HEMATOLOGY
&LYMPH SYSTEM

Biochemistry sheet

Number	2
Done BY	Jumana Jiham
Correction	Abdullah Alzibdeh
Doctor	Nayef

(ابدأ واضعاً هدفك نصب عينيك)

First lecture review

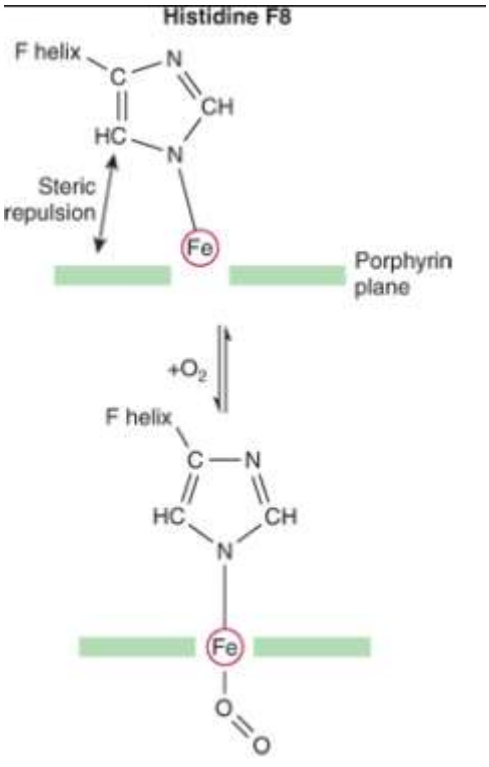
Structure of hemoglobin:

-Hemoglobin is a tetramer composed of two (αβ) dimers. The two polypeptide chains within each dimer are held tightly by hydrophobic interaction and between the polar groups of the two dimers weak ionic and hydrogen bonds occur. When oxygen bind to the deoxy state (T state or tight state) the molecule undergoes a conformational change to a more relaxed state (R form), some of the ionic and hydrogen bonds between the dimers are broken or become weaker allowing movement and change in structure.

-Myoglobin can bind only one molecule of O2, because it contains only one heme group,While Hb can bind four O2.

Cooperativity of hemoglobin:

In deoxyhemoglobin, there is steric repulsion between proximal histideine and the porphyrin. Iron is situated in the plane of the heme when heme is alone (out of the globin protein), but because of this steric repulsion mentioned above Iron bulges (pyramidal shape).



-Oxygenation moves the iron atom into the plane of porphyrine.

The binding of O2 to the heme iron pulls the iron into the plan of the heme.

Because the iron is also linked to the proximal histidine (F8), (F helix) will move, which pulls the whole structure. These changes will alter the interaction between (αβ) dimers. This is how this

movement is initiated and transmitted to neighboring subunits. And that explains how some hydrogen bonds between the (αβ) dimers are broken in the oxygenated state.

12c

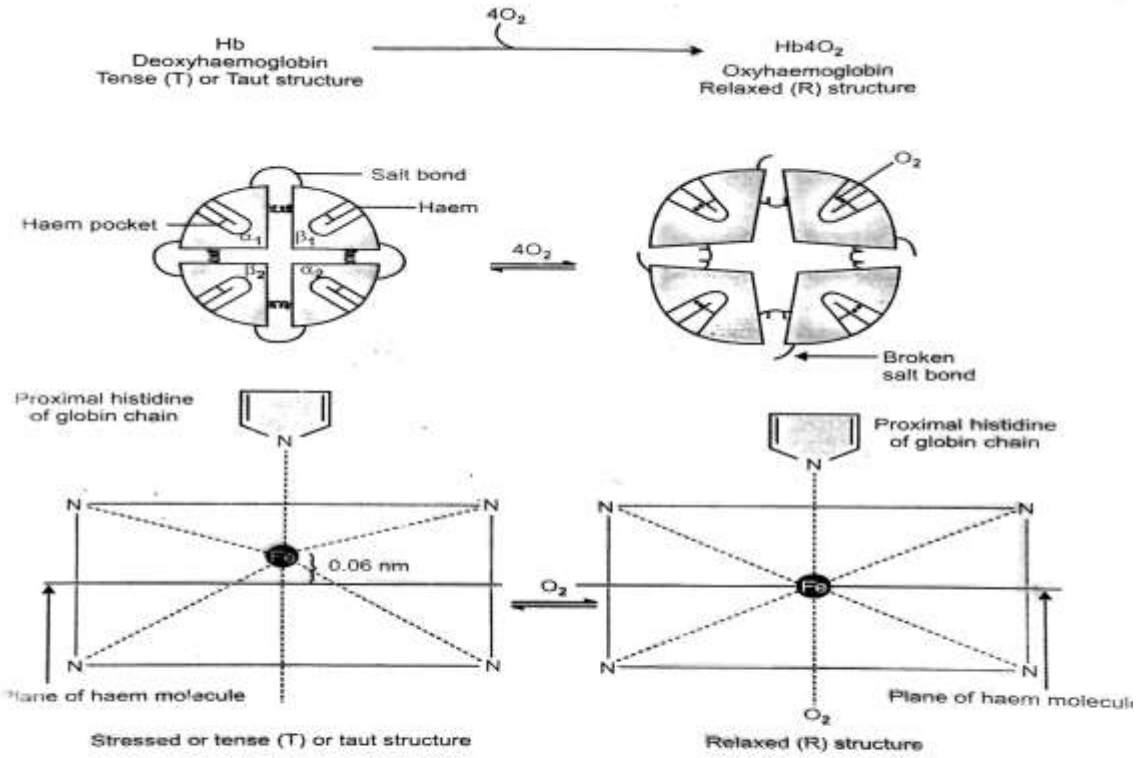


Figure 8.5: Schematic representation of changes during oxygenation of deoxy haemoglobin

Binding of O_2 is revisable:

Binding of oxygen should be reversible (oxygen should bind to Hb at high oxygen pressure (lungs) but it should also be released in low oxygen pressure (peripheral tissues)).

You know that Hb has a hydrophobic heme pocket. This pocket does not allow charge separation (Fe^{+2} to Fe^{+3}):

$$Fe\text{-}O_2 \longrightarrow Fe^{3+} + O_2^{-}$$ **(should not happen)**

Fe^{3+} : ferric O_2^{-} : superoxide (a free radical—dangerous!)
- THIS REACTION SHOULD NOT HAPPEN!!
We are protected against it and ferrous iron is preserved by the hydrophobic environment of the heme pocket so no charge separation takes place (if the pocket was hydrophilic then we will have water, which can separate charges-- remember from school chemistry that when you dissolve NaCl in water, they separate into Na+ and Cl- and that is because water molecules surround these particles and keep them separated; because of the polarity of water molecules which makes charge separation stable).

However, this reaction happens but only to a small extent.
If a mutation occurs in the hydrophobic pocket and water was allowed in, charge separation will take place and we will have ferric iron.
<u>Hemoglobin + ferric</u> iron (instead of ferrous) = Methemoglobin; which does not bind oxygen. Methemoglobin increases in the blood when exposed to oxidizing drugs, or if you have a mutation in the heme pocket as mentioned earlier.

From the slides:

Causes of Methemoglobin formation:
1-chemicals and drugs
2-endogenous production of H_2O_2 and free radicals
3-inhereted defect in the α or β chains

-NADH-cytb5 reductase is the enzyme that protects us from methemoglobin accumulation in the blood; through reducing the ferric iron back to ferrous, and therefore methemoglobin to hemoglobin. This enzyme -as its name implies- needs NADH to function.

-Newborns have only half of the amount of the enzyme compared to adults. That is why newborns should not be given some of the oxidizing drugs. Rare deficiency of this enzyme is present around the world and these patients should be careful not to take oxidizing drugs. Methemoglobinemia is treated with methylene blue to reduce methemoglobin back to hemoglobin. Methylene blue needs NADPH to work (Remember that G6PD enzyme is the source of NADPH, an enzyme of high incidence of deficiency in our area). If the patient was also G6PD deficient; give Ascorbate- which is also a reducing agent but is less effective.

(If the patient has a G6PD deficiency, DO NOT give methylene blue)

Cyanide poisoning;

Cyanide is a deadly poison because it inhibits complex IV (of the electron transport chain in the mitochondria) Cyanide loves ferric iron (Fe3+).

And by the way complex IV has ferric iron in it and that is why it hits it. Oxidative phosphorylation is essential for life. So when someone is poisoned with cyanide you give them a drug which converts hemoglobin to methemoglobin (oxidizing drugs). i.e. we create MetHb in purpose! So cyanide binds methemoglobin; forming cynomethemoglobin in the blood before reaching tissues. And you SAVE the patient's life.

Hemoglobin allosteric effectors:

The ability of Hb to reversibly bind oxygen is affected by

1. **The PO2**
2. **The ph of the environment**
3. **The partial pressure of CO2**
4. **Avalibility of 2,3-bisphosphoglycerate**

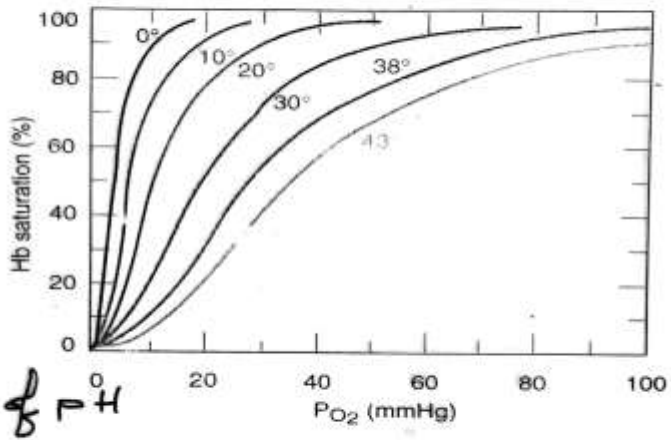
Alloseteric (other site)-effectors, because their interaction at one site on the Hb affects the binding of oxygen to another site; which is the heme group.

- Because saturation happens in the lungs where there is very high partial oxygen pressure (pO2) and high concentration of O2; so allosteric effectors have no effect. These allosteric effectors show their effect on lower pO2 (in peripheral tissue) increasing or decreasing oxygen delivery to tissues.

In pure Hb the affinity for O2 is higher than the Hb in the physiological condition as shown in the figure.

15

Effect of Temp.



Effect of pH

These effectors (CO_2,2,3-BPG,temp) all shifts the curve to the right decreasing the affinity of the Hb to O_2. This shift will allow for unloading of O2 to the tissues.

While exercising, the temperature of the body increases; this will reduce the affinity, so it will help the muscles to get more oxygen.

-Note the shift in the curve when temp is increased

The release of O_2 by Hb is enhanced when the PH decrease (more protons) or in the presence of an increased PCO_2. Both result in a shift to the right.

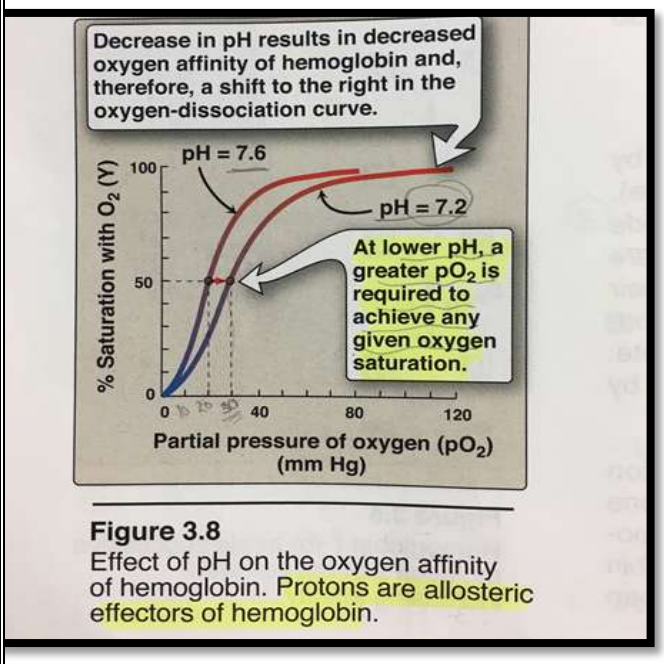


Figure 3.8
Effect of pH on the oxygen affinity of hemoglobin. Protons are allosteric effectors of hemoglobin.

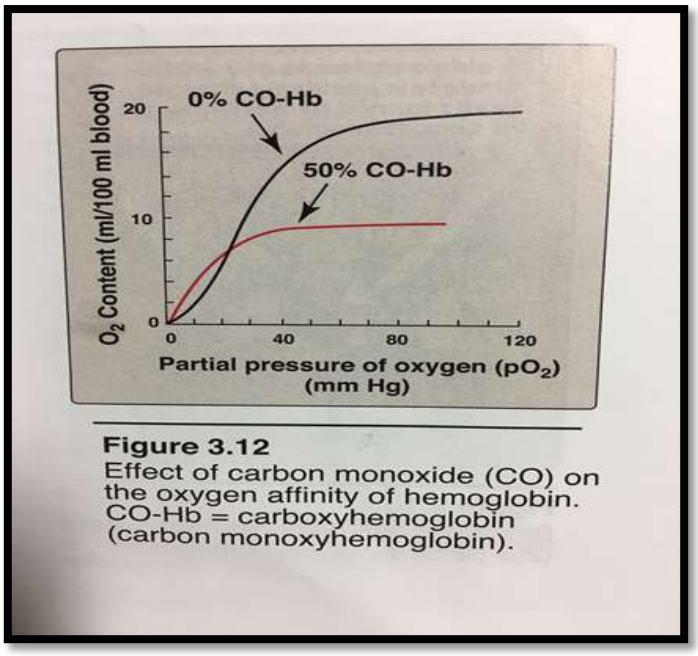


Figure 3.12
Effect of carbon monoxide (CO) on the oxygen affinity of hemoglobin. CO-Hb = carboxyhemoglobin (carbon monoxyhemoglobin).

3-Efect of 2,3-BPG on O2 affinity of Hb:

- **Erythrocytes have the enzyme Mutase, which produces 2,3-bisphosphoglyerate from 1,3- bisphosphoglycerate (a high energy compound and a source of ATP) in glycolysis. So this compound is present considerably higher amounts in erythrocytes than in other**

cells. it is an important regulator and has a special role in the RBC's . 2,3-BPG (has 5 negative charges) binds in a pocket formed by the two βchains of <u>deoxygenated hemoglobin</u> A. These β chains have several positive charges to interact with the negative charges of 2,3-BPG.

- **2,3BPG normal molar concentration inside the cell is around 4.5millimolar and that is approximately that of hemoglobin.**

-At high altitudes the PO_2 is less and the O_2 CONCENTRATION is less so as a compensatory mechanism the concentration of 2,3-BPG will increase this increase will reduces the affinity of hemoglobin for oxygen and will enable hemoglobin to release oxygen efficiently at the partial pressures found in the tissues.(To deliver more oxygen)

So 2,3-BPG = 8mmol/l and the P50 = 37aprximitly

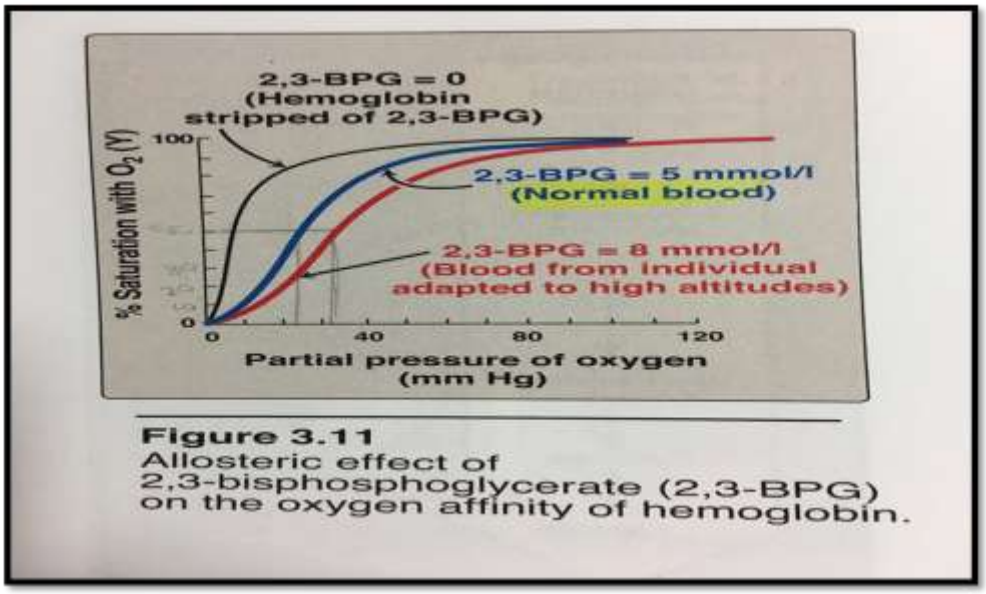
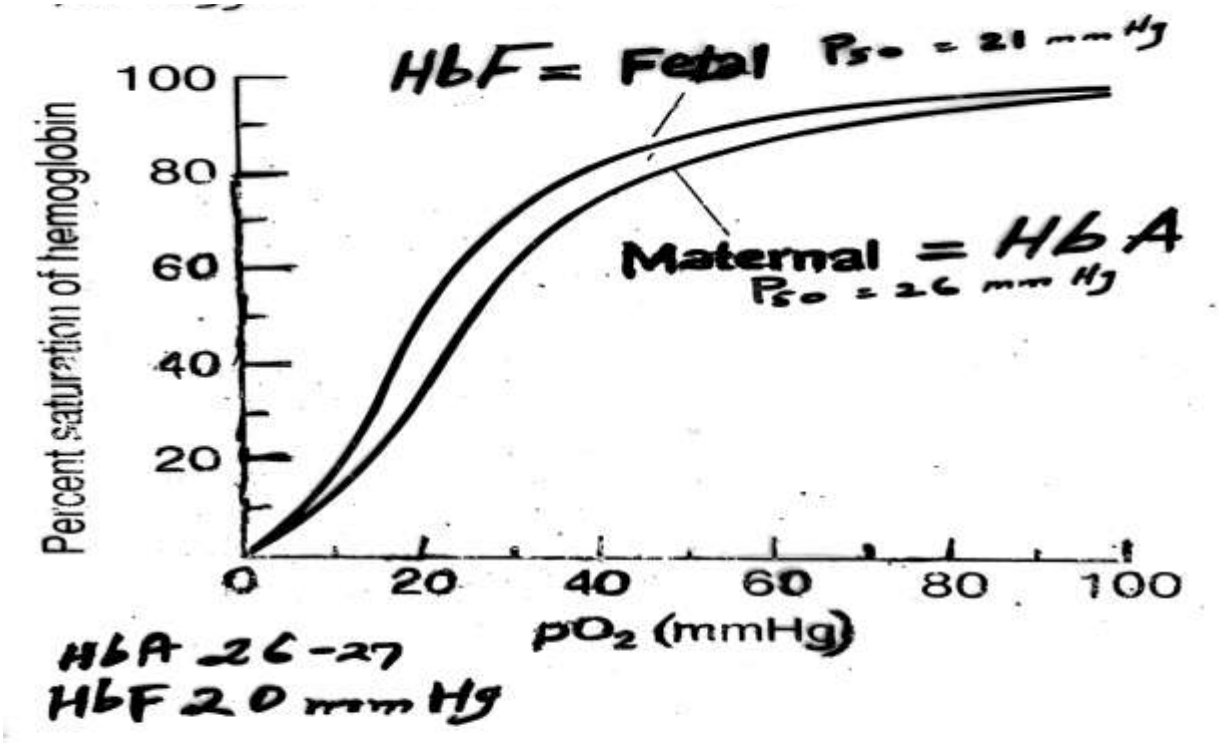


Figure 3.11
Allosteric effect of 2,3-bisphosphoglycerate (2,3-BPG) on the oxygen affinity of hemoglobin.

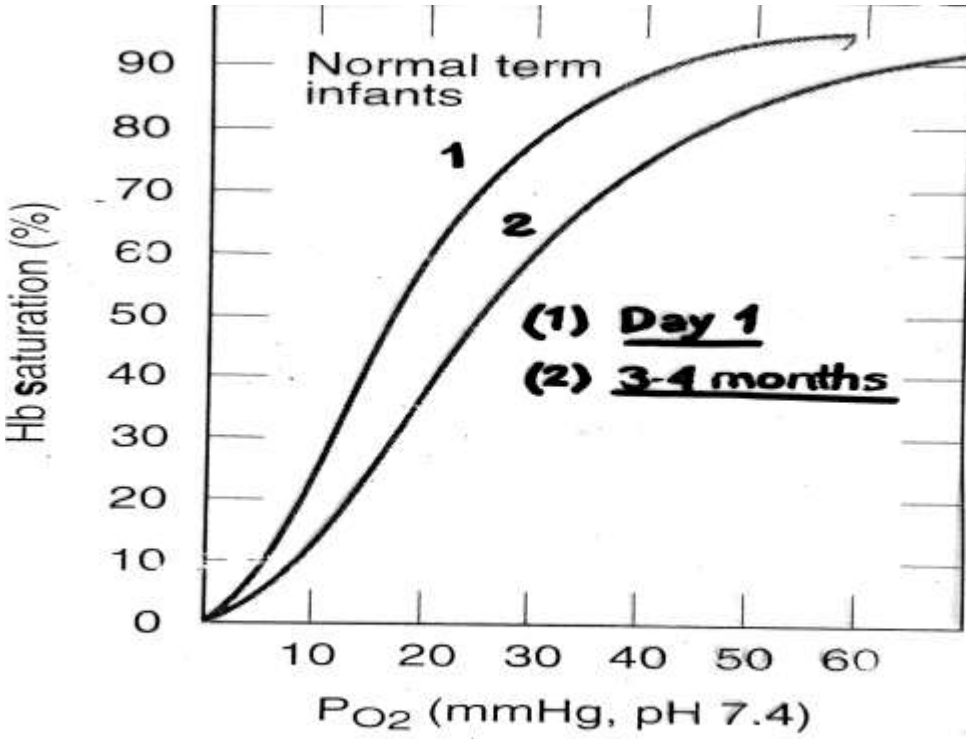
In the picture above we can see that in normal blood the concentration of 2,3-BPG = 5mmol/l and the P_{50} will be 26.

If we have zero concentration of BPG the P_{50} =7 -10 only.

• **Fetal hemoglobin has two alpha and two gamma chains (instead of the beta chains); one of the differences between gamma and beta chain is that histidine143 residue in the beta chain is replaced for a serine residue on the gamma chain and this causes a weaker binding to 2,3-BPG. The result is that HbF has a higher affinity for oxygen than maternal HbA, allowing transplacental passage of oxygen from mother to fetus.**



If we take a pure HbA and HbF there will be no difference in the affinity so the concentration of 2,3-BPG is what makes the difference between them



This figure show the oxygen dissociation curve for a new born that still have a considerable amount of HbF and compare it after 3-4 moths curve where it declined to form only 1% of total hemoglobin

Summary:

1. **2,3-BPG is an important regulator of oxygen binding, it is the most abundant organic phosphate in the RBC; has the same concentration as Hb.**
2. **It is an intermediate of glycolysis.**
3. **HbA has high affinity to oxygen without BPG.**
4. **BPG stabilizes the T state. It does not bind the oxygenated Hb (R form).**
5. **2,3-BPG concertation is increased in response to:**

- Chronic hypoxia; such as the hypoxia caused by chronic obstructive pulmonary diseases (COPD, emphysema, etc.)
- Being at high altitudes (low PO_2 ⟶ lower oxygen carrying capacity ⟶ adapt by increasing BPG so increasing delivery)
-Pyruvate kinase deficiency; causes a chronic anemia; lower oxygen carrying capacity ⟶ replenished by increasing 2,3-BPG

6. 2,3-BPG binds to a pocket, formed by the 2 β-globin chains at (1:1) ratio where the pocket contains several +vely charged amino acid that form ionic bonds with the negatively charged phosphate groups of 2,3-BPG.

Stored blood in blood bank:

Blood could be stored for 30 to 42 days in the blood bank.

***Trans*fused blood was noticed to need few hours to restore 2,3-BPG. If the patient is severely ill transfused blood will be useless (or might even compromise the patient). Because there is yet no BPG, delivery of oxygen is very low. So RBCs trap oxygen instead of delivering it. Now, stored blood is supplemented with phosphate, hexose sugar and adenine to avoid depletion of BPG.**

So this will increase storage time from 21 d to 42d

NEXT LECTRE : CO2 TRANSPOR AND BOHER EFFECT

Sorry for any mistake
GOOD LUCK