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Robbins
BASIC PATHOLOGY

NINTH EDITION

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ROBBINS

Basic Pathology

NINTH EDITION

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DEDICATION

To
Our children and a special grandchild
Kiera Chapman Kumar

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FORTY YEARS OF BASIC PATHOLOGY

As we reach the 40th year of the publication of *Robbins Basic Pathology,* it is useful to quote Stanley Robbins from the Preface of the first edition (1971):

"Of books as well as men, it may be observed that fat ones contain thin ones struggling to get out. In a sense, this book bears such a relationship to its more substantial progenitor, *Robbins Pathology*. It arose from an appreciation of the modern medical student's dilemma. As the curriculum has become restructured to place greater emphasis on clinical experience, time for reading is correspondingly curtailed. … In writing this book, rare and esoteric lesions are omitted without apology, and infrequent or trivial ones described only briefly. We felt it important, however, to consider rather fully the major disease entities."

The goals of this edition of "baby Robbins" remain true to this vision of Stanley Robbins.

This is an exciting time for students of medicine because the fundamental mechanisms of disease are being unveiled at a breathtaking pace. Pathology is central to understanding the molecular basis of disease, and we have tried to capture the essence of this new knowledge in the ninth edition of *Robbins Basic Pathology*. We firmly believe that pathology forms the scientific foundation of medicine, and advances in the basic sciences ultimately help us in understanding diseases in the individual patient. Thus, while many of the new discoveries in genomics and personalized medicine are covered in the initial chapters on general pathology, we have strived to include the impact of scientific advances on diseases of organ systems described throughout the text. To emphasize the importance of disease mechanisms in the practice of medicine, we have highlighted sections dealing with pathogenesis. In recent years an understanding of the molecular basis of disease has led to the development of "targeted therapies." These are highlighted in the form of "Targeted Therapy" boxes in the online edition of this book. We hope that this new feature will provide examples of "bench-to-bedside" medicine. Although many of the "breakthroughs" in the laboratory have not yet reached the bedside, we have included them in measured "doses" so that students can begin to experience the excitement that is ahead in their careers.

Realizing that the modern medical student feels inundated in trying to synthesize the essentials with the "state of the art," we have continued the use of Summary boxes designed to provide the students with key "take home" messages. These have been retained at the risk of adding a few additional pages to the book since students have uniformly told us that they find them useful.

Many new pieces of four-color art—schematics, flow charts, and diagrammatic representations of disease—have been added to facilitate the understanding of difficult concepts such as the control of the cell cycle, functions of cancer genes, interactions of HIV with its receptors, and the biochemical basis of apoptotic cell death. More illustrations have been added, bringing the total to more than 1,000. Formatting and color palettes of the tables have been changed for greater clarity.

Despite the extensive changes and revisions, our goals remain essentially unaltered. Although we have entered the genomic era, the time-honored tools of gross and microscopic analysis remain useful and morphologic changes are highlighted for ready reference. The strong emphasis on clinicopathologic correlations is maintained, and wherever understood, the impact of molecular pathology on the practice of medicine is emphasized. We are pleased that all of this was accomplished without any "bulge" in the waistline of the text.

We continue to firmly believe that clarity of writing and proper use of language enhance comprehension and facilitate the learning process. Generations of students have told us that they enjoy reading this book. We hope that this edition will be worthy of and possibly enhance the tradition of its forebears.

Acknowledgments

Contents

Chapters without author names were written by the editors.

Cell Injury, Cell Death, and Adaptations

INTRODUCTION TO PATHOLOGY

Literally translated, *pathology* is the study (*logos*) of disease (*pathos,* suffering). It involves the investigation of the causes of disease and the associated changes at the levels of cells, tissues, and organs, which in turn give rise to the presenting signs and symptoms of the patient. There are two important terms that students will encounter throughout their study of pathology and medicine:

- *Etiology* is the origin of a disease, including the underlying causes and modifying factors. It is now clear that most common diseases, such as hypertension, diabetes, and cancer, are caused by a combination of inherited genetic susceptibility and various environmental triggers. Understanding the genetic and environmental factors underlying diseases is a major theme of modern medicine.
- *Pathogenesis* refers to the steps in the development of disease. It describes how etiologic factors trigger cellular and molecular changes that give rise to the specific functional and structural abnormalities that characterize the disease. Whereas etiology refers to *why* a disease arises, pathogenesis describes *how* a disease develops.

Defining the etiology and pathogenesis of disease not only is essential for understanding a disease but is also the basis for developing rational treatments. Thus, by explaining the causes and development of disease *pathology provides the scientific foundation for the practice of medicine*.

To render diagnoses and guide therapy in clinical practice, pathologists identify changes in the gross or microscopic appearance (*morphology*) of cells and tissues, and biochemical alterations in body fluids (such as blood and urine). Pathologists also use a variety of morphologic, molecular, microbiologic, and immunologic techniques to define the biochemical, structural, and functional changes that occur in cells, tissues, and organs in response to injury. Traditionally, the discipline is divided into general pathology and systemic pathology; the former focuses on the cellular and tissue alterations caused by pathologic stimuli in most tissues, while the latter examines the reactions and abnormalities of different specialized organs. In this book we first cover the broad principles of general pathology and then progress to specific disease processes in individual organs.

OVERVIEW OF CELLULAR RESPONSES TO STRESS AND NOXIOUS STIMULI

Cells are active participants in their environment, constantly adjusting their structure and function to accommodate changing demands and extracellular stresses. Cells normally maintain a steady state called *homeostasis* in which the intracellular milieu is kept within a fairly narrow range of physiologic parameters. As cells encounter physiologic stresses or pathologic stimuli, they can undergo

adaptation, achieving a new steady state and preserving viability and function. The principal adaptive responses are *hypertrophy, hyperplasia, atrophy,* and *metaplasia.* If the adaptive capability is exceeded or if the external stress is inherently harmful, *cell injury* develops (Fig. 1–1). Within certain limits, injury is *reversible,* and cells return to a stable baseline; however, if the stress is severe, persistent and rapid in onset, it results in *irreversible injury* and death of the affected cells. *Cell death* is one of the most crucial events in the evolution of disease in any tissue or organ. It results from diverse causes, including ischemia (lack of blood flow), infections, toxins, and immune reactions. Cell death also is a normal and essential process in embryogenesis, the development of organs, and the maintenance of homeostasis.

The relationships among normal, adapted, and reversibly and irreversibly injured cells are well illustrated by the responses of the heart to different types of stress (Fig. 1–2). Myocardium subjected to persistent increased load, as in hypertension or with a narrowed (stenotic) valve, adapts by undergoing *hypertrophy*—an increase in the size of the individual cells and ultimately the entire heart—to generate the required higher contractile force. If the increased demand is not relieved, or if the myocardium is subjected to reduced blood flow (*ischemia*) from an occluded coronary artery, the muscle cells may undergo injury. Myocardium may be reversibly injured if the stress is mild or the arterial occlusion is incomplete or sufficiently brief, or it may undergo irreversible injury and cell death (*infarction*) after complete or prolonged occlusion. Also of note, stresses
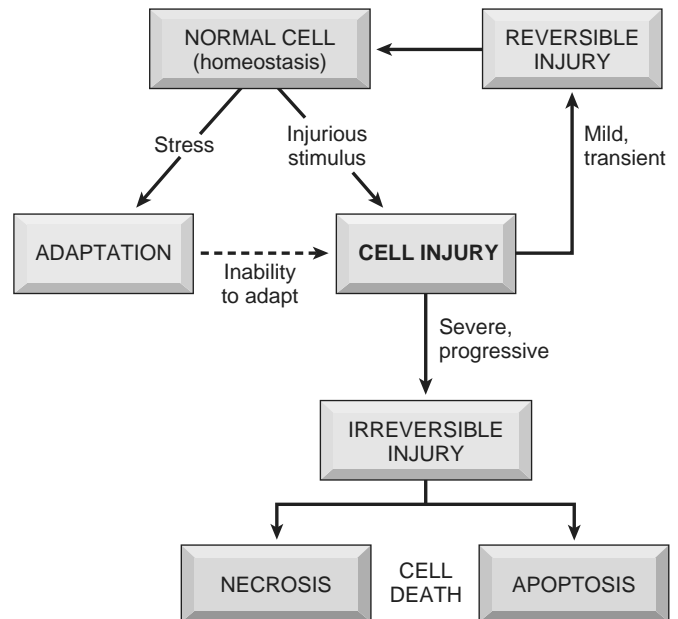
Figure 1–1 Stages in the cellular response to stress and injurious stimuli.

and injury affect not only the morphology but also the functional status of cells and tissues. Thus, reversibly injured myocytes are not dead and may resemble normal myocytes morphologically; however, they are transiently noncontractile, so even mild injury can have a significant
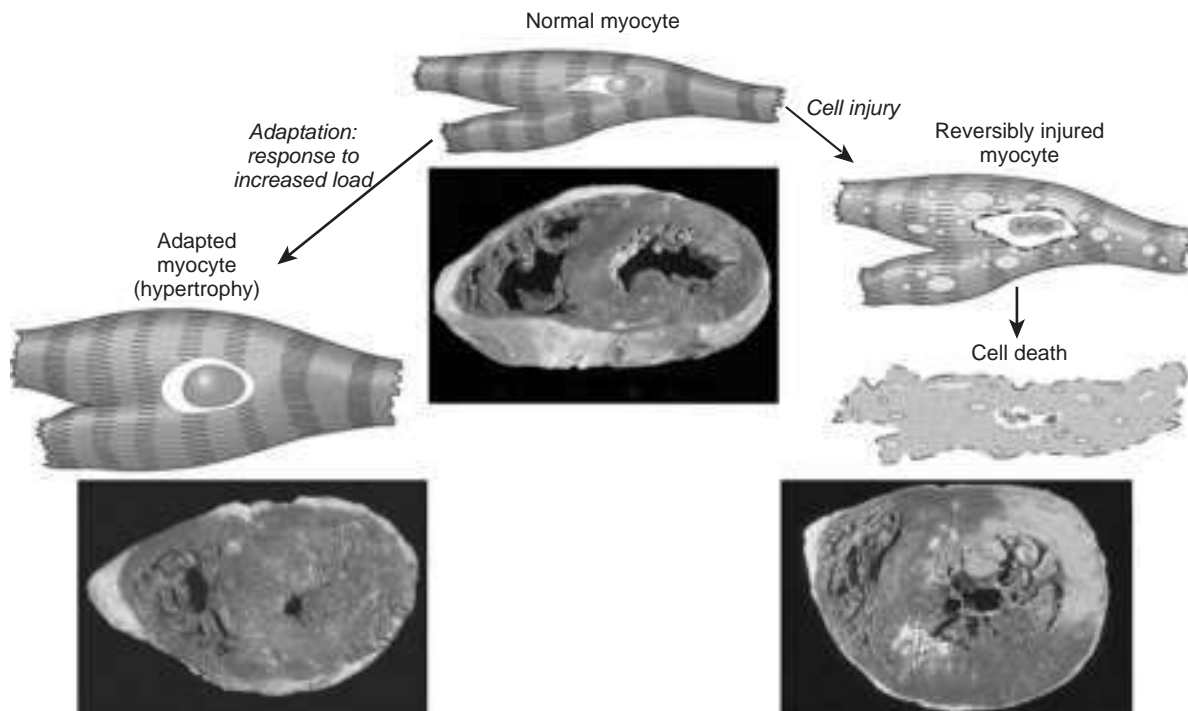
Figure 1–2 The relationship among normal, adapted, reversibly injured, and dead myocardial cells. The cellular adaptation depicted here is hypertrophy, the type of reversible injury is ischemia, and the irreversible injury is ischemic coagulative necrosis. In the example of myocardial hypertrophy (*lower left*), the left ventricular wall is thicker than 2 cm (normal, 1–1.5 cm). Reversibly injured myocardium shows functional effects without any gross or light microscopic changes, or reversible changes like cellular swelling and fatty change (*shown here*). In the specimen showing necrosis (*lower right*) the transmural light area in the posterolateral left ventricle represents an acute myocardial infarction. All three transverse sections of myocardium have been stained with triphenyltetrazolium chloride, an enzyme substrate that colors viable myocardium magenta. Failure to stain is due to enzyme loss after cell death.

clinical impact. Whether a specific form of stress induces adaptation or causes reversible or irreversible injury depends not only on the nature and severity of the stress but also on several other variables, including basal cellular metabolism and blood and nutrient supply.

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 reversible cell injury, subcellular alterations, and cell death. We conclude with three other processes that affect cells and tissues: intracellular accumulations, pathologic calcification, and cell aging.

CELLULAR ADAPTATIONS TO STRESS

Adaptations are reversible changes in the number, size, phenotype, metabolic activity, or functions of cells in response to changes in their environment. *Physiologic adaptations* usually represent responses of cells to normal stimulation by hormones or endogenous chemical mediators (e.g., the hormone-induced enlargement of the breast and uterus during pregnancy). *Pathologic adaptations* are responses to stress that allow cells to modulate their structure and function and thus escape injury. Such adaptations can take several distinct forms.

Hypertrophy

Hypertrophy is an increase in the size of cells resulting in increase in the size of the organ. In contrast, hyperplasia (discussed next) is characterized by an increase in cell number because of proliferation of differentiated cells and replacement by tissue stem cells. Stated another way, in pure hypertrophy there are no new cells, just bigger cells containing increased amounts of structural proteins and organelles. Hyperplasia is an adaptive response in cells capable of replication, whereas hypertrophy occurs when cells have a limited capacity to divide. Hypertrophy and hyperplasia also can occur together, and obviously both result in an enlarged (*hypertrophic*) organ.

Hypertrophy can be physiologic or pathologic and is caused either by increased functional demand or by growth factor or hormonal stimulation.

- The massive physiologic enlargement of the uterus during pregnancy occurs as a consequence of estrogen-stimulated smooth muscle hypertrophy and smooth muscle hyperplasia (Fig. 1–3). In contrast, in response to increased demand the striated muscle cells in both the skeletal muscle and the heart can undergo only hypertrophy because adult muscle cells have a limited capacity to divide. Therefore, the chiseled physique of the avid weightlifter stems solely from the hypertrophy of individual skeletal muscles.
- An example of pathologic cellular hypertrophy is the cardiac enlargement that occurs with hypertension or aortic valve disease (Fig. 1–2).

The mechanisms driving cardiac hypertrophy involve at least two types of signals: *mechanical triggers,* such as stretch, and *trophic triggers,* which typically are soluble mediators that stimulate cell growth, such as growth factors and adrenergic hormones. These stimuli turn on signal transduction pathways that lead to the induction of a number of genes, which in turn stimulate synthesis of many cellular proteins, including growth factors and structural proteins. The result is the synthesis of more proteins and myofilaments per cell, which increases the force generated with each contraction, enabling the cell to meet increased work demands. There may also be a switch of contractile proteins from adult to fetal or neonatal forms. For example, during muscle hypertrophy, the α-myosin heavy chain is replaced by the β form of the myosin heavy chain, which produces slower, more energetically economical contraction.

Whatever the exact mechanisms of hypertrophy, a limit is reached beyond which the enlargement of muscle mass
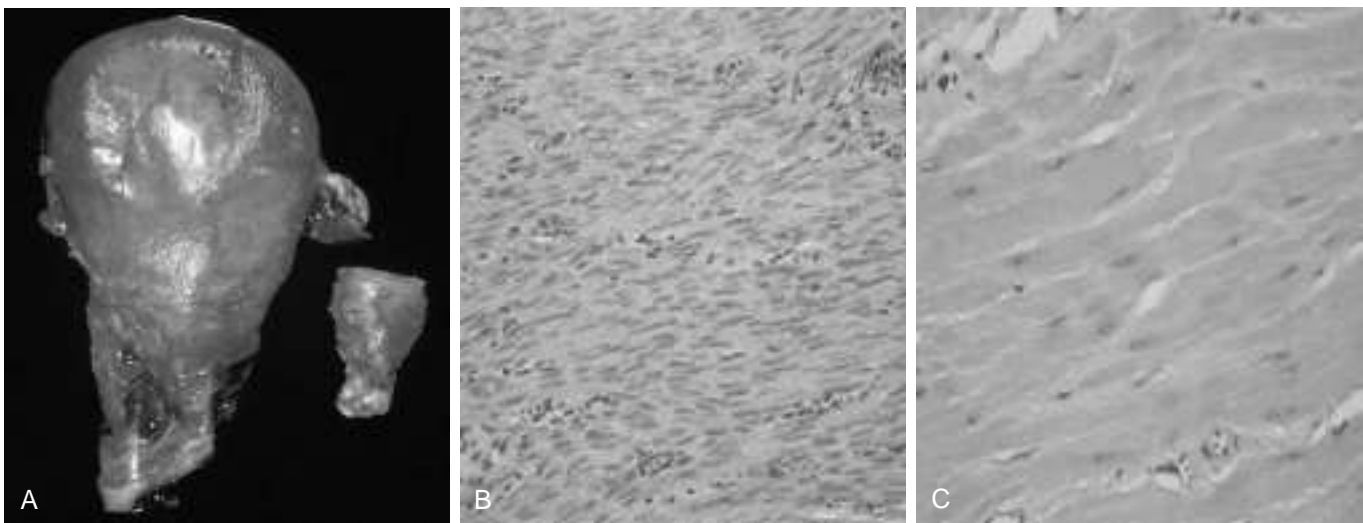
Figure 1–3 Physiologic hypertrophy of the uterus during pregnancy. **A,** Gross appearance of a normal uterus (*right*) and a gravid uterus (*left*) that was removed for postpartum bleeding. **B,** Small spindle-shaped uterine smooth muscle cells from a normal uterus. **C,** Large, plump hypertrophied smooth muscle cells from a gravid uterus; compare with **B.** (**B** and **C,** Same magnification.)

can no longer compensate for the increased burden. When this happens in the heart, several "degenerative" changes occur in the myocardial fibers, of which the most important are fragmentation and loss of myofibrillar contractile elements. The variables that limit continued hypertrophy and cause the regressive changes are incompletely understood. There may be finite limits of the vasculature to adequately supply the enlarged fibers, of the mitochondria to supply adenosine triphosphate (ATP), or of the biosynthetic machinery to provide the contractile proteins or other cytoskeletal elements. The net result of these changes is ventricular dilation and ultimately cardiac failure, a sequence of events that illustrates how *an adaptation to stress can progress to functionally significant cell injury if the stress is not relieved.*

Hyperplasia

As discussed earlier, hyperplasia takes place if the tissue contains cell populations capable of replication; it may occur concurrently with hypertrophy and often in response to the same stimuli.

Hyperplasia can be physiologic or pathologic. In both situations, cellular proliferation is stimulated by growth factors that are produced by a variety of cell types.

- The two types of *physiologic hyperplasia* are (1) *hormonal hyperplasia,* exemplified by the proliferation of the glandular epithelium of the female breast at puberty and during pregnancy, and (2) *compensatory hyperplasia,* in which residual tissue grows after removal or loss of part of an organ. For example, when part of a liver is resected, mitotic activity in the remaining cells begins as early as 12 hours later, eventually restoring the liver to its normal weight. The stimuli for hyperplasia in this setting are polypeptide growth factors produced by uninjured hepatocytes as well as nonparenchymal cells in the liver (Chapter 2). After restoration of the liver mass, cell proliferation is "turned off" by various growth inhibitors.
- Most forms of *pathologic hyperplasia* are caused by excessive hormonal or growth factor stimulation. For example,

after a normal menstrual period there is a burst of uterine epithelial proliferation that is normally tightly regulated by stimulation through pituitary hormones and ovarian estrogen and by inhibition through progesterone. However, a disturbed balance between estrogen and progesterone causes endometrial hyperplasia, which is a common cause of abnormal menstrual bleeding. Hyperplasia also is an important response of connective tissue cells in wound healing, in which proliferating fibroblasts and blood vessels aid in repair (Chapter 2). In this process, growth factors are produced by white blood cells (leukocytes) responding to the injury and by cells in the extracellular matrix. Stimulation by growth factors also is involved in the hyperplasia that is associated with certain viral infections; for example, papillomaviruses cause skin warts and mucosal lesions composed of masses of hyperplastic epithelium. Here the growth factors may be encoded by viral genes or by the genes of the infected host cells.

An important point is that in all of these situations, *the hyperplastic process remains controlled; if the signals that initiate it abate, the hyperplasia disappears.* It is this responsiveness to normal regulatory control mechanisms that distinguishes pathologic hyperplasias from cancer, in which the growth control mechanisms become dysregulated or ineffective (Chapter 5). Nevertheless, in many cases, pathologic hyperplasia constitutes a fertile soil in which cancers may eventually arise. For example, patients with hyperplasia of the endometrium are at increased risk of developing endometrial cancer (Chapter 18).

Atrophy

Shrinkage in the size of the cell by the loss of cell substance is known as atrophy. When a sufficient number of cells are involved, the entire tissue or organ diminishes in size, becoming atrophic (Fig. 1–4). Although atrophic cells may have diminished function, they are not dead.

Causes of atrophy include a decreased workload (e.g., immobilization of a limb to permit healing of a fracture),
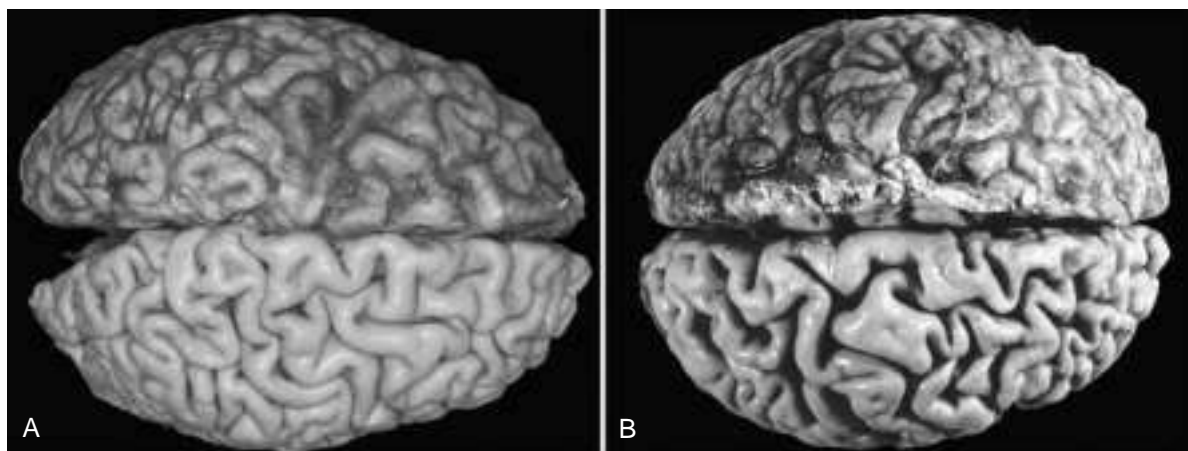
Figure 1–4 Atrophy as seen in the brain. **A,** Normal brain of a young adult. **B,** Atrophy of the brain in an 82-year-old man with atherosclerotic disease. Atrophy of the brain is due to aging and reduced blood supply. Note that loss of brain substance narrows the gyri and widens the sulci. The meninges have been stripped from the bottom half of each specimen to reveal the surface of the brain.

loss of innervation, diminished blood supply, inadequate nutrition, loss of endocrine stimulation, and aging (senile atrophy). Although some of these stimuli are physiologic (e.g., the loss of hormone stimulation in menopause) and others pathologic (e.g., denervation), the fundamental cellular changes are identical. They represent a retreat by the cell to a smaller size at which survival is still possible; a new equilibrium is achieved between cell size and diminished blood supply, nutrition, or trophic stimulation.

The mechanisms of atrophy consist of a combination of decreased protein synthesis and increased protein degradation in cells.

- Protein synthesis decreases because of reduced metabolic activity.
- The degradation of cellular proteins occurs mainly by the *ubiquitin-proteasome pathway.* Nutrient deficiency and disuse may activate ubiquitin ligases, which attach multiple copies of the small peptide ubiquitin to cellular proteins and target them for degradation in proteasomes. This pathway is also thought to be responsible for the accelerated proteolysis seen in a variety of catabolic conditions, including the cachexia associated with cancer.
- In many situations, atrophy is also accompanied by increased *autophagy,* with resulting increases in the number of *autophagic vacuoles.* Autophagy ("self-eating") is the process in which the starved cell eats its own components in an attempt to survive. We describe this process later in the chapter.

Metaplasia

Metaplasia is a reversible change in which one adult cell type (epithelial or mesenchymal) is replaced by another adult cell type. In this type of cellular adaptation, a cell type sensitive to a particular stress is replaced by another cell type better able to withstand the adverse environment. Metaplasia is thought to arise by reprogramming of stem cells to differentiate along a new pathway rather than a phenotypic change (transdifferentiation) of already differentiated cells.

Epithelial metaplasia is exemplified by the squamous change that occurs in the respiratory epithelium of habitual cigarette smokers (Fig. 1–5). The normal ciliated columnar epithelial cells of the trachea and bronchi are focally or widely replaced by stratified squamous epithelial cells. The rugged stratified squamous epithelium may be able to survive the noxious chemicals in cigarette smoke that the more fragile specialized epithelium would not tolerate. *Although the metaplastic squamous epithelium has survival advantages, important protective mechanisms are lost,* such as mucus secretion and ciliary clearance of particulate matter. Epithelial metaplasia is therefore a double-edged sword. Moreover, *the influences that induce metaplastic change, if persistent, may predispose to malignant transformation of the epithelium.* In fact, squamous metaplasia of the respiratory epithelium often coexists with lung cancers composed of malignant squamous cells. It is thought that cigarette smoking initially causes squamous metaplasia, and cancers arise later in some of these altered foci. Since vitamin A is essential for normal epithelial differentiation, its deficiency may also induce squamous metaplasia in the respiratory
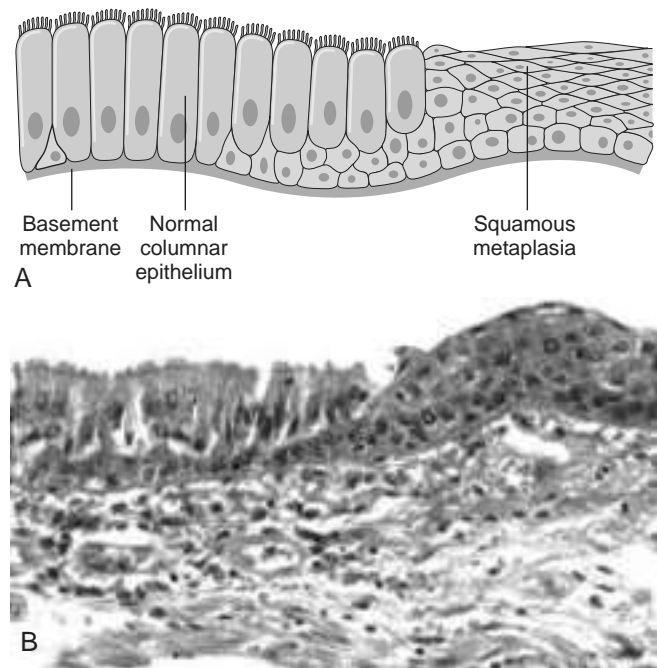
Figure 1–5 Metaplasia of normal columnar (*left*) to squamous epithelium (*right*) in a bronchus, shown schematically (**A**) and histologically (**B**).

Labels: Basement membrane; Normal columnar epithelium; Squamous metaplasia; A; B

epithelium. Metaplasia need not always occur in the direction of columnar to squamous epithelium; in chronic gastric reflux, the normal stratified squamous epithelium of the lower esophagus may undergo metaplastic transformation to gastric or intestinal-type columnar epithelium. Metaplasia may also occur in mesenchymal cells but in these situations it is generally a reaction to some pathologic alteration and not an adaptive response to stress. For example, bone is occasionally formed in soft tissues, particularly in foci of injury.

SUMMARY

Cellular Adaptations to Stress

- *Hypertrophy:* increased cell and organ size, often in response to increased workload; induced by growth factors produced in response to mechanical stress or other stimuli; occurs in tissues incapable of cell division
- *Hyperplasia:* increased cell numbers in response to hormones and other growth factors; occurs in tissues whose cells are able to divide or contain abundant tissue stem cells
- *Atrophy:* decreased cell and organ size, as a result of decreased nutrient supply or disuse; associated with decreased synthesis of cellular building blocks and increased breakdown of cellular organelles
- *Metaplasia:* change in phenotype of differentiated cells, often in response to chronic irritation, that makes cells better able to withstand the stress; usually induced by altered differentiation pathway of tissue stem cells; may result in reduced functions or increased propensity for malignant transformation

OVERVIEW OF CELL INJURY AND CELL DEATH

As stated at the beginning of the chapter, cell injury results when cells are stressed so severely that they are no longer able to adapt or when cells are exposed to inherently damaging agents or suffer from intrinsic abnormalities (e.g., in DNA or proteins). Different injurious stimuli affect many metabolic pathways and cellular organelles. Injury may progress through a reversible stage and culminate in cell death (Fig. 1–1).

- *Reversible cell injury.* In early stages or mild forms of injury the functional and morphologic changes are reversible if the damaging stimulus is removed. At this stage, although there may be significant structural and functional abnormalities, the injury has typically not progressed to severe membrane damage and nuclear dissolution.
- *Cell death.* With continuing damage, the injury becomes irreversible, at which time the cell cannot recover and it dies. *There are two types of cell death—necrosis and apoptosis—which differ in their mechanisms, morphology, and roles in disease and physiology* (Fig. 1–6 and Table 1–1). When damage to membranes is severe, enzymes leak out of lysosomes, enter the cytoplasm, and digest the

cell, resulting in *necrosis.* Cellular contents also leak through the damaged plasma membrane into the extracellular space, where they elicit a host reaction (inflammation). Necrosis is the major pathway of cell death in many commonly encountered injuries, such as those resulting from ischemia, exposure to toxins, various infections, and trauma. When a cell is deprived of growth factors, or the cell's DNA or proteins are damaged beyond repair, typically the cell kills itself by another type of death, called *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 pathologic cell injury. Furthermore, in keeping with its role in certain physiologic processes, apoptosis does not elicit an inflammatory response.* The morphologic features, mechanisms, and significance of these two death pathways are discussed in more detail later in the chapter.

CAUSES OF CELL INJURY

The causes of cell injury range from the gross physical trauma of a motor vehicle accident to the single gene defect that results in a nonfunctional enzyme underlying a

Figure 1–6 Cellular features of necrosis (*left*) and apoptosis (*right*).

Table 1–1 Features of Necrosis and Apoptosis

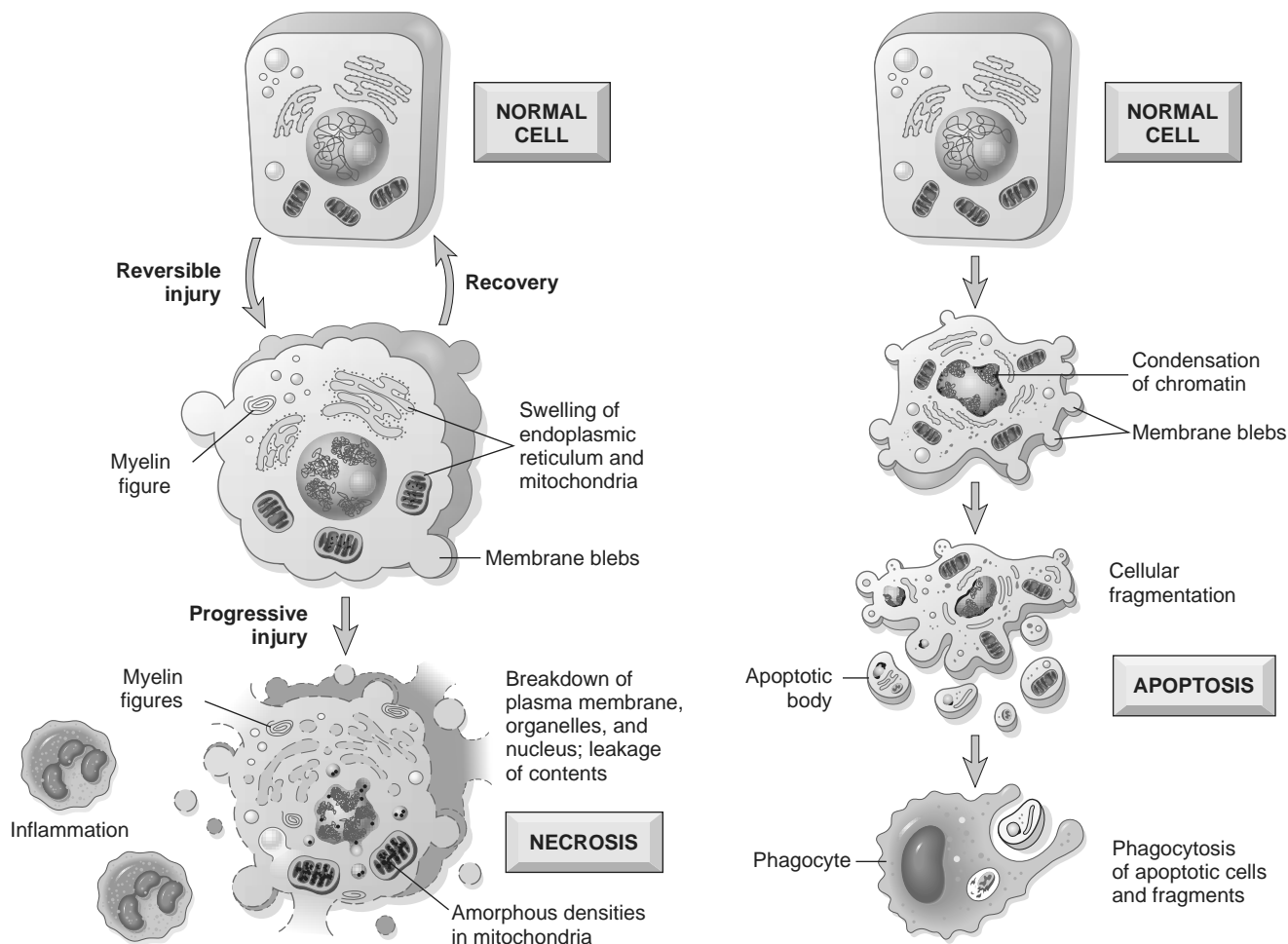| Feature	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis → karyorrhexis → karyolysis	Fragmentation into nucleosome size fragments
Plasma membrane	Disrupted	Intact; altered structure, especially orientation of lipids
Cellular contents	Enzymatic digestion; may leak 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 and protein damage

DNA, deoxyribonucleic acid.

specific metabolic disease. Most injurious stimuli can be grouped into the following categories.

Oxygen Deprivation
Hypoxia, or oxygen deficiency, interferes with aerobic oxidative respiration and is an extremely important and common cause of cell injury and death. Hypoxia should be distinguished from *ischemia,* which is a loss of blood supply in a tissue due to impeded arterial flow or reduced venous drainage. While ischemia is the most common cause of hypoxia, oxygen deficiency can also result from inadequate oxygenation of the blood, as in pneumonia, or from reduction in the oxygen-carrying capacity of the blood, as in blood loss anemia or carbon monoxide (CO) poisoning. (CO forms a stable complex with hemoglobin that prevents oxygen binding.)

Chemical Agents
An increasing number of chemical substances that can injure cells are being recognized; even innocuous substances such as glucose, salt, or even water, if absorbed or administered in excess, can so derange the osmotic environment that cell injury or death results. Agents commonly known as poisons cause severe damage at the cellular level by altering membrane permeability, osmotic homeostasis, or the integrity of an enzyme or cofactor, and exposure to such poisons can culminate in the death of the whole organism. Other potentially toxic agents are encountered daily in the environment; these include air pollutants, insecticides, CO, asbestos, and "social stimuli" such as ethanol. Many therapeutic drugs can cause cell or tissue injury in a susceptible patient or if used excessively or inappropriately (Chapter 7). Even oxygen at sufficiently high partial pressures is toxic.

Infectious Agents
Agents of infection range from submicroscopic viruses to meter-long tapeworms; in between are the rickettsiae, bacteria, fungi, and protozoans. The diverse ways in which infectious pathogens cause injury are discussed in Chapter 8.

Immunologic Reactions
Although the immune system defends the body against pathogenic microbes, immune reactions can also result in cell and tissue injury. Examples are autoimmune reactions against one's own tissues and allergic reactions against environmental substances in genetically susceptible individuals (Chapter 4).

Genetic Factors
Genetic aberrations can result in pathologic changes as conspicuous as the congenital malformations associated with Down syndrome or as subtle as the single amino acid substitution in hemoglobin S giving rise to sickle cell anemia (Chapter 6). Genetic defects may cause cell injury as a consequence of deficiency of functional proteins, such as enzymes in inborn errors of metabolism, or accumulation of damaged DNA or misfolded proteins, both of which trigger cell death when they are beyond repair. Genetic variations (polymorphisms) contribute to the development of many complex diseases and can influence the susceptibility of cells to injury by chemicals and other environmental insults.

Nutritional Imbalances
Even in the current era of burgeoning global affluence, nutritional deficiencies remain a major cause of cell injury. Protein–calorie insufficiency among underprivileged populations is only the most obvious example; specific vitamin deficiencies are not uncommon even in developed countries with high standards of living (Chapter 7). Ironically, disorders of nutrition rather than lack of nutrients are also important causes of morbidity and mortality; for example, obesity markedly increases the risk for type 2 diabetes mellitus. Moreover, diets rich in animal fat are strongly implicated in the development of atherosclerosis as well as in increased vulnerability to many disorders, including cancer.

Physical Agents
Trauma, extremes of temperature, radiation, electric shock, and sudden changes in atmospheric pressure all have wide-ranging effects on cells (Chapter 7).

Aging
Cellular senescence leads to alterations in replicative and repair abilities of individual cells and tissues. All of these changes result in a diminished ability to respond to damage and, eventually, the death of cells and of the organism. The mechanisms underlying cellular aging are discussed separately at the end of the chapter.

THE MORPHOLOGY OF CELL AND TISSUE INJURY

It is useful to describe the structural alterations that occur in damaged cells before we discuss the biochemical mechanisms that bring about these changes. All stresses and noxious influences exert their effects first at the molecular or biochemical level. *Cellular function may be lost long before cell death occurs, and the morphologic changes of cell injury (or death) lag far behind both* (Fig. 1–7). For example, myocardial cells become noncontractile after 1 to 2 minutes of ischemia, although they do not die until 20 to 30 minutes of ischemia have elapsed. These myocytes may not appear dead by electron microscopy for 2 to 3 hours, or by light microscopy for 6 to 12 hours.

The cellular derangements of reversible injury can be corrected, and if the injurious stimulus abates, the cell can return to normalcy. Persistent or excessive injury, however, causes cells to pass the nebulous "point of no return" into *irreversible injury* and *cell death.* The events that determine when reversible injury becomes irreversible and progresses to cell death remain poorly understood. The clinical relevance of this question is obvious; if the biochemical and molecular changes that predict cell death can be identified with precision, it may be possible to devise strategies for preventing the transition from reversible to irreversible cell injury. Although there are no definitive morphologic or biochemical correlates of irreversibility, *two phenomena consistently characterize irreversibility: the inability to correct mitochondrial dysfunction* (lack of oxidative phosphorylation and ATP generation) even after resolution of the original injury, and *profound disturbances in membrane function.* As mentioned earlier, injury to lysosomal membranes results in the enzymatic dissolution of the injured cell, which is the culmination of injury progressing to necrosis.

As mentioned earlier, different injurious stimuli may induce death by necrosis or apoptosis (Fig. 1–6 and Table 1–1). Below we describe the morphology of reversible cell injury and necrosis; the sequence of morphologic alterations in these processes is illustrated in Figure 1–6, *left.* Apoptosis has many unique features, and we describe it separately later in the chapter.

Reversible Injury

The two main morphologic correlates of reversible cell injury are *cellular swelling* and *fatty change.* Cellular swelling is the result of failure of energy-dependent ion pumps in the plasma membrane, leading to an inability to maintain ionic and fluid homeostasis. Fatty change occurs in hypoxic injury and in various forms of toxic or metabolic injury and is manifested by the appearance of small or large lipid vacuoles in the cytoplasm. The mechanisms of fatty change are discussed in Chapter 15.

In some situations, potentially injurious insults induce specific alterations in cellular organelles, like the ER. The smooth ER is involved in the metabolism of various chemicals, and cells exposed to these chemicals show hypertrophy of the ER as an adaptive response that may have important functional consequences. For instance, barbiturates are metabolized in the liver by the cytochrome P-450 mixed-function oxidase system found in the smooth ER. Protracted use of barbiturates leads to a state of tolerance, with a decrease in the effects of the drug and the need to use increasing doses. This adaptation is due to increased volume (hypertrophy) of the smooth ER of hepatocytes and consequent increased P-450 enzymatic activity. Although P-450–mediated modification is often thought of as "detoxification," many compounds are rendered *more* injurious by this process; one example is carbon tetrachloride (CCl_4), discussed later. In addition, the products formed by this oxidative metabolism include reactive oxygen species (ROS), which can injure the cell. Cells adapted to one drug have increased capacity to metabolize other compounds handled by the same system. Thus, if patients taking phenobarbital for epilepsy increase their alcohol intake, they may experience a drop in blood concentration of the anti-seizure medication to subtherapeutic levels because of induction of smooth ER in response to the alcohol.
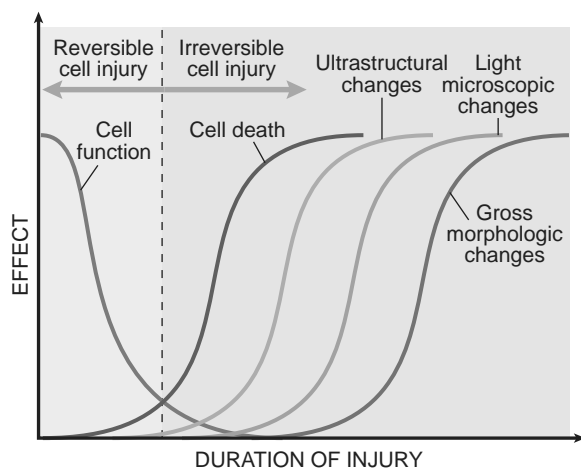
Figure 1–7 The relationship among cellular function, cell death, and the morphologic changes of cell injury. Note that cells may rapidly become nonfunctional after the onset of injury, although they are still viable, with potentially reversible damage; with a longer duration of injury, irreversible injury and cell death may result. Note also that cell death typically precedes ultrastructural, light microscopic, and grossly visible morphologic changes.

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Cellular swelling (Fig. 1–8, *B*), the first manifestation of almost all forms of injury to cells, is a reversible alteration that may be difficult to appreciate with the light microscope, but it may be more apparent at the level of the whole organ. When it affects many cells in an organ, it causes some pallor (as a result of compression of capillaries), increased turgor, and increase in weight of the organ. Microscopic examination may reveal small, clear vacuoles within the cytoplasm; these represent distended and pinched-off segments of the endoplasmic reticulum (ER). This pattern of nonlethal injury is sometimes called **hydropic change** or **vacuolar degeneration. Fatty change** is manifested by the appearance of lipid vacuoles in the cytoplasm. It is principally encountered in cells participating in fat metabolism (e.g., hepatocytes, myocardial cells) and is also reversible. Injured cells may also show increased eosinophilic staining, which becomes much
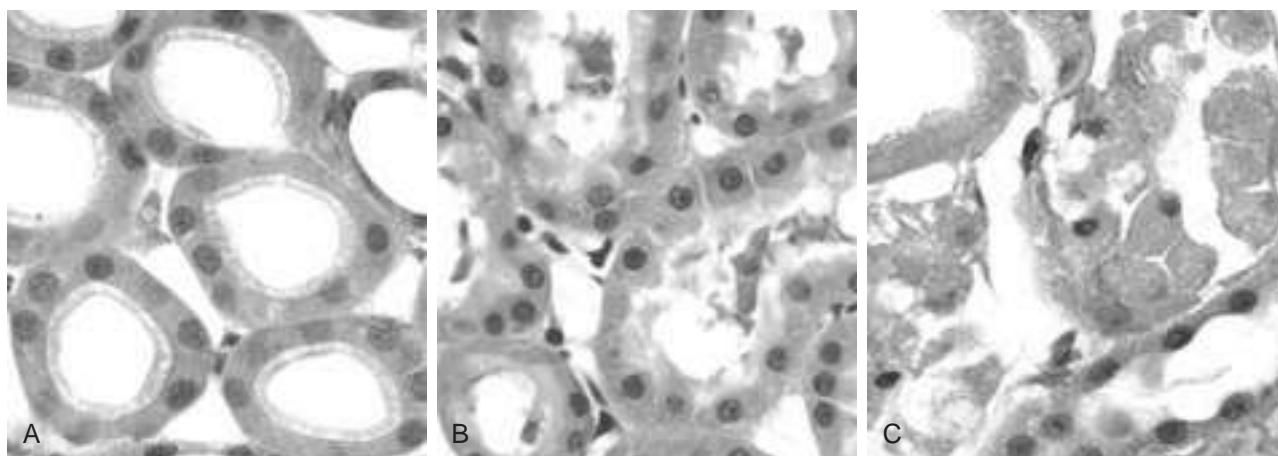
Figure 1–8 Morphologic changes in reversible and irreversible cell injury (necrosis). **A,** Normal kidney tubules with viable epithelial cells. **B,** Early (reversible) ischemic injury showing surface blebs, increased eosinophilia of cytoplasm, and swelling of occasional cells. **C,** Necrotic (irreversible) injury of epithelial cells, with loss of nuclei and fragmentation of cells and leakage of contents.
(Courtesy of Drs. Neal Pinckard and M.A. Venkatachalam, University of Texas Health Sciences Center, San Antonio, Tex.)

more pronounced with progression to necrosis (described further on).

The intracellular changes associated with reversible injury (Fig. 1–6) include (1) plasma membrane alterations such as blebbing, blunting, or distortion of microvilli, and loosening of intercellular attachments; (2) mitochondrial changes such as swelling and the appearance of phospholipid-rich amorphous densities; (3) dilation of the ER with detachment of ribosomes and dissociation of polysomes; and (4) nuclear alterations, with clumping of chromatin. The cytoplasm may contain phospholipid masses, called myelin figures, which are derived from damaged cellular membranes.

Necrosis

Necrosis is the type of cell death that is associated with loss of membrane integrity and leakage of cellular contents culminating in dissolution of cells, largely resulting from the degradative action of enzymes on lethally injured cells. The leaked cellular contents often elicit a local host reaction, called *inflammation,* that attempts to eliminate the dead cells and start the subsequent repair process (Chapter 2). The enzymes responsible for digestion of the cell may be derived from the lysosomes of the dying cells themselves and from the lysosomes of leukocytes that are recruited as part of the inflammatory reaction to the dead cells.

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Necrosis is characterized by changes in the cytoplasm and nuclei of the injured cells (Figs. 1–6, *left,* and 1–8, *C*).

- **Cytoplasmic changes.** Necrotic cells show **increased eosinophilia** (i.e., pink staining from the eosin dye—the E in the hematoxylin and eosin [H&E] stain), attributable in part to increased binding of eosin to denatured cytoplasmic proteins and in part to loss of the basophilia that is normally imparted by the ribonucleic acid (RNA) in the cytoplasm (basophilia is the blue staining from the hematoxylin dye—the H in "H&E"). Compared with viable cells,

the cell may have a more glassy, homogeneous appearance, mostly because of the loss of glycogen particles. Myelin figures are more prominent in necrotic cells than during reversible injury. When enzymes have digested cytoplasmic organelles, the cytoplasm becomes vacuolated and appears "moth-eaten." By electron microscopy, necrotic cells are characterized by discontinuities in plasma and organelle membranes, marked dilation of mitochondria with the appearance of large amorphous densities, disruption of lysosomes, and intracytoplasmic myelin figures.

- **Nuclear changes.** Nuclear changes assume one of three patterns, all due to breakdown of DNA and chromatin. The basophilia of the chromatin may fade **(karyolysis),** presumably secondary to deoxyribonuclease (DNase) activity. A second pattern is **pyknosis,** characterized by nuclear shrinkage and increased basophilia; the DNA condenses into a solid shrunken mass. In the third pattern, **karyorrhexis,** the pyknotic nucleus undergoes fragmentation. In 1 to 2 days, the nucleus in a dead cell may completely disappear. Electron microscopy reveals profound nuclear changes culminating in nuclear dissolution.
- **Fates of necrotic cells.** Necrotic cells may persist for some time or may be digested by enzymes and disappear. Dead cells may be replaced by myelin figures, which are either phagocytosed by other cells or further degraded into fatty acids. These fatty acids bind calcium salts, which may result in the dead cells ultimately becoming **calcified.**

Patterns of Tissue Necrosis

There are several morphologically distinct patterns of tissue necrosis, which may provide clues about the underlying cause. Although the terms that describe these patterns do not reflect underlying mechanisms, such terms are in common use, and their implications are understood by both pathologists and clinicians. Most of these types of necrosis have distinct gross appearance; fibrinoid necrosis is detected only by histologic examination.
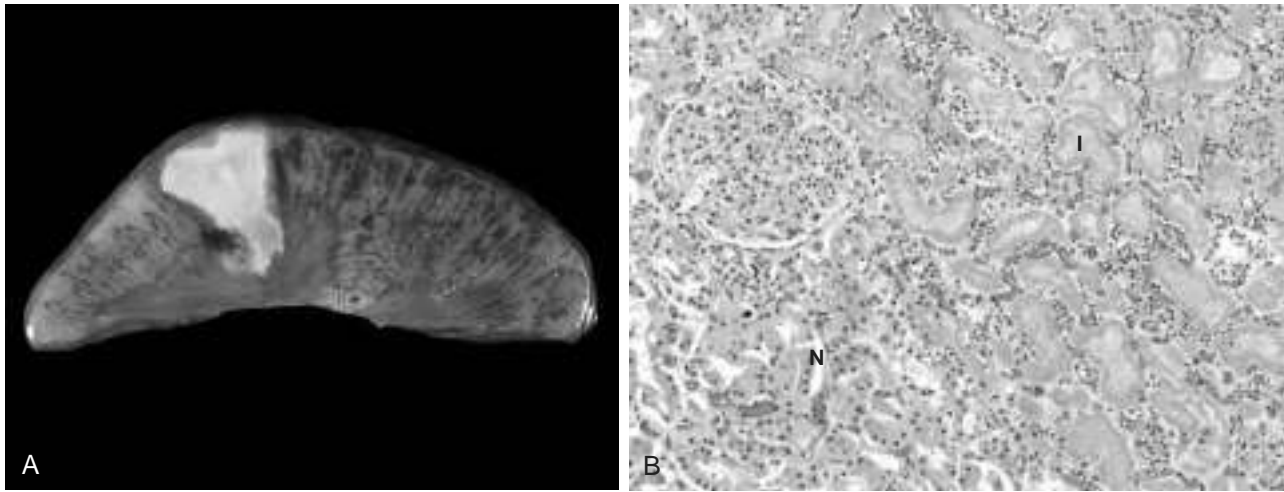
Figure 1–9 Coagulative necrosis. **A,** A wedge-shaped kidney infarct *(yellow)* with preservation of the outlines. **B,** Microscopic view of the edge of the infarct, with normal kidney *(N)* and necrotic cells in the infarct *(I)*. The necrotic cells show preserved outlines with loss of nuclei, and an inflammatory infiltrate is present (difficult to discern at this magnification).

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- **Coagulative necrosis** is a form of necrosis in which the underlying tissue architecture is preserved for at least several days (Fig. 1–9). The affected tissues take on a firm texture. Presumably the injury denatures not only structural proteins but also enzymes, thereby blocking the proteolysis of the dead cells; as a result, eosinophilic, anucleate cells may persist for days or weeks. Leukocytes are recruited to the site of necrosis, and the dead cells are digested by the action of lysosomal enzymes of the leukocytes. The cellular debris is then removed by phagocytosis. Coagulative necrosis is characteristic of **infarcts** (areas of ischemic necrosis) in all of the solid organs except the brain.
- **Liquefactive necrosis** is seen in focal bacterial or, occasionally, fungal infections, because microbes stimulate the accumulation of inflammatory cells and the enzymes of leukocytes digest ("liquefy") the tissue. For obscure reasons, hypoxic death of cells within the central nervous system often evokes liquefactive necrosis (Fig. 1–10). Whatever the pathogenesis, the dead cells are completely digested, transforming the tissue into a liquid viscous mass. Eventually, the digested tissue is removed by phagocytes. If the process was initiated by acute inflammation, as in a bacterial infection, the material is frequently creamy yellow and is called pus (Chapter 2).
- Although **gangrenous necrosis** is not a distinctive pattern of cell death, the term is still commonly used in clinical practice. It usually refers to the condition of a limb, generally the lower leg, that has lost its blood supply and has undergone coagulative necrosis involving multiple tissue layers. When bacterial infection is superimposed, coagulative necrosis is modified by the liquefactive action of the bacteria and the attracted leukocytes (resulting in so-called **wet gangrene**).
- **Caseous necrosis** is encountered most often in foci of tuberculous infection. **Caseous** means "cheese-like," referring to the friable yellow-white appearance of the area of necrosis (Fig. 1–11). On microscopic examination, the necrotic focus appears as a collection of fragmented or lysed cells with an amorphous granular pink appearance in the usual H&E-stained tissue. Unlike with coagulative necrosis, the tissue architecture is completely obliterated and cellular outlines cannot be discerned. The area of caseous necrosis is often enclosed within a distinctive inflammatory border; this appearance is characteristic of a focus of inflammation known as a **granuloma** (Chapter 2).
- **Fat necrosis** refers to focal areas of fat destruction, typically resulting from release of activated pancreatic lipases into the substance of the pancreas and the peritoneal cavity. This occurs in the calamitous abdominal emergency known as acute pancreatitis (Chapter 16). In this disorder, pancreatic enzymes that have leaked out of acinar cells
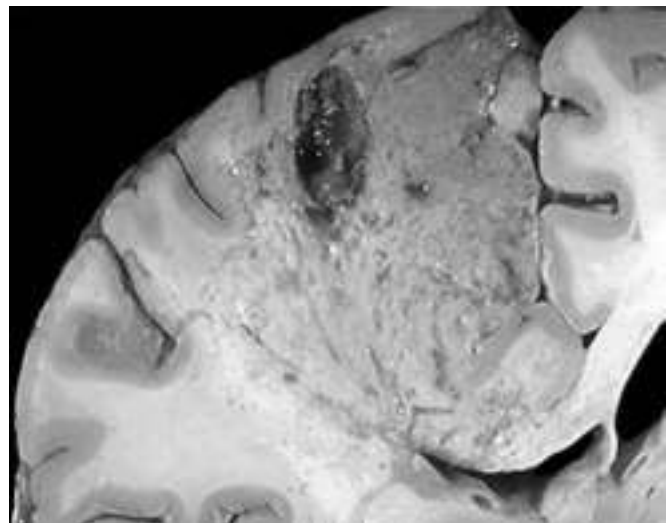

Figure 1–10 Liquefactive necrosis. An infarct in the brain showing dissolution of the tissue.
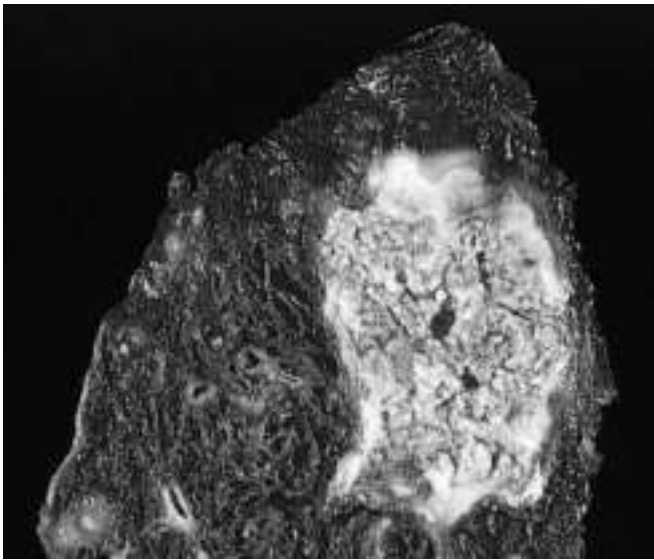
Figure 1–11 Caseous necrosis. Tuberculosis of the lung, with a large area of caseous necrosis containing yellow-white (cheesy) debris.

and ducts liquefy the membranes of fat cells in the peritoneum, and lipases split the triglyceride esters contained within fat cells. The released fatty acids combine with calcium to produce grossly visible chalky white areas (fat saponification), which enable the surgeon and the pathologist to identify the lesions (Fig. 1–12). On histologic examination, the foci of necrosis contain shadowy outlines of necrotic fat cells with basophilic calcium deposits, surrounded by an inflammatory reaction.

- **Fibrinoid necrosis** is a special form of necrosis, visible by light microscopy, usually in immune reactions in which complexes of antigens and antibodies are deposited in the walls of arteries. The deposited immune complexes, together with fibrin that has leaked out of vessels, produce a bright pink and amorphous appearance on H&E preparations called **fibrinoid** (fibrin-like) by pathologists (Fig. 1–13). The immunologically mediated diseases (e.g., polyarteritis nodosa) in which this type of necrosis is seen are described in Chapter 4.
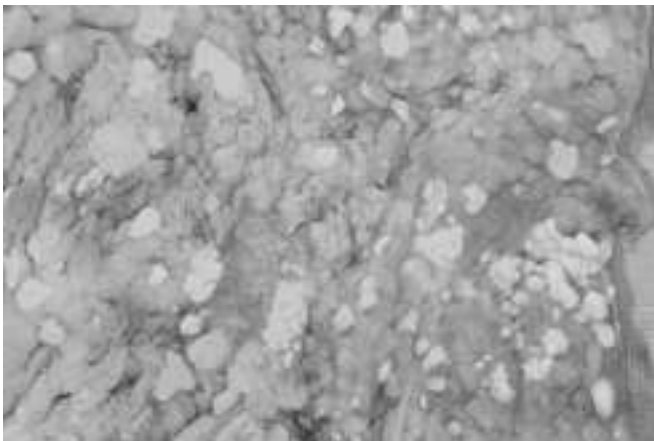

Figure 1–12 Fat necrosis in acute pancreatitis. The areas of white chalky deposits represent foci of fat necrosis with calcium soap formation (saponification) at sites of lipid breakdown in the mesentery.
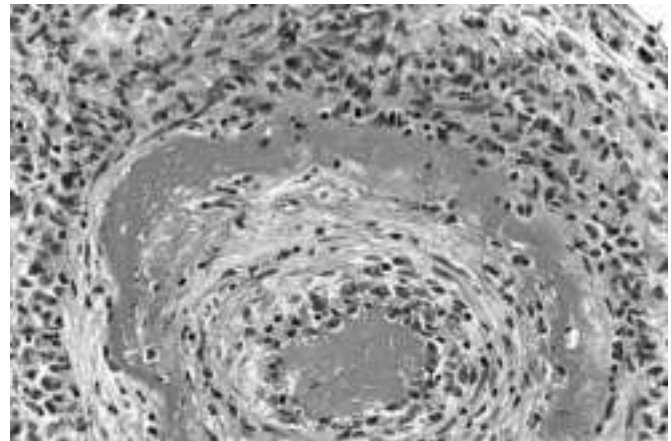
Figure 1–13 Fibrinoid necrosis in an artery in a patient with polyarteritis nodosa. The wall of the artery shows a circumferential bright pink area of necrosis with protein deposition and inflammation.

Leakage of intracellular proteins through the damaged cell membrane and ultimately into the circulation provides a means of detecting tissue-specific necrosis using blood or serum samples. Cardiac muscle, for example, contains a unique isoform of the enzyme creatine kinase and of the contractile protein troponin, whereas hepatic bile duct epithelium contains a temperature-resistant isoform of the enzyme alkaline phosphatase, and hepatocytes contain transaminases. Irreversible injury and cell death in these tissues result in increased serum levels of such proteins, and measurement of serum levels is used clinically to assess damage to these tissues.

SUMMARY

Morphologic Alterations in Injured Cells and Tissues

- *Reversible cell injury:* cell swelling, fatty change, plasma membrane blebbing and loss of microvilli, mitochondrial swelling, dilation of the ER, eosinophilia (due to decreased cytoplasmic RNA)
- *Necrosis:* increased eosinophilia; nuclear shrinkage, fragmentation, and dissolution; breakdown of plasma membrane and organellar membranes; abundant myelin figures; leakage and enzymatic digestion of cellular contents
- *Patterns of tissue necrosis:* Under different conditions, necrosis in tissues may assume specific patterns: coagulative, liquefactive, gangrenous, caseous, fat, and fibrinoid.

MECHANISMS OF CELL INJURY

Now that we have discussed the causes of cell injury and the morphologic changes in necrosis, we next consider in more detail the molecular basis of cell injury, and then illustrate the important principles with a few selected examples of common types of injury.

The biochemical mechanisms linking any given injury with the resulting cellular and tissue manifestations are complex, interconnected, and tightly interwoven with many intracellular metabolic pathways. Nevertheless,

several general principles are relevant to most forms of cell injury:

- *The cellular response to injurious stimuli depends on the type of injury, its duration, and its severity.* Thus, low doses of toxins or a brief duration of ischemia may lead to reversible cell injury, whereas larger toxin doses or longer ischemic intervals may result in irreversible injury and cell death.

- *The consequences of an injurious stimulus depend on the type, status, adaptability, and genetic makeup of the injured cell.* The same injury has vastly different outcomes depending on the cell type; thus, striated skeletal muscle in the leg accommodates complete ischemia for 2 to 3 hours without irreversible injury, whereas cardiac muscle dies after only 20 to 30 minutes. The nutritional (or hormonal) status can also be important; clearly, a glycogen-replete hepatocyte will tolerate ischemia much better than one that has just burned its last glucose molecule. Genetically determined diversity in metabolic pathways can contribute to differences in responses to injurious stimuli. For instance, when exposed to the same dose of a toxin, individuals who inherit variants in genes encoding cytochrome P-450 may catabolize the toxin at different rates, leading to different outcomes. Much effort is now directed toward understanding the role of genetic polymorphisms in responses to drugs and toxins. The study of such interactions is called pharmacogenomics. In fact, genetic variations influence susceptibility to many complex diseases as well as responsiveness to various therapeutic agents. Using the genetic makeup of the individual patient to guide therapy is one example of "personalized medicine."

- *Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components* (Fig. 1–14). The principal targets and biochemical mechanisms of cell injury are: (1) mitochondria and their ability to generate ATP and ROS under pathologic conditions; (2) disturbance in calcium homeostasis; (3) damage to cellular (plasma and lysosomal) membranes; and (4) damage to DNA and misfolding of proteins.

- *Multiple biochemical alterations may be triggered by any one injurious insult.* It is therefore difficult to assign any one mechanism to a particular insult or clinical situation in which cell injury is prominent. For this reason, therapies that target individual mechanisms of cell injury may not be effective.

With this background, we can briefly discuss the major biochemical mechanisms of cell injury.

Depletion of ATP

ATP, the energy store of cells, is produced mainly by oxidative phosphorylation of adenosine diphosphate (ADP) during reduction of oxygen in the electron transport system of mitochondria. In addition, the glycolytic pathway can generate ATP in the absence of oxygen using glucose derived either from the circulation or from the hydrolysis of intracellular glycogen. The major causes of ATP depletion are reduced supply of oxygen and nutrients, mitochondrial damage, and the actions of some toxins (e.g., cyanide). Tissues with a greater glycolytic capacity (e.g., the liver) are able to survive loss of oxygen and decreased oxidative phosphorylation better than are tissues with limited capacity for glycolysis (e.g., the brain). High-energy phosphate in the form of ATP is required for virtually all synthetic and degradative processes within the cell, including membrane transport, protein synthesis, lipogenesis, and the deacylation-reacylation reactions necessary for phospholipid turnover. It is estimated that in total, the cells of a healthy human burn 50 to 75 kg of ATP every day!

Significant depletion of ATP has widespread effects on many critical cellular systems (Fig. 1–15):

- The activity of *plasma membrane ATP-dependent sodium pumps* is reduced, resulting in intracellular accumulation of sodium and efflux of potassium. The net gain of solute is accompanied by iso-osmotic gain of water, causing *cell swelling* and dilation of the ER.

- There is a compensatory *increase in anaerobic glycolysis* in an attempt to maintain the cell's energy sources. As a consequence, intracellular glycogen stores are rapidly depleted, and lactic acid accumulates, leading to decreased intracellular pH and decreased activity of many cellular enzymes.

- *Failure of ATP-dependent Ca^{2+} pumps* leads to influx of Ca^{2+}, with damaging effects on numerous cellular components, described later.
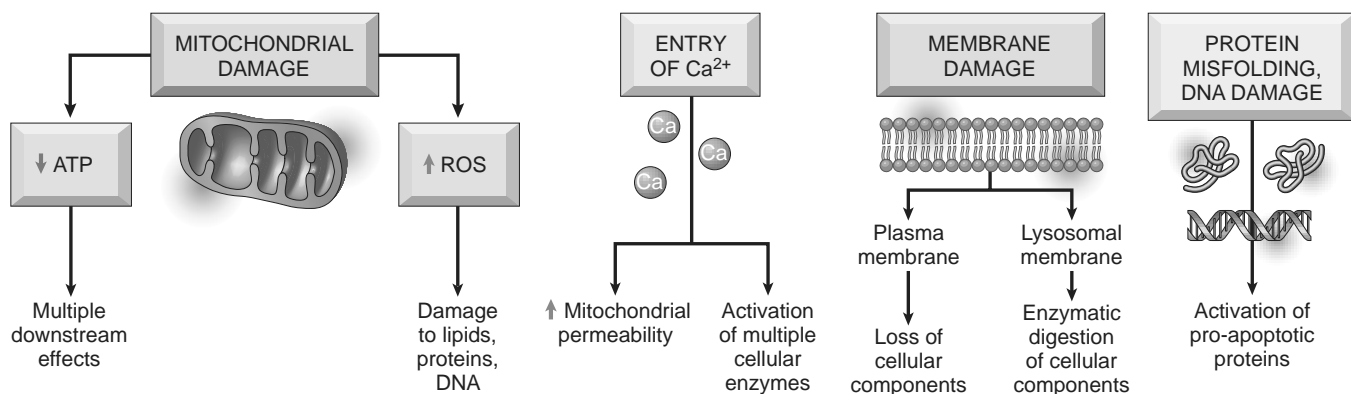

Figure 1–14 The principal biochemical mechanisms and sites of damage in cell injury. ATP, adenosine triphospate; ROS, reactive oxygen species.
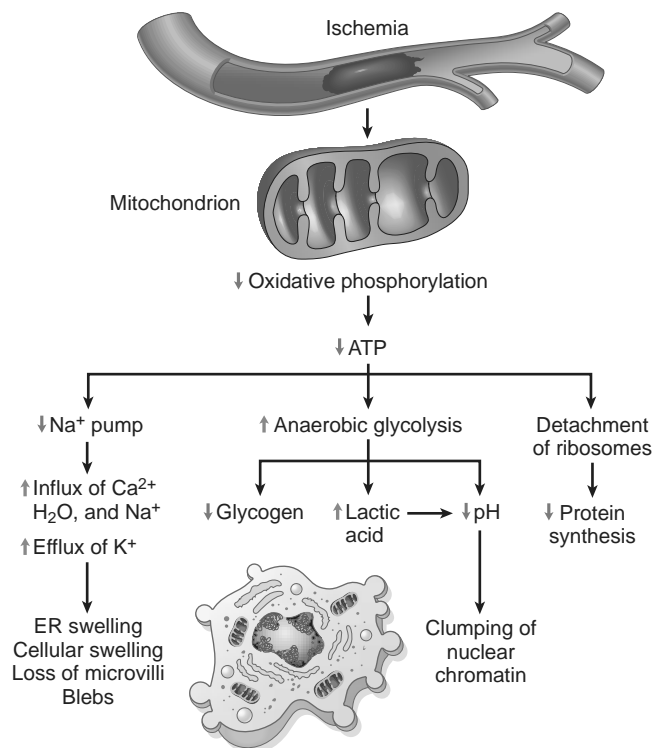
Figure 1–15 The functional and morphologic consequences of depletion of intracellular adenosine triphosphate (ATP). ER, endoplasmic reticulum.

and pH changes, further compromising oxidative phosphorylation.
• The mitochondria also contain several proteins that, when released into the cytoplasm, tell the cell there is internal injury and activate a pathway of apoptosis, discussed later.

Influx of Calcium

The importance of Ca^{2+} in cell injury was established by the experimental finding that depleting extracellular Ca^{2+} delays cell death after hypoxia and exposure to some toxins. Cytosolic free calcium is normally maintained by ATP-dependent calcium transporters at concentrations as much as 10,000 times lower than the concentration of extracellular calcium or of sequestered intracellular mitochondrial and ER calcium. Ischemia and certain toxins cause an increase in cytosolic calcium concentration, initially because of release of Ca^{2+} from the intracellular stores, and later resulting from increased influx across the plasma membrane. *Increased cytosolic Ca^{2+} activates a number of enzymes,* with potentially deleterious cellular effects (Fig. 1–17). These enzymes include phospholipases (which cause membrane damage), proteases (which break down both membrane and cytoskeletal proteins), endonucleases (which are responsible for DNA and chromatin fragmentation), and adenosine triphosphatases (ATPases) (thereby hastening ATP depletion). Increased intracellular Ca^{2+} levels may also induce apoptosis, by direct activation of caspases and by increasing mitochondrial permeability.

• Prolonged or worsening depletion of ATP causes *structural disruption of the protein synthetic apparatus,* manifested as detachment of ribosomes from the rough ER (RER) and dissociation of polysomes into monosomes, with a consequent reduction in protein synthesis. Ultimately, there is irreversible damage to mitochondrial and lysosomal membranes, and the cell undergoes necrosis.

Mitochondrial Damage and Dysfunction

Mitochondria may be viewed as "mini-factories" that produce life-sustaining energy in the form of ATP. Not surprisingly, therefore, they are also critical players in cell injury and death (Fig. 1–16). Mitochondria are sensitive to many types of injurious stimuli, including hypoxia, chemical toxins, and radiation. Mitochondrial damage may result in several biochemical abnormalities:

• Failure of oxidative phosphorylation leads to progressive depletion of ATP, culminating in necrosis of the cell, as described earlier.
• Abnormal oxidative phosphorylation also leads to the formation of reactive oxygen species, which have many deleterious effects, described below.
• Damage to mitochondria is often associated with the formation of a high-conductance channel in the mitochondrial membrane, called the mitochondrial permeability transition pore. The opening of this channel leads to the loss of mitochondrial membrane potential
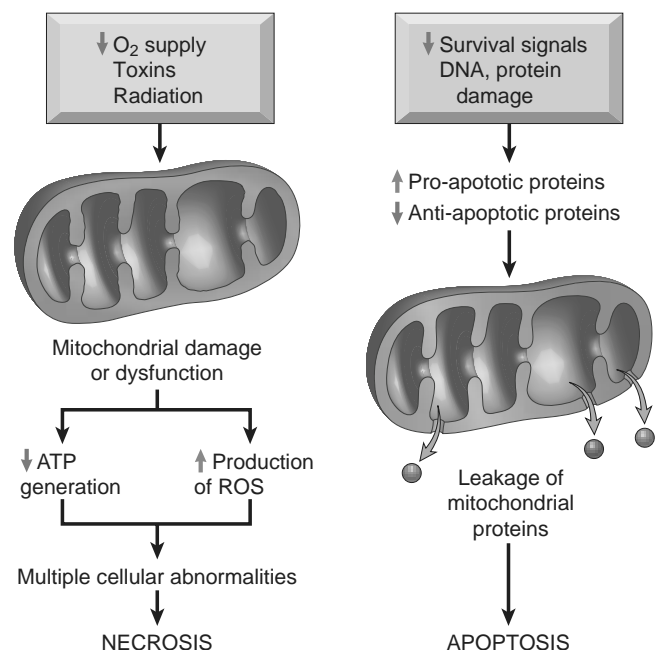

Figure 1–16 Role of mitochondria in cell injury and death. Mitochondria are affected by a variety of injurious stimuli and their abnormalities lead to necrosis or apoptosis. This pathway of apoptosis is described in more detail later. ATP, adenosine triphosphate; ROS, reactive oxygen species.
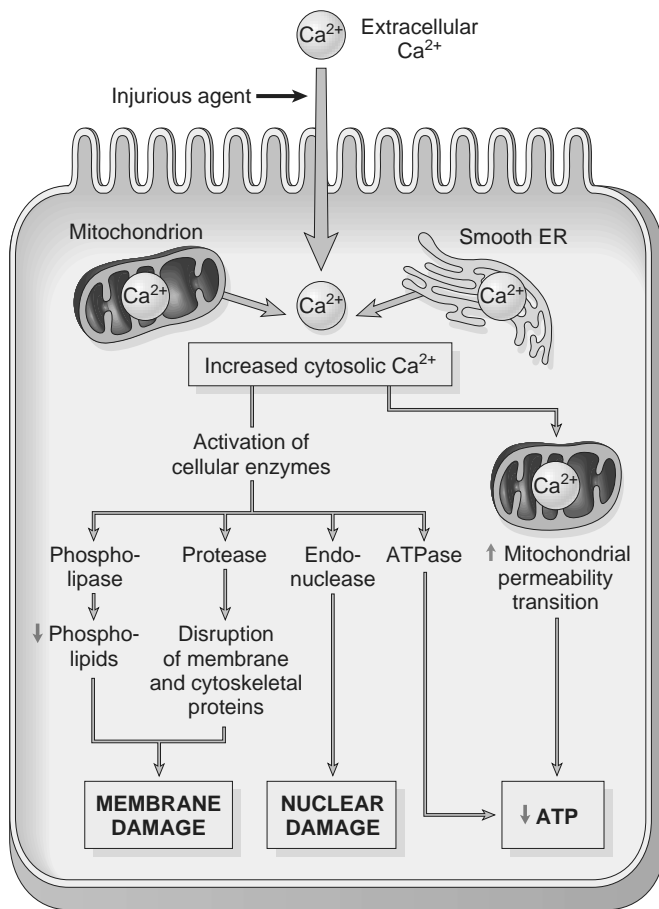
Figure 1–17 Sources and consequences of increased cytosolic calcium in cell injury. ATP, adenosine triphosphate; ATPase, adenosine triphosphatase.

Accumulation of Oxygen-Derived Free Radicals (Oxidative Stress)

Free radicals are chemical species with a single unpaired electron in an outer orbital. Such chemical states are extremely unstable, and free radicals readily react with inorganic and organic chemicals; when generated in cells, they avidly attack nucleic acids as well as a variety of cellular proteins and lipids. In addition, free radicals initiate reactions in which molecules that react with free radicals are themselves converted into other types of free radicals, thereby propagating the chain of damage.

Reactive oxygen species (ROS) are a type of oxygen-derived free radical whose role in cell injury is well established. Cell injury in many circumstances involves damage by free radicals; these situations include ischemia-reperfusion (discussed later on), chemical and radiation injury, toxicity from oxygen and other gases, cellular aging, microbial killing by phagocytic cells, and tissue injury caused by inflammatory cells.

There are different types of ROS, and they are produced by two major pathways (Fig. 1–18).

- *ROS are produced normally in small amounts in all cells during the reduction-oxidation (redox) reactions* that occur during mitochondrial respiration and energy generation. In this process, molecular oxygen is sequentially reduced in mitochondria by the addition of four electrons to generate water. This reaction is imperfect, however, and small amounts of highly reactive but short-lived toxic intermediates are generated when oxygen is only partially reduced. These intermediates include superoxide ($O_2^{\cdot -}$), which is converted to hydrogen peroxide (H_2O_2) spontaneously and by the action of the enzyme superoxide dismutase. H_2O_2 is more stable than $O_2^{\cdot -}$ and can cross biologic membranes. In the presence of metals, such as Fe^{2+}, H_2O_2 is converted to the highly reactive hydroxyl radical $^{\cdot}OH$ by the Fenton reaction.
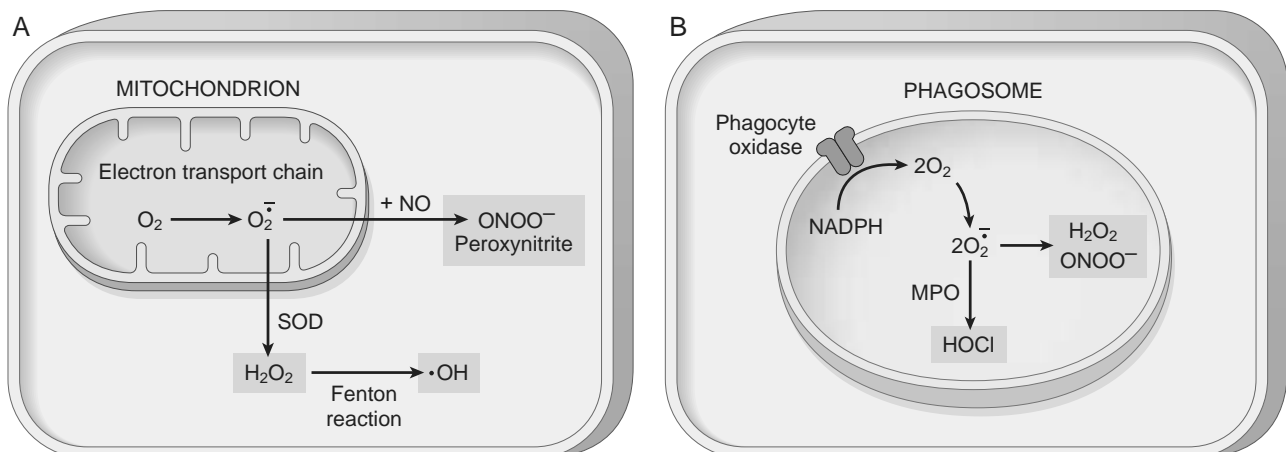
Figure 1–18 Pathways of production of reactive oxygen species. **A,** In all cells, superoxide ($O_2^{\cdot}$) is generated during mitochondrial respiration by the electron transport chain and may be converted to H_2O_2 and the hydroxyl ($^{\cdot}OH$) free radical or to peroxynitrite ($ONOO^-$). **B,** In leukocytes (mainly neutrophils and macrophages), the phagocyte oxidase enzyme in the phagosome membrane generates superoxide, which can be converted to other free radicals. Myeloperoxidase (MPO) in phagosomes also generates hypochlorite from reactive oxygen species (ROS). NO, nitric oxide; SOD, superoxide dismutase.

- *ROS are produced in phagocytic leukocytes, mainly neutrophils and macrophages,* as a weapon for destroying ingested microbes and other substances during inflammation and host defense (Chapter 2). The ROS are generated in the phagosomes and phagolysosomes of leukocytes by a process that is similar to mitochondrial respiration and is called the *respiratory burst* (or oxidative burst). In this process, a phagosome membrane enzyme catalyzes the generation of superoxide, which is converted to H_2O_2. H_2O_2 is in turn converted to a highly reactive compound hypochlorite (the major component of household bleach) by the enzyme myeloperoxidase, which is present in leukocytes. The role of ROS in inflammation is described in Chapter 2.

- *Nitric oxide (NO)* is another reactive free radical produced in leukocytes and other cells. It can react with $O_2^{\bullet-}$ to form a highly reactive compound, peroxynitrite, which also participates in cell injury.

The damage caused by free radicals is determined by their rates of production and removal (Fig. 1–19). When the production of ROS increases or the scavenging systems are ineffective, the result is an excess of these free radicals, leading to a condition called *oxidative stress.*

The generation of free radicals is increased under several circumstances:

- The absorption of radiant energy (e.g., ultraviolet light, x-rays). Ionizing radiation can hydrolyze water into hydroxyl ($^{\bullet}OH$) and hydrogen ($H^{\bullet}$) free radicals.

- The enzymatic metabolism of exogenous chemicals (e.g., carbon tetrachloride—see later)

- Inflammation, in which free radicals are produced by leukocytes (Chapter 2)

Cells have developed many *mechanisms to remove free radicals* and thereby minimize injury. Free radicals are inherently unstable and decay spontaneously. There are also nonenzymatic and enzymatic systems that contribute to inactivation of free radicals (Fig. 1–19).

- The rate of decay of superoxide is significantly increased by the action of superoxide dismutases (SODs) found in many cell types.

- Glutathione (GSH) peroxidases are a family of enzymes whose major function is to protect cells from oxidative damage. The most abundant member of this family, glutathione peroxidase 1, is found in the cytoplasm of all cells. It catalyzes the breakdown of H_2O_2 by the reaction 2 GSH (glutathione) + $H_2O_2 \rightarrow$ GS-SG + 2 H_2O. The intracellular ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) is a reflection of this enzyme's activity and thus of the cell's ability to catabolize free radicals.

- Catalase, present in peroxisomes, catalyzes the decomposition of hydrogen peroxide ($2H_2O_2 \rightarrow O_2 + 2H_2O$). It is one of the most active enzymes known, capable of degrading millions of molecules of H_2O_2 per second.

- Endogenous or exogenous antioxidants (e.g., vitamins E, A, and C and β-carotene) may either block the formation of free radicals or scavenge them once they have formed.

Reactive oxygen species cause cell injury by three main reactions (Fig. 1–19):

- *Lipid peroxidation of membranes.* Double bonds in membrane polyunsaturated lipids are vulnerable to attack by oxygen-derived free radicals. The lipid–radical interactions yield peroxides, which are themselves unstable and reactive, and an autocatalytic chain reaction ensues.

- *Cross-linking and other changes in proteins.* Free radicals promote sulfhydryl-mediated protein cross-linking, resulting in enhanced degradation or loss of enzymatic activity. Free radical reactions may also directly cause polypeptide fragmentation.

- *DNA damage.* Free radical reactions with thymine in nuclear and mitochondrial DNA produce single-strand breaks. Such DNA damage has been implicated in cell death, aging, and malignant transformation of cells.

In addition to the role of ROS in cell injury and killing of microbes, low concentrations of ROS are involved in numerous signaling pathways in cells and thus in many physiologic reactions. Therefore, these molecules are produced normally but, to avoid their harmful effects, their intracellular concentrations are tightly regulated in healthy cells.
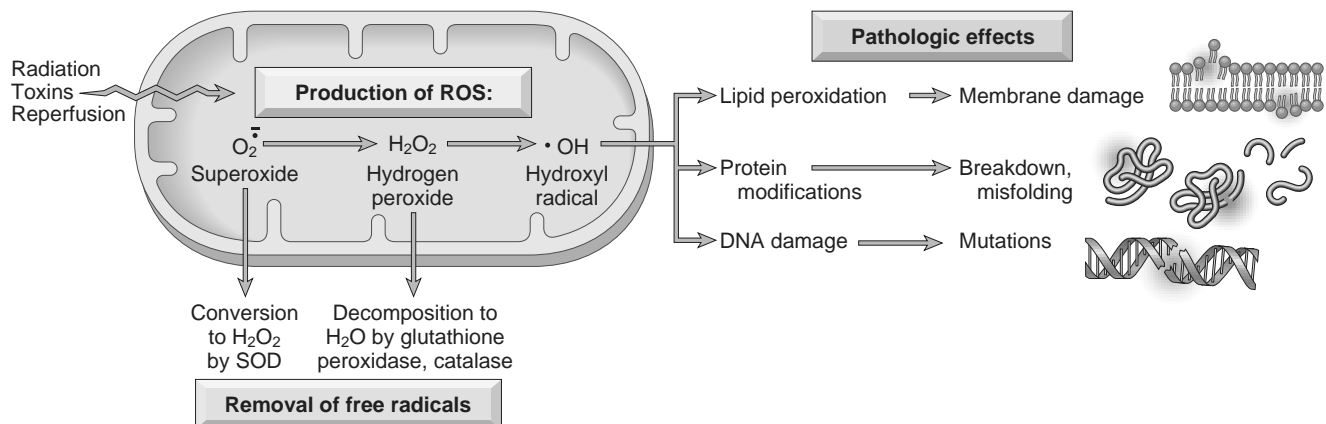
Figure 1–19 The generation, removal, and role of reactive oxygen species (ROS) in cell injury. The production of ROS is increased by many injurious stimuli. These free radicals are removed by spontaneous decay and by specialized enzymatic systems. Excessive production or inadequate removal leads to accumulation of free radicals in cells, which may damage lipids (by peroxidation), proteins, and deoxyribonucleic acid (DNA), resulting in cell injury.

Defects in Membrane Permeability

Increased membrane permeability leading ultimately to overt membrane damage is a consistent feature of most forms of cell injury that culminate in necrosis. The plasma membrane can be damaged by ischemia, various microbial toxins, lytic complement components, and a variety of physical and chemical agents. Several biochemical mechanisms may contribute to membrane damage (Fig. 1–20):

- *Decreased phospholipid synthesis.* The production of phospholipids in cells may be reduced whenever there is a fall in ATP levels, leading to decreased energy-dependent enzymatic activities. The reduced phospholipid synthesis may affect all cellular membranes, including the membranes of mitochondria, thus exacerbating the loss of ATP.
- *Increased phospholipid breakdown.* Severe cell injury is associated with increased degradation of membrane phospholipids, probably owing to activation of endogenous phospholipases by increased levels of cytosolic Ca^{2+}.
- *ROS.* Oxygen free radicals cause injury to cell membranes by lipid peroxidation, discussed earlier.
- *Cytoskeletal abnormalities.* Cytoskeletal filaments act as anchors connecting the plasma membrane to the cell interior, and serve many functions in maintaining normal cellular architecture, motility, and signaling. Activation of proteases by increased cytosolic Ca^{2+} may cause damage to elements of the cytoskeleton, leading to membrane damage.
- *Lipid breakdown products.* These include unesterified free fatty acids, acyl carnitine, and lysophospholipids, all of which accumulate in injured cells as a result of phospholipid degradation. These catabolic products have a detergent effect on membranes. They may also either insert into the lipid bilayer of the membrane or exchange with membrane phospholipids, causing changes in permeability and electrophysiologic alterations.
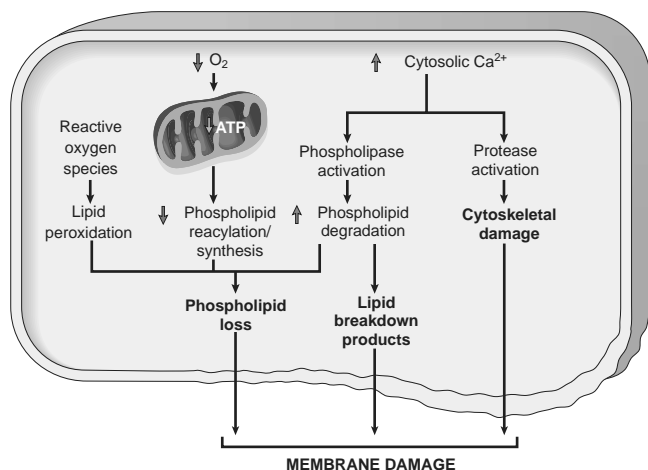
The most important sites of membrane damage during cell injury are the mitochondrial membrane, the plasma membrane, and membranes of lysosomes.

- *Mitochondrial membrane damage.* As discussed earlier, damage to mitochondrial membranes results in decreased production of ATP, with many deleterious effects culminating in necrosis.
- *Plasma membrane damage.* Plasma membrane damage leads to loss of osmotic balance and influx of fluids and ions, as well as loss of cellular contents. The cells may also leak metabolites that are vital for the reconstitution of ATP, thus further depleting energy stores.
- *Injury to lysosomal membranes* results in leakage of their enzymes into the cytoplasm and activation of the acid hydrolases in the acidic intracellular pH of the injured (e.g., ischemic) cell. Lysosomes contain ribonucleases (RNases), DNases, proteases, glucosidases, and other enzymes. Activation of these enzymes leads to enzymatic digestion of cell components, and the cells die by necrosis.

Damage to DNA and Proteins

Cells have mechanisms that repair damage to DNA, but if this damage is too severe to be corrected (e.g., after radiation injury or oxidative stress), the cell initiates its suicide program and dies by apoptosis. A similar reaction is triggered by the accumulation of improperly folded proteins, which may result from inherited mutations or external triggers such as free radicals. Since these mechanisms of cell injury typically cause apoptosis, they are discussed later in the chapter.

SUMMARY

Mechanisms of Cell Injury

- *ATP depletion:* failure of energy-dependent functions → reversible injury → necrosis
- *Mitochondrial damage:* ATP depletion → failure of energy-dependent cellular functions → ultimately, necrosis; under some conditions, leakage of mitochondrial proteins that cause apoptosis
- *Influx of calcium:* activation of enzymes that damage cellular components and may also trigger apoptosis
- *Accumulation of reactive oxygen species:* covalent modification of cellular proteins, lipids, nucleic acids
- *Increased permeability of cellular membranes:* may affect plasma membrane, lysosomal membranes, mitochondrial membranes; typically culminates in necrosis
- *Accumulation of damaged DNA and misfolded proteins:* triggers apoptosis

CLINICOPATHOLOGIC CORRELATIONS: EXAMPLES OF CELL INJURY AND NECROSIS

To illustrate the evolution and biochemical mechanisms of cell injury, we conclude this section by discussing some commonly encountered examples of reversible cell injury and necrosis.

Figure 1–20 Mechanisms of membrane damage in cell injury. Decreased O_2 and increased cytosolic Ca^{2+} typically are seen in ischemia but may accompany other forms of cell injury. Reactive oxygen species, which often are produced on reperfusion of ischemic tissues, also cause membrane damage (*not shown*).