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PATHOLOGY

MID | Lecture 3&4

Mechanism of Cell Injury

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Color coding used in the modified:



Black: the original slides



Magenta: the doctor's explanation/words



Gray: additional information and explanation



Red: important information

MECHANISMS OF CELL INJURY

cell injury and adaptations

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MECHANISMS OF CELL INJURY

Principles

- The cellular response to injury depends on:

type of injury

Hypoxia, Ischemia (could cause coagulative necrosis), infections (could cause caseous necrosis) and other types of injuries each one has its own consequences

duration (determines if the cell will enter reversible or irreversible injury)

For example ischemia to the heart (cardiac muscle) :for 1-3 minutes causes angina (chest pain or pressure) (reversible) which is different from ischemia for 20 minutes that causes myocardial infarction (irreversible)

The same for brain cells and skeletal muscles; ischemia for half an hour to a skeletal muscle doesn't cause death to it but if ischemia continues for 3 hours it causes necrosis (death)

Severity *is important in determination of consequences of cell injury* (ischemia and hypoxia are the most common cause of cell injury)

- I. Hypoxia (decrease oxygen supply) is different from ischemia (cut of blood and nutrients supply) with regards to severity
- II. Decreasing the blood supply (causes angina which is reversible) is different from cutting the blood supply (leads to myocardial infarction which is irreversible)

MECHANISMS OF CELL INJURY

Principles

- The consequences of injury also depend on:
type,

For example, myocardial infarction happens after 30 minutes of ischemia in the heart whereas the skeletal muscles withstand ischemia for 3 hours because they have higher adaptability while brain cells can barely withstand 2-3 minutes before developing a stroke (cerebral infarction)

status, *(was the cell diseased or sound before the injury)*

The same injury will cause **different** consequences on healthy cells from previously diseased cells, a cell in starvation would face injury faster than a nourished one (a cell in a reversible injury might undergo necrosis if it is affected by another injury)

adaptability, and genetic makeup of the injured cell (precision medicine concept)

The genetic makeup differs from one person to another, so the response to the same injury differs between individuals
One patient may require a higher dose than another, and some patients' cells may respond to certain drugs while others'

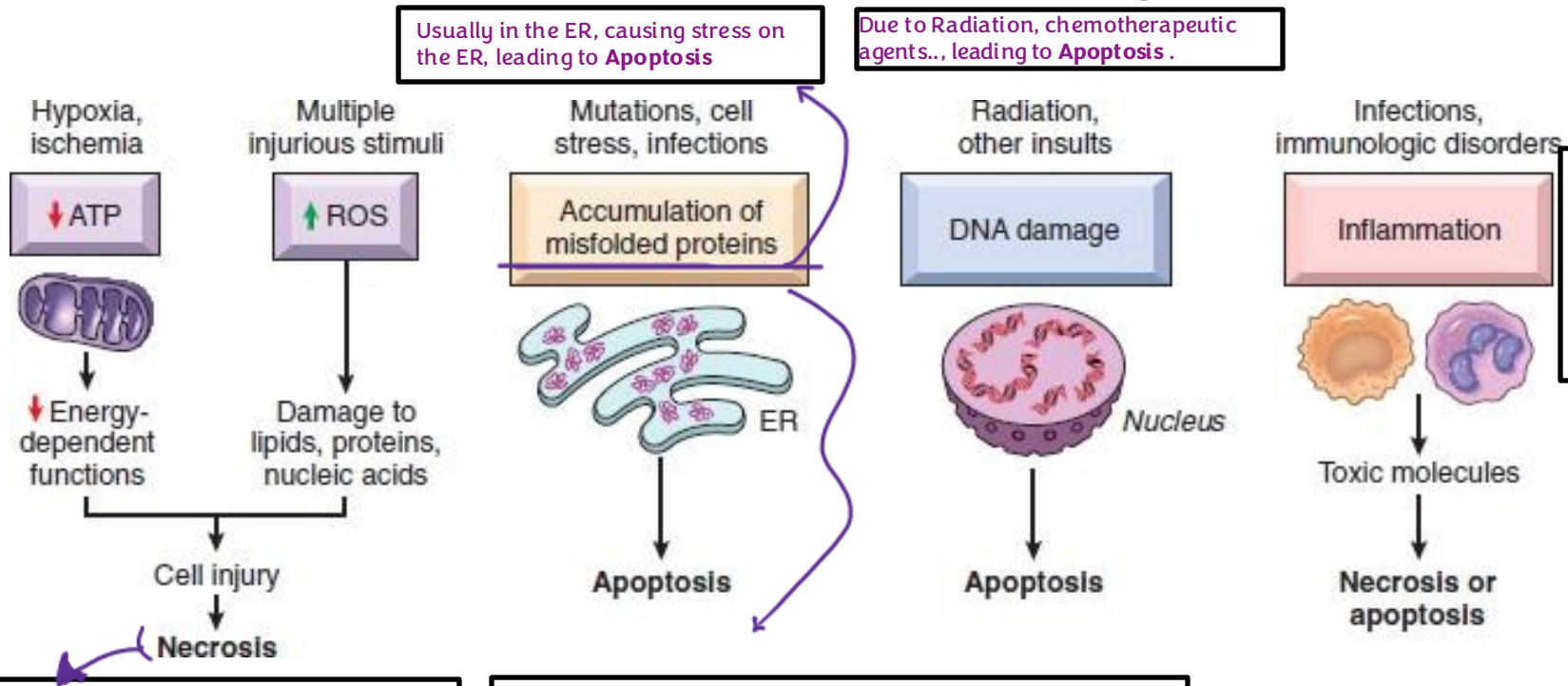
cells do not. (The genetic makeup : the complete set of genes an organism has, inherited from both parents, which contains the instructions for building and maintaining the body)

- Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components.
- Same injury may trigger more than one mechanism.

The same injury can activate more than one mechanism, the same cell can undergo more than one mechanism, the same mechanism can be triggered by more than one type of injury.

-Notice that some of these processes induce **necrosis**, some induce **apoptosis**, and some induce **both mechanisms which is called necroptosis**

The principal biochemical mechanisms and sites of damage in cell injury



Due to Radiation, chemotherapeutic agents..., leading to **Apoptosis**.

Usually in the ER, causing stress on the ER, leading to **Apoptosis**

Can also be triggered by **necrosis**. While the end result is the release of toxic metabolites and cytokines, cells death here is due to necrosis and sometimes apoptosis

(The end result of irreversible cell injury, whether by decreased ATP production or by ROS)

Due to different reasons such as **mutations**, which could occur as a result of skin exposure to UV light. This accumulation could also be due to **cell stress**, or even **viral infections**

Reactive Oxygen Species (ROS) – free radicals - : particles that have one extra single unpaired electron in the outer shell which gives them very high energy(they cause cell damage by converting the molecules they interact with into free radicals, initiating a chain of reactions that will damage everything in the cell (nucleus, proteins, membrane.....)

Hypoxia and Ischemia

The most common cause of Hypoxia is **Ischemia**. Other reasons include **carbon monoxide poisoning, anemia**, high altitudes, and respiratory diseases could lead to hypoxia as well

Ischemia is mostly a result of **vasospasm**(narrowing in blood vessels) or a result of a **thrombus**(خثرة)

- One of the most frequent causes of injury.
- Defective oxidative phosphorylation (in the mitochondria)>>Failure of ATP generation>>>depletion of ATP in cells
- Failure of energy dependent pathways (membrane transport, protein synthesis, lipogenesis and phospholipid turnover)

Like Na^+/K^+ pump which needs ATP, and lack of ATP needed for the pump leads to accumulation of Na^+ inside and attraction of water → swelling of the cell and organ

- Anaerobic glycolysis. (Compensates for lack of ATP in little amounts, but it also produces lactic acid which causes acidosis in the tissues)
- Liver cells and skeletal muscle cells Vs brain and heart.



Can do anaerobic glycolysis



Can not do anaerobic glycolysis

Hypoxia effects:

- Reduced activity of membrane ATP dependent sodium pumps>> cell swelling>>organ swelling
- Lactic acid accumulation >> decreased PH>> failure of enzymes .(Acidosis)
- Disruption of the ribosomes>> decreased protein synthesis. (Swelling occurs in mitochondria and ER, leading to ribosomes detachment)
- Accumulation of ROS (inefficient oxidative phosphorylation produces ROS)
- Damage to mitochondrial and lysosomal membranes .(Due to ROS)
- Necrosis is the end result. (And sometimes apoptosis, but ischemia is mostly related to necrosis)
- Apoptosis can contribute. (according to severity; in severe injuries death mainly happens by necrosis but sometimes in some cells by apoptosis)

Any cellular injury makes the cell non functional , regardless of whether the damage is reversible or irreversible.”

blood supply returns to tissue after a period of ischemia or lack of oxygen

Ischemia-Reperfusion Injury

(Although reperfusion is expected to restore cell viability after ischemia, it paradoxically increases injury, mainly due to free radical generation from dysfunctional mitochondrial redox reactions during the reversible stage of injury.)

- Paradoxical cell injury after restoration of blood flow to ischemic but viable tissues. Paradoxical cell injury is unexpected and can't be predicted (the cell can return to its normal state after reperfusion)
- After myocardial and cerebral ischemia. (Infarction)
- **Increased generation of ROS from:**
- Injured cells with damaged mitochondria (defective oxidative phosphorylation) & defective antioxidant mechanisms.
- Infiltrating new leukocytes. (White blood cells)
- **Inflammation induced by influx of leukocytes, plasma proteins and complement** etareneg dna aera eht naelc (sllec doolb etihw) maertsdoolb eht ni sllec yrotammalfnl) noisufreper gnirud SORlanoitidda so the damage is actually increased after Reperfusion(.

Oxidative Stress

Oxidative stress occurs when ROS accumulate in the cell. The cell is exposed to high stress, resulting in a lot of damage to every single component of the cell

- Cellular abnormalities induced by ROS (free radicals)
- Chemical species with single unpaired electron (extremely unstable)

- **ROS generated in:**

- Chemical injury (CCL4)
- Radiation injury (UV, Xray)
- Hypoxia
- Cellular aging
- Inflammation (WBCs contain enzymes that produce ROS)
- Ischemia-reperfusion injury.

ROS Once they are produced in large number, they enter a closed chain and expose the cell to oxidative stress, which leads to damage in every single component of the cell: the membrane, proteins, and DNA. These are the three components targeted by ROS

ROS cause the cell to enter a vicious circle (حلقة غير منتهية) each ROS cause cellular damage and produce more ROS and so on

Generation and Removal of Reactive Oxygen Species

In a way that prevents damage to the cell (they are toxic intermediates that are usually degraded or removed by specific enzymes under normal conditions)

- **1-Normally produced in all amounts in all cells during the redox reactions.**
- Oxygen is reduced to produce water.
- Small amounts of highly reactive but short-lived toxic intermediates are generated.
- Superoxide ($O_2 \cdot^-$), hydrogen peroxide (H_2O_2), hydroxyl radical $\cdot OH$.

ROS is
 O_2 converted to
superoxide $O_2 \cdot^-$
in mitochondria



Hydrogen
peroxide
 H_2O_2



Water (normal)

Hydroxyl radical (in injury state)

Phagocytes are white blood cells that phagocytose microbes or foreign substances.

- **2-Produced in phagocytic leukocytes (neutrophils and macrophages) during inflammation.**
- In phagosomes and phagolysosomes to kill microbes.
- $O_2 \gg \text{superoxide} \gg H_2O_2 \gg \text{hypochlorite}$.
Is produced by myeloperoxidase
- Myeloperoxidase (H_2O_2 into hypochlorite).

↓
Found normally in
phagocytes

To confirm under the microscope whether these are phagocytes or macrophages, we can use myeloperoxidase immunostaining to highlight these cells
Hypochlorite is a potent free radical that is
Bactericidal(capable of killing bacteria)

- It is interesting that ROS are normally produced in the cell and removed. ROS are produced during inflammation and microbial infection, but there is always a removal mechanism. They undergo spontaneous decay; although they have high energy, they are short-lived, and the cell contains certain enzymes to manage ROS.

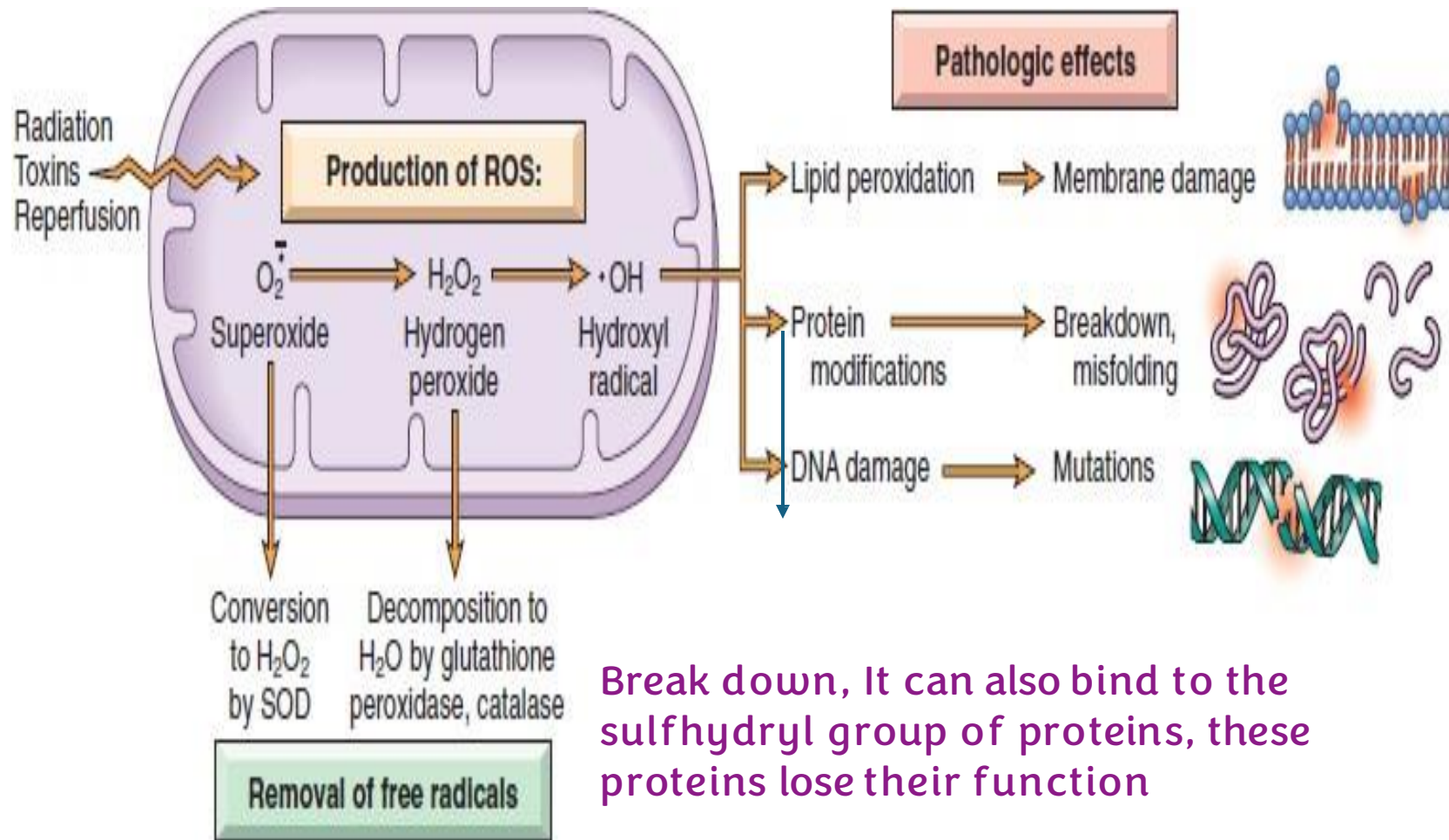
Removal of free radicals

If SOD isn't present the superoxide will continue to produce free radicals and damage the cell.

- Decay spontaneously (short lived)
- Superoxide dismutase (SOD). Convert superoxide to H_2O_2 which is a potent ROS
- Glutathione (GSH) peroxidases.
- Catalase (one of most active enzymes known)
- Endogenous or exogenous anti-oxidants (e.g., vitamins E, A, and C and β -carotene) Natural anti-oxidants used to prevent aging (aging causes increased production of free radicals)

H_2O_2 must be reduced to water by glutathione peroxidase and catalase (if they are not present H_2O_2 will be converted to hydroxyl radical and cause injury). Catalase is one of the most important enzymes known ever

Protective for the cell because they always can protect the cell from the damaging effects of free radical



Not only the cell membrane, but also the mitochondrial membrane and lysosomal membrane are affected and all cell membrane

Break down, It can also bind to the sulfhydryl group of proteins, these proteins lose their function

Effects of ROS:

- **1-Lipid peroxidation of membranes.**
- (plasma, lysosomal & mitochondrial membranes and all other intracellular membranes) [See the last slide](#) [Click here](#)
- **2-Crosslinking and other changes in proteins.**
- (degradation, fragmentation, loss of enzymatic activity & misfolding).
- **3-DNA damage.** [And mutations](#)
- Single strand breaks, mediate: apoptosis, aging, malignant transformation
- **4-Killing of microbes.**
[and degradation of dead cells](#) (eg. Necrotic cells' clearance by inflammatory cells)

[Accumulation of misfolded proteins may lead to apoptosis.](#) [Click here](#)



[When a person is exposed to UV or X-rays, which increase the amounts of ROS, the continuous exposure and continuous DNA damage can cause malignant transformation.](#) The end result is not always cell death or apoptosis. Sometimes, if the effect is minor but continues for a long period of time, it causes mutations that lead to malignant transformation

Cell Injury Caused by Toxins

- Environmental chemicals & substances **or** produced by infectious pathogens.
- **Direct-acting toxins**
- **Latent toxins.** **Indirect-acting**

Direct-acting toxins

- Act **directly** by combining **with a critical** molecular **component** or cellular organelle.
- **Mercuric chloride poisoning** It binds to sulfhydryl groups of membrane proteins, **such as Na⁺/ K⁺ pump**, inhibiting this ATP-dependent transport and this increases the permeability to sodium and water which causes swelling
- Contaminated seafood
- Mercury binds to sulfhydryl groups of membrane proteins >> inhibit ATP-dependent transport and increase permeability.
- **Chemotherapeutic agents** The chemotherapeutic agent directly targets and damages malignant cells –this is a desirable effect.
- **Toxins from microorganisms.** **cholera toxin, or other certain bacterial toxins**

Latent toxins

- Not intrinsically active
- Must be converted to reactive metabolites, then act on target cells.
- **Via cytochrome P-450 in SER of the liver.**
- **Damage mainly by formation of free radicals>>membrane phospholipid peroxidation.**

Protein damage, or DNA damage
dependent on the toxin type

- **CCl₄ and acetaminophen.** Paracetamol (in overdose)
- Membrane peroxidation>>>>damage
- ER membranes >> detachment of ribosomes>>decline in synthesis of enzymes and proteins
+decreased synthesis of apoproteins >> fatty liver
- Mitochondrial membranes>> decreased ATP >> cell swelling >> cell death.

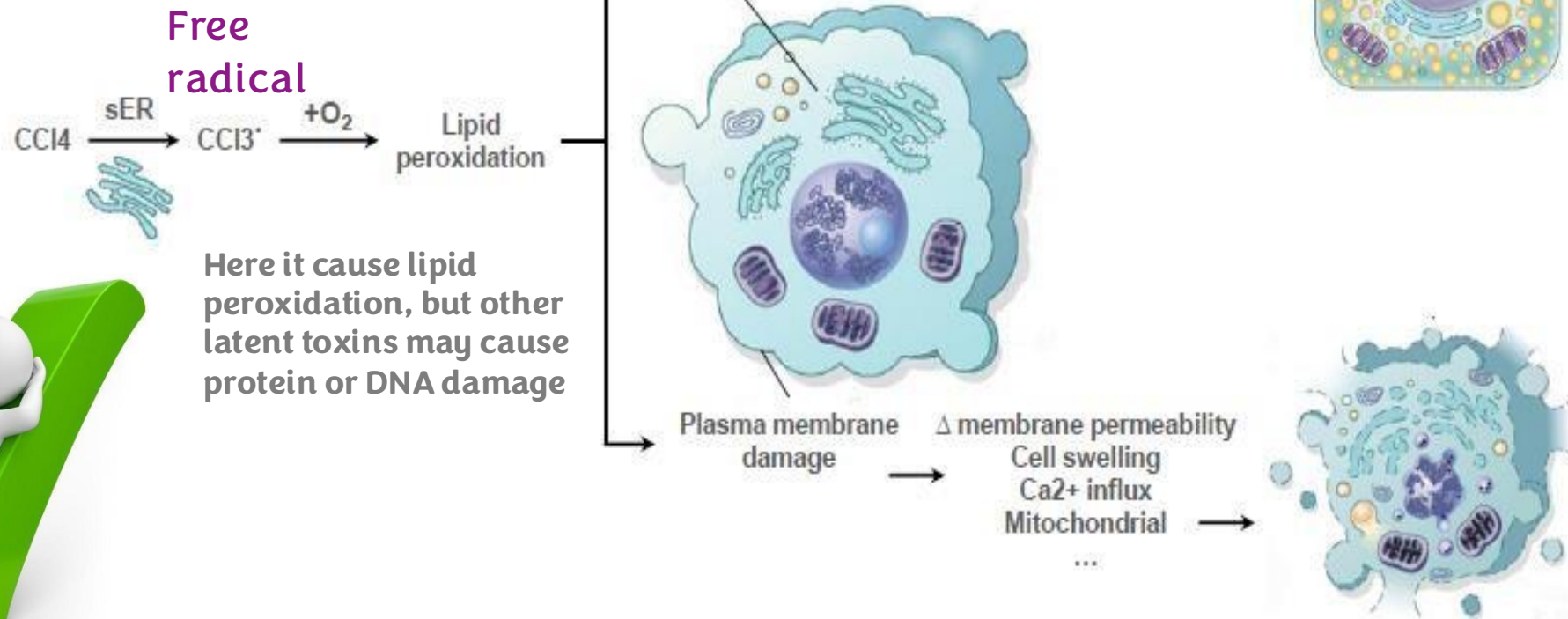
ATP- dependent processes

According to severity, it may be
necrosis and apoptosis, (usually, it is
not a pure type of death to all of the
tissue, but the predominant).

CCL4 toxicity

When protein synthesis in the liver decreases, what happens to lipids? They remain in the liver because **lipids need to bind to apoproteins to be transported in the blood**. Decrease synthesis of proteins lead to accumulate the lipid in the liver acute fatty liver (fatty change and liver swelling due to mitochondrial damage) yellowish (this happens in the case of acetaminophen overdose)

▶ CCl₄



Endoplasmic Reticulum Stress

- Chaperones in ER control proper protein folding
After protein folding the protein gets excreted outside of the ER
-> Folding happens so that the protein becomes functional
- Misfolded proteins >> ubiquitinated >> targeted to proteolysis

In the case of accumulation of misfolded protein:
- **Unfolded protein response (adaptive response):** increase chaperones production, decrease protein translation and increase destruction.
- If failed >> proapoptotic sensor activation (BH3-only family) + direct activation of caspases >> **apoptosis by the mitochondrial pathway.**

When ribosomes synthesize proteins, they are transferred to the endoplasmic reticulum (ER), where they undergo a process called protein folding.

This folding is essential because it allows the protein to acquire its proper three-dimensional conformation and become functionally active.

Proper folding enables the protein to be transported from the ER to its final destination, whether within the cytoplasm or outside the cell.

If proper folding fails, misfolded proteins accumulate in the ER, leading to a condition known as ER stress.

To cope with this stress, the cell activates a protective mechanism called the unfolded protein response (UPR).

The UPR increases the synthesis of molecular chaperones (which assist in refolding misfolded proteins), reduces overall protein synthesis, and enhances the degradation of abnormal proteins.

These combined actions decrease the burden on the ER and help restore cellular homeostasis.

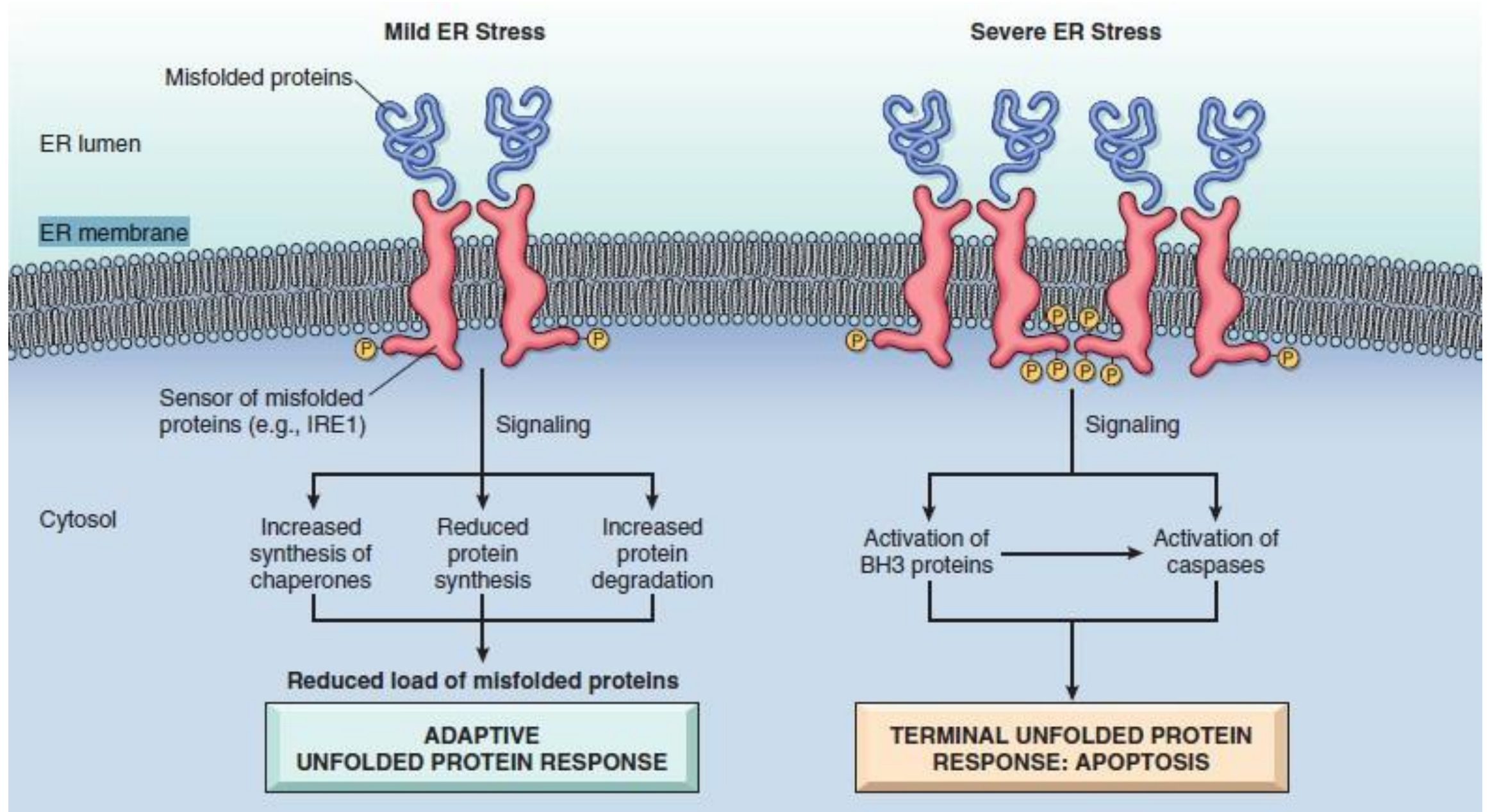
If the adaptive response is successful, the cell survives.

However, if misfolded proteins continue to accumulate and exceed the cell's capacity to adapt, the response fails.

In this situation, the pro-apoptotic pathways are activated through members of the BH3-only family, leading to the activation of caspases—the enzymes responsible for apoptosis.

This triggers the mitochondrial (intrinsic) pathway of apoptosis, resulting in programmed cell death.





Causes of misfolding

سبحان الله وبحمده
سبحان الله العظيم

- Gene mutations Cystic fibrosis -> leading to accumulation of misfolded proteins
- Aging (decreased capacity to correct misfolding) Aging -> reduced ability to fold proteins correctly -> misfolded proteins accumulate -> ER stress
- Infections, especially viral infections (microbial proteins)
- Increased demand for secretory proteins such as insulin in insulin-resistant states(
NEXT SLIDE)
- Changes in intracellular pH Enzymes will be non - functional
- Neurodegenerative diseases
- Deprivation of glucose and oxygen in ischemia and hypoxia.

Increased demand for secretory proteins, such as insulin in insulin-resistant states: Insulin resistance occurs when insulin is present, but cells respond poorly to it. Consequently, pancreatic β cells increase insulin synthesis, which can overwhelm the ER's protein-folding capacity. Over time, misfolded proteins may accumulate, triggering ER stress and apoptosis. This can contribute to decreased insulin production in type 2 diabetes, and some patients may require insulin injections.

Neurodegenerative diseases: Alzheimer's, Parkinson's, and Huntington's diseases involve the accumulation of misfolded proteins, which contributes to neuronal dysfunction and death. Ultimately, this process can result in atrophy of brain.

Protein misfolding causes disease by:

- **Deficiency of an essential protein due to degradation**
- Cystic fibrosis
- **Inducing apoptosis of the affected cells**
- Neurodegenerative disorders (Alzheimer disease, Huntington disease & Parkinson disease), type 2 diabetes and prions disease.
- **Inducing both:**
- Alpha 1 antitrypsin deficiency.
- **Improperly folded proteins accumulation in extracellular tissues**
- Amyloidosis

Sometimes, misfolded proteins may become inactive, preventing them from being secreted outside the cell and hindering their proper function.

DNA Damage

- Radiation As UV light
- Chemotherapeutic agents
- Intracellular generation of ROS
- Mutations

What happens when DNA damage occurs?

Tumor suppressor gene

- DNA damage >> p53 activation >> arrest cell cycle at G1 phase for repair >> if repair is impossible >> apoptosis.
- In P53 mutations >> mutated cells replicate >> neoplastic change.

The patient will be exposed to tumors

If the DNA damage is repaired while being arrested in G1 phase, it will continue the cell cycle and proliferate. Otherwise, apoptosis is supposed to happen.

This mechanism will protect us from tumors

Inflammation

- Pathogens As viruses, bacteria, and protozoa.
- Necrotic cells, Inflammation happens because of the accumulation of cell debris
- Dysregulated immune responses (autoimmune diseases and allergies)
- Inflammatory cells (neutrophils, macrophages, lymphocytes) secrete products that destroy microbes and damage host tissues.

Inflammation itself mediates cell injury, but it is an intended process to protect our bodies from foreign substances and to clear the necrotic debris when necrosis occurs. Cell injury occurs because of the production of cytokines, mediators and reactive Oxygen species (ROS) by the inflammatory cells.

Common Events in Cell Injury From Diverse Causes

All mechanisms of cell injury end up with one of the 2 following damages:

- Mitochondrial Dysfunction Which causes decrease in ATP, formation of ROS or activation of mitochondrial apoptosis pathway.
- Defects in Membrane Permeability **Whether the plasma membrane, mitochondrial, lysosomal or endoplasmic reticulum membranes.**

Mitochondrial Dysfunction

- Energy factory
- Hypoxia, toxins, radiation. **All decrease the efficiency of the mitochondria → decrease ATP production leading to either apoptosis or necrosis**
- In necrosis and apoptosis.

- **Consequences:**

- Failure of oxidative phosphorylation, ATP depletion.
- Abnormal oxidative phosphorylation, formation of ROS
- Mitochondrial permeability transition pores, loss of membrane potential.
- Release of cytochrome c >> apoptosis

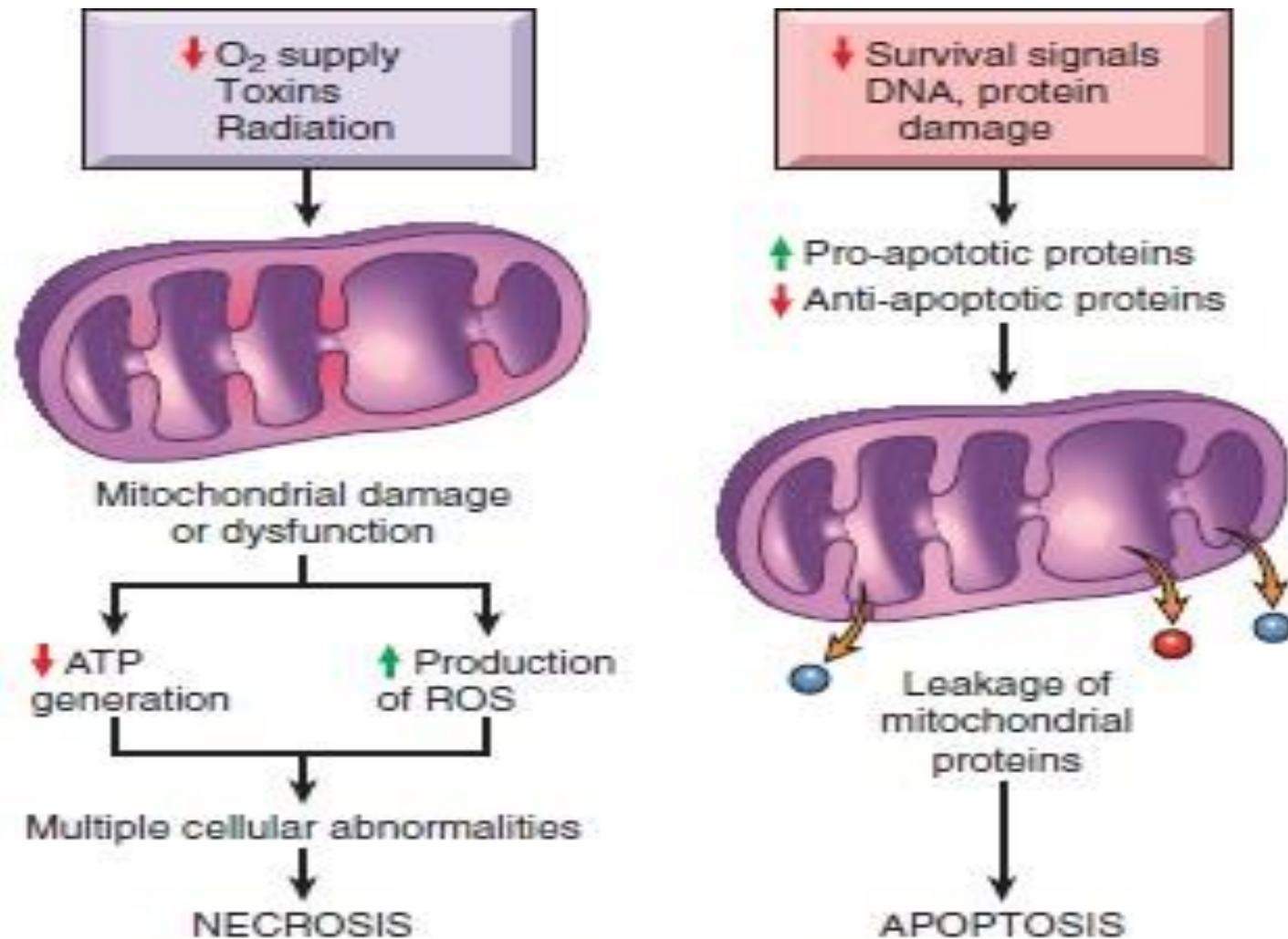
ATP pathways in the cell will be deactivated like the Na⁺/K⁺ pump which will cause cell swelling.

Causes peroxidation, protein and DNA damage.

Which leads to the leak of cytochrome c to the cytoplasm, activating caspases and initiating apoptosis

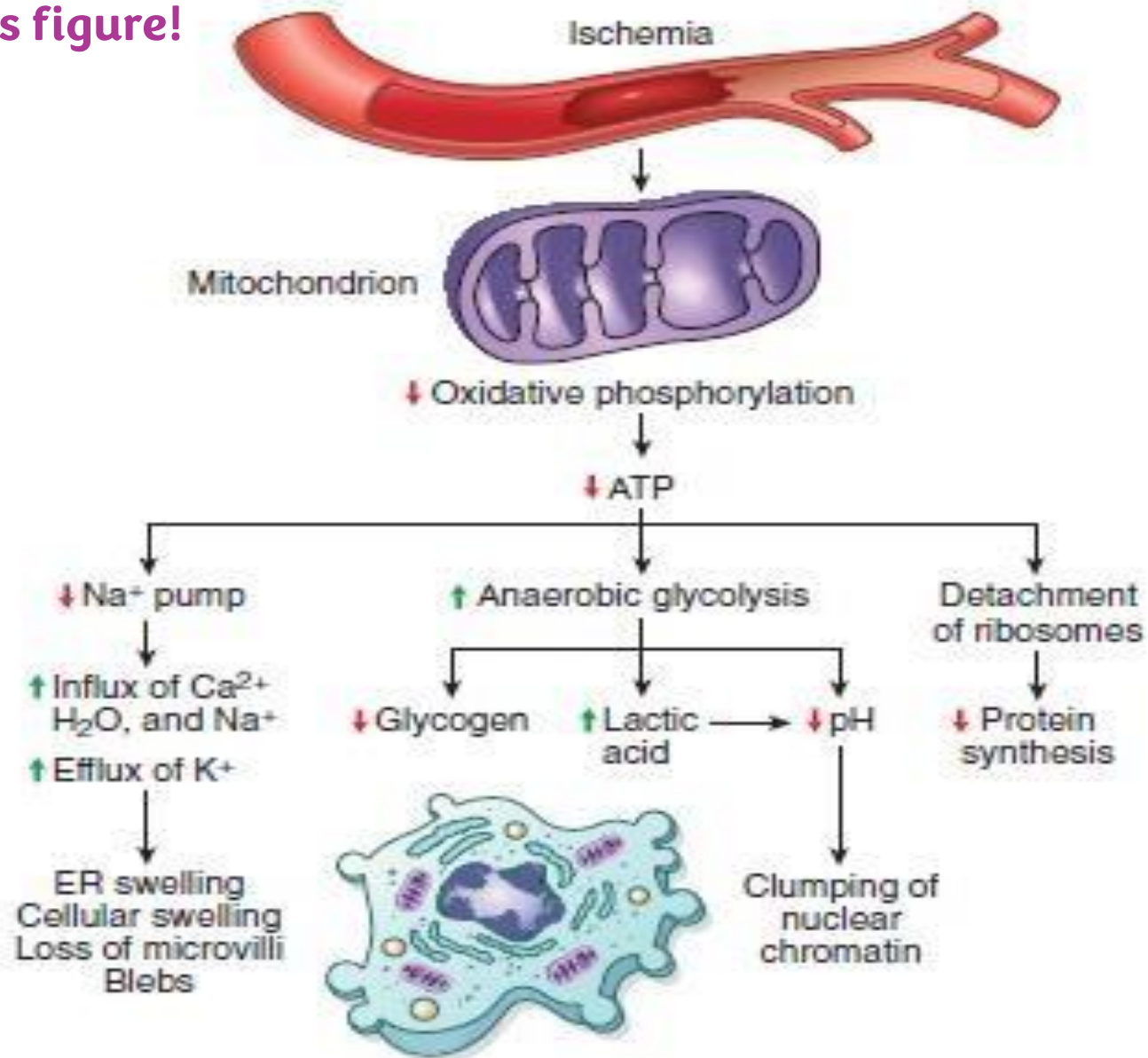
Mitochondrial Damage and Dysfunction

Important to study this figure!



Depletion of ATP

Important to study this figure!



Defects in Membrane Permeability

- Mitochondrial membrane damage: decreased ATP
in addition to the release of cytochrome c to the cytoplasm because of the increased permeability
- Plasma membrane damage: loss of osmotic balance, influx of fluids, leak of contents
- Lysosomal membranes: leakage of enzymes outside the cell >> cellular digestion.



For any feedback, scan the code or click on it.



Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional resources:

References Used:

Prof. Manar Hajeer recorded lecture.

Extra References for The Reader to Use:

Robbin's Basic Pathology (10th Edition).



رسالة من الفريق العلمي: