Regulation of hemoglobin function

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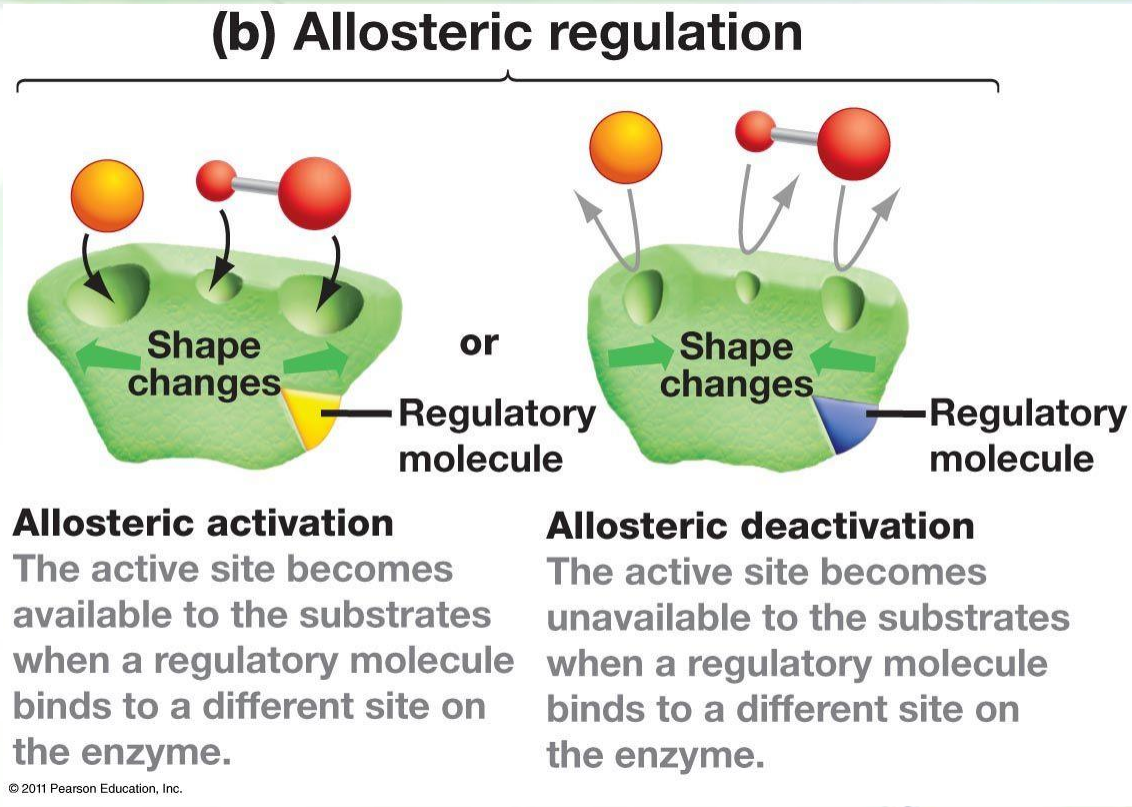
Hematopoietic-lymphatic system

Allosteric regulation

- Ligands that induce conformational changes in *allosteric* proteins are referred to as allosteric modulators or effectors.
- Allosteric modulators may be inhibitors or activators.
- They can be either:
- Homotropic modulators: are the same as the ligand itself, such as O_2
- Heterotropic modulators: are different from the ligand (next slide)



Allosteric effectors

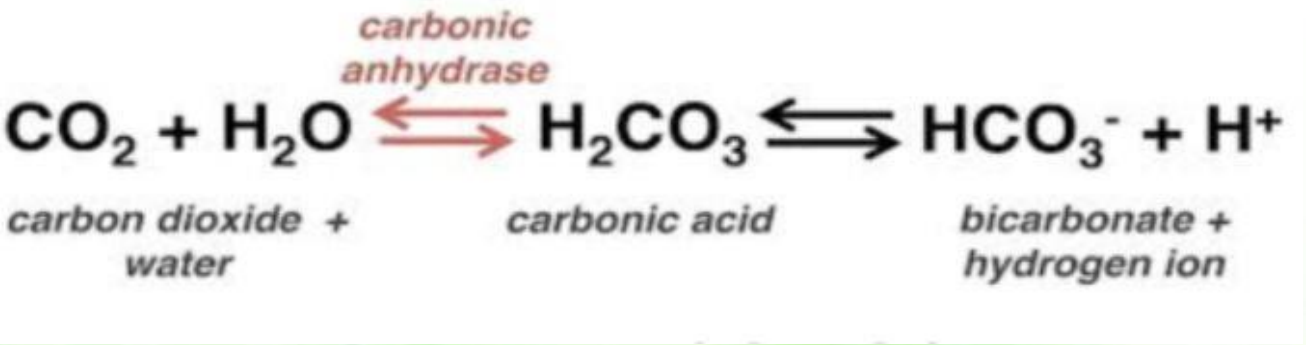
- The major heterotropic effectors of hemoglobin
  - Hydrogen ion, Bohr effect
  - Carbon dioxide
  - 2,3-Bisphosphoglycerate
  - Chloride ions

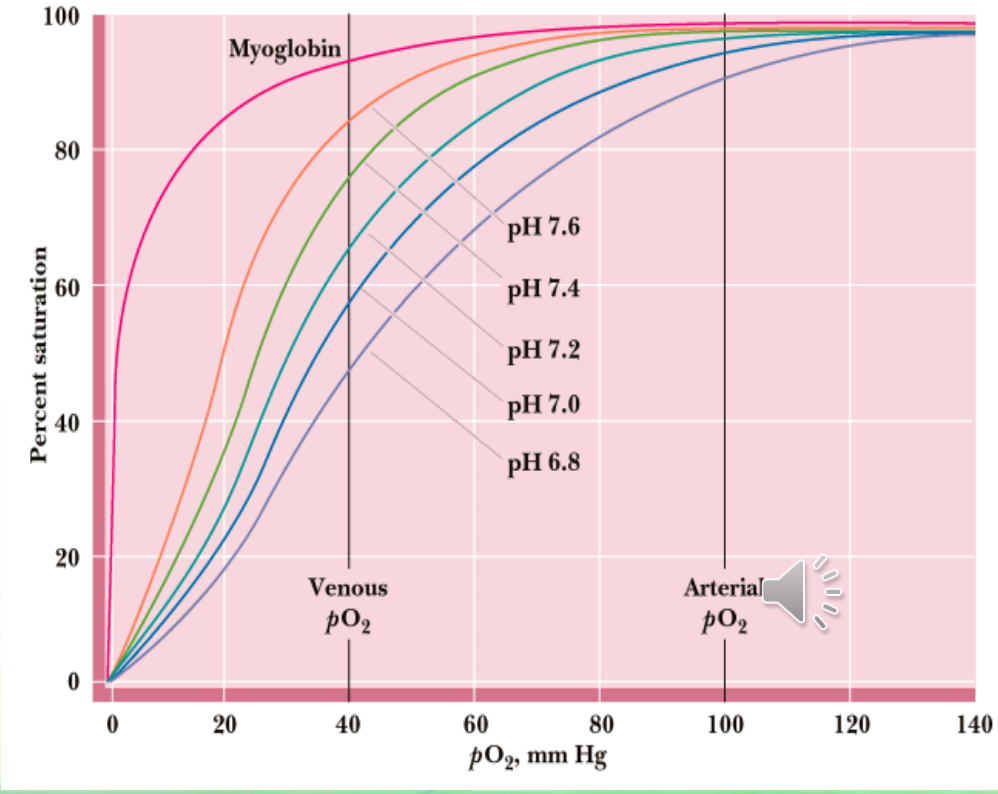
- A competitive inhibitor
  - Carbon monoxide

1- The effect of pH and H^+

The effect of pH

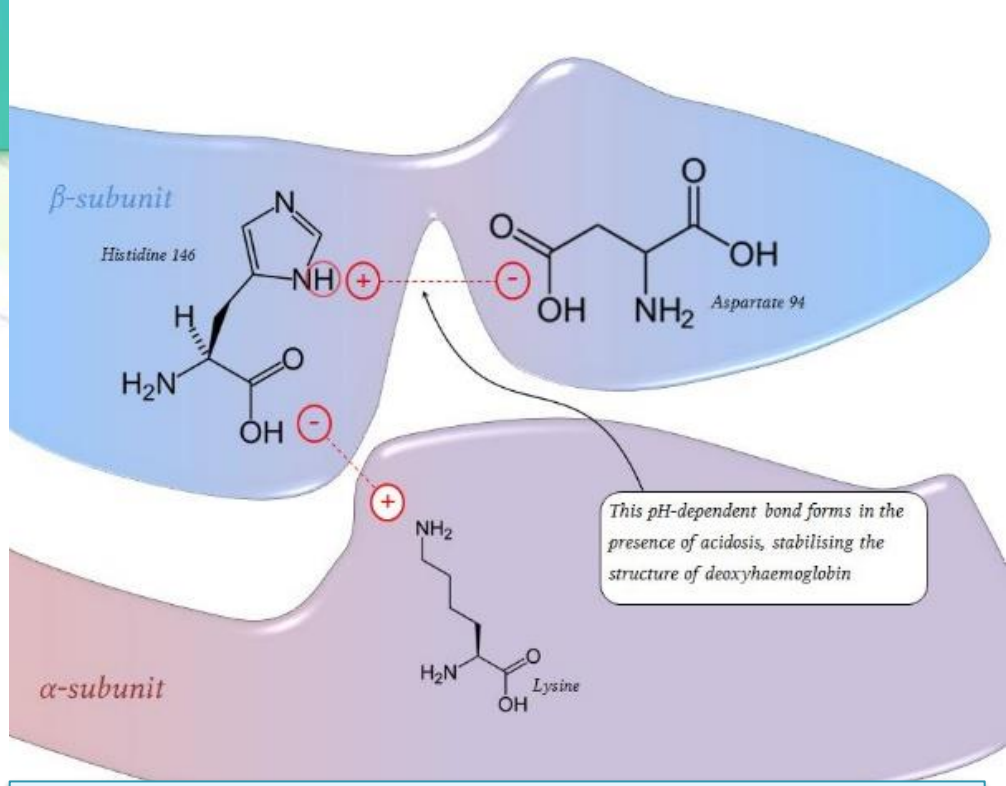
- The binding of H^+ to hemoglobin promotes the release of O_2 from hemoglobin, and the binding of O_2 at the lungs releases the H+.
- This phenomenon is known as the **Bohr effect**.





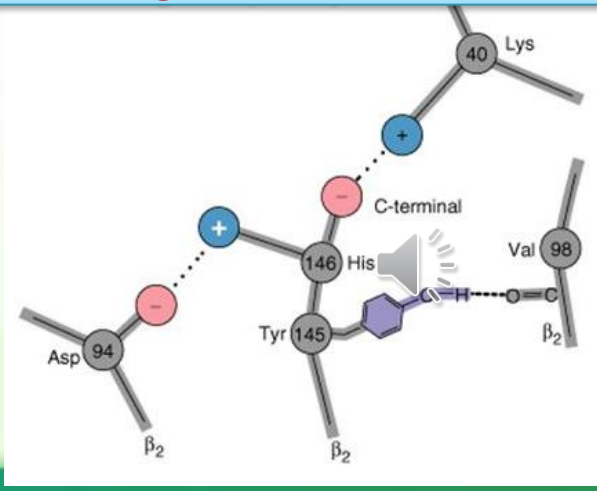
Mechanism of Bohr effect

- Increasing H^+ (in tissues) causes the protonation of key amino acids, including the last histidine residue of the β chains (**His146**).
- Once the Hemoglobin is at the tissues, it starts losing the Oxygen and starts changing to T state. The protonation of His146 speeds up the process as electrostatic interaction occur between the carboxylic group of His146 and a lysine of the α and a salt bridge forms between the same His and Asp94 within the same chain. These interactions stabilize the T state and keep His in an environment that increases its pKa leading to further stabilizing of the salt bridges.
- The reason behind the increase in the pKa is the movement of the His to a more negative environment. Although this should decrease its pKa because it would be more likely to lose the H+, nonetheless, it seems that there are multiple negative charges that bind to this H+ so none can pull it away from the rest. Furthermore, pulling this H+ away would add more negative charge to the environment, thus increasing the repulsion.
- When hemoglobin reaches lungs, Oxygen binds to it changing it from the T state to the R state, causing a destabilization in the whole structure which leads to breaking of the electrostatic bonds and changing to the position of the His to a place where its pKa becomes lower which causes it to loose the H+.



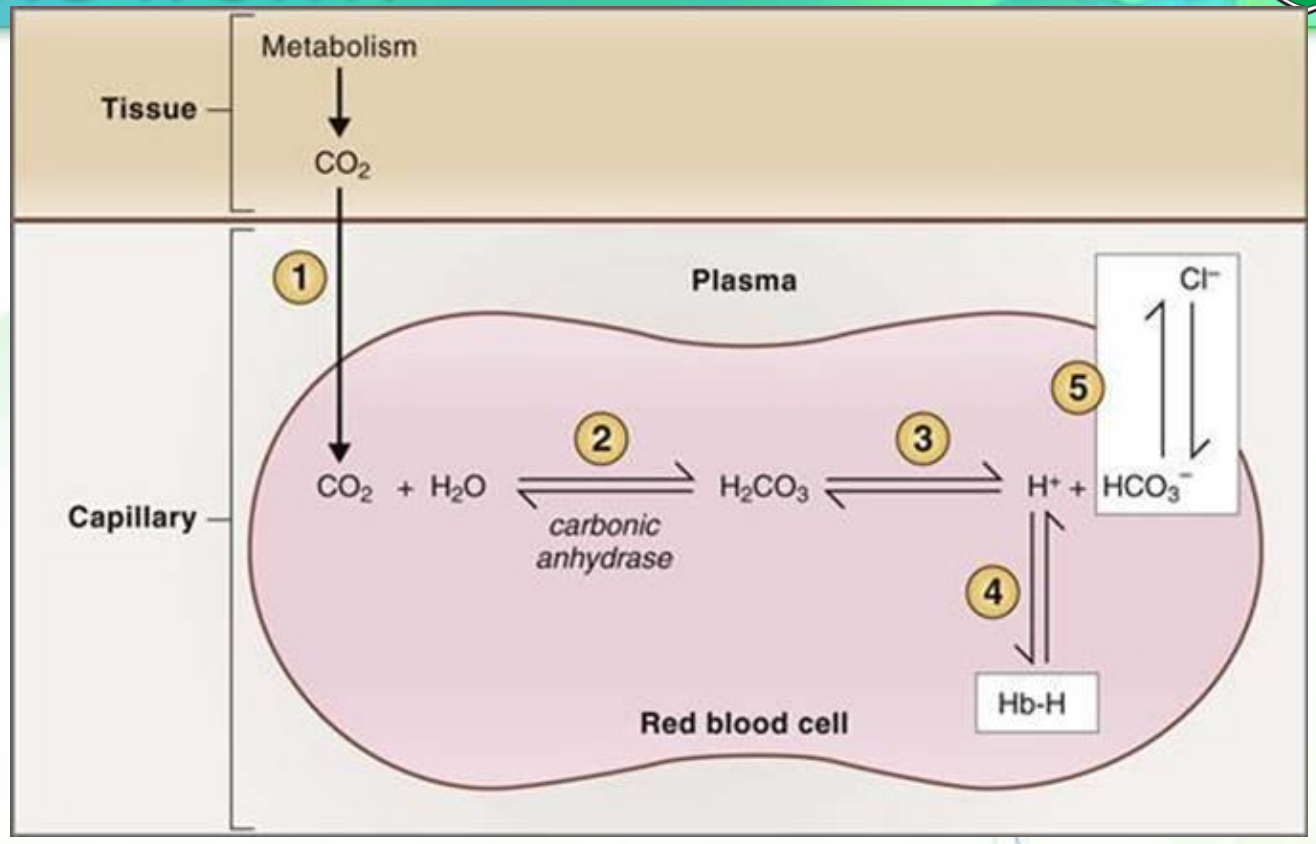
Note

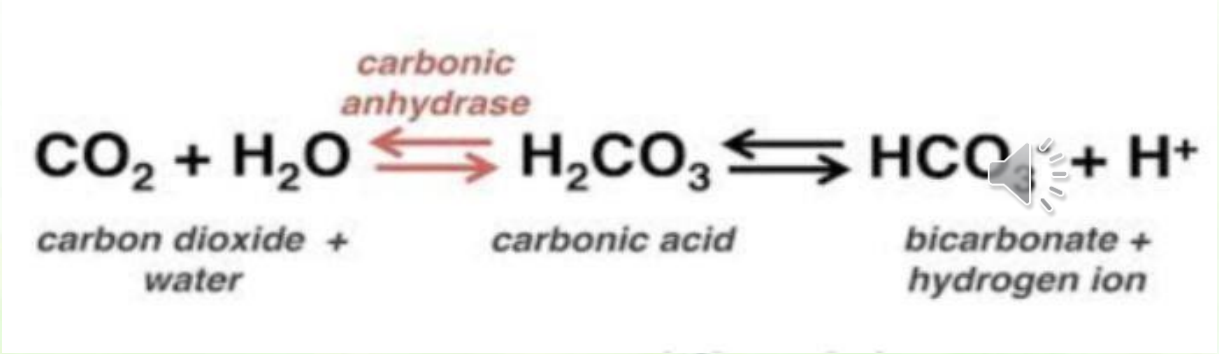
- **When pH> pKa, the group is deprotonated.**
- **When pH < pKa, the group is protonated.**



Where do protons come from?

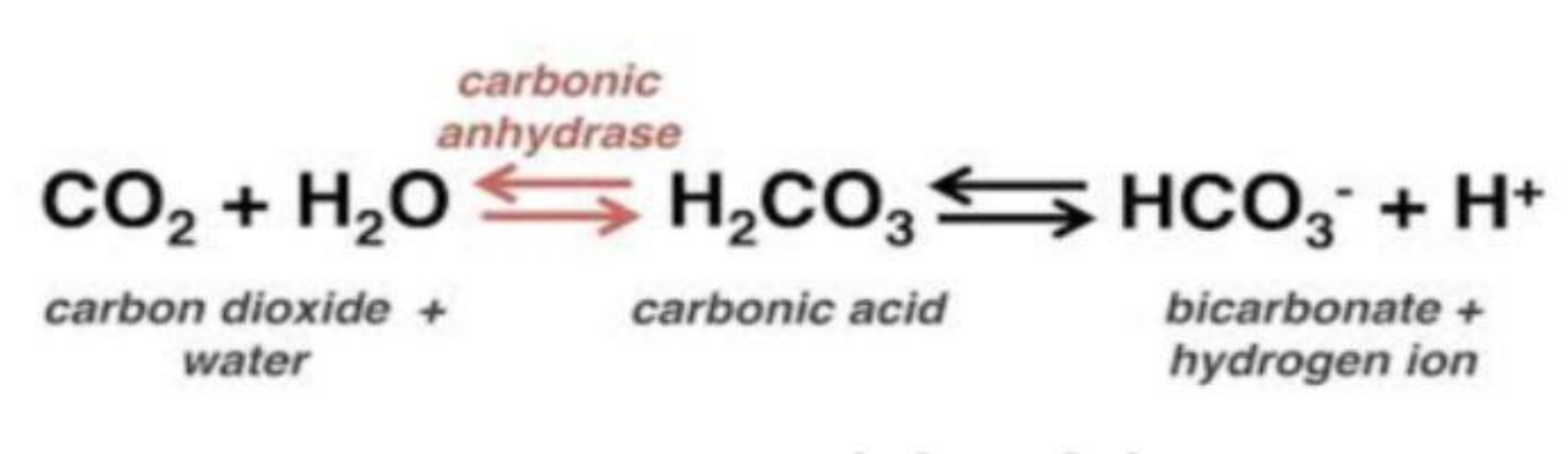
- H^+ is produced at high levels in metabolically active tissues. Then it enters the RBCs and reacts with H2O through carbonic anhydrase as shown in the figure and the equation to the right to produce H2CO3 (carbonic acid) which breaks to H+ and HCO3- (bicarbonate).
- In the lungs, the binding of O2 to the hemoglobin causes the H+ to be released as described in the previous slide.





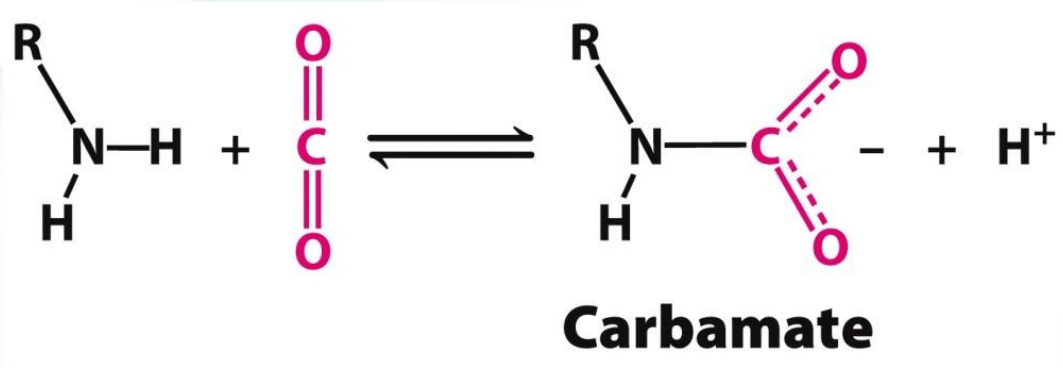
2- The effect of CO_2

Mechanism #1 - production of protons



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 and producing negatively-charged groups

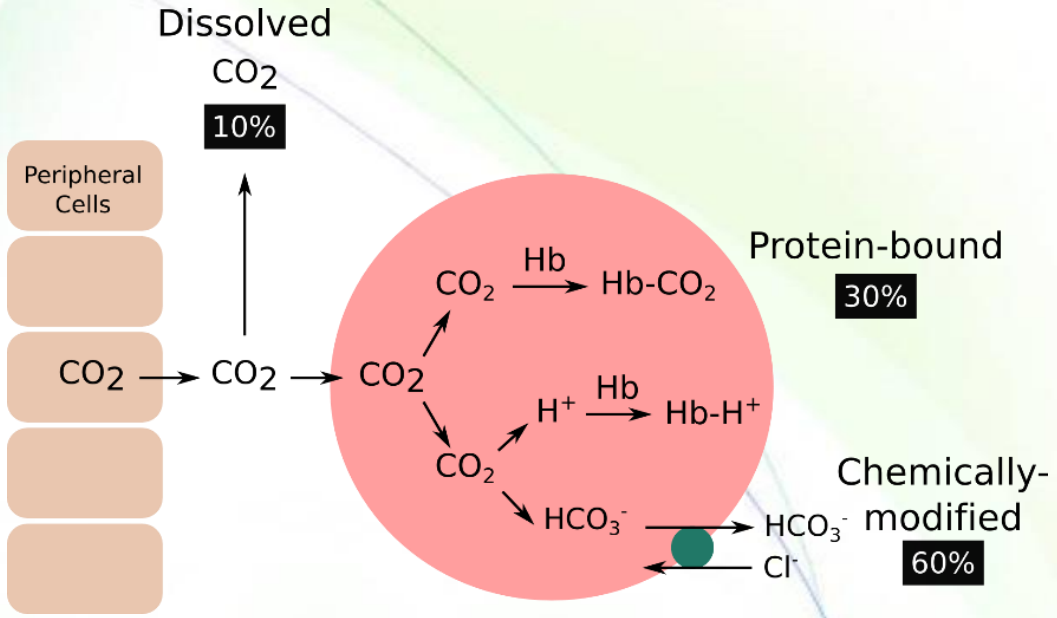


Carbamate

- The increased number of negatively-charged residues increases the number of electrostatic interactions that stabilize the T-state of hemoglobin.

Transport of CO_2 into lungs

- Approximately 60% of CO_2 is transported as bicarbonate ion, which diffuses out of the RBC.
- About 30% of CO_2 is transported bound to N-terminal amino groups of the T form of hemoglobin .
- A small percentage of CO_2 is transported as a dissolved gas.



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 in binding to the H+.

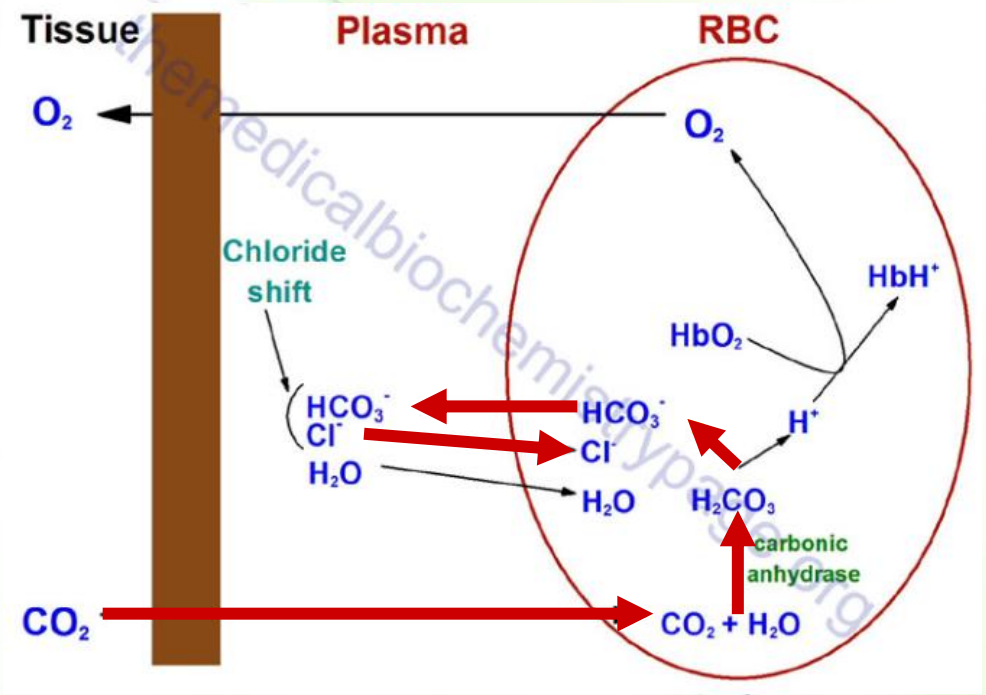
Which mechanism has a stronger effect in lowering hemoglobin's affinity to O2?

- About 75% of the shift is caused by H^+.
- About 25% of the effect is due to the formation of the carbamino compounds.
- How do we know that? By changing one factor at a time and keeping the others constant. We saturate the hemoglobins with O_2, then we start reducing it (the O_2) without any effector (control) and we record the p50. Then we saturate the hemoglobin again and we start reducing the Oxygen in the same rate as above but now we perform the test at low pH (different pHs can be measured). Then we do the same but with varying CO_2 concentrations instead of pH, then we compare which causes bigger shift to the right.

3- Effect of Chloride ion

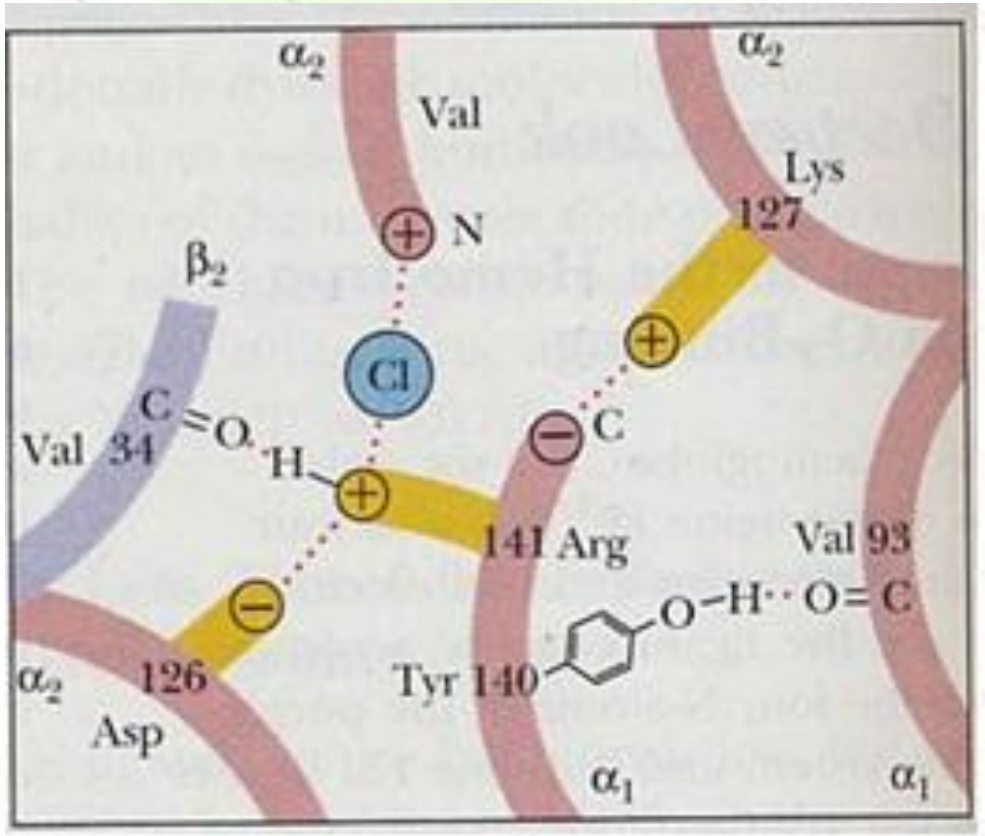
Chloride shift

- Bicarbonate diffuses out of the red blood cells into the plasma when the RBCs are near active tissues, but enters into the RBC near the lungs in order to be converted to CO_2 then released into the lungs.
- Chloride ion always diffuses in an opposite direction of bicarbonate ion in order to maintain a charge balance.
- This is referred to as the "chloride shift“.



Effect of chloride ions

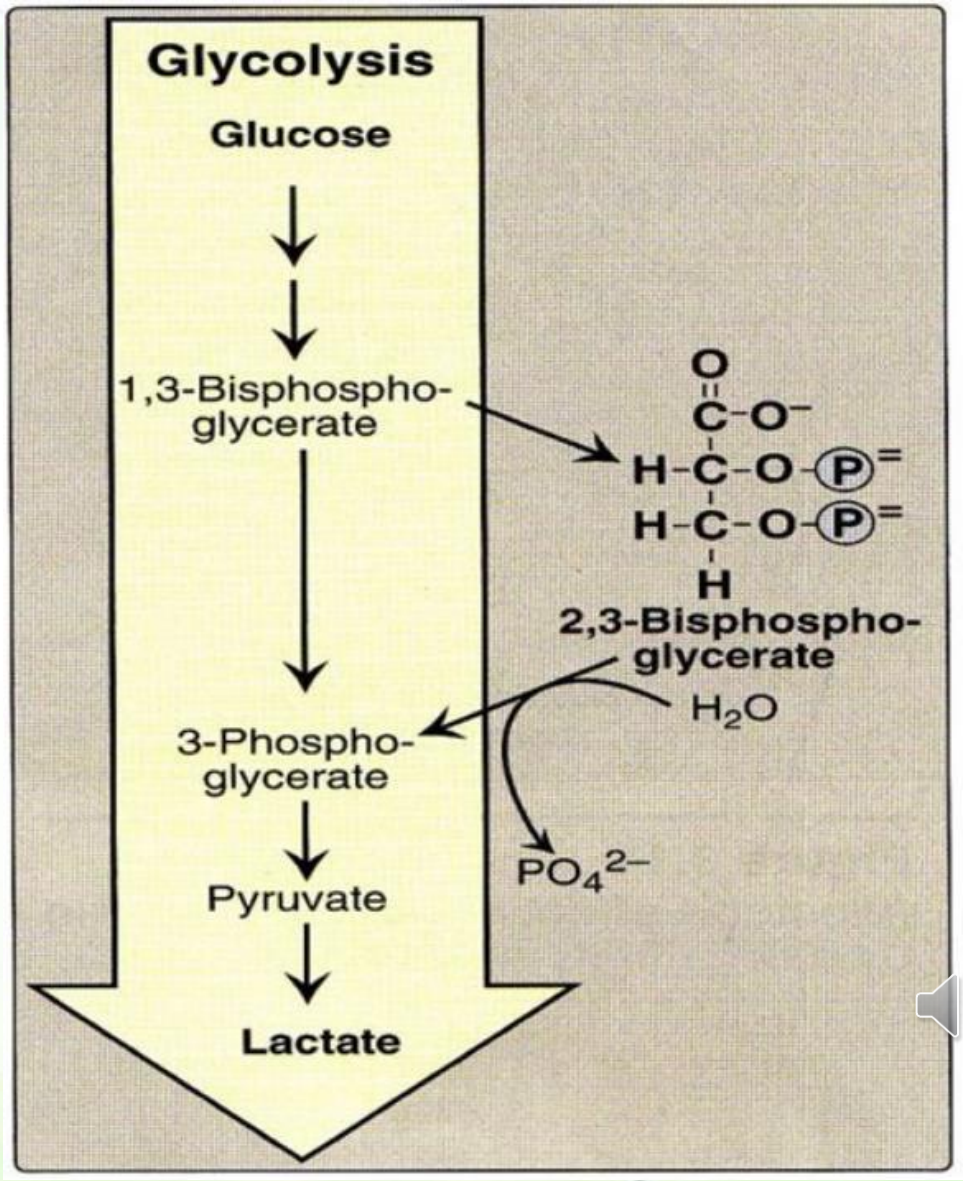
- Chloride ions interact with both the N-terminus of α2 chain and Arg141 of α1 chain stabilizing the T-state of hemoglobin.
- Increasing the concentration of chloride ions (Cl^-) shifts the oxygen dissociation curve to the right (lower affinity).



4- Effect of 2,3-bisphosphoglycerate

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

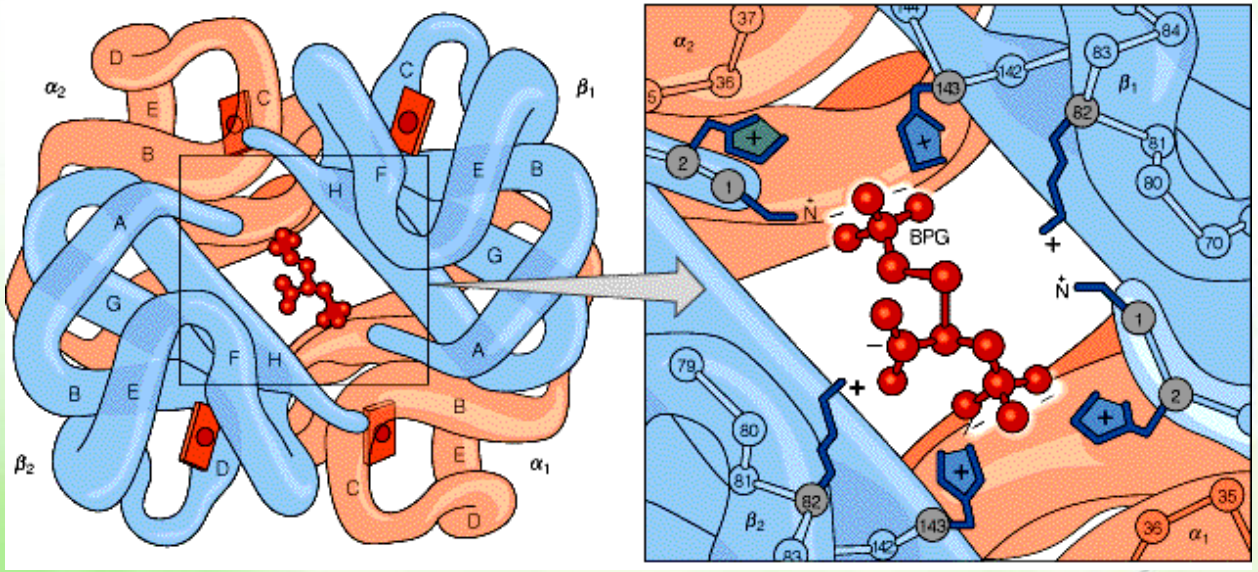
- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- It binds to hemoglobin and reduces its affinity towards oxygen.



2,3-BPG –hemoglobin interaction

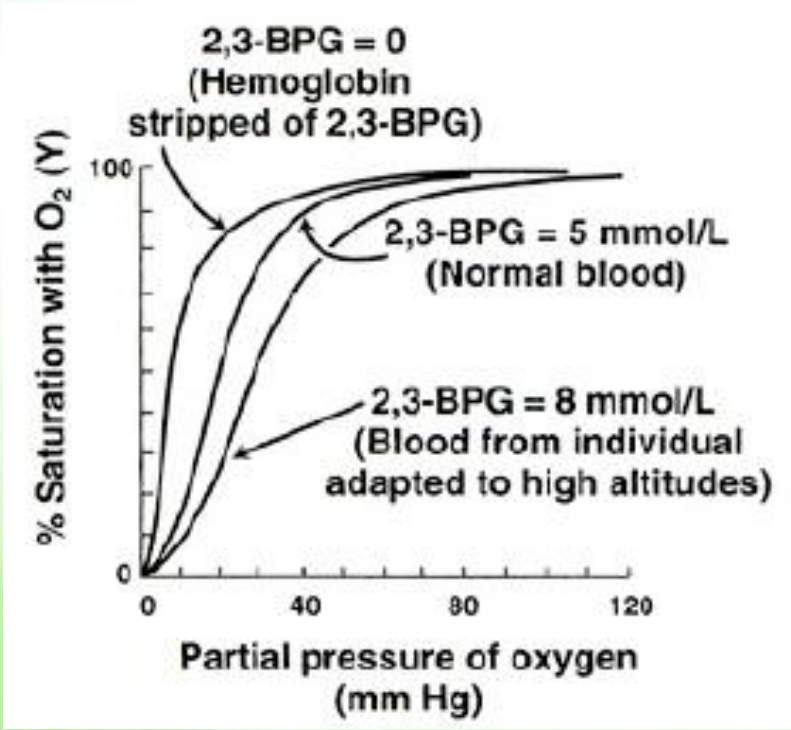
- 2,3-BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 2,3-BPG/hemoglobin tetramer.
- This binding stabilizes the T-state hemoglobin reducing the binding of oxygen to hemoglobin and facilitating oxygen release.

2,3-BPG forms salt bridges with the terminal amino groups of both β chains and with a lysine and His143.



Effect of 2,3-BPG on oxygen binding

- In the presence of 2,3-BPG, the p50 of oxyhemoglobin is 26 torr.
- If 2,3-BPG were not present, p50 is close to 1 torr.
- Notice the big drop of saturation with and without oxygen at the tissues.
- The concentration of 2,3-BPG increases at high altitudes (low O_2) and in certain metabolic conditions making hemoglobin more efficient at delivering oxygen to tissues.



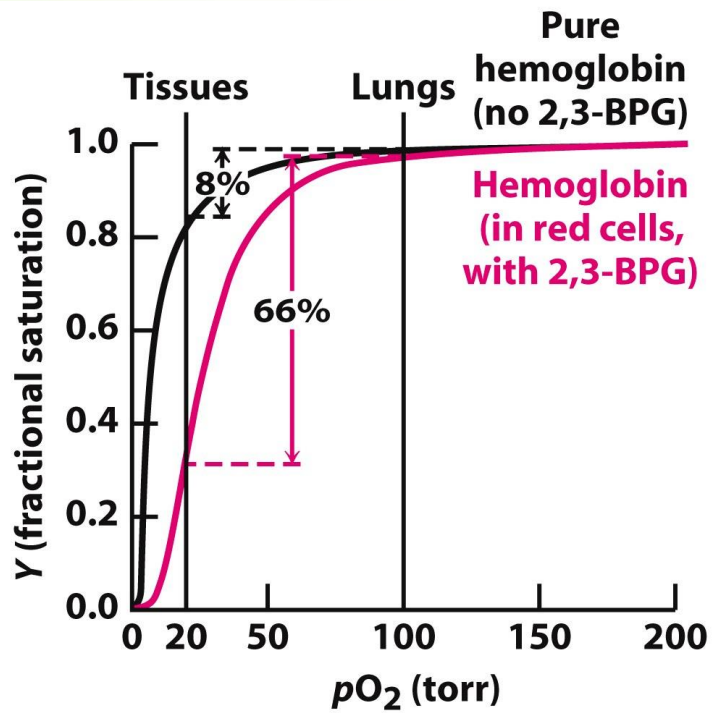
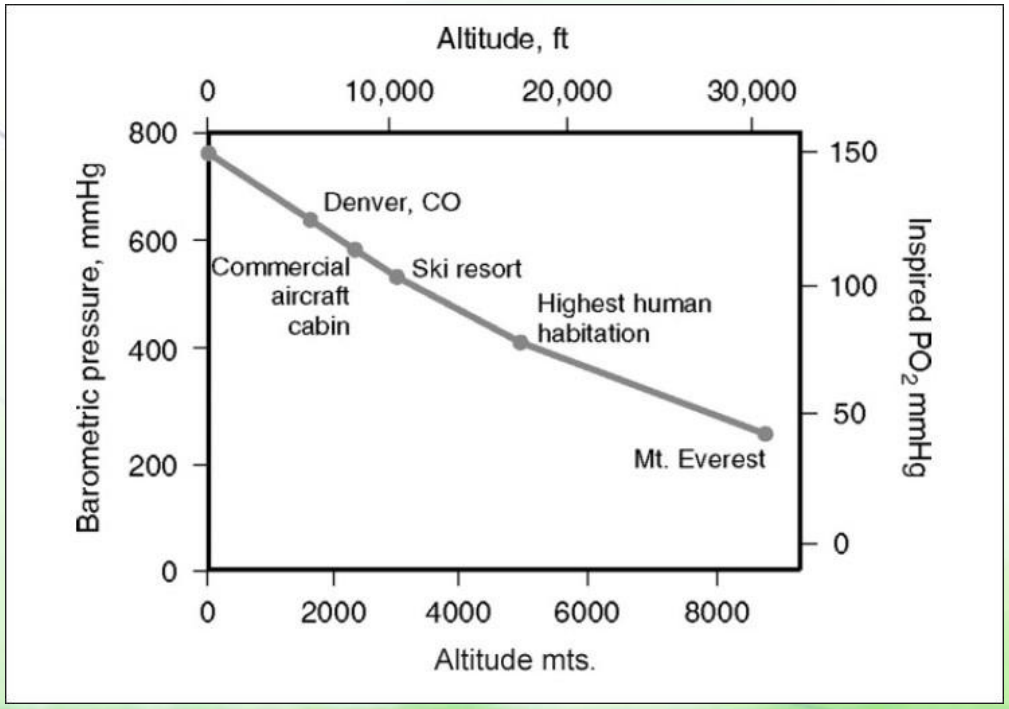


Figure 7.16
Biochemistry, Seventh Edition

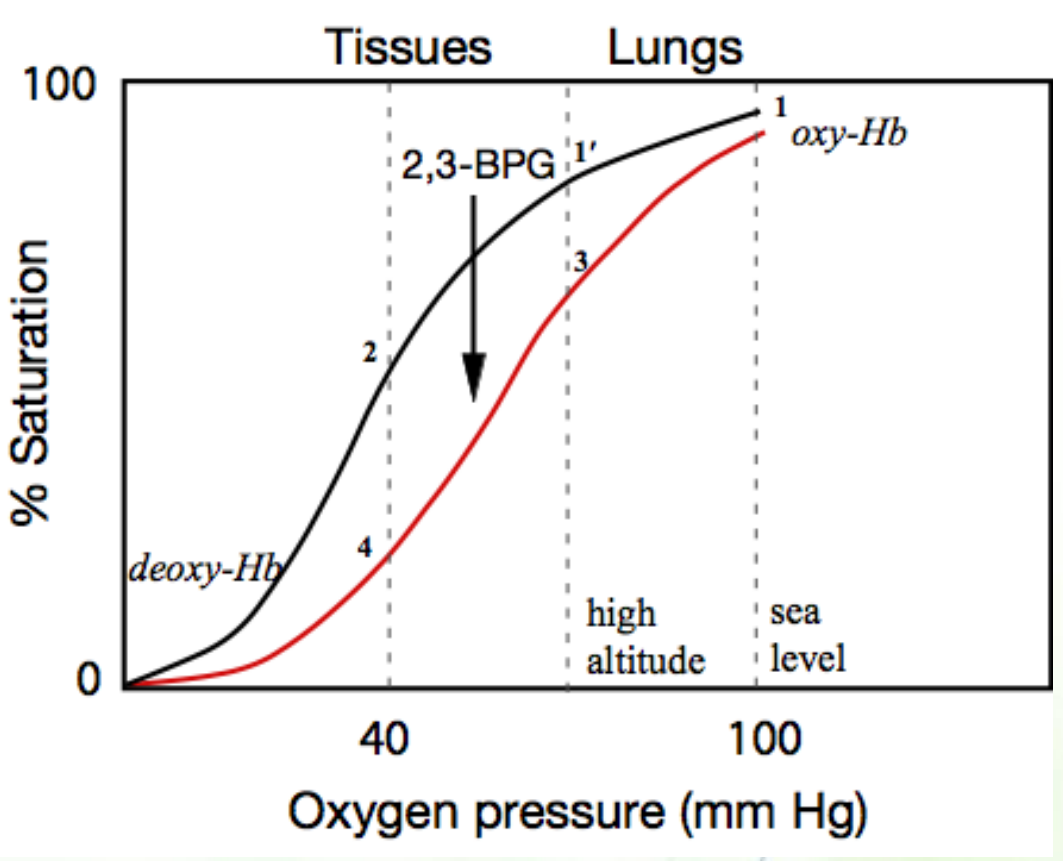
But pO_2 is low at high altitudes!!!

Altitude (feet)	Atmospheric Pressure (mm/Hg)	PAO_2 (mm/Hg)	PVO_2 (mm/Hg)	Pressure Differential (mm/Hg)	Blood Saturation (%)
Sea Level	760	100	40	60	98
10,000	523	60	31	29	87
18,000	380	38	26	12	72
22,000	321	30	22	8	60
25,000	282	7	4	3	9
35,000	179	0	0	0	0



Better explanation of the role of 2,3-BPG

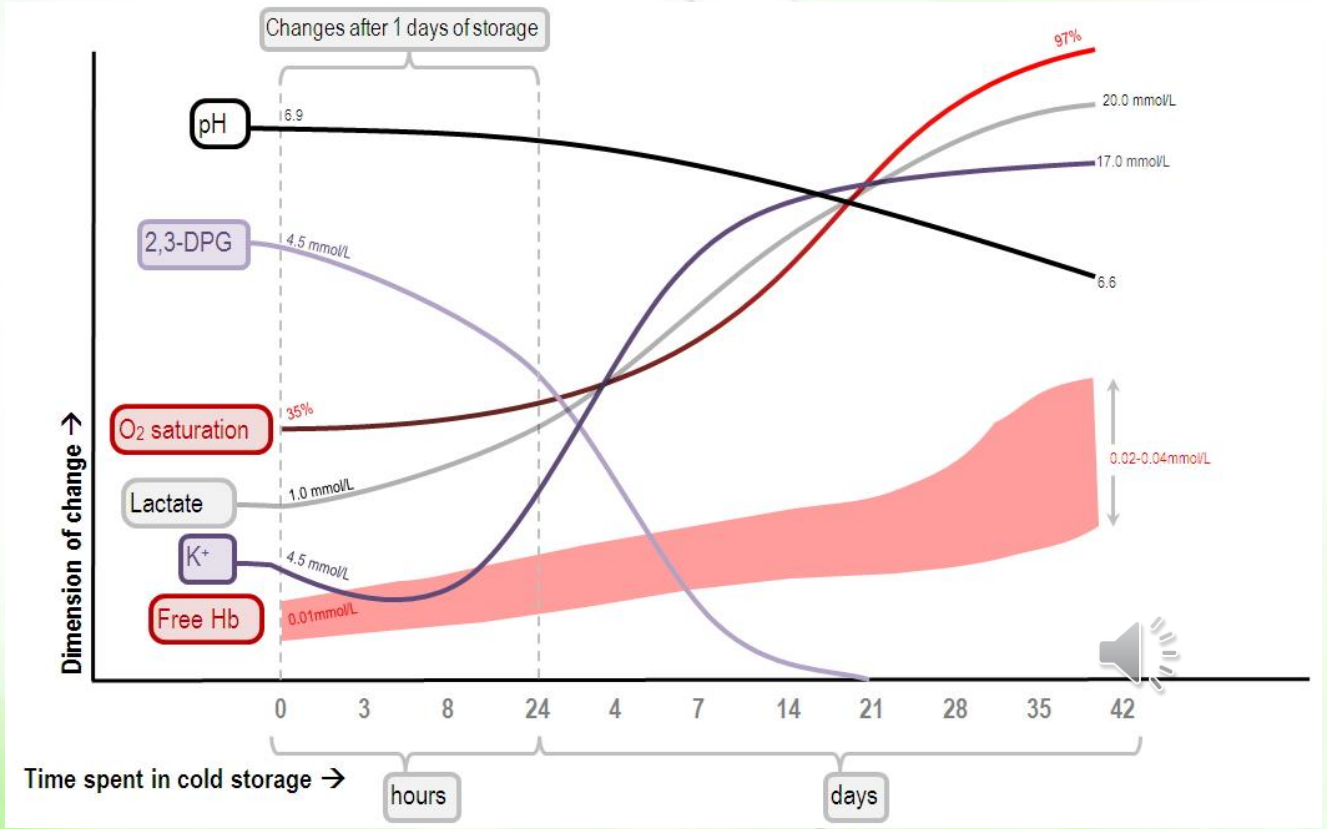
- At sea level the lungs pick up oxygen with 100% saturation of Hb (1) and when the oxygen pressure drops to 40 mm Hg in the tissues (2) the Hb will be 55% saturated.
  - They have released 45% of bound oxygen.
- At high altitudes (in case of no adaptation), Hb is only 80% saturated (1'). Thus at 40 mm Hg in the tissues (2) when Hb is only 55% saturated, it will only have released 25% of its oxygen.
- At high altitude (with increased 2,3-BPG production- in red), At the lungs (3) the Hb will be less bound with oxygen — only 70% saturation — but at 40mm Hg in the tissues (4) it will be much less saturated than on the black curve — 30%. Thus, it will have made available 40% of its oxygen.
- This is not a perfect solution, but over time there is increased production of red blood cells to provide more hemoglobin to compensate for the smaller amount of oxygen it can bind.



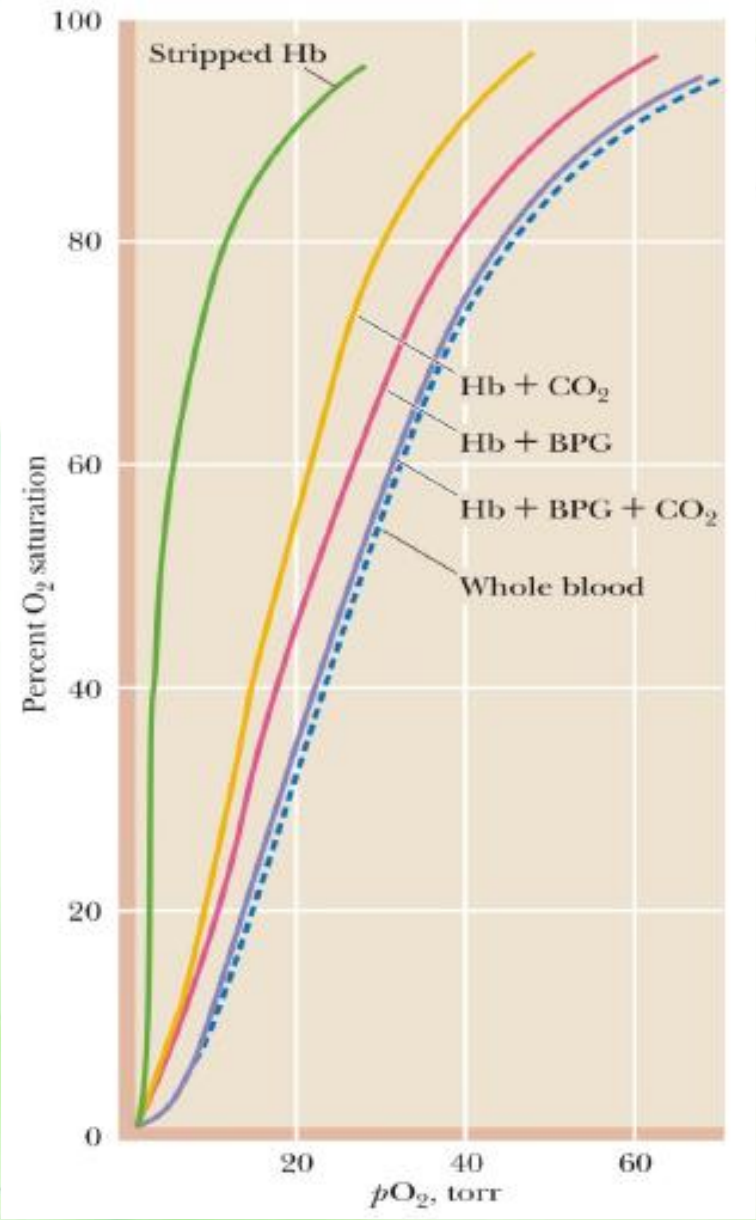
2,3-BPG in transfused blood

- Storing blood results in a decrease in 2,3-PBG (and ATP), hence hemoglobin acts as an oxygen “trap”, not an oxygen transporter.
- Transfused RBCs are able to restore the depleted supplies of 2,3-BPG in 6–24 hours.

- Severely ill patients may be compromised.
- Both 2,3-PBG and ATP are rejuvenated.



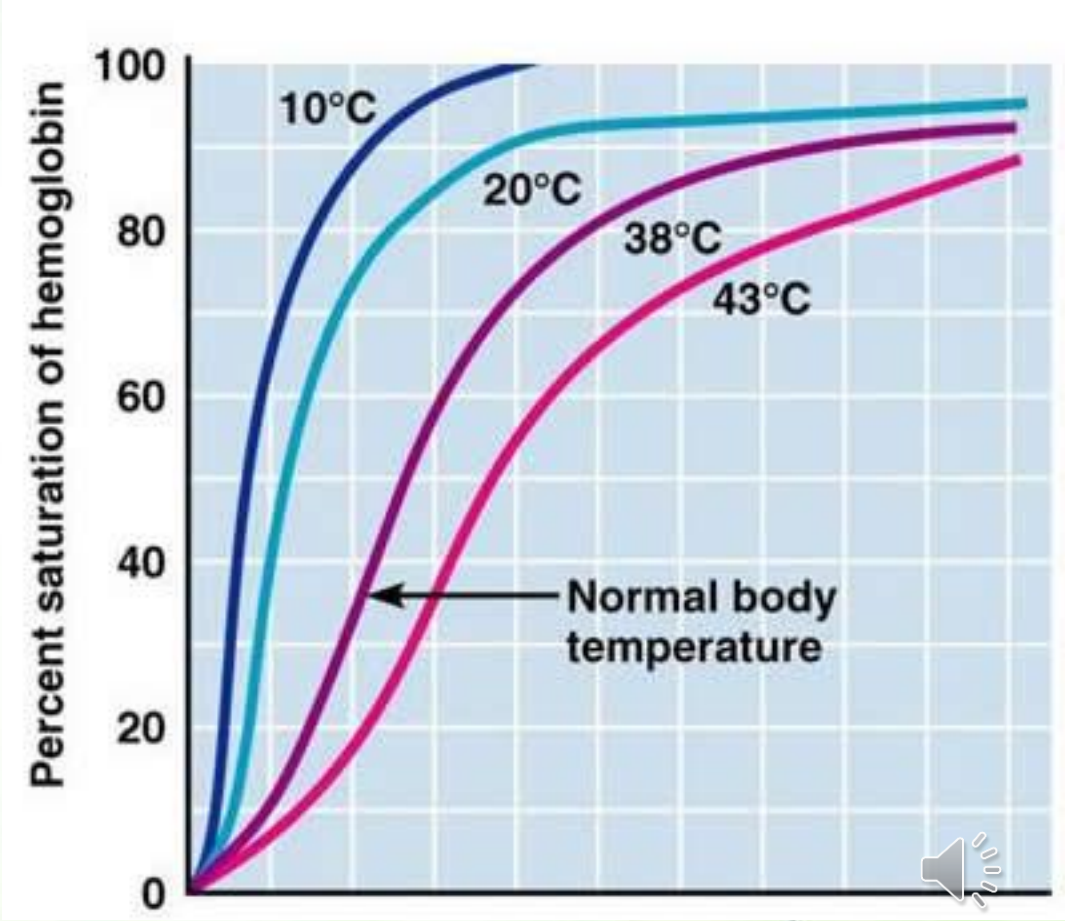
2,3-BPG and CO2 are important players



Other factors: A-Effect of temperature

Effect of temperature

- An increase in temperature decreases oxygen affinity and therefore increases the P50.
- Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading from HbO_2.

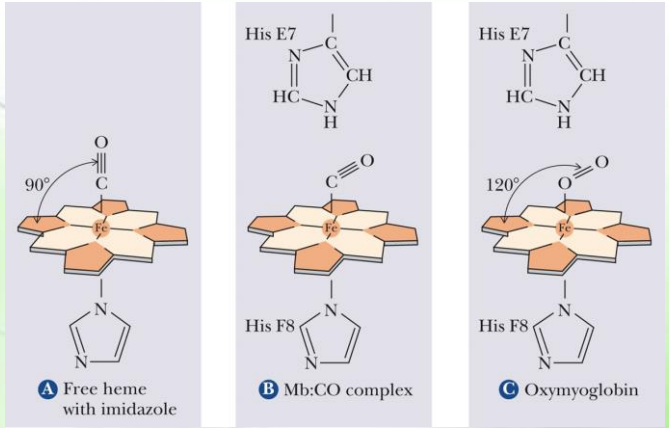


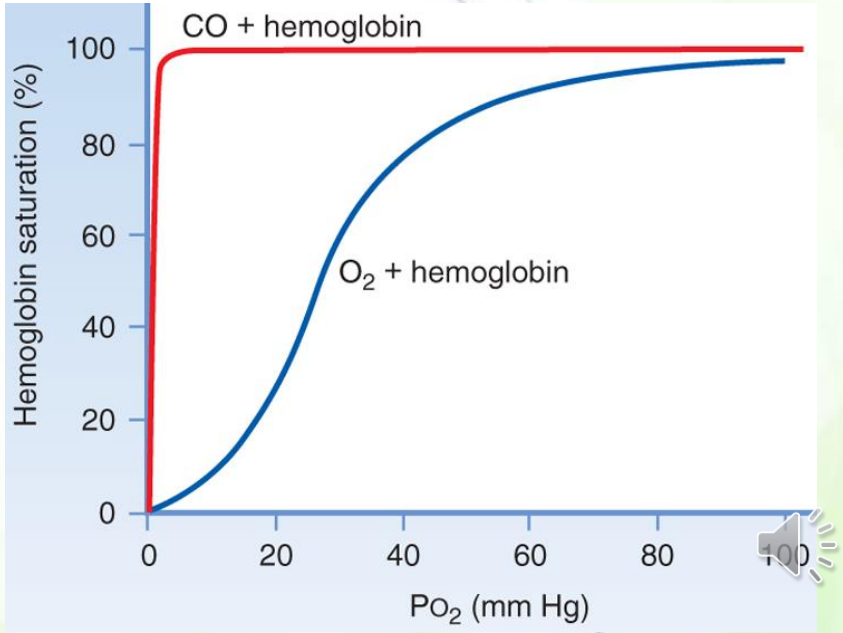
Other factors: B- Effect of CO

Effect of CO

- In addition to competing with oxygen in binding to hemoglobin, the affinity of Hb-CO towards oxygen <u>increases</u> resulting in less oxygen <u>unloading</u> in peripheral tissues.

<u>(Hb + O_2) versus (Hb + CO)→ the curve illustrates the release of oxygen in the presence and in the absence of CO.</u>





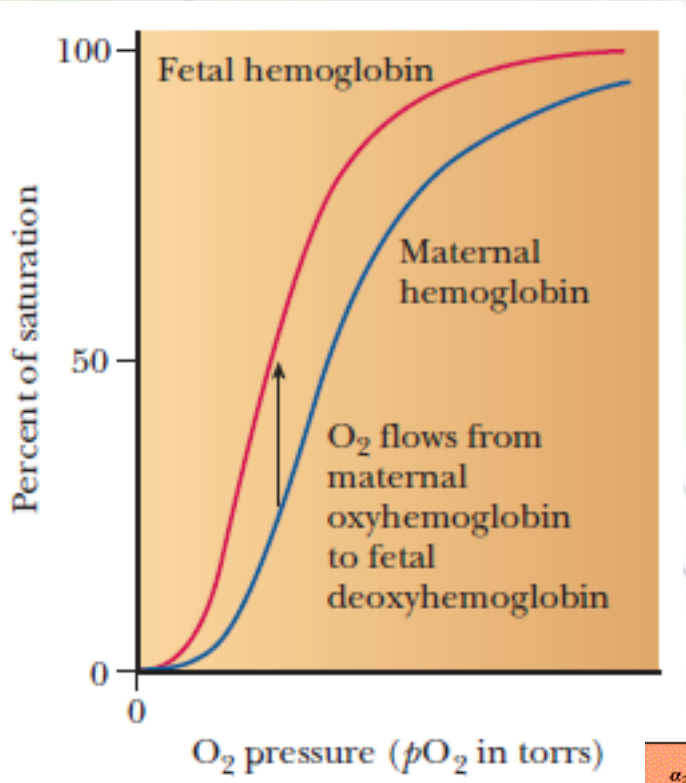
Relevant information

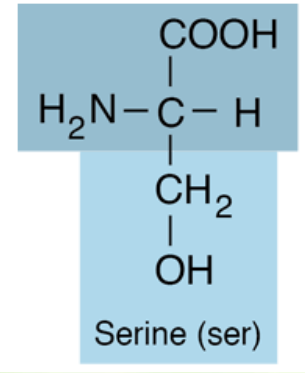
- Due to pollutants, the concentration of CO-Hb in the blood is usually 1% in a non-smoker.
- In smokers, CO-Hb can reach up to 10% in smokers.
- If this concentration of CO-Hb in the blood reaches 40% it would cause unconsciousness initially, followed by death.
- Increasing the amount of CO in inspired air to 1% and above would be fatal in minutes as that would increase the CO-Hb in blood to 40%

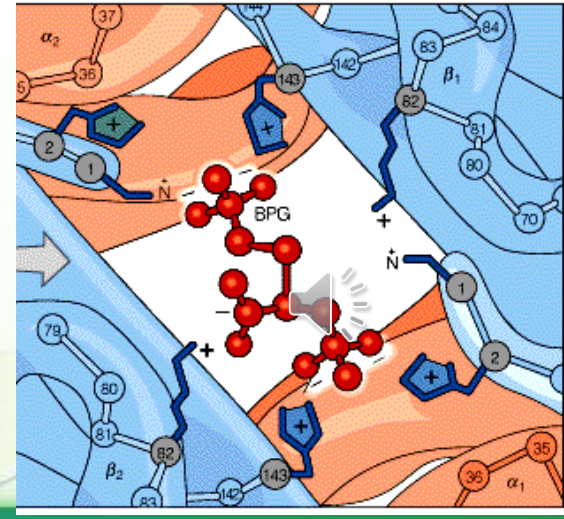
Other considerations

Fetal hemoglobin

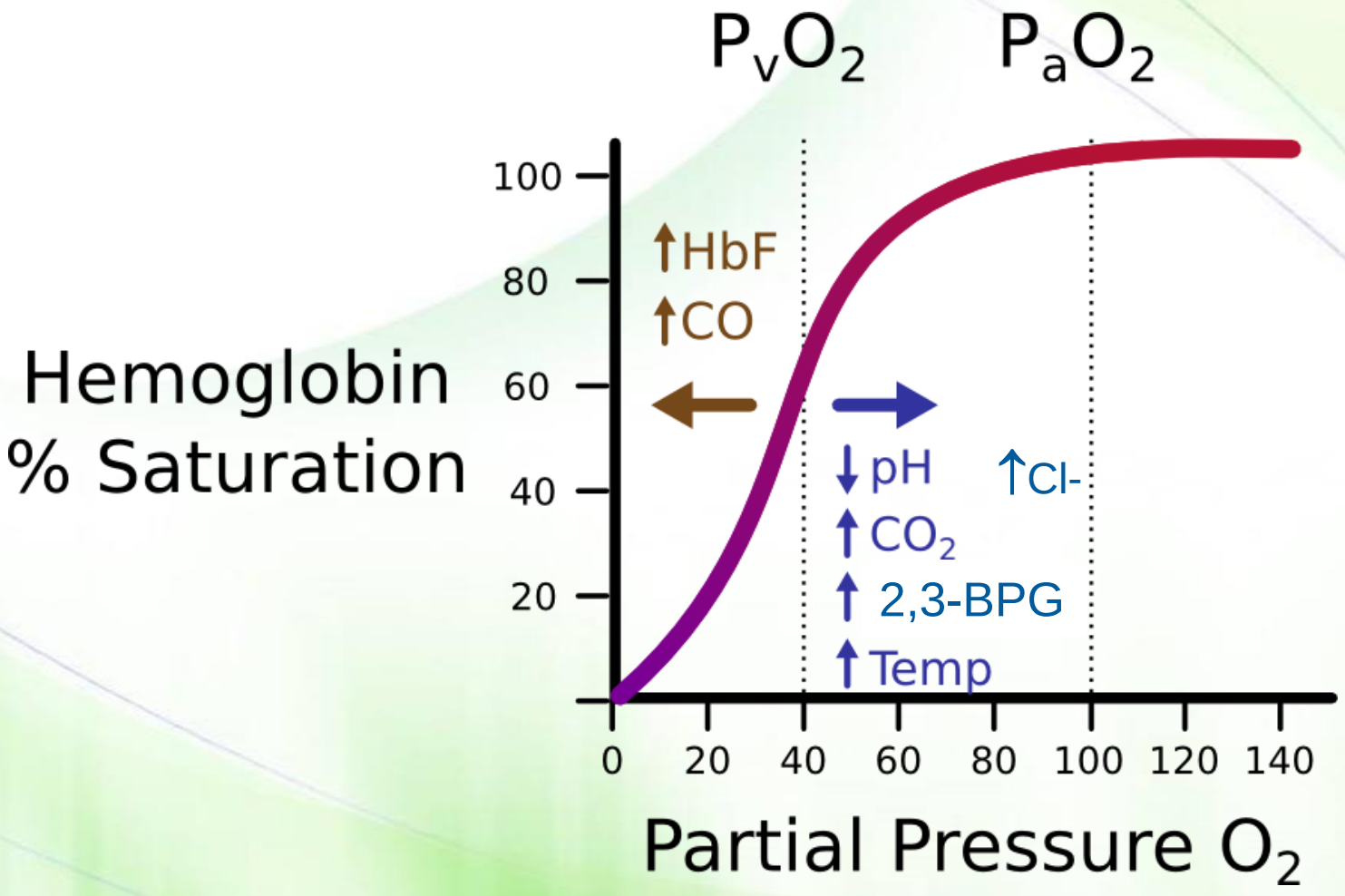
- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HBA).
  - HbA = $\alpha 2 \beta 2$
  - HbF = $\alpha 2 \gamma 2$
- His143 residue in the β subunit is replaced by a serine residue in the γ subunit of HbF.
  - Since serine cannot form a salt bridge with 2,3-BPG, it binds weaker to HbF than to HbA.







Summary


P_vO_2
P_aO_2
Hemoglobin % Saturation
100
80
60
40
20
↑HbF
↑CO
↓pH
↑CO_2
↑ 2,3-BPG
↑Temp
↑Cl-
0
20
40
60
80
100
120
140
Partial Pressure O_2