



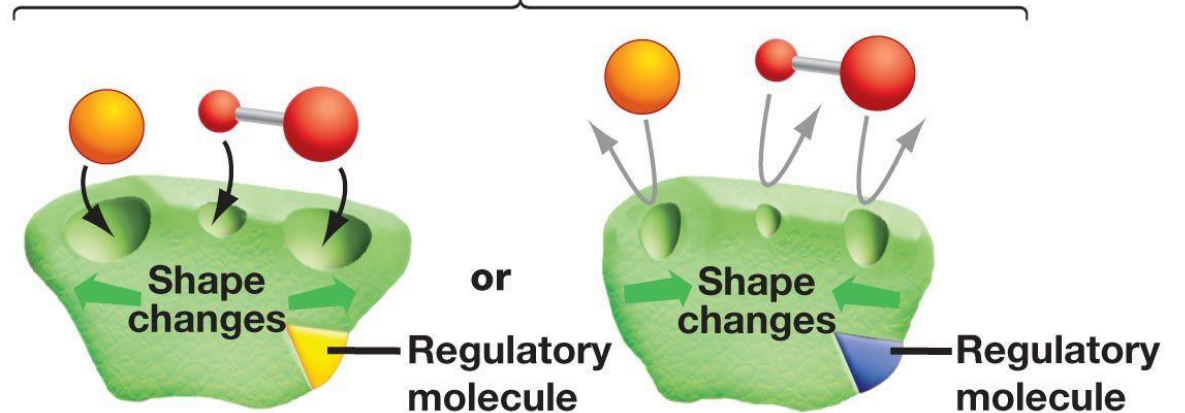
Regulation of hemoglobin function

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Allosteric regulation

- Ligands that induce conformational changes in allosteric proteins are referred to as allosteric modulators or effectors.
- Modulators may be inhibitors or activators.
 - Homotropic modulators are the same as the ligand itself.
 - Heterotropic modulators are different from the ligand.

(b) Allosteric regulation



Allosteric activation

The active site becomes available to the substrates when a regulatory molecule binds to a different site on the enzyme.

Allosteric deactivation

The active site becomes unavailable to the substrates when a regulatory molecule binds to a different site on the enzyme.

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Allosteric effectors

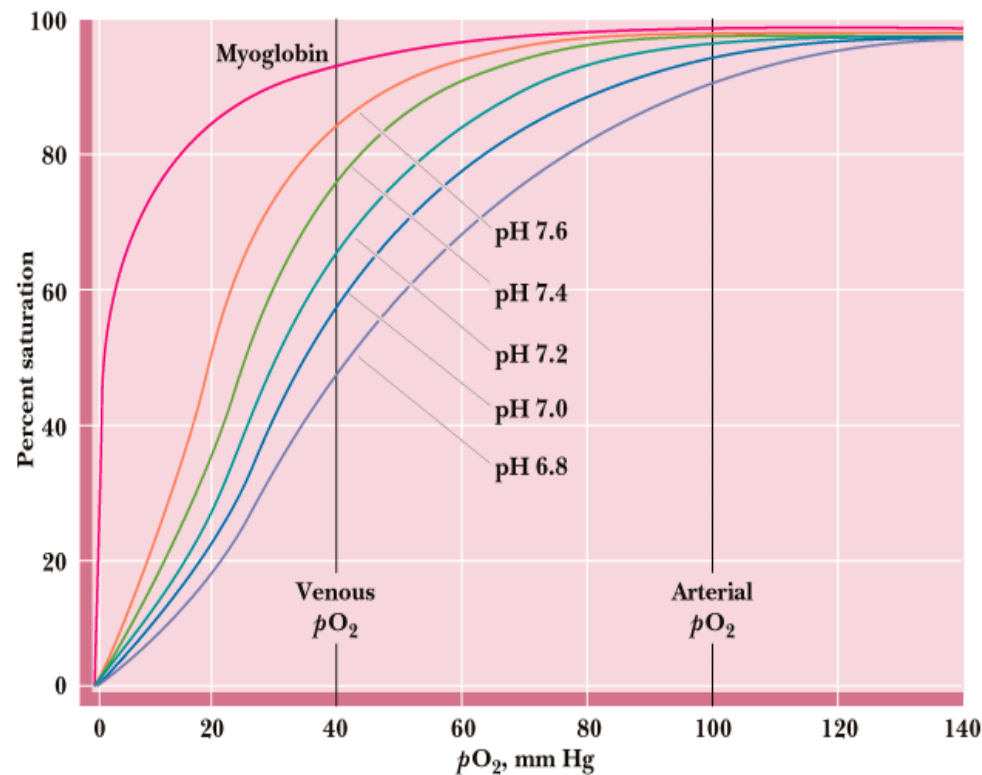
- The major heterotropic effectors of hemoglobin
 - Hydrogen ion,
 - Carbon dioxide
 - 2,3-Bisphosphoglycerate
 - Chloride ions
- A competitive inhibitor
 - Carbon monoxide

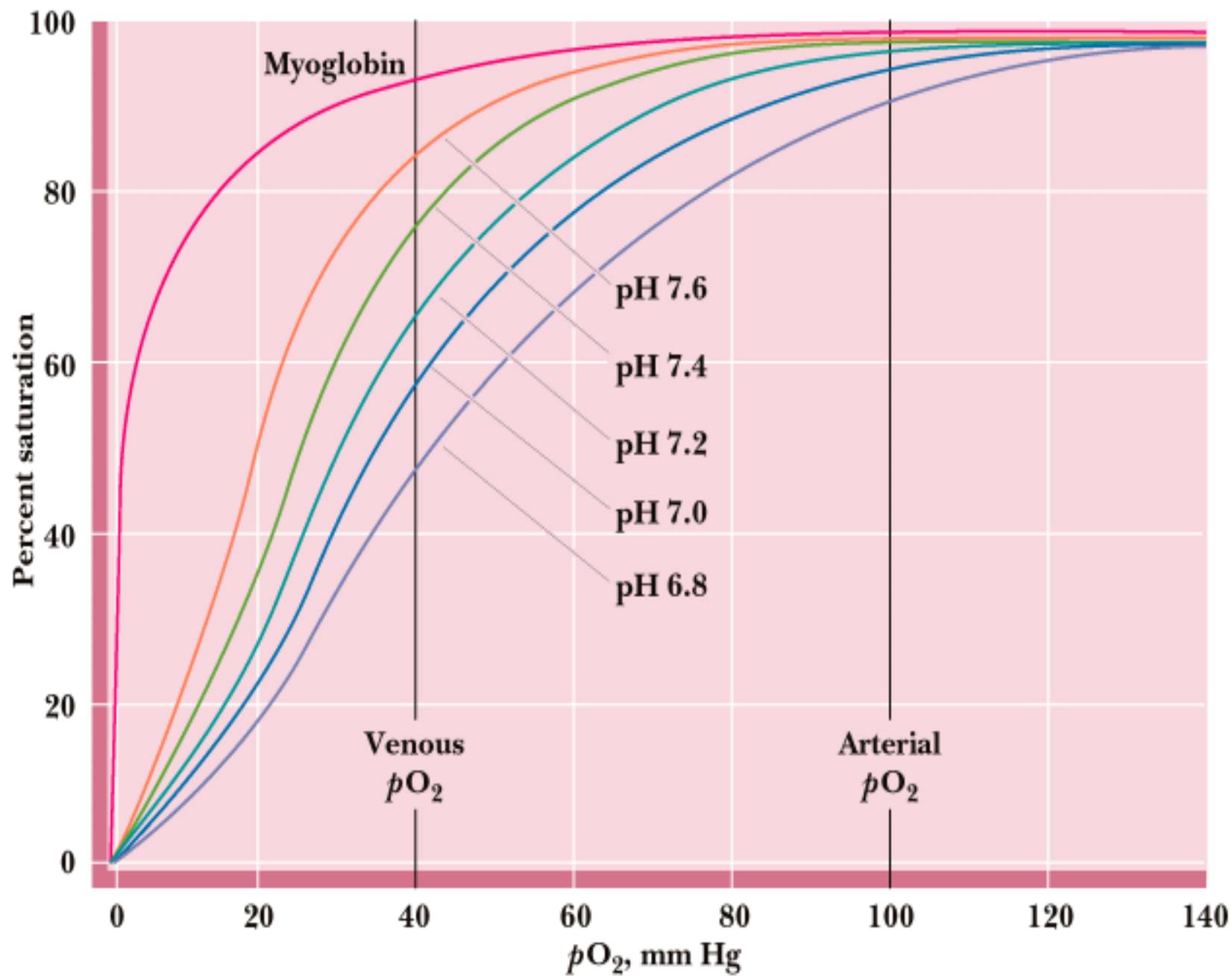
The effect of pH



The effect of pH

- 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**.



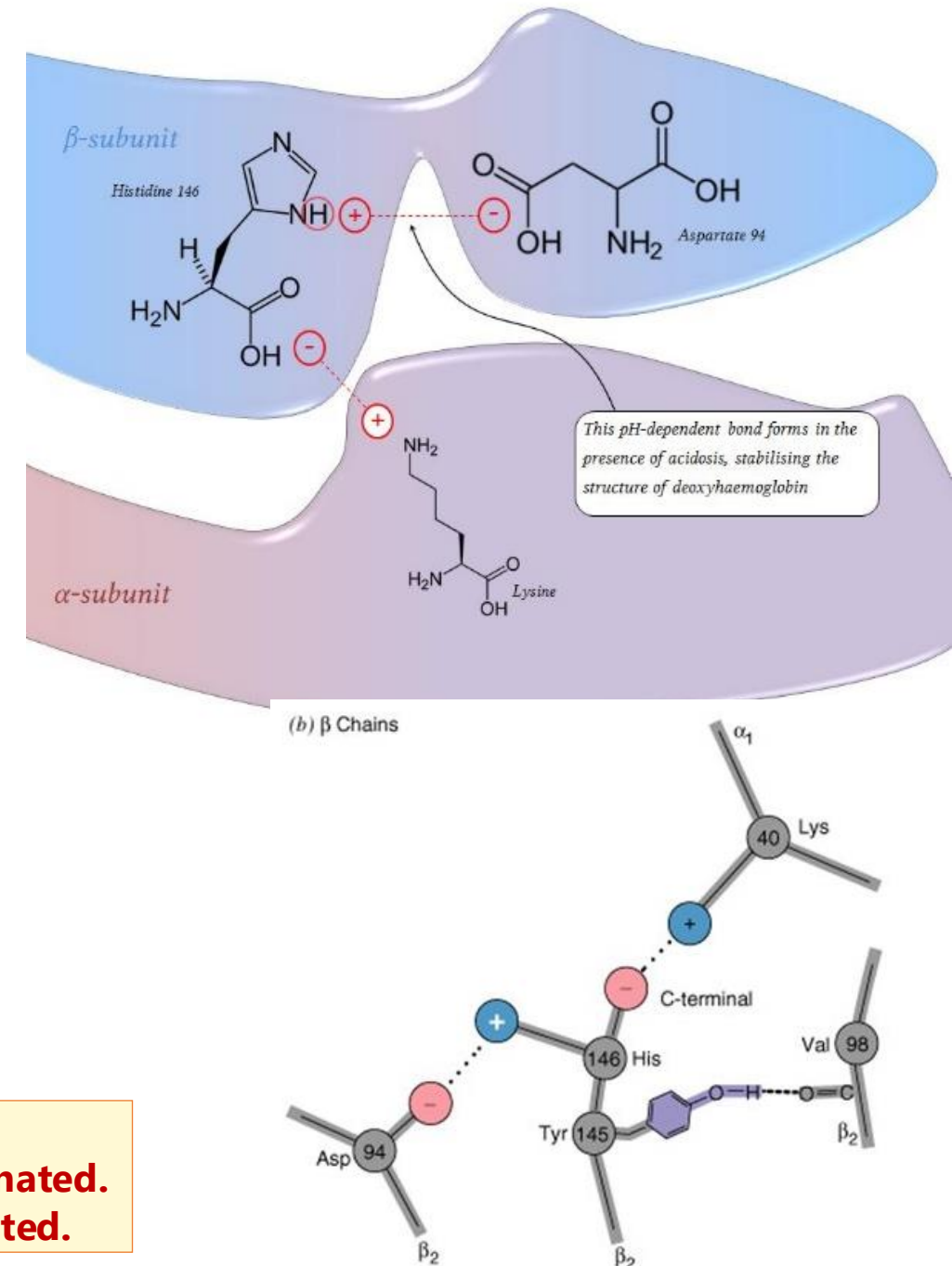


Mechanism of Bohr effect

- Increasing H^+ (in tissues) causes the protonation of key amino acids, including the last histidine residue of the β chains (**His146**).
- Electrostatic interaction occurs between the carboxylic group of His146 and a lysine of the α chain, stabilizing the T form of hemoglobin.
- The protonated His146 also forms a salt bridge to Asp94 within the same chain.
 - The pK_a of the imidazole ring of His146 is increased from 7.3 in the R-state to 7.7 in the T-state, meaning that it is deprotonated (charged) in the R-state and protonated (charged) in the T-state, meaning that at low pH (in tissues), it is easier for hemoglobin to be in the T state.
- This favors the deoxygenated T-form of hemoglobin.

Note

- When $pH > pK_a$, the group is deprotonated.
- When $pH < pK_a$, the group is protonated.





Where do protons come from?

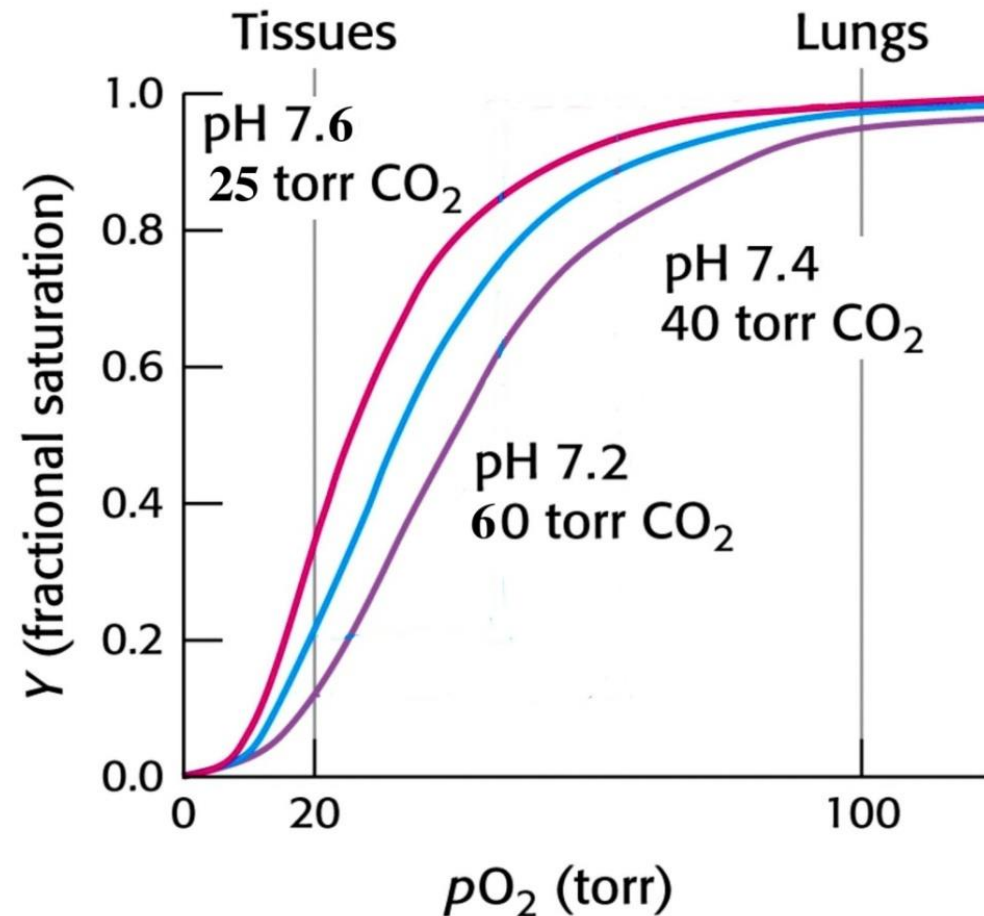
- Metabolism of glucose to CO₂ and lactic acid.
- H⁺'s are produced at high levels in metabolically active tissues from CO₂ by carbonic anhydrase.
 - In the lungs, the reverse effect occurs, where the high levels of O₂ cause the release of CO₂ from hemoglobin.



The effect of CO₂



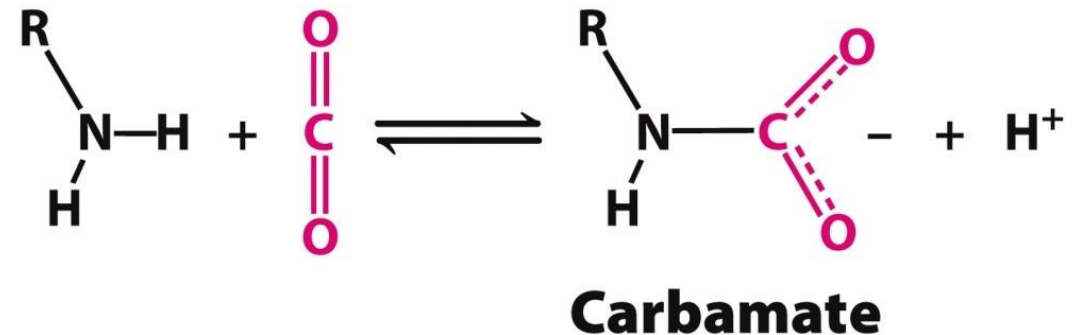
Mechanism #1 - production of protons





Mechanism #2- formation of carbamates

- Hemoglobin transports some CO₂ directly.
- CO₂ combines with the free α -amino terminal groups, forming carbamate and producing negatively charged groups.



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



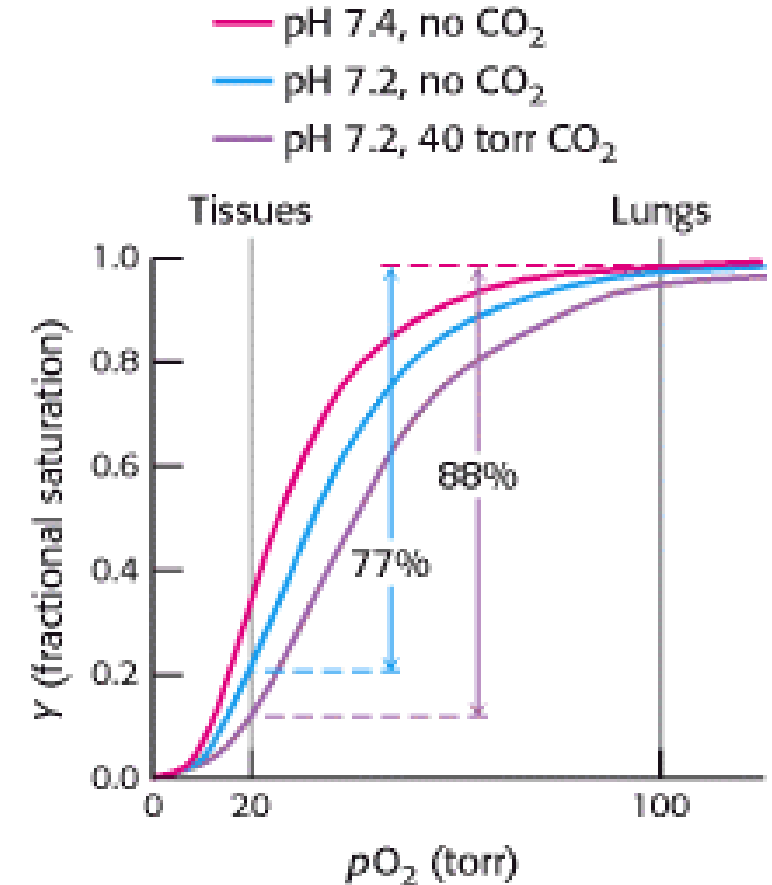
Which mechanism has a stronger effect?

- 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 and keeping the other constant.

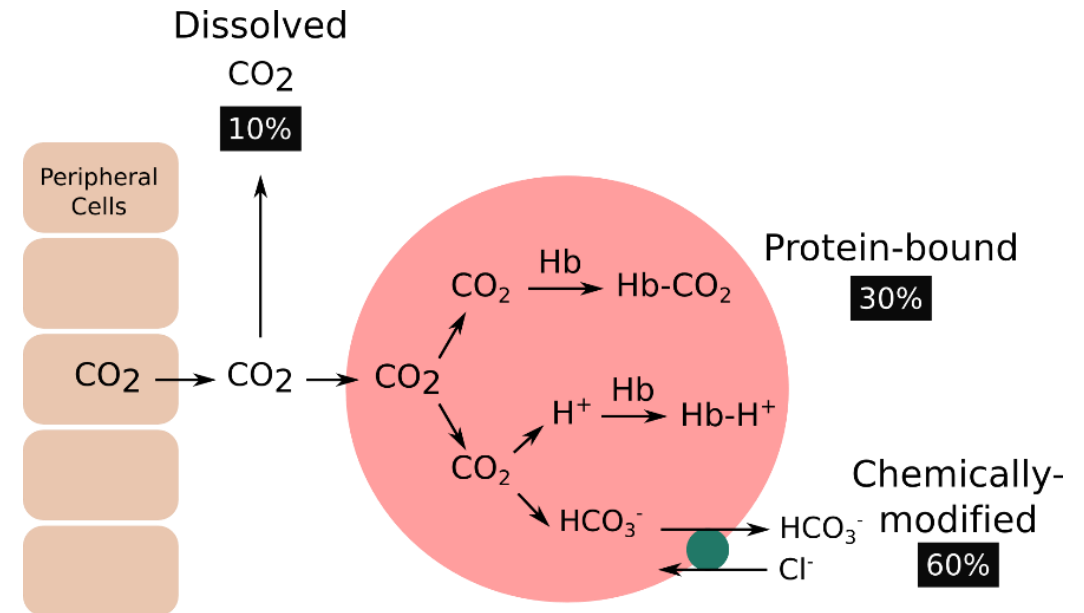
An increase in CO_2 tension shifts the oxygen dissociation curve to the right, even when the pH is held constant.





Transport of CO₂ into lungs

- Approximately 60% of CO₂ is transported as bicarbonate ion, which diffuses out of the RBC.
- About 30% of CO₂ is transported bound to N-terminal amino groups of the T form of hemoglobin .
- A small percentage of CO₂ is transported as a dissolved gas.



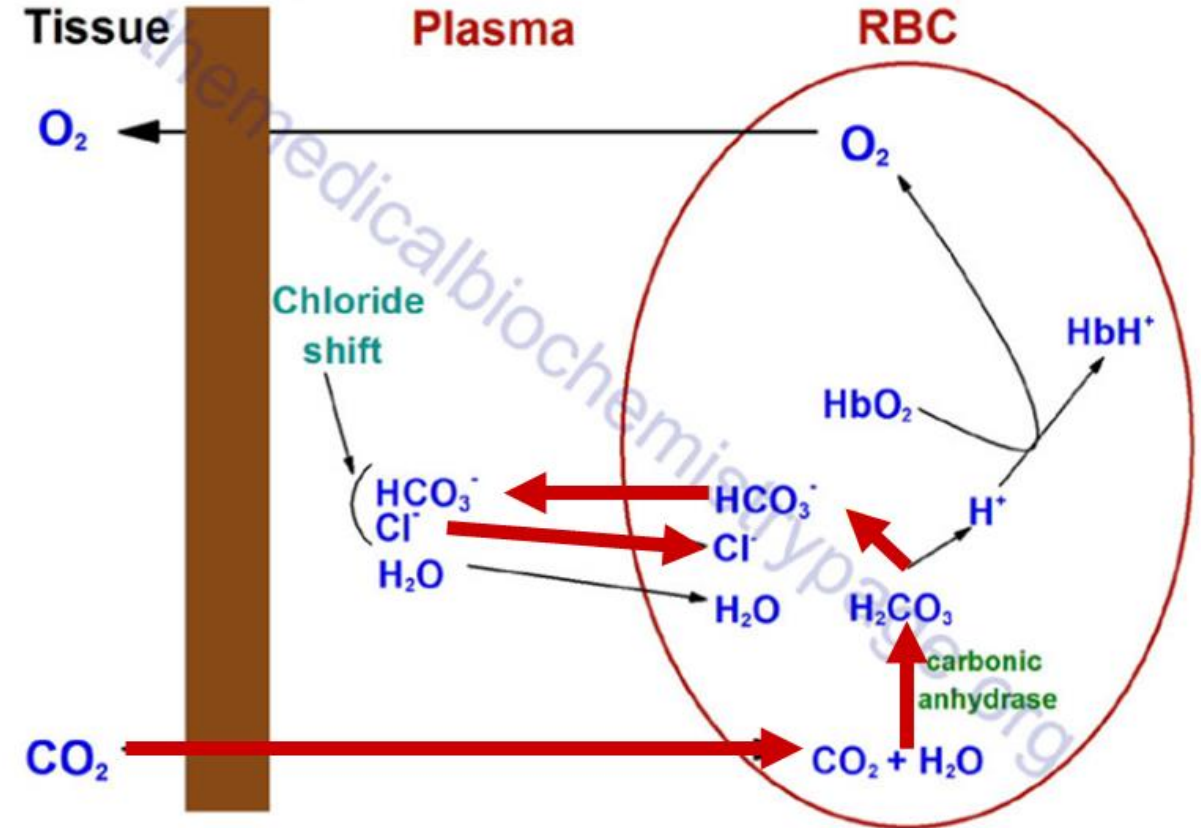
The isohydric shift is the movement of CO₂ in/out of cells without changing the pH as a result of hemoglobin acting as an effective buffer.

Effect of Chloride ion



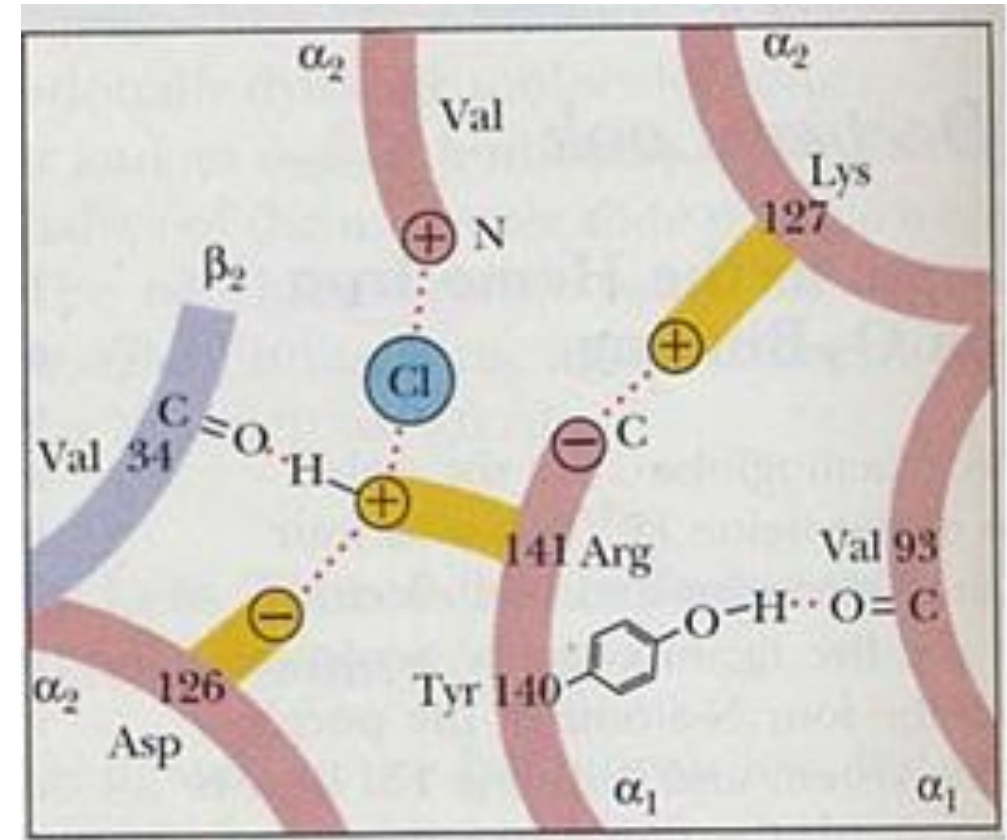
Chloride shift

- Bicarbonate diffuses out of the red blood cells into the plasma in venous blood and visa versa in arterial blood.
- 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 α_2 chain and Arg141 of α_1 chain stabilizing the T-state of hemoglobin.
- Increasing the concentration of chloride ions (Cl^-) shifts the oxygen dissociation curve to the right (lower affinity)

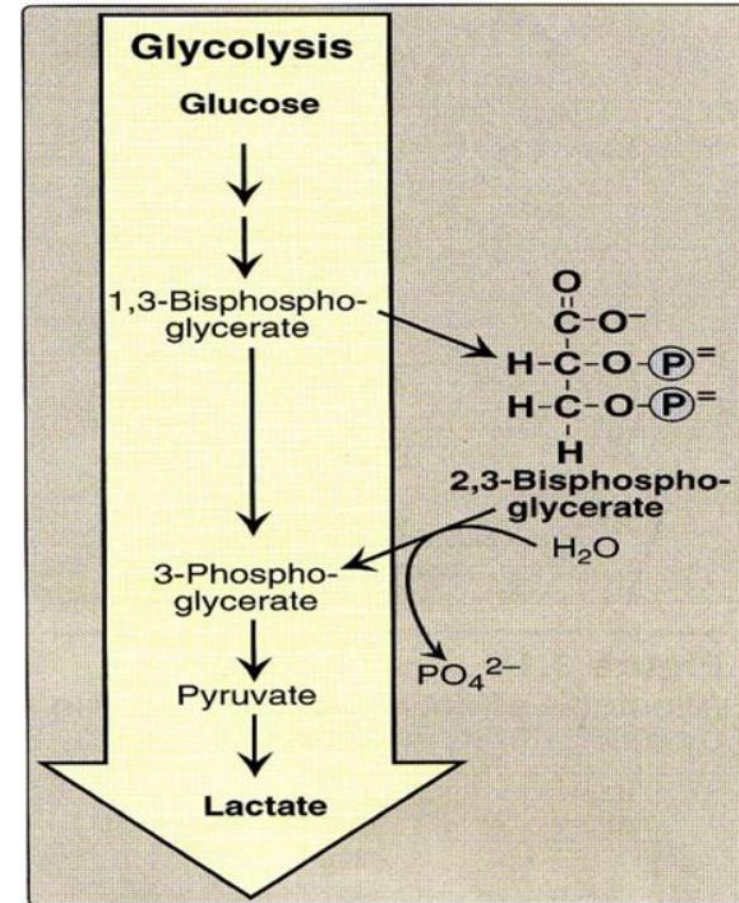


Effect of 2,3-bisphosphoglycerate



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

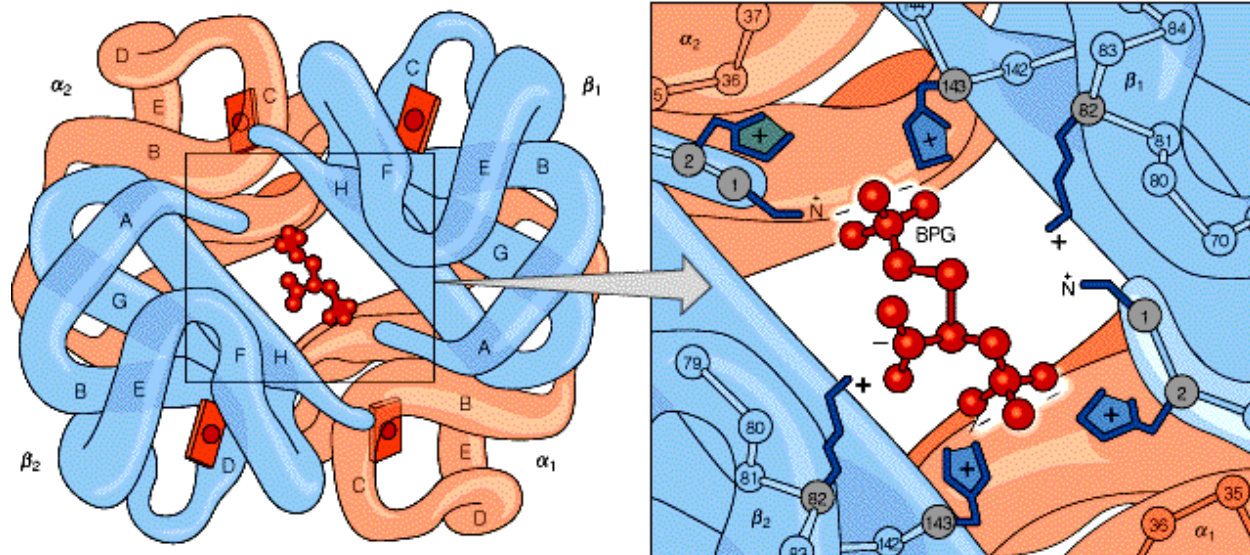
- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- It binds to hemoglobin and reduces its affinity towards oxygen.



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.
- The concentration of 2,3-BPG increases at high altitudes (low O₂) and in certain metabolic conditions making hemoglobin more efficient at delivering oxygen to tissues.

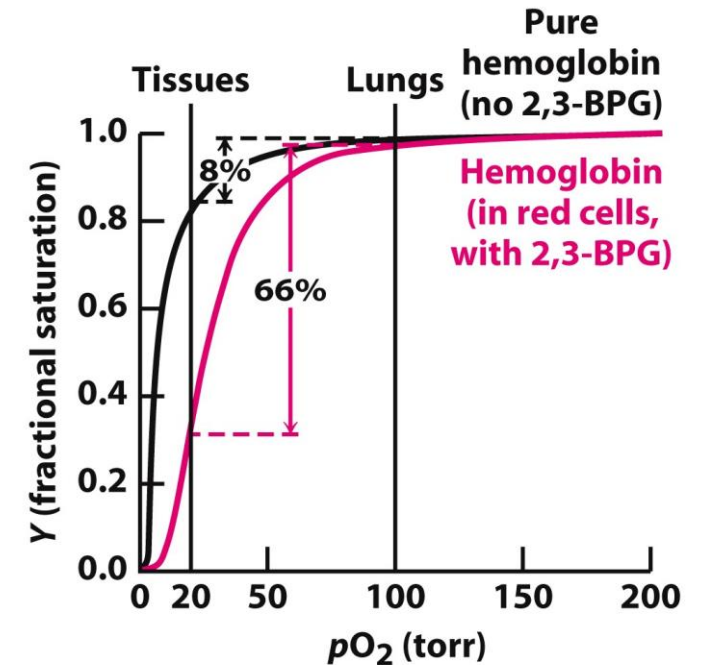
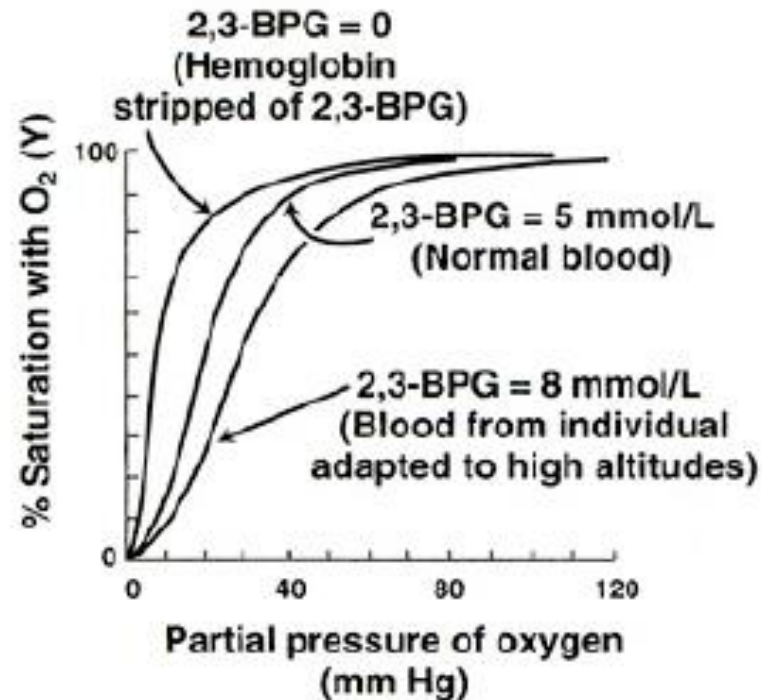
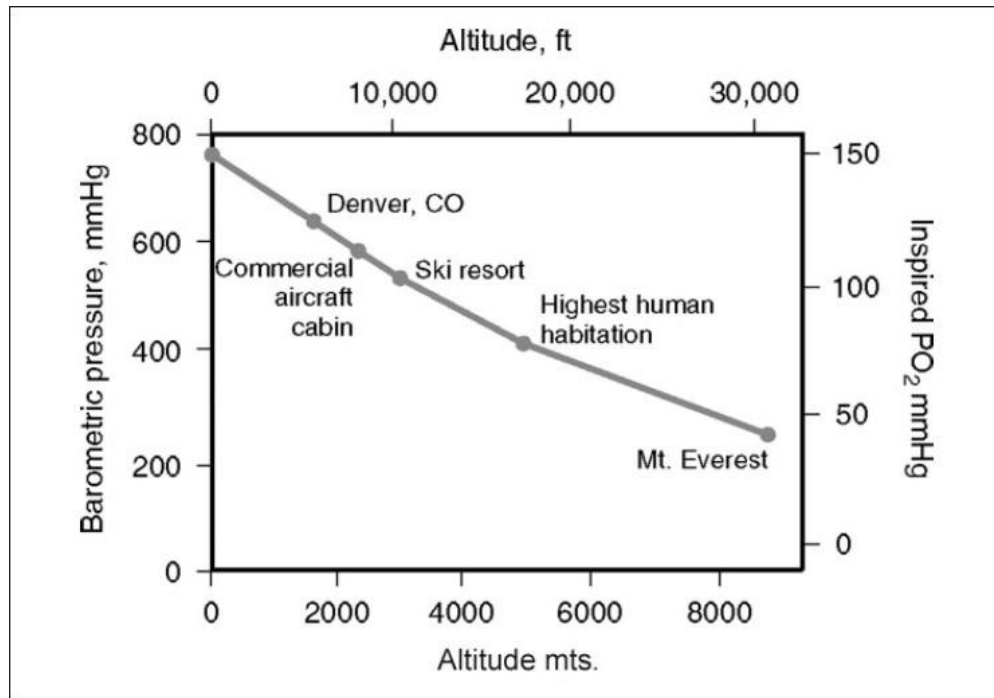


Figure 7.16
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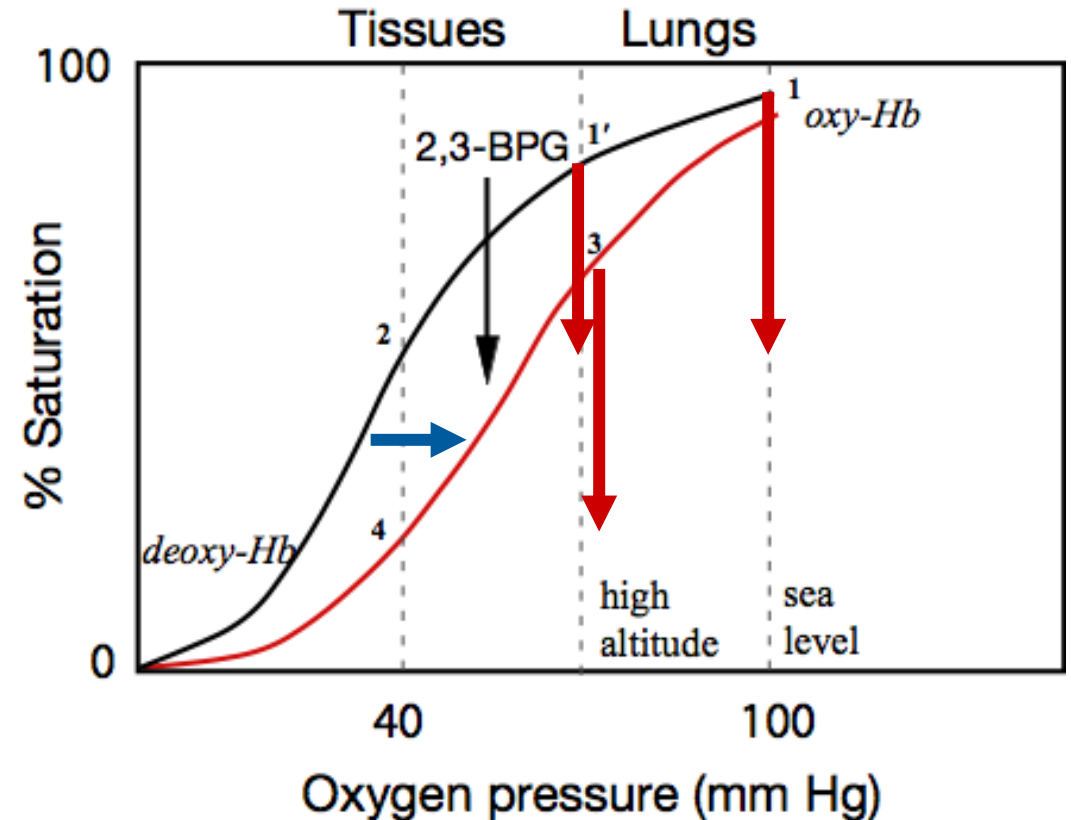
But pO_2 is low at high altitudes!!!

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.

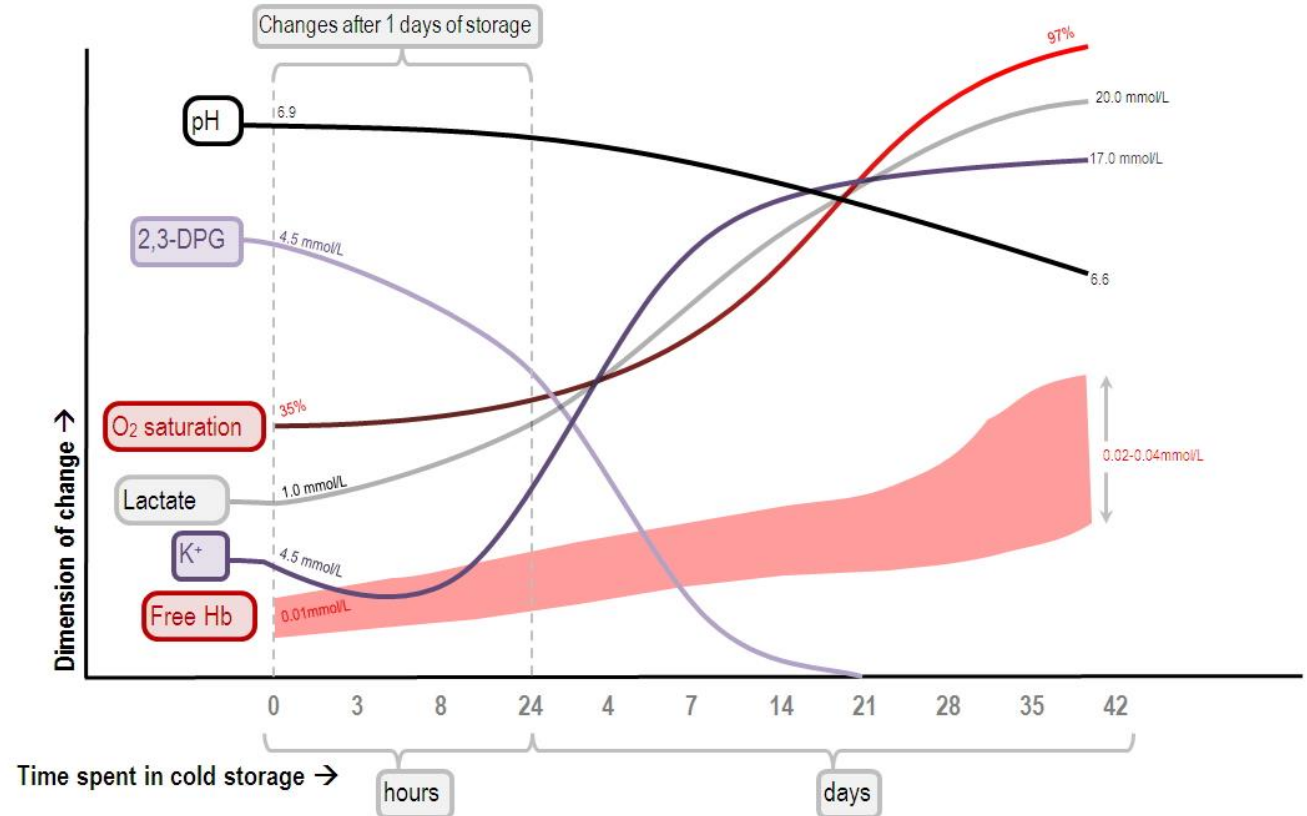


It is not a perfect solution, but over time, there is increased production of red blood cells to provide more hemoglobin and compensate for the smaller amount of oxygen it can bind.

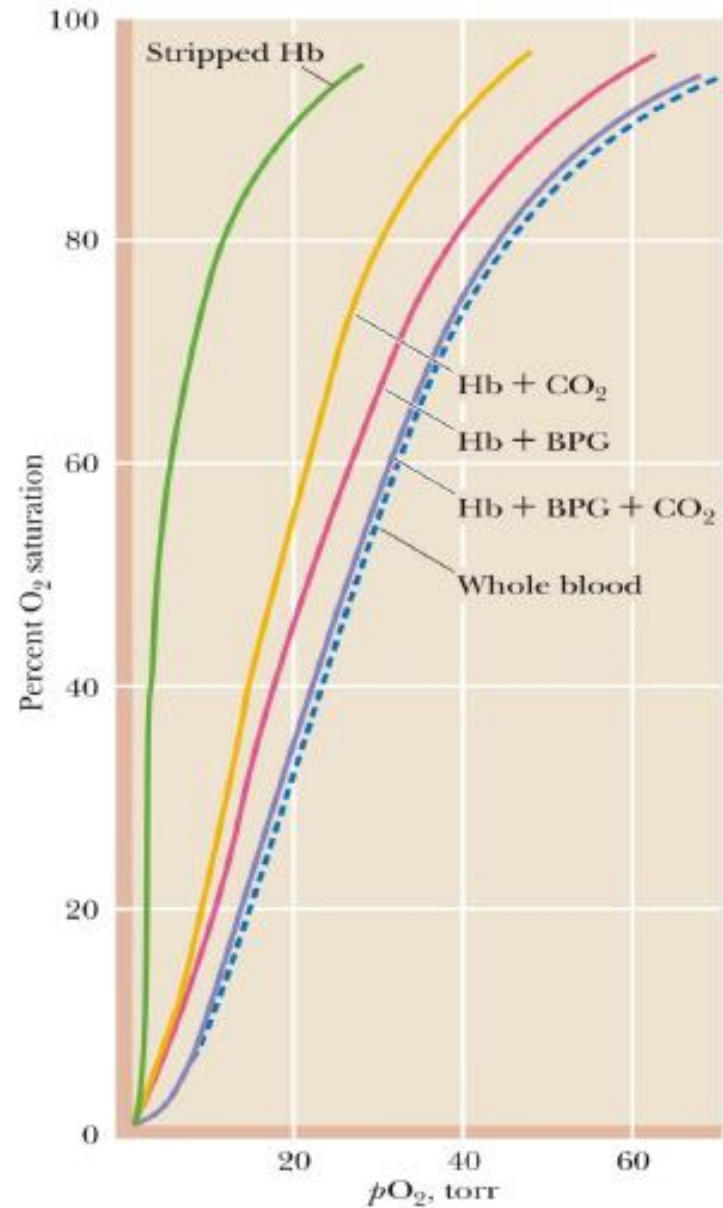
2,3-BPG in transfused blood

- Storing blood results in a decrease in 2,3-BPG (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-BPG and ATP are rejuvenated.



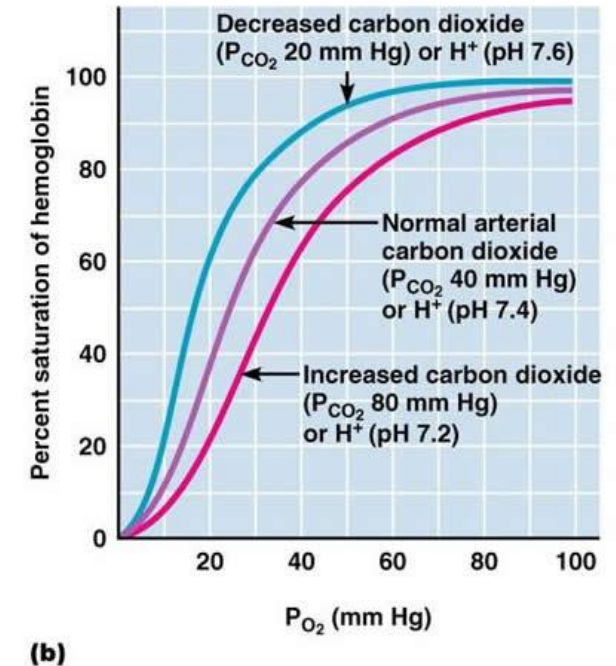
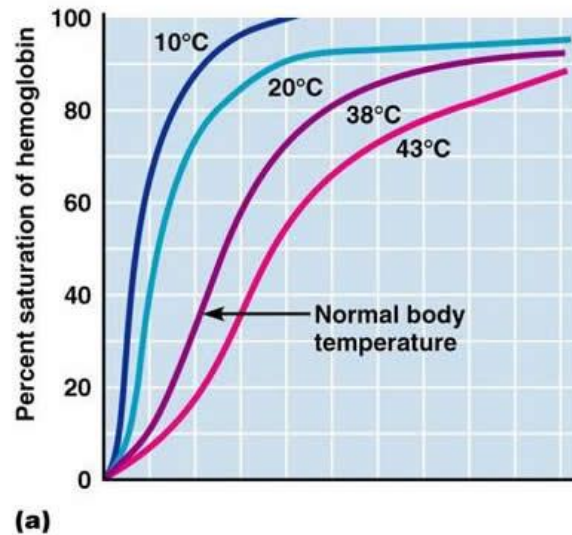
2,3-BPG and CO_2 are important players



Effect of temperature

Effect of temperature

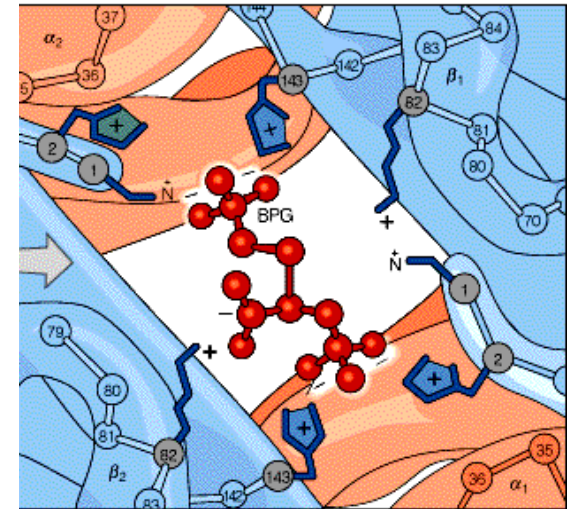
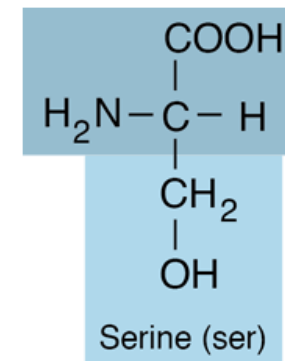
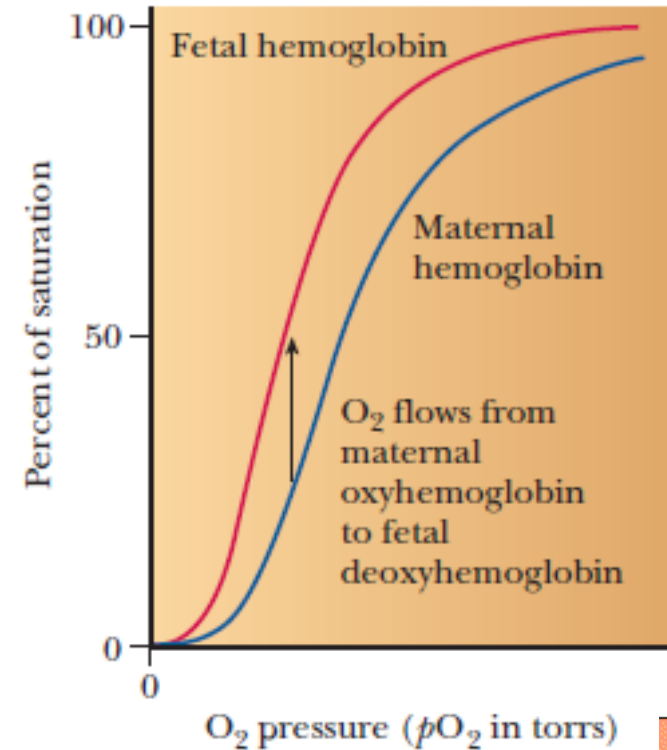
- An increase in temperature decreases oxygen affinity and, therefore, increases the P50.
- Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading from HbO₂.



Other considerations

Fetal hemoglobin

- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HbA).
 - $\text{HbA} = \alpha_2\beta_2$
 - $\text{HbF} = \alpha_2\gamma_2$
- 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.



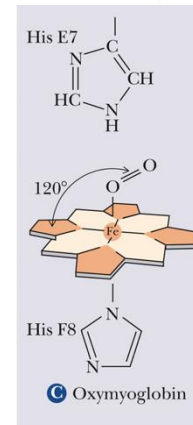
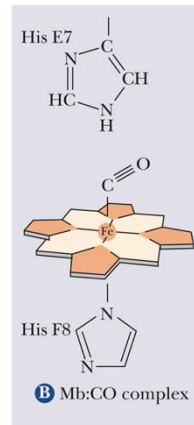
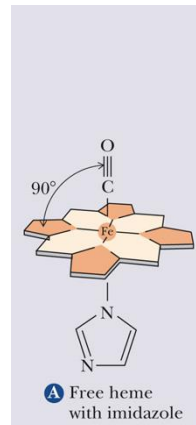
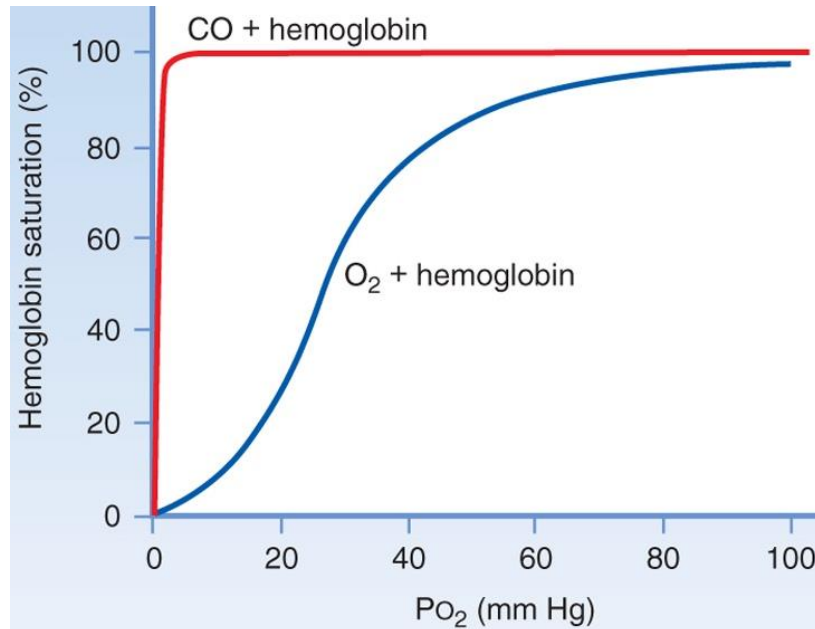
Effect of CO



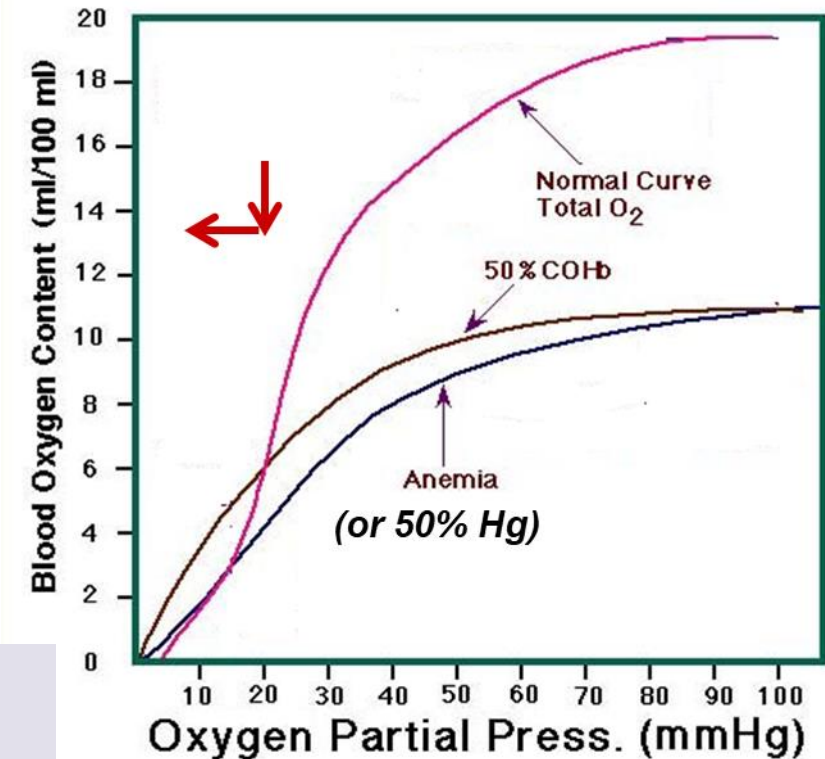
Effect of CO

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

(Hb + O₂) versus (Hb + CO)

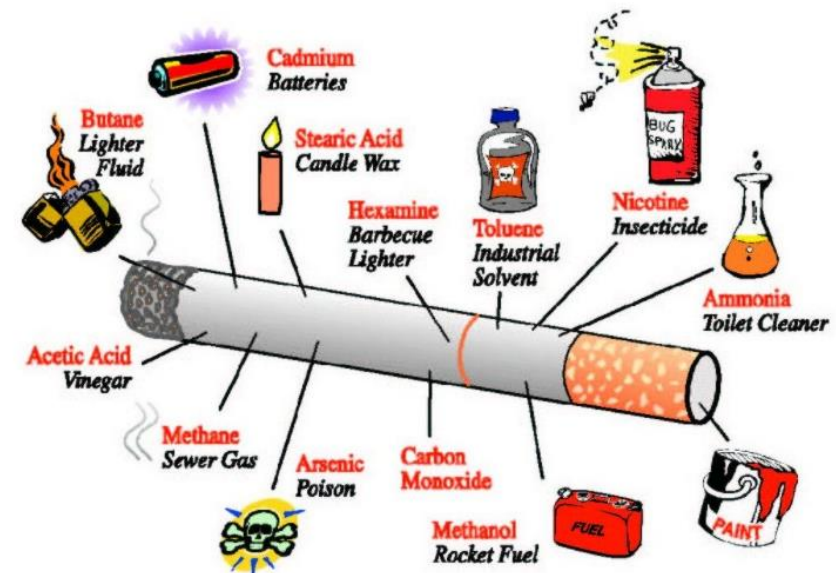


(Hb + O₂) versus (Hb + O₂ + CO)



Relevant information

- Increasing the amount of CO in inspired air to 1% and above would be fatal in minutes.
- 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% (as is caused by 1% of CO in inspired air), it would cause unconsciousness initially, followed by death.





Summary

