Metabolism in erythrocytes

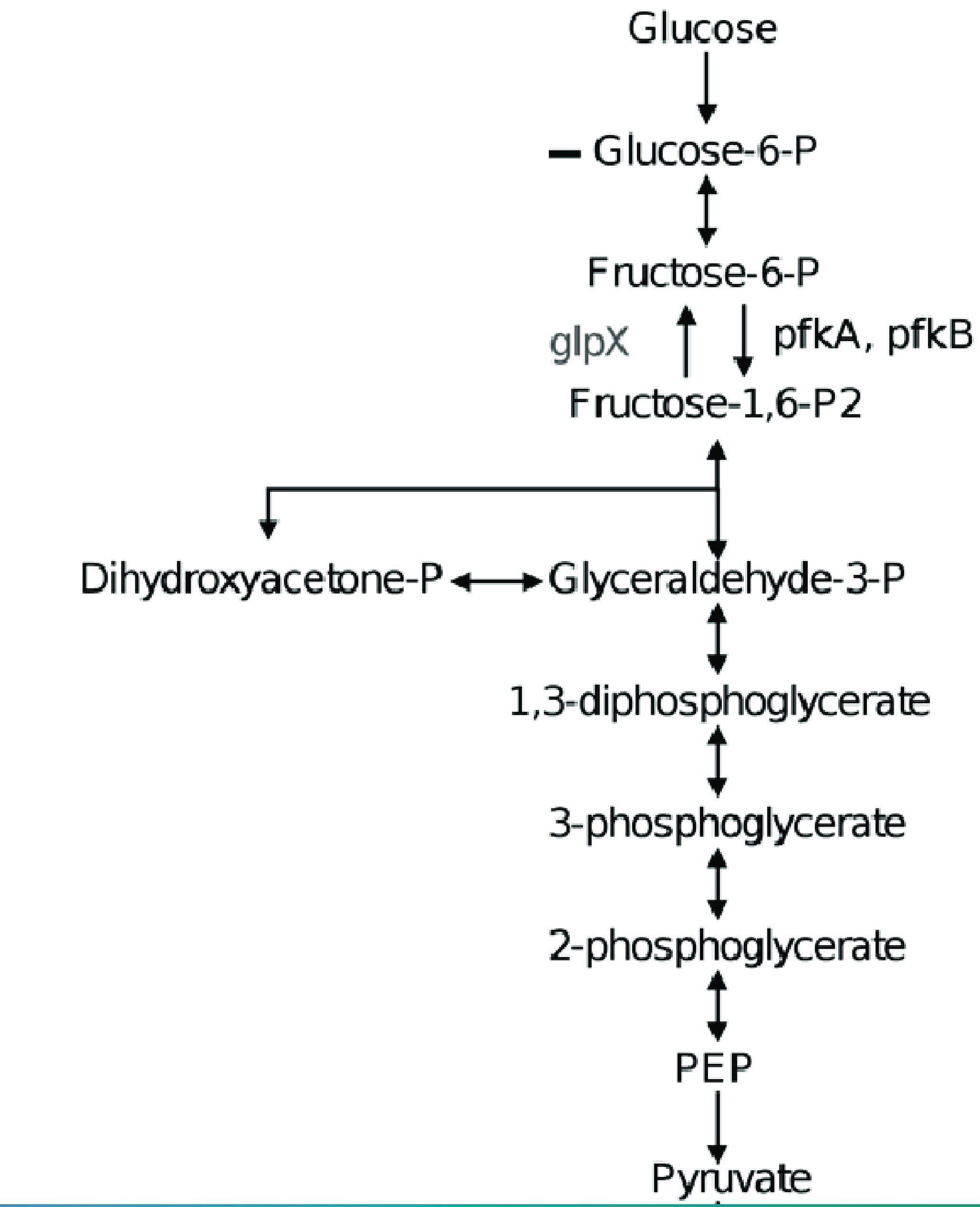
Prof. Mamoun Ahram
Hematopoietic-lymphatic system

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

- Glycolysis (Directly)
  - 1- ATP
  - 2- NADH
  - 3- Pyruvate kinase
- Glycolysis (Indirectly)
  - 4- 2,3 bisphosphoglycerate
  - 5- Pentose phosphate pathway
    - A- NADPH
    - B- G6PD

Carbohydrate metabolism in RBC

- Glycolysis (Directly)



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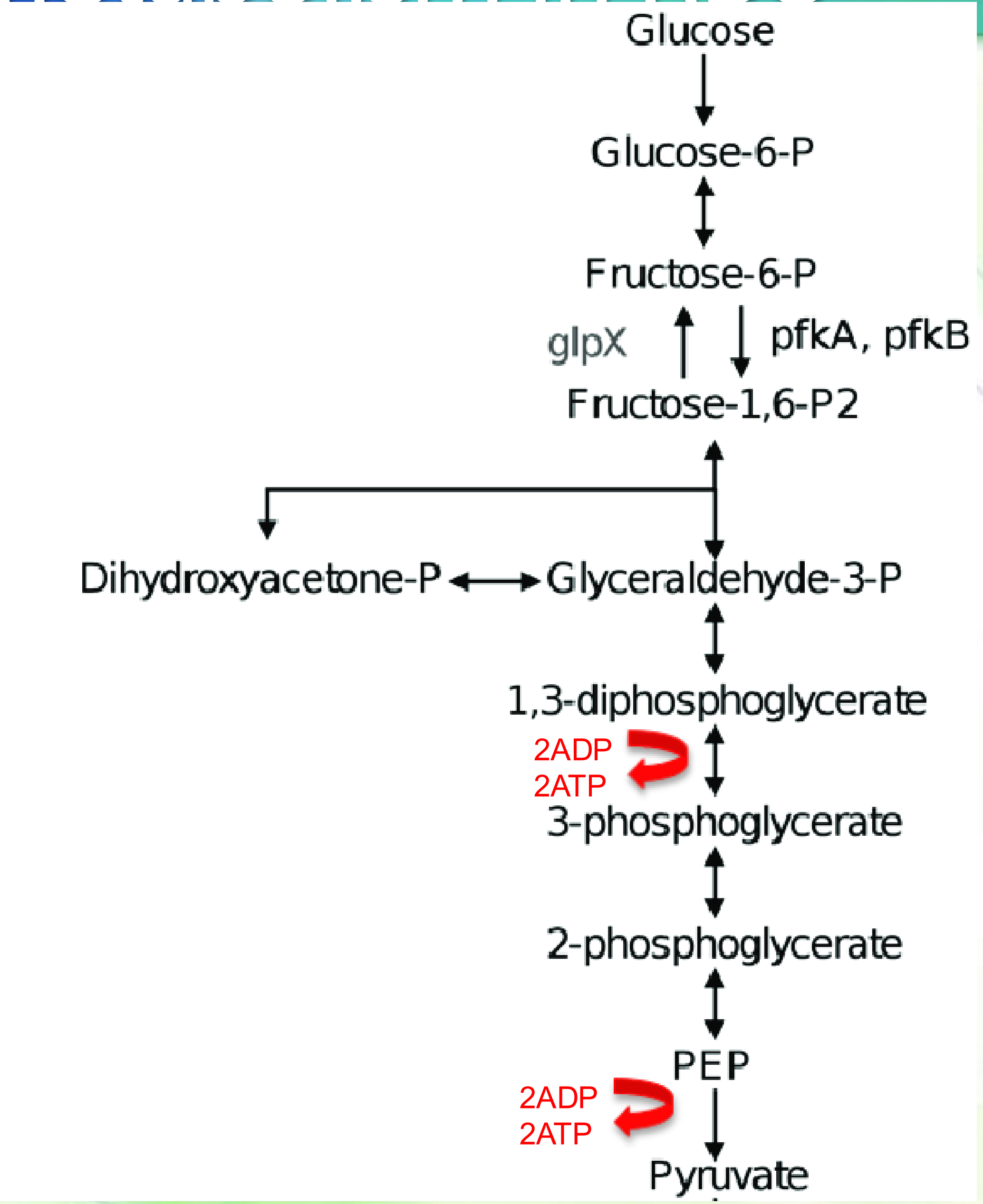

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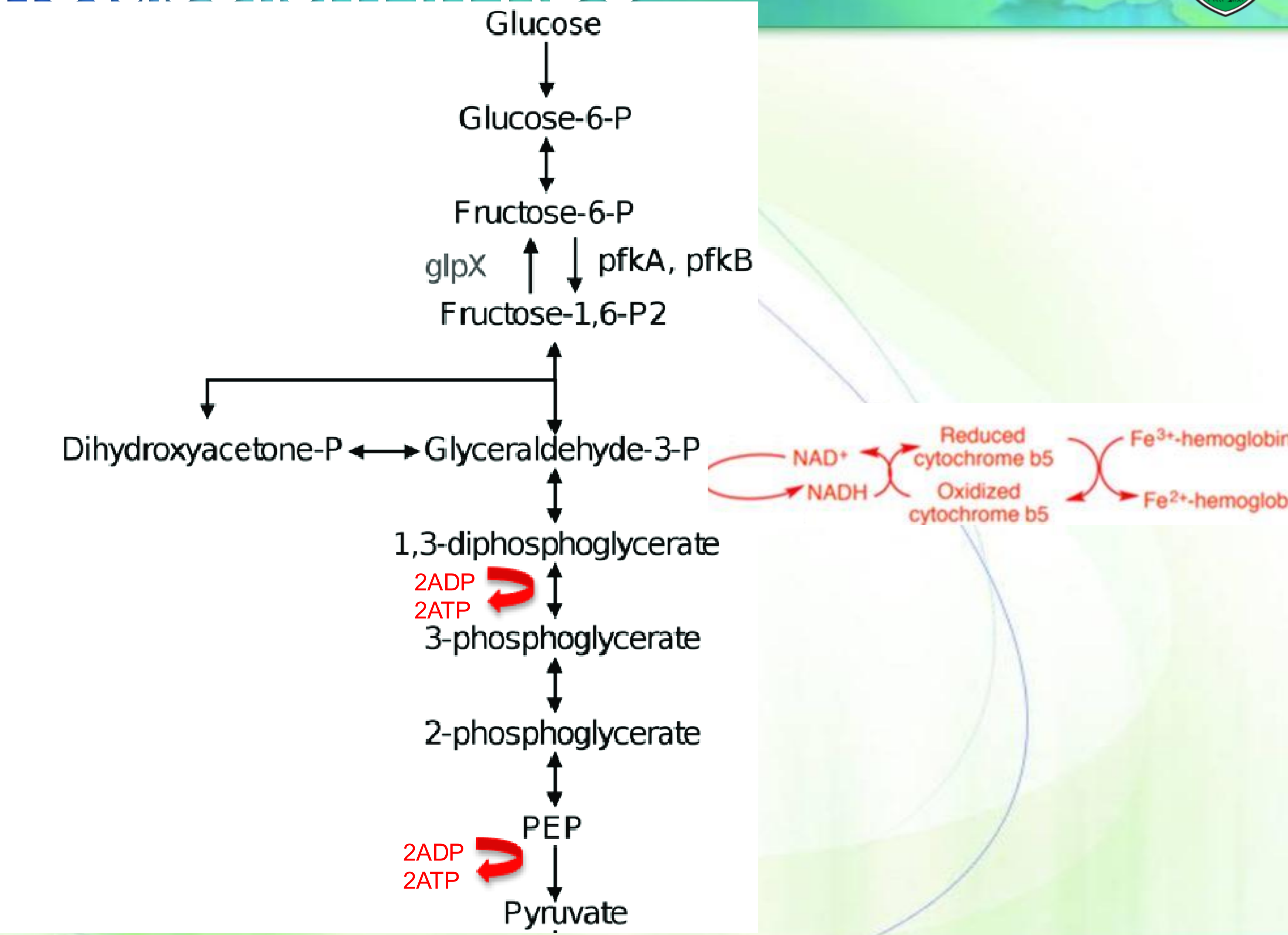

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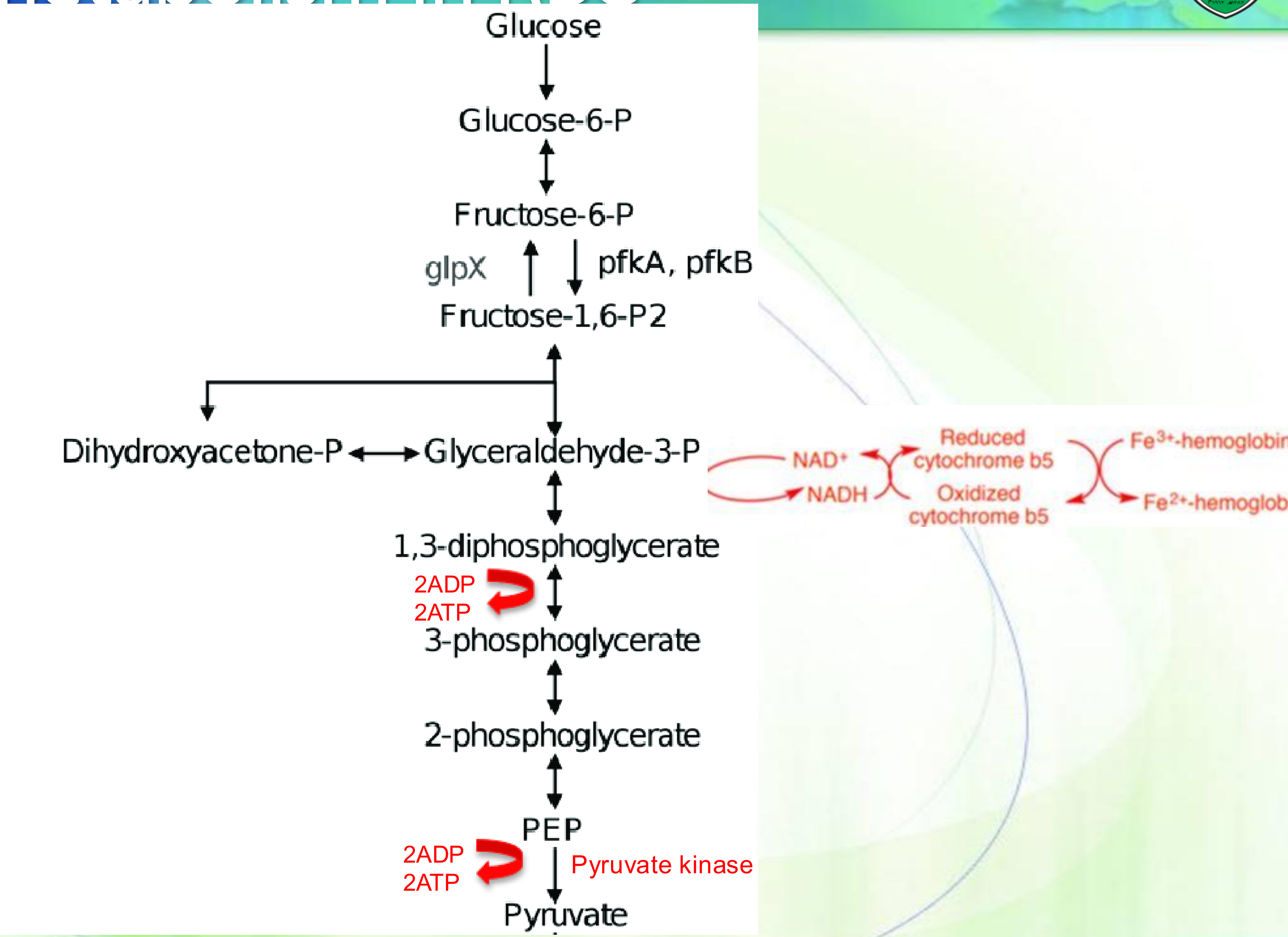

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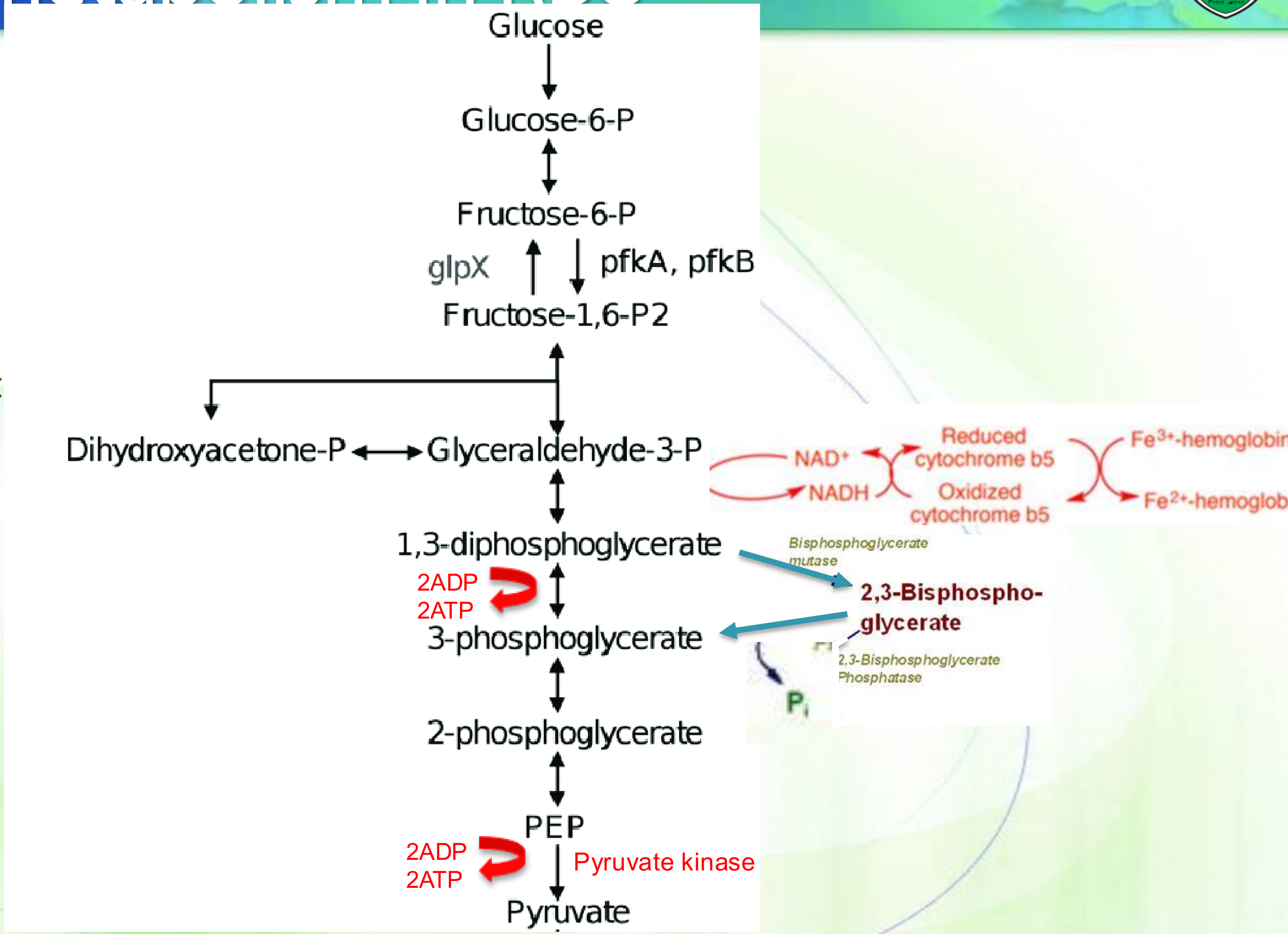

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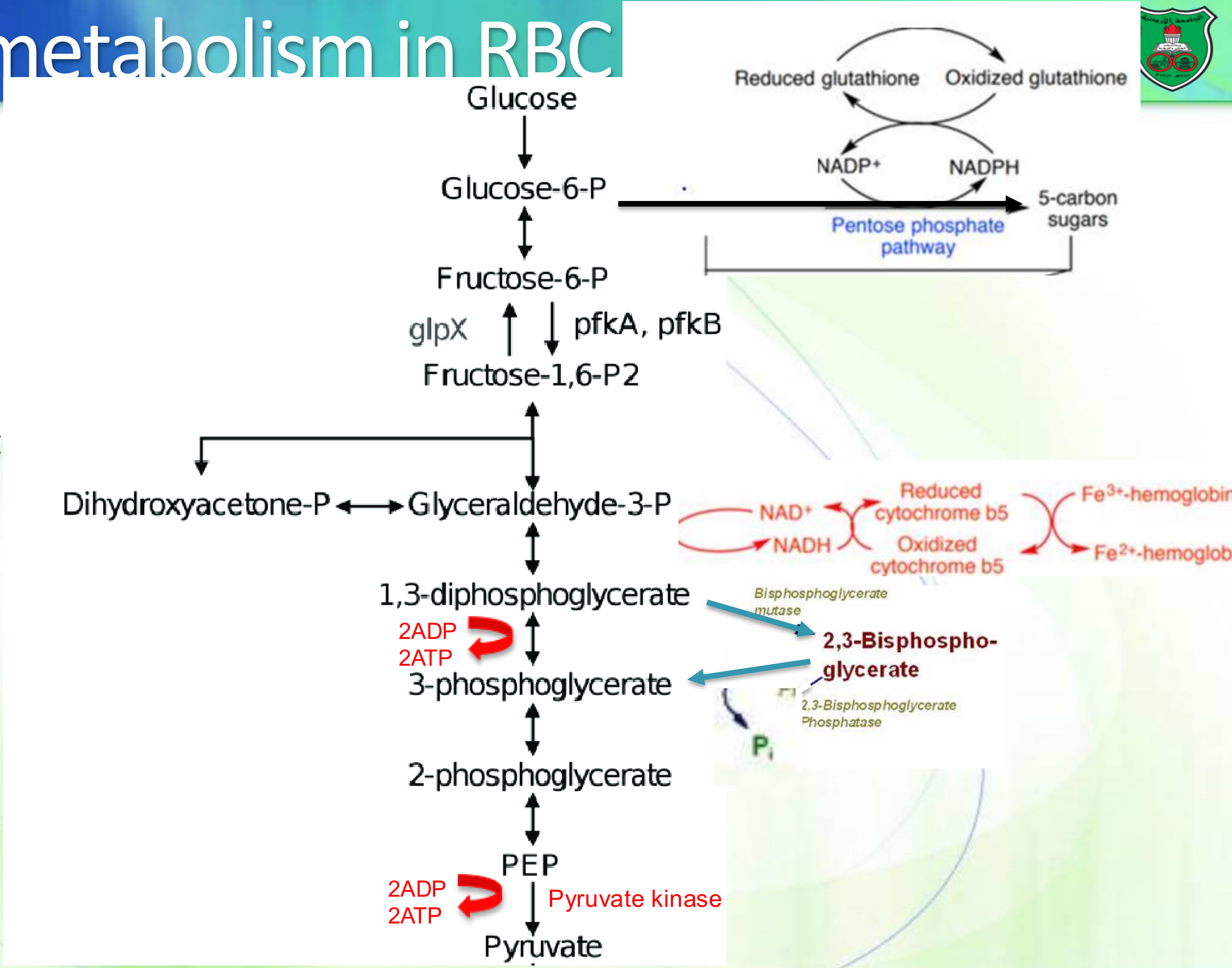
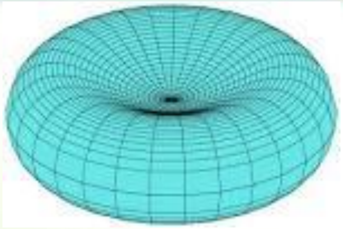


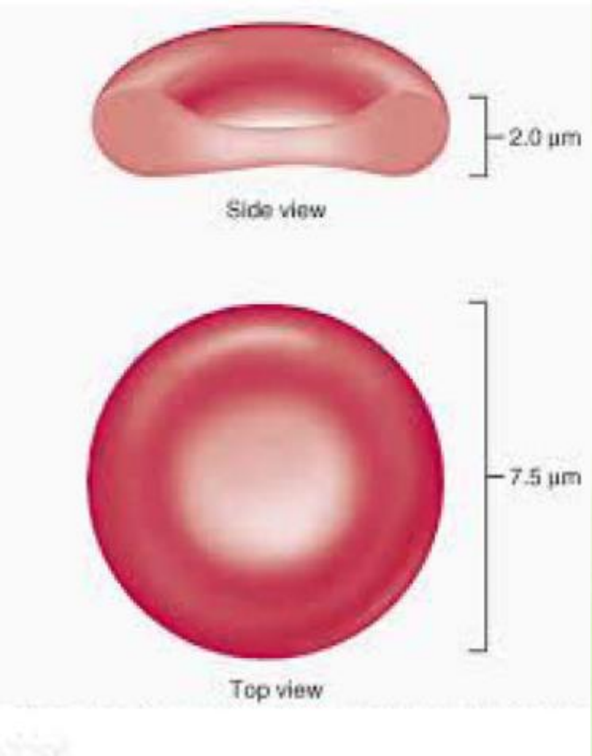
1- Glycolysis and the production ATP

Main purpose

- Glycolysis is the only form of ATP in the RBC:
- 1- Modifying sugars and proteins
- 2- Maintaining membrane asymmetry
- 3- Functioning of membrane ion pumps
- 4- Regulating cytoskeletal proteins
  - Maintenance of the discocytic shape, which is critical for the optimal viability and functional capacity.
- 5- The major source of energy



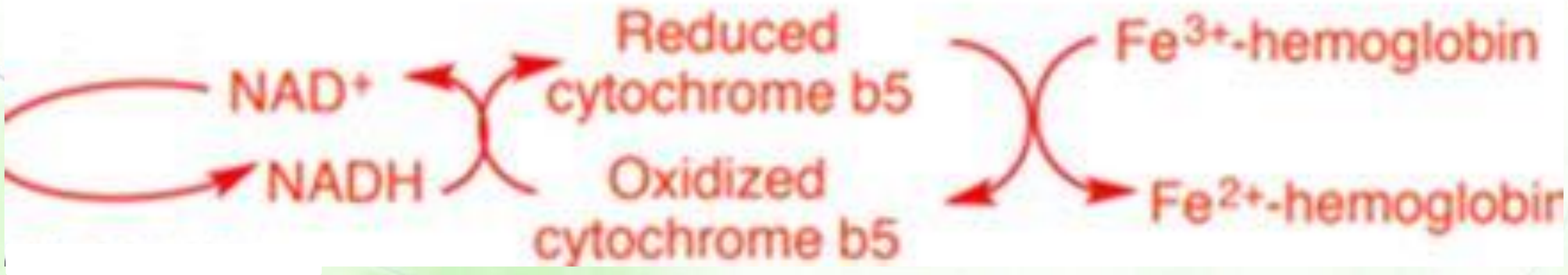
Discocyte



2- Glycolysis and the production NADH

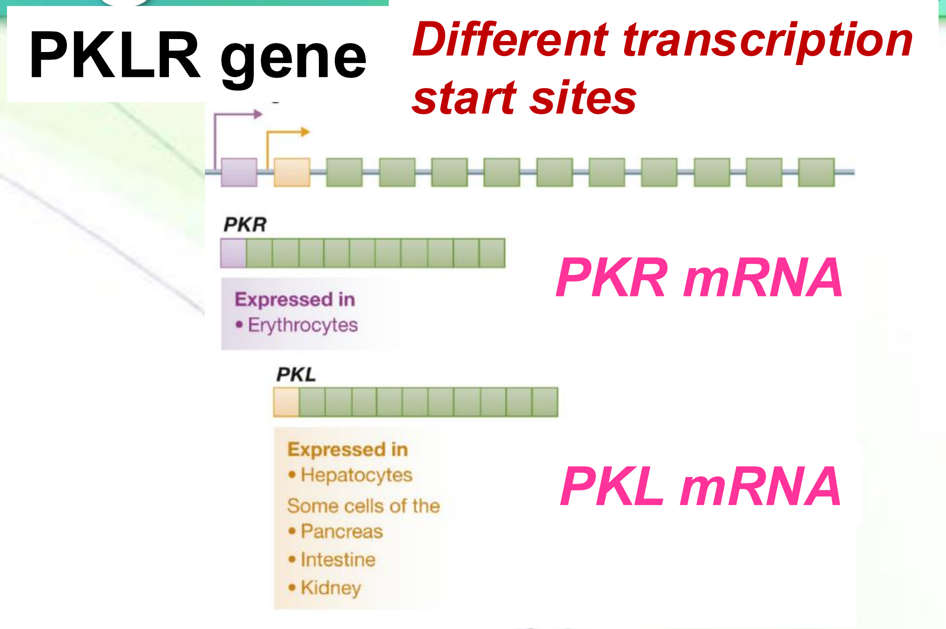
Carbohydrate metabolism in RBC

- 2- Glycolysis and NADH
- Glycolysis provides NADH for reduction of methemoglobin (hemoglobin with oxidized Fe^{3+} in heme)

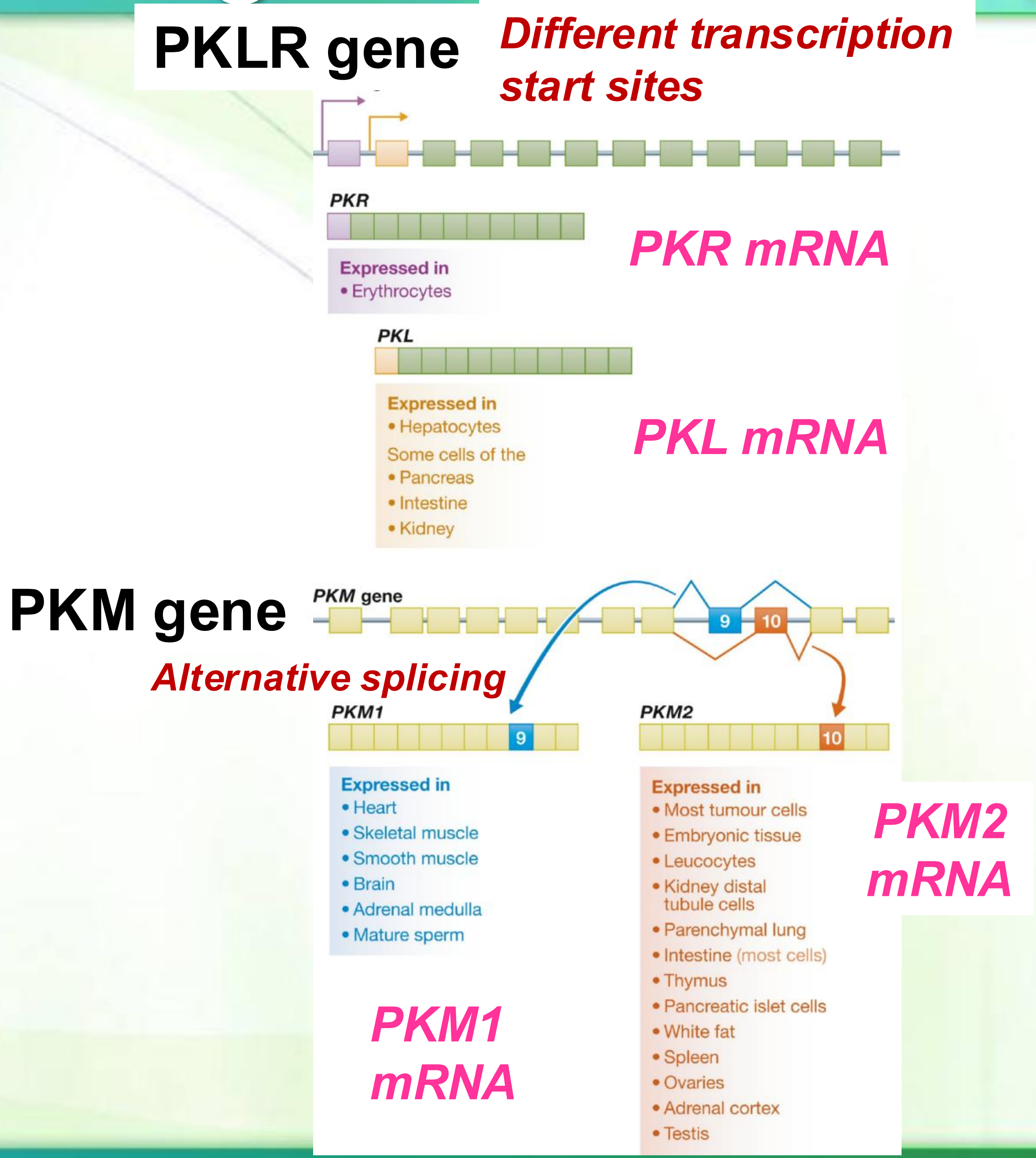


3- Pyruvate Kinase

Pyruvate kinase isozymes and regulation

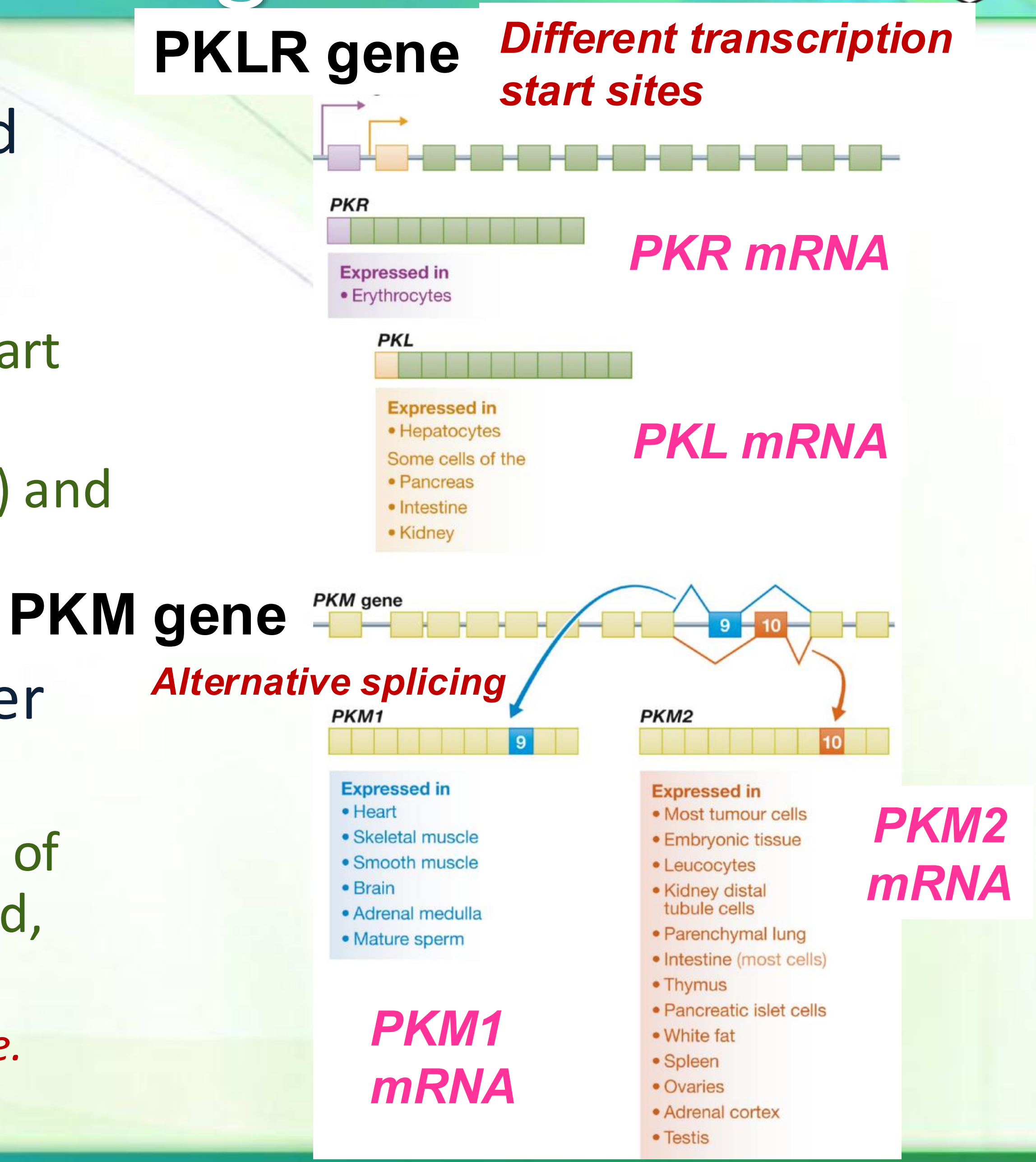


Pyruvate kinase isozymes and regulation



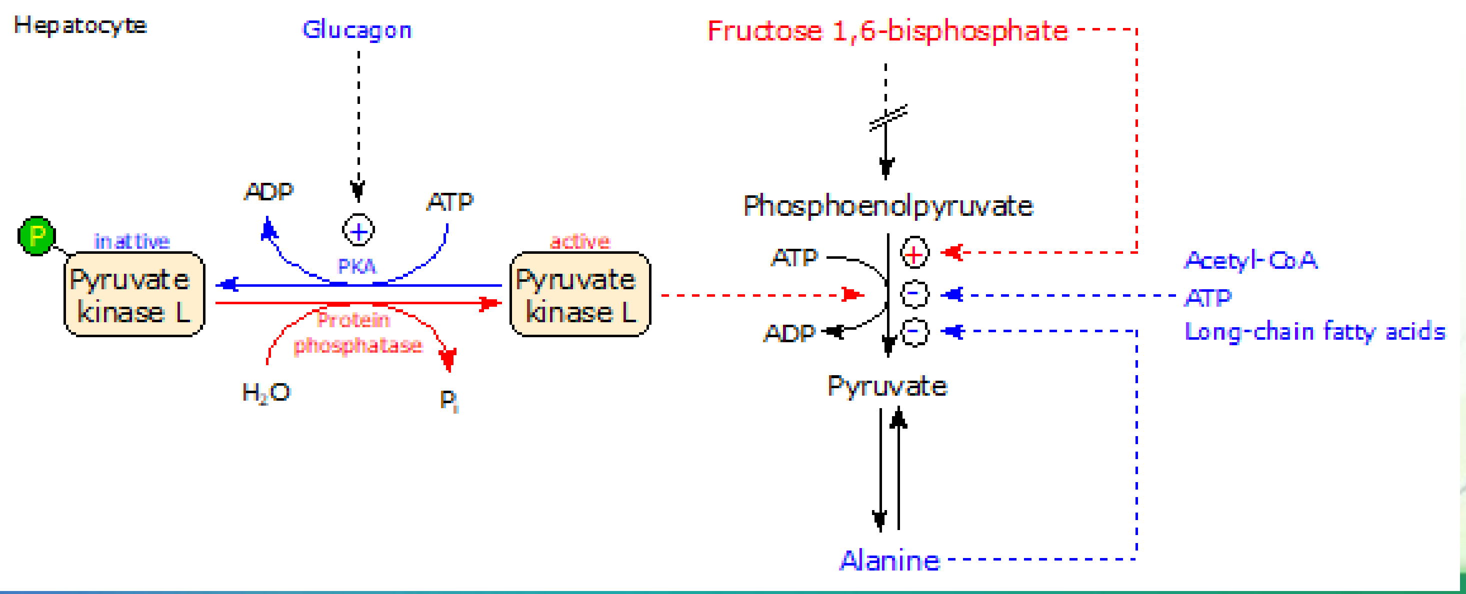
Pyruvate kinase isozymes and regulation

- 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.*



Regulation of PKL

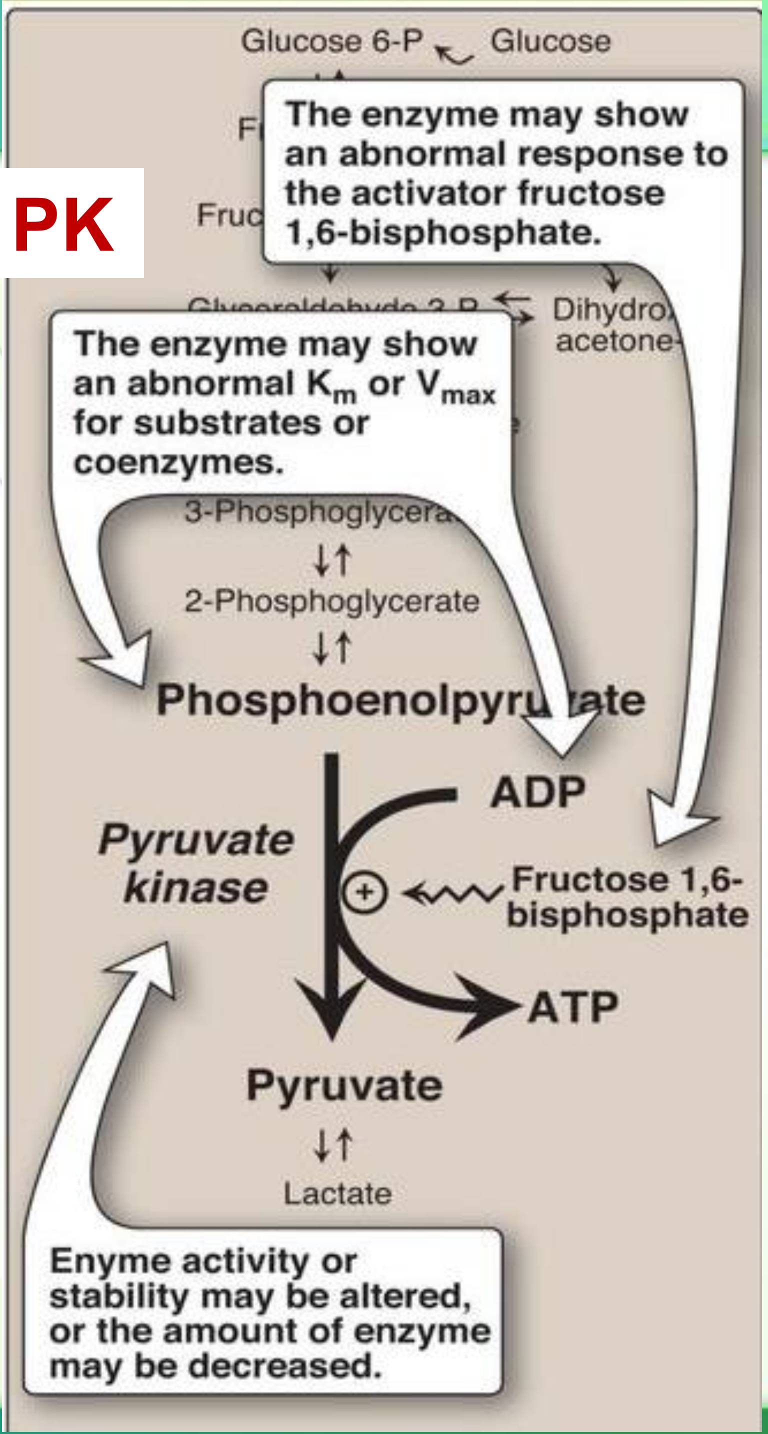
- The liver enzyme (PKL) is allosterically regulated:
  - inhibited by ATP, acetyl-CoA, alanine, and long-chain fatty acids and by phosphorylation by protein kinase A.
  - activated by F1,6-BP.
- The liver (PKL) gene is also controlled at the level of synthesis.
  - Increased carbohydrate ingestion induces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



Genetic diseases of PK deficiency

Alterations observed in PK

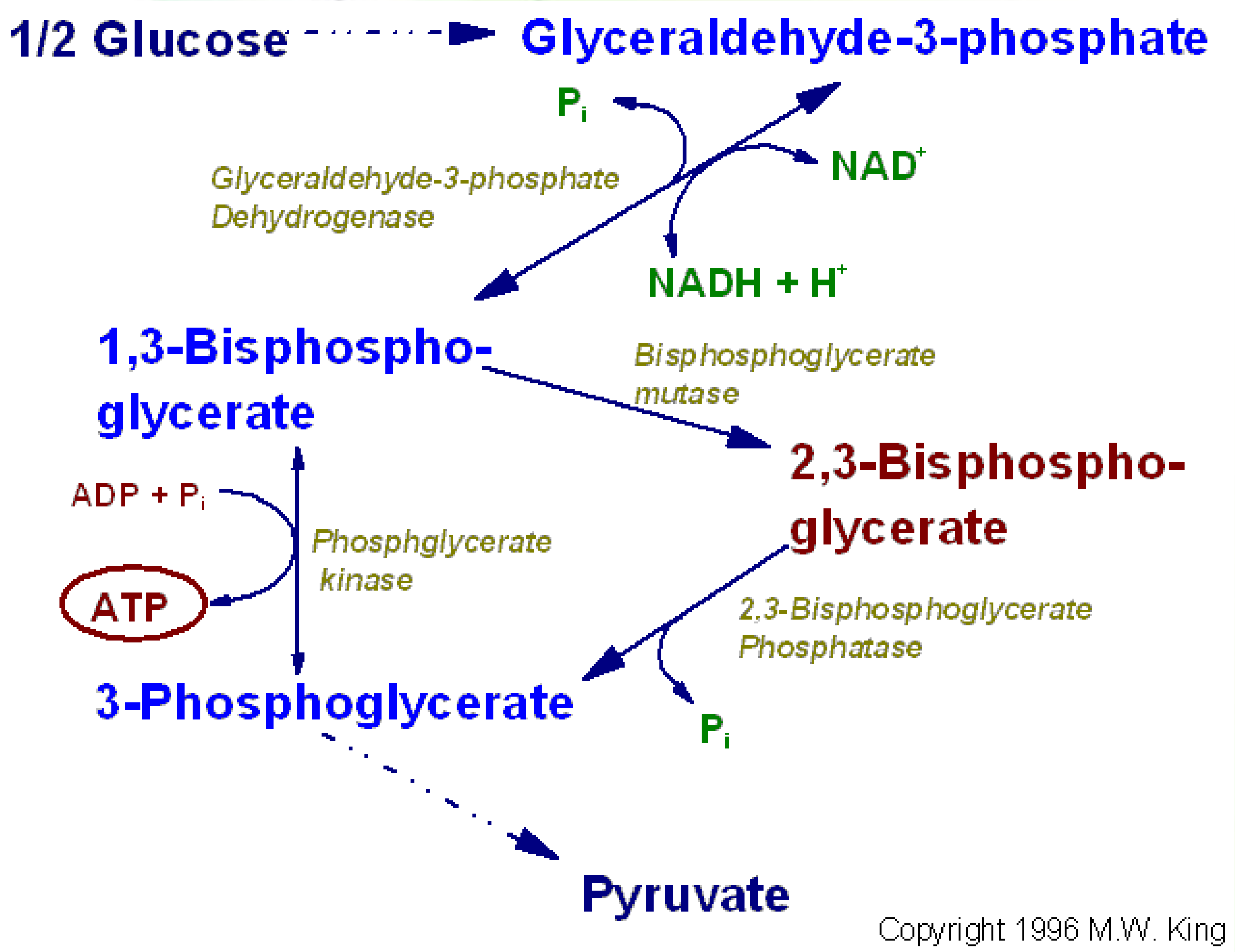
- No mitochondria in the RBC, so it completely counts on glycolysis to provide ATP
- 95% of the genetic diseases in the glycolytic pathway are related to PK.
- The adult erythrocyte PK is virtually inactive.
  - Reduced capacity to make ATP → hereditary hemolytic anemia
- The severity of the disease depends on
  - The degree of enzyme deficiency (5-35%)
- The disease can affect the enzyme’s Vmax or Km, its stability or its response to activators.
- Patients are resistant to malaria.



4- Glycolysis and the production of 2,3 Bisphosphoglycerate

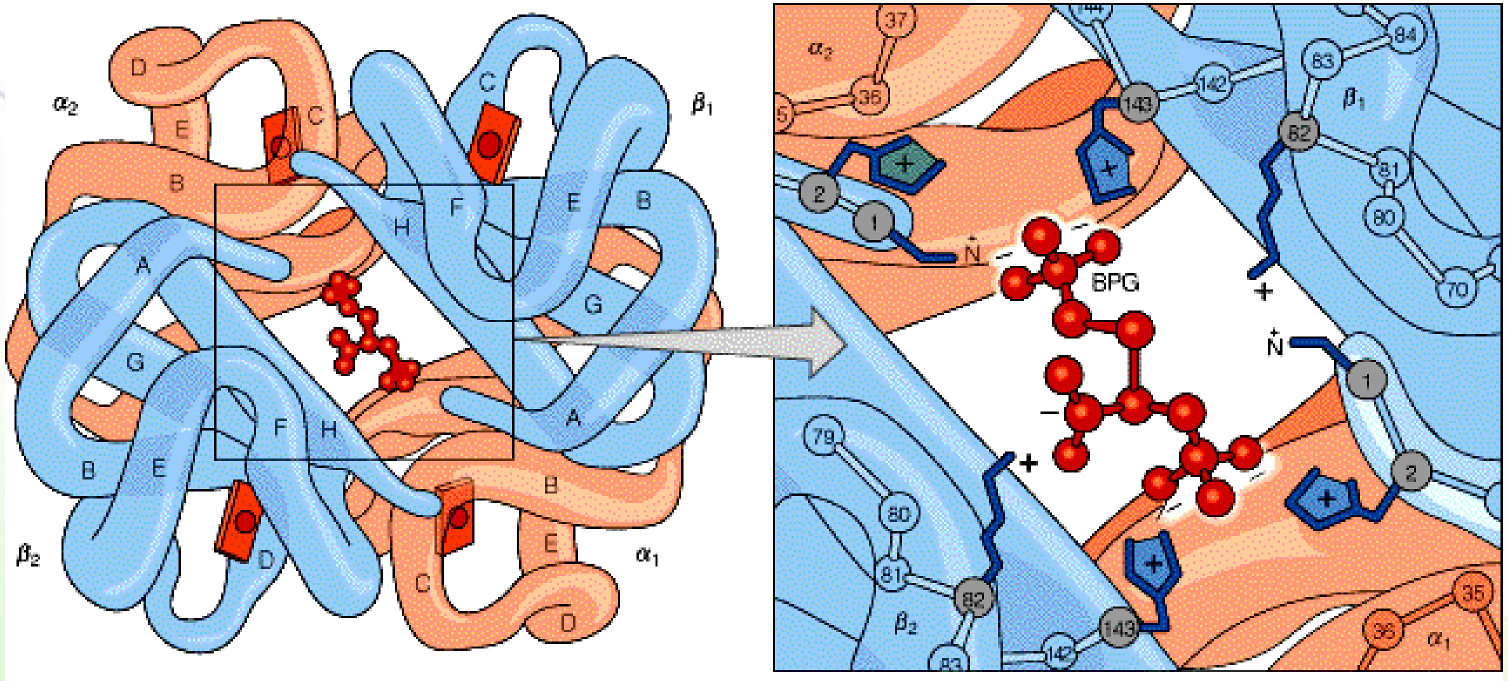
Generation of 2,3-BPG

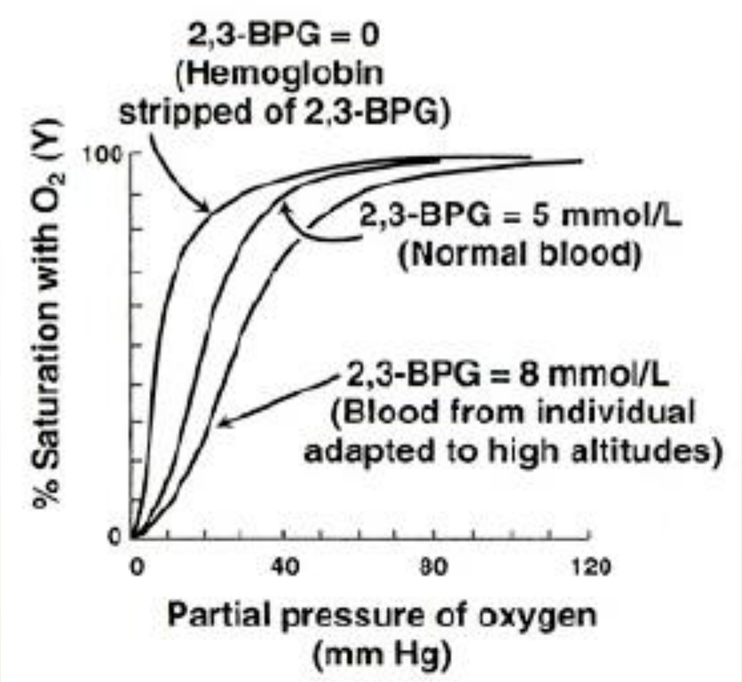
- 2,3-bisphosphoglycerate (2,3-BPG) is derived in small amounts from the glycolytic intermediate 1,3-bisphosphoglycerate.
- It can re-enter the glycolytic pathway.
  - The erythrocyte loses 2 ATPs.



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.

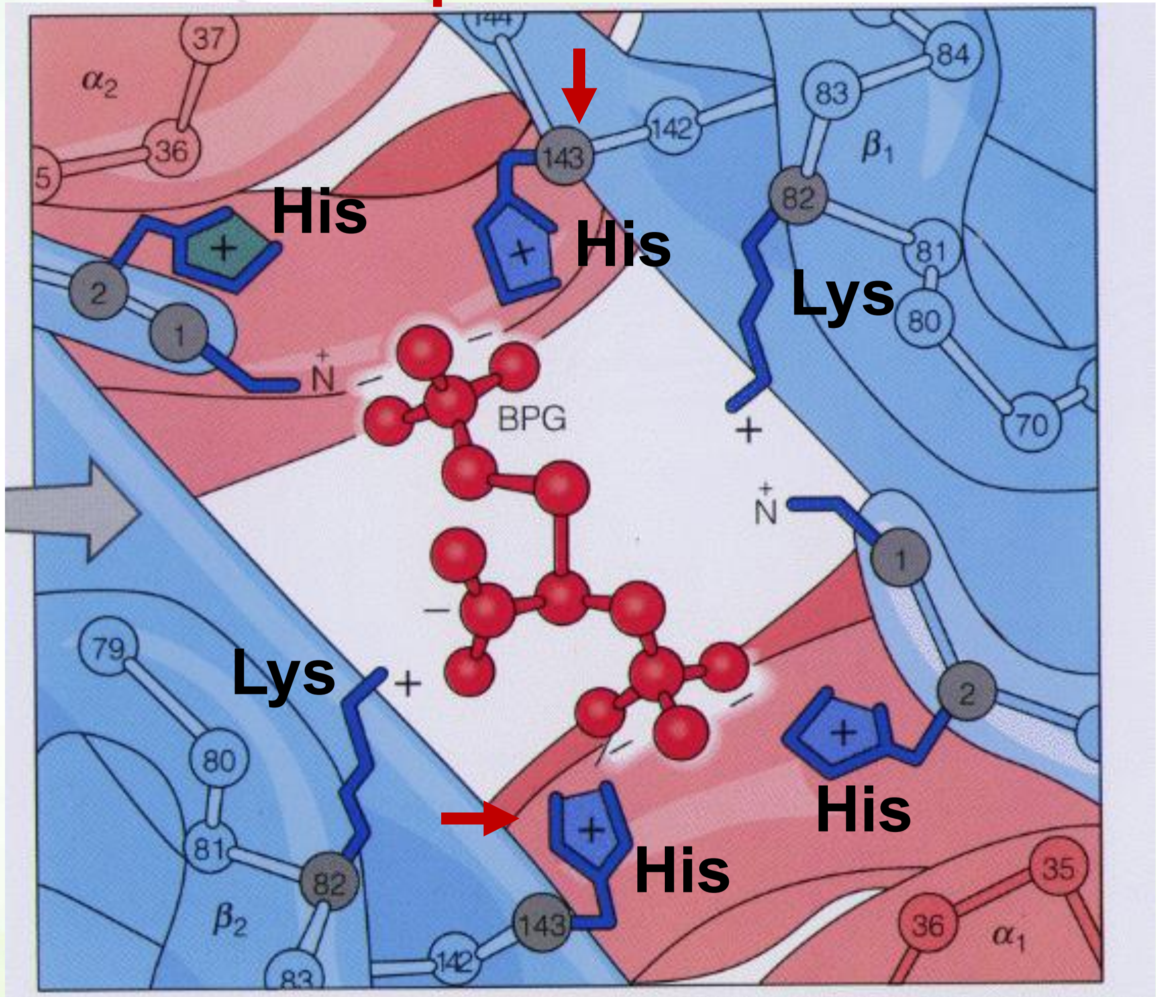


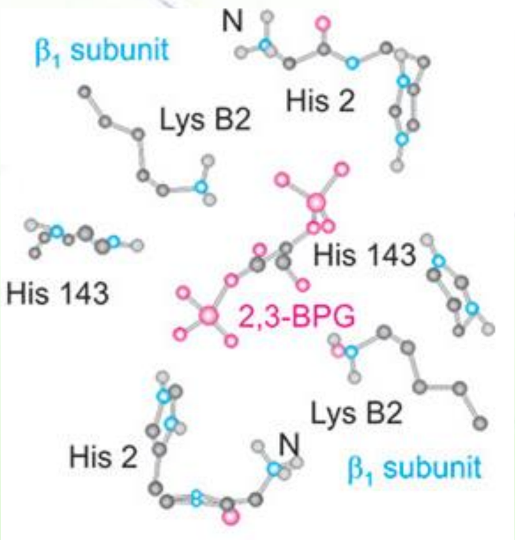


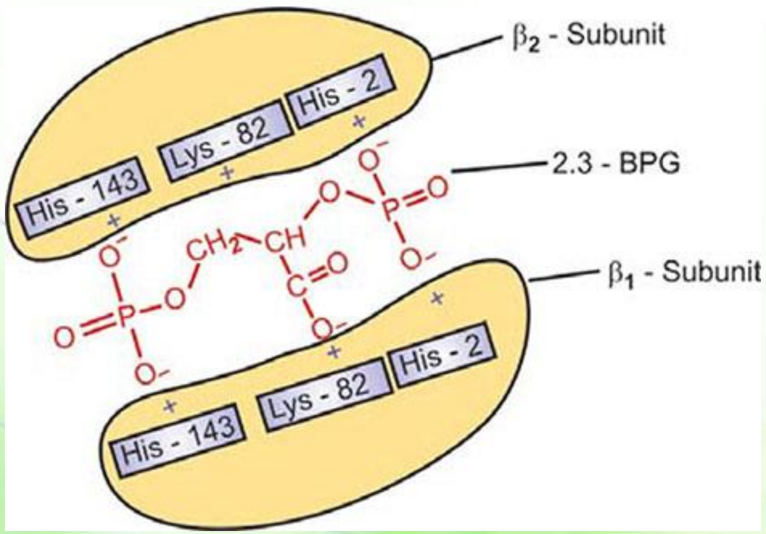
2,3-BPG and HbF

- 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.





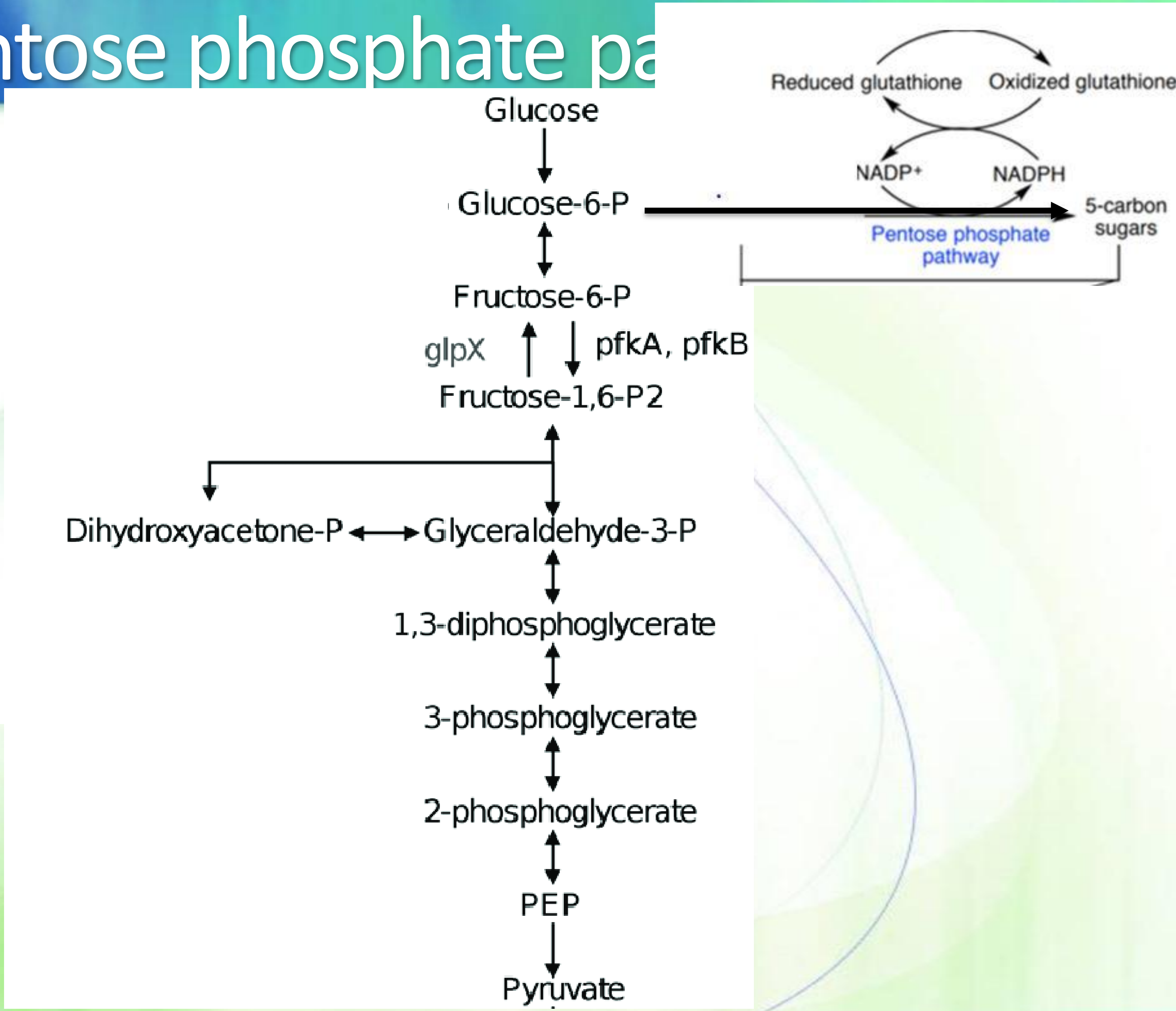


5- The pentose phosphate pathway→ NADPH and G6PD

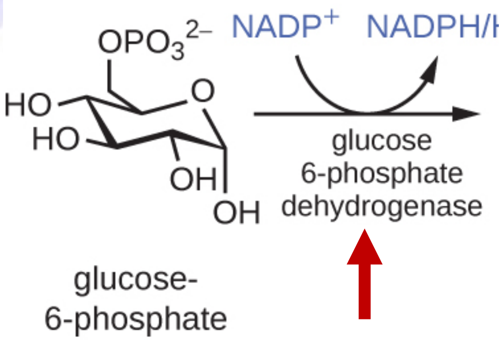
Two phases of pentose phosphate pa

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

$$\text{Glucose 6-phosphate} + 2\,NADP^+ + H_2O \longrightarrow \text{ribose 5-phosphate} + 2\,NADPH + 2\,H^+ + CO_2$$



The first step



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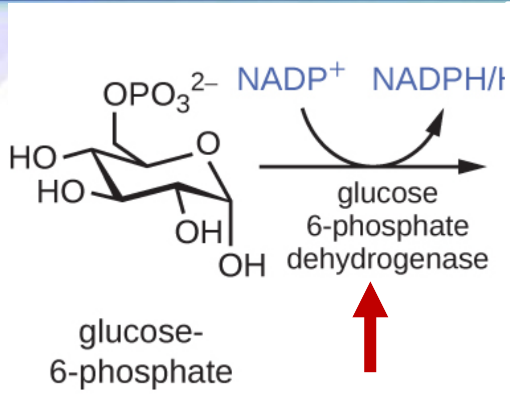

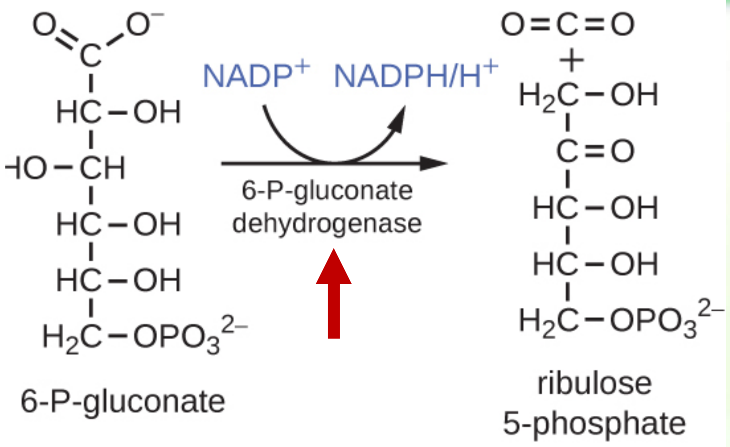

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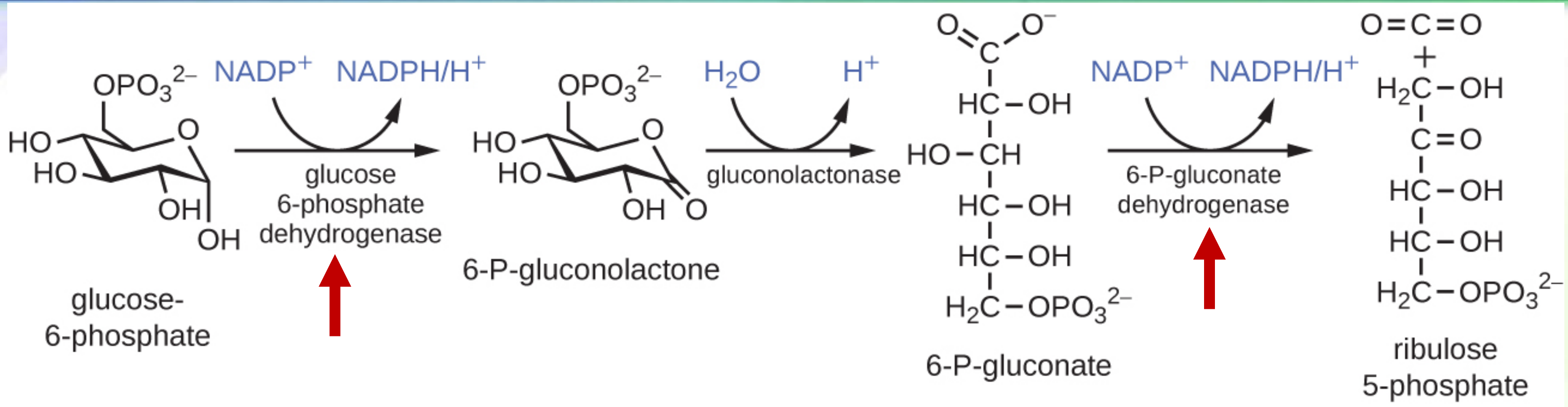
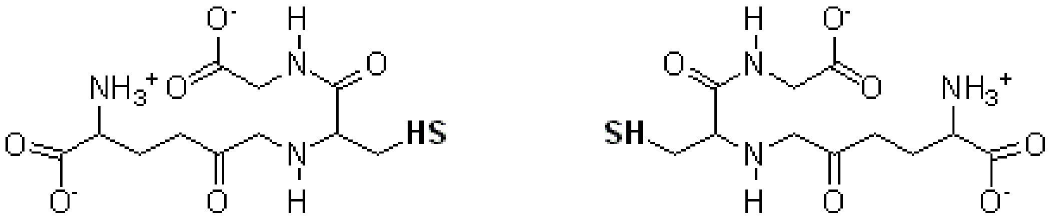


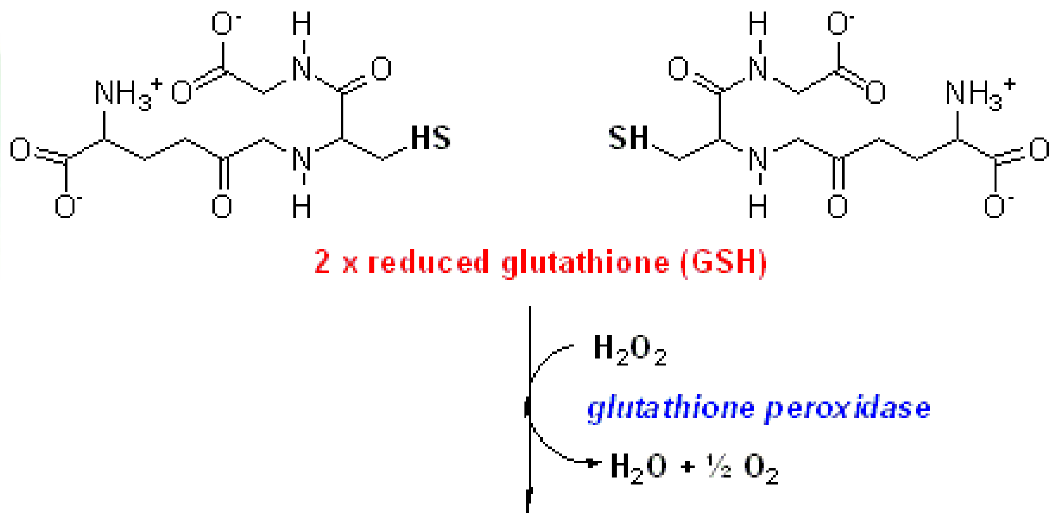
- The oxidative phase of the pentose phosphate pathway starts with the dehydrogenation of glucose 6-phosphate by glucose 6-phosphate dehydrogenase (G6PD).
- G6PD is highly specific for NADP+, relative to NAD+
- The reaction is irreversible and is the rate-limiting reaction.
- High levels of NADP+ stimulate the reaction .

Oxidative stress and glutathione



2 x reduced glutathione (GSH)

Oxidative stress and glutathione



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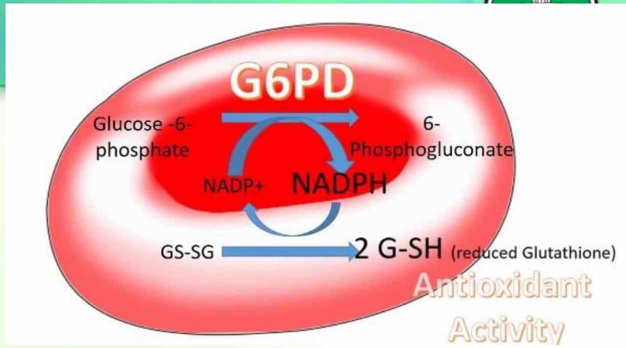

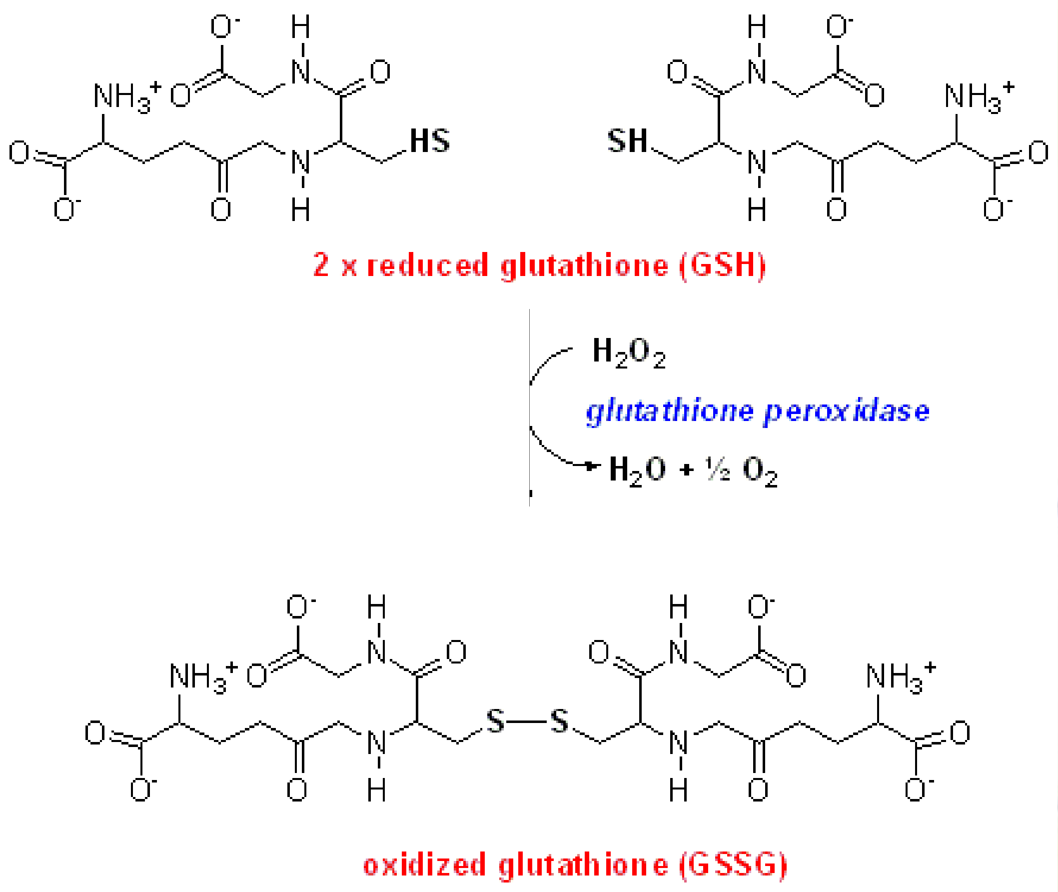

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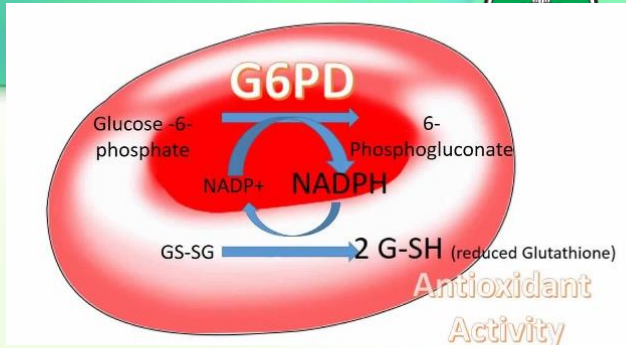
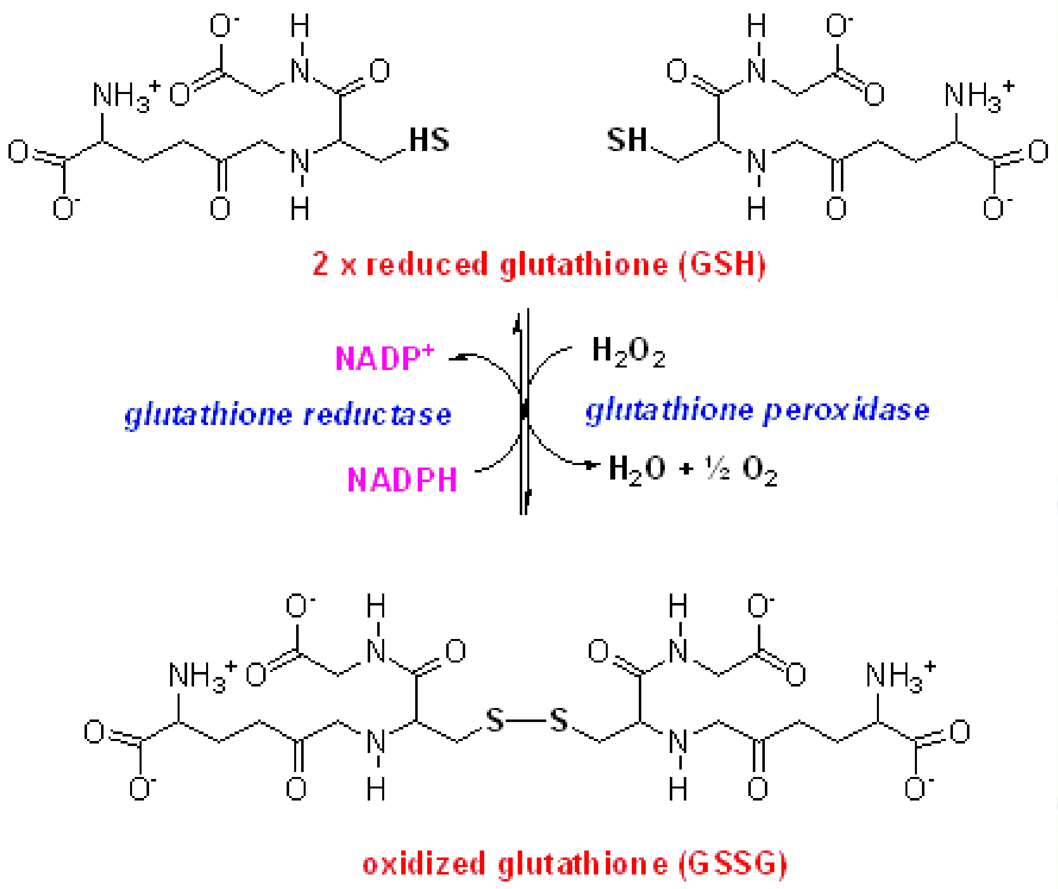


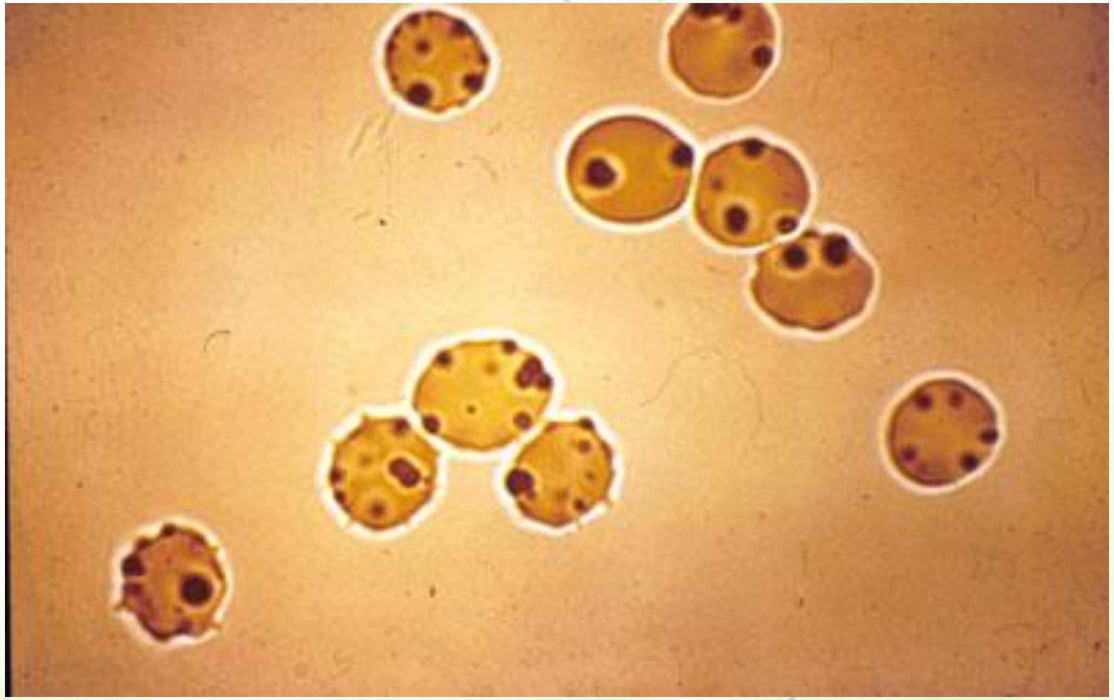
- **Oxidative stress within cells is controlled by the action of glutathione (GSH).**
- GSH reduces peroxides via glutathione peroxidase.
- GSH is regenerated via NADPH-dependent glutathione reductase.
- The PPP in erythrocytes is the only pathway to produce NADPH.

  PPP consumes almost 10% of glucose by erythrocytes.



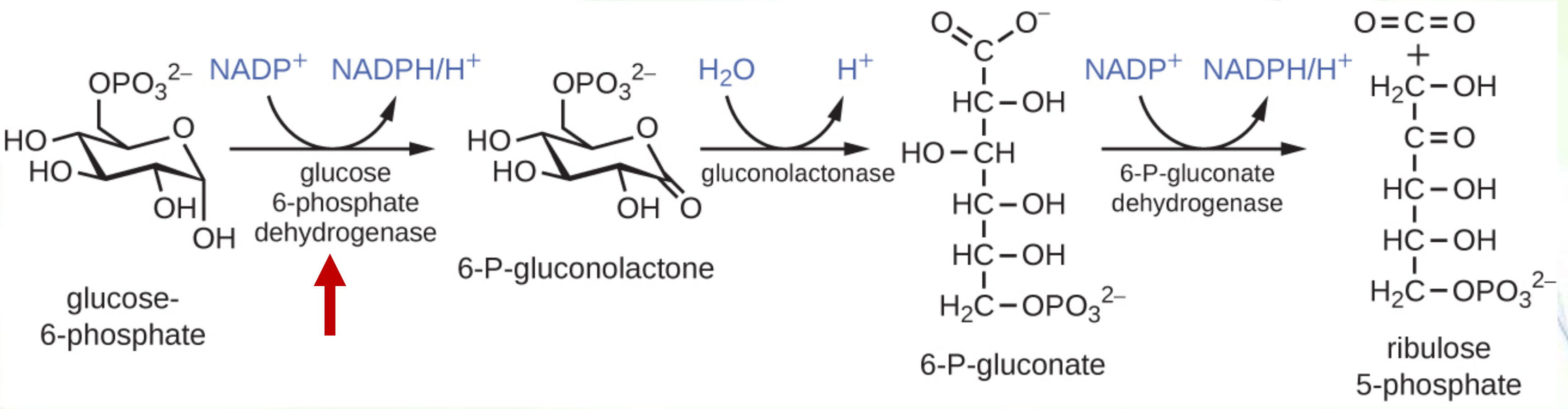
Low GSH levels

- 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



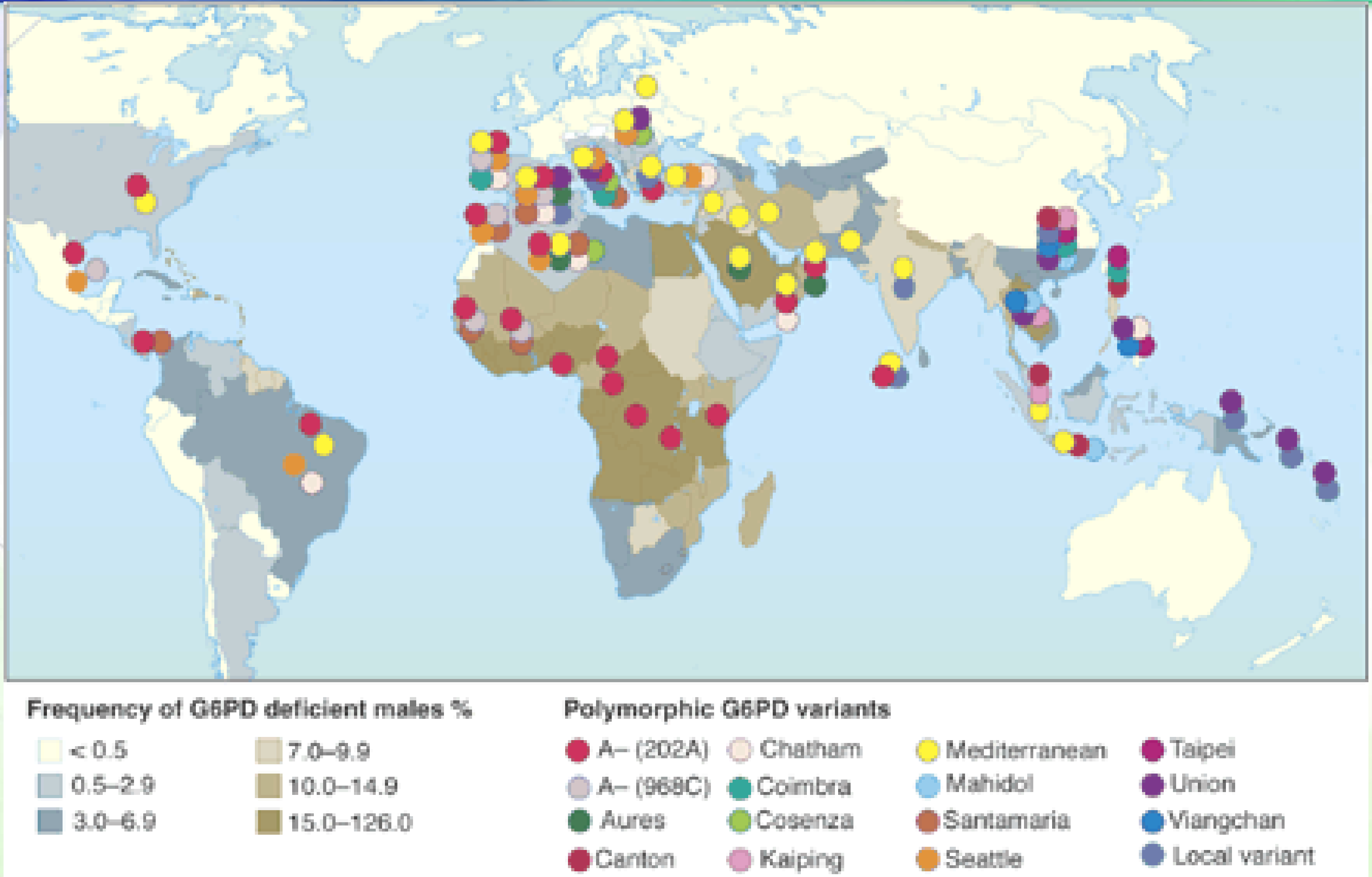
Glucose-6-phosphate dehydrogenase deficiency

G6PD deficiency



G6PD deficiency

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a group of heterogeneous disease with significantly reduced activity.
- 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 oxidization from the free radicals.



Frequency of G6PD deficient males %
< 0.5
0.5–2.9
3.0–6.9
7.0–9.9
10.0–14.9
15.0–126.0
Polymorphic G6PD variants
A– (202A)
A– (968C)
Aures
Canton
Chatham
Coimbra
Cosenza
Kaiping
Mediterranean
Mahidol
Santamaria
Seattle
Taipei
Union
Viangchan
Local variant

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

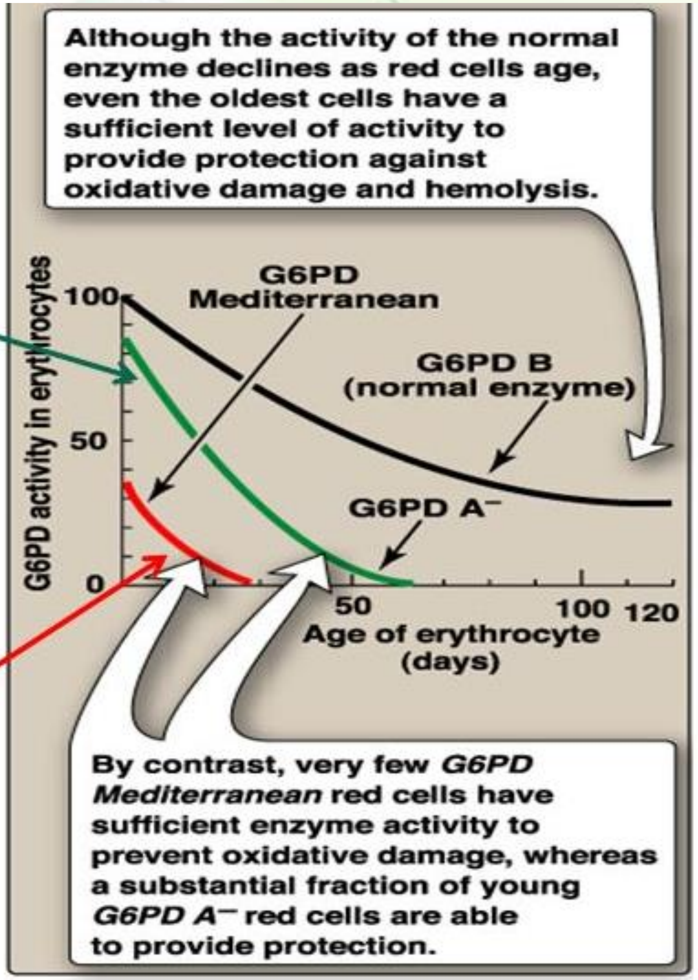
Class	Clinical symptoms	Residual enzyme activity
I	Very severe (chronic hemolytic anemia)	<2%
II	Severe (episodic hemolytic anemia)	<10%
III	Moderate	10%-60%
IV	None	>60%

- G6PD B (Normal)
- Abnormal G6PDs
  - Class IV: 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 10-60% of normal activity.
    - The disease is moderate.
  - G6PD Mediterranean (group II or class II)
    - Severe
    - The enzyme has normal stability, but negligible activity.
  - Class I: the most severe and rare.

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)

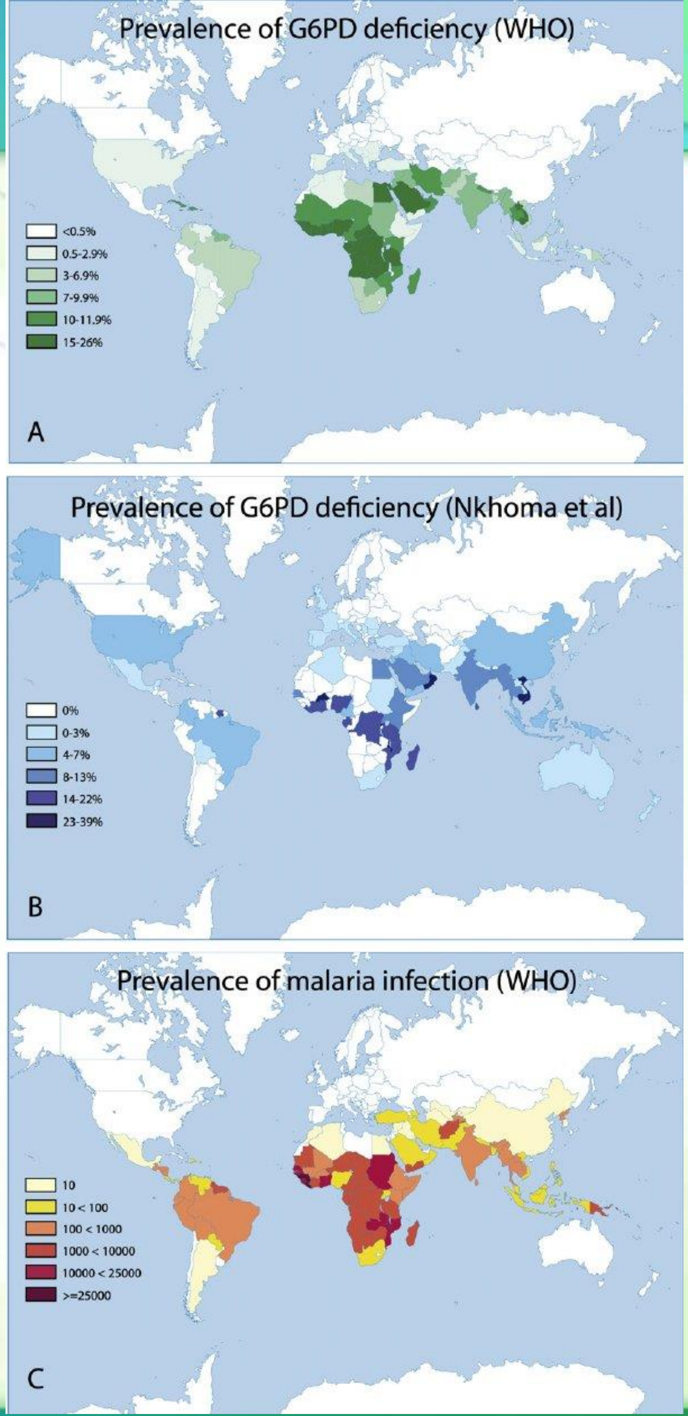


Inducers of G6PD deficiency symptoms

- 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.



- Glycolysis
  - 1- ATP→ Hemolytic anemia
  - 2- NADH→ Methemoglobin
  - 3- Pyruvate kinase→ Affects ATP production which could cause anemia, and affects 2,3 BPG production.
- 4- 2,3 bisphosphoglycerate→ R to T state
- 5- Pentose phosphate pathway
  - A- NADPH→ For removal of peroxidases and free radicals
  - B- G6PD→ Produces NADPH