

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



Biochemistry

MID | Lecture 4

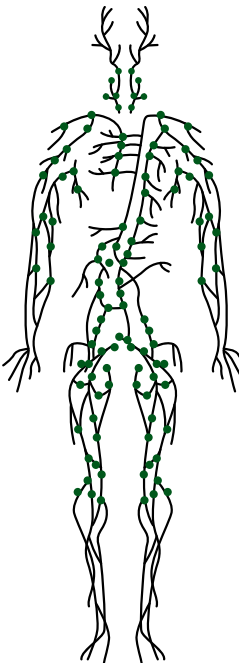
Metabolism of Erythrocytes

﴿وَقُلْ رَبِّ أَدْخِلْنِي مُدْخَلَ صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَلْ لِي مِنْ لَدُنْكَ سُلْطَانًا نَصِيرًا﴾
ربنا آتانا من لَدُنْكَ رَحْمَةً وَهَيِّئْ لَنَا مِنْ أَمْرِنَا رَشَدًا



Written by: Omar Ibrahim

Reviewed by:
Almothana Khalil



Pre-studying Notice

- Dr. Motamed focuses on general concepts, yet important details are pointed and emphasized throughout the file.
- Some originally **green** text is switched to **dark red** to not confuse it with added text.
- Read the full content of the slides; the doctor sometimes explains things in his own words while they are written in the slides, so we may not rewrite them if they are clear. Make sure to read everything.

Carbohydrate metabolism in RBC

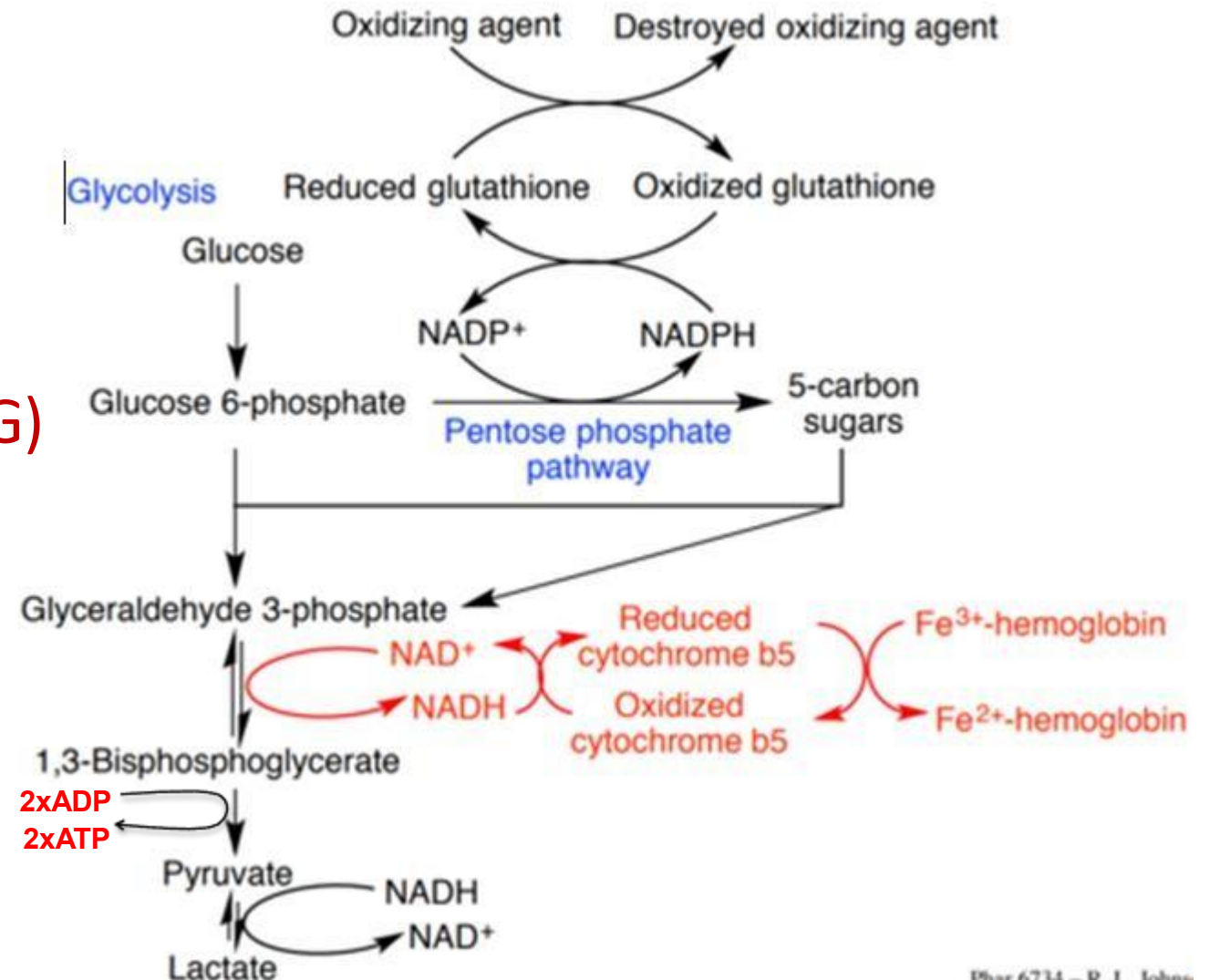
You are expected to know the general overview of glycolysis; relevant details will be pointed out.

- Glycolysis (Directly):

- ATP
- NADH
- Pyruvate Kinase

- Glycolysis (Indirectly):

- 2,3-bisphosphoglycerate (2,3-BPG)
- Pentose phosphate pathway:
 - a) NADPH
 - b) G6PD



These points are virtually THE LECTURE; they will be discussed in detail in the coming slides.

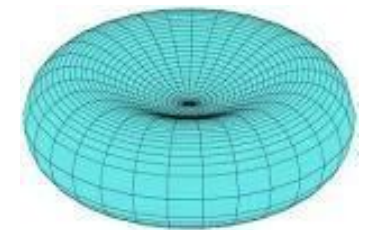
Glycolysis direct contribution:

1 - ATP

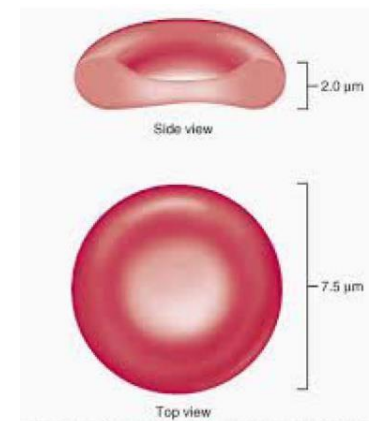
Main purpose

Glycolysis is the only form of ATP production in the RBC, which is used for:

- Modifying sugars and proteins
- Maintaining membrane asymmetry
- Functioning of membrane ion pumps
- Regulating cytoskeletal proteins
 - Maintenance of the discocytic shape, which is critical for the optimal viability and functional capacity.
- The major source of energy



Discocyte

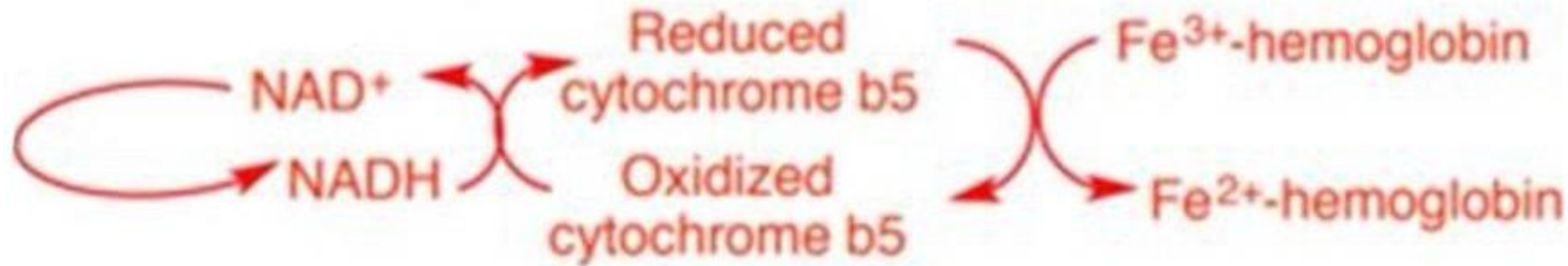


ATP is utilized by the cytoskeletal proteins of the RBC in order to maintain their function; inadequate ATP levels in the RBC would impair the cytoskeleton and lead to the loss of the RBC's flexible discocytic (biconcave) shape → Hemolytic Anemia.

Glycolysis direct contribution:

2- NADH

Glycolysis provides NADH for the reduction of methemoglobin (hemoglobin with an oxidized iron (Fe^{+3}) in heme).



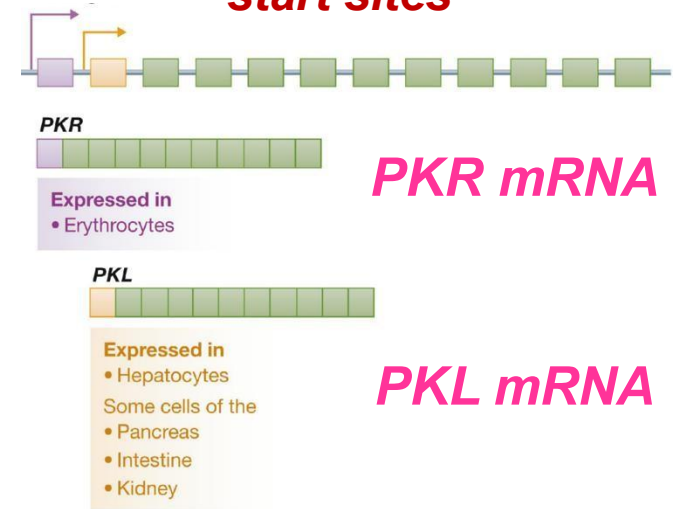
Glycolysis direct contribution:

3- Pyruvate Kinase

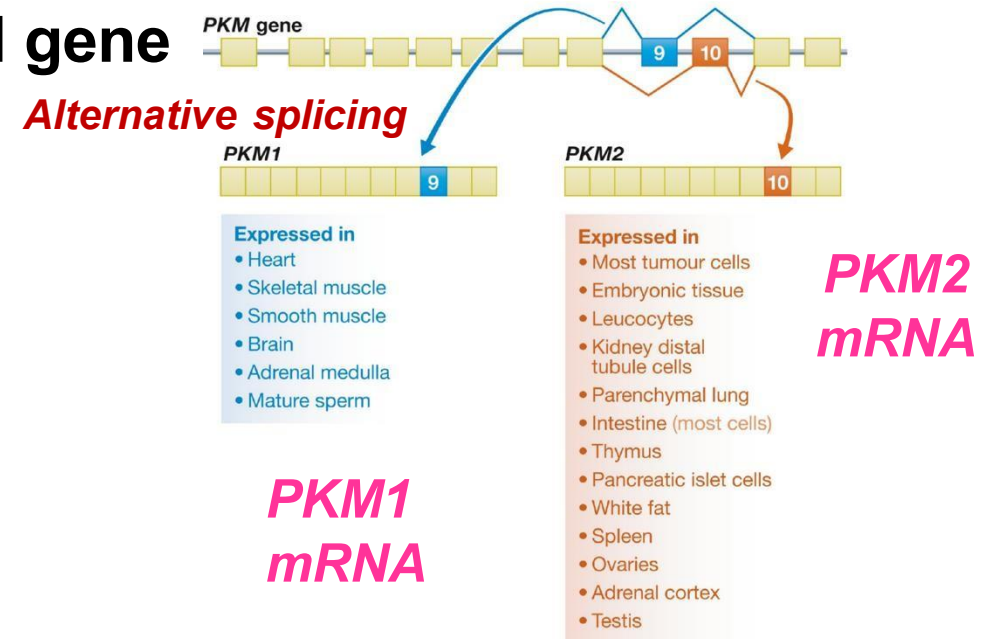
Pyruvate kinase isozymes and regulation

- There are two isoenzyme genes of PK and each produces two isoforms (so, 4 total):
 - PKLR gene** produces PKL (liver) and PKR (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.*

PKLR gene *Different transcription start sites*



PKM gene



Reasons HbF Has Higher Oxygen Affinity?

1- Structural Difference (γ -chain substitution):

- The γ -globin chain of HbF lacks the histidine at position 143 that is present in the β -globin chain of HbA.
- Histidine-143 in HbA normally binds 2,3-bisphosphoglycerate (2,3-BPG), which stabilizes the T-state and lowers O₂ affinity.
- Its replacement by serine in HbF reduces 2,3-BPG binding → less T-state stabilization → higher O₂ affinity.

2- Metabolic Difference (↓ 2,3-BPG production):

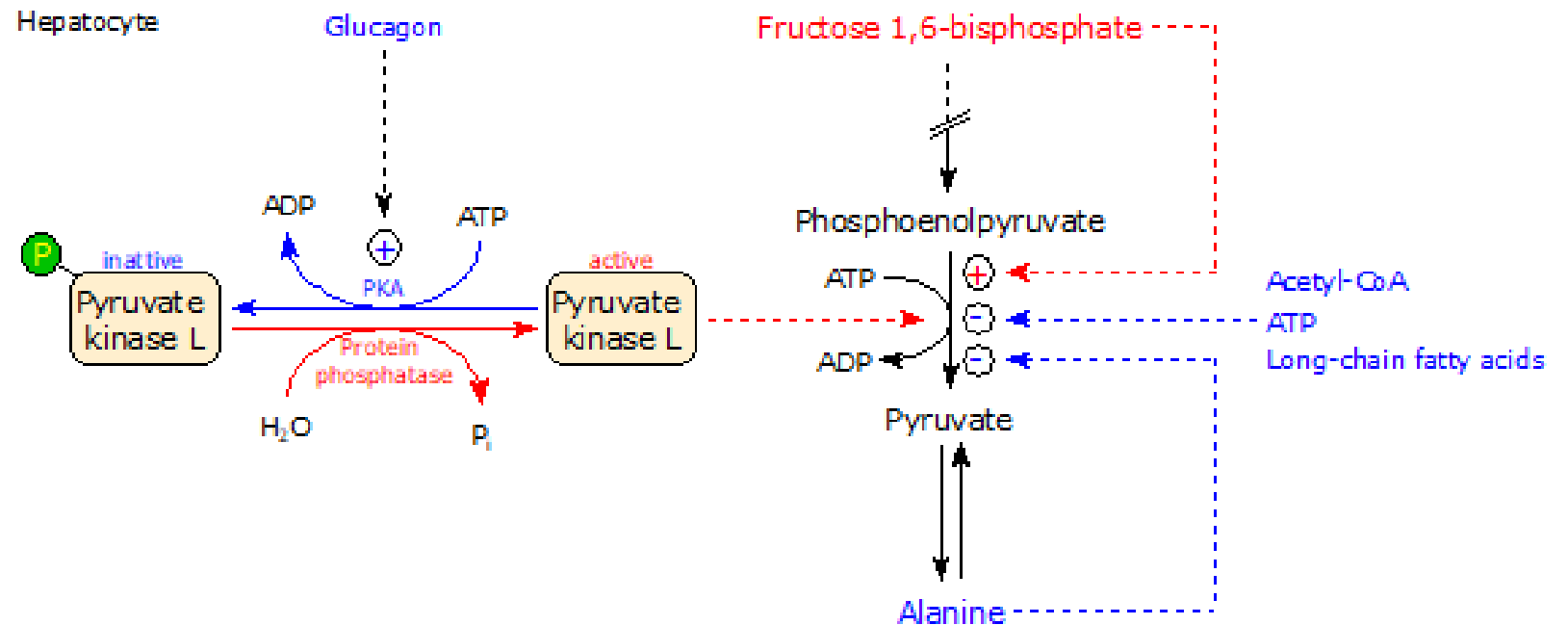
- Fetal red blood cells contain the PKM2 isoform of pyruvate kinase, which is more active than the adult enzyme.
- This drives glycolysis forward toward pyruvate and prevents any accumulation of 2,3-BPG.
- Consequently, 2,3-BPG levels are lower in fetal RBCs → less T-state stabilization → higher O₂ affinity.

In a Nutshell: **less 2,3-BPG + less Binding to 2,3-BPG**

Regulation of PKL

- The liver enzyme (PKL) is allosterically regulated:
 - **Inhibited by:**
ATP, acetyl-CoA, alanine, and long-chain fatty acids and **phosphorylation by protein kinase A.**
 - **Activated by:**
F1,6-BP (Feed Forward Activation)
- The liver (PKL) gene is also controlled at the level of synthesis.
 - Increased carbohydrate ingestion induces the synthesis of PKL.

So, both the enzyme itself and the levels of mRNA coding for it are regulated.

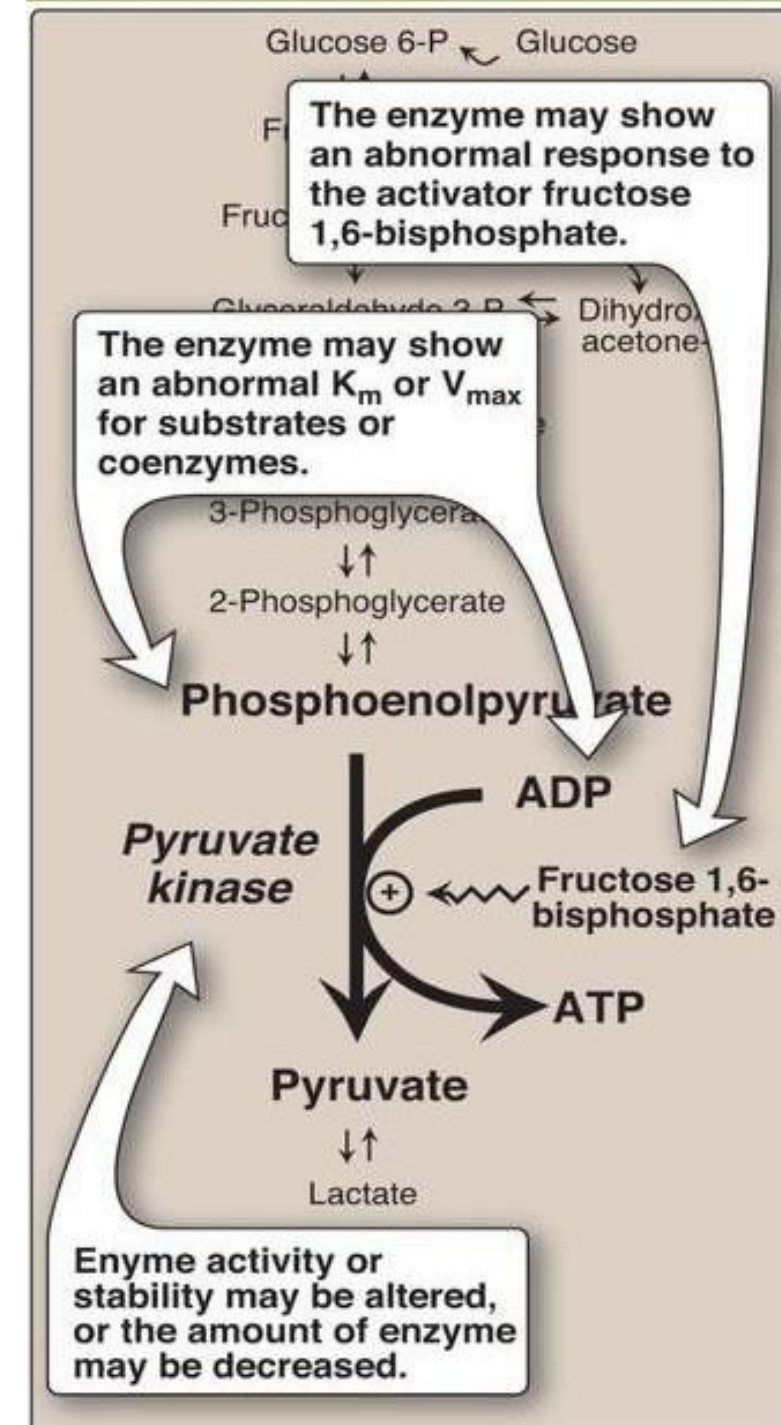


Genetic diseases of PK deficiency

(Alterations observed in PK)

- **No mitochondria in the RBC**, so it completely counts on glycolysis to provide ATP.
- **95% of the genetic diseases in the glycolytic pathway** are related to **PK**.
- If The **adult erythrocyte PK is virtually inactive**:
This would lead to **Reduced capacity to make ATP** → **hereditary hemolytic anemia**.
- The **severity of the disease depends on**:
The degree of enzyme deficiency (5–35%)
- The disease can affect the enzyme's **V_{max} or K_m**, its stability, or its response to activators.
- **Patients are resistant to malaria**.

More fragile cytoskeleton → Hemolytic Anemia:
Shorter RBC Life-Span → Parasites don't get to complete their life cycles in the RBC before it gets destroyed by the macrophages.

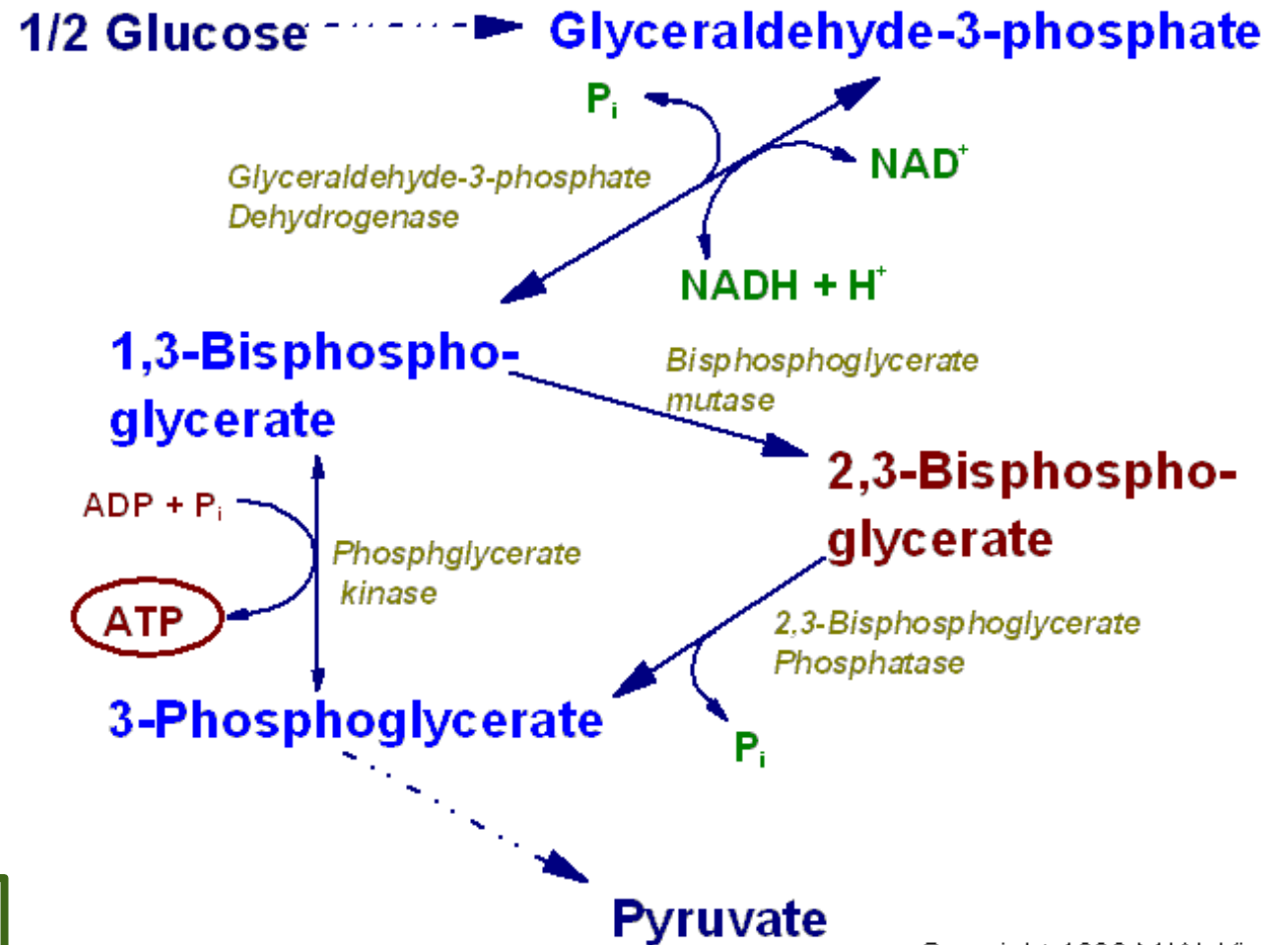


Glycolysis Indirect contribution:

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

Generation of 2,3-BPG

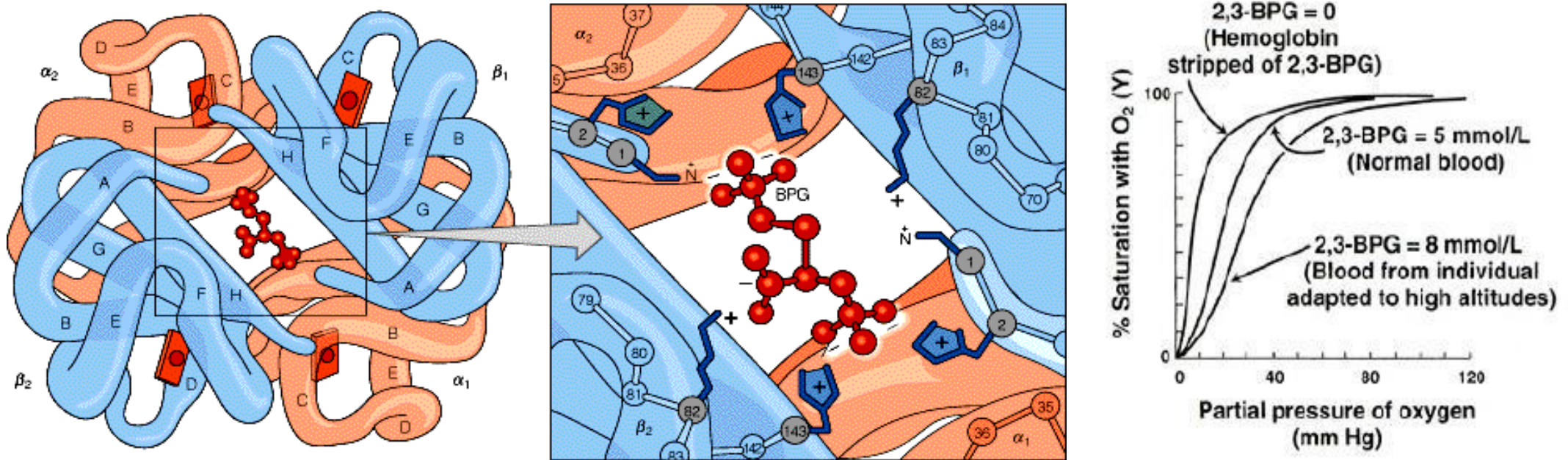
- 2,3-bisphosphoglycerate (2,3-BPG) is derived in small amounts from the glycolytic intermediate 1,3-bisphosphoglycerate.
- It can re-enter the glycolytic pathway.
 - The erythrocyte loses 2 ATPs.



A Mutase Enzyme turns the glycolytic intermediate (1,3 BPG) into 2,3BPG.

Effect of 2,3-BPG on Hb

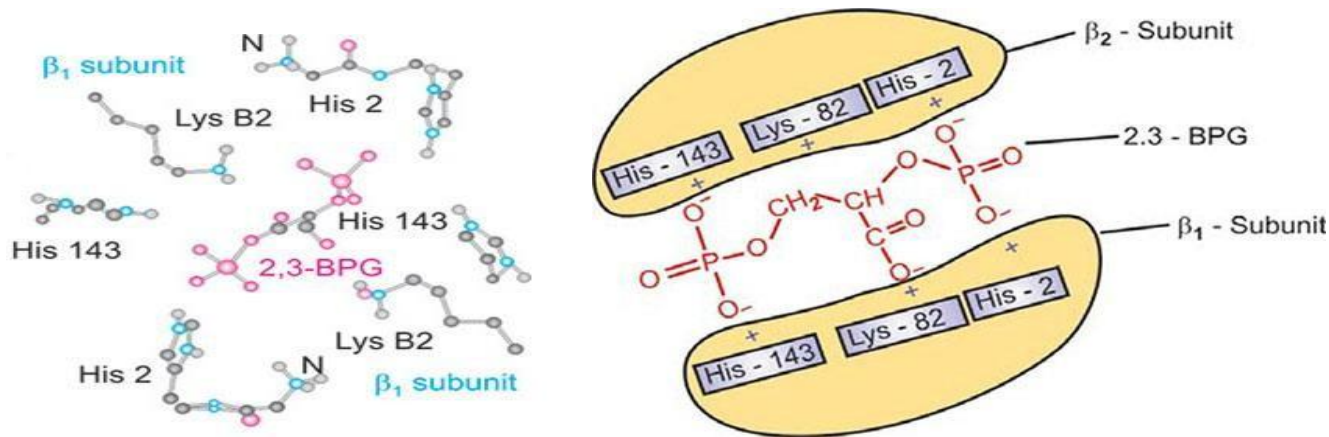
- 2,3-BPG occupies the center of deoxygenated Hb stabilizing it in the T structure.
- When 2,3-BPG is not available (not bound), Hb can be easily converted to the R-structure.



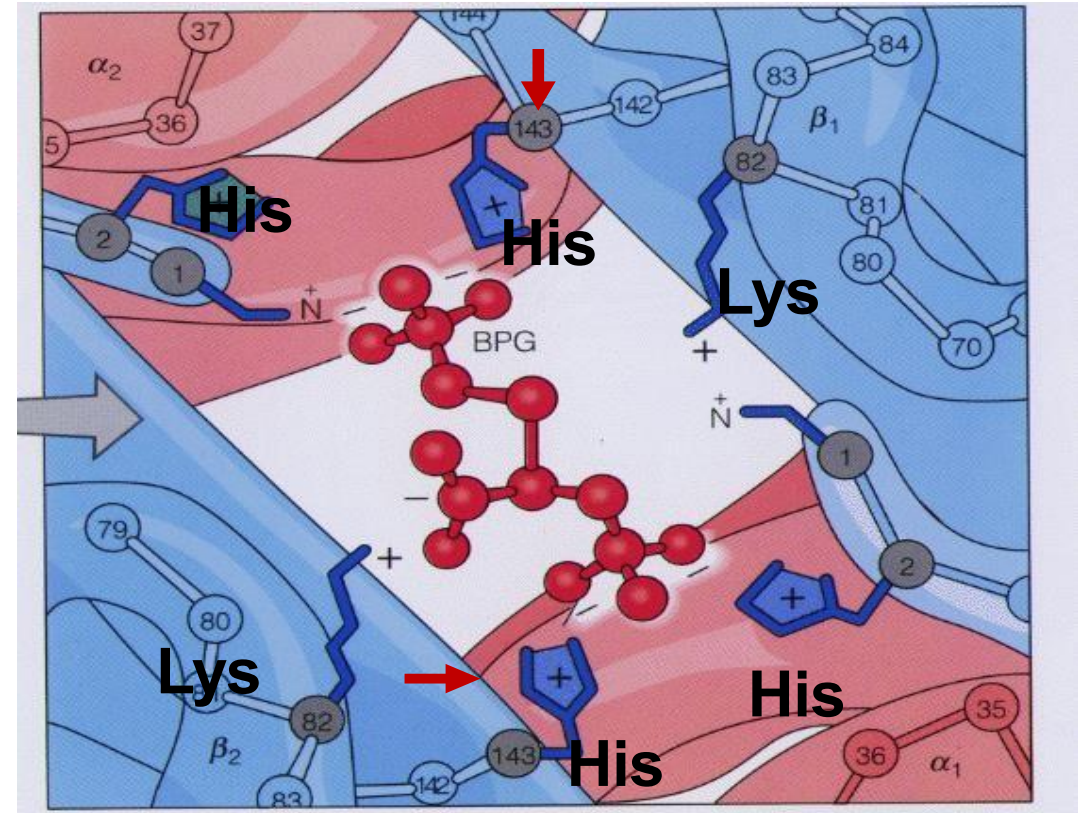
The 2,3BPG has multiple **Negatively Charged Ions** that interact with **Positive Charges** in the center of the hemoglobin forming **Electrostatic Bonds** that **Stabilize the T-state**

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.



As mentioned before, Fetal Hemoglobin has **Serine** instead of the **Histidine** (143 position, B chain). Unlike Histidine which is Positively Charged, **Serine** is partially negatively charged causing **Repulsion** of the **2,3BPG** when it tries to bind to the ceter of hemoglobin.

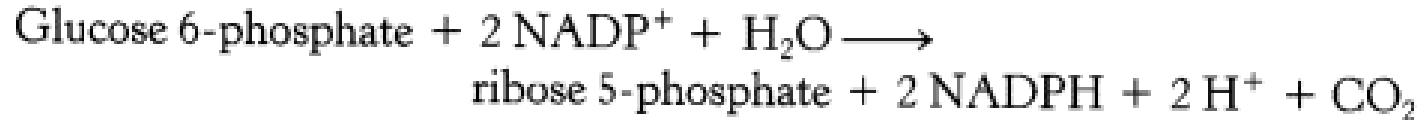
Glycolysis Indirect contribution:

**The pentose phosphate pathway:
NADPH Production through G6PD**

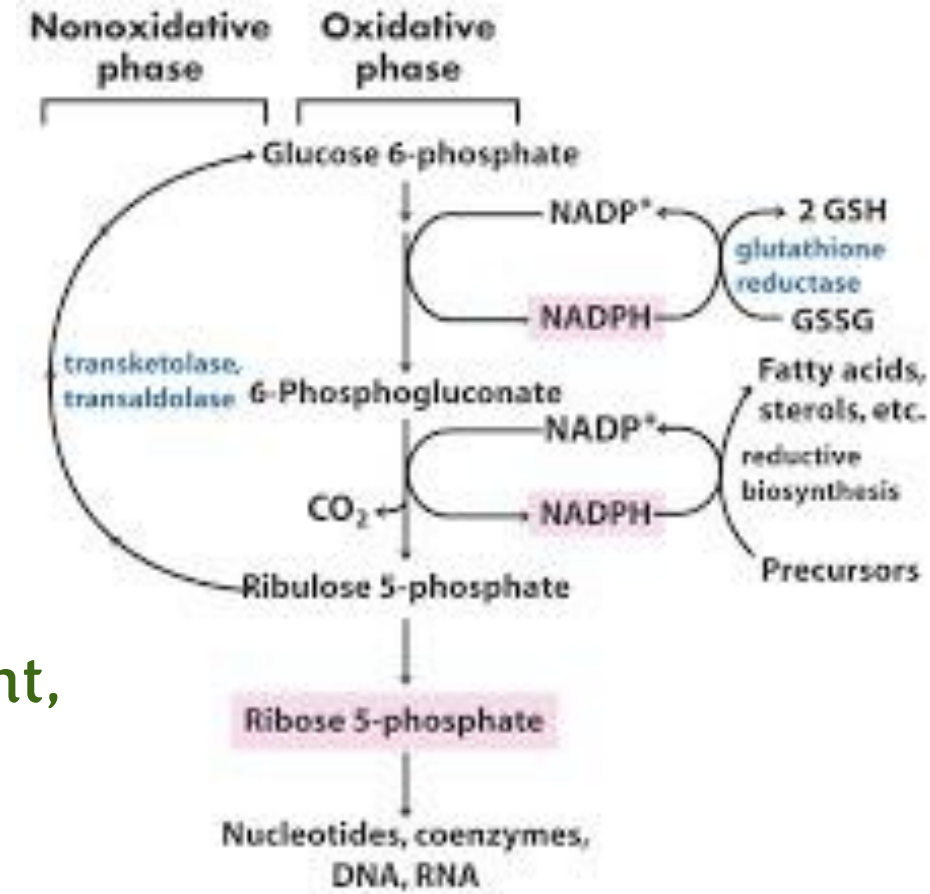
Two phases of pentose phosphate pathway

The oxidative generation of NADPH :

NADPH is generated when **glucose 6- phosphate** is **oxidized** to **ribulose 5- phosphate** through the enzyme **G6PD** (Glucose 6-Phosphate Dehydrogenase).

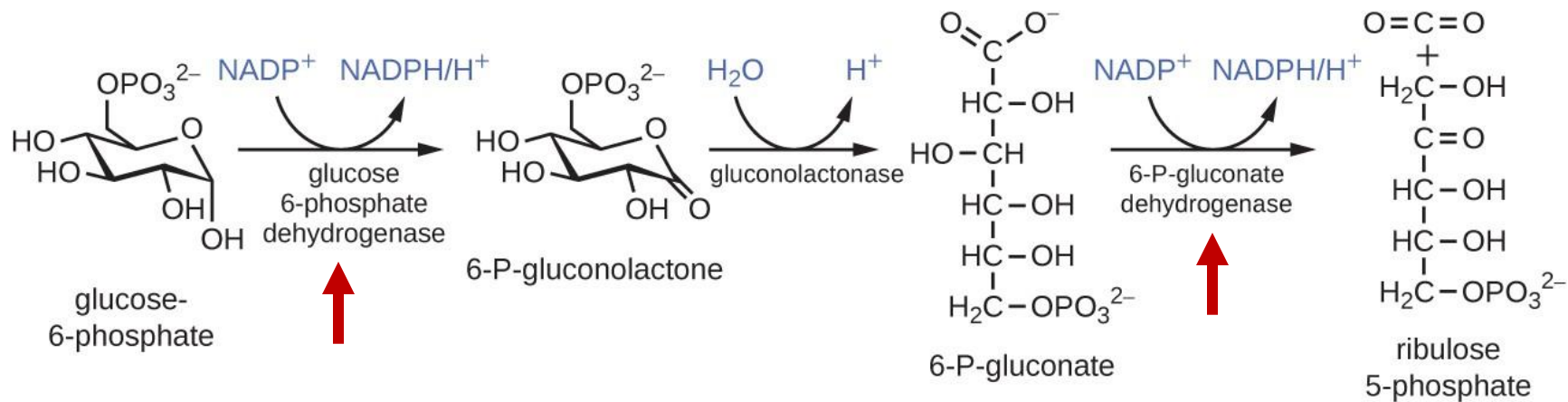


NADPH could then be used as an Anti-oxidating agent, or reused in the non-oxidative interconversion of sugars.



The first step

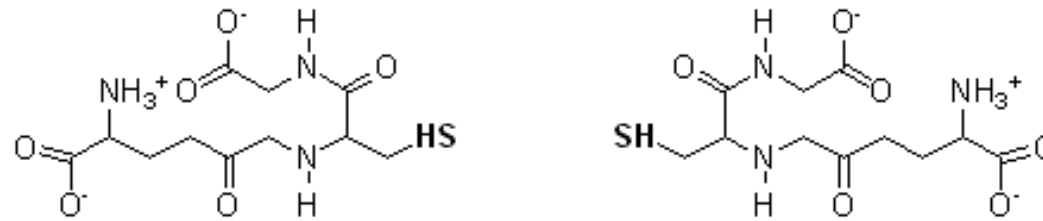
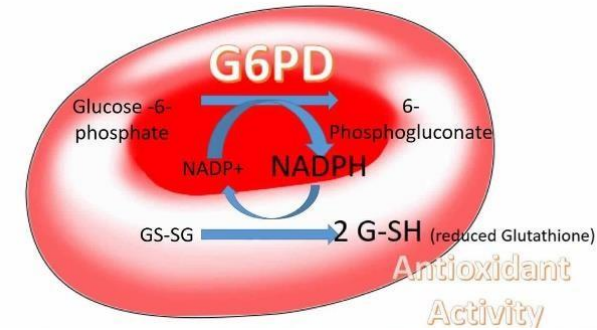
- The oxidative phase of the pentose phosphate pathway starts with the dehydrogenation of glucose 6-phosphate by glucose 6-phosphate dehydrogenase (G6PD).



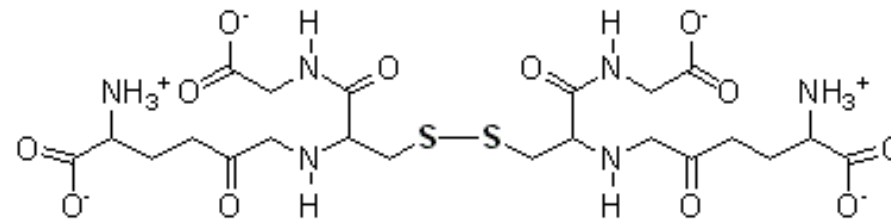
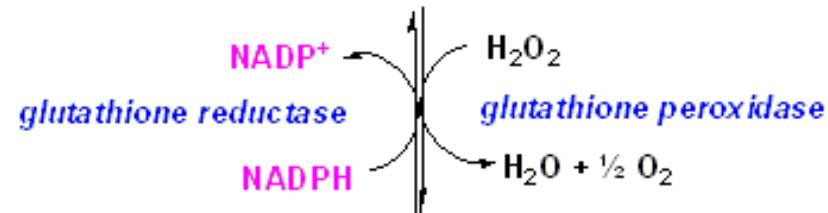
- G6PD is highly specific for NADP^+ , relative to NAD^+ (ie: Prefers NADP^+ as an Electron Acceptor)
- The reaction is irreversible and is the rate-limiting reaction.
- High levels of NADP^+ stimulate the reaction .

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.



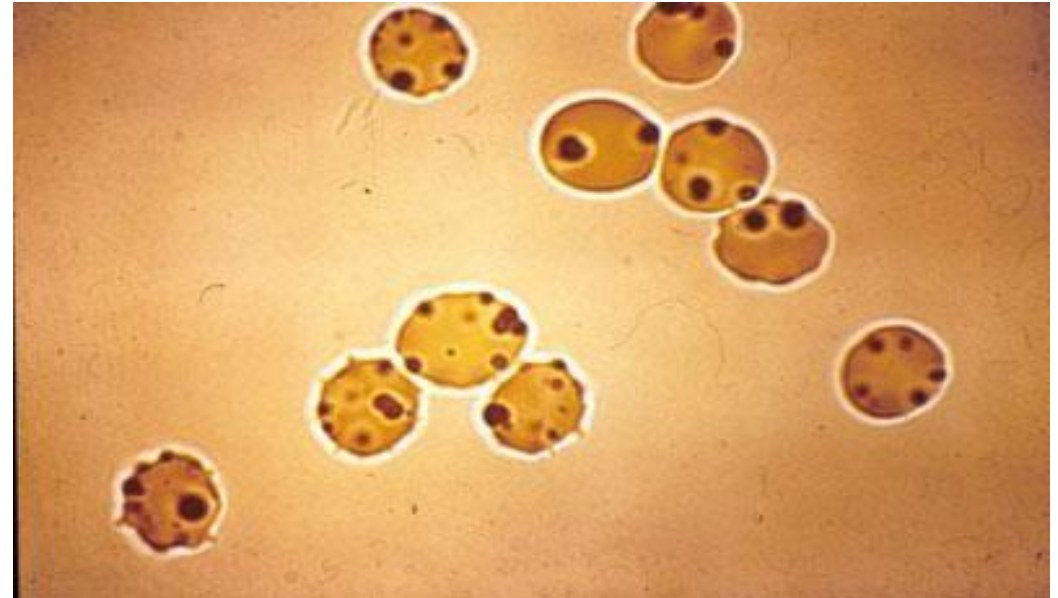
2 x reduced glutathione (GSH)



oxidized glutathione (GSSG)

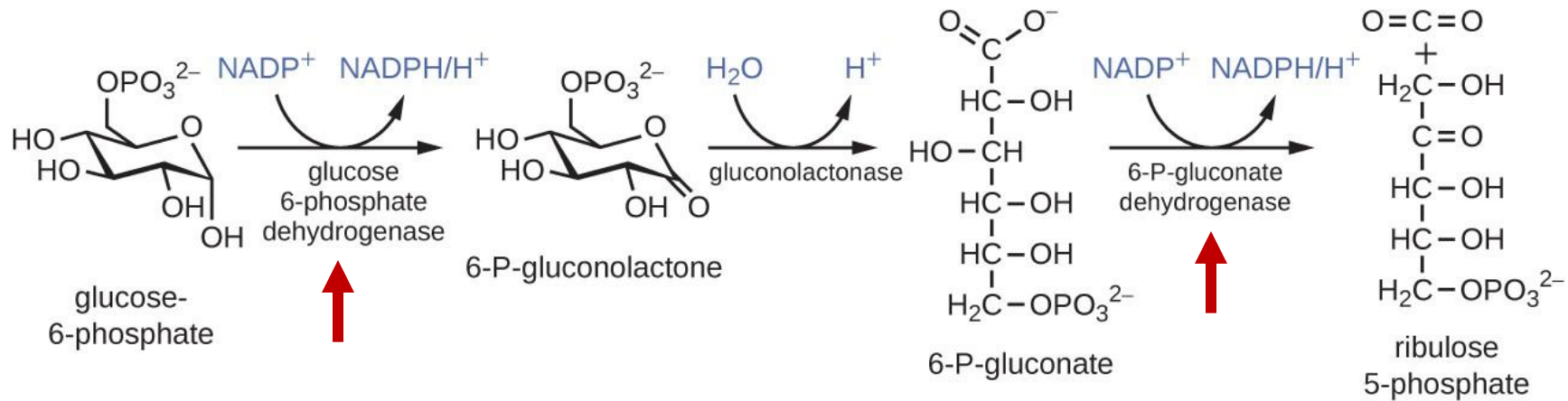
Low GSH levels

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



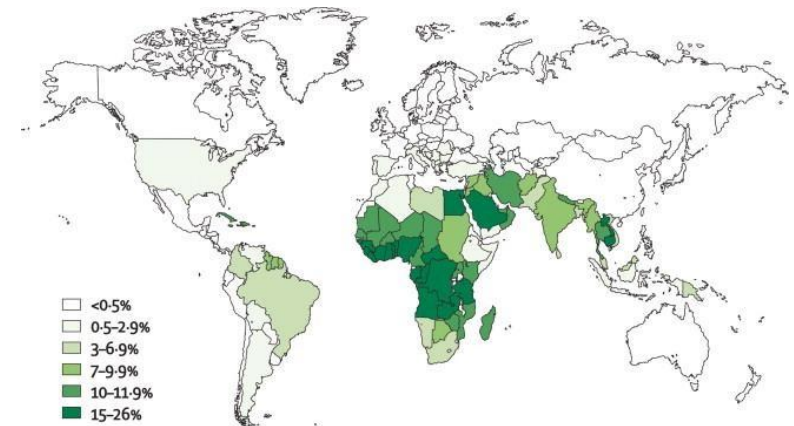
No Sufficient NADPH Production → Inability to Regenerate Reduced Glutathione = Insufficient Reduction of Peroxidases → Accumulation of Peroxidases → Destruction of the cellular components (Destroyed cellular components appear as Heinz Bodies) → Hemolytic Anemia

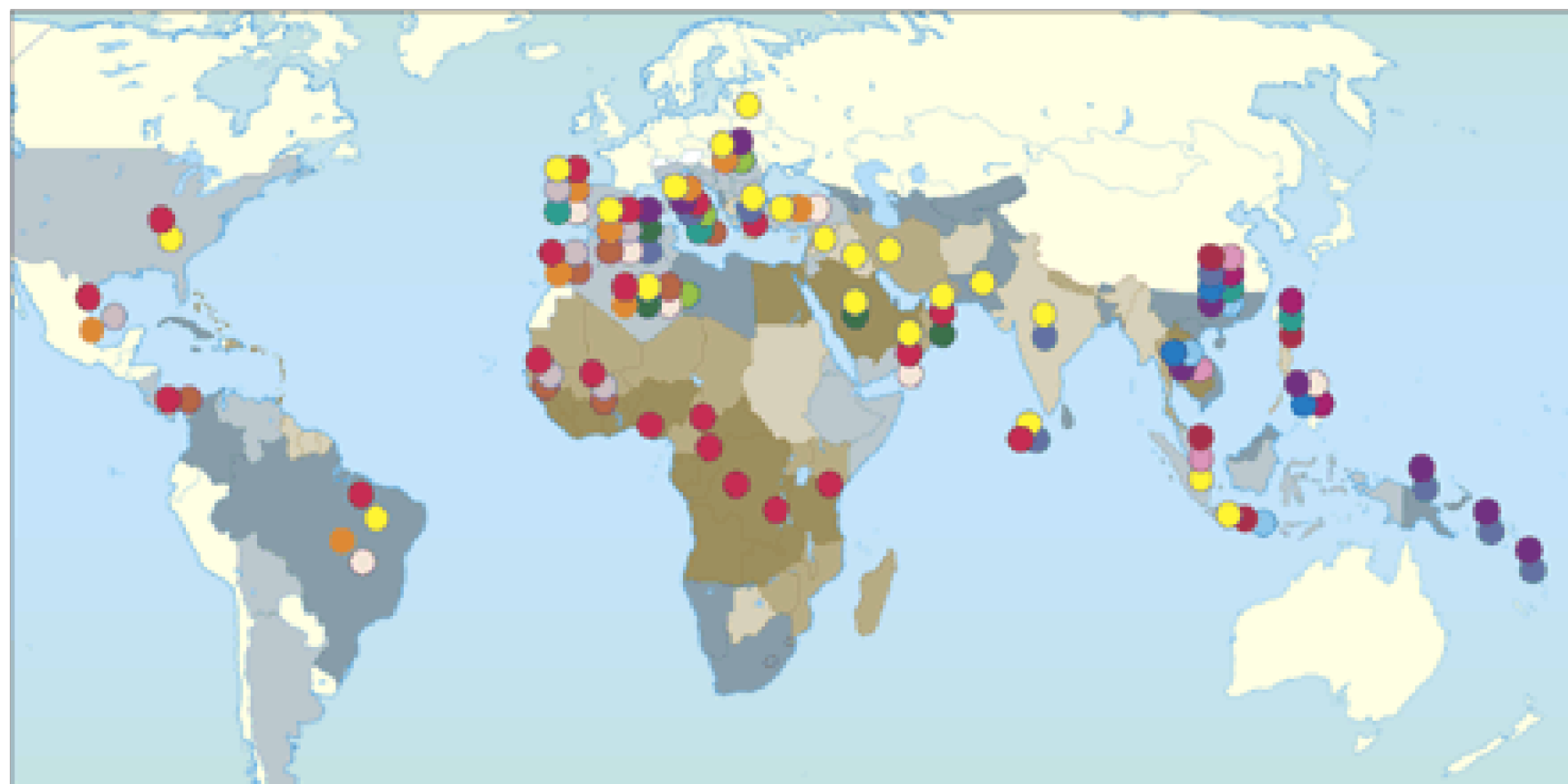
Glucose-6-phosphate dehydrogenase deficiency



G6PD deficiency

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a group of heterogeneous disease with significantly reduced activity.
 - **G6PD deficiency leads to Hemolytic anemia**
- 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.**
- The disease causes hemolytic anemia because the membranes of the cells become prone to oxidation from the free radicals.

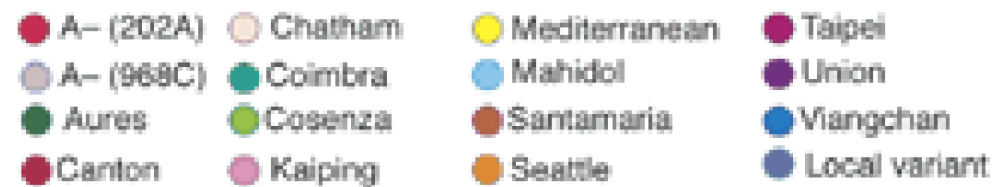




Frequency of G6PD deficient males %



Polymorphic G6PD variants



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?

The four classes of G6PD deficiency

- G6PD B (Normal)
- Abnormal G6PDs:
 - Class IV (>60% Enzymatic Activity): no clinical symptoms
 - 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.
 - G6PD Mediterranean (group II or class II) (<10% Enzymatic Activity):
 - Severe
 - The enzyme has normal stability, but negligible activity.
 - Class I: The most Severe and rare.

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%

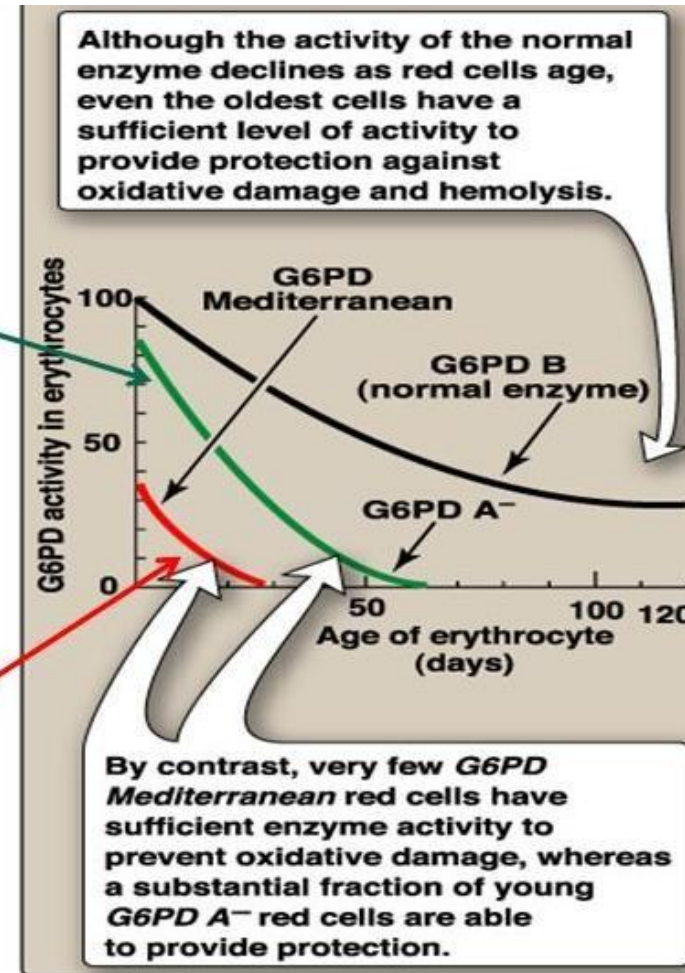
Class II vs. Class III

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)



Inducers of G6PD deficiency symptoms

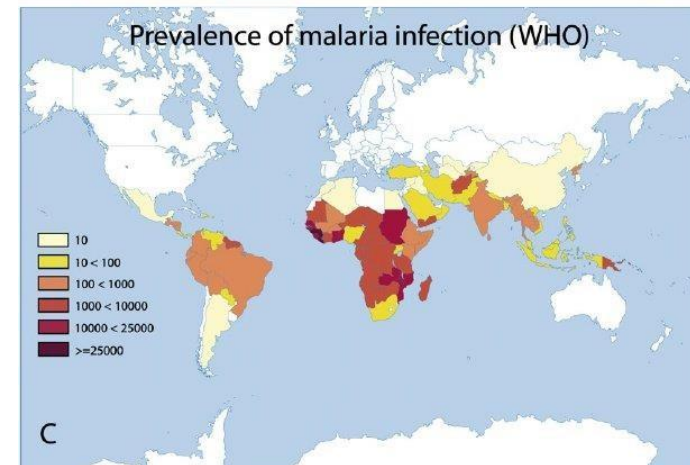
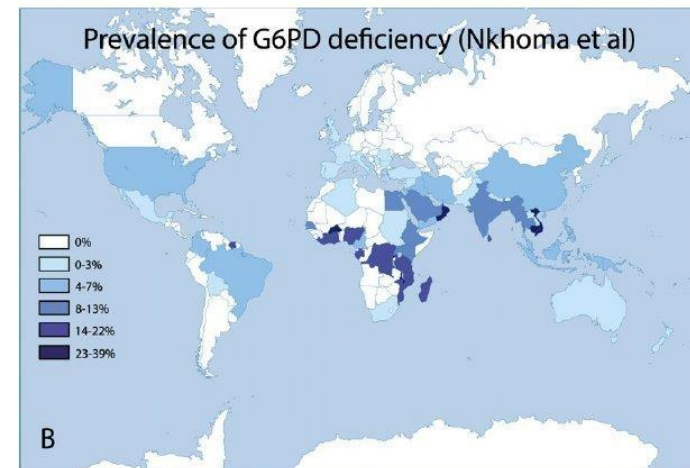
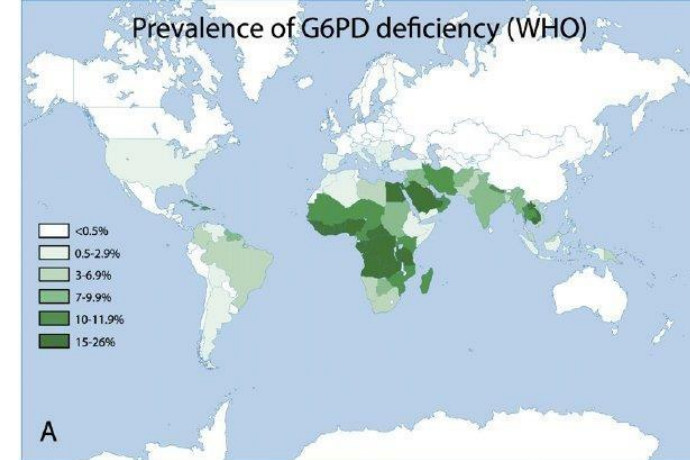
(G6PD Deficiency Flare Ups)

- Any increase in oxidizing agents intensifies oxidative stress on erythrocytes, which are already vulnerable due to G6PD deficiency, potentially triggering severe oxidative flare-ups and hemolysis.
- An increase in all of the following would lead to increased production of Oxidating Agents:
 - 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.

Remember: Any condition that compromises the integrity of red blood cells and causes their premature hemolysis provides **partial protection against malaria**, as the **parasite cannot complete its life cycle before the RBCs are destroyed**.



Summary

- **Glycolysis**

- 1- **ATP** → Hemolytic anemia

- 2- **NADH** → Methemoglobin

- 3- **Pyruvate kinase** → Affects ATP production which could cause anemia, and affects 2,3-BPG production.

- **2,3-bisphosphoglycerate** → R to T state

- **Pentose phosphate pathway:**

- A- **NADPH** → For removal of peroxidases and free radicals

- B- **G6PD** → Produces NADPH

"فَبِمَا رَحْمَةٍ مِّنَ اللَّهِ لِنْتَ لَهُمْ وَلَوْ كُنْتَ فَظًّا غَلِيظَ الْقَلْبِ لَانْفَضُّوا مِنْ حَوْلِكَ فَاعْفُ عَنْهُمْ
وَاسْتَغْفِرْ لَهُمْ وَشَاوِرْهُمْ فِي الْأَمْرِ فَإِذَا عَزَمْتَ فَتَوَكَّلْ عَلَى اللَّهِ إِنَّ اللَّهَ يُحِبُّ الْمُتَوَكِّلِينَ"

يقول الإمام الشافعي رحمه الله: "الحرّ من راعي وِدَادٍ لحظة، وانتِمْ لمن أفاده لفظة."

Biochemistry Quiz



For any feedback, scan the code or click on it.



Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	16; 2 instances 16	Histamine Serine is negatively charged	Histidine Serine is partially negatively charged
V1 → V2			