Regulation of hemoglobin function

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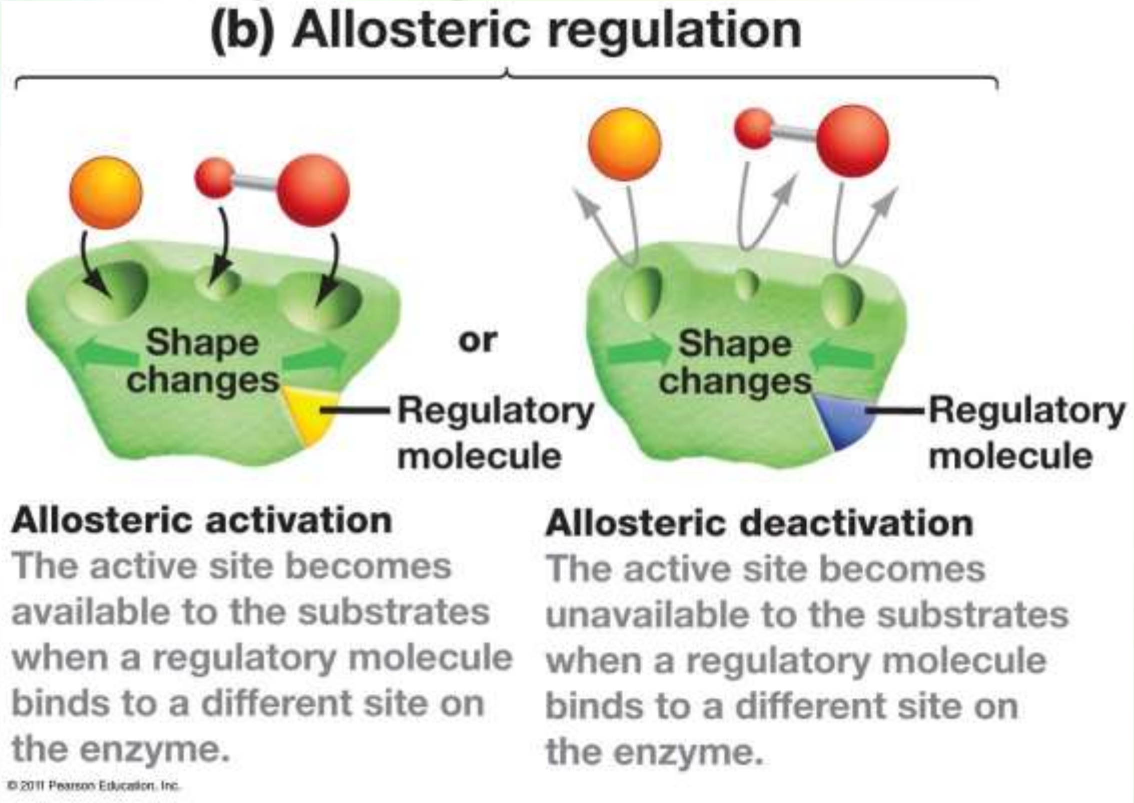
Hematopoietic-lymphatic system

Allosteric regulation

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



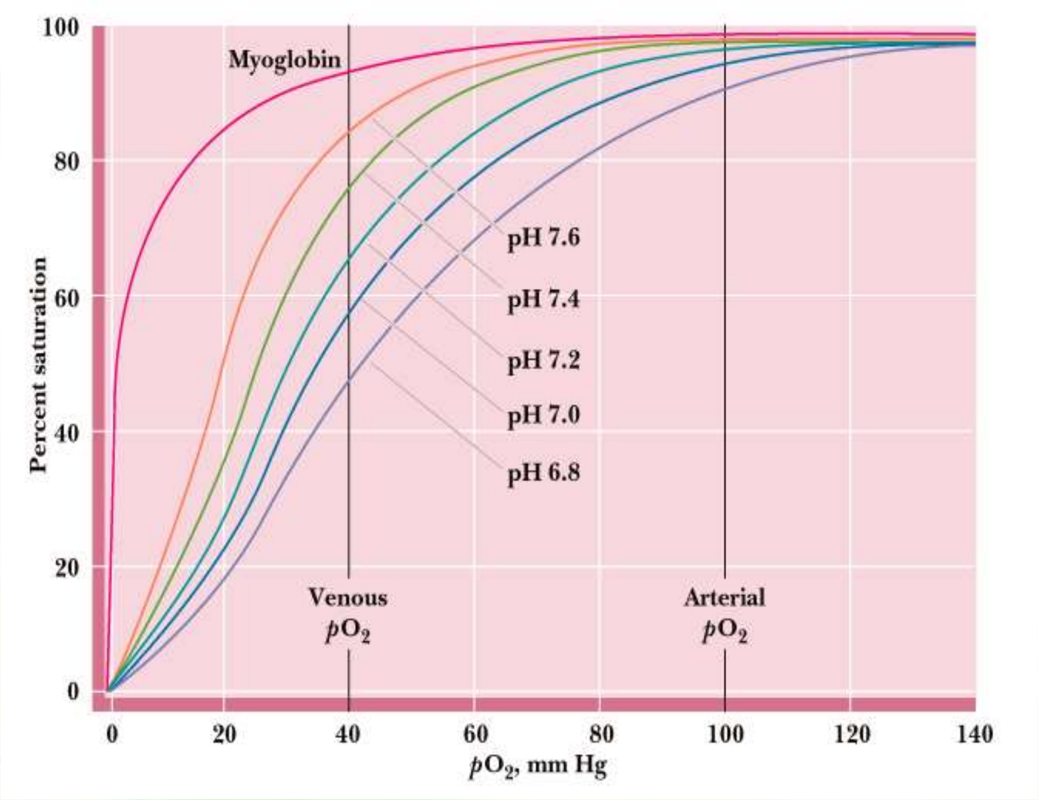
Allosteric effectors

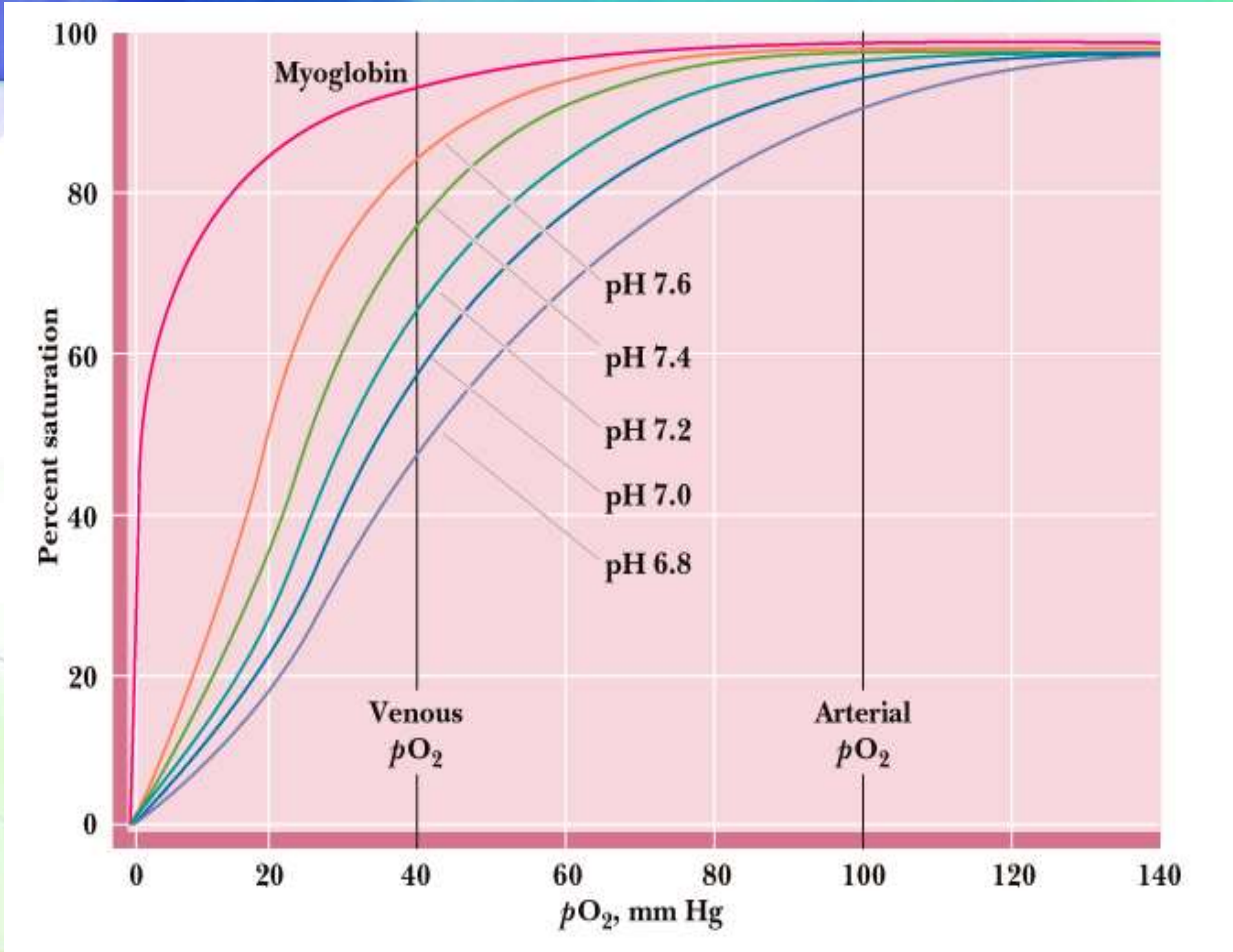
- The major heterotropic effectors of hemoglobin
  - Hydrogen ion,
  - Carbon dioxide
  - 2,3-Bisphosphoglycerate
  - Chloride ions

- A competitive inhibitor
  - Carbon monoxide

The effect of pH

- The binding of H+ to hemoglobin promotes the release of O_2 from hemoglobin and vice versa.
- This phenomenon is known as the Bohr effect.

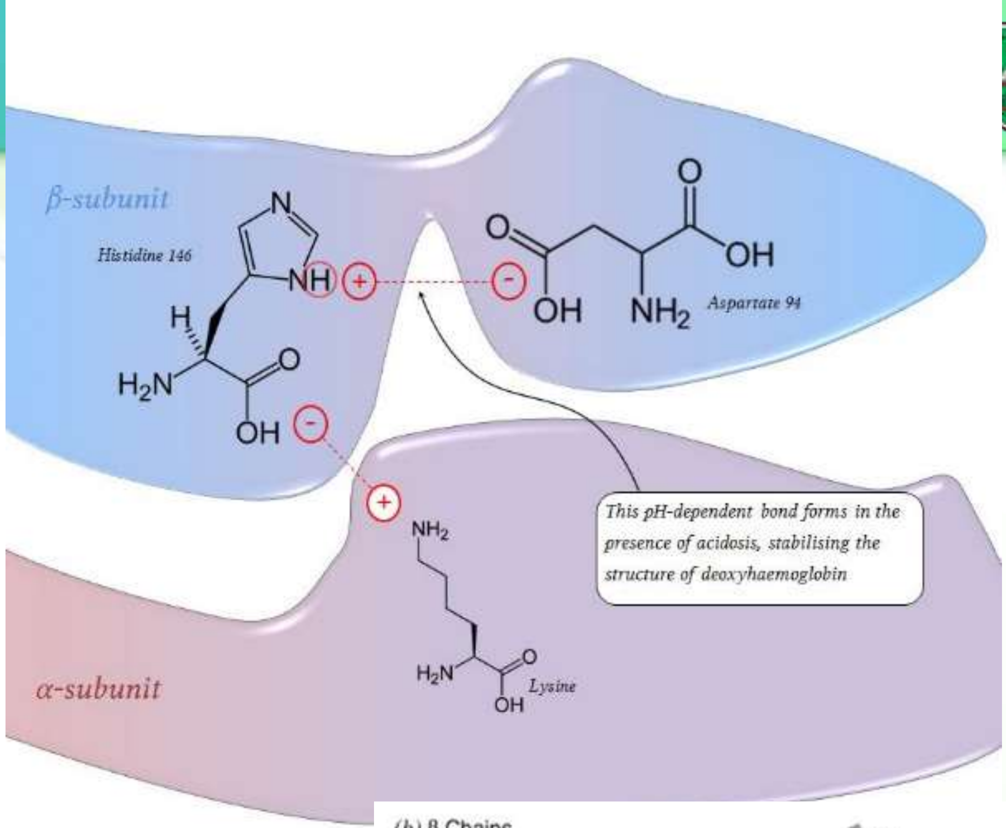


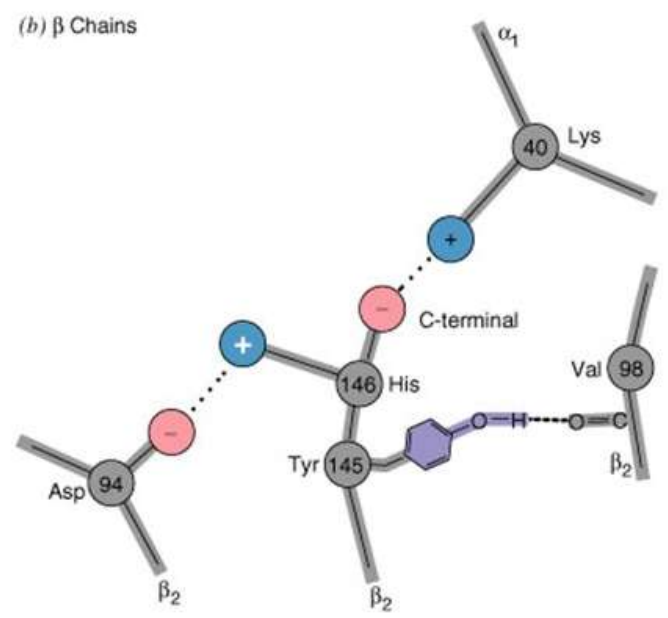


Myoglobin
pH 7.6
pH 7.4
pH 7.2
pH 7.0
pH 6.8
Venous
pO_2
Arterial
pO_2
Percent saturation
100
80
60
40
20
0
0
20
40
60
80
100
120
140
pO_2, mm Hg

Mechanism of Bohr effect

- Increasing H+ 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.
- The protonated histidine also forms a salt bridge to Asp94 within the same chain.
    - The pKa of His146 is reduced from 7.7 in the T state to 7.3 in the R state allowing for deprotonation.
- This favors the deoxygenated T form of hemoglobin.





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

Where do protons come from?

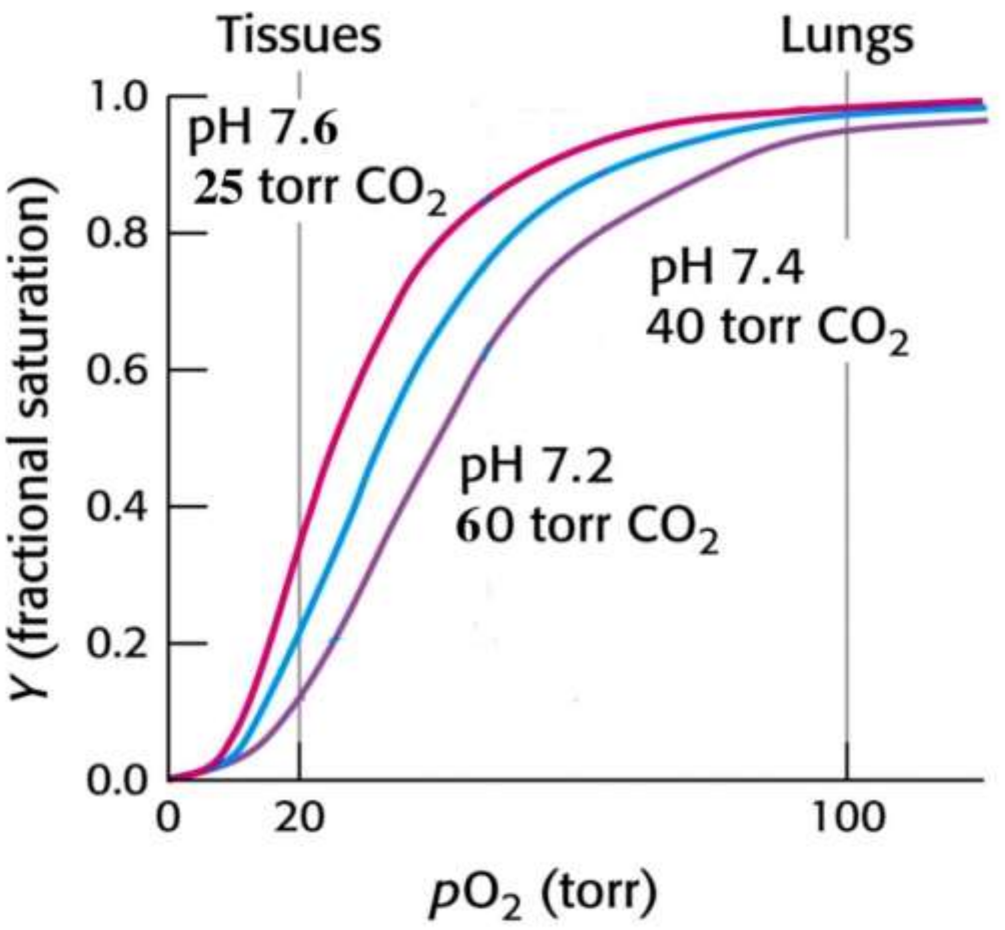
$$CO_2 + H_2O \Longleftrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$$

- CO_2 and H+ are produced at high levels in metabolically active tissues by carbonic anhydrase.
- This is accompanied by generation of H+, facilitating the release of O_2.
- In the lungs, the reverse effect occurs and high levels of O2 cause the release of CO_2 from hemoglobin.

Effect of CO2

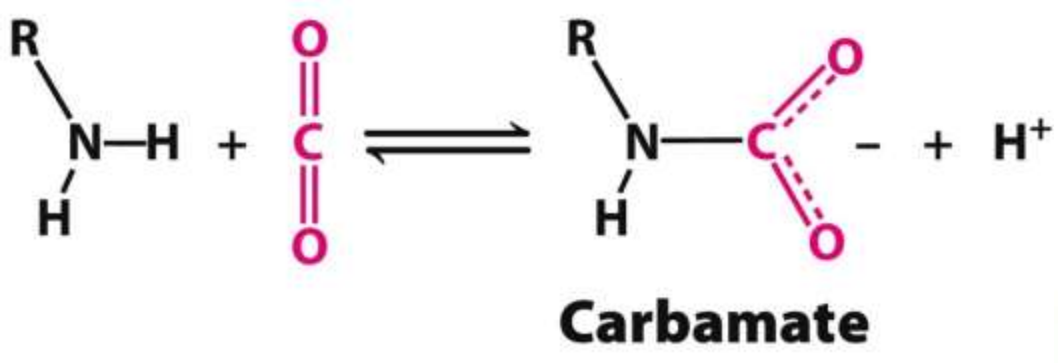
(Mechanism #1 - production of protons)

$$CO_2 + H_2O \Longleftrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$$



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

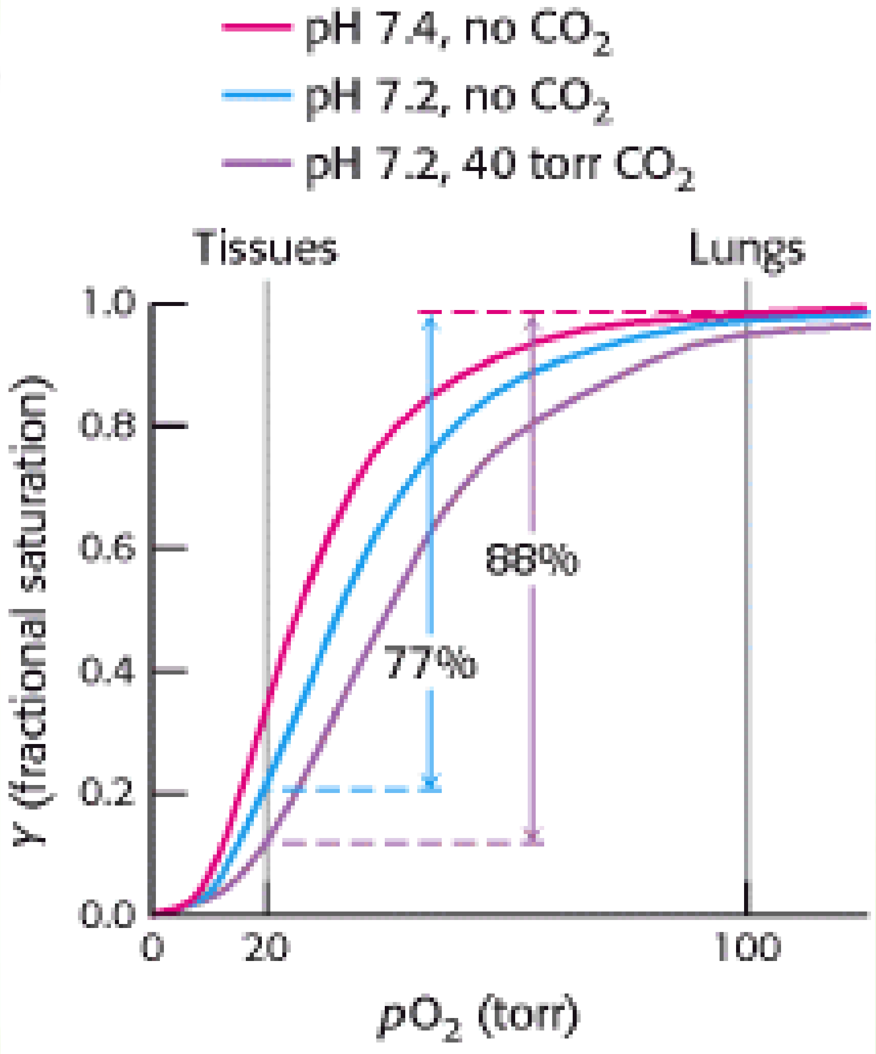


- The increased number of negatively-charged residues increases the number of electrostatic interactions that stabilize the T-state of hemoglobin.

Contribution of both mechanisms

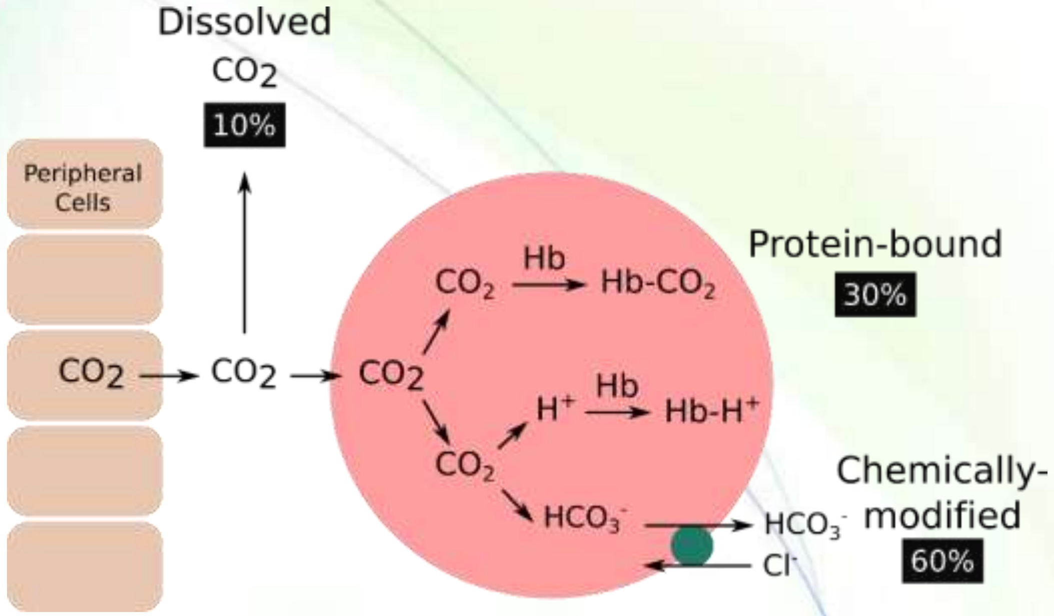
- About 75% of the shift is caused by H+.
- About 25% of the effect is due to the formation of the carbamino compounds.

How do we know that?
An increase in CO_2 tension will shift the oxygen dissociation curve to the right, even when the pH is held constant.



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.

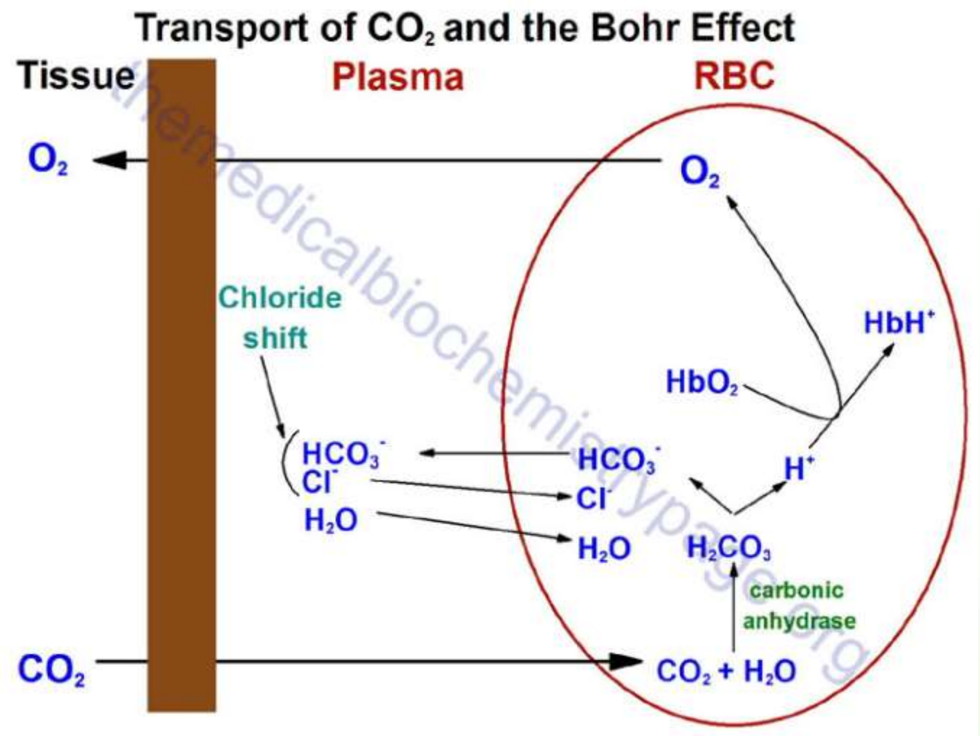


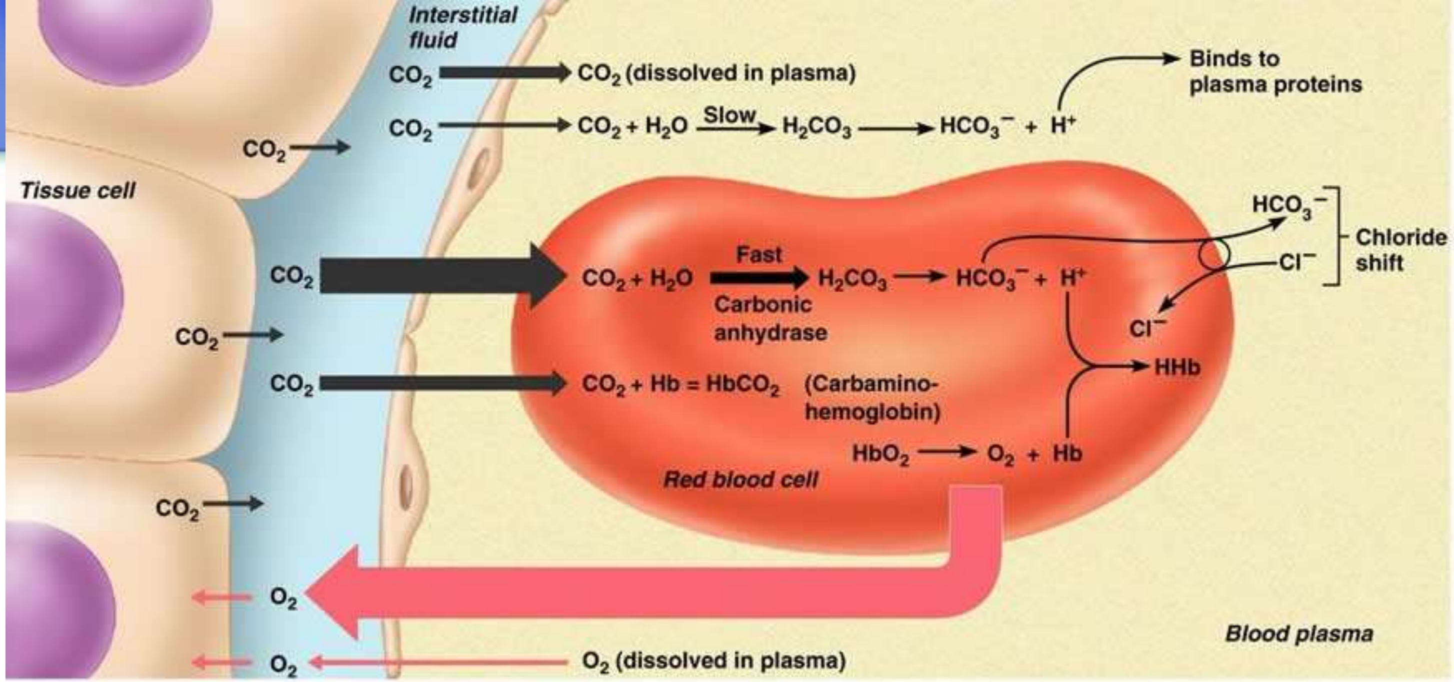
The movement of CO_2 in/out of cells does not change the pH, a phenomenon called <u>isohydric shift</u>, which is partially a result of hemoglobin being an effective buffer.

Other allosteric effectors

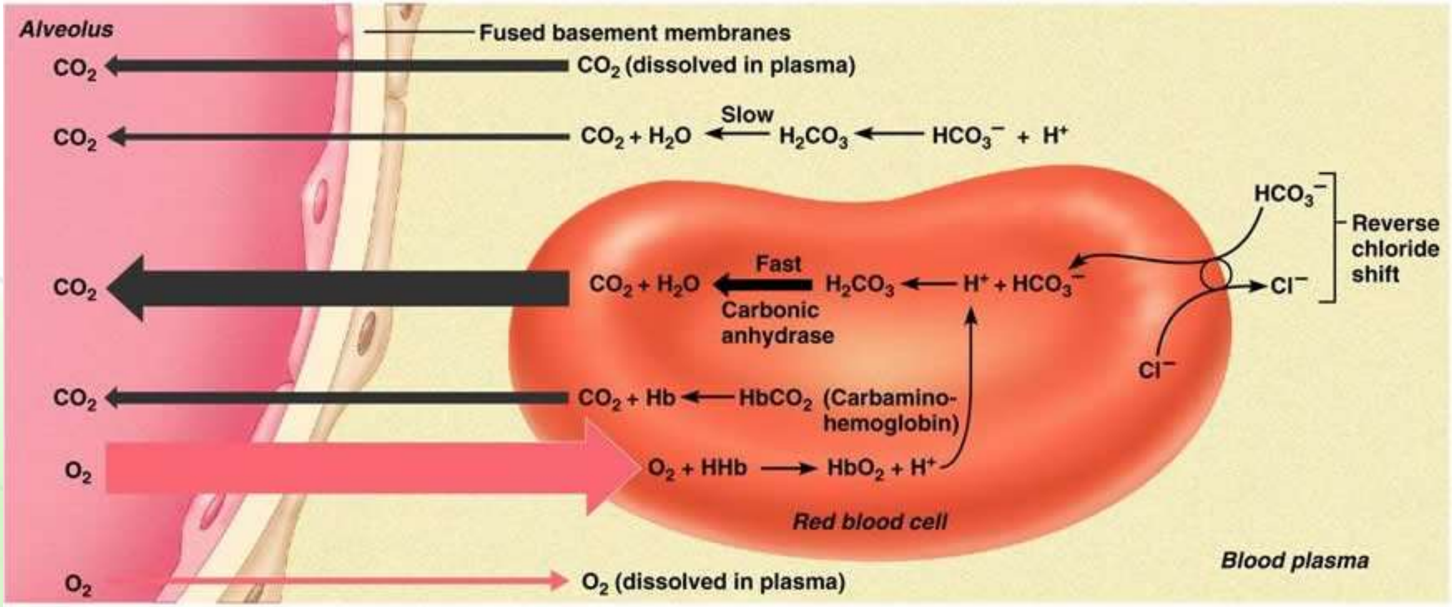
Chloride shift

- 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“.





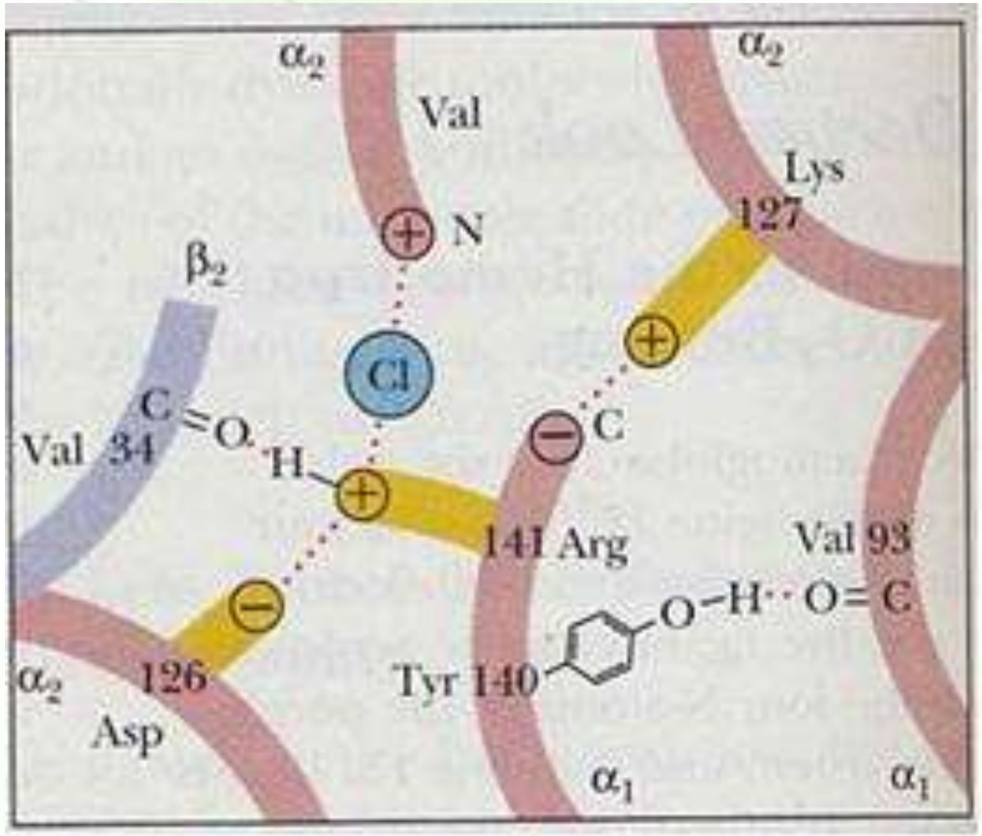
(a) Oxygen release and carbon dioxide pickup at the tissues



(b) Oxygen pickup and carbon dioxide release in the lungs

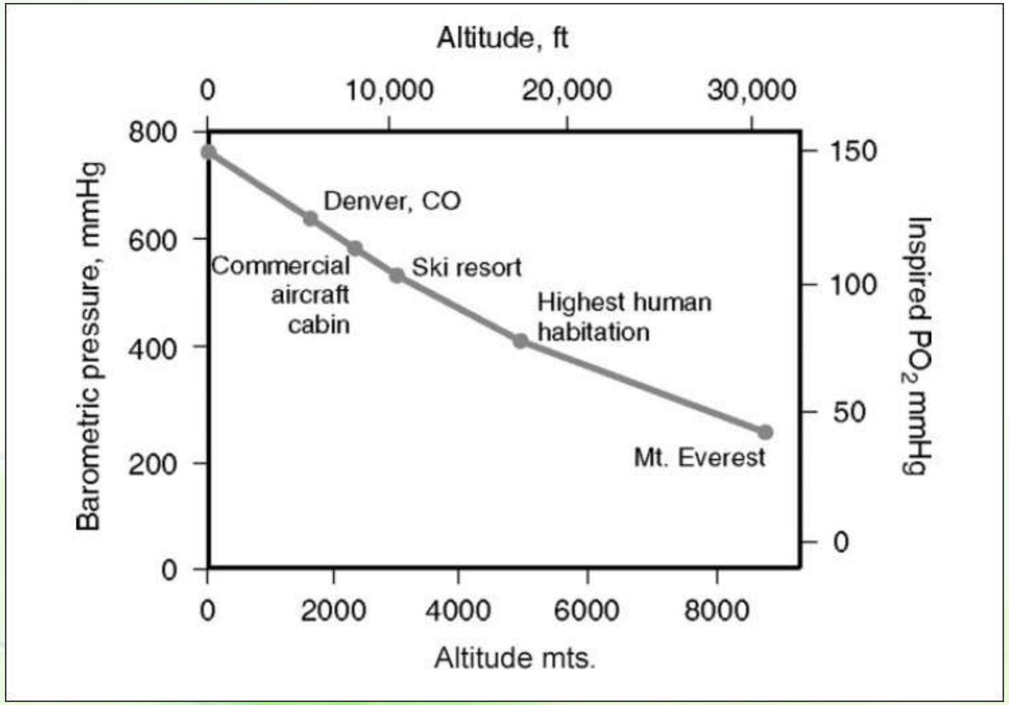
Effect of chloride ions

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



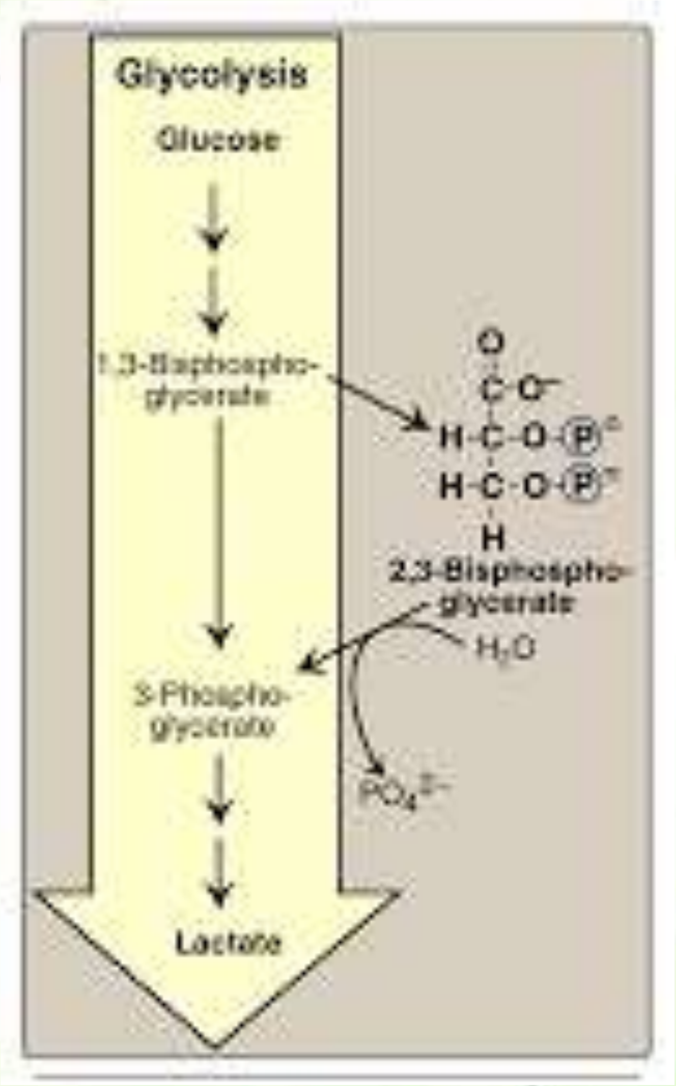
pO_2 at different altitudes

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



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

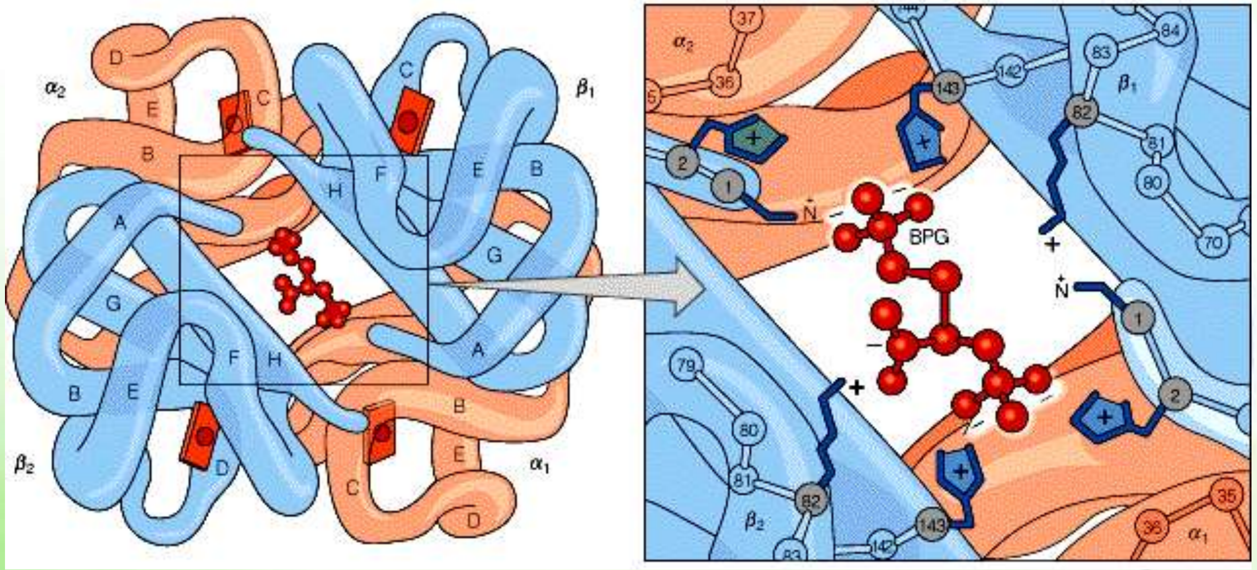
- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- BPG binds to hemoglobin and reduces its affinity towards oxygen.



BPG –hemoglobin interaction

- BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 BPG/hemoglobin tetramer.
- This binding increases the energy needed to transform hemoglobin from the T state to R state.
- Bound, 2,3-BPG reduces binding of oxygen to hemoglobin and facilitates oxygen release.

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 O_2) and in certain metabolic conditions making hemoglobin more efficient at delivering oxygen to tissues.

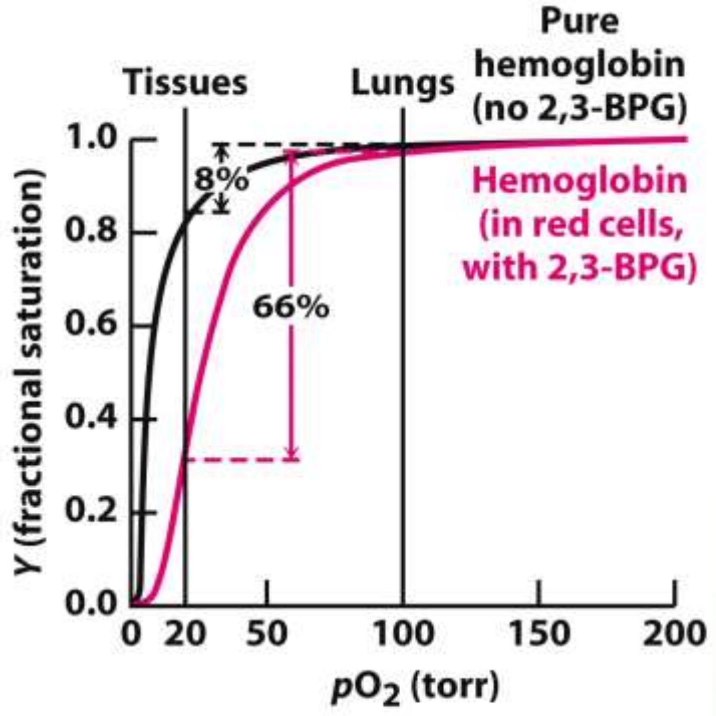


Figure 7.16
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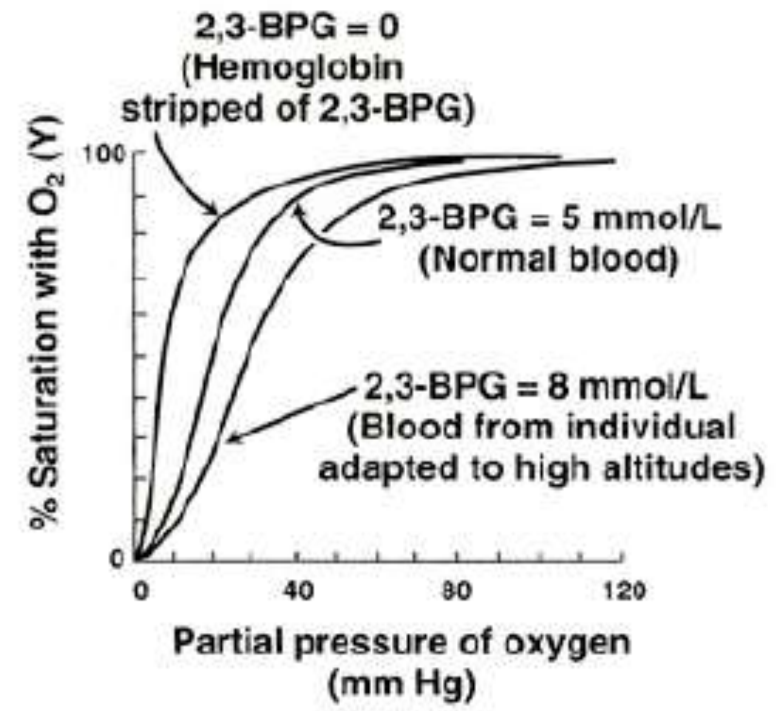
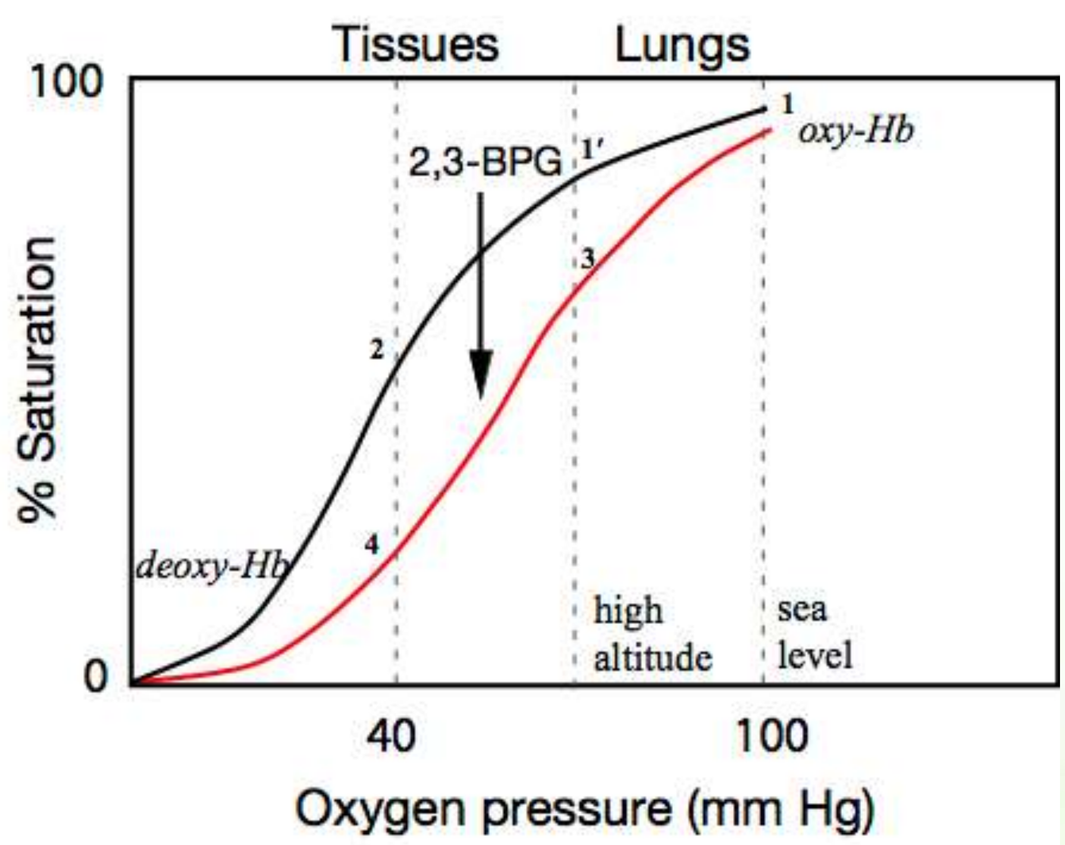


Better explanation of role of 2,3-BPG

- 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 it oxygen.
- At high altitude (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 charged 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.

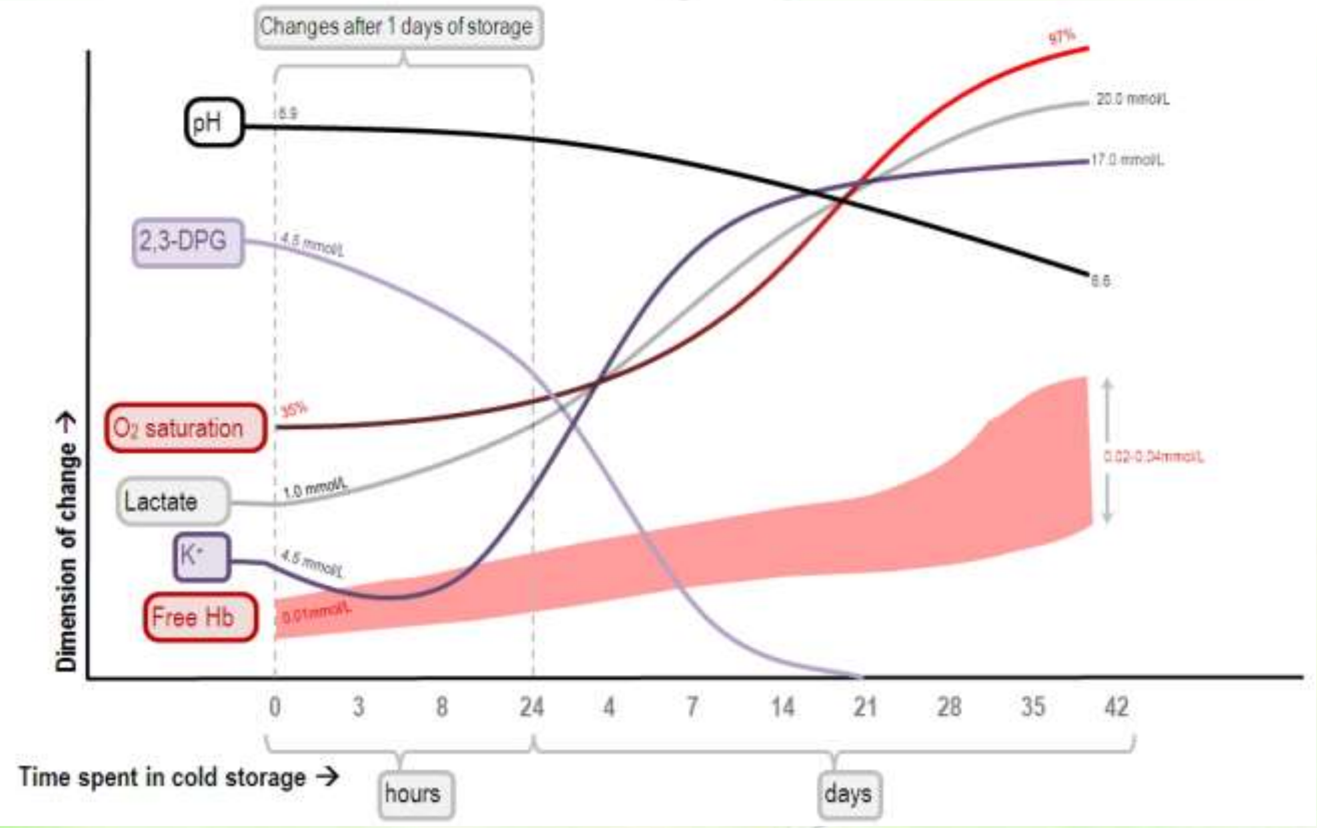
- This is not a perfect solution, but over time there is increased production of red blood cells to provide more hemoglobin to compensate for the smaller amount of oxygen it can bind.



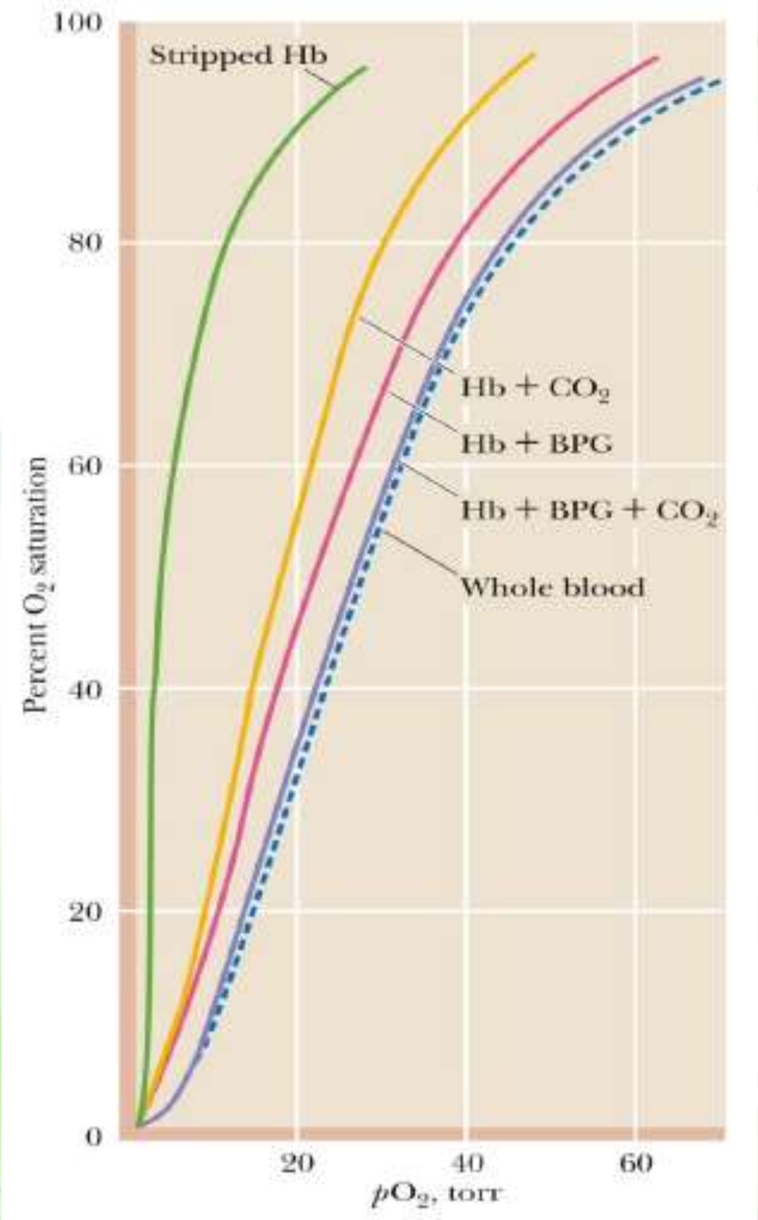
2,3-BPG in transfused blood

- 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 their depleted supplies of 2,3-BPG in 6–24 hours.

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

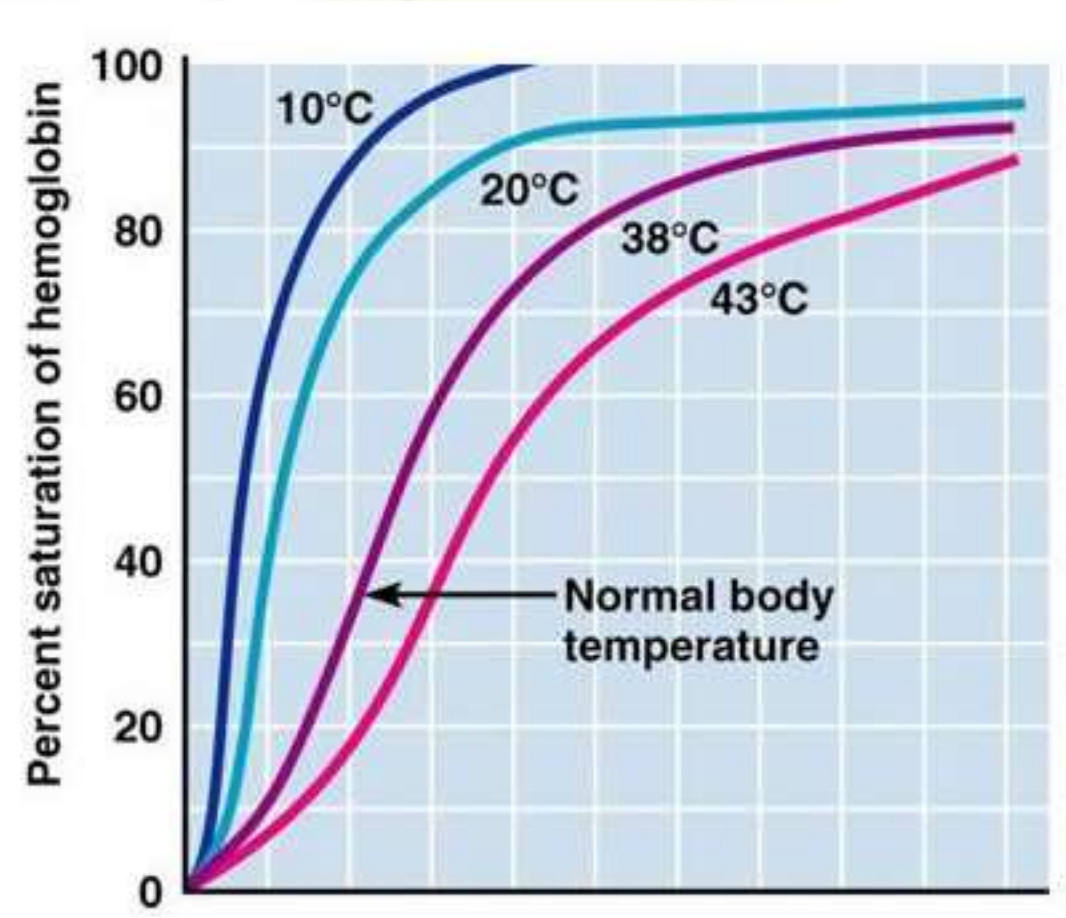


2,3-BPG and CO2 are important players



Effect of temperature

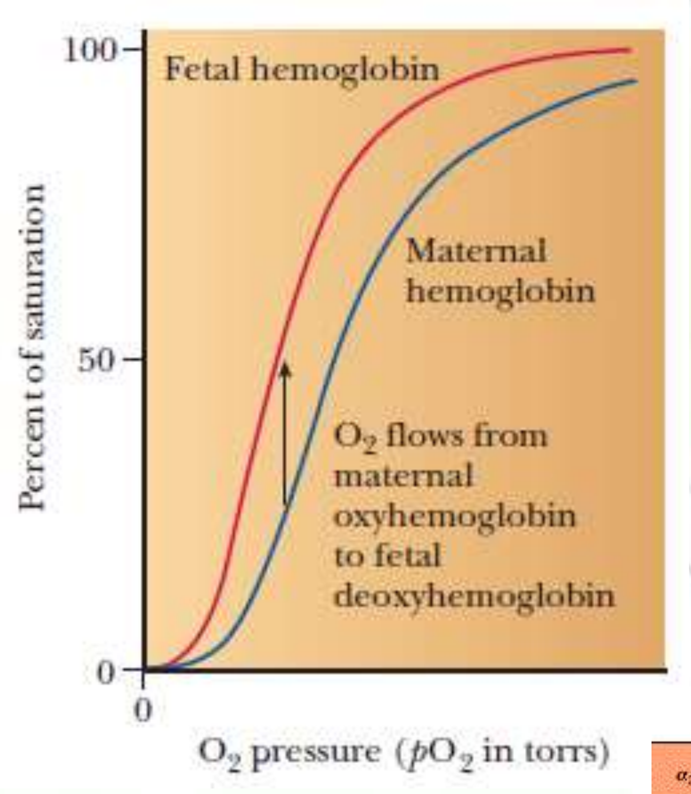
- An increase in temperature decreases oxygen affinity and therefore increases the P50.
- Temperature affects the O_2 binding of both myoglobin and hemoglobin.
- Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading from HbO_2.

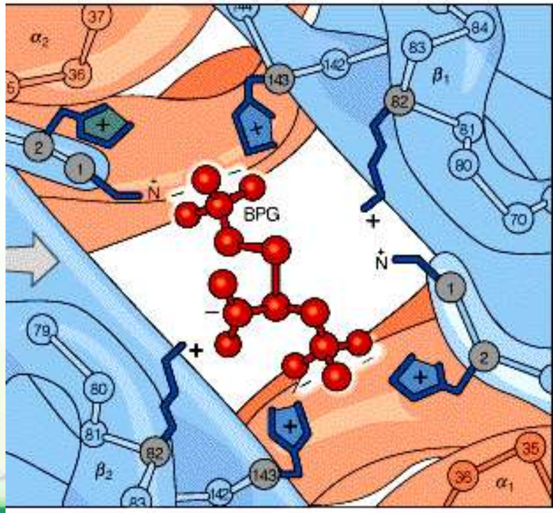


Other considerations

Fetal hemoglobin

- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HBA).
  - HbA = $\alpha2\beta2$
  - HbF = $\alpha2\gamma2$
- His143 residue in the β subunit is replaced by a Ser in the γ subunit of HbF.
  - Since Ser cannot form a salt bridge with 2,3-BPG, it binds weaklier to HbF than to HbA.

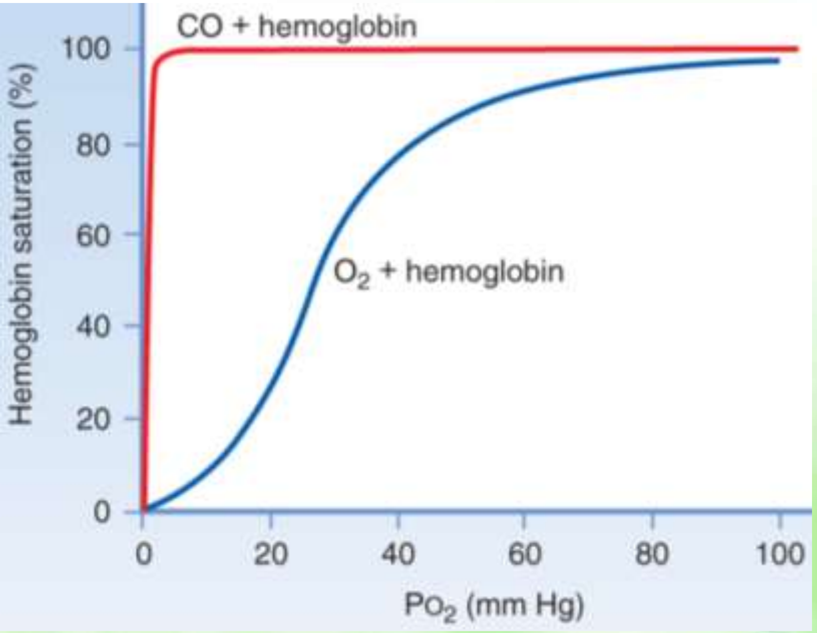


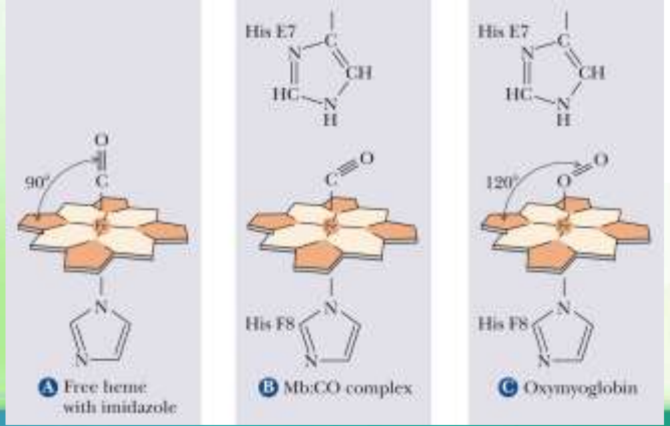


Effect of CO

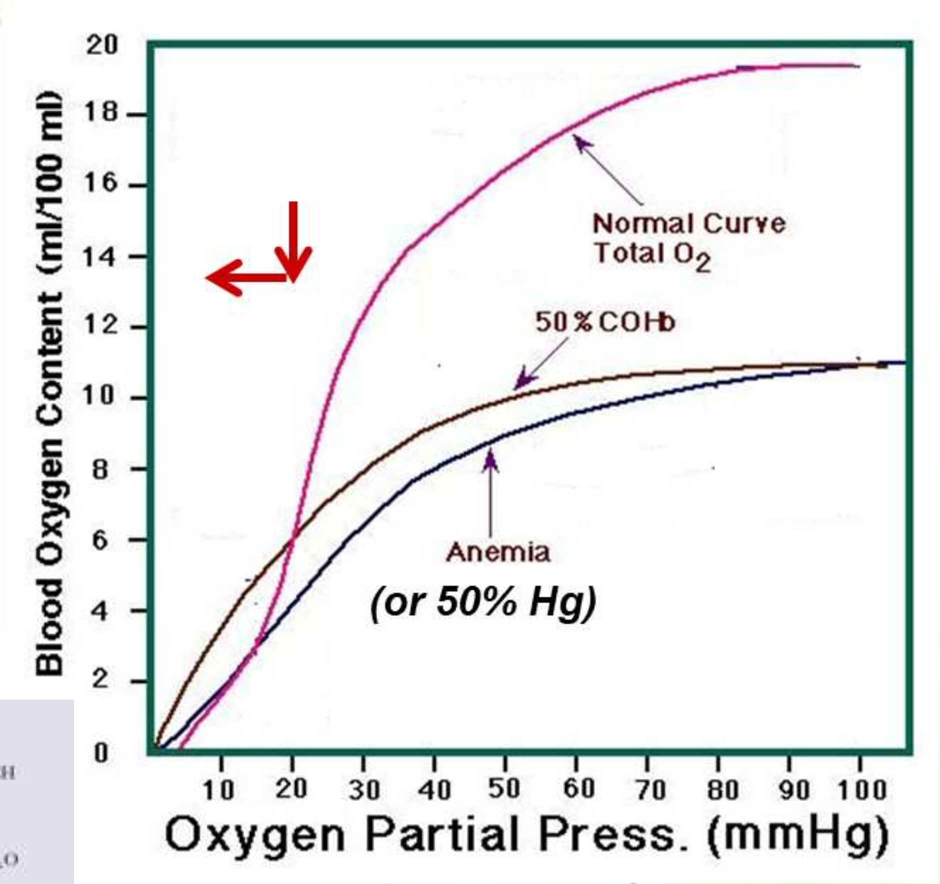
- In addition to competing with oxygen in binding to hemoglobin, affinity of Hb-CO towards oxygen increases resulting in less oxygen unloading in peripheral tissues.

(Hb + O_2) versus (Hb + CO)





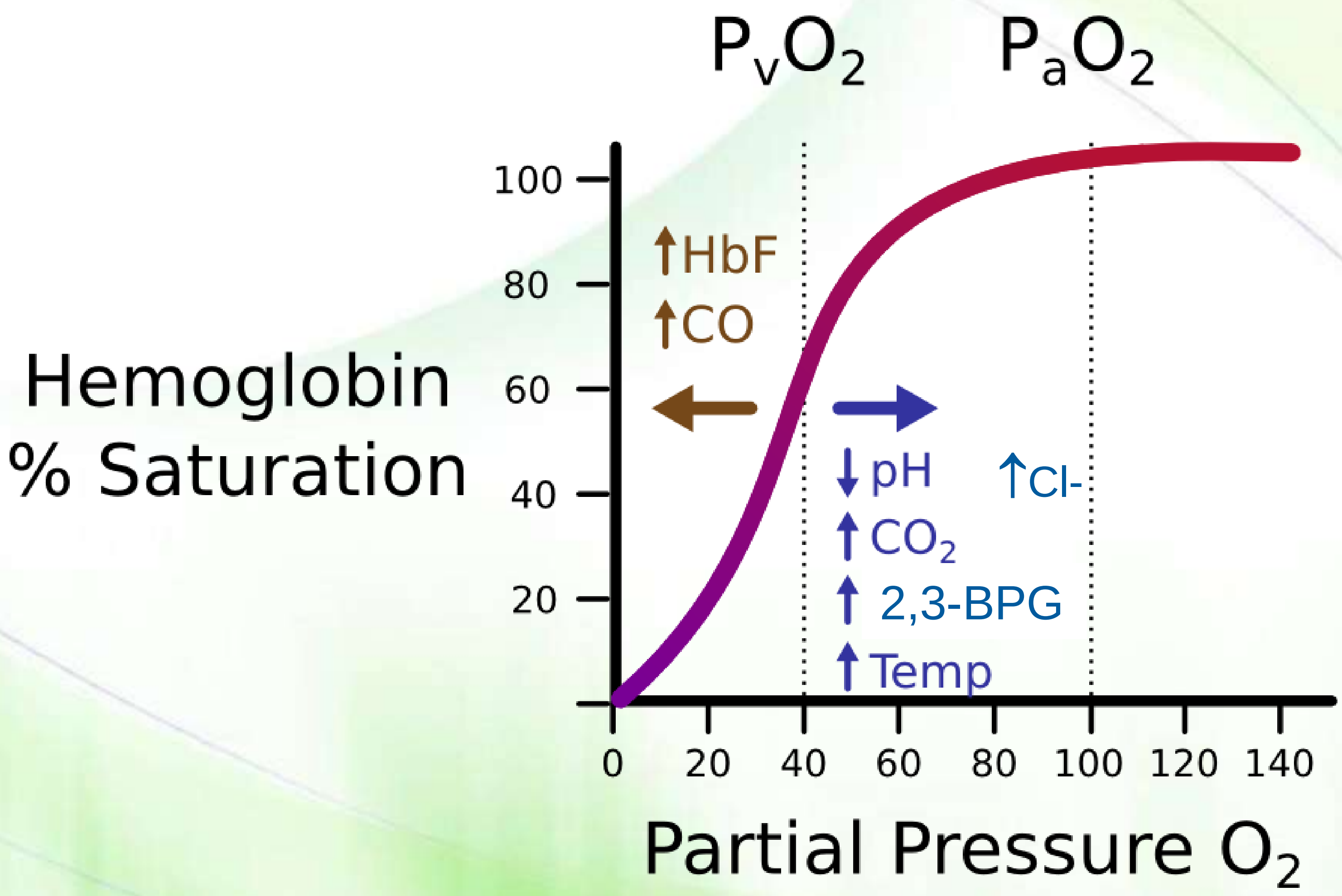
(Hb + O_2) versus (Hb + O_2 + CO)



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 COHb in the blood is usually 1% in a non-smoker.
- In smokers, COHb can reach up to 10% in smokers.
- If this concentration of COHb in the blood reaches 40% (as is caused by 1% of CO in inspired air), it would cause unconsciousness initially, followed by death.

Summary



PvO2
PaO2
Hemoglobin
% Saturation
100
80
60
40
20
↑HbF
↑CO
↓pH
↑CO2
↑ 2,3-BPG
↑Temp
↑Cl-
0
20
40
60
80
100
120
140
Partial Pressure O2