Sheet No.
4

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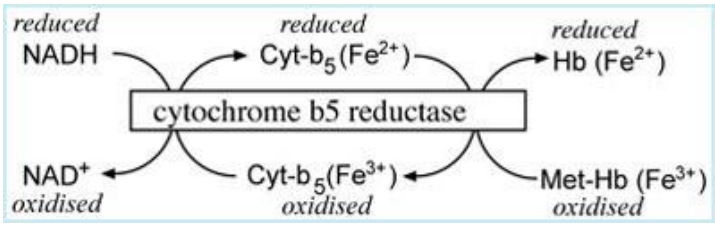
We didn't finish all hemoglobinopathies in the last sheet, so we will complete them first, then we gonna talk about RBCs metabolism

We will start talking about another condition of qualitative hemoglobinopathies which alters oxygen transport

Methemoglobinemia

Methemoglobin is a hemoglobin molecule that is bound to iron in the ferric state rather than the ferrous, so it will become unable to bind O2.

Normal people have some HbM, but in very low quantity because we are protected by some factors such as: hydrophobic pocket surrounding the heme molecule decreases the probability of oxidizing heme iron from Fe2+ to Fe3+ due to electron rearrangement between amino acid residues and there are protective reductase systems that convert HbM to HbA.



Methemoglobin reductase AKA

NADH-Cytochrome b5 reductase

When HbM is present in excess amount, a condition known as Methemoglobinemia develops which appears in the patients as cyanosis (bluish)

HbM can increase in some individuals due to:

1. Some mutant globins (alpha and beta) bond hemes in such a way as to resist the reductase:

- Hb Boston: distal histidine is mutated into a tyrosine resulting in oxidation of ferrous iron by tyrosine's oxygen. It also attracts H2O into the pocket.
- HbM Iwate: proximal histidine is replaced by a tyrosine.

2. A deficiency of the reductase enzyme.

3. Certain drugs or drinking water containing nitrates.

There are 2 ways to reduce HbM:

1.The major one using an enzyme for methemoglobin reduction is cytochrome b5 reductase (NADH-methemoglobin reductase). It uses cytochrome b5 as an electron acceptor and reduces it by giving it hydrogen atoms from NADH. Then, the reduced Cyt b5 is used to convert methemoglobin to hemoglobin.

2. There's an alternative enzyme called NADPH-methemoglobin reductase, which requires an exogenous electron acceptor (like methylene blue) and reduces it using NADPH. This reaction produces a compound called leukomethylene blue, which is

then oxidized to reduce methemoglobin to hemoglobin. That's why methylene blue can be used to treat the disease.

Hereditary persistence of fetal hemoglobin (HPFH)

Persons with HPFH continue to make HbF as adults(no transition from gamma to beta).

Because the syndrome is benign most individuals do not even know they carry a hemoglobin abnormality. (HbF is efficient)

Although HbF has a higher affinity to oxygen, it has no significance or advantage in these people

Many HPFH individuals harbor large deletions of the δ- and β-coding region of the cluster.

There is no deletion of the fetal globin genes.

We can use it as an advantage to treat beta thalassemia.

Diagnosing hemoglobinopathies

Gel electrophoresis: It's a technique where molecules such as proteins can be separated according to their size, charge (here we depend on the charge).

The same thing can be done with hemoglobin especially those who have amino acid substitution results in an overall change in the charge of the molecule.so it doesn't depend on the size rather it depends on the charge.

Ex. HbF molecules have a more positive charge than HbA, so it would migrate away from the anode (+).

HbF has more positive charges (has high isoelectric point), so it will be closer to the cathode (-).

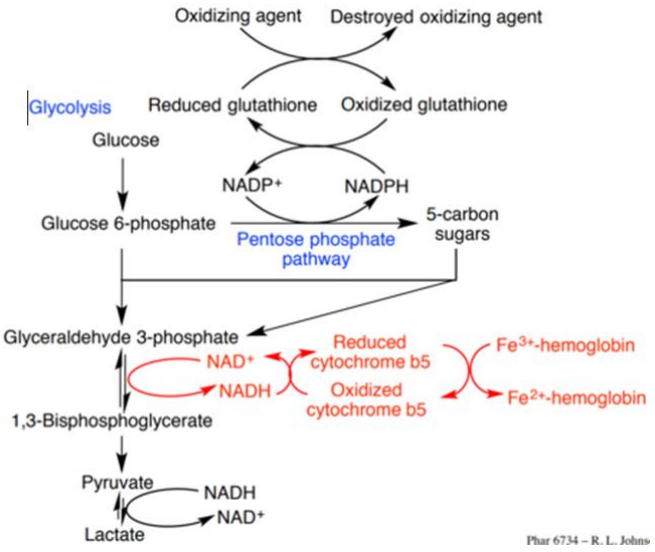
HbA has more negative charges (has less isoelectric point), so it will be closer to the anode (+).

Isoelectric point: pH that makes the protein neutrally charged.

Note: protein fragments usually move from anode to cathode.in some cases, these pieces can move in the opposite way

Results: If the individual is heterozygous then the Hb will appear as 2 bands each representing its charge. As we can see from the test results the migration pattern is different, why is it? Because we have different charges.

In this sheet we will go through the major metabolic pathways in the RBCs: (RBCs have no nucleus, so precursor cells 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 below pathways as primary sources not as other cells).



1. Glycolysis (glucose to pyruvate thus generating 2 ATP, 2 NADH).

2. Pentose phosphate pathway PPP.

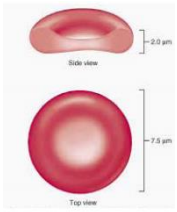
Starting with glycolysis in RBCs and major products formed from it:

ATP: The importance of ATP to RBCs:

A- Modifying sugars and proteins (cytoskeletal importance).

B- Maintaining membrane asymmetry.

C- Function of membrane ion pumps.

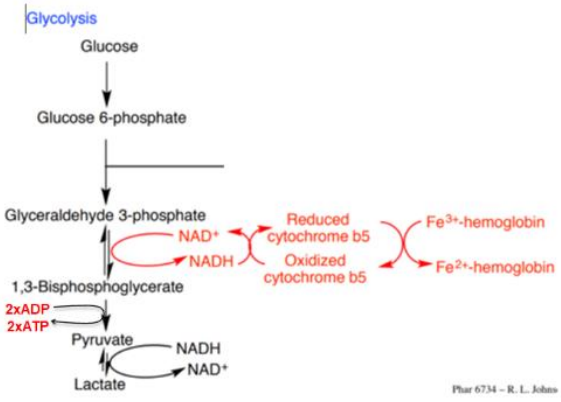


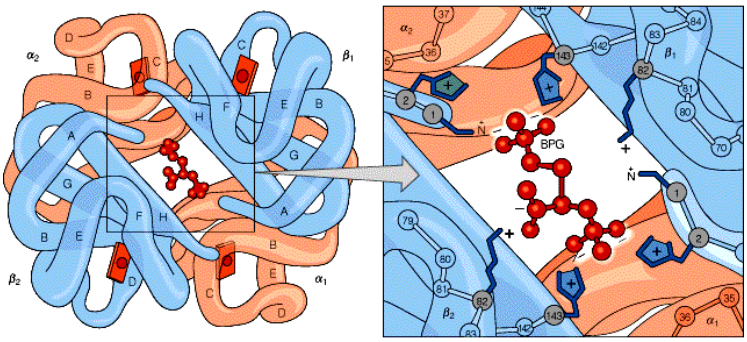
D- Regulating cytoskeletal proteins (Maintenance of the discocyte shape-biconcave disc-, which is critical for the optimal viability and functional capacity) Which makes them able to be squeezed in capillaries.

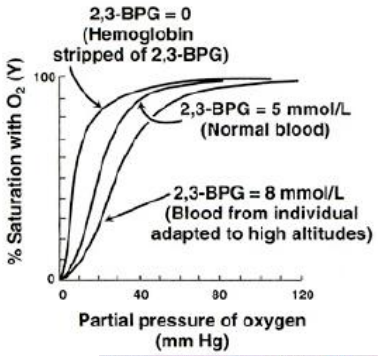
NADH: NADH is important for the reduction of methemoglobin into hemoglobin which is catalyzed by methemoglobin's reductase.

2,3-BPG: Highly negatively charged molecule interacted with the positively charged amino acids (histidine and lysine) in the center of adult deoxygenated hemoglobin (by 1:1 ratio) and stabilize the T state and reduce the affinity to oxygen (shift to the right / increase p50).

When 2,3-BPG is not available (not bound), Hb can be easily converted to the R-structure.

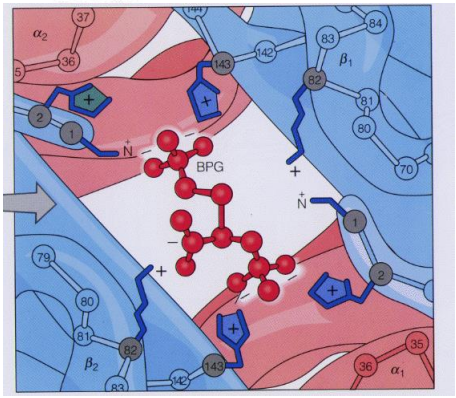






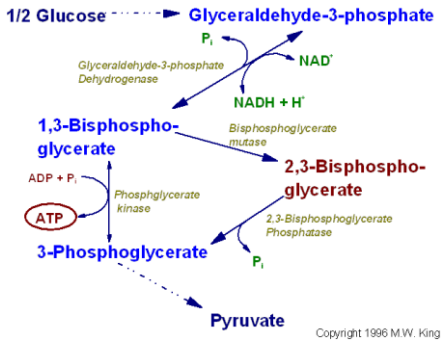
*2,3 BPG interacts with several groups including a lysine, His143, His2 of the β chain and N-terminus of the β chain.

*Fetal hemoglobin (HbF) doesn't have His143 -replaced by serine-, so it binds 2,3-BPG much less strongly than HbA, so it has higher affinity toward O_2 than adult Hb.



2,3 BPG is formed by the reaction in the right photo below

Glycolysis in cells other than 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 go 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.

   Note: the regulation between the 2 ways is not well understood.

The last step in glycolysis is converting **phosphoenol pyruvate (PEP)** to **pyruvate** and generating ATP by enzyme called **pyruvate kinase (PK).**

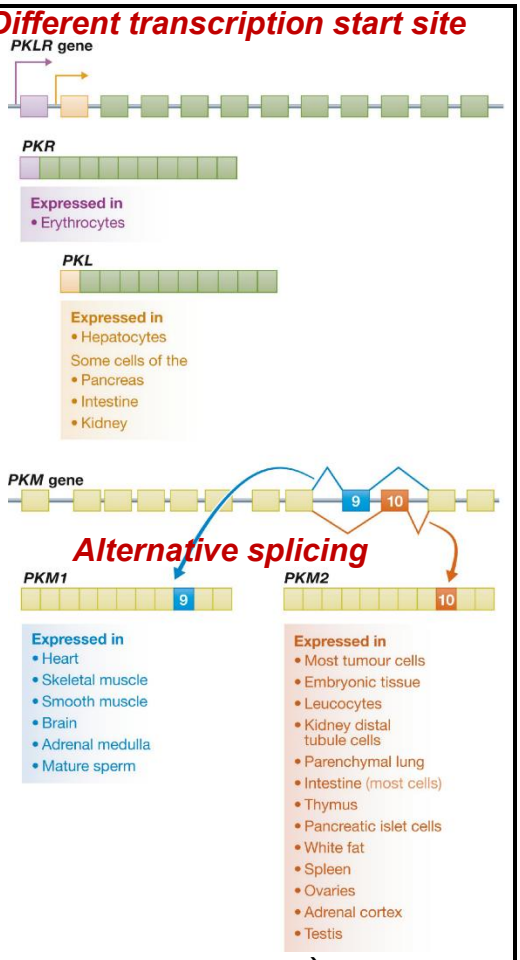
There are 2 isoenzyme genes which produce two isoforms of PK gene:

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.

1. **PKLR**: (L for liver, R for RBC/ R and L are isoforms) they differ in the transcription start sites →L is the same as R but R isoform has additional unit at the beginning (look at the image).

2. **PKM**: (has 2 isoforms: *PKM1* in muscles and brain and *PKM2* in fetus and most tissues) by alternative splicing.



PKM2 has much greater activity than the adult isozyme, which gives the fetal RBCs an advantage. since the PK is very active >> reduced amounts of glycolytic pathway intermediates (such as 1,3-BPG, 2,3-BPG in RBCs) >> high amounts of hemoglobin in R state >> higher affinity for oxygen.

So 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).

Pyruvate kinase regulation:

- Both PKL and PKR are allosterically regulated:

➢ Activated by **fructose 1,6-BP**:

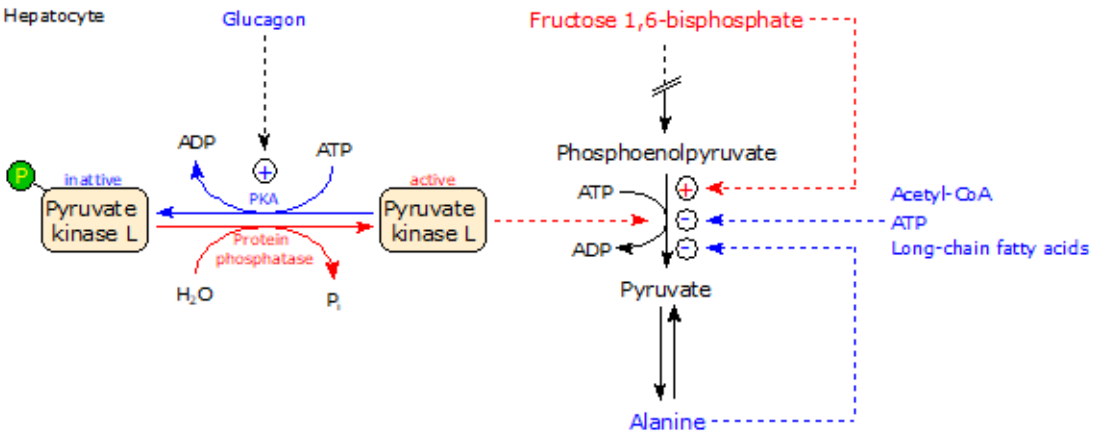
The committed step in glycolysis (**fructose 6-phosphate → fructose 1,6- bisphosphate**), so increasing the product of the committed step will activate PK.

➢ Inhibited by acetyl co-A, ATP, alanine, long chain fatty acids (indicators of high or enough energy state).

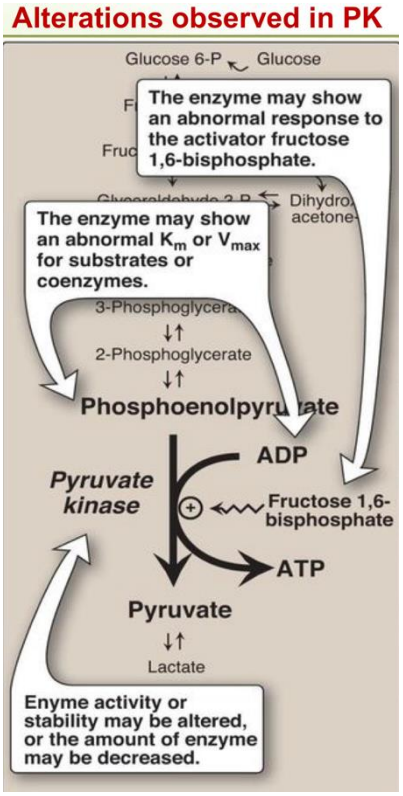
PKL is also inhibited by phosphorylation of Protein kinase A; High glucagon or epinephrin levels (starvation mood) >> activate the phosphorylation of PKA >> inactivate PKL (phosphorylated PKL=inactive PKL).

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

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



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



Can be mutated in any aspect (regulation, activity, synthesis, affinity).

It is a hereditary genetic disease (single point mutation) 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.

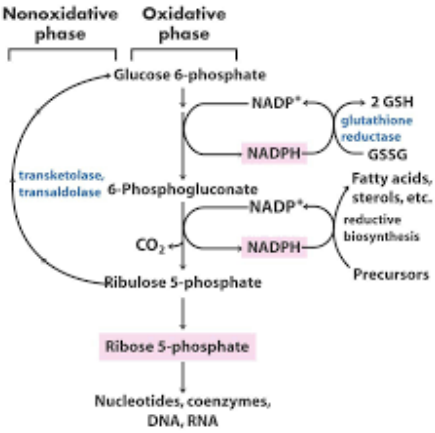
Liver is not affected since expression can be stimulated (synthesis covers the deficiency)

Recall that PKL is also controlled at the level of synthesis thus low levels of pyruvate in liver stimulate the synthesis of more PK accordingly compensate activity loss.

Patients are resistant to malaria.

Pentose Phosphate Pathway

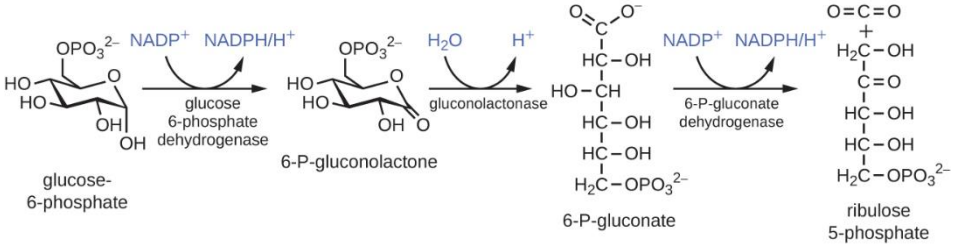
Now we are going to talk about the next major metabolic pathway which is <u>pentose phosphate pathway (PPP)</u>, it has two phases: **<u>oxidative</u>** and **<u>non-oxidative</u>** phases



1. **<u>Oxidative phase:</u>** converting Glucose-6-p to Ribulose- 5-p which eventually will be converted to ribose -5- phosphate. These reactions are irreversible and accompany NADPH synthesis (which is an important molecule for reducing glutathione which protects RBCs from oxidative stress. Also it works as a coenzyme)

G-6-P +2NADP+ + H2O —> Ribose-5-P +2NADPH +2H+ +CO2

The first RXN is a dehydrogenation reaction, and it is the most important one in the PPP (rate limiting step & irreversible) that is catalyzed by **glucose -6- phosphate dehydrogenase** which is highly regulated (regulated by levels of NADP+).

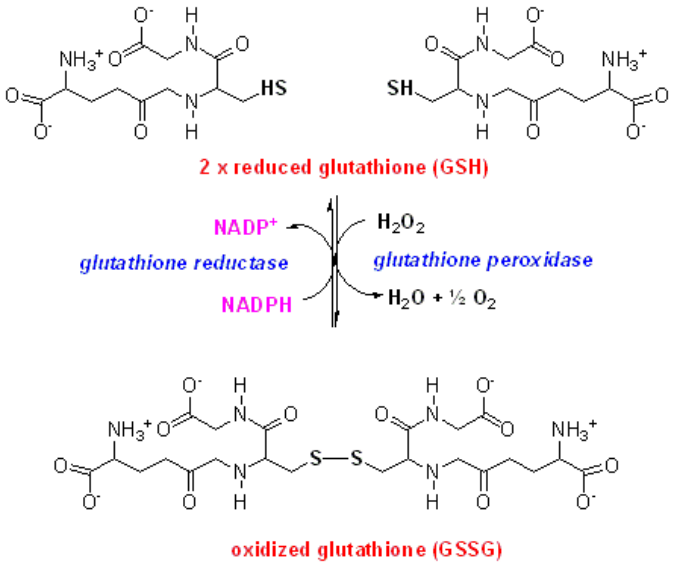


G6PD has a high affinity and is highly specific for NADP+ relative to NAD+.

High levels of NADP+ increase the activity of the G6PD and stimulate the reaction.

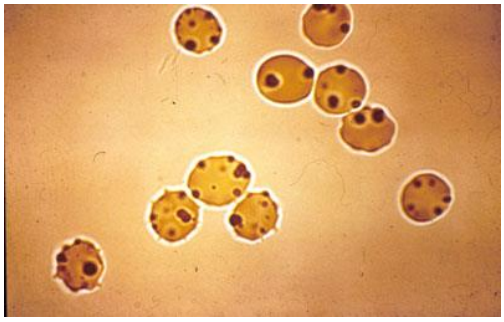
As we mentioned above NADPH is responsible for regeneration of glutathione (GSH).

- Glutathione is a tripeptide that consists of glycine, **cystine (functional one; contains thiol group)**, glutamate and it is an important molecule for controlling the oxidative stress in the cell.
- Two GSH reduce hydrogen peroxide H_2O_2 by glutathione peroxidase into oxygen and water, producing oxidized glutathione molecule (GSSG), that consists of two glutathione which are connected by disulfide bond.
- GSSG (oxidized) is converted to GSH (reduced) via NADPH-dependent glutathione reductase (as the name implies, the source of electrons is NADPH)
- The PPP in erythrocytes is the only pathway to produce NADPH.
- PPP consumes almost 10% of glucose by erythrocytes.



The inability to maintain reduced glutathione in RBCs leads to increased accumulation of peroxides (which have high affinity to draw electrons from other molecules either from erythrocytic plasma membrane or from Hb), predominantly H_2O_2, resulting in:

1. Weakening of the cell membrane by peroxidizing erythrocyte membrane lipids, and simultaneous hemolysis.
2. increasing rates of oxidation of hemoglobin to methemoglobin and other proteins including membrane proteins, insolubilizing them forming **Heinz bodies** and, further, weakening the cell membrane.



Heinz bodies are a cluster of proteins on the plasma membrane protein including band 3 which result in weaking the erythrocytic plasma membrane.

Glucose 6 phosphate dehydrogenase deficiency

*It is prevalent in the old world, and it is the most common enzyme deficiency worldwide.

*Heterogeneous disease with reduced activity.

*Inheritance of G6PD deficiency is sex linked because G6PD gene is carried on X chromosome (more common in males).

*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 →

(Mainly these mutations alter the kinetic properties, stability, or binding affinity to NADP+ or G6P).

***No large deletions or frameshift mutations. Why?** This is an indicator that G6PD is an essential enzyme. If it is absent, the patient will die.

G6PD has four classes: **from class I** (most severe) **to class IV** (asymptomatic).

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%

*G6PD B is Normal.

Abnormal ones are:

G6PD A- is abnormal (group III or **class III**) (Among persons of African descent):

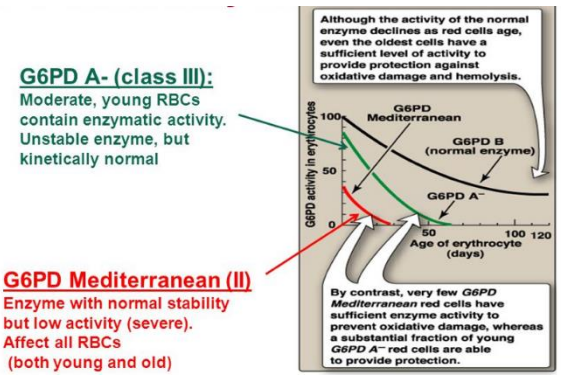
It is caused by a single amino acid substitution of Asn to Asp that decreases enzyme stability with 5-15% of normal activity, (quantitative decrease in enzyme amount rather than in its function) thus moderate diseases and symptoms.

G6PD Mediterranean (group II or **class II**) (severe) The enzyme has normal stability, but negligible activity (opposite to G6PD A-: normal enzyme levels with a defect in enzyme function).

Class II vs Class IV:

The normal activity of the enzyme declines with age in normal blood cells. However, even the oldest has enough activity to survive

In G6PD Mediterranean they are less active and the activity declines more rapidly → that justifies the reason behind why G6PD II is more sever than class IV.



It induces hemolytic anemia due to accumulation of oxidants that disrupt the cell. There are some inducers that increase the oxidative stress such as:

- Oxidant drugs (Antibiotics, anti-malarial, and anti-pyretics (except 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 people 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).

G6PD are resistant 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.