Cellular Responses to Stress and Noxious Stimuli

Cell injury

- The cellular response to injurious stimuli depends on the nature of the injury, its duration, its severity and Type of cell
- Small doses of a chemical toxin or brief periods of ischemia may induce reversible injury, whereas large doses of the same toxin or more prolonged ischemia might result either in instantaneous cell death or in slow, irreversible injury leading in time to cell death
- -neurons can withstand hypoxia only for 3-5mins.while Skeletal muscle can withstand for a very long time(many hours)
- The cell's nutritional and hormonal status and its metabolic needs are important in its response to injury
- Exposure of two individuals to identical concentrations of a toxin, such as carbon tetrachloride, may produce no effect in one and cell death in the other. This may be due to genetic variations affecting the amount and activity of hepatic enzymes that convert carbon tetrachloride (CCl_4) to toxic by-products
- **Forms of cellular responses to cell injury** :

1. When there is increased functional demand, the cell may adapt to the changes and then revert back to normal after the stress is removed (cellular adaptations,).

2. When the stress is mild to moderate, the injured cell may recover (reversible cell injury),its include degenerations, infiltrations, or disturbances in cellular growth, etc.

3- when the injury is severe and persistent the cell death may occur (irreversible cell injury-necrosis or apoptosis).

The normal cell is confined to a fairly narrow range of function and structure by its state of metabolism, differentiation, and specialization; by constraints of neighboring cells; and by the availability of metabolic substrates

Adaptations are reversible functional and structural responses to more severe physiologic stresses and some pathologic stimuli

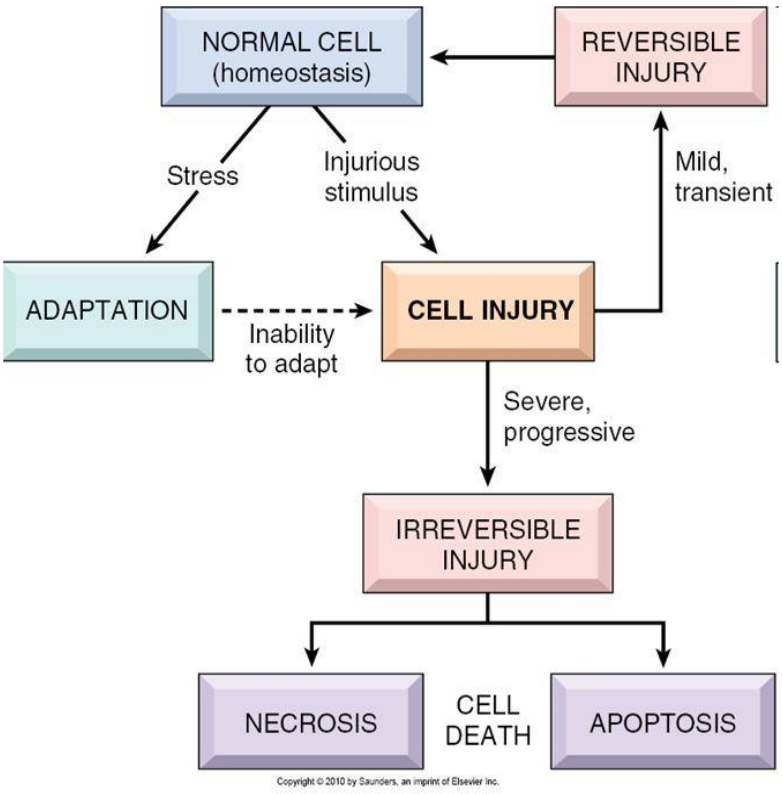
If the limits of adaptive responses are exceeded or if cells are exposed to injurious agents or stress, deprived of essential nutrients, or become compromised by mutations that affect essential cellular constituents, a sequence of events follows that is termed cell injury

A cell that suffers reversible damage can return to its normal state or reach cellular death, while a cell that suffers cellular death cannot return to its normal state

-Cell injury is reversible up to a certain point, but if the stimulus persists or is severe enough from the beginning, the cell suffers irreversible injury and ultimately cell death

All harmful effects initially lead to a change at the molecular level and at the biochemical level that may not be detected early

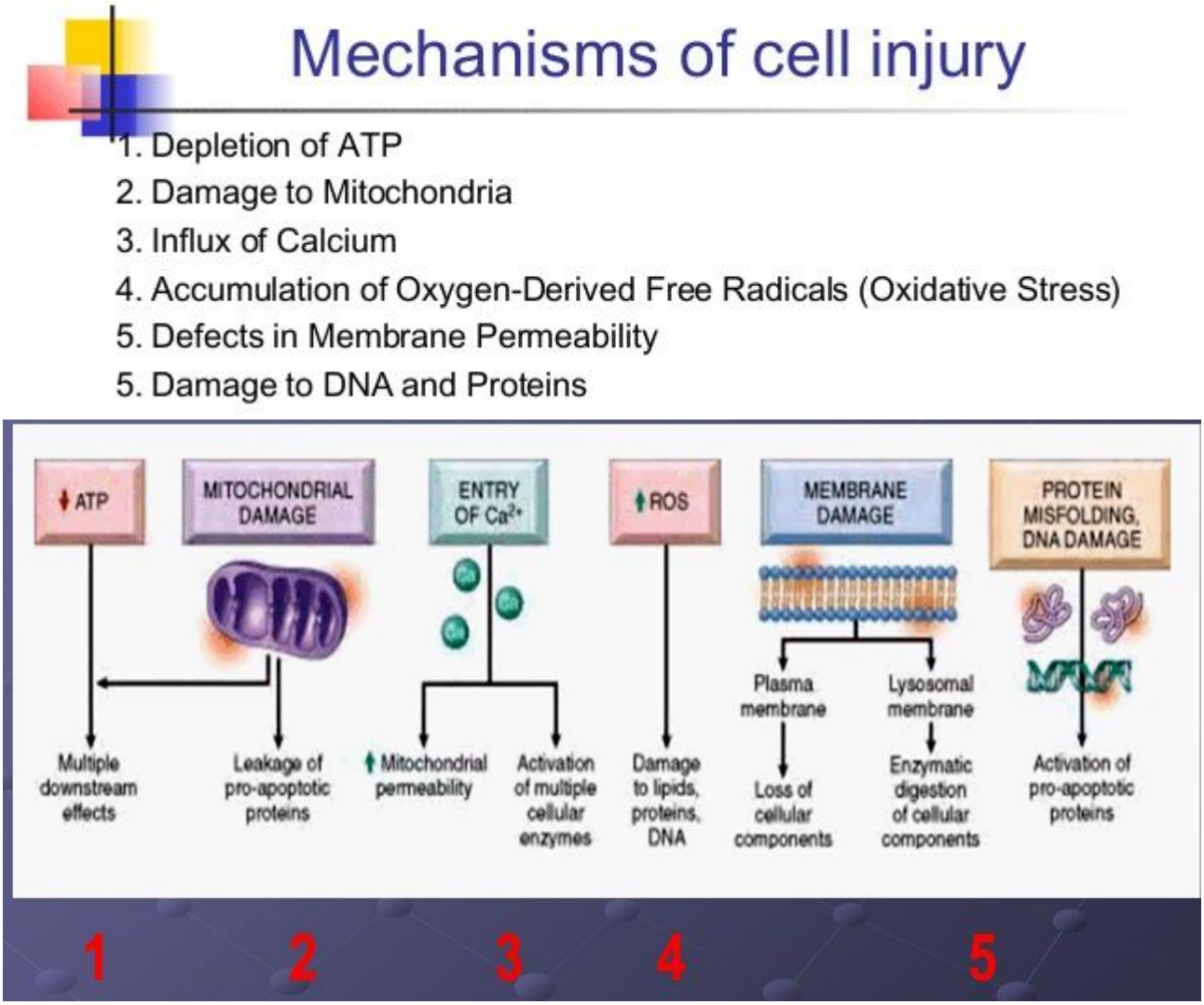
But when the changes become at the phenotypic and histological levels, it is possible to determine the type of pathological change in the cell and whether the cell is adaptive, damaged or dead



Mechanisms of Cell injury

Cell injury results from different biochemical mechanisms acting on several essential cellular components.

The cellular components that are most frequently damaged by injurious stimuli include mitochondria, cell membranes, the machinery of protein synthesis and packaging, and the DNA in nuclei



DEPLETION OF ATP

-ATP depletion and decreased ATP synthesis are frequently associated with hypoxia ,ischemia and chemical (toxic) injury

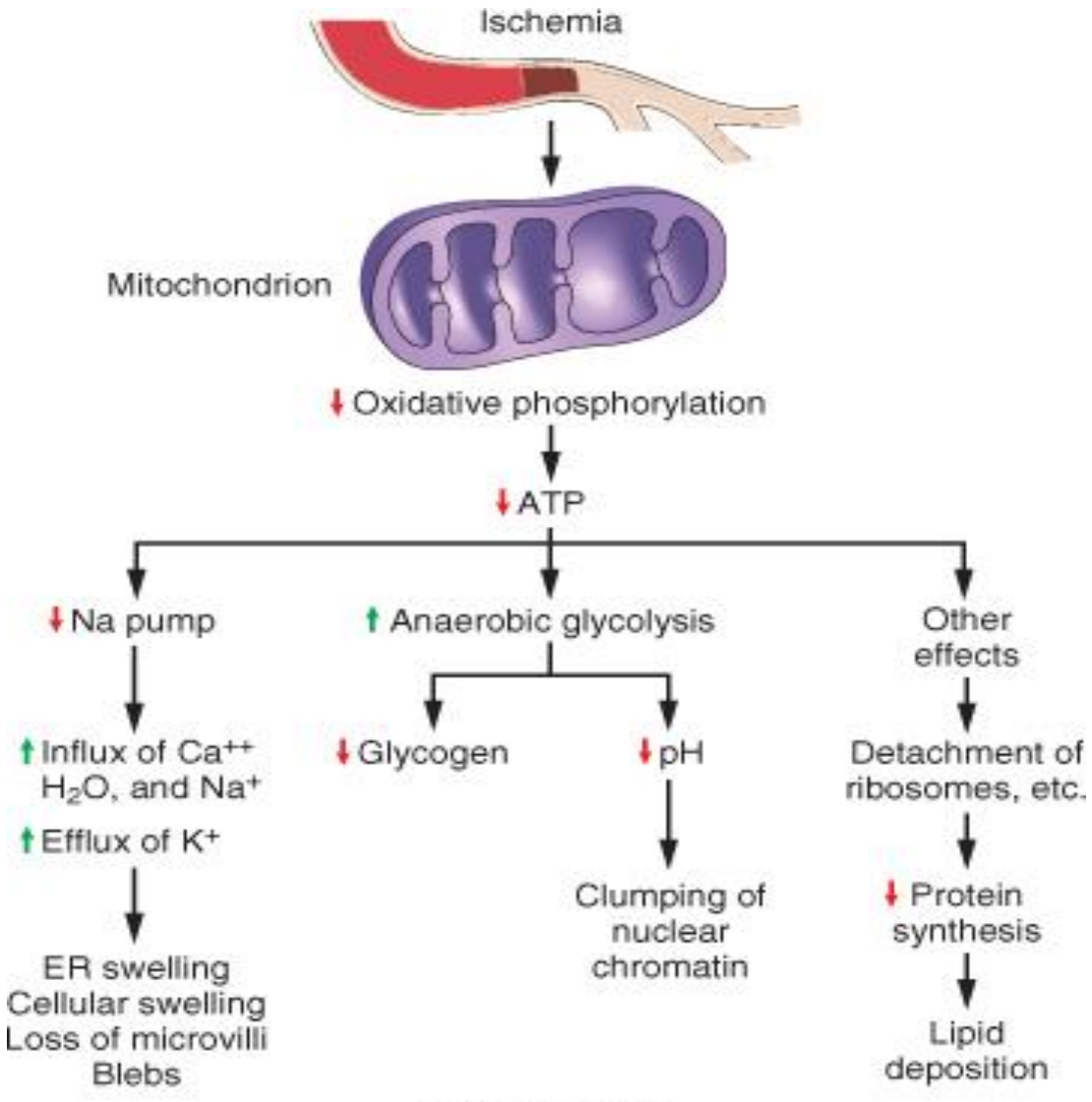
-ATP is produced in two ways:

1-The major pathway in mammalian cells is oxidative phosphorylation of adenosine diphosphate, in a reaction that results in reduction of oxygen by the electron transfer system of mitochondria.

2- The second is the glycolytic pathway, which can generate ATP in the absence of oxygen using glucose derived either from body fluids or from the hydrolysis of glycogen.

- High-energy phosphate in the form of ATP is required for virtually all synthetic and degradative processes within the cell. These include membrane transport, protein synthesis, lipogenesis

- The major causes of ATP depletion are reduced supply of oxygen and nutrients, mitochondrial damage, and the actions of some toxins (e.g., cyanide).



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

1-The activity of the plasma membrane energy-dependent sodium pump (Na+, K+-ATPase) is reduced.

Failure of this active transport system causes sodium to enter and accumulate inside cells and potassium to diffuse out.

The net gain of solute is accompanied by isosmotic gain of water, causing cell swelling, and dilation of the ER

2- Cellular energy metabolism is altered. If the supply of oxygen to cells is reduced, as in ischemia, oxidative phosphorylation ceases, resulting in a decrease in cellular ATP and associated increase in adenosine monophosphate.

These changes stimulate phosphofructokinase and phosphorylase activities, leading to an increased rate of anaerobic glycolysis,

Anaerobic glycolysis results in the accumulation of lactic acid and inorganic phosphates from the hydrolysis of phosphate esters.

This reduces the intracellular pH, resulting in decreased activity of many cellular enzymes

- Failure of the Ca^{2+} pump leads to influx of Ca^{2+}, with damaging effects on numerous cellular components
- With prolonged depletion of ATP, structural disruption of the protein synthetic apparatus occurs: detachment of ribosomes from the rough ER and dissociation of polysomes, with a consequent *reduction in protein synthesis*.
- In cells deprived of oxygen or glucose, proteins may become misfolded, and this cellular reaction called the *unfolded protein response* that lead to cell death

MITOCHONDRIAL DAMAGE

- Mitochondria are the cell's suppliers of energy in the form of ATP, but they are also critical players in cell injury and death
- Mitochondria can be damaged by increases of cytosolic Ca^{2+}, reactive oxygen species .,oxygen deprivation , hypoxia and toxins. In addition, mutations in mitochondrial genes
- There are two major *consequences of mitochondrial damage*:
- 1- Mitochondrial damage often results in the formation of a high-conductance channel in the mitochondrial membrane, called the *mitochondrial permeability transition pore* .
- The opening of this conductance channel leads to the loss of mitochondrial membrane potential, resulting in failure of oxidative phosphorylation and progressive depletion of ATP, culminating in necrosis of the cell.

2- The mitochondria also sequester between their outer and inner membranes proteins that are capable of activating apoptotic pathways;

- these include cytochrome *c* and proteins that indirectly activate apoptosis inducing enzymes called caspases.
- Increased permeability of the outer mitochondrial membrane may result in leakage of these proteins into the cytosol, and death by apoptosis

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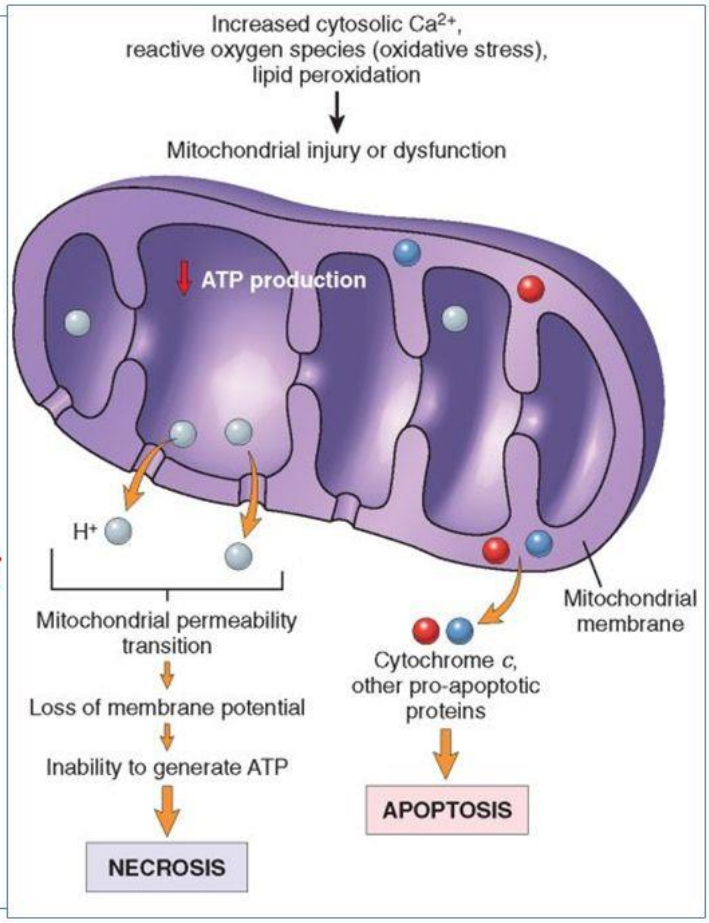

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INFLUX OF CALCIUM AND LOSS OF CALCIUM HOMEOSTASIS

- Cytosolic free calcium is normally maintained at very low concentrations (-0.1 μmol) compared with extracellular levels of 1.3 mmol,
– most intracellular calcium is sequestered in mitochondria and the ER
-Ischemia and certain toxins cause an increase in cytosolic calcium concentration, initially because of release of Ca^{2+} from intracellular stores, and later resulting from increased influx across the plasma membrane

Increased intracellular Ca^{2+} causes cell injury by several mechanisms.:

1-The accumulation of Ca^{2+} in mitochondria results in opening of the mitochondrial permeability transition pore and, failure of ATP generation.
2-Increased cytosolic Ca^{2+} activates a number of enzymes, with potentially deleterious cellular effects. These enzymes include *phospholipases* (which cause membrane damage), *proteases* (which break down both membrane and cytoskeletal

proteins), *endonucleases* (which are responsible for DNA and chromatin fragmentation), and *ATPases* (thereby hastening ATP depletion).
3- Increased intracellular Ca^{2+} levels also result in the induction of apoptosis, by direct activation of caspases and by increasing mitochondrial permeability

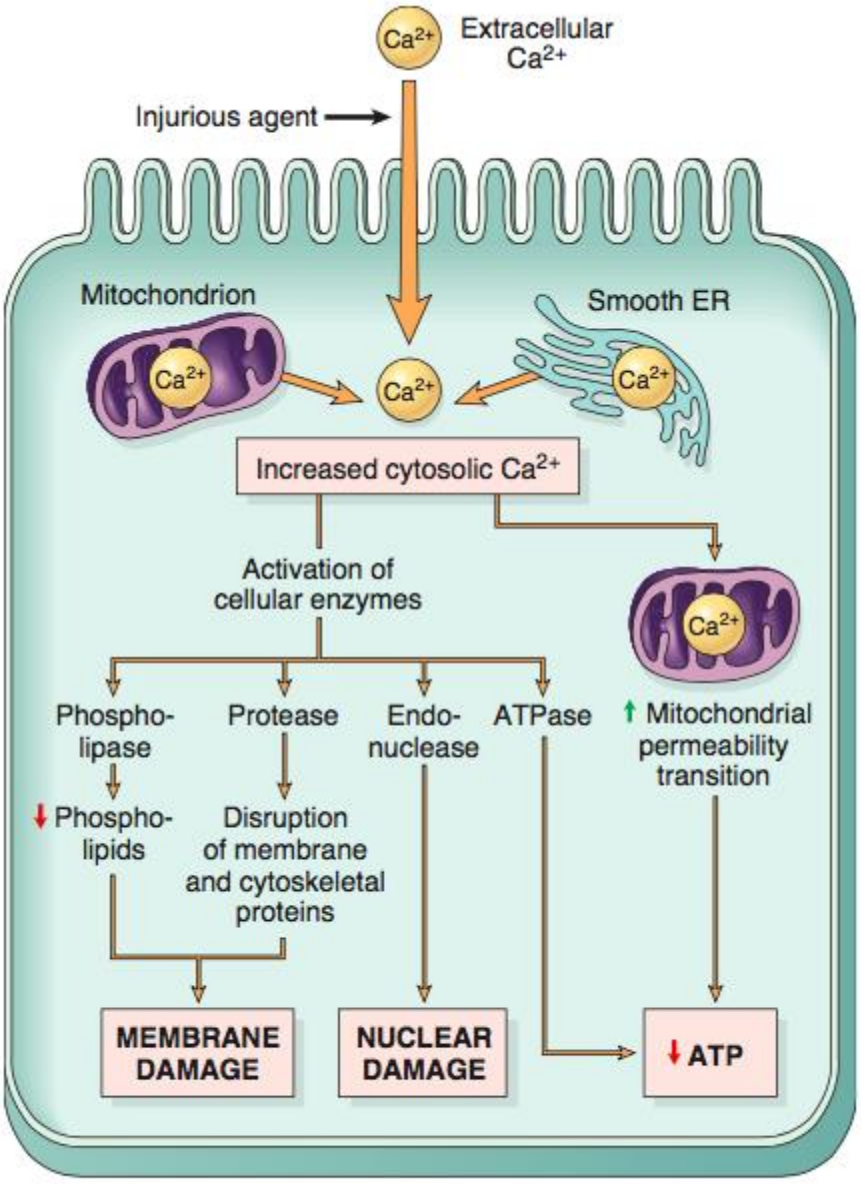
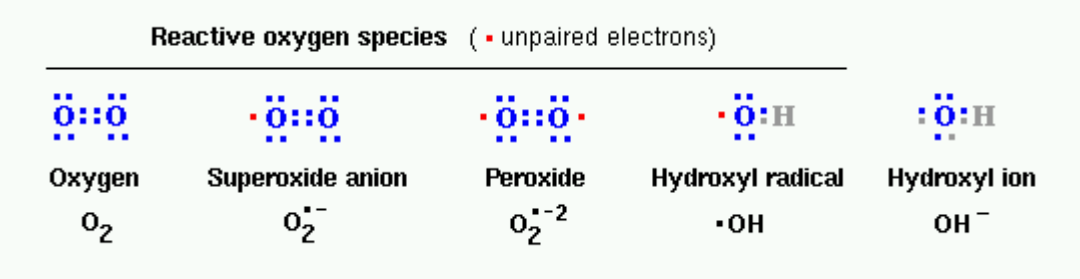


Figure 2-19 The role of increased cytosolic calcium in cell injury. ER, Endoplasmic reticulum.

ACCUMULATION OF OXYGEN-DERIVED FREE RADICALS (OXIDATIVE STRESS)

- Cell injury induced by free radicals, particularly reactive oxygen species(ROS)
-Free radicals are chemical species that have a single unpaired electron in an outer orbit



- ROS are produced normally in cells during mitochondrial respiration and energy generation, but they are degraded and removed by cellular defense systems.
- Thus, cells are able to maintain a steady state in which free radicals may be present transiently at low concentrations but do not cause damage
- When the production of ROS increases or the scavenging systems are ineffective, the result is an excess of these free radicals, leading to a condition called oxidative stress
- Oxidative stress has been implicated in a wide variety of pathologic processes, including cell injury, cancer, aging, and some degenerative diseases such as Alzheimer disease

Generation of Free Radicals

1- During normal respiration, molecular O2 is reduced by the transfer of four electrons to H2 to generate two water molecules. This conversion is catalyzed by oxidative enzymes in the ER, cytosol, mitochondria, peroxisomes, and lysosomes

2-ROS are produced in activated leukocytes during inflammation particularly neutrophils and macrophages, as mediators for destroying microbes, dead tissue, and other unwanted substances. This occurs by reaction in a plasma membrane multiprotein complex that uses NADPH oxidase for the redox reaction . In addition, some intracellular oxidases (such as xanthine oxidase)

3-Enzymatic metabolism of exogenous chemicals or drugs can generate free radicals that are not ROS but have similar effects (e.g., CCl4 can generate CCl3

4-Transition metals such as iron and copper donate or accept free electrons during intracellular reactions and catalyze free radical formation, as in the Fenton reaction (H2O2 + Fe2+ → Fe3+ + OH + OH-).

- Because most of the intracellular free iron is in the ferric (Fe3+) state, it must be reduced to the ferrous (Fe2+) form to participate in the Fenton reaction

This reduction can be enhanced by , and thus sources of iron and may cooperate in oxidative cell damage

5-Nitric oxide (NO), an important chemical mediator generated by endothelial cells, macrophages, neurons, and other cell types , can act as a free radical and can also be converted to highly reactive peroxynitrite anion ($ONOO^-$) as well as NO_2 and NO_3^-.

Removal of Free Radicals

- cells have developed multiple nonenzymatic and enzymatic mechanisms to remove free radicals and thereby minimize injury . These include the following:

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

2-iron and copper can catalyze the formation of ROS. The levels of these reactive metals are minimized by binding of the ions to storage and transport proteins (e.g., transferrin, ferritin, lactoferrin, and ceruloplasmin), thereby minimizing the formation of ROS.

3- A series of enzymes acts as free radical–scavenging systems and breaks down H2O2 and , These enzymes are lo-cated near the sites of generation of the oxidants and include the following:

- Catalase, present in peroxisomes, decomposes H2O2 ($2H_2O_2 \rightarrow O_2 + 2H_2O$).
- Superoxide dismutases (SODs) are found in many cell types and convert to H2O2 ($2 + 2H \rightarrow H_2O_2 + O_2$). This group includes both manganese–SOD, which is localized in mitochondria, and copper-zinc–SOD, which is found in the cytosol.
- Glutathione peroxidase also protects against injury by catalyzing free radical breakdown ($H_2O_2 + 2GSH \rightarrow GSSG$ [glutathione homodimer] + $2H_2O$, or $2OH + 2GSH \rightarrow GSSG + 2H_2O$). The intracellular ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) is a reflection of the oxidative state of the cell and is an important indicator of the cell's ability to detoxify ROS.

Pathologic Effects of Free Radicals

1- Lipid peroxidation in membranes.

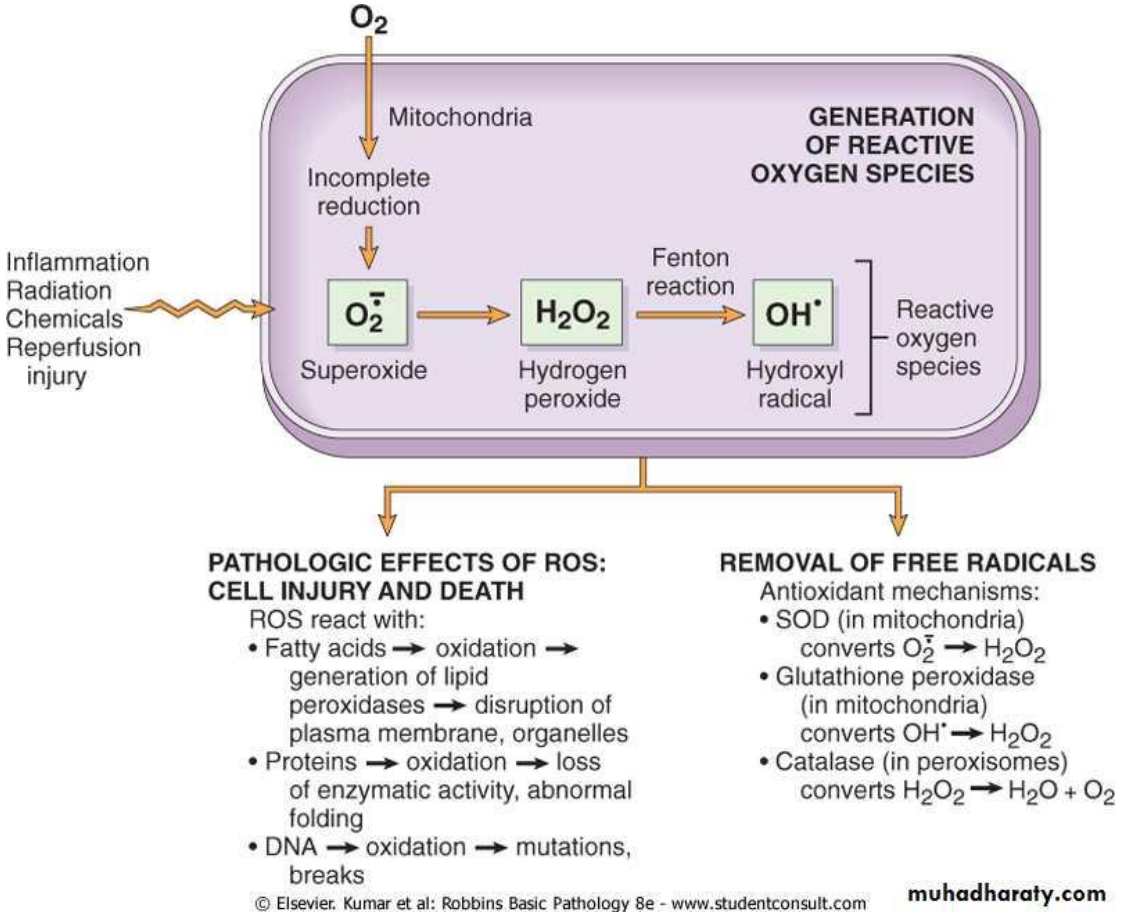
In the presence of O2, free radicals may cause peroxidation of lipids within plasma and organellar membranes

2-Oxidative modification of proteins.

Free radicals promote oxidation of amino acid side chains, formation of protein-protein cross-linkages (e.g., disulfide bonds), and oxidation of the protein backbone

3- Oxidative DNA damage (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.



DEFECTS IN MEMBRANE PERMEABILITY

- Reactive oxygen species.

Oxygen free radicals cause injury to cell membranes by lipid peroxidation

- 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 enzymatic activities.

- Increased phospholipid breakdown.

probably due to activation of endogenous phospholipases by increased levels of cytosolic and mitochondrial Ca2+.

Phospholipid breakdown leads to the accumulation of lipid breakdown products, including unesterified free fatty acids, acylcarnitine, 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

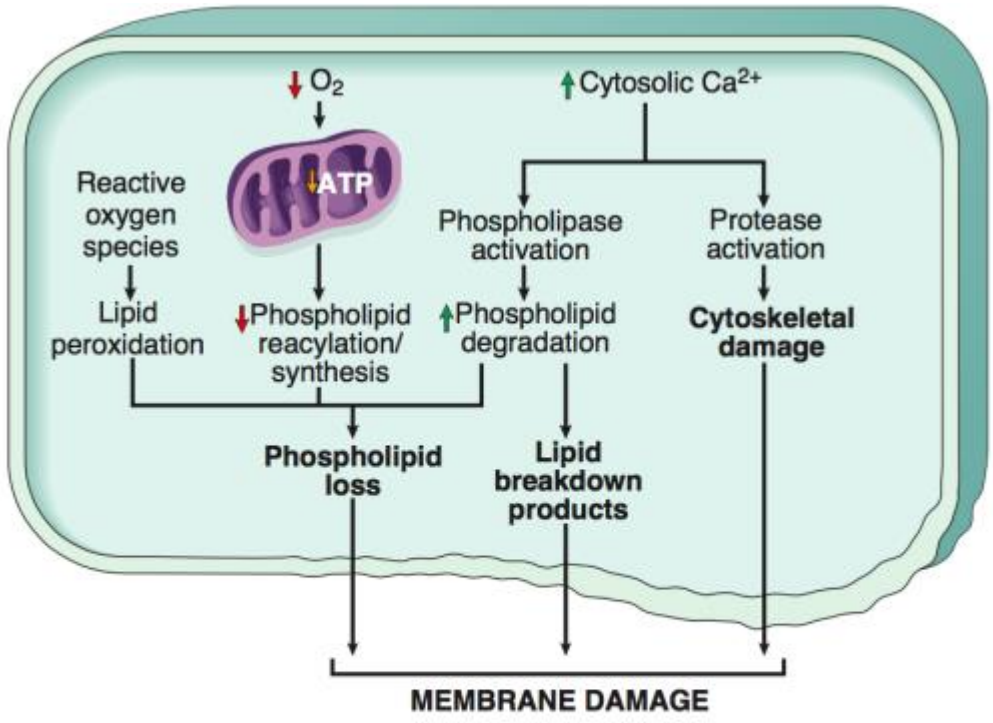


Figure 2-21 Mechanisms of membrane damage in cell injury. Decreased O_2 and increased cytosolic Ca^{2+} are typically seen in ischemia but may accompany other forms of cell injury. Reactive oxygen species, which are often produced on reperfusion of ischemic tissues, also cause membrane damage (not shown).

Sites of membrane damage during cell injury

- Mitochondrial membrane damage

damage to mitochondrial membranes results in opening of the mitochondrial permeability transition pore leading to decreased ATP, 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 also leak metabolites that are vital for the reconstitution of ATP, thus further depleting energy stores.

Leakage of intracellular proteins through the damaged cell membrane and ultimately into the circulation provides a means of detecting tissue-specific cellular injury and necrosis using blood serum samples. Cardiac muscle, for example, contains a specific isoform of the enzyme creatine kinase and of the contractile protein troponin; liver (and specifically bile duct epithelium) contains an isoform of the enzyme alkaline phosphatase; and hepatocytes contain transaminases. Irreversible injury and cell death in these tissues are reflected in increased levels of such proteins in the blood, and measurement of these biomarkers is used clinically to assess damage to these tissues.

- Injury to lysosomal membranes results in leakage of their enzymes into the cytoplasm and activation of the acid hydrolases in the acidic intracellular pH of the injured (e.g., ischemic) cell.

Lysosomes contain RNases, DNases, proteases, phosphatases, glucosidases, and cathepsins. Activation of these enzymes leads to enzymatic digestion of proteins, RNA, DNA, and glycogen, and the cells die by necrosis.
injury to lysosomal membranes results in the enzymatic dissolution of the injured cell that is characteristic of necrosis.

DAMAGE TO DNA AND PROTEINS

Cells have mechanisms that repair damage to DNA, but if this damage is too severe to be corrected (e.g., after exposure to DNA damaging drugs, radiation, or oxidative stress), the cell initiates a suicide program that results in death by apoptosis. A similar reaction is triggered by improperly folded proteins, which may be the result of inherited mutations or external triggers such as free radicals. these mechanisms of cell injury typically cause apoptosis