Mechanisms of cell injury

DR. SAIMA NADEEM
Assist Professor
Pathology


وَمَا تَوْفِيقِيٓ إِلَّا بِٱللَّهِ
My success is only by Allah
QUR'AN [11,88]


بسم الله الرحمن الرحيم

Knowledge gained up till now about Cell injury

Etiology or Causes of cell injury

Learning Objectives:

- By the end of this lecture student should be able to;

1. Enumerate the mechanisms of cell injury
2. Describe each mechanism
3. Understand the relation of different causes of cell injury to these mechanisms.
4. Discuss Free radical injury.

- The biochemical mechanisms responsible for cell injury can be grouped as:

1. ATP depletion
2. Mitochondrial damage
3. Net influx of extracellular calcium
4. Accumulation of oxygen-derived free radicals
5. Defects in membrane permeability
6. Damage to DNA and proteins

•ATP depletion:

- ATP depletion and decreased ATP synthesis frequently associated with hypoxic and chemical injury.
- Can occur due to

a. Reduced supply of oxygen and nutrients

b. Mitochondrial damage.

c. Actions of some toxins (e.g. Cyanide).

- Fundamental cause of necrotic cell death

- ATP required for many processes within cell (Membrane transport, protein synthesis and lipogenesis etc).
- ATP is produced in two ways.

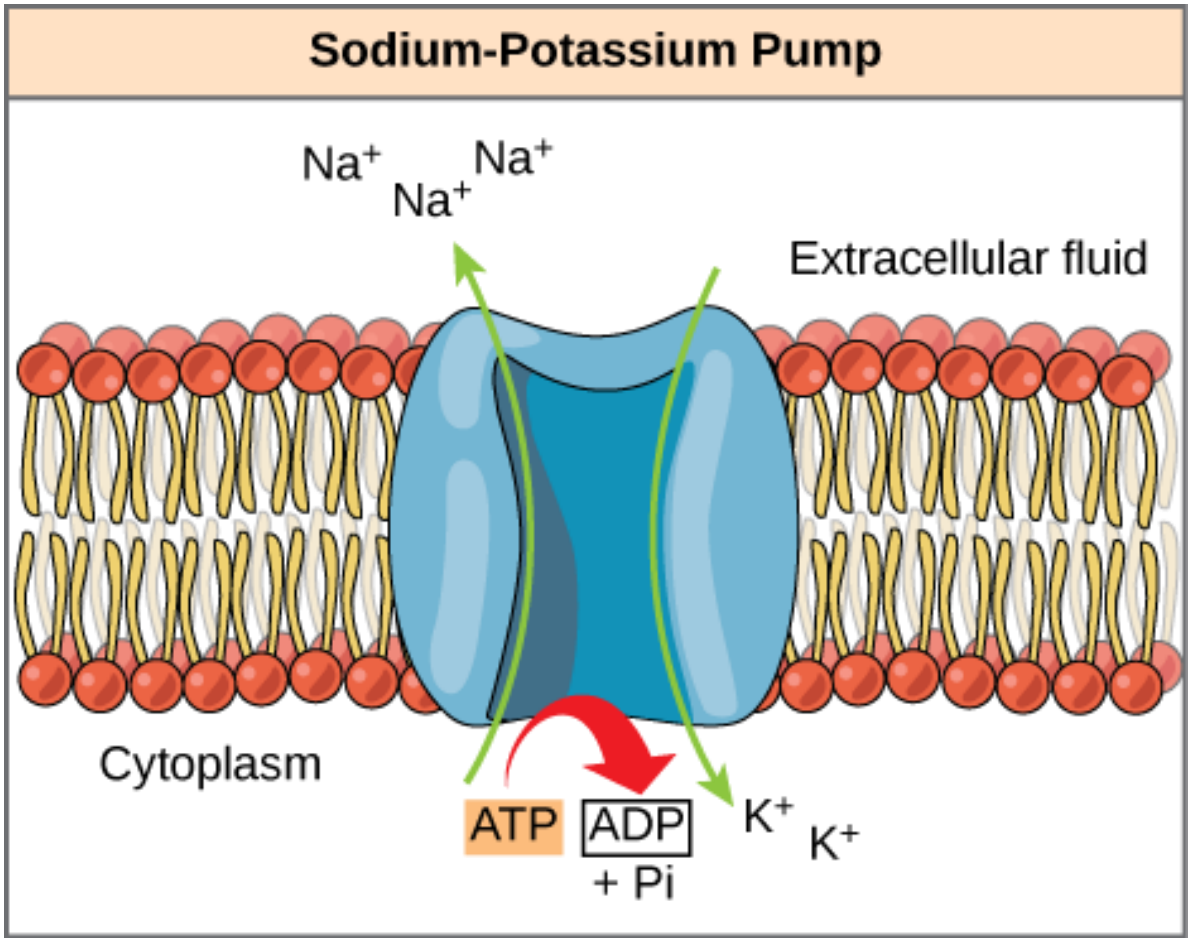
a. Oxidative phosphorylation of adenosine diphosphate.

b. Anaerobic glycolysis.

- Depletion of ATP to <5% to 10% of normal levels has serious effects on many cellular systems.

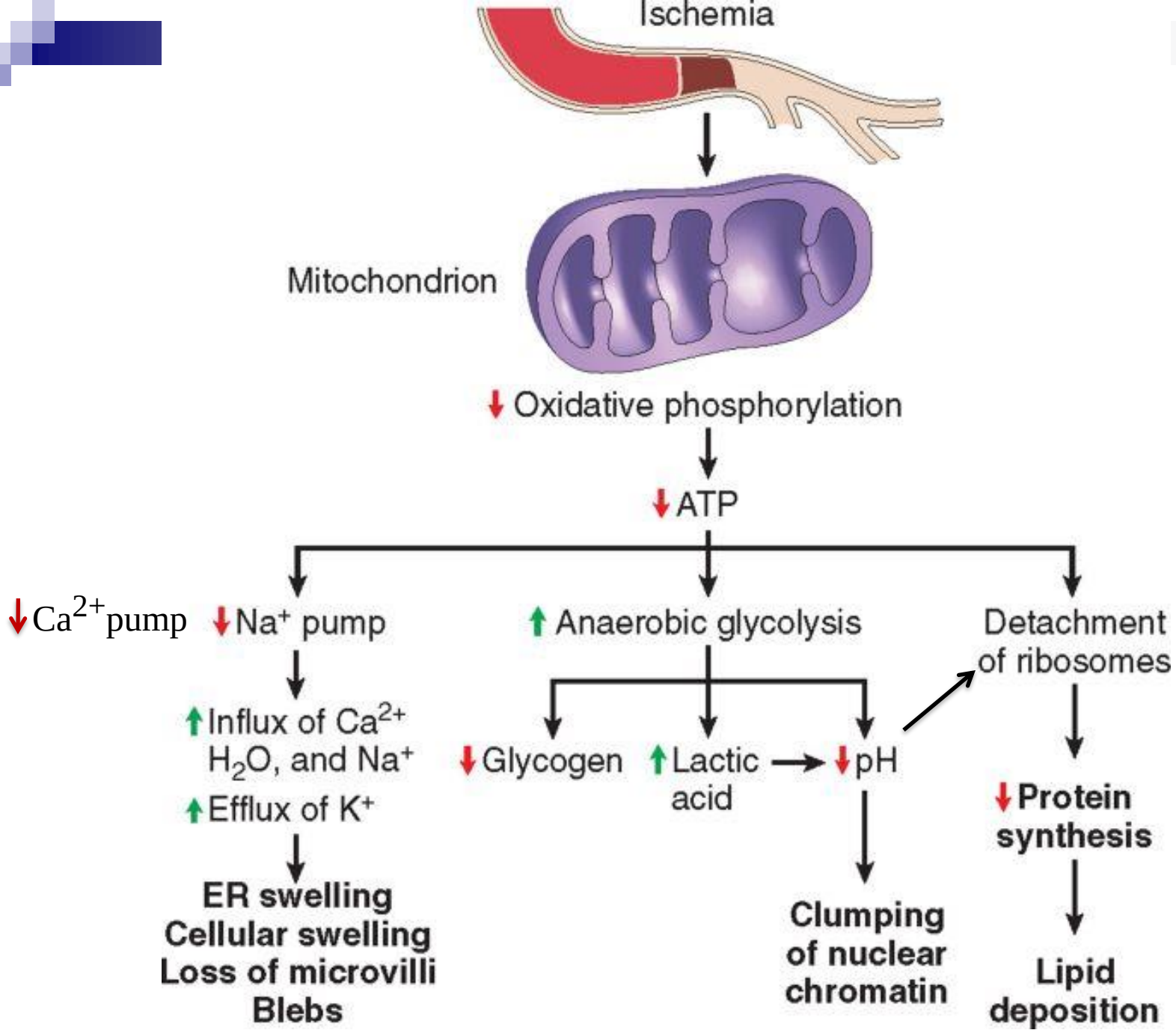
1. Failure of "sodium potassium pump causing accumulation of extracellular sodium and the diffusion of potassium out of the cell.

- The net gain of sodium accompanied by gain of water, producing acute cellular swelling.



2. Failure of Ca^{2+} pump causing Ca^{2+} influx with damaging effects on numerous cellular components.
3. Anaerobic glycolysis increases due to decreased oxygen leading to increased lactic acid and a fall in PH.

- Decreasing pH and ATP levels cause ribosomes to detach from the rough endoplasmic reticulum (RER) causing reduction in protein synthesis.
- Decreased PH also leads to clumping of chromatin.


Ischemia
Mitochondrion
↓ Oxidative phosphorylation
↓ ATP
↓ Ca^{2+} pump
↓ Na^+ pump
↑ Influx of Ca^{2+}, H_2O, and Na^+
↑ Efflux of K^+
ER swelling
Cellular swelling
Loss of microvilli
Blebs
↑ Anaerobic glycolysis
↓ Glycogen
↑ Lactic acid
↓ pH
Clumping of nuclear chromatin
Detachment of ribosomes
↓ Protein synthesis
Lipid deposition

•Mitochondrial Damage:

- Cell injury frequently accompanied by morphologic changes in mitochondria.
- Causes:

a. Increase in Cytoplasmic/cytosolic Ca^{+2}. Cytolsolic calcium leads to activation of enzymes like phospholipases and formation of channels in mitochondria.

b. Increased production of ROS.

c. Oxygen deprivation.

- Three major results of mitochondrial damage.

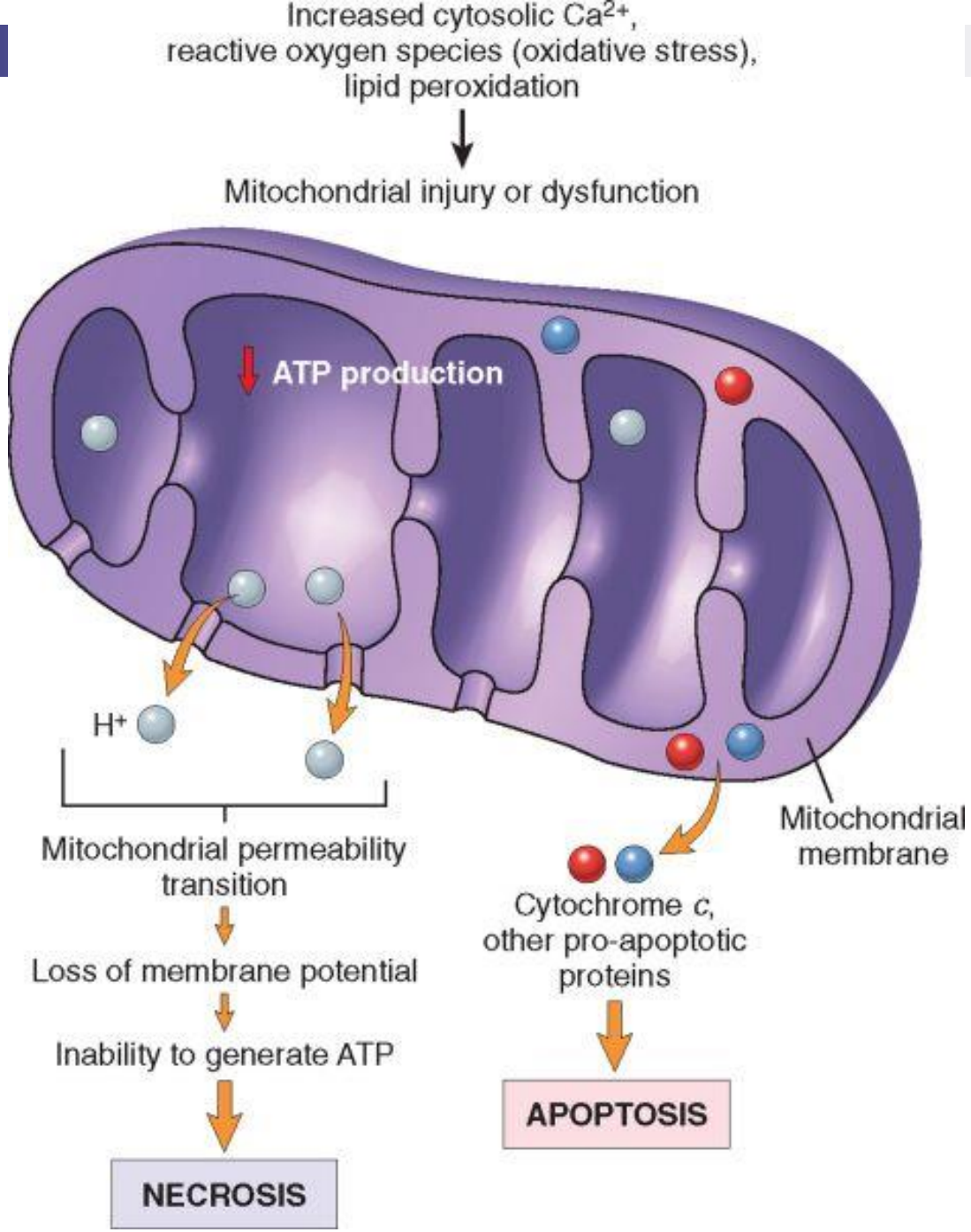
1. Mitochondrial damage causes formation of high-conductance channel(mitochondrial permeability transition pore or MPTP) in the mitochondrial membrane leading to loss of membrane potential and thus ↓ oxidative phosphorylation ⟶ necrosis.

- The mitochondrial membrane potential generated by proton pumps is an essential component in the process of energy storage during oxidative phosphorylation
- Mitochondria membrane potential (MMP) is required for ATP production. MMP decrease results in ATP depletion

- Mitochondrial permeability transition pore (MPTP) is a transmembrane protein residing in the mitochondrial inner membrane.
- Normally closed, this large protein pore opens when stimulated by mitochondrial matrix Ca2+ accumulation, adenine nucleotide depletion, increased phosphate concentration or oxidative stress.

2. Abnormal oxidative phosphorylation leads to ROS production which cause damage to mitochondria.
3. The mitochondria contain several proteins (like cytochrome c) capable of activating apoptosis.

- These proteins leak into the cytoplasm and cause death by apoptosis.


Increased cytosolic Ca^{2+},
reactive oxygen species (oxidative stress),
lipid peroxidation
Mitochondrial injury or dysfunction
ATP production
H^+
Mitochondrial permeability transition
Loss of membrane potential
Inability to generate ATP
NECROSIS
Cytochrome *c*, other pro-apoptotic proteins
APOPTOSIS
Mitochondrial membrane

Influx of calcium:

- Ischemia and certain toxins cause increase in cytosolic calcium because of

1. Release of Ca^{+2} from intracellular stores.

2. Increased influx across the plasma membrane.

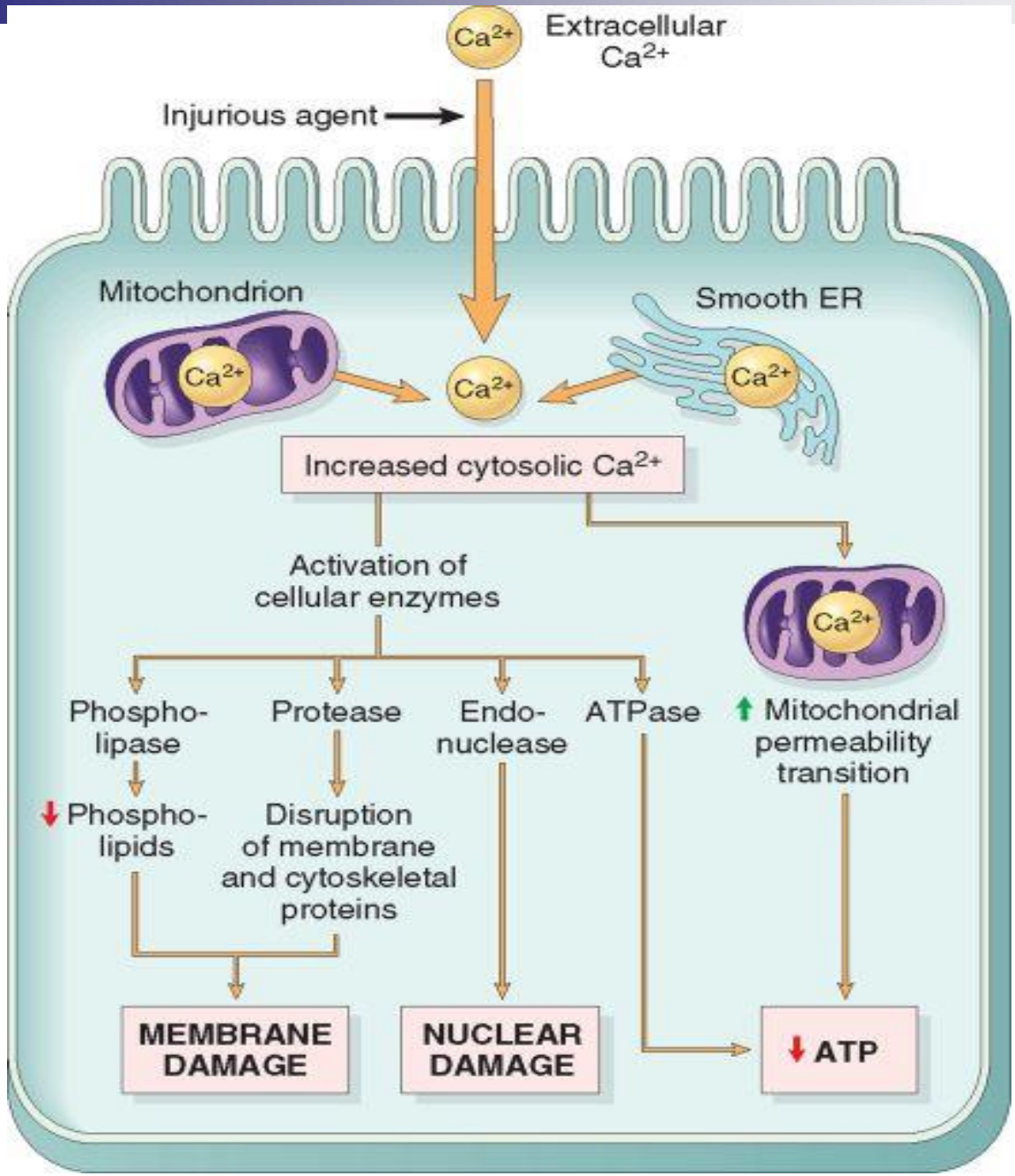
- Increased cytosolic Ca^{+2} causes cell damage in three ways.

1. Activates enzymes. These enzymes include phospholipases, proteases, endonucleases, and Adenosine triphosphatases (ATPases).

- These enzymes perform their respective functions.

2. Opening of mitochondrial permeability transition pore (MPTP).

3. Cytosolic calcium directly activates enzymes responsible for apoptosis i.e. caspases


Ca2+
Extracellular Ca2+
Injurious agent
Mitochondrion
Ca2+
Smooth ER
Ca2+
Ca2+
Increased cytosolic Ca2+
Activation of cellular enzymes
Ca2+
Phospho-lipase
Protease
Endo-nuclease
ATPase
Mitochondrial permeability transition
Phospho-lipids
Disruption of membrane and cytoskeletal proteins
MEMBRANE DAMAGE
NUCLEAR DAMAGE
ATP

OXIDATIVE STRESS

- Oxidative stress refers to cellular abnormalities that are induced by ROS, which belong to a group of molecules known as free radicals.
- Free radical-mediated cell injury is seen in many circumstances, including chemical and radiation injury, hypoxia, cellular aging, tissue injury caused by inflammatory cells, and ischemic reperfusion injury.
- In all these cases, cell death may be by necrosis, apoptosis, or the mixed pattern of necroptosis

•Accumulation of Oxygen-Derived Free Radicals:

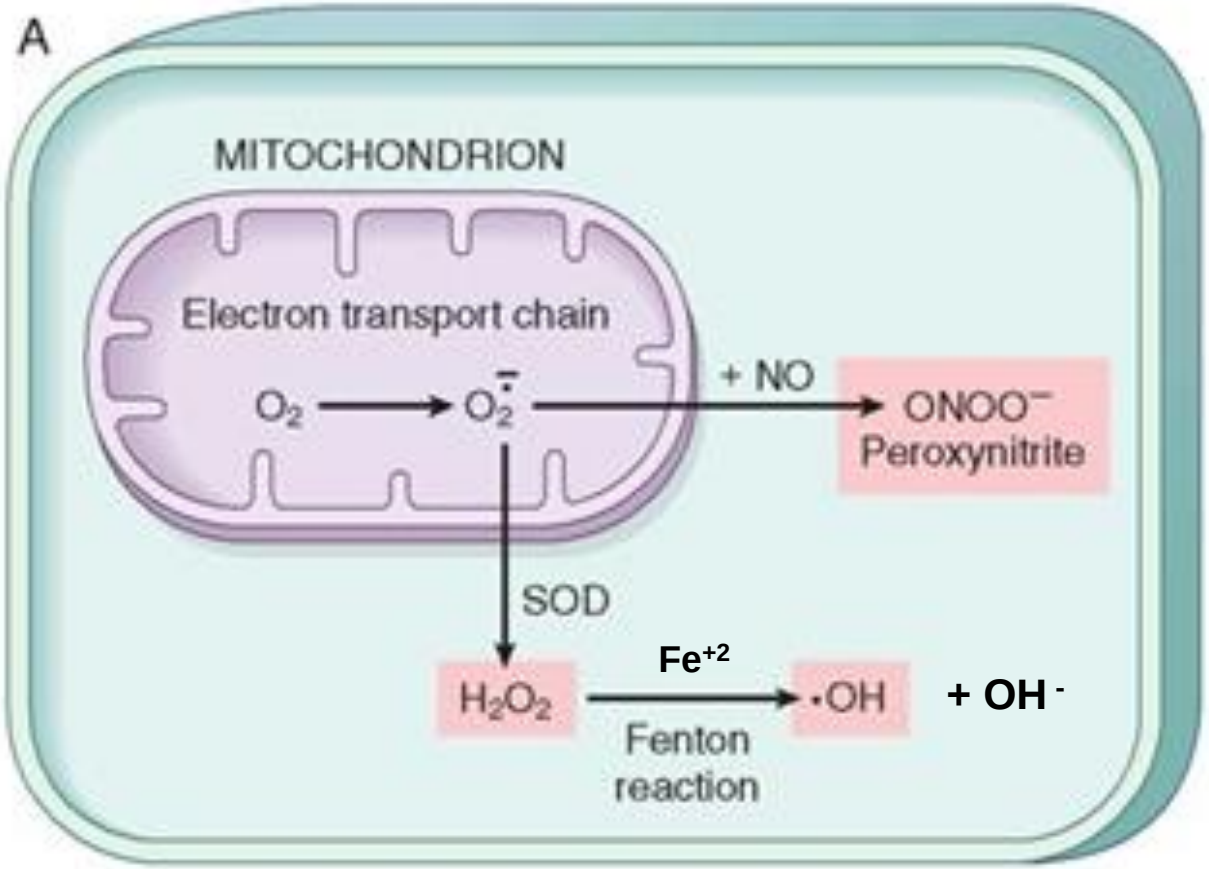
- Free radicals are chemical species with a single unpaired electron in their outer orbit.
- Extremely unstable
- Attack nucleic acids, variety of cellular proteins and lipids to give or take an electron.

- Molecules that react with free radicals are in turn converted into free radicals.
- Reactive oxygen species (ROS) are type of free radicals derived from oxygen.
- The common ROS include superoxide radical ($O^{-\bullet}_2$), hydrogen peroxide (H_2O_2), and hyroxyl (•OH) radical.

- ROS are produced by two major pathways

1. Produced in small amounts in all cells during oxidative phosphorylation ($O^{-}\bullet_2$).

- $O^{-}\bullet_2$ is converted spontaneously into H_2O_2 and Peroxynitrite ($ONOO^-$) another free radical.
- In presence of Fe^{2+}, H_2O_2 converted to highly reactive •OH and ^{-}OH by Fenton reaction.



$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + OH^-$$

(Fenton reaction)

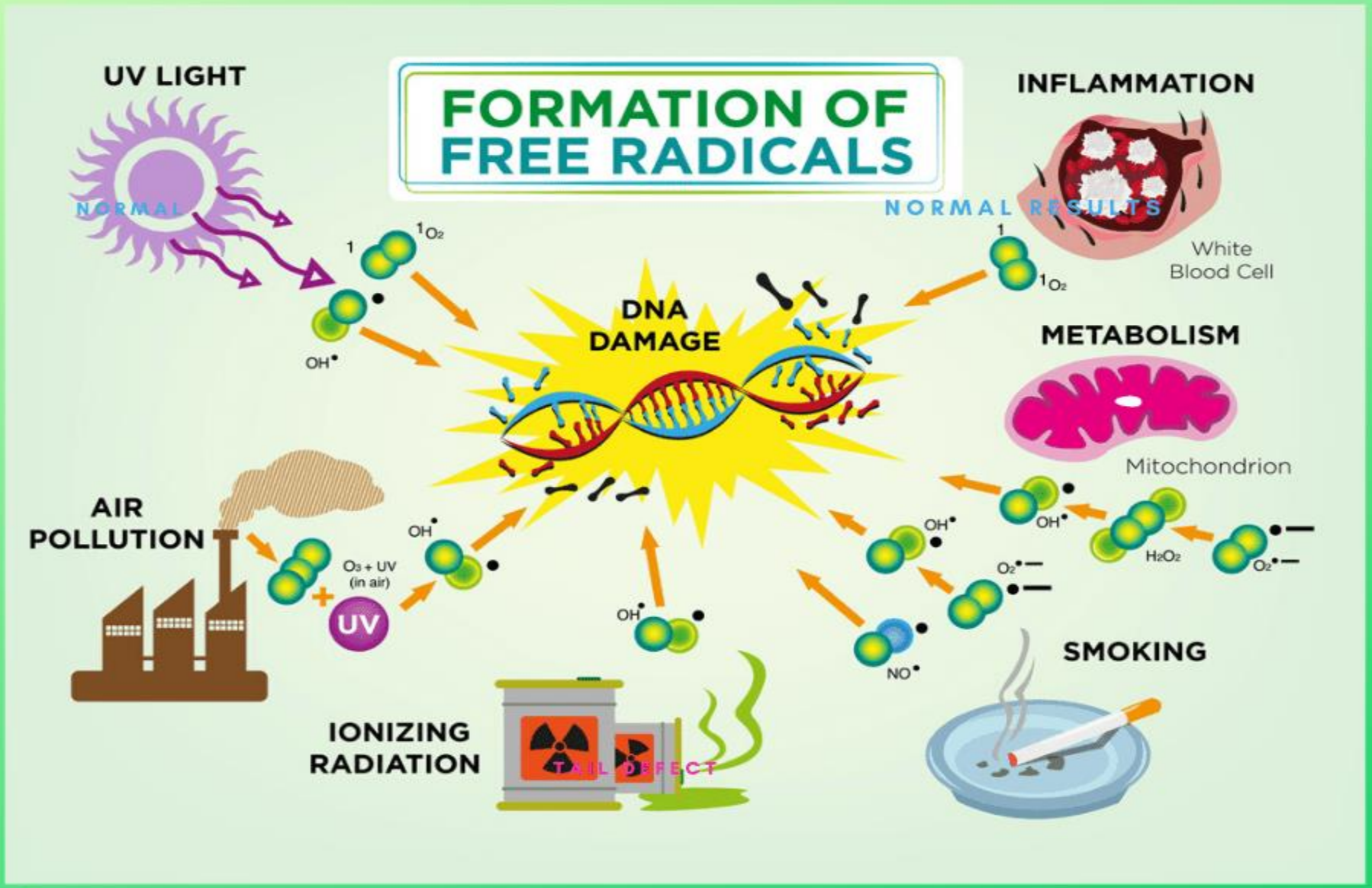
2. ROS are produced in neutrophils and macrophages during inflammation.

- The ROS are generated in leukocytes in a process called oxidative burst (enormous ROS production).
- First of all $O^{-}\bullet_2$ is produced which is converted into hypochlorite (HOCl), H_2O_2 and $ONOO^-$ (Peroxynitrite)

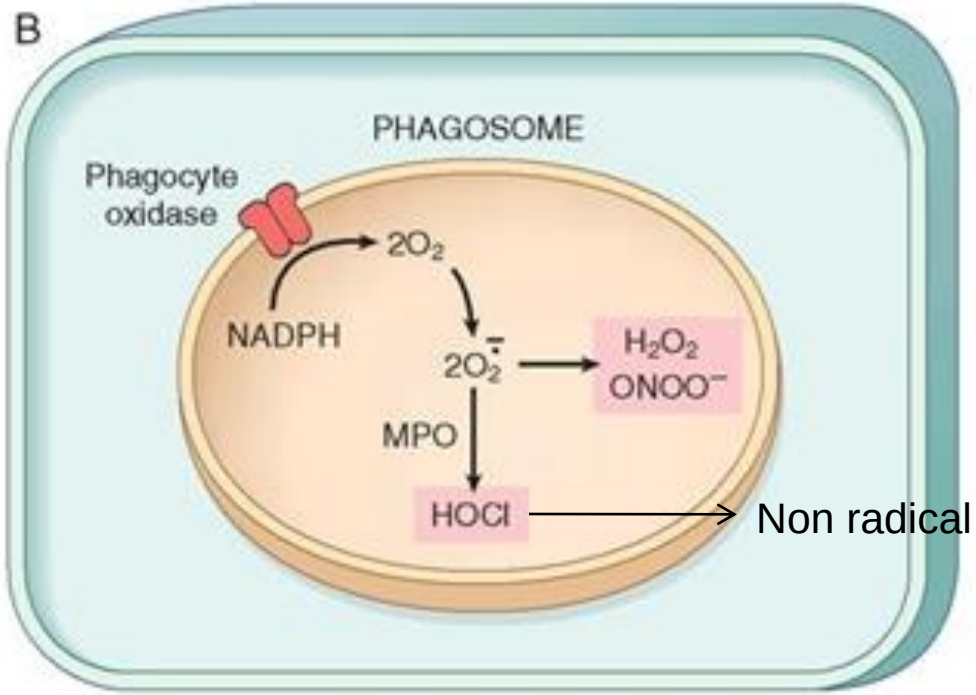
- The generation of free radicals is increased under several circumstances:
- The absorption of radiant energy (e.g., ultraviolet (UV)light, x-rays).
- Ionizing radiation can hydrolyze water into hydroxyl (•OH) and hydrogen (H•) free radicals.

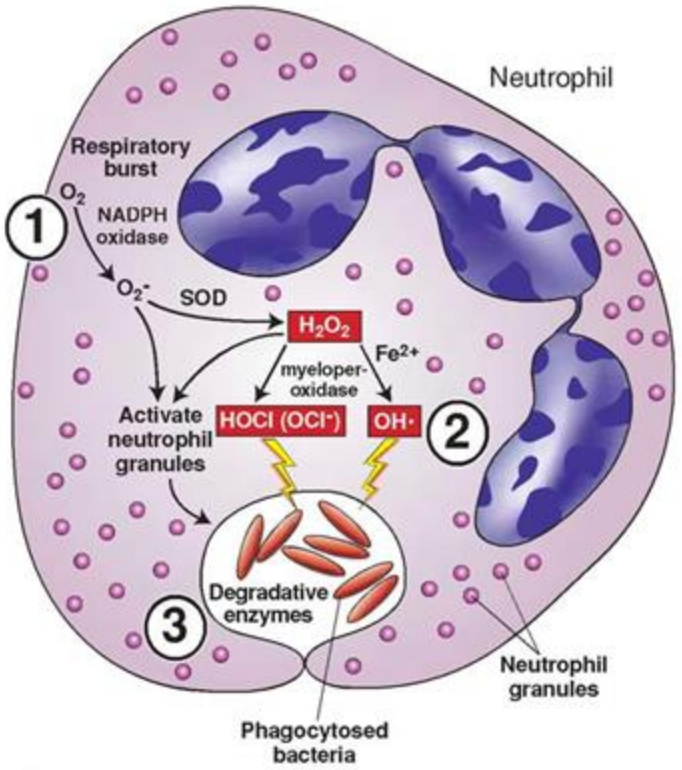
- The enzymatic metabolism of exogenous chemicals(e.g., carbon tetrachloride.
- Inflammation, in which free radicals are produced by leukocytes.
- Reperfusion of ischemic tissues

FREE RADICALS CAUSE DNA DAMAGE



ecosh





- Free radicals are unstable and decay spontaneously. There are also enzymatic systems that contribute to inactivation of free radicals.

- Superoxide dismutase, Glutathione peroxidase and Catalase

1. $O^{-\bullet}_2 \xrightarrow{SOD} H_2O_2$

2. $2\ GSH + H_2O_2 \xrightarrow[\text{peroxidase}]{\text{Glutathione}} GSSG + 2\ H_2O$

- GSH (reduced glutathione) and GSSG(oxidized glutathione)

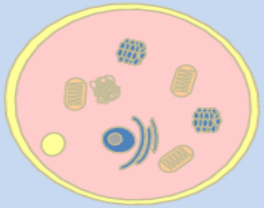
3. $2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O$

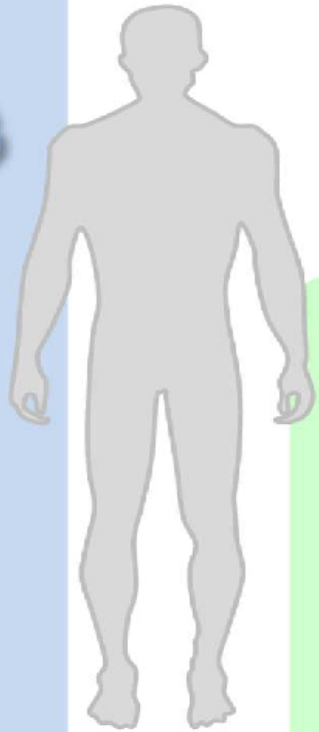
- Endogenous or exogenous anti-oxidants (e.g., vitamins E,A, and C and β-carotene) may either block the formation of free radicals or scavenge them after they have formed.

Antioxidants

Endogenous Antioxidants

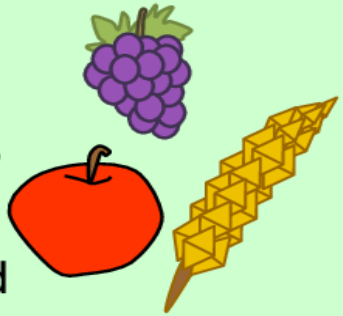
- glutathione
- superoxide dismutase
- peroxiredoxin
- catalase
- thioredoxin
- uric acid
- albumin
- bilirubin
- glucose
- Fe- and Cu-binding proteins (Ferritin, Transferrin, etc.)
- coenzyme Q
- metallothioneins
- melatonin
- L-carnitine





Exogenous Antioxidants

- vitamin E
- vitamin C
- carotenoids
- ubiqinol
- α-lipoic acid
- flavonoids, polyphenols, anthocyanidins, isoflavones
- trace elements (Zn, Se)



- ROS cause cell damage by

1. Lipid peroxidation of cell membranes.

2. Cross linking of proteins.

3. Direct protein and DNA damage.

DEFECTS IN MEMBRANE PERMEABILITY:

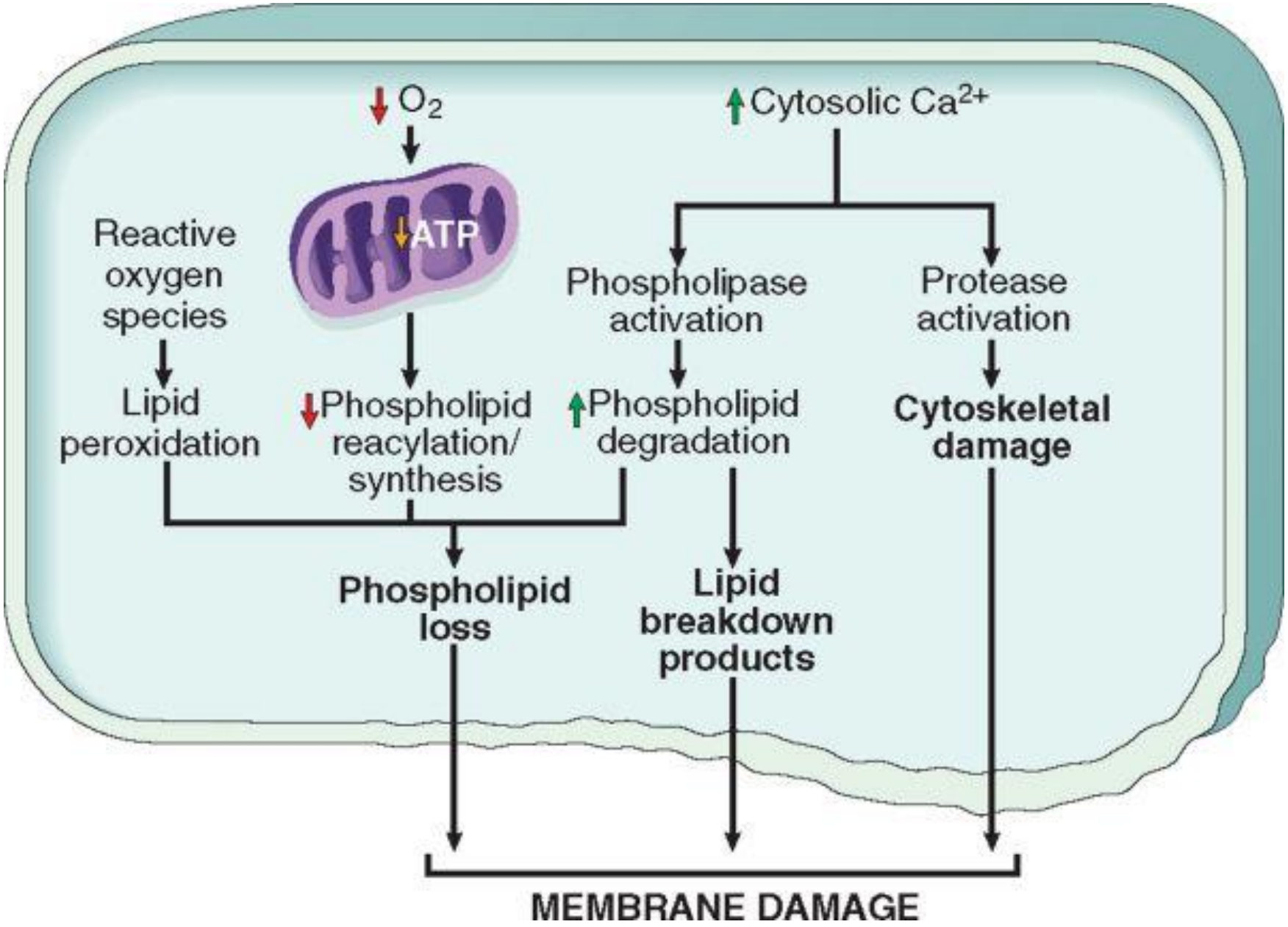
- Loss of membrane permeability leads to membrane damage.
- Mechanisms of Membrane Damage.

a. Lipid peroxidation by ROS.

b. Decreased phospholipid synthesis due to ↓ ATP

c. Increased phospholipid breakdown (Phospholipases)

d. Cytoskeletal abnormalities/ damage due to proteases (activated by Ca^{2+} influx)


↓O2
↑Cytosolic Ca2+
↓ATP
Reactive oxygen species
Phospholipase activation
Protease activation
Lipid peroxidation
↓Phospholipid reacylation/ synthesis
↑Phospholipid degradation
Cytoskeletal damage
Phospholipid loss
Lipid breakdown products
MEMBRANE DAMAGE

Consequences of Membrane Damage:

a. Mitochondrial membrane damage → ↓ATP due to MPTP formation.

b. 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.

c. Lysosomal membrane damage → release of degradative enzymes

- **DAMAGE TO DNA AND PROTEINS:**
- Cells have mechanisms that repair damage to DNA, but if damage is too severe to be corrected the cell initiates death by apoptosis.
- Similarly damage to proteins (by ROS) leads to their misfolding which initiates apoptosis.

surgeons after operation..and medical students after exams tell the same thing..

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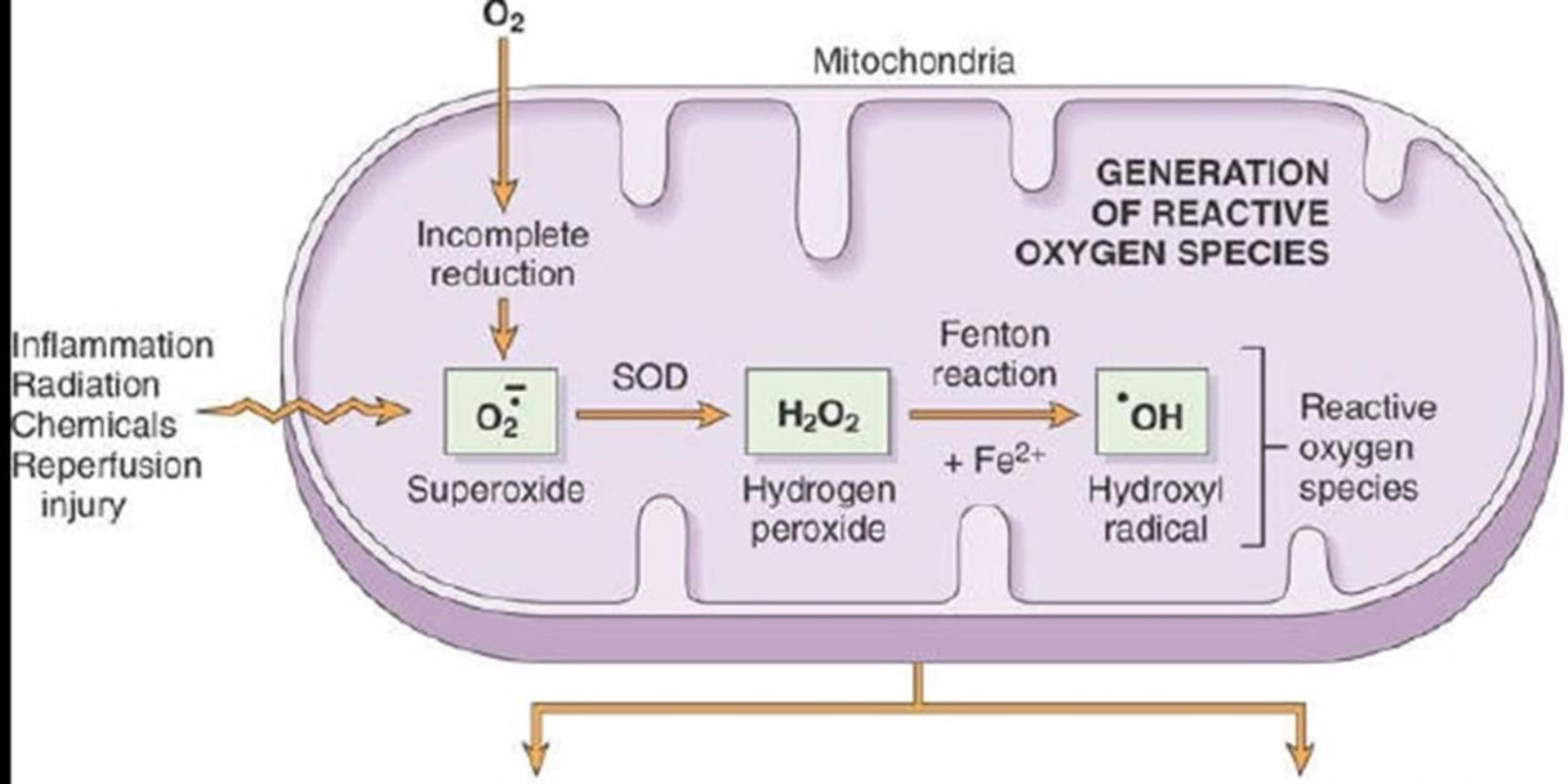
we tried our best cant say anything right now..!



PATHOLOGIC EFFECTS OF ROS: CELL INJURY AND DEATH

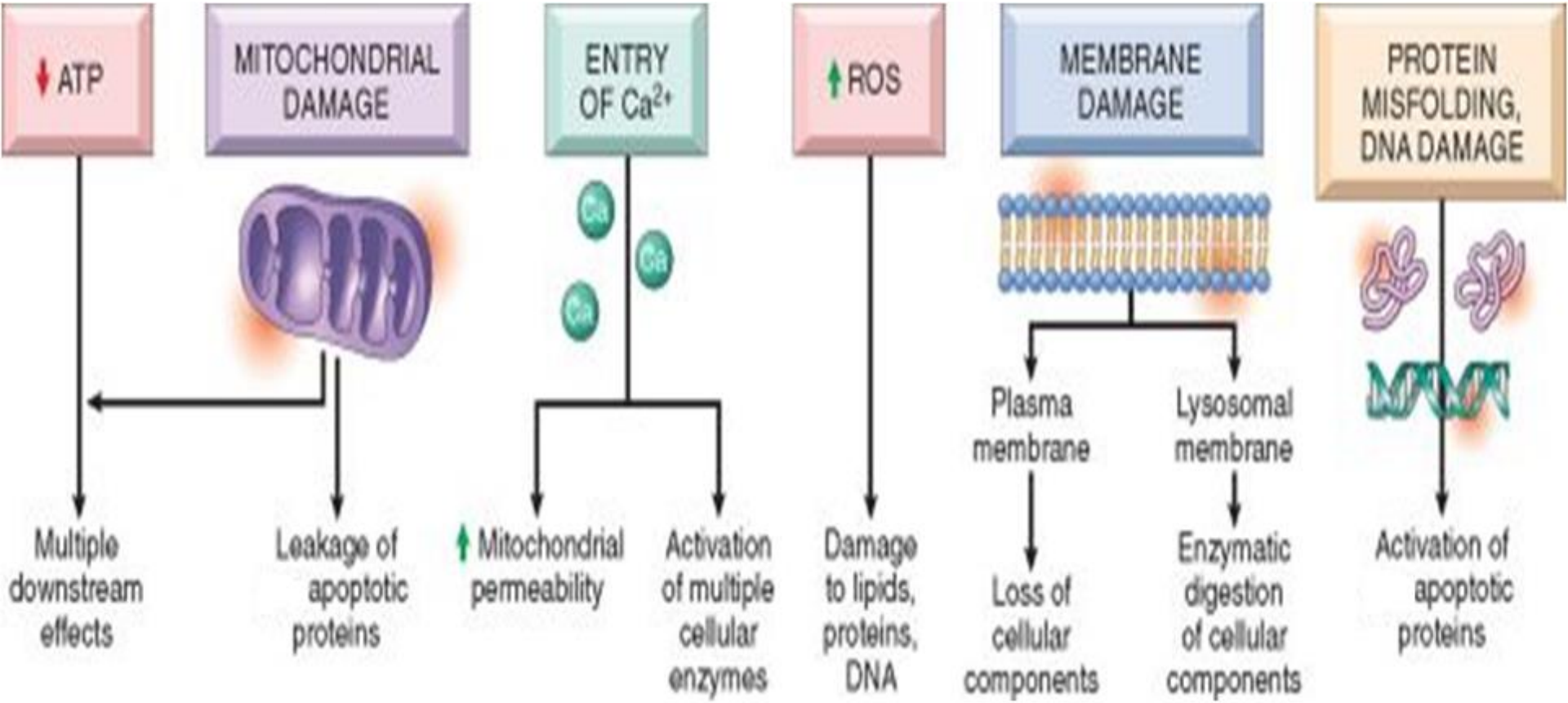
ROS react with:

- Fatty acids → oxidation → generation of lipid peroxidases → disruption of plasma membrane, organelles
- Proteins → oxidation → loss of enzymatic activity, abnormal folding
- DNA → oxidation → mutations, breaks

REMOVAL OF FREE RADICALS

Antioxidant mechanisms:

- SOD (in mitochondria) converts $O_2^{\cdot -} \rightarrow H_2O_2$
- Glutathione peroxidase (in mitochondria) converts $^{\cdot}OH \rightarrow H_2O_2 \rightarrow H_2O + O_2$
- Catalase (in peroxisomes) converts $H_2O_2 \rightarrow H_2O + O_2$


ATP
MITOCHONDRIAL DAMAGE
ENTRY OF Ca^{2+}
ROS
MEMBRANE DAMAGE
PROTEIN MISFOLDING, DNA DAMAGE
Multiple downstream effects
Leakage of apoptotic proteins
Mitochondrial permeability
Activation of multiple cellular enzymes
Damage to lipids, proteins, DNA
Plasma membrane
Lysosomal membrane
Loss of cellular components
Enzymatic digestion of cellular components
Activation of apoptotic proteins

	Reversible Injury	Irreversible Injury
Mitochondria) 1	Swelling with Few small . amorphous densities	Severe mitochondrial membrane swollen & Large amorphous densities
Plasma) 2 Membrane	Intact with few Blebbing, Blunting, with loss of microvillus	Extensive damage to plasma membrane with loss cellular .organelle
Lysosome) 3	Membrane intact	Membrane damaged with .vacuoles
Endoplasmic) 4 Reticulum	Smoothening with detachments of .ribosome	Lysis of ER with dilatation .with detachment of ribosome
Nucleus) 5	Clumping of chromatin	Pyknosis->Karyorrhexis- .>Karyolysis
Cytoplasm) 6	Eosinophilic with Fine Myelin figure	Shows Course Myelin figure

Necroptosis

- . In some instances, regulated cell death shows features of both necrosis and apoptosis, and has been called necroptosis.

Autophagy

- Autophagy (“self-eating”) refers to lysosomal digestion of the cell’s own components.
- It is a survival mechanism in times of nutrient deprivation, so that the starved cell can live by eating its own contents and recycling these contents to provide nutrients and energy.
- In this process, intracellular organelles and portions of cytosol are first sequestered within an ER-derived autophagic vacuole, whose formation is initiated by cytosolic proteins that sense nutrient deprivation

- The vacuole fuses with lysosomes to form an autophagolysosome, in which lysosomal enzymes digest the cellular components.
- In some circumstances, autophagy may be associated with atrophy of tissues and may represent an adaptation that helps cells survive such times.
- If, however, the starved cell can no longer cope by consuming its contents, autophagy may eventually lead to apoptotic cell death

Endocytosis

- Endocytosis is the process by which cells take in substances from outside of the cell by engulfing them in a vesicle. These can include things like nutrients to support the cell or pathogens that immune cells engulf and destroy.

Heterophagy

- The transport of materials from the extracellular medium into the interior of the cell by endocytosis, and the subsequent digestion of the contents of endocytotic vacuoles by lysosomal enzymes, is known as heterophagy.

Pinocytosis

- Pinocytosis is an active, energy consuming process where extracellular fluid and solutes are taken up into a cell via small vesicles. It is a type of endocytosis, which refers to the uptake of substances by a cell.

Pyroptosis

- This form of cell death is associated with activation of a cytosolic danger-sensing protein complex called the inflammasome .
- The net result of inflammasome activation is the activation of caspases, some of which induce the production of cytokines that induce inflammation, often manifested by fever, and others trigger apoptosis.
- Thus, apoptosis and inflammation coexist. The name pyroptosis stems from the association of apoptosis with fever (Greek, pyro = fire).

Subcellular responses to injury

1. **Lysosomal Catabolism**
   - ***Heterophagy***
   - **Autophagy**
2. **Induction (Hypertrophy) of Smooth Endoplasmic Reticulum**
3. **Mitochondrial Alterations**
4. **Cytoskeletal Abnormalities**
5. **Heat Shock Proteins**

In an experiment, a large amount of a drug is administered to experimental organisms and is converted by cytochrome P-450 to a toxic metabolite. Accumulation of this metabolite leads to increased intracellular lipid peroxidation. Depletion of which of the following intracellular substances within the cytosol exacerbates this form of cellular injury by this mechanism?

ADP
Glutathione
NADPH oxidase
Nitric oxide synthase
Sodium

In an experiment, metabolically active cells are subjected to radiant energy in the form of x-rays. This results in cell injury caused by hydrolysis of water leading to production of H_2O_2. Which of the following intracellular enzymes helps to protect the cells from this type of injury?

Endonuclease
Glutathione peroxidase
Lactate dehydrogenase
Phospholipase
Protease


جزاك اللهُ خيرً
JazakAllah Khairn
May Allah reward
you with good