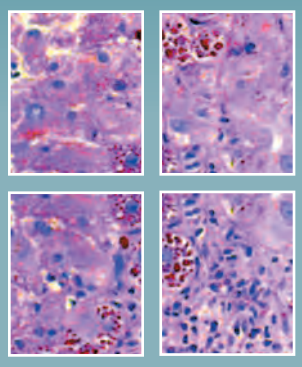

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Cell Injury

David S. Strayer
Emanuel Rubin

Pathology *is basically the study of structural and functional abnormalities that are expressed as diseases of organs and systems.* Classic theories of disease attributed disease to imbalances or noxious effects of humors on specific organs. In the 19th century, Rudolf Virchow, often referred to as the father of modern pathology, proposed that injury to the smallest living unit of the body, the cell, is the basis of all disease. To this day, clinical and experimental pathology remain rooted in this concept.

Teleology—the study of design or purpose in nature—has long since been discredited as part of scientific investigation. Although facts can only be established by observation, to appreciate the mechanisms of injury to the cell, teleologic thinking can be useful in framing questions. As an analogy, it would be impossible to understand a chess-playing computer without an understanding of the goals of chess and prior knowledge that a particular computer is programmed to play it: it would be futile to search for the sources of defects in the specific program or overall operating system without an appreciation of the goals of the device. In this sense, it is helpful to understand the problems with which the cell is confronted and the strategies that have evolved to cope with them.

A living cell must maintain the ability to produce energy. Thus, the most pressing need for any free living cell, prokaryotic or eukaryotic, is to establish a structural and functional barrier between its internal milieu and a hostile environment. The plasma membrane serves this purpose in several ways:

- It maintains a constant internal ionic composition against very large chemical gradients between the interior and exterior compartments.
- It selectively admits some molecules while excluding or extruding others.
- It provides a structural envelope to contain the informational, synthetic, and catabolic constituents of the cell.
- It provides an environment to house signal transduction molecules that mediate communication between the external and internal milieus.

At the same time, to survive, a cell must be able to adapt to adverse environmental conditions, such as changes in temperature, solute concentrations, or oxygen supply; the presence of noxious agents; and so on. The evolution of multicellular organisms eased the hazardous lot of individual cells by establishing a controlled extracellular environment in which temperature, oxygenation, ionic content, and nutrient supply are relatively constant. It also permitted the luxury of cell differentiation for such widely divergent functions as energy storage (liver cell glycogen and adipocytes), communication (neurons), contractile activity

(heart muscle), synthesis of proteins or peptides for export (liver, pancreas, and endocrine cells), absorption (intestine), and defense from foreign invaders (polymorphonuclear leukocytes, lymphocytes, and macrophages).

Cells encounter many stresses as a result of changes in their internal and external environments. *Patterns of response to such stresses is the cellular basis of disease.* If an injury exceeds the adaptive capacity of the cell, the cell dies. A cell exposed to persistent sublethal injury has limited available responses, the expression of which we interpret as evidence of cell injury. In general, mammalian cells adapt to injury by conserving resources: decreasing or ceasing differentiated functions and focusing exclusively on its own survival. *From this perspective, pathology is the study of cell injury and the expression of a cell's preexisting capacity to adapt to such injury.* Such an orientation leaves little room for the concept of parallel—normal and pathologic—biologies.

Reactions to Persistent Stress and Cell Injury

Persistent stress often leads to chronic cell injury. In general, permanent organ injury is associated with the death of individual cells. By contrast, the cellular response to persistent sublethal injury, whether chemical or physical, reflects adaptation of the cell to a hostile environment. Again, these changes are, for the most part, reversible on discontinuation of the stress. In response to persistent stress, a cell dies or adapts. It is thus our view that at the cellular level it is more appropriate to speak of chronic adaptation than of chronic injury. The major adaptive responses are atrophy, hypertrophy, hyperplasia, metaplasia, dysplasia, and intracellular storage. In addition, certain forms of neoplasia may follow adaptive responses.

Proteasomes are Key Participants in Cell Homeostasis, Response to Stress, and Adaptation to Altered Extracellular Environment

Cellular responses to alterations in their milieu were once studied exclusively by analyzing changes in gene expression and protein production. The issue of protein degradation was either ignored or relegated to the nonspecific proteolytic activities of lysosomes. However it has become clear that cellular homeostasis requires mechanisms that allow the cell to destroy certain proteins selectively. Although there is evidence that more than one such pathway may exist, the best understood mechanism by which cells target specific proteins for elimination is the ubiquitin (Ub)-proteasomal apparatus.

Proteasomes

There are two different types of these cellular organelles, 20S and 26S. The degradative unit is the 20S core, to which two additional 19S "caps" may be attached to make a 26S proteasome. There are at least 32 different proteins in the 2.5 MDa proteasomal complex. They are arrayed, as shown in Fig. 1-1, with one 19S subunit at either end of the barrel-like 20S degradative center.

Proteins targeted for destruction are modified as described below, and recognized by one 19S subunit. They are then degraded in an adenosine triphosphate (ATP)-requiring process, by the 20S subunit. The products of this process are peptides that are 3 to 25 amino acids in size, which are released through the lower 19S subunit.

The importance of this structure is underscored by the fact that it may comprise up to 1% of the total protein of the cell. Proteasomes are evolutionarily highly conserved, and are described in all eukaryotic cells. Mutation in key proteins, leading to interference with normal proteasomal function, are lethal.

The 20S proteasomes are important in degradation of oxidized proteins (see below). In 26S proteasomes, ubiquitinated proteins are degraded.

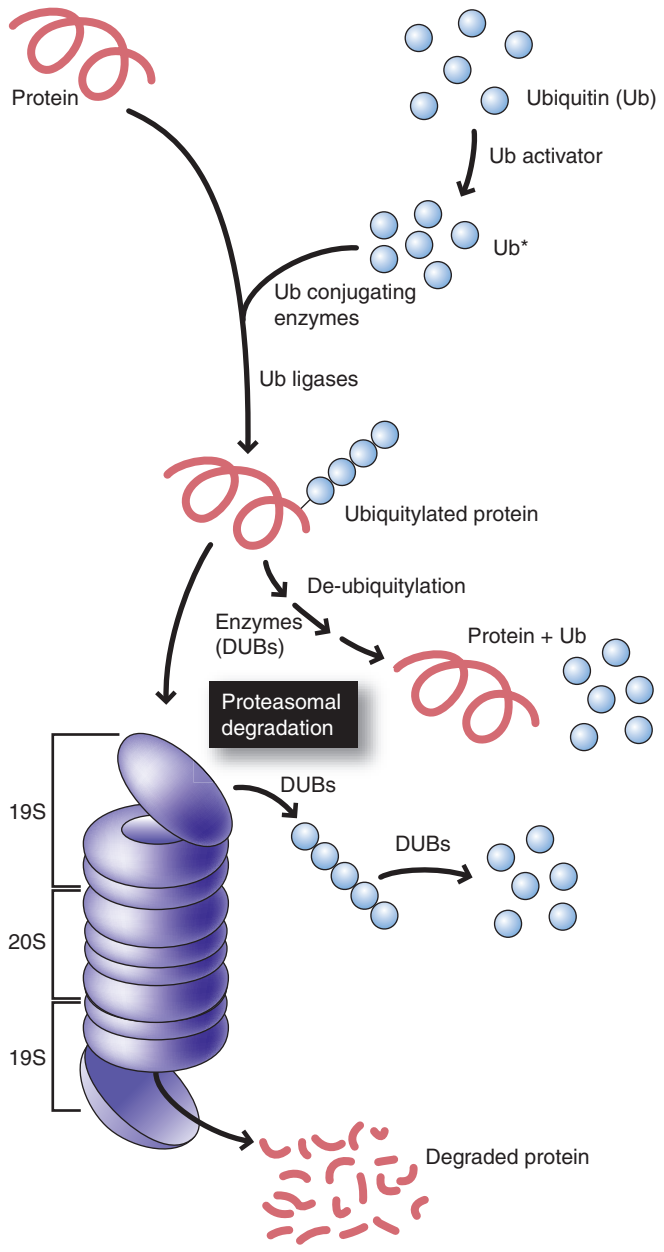


FIGURE 1-1. **Ubiquitin-proteasome pathways.** The mechanisms by which ubiquitin (Ub) targets proteins for specific elimination in proteasomes are shown here. Ub is activated (Ub*) by E1 ubiquitin activating enzyme, then transferred to an E2 (ubiquitin conjugating enzyme). The E2-Ub* complex interacts with an E3 (ubiquitin ligase) to bind a particular protein. The process may be repeated multiple times to append a chain of Ub moieties. These complexes may be deubiquitinated by de-ubiquitinating enzymes (DUBs). If degradation is to proceed, 26S proteasomes recognize the poly-Ub-conjugated protein via their 19S subunit and degrade it into oligopeptides. In the process, Ub moieties are returned to the cell pool of ubiquitin monomers.

Ubiquitin and Ubiquitination

Proteins to be degraded are flagged by attaching small chains of ubiquitin molecules to them. Ub is a 76-amino acid protein that is almost identical in yeast as in humans. It is the key to selective protein elimination: it is conjugated to proteins as a flag to identify those proteins to be destroyed. The process of attaching Ub to proteins is called ubiquitination.

A cascade of enzymes is involved (see Fig. 1-1). Ub activating enzyme, E1, binds Ub then transfers it to one of dozens of Ub conjugating enzymes (E2). These bind one of over 500 Ub ligating enzymes (E3), which add the Ub to an ϵ-amino group of a lysine on the doomed protein. In the multiple cycles of this reaction, subsequent Ub moieties are added to the original, forming a polybuiquitin chain (at least 4 Ubs). The specificity of the process resides in the combinations of E2 and E3 enzymes. Proteins to be degraded have specific structures called degrons, that are recognized by E2-E3 combinations. Degrons are two-part structures with a recognition unit and the site of Ub conjugation.

The system is complex, however. First there are de-ubiquitinating enzymes (DUBs) which can reverse the process. As well, addition of only one or two Ub moieties may occur as part of other cellular functions. Such ubiquitination is important in cell membrane budding, vesicular transport, and protein sorting within cellular compartments.

Further, some modifications of proteins may protect them from ubiquitination. When the tumor suppressor protein, p53, is phosphorylated in response to DNA damage, it is protected from Ub-mediated degradation.

Ubiquitin-like Proteins

There are a number of proteins that resemble, but are structurally distinct from, Ub, and that subserve somewhat different functions. Sentrin/SUMO may be added to proteins similarly to Ub, but is not known to be involved with protein degradation. NEDD8 is a closer homolog of Ub. It may participate in forming some E3 complexes and may substitute for Ub in poly-Ub chains.

How Ubiquitination Matters

The importance of ubiquitination and specific protein elimination is fundamental to cellular adaptation to stress and injury, as the following sections will show. It is also involved in disease processes. Defective ubiquitination may play a role in several important neurodegenerative diseases. Mutations in parkin, a ubiquitin ligase, and a combined E3-DUB enzyme, are implicated in two hereditary forms of Parkinson disease. Manipulation of ubiquitination may be important in tumor development. Thus, human papilloma virus strains that are associated with human cervical cancer (see Chapters 5, 18) produce E6 protein, which inactivates the p53 tumor suppressor. This inactivation is implicated in the genesis of cervical cancer. E6 accomplishes this by binding an E3 (ubiquitin ligase), thereby facilitating its association with p53, leading to increased p53 ubiquitination and accelerating p53 degradation. Finally, there is increasing evidence suggesting that impaired ubiquitination may be involved in some cellular degenerative changes that occur in aging and in some storage diseases.

Ubiquitination also plays a role in gene expression. Nuclear factor-κB (NFκB) is an important transcriptional activator. The inhibitor of NFκB, called IκB, is degraded by ubiquitination. This step appears to be important in NFκB-mediated gene expression since proteasome inhibition greatly decreases NFκB-induced transcriptional activation.

Other Specific Protein Degradation Pathways

There are other mechanisms by which cells may selectively eliminate particular proteins. For example, in chaperone-mediated autophagy (CMA) a chaperone related to heat shock protein-70 (called hsc70) binds particular sequences in proteins and directs them to lysosomes for destruction. CMA is activated as an adaptive response in some stress situations, such as starvation. Defects in this pathway occur during aging and have been related to some lysosomal storage diseases, but the extent to which CMA mechanisms may be involved in other disease processes remains conjectural.

Atrophy Is an Adaptation to Diminished Need or Resources for a Cell's Activities

Clinically, atrophy is often noted as decreased size or function of an organ. It may occur under both pathologic and physiologic circumstances. Thus, atrophy may result from disuse of skeletal muscle or from loss of trophic signals as part of normal aging. Atrophy may be thought of as an adaptive response whereby a cell accommodates to changes in its environment, all the while remaining viable.

One must distinguish atrophy of an organ from cellular atrophy. Reduction in an organ's size may reflect reversible cell atrophy or irreversible loss of cells. For example, atrophy of the brain in Alzheimer disease is secondary to extensive cell death; the size of the organ cannot be restored (Fig. 1-2). Atrophy occurs under a variety of conditions outlined below.

Reduced Functional Demand

The most common form of atrophy follows reduced functional demand. For example, after immobilization of a limb in a cast as treatment for a bone fracture or after prolonged bed rest, the limb's muscle cells atrophy and muscular strength is reduced. When normal activity resumes, the muscle's size and function return.

Inadequate Supply of Oxygen

Interference with blood supply to tissues is called **ischemia**. Total cessation of oxygen perfusion of tissues results in cell death. However, when oxygen deprivation is insufficient to kill cells,

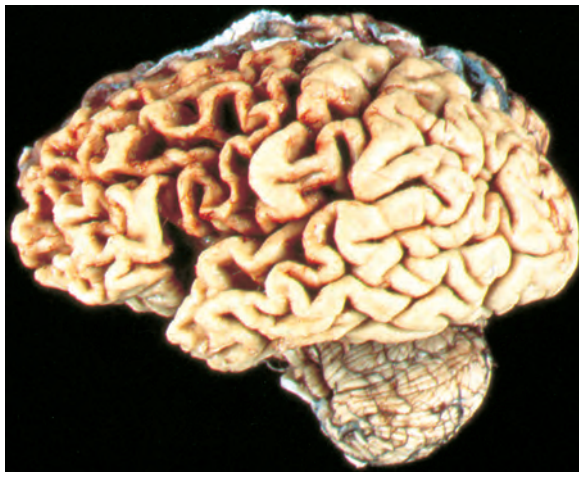


FIGURE 1-2. **Atrophy of the brain.** Marked atrophy of the frontal lobe is noted in this photograph of the brain. The gyri are thinned and the sulci conspicuously widened.

resulting partial ischemia is often compatible with cell viability. Under such circumstances, cell atrophy is common. It is frequently seen around the inadequately perfused margins of ischemic necrosis (infarcts) in the heart, brain, and kidneys following vascular occlusion in these organs.

Insufficient Nutrients

Starvation or inadequate nutrition associated with chronic disease leads to cell atrophy, particularly in skeletal muscle. It is striking that reduction in mass is particularly prominent in cells that are not vital to the survival of the organism. One cannot dismiss the possibility that a portion of the cell atrophy attributed to partial ischemia reflects a lack of nutrients.

Interruption of Trophic Signals

The functions of many cells depend on signals transmitted by chemical mediators. The endocrine system and neuromuscular transmission are the best examples. The actions of hormones or, for skeletal muscle, synaptic transmission, place functional demands on cells. These can be eliminated by removing the source of the signal, e.g., via ablation of an endocrine gland or denervation. If the anterior pituitary is surgically resected, loss of thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH, also termed corticotropin), and follicle-stimulating hormone (FSH) results in atrophy of the thyroid, adrenal cortex, and ovaries, respectively. Atrophy secondary to endocrine insufficiency is not restricted to pathologic conditions: the endometrium atrophies when estrogen levels decrease after menopause (Fig. 1-3). Even cancer cells may undergo atrophy, to some extent, by hormonal deprivation. Androgen-dependent prostatic cancer partially regresses after administration of testosterone antagonists. Certain types of thyroid cancer may stop growing if one inhibits pituitary TSH secretion by administering thyroxine. If neurologic damage, e.g., from poliomyelitis or traumatic spinal cord injury, leads to denervation of muscle, the neuromuscular transmission necessary for muscle tone is lost and affected muscles atrophy.

Persistent Cell Injury

Persistent cell injury is most commonly caused by chronic inflammation associated with prolonged viral or bacterial infections. Chronic inflammation may be seen in a variety of other circumstances, including immunologic and granulomatous disorders. A good example is the atrophy of the gastric mucosa that occurs in association with chronic gastritis (see Chapter 13). Similarly, villous atrophy of the small intestinal mucosa follows the chronic inflammation of celiac disease. Even physical injury, such as prolonged pressure in inappropriate locations, produces atrophy. Heart failure leads to increased pressure in sinusoids of the liver because the heart cannot pump the venous return from that organ efficiently. Accordingly, the cells in the center of the liver lobule, which are exposed to the greatest pressure, become atrophic.

Aging

One of the hallmarks of aging, particularly in nonreplicating cells such as those of the brain and heart, is cell atrophy. The size of all parenchymal organs decreases with age. The size of the brain is invariably decreased, and in the very old the size of the heart may be so diminished that the term **senile atrophy** has been used.

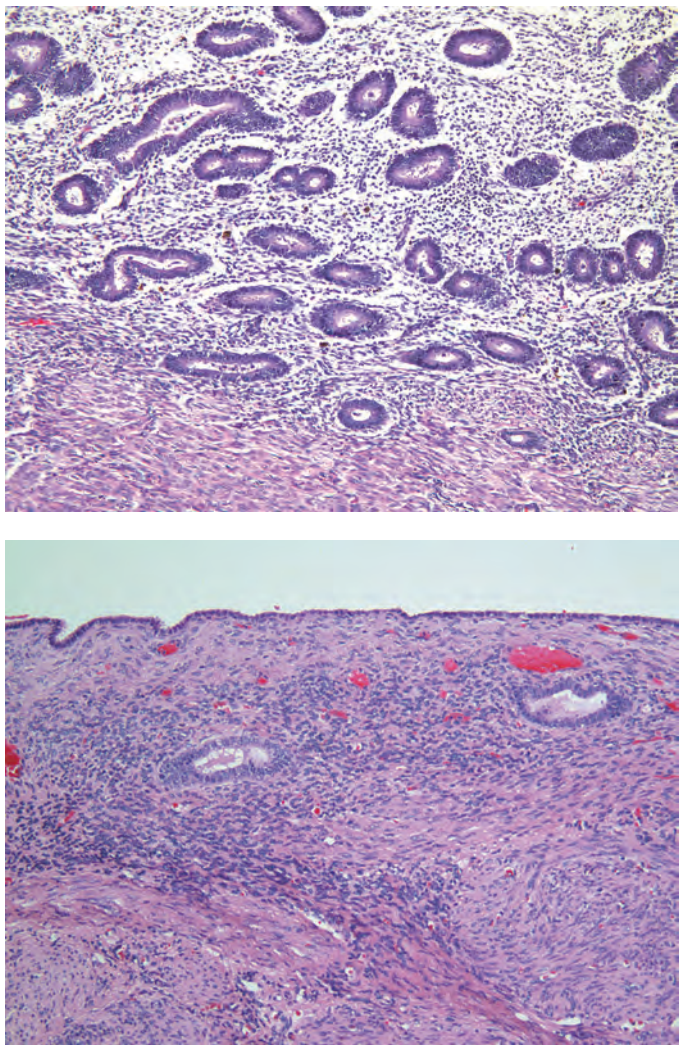


FIGURE 1-3. **Proliferative endometrium. A.** A section of the uterus from a woman of reproductive age reveals a thick endometrium composed of proliferative glands in an abundant stroma. **B.** The endometrium of a 75-year-old woman (shown at the same magnification) is thin and contains only a few atrophic and cystic glands.

Atrophy Is an Active Process

Atrophy involves changes both in production and destruction of cellular constituents. In its most basic sense, atrophy is a cell's reversible restructuring of its activities to facilitate its own survival and adapt to conditions of diminished use.

This process has been most extensively studied in skeletal muscle, which responds rapidly to changes in demand for contractile force. Since striated muscle cells are terminally differentiated, they allow study of atrophy and hypertrophy (see below) without the confounding influence of cell proliferation. This system is used here to illustrate the mechanisms of cell atrophy. When the need for contraction decreases ("unloading," see below), cells institute several selective adaptive mechanisms.

- **Protein synthesis.** Shortly after unloading, protein synthesis decreases. This effect appears mainly to reflect decreased protein elongation at the ribosomes. In the immediate term, RNA levels for contractile proteins are not altered.

- **Protein degradation.** Ubiquitin-related specific protein degradation pathways are activated. These mediate the atrophic response in several ways. They lead to decreases in specific contractile proteins. Ubiquitination of specific transcription factors (e.g., myoD) that increase expression of contractile protein genes further steers the cells adaptive atrophy. The increase in specific protein elimination is transient and if the atrophied state is maintained, the cells reach a new steady state in which mass remains decreased and rates of protein synthesis and degradation re in alignment.
- **Gene expression.** There are selective decreases in transcription of genes for, among other things, contractile activities. Transcription of some genes is upregulated, particularly genes encoding specific E3 enzymes (Ub-ligases) involved in ubiquitinating contractile proteins. Proteasome subunit synthesis is also increased.
- **Signaling.** More complex, and less well understood, changes in intracellular signaling occur. Atrophy-related increased Ub activity causes ubiquitination and elimination of inhibitors of NFκB. This in turn leads to increased NFκB activity. The ramifications of this aspect of the atrophic response are not yet clear.
- **Energy utilization.** A selective decrease in use of free fatty acids as an energy source has been noted during the response to unloading.

Atrophy is thus an active adaptive response, not a passive shutdown of cellular processes, to which the Ub pathway and specific protein elimination by proteasomes are fundamental. The atrophic response is reversible: restoring the pre-atrophy environment allows cells to return to their pre-atrophy functionality.

Hypertrophy Is an Increase in Cell Size and Functional Capacity

When trophic signals or functional demand increase, adaptive changes to satisfy these needs lead to increased cellular size (hypertrophy) and, in some cases, increased cellular number (hyperplasia, see below).

In organs made of terminally differentiated cells (e.g., heart, skeletal muscle), such adaptive responses are accomplished solely by increased cell size (Fig. 1-4). In other organs (e.g., kidney, thyroid) cell numbers and cell size may both increase. This section deals with the mechanisms and consequences when cells enlarge to meet increased functional demands on them. Some mechanisms involved in the hypertrophic response are cell type-specific and some are more general. Also, cells that can divide use some of the same mechanisms to stimulate mitosis that non-dividing cells use to increase their size.

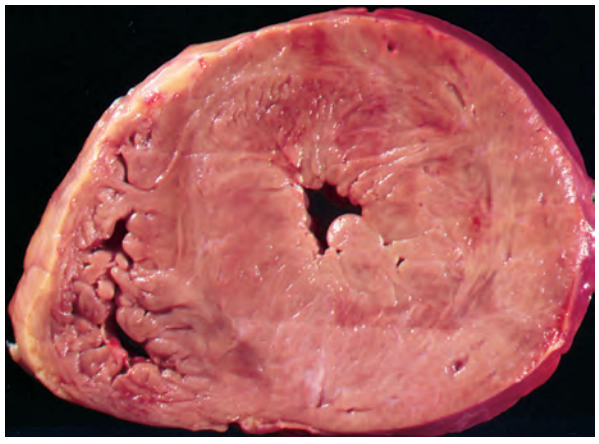


FIGURE 1-4. **Myocardial hypertrophy.** Cross-section of the heart of a patient with long-standing hypertension shows pronounced, concentric left ventricular hypertrophy.

Mechanisms of Cellular Hypertrophy

Whether the stimulus to enlarge is increased work load or increased endocrine or neuroendocrine mediators, there are certain processes that usually contribute to generating cellular hypertrophy.

Cellular Remodeling in Hypertrophy

When cells are stimulated to increase mass one of the first activities is increased proteasomal degradation of selected cellular macromolecular constituents. Thus, proteins that do not contribute to the specific functional need to be expanded are degraded, even as production of proteins that do so contribute increases.

Signaling Mechanisms in Hypertrophy

Although signals that elicit hypertrophic responses vary, depending on the cell type and circumstances, the example of skeletal muscle hypertrophy illustrates some critical general principles. Thus, many types of signaling may lead to cell hypertrophy:

- **Growth factor stimulation.** Each tissue responds to different signals. In many cases certain growth factors appear to be key initiators of hypertrophy. Thus, insulin-like growth factor-I (IGF-I) is increased in load-induced muscle hypertrophy and in experimental settings may elicit hypertrophy even if load does not increase.
- **Neuroendocrine stimulation.** In some tissues, adrenergic or noradrenergic signaling may be important in initiating and/or facilitating hypertrophy.
- **Ion channels.** Ion fluxes may activate adaptation to increased demand. Calcium channel activity, in particular, may stimulate a host of downstream enzymes (e.g., calcineurin) to produce hypertrophy.
- **Other chemical mediators.** Depending on the particular tissue, such factors as nitric oxide (NO•), angiotensin II, and bradykinin may support cell hypertrophic responses.
- **Oxygen supply.** Clearly increased functional demand on cells requires increased energy supply. Angiogenesis is stimulated when a tissue oxygen deficit is sensed and may be an indispensable component of adaptive hypertrophy.
- **Hypertrophy antagonists.** Just as some mechanisms foster cellular hypertrophy, others inhibit it. Atrial and B-type natriuretic factors, high concentrations of NO• and many other factors either brake or prevent cell adaptation by hypertrophy.

Effector Pathways

Whatever mechanisms initiate the signaling process to stimulate hypertrophy, there are a limited number of downstream pathways that mediate the effects of such signaling.

- **Increased protein degradation.** This was discussed above.
- **Increased protein translation.** Shortly after a prohypertrophic signal is received, production of certain proteins increases. This occurs very quickly and without changes in

RNA levels, via increased translational efficiency. Activities of translational initiators and elongation factors are often stimulated in the early phases of hypertrophy, to provide a rapid increase in the proteins needed to meet the increased functional demand.

- **Increased gene expression.** Concentrations of key proteins are also increased by upregulation of transcription of genes that encode those proteins. Many of the signaling pathways activated by cytokines, neurotransmitters, and so forth, activate an array of transcription factors. Thus, for example, calcineurin dephosphorylates the misnamed nuclear factor of activated T lymphocytes (NFAT), facilitating NFAT movement to the nucleus by unmasking its nuclear localization signal and leading to increased transcription of target genes. Hypertrophy may involve increased transcription of genes encoding growth-promoting transcription factors, such as Fos and Myc.
- **Survival.** Among the functions activated during hypertrophy is inhibition of cell death. Thus, stimulation of several receptors increases the activity of several kinases (Akt, PI3K and others) (see below). These in turn promote cell survival, largely by inhibiting programmed cell death (apoptosis, see below).
- **Ancillary functions.** In some situations hypertrophy may involve changes in a cell's relation to its environment, such as remodelling extracellular matrix. It has been suggested that skeletal muscle hypertrophy may include recruiting perimuscular satellite cells to fuse with the muscle syncytia, providing additional nuclei to the expanding muscle.

In sum, the diverse stimuli that lead to cell hypertrophy stimulate adaptive cellular remodeling, increase protein production, facilitate cell function, and promote cell survival.

Hyperplasia Is an Increase in the Number of Cells in an Organ or Tissue

Hypertrophy and hyperplasia are not mutually exclusive and often occur concurrently. The specific stimuli that induce hyperplasia and the mechanisms by which they act vary greatly from one tissue and cell type to the next. Diverse agents that elicit hyperplastic responses in one tissue may do so by entirely different mechanisms. Basically, however it is evoked, hyperplasia involves stimulating resting (G0) cells to enter the cell cycle (G1) and then to multiply. This may be a response to altered endocrine milieu, increased functional demand or chronic injury.

Hormonal Stimulation

Changes in hormone concentrations can elicit proliferation of responsive cells. These changes may reflect developmental, pharmacologic, or pathologic influences. Thus, the normal increase in estrogens at puberty or early in the menstrual cycle leads to increased numbers of endometrial and uterine stromal cells. Estrogen administration to postmenopausal women has the same effect. Enlargement of the male breast, called gynecomastia, may occur in liver failure when the liver's inability to metabolize endogenous estrogens leads to their accumulation, or in men given estrogens as therapy for prostate cancer. Ectopic hormone production, e.g., erythropoietin by renal tumors, may lead to hyperplasia (in this case, of erythrocytes in the bone marrow), and may be a tumor's first presenting symptom.

Increased Functional Demand

Hyperplasia, like hypertrophy, may be a response to increased physiologic demand. At high altitudes low atmospheric oxygen content leads to compensatory hyperplasia of erythrocyte precursors in the bone marrow and increased erythrocytes in the blood (secondary polycythemia) (Fig. 1-5). In this fashion, increased numbers of cells compensate for the decreased oxygen carried by each erythrocyte. The number of erythrocytes promptly falls to normal on return to sea level. Similarly, chronic blood loss, as in excessive menstrual bleeding, also causes hyperplasia of erythrocytic elements.

Immune responsiveness to many antigens may lead to lymphoid hyperplasia, e.g., the enlarged tonsils and swollen lymph nodes that occur with streptococcal pharyngitis. The hypocalcemia that occurs in chronic renal failure leads to increased demand for parathyroid hormone in order to increase blood calcium. The result is hyperplasia of the parathyroid glands.

Chronic Injury

Persistent injury may lead to hyperplasia. Long-standing inflammation or chronic physical or chemical injury often results in a hyperplastic response. For instance, pressure from ill-fitting shoes causes hyperplasia of the skin of the foot, so-called corns or calluses. If one considers that a key function of the skin is to protect underlying structures, such hyperplasia results in thickening of the skin and enhances the skin's functional capacity. Chronic inflammation of the bladder (chronic cystitis) often causes hyperplasia of the bladder epithelium, visible as white plaques on the bladder lining.

Inappropriate hyperplasia can itself be harmful—witness the unpleasant consequences of psoriasis, which is characterized by conspicuous hyperplasia of the skin (see Fig. 1-5D). Excessive estrogen stimulation, whether from endogenous or exogenous sources, may lead to endometrial hyperplasia.

The cellular and molecular mechanisms responsible for hyperplastic responses clearly relate to control of cell proliferation. These topics are discussed in Chapters 3 and 5, and under the heading of liver regeneration in Chapter 14.

Metaplasia Is Conversion of One Differentiated Cell Type to Another

Metaplasia is usually an adaptive response to chronic, persistent injury. That is, a tissue will assume the phenotype that provides it the best protection from the insult. Most commonly, glandular epithelium is replaced by squamous epithelium. Columnar or cuboidal lining cells may be committed to mucus production, but may not be adequately resistant to the effects of chronic irritation or a pernicious chemical. For example, prolonged exposure of the bronchial epithelium to tobacco smoke leads to squamous metaplasia. A similar response occurs in the endocervix, associated with chronic infection (Fig. 1-6). In molecular terms, metaplasia involves replacing the expression of one set of differentiation genes with another.

The process is not restricted to squamous differentiation. When highly acidic gastric contents reflux chronically into the lower esophagus, the squamous epithelium of the esophagus may be replaced by stomach-like glandular mucosa (Barrett epithelium). This can be thought of as an adaption to protect the esophagus from injury by gastric acid and pepsin, to which the normal gastric mucosa is resistant. Metaplasia may also consist of replacement of one glandular epithelium by another. In chronic gastritis, a disorder of the stomach characterized by chronic inflammation, atrophic gastric glands are replaced by cells resembling those of the small intestine. The adaptive value of this condition, known as intestinal metaplasia, is not clear. Metaplasia of transitional epithelium to glandular epithelium occurs when the bladder is chronically inflamed (cystitis glandularis).

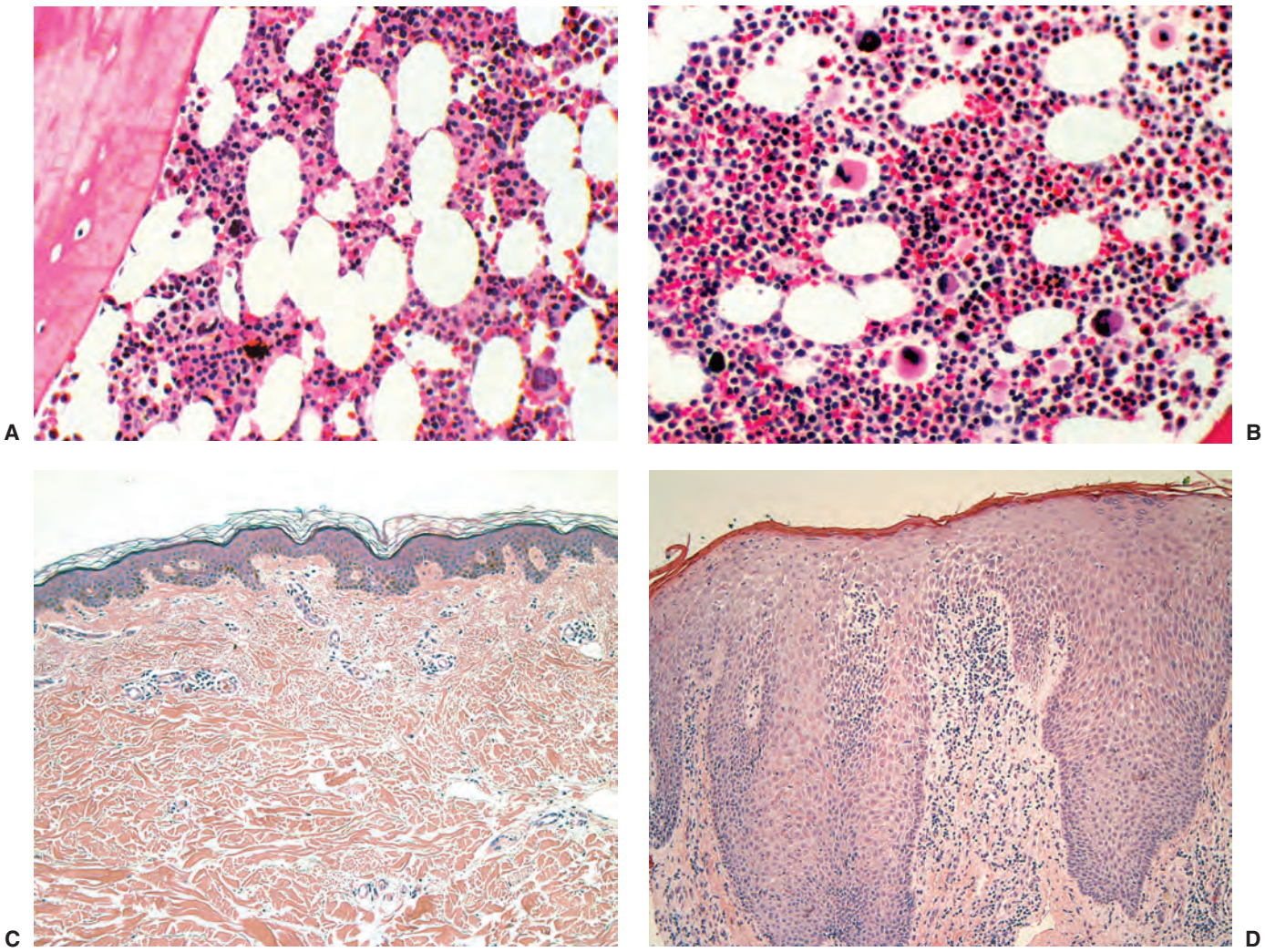


FIGURE 1-5. **Hyperplasia. A.** Normal adult bone marrow. **B.** Hyperplasia of the bone marrow. Cellularity is increased, fat is decreased. **C.** Normal epidermis. **D.** Epidermal hyperplasia in psoriasis, shown at the same magnification as in C. The epidermis is thickened, owing to an increase in the number of squamous cells.

Although this response may be thought of as adaptive, metaplasia is not necessarily innocuous. For example, squamous metaplasia may protect a bronchus from injury due to tobacco smoke, but it also impairs mucous production and ciliary clearance. Neoplastic transformation may occur in metaplastic epithelium; cancers of the lung, cervix, stomach, and bladder often arise in such areas. However, if the chronic injury stops, there is little stimulus for cells to proliferate, and the epithelium does not become cancerous.

Metaplasia is usually fully reversible. If the noxious stimulus is removed (e.g., when one stops smoking), the metaplastic epithelium eventually returns to normal.

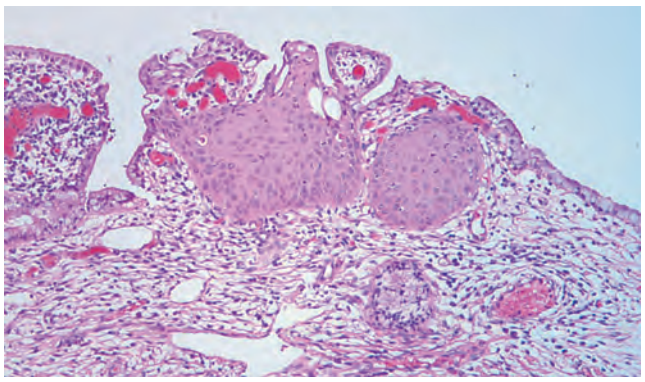


FIGURE 1-6. **Squamous metaplasia.** A section of endocervix shows the normal columnar epithelium at both margins and a focus of squamous metaplasia in the center.

Dysplasia is Disordered Growth and Maturation of the Cellular Components of a Tissue

The cells that compose an epithelium normally exhibit uniformity of size, shape, and nucleus. Moreover, they are arranged in a regular fashion, as, for example, a squamous epithelium progresses from plump basal cells to flat superficial cells. In dysplasia, this monotonous appearance is disturbed by (1) variation in cell size and shape; (2) nuclear enlargement, irregularity, and hyperchromatism; and (3) disarray in the arrangement of cells within the epithelium (Fig. 1-7). Dysplasia occurs most often in hyperplastic squamous epithelium, as seen in epidermal actinic keratosis (caused by sunlight) and in areas of squamous metaplasia, such as in the bronchus or the cervix. It is not, however, exclusive to squamous epithelium. Ulcerative

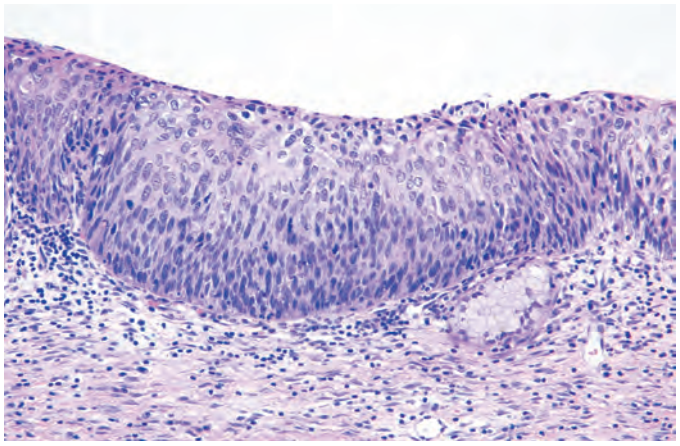


FIGURE 1-7. **Dysplasia.** The dysplastic epithelium of the uterine cervix lacks the normal polarity, and the individual cells show hyperchromatic nuclei, a larger nucleus-to-cytoplasm ratio, and a disorderly arrangement.

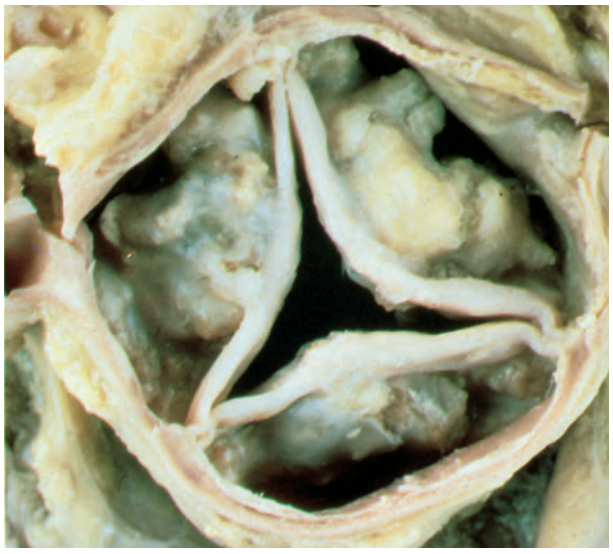


FIGURE 1-8. **Calcific aortic stenosis.** Large deposits of calcium salts are evident in the cusps and the free margins of the thickened aortic valve, as viewed from above.

colitis, an inflammatory disease of the large intestine, is often complicated by dysplastic changes in the columnar mucosal cells.

Like metaplasia, dysplasia is a response to a persistent injurious influence and will usually regress, for example, on cessation of smoking or the disappearance of human papillomavirus from the cervix. However, dysplasia shares many cytologic features with cancer, and the line between the two may be very fine indeed. For example, it may be difficult to distinguish severe dysplasia from early cancer of the cervix. *Dysplasia is a preneoplastic lesion, in that it is a necessary stage in the multistep cellular evolution to cancer.* In fact, dysplasia is included in the morphologic classifications of the stages of intraepithelial neoplasia in a variety of organs (e.g., cervix, prostate, bladder). Severe dysplasia is considered an indication for aggressive preventive therapy to cure the underlying cause, eliminate the noxious agent, or surgically remove the offending tissue.

As in the development of cancer (see Chapter 5), dysplasia results from sequential mutations in a proliferating cell population. The fidelity of DNA replication is imperfect, and occasional mutations are inevitable. When a particular mutation confers a growth or survival advantage, progeny of the affected cell will tend to predominate. In turn, their continued proliferation provides greater opportunity for additional mutations. Accumulation of such mutations progressively distances the cell from normal regulatory constraints. *Dysplasia is the morphologic expression of the disturbance in growth regulation.* However, unlike cancer cells, dysplastic cells are not entirely autonomous, and with intervention, tissue appearance may still revert to normal.

Calcification Is a Normal or Abnormal Process

The deposition of mineral salts of calcium is, of course, a normal process in the formation of bone from cartilage. As we have learned, calcium entry into dead or dying cells is usual, owing to the inability of such cells to maintain a steep calcium gradient. This cellular calcification is not ordinarily visible except as inclusions within mitochondria.

Dystrophic calcification *refers to the macroscopic deposition of calcium salts in injured tissues*. This type of calcification does not simply reflect an accumulation of calcium derived from the bodies of dead cells but rather represents an extracellular deposition of calcium from the circulation or interstitial fluid. Dystrophic calcification apparently requires the persistence of necrotic tissue; it is often visible to the naked eye and ranges from gritty, sandlike grains to firm, rock-hard material. In many locations, such as in cases of tuberculous caseous necrosis in the lung or lymph nodes, calcification has no functional consequences. However, dystrophic calcification may also occur in crucial locations, such as in the mitral or aortic valves (Fig. 1-8). In such instances, calcification leads to impeded blood flow because it produces inflexible valve leaflets and narrowed valve orifices (mitral and aortic stenosis). Dystrophic calcification in atherosclerotic coronary arteries contributes to narrowing of those vessels. Although molecules involved in physiologic calcium deposition in bone—e.g., osteopontin, osteonectin, and osteocalcin—are reported in association with dystrophic calcification, the underlying mechanisms of this process remain obscure.

Dystrophic calcification also plays a role in diagnostic radiography. Mammography is based principally on the detection of calcifications in breast cancers; congenital toxoplasmosis, an infection involving the central nervous system, is suggested by the visualization of calcification in the infant brain.

Metastatic calcification *reflects deranged calcium metabolism, in contrast to dystrophic calcification, which has its origin in cell injury*. Metastatic calcification is associated with an increased serum calcium concentration (hypercalcemia). In general, almost any disorder that increases the serum calcium level can lead to calcification in such inappropriate locations as the alveolar septa of the lung, renal tubules, and blood vessels. Calcification is seen in various disorders, including chronic renal failure, vitamin D intoxication, and hyperparathyroidism.

The formation of stones containing calcium carbonate in sites such as the gallbladder, renal pelvis, bladder, and pancreatic duct is another form of pathologic calcification. Under certain circumstances, the mineral salts precipitate from solution and crystallize about foci of organic material. Those who have suffered the agony of gallbladder or renal colic will attest to the unpleasant consequences of this type of calcification.

Hyaline Refers to Any Material That has a Reddish, Homogeneous Appearance When Stained with Hematoxylin and Eosin

The student will encounter the term **hyaline** in classic descriptions of diverse and unrelated lesions. Standard terminology includes hyaline arteriolosclerosis, alcoholic hyaline in the liver, hyaline membranes in the lung, and hyaline droplets in various cells. The various lesions called hyaline actually have nothing in common. Alcoholic hyaline is composed of cytoskeletal filaments; the hyaline found in arterioles of the kidney is derived from basement membranes; and hyaline membranes consist of plasma proteins deposited in alveoli. The term is anachronistic and of questionable value, although it is still used as a morphologic descriptor.

Mechanisms and Morphology of Cell Injury

All cells have efficient mechanisms to deal with shifts in environmental conditions. Thus, ion channels open or close, harmful chemicals are detoxified, metabolic stores such as fat or glycogen may be mobilized, and catabolic processes lead to the segregation of internal particulate materials. It is when environmental changes exceed the cell's capacity to maintain normal homeostasis that we recognize acute cell injury. If the stress is removed in time or if the cell can withstand the assault, cell injury is reversible, and complete structural and functional integrity is restored. For example, when circulation to the heart is interrupted for less than 30 minutes, all structural and functional alterations prove to be reversible. The cell can also be exposed to persistent sublethal stress, as in mechanical irritation of the skin or exposure of the bronchial mucosa to tobacco smoke. In such instances, the cell has time to adapt to reversible injury in a number of ways, each of which has its morphologic counterpart. On the other hand, if the stress is severe, irreversible injury leads to death of the cell. The precise moment at which reversible injury gives way to irreversible injury, the "point of no return," cannot be identified at present.

Hydropic Swelling Is a Reversible Increase in Cell Volume

Hydropic swelling is characterized by a large, pale cytoplasm and a normally located nucleus (Fig. 1-9). The greater volume reflects an increased water content. Hydropic swelling reflects acute, reversible cell injury and may result from such varied causes as chemical and biological toxins, viral or bacterial infections, ischemia, excessive heat or cold, and so on.

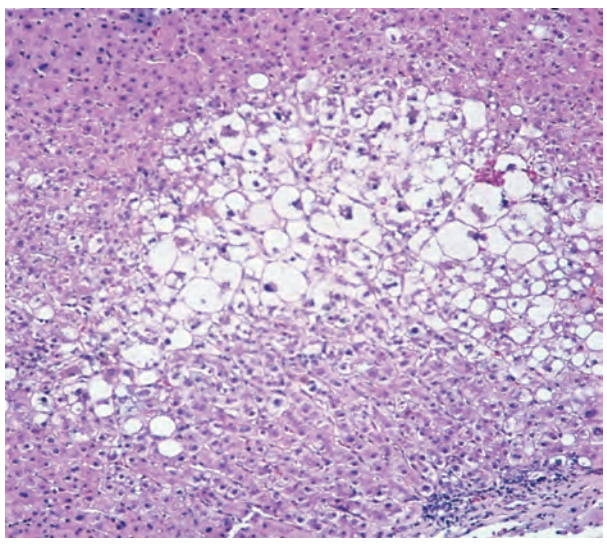
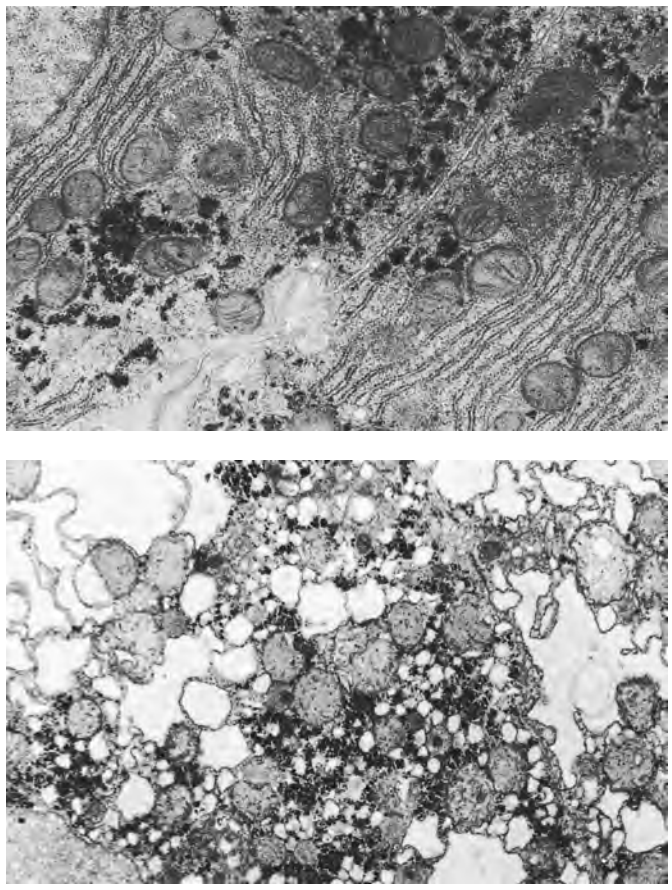


FIGURE 1-9. **Hydropic swelling.** A needle biopsy of the liver of a patient with toxic hepatic injury shows severe hydropic swelling in the centrilobular zone. The affected hepatocytes exhibit central nuclei and cytoplasm distended (ballooned) by excess fluid.



A

B

FIGURE 1-10. **Ultrastructure of hydropic swelling of a liver cell. A.** Two apposed normal hepatocytes with tightly organized, parallel arrays of rough endoplasmic reticulum. **B.** Swollen hepatocyte in which the cisternae of the endoplasmic reticulum are dilated by excess fluid.

By electron microscopy, the number of organelles is unchanged, although they appear dispersed in a larger volume. The excess fluid accumulates preferentially in the cisternae of the endoplasmic reticulum, which are conspicuously dilated, presumably because of ionic shifts into this compartment (Fig. 1-10). Hydropic swelling is entirely reversible when the cause is removed.

Hydropic swelling results from impairment of cellular volume regulation, a process that controls ionic concentrations in the cytoplasm. This regulation, particularly for sodium, involves three components: (1) the plasma membrane, (2) the plasma membrane sodium pump, and (3) the supply of ATP. The plasma membrane imposes a barrier to the flow of sodium (Na^+) down a concentration gradient into the cell and prevents a similar efflux of potassium (K^+) from the cell. The barrier to sodium is imperfect and the relative leakiness to that ion permits the passive entry of sodium into the cell. To compensate for this intrusion, the energy-dependent plasma membrane sodium pump (Na^+/K^+-ATPase), which is fueled by ATP, extrudes sodium from the cell. Injurious agents may interfere with this membrane-regulated process by (1) increasing the permeability of the plasma membrane to sodium, thereby

exceeding the capacity of the pump to extrude sodium; (2) damaging the pump directly; or (3) interfering with the synthesis of ATP, thereby depriving the pump of its fuel. In any event, the accumulation of sodium in the cell leads to an increase in water content to maintain isosmotic conditions, and the cell then swells.

Subcellular Changes Occur in Reversibly Injured Cells

- **Endoplasmic reticulum:** The cisternae of the endoplasmic reticulum are distended by fluid in hydropic swelling (see Fig. 1-10). In other forms of acute, reversible cell injury, membrane-bound polysomes may undergo disaggregation and detach from the surface of the rough endoplasmic reticulum (Fig. 1-11).
- **Mitochondria:** In some forms of acute injury, particularly ischemia, mitochondria swell (Fig. 1-12). This enlargement reflects the dissipation of the energy gradient and consequent impairment of mitochondrial volume control. Amorphous densities rich in phospholipid may appear, but these effects are fully reversible on recovery.
- **Plasma membrane:** Blebs of the plasma membrane—that is, focal extrusions of the cytoplasm—are occasionally noted. These can detach from the membrane into the external environment without the loss of cell viability.
- **Nucleus:** In the nucleus, reversible injury is reflected principally in nucleolar change. The fibrillar and granular components of the nucleolus may segregate. Alternatively, the granular component may be diminished, leaving only a fibrillar core.

These changes in cell organelles (Fig. 1-13) are reflected in functional derangements (e.g., reduced protein synthesis and impaired energy production). *After withdrawal of an acute stress that has led to reversible cell injury, by definition, the cell returns to its normal state.*

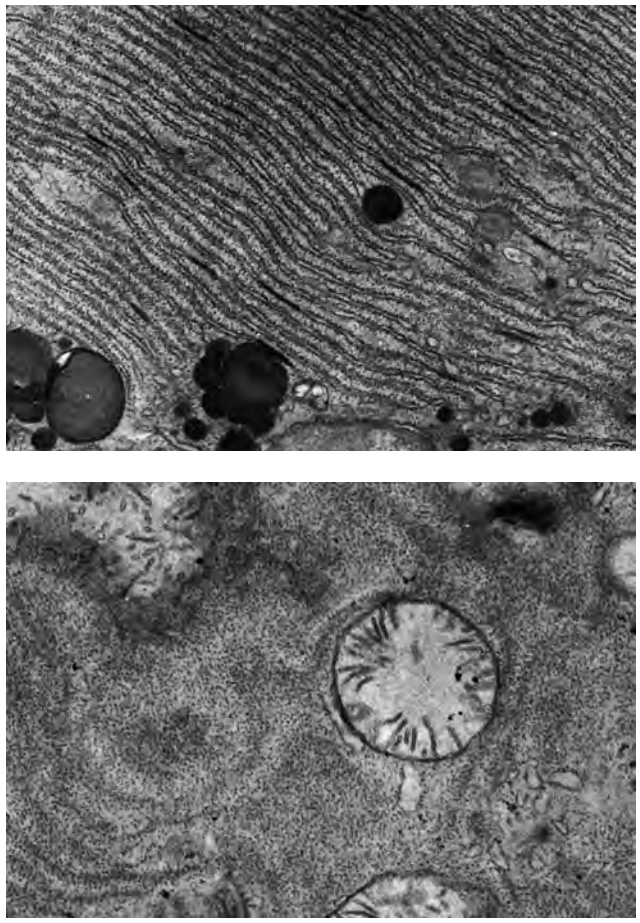


FIGURE 1-11. **Disaggregation of membrane-bound polyribosomes in acute, reversible liver injury. A.** Normal hepatocyte, in which the profiles of endoplasmic reticulum are studded with ribosomes. **B.** An injured hepatocyte, showing detachment of ribosomes from the membranes of the endoplasmic reticulum and the accumulation of free ribosomes in the cytoplasm.

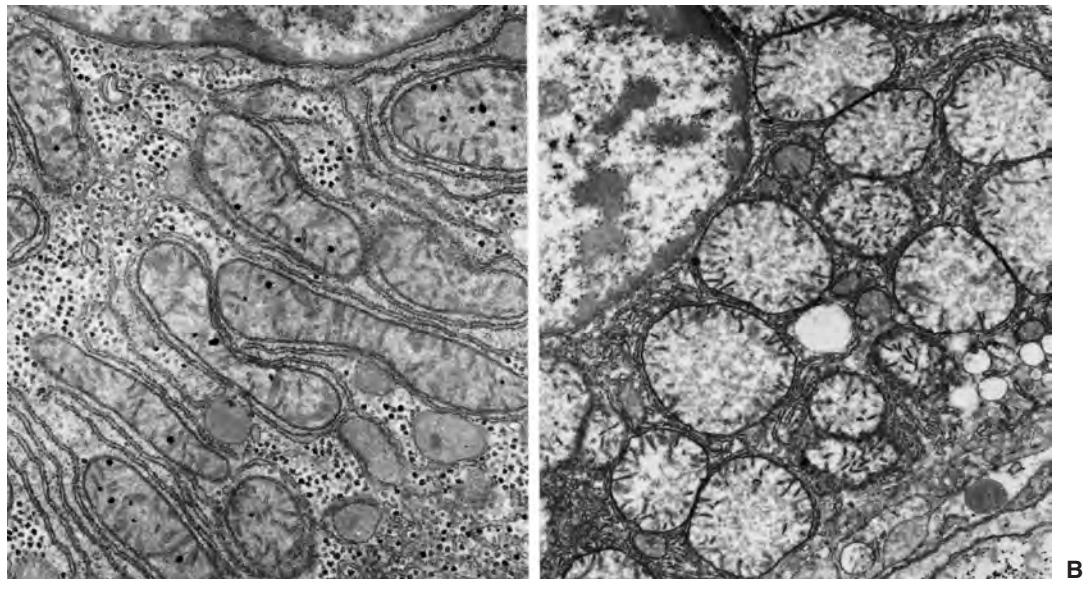


FIGURE 1-12. **Mitochondrial swelling in acute ischemic cell injury. A.** Normal mitochondria are elongated and display prominent cristae, which traverse the mitochondrial matrix. **B.** Mitochondria from an ischemic cell are swollen and round and exhibit a decreased matrix density. The cristae are less prominent than in the normal organelle.

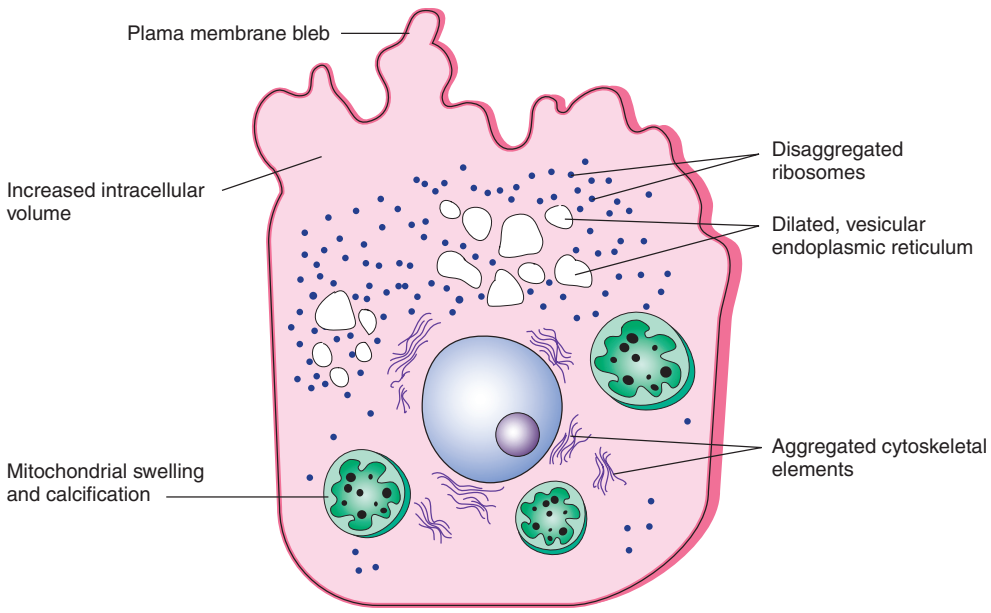


FIGURE 1-13. **Ultrastructural features of reversible cell injury.**

Ischemic Cell Injury Usually Results from Obstruction to the Flow of Blood

When tissues are deprived of oxygen, ATP cannot be produced by aerobic metabolism and is instead generated inefficiently by anaerobic metabolism. Ischemia initiates a series of chemical and pH imbalances, which are accompanied by enhanced generation of injurious free radical species. The damage produced by short periods of ischemia tends to be reversible if the circulation is restored. However, cells subjected to long episodes of ischemia become irreversibly injured and die. The mechanisms of cell damage are discussed below.

Oxidative Stress Leads to Cell Injury in Many Organs

For human life oxygen is both a blessing and a curse. Without it, life is impossible, but its metabolism can produce partially reduced oxygen species that react with virtually any molecule they reach.

Reactive Oxygen Species (ROS)

ROS have been identified as the likely cause of cell injury in many diseases (Fig. 1-14). The inflammatory process, whether acute or chronic, can cause considerable tissue destruction. In such circumstances partially reduced oxygen species produced by phagocytic cells are important mediators of cell injury. Damage to cells resulting from oxygen radicals formed by inflammatory cells has been implicated in diseases of the joints and of many organs, including the kidneys, lungs, and heart. The toxicity of many chemicals may reflect the formation of toxic oxygen species. For example, the killing of cells by ionizing radiation is most likely the result of the direct formation of hydroxyl (•OH) radicals from the radiolysis of water (H_2O). There is also evidence of a role for oxygen species in the formation of mutations during chemical carcinogenesis. Finally, oxidative damage has been implicated in biological aging (see below).

Cells also may be injured when oxygen is present at concentrations greater than normal. In the past, this occurred largely under therapeutic circumstances in which oxygen was given to patients at concentrations greater than the normal 20% of inspired air. The lungs of adults and the eyes of premature newborns were the major targets of such oxygen toxicity.

Oxygen (O_2) has a major metabolic role as the terminal acceptor for mitochondrial electron transport. Cytochrome oxidase catalyzes the four-electron reduction of O_2 to H_2O. The resultant energy is harnessed as an electrochemical potential across the mitochondrial inner membrane.

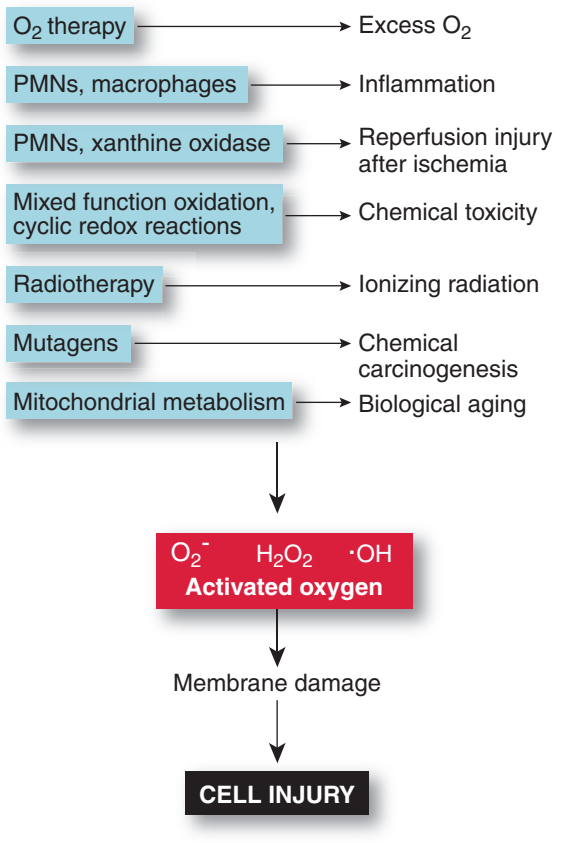


FIGURE 1-14. **The role of activated oxygen species in human disease.** H_2O_2 = hydrogen peroxide; O_2 = oxygen; O_2^- = superoxide; •OH = hydroxyl radical; PMNs = polymorphonuclear neutrophils.

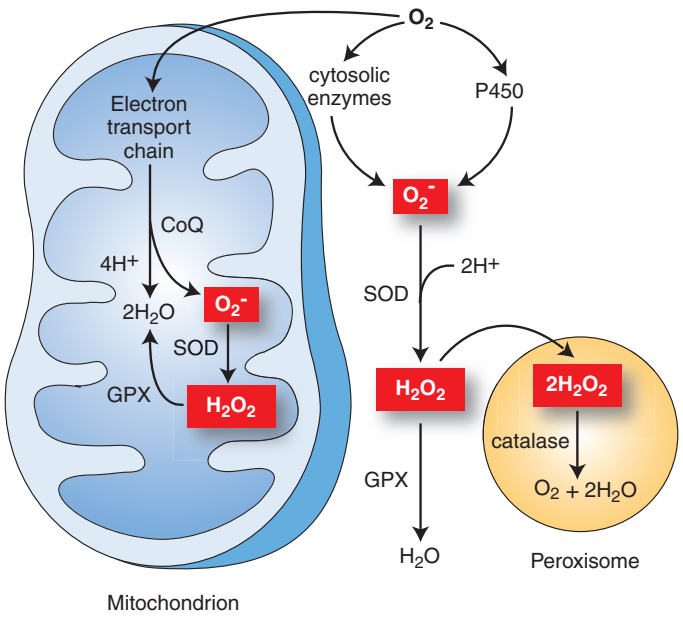


FIGURE 1-15. **Mechanisms by which reactive oxygen radicals are generated from molecular oxygen and then detoxified by cellular enzymes.** CoQ = coenzyme Q; GPX = glutathione peroxidase; H^+ = hydrogen ion; H_2O = water; H_2O_2 = hydrogen peroxide; O_2 = oxygen; O_2^- = superoxide; SOD = superoxide dismutase.

Complete reduction of O_2 to H_2O involves the transfer of four electrons. There are three partially reduced species that are intermediate between O_2 and H_2O, representing transfers of varying numbers of electrons (Fig. 1-15). They are O_2^-, superoxide (one electron); H_2O_2, hydrogen peroxide (two electrons); and •OH, the hydroxyl radical (three electrons). For the most part these ROS are produced principally by leaks in mitochondrial electron transport, with an additional contribution from the mixed-function oxygenase (P450) system. The major forms of ROS are listed in Table 1-1.

Superoxide

The superoxide anion (O_2^-) is produced principally by leaks in mitochondrial electron transport or as part of the inflammatory response. In the first instance, the promiscuity of coenzyme Q (CoQ) and other imperfections in the electron transport chain allows the transfer of electrons to O_2 to yield O_2^-. In the case of phagocytic inflammatory cells, activation of a plasma membrane oxidase produces O_2^-, which is then converted to H_2O_2 and eventually to other ROS (Fig. 1-16). These ROS have generally been viewed as the principal effectors of cellular oxidative defenses that destroy pathogens, fragments of necrotic cells or other phagocytosed material (see Chapter 2). There is now evidence to suggest that their main role in cellular defenses may be as signaling intermediates, to elicit release of proteolytic and other degradative enzymes. It is these enzymes that are probably the most critical effectors of neutrophil-mediated destruction of bacteria and other foreign materials.

Hydrogen Peroxide

O_2^- anions are catabolized by SOD to produce H_2O_2. Hydrogen peroxide is also produced directly by a number of oxidases in cytoplasmic peroxisomes (see Fig. 1-15). By itself, H_2O_2 is not particularly injurious, and it is largely metabolized to H_2O by catalase. However, when produced in excess, it is converted to highly reactive •OH. In neutrophils, myeloperoxidase transforms H_2O_2 to the potent radical hypochlorite (OCl^-), which is lethal for microorganisms and cells.

Most cells have efficient mechanisms for removing H_2O_2. Two different enzymes reduce H_2O_2 to water: (1) catalase within the peroxisomes and (2) glutathione peroxidase (GPX) in both the cytosol and the mitochondria (see Fig. 1-15). GPX uses reduced glutathione (GSH) as a cofactor, producing two molecules of oxidized glutathione (GSSG) for every molecule of H_2O_2 reduced to water. GSSG is re-reduced to GSH by glutathione reductase, with reduced nicotinamide adenine dinucleotide phosphate (NADPH) as the cofactor.

TABLE 1–1

Reactive Oxygen Species (ROS)

Molecule	Attributes
Hydrogen peroxide (H_2O_2)	Forms free radicals via Fe^{2+}-catalyzed Fenton reaction Diffuses widely within the cell
Superoxide anion (O_2^-)	Generated by leaks in the electron transport chain and some cytosolic reactions Produces other ROS Does not readily diffuse far from its origin
Hydroxyl radical (•OH)	Generated from H_2O_2 by Fe^{2+}-catalyzed Fenton reaction The intracellular radical most responsible for attack on macromolecules
Peroxynitrite (ONOO•)	Formed from the reaction of nitric oxide (NO) with O_2^- damages macromolecules
Lipid peroxide radicals (RCOO•)	Organic radicals produced during lipid peroxidation
Hypochlorous acid (HOCl)	Produced by macrophages and neutrophils during respiratory burst that accompanies phagocytosis Dissociates to yield hypochlorite radical (OCl^-)

Fe^{2+} = ferrous iron

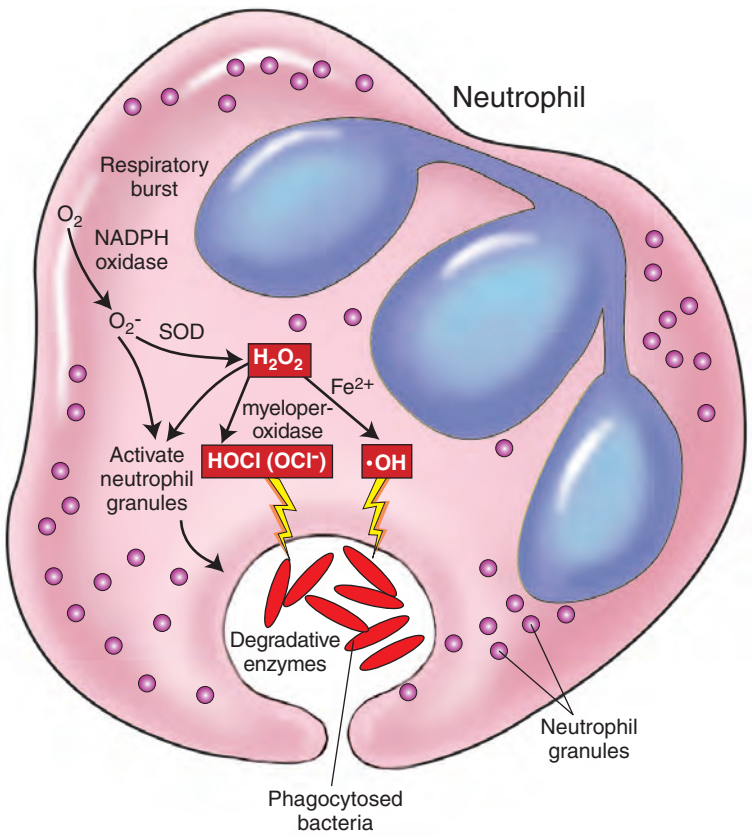


FIGURE 1-16. **Generation of reactive oxygen species in neutrophils as a result of phagocytosis of bacteria.** Fe^{2+} = ferrous iron; H_2O_2 = hydrogen peroxide; HOCl = hypochlorous acid; NADPH = nicotinamide adenine dinucleotide phosphate; OCl^- = hypochlorite radical; •OH = hydroxyl radical; SOD = superoxide dismutase.

Hydroxyl Radical

Hydroxyl radicals (•OH) are formed by (1) the radiolysis of water, (2) the reaction of H_2O_2 with ferrous iron (Fe^{2+}) (the Fenton reaction), and (3) the reaction of O_2^- with H_2O_2 (the Haber-Weiss reaction) (Fig. 1-17). The hydroxyl radical is the most reactive molecule of ROS and there are several mechanisms by which it can damage macromolecules.

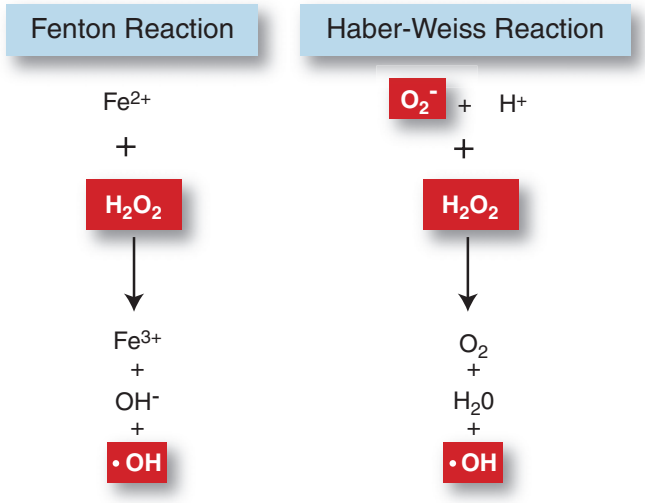


FIGURE 1-17. **Fenton and Haber-Weiss reactions to generate the highly reactive hydroxyl radical.** Reactive species are shown in red. Fe^{2+} = ferrous iron; Fe^{3+} = ferric iron; H^+ = hydrogen ion; H_2O_2 = hydrogen peroxide; OH^- = hydroxide; •OH = hydroxyl radical.

Iron is often an active participant in oxidative damage to cells (see below) by virtue of the Fenton reaction. Many lines of experimental evidence now suggest that in a number of different cell types H_2O_2 stimulates iron uptake and so increases production of hydroxyl radicals.

- **Lipid peroxidation:** The hydroxyl radical removes a hydrogen atom from the unsaturated fatty acids of membrane phospholipids, a process that forms a free lipid radical (Fig. 1-18). The lipid radical, in turn, reacts with molecular oxygen and forms a lipid peroxide radical. This peroxide radical can, in

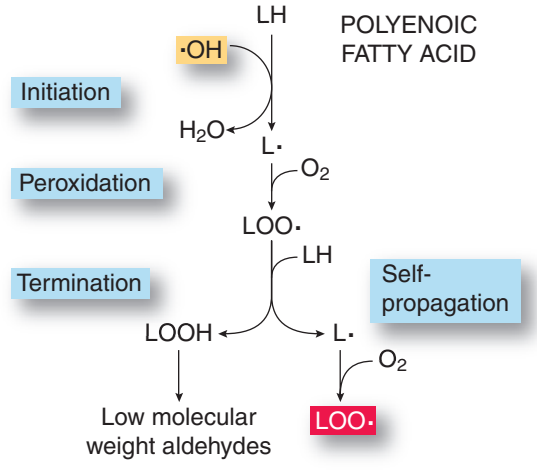


FIGURE 1-18. **Lipid peroxidation initiated by the hydroxyl radical (•OH). H_2O 5 water; O_2 5 oxygen. L•, lipid radical; LOO•, lipid peroxy radical; LOOH, lipid peroxide.**

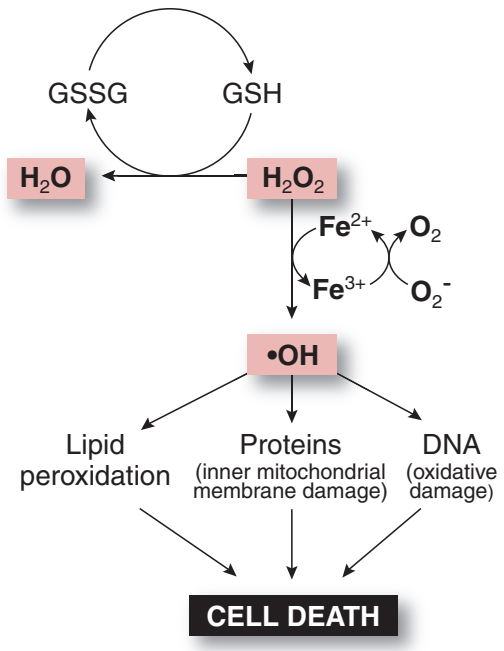


FIGURE 1-19. **Mechanisms of cell injury by activated oxygen species.** Fe^{2+} = ferrous iron; Fe^{3+} = ferric iron; GSH = glutathione; GSSG = glutathione; H_2O_2 = hydrogen peroxide; O_2 = oxygen, O_2^- = superoxide anion; •OH = hydroxyl radical.

turn, function as an initiator, removing another hydrogen atom from a second unsaturated fatty acid. A lipid peroxide and a new lipid radical result and a chain reaction is initiated. Lipid peroxides are unstable and break down into smaller molecules. The destruction of the unsaturated fatty acids of phospholipids results in a loss of membrane integrity.

- **Protein interactions:** Hydroxyl radicals may also attack proteins. The sulfur-containing amino acids cysteine and methionine, as well as arginine, histidine, and proline, are especially vulnerable to attack by •OH. As a result of oxidative damage, proteins undergo fragmentation, cross-linking, aggregation, and eventually degradation.
- **DNA damage:** DNA is an important target of the hydroxyl radical. A variety of structural alterations include strand breaks, modified bases, and cross-links between strands. In most cases, the integrity of the genome can be reconstituted by the various DNA repair pathways. However, if oxidative damage to DNA is sufficiently extensive, the cell dies.

Figure 1-19 summarizes the mechanisms of cell injury by activated oxygen species.

Peroxynitrite

Peroxynitrite ($ONOO^-$) is formed by the interaction of superoxide (O_2^-) with nitric oxide (NO•).

$$NO\bullet + O_2^- \rightarrow ONOO^-$$

The free radical $ONOO^-$ attacks a wide range of biologically important molecules, including lipids, proteins, and DNA. Nitric oxide, a molecule generated in many tissues, is a potent vasodilator and mediator of a number of important biological processes. Thus, the formation of peroxynitrite occupies an important place in free radical toxicology.

Cellular Defenses against Oxygen Free Radicals

Cells manifest potent antioxidant defenses against ROS, including detoxifying enzymes and exogenous free radical scavengers (vitamins). The major enzymes that convert ROS to less reactive molecules are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX).

Detoxifying Enzymes

- **SOD** is the first line of defense against O_2^-, converting it to H_2O_2 and O_2. (H^+ = hydrogen ion)

$$2\ O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2.$$

- **Catalase**, principally located in peroxisomes, is one of two enzymes that complete the dissolution of O_2^- by eliminating H_2O_2 and, therefore, its potential conversion to •OH.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

- **GPX** catalyzes the reduction of H_2O_2 and lipid peroxides in mitochondria and the cytosol.

$$H_2O_2 + 2\ GSH \rightarrow 2\ H_2O + GSSG$$

Scavengers of ROS

- **Vitamin E** (**α-tocopherol**) is a terminal electron acceptor and, therefore, blocks free-radical chain reactions. Given that it is fat soluble, it exerts its activity in lipid membranes, protecting them against lipid peroxidation.
- **Vitamin C** (**ascorbate**) is water soluble and reacts directly with O_2, •OH, and some products of lipid peroxidation. It also serves to regenerate the reduced form of vitamin E.
- **Retinoids,** the precursors of vitamin A, are lipid soluble and function as chain-breaking antioxidants.
- **NO•** is the product of constitutive or inducible nitric oxide synthases (NOSs). Although it may bind to superoxide to form highly reactive peroxynitrite, nitric oxide is also a major mechanism by which ROS are contained. NO• may accomplish this in several ways. Chelation of iron and scavenging of free radicals have been suggested, but recent studies suggest that the ability of NO• to increase proteasomal activity, and thus decrease cellular iron uptake by the transferrin receptor, may be involved.

Mutations May Impair Cell Function Without Causing Cell Death

There is evidence that mutations in genes which encode proteins that mediate certain cellular activities, but are not necessarily lethal to affected cells, may lead to a wide array of clinical syndromes. Increasingly, such mutations provide pathogenetic links among seemingly unrelated clinical diseases.

Chaperonopathies

As indicated above, molecular chaperones are important in maintaining correct protein folding, recognizing misfolded or improperly modified proteins, and providing for their degradation. An array of diseases, called **chaperonopathies**, have been linked to mutations in the genes which encode these chaperones or other molecules that participate in these processes. The organ systems affected and the presenting symptom complexes of these diseases are diverse. A mutation in a chaperone cofactor is responsible for a form of X-linked retinitis pigmentosa. Hereditary spastic

paraplegia is related to a mutation in heat shock protein (hsp)60, a mitochondrial chaperone. In von Hippel-Lindau disease, a mutation in the gene for VHL protein leads to poor chaperone binding to VHL. Consequent VHL protein misfolding inactivates the tumor suppressor activity of the complex of which it is a part and leads to development of tumors of the adrenal, kidney, and brain.

Channelopathies

Ion channels are transmembrane pore-forming proteins that allow ions, principally Na^+, K^+, calcium (Ca^{2+}), and chloride (Cl^-), to flow in or out of the cell. These functions are critical for numerous physiological processes, such as control of the heartbeat, muscular contraction and relaxation, and regulation of insulin secretion in pancreatic beta cells. For example activation and inactivation of sodium and potassium channels are responsible for the action potential in neurons, and calcium channels are important in contraction and relaxation of cardiac and skeletal muscle. Congenital defects caused by mutations in genes that encode ion channel proteins are now termed **channelopathies.** Mutations in over 60 ion channel genes are known to cause a variety of diseases, including cardiac arrhythmias (e.g., short and long QT syndromes) and neuromuscular syndromes (e.g. myotonias, familial periodic paralysis). As an example, a number of inherited human disorders affecting skeletal muscle contraction, heart rhythm, and function of the nervous system are attributable to mutations in genes that encode voltage-gated sodium channels. Channelopathies have also been implicated in certain pediatric epilepsy syndromes. In addition, non-excitable tissues may also be affected. In pancreatic beta cells, ATP-sensitive potassium channels regulate insulin secretion, and mutations in these channel genes lead to certain forms of diabetes.

Intracellular Storage Is Retention of Materials within the Cell

The substance that accumulates may be normal or abnormal, endogenous or exogenous, harmful or innocuous.

- **Nutrients,** such as fat, glycogen, vitamins, and minerals, are stored for later use.
- **Degraded phospholipids,** which result from turnover of endogenous membranes, are stored in lysosomes and may be recycled.
- **Substances that cannot be metabolized** accumulate in cells. These include (1) endogenous substrates that are not further processed because a key enzyme is missing (hereditary storage diseases), (2) insoluble endogenous pigments (e.g., lipofuscin and melanin), (3) aggregates of normal or abnormal proteins, and (4) exogenous particulates, such as inhaled silica and carbon or injected tattoo pigments.
- **Overload of normal body constituents,** including iron, copper, and cholesterol, injures a variety of cells.
- **Abnormal proteins** may be toxic when they are retained within a cell. Examples are Lewy bodies in Parkinson disease and mutant α_1-antitrypsin.

Fat

Bacteria and other unicellular organisms continuously ingest nutrients. By contrast, mammals do not need to eat continuously. They eat periodically and can survive a prolonged fast because they store nutrients in specialized cells for later use—fat in adipocytes and glycogen in the liver, heart, and muscle.

Abnormal accumulation of fat is most conspicuous in the liver, a subject treated in detail in Chapter 14. Briefly, liver cells always contain some fat, because free fatty acids released from adipose tissue are taken up by the liver. There, they are oxidized or converted to triglycerides. Most of newly synthesized triglycerides are secreted by the liver as lipoproteins. When delivery of free fatty acids to the liver is increased, as in diabetes or when intrahepatic lipid metabolism is disturbed, as in alcoholism, triglycerides accumulate in liver cells. Fatty liver is identified morphologically as lipid globules in the cytoplasm. Other organs, including the heart, kidney, and skeletal muscle, also store fat. One must recognize that fat storage is always reversible and there is no evidence that the excess fat in the cytoplasm interferes with cell function.

Glycogen

Glycogen is a long-chain polymer of glucose, formed and largely stored in the liver and to a lesser extent in muscles. It is depolymerized to glucose and liberated as needed. Glycogen is degraded in steps by a series of enzymes, each of which may be deficient as a result of an inborn error of metabolism. Regardless of the specific enzyme deficiency, the result is a glycogen storage disease (see Chapter 6). These inherited disorders affect the liver, heart, and skeletal muscle and range from mild and asymptomatic conditions to inexorably progressive and fatal diseases (see Chapters 11, 14, and 27).

The amount of glycogen stored in cells is normally regulated by the blood glucose concentration, and hyperglycemic states are associated with increased glycogen stores. Thus, in uncontrolled diabetes, hepatocytes and epithelial cells of the renal proximal tubules are enlarged by excess glycogen.

Inherited Lysosomal Storage Diseases

Like glycogen catabolism, breakdown of certain complex lipids and mucopolysaccharides (glycosaminoglycans) takes place by a sequence of enzymatic steps. Since these enzymes are located in the lysosomes, their absence results in lysosomal storage of incompletely degraded lipids, such as cerebrosides (e.g., Gaucher disease) and gangliosides (e.g., Tay-Sachs disease) or products of mucopolysaccharide catabolism (e.g., Hurler and Hunter syndromes). These disorders are all progressive but vary from asymptomatic organomegaly to rapidly fatal brain disease. See Chapter 6 for the metabolic bases of these disorders and Chapters 26 and 28 for specific organ pathology.

Cholesterol

The human body has a love–hate relationship with cholesterol. On the one hand, it is a critical component of all plasma membranes. On the other hand, when stored in excess, it is closely associated with atherosclerosis and cardiovascular disease, the leading cause of death in the Western world (see Chapter 10).

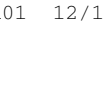
Briefly, the initial lesion of atherosclerosis (fatty streak) reflects accumulation of cholesterol and cholesterol esters in macrophages within the arterial intima. As the disease progresses, smooth muscle cells also store cholesterol. Advanced lesions of atherosclerosis are characterized by extracellular deposition of cholesterol (see Fig 1- 22B).

In a number of disorders characterized by elevated blood levels of cholesterol (e.g., familial hypercholesterolemia or primary biliary cirrhosis), macrophages store cholesterol. When clusters of these cells in subcutaneous tissues become grossly visible, they are termed **xanthomas** (see Fig 1- 22A).

Abnormal Proteins

Several acquired and inherited diseases are characterized by intracellular accumulation of abnormal proteins. The deviant tertiary structure of the protein may result from an inherited mutation that alters the normal primary amino acid sequence or may reflect an acquired defect in protein folding. The following are examples:

- **α_1-Antitrypsin deficiency** is a heritable disorder in which mutations in the coding gene for α_1-antitrypsin yield an insoluble protein. Mutant protein is not easily exported. It accumulates in liver cells (see Fig. 1-22C), causing cell injury and cirrhosis (see Chapter 14).
- **Prion diseases** comprise a group of neurodegenerative disorders (spongiform encephalopathies) caused by the accumulation of abnormally folded prion proteins. The anomaly reflects the conversion of the normal α-helical structure to a β-pleated sheet. Abnormal prion proteins may result from an inherited mutation or from exposure to the aberrant form of the protein (see Chapter 28). The function of normal prion protein is not yet clear. It has been reported to have SOD-like antioxidant activity, a role in T lymphocyte-dendritic cell interactions, the ability to enhance neural progenitor proliferation, and a key role in development of in long- term memory.
- **Lewy bodies** (α-synuclein) are seen in neurons of the substantia nigra in Parkinson disease (Chapter 28).
- **Neurofibrillary tangles** (tau protein) characterize cortical neurons in Alzheimer disease (Chapter 28).
- **Mallory bodies** (intermediate filaments) are hepatocellular inclusions in alcoholic liver injury (Chapter 14).



PATHOGENESIS: After nascent polypeptides emerge from the ribosomes, they fold to assume the tertiary configuration of mature proteins. Correct folding requires proteins to assume one particular structure from a constellation of possible but incorrect conformations. Curiously, it is energetically more favorable for the cell to produce many foldings and then edit the protein repertoire than to produce only a single correct conformation. Molecular chaperones associate with polypeptides in the endoplasmic reticulum and promote correct folding, after which they dissociate from those proteins that have assumed the correct conformation (Fig. 1-20). By contrast, incorrectly folded proteins remain bound to their chaperones and are subsequently degraded by the ubiquitin–proteasome system (see above). Evolutionary preference for energy conservation has dictated that a substantial proportion of newly formed proteins are rogues unsuitable for the society of civilized cells.

Numerous hereditary and acquired diseases are caused by evasion of the quality control system designed to promote correct folding and eliminate faulty proteins. Misfolded proteins can injure the cell in a number of ways.

- **Loss of function:** Certain mutations prevent correct folding of crucial proteins, which then do not function properly or cannot be incorporated into the correct site. For example, some mutations that lead to cystic fibrosis cause misfolding of an ion channel protein, which is then degraded. The protein does not reach its destination at the cell membrane, leading to a defect in chloride transport that produces the disease cystic fibrosis. Other examples of loss of function include mutations of the low-density lipoprotein (LDL) receptor in certain types of hypercholesterolemia and mutations of a copper transport adenosine triphosphatase (ATPase) in Wilson disease.
- **Formation of toxic protein aggregates:** Defects in protein structure may be acquired as well as genetic. Thus, particularly in nondividing cells, age-related impairment of cellular antioxidant defenses leads to protein oxidation, which commonly alters protein tertiary structure, exposing interior hydrophobic amino acids that are normally hidden. In situations of mild to moderate oxidative stress, 20S proteasomes recognize the exposed hydrophobic moieties and degrade these proteins. However if oxidative stress is severe, these proteins aggregate by virtue of a combination of hydrophobic and ionic bonds. Such aggregates are insoluble and tend to sequester Fe^{2+} ions, which in turn help generate additional ROS (see above) and aggregate size increases. Whether or not the proteins contained in the aggregates are ubiquitinated, the aggregates are indigestible (Fig. 1- 21). Any Ub bound to them is lost, which may cause a cellular deficit in Ub and impair protein degradation in general. Both by virtue of their generation of toxic ROS and their inhibition of proteasomal degradation, these aggregates may lead to cell death. Accumulation of amyloid β protein in Alzheimer's disease and α-synuclein in Parkinson's disease may occur by this type of mechanism.
- **Retention of secretory proteins:** Many proteins that are destined to be secreted from the cell require a correctly folded conformation to be transported through cellular compartments and released at the cell membrane. Mutations in genes that encode such proteins (e.g., α_1-antitrypsin) lead to cell injury because of massive accumulation of misfolded proteins within the liver cell. Failure to secrete this antiprotease into the circulation also leads to unregulated proteolysis of connective tissue in the lung and loss of pulmonary elasticity (emphysema).
- **Extracellular deposition of aggregated proteins:** Misfolded proteins tend to exhibit a β-pleated conformation in place of random coils or α helices. These abnormal proteins often form insoluble aggregates, which may be visualized as extracellular deposits, the appearance depending upon the specific disease. These accumulations often assume the forms of various types of amyloid and produce cell injury in systemic amyloidoses (see Chapter 23) and a variety of neurodegenerative diseases (see Chapter 28).

Lipofuscin

Lipofuscin is a mixture of lipids and proteins containing a golden-brown pigment called ceroid. Lipofuscin tends to accumulate by accretion of oxidized, cross-linked proteins (see Fig. 1- 21*) and is indigestible. It occurs mainly in terminally differentiated cells (neurons and cardiac myocytes) or in cells that cycle infrequently (hepatocytes)* (see Fig. 1- 22D). It is often more conspicuous in conditions associated with atrophy of an organ.

Although it was previously thought to be benign, there is increasing evidence that lipofuscin may be both a result and a cause of increasing oxidant stress in cells. It may impair both proteasomal function and lysosomal degradation of senescent or

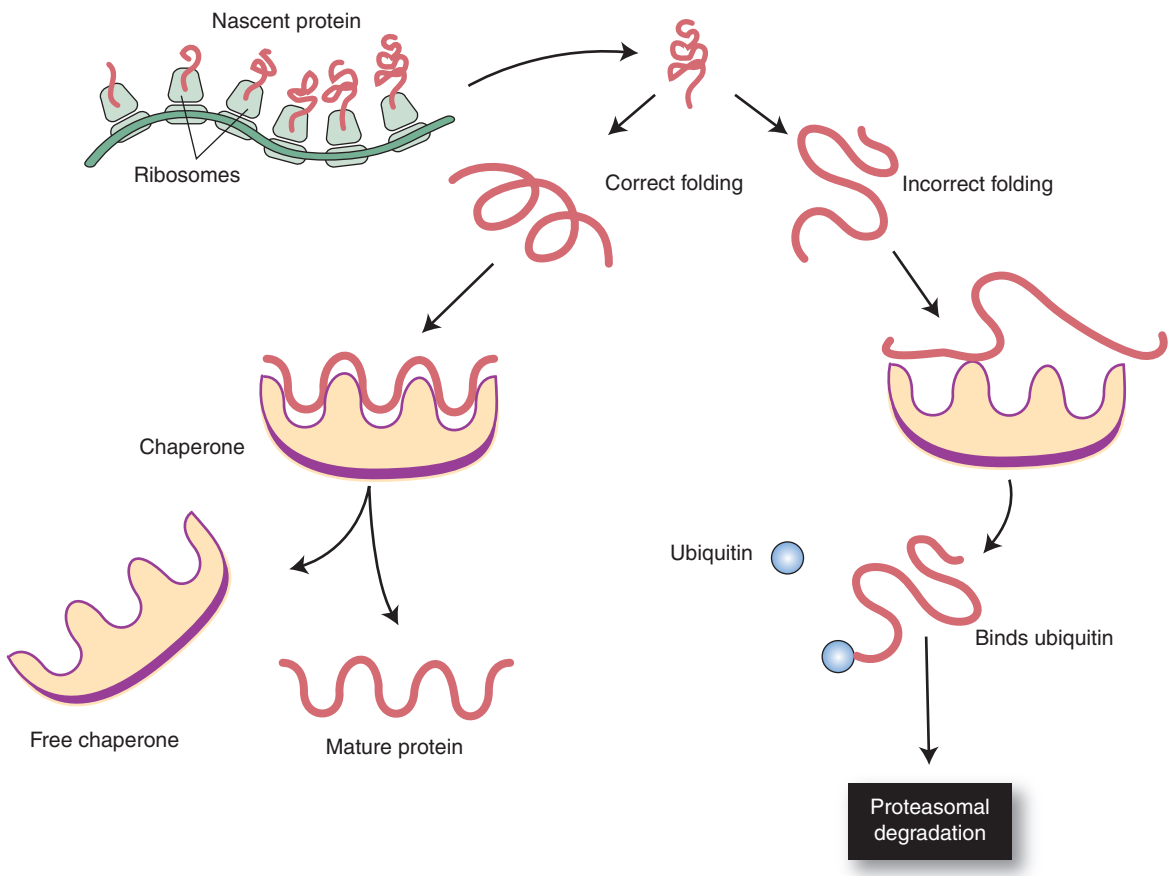


FIGURE 1-20. **Differential handling of protein that is correctly folded *(left arrows)* and protein that is incorrectly folded *(right arrows)*.** Correctly folded proteins are chaperoned from the ribosomes that produce them to their ultimate cellular destination. Incorrectly folded proteins bind to ubiquitin, an association that directs the protein to proteasomes, where the misfolded protein is degraded.

poorly functioning organelles. Consequently, inefficient or poorly functioning mitochondria may accumulate, generate more ROS and perpetuate the cycle.

Melanin

Melanin is an insoluble, brown-black pigment found principally in the epidermal cells of the skin, but also in the eye and other organs (see Fig. 1-22E). It is located in intracellular organelles known as melanosomes and results from the polymerization of certain oxidation products of tyrosine. The amount of melanin is responsible for the differences in skin color among the various races, as well as the color of the eyes. It serves a protective function owing to its ability to absorb ultraviolet light. In white persons, exposure to sunlight increases melanin formation (tanning). The hereditary inability to produce melanin results in the disorder known as **albinism.** The presence of melanin is also a marker of the cancer that arises from melanocytes (melanoma). Melanin is discussed in detail in Chapter 24.

Exogenous Pigments

Anthracosis refers to the storage of carbon particles in the lung and regional lymph nodes (see Fig. 1- 22F). Virtually all urban dwellers inhale particulates of organic carbon generated by the burning of fossil fuels. These particles accumulate in alveolar macrophages and are also transported to hilar and mediastinal lymph nodes, where the indigestible material is stored indefinitely within macrophages. Although the gross appearance of the lungs of persons with anthracosis may be alarming, the condition is innocuous.

Tattoos are the result of the introduction of insoluble metallic and vegetable pigments into the skin, where they are engulfed by dermal macrophages and persist for a lifetime.

Iron and Other Metals

About 25% of the body's total iron content is in an intracellular storage pool composed of the iron-storage proteins **ferritin** and **hemosiderin**. The liver and bone marrow are particularly rich in ferritin, although it is present in virtually all cells. Hemosiderin is a partially denatured form of ferritin that aggregates easily and is recognized microscopically as yellow-brown granules in the cytoplasm. Normally, hemosiderin is found mainly in the spleen, bone marrow, and Kupffer cells of the liver.

Total body iron may be increased by enhanced intestinal iron absorption, as in some anemias, or by administration of iron-containing erythrocytes in a transfusion. In either case, the excess iron is stored intracellularly as ferritin and hemosiderin. Increasing the body's total iron content leads to progressive accumulation of hemosiderin, a condition termed **hemosiderosis.** In this condition, iron is present not only in the organs in which it is normally found but also throughout the body, in such places as the skin, pancreas, heart, kidneys, and endocrine organs. Intracellular accumulation of

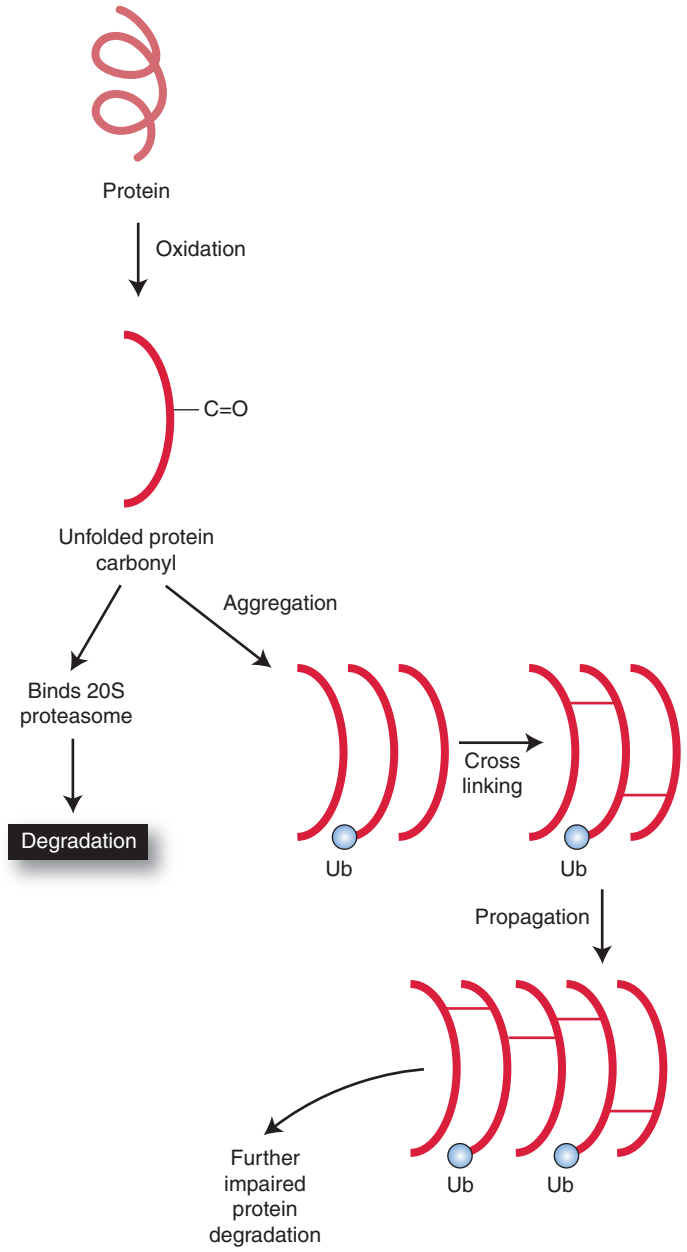


FIGURE 1-21. **Mechanism of accumulation and elimination of oxidized proteins.** On exposure to reactive oxygen species (ROS), proteins become oxidized to protein carbonyls. This forces a change in protein tertiary structure, exposing hydrophobic residues ordinarily on the protein interior. These protein carbonyls may be eliminated by 20S proteasomes without ubiquitination or, especially if the oxidant stress is heavy, they may aggregate on the basis of hydrophobic interactions. Some aggregated proteins may be ubiquitinated, but such aggregates cannot be degraded. With further accretion, aggregate substituents undergo crosslinking and continue to enlarge. Ub = ubiquitin

iron in hemosiderosis does not usually injure cells. However, if the increase in total body iron is extreme; we speak of **iron overload syndromes** (see Chapter 14), in which iron deposition is so severe that it damages vital organs—the heart, liver, and pancreas. Severe iron overload can result from a genetic abnormality in iron absorption, **hereditary hemochromatosis** (see Fig. 1- 22G). Alternatively, severe iron overload may occur after multiple blood transfusions, such as in treating hemophilia or certain hereditary anemias.

Excessive iron storage in some organs is also associated with increased risk of cancer. The pulmonary siderosis encountered among certain metal polishers is accompanied by increased risk of lung cancer. Hemochromatosis leads to a higher incidence of liver cancer.

Excess accumulation of lead, particularly in children, causes mental retardation and anemia. The storage of other metals also presents dangers. In Wilson disease, a hereditary disorder of copper metabolism, storage of excess copper in the liver and brain may lead to severe chronic disease of those organs.

Ischemia/Reperfusion Injury Reflects Oxidative Stress

Ischemia/reperfusion (I/R) injury is a common clinical problem that arises in occlusive cardiovascular disease, infection, shock, and many other settings. I/R injury reflects the interplay of transient ischemia, consequent tissue damage, and exposure of damaged tissue to the oxygen that arrives when blood flow is reestablished (reperfusion). Initially, ischemic cellular damage leads to generation of free radical species. Reperfusion then provides abundant molecular O_2 to combine with free radicals to form ROS. Evolution of I/R injury also involves many other factors, including inflammatory mediators [tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1)], platelet activating factor (PAF), NOS and NO•, intercellular adhesion molecules, and many more.

Xanthine Oxidase

Xanthine dehydrogenase may be converted by proteolysis during a period of ischemia into xanthine oxidase. On reperfusion, oxygen returns and the abundant purines derived from ATP catabolism during ischemia provide substrates for xanthine oxidase. This enzyme requires oxygen to catalyze the formation of uric acid; activated oxygen species are byproducts of this reaction.

The Role of Neutrophils

An additional source of ROS during reperfusion is the neutrophil. Alterations in the cell surface that occur during ischemia and on reperfusion induce the adhesion and activation of circulating neutrophils. These cells release large quantities of activated oxygen species and hydrolytic enzymes, both of which may injure the previously ischemic cells.

Reperfusion also prompts endothelial cells to move preformed P-selectin to the cell surface, allowing neutrophils to bind endothelial membrane intercellular adhesion molecule-1 (ICAM-1) and roll along endothelial cells (see Chapter 2). Recruitment of these inflammatory cells to affected areas increases local production of oxygen free radicals.

The Role of Nitric Oxide

There are two major forms of NOS: a constitutive form, which is common to endothelial cells and parenchymal cells (e.g., hepatocytes, neurons) and an inducible form (iNOS), mostly found in inflammatory cells. Nitric oxide dilates the microvasculature by relaxing smooth muscle, inhibits platelet aggregation, and decreases adhesion between leukocytes and the endothelial surface. These activities are all mediated by the ability of NO• to decrease cytosolic Ca^{2+}, both by extrusion of calcium from the cell and by its sequestration within intracellular stores.

NO• also reacts with O_2^- to form the highly reactive species, $ONOO^-$. Normally, O_2^- is detoxified by SOD and little $ONOO^-$ is produced. However, I/R stimulates iNOS and the NO• produced inactivates SOD, thereby increasing the amount of NO• and favoring production of $ONOO^-$ The free radical

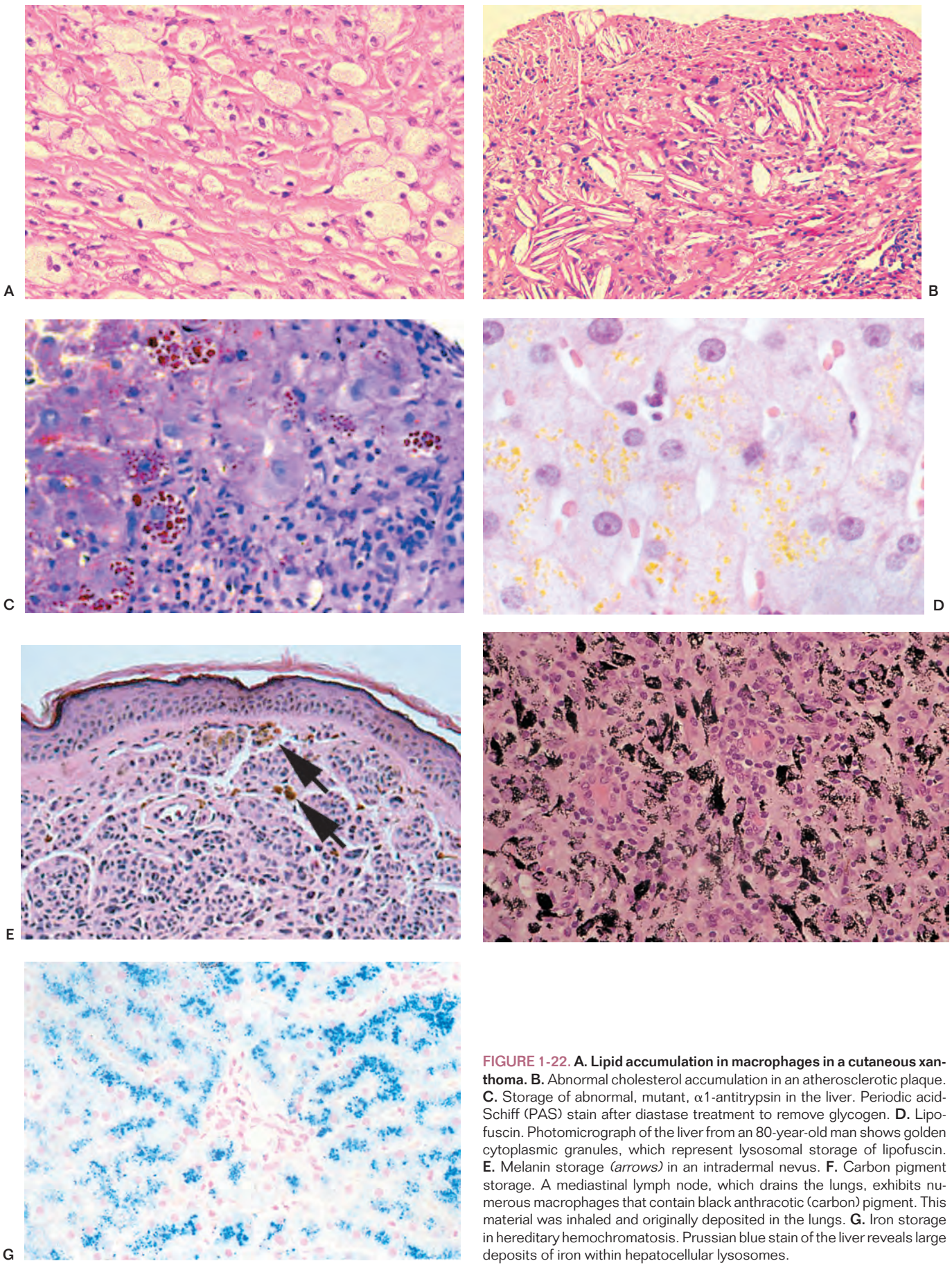


FIGURE 1-22. **A. Lipid accumulation in macrophages in a cutaneous xanthoma. B.** Abnormal cholesterol accumulation in an atherosclerotic plaque. **C.** Storage of abnormal, mutant, α1-antitrypsin in the liver. Periodic acid-Schiff (PAS) stain after diastase treatment to remove glycogen. **D.** Lipofuscin. Photomicrograph of the liver from an 80-year-old man shows golden cytoplasmic granules, which represent lysosomal storage of lipofuscin. **E.** Melanin storage *(arrows)* in an intradermal nevus. **F.** Carbon pigment storage. A mediastinal lymph node, which drains the lungs, exhibits numerous macrophages that contain black anthracotic (carbon) pigment. This material was inhaled and originally deposited in the lungs. **G.** Iron storage in hereditary hemochromatosis. Prussian blue stain of the liver reveals large deposits of iron within hepatocellular lysosomes.

gives rise to DNA strand breaks and lipid peroxidation in cell membranes.

NO• is a double-edged sword in I/R injury, however. Fe^{2+} ion plays an important role in continuing to generate ROS during I/R injury. As noted above, NO• decreases transferrin-mediated iron uptake, and so may also partly protect cells from I/R injury.

Inflammatory Cytokines

I/R injury leads to the release of cytokines that (1) promote vasoconstriction, (2) stimulate the adherence of inflammatory cells and platelets to endothelium, and (3) have effects at sites distant from the ischemic insult itself.

Local release of TNF-α at the site of I/R injury results in chemotaxis and sequestration of neutrophils by upregulating the expression of cell adhesion molecules on both neutrophils and endothelial cells. This cytokine is also responsible for increases in neutrophil trafficking and neutrophil-related damage at locations distant from the site of I/R injury itself, thereby causing systemic effects. By increasing the levels of PAF, I/R injury cripples vascular function both locally and systemically. In addition, augmented release of endothelin during I/R injury promotes the adherence of inflammatory cells and increases vascular tone and permeability.

We can put reperfusion injury in perspective by emphasizing that there are three different degrees of cell injury, depending on the duration of the ischemia:

- With short periods of ischemia, reperfusion (and, therefore, the resupply of oxygen) completely restores the structural and functional integrity of the cell. Cell injury in this case is completely reversible.
- With longer periods of ischemia, reperfusion is not associated with restoration of cell structure and function but rather with deterioration and death of the cells. In this case, lethal cell injury occurs during the period of reperfusion.
- Lethal cell injury may develop during the period of ischemia itself, in which case reperfusion is not a factor. A longer period of ischemia is needed to produce this third type of cell injury. In this case, cell damage does not depend on the formation of activated oxygen species.

Proteasomes and Ischemia/Reperfusion Injury

Many of the cytokines that mediate tissue damage in I/R injury are expressed under the control of NFκB. In a number of experimental models, proteasomal activity is decreased after I/R. This stabilizes the NFκB-IκB complex (see above), and limits tissue damage.

Ionizing Radiation Causes Oxidative Stress

The term "ionizing radiation" connotes an ability to cause radiolysis of water, thereby directly forming hydroxyl radicals. As noted above, hydroxyl radicals interact with DNA and inhibit DNA replication. For a nonproliferating cell, such as a hepatocyte or a neuron, the inability to divide is of little consequence. For a proliferating cell, however, the prevention of mitosis is a catastrophic loss of function. Once a proliferating cell can no longer divide, it dies by **apoptosis,** which rids the body of those cells that have lost their prime function. Direct mutagenic effects of ionizing radiation on DNA are also important. The cytotoxic effects of ionizing radiation are also dose-dependent. Whereas exposure to significant sources of radiation impairs the replicating capacity of cycling cells, massive doses of radiation may kill both proliferating and quiescent cells directly. Figure 1-23 summarizes the mechanisms of cell killing by ionizing radiation.

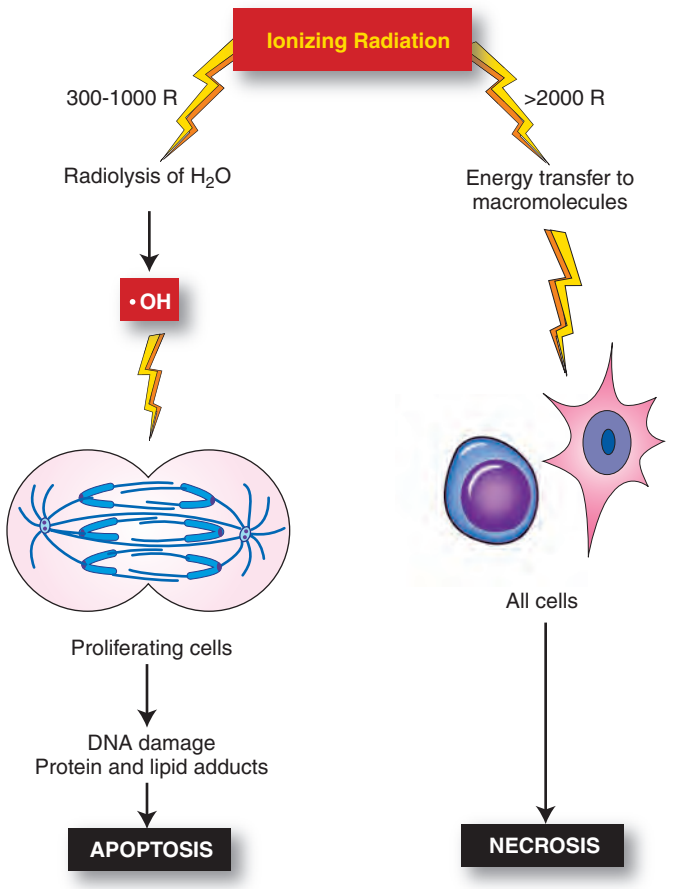
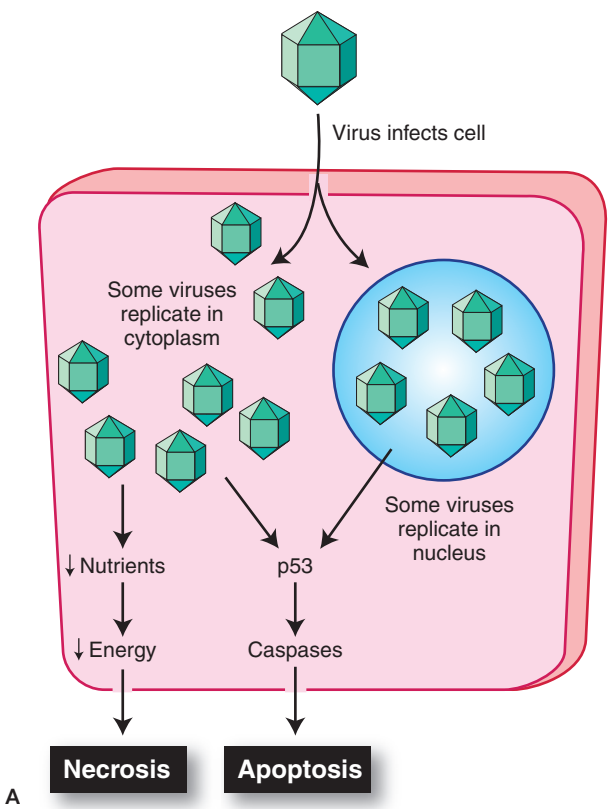


FIGURE 1-23. **Mechanisms by which ionizing radiation at low and high doses causes cell death.** H_20 = water, •OH = hydroxyl radical; R = rads.

Viral Cytotoxicity Is Direct or Immunologically Mediated

The means by which viruses cause cell injury and death are as diverse as viruses themselves. Unlike bacteria, a virus requires a cellular host to (1) house it; (2) provide enzymes, substrates, and other resources for viral replication; and (3) serve as a source for dissemination when mature virions are ready to be spread to other cells. Viruses have evolved mechanisms by which they avoid biting the hand that feeds them (at least until they are ready for other hands). The ability of a virus to persist in an infected cell necessitates a parasitic, albeit temporary, relationship with the host cell. During this vulnerable phase, the virus plays a game of cat and mouse with the immune system as a device to evade elimination of the infected cell. This period is followed by a phase in which the virus disseminates, either by budding (which does not necessarily destroy the cell) or by lysis (which does). In some viral infections (e.g., herpes simplex, measles, zoster-varicella), infection of a host cell may last for many years or a lifetime, in which case the cell is not destroyed. There are patterns of cellular injury related to viral infections that deserve a brief mention:

- **Direct toxicity:** Viruses may injure cells directly by subverting cellular enzymes and depleting the cell's nutrients, thereby disrupting the normal homeostatic mechanisms. The mechanisms underlying virus-induced lysis of cells, however, are probably more complex (Fig. 1-24A).



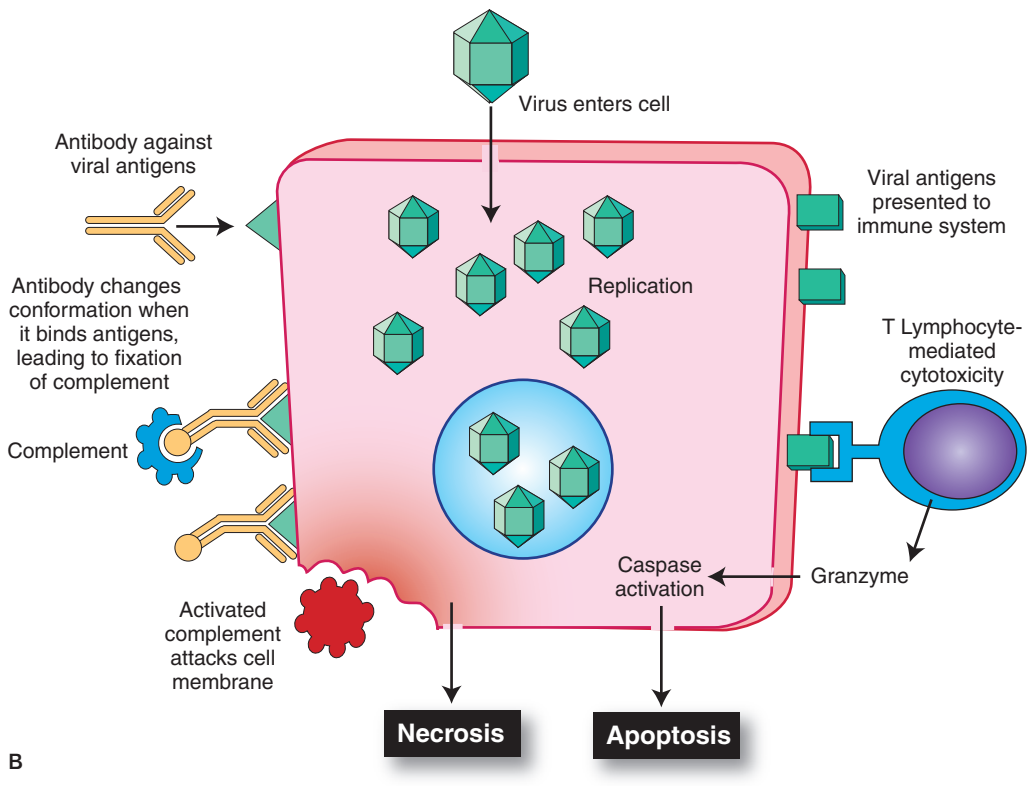


FIGURE 1-24. **Cell injury caused by virus infection. A.** Direct injury caused by virus infection, involving both depletion of cellular resources and activation of apoptotic signaling mechanisms. **B.** Mechanisms that lead to immunologically mediated destruction of virus-infected cells.

- **Manipulation of apoptosis:** During their replicative cycle, and before virion assembly is complete, there are many viral activities that can elicit apoptosis. For example, apoptosis is activated when the cell detects episomal (extrachromosomal) DNA replication. Since viruses must avoid cell death before they have produced infectious progeny, they have evolved mechanisms to counteract this effect by upregulating anti-apoptotic proteins and inhibiting proapoptotic ones. Some viruses also encode proteins that induce apoptosis once daughter virions are mature (see Fig. 1-24A).
- **Immunologically mediated cytotoxicity:** Both humoral and cellular arms of the immune system protect against the harmful effects of viral infections by eliminating infected cells. Thus, presentation of viral proteins to the immune system in the context of a self major histocompatibility complex (MHC) on cell surfaces leads to immune responses against the invader and elicits killer cells and antiviral antibodies. These arms of the immune system eliminate virus-infected cells by inducing apoptosis or by lysing the cell with complement (see Fig. 1-24B) (see Chapter 4).

Chemicals Injure Cells Directly and Indirectly

Innumerable chemicals can damage almost any cell in the body. The science of toxicology attempts to define the mechanisms that determine both target cell specificity and the mechanism of action of such chemicals. Toxic chemicals either: (1) interact directly with cellular constituents without requiring metabolic activation or (2) are themselves not toxic but are metabolized to yield an ultimate toxin that interacts with the target cell. Whatever the mechanism, the result is usually necrotic cell death (see below).

Liver Necrosis Caused by the Metabolic Products of Chemicals

Studies of a few compounds that produce liver cell injury in rodents have enhanced our understanding of how chemicals injure cells. These studies have focused principally on those compounds that are converted to toxic metabolites. Carbon tetrachloride (CCl_4) and acetaminophen are well-studied hepatotoxins. Each is metabolized by the mixed-function oxidase system of the endoplasmic reticulum and each causes liver cell necrosis. These hepatotoxins are metabolized differently, and it is possible to relate the subsequent evolution of lethal cell injury to the specific features of this metabolism.

Carbon Tetrachloride

CCl_4 metabolism is a model system for toxicological studies. CCl_4 is metabolized via the hepatic mixed function oxygenase system (P450) to a chloride ion and a highly reactive trichloromethyl free radical ($CCl_3\bullet$).

$$CCl_4 + e^- \xrightarrow{P450} CCl_3\bullet + Cl^-$$

Like the hydroxyl radical, the trichloromethyl radical is a potent initiator of lipid peroxidation, although it may also interact with other macromolecules. However, in view of the rapidity with which CCl_4 kills cells (hours), peroxidative damage to the plasma membrane is the most likely culprit.

Acetaminophen

Acetaminophen, an important constituent of many analgesics, is innocuous in recommended doses, but when consumed to excess it is highly toxic to the liver. Most acetaminophen is enzymatically converted in the liver to nontoxic glucuronide or sulfate metabolites. Less than 5% of acetaminophen is ordinarily metabolized by isoforms of cytochrome P450 to NAPQI (*N*-acetyl-*p*-benzoquinone imine), a highly reactive quinone (Fig. 1-25). However, when large doses of acetaminophen overwhelm the glucuronidation pathway, toxic amounts of NAPQI are formed. NAPQI is responsible for acetaminophen-related toxicity by virtue of its conjugation with either GSH or sulfhydryl groups on liver proteins to form thiol esters. The latter cause extensive cellular dysfunction and lead to injury. At the same time, NAPQI depletes the antioxidant GSH, rendering the cell more susceptible to free radical-induced injury. Thus, conditions that deplete GSH (e.g., starvation) enhance the toxicity of acetaminophen. In addition, acetaminophen metabolism is accelerated by chronic alcohol consumption, an effect mediated by an ethanol-induced increase in the 3A4 isoform of P450. As a result, toxic amounts of NAPQI rapidly accumulate and may destroy the liver.

To summarize, metabolism of hepatotoxic chemicals by mixed-function oxidation leads to cell injury through covalent binding of reactive metabolites and peroxidation of membrane phospholipids. Lipid peroxidation is initiated by (1) a metabolite of the original compound (as with CCl_4) or (2) by activated oxygen species formed during the metabolism of the toxin (as with acetaminophen), the latter augmented by weakened antioxidant defenses.

Chemicals That Are Not Metabolized

Directly cytotoxic chemicals interact with cellular constituents: prior metabolic conversion is not needed. The critical cellular

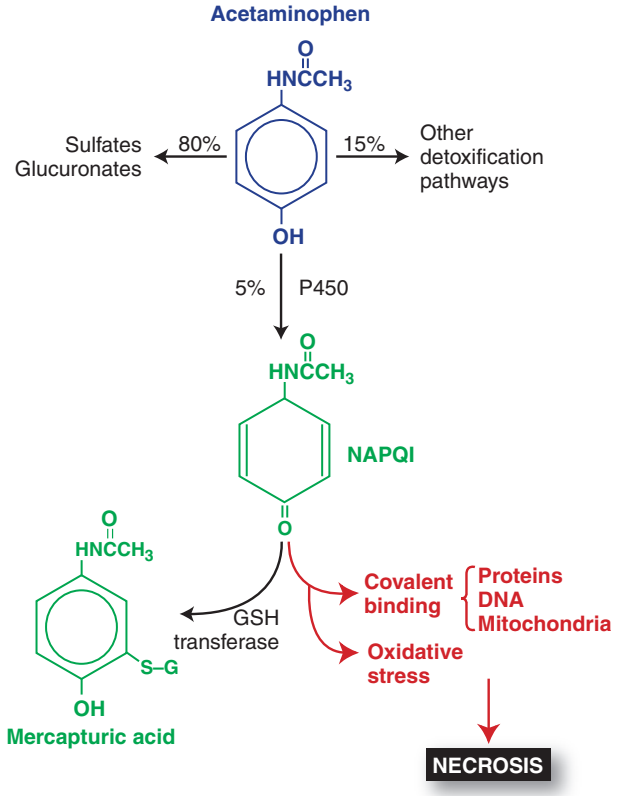


FIGURE 1-25. **Chemical reactions involved in acetaminophen hepatotoxicity.** GSH = glutathione; NAPQI = *N*-acetyl-*p*-benzoquinone imine.

targets are diverse and include, for example, mitochondria (heavy metals and cyanide), cytoskeleton (phalloidin, paclitaxel), and DNA (chemotherapeutic alkylating agents). As well, interaction of directly cytotoxic chemicals with glutathione (alkylating agents) weakens the cell's antioxidant defenses.

Abnormal G Protein Activity Leads to Functional Cell Injury

Normal cell function requires the coordination of numerous activating and regulatory signaling cascades. Hereditary or acquired interference with correct signal transduction can result in significant cellular dysfunction, as illustrated by diseases associated with faulty G proteins. A variety of membrane receptors (e.g., adrenergic or vasopressin receptors) are linked to intracellular G proteins, which activate downstream signaling. Inherited defects in G protein subunits can lead to constitutive activation of the protein. In one such hereditary syndrome, endocrine manifestations predominate, including multiple tumors in the pituitary and thyroid glands. Another G protein mutation appears to predominate in many cases of essential hypertension, in which exaggerated activation of G protein signaling results in increased vascular responsiveness to stimuli that cause vasoconstriction. Certain microorganisms (e.g., *Vibrio cholerae* and *Escherichia coli*) produce their effects by elaborating toxins that activate G proteins.

Decreased responsiveness of G proteins to ligand–receptor interactions may also be caused by certain mutations in G protein subunits. In addition, G protein activity can be inhibited by certain bacterial products, the most important example being pertussis toxin, the cause of whooping cough.

Cell Death

An understanding of the mechanisms underlying cell death is not simply an academic exercise; manipulation of cell viability by biochemical and pharmacologic intervention is currently a major area of research. For example, if we understand the biochemistry of ischemic death of cardiac myocytes, which is responsible for the leading cause of death in the Western world, we may be able to prolong myocyte survival after a coronary occlusion until circulation is restored.

Paradoxically, an organism's survival requires sacrifice of individual cells. Physiologic cell death is integral to the transformation of embryonic anlagen to fully developed organs. It is also crucial for regulation of cell numbers in a variety of tissues, including the epidermis, gastrointestinal tract, and hematopoietic system. Physiological cell death involves activation of an internal suicide program, which results in cell killing by a process termed **apoptosis.**

By contrast, pathologic cell death is not regulated and is invariably injurious to the organism. It may result from a variety of insults to cellular integrity (e.g., ischemia, burns, and toxins). **Necrosis** occurs when an insult interferes with a vital structure or function of an organelle (plasma membrane, mitochondria, etc.) and does not trigger apoptosis. Pathologic cell death, however, can also result from apoptosis, as exemplified by viral infections and ionizing radiation.

Necrosis Results from Exogenous Cell Injury and Is Reflected in Geographic Areas of Cell Death

At the cellular level, necrosis is characterized by cell and organelle swelling, ATP depletion, increased plasma membrane permeability, release of macromolecules, and eventually inflammation. Although the mechanisms responsible for necrosis vary according to the nature of the insult and the organ involved, most instances of necrosis share certain mechanistic similarities. The model of necrotic cell death that has been studied most extensively in mechanistic terms is ischemic injury to cardiac myocytes. The sequence of events is admittedly unique to cardiac myocytes, but most features are pertinent to other cell types and injurious agents.

Necrosis is the Process by Which Exogenous Stress Kills the Cell

Cells exist in a skewed equilibrium with their external environment. The plasma membrane is the barrier that separates the extracellular fluid from the internal cellular milieu. Whatever the nature of the lethal insult, cell necrosis is heralded by disruption of the permeability barrier function of the plasma membrane. Normally, extracellular concentrations of sodium and calcium are orders of magnitude greater than intracellular concentrations. The opposite holds for potassium. The selective ion permeability requires (1) considerable energy, (2) structural integrity of the lipid bilayer, (3) intact ion channel proteins, and (4) normal association of the membrane with cytoskeletal constituents. When one or more of these elements is severely damaged, the resulting disturbance of the internal ionic balance is thought to represent the "point of no return" for the injured cell.

The role of calcium in the pathogenesis of cell death deserves special mention. Ca^{2+} concentration in extracellular fluids is in the millimolar range (10^{-3} M). By contrast, cytosol Ca^{2+} concentration is 10,000-fold lower, on the order of 10^{-7} M. Many crucial cell functions are exquisitely regulated by minute fluctuations in cytosol free calcium concentration. Thus massive influx of Ca^{2+} through a damaged plasma membrane ensures loss of cell viability.

Coagulative Necrosis

Coagulative necrosis refers to light microscopic alterations in a dead or dying cell (Fig. 1-26). Shortly after a cell's death, its outline is maintained. When stained with the usual combination of hematoxylin and eosin, the cytoplasm of a necrotic cell is more deeply eosinophilic than usual. In the nucleus chromatin is initially clumped, and then is redistributed along the nuclear membrane.

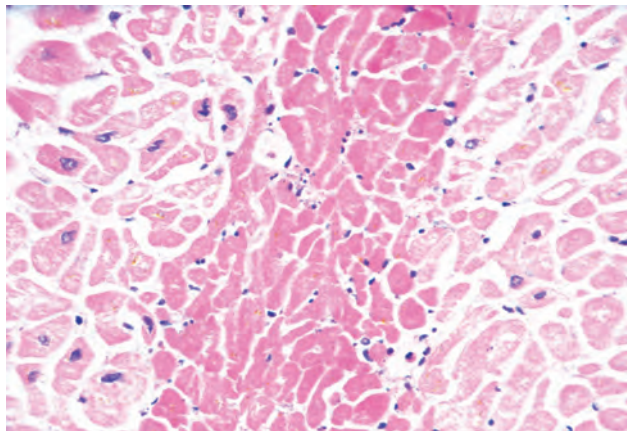


FIGURE 1-26. **Coagulative necrosis.** Photomicrograph of the heart in a patient with an acute myocardial infarction. In the center, the deeply eosinophilic necrotic cells have lost their nuclei. The necrotic focus is surrounded by paler-staining, viable cardiac myocytes.

Three morphologic changes follow:

- **Pyknosis:** The nucleus becomes smaller and stains deeply basophilic as chromatin clumping continues.
- **Karyorrhexis:** The pyknotic nucleus breaks up into many smaller fragments scattered about the cytoplasm.
- **Karyolysis:** The pyknotic nucleus may be extruded from the cell or it may manifest progressive loss of chromatin staining.

Early ultrastructural changes in a dying or dead cell reflect an extension of alterations associated with reversible cell injury (see Fig. 1-11, and Fig. 1-12). In addition to the nuclear changes described above, the dead cell features dilated endoplasmic reticulum, disaggregated ribosomes, swollen and calcified mitochondria, aggregated cytoskeletal elements, and plasma membrane blebs.

After a variable time, depending on the tissue and circumstances, a dead cell is subjected to the lytic activity of intracellular and extracellular enzymes. As a result, the cell disintegrates. This is particularly the case when necrotic cells have elicited an acute inflammatory response.

The appearance of the necrotic cell has traditionally been termed **coagulative necrosis** because of its similarity to coagulation of proteins that occurs upon heating. However, the usefulness of this historical term today is questionable.

Whereas the morphology of individual cell death tends to be uniform across different cell types, the tissue responses are more variable. This diversity is described by a number of terms that reflect specific histologic patterns that depend upon the organ and the circumstances.

Liquefactive Necrosis

When the rate of dissolution of the necrotic cells is considerably faster than the rate of repair, the resulting morphologic appearance is termed **liquefactive necrosis.** The polymorphonuclear leukocytes of the acute inflammatory reaction contain potent hydrolases capable of digesting dead cells. A sharply localized collection of these acute inflammatory cells, generally in response to bacterial infection, produces rapid cell death and tissue dissolution. The result is often an **abscess** (Fig. 1-27), which is a cavity formed by liquefactive necrosis in a solid tissue. Eventually an abscess is walled off by a fibrous capsule that contains its contents.

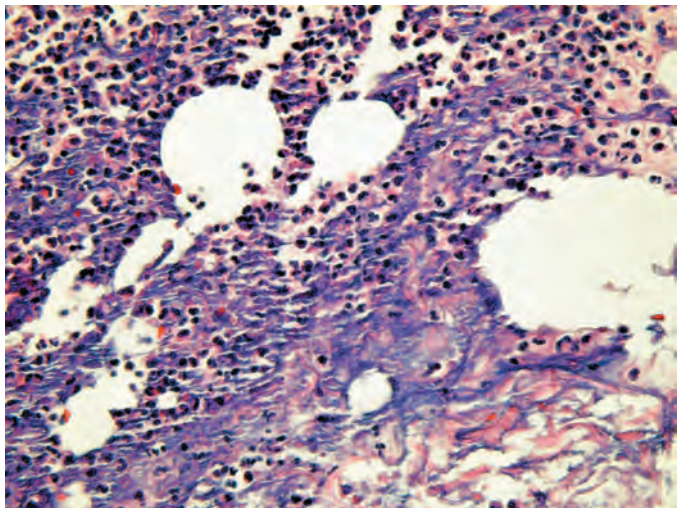


FIGURE 1-27. **Liquefactive necrosis in an abscess of the skin.** The abscess cavity is filled with polymorphonuclear leukocytes.

Coagulative necrosis of the brain may occur after cerebral artery occlusion, and is often followed by rapid dissolution—liquefactive necrosis—of the dead tissue by a mechanism that cannot be attributed to the action of an acute inflammatory response. It is not clear why coagulative necrosis in the brain and not elsewhere, is followed by dissolution of the necrotic cells, but the phenomenon may be related to the presence of more abundant lysosomal enzymes or different hydrolases specific to the cells of the central nervous system. Liquefactive necrosis of large areas of the central nervous system can lead to an actual cavity or cyst that will persist for the life of the person.

Fat Necrosis

Fat necrosis specifically affects adipose tissue and most commonly results from pancreatitis or trauma (Fig. 1-28). The unique feature determining this type of necrosis is the presence of triglycerides in adipose tissue. The process begins when digestive enzymes, normally found only in the pancreatic duct and small intestine, are released from injured pancreatic acinar cells and ducts into the extracellular spaces. On extracellular activation, these enzymes digest the pancreas itself as well as surrounding tissues, including adipose cells:

1. Phospholipases and proteases attack the plasma membrane of fat cells, releasing their stored triglycerides.
2. Pancreatic lipase hydrolyzes the triglycerides, a process to produce free fatty acids.
3. Free fatty acids bind calcium and are precipitated as calcium soaps. These appear as amorphous, basophilic deposits at the edges of irregular islands of necrotic adipocytes.

Grossly, fat necrosis appears as an irregular, chalky white area embedded in otherwise normal adipose tissue. In the case of traumatic fat necrosis, we presume that triglycerides and lipases are released from the injured adipocytes. In the breast, fat

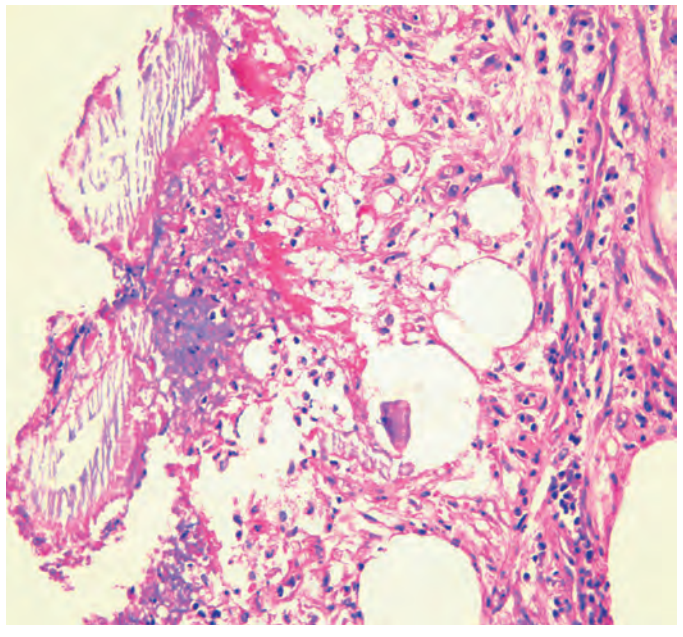
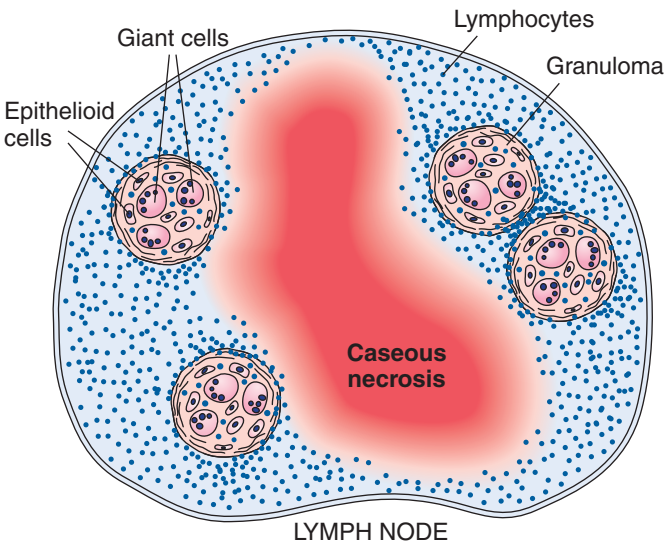


FIGURE 1-28. **Fat necrosis.** Photomicrograph of peripancreatic adipose tissue from a patient with acute pancreatitis shows an island of necrotic adipocytes adjacent to an acutely inflamed area. Fatty acids are precipitated as calcium soaps, which accumulate as amorphous, basophilic deposits at the periphery of the irregular island of necrotic adipocytes.

necrosis secondary to trauma is not uncommon and may mimic a tumor.

Caseous Necrosis

Caseous necrosis is characteristic of tuberculosis (Fig. 1-29). The lesions of tuberculosis are tuberculous granulomas, or tubercles. In the center of such granulomas, the accumulated mononuclear cells that mediate the chronic inflammatory reaction to the offending mycobacteria are killed. In caseous necrosis, unlike coagulative necrosis, the necrotic cells fail to retain their cellular outlines. They do not, however, disappear by lysis, as in liquefactive necrosis. Rather, the dead cells persist indefinitely as amorphous, coarsely granular, eosinophilic debris. Grossly, this debris is grayish white, soft and friable. It resembles clumpy cheese, hence the name **caseous necrosis.** This distinctive type of necrosis is generally attributed to the toxic effects of the mycobacterial cell wall, which contains complex waxes (peptidoglycolipids) that exert potent biological effects.



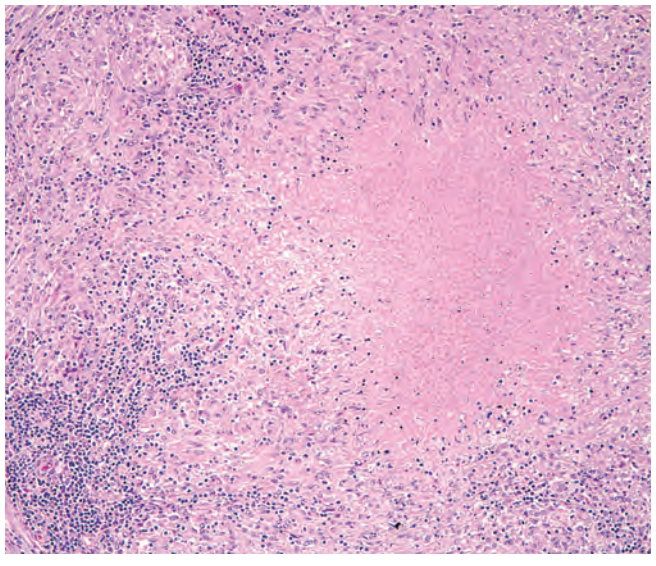


FIGURE 1-29. **Caseous necrosis in a tuberculous lymph node. A.** The typical amorphous, granular, eosinophilic, necrotic center is surrounded by granulomatous inflammation. **B.** Photomicrograph showing a tuberculous granuloma with central caseous necrosis.

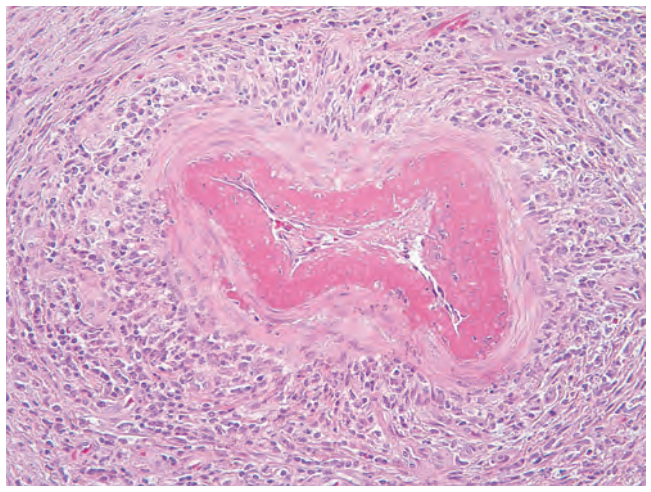


FIGURE 1-30. **Fibrinoid necrosis.** An inflamed muscular artery in a patient with systemic arteritis shows a sharply demarcated, homogeneous, deeply eosinophilic zone of necrosis.

Fibrinoid Necrosis

Fibrinoid necrosis is an alteration of injured blood vessels, in which insudation and accumulation of plasma proteins cause the wall to stain intensely with eosin (Fig. 1-30). The term is something of a misnomer, however, because the eosinophilia of the accumulated plasma proteins obscures the underlying alterations in the blood vessel, making it difficult, if not impossible, to determine whether there truly is necrosis in the vascular wall.

Necrosis Usually Involves Accumulation of a Number of Intracellular Insults

The processes by which cells undergo death by necrosis vary according to the cause, organ, and cell type. The best studied and most clinically important example is ischemic necrosis of cardiac myocytes. The mechanisms underlying the death of cardiac myocytes are in part unique, but the basic processes that are involved are comparable to those in other organs. Some of the unfolding events may occur simultaneously; others may be sequential (Fig. 1-31).

1. **Interruption of blood supply decreases delivery of O_2 and glucose.** Anoxia, whether it arises from ischemia (e.g., atherosclerosis) or other causes (e.g., blood loss from trauma), decreases delivery of both oxygen and glucose to myocytes. For most cells, but especially for cardiac myocytes (which do not store much energy), this combined insult is formidable.
2. **Anaerobic glycolysis leads to overproduction of lactate and decreased intracellular pH.** The lack of O_2 during myocardial ischemia both blocks production of ATP and inhibits mitochondrial oxidation of pyruvate. Instead, pyruvate is reduced to lactate in the cytosol, where its accumulation lowers intracellular pH. The acidification of the cytosol initiates a downward spiral of events that propels the cell towards disaster.
3. **Distortion of the activities of pumps in the plasma membrane skews the ionic balance of the cell.** Na^+ accumulates because the lack of ATP renders the Na^+/K^+ ion exchanger inactive, an effect that leads to activation of the Na^+/H^+ ion exchanger. This pump is normally quiescent, but when intracellular acidosis threatens, it pumps H^+ out of the cell in

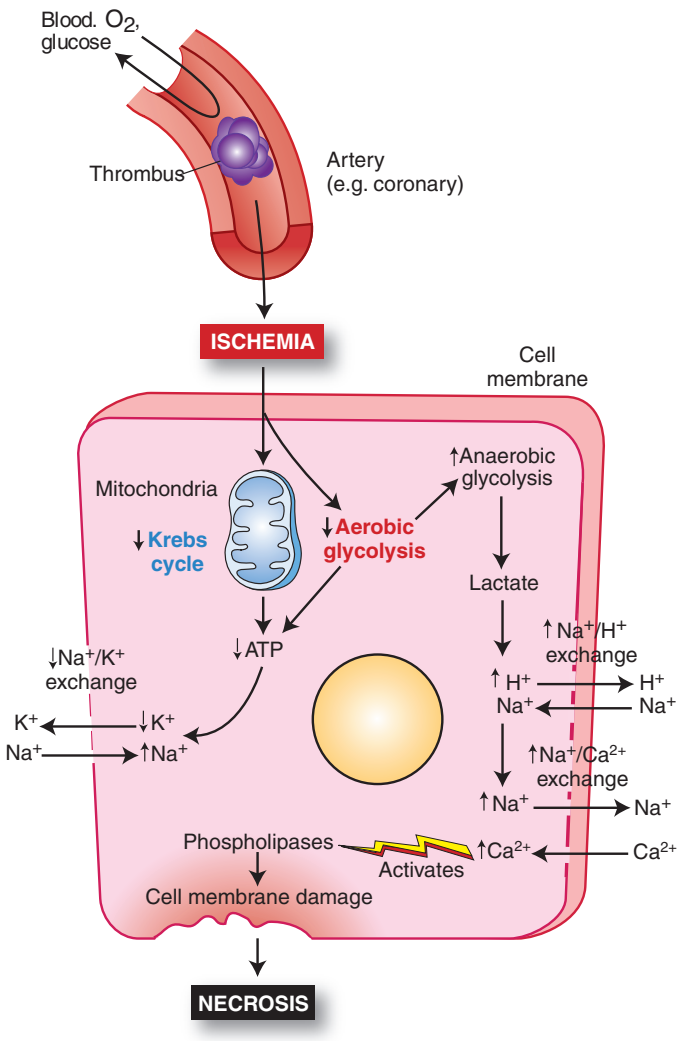


FIGURE 1-31. **Mechanisms by which ischemia leads to cell death.** ATP = adenosine triphosphate; Ca^{2+} = calcium ion; H^+ = hydrogen ion; K^+ = potassium ion; Na^{2+} = sodium ion; O_2 = oxygen.

exchange for Na^+ to maintain proper intracellular pH. The resulting increase in intracellular sodium activates the Na^+/Ca^{2+} ion exchanger, which increases calcium entry. Ordinarily, excess intracellular Ca^{2+} is extruded by an ATP-dependent calcium pump. However, with ATP in very short supply and Ca^{2+} accumulates in the cell.

4. **Activation of phospholipase A_2 (PLA_2) and proteases disrupts the plasma membrane and cytoskeleton.** High calcium concentrations in the cytosol of an ischemic cell activate PLA_2, leading to degradation of membrane phospholipids and consequent release of free fatty acids and lysophospholipids. The latter act as detergents that solubilize cell membranes. Both fatty acids and lysophospholipids are also potent mediators of inflammation (see Chapter 2), an effect that may further disrupt the integrity of the already compromised cell.

   Calcium also activates a series of proteases that attack the cytoskeleton and its attachments to the cell membrane. As the cohesion between cytoskeletal proteins and the plasma membrane is disrupted, membrane blebs form, and the shape of the cell is altered. The combination of electrolyte imbalance and increased cell membrane permeability causes the cell to swell, a frequent morphologic prelude to dissolution of the cell.

5. **The lack of O_2 impairs mitochondrial electron transport, thus decreasing ATP synthesis and facilitating production of ROS.** Under normal circumstances, about 3% of the oxygen entering the mitochondrial electron transport chain is converted to ROS. During ischemia, generation of ROS increases because of (1) decreased availability of favored substrates for the electron transport chain, (2) damage to elements of the chain, and (3) reduced activity of mitochondrial SOD. ROS cause peroxidation of cardiolipin, a membrane phospholipid that is unique to mitochondria and is sensitive to oxidative damage by virtue of its high content of unsaturated fatty acids. This attack inhibits the function of the electron transport chain and decreases its ability to produce ATP.

6. **Mitochondrial damage promotes the release of cytochrome c to the cytosol.** In normal cells the mitochondrial permeability transition pore (MPTP) opens and closes sporadically. Ischemic injury to mitochondria causes sustained opening of the MPTP, with resulting loss of cytochrome c from the electron transport chain. This process further diminishes ATP synthesis and may, under some circumstances, also trigger apoptotic cell death (see below).

7. **The cell dies.** When the cell can no longer maintain itself as a metabolic unit, necrotic cell death occurs. The line between reversible and irreversible cell injury (i.e., the "point of no return,") is not precisely defined, but it is probably reached at about the time that the MPTP opens. Although this event by itself is not necessarily lethal, by the time it occurs, disruption of the electron transport chain has become irreparable and eventually necrotic cell death is inevitable.

Ample data from experimental and clinical studies indicate that pharmacologic interference with a number of events involved in the pathogenesis of cell necrosis can preserve cell viability after an ischemic insult. The Na^+/H^+ exchanger has become an interesting target for therapeutic intervention to maintain the viability of cardiac myocytes during acute ischemia. Treatments that increase glucose uptake and redress some of the ionic imbalances may preserve myocyte viability during ischemia. Indeed, some success has been reported with simple administration of a combination of glucose, insulin, and potassium.

Apoptosis, or Programmed Cell Death, Refers to a Cellular Suicide Mechanism

Apoptosis is a prearranged pathway of cell death triggered by a variety of extracellular and intracellular signals. It is part of the balance between the life and death of cells and determines that a cell dies when it is no longer useful or when it may be harmful to the larger organism. It is also a self-defense mechanism, cells that are infected with pathogens or in which genomic alterations have occurred are destroyed. In this context, many pathogens have evolved mechanisms to inactivate key components of the apoptotic signaling cascades. Apoptosis detects and destroys cells that harbor dangerous mutations, thereby maintaining genetic consistency and preventing the development of cancer. By contrast, as in the case of infectious agents, successful clones of tumor cells often devise mechanisms to circumvent apoptosis.

The Morphology of Apoptosis

Apoptotic cells are recognized by nuclear fragmentation and pyknosis, generally against a background of viable cells. Importantly, individual cells or small groups of cells undergo apoptosis, whereas necrosis characteristically involves larger geographic areas of cell death. Ultrastructural features of apoptotic cells include (1) nuclear condensation and fragmentation; (2) segregation of cytoplasmic organelles into distinct regions; (3) blebs of the plasma membrane; and (4) membrane-bound cellular fragments, which often lack nuclei (Fig. 1-32).

Cells that have undergone necrotic cell death tend to elicit strong inflammatory responses. Inflammation, however, is not generally seen in the vicinity of apoptotic cells (Fig. 1-33). Mononuclear phagocytes may contain cellular debris from apoptotic cells, but recruitment of neutrophils or lymphocytes is uncommon (see Chapter 2). In view of the numerous developmental, physiologic and protective functions of apoptosis, the lack of inflammation is clearly beneficial to the organism.

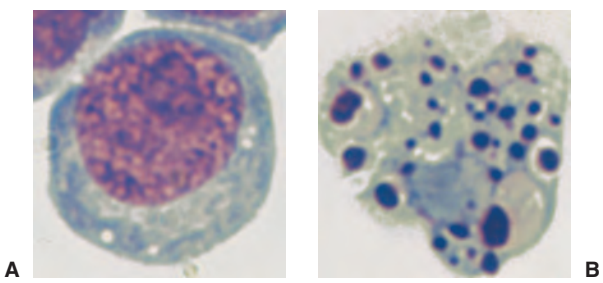
Apoptosis in Developmental and Physiologic Processes

Fetal development involves the sequential appearance and regression of many anatomical structures: some aortic arches do not persist, the mesonephros regresses in favor of the metanephros, interdigital tissues disappear to allow discrete fingers and toes, and excess neurons are pruned from the developing brain. In the generation of immunologic diversity, clones of cells that recognize normal self antigens are deleted by apoptosis.

Physiologic apoptosis principally involves the progeny of stem cells that are continuously dividing (e.g., stem cells of the hematopoietic system, gastrointestinal mucosa, and epidermis). Apoptosis of mature cells in these organs prevents overpopulation of the respective cell compartments by removing senescent cells and thus maintaining the normal architecture and size of the organ systems.

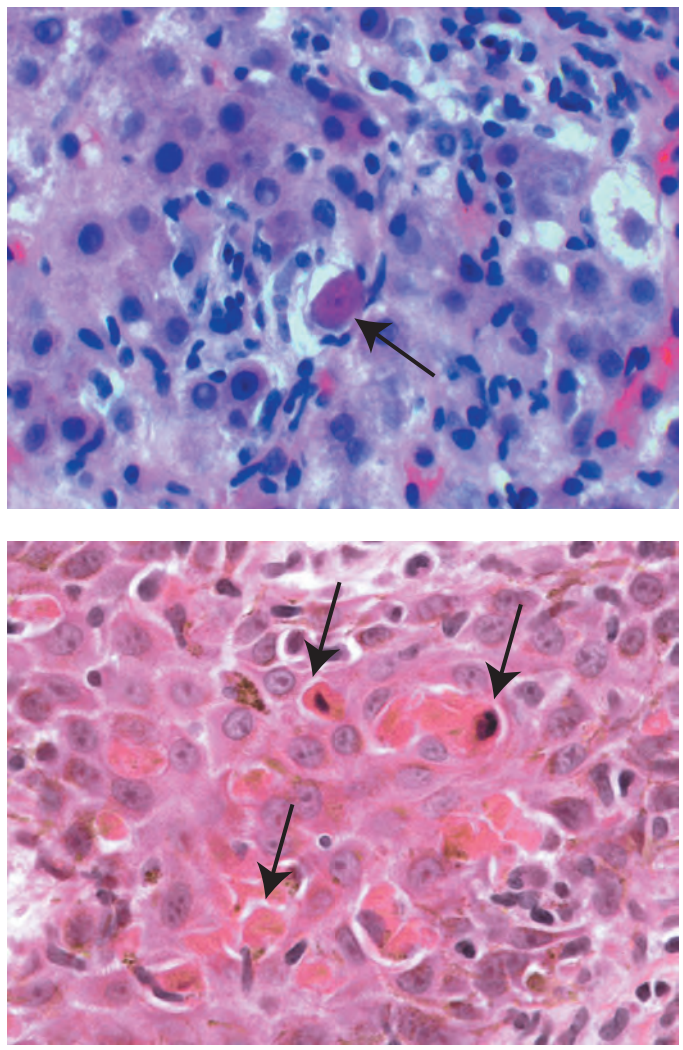
Apoptosis Eliminates Obsolescent Cells

A normal turnover of cells in many organs is essential to maintain the size and function of that cellular compartment. For example, as cells are continuously supplied to the circulating blood, older and less functional white blood cells must be eliminated to maintain the normal complement of the cells. Indeed, the pathologic accumulation of polymorphonuclear leukocytes in chronic myelogenous leukemia results from a mutation that inhibits apoptosis and therefore allows these cells to persist. In the mucosa of the small intestine, cells migrate from the depths of the crypts to the tips of the villi, where they undergo apoptosis and are sloughed into the lumen.



A B

FIGURE 1-32. **Apoptosis.** A viable leukemic cell (**A**) contrasts with an apoptotic cell (**B**) in which the nucleus has undergone condensation and fragmentation.



A

B

FIGURE 1-33. **Histopathologic illustrations of apoptosis in the liver in viral hepatitis** (**A**) and in the skin in erythema multiforme (**B**). Apoptotic cells are highlighted by *arrows*.

Apoptosis also maintains the balance of cellularity in organs that respond to trophic stimuli, such as hormones. An illustration of such an effect is the regression of lactational hyperplasia of the breast in women who have stopped nursing their infants. On the other side of the reproductive divide, postmenopausal women suffer atrophy of the endometrium after hormonal support has withered.

Apoptosis Deletes Mutant Cells

The integrity of an organism requires that it be able to recognize irreparable damage to DNA, after which the damaged cells must be eliminated by apoptosis. There is a finite error rate in DNA replication, owing to the infidelity of DNA polymerases. In addition, environmental stresses such as ultraviolet (UV) light, ionizing radiation, and DNA-binding chemicals may also alter DNA structure. There are several means, the most important of which is probably p53, by which the cell recognizes genomic abnormalities and "assesses" whether they can be repaired. If the DNA damage is too severe to be repaired, a cascade of events leading to apoptosis is activated and the cell dies. This process protects an organism from the consequences of a nonfunctional cell or one that cannot control its own proliferation (e.g., a cancer cell).

Apoptosis as a Defense against Dissemination of Infection

When a cell "detects" episomal (extrachromosomal) DNA replication, as in a viral infection, it tends to initiate apoptosis. This effect can be viewed as a means to eliminate infected cells before they can spread the virus. Many viruses have evolved protective mechanisms to manipulate cellular apoptosis. Viral gene products that inhibit apoptosis have been identified for many viruses, including human immunodeficiency virus (HIV), human papillomavirus, adenovirus, and many others. In some cases these viral proteins bind and inactivate certain cellular proteins (e.g., p53) that are important in signaling apoptosis. In other instances, they may act at various points in the signaling pathways that activate apoptosis.

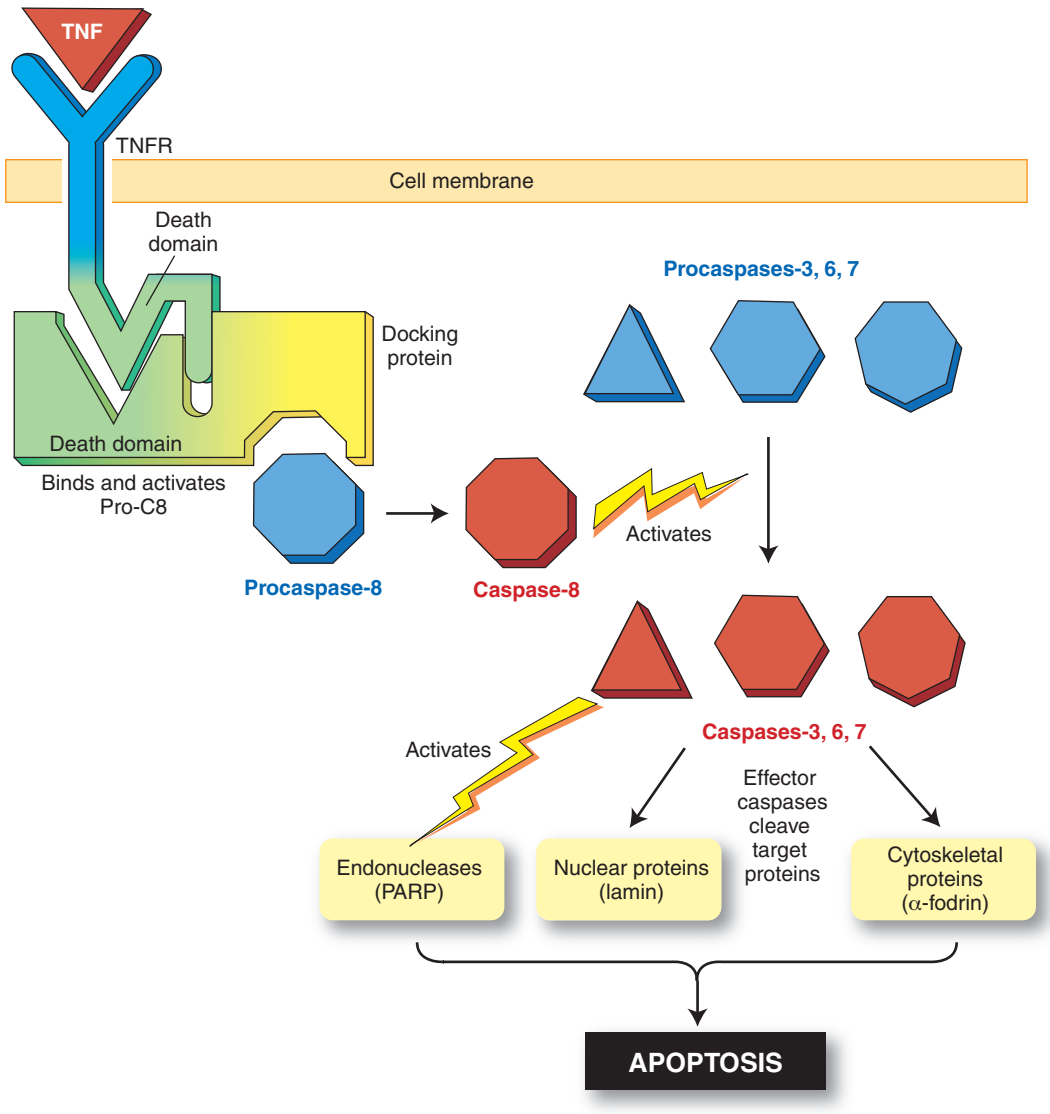
Signaling Apoptosis

Apoptosis is a final effector mechanism that can be initiated by many different stimuli, whose signals are propagated by a number of pathways. Unlike necrosis, apoptosis engages the cell's own signaling cascades. That is, a cell that undergoes apoptosis is an active participant in its own death (suicide). Most intermediate enzymes that transduce proapoptotic signals belong to a family of cysteine proteases called **caspases**.

Apoptosis May be Initiated by Receptor–Ligand Interactions at the Cell Membrane

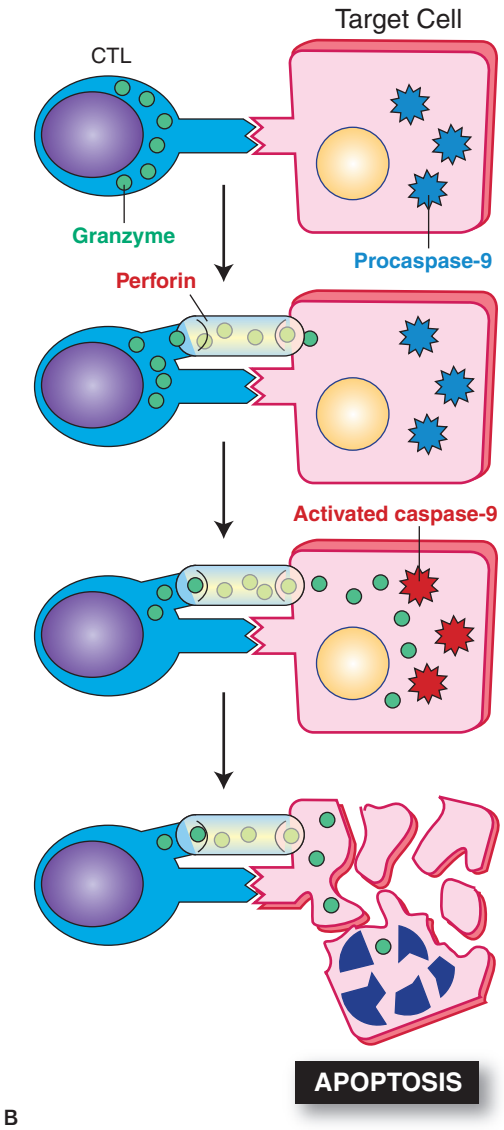
The best understood initiators of apoptosis at the cell membrane are the binding of TNF-α to its receptor (TNFR), and that of the Fas ligand to its receptor (Fas, or Fas receptor). TNF-α is most often a free cytokine, whereas Fas ligand is located at the plasma membrane of certain cells, such as cytotoxic effector lymphocytes.

The receptors for TNF-α and Fas ligand become activated when they bind their ligands. These transmembrane proteins have specific amino acid sequences, termed **death domains,** in their cytoplasmic tails that act as docking sites for death domains of other proteins that participate in the signaling process leading to apoptosis (Fig. 1-34A). After binding to the receptors, the latter proteins activate downstream signaling molecules, especially procaspase-8, which is converted to caspase-8. In turn, caspase-8 initiates an activation cascade of other downstream caspases in

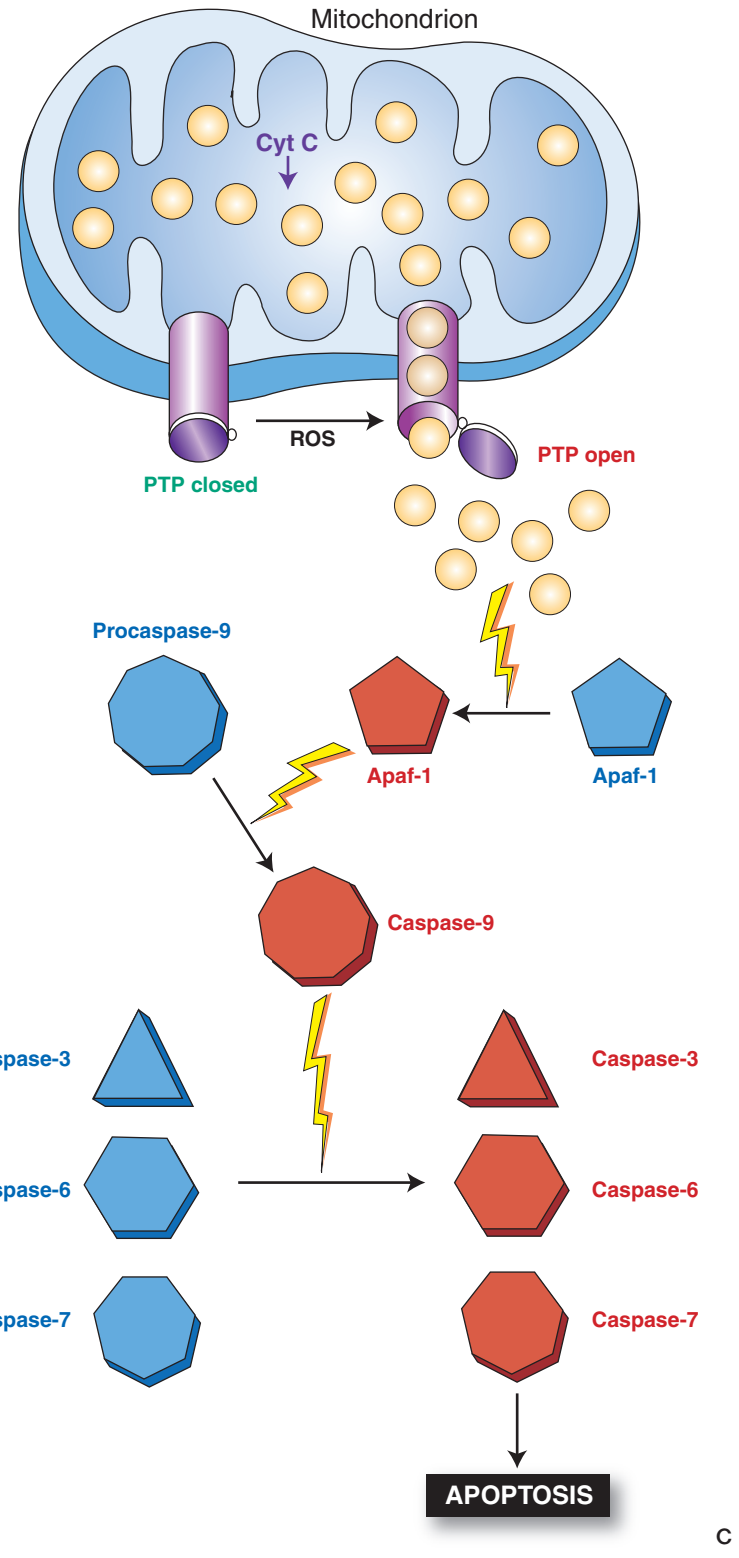


A

FIGURE 1-34. **Mechanisms by which apoptosis may be initiated, signaled, and executed. A.** Ligand–receptor interactions that lead to caspase activation. TNF = tumor necrosis factor; TNFR = tumor necrosis factor receptor.



B



C

FIGURE 1-34. *(Continued)* **B.** Immunologic reactions in which granzyme released by cytotoxic lymphocytes (CTLs) causes apoptosis. Procaspase-9 is shown as an example of procaspases that are activated by granzyme B. **C.** Opening of the mitochondrial permeability transition pore, leading to Apaf-1 activation, thereby triggering the apoptotic cascade. Cyt C = cytochrome C; PTP = permeability transition pores; ROS = reactive oxygen species.

the apoptosis pathway. These caspases, (3, 6, and 7) activate a number of nuclear enzymes (e.g., poly-adenosine diphosphate [ADP]-ribosyl polymerase [PARP]) that mediate the nuclear fragmentation of apoptotic cell death.

Activation of caspase signaling also occurs when killer lymphocytes, mainly cytotoxic T cells, recognize a cell as foreign. These lymphocytes release perforin and granzyme B. Perforin, as its name suggests, punches a hole in the plasma membrane of a target cell, through which granzyme B enters and activates procaspases-3, -8, and -9 directly (see Fig. 1-34B).

Apoptosis and Mitochondrial Proteins

The mitochondrial membrane is a key regulator of the balance between cell death and cell survival. Proteins of the Bcl-2 family reside in the mitochondrial inner membrane and are either proapoptotic or anti-apoptotic (prosurvival). Proapoptotic proteins in this family include Bax, Bak, Bad, Bid, and Bik; anti-apoptotic proteins are Bcl-2, Bcl-X_L, and A1. These members of the Bcl-2 family form homo- and heterodimers in the mitochondrial membrane. Heterodimers of pro- and anti-apoptotic Bcl-2 proteins, or homodimers of anti-apoptotic proteins, promote cell survival. If the balance shifts to homodimers of proapoptotic proteins, the apoptotic cascade is activated (see Fig. 1-34C).

Cytochrome c is a key player in apoptosis. It is normally bound by a phospholipid, **cardiolipin** (CL), to the inner mitochondrial membrane. Cytochrome c participates in many mitochondrial processes, including electron transport and membrane fluidity. ROS (e.g., superoxide) cause apoptosis by opening the mitochondrial permeability transition pores (PTP). This process may involve oxidation of CL, resulting in cytochrome c release through the PTP (see below).

The formation of nitric oxide radical (NO•) has similar consequences. ROS also activate neutral sphingomyelinase, a cytosolic enzyme that releases ceramide from sphingomyelin in the plasma membrane. In turn, ceramide stimulates cellular stress responses (stress-activated protein kinases), which then activate procaspase-8.

Activation of p53 by DNA damage or by other means also initiates apoptotic signaling through the mitochondria. As a transcription factor, p53 increases the production of the proapoptotic mitochondrial protein Bax and the proapoptotic FasR. Apoptosis is also initiated by p53 through means unrelated to transcriptional activation. The mechanisms by which mitochondria exert such a powerful effect on apoptosis have recently been elucidated (see Fig. 1-34C). Bcl-2 dimers at the mitochondrial membrane bind the protein Apaf-1. A surfeit of proapoptotic constituents of the Bcl-2 family leads to the release of Apaf-1. At the same time, PTP open and cytochrome c leaks through the mitochondrial membrane. Cytosolic cytochrome c activates Apaf-1, which in turn converts procaspase-9 to caspase-9. Caspase-9 activates downstream caspases (3, 6, and 7) in the same manner as caspase-8.

The Equilibrium between Pro- and Anti-apoptotic Signals

Apoptosis can be viewed as a default pathway and the survival of many cells is contingent upon constant anti-apoptotic (prosurvival) signals. In other words, a cell must actively choose life rather than succumbing to the despair of apoptosis. Survival signals are transduced through receptors linked to phosphatidylinositol 3-kinase (PI3K), the enzyme that phosphorylates PIP_2 and PIP_3 (phosphatidyl inositol bis- and triphosphates). By antagonizing apoptosis, PI3K plays a critical role in cell viability. A prototypical receptor that signals via this mechanism is insulin-like growth factor-I receptor (IGF-IR). Paradoxically, PI3K is also activated by TNFR after binding TNF-α.Thus, the same cell membrane receptor that induces apoptosis in some circumstances can also initiate anti-apoptotic signaling in other situations.

PI3K exerts antiapoptotic effects through intracellular mediators, which favor survival by activating protein kinase B (PKB), also called Akt. The latter then inactivates several important proapoptotic proteins (e.g., the Bcl-2 family member, Bad). More importantly, PKB activates NFκB (nuclear factor κB), an important transcription factor that promotes the expression of proteins (A1 and Bcl-X_L) that prevent the loss of cytochrome c from mitochondria and promote cell survival. PKB also stimulates signaling mechanisms that activate cell division.

Apoptosis Activated by p53

A pivotal molecule in the cell's life-and-death dance is the versatile protein p53, which preserves the viability of an injured cell when DNA damage can be repaired, but propels it toward apoptosis after irreparable harm has occurred (p53 is discussed in greater detail in Chapter 5).

Homeostasis of p53

A delicate balance exists between the stabilization and destruction of p53. Thus, p53 binds to several proteins (e.g., Mdm2), which promote its degradation via ubiquitination. The ability of p53 to avoid this pernicious association depends upon certain structural changes in the protein in response to stress, DNA damage, and so forth. These molecular modifications decrease its interaction with Mdm2, thereby enhancing survival of p53 and permitting its accumulation.

Function of p53

After it binds to areas of DNA damage, p53 activates proteins that arrest the cell in stage G1 of the cell cycle, allowing time for DNA repair to proceed. It also directs DNA repair enzymes to the site of injury. If DNA damage cannot be repaired, p53 activates mechanisms that lead to apoptosis.

There are several pathways by which p53 induces apoptosis. It downregulates transcription of the antiapoptotic protein Bcl-2, while it upregulates transcription of the proapoptotic genes *bax* and *bak*. In addition, certain DNA helicases and other enzymes are activated by p53-mediated recognition of DNA damage, an effect that leads to translocation of a number of proapoptotic proteins (e.g., Fas) from the cell membrane to the cytosol.

Stress also leads to accumulation of p53. Activation of certain oncogenes, such as *c-myc,* increases the amount of an Mdm2-binding protein ($p14^{arf}$), thereby protecting p53 from Mdm2-induced destruction. Additional forms of stress that lead to p53 accumulation include hypoxia, depletion of ribonucleotides, and loss of cell–cell adhesion during oncogenesis.

Inactivation of p53

Proteins of a number of oncogenic viruses inactivate p53 by binding to it. In fact, p53 was first identified as a cellular protein that coprecipitated with one such protein (SV40 large T antigen). Inactivating mutations of p53 are the most common DNA alterations in human cancer, which underscores its role as a switch that allows repair of DNA but triggers cellular suicide if that proves to be impossible.

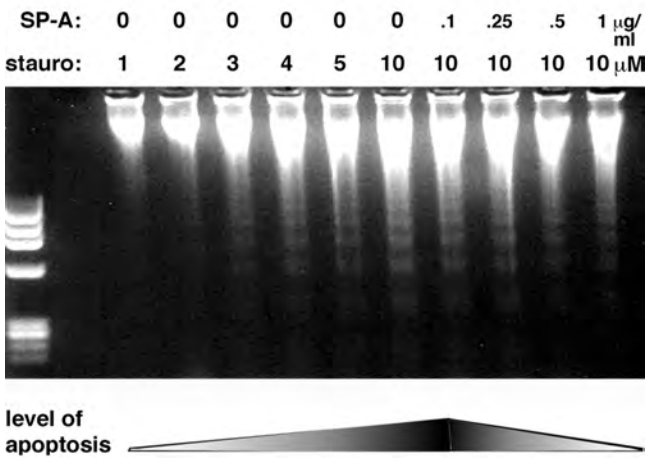


FIGURE 1-35. **DNA fragmentation in apoptosis.** Agarose gel electrophoresis of DNA isolated from lung epithelial cells treated with different amounts of staurosporine, which induces apoptosis, and with different amounts of surfactant protein-A (SP-A), which protects these cells from apoptosis. The schematic at the bottom illustrates the degree of apoptosis observed in these cells as a function of the concentrations of these two agents. At low concentrations of staurosporine or at high concentrations of SP-A, genomic DNA is largely unfragmented and thus remains at the top of the gel. By contrast, internucleosomal cleavage of DNA, such as occurs in apoptosis, is reflected in multiple, regularly spaced genomic DNA fragments, resembling a ladder. This phenomenon is called "laddering."

Quantitative Assays for Apoptosis

Apoptotic cells can be detected by demonstrating fragmented DNA. A popular method involves the demonstration of nucleosomal "laddering." This virtually diagnostic pattern of DNA degradation, which is characteristic of apoptotic cell death, results from cleavage of chromosomal DNA at nucleosomes by activated endonucleases. Since nucleosomes are regularly spaced along the genome, a pattern of regular bands can be seen when fragments of cellular DNA are separated by electrophoresis (Fig. 1-35).

Other assays are also used to detect and quantitate apoptosis. One is the TUNEL assay (terminal deoxyribonucleotidyl transferase [TdT]-mediated deoxyuridine triphosphate [dUTP]-digoxigenin nick end labeling) in which TdT transfers a fluorescent nucleotide to exposed breakpoints in DNA. Apoptotic cells that incorporate the labeled nucleotide are visualized by fluorescence microscopy or flow cytometry. Apoptotic cells that have extruded some of the DNA have less than their normal diploid content. Automated measurement of DNA content in individual cells by flow cytometry thus produces a population distribution according to DNA content (cytofluorography). Other means of detecting apoptosis depend upon quantitating the activated forms of enzymes that signal apoptosis, including the nuclear proteins PARP and lamin A.

In summary, cells are continually poised between survival and apoptosis: their fate rests on the balance of powerful intracellular and extracellular forces, whose signals constantly act upon and counteract each other. Often, apoptosis functions as a self-protective programmed mechanism that leads to a cell's suicide when its survival may be detrimental to the organism. At other times, apoptosis is a pathologic process that contributes to many disorders, especially degenerative diseases. Thus, pharmacologic manipulation of apoptosis is an active frontier of drug development.

Biological Aging

Old age is a consequence of civilization; it is a condition rarely encountered in the animal kingdom or in primitive societies. From an evolutionary perspective, the aging process presents conceptual difficulties. Since animals in the wild do not attain their maximum longevity, how did aging evolve? The consequences of aging arise after the reproductive period and thus should not have an evolutionary impact.

Aging must be distinguished from mortality on the one hand and from disease on the other. Death is a random event; an aged person who does not succumb to the most common cause of death will die from the second, third, or tenth most common cause. Although the increased vulnerability to disease among the elderly is an interesting problem, disease itself is entirely distinct from aging.

Maximal Life Span Has Remained Unchanged

Millennia ago the psalmist sang of a natural life span of 70 years, which with vigor may extend to 80. By contrast, it is estimated that the usual age at death of Neolithic humans was 20 to 25 years, and the average life span today in some regions is often barely 10 years more.

The difference between humans in primitive and in civilized environments is analogous to that observed between animals in their natural habitat and those in a zoo (Fig. 1-36). For animals in the wild, after an initial high mortality during maturation, a progressive linear decline in survival is noted, ending at the maximum life span of the species. This steady decrease in the number of mature animals does not reflect aging but rather sporadic events, such as encounters with beasts of prey, accidental trauma, infection, starvation, and so on. On the other hand, survival in the protected environment of a zoo is characterized by slow attrition until old age, at which time the steep decline in numbers is attributable to the aging process. *Interestingly, the maximum life span attained is not significantly altered by a protected environment.* An analogous situation is seen in studies of human mortality. Less than a century ago, the steep linear slope of mortality in human adults principally reflected random accidents and infections. With improved safety and sanitation, antibiotics and other drugs, and better diagnostic and therapeutic methods, the age-adjusted death rate in the United States has declined by 40% since 1970 and in 2004, life expectancy at

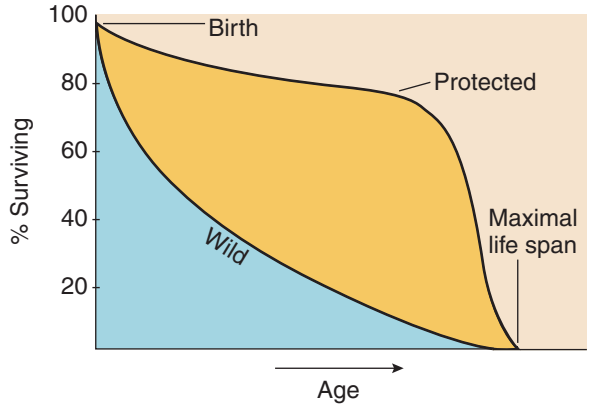


FIGURE 1-36. **Life span of animals in their natural environment compared with that in a protected habitat.** Note that both curves reach the same maximal life span.

the time of birth was 80.4 years for females and 75.2 years for males. At age 60, life expectancy was 20 additional years and 24 additional years, respectively.

Yet the maximum human life span has remained constant at about 110 years. Even if diseases associated with old age, such as cardiovascular disease and cancer, were eliminated, only a modest increase in average life expectancy would be seen. A long period of good health and low mortality rate would be followed by a precipitously increased mortality owing to aging itself; the life span would, for practical purposes, remain on the lower side of 100 years. Given the current life expectancy, the prevention or cure of the causes of premature death would have little impact on mean longevity.

Why do women live longer than men? The male-to-female ratio is 106:100 at birth, but from that time on, more women than men survive at every age, and at age 75 the male-to-female ratio is 2:3. Interestingly, greater female longevity is almost universal in the animal kingdom. At the cellular level, somatic cells with the female genotype are no hardier than those with the male pattern. Factors involved in the difference in average human longevity include the greater male mortality from violent causes and greater susceptibility to cardiovascular disease, cancer, respiratory illness, and cirrhosis in middle and old age. Historical differences between the sexes in cigarette smoking and alcohol consumption are also important in the gender gap in longevity. Indeed, smoking alone has been estimated to account for 4 of the 7 years of sex differential in longevity at birth. Thus, if men escape from these hazards, the gap in longevity between the sexes is progressively reduced with advancing age to just over 1 year beyond age 85.

Functional and Structural Changes Accompany Aging

The insidious effects of aging can be detected in otherwise healthy persons. The great leaps of imagination in theoretical physics and mathematics are almost exclusively made by the young. In many sports, an athlete in his or her 30s may be referred to as "aged." Even in the absence of specific diseases or vascular abnormalities, beginning in the fourth decade of life there is a progressive decline in many physiologic functions (Fig. 1-37), including such easily measurable parameters as muscular strength, cardiac reserve, nerve conduction time, pulmonary vital capacity, glomerular filtration, and vascular elasticity. These functional deteriorations are accompanied by structural changes. Lean body mass decreases and the proportion of fat rises. Constituents of the connective tissue matrix are progressively cross-linked. Lipofuscin ("wear and tear") pigment accumulates in organs such as the brain, heart, and liver.

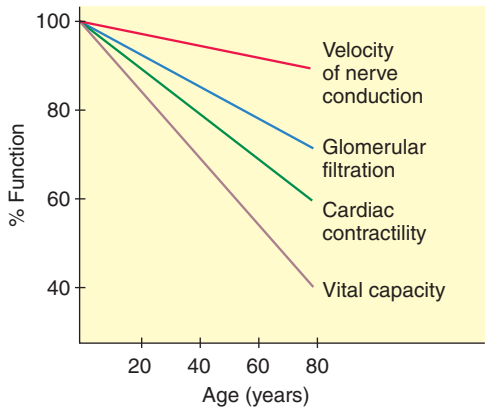


FIGURE 1-37. **Decrease in human physiologic capacities as a function of age.**

The salient characteristic of aging is not so much a decrease in basal functional capacity as it is a reduced ability to adapt to environmental stresses. Although resting pulse is unchanged, the maximal increase with exercise is reduced with age and the time required to return to normal heart rate is prolonged. Similarly, the aged show impaired adaptation to ingested carbohydrates: fasting blood glucose levels are often normal compared with younger people, but they rise higher after a carbohydrate meal and decline more slowly.

The Cellular Basis of Aging Is Studied in Culture

Although the biological basis for aging is obscure, there is general agreement that its elucidation, as in all pathologic conditions, should be sought at the cellular level. Various theories of cellular aging have been proposed, but the evidence adduced for each is at best indirect and is often derived from data obtained in cultured cells. An adequate theory should be parsimonious, compatible with the species-specific differences in life spans and consistent with the fact that most noncycling cells, such as neurons and myocytes, undergo a linear, relatively uniform functional decline with age. Below, we review the major considerations in this controversial field of investigation.

Support for the concept of a genetically programmed life span comes from studies of replicating cells in tissue culture. Unlike cancer cells, normal cells in tissue culture do not exhibit an unrestrained capacity to replicate. Cultured human fibroblasts undergo about 50 population doublings, after which they are irreversibly arrested in the G1 phase of the cell cycle and no longer divide. If they are exposed to an oncogenic virus or a chemical carcinogen, they may continue to replicate; in a sense, they become immortal. A rough correlation between the number of population doublings in fibroblasts and life span has been reported in several species. For example, rat fibroblasts exhibit considerably fewer doublings than do human ones. Moreover, cells obtained from persons afflicted with a syndrome of precocious aging, such as progeria (see below), also display a conspicuously reduced number of population doublings in vitro.

In Vivo Studies

There is no demonstrable age-related change in vivo in the replicative capacity of rapidly cycling cells (e.g., epithelial cells of the intestine). One is, therefore, left with the apparent paradox that replicating cells in culture have a limited life span, whereas aging in vivo seems mainly to affect the functional capacity of postmitotic cells. In other words, persons do not age because cells of the intestinal tract or bone marrow fail to replicate. However, if one considers that a function of cells in vitro is to proliferate, then they indeed display a major failure in functional capacity, and in many studies cells in culture are used as a model for the study of aging.

Just as Greek mythology postulates that the offspring of unions between immortal gods and mortal humans are mortal (e.g., Hercules), cellular senescence in vitro is also a dominant genetic trait. Thus, hybrids between normal human cells in vitro, which exhibit a limited number of cell divisions, and immortalized cells with an indefinite capacity to divide, undergo senescence. This finding shows that senescence is dominant over

immortality. Replicative senescence–related genes have been identified on a number of human chromosomes, but the precise function(s) encoded by most of them have not been elucidated.

Telomerase and Senescence

An attractive explanation for cell senescence in vitro centers on the genetic elements at the tips of chromosomes, termed **telomeres.** These are series of short repetitive nucleotide sequences (TTAGGG in vertebrates) that vary in size from 70 in *Tetrahymena* (a protozoan) to 2000 in human chromosomes. Since DNA polymerase cannot copy the linear chromosomes all the way to the tip, the telomeres tend to shorten with each cell division until a critical diminution in size interfered with replication. Moreover, oxidative stress induces single-stranded damage in telomeric DNA, and this defect cannot be repaired in telomeres.

To overcome this "end-replication" problem, most eukaryotic cells use a ribonucleoprotein enzyme termed **telomerase,** which can extend chromosome ends. It has thus been proposed that telomere shortening acts as a molecular clock ("replicometer"), which produces senescence after a defined number of cell divisions in vitro. In this context, ectopic expression of telomerase reverses the senescent phenotype, and after immortalization of cells in vitro, telomerase activity can also be demonstrated.

Senescence also functions as a tumor-suppressing mechanism, limiting cell proliferative capacity in vivo. This idea implies that replicative senescence related to telomere shortening did not evolve to cause aging, but is rather a consequence of a biological device that suppresses tumor formation. Thus, shortening of telomeres to a critical length activates a p53-dependent check point system in the cell cycle. Mice that are mutant for an activated form of p53 display an early onset of phenotypes associated with aging, including a shortened life span, generalized organ atrophy, osteoporosis, and diminished tolerance to a variety of stresses. These data are consistent with the observation that mutant mice that are deficient in telomerase exhibit high levels of activated p53 and also suffer reduced longevity and early senescence-related phenotypes. Other tumor suppressor genes also appear to be activated by telomere shortening and cyclin-dependent kinase inhibitors (p16, p21, and p27) are regarded as the key effectors of replicative senescence. There is also evidence for a telomere-independent pathway for growth arrest in humans. In view of these data, current concepts hold that growth arrest suppresses tumorigenesis but that the functional changes contribute to aging.

Genetic Factors Influence Aging

Experimental Models

Invertebrates, including roundworms and flies, represent a level of biological complexity beyond that afforded by tissue culture. The short generation times of these organisms have been exploited to study genetic influences on aging and longevity.

Caenorhabditis elegans is a worm in which single-gene mutations that extend life span have been identified. A variety of such mutations (*Age* mutations) increase the life spans of these nematode up to fivefold, a greater increase than has been reported for any other model. In addition to prolonging the life span, *Age* mutations in *C. elegans* also confer a complex array of other phenotypes. For example, the so-called clock *(clk)* mutations slow most functions that relate to the overall metabolic rate (cell cycle progression, swimming, food pumping, etc.). *Age* mutations also confer resistance to both environmental (extrinsic) and intrinsic stresses, including oxygen free radicals, heat shock, and ultraviolet radiation. Thus, genes that prolong life in *C. elegans* apparently act to reduce the accumulation of cellular "injuries" that impair homeostatic mechanisms and, thereby, shorten life span.

In experiments with *Drosophila,* strains of long-lived flies can be readily created by using the oldest flies for breeding. In such studies, the better health of the aged flies is associated with a "trade-off" of decreased fitness in the young flies, as evidenced by lesser activity and fertility than in wild-type flies. Thus, the original population must have had a set of alleles that yields greater fitness at a young age and decreased fitness at an older one, a phenomenon termed "antagonistic pleiotropy." This doctrine also applies to the protection against cancer by tumor suppressor mechanisms at the price of promoting the aging process.

Diseases of Premature Aging

In humans, the modest correlation in longevity between related persons and the excellent concordance of life span among identical twins lend credence to the concept that aging is influenced by genetic factors. The existence of heritable diseases associated with accelerated aging buttresses this notion. The entire process of aging, including features such as male-pattern baldness, cataracts, and coronary artery disease, is compressed into a span of less than 10 years in a genetic syndrome termed **Hutchinson-Guilford progeria** (Fig. 1-38). The cause of progeria is apparently a mutation in the LMNA gene, whose product is a protein termed lamin A. The mutant gene codes for a defective precursor of the lamin A protein, which has been termed **progerin.** This abnormal protein accumulates in the nucleus from one cell generation to the next, thereby interfering with the structural

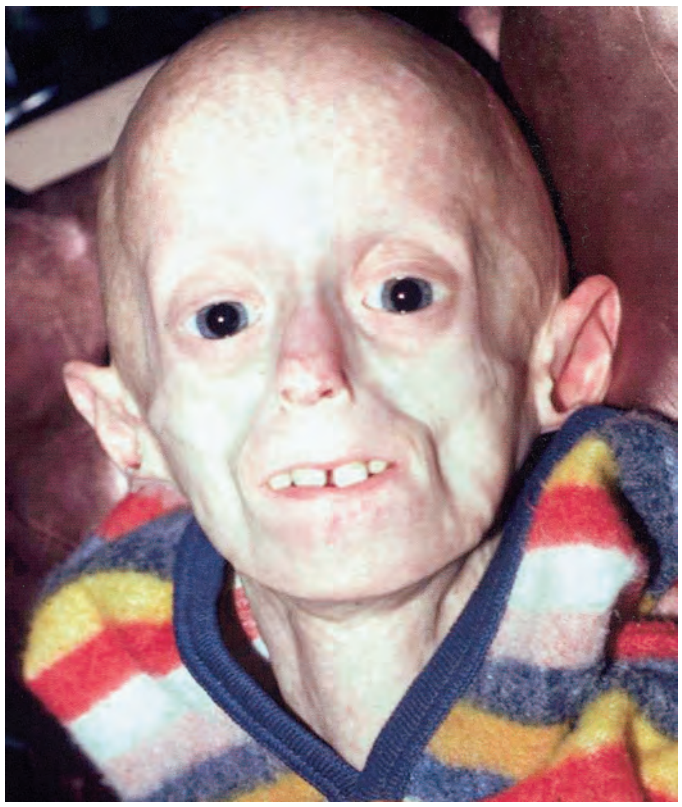


FIGURE 1-38. **Progeria.** A 10-year old girl shows the typical features of premature aging associated with progeria.

integrity of the nucleus and resulting in a lobulated shape. The buildup of progerin also interferes with the organization of nuclear heterochromatin, a component that is thought to regulate the expression of numerous genes. Interestingly, the nuclear changes in cells from patients with progeria were corrected by treating the cells with inhibitors of farnesyltransferase, which prevents progerin from becoming farnesylated. Experimental suppression of the production of progerin has also corrected the nuclear changes in cultured cells from patients with progeria.

Hutchinson-Guilford progeria is now recognized to be one of about 10 disorders associated with mutations in the LMNA gene, comprising a group termed "laminopathies". It is not known whether changes in lamin A contribute to normal aging, but cell nuclei from aged persons have been shown to acquire defects similar to those seen in cells from patients with progeria.

Werner syndrome (**WS**) is a rare autosomal recessive disease characterized by early cataracts, hair loss, atrophy of the skin, osteoporosis, and atherosclerosis. Affected persons are also at increased risk for the development of a variety of cancers. Patients typically die in the fifth decade from either cancer or cardiovascular disease. This phenotype of patients with WS gives the impression of premature aging. WS is caused by loss of function of the Werner (WRN) gene, which codes for a protein with multiple DNA-dependent enzymatic activities, including ATPase, helicase, exonuclease, and strand annealing. There is experimental evidence that WRN plays a role in the resolution of replication blockage and in telomere maintenance. Its loss leads to defective processing of DNA damage and replication. Experimentally, epigenetic inactivation of WRN by transcriptional silencing associated with promoter hypermethylation results in chromosomal instability and increased apoptosis. It is thought that the increased incidence of cancer in WS may reflect chromosomal changes, whereas accelerated aging probably reflects telomere dysfunction.

Aging May Reflect Accumulated Somatic Damage

Oxidative stress is an invariable consequence of life in an atmosphere rich in oxygen. An important hypothesis holds that the loss of function that is characteristic of aging is caused by progressive and irreversible accrual of molecular oxidative damage. Such lesions would be manifested as (1) peroxidation of membrane lipids, (2) DNA modifications (strand breaks, base alterations, DNA–protein cross-linking), and (3) protein oxidation (loss of sulfhydryl groups, carbonylation). Oxidative stress in normal cells is hardly trivial, since up to 3% of total oxygen consumption generates superoxide anions and hydrogen peroxide. It has been estimated that a single cell undergoes some 100,000 attacks on DNA a day by oxygen free radicals and that at any one time 10% of protein molecules are modified by carbonyl adducts. Thus, antioxidant defenses are not fully efficient and progressive oxidative damage to the cell may be responsible, at least in part, for the aging process.

The rate of generation of ROS correlates with an organism's overall metabolic rate. The theory that aging is related to oxidative stress is based on several observations: (1) larger animals usually live longer than smaller ones, (2) metabolic rate is inversely related to body size (the larger the animal, the lower the metabolic rate), and (3) generation of activated oxygen species correlates inversely with body size.

The role of oxidative stress in aging has been emphasized by experiments in *Drosophila* in which overexpression of genes for SOD or catalase significantly prolongs the fly's life span. Furthermore, as discussed above, virtually all long-lived worms and flies display increased antioxidant defenses. SOD activity in livers of different primates has also been reported to be proportional to maximal life span. The correlation of oxidative damage with aging is further exemplified by the demonstration of increased oxidative damage to lipids, proteins, and DNA in aged animals.

Additional evidence for progressive oxidative damage with aging is the deposition of aggregated proteins and lipofuscin pigment, principally in postmitotic cells of organs such as the brain, heart, and liver (see above). The emerging appreciation of the role of protein and lipid oxidation to the formation of such aggregates and the potential impairment of cell function that results highlight the role of oxidative damage in the decline of cell activities that accompanies aging.

Oxidative damage to mitochondria has also been proposed to play a major role in aging. Aerobic respiration in mitochondria is the richest source of ROS in the cell. Mitochondrial DNA is extremely sensitive to hydroxyl radical damage and progressively develops more than 100 different DNA deletions over the course of a human life. In turn, these DNA defects may lead to further increases in the mitochondrial generation of toxic oxygen species, thereby establishing a vicious circle.

There is increasing evidence that molecular chaperones (see above) decline both in activity and concentration with age, for unknown reasons. Since chaperones such as hsp70 are important in targeting misfolded proteins, loss of chaperone function affects many aspects of organ function and cell repair. Hsp70 influences several facets of the immune response, such as cytokine production and antigen presentation. There are, further, data suggesting that allelic polymorphisms of hsp70 genes may influence longevity in humans. For example, people in whom the amino acid at position #493 in the protein-binding region of HSP70-HOM protein is methionine have been reported to live longer on the average than those in whom this amino acid is threonine. The potential involvement of molecular chaperone function in aging is an active area of investigation.

Caloric restriction in rodents and lower species has long been known to increase longevity. However alluring it may be to extrapolate from lower species to humans, current mathematical models suggest that human life span may not be greatly increased by such severe caloric restriction. There is evidence to indicate that extension of rodent life span by caloric restriction is associated with a hypometabolic state, analogous to the effect of the "clock" mutations in *C. elegans*. Animals subjected to caloric restriction show attenuation of age-related increases in rates of mitochondrial generation of ROS, slower accrual of oxidative damage, and decreased evidence of lipid peroxidation and oxidative alterations of proteins.

Summary Hypothesis of Aging

After the reproductive period, evolution loses interest in an individual and abandons the organism to events against which nature confers no protection. As reviewed above, the doctrine of antagonistic pleiotropy posits the existence of genes that are beneficial during development and the reproductive period but exert baleful influences later in life. The alternative hypothesis of mutation accumulation holds that the evolutionary suppression of genes that are harmful to young individuals of a species creates pressure favoring alleles that defer the attainment of a deleterious phenotype until the postreproductive period. Finally, the major non-

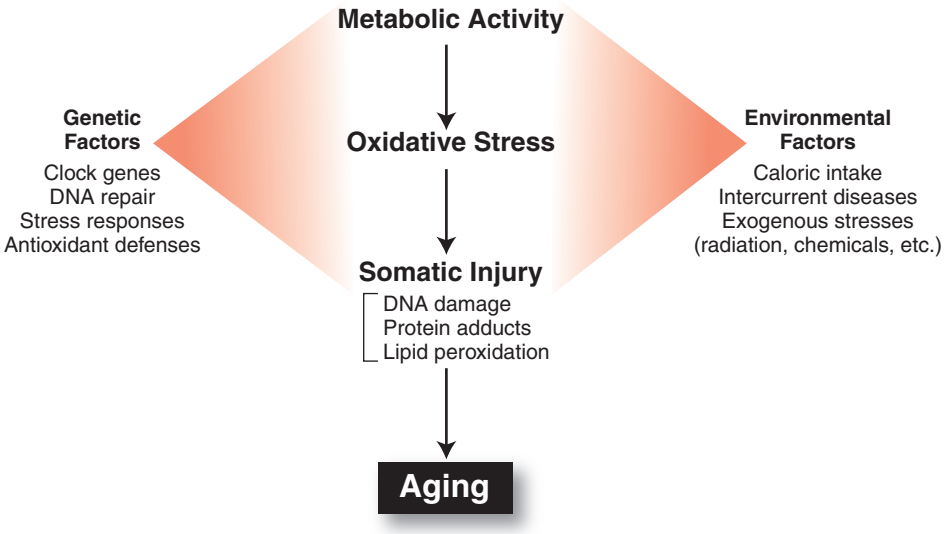


FIGURE 1-39. **Factors that influence the development of biological aging.**

genetic theories postulate that simple accumulation of various cell injuries eventuates in senescence. Current evidence supports the notion that these hypotheses are not mutually contradictory and that all may contribute to aging (Fig. 1-39). *According to this concept, although aging is under some measure of genetic control, it is unlikely that a predetermined genetic program for aging exists.* It is likely that the combined effects of a number of genes eventually lead to the accumulation of somatic mutations, deficiencies in DNA repair, the accretion of oxidative damage to macromolecules, and a variety of other defects in cell function, all culminating in the progressive failure of homeostatic mechanisms characteristic of aging. As Maimonides said, "The same forces that operate in the birth and temporal existence of man also operate in his destruction and death."