EMATO
YMPHATIC
YSTEM

BIOCHEMISTRY

Sawsan alqeam+018 sheet

Writer: Anwar Abdullah

Corrector: Ma’moun ahram

Doctor:

In this sheet we will go through the major metabolic pathways in the RBCs :

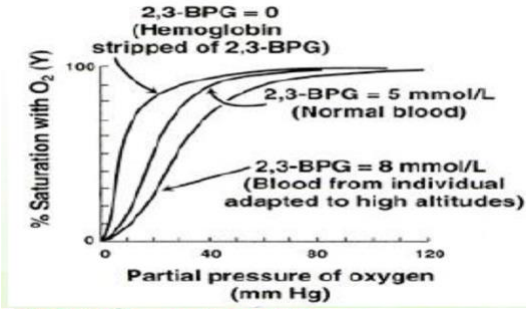
1. Glycolysis
2. Pentose phosphate pathway PPP

Let's start our journey with a revision of glycolysis pathway products and there importance to the erythrocytes and the whole system :

1. ATP:
   a. Modifying sugars and proteins.
   b. Maintaining membrane asymmetry.
   c. Function of membrane ion pumps .
   d. Modifying and regulating cytoskeletal proteins .
2. NADH:
   NADH is important for the reoxidization of methemoglobin into haemoglobin which is catalysed by methemoglobin's re-educates.
3. 2,3-BPG:
   Highly negatively charged molecule interacted with the positively charged amino acids like histidine and lysine in the centre of deoxygenated haemoglobin and stabilise the T state and reduce the affinity to oxygen.

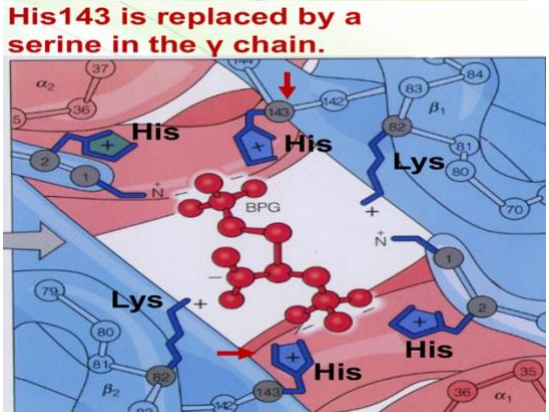
Now let's talk about the effect of 2,3-BPG on the affinity to oxygen:

1. When there is no 2,3-BPG :Hb will be in the R State which increase the affinity for O2.
2. When the concentration of 2,3- BPG is higher than the normal , we can see the curve is shifted to the right which means the affinity is reduced



In adult hemoglobin Hb A, 2,3-BPG interacts with lysine ,histidine 143,histidine 2 and N termini of beta chains.

2,3-BPG interacts with the fetal hemoglobin in a weaker Manner in the adult hemoglobin and that's because of the replacement that happened to the histidine 143 by serine in the beta chain which reduces the electrostatic interactions.



And now we are going to talk about pyruvate kinase :

As we know ,pyruvate kinase converts phosphoenolpyruvate to pyruvate yielding ATP.

There are 2 isoenzyme genes which produces two I so forms of PK gene

1. PKLR :
    a. PKL :liver
    b. PKR: Erythrocytes

Both PKL and PKR differs in the transcription start sites.

2. PKM:
    a. PKM1: in muscles and brain
    b. PKM2: in fetal tissue and most tissues of the body

PKM 2 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 (1,2-BPG , 2,3-BPG) >> high amounts of hemoglobin in R state >> higher affinity for oxygen.

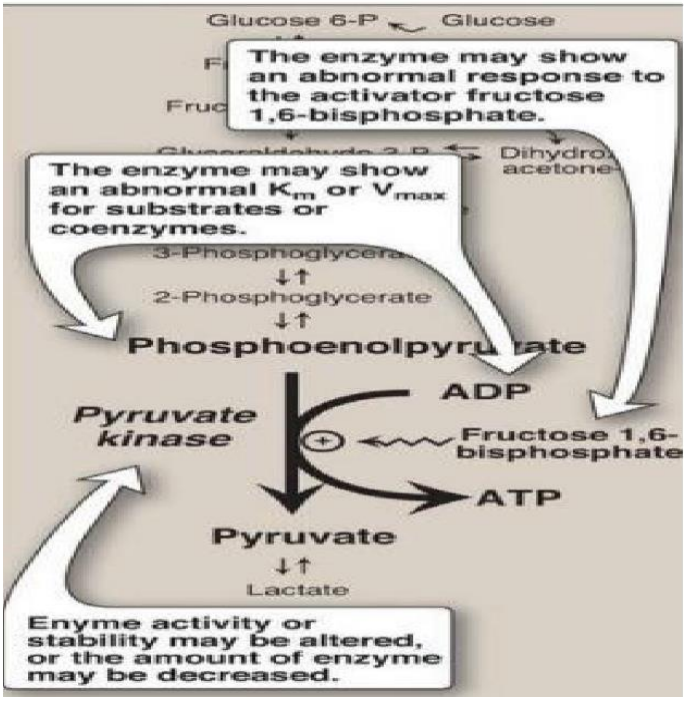
Pyruvate kinase regulation

Both PKL and PKR are allosterically regulated:

- Activated by : fructose 1,6-BP
- Inhibited by : acetyl co-A , ATP , alanine ,long chain fatty acids (when their levels are high indicates high levels of energy so it will inhibit the glycolysis by inhibiting PK)
- It's also inhibited by phosphorylation of Protein kinase A

High glucagon levels >> activate the phosphorylation of PKA >> down regulation of PK

Only PKL controlled at the level of synthesise



Pyruvate kinase deficiency:

- It is a hereditary genetic disease (single point mutation) reduces the erythrocytes ability to produce ATP leading to haemolytic anemia.
- The severity of the disease depends on how much the enzyme is deficient.
- When we say the enzyme deficiency is 50% we mean that the enzyme is active by 50% of its normal capacity but no symptoms will appear
- And when we say the enzyme deficiency 5-35% then symptoms will appear.
- Liver is not affected since expression is stimulated (synthesis covers for deficiency)

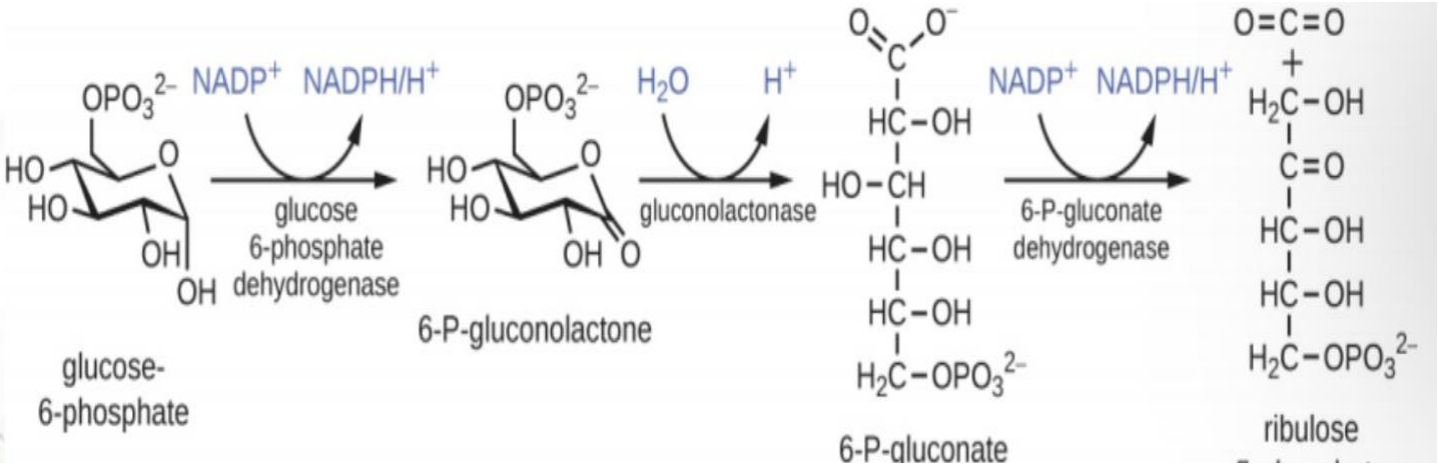
Now we are going to talk about the next major metabolic pathway which is **pentose phosphate pathway:**

As we know PPP has two phases , oxidative and non-oxidative phases

1. Oxidative phase : it's main purpose is to convert Glucose-6-p to Ribulose- 5- p which eventually will be converted to ribose -5- phosphate
2. As we know it's an irreversible reaction and the main purpose of it is to produce NADPH.

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

- The first RXN is the most important one in the PPP which is catalysed by glucose -6-phosphate dehydrogenase which is highly regulated.
- G6PD has a high affinity and is highly specific for NADP+ relative to NAD+.
- High levels of NADP+ increases the activity of the G6PD.



Non-oxidative Phase:

This phase main purpose is to convert ribulose -6-p to different type of sugars including G6P.

*Why NADPH is important?

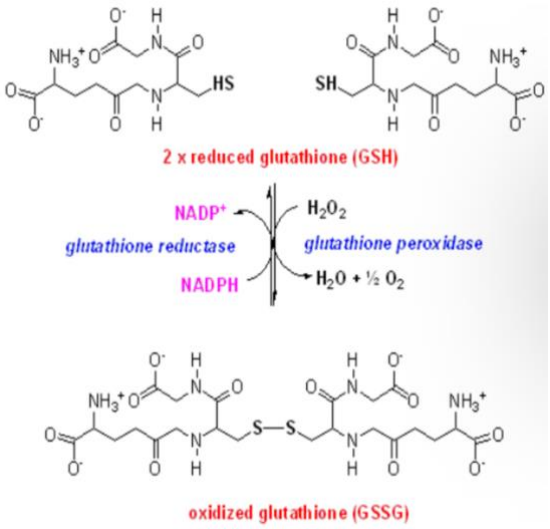
It's responsible of regeneration of glutathione (GSH).

- Glutathione is important to control the oxidative stress in the cell.
- Two GSH reduce hydrogen peroxide H2O2 by glutathione peroxidase into oxygen and water ,producing oxidised glutathione molecule, in which these two molecules are connected by disulphide bond.
- In order for RBCs or any other cells to fight more of these oxidising agents the reduced glutathione must be regenerated by NADPH- dependent glutathione reductase.
- The source of electrons is NADPH.
- PPP is the main source of NADPH in RBCs and consumes about 10% of glucose by erythrocytes.

What if we have low GSH levels in RBCs ?

Increased accumulation of peroxide resulting in:

- Weakening of the cell membrane and concomitant hemolysis because H2O2 will start to oxidise fatty acids in plasma membrane
- Increasing rates of oxidation of hemoglobin to methemoglobin and other proteins insolubilizing them forming HEINZ BODIES which weakening the cell membrane.



Glucose 6 phosphate dehydrogenase deficiency

- Is most prevalent in individuals of African Mediterranean and oriental ethnic origins.
- Is the most common enzyme deficient world wide.
- Inheritance of G6PD deficiency is sex linked because G6PD gene is carried on X chromosome.
- Heterogeneous disease with reduced activity.
- It induces haemolytic anemia ;due to compromised plasma membrane by high level of H2O2 due to the low levels of GSH , **WHEN?**
  1.after the administration of drugs
  2. During infections
  3. In neonatal period.

G6PD mutations

- Several G6PD variants identified but most of the are asymptomatic.
- Almost all G6PD deficiencies are caused by point mutations in the gene.
  ** the idea of point mutations in this gene rather than large deletion or frameshift will give you the importance of this enzyme to the body .
- Mainly these mutations alter the kinetic properties, stability or binding affinity to NADP+ or G6P .

WE HAVE FOUR CLASSES OF G6PD DEFICIENCY:

A. G6PD B is normal with 100% activity.

B. G6PD IS ABNORMAL

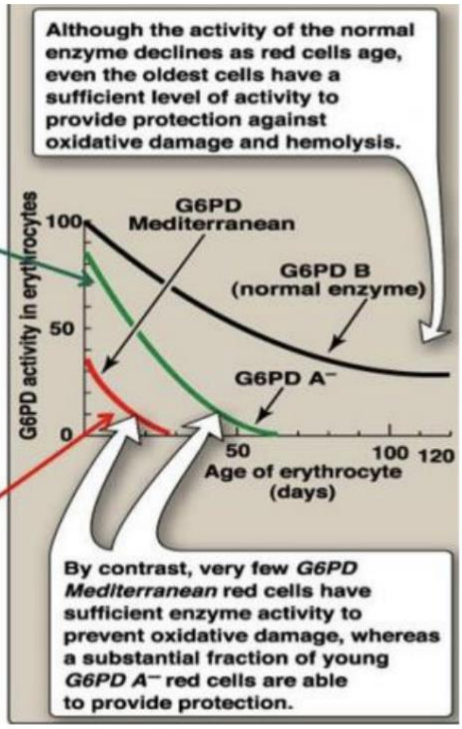
1. Class 1: most sever and rare (very little activity for G6PD) >> chronic haemolytic anemia.
2. Class IV: high activity of G6PD ,asymptomatic.
3. G6PD A (class III):
   *among people of African descent.
   * caused by single amino acid substitution (ASN to ASP) >> decreases enzyme stability with 5-15% activity.
   * the disease is moderate .

4. G6PD Mediterranean (class II):

   * common in Mediterranean people.

   * sever ; normal stability but negligible activity.

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)

✓Notice that in normal individual (in black)→as RBCs increase in age the activity of G6PD decreases.

✓In dass III→ the enzymatic activity is high, it reduces as RBCs increase in age but still high, that's why the severity of this condition is moderate.

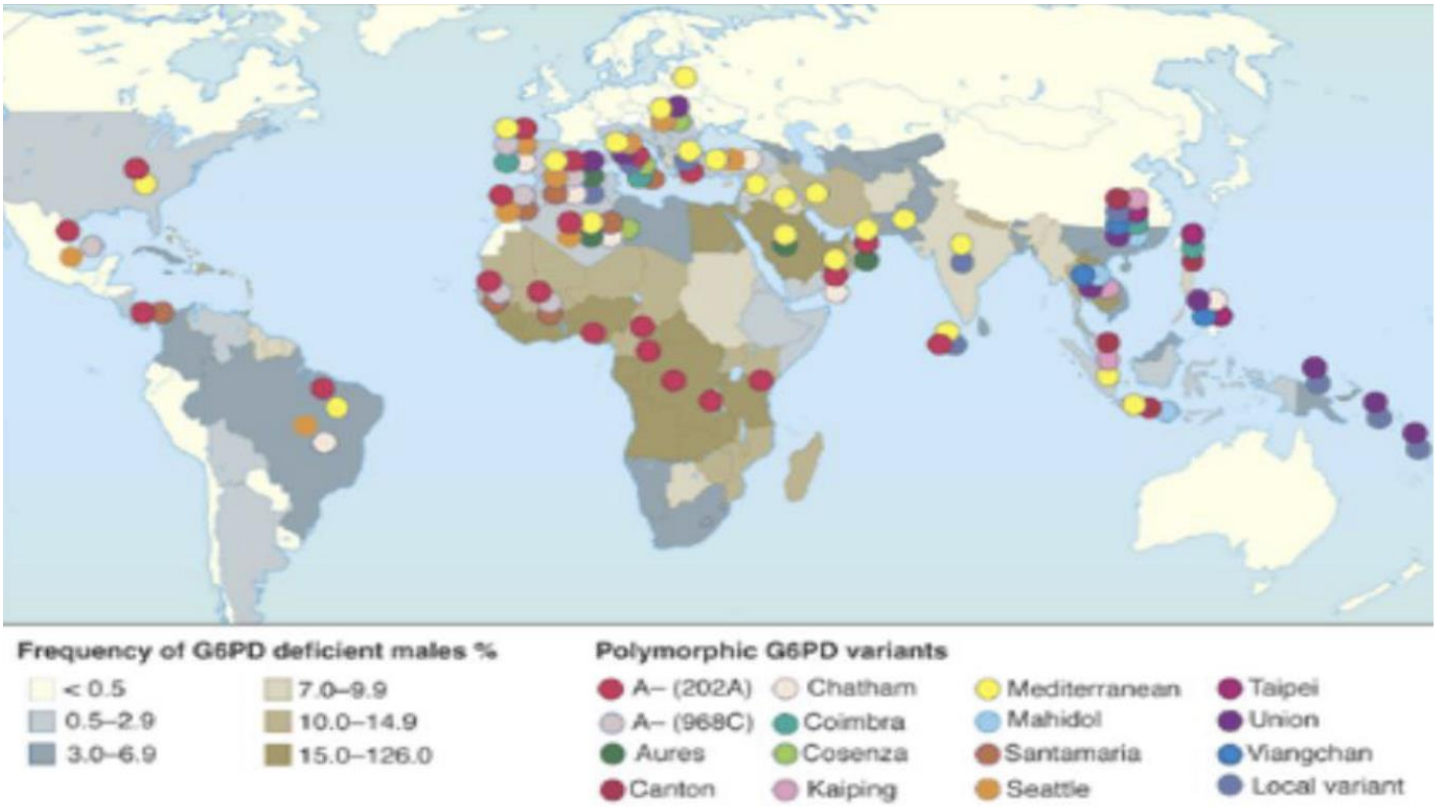
✓ In dass II→ although the enzymatic stability is high, its activity gets reduced early on as RBCs increase in age.

❖ **Inducers of G6PD deficiency symptoms:**

- **Oxidant drugs:**
  Antibiotics, anti- malarial drugs ,anti- pyritcs >> can exaggerate symptoms.
- **Fava beans (favism):**
  - Substances capable of destroying red cell GSH have been isolated from fava beans (fool)→ reducing the half-life of RBCs.
  - 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.
- **Infection:**
  The most common inducer due to the production of free radical by immune system.

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.



Distribution of G6PD deficiency.

Good luck , wish you all the best