Hematology

BIOCHEMISTRY

☑ Sheet ☐ Slide ☐ Handout

Number: 3

Subject: BPG + CO_2 transport

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Price:

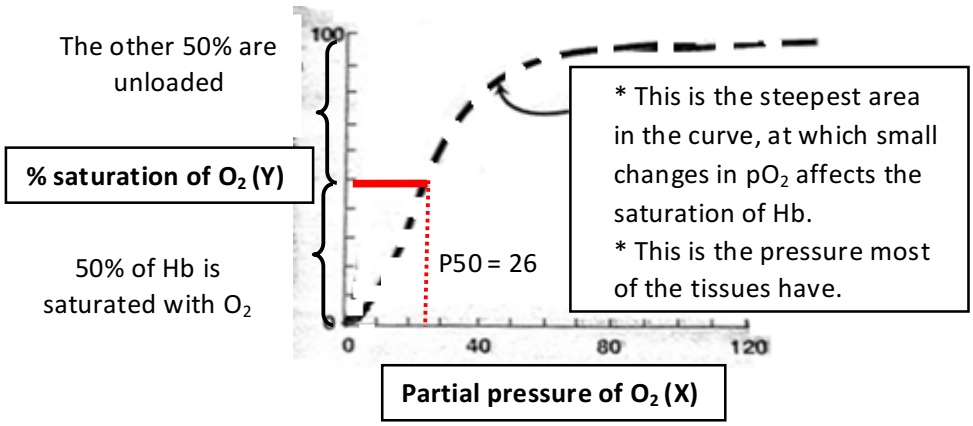
Review:

- Oxygen-dissociation curve for Hemoglobin is **sigmoidal**
- Its affinity of binding oxygen is affected by **allosteric effectors**
- Positive allosteric effectors: shift the curve to the left, favoring more binding of O_2.
- Negative allosteric effectors: shift the curve to the right favoring less binding and more unloading of O_2.

BPG as a negative allosteric effector:

- BPG is an intermediate of glycolytic pathway, that's profoundly produced by the RBCs.
- BPG concentration differs according to the overall state and concentration of O_2 to help the body undergo adaptation.
- When you have a situation of chronic hypoxia (like high altitudes, COMP or anemia) the delivery of oxygen can be adjusted by changing the concentration of BPG.

To have a full understanding study these figures:



*At normal conditions, the dissociation curve for Hb has a P50 of 26 mmHg.

The figure above is the normal saturation curve for hemoglobin under the prevailing conditions in RBCs:

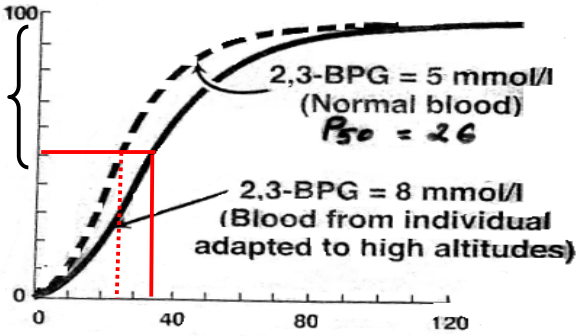
- 50% of Hb is saturated when the pressure of O_2 equals 26 (the doctor said 27 though).

- The steepest area in the curve is the area affected by the allosteric activators/inhibitors, so any increase or decrease in oxygen will affect this area.

The other 50% are unloaded

% saturation of O_2 (Y)

50% of Hb is saturated with O_2



Partial pressure of O_2 (X)

Oxygen-dissociation curve for Hb when **a negative** allosteric effector is added (let it be BPG in this example).

*The shift of the curve toward the right indicate that this will unload the same amount of oxygen under hypoxic conditions, since the whole curve is brought to a new higher p50 and 100% saturation at low amounts of O_2.

In hypoxic conditions (↑altitudes, pulmonary diseases, anemia..):

- Partial pressure of O_2 is going to be less (it is not going to be 100mmHg)
- Hb is not going to be fully saturated but about 80% saturated.
- In this case BPG will increase shiftting the curve to the right.

When BPG increases it will decrease the affinity of Hb binding to O_2 releasing more amount of O_2 to the tissue, so the amount unloaded will be the same as in normal situations.

Transport of CO2:

Hemoglobin is important for the transport of O_2. Plasma without hemoglobin can carry only 2ml/L due to the low solubility of oxygen, but with hemoglobin the amount of O_2 carried is 200 ml/L (100 times more). Also, hemoglobin is involved **directly** and **indirectly** in the transport of CO_2.

CO_2 is transported from tissues to the lungs by three mechanisms:

1. Bicarbonate ion.
2. Dissolved CO_2 in the plasma.

3. Carbaminohemoglobin.

1- Bicarbonate ion:

- Some of The CO_2 that is produced in the tissue is dissolved in plasma as it diffuses across the membrane easily.
- In the plasma when its concentration becomes high it enters the RBCs.
- In the RBCs carbonic anhydrase enzyme**,** which has a very high catalytic power, is going to hydrate carbon dioxide into carbonic acid.
  $CO_2 + H_2O \rightleftharpoons H_2CO_3$
- Carbonic acid is a very weak acid (pKa is less than the pH prevailing inside the RBCs) so it will dissociate to form bicarbonate & protons.
  $H_2CO_3 \rightleftharpoons HCO_3^- + H^+$
- Bicarbonate ions are going to leave the RBCs to the plasma.

2- Dissolved in plasma (10%):

- Most of the dissolved CO_2 becomes bicarbonate producing lots of protons. However, some of the CO_2 remains in the solution rather than entering RBCs and producing H_2CO_3.
- This CO_2 that remains in the blood is higher in **venous** blood than arterial blood.
- The difference in concentration between both venous and arterial blood actually represents the amount of CO_2 that has been expelled out by this mechanism (which is 10%).

3- Carbaminohemoglobin (15%):

- Some CO_2 react covalently with hemoglobin forming carbaminohemoglobin.
- Although most of the amino groups in hemoglobin are charged, CO_2 reacts with the uncharged **NH_2-terminal of hemoglobin**. Which means that after this binding the equilibrium will be pushed to convert more of the charged to the uncharged form.
  $Hb–NH_2 + CO_2 \rightleftharpoons Hb–NH–COO^- + H^+$
- Deoxyhemoglobin (T form) has more affinity for forming the carbaminohemoglobin than the oxy form of hemoglobin. This binding stabilizes the T form, resulting in a decrease in its affinity for oxygen and a

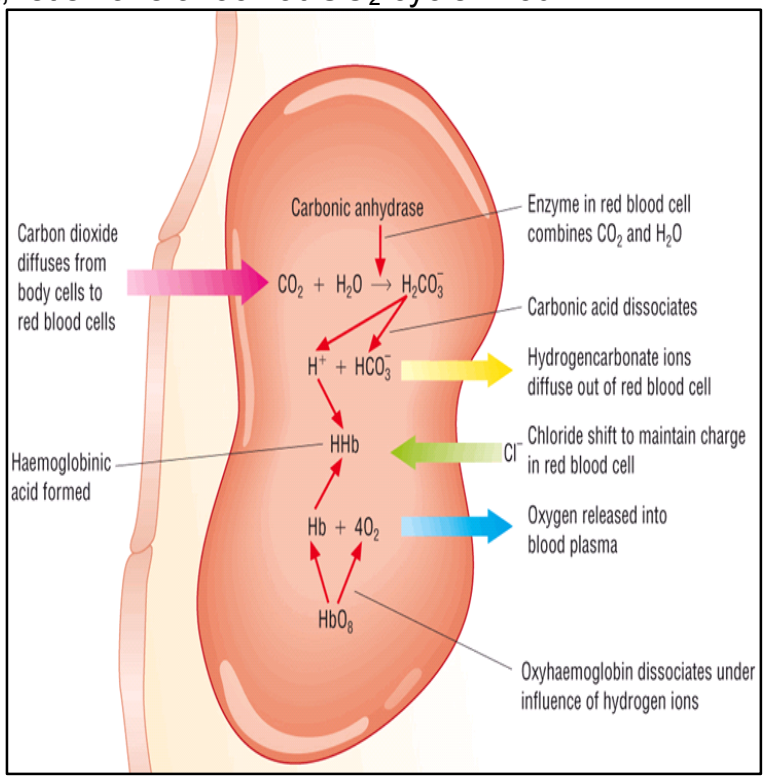
right shift in the oxygen dissociation curve. In the lungs, CO_2 dissociates from the hemoglobin, and is released in the breath.

- The difference between the oxyhemoglobin and deoxy affinity equals the amount of CO_2 removed when hemoglobin undergoes oxygenation in the lungs.
- This contributes to about 15% of the removal of CO_2.
- Don't forget that the bulk of CO_2 is removed by the bicarbonate system.

The Bohr Effect:

- As we said earlier, most of CO_2 produced in metabolism is hydrated and transported as bicarbonate ion. This process releases huge amount of protons, however, the pH in plasma remains constant (7.4). How does that happen?
- When those protons are released, they will be neutralized (buffered) by 3 different buffers:
  1- Hemoglobin (50%)
  2- Other classical buffers (10%)
  3- Isohydric system (40%): This system makes the base of the Bohr Effect.

- To understand the Bohr effect, let's have a look at CO_2 cycle in our bodies:



At tissue side, the bulk of CO_2 is produced by Kreb's cycle. CO_2 diffuses to plasma through cell membranes. When its concentration becomes high in the plasma it diffuses to RBCs.

In the RBCs we have our powerful enzyme (carbonic anhydrase), CO_2 undergoes hydration mainly by this enzyme. Without carbonic anhydrase hydration occurs slowly (takes about 100 sec while the whole blood cycle takes about 60 sec).

Carbonic acid, produced in the previous step, dissociates forming protons. When oxyhemoglobin arrives, these protons work as negative effectors

shifting the curve to the right so that oxygen will be released (remember: lower pH favors unloading of O_2 & the more protons produced the more oxygen released).

But why do protons act as negative allosteric effectors?

The Bohr Effect reflects the fact that the deoxy form of hemoglobin has a greater affinity for protons than does oxyhemoglobin. This effect is caused by ionizable groups, such as specific histidine side chains (imidazole group of His146 mainly). This group has a higher pKa **in deoxyhemoglobin** than in oxyhemoglobin. Higher pKa means weaker acid that favors **accepting protons** (more basic).

Therefore, an increase in the concentration of protons (resulting in a decrease in pH) causes these groups to become protonated (charged) and able to form ionic bonds (also called salt bridges). These bonds preferentially stabilize the deoxy form of hemoglobin, producing a decrease in oxygen affinity (thus O_2 release).

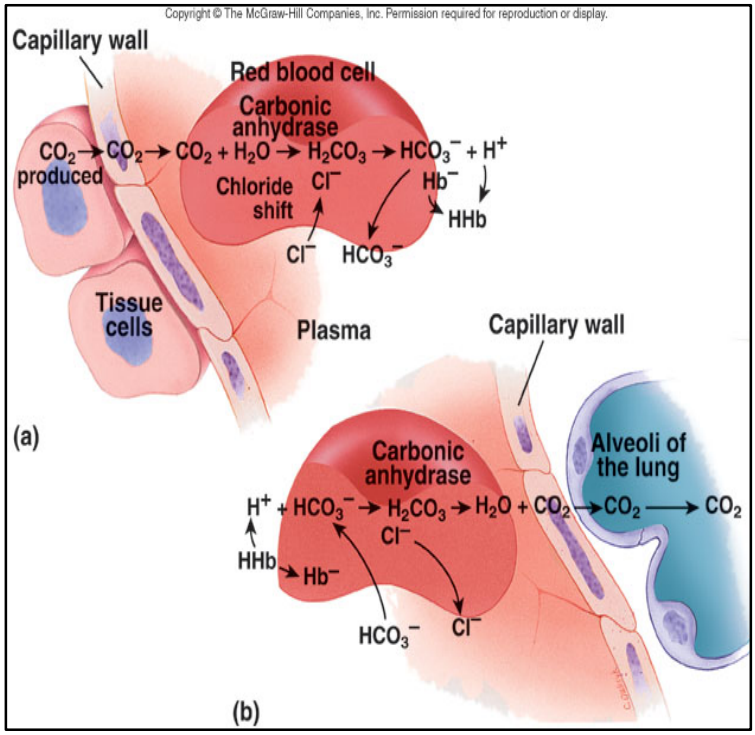
$$HbO_2 + H^+ \rightleftharpoons HbH + O_2$$

Oxyhemoglobin Deoxyhemoglobin

This deoxyhemoglobin can accept from 1.2 to 2.7 protons. The exact amount of protons added depend on the condition:

1- H^+ concentration.
2- HCO_2
3- Chloride (discussed later)
4- Temperature

This mechanism is different from other classical buffers.



At Lungs side: Deoxyhemoglobin comes to the lung carrying CO_2, CO_2 dissociates giving the chance to O_2 to bind and be transferred to the tissue where CO_2 will bind again. How? Please follow the figure!

1- Venous blood that arrives to the lungs has a higher concentration of CO_2 than alveoli, so CO_2 will be removed by exhalation. This removal of CO_2 will lower its concentration

in the plasma. CO_2 will diffuse out of RBCs to the plasma. Then, bicarbonate concentration becomes low inside the RBCs, so bicarbonate diffuses from the plasma to the RBC.

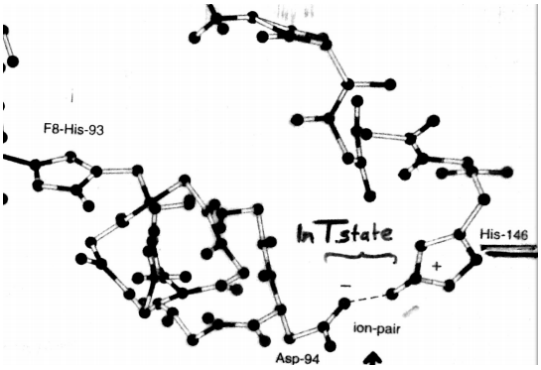
2- Oxygen pressure is high in the lung (approximately 100 mmHg) so hemoglobin will undergo oxygenation. Oxygenation leads to conformational changes that lower the pKa of His residue so it becomes more acidic releasing protons.
3- These new protons will interact with bicarbonate (from step 2) in the cytoplasm forming more carbonic acid.

(Be aware that what happens at the lung side is opposite to what happens at the tissue side).

How do protons act with hemoglobin?

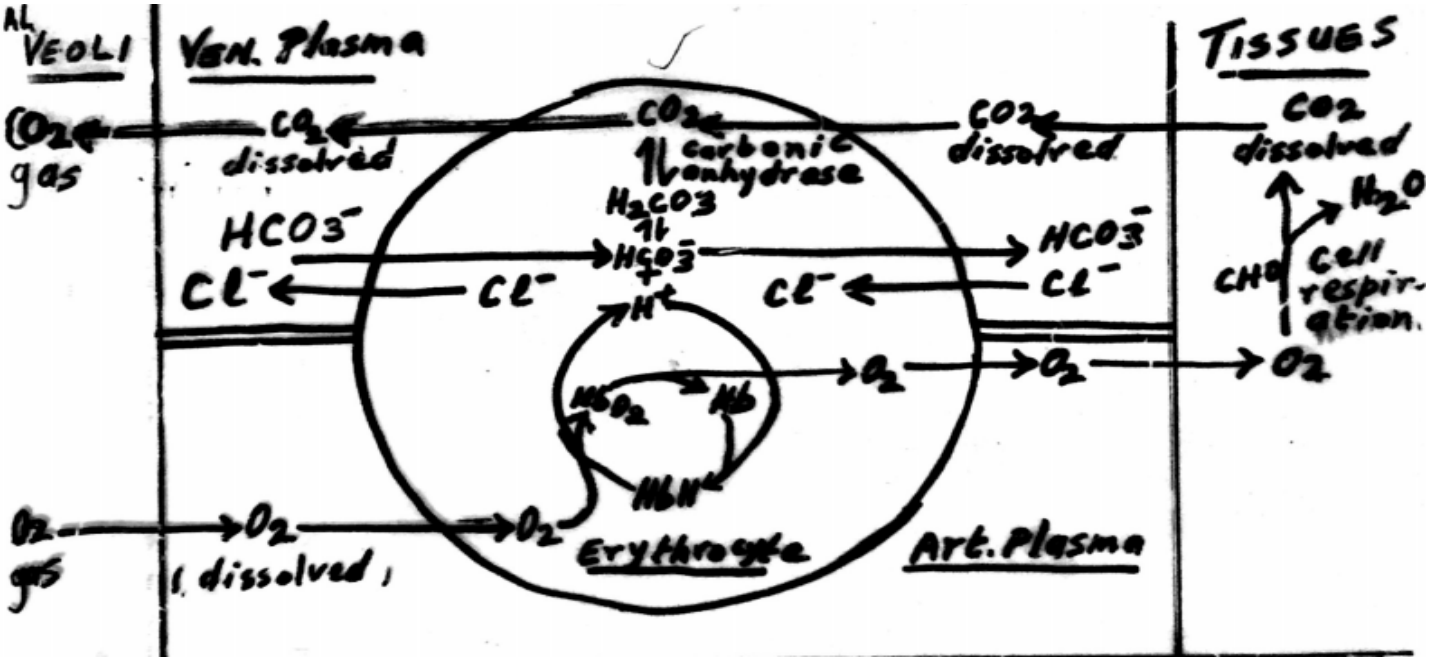
Hemoglobin accepts a proton in deoxy state, and releases a proton in oxy state.

- We have 7 ionic groups that contribute to accepting/ releasing of protons, the most important one is the imidazolium of the c-terminal histidine (His146) of the β-chain.
- When hemoglobin is in the deoxy state, imidazole will become more basic with a higher pKa. This will favor accepting protons.
- When His146 is protonated, it will be charged. So it will form ionic bonds with aspartate residue. This ionic bond has a major role in stabilizing the T state (reduced state).
- When Hb becomes oxygenated, some electrostatic bonds will be weakened and broken. Also, the pKa will be lowered becoming more acidic and releasing the proton, which will break the ionic bond.
- His146 residue is responsible for 50% of the Bohr Effect, terminal amino group and many other groups that we don't know also play a role in Bohr Effect.



- pKa of deoxyhemoglobin is 7.7 while for oxyhemoglobin is 7.3.

Summary:



On the right (tissue side):

1- The cell undergoes respiration producing CO_2, when its concentration increases it will diffuse to the **plasma**. Then, it will diffuse to the **RBC**.
2- Inside the RBC, CO_2 undergoes hydration by **carbonic anhydrase** to form **carbonic acid**. Carbonic acid dissociates into $\mathbf{H^+}$ and **bicarbonate** ion.
3- **Bicarbonate** accumulates and exits the cells toward the **plasma**.
4- In order to achieve electrical equilibrium, some negatively charged chloride enters the RBC. We call this **<u>chloride shift.</u>** (Chloride shift happens in venous blood).
5- Elevated levels of chloride and protons (produced in step 2) favor shifting the curve to the right causing release of O_2 to the tissues.

On the left (lungs side):

1. Deoxyhemoglobin goes to the alveolar membrane in the lung where it undergoes oxygenation releasing its proton.
2. CO_2 will diffuse among its concentration gradient from the plasma to the lungs, then from the RBC to the plasma.
3. Bicarbonate will react with H^+ to form H_2CO_3, H_2CO_3 dissociates to produce CO_2 again. This brings more bicarbonate from plasma.
4. Chloride, which entered previously, will exit the RBC to maintain equilibrium. (**<u>Chloride shift</u>**)

❖ Isohydric shift is the movement of the proton, which is the Bohr Effect.

"Twenty years from now you will be more disappointed by the things that you didn’t do than by the ones you did do, so throw off the bowlines, sail away from safe harbor, catch the trade winds in your sails. Explore, Dream, Discover"

–Mark Twain

سامحونا على التأخير، موفقين جميعًا دكاترة..