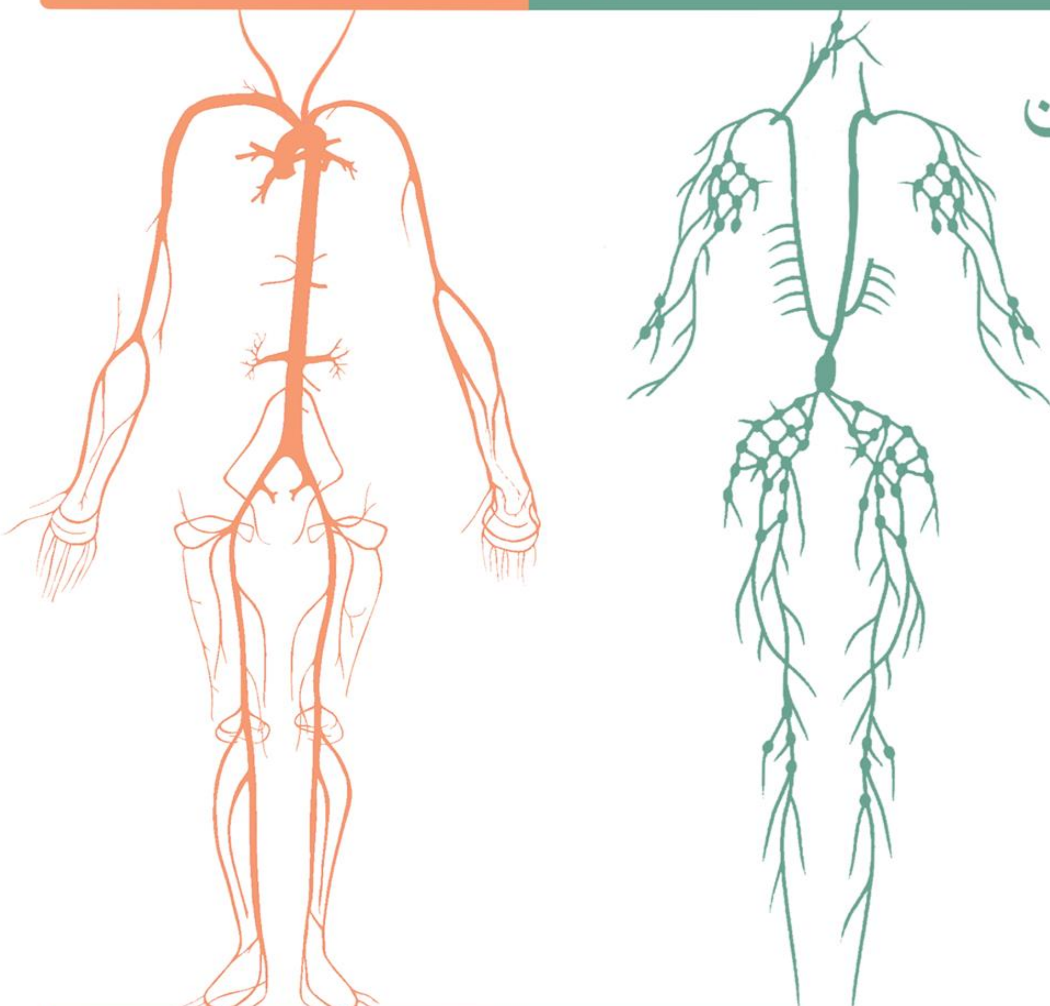
Biochemistry

HematoLymphatic



Title: Sheet 2 - Regulation of hemoglobin function

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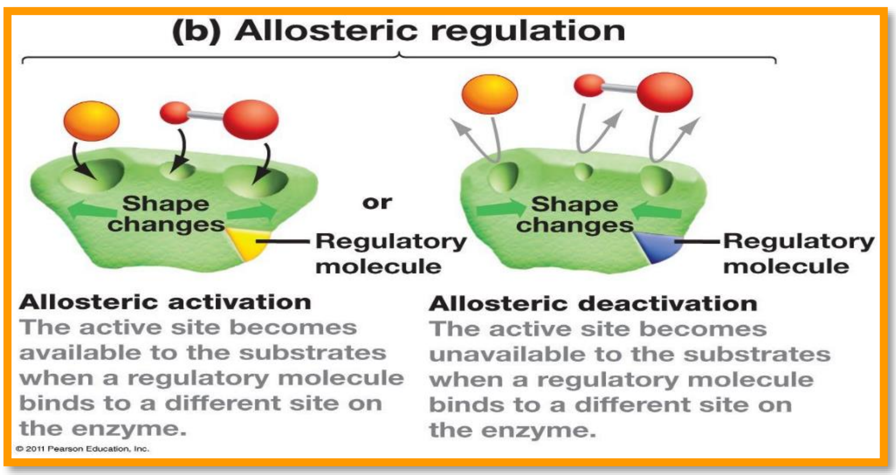
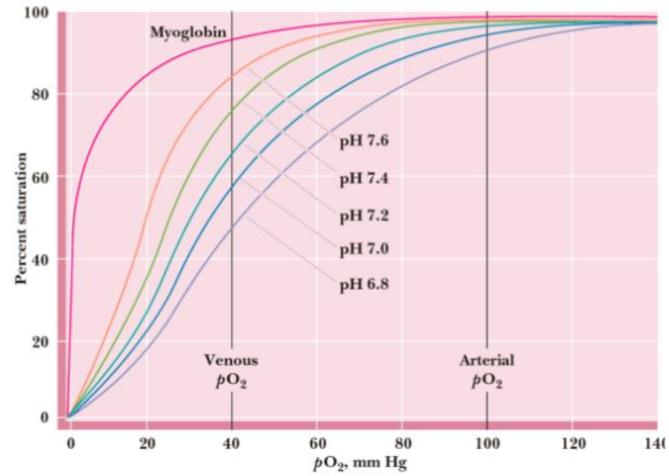
- In the previous lecture we mentioned the following concepts:
  - Hemoglobin is an **allosteric protein** (has two conformations).
  - **T(taut)** is the low-oxygen affinity form, and **R(relaxed)** is the high-affinity form of hemoglobin.
  - **Allosteric effect** a change in the shape and activity of a protein, resulting from binding of a regulatory substance.
  - **Cooperative process:** The binding of the first O_2 to Hb enhances the binding of further O_2 molecules until all 4 are saturated.
- In this sheet we will go through the regulation process of hemoglobin and its allosteric effectors.
  - Allosteric proteins have sigmoidal (S shaped) curve.
  - Allosteric effectors bind to regulatory sites other than the active site (the oxygen binding site).



- Heterotropic effector: a regulatory molecule which is not the substrate for the protein.
  - The major heterotropic effectors of hemoglobin:
    1. Hydrogen ion.
    2. Carbon dioxide.
    3. 2,3-Bisphosphoglycerate.
    4. Chloride ions.
    5. Carbon monoxide.

Hydrogen ions (pH)

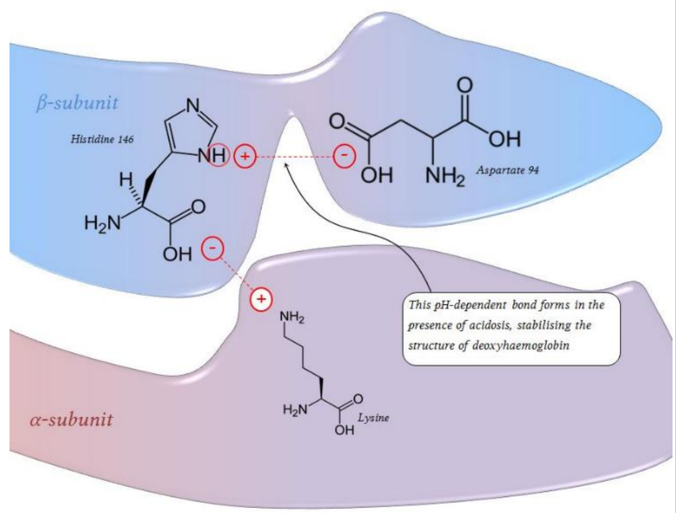
- A change in pH alters the structure of hemoglobin and the accessibility of oxygen to the binding sites on heme. The binding of H^+ to hemoglobin promotes the release of O_2 from hemoglobin and vice versa. This phenomenon is known as the **Bohr effect.** The lowering of pH shifts the sigmoidal curve to the right, and the result is a decreased affinity.



- You can notice from the plot that when pH is lowered P50 is increased, indicating that more oxygen is required to saturate 50% of hemoglobin (lowered affinity).

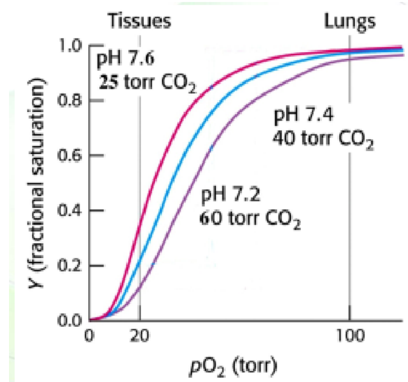
Mechanism of Bohr effect:

- At low pH (high [H^+]), His146 from the β-subunits becomes protonated (positively charged) and can make a salt-bridge with Asp from the same chain. Electrostatic interaction also occurs between the carboxylic group of His146 and a lysine of the α chain. This favors the deoxygenated form of hemoglobin (T-form).
- The pKa of His146 is increased from 7.3 in the R state to 7.7 in the T state allowing for protonation.



To sum up: At low pH His146 (β-chain) is more frequently protonated → salt bridges are formed with Asp(β-chain) and Lys (a-chain) → T-state is stabilized.

Effect of CO_2: (Mechanism #1 Where do protons come from?)



- Tissues produce CO_2 as by-product of metabolism that reacts with water to form the weak carbonic acid by the enzyme carbonic anhydrase. Carbonic acid dissociates spontaneously and generates protons (H^+) as well as bicarbonate (the major blood buffer). These protons are taken up by Hb. The dissociation curve is shifted to the right by ↑PCO_2 to enhance the release of O_2 in tissues.

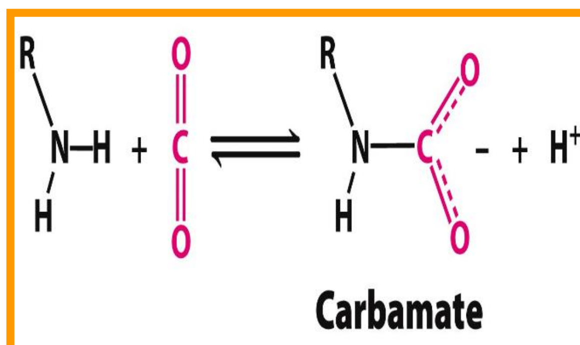
$$\underset{\textit{carbon dioxide + water}}{CO_2 + H_2O} \rightleftarrows \underset{\textit{carbonic acid}}{H_2CO_3} \rightleftarrows \underset{\textit{bicarbonate + hydrogen ion}}{HCO_3^- + H^+}$$

- The reverse mechanism occurs in the lungs where O_2 concentration is high and CO_2 concentration is low.

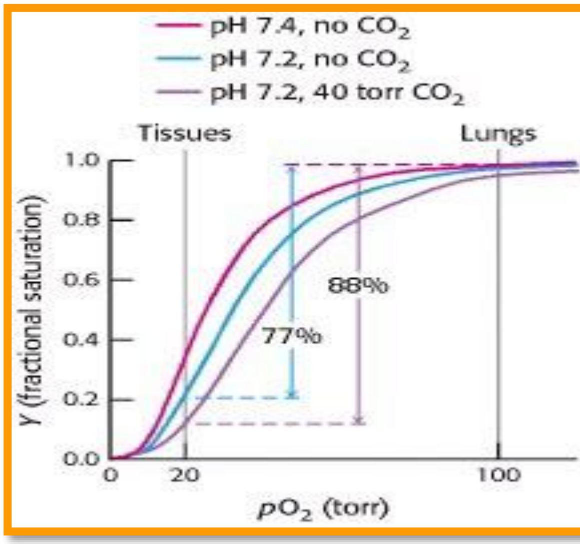
↓[H2CO3] →↓[H+] → shift of O2-Hb curve to the left →more binding of O2 to Hb → more O2 is transported to tissues.

Mechanism #2- formation of carbamates

- Hemoglobin transports some CO_2 directly, when the CO_2 concentration is high, it combines with the free α-amino terminal groups to form carbamate producing negatively charged groups. The increased number of negatively-charged residues increases the number of electrostatic interactions that stabilize the T-state of hemoglobin.

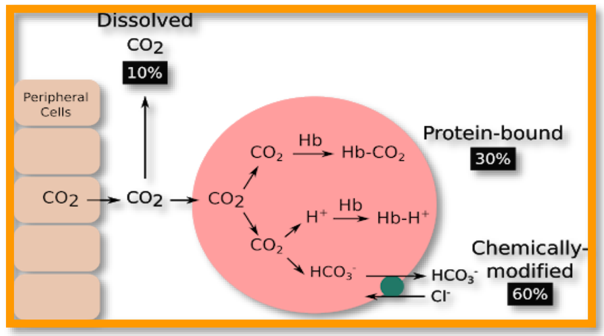


⇘ Contribution of both mechanisms:



- First situation (no CO_2): Lowering the pH from 7.4 (pink curve) to 7.2 (blue curve) results in the release of O_2 from oxyhemoglobin.
- Second situation (pH is held constant): Raising the CO_2 partial pressure from 0 to 40 torr (purple curve) will shift the oxygen dissociation curve to the right also favoring the release of O_2 from oxyhemoglobin.
- About 75% of the shift is caused by H^+. About 25% of the effect is due to the formation of the carbamino compounds.

⇘ Transport of CO_2 into lungs:

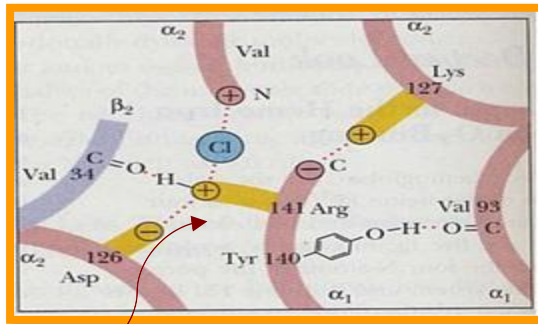


Carbon dioxide is transported to the lungs in three distinct chemical forms. Roughly 10% is transported as a simple dissolved gas. The remainder enters erythrocytes and encounters one of two fates:

1. A proportion binds directly to hemoglobin, generating carbamino-hemoglobin, and is transported within RBCs to the lung, accounting for 30% of CO_2 transport.
2. A much larger proportion is converted to bicarbonate and hydrogen by carbonic anhydrase. The bicarbonate is exported into the blood and travels to the lungs, accounting for 60% of total CO_2 transport. Hemoglobin binds the remaining H_+ so that it will not alter the pH of blood.

- The movement of CO_2 in/out of cells does not change the pH, a phenomenon called isohydric shift, which is partially a result of hemoglobin being an effective buffer.

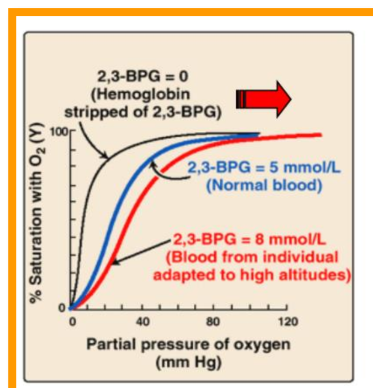
Chloride shift:



- Bicarbonate diffuses from tissues into the plasma and chloride shifts into RBCS. The process is reversed in pulmonary capillaries, chloride ion always diffuses in an opposite direction of bicarbonate ions in order to maintain an electrical balance.
  - Chloride ions interact with N-terminus of Val (α2) chain and the R side chain of Arg141 (α1) chain via charge-charge interactions, stabilizing the T-state.
  - Increasing the concentration of chloride ions (Cl^-) shifts the oxygen dissociation curve to the right (lower affinity).

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

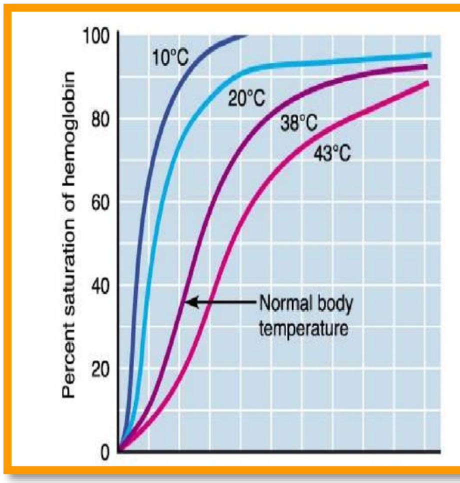
- A by-product of glycolysis. It stimulates the release of oxygen from erythrocytes. When a single molecule of 2,3-bisphosphoglycerate binds a hemoglobin tetramer, it forms salt bridges with the terminal amino groups of both β chains and with a lysine as well as a histidine stabilizing the T form of hemoglobin. This stabilization shifts the oxygen dissociation curve to the right, promoting the release of oxygen from hemoglobin in the tissues.
- Significance of BPG: At higher altitudes such as on top of a mountain, the partial pressure of oxygen is lower. The physiological consequence of this is that less oxygen will ultimately reach the tissues of our body. Our body begins to adapt by producing more 2,3-BGP. This will shift the oxygen-binding curve to the right lowering the affinity for oxygen, thus hemoglobin will be able to release more oxygen to the tissues of our body.



- 2,3-BPG in transfused blood:
  Storing blood results in a decrease in 2,3-BPG, hence hemoglobin acts as an oxygen "trap" not an oxygen transporter due to the abnormal high oxygen affinity. Transfused RBCs are able to restore their depleted supplies of

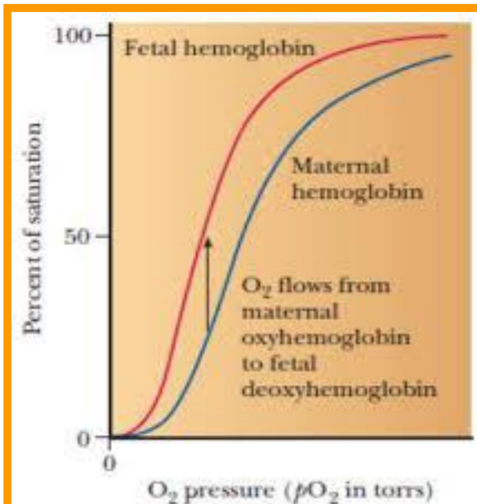
2,3BPG in 6–24 hours. However, severely ill patients may be compromised, so it is necessary to add ATP and BPG to the transfused blood in severe cases.

Effect of temperature:



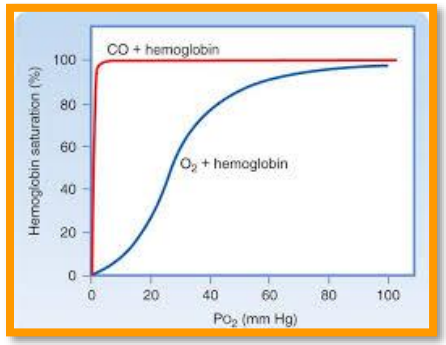
- An increase in temperature decreases oxygen affinity and therefore increases the P_{50}.
  Temperature has a dual effect:
  1. Temperature affects the O_2 binding of both myoglobin and hemoglobin.
  2. Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading from Hb-O_2.

Fetal hemoglobin HbF = α2γ2



- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HbA) because it contains gamma subunits instead of beta subunits. A His residue (needed for 2,3BPG binding) in the β subunit is replaced with a Ser in the γ subunit of HbF. Therefore, 2,3-BPG binds to fetal hemoglobin less strongly than to adult hemoglobin. The fact that adult hemoglobin can bind to 2,3BPG but HbF cannot is beneficial because HbF would have higher affinity for O2 than the mother; so oxygen is transferred to the fetus by diffusion since maternal bloodstream is richer in oxygen than the fetus'.

Effect of CO:

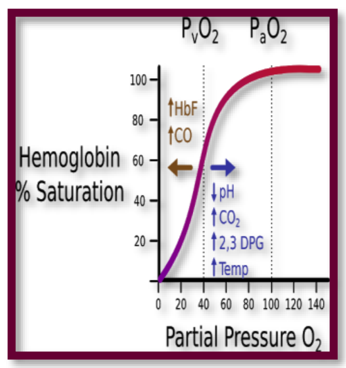


- The affinity of hemoglobin for CO is much greater than for O2, thus carbon monoxide combines with hemoglobin at the oxygen-binding sites. It also increases the oxygen affinity of hemoglobin, reducing the ability of the hemoglobin molecule to release oxygen bound to other oxygen-binding sites.
  - Relevant information:
    1. Increasing the amount of CO in inspired air to 1% and above would be fatal in minutes.
    2. Due to pollutants, the concentration of CO-Hb in the blood is usually 1% in a non-smoker.
    3. In smokers, CO-Hb can reach up to 10% in smokers. If this concentration of CO-Hb in the blood reaches 40% (as is caused by 1% of CO in inspired air), it would cause unconsciousness initially, followed by death.

Summary

Decreased pH
Increased pCO2
Increased 2,3-BPG
Increased Cl-
Increased Temperature

The curve will shift to the right → LOWERED O2 AFFINITY→O2 is released.



Quiz

1. Oxygen HB-dissociation curve shifts to the left due to?
   a. increased CO2
   b. decreased PH
   c. high altitude
   d. carbon monoxide poisoning.
2. Fetal hemoglobin has greater affinity than adult hemoglobin because
   a. It binds 2,3 BPG with less affinity by Gamma polypeptide chain than HbA.
   b. Its concentration is very high.
   c. Fetal blood gets oxygen from the mother.
   d. Its polypeptide chains bind very fast with Oxygen.

3. The following small molecules affect hemoglobin as indicated:
   a. Decreased [H^+] and [N_3^-] decrease Hb affinity for O2.
   b. Increased [H^+] and [Cl^-] increase Hb affinity for O2.
   c. Increased [CO] and [Cl^-] increase Hb affinity for O2.
   d. Increased [H^+] and [Cl^-] decrease Hb affinity for O2.
   e. Decreased [bisphosphoglycerate] (BPG) decreases the affinity for O2.

4. β-Lysine 82 in hemoglobin A is important for the binding of 2,3-BPG. In Hb Helsinki, this amino acid has been replaced by methionine. Which of the following should be true concerning Hb Helsinki?
   a. It should be stabilized in the T, rather than the R form.
   b. It should have increased O2 affinity and, consequently, decreased delivery of O2 to tissues.
   c. Its O2 dissociation curve should be shifted to the right relative to Hb.
   d. It results in anemia.

ANSWERS:

1. D
2. A
3. D
4. B