Doctor 021

# PATHOLOGY

## Sheet no. 13


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During the last two lectures, we have talked about the adaptive mechanisms in response to stress, reversible and irreversible cell injury, and causes of cell injury in general. In this lecture, we will talk about the mechanisms, and the molecular basis of cell injury.

## Principles of cellular response, and consequences regarding injurious stimulus:

- **The cellular response to injury depends on:**

1. **The type of injury**. (ischemia , trauma , chemicals )

2. **Its duration**. (ischemia , long time leads to necrosis)

For example if we talked about ischemia (a complete cut of blood supply )for 3-5 min(reversible injury) the consequences will differ if the duration more than 30 min(will be irreversible injury).

3. **Its severity** ,Ex: low doses of toxins or a partial cut of blood supply may lead to reversible cell injury, whereas larger doses of toxins or complete cut of blood supply may result in irreversible injury and cell death.

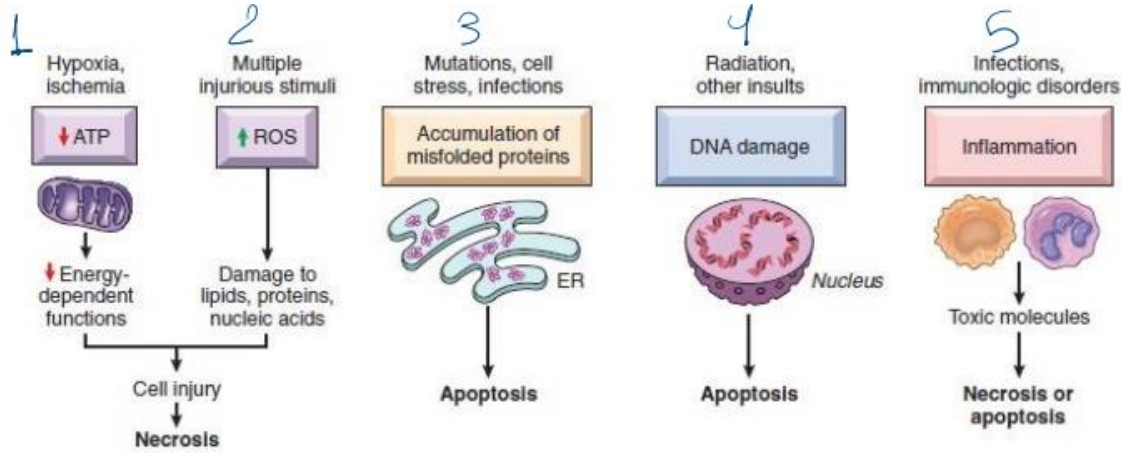
- **The consequences of an injurious stimulus depend on:**

1. The **type** of the cells determine the outcome of injury (status of cells adaptability) , Ex: a skeletal muscle accommodates ischemia for 2-3 hours without irreversible injury/necrosis, whereas cardiac muscle dies because of myocardial infraction after only 20-30 minutes, also, in the brain(highly sensitive for ischemia and hypoxia) an ischemia for 2 minutes is enough to cause death.
2. **status of the cells**, meaning that whether a cell is diseased from the beginning or normal looking, determines the injury consequences.
3. **Adaptability**, For a period of hypoxia, some tissues can adapt, and some are highly susceptible to it.
4. **genetic makeup of the injured cell**. Sometimes cells among different individuals may develop different consequences to the same toxin/drug, ex: when exposed to a toxin, individuals who inherit differences in genes encoding cytochrome P-450 in the liver, may catabolize the toxin at different rates, some could respond exaggeratedly, and some respond by the minimal effect. And this is the definition of Pharmacogenomics.

**-Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components**

Cell injury disrupts cellular homeostasis. Cells are injured by numerous and diverse causes (etiologic agents) from intrinsic and extrinsic sources; however, all of these causes, activate one or more biochemical mechanisms that may act together at the same time cooperating in the same scenario leading to cell injury.

# Principle of biochemical mechanisms and sites of damage in cell injury:




A brief discussion about the figure above:

1. Several causes can lead to the same mechanism of cell injury, ex: Hypoxia lead to ATP depletion (since oxygen is needed in ATP generation in mitochondria) then, every energy-dependent function, like protein synthesis, plasma membrane pumps, will be decreased, leading to necrosis in the end.
2. Multiple injury stimuli increases reactive oxygen species(free radicals: highly unstable substances, and they have a strong damaging capacity ) in cell which leads to damage in lipids proteins and nucleic acid, leading to cell necrosis(cell death).
3. Multiple mutations , cell stress and infections (viral infection) lead to accumulation of misfolded protein which leads to apoptosis.
4. Radiation (Sun UV) may lead to DNA damage which also lead to apoptosis(if the cell continues proliferation with damaged DNA that will lead to cause cancer).
5. Also, Immunologic disorders (ex: allergy, autoimmune disease), cause inflammation to the cell, by the high accumulation of leukocytes, generating toxic molecules, and leading to necrosis “mainly” and Apoptosis “some”.

-The stimuli of cell injury with the mechanism of cell injury determine whether the cell will die by Apoptosis or by Necrosis.

## Mechanisms of Cell injury:

**First event**: **Hypoxia and Ischemia**

-As we all know, oxygen is needed to all cells in producing energy in the form of ATP, by oxidative phosphorylation which occurs in the mitochondria. -So, Hypoxia due to ischemia (which is the most common cause) or any other cause of reduction in oxygen supply to the cell→**leads to Defective oxidative phosphorylation →failure of ATP generation → depletion of ATP in cells.** Depletion of energy source (ATP) in the cell causes **failure of energy dependent pathways** such as; **plasma membrane transportation** (Na+/K+ pump)when it fails the Na+ will accumulate inside the cell and attract water so the cell will swell, **protein synthesis** in ribosomes, **lipogenesis** (production of lipids) and **phospholipid turnover** (degradation of phospholipids every 1-2 cell division). All of these pathways will get decreased in an attempt of helping the cell to withstand the period of hypoxia, and go back to normal state.

-A compensatory pathway of generating ATP, could be followed**: Anaerobic Glycolysis** can help the cell to withstand hypoxia for a longer period of time. This pathway is less efficient than Oxidative phosphorylation, since it produces only 2 ATP molecules, and causes muscle fatigue, due to Lactic acid accumulation which will decrease the PH of the cell leading to protein degradation.

**-Liver cells and skeletal muscle cells Vs brain and heart:**

That's why skeletal muscles takes 2-3 hours after hypoxia before death (the skeletal muscles and liver cells ,they good stand hypoxia for a longer period of time ).while brain and heart don't tolerate (Heart takes from 20-30 mins while brain cant tolerate it takes 1-2 mins and die).

Refer to the cellular response depends on >>>

**-The net effect of hypoxia** on the cells explain the ultrastructural and microscopic changes that we see in these cells due to injury, and hypoxia have several effects illustrated below:

**1) Reduced activity of membrane ATP-dependent sodium pumps** →sodium and water accumulation inside the cell → **cellular swelling**.

**2)** **Lactic acid accumulation**(anaerobic respiration) → **decreased PH** (it's an acid) → **failure of enzymes in the cell.**

**3)** **Disruption of the ribosomes** from the RER membrane → **decreased protein synthesis.**

**4)** **Accumulation of ROS**, due to ineffective oxidative phosphorylation ,and they are more produced in the case of reperfusion after ischemia. Will be discussed later.

**5)** **Damage to mitochondrial and lysosomal membranes** mainly due to production of ROS and leakage of destructive lysosomal enzymes to the cytosol of the cell.

**6)** Leading to **necrosis.**

**Second Event:** **Ischemia -Reperfusion Injury**

**Paradoxical cell injury after restoration of blood flow to ischemic but viable tissues:**

Ischemia-Reperfusion injury The tissue damage when blood supply returns to tissue after a period of ischemia or lack of oxygen. Explanation: If there was a blockage in a blood vessel and after a while it was removed, which means the blood flow is back to the tissue (reperfusion), we might think that the tissue will get back to its normal state and nourish immediately, but in certain scenarios it won't. If a tissue undergoes ischemia and it's still viable (didn't undergo irreversible cell injury), Reperfusion will cause Paradoxical cell injury sometimes. An example is: **After myocardial or cerebral ischemia**.

Why the ischemia-reperfusion injury happen?

**1 -Increased generation of ROS from:**

**-injured cells with damaged mitochondria** leads to incomplete reduction of oxygen in oxidative phosphorylation mechanisms**.( defective antioxidant mechanisms).**

**- Infiltrating new leukocytes** will add ROS, causing the production of (free radicals).

**2- Increased inflammation by influx of leukocytes and plasma proteins complement**

After the cut of blood supply the blood come back with WBCs and they can produce ROS, cytokines and other proteins which will lead to tissue damage.

**Third event: Oxidative stress :**

**Cellular abnormalities induced by ROS (free radicals), which are chemical species with single unpaired electron (extremely unstable, and have high energy),** that if the they bind to any organic/inorganic molecule, they will convert them to a free radical too, and they will induce damage to cellular proteins/lipids and nucleic acids.

**ROS generated in:**

**1.Chemical injury (CCL4)**

**2.Radiation injury (UV, Xray)**

**3.Hypoxia**

**4. Cellular aging**

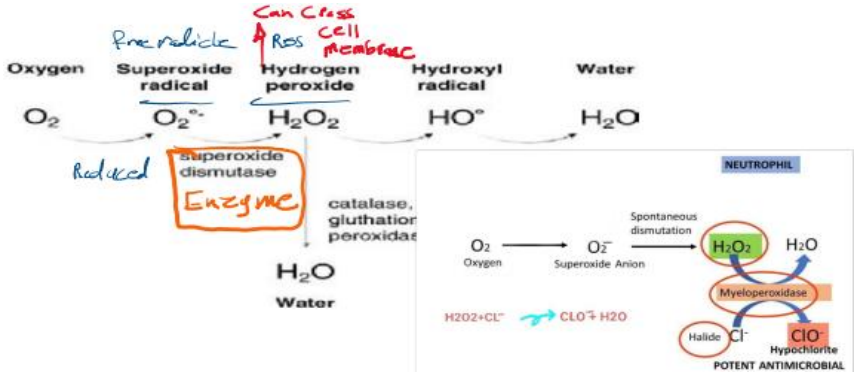
**5. Inflammation**

**6.Ischemia-reperfusion injury**

## Generation and Production of ROS:

1- **Normally produced in small amounts in all cells during the redox reactions, in the mitochondria when oxygen is reduced to produce water.** But because these small amounts of highly reactive short-lived toxic intermediates are generated, the cell is easily able to get rid of them. Thus, there will be no net effect of damage on the cell, unless their amount is increased. Free radicals production reaction:

**Superoxide (O2 • ), hydrogen peroxide (H2O2), hydroxyl radical •OH.**




2- **Produced in phagocytic leukocytes (neutrophils and macrophages) during inflammation, in an attempt to kill the microbe or to phagocytose the bacteria by phagosomes and phagolysosomes.**

**O2 >> superoxide**

**superoxide >> H2O2 (** through enzyme superoxide dismutase**)**

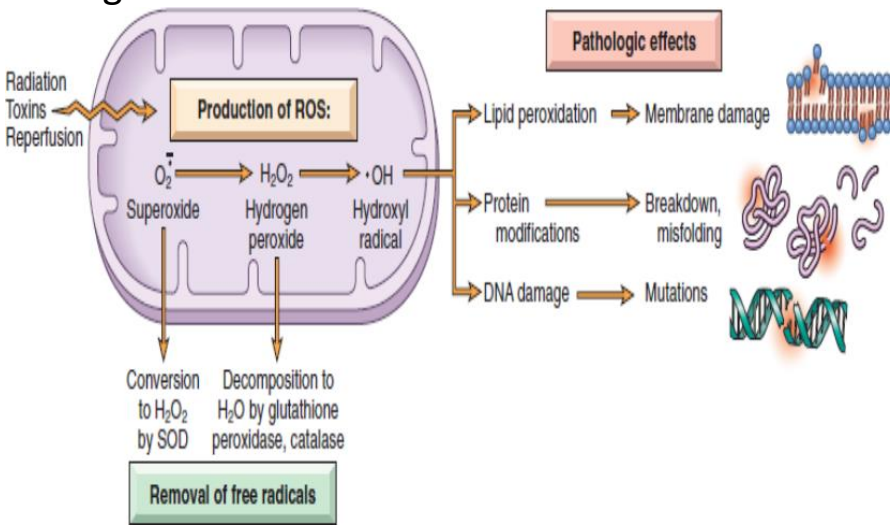
**H2O2 >> hypochlorite(**presence in cleaning detergents**). The reaction of free radicals production is the same but H2O2 is converted into Hypochlorite in phagocytes**(macrophages, neutrophiles**) catalyzed by Myeloperoxidase(MPO).**

## Removal Mechanisms of removing free radicals:

limit the damaging effect of free radicals on the cell, and below are some:
**1-**sometimes the H2O2 converted into another free radical which is hydroxyl group that will **Decay spontaneously** .

**2-Superoxide dismutase**, (catalyzes the conversion: O2 superoxide into H2O2 which is converted to water by **Glutathione (GSH) peroxidases.** GSH peroxidase (mostly GSH type 1) catalyzes the conversion: H2O2 into water. ALSO **Catalase** which converts H2O2 to water **(one of most active enzymes known, also contributes in catalyzing the previous reaction)**

**3- Endogenous or Exogenous anti-oxidants (e.g., vitamins E, A and C and βCarotene),** antioxidants either block free radicals' production or scavenge them as soon as they are produced Net removal decide the damage.




## Effects of ROS :

**1-Lipid peroxidation of membranes**. it will do lipid peroxidation for the lipid residues in the membranes, this peroxidation happen when the reactive oxygen species react with the lipid present in the **plasma, lysosomal, mitochondrial membranes** leading to the production of peroxides these peroxides themselves are unstable and will mediate destructive process to these membranes which lead to leakage of substances inside these membranes .

**2**-**Crosslinking(**they attach like sulfhydryl groups to the protein**) and other changes in proteins. It will lead to degradation, fragmentation, with loss of enzymatic activities in addition to improper folding of these proteins** (non-functional proteins)leading to accumulation of misfolded protein in the cell which may also predispose (تستعد ) to activate apoptosis.

**3**-**DNA damage.** it will do damage to the DNA and mutations at the level of mitochondrial and nuclear DNA leading to **single strand breaks, mediate: apoptosis, aging, malignant transformation.**

**4**-**Killing of microbes**. this is the beneficial role of ROS.

**Note:** ROS are produced in small amounts in normal cells in physiological conditions, however in pathologic conditions they are produced at large amounts and leading to catastrophic (كارثي) effect to the cell.

**Fourth Event: Cell injury caused by toxins**

**Toxins are mainly: Environmental chemicals & substances produced by infectious pathogens.**

their effect could be: Direct-acting toxins or latent-acting toxins.

**1- Direct acting of toxins:**

**They act directly by combining with a critical molecular component or cellular organelle** or cellular membrane component leading to change in those membranes Like binding with phospholipids in the brain.

**Examples:**

**1. Mercuric, chloride poisoning, occurs due to ingestion of contaminated seafood. Process→Mercury binds to sulfhydryl groups of membrane proteins→→inhibit ATP-dependent transport and increase permeability**.

**2. Chemotherapeutic agents, antineoplastic**.

**2- Latent acting toxins(indirect):**

1-the indirect acting toxins

2-**not intrinsically active by themselves**

3-**must be converted to reactive metabolites**(the substance by itself is inactive) **that then act on targeted cells, and this conversion usually happens in cells that contain Cytochrome p450 in SER, mainly in liver cells.**

4-**they cause cells damage by formation of free radicals which will cause membrane phospholipid peroxidation ,protein damage and DNA damage.**

**Examples:**

**CCL4 and acetaminophen (Panadol ,** the toxic doses**)**

**They both cause membrane peroxidation, thus damaging the cell.**

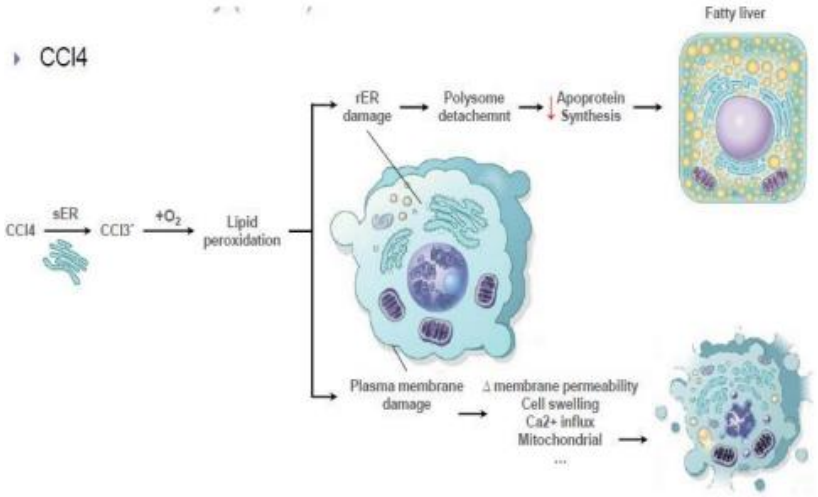
They also cause **ER membrane damage** leading to swelling of ER which will lead to decline and detachment of ribosomes which will cause a **decline in the synthesis of enzymes and proteins**(one of the protein function is to form lipoprotein so if the proteins decreased the lipids will accumulate)and **decreased synthesis of apoproteins** which will cause **fatty liver**.

**They also cause mitochondrial membrane injury→ decreased ATP →cell swelling** (due to reduced ATP-dependent membrane function) → **cell death**.

**NOTE: ***Apoproteins: they are especially produced in the liver, responsible of carrying lipids and excreting them out of the hepatocytes to the circulation. [ Their low concentrations cause accumulation of lipids and triglycerides inside hepatocytes, thus causing fatty liver]

## The Mechanism of ccl4 and acetaminophen(CCL4 toxicity)

CCL4 is converted to ccl3(free radical with unpaired electron) By the action of Cytochrome p450 In the liver.




Fatty liver is the consequence of CCL4 and Acetaminophen toxicity.

**Fifth Event : Endoplasmic Reticulum Stress:**

proteins are usually synthesized in the ER, after synthesis they are folded in a proper way to function properly. This folding process needs specific molecules called **chaperones.**

**Improper folding can occur due to:**

**A-Gene mutations**, leading to production of proteins that can't be folded properly.

**B-Aging**, ↓ cell's ability to produce chaperons, ↓ cell's capacity to fold protein.

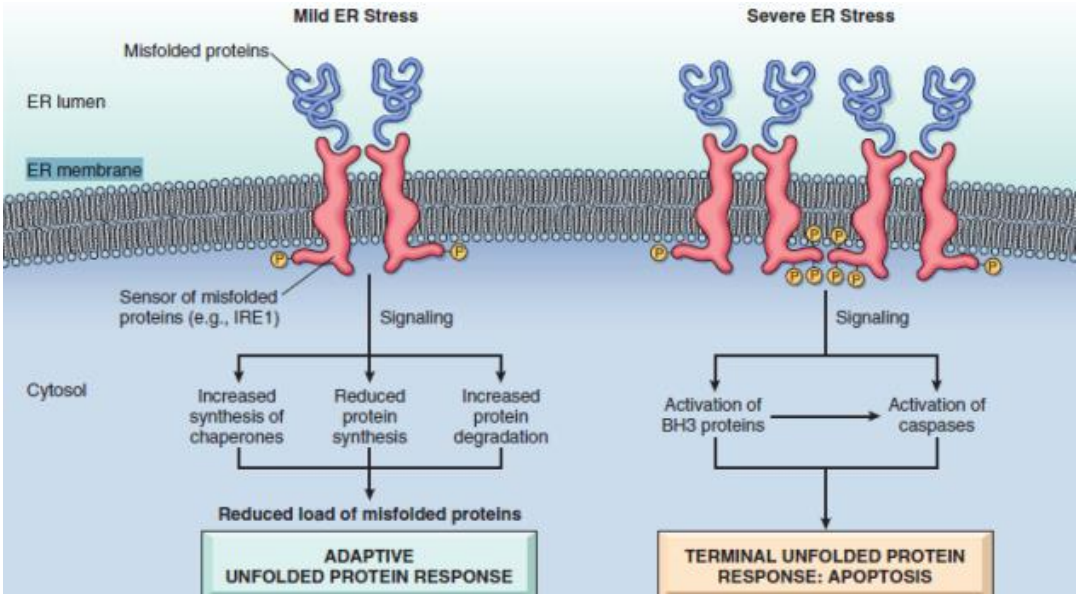
**C-infections**, especially viral infections, due to increased production and misfolding of viral proteins will lead to accumulation of viral proteins causing stress to the cell.

**D-Increased demand for secretory proteins such as insulin in insulin-resistant patients**(diabetes type 2) the body respond as if there is no insulin, so the pancreas respond by secreting more insulin, which will accumulate in cells, as there is no capacity to fold this insulin properly, so it will accumulate in a misfolded form in the pancreatic cells leading to their death and damage by apoptosis.

**E- Changes in intracellular PH in ischemia and hypoxia**.

**F-neurodegenerative disease**, like Alzheimer, Parkinson disease

Cell's response against misfolded proteins production:



**A-**If their amount was not high, they will be targeted for proteolysis**(Misfolded proteins >> ubiquinated >> targeted to proteolysis).**

**B-**If the misfolded proteins were found in high amounts, the cell will try to adapt by the activation of Unfolded Protein Response (adaptive response).

**Unfolded Protein Response (adaptive response): increase chaperons' production, decrease protein translation, increase protein destruction.** By this, unfolded protein concentration is decreased in the cell. by this, the unfolded proteins concentration is decreased in the cell.

**C-**If the previous responses weren't efficient, there will be activation of apoptosis (cell suicide), but, HOW?

**By Activation of → 'proapoptotic sensor activation (BH3-protein family) → activation of specific caspases (apoptotic enzymes),which will eventually cause apoptosis by the mitochondrial pathway.**

- We said that misfolded-proteins accumulation may turn on the apoptosis pathway and lead to cell death thus diseases, but not always. Diseases could result from the **deficiency of a specific protein that was produced misfolded and degraded, some examples:**

**1) Cystic Fibrosis:** Genetic mutation in a protein that function as a membrane transporter, so when we Produce misfolded protein we degrade it so we will have a deficiency of this transporter. The cystic fibrosis a disease which affect the lungs and the GI tract with a thick secretions which can cause obstruction in the GI tract and bronchi .

**2)Neurodegenerative disorders: Apoptosis of the diseased cell is the result Ex: Alzheimer, Huntington, Parkinson disease**

**3)Type 2 diabetes: also result from apoptosis of the diseased cell.**

**4)Amyloidosis: Improperly folded proteins accumulation in the extracellular tissues.**

**Sixth Event: DNA damage:**

Causes:

- **Radiation**
- **Chemotherapeutic agents**
- **Intracellular generation of ROS**
- **Mutation.**

**Any DNA damage in the cell, is sensed by P53 gene "guardian of the genome" (tumor suppressor gene), leading to its activation.**

**P53 arrests cell's replication at G1 phase for repair:**

- **if Repair is possible, the cell will continue replication.**
- **if repair is impossible, apoptosis is induced**.
- **If P53 gene is mutated**, there will be no stop for the **mutated cell from completing replication**, leading to **neoplastic change**(cancer).

**Ex:** sometimes cells develop DNA damage after exposure to sun for a long time, and this leads to P53 activation, thus arresting the cell at G1 phase until repair is done. BUT if P53 was mutated, abnormal cells will be produced, leading to skin cancer, like: basal cell carcinoma or squamous cell carcinoma.

**Seventh Event : Inflammation:**

Normal reaction in the body elicited by:

**o Pathogens, ex: microbes.**

**o Necrotic cells**

**o Dysregulated immune responses (autoimmune diseases and allergies)**

when inflammation occurs, there will be accumulation of**: Inflammatory cells (neutrophils, macrophages, lymphocytes) that will secrete products that destroy microbes and damage host tissues**, leading to cell injury.

**Remember :all of these mechanisms work together, for example the ROS may generate and at the same time hypoxia or inflammation takes place.**

## After studying mechanisms of cell injury, we end up with events that are common in cell injury despite their different causes:

So, most of the causes that we have talked about (hypoxia/ischemia/toxins…) lead to:

**o Mitochondrial Dysfunction**

**o Defects in membrane permeability**

**Mitochondrial Dysfunction:** can result from **Hypoxia, toxins, radiation**, and since it's the **energy factory in the cell**, its dysfunction causes either **Apoptosis or Necrosis.**

Consequences:

**1. Failure of oxidative phosphorylation, leading to ATP depletion.**

**2. Abnormal/insufficient oxidative phosphorylation, leading to formation of ROS.**

**3. mitochondrial permeability transition pores, leading to loss of membrane potential** and insufficient oxidative phosphorylation.

**4. Release of cytochrome c** which is usually stored in mitochondria, to the cytoplasm which will activate specific cascades >> stimulating **apoptosis.**

## Mitochondrial Damage and Dysfunction

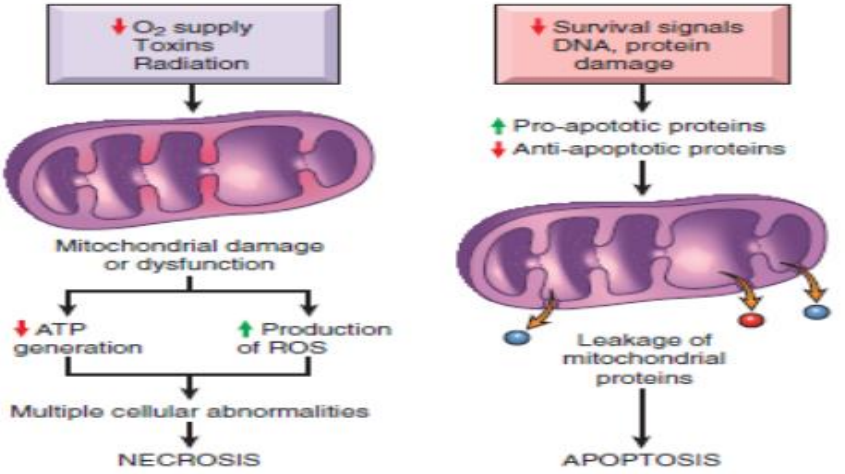



**Figure 1–16** Role of mitochondria in cell injury and death. Mitochondria are affected by a variety of injurious stimuli and their abnormalities lead to necrosis or apoptosis. This pathway of apoptosis is described in more detail later. ATP, adenosine triphosphate; ROS, reactive oxygen species.

## Depletion of ATP

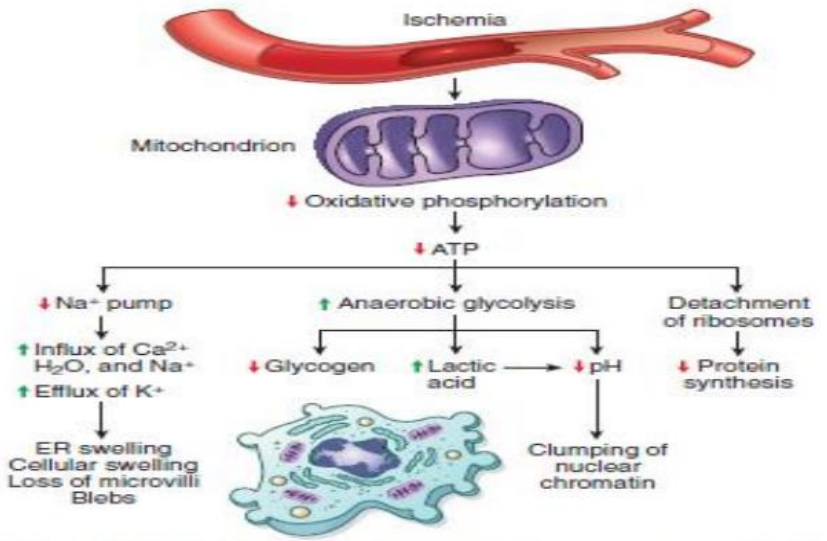



**Figure 1–15** The functional and morphologic consequences of depletion of intracellular adenosine triphosphate (ATP). ER, endoplasmic reticulum.

**Defects in membrane permeability :**

- **Mitochondrial membrane damage: decreased ATP**
- **Plasma membrane damage: loss of osmotic balance, influx of fluids** due to attraction of water**, leak of contents**
- **Lysosomal membranes: leakage of enzymes into cytosol >> leading to cellular digestion.**

V1