HLS

MODIFIED NO. 4

BIOCHEMISTRY

كتابة: ميس قشّوع وشَهْد العَرب

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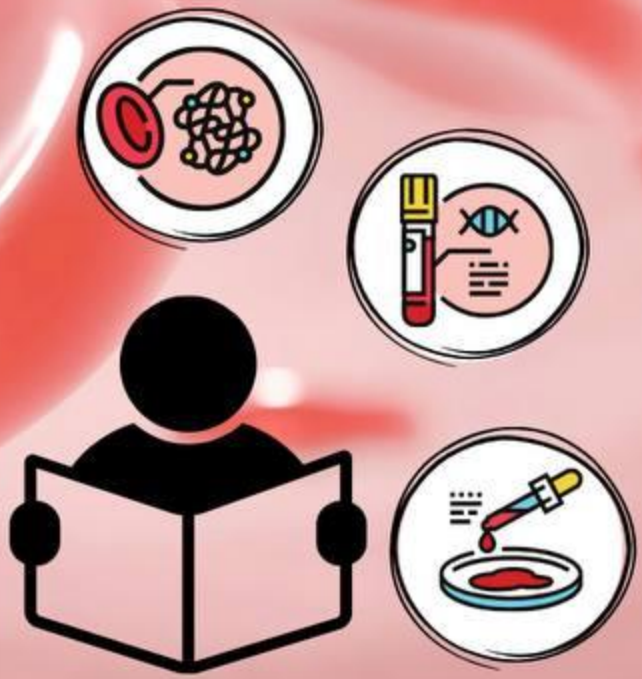


Metabolism in erythrocytes

Prof. Mamoun Ahram
Hematopoietic-lymphatic system

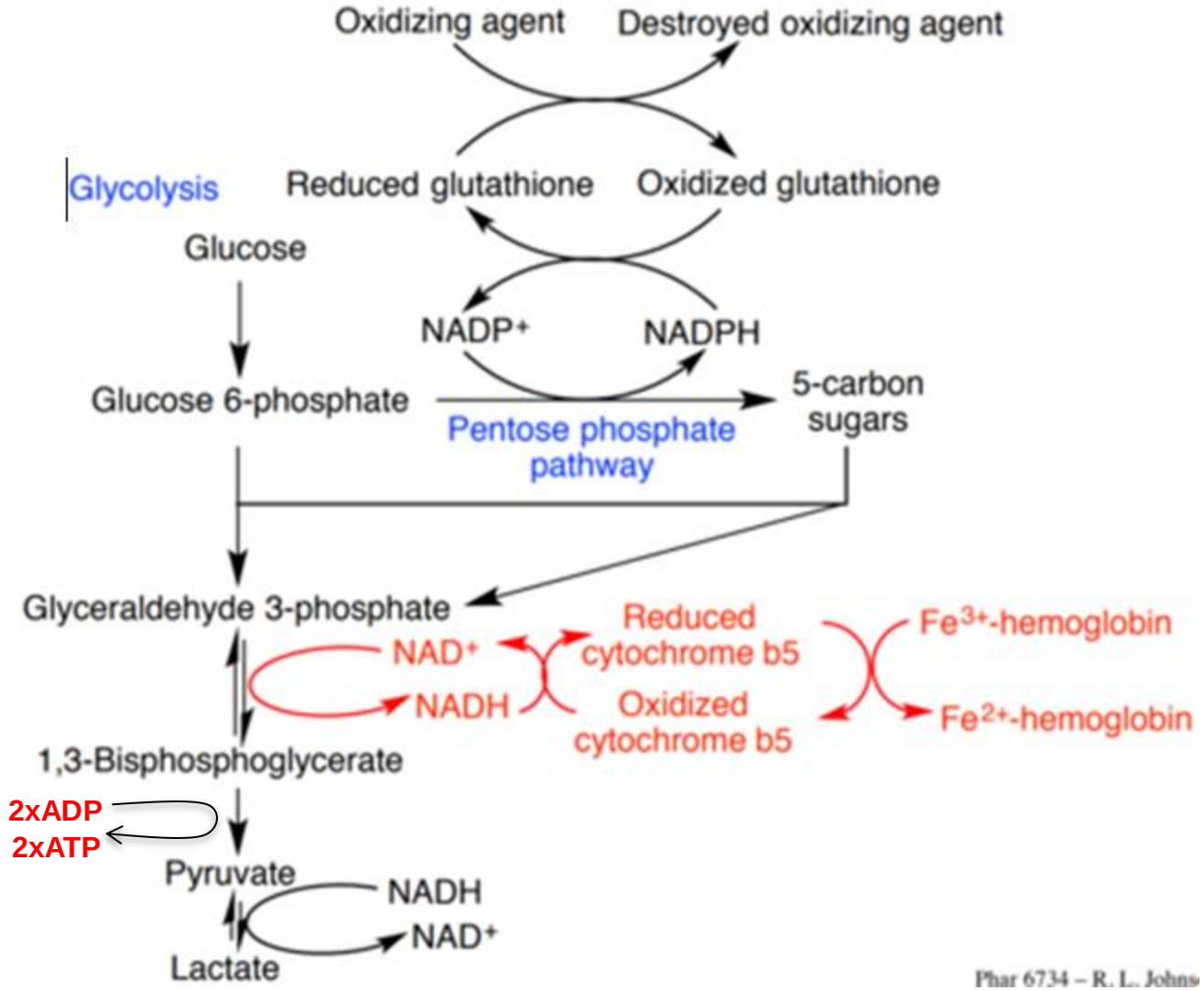
Color code

Slides
Doctor
Additional info
Important

This lecture
Lippincott's Biochemistry, 8th edition
The Medical Biochemistry Page
(https://themedicalbiochemistrypage.org/)

Carbohydrate metabolism in RBC

- Glycolysis
  - 2,3-bisphosphoglycerate (2,3-BPG)
  - NADH
- Pentose phosphate pathway
  - NADPH

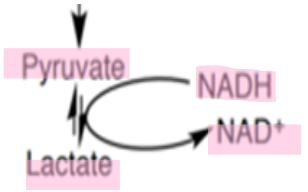


❑ There are two main metabolic pathways starting from glucose:

1.Glycolysis: if cells need energy, they break down glucose into pyruvate.

- Aerobically, glucose is converted to Acetyl-CoA, which then enters the Krebs cycle, followed by the electron transport chain and oxidative phosphorylation, leading to the production of a lot of ATP.

- Anaerobically, glucose is converted to pyruvate and then to lactate. The purpose of this reaction (converting pyruvate to lactate) is to regenerate NAD by oxidizing NADH, allowing glycolysis to continue to produce energy , because if NAD is depleted, the pathway will stop. Another important product produced form glycolysis is 2,3-bisphosphoglycerate (2,3-BPG).



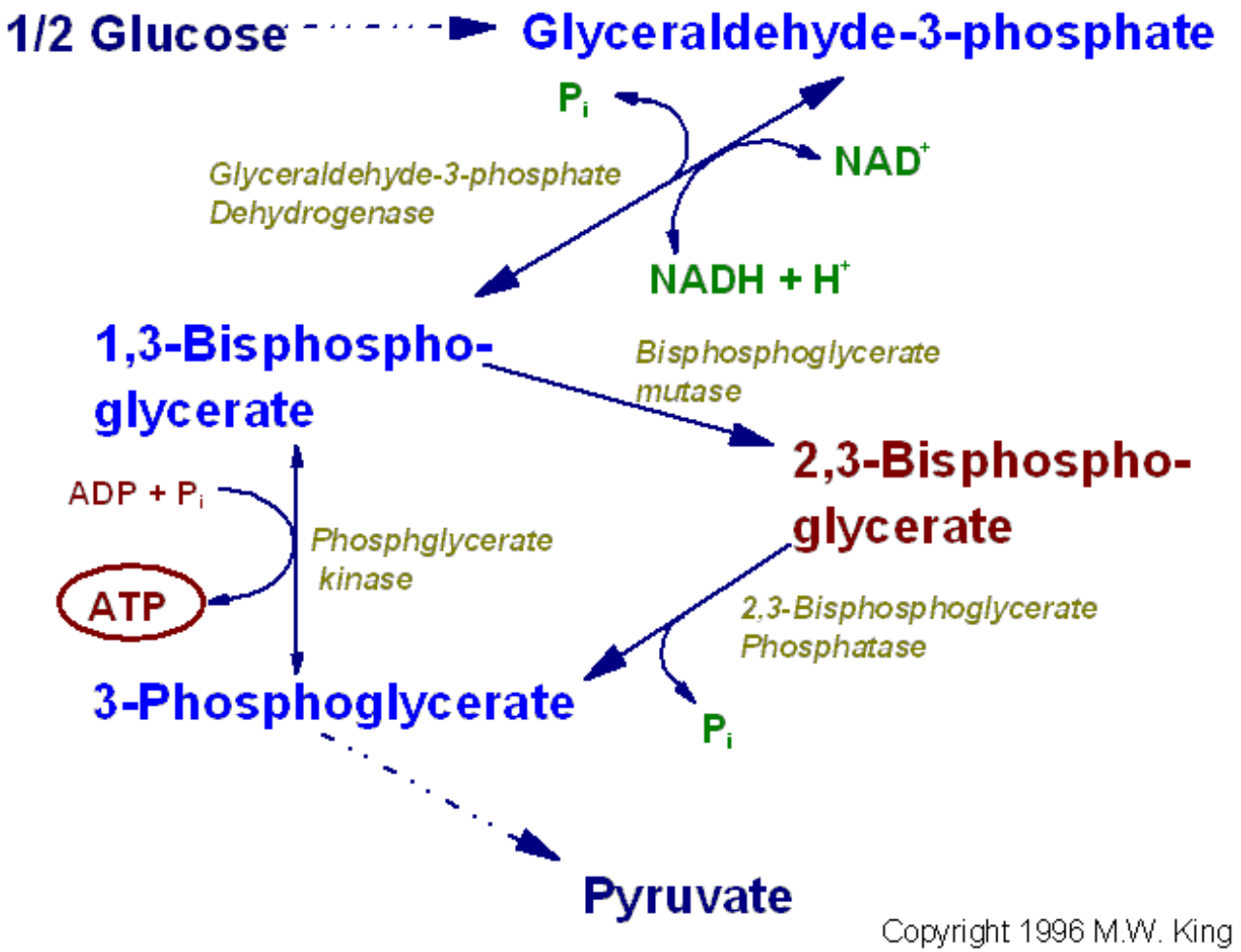
2. Pentose phosphate pathway: glucose is phosphorylated to glucose 6-phosphate and then converted to 5-carbon sugars.

- The purpose of this reaction is to produce NADPH and 5-carbon sugars which are important in DNA synthesis.

2,3-bisphosphoglycerate (2,3-BPG)

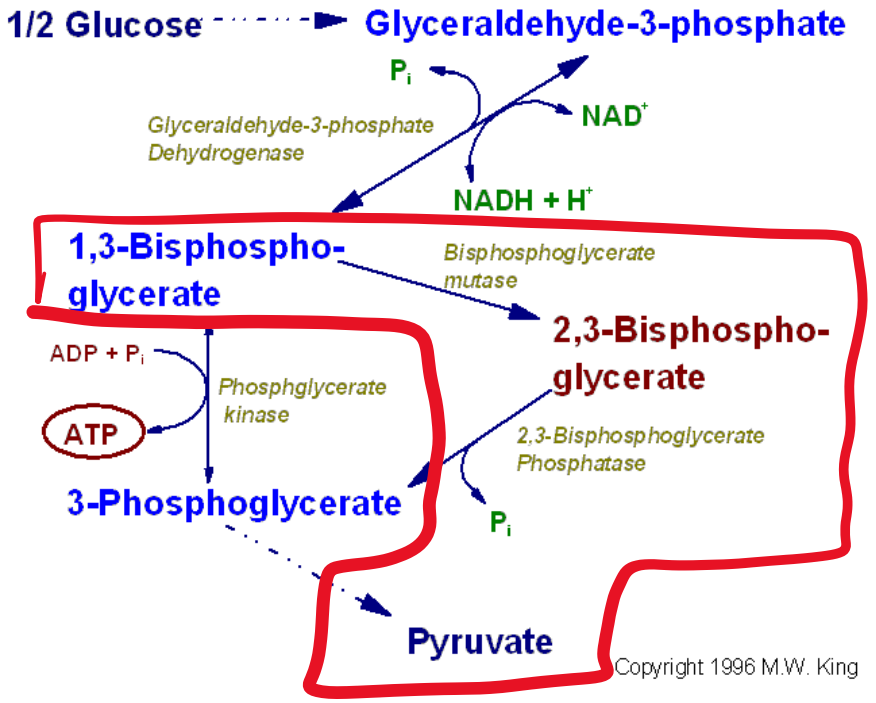
Generation of 2,3-BPG

- 2,3-bisphosphoglycerate (2,3-BPG) is derived in small amounts from the glycolytic intermediate 1,3-bisphosphoglycerate.
- It can re-enter the glycolytic pathway.
  - The erythrocyte loses 2 ATPs.



- Glucose would be broken up into 2 molecules of glyceraldehyde 3-phosphate. This molecule will be phosphorylated, resulting in 1,3-bisphosphoglycerate. Then, by a kinase enzyme, phosphate group will be removed from 1,3-bisphosphoglycerate, resulting in 3-phosphoglycerate and the phosphate group will transfer to ADP, making ATP.

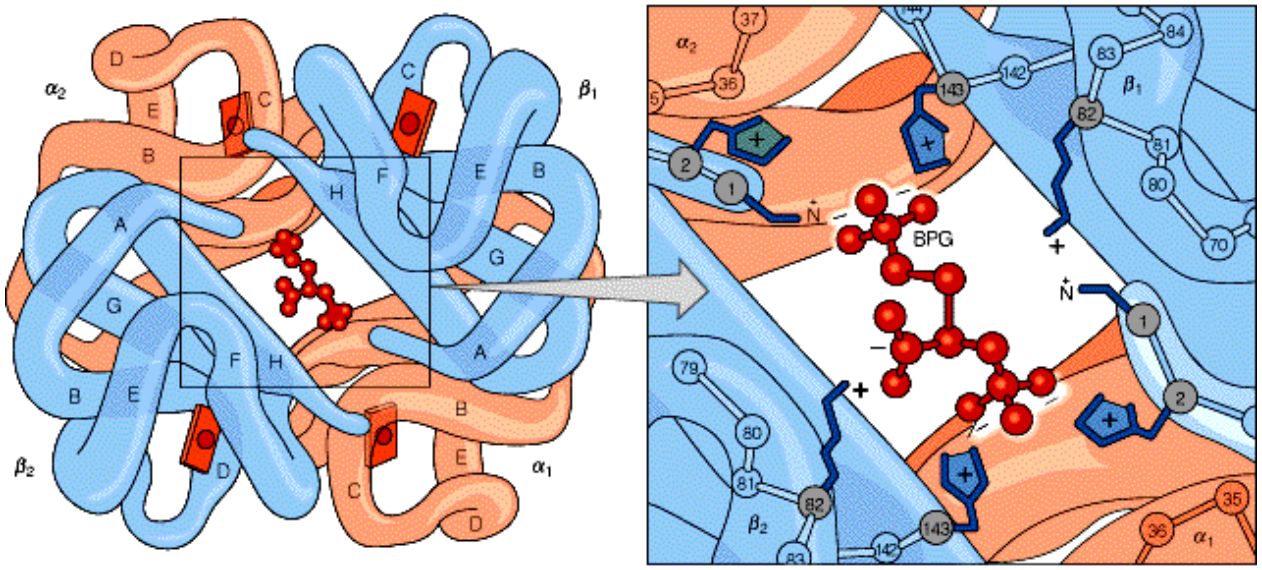
- 1,3-bisphosphoglycerate may be isomerized to 2,3-bisphosphoglycerate by transferring the phosphate group from C1 to C2. Then, this 2,3-BFG is dephosphorylated, producing 3-phosphoglycerate, but this reaction does not produce ATP, and this is what happens in erythrocytes so that erythrocyte loses 2 ATPs. [In erythrocytes , the conversion of 1,3-BPG to 2,3-BPG and then to 3-PG results in a net loss of 2 ATPs during glycolysis because the dephosphorylation step does not generate ATP].

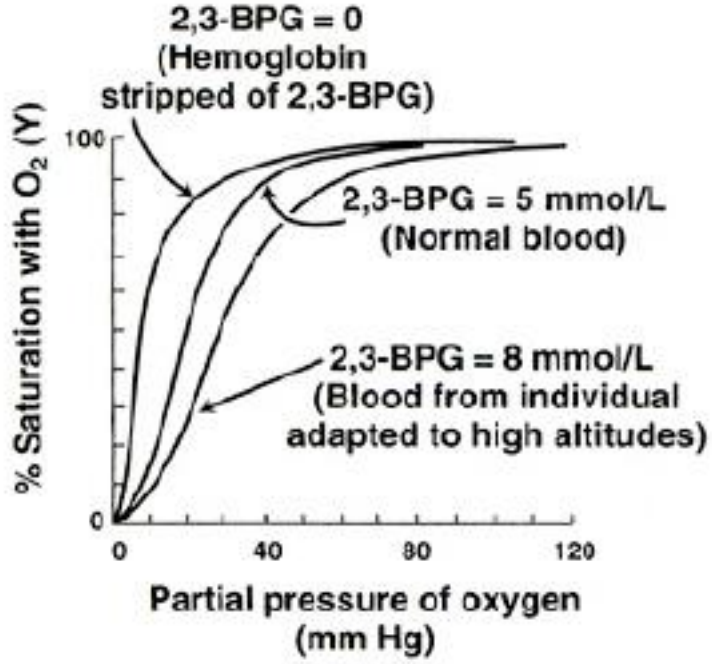


NO ATP is generated

Effect of 2,3-BPG on Hb

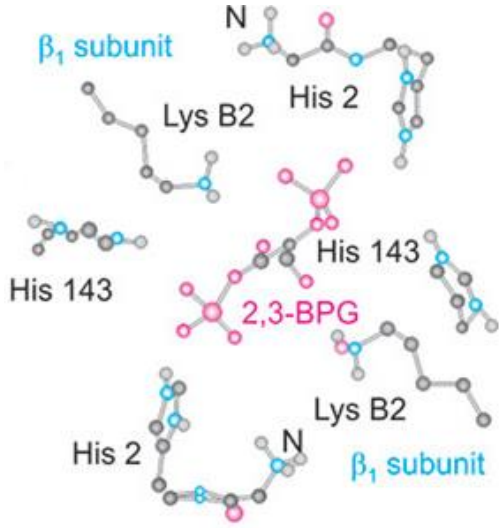
- 2,3-BPG occupies the center of deoxygenated Hb stabilizing it in the T structure.
- When 2,3-BPG is not available (not bound), Hb can be easily converted to the R-structure.

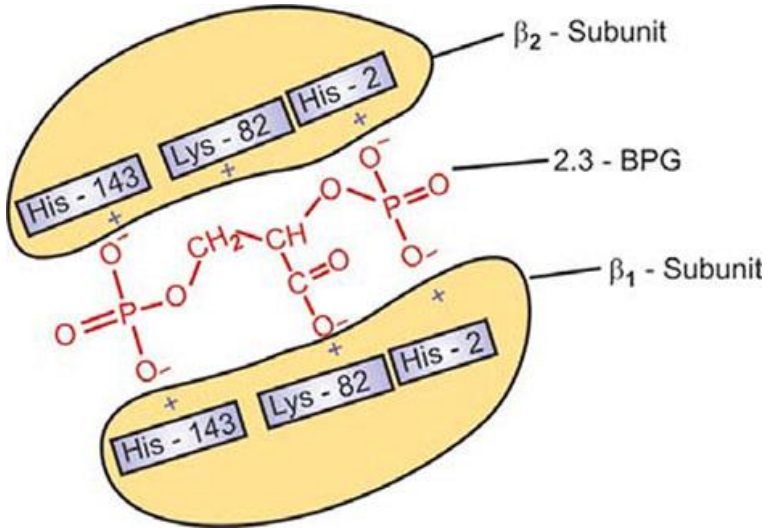




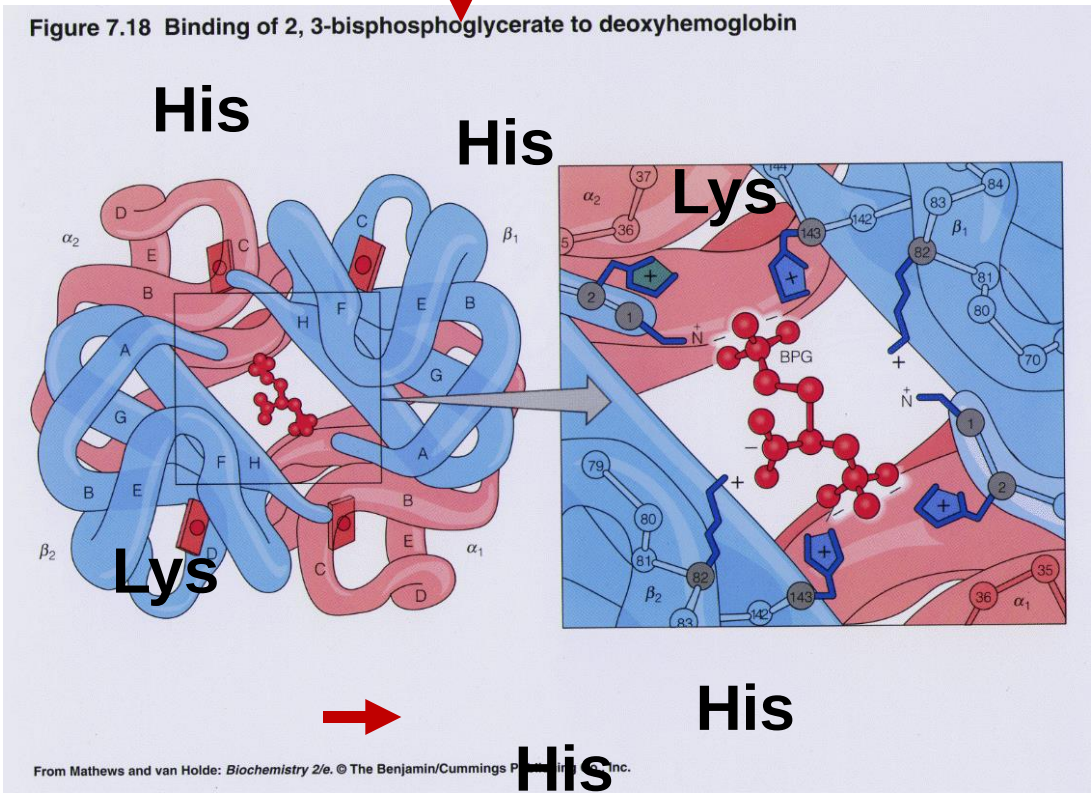
2,3-BPG and HbF

- 2,3-BPG interacts with several groups including His143.
- Fetal hemoglobin (HbF) binds 2,3-BPG much less strongly than HbA.
- Why?





His143 is replaced by a serine in the γ chain.



- Remember that one molecule of 2,3-BPG binds to one Hb molecule, the binding is in the middle of hemoglobin.
- Because 2,3-BPG molecule has a lot of negative charges it will make hydrostatic interactions with amino acid residues around it (specifically with His143 amino acid on beta chain). These electrostatic interactions stabilize the Hb molecule in T-state (low Hb-O2 affinity) so 2,3-BPG decreases Hb affinity to oxygen helping Hb to release oxygen in tissues.

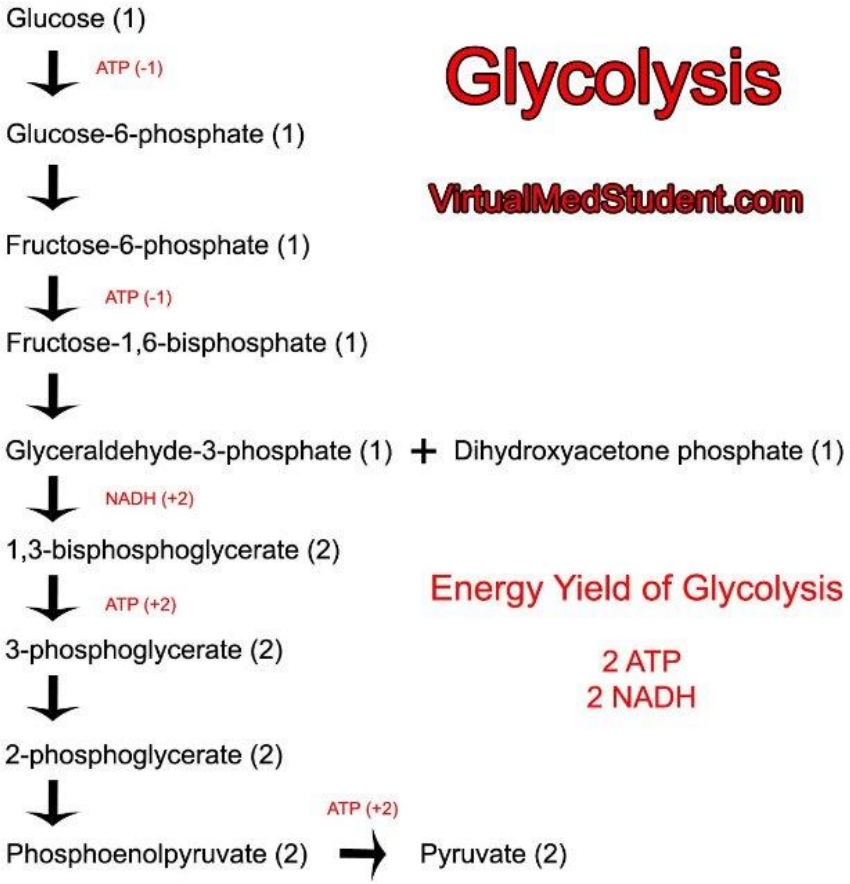
- The normal amount of 2,3-BPG is 5mmol/L.
- If 2,3-BPG was zero, Hb molecule will have really high affinity to oxygen.
- People living at high altitudes adapt by increasing 2,3-BPG levels, which decreases hemoglobin's affinity for oxygen and enhances oxygen release to the tissues (we took that in details in lecture 2).

- Remember fetal hemoglobin has a higher affinity towards oxygen , because gamma globin molecule in fetal hemoglobin doesn't have His143 (which makes electrostatic interactions with 2,3-BPG stabilizing Hb in T-state). Instead of His143, fetal hemoglobin has serine, and the absence of His143 results in a much higher oxygen affinity compared to adult hemoglobin (HbA1).[because the absence of His143 eliminates the electrostatic interactions with 2,3-BPG, preventing stabilization of the T-state and allowing fetal hemoglobin to stay more easily in the oxygen-binding R-state].

Remember glycolysis pathway

The First carbohydrate metabolic pathway in RBCs :

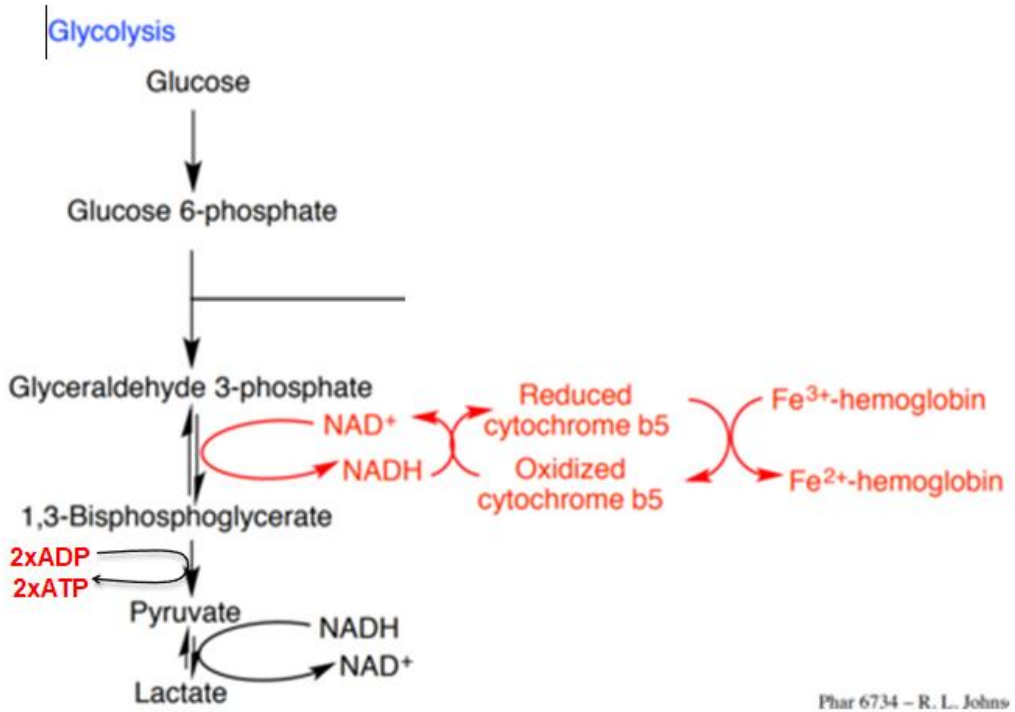
1. Glycolysis

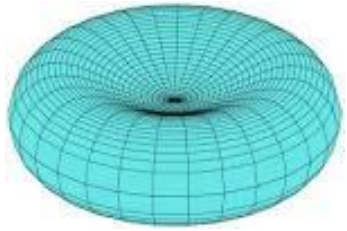


Main purpose

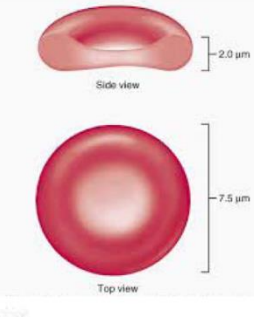
- Glycolysis provides

1. NADH for reduction of methemoglobin (hemoglobin with oxidized Fe^{3+} in heme)
2. ATP for
    - Modifying sugars and proteins
    - Maintaining membrane asymmetry (outer leaflet is different from inner leaflet of the membrane).
    - Functioning of membrane ion pumps
    - Regulating cytoskeletal proteins(which use ATP to alter the shape of the cells, enabling them to move and squeez through blood vessels).
        - Maintenance of the discocytic shape, w which is critical for the optimal viability and functional capacity.





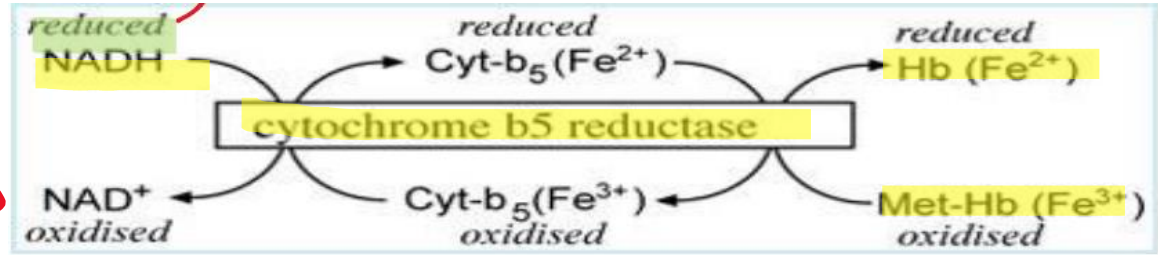
Discocyte



- Glycolysis is an important anaerobic metabolic pathway that converts glucose into pyruvate, producing 2 ATP molecules and regenerating NADH in the process. Also, 2,3-bisphosphoglycerate is formed as a byproduct.

- Two important products result from glycolysis: 2 ATP molecules and NADH.
- NADH is important because it prevents the formation of methemoglobin (hemoglobin with oxidized Fe3+ in heme) by supporting the action of the enzyme methemoglobin reductase. This enzyme uses NADH to reduce Ferric iron (Fe^{3+}) in methemoglobin, converting it back into functional hemoglobin.

Remember from lecture 3:
Methemoglobin reductase requires NADH to perform its function.
NADH is produced by glycolysis in RBC.



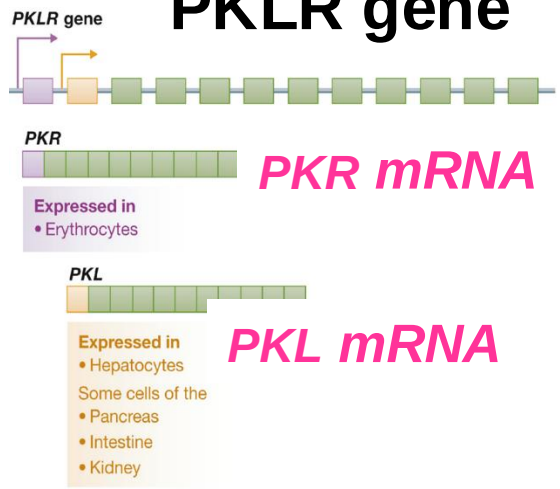
Methemoglobin reductase AKA
NADH-Cytochrome b5 reductase

Pyruvate kinase isozymes and regulation

- There are two <u>isoenzyme</u> genes of PK and each produces two isoforms:
  - PKLR gene produces PK**L** (liver) and PK**R** (erythrocytes) using different transcription start sites.
  - PKM gene produces PKM1 (muscle and brain) and PKM2 (fetal and most tissues) by alternative splicing.
- 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.*

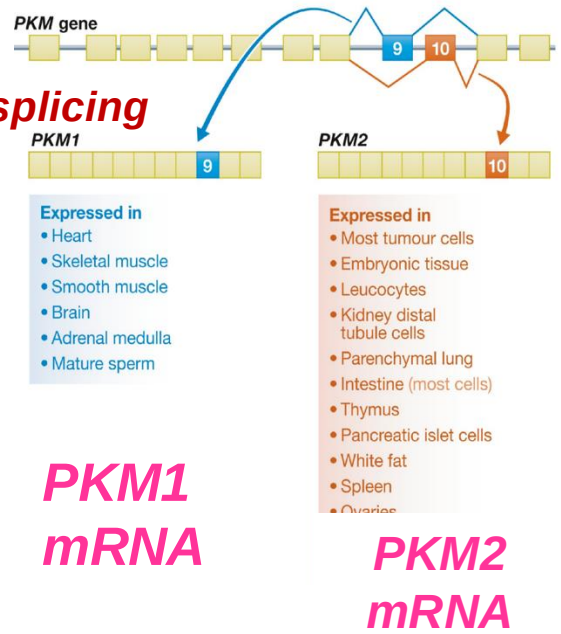
Different transcription start sites

PKLR gene



PKM gene

Alternative splicing



❖ The final reaction in the glycolytic pathway involves the dephosphorylation of phosphoenolpyruvate resulting in pyruvate. The released phosphate group is transferred to ADP, forming ATP. This reaction is catalyzed by the enzyme pyruvate kinase (PK) enzyme.

❖ There are two isoenzymes of pyruvate kinase (PK) [isoenzymes are enzymes that catalyze the same reaction, using the same substrate and producing the same product, but they are encoded by different genes, exhibit different kinetics, regulated differently, and can be expressed in various tissues]. And each isoenzyme can have two isoforms.

❖ There are two isoenzyme genes of PK and each produces two isoforms:
- The two isoenzymes of pyruvate kinase are produced by the PKLR and PKM genes.
- The PKLR gene gives rise to two isoforms, PKL (liver) and PKR (erythrocytes).
- The PKM gene also produces two isoforms, PKM1 (muscle and brain) and PKM2 (fetal and most tissues).

❖ 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 exon.
➢ While in PKM there is an 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.

- The fetal pyruvate kinase isozyme (PKM2) has significantly higher activity than the adult isozymes (PKLR and PKM1). This causes the final reaction in glycolysis to proceed rapidly, which in turn pulls all preceding reactions forward (فرطت المسبحة), leading to a decrease in intermediates such as 1,3-BPG , PEP, 2,3-BPG and glucose. As a result, the levels of 2,3-BPG (2,3-bisphosphoglycerate) in fetal tissues are lower than in adult tissues. This lower concentration of 2,3-BPG is another reason why fetal hemoglobin has a much higher affinity for oxygen compared to adult hemoglobin.
- [Remember the first reason was >> in fetal hemoglobin (HbF), the gamma globin chain lacks the His143 residue found in adult hemoglobin (HbA1), which plays a crucial role in forming electrostatic interactions with 2,3-BPG to stabilize the deoxygenated T-state of hemoglobin. Instead of His143, HbF contains serine, which does not interact with 2,3-BPG in the same way. This absence of His143 contributes to a significantly higher affinity for oxygen in fetal hemoglobin compared to adult hemoglobin].

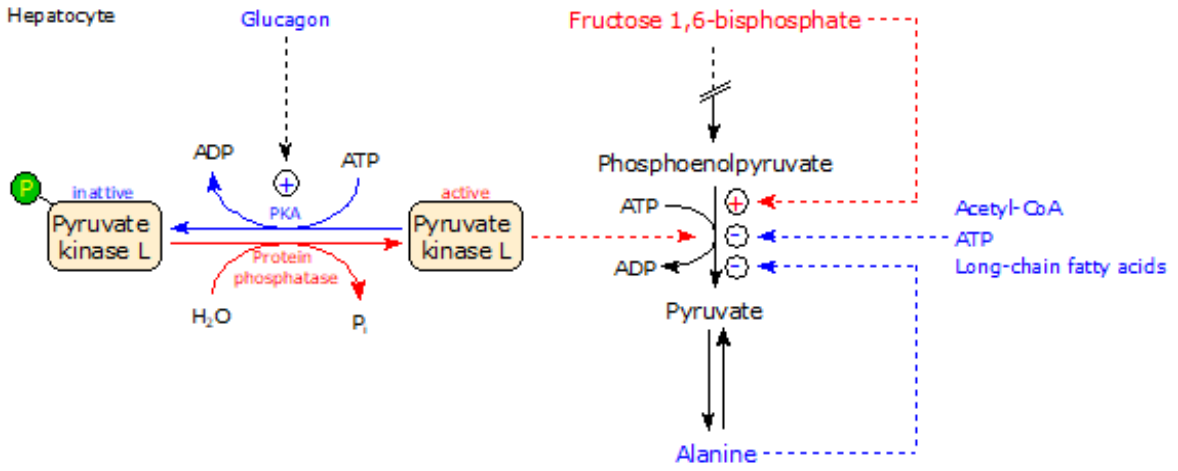
- To recap: Fetus increases his affinity towards O2 by two ways:

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

- 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 F1,6-BP.
- The liver (PKL) gene is also controlled at the level of synthesis.
  - Increased carbohydrate ingestion induces the synthesis of PKL.

- Note: When we have a crucial enzyme, it will be regulated at multiple levels, including transcriptional, post-transcriptional, translational, and post-translational levels.



➢ We do not want the liver to consume glucose through glycolysis, especially in situations where there is no glucose available in the tissues. In this case, pyruvate kinase in the liver may remain active.

The liver isoenzyme (PKL) is allosterically regulated by several factors:

✋ It is inhibited by high concentrations of:

1. ATP: indicates sufficient energy, reducing glucose metabolism.
2. Acetyl-CoA: Suggests reliance on fat for energy, prompting glucose conservation.
3. Alanine: High levels of alanine indicate that the body is in a state of amino acid catabolism rather than glucose metabolism, prompting the inhibition of pyruvate kinase.
4. Long-Chain Fatty Acids: Indicates fat utilization for energy, inhibiting glucose consumption.
5. Phosphorylation by Protein Kinase A: When protein kinase A phosphorylates pyruvate kinase, it typically leads to the enzyme's inactivation. This phosphorylation is often part of a broader signaling response to low insulin levels or high glucagon levels, indicating that the liver should conserve glucose

😊 It is activated by:

- fructose-1,6-bisphosphate (F1,6-BP), which indicates a low energy status in the cells promoting glucose metabolism to meet energy needs.

✓ Remember that any text in this colour is additional information for further understanding.

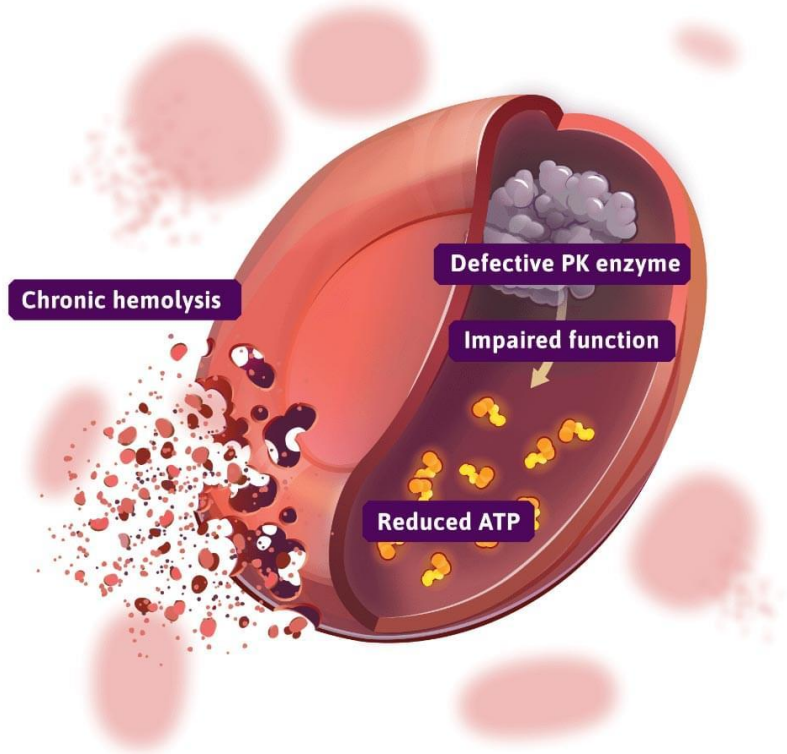
Conditions related to PK deficiency:

1) They are hereditary
2) The severity of the disease depend on "mutations" :

a) the degree of enzyme deficiency: for example if the enzyme activity is 5% of normal levels, the person will be symptomatic. (the person can survive, but with symptoms)

It can never be 0% (the person will not survive, because PK produce energy, and energy is important for living)

b) The ability to produce 2,3 BPG, (will affect the Hb affinity to O2)



The pathogenesis of these conditions:

the adult erythrocyte PK is virtually inactive (not totally, but the activity is very low) --> and this will affect the erythrocyte (they will not be able to produce ATP at sufficient levels causing cell damage).

REMEMBER the importance of ATP in maintaining the integrity of RBC cell membrane, shape, cytoskeletal proteins, ion pumps. (so if we have PK deficiency, RBC will not produce sufficient amount of ATP and that will cause hemolytic anemia,

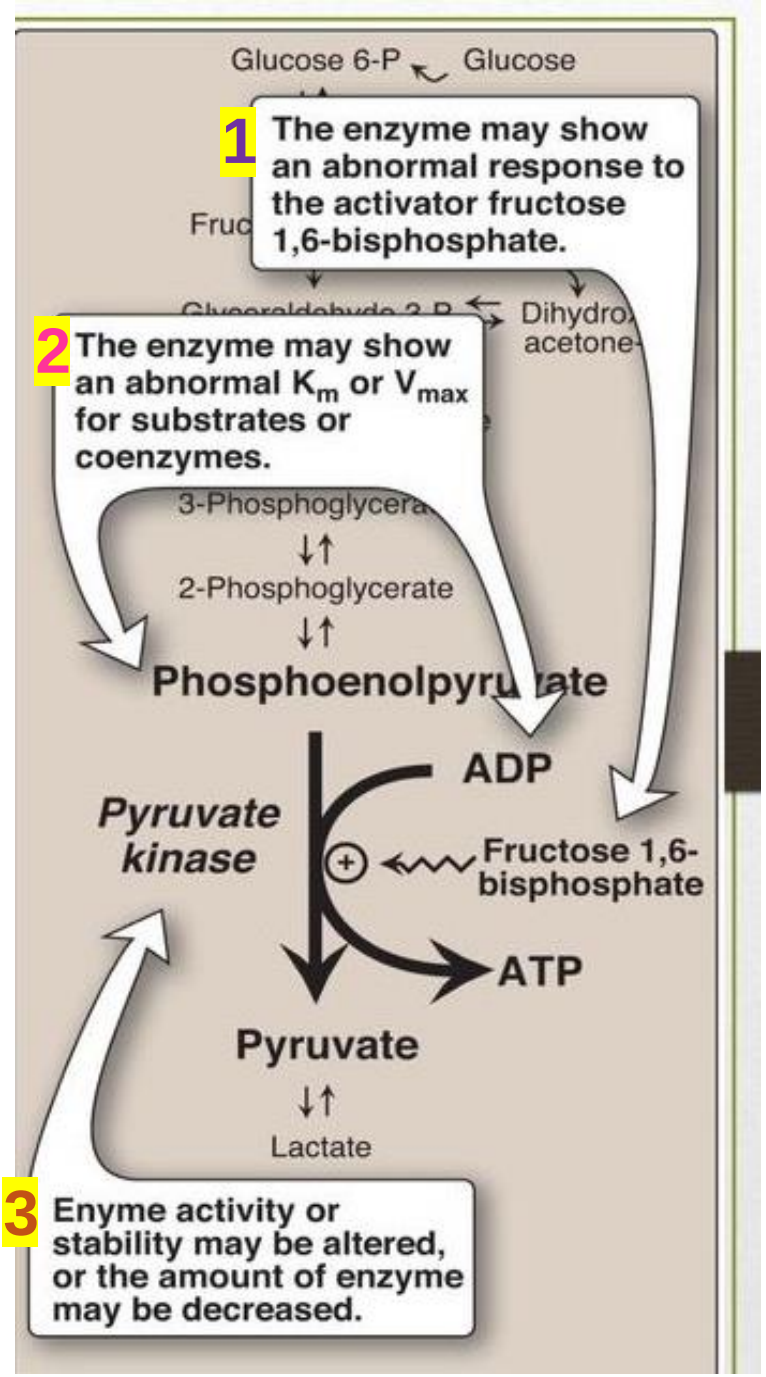
Alterations observed in PK

Different mutations have been identified that affect the PK enzyme:

1) Abnormal response to the activator (Fructose1,6-bisphosphate). they may not respond well to the presence of it, so the enzyme will not be as active as in normal cell)

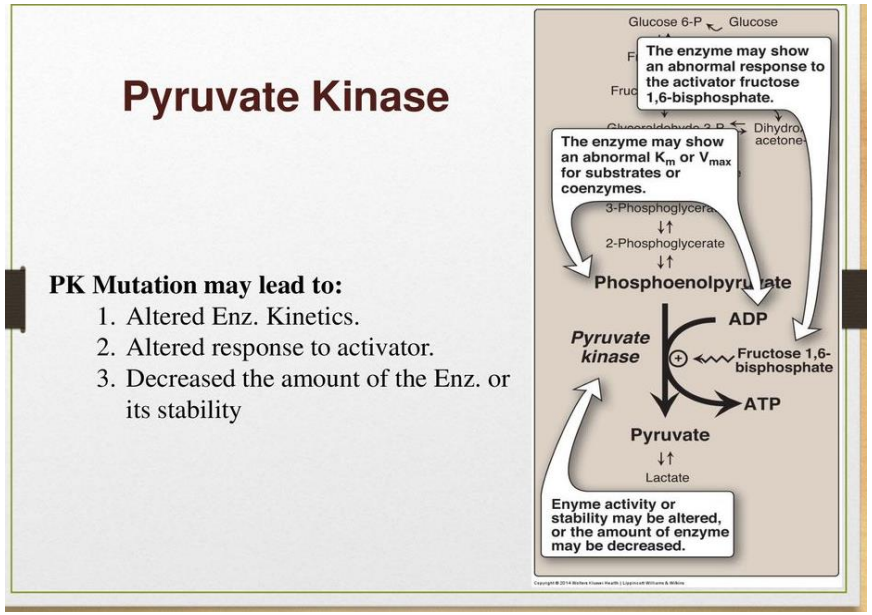
2) Abnormal affinity (Km) or Vmax (catalytic activity) for substrates or coenzymes.
-Substrates: Phosphoenolpyruvate + ADP

3) Enzyme activity or stability may be altered, or the amount of enzyme may be compromised (lower than normal)



Genetic diseases of PK deficiency

- The adult erythrocyte PK is virtually inactive.
  - Reduced capacity to make ATP → hereditary hemolytic anemia
- The severity of the disease depends on
  - The degree of enzyme deficiency (5-35%)
  - The ability to produce 2,3-BPG.
- The liver is not affected since expression is stimulated.
- Patients are resistant to malaria.



The integrity of RBCs will be affected --> hemolytic anemia --> the person will be resistant to malaria.
(because the parasites life cycle happens inside the RBC, so in hemolytic anemia the parasite will not complete their cycle because of hemolysis and premature destruction of RBC
(as explained in hemoglobinopathies lec)

The other carbohydrate metabolic pathway in RBC :

2. The pentose phosphate pathway

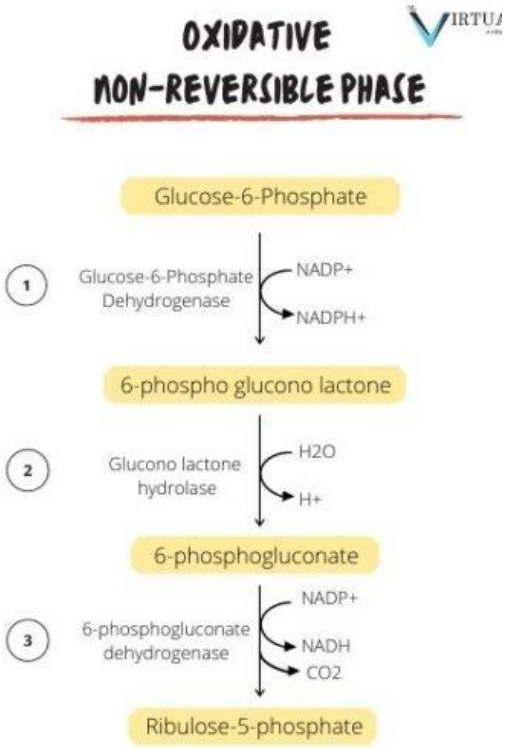
Why it's important in RBCs ?

Note: Everything written in this slide is from doctor but in different context, (with external images)

REMEMBER: The pentose phosphate pathway (PPP) consists of two main phases:

1. Oxidative Phase:
Function: This phase generates NADPH and ribose-5-phosphate.
Key Steps:
Glucose-6-phosphate is oxidized, producing NADPH and 6-phosphoglucono-S-lactone. 6-phosphoglucono-S-lactone is further oxidized to ribulose-5-phosphate (5 carbon"pentose" sugar), producing another NADPH in the process.

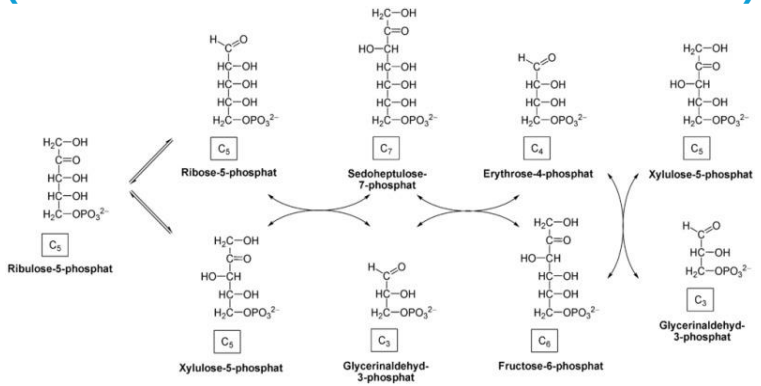
End Products: NADPH (used in biosynthetic reactions and for combating oxidative stress) and ribulose-5-phosphate.
what's important in this reaction for RBCs is: the production of NADPH



*All reactions are irreversible.
*step 1 is the major rate- limiting step.
*oxidative phase is controlled by level of NADP+

non oxidative phase

(remember from metabolism with Dr.Diala)



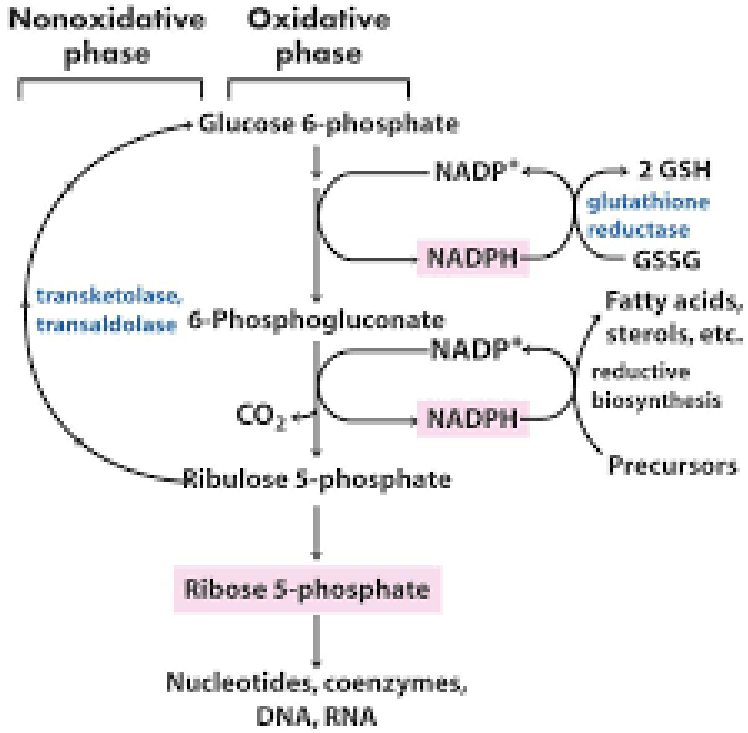
2. Non-Oxidative Phase:
Function: This phase is involved in the interconversion of sugar phosphates and the production of ribose-5-phosphate (pentose sugar) for nucleotide, DNA and RNA synthesis in different cells

Two phases of pentose phosphate pathway

- The oxidative generation of NADPH
  - NADPH is generated when glucose 6-phosphate is oxidized to ribulose 5-phosphate.

$$\text{Glucose 6-phosphate} + 2\,\text{NADP}^+ + \text{H}_2\text{O} \longrightarrow \text{ribose 5-phosphate} + 2\,\text{NADPH} + 2\,\text{H}^+ + \text{CO}_2$$

- The non-oxidative interconversion of sugars



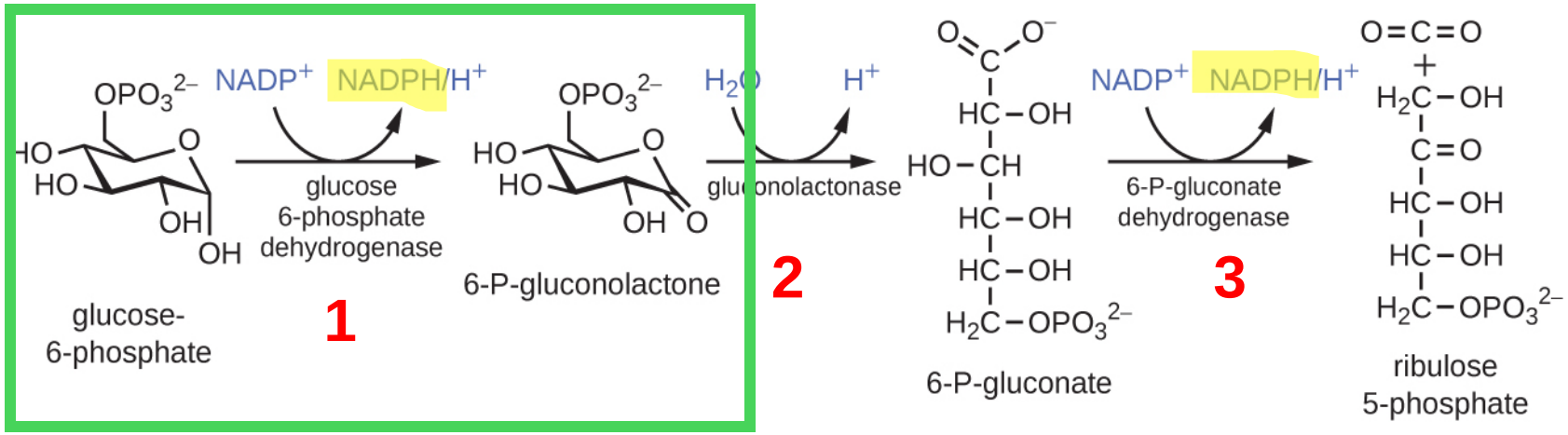
How is this reaction (the production of NADPH) catalyzed ?

1) first of all, the glu-6-phosphate is oxidized to 6-P-gluconolactone

2) 6-P-glucolactone is converted to 6-P-gluconate.

3) 6-P-gluconate is oxidized to ribulose-5-phosphate (a 5 carbon sugar).

(1+3): in these 2 reactions, NADPH will be produced.

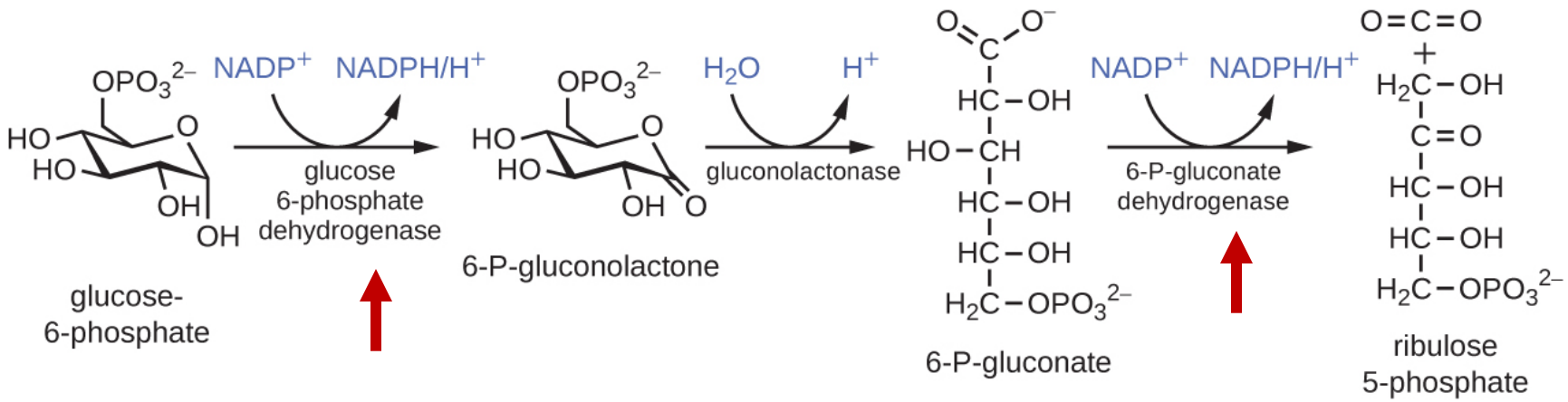


the first reaction : (the rectangle)

- **Is the rate limiting reaction.**
- **it's highly regulated.**
- **catalyzed by the enzyme G6PD.**
- **It's an impoerant reaction**

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).



- 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 . (it indicate that cells need NADPH, why cells need NADPH? The answer in the next slides)

why we need NADPH?
-remember that RBCs have a huge amount of O2, and it may converted to reactive oxygen species (free radicals and hydrogen peroxide H2O2).

-RBCs produce H2O2 that would damage membranes, so cells will rupture and die (hemolysis).

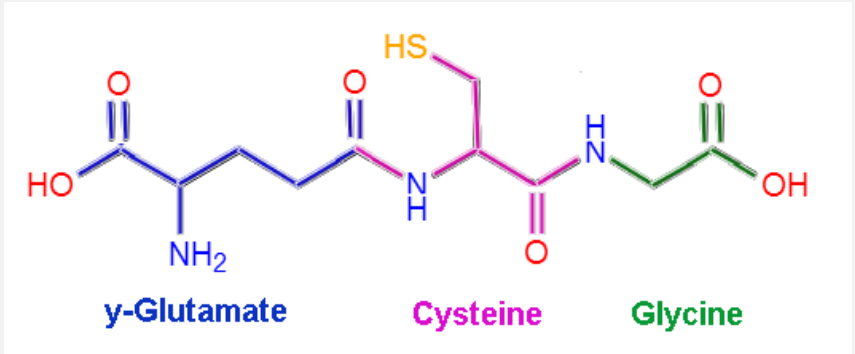
The solution:

- RBCs have glutathione (notice the structure at the right).

- 2 molecules of GSH will donate electrons for H2O2, converting it to water and oxygen protecting the RBCs. (this reaction is catalyzed by GSH peroxidase).

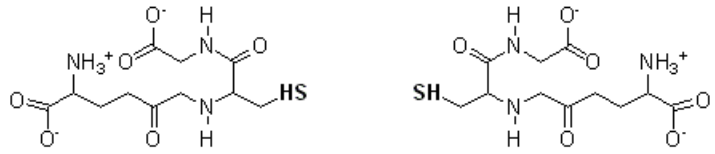
- regeneration of reduced GSH molecules, using NADPH (as a source for electrons), so electrons will transfer to the oxidized form GSSG producing the reduced form GSH, and NADPH will be oxidized to NADP+. (catalyzed by GSH reductase). GSH is important and it has to be regenerated. if it's not regenerated, RBCs would have a huge amount of H2O2, and that would cause hemolysis.

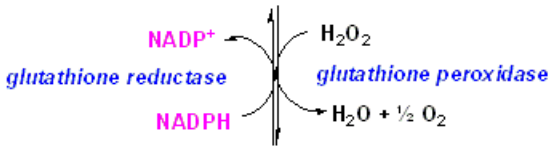
REMEMBER the glutathione structure:

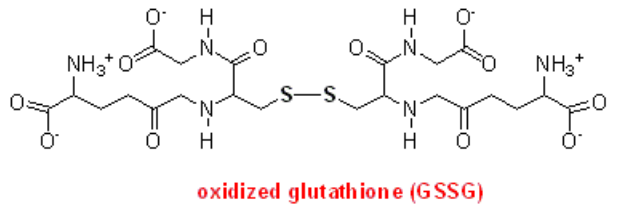


- GSH is a tripeptide: GLU , CYS, GLY.

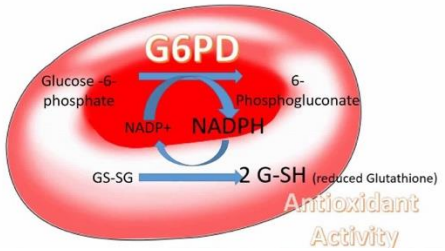
- notice the thiol group in CYS.







Oxidative stress and glutathione

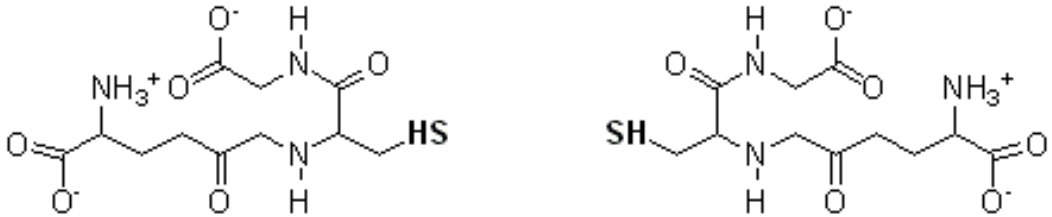


- Oxidative stress within cells is controlled by the action of glutathione (GSH).

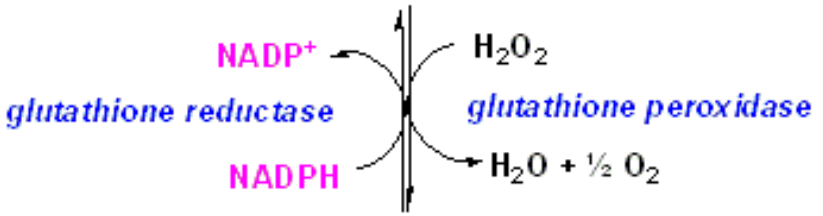
- GSH reduces peroxides via glutathione peroxidase.
- GSH is regenerated via NADPH-dependent glutathione reductase.
- The PPP in erythrocytes is the only pathway to produce NADPH.

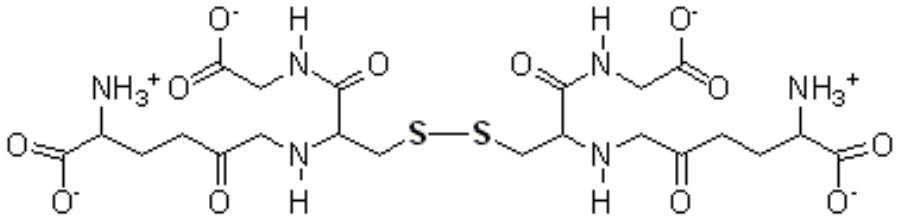
  PPP consumes almost 10% of glucose by erythrocytes.

  (to produce NADPH)



2 x reduced glutathione (GSH)





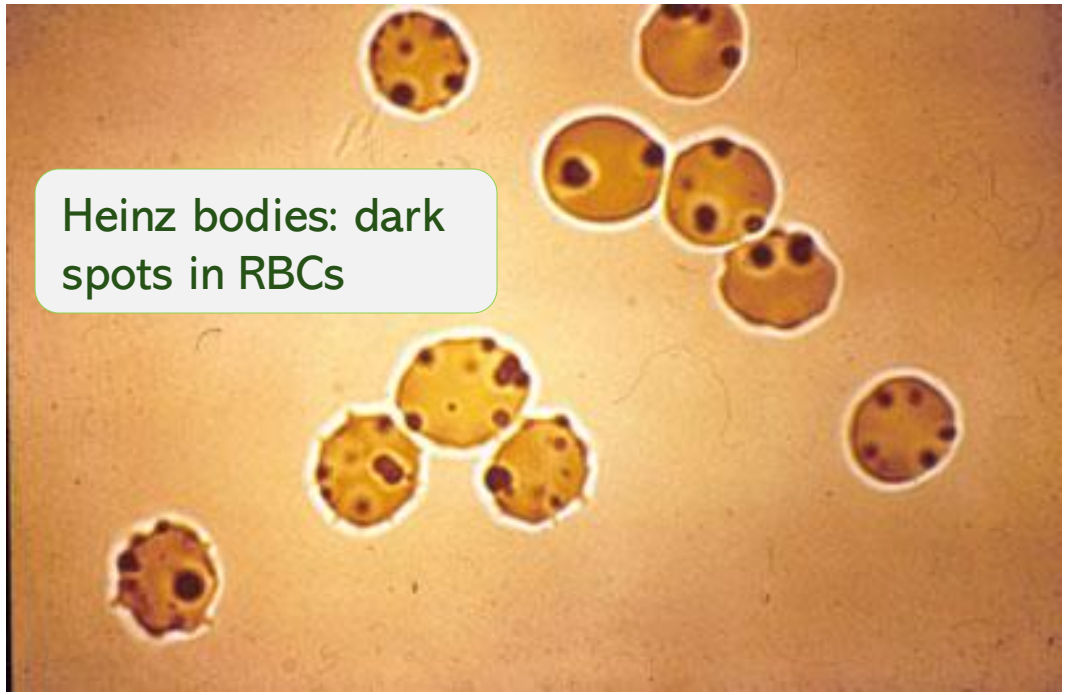
oxidized glutathione (GSSG)

Low GSH levels

- The inability to maintain reduced glutathione in RBCs leads to increased accumulation of peroxides, predominantly H_2O_2, resulting in weakening of the cell membrane due to:

The effects of high level of H2O2 in RBCs:

1. peroxidizing membrane lipids leading to hemolysis
2. oxidizing proteins including: hemoglobin (to methemoglobin) and membrane proteins, insolubilizing them, and forming Heinz bodies



In addition to damaging the cell membrane, H2O2 also damage the proteins, eg) Hemoglobin (the predominant protein in RBCs) oxidizing it to produce the Methemoglobin (Hb-Fe3+), resulting in clustering, aggregation of these damaged proteins (mainly hemoglobin), forming HEINZ BODIES

Glucose-6-phosphate dehydrogenase deficiency

Is a group of heterogenous disease (The term "heterogeneous disease" refers to a group of diseases that have diverse causes, characteristics, and clinical manifestations. This means that the diseases within this group can vary widely in terms of their underlying mechanisms, symptoms, progression, and responses to treatment.). But overall we have reduced activity of this enzyme resulting in hemolytic anemia.

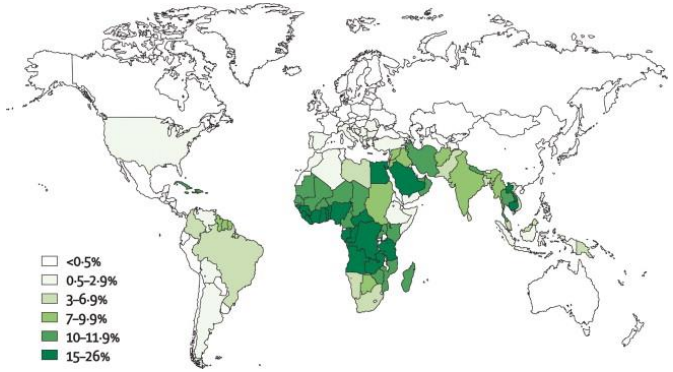
G6PD deficiency

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a group of heterogeneous disease with significantly reduced activity.
  - Hemolytic anemia
    - particularly after the administration of drugs, during infections and in the neonatal period (jaundice)

Because of large amount of hemoglobin released from RBCs hemolysis, and converting hemoglobin into bilirubin, resulting in jaundice

- Deficiency of G6PD is most prevalent in individuals of African, Mediterranean, and Oriental ethnic origins.
- It is the most common enzyme deficiency worldwide.

This disease is prevalent in old world (Africa, Middle east and Asia)
Common in Jordan (more than other Eastren countries)



- G6PD gene is located on the X chromosome.
  - Inheritance of G6PD deficiency is sex-linked.

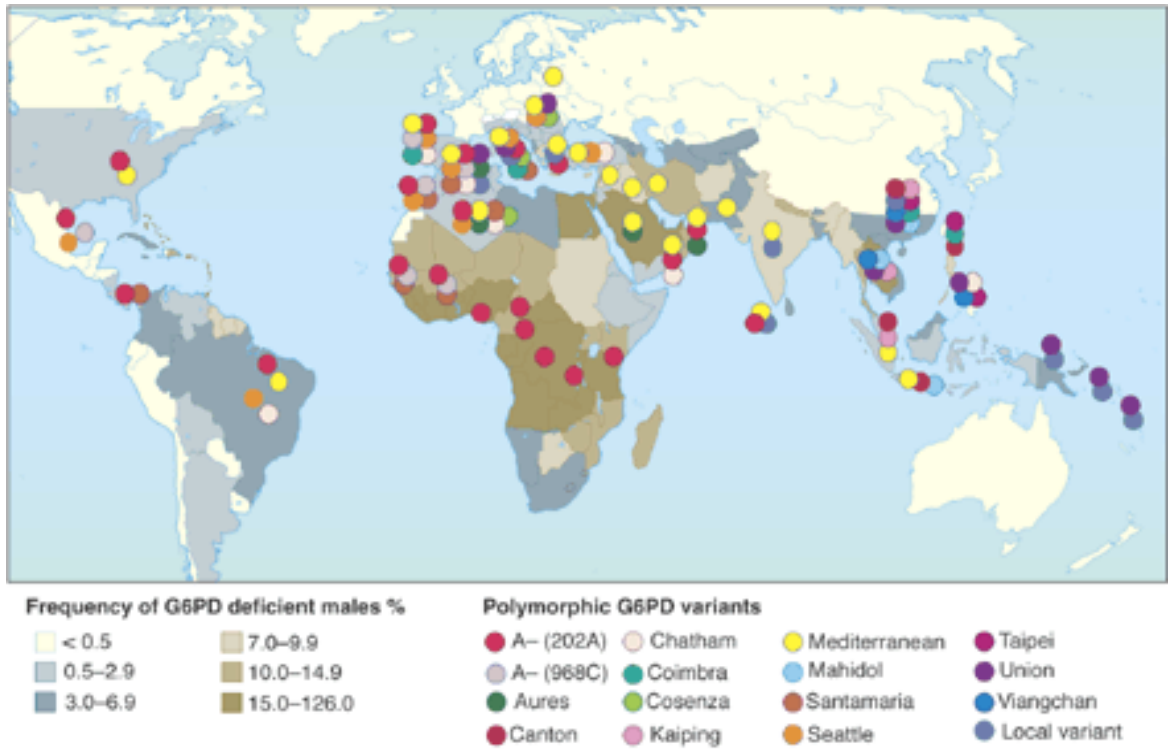
More common in males

There are many variants of the disease, (African, Mediterranean, and so on).
We will talk about these variants in the next slides.

Mutations can exist anywhere in the protein:
1) usually these mutations have little effect, because it's not nessessory for enzyme activity to be100% to work effectively.

eg) if the enzyme activity was 40% it's fine! There will not be any symptoms.

2) but some mutations can have an effect. (symptomatic), they alter:
- the kinetic properties
- the stability of the enzyme
-the ability to bind substrates (NADP+ or G6P)



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.
  - These mutations mainly alter the kinetic properties, stability, or binding affinity to $NADP^+$ or G6P.
- No large deletions or frameshift mutations. Why?

Because the effect of deletion mutation is huge, and that would really affect the human being, resulting in Stillbirth! Because this gene is critical for living.

The four classes of G6PD deficiency

- G6PD B (Normal) they named as "B"
- Abnormal G6PDs
  1. Class I: the most severe and rare.
  4. Class IV: no clinical symptoms
  3. G6PD A- (group III or class III)
     - 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 disease is moderate.
  2. G6PD Mediterranean (group II or class II)
     - Severe
     - The enzyme has normal stability, but negligible activity.

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%

2+3 classes are the most important

Less stability
Normal activity

Notice that even when the enzyme activity is 10% of normal activity, the person is fine ! The disease is moderate.

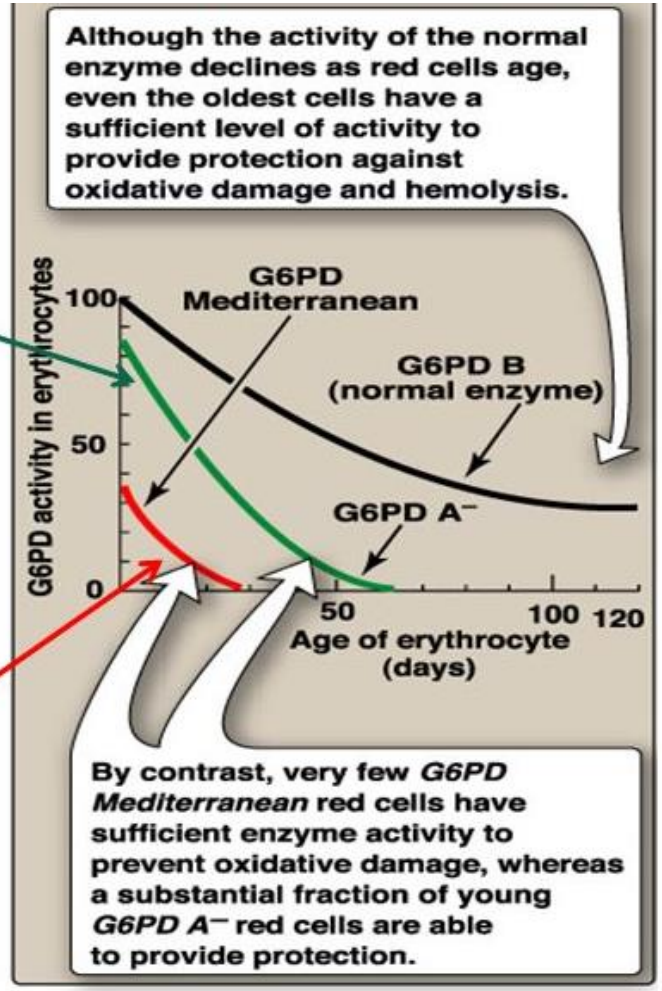
Normal stability
negligible activity

- Common in Jordan and Mediterranean region
- Non existed activity

Class II vs. class III

G6PD A- (class III):
Moderate, young RBCs contain enzymatic activity.
Unstable enzyme, but kinetically normal

G6PD Mediterranean (II)
Enzyme with normal stability but low activity (severe).
Affect all RBCs (both young and old)



As you see in the figure:
- the activity of G6PD declines with age of erythrocyte

- G6PD B:

The life span of RBC is 120.
At day 120 (the oldest cell), even the activity of the enzyme is < 50%, but it's fine.

- G6PD A- :

the activity of the enzyme is reduced at younger age (the life span is about 50-60 days).

- G6PD mediterranean: هون المشكلة

the life span of RBCs is 20-25 days (very very low), that will result in premature death of RBCs.

Inducers of G6PD deficiency symptoms

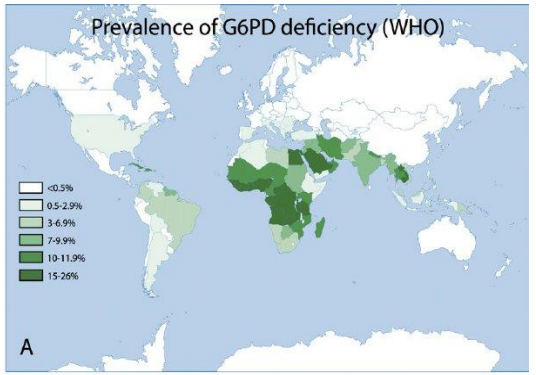
There are external factors that would exaggerate the symptoms of G6PD deficiency. by increasing the oxidative stress, so RBCs can not get rid of them, leading to hemolysis

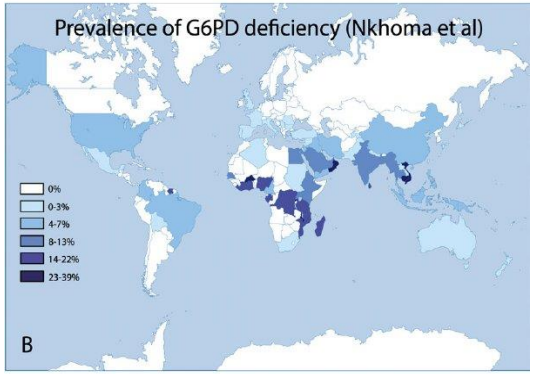
- Oxidant drugs
  - Antibiotics, anti-malarial, and anti-pyretics (not acetaminophen)
- Fava beans (favism) التفول
  - Fava beans are presumed to cause oxidative damage.
  - Substances capable of destroying red cell GSH have been isolated from fava beans (fool).
  - Favism is most common in persons with 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.

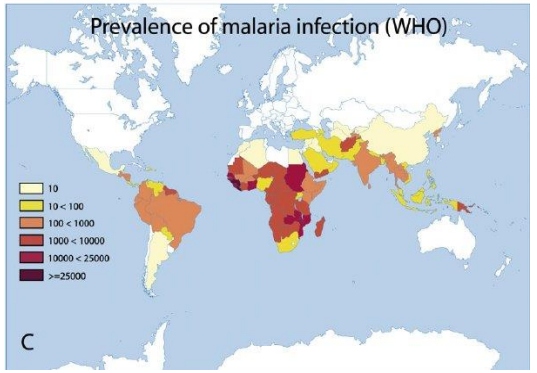
Connection to malaria

Notice the overlapping !
سبحان الله

- 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.







Remember when we talked about the overlap between the distribution of malarial disease and Sickle Cell Anemia, also here we have overlap between the distribution of G6PD deficiency and the prevelance of malaria.

سُبْحانَ اللَّهِ وبِحَمْدِهِ، سُبْحانَ اللَّهِ العَظِيمِ ♡

﴿ إِنَّ اللَّهَ وَمَلَائِكَتَهُ يُصَلُّونَ عَلَى النَّبِيِّ ۚ يَا أَيُّهَا الَّذِينَ آمَنُوا صَلُّوا عَلَيْهِ وَسَلِّمُوا تَسْلِيمًا﴾

VERSIONS	SLIDE #	BEFORE CORRECTION	AFTER CORRECTION
V1→ V2			
V2→V3			

امسح الرمز و شاركنا بأفكارك لتحسين أدائنا !!