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Bio chemistry 2

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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In the previous lecture…
we discussed the **pentose phosphate pathway,** and we said that it's the major producer of **NADPH**. Now, what are the functions of this molecule and how is it utilized?

Anything in *Italic*, I consider inessential for the lecture, but the doctor mentioned.

1.Reductive Biosynthesis:

To reduce a substance that is **more oxidized** into another that is **more reduced** during biosynthesis. This reaction requires a high energy reducing substance (e^- donor), and the man for the job here is **NADPH**.

(**NADH** can't be used for this job ☹)

e.g. 8 CH_3COO → $C_{15}H_{33}COO$
(Acetate) (the fatty acid palmitate)

→notice that the end product is more reduced (it contains less oxygen), so in this kind of synthesis we use a mechanism that reduces the monomers into the required macromolecule, NADPH is needed to accomplish that.

Highest percentage of NADPH in the cell is present in the reduced form (NADPH) , so it can do the job of reducing other substances by getting oxidized.

2. Reduction of H_2O_2:

Substances such as H_2O_2 are formed continuously during metabolism, whether **accidentally** as **byproducts**, or **purposefully** to carry out certain functions. (still, they are harmful to the components of the cell, by **oxidizing** them)

H_2O_2 belongs to a group of substances called $R_{eactive}O_{xygen}S_{pecies}$. Other members include:(•OH, O_2● -)

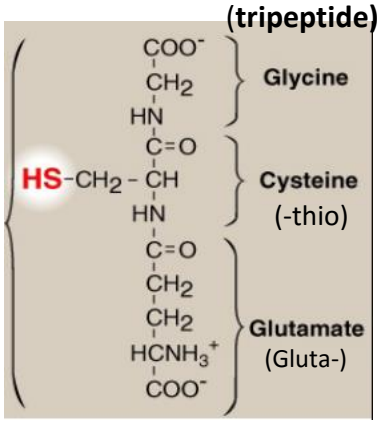
•they Can cause chemical damage to proteins, lipids and DNA which may lead to cancer, inflammatory diseases and ultimately cell death

•they Interact with drugs and are environmental toxins.

To fix that we have Enzymes that catalyze antioxidation:

First, Glutathione peroxidase:

(tripeptide)



(**G-SH**) is the reduced form

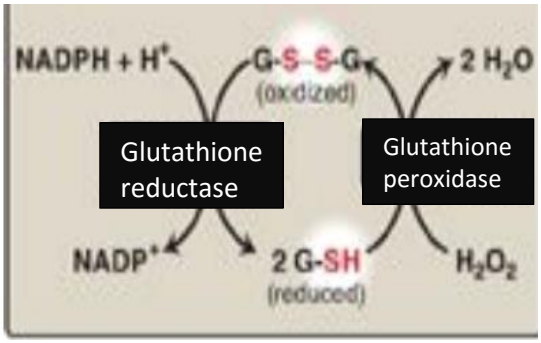
The antioxidant effect:

It gets **oxidized** by **H2O2** into **GS-SG** (two glutathione molecules are joined by a disulphide bond) which in turn **becomes H_2O**, losing its damaging effect. **Reduced** form of glutathione can reduce **H_2O_2** into **H_2O**, but the oxidized form cannot, that's why we need glutathione to be reduced again.

-peptide bond between glu and cys is formed through the side chain, the carboxyl group of glutamate backbone is not involved in the peptide bond as usual.

To reduce glutathione again, an enzyme called **Glutathione reductase** (reductase uses NADPH. we look at it only in the reduction direction, unlike dehydrogenases) reduces it back to its reduced form that can act as antioxidant.

***Note**: enzymes that utilize NAD are called dehydrogenases*.



This enzyme **requires selenium** which is an essential trace element required in little amounts to supply this reaction.

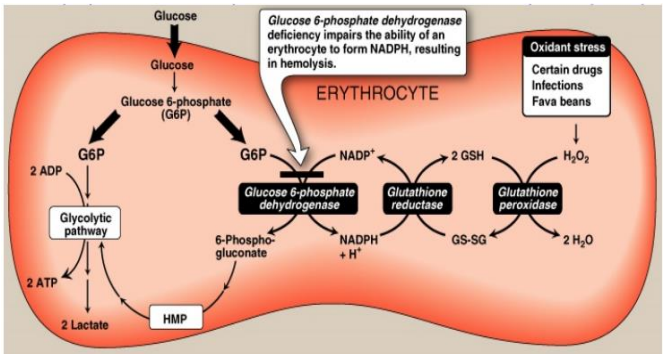
Glucose-6-phosphate dehydrogenase deficiency:

- **Common** disease
- characterized by **hemolytic anemia**
- affects **200 – 400 million individuals** worldwide
- Highest prevalence in **Middle East**, **S.E. Asia**, **Mediterranean**, common in our region.
- **X-linked** inheritance affects **males more** *because they have only one X chromosome*.
- > 400 different mutations, which means it has **many types**.
- advantage: Deficiency provides **resistance to falciparum malaria.**

'Some period of the malaria's parasite's life occurs in RBCs. If red blood cells don't live for a long period of time, malaria can't continue its life cycle, that's why people with G6PD deficiency can survive it better. maybe that's why there's an abundant deficiency in our region, to survive malaria better'

This disease **can be lived with**, unless patient faces **oxidative stress** (many free radicals)

Elaboration: RBCs are the most affected, because **1.the only source of NADPH** in RBCs is **PPP** which utilizes **G6PD**. **2.** Since **RBCs are semi-dead** cells (no nucleus, ribosomes, etc.) they **can't compensate** by synthesizing more of the enzyme, unlike other cells.



$H_2O_2 + GSH \longrightarrow G\text{-}S\text{-}S\text{-}G + 2H_2O$

$G\text{-}S\text{-}S\text{-}G + NADPH \longrightarrow 2GSH + NADP+$

We said this deficiency leads to hemolysis, **How**?

Since this deficiency causes **inability to synthesize NADPH**, reduction of **GSSG** back to **Glutathione** is not possible.

Remember: **glutathione** helps in preventing the -SH groups in proteins from being oxidized into unwanted forms (joined by unwanted disulfide bonds).

•A **decrease in the amount of reduced glutathione** leads to oxidation of these proteins →which leads to their denaturation.
If these **proteins** are **in the membrane**, it affects flexibility of the cell (causes **rigidity in cell membrane**), thus cells **can't go through the capillaries** and they would be removed by *reticuloendothelial systems*. **life span of RBCs will be reduced to less than 120 days.**

Person with deficiency can be normal until exposed to **precipitating factors** that can cause the hemolysis. So they might not suffer from hemolysis everyday (only upon exposure to some drugs, or eating some types food)

Examples:
• **Oxidant drugs** (can increase the amount of ROS)
– Antibiotics e.g. Sulfamethoxazole
– Antimalaria e.g. Primaquine
– Antipyretics e.g. Acetanilide (not used any longer)
• **Favism** Ingestion of fava beans, can increase oxidative stress → leads to hemolysis
• **Infection** can lead to ROS production.
• **Neonatal Jaundice**

G6PD Deficiency Variants:

• Wild type B → normal allele, normal enzyme activity.
• Mediterranean Variant B^- (Class II): 563 C→T (mutation)
• African Variant A^- (Class III); two point mutations
• African Variant A; Normal activity 80%
• **Very severe deficiency** (**Class I**) {notice red box}

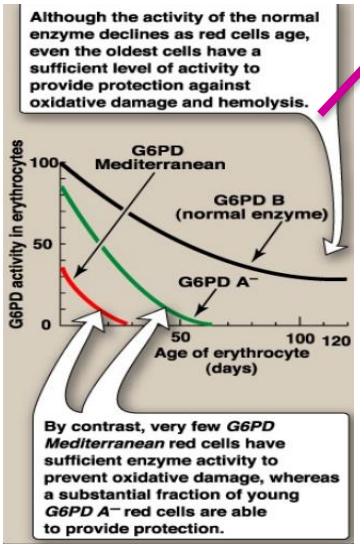
Increasing severity

Class	Clinical symptoms	Residual enzyme activity
I	Very severe	<2%
II	Severe	<10%
III	Moderate	10-50%
IV	None	> 60%

• Majority of mutations are **missense point mutations**. Like replacement of one N base by another (e.g. C→T in class II)

• Large deletions or frame shift mutations are **Not Observed**; simply because they're **not compatible with life**, people can't survive them to be recorded as cases.

In all of these cases, the enzyme **isn't absent**. It's present but it could be less stable, having lower affinity, or achieving lower vMax (less active).



As the **severity** of the case increases, the **life span** of cells decreases. Also, **enzyme's activity is reduced, it declines rapidly with time,** because of decreased stability with time.

If someone is exposed to hemolysis it'll be self-limiting, because **older RBCs will be affected**. However, the **newly synthesized ones** won't be affected by the precipitating factor when their age is still a few days. **So, there's no need for continuous blood replacement.**

Conclusion: It'll be limited with time because it affects only aged RBCs.

Antioxidant reactions' Enzymes:

1. superoxide dismutase: $O_2.^-$ is a very strong oxidizing agent. This is fixed by **turning it into H_2O_2**, which is less vicious. Then **H_2O_2** is **turned into H_2O** by the enzyme **catalase**.

Super oxide dismutase (**SOD**)

$$2O_2\bullet^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

Catalase

$$2H_2O_2 \longrightarrow O_2 + 2H_2O$$

2. chemicals: such as vitamins, **E (tocopherol)** and **C (ascorbic acid)**, and **Carotenoids** (e.g. alpha carotene, beta carotene). **E is stronger than C** because E's reduced form can't be regenerated once it's oxidized. These were proved to protect against some diseases like cancer, vascular diseases. However, *ingestion of these chemicals in a purified form wasn't proved to fix anything.*

Sources of ROS:

1. Oxidases: major producers of H_2O_2, they're found in compartments of the cell that **also contain antioxidant enzymes**.

2.oxygenases:

¬ **monooxygenases (hydroxylases)** add a **hydroxyl group** to their substrate.

¬ **dioxygenases** in synthesis of prostaglandins, thromboxane, leukotrienes (they get oxidized by it)

3.CoQ in **respiratory chain** can accidentally produce reactive oxygen species.

4. purposefully, during phagocytosis known as **respiratory burst.**

(Res. Burst: rapid production of so many **O_2^-, H_2O_2, $OH^\bullet$, NO, HOCl**)

5. **Ionizing Radiation** would form $OH^\bullet$

Cytochrome p450 is an example of **monooxygenases**.

Monooxygenases are known as **mixed function enzymes**: **mono-** because they **only use one oxygen** atom to be added to the substrate. **mixed function** because the other oxygen would be used to oxidize NADPH.

$R\text{-}H + O_2 + NADPH + H^+ \rightarrow \underline{R\text{-}OH} + H_2O + \underline{NADP^+}$ (Notice how the substrate got hydroxylated and NADPH was oxidized)

→**Advantage** of adding OH is to make the substrate more soluble (mostly for excretion and stabilization) and detoxicated.
(hydroxylation can transform substances from <u>active → inactive</u> or the opposite)

Sometimes, OH is added to **make addition of glucuronic acid possible**, which in turn <u>makes the substance more soluble.</u>

There are **two systems** that contain the monooxygenase:

1. **the mitochondrial system**, for Hydroxylation of **steroids, bile acids, active form of Vit. D.**

2.the microsomal system for **1.** detoxification of substances (First step of detoxification occurs by adding the OH group) <u>e.g.</u> pesticides. **2.** activation or inactivation of Drugs. **3.** solubilization

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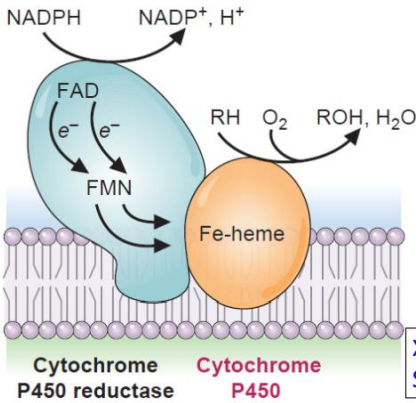
The action of CyCh P450 (450 → wave length where absorption of light is maximized):
Presenting hydroxyl group to substrate.



It's complexed with another enzyme called **CyCh P450 reductase**, to achieve certain things. Cytochrome contains a heme group, and <u>to reduce this heme group we need reductase</u>.

NADPH donates an **e**$^-$ <u>through reductase</u> to **FAD**, which gives it **to FMN** which then gives it **to the iron of heme** (reducing it from **fe**$^{3+}$ to **fe**$^{2+}$). The **e**$^-$ is then added to **oxygen of hydroxyl** group in the substrate, the other oxygen is reduced producing H2O.

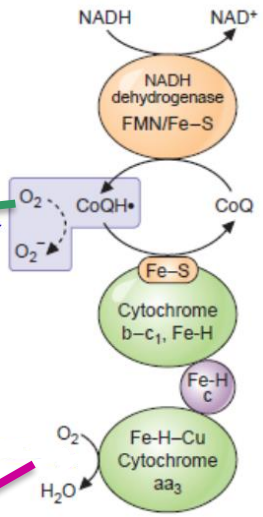
Accidental release of free radical intermediates may occur, because addition of e$^-$ to oxygen makes it very reactive.

Reaction:

$$O_2 + S + XH_2 \longrightarrow H_2O + SOH + X$$

XH_2: electron donor ~ S: substrate ~ SOH: hydroxylated substrate ~ X: oxidized form of reducing substance

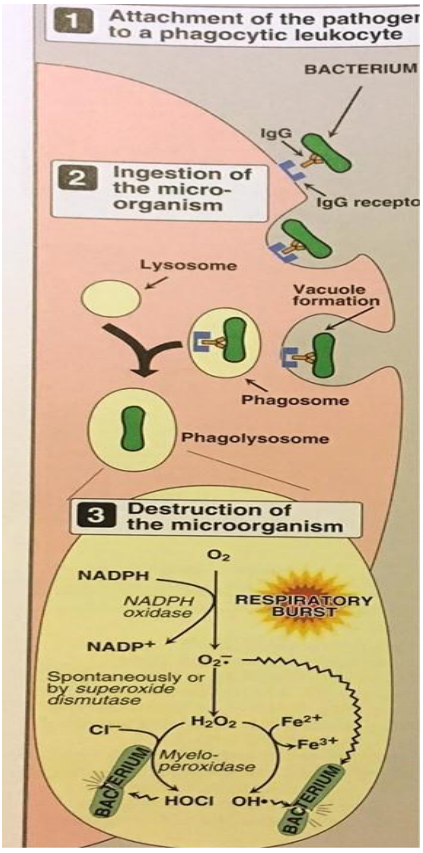
Generation of O_2^- by the respiratory chain of oxidative phosphorylation:



CoQ can hold one or two electrons, if the **reduced form** of it **donates one electron**, it'll result in a **partially reduced CoQ** formation. This **partially reduced CoQ** may <u>accidentally</u> **reduce oxygen into superoxide.**
'It's a minor pathway but it occurs all the time, superoxide here is a byproduct'

→If superoxide is produced it has to be taken care of by superoxide dismutase, and catalase.

However, in **cytochrome oxidase (complex IV)** that reacts with oxygen. superoxide and peroxide won't be formed because oxygen would be bound to copper and iron at the same time (binuclear center).



Phagocytosis:

We know the mechanism by which leukocytes fight off bacteria.
Pathogen recognized by IgG then → bind to a receptor → endocytosis → lysosome binding → destruction of pathogen, which **can occur in 2 ways**:

Oxygen independent	**lysosome acidity along with many reactions by hydrolases and proteases that degrade it.**
Oxygen dependent	**by super oxide. oxygen is used to oxidize NADPH purposefully, Losing the electron from NADPH to the oxygen will produce superoxide. Which spontaneously or by dismutase yields hydrogen peroxide, these both can kill the bacteria. Respiratory burst occurs here.**

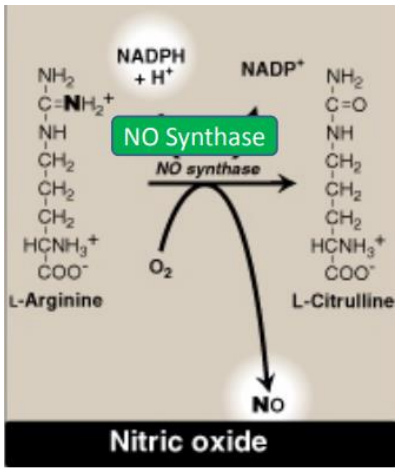
There's an enzyme called **myeloperoxidase**, which **adds chloride to H_2O_2 forming OCl^- (**hypochlorite). It's very active against bacteria and is present in bleach.

If **Nitrogen** is added we have what's called Reactive Nitrogen Oxygen Species, these are also free radicals.

NO:

- A Free radical that diffuses readily
- Essential for life, but can be toxic easily.
- Works as Neurotransmitter, vasodilator
- NO decreases Platelet aggregation
- At high concentration NO combines with $O_2^{\bullet -}$ or O2 to form RNOS that can kill bacteria.
- RNOS are involved in neurodegenerative diseases and inflammatory diseases

NO synthesis:

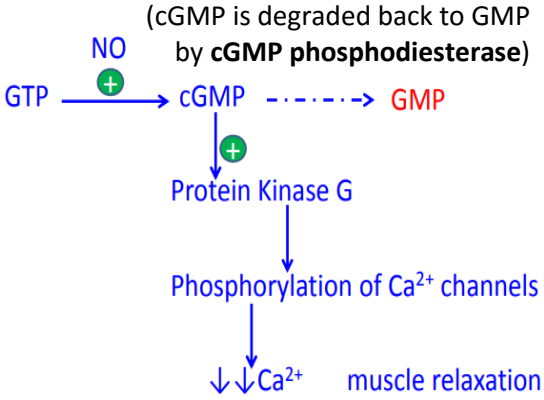


It's derived from arginine with NADPH which causes oxidation of nitrogen to produce NO. The amino acid after losing this NH2 turns into L-citrulline, which is not found in proteins.

Through an enzyme called **NO synthase**: has three isoforms:

nNOS neural and **eNOS endothelial** → both constitutive, meaning their levels remain the same, not increased by signals. **iNOS** → this isoform is **inducible in immune cells**. Its induction leads to **production of more RNOS** to kill invading bacteria.

Action of NO in vascular endothelium:



NO synthesized in **endothelial cells** defuses into smooth muscle cells causing relaxation in blood vessels.

How does it act? NO Converts GTP into cGMP, which stimulates protein kinase G → phosphorylates calcium channels → causes a decrease in calcium level, which leads to muscle relaxation.

Inhibition of cGMP phosphodiesterase and up regulation (activation) of this mechanism can be done by Viagra, for sexual dysfunction**. How does it act?**

It inhibits **the phosphodiesterase** leading to **cGMP elevation** and for **muscle relaxation**. same drug was used for treating pulmonary hyper tension, it causes relaxation of the pulmonary artery leading to a decrease in the pressure in the pulmonary artery.

And guess what? There isn’t an eighth page, it’s just here to say enjoy and

Good luck