ISCHEMIC AND HYPOXIC INJURY

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

Types of Ischemic Injury.

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

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

Mechanisms of Ischemic Cell Injury.
The sequence of events following hypoxia was described earlier and is summarized in Figure 1-22. Briefly, as the oxygen tension within the cell decreases, there is loss of oxidative phosphorylation and decreased generation of ATP. The depletion of ATP results in failure of the sodium pump, with loss of potassium, influx of sodium and water, and cell swelling. There is progressive loss of glycogen and decreased protein synthesis. There may be severe functional consequences at this stage. For instance, heart muscle ceases to contract within 60 seconds of coronary occlusion. Note, however, that loss of contractility does not mean cell death. If hypoxia continues, worsening ATP depletion causes further morphologic deterioration. The cytoskeleton disperses, resulting in the loss of ultrastructural features such as microvilli and the formation of "blebs" at the cell surface (see Fig. 1-17). "Myelin figures," derived from plasma as well as organelar membranes, may be seen within the cytoplasm or extracellularly. They are thought to result from dissociation of lipoproteins with unmasking of phosphatide groups, promoting the uptake and intercalation of water between the lamellar stacks of membranes. At this time, the mitochondria are usually swollen, owing to loss of volume control by these organelles; the endoplasmic reticulum remains dilated; and the entire cell is markedly swollen, with increased concentrations of water, sodium, and chloride and a decreased concentration of potassium. If oxygen is restored, all of these disturbances are reversible.

Figure 1-22  Postulated sequence of events in reversible and irreversible ischemic cell injury. Note that although reduced oxidative phosphorylation and ATP levels have a central role, ischemia can cause direct membrane damage. ER, endoplasmic reticulum; CK, creatine kinase; LDH, lactate dehydrogenase; RNP, ribonucleoprotein.
If ischemia persists, irreversible injury and necrosis ensue. Irreversible injury is associated morphologically with severe swelling of mitochondria, extensive damage to plasma membranes, and swelling of lysosomes (see Fig. 1-17C). Large, flocculent, amorphous densities develop in the mitochondrial matrix. In the myocardium, these are indications of irreversible injury and can be seen as early as 30 to 40 minutes after ischemia. Massive influx of calcium into the cell then occurs, particularly if the ischemic zone is reperfused. Death is mainly by necrosis, but apoptosis also contributes; the apoptotic pathway is activated probably by release of proapoptotic molecules from leaky mitochondria. After death, cell components are progressively degraded, and there is widespread leakage of cellular enzymes into the extracellular space and, conversely, entry of extracellular macromolecules from the interstitial space into the dying cells. Finally, the dead cell may become replaced by large masses composed of phospholipids in the form of myelin figures. These are then either phagocytosed by other cells or degraded further into fatty acids. Calcification of such fatty acid residues may occur with the formation of calcium soaps.

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

ISCHEMIA-REPERFUSION INJURY

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

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

? New damage may be initiated during reoxygenation by increased generation of oxygen free radicals from parenchymal and endothelial cells and from infiltrating leukocytes. Superoxide anions can be produced in reperfused tissue as a result of incomplete and vicarious reduction of oxygen by damaged mitochondria or because of the action of oxidases derived from leukocytes, endothelial cells, or parenchymal cells. Antioxidant defense mechanisms may also be compromised by ischemia, favoring the accumulation of radicals. Free radical scavengers may be of therapeutic benefit.

? Reactive oxygen species can further promote the mitochondrial permeability transition, referred to earlier, which, when it occurs, precludes mitochondrial energization and cellular
ATP recovery and leads to cell death.\(^{[25]}\)

- Ischemic injury is associated with inflammation as a result of the production of cytokines and increased expression of adhesion molecules by hypoxic parenchymal and endothelial cells.\(^{[31]}\) These agents recruit circulating polymorphonuclear leukocytes to reperfused tissue; the ensuing inflammation causes additional injury (Chapter 2). The importance of neutrophil influx in reperfusion injury has been demonstrated by experimental studies that have used anti-inflammatory interventions, such as antibodies to cytokines or adhesion molecules, to reduce the extent of the injury.\(^{[29]}\)

- Recent data suggest that activation of the complement pathway may contribute to ischemia-reperfusion injury.\(^{[34]}\) The complement system is involved in host defense and is an important mechanism of immune injury (Chapter 6). Some IgM antibodies have a propensity to deposit in ischemic tissues, for unknown reasons, and when blood flow is resumed, complement proteins bind to the antibodies, are activated, and cause cell injury and inflammation. Knockout mice lacking several complement proteins are resistant to this type of injury.\(^{[39]}\)

**CHEMICAL INJURY**

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

Chemicals induce cell injury by one of two general mechanisms.\(^{[36]}\)

- Some chemicals can act *directly* by combining with some critical molecular component or cellular organelle. For example, in mercuric chloride poisoning, mercury binds to the sulfhydryl groups of the cell membrane and other proteins, causing increased membrane permeability and inhibition of ATPase-dependent transport. In such instances, the greatest damage is usually to the cells that use, absorb, excrete, or concentrate the chemicals — in the case of mercuric chloride, the cells of the gastrointestinal tract and kidney (Chapter 9). Cyanide poisons mitochondrial cytochrome oxidase and blocks oxidative phosphorylation. Many antineoplastic chemotherapeutic agents and antibiotic drugs who induce cell damage by direct cytotoxic effects.

- Most other chemicals are not biologically active but must be converted to reactive toxic metabolites, which then act on target cells. This modification is usually accomplished by the P-450 mixed function oxidases in the smooth endoplasmic reticulum of the liver and other organs.\(^{[37]}\) Although these metabolites might cause membrane damage and cell injury by *direct covalent binding* to membrane protein and lipids, by far the most important mechanism of membrane injury involves the information of *reactive free radicals* and subsequent lipid peroxidation.
The diverse mechanisms of chemical injury are well illustrated by carbon tetrachloride and acetaminophen. *Carbon tetrachloride* (CCl₄) was once used widely in the dry-cleaning industry. The toxic effect of CCl₄ is due to its conversion by P-450 to the *highly reactive toxic free radical* CCl₃ (CCl₄ + e⁻ CCl₃ + Cl⁻) (Fig. 1-23). The free radicals produced locally cause autooxidation of the polyenoic fatty acids present within the membrane phospholipids. There, oxidative decomposition of the lipid is initiated, and organic peroxides are formed after reacting with oxygen (lipid peroxidation). This reaction is *autocatalytic* in that new radicals are formed from the peroxide radicals themselves. Thus, rapid breakdown of the structure and function of the endoplasmic reticulum is due to decomposition of the lipid. It is no surprise, therefore, that CCl₄-induced liver cell injury is both severe and extremely rapid in onset. Within less than 30 minutes, there is a decline in hepatic protein synthesis; within 2 hours, there is swelling of smooth endoplasmic reticulum and dissociation of ribosomes from the rough endoplasmic reticulum. Lipid export from the hepatocytes is reduced owing to their inability to synthesize apoprotein to complex with triglycerides and thereby facilitate lipoprotein secretion. The result is the fatty liver of CCl₄ poisoning (Fig. 1-24). (Fatty liver is discussed later in the chapter.) Mitochondrial injury then occurs, and this is followed by progressive swelling of the cells due to increased permeability of the plasma membrane. Plasma membrane damage is through to be caused by relatively stable fatty aldehydes, which are produced by lipid peroxidation in the smooth endoplasmic reticulum but are able to act at distant sites. This is followed by massive influx of calcium and cell death (see Fig. 1-23).
Figure 1-23  Sequence of events leading to fatty change and cell necrosis in carbon tetrachloride (CCl₄) toxicity. RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum.
Acetaminophen (Tylenol), a commonly used analgesic drug, is detoxified in the liver through sulfation and glucuronidation, and small amounts are converted by cytochrome P-450-catalyzed oxidation to an electrophilic, highly toxic metabolite. This metabolite itself is detoxified by interaction with GSH. When large doses of the drug are ingested, GSH is depleted, and thus the toxic metabolites accumulate in the cell, destroy nucleophilic macromolecules, and covalently bind proteins and nucleic acids. The decrease in GSH concentration, coupled with covalent binding of toxic metabolites, increases drug toxicity, resulting in massive liver cell necrosis, usually 3 to 5 days after the ingestion of toxic doses. This hepatotoxicity correlates with lipid peroxidation and can be reduced by administration of antioxidants, suggesting that the oxidative damage may be more important than covalent binding in the ultimate toxicity of the drug.