Doctor.021

no. 4

HLS BIOCHEMISTRY

Writer: Eman Kittaneh & Rama Harb

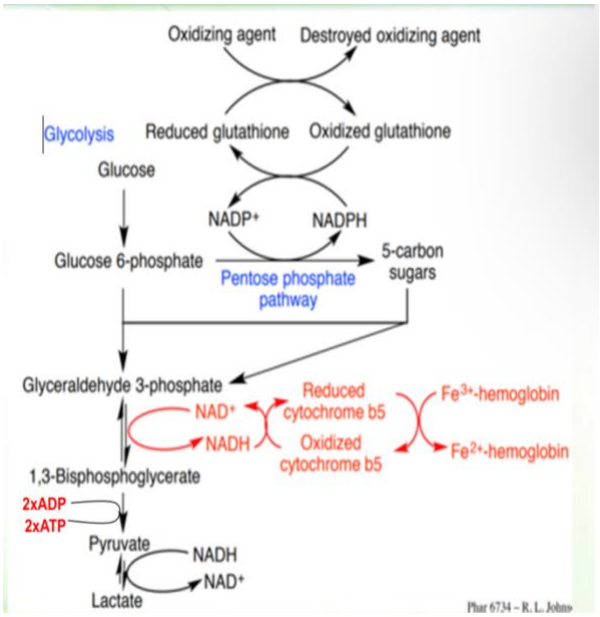
Corrector: Eman Amjad

Doctor: DR. Mamoun

METABOLISM IN ERYTHROCYTES

❖ **There are 2 main metabolic pathways in RBC's:**

❖ (recall that RBCs have no nucleus, so <u>precursor cells</u> will produce everything needed by RBCs to survive like enzymes and hemoglobin. Also, they lack mitochondria, so there won't be metabolic pathways as Krebs cycle or oxidative phosphorylation, and they depend on the following pathways as primary sources not as other cells).



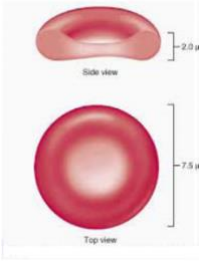
1. GLYCOLYSIS

- **(glucose → pyruvate → lactate),since there is no mitochondria in RBC's, pyruvate will be converted to lactate -anaerobic metabolism-**

❖ **Glycolysis Products:**

A. 2,3-bisphosphoglycerate (2,3-BPG) -more about it below-

B. NADH for reduction of methemoglobin (hemoglobin with oxidized Fe3+ in heme) into hemoglobin which is catalyzed by methemoglobin's reductase.



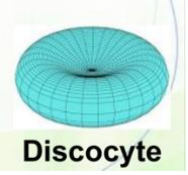
C. ATP for:

➢ **Modifying sugars and proteins**

➢ **Maintaining membrane asymmetry (biconcave shape)**

➢ **Functioning of membrane ion pumps (like HCO3- and Cl- ions exchange)**

➢ **Regulating cytoskeletal proteins (Maintenance of the discocyte shape-biconcave disc-, which is critical for the optimal viability and functional capacity) Which makes them able to be squeezed in capillaries.**

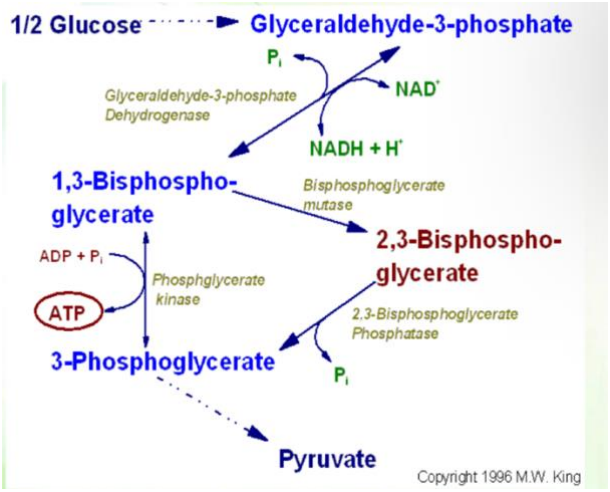


2.PENTOSE-PHOSPHATE PATHWAY (PPP)

- ❖ (Starting with glycolysis in RBCs and major products formed from it)
- ➢ (Glucose 6-phosphate→ 5 carbon-sugar)
- ➢ Producing:
  - NADPH -helps in getting rid of oxidizing agents-
  - ❖ (Glutathione plays an important role in protecting RBC's against oxidative damage, and glutathione needs NADPH to function)

2,3-BISPHOSPHOGLYCERATE (2,3-BPG)

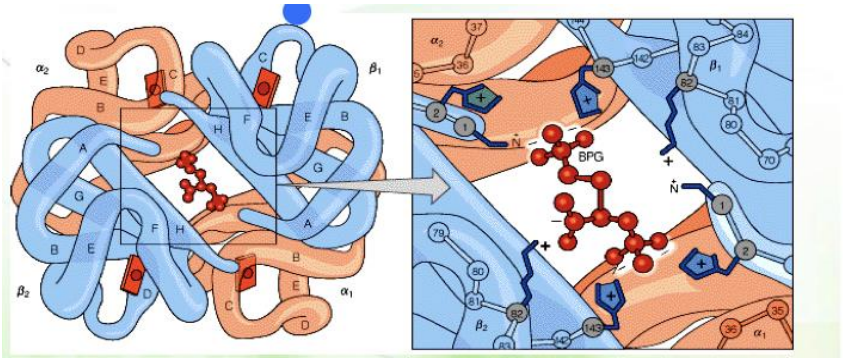
- is derived in small amounts from the glycolytic intermediate 1,3-bisphosphoglycerate. (not much in quantity but has huge effect on Hb)
- It can re-enter the glycolytic pathway.
- The erythrocyte loses 2 ATPs.

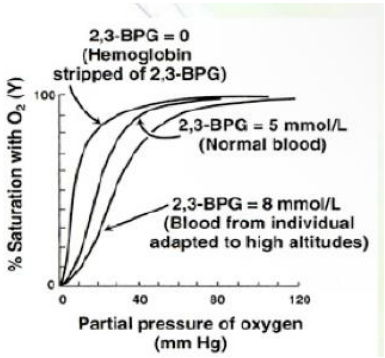


- ➢ notice the reactions in the picture :
  - ❖ Glycolysis in other cells, except RBCs, does NOT form 2,3 BPG at all, it goes directly from 1,3 BPG to 3-phosphoglycerate thus generating 2 ATPs
  - ❖ while in RBCs, it can goes in 2 ways:
- ➢ 1. As other cells (the direct way→generating ATP).
- ➢ 2. 1,3 BPG is isomerized to 2,3 BPG then to 3-phosphoglycerate and this reaction will NOT generate ATP.
- ➢ Thus the ATP net result in glycolysis, in case of using 2,3 BPG pathway is zero (2ATp is used in glycolysis first half and no ATP generated by 2,3BPG reaction)
- ➢ Normal glycolysis net result→ 2 net molecules of ATP per 1 molecule of glucose

- 2,3-BPG occupies the center of deoxygenated Hb stabilizing it in the T structure. (one molecule only)
  - ❖ Only 1molecule of 2,3BPG binds right in the middle of the Hb - interacting with the four chains-
  - ❖ 2,3BPG stabilizes Hb in T-state and releases o2 into tissue

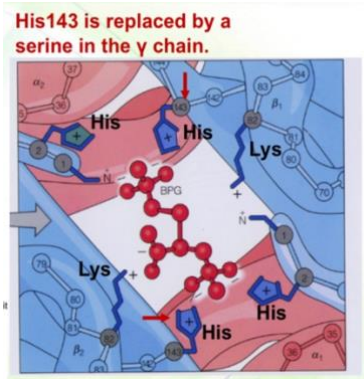
- **when 2,3-BPG is not available (not bound), Hb can be easily converted to the R-structure.**
  - ❖ **Remmeber: R-structure→oxygen is bound, T-structure→ oxygen is released**
  - ❖ **Normally we have sigmoidal shape with normal release of o2, In the case of 2,3BPG absence the affinity with o2 will be really high, thus hardly any release of o2 in tissues**
  - ▪ **Explaining why in high attitude 2,3 BPG increases , to increase o2 releasing**

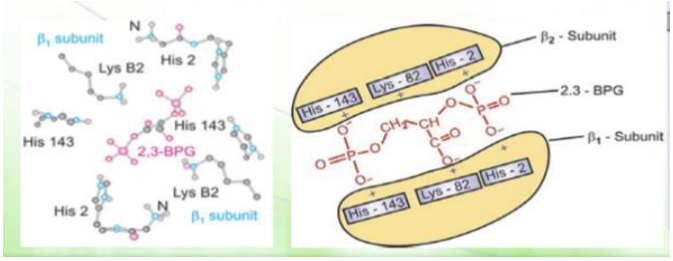




2,3-BPG AND HBF

- **2,3-BPG interacts with several groups including His143.**
- **Fetal hemoglobin (HbF) binds 2,3- BPG much less strongly than HbA.**
- **Why?**
  - ❖ **Just to remind you: 2,3 BPG depends on His143 of the β chain in order to make good interaction with the Hb molecule**
  - ❖ **While Fetal hemoglobin (HbF) doesn't have His143 -replaced by serine-,as a result there is no good interaction with the Hb molecule, so HbF binds 2,3-BPG much less strongly than HbA, explaining HbF higher affinity toward O2**



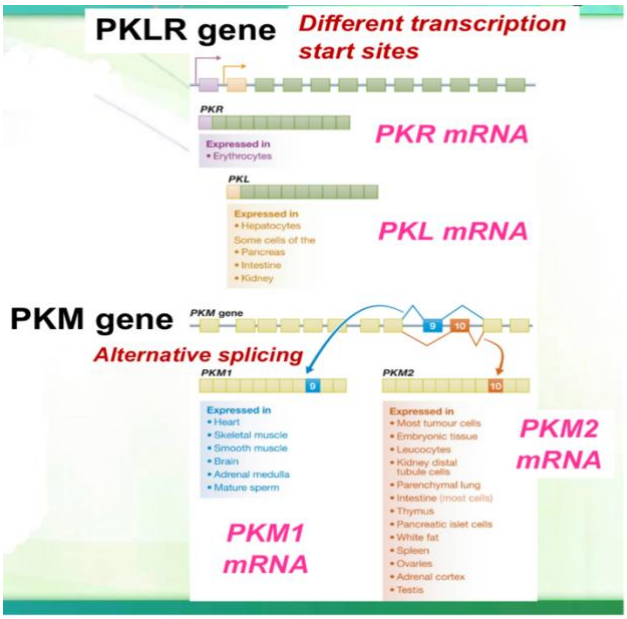


PYRUVATE KINASE ISOZYMES AND REGULATION

Recall :

- **The last step in glycolysis is converting phosphoenol pyruvate (PEP) to pyruvate and generating ATP by enzyme called pyruvate kinase (PK).**
- **Isoenzymes: enzymes that catalyze the same reaction, but they are produced by different genes and have different regulations, kinetics, and tissue distribution**
- **Isoforms: proteins or enzymes that are produced from the same gene, but have different regulations, kinetics, and tissue distribution.**

- **There are two isoenzyme genes of PK and each produces two isoforms:**
  - **The 2 isoenzyme: 1-PKLR, 2-PKM**
  - **The isoforms of each isoenzyme:**
    1. **PKLR: PKLR gene produces PKL (liver) and PKR (erythrocytes) using different transcription start sites.**
    2. **PKM: PKM gene produces PKM1 (muscle and brain) and PKM2 (fetal and most tissues) by alternative splicing**
  - **How is the isoforms produced for each?**



- **In PKLR gene it is transcribed on different sites -only exon 1 differs between PKL and PKR, while everything else is the same-**
  - **PKR(erythrocytes) uses exon 1, PKL(liver) uses the second exons as exon 1**
- **While in PKM -we have alternative splicing, so one of the exons is different producing 2 different mRNA molecules and enzymes(PKM1, PKM2), notice that the tissue distribution is also different.**

- **Fetal PK isozyme (PKM2) has much greater activity than the adult isozymes.**

- Fetal erythrocytes have lower concentrations of glycolytic intermediates including 1,3-BPG and, hence, 2,3-BPG).
- Remember: lower 2,3BPG means more Hb in R-state.

❖ The higher activity of PKM2 in fetus will accelerate the pathway→producing lower levels of 1,3BPG → lower2,3BPG than the adults erythrocytes→ high amounts of hemoglobin in R state→ higher affinity toward oxygen

❖ To recap: Fetus increases his affinity towards O2 by 2 ways:

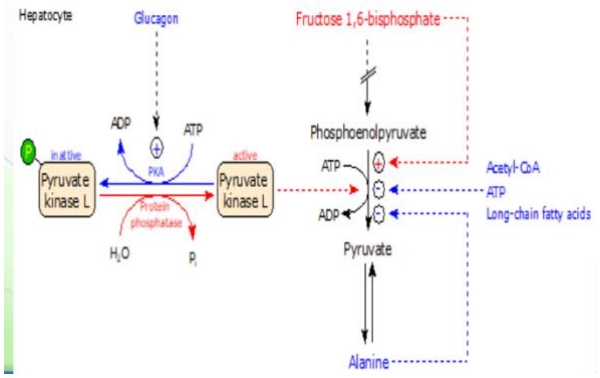
1. HbF has lower affinity to 2,3 BPG (due to the absence of His143).
2. He has lower amounts of 2,3 BPG (Fetus has PKM2 isoform of PK).

REGULATION OF PKL

❖ remember that PK is allosteric enzyme

- The liver enzyme (PKL) is allosterically regulated:
- inhibited by ATP, acetyl-CoA, alanine, and long-chain fatty acids and by phosphorylation by protein kinase A.
- activated by Fructose 1,6-BP.

❖ When there's enough glucose in body → there's Fructose 1,6-BP in liver → PKL is activated



- The liver (PKL) gene is also controlled at the level of synthesis. (Increased carbohydrate ingestion induces the synthesis of PKL)

❖ The gene itself (the transcription), not only the enzyme, could be regulated by increased carbohydrate.

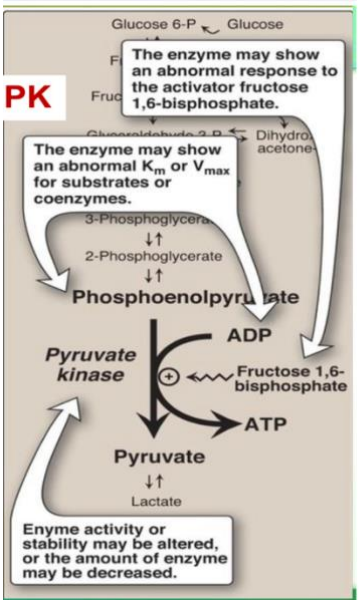
❖ When the liver needs more glycolysis, it can increase the expression of the enzyme by itself.

GENETIC DISEASES OF PK DEFICIENCY

❖ PK deficiency: it is an essential enzyme (cannot be lost completely but can have very low activity).

❖ Can be affected in any aspect (regulation, activity, synthesis, affinity, Abnormal response to F1,6BP, Binding to substrate and ADP).

- **It is a hereditary genetic disease (single point mutation) : The adult erythrocyte PK is virtually inactive which reduces the erythrocytes' ability to produce ATP leading to hereditary hemolytic anemia.**



- **The severity of the disease depends on the degree of enzyme deficiency (5-35%) and ability to produce 2,3-BPG.**
  - ❖ **And when we say the enzyme deficiency 5-35% then symptoms will appear, Little deficiency is fine, notice that in general the enzymatic activity doesn't necessarily have to be 100%**
    - **Liver is not affected since expression can be stimulated (synthesis covers the deficiency)**
  - ❖ **as said, liver can compensate for any loss of PK by controlling the level of synthesis thus low levels of pyruvate in liver stimulate the synthesis of more PK accordingly compensate activity loss, increasing production, while the opposite to erythrocytes they would be affected-the gene isn't regulated at the transcriptional level-)**

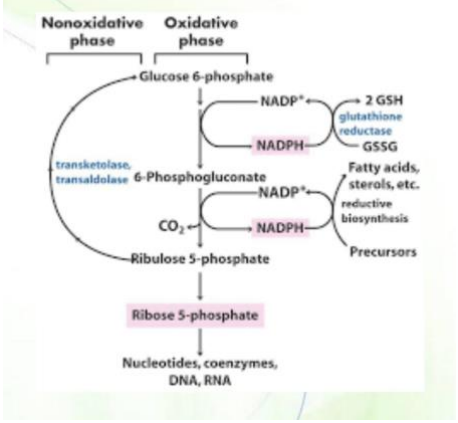
- **Patients are resistant to malaria.**

The pentose phosphate pathway

❖ **It is basically converting glu6-phosphate to 5-carbon sugars**

Two phases of pentose phosphate pathway:

- **The oxidative generation of NADPH**
- **NADPH is generated when glucose 6- phosphate is oxidized to ribulose 5-phosphate. Glu6-phosphate+2NADP+H2O→ribose5-phosphate+2NADPH+2H+CO2**



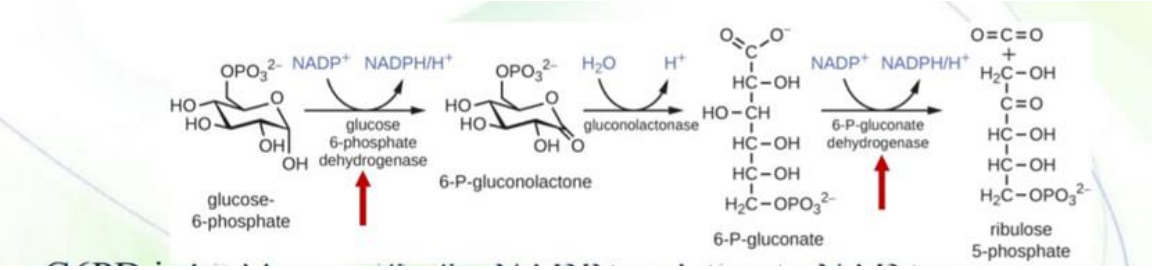
Why NADPH is important? It is important in regeneration of glutathione-which protects erythrocytes from oxidizing agents-by the reductase enzyme.

- **The non-oxidative interconversion of sugars→that is when 5-carbon sugars exchange carbons which make a 7-carbon sugar, 6-carbon sugars and so on.**

The first step

- **The oxidative phase of the pentose phosphate pathway starts with the dehydrogenation of glucose 6-phosphate by glucose 6-phosphate dehydrogenase (G6PD).**

The two reactions that catalyzed by G6PD are irreversible reaction and both of then produce NADPH.



- **G6PD is highly specific for NADP+, relative to NAD+**
- **The reaction is irreversible and is the rate-limiting reaction.**
- **High levels of NADP+ stimulate the reaction.**

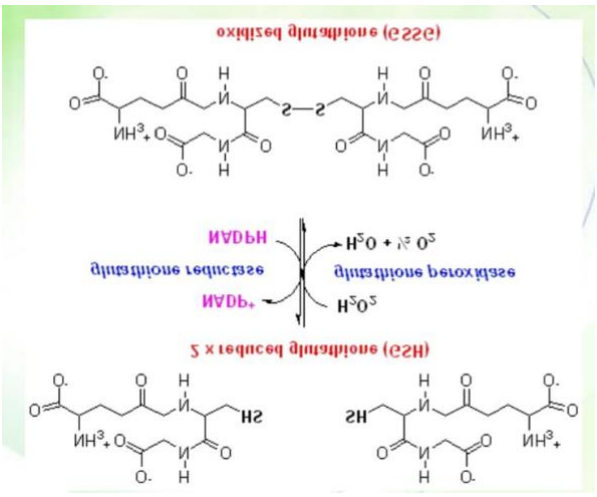
Oxidative stress and glutathione

- **Oxidative stress within cells is controlled by the action of glutathione (GSH).**
- **GSH reduces peroxides via glutathione peroxidase.**

- **GSH is regenerated via NADPH-dependent glutathione reductase.**
- **The PPP in erythrocytes is the only pathway to produce NADPH.**

PPP consumes almost 10% of glucose by erythrocytes.

90% by glycolysis



Low GSH levels

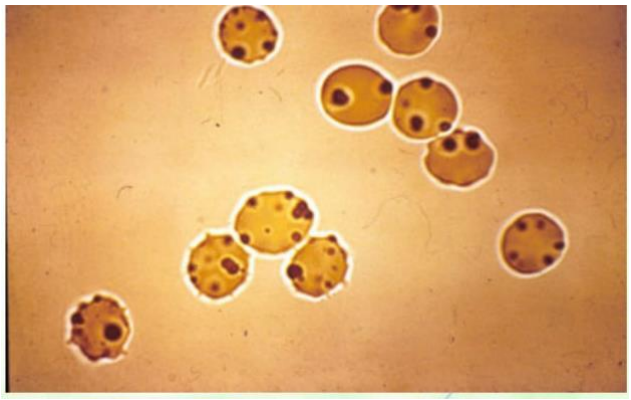
When there is a low level of GSH and the cell contains oxidizing agent as myeloid peroxide at high level it gonna attack whatever molecule present in the erythrocytes.

Well, what is the most abundant molecule in erythrocytes? Right, it is hemoglobin molecule.

Then hemoglobin will be damaged and undergoes denaturation.

• The inability to maintain reduced glutathione in RBCs leads to increased accumulation of peroxides, predominantly H2O2, resulting in weakening of the cell membrane due to:

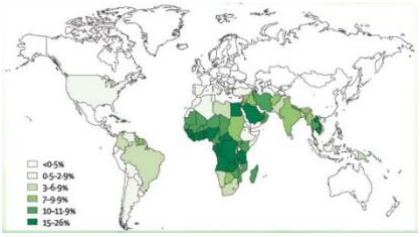
- **peroxidizing the membrane lipids leading to hemolysis**
- **oxidizing the proteins including hemoglobin (to methemoglobin) and membrane proteins, insolubilizing them, and forming Heinz bodies.**



Glucose-6-phosphate dehydrogenase deficiency

G6PD deficiency

• Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a group of heterogeneous disease with significantly reduced activity.



Causes:

• Hemolytic anemia

• particularly after the administration of drugs, during infections and in the neonatal period (jaundice)

• Deficiency of G6PD is most prevalent in individuals of African, Mediterranean, and Oriental ethnic origins.

• It is the most common enzyme deficiency worldwide.

• G6PD gene is located on the X chromosome.

- Inheritance of G6PD deficiency is sex-linked.--> so males is more affected than females

G6PD mutations

- Several hundred G6PD genetic variants have been identified, but most have no clinical symptoms.
- Almost all G6PD deficiency variants are caused by point mutations in the gene. Not deletion as the effect of deletion is huge even at fetal level.
- These mutations mainly alter the kinetic properties, stability, or binding affinity to NADP+ or G6P.
- No large deletions or frameshift mutations. Why? Deletion has a huge effect even on fetal stage so embryo will not survive.

The four classes of G6PD deficiency

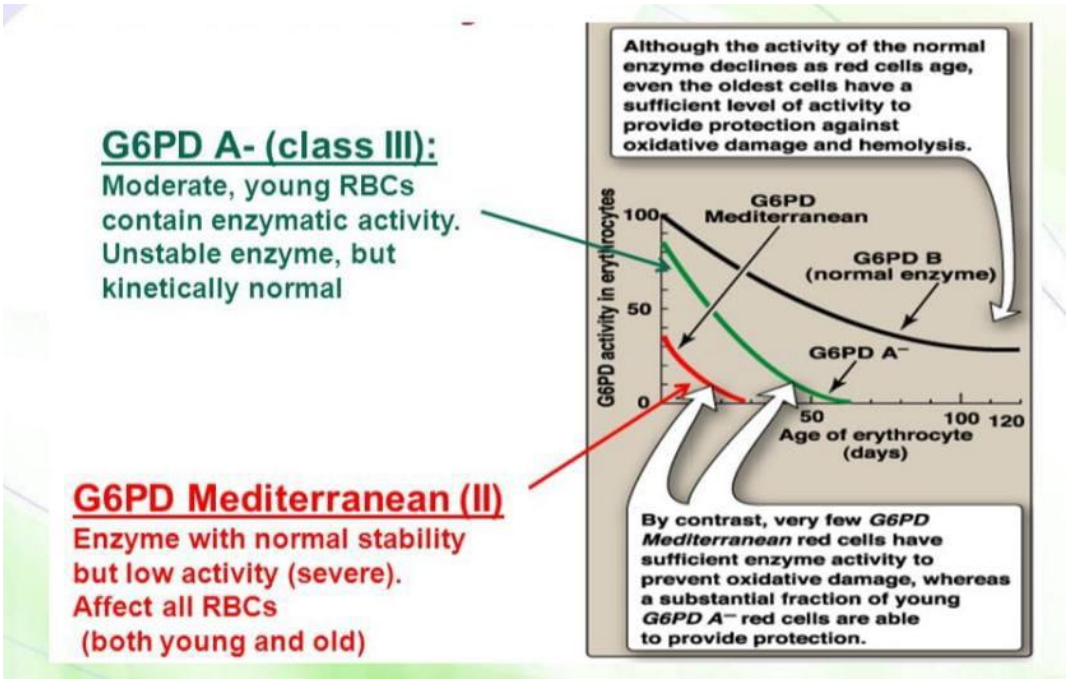
- G6PD B (Normal)
- Abnormal G6PDs
  - Class I: the most severe and rare.
  - Class IV: no clinical symptoms →there are a mutation and the person is affected but there is an activity reach 50% nearly so the person is fine
  - G6PD A- (group III or class III)

Class	Clinical symptoms	Residual enzyme activity
I	Very severe (chronic hemolytic anemia)	<2%
II	Severe (episodic hemolytic anemia)	<10%
III	Moderate	10%-60%
IV	None	>60%

- Among persons of African descent
- It is caused by a single amino acid substitution that decreases enzyme (protein) stability but has 5-15% of normal activity. The stability of protein is compromised.
- The disease is moderate.

- G6PD Mediterranean (group II or class II)
  - Severe
  - The enzyme has normal stability, but negligible activity.

Class II vs. class III



Inducers of G6PD deficiency symptoms

- **Oxidant drugs**

➢ **Antibiotics, anti-malarial, and anti-pyretics (not acetaminophen)**

- **Fava beans (favism)**

➢ **Fava beans are presumed to cause oxidative damage.**

➢ **Substances capable of destroying red cell GSH have been isolated from fava beans (fool).**

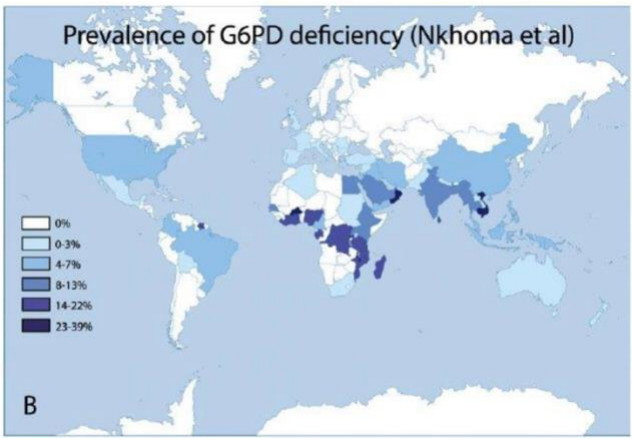
➢ **Favism is most common in persons with G6PD class II variants, but rarely can occur in patients with the G6PD A- variant.**

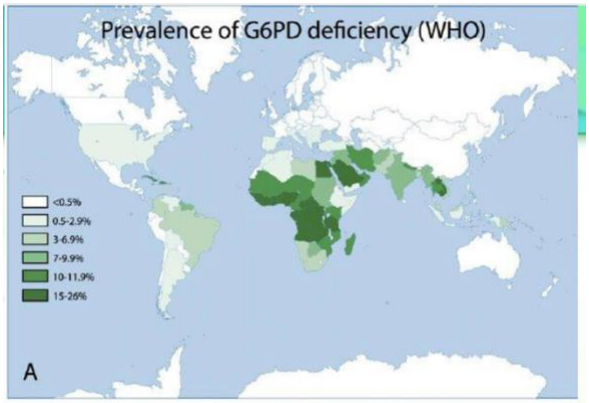
- **Infection**

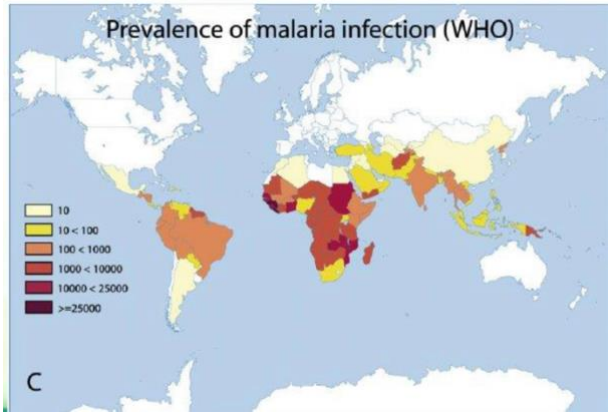
The most common inducer due to production of free radicals.

Connection to malaria

- **Several G6PD deficiencies are associated with resistance to the malarial parasite, Plasmodium falciparum, among individuals of Mediterranean and African descent.**
- **The basis for this resistance is the weakening of the red cell membrane (the erythrocyte is the host cell for the parasite) such that it cannot sustain the parasitic life cycle long enough for productive growth.**







At the end, while your studying your best to become the greatest doctor :)
don't forget our people in G*aza, and don't forget your purpose

"وَلا تَهِنُوا وَلا تَحْزَنُوا وَأَنْتُمُ الأَعْلَوْنَ إِنْ كُنْتُمْ مُؤْمِنِينَ"

اللهم احفظ أرواحهم وأبناءهم وردهم إلى ديارهم مردًا كريمًا آمنًا. اللهم انصر أهل غ*زة على من عاداهم اللهم صوب رميهم اللهم ثبت الأرض تحت أقدامهم. اللهمَّ اجعل الأطفال في فلسطين محميين وآمنين، وارزقهم الفرحة والطمأنينة في حياتهم.