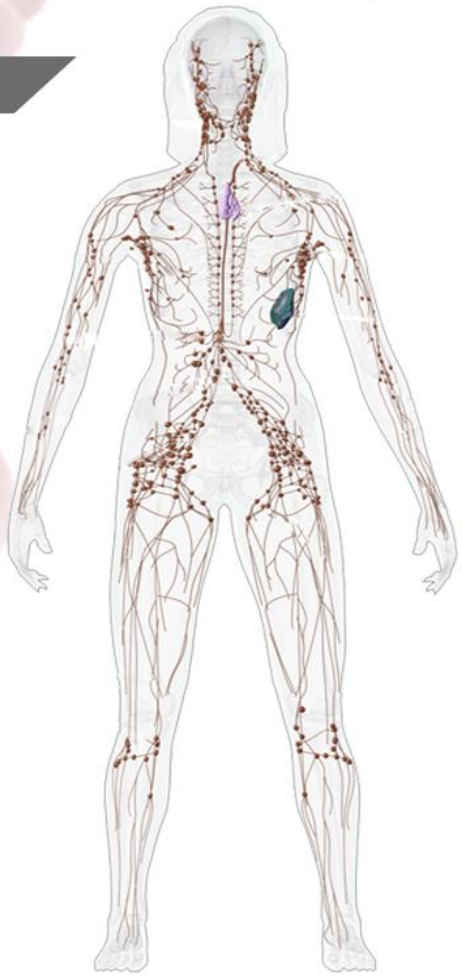
Sheet 4

Hematology and Lymphatic system

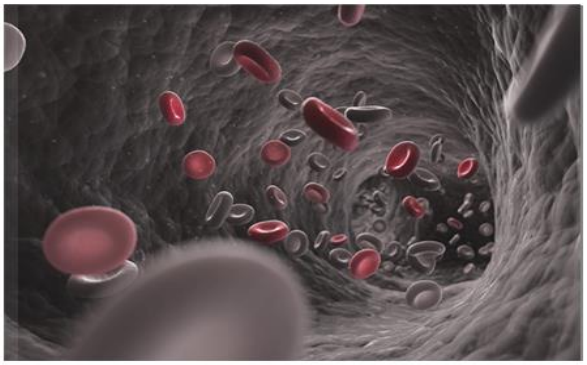
Subject | Biochemistry



Done by | Tala Saleh

Corrected by | Razi Kittaneh

Doctor | Ma'moun



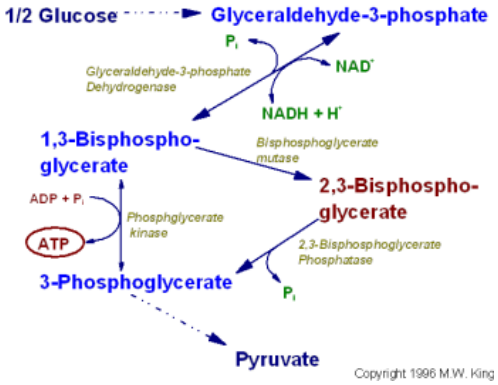
We will discuss different metabolic pathways and metabolites associated with carbohydrates metabolism in RBCs:

2,3-bisphosphoglycerate (2,3-BPG), Glycolysis, G6PD, and PPP.

1- 2,3-Bisphosphoglycerate (2,3-BPG)

Generation of 2,3-BPG

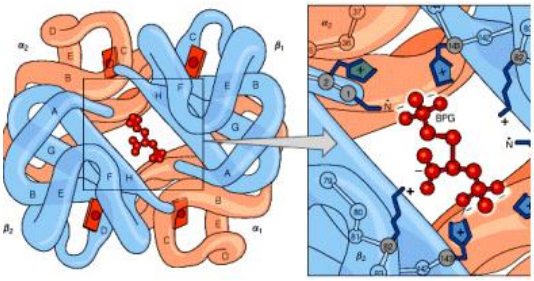
- 2,3-bisphosphoglycerate (2,3-BPG) is a **byproduct** of the glycolytic pathway derived from the intermediate 1,3-bisphosphoglycerate.
- It can **re-enter** the glycolytic pathway. However, the erythrocyte would gain **1 ATP** instead of 2 'a problem of energy wasting'.



Effect of 2,3-BPG on Hemoglobin (Hb)

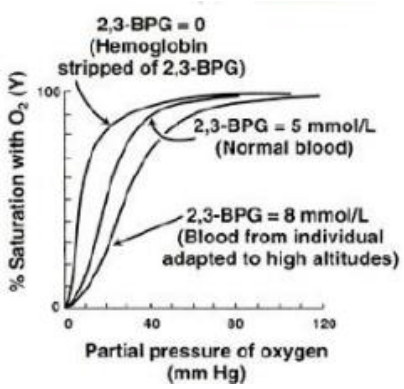
- 2,3-BPG occupies the **center** of deoxygenated Hb where it interacts and bonds with several groups including a lysine, His143, His2, and N-terminal ends.

  This contributes in **stabilizing** the Hb in the **T** structure **lowering** its **affinity to O_2**.



- When 2,3-BPG is not available (not bound), Hb can be easily converted to the **R-structure** thus **increasing** the affinity of Hb to O_2.

  ⇨ **BPG** is considered an **allosteric inhibitor** of Hemoglobin. Notice the **shift** to the **right** in the curve when 2,3-BPG is **bound**; p50 is **higher** indicating **decreased** affinity.



- **Fetal hemoglobin** (HbF) does **not** contain **His143,** instead **serine** is present in the γ chain *(this is a variant not a mutation).* This causes 2,3-BPG to have a much **weaker** interaction with **HbF** compared to **HbA** *(the adult form of hemoglobin).* As a result, **HbF** has a **stronger affinity t**o oxygen.

2- Glycolysis

Main purpose

- Glycolysis is the main metabolic pathway in erythrocytes; as there is **no mitochondria** in them. Therefore, it is the **only source of energy**.
- Glycolysis provides:

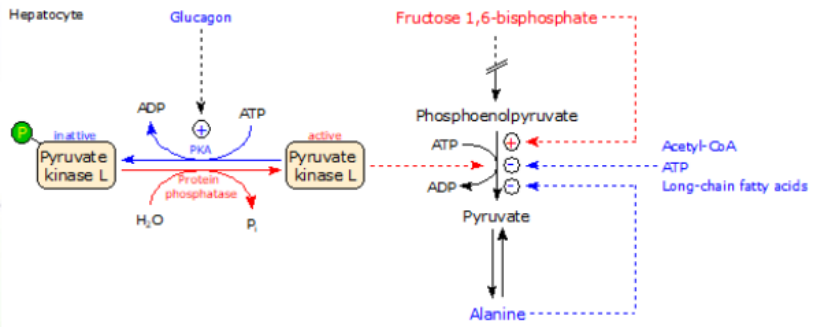
1- **NADH, for reducing methemoglobin back to hemoglobin**.
Methemoglobin is a hemoglobin in which the iron in the heme group is in the oxidized ferric state Fe^{+3}.

2- **ATP, for:**
   - **a-** Sugars and proteins
   - **b-** Membrane asymmetry
   - **c-** Membrane ion pumps
   - **d-** Cytoskeletal proteins; to maintain the discocyte shape, which is critical for the optimal viability and functional capacity.

Pyruvate kinase isozymes and regulation

- In the last steps of glycolysis, PEP is converted into Pyruvate generating **ATP**. This important reaction is catalyzed by **Pyruvate Kinase (PK)**.
- PK has 4 different isozymes:

1- **The liver (PKL) and erythrocyte (PKR) isoenzymes** are produced from the **PKLR gene.** They are **inhibited** by phosphorylation by **protein kinase A**, **ATP**, **acetyl-CoA**, **alanine**, and **long-chain fatty acids** and are **activated** by **F1, 6-BP.**



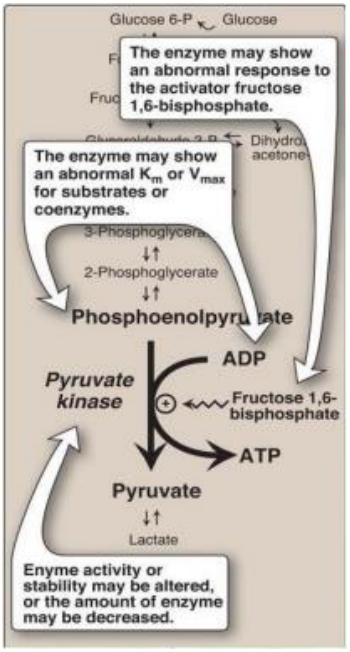
The liver isozyme (PKL) is also controlled at the level of **synthesis**, in which increased **carbohydrate** ingestion **induces** the synthesis of PK.

2- **The muscle and brain (PKM1), fetal and most tissues (PKM2) isoenzymes** are produced by the **PKM gene.** This gene is **not** inhibited by PKA.

3- **Fetal PK.** In erythrocytes, the **fetal PK** isozyme has much **greater activity** than the **adult isozyme**.

- Since the reaction is going **fast** (higher activity), fetal erythrocytes have **lower** concentrations of glycolytic intermediates including 1,3-BPG, and 2,3-BPG, contributing to a **higher** affinity to oxygen as discussed before.

PK deficiency



- Different mutations may affect PK's stability, affinity, etc.
- Genetic diseases of adult erythrocyte PK where the kinase is virtually inactive causes the erythrocytes to have a greatly **reduced** capacity to make **ATP**, which causes hereditary **hemolytic anemia**.
- The severity of the disease depends on the degree of **enzyme deficiency** (5-35%) and ability to **produce 2,3-BPG**.
- PK deficiency in **RBCs** has a **great** impact since they are **dependent** on it. However, the **liver** is **not** much affected; because the liver compensates by producing energy from **other** pathways. Also, PK's expression is **stimulated** when deficient in the **liver**.

3- The pentose phosphate pathway

Two phases of PPP:

1- The oxidative phase:

Consists of **irreversible** reactions generating **NADPH** molecules. It starts with the dehydrogenation of glucose 6-phosphate by glucose 6-phosphate dehydrogenase (**G6PD**) forming, eventually, ribose 5-phosphate. This is the **only way** RBCs form NADPH.

⇨ G6PD catalyzes the **first step** in the oxidative phase which is the **rate limiting step**.
⇨ G6PD enzyme is **highly specific** for NADP+.
⇨ High levels of **NADP+ stimulate** the reaction, while **NADPH** causes **feedback inhibition**.

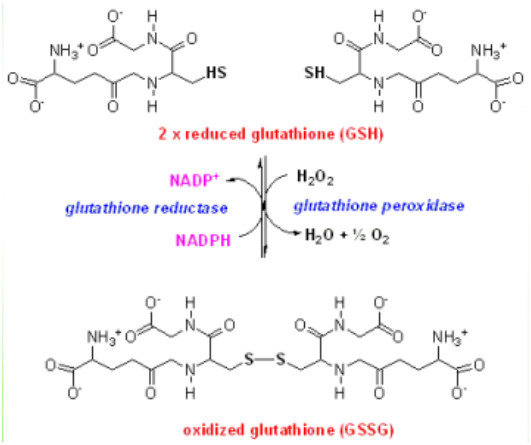
$$\text{Glu 6-P} + 2\ NADP^+ + H_2O \rightarrow \text{Ribose 5-P} + 2\ NADPH + 2\ H^+ + CO_2$$

2- The non-oxidative phase:

Reversible reactions where sugars are interconverted forming **5 carbon** sugars with **no** production of NADPH.

Oxidative stress and glutathione

- **NADPH** in erythrocytes are **necessary**, and the **only way**, for controlling oxidative stress.
- Glutathione (**GSH**) reduces **peroxides** via **glutathione peroxidase** → GSH is then regenerated via NADPH-dependent glutathione reductase.
- PPP consumes almost 10% of glucose in erythrocytes.
  - ⇨ Since PPP is the only source of NADPH in erythrocytes, this shows how important G6PD is, which catalyzes the first step in PPP.

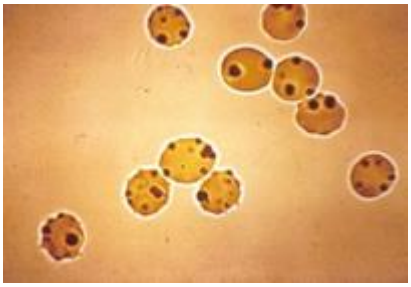


Low GSH levels

The inability to maintain **reduced glutathione** in RBCs leads to **increased** accumulation of peroxides, predominantly H_2O_2, resulting in:

a- Weakening of the cell membrane and associated hemolysis

b- Increasing rates of oxidation of hemoglobin to **methemoglobin** and other proteins including membrane proteins **insolubilizing them**, where proteins would then cluster forming **Heinz bodies**, and attach to the plasma membrane weakening it causing **hemolytic anemia**.



G6PD deficiency

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a group of heterogeneous disease with significantly reduced activity.
- G6PD is the most common enzyme deficiency **worldwide**. It is prevalent among individuals of African, Mediterranean, and Oriental ethnic origins.

- G6PD gene is located on the **X chromosome**, thus it is more prevalent among **males**. Inheritance of G6PD deficiency is **sex-linked.**
- It induces **Hemolytic anemia**, particularly after the administration of drugs, during infections, and in the neonatal period (jaundice).

G6PD mutations

- Several hundred G6PD genetic variants have been identified, but most have no clinical symptom.
- G6PD is **critical for life**, therefore, large deletions or frameshift mutations would cause **death**. That's why 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.

The four classes of G6PD deficiency:

- Normal enzyme → **G6PD B variant**
- Abnormal enzymes → G6PDs:

1- **Class I:** are most severe and rare.

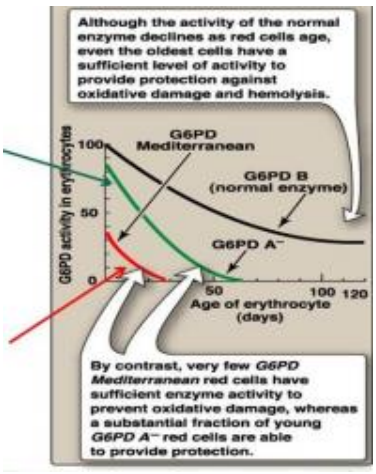
2- **Class IV:** mild with no clinical symptoms.

3- **Class III (G6PD A-):** moderate and common among persons of African descent. It is caused by a single amino acid substitution of Asn to Asp that **decreases enzyme stability** but remains **kinetically normal**. Young RBCs contain 5-15% of normal enzyme 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%

4- **Class II (G6PD Mediterranean):** severe where the enzyme has **normal stability**, but **negligible activity** affecting **all** cells; young and old.

- **Normally (G6PD B),** starts with a maximal activity which declines with age. However, even the oldest cells would still have enough level of activity to provide protection.
- A fraction of young **G6PD A-** cells are able to provide protection against oxidative stress.
- Whereas, very few **G6PD Mediterranean** cells have the activity to provide such protection.



Inducers of G6PD deficiency symptoms 'factors that would make it worse'

1- **Oxidant drugs:**
   Antibiotics, anti-malarial, and anti-pyritic (not acetaminophen).

2- **Fava beans (favism):**
   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.

   Fava beans are presumed to cause **oxidative damage** by an unknown component

3- **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.

Good Luck