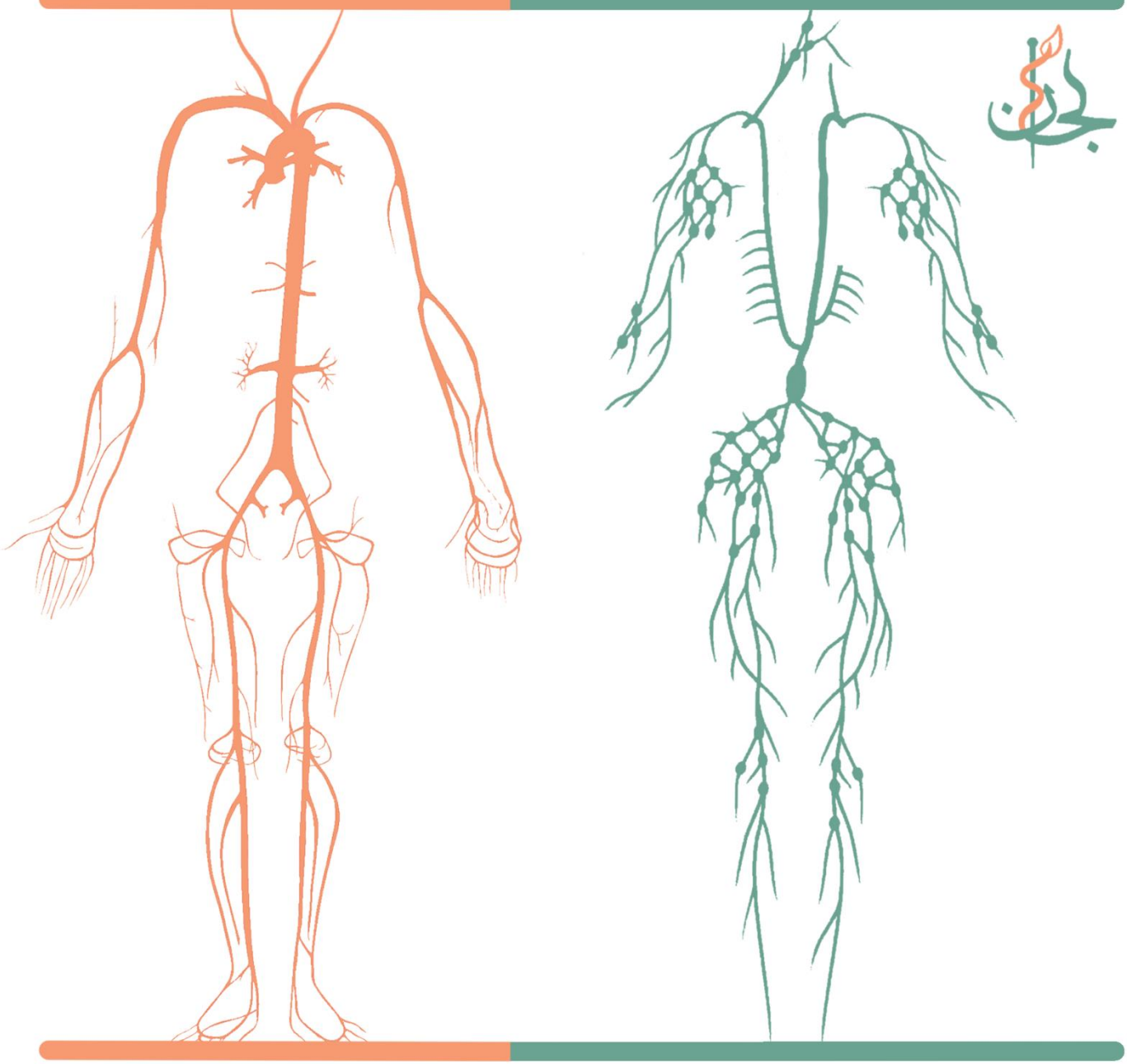
Biochemistry

HematoLymphatic



Title: Sheet 4 – Metabolism in erythrocytes

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In this sheet we are going to discuss the major metabolic pathways in erythrocytes and their importance for the cell and the whole system.

1) Glycolysis → Produces 2,3-bisphosphoglycerate, NADH and ATP.
2) Pentose Phosphate Pathway → Produces NADPH.

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

- ✓ 2,3-BPG is a byproduct of the glycolytic pathway and it's an isomer of the glycolytic intermediate 1,3-bisphosphoglycerate.
- ✓ Each **glucose** molecule will eventually give two molecules of 1,3-BPG which will be converted into two 3-Phosphoglycerate yielding two ATP.
- ✓ 2,3-BPG can be converted to 3-Phosphoglycerate but without gaining two ATP.
- ✓ Since 2,3-BPG is a highly negatively charged molecule, it interacts electrostatically with positively charged amino acids, like **histidine** & **lysine** in the center of deoxygenated hemoglobin which stabilizes the T state of Hb reducing its affinity for O_2.
- ✓ In the absence of 2,3-BPG (not bound) Hb will be in the R state more readily which increases the affinity for O_2 (looks like myoglobin O_2 saturation curve).
- ✓ In **adult hemoglobin** HbA, 2,3-BPG interacts with lysine, His 143, His 2 and N-termini of β chains.
- ✓ 2,3-BPG interacts with **fetal hemoglobin** HbF in a weaker manner than HbA, and that's due to His 143 in the β chain of HbA being **replaced** by a serine in HbF which reduces electrostatic interactions.

B) NADH

NADH is important for the re-oxidation of **methemoglobin** into **hemoglobin** which is catalysed by NADH-Cytochrome b5 reductase (Methemoglobin reductase).

C) ATP

- ✓ Modifying sugars and proteins.
- ✓ Maintaining membrane asymmetry.
- ✓ Function of membrane ion pumps.
- ✓ Modifying and regulating cytoskeletal proteins.

Cytoskeletal proteins are important for changing the morphology of RBCs so they can squeeze into narrow capillaries and they also maintain RBC discocytic shape.

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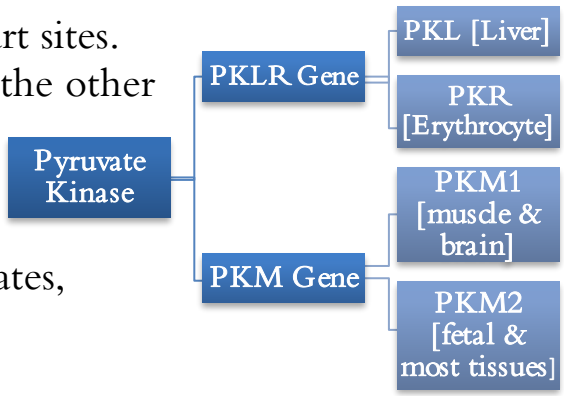
***We aren't required to memorize the glycolytic intermediates and enzymes.

❖ Pyruvate Kinase (PK)

- ✓ It converts **Phosphoenolpyruvate** into **Pyruvate** yielding **ATP**.
- ✓ 2 genes produce PK and each one of them can produce 2 isoforms.

- ✓ PKL & PKR differ in transcription start sites.
- ✓ PKM2 has much greater activity than the other adult isozymes, this increased activity in fetal tissues, including erythrocytes, leads to reduced concentrations of glycolytic intermediates, including 1,3-BPG & 2,3-BPG.



- ✓ Since fetal erythrocytes have lower 2,3-BPG, they have higher affinity for O_2 and more HbF in the R state.

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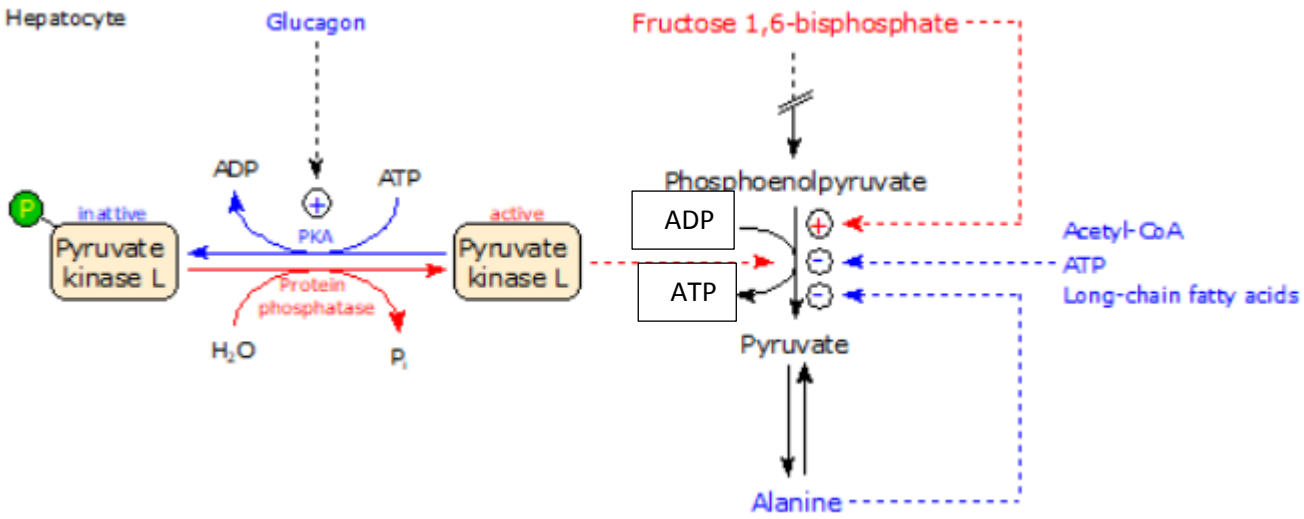
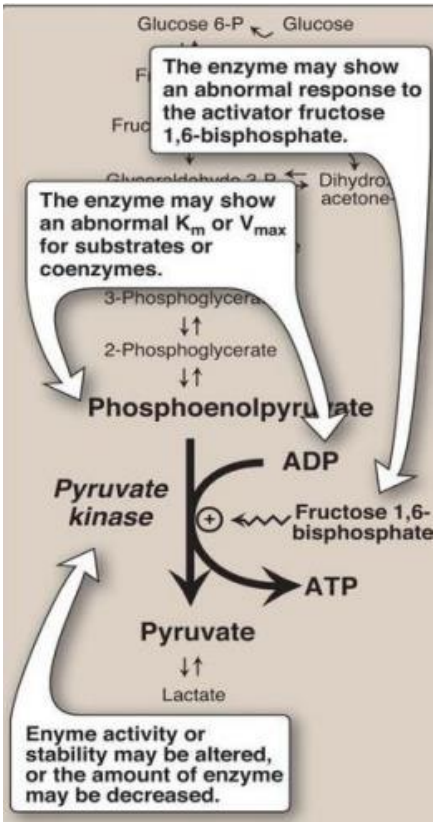
Pyruvate Kinase Regulation

-Both PKL and PKR are allosterically regulated as follows:

- ✓ **Activated** by ➔ Fructose 1,6-bisphosphate.
- ✓ **Inhibited** by ➔ Acetyl-CoA, ATP, alanine and long-chain fatty acids (high energy markers).
- ✓ **Inhibited** by ➔ Phosphorylation by Protein Kinase A (PKA) (high Glucagon activates PKA).



✓ Only the liver isozyme (PKL) is controlled at the level of synthesis:
➔ Increased carbohydrate ingestion induces synthesis of **PK**.



Pyruvate Kinase Deficiency

✓ It is a set of hereditary genetic diseases of adult erythrocyte PK (**PKR**) in which the kinase is **inactive**.
✓ They are mainly caused by **single point mutations**.
✓ Erythrocytes ability to produce ATP is greatly reduced →Hereditary hemolytic anemia.

✓ The **severity of the disease** depends on the degree of enzyme deficiency and ability to produce 2,3-BPG.
*5-35% of the normal activity then symptoms will appear.

✓ **Liver** is not affected since expression is stimulated (synthesis covers for deficiency).
✓ **Enzyme activity** is reduced because of inability to bind to substrate or activator, or due to decreased stability, so the **amount of enzyme is reduced.**

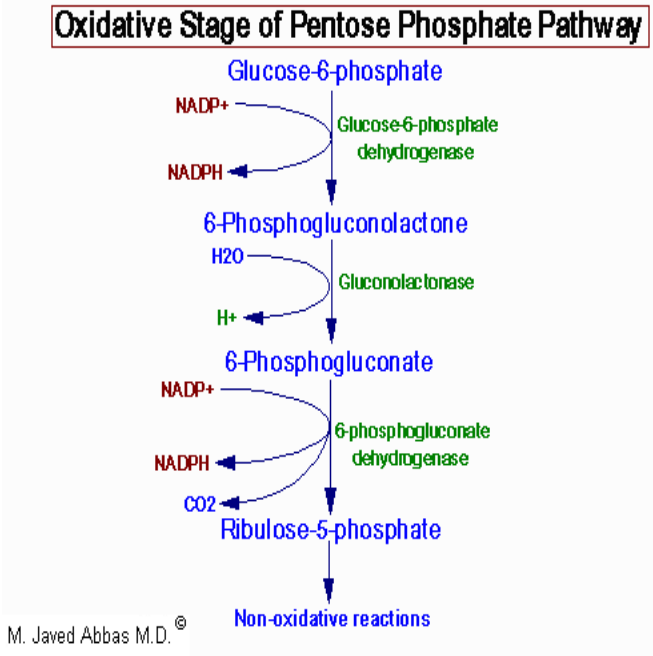
The pentose phosphate pathway

The PPP is divided into two phases:

1-Oxidative phase

✓ The main purpose of oxidative phase is to convert Glucose-6-phosphate to Ribulose-5-phosphate forming, eventually, it would be converted to ribose 5-phosphate.

✓The main purpose of these **irreversible** reactions is to produce NADPH.

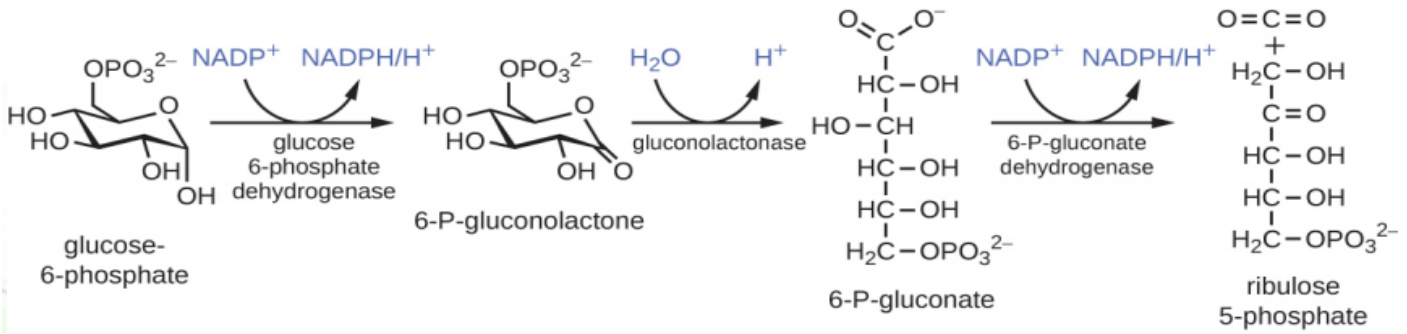


G 6-P + 2NADP+ + H2O → Ribose 5-P + 2NADPH + 2H+ + CO2

✓The first reaction **(rate limiting reaction)** is the most important reaction in PPP **(irreversible)**, which is catalyzed by the enzyme Glucose-6-phospate dehydrogenase **(highly regulated)**.

✓G6PD enzyme has a **high affinity** and is **highly specific** for **NADP+** relative to **NAD+**.

✓High level of **NADP+** increase the activity of the G6PD **(stimulate the reaction)**.



2-Nonoxidative phase

✓This phase coverts Ribulose-5-phosphate to different type of suger including Glucose-6-phosphate.

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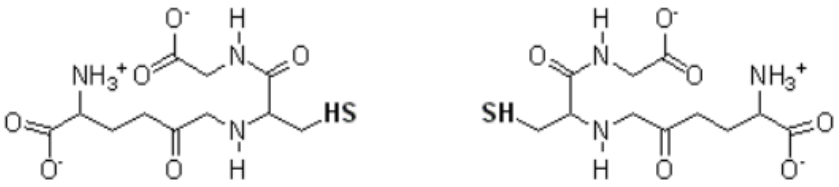
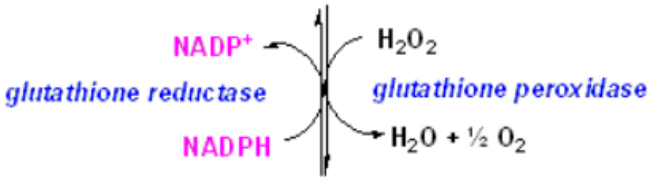
Why **NADPH** is important?

It is responsible of regenerating Glutathione (GSH).

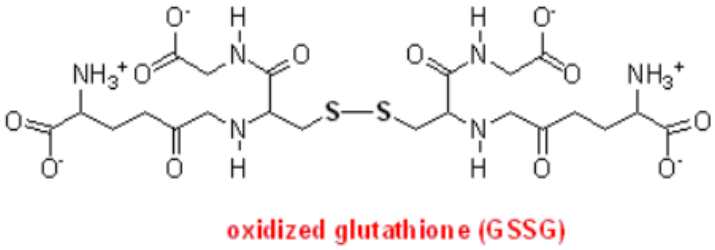
✓Glutathione control the oxidative stress within the cell, it's a small peptide molecule consists of 3 amino acids; Glycine, Cysteine and Glutamate.

✓ Two GSH reduce **Hydrogen peroxide(H2O2)** by Glutathione peroxidase into oxygen and water producing Oxidized glutathione (GSSG) molecule where these two molecules are connected to each other by a **disulphide bond**.





✓In order for RBCS or any other cells to fight more of these oxidizing agents the **Reduced glutathione (GSH)** must be regenerated by NADPH-dependent glutathione reductase.



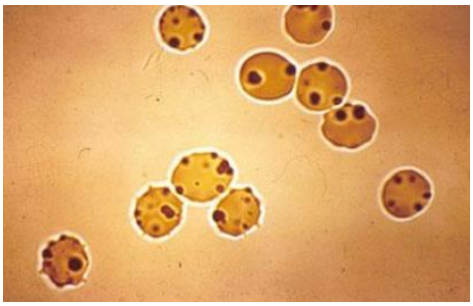
✓The source of electrons is **NADPH**; electrons are taken from **NADPH** to reduce the **oxidized glutathione** producing two molecules **(GSH).**

✓PPP is the main source of **NADPH** in RBCs; PPP consumes about almost 10% of glucose by erythrocytes.

✓ **What if you have low GSH levels in cells like erythrocytes?** (inability to maintain reduced glutathione in RBCs)
This leads to increased accumulation of peroxide, predominantly **H2O2, resulting in:**

- Weakening of the cell membrane and concomitant hemolysis of RBCs so the membrane would be compromised because H2O2 starts to oxidize fatty acids in plasma membrane.

- Increasing rates of oxidation of **hemoglobin** to **methemoglobin** (higher level of methemoglobin) and other proteins including membrane proteins, insolubilizing them forming Heinz bodies, weakening the cell membrane.



Heinz bodies

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Glucose-6-phosphate dehydrogenase deficiency

✓ 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**.

✓ It is a group of **heterogeneous diseases** with significantly reduced activity.

✓It induces Hemolytic anemia; plasma membrane is compromised by high level of H2O2 as a result of low level of GSH, 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.

✓Almost all **G6PD deficiency** variants are caused by point mutations in the gene rather than large deletions or frameshift mutations. This tell you something about the importance of this enzyme for the whole individual.

✓Mainly these mutations alter the kinetic properties, stability, or binding affinity to NADP+ or G6P.

The four classes of G6PD deficiency

a. G6PD B (Normal) → 100% activity.

b. Abnormal G6PDs:

- **Class I** are most severe and rare → it has a very little activity for G6PD, resulting in chronic hemolytic anemia.
- **Class IV**: no clinical symptoms→ it has high activity of G6PD with no symptoms.
- **G6PD A-** (group III or **class III**):

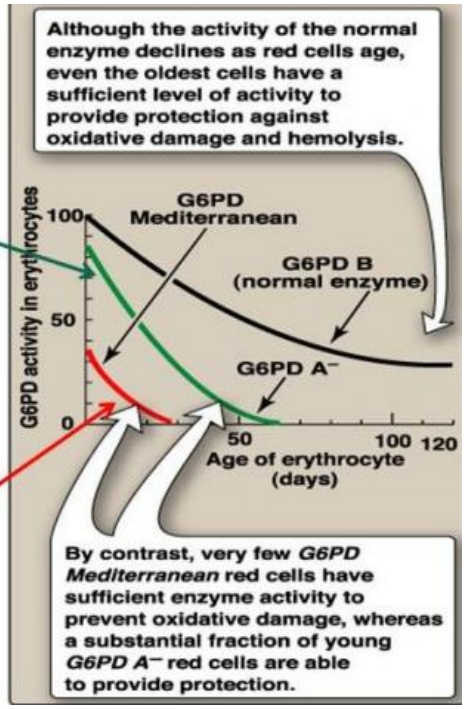
✓ Among persons of African descent.
✓ It is caused by a single amino acid substitution of **Asn** to **Asp** that decreases enzyme stability, but 5-15% of normal activity.
✓ The disease is **moderate.**

- **G6PD Mediterranean** (group II or **class II**):

✓ Common in Mediterranean individuals.
✓ **Severe**.
✓ The enzyme has 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.

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Inducers of G6PD deficiency symptoms

✓Oxidant drugs:

-Antibiotics, anti-malarial, and anti-pyritcs (not acetaminophen)→these 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.

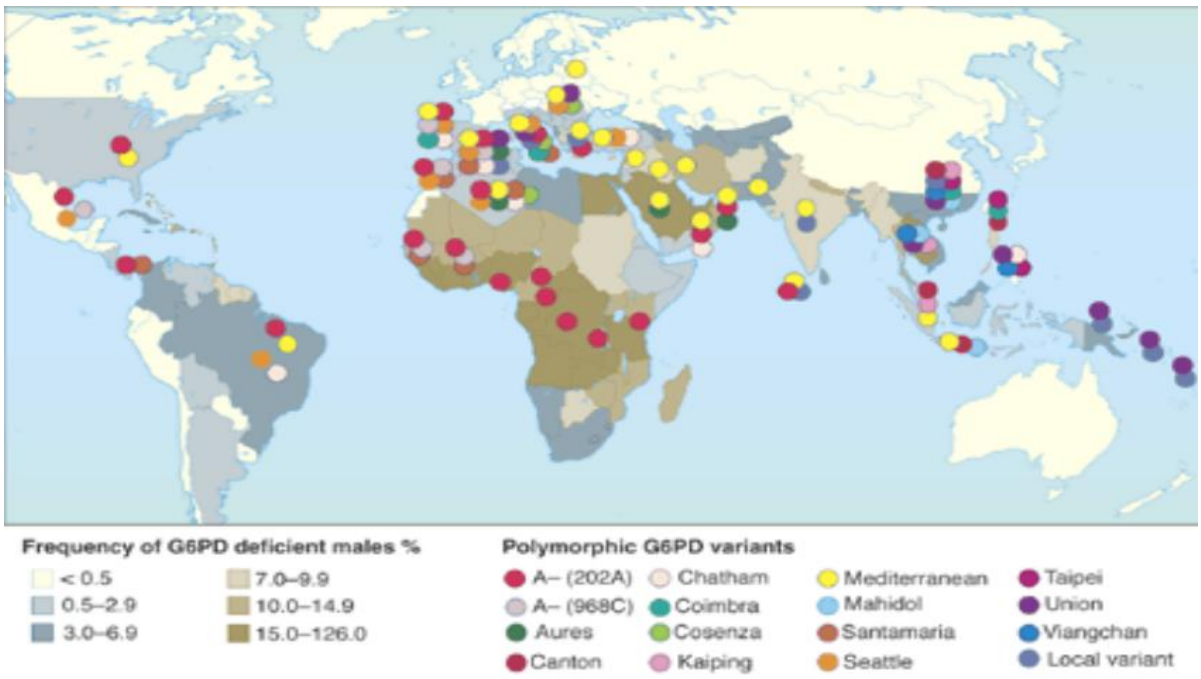
-**Fava beans** are presumed to cause oxidative damage by an unknown component

✓Infection:

-the most common inducer due to the production of free radical by immune cells.

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