Pathologic Effects of Free Radicals. The effects of ROS and other free radicals are wide-ranging, but three reactions are particularly relevant to cell injury (Fig. 2-20):

- *Lipid peroxidation in membranes.* In the presence of O_2, free radicals may cause peroxidation of lipids within plasma and organellar membranes. Oxidative damage is initiated when the double bonds in unsaturated fatty acids of membrane lipids are attacked by O_2-derived free radicals, particularly by $^{\bullet}OH$. The lipid-free radical interactions yield peroxides, which are themselves unstable and reactive, and an autocatalytic chain reaction ensues (called *propagation*) that can result in extensive membrane damage.
- *Oxidative modification of proteins*. Free radicals promote oxidation of amino acid side chains, formation of covalent protein-protein cross-lins (e.g., disulfide bonds), and oxidation of the protein backbone. Oxidative modification of proteins may damage the active sites of enzymes, disrupt the conformation of structural proteins, and enhance proteasomal degradation of unfolded or misfolded proteins, raising havoc throughout the cell.
- *Lesions in DNA*. Free radicals are capable of causing single- and double-strand breaks in DNA, cross-linking of DNA strands, and formation of adducts. Oxidative DNA damage has been implicated in cell aging (discussed later in this chapter) and in malignant transformation of cells (Chapter 7).

The traditional thinking about free radicals was that they cause cell injury and death by necrosis, and, in fact, the production of ROS is a frequent prelude to necrosis. However, it is now clear that free radicals can trigger apoptosis as well. Recent studies have also revealed a role of ROS in signaling by a variety of cellular receptors and biochemical intermediates. In fact, according to one hypothesis, the major actions of $O_2^{\cdot}$ stem from its ability to stimulate the production of degradative enzymes rather than direct damage of macromolecules. It is also possible that these potentially deadly molecules, when produced under physiologic conditions in the "right" dose, serve important physiologic functions.

Defects in Membrane Permeability

Early loss of selective membrane permeability, leading ultimately to overt membrane damage, is a consistent feature of most forms of cell injury (except apoptosis). Membrane damage may affect the functions and integrity of all cellular membranes. The following paragraphs discuss the mechanisms and pathologic consequences of membrane damage.

Mechanisms of Membrane Damage. In ischemic cells, membrane defects may be the result of ATP depletion and calcium-mediated activation of phospholipases. The plasma membrane can also be damaged directly by various bacterial toxins, viral proteins, lytic complement components, and a variety of physical and chemical agents. Several biochemical mechanisms may contribute to membrane damage (Fig. 2-21):

- *Reactive oxygen species*. Oxygen free radicals cause injury to cell membranes by lipid peroxidation, discussed earlier.

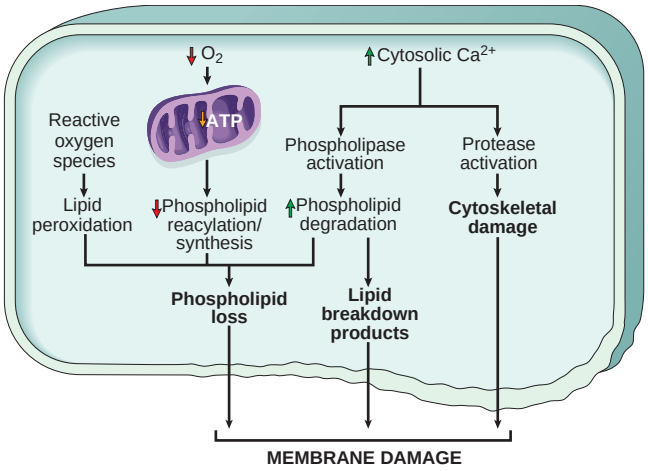

Figure 2-21 Mechanisms of membrane damage in cell injury. Decreased O_2 and increased cytosolic Ca^{2+} are typically seen in ischemia but may accompany other forms of cell injury. Reactive oxygen species, which are often produced on reperfusion of ischemic tissues, also cause membrane damage (not shown).

- *Decreased phospholipid synthesis*. The production of phospholipids in cells may be reduced as a consequence of defective mitochondrial function or hypoxia, both of which decrease the production of ATP and thus affect energy-dependent biosynthetic pathways. The decreased phospholipid synthesis may affect all cellular membranes, including the mitochondria themselves.
- *Increased phospholipid breakdown.* Severe cell injury is associated with increased degradation of membrane phospholipids, probably due to activation of calcium-dependent phospholipases by increased levels of cytosolic and mitochondrial $Ca2^{+}$. Phospholipid breakdown leads to the accumulation of *lipid breakdown products*, including unesterified free fatty acids, acyl carnitine, and lysophospholipids, which have a detergent effect on membranes. They may also either insert into the lipid bilayer of the membrane or exchange with membrane phospholipids, potentially causing changes in permeability and electrophysiologic alterations.
- *Cytoskeletal abnormalities*. Cytoskeletal filaments serve as anchors connecting the plasma membrane to the cell interior. Activation of proteases by increased cytosolic calcium may cause damage to elements of the cytoskeleton. In the presence of cell swelling, this damage results, particularly in myocardial cells, in detachment of the cell membrane from the cytoskeleton, rendering it susceptible to stretching and rupture.

Consequences of Membrane Damage. The most important sites of membrane damage during cell injury are the mitochondrial membrane, the plasma membrane, and membranes of lysosomes.

- *Mitochondrial membrane damage*. As discussed earlier, damage to mitochondrial membranes results in opening of the mitochondrial permeability transition pore, leading to decreased ATP generation and release of proteins that trigger apoptotic death.
- *Plasma membrane damage*. Plasma membrane damage results in loss of osmotic balance and influx of fluids and ions, as well as loss of cellular contents. The cells may