CHAPTER 1

Cellular Adaptations, Cell Injury, and Cell Death

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Introduction to Pathology

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

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

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

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

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

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

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

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

Overview: Cellular Responses to Stress and Noxious Stimuli

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

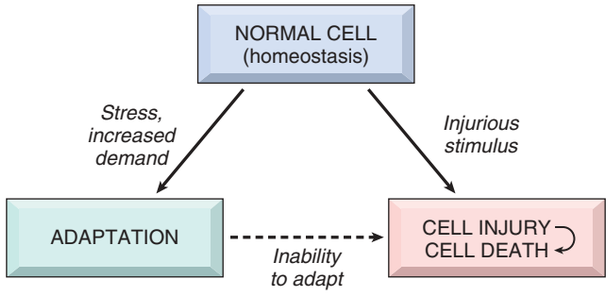


FIGURE 1–1 Stages in the cellular response to stress and injurious stimuli.

If the limits of adaptive response to a stimulus are exceeded, or in certain instances when the cell is exposed to an injurious agent or stress, a sequence of events follows that is loosely termed *cell injury*. Cell injury is *reversible* up to a certain point, but if the stimulus persists or is severe enough from the beginning, the cell reaches a "point of no return" and suffers *irreversible* cell injury and ultimately *cell death*. *Adaptation, reversible injury*, and *cell death* can be considered stages of progressive impairment of the cell's normal function and structure (see Fig. 1–1). For instance, in response to increased hemodynamic loads, the heart muscle first becomes enlarged, a form of adaptation. If the blood supply to the myocardium is insufficient to cope with the demand, the muscle becomes reversibly injured and finally undergoes cell death (Fig. 1–2).

Cell death, the ultimate result of cell injury, is one of the most crucial events in the evolution of disease of any tissue or organ. It results from diverse causes, including ischemia (lack of blood flow), infection, toxins, and immune reactions. In addition, cell death is a normal and essential part of embryogenesis, the development of organs, and the maintenance of homeostasis, and is the aim of cancer therapy. There are two principal patterns of cell death, necrosis and apoptosis. *Necrosis* is the type of cell death that occurs after such abnormal stresses as ischemia and chemical injury, and it is always pathologic. *Apoptosis* occurs when a cell dies through activation of an internally controlled suicide program. It is designed to eliminate unwanted cells during embryogenesis and in various physiologic processes, such as involution of hormone-responsive tissues upon withdrawal of the hormone. It also occurs in certain pathologic conditions, when cells are damaged beyond repair, and especially if the damage affects the cell's nuclear DNA. We will return to a detailed discussion of these pathways of cell death later in the chapter.

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

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

Cellular Adaptations of Growth and Differentiation

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

TABLE 1–1 Cellular Responses to Injury

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

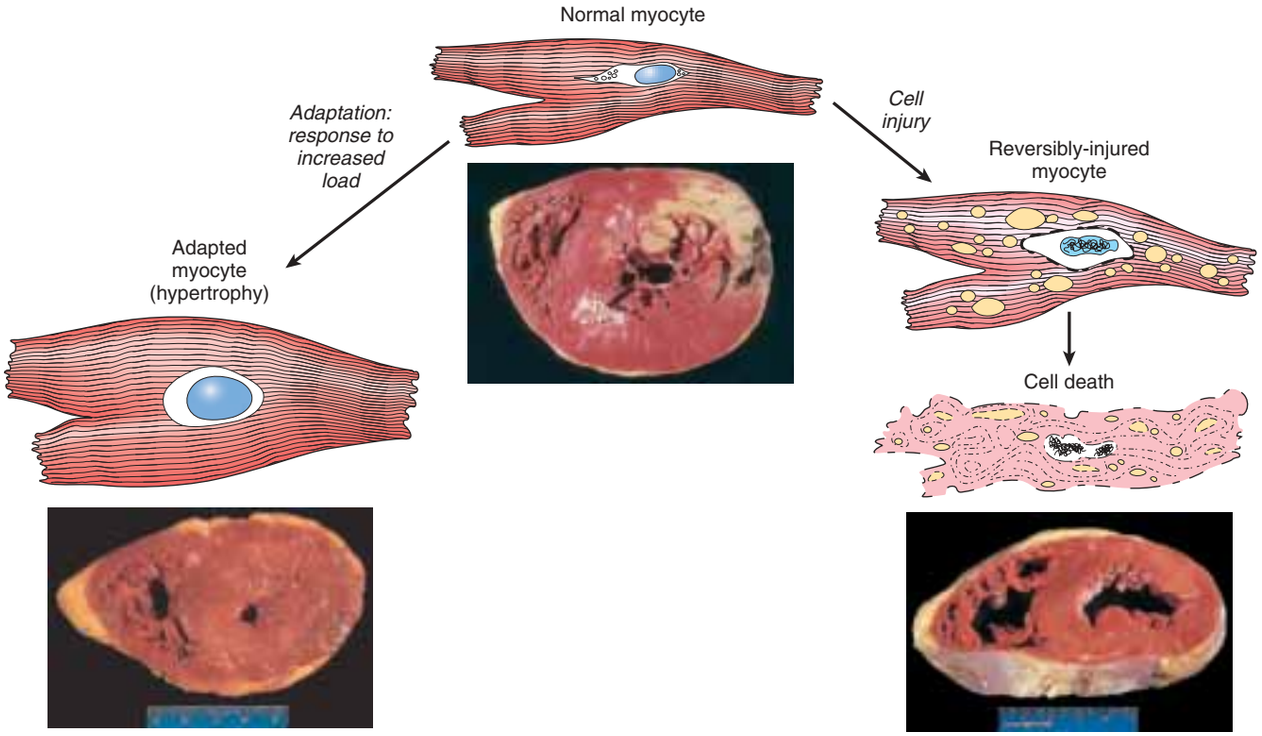


FIGURE 1–2 The relationships between normal, adapted, reversibly injured, and dead myocardial cells. The cellular adaptation depicted here is hypertrophy, and the type of cell death is ischemic necrosis. In reversibly injured myocardium, generally effects are only functional, without any readily apparent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

HYPERPLASIA

Hyperplasia is an increase in the number of cells in an organ or tissue, usually resulting in increased volume of the organ or tissue. Although hyperplasia and hypertrophy are two distinct processes, frequently both occur together, and they may be triggered by the same external stimulus. For instance, hormone-induced growth in the uterus involves both increased numbers of smooth muscle and epithelial cells and the enlargement of these cells. Hyperplasia takes place if the cellular population is capable of synthesizing DNA, thus permitting mitotic division; by contrast, hypertrophy involves cell enlargement without cell division. Hyperplasia can be physiologic or pathologic.

Physiologic Hyperplasia

Physiologic hyperplasia can be divided into: (1) *hormonal hyperplasia*, which increases the functional capacity of a tissue when needed, and (2) *compensatory hyperplasia*, which increases tissue mass after damage or partial resection. Hormonal hyperplasia is best exemplified by the proliferation of the glandular epithelium of the female breast at puberty and during pregnancy and the physiologic hyperplasia that occurs in the pregnant uterus. The classical illustration of compensatory hyperplasia comes from the myth of Prometheus, which shows that the ancient Greeks recognized the capacity of the liver to regenerate. As punishment for having stolen the secret of fire from the gods, Prometheus was chained to a mountain, and his liver was devoured daily by a vulture, only to regenerate anew every night.[1] The experimental model of partial hepatectomy has been especially useful in examining the mechanisms that stimulate proliferation of residual liver cells and regeneration of the liver (Chapter 3). Similar mechanisms are likely involved in other situations when remaining tissue grows to make up for partial tissue loss (e.g., after unilateral nephrectomy, when the remaining kidney undergoes compensatory hyperplasia).

Mechanisms of Hyperplasia. Hyperplasia is generally caused by increased local production of growth factors, increased levels of growth factor receptors on the responding cells, or activation of particular intracellular signaling pathways. All these changes lead to production of transcription factors that turn on many cellular genes, including genes encoding growth factors, receptors for growth factors, and cell cycle regulators, and the net result is cellular proliferation.[2] In hormonal hyperplasia, the hormones may themselves act as growth factors and trigger the transcription of various cellular genes. The source of growth factors in compensatory hyperplasia and the stimuli for the production of these growth factors are less well defined. The increase in tissue mass after some types of cell loss is achieved not only by proliferation of

the remaining cells but also by the development of new cells from *stem cells*.[3,4] For instance, in the liver, intrahepatic stem cells do not play a major role in the hyperplasia that occurs after hepatectomy but they may participate in regeneration after certain forms of liver injury, such as chronic hepatitis, in which the proliferative capacity of hepatocytes is compromised. Recent data from clinical observations and experimental studies have demonstrated that the bone marrow contains stem cells that may be able to give rise to many types of differentiated, specialized cell types, including some liver cells.[5] These observations highlight the plasticity of adult stem cells and raise the potential of repopulating damaged tissues with bone marrow-derived stem cells. We will return to a discussion of stem cells, their biology, and their clinical relevance in Chapter 3.

Pathologic Hyperplasia

Most forms of pathologic hyperplasia are caused by excessive hormonal stimulation or growth factors acting on target cells. Endometrial hyperplasia is an example of abnormal hormone-induced hyperplasia. After a normal menstrual period, there is a rapid burst of proliferative activity that is stimulated by pituitary hormones and ovarian estrogen. It is brought to a halt by the rising levels of progesterone, usually about 10 to 14 days before the anticipated menstrual period. In some instances, however, the balance between estrogen and progesterone is disturbed. This results in absolute or relative increases in the amount of estrogen, with consequent hyperplasia of the endometrial glands. This form of hyperplasia is a common cause of abnormal menstrual bleeding. Benign prostatic hyperplasia is another common example of pathologic hyperplasia induced by responses to hormones, in this case, androgens. Although these forms of hyperplasia are abnormal, the process remains controlled, because the hyperplasia regresses if the hormonal stimulation is eliminated. As is discussed in Chapter 7, it is this response to normal regulatory control mechanisms that distinguishes benign pathologic hyperplasias from cancer, in which the growth control mechanisms become defective. *Pathologic hyperplasia, however, constitutes a fertile soil in which cancerous proliferation may eventually arise*. Thus, patients with hyperplasia of the endometrium are at increased risk for developing endometrial cancer (Chapter 22).

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). Under these circumstances, growth factors are responsible for the hyperplasia. Stimulation by growth factors is also involved in the hyperplasia that is associated with certain *viral infections*, such as papillomaviruses, which cause skin warts and a number of mucosal lesions composed of masses of hyperplastic epithelium.

HYPERTROPHY

Hypertrophy refers to an increase in the size of cells, resulting in an increase in the size of the organ. Thus, the hypertrophied organ has no new cells, just larger cells. The increased size of the cells is due not to cellular swelling but to the synthesis of more structural components. As mentioned above, cells capable of division may respond to stress by undergoing both hyperplasia and hypertrophy, whereas in *nondividing cells* (e.g., myocardial fibers), hypertrophy occurs. Nuclei in hypertrophied cells may have a higher DNA content than in normal cells, probably because the cells arrest in the cell cycle without undergoing mitosis.

Hypertrophy can be *physiologic* or *pathologic* and is caused by increased functional demand or by specific hormonal stimulation. The striated muscle cells in both the heart and the skeletal muscles are capable of tremendous hypertrophy, perhaps because they cannot adequately adapt to increased metabolic demands by mitotic division and production of more cells to share the work. The most common stimulus for hypertrophy of muscle is increased workload. For example, the bulging muscles of bodybuilders engaged in "pumping iron" result from an increase in size of the individual muscle fibers in response to increased demand. The workload is thus shared by a greater mass of cellular components, and each muscle fiber is spared excess work and so escapes injury. The enlarged muscle cell achieves a new equilibrium, permitting it to function at a higher level of activity. In the heart, the stimulus for hypertrophy is usually *chronic hemodynamic overload*, resulting from either hypertension or faulty valves. Synthesis of more proteins and filaments occurs, achieving a balance between the demand and the cell's functional capacity. The greater number of myofilaments per cell permits an increased workload with a level of metabolic activity per unit volume of cell not different from that borne by the normal cell.

The massive physiologic growth of the uterus during pregnancy is a good example of hormone-induced increase in the size of an organ that results from both hypertrophy and hyperplasia (Fig. 1–3*A*). The cellular hypertrophy is stimulated by estrogenic hormones acting on smooth muscle estrogen receptors, eventually resulting in increased synthesis of smooth muscle proteins and an increase in cell size (Fig. 1–3*B*). Similarly, prolactin and estrogen cause hypertrophy of the breasts during lactation. These are examples of physiologic hypertrophy induced by hormonal stimulation.

Although the traditional view of cardiac and skeletal muscle is that these tissues are incapable of proliferation and, therefore, their enlargement is entirely a result of hypertrophy, recent data suggest that even these cell types are capable of limited proliferation as well as repopulation from precursors.[6] This view emphasizes the concept, mentioned earlier, that hyperplasia and hypertrophy often occur concomitantly during the responses of tissues and organs to increased stress and cell loss.

Mechanisms of Hypertrophy. Much of our understanding of hypertrophy is based on studies of the heart. The mechanisms of cardiac muscle hypertrophy involve many signal transduction pathways, leading to the induction of a number of genes, which in turn stimulate synthesis of numerous cellular proteins (Fig. 1–4).[7,8] The genes that are induced during hypertrophy include those encoding transcription factors (such as c-*fos*, c-*jun*); growth factors (TGF-β, insulin-like growth factor-1 [IGF-1], fibroblast growth factor); and vasoactive agents (α-adrenergic agonists, endothelin-1, and angiotensin II). These factors are discussed in detail in Chapter 3. 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 leads to

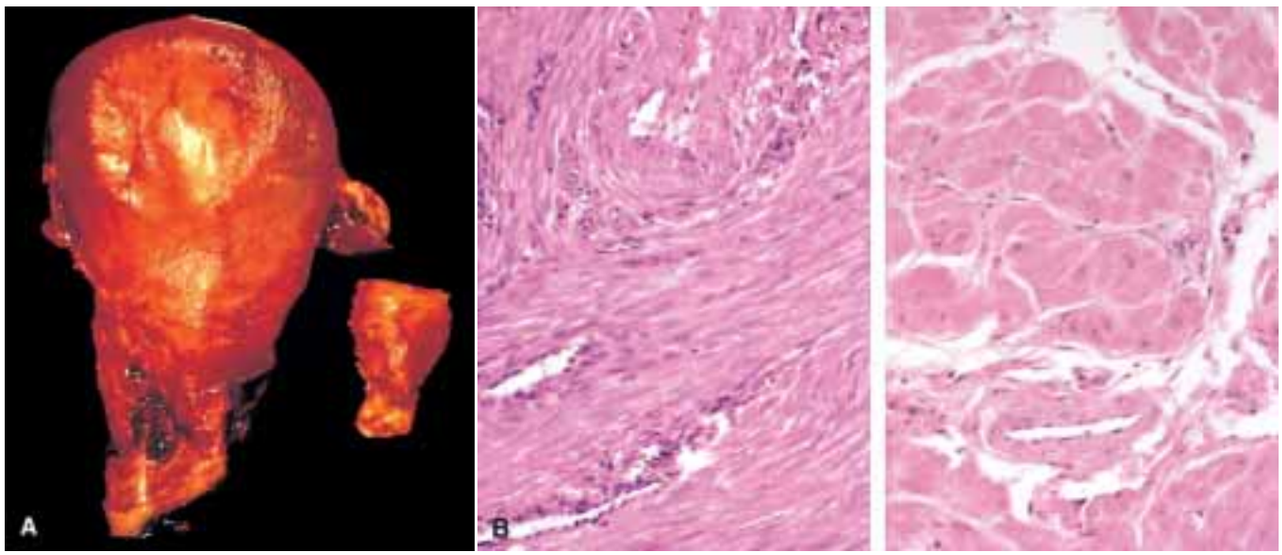


FIGURE 1–3 Physiologic hypertrophy of the uterus during pregnancy. *A,* Gross appearance of a normal uterus *(right)* and a gravid uterus (removed for postpartum bleeding) *(left). B,* Small spindle-shaped uterine smooth muscle cells from a normal uterus *(left)* compared with large plump cells in gravid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

decreased myosin adenosine triphosphatase (ATPase) activity and a slower, more energetically economical contraction. In addition, some genes that are expressed only during early development are re-expressed in hypertrophic cells, and the products of these genes participate in the cellular response to stress. For example, in the embryonic heart, the gene for atrial natriuretic factor (ANF) is expressed in both the atrium and the ventricle. After birth, ventricular expression of the gene is down-regulated. Cardiac hypertrophy, however, is associated with reinduction of ANF gene expression.[9] ANF is a peptide hormone that causes salt secretion by the kidney, decreases blood volume and pressure, and therefore serves to reduce hemodynamic load.

What are the triggers for hypertrophy and for these changes in gene expression? In the heart, there are at least two groups of signals: *mechanical triggers*, such as stretch, and *trophic triggers*, such as polypeptide growth factors (IGF-1) and vasoactive agents (angiotensin II, α-adrenergic agonists). Current models suggest that growth factors or vasoactive agents produced by cardiac nonmuscle cells or by myocytes themselves in response to hemodynamic stress stimulate the expression of various genes, leading to myocyte hypertrophy. The size of

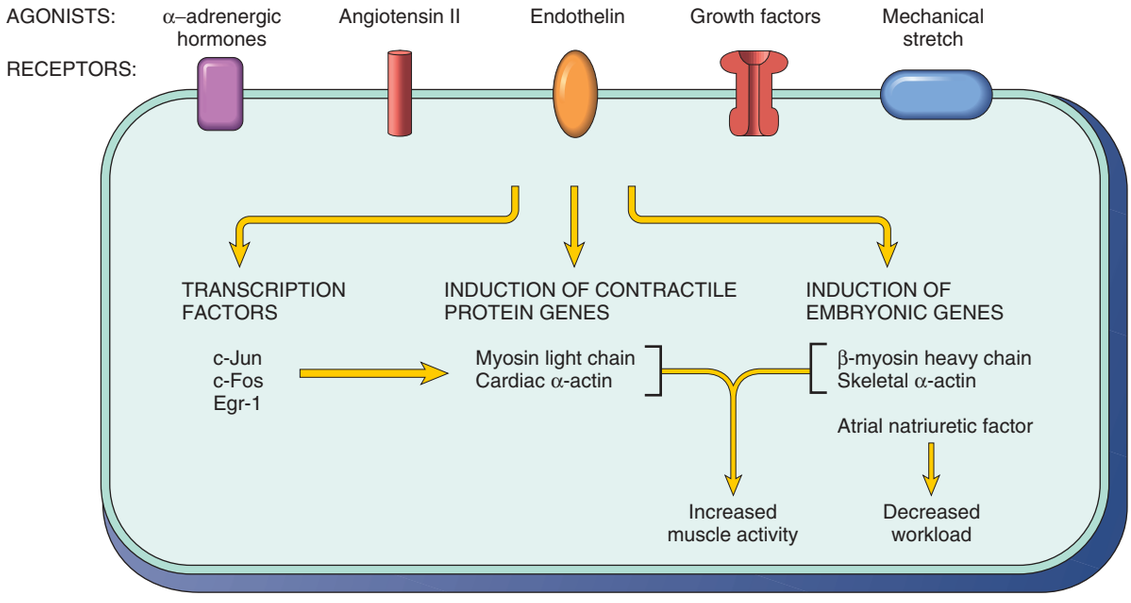


FIGURE 1–4 Changes in the expression of selected genes and proteins during myocardial hypertrophy.

cells is regulated by nutrients and environmental cues and involves several signal transduction pathways that are being unraveled.[10]

Whatever the exact mechanism of cardiac hypertrophy, it eventually reaches a limit beyond which enlargement of muscle mass is no longer able to compensate for the increased burden, and cardiac failure ensues. At this stage, a number of *degenerative* changes occur in the myocardial fibers, of which the most important are lysis and loss of myofibrillar contractile elements. Myocyte death can occur by either apoptosis or necrosis.[11] The limiting factors for continued hypertrophy and the causes of the cardiac dysfunction are poorly understood; they may be due to limitation of the vascular supply to the enlarged fibers, diminished oxidative capabilities of mitochondria, alterations in protein synthesis and degradation, or cytoskeletal alterations.

ATROPHY

Shrinkage in the size of the cell by loss of cell substance is known as atrophy. It represents a form of adaptive response and may culminate in cell death. When a sufficient number of cells are involved, the entire tissue or organ diminishes in size, or becomes atrophic. Atrophy can be physiologic or pathologic. *Physiologic atrophy* is common during early development. Some embryonic structures, such as the notochord and thyroglossal duct, undergo atrophy during fetal development. The uterus decreases in size shortly after parturition, and this is a form of physiologic atrophy. *Pathologic atrophy* depends on the underlying cause and can be local or generalized. The common causes of atrophy are the following:

- *Decreased workload (atrophy of disuse).* When a broken limb is immobilized in a plaster cast or when a patient is restricted to complete bed rest, skeletal muscle atrophy rapidly ensues. The initial rapid decrease in cell size is reversible once activity is resumed. With more prolonged disuse, skeletal muscle fibers decrease in number as well as in size; this atrophy can be accompanied by increased bone resorption, leading to osteoporosis of disuse.
- *Loss of innervation (denervation atrophy).* Normal function of skeletal muscle is dependent on its nerve supply. Damage to the nerves leads to rapid atrophy of the muscle fibers supplied by those nerves (Chapter 27).
- *Diminished blood supply.* A decrease in blood supply (ischemia) to a tissue as a result of arterial occlusive disease results in atrophy of tissue owing to progressive cell loss. In late adult life, the brain undergoes progressive atrophy, presumably as atherosclerosis narrows its blood supply (Fig. 1–5).
- *Inadequate nutrition.* Profound protein-calorie malnutrition (marasmus) is associated with the use of skeletal muscle as a source of energy after other reserves such as adipose stores have been depleted. This results in marked muscle wasting *(cachexia)*. Cachexia is also seen in patients with chronic inflammatory diseases and cancer. In the former, chronic overproduction of the inflammatory cytokine tumor necrosis factor (TNF) is thought to be responsible for appetite suppression and muscle atrophy.
- *Loss of endocrine stimulation.* Many endocrine glands, the breast, and the reproductive organs are dependent on endocrine stimulation for normal metabolism and function. The loss of estrogen stimulation after menopause results in physiologic atrophy of the endometrium, vaginal epithelium, and breast.
- *Aging (senile atrophy).* The aging process is associated with cell loss, typically seen in tissues containing permanent cells, particularly the brain and heart.
- *Pressure.* Tissue compression for any length of time can cause atrophy. An enlarging benign tumor can cause atrophy in the surrounding compressed tissues. Atrophy in this setting is probably the result of ischemic changes caused by compromise of the blood supply to those tissues by the expanding mass.

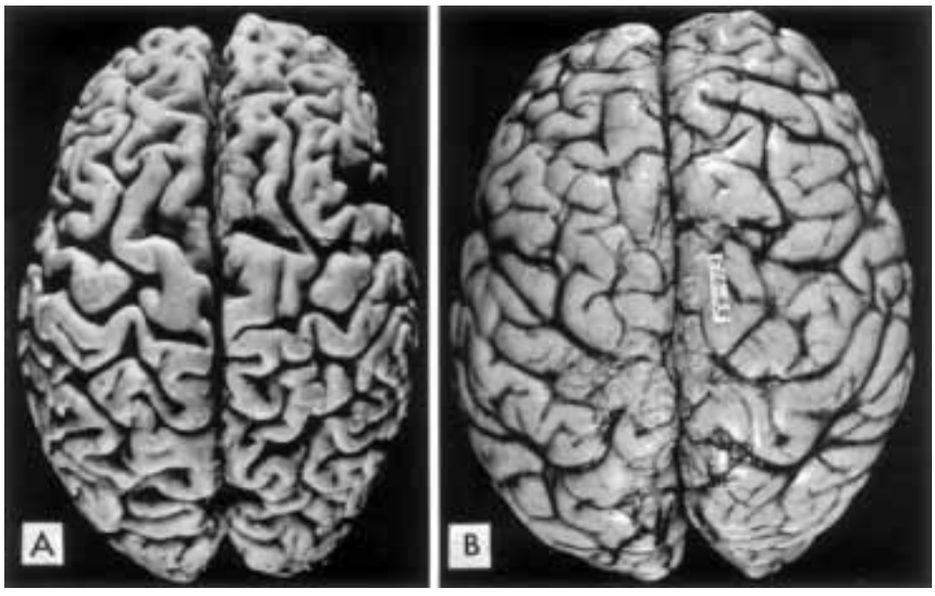


FIGURE 1–5 *A,* Physiologic atrophy of the brain in an 82-year-old male. The meninges have been stripped. *B,* Normal brain of a 36-year-old male. Note that loss of brain substance with aging narrows the gyri and widens the sulci.

The fundamental cellular changes associated with atrophy are identical in all of these settings, representing a retreat by the cells to a smaller size at which survival is still possible. Atrophy results from a reduction in the structural components of the cell. In atrophic muscle, the cells contain fewer mitochondria and myofilaments and a reduced amount of endoplasmic reticulum. By bringing into balance cell volume and lower levels of blood supply, nutrition, or trophic stimulation, a new equilibrium is achieved. *Although atrophic cells may have diminished function, they are not dead.* However, atrophy may progress to the point at which cells are injured and die. In ischemic tissues, if the blood supply is inadequate even to maintain the life of shrunken cells, injury and cell death may supervene. Furthermore, apoptosis may be induced by the same signals that cause atrophy and thus may contribute to loss of organ mass. For example, apoptosis contributes to the regression of endocrine organs after hormone withdrawal.

Mechanisms of Atrophy. The biochemical mechanisms responsible for atrophy are incompletely understood but are likely to affect the balance between protein synthesis and degradation. Increased protein degradation probably plays a key role in atrophy. Mammalian cells contain multiple proteolytic systems that serve distinct functions. *Lysosomes* contain acid hydrolases (e.g., cathepsins) and other enzymes that degrade endocytosed proteins from the extracellular environment and the cell surface as well as some cellular components. The *ubiquitin-proteasome pathway* is responsible for the degradation of many cytosolic and nuclear proteins.[12] Proteins to be degraded by this process are first conjugated to ubiquitin and then degraded within a large cytoplasmic proteolytic organelle called the *proteasome.* This pathway is thought to be responsible for the accelerated proteolysis seen in a variety of catabolic conditions, including cancer cachexia. Hormones, particularly glucocorticoids and thyroid hormone, stimulate proteasome-mediated protein degradation; insulin opposes these actions. Additionally, cytokines, such as TNF, are capable of signaling accelerated muscle proteolysis by way of this mechanism.

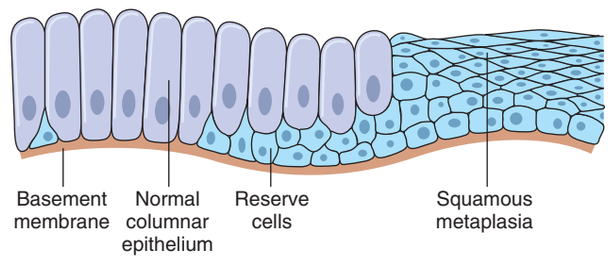
In many situations, atrophy is also accompanied by marked increases in the number of *autophagic vacuoles*. These are membrane-bound vacuoles within the cell that contain fragments of cell components (e.g., mitochondria, endoplasmic reticulum) that are destined for destruction and into which the lysosomes discharge their hydrolytic contents. The cellular components are then digested. Some of the cell debris within the autophagic vacuole may resist digestion and persist as membrane-bound residual bodies that may remain as a sarcophagus in the cytoplasm. An example of such *residual bodies* is the *lipofuscin granules*, discussed later in the chapter. When present in sufficient amounts, they impart a brown discoloration to the tissue *(brown atrophy).*

METAPLASIA

Metaplasia is a reversible change in which one adult cell type (epithelial or mesenchymal) is replaced by another adult cell type.[13] It may represent an adaptive substitution of cells that are sensitive to stress by cell types better able to withstand the adverse environment.

The most common epithelial metaplasia is *columnar* to *squamous* (Fig. 1–6*A*), as occurs in the respiratory tract in response to chronic irritation. In the habitual cigarette smoker, the normal ciliated columnar epithelial cells of the trachea and bronchi are often replaced focally or widely by stratified squamous epithelial cells. Stones in the excretory ducts of the salivary glands, pancreas, or bile ducts may cause replacement of the normal secretory columnar epithelium by nonfunctioning stratified squamous epithelium. A deficiency of vitamin A (retinoic acid) induces squamous metaplasia in the respiratory epithelium, and vitamin A excess suppresses keratinization (Chapter 9). In all these instances, the more rugged stratified squamous epithelium is able to survive under circumstances in which the more fragile specialized columnar epithelium most likely would have succumbed. Although the metaplastic squamous cells in the respiratory tract, for example, are capable of surviving, an important protective mechanism—mucus secretion—is lost. Thus, epithelial metaplasia is a two-edged sword and, in most circumstances, represents an undesirable change. Moreover, *the influences that predispose to metaplasia, if persistent, may induce malignant transformation in metaplastic epithelium*. Thus, the common form of cancer in the respiratory tract is composed of squamous cells, which arise in areas of metaplasia of the normal columnar epithelium into squamous epithelium.

Metaplasia from squamous to columnar type may also occur, as in *Barrett esophagus*, in which the esophageal squamous epithelium is replaced by intestinal-like columnar cells under the influence of refluxed gastric acid (Fig. 1–6*B*). Cancers may arise in these areas, and these are typically glandular (adeno)carcinomas (Chapter 17).



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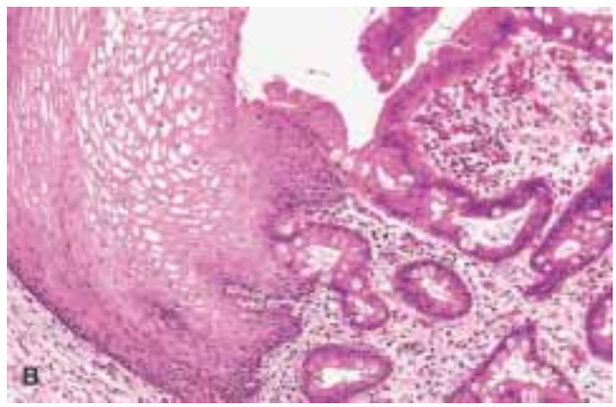


FIGURE 1–6 Metaplasia. *A,* Schematic diagram of columnar to squamous metaplasia. *B,* Metaplastic transformation of esophageal stratified squamous epithelium (*left)* to mature columnar epithelium (so-called Barrett metaplasia).

Connective tissue metaplasia is the formation of cartilage, bone, or adipose tissue (mesenchymal tissues) in tissues that normally do not contain these elements. For example, bone formation in muscle, designated *myositis ossificans*, occasionally occurs after bone fracture. This type of metaplasia is less clearly seen as an adaptive response.

Mechanisms of Metaplasia. Metaplasia does not result from a change in the phenotype of a differentiated cell type; instead it is the result of a reprogramming of stem cells that are known to exist in normal tissues, or of undifferentiated mesenchymal cells present in connective tissue. In a metaplastic change, these precursor cells differentiate along a new pathway. The differentiation of stem cells to a particular lineage is brought about by signals generated by cytokines, growth factors, and extracellular matrix components in the cell's environment. Tissue-specific and differentiation genes are involved in the process, and an increasing number of these are being identified.[14] For example, bone morphogenetic proteins, members of the TGF-β superfamily, induce chondrogenic or osteogenic expression in stem cells while suppressing differentiation into muscle or fat.[15] These growth factors, acting as external triggers, then induce specific transcription factors that lead the cascade of phenotype-specific genes toward a fully differentiated cell. How these normal pathways run amok to cause metaplasia is unclear in most instances. In the case of vitamin A deficiency or excess, it is known that retinoic acid regulates cell growth, differentiation, and tissue patterning and may thus influence the differentiation pathway of stem cells.[16] Certain *cytostatic* drugs cause a disruption of DNA methylation patterns and can transform mesenchymal cells from one type (fibroblast) to another (muscle, cartilage).

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. Injury may progress through a reversible stage and culminate in cell death (Fig. 1–7). An overview of the morphologic changes in cell injury is shown in Figure 1–8. The biochemical alterations and the associated morphologic abnormalities are described later, under "Mechanisms of Cell Injury." These alterations may be divided into the following stages:

- *Reversible cell injury.* Initially, injury is manifested as functional and morphologic changes that are reversible if the damaging stimulus is removed. The hallmarks of reversible injury are reduced oxidative phosphorylation, adenosine triphosphate (ATP) depletion, and cellular swelling caused by changes in ion concentrations and water influx.
- *Irreversible injury and cell death.* With continuing damage, the injury becomes irreversible, at which time the cell cannot recover. Is there a critical biochemical event (the "lethal hit") responsible for the point of no return? There are no clear answers to this question. However, as discussed later, in ischemic tissues such as the myocardium, certain structural changes (e.g., amorphous densities in mitochondria, indicative of severe mitochondrial damage) and functional changes (e.g., loss of membrane permeability) are indicative of cells that have suffered irreversible injury.
- *Irreversibly injured cells invariably undergo morphologic changes that are recognized as cell death*. There are two types of cell death, necrosis and apoptosis, which differ in their morphology, mechanisms, and roles in disease and physiology (Fig. 1–9 and Table 1–2). When damage to membranes is severe, lysosomal enzymes enter the cytoplasm and digest the cell, and cellular contents leak out, resulting in *necrosis*. Some noxious stimuli, especially those that damage DNA, induce another type of death, *apoptosis*, which is characterized by nuclear dissolution without complete loss of membrane integrity. *Whereas necrosis is always a pathologic process, apoptosis serves many normal functions and is not necessarily associated with cell injury*. Although we emphasize the distinctions between necrosis and apoptosis, there may be some overlaps and common mechanisms between these two pathways. In addition, at least some types of stimuli may induce either apoptosis or necrosis, depending on the intensity and duration of the stimulus, the rapidity of the death process, and the biochemical derangements induced in the injured cell. The mechanisms and significance of these two death pathways are discussed later in the chapter.

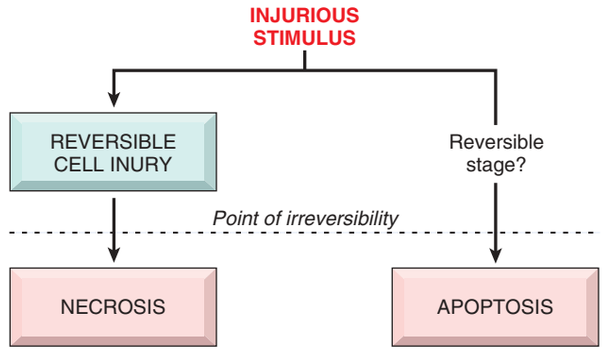


FIGURE 1–7 Stages in the evolution of cell injury and death.

In the following sections, we will discuss the causes and mechanisms of cell injury. We first describe the sequence of events in cell injury and its common end point, necrosis, and discuss selected illustrative examples of cell injury and necrosis. We conclude with a discussion of the unique pattern of cell death represented by apoptosis.

Causes of Cell Injury

The causes of cell injury range from the external gross physical violence of an automobile accident to internal endogenous causes, such as a subtle genetic mutation causing lack of a vital enzyme that impairs normal metabolic function. Most injurious stimuli can be grouped into the following broad categories.

Oxygen Deprivation. *Hypoxia* is a deficiency of oxygen, which causes cell injury by reducing aerobic oxidative respiration. Hypoxia is an extremely important and common cause of cell injury and cell death. It should be distinguished from *ischemia*, which is a loss of blood supply from impeded arterial flow or reduced venous drainage in a tissue. Ischemia

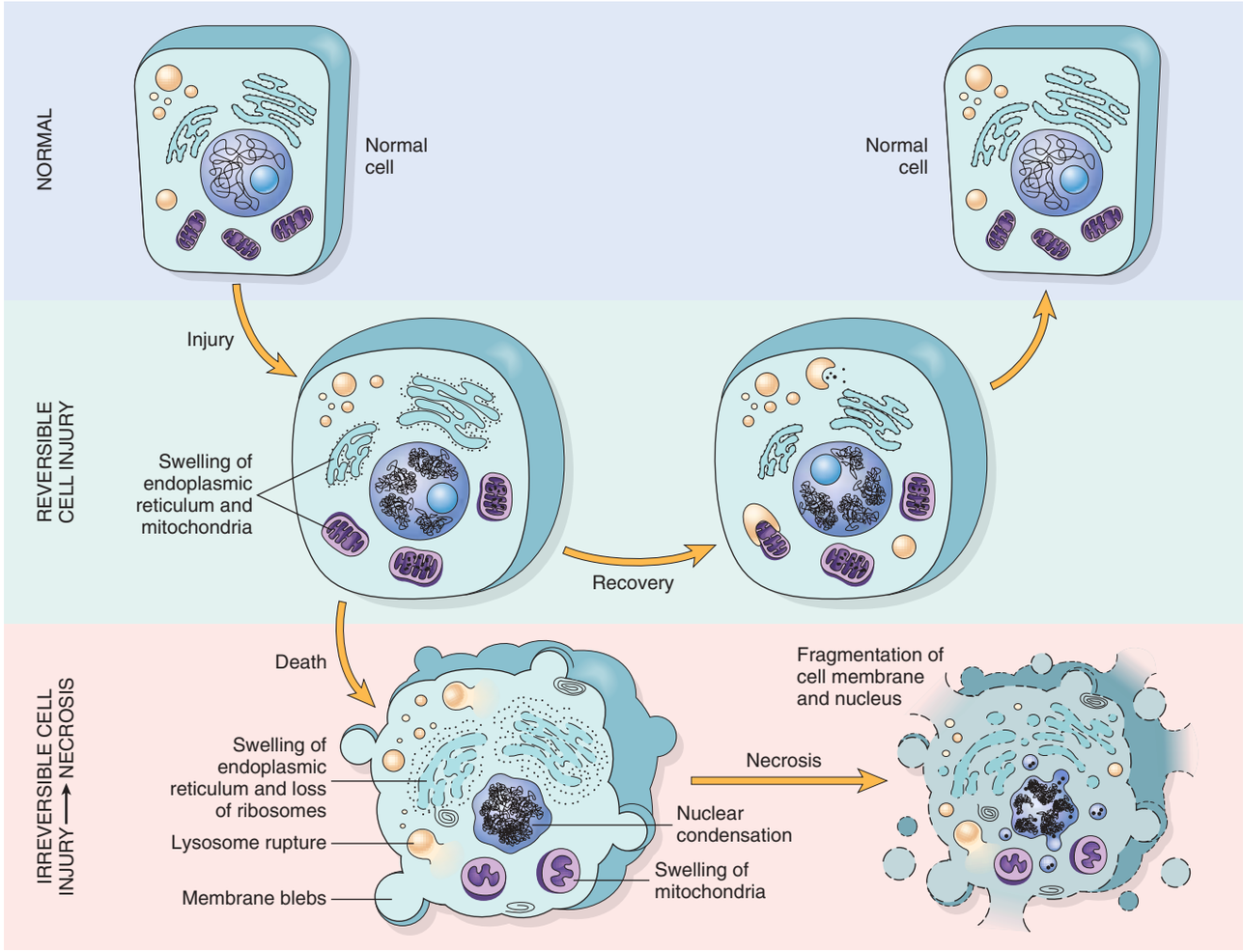


FIGURE 1–8 Schematic representation of a normal cell and the changes in reversible and irreversible cell injury. Depicted are morphologic changes, which are described in the following pages and shown in electron micrographs in Figure 1–17. Reversible injury is characterized by generalized swelling of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

TABLE 1–2 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

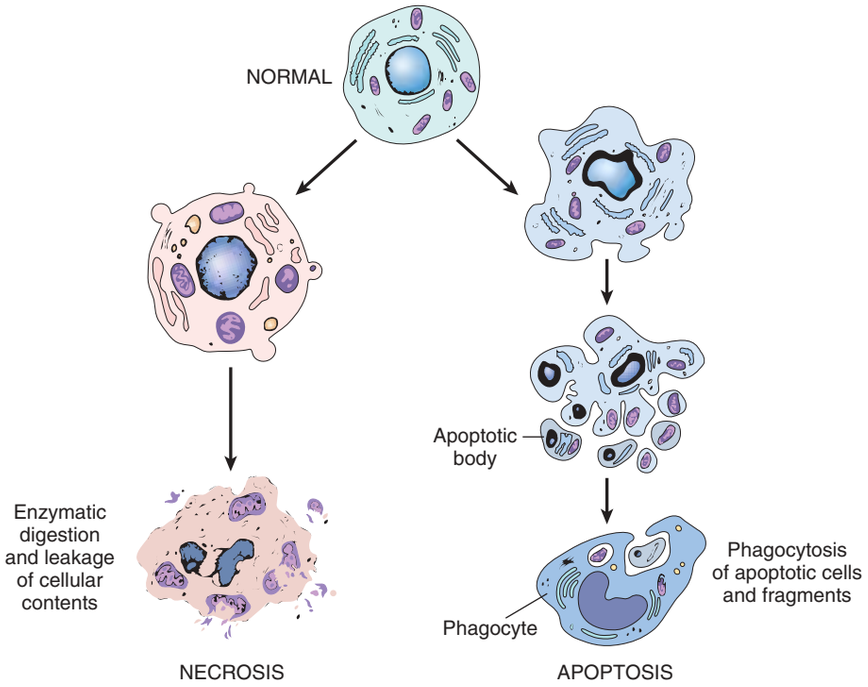


FIGURE 1–9 The sequential ultrastructural changes seen in necrosis *(left)* and apoptosis *(right)*. In apoptosis, the initial changes consist of nuclear chromatin condensation and fragmentation, followed by cytoplasmic budding and phagocytosis of the extruded apoptotic bodies. Signs of cytoplasmic blebs,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

compromises the supply not only of oxygen, but also of metabolic substrates, including glucose (normally provided by flowing blood). Therefore, ischemic tissues are injured more rapidly and severely than are hypoxic tissues. One cause of hypoxia is inadequate oxygenation of the blood due to cardiorespiratory failure. Loss of the oxygen-carrying capacity of the blood, as in anemia or carbon monoxide poisoning (producing a stable carbon monoxyhemoglobin that blocks oxygen carriage), is a less frequent cause of oxygen deprivation that results in significant injury. Depending on the severity of the hypoxic state, cells may adapt, undergo injury, or die. For example, if the femoral artery is narrowed, the skeletal muscle cells of the leg may shrink in size (atrophy). This reduction in cell mass achieves a balance between metabolic needs and the available oxygen supply. More severe hypoxia induces injury and cell death.

Physical Agents. Physical agents capable of causing cell injury include mechanical trauma, extremes of temperature (burns and deep cold), sudden changes in atmospheric pressure, radiation, and electric shock (Chapter 9).

Chemical Agents and Drugs. The list of chemicals that may produce cell injury defies compilation. Simple chemicals such as glucose or salt in hypertonic concentrations may cause cell injury directly or by deranging electrolyte homeostasis of cells. Even oxygen, in high concentrations, is severely toxic. Trace amounts of agents known as *poisons*, such as arsenic, cyanide, or mercuric salts, may destroy sufficient numbers of cells within minutes to hours to cause death. Other substances, however, are our daily companions: environmental and air pollutants, insecticides, and herbicides; industrial and occupational hazards, such as carbon monoxide and asbestos; social stimuli, such as alcohol and narcotic drugs; and the ever-increasing variety of therapeutic drugs.

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

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

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

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

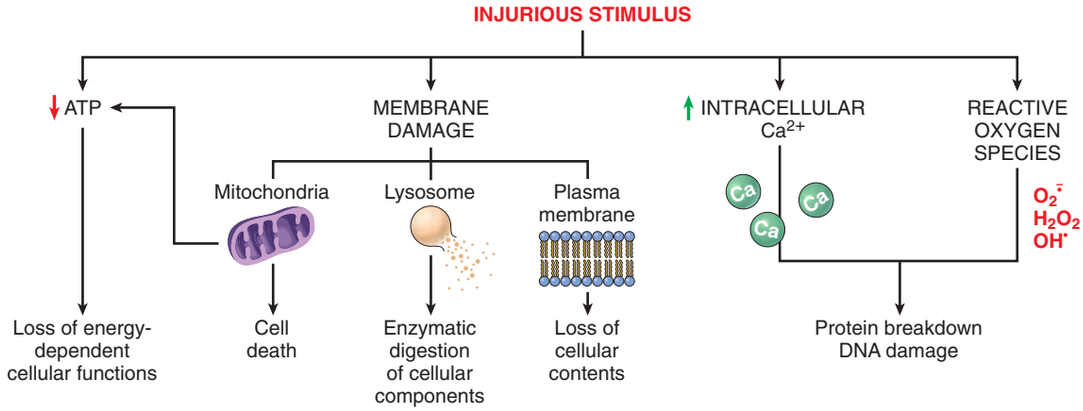


FIGURE 1–10 Cellular and biochemical sites of damage in cell injury.

contribution to a number of diseases. Metabolic diseases such as diabetes also cause severe cell injury.

Mechanisms of Cell Injury

The biochemical mechanisms responsible for cell injury are complex. There are, however, a number of principles that 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, small doses of a chemical toxin or brief periods of ischemia may induce reversible injury, whereas large doses of the same toxin or more prolonged ischemia might result either in instantaneous cell death or in slow, irreversible injury leading in time to cell death.
- *The consequences of cell injury depend on the type, state, and adaptability of the injured cell.* The cell's nutritional and hormonal status and its metabolic needs are important in its response to injury. How vulnerable is a cell, for example, to loss of blood supply and hypoxia? The striated muscle cell in the leg can be placed entirely at rest when it is deprived of its blood supply; not so the striated muscle of the heart. Exposure of two individuals to identical concentrations of a toxin, such as carbon tetrachloride, may produce no effect in one and cell death in the other. This may be due to genetic variations affecting the amount and activity of hepatic enzymes that convert carbon tetrachloride to toxic byproducts (Chapter 9). With the complete mapping of the human genome, there is great interest in identifying genetic polymorphisms that affect the cell's response to injurious agents.
- *Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components* (Fig. 1–10). The most important targets of injurious stimuli are: (1) aerobic respiration involving mitochondrial oxidative phosphorylation and production of ATP; (2) the integrity of 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 integrity of the genetic apparatus of the cell.

In the following section, we describe each of the biochemical mechanisms that are responsible for cell injury induced by different stimuli. It should be noted that with most stimuli, multiple mechanisms contribute to injury, and in the case of many injurious stimuli, the actual biochemical locus of injury remains unknown.

DEPLETION OF ATP

ATP depletion and decreased ATP synthesis are frequently associated with both hypoxic and chemical (toxic) injury (Fig. 1–11). High-energy phosphate in the form of ATP is required for many synthetic and degradative processes within the cell. These include membrane transport, protein synthesis, lipogenesis, and the deacylation–reacylation reactions necessary for phospholipid turnover. ATP is produced in two ways. The major pathway in mammalian cells is *oxidative phosphorylation* of adenosine diphosphate, in a reaction that results in reduction of oxygen by the electron transfer system of mito-

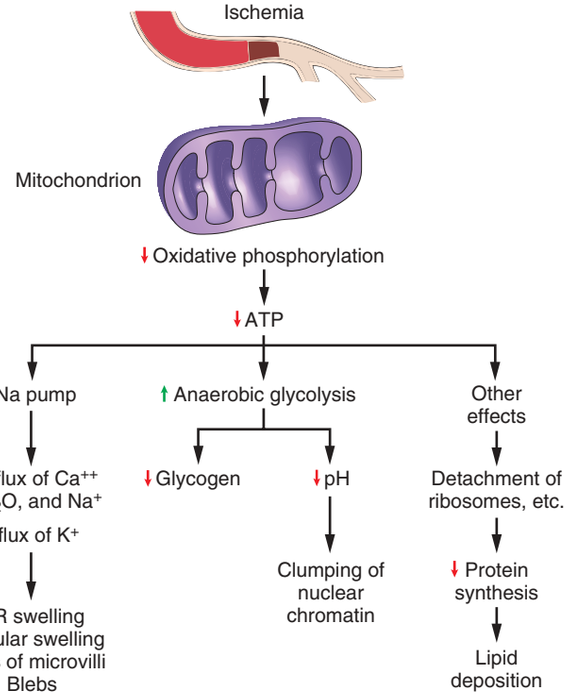


FIGURE 1–11 Functional and morphologic consequences of decreased intracellular ATP during cell injury.

chondria. The second is the *glycolytic pathway*, which can generate ATP in the absence of oxygen using glucose derived either from body fluids or from the hydrolysis of glycogen. Thus, tissues with greater glycolytic capacity (e.g., liver) have an advantage when ATP levels are falling because of inhibition of oxidative metabolism by injury.

Depletion of ATP to <5% to 10% of normal levels has widespread effects on many critical cellular systems:

- The activity of the *plasma membrane energy-dependent sodium pump* (ouabain-sensitive Na^+, K^+-ATPase) is reduced. Failure of this active transport system, due to diminished ATP concentration and enhanced ATPase activity, causes sodium to accumulate intracellularly and potassium to diffuse out of the cell. The net gain of solute is accompanied by isosmotic gain of water, causing *cell swelling*, and dilation of the endoplasmic reticulum (see Fig. 1–8).
- *Cellular energy metabolism is altered*. If the supply of oxygen to cells is reduced, as in ischemia, oxidative phosphorylation ceases and cells rely on glycolysis for energy production. This switch to anaerobic metabolism is controlled by energy pathway metabolites acting on glycolytic enzymes. The decrease in cellular ATP and associated increase in adenosine monophosphate stimulate phosphofructokinase and phosphorylase activities. These result in an increased rate of *anaerobic glycolysis* designed to maintain the cell's energy sources by generating ATP through metabolism of glucose derived from glycogen. As a consequence, *glycogen stores are rapidly depleted*. Glycolysis results in the accumulation of *lactic acid* and inorganic phosphates from the hydrolysis of phosphate esters. This reduces the intracellular pH, resulting in 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.
- With prolonged or worsening depletion of ATP, structural disruption of the protein synthetic apparatus occurs, manifested as detachment of ribosomes from the rough endoplasmic reticulum 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 (see Fig. 1–8).
- In cells deprived of oxygen or glucose, proteins may become misfolded, and misfolded proteins trigger a cellular reaction called the *unfolded protein response* that may lead to cell injury and even death. This process is described later in the chapter. Protein misfolding is also seen in cells exposed to stress, such as heat, and when proteins are damaged by enzymes (such as Ca^{2+}-responsive enzymes, described below) and free radicals.

MITOCHONDRIAL DAMAGE

Mitochondria are important targets for virtually all types of injurious stimuli, including hypoxia and toxins. Cell injury is frequently accompanied by morphologic changes in mitochondria. Mitochondria can be damaged by increases of cytosolic Ca^{2+}, by oxidative stress, by breakdown of phospholipids through the phospholipase A_2 and sphingomyelin pathways, and by lipid breakdown products derived therefrom, such as free fatty acids and ceramide. Mitochondrial damage often results in the formation of a high-conductance channel, the so-called mitochondrial permeability transition, in the inner mitochondrial membrane (Fig. 1–12).[17] Although reversible in its early stages, this nonselective pore becomes permanent if the inciting stimuli persist, precluding maintenance of mitochondrial proton motive force, or potential. Because maintenance of membrane potential is critical for mitochondrial oxidative phosphorylation, it follows that irreversible mitochondrial permeability transition is a deathblow to the cell. Mitochondrial damage can also be associated with leakage of cytochrome *c* into the cytosol. Because cytochrome *c* is an integral component of the electron transport chain and can trigger apoptotic death pathways in the cytosol (see later), this pathologic event is also likely to be a key determinant of cell death.

INFLUX OF INTRACELLULAR CALCIUM AND LOSS OF CALCIUM HOMEOSTASIS

Calcium ions are important mediators of cell injury.[18] Cytosolic free calcium is maintained at extremely low concentrations (<0.1 μmol) compared with extracellular levels of 1.3 mmol, and most intracellular calcium is sequestered in mitochondria and endoplasmic reticulum. Such gradients are modulated by membrane-associated, energy-dependent Ca^{2+}, Mg^{2+}-ATPases. Ischemia and certain toxins cause an early increase in cytosolic calcium concentration, owing to the net influx of Ca^{2+} across the plasma membrane and the release of Ca^{2+} from mitochondria and endoplasmic reticulum (Fig. 1–13). Sustained rises in intracellular Ca^{2+} subsequently result from nonspecific increases in membrane permeability. Increased Ca^{2+} in turn activates a number of enzymes, with potential deleterious cellular effects. The enzymes known to be activated by calcium include *ATPases* (thereby hastening ATP depletion), *phospholipases* (which cause membrane

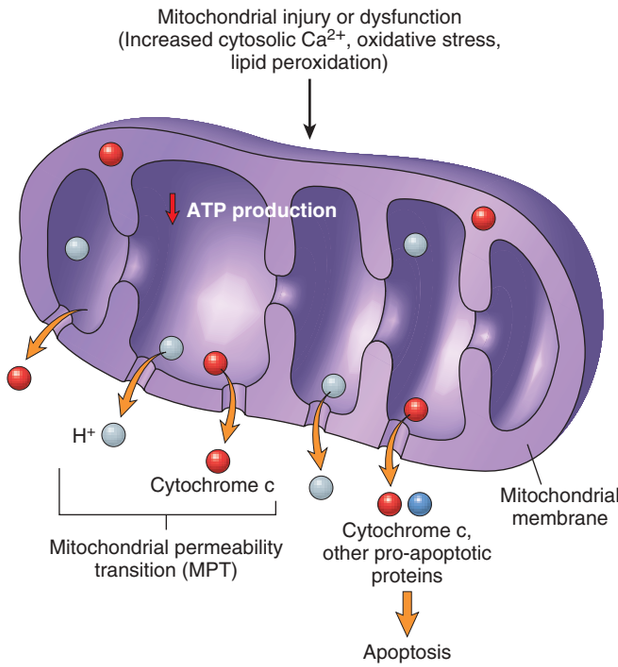


FIGURE 1–12 Mitochondrial dysfunction in cell injury.

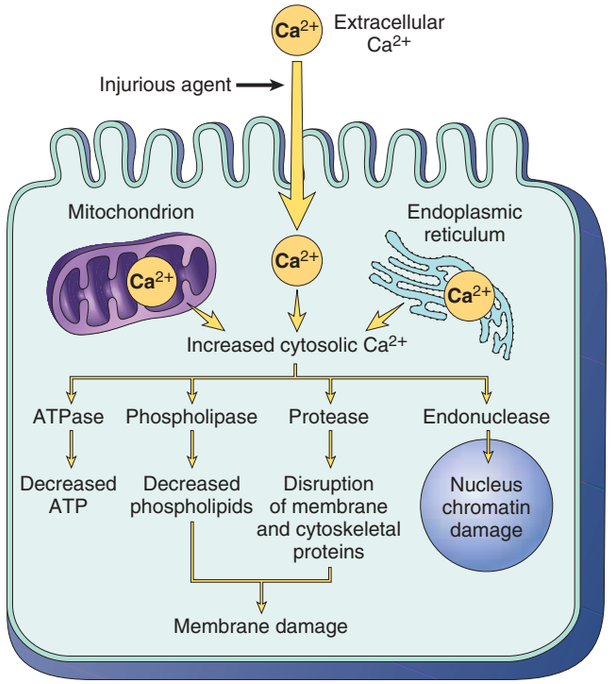


FIGURE 1–13 Sources and consequences of increased cytosolic calcium in cell injury. ATP, adenosine triphosphate.

damage), *proteases* (which break down both membrane and cytoskeletal proteins), and *endonucleases* (which are responsible for DNA and chromatin fragmentation). Increased intracellular Ca^{2+} levels also result in increased mitochondrial permeability and the induction of apoptosis.[18–20] Although cell injury often results in increased intracellular calcium and this in turn mediates a variety of deleterious effects, including cell death, loss of calcium homeostasis is not always a proximal event in irreversible cell injury.

ACCUMULATION OF OXYGEN-DERIVED FREE RADICALS (OXIDATIVE STRESS)

Cells generate energy by reducing molecular oxygen to water. During this process, small amounts of partially reduced reactive oxygen forms are produced as an unavoidable byproduct of mitochondrial respiration. Some of these forms are free radicals that can damage lipids, proteins, and nucleic acids. They are referred to as *reactive oxygen species*. Cells have defense systems to prevent injury caused by these products. An imbalance between free radical-generating and radical-scavenging systems results in *oxidative stress*, a condition that has been associated with the cell injury seen in many pathologic conditions. Free radical–mediated damage contributes to such varied processes as chemical and radiation injury, ischemia-reperfusion injury (induced by restoration of blood flow in ischemic tissue), cellular aging, and microbial killing by phagocytes.[21–24]

Free radicals are chemical species that have a single unpaired electron in an outer orbit. Energy created by this unstable configuration is released through reactions with adjacent molecules, such as inorganic or organic chemicals—proteins, lipids, carbohydrates—particularly with key molecules in membranes and nucleic acids. Moreover, free radicals initiate autocatalytic reactions, whereby molecules with which they react are themselves converted into free radicals to propagate the chain of damage.

Free radicals may be *initiated* within cells in several ways (Fig. 1–14):

- *Absorption of radiant energy* (e.g., ultraviolet light, x-rays). For example, ionizing radiation can hydrolyze water into hydroxyl (OH) and hydrogen (H) free radicals.
- *Enzymatic metabolism of exogenous chemicals or drugs* (e.g., carbon tetrachloride [CCl_4] can generate CCl_3, described later in this chapter).
- *The reduction-oxidation reactions that occur during normal metabolic processes.* During normal respiration, molecular oxygen is sequentially reduced by the addition of four electrons to generate water. Such conversion occurs by oxidative enzymes in the endoplasmic reticulum, cytosol, mitochondria, peroxisomes, and lysosomes. In this process, small amounts of toxic intermediates are produced; these include superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl ions (OH). Rapid bursts of superoxide production occur in activated polymorphonuclear leukocytes during inflammation. This occurs by a precisely controlled reaction in a plasma membrane multiprotein complex that uses NADPH oxidase for the redox reaction (Chapter 2). In addition, some intracellular oxidases (such as xanthine oxidase) generate superoxide radicals as a consequence of their activity.
- *Transition metals* such as iron and copper donate or accept free electrons during intracellular reactions and catalyze free radical formation, as in the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + OH^-$). Because most of the intracellular free iron is in the ferric (Fe^{3+}) state, it must be first reduced to the ferrous (Fe^{2+}) form to participate in the Fenton reaction. This reduction can be enhanced by superoxide, and thus sources of iron and superoxide are required for maximal oxidative cell damage.
- *Nitric oxide* (NO), an important chemical mediator generated by endothelial cells, macrophages, neurons, and other cell types (Chapter 2), can act as a free radical and can also be converted to highly reactive peroxynitrite anion ($ONOO^-$) as well as NO_2 and NO_3^-.

The effects of these reactive species are wide-ranging, but three reactions are particularly relevant to cell injury (see Fig. 1–14):

- *Lipid peroxidation of membranes.* Free radicals in the presence of oxygen may cause peroxidation of lipids within plasma and organellar membranes. Oxidative damage is initiated when the double bonds in unsaturated fatty acids of membrane lipids are attacked by oxygen-derived free radicals, particularly by OH. The lipid–free radical interactions yield peroxides, which are themselves unstable and reactive, and an autocatalytic chain reaction ensues (called *propagation*), which can result in extensive membrane, organellar, and cellular damage. Other more favorable termination options take place when the free radical is captured by a scavenger, such as vitamin E, embedded in the cell membrane.
- *Oxidative modification of proteins.* Free radicals promote oxidation of amino acid residue side chains, formation of protein-protein cross-linkages (e.g., disulfide bonds), and

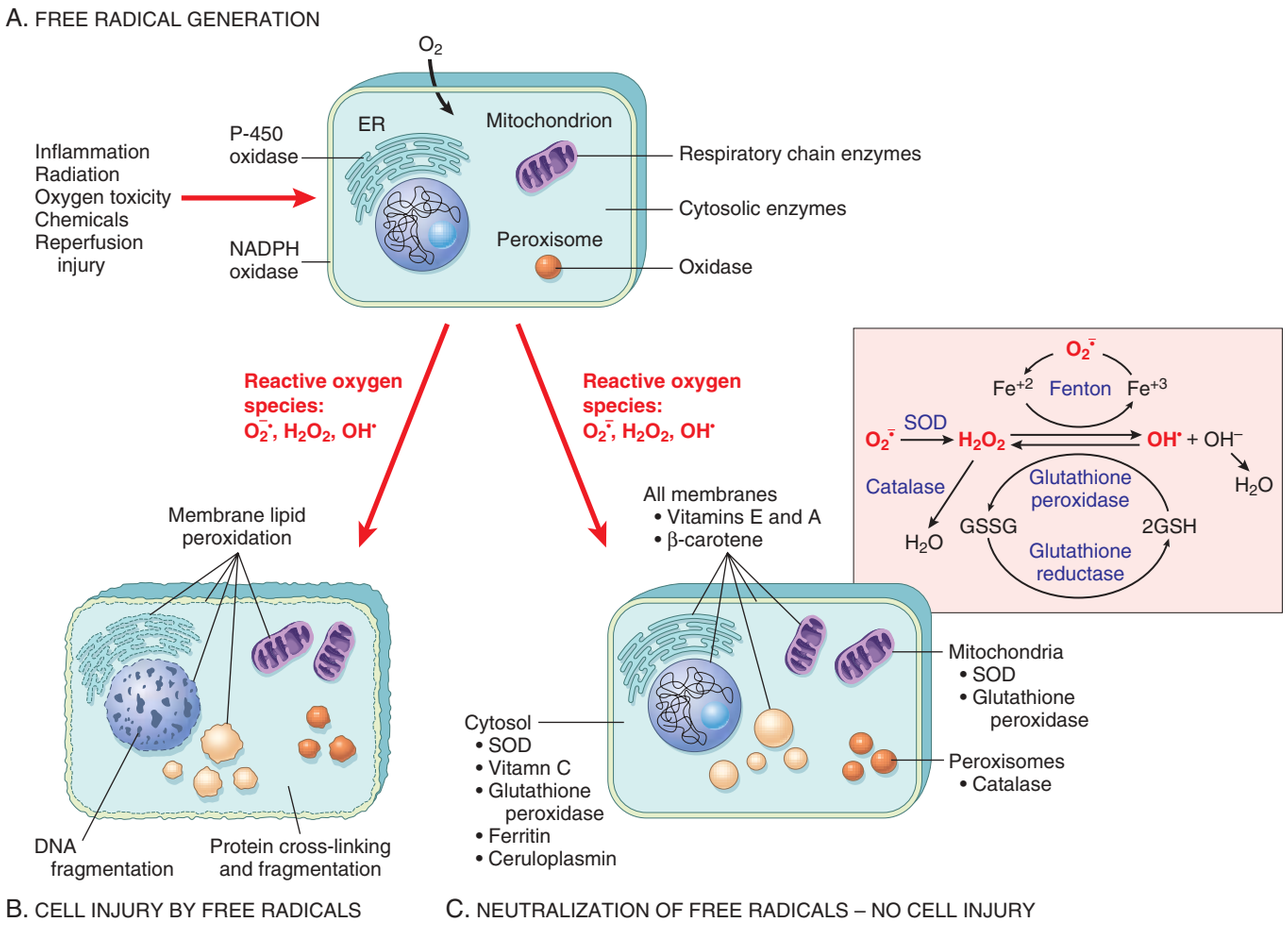


FIGURE 1–14 The role of reactive oxygen species in cell injury. O_2 is converted to superoxide (O_2^-) by oxidative enzymes in the endoplasmic reticulum (ER), mitochondria, plasma membrane, peroxisomes, and cytosol. O_2^- is converted to H_2O_2 by dismutation and thence to OH by the Cu^{2+}/Fe^{2+}-catalyzed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

oxidation of the protein backbone, resulting in protein fragmentation. Oxidative modification enhances degradation of critical proteins by the multicatalytic proteasome complex, raising havoc throughout the cell.

■ *Lesions in DNA*. Reactions with thymine in nuclear and mitochondrial DNA produce single-stranded breaks in DNA. This DNA damage has been implicated in cell aging (discussed later in this chapter) and in malignant transformation of cells (Chapter 7).

Cells have developed multiple mechanisms to remove free radicals and thereby minimize injury. Free radicals are inherently unstable and generally decay spontaneously. Superoxide, for example, is unstable and decays (dismutes) spontaneously into oxygen and hydrogen peroxide in the presence of water. There are, however, several nonenzymatic and enzymatic systems that contribute to inactivation of free radical reactions (see Fig. 1–14). These include the following:

■ *Antioxidants* either block the initiation of free radical formation or inactivate (e.g., scavenge) free radicals and terminate radical damage. Examples are the lipid-soluble vitamins E and A as well as ascorbic acid and glutathione in the cytosol.

■ As we have seen, *iron* and *copper* can catalyze the formation of reactive oxygen species. The levels of these reactive forms are minimized by binding of the ions to storage and transport proteins (e.g., transferrin, ferritin, lactoferrin, and ceruloplasmin), thereby minimizing OH formation.

■ A series of *enzymes* acts as free radical–scavenging systems and break down hydrogen peroxide and superoxide anion. These enzymes are located near the sites of generation of these oxidants and include the following:

- *Catalase*, present in peroxisomes, which decomposes H_2O_2 ($2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$).
- *Superoxide dismutases* are found in many cell types and convert superoxide to H_2O_2 ($2\ O_2^- + 2\ H \rightarrow H_2O_2 + O_2$).[23] This group includes both manganese–superoxide dismutase, which is localized in mitochondria, and copper-zinc–superoxide dismutase, which is found in the cytosol.

- *Glutathione peroxidase* also protects against injury by catalyzing free radical breakdown (H_2O_2 + 2 GSH → GSSG [glutathione homodimer] + 2 H_2O, or 2 OH + 2 GSH → GSSG + 2 H_2O). The intracellular ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) is a reflection of the oxidative state of the cell and is an important aspect of the cell's ability to detoxify reactive oxygen species.

In many pathologic processes, the final effects induced by free radicals depend on the net balance between free radical formation and termination. As stated earlier, free radicals are thought to be involved in many pathologic and physiologic processes, to be mentioned throughout this book.

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. Membrane damage may affect the mitochondria, the plasma membrane, and other cellular membranes. In ischemic cells, membrane defects may be the result of a series of events involving ATP depletion and calcium-modulated activation of phospholipases (see below). The plasma membrane, however, can also be damaged directly by certain bacterial toxins, viral proteins, lytic complement components, and a variety of physical and chemical agents. Several biochemical mechanisms may contribute to membrane damage (Fig. 1–15):

- *Mitochondrial dysfunction*. Defective mitochondrial function results in decreased phospholipid synthesis, which affects all cellular membranes, including the mitochondria themselves. At the same time, increase of cytosolic calcium associated with ATP depletion results in increased uptake of Ca^{2+} into the mitochondria, activating phospholipases and leading to breakdown of phospholipids. The net result is depletion of phospholipids from the mitochondria and other cellular membranes, and accumulation of free fatty acids. In the mitochondria, these changes cause permeability defects, such as the mitochondrial permeability transition, [17,26] leading to progresive cell injury (see Fig. 1–12).
- *Loss of membrane phospholipids*. Severe cell injury is associated with a decrease in the content of membrane phospholipids, because of degradation likely due to activation of endogenous phospholipases by increased levels of cytosolic calcium.[26] Phospholipid loss can also occur secondary to decreased ATP-dependent reacylation or diminished de novo synthesis of phospholipids.
- *Cytoskeletal abnormalities*. Cytoskeletal filaments serve as anchors connecting the plasma membrane to the cell interior. Activation of proteases by increased cytosolic calcium may cause damage to elements of the cytoskeleton. In the presence of cell swelling, this damage results, particularly in myocardial cells, in detachment of the cell membrane from the cytoskeleton, rendering it susceptible to stretching and rupture.
- *Reactive oxygen species*. Partially reduced oxygen free radicals cause injury to cell membranes and other cell constituents, by mechanisms that were discussed earlier.
- *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.[17]

Damage to mitochondrial membranes has consequences that were described above. Plasma membrane damage results in loss of osmotic balance and influx of fluids and ions, as well as loss of proteins, enzymes, coenzymes, and ribonucleic acids. The cells may also leak metabolites, which are vital for the reconstitution of ATP, thus further depleting net intracellular high-energy phosphates. *Injury to lysosomal membranes results in leakage of their enzymes into the cytoplasm and activation of these enzymes*. Lysosomes contain RNases, DNases, proteases,

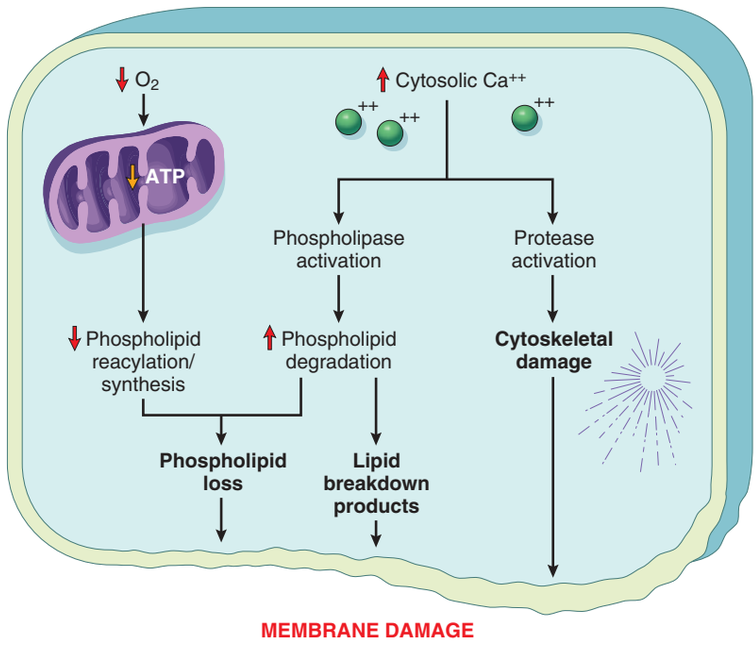


FIGURE 1–15 Mechanisms of membrane damage in cell injury. Decreased O_2 and increased cytosolic Ca^{2+} are typically seen in ischemia but may accompany other forms of cell injury. Reactive oxygen species, which are often produced on reperfusion of ischemic tissues, also cause membrane damage (not shown).

phosphatases, glucosidases, and cathepsins. Activation of these enzymes leads to enzymatic digestion of cell components, resulting in loss of ribonucleoprotein, deoxyribonucleoprotein, and glycogen, and the cells die by necrosis.

Reversible and Irreversible Cell Injury

Up to this point, we have focused on the causes and general biochemical mechanisms of cell injury. In this section, we will turn our attention to the pathways underlying the sequence of events whereby *reversible injury becomes irreversible*, leading to *cell death*, principally *necrosis*.

As discussed previously, the earliest changes associated with various forms of cell injury are decreased generation of ATP, loss of cell membrane integrity, defects in protein synthesis, cytoskeletal damage, and DNA damage. Within limits, the cell can compensate for these derangements and, if the injurious stimulus abates, will return to normalcy. Persistent or excessive injury, however, causes cells to pass the threshold into *irreversible injury* (see Fig. 1–8). This is associated with extensive damage to all cellular membranes, swelling of lysosomes, and vacuolization of mitochondria with reduced capacity to generate ATP. Extracellular calcium enters the cell and intracellular calcium stores are released, resulting in the activation of enzymes that can catabolize membranes, proteins, ATP, and nucleic acids. Following this, there is continued loss of proteins, essential coenzymes, and ribonucleic acids from the hyperpermeable plasma membrane, with cells leaking metabolites vital for the reconstitution of ATP and further depleting intracellular high-energy phosphates.

The molecular mechanisms connecting most forms of cell injury to ultimate cell death have proved elusive, for several reasons. First, there are clearly many ways to injure a cell, not all of them invariably fatal. Second, the numerous macromolecules, enzymes, and organelles within the cell are so closely interdependent that it is difficult to distinguish a primary injury from secondary (and not necessarily relevant) ripple effects. Third, the "point of no return," at which irreversible damage has occurred, is still largely undetermined; thus, we have no precise cut-off point to establish cause and effect. Finally, there is probably no single common final pathway by which cells die. It is, therefore, difficult to define the stage beyond which the cell is irretrievably doomed to destruction. And when does the cell actually die? Two phenomena consistently characterize irreversibility. *The first is the inability to reverse mitochondrial dysfunction* (lack of oxidative phosphorylation and ATP generation) even after resolution of the original injury. *The second is the development of profound disturbances in membrane function*. As mentioned earlier, injury to the lysosomal membranes results in leakage of their enzymes into the cytoplasm; the acid hydrolases are activated in the reduced intracellular pH of the ischemic cell and degrade cytoplasmic and nuclear components. This dissolution of the injured cell is characteristic of *necrosis*, one of the recognized patterns of cell death. There is also widespread leakage of potentially destructive cellular enzymes into the extracellular space, with damage to adjacent tissues and a host response (Chapter 2). Whatever the mechanism(s) of membrane damage, the end result is a massive leak of intracellular materials and a massive influx of calcium, with the consequences described above.

It is worth noting that leakage of intracellular proteins across the degraded cell membrane into the peripheral circulation provides a means of detecting tissue-specific cellular injury and death using blood serum samples. Cardiac muscle, for example, contains a specific isoform of the enzyme creatine kinase and of the contractile protein troponin; liver (and specifically 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 consequently reflected in increased levels of such proteins in the blood.

Morphology of Cell Injury and Necrosis

Cells undergo sequential biochemical and morphologic changes as they are progressively injured and ultimately die by necrosis. All stresses and noxious influences exert their effects first at the molecular or biochemical level. There is a time lag between the stress and the morphologic changes of cell injury or death; the duration of this delay may vary with the sensitivity of the methods used to detect these changes (Fig. 1–16). With histochemical or ultrastructural techniques, changes may be seen in minutes to hours after ischemic injury; however, it may take considerably longer (hours to days) before changes can be seen by light microscopy or on gross examination. As would be expected, the morphologic manifestations of necrosis take more time to develop than those of reversible damage. For example, cell swelling is a reversible morphologic change, and this may occur in a matter of minutes. Unmistakable light microscopic changes of cell death, however, do not occur in the myocardium until 4 to 12 hours after total ischemia, yet we know that irreversible injury occurs within 20 to 60 minutes.

Reversible Injury

Two patterns of reversible cell injury can be recognized under the light microscope: *cellular swelling* and *fatty change*.

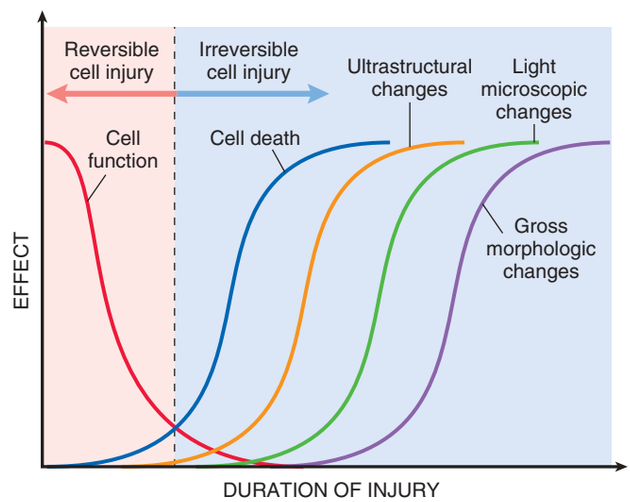


FIGURE 1–16 Timing of biochemical and morphologic changes in cell injury.

Cellular swelling appears whenever cells are incapable of maintaining ionic and fluid homeostasis and is the result of loss of function of plasma membrane energy-dependent ion pumps. Fatty change occurs in hypoxic injury and various forms of toxic or metabolic injury. It is manifested by the appearance of small or large lipid vacuoles in the cytoplasm and occurs in hypoxic and various forms of toxic injury. It is principally encountered in cells involved in and dependent on fat metabolism, such as the hepatocyte and myocardial cell. The mechanisms of fatty change are discussed in more detail later in the chapter.

Morphology. Cellular swelling is the first manifestation of almost all forms of injury to cells. It is a difficult morphologic change 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. On microscopic examination, small clear vacuoles may be seen within the cytoplasm; these represent distended and pinched-off segments of the endoplasmic reticulum. This pattern of nonlethal injury is sometimes called hydropic change or vacuolar degeneration. Swelling of cells is reversible.

The ultrastructural changes of reversible cell injury (Fig. 1–17) include:

1. **plasma membrane alterations,** such as blebbing, blunting, and distortion of microvilli; creation of myelin figures; and loosening of intercellular attachments
2. **mitochondrial changes,** including swelling, rarefaction, and the appearance of small phospholipid-rich amorphous densities
3. **dilation of the endoplasmic reticulum,** with detachment and disaggregation of polysomes
4. **nuclear alterations,** with disaggregation of granular and fibrillar elements.

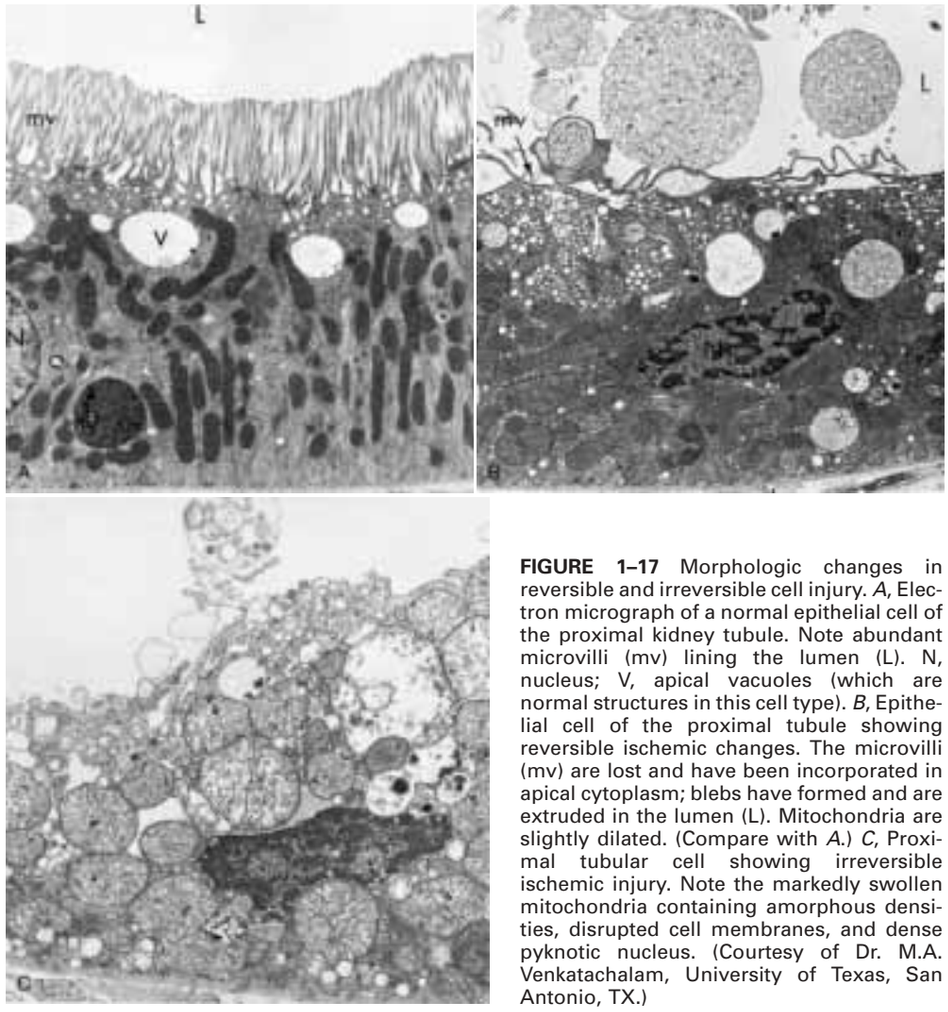


FIGURE 1–17 Morphologic changes in reversible and irreversible cell injury. *A*, Electron micrograph of a normal epithelial cell of the proximal kidney tubule. Note abundant microvilli (mv) lining the lumen (L). N, nucleus; V, apical vacuoles (which are normal structures in this cell type). *B*, Epithelial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

Necrosis

Necrosis refers to a spectrum of morphologic changes that follow cell death in living tissue, largely resulting from the progressive degradative action of enzymes on the lethally injured cell (cells placed immediately in fixative are dead but not necrotic). As commonly used, necrosis is the gross and histologic correlate of cell death occurring in the setting of irreversible exogenous injury. Necrotic cells are unable to maintain membrane integrity and their contents often leak out. This may elicit inflammation in the surrounding tissue.

The morphologic appearance of necrosis is the result of denaturation of intracellular proteins and enzymatic digestion of the cell. The enzymes are derived either from the lysosomes of the dead cells themselves, in which case the enzymatic digestion is referred to as *autolysis*, or from the lysosomes of immigrant leukocytes, during inflammatory reactions. These processes require hours to develop, and so there would be no detectable changes in cells if, for example, a myocardial infarct caused sudden death. The only telling evidence might be occlusion of a coronary artery. The earliest histologic evidence of myocardial necrosis does not become manifest until 4 to 12 hours later, but cardiac-specific enzymes and proteins that are released from necrotic muscle can be detected in the blood as early as 2 hours after myocardial cell death.

Morphology. Necrotic cells show **increased eosinophilia** attributable in part to loss of the normal basophilia imparted by the RNA in the cytoplasm and in part to the increased binding of eosin to denatured intracytoplasmic proteins (Fig. 1–18). The necrotic cell may have a more glassy homogeneous appearance than that of normal cells, mainly as a result of the loss of glycogen particles. When enzymes have digested the cytoplasmic organelles, the cytoplasm becomes vacuolated and appears moth-eaten. Finally, calcification of the dead cells may occur. Dead cells may ultimately be replaced by large, whorled phospholipid masses called **myelin figures**. 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. By electron microscopy, necrotic cells are characterized by overt discontinuities in plasma and organelle membranes, marked dilation of mitochondria with the appearance of large amorphous densities, intracytoplasmic myelin figures, amorphous osmiophilic debris, and aggregates of fluffy material probably representing denatured protein (see Fig. 1–17*C*).

Nuclear changes appear in the form of one of three patterns, all due to nonspecific breakdown of DNA (see Figs. 1–8 and 1–17*C*). The basophilia of the chromatin may fade **(karyolysis)**, a change that presumably reflects DNase activity. A second pattern (also seen in apoptotic cell death) is **pyknosis**, characterized by nuclear shrinkage and increased basophilia. Here the DNA apparently condenses into a solid, shrunken basophilic mass. In the third pattern, known as **karyorrhexis**, the pyknotic or partially pyknotic nucleus undergoes fragmentation. With the passage of time (a day or two), the nucleus in the necrotic cell totally disappears.

Once the necrotic cells have undergone the early alterations described, the mass of necrotic cells may have several morphologic patterns. Although the terms are somewhat outmoded, they are routinely used and their meanings are understood by both pathologists and clinicians. When denaturation is the primary pattern, **coagulative necrosis** develops. In the instance of dominant enzyme digestion, the result is **liquefactive necrosis;** in special circumstances, **caseous necrosis** and **fat necrosis** may occur.

Coagulative necrosis implies preservation of the basic outline of the coagulated cell for a span of at least some days (Fig. 1–19*A*). The affected tissues exhibit a firm texture. Presumably, the injury or the subsequent increasing intracellular acidosis denatures not only structural proteins but also enzymes and so blocks the proteolysis of the cell. The myocardial infarct is an excellent example in which acidophilic, coagulated, anucleate cells may persist for weeks. Ultimately, the necrotic myocardial cells are removed by fragmentation and phagocytosis of the cellular debris by scavenger white cells and by the action of proteolytic lysosomal enzymes brought in by the immigrant white cells. The process of coagulative

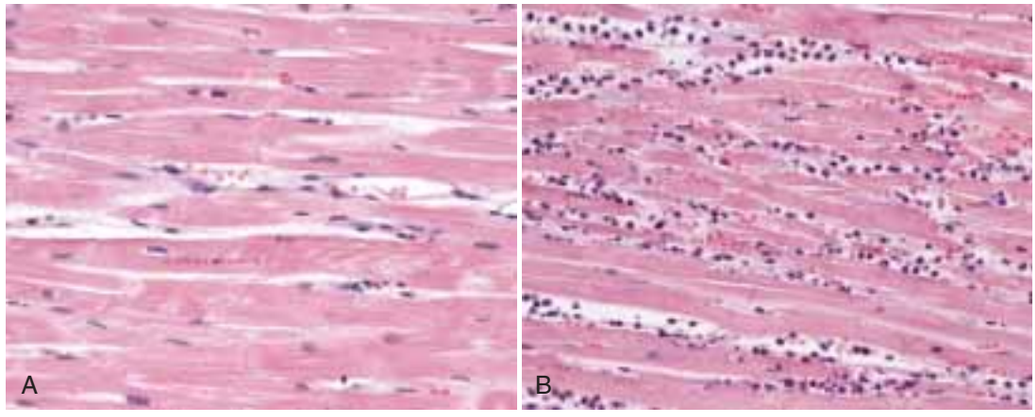


FIGURE 1–18 Ischemic necrosis of the myocardium. *A*, Normal myocardium. *B*, Myocardium with coagulation necrosis (upper two thirds of figure), showing strongly eosinophilic anucleate myocardial fibers. Leukocytes in the interstitium are an early reaction to necrotic muscle. Compare with *A* and with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

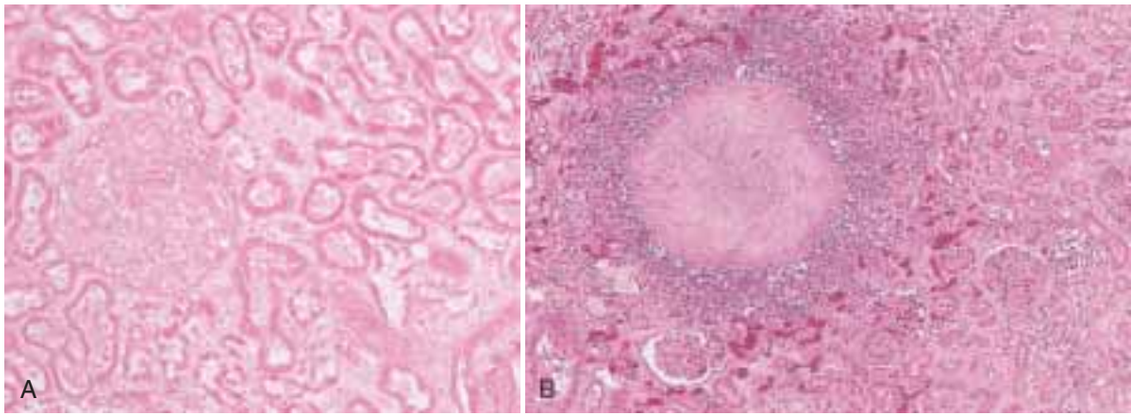


FIGURE 1–19 Coagulative and liquefactive necrosis. *A*, Kidney infarct exhibiting coagulative necrosis, with loss of nuclei and clumping of cytoplasm but with preservation of basic outlines of glomerular and tubular architecture. *B*, A focus of liquefactive necrosis in the kidney caused by fungal infection.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

necrosis, with preservation of the general tissue architecture, is characteristic of hypoxic death of cells in all tissues except the brain.

Liquefactive necrosis is characteristic of focal bacterial or, occasionally, fungal infections, because microbes stimulate the accumulation of inflammatory cells (Fig. 1–19*B*). For obscure reasons, hypoxic death of cells within the central nervous system often evokes liquefactive necrosis. Whatever the pathogenesis, liquefaction completely digests the dead cells. The end result is transformation of the tissue into a liquid viscous mass. If the process was initiated by acute inflammation (Chapter 2), the material is frequently creamy yellow because of the presence of dead white cells and is called *pus.* Although **gangrenous necrosis** is not a distinctive pattern of cell death, the term is still commonly used in surgical clinical practice. It is usually applied to a limb, generally the lower leg, that has lost its blood supply and has undergone coagulation necrosis. 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, a distinctive form of coagulative necrosis, is encountered most often in foci of tuberculous infection (Chapter 8). The term caseous is derived from the cheesy white gross appearance of the area of necrosis (Fig. 1–20). On microscopic examination, the necrotic focus appears as amorphous granular debris seemingly composed of fragmented, coagulated cells and amorphous granular debris enclosed within a distinctive inflammatory border known as a granulomatous reaction (Chapter 2). Unlike coagulative necrosis, the tissue architecture is completely obliterated.

Fat necrosis is a term that is well fixed in medical parlance but does not in reality denote a specific pattern of necrosis. Rather, it is descriptive of focal areas of fat destruction, typically occurring as a result of 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 19). In this disorder, activated pancreatic enzymes escape from acinar cells and ducts, the activated enzymes liquefy fat cell membranes, and the activated 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. 1–21). On histologic examination, the necrosis takes the form of foci of shadowy outlines of necrotic fat cells, with basophilic calcium deposits, surrounded by an inflammatory reaction.

Ultimately, in the living patient, most necrotic cells and their debris disappear by a combined process of enzymatic digestion and fragmentation, followed by phagocytosis of the particulate debris by leukocytes. If necrotic cells and cellular debris are not promptly destroyed and reabsorbed, they tend to attract calcium salts and other minerals and to become calcified. This phenomenon, called *dystrophic calcification*, is considered later in the chapter.

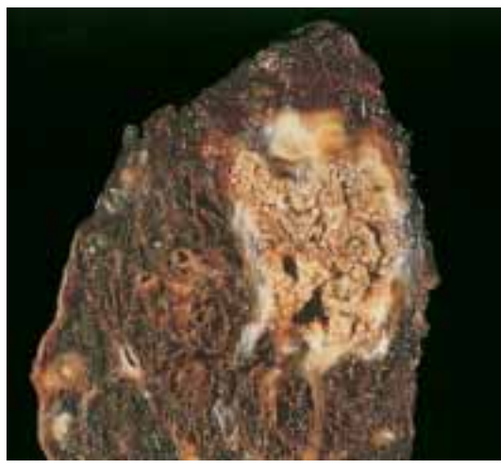


FIGURE 1–20 A tuberculous lung with a large area of caseous necrosis. The caseous debris is yellow-white and cheesy.

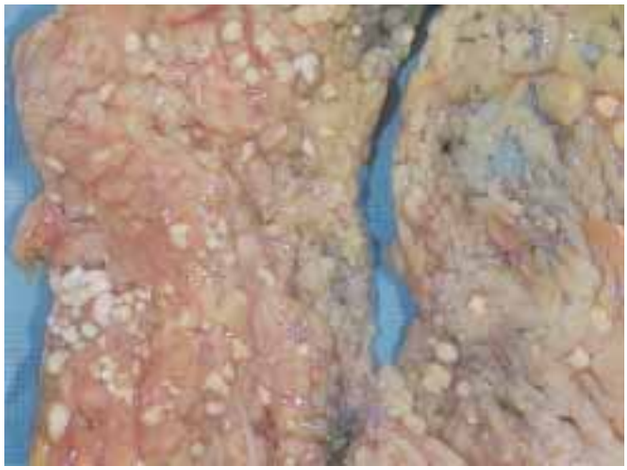


FIGURE 1–21 Foci of fat necrosis with saponification in the mesentery. The areas of white chalky deposits represent calcium soap formation at sites of lipid breakdown.

Examples of Cell Injury and Necrosis

Having briefly reviewed the general causes, mechanisms, and morphology of cell injury and necrotic cell death, we now describe some common forms of cell injury, namely ischemic and hypoxic injury, and some types of toxic injury. Although apoptosis contributes to cell death in some of these conditions, it has many unique features and is therefore discussed later in the chapter.

ISCHEMIC AND HYPOXIC INJURY

This is the most common type of cell injury in clinical medicine and has been studied extensively in humans, in experimental animals, and in culture systems.[27–29] Reasonable scenarios concerning the mechanisms underlying the morphologic changes have emerged. Hypoxia refers to any state of reduced oxygen availability. It may be caused by reduced amounts or saturation of hemoglobin. Ischemia, on the other hand, is brought about by reduced blood flow, usually as a consequence of a mechanical obstruction in the arterial system but sometimes as a result of a catastrophic fall in blood pressure or loss of blood. In contrast to hypoxia, during which glycolytic energy production can continue, ischemia compromises the delivery of substrates for glycolysis. Thus, in ischemic tissues, anaerobic energy generation stops after glycolytic substrates are exhausted, or glycolytic function becomes inhibited by the accumulation of metabolites that would have been removed otherwise by blood flow. For this reason, *ischemia tends to injure tissues faster than does hypoxia*.

Types of Ischemic Injury. Ischemic injury is the most common clinical expression of cell injury by oxygen deprivation. The most useful models for studying ischemic injury involve complete occlusion of one of the end-arteries to an organ (e.g., a coronary artery) and examination of the tissue (e.g., cardiac muscle) in areas supplied by the artery. Complex pathologic changes occur in diverse cellular systems during ischemia. Up to a certain point, for a duration that varies among different types of cells, the injury is amenable to repair, and the affected cells can recover if oxygen and metabolic substrates are again made available by restoration of blood flow. With further extension of the ischemic duration, cell structure continues to deteriorate, owing to relentless progression of ongoing injury mechanisms. With time, the energetic machinery of the cell—the mitochondrial oxidative powerhouse and the glycolytic pathway—becomes irreparably damaged, and restoration of blood flow (reperfusion) cannot rescue the damaged cell. Even if the cellular energetic machinery were to remain intact, irreparable damage to the genome or to cellular membranes will ensure a lethal outcome regardless of reperfusion. This irreversible injury is usually manifested as *necrosis*, but apoptosis may also play a role.

Under certain circumstances, when blood flow is restored to cells that have been previously made ischemic but have not died, injury is often paradoxically exacerbated and proceeds at an accelerated pace. As a consequence, reperfused tissues may sustain *loss of cells in addition to cells that are irreversibly damaged at the end of ischemia*. This is a clinically important process that contributes to net tissue damage during myocardial and cerebral infarction, as described in Chapters 12 and 28. This so-called *ischemia–reperfusion injury* (discussed later) is particularly significant because appropriate medical treatment can decrease the fraction of cells that may otherwise be destined to die in the "area at risk."

Mechanisms of Ischemic Cell Injury. The sequence of events following hypoxia was described earlier and is summarized in Figure 1–22.[28,29] Briefly, as the oxygen tension within the cell decreases, there is loss of oxidative phosphorylation and decreased generation of ATP. The depletion of ATP results in failure of the sodium pump, with loss of potassium, influx of sodium and water, and cell swelling. There is progressive loss of glycogen and decreased protein synthesis. There may be severe functional consequences at this stage. For instance, heart muscle ceases to contract within 60 seconds of coronary occlusion. Note, however, that loss of contractility does not mean cell death. If hypoxia continues, worsening ATP depletion causes further morphologic deterioration. The cytoskeleton disperses, resulting in the loss of ultrastructural features such as microvilli and the formation of "blebs" at the cell surface (see Fig. 1–17). "Myelin figures," derived from plasma as well as organellar membranes, may be seen within the cytoplasm or extracellularly. 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. At this time, the mitochondria are usually swollen, owing to loss of volume control by these organelles; the endoplasmic reticulum remains dilated; and the entire cell is 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 morphologically with severe swelling of mitochondria, extensive damage to plasma membranes, and swelling of lysosomes (see Fig. 1–17*C*). Large, flocculent, amorphous densities develop in the mitochondrial matrix. In the myocardium, these are indications of irreversible injury and can be seen as early as 30 to 40 minutes after ischemia. Massive influx of calcium into the cell then occurs, particularly if the ischemic zone is reperfused. Death

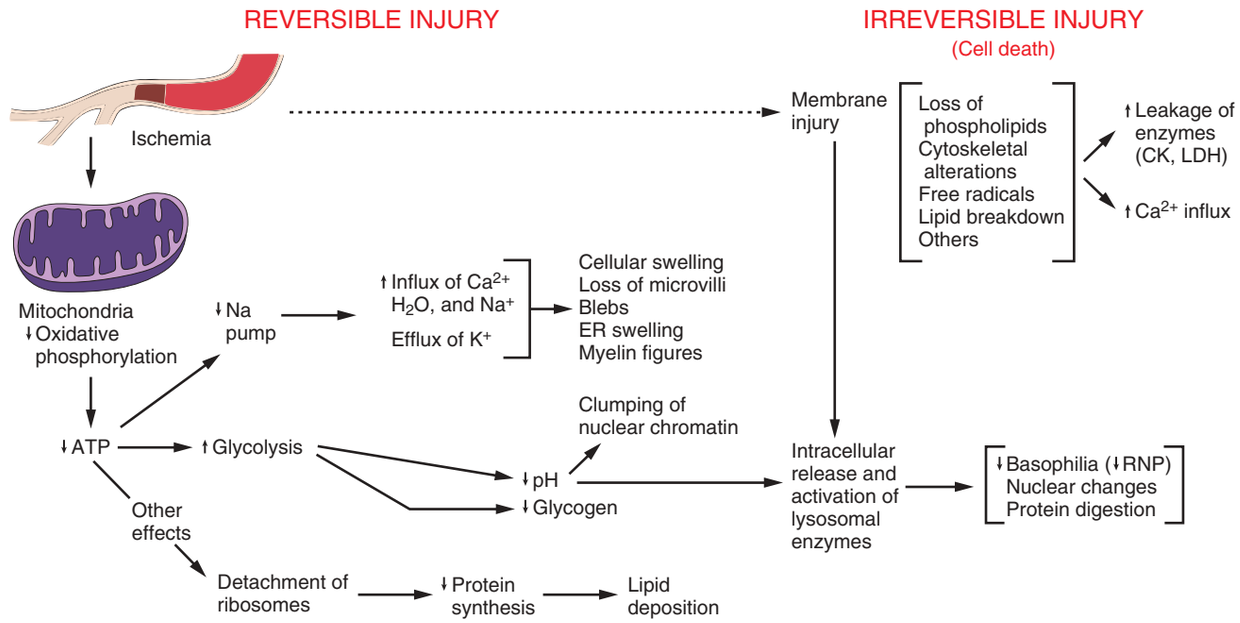


FIGURE 1–22 Postulated sequence of events in reversible and irreversible ischemic cell injury. Note that although reduced oxidative phosphorylation and ATP levels have a central role, ischemia can cause direct membrane damage. ER, endoplasmic reticulum; CK, creatine kinase; LDH, lactate dehydrogenase;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

is mainly by necrosis, but apoptosis may also contribute; the apoptotic pathway is activated probably by release of pro-apoptotic molecules from leaky mitochondria. After death, cell components are progressively degraded, and there is widespread leakage of cellular enzymes into the extracellular space and, conversely, entry of extracellular macromolecules from the interstitial space into the dying cells. Finally, the dead cell may become replaced by large masses composed of phospholipids in the form of myelin figures. These are then either phagocytosed by other cells or degraded further into fatty acids. *Calcification* of such fatty acid residues may occur with the formation of calcium soaps.

As we mentioned previously, leakage of intracellular enzymes and other proteins across the abnormally permeable plasma membrane and into the plasma provides important clinical parameters of cell death. For example, elevated serum levels of cardiac muscle creatine kinase MB and troponin are valuable clinical indicators of myocardial infarction, an area of cell death in heart muscle (Chapter 12).

ISCHEMIA–REPERFUSION INJURY

Restoration of blood flow to ischemic tissues can result in recovery of cells if they are reversibly injured, or not affect the outcome if irreversible cell damage has occurred. However, depending on the intensity and duration of the ischemic insult, variable numbers of cells may proceed to die *after* blood flow resumes, by necrosis as well as by apoptosis.[30] The affected tissues often show neutrophilic infiltrates. As noted earlier, this ischemia–reperfusion injury is a clinically important process in such conditions as myocardial infarction and stroke and may be amenable to therapeutic interventions.

How does reperfusion injury occur? The likely answer is that *new damaging processes* are set in motion during reperfusion, causing the death of cells that might have recovered otherwise.[31,32] Several mechanisms have been proposed:

- New damage may be initiated during reoxygenation by increased generation of *oxygen free radicals* from parenchymal and endothelial cells and from infiltrating leukocytes.[31,33] Superoxide anions can be produced in reperfused tissue as a result of incomplete and vicarious reduction of oxygen by damaged mitochondria or because of the action of oxidases derived from leukocytes, endothelial cells, or parenchymal cells.[32] Cellular antioxidant defense mechanisms may also be compromised by ischemia, favoring the accumulation of radicals. Free radical scavengers may be of therapeutic benefit.
- Reactive oxygen species can further promote the *mitochondrial permeability transition*, referred to earlier, which, when it occurs, precludes mitochondrial energization and cellular ATP recovery and leads to cell death.[25]
- Ischemic injury is associated with *inflammation* as a result of the production of cytokines and increased expression of adhesion molecules by hypoxic parenchymal and endothelial cells.[31, 33] These agents recruit circulating polymorphonuclear leukocytes to reperfused tissue; the ensuing inflammation causes additional injury (Chapter 2). The importance of neutrophil influx in reperfusion injury has been demonstrated by experimental studies that have used anti-inflammatory interventions, such as antibodies to cytokines or adhesion molecules, to reduce the extent of the injury.[30,32]
- Recent data suggest that activation of the *complement pathway* may contribute to ischemia–reperfusion injury.[34] The complement system is involved in host defense and is an important mechanism of immune injury (Chapter 6). Some IgM antibodies have a propensity to deposit in

ischemic tissues, for unknown reasons, and when blood flow is resumed, complement proteins bind to the antibodies, are activated, and cause cell injury and inflammation. Knockout mice lacking several complement proteins are resistant to this type of injury.[35]

CHEMICAL INJURY

The mechanisms by which chemicals, certain drugs, and toxins produce injury are described in greater detail in Chapter 9 in the discussion of environmental disease. Here we will describe two forms of chemically induced injury as examples of the sequence of events leading to cell death.

Chemicals induce cell injury by one of two general mechanisms:[36]

- Some chemicals can act *directly* by combining with some critical molecular component or cellular organelle. For example, in *mercuric chloride poisoning*, mercury binds to the sulfhydryl groups of the cell membrane and other proteins, causing increased membrane permeability and inhibition of ATPase-dependent transport. In such instances, the greatest damage is usually to the cells that use, absorb, excrete, or concentrate the chemicals — in the case of mercuric chloride, the cells of the gastrointestinal tract and kidney (Chapter 9). *Cyanide* poisons mitochondrial cytochrome oxidase and blocks oxidative phosphorylation. Many antineoplastic chemotherapeutic agents and antibiotic drugs also induce cell damage by direct cytotoxic effects.
- Most other chemicals are not biologically active but must be 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.[37,38] Although these metabolites might cause membrane damage and cell injury by *direct covalent binding* to membrane protein and lipids, by far the most important mechanism of membrane injury involves the formation of *reactive free radicals* and subsequent lipid peroxidation.

The diverse mechanisms of chemical injury are well illustrated by carbon tetrachloride and acetaminophen. *Carbon tetrachloride* (CCl_4) was once used widely in the dry-cleaning industry.[39] The toxic effect of CCl_4 is due to its conversion by P-450 to the *highly reactive toxic free radical* CCl_3 ($CCl_4 + e \rightarrow CCl_3 + Cl^-$) (Fig. 1–23). The free radicals produced locally cause autooxidation of the polyenoic fatty acids present within the membrane phospholipids. There, oxidative decomposition of the lipid is initiated, and organic peroxides are formed after reacting with oxygen (lipid peroxidation). This *reaction is autocatalytic* in that new radicals are formed from the peroxide radicals themselves. Thus, rapid breakdown of the structure and function of the endoplasmic reticulum is due to decomposition of the lipid. It is no surprise, therefore, that *CCl_4-induced liver cell injury is both severe and extremely rapid in onset*. Within less than 30 minutes, there is a decline in hepatic protein synthesis; within 2 hours, there is swelling of smooth endoplasmic reticulum and dissociation of ribosomes from the rough endoplasmic reticulum. Lipid export from the hepatocytes is reduced owing to their inability to synthesize apoprotein to complex with triglycerides and thereby facilitate

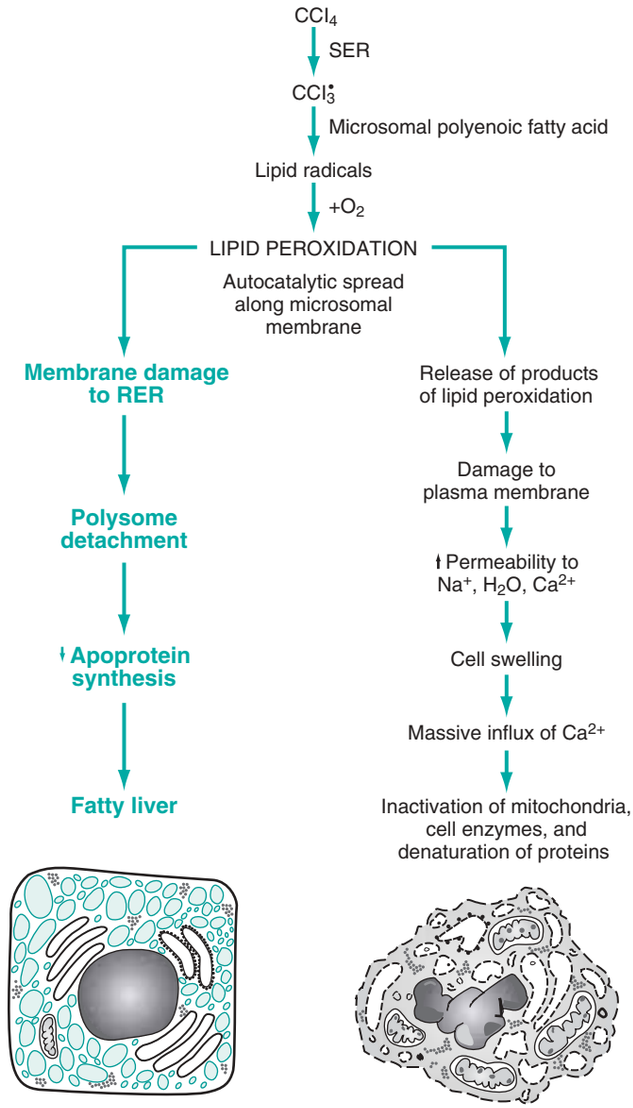


FIGURE 1–23 Sequence of events leading to fatty change and cell necrosis in carbon tetrachloride (CCl_4) toxicity. RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum.

lipoprotein secretion. The result is the fatty liver of CCl_4 poisoning (Fig. 1–24). (Fatty liver is discussed later in the chapter.) Mitochondrial injury then occurs, and this is followed by progressive swelling of the cells due to increased permeability of the plasma membrane. Plasma membrane damage is thought to be caused by relatively stable fatty aldehydes, which are produced by lipid peroxidation in the smooth endoplasmic reticulum but are able to act at distant sites. This is followed by massive influx of calcium and cell death (see Fig. 1–23).

Acetaminophen (Tylenol), a commonly used analgesic drug, is detoxified in the liver through sulfation and glucuronidation, and small amounts are converted by cytochrome P-450–catalyzed oxidation to an electrophilic, highly toxic metabolite.[40] This metabolite itself is detoxified by interaction with GSH. When large doses of the drug are ingested, GSH is depleted, and thus the toxic metabolites accumulate in the cell,

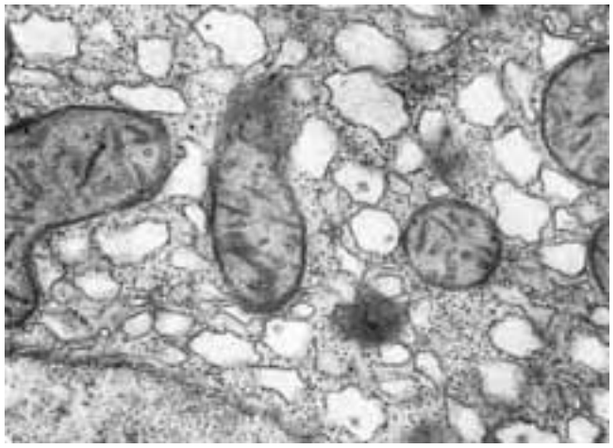


FIGURE 1–24 Rat liver cell 4 hours after carbon tetrachloride intoxication, with swelling of endoplasmic reticulum and shedding of ribosomes. At this stage, mitochondria are unaltered. (Courtesy of Dr. O. Iseri, University of Maryland, Baltimore, MD.)

destroy nucleophilic macromolecules, and covalently bind proteins and nucleic acids. The decrease in GSH concentration, coupled with covalent binding of toxic metabolites, increases drug toxicity, resulting in massive liver cell necrosis, usually 3 to 5 days after the ingestion of toxic doses. This hepatotoxicity correlates with lipid peroxidation and can be reduced by administration of antioxidants, suggesting that the oxidative damage may be more important than covalent binding in the ultimate toxicity of the drug.[41]

Apoptosis

Apoptosis is a pathway of cell death that is induced by a tightly regulated intracellular program in which cells destined to die activate enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins. The cell's plasma membrane remains intact, but its structure is altered in such a way that the apoptotic cell becomes an avid target for phagocytosis. 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 is fundamentally different from necrosis, which is characterized by loss of membrane integrity, enzymatic digestion of cells, and frequently a host reaction (see Fig. 1–9 and Table 1–2). However, apoptosis and necrosis sometimes coexist, and they may share some common features and mechanisms.

CAUSES OF APOPTOSIS

Apoptosis was initially recognized in 1972 by its distinctive morphology and named after the Greek designation for "falling off."[42] It occurs normally in many situations, and serves to eliminate unwanted or 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; in these situations, the irreparably damaged cell is eliminated. Apoptosis is responsible for numerous physiologic, adaptive, and pathologic events, listed next.

Apoptosis in Physiologic Situations

Death by apoptosis is a normal phenomenon that serves to eliminate cells that are no longer needed, as, for example, during development, 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 development.[43] 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."
- *Hormone-dependent involution in the adult*, such as endometrial cell breakdown during the menstrual cycle, ovarian follicular atresia in the menopause, the regression of the lactating breast after weaning, and prostatic atrophy after castration.
- *Cell deletion in proliferating cell populations*, such as intestinal crypt epithelia, in order to maintain a constant number.
- Death of host 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 (Chapter 6).
- *Cell death induced by cytotoxic T cells*, a defense mechanism against viruses and tumors that serves to eliminate virus-infected and neoplastic cells. The same mechanism is responsible for cellular rejection of transplants (Chapter 6).

Apoptosis in Pathologic Conditions

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

- *Cell death produced by a variety of injurious stimuli*. For instance, radiation and cytotoxic anticancer drugs damage DNA, and if repair mechanisms cannot cope with the injury the cell kills itself by apoptosis. In these situations, elimination of the cell may be a better alternative than risking mutations and translocations in the damaged DNA, which may result in malignant transformation. These injurious stimuli, as well as heat and hypoxia, can induce apoptosis if the insult is mild, but large doses of the same stimuli result in necrotic cell death. Endoplasmic reticulum (ER) stress, which is induced by the accumulation of unfolded proteins, also triggers apoptotic death of cells (described later in the chapter).
- *Cell injury in certain viral diseases*, such as viral hepatitis, in which loss of infected cells is largely because of apoptotic death.
- *Pathologic atrophy in parenchymal organs after duct obstruction*, such as occurs in the pancreas, parotid gland, and kidney.
- *Cell death in tumors*, most frequently during regression but also in actively growing tumors.

■ As we mentioned earlier, even in situations in which cell death is mainly by necrosis, the pathway of apoptosis may contribute. For instance, injurious stimuli that cause increased mitochondrial permeability trigger apoptosis.

Before the mechanisms of apoptosis are discussed, we describe the morphologic and biochemical characteristics of this process.

Morphology. The following morphologic features, some best seen with the electron microscope, characterize cells undergoing apoptosis (Fig. 1–25).

- **Cell shrinkage.** The cell is smaller in size; the cytoplasm is dense; and the organelles, although relatively normal, are more tightly packed.
- **Chromatin condensation.** This is the most characteristic feature of apoptosis. The chromatin aggregates peripherally, under the nuclear membrane, into dense masses of various shapes and sizes. The nucleus itself may break up, producing two or more fragments.
- **Formation of cytoplasmic blebs and apoptotic bodies.** The apoptotic cell first shows extensive surface blebbing, then undergoes fragmentation into membrane-bound apoptotic bodies composed of cytoplasm and tightly packed organelles, with or without nuclear fragments.
- **Phagocytosis of apoptotic cells or cell bodies, usually by macrophages.** The apoptotic bodies are rapidly degraded within lysosomes, and the adjacent healthy cells migrate or proliferate to replace the space occupied by the now deleted apoptotic cell.

Plasma membranes are thought to remain intact during apoptosis, until the last stages, when they become permeable to normally retained solutes. This classical description is accurate with respect to apoptosis during physiologic conditions such as embryogenesis and deletion of immune cells. However, forms of cell death with features of necrosis as well as of apoptosis are not uncommon after injurious stimuli.[44] Under such conditions, the severity, rather than the specificity, of stimulus determines the form in which death is expressed. If necrotic features are predominant, early plasma membrane damage occurs, and cell swelling, rather than shrinkage, is seen.

On histologic examination, in tissues stained with hematoxylin and eosin, apoptosis involves single cells or small clusters of cells. The apoptotic cell appears as a round or oval mass of intensely eosinophilic cytoplasm with dense nuclear chromatin fragments (Fig. 1–26). Because the cell shrinkage and formation of apoptotic bodies are rapid and the fragments are quickly phagocytosed, considerable apoptosis may occur in tissues before it becomes apparent in histologic sections. In addition, apoptosis—in contrast to necrosis—does not elicit inflammation, making it more difficult to detect histologically.

BIOCHEMICAL FEATURES OF APOPTOSIS

Apoptotic cells usually exhibit a distinctive constellation of biochemical modifications that underlie the structural changes described above. Some of these features may be seen in necrotic cells also, but other alterations are more specific.

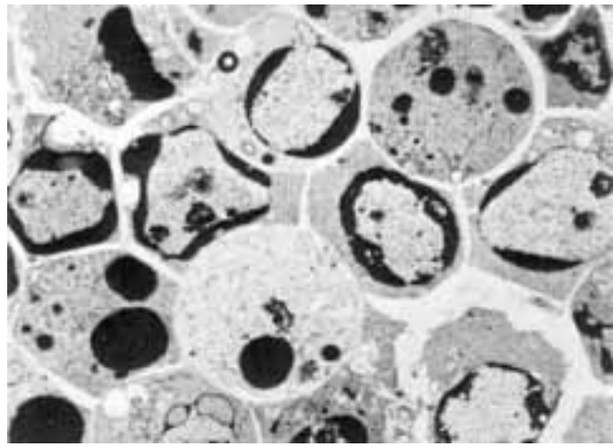


FIGURE 1–25 Ultrastructural features of apoptosis. Some nuclear fragments show peripheral crescents of compacted chromatin, whereas others are uniformly dense. (From Kerr JFR, Harmon BV: Definition and incidence of apoptosis: a historical perspective. In Tomei LD, Cope FO (eds): Apoptosis: The Molecular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

Protein Cleavage. A specific feature of apoptosis is protein hydrolysis involving the activation of several members of a family of cysteine proteases named *caspases*.[45] Many caspases are present in normal cells as inactive pro-enzymes, and they need to be activated to induce apoptosis. Active caspases cleave many vital cellular proteins, such as lamins, and thus break up the nuclear scaffold and cytoskeleton; in addition, caspases activate DNAses, which degrade nuclear DNA. These changes underlie the nuclear and cytoplasmic structural alterations seen in apoptotic cells.

DNA Breakdown. Apoptotic cells exhibit a characteristic breakdown of DNA into large 50- to 300-kilobase pieces.[46] Subsequently, there is internucleosomal cleavage of DNA into oligonucleosomes, in multiples of 180 to 200 base pairs, by Ca^{2+}- and Mg^{2+}-dependent endonucleases. The fragments may be visualized by agarose gel electrophoresis as DNA ladders (Fig. 1–27). Endonuclease activity also forms the basis for detecting cell death by cytochemical techniques that recognize double-stranded breaks of DNA.[47] However, internucleosomal DNA cleavage is not specific for apoptosis. A "smeared" pattern of DNA fragmentation is thought to be indicative of necrosis, but this may be a late autolytic phenomenon, and typical DNA ladders may be seen in necrotic cells as well.[47]

Phagocytic Recognition. Apoptotic cells express phosphatidylserine in the outer layers of their plasma membranes, the phospholipid having "flipped" out from the inner layers. (Because of these changes, apoptotic cells can be identified by binding of special dyes, such as Annexin V.) In some types of apoptosis, thrombospondin, an adhesive glycoprotein, is also expressed on the surfaces of apoptotic bodies, and other proteins secreted by phagocytes may bind to apoptotic cells and opsonize the cells for phagocytosis.[48] These alterations permit the early recognition of dead cells by macrophages, resulting in phagocytosis without the release of proinflammatory cellular components.[49] In this way, the apoptotic response dis-

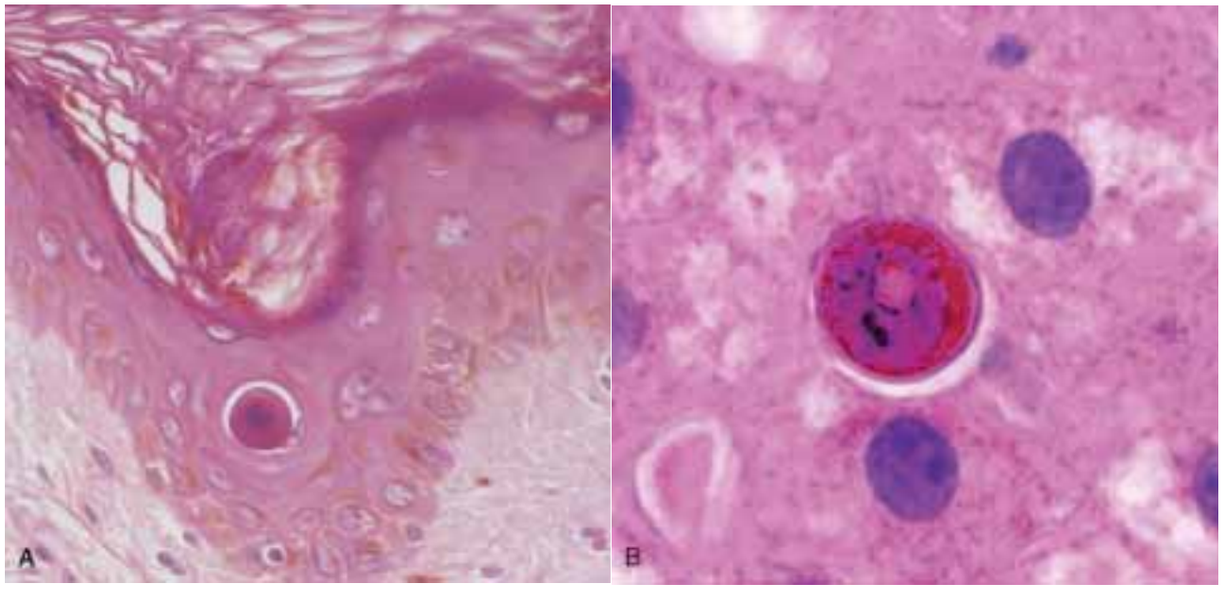


FIGURE 1–26 *A,* Apoptosis of epidermal cells in an immune-mediated reaction. The apoptotic cells are visible in the epidermis with intensely eosinophilic cytoplasm and small, dense nuclei. H&E stain. (Courtesy of Dr. Scott Granter, Brigham and Women's Hospital, Boston, MA.) *B,* High power of apoptotic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

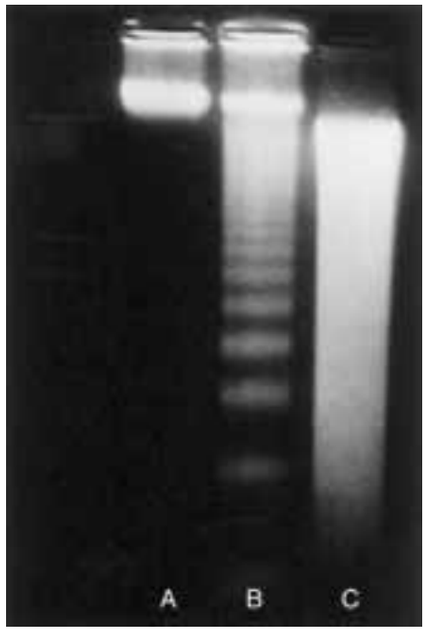


FIGURE 1–27 Agarose gel electrophoresis of DNA extracted from culture cells. Ethidium bromide stain; photographed under ultraviolet illumination. *Lane A,* Control culture. *Lane B,* Culture of cells exposed to heat showing extensive apoptosis; note ladder pattern of DNA fragments, which represent multiples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

poses of cells with minimal compromise to the surrounding tissue.

MECHANISMS OF APOPTOSIS

Apoptosis is induced by a cascade of molecular events that may be initiated in distinct ways and culminate in the activation of caspases (Fig. 1–28).[45] Because too much or too little apoptosis is thought to underlie many diseases, such as degenerative diseases and cancer, there is great interest in elucidating the mechanisms of this form of cell death. Tremendous progress has been made in our understanding of apoptosis. One of the remarkable facts to emerge is that the basic mechanisms of apoptosis are conserved in all metazoans.[50] In fact, some of the major breakthroughs came from observations made in the nematode *Caenorhabditis elegans*, whose development proceeds by a highly reproducible, programmed pattern of cell growth followed by cell death. Studies of mutant worms have allowed the identification of specific genes (called *ced* genes, for *c*ell *d*eath abnormal) that initiate or inhibit apoptosis and for which there are defined mammalian homologues.

The process of apoptosis may be divided into an initiation phase, during which caspases become catalytically active, and an execution phase, during which these enzymes act to cause cell death. Initiation of apoptosis occurs principally by signals from two distinct but convergent pathways — the extrinsic, or receptor-initiated, pathway and the intrinsic, or mitochondrial, pathway. Both pathways converge to activate caspases. We will describe these two pathways separately because they involve largely distinct molecular interactions, but it is important to remember that they may be interconnected at numerous steps.

The Extrinsic (Death Receptor-Initiated) Pathway. This pathway is initiated by engagement of cell surface death recep-

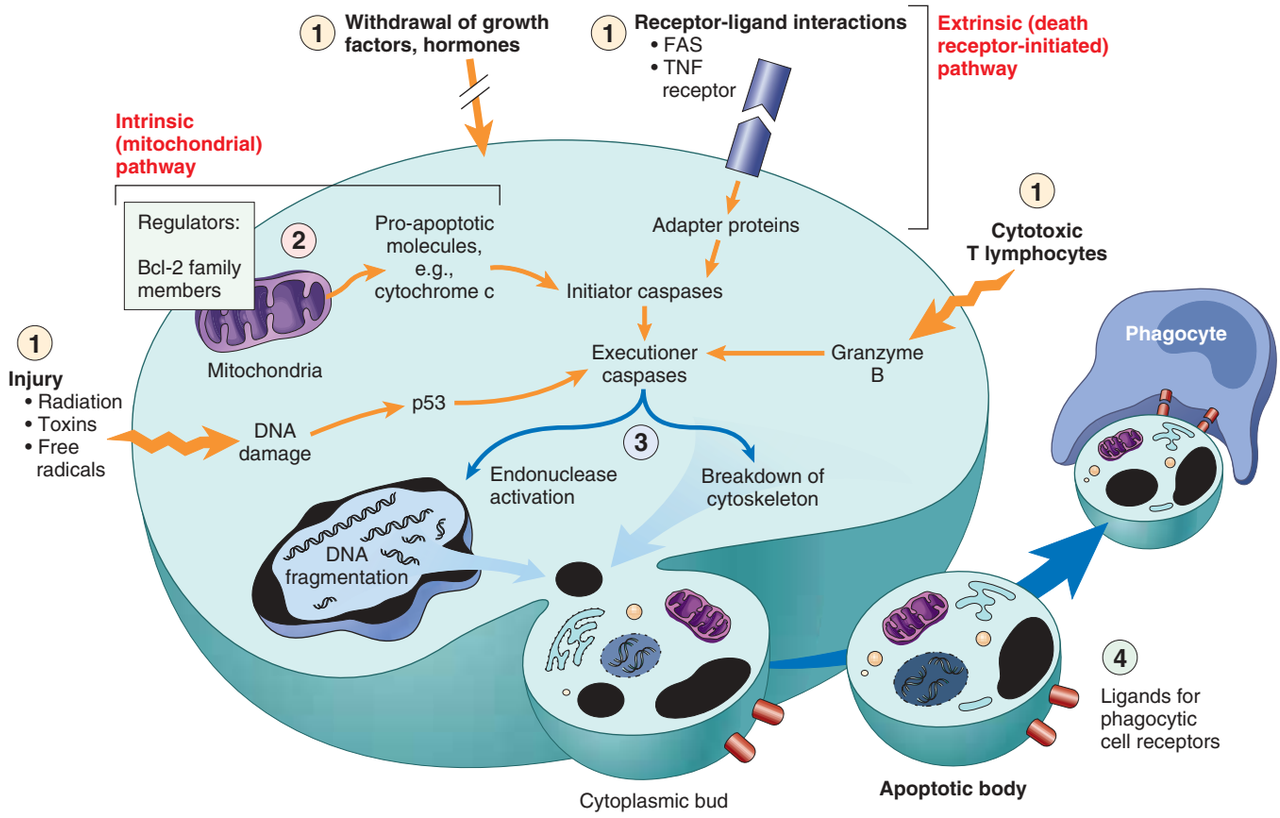


FIGURE 1–28 Mechanisms of apoptosis. Labeled (1) are some of the major inducers of apoptosis. These include specific death ligands (tumor necrosis factor [TNF] and Fas ligand), withdrawal of growth factors or hormones, and injurious agents (e.g., radiation). Some stimuli (such as cytotoxic cells) directly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

tors on a variety of cells.[51] Death receptors are members of the tumor necrosis factor receptor family that contain a cytoplasmic domain involved in protein-protein interactions that is called the *death domain* because it is essential for delivering apoptotic signals. (Some TNF receptor family members do not contain cytoplasmic death domains; their role in triggering apoptosis is much less established). The best-known death receptors are the type 1 TNF receptor (TNFR1) and a related protein called Fas (CD95), but several others have been described. The mechanism of apoptosis induced by these death receptors is well illustrated by Fas (Fig. 1–29). When Fas is cross-linked by its ligand, membrane-bound Fas ligand (FasL), three or more molecules of Fas come together, and their cytoplasmic death domains form a binding site for an adapter protein that also contains a death domain and is called FADD (*F*as-*a*ssociated *d*eath *d*omain). FADD that is attached to the death receptors in turn binds an inactive form of caspase-8 (and, in humans, caspase-10), again via a death domain. Multiple pro-caspase-8 molecules are thus brought into proximity, and they cleave one another to generate active caspase-8. The enzyme then triggers a cascade of caspase activation by cleaving and thereby activating other pro-caspases, and the active enzymes mediate the execution phase of apoptosis (discussed below). This pathway of apoptosis can be inhibited by a protein called FLIP, which binds to pro-caspase-8 but cannot cleave and activate the enzyme because it lacks enzymatic activity.[52] Some viruses and normal cells produce FLIP and use this inhibitor to protect infected and normal cells from Fas-mediated apoptosis. The sphingolipid ceramide has been implicated as an intermediate between death receptors and caspase activation, but the role of this pathway is unclear and remains controversial.[53]

The Intrinsic (Mitochondrial) Pathway. This pathway of apoptosis is the result of increased mitochondrial permeability and release of pro-apoptotic molecules into the cytoplasm, without a role for death receptors.[54,55] Growth factors and other survival signals stimulate the production of anti-apoptotic members of the Bcl-2 family of proteins.[56] This family is named after Bcl-2, which was identified as an oncogene in a B cell lymphoma and is homologous to the *C. elegans* protein, Ced-9. There are more than 20 proteins in this family, all of which function to regulate apoptosis; the two main anti-apoptotic ones are Bcl-2 and Bcl-x. These anti-apoptotic proteins normally reside in mitochondrial membranes and the cyto-

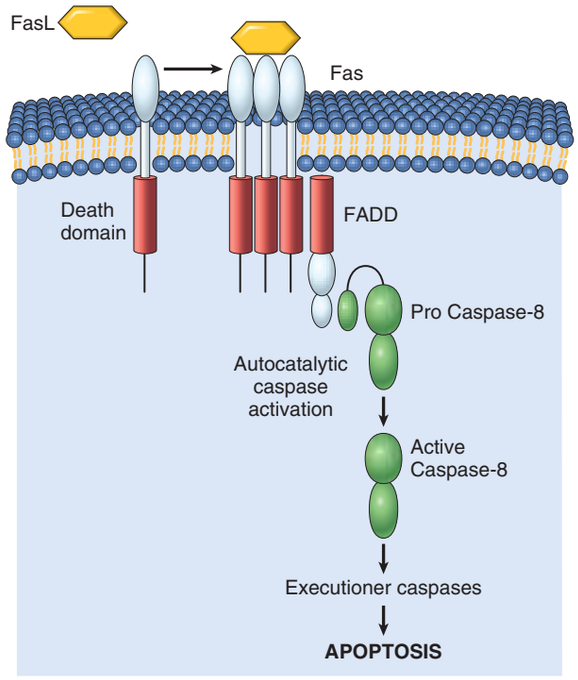


FIGURE 1–29 The extrinsic (death receptor-initiated) pathway of apoptosis, illustrated by the events following Fas engagement (see text).

plasm. When cells are deprived of survival signals or subjected to stress, Bcl-2 and/or Bcl-x are lost from the mitochondrial membrane and are replaced by pro-apoptotic members of the family, such as Bak, Bax, and Bim. When Bcl-2/Bcl-x levels decrease, the permeability of the mitochondrial membrane increases, and several proteins that can activate the caspase cascade leak out (Fig. 1–30). One of these proteins is cytochrome *c*, well known for its role in mitochondrial respiration. In the cytosol, cytochrome *c* binds to a protein called Apaf-1 (apoptosis activating factor-1, homologous to Ced-4 in *C. elegans*), and the complex activates caspase-9.[57] (Bcl-2 and Bcl-x may also directly inhibit Apaf-1 activation, and their loss from cells may permit activation of Apaf-1). Other mitochondrial proteins, such as apoptosis inducing factor (AIF), enter the cytoplasm, where they bind to and neutralize various inhibitors of apoptosis, whose normal function is to block caspase activation.[58] The net result is the initiation of a caspase cascade. Thus, *the essence of this intrinsic pathway is a balance between pro-apoptotic and protective molecules that regulate mitochondrial permeability and the release of death inducers that are normally sequestered within the mitochondria.*

There is quite a lot of evidence that the intrinsic pathway of apoptosis can be triggered without a role for mitochondria.[59] Apoptosis may be initiated by caspase activation upstream of mitochondria, and the subsequent increase in mitochondrial permeability and release of pro-apoptotic molecules amplify the death signal.[46] However, these pathways of apoptosis involving mitochondria-independent initiation are not well defined. We have described the extrinsic and intrinsic pathways for initiating apoptosis as distinct, but there may be overlaps between them. For instance, in hepatocytes, Fas signaling activates a pro-apoptotic member of the Bcl family called Bid, which then activates the mitochondrial pathway. It is not known if such cooperative interactions between apoptosis pathways are active in most other cell types.

The Execution Phase. This final phase of apoptosis is mediated by a proteolytic cascade, toward which the various initiating mechanisms converge. The proteases that mediate the execution phase are highly conserved across species and belong to the caspase family, as previously mentioned. They are mammalian homologues of the *ced-3* gene in *C. elegans*.[44] The term *caspase* is based on two properties of this family of enzymes: the "c" refers to a cysteine protease (i.e., an enzyme with cysteine in its active site), and "aspase" refers to the unique ability of these enzymes to cleave after aspartic acid residues.[60] The caspase family, now including more than 10

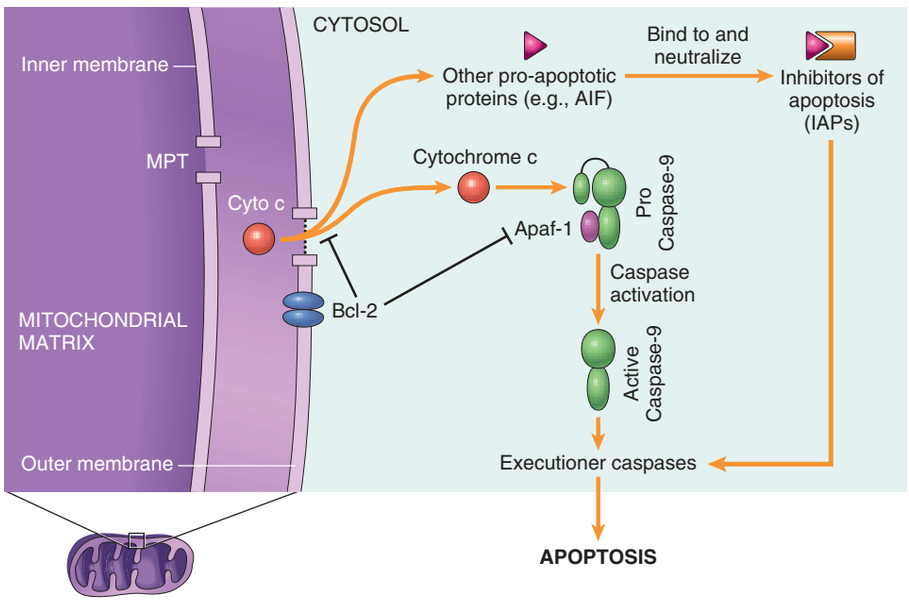


FIGURE 1–30 The intrinsic (mitochondrial) pathway of apoptosis. Death agonists cause changes in the inner mitochondrial membrane, resulting in the mitochondrial permeability transition (MPT) and release of cytochrome *c* and other pro-apoptotic proteins into the cytosol, which activate caspases (see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

members, can be divided functionally into two basic groups—initiator and executioner—depending on the order in which they are activated during apoptosis.[60] Initiator caspases, as we have seen, include caspase-8 and caspase-9. Several caspases, including caspase-3 and caspase-6, serve as executioners.

Like many proteases, caspases exist as inactive pro-enzymes, or zymogens, and must undergo an activating cleavage for apoptosis to be initiated. Caspases have their own cleavage sites that can be hydrolyzed not only by other caspases but also autocatalytically. After an initiator caspase is cleaved to generate its active form, the enzymatic death program is set in motion by rapid and sequential activation of other caspases. Execution caspases act on many cellular components. They cleave cytoskeletal and nuclear matrix proteins and thus disrupt the cytoskeleton and lead to breakdown of the nucleus.[60] In the nucleus, the targets of caspase activation include proteins involved in transcription, DNA replication, and DNA repair. In particular, caspase-3 activation converts a cytoplasmic DNase into an active form by cleaving an inhibitor of the enzyme; this DNase induces the characteristic internucleosomal cleavage of DNA, described earlier.

Removal of Dead Cells. At early stages of apoptosis, dying cells secrete soluble factors that recruit phagocytes.[61] This facilitates prompt dearance of apoptotic cells before they undergo secondary necrosis and release their cellular contents (which can result in inflammation). As already alluded to, apoptotic cells and their fragments have marker molecules on their surfaces, which facilitates early recognition by adjacent cells or phagocytes for phagocytic uptake and disposal. Numerous macrophage receptors have been shown to be involved in the binding and engulfment of apoptotic cells. In addition, macrophages can also secrete substances that bind specifically to apoptotic but not live cells and opsonize these cells for phagocytosis. In contrast to markers on apoptotic cells, viable cells appear to prevent their own engulfment by macrophages through expression of certain surface molecules (such as CD31). This process of phagocytosis of apoptotic cells is so efficient that dead cells disappear without leaving a trace, and inflammation is virtually absent.

EXAMPLES OF APOPTOSIS

The signals that induce apoptosis include *lack of growth factor or hormone*, *specific engagement of death receptors*, and particular *injurious agents*. Although the classic example of apoptosis has been programmed death of cells during embryogenesis, we still do not know what triggers apoptosis in this situation. However, many other well-defined examples of apoptosis are known.

Apoptosis After 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.[62] In all these situations, apoptosis is triggered by the intrinsic (mitochondrial) pathway and is attributable to an excess of pro-apoptotic members of the Bcl family relative to anti-apoptotic members.

DNA Damage–Mediated Apoptosis. Exposure of cells to radiation or chemotherapeutic agents induces apoptosis by a mechanism that is initiated by DNA damage (genotoxic stress) and that involves the tumor-suppressor gene *p53*.[63] p53 accumulates when DNA is damaged and arrests the cell cycle (at the G_1 phase) to allow time for repair (Chapter 7). However, if the DNA repair process fails, p53 triggers apoptosis. When p53 is mutated or absent (as it is in certain cancers), it is incapable of inducing apoptosis and it favors cell survival. Thus, p53 seems to serve as a critical "life or death" switch in the case of genotoxic stress. The mechanism by which p53 triggers the distal death effector machinery—the caspases—is complex but seems to involve its well-characterized function in transcriptional activation. Among the proteins whose production is stimulated by p53 are several pro-apoptotic members of the Bcl family, notably Bax and Bak, as well as Apaf-1, mentioned earlier. These proteins activate caspases and cause apoptosis.

Apoptosis Induced by Tumor Necrosis Factor Family of Receptors. As discussed above, the cell surface receptor Fas (CD95) induces apoptosis when it is engaged by Fas ligand (FasL or CD95L), which is produced by cells of the immune system. This system is important in the elimination of lymphocytes that recognize self-antigens, and mutations in Fas or FasL result in autoimmune diseases in humans and mice (Chapter 6).[64]

The cytokine TNF is an important mediator of the inflammatory reaction (Chapter 2), but it is also capable of inducing apoptosis. (The name "tumor necrosis factor" arose not because the cytokine kills tumor cells directly, but because it induces thrombosis of tumor blood vessels, resulting in ischemic death of the tumor.) The binding of TNF to TNFR1 leads to association of the receptor with the adapter protein TRADD (*T*NF *r*eceptor-*a*ssociated *d*eath *d*omain containing protein). TRADD in turn binds to FADD and leads to apoptosis through caspase activation, as with Fas–FasL interactions.[52] The major functions of TNF, however, are mediated not by inducing apoptosis but by activation of the important transcription factor nuclear factor-κB (NF-κB). TNF-mediated signals accomplish this by stimulating degradation of its inhibitor (IκB).[65] The NF-κB/IκB transcriptional regulatory system is important for cell survival and, as we shall see in Chapter 2, for a number of inflammatory responses. Since TNF can induce cell death and promote cell survival, what determines this yin and yang of its action? The answer is unclear, but it probably depends on which adapter protein attaches to the TNF receptor after binding of the cytokine. TRADD and FADD favor apoptosis, and other adapter proteins, called TRAFs (TNF receptor associated factors) favor NF-κB activation and survival.

Cytotoxic T-Lymphocyte–Stimulated Apoptosis. Cytotoxic T lymphocytes (CTLs) recognize foreign antigens presented on the surface of infected host cells (Chapter 6). On recognition, CTLs secrete *perforin*, a transmembrane pore-forming molecule, which allows entry of the CTL granule serine protease called *granzyme B*. Granzyme B has the ability to cleave proteins at aspartate residues and is able to activate a variety of cellular caspases.[66] *In this way, the CTL kills target cells through bypassing the upstream signaling events and directly induces the effector phase of apoptosis.* CTLs also express FasL on their surfaces and kill target cells by ligation of Fas receptors, as described earlier.

Dysregulated apoptosis ("too little or too much") has been postulated to explain components of a wide range of diseases.[67] In essence, two groups of disorders may result from such dysregulation:

- *Disorders associated with defective apoptosis and increased cell survival.* Here, an inappropriately low rate of apoptosis may prolong the survival or reduce the turnover of abnormal cells. These accumulated cells can give rise to: (1) *cancers*, especially tumors with p53 mutations, or hormone-dependent tumors, such as breast, prostate, or ovarian cancers (Chapter 7); and (2) *autoimmune disorders,* which could arise if autoreactive lymphocytes are not eliminated after encounter with self antigens (Chapter 6).
- *Disorders associated with increased apoptosis and excessive cell death*. These diseases are characterized by a marked loss of normal or protective cells and include: (1) *neurodegenerative diseases*, manifested by loss of specific sets of neurons, such as in the spinal muscular atrophies (Chapter 27); (2) *ischemic injury*, as in myocardial infarction (Chapter 12) and stroke (Chapter 28); and (3) *death of virus-infected cells*, in many viral infections (Chapter 8).

Subcellular Responses to Injury

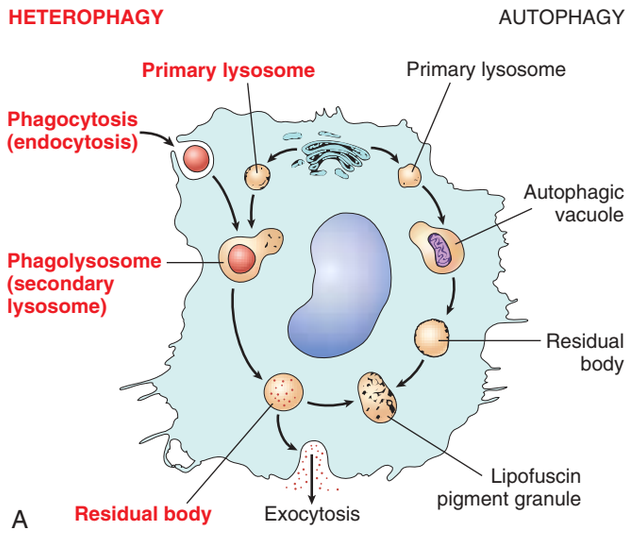
To this point in the chapter, the focus has been on the cell as a unit. Certain conditions, however, are associated with distinctive alterations in cell organelles or the cytoskeleton. Some of these alterations coexist with those described for acute lethal injury; others represent more chronic forms of cell injury; still others are adaptive responses that serve to maintain homeostasis. Here we touch on only some of the more common or interesting of these reactions.

LYSOSOMAL CATABOLISM

Primary lysosomes are membrane-bound intracellular organelles that contain a variety of hydrolytic enzymes, including acid phosphatase, glucuronidase, sulfatase, ribonuclease, and collagenase. These enzymes are synthesized in the rough endoplasmic reticulum and then packaged into vesicles in the Golgi apparatus. Primary lysosomes fuse with membrane-bound vacuoles that contain material to be digested, forming *secondary lysosomes* or *phagolysosomes*. Lysosomes are involved in the breakdown of phagocytosed material in one of two ways: heterophagy and autophagy (Fig. 1–31).

- **Heterophagy.** Heterophagy is the process of lysosomal digestion of materials ingested from the extracellular environment. Extracellular materials are taken up by cells through the general process of *endocytosis*. Uptake of particulate matter is known as *phagocytosis*; uptake of soluble smaller macromolecules is called *pinocytosis*. Extracellular materials are endocytosed into vacuoles (endosomes or phagosomes), which eventually fuse with lysosomes to form phagolysosomes, where the engulfed material is digested. Heterophagy is most common in the "professional" phagocytes, such as neutrophils and macrophages, although it may also occur in other cell types. Examples of heterophagocytosis include the uptake and digestion of bacteria by neutrophils and the removal of apoptotic cells by macrophages.
- **Autophagy.** Autophagy refers to lysosomal digestion of the cell's own components. 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 endoplasmic reticulum. The vacuole fuses with lysosomes or Golgi elements to form an *autophagolysosome*.[68,69] Autophagy is a common phenomenon involved in the removal of damaged organelles during cell injury and the cellular remodeling of differentiation, and it is particularly pronounced in cells undergoing atrophy induced by nutrient deprivation or hormonal involution.

The enzymes in lysosomes are capable of degrading most proteins and carbohydrates, but some lipids remain undigested. Lysosomes with undigested debris may persist within cells as *residual bodies* or may be extruded. *Lipofuscin pigment* granules represent undigested material derived from intracel-



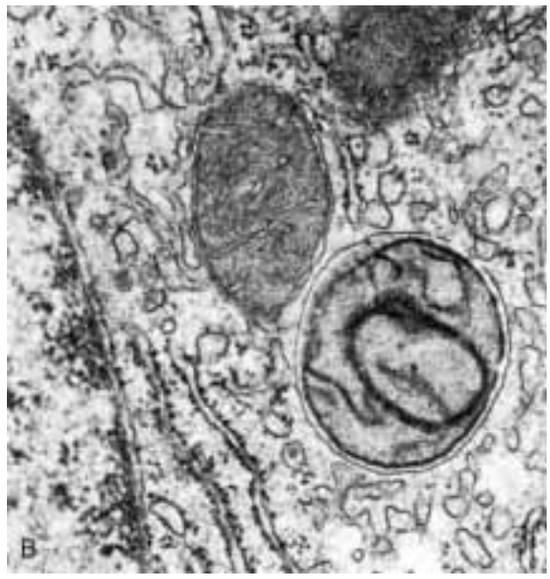


FIGURE 1–31 *A,* Schematic representation of heterophagy *(left)* and autophagy *(right).* (Redrawn from Fawcett DW: A Textbook of Histology, 11th ed. Philadelphia, WB Saunders, 1986, p 17.) *B,* Electron micrograph of an autophagolysosome containing a degenerating mitochondrion and amorphous material.

lular 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.

Lysosomes are also repositories where cells sequester abnormal substances that cannot be completely metabolized. Hereditary *lysosomal storage disorders*, caused by deficiencies of enzymes that degrade various macromolecules, result in the accumulation of abnormal amounts of these compounds in the lysosomes of cells all over the body, particularly neurons, leading to severe abnormalities (Chapter 5). Certain drugs can disturb lysosomal function and cause *acquired or drug-induced (iatrogenic) lysosomal diseases*. Drugs in this group include chloroquine, an antimalarial agent that raises the internal pH of the lysosome, thus inactivating its enzymes. By inhibiting lysosomal enzymes, chloroquine reduces tissue damage in inflammatory reactions, which are mediated in part by enzymes released from leukocytes; this action is the basis of the use of the drug in autoimmune diseases like rheumatoid arthritis. The same inhibition of enzymes, however, can result in abnormal accumulation of glycogen and phospholipids in lysosomes, causing toxic myopathy.

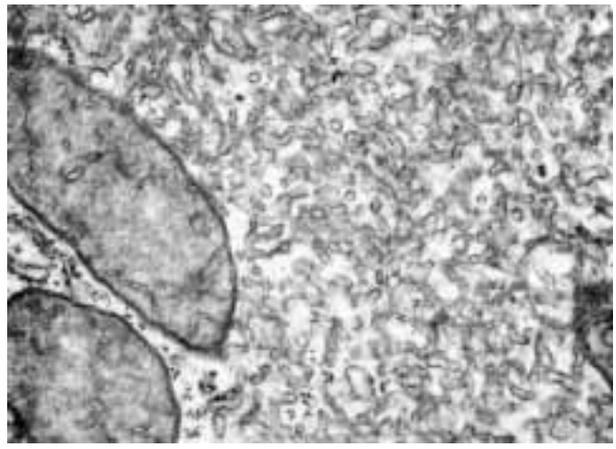


FIGURE 1–32 Electron micrograph of liver from phenobarbital-treated rat showing marked increase in smooth endoplasmic reticulum. (From Jones AL, Fawcett DW: Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by Phenobarbital. J Histochem Cytochem 14:215, 1966. Courtesy of Dr.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

INDUCTION (HYPERTROPHY) OF SMOOTH ENDOPLASMIC RETICULUM

The smooth ER is involved in the metabolism of various chemicals, and cells exposed to these chemicals show hypertrophy of the ER as an adaptive response that may have important functional consequences. Protracted use of barbiturates leads to a state of tolerance, with a decrease in the effects of the drug and the need to use increasing doses. Patients are said to have "adapted" to the medication. This adaptation is due to increased volume (hypertrophy) of the smooth ER of hepatocytes, which metabolizes the drug (Fig. 1–32). Barbiturates are modified in the liver by oxidative demethylation, which involves the P-450 mixed function oxidase system found in the smooth ER. The role of these enzyme modifications is to increase the solubility of a variety of compounds (e.g., alcohol, steroids, eicosanoids, and carcinogens as well as insecticides and other environmental pollutants) and thereby facilitate their secretion.[37] Although this is often thought of as "detoxification," many compounds are rendered *more* injurious by P-450 modification. In addition, the products formed by this oxidative metabolism include reactive oxygen species, which can cause injury of the cell. With prolonged use, the barbiturates (and many other agents) stimulate the synthesis of more enzymes, as well as more smooth ER. In this manner, the cell is better able to modify the drugs and adapt to its altered environment. Cells adapted to one drug have increased capacity to metabolize other compounds handled by the system. Thus, if patients taking phenobarbital for epilepsy increase their alcohol intake they may have subtherapeutic levels of the antiseizure medication because of induction of smooth ER in response to the alcohol.

MITOCHONDRIAL ALTERATIONS

We have seen that mitochondrial dysfunction plays an important role in cell injury and apoptosis. In addition, various alterations in the number, size, and shape of mitochondria occur in some pathologic conditions. For example, in cell hypertrophy and atrophy, there is an increase and decrease, respectively, in the number of mitochondria in cells. Mitochondria may assume extremely large and abnormal shapes (megamitochondria), as can be seen in the liver in alcoholic liver disease and in certain nutritional deficiencies (Fig. 1–33). Abnormalities of mitochondria are now recognized as the basis of many genetic diseases[70] (Chapter 5). In certain inherited metabolic diseases of skeletal muscle, the *mitochondrial myopathies*, defects in mitochondrial metabolism are associated with increased numbers of mitochondria that are often unusually large, have abnormal cristae, and contain crystalloids (Chapter 27). In addition, certain benign tumors found in salivary glands, thyroid, parathyroids, and kidneys consist of cells (sometimes called "oncocytes") with abundant enlarged mitochondria, giving the cell a distinctly eosinophilic appearance.

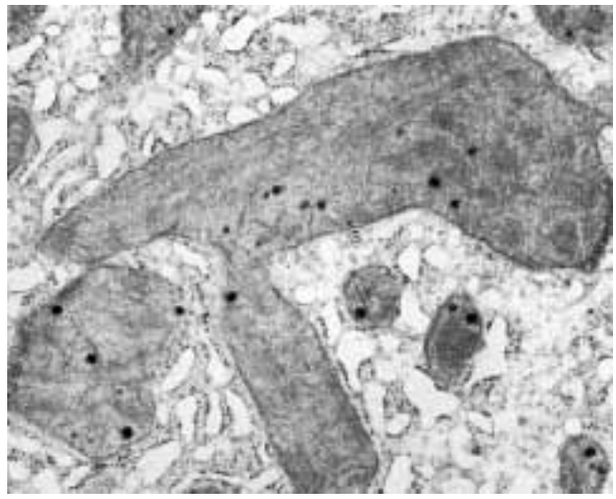


FIGURE 1–33 Enlarged, abnormally shaped mitochondria from the liver of a patient with alcoholic cirrhosis. Note also crystalline formations in the mitochondria.

CYTOSKELETAL ABNORMALITIES

Abnormalities of the cytoskeleton underlie a variety of pathologic states. The *cytoskeleton* consists of microtubules (20 to 25 nm in diameter), thin actin filaments (6 to 8 nm), thick myosin filaments (15 nm), and various classes of intermediate filaments (10 nm). Several other nonpolymerized and nonfilamentous forms of contractile proteins also exist. Cytoskeletal abnormalities may be reflected by: (1) defects in cell function, such as cell locomotion and intracellular organelle movements, and (2) in some instances by intracellular accumulations of fibrillar material. Only a few examples are cited.

- *Thin filaments.* Thin filaments are composed of actin, myosin, and their associated regulatory proteins.[71] Functioning thin filaments are essential for various stages of leukocyte movement or the ability of such cells to perform phagocytosis adequately. Some drugs and toxins target actin filaments and thus affect these processes. For example, cytochalasin B prevents polymerization of actin filaments, and phalloidin, a toxin of the mushroom *Amanita phalloides*, also binds actin filaments.
- *Microtubules.* Defects in the organization of microtubules can inhibit sperm motility, causing male sterility, and can immobilize the cilia of respiratory epithelium, causing interference with the ability of this epithelium to clear inhaled bacteria, leading to bronchiectasis (Kartagener's syndrome, or the *immotile cilia syndrome*; Chapter 15). Microtubules, like microfilaments, are essential for leukocyte migration and phagocytosis. Drugs such as colchicine bind to tubulin and prevent the assembly of microtubules. The drug is used in acute attacks of gout to prevent leukocyte migration and phagocytosis in response to deposition of urate crystals. Microtubules are an essential component of the mitotic spindle, which is required for cell division. Drugs that bind to microtubules (e.g., vinca alkaloids) can be antiproliferative and therefore act as antitumor agents.
- *Intermediate filaments.* These components provide a flexible intracellular scaffold that organizes the cytoplasm and resists forces applied to the cell.[72] The intermediate filaments are divided into five classes, including keratin filaments (characteristic of epithelial cells), neurofilaments (neurons), desmin filaments (muscle cells), vimentin filaments (connective tissue cells), and glial filaments (astrocytes). Accumulations of keratin filaments and neurofilaments are associated with certain types of cell injury. For example, the *Mallory body*, or "alcoholic hyalin," is an eosinophilic intracytoplasmic inclusion in liver cells that is characteristic of alcoholic liver disease,[73] although it can be present in other conditions. Such inclusions are composed predominantly of *keratin* intermediate filaments (Fig. 1–34). In the nervous system, neurofilaments are present in the axon, where they provide structural support. The *neurofibrillary tangle* found in the brain in Alzheimer's disease contains microtubule-associated proteins and neurofilaments, a reflection of a disrupted neuronal cytoskeleton (Chapter 28). Mutations in intermediate filament genes cause multiple human disorders, including myopathies, neurologic diseases, and skin diseases.

Much of the emphasis on the functions of the cytoskeleton has been on its mechanical role, in maintaining cellular architecture and in cell attachment and locomotion. It has recently been appreciated that *cytoskeletal proteins are linked to many cellular receptors*, such as lymphocyte receptors for antigens, and are active participants in signal transduction by these receptors (Chapter 3). Therefore, defects in the links between receptors and cytoskeletal proteins may affect many cellular responses. The *Wiskott-Aldrich syndrome* is an inherited disease characterized by eczema, platelet abnormalities, and immune deficiency. The protein that is mutated in this disease is involved in linking lymphocyte antigen receptors (and perhaps other receptors) to the cytoskeleton, and defects in the protein interfere with diverse cellular responses (Chapter 6).[74]

Intracellular Accumulations

One of the manifestations of metabolic derangements in cells is the intracellular accumulation of abnormal amounts of various substances. The stockpiled substances fall into three categories: (1) a *normal cellular constituent* accumulated in excess, such as water, lipids, proteins, and carbohydrates; (2)

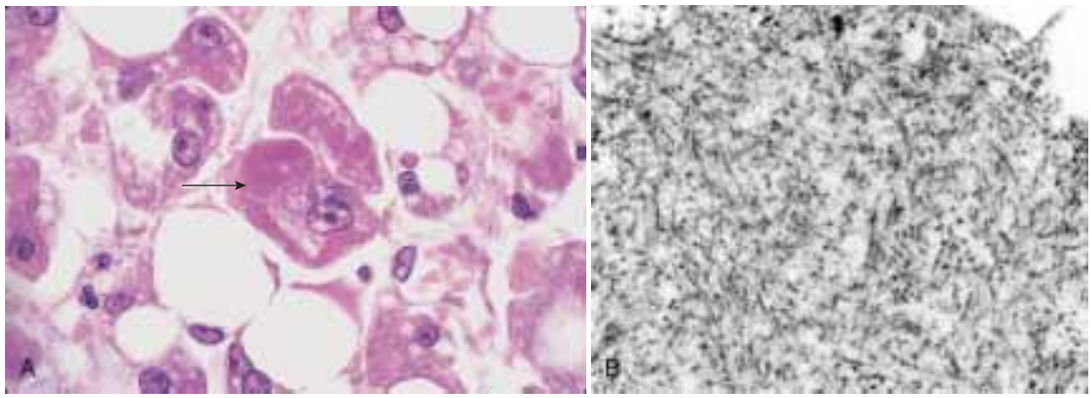


FIGURE 1–34 *A,* The liver of alcohol abuse (chronic alcoholism). Hyaline inclusions in the hepatic parenchymal cell in the center appear as eosinophilic networks disposed about the nuclei *(arrow). B,* Electron micrograph of alcoholic hyalin. The material is composed of intermediate (prekeratin) filaments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

an *abnormal substance*, either exogenous, such as a mineral or products of infectious agents, or endogenous, such as a product of abnormal synthesis or metabolism; and (3) a *pigment*. These substances may accumulate either transiently or permanently, and they may be harmless to the cells, but on occasion they are severely toxic. The substance may be located in either the cytoplasm (frequently within phagolysosomes) or the nucleus. In some instances, the cell may be producing the abnormal substance, and in others it may be merely storing products of pathologic processes occurring elsewhere in the body.

Many processes result in abnormal intracellular accumulations, but most accumulations are attributable to three types of abnormalities (Fig. 1–35).

1. *A normal endogenous substance is produced at a normal or increased rate, but the rate of metabolism is inadequate to remove it*. An example of this type of process is fatty change in the liver because of intracellular accumulation of triglycerides (see later). Another is the appearance of reabsorption protein droplets in renal tubules because of increased leakage of protein from the glomerulus.
2. *A normal or abnormal endogenous substance accumulates because of genetic or acquired defects in the metabolism, packaging, transport, or secretion of these substances*. One example is the group of conditions caused by genetic defects of specific enzymes involved in the metabolism of lipid and carbohydrates resulting in intracellular deposition of these substances, largely in lysosomes. These so-called storage diseases are discussed in Chapter 5. Another is alpha$_1$-antitrypsin deficiency, in which a single amino acid substitution in the enzyme results in defects in protein folding and accumulation of the enzyme in the endoplasmic reticulum of the liver in the form of globular eosinophilic inclusions (see later and Chapter 18).
3. *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 particles and such nonmetabolizable chemicals as silica particles are examples of this type of alteration.

Whatever the nature and origin of the intracellular accumulation, it implies the storage of some product by individual cells. If the overload is due to a systemic derangement and can be brought under control, the accumulation is reversible. In genetic storage diseases, accumulation is progressive, and the cells may become so overloaded as to cause secondary injury, leading in some instances to death of the tissue and the patient.

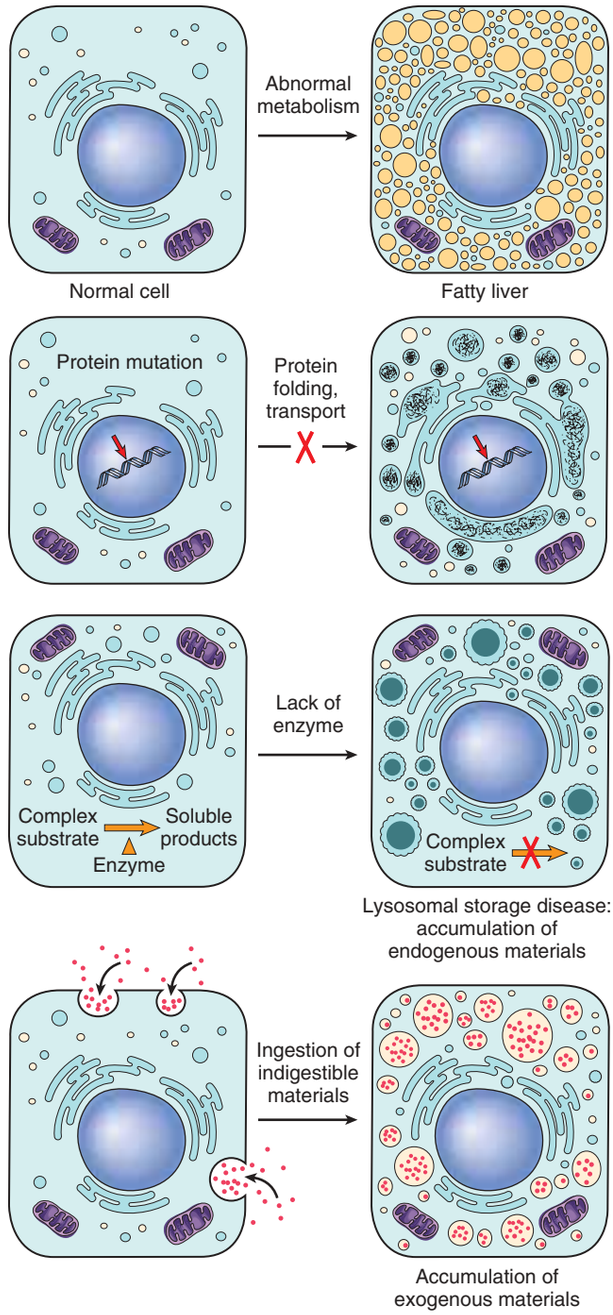


FIGURE 1–35 Mechanisms of intracellular accumulations: (1) abnormal metabolism, as in fatty change in the liver; (2) mutations causing alterations in protein folding and transport, as in alpha$_1$-antitrypsin deficiency; (3) deficiency of critical enzymes that prevent breakdown of substrates that accumulate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

LIPIDS

All major classes of lipids can accumulate in cells: triglycerides, cholesterol/cholesterol esters, and phospholipids. Phospholipids are components of the myelin figures found in necrotic cells. In addition, abnormal complexes of lipids and carbohydrates accumulate in the lysosomal storage diseases (Chapter 5). Here we concentrate on triglyceride and cholesterol accumulations.

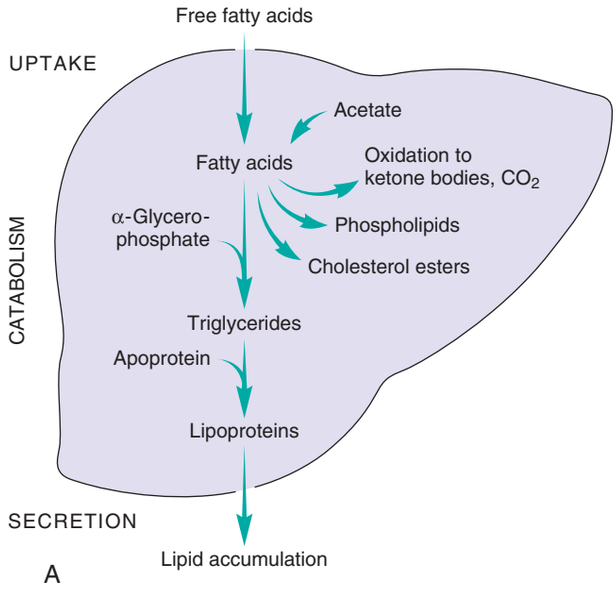
Steatosis (Fatty Change)

The terms *steatosis* and *fatty change* describe abnormal accumulations of triglycerides within parenchymal cells. Fatty change is often seen in the liver because it is the major organ involved in fat metabolism, but it also occurs in heart, muscle,

and kidney. The causes of steatosis include toxins, protein malnutrition, diabetes mellitus, obesity, and anoxia. *In industrialized nations, by far the most common cause of significant fatty change in the liver (fatty liver) is alcohol abuse* (Chapter 18).[75]

Different mechanisms account for triglyceride accumulation in the liver. Free fatty acids from adipose tissue or ingested food are normally transported into hepatocytes. In the liver, they are esterified to triglycerides, converted into cholesterol or phospholipids, or oxidized to ketone bodies. Some fatty acids are synthesized from acetate as well. Release of triglycerides from the hepatocytes requires association with apoproteins to form lipoproteins, which may then traverse the circulation (Chapter 4). *Excess accumulation of triglycerides within the liver may result from defects in any one of the events in the sequence from fatty acid entry to lipoprotein exit* (Fig. 1–36*A*). A number of such defects are induced by alcohol, a hepatotoxin that alters mitochondrial and microsomal functions. CCl_4 and protein malnutrition act by decreasing synthesis of apoproteins. Anoxia inhibits fatty acid oxidation. Starvation increases fatty acid mobilization from the 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 impair cellular function, typically when some vital intracellular process is also impaired (e.g., in CCl_4 poisoning). As a severe form of injury, fatty change may be a harbinger of cell death. In recent years, nonalcoholic steatohepatitis and nonalcoholic fatty liver disease have been recognized as fairly common disease entities that may lead to cirrhosis and even hepatocellular cancer (Chapter 18).



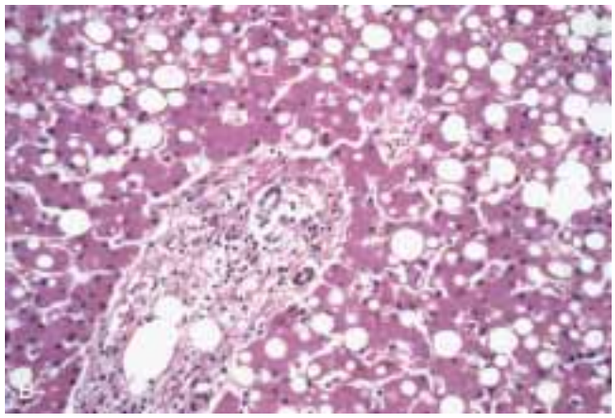


FIGURE 1–36 Fatty liver. *A*, Schematic diagram of the possible mechanisms leading to accumulation of triglycerides in fatty liver. Defects in any of the steps of uptake, catabolism, or secretion can result in lipid accumulation. *B*, High-power detail of fatty change of the liver. In most cells, the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

Morphology. Fatty change is most often seen in the liver and heart. In all organs, fatty change appears as clear vacuoles within parenchymal cells. Intracellular accumulations of water or polysaccharides (e.g., glycogen) may also produce clear vacuoles, and it becomes necessary to resort to special techniques to distinguish these three types of clear vacuoles. The identification of lipids requires the avoidance of fat solvents commonly used in paraffin embedding for routine hematoxylin and eosin stains. To identify the fat, it is necessary to prepare frozen tissue sections of either fresh or aqueous formalin-fixed tissues. The sections may then be stained with Sudan IV or Oil Red-O, both of which impart an orange-red color to the contained lipids. The periodic acid-Schiff (PAS) reaction is commonly employed to identify glycogen, although it is by no means specific. When neither fat nor polysaccharide can be demonstrated within a clear vacuole, it is presumed to contain water or fluid with a low protein content.

Liver. In the liver, mild fatty change may not affect the gross appearance. With progressive accumulation, the organ enlarges and becomes increasingly yellow until, in extreme instances, the liver may weigh 3 to 6 kg and be transformed into a bright yellow, soft, greasy organ.

Fatty change begins with the development of minute, membrane-bound inclusions (liposomes) closely applied to the endoplasmic reticulum. Fatty change is first seen by light microscopy as small vacuoles in the cytoplasm around the nucleus. As the process progresses, the vacuoles coalesce, creating cleared spaces that displace the nucleus to the periphery of the cell (Fig. 1–36*B*). Occasionally, contiguous cells rupture, and the enclosed fat globules coalesce, producing so-called fatty cysts.

Heart. Lipid is found in cardiac muscle in the form of small droplets, occurring in two patterns. In one, prolonged moderate hypoxia, such as that produced by profound anemia, causes intracellular deposits of fat, which create grossly apparent bands of yellowed myocardium alternating with bands of darker, red-brown, uninvolved myocardium **(tigered effect)**. The other pattern of hypoxia is produced by more profound hypoxia or by some forms of myocarditis (e.g., diphtheria) and shows more uniformly affected myocytes.

Cholesterol and Cholesterol Esters

The cellular metabolism of cholesterol (discussed in detail in Chapter 5) is tightly regulated such that most cells use cholesterol for the synthesis of cell membranes without intracellular accumulation of cholesterol or cholesterol esters. Accumulations, however, manifested histologically by intracellular vacuoles, are seen in several pathologic processes.

- *Atherosclerosis.* In atherosclerotic plaques, smooth muscle cells and macrophages within the intimal layer of the aorta and large arteries are filled with lipid vacuoles, most of which are made up of cholesterol and cholesterol esters. Such cells have a foamy appearance (foam cells), and aggregates of them in the intima produce the yellow cholesterol-laden atheromas characteristic of this serious disorder. Some of these fat-laden cells rupture, releasing lipids into the extracellular space. The mechanisms of cholesterol accumulation in both cell types in atherosclerosis are discussed in detail in Chapter 11. The extracellular cholesterol esters may crystallize in the shape of long needles, producing quite distinctive clefts in tissue sections.
- *Xanthomas.* Intracellular accumulation of cholesterol within macrophages is also characteristic of acquired and hereditary hyperlipidemic states. Clusters of foamy cells are found in the subepithelial connective tissue of the skin and in tendons, producing tumorous masses known as xanthomas.
- *Inflammation and necrosis.* Foamy macrophages are frequently found at sites of cell injury and inflammation, owing to phagocytosis of cholesterol from the membranes of injured cells, including parenchymal cells, leukocytes, and erythrocytes. Phospholipids and myelin figures are also found in inflammatory foci. When abundant, the cholesterol-laden macrophages impart a yellowish discoloration to such inflammatory foci.
- *Cholesterolosis.* This refers to the focal accumulations of cholesterol-laden macrophages in the lamina propria of the gallbladder (Fig. 1–37). The mechanism of accumulation is unknown.
- *Niemann-Pick disease, type C.* In this lysosomal storage disease, an enzyme involved in cholesterol trafficking is mutated, and hence cholesterol accumulates in multiple organs (Chapter 5).

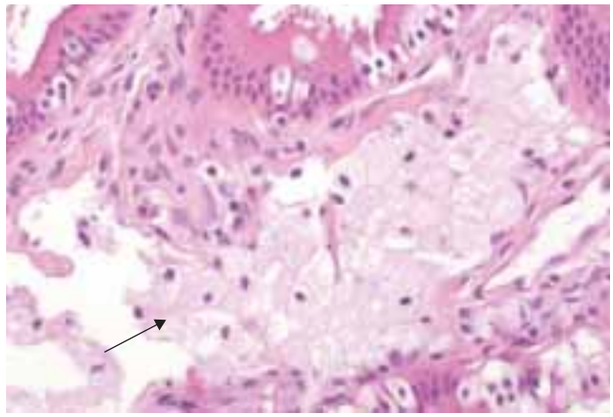


FIGURE 1–37 Cholesterolosis. Cholesterol-laden macrophages (foam cells) from a focus of gallbladder cholesterolosis (*arrow*). (Courtesy of Dr. Matthew Yeh, University of Washington)

PROTEINS

Intracellular accumulations of proteins usually appear as rounded, eosinophilic droplets, vacuoles, or aggregates in the cytoplasm. By electron microscopy, they can be amorphous, fibrillar, or crystalline in appearance. In some disorders, such as certain forms of amyloidosis, abnormal proteins deposit primarily in the extracellular space (Chapter 6).

Excesses of proteins within the cells sufficient to cause morphologically visible accumulation have diverse causes.

- *Reabsorption droplets in proximal renal tubules* are seen in renal diseases associated with protein loss in the urine (proteinuria). In the kidney, small amounts of protein filtered through the glomerulus are normally reabsorbed by pinocytosis in the proximal tubule. In disorders with heavy protein leakage across the glomerular filter, there is increased reabsorption of the protein into vesicles. These vesicles fuse with lysosomes to produce phagolysosomes, which appear as pink hyaline droplets within the cytoplasm of the tubular cell (Fig. 1–38). The process is reversible; if the proteinuria diminishes, the protein droplets are metabolized and disappear.
- A second cause is synthesis of excessive amounts of normal secretory protein, as occurs in certain plasma cells engaged in active synthesis of immunoglobulins. The ER becomes hugely distended, producing large, homogeneous eosinophilic inclusions called *Russell bodies*.
- *Defects in protein folding* may underlie some of these depositions in a variety of unrelated diseases.[76] Nascent polypeptide chains of proteins, made on ribosomes, are ultimately arranged into either α helices or β sheets, and the proper configuration of these arrangements (protein folding) is critical to the individual protein's function and its transport into cell organelles.[77] In the process of folding, partially folded intermediates arise, and these may form intracellular aggregates among themselves or by entangling other proteins. Under normal conditions, however, these intermediates are stabilized by a number of molecular chaperones, which interact with proteins directly.[78] Chaperones aid in proper folding and in transport across the ER, Golgi complex, and beyond (Fig. 1–39). Some chaperones are synthesized constitutively and affect normal intracellular protein trafficking, whereas others

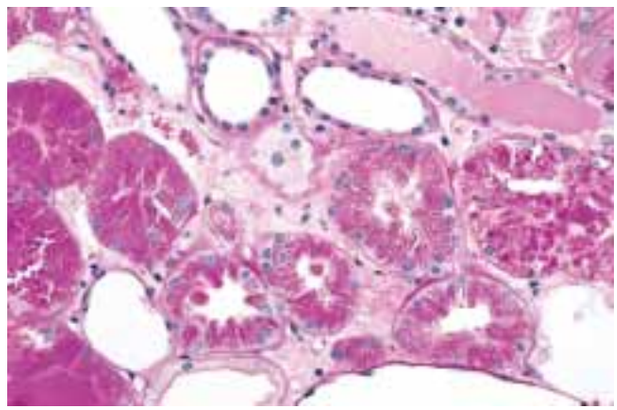


FIGURE 1–38 Protein reabsorption droplets in the renal tubular epithelium. (Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital.)

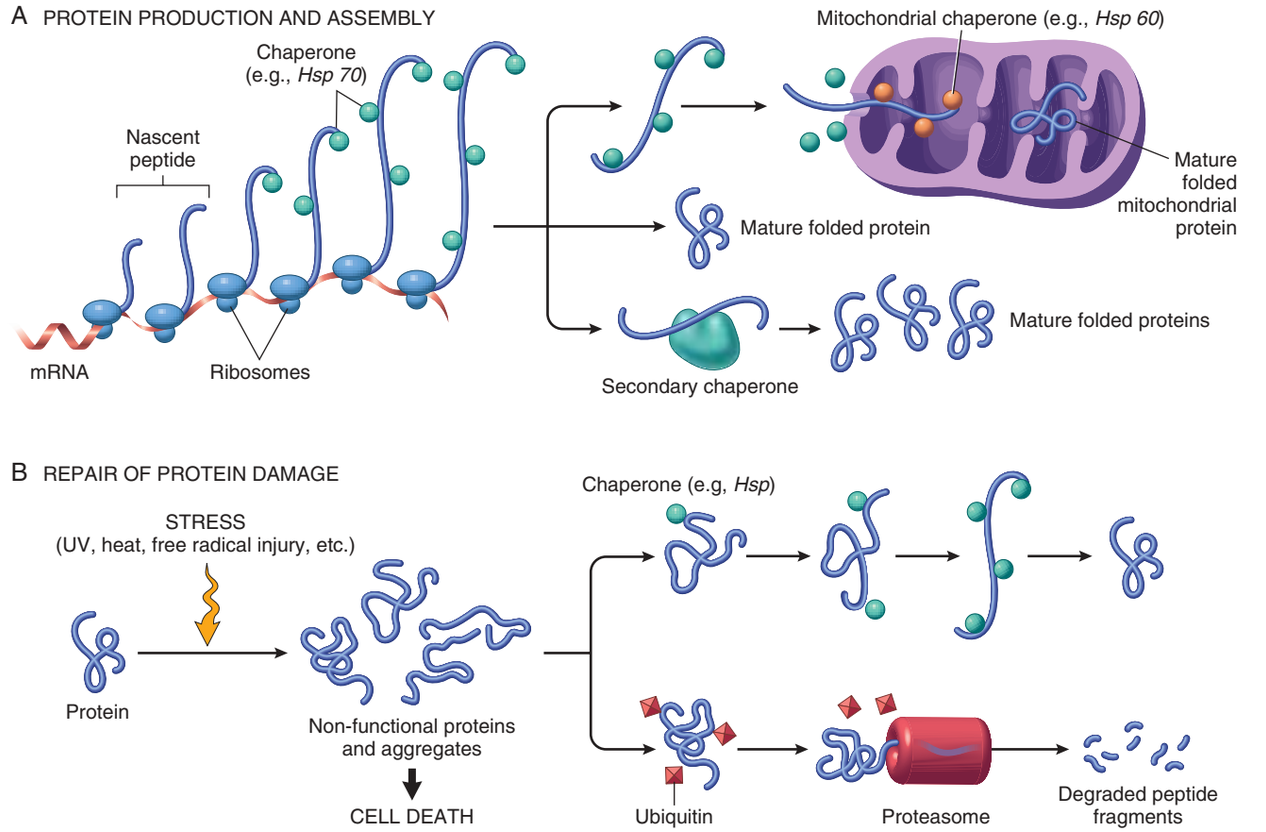


FIGURE 1–39 Mechanisms of protein folding and the role of chaperones. *A,* Chaperones, such as heat shock proteins (Hsp), protect unfolded or partially folded protein from degradation and guide proteins into organelles. *B,* Chaperones repair misfolded proteins; when this process is ineffective, proteins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

are induced by stress, such as heat (heat-shock proteins, e.g., hsp70, hsp90), and "rescue" shock-stressed proteins from misfolding. If the folding process is not successful, the chaperones facilitate degradation of the damaged protein. This degradative process often involves ubiquitin (also a heat-shock protein), which is added to the abnormal protein and marks it for degradation by the proteasome complex. There are several mechanisms by which protein folding defects can cause intracellular accumulations or result in disease.

- *Defective intracellular transport and secretion of critical proteins.* In *α_1-antitrypsin deficiency*, mutations in the protein significantly slow folding, resulting in the build-up of partially folded intermediates, which aggregate in the ER of the liver and are not secreted. The resultant deficiency of the circulating enzyme causes emphysema (Chapter 15). In *cystic fibrosis,* mutation delays dissociation of a chloride channel protein from one of its chaperones, resulting in abnormal folding and loss of function (Chapter 10). In *familial hypercholesterolemia*, mutations in low-density lipoprotein receptors interfere with proper folding of receptor proteins (Chapter 5).
- *ER stress induced by unfolded and misfolded proteins.* Unfolded or misfolded proteins accumulate in the ER and trigger a number of cellular responses, collectively called the *unfolded protein response*.[79–81] The unfolded protein response is mediated by several proteins that reside in and span the ER membrane. The luminal domains of these proteins sense perturbations in protein folding, and the cytoplasmic domains activate signaling pathways that reduce the levels of misfolded proteins in the cell, by increasing the production of chaperones and slowing down protein translation. Paradoxically, the activation of the unfolded protein response also leads to cell death by activating caspases, particularly an ER-resident caspase called caspase-12. Thus, misfolded proteins initially trigger the cytoprotective function of this response, but if these abnormal proteins persist, the pro-apoptotic cytotoxic functions take over. Aggregation of abnormally folded proteins, caused by genetic mutations, aging, or unknown environmental factors, is now recognized as a feature of a number of neurodegenerative diseases, including Alzheimer's, Huntington's, and Parkinson's diseases (Chapter 28), and possibly type II diabetes. Deprivation of glucose and oxygen, and stress such as heat, also result in protein misfolding and trigger the unfolded protein response, culminating in cell injury and death.

- *Aggregation of abnormal proteins.* Abnormal or misfolded proteins may deposit in tissues and interfere with normal functions. The deposits can be intracellular, extracellular, or both, and there is accumulating evidence that the aggregates may either directly or indirectly cause the pathologic changes. Certain forms of *amyloidosis* (Chapter 6) fall in this category of diseases. These disorders are sometimes called *proteinopathies* or *protein-aggregation diseases*.

HYALINE CHANGE

The term *hyaline* usually refers to an alteration within cells or in the extracellular space, which gives a homogeneous, glassy, pink appearance in routine histologic sections stained with hematoxylin and eosin. It is widely used as a descriptive histologic term rather than a specific marker for cell injury. This tinctorial change is produced by a variety of alterations and does not represent a specific pattern of accumulation. Intracellular accumulations of protein, described earlier (reabsorption droplets, Russell bodies, Mallory alcoholic hyalin), are examples of intracellular hyaline deposits.

Extracellular hyalin has been somewhat more difficult to analyze. Collagenous fibrous tissue in old scars may appear hyalinized, but the physiochemical mechanism underlying this change is not clear. In long-standing hypertension and diabetes mellitus, the walls of arterioles, especially in the kidney, become hyalinized, owing to extravasated plasma protein and deposition of basement membrane material.

GLYCOGEN

Glycogen is a readily available energy store that is present in the cytoplasm. Excessive intracellular deposits of glycogen are seen in patients with an abnormality in either glucose or glycogen metabolism. Whatever the clinical setting, the glycogen masses appear as clear vacuoles within the cytoplasm. Glycogen is best preserved in nonaqueous fixatives; for its localization, tissues are best fixed in absolute alcohol. Staining with Best carmine or the periodic acid schiff (PAS) reaction imparts a rose-to-violet color to the glycogen, and diastase digestion of a parallel section before staining serves as a further control by hydrolyzing the glycogen.

Diabetes mellitus is the prime example of a disorder of glucose metabolism. In this disease, glycogen is found in the epithelial cells of the distal portions of the proximal convoluted tubules and sometimes in the descending loop of Henle, as well as within liver cells, β cells of the islets of Langerhans, and heart muscle cells.

Glycogen also accumulates within the cells in a group of closely related disorders, all genetic, collectively referred to as the *glycogen storage diseases*, or *glycogenoses* (Chapter 5). In these diseases, enzymatic defects in the synthesis or breakdown of glycogen result in massive accumulation, with secondary injury and cell death.

PIGMENTS

Pigments are colored substances, some of which are normal constituents of cells (e.g., melanin), whereas others are abnormal and collect in cells only under special circumstances. Pigments can be exogenous, coming from outside the body, or endogenous, synthesized within the body itself.

Exogenous Pigments. The most common *exogenous pigment* is *carbon* or coal dust, which is a ubiquitous air pollutant of urban life. When inhaled, it is picked up by macrophages within the alveoli and is then transported through lymphatic channels to the regional lymph nodes in the tracheobronchial region. Accumulations of this pigment blacken the tissues of the lungs *(anthracosis)* and the involved lymph nodes. In coal miners, the aggregates of carbon dust may induce a fibroblastic reaction or even emphysema and thus cause a serious lung disease known as *coal worker's pneumoconiosis* (Chapter 15). *Tattooing* is a form of localized, exogenous pigmentation of the skin. The pigments inoculated are phagocytosed by dermal macrophages, in which they reside for the remainder of the life of the embellished (sometimes with embarrassing consequences for the bearer of the tattoo!). The pigments do not usually evoke any inflammatory response.

Endogenous Pigments. *Lipofuscin* is an insoluble pigment, also known as lipochrome and wear-and-tear or aging pigment. Lipofuscin is composed of polymers of lipids and phospholipids complexed with protein, suggesting that it is derived through lipid peroxidation of polyunsaturated lipids of subcellular membranes. Lipofuscin is not injurious to the cell or its functions. Its importance lies in its being the telltale sign of free radical injury and lipid peroxidation. The term is derived from the Latin (*fuscus* = brown), thus brown lipid. In tissue sections, it appears as a yellow-brown, finely granular intracytoplasmic, often perinuclear pigment (Fig. 1–40). It is seen in cells undergoing slow, regressive changes and is particularly prominent in the liver and heart of aging patients or patients with severe malnutrition and cancer cachexia. On electron microscopy, the granules are highly electron dense, often have membranous structures in their midst, and are usually in a perinuclear location.

Melanin, derived from the Greek (*melas* = black), is an endogenous, non-hemoglobin-derived, brown-black pigment formed when the enzyme tyrosinase catalyzes the oxidation of tyrosine to dihydroxyphenylalanine in melanocytes. It is discussed further in Chapter 25. For all practical purposes, melanin is the *only endogenous brown-black pigment*. The only other that could be considered in this category is homogentisic acid, a black pigment that occurs in patients with *alkaptonuria*, a rare metabolic disease. Here the pigment is deposited in the skin, connective tissue, and cartilage, and the pigmentation is known as *ochronosis* (Chapter 5).

Hemosiderin is a hemoglobin-derived, golden yellow-to-brown, granular or crystalline pigment in which form iron is stored in cells. Iron metabolism and the synthesis of ferritin and hemosiderin are considered in detail in Chapter 13. Iron is normally carried by specific transport proteins, transferrins. In cells, it is stored in association with a protein, apoferritin, to form ferritin micelles. Ferritin is a constituent of most cell types. *When there is a local or systemic excess of iron, ferritin forms hemosiderin granules*, which are easily seen with the light microscope (Fig. 1–41). Thus, hemosiderin pigment represents aggregates of ferritin micelles. Under normal conditions, small amounts of hemosiderin can be seen in the mononuclear phagocytes of the bone marrow, spleen, and liver, all actively engaged in red cell breakdown.

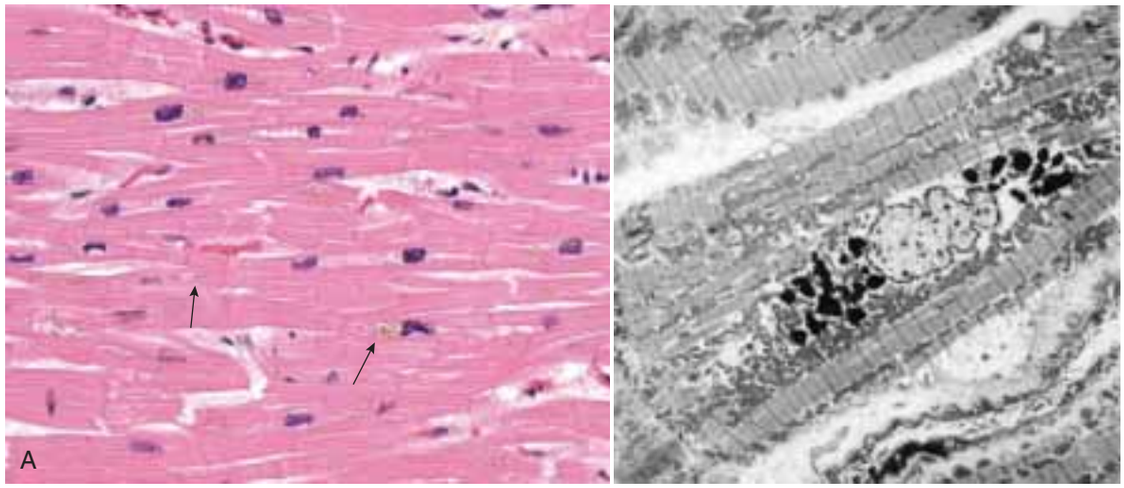


FIGURE 1–40 Lipofuscin granules in a cardiac myocyte as shown by *A,* light microscopy (deposits indicated by *arrows*), and *B,* electron microscopy (note the perinuclear, intralysosomal location).

Excesses of iron cause hemosiderin to accumulate within cells, either as a localized process or as a systemic derangement. *Local excesses* of iron and hemosiderin result from gross hemorrhages or the myriad minute hemorrhages that accompany severe vascular congestion. The best example of localized hemosiderosis is the common bruise. After local hemorrhage, the area is at first red-blue. With lysis of the erythrocytes, the hemoglobin eventually undergoes transformation to hemosiderin. Macrophages take part in this process by phagocytiosing the red cell debris, and then lysosomal enzymes eventually convert the hemoglobin, through a sequence of pigments, into hemosiderin. The play 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, comprising the local formation of biliverdin (green bile), then bilirubin (red bile), and thereafter the iron moiety of hemoglobin is deposited as golden yellow hemosiderin.

Whenever there are causes for *systemic overload of iron*, hemosiderin is deposited in many organs and tissues, a condition called *hemosiderosis*. It is seen with: (1) increased absorption of dietary iron, (2) impaired use of iron, (3) hemolytic anemias, and (4) transfusions because the transfused red cells constitute an exogenous load of iron. These conditions are discussed in Chapter 18.

Morphology. Iron pigment appears as a coarse, golden, granular pigment lying within the cell's cytoplasm. When the basic cause is the localized breakdown of red cells, the pigmentation is found at first in the phagocytes in the area. In systemic hemosiderosis, 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 such as the skin, pancreas, and kidneys. With progressive accumulation, parenchymal cells throughout the body (principally in the liver, pancreas, heart, and endocrine organs) become pigmented. Iron can be visualized in tissues by the Prussian blue histochemical reaction, in which colorless potassium ferrocyanide is converted by iron to blue-black ferric ferrocyanide (Fig. 1–41*B*).

In most instances of systemic hemosiderosis, the pigment does not damage the parenchymal cells or

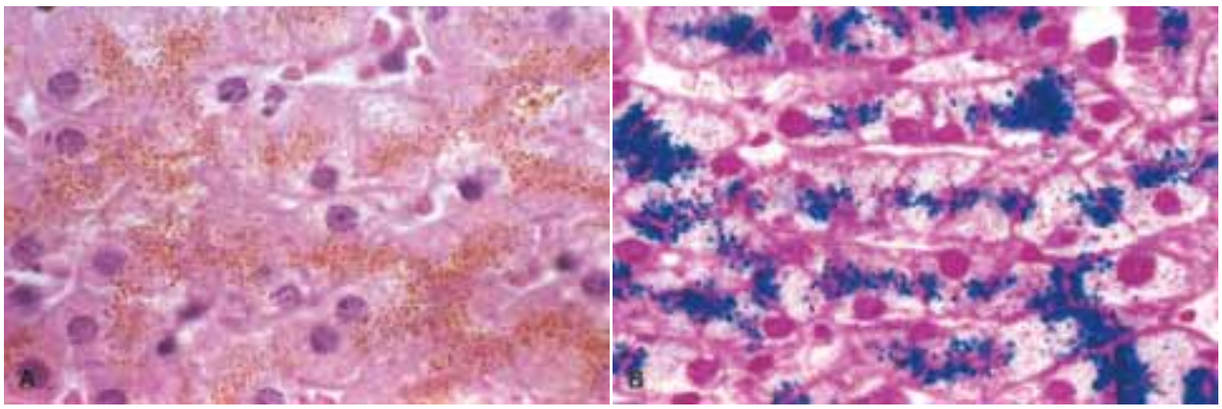


FIGURE 1–41 Hemosiderin granules in liver cells. *A,* H&E section showing golden-brown, finely granular pigment. *B,* Prussian blue reaction, specific for iron.

impair organ function. The more extreme accumulation of iron, however, in a disease called **hemochromatosis,** is associated with liver, heart, and pancreatic damage, resulting in liver fibrosis, heart failure, and diabetes mellitus (Chapter 18).

Bilirubin is the normal major pigment found in bile. It is derived from hemoglobin but contains no iron. Its normal formation and excretion are vital to health, and jaundice is a common clinical disorder caused by excesses of this pigment within cells and tissues. Bilirubin metabolism and jaundice are discussed in Chapter 18.

Pathologic Calcification

Pathologic calcification is the abnormal tissue deposition of calcium salts, together with smaller amounts of iron, magnesium, and other mineral salts. It is a common process occurring in a variety of pathologic conditions. There are two forms of pathologic calcification. When the deposition occurs locally in dying tissues, it is known as *dystrophic calcification*; it occurs despite normal serum levels of calcium and in the absence of derangements in calcium metabolism. In contrast, the deposition of calcium salts in otherwise normal tissues is known as *metastatic calcification*, and it almost always results from hypercalcemia secondary to some disturbance in calcium metabolism.

DYSTROPHIC CALCIFICATION

Dystrophic calcification is encountered in areas of necrosis, whether they are of coagulative, caseous, or liquefactive type, and in foci of enzymatic necrosis of fat. Calcification is almost inevitable in the atheromas of advanced atherosclerosis. It also commonly develops in aging or damaged heart valves, further hampering their function (Fig. 1–42). Whatever the site of deposition, the calcium salts appear macroscopically as fine, white granules or clumps, often felt as gritty deposits. Sometimes a tuberculous lymph node is virtually converted to stone.

Morphology. Histologically, with the usual hematoxylin and eosin stain, the calcium salts have a basophilic, amorphous granular, sometimes clumped, appearance. They can be intracellular, extracellular, or in both locations. In the course of time, **heterotopic** bone may be formed in the focus of calcification. On occasion, single necrotic cells may constitute seed crystals that become encrusted by the mineral deposits. The progressive acquisition of outer layers may create lamellated configurations, called **psammoma bodies** because of their resemblance to grains of sand. Some types of papillary cancers (e.g., thyroid) are apt to develop psammoma bodies. Strange concretions emerge when calcium iron salts gather about long slender spicules of asbestos in the lung, creating exotic, beaded dumbbell forms.

Pathogenesis. In the pathogenesis of dystrophic calcification, the final common pathway is the formation of crystalline calcium phosphate mineral in the form of an apatite similar to the hydroxyapatite of bone. The process has two major phases: *initiation* (or nucleation) and *propagation*; both can occur intracellularly and extracellularly. Initiation of *intracellular calcification* occurs in the *mitochondria* of dead or dying cells that accumulate calcium. Initiators of *extracellular dystrophic calcification* include phospholipids found in membrane-bound *vesicles* about 200 nm in diameter; in cartilage and bone, they are known as *matrix vesicles,* and in pathologic calcification, they are derived from degenerating or aging cells. It is thought that calcium is concentrated in these vesicles by a process of membrane-facilitated calcification, which has several steps: (1) calcium ion binds to the phospholipids present in the vesicle membrane, (2) phosphatases associated with the membrane generate phosphate groups, which bind to the calcium, (3) the cycle of calcium and phosphate binding is repeated, raising the local concentrations and producing a deposit near the membrane, and (4) a structural change occurs in the arrangement of calcium and phosphate groups, generating a microcrystal, which can then propagate and perforate the membrane. Propagation of crystal formation depends on the concentration of Ca^{2+} and PO_4 and the presence of inhibitors and other proteins in the extracellular space, such as the connective tissue matrix proteins.

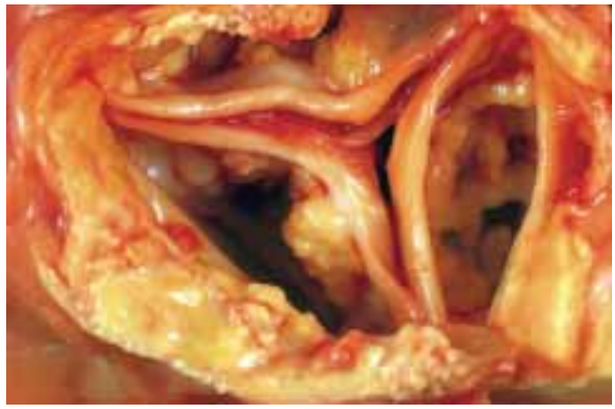


FIGURE 1–42 View looking down onto the unopened aortic valve in a heart with calcific aortic stenosis. The semilunar cusps are thickened and fibrotic. Behind each cusp are seen irregular masses of piled-up dystrophic calcification.

Although dystrophic calcification may be simply a telltale sign of previous cell injury, it is often a cause of organ dysfunction. Such is the case in calcific valvular disease and atherosclerosis, as becomes clear in further discussion of these diseases.

METASTATIC CALCIFICATION

Metastatic calcification may occur in normal tissues whenever there is hypercalcemia. Hypercalcemia also accentuates dystrophic calcification. There are four principal causes of hypercalcemia: (1) increased secretion of parathyroid hormone (PTH) with subsequent bone resorption, as in hyperparathyroidism due to parathyroid tumors, and ectopic secretion of PTH-related protein by malignant tumors (Chapter 7); (2) *destruction of bone tissue*, occurring with primary tumors of bone marrow (e.g., multiple myeloma, leukemia) or diffuse skeletal metastasis (e.g., breast cancer),

accelerated bone turnover (e.g., Paget disease), or immobilization; (3) *vitamin D–related disorders*, including vitamin D intoxication, sarcoidosis (in which macrophages activate a vitamin D precursor), and idiopathic hypercalcemia of infancy (Williams syndrome), characterized by abnormal sensitivity to vitamin D; and (4) *renal failure*, which causes retention of phosphate, leading to secondary hyperparathyroidism. Less common causes include aluminum intoxication, which occurs in patients on chronic renal dialysis, and milk-alkali syndrome, which is due to excessive ingestion of calcium and absorbable antacids such as milk or calcium carbonate.

Metastatic calcification may occur widely throughout the body but principally affects the interstitial tissues of the gastric mucosa, kidneys, lungs, systemic arteries, and pulmonary veins. Although quite different in location, all of these tissues *lose acid* and therefore have an internal alkaline compartment that predisposes them to metastatic calcification. In all these sites, the calcium salts morphologically resemble those described in dystrophic calcification. Thus, they may occur as noncrystalline amorphous deposits or, at other times, as hydroxyapatite crystals.

Usually, the mineral salts cause no clinical dysfunction, but, on occasion, massive involvement of the lungs produces remarkable x-ray films and respiratory deficits. Massive deposits in the kidney (nephrocalcinosis) may in time cause renal damage (Chapter 20).

Cellular Aging

Shakespeare probably characterized aging best in his elegant description of the seven ages of man. It begins at the moment of conception, involves the differentiation and maturation of the organism and its cells, at some variable point in time leads to the progressive loss of functional capacity characteristic of senescence, and ends in death.

With age, there are physiologic and structural alterations in almost all organ systems. Aging in individuals is affected to a great extent by genetic factors, diet, social conditions, and occurrence of age-related diseases, such as atherosclerosis, diabetes, and osteoarthritis. In addition, there is good evidence that aging-induced alterations in cells are an important component of the aging of the organism. Here we discuss cellular aging because it could represent the progressive accumulation over the years of sublethal injury that may lead to cell death or at least to the diminished capacity of the cell to respond to injury.

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 influences that result in the progressive accumulation of cellular and molecular damage (Fig. 1–43). These processes are reviewed next.

Structural and Biochemical changes with cellular Aging. A number of cell functions decline progressively with age. Oxidative phosphorylation by mitochondria is reduced, as is synthesis of nucleic acids and structural and enzymatic proteins, cell receptors, and transcription factors. Senescent cells have a decreased capacity for uptake of nutrients and for repair of chromosomal damage. The morphologic alterations in aging cells include irregular and abnormally lobed nuclei, pleomorphic vacuolated mitochondria, decreased endoplasmic reticulum, and distorted Golgi apparatus. Concomitantly, there is a steady accumulation of the pigment lipofuscin, which, as we have seen, represents a product of lipid peroxidation and evidence of *oxidative damage; advanced glycation end products*, which result from nonenzymatic glycosylation and are capable of cross-linking adjacent proteins; and the accumulation of *abnormally folded proteins.* The role of oxidative damage is discussed later. Advanced glycation end products are important in the pathogenesis of diabetes mellitus and are discussed in Chapter 24, but they may also participate in aging. For example, age-related glycosylation of lens proteins may underlie senile cataracts. The nature of abnormally folded proteins was discussed earlier in the chapter.

Replicative Senescence. The concept that cells have a limited capacity for replication was developed from a simple experimental model for aging. Normal human fibroblasts, when placed in tissue culture, have limited division potential.[82] Cells from children undergo more rounds of replication than cells from older people (Fig. 1–44). In contrast, cells from patients with *Werner syndrome*, a rare disease characterized by premature aging, have a markedly reduced in vitro life span. After a fixed number of divisions, all cells become arrested in a terminally nondividing state, known as *cellular senescence*. Many changes in gene expression occur during cellular aging, but a key question is which of these are causes and which are effects of cellular senescence.[83] For example, some of the proteins that inhibit progression of the cell growth cycle

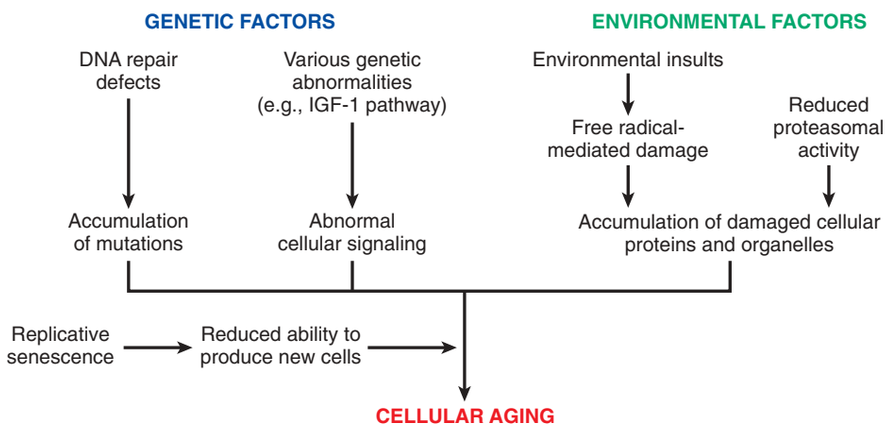


FIGURE 1–43 Mechanisms of cellular aging. Genetic factors and environmental insults combine to produce the cellular abnormalities characteristic of aging.

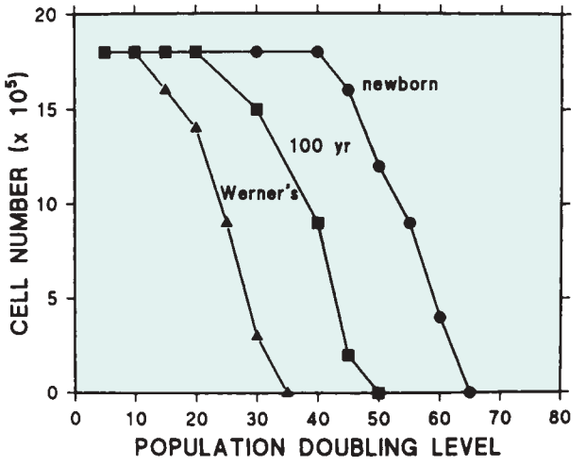


FIGURE 1–44 Finite population doublings of primary human fibroblasts derived from a newborn, a 100-year-old person, and a 20-year-old patient with Werner's syndrome. The ability of cells to grow to a confluent monolayer decreases with increasing population-doubling levels. (From Dice JF: Cellular and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

(as detailed in Chapter 7)—such as the products of the cyclin-dependent kinase inhibitor genes (e.g., *p21*)—are overexpressed in senescent cells.

How dividing cells can *count* their divisions is under intensive investigation. One likely mechanism is that with each cell division, there is *incomplete replication of chromosome ends (telomere shortening), which ultimately results in cell cycle arrest*. *Telomeres* are short repeated sequences of DNA (TTAGGG) present at the linear ends of chromosomes that are important for ensuring the complete replication of chromosome ends and protecting chromosomal termini from fusion and degradation.[84,85]

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 (Fig. 1–45). The activity of telomerase is repressed by regulatory proteins, which restrict telomere elongation, thus providing a length sensing mechanism. 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. 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.[85] Despite such alluring observations, however, the relationship of telomerase activity and telomeric length to aging and cancer still needs to be fully established.[86]

Genes That Influence the Aging Process. Studies in *Drosophila, C. elegans,* and mice are leading to the discovery of genes that influence the aging process.[87] One interesting set of genes involves the insulin/insulin growth factor-1 pathway. Decreased signaling through the IGF-1 receptor as a result of decreased caloric intake, or mutations in the receptor, result in prolonged life span in *C. elegans*. The signals downstream of the IGF-1 receptor involve a number of kinases and may lead to the silencing of particular genes, thus promoting aging. Analyses of humans with premature aging are also establishing the fundamental concept that aging is not a random process but is regulated by specific genes, receptors, and signals.[88]

Accumulation of Metabolic and Genetic Damage. In addition to the importance of timing and a genetic clock, cellular life span may also be determined by the balance between cellular damage resulting from *metabolic events* occurring within the cell and counteracting molecular responses that can repair the damage. Smaller animals have generally shorter life spans and faster metabolic rates, suggesting that the life span of a species is limited by fixed total metabolic consumption over a lifetime.[89] One group of products of normal metabolism are reactive oxygen species. As we have seen, these byproducts of oxidative phosphorylation cause covalent modifications of proteins, lipids, and nucleic acids. The amount of oxidative damage, which increases as an organism ages, may be an important component of senescence, and the accumulation of lipofuscin in aging cells is seen as the telltale sign of such damage. Consistent with this proposal are the following observations: (1) variation in the longevity among different species is inversely correlated with the rates of mitochondrial generation of superoxide anion radical, and (2) overexpression of the antioxidative enzymes superoxide dismutase (SOD) and catalase extends life span in transgenic forms of *Drosophila*. Thus, part of the mechanism that times aging may be the cumulative damage that is generated by toxic byproducts of metabolism, such as oxygen radicals. Increased oxidative damage could result from repeated environmental exposure to such influences as ionizing radiation, progressive reduction of antioxidant defense mechanisms (e.g., vitamin E, glutathione peroxidase), or both.

A number of protective responses counterbalance progressive damage in cells, and an important one is the recognition and repair of damaged DNA.[90] Although most DNA damage is repaired by endogenous DNA repair enzymes, some persists and accumulates as cells age. Several lines of evidence point to the importance of DNA repair in the aging process. Patients with *Werner syndrome* show premature aging, and the defective gene product is a DNA helicase — a protein involved in DNA replication and repair and other functions requiring DNA unwinding.[91] A defect in this enzyme causes rapid accumulation of chromosomal damage that mimics the injury that normally accumulates during cellular aging. Genetic instability in somatic cells is also characteristic of other disorders in which patients display some of the manifestations of aging at an increased rate, such as *ataxia-telangiectasia*, in which the mutated gene encodes a protein involved in repairing double strand breaks in DNA (Chapter 7). Studies of mutants of budding yeast and *C. elegans* show that life span is increased if responses to DNA damage are enhanced. Thus, the balance between cumulative metabolic damage and the response to that damage could determine the rate at which we age. In this scenario, aging can be delayed by decreasing the accumulation of damage or by increasing the response to that damage.

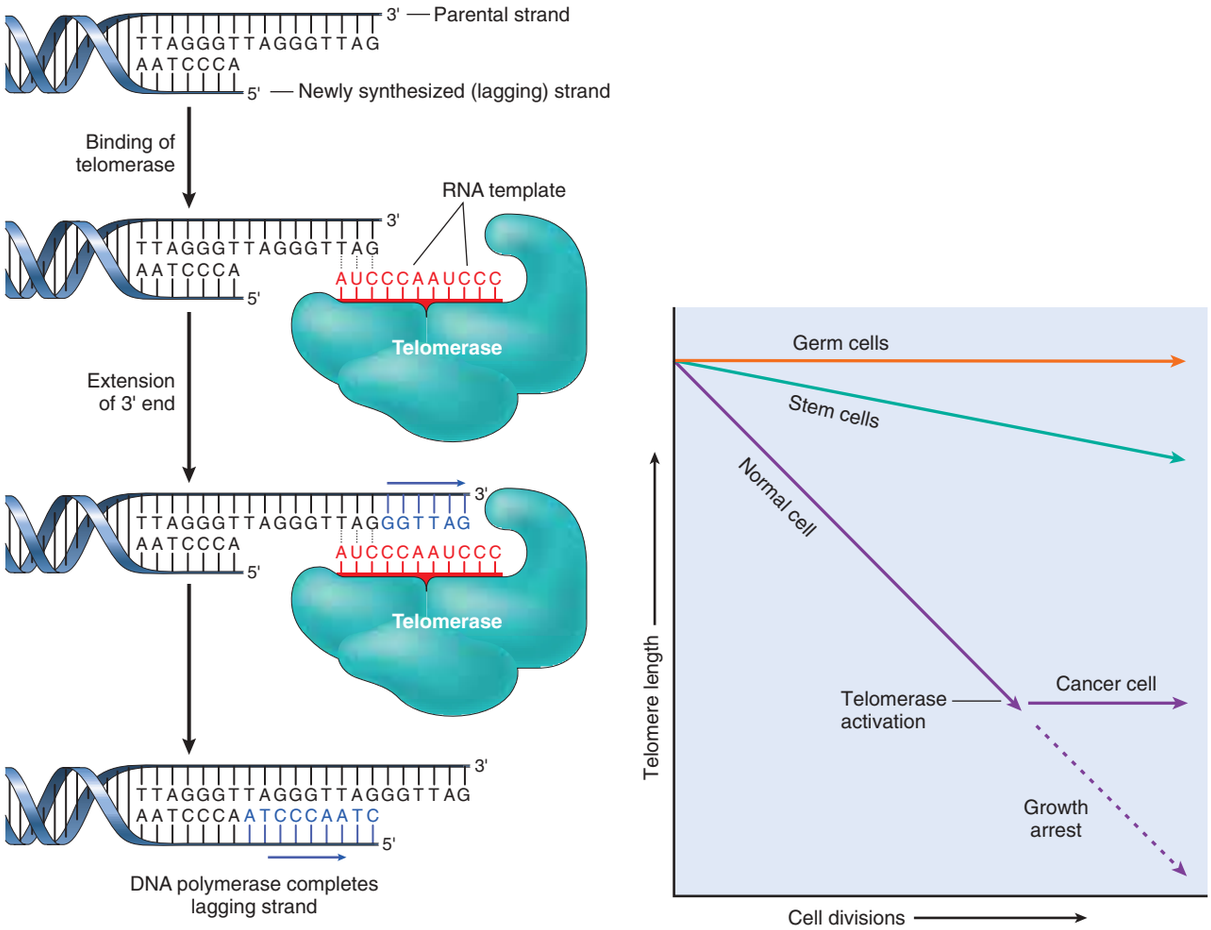


FIGURE 1–45 The role of telomeres and telomerase in replicative senescence of cells. *A,* Telomerase directs RNA template-dependent DNA synthesis, in which nucleotides are added to one strand at the end of a chromosome. The lagging strand is presumably filled in by DNA polymerase α. The RNA sequence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

Not only damaged DNA but damaged cellular organelles also accumulate as cells age. In part, this may be the result of declining function of the proteasome, the proteolytic machine that serves to eliminate abnormal and unwanted intracellular proteins.[92]

In conclusion, 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; to the pathologic alterations in cell organelles; and to the less ominous forms of intracellular accumulations, including pigmentations. 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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