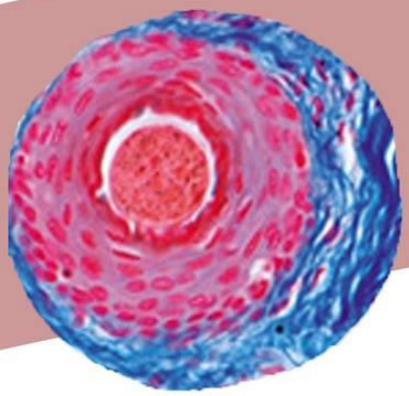
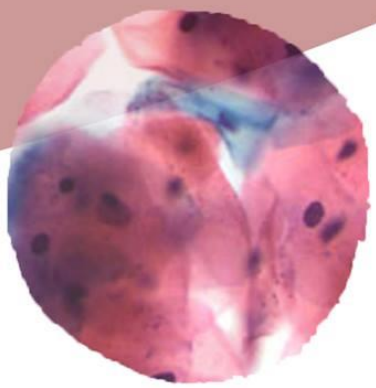
INTRODUCTION TO PATHOLOGY





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Sheet# 3

Revision:

- Differences between Apoptosis and Necrosis :

Apoptosis	*Necrosis*
The membranes remain intact	Loss of membranes integrity (membrane damaged)
Physiologic often	Pathologic always
Shrinkage of the cell	Swelling of the cell
No leakage of cellular contents	Leakage of cellular contents
No inflammation	Inflammation

-The changes that we see under the microscope are cellular swelling and the formation of lipid vacuoles.

-Swelling happens because of the failure of ion pumps due to mitochondrial damage (no ATP = ion pumps can't work so molecules move *down* their concentration gradient)

-Influx of Ca^{++} , Na^{+} , and water . and efflux of K^{+}.

Causes of cell injury :

1- ***Hypoxia*** : lack of oxygen & ***Ischemia*** : lack of blood supply .

- Ischemia is the most common cause of hypoxia.
- The causes of Non-ischemic hypoxia :

-carbon monoxide (CO) poisoning :because it has higher affinity to hemoglobin than O_2.

-high altitude : low partial pressure of oxygen thus it can't bind to hemoglobin.

-pulmonary problems .

-Anemia (فقرالدم) .

- Ischemia is more severe than hypoxia because blood carries not only oxygen , but nutrients as well .

2. **Chemical agents :** -ex : cyanide , acids and any chemicals in the lab .

   CN : it disrupts the electron transport chain ; and stops the production of ATP in the mitochondria . and it affects all cells in the body .

   Note : any excess in any material even water and nutrients can cause cellular damage .

3.physical agents : (Trauma , Radiation , Temperature and electric shock)

4.Infections : mainly by bacteria and viruses .

-two ways that infection causes cellular damage :

A- when you have an infection , WBC will cause inflammation , and this inflammation may cause collateral damage to the neighboring tissues .

- Other immunological reaction is " Auto immune disease " . where your own immune system attacks your body and causes cellular damage .
- **Why** auto-immune disease occurs ?
  Some T cells (a type of WBC) ,when they develop they become reactive towards your own body , if those cells stay alive you will end up with auto-immune disease , However in order to avoid this ; these cells must kill themselves by Apoptosis .

B- The bacteria and viruses can directly damage the cells.

5. Immunologic reactions : like autoimmune reactions (discussed above) and allergic reactions against environmental substance in genetically susceptible individuals.

6. Genetic defects :

Ex: sickle cell anemia : the change here is replacing one amino acid in hemoglobin (Val instead of Arg) , the cell become sickle shaped , and this causes accumulation of RBC in the small vessels .

7. Nutritional defects :

Ex: 1-vitamin C deficiency causing Scurvy .

2-high levels of LDL (too much fats).

3-high levels of sugars (causing diabetes).

8. Aging: as you get older , your cells do not heal as they used to .

-*what is healing in the first place?* It's regeneration of cells (replication) , and cells are programed for a certain number of replications , and after this number they stop replicating . This is known as "cellular senescence ".

Note: cancer cells do not follow this rule; they ultimately replicate and grow!

Principles of cell injury

We will discuss the principles with regard to the **injury** and with regard to the **affected cell**.

A- The injury depends on :
   1- Type of injury .
   2- Duration : if the injury is short , the cell may adapt and survive . but if it was long and extended it will lead to cell death .
   3- Dose : it's how severe the injury is .

B- The affected cell :
   1- *Type of the cell* ; some cells can survive more than others
      Ex: heart cells can resist Ischemia for 20-30 minutes , while skeletal muscle cells can resist Ischemia for 2-3 hours .
   2- *Adaptability :* (including the basal state of the cell).
      Ex: if you expose two cardiac muscles to ischemia , one from a heart of twenty year old healthy person , the second is from hypertrophic heart of a diseased person . which one is going to die first ??
      The diseased one , of course , because his cell is *already* exhausted . this is called the basal state .

*Let's talk a bit about **polymorphism** :* it's a mutation , but it's more frequent in the population , and less likely to cause diseases (change in the form of a protein to become more or less active).

Ex: " cytochrome p-450 " :

-It's present in the liver (SER)

-it's responsible for detoxification of drugs and

and toxics .

-if you have a polymorphism that makes P-450 more active , your body will get rid of toxics faster (more reactive form).

Couple of important things you should know befor we start talking about the mechanisms:

1. Ca^{+2} found in high concentrations outside the cell (10,000 times more)
2. There are intracellular stores of calcium inside the smooth ER , and the mitochondria , and they release Ca^{+2} in small amounts when needed .
3. Cells produce ATP in the presence of oxygen (aerobically) through aerobic *Respiration* ; which produces about 32-34 molecules of ATP per one glucose, but in the absence of oxygen (anaerobically)cells produce ATP through glycolysis which produces only 2 ATP molecules per one glucose.
4. The product of anaerobic respiration is Lactic acid .

Let's get back to the mechanisms of cell injury .

Mechanism of cell injury

There are 4 mechanisms of cell injury, but they are very very interrelated.

1-mitochondria:

- Mitochondria produces ATP.
- Mitochondrial damage → no ATP → a lot of cell functions will stop including ion pumps which causes swelling of the cell and this will cause influx of Na+ , Ca+2 and water and efflux of K+ (action potential could be effected)

* Mitochondria produces ROS in small amounts (reactive oxygen species) as a by-product of electron transport chain. However when the mitochondria are damaged; they will produce more ROS because you are disrupting the electron transport chain.

*Normally we get red of ROS by the action of many enzymes (like superoxide dismutase that catalase the conversion of superoxide to hydrogen peroxide)

* Damaging the mitochondria will also release Ca^{+2} into the cell, and as we know; calcium *activates* several enzymes within the cell. And if you have a massive influx of calcium, all these enzymes will be activated.

(We will talk about calcium in a moment)

- ***So reviewing the Mitochondrial damage :***

A) ATP depletion → failure of ion pumps → swelling of the cell and loss of its microvilli .

B) if the cell has no oxygen it will go throw glycolysis which produces 2 ATP molecules only, comparing with full Respiration process that produces much larger amounts of ATP. (Per on glucose molecule)

Not only that ; when anaerobic respiration occurs you have the formation of *Lactic acid* , and by the accumulation of lactic acid ,the acidity of the cell increases (decreasing pH) .

This decrease in pH causes "chromatic clumping".

C) NO ATP → detachment of ribosomes from the ER and dissociation of polysomes into monosomes and this will cause reduction in protein synthesis. Ultimately, there is irreversible damage to mitochondrial membrane and lysosomal membrane and the cell undergoes necrosis

D) Damaging the mitochondria will increase the production of ROS.

Now, ROS are free radicals (missing an electron) so they react with everything trying to *steal* an electron, so they damage: proteins, lipids, DNA.

Also the affected target (ex: protein) will become a free radical … and so on. So they create a *chain of destruction.*

Normally cells have enzymes that remove free radicals, **but** these enzymes require ATP, and we already have ATP depletion ☹ **.**

Q: EXPLAIN "the mitochondria is a crossroad between necrosis and apoptosis "

Answer: if the injurious stimuli are other than hypoxia, chemical toxins and radiation but they are reduction in survival signals (which will reduce growth factors for example) or DNA or protein damage, this will give a signal to mitochondria to change their permeability to certain proteins (cytochrome C for example) this will increase the pro-apoptotic proteins in the cytosol and inhibition of anti-apoptotic proteins and this will lead to apoptosis

2- Ca^{+2}:

Again; no ATP ⟶ failure of ion pumps ⟶ increasing the intracellular calcium ⟶ causing swelling of the cell and activating several enzymes.

- The enzymes that get activated are from "ase" family, and they are hydrolases (digestive enzymes).
- Some of these enzymes :
  1-phosphlipases: hydrolysis of phospholipids causing membrane damage.
  2-proteases: hydrolysis of proteins causing damage to the membrane and the cytoskeleton.
  3-Endonuceases: nuclear damage.

  4-ATPase: break down of ATP.

-other thing about calcium that it affects the permeability of the mitochondria. **HOW?** By the influx of calcium, the permeability of the mitochondria will increase, thus ruining the protons gradient, so the mitochondria can't produce ATP as they should even in the presence of oxygen.

3- Free Radicals

-they are produced as a by-product from most cells.

-our body can get rid of them by many ways:

1-without using enzymes (endogenous and exogenous antioxidants),**Ex**: beta-carotene , vitamin C ,A and E that may either block the formation of free radicals or scavenge them once they have formed

2-using enzymes: (peroxidases), ex: catalase,

Glutathione peroxidase.
And guess what these enzymes need!! ATP. ★

So, you don't have ATP, you can't get rid of free radicals, more damage to the cell.

*pathological effects of free radicals:

1 - On lipids: membrane damage.

2 - Proteins: misfolding and breakdown.

3 - DNA damage causing mutations.

*the effect of ROS on the cell is called oxidative stress

*Some cells produce oxygen free radicals on purpose! These cells are WBC and phagocytes (mainly neutrophils and macrophages) during a process called "oxidative burst "or "respiration burst".

-Leukocytes contain enzyme is called (myeloperoxidase), that actually converts these
Oxygen free radicals into (hypochlorite HOCL).
-Hypochlorite: is the major component of household *bleach*
-so WBCs are actually producing bleach

In order to get rid of any foreign microorganism. However, these ROS are indiscriminate which means that these ROS will affect the surrounding tissues also and this is an example of the indiscriminate damage because of inflammation.

4 – Membranes damage

So the mitochondria are not producing ATP and you need ATP to build phospholipids, also the mitochondria produce ROS which can damage phospholipids, also we have huge influx of Ca+2 which activates phospholipases.
So you are not producing phospholipids, you are damaging phospholipids, and you are degrading phospholipids. To add further, you are damaging proteins, and the only reason that the cell keeps its shape is not because of membranes, but because of its cytoskeleton, so when proteases get activated, the cell will lose its shape.

Because of activation of phospholipases, anything that has membrane will be damaged, and the most important sites of membrane damage during cell Injury are the mitochondrial membrane (decrease the production of ATP and produce ROS), the plasma membrane (loss of osmotic balance and influx of water and ions, as well as loss of cellular contents), and membranes of lysosomes (leakage of their enzymes into the cytoplasm and damage the cellular contents)

Causes and mechanisms of cell injury in practice: (see page 17 in the book)

Ischemia and hypoxia:

*No/less oxygen causes depletion of ATP which in turn will cause: 1- pumps failure and swelling of the cell 2-increase glycolysis rate which leads to accumulation of lactic acid and decrease of pH 3- detachment of ribosomes from the rough

ER (RER) and dissociation of polysomes into monosomes which will reduce the rate of protein synthesis

-These(1+2+3) are all early responses, still reversible injury.

-Once there is a massive (1)influx of Ca^{+2} , and (2)change in the mitochondrial permeability , and (3) increasing the activity of cellular enzymes (like phospholipases, proteases, etc),this will lead to Irreversible injury . Also accumulation of ROS(4) which causes phospholipid damage can be a sign of Irreversible cell injury .

Ischemia-Reperfusion Injury

“It's when you try to fix something, but you’re only making it worse! “ (بدو يكحلها عماها)

-It happens when there is an Ischemia, and you try to fix this ischemia by allowing the blood flow to come back to that ischemic organ, you’re causing more damage than before.

But WHY? Because blood carries O_2, WBC, and Ca^{+2}.

1 - With regard to oxygen: the mitochondria now produces ATP by oxidative phosphorylation, thus producing more ROS, and you can’t get rid of them because you don’t have enough ATP.

2 - Ca^{+2}: adding more calcium to the cell that already has a lot of it will make things much worse by causing all kinds of damage mentioned before.

3 - WBC: containing inflammatory proteins, anti-bodies, complement system. They will attack the Ischemic cell thinking it's foreign, and the immune system will target this cell to destruction, and will start inflammatory processes that further increase the damage that is already there.

**so if a cell was at reversible injury, reperfusion will push it towards irreversible injury.

Chemical Injuries

-Two types of chemical injury: 1-direct

2-indirect (metabolic)

1-direct toxicity: the chemical itself is toxic to the cell

Ex: A) Cyanide: it affects all cells in our bodies by blocking the oxidative phosphorylation and electron transport chain.

B) Mercuric chloride: it's a sea *food* toxic, so it affects the cells that are responsible for absorption, digestion and excretion. (GI system and the kidney).

2- Indirect (metabolite) toxicity: the product of the metabolism of the drug is toxic.

Ex:

1.CCL_4 : it's used to be used for dry cleaning industry , but now its banned .

*CCL_4 itself is not toxic , but when its metabolized in the liver ; it produces CCL_3 , which is a free radical .

This free radical causes several types of damage :

A) Damage to the rough ER where cells produce proteins , and hepatocytes produce proteins called "Apo-proteins" and these proteins are required to transport lipids from and into the liver (LDL , HDL) , (they are part of the circulation system because lipids can't move alone in the blood stream . If a damage is done to the liver's rough ER , the lipids will accumulate in the liver causing what is called " Fatty liver ".

B) The CCL_3 free radical also damages the membrane of mitochondria causing change its membrane permeability , cell swelling , influx of calcium and all types of damage mentioned before …….. .

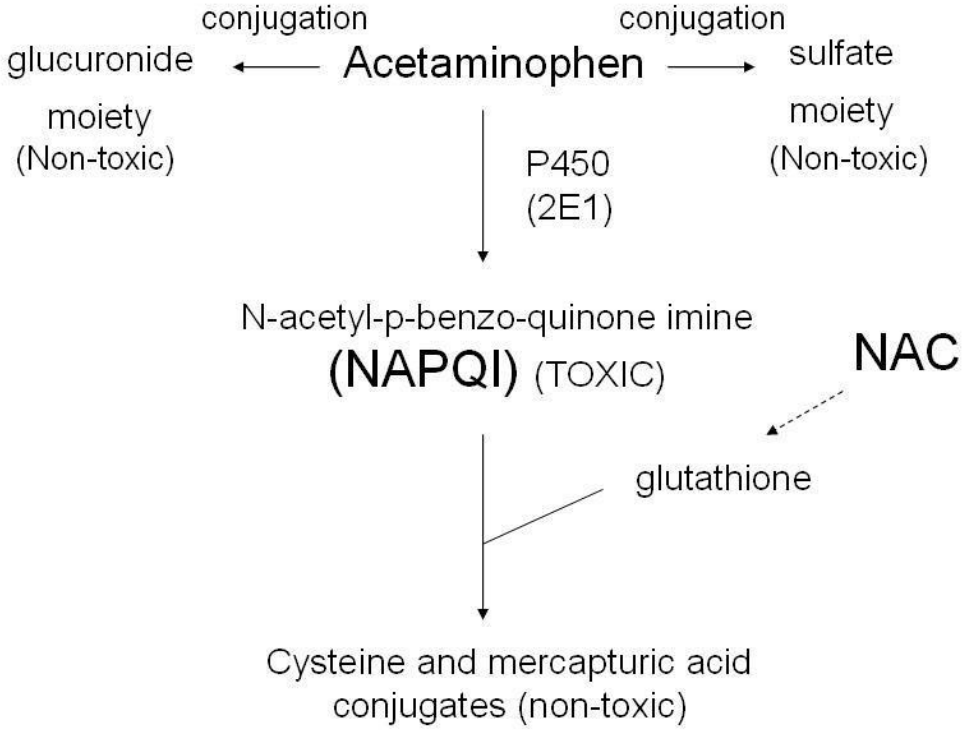
2. Acetaminophen (Paracetamol or Panadol) : It is a non-toxic material, and normally it is conjugated in the liver to non-toxic moieties. However, if you take an over dose of acetaminophen, your cytochrome P-450 system will metabolize it and produce a toxic intermediate which is then conjugated with glutathione to produce a non-toxic material. However, you only have a limited amount of glutathione and if the dose is big enough to consume these stores, this toxic material will accumulate in your tissues and cause many damages to you.

*Alcoholics are more susceptible to paracetamol overdose than a normal person (have less amount of glutathione in their stores than others)

* we replenish the glutathione by giving them (acetyl-cysteine) or directly give glutathione

*Polymorphisms may change the activity of cytochrome P-450 (increase or decrease the activity), if this polymorphism increase the activity of cytochrome P-450, are you going to be more or less susceptible to Acetaminophen overdose?

You will be more susceptible to the overdose because you metabolize a non-toxic material to a toxic one. *(for more info. Refer to the book page 284)*



Test yourself:

Q1: One of the following indicates Irreversible cell injury :

A- Pumps failure.
B- Cell swelling.
C- ATP depletion.

D-change in the mitochondria permeability .

Q2: Choose the correct statement :

A) Ca^{+2} influx causes mitochondrial damage
B) Mitochondrial damage induces Ca^{+2} influx
C) Leakage of mitochondrial proteins leads to Apoptosis

D) All of the above .

Q3: The toxic that affects all cells in the body is :

A) CCL_4
B) CCL_3
C) Cyanide

D)Mercuric chloride