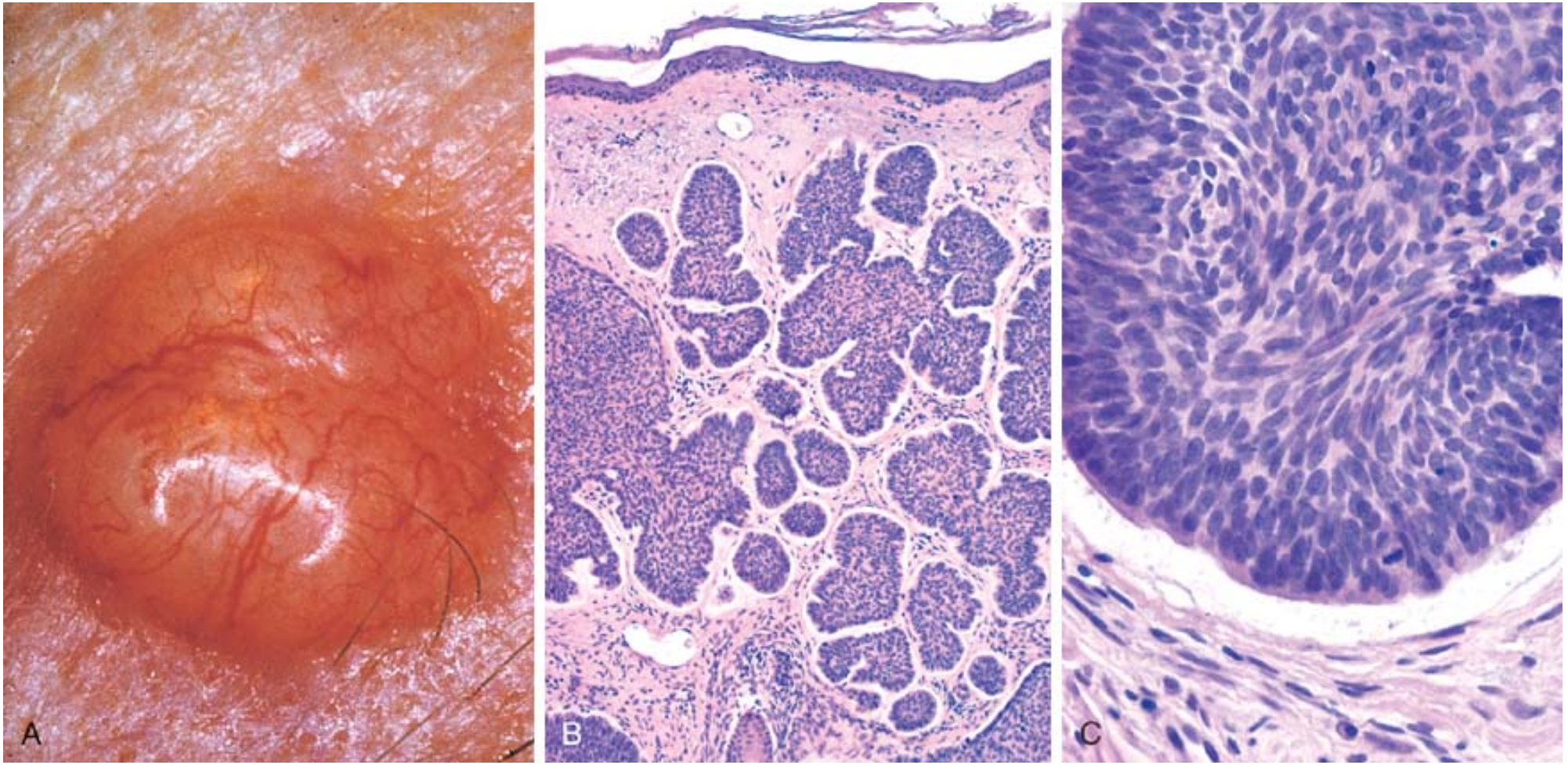


TABLE 25-3 -- A Survey of Familial Cancer Syndromes with Cutaneous Manifestations[44]

Disease	Inheritance	Chromosomal Location	Gene/Protein	Function/Manifestation
Ataxia-telangiectasia	AR	11q22.3	*AT*/AT †	DNA repair after radiation injury; p53 signaling/neurologic and vascular lesions
Nevoid basal cell carcinoma syndrome	AD	9q22.3	*PTCH*//PTCH	Developmental gene/multiple basal cell carcinomas; jaw cysts, etc.
Cowden syndrome	AD	10q23	*PTEN*, *MMAC1*/PTEN, TEP1, MMAC1	Lipid/protein phosphatase/benign follicular appendage tumors (trichilemmomas); internal adenocarcinoma
Familial melanoma syndrome	AD	9p21	*CDKN2*/p16INK4	Inhibits CDKs from phosphorylating Rb, thus arresting cell cycle/ melanoma

			CDKN2/p14ARF	Binds MDM2 and thus, preserves p53/melanoma
Muir-Torre syndrome	AD	2p22	*hMSH2*/hMSH2	Involved in DNA mismatch repair/benign and malignant sebaceous tumors; internal adenocarcinoma
Neurofibromatosis I	AD	17q11.2	*NF1*/neurofibromin	Negatively regulates Ras family of signal molecules/neurofibromas
Neurofibromatosis II	AD	22q12.2	*NF2*/merlin	Integrates cytoskeletal signaling/neurofibromas and acoustic neuromas
Tuberous sclerosis	AD	9q34	*TSC1*/hamartin	Interacts with tuberin; function unknown
		16p13.3	*TSC2*/tuberin	Interacts with hamartin; may regulate ras proteins/angiofibromas, mental retardation
Xeroderma pigmentosum	AR	9q22 and others	*XPA*/XPA and others	Nucleotide excision repair/melanoma and nonmelanoma skin cancers

AD, Autosomal dominant; AR, autosomal recessive.

†By convention, genes are italicized and proteins are not italicized.

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mutations are found in approximately 30% of sporadic basal cell carcinomas, and of these, about one-third have mutations (C to T transitions) that are considered to be the hallmark of UV damage. Mutations in *p53* occur in 40% to 60% of basal cell carcinomas, and 60% of these have such a "UV signature." [54] Xeroderma pigmentosum, a disorder of DNA repair, presents a striking example of the connection between sun exposure and defects in *PTCH* and *p53*.[55] In these tumors, the frequency of mutations in *PTCH* and *p53* are, respectively, 90% and 40%, and the majority of these bear the UV signature.

In summary, mutations of PTCH are the cause of NBCCS. In sporadic basal cell carcinomas there are frequent mutations in PTCH and p53, most mutations being the product of unrepaired DNA damage by UV light.

Squamous Cell Carcinoma.

By contrast to basal cell carcinomas, there is no inherited single gene defect associated with squamous cell carcinomas. Thus, most studies of the molecular genetics of squamous cell carcinoma have examined defects in sporadic tumors and their precursors (actinic keratoses), and the relationships between these defects and sun exposure.[56] The incidence of mutations in *p53* in Caucasian patients with actinic keratoses is high, suggesting that sunlight causes alterations at the early stages of carcinogenesis. [57] However it is not known whether lesions with *p53* mutations are more likely to progress to carcinomas than lesions without the mutation. The immediate effects of UV light on *p53* are positive, involving its induction, resulting in cell-cycle arrest in G_1 to permit DNA repair, or elimination by apoptosis of the damaged cells that are beyond repair (see Chapter 7). When this protective function of *p53* is lost because of unrepaired UV-induced damage of the gene through production of pyrimidine dimers, [58] continuous division of the mutant cells is favored. Such mutations are found in some but not all invasive squamous cell carcinomas. Unlike basal cell carcinomas, aneuploidy is very common in squamous cell carcinomas, and loss of heterozygosity involving chromosomes 3, 9, and 17 occurs in approximately 30% of cases. With the exception of the *p53* locus on chromosome 17, putative tumor suppressor genes localized in the other chromosomes have not been identified.

HPV types 5 and 8, among others (see Chapter 7), are involved in the molecular pathogenesis of a rare condition, epidermodysplasia verruciformis, associated with formation of numerous cutaneous squamous cell carcinomas. A decreased immune response also seems to play a role, since this condition may be found in severely immunosuppressed individuals. [59]

Melanomas.

It is estimated that 10% to 15% of melanomas arise in a familial setting.[60] This was first observed in families whose members have large numbers of dysplastic nevi. As already mentioned, some dysplastic nevi develop into melanoma, as in the familial BK mole syndrome, also known

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as *dysplastic nevus syndrome*, *familial atypical multiple mole-melanoma syndrome*, or simply *familial melanoma syndrome* (FMS). Unlike NBCCS, which has a clear Mendelian mode of inheritance, FMS does not display distinct and predictable inherited phenotypic abnormalities.[44] The situation is even more complex because not all familial melanomas develop in the setting of multiple dysplastic nevi; conversely, melanomas can occur in patients with multiple sporadic dysplastic nevi. To complicate matters, there is no complete agreement about the histopathologic criteria for the diagnosis of dysplastic nevi. [61] [62] [63]

The main locus associated with familial predisposition to melanomas has been mapped to chromosome 9p21[64] and it encodes *p16INK4A* (also known as cyclin-dependent kinase inhibitor 2, or *CDNK2*). It is frequently deleted in melanomas. [65] [66] As you may recall from the discussions of the cell cycle, lack of functional p16INK4A leads to unrestricted phosphorylation of RB, release of E2F, and uncontrolled cell growth. *p16INK4A* is the most commonly mutated gene in

Figure 25-16 Model of the hedgehog signaling pathway. PTCH and SMD form a receptor complex that binds Sonic Hedge Hog (SHH). In the absence of SHH, the PTCH protein prevents SMO from activating signal transduction. Binding of the SHH to the two large extracellular domains of PTCH releases SMO from its association with PTCH and allows downstream activation of hedgehog target genes via an intracytoplasmic signal cascade and generation of transcription factors, the most notable one being GLI1. Unopposed gene expression leads to basal cell carcinoma and development of anomalies seen in the nevoid basal cell carcinoma syndrome (NBCCS). Perturbations of this pathway are also important in sporadic forms of basal cell carcinoma.

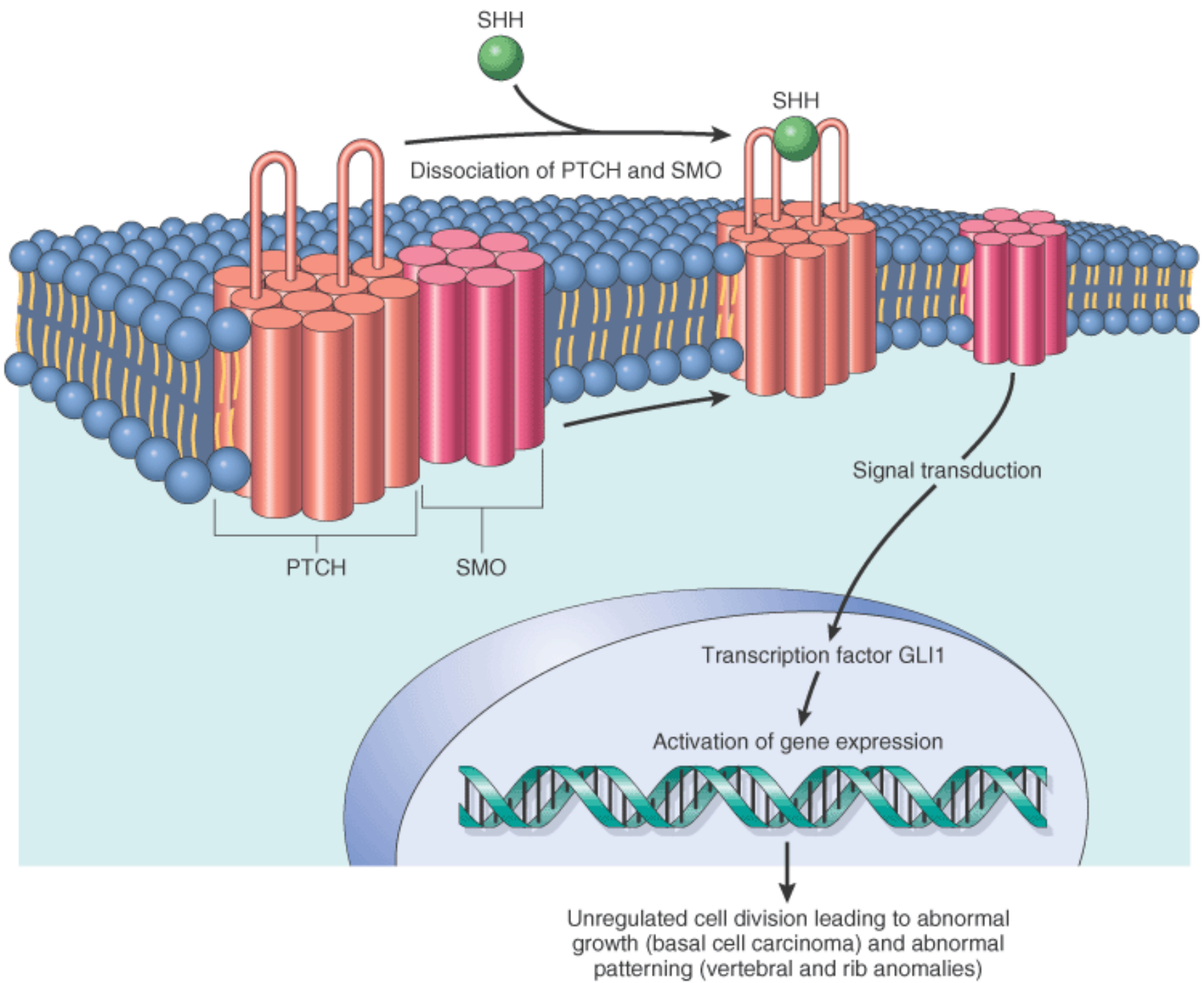


Figure 25-17 *A*, Benign fibrous histiocytoma (dermatofibroma). *B, C,* On excision, this firm, tan papule on the leg shows a localized nodular proliferation of benign-appearing fibroblasts within the dermis. Note the characteristic overlying epidermal hyperplasia and the tendency of fibroblasts to surround individual collagen bundles.

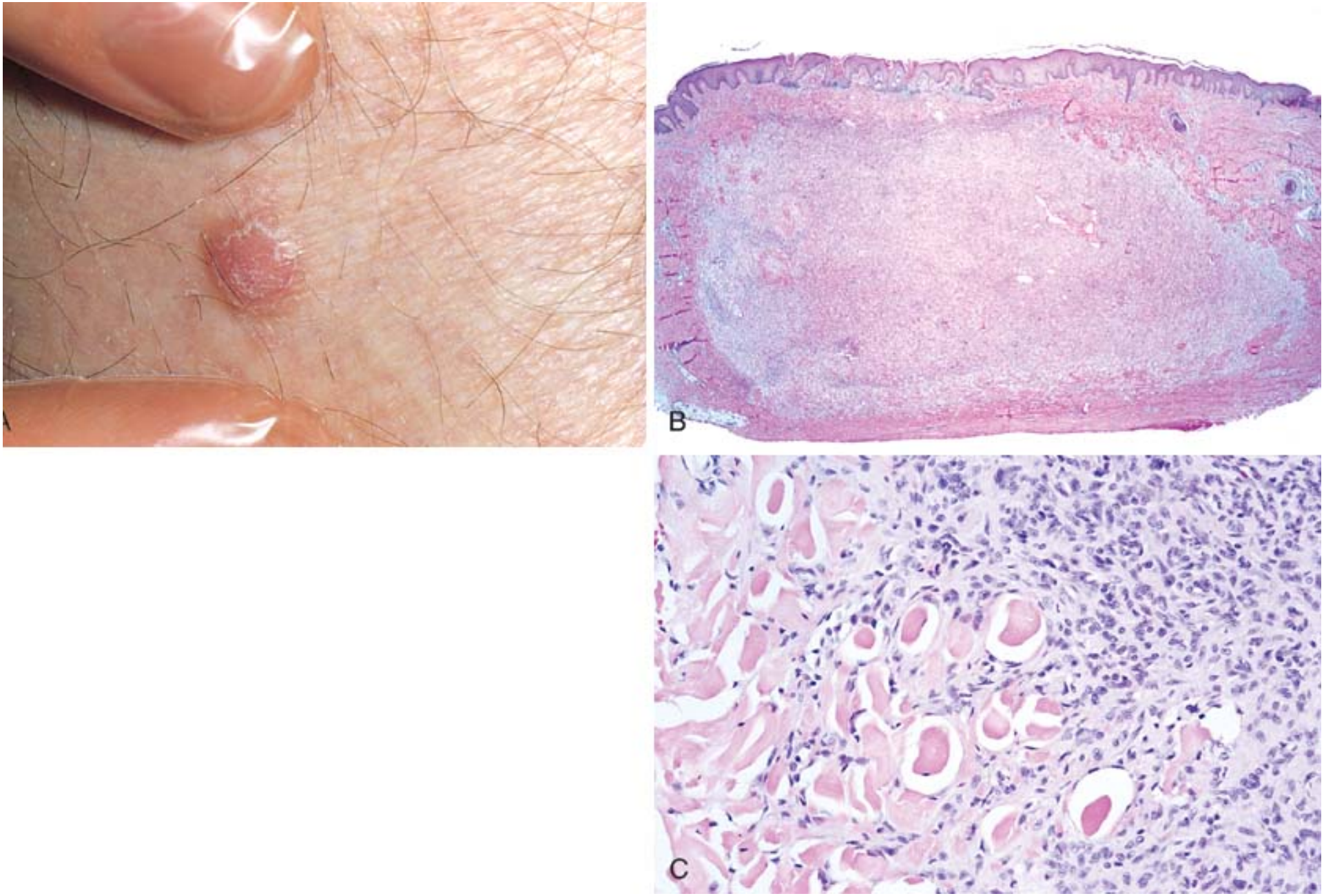


Figure 25-18 Langerhans cell histiocytosis. *A*, Lesions may appear clinically as papules and nodules or, as in this case, as erythematous scaling plaques mimicking the infantile form of seborrheic dermatitis. *B*, Dermal infiltration by bland mononuclear cells with infolded nuclei presents a nonspecific histologic pattern. *C*, Immunohistochemical demonstration of CD1a antigen confirms the origin of these mononuclear cells from Langerhans cells.

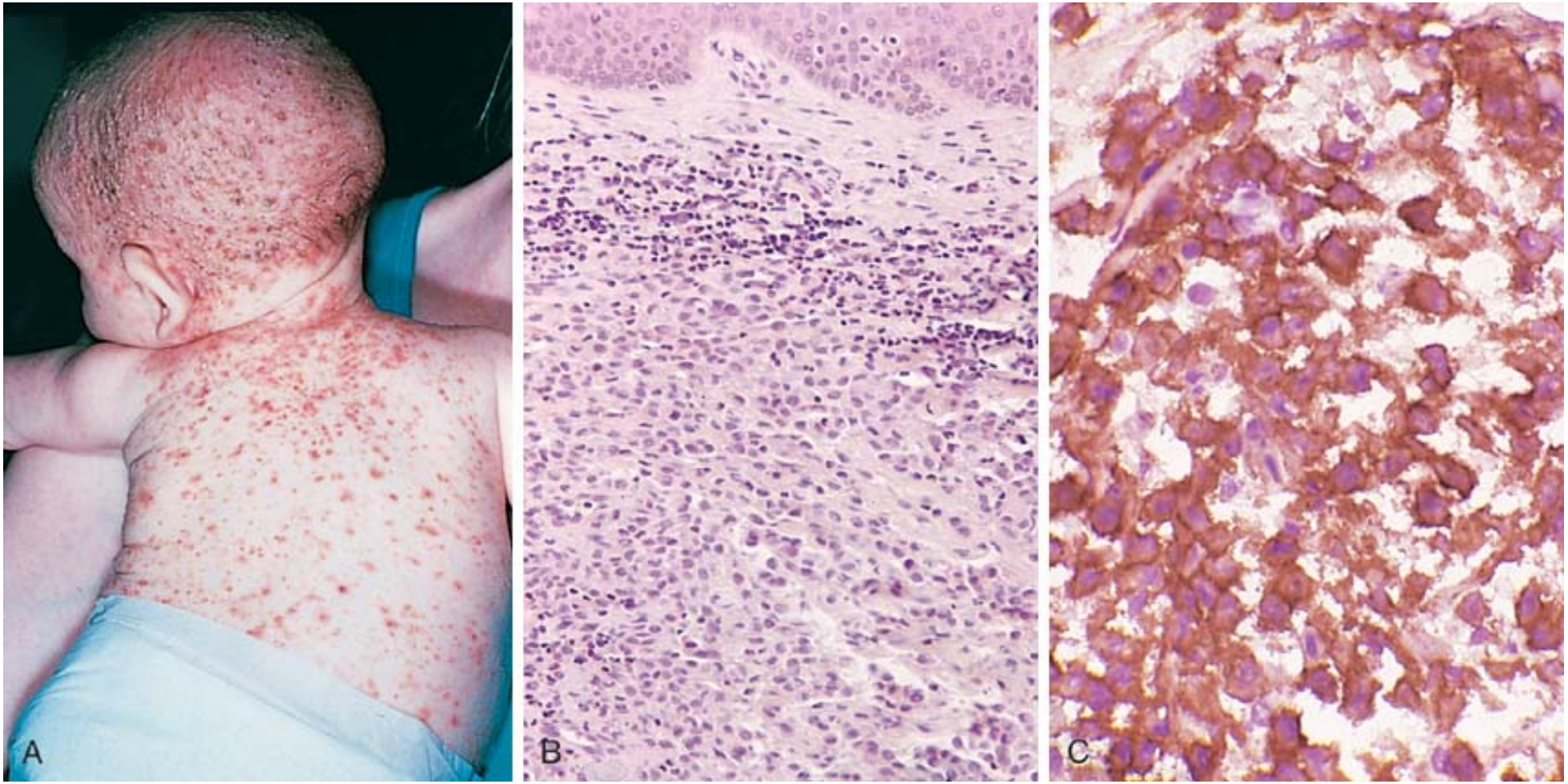


Figure 25-19 Cutaneous T-cell lymphoma. The histologic correlate of ill-defined, erythematous, often scaling, and occasionally ulcerated plaques *(A)* is an infiltrate of atypical lymphocytes that show a tendency to accumulate beneath the epidermal layer *(B)* and to invade the epidermis as small microabscesses.

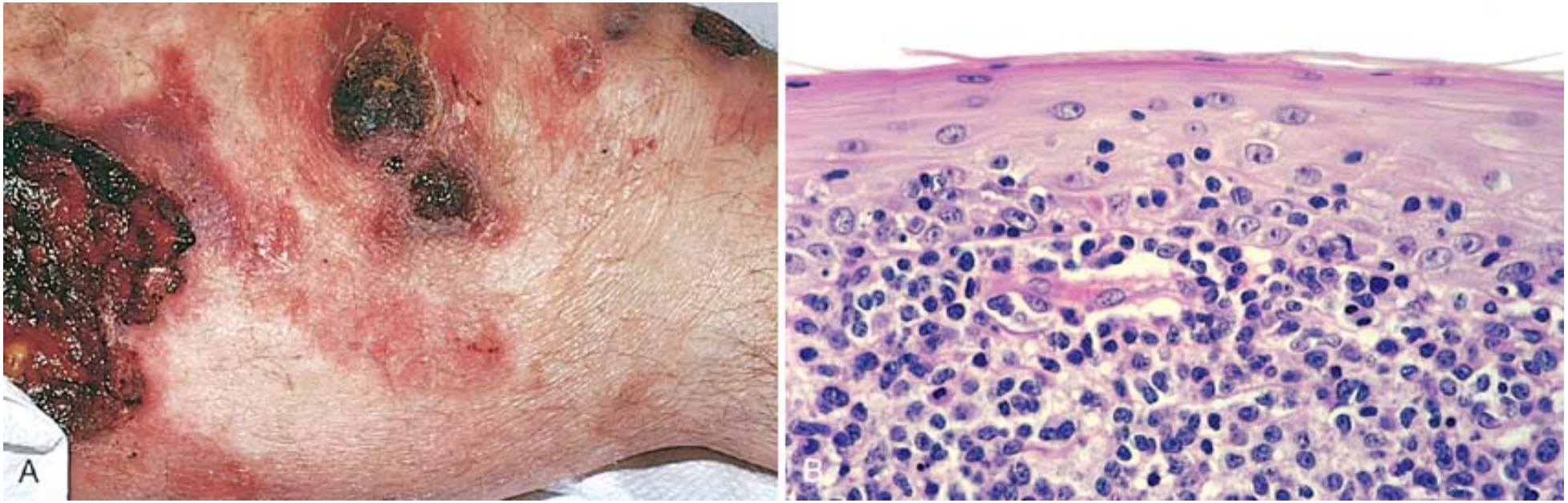


Figure 25-20 Mastocytosis. *A*, Solitary mastocytoma in a 1-year-old child. *B*, By routine histology, numerous ovoid cells with uniform, centrally located nuclei are observed in the dermis. *C*, Giemsa staining reveals purple, "metachromatic" granules within the cytoplasm of the cells.

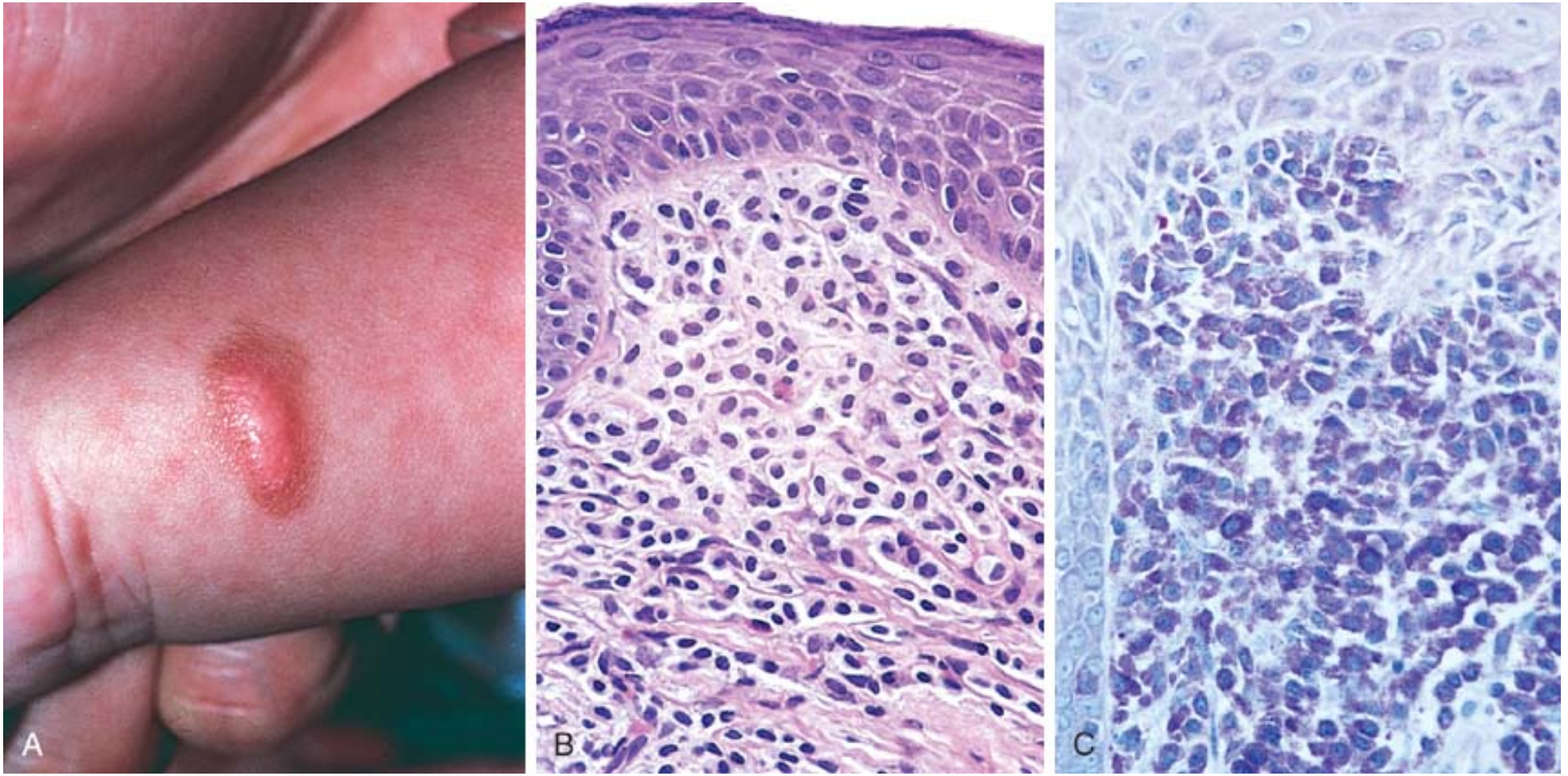


Figure 25-21 Ichthyosis. Note prominent fishlike scales *(A)* and compacted stratum corneum *(B)*.

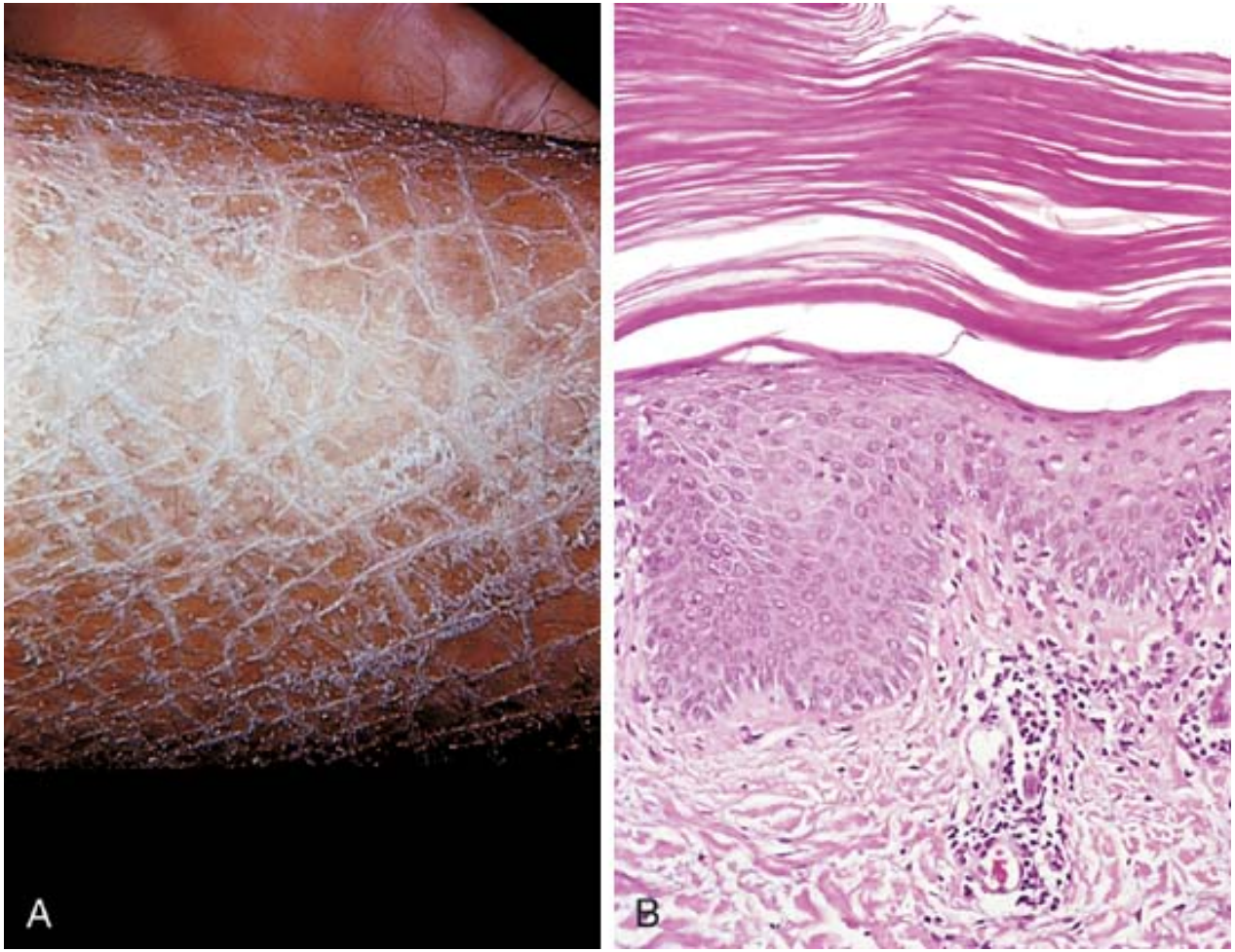


Figure 25-22 Urticaria. Clinically, there are erythematous, edematous, often circular plaques covered by a normal epidermal surface.

Figure 25-23 Urticaria. Histologically, there is superficial dermal edema and dilated lymphatic and blood-filled vascular spaces. The edema is manifested by widening of spaces that separate the collagen bundles.

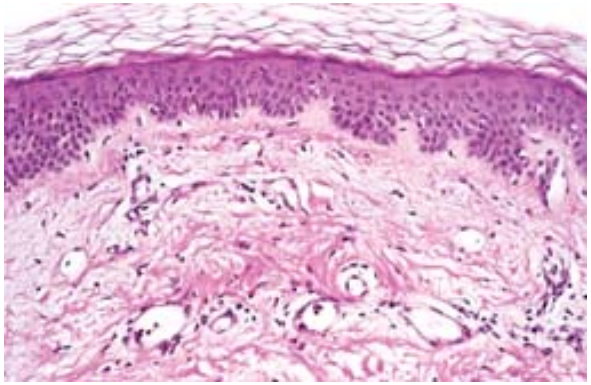


Figure 25-24 Stages of eczema development. *A*, Initial dermal edema and perivascular infiltration by inflammatory cells is followed within 24 to 48 hours by epidermal spongiosis and microvesicle formation *(B). C*, Abnormal scale, including parakeratosis, follows, along with progressive epidermal hyperplasia *(D)* and hyperkeratosis *(E)* as the lesion enters into a more chronic stage.

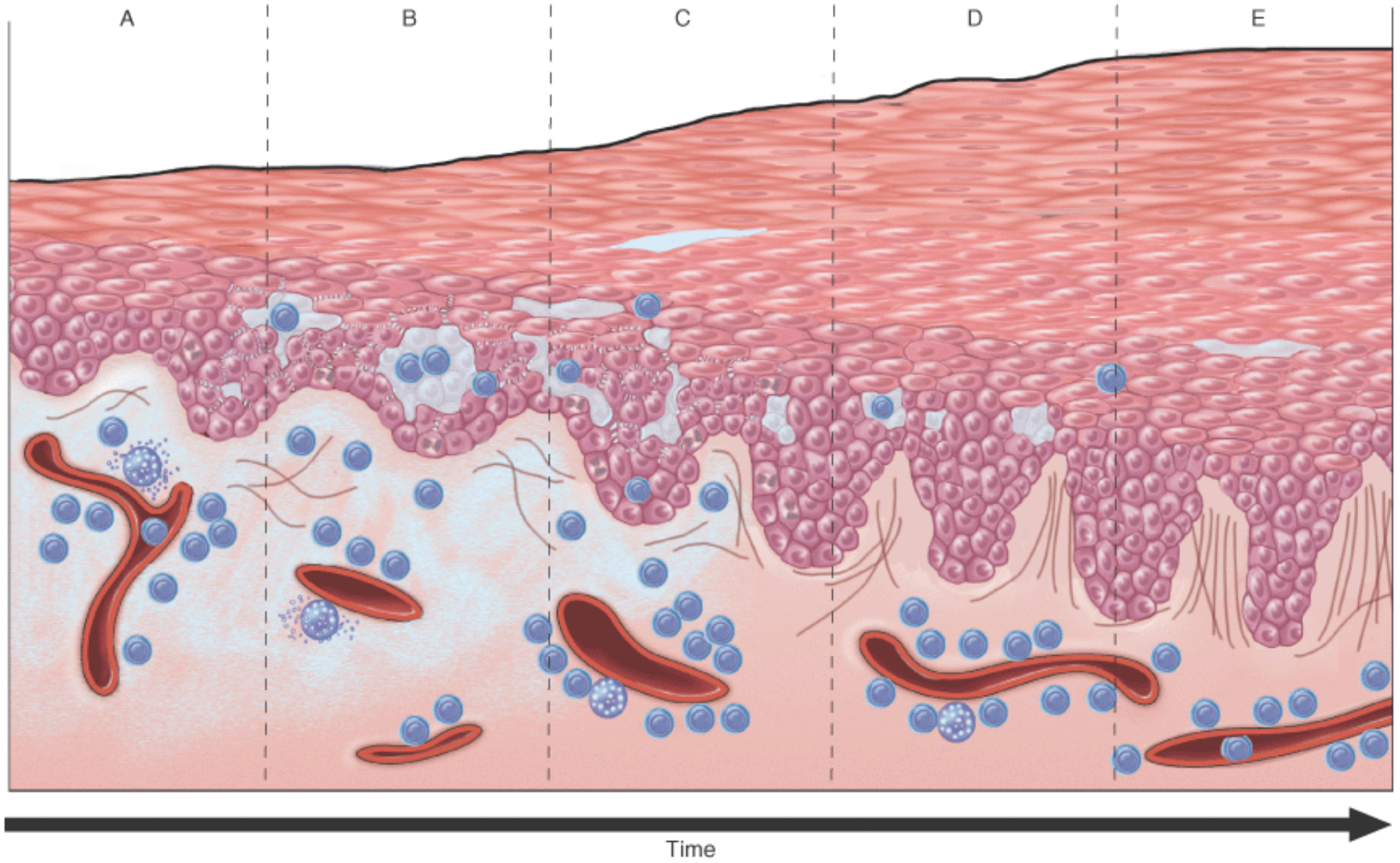


TABLE 25-4 -- Classification of Eczematous Dermatitis

Type	Cause or Pathogenesis	Histology *	Clinical Features
Contact dermatitis	Topically applied antigens	Spongiotic dermatitis	Marked itching, burning, or both; requires antecedent exposure

Atopic dermatitis	Unknown; may be heritable	Spongiotic dermatitis	Erythematous plaques in flexural areas; family history of eczema, hay fever, or asthma
Drug-related eczematous dermatitis	Systemically administered antigens or haptens (e.g. penicillin)	Spongiotic dermatitis; infiltrate often deeper with abundant eosinophils	Temporal relationship to drug administration; remits with cessation of drug
Eczematous insect bite reaction	Locally injected antigen or toxin	Spongiotic dermatitis; wedge-shaped infiltrate; many eosinophils	Papules, nodules, and plaques with vesicles; may be linear when multiple
Photoeczematous eruption	Ultraviolet light	Spongiotic dermatitis; infiltrate that diminishes gradually with depth	Occurs at sites of sun exposure; may require associated exposure to systemic or topical antigen; photopatch testing may help in diagnosis
Primary irritant dermatitis	Repeated trauma or chemical irritants (as in detergent)	Spongiotic dermatitis in early stages; acanthosis predominates in later stages	Localized mechanical or chemical irritants (nonimmunologic)

*All types, with time, may develop chronic changes, with prominent acanthosis of the epidermal layer.

The most obvious example is an acute contact reaction to topical antigens such as poison ivy, characterized by pruritic, edematous, oozing plaques, often containing small and large blisters (vesicles and bullae) (Fig. 25-25*A*). Such lesions are prone to bacterial superinfection, which produces a yellow crust (impetiginization). With time, persistent lesions become less "wet" (fail to ooze or form vesicles) and become progressively scaly (hyperkeratotic) as the epidermis thickens (acanthosis).

Pathogenesis.

This has been well studied in dermatitis due to contact hypersensitivity (e.g., poison ivy dermatitis). Initially, antigens at the epidermal surface are taken up by dendritic Langerhans cells, which then migrate by way of dermal lymphatics to draining lymph nodes (Fig. 25-26). Here, antigens, now processed by the Langerhans cell, are presented to naive CD4 T cells, which are activated and develop into effector and memory cells (Chapter 6). On antigen re-exposure,

Figure 25-25 Eczematous dermatitis. *A*, In an acute allergic contact dermatitis, numerous vesicles appear at the site of antigen exposure (in this case, laundry detergent that persisted in clothing). *B*, Histologically, intercellular edema produces widened intercellular spaces within the epidermis, eventually resulting in small, fluid-filled intraepidermal vesicles.

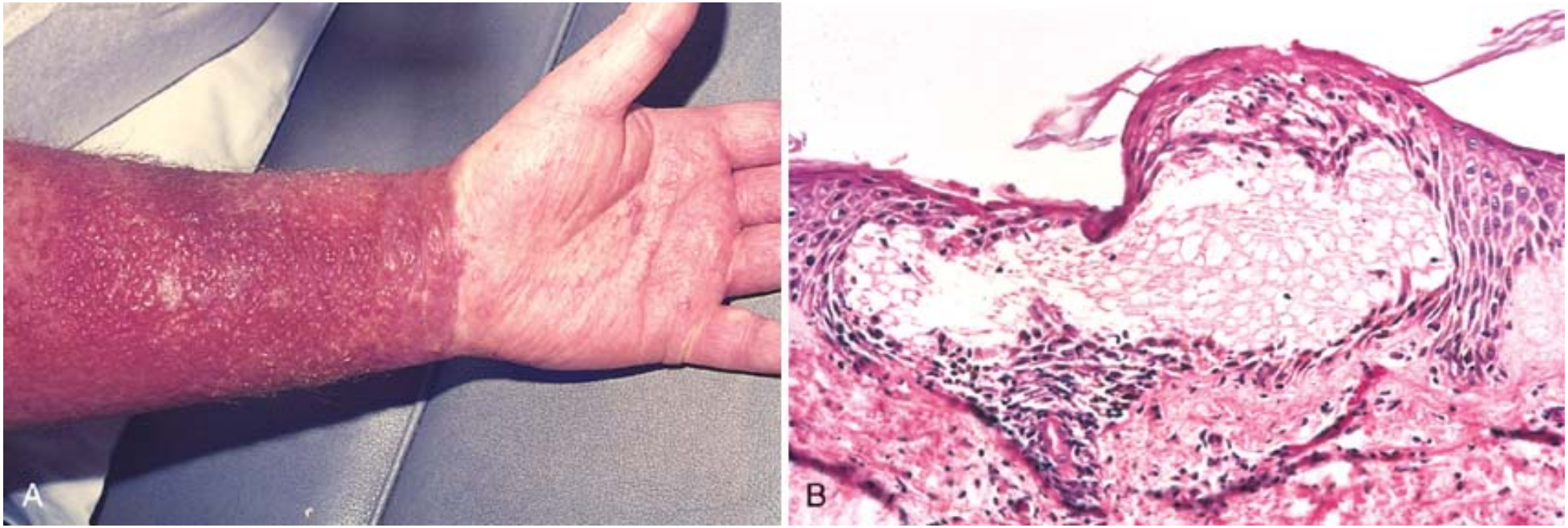


Figure 25-26 Schematic diagram of mechanisms of allergic contact dermatitis. Δ, antigen; Ln, naive T lymphocyte; Lm, memory T lymphocyte.

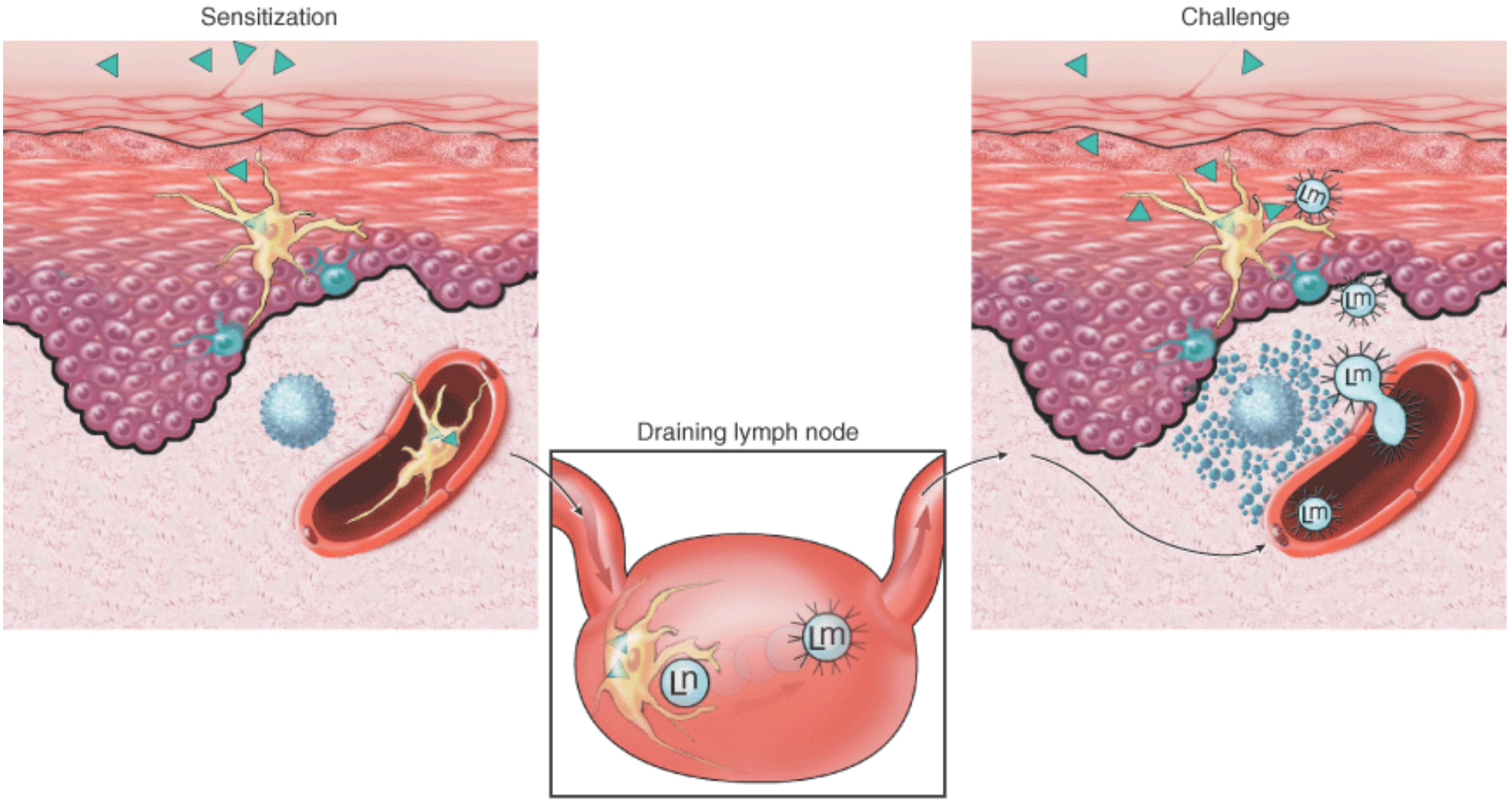


Figure 25-27 Erythema multiforme. *A*, The target-like clinical lesions consist of a central blister or zone of epidermal necrosis surrounded by macular erythema. *B*, Early lesions show lymphocytes collecting along the dermal epidermal junction where basal keratinocytes have begun to become vacuolated.

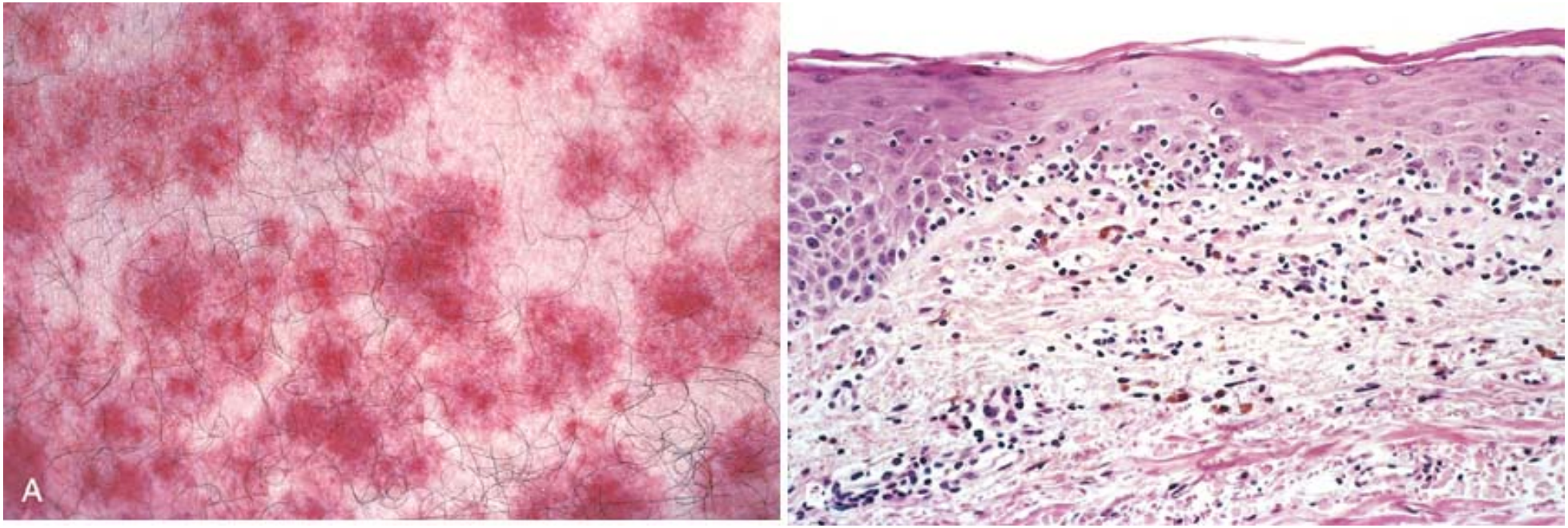


Figure 25-28 Clinical evolution of psoriasis. Early and eruptive lesions may be dominated by signs of inflammation and erythema *(left)*. Established, chronic lesions demonstrate erythema surmounted by characteristic silver-white scale *(right)*. Rarely, the early inflammatory phase predominates throughout the course of the disease (pustular psoriasis).

Figure 25-29 Psoriasis. Histologically, established lesions demonstrate marked epidermal hyperplasia, parakeratotic scale, and, importantly, minute microabscesses of neutrophils within the superficial epidermal layers.

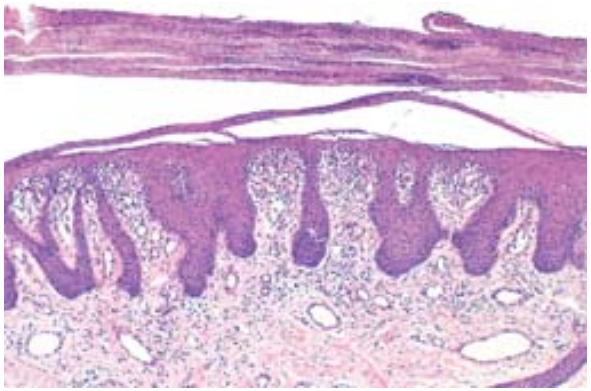


Figure 25-30 Lichen planus. *A,* A solitary lesion of lichen planus (glistening surface is due to application of mineral oil, rendering the scale transparent). This flat-topped pink-purple, polygonal papule shows prominent Wickham striae that are more easily appreciated through the transparent scale. *B,* Biopsy of one of the lesions demonstrates the bandlike infiltrate of lymphocytes at the dermoepidermal junction and pointed rete ridges (saw-toothing; compare with Fig. 25-1).

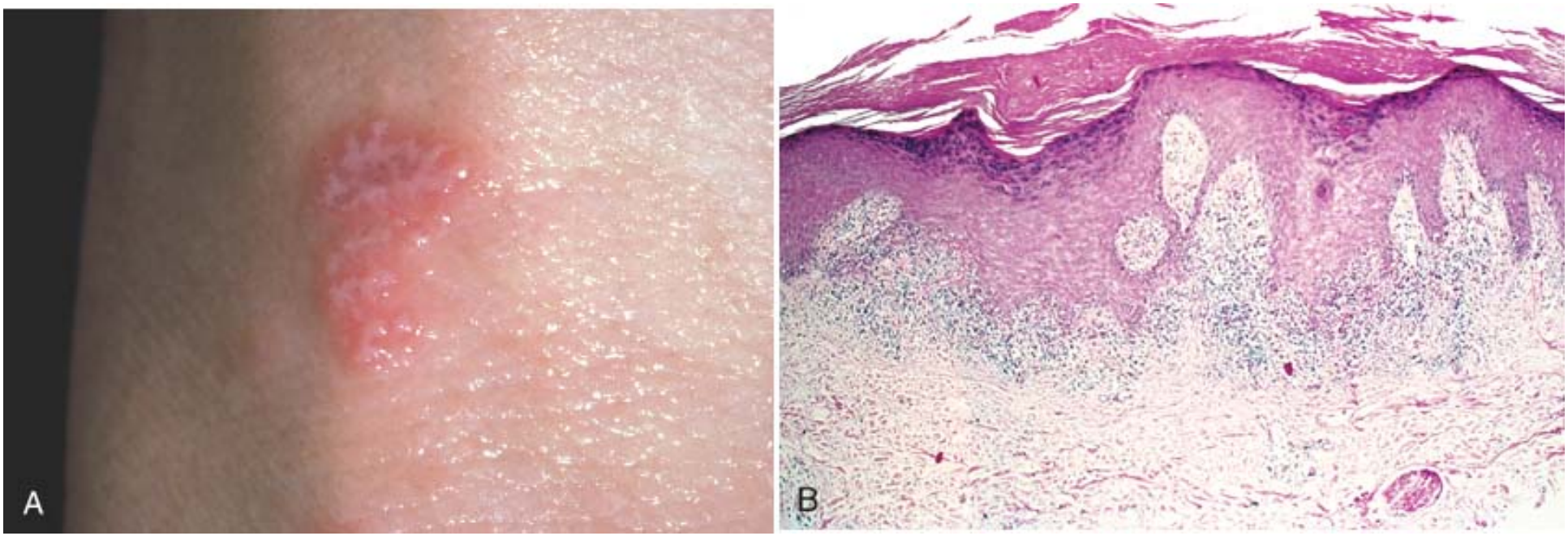


Figure 25-31 Lupus erythematosus. *A,* These chronic plaques show a thinned and glistening (atrophic) epidermis, areas where dilated and tortuous dermal vessels are apparent, and central hypopigmentation surrounded by peripheral hyperpigmentation. Note areas of early hair loss due to follicular involvement. *B,* There is an infiltrate of lymphocytes within the superficial and deep dermis, marked thinning of the epidermis with loss of normal rete ridges, and hyperkeratosis.

Figure 25-32 Granular deposits of immunoglobulin (here IgG) and complement at the dermoepidermal junction constitute a positive "band test" in lupus erythematosus.

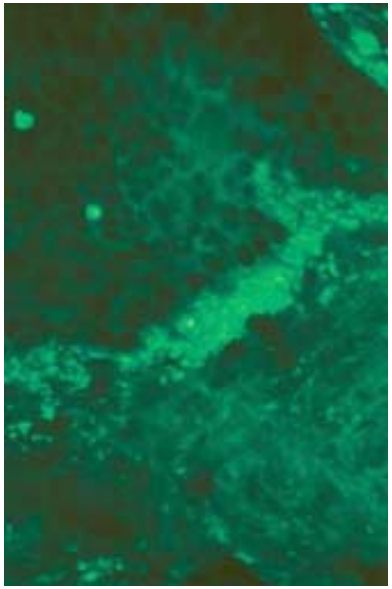


Figure 25-33 Schematic representation of sites of blister formation. *A*, In a subcorneal blister, the stratum corneum forms the roof of the bulla (as in impetigo or pemphigus foliaceus). *B*, In a suprabasal blister, a portion of the epidermis including the stratum corneum forms the roof (as in pemphigus vulgaris). *C*, In a subepidermal blister, the entire epidermis separates from the dermis (as in bullous pemphigoid and dermatitis herpetiformis).

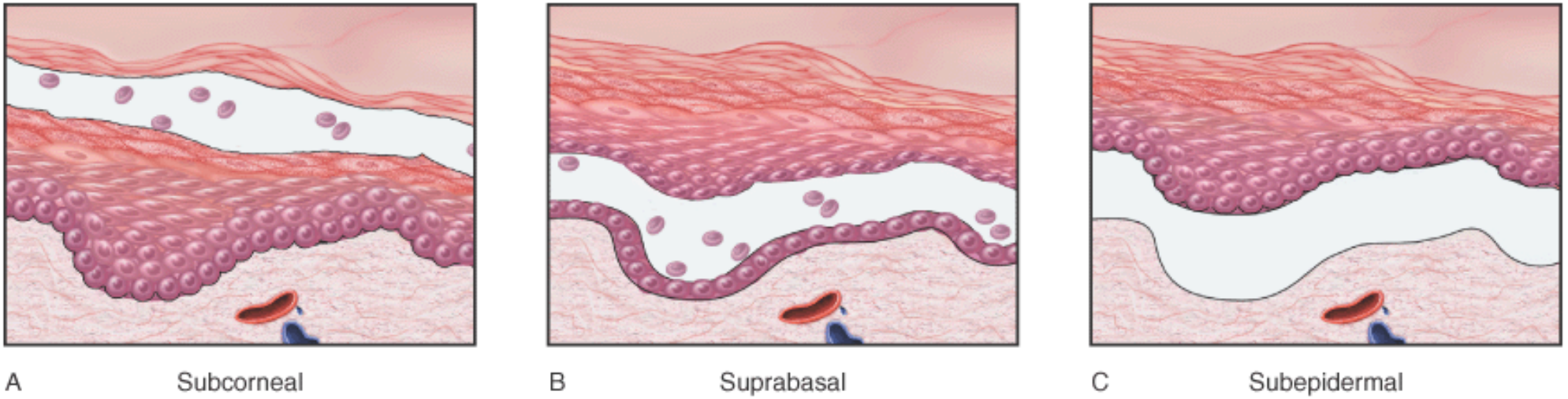


Figure 25-34 Pemphigus vulgaris. *A*, Eroded plaques are formed on rupture of confluent, thin-roofed bullae, here affecting axillary skin. *B*, Suprabasal acantholysis results in an intraepidermal blister in which rounded (acantholytic) epidermal cells are identified *(inset)*.

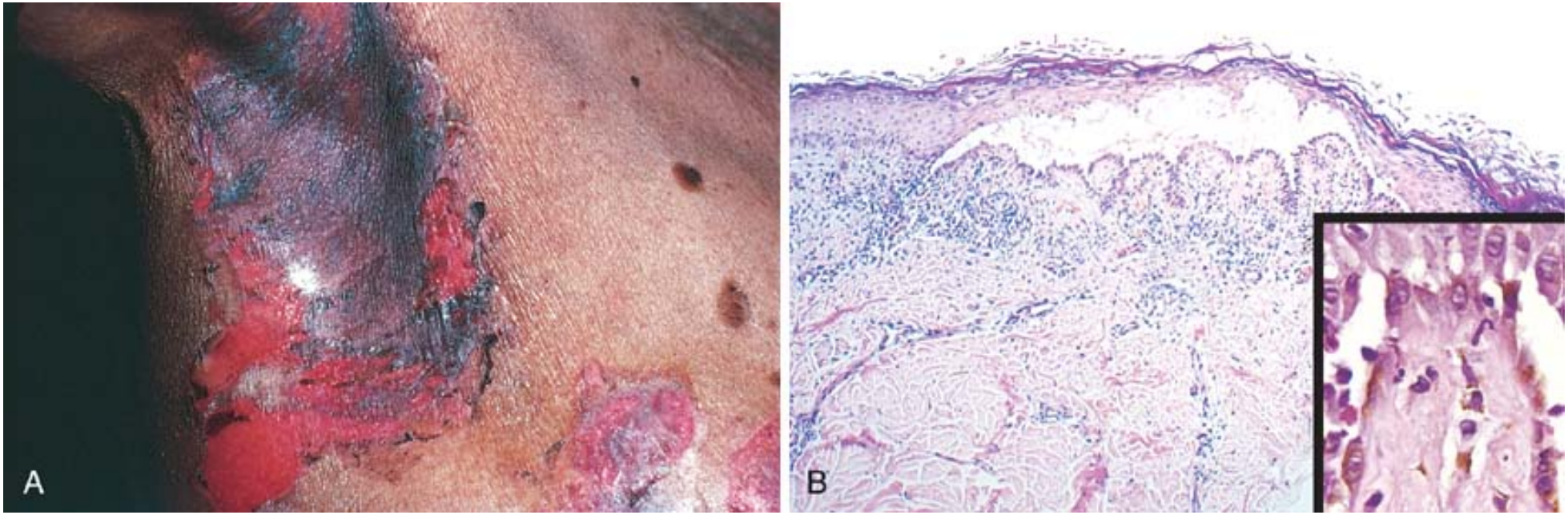


Figure 25-35 Direct immunofluorescence of pemphigus vulgaris. There is deposition of immunoglobulin along the plasma membranes of epidermal keratinocytes in a fishnet-like pattern. Also note the early suprabasal separation due to loss of cell-to-cell adhesion (acantholysis).

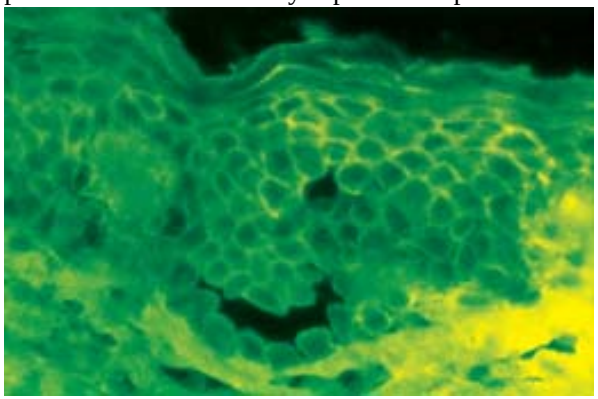


Figure 25-36 Bullous pemphigoid. Clinical bullae *(A)* result from basal cell layer vacuolization, producing a subepidermal blister *(B)*. Histopathology of the edge of an early lesion showing the onset of epidermal separation from the underlying dermis. Eosinophils, as well as lymphocytes and occasional neutrophils, may be intimately associated with basal cell layer destruction, creating the subepidermal cleft.

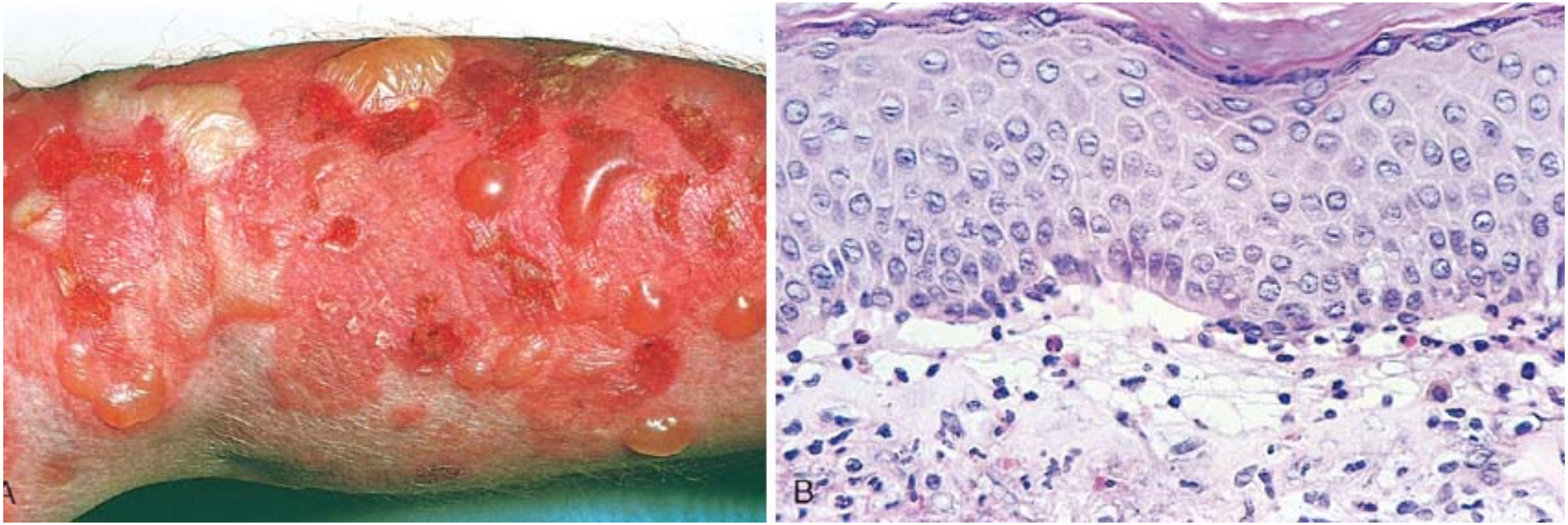


Figure 25-37 *A*, Linear deposition of complement along the dermoepidermal junction in bullous pemphigoid; the pattern has been likened to ribbon candy. *B*, Bullous pemphigoid antigen is located in the lowermost portion of the basal cell cytoplasm in association with hemidesmosomes (HD), with blister formation affecting the lamina lucida (LL) of the basement membrane zone. LD, lamina densa; AF, anchoring fibrils.

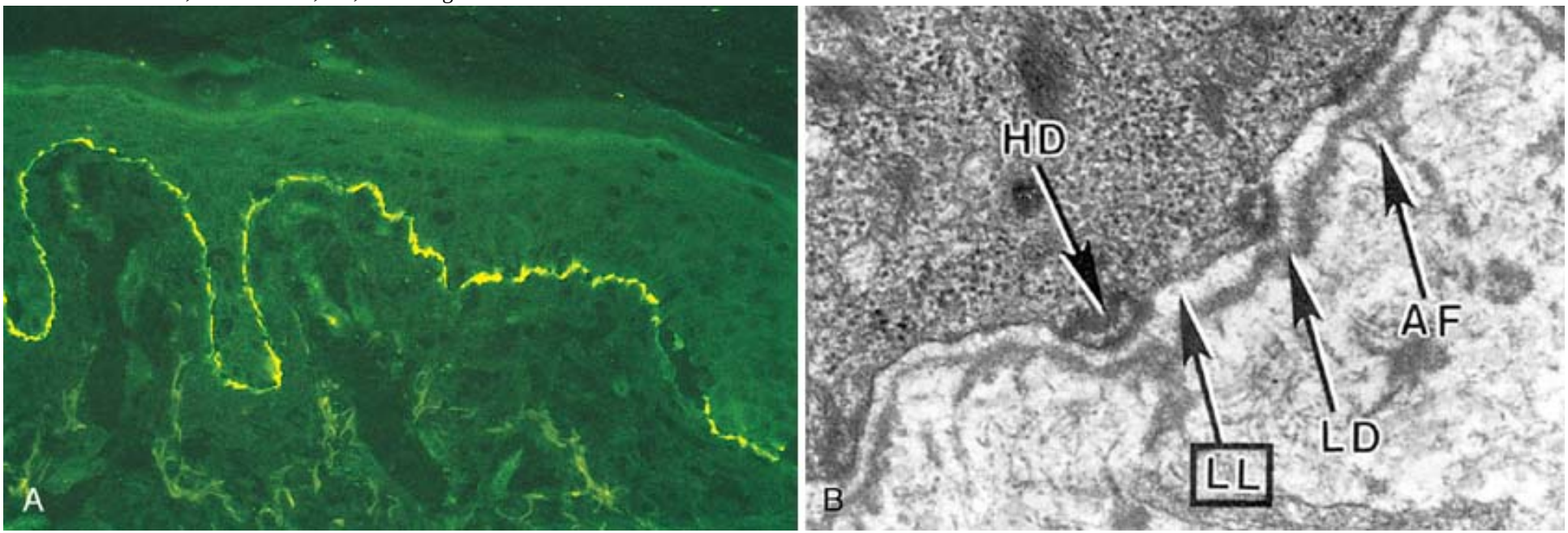


Figure 25-38 Dermatitis herpetiformis. *A*, Clinical lesions consist of intact and eroded erythematous blisters that are often grouped together. *B*, Histologically, neutrophilic microabscesses selectively involve the dermal papilla.

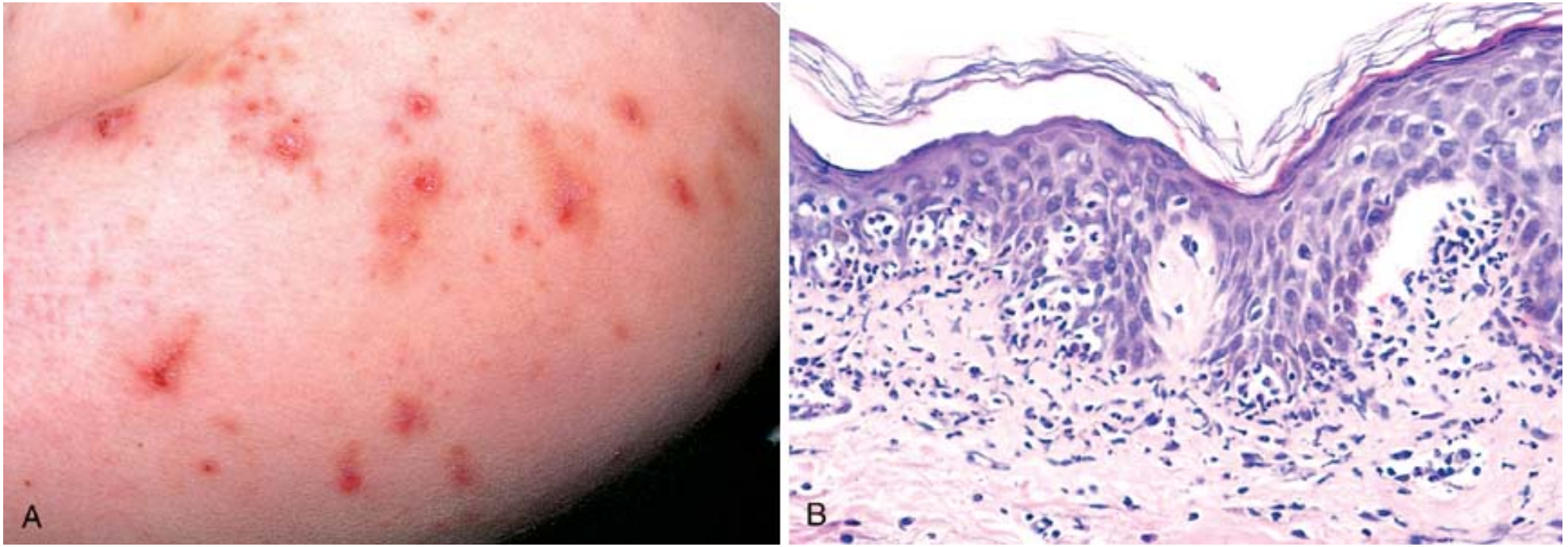


Figure 25-39 Dermatitis herpetiformis. *A*, Papillary dermal microabscesses are associated with zones of dermoepidermal cleavage that eventually coalesce to form a clinical blister. *B*, By direct immunofluorescence, these abscesses are rich in IgA and fibrin deposits.

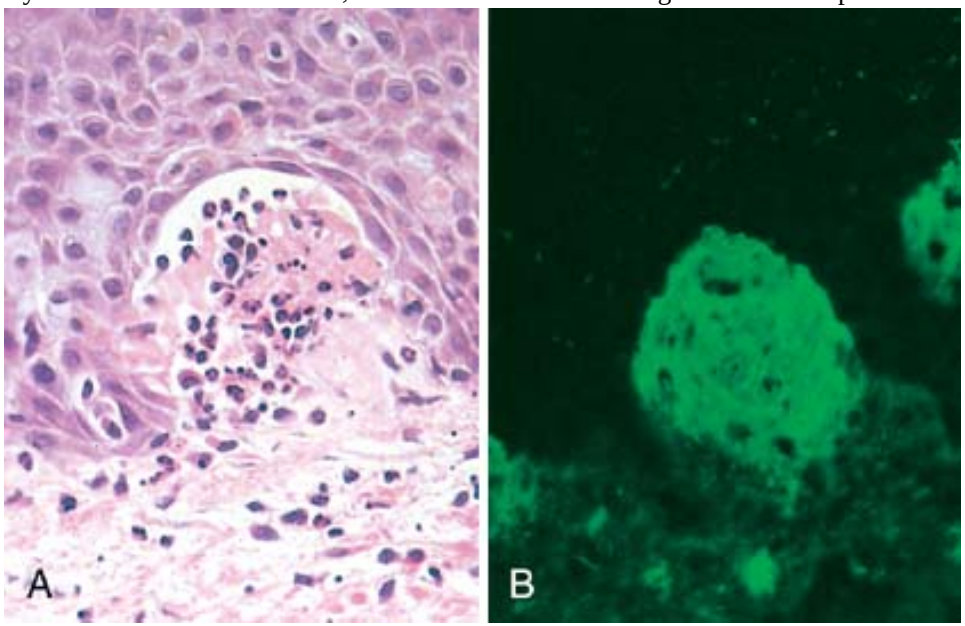


Figure 25-40 Epidermolysis bullosa. *A*, Junctional epidermolysis bullosa showing typical erosions in flexural creases. *B*, A noninflammatory subepidermal blister in this case has formed at the level of the lamina lucida (Giemsa-stained section).

Figure 25-41 Porphyria. A noninflammatory blister is forming at the dermoepidermal junction; note the seemingly rigid dermal papillae at the base that contain the altered superficial vessels.

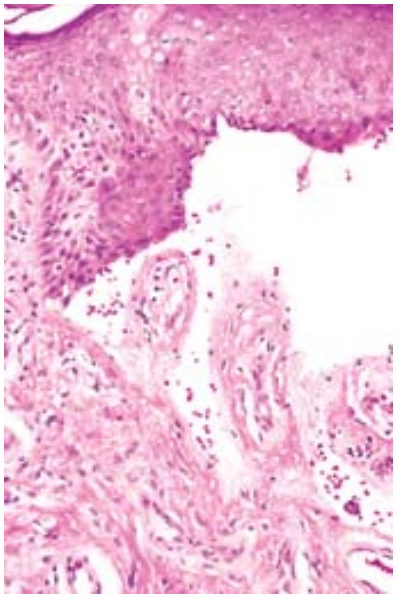


Figure 25-42 Acne. *A*, Inflammatory acne is characterized clinically by erythematous papules and pustules, with the possibility of eventual scarring. *B*, A portion of a hair shaft piercing the follicular epithelium and eliciting an inflammatory response and fibrosis.

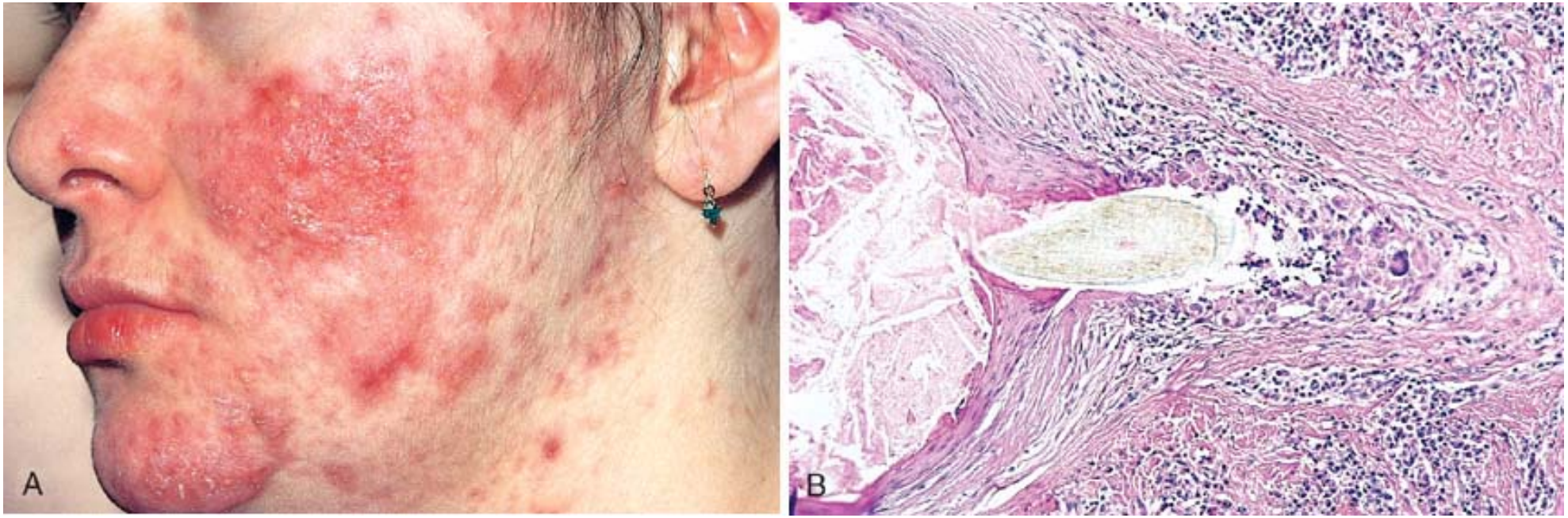


Figure 25-43 Verruca vulgaris. *A*, Multiple papules with rough pebble-like surfaces. *B* (low power) and *C* (high power), histology of the lesions show papillomatous epidermal hyperplasia and cytopathic alterations that include nuclear pallor and prominent keratohyaline granules. *D*, In situ hybridization showing viral DNA within epidermal cells.

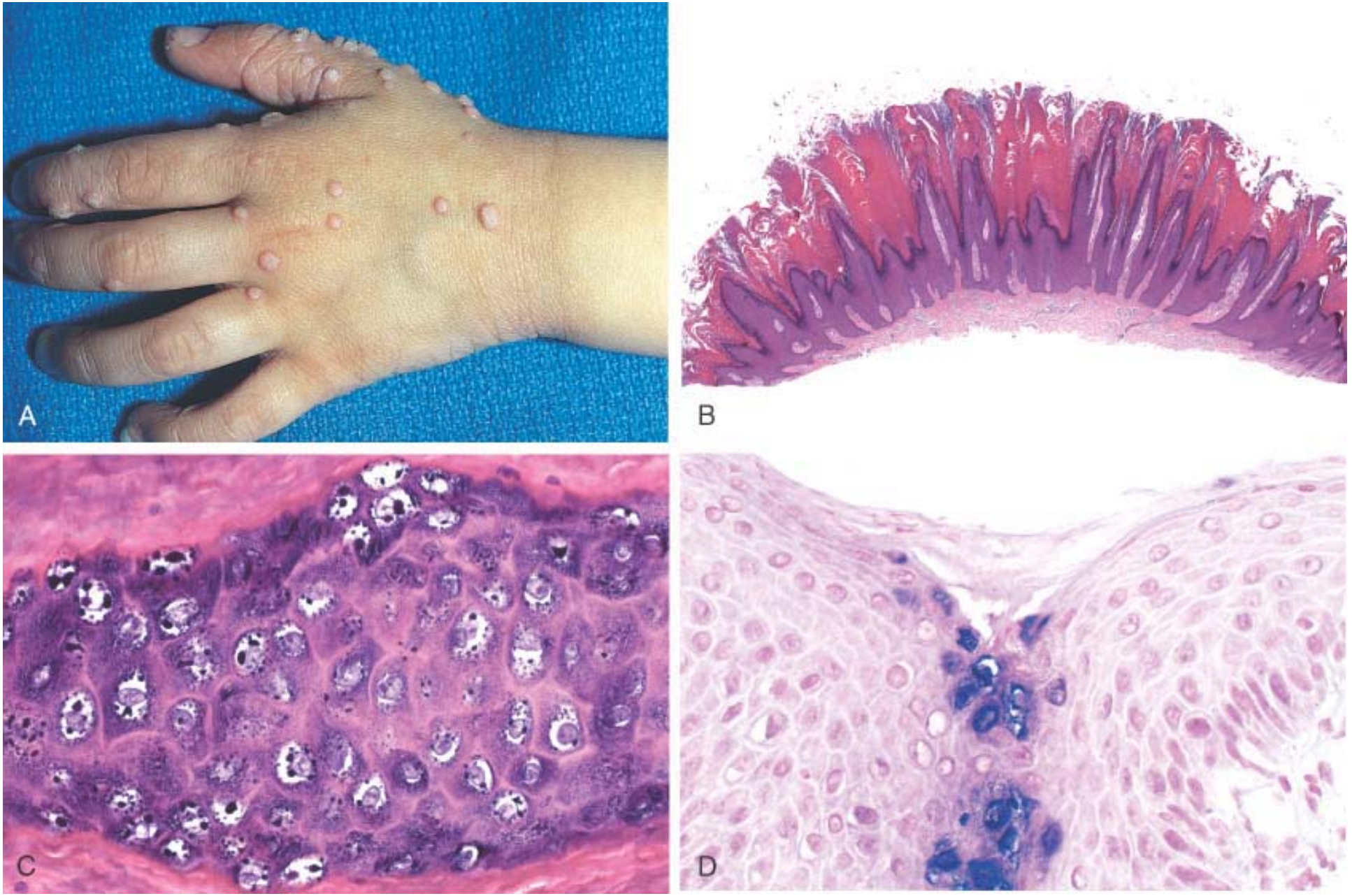


Figure 25-44 Molluscum contagiosum. A focus of verrucous epidermal hyperplasia contains numerous cells with ellipsoid cytoplasmic inclusions (molluscum bodies) within the stratum granulosum and stratum corneum.

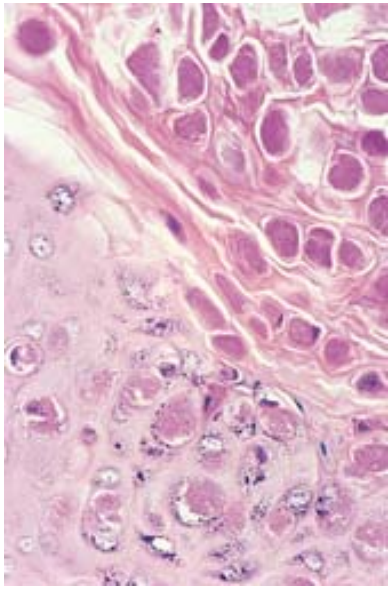


Figure 25-45 Tinea. *A*, Characteristic plaque of tinea corporis. Routine histology *(B)* shows the picture of mild eczematous (spongiotic) dermatitis, and periodic acid-Schiff stain reveals deep red hyphae and yeast forms *(C)* within the stratum corneum.

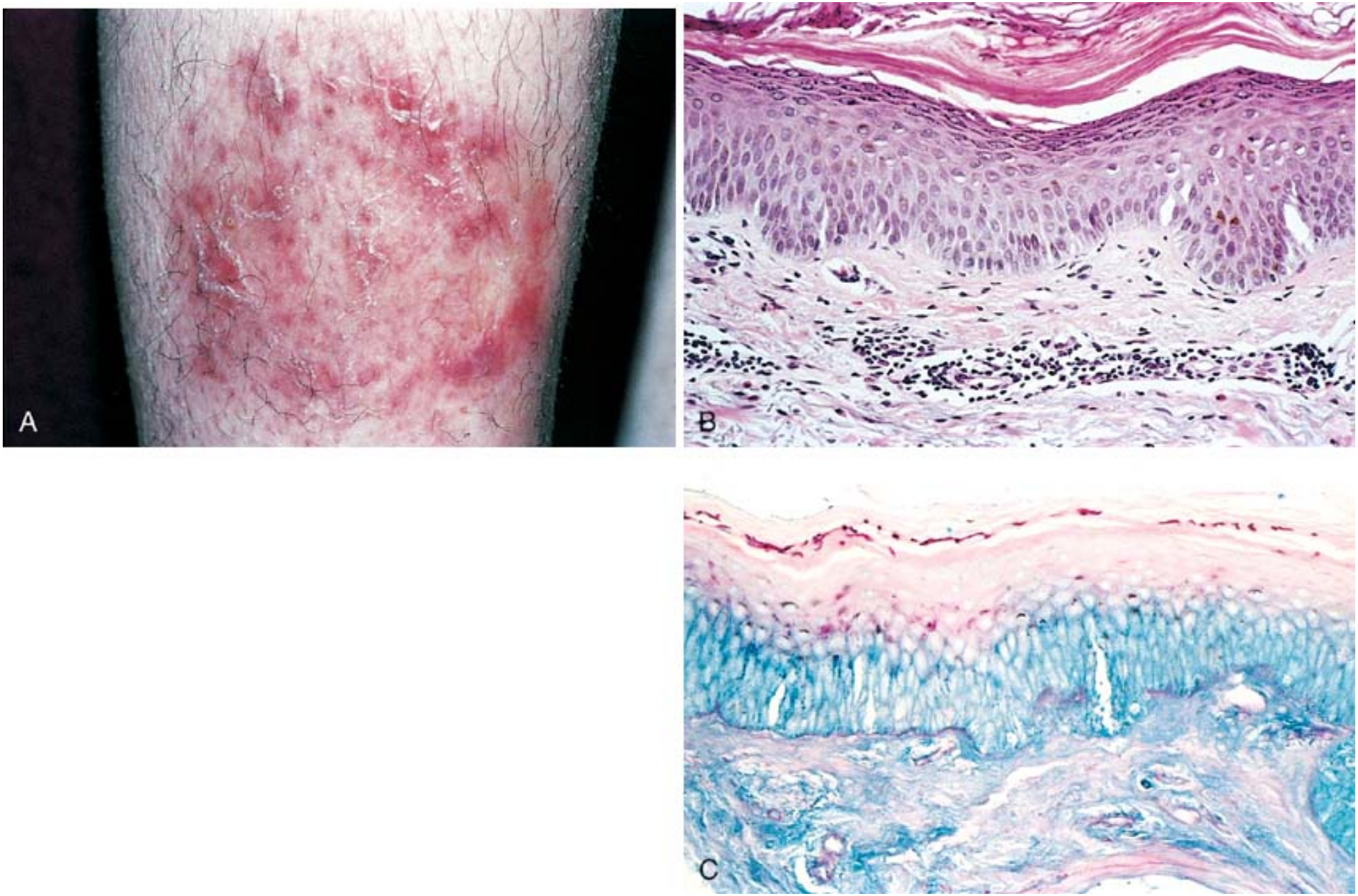
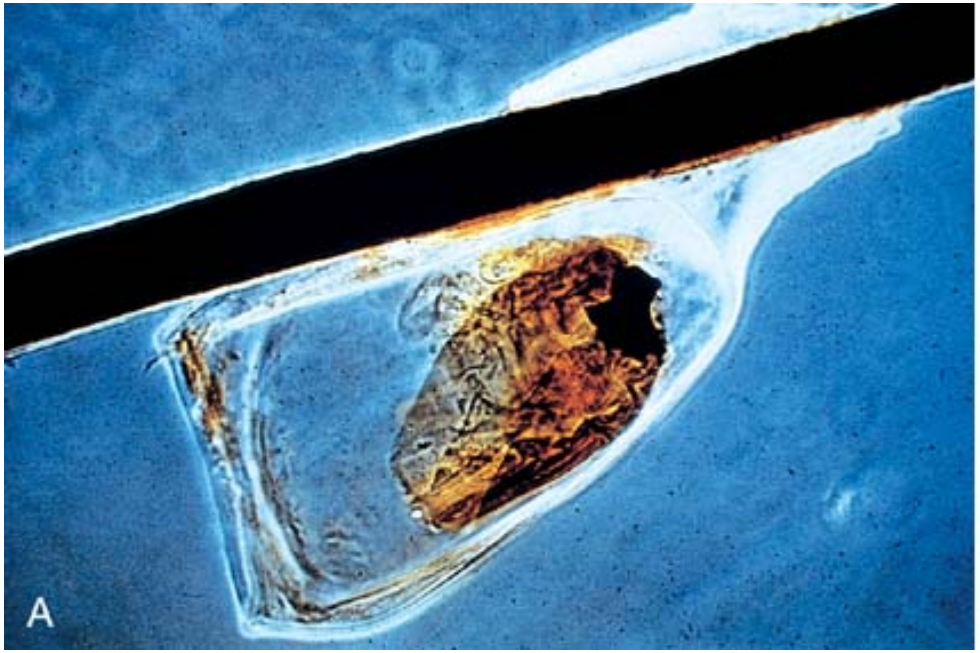
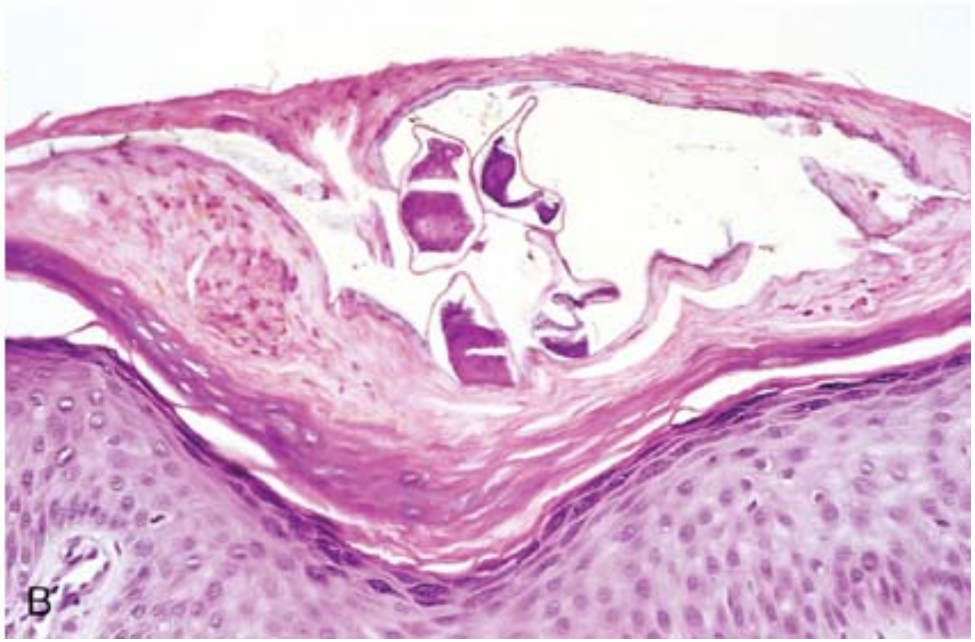


Figure 25-46 *A*, Pediculosis. Egg case (nit) of head louse attached to hair shaft. *B*, Portions of a scabies mite within a burrow involving the stratum corneum.





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Chapter 26 - Bones, Joints, and Soft Tissue Tumors

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Bones

Normal

The skeletal system is as vital to life as any organ system because of its essential roles in mechanical support and mineral homeostasis. Importantly, the skeleton also houses the hematopoietic elements, protects viscera, and determines body size and shape. The skeletal system is composed of 206 bones that vary in size and shape (tubular, flat, cuboid). The bones are interconnected by a variety of joints that allow for a wide range of movement while maintaining structural stability.

Bone is a type of connective tissue, and it is unique because it is one of the few tissues that normally undergo mineralization. Biochemically, it is defined by its distinctive admixture of inorganic elements (65%) and organic matrix (35%). The inorganic component, calcium hydroxyapatite [$10Ca:6(PO_4):(OH)_2$], is the mineral that gives bone strength and hardness, and is the storehouse for 99% of the body's calcium, 85% of the body's phosphorus, and 65% of the body's sodium and magnesium. The formation of hydroxyapatite crystal in bone is a phase transformation from liquid to solid analogous to the conversion of water to ice. The process involves the initiation and induction of mineralization by the organic matrix and it is tightly regulated by numerous factors.[1] The rate of mineralization can vary, but normally there is a 12- to 15-day lag time between the formation of the matrix and its mineralization. Bone that is unmineralized is known as *osteoid*.

The organic component includes the cells of bone and the proteins of the matrix. The bone-forming cells include the osteoprogenitor cells, osteoblasts, and osteocytes. The generation and stimulation of these cells are regulated by cytokines and growth factors such as bone morphogenic proteins (BMPs), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor, and transforming growth factor-β (TGF-β).[2]

- *Osteoprogenitor* cells are pluripotent mesenchymal stem cells that are located in the vicinity of all bony surfaces. When appropriately stimulated by growth factors such as bone morphogenic proteins, which are members of the TGF-β superfamily, they undergo cell division and produce offspring that differentiate into osteoblasts. The process of osteoblastic differentiation is initiated and governed by the transcription factor core binding factor a1, which activates osteoblast-specific gene expression.[3] The generation of osteoblasts from osteoprogenitor cells is vital to growth, remodeling, and repair of bone throughout life.
- *Osteoblasts and surface lining cells* are located on the surface of bone and synthesize, transport, and arrange

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the many proteins of matrix detailed later (Fig. 26-1). They also initiate the process of mineralization. Osteoblasts express cell-surface receptors that bind many hormones (parathyroid hormone [PTH], vitamin D, and estrogen), cytokines, growth factors, and extracellular matrix proteins. Recently, the hormone leptin and low-density lipoprotein receptor-related protein 5 have been shown to play an important role in determining osteoblastic activity, and they may represent evidence of central nervous system and cell-autonomous control of bone mass, respectively.[4][5][6]Metabolically active osteoblasts have a life span of approximately 3 months and then either undergo apoptosis, become surrounded by matrix and transform into *osteocytes*, or become quiescent, flattened, bone surface-lining cells.

- *Osteocytes* are more numerous than any other bone-forming cell and outnumber osteoblasts by about 10:1. Although encased by bone, they communicate with each other and with surface cells via an intricate network of tunnels through the matrix known as *canaliculi*. The osteocytic cell processes traverse the canaliculi, and their contacts along gap junctions allow the transfer of surface membrane potentials and substrates. The large number of osteocytic processes and their distribution throughout bone tissue enable them to be the key cells in several biologic processes. Studies have shown that this network may be important in controlling the second-to-second fluctuations in serum calcium and phosphorus levels by altering the concentration of these minerals in the local extracellular fluid compartment. Osteocytes also can detect mechanical forces and translate them into biologic activity, including the release of chemical mediators by signal transduction pathways, which activate second messengers such as cyclic adenosine monophosphate (cAMP).[7]
- The *osteoclast* is the cell responsible for bone resorption. It is derived from hematopoietic progenitor cells that also give rise to monocytes and macrophages. Information regarding the molecular regulation of osteoclast formation in humans is limited. In mice, a number of transcription factors, including PU.1 and Fos, are essential for developing an osteoclast phenotype. [8] The cytokines and growth factors crucial for osteoclast differentiation and maturation in humans include interleukin (IL)-1, IL-3, IL-6, IL-11, tumor

necrosis factor (TNF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and macrophage colony-stimulating factor (M-CSF).[9] These factors work by either stimulating osteoclast progenitor cells or participating in a paracrine system in which osteoblasts and marrow stromal cells play a central role. This paracrine system is essential to bone metabolism, and its mediators include the molecules RANK (Receptor Activator for Nuclear factor κB), RANK ligand (RANKL), and osteoprotegerin (OPG).[9] [10] RANK is a member of the TNF family of receptors expressed mainly on cells of macrophage/monocytic lineage such as preosteoclasts. When this receptor binds its specific ligand (RANKL) through cell-to-cell contact, osteoclastogenesis is initiated. RANKL is produced by and expressed on the cell membranes of osteoblasts and marrow stromal cells; its major role in bone metabolism is stimulation of osteoclast formation, fusion, differentiation, activation, and survival. The actions of RANKL can be blocked by another member of the TNF family of receptors, osteoprotegrin (OPG), which is a soluble protein produced by a number of tissues, including bone, hematopoietic marrow cells, and immune cells. OPG inhibits osteoclastogenesis by acting as a decoy receptor that binds to RANKL, thus preventing the interaction of RANK with RANKL. Therefore, interplay between bone cells and these molecules permits osteoblasts and stromal cells to control osteoclast development[10] (Fig. 26-2). This ensures the tight coupling of bone formation and resorption vital to the success of the skeletal system, and provides a mechanism for a wide variety of biologic mediators (hormones, cytokines, growth factors) to influence the homeostasis of bone tissue.

Mature multinucleated osteoclasts (containing 6 to 12 nuclei) form from fusion of circulating mononuclear precursors and have a limited life span (approximately 2 weeks). They are intimately related to the bone surface (Fig. 26-3), where their activity is initiated by binding to matrix adhesion proteins. The scalloped *resorption pits* they produce, and frequently reside in, are known as *Howship lacunae*. The portion of the osteoclast cell membrane overlying the resorption surface is modified by numerous villous extensions, known as the *ruffled border*, which serve to increase the membrane surface area. The plasmalemma bordering this region is specialized and forms a seal with the underlying bone, preventing leakage of digestion products. This self-contained extracellular space is analogous to a secondary lysosome, and the osteoclast acidifies it with a hydrogen pump system that solubilizes the mineral. The osteoclast also releases into this space a multitude of enzymes that help disassemble the matrix proteins into amino acids and liberate and activate growth factors, cytokines, and enzymes (such as collagenase), which have been previously deposited and bound to the matrix by osteoblasts. *Thus, as bone is broken down to its elemental units, substances are released into the microenvironment that initiate its renewal* (Fig. 26-4).

• The *proteins of bone* include type 1 collagen and a family of noncollagenous proteins that are derived mainly from osteoblasts. Type 1 collagen forms the backbone of matrix and accounts for 90% of the weight of the organic component. Osteoblasts deposit collagen either in a random weave known as *woven bone* or in an orderly layered manner designated

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lamellar bone(Fig. 26-5). Normally, woven bone is seen in the fetal skeleton and is formed at growth plates. Its advantages are that it is produced quickly and resists forces equally from all directions. The presence of woven bone in the adult is always indicative of a pathologic state; however, it is not diagnostic of a particular disease. For instance, in circumstances requiring rapid reparative stability, such as a fracture, woven bone is produced. It is also formed around sites of infection and composes the matrix of bone-forming tumors. *Lamellar bone, which gradually replaces woven bone during growth, is deposited much more slowly and is stronger than woven bone.*There are four different types of lamellar bone. Three are present only in the cortex—circumferential, concentric, and interstitial (Fig. 26-6). The fourth type, trabecular lamellae, composes the bone trabeculae in which the lamellae are oriented parallel to the long axis of the trabeculum.

The noncollagenous proteins of bone are bound to the matrix and grouped according to their function as adhesion proteins, calcium-binding proteins, mineralization proteins, enzymes, cytokines, and growth factors (Table 26-1).[11]Of these, only osteocalcin is unique to bone. It is used as a sensitive and specific serum marker for osteoblast activity. Cytokines and growth factors control bone cell proliferation, maturation, and metabolism.[12]They serve an important messenger function in translating mechanical and metabolic signals into local bone cell activity and eventual skeletal adaptation. In this fashion the skeleton is uniquely able to change its structure in response to new physical forces; witness the repositioning of teeth by the forces of braces.

Figure 26-1 Active osteoblasts synthesizing bone matrix. The surrounding spindle cells represent osteoprogenitor cells.

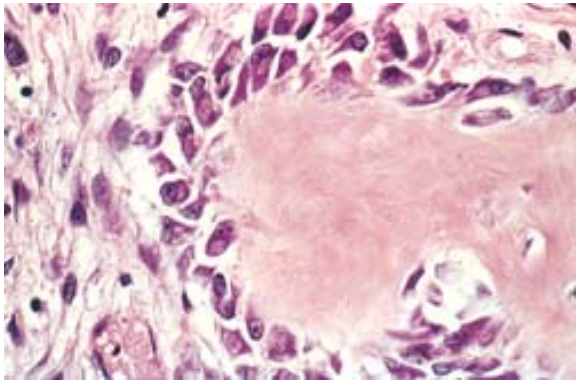

Figure 26-2 Paracrine molecular mechanisms that regulate osteoclast formation and function. Osteoclasts are derived from the same stem cells that produce macrophages. Osteoblast/ stromal cell membrane-associated RANK ligand (RANKL) binds to its receptor RANK located on the cell surface of osteoclast precursors. This interaction in the background of macrophage colony-stimulating factor (M-CSF) causes the precursor cells to produce functional osteoclasts. Stromal cells also secrete osteoprotegerin (OPG) which acts as a decoy receptor for RANKL, preventing it from binding the RANK receptor on osteoclast precursors. Consequently OPG prevents bone resorption by inhibiting osteoclast differentiation.

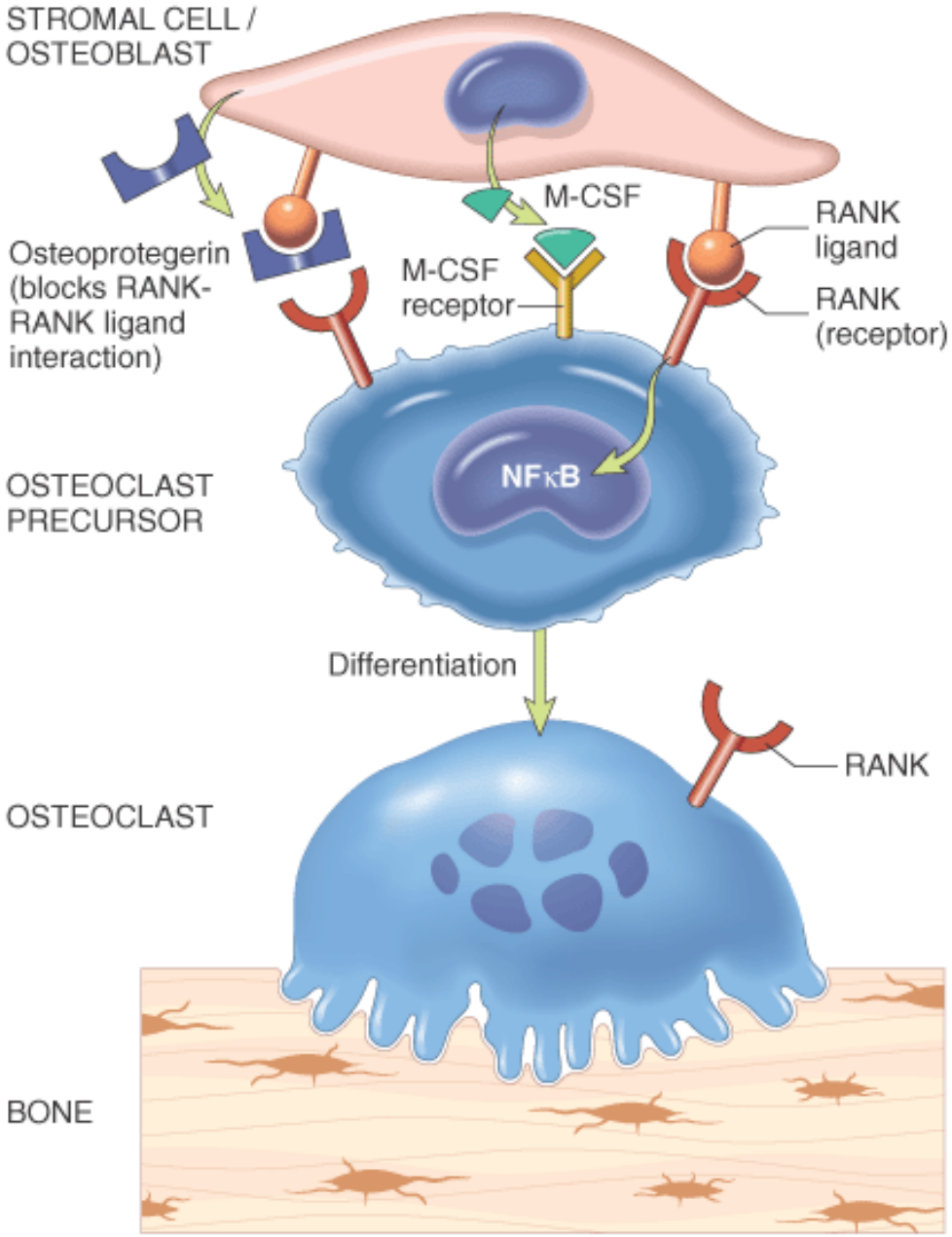

Figure 26-3 Two osteoclasts resorbing bone.

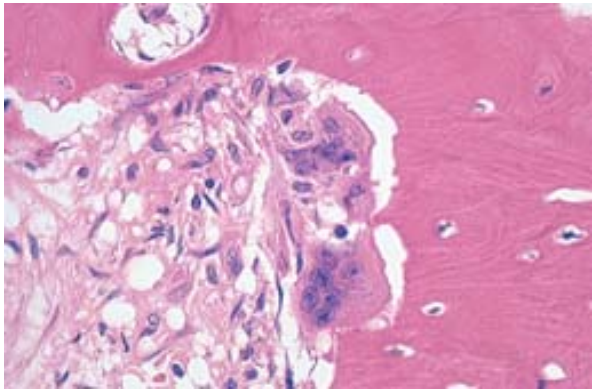

Figure 26-4 Bone resorption and formation are coupled processes that are controlled by systemic factors and local cytokines and growth factors, some of which are deposited in the bone matrix. Cytokines, growth factors, and signal-transducing molecules are key in the communication between osteoblasts and osteoclasts.

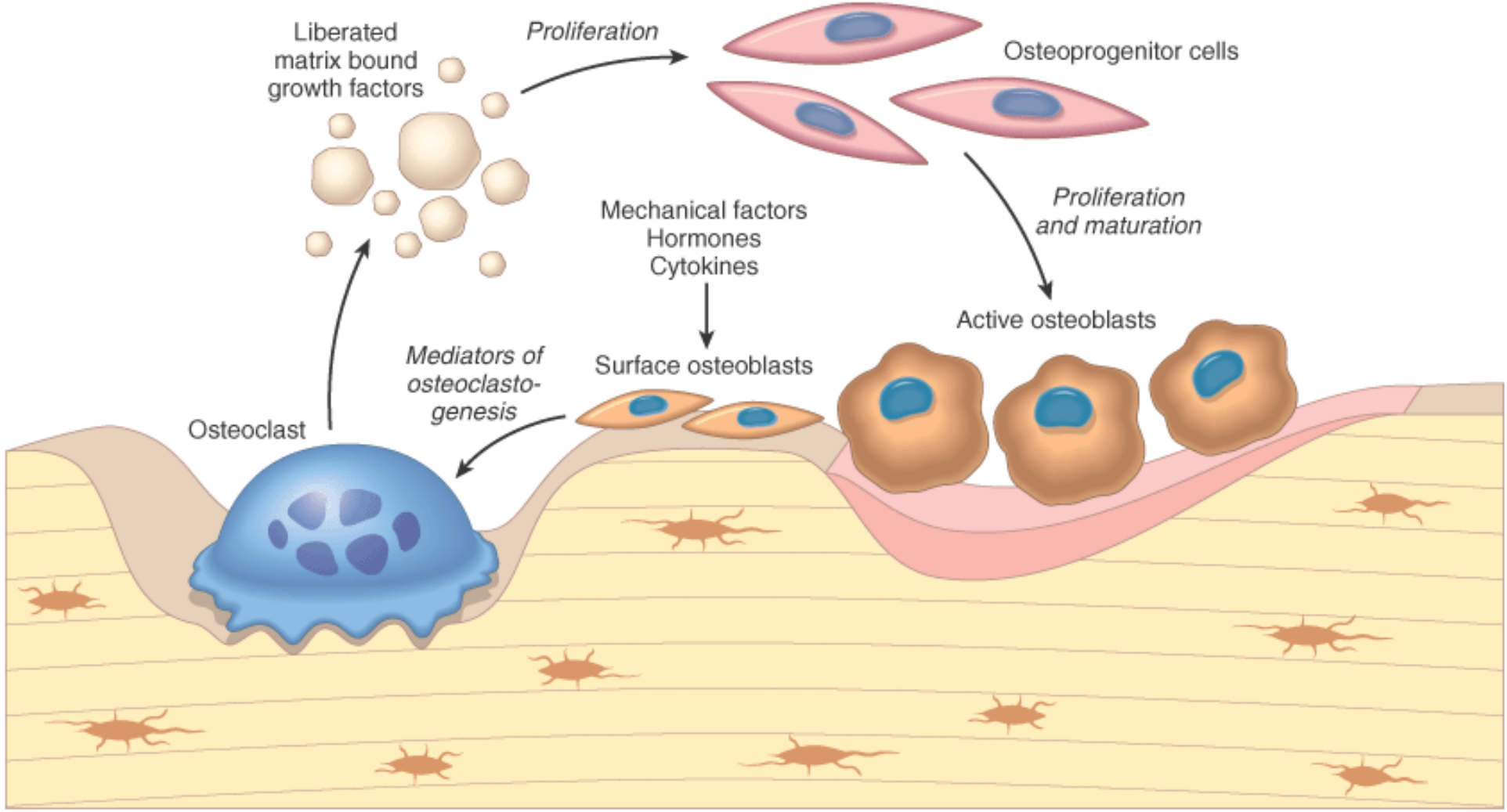

Figure 26-5 Woven bone *(top)* deposited on the surface of pre-existing lamellar bone *(bottom)*.

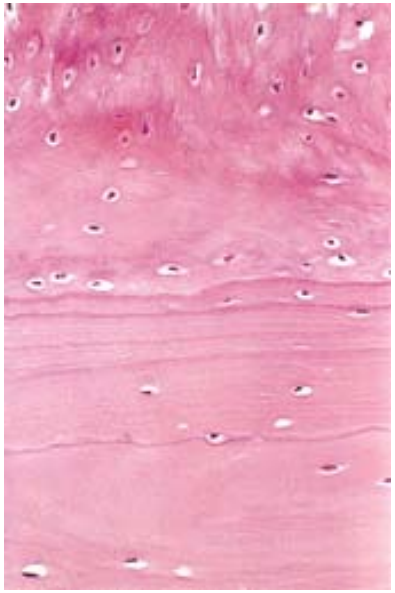

Figure 26-6 The schematic of normal bone structure reveals the subperiosteal and endosteal circumferential lamellae, concentric lamellae about vascular cores creating haversian systems, and the interstitial lamellae that fill the spaces in between the haversian systems. The trabecular lamellae extend from the endosteal surface. The individual lamellae are punctuated by osteocytic lacunae with their finely ramifying and interconnecting canals, which contain cell processes.

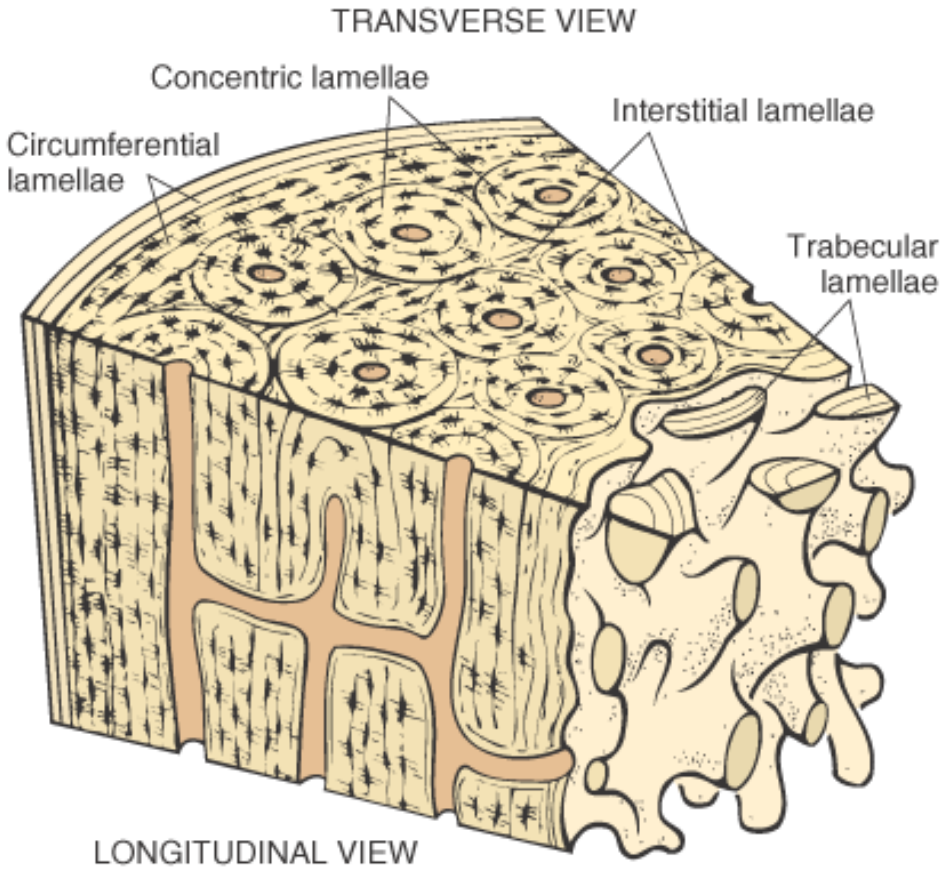

TABLE 26-1 -- Proteins of Bone Matrix

Osteoblast-Derived Proteins
Type 1 collagen
Cell adhesion proteins
••Osteopontin, fibronectin, thrombospondin
Calcium-binding proteins
••Osteonectin, bone sialoprotein
Proteins involved in mineralization
••Osteocalcin
Enzymes
••Collagenase, alkaline phosphatase
Growth factors

••IGF-1, TGF-β, PDGF
Cytokines
••Prostaglandins, IL-1, IL-6, RANKL
Proteins Concentrated from Serum
β_2 -microglobulin
Albumin
IGF, insulin-like growth factor; TGF, transforming growth factor; PDGF, platelet-derived growth factor; IL, interleukin; RANKL, RANK ligand.

degradative changes, mineralizes, and is removed by osteoclast-type cells. This process, which progresses up and down the length of the bone, allows for the ingrowth of blood vessels and osteoprogenitor cells that provide the bone-forming cells. Concurrently, the periosteum in the midshaft of the anlage produces osteoblasts that deposit the beginnings of the cortex. This region is known as the *primary center of ossification*. In the epiphyses, a similar sequence of events leading to the removal of cartilage occurs *(secondary center of ossification)* such that a plate of the cartilage model becomes entrapped between the expanding centers of ossification; this structure is known as the *physis*, or *growth plate* (Fig. 26-7). The chondrocytes within the growth plate undergo a series of events, including proliferation, growth, maturation, and necrosis. Important regulators of this sequence of chondrocyte growth and maturation are the *Indian hedgehog* gene and parathyroid hormone (PTH)-related protein (PTHRP).[5] [9] Eventually, the cartilage matrix mineralizes and this acts as a signal for its resorption by osteoclasts; however, remnant struts persist and act as scaffolding for the deposition of bone on their surfaces. These structures, composed of a core of cartilage covered by a layer of bone, are known as *primary spongiosa*. The process of enchondral ossification also occurs at the base of articular cartilage, and by this mechanism bones increase in length and articular surfaces increase in diameter. In contrast, bones derived from *intramembranous formation*, such as the cranium and portions of the clavicles, are formed by osteoblasts directly from a fibrous layer of tissue derived from mesenchyme. Because bone tissue is made only by osteoblasts, the enlargement of bones is achieved only by the deposition of new bone on a pre-existing surface. This mechanism of *appositional growth* is key to understanding the facets of bone growth and modeling.

Pathology

The skeletal system is susceptible to circulatory, inflammatory, neoplastic, metabolic, and congenital disorders, similar to the other organ systems of the body. The complexity of

Figure 26-7 Active growth plate with ongoing enchondral ossification. *1*, Reserve zone. *2*, Zone of proliferation. *3*, Zone of hypertrophy. *4*, Zone of mineralization. *5*, Primary spongiosa.

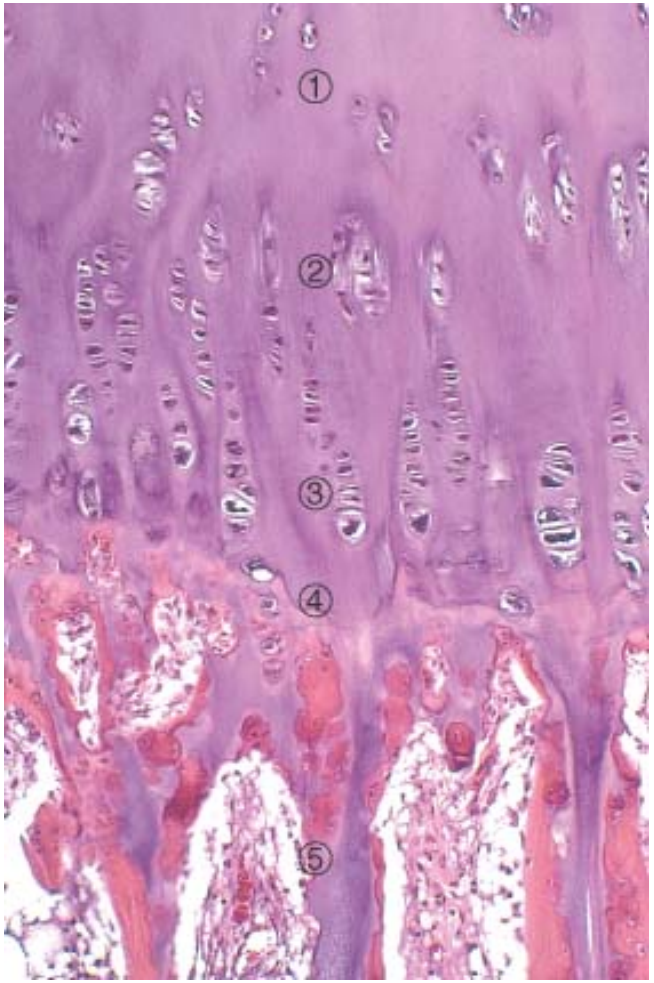

TABLE 26-2 -- Molecular Genetics of Diseases of the Skeleton

Human Disorder	Gene Mutation	Affected Molecule	Phenotype
Defects in Transcription Factors Producing Abnormalities in Mesenchymal Condensation and Related Cell Differentiation			
Synpolydactyly	HOXD-13	Transcription factor	Extra digit with fusion
Waardenburg syndrome	PAX-3	Transcription factor	Hearing loss, abnormal pigmentation, craniofacial abnormalities
Greig syndrome	GL13	Transcription factor	Synpolydactyly, craniofacial abnormalities
Campomelic dysplasia	SOX9	Transcription factor	Sex reversal, abnormal skeletal development
Oligodontia	PAX9	Transcription factor	Congenital absence of teeth
Nail-patella syndrome	LMX1B	Transcription factor	Hypoplastic nails, hypoplastic or aplastic patellae, dislocated radial head, progressive nephropathy

Holt-Oram syndrome	TBX5	Transcription factor	Congenital abnormalities, forelimb anomalies
Ulnar-mammary syndrome	TBX3	Transcription factor	Hypoplasia or absent ulna, 3rd–5th digits, breast, and teeth, delayed puberty
Cleidocranial dysplasia	CBFA1	Transcription factor	Abnormal clavicles, wormian bones, supernumerary teeth
Defects in Extracellular Structural Proteins			
Osteogenesis imperfecta types 1–4	COL1A1	Type 1 collagen	Bone fragility, hearing loss, blue sclerae
	COL1A2		Dentinogenesis imperfecta
Achondrogenesis II	COL2A1	Type 2 collagen	Short trunk, severely shortened extremities, relatively enlarged cranium, flattened face
Hypochondrogenesis	COL2A1		Short trunk, shortened extremities, relatively enlarged cranium, flattened face
Stickler syndrome	COL2A1	Type 2 collagen	Myopia, retinal detachment, hearing loss, flattened face, premature osteoarthritis
Multiple epiphyseal dysplasia	COL9A2	Type 9 collagen	Short or normal stature, small epiphyses, early onset osteoarthritis
Schmid metaphyseal chondrodysplasia	COL10A1	Type 10 collagen	Mild short stature, bowing of lower extremities, coxa vara, metaphyseal flaring
Defects in Hormones and Signal Transduction Mechanisms Producing Abnormal Proliferation or Maturation of Chondrocytes and Osteoblasts			
Brachydactyly type C	CDMP1	Signaling molecule	Shortened metacarpals and phalanges
Jansen metaphyseal chondroplasia	PTHrp receptor	Receptor	Short bowed limbs, clinodactyly, facial abnormalities, hypercalcemia, hypophosphatemia
Achondroplasia	FGFR3	Receptor	Short stature, rhizomelic shortening of limbs, frontal bossing, midface deficiency
Hypochondroplasia	FGFR3	Receptor	Disproportionate short stature, micromelia, relative macrocephaly
Thanatophoric dwarfism	FGFR3	Receptor	Severe limb shortening and bowing, frontal bossing, depressed nasal bridge
Crouzon syndrome	FGFR2	Receptor	Craniosynostosis

Adapted from Mundlos S, Olsen BR: Heritable diseases of the skeleton. Part I: Molecular insights into skeletal development—transcription factors and signaling pathways. Faseb J 11:125–132, 1997; Mundlos S, Olsen BR: Heritable diseases of the skeleton. Part II: Molecular insights into skeletal development—matrix components and their homeostasis. Faseb J 11:227–233, 1997; Superti-Furga A, Bonafe L, Rimoin DL: Molecular-pathogenetic classification of genetic disorders of the skeleton. Am J Med Genet 106:282–293, 2001.

(Table 26-3). The *type II variant* is at one end of the spectrum and is uniformly fatal in utero or during the perinatal period. It is characterized by extraordinary bone fragility with multiple fractures occurring when the fetus is still within the womb (Fig. 26-8). In contrast, the *type I form*, which is more often due to an acquired rather than an inherited mutation, permits a normal life span but with an increased number of fractures during childhood that decrease in frequency after puberty. Other findings include *blue sclerae* caused by a decrease in collagen content, making the sclera translucent and allowing partial visualization of the underlying choroid; *hearing loss* related to both a sensorineural deficit and impaired conduction owing to abnormalities in the bones of the middle and inner ear; and *dental imperfections* (small, misshapen, and blue-yellow teeth) secondary to a deficiency in dentin. In some variants, the skeleton fails to model properly, and there are persistent foci of hypercellular woven bone.[18] New and less well-characterized variants are still being identified. The recognition of particular variants and their modes of inheritance is important in genetic counseling.

Types 2, 10, and 11 Collagen Diseases

Types 2, 10, and 11 collagens are important structural components of hyaline cartilage. Mutations that result in their abnormal metabolism, although uncommon, produce a spectrum of

disorders ranging from those that are fatal to those compatible with life but associated with early destruction of joints (see Table 26-2). More than 30 mutations have been identified in the type 2 collagen gene, and all have affected the triple helical component of the molecule. In severe disorders, the type 2 collagen molecules are not secreted by the chondrocytes, and insufficient bone formation occurs. In the milder phenotypes, a nonfunctioning or null collagen gene

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TABLE 26-3 -- Osteogenesis Imperfecta

	Subtype	Inheritance	Collagen Defect	Major Clinical Features
OI I	Postnatal fractures, blue sclerae	Autosomal dominant	Decreased synthesis pro-α1(1) chain	Compatible with survival
			Abnormal pro-α1(1) or pro-α2(1) chains	Normal stature
				Skeletal fragility
				Dentinogenesis imperfecta
				Hearing impairment
				Joint laxity
				Blue sclerae
OI II	Perinatal lethal	Most autosomal recessive	Abnormally short pro-α1(1) chain	Death in utero or within days of birth
		Some autosomal dominant	Unstable triple helix	Skeletal deformity with excessive fragility and multiple fractures
		?New mutations	Abnormal or insufficient pro-α2(1)	Blue sclerae
OI III	Progressive deforming	Autosomal dominant (75%)	Altered structure of pro-peptides of pro-α2(1)	Compatible with survival
		Autosomal recessive (25%)	Impaired formation of triple helix	Growth retardation
				Multiple fractures
				Progressive kyphoscoliosis
				Blue sclerae at birth that become white
				Hearing impairment
				Dentinogenesis imperfecta
OI IV	Postnatal fractures, normal sclerae	Autosomal dominant	Short pro-α2(1) chain	Compatible with survival
			Unstable triple helix	Moderate skeletal fragility

				Short stature
				Sometimes dentinogenesis imperfecta

OI, osteogenesis imperfecta.

allele is formed, leading to a reduced content of type 2 collagen in the cartilage. [12]

DISEASES ASSOCIATED WITH DEFECTS IN FOLDING AND DEGRADATION OF MACROMOLECULES

Mucopolysaccharidoses

The mucopolysaccharidoses, as discussed in Chapter 5 , are a group of lysosomal storage diseases caused by deficiencies in the enzymes that degrade dermatan sulfate, heparan sulfate, and keratan sulfate. The implicated enzymes are mainly acid hydrolases. Mesenchymal cells, especially chondrocytes, play an important role in the metabolism of extracellular matrix mucopolysaccharides and therefore are most severely affected. Consequently, many of the skeletal manifestations of the mucopolysaccharidoses result from abnormalities in hyaline cartilage, including the cartilage anlage, growth plates, costal cartilages, and articular surfaces. It is not surprising therefore that patients with mucopolysaccharidoses are frequently of short stature and have chest wall abnormalities and malformed bones.

DISEASES ASSOCIATED WITH DEFECTS IN METABOLIC PATHWAYS (ENZYMES, ION CHANNELS, AND TRANSPORTERS)

Osteopetrosis

Osteopetrosis refers to a group of rare genetic diseases that are characterized by reduced osteoclast bone resorption, resulting in diffuse symmetric skeletal sclerosis (Fig. 26-9). The term *osteopetrosis* was coined because of the stonelike quality of the bones; however, the bones are abnormally brittle and fracture like a piece of chalk. Osteopetrosis, which is also known as *marble bone disease* and *Albers-Schönberg disease,* is classified into variants based on both the mode of inheritance and the clinical findings. The autosomal recessive malignant type and the autosomal dominant benign type are the most common variants.

Pathogenesis.

Four types of osteopetrosis have been identified: infantile malignant osteopetrosis, type II carbonic anhydrase deficiency, and autosomal-dominant types I and II. However, the precise nature of the osteoclast dysfunction in many cases remains unknown. An example of a form of the disease in which the molecular mechanism is understood is the variant associated with *carbonic anhydrase II* deficiency.[19] Carbonic anhydrase II is required by osteoclasts and renal tubular cells to excrete hydrogen ions and acidify their environment. The absence of this enzyme prevents osteoclasts from acidifying the resorption pit and solubilizing the hydroxyapatite crystals and also blocks the acidification of urine by renal tubular cells. In another form of the disease, a mutation in the *ClC-7* chloride channel gene causes osteoclast dysfunction by interfering with the chloride channel that is important in the proton pump of the H^+ -ATPase located on the osteoclast ruffled border. Consequently, osteoclasts cannot acidify the resorption pit, thus preventing the digestion of bone.[20] In mice, osteopetrosis can also be caused by targeted mutations in the genes coding for M-CSF, *c-src,* RANK, and OPG.[19] It is possible that some of these genes will be linked to the human disease.

Morphology.

The morphologic changes of osteopetrosis are explained by deficient osteoclast activity. Grossly the bones lack a medullary canal, and the

Figure 26-8 Skeletal radiograph of a fetus with lethal type II osteogenesis imperfecta. Note the numerous fractures of virtually all bones, resulting in accordion-like shortening of the limbs.

Figure 26-9 Radiograph of the upper extremity in a patient with osteopetrosis. The bones are diffusely sclerotic, and the distal metaphyses of the ulna and radius are poorly formed (Erlenmeyer flask deformity).

Figure 26-10 Section of proximal tibial diaphysis from a fetus with osteopetrosis. The cortex *(1)* is being formed, and the medullary cavity *(2)* is abnormally filled with primary spongiosa replacing the hematopoietic elements.

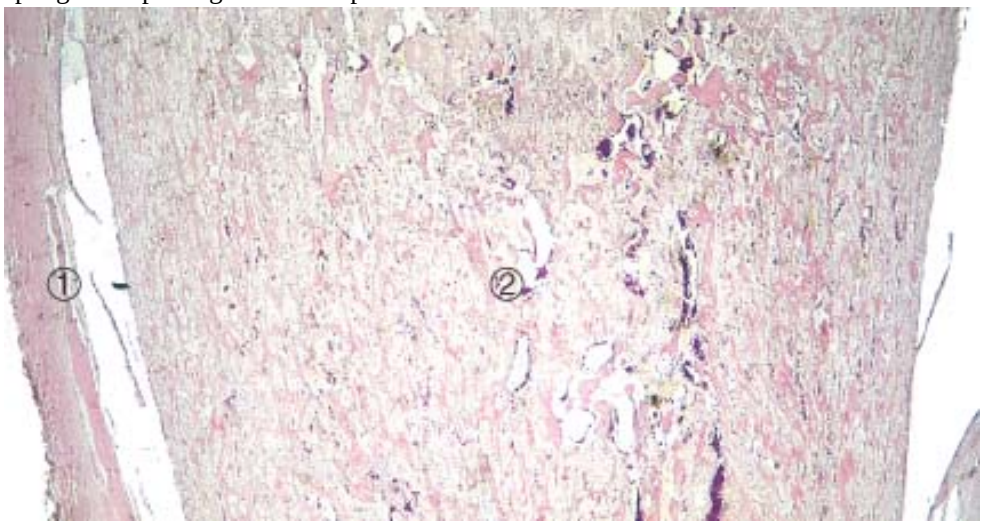


TABLE 26-4 -- Categories of Generalized Osteoporosis

Primary	
Postmenopausal	••Idiopathic

Senile	
Secondary	
Endocrine disorders	**Rheumatologic disease**
••Hyperparathyroidism	**Drugs**
••Hypo-hyperthyroidism	••Anticoagulants
••Hypogonadism	••Chemotherapy
••Pituitary tumors	••Corticosteroids
••Diabetes, type 1	••Anticonvulsants
••Addison disease	••Alcohol
Neoplasia	**Miscellaneous**
••Multiple myeloma	••Osteogenesis imperfecta
••Carcinomatosis	••Immobilization
Gastrointestinal	••Pulmonary disease
••Malnutrition	••Homocystinuria
••Malabsorption	••Anemia
••Hepatic insufficiency	
••Vitamin C, D deficiencies	

new hypotheses in the pathogenesis of osteoporosis (Fig. 26-11):

• *Age-related changes* in bone cells and matrix have a strong impact on bone metabolism. Osteoblasts from elderly individuals have reduced replicative and biosynthetic potential when compared with osteoblasts from younger individuals.[22] Also, proteins bound to the extracellular matrix (such as growth factors, which are mitogenic to osteoprogenitor cells and stimulate osteoblastic synthetic activity) lose their biologic potency over time. The end result is a skeleton populated by bone-forming cells that have a diminished capacity to make bone. This form of osteoporosis, also known as *senile osteoporosis*, is categorized as a *low turnover variant*.
• *Reduced physical activity* increases the rate of bone loss in experimental animals and humans because mechanical forces are important stimuli for normal bone remodeling. The bone loss seen in an immobilized or paralyzed extremity, the reduction of skeletal mass observed in astronauts subjected to a gravity-free environment for prolonged periods, and the higher bone density in athletes as compared with nonathletes all support a role for physical activity in preventing bone loss. The type of exercise is important because load magnitude influences bone density more than the number of load cycles. Because muscle contraction is the dominant source of skeletal loading, it is logical that resistance exercises such as weight training are more effective stimuli for increasing bone mass than repetitive endurance activities such as jogging. Certainly the decreased physical activity that is associated with aging contributes to senile osteoporosis.
• *Genetic factors are also important*, as noted previously. The type of vitamin D receptor molecule that is inherited accounts for approximately 75% of the maximal peak mass achieved. Polymorphism in the vitamin D receptor molecule is associated with either a higher or lower maximal bone mass. Calcium deficiency, increased PTH levels, and reduced levels of vitamin D also may play a role in the development of senile osteoporosis.

• The body's calcium *nutritional state* is important. It has been shown that adolescent girls (but not boys) have insufficient calcium intake in the diet. This calcium deficiency occurs during a period of rapid bone growth, stunting the peak bone mass ultimately achieved; thus, these individuals are at greater risk of developing osteoporosis.
• *Hormonal influences.* In the decade after menopause, yearly reductions in bone mass may reach up to 2% of cortical bone and 9% of cancellous bone. Women may lose as much as 35% of their cortical bone and 50% of their trabecular bone within the 30 to 40 years after menopause. It is thus no surprise that 1 out of every 2 women suffers an osteoporotic fracture, in contrast to 1 in 40 men. *Postmenopausal osteoporosis* is characterized by a hormone-dependent acceleration of bone loss that occurs during the decade after menopause. *Estrogen deficiency plays the major role in this phenomenon, and estrogen replacement at menopause is protective against bone loss.* The effects of estrogen on bone mass are mediated by cytokines. Decreased estrogen levels result in increased secretion of IL-1, IL-6, and TNF by blood monocytes and bone marrow cells.[23] These cytokines are potent stimulators of osteoclast recruitment and activity; they act, in part, by increasing the levels of RANK and RANKL and diminishing the quantity of OPG. Compensatory osteoblastic activity occurs, but it does not keep pace, leading to what is classified as a *high turnover form* of osteoporosis.

Figure 26-11 Pathophysiology of postmenopausal and senile osteoporosis (see text).

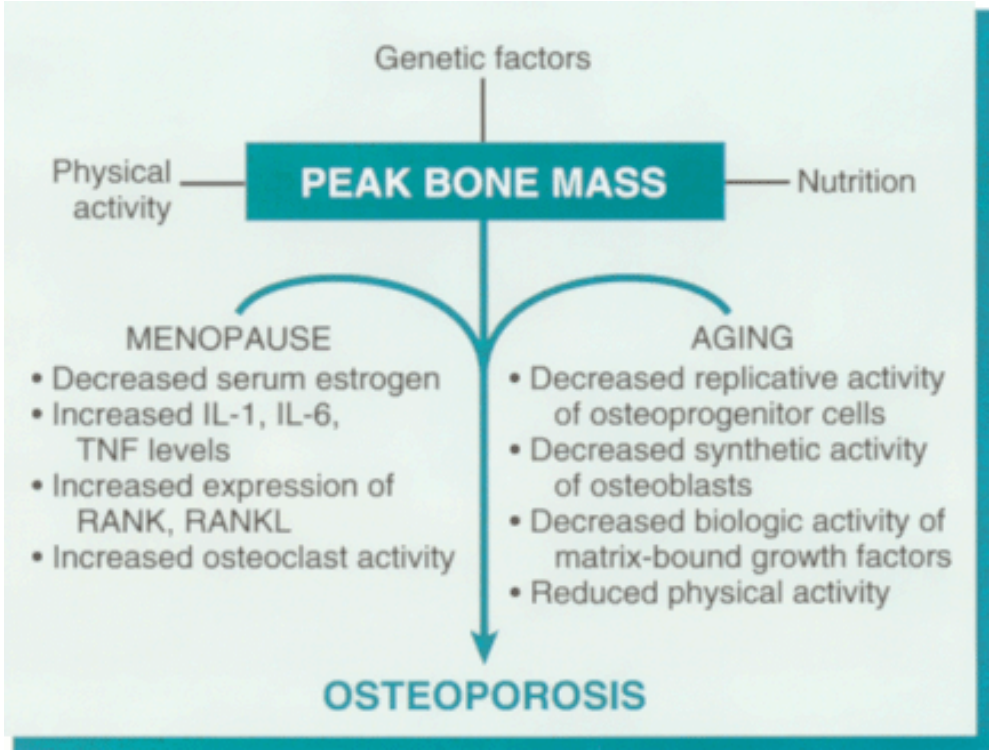
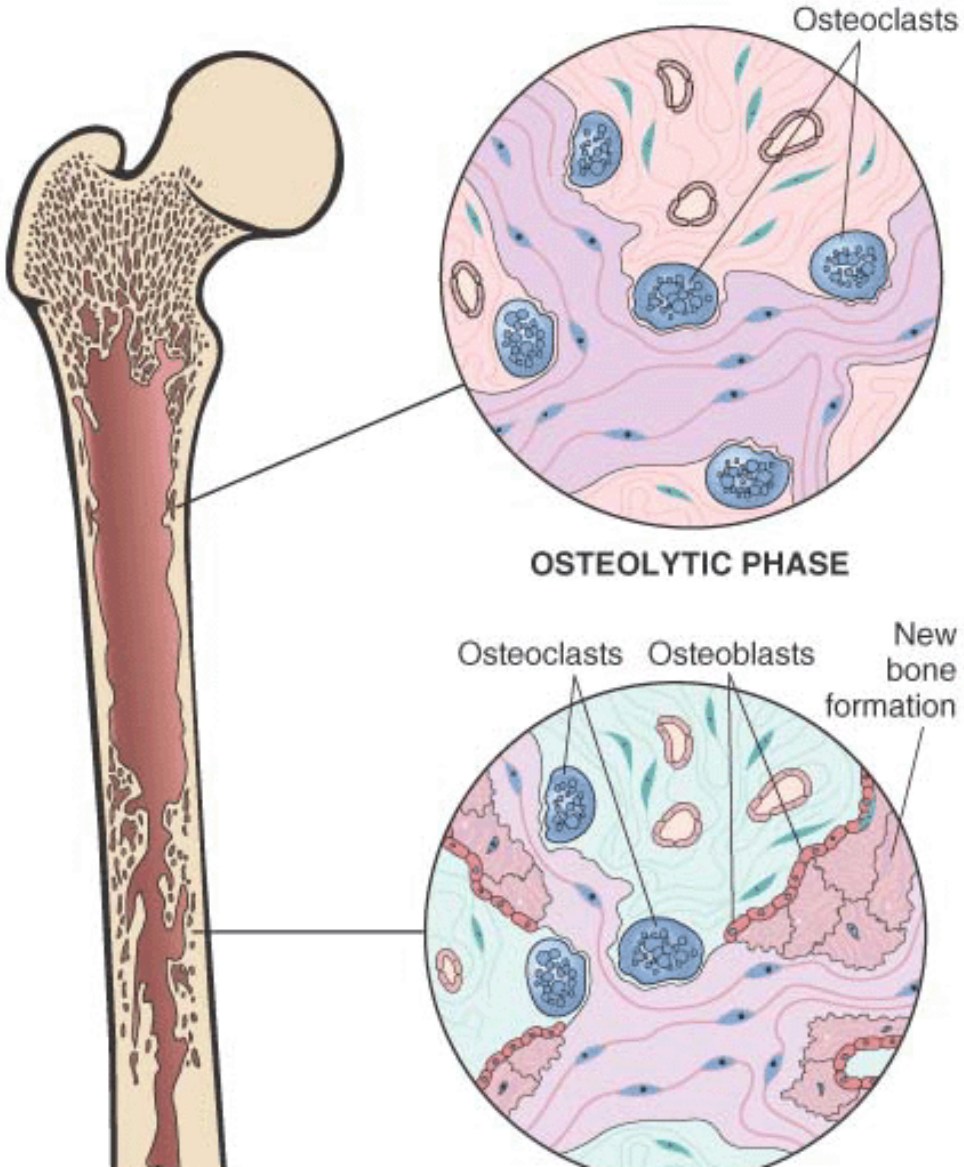


Figure 26-12 Osteoporotic vertebral body *(right)* shortened by compression fractures, compared with a normal vertebral body. Note that the osteoporotic vertebra has a characteristic loss of horizontal trabeculae and thickened vertical trabeculae.

Figure 26-13 Diagrammatic representation of Paget disease of bone, demonstrating the three phases in the evolution of the disease.



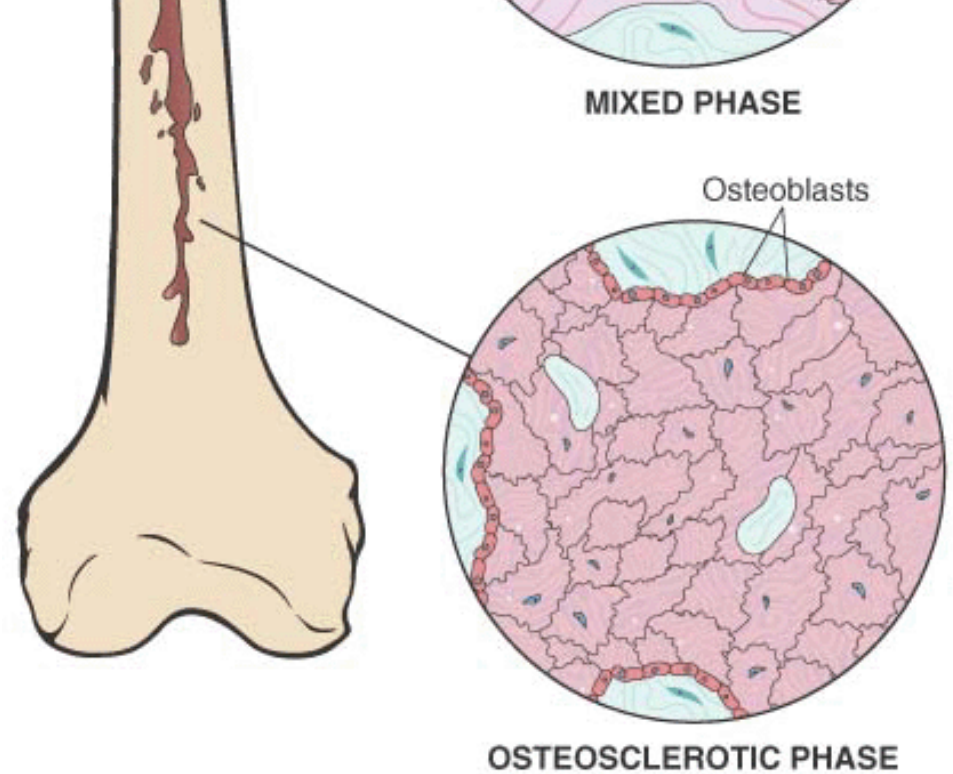


Figure 26-14 Mosaic pattern of lamellar bone pathognomonic of Paget disease.

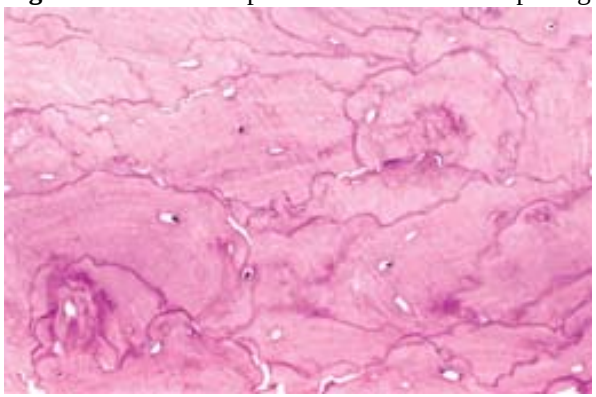


Figure 26-15 Paget disease of the humerus. *A*, The three sequential stages: *(1)* lytic, *(2)* mixed, and *(3)* sclerotic. *B*, Area 1, the lytic stage, is seen in close-up. Area 2, the mixed stage *(upper portion of B)* reveals central and endosteal cortical resorption and replacement by less compact new bone. *C*, Area 3, the sclerotic stage, with irregular thickening of both cortical and trabecular bone. *(From Maldague B, Malghem J: Dynamic radiologic pattern of Paget's disease of bone. Clin Orthop 217:127, 1987.)*

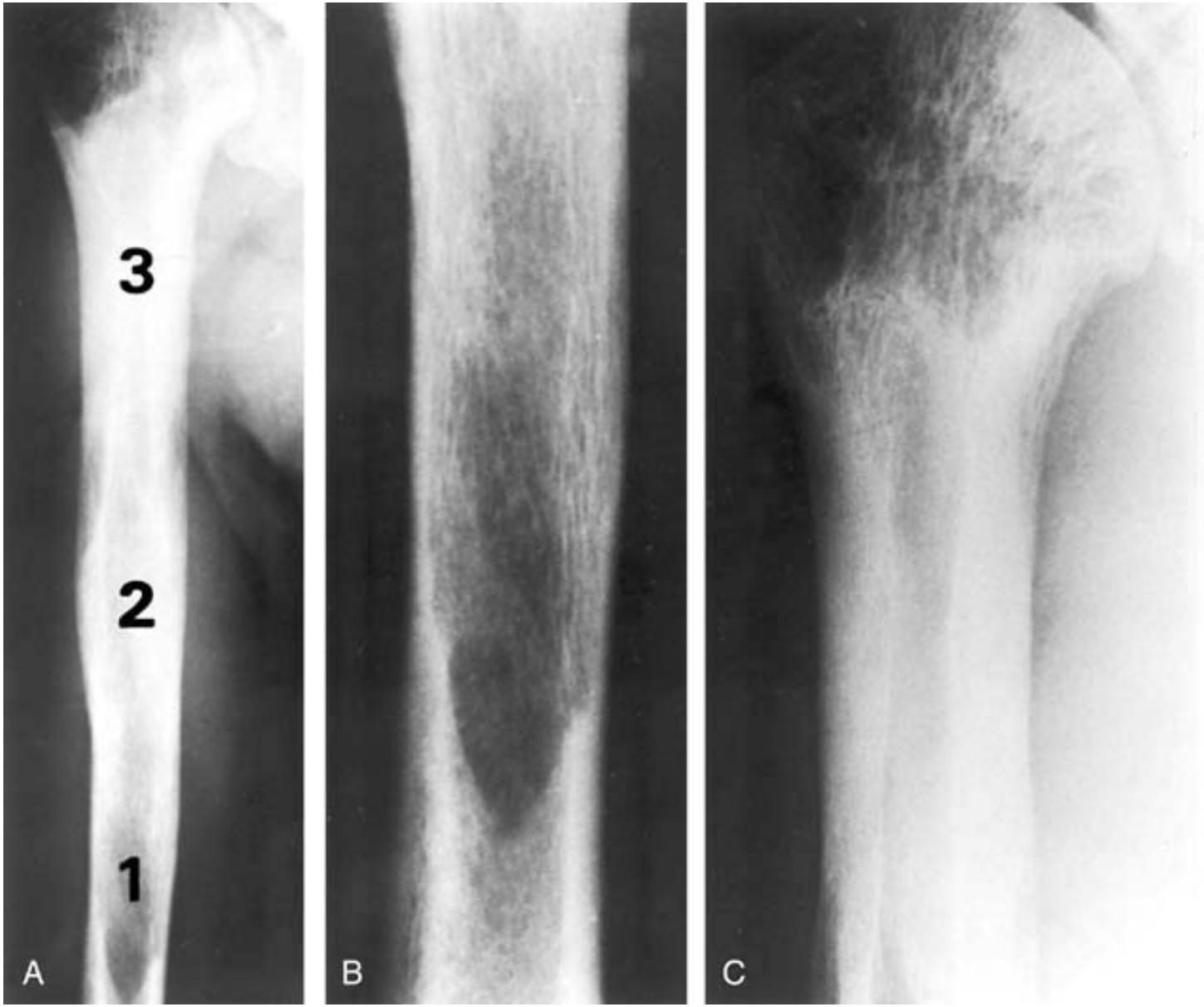


Figure 26-16 Hyperparathyroidism with osteoclasts boring into the center of the trabeculum (dissecting osteitis).

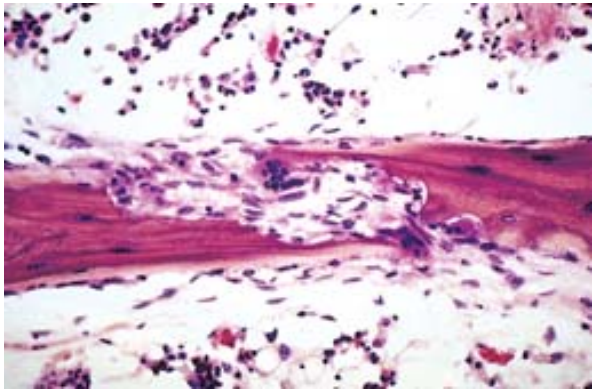


Figure 26-17 Resected rib, harboring an expansile brown tumor adjacent to the costal cartilage.

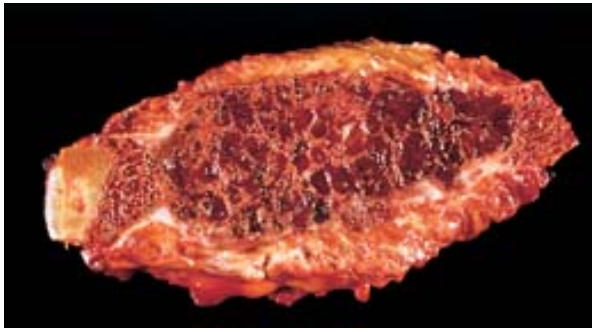


Figure 26-18 *A*, Recent fracture of the fibula. *B*, Marked callus formation 6 weeks later. *(Courtesy of Dr. Barbara Weissman, Brigham and Women's Hospital, Boston, MA.)*

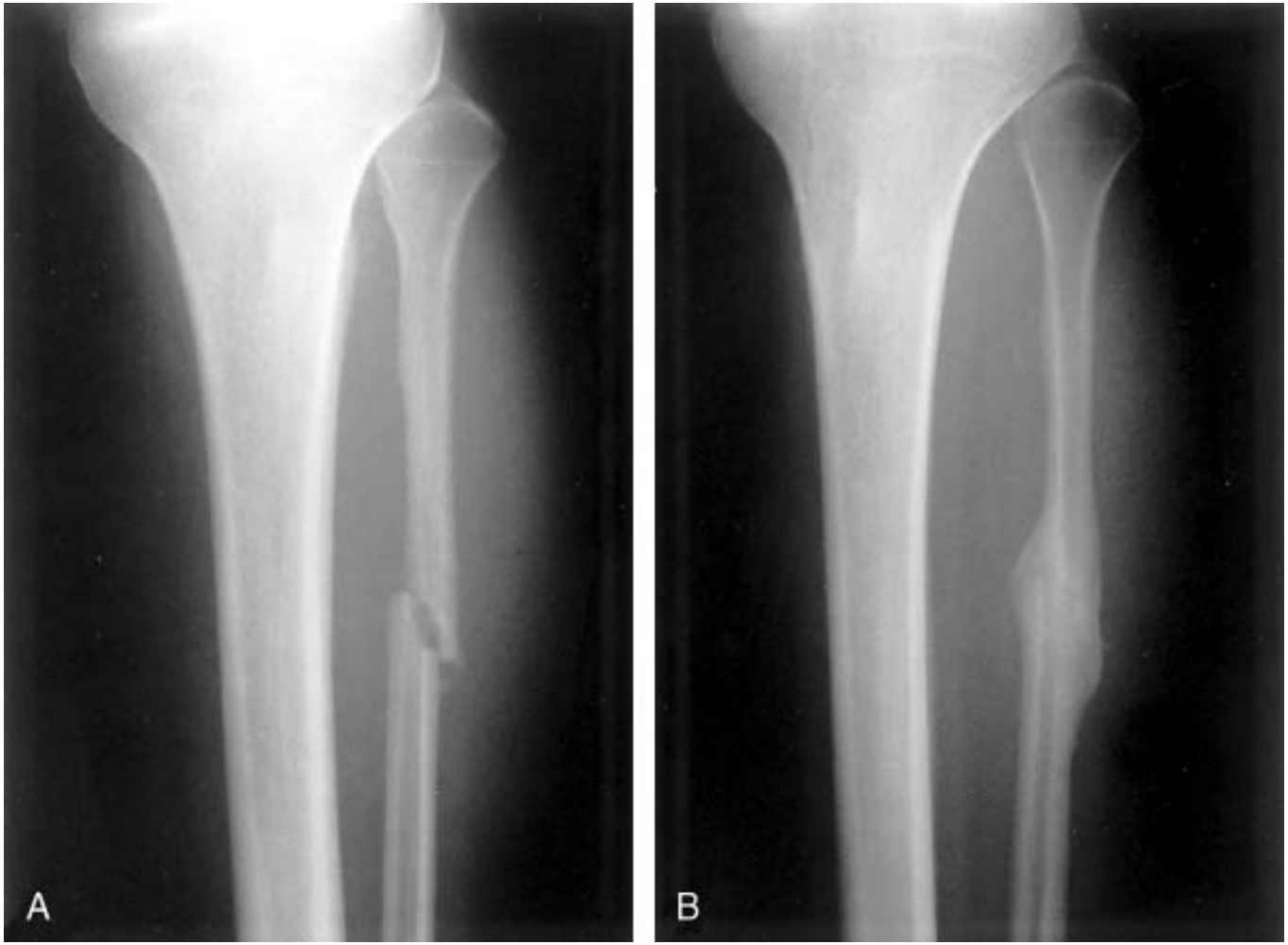


TABLE 26-5 -- Disorders Associated with Osteonecrosis

Idiopathic	Pregnancy
Trauma	Gaucher disease
Corticosteroid administration	Sickle cell and other anemias
Infection	Alcohol abuse
Dysbarism	Chronic pancreatitis
Radiation therapy	Tumors
Connective tissue disorders	Epiphyseal disorders

pathophysiology underlying steroid-induced bone infarcts is obscure. The infarcts follow high-dose steroid therapy for short periods, long-term administration of smaller doses, and even intra-articular injections.

Morphology.

The pathologic features of bone necrosis are the same regardless of the cause. In medullary infarcts, the necrosis is geographic and involves the cancellous bone and marrow. The cortex is usually not affected because of its collateral blood flow. In subchondral infarcts, necrosis involves a triangular or wedge-shaped segment of tissue that has the subchondral bone plate as its base and the center of the epiphysis as its apex. The overlying articular cartilage remains viable because it receives nutrition from the synovial fluid. The dead bone, recognized by its empty lacunae, is surrounded by necrotic adipocytes that frequently rupture, releasing their fatty acids, which bind calcium and form insoluble calcium soaps that may remain for life. In the healing response, osteoclasts resorb the necrotic trabeculae; however, those that remain act as scaffolding for the deposition of new living bone in a process known as **creeping substitution**. In subchondral infarcts, the pace of creeping substitution is too slow to be effective so there is eventual collapse of the necrotic cancellous bone and distortion, fracture, and even sloughing of the articular cartilage (Fig. 26-19).

Clinical Course.

The symptoms depend on the location and extent of infarction. Typically, subchondral infarcts cause chronic pain that is initially associated only with physical activity but then becomes progressively more constant as secondary changes supervene. In contrast, medullary infarcts are clinically silent except for large ones occurring in Gaucher disease, dysbarism, and hemoglobinopathies. Medullary infarcts usually remain stable over time and rarely are the site of malignant transformation. Subchondral infarcts, however, often collapse and may predispose to severe, secondary

Figure 26-19 Femoral head with a subchondral, wedge-shaped pale yellow area of osteonecrosis. The space between the overlying articular cartilage and bone is caused by trabecular compression fractures without repair.

Figure 26-20 Resected femur in a patient with draining osteomyelitis. The drainage tract in the subperiosteal shell of viable new bone (involucrum) reveals the inner native necrotic cortex (sequestrum).

TABLE 26-6 -- Classification of Primary Tumors Involving Bones

Histologic Type	Benign	Malignant
Hematopoietic (40%)		Myeloma
		Malignant lymphoma
Chondrogenic (22%)	Osteochondroma	Chondrosarcoma
	Chondroma	Dedifferentiated chondrosarcoma
	Chondroblastoma	Mesenchymal chondrosarcoma
	Chondromyxoid fibroma	
Osteogenic (19%)	Osteoid osteoma	Osteosarcoma
	Osteoblastoma	
Unknown origin (10%)	Giant cell tumor	Ewing tumor
		Giant cell tumor
		Adamantinoma
Histiocytic origin	Fibrous histiocytoma	Malignant fibrous histiocytoma
Fibrogenic	Metaphyseal fibrous defect (fibroma)	Desmoplastic fibroma
		Fibrosarcoma
Notochordal		Chordoma
Vascular	Hemangioma	Hemangioendothelioma
		Hemangiopericytoma

Lipogenic	Lipoma	Liposarcoma
Neurogenic	Neurilemmoma	

Data on percentage of each type from Unni KK: Dahlin's Bone Tumors, 5th ed. Philadelphia, Lippincott-Raven, 1996, p 4; by permission of Mayo Foundation.

acquired syphilis. Gummata also occur in the acquired disease. The spirochetes can be demonstrated in the inflammatory tissue with special silver stains.

Bone Tumors and Tumor-Like Lesions

Bone tumors are diverse in their gross and morphologic features and range in their biologic potential from the innocuous to the rapidly fatal. This diversity makes it critical to accurately diagnose and stage tumors, and treat them appropriately, so that the patients can not only survive, but also maintain optimal function of the affected body parts.

Most bone tumors are classified according to the normal cell or tissue of origin. Lesions that do not have normal tissue counterparts are grouped according to their distinct clinicopathologic features (Table 26-6). Overall, matrix-producing and fibrous tumors are the most common, and among the benign tumors, osteochondroma and fibrous cortical defect are most frequent. Excluding malignant neoplasms of marrow origin (myeloma, lymphoma, and leukemia), osteosarcoma is the most common primary cancer of bone, followed by chondrosarcoma and Ewing sarcoma.

The precise incidence of different bone tumors is not known because many benign lesions are not biopsied. Benign tumors outnumber their malignant counterparts, however, by at least several hundredfold. Benign tumors have their greatest frequency within the first three decades of life, whereas in the elderly a bone tumor is likely to be malignant. In the United States, about 2,100 new cases of bone sarcoma are

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diagnosed annually, and approximately 1,300 deaths from bone sarcoma occur each year.

As a group these neoplasms affect all ages and arise in virtually every bone, but most develop during the first several decades of life and have a propensity to originate in the long bones of the extremities. However, specific types of tumors target certain age groups and anatomic sites.[32] For instance, most osteosarcomas occur during adolescence, and about half of them arise in the metaphysis around the knee, either in the distal femur or proximal tibia. These are the sites of greatest skeletal growth activity. In contrast, chondrosarcomas tend to develop during mid- to late adulthood and frequently involve the trunk, limb girdles, and proximal long bones. Chondro-blastomas and giant cell tumors almost always arise in the epiphysis of long bones; by comparison, Ewing sarcoma, osteofibrous dysplasia, and adamantinoma most often are centered in the diaphysis. Thus, the location of a tumor provides important diagnostic information.

Although the cause of most bone tumors is unknown, genetic alterations similar to those that occur in other tumors clearly play a role. For instance, bone sarcomas occur in the Li-Fraumeni and hereditary retinoblastoma cancer syndromes, which are linked to mutations in *p53* and *RB* (Chapter 7). Bone infarcts, chronic osteomyelitis, Paget disease, radiation, and metal prostheses are also associated with an increased incidence of bone neoplasia. Such secondary neoplasms, however, account for only a small fraction of all skeletal tumors.

Clinically, bone tumors present in various ways. The more common benign lesions are frequently asymptomatic and are detected as incidental findings. Many tumors, however, produce pain or are noticed as a slow-growing mass. Sometimes, the first hint of a tumor's presence is a sudden pathologic fracture. Radiographic analysis plays an important role in diagnosing these lesions. In addition to providing the exact location and extent of the tumor, imaging studies can detect features that help limit diagnostic possibilities and give clues to the aggressiveness of the tumor. Ultimately, in most instances, biopsy and histologic study are necessary. In addition to classifying the tumor, histologic grade must also be determined in

most primary malignancies. *The histologic grade has been shown to be the most important prognostic feature of a bone sarcoma* and is a key component of the major staging systems of bone neoplasms.

BONE-FORMING TUMORS

Common to all these neoplasms is the production of bone by the neoplastic cells. The tumor bone is usually deposited as woven trabeculae (except in osteomas) and is variably mineralized.

Osteoma

Osteomas are bosselated, round to oval sessile tumors that project from the subperiosteal or endosteal surfaces of the cortex. Subperiosteal osteomas most often arise on or inside the skull and facial bones. They are usually solitary and are detected in middle-aged adults. Multiple osteomas are seen in the setting of *Gardner syndrome* (Chapter 17). They are composed of woven and lamellar bone that is frequently deposited in a cortical pattern with haversian-like systems. Some variants contain a component of trabecular bone in which the intertrabecular spaces are filled with hematopoietic marrow. Histologically the reactive bone induced by infection, trauma, or hemangiomas may simulate an osteoma and should be considered in the differential diagnosis.

Osteomas are generally slow-growing tumors of little clinical significance except when they cause obstruction of a sinus cavity, impinge on the brain or eye, interfere with function of the oral cavity, or produce cosmetic problems. Osteomas do not transform into osteosarcoma.

Osteoid Osteoma and Osteoblastoma

Osteoid osteoma and *osteoblastoma* are terms used to describe benign bone tumors that have identical histologic features but that differ in size, sites of origin, and symptoms. *Osteoid osteomas* are, by definition, less than 2 cm in greatest dimension and usually occur in patients in their teens and twenties. Seventy-five per cent of patients are under age 25, and men outnumber women 2:1. Osteoid osteomas can arise in any bone but have a predilection for the appendicular skeleton. Fifty percent of cases involve the femur or tibia, where they commonly arise in the cortex and less frequently within the medullary cavity. Osteoid osteomas are painful lesions. The pain, which is caused by excess prostaglandin E_2 produced by the proliferating osteoblasts, is severe in relation to the small size of the lesion, is characteristically nocturnal, and is dramatically relieved by aspirin.[33] *Osteoblastoma* differs from osteoid osteoma in that it more frequently involves the spine; the pain is dull, achy, and not responsive to salicylates; and it does not induce a marked bony reaction.

Morphology.

Grossly, both osteoid osteoma and osteoblastoma are round to oval masses of hemorrhagic gritty tan tissue. Histologically, they are well circumscribed and composed of a morass of randomly interconnecting trabeculae of woven bone prominently rimmed by osteoblasts (Fig. 26-21). The stroma surrounding the tumor bone consists of loose connective tissue that contains many dilated and congested capillaries. The relatively small size and well-defined margins of these tumors in combination

Figure 26-21 Osteoid osteoma composed of haphazardly interconnecting trabeculae of woven bone that are rimmed by prominent osteoblasts. The intertrabecular spaces are filled by vascular loose connective tissue.

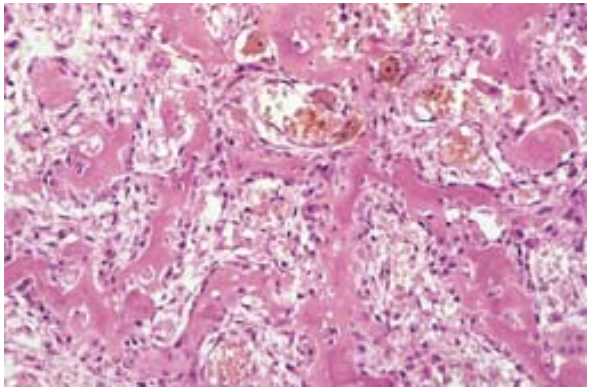

Figure 26-22 Specimen radiograph of intracortical osteoid osteoma. The round radiolucency with central mineralization represents the lesion and is surrounded by abundant reactive bone that has massively thickened the cortex.

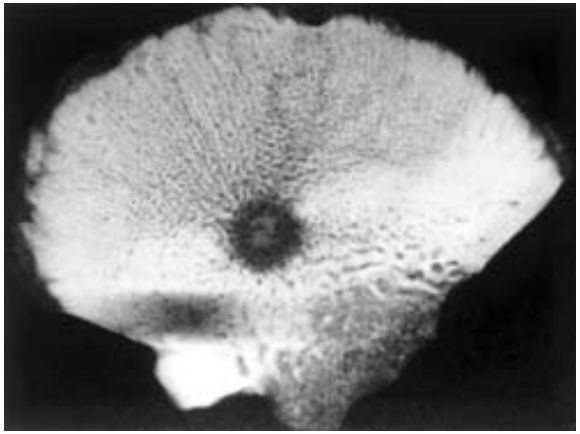

Figure 26-23 Major sites of origin of osteosarcomas. The numbers are approximate percentages.

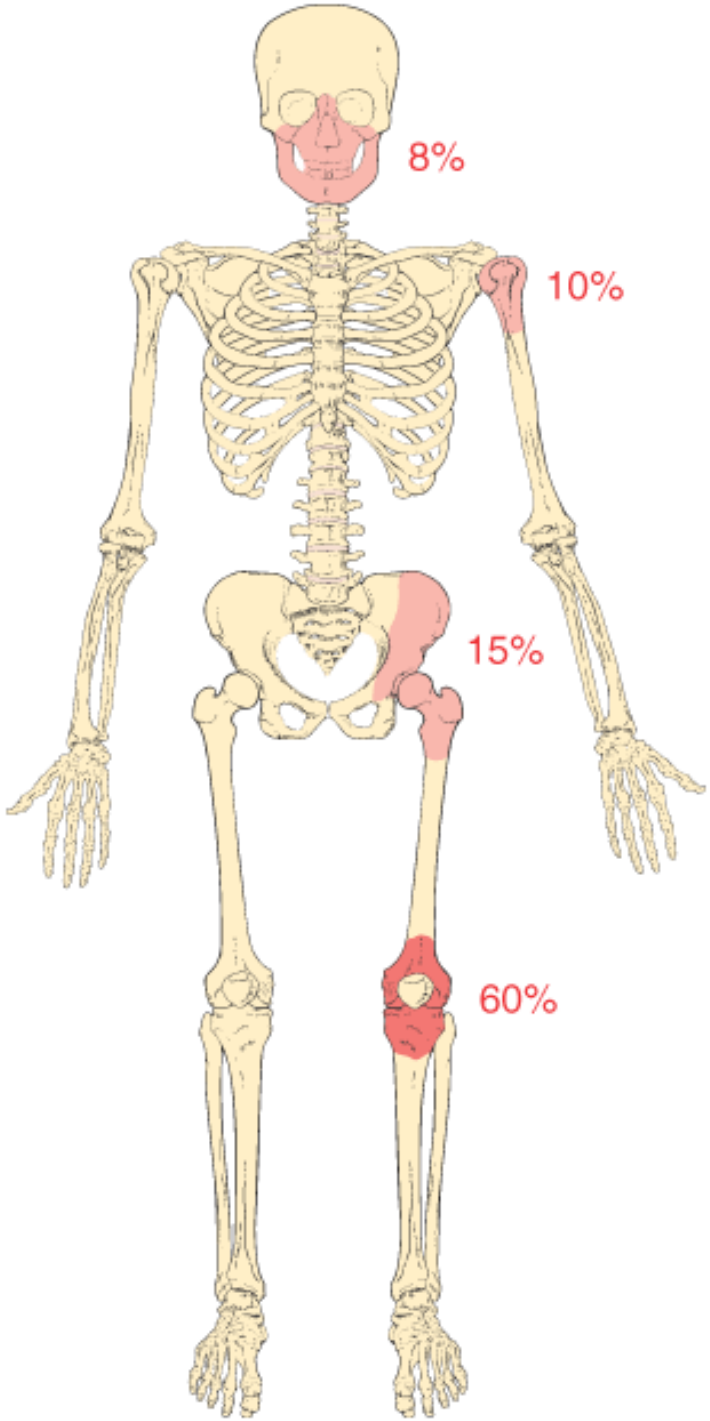

Figure 26-24 Osteosarcoma of the upper end of the tibia. The tan-white tumor fills most of the medullary cavity of the metaphysis and proximal diaphysis. It has infiltrated through the cortex, lifted the periosteum, and formed soft tissue masses on both sides of the bone.

Figure 26-25 Osteosarcoma. Coarse, lacelike pattern of neoplastic bone produced by anaplastic malignant tumor cells. Note the mitotic figures.

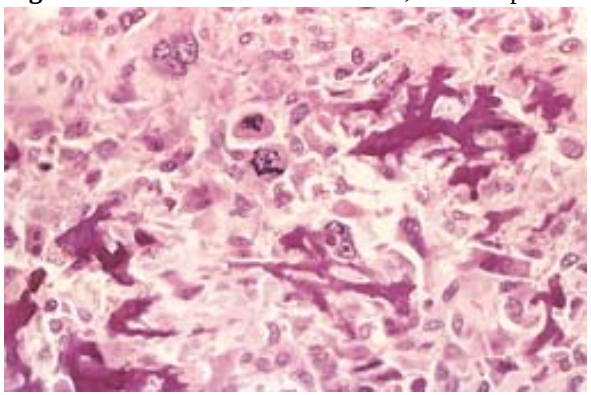

Figure 26-26 Distal femoral osteosarcoma with prominent bone formation extending into the soft tissues. The periosteum, which has been lifted, has laid down a proximal triangular shell of reactive bone known as a Codman triangle *(arrow)*.

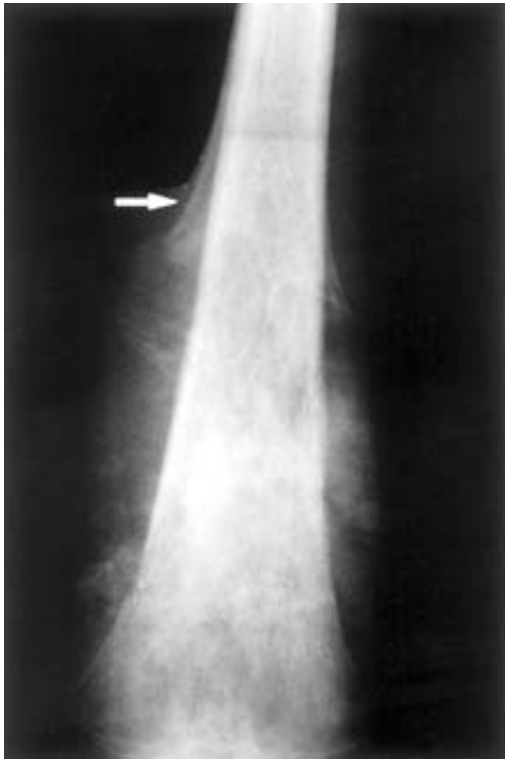

Figure 26-27 Schematic of the development over time of an osteochondroma, beginning with an outgrowth from the epiphyseal cartilage.

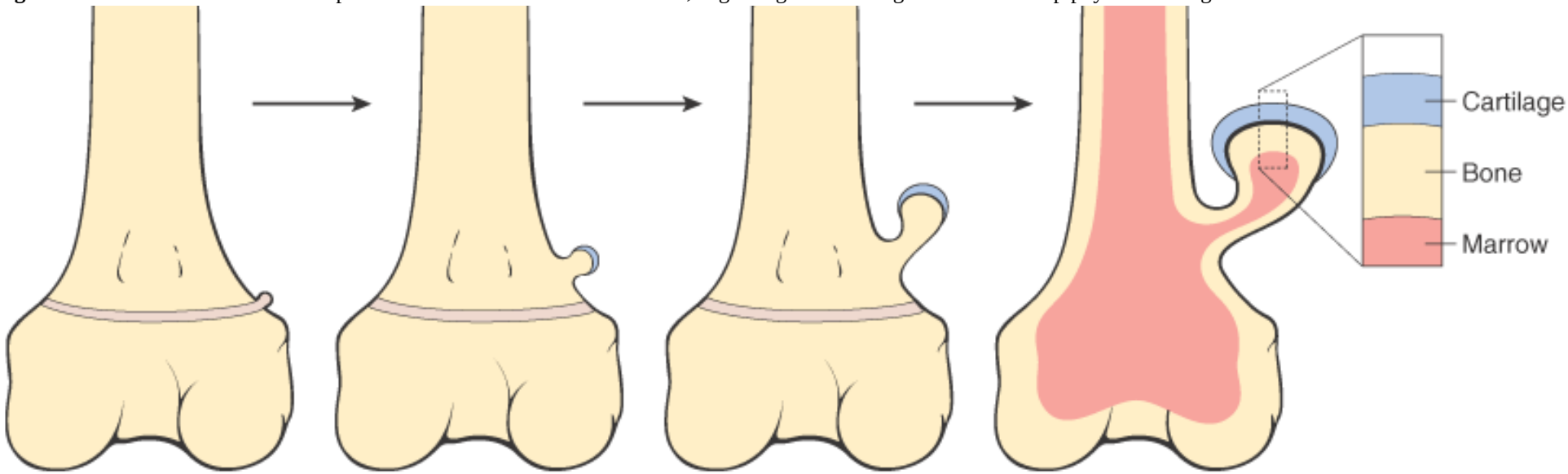

Figure 26-28 Enchondroma with a nodule of hyaline cartilage encased by a thin layer of reactive bone.

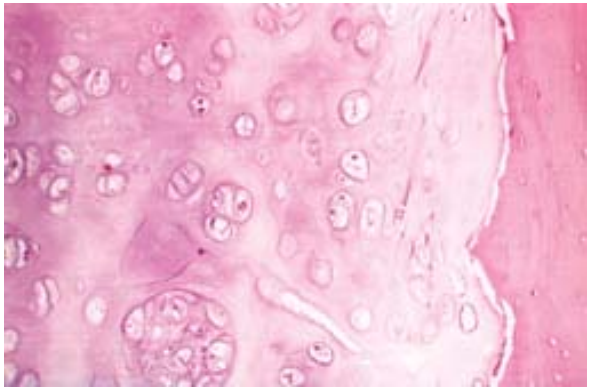

Figure 26-29 Enchondroma of the phalanx with a pathologic fracture. The radiolucent nodules of hyaline cartilage scallop the endosteal surface.

Figure 26-30 Chondroblastoma with scant mineralized matrix surrounding chondroblasts in a chicken wire-like fashion.

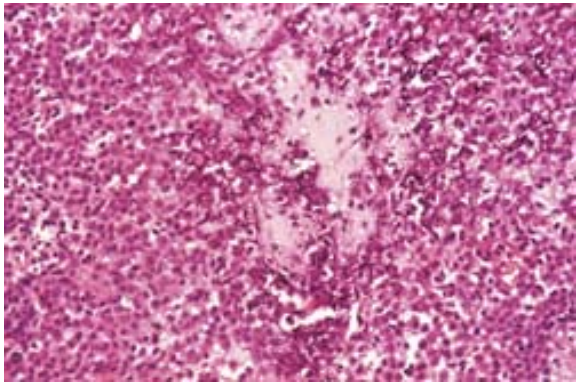

Figure 26-31 Chondromyxoid fibroma with prominent stellate and spindle cells surrounded by myxoid matrix. Occasional osteoclast-type giant cells are also present.

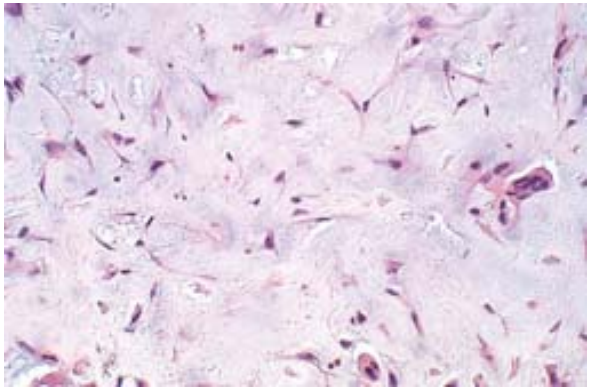

Figure 26-32 Chondrosarcoma with lobules of hyaline and myxoid cartilage permeating throughout the medullary cavity, growing through the cortex, and forming a relatively well-circumscribed soft tissue mass.

Figure 26-33 Anaplastic chondrocytes within a chondrosarcoma.

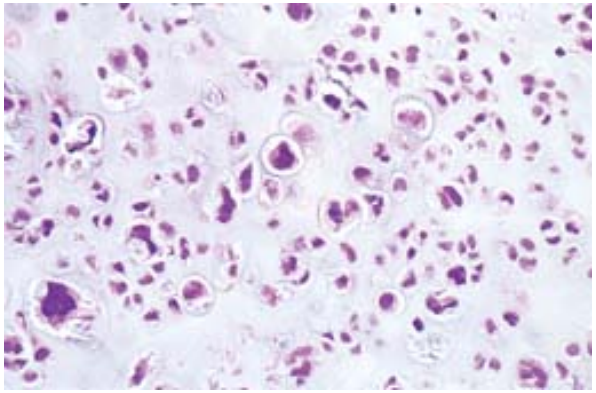

Figure 26-34 Nonossifying fibromas of the distal tibial metaphysis, producing an eccentric lobulated radiolucency surrounded by a sclerotic margin.

Figure 26-35 Storiform pattern created by benign spindle cells with scattered osteoclast-type giant cells characteristic of a fibrous cortical defect and nonossifying fibroma.

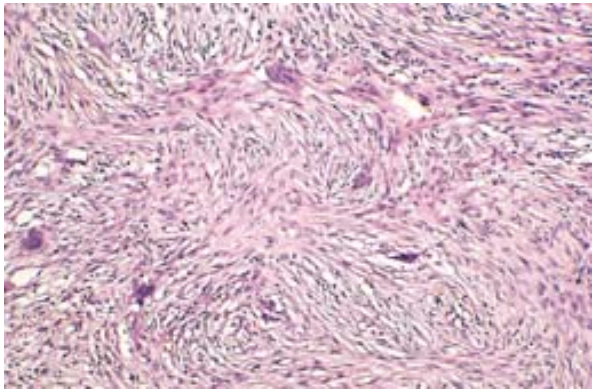

Figure 26-36 Fibrous dysplasia composed of curvilinear trabeculae of woven bone that lack conspicuous osteoblastic rimming and arise in a background of fibrous tissue.

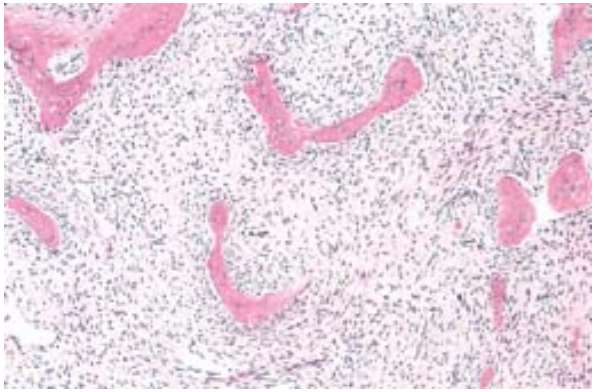

Figure 26-37 Ewing sarcoma composed of sheets of small round cells with small amounts of clear cytoplasm.

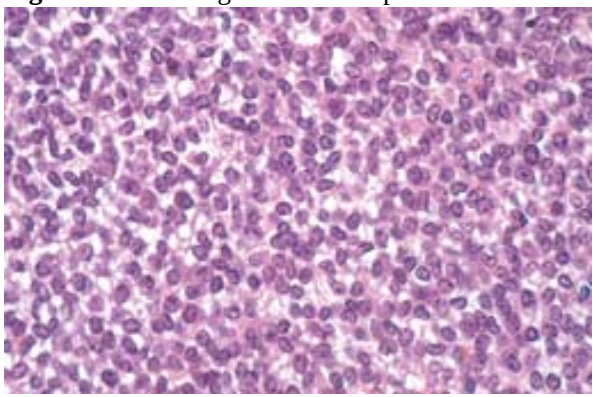

Figure 26-38 Benign giant cell tumor illustrating an abundance of multinucleated giant cells with background mononuclear stromal cells.

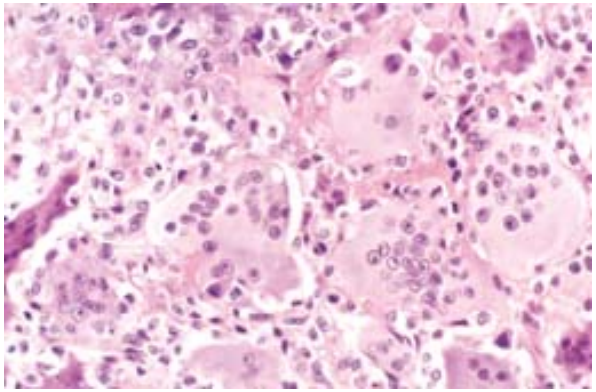

Figure 26-39 Magnetic resonance image of a giant cell tumor that replaces most of the femoral condyle and extends to the sub-chondral bone plate.

Figure 26-40 Severe osteoarthritis with small islands of residual articular cartilage next to exposed subchondral bone. *1*, Eburnated articular surface. *2*, Subchondral cyst. *3*, Residual articular cartilage.

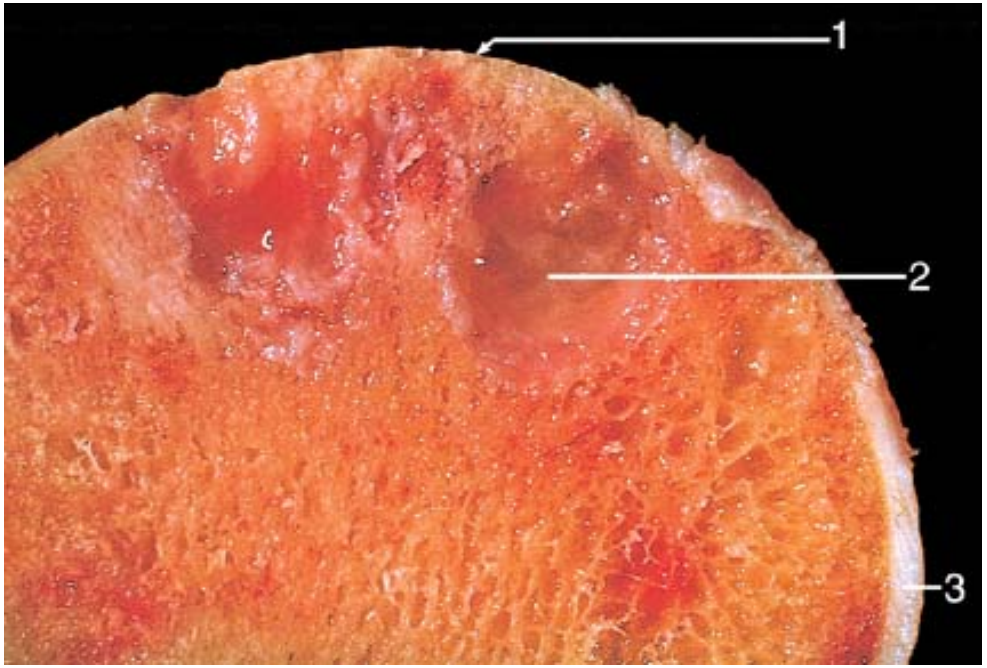

Figure 26-41 Severe osteoarthritis of the hip. The joint space is narrowed, and there is subchondral sclerosis with scattered oval radiolucent cysts and peripheral osteophyte lipping *(arrows).*

Figure 26-42 Rheumatoid arthritis. *A,* Schematic view of the joint lesion. *(Modified from Feldmann M: Development of anti-TNF therapy for rheumatoid arthritis. Nat Rev Immunol 2:364, 2002.) B,* Low magnification reveals marked synovial hypertrophy with formation of villi. *C,* At higher magnification, subsynovial tissue containing a dense lymphoid aggregate is seen.

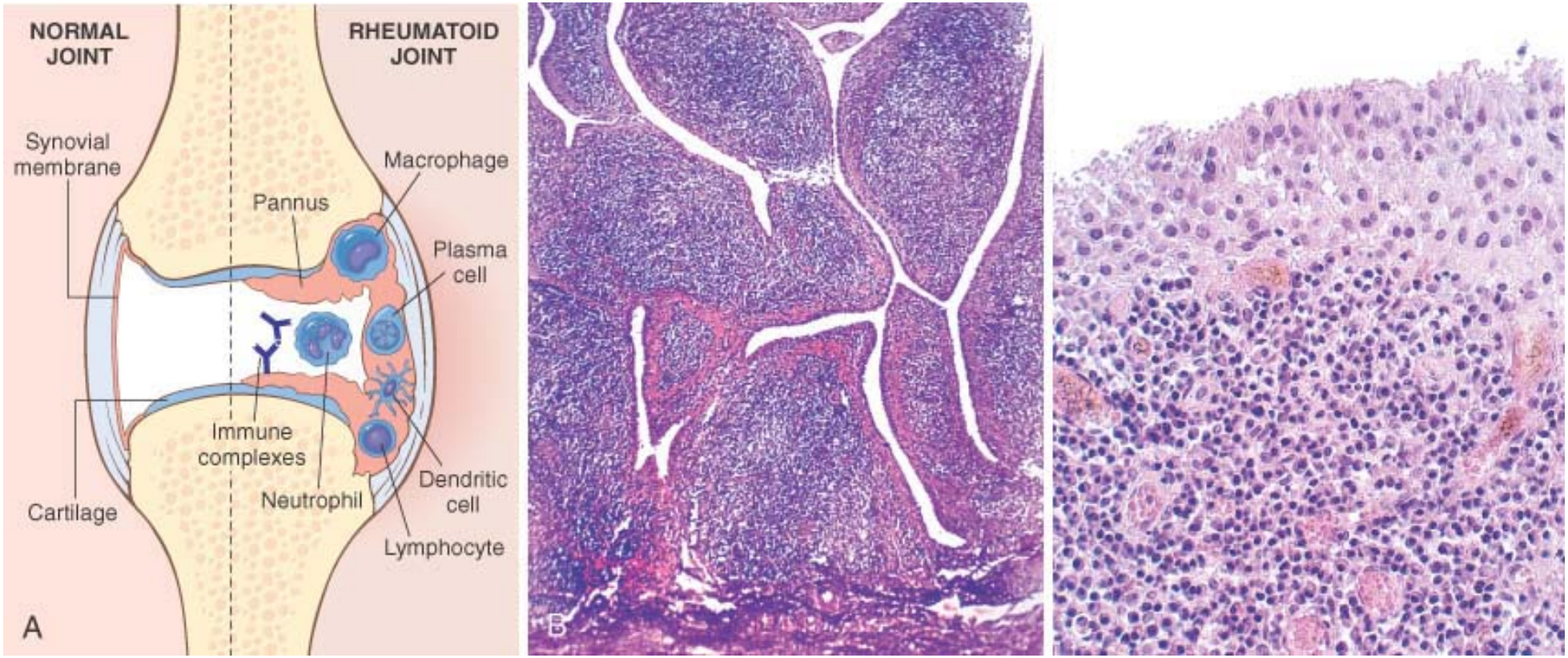

Figure 26-43 Subcutaneous rheumatoid nodule with an area of necrosis *(top)* surrounded by a palisade of macrophages and scattered chronic inflammatory cells.

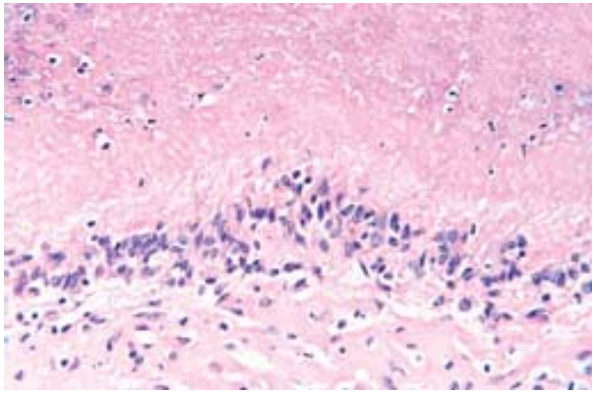

Figure 26-44 Immunopathogenesis of rheumatoid arthritis.

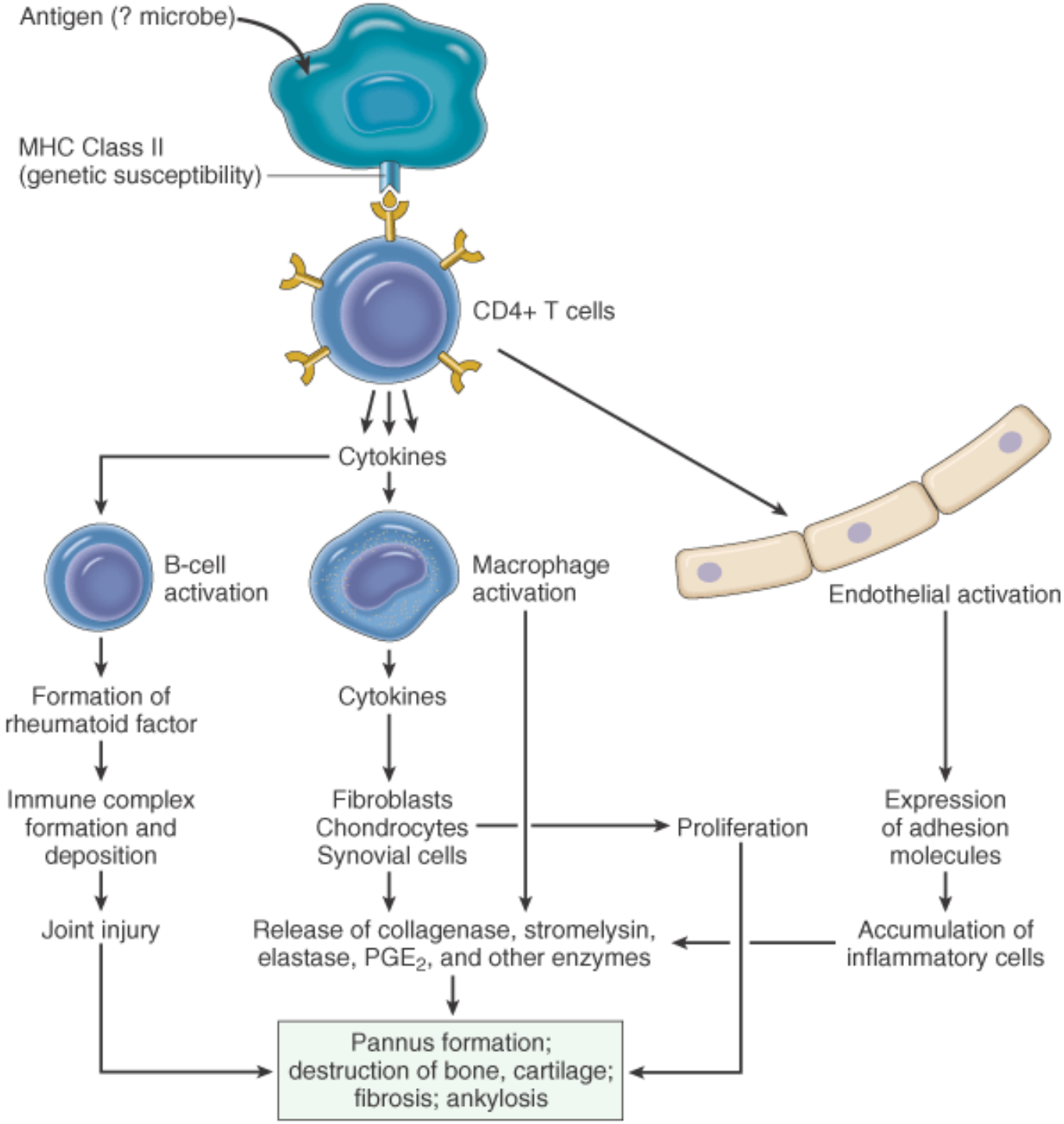

Figure 26-45 Rheumatoid arthritis. *A*, Early disease, most marked in the second metacarpophalangeal joint, where there is narrowing of joint space and marginal erosions on both radial

and ulnar aspects of the proximal phalanx *(see inset)*. *B,* More advanced disease with loss of articular cartilage, narrowing of joint spaces of virtually all the small joints, and ulnar deviation of the fingers. There is dislocation of the second, third, and fourth proximal phalanges produced by advanced articular disease. *(Courtesy of Dr. John O'Connor, Boston University Medical Center, Boston, MA.)*

TABLE 26-7 -- Classification of Gout

Clinical Category	Metabolic Defect
Primary Gout (90% of cases)	
Enzyme defects unknown (85%–90% of primary gout)	■ Overproduction of uric acid
	••Normal excretion (majority)
	••Increased excretion (minority)
	••Underexcretion of uric acid with normal production

Known enzyme defects—e.g., partial HGPRT deficiency (rare)	■ Overproduction of uric acid
Secondary Gout (10% of cases)	
Associated with increased nucleic acid turnover—e.g., leukemias	■ Overproduction of uric acid with increased urinary excretion
Chronic renal disease	■ Reduced excretion of uric acid with normal production
Inborn errors of metabolism—e.g., complete HGPRT deficiency (Lesch-Nyhan syndrome)	■ Overproduction of uric acid with increased urinary excretion
HGPRT, hypoxanthine guanine phosphoribosyl transferase.	

and in some cases gouty arthritis. Less severe deficiencies of the enzyme may also induce hyperuricemia and gouty arthritis with only mild neurologic deficits, but together these causes of gout are uncommon. The great majority of cases of gout are primary, in which the metabolic defect underlying the increased levels of uric acid is unknown.

As stated earlier, hyperuricemia does not necessarily lead to gouty arthritis. Many factors contribute to the conversion of asymptomatic hyperuricemia into primary gout, including the following:

- *Age* of the individual and duration of the hyperuricemia are factors. Gout rarely appears before 20 to 30 years of hyperuricemia.
- *Genetic predisposition* is another factor. In addition to the well-defined X-linked abnormalities of HGPRT, primary gout follows multifactorial inheritance and runs in families.
- Heavy *alcohol* consumption predisposes to attacks of gouty arthritis.
- *Obesity* increases the risk of asymptomatic gout.
- Certain *drugs* (e.g., thiazides) predispose to the development of gout.
- *Lead toxicity* increases the tendency to develop saturnine gout (Chapter 9).

Central to the pathogenesis of the arthritis is precipitation of monosodium urate crystals into the joints (Fig. 26-47). Synovial fluid is a poorer solvent for monosodium urate than plasma, and so with hyperuricemia the urates in the joint fluid become supersaturated, particularly in the peripheral joints (ankle), which may have temperatures as low as 20°C. With prolonged hyperuricemia, crystals and microtophi of urates develop in the synovium and in the joint cartilage. Some unknown event, possibly trauma, then initiates release of crystals into the synovial fluid, which begins a cascade of events. The released crystals are chemotactic to leukocytes and also activate complement,

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Figure 26-46 Purine metabolism. The conversion of PRPP to purine nucleotides is catalyzed by amido PRT in the de novo pathway and by APRT and HGPRT in the salvage pathway. APRT, amido-phosphoribosyltransferase; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; PRPP, phosphoribosyl pyrophosphate; PRT, phosphoribosyltransferase.

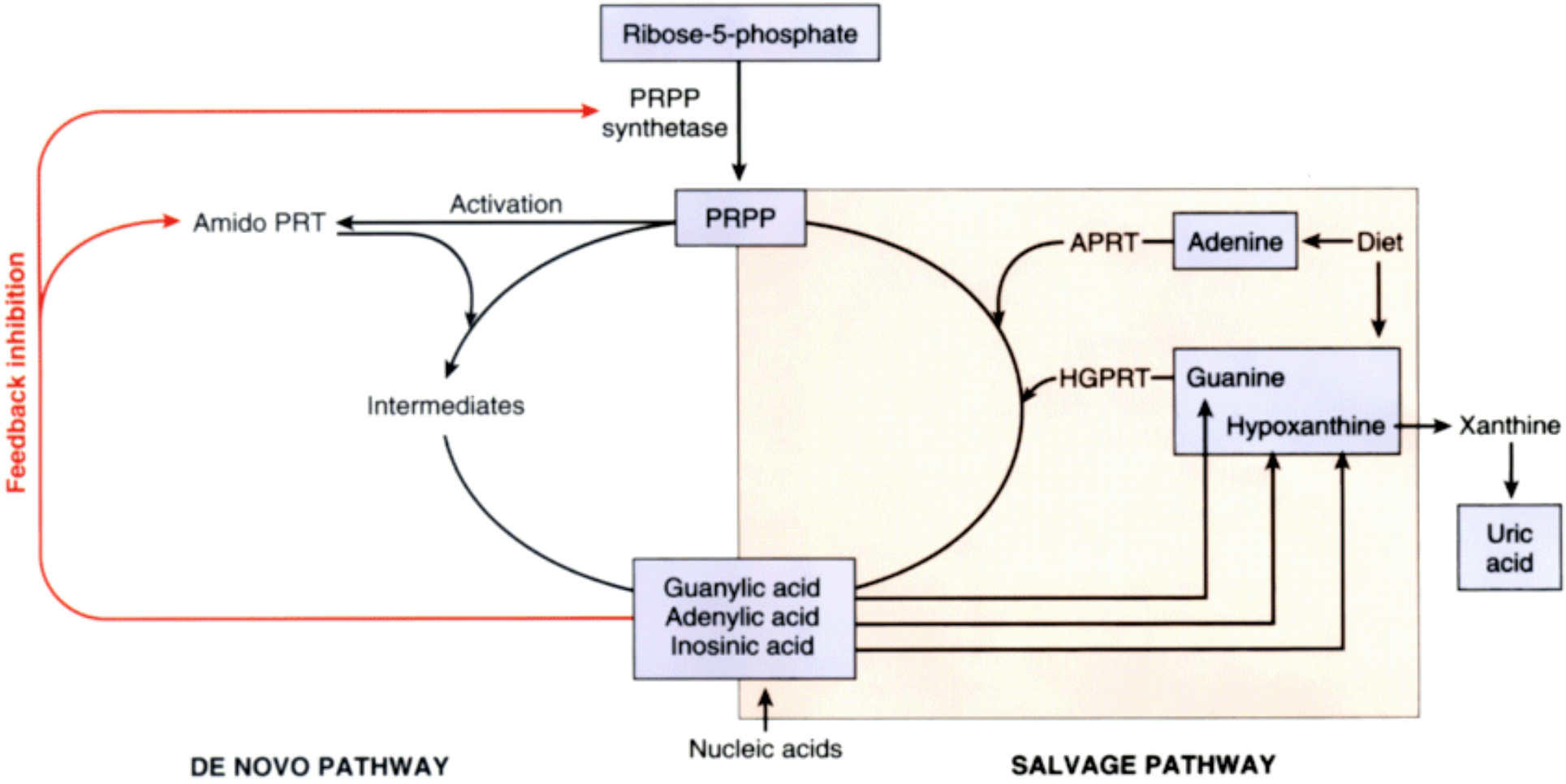

Figure 26-47 Pathogenesis of acute gouty arthritis.

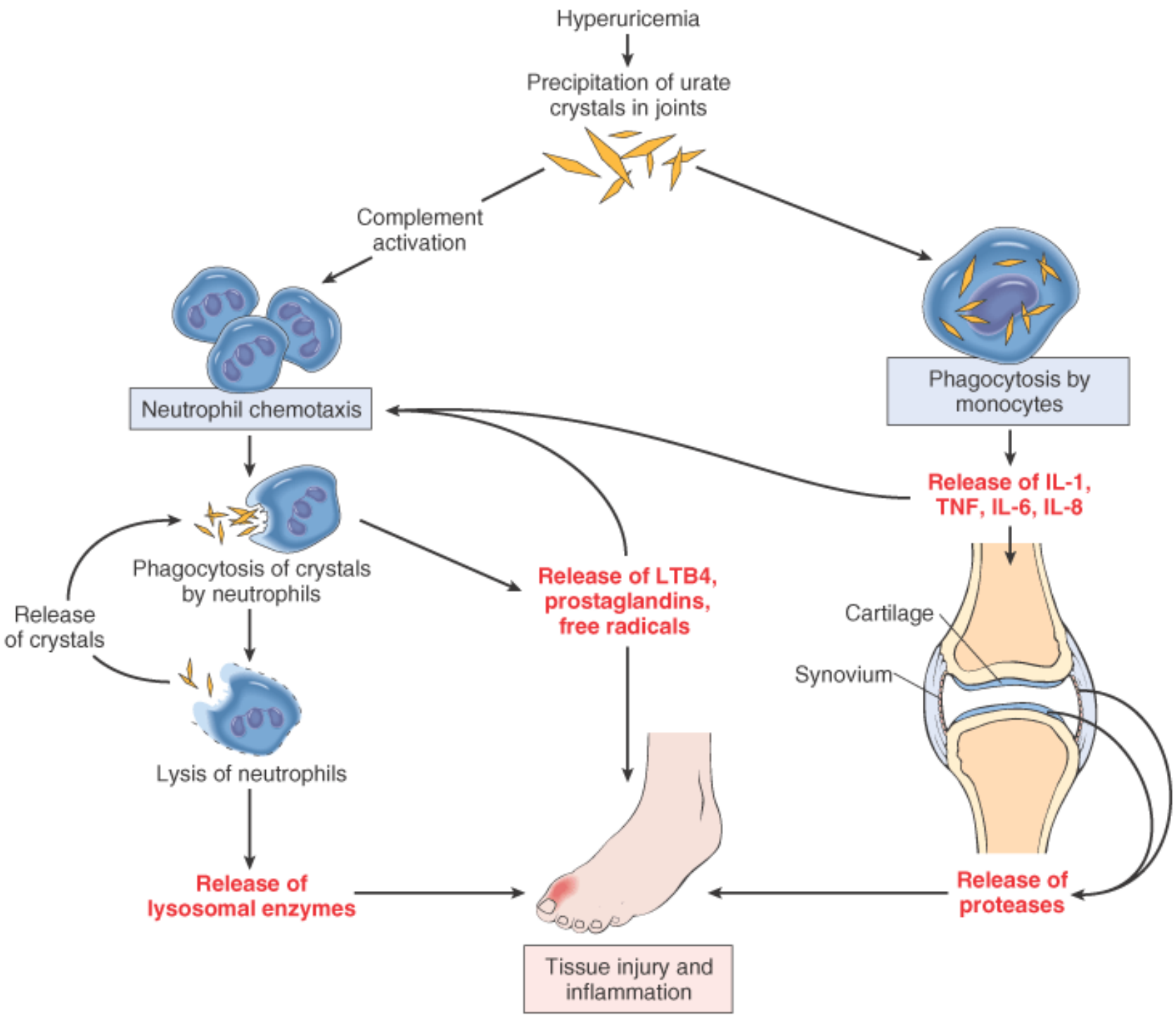

Figure 26-48 Amputated great toe with white tophi involving the joint and soft tissues.

Figure 26-49 Photomicrograph of a gouty tophus. An aggregate of dissolved urate crystals is surrounded by reactive fibroblasts, mononuclear inflammatory cells, and giant cells.

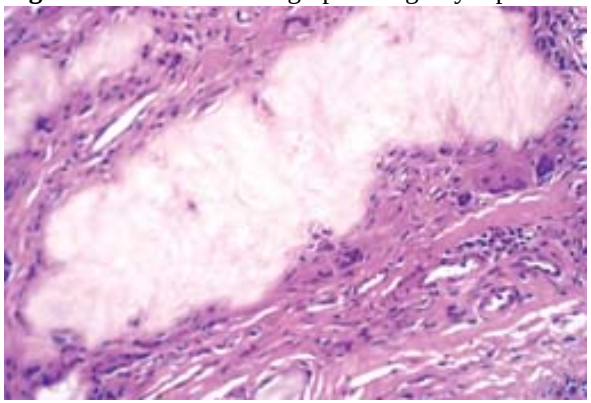

Figure 26-50 Smear preparation of synovial fluid containing calcium pyrophosphate crystals.

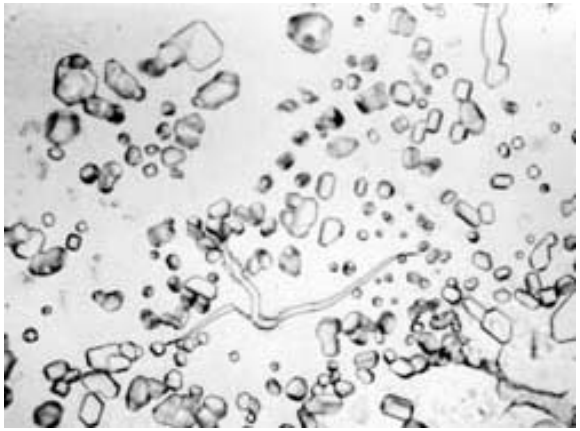

Figure 26-51 Excised synovium with fronds and nodules typical of pigmented villonodular synovitis (PVNS) *(arrow)*.

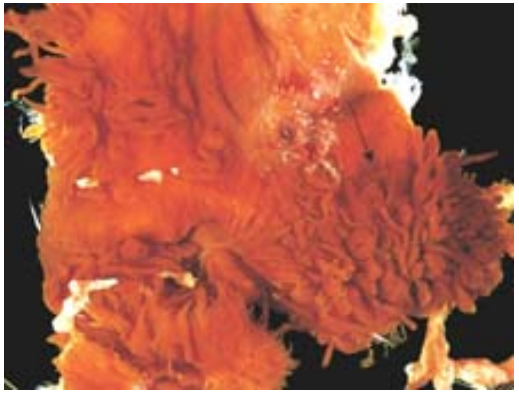

Figure 26-52 Sheets of proliferating cells in PVNS bulging the synovial lining.

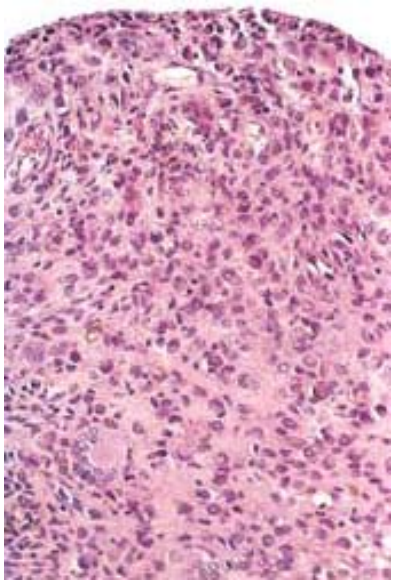

TABLE 26-8 -- Soft Tissue Tumors

■ ***Tumors of adipose tissue***
••Lipomas
••Liposarcoma
■ ***Tumors and tumor-like lesions of fibrous tissue***
••Nodular fasciitis
••Fibromatoses
••••Superficial fibromatoses
••••Deep fibromatoses
••Fibrosarcoma
■ ***Fibrohistiocytic tumors***
••Fibrous histiocytoma
••Dermatofibrosarcoma protuberans
••Malignant fibrous histiocytoma
■ ***Tumors of skeletal muscle***
••Rhabdomyoma
••Rhabdomyosarcoma

■ ***Tumors of smooth muscle***
••Leiomyoma
••Smooth muscle tumors of uncertain malignant potential
••Leiomyosarcoma
■ ***Vascular tumors***
••Hemangioma
••Lymphangioma
••Hemangioendothelioma
••Hemangiopericytoma
••Angiosarcoma
■ ***Peripheral nerve tumors***
••Neurofibroma
••Schwannoma
••Granular cell tumor
••Malignant peripheral nerve sheath tumors
■ ***Tumors of uncertain histogenesis***
••Synovial sarcoma
••Alveolar soft part sarcoma
••Epithelioid sarcoma

at least 100:1. In the United States, little more than 8000 sarcomas are diagnosed annually (0.8% of invasive malignancies), yet they are responsible for 2% of all cancer deaths, reflecting their lethal nature.

Pathogenesis and General Features

The cause of most soft tissue tumors is unknown. There are documented associations, however, between radiation therapy and rare instances in which chemical burns, thermal burns, or trauma were associated with subsequent development of a sarcoma. Exposure to phenoxyherbicides and chlorophenols has also been implicated in some cases. Kaposi sarcoma is causally associated with the human herpesvirus 8; however, viruses are probably not important in the pathogenesis of most sarcomas. The majority of soft tissue tumors occur sporadically, but a small minority is associated with genetic syndromes, the most notable of which are neurofibromatosis type 1 (neurofibroma, malignant schwannoma), Gardner syndrome (fibromatosis), Li-Fraumeni syndrome (soft tissue sarcoma), and Osler-Weber-Rendu syndrome (telangiectasia). Cytogenetic and molecular analyses of soft tissue tumors have provided significant insight into their biology. Specific chromosomal abnormalities and genetic derangements can not only be used as diagnostic markers, but also provide important clues about the genesis of the neoplasms.[60] For example, many of the mutations target oncogenes that encode transcription factors or cell-cycle regulators, and their dysfunction results in uncontrolled cell proliferation (Table 26-9).

Soft tissue tumors may arise in any location, although approximately 40% occur in the lower extremities, especially the thigh; 20% in the upper extremities; 10% in the head and neck; and 30% in the trunk and retroperitoneum. Regarding sarcomas, males are affected more frequently than females (1.4:1), and the incidence generally increases with age. Fifteen per cent arise in children; they constitute the fourth most common malignancy in this age group, following brain tumors, hematopoietic cancers, and Wilms tumor in frequency. Specific sarcomas tend to appear in certain age groups (e.g., rhabdomyosarcoma in children, synovial sarcoma in young adulthood, and liposarcoma and malignant fibrous histiocytoma in mid- to late adult life).

Several features of soft tissue tumors influence their prognosis:

- Accurate histologic classification contributes significantly to establishing the prognosis of a sarcoma. Important diagnostic features are cell morphology and architectural arrangement (Table 26-10 and Table 26-11). Often these features are not sufficient to distinguish one sarcoma

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from another, particularly with poorly differentiated aggressive tumors. Great reliance must, therefore, be placed on immunohistochemistry, electron microscopy, cytogenetics, and molecular genetics.

- Whatever the type, the *grade* of a soft tissue sarcoma is important for predicting its behavior. Grading, usually I to III, is based largely on the degree of differentiation, the average number of mitoses per high-power field, cellularity, pleomorphism, and an estimate of the extent of necrosis (presumably a reflection of rate of growth). Mitotic activity and extent of necrosis are thought to be particularly significant. The size, depth, and stage of the tumor also provide important diagnostic and prognostic information.[61]
- Staging helps determine the prognosis and chance of successful excision of a tumor. Several staging systems are utilized in treating sarcomas.
- In general, tumors arising in superficial locations (e.g., skin and subcutis) have a better prognosis than deep-seated lesions. In patients with deep-seated, high-grade sarcomas, metastatic disease develops in 80% of those with a tumor larger than 20 cm and 30% of those with a tumor larger than 5 cm. Overall the 10-year survival rate for sarcomas is approximately 40%.

TABLE 26-9 -- Chromosomal and Genetic Abnormalities in Soft Tissue Sarcomas

Tumor	Cytogenetic Abnormality	Genetic Abnormality
Extraosseous Ewing sarcoma and primitive neuroectodermal tumor	t(11:22)(q24;q12)	FLI-1-EWS fusion gene
	t(21:22)(q22;q12)	ERG-EWS fusion gene
	t(7;22)(q22;q12)	ETV1-EWS fusion gene
Liposarcoma—myxoid and round cell type	t(12:16)(q13;p11)	CHOP/TLS fusion gene
Synovial sarcoma	t(x;18)(p11;q11)	SYT-SSX fusion gene
Rhabdomyosarcoma—alveolar type	t(2;13)(q35;q14)	PAX3-FKHR fusion gene
	t(1;13)(p36;q14)	PAX7-FKHR fusion gene
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	CHN-EWS fusion gene
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	EWS-WT1 fusion gene
Clear cell sarcoma	t(12;22)(q13;q12)	EWS-ATF1 fusion gene

Dermatofibrosarcoma protuberans	t(17:22)(q22;q15)	COLA1-PDGFB fusion gene
Alveolar soft part sarcoma	t(X;17)(p11.2;q25)	TFE3-ASPL fusion gene
Congenital fibrosarcoma	t(12;15)(p13;q23)	ETV6-NTRK3 fusion gene

TABLE 26-10 -- Morphology of Cells in Soft Tissue Tumors

Cell Type	Features	Tumor Type
Spindle cell	Rod-shaped, long axis twice as great as short axis	Fibrous, fibrohistiocytic, smooth muscle, Schwann cell
Small round cell	Size of a lymphocyte with little cytoplasm	Rhabdomyosarcoma, primitive neuroectodermal tumor
Epithelioid	Polyhedral with abundant cytoplasm, nucleus is centrally located	Smooth muscle, Schwann cell endothelial, epithelioid sarcoma

TABLE 26-11 -- Architectural Patterns in Soft Tissue Tumors

Pattern	Tumor Type
Fascicles of eosinophilic spindle cells intersecting at right angles	Smooth muscle
Short fascicles of spindle cells radiating from a central point (like spokes on a wheel)—storiform	Fibrohistiocytic
Nuclei arranged in columns—palisading	Schwann cell
Herringbone	Fibrosarcoma
Mixture of fascicles of spindle cells and groups of epithelioid cells—biphasic	Synovial sarcoma

With this brief background, we now turn to the individual tumors and tumor-like lesions. Some of the soft tissue tumors are presented elsewhere—tumors of peripheral nerve (Chapter 27); and tumors of vascular origin, including Kaposi sarcoma (Chapter 11).

Fatty Tumors

LIPOMA

Benign tumors of fat, known as *lipomas*, are the most common soft tissue tumor of adulthood. They are subclassified according to particular morphologic features as conventional lipoma, fibrolipoma, angiolipoma, spindle cell lipoma, myelolipoma, and pleomorphic lipoma. Some of the variants have characteristic chromosomal abnormalities; for example, conventional lipomas often show rearrangements of 12q14-15, 6p, and 13q, and spindle cell and pleomorphic lipomas have rearrangements of 16q and 13q.

Morphology.

The conventional lipoma, the most common subtype, is a well-encapsulated mass of mature adipocytes that varies considerably in size. It arises in the subcutis of the proximal extremities and trunk, most frequently during mid-adulthood. Infrequently, lipomas are large, intramuscular, and poorly circumscribed. Histologically, they consist of mature white fat cells with no pleomorphism.

Lipomas are soft, mobile, and painless (except angiolipoma) and are usually cured by simple excision.

LIPOSARCOMA

Liposarcomas are one of the most common sarcomas of adulthood and appear in those in their forties to sixties; they are uncommon in children. They usually arise in the deep soft tissues of the proximal extremities and retroperitoneum and are notorious for developing into large tumors.

Morphology.

Histologically, liposarcomas can be divided into well-differentiated, myxoid, round cell, and pleomorphic variants. The cells in well-differentiated liposarcomas are readily recognized as lipocytes. In the other variants, most of the tumor cells are not obviously adipogenic, but some cells indicative of fatty differentiation are almost always present. These cells are known as **lipoblasts;** they mimic fetal fat cells and contain round clear cytoplasmic vacuoles of lipid that scallop the nucleus (Fig. 26-53). The myxoid and round cell variant of liposarcoma has a t(12;16) chromosomal abnormality in most cases (Table 26-9).

The well-differentiated variant is relatively indolent, the myxoid type is intermediate in its malignant behavior, and the round cell and pleomorphic variants usually are aggressive and frequently metastasize. All types of liposarcoma recur locally and often repeatedly unless adequately excised.

Fibrous Tumors and Tumor-Like Lesions

REACTIVE PSEUDOSARCOMATOUS PROLIFERATIONS

Reactive pseudosarcomatous proliferations are nonneoplastic lesions that either develop in response to some form of local trauma (physical or ischemic) or are idiopathic. They are composed of plump reactive fibroblasts or related mesenchymal cells. Clinically, they are alarming because they develop suddenly and grow rapidly; histologically, they cause concern because they mimic sarcomas owing to their hypercellularity,

Figure 26-53 Myxoid liposarcoma with abundant ground substance in which are scattered adult-appearing fat cells and more primitive cells, some containing small lipid vacuoles (lipoblasts).

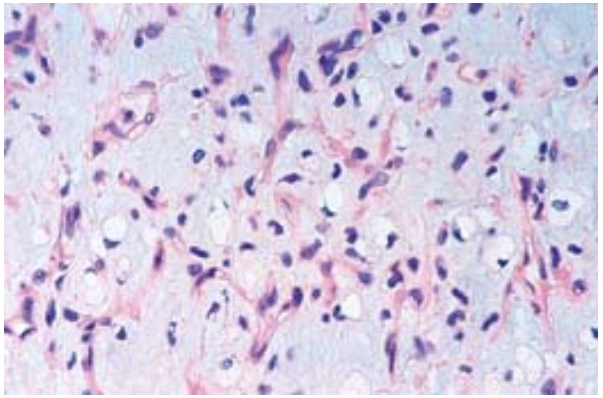


Figure 26-54 Nodular fasciitis with plump, randomly oriented spindle cells surrounded by myxoid stroma. Note the mitotic activity and extravasated red blood cells.

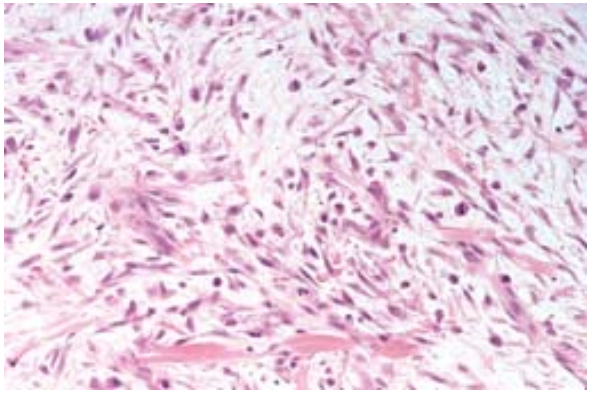


Figure 26-55 Peripherally mineralized myositis ossificans (*arrows*) involving the posterior thigh.

Figure 26-56 Fibromatosis infiltrating between skeletal muscle cells.

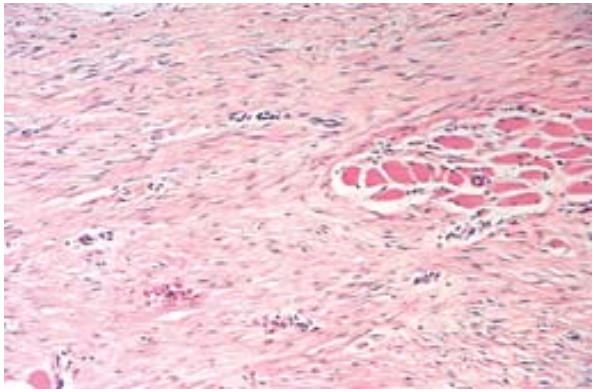


Figure 26-57 Fibrosarcoma composed of malignant spindle cells arranged in a herringbone pattern.

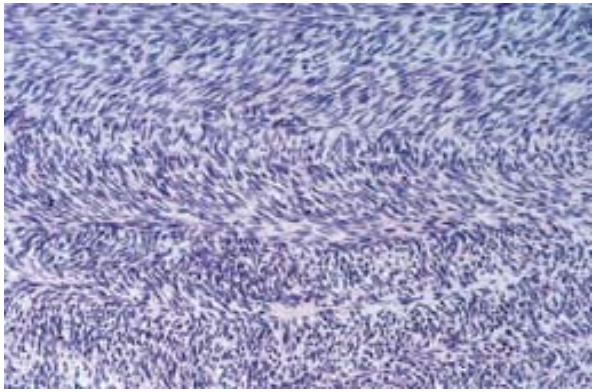


Figure 26-58 Malignant fibrous histiocytoma revealing fascicles of plump spindle cells in a swirling (storiform) pattern, typical but not pathognomonic of this neoplasm. *(Courtesy of Dr. J. Corson, Brigham and Women's Hospital, Boston, MA.)*

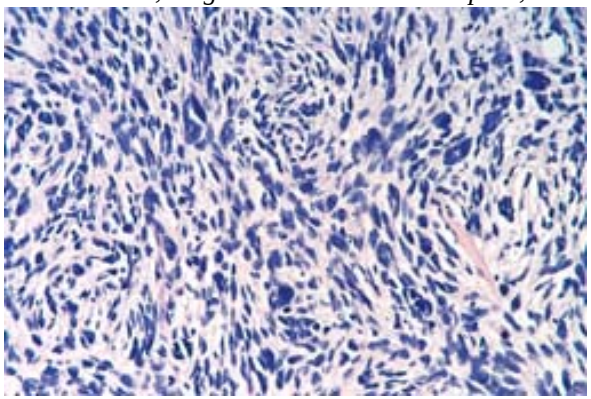


Figure 26-59 Rhabdomyosarcoma composed of malignant small round cells. The rhabdomyoblasts are large and round and have abundant eosinophilic cytoplasm; no cross-striations are evident.

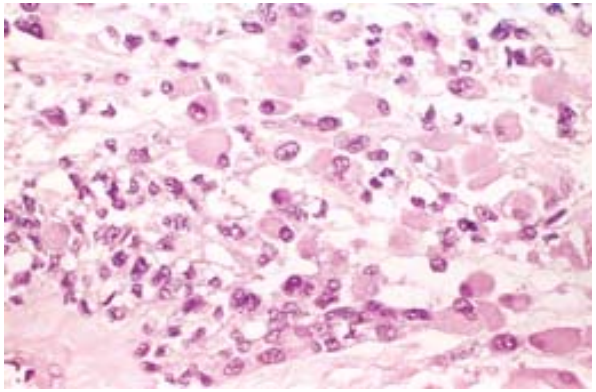


Figure 26-60 Alveolar rhabdomyosarcoma with numerous spaces lined by tumor cells.

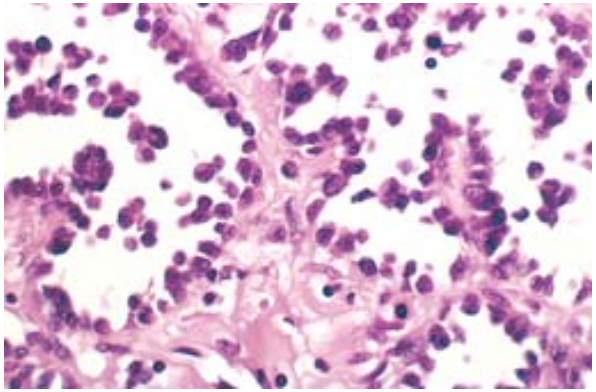
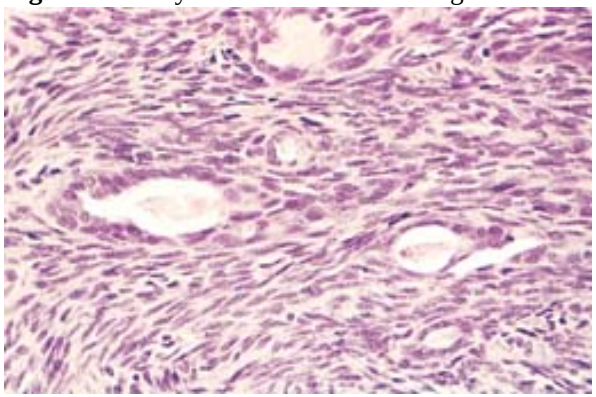


Figure 26-61 Synovial sarcoma revealing the classic biphasic spindle cell and glandular-like histologic appearance.



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Chapter 27 - Peripheral Nerve and Skeletal Muscle

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Normal

The functional unit of the neuromuscular system is the *motor unit*, which consists of (1) a *lower motor neuron* in the anterior horn of the spinal cord or cranial nerve motor nucleus in the brain stem, (2) the *axon* of that neuron, and (3) the multiple *muscle fibers* it innervates (Fig. 27-1). Lower motor neurons are distributed in the anterior horns of the spinal cord in columns or groups; they are arranged somatotopically so that cells lying medially innervate proximal muscles and those lying laterally supply the distal musculature. The number of muscle fibers within each unit varies considerably. Muscles with highly refined movements, such as the extrinsic muscles of the eye, have a high neuron-to-muscle-fiber ratio (1:10); those with relatively coarse and stereotyped movements, such as calf muscles, have a much lower ratio (1:1800).[1]

NORMAL PERIPHERAL NERVE

The principal structural component of peripheral nerve is the *nerve fiber* (an axon with its Schwann cells and myelin sheath). A nerve consists of numerous fibers that are grouped

Figure 27-1 Normal and abnormal motor units. *Normal motor units*: Two adjacent motor units are shown. *Segmental demyelination:* Random internodes of myelin are injured and are remyelinated by multiple Schwann cells, while the axon and myocytes remain intact. *Axonal degeneration:* The axon and its myelin sheath undergo anterograde degeneration (shown for the green neuron), with resulting denervation atrophy of the myocytes within its motor unit. *Reinnervation of muscle:* Sprouting of adjacent (red) uninjured motor axons leads to fiber type grouping of myocytes, while the injured axon attempts axonal sprouting. *Myopathy:* Scattered myocytes of adjacent motor units are small (degenerated or regenerated), whereas the neurons and nerve fibers are normal.

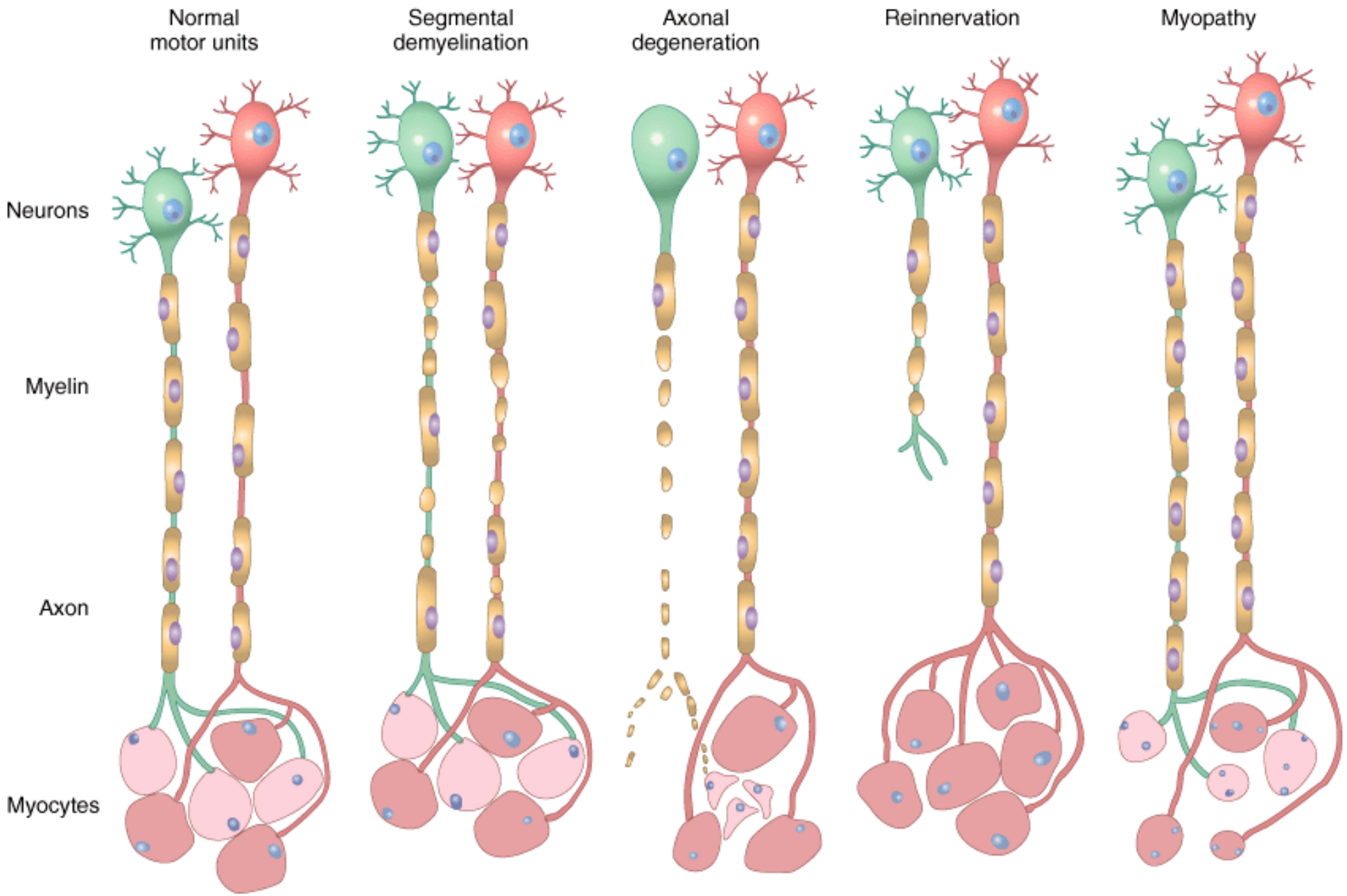

Figure 27-2 Electron micrograph of myelinated *(arrow)* and unmyelinated *(arrowhead)* fibers in human sural nerve. One Schwann cell nucleus is present.

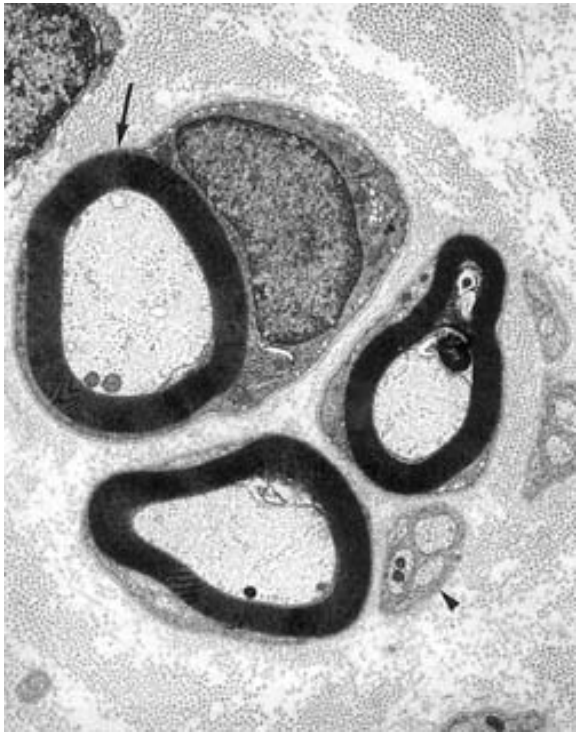

Figure 27-3 Electron micrograph of skeletal muscle in the longitudinal plane. A nucleus is located at the top of the illustration and the sarcomeres of two myofibrils are located below. The principal components of the sarcomere are identified, creating the pattern of cross-striations. *(From Bloom W, Fawcett DW: A Textbook of Histology, 11th ed. Philadelphia, WB Saunders, 1986.)*

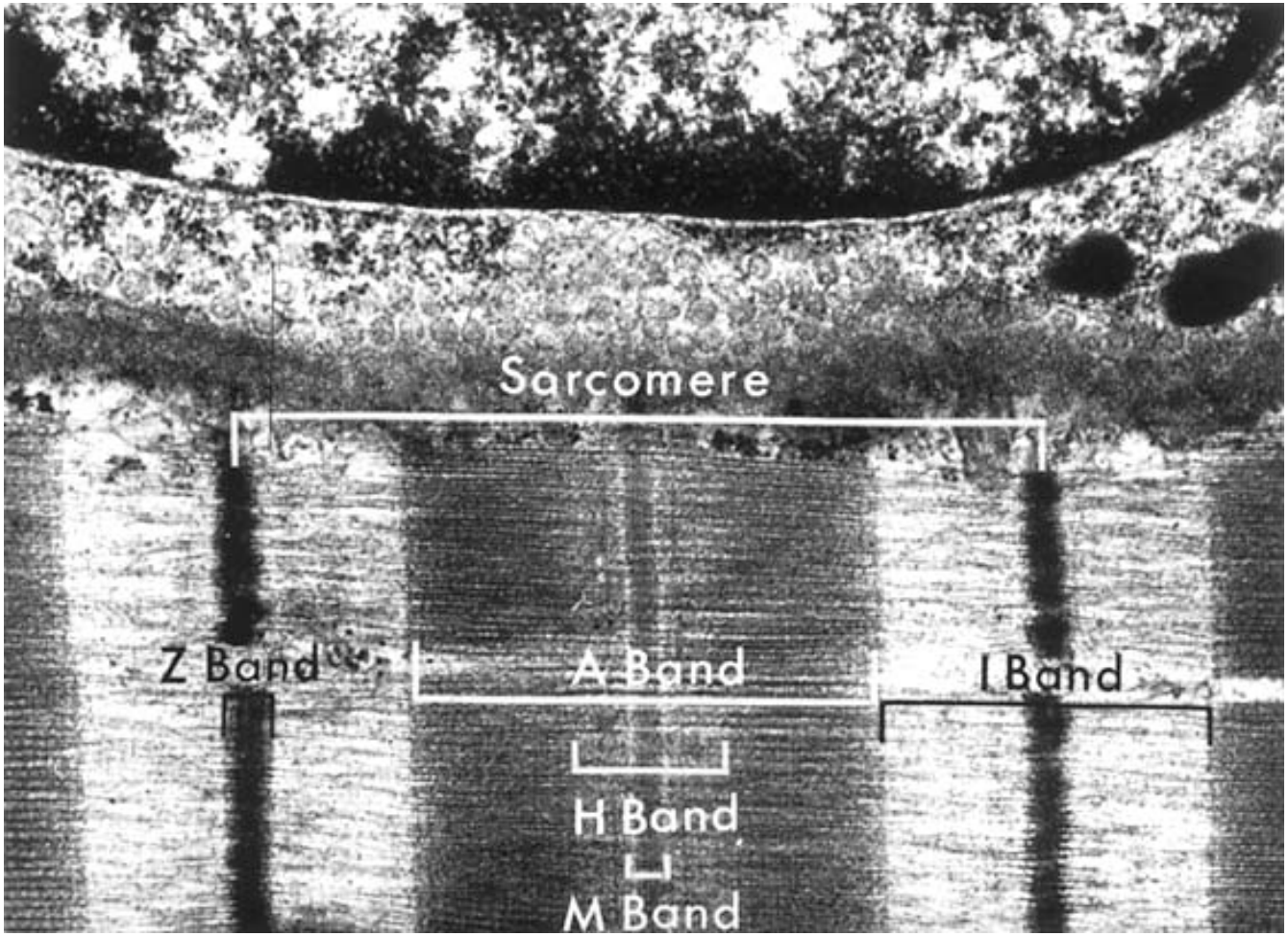

TABLE 27-1 -- Muscle Fiber Types

	Type 1	Type 2
Action	Sustained force	Sudden movements
Strength	Weight-bearing	Purposeful motion
Enzyme content	NADH dark staining	NADH light staining
	ATPase at pH 4.2, dark staining	ATPase at pH 4.2, light staining
	ATPase at pH 9.4, light staining	ATPase at pH 9.4, dark staining
Lipids	Abundant	Scant
Glycogen	Scant	Abundant
Ultrastructure	Many mitochondria	Few mitochondria

	Wide Z-band	Narrow Z-band
Physiology	Slow-twitch	Fast-twitch
Color	Red	White
Prototype	Soleus (pigeon)	Pectoral (pigeon)

consist of specialized muscle and nerve fibers, delimited by a connective tissue capsule.

The connective tissue sheath of muscles includes the *endomysium*, which surrounds individual muscle fibers; the *perimysium*, which groups muscle fibers into primary and secondary bundles (fasciculi); and the *epimysium*, which envelops single muscles or large groups of fibers.

Pathology

General Reactions of the Motor Unit

The two main responses of peripheral nerve to injury are based on the target of the insult: either the Schwann cell or the axon. Diseases that affect primarily the Schwann cell lead to a loss of myelin, referred to as *segmental demyelination*. In contrast, primary involvement of the neuron and its axon leads to axonal degeneration. In some diseases, axonal degeneration may be followed by *axonal regeneration* and *reinnervation* of muscle. The two principal pathologic processes seen in skeletal muscle are denervation atrophy, which follows loss of axons, and those due to a primary abnormality of the muscle fiber itself, referred to as *myopathy*. We now consider the general features of these processes.

SEGMENTAL DEMYELINATION

Segmental demyelination occurs when there is dysfunction of the Schwann cell (as in Guillain-Barré Syndrome) or damage to the myelin sheath (e.g., in hereditary motor and sensory neuropathy); there is no primary abnormality of the axon. The process affects some Schwann cells and their corresponding internodes while sparing others (see Fig. 27-1). The disintegrating myelin is engulfed initially by Schwann cells

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Figure 27-4 *A*, ATPase histochemical staining, at pH 9.4, of normal muscle showing checkerboard distribution of intermingled type 1 *(light)* and type 2 *(dark)* fibers. *B*, In contrast, fibers of either histochemical type are grouped together after reinnervation of muscle. *C*, A cluster of atrophic fibers (group atrophy) in the center *(arrow)*.

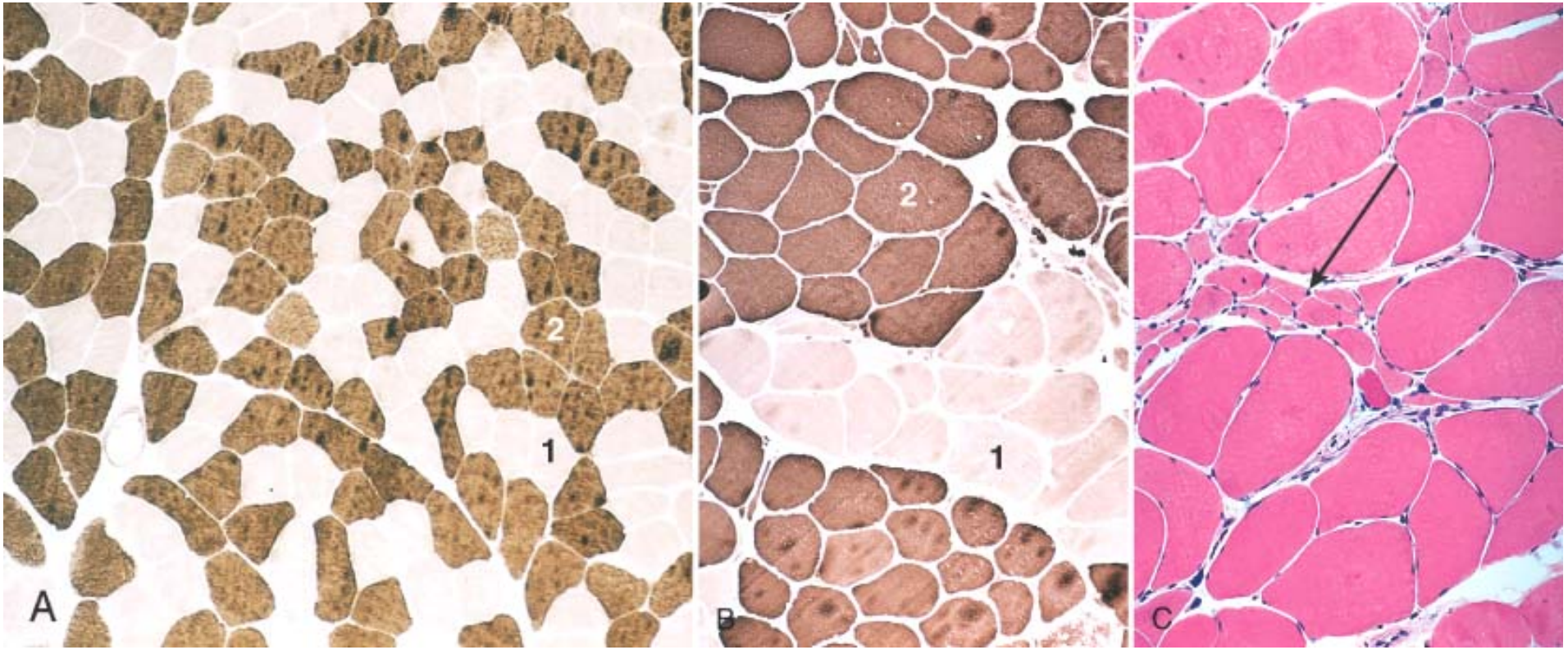

Figure 27-5 Electron micrograph of a single, thinly myelinated axon *(arrow)* surrounded by concentrically arranged Schwann cells, forming an onion bulb. *(Courtesy of G. Richard Dickersin, MD, from Diagnostic Electron Microscopy: A Text-Atlas. New York, Igaku-Shoin Medical Publishers, 2000, p. 984.) Inset,* Light microscopic appearance of an onion bulb neuropathy, characterized by "onion bulbs" surrounding axons.

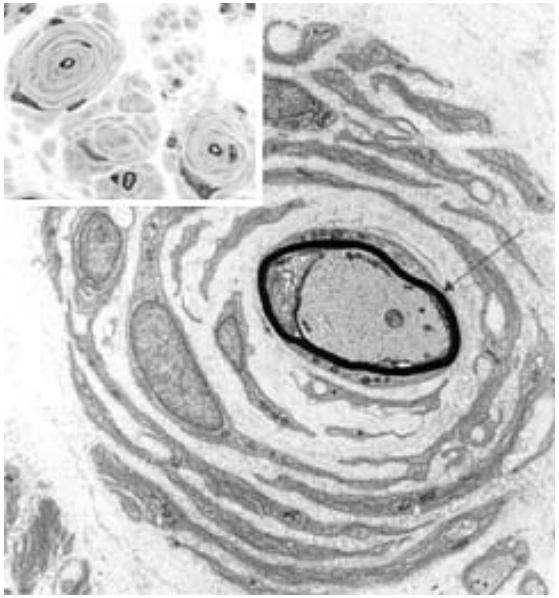

Figure 27-6 Electron micrograph of a degenerating axon *(arrow)* adjacent to several intact unmyelinated fibers *(arrow-heads).* The axon is markedly distended and contains numerous degenerating organelles and dense bodies.

TABLE 27-2 -- Hereditary Sensory and Autonomic Neuropathies

Disease and Inheritance	Gene and Locus	Clinical and Pathologic Findings
HSAN I; autosomal-dominant	Serine palmitoyl transferase, long-chain base, subunit 1 (SPTLC1) gene; 9q22.1-q22.3	Predominantly sensory neuropathy, presenting in young adults; axonal degeneration (mostly myelinated fibers)
HSAN II; autosomal-recessive (some cases are sporadic)	Unknown	Predominantly sensory neuropathy, presenting in infancy; axonal degeneration (mostly myelinated fibers)
HSAN III (Riley-Day syndrome; familial dysautonomia; most often in Jewish children); autosomal-recessive	Inhibitor of kappa light polypeptide gene enhancer in B cells, kinase complex-associated protein (IKBKAP or IKAP) gene; 9q31-q33	Predominantly autonomic neuropathy, presenting in infancy; axonal degeneration (mostly unmyelinated fibers); atrophy and loss of sensory and autonomic ganglion cells

The pathologic findings of many of the hereditary neuropathies are those of an axonal neuropathy. Fiber loss is the most prominent finding.

Hereditary Motor and Sensory Neuropathy Type I

The most common hereditary peripheral neuropathy, *Charcot-Marie-Tooth (CMT) disease, hypertrophic form (HMSN I)*, usually presents in childhood or early adulthood. A characteristic progressive muscular atrophy of the calf seen in these patients gives rise to the common clinical term *peroneal muscular atrophy*. Patients may be asymptomatic, but when they present, it is often with symptoms such as distal muscle weakness, atrophy of the calf, or secondary orthopedic problems of the foot (such as *pes cavus*).

Molecular Genetics.

The disease is genetically heterogeneous. In most pedigrees (known as HMSN IA or CMT1A), there is a duplication of a large region of chromosome 17p11.2-p12, resulting in "segmental trisomy" of the duplicated region. The duplicated segment includes the gene for

TABLE 27-3 -- Hereditary Neuropathies Accompanying Inherited Metabolic Disease

Disease	Metabolic Defect	Inheritance	Clinical Findings	Pathologic Findings
Adrenoleukodystrophy	ATP-binding cassette, or ABC, transporter protein, subfamily D, member 1 (ALD protein, or ABCD1) gene; Xq28	X-linked; 4% of female carriers are symptomatic	Mixed motor and sensory neuropathy, adrenal insufficiency, spastic paraplegia; onset between 10 and 20 years for males with leukodystrophy, between 20 and 40 years for females with myeloneuropathy	Segmental demyelination, with onion bulbs; axonal degeneration (myelinated and unmyelinated); electron microscopy; linear inclusions in Schwann cells
Familial amyloid polyneuropathies	Transthyretin (TTR) gene (rarely other genes); 18q11.2-q12.1	Autosomal-dominant	Sensory and autonomic dysfunction; age at onset varies with site of mutation	Amyloid deposits in vessel walls and connective tissue with axonal degeneration
Porphyria, acute intermittent (AIP) or variegate coproporphyria	Enzymes involved in heme synthesis (acute intermittent porphyria—porphobilinogen deaminase deficiency; 11q24.1-q24.2)	Autosomal-dominant	Acute episodes of neurologic dysfunction, psychiatric disturbances, abdominal pain, seizures, proximal weakness, autonomic dysfunction; attacks may be precipitated by drugs	Acute and chronic axonal degeneration; regenerating clusters
Refsum disease	Peroxisomal enzyme phytanoyl CoA α-hydroxylase (PAHX) gene; 10pter-p11.2	Autosomal-recessive	Mixed motor and sensory neuropathy with palpable nerves; ataxia, night blindness, retinitis pigmentosa, ichthyosis; age at onset before 20 years (a genetically distinct infantile form also exists)	Severe onion bulb formation

peripheral myelin protein 22 (PMP22), but whether the disease is caused by overexpression of PMP22, by gene dosage effect,[28] [29] or by duplication of other adjacent genes is not clear. A separate genetic locus on chromosome 1 involves myelin protein zero (MPZ) but produces an identical clinical phenotype (HMSN IB).[26] A third set of pedigrees shows linkage to chromosome 16p, and is associated with mutations in a gene whose product is involved in protein degradation pathways.[30] In addition, some pedigrees are associated with mutations in the gene for the gap junction protein connexin-32, which is located on the X chromosome.[31]

Morphology.

CMT1 is a demyelinating neuropathy, both by nerve conduction velocity studies and pathologically. Histologic examination shows the consequences of repetitive demyelination and remyelination, with multiple onion bulbs, more pronounced in distal nerves than in proximal nerves (see Fig. 27-5). The axon is often present in the center of the onion bulb, and the myelin sheath is usually thin or absent. The redundant layers of Schwann cell hyperplasia surrounding individual axons are associated with enlargement of individual peripheral nerves that may be individually palpable, which has led to the term **hypertrophic neuropathy**. In the longitudinal plane, individual segments of the axon may show evidence of segmental demyelination. Autopsy studies of affected individuals have shown degeneration of the posterior columns of the spinal cord.

Clinical Course.

The disorder is usually autosomal-dominant, and although it is slowly progressive, the disability of sensorimotor deficits and associated orthopedic problems such as pes cavus are usually limited in severity, and a normal life span is typical. The relationship between the molecular events and the observed peripheral nerve pathology is not well understood.

Other Hereditary Motor and Sensory Neuropathies

HMSN II

This is a neuronal form of autosomal-dominant Charcot-Marie-Tooth disease that presents with signs and symptoms similar to those of HMSN I, although nerve enlargement is not seen and the disease presents at a slightly later age. This form is less common than HMSN I, and in some families (designated CMT2A), it is linked to chromosome 1p35-p36.[26] Additional, less common loci have been linked to forms CMT 2B to 2G.[26] Nerve biopsy specimens in this disorder show loss of myelinated axons as the predominant finding. Segmental demyelination of internodes is infrequent. These findings suggest that the site of primary cellular dysfunction is the axon or neuron.

Dejerine-Sottas Disease (HMSN III)

Dejerine-Sottas disease is a slowly progressive, autosomal-recessive disorder that begins in early childhood, manifested by delay in developmental milestones, such as the acquisition of motor skills. In contrast to HMSN I and HMSN II, in which muscular atrophy is limited to the leg, both trunk and limb muscles are involved in Dejerine-Sottas disease. On physical examination, *enlarged peripheral nerves* can be detected by inspection and palpation. The deep tendon reflexes are depressed or absent, and nerve conduction velocity is slowed. HMSN III is genetically heterogeneous, and arises from distinct mutations in the same myelin-associated genes that are mutated in HMSN I. These include genes encoding peripheral

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myelin protein 22 (PMP22), myelin protein zero (MPZ), periaxin (PRX), and early growth response 2 (EGR2).[32] [33] [34] Morphologically, the size of individual peripheral nerve fascicles is increased, often dramatically, with abundant onion bulb formation as well as segmental demyelination. There is usually evidence of axonal loss, and the axons that remain are often of diminished caliber. These findings are most severe in the distal portions of the peripheral nervous system; however, autopsy studies have shown that similar findings may be present in spinal roots.

Other less common forms of HMSN are characterized by additional neurologic and ophthalmologic abnormalities, such as retinitis pigmentosa and deafness.

ACQUIRED METABOLIC AND TOXIC NEUROPATHIES

Functional and structural changes in peripheral nerve develop in response to various metabolic alterations—either from endogenous disorders or from exogenous agents. The most common of these processes are discussed here.

Peripheral Neuropathy in Adult-Onset Diabetes Mellitus

The prevalence of peripheral neuropathy in patients with diabetes mellitus depends on the duration of the disease, with up to 50% of diabetic patients having peripheral neuropathy clinically after 25 years of diabetes and nearly 100% having conduction abnormalities electrophysiologically.[35] Several distinct clinicopathologic patterns of diabetes-related peripheral nerve abnormalities have been recognized (Chapter 24). They are categorized as *distal symmetric sensory or sensorimotor neuropathy*, *autonomic neuropathy*, and *focal or multifocal asymmetric neuropathy*. Individuals may develop any combination of these lesions; in fact, the first two (sensorimotor and autonomic) are often found together.

Morphology.

In patients with a distal symmetric sensorimotor neuropathy, the predominant pathologic finding is an axonal neuropathy. As with other chronic axonal neuropathies, there is often some segmental demyelination. There is a relative loss of small myelinated fibers and of unmyelinated fibers, but large fibers are also affected. Endoneurial arterioles show thickening, hyalinization, and intense periodic acid-Schiff (PAS) positivity in their walls and extensive reduplication of the basement membrane[36] (Fig. 27-7). Whether the lesions are due to ischemia[37] or metabolic derangement is unclear.

Clinical Course.

The most common peripheral neuropathy is the symmetric neuropathy that involves distal sensory and motor nerves. Patients with the neuropathy develop decreased sensation in the distal extremities with less evident motor abnormalities. The loss of pain sensation can result in the development of ulcers that heal poorly because of the diffuse vascular injury in diabetes, and are a major cause of morbidity. Another manifestation of diabetic neuropathy is *dysfunction of the autonomic nervous system*; this affects 20% to 40% of diabetics, nearly always in association with a distal sensorimotor neuropathy.[38] Some patients, especially elderly

Figure 27-7 Diabetic neuropathy with marked loss of myelinated fibers, a thinly myelinated fiber *(arrowheads)*, and thickening of endoneurial vessel wall *(arrow)*.

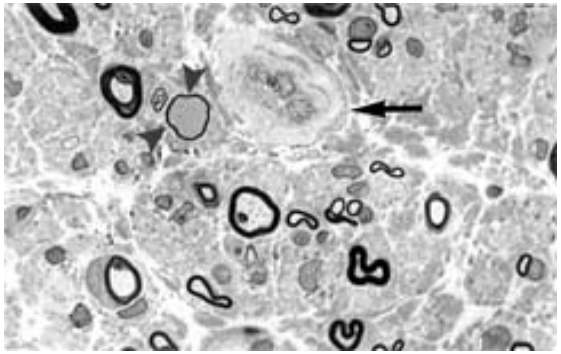


Figure 27-8 Traumatic neuroma showing disordered orientation of nerve fiber bundles *(purple)* intermixed with connective tissue *(blue)*.

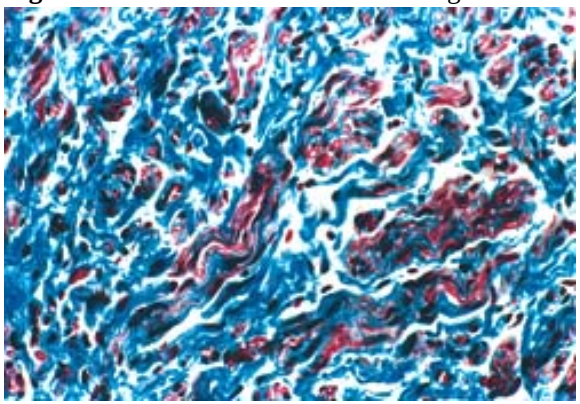


Figure 27-9 Spinal muscular atrophy with groups of atrophic muscle fibers resulting from denervation atrophy of muscle in early childhood.

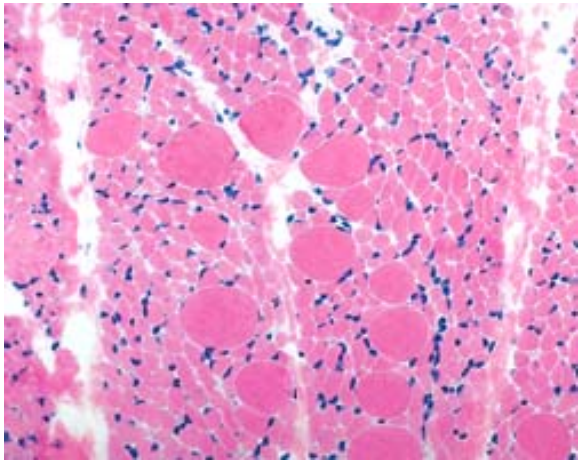


Figure 27-10 Diagram showing the relationship between the cell membrane (sarcolemma) and the sarcolemmal associated proteins. Dystrophin, an intracellular protein, forms an interface between the cytoskeletal proteins and a group of transmembrane proteins, the dystroglycans and the sarcoglycans. These transmembrane proteins have interactions with the extracellular matrix, including the laminin proteins. Dystrophin also interacts with dystrobrevin and the syntrophins, which form a link with neuronal-type nitric oxide synthetase (nNOS) and caveolin. Mutations in dystrophin are associated with the X-linked muscular dystrophies, mutations in caveolin and the sarcoglycan proteins with the autosomal limb girdle muscular dystrophies, and mutations in the α2-laminin (merosin) with a form of congenital muscular dystrophy.

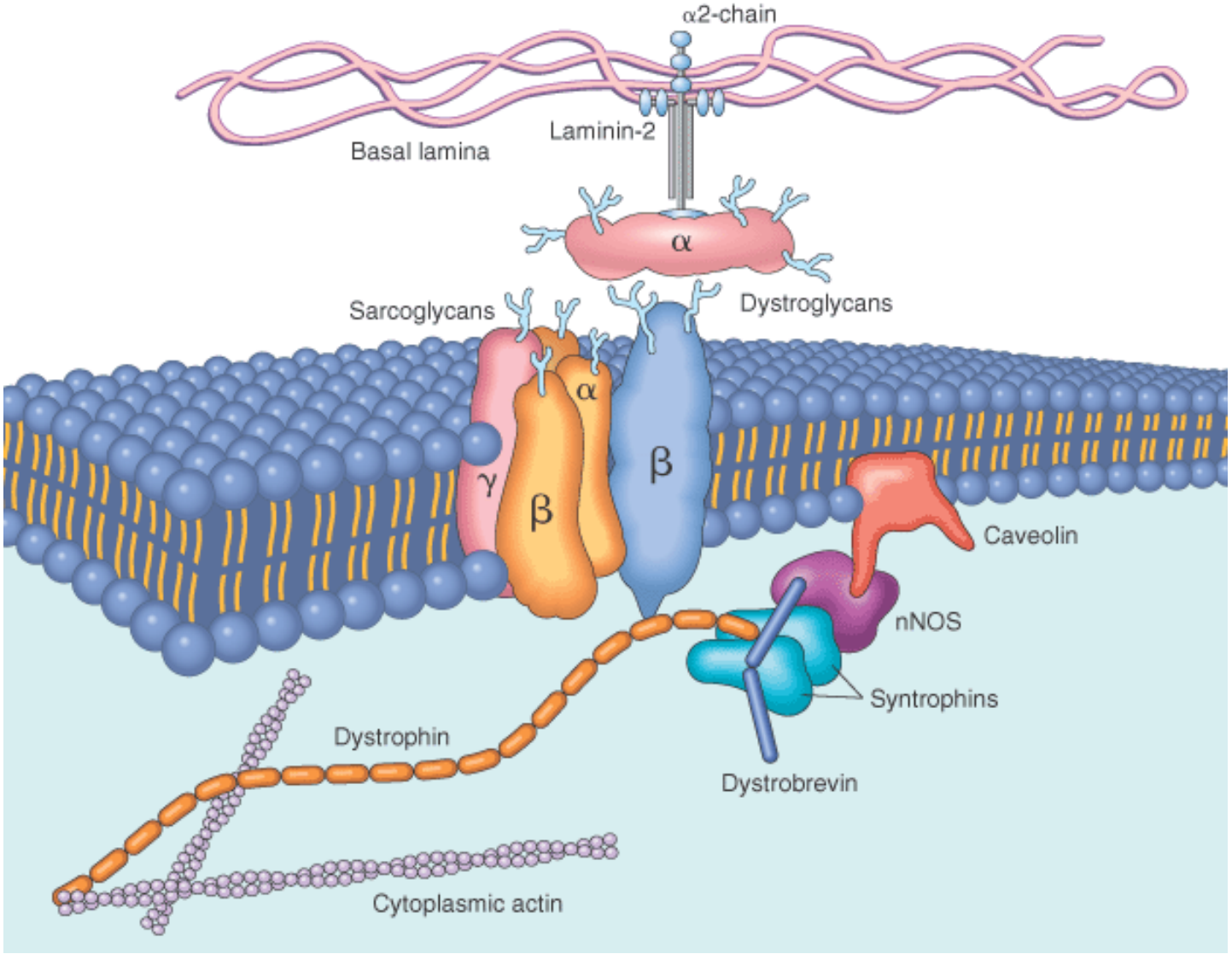


Figure 27-11 *A*, Duchenne muscular dystrophy (DMD) showing variation in muscle fiber size, increased endomysial connective tissue, and regenerating fibers *(blue hue)*. *B*, Western blot showing absence of dystrophin in DMD and altered dystrophin size in Becker muscular dystrophy (BMD) compared with control (Con). *(Courtesy of Dr. L. Kunkel, Children's Hospital, Boston, MA.)*

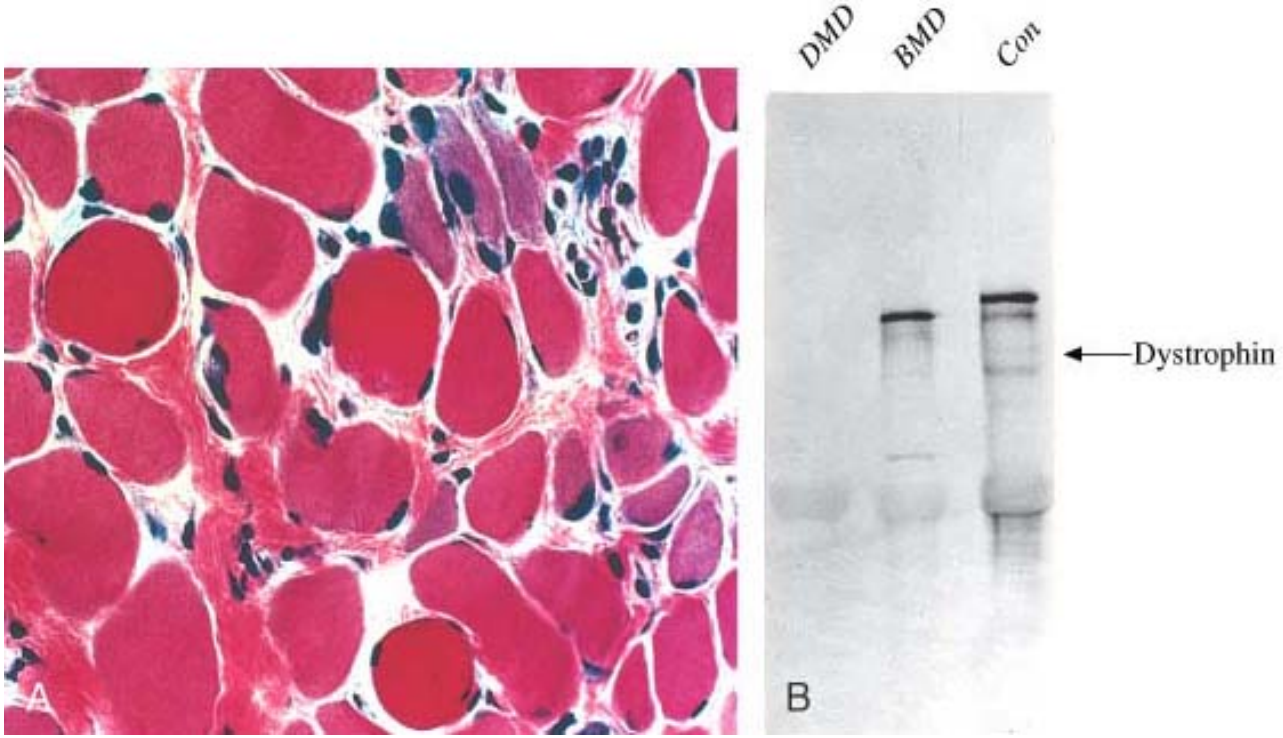


TABLE 27-4 -- Other Muscular Dystrophies

Disease and Inheritance	Gene and Locus	Clinical Findings	Pathologic Findings
Facioscapulohumeral muscular dystrophy; autosomal-dominant	Type 1A—deletion of variable number of 3.3-kB subunits of a tandemly arranged repeat (D4Z4) on 4q35	Variable age at onset (most commonly 10–30 years); Weakness of muscles of face, neck, and shoulder girdle	Dystrophic myopathy, but also often including inflammatory infiltrates of muscle.
	Type 1B (FSHMD1B)—locus unknown		
Oculopharyngeal muscular dystrophy; autosomal-dominant	Poly(A)-binding protein-2 (PABP2) gene; 14q11.2-q13	Onset in midadult life; ptosis and weakness of extraocular muscles; difficulty in swallowing	Dystrophic myopathy, but often including rimmed vacuoles in type 1 fibers
Emery-Dreifuss muscular dystrophy; X-linked (mostly)	Emerin (EMD1) gene; Xq28	Variable onset (most commonly 10–20 years); prominent contractures, especially of elbows and ankles	Mild myopathic changes; absent emerin by immunohistochemistry
Congenital muscular dystrophies; autosomal-recessive (Also called muscular dystrophy, congenital, subtypes MDC1A, MDC1B, MDC1C)	Type 1A (merosin-deficient type)—laminin α2 (merosin) gene; 6q22-q23	Neonatal hypotonia, respiratory insufficiency, delayed motor milestones	Variable fiber size and extensive endomysial fibrosis
	Type 1B—locus at 1q42; gene unknown		

	Type 1C; fukutin-related protein gene; 19q13.3		

muscle groups, and the specific diagnosis is based largely on the pattern of clinical muscle weakness (Table 27-4). Several autosomal muscular dystrophies, however, affect the proximal musculature of the trunk and limbs, similar to the X-linked muscular dystrophies, and are termed *limb girdle muscular dystrophies*.

Limb girdle muscular dystrophies are inherited in either an autosomal-dominant (type 1) or an autosomal-recessive (type 2) pattern (Table 27-5). Six subtypes of the dominant dystrophies (1A to 1F) and ten subtypes of the recessive limb girdle dystrophies (2A to 2J) have been identified. Mutations of the *sarcoglycan complex of proteins* have been identified in four of the limb girdle muscular dystrophies[52] (2C, 2D, 2E, and 2F). These membrane proteins interact with dystrophin through another transmembrane protein, β-dystroglycan (Fig. 27-10).

Myotonic Dystrophy

Myotonia, the sustained involuntary contraction of a group of muscles, is the cardinal neuromuscular symptom in this disease.[53] Patients often complain of "stiffness" and have difficulty in releasing their grip, for instance, after a handshake. Myotonia can often be elicited by percussion of the thenar eminence.

Pathogenesis.

Inherited as an autosomal-dominant trait, the disease tends to increase in severity and appear at a younger age in succeeding generations, a phenomenon termed *anticipation*. Myotonic dystrophy is associated with a trinucleotide CTG repeat expansion on chromosome 19q13.2-13.3. This expansion affects the mRNA for the dystrophila myotonia-protein kinase (DMPK). [54] In normal subjects, fewer than 30 repeats are present; disease develops with expansion of this repeat, and in severely affected individuals, several thousand repeats may be present. [54] The mutation is not stable within a pedigree; with each generation, more repeats accumulate, and this appears to correspond to the clinical

TABLE 27-5 -- Limb Girdle Muscular Dystrophies

Type	Inheritance	Locus	Gene	Clinicopathologic Features
1A	Autosomal-dominant	5q31	Myotilin	Onset in adult life with slow progression of limb weakness, but sparing of facial muscles; dysarthric speech
1B	Autosomal-dominant	1q21	Lamin A/C	Onset before the age of 20 years in lower limbs, progression during many years with cardiac involvement
1C	Autosomal-dominant	3p25	Caveolin-3 (M-caveolin)	Onset before the age of 20, clinically similar to type 1B
1D	Autosomal-dominant	7p	Unknown	Limb girdle muscle weakness, adult onset
2A	Autosomal-recessive	15q15.1-21.1	Calpain 3	Onset in late childhood to middle age; slow progression during 20–30 years
2B	Autosomal-recessive	2p13.3-q13.1	Dysferlin	Mild clinical course with onset in early adulthood

2C	Autosomal-recessive	13q12	γ-Sarcoglycan	Severe weakness during childhood, rapid progression; dystrophic myopathy on muscle biopsy
2D	Autosomal-recessive	17q21	α-Sarcoglycan (adhalin)	Severe weakness during childhood, rapid progression; dystrophic myopathy on muscle biopsy
2E	Autosomal-recessive	4q12	β-Sarcoglycan	Onset in early childhood, with Duchenne-like clinical course
2F	Autosomal-recessive	5q33	δ-Sarcoglycan	Early onset and severe myopathy; dystrophic myopathy on muscle biopsy
2G	Autosomal-recessive	17q11-q12	Telethonin	Distal weakness with limb-girdle weakness in late childhood to adulthood; rimmed vacuoles in muscle cells
2H	Autosomal-recessive	9q31-q34.1	Tripartite motif-containing protein 32 (TRIM32)	Limb-girdle and facial weakness with onset in childhood, mild, slowly progressive course

feature of anticipation. Expansion of the trinucleotide repeat influences the eventual level of protein product.

The pathologic features of the disease relate only in part to altered DMPK function. RNA that contains trinucleotide repeat expansions can directly affect splicing of other RNAs, including those for the ClC-1 chloride channel.[55] A second form of myotonic dystrophy is associated with untranslated CCTG expansion in a gene called *ZNF9* on chromosome 3.[56]

Morphology.

Skeletal muscle may show variation in fiber size. In addition, there is a striking increase in the number of internal nuclei, which on longitudinal section may form conspicuous chains. Another well-recognized abnormality is the **ring fiber**, with a subsarcolemmal band of cytoplasm that appears distinct from the center of the fiber. The rim contains myofibrils that are oriented circumferentially around the longitudinally oriented fibrils in the rest of the fiber. The ring fiber may be associated with an irregular mass of sarcoplasm **(sarcoplasmic mass)** extending outward from the ring. These sarcoplasmic masses stain blue with hematoxylin and eosin, red with Gomori trichrome, and intensely blue with the nicotinamide adenine dinucleotide-tetrazolium reductase (NADHTR) histochemical reaction. Histochemical techniques have demonstrated a relative atrophy of type 1 fibers early in the course of the disease in some cases. Of all the dystrophies, only myotonic dystrophy shows pathologic changes in the intrafusal fibers of muscle spindles, with fiber splitting, necrosis, and regeneration.

Clinical Course.

The disease often presents in late childhood with abnormalities in gait secondary to weakness of foot dorsiflexors and subsequently progresses to weakness of the hand intrinsic muscles and wrist extensors. Atrophy of muscles of the face and ptosis ensue, leading to the typical facial appearance. Cataracts, which are present in virtually every patient, may be detected early in the course of the disease with slit-lamp examination. Other associated abnormalities include frontal balding, gonadal atrophy, cardiomyopathy, smooth muscle involvement, decreased plasma immunoglobulin G, and an abnormal glucose tolerance test response. Dementia has been reported in some cases.

ION CHANNEL MYOPATHIES (CHANNELOPATHIES)

The *ion channel myopathies*, or channelopathies, are a group of familial diseases characterized clinically by myotonia, relapsing episodes of hypotonic paralysis (induced by vigorous exercise, cold, or a high-carbohydrate meal), or both. Hypotonia variants associated with elevated, depressed, or normal serum potassium levels at the time of the attack are called *hyperkalemic, hypokalemic, and normokalemic periodic paralysis*, respectively.

Pathogenesis.

As their name indicates, at the molecular level these diseases are caused by mutations in genes that encode ion channels.[57] [58] Hyperkalemic periodic paralysis results from mutations in the gene that encodes a skeletal muscle sodium channel protein (SCN4A), which regulates the entry of sodium into muscle during contraction. The gene for hypokalemic periodic paralysis encodes a voltage-gated calcium channel.

TABLE 27-6 -- Congenital Myopathies

Disease and Inheritance	Gene and Locus	Clinical Findings	Pathologic Findings
Central core diseases; autosomal-dominant	Ryanodine receptor-1 (RYR1) gene; 19q13.1	Early-onset hypotonia and nonprogressive weakness; associated skeletal deformities; may develop malignant hyperthermia	Cytoplasmic cores are lightly eosinophilic and distinct from surrounding sarcoplasm; Found only in type 1 fibers, which usually predominate, best seen on NADH stain
Nemaline myopathy; autosomal-dominant or autosomal-recessive	Autosomal-dominant (NEM1)—Tropomyosin 3 (TPM3) gene; Autosomal-recessive (NEM2)—nebulin (NEB) gene; 2q22	Weakness, hypotonia, and delayed motor development in childhood; may also be seen in adults; usually nonprogressive; involves proximal limb muscles most severely; skeletal abnormalities may be present	Aggregates of subsarcolemmal spindle-shaped particles *(nemaline rods)*; occur predominantly in type 1 fibers; derived from Z-band material (α-actinin) and best seen on modified Gomori stain
	Autosomal-dominant or recessive—skeletal muscle actin, α chain (ACTA1) gene; 1q42.1		
Myotubular (centronuclear) myopathy; X-linked (MTM1), autosomal-recessive, or autosomal-dominant	X-linked—myotubularin (MTM1) gene; Xq28	X-linked form presents in infancy with prominent hypotonia and poor prognosis; autosomal forms have limb weakness and are slowly progressive; autosomal-recessive form is intermediate in severity and prognosis	Abundance of centrally located nuclei involving the majority of muscle fibers; central nuclei are usually confined to type 1 fibers, which are small in diameter, but can occur in both fiber types
	Autosomal-dominant—myogenic factor 6 (MYF6) gene; 12q21		
	Autosomal-recessive—locus and gene unknown		

Malignant hyperpyrexia (malignant hyperthermia) is a rare clinical syndrome characterized by a dramatic hypermetabolic state (tachycardia, tachypnea, muscle spasms, and later hyperpyrexia) triggered by the induction of anesthesia, usually with halogenated inhalational agents and succinylcholine. The clinical syndrome may also occur in predisposed individuals with hereditary muscle diseases, including congenital myopathies, dystrophinopathies, and metabolic myopathies. The only reliable method of diagnosis is contraction of biopsied muscle on exposure to anesthetic. Mutations in different genes have been identified in families with susceptibility to malignant hyperthermia, including genes encoding a voltage-gated calcium channel (lq32), an L-type voltage-dependent calcium channel (7q21-q22), and a ryanodine receptor (19q13.1).[59]

Figure 27-12 *A,* Nemaline myopathy with numerous rod-shaped, intracytoplasmic inclusions *(dark purple structures). B,* Electron micrograph of subsarcolemmal nemaline bodies, showing material of Z-band density.

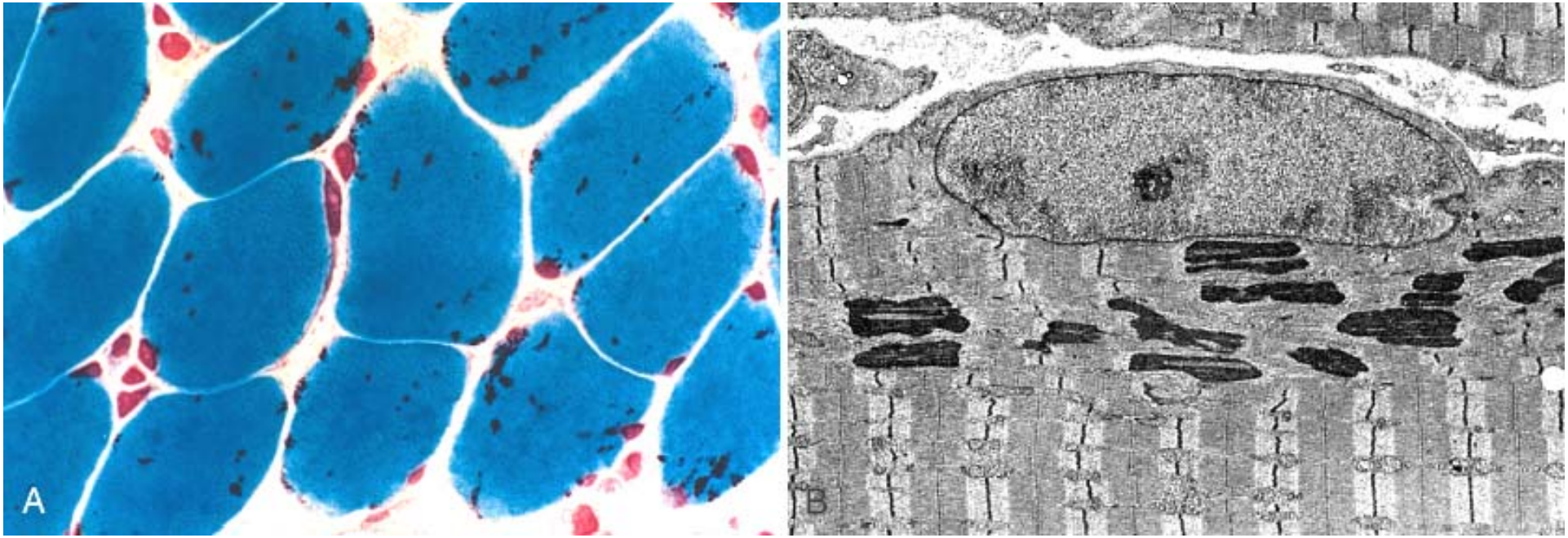


Figure 27-13 *A,* Mitochondrial myopathy showing an irregular fiber with subsarcolemmal collections of mitochondria that stain red with the modified Gomori trichrome stain *(ragged red fiber). B,* Electron micrograph of mitochondria from biopsy specimen in *A* showing "parking lot" inclusions.

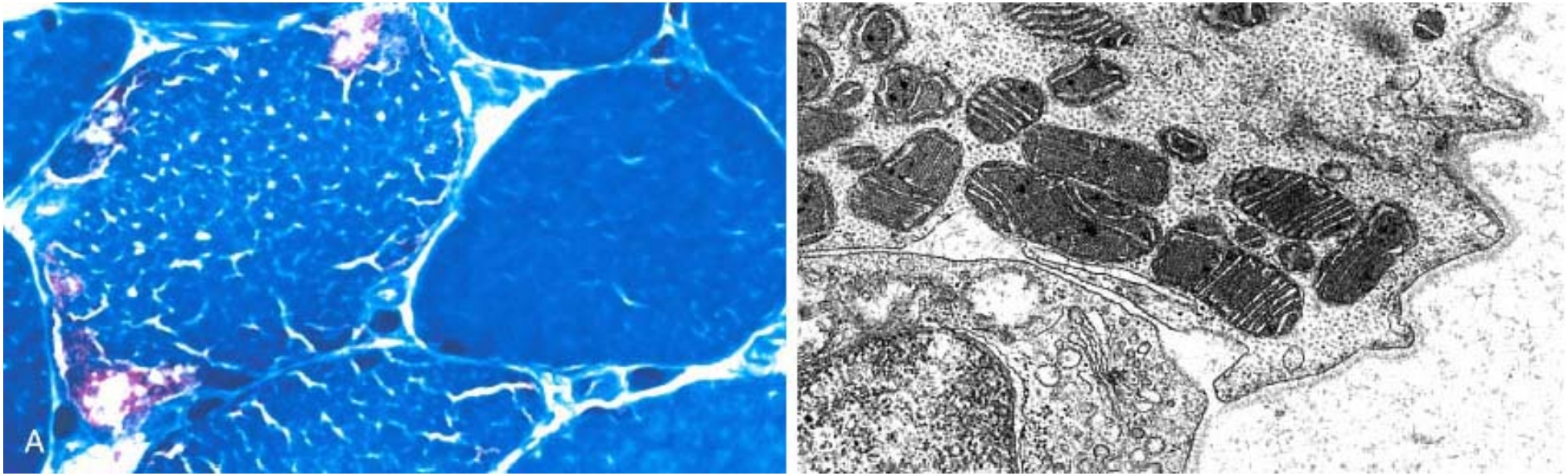
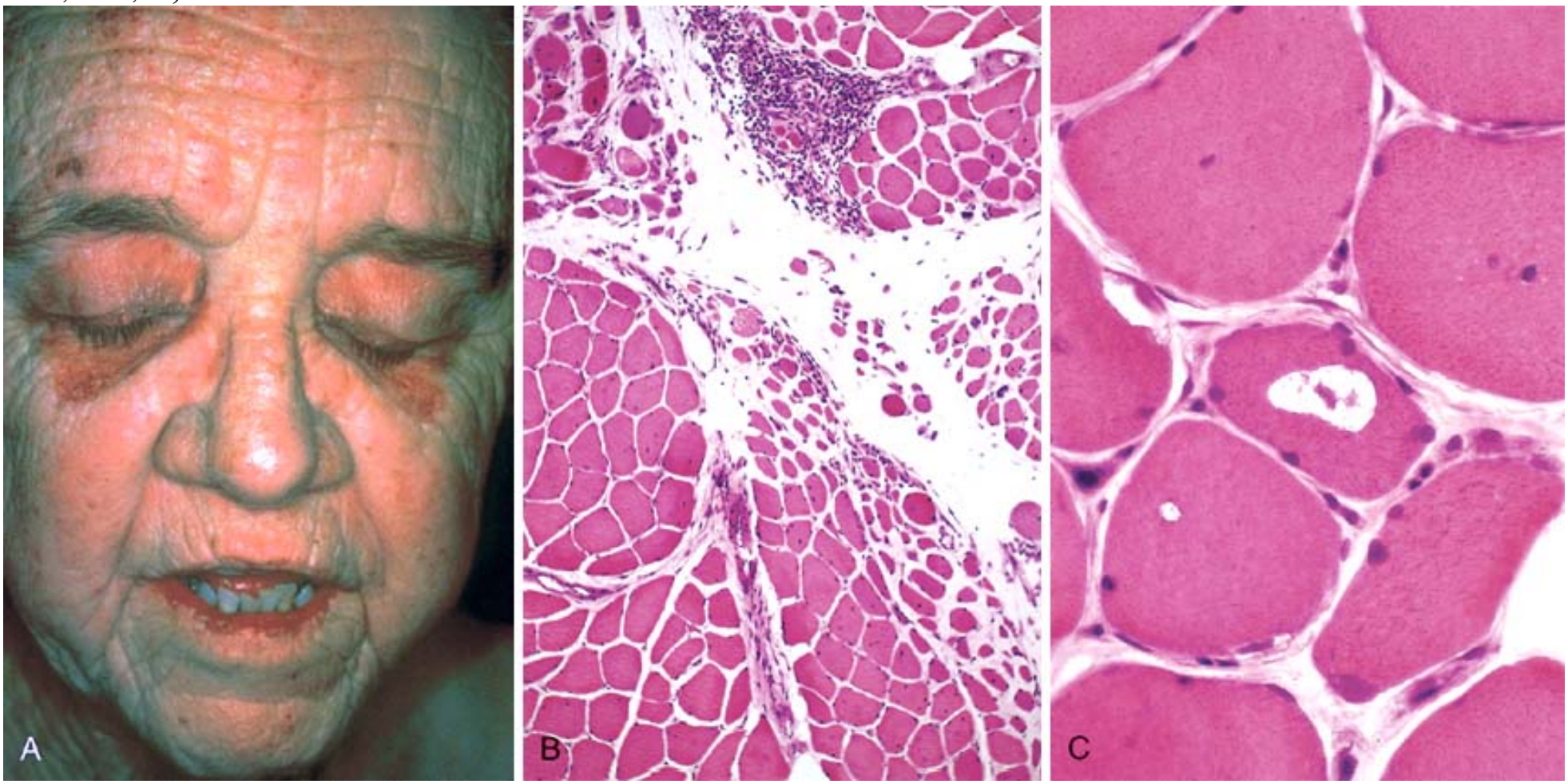


Figure 27-14 *A,* Dermatomyositis. Note the rash affecting the eyelids. *B,* Dermatomyositis. The histologic appearance of muscle shows perifascicular atrophy of muscle fibers and inflammation. *C,* Inclusion body myositis showing a vacuole within a myocyte. *(Courtesy of Dr. Dennis Burns, Department of Pathology, University of Texas Southwestern Medical*

School, Dallas, TX.)



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Chapter 28 - The Central Nervous System

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The human central nervous system (CNS) is an enormously complex tissue serving the organism as a processing center linking information between the outside world and the body. The principal functional unit of the CNS is the neuron; the best estimates are that there are about 10^{11} neurons in the human brain. Neurons, although similar in many ways to other cells in the body, are unique in their ability to receive, store, and transmit information. Neurons differ greatly from one another in many important properties: their functional roles (e.g., sensory, motor, autonomic), the distribution of

their connections, the neurotransmitters they use for synaptic transmission, their metabolic requirements, and their levels of electrical activity at a given moment. A set of neurons, not necessarily clustered together in a region of the brain, may thus be singled out for destruction in a pathologic condition—*selective vulnerability*—because it shares one or more of these properties. Furthermore, and of particular importance in medicine, most mature neurons are postmitotic cells that are incapable of cell division, so destruction of even a small number of neurons responsible for a specific function may leave the patient with a severe clinical neurologic deficit. Stem cell populations have been described in several areas of the brain and represent a potential mechanism for repair after injury.[1] In comparison to other organ systems of the body, the nervous system has several unique anatomic and physiologic characteristics: the protective bony enclosure of the skull and spinal column that contains it, a specialized system of autoregulation of cerebral blood flow, metabolic substrate requirements, the absence of a conventional lymphatic system, a special cerebrospinal fluid (CSF) circulation, limited immunologic surveillance, and distinctive responses to injury and wound healing. As a result of these special characteristics, the CNS is vulnerable to unique pathologic processes, and the reactions of CNS tissue to injury differ considerably from those encountered elsewhere.[2] [3]

Normal Cells

The principal cells of the CNS are neurons, glia, and the cells that compose the meninges and blood vessels.

Neurons

In the CNS, neurons are topographically organized either as aggregates (nuclei, ganglia) or as elongated columns or layers (such as the intermediolateral gray column of the spinal cord or the six-layered cerebral cortex).[4] Functional domains are located in many of these anatomically defined regions (such as the hypoglossal nucleus of the medulla for motor fibers of the twelfth cranial nerve; calcarine cortex of the occipital lobe for primary visual cortex). In addition, as a further dimension of anatomic-functional specificity, some cortical and subcortical neurons and their projections are arranged somatotopically (such as motor and sensory homunculi).[5] Neurons vary considerably in structure and size throughout the nervous system and within a given brain region. With conventional histologic preparations, an anterior horn neuron in the spinal cord has a cell body (perikaryon) that is about 50 µm wide, a relatively large and somewhat eccentrically placed nucleus, a prominent nucleolus, and abundant Nissl substance; the nucleus of a granule cell neuron of the cerebellar cortex is about 10 µm across, and its perikaryon and nucleolus are not readily visible by light microscopy. Electron microscopic study reveals further variability among neurons in cytoplasmic content and the shape of the cells and their processes.[6] Characteristic ultrastructural features common to many neurons include microtubules, neurofilaments, prominent Golgi apparatus and rough endoplasmic reticulum, and synaptic specializations. Despite these shared structures, axon length may vary greatly (hundreds of microns for interneurons versus a meter for an upper motor neuron). Immunohistochemical markers for neurons and their processes commonly used in diagnostic work include neurofilament protein, NeuN, and synaptophysin.[7]

Glia

Glial cells are derived from neuroectoderm (macroglia: astrocytes, oligodendrocytes, ependyma) or from bone marrow (microglia). Glial cells have important structural and metabolic interactions with neurons and their dendritic and axonal processes; they also have a primary role in a wide range of normal functions and reactions to injury, including inflammation, repair, fluid balance, and energy metabolism. The size and shape of the nucleus helps in the light microscopic distinction of one glial cell type from another, as their cytoplasmic processes are often not apparent on H&E preparations and can be demonstrated only with the use of metallic impregnation, immunohistochemical, or electron microscopic methods. *Astrocytes* typically have round to oval nuclei (10 µm wide) with evenly dispersed, pale chromatin; *oligodendrocytes* have a denser, more homogeneous chromatin in a rounder and smaller nucleus (8 µm); and *microglia* have an elongated, irregularly shaped nucleus (5 to 10 µm) with clumped chromatin. *Ependymal cells*, on the other hand, do have visible cytoplasm; seen with H & E, they are columnar epithelial-like cells with a ciliated/microvillous border facing the ventricular surface with pale, vesiculated nuclei (each about 8 µm) located at the abluminal end of the cell.

ASTROCYTES

This glial cell is found throughout the CNS in both gray and white matter. *Protoplasmic* astrocytes occur mainly in the gray matter; *fibrous* astrocytes occur in white and gray matter. The cell derives its name from its star-shaped appearance, which is imparted by the multipolar, branching cytoplasmic processes that emanate from the cell body containing the characteristic cytoplasmic intermediate filament protein called glial fibrillary acidic protein (GFAP). These are seen well in tissue sections only with metallic impregnation techniques (e. g., the Golgi method) (Fig. 28-1*A*) or immunohistochemical preparations (Fig. 28-1*B*). The filaments are either aggregated in fascicles (in protoplasmic astrocytes) or dispersed diffusely throughout the cytoplasm (in fibrous astrocytes). Some astrocytic processes are directed toward neurons and their processes and synapses, where they are believed to act as metabolic buffers or detoxifiers, suppliers of nutrients, and electrical insulators. Others surround capillaries or extend to the subpial and subependymal zones, where they contribute to barrier functions controlling the flow of macromolecules between the blood, the CSF, and the brain. Astrocytes are also the principal cells responsible for repair and scar formation in the brain. Fibroblasts, which have a major role in wound healing elsewhere, are located mainly around large CNS blood vessels and in the meninges; they participate in wound healing only to a limited extent (primarily in the organization of subdural hematomas and the formation of abscess cavities).

Figure 28-1 *A,* Astrocytes and their processes. Some processes extend toward blood vessels (Golgi). *B,* Immunoperoxidase staining for glial fibrillary acidic protein shows astrocytic perinuclear cytoplasm and well-developed processes (*brown*). *(Courtesy of Dr. J. Corbo, Brigham and Women's Hospital, Boston, MA.)*

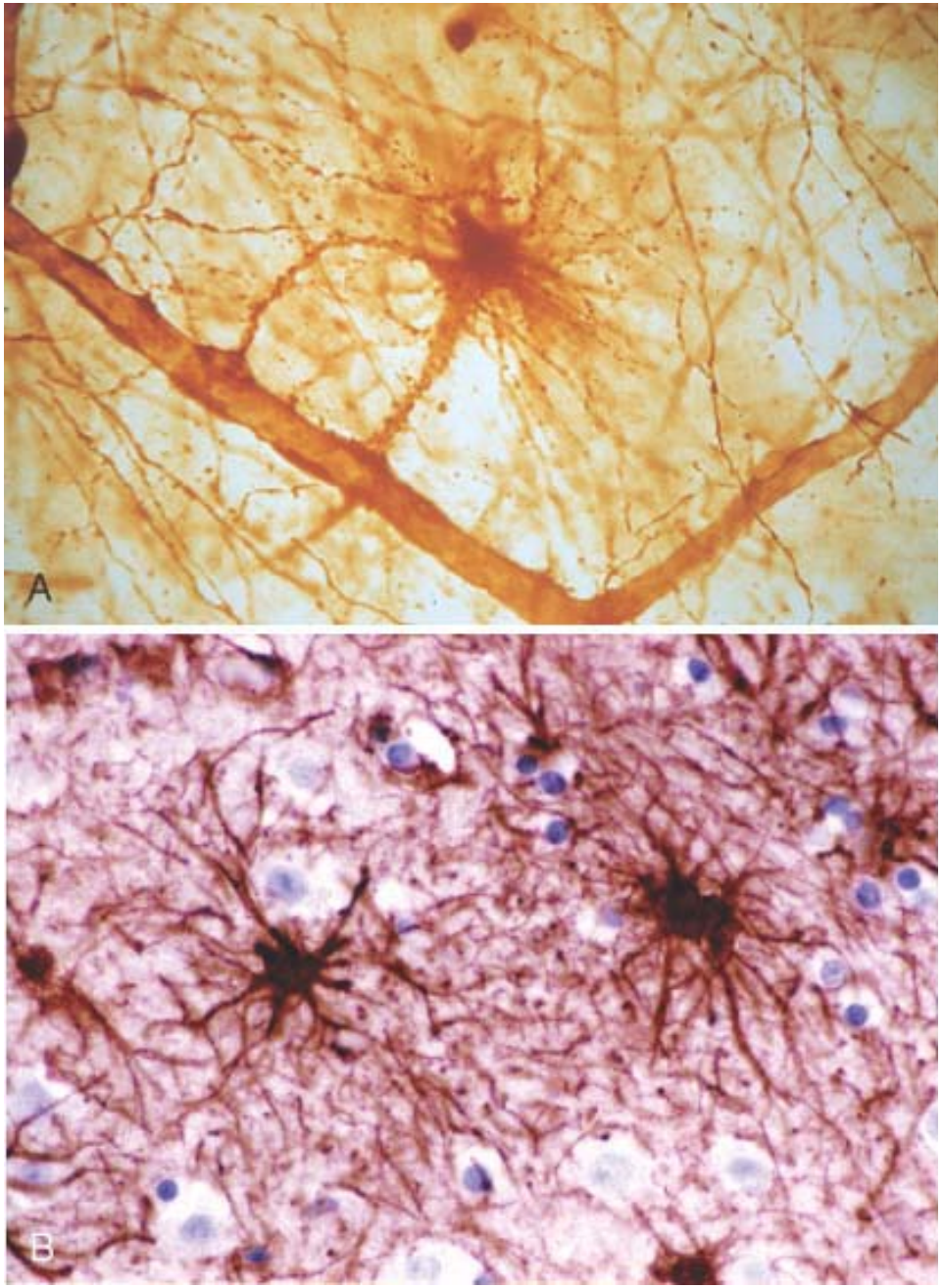

TABLE 28-1 -- Neurodegenerative Diseases Associated with Aggregated Proteins

Disease	Protein	Normal Structure	Aggregate/Inclusion	Location

Transmissible spongiform encephalopathies (Prion disease) (see Fig. 28-31)	Prion protein (PrP)	α-Helix and random coil	β-pleated sheet, proteinase K-resistant	Extracellular
Alzheimer disease (see Fig. 28-35*C*)	Amyloid precursor protein (APP)	α-Helix and random coil	β-pleated sheet, amyloid (fragment of APP)	Extracellular
Tauopathies and Alzheimer disease	Tau (microtubule binding protein)	3 and 4 repeat isoforms	Hyperphosphorylated aggregated protein	Intracellular
Parkinson disease (see Fig. 28-37*C*)	α-Synuclein	Random coil, repeats	Aggregated, Lewy bodies	Cytoplasmic
Multiple system atrophy	α-Synuclein	Random coil, repeats	Aggregated, Glial cytoplasmic inclusions	Cytoplasmic
Huntington disease	Huntingtin	Trinucleotide repeats	Insoluble aggregates	Nuclear
Spinocerebellar ataxias	Ataxins	Trinucleotide repeats	Insoluble aggregates	Nuclear

Modified from Welch WJ, Gambetti P: Chaperoning brain diseases. Nature 392:23–24, 1998.

REACTIONS OF ASTROCYTES TO INJURY

The cellular pathology of astrocytes may be subdivided into reactive responses that accompany the cells' proliferation (*gliosis*) and the sets of reactions to injury that lead to their death. In addition, morphologically diverse intracellular inclusions and deposits are seen in injured astrocytes.

- *Gliosis* is the most important histopathologic indicator of CNS injury, regardless of etiology. Astrocytes participate in this process by undergoing both hypertrophy and hyperplasia. The nucleus enlarges and becomes vesicular, and the nucleolus is prominent. The previously scant cytoplasm expands to a bright pink, somewhat irregular swath around an eccentric nucleus, from which emerge numerous stout, ramifying processes (*gemistocytic astrocyte*). Immunohistochemistry for glial fibrillary acidic protein (GFAP) splendidly demonstrates the extraordinary metamorphosis. In long-standing lesions, the nuclei become small and dark and lie in a dense net of processes. These cell processes, *glial "fibrils,"* are not true extracellular fibers. Proliferation of astrocytes residing between the molecular and granule cell layers of the cerebellum is a regular accompaniment of anoxic injury and other conditions associated with death of Purkinje cells, termed *Bergmann gliosis*.
- *Cellular swelling*, or swelling of the astrocyte cytoplasm, occurs regularly in acute insults when there is a failure of the cell's pump systems, as occurs in hypoxia, hypoglycemia, and toxic injuries.[13]
- *Rosenthal fibers* are thick, elongated, brightly eosinophilic structures that are somewhat irregular in contour and occur within astrocytic processes. Ultrastructurally, they exhibit dense osmiophilic deposits that contain two heat-shock proteins (αB-crystallin and hsp27) and ubiquitin. Rosenthal fibers are typically found in regions of long-standing gliosis; they are also characteristic of cerebellar pilocytic astrocytoma (see later), as well as the reactive brain adjacent to craniopharyngioma or syrinx cavities. In *Alexander*

disease, a leukodystrophy due to a mutation in the gene for GFAP,[14] abundant Rosenthal fibers are found in periventricular, perivascular, and subpial locations.

- *Corpora amylacea*, or polyglucosan bodies, are round, faintly basophilic, periodic acid-Schiff (PAS)-positive, concentrically lamellated structures ranging between 5 and 50 μm in diameter and located wherever there are astrocytic end processes, especially in the subpial and perivascular zones. Although consisting primarily of glycosaminoglycan polymers, they also contain heat-shock proteins and ubiquitin. They represent a degenerative change in the astrocyte, and they occur in increasing numbers with advancing age and in a rare condition called *adult polyglucosan body disease*. The *Lafora bodies* that are seen in the cytoplasm of neurons (as well as hepatocytes, myocytes, and other cells) in myoclonic epilepsy (Lafora body myoclonus with epilepsy) are of similar structure and biochemical composition.
- *Glial cytoplasmic inclusions* consisting of silver-positive meshes of 20- to 40-nm intermediate filaments that contain the protein α-synuclein[15] are characteristic of a number

of CNS degenerative diseases, collectively known as multiple system atrophy. [16]

• The *Alzheimer type II astrocyte* is a gray matter astrocyte with a large (two to three times normal) nucleus, pale-staining central chromatin, an intranuclear glycogen droplet, and a prominent nuclear membrane and nucleolus. Despite its name, it is unrelated to Alzheimer disease; rather, it occurs especially in patients with long-standing hyperammonemia due to chronic liver disease, Wilson disease, or hereditary metabolic disorders of the urea cycle.

Cerebral Edema, Raised Intracranial Pressure and Herniation, and Hydrocephalus

The brain and spinal cord exist within a rigid compartment defined by the skull, vertebral bodies, and dura mater. The advantage of housing as vital and delicate a structure as the CNS in a protective environment is obvious. On the other hand, such rigid confines provide little room for brain parenchymal expansion in disease states. A number of disorders may upset the delicate balance between brain volume and the fixed boundaries of the intracranial vault. These conditions include generalized brain edema, hydrocephalus, and focally expanding mass lesions.

CEREBRAL EDEMA

Cerebral edema or, more precisely, brain parenchymal edema may arise in the setting of a number of diseases. Two principal types are recognized:

• *Vasogenic edema* occurs when the integrity of the normal blood-brain barrier is disrupted and increased vascular permeability occurs, allowing fluid to escape from the intravascular compartment predominantly into the intercellular spaces of the brain. The paucity of conventional lymphatics and the close apposition of cell processes of neurons and glia in the brain greatly impairs the resorption of excess extracellular fluid. Vasogenic edema may be either localized, as when it results from abnormally permeable vessels adjacent to inflammatory disease or neoplasms, or generalized.

• *Cytotoxic edema*, in contrast, implies an increase in intracellular fluid secondary to neuronal, glial or endothelial cell membrane injury, as might be encountered in a patient with a generalized hypoxic/ischemic insult or with some intoxications.

In practice, conditions associated with generalized edema often have elements of both vasogenic and cytotoxic edema.

Interstitial edema (hydrocephalic edema) occurs especially around the lateral ventricles when there is an abnormal flow of fluid from the intraventricular CSF across the ependymal lining to the periventricular white matter in a setting of increased intraventricular pressure.

Morphology.

The edematous brain is softer than normal and often appears to "overfill" the cranial vault. In generalized edema, the gyri are flattened, the intervening sulci are narrowed, and the ventricular cavities are compressed. As the brain expands, herniation may occur.

RAISED INTRACRANIAL PRESSURE AND HERNIATION

Raised intracranial pressure is an increase in mean CSF pressure above 200 mm water with the patient recumbent. It occurs when the volume of brain tissue increases beyond the limit permitted by compression of veins and displacement of CSF. Most cases are associated with a mass effect, either diffuse, as in generalized brain edema, or focal, as with tumors, abscesses, or hemorrhages. Because the cranial vault is subdivided by rigid dural folds (the falx and tentorium), a focal expansion of the brain causes it to be displaced in relation to these partitions. If the expansion is sufficiently severe, a *herniation* of the brain will occur (Fig. 28-2).

• *Subfalcine (cingulate) herniation* occurs when unilateral or asymmetric expansion of a cerebral hemisphere displaces the cingulate gyrus under the falx cerebri. This may be associated with compression of branches of the anterior cerebral artery.

• *Transtentorial (uncinate, mesial temporal) herniation* occurs when the medial aspect of the temporal lobe is compressed against the free margin of the tentorium cerebelli.

With increasing displacement of the temporal lobe, the third cranial nerve is compromised, resulting in pupillary dilation and impairment of ocular movements on the side of the lesion. The posterior cerebral artery may also be compressed, resulting in ischemic injury to the territory supplied by that vessel, including the primary visual cortex. When the extent of herniation is large enough, the contralateral cerebral peduncle may be compressed, resulting in hemiparesis ipsilateral to the side of the herniation; the changes in the peduncle in this setting are known as *Kernohan's notch*. Progression of transtentorial herniation is often accompanied by hemorrhagic lesions in the midbrain and pons, termed *secondary brainstem*, or *Duret, hemorrhages* (Fig. 28-3). These linear or flame-shaped lesions usually occur in the midline and paramedian regions and

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are believed to be due to tearing of penetrating veins and arteries supplying the upper brainstem.

• *Tonsillar herniation* refers to displacement of the cerebellar tonsils through the foramen magnum. This pattern of herniation is life-threatening because it causes brainstem compression and compromises vital respiratory and cardiac centers in the medulla oblongata.

Figure 28-2 Major herniations of the brain: subfalcine, transtentorial, and tonsillar. *(Adapted from Fishman RA: Brain edema. N Engl J Med 293:706, 1975. Copyright © 1975, Massachusetts Medical Society. All rights reserved.)*

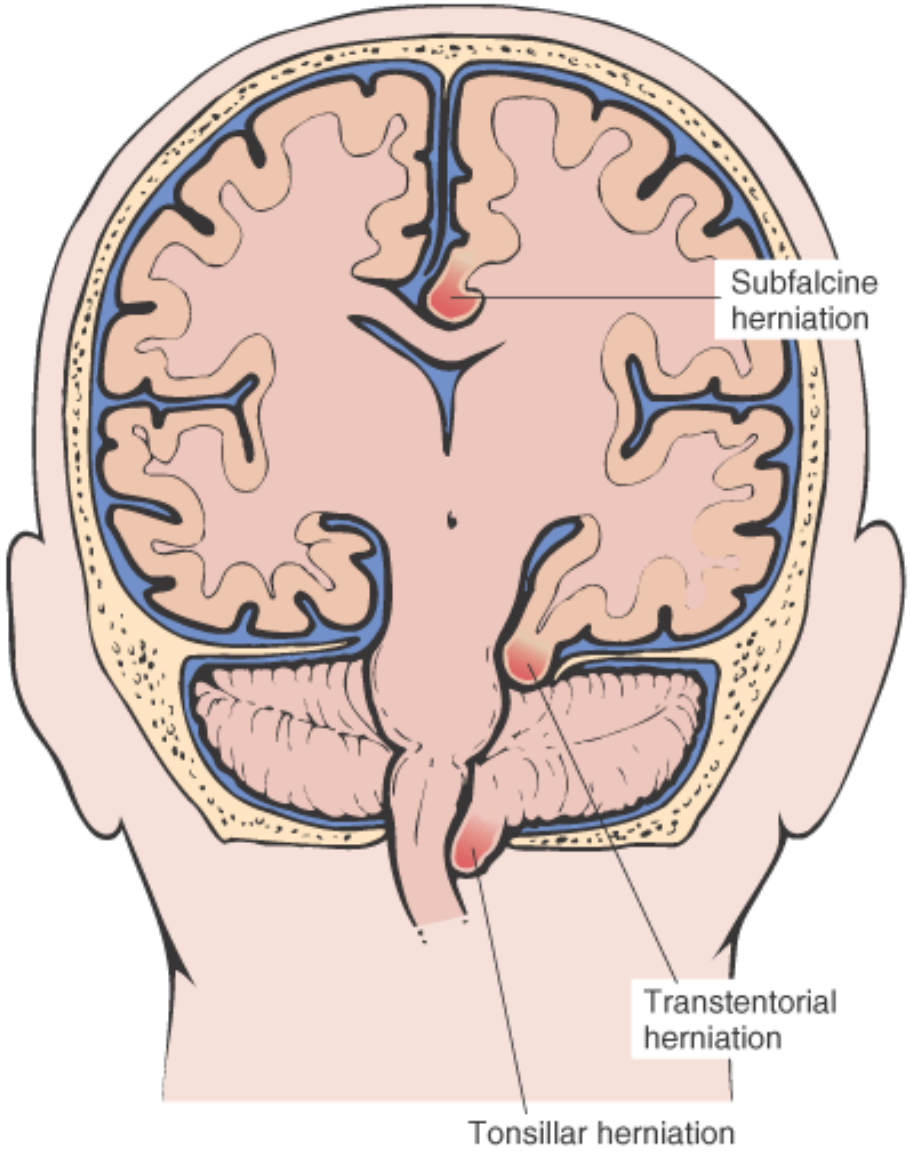


Figure 28-3 Duret hemorrhage involving the brainstem at the junction of the pons and midbrain.

Figure 28-4 *A*, Hydrocephalus. Dilated lateral ventricles seen in a coronal section through the midthalamus. *B*, Midsagittal plane T1-weighted magnetic resonance image of a child with communicating hydrocephalus, involving all ventricles. *(B, courtesy of Dr. P. Barnes, Stanford University Medical Center, CA.)*

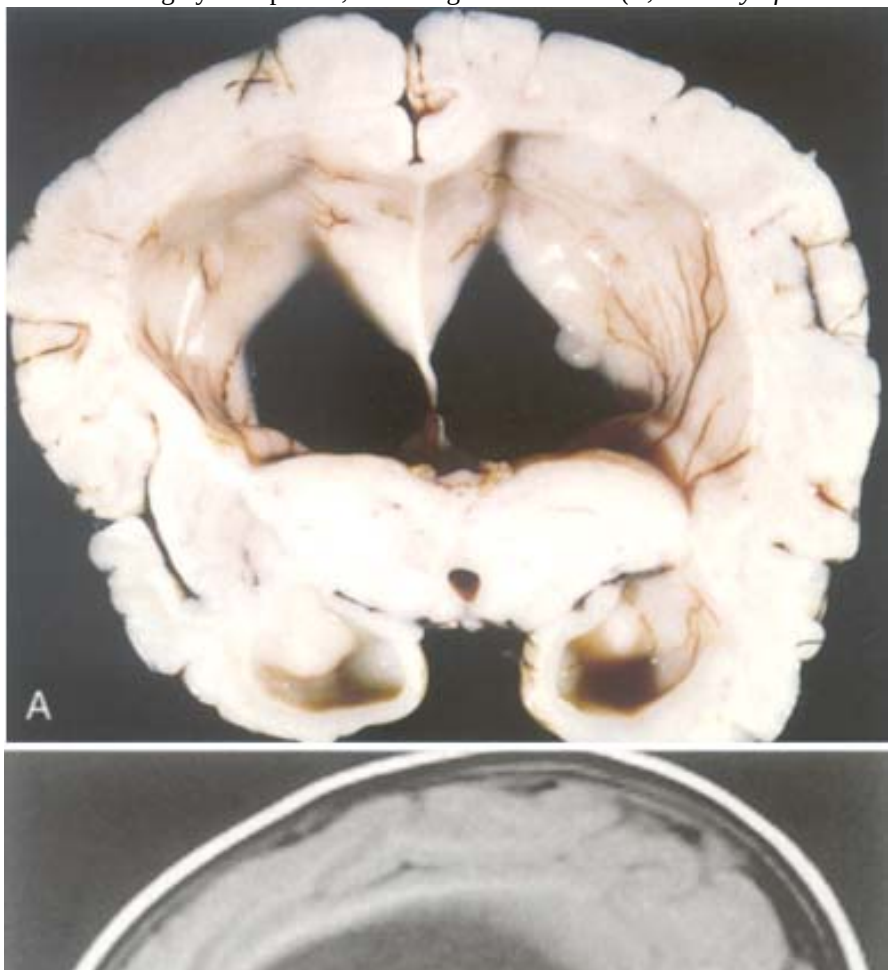


Figure 28-5 Holoprosencephaly (severe alobar form). View of the dorsal surface showing a lack of separation of cerebral hemispheres, a single ventricle, and fused basal ganglia.

Figure 28-6 Agenesis of the corpus callosum. The midsagittal view of the left hemisphere shows the lack of a corpus callosum and cingulate gyrus above the third ventricle.

Figure 28-7 Arnold-Chiari malformation. Midsagittal section showing small posterior fossa contents, downward displacement of the cerebellar vermis, and deformity of the medulla (arrows indicate the approximate level of the foramen magnum).

Figure 28-8 Periventricular leukomalacia. Central focus of white matter necrosis with a peripheral rim of mineralized axonal processes (staining blue).

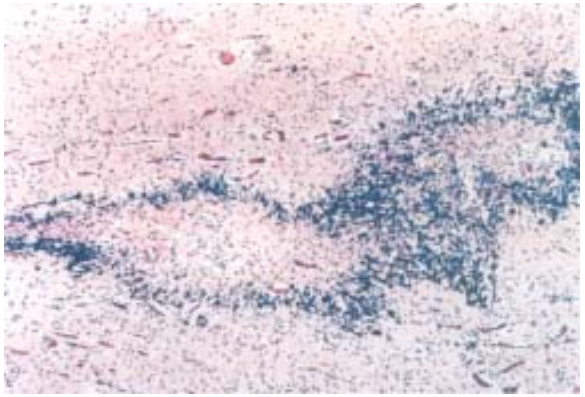
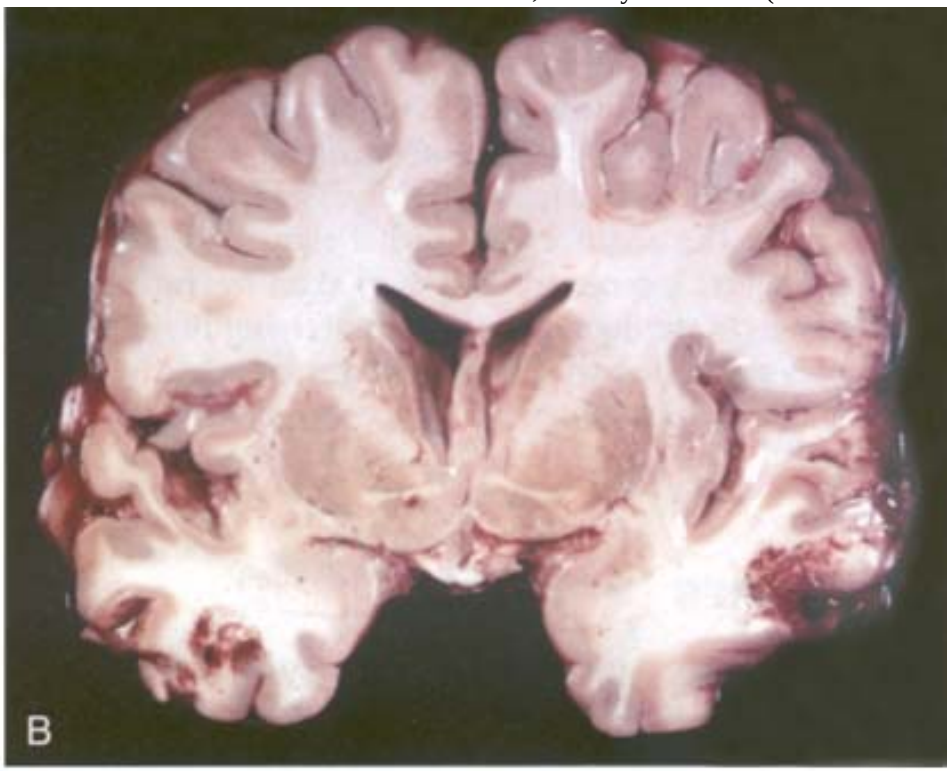


Figure 28-9 *A,* Multiple contusions involving the inferior surfaces of frontal lobes, anterior temporal lobes, and cerebellum. *B,* Acute contusions are present in both temporal lobes, with areas of hemorrhage and tissue disruption. *C,* Remote contusions are present on the inferior frontal surface of this brain, with a yellow color (associated with the term *plaque jaune*).



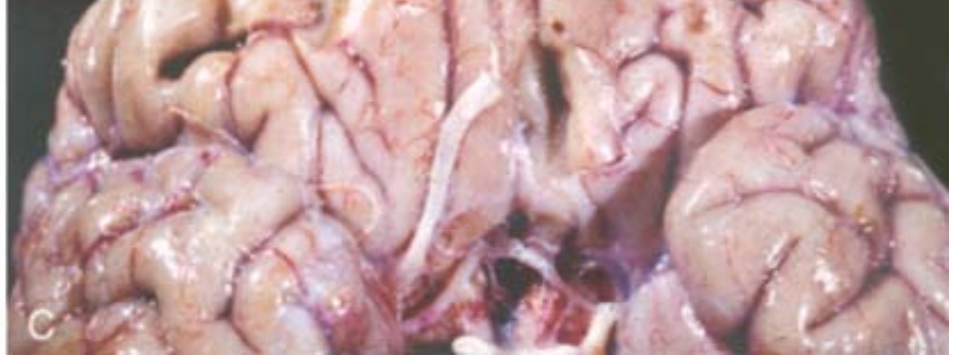


Figure 28-10 Epidural hematoma covering a portion of the dura. Multiple small contusions are seen in the temporal lobe. *(Courtesy of Dr. Raymond D. Adams, Massachusetts General Hospital, Boston, MA.)*

Figure 28-11 Epidural hematoma *(left)* in which rupture of a meningeal artery, usually associated with a skull fracture, leads to accumulation of arterial blood between the dura and the skull. In a subdural hematoma *(right)*, damage to bridging veins between the brain and the superior sagittal sinus leads to the accumulation of blood between the dura and the arachnoid.

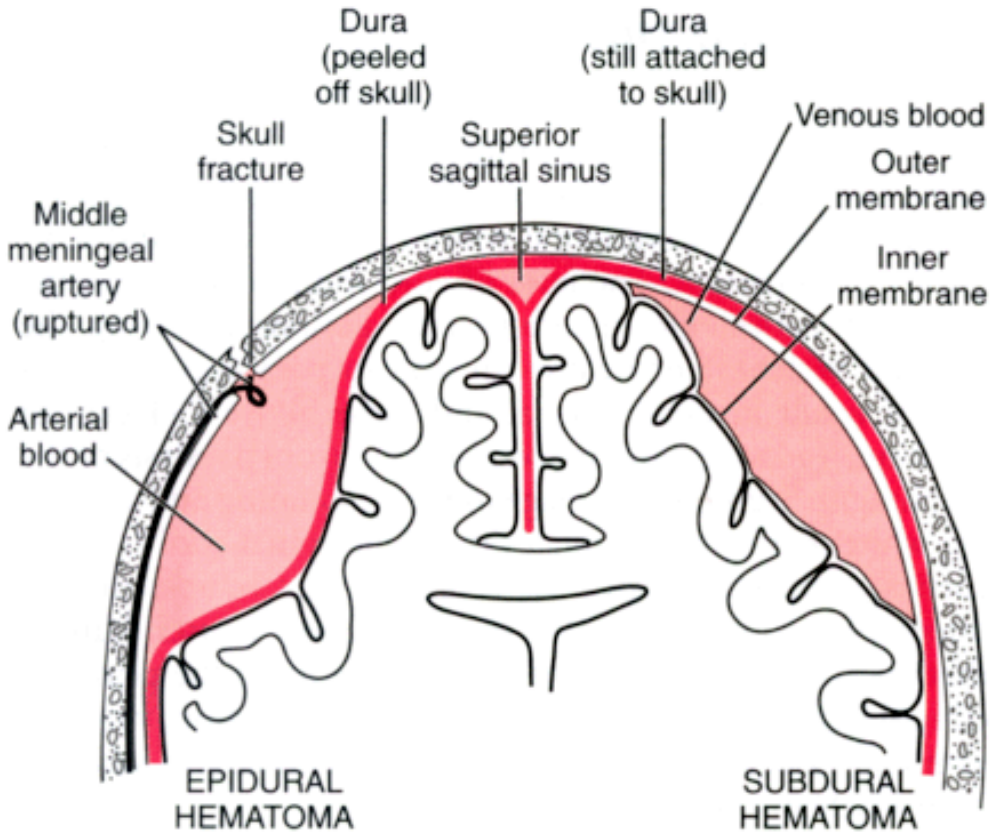
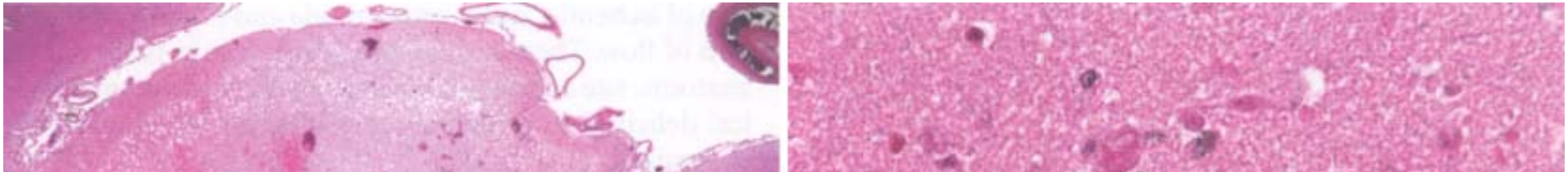


Figure 28-12 *A,* Large organizing subdural hematoma attached to the dura. *B,* Coronal section of the brain showing compression of the hemisphere underlying the hematoma.

Figure 28-13 Cerebral infarction. *A,* At low magnification, it is possible to see the demarcated areas of an acute infarction. In the underlying white matter, the areas of infarction are well shown by the myelin stain. *B,* Acute ischemic injury causes diffuse eosinophilia of neurons, which are beginning to shrink. *C,* Infiltration of a cerebral infarct by neutrophils begins at the edges of the lesion where vascular supply has remained intact. *D,* After about 10 days, an area of infarction is characterized by the presence of macrophages and surrounding reactive gliosis. *E,* Remote small intracortical infarcts are seen as areas of tissue loss with a small amount of residual gliosis.



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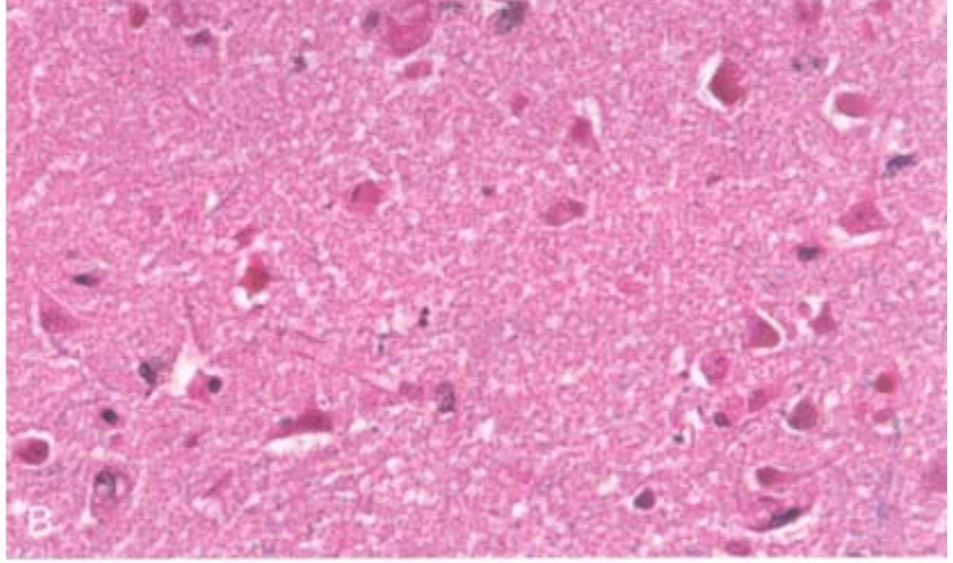
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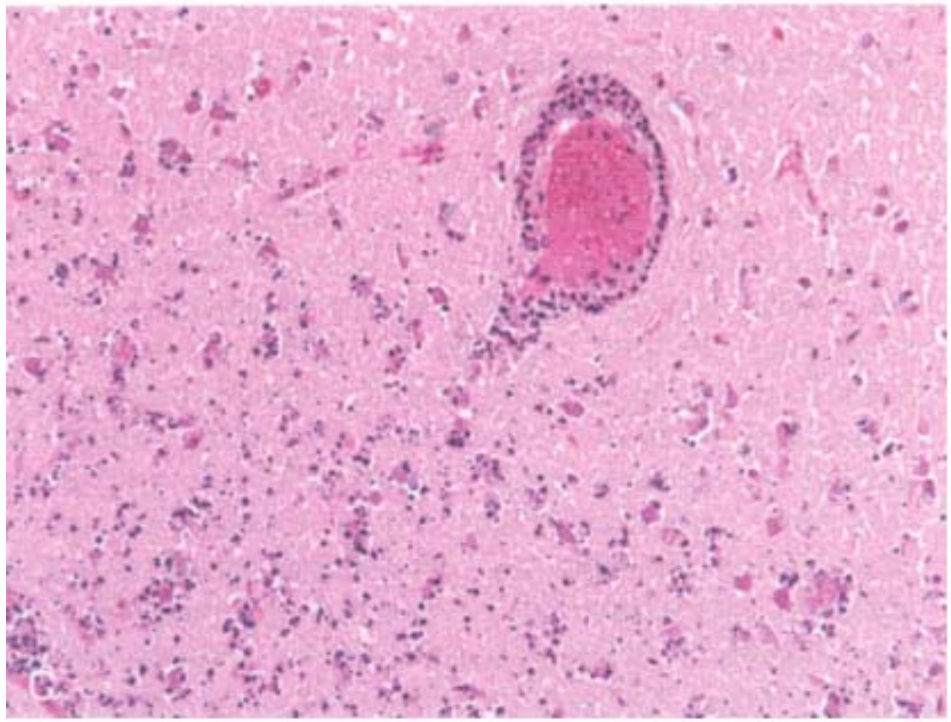

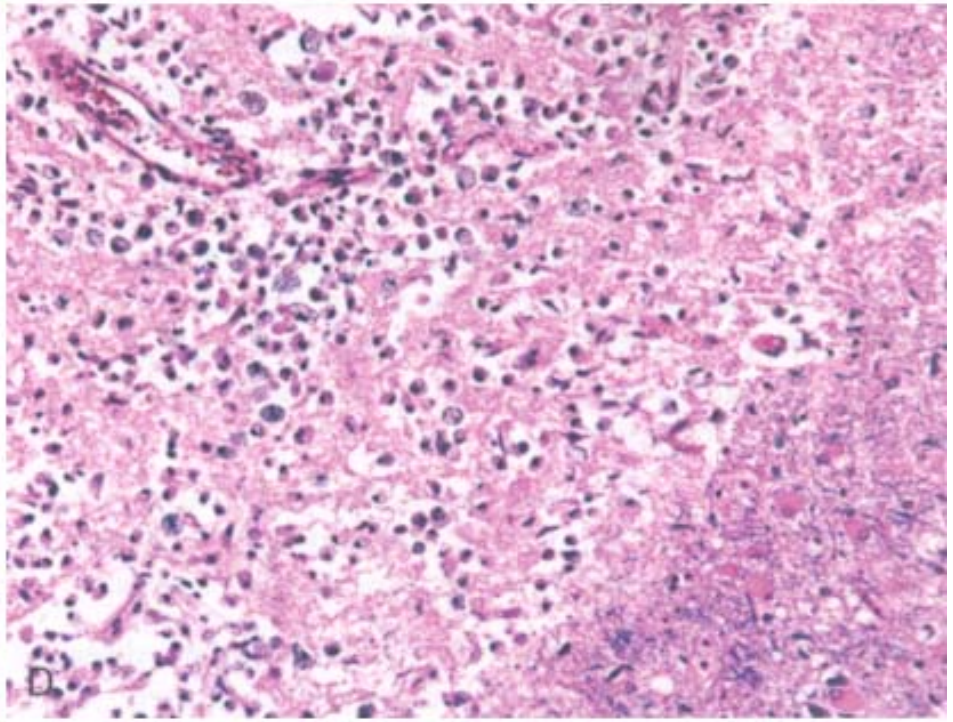
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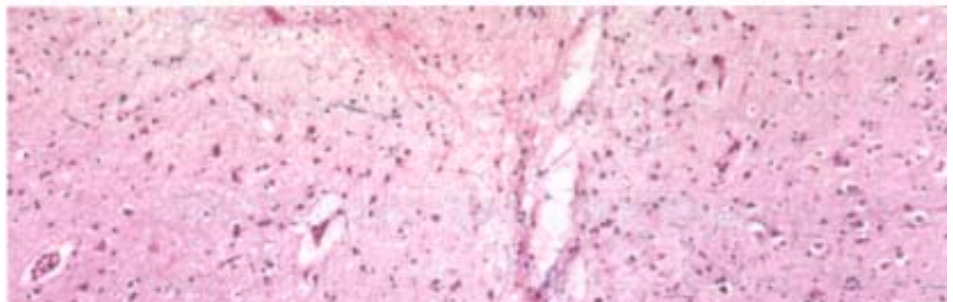

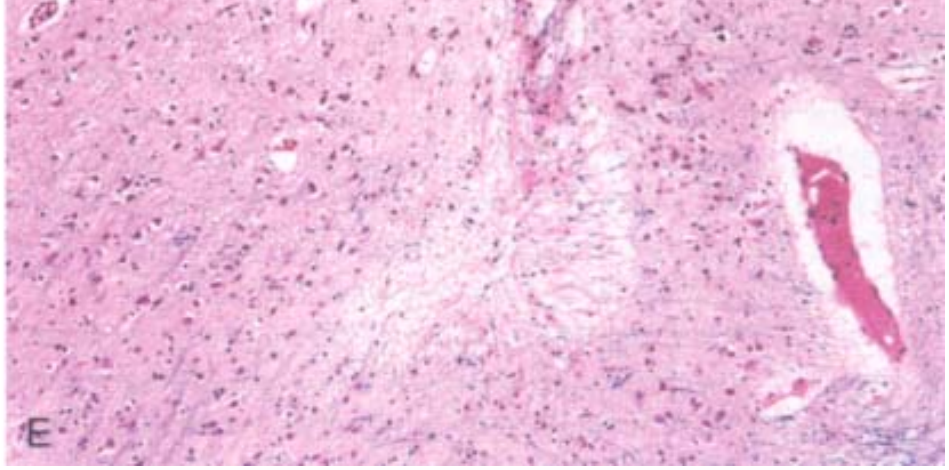

Figure 28-14 Widespread white matter hemorrhages are characteristic of bone marrow embolization.

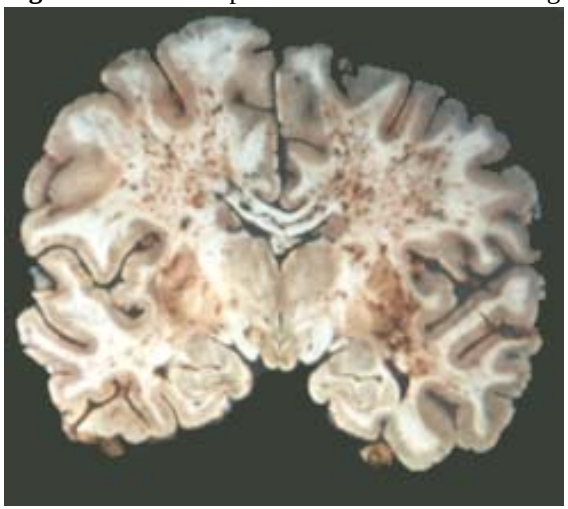

Figure 28-15 *A,* Sections of the brain showing a large, discolored, focally hemorrhagic region in the left middle cerebral artery distribution (hemorrhagic, or red, infarction). *B,* A hemorrhagic infarction is present in the inferior temporal lobe of the left side of this brain. *C,* A bland infarct with punctate hemorrhages, consistent with ischemia-reperfusion injury, is present in the temporal lobe.

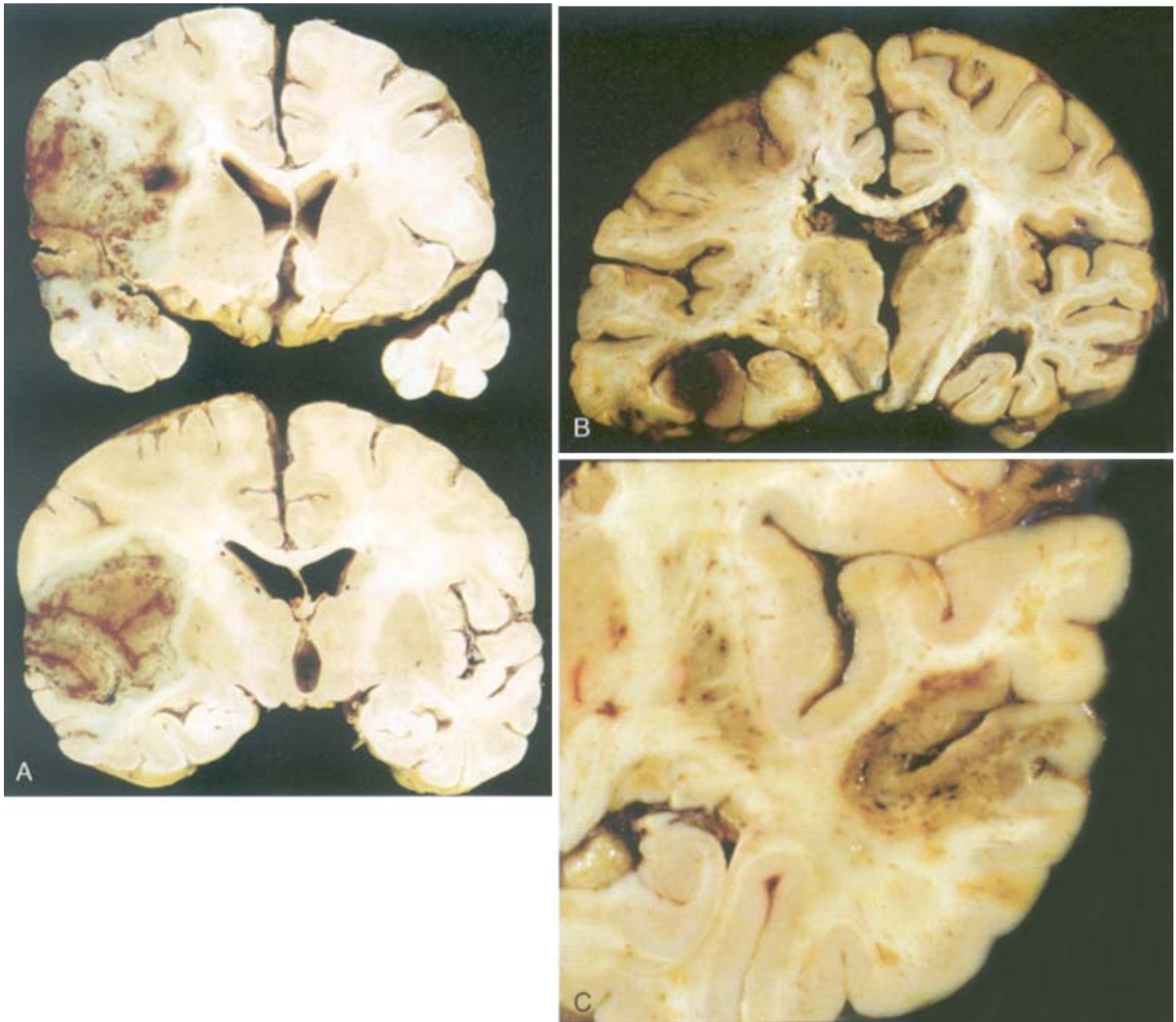
A
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Figure 28-16 Old cystic infarct. Destruction of cortex and surrounding gliosis.

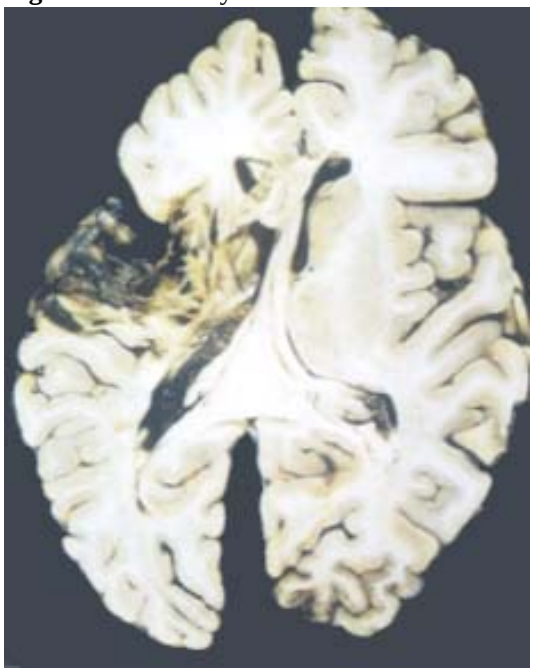


Figure 28-17 *A,* Massive hypertensive hemorrhage rupturing into a lateral ventricle. *B,* Hypertensive hemorrhage in the pons, with extension to fill the fourth ventricle.

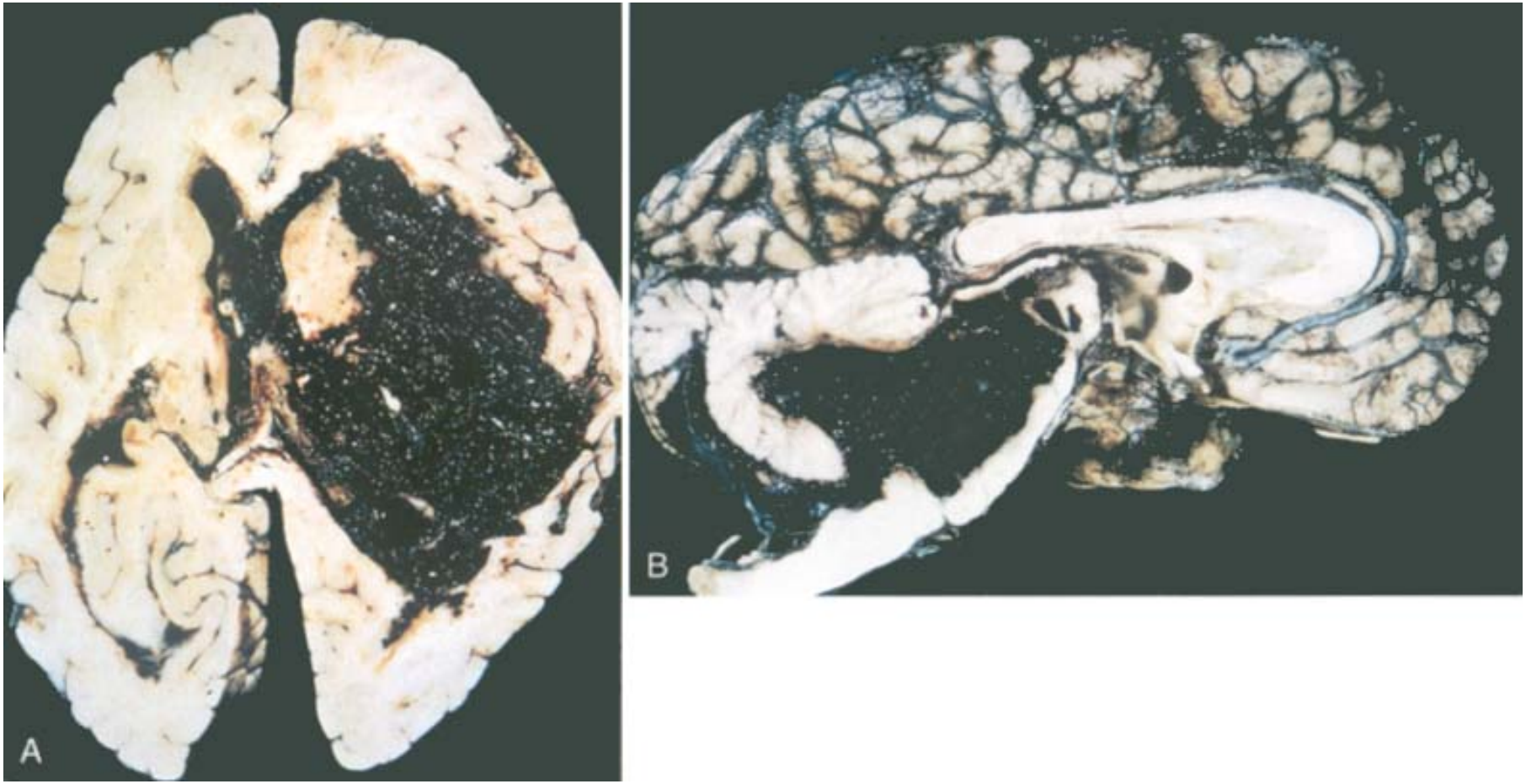


Figure 28-18 Common sites of saccular (berry) aneurysms in the circle of Willis.

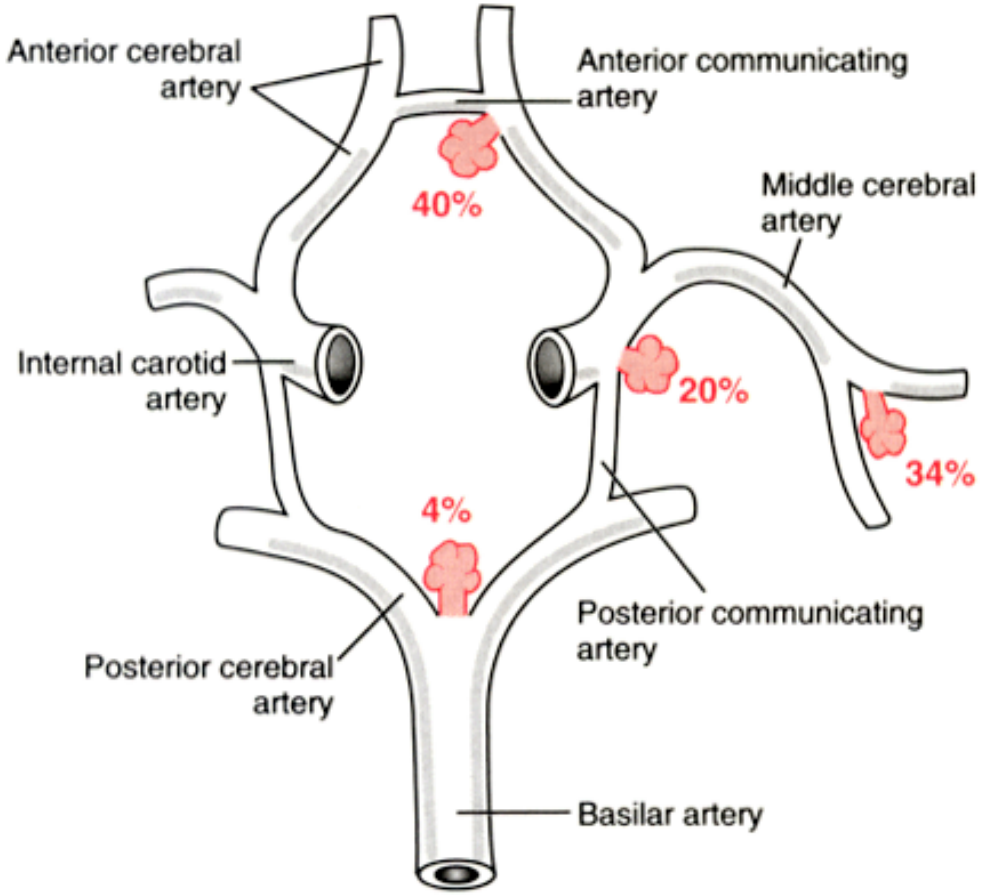


Figure 28-19 *A*, View of the base of the brain, dissected to show the circle of Willis with an aneurysm of the anterior cerebral artery *(arrow)*. *B*, Dissected circle of Willis to show large aneurysm. *C*, Section through a saccular aneurysm showing the hyalinized fibrous vessel wall (H & E).

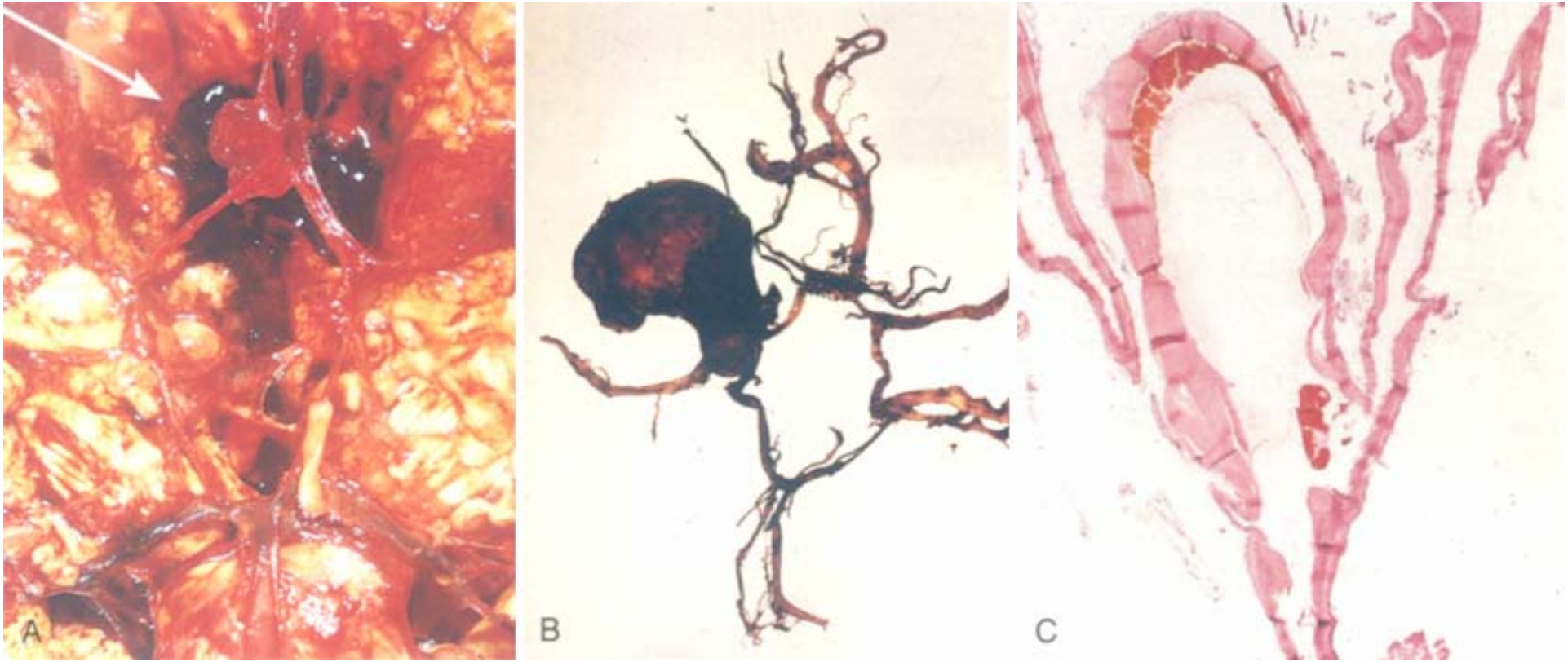


Figure 28-20 Lacunar infarcts in the caudate and putamen.

Figure 28-21 Pyogenic meningitis. A thick layer of suppurative exudate covers the brain stem and cerebellum and thickens the leptomeninges. *(From Golden JA, Louis DN: Images in clinical medicine: Acute bacterial meningitis. N Engl J Med 333:364, 1994.)*

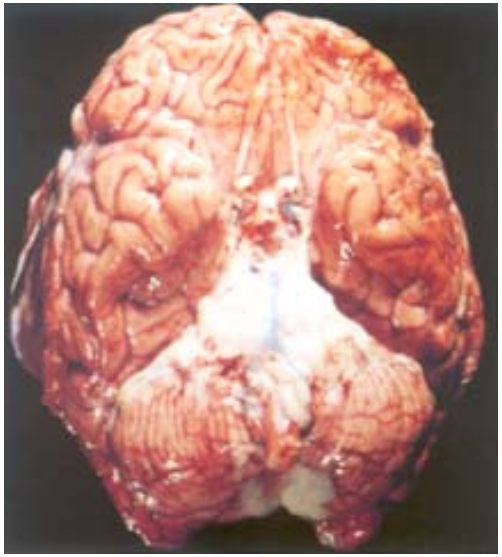


Figure 28-22 Frontal abscesses (*arrows*).

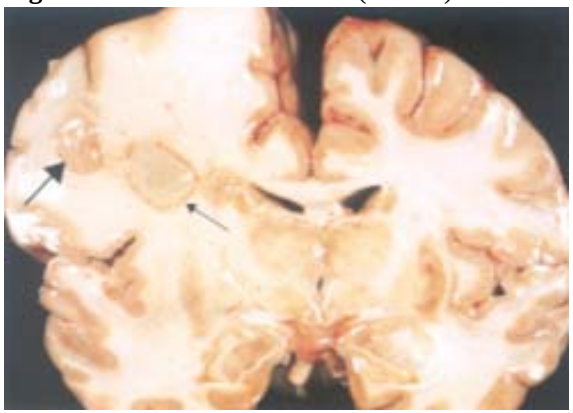


Figure 28-23 Characteristic findings of viral meningitis include perivascular cuffs of lymphocytes *(A)* and microglial nodules *(B)*.

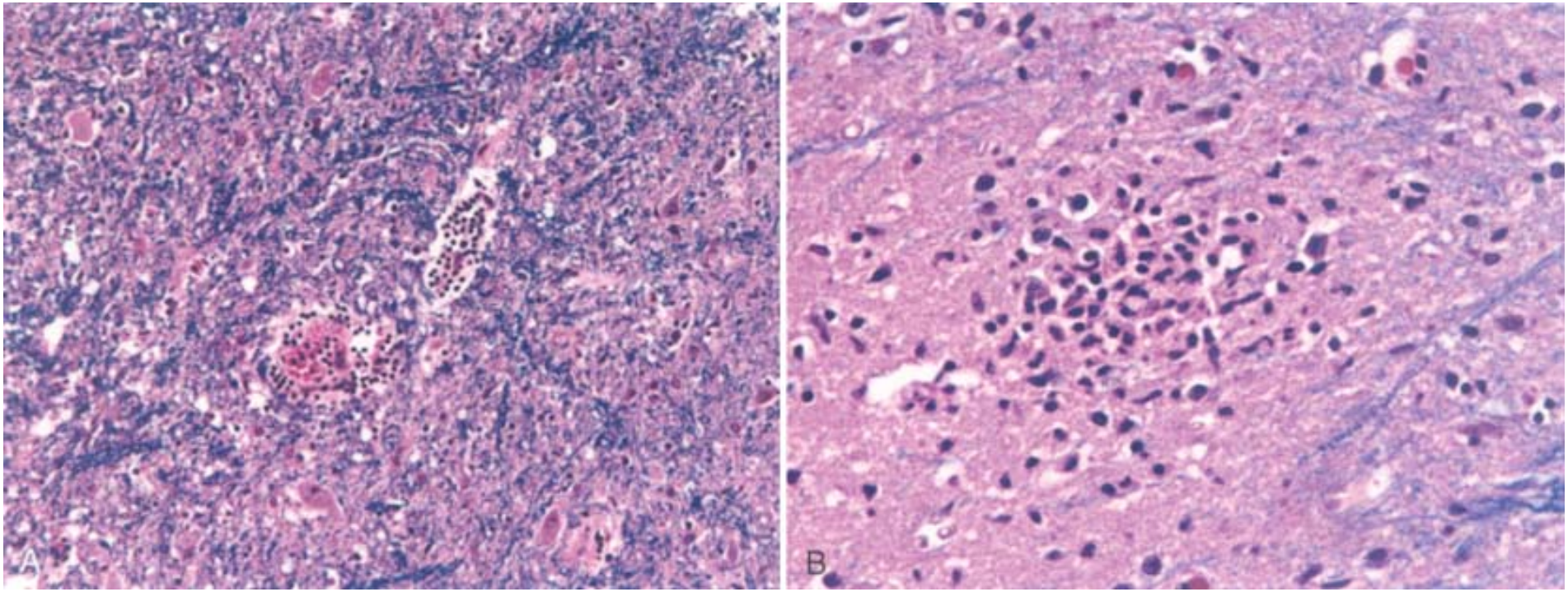


Figure 28-24 *A,* Herpes encephalitis showing extensive destruction of inferior frontal and anterior temporal lobes. *(Courtesy of Dr. T.W. Smith, University of Massachusetts Medical School, Worcester, MA.) B,* Necrotizing inflammatory process characterizes the acute herpes encephalitis.

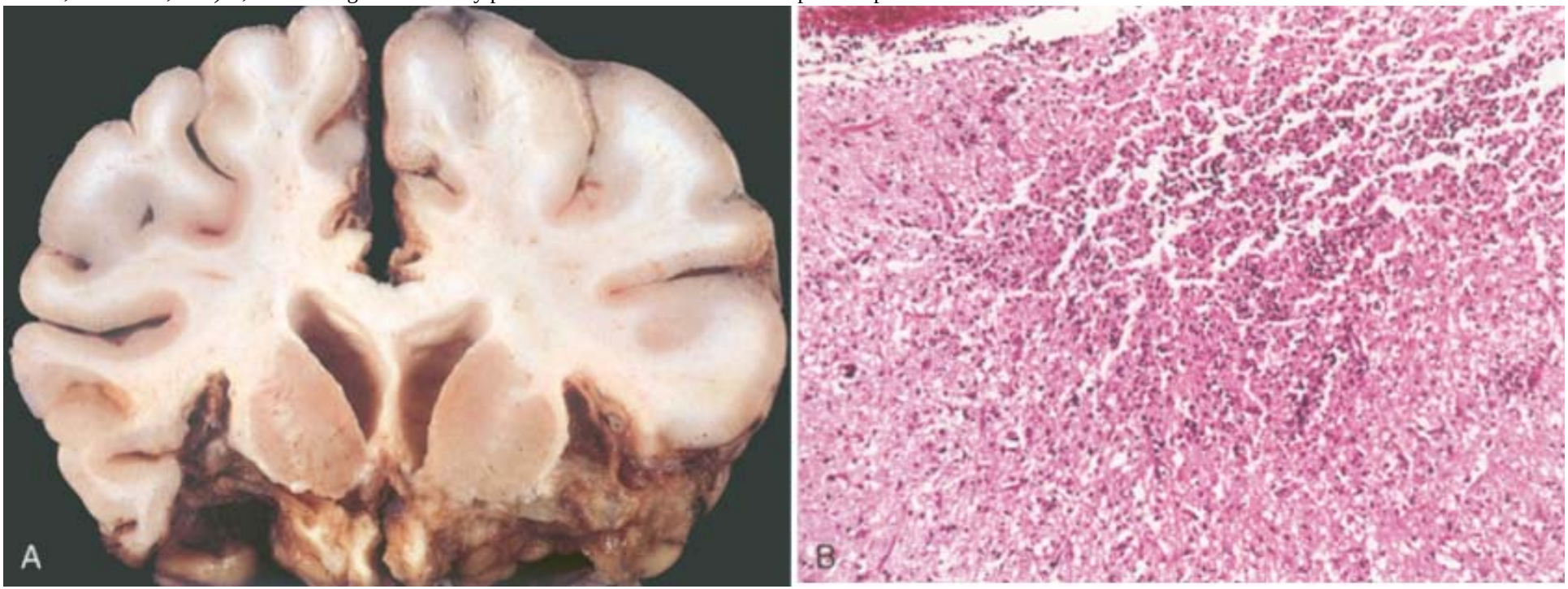


Figure 28-25 The diagnostic histologic finding in rabies is the eosinophilic Negri body, as seen here in a Purkinje cell *(arrows)*.

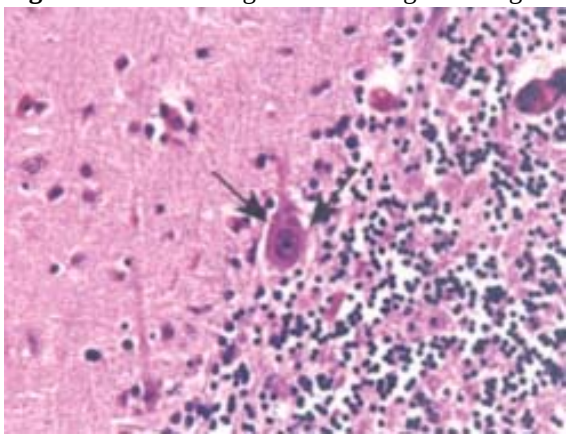


Figure 28-26 HIV encephalitis. Note the microglial nodule and multinucleated giant cells.

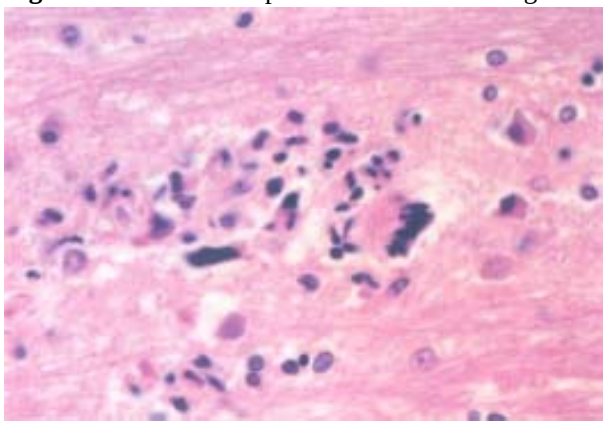
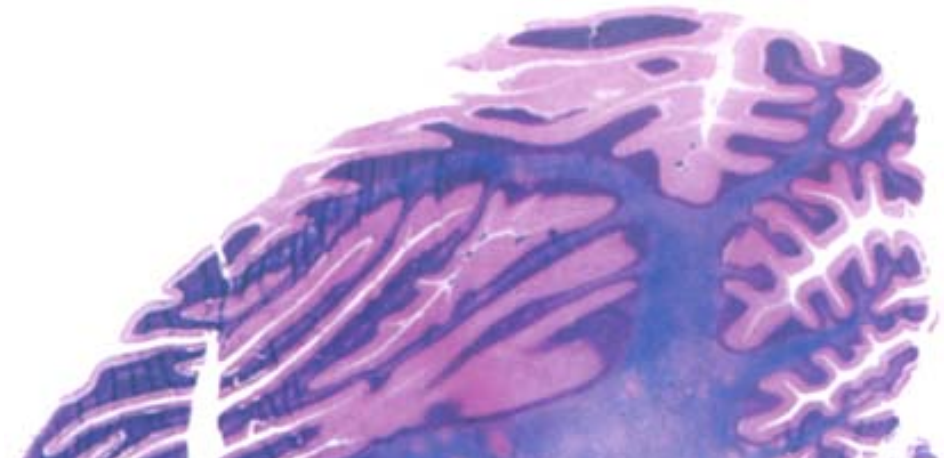


Figure 28-27 Progressive multifocal leukoencephalopathy. *A,* Section stained for myelin showing irregular, poorly defined areas of demyelination, which become confluent in places. *B,* Enlarged oligodendrocyte nuclei stained for viral antigens surround an area of early myelin loss.



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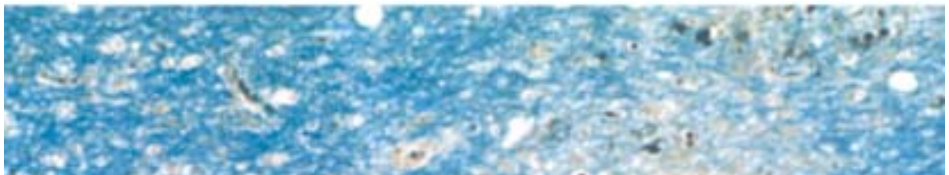
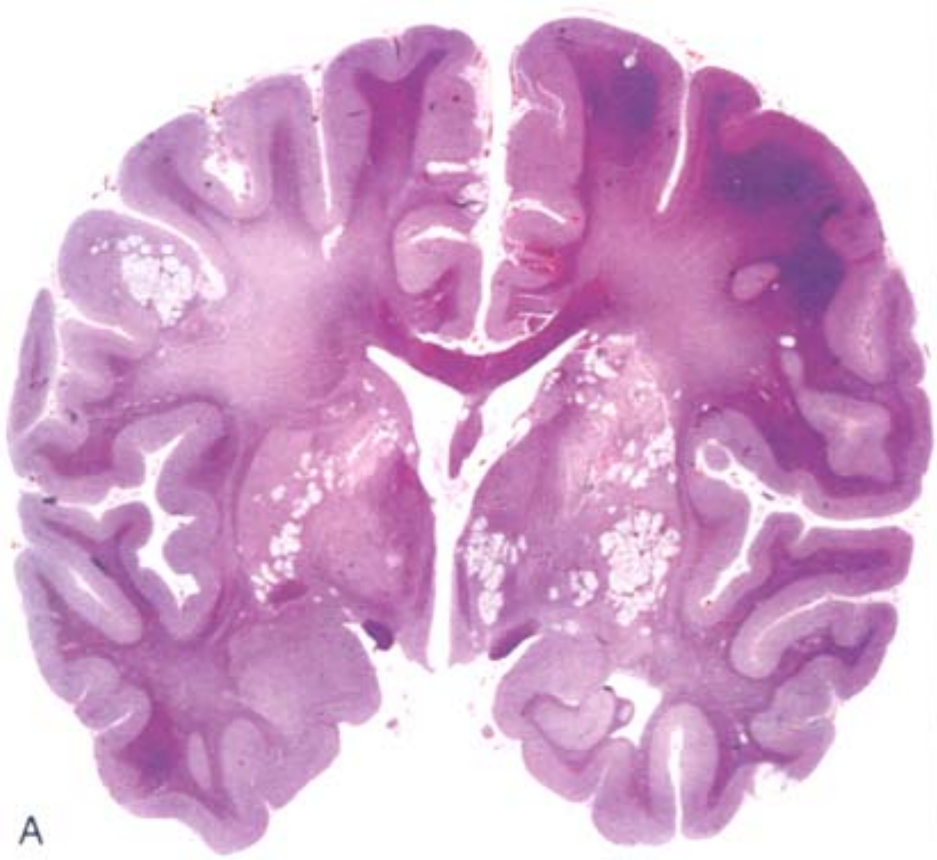


Figure 28-28 Cryptococcal infection. *A,* Whole brain section showing the numerous areas of tissue destruction associated with the spread of organisms in the perivascular spaces. *B,* At higher magnification, it is possible to see the cryptococci in the lesions.



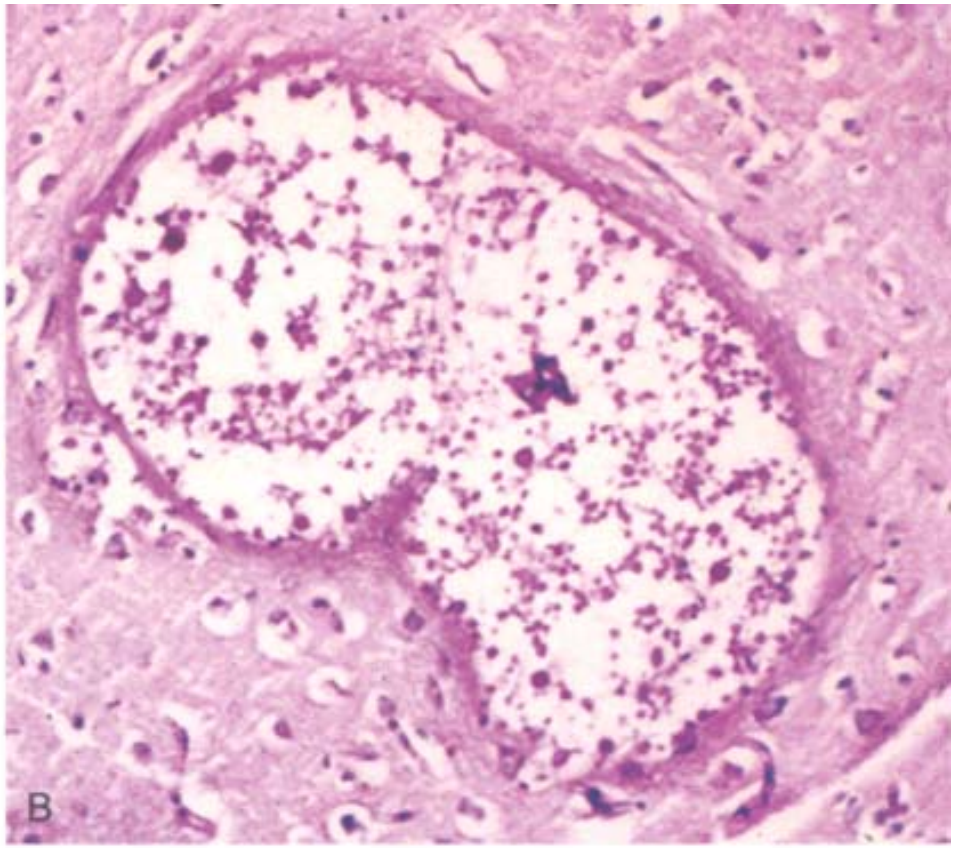
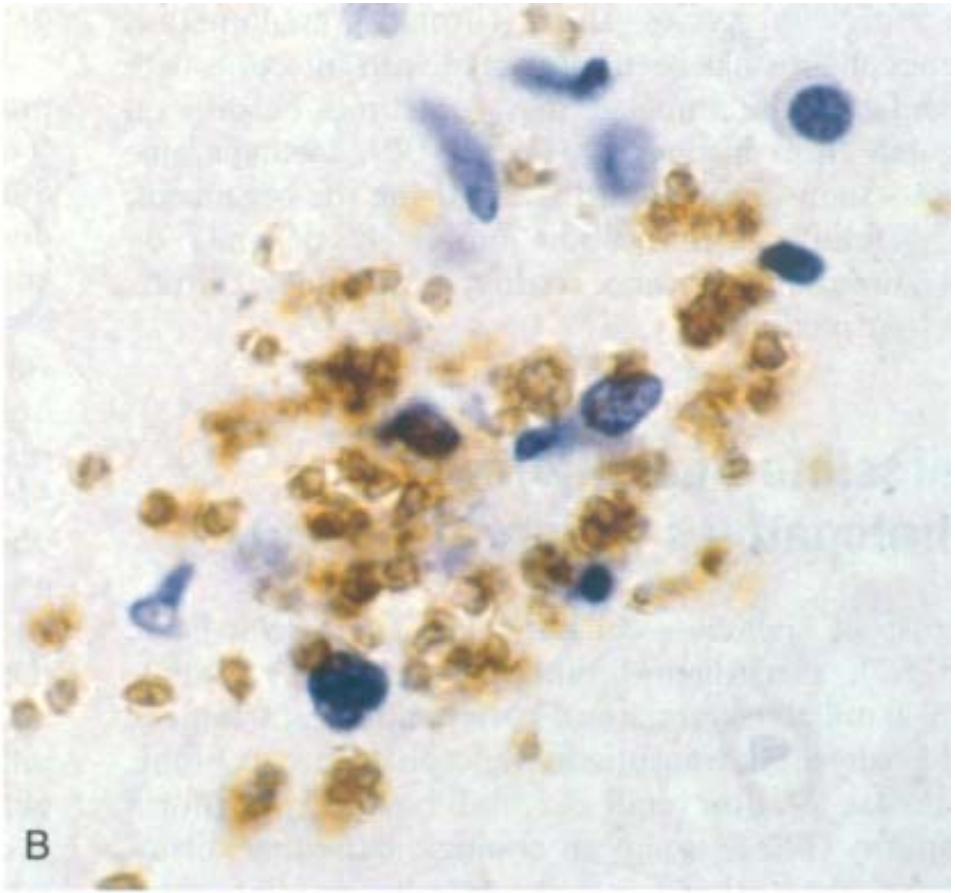
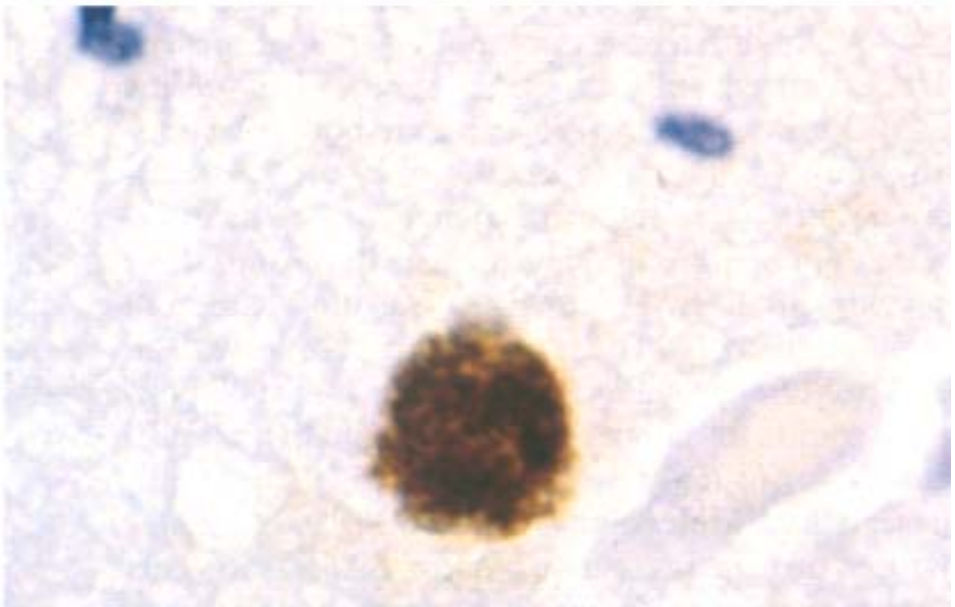


Figure 28-29 *A*, *Toxoplasma* abscesses in the putamen and thalamus. *B*, Free tachyzoites demonstrated by immunostaining. *C*, *Toxoplasma* pseudocyst with bradyzoites highlighted by immunostaining.





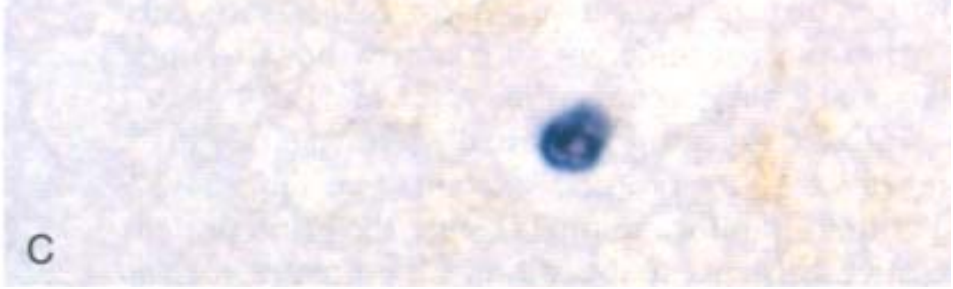


Figure 28-30 Necrotizing amoebic meningoencephalitis involving the cerebellum *(organism highlighted by arrow)*.

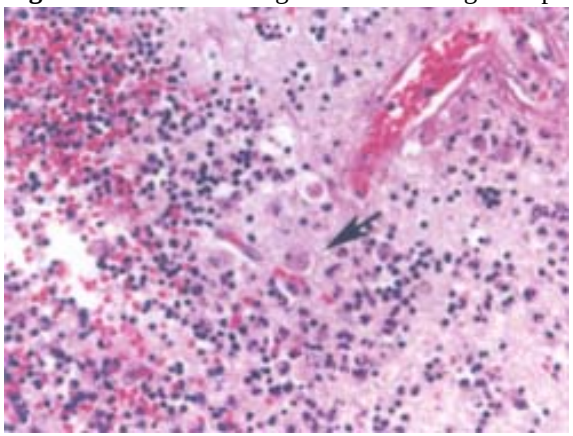
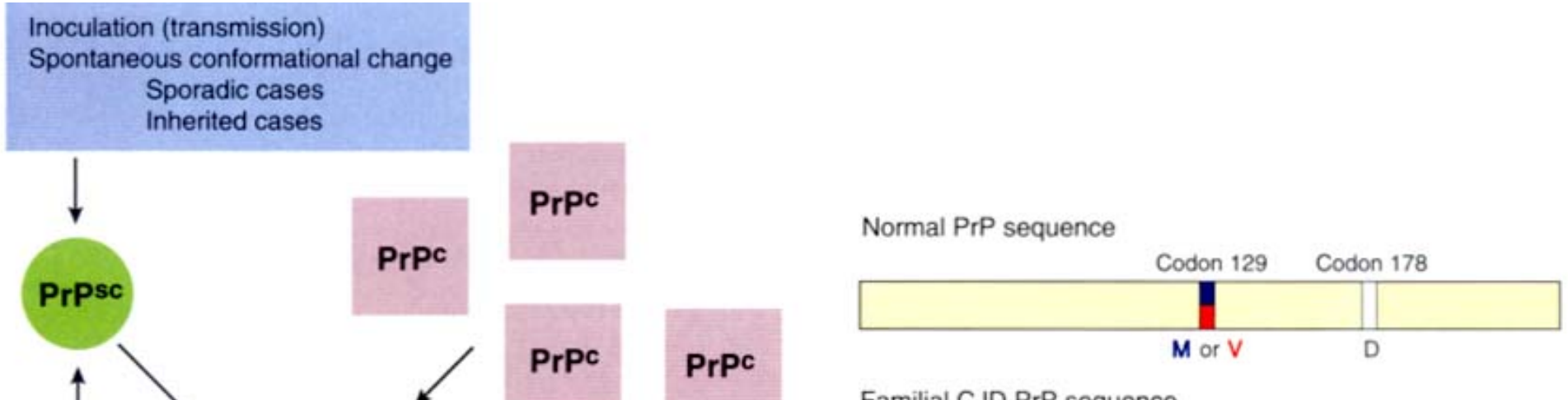
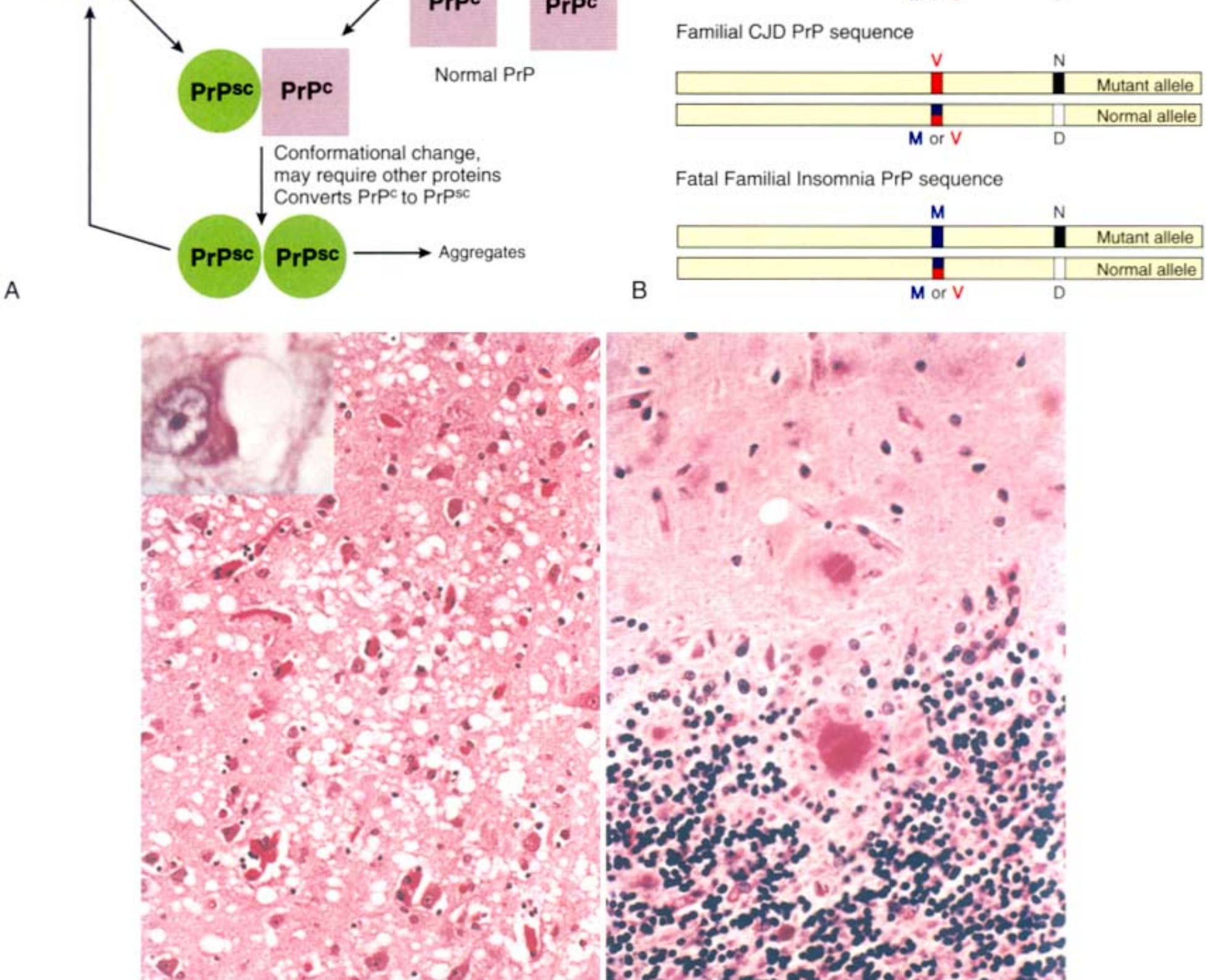


Figure 28-31 Mechanism and pathology of prion disease. *A*, Proposed mechanism for the conversion of PrP^{c} through protein-protein interactions. The initiating molecules of PrP^{sc} may arise through inoculation (as in directly transmitted cases) or through an extremely low-rate spontaneous conformational change. The effect of the mutations in PrP (see *B*) is to increase the rate of the conformational change once PrP^{sc} is able to recruit and convert other molecules of PrP^{c} into the abnormal form of the protein. Although the model is drawn with no other proteins involved, it is possible that other proteins play critical roles in the conversion of Prp^{c} to PrP^{sc} . *B*, The basic structure of the PrP protein with important sites of mutation (codon 178) and disease-associated polymorphism (codon 129). In normal individuals, codon 178 encodes Asp (D), and codon 129 encodes either Met (M) or Val (V). In some familial forms of disease, the mutation changes codon 178 to Asn (D178N). When the allele containing the D178N mutation also has a Val at codon 129, the patient develops Creutzfeldt-Jakob disease (CJD). In contrast, when the D178N allele has Met at codon 129, the clinical disorder is fatal familial insomnia. *C*, Histology of CJD showing spongiform change in the cerebral cortex. *Inset*, High magnification of neuron with vacuoles. *D*, Cerebellar cortex showing *kuru plaques* (periodic acid-Schiff [PAS] stain) representing aggregated PrP^{sc} .




PrPsc
PrPc
Normal PrP
Conformational change,
may require other proteins
Converts PrPc to PrPsc
PrPsc
PrPsc
Aggregates
A
Familial CJD PrP sequence
V
N
Mutant allele
Normal allele
M or V
D
Fatal Familial Insomnia PrP sequence
M
N
Mutant allele
Normal allele
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B

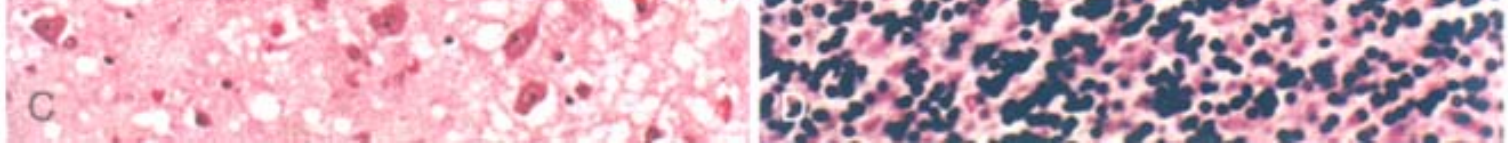


Figure 28-32 Multiple sclerosis. Section of fresh brain showing brown plaque around occipital horn of the lateral ventricle.

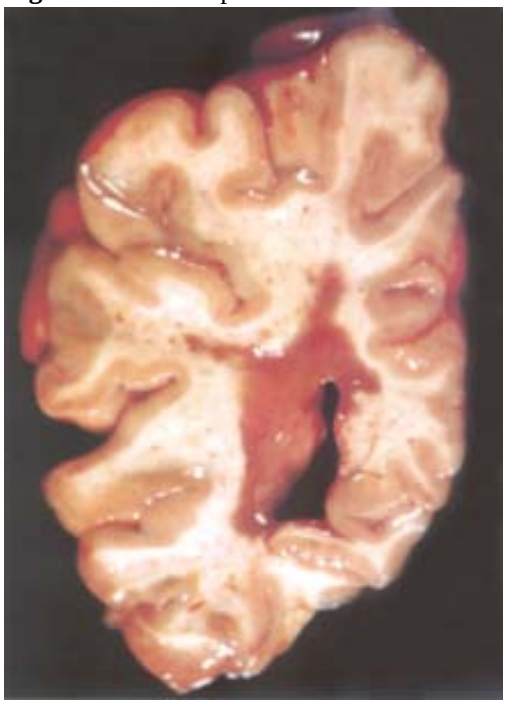


Figure 28-33 Multiple sclerosis. *A,* Unstained regions of demyelination (MS plaques) around the fourth ventricle. (Luxol fast blue PAS stain for myelin). *B,* Myelin-stained section shows the sharp edge of a demyelinated plaque and perivascular lymphocytic cuffs. *C,* The same lesion stained for axons shows relative preservation.

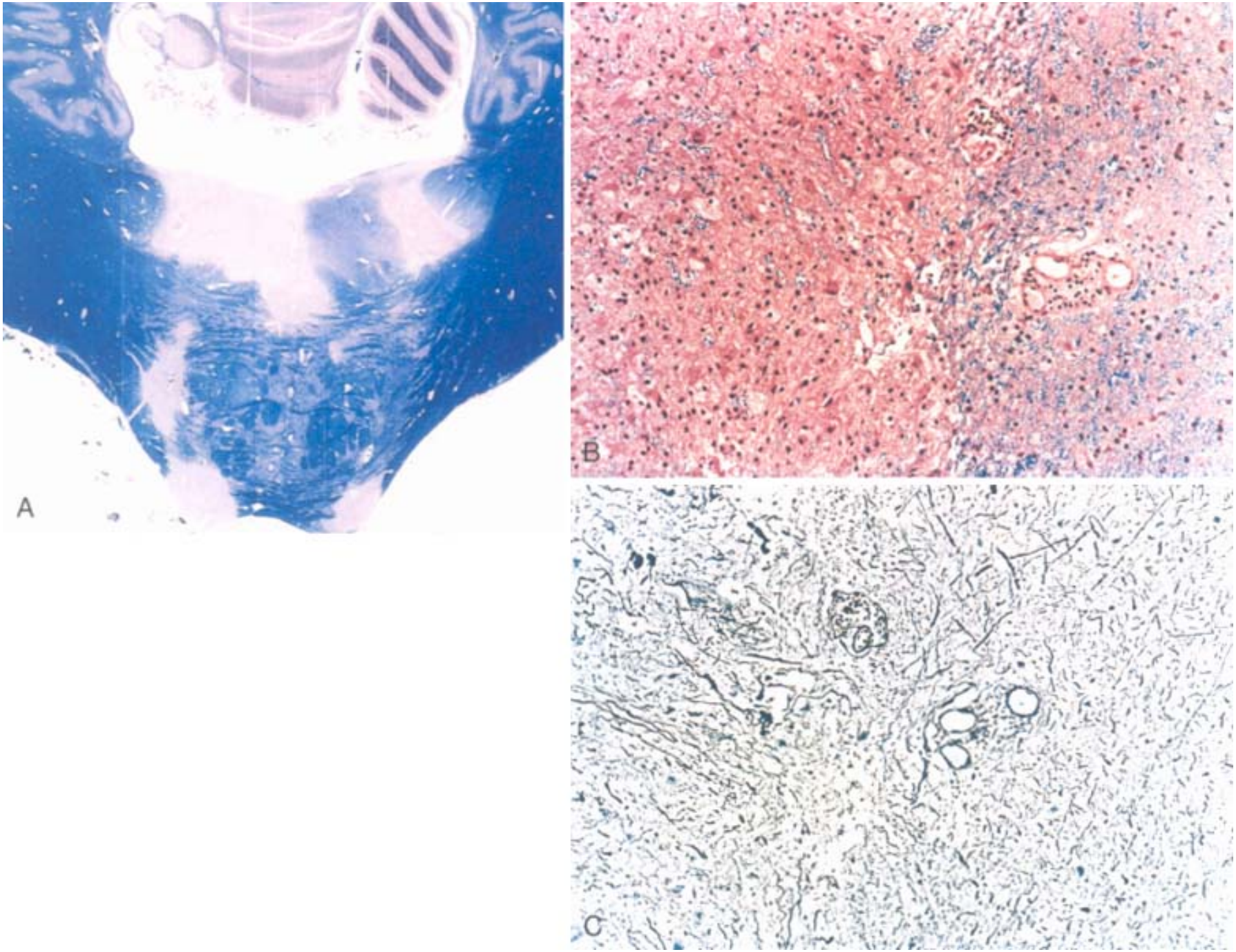


Figure 28-34 Alzheimer disease with cortical atrophy most evident on the right, where meninges have been removed. *(Courtesy of Dr. E.P. Richardson, Jr., Massachusetts General Hospital, Boston, MA.)*

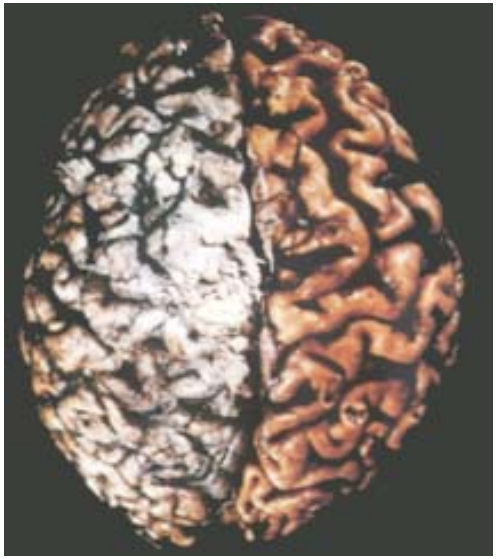
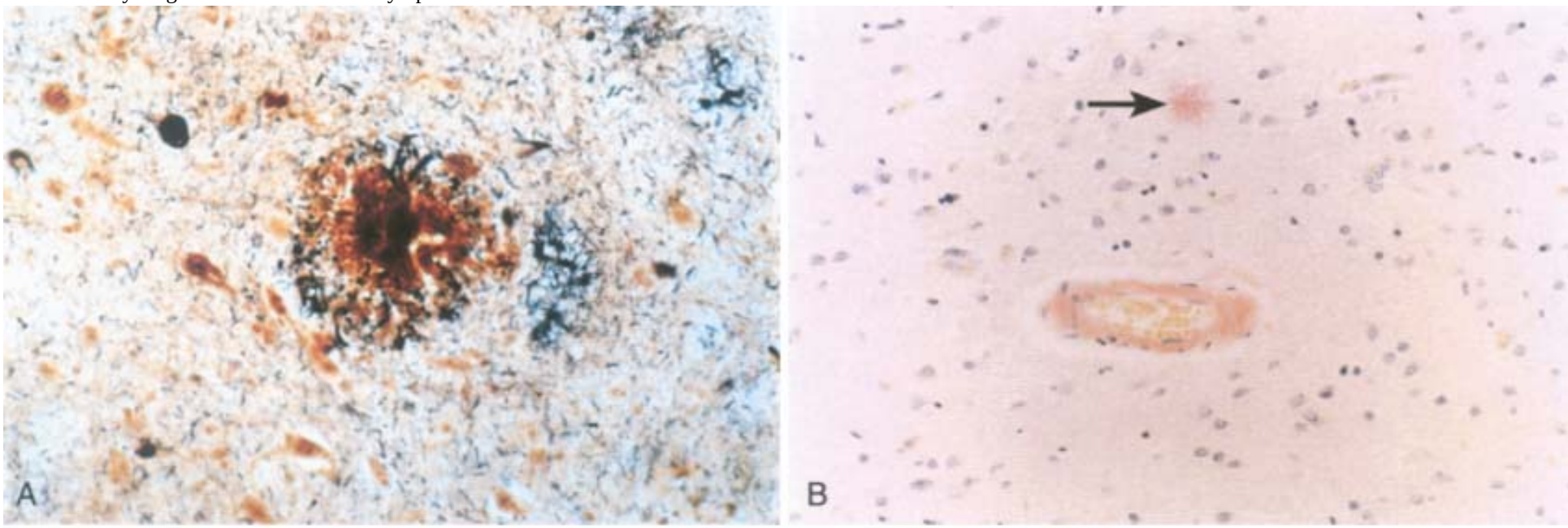


Figure 28-35 Alzheimer disease. *A*, Neuritic plaque with a rim of dystrophic neurites surrounding an amyloid core. *B*, Congo red stain of the cerebral cortex showing amyloid deposition in the blood vessels and the amyloid core of the neuritic plaque *(arrow)*. *C*, Neurofibrillary tangles *(arrowheads)* are present within the neurons (H & E). *D*, Silver stain showing a neurofibrillary tangle within the neuronal cytoplasm.



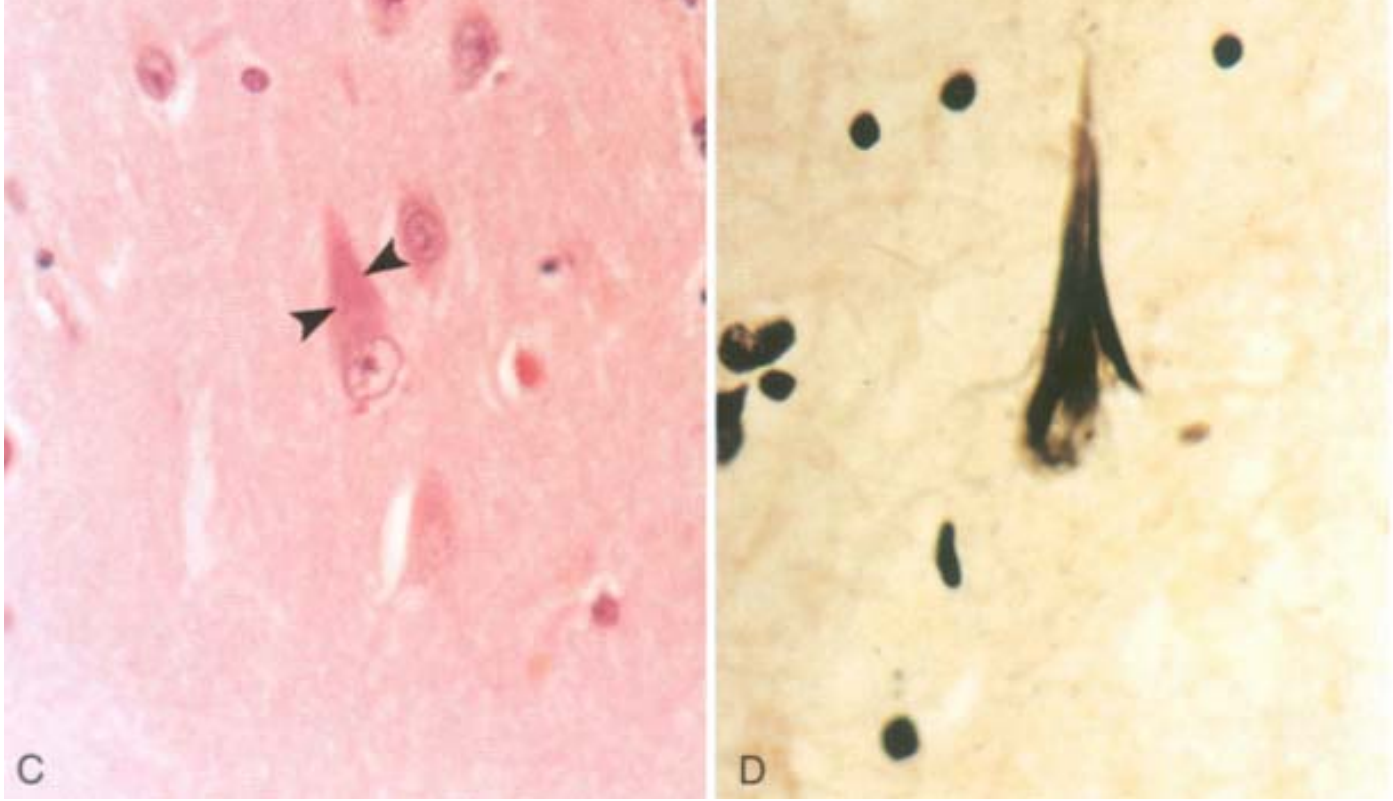


Figure 28-36 Mechanism of amyloid generation in Alzheimer disease. Amyloid precursor protein (APP) is a transmembrane protein, with potential cleavage sites for three distinct enzymes (α-, β- and γ-secretases) as shown in *A*. The Aβ domain extends from the extracellular side of the protein into the transmembrane domain. When APP is cleaved by α-secretase (*B*), subsequent cleavage by γ-secretase does not yield Aβ. In contrast, cleavage by β-secretase followed by γ-secretase (*C*) results in production of Aβ, which can then aggregate and form fibrils. In either pathway, intramembranous cleavage by γ-secretase follows cleavage at a site located closer to the N-terminus of the protein.

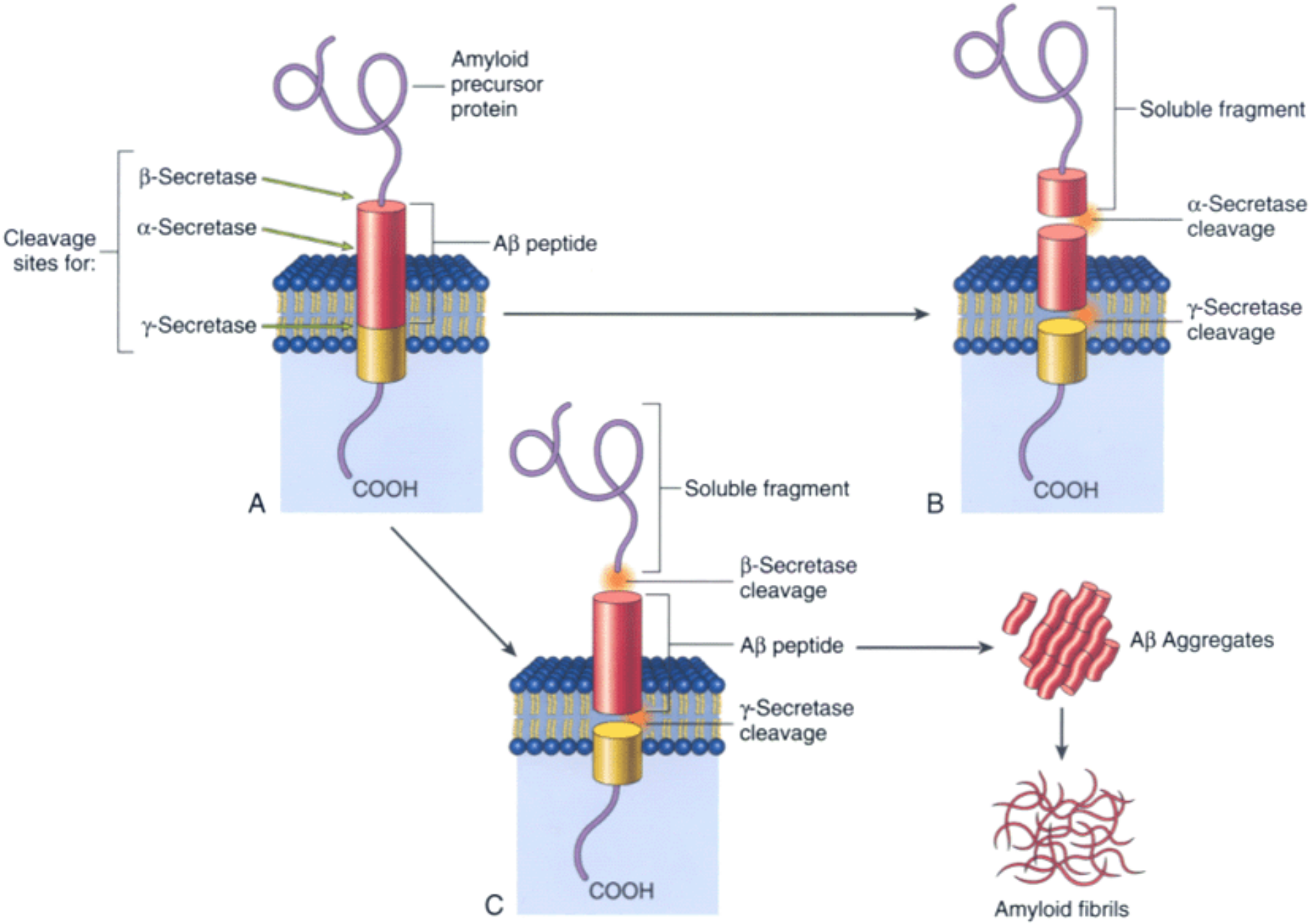


TABLE 28-2 -- Genetics of Alzheimer Disease

Chromosome	Gene	Mutations/Alleles	Consequences
21	Amyloid precursor protein (*APP*)	• Single missense mutations	• Early-onset FAD

		••Double missense mutation	••Increased Aβ production
		••Trisomy 21 (gene dosage effect)	
14	Presenilin-1 (*PS1*)	• Missense mutations	• Early-onset FAD
		••Splice site mutations	••Increased Aβ production
1	Presenilin-2 (*PS2*)	• Missense mutations	• Early-onset FAD
			••Increased Aβ production
19	Apolipoprotein E (*ApoE*)	• Allele epsilon4	• Increased *risk* of development of AD
			••Decreased age at onset of AD

AD, Alzheimer disease; FAD, familial Alzheimer disease.

How Aβ is related to the neurodegeneration of AD, how it is linked to the other pathologic features of AD such as tangles and abnormal hyperphosphorylation of tau, and what controls the stereotypic pattern of involvement of brain regions and the pattern of progression all remain open questions. There are various lines of evidence indicating that the small aggregates of Aβ as well as larger fibrils are directly neurotoxic and can elicit various cellular responses, including oxidative damage and alterations in calcium homeostasis. In addition, the reactions of other cell types in the brain influence the disease. There is evidence that the inflammatory response that accompanies Aβ deposition may have both protective effects (through assisting clearance of the aggregated peptide) and injurious effects.[153] [154] [155] [156]

Clinical Features.

The progression of Alzheimer disease is slow but relentless, with a symptomatic course often running more than 10 years. Initial symptoms are forgetfulness and other memory disturbances; with progression of the disease, other symptoms emerge, including language deficits, loss of mathematical skills, and loss of learned motor skills. In the final stages of Alzheimer disease, patients may become incontinent, mute, and unable to walk. Intercurrent disease, often pneumonia, is usually the terminal event in these individuals. While biomarkers for AD are still unavailable, there are indicators that structural imaging can suggest which individuals are at increased risk of progressing from a mild memory disturbance to a diagnosis of probable AD.[157]

Frontotemporal Dementias

These are a group of disorders that were first gathered under a single broad term because they shared clinical features (progressive deterioration of language and changes in personality) that corresponded to degeneration and atrophy of temporal and frontal lobes. These entities have recently been better understood through a combination of immunohistochemical and biochemical studies as well as genetic insights.[158] [159]

Frontotemporal Dementia with Parkinsonism Linked to Chromosome 17 (FTD(P)-17)

As the name implies, this is a genetically determined disorder in which the clinical syndrome of a frontotemporal dementia is often accompanied by parkinsonian symptoms. In these families, the disease has been mapped to chromosome 17; in particular, it has been linked to a variety of mutations

in the *tau* gene. Tau is a microtubule binding protein that has numerous sites of potential phosphorylation and exists in six splice forms as the result of alternative splicing of exons 2, 3 and 10.[160] The protein contains either three or four copies of the microtubule binding domain depending on whether exon 10 is included (4 repeat tau) or not (3 repeat tau).

Morphology.

There is evidence of atrophy of frontal and temporal lobes in various combinations and to various degrees. The pattern of atrophy can often be predicted in part by the clinical symptomatology. The atrophic regions of cortex are marked by neuronal loss and gliosis as well as the presence of taucontaining neurofibrillary tangles. These tangles may contain either 4 repeat tau or a mixture of 3 and 4 repeat tau, depending on the underlying genetic basis for the disease. Nigral degeneration may also occur. Inclusions can also be found in glial cells in some forms of the disease.

Pathogenesis and Molecular Genetics.

The study of families with frontotemporal dementia led to the recognition that in some, but not all, pedigrees, there is linkage to mutations in the *tau* gene. The mutations fall into several broad categories: coding region mutations and intronic mutations that affect the splicing of exon 10.[161] The intronic mutations result in increased production of 4 repeat forms of tau. Coding region mutations appear to have several different consequences, including alterations in the interaction of tau with microtubules (mutations in exon 10 will change this interaction only for 4 repeat tau) and altering the intrinsic tendency to aggregate.

Pick Disease

Pick disease (lobar atrophy) is a rare, distinct, progressive dementia characterized clinically by early onset of behavioral changes together with alterations in personality (frontal lobe signs) and language disturbances (temporal lobe signs).[162] While most cases of Pick disease are sporadic, there have been some familial forms identified and linked to mutations in *tau*.

Morphology.

The brain invariably shows a pronounced, frequently asymmetric, atrophy of the frontal and temporal lobes with conspicuous sparing of the posterior two thirds of the superior temporal gyrus and only rare involvement of either the parietal or occipital lobe. The atrophy can be severe, reducing the gyri to a thin wafer ("knife-edge" appearance). This pattern of **lobar atrophy** is often prominent enough to distinguish Pick disease from Alzheimer disease on macroscopic examination. In addition to the localized cortical atrophy, there may also be bilateral atrophy of the caudate nucleus and putamen.

On microscopic examination, neuronal loss is most severe in the outer three layers of the cortex. Some of the surviving neurons show a characteristic swelling (**Pick cells**) or contain **Pick bodies,** which are cytoplasmic, round to oval, filamentous inclusions that are only weakly basophilic but stain strongly with silver methods. Ultrastructurally, these are composed of straight filaments, vesiculated endoplasmic reticulum, and paired helical filaments that are immunocytochemically similar to those found in Alzheimer disease and contain 3 repeat tau. Unlike the neurofibrillary tangles of Alzheimer disease, Pick bodies do not survive the death of their host neuron and do not remain as markers of the disease.

Progressive Supranuclear Palsy (PSP)

This is an illness characterized clinically by truncal rigidity with dysequilibrium and nuchal dystonia; pseudobulbar palsy and abnormal speech; ocular disturbances, including vertical gaze palsy progressing to difficulty with all eye movements; and mild progressive dementia in most patients. The onset of the disease is usually between the fifth and seventh decades, and males are affected approximately twice as frequently as are females. The disease is often fatal within 5 to 7 years of onset.

Morphology.

There is widespread neuronal loss in the globus pallidus, subthalamic nucleus, substantia nigra, colliculi, periaqueductal gray matter, and dentate nucleus of the cerebellum. Globose neurofibrillary tangles are found in these affected regions, in neurons as well as in glia. Ultrastructural analysis reveals 15-nm straight filaments that are composed of 4 repeat tau.

Mutations in *tau* have not been found in PSP. Analysis of the *tau* gene has shown that there is an extended haplotype (a series of polymorphic markers spread out along the gene that are in complete linkage disequilibrium; that is, recombination events do not appear to occur between the sites). Of the two haplotypes, one of them is strongly overrepresented in PSP patients.[163] How this haplotype influences the risk of PSP is unknown.

Corticobasal Degeneration (CBD)

This is a disease of the elderly, with considerable clinical and neuropathologic heterogeneity. The extrapyramidal signs and symptoms result in this disorder's also being grouped with syndromes of basal ganglia dysfunction.

Morphology.

On macroscopic examination, there is cortical atrophy, mainly of the motor, premotor, and anterior parietal lobes. The regions of cortex show severe loss of neurons, gliosis, and *"ballooned" neurons* (neuronal achromasia) that can be highlighted with immunocytochemical methods for phosphorylated neurofilaments. Tau immunoreactivity has been found in astrocytes ("tufted astrocytes"), oligodendrocytes ("coiled bodies"), basal ganglionic neurons, and, variably, cortical neurons.[164] [165] Clusters of tau-positive processes around an astrocyte ("astrocytic plaques") and the presence of tau-positive threads in gray and white matter may be the most specific pathologic findings of CBD.[166] The substantia nigra and locus ceruleus show loss of pigmented neurons, neuronal achromasia, and tangles. Similar to

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PSP, the tau deposits in CBD contain predominantly 4 repeat tau. Recently proposed consensus diagnostic criteria for CBD focus on the presence of tau-positive inclusions in neurons and glia of the cortex and striatum, including astrocytic plaques, associated with neuronal loss from substantia nigra and cortex.[167]

Clinical Features.

The disease is characterized by extrapyramidal rigidity, asymmetric motor disturbances (jerking movements of limbs: "alien hand"), and sensory cortical dysfunction (apraxias, disorders of language); cognitive decline occurs, and may be prominent in some cases.[168] [169] The same extended *tau* haplotype is linked to CBD as to PSP.[170] Although tau deposits are a hallmark of CBD, it is rare to find CBD pathology in individuals with mutations in the *tau* gene.[171]

Frontotemporal Dementias Without Tau Pathology

Some cases with clinical and pathologic findings involving these brain regions do not show evidence of Tau deposition. Some cases with this pattern are found in association with motor neuron disease (see below); in this setting, Tau-negative, ubiquitin-positive inclusions can be found in superficial cortical layers in temporal and frontal lobe and in the dentate gyrus. This pattern of pathology is termed *motor neuron disease inclusion dementia*. It has also been described in the absence of ALS-like pathology. Other cases show no specific inclusions but rather have cortical atrophy and some thalamic gliosis. This pattern of injury has been termed dementia lacking distinctive histology (DLDH). [172] With time and increased biochemical and molecular investigations, these unusual entities are likely to be reclassified.

Vascular Dementia

It is now clear from autopsy studies of individuals who were carefully studied during life that various types of vascular injury to the brain can result in dementia.[173] Some individuals with a rapidly progressive cognitive decline have vasculitis (discussed above) and will often show improvement with treatment. Among the irreversible disorders, several specific entities have been identified, which can be separated in part by their clinical course (typically a stepwise progression rather than a gradual decline) and imaging features. Various etiologies include small areas of infarction (granular atrophy from cortical microinfarcts, multiple lacunar infarcts, cortical laminar necrosis associated with reduced perfusion/oxygenation) and diffuse white matter injury (Binswanger disease, CADASIL). Additionally, dementia has been associated with so-called strategic infarcts, which are usually embolic and involve brain regions such as the hippocampus, dorsomedial thalamus, or frontal cortex including cingulate gyrus. Many individuals, in fact, will demonstrate a combination of pathologic changes. There is also an interaction between vascular injury and other dementing disorders, such as AD. It has been found that patients with vascular changes above a certain threshold have a lower burden of plaques and tangles for their level of cognitive impairment than do those without vascular-based cerebral pathology.[174]

DEGENERATIVE DISEASES OF BASAL GANGLIA AND BRAINSTEM

Diseases affecting these regions of the brain are frequently associated with movement disorders, including rigidity, abnormal posturing, and chorea. In general, they can be categorized as manifesting either a reduction of voluntary movement or an abundance of involuntary movement. The basal ganglia and especially the nigrostriatal pathway play an important role in the system of positive and negative regulatory synaptic pathways that serve to modulate feedback from the thalamus to the motor cortex. The most important disorders in this group are those associated with parkinsonism and Huntington chorea.

Parkinsonism

Parkinsonism is a clinical syndrome characterized by diminished facial expression, stooped posture, slowness of voluntary movement, festinating gait (progressively shortened, accelerated steps), rigidity, and a "pill-rolling" tremor. This type of motor disturbance is seen in a number of conditions that have in common damage to the nigrostriatal dopaminergic system. Parkinsonism may also be induced by drugs that affect this system, particularly dopamine antagonists and toxins. The principal diseases to be discussed here that involve the nigrostriatal system are as follows:

- Parkinson disease (PD)
- Multiple system atrophy, a disorder that may have parkinsonism as a prominent symptom (clinical presentation as striatonigral degeneration) as well as other symptoms (cerebellar ataxia and autonomic dysfunction)
- Postencephalitic parkinsonism, which was observed in the wake of the influenza pandemic that occurred between 1914 and 1918 and is now vanishingly rare
- Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), which are movement disorders that may also exhibit cognitive impairment; they share some pathologic and genetic features with each other and with other tauopathies (see the discussion above in the section on frontotemporal dementias).

Parkinson Disease

This diagnosis is made in patients with progressive parkinsonism in the absence of a toxic or other known underlying etiology. Familial forms with autosomal-dominant or autosomal-recessive inheritance exist. Although these make up a limited number of cases, they have contributed to our understanding of the pathogenesis of the disease. In addition to the movement disorder, there are other, less well-characterized changes in mental function, which may include dementia, in a subset of individuals with PD.

Morphology.

On pathologic examination, the typical macroscopic findings are **pallor of the substantia nigra** (Fig. 28-37) and locus ceruleus. On microscopic examination, there is loss of the pigmented, catecholaminergic neurons in these regions associated with gliosis. Lewy bodies (Fig. 28-37*C*) may be found in some of the remaining neurons.

Figure 28-37 Parkinson disease (PD). *A,* Normal substantia nigra. *B,* Depigmented substantia nigra in idiopathic PD. *C,* Lewy bodies in a substantia nigra neuron stain bright pink. *(C, courtesy of Dr. R. Kim, V.A. Medical Center, Long Beach, CA.)*

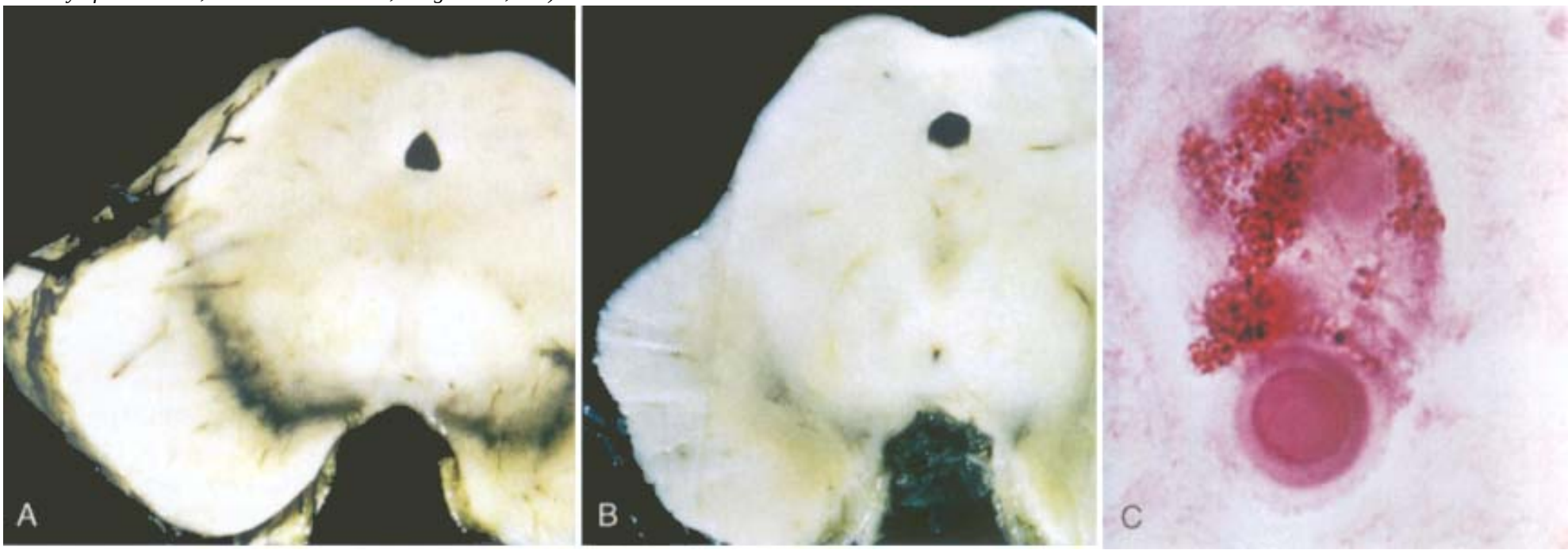

Figure 28-38 Huntington disease (HD). Normal hemisphere on the left compared with the hemisphere with HD on the right showing atrophy of the striatum and ventricular dilation. *(Courtesy of Dr. J.-P. Vonsattel, Columbia University, New York, NY.)*

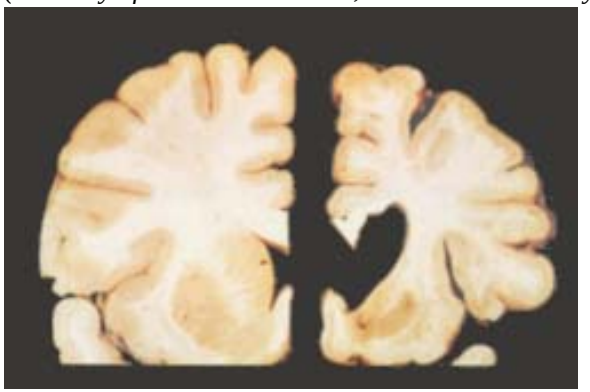

TABLE 28-3 -- Spinocerebellar Ataxias

Disease	Chromosome	Gene Product	Inheritance	Mutation
SCA1	6p23	ataxin-1	AD	CAG in coding region
SCA2	12q24.1	ataxin-2	AD	CAG in coding region
SCA3	14q21	ataxin-3	AD	CAG in coding region
SCA4	16q22.1	?	AD	?
SCA5	11	?	AD	?
SCA6	19p13.1-2	α_{1A} voltage-dependent calcium channel subunit	AD	CAG in coding region
SCA7	3p21.1-p12	ataxin7	AD	CAG in coding region
SCA8	13q21	?	AD	Untranslated
				CTG repeat on antisense strand
SCA10	22q13	ataxin-10	AD	Intronic
				ATTTC repeat
SCA11	15q14-21	?	AD	
SCA12	5q31	protein	AD	CAG in 5' UTR region
		phosphatase 2A		
SCA13	19q13	?	AD	?
SCA14	19q13.4	?	AD	?
SCA15	3pter-24.2	?	AD	?
SCA16	8q22.24	?	AD	?
SCA17	6q27	TATA-binding protein	AD	CAG in coding region
FA	9q13-21	frataxin-1	AR	Intronic GAA repeat
Ataxia-telangiectasia	11q22-23	ATM	AR	Point mutations

Friedreich Ataxia

This is an autosomal-recessive progressive illness, generally beginning in the first decade of life with gait ataxia, followed by hand clumsiness and dysarthria. Deep tendon reflexes are depressed or absent, but an extensor plantar reflex is typically present. Joint position and vibratory sense are impaired, and there is sometimes loss of pain and temperature sensation and light touch. Most patients develop pes cavus and kyphoscoliosis. There is a high incidence of cardiac disease with arrhythmias and congestive heart failure. Concomitant diabetes is found in about 10% of patients. Most patients become wheelchair-bound within about 5 years of onset; the cause of death is intercurrent pulmonary infections and cardiac disease.

The gene for Friedreich ataxia has been mapped to chromosome 9q13, and in most cases, there is a GAA trinucleotide repeat expansion in the first intron of a gene encoding a protein named *frataxin*.[218] Affected individuals inherit abnormal forms of the *frataxin* gene from both parents and have extremely low levels of the protein. In some cases of Friedreich ataxia,

one of the mutant alleles harbors a missense or nonsense mutation. Frataxin undergoes processing and ends up in the inner mitochondrial membrane, where it has been suggested to play a role in regulation of iron levels.[219] [220] Because of the need for this metal in many of the complexes of the oxidative phosphorylation chain, mutations in frataxin have been suggested to result in generalized mitochondrial dysfunction. Thus, Friedreich ataxia shares biologic features with other spinocerebellar ataxias (anatomic distribution of pathology, trinucleotide repeat expansion) and the mitochondrial encephalopathies.

Morphology.

The spinal cord shows loss of axons and gliosis in the posterior columns, the distal portions of corticospinal tracts, and the spinocerebellar tracts. There is degeneration of neurons in the spinal cord (Clarke column), the brainstem (cranial nerve nuclei VIII, X, and XII), the cerebellum (dentate nucleus and the Purkinje cells of the superior vermis), and to some extent the Betz cells of the motor cortex. Large dorsal root ganglion neurons are also decreased in number; their large myelinated axons, traveling first in the dorsal roots and then in dorsal columns, therefore undergo secondary degeneration. The heart is enlarged and may have pericardial adhesions. Multifocal destruction of myocardial fibers with inflammation and fibrosis is detectable in about half the patients who come to autopsy examination.

Ataxia-Telangiectasia

Ataxia-telangiectasia (Chapter 7) is an autosomal-recessive disorder characterized by an ataxic-dyskinetic syndrome beginning in early childhood, caused by neuronal degeneration predominantly in the cerebellum, the subsequent development of telangiectasias in the conjunctiva and skin, and immunodeficiency. Cells from patients with the disease show increased sensitivity to x-ray-induced chromosome abnormalities; these cells continue to replicate damaged DNA

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rather than stopping to allow repair or apoptosis. The ataxia-telangiectasia locus on chromosome 11q22-23 has been identified as a large gene, *ATM*, that encodes a protein with a kinase domain; the protein orchestrates the cellular response to double-stranded DNA breaks.[221] [222] The carrier frequency of ataxia-telangiectasia has been estimated at 1%; in these individuals, the mutated ataxia-telangiectasia allele may underlie an increased risk of cancer, specifically breast cancer.

Morphology.

The abnormalities are predominantly in the cerebellum, with loss of Purkinje and granule cells; there is also degeneration of the dorsal columns, spinocerebellar tracts, and anterior horn cells and a peripheral neuropathy. Telangiectatic lesions have been reported in the CNS as well as in the conjunctiva and skin of the face, neck, and arms. The nuclei of cells in many organs (e.g., Schwann cells in dorsal root ganglia and peripheral nerves, endothelial cells, pituicytes) show a bizarre enlargement of the cell nucleus to two to five times normal size and are referred to as amphicytes. The lymph nodes, thymus, and gonads are hypoplastic.

Clinical Features.

The disease relentlessly progresses to death early in the second decade. Patients first come to medical attention because of recurrent sinopulmonary infections and unsteadiness in walking. Later on, speech is noted to become dysarthric, and eye movement abnormalities develop. Many affected individuals develop lymphoid malignant disease (T-cell leukemia, T-cell lymphoma); gliomas and carcinomas have been reported in some.

DEGENERATIVE DISEASES AFFECTING MOTOR NEURONS

These are a group of inherited or sporadic diseases that, in variable degrees of severity, affect:

- *Lower motor neurons* in the anterior horns of the spinal cord
- *Lower motor neurons* in certain cranial nerve motor nuclei (V, VII, IX, XII) but not those that control eye movements (III, IV, VI)
- *Upper motor neurons* (Betz cells) in the motor cortex

The disorders occur in all age groups, and the course of the illness is extremely variable, ranging from slowly progressive or nonprogressive to rapidly progressive and fatal in a period of months or a few years. Denervation of muscles from loss of lower motor neurons and their axons results in muscular atrophy, weakness, and fasciculations; the corresponding histologic changes in nerve and muscle are discussed in Chapter 27 . The clinical manifestations include paresis, hyperreflexia, spasticity, and extensor plantar responses (Babinski sign). Sensory systems and cognitive functions are unaffected, but types with dementia do occur.

Amyotrophic Lateral Sclerosis (Motor Neuron Disease)

Amytrophic lateral sclerosis (ALS) is characterized by neuronal muscle atrophy (amyotrophy) and hyperreflexia due to loss of lower motor neurons in the anterior horns of the spinal cord and upper motor neurons that project in corticospinal tracts, respectively.[223] The disease affects men slightly more frequently than women and becomes clinically manifest in the fifth decade or later. Five per cent to 10% of cases are familial, mostly with autosomal-dominant inheritance.[224]

Pathogenesis.

The etiology and pathogenesis of amyotrophic lateral sclerosis are unknown. For a subset of the familial cases, the genetic locus has been mapped to the copper-zinc superoxide dismutase gene (*SOD1*) on chromosome 21.[225] A wide variety of missense mutations have been identified that appear to generate an adverse gain-of-function phenotype. Among the mutations, the A4V mutation is the most common (approaching 50% of cases), is associated with a rapid course, and rarely has upper motor neuron signs.[226] [227] A recessive locus on chromosome 2 has been mapped to a gene encoding a protein termed alsin that has structural homology to proteins involved in GTPase regulation.[228] [229] Other genetic loci for ALS have been mapped but not yet cloned. There is also evidence of roles of glutamate toxicity and protein nitration in the development of ALS pathology.[224] The basis for the selective involvement of motor neurons remains uncertain.

Morphology.

On macroscopic examination, the anterior roots of the spinal cord are thin; the precentral gyrus may be atrophic in especially severe cases. Microscopic examination demonstrates a reduction in the number of anterior horn neurons throughout the length of the spinal cord with associated reactive gliosis and loss of anterior root myelinated fibers. Similar findings are found with involvement of the hypoglossal, ambiguus, and motor trigeminal cranial nerve nuclei. Remaining neurons often contain Bunina bodies: PAS-positive cytoplasmic inclusions that appear to be remnant of autophagic vacuoles. Skeletal muscles innervated by the degenerated lower motor neurons show neurogenic atrophy. Destruction of the upper motor neurons leads to degeneration of myelin in the corticospinal tracts, resulting in pale staining that is particularly evident at the lower segmental levels but traceable throughout the corticospinal system with special studies (Fig. 28-39).

Clinical Features.

Early symptoms include asymmetric weakness of the hands, manifested as dropping objects and difficulty in performing fine motor tasks, and cramping and

Figure 28-39 Amyotrophic lateral sclerosis. Spinal cord showing loss of myelinated fibers (lack of stain) in corticospinal tracts. The anterior roots are smaller than the posterior roots.

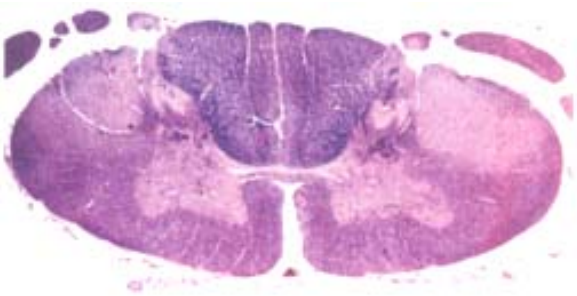


Figure 28-40 Metachromatic leukodystrophy. Demyelination is extensive. The subcortical fibers in the cerebral hemisphere are spared (Luxol fast blue PAS stain for myelin).

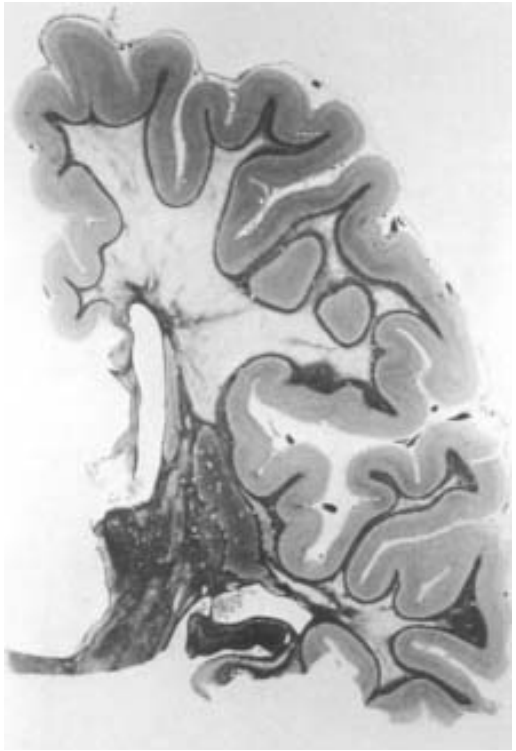


Figure 28-41 Alcoholic cerebellar degeneration. The anterior portion of the vermis *(upper portion of figure)* is atrophic with widened spaces between the folia.

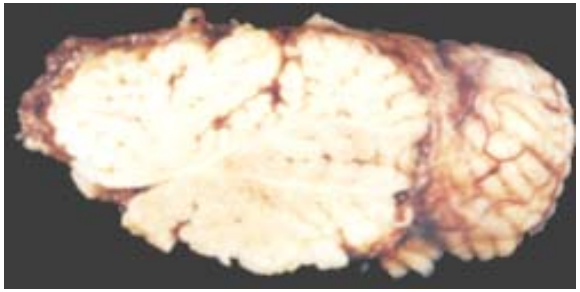


Figure 28-42 Well-differentiated astrocytoma. *A*, The right frontal tumor has expanded gyri, which led to flattening *(arrows)*. *B*, Expanded white matter of the left cerebral hemisphere and thickened corpus callosum and fornices.

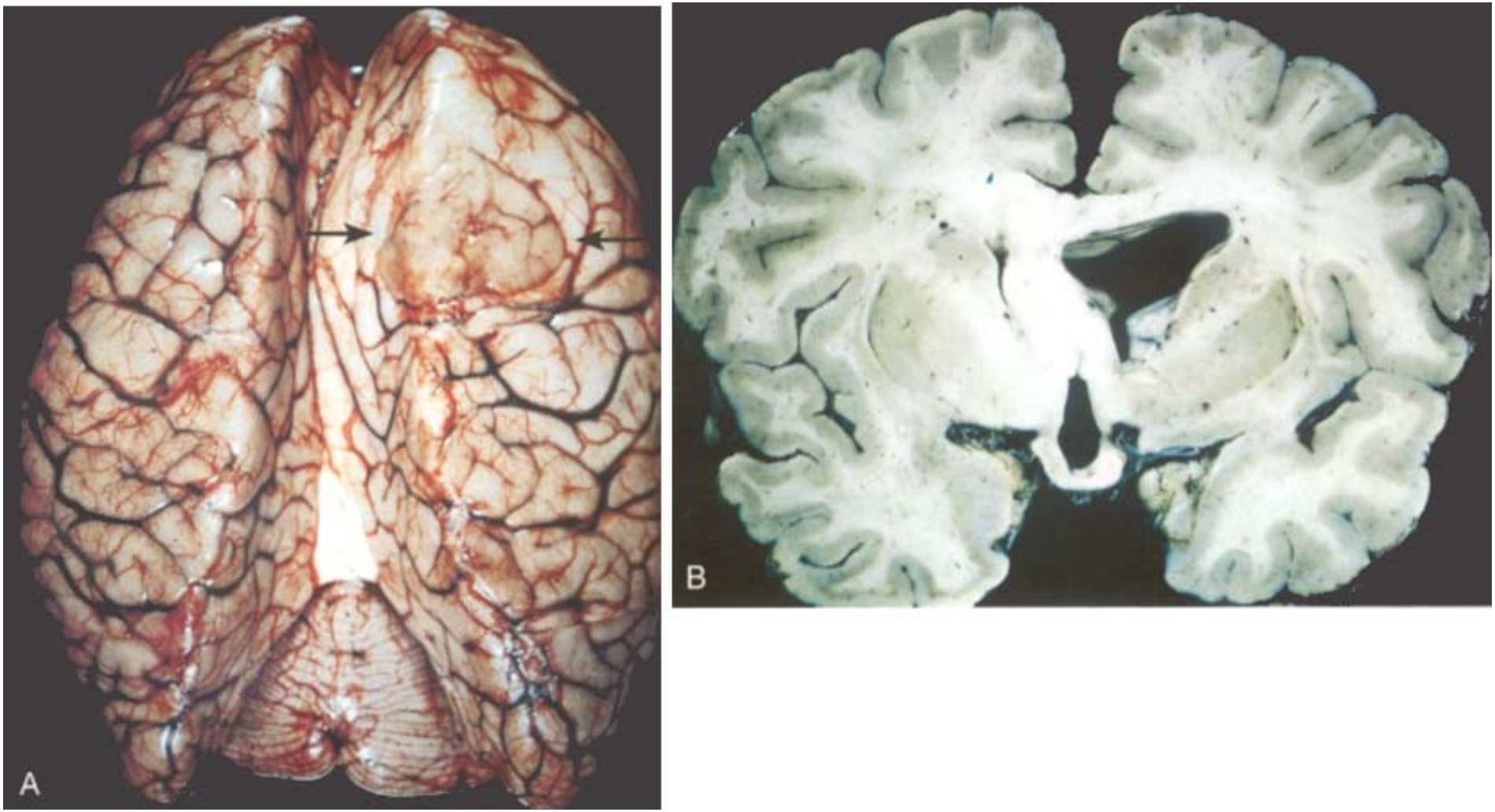


Figure 28-43 *A*, Computed tomographic (CT) scan of a large tumor in the cerebral hemisphere showing signal enhancement with contrast material and pronounced peritumoral edema. *B*, Glioblastoma multiforme appearing as a necrotic, hemorrhagic, infiltrating mass.

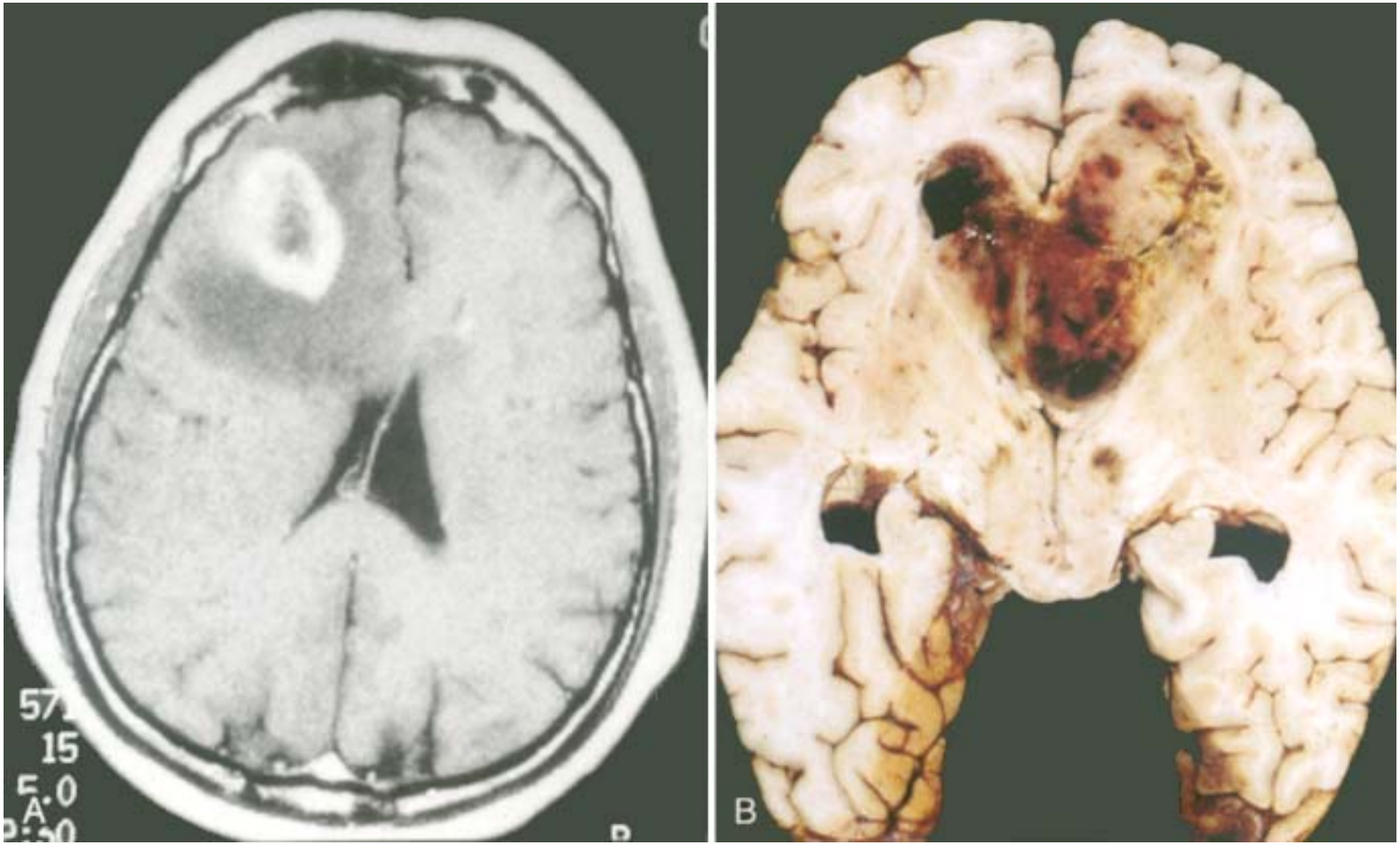


Figure 28-44 Glioblastoma. Foci of necrosis with pseudopalisading of malignant nuclei.

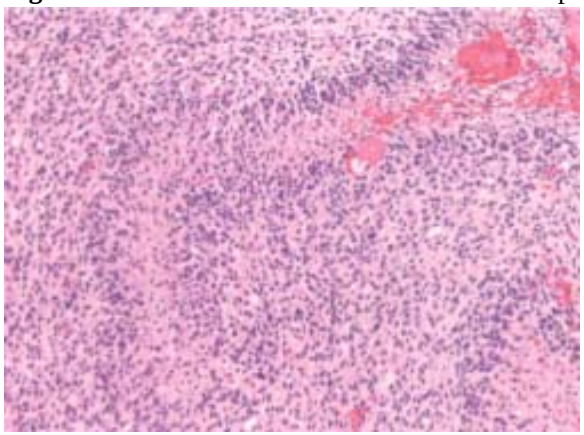


Figure 28-45 Pilocytic astrocytoma in the cerebellum with a nodule of tumor in a cyst.

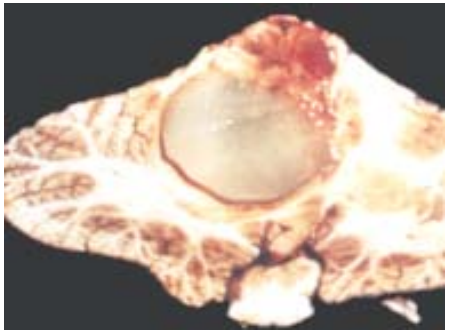
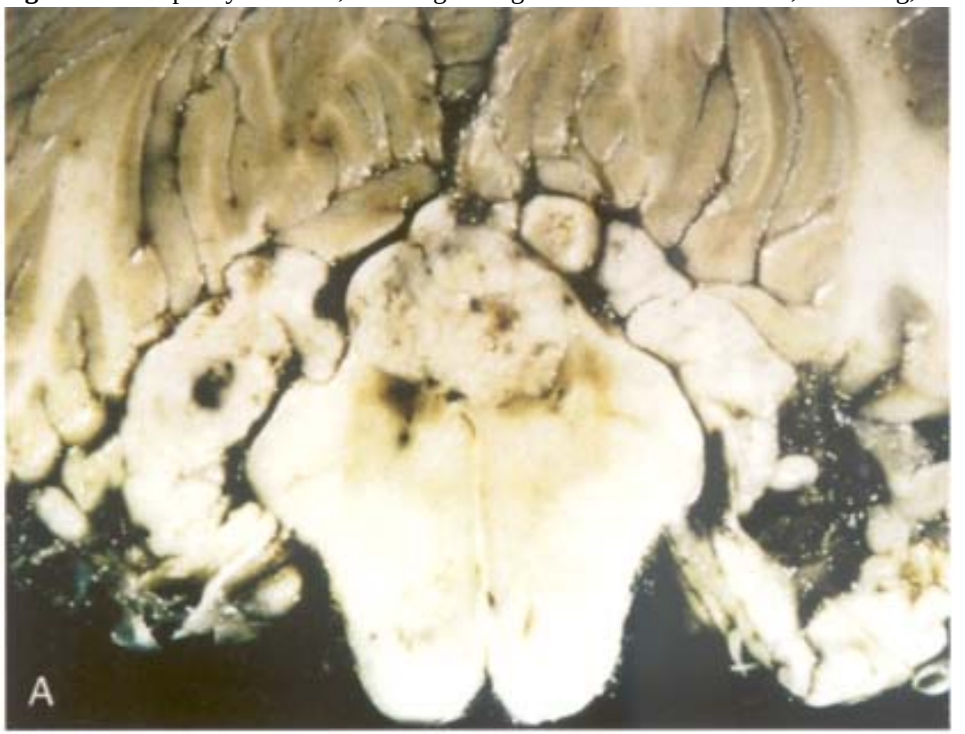
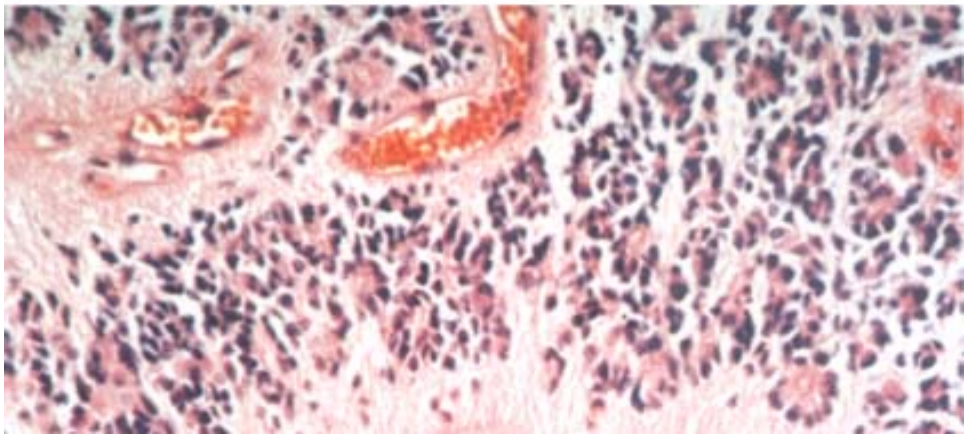


Figure 28-46 Ependymoma. *A*, Tumor growing into the fourth ventricle, distorting, compressing, and infiltrating surrounding structures. *B*, Microscopic appearance of ependymoma.





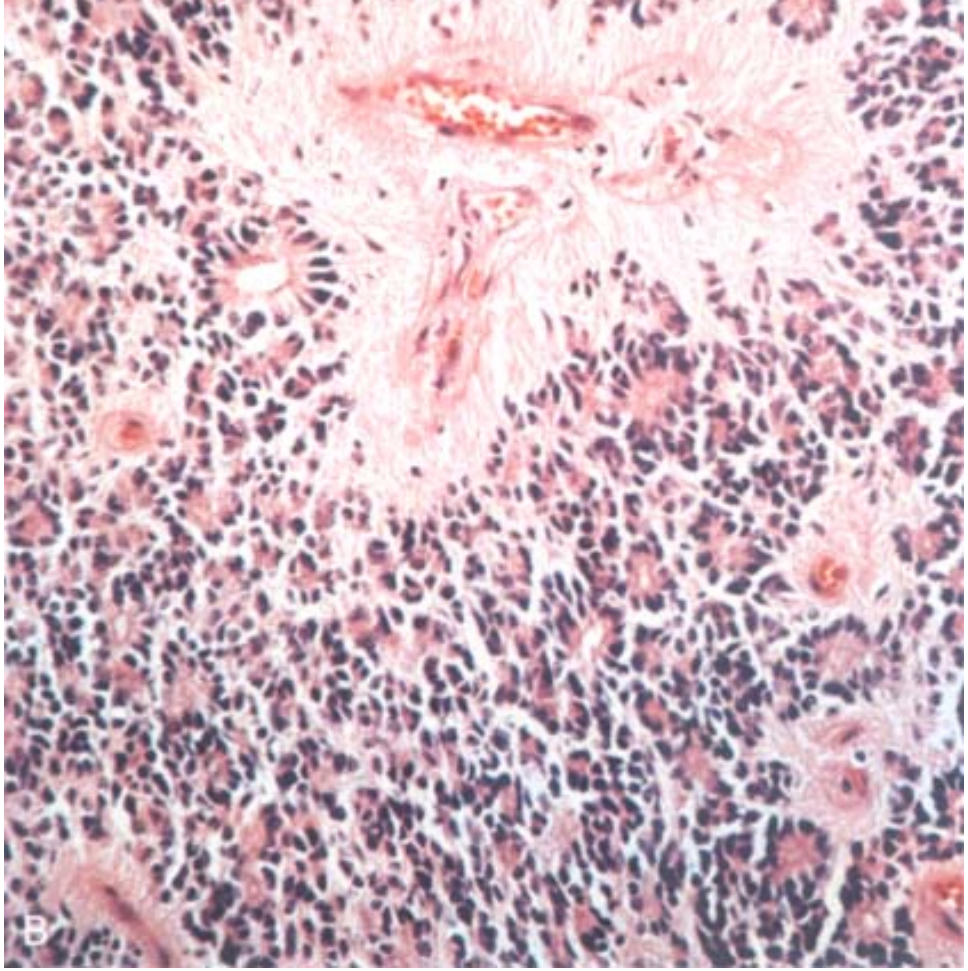


Figure 28-47 Medulloblastoma. *A*, CT scan showing a contrast-enhancing midline lesion in the posterior fossa. *B*, Sagittal section of brain showing medulloblastoma destroying the superior midline cerebellum. *C*, Microscopic appearance of medulloblastoma.

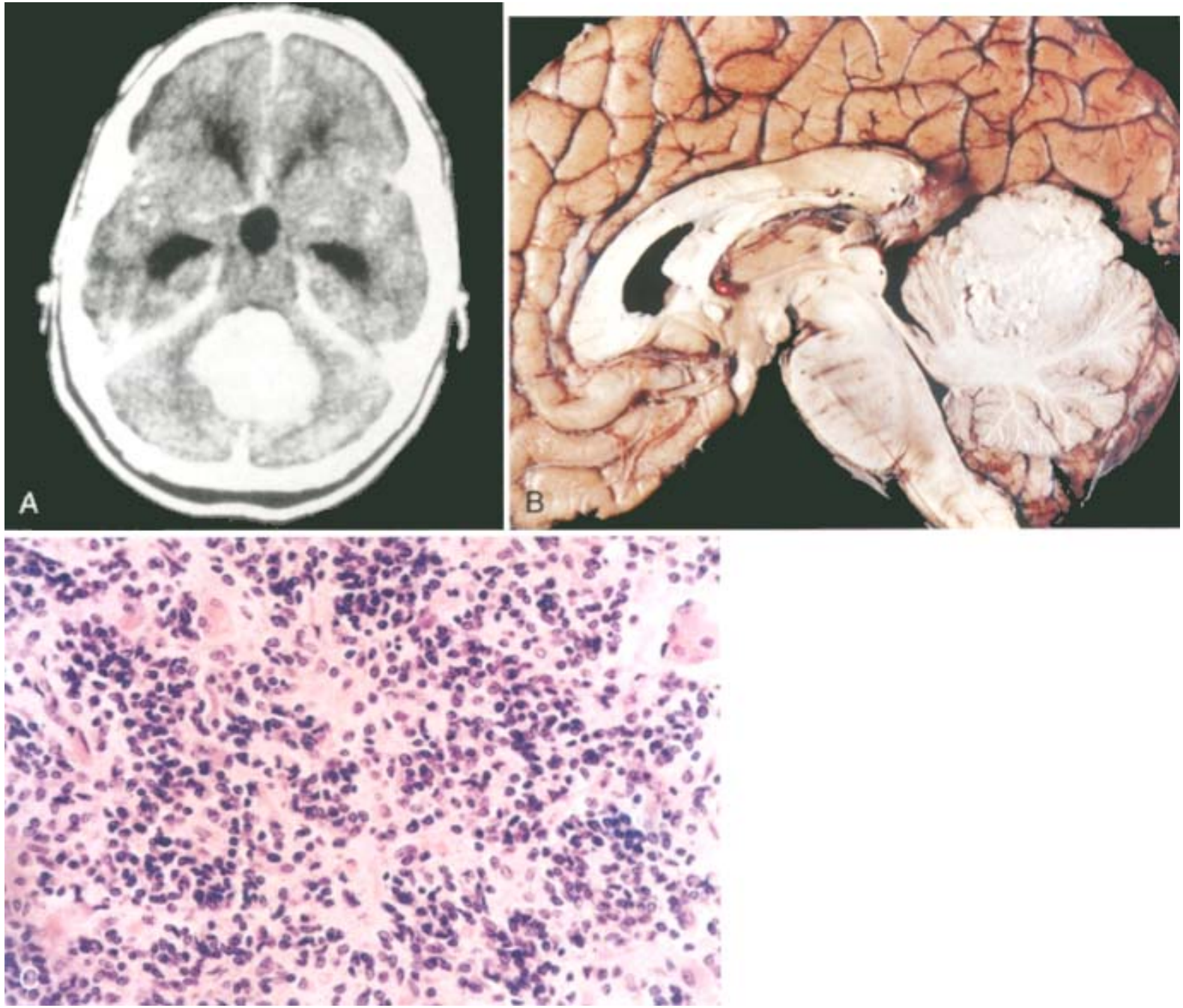
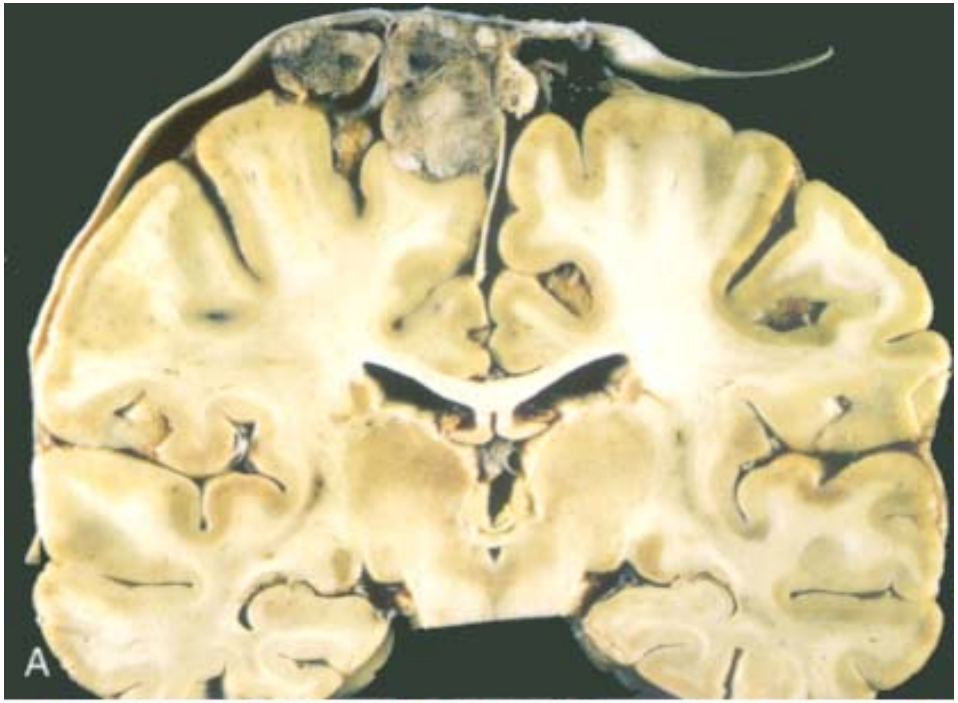


Figure 28-48 *A*, Parasagittal multilobular meningioma attached to the dura with compression of underlying brain. *B*, Meningioma with a whorled pattern of cell growth and psammoma bodies.



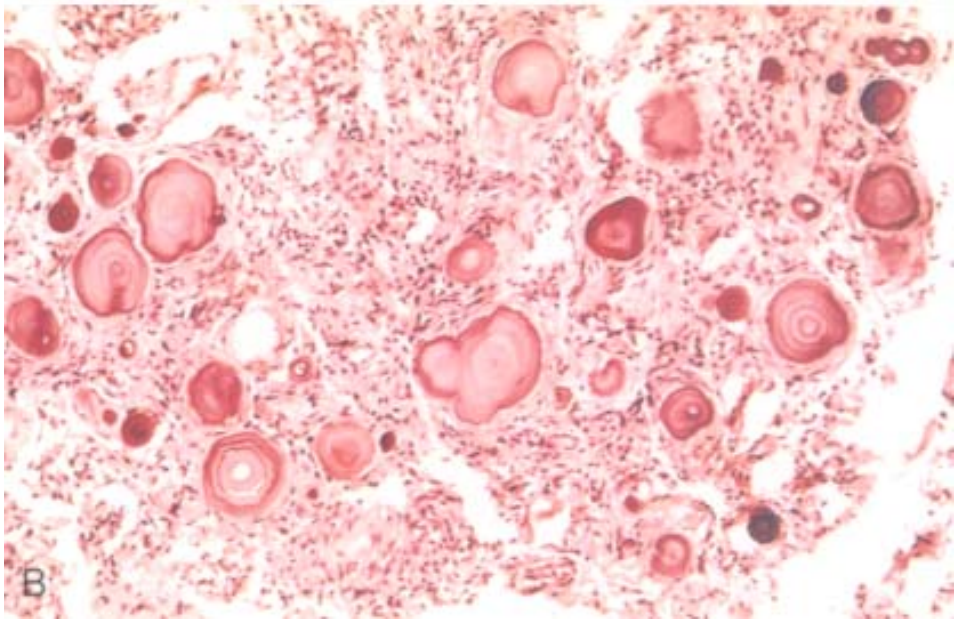


TABLE 28-4 -- Paraneoplastic Syndromes

Syndrome	Target	Tumor	Antigen
Subacute cerebellar degeneration	Purkinje cells	Hodgkin lymphoma	Tr

		Breast, GYN	Yo
		SCLC, neuroblastoma	Hu
Limbic encephalitis; brainstem encephalitis	Various neurons in mesial temporal lobe, brainstem	SCLC, neuroblastoma	Hu
		Testicular, others	Ma
Subacute sensory neuropathy	Dorsal root ganglion neurons	SCLC, neuroblastoma	Hu
Opsoclonus myoclonus	Unknown (presumed brain stem)	Neuroblastoma, Breast	Ri
Retinal degeneration	Photoreceptors	SCLC	Recoverin
Stiff-man syndrome	Spinal interneurons	Breast	Amphiphysin
Lambert-Eaton myasthenic syndrome	Presynaptic terminals at neuromuscular junction	SCLC	Presynaptic calcium channel
SCLC, Small cell lung carcinoma.			

The major underlying mechanism of these diseases involves the systemic development of an immune response against tumor antigens that can cross-react with antigens in the central or peripheral nervous systems.[280] [281] The relationship among the underlying malignant process, the clinical features, and the antigens underlying the syndrome are complex. Some tumor types are associated with multiple types of autoantibodies, and the same antibodies can be present in different clinical syndromes. What allows these antibodies access to the nervous system and how this immune response to intracellular proteins (for the most part) elicits disease remain unanswered questions. There may also be a component of T cell-mediated neuronal injury in some settings.[282]

PERIPHERAL NERVE SHEATH TUMORS

These tumors arise from cells of the peripheral nerve, including Schwann cells, perineurial cells, and fibroblasts. Many express Schwann cell characteristics, including the presence of S-100 antigen as well as the potential for melanocytic differentiation. As nerves exit the brain and spinal cord, there is a transition between myelination by oligodendrocytes and myelination by Schwann cells. This occurs within several millimeters of the substance of the brain; thus, peripheral nerve tumors can arise within the dura and may cause changes in adjacent brain or spinal cord. Tumors of comparable histogenesis and biologic behavior also arise along the peripheral course of nerves.

Schwannoma

These benign tumors arise from the neural crest-derived Schwann cell and are associated with neurofibromatosis type 2. Symptoms are referable to local compression of the involved nerve or to compression of adjacent structures (such as brain stem or spinal cord). Sporadic schwannomas are associated with mutations in the *NF2* gene on chromosome 22; there is usually absence of the *NF2* gene product by Western blotting or immunostaining, even if there is no evidence of a mutation in the gene.[283]

Morphology.

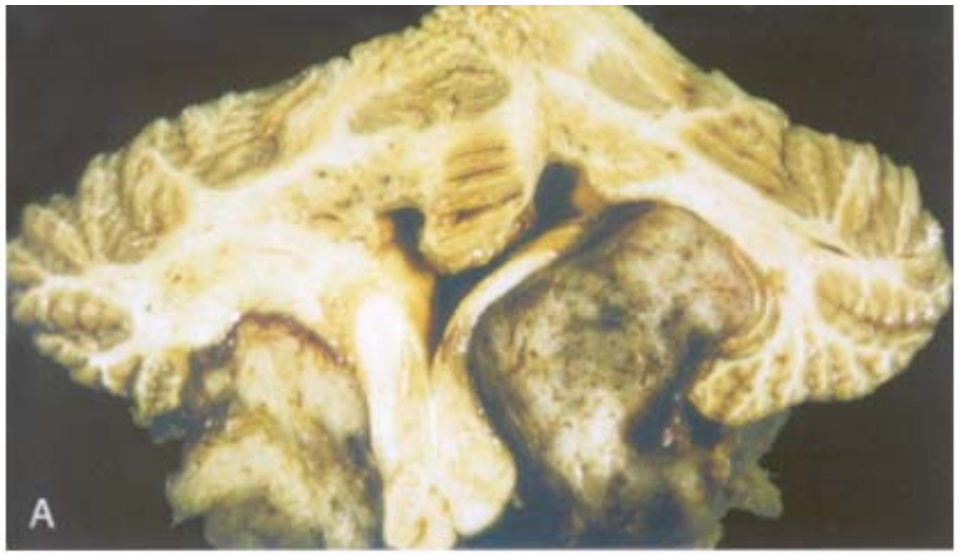
Schwannomas are well-circumscribed, encapsulated masses that are attached to the nerve but can be separated from it (Fig. 28-49*A*). Tumors form firm, gray masses but may also have areas of cystic and xanthomatous change. On microscopic examination, tumors show a mixture of two growth patterns (Fig. 28-49*B*). In the **Antoni A** pattern of growth, elongated cells with cytoplasmic processes are arranged in fascicles in areas of moderate to high cellularity with little stromal matrix; the "nuclear-free zones" of processes that lie between the regions of nuclear palisading are termed Verocay bodies. In the **Antoni B** pattern of growth, the tumor is less densely cellular with a loose meshwork of cells along with microcysts and myxoid changes. In both areas, the cytology of the individual cells is similar, with elongated cell shape and regular oval nuclei. Electron microscopy shows basement membrane deposits encasing single cells and long-spacing collagen. Because the lesion displaces the nerve of origin as it grows, silver stains or immunostains for neurofilament proteins demonstrate that

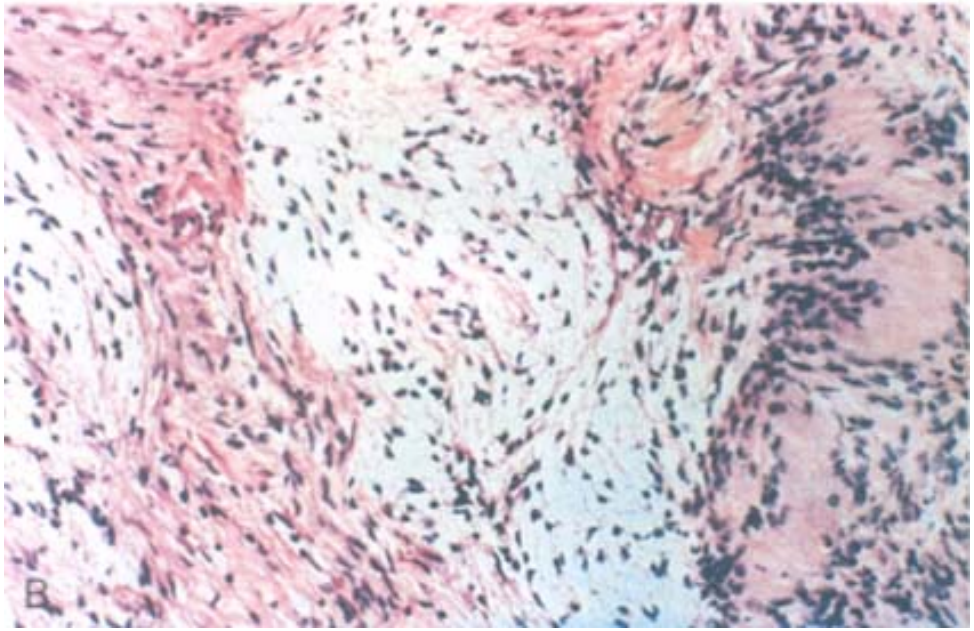
axons are largely excluded from the tumor, although they may become entrapped in the capsule. The Schwann cell origin of these tumors is borne out by their S-100 immunoreactivity. A variety of degenerative changes may be found in schwannomas, including nuclear pleomorphism, xanthomatous change, and vascular hyalinization. Malignant change is extremely rare in schwannomas, although local recurrence can follow incomplete resection.

Clinical Features.

Within the cranial vault, the most common location of schwannomas is in the cerebellopontine angle, where they are attached to the vestibular branch of the eighth nerve (Fig. 28-49). Patients often present with tinnitus and hearing loss, and the tumor is often referred to as an acoustic neuroma, although it is more accurately called a vestibular schwannoma. Elsewhere within the dura, sensory nerves are preferentially involved, including branches of the trigeminal nerve and dorsal roots. When extradural, schwannomas are most commonly found in association with large nerve trunks, where motor and sensory modalities are intermixed.

Figure 28-49 Schwannoma. *A*, Bilateral eighth nerve schwannomas. *(Courtesy of Dr. K.M. Earle.) B*, Tumor showing cellular areas (Antoni A), including Verocay bodies *(far right)*, as well as looser, myxoid regions (Antoni B).





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Chapter 29 - The Eye

Robert Folberg MD

Although this chapter comes at the end of the book, it is not least important. Vision is a major quality-of-life issue for individuals. In the mid-1960s and again in the mid-1970s, the Gallup Organization polled Americans and asked the following question: "Which disease do you fear most?" Before the public awareness of AIDS and Alzheimer disease, the most feared disease among Americans was cancer. The second most feared disease was blindness. So great is the fear of blindness that even today, patients often tell their physicians, "Doctor, I'd rather be dead than be blind!"

In general, diseases that produce loss of vision do not attract as much of our attention as do many of the conditions described in this book that are life-threatening. Typically, loss of vision is enacted in the theater of the mundane. Age-related macular degeneration (ARMD) is the most common cause of irreversible visual loss in the United States. ARMD is not life-threatening and most patients do not even suffer from a total loss of vision—an immersion into total darkness. The pathology is quantitatively and qualitatively unspectacular: Small scars develop in the macula. But consider the effect of these tiny scars perhaps in a retired schoolteacher with ARMD. The small macular scars make it impossible for her to see anything clearly in the central portion of her vision. She looks directly at her life-long companion, her spouse, and cannot see his face. She cannot read a book or newspaper or look up telephone numbers. She, who raised children, had a career, and was the model of independence, can no longer drive a car and must ask people to take her where she wishes to go: true, her life is not threatened by the small scars in the macula of her

Figure 29-1 Anatomy of the eye.

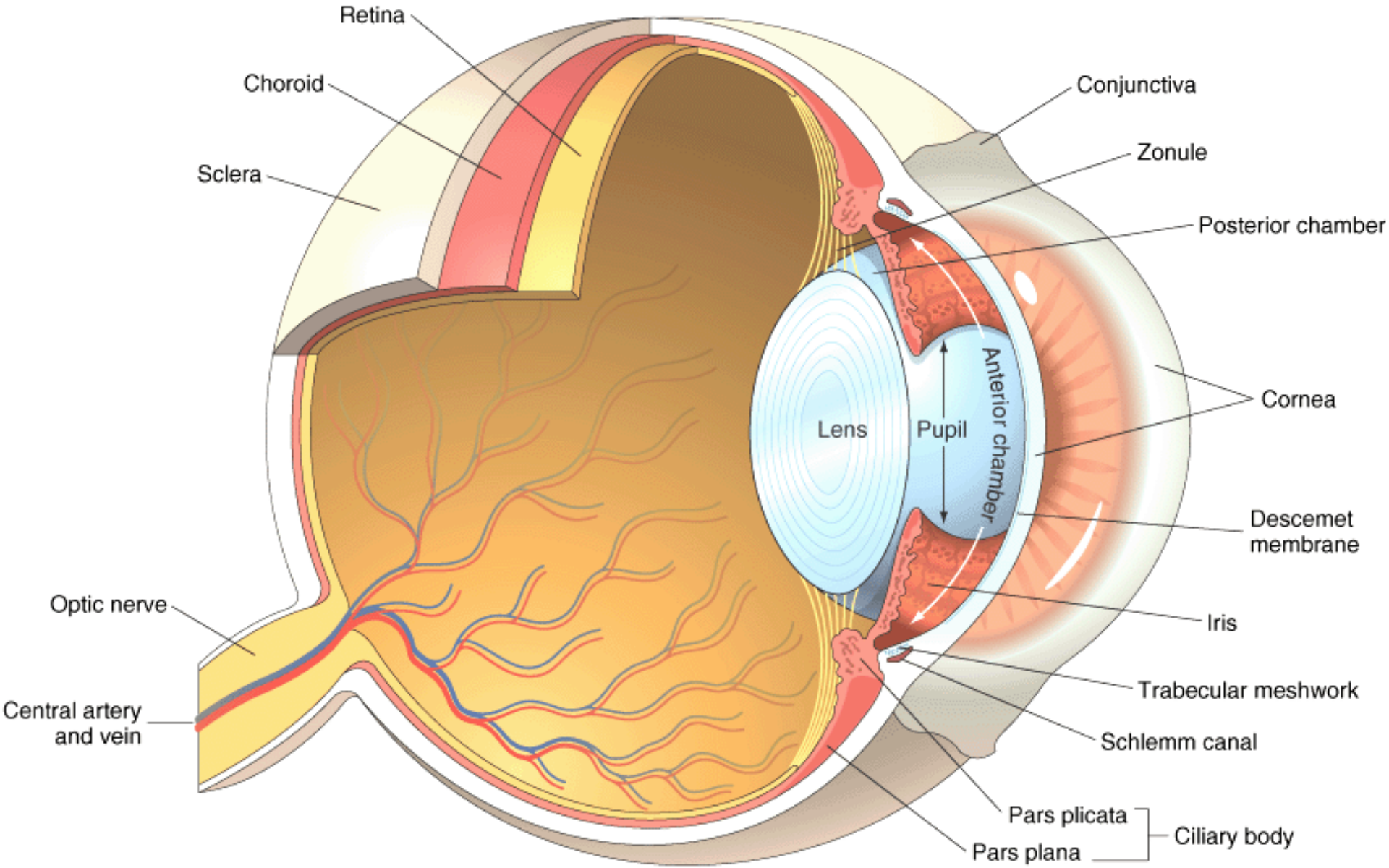


Figure 29-2 The extraocular muscles are greatly distended in this postmortem dissection of tissues from a patient with thyroid (Graves) ophthalmopathy. Note that the tendons of the muscles are spared involvement. *(Courtesy of Dr. Ralph C. Eagle Jr, Wills Eye Hospital, Philadelphia, PA.)*

Figure 29-3 In idiopathic orbital inflammation (orbital inflammatory pseudotumor), the orbital fat is replaced by fibrosis. Note the chronic inflammation, accompanied in this case by eosinophils.

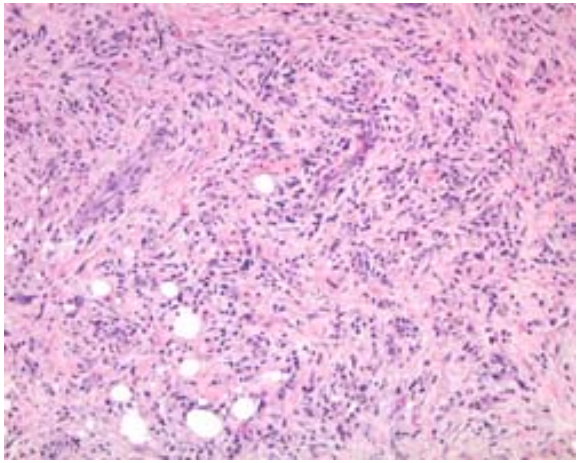


Figure 29-4 Anatomy of the conjunctiva and eyelids.

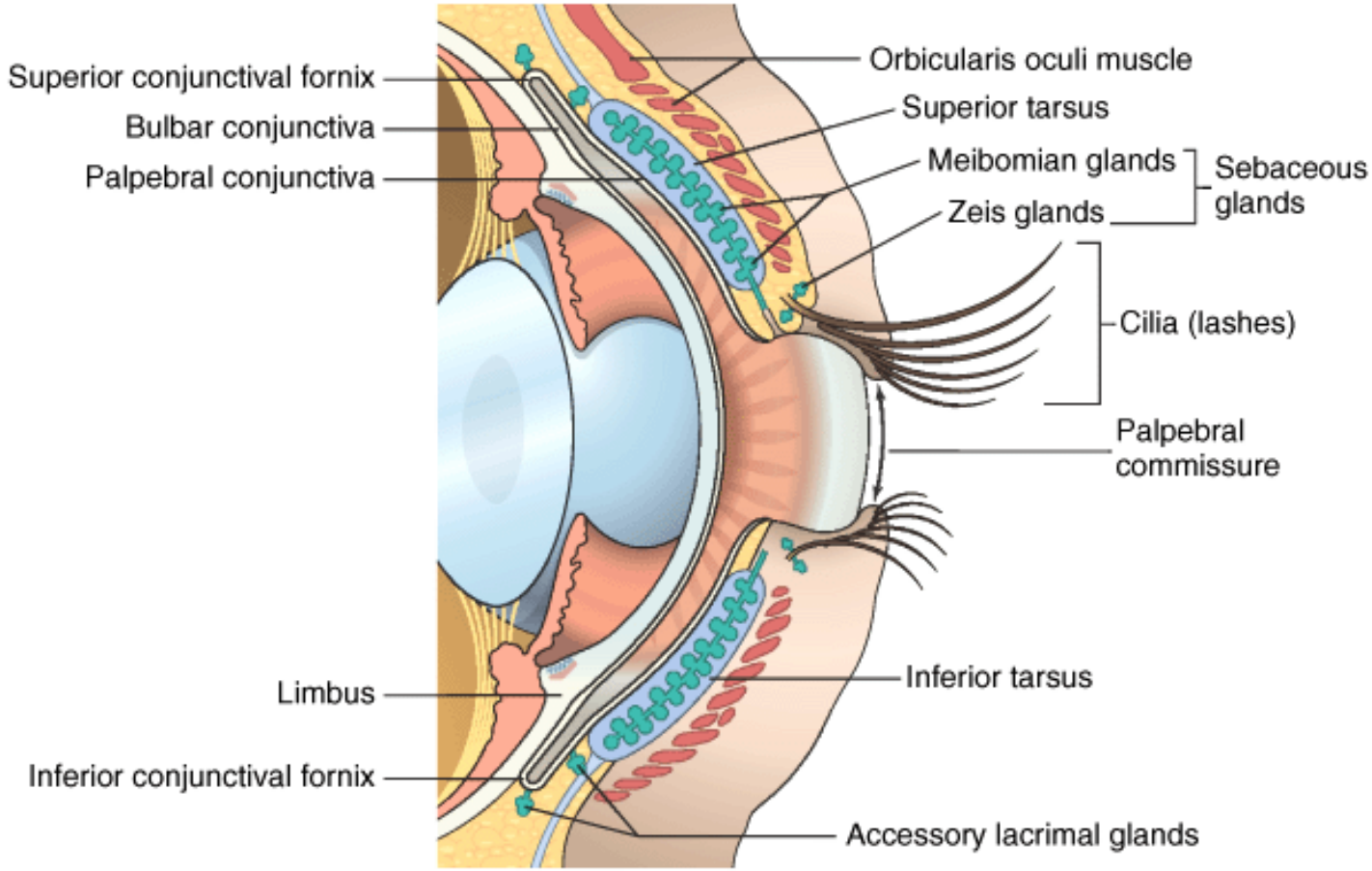


Figure 29-5 Pagetoid spread of sebaceous carcinoma. Neoplastic cells with foamy cytoplasm are detected within the epidermis. Invasive sebaceous carcinoma was identified elsewhere in this biopsy sample.

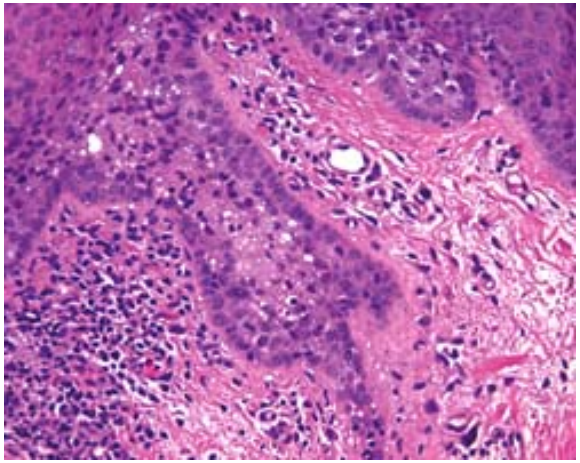


Figure 29-6 *A, B,* Cystic compound nevus of the conjunctiva. *(From Folberg R, Jakobiec FA, Bernardino VB, Iwamoto T: Benign conjunctival melanocytic lesions: clinicopathologic features. Ophthalmology 96:436–461, 1989.) C, D,* Conjunctival malignant melanoma. In *C,* note the deflection of the beam of the slit lamp over the surface of the lesion, indicative of invasion.

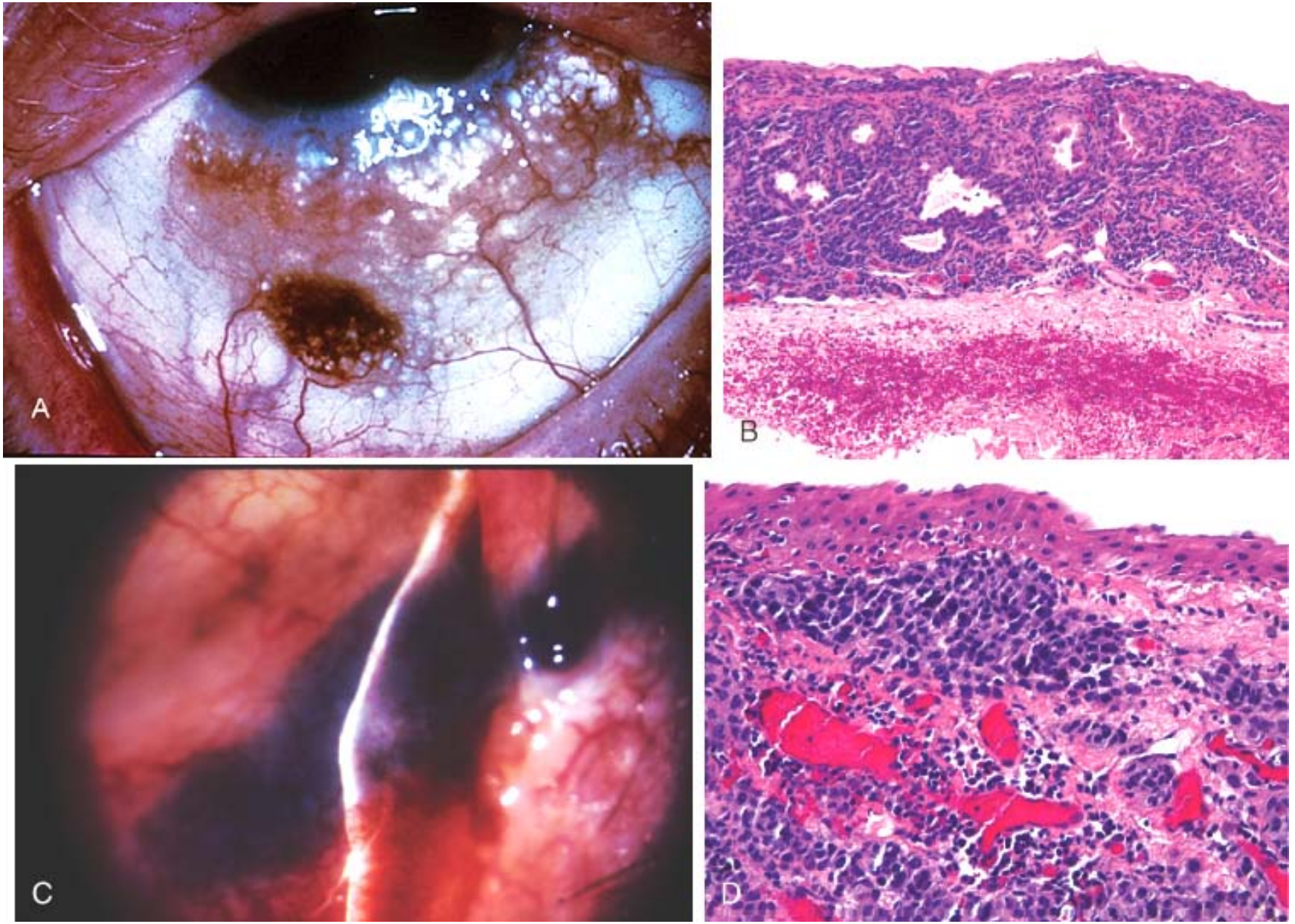


Figure 29-7 Normal corneal microarchitecture. The corneal tissue is stained by periodic acid-Schiff (PAS) to highlight basement membranes. The inset at the upper left is a high magnification of the anterior layers of the cornea: the epithelium *(e)*, Bowman's layer *(b)*, and the stroma *(s)*. A very thin PAS-positive basement membrane separates the epithelium from Bowman's layer. Note that Bowman's layer is acellular. The inset at the lower right is a high magnification of the PAS-positive Descemet membrane and the corneal endothelium. The "holes" in the stroma are artifactitious spaces between parallel collagenous stromal lamellae.

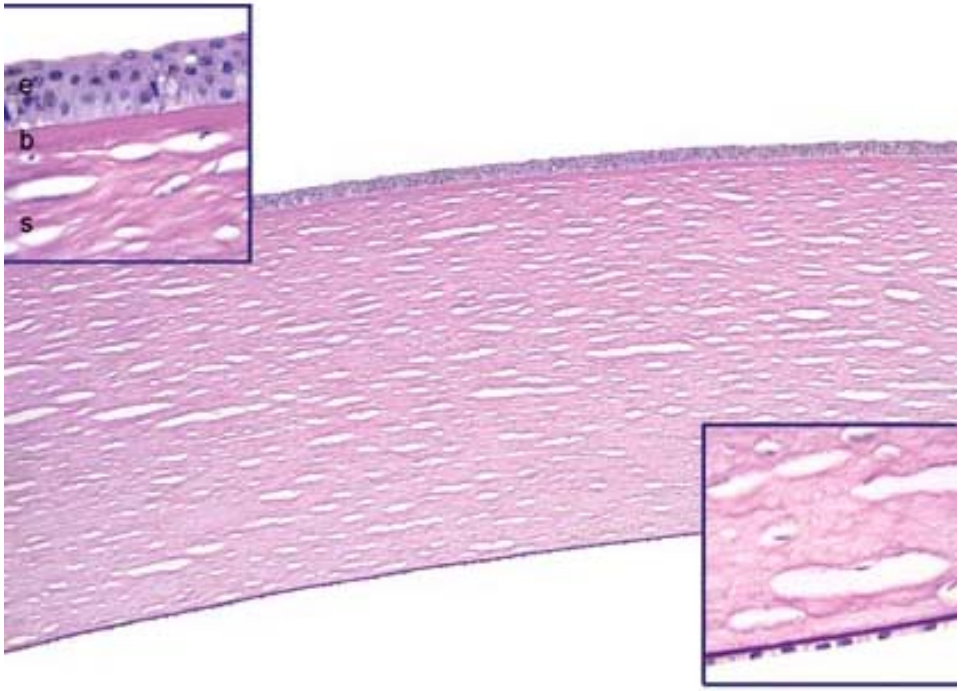


Figure 29-8 Chronic herpes simplex keratitis. The cornea is thin and scarred (note the increased number of fibroblast nuclei). Granulomatous reaction to Descemet's membrane, illustrated in this photomicrograph (*arrows*), is a histologic hallmark or chronic herpes simplex keratitis.

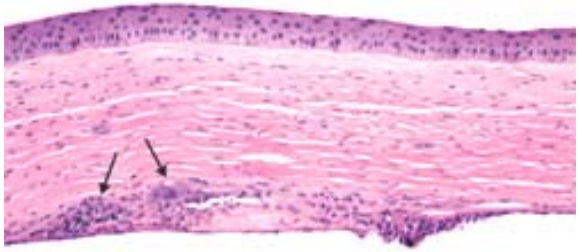


Figure 29-9 Keratoconus. This high-magnification photomicrograph captures the epithelium, Bowman's layer, and the superficial layers of the corneal stroma; the posterior layers of the stroma, Descemet's membrane, and the endothelium are not included. The tissue section is stained by periodic acid-Schiff to highlight the epithelial basement membrane (ebm), which is intact. Bowman's layer (bl), situated between the epithelial basement membrane and the stroma (s), is not a basement membrane. By tracing Bowman's layer from the right side of the photomicrograph toward the center, one notices a discontinuity in this layer, diagnostic of keratoconus. The epithelial separation just to the left of the Bowman's layer break resulted from an episode of corneal hydrops, secondary to a break in Descemet's membrane (not shown).

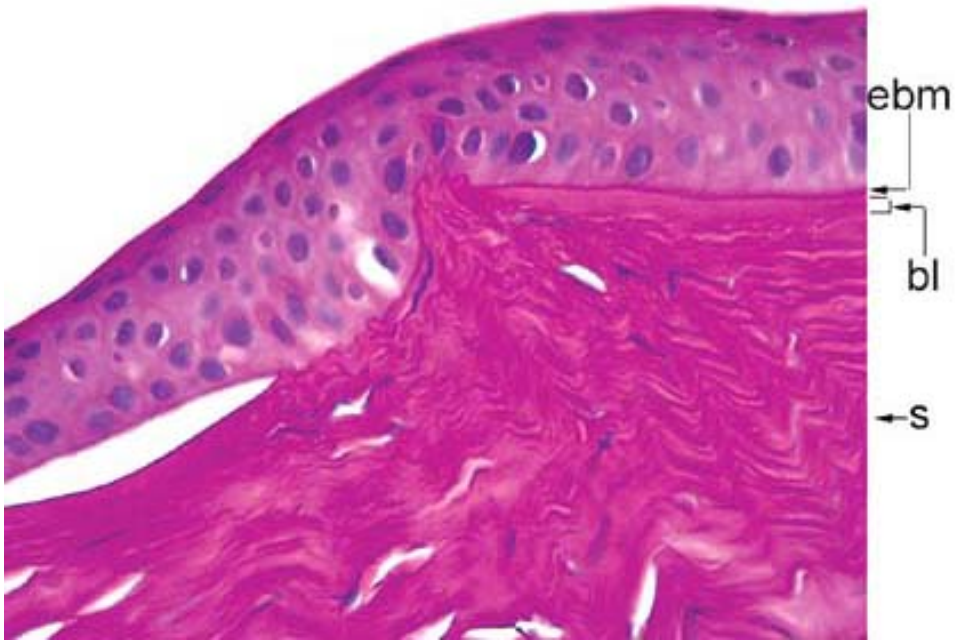
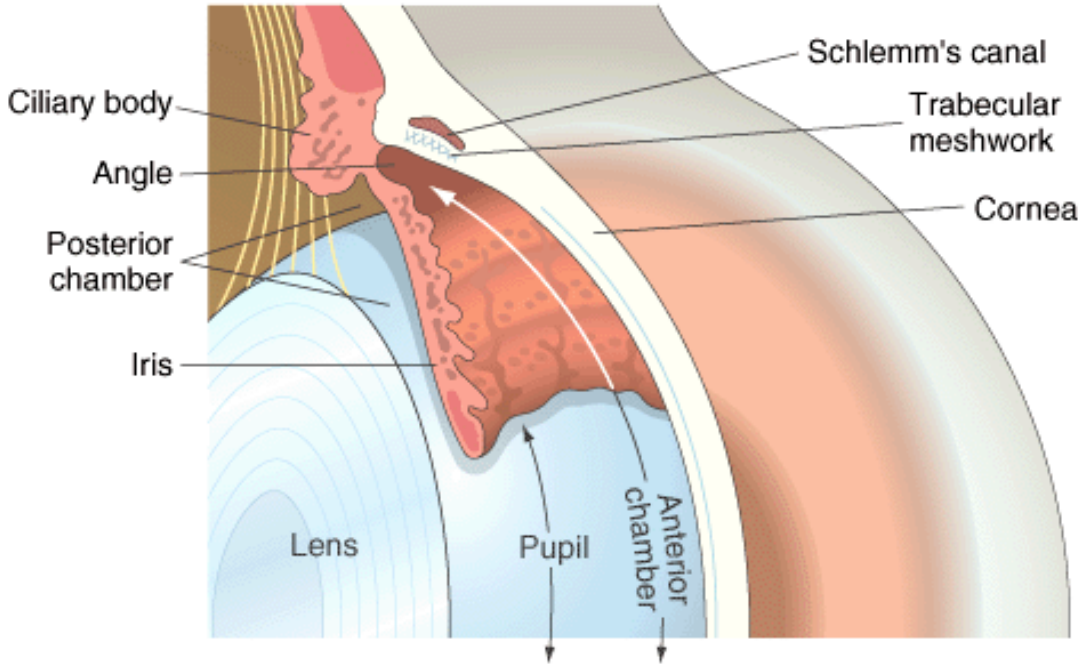


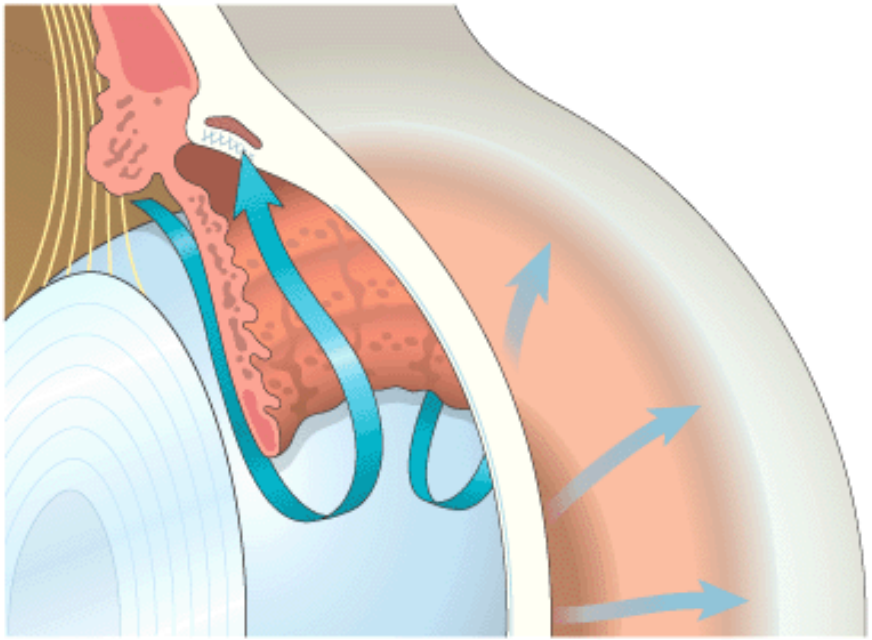
Figure 29-10 Fuchs dystrophy. This tissue section is stained by periodic acid-Schiff to highlight the Descemet's membrane, which is thick. Numerous droplike excrescences—guttata—protrude downward from Descemet's membrane. Endothelial cell nuclei are not seen. Epithelial bullae, not shown in this micrograph, were present, reflecting corneal edema.

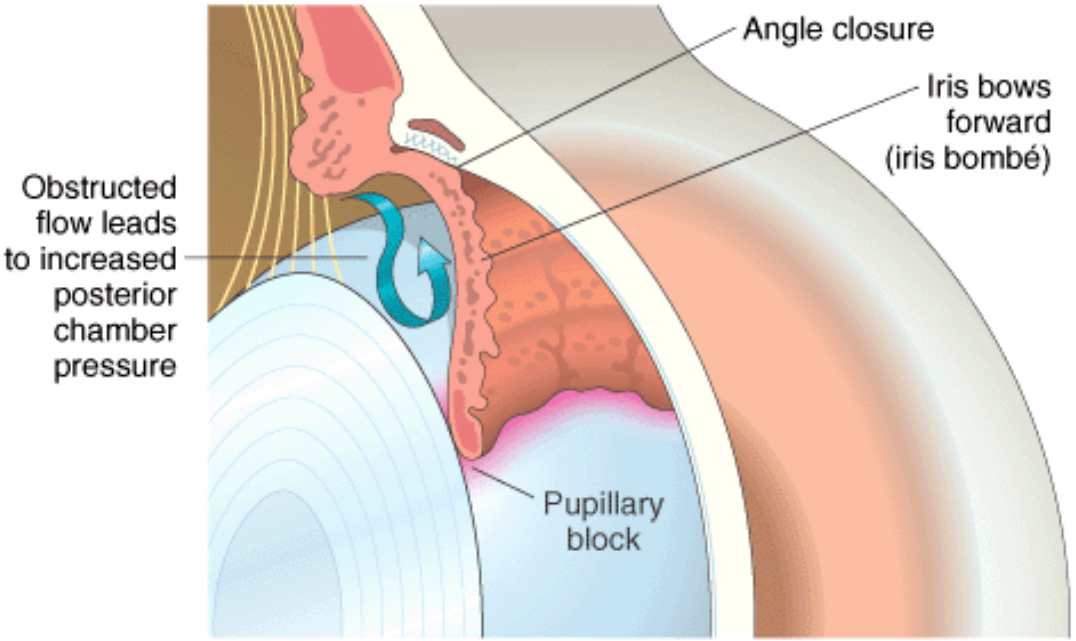
Figure 29-11 *Upper left*, The normal eye. Note that the surface of the iris is highly textured with crypts and folds. *Upper right*, The normal flow of aqueous humor. Aqueous humor, produced in the posterior chamber, flows through the pupil into the anterior chamber. The major pathway for the egress of aqueous humor is through the trabecular meshwork, into Schlemm's canal. Minor outflow pathways (uveoscleral and iris, not depicted) contribute to a limited extent to aqueous outflow. *Lower left*, Primary angle closure glaucoma. In anatomically predisposed eyes, transient apposition of the iris at the pupillary margin to the lens blocks the passage of aqueous humor from the posterior chamber to the anterior chamber. Pressure builds in the posterior chamber, bowing the iris forward (iris bombé) and occluding the trabecular meshwork. *Lower right*, A neovascular membrane has grown over the surface of the iris, smoothing the iris folds and crypts. Myofibroblasts within the neovascular membrane cause the membrane to contract and to become apposed to the trabecular meshwork (peripheral anterior synechiae). Outflow of aqueous humor is blocked, and the intraocular pressure becomes elevated.

ANTERIOR AND POSTERIOR CHAMBERS

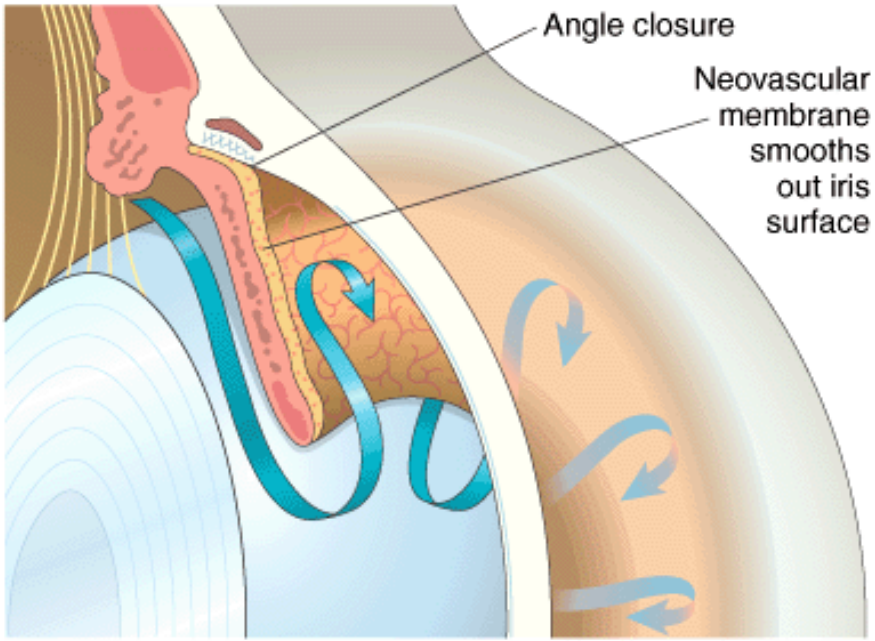


MAJOR AQUEOUS OUTFLOW PATHWAY





PRIMARY ANGLE CLOSURE GLAUCOMA



NEOVASCULAR GLAUCOMA

Figure 29-12 Sequelae of anterior segment inflammation. This eye was removed for complications of chronic corneal inflammation (which cannot be discerned at this magnification). The exudate (e) present in the anterior chamber would have been visualized at the slit lamp as an optical "flare." The iris is adherent focally to the cornea, obstructing the trabecular meshwork (anterior synechia, *arrow*), and adheres to the lens (posterior synechiae, *arrowheads*). An anterior subcapsular cataract (asc) has formed. The radial folds in the lens are artifacts.

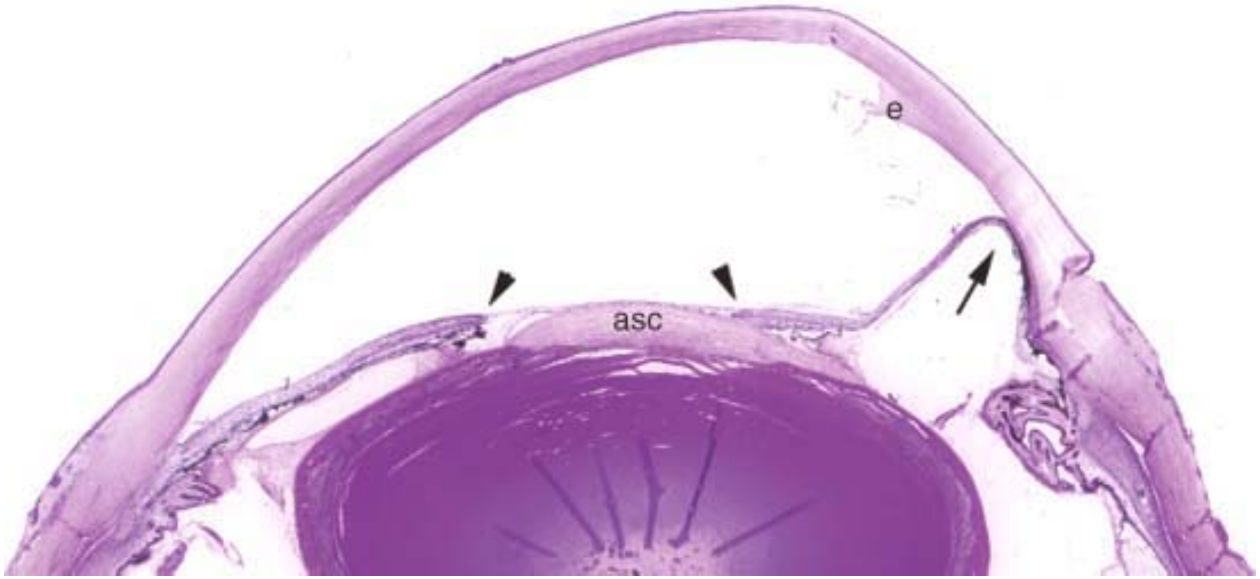


Figure 29-13 Exogenous panophthalmitis. This eye was removed after a foreign body injury. Note the suppurative inflammation behind the lens and drawn up to the right of the lens to the cornea, the site of the wound. The central portion of the vitreous humor was extracted surgically (by vitrectomy). Note the adhesions to the surface of the eye at the eight o'clock position, indicating that the intraocular inflammation has spread through the sclera into the orbit: panophthalmitis. *(From Folberg R: The Eye. In Spencer WH (ed.) Ophthalmic Pathology—An Atlas and Textbook (4th Edition). Philadelphia, WB Saunders, 1985.)*

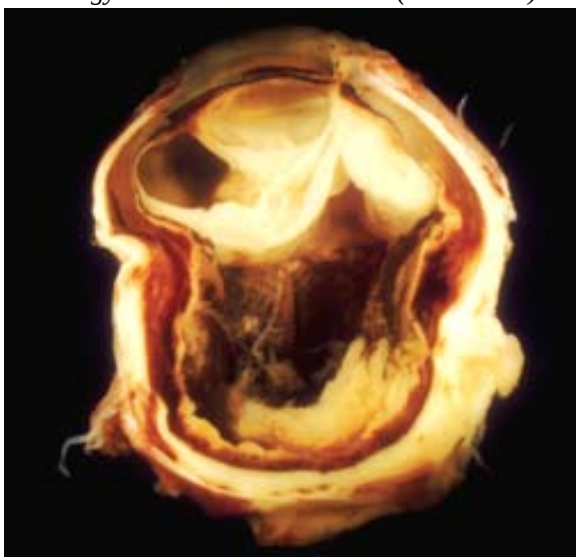


Figure 29-14 Sympathetic ophthalmia. The granulomatous inflammation depicted here was identified diffusely throughout the uvea. The uveal granulomas may contain melanin pigment

and may be accompanied by eosinophils.

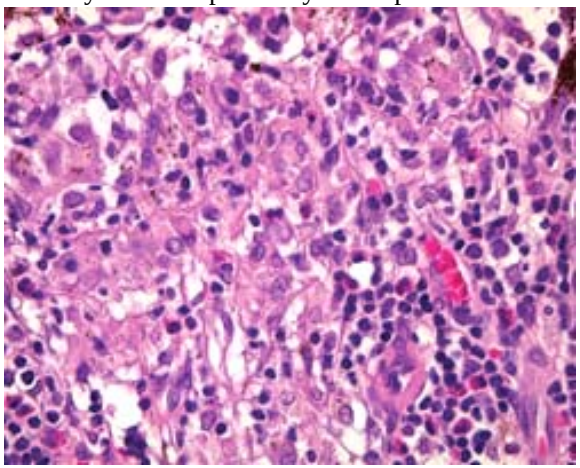
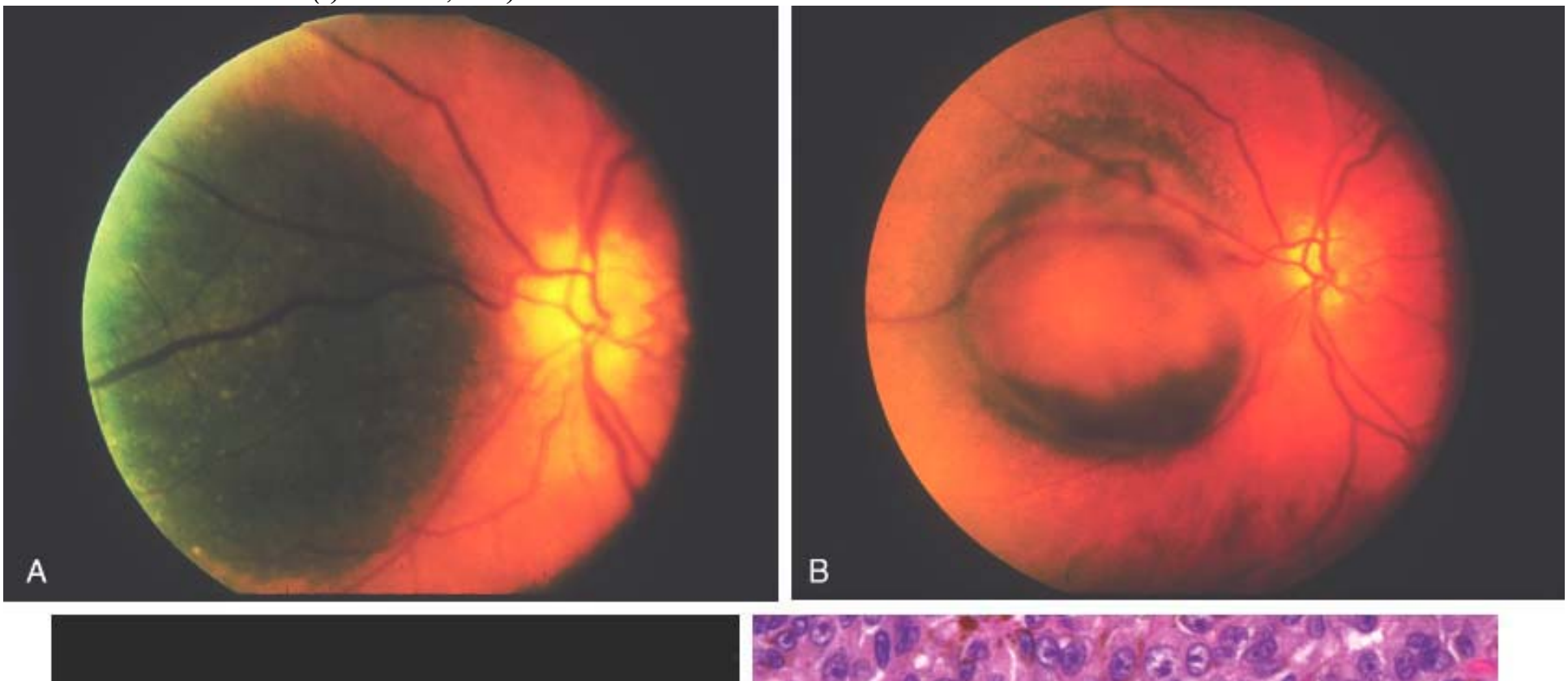
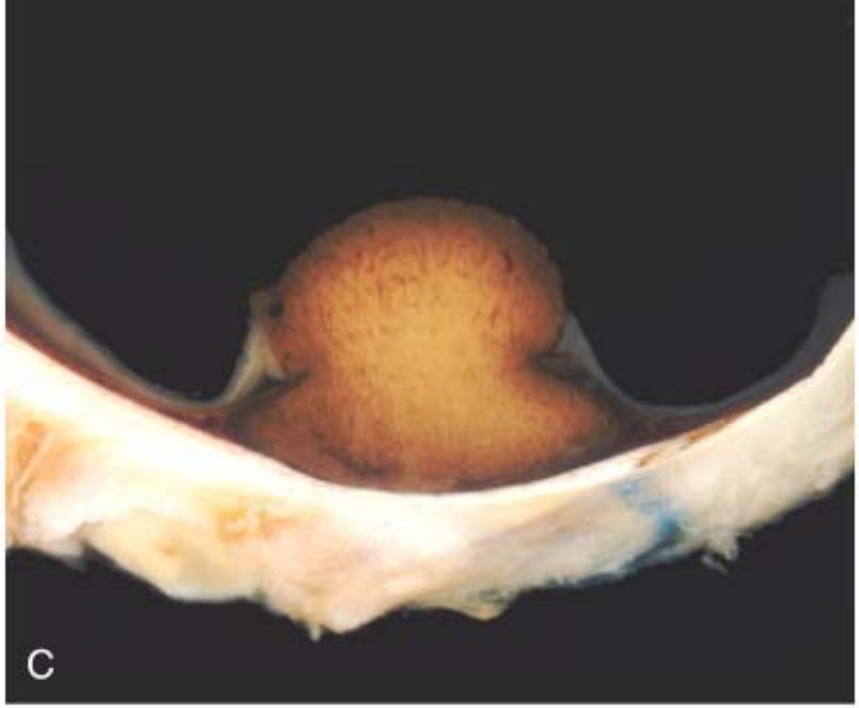


Figure 29-15 Uveal melanoma. *A*, Fundus photograph from a patient with a relatively flat pigmented lesion of the choroid near the optic disc. *B*, Fundus photograph of the same patient several years later; the tumor has grown and has ruptured through Bruch's membrane. *C*, Gross photograph of a choroidal melanoma that has ruptured Bruch's membrane. The overlying retina is detached. *D*, Epithelioid melanoma cells are associated with an adverse outcome. *E*, Patterns rich in laminin (that are periodic acid-Schiff positive) surround aggregates of melanoma cells; these patterns form a "fluid-conducting meshwork" in uveal melanoma and are associated with an adverse outcome. *(A to C from Folberg R: Pathology of the eye—an interactive CD-ROM program. Philadelphia, Mosby, 1996; E from Maniotis AJ, Chen X, Garcia C, et al: Control of melanoma morphogenesis, endothelial survival, and perfusion by extracellular matrix. Lab Invest 82(8):1031–1043, 2002.)*





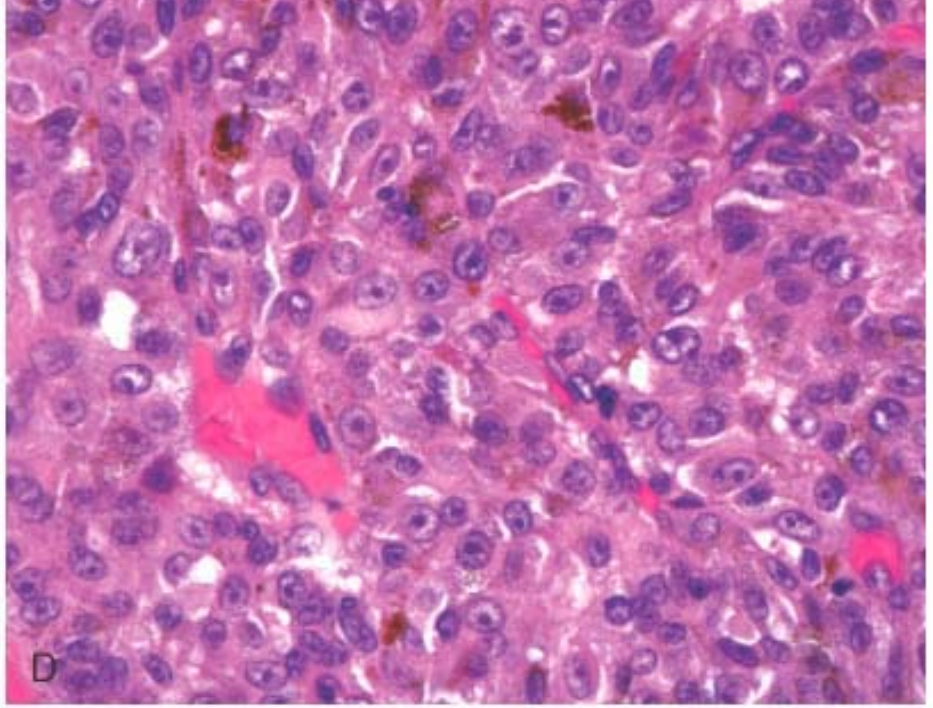


Figure 29-16 Clinicopathologic correlations of retinal hemorrhages and exudates. The location of the hemorrhage within the retina determines its appearance by ophthalmoscopy. The retinal nerve fiber layer is oriented parallel to the internal limiting membrane, and hemorrhages of this layer appear to be flame-shaped ophthalmoscopically. The deeper retinal layers

are oriented perpendicular to the internal limiting membrane and hemorrhages in this location appear as cross-sections of a cylinder or "dot" hemorrhages. Exudates that originate from leaky retinal vessels accumulate in the outer plexiform layer.

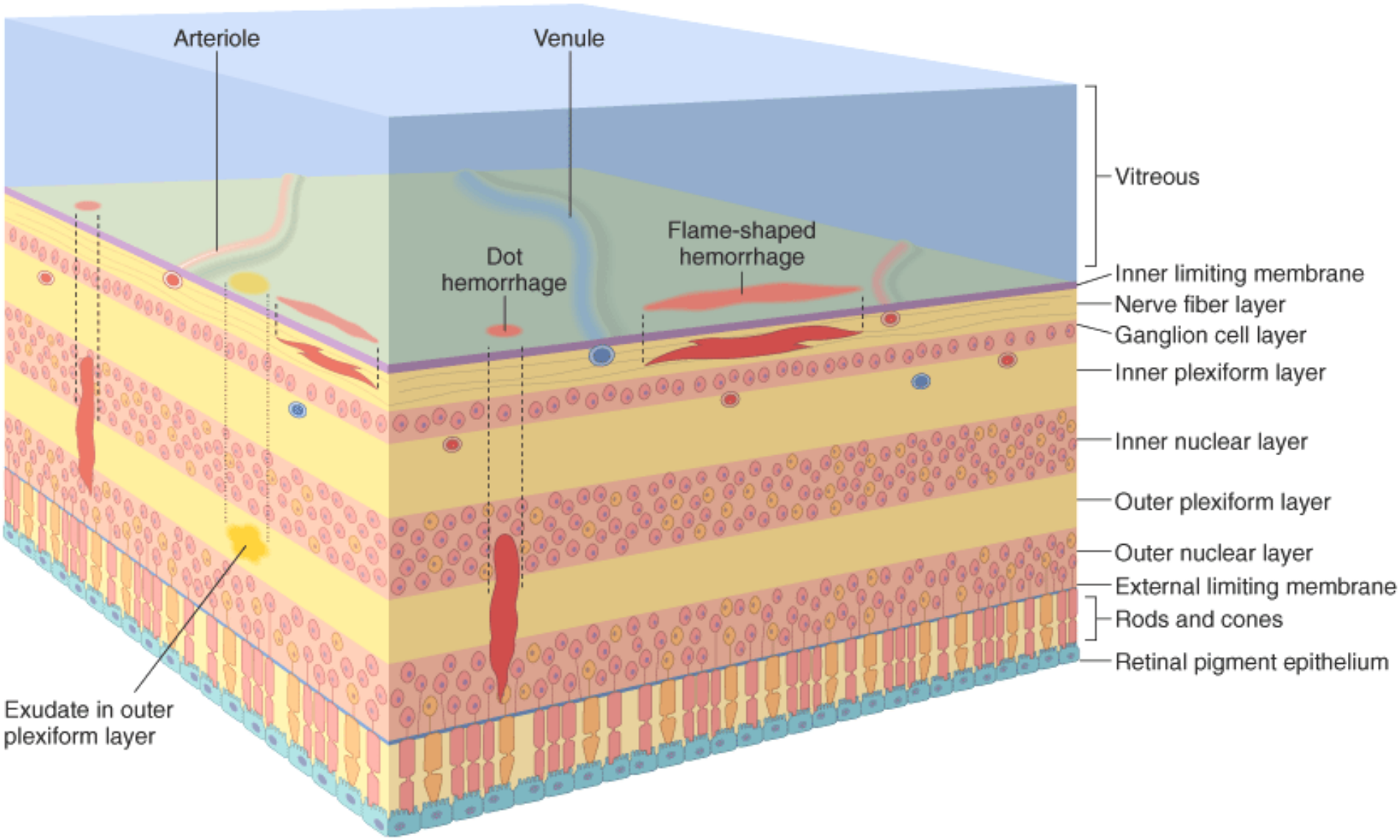
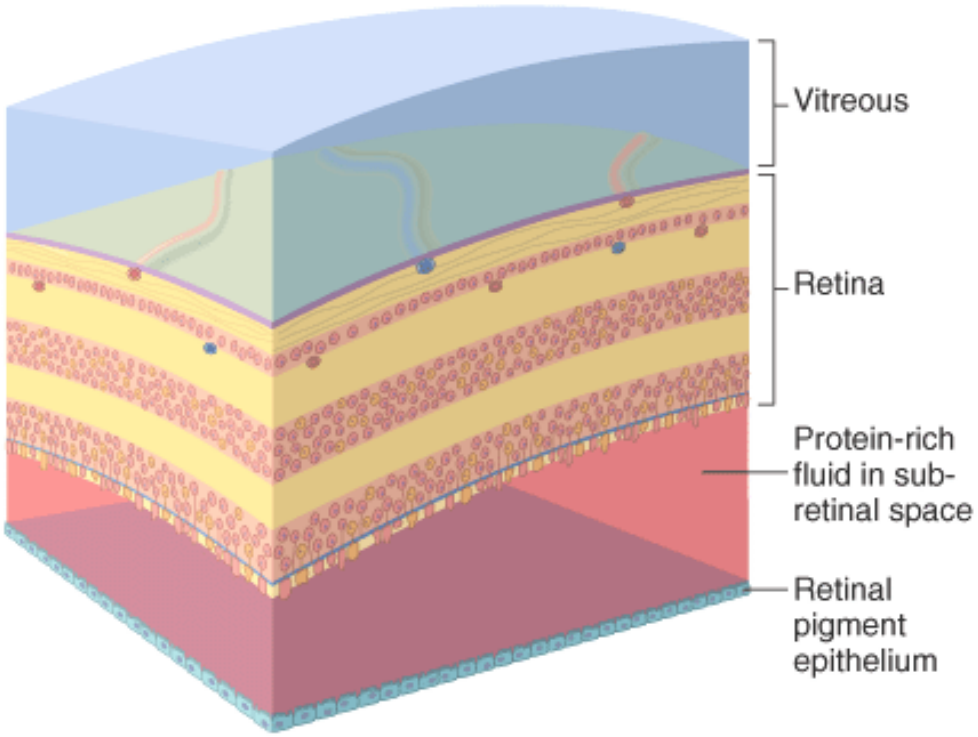


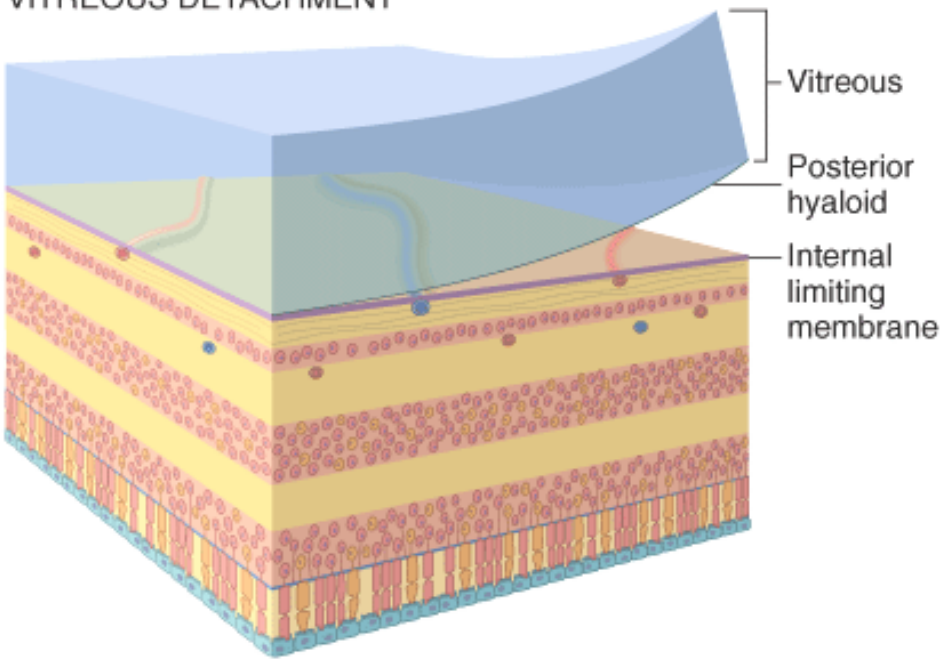
Figure 29-17 Retinal detachment is defined as the separation of the neurosensory retina from the retinal pigment epithelium. Retinal detachments are classified broadly into non-rhegmatogenous (without a retinal break) and rhegmatogenous (with a retinal break) types. *Top*, In non-rhegmatogenous retinal detachment, the subretinal space is filled with protein-rich exudate. Note in this sketch that the outer segments of the photoreceptors are missing. This indicates a chronic retinal detachment, a finding that can be seen in both non-rhegmatogenous and rhegmatogenous detachments. *Middle*, Posterior vitreous detachment involves the separation of the posterior hyaloid from the internal limiting membrane of the retina and is a normal occurrence in the aging eye. *Bottom*, If, during a posterior vitreous detachment, the posterior hyaloid does not separate cleanly from the internal limiting membrane of the retina, the vitreous humor will exert traction on the retina which will be torn at this point. Liquefied vitreous humor seeps through the retinal defect, and the retina is separated from the retinal pigment epithelium. Note in this sketch that the photoreceptor outer segments are intact, suggesting that an acute detachment is being illustrated.

NON-RHEGMATOGENOUS
RETINAL DETACHMENT

NON-RHEGMATOGENOUS
RETINAL DETACHMENT



VITREOUS DETACHMENT



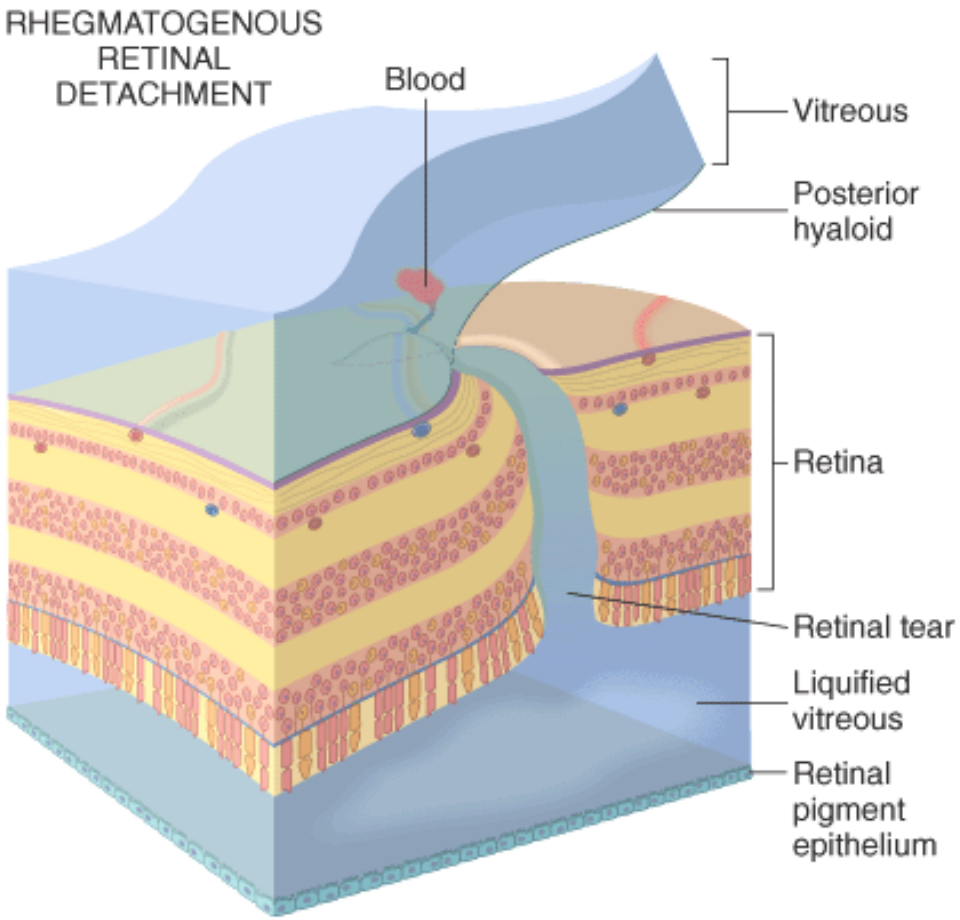


Figure 29-18 The retina in hypertension. *A*, The wall of the retinal arteriole *(arrow)* is thick. Note the exudate (e) in the retinal outer plexiform layer. *B*, The fundus in hypertension. The diameter of the arterioles is reduced, and the color of the blood column appears to be less saturated (copper wire-like). The retinal venule is compressed at a point where the artery and vein cross. If the wall of the vessel were thicker still, the degree of red color would diminish such that the vessels might appear clinically to have a "silver-wire" appearance. In this fundus photograph, note that the vein is compressed where the sclerotic arteriole crosses over it. *(B, courtesy of Dr. Thomas A. Weingeist, Department of Ophthalmology & Visual Science, University of Iowa, Iowa City, IA.)*

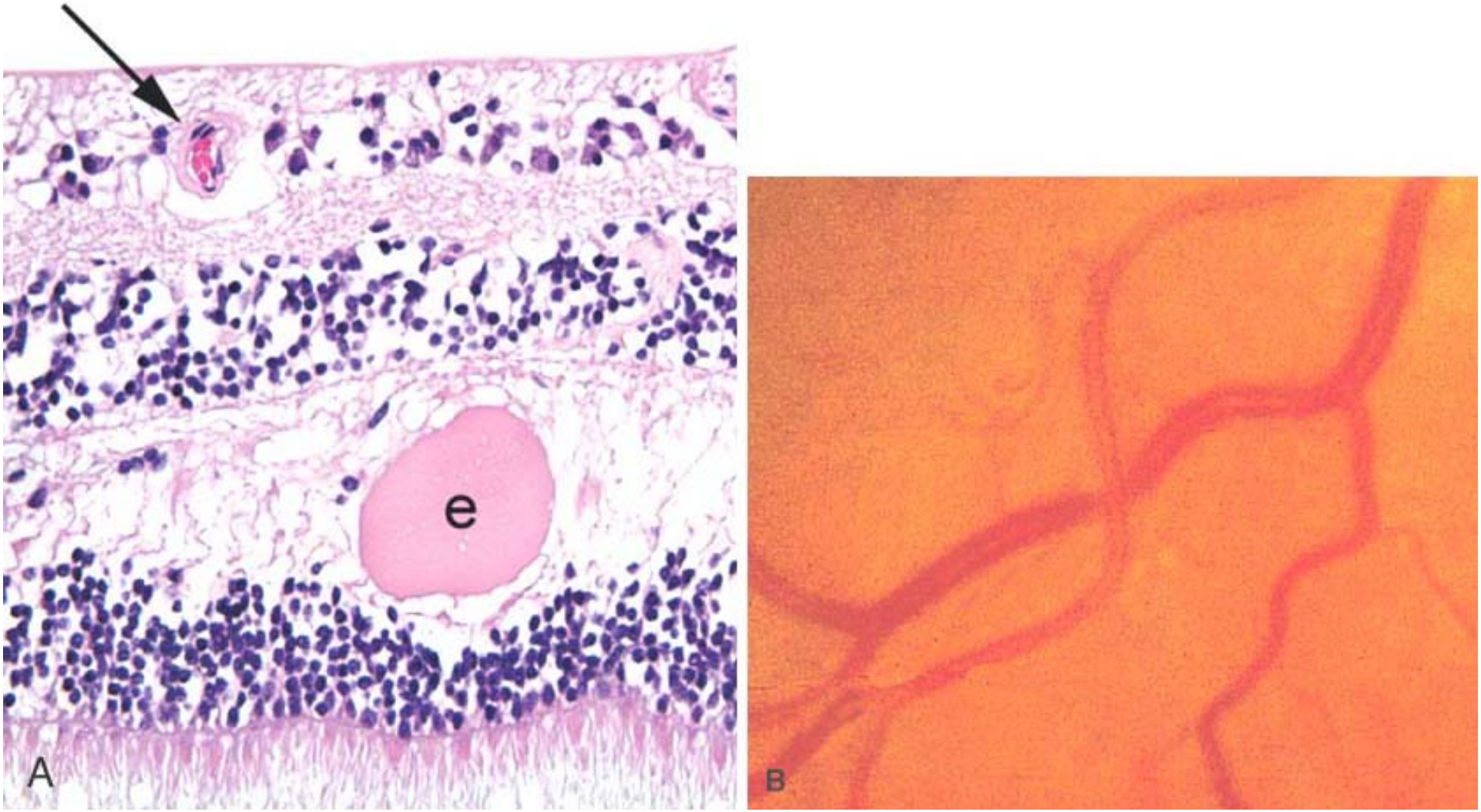


Figure 29-19 Nerve fiber layer infarct. A "cotton-wool spot" is illustrated in the inset, adjacent to a flame-shaped (nerve fiber layer) hemorrhage. The histology of a cotton-wool spot—an infarct of the nerve fiber layer of the retina—is illustrated in the photomicrograph. A focal swelling of the nerve fiber layer is occupied by numerous red to pink cytoid bodies *(arrowheads)*, bulbous ends of severed axons. Hemorrhage *(arrows)* surrounding the nerve fiber layer infarct as illustrated here is a variable and inconsistent finding. *(The fundus photo courtesy of Dr. Thomas A. Weingeist, Department of Ophthalmology & Visual Science, University of Iowa, Iowa City, IA.)*

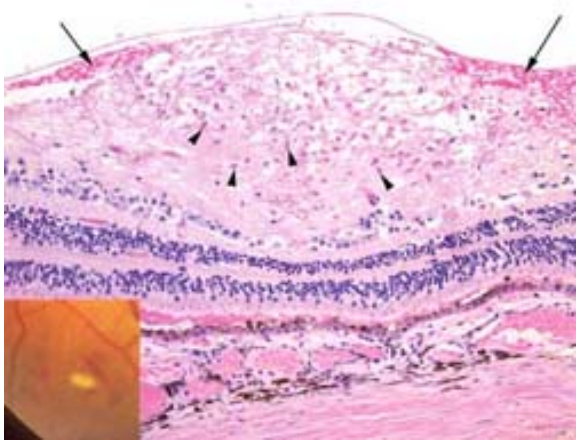


Figure 29-20 The ciliary body in chronic diabetes mellitus, periodic acid-Schiff stain. Note the massive thickening of the basement membrane of the ciliary body epithelia, reminiscent of changes in the mesangium of the renal glomerulus.

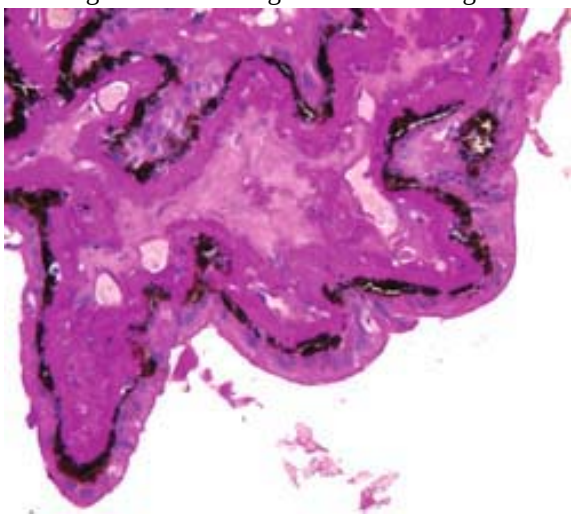


Figure 29-21 The retina in diabetes mellitus. *A*, Note the tangle of abnormal vessels just beneath the internal limiting membrane of the retina on the right half of the photomicrograph *(between arrows)*. This is an example of intraretinal angiogenesis known as intraretinal microangiopathy (IRMA). Note the retinal hemorrhage in the outer plexiform layer in the left half of this photomicrograph. Ophthalmoscopically, this outer retinal layer hemorrhage would appear as a "dot" hemorrhage. Finally, note that there are only two well-defined layers of retinal nuclei: the outer nuclear layer and the inner nuclear layer. The ganglion cell layer is absent, and the nerve fiber layer—the axons of the ganglion cells—is also absent. The rarefied space beneath internal limiting membrane to the left of the focus of IRMA consists largely of elements of retinal glial (Müller) cells. Absence of the ganglion cell and nerve fiber layers is a hallmark of glaucoma. The chronic diabetes mellitus in this patient was complicated by the development of iris neovascularization and secondary angle closure glaucoma (neovascular glaucoma). *B*, In this histologic section, stained by periodic acid-Schiff, the internal limiting membrane is noted by the long, thick arrows and the posterior hyaloid of the vitreous by the thin, short arrows. Note the vessels in the potential space between these two landmarks. The vessels to the left of the short arrow are invested with a fibrous-glial stroma and would appear ophthalmoscopically as a white neovascular membrane. However, the thin-walled vessel to the right of the short arrow is not invested with a connective tissue stroma and would appear ophthalmoscopically as merely a very thin vessel. A posterior vitreous detachment in an eye such as this might exert traction on these new vessels and precipitate a massive vitreous hemorrhage. *C*, Ophthalmoscopic view of retinal neovascularization (known clinically as neovascularization "elsewhere" in contrast with neovascularization of the optic disc). Note the blush of thin-walled vessels that are not accompanied by a fibrous stroma (analogous to the thin walled vessel in *B*). Even without a connective tissue stroma, this qualifies clinically as a neovascular membrane and is reminiscent of the appearance of experimental angiogenesis on a chick chorioallantoic membrane.

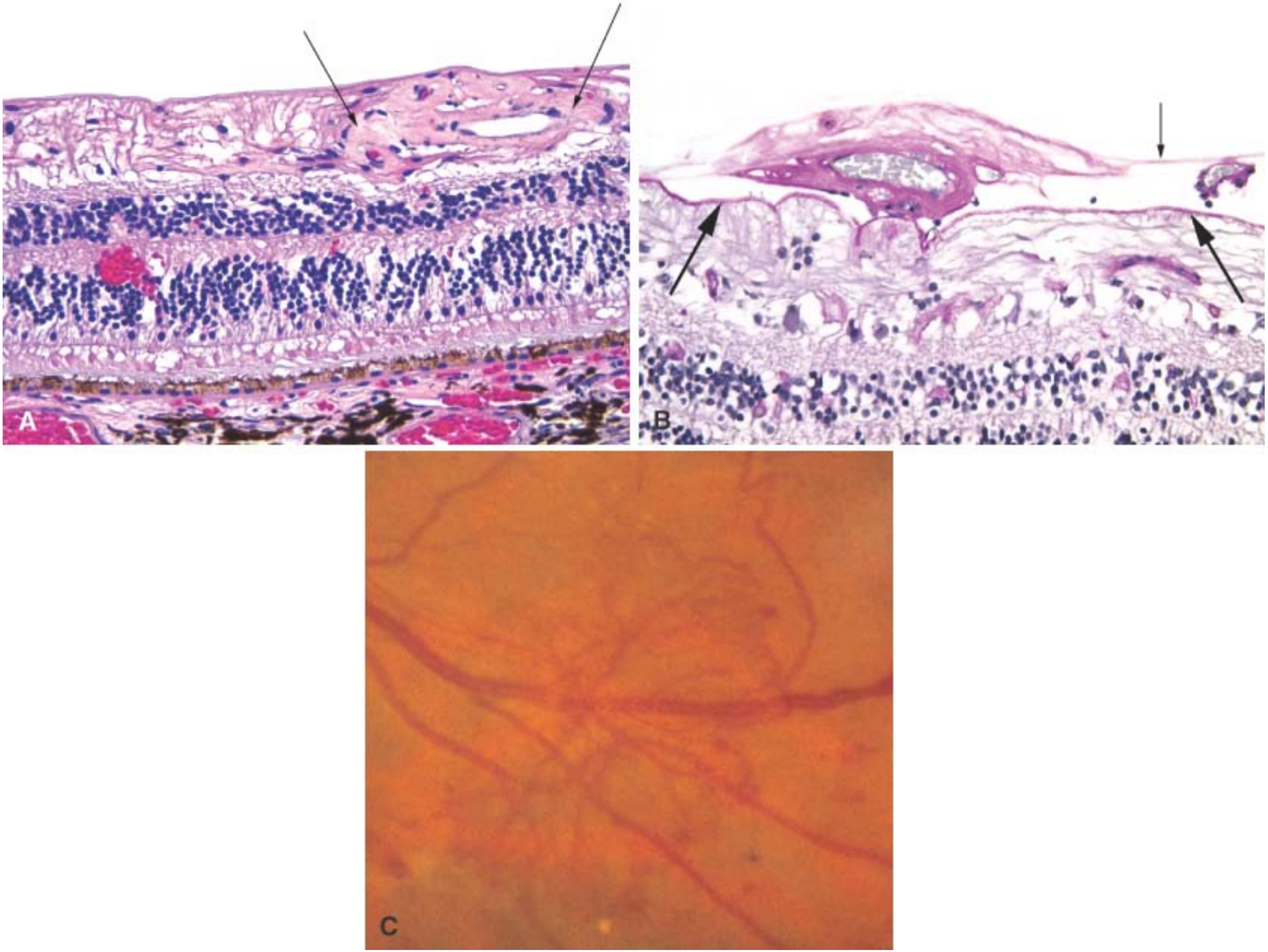


Figure 29-22 The cherry-red spot in Tay-Sachs disease. *A*, Fundus photograph of the cherry-red spot in Tay-Sachs disease. *B*, Photomicrograph of the macula in a patient with Tay-

Sachs disease, stained with periodic acid-Schiff to highlight the accumulation of ganglioside material in the retinal ganglion cells. The presence of ganglion cells filled with gangliosides outside the fovea blocks the transmission of the normal orange-red color of the choroid, but absence of ganglion cells within the fovea (to the right of the vertical bar) permits the normal orange-red color to be visualized, accounting for the so-called cherry-red spot. *(A courtesy of Dr. Thomas A. Weingeist, Department of Ophthalmology & Visual Science, University of lowa, lowa City, IA; B originates from the teaching collection of the Armed Forces Institute of Pathology.)*

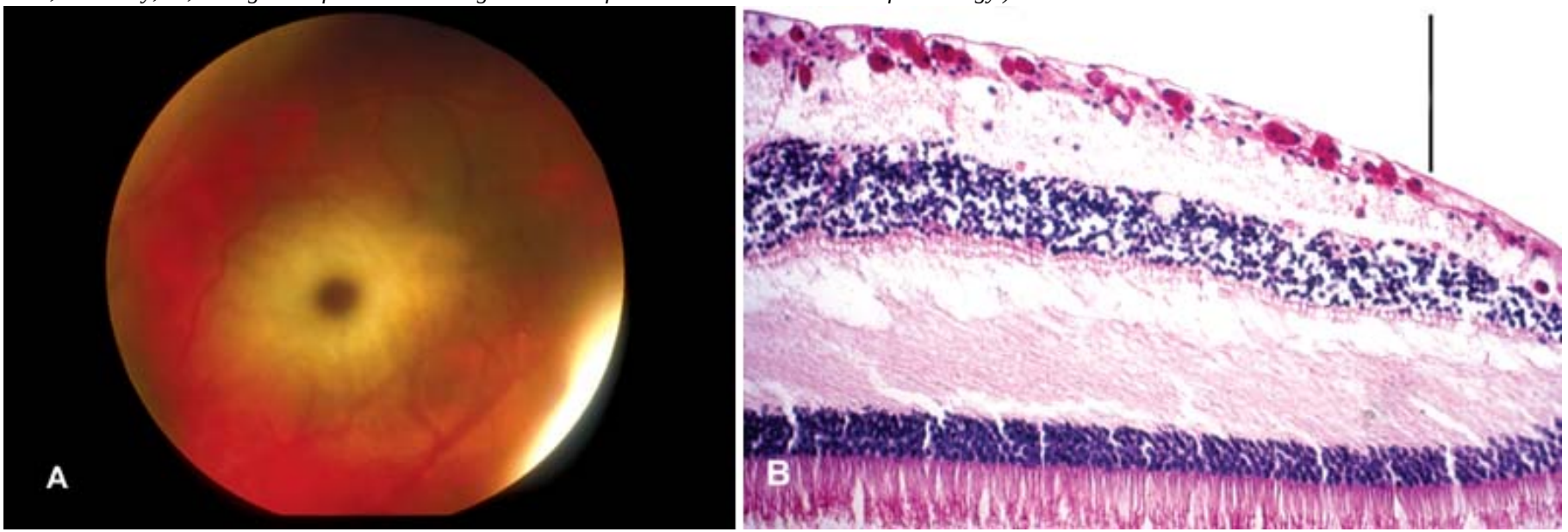


Figure 29-23 Age-related macular degeneration. A neovascular membrane is positioned between the retinal pigment epithelium (RPE) and Bruch's membrane (BM). Note the blue discoloration of Bruch's membrane to the right of the label, indicating focal calcification.

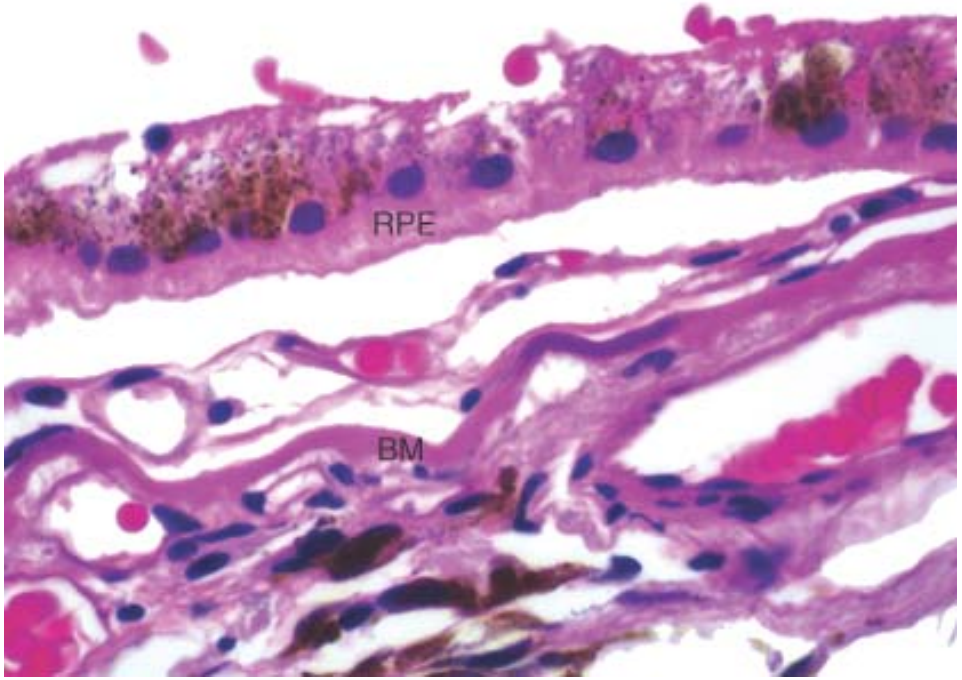


Figure 29-24 Retinoblastoma. *A*, Gross photograph of retinoblastoma. *B*, Tumor cells appear viable when in proximity to blood vessels, but necrosis is seen as the distance from the vessel increases. Dystrophic calcification *(dark arrow)* is present in the zones of tumor necrosis. Flexner-Wintersteiner rosettes—arrangements of a single layer of tumor cells around an apparent "lumen"—are seen throughout the tumor, and one such rosette is indicated by the white arrow.

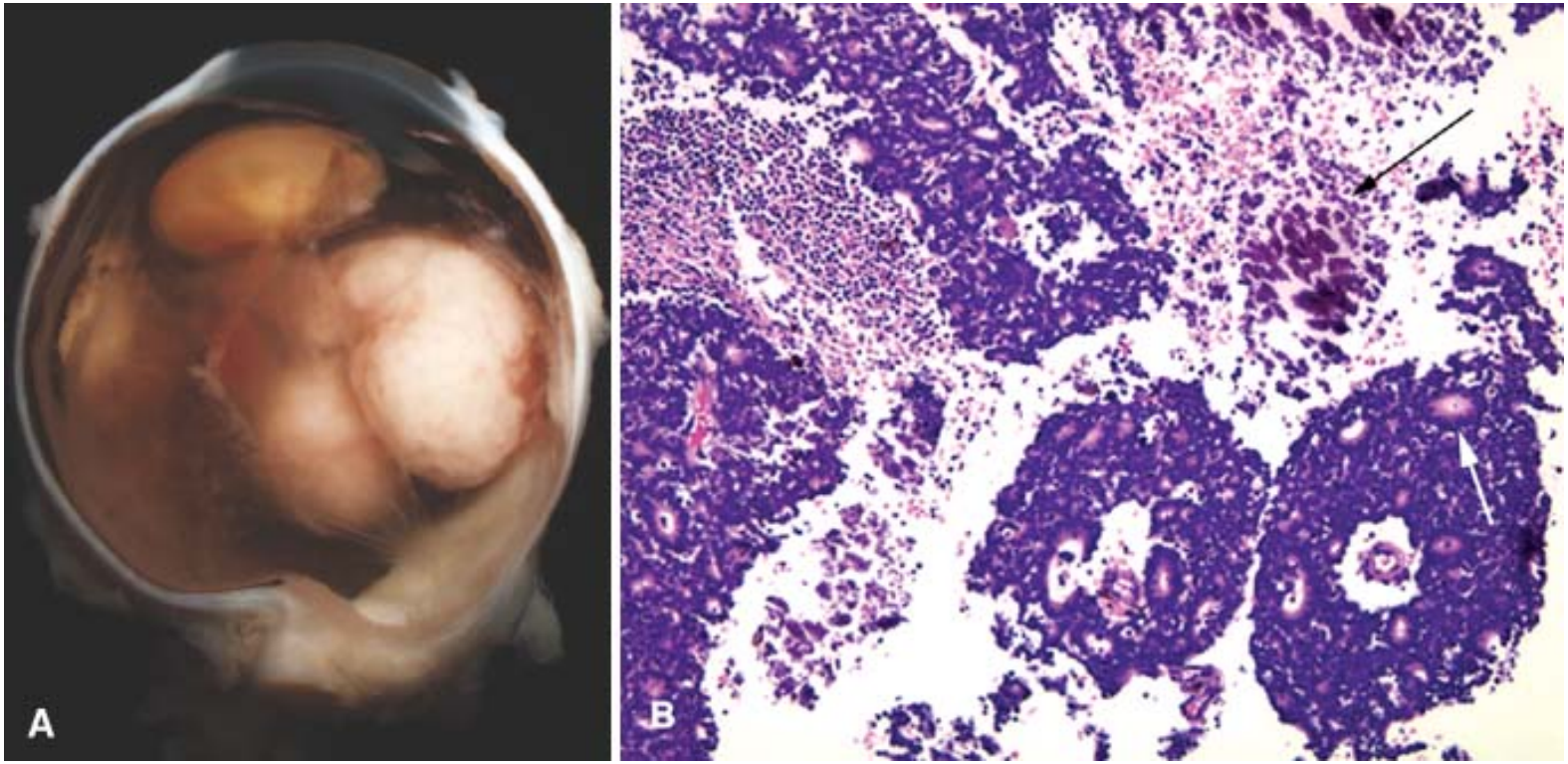


Figure 29-25 The optic nerve in anterior ischemic optic neuropathy (AION) and papilledema. *A*, In the relatively acute phases of AION, the optic nerve may be swollen, but it is relatively pale because of decreased perfusion. *B*, In papilledema secondary to increased intracranial pressure, the optic nerve is typically swollen and hyperemic. *C*, Normally, the termination of Bruch's membrane *(arrowhead)* is aligned with the beginning of the neurosensory retina, as indicated by the presence of stratified nuclei *(arrow)*, but in papilledema, the optic nerve is swollen, and the retina is displaced laterally. This is the histologic explanation for the blurred margins of the optic nerve head seen clinically in this condition. *(A and B courtesy of Dr. Sohan S. Hayreh, Department of Ophthalmology & Visual Science, University of lowa, lowa City, IA; C originates from the teaching collection of the Armed Forces Institute of Pathology.)*

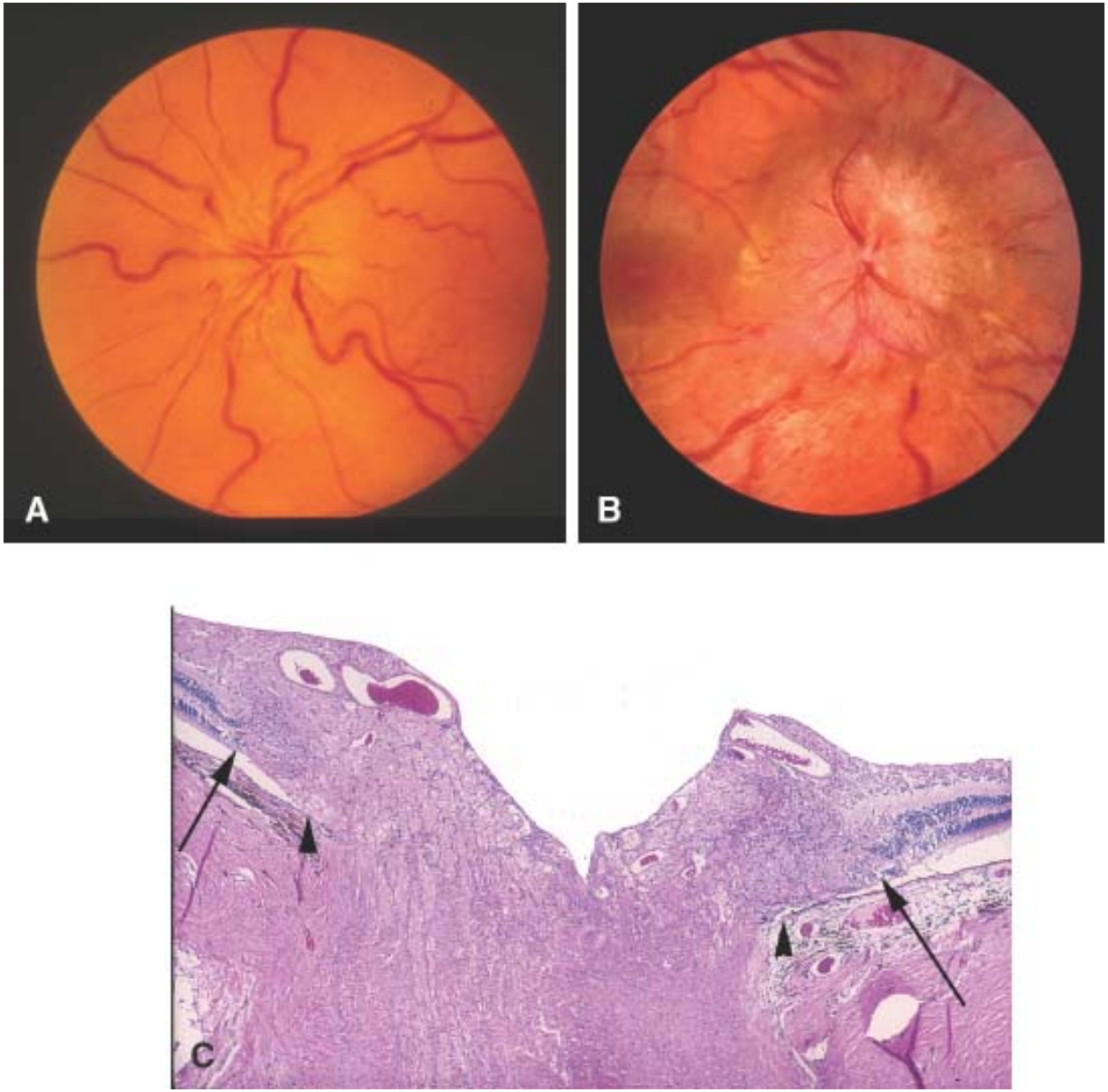
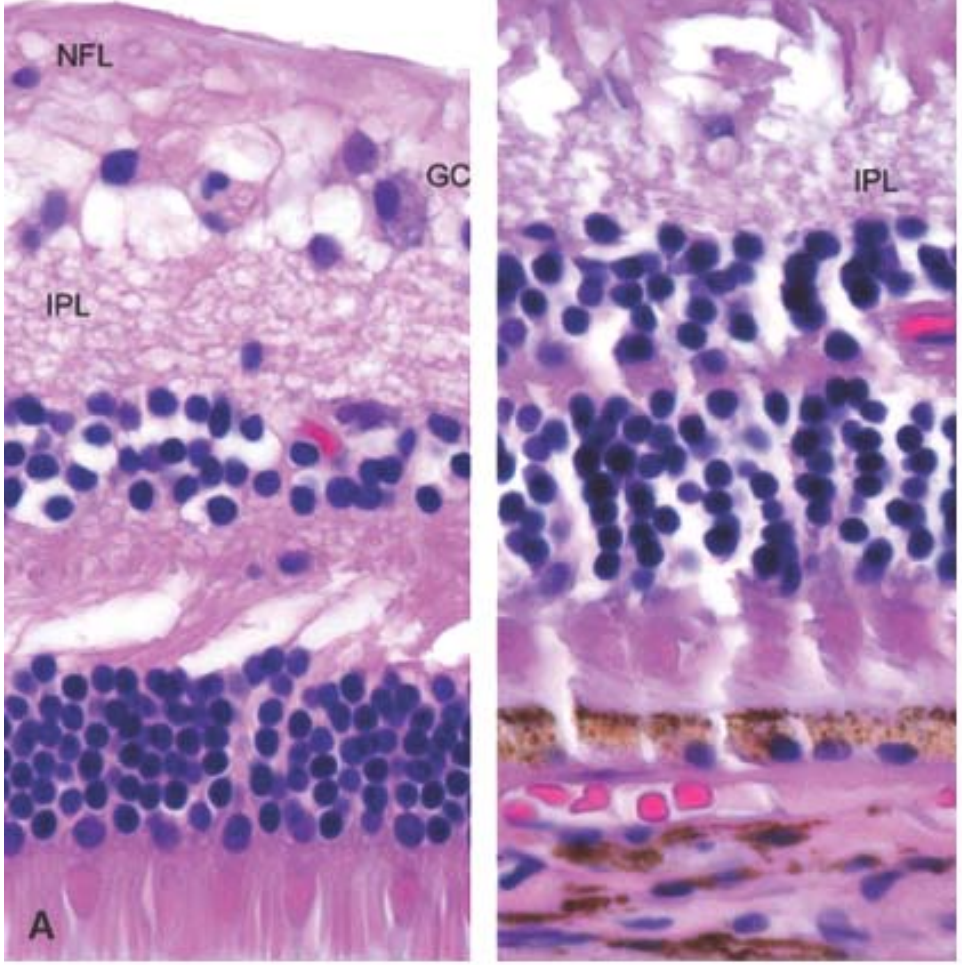


Figure 29-26 The retina and optic nerve in glaucoma. *A*, The normal retina is illustrated in the left panel, and the retina in long-standing glaucoma is in the right panel. Both pictures were taken at the same magnification. Note that the full thickness of the glaucomatous retina is captured (right), whereas only a portion of the normal retina (left) can be seen—a reflection of the thinning of the retina in glaucoma. In the glaucomatous retina, the areas corresponding to the nerve fiber layer (NFL) and ganglion cell layer (GC) are atrophic; the inner plexiform layer (IPL) is labeled for a point of reference. *B*, Glaucomatous optic nerve cupping results, in part, from loss of retinal ganglion cells, the axons of which populate the optic nerve. *C*, The arrows point to the dura of the optic nerve. Notice the wide subdural space, a result of atrophy of the substance of the optic nerve. The degree of cupping on the surface of the nerve is striking in this eye, which was removed because of complications of long-standing glaucoma.


NFL
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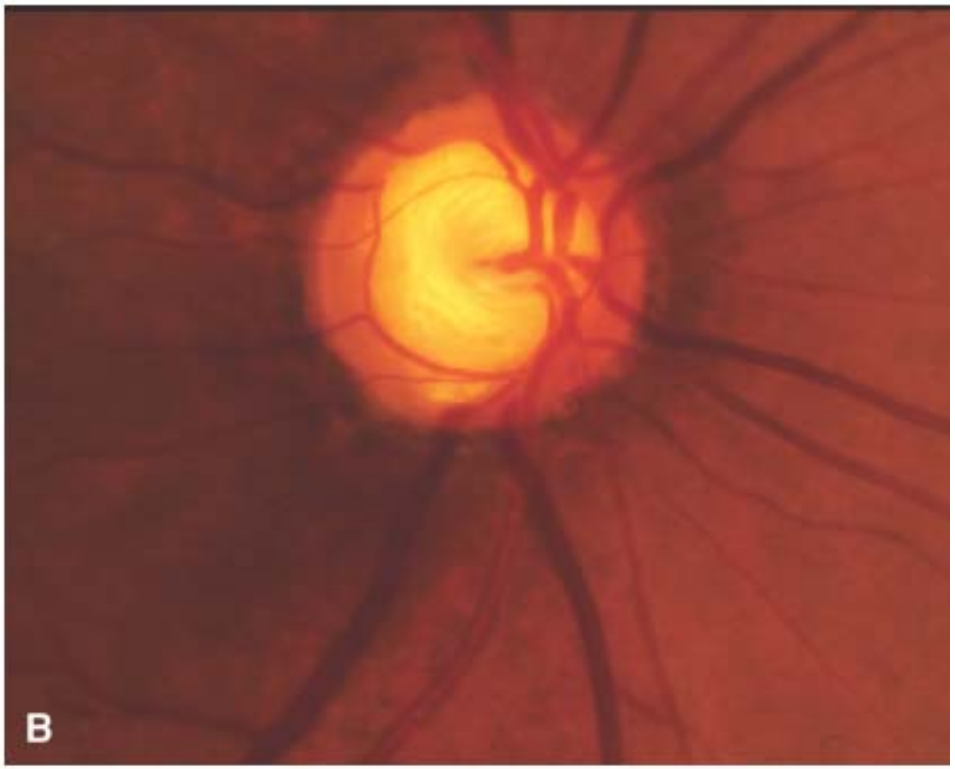
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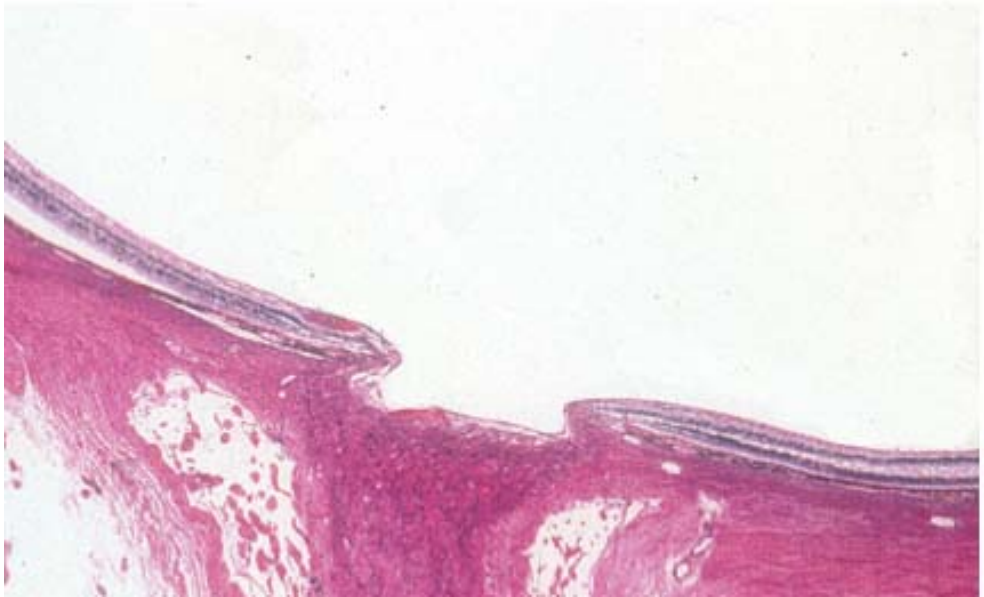


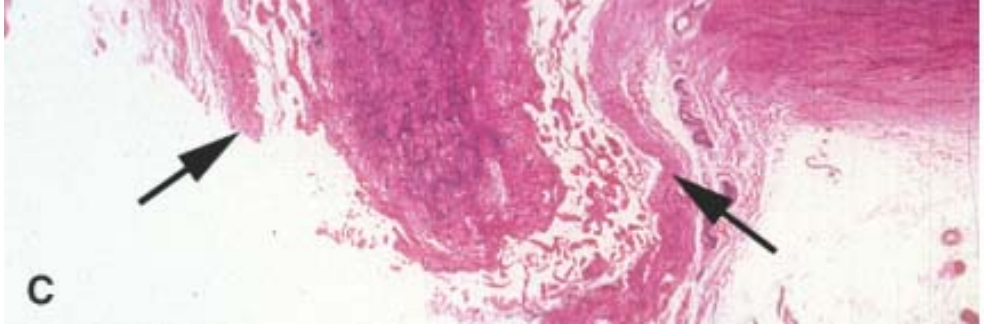
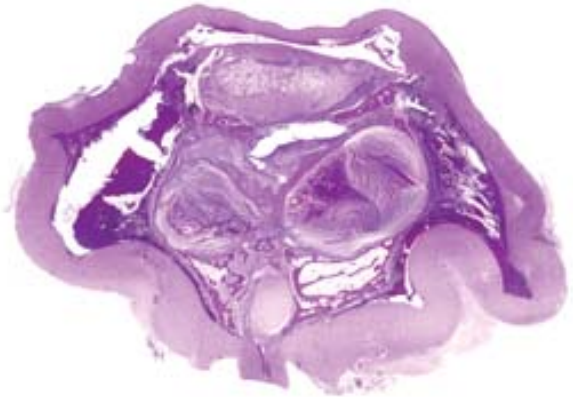


Figure 29-27 Phthisis bulbi. The eye is small and internally disorganized. The tension exerted on this hypotonic eye by the extraocular muscles contributes to a square rather than round shape. Note the atrophic optic nerve and the presence of intraocular bone posteriorly and anteriorly.



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