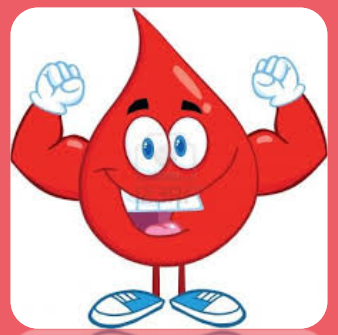
Lectures 1-2

" Hemoglobin + G-6PD "



Biochemistry Team

The Objectives

- The biochemical basis of G6PD deficiency anemia
- The precipitating factors for G6PD deficiency anemia
- Classes of G6PD deficiency anemia (variant enzyme)
- Diagnosis of G6PD deficiency anemia

Red = Import-ant

Blue = explain

Green = addition notes

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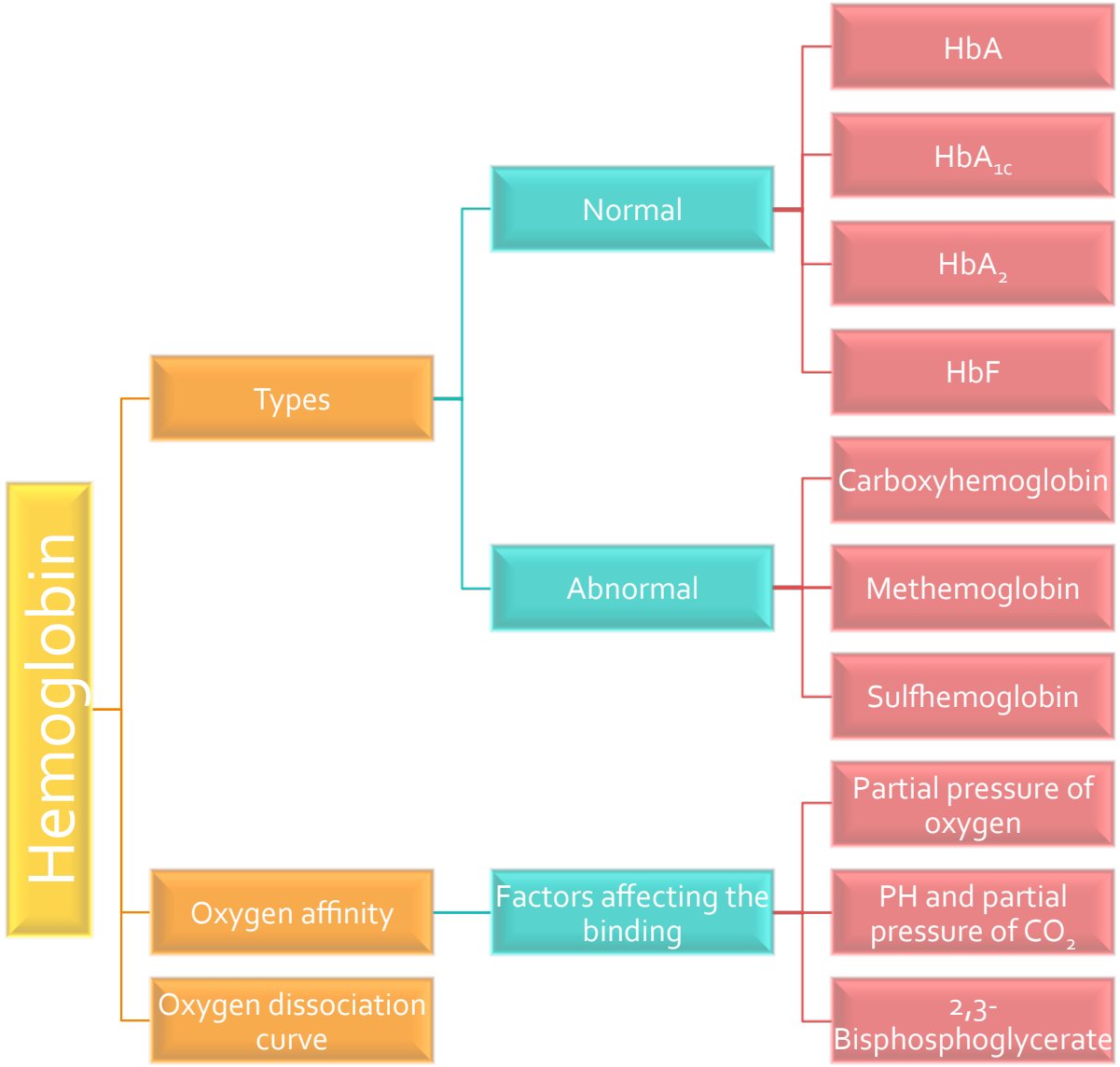
Mind Map



Hemoglobin (Hb):

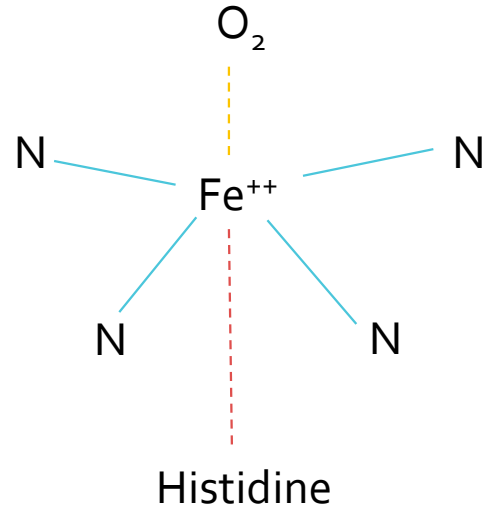
- A hemeprotein found only in RBCs
- Contains Heme as the prosthetic group

A cofactor required for activation and is permanently bound.

- Oxygen reversibly binds to Heme in order to transport Oxygen from the lungs to the tissues and carry Carbon dioxide from tissues back to the lungs.
- Normal levels:

Males ➔ 14-16 g/dL

Females ➔ 13-15 g/dL



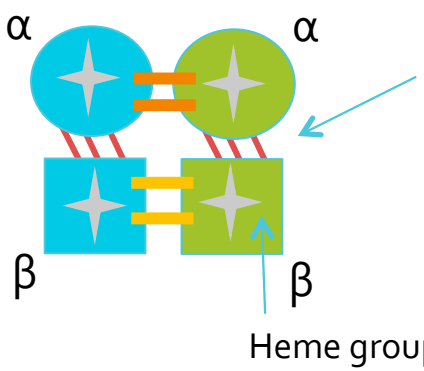
The Heme group:

Components:	Protoporphyrin IX	Ferrous Iron (Fe^{++})
Bonds:	4 Nitrogens bind to Fe^{++}	1. 4 Nitrogens from Porphyrin ring 2. Histidine from globin chain 3. Oxygen

Types of Hemoglobin

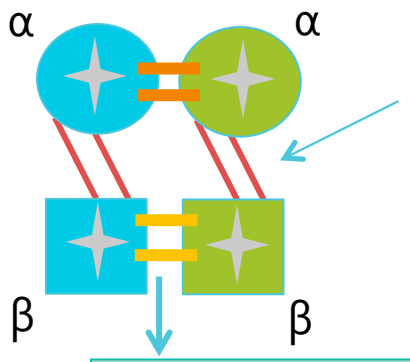
	Normal			
Type	A	A_{1c}	A_2	F
	T-form (Taut-form) / R-form (Relaxed-form)			
Amount in blood	97%	Depends on plasma glucose levels	2%	1%
Found in	Major Hb in adults	High in patients with diabetes millitus	Appears ~12 weeks after birth	Major Hb in fetus and newborn
Structure	• Four polypeptide chains, 2 α and 2 β • Each subunit has a heme group carrying O_2 • Contains 2 dimers, each dimer has 1 α and 1 β subunits bound with noncovalent interactions Net: **4 subunits, 4 heme groups, 4 O_2 molecules**	Non Enzymatically glycosylated HbA	4 subunits, 2 α and 2 δ globin chains	2 α and 2 γ chains
Features	• Deoxy form of Hb • Low oxygen affinity (To allow delivery of O_2 to tissue) • Movement of dimers is constricted. (T.form) • Oxygenated form • High oxygen affinity • Free dimer movement (R.form)	Glycosylation depends on plasma glucose levels		• Higher affinity to O_2 than HbA • Transfers O_2 from maternal to fetal circulation across placenta

Taut Form of HbA



Weak ionic and hydrogen bonds

Relaxed Form of HbA



Weak interactions (some ionic and hydrogen bonds are broken).

Strong hydrophobic interaction between dimers

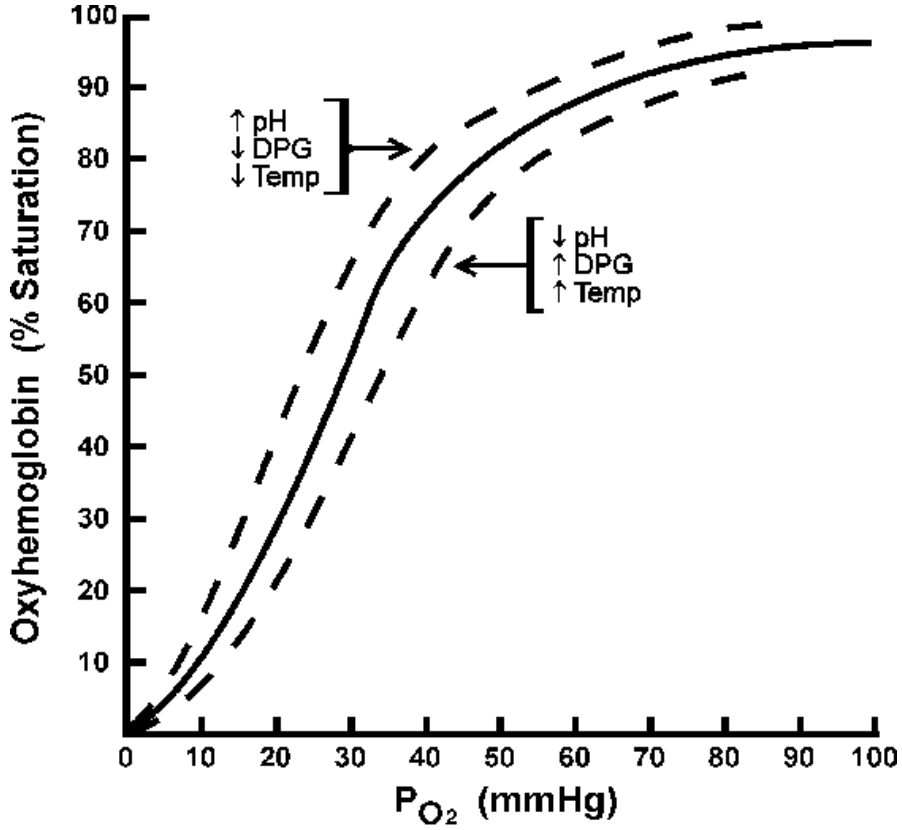
Types of Hemoglobin

Abnormal			
Type	Carboxy-Hb	Met-Hb	Sulf-Hb
Percentage in blood		0%	
Caused by	Smoking	Oxidative stress	Administration of Sulfa drugs (high sulfur levels in blood)
Structure	CO replaces O_2	Contains oxidized iron (Fe^{+3})	Sulfur
Features	CO binds 200× tighter than O_2		Irreversible reaction
	Unable to transport oxygen due to abnormal hemoglobin structure		

Oxygen Dissociation Curve:

The graph that shows the percent of saturation of hemoglobin at various partial pressures of oxygen.

(At high partial pressures of oxygen (in lungs) hemoglobin binds to oxygen to form oxyhemoglobin. The blood is fully saturated when all the erythrocytes are in the form of oxyhemoglobin. As the erythrocytes travel to the tissues, the partial pressure of oxygen decreases which will result in the release of oxygen and the formation of the curve)



- By heme-heme interaction, the binding and of O_2 to a subunit increases O_2 affinity to the other subunits in the heme group (Cooperative binding)
- The curve is sigmoidal
- It is the steepest at the oxygen concentrations in the tissue which allows oxygen delivery to respond to small changes in PO_2.

Factors Affecting Oxygen Binding:

A. PO_2 (Partial Oxygen Pressure)
B. PH of the Environment and PCO_2 (Partial carbon dioxide pressure)
C. Availability of 2,3-bisphosphoglycerate

A) Partial Oxygen Pressure (P50):

The pressure at which hemoglobin is 50% saturated with oxygen.

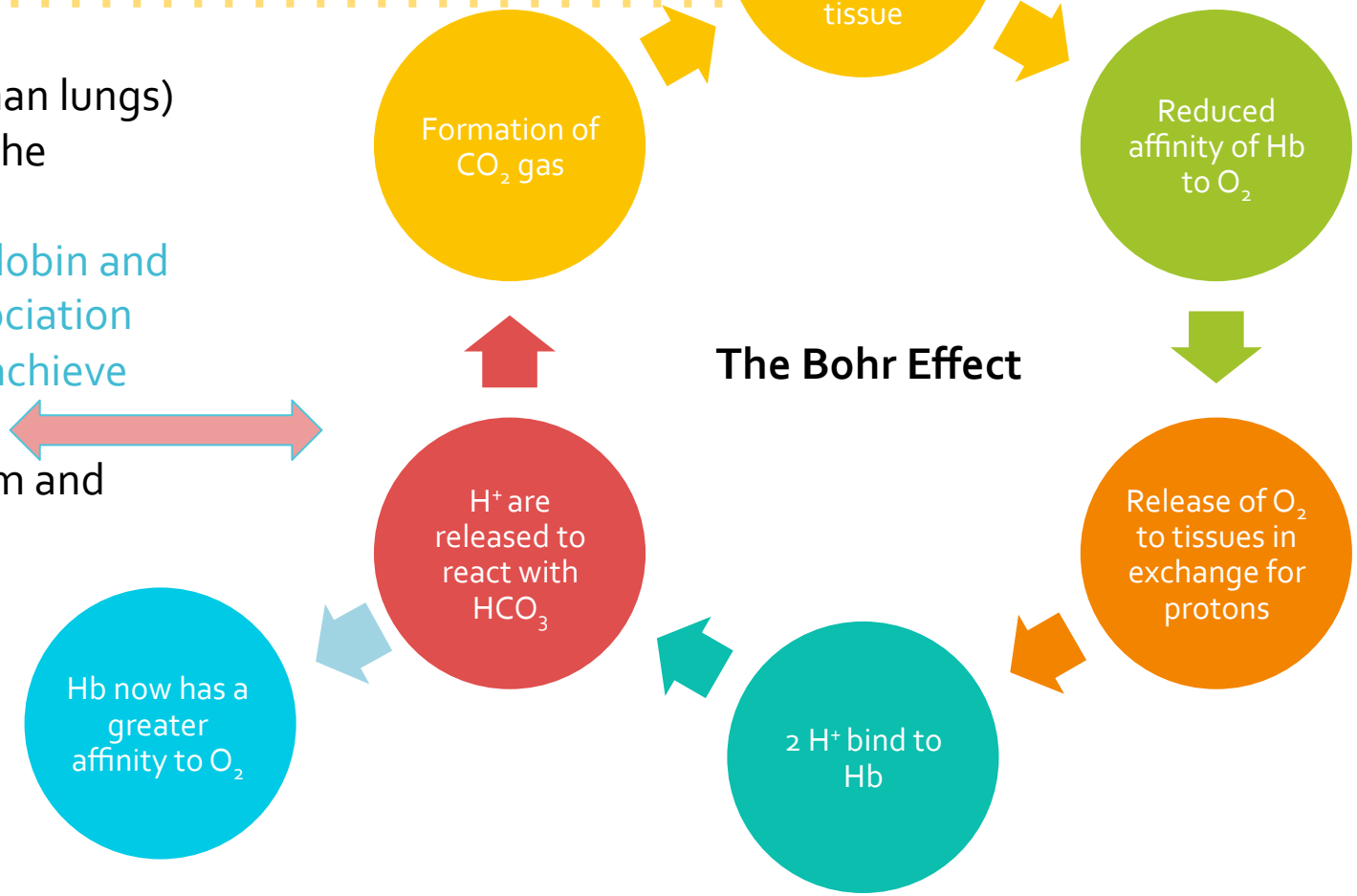
- At 100% saturation (in lung PO_2 is 100 mmHg) ➔ High affinity to oxygen, slow release
- When the saturation decreases (at tissue PO_2 is 40 mmHg) ➔ Low affinity, fast unloading

B) The Bohr Effect:

Definition: The effect of PH (at tissue is lower than lungs) and CO_2 on oxygenation of Hb in the lungs and the deoxygenation of it at the tissues.

(Low PH results in low oxygen affinity of hemoglobin and therefore, a shift to the right in the oxygen dissociation curve. That means: a greater PO_2 is required to achieve any given oxygen saturation)

Effect: Removes insoluble CO_2 from bloodstream and produces soluble bicarbonate.



C) Availability of 2,3-bisphosphoglycerate:

Binds to deoxyhemoglobin and stabilizes the T-form. As soon as oxygen binds to Hb, BPG is released.

BPG(Bisphosphoglycerate)

Oxygen Affinity

High

Occurs due to:

- Alkalosis (High oxygen)
- High Hb F
- Multiple transfusions of 2,3- DPG-depleted blood

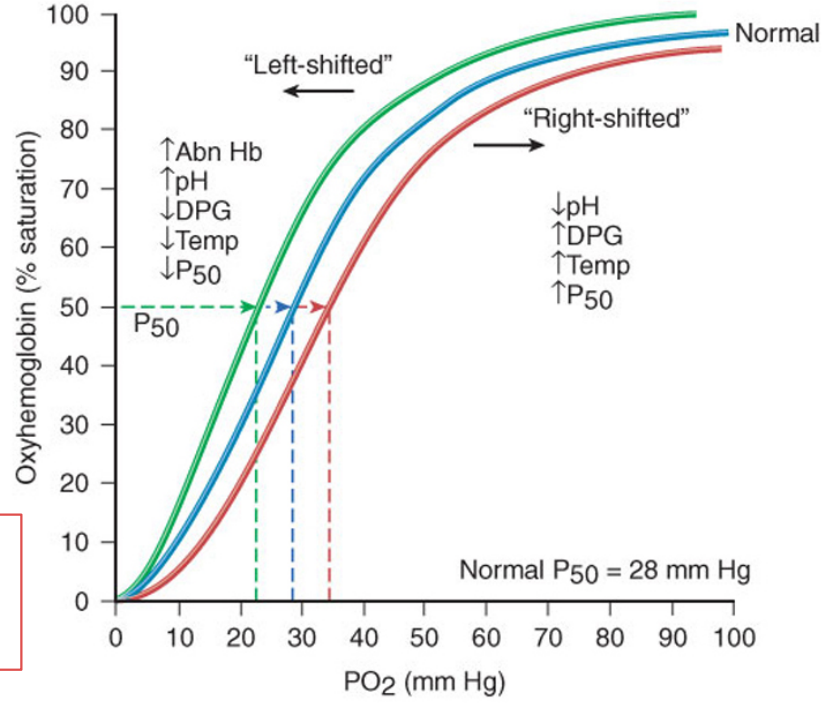
(DPG diphosphoglycerate)

Low

Occurs due to **Hypoxia** and **living in high altitude**.

It results in:

- Increased 2,3-BPG *
- Increases oxygen delivery to tissue



In high altitude there is an increase in RBCs, concentration of Hb, and 2,3 BPG

* "2,3-Bisphosphoglycerate or 2,3-BPG, also known as 2,3-diphosphoglycerate or 2,3-DPG".

Summary

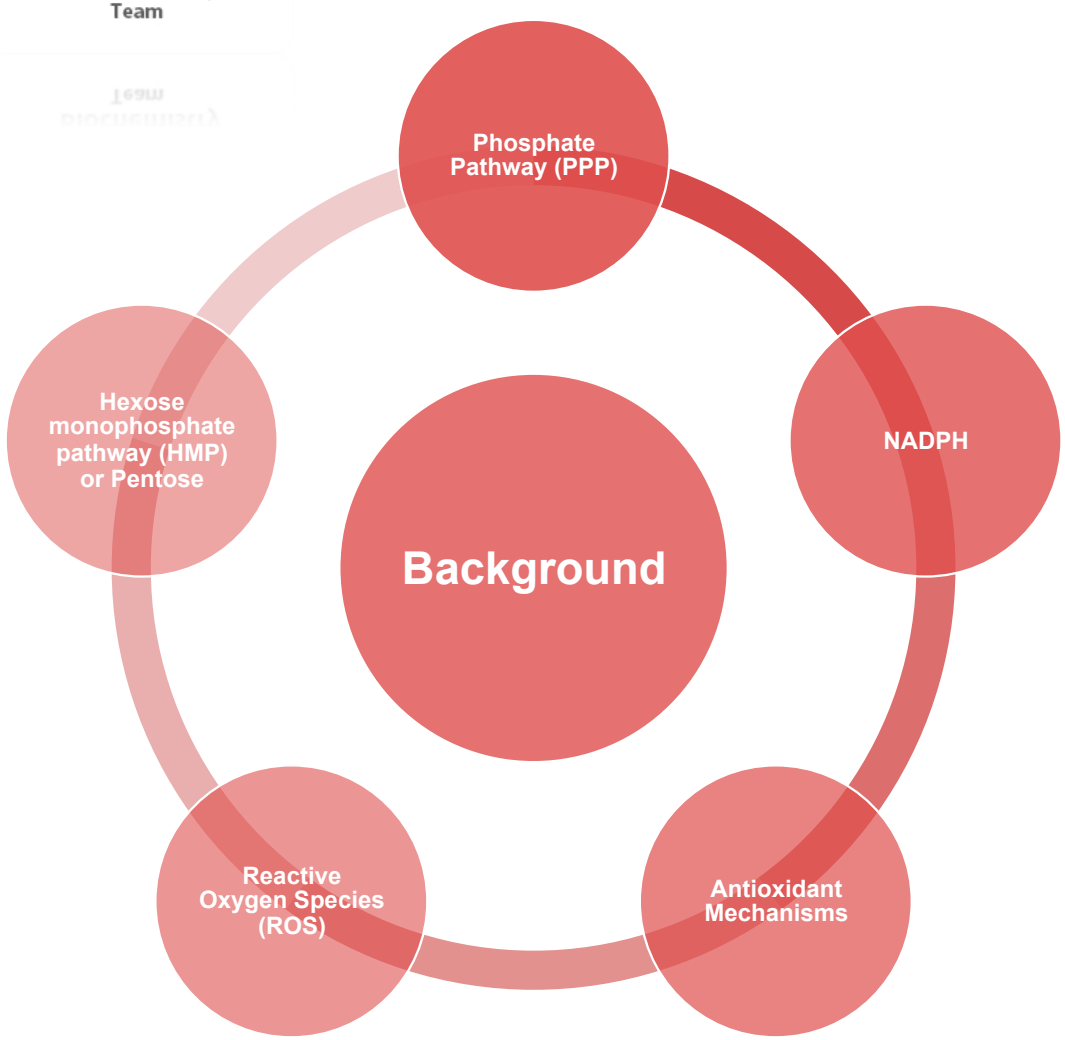
- Hemoglobin is a heme protein essential for maintaining gas exchange
- HbA is the major type of Hemoglobin in adults
- Increasing oxygen affinity occurs at high partial oxygen pressures and results in the binding of oxygen to Hb
- Decreasing oxygen affinity occurs at low partial oxygen pressure to allow delivering oxygen to tissue
- Any change in the normal structure of hemoglobin makes it unable to transport oxygen and causes hypoxia to the tissue

Lecture 2

Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency Anemia

Biochemistry Team

Mind Map



G6PD Deficiency Hemolytic Anemia

- Definition
- Biochemical Basis
- Precipitating Factors
- Different Classes
- Variant Enzymes
- Diagnosis

Background :-

- RBCs has one source for H+ NADPH and it is produced by PPP

1- NADPH has a reductive power "reduction of fatty acids and steroidal hormones synthesis"

2- Act as H donor for Nitric oxide synthesis and for the G-S-S-G "antioxidant (part of glutathione system) "

3- Oxygen-dependent phagocytosis by WBCs

* Although PPP provide RBCs by NADPH, it also provide for it the Ribose-5-Phosphate which is responsible for synthesis of nucleic acid :)

*Oxidative damage to:

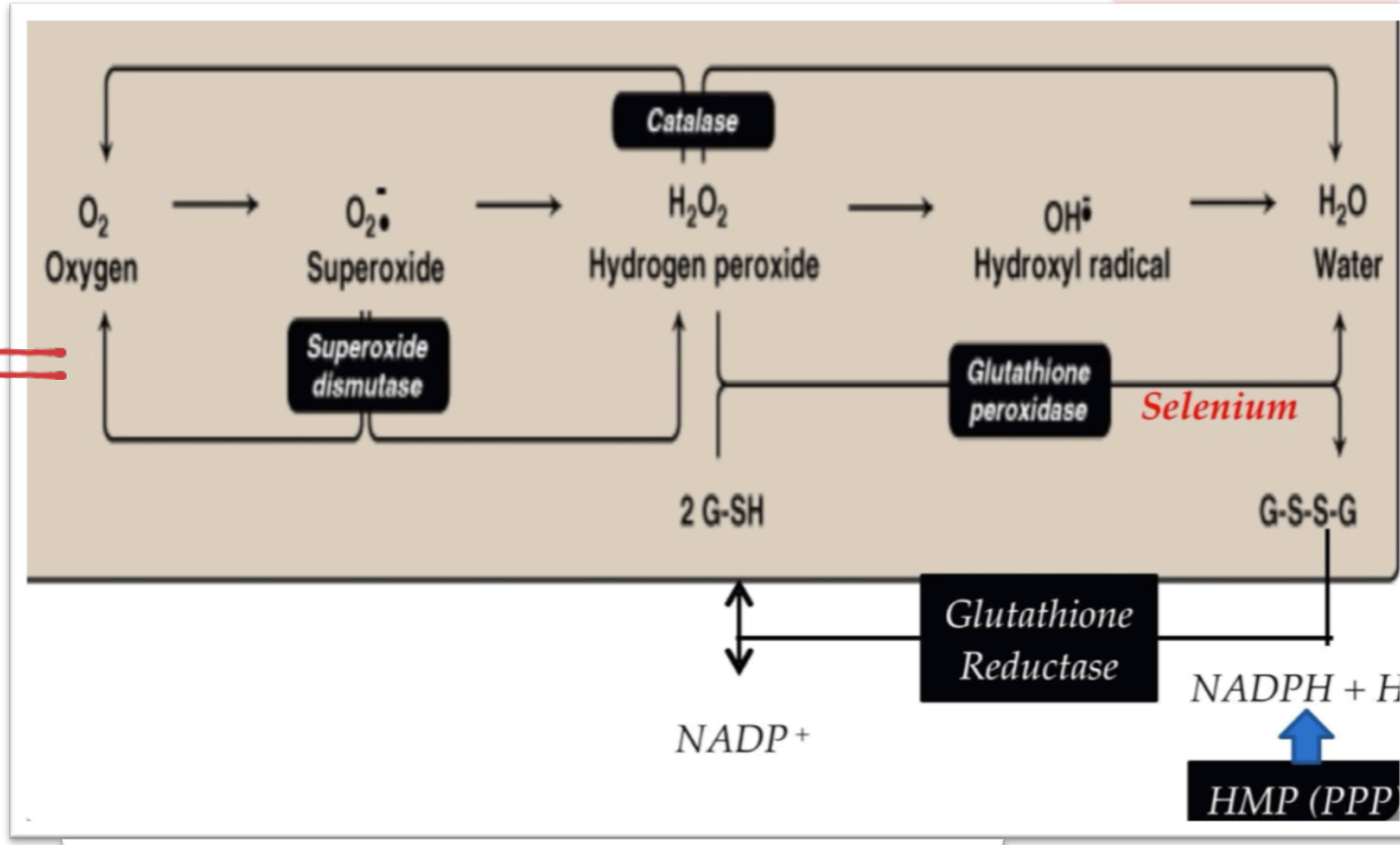
- -DNA
- -Proteins
- -Lipids (unsaturated fatty acids)

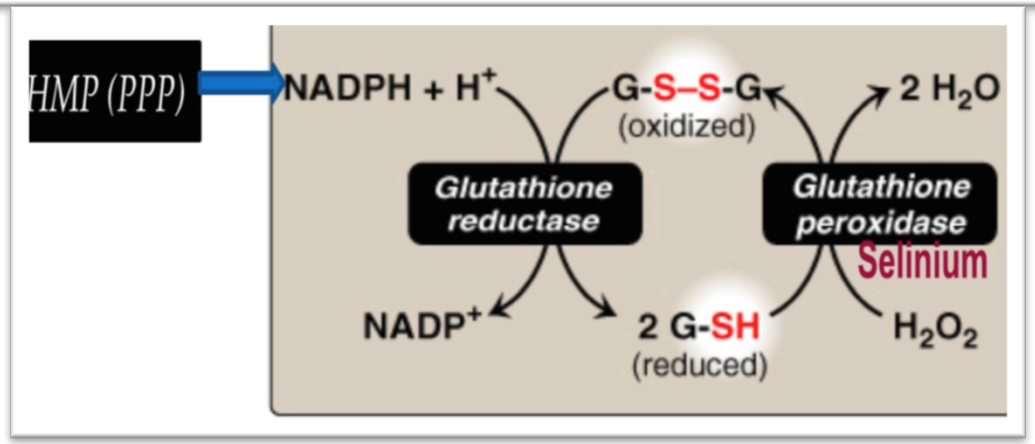
*Oxidative stress and diseases:

- -Inflammatory conditions e.g., Rheumatoid arthritis
- -Atherosclerosis and coronary heart diseases
- -Obesity
- -Cancers
- -G6PD deficiency hemolytic anemia

Oxidative stress: Imbalance between oxidant production and antioxidant mechanisms

* Those antioxidant are essential to get rid of ROS :
* Role of NADH+ :-
It donate H to "G-S-S-G"
NADH + G-S-S-G => 2G-SH
G-S-S-G "Oxidized" will be 2G-SH "Reduced" via Glutathione reducates
2G-SH is part of Glutathione system which react with H2O2 to convert it to "water+G-s-s-G" via Glutathione peroxidase
Then, G-S-S-G will be recycled by NADH with the help of Selenium .....



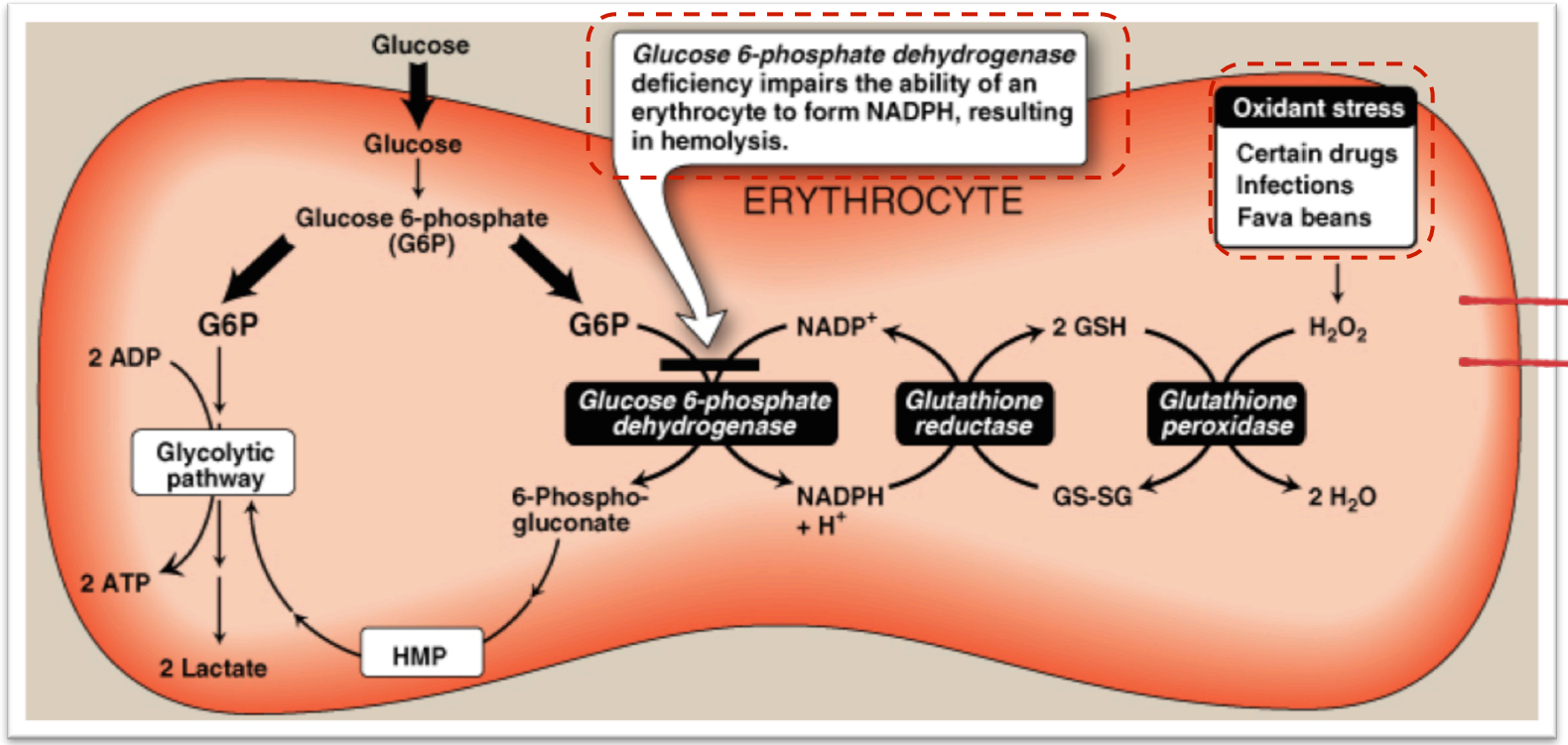


G6PD dificiency

* Inherited X-linked recessive disease
* Most common enzyme-related hemolytic anemia
* Highest prevalence: Middle East, Tropical Africa, Asia and Mediterranean
* ~400 different mutations affect G6PD gene, but only some can cause clinical hemolytic anemia
* G6PD deficient patients have increased resistance to infestation by falciparum malaria >> since the RBCs are abnormal , so they are not suitable for malaria to live

*Although G6PD deficiency affects all cells, it is most severe in RBCs ..... Why?

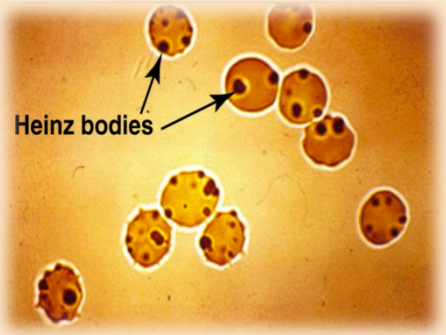
Other cells have other sources for NADPH production: e.g., Malic enzyme that converts malate into pyruvate



Glucose-6-Phosphate+ NADP "via G6PD" will donate H "G-S-S-G" to be transformed to 2GSH which will join H2O2 "one of the ROS"

Biochemical Basis of G6PD Deficiency Hemolytic Anemia

Oxidation of sulfhydryl (SH) groups of proteins inside RBCs causes protein denaturation and formation of insoluble masses (Heinz bodies) that attach to RBCs membranes



Precipitating Factors for G6PD Deficiency Hemolytic Anemia

1. Intake of oxidant drugs (AAA):
Antibiotics e.g., sulfa preparation
Antimalarial: e.g., Primaquine
Antipyretics
2. Exposure to infection
3. Ingestion of fava beans (favism, Mediterranean variant)> even inhalation

*Chronic nonspherocytic anemia: Hemolytic attack in absence of precipitating factors. Severe form due to class I mutation..

Diagnosis of G6PD Deficiency Hemolytic Anemia

*Diagnosis :
Complete Blood Count (CBC) & reticulocytic count
*Screening:
Qualitative assessment of G6PD enzymatic activity (UV-based test)
*Confirmatory test:
Quantitative measurement of G6PD enzymatic activity
*Molecular test:
Detection of G6PD gene mutation

Decreasing the G6PD enzyme activity will increase the severity

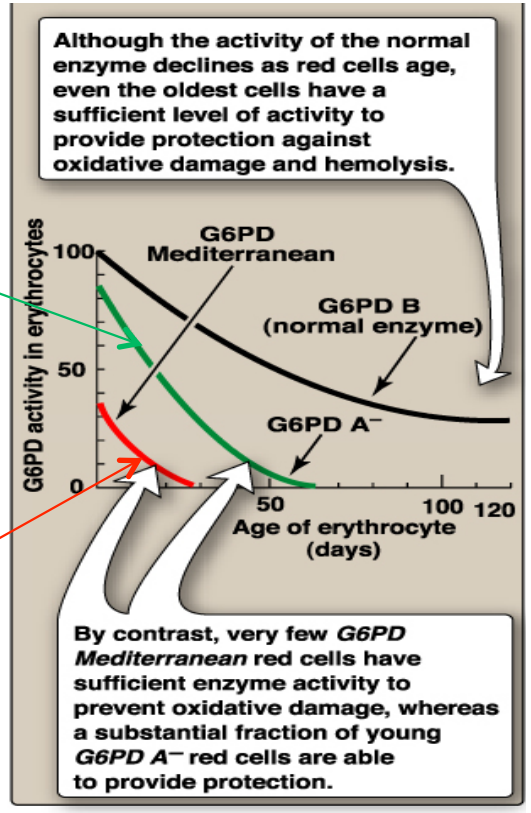
Mediterranean G6PD A- Normal

Class	Clinical symptoms	Residual enzyme activity
I	Very severe	<2%
II	Severe	<10%
III	Moderate	10–50%
IV	None	60–150%

Normal RBCs still functioning even the last days of its life span with decrease its activity ..
Decreasing the G6PD enzyme activity will increase the severity

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)



	Diagnosis of hemolytic anemia	Screening	Confirmatory test	Molecular test:
Diagnosis of G6PD Deficiency Hemolytic Anemia	CBC and reticulocytic count.	Qualitative assessment of G6PD enzymatic activity (UV-based test).	Quantitative measurement of G6PD enzymatic activity.	Detection of G6PD gene mutation.

Summary

* RBCs has one source for H+ NADPH and it is produced by PPP
*NADPH has a reductive power "reduction of fatty acids and steroidal hormones synthesis“
*Glucose-6-Phosphate+ NADP “via G6PD” will donate H “G-S-S-G” to be transformed to 2GSH which will join H_2O_2 “one of the ROS”
*Heinz bodies : seen in G6PD deficiency
***Precipitating Factors** :
1- Intake of Antibiotics, Antimalarial and Antipyretics
2- Exposure to infection
Ingestion of fava beans (fauvism, Mediterranean variant)
*G6PD A- (class III) >> Moderate
*G6PD Mediterranean (II) >> Enzyme with normal stability but low activity (severe).

Test your knowledge ..!

1. A 25-year-old female was brought to the emergency unit apneic and cyanotic, the physician on-call learned that she has Crohn's disease and is on Sulfasalazine. She told him that she took a few more pills than the dose her doctor prescribed because she was in pain. What is the condition associated with her symptoms?

a) Thalassemia
b) Sickle Cell Anemia
c) Methemoglobinemia
d) Sulfhemoglobinemia

2. A person was found to have 8 mmol/L of 2,3-BPG in his blood while a normal person is found to have 5 mmol/L, which one of the following is a possible cause for this increase:

a) He is adapted to high altitude
b) Alkalosis
c) He has high levels of HbF
d) Multiple transfusions of 2,3 DPG-depleted blood

3. Which of the following is considered the major type of Hb in fetus and newborn:

a) HbA
b) HbF
c) MetHb
d) SulfHb

Answers:
1. D
2. A
3. B

Test your knowledge ..!

4. In male patients who are homozygous for glucose 6-phosphate dehydrogenase (G6PD) deficiency, pathophysiologic consequences are more apparent in erythrocytes (RBC) than in other cells, such as, in the liver. Which one of the following provides the most reasonable explanation for this different response by these individual tissue types?

a) Excess glucose 6-phosphate in the liver, but not in RBCs, can be channeled to glycogen, thus averting cellular damage.
b) Liver cells, in contrast to RBCs, have alternative mechanisms for supplying the NADPH required for keeping metabolic and cellular integrity.
c) Glucose 6-phosphatase activity in RBCs removes the excess glucose 6-phosphate, thus resulting in cell damage. This does not happen in the hepatocyte.
d) Because RBCs do not have mitochondria, production of ATP required to keep cell integrity depends exclusively on the routing of glucose 6-phosphate to the pentose phosphate pathway.
e) The catalytic properties of the liver enzyme are significantly different than those of the RBC enzyme.

5. Which of the following is correct about G6PD deficiency hemolytic anemia?

a) Autosomal recessive disease
b) Affects Females more than males
c) Class I of the disease is mild
d) Inherited X-linked recessive disease

Answers:
4. B
5. D

Biochemistry
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If you find any mistake, please contact us:)
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Thank you