

Chapter 1

Cell Injury, Cell Death, and Adaptations

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INTRODUCTION TO PATHOLOGY

Literally translated, *pathology* is the study (*logos*) of suffering (*pathos*). It is a discipline that bridges clinical practice and basic science, and it involves the investigation of the causes (*etiology*) of disease as well as the underlying mechanisms (*pathogenesis*) that result in the presenting signs and symptoms of the patient. Pathologists use a variety of molecular, microbiologic, and immunologic techniques to understand the biochemical, structural, and functional changes that occur in cells, tissues, and organs. To render diagnoses and guide therapy, 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). Traditionally, the discipline is divided into general pathology and systemic pathology; the former focuses on the fundamental cellular and tissue responses to pathologic stimuli, while

the latter examines the particular responses of 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 tend to maintain their intracellular milieu within a fairly narrow range of physiologic parameters; that is, they maintain normal *homeostasis*. 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, severe or persistent stress 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 is also a normal and essential process in embryogenesis, the development of organs, and the maintenance of homeostasis.

The relationships between 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 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

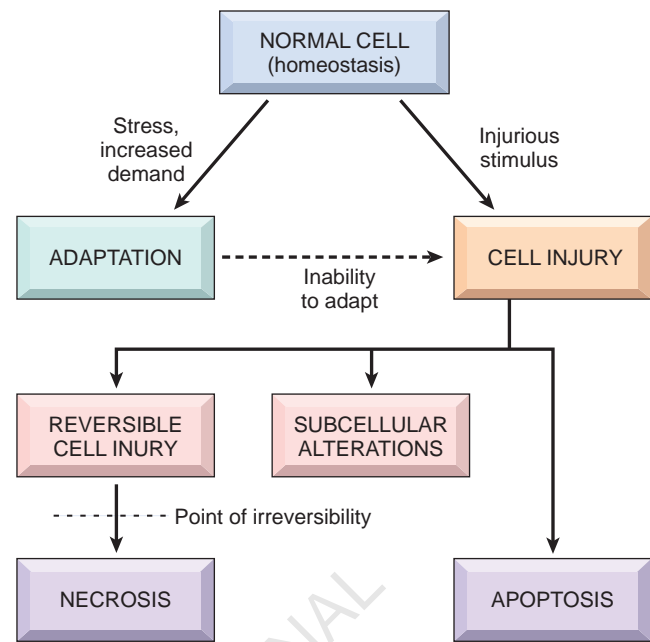


Figure 1–1

Stages in the cellular response to stress and injurious stimuli.

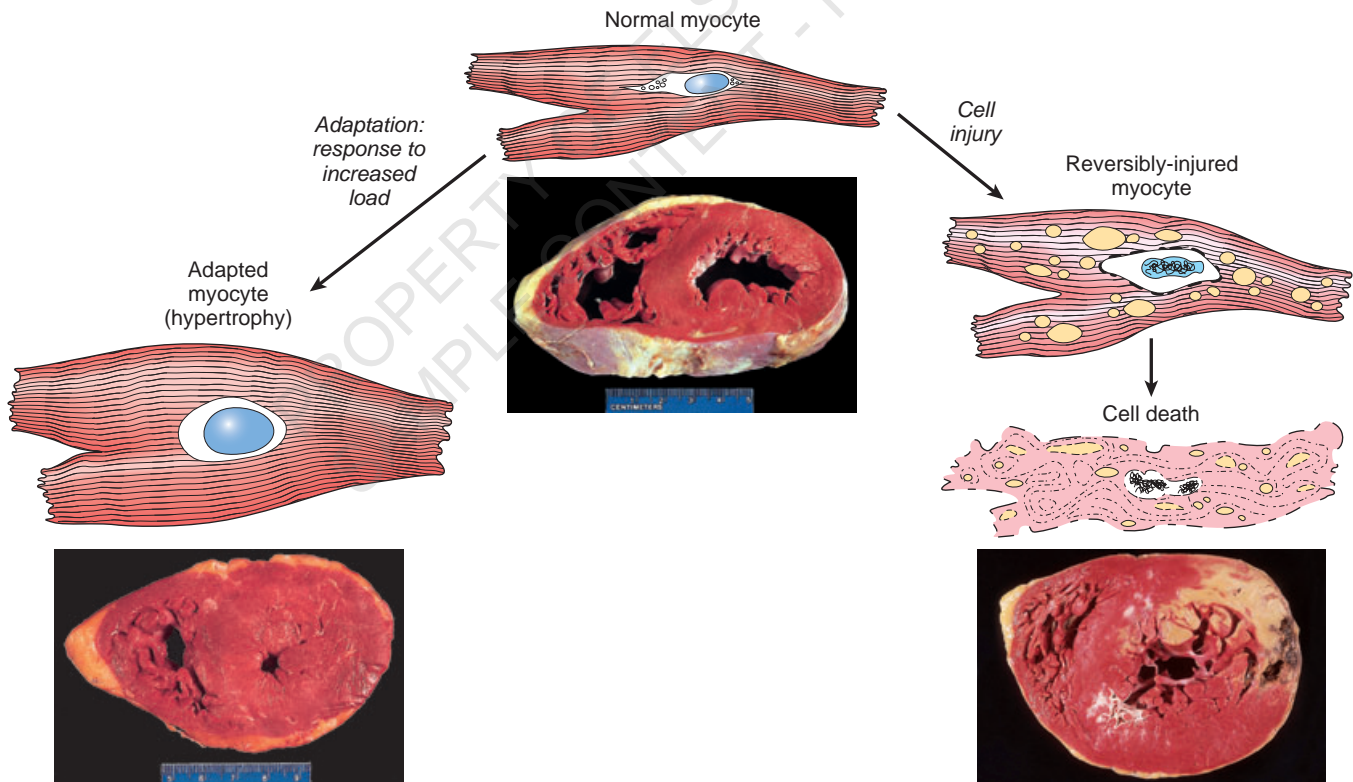


Figure 1–2

The relationship between 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.

is mild or the arterial occlusion is incomplete or sufficiently brief, or it may undergo irreversible injury (*infarction*) after complete or prolonged occlusion. Note, too, that stresses 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, and therefore, even mild injury can have a lethal 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 cellular metabolism, blood supply, and nutritional status.

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. Stated another way, in pure hypertrophy there are no new cells, just bigger cells, enlarged by an increased amount of structural proteins and organelles. Hyperplasia is an adaptive response in cells capable of replication, whereas hypertrophy occurs when cells are incapable of dividing. *Hypertrophy can be physiologic or pathologic* and is caused either by increased functional demand or by specific hormonal stimulation. Hypertrophy and hyperplasia can also occur together, and obviously both result in an enlarged (*hypertrophic*) organ. Thus, 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, the striated muscle cells in both the skeletal muscle and the heart can undergo only hypertrophy in response to increased demand because in the adult they have limited capacity to divide. Therefore, the avid weightlifter can develop a rippled physique only by hypertrophy of individual skeletal muscle cells induced by an increased workload. Examples of pathologic cellular hypertrophy include the cardiac enlargement that occurs with hypertension or aortic valve disease (see Fig. 1–2).

The mechanisms driving cardiac hypertrophy involve at least two types of signals: *mechanical triggers*, such as stretch, and *trophic triggers*, such as activation of α -adrenergic receptors. These stimuli turn on signal transduction pathways that lead to the induction of a number of genes, which in turn stimulate synthesis of numerous cellular proteins, including growth factors and structural proteins. The result is the synthesis of more proteins and

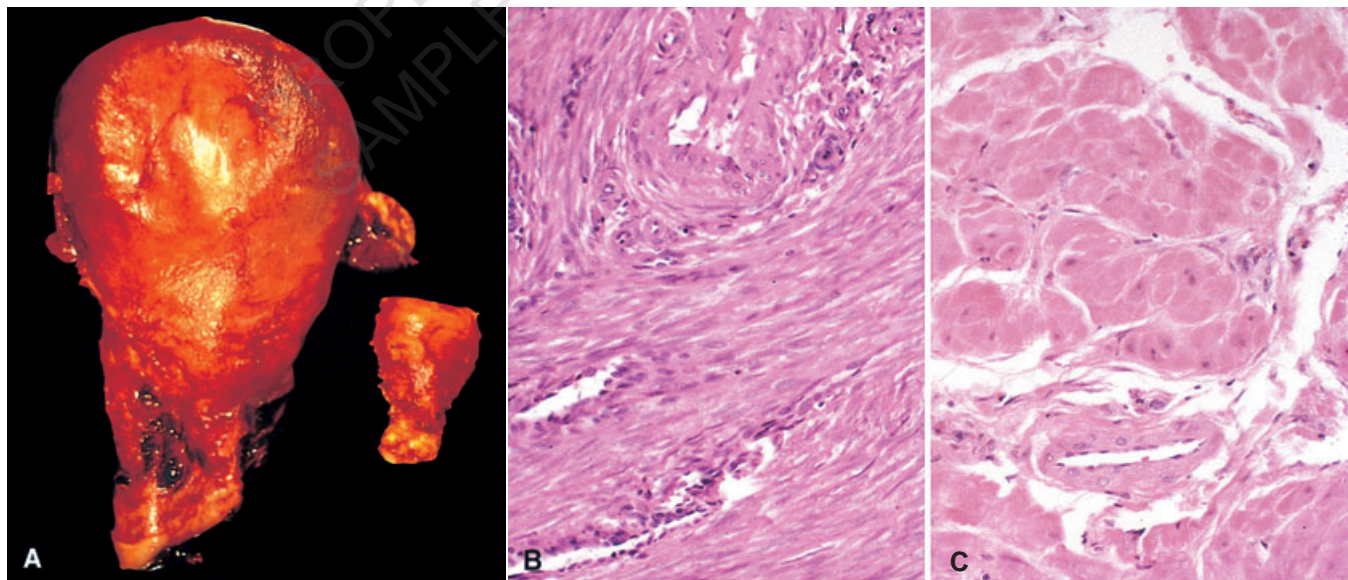


Figure 1–3

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. Compare this with (**C**) large, plump hypertrophied smooth muscle cells from a gravid uterus (**B** and **C**, same magnification).

myofilaments per cell, which achieves improved performance and thus a balance between the demand and the cell's functional capacity. 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 has a slower, more energetically economical contraction. Whatever the exact mechanisms of hypertrophy, a limit is reached beyond which the enlargement of muscle mass 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 above, hyperplasia takes place if the cell population is capable of replication; it may occur with hypertrophy and often in response to the same stimuli.

Hyperplasia can be physiologic or pathologic.

- 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*, that is, hyperplasia that occurs when a portion of the tissue is removed or diseased. For example, when a liver is partially 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 remnant hepatocytes as well as nonparenchymal cells in the liver. After restoration of the liver mass, cell proliferation is "turned off" by various growth inhibitors (Chapter 3).
- 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, if the balance between estrogen and progesterone is disturbed, endometrial hyperplasia ensues, a common cause of abnormal menstrual bleeding. Hyperplasia is also an important response of connective tissue cells in wound healing, in which proliferating fibroblasts and blood vessels aid in repair

(Chapter 3). 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 is also 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 produced by the virus or by infected cells. It is important to note that in all these situations, the hyperplastic process remains controlled; if hormonal or growth factor stimulation abates, the hyperplasia disappears. It is this sensitivity to normal regulatory control mechanisms that distinguishes benign pathologic hyperplasias from cancer, in which the growth control mechanisms become dysregulated or ineffective (Chapter 6). Nevertheless, pathologic hyperplasia constitutes a fertile soil in which cancerous proliferation may eventually arise. Thus, patients with hyperplasia of the endometrium are at increased risk of developing endometrial cancer, and certain papillomavirus infections predispose to cervical cancers (Chapter 19).

Atrophy

Shrinkage in the size of the cell by the loss of cell substance is known as atrophy. When a sufficient number of cells is involved, the entire tissue or organ diminishes in size, becoming atrophic (Fig. 1–4). It should be emphasized that *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), 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.

Atrophy results from 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 these proteins for degradation in proteasomes. This pathway is also thought to be responsible for the accelerated proteolysis seen in a variety of catabolic conditions, including cancer cachexia.

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 find nutrients and survive. We will describe this process later.

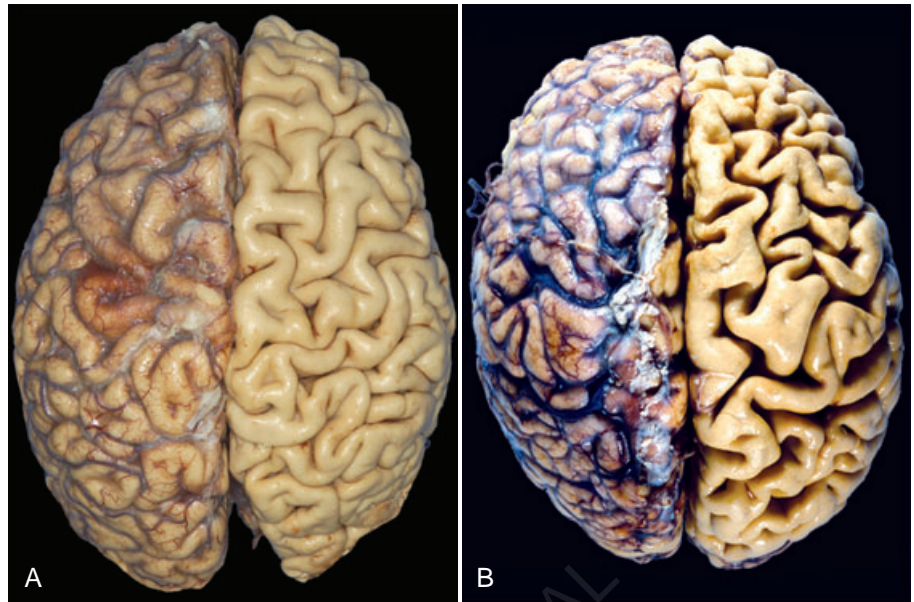


Figure 1-4

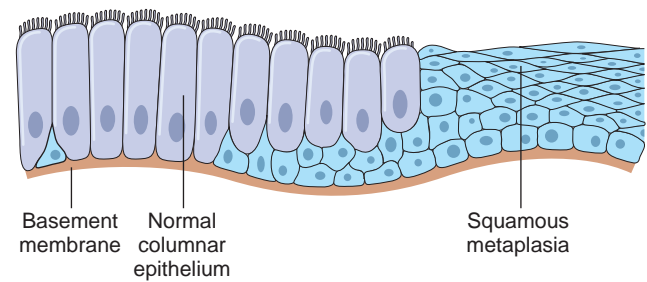
Atrophy. **A**, Normal brain of a young adult. **B**, Atrophy of the brain in an 82-year-old male with atherosclerotic disease. Atrophy of the brain is due to aging and reduced blood supply. Note that loss of brain substance narrows the gyri and widens the sulci. The meninges have been stripped from the right half of each specimen to reveal the surface of the brain.

Metaplasia

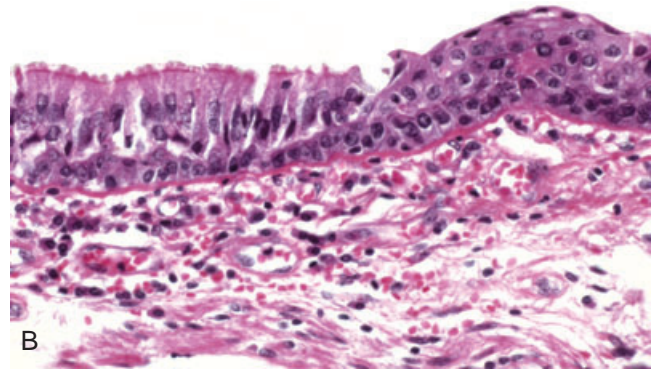
Metaplasia is a reversible change in which one adult cell type (epithelial or mesenchymal) is replaced by another adult cell type. In this type of cellular adaptation, cells sensitive to a particular stress are replaced by other cell types better able to withstand the adverse environment. Metaplasia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

Epithelial metaplasia is exemplified by the squamous change that occurs in the respiratory epithelium in 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. Vitamin A deficiency may also induce squamous metaplasia in the respiratory epithelium. The “rugged” stratified squamous epithelium may be able to survive under circumstances 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 transformation, if persistent, may predispose to malignant transformation of the epithelium.* In fact, in a common form of lung cancer, squamous metaplasia of the respiratory epithelium often coexists with 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. 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 less clearly as an adaptive response. For example, bone is occasionally formed in soft tissues, particularly in foci of injury.



A



B

Figure 1-5

Metaplasia of normal columnar (left) to squamous epithelium (right) in a bronchus, shown (A) schematically and (B) histologically.

SUMMARY

Cellular Adaptations to Stress

- **Hypertrophy:** increased cell and organ size, often in response to increased workload; induced by mechanical stress and by growth factors; 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
- **Atrophy:** decreased cell and organ size, as a result of decreased nutrient supply or disuse; associated with decreased synthesis and increased proteolytic breakdown of cellular organelles
- **Metaplasia:** change in phenotype of differentiated cells, often a 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. Different injurious stimuli affect many metabolic pathways and cellular organelles. Injury may progress through a reversible stage and culminate in cell death (see 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 morphol-*

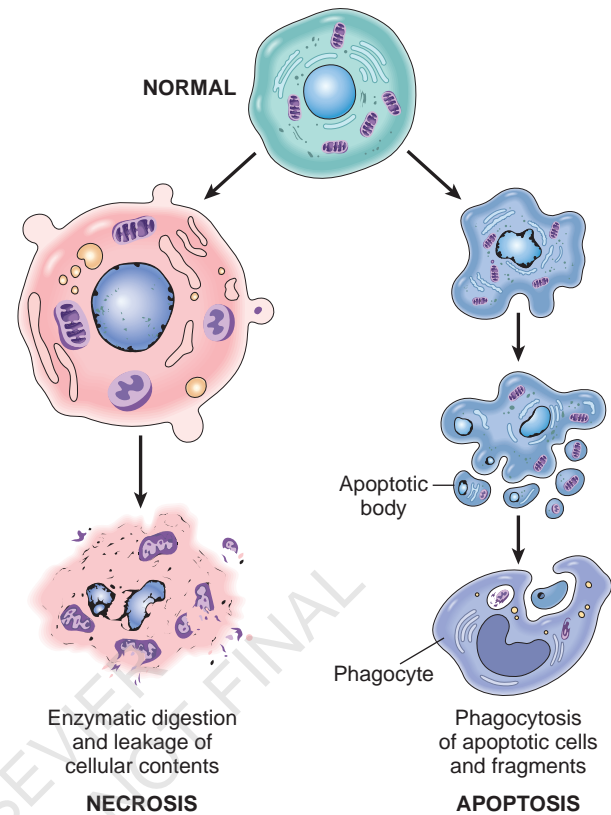


Figure 1-6

Cellular features of necrosis (left) and apoptosis (right). (Adapted from Walker NI, et al: Patterns of cell death. *Methods Archiv Exp Pathol* 13:18–32, 1988. With permission of S. Karger, Basel, Switzerland.)

ogy, mechanisms, 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 out through the damaged plasma membrane and 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, the cell kills itself by another

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 damage

type of death, called *apoptosis*, which is characterized by nuclear dissolution without complete loss of membrane integrity. Apoptosis is an active, energy-dependent, tightly regulated type of cell death that is seen in some specific situations. *Whereas necrosis is always a pathologic process, apoptosis serves many normal functions and is not necessarily associated with pathologic cell injury.* 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 defective enzyme underlying a 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 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 enormous number of chemical substances can injure cells; even innocuous substances such as glucose or salt, if sufficiently concentrated, can so derange the osmotic environment that cell injury or death results. Oxygen at sufficiently high partial pressures is also toxic. 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 these poisons can culminate in the death of the whole organism. Other potentially toxic agents are encountered daily in our environment; these include air pollutants, insecticides, CO, asbestos, and social “stimuli” such as ethanol. Even therapeutic drugs can cause cell or tissue injury in a susceptible patient or if used excessively or inappropriately (Chapter 8).

Infectious Agents. These range from submicroscopic viruses to meter-long tapeworms; in between are the rickettsiae, bacteria, fungi, and protozoans. The diverse ways by which infectious pathogens cause injury are discussed in Chapter 9.

Immunologic Reactions. Although the immune system defends the body against pathogenic microbes, immune reactions can also result in cell and tissue injury. Examples include autoimmune reactions against one’s own tissues and allergic reactions against environmental substances in genetically susceptible individuals (Chapter 5).

Genetic Defects. Genetic defects 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. Genetic defects may cause cell injury because 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. Variations in the genetic makeup can also 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 8). Ironically, excesses of nutrition 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 temperatures, radiation, electric shock, and sudden changes in atmospheric pressure all have wide-ranging effects on cells (Chapter 8).

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 at the end of this chapter.

THE MORPHOLOGY OF CELL AND TISSUE INJURY

It is useful to describe the basic 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 do not appear dead by electron microscopy for 2 to 3 hours, and by light microscopy for 6 to 12 hours.

The cellular derangements of reversible injury can be repaired and, if the injurious stimulus abates, the cell will 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 we can answer it we may be able to devise strategies for preventing cell injury from having permanent deleterious consequences. Although there are no definitive morphologic or biochemical correlates of irreversibility, *two phenomena*

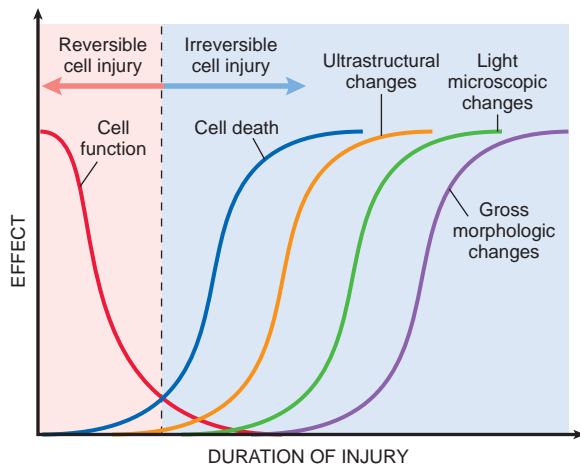


Figure 1-7

The relationship between 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; a longer duration of injury may eventually lead to irreversible injury and cell death. Note also that cell death typically precedes ultrastructural, light microscopic, and grossly visible morphologic changes.

consistently characterize irreversibility: the inability to reverse 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 that is characteristic of necrosis.

Different injurious stimuli may induce death by necrosis or apoptosis (see Fig. 1-6 and Table 1-1). As mentioned above and detailed later, severe depletion of ATP and loss of membrane integrity are typically associated with necrosis. Apoptosis is an active and regulated process not associated with ATP depletion and it has many unique features, which we will describe 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 various forms of toxic or metabolic injury, and is manifested by the appearance of small or large lipid vacuoles in the cytoplasm. It occurs mainly in cells involved in and dependent on fat metabolism, such as hepatocytes and myocardial cells. The mechanisms of fatty change are discussed later in the chapter.

Morphology

Cellular swelling (Fig. 1-8B), the first manifestation of almost all forms of injury to cells, is difficult to appreciate with the light microscope; it may be more apparent at the level of the whole organ. When it affects many cells in an organ it causes some pallor, 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 ER. This pattern of nonlethal injury is sometimes called **hydropic change** or **vacuolar degeneration**. Swelling of cells is reversible. **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 and myocardial cells) and is also reversible. Injured cells may also show increased eosinophilic staining, which becomes much more pronounced with progression to necrosis (described below).

The ultrastructural changes of reversible cell injury are illustrated schematically in Figure 1-9 and 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.

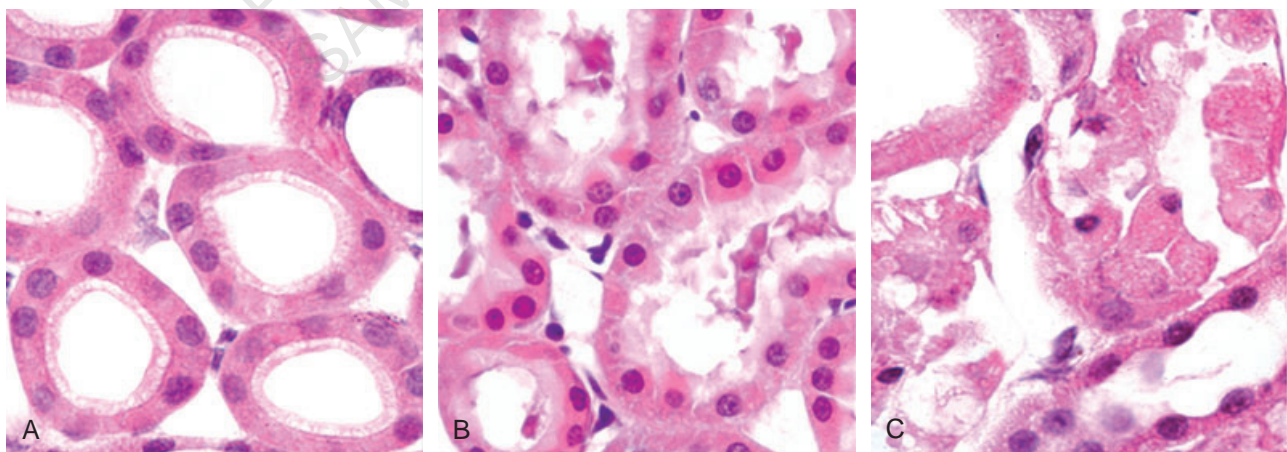


Figure 1-8

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. The ultrastructural features of these stages of cell injury are shown in Fig. 1-9. (Courtesy of Drs. Neal Pinckard and M.A. Venkatachalam, University of Texas Health Sciences Center San Antonio.)

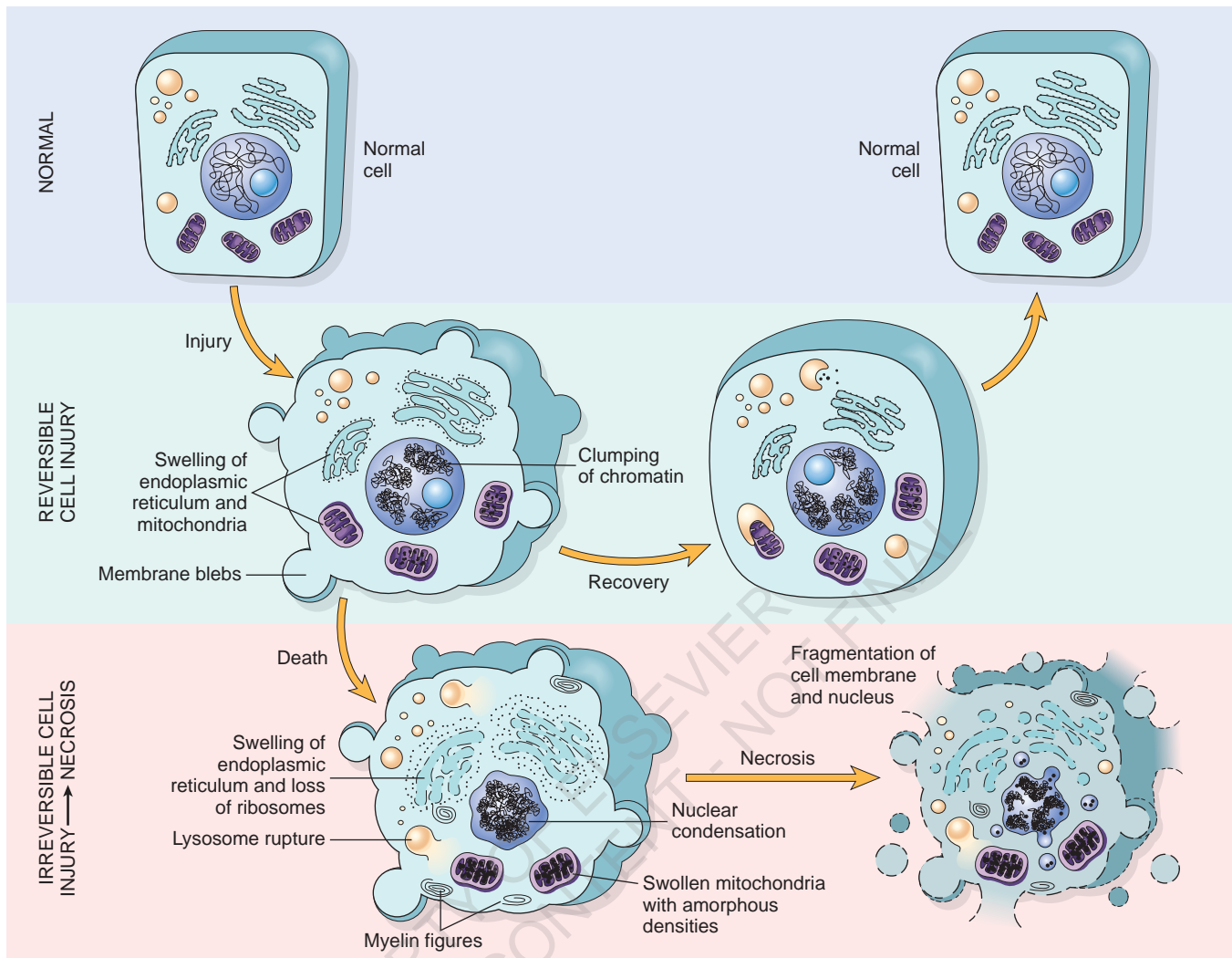


Figure 1-9

A normal cell and the changes in reversible and irreversible cell injury (necrosis).

Necrosis

The term *necrosis* was first used by morphologists to refer to a series of changes that accompany cell death, largely resulting from the degradative action of enzymes on lethally injured cells. Necrotic cells are unable to maintain membrane integrity, and their contents often leak out. The enzymes responsible for digestion of the cell are derived either from the lysosomes of the dying cells themselves or from the lysosomes of leukocytes that are recruited as part of the inflammatory reaction to the dead cells.

Morphology

In one common pattern of cell death resulting from lack of oxygen, the necrotic cells show **increased eosinophilia** (i.e., pink staining from the eosin dye, the "E" in "H&E"). This is 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"). The cell may have a more glassy

homogeneous appearance than viable cells, mostly because of the loss of glycogen particles. When enzymes have digested the cytoplasmic organelles, the cytoplasm becomes vacuolated and appears moth-eaten. Dead cells may be replaced by large, whorled phospholipid masses, called **myelin figures**, that are derived from damaged cellular membranes. They are thought to result from dissociation of lipoproteins with unmasking of phosphatide groups, promoting the uptake and intercalation of water between the lamellar stacks of membranes. These phospholipid precipitates are then either phagocytosed by other cells or further degraded into fatty acids; calcification of such fatty acid residues results in the generation of calcium soaps. Thus, the dead cells may ultimately become **calcified**. By electron microscopy (see Fig. 1-9), 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, intracytoplasmic myelin figures, and profound nuclear changes culminating in nuclear dissolution.

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 completely disappears.

SUMMARY

Morphologic Alterations in Injured Cells

- *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; myelin figures; leakage and enzymatic digestion of cellular contents
- *Apoptosis*: nuclear chromatin condensation; formation of apoptotic bodies (fragments of nuclei and cytoplasm)

Patterns of Tissue Necrosis

Necrosis of a collection of cells in a tissue or an organ, for instance in the ischemic myocardium, results in death of the entire tissue and sometimes an entire organ. 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, the terms are used often and their implications are understood by both pathologists and clinicians.

Morphology

Coagulative necrosis is a form of tissue necrosis in which the component cells are dead but the basic tissue architecture is preserved for at least several days (Fig. 1–10). The affected tissues take on a firm texture. Presumably the injury denatures not only structural proteins but also enzymes and so blocks the proteolysis of the dead cells; as a result, eosinophilic, anucleate cells may persist for days or weeks. Ultimately, the necrotic cells are removed by phagocytosis of the cellular debris by infiltrating leukocytes and by digestion of the dead cells by the action of lysosomal enzymes of the leukocytes. Coagulative necrosis is characteristic of **infarcts** (areas of ischemic necrosis) in all 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. 1–11). Whatever the pathogenesis, liquefaction completely digests the dead cells, resulting in transformation of the tissue into a liquid viscous mass. If the process was initiated by acute inflammation, 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 is usually applied to 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 (so-called **wet gangrene**).

Caseous necrosis is encountered most often in foci of tuberculous infection. The term “caseous” (cheese-like) is derived from the friable yellow-white appearance of the area of necrosis (Fig. 1–12). On microscopic examination, the necrotic focus appears as a collection of fragmented or lysed cells with an amorphous granu-

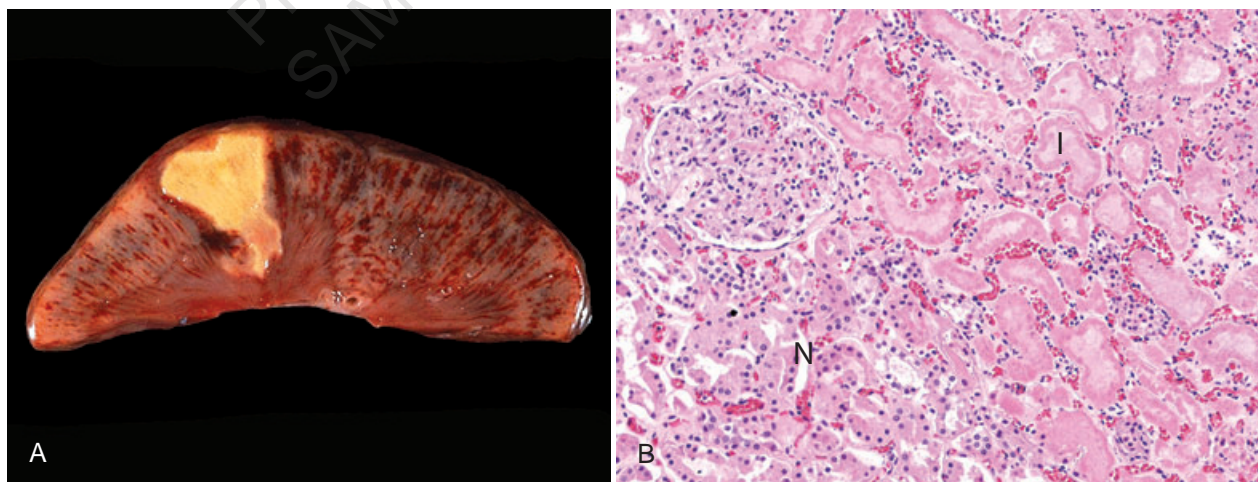


Figure 1–10

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).

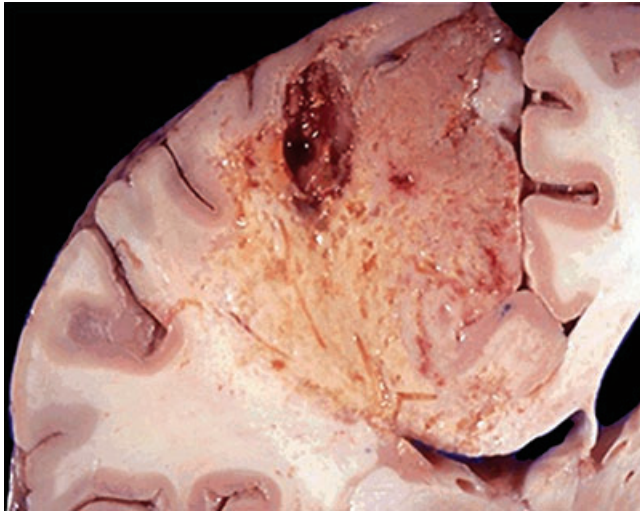


Figure 1-11

Liquefactive necrosis. An infarct in the brain, showing dissolution of the tissue.

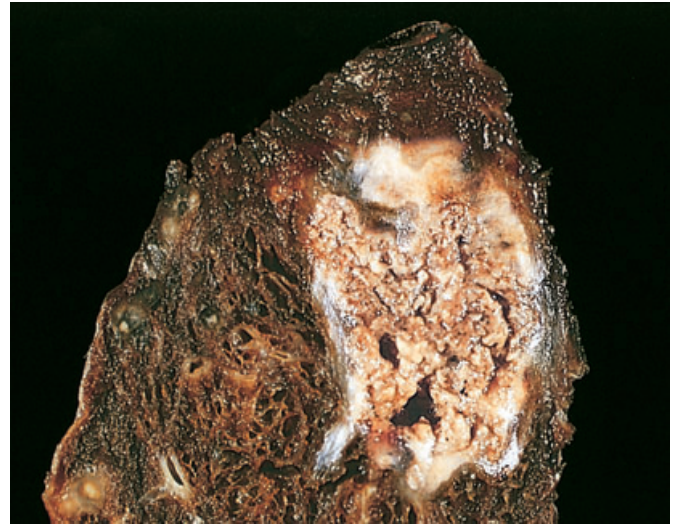


Figure 1-12

Caseous necrosis. A tuberculous lung with a large area of caseous necrosis containing yellow-white and cheesy debris.

lar appearance. Unlike coagulative necrosis, the tissue architecture is completely obliterated and cellular outlines cannot be discerned. 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, a term that is well fixed in medical parlance, 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 17). In this disorder, pancreatic enzymes that have leaked out of acinar cells 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. 1-13). 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 usually seen in immune reactions involving blood vessels. This pattern of necrosis is prominent when complexes of antigens and antibodies are deposited in the walls of arteries. Deposits of these “immune complexes,” together with fibrin that has leaked out of vessels, result in a bright pink and amorphous appearance in H&E stains, called “fibrinoid” (fibrin-like) by pathologists (Fig. 1-14). The immunologically mediated diseases (e.g., polyarteritis nodosa) in which this type of necrosis is seen are described in Chapter 5.

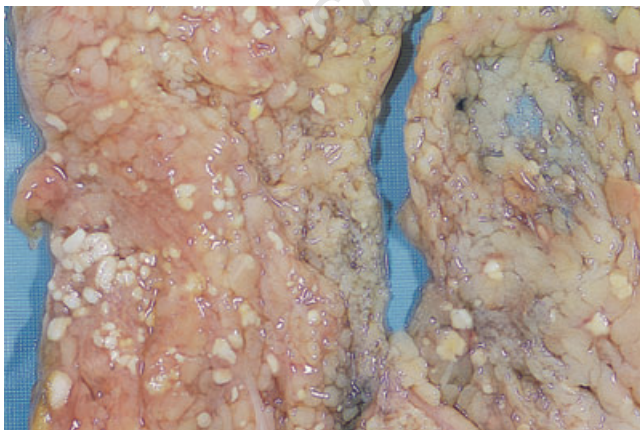


Figure 1-13

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.

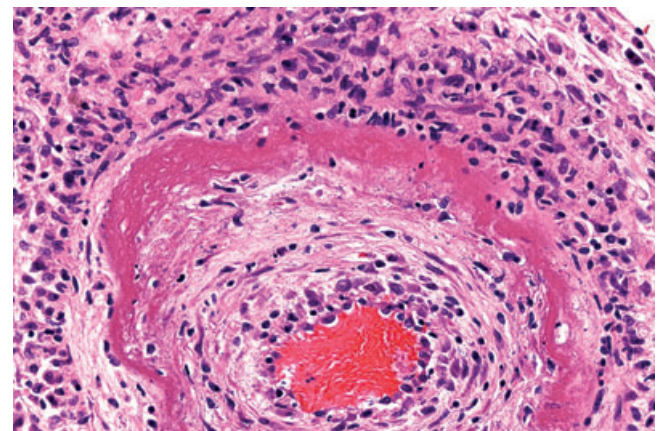


Figure 1-14

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 (dark nuclei of neutrophils).

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 are reflected in increased serum levels of such proteins, and measurement of serum levels is used clinically to assess damage to these tissues.

Subcellular Responses to Injury

Thus far we have mainly focused on the whole tissue or the cell as a unit. However, certain agents and stresses induce distinctive alterations involving only subcellular organelles. Although some of these alterations occur in acute lethal injury, others are seen in chronic forms of cell injury, and still others are adaptive responses. In this section, some of the more common and interesting of these reactions are discussed.

Autophagy. Autophagy refers to lysosomal digestion of the cell's own components and is contrasted with *heterophagy*, in which a cell (usually a macrophage) ingests substances from the outside for intracellular destruction (Fig. 1–15). Autophagy is thought to be a survival mechanism in times of nutrient deprivation, such that the starved cell lives by eating its own contents. In this process, intracellular organelles and portions of cytosol are first sequestered from the cytoplasm in an *autophagic vacuole* formed from ribosome-free regions of the rough ER (RER). The vacuole fuses with lysosomes to form an *autophagolysosome*, and the cellular components are digested by lysosomal enzymes. Autophagy is initiated by

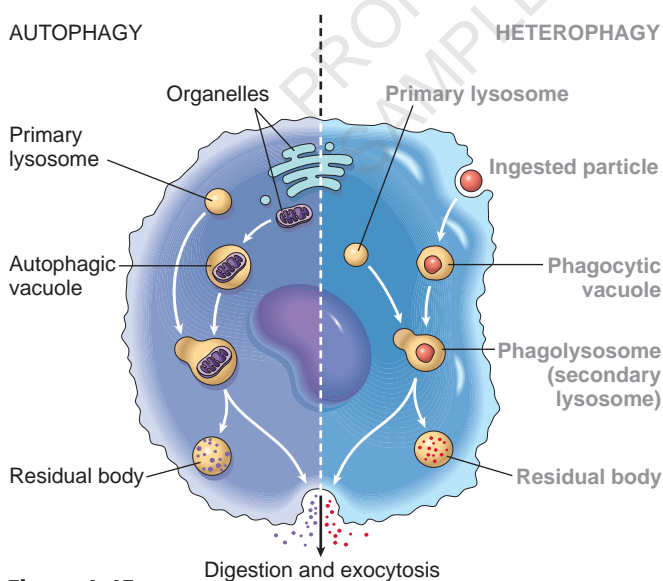


Figure 1-15

Autophagy (right) and heterophagy (left). (Redrawn from Fawcett DW: A Textbook of Histology, 11th ed. Philadelphia, WB Saunders, 1986, p 17.)

several proteins that sense nutrient deprivation. If it is not corrected, autophagy may also signal cell death by apoptosis, a way of telling a stressed or starved cell that it can no longer cope by living on its own organelles.

The enzymes in lysosomes can break down most proteins and carbohydrates, although some lipids remain undigested. Lysosomes with undigested debris may persist within cells as *residual bodies* or may be extruded. *Lipofuscin pigment* granules represent indigestible material resulting from free radical-mediated lipid peroxidation. Certain indigestible pigments, such as carbon particles inhaled from the atmosphere or inoculated pigment in tattoos, can persist in phagolysosomes of macrophages for decades (discussed later).

Lysosomes are also repositories wherein cells sequester materials that cannot be completely degraded. Hereditary *lysosomal storage disorders*, caused by deficiencies of enzymes that degrade various macromolecules, result in abnormal collections of intermediate metabolites in the lysosomes of cells all over the body; neurons are particularly susceptible to lethal injury from such accumulations (Chapter 7).

Induction (Hypertrophy) of Smooth ER. The smooth ER (SER) 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 SER. Prolonged 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 SER of hepatocytes and 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, 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 have subtherapeutic levels of the anti-seizure medication because of induction of SER in response to the alcohol.

Mitochondrial Alterations. As described later, mitochondrial dysfunction plays an important role in acute cell injury and death. In some nonlethal pathologic conditions, however, there may be alterations in the number, size, shape, and presumably function of mitochondria. For example, in cellular hypertrophy there is an increase in the number of mitochondria in cells; conversely, mitochondria decrease in number during cellular atrophy (probably via autophagy). Mitochondria may assume extremely large and abnormal shapes (*megamitochondria*), as seen in hepatocytes in various nutritional deficiencies and alcoholic liver disease. In certain inherited metabolic diseases of skeletal muscle, the *mitochondrial myopathies*, defects in mitochondrial metabolism are

associated with increased numbers of unusually large mitochondria containing abnormal cristae.

Cytoskeletal Abnormalities. The cytoskeleton consists of actin and myosin filaments, microtubules, and various classes of intermediate filaments; several other nonpolymerized and nonfilamentous forms of contractile proteins also contribute to the cellular scaffold. The cytoskeleton is important for many cellular functions, including

- Intracellular transport of organelles and molecules
- Maintenance of basic cell architecture (e.g., cell polarity, distinguishing up and down)
- Transmission of cell-cell and cell–extracellular matrix signals to the nucleus
- Maintenance of mechanical strength for tissue integrity
- Cell mobility
- Phagocytosis

Cells and tissues respond to environmental stresses (e.g., shear stress in blood vessels or increased pressures in the heart) by constantly remodeling their intracellular scaffolding. Abnormalities of the cytoskeleton occur in a variety of pathologic states. These abnormalities may be manifested as an abnormal appearance and function of cells (hypertrophic cardiomyopathy; Chapter 11), aberrant movements of intracellular organelles, defective cell locomotion, or intracellular accumulations of fibrillar material as in alcoholic liver disease (Chapter 16). Perturbations in the organization of *microtubules* can cause sterility by inhibiting sperm motility, as well as defective mobility of cilia in the respiratory epithelium, resulting in chronic infections due to impaired clearance of inhaled bacteria (*Kartagener*, or the *immotile cilia, syndrome*). Microtubules are also essential for leukocyte migration and phagocytosis. Drugs that prevent microtubule polymerization (e.g., colchicine) are useful in treating gout, in which symptoms are due to movement of macrophages toward urate crystals with subsequent frustrated attempts at phagocytosis and inflammation. Since microtubules form the mitotic spindle, drugs that bind to microtubules (e.g., vinca alkaloids) are also antiproliferative and may therefore be useful as antitumor agents.

SUMMARY

Subcellular Alterations in Cell Injury: Effects of Injurious Agents on Organelles and Cellular Components

Some forms of cell injury affect particular organelles and have unique manifestations.

- **Autophagy:** In nutrient-deprived cells, organelles are enclosed in vacuoles that fuse with lysosomes. The organelles are digested but in some cases indigestible pigment (e.g. lipofuscin) remains.
- **Hypertrophy of SER:** Cells exposed to toxins that are metabolized in the SER show hypertrophy of the ER, a compensatory mechanism to maximize removal of the toxins.

- **Mitochondrial alterations:** Changes in the number, size, and shape of mitochondria are seen in diverse adaptations and responses to chronic injury.
- **Cytoskeletal alterations:** Some drugs and toxins interfere with the assembly and functions of cytoskeletal filaments or result in abnormal accumulations of filaments.

MECHANISMS OF CELL INJURY

Now that we have discussed the causes of cell injury and necrosis and their morphologic and functional correlates, 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. It is therefore often difficult to pinpoint specific molecular alterations caused by a particular insult. 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 also be important. 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 and in disease susceptibility. The study of such interactions is called pharmacogenomics.
- *Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components* (Fig. 1–16). The most important targets of injurious stimuli are (1) mitochondria, the sites of ATP generation; (2) cell membranes, on which the ionic and osmotic homeostasis of the cell and its organelles depends; (3) protein synthesis; (4) the cytoskeleton; and (5) the genetic apparatus of the cell.

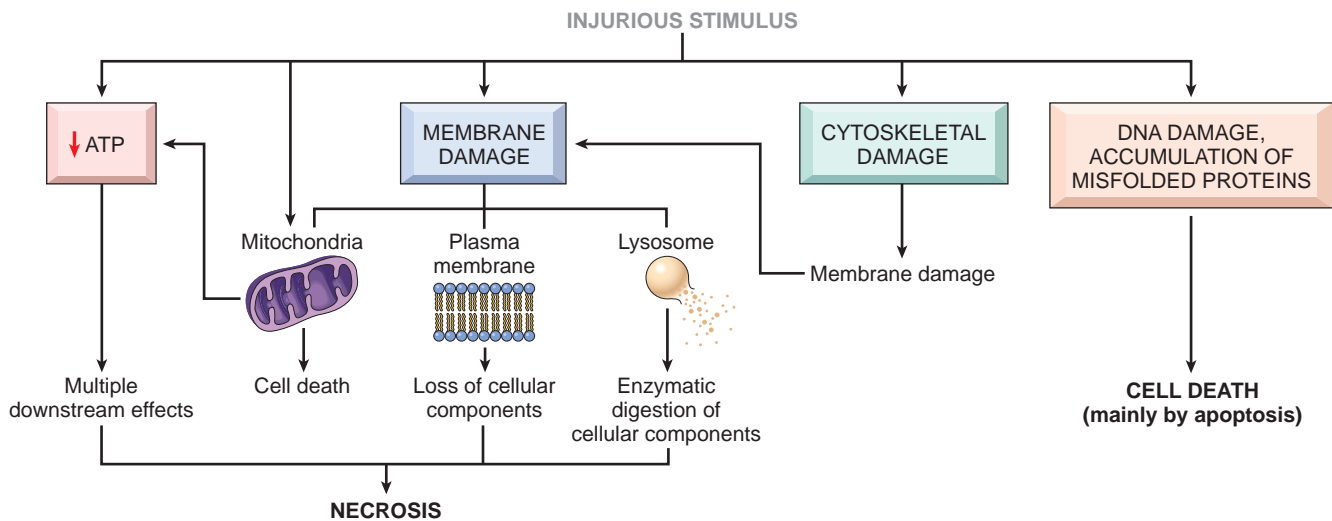


Figure 1-16

The principal cellular and biochemical sites of damage in cell injury. Note that loss of adenosine triphosphate (ATP) results first in reversible injury (not shown) and culminates in necrosis. Mitochondrial damage may lead to reversible injury and death by necrosis or apoptosis.

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. *Depletion of ATP to less than 5% to 10% of normal levels has widespread effects on many critical cellular systems* (Fig. 1-17).

- The activity of the *plasma membrane energy-dependent sodium pump* 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 the Ca^{2+} pump* leads to influx of Ca^{2+} , with damaging effects on numerous cellular components, described below.

- Prolonged or worsening depletion of ATP causes *structural disruption of the protein synthetic apparatus*, manifested as detachment of ribosomes from the rough endoplasmic reticulum (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.

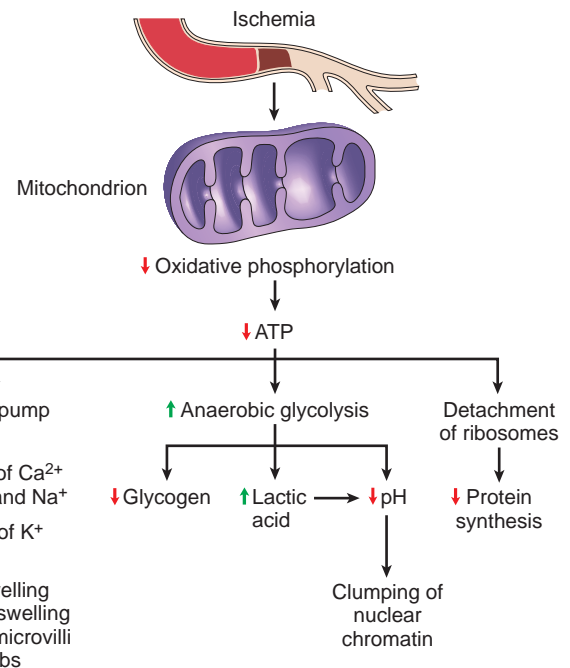


Figure 1-17

The initial functional and morphologic consequences of decreased intracellular adenosine triphosphate (ATP) during cell injury. ER, Endoplasmic reticulum.

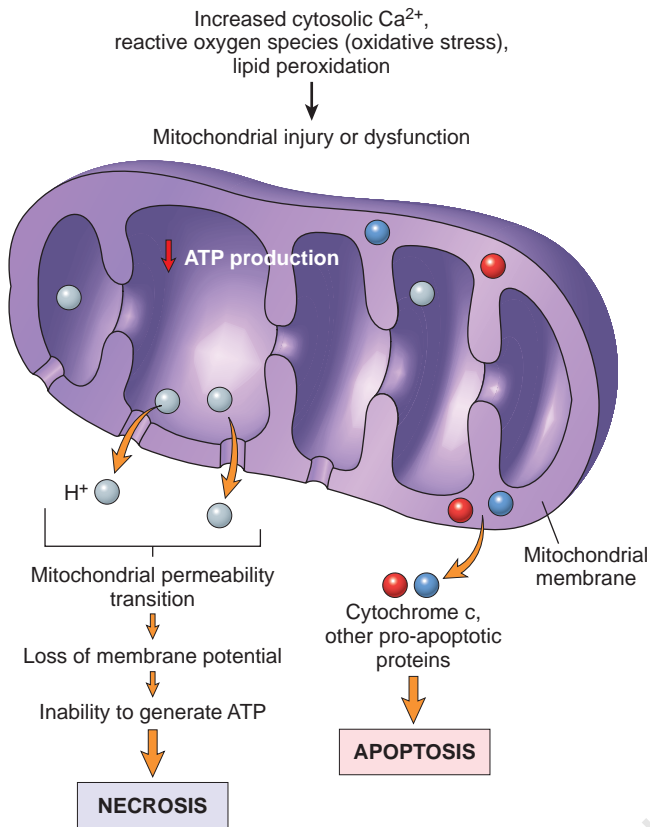


Figure 1-18

Consequences of mitochondrial dysfunction, culminating in cell death by necrosis or apoptosis. ATP, Adenosine triphosphate.

Damage to Mitochondria

Mitochondria are the cell's suppliers of life-sustaining energy in the form of ATP, but they are also critical players in cell injury and death. Mitochondria can be damaged by increases of cytosolic Ca^{2+} , reactive oxygen species (discussed below), and oxygen deprivation, and so they are sensitive to virtually all types of injurious stimuli, including hypoxia and toxins. There are two major consequences of mitochondrial damage (Fig. 1-18):

- Mitochondrial damage often results in 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 and pH changes, resulting in *failure of oxidative phosphorylation and progressive depletion of ATP*, culminating in necrosis of the cell.
- The mitochondria also contain several proteins that are capable of activating apoptotic pathways, including cytochrome *c* (the major protein involved in electron transport). Increased permeability of the mitochondrial membrane may result in leakage of these proteins into the cytosol and *death by apoptosis*. Thus, cytochrome *c* plays a key dual role in cell survival and death; in its normal location inside mitochondria, it is essential for energy generation and the life of the cell, but when mitochondria are damaged so severely that cytochrome *c* leaks out, it signals cells to die.

Influx of Calcium

Cytosolic free calcium is normally maintained by ATP-dependent calcium transporters at concentrations that are 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. 1-19). 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 also result in the induction of apoptosis, by direct activation of caspases and by increasing mitochondrial permeability. The importance of Ca^{2+} in cell injury was established by the finding that depleting extracellular Ca^{2+} delays cell death after hypoxia and exposure to some toxins.

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 readily react with inorganic and

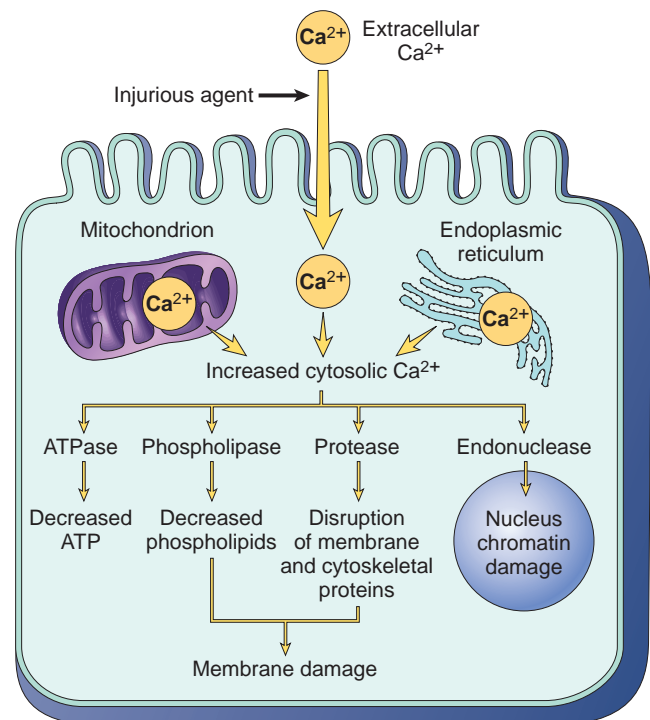


Figure 1-19

Sources and consequences of increased cytosolic calcium in cell injury. ATP, Adenosine triphosphate; ATPase, adenosine triphosphatase.

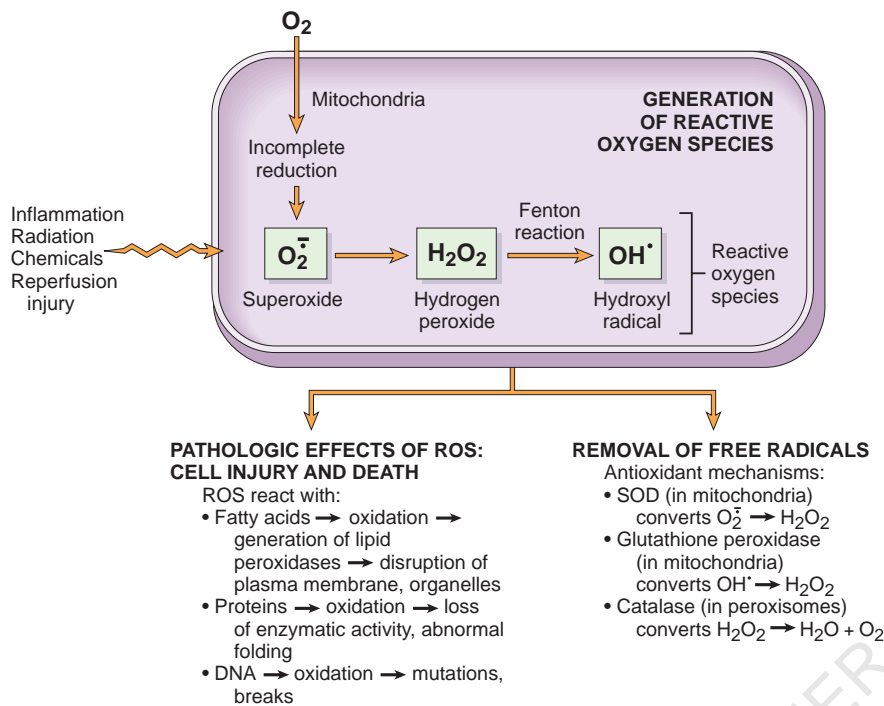


Figure 1–20

The role of reactive oxygen species (ROS) in cell injury. O_2 is converted to superoxide (O_2^-) by oxidative enzymes in the endoplasmic reticulum, 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). Also not shown is another potentially injurious free radical, singlet oxygen. Resultant free-radical damage to lipid (by peroxidation), proteins, and deoxyribonucleic acid (DNA) leads to various forms of cell injury. The major antioxidant enzymes are superoxide dismutase (SOD), catalase, and glutathione peroxidase.

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 autocatalytic reactions; molecules that react with free radicals are in turn converted into free radicals, thus 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. They are produced normally in cells during mitochondrial respiration and energy generation, but they are degraded and removed by cellular defense systems. 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*. Cell injury in many circumstances involves damage by free radicals; these situations include ischemia-reperfusion (discussed below), chemical and radiation injury, toxicity from oxygen and other gases, cellular aging, microbial killing by phagocytic cells, and tissue injury caused by inflammatory cells.

The accumulation of free radicals is determined by their rates of production and removal (Fig. 1–20). Several reactions are responsible for the *generation of free radicals*.

- The reduction-oxidation (redox) reactions that occur during normal mitochondrial metabolism. During normal respiration, for example, molecular oxygen is sequentially reduced in mitochondria by the addition of four electrons to generate water. In the process, small amounts of toxic intermediate species are generated by partial reduction of oxygen; these include superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and OH^* . Transition metals such as copper and iron also accept or donate free electrons during certain intracellular reactions and thereby catalyze free-radical formation, as in the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^* + OH^-$).

- The absorption of radiant energy (e.g., ultraviolet light, x-rays). Ionizing radiation can hydrolyze water into hydroxyl (OH^*) and hydrogen (H^*) free radicals.
- The enzymatic metabolism of exogenous chemicals (e.g., carbon tetrachloride; see later)
- Inflammation, since free radicals are produced by leukocytes that enter tissues (see Chapter 2)
- Nitric oxide (NO), an important chemical mediator normally synthesized by a variety of cell types (Chapter 2), can act as a free radical or can be converted into highly reactive nitrite species

Cells have developed many *mechanisms to remove free radicals* and thereby minimize injury. Free radicals are inherently unstable and decay spontaneously. There are also several nonenzymatic and enzymatic systems that contribute to inactivation of free-radical reactions (see Fig. 1–20).

- The rate of spontaneous decay of superoxide is significantly increased by the action of superoxide dismutases (SODs) found in many cell types (catalyzing the reaction $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$).
- Glutathione (GSH) peroxidase also protects against injury by catalyzing free-radical breakdown: $2OH^* + 2GSH \rightarrow 2H_2O + GSSG$ (glutathione homodimer). The intracellular ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) is a reflection of the oxidative state of the cell and an important aspect of the cell's ability to catabolize free radicals.
- Catalase, present in peroxisomes, directs the degradation of hydrogen peroxide ($2H_2O_2 \rightarrow O_2 + 2H_2O$).
- 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.

- As mentioned above, *iron* and *copper* can catalyze the formation of ROS. The levels of these reactive metals are reduced by binding of the ions to storage and transport proteins (e.g., transferrin, ferritin, lactoferrin, and ceruloplasmin), thereby decreasing the formation of ROS.

ROS have many diverse effects on cells and have even been implicated in activation of cells by a variety of physiologic stimuli. However, three reactions are particularly relevant to *cell injury mediated by free radicals* (see Fig. 1–20):

- *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 of 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 fragmentation.* 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.

Defects in Membrane Permeability

Early loss of selective membrane permeability leading ultimately to overt membrane damage is a consistent feature of most forms of cell injury (except apoptosis). 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. 1–21):

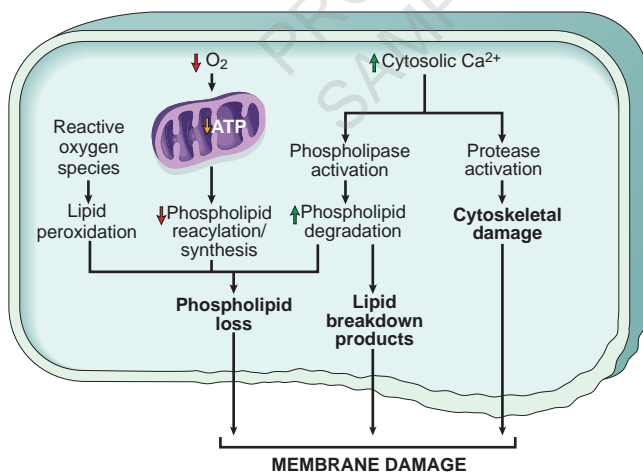


Figure 1–21

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).

- *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 mitochondria themselves, thus exacerbating the loss of ATP.

- *Increased phospholipid breakdown.* Severe cell injury is associated with increased degradation of membrane phospholipids, probably due 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 serve as anchors connecting the plasma membrane to the cell interior. Activation of proteases by increased cytosolic Ca^{2+} may cause damage to elements of the cytoskeleton.

- *Lipid breakdown products.* These include unesterified free fatty acids, acyl carnitine, and lysophospholipids, catabolic products that are known to accumulate in injured cells as a result of phospholipid degradation. They have a detergent effect on membranes. They also either insert into the lipid bilayer of the membrane or exchange with membrane phospholipids, potentially 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 above, damage to mitochondrial membranes results in decreased production of ATP, culminating in necrosis, and release of proteins that trigger apoptotic death.

- *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 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 improperly folded proteins, which may be the result of 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 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

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.

Ischemic and Hypoxic Injury

Ischemia, or diminished blood flow to a tissue, is the most common cause of cell injury in clinical medicine. In contrast to hypoxia, in which energy generation by anaerobic glycolysis can continue (albeit less efficiently than by oxidative pathways), ischemia also compromises the delivery of substrates for glycolysis. Consequently, anaerobic energy generation also ceases in ischemic tissues after potential substrates are exhausted or when glycolysis is inhibited by the accumulation of metabolites that would normally be removed by blood flow. Therefore, *ischemia injures tissues faster than does hypoxia*. The biochemical and structural changes in oxygen-deprived cells were discussed in detail earlier and the sequence of events is recapitulated below.

The fundamental biochemical abnormality in hypoxic cells that leads to cell injury is reduced intracellular generation of ATP, as a consequence of reduced supply of oxygen. As described above, loss of ATP leads to the *failure of many energy-dependent cellular systems, including* (1) *ion pumps* (leading to cell swelling, and influx of Ca^{2+} , with its deleterious consequences); (2) *depletion of glycogen stores*, apparent histologically by reduced staining for carbohydrates (e.g., by the periodic acid–Schiff stain), with accumulation of lactic acid, thus lowering the intracellular pH; and (3) *reduction in protein synthesis*.

The functional consequences may be severe at this stage. For instance, heart muscle ceases to contract within 60 seconds of coronary occlusion. However, loss of contractility does not mean cell death. If hypoxia continues, worsening ATP depletion causes further deterioration, with loss of microvilli and the formation of “blebs” (see Fig. 1–9). At this time, the entire cell and its organelles (mitochondria, ER) are markedly swollen, with increased concentrations of water, sodium, and chloride and a decreased concentration of potassium. *If oxygen is restored, all of these disturbances are reversible.*

If ischemia persists, irreversible injury and necrosis ensue. Irreversible injury is associated with severe swelling of mitochondria, extensive damage to plasma membranes, and swelling of lysosomes (see Fig. 1–9). Massive influx of calcium into the cell may occur. Death is mainly by necrosis, but apoptosis also contributes; the apoptotic pathway is activated probably by release of pro-apoptotic molecules from leaky mitochondria. The cell's components are progressively degraded, and there is widespread leakage of cellular enzymes into the extracellular space. Finally, the dead cells may become replaced by large masses composed of phospholipids in the form of myelin figures. These are then either phagocytosed by leukocytes or degraded further into fatty acids that may become calcified.

Ischemia-Reperfusion Injury

If cells are reversibly injured, the restoration of blood flow can result in cell recovery. However, under certain circumstances, the restoration of blood flow to ischemic but otherwise viable tissues results, paradoxically, in exacerbated and accelerated injury. As a result, tissues sustain the loss of cells *in addition to those that are irreversibly damaged at the end of the ischemic episode*. This so-called *ischemia-reperfusion injury* is a clinically important process that may contribute significantly to tissue damage in myocardial and cerebral infarctions.

Several mechanisms may account for the exacerbation of cell injury resulting from reperfusion into ischemic tissues:

- New damage may be initiated during reoxygenation by increased generation of ROS from parenchymal and endothelial cells and from infiltrating leukocytes. When the supply of oxygen is increased, there may be a corresponding increase in the production of ROS, especially because mitochondrial damage leads to incomplete reduction of oxygen, and because of the action of oxidases in leukocytes, endothelial cells, or parenchymal cells. Cellular antioxidant defense mechanisms may also be compromised by ischemia, favoring the accumulation of free radicals.
- Ischemic injury is associated with *inflammation*, which may increase with reperfusion because of increased influx of leukocytes and plasma proteins. The products of activated leukocytes may cause additional tissue injury (Chapter 2). Activation of the *complement system* may also contribute to ischemia-reperfusion injury. Some antibodies have a propensity to deposit in ischemic tissues for unknown reasons, and when blood

flow is resumed, complement proteins bind to the deposited antibodies, are activated, and exacerbate the cell injury and inflammation.

Chemical (Toxic) Injury

Chemicals induce cell injury by one of two general mechanisms.

- *Some chemicals act directly by combining with a critical molecular component or cellular organelle.* For example, in mercuric chloride poisoning, mercury binds to the sulfhydryl groups of various cell membrane proteins, causing inhibition of ATP-dependent transport and increased membrane permeability. Many anti-neoplastic chemotherapeutic agents also induce cell damage by direct cytotoxic effects. In such instances, *the greatest damage is sustained by the cells that use, absorb, excrete, or concentrate the compounds.*
- *Many other chemicals are not intrinsically biologically active but must be first converted to reactive toxic metabolites, which then act on target cells.* This modification is usually accomplished by the P-450 mixed-function oxidases in the smooth endoplasmic reticulum of the liver and other organs. Although the metabolites might cause membrane damage and cell injury by direct covalent binding to protein and lipids, the most important mechanism of cell injury involves the formation of free radicals. *Carbon tetrachloride* (CCl_4 , which was used widely in the dry cleaning industry but is now banned) and the analgesic *acetaminophen* belong in this category. CCl_4 , for example, is converted to the toxic free radical $\text{CCl}_3\cdot$, principally in the liver. The free radicals cause autocatalytic membrane phospholipid peroxidation, with rapid breakdown of the ER. In less than 30 minutes after exposure to CCl_4 , there is a decline in hepatic protein synthesis of enzymes and plasma proteins; within 2 hours, swelling of the smooth endoplasmic reticulum and dissociation of ribosomes from the smooth endoplasmic reticulum have occurred. There is reduced lipid export from the hepatocytes, as a result of their inability to synthesize apoprotein to form complexes with triglycerides and thereby facilitate lipoprotein secretion; the result is the “fatty liver” of CCl_4 poisoning. Mitochondrial injury follows, and subsequently diminished ATP stores result in defective ion transport and progressive cell swelling; the plasma membranes are further damaged by fatty aldehydes produced by lipid peroxidation in the ER. The end result can be calcium influx and eventually cell death.

APOPTOSIS

Apoptosis is a pathway of cell death that is induced by a tightly regulated suicide program in which cells destined to die activate enzymes capable of degrading the cells' own nuclear DNA and nuclear and cytoplasmic proteins. Fragments of the apoptotic cells then break off, giving the appearance that is responsible for the name (*apoptosis*, “falling off”). The plasma membrane of the apoptotic cell remains intact, but the membrane is altered in such a way

that the cell and its fragments become avid targets for phagocytes. The dead cell is rapidly cleared before its contents have leaked out, and therefore cell death by this pathway does not elicit an inflammatory reaction in the host. Thus, apoptosis differs from necrosis, which is characterized by loss of membrane integrity, enzymatic digestion of cells, leakage of cellular contents, and frequently a host reaction (see Fig. 1–6 and Table 1–1). However, apoptosis and necrosis sometimes coexist, and apoptosis induced by some pathologic stimuli may progress to necrosis.

Causes of Apoptosis

Apoptosis occurs normally in many situations, and serves to eliminate potentially harmful cells and cells that have outlived their usefulness. It is also a pathologic event when cells are damaged beyond repair, especially when the damage affects the cell's DNA or proteins; in these situations, the irreparably damaged cell is eliminated.

Apoptosis in Physiologic Situations

Death by apoptosis is a normal phenomenon that serves to eliminate cells that are no longer needed and to maintain a steady number of various cell populations in tissues. It is important in the following physiologic situations:

- *The programmed destruction of cells during embryogenesis*, including implantation, organogenesis, developmental involution, and metamorphosis. The term “programmed cell death” was originally coined to denote death of specific cell types at defined times during the development of an organism. Apoptosis is a generic term for this pattern of cell death, regardless of the context, but it is often used interchangeably with “programmed cell death.”
- *Involution of hormone-dependent tissues upon hormone deprivation*, such as endometrial cell breakdown during the menstrual cycle, and regression of the lactating breast after weaning
- *Cell loss in proliferating cell populations*, such as intestinal crypt epithelia, so as to maintain a constant number
- *Death of cells that have served their useful purpose*, such as neutrophils in an acute inflammatory response, and lymphocytes at the end of an immune response. In these situations, cells undergo apoptosis because they are deprived of necessary survival signals, such as growth factors.
- *Elimination of potentially harmful self-reactive lymphocytes*, either before or after they have completed their maturation, in order to prevent reactions against one's own tissues (Chapter 5)
- *Cell death induced by cytotoxic T lymphocytes*, a defense mechanism against viruses and tumors that serves to kill and eliminate virus-infected and neoplastic cells (Chapter 5)

Apoptosis in Pathologic Conditions

Apoptosis eliminates cells that are genetically altered or injured beyond repair without eliciting a severe host reaction, thus keeping the damage as contained as possible.

Death by apoptosis is responsible for loss of cells in a variety of pathologic states:

- **DNA damage.** Radiation, cytotoxic anticancer drugs, extremes of temperature, and even hypoxia can damage DNA, either directly or via production of free radicals. If repair mechanisms cannot cope with the injury, the cell triggers intrinsic mechanisms that induce apoptosis. In these situations, elimination of the cell may be a better alternative than risking mutations in the damaged DNA, which may progress to malignant transformation. These injurious stimuli cause apoptosis if the insult is mild, but larger doses of the same stimuli result in necrotic cell death. Inducing apoptosis of cancer cells is a desired effect of chemotherapeutic agents, many of which work by damaging DNA.
- **Accumulation of misfolded proteins.** Improperly folded proteins may arise because of mutations in the genes encoding these proteins or because of extrinsic factors, such as damage caused by free radicals. Excessive accumulation of these proteins in the ER leads to a condition called *ER stress*, which culminates in apoptotic death of cells.
- **Cell injury in certain infections,** particularly viral infections, in which loss of infected cells is largely due to apoptotic death that may be induced by the virus (as in adenovirus and human immunodeficiency virus infections) or by the host immune response (as in viral hepatitis)
- **Pathologic atrophy in parenchymal organs after duct obstruction,** such as occurs in the pancreas, parotid gland, and kidney

Mechanisms of Apoptosis

Apoptosis is an active enzymatic process in which nucleoproteins are broken down and then the cell is fragmented. Before discussing the molecular mechanisms, it is useful to review the morphology of this pathway of cell death.

Morphology

In H&E-stained tissue sections, apoptotic cells may appear as round or oval masses with intensely eosinophilic cytoplasm (Fig. 1–22). Nuclei show various stages of chromatin condensation and aggregation and, ultimately, karyorrhexis; at the molecular level this is reflected in fragmentation of DNA into nucleosome-sized pieces. The cells rapidly shrink, form cytoplasmic buds, and fragment into **apoptotic bodies** composed of membrane-bound vesicles of cytosol and organelles (see Fig. 1–6). Because these fragments are quickly extruded and phagocytosed without eliciting an inflammatory response, even substantial apoptosis may be histologically undetectable.

The fundamental event in apoptosis is the activation of enzymes called *caspases* (so named because they are cysteine proteases that cleave proteins after *aspartic* residues). Activated caspases cleave numerous targets,

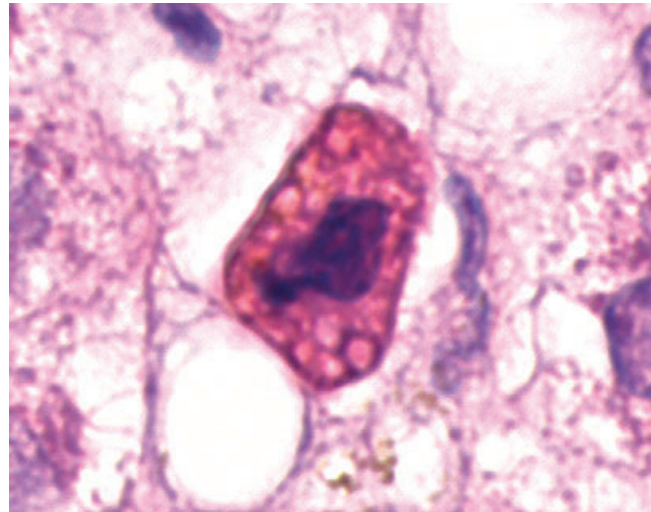
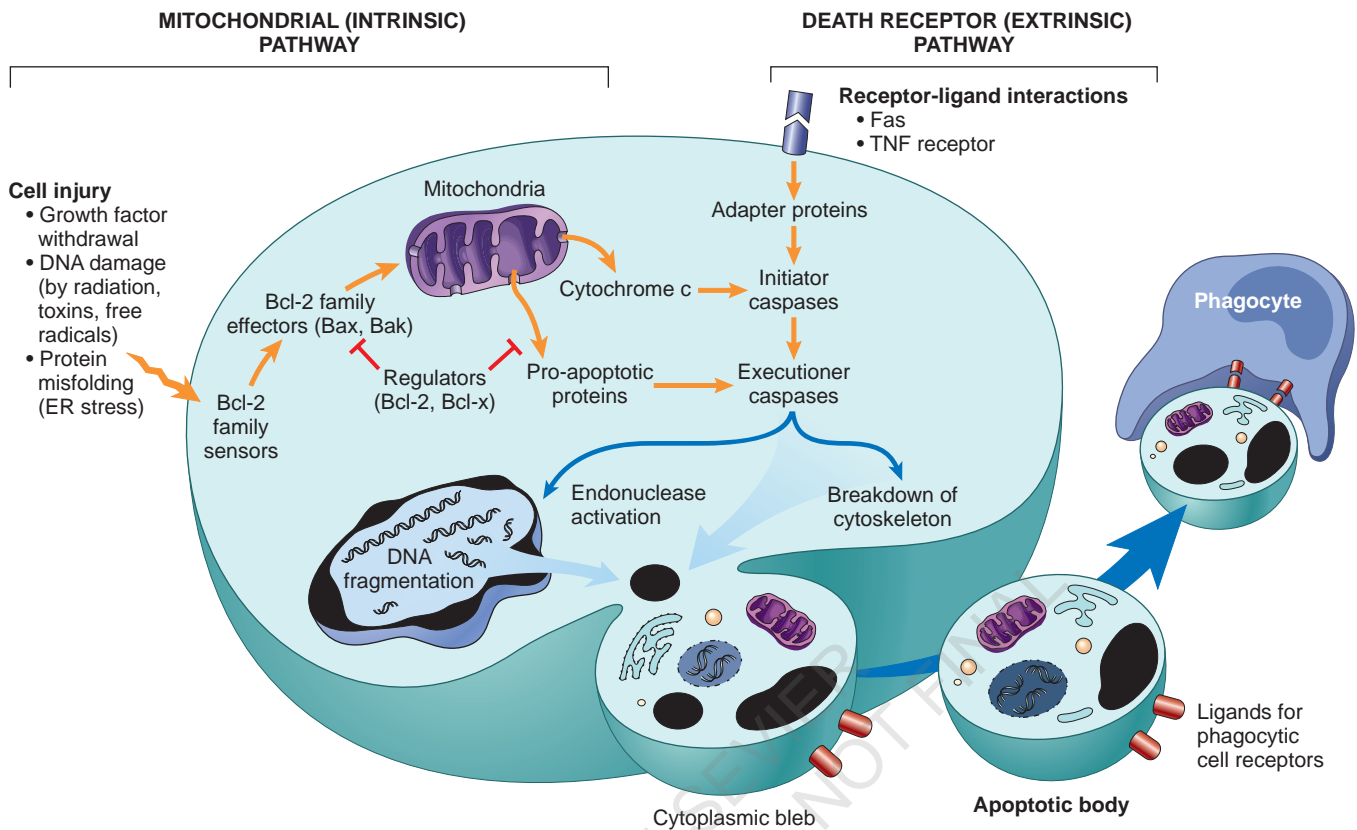


Figure 1–22

Apoptosis of a liver cell in viral hepatitis. The cell is reduced in size and contains brightly eosinophilic cytoplasm and a condensed nucleus.

culminating in activation of nucleases that degrade DNA and other enzymes that presumably destroy nucleoproteins and cytoskeletal proteins. The activation of caspases depends on a finely tuned balance between pro- and anti-apoptotic molecular pathways. Two distinct pathways converge on caspase activation, called the *mitochondrial pathway* and the *death receptor pathway*. Although these pathways can interact, they are generally induced under different conditions, involve different molecules, and serve distinct roles in physiology and disease (Fig. 1–23).

The Mitochondrial (Intrinsic) Pathway of Apoptosis. Mitochondria contain several proteins that are capable of inducing apoptosis; these proteins include cytochrome *c* and antagonists of endogenous cytosolic inhibitors of apoptosis. The choice between cell survival and death is determined by the permeability of mitochondria, which is controlled by a family of more than 20 proteins, the prototype of which is Bcl-2. When cells are deprived of growth factors and trophic hormones, or are exposed to agents that damage DNA, or accumulate unacceptable amounts of misfolded proteins, a group of sensors is activated. Some of these sensors, which are members of the Bcl-2 family, in turn activate two pro-apoptotic members of the family called Bax and Bak, which dimerize, insert into the mitochondrial membrane, and form channels through which cytochrome *c* and other mitochondrial proteins escape into the cytosol. Other related sensors inhibit the anti-apoptotic molecules Bcl-2 and Bcl-x_L (see below), with the same end result—the leakage of mitochondrial proteins. Cytochrome *c*, together with some cofactors, activates caspase-9, while other proteins block the activities of caspase antagonists that function as physiologic inhibitors of apoptosis. The net result is the activation of the caspase cascade, ultimately leading to nuclear fragmentation. If cells are exposed to growth factors and other survival signals, they synthesize anti-apoptotic members of the Bcl-2 family, the two main ones of which are Bcl-2 itself and Bcl-x_L. These proteins antag-

**Figure 1-23**

Mechanisms of apoptosis. The two pathways of apoptosis differ in their induction and regulation, and both culminate in the activation of “executioner” caspases. The induction of apoptosis is dependent on a balance between pro- and anti-apoptotic signals and intracellular proteins. The figure shows the pathways that induce apoptotic cell death, and the anti-apoptotic proteins that inhibit mitochondrial leakiness and cytochrome *c*-dependent caspase activation and thus function as regulators of mitochondrial apoptosis.

onize Bax and Bak, and thus limit the escape of mitochondrial pro-apoptotic proteins. Cells deprived of growth factors not only activate the pro-apoptotic proteins but also show reduced levels of Bcl-2 and Bcl-x_L, thus further tilting the balance toward death. The mitochondrial pathway seems to be the pathway that is responsible for most situations of apoptosis, as we shall discuss below.

The Death Receptor (Extrinsic) Pathway of Apoptosis. Many cells express surface molecules, called death receptors, that trigger apoptosis. Most of these are members of the tumor necrosis factor (TNF) receptor family that contain in their cytoplasmic regions a conserved “death domain,” so named because it mediates interaction with other proteins. The prototypic death receptors are the type I TNF receptor and Fas (CD95). Fas-ligand (FasL) is a membrane protein expressed mainly on activated T lymphocytes. When these T cells recognize Fas-expressing targets, Fas molecules are cross-linked by the FasL and they bind adapter proteins, which in turn bind caspase-8. Clustering of many caspase molecules leads to their activation, thus initiating the caspase cascade. In many cell types caspase-8 may cleave and activate a pro-apoptotic member of the Bcl-2 family called Bid, thus feeding into the mitochondrial pathway. The combined activation of both pathways delivers a lethal blow to the cell. Cellular

proteins, notably a caspase antagonist called FLIP, block activation of caspases downstream of death receptors. Interestingly, some viruses produce homologues of FLIP, and it is suggested that this is a mechanism that viruses use to keep infected cells alive. The death receptor pathway is involved in elimination of self-reactive lymphocytes and in killing of target cells by some cytotoxic T lymphocytes.

Clearance of Apoptotic Cells. Apoptotic cells undergo several changes in their membranes that promote their phagocytosis. In normal cells phosphatidylserine is present on the inner leaflet of the plasma membrane, but in apoptotic cells this phospholipid “flips” out and is expressed on the outer layer of the membrane, where it is recognized by macrophages. Cells that are dying by apoptosis also secrete soluble factors that recruit phagocytes. This facilitates prompt clearance of the dead cells before they undergo secondary membrane damage and release their cellular contents (which can result in inflammation). Some apoptotic bodies express adhesive glycoproteins that are recognized by phagocytes, and macrophages themselves may produce proteins that bind to apoptotic cells (but not to live cells) and target the dead cells for engulfment. Numerous macrophage receptors have been shown to be involved in the binding and engulfment of apoptotic cells. This process of phagocytosis

osis of apoptotic cells is so efficient that dead cells disappear without leaving a trace, and inflammation is virtually absent.

Although we have emphasized the distinctions between necrosis and apoptosis, these two forms of cell death may coexist and be related mechanistically. For instance, DNA damage (seen in apoptosis) activates an enzyme called poly-ADP(ribose) polymerase, which depletes cellular supplies of nicotinamide adenine dinucleotide, leading to a fall in ATP levels and ultimately necrosis. In fact, even in common situations such as ischemia, it has been suggested that early cell death can be partly attributed to apoptosis, and necrosis is the dominant type of cell death late, with worsening ischemia.

Examples of Apoptosis

Cell death in many situations is known to be caused by apoptosis, and the selected examples listed below illustrate the role of this death pathway in normal physiology and in disease.

Growth Factor Deprivation. Hormone-sensitive cells deprived of the relevant hormone, lymphocytes that are not stimulated by antigens and cytokines, and neurons deprived of nerve growth factor die by apoptosis. In all these situations, apoptosis is triggered by the mitochondrial pathway and is attributable to activation of proapoptotic members of the Bcl-2 family and decreased synthesis of Bcl-2 and Bcl-x_L.

DNA Damage. Exposure of cells to radiation or chemotherapeutic agents induces DNA damage, and if this is too severe to be repaired it triggers apoptotic death. When DNA is damaged, the p53 protein accumulates in cells. It first arrests the cell cycle (at the G₁ phase) to allow time for repair (Chapter 6). However, if the damage is too great to be repaired successfully, p53 triggers apoptosis, mainly by activating sensors that ultimately activate Bax and Bak, and by stimulating synthesis of proapoptotic members of the Bcl-2 family. When p53 is mutated or absent (as it is in certain cancers), it is incapable of inducing apoptosis, so that cells with damaged DNA are allowed to survive. In such cells, the DNA damage may result in mutations or translocations that lead to neoplastic transformation (Chapter 6).

Accumulation of Misfolded Proteins. During normal protein synthesis, chaperones in the ER control the proper folding of newly synthesized proteins, and misfolded polypeptides are ubiquitinated and targeted for proteolysis. If, however, unfolded or misfolded proteins accumulate in the ER because of inherited mutations or stresses, they induce “ER stress” that triggers a number of cellular responses, collectively called the *unfolded protein response*. This response activates signaling pathways that increase the production of chaperones and retard protein translation, thus reducing the levels of misfolded proteins in the cell. However, if this response is unable to cope with the accumulation of misfolded proteins, the result is the activation of caspases that lead to apoptosis. Intracellular accumulation of abnormally folded proteins, caused by mutations, aging, or unknown environmental factors, is now recognized as a feature of

a number of neurodegenerative diseases, including Alzheimer, Huntington, and Parkinson diseases, and possibly type II diabetes. Deprivation of glucose and oxygen, and stress such as heat, also result in protein misfolding, culminating in cell injury and death.

Apoptosis of Self-Reactive Lymphocytes. Lymphocytes capable of recognizing self antigens are normally produced in all individuals. If these lymphocytes encounter self antigens, the cells die by apoptosis. Both the mitochondrial pathway and the Fas death receptor pathway have been implicated in this process (Chapter 5). Failure of apoptosis of self-reactive lymphocytes is one of the causes of autoimmune diseases.

Cytotoxic T Lymphocyte–Mediated Apoptosis. Cytotoxic T lymphocytes (CTLs) recognize foreign antigens presented on the surface of infected host cells and tumor cells (Chapter 5). Upon activation, CTL granule proteases called *granzymes* enter the target cells. Granzymes cleave proteins at aspartate residues and are able to activate cellular caspases. In this way, the CTL kills target cells by directly inducing the effector phase of apoptosis, without engaging mitochondria or death receptors. CTLs also express FasL on their surface and may kill target cells by ligation of Fas receptors.

SUMMARY

Apoptosis

- Regulated mechanism of cell death that serves to eliminate unwanted and irreparably damaged cells, with the least possible host reaction
- Characterized by: enzymatic degradation of proteins and DNA, initiated by caspases; and recognition and removal of dead cells by phagocytes
- Initiated by two major pathways:
 - *Mitochondrial (intrinsic) pathway* is triggered by loss of survival signals, DNA damage and accumulation of misfolded proteins (ER stress); associated with leakage of pro-apoptotic proteins from mitochondrial membrane into the cytoplasm, where they trigger caspase activation; inhibited by anti-apoptotic members of the Bcl family, which are induced by survival signals including growth factors.
 - *Death receptor (extrinsic) pathway* is responsible for elimination of self-reactive lymphocytes and damage by cytotoxic T lymphocytes; is initiated by engagement of death receptors (members of the TNF receptor family) by ligands on adjacent cells.

This description of apoptosis concludes the discussion of cell injury and cell death. As we have seen, these processes are the root cause of many common diseases. We end this chapter with brief considerations of three other processes: intracellular accumulations of various substances and extracellular deposition of calcium, both of which are often associated with cell injury, and aging.

INTRACELLULAR ACCUMULATIONS

Under some circumstances cells may accumulate abnormal amounts of various substances, which may be harmless or associated with varying degrees of injury. The substance may be located in the cytoplasm, within organelles (typically lysosomes), or in the nucleus, and it may be synthesized by the affected cells or may be produced elsewhere.

There are three main pathways of abnormal intracellular accumulations (Fig. 1–24):

- A normal substance is produced at a normal or an increased rate, but the metabolic rate is inadequate to remove it. An example of this type of process is fatty change in the liver.
- A normal or an abnormal endogenous substance accumulates because of genetic or acquired defects in its folding, packaging, transport, or secretion. Mutations that cause defective folding and transport may lead to accumulation of proteins (e.g., α_1 -antitrypsin deficiency).
- An inherited defect in an enzyme may result in failure to degrade a metabolite. The resulting disorders are called storage diseases (Chapter 7).
- An abnormal exogenous substance is deposited and accumulates because the cell has neither the enzymatic machinery to degrade the substance nor the ability to transport it to other sites. Accumulations of carbon or silica particles are examples of this type of alteration.

Fatty Change (Steatosis). *Fatty change* refers to any abnormal accumulation of triglycerides within parenchymal cells. It is most often seen in the liver, since this is the major organ involved in fat metabolism, but it may also occur in heart, skeletal muscle, kidney, and other organs. Steatosis may be caused by toxins, protein malnutrition, diabetes mellitus, obesity, and anoxia. *Alcohol abuse and diabetes associated with obesity are the most common causes of fatty change in the liver (fatty liver) in industrialized nations.*

Free fatty acids from adipose tissue or ingested food are normally transported into hepatocytes, where they are esterified to triglycerides, converted into cholesterol or phospholipids, or oxidized to ketone bodies (Fig. 1–25A). Some fatty acids are synthesized from acetate within the hepatocytes as well. Egress of the triglycerides from the hepatocytes requires the formation of complexes with apoproteins to form lipoproteins, which are able to enter the circulation (Chapter 7). Excess accumulation of triglycerides may result from defects at any step from fatty acid entry to lipoprotein exit, thus accounting for the occurrence of fatty liver after diverse hepatic insults. Hepatotoxins (e.g., alcohol) alter mitochondrial and SER function and thus inhibit fatty acid oxidation; CCl_4 and protein malnutrition decrease the synthesis of apoproteins; anoxia inhibits fatty acid oxidation; and starvation increases fatty acid mobilization from peripheral stores.

The significance of fatty change depends on the cause and severity of the accumulation. When mild it may have no effect on cellular function. More severe fatty change may transiently impair cellular function, but unless some vital intracellular process is irreversibly impaired (e.g., in

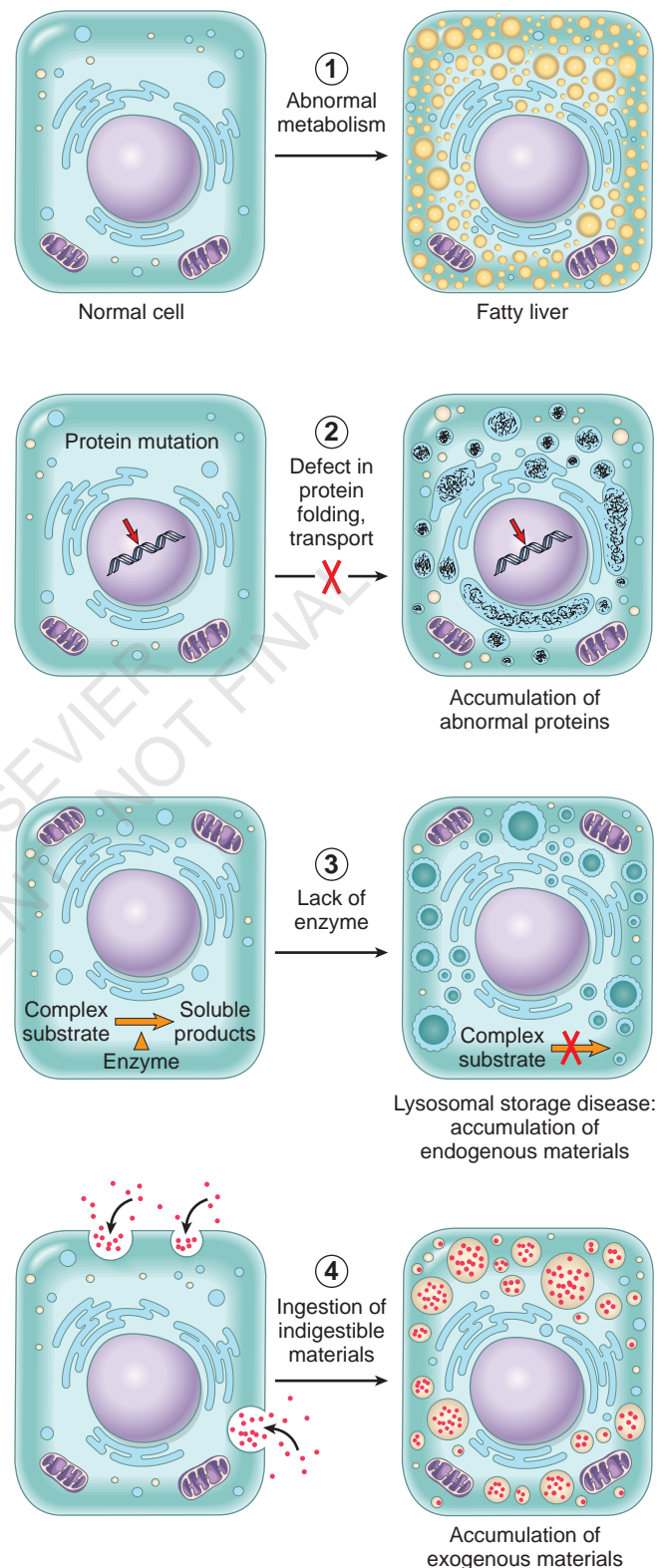


Figure 1–24

Mechanisms of intracellular accumulation. (1) Abnormal metabolism, as in fatty change in the liver. (2) Mutations causing alterations in protein folding and transport, so that defective molecules accumulate intracellularly. (3) A deficiency of critical enzymes responsible for breaking down certain compounds, causing substrates to accumulate in lysosomes, as in lysosomal storage diseases. (4) An inability to degrade phagocytosed particles, as in carbon pigment accumulation.

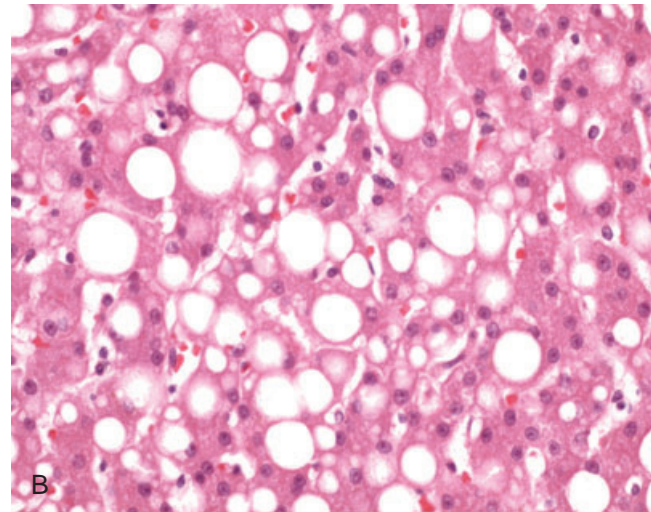
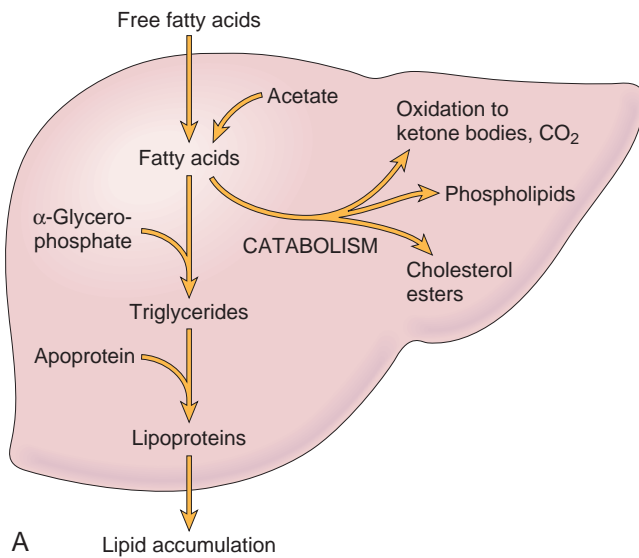


Figure 1-25

Fatty liver. **A**, The possible mechanisms leading to accumulation of triglycerides in fatty liver. Defects in any of the steps of uptake, catabolism, or secretion can lead to 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, University of Florida School of Medicine, Gainesville.)

CCl_4 poisoning), fatty change is reversible. In the severe form, fatty change may precede cell death, and may be an early lesion in a serious liver disease called nonalcoholic steatohepatitis (Chapter 16).

Morphology

In any site, fatty accumulation appears as clear vacuoles within parenchymal cells. Special staining techniques are required to distinguish fat from intracellular water or glycogen, which can also produce clear vacuoles but have a different significance. To identify fat microscopically, tissues must be processed for polysaccharides using the periodic acid–Schiff stain (which stains glycogen red-violet). If vacuoles do not stain for either fat or glycogen, they are presumed to be composed mostly of water.

Fatty change is most commonly seen in the liver and the heart. Mild fatty change in the **liver** may not affect the gross appearance. With increasing accumulation, the organ enlarges and becomes progressively yellow until, in extreme cases, it may weigh 3 to 6 kg (1.5–3 times the normal weight) and appear bright yellow, soft, and greasy. Early fatty change is seen by light microscopy as small fat vacuoles in the cytoplasm around the nucleus. In later stages, the vacuoles coalesce to create cleared spaces that displace the nucleus to the cell periphery (Fig. 1–25B). Occasionally contiguous cells rupture, and the enclosed fat globules unite to produce so-called fatty cysts.

In the **heart**, lipid is found in the form of small droplets, occurring in one of two patterns. Prolonged moderate hypoxia (as in profound anemia) results in focal intracellular fat deposits, creating grossly appar-

ent bands of yellowed myocardium alternating with bands of darker, red-brown, uninvolved heart (“tigered effect”). The other pattern of fatty change is produced by more profound hypoxia or by some forms of toxic injury (e.g., diphtheria) and shows more uniformly affected myocytes.

Cholesterol and Cholesteryl Esters. Cellular cholesterol metabolism is tightly regulated to ensure normal cell membrane synthesis without significant intracellular accumulation. However, phagocytic cells may become overloaded with lipid (triglycerides, cholesterol, and cholesteryl esters) in several different pathologic processes.

Macrophages in contact with the lipid debris of necrotic cells or abnormal (e.g., oxidized) forms of lipoproteins may become stuffed with phagocytosed lipid. These macrophages may be filled with minute, membrane-bound vacuoles of lipid, imparting a foamy appearance to their cytoplasm (*foam cells*). In *atherosclerosis*, smooth muscle cells and macrophages are filled with lipid vacuoles composed of cholesterol and cholesteryl esters; these give atherosclerotic plaques their characteristic yellow color and contribute to the pathogenesis of the lesion (Chapter 10). In hereditary and acquired hyperlipidemic syndromes, macrophages accumulate intracellular cholesterol; when present in the subepithelial connective tissue of skin or in tendons, clusters of these foamy macrophages form masses called *xanthomas*.

Proteins. Morphologically visible protein accumulations are much less common than lipid accumulations; they may occur because excesses are presented to the cells or because the cells synthesize excessive amounts. In the kidney, for example, trace amounts of albumin filtered through the glomerulus are normally reabsorbed by pinocytosis in the proximal convoluted tubules. However, in disorders with heavy protein leakage across the glomerular filter (e.g.,

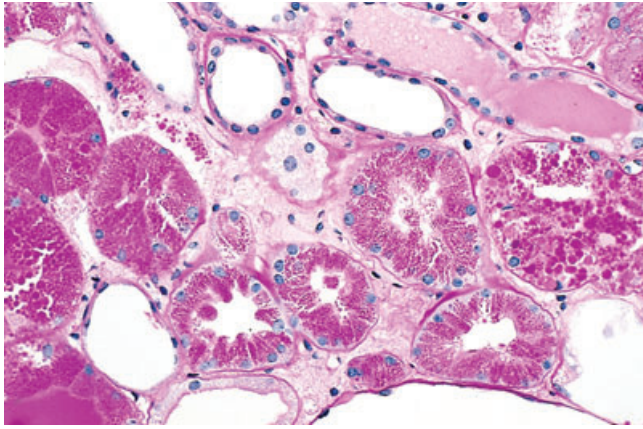


Figure 1-26

Protein reabsorption droplets in the renal tubular epithelium. (Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)

nephrotic syndrome), there is a much larger reabsorption of the protein. Pinocytic vesicles containing this protein fuse with lysosomes, resulting in the histologic appearance of pink, hyaline cytoplasmic droplets (Fig. 1-26). The process is reversible; if the proteinuria abates, the protein droplets are metabolized and disappear. Another example is the marked accumulation of newly synthesized immunoglobulins that may occur in the RER of some plasma cells, forming rounded, eosinophilic *Russell bodies*.

Accumulations of intracellular proteins are also seen in certain types of cell injury. For example, the Mallory body, or “alcoholic hyalin,” is an eosinophilic cytoplasmic inclusion in liver cells that is highly characteristic of alcoholic liver disease (Chapter 16). Such inclusions are composed predominantly of aggregated intermediate filaments that presumably resist degradation. The neurofibrillary tangle found in the brain in Alzheimer disease is an aggregated protein inclusion that contains microtubule-associated proteins and neurofilaments, a reflection of a disrupted neuronal cytoskeleton (Chapter 23).

Glycogen. Excessive intracellular deposits of glycogen are associated with abnormalities in the metabolism of either glucose or glycogen. In poorly controlled diabetes mellitus, the prime example of abnormal glucose metabolism, glycogen accumulates in renal tubular epithelium, cardiac myocytes, and β cells of the islets of Langerhans. Glycogen also accumulates within cells in a group of closely related genetic disorders collectively referred to as *glycogen storage diseases*, or *glycogenoses* (Chapter 7). In these diseases, enzymatic defects in the synthesis or breakdown of glycogen result in massive stockpiling, with secondary injury and cell death.

Pigments. Pigments are colored substances that are either exogenous, coming from outside the body, or endogenous, synthesized within the body itself.

- The most common exogenous pigment is *carbon* (an example is coal dust), a ubiquitous air pollutant of urban life. When inhaled, it is phagocytosed by alveolar macrophages and transported through lymphatic channels to the regional tracheobronchial lymph nodes. Aggregates of the pigment blacken the draining lymph nodes and pulmonary parenchyma (*anthracosis*). Heavy accumulations may induce emphysema or a fibroblastic reaction that can result in a serious lung disease called coal workers' pneumoconiosis (Chapter 13).
- Endogenous pigments include lipofuscin, melanin, and certain derivatives of hemoglobin. *Lipofuscin*, or “wear-and-tear pigment,” is an insoluble brownish-yellow granular intracellular material that accumulates in a variety of tissues (particularly the heart, liver, and brain) as a function of age or atrophy. Lipofuscin represents complexes of lipid and protein that derive from the free radical-catalyzed peroxidation of polyunsaturated lipids of subcellular membranes. It is not injurious to the cell but is important as a marker of past free-radical injury. The brown pigment (Fig. 1-27), when present in large amounts, imparts an appearance to the tissue that is called *brown atrophy*. By electron microscopy, the pigment appears as perinuclear electron-dense granules (Fig. 1-27B).

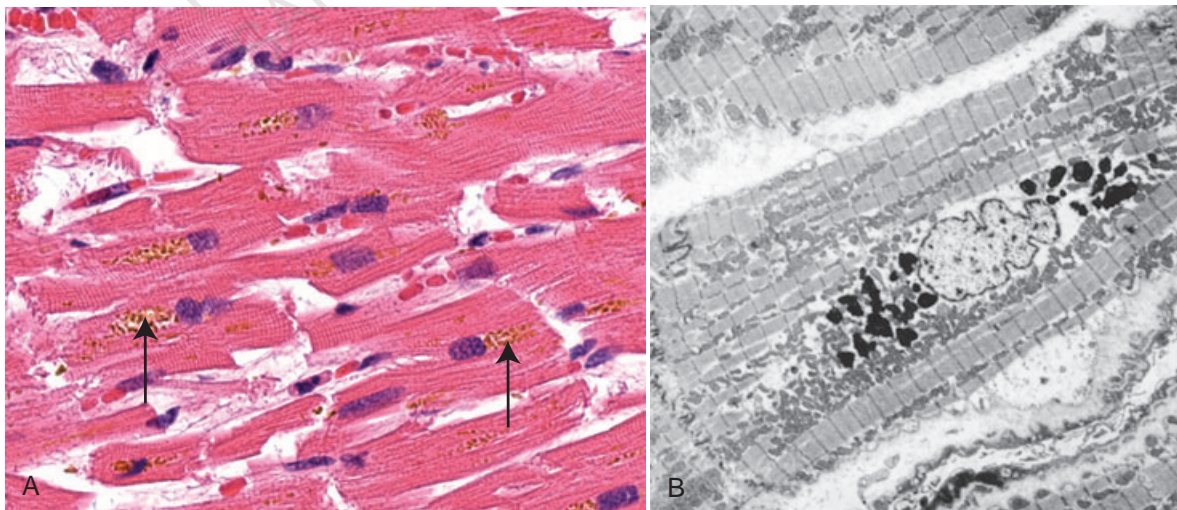


Figure 1-27

Lipofuscin granules in a cardiac myocyte. **A**, Light microscopy (deposits indicated by *arrows*). **B**, Electron microscopy. Note the perinuclear, intralysosomal location.

- *Melanin* is an endogenous, brown-black pigment produced in melanocytes following the tyrosinase-catalyzed oxidation of tyrosine to dihydroxyphenylalanine. It is synthesized exclusively by melanocytes located in the epidermis and acts as a screen against harmful ultraviolet radiation. Although melanocytes are the only source of melanin, adjacent basal keratinocytes in the skin can accumulate the pigment (e.g., in freckles), as can dermal macrophages.
- *Hemosiderin* is a hemoglobin-derived granular pigment that is golden yellow to brown and accumulates in tissues when there is a local or systemic excess of iron. Iron is normally stored within cells in association with the protein *apoferritin*, forming ferritin micelles. Hemosiderin pigment represents large aggregates of these ferritin micelles, readily visualized by light and electron microscopy; the iron can be unambiguously identified by the Prussian blue histochemical reaction (Fig. 1–28). Although hemosiderin accumulation is usually pathologic, small amounts of this pigment are normal in the mononuclear phagocytes of the bone marrow, spleen, and liver, where there is extensive red cell breakdown.
- Local excesses of iron, and consequently of hemosiderin, result from hemorrhage. The best example is the common bruise. After lysis of the erythrocytes at the site of hemorrhage, the red cell debris is phagocytosed by macrophages; the hemoglobin content is then catabolized by lysosomes with accumulation of the heme iron in hemosiderin. The array of colors through which the bruise passes reflects these transformations. The original red-blue color of hemoglobin is transformed to varying shades of green-blue by the local formation of biliverdin (green bile) and bilirubin (red bile) from the heme moiety; the iron ions of hemoglobin accumulate as golden-yellow hemosiderin.
- Whenever there is systemic overload of iron, hemosiderin is deposited in many organs and tissues, a condition called *hemosiderosis* (Chapter 12). It is found at first in the mononuclear phagocytes of the liver, bone marrow, spleen, and lymph nodes and in scattered macrophages throughout other organs. With pro-

gressive accumulation, parenchymal cells throughout the body (but principally the liver, pancreas, heart, and endocrine organs) become “bronzed” with accumulating pigment. Hemosiderosis occurs in the setting of (1) increased absorption of dietary iron, (2) impaired utilization of iron, (3) hemolytic anemias, and (4) transfusions (the transfused red cells constitute an exogenous load of iron). In most instances of systemic hemosiderosis, the iron pigment does not damage the parenchymal cells or impair organ function despite an impressive accumulation (Fig. 1–28). However, more extensive accumulations of iron are seen in hereditary *hemochromatosis* (Chapter 16), with tissue injury including liver fibrosis, heart failure, and diabetes mellitus.

PATHOLOGIC CALCIFICATION

Pathologic calcification is a common process in a wide variety of disease states; it implies the abnormal deposition of calcium salts, together with smaller amounts of iron, magnesium, and other minerals. When the deposition occurs in dead or dying tissues, it is called *dystrophic calcification*; it occurs in the absence of calcium metabolic derangements (i.e., with normal serum levels of calcium). In contrast, the deposition of calcium salts in normal tissues is known as *metastatic calcification* and almost always reflects some derangement in calcium metabolism (*hypercalcemia*). It should be noted that while hypercalcemia is not a prerequisite for dystrophic calcification, it can exacerbate it.

Dystrophic Calcification. Dystrophic calcification is encountered in areas of necrosis of any type. It is virtually inevitable in the *atheromas* of advanced atherosclerosis, associated with intimal injury in the aorta and large arteries and characterized by accumulation of lipids (Chapter 10). Although dystrophic calcification may be an incidental finding indicating insignificant past cell injury, it may also be a cause of organ dysfunction. For example, calcification can develop in aging or damaged heart valves, resulting in severely compromised valve

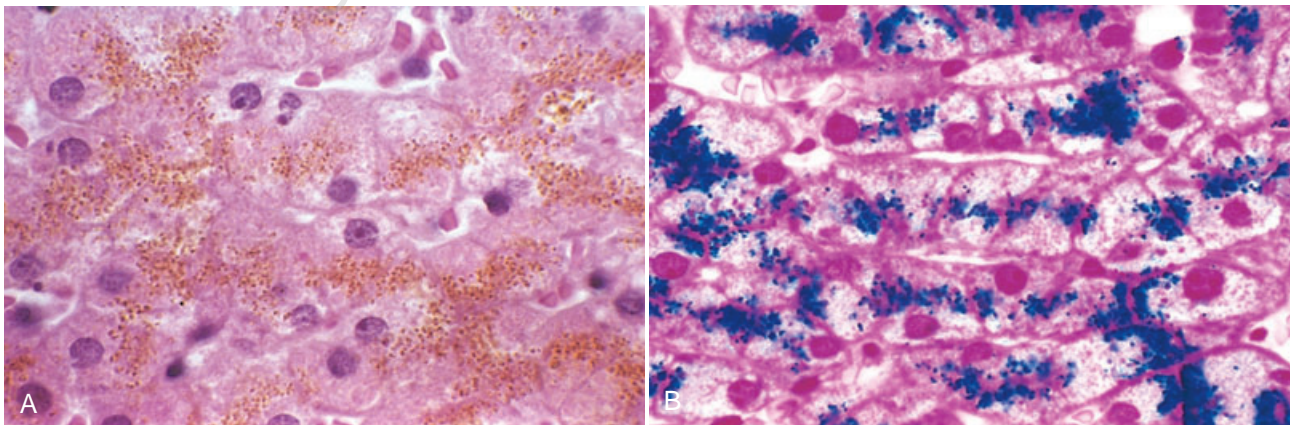


Figure 1–28

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



Figure 1-29

Calcification of the aortic valve. A 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 large, irregular masses of dystrophic calcification that will prevent normal opening of the cusps.

motion. Dystrophic calcification of the aortic valves is an important cause of aortic stenosis in the elderly (Fig. 1-29).

Morphology

Regardless of the site, calcium salts are grossly seen as fine white granules or clumps, often felt as gritty deposits. Sometimes a tuberculous lymph node is essentially converted to radio-opaque stone. Histologically, calcification appears as intracellular and/or extracellular basophilic deposits. In time, heterotopic bone may be formed in the focus of calcification.

The pathogenesis of dystrophic calcification involves *initiation* (or nucleation) and *propagation*, both of which may be either intracellular or extracellular; the ultimate end product is the formation of crystalline *calcium phosphate*. Initiation in extracellular sites occurs in membrane-bound vesicles about 200nm in diameter; in normal cartilage and bone they are known as *matrix vesicles*, and in pathologic calcification they derive from degenerating cells. It is thought that calcium is initially concentrated in these vesicles by its affinity for membrane phospholipids, while phosphates accumulate as a result of the action of membrane-bound phosphatases. Initiation of intracellular calcification occurs in the mitochondria of dead or dying cells that have lost their ability to regulate intracellular calcium. After initiation in either location, propagation of crystal formation occurs. This is dependent on the concentration of Ca^{2+} and PO_4^- in the extracellular spaces, the presence of mineral inhibitors, and the degree of collagenization, which enhances the rate of crystal growth.

Metastatic Calcification. Metastatic calcification can occur in normal tissues whenever there is hypercalcemia. The four major causes of hypercalcemia are (1) *increased secretion of parathyroid hormone*, due to either primary

parathyroid tumors or production of parathyroid hormone-related protein by other malignant tumors; (2) *destruction of bone* due to the effects of accelerated turnover (e.g., *Paget disease*), immobilization, or tumors (increased bone catabolism associated with multiple myeloma, leukemia, or diffuse skeletal metastases); (3) *vitamin D-related disorders* including vitamin D intoxication and *sarcoidosis* (in which macrophages activate a vitamin D precursor); and (4) *renal failure*, in which phosphate retention leads to *secondary hyperparathyroidism*.

Morphology

Metastatic calcification can occur widely throughout the body but principally affects the interstitial tissues of the vasculature, kidneys, lungs, and gastric mucosa. The calcium deposits morphologically resemble those described in dystrophic calcification. Although they do not generally cause clinical dysfunction, extensive calcifications in the lungs may produce remarkable radiographs and respiratory deficits, and massive deposits in the kidney (**nephrocalcinosis**) can cause renal damage.

SUMMARY

Abnormal Intracellular Depositions and Calcifications

- Abnormal deposits of materials in cells and tissues are the result of excessive intake or defective transport or catabolism.
- Depositions of *lipids*:
 - *Fatty change*: accumulation of free triglycerides in cells, resulting from excessive intake or defective transport (often because of defects in synthesis of transport proteins); manifestation of reversible cell injury
 - *Cholesterol deposition*: result of defective catabolism and excessive intake; in macrophages and smooth muscle cells of vessel walls in atherosclerosis
- Deposition of *proteins*: reabsorbed proteins in kidney tubules; immunoglobulins in plasma cells
- Deposition of *glycogen*: in macrophages of patients with defects in lysosomal enzymes that break down glycogen (glycogen storage diseases)
- Deposition of *pigments*: typically indigestible pigments, such as carbon, lipofuscin (breakdown product of lipid peroxidation), iron (usually due to overload, as in hemosiderosis)
- Pathologic calcifications:
 - *Dystrophic calcification*: deposition of calcium at sites of cell injury and necrosis
 - *Metastatic calcification*: deposition of calcium in normal tissues, caused by hypercalcemia (usually a consequence of parathyroid hormone excess)

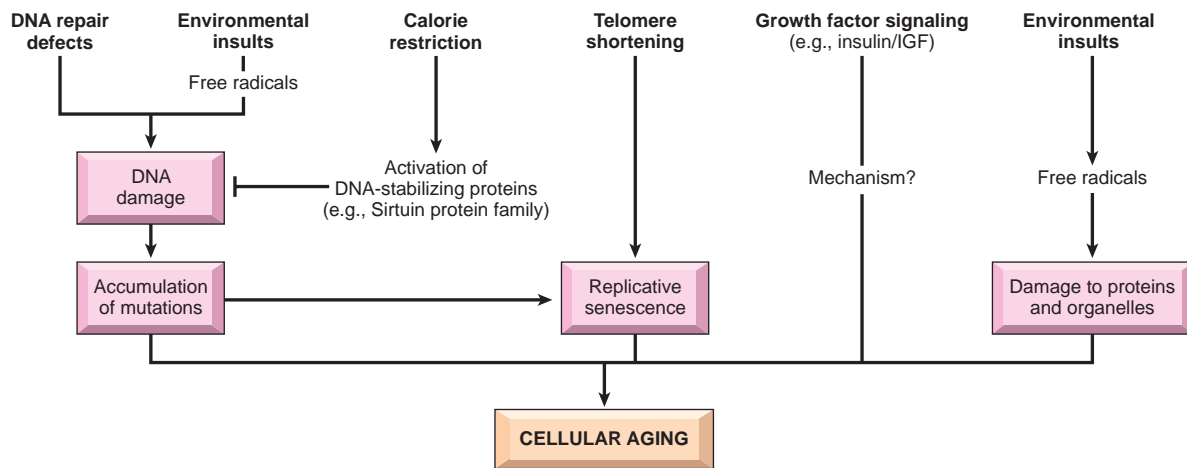


Figure 1-30

Mechanisms of cellular aging. Among the several pathways contributing to aging of cells and organisms, many have been defined in simple model organisms, and their relevance to aging in humans remains an area of active investigation. IGF, insulin-like growth factor.

CELLULAR AGING

Cellular aging is the result of a progressive decline in the proliferative capacity and life span of cells and the effects of continuous exposure to exogenous factors that cause accumulation of cellular and molecular damage (Fig. 1–30). The process of aging is conserved from yeast to humans, and—at least in simple model organisms—seems to be regulated by a limited number of genes. The idea that aging is controlled by particular genes has spurred enormous interest in defining its molecular pathways and in devising ways to manipulate a process that was once considered inexorable. Several mechanisms are known or suspected to be responsible for cellular aging.

- **DNA damage.** Cellular aging is associated with increasing DNA damage, which may happen during normal DNA replication and can be enhanced by free radicals. Although most DNA damage is repaired by DNA repair enzymes, some persists and accumulates as cells age. Some aging syndromes are associated with defects in DNA repair mechanisms, and the life span of model animals can be increased if responses to DNA damage are enhanced or proteins that stabilize DNA are introduced. In fact, the intervention that has most consistently prolonged life span in most species is *calorie restriction*. Recently, it has been proposed that calorie restriction imposes a level of stress that activates proteins of the Sirtuin family, such as Sir2, that function as histone deacetylases. These proteins may deacetylate and thereby activate DNA repair enzymes, thus stabilizing the DNA; in the absence of these proteins, DNA is prone to damage.
- **Decreased cellular replication.** All normal cells have a limited capacity for replication, and after a fixed number of divisions cells become arrested in a terminally nondividing state, known as *replicative senescence*. Aging is associated with progressive replicative

senescence of cells. Cells from children have the capacity to undergo more rounds of replication than do cells from older people. In contrast, cells from patients with *Werner syndrome*, a rare disease characterized by premature aging, have a markedly reduced in vitro life span. In human cells, the mechanism of replicative senescence involves incomplete replication and progressive shortening of telomeres, which ultimately results in cell cycle arrest. *Telomeres* are short repeated sequences of DNA present at the linear ends of chromosomes that are important for ensuring the complete replication of chromosome ends and for protecting the ends from fusion and degradation. When somatic cells replicate, a small section of the telomere is not duplicated, and telomeres become progressively shortened. As the telomeres become shorter, the ends of chromosomes cannot be protected and are seen as broken DNA, which signals cell cycle arrest. The lengths of the telomeres are normally maintained by nucleotide addition mediated by an enzyme called *telomerase*. Telomerase is a specialized RNA-protein complex that uses its own RNA as a template for adding nucleotides to the ends of chromosomes. Telomerase activity is expressed in germ cells and is present at low levels in stem cells, but it is usually absent in most somatic tissues (Fig. 1–31). Therefore, as cells age their telomeres become shorter and they exit the cell cycle, resulting in an inability to generate new cells to replace damaged ones. Conversely, in immortal cancer cells, telomerase is reactivated and telomeres are not shortened, suggesting that telomere elongation might be an important—possibly essential—step in tumor formation. This is discussed more fully in Chapter 6. Despite such alluring observations, however, the relationship of telomerase activity and telomere length to aging and cancer has yet to be fully established.

- **Reduced regenerative capacity of tissue stem cells.** Recent studies suggest that with age, the p16

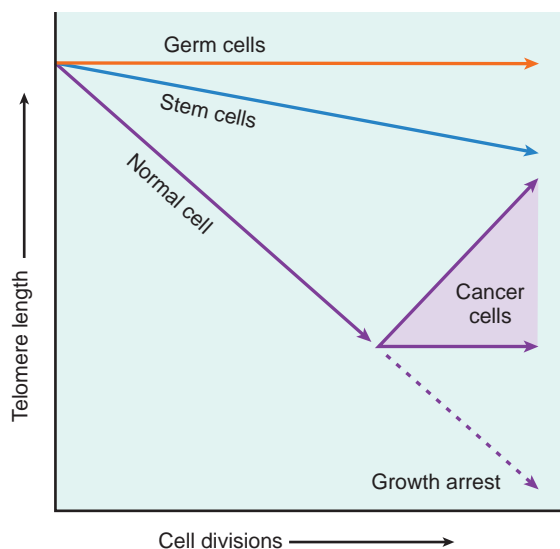


Figure 1-31

The role of telomeres and telomerase in replicative senescence of cells. 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. In cancer cells, telomerase is often reactivated. (Modified from Holt SE, et al: Refining the telomer-telomerase hypothesis of aging and cancer. *Nat Biotechnol* 14:836, 1996.)

(CDKN2A) protein accumulates in stem cells, and they progressively lose their capacity to self-renew. p16 is a physiological inhibitor of cell cycle progression; as we discuss in Chapter 6, deletion or loss-of-function mutations of p16 are associated with cancer development.

- **Accumulation of metabolic damage.** Cellular life span is also determined by a balance between damage resulting from metabolic events occurring within the cell and counteracting molecular responses that can repair the damage. One group of potentially toxic products of normal metabolism is reactive oxygen species. As we have discussed earlier in the chapter, these byproducts of oxidative phosphorylation cause covalent modifications of proteins, lipids, and nucleic acids. Increased oxidative damage could result from repeated environmental exposure to such influences as ionizing radiation along with progressive reduction of antioxidant defense mechanisms. Damaged cellular organelles accumulate as cells age. This may also be the result of declining function of the proteasome, the proteolytic machine that serves to eliminate abnormal and unwanted intracellular proteins.

- Studies in model organisms, like the worm *Caenorhabditis elegans*, have shown that growth factors, such as insulin-like growth factor, and intracellular signaling pathways triggered by these hormones, tend to reduce life span. The underlying mechanisms are not fully understood, but these growth factors may attenuate Sir2 responses to cellular stress and thus reduce the stability of the DNA.

SUMMARY

Cellular Aging

- Results from combination of accumulating cellular damage (e.g., by free radicals), reduced capacity to divide (replicative senescence), and reduced ability to repair damaged DNA
- **Accumulation of DNA damage:** defective DNA repair mechanisms; DNA repair may be activated by calorie restriction (known to prolong aging in model organisms)
- **Replicative senescence:** reduced capacity of cells to divide because of decreasing amounts of telomerase and progressive shortening of chromosomal ends (telomeres)
- **Other factors:** progressive accumulation of metabolic damage; possible roles of growth factors that promote aging in simple model organisms

It should be apparent that the various forms of cellular derangements and adaptations described in this chapter cover a wide spectrum, ranging from adaptations in cell size, growth, and function; to the reversible and irreversible forms of acute cell injury; to the regulated type of cell death represented by apoptosis. Reference is made to all these alterations throughout this book because all organ injury and ultimately all clinical disease arise from derangements in cell structure and function.

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