Regulation of hemoglobin function

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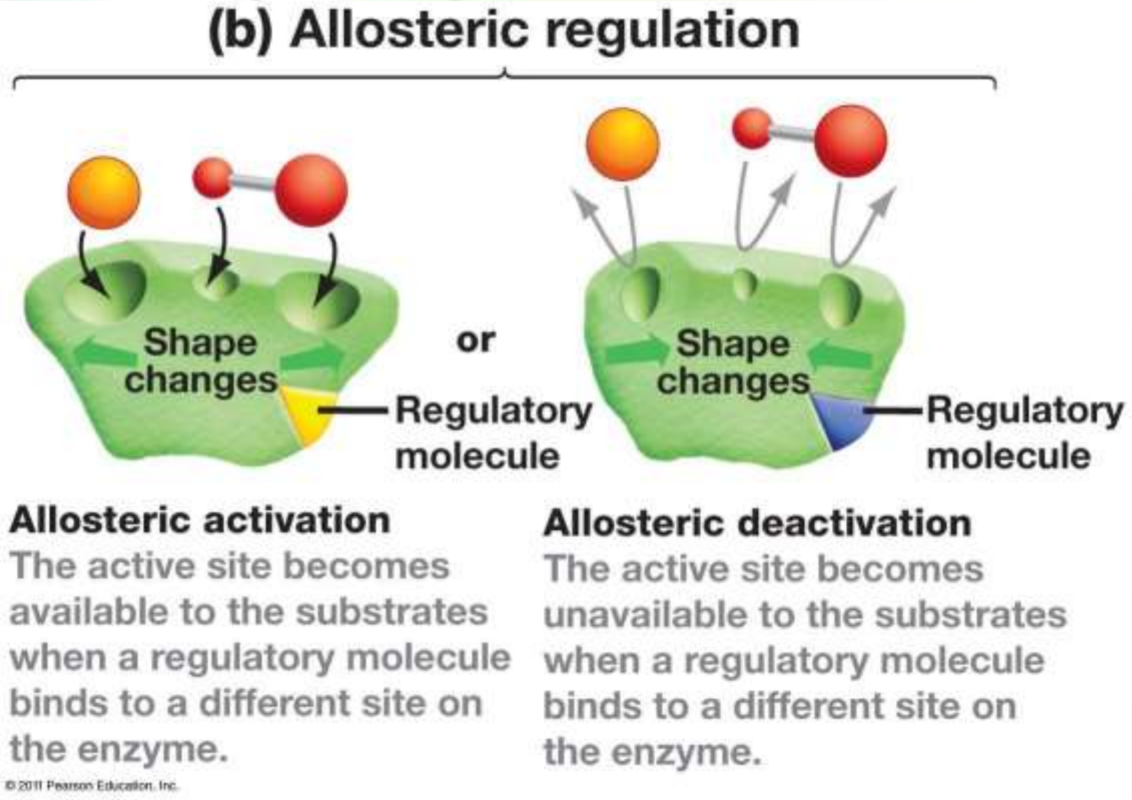
Hematopoietic-lymphatic system

Allosteric regulation

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



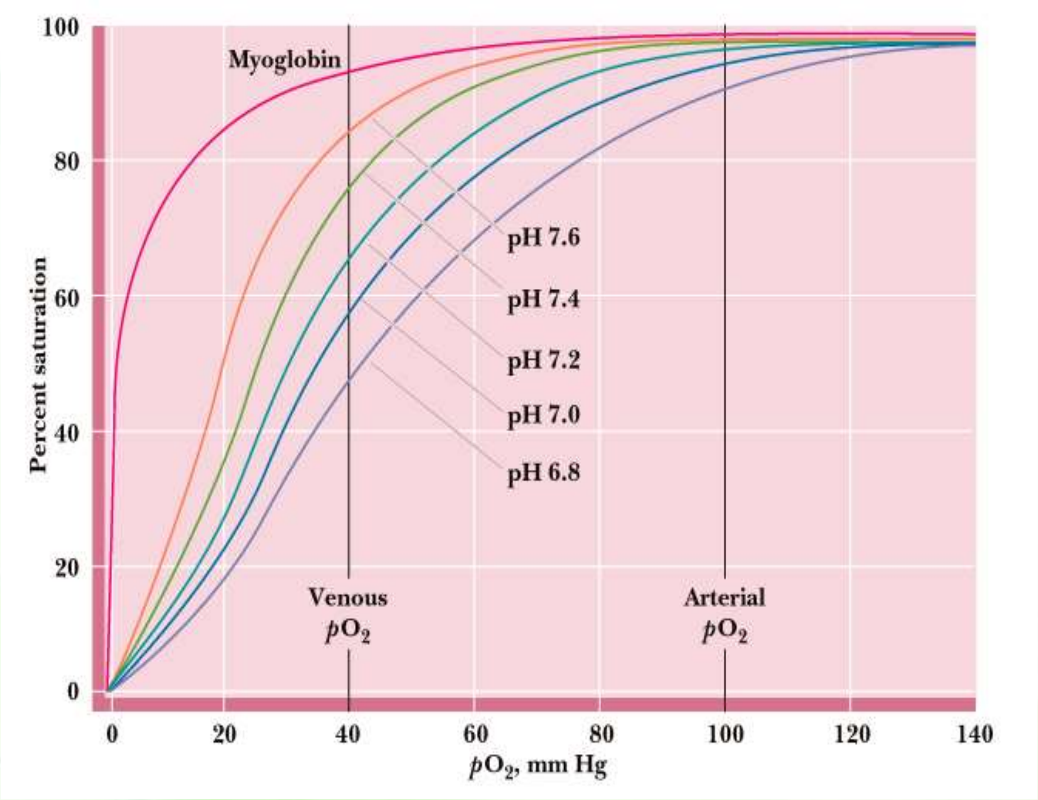
Allosteric effectors

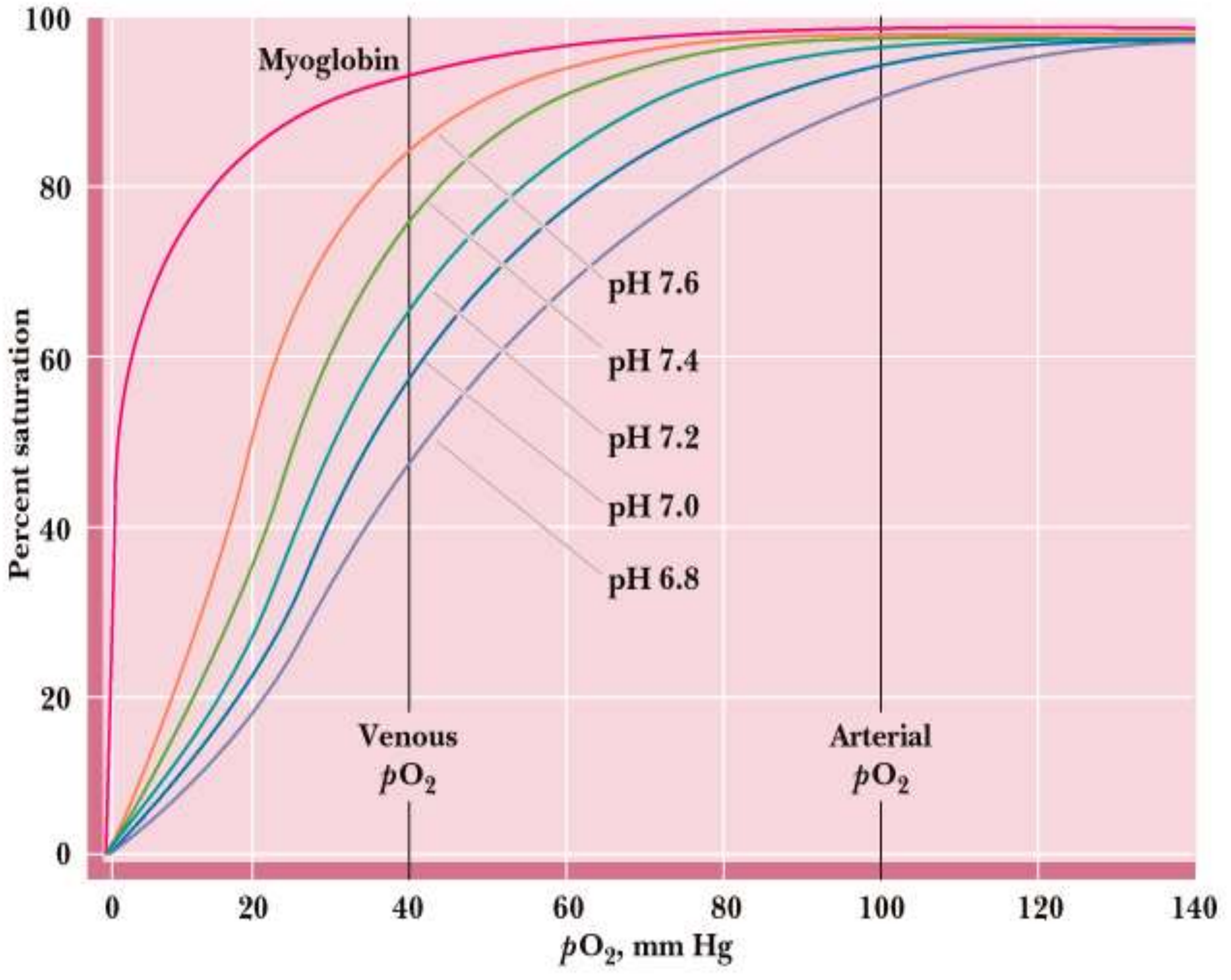
- The major heterotropic effectors of hemoglobin
  - Hydrogen ion,
  - Carbon dioxide
  - 2,3-Bisphosphoglycerate
  - Chloride ions
- A competitive inhibitor
  - Carbon monoxide

The effect of pH and H^+

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**.

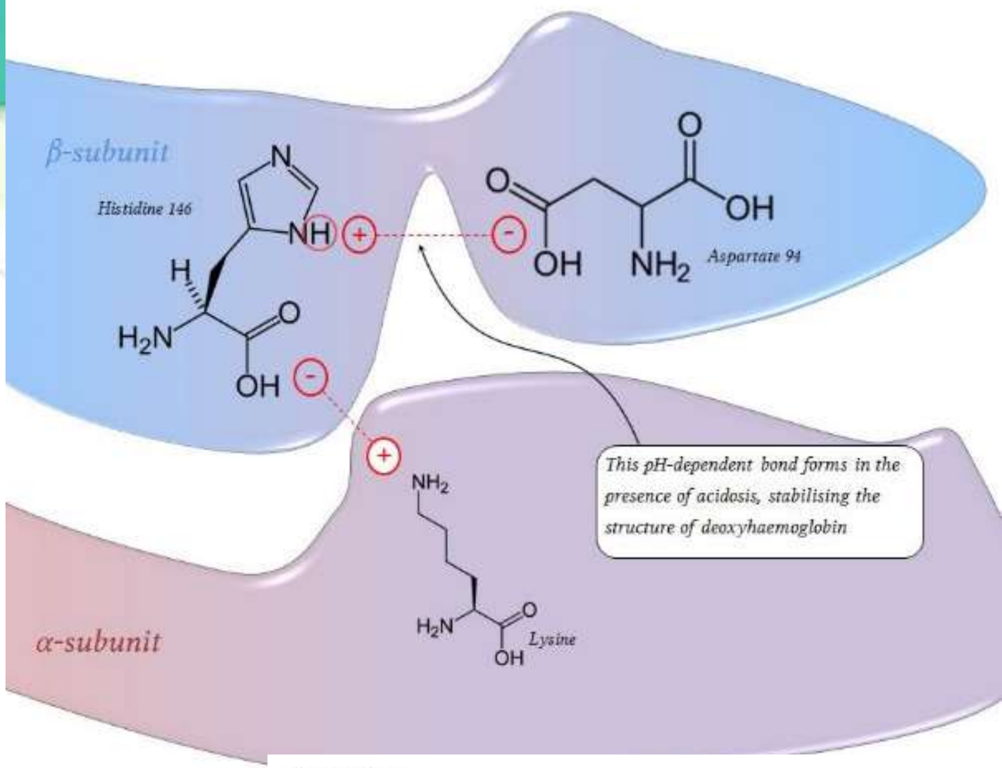


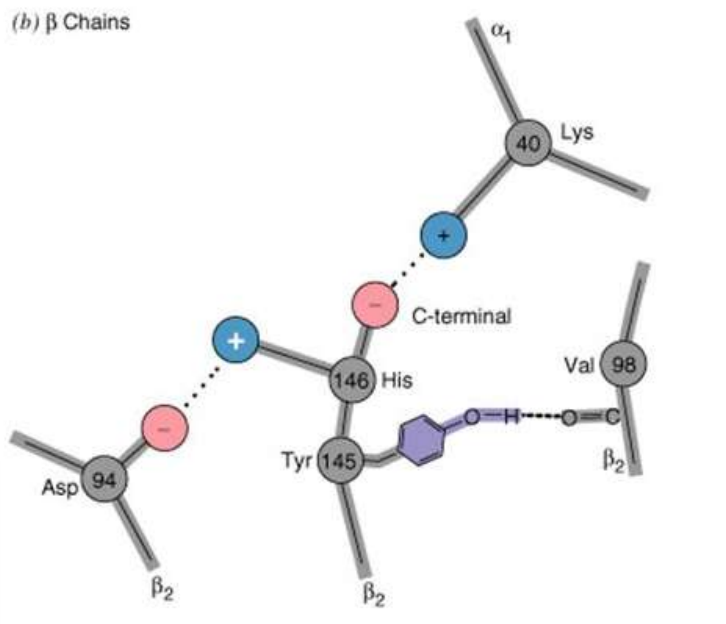


100
80
60
40
20
0
Percent saturation
Myoglobin
pH 7.6
pH 7.4
pH 7.2
pH 7.0
pH 6.8
Venous
pO_2
Arterial
pO_2
0
20
40
60
80
100
120
140
pO_2, mm Hg

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.
- The protonated histidine also forms a salt bridge to Asp94 within the same chain.
  - The pKa of the imidazole ring of His146 is reduced from 7.7 in the T-state to 7.3 in the R-state, meaning that it is protonated (charged) in the T-state and deprotonated (uncharged) in the R-state.
- 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?

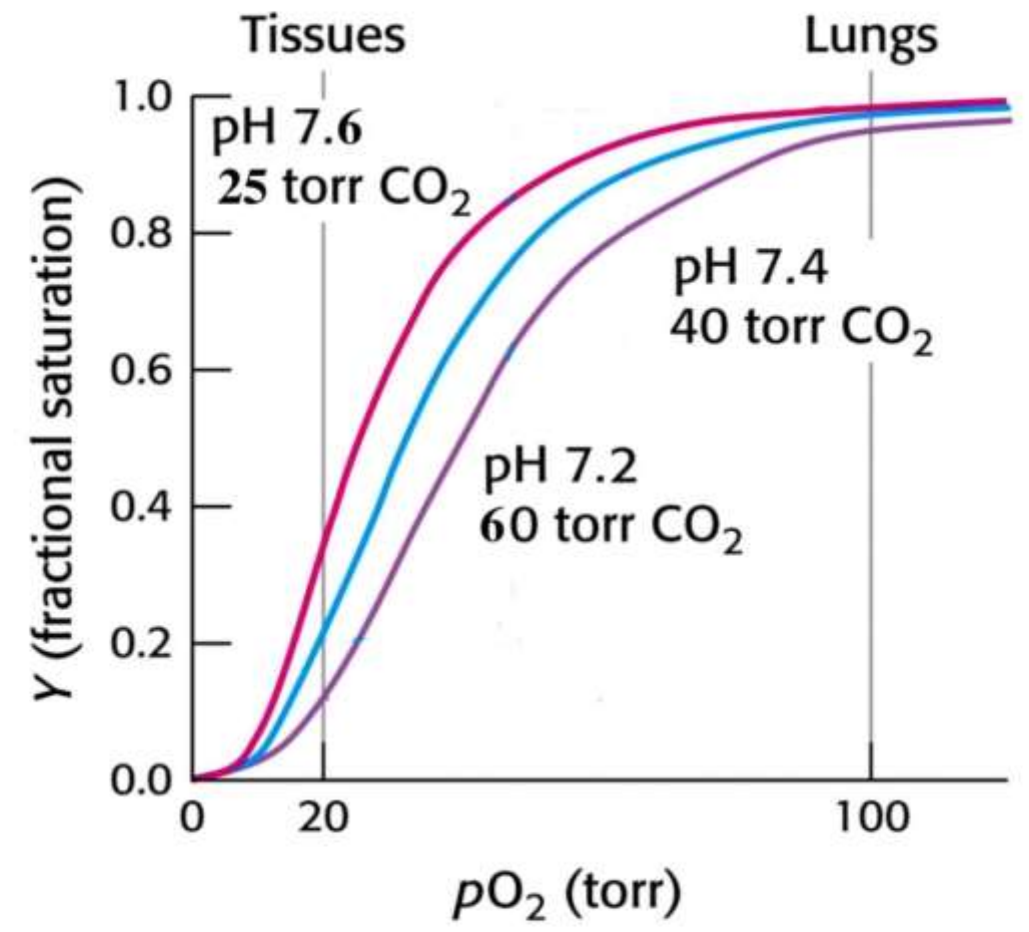
$$CO_2 + H_2O \Longleftrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$$

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

The effect of CO_2

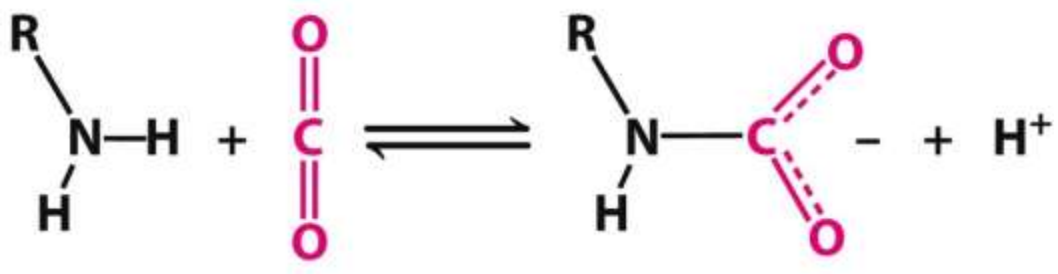
Mechanism #1 - production of protons

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



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



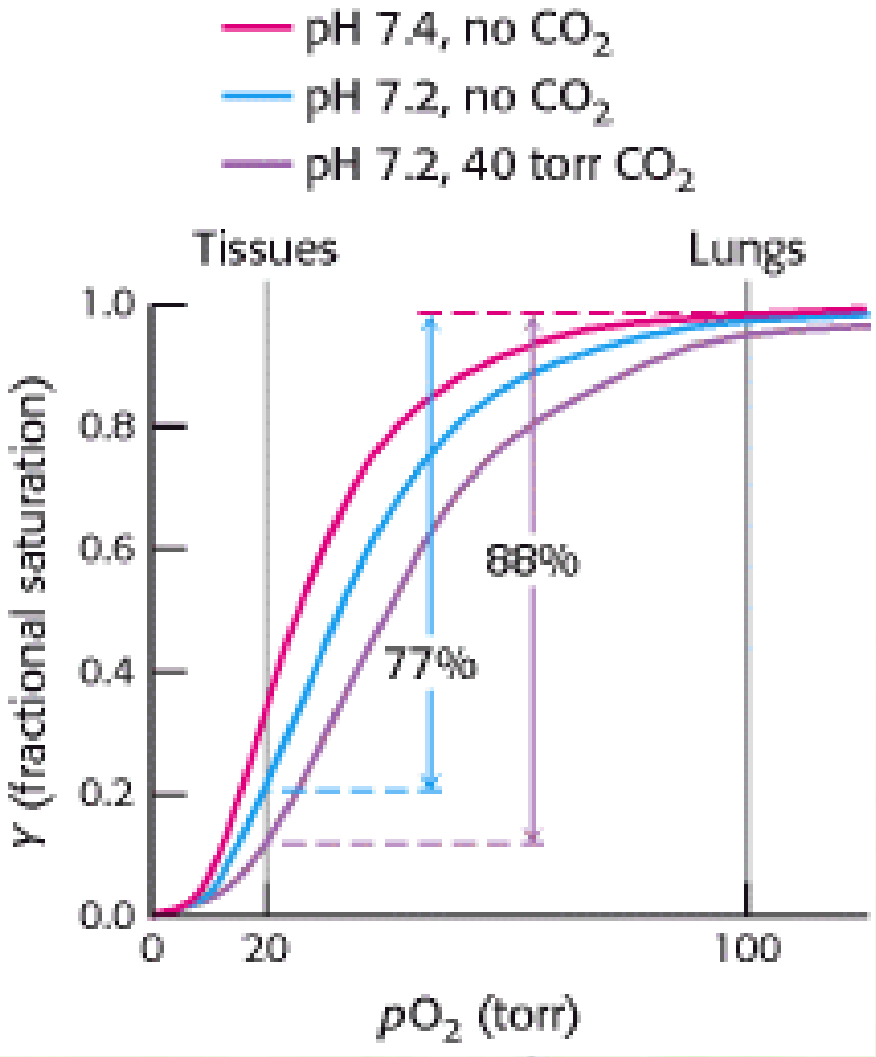
Carbamate

- 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?

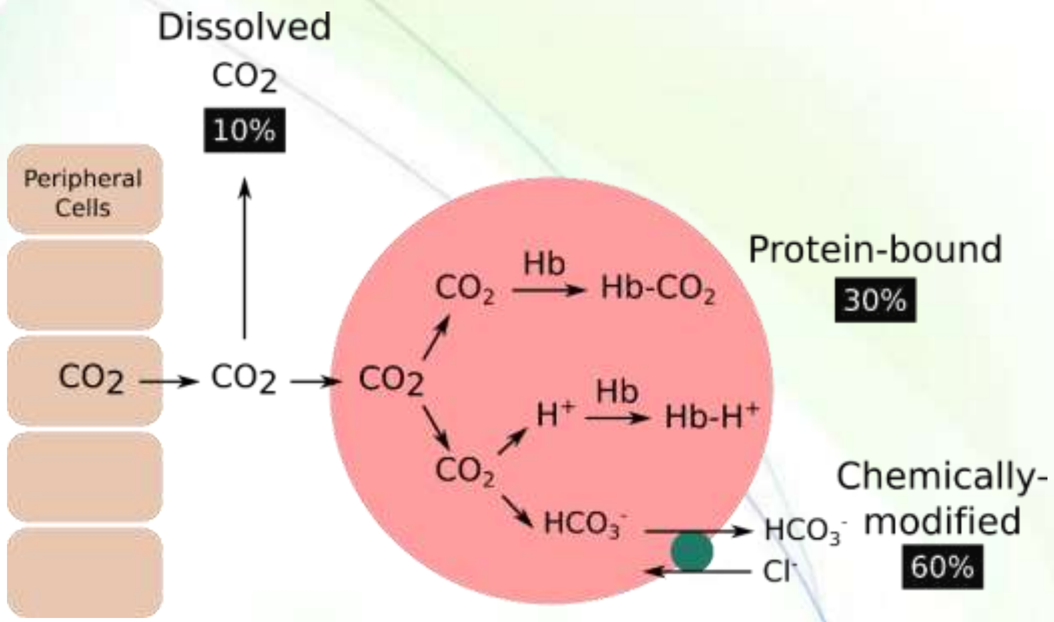
- About 75% of the shift is caused by H^+.
- About 25% of the effect is due to the formation of the carbamino compounds.

How do we know that?
By changing one factor and keeping the other constant.
An increase in CO_2 tension 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.

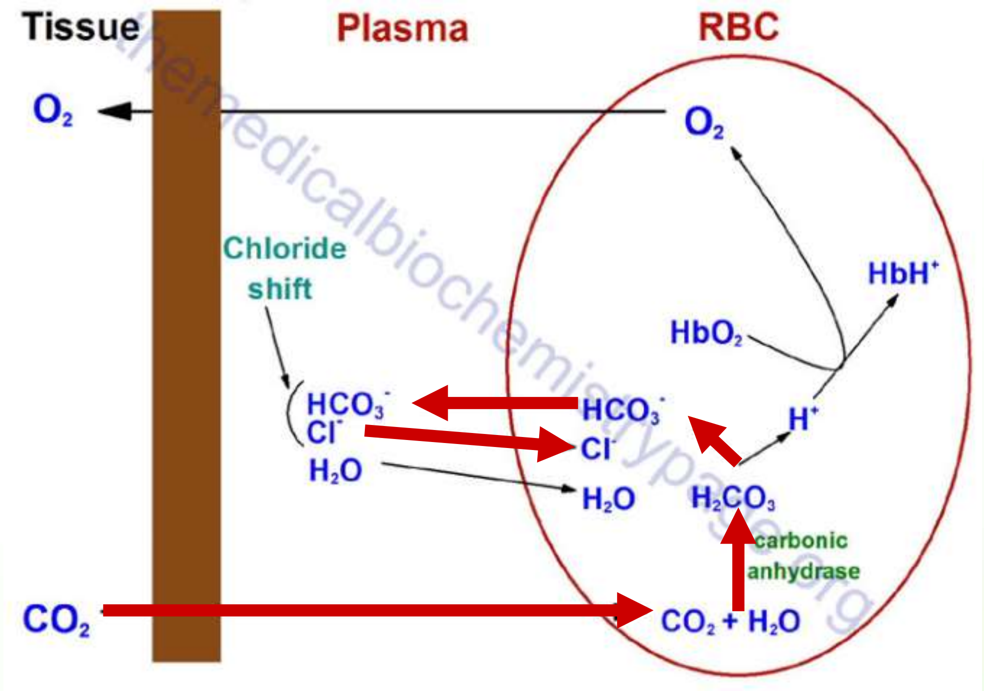


The movement of CO_2 in/out of cells does not change the pH, a phenomenon called <u>isohydric shift</u>, which is partially a result of hemoglobin being an effective buffer.

Effect of Chloride ion

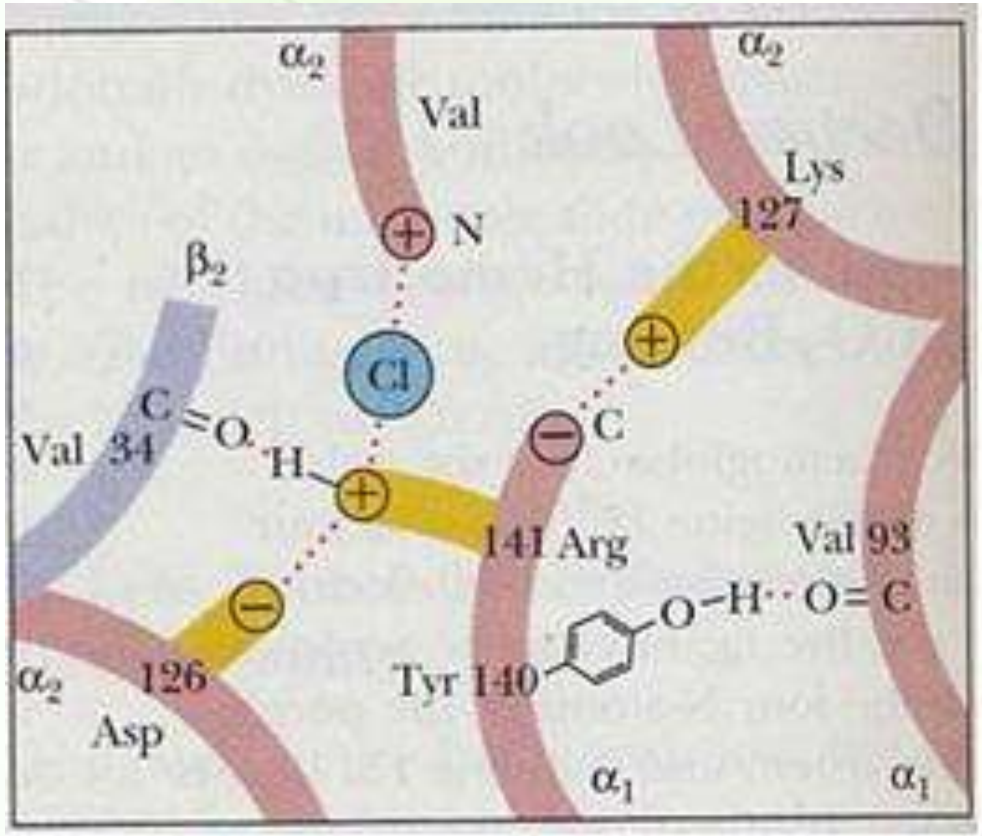
Chloride shift

- 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

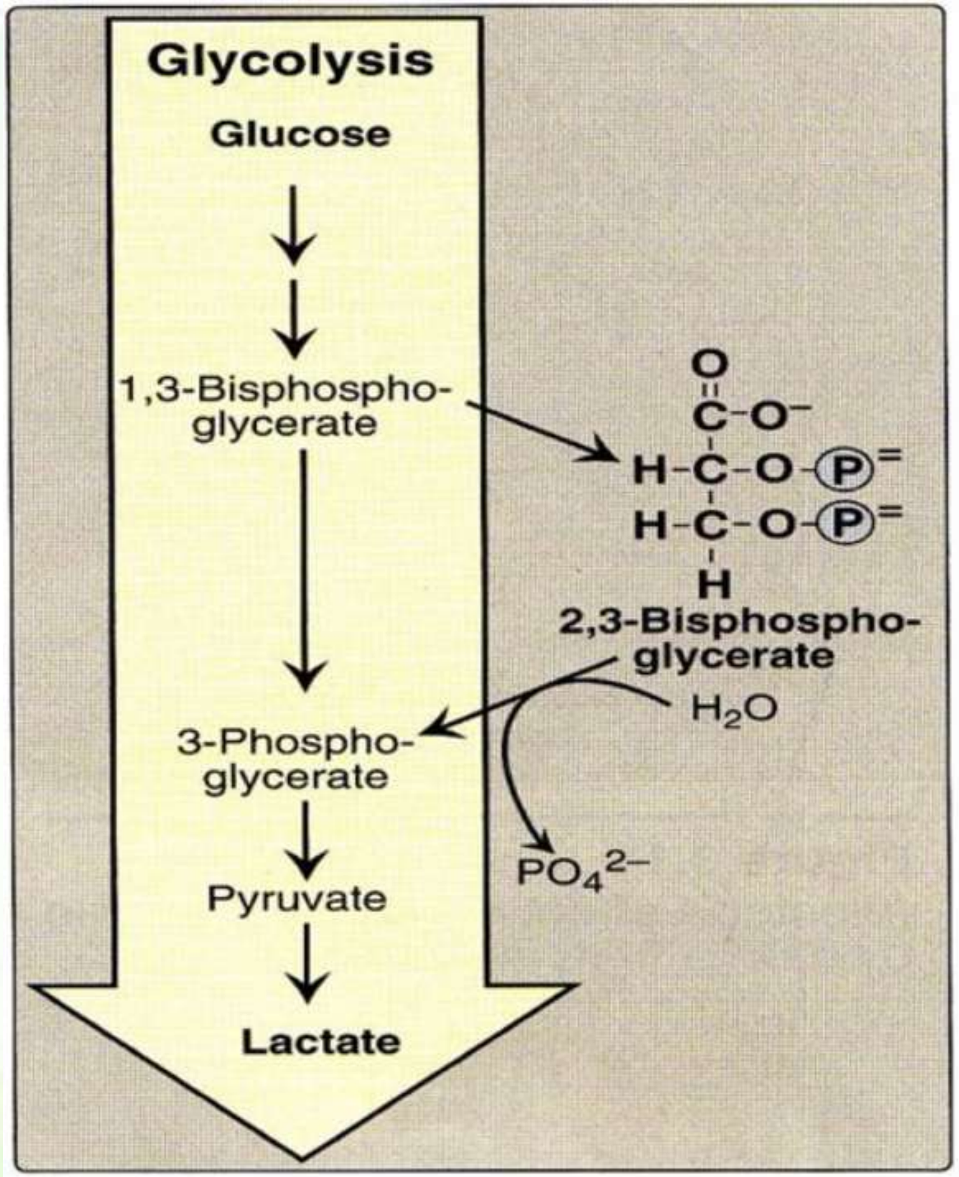
- Chloride ions interact with both the N-terminus of α2 chain and Arg141 of α1 chain stabilizing the T-state of hemoglobin.
- Increasing the concentration of chloride ions (Cl^-) shifts the oxygen dissociation curve to the right (lower affinity)



Effect of 2,3-bisphosphoglycerate

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

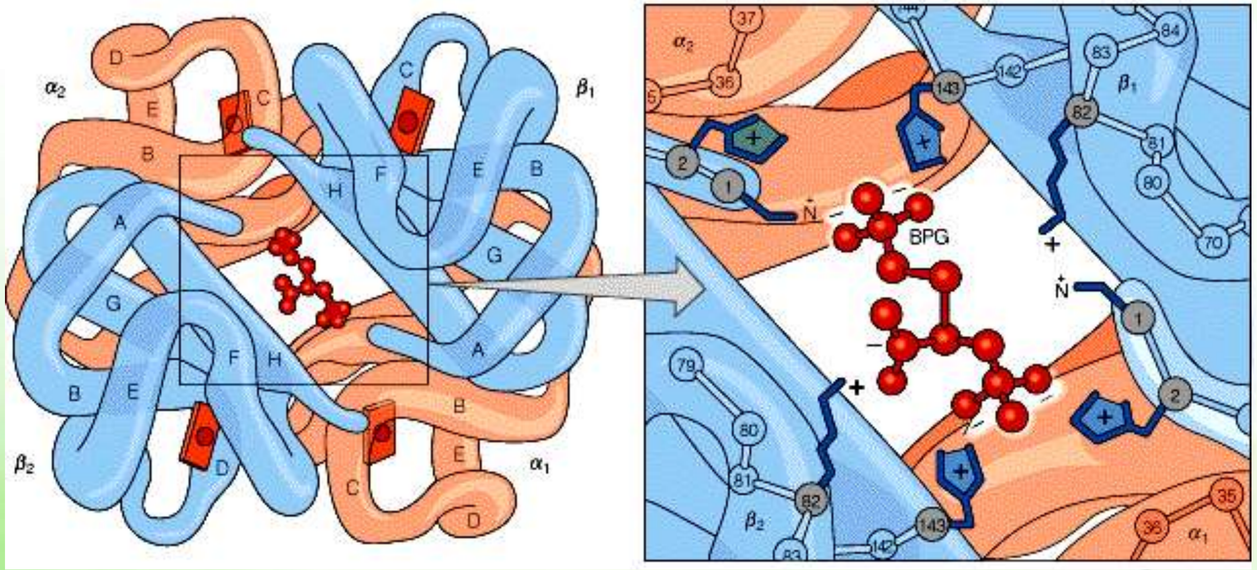
- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- It binds to hemoglobin and reduces its affinity towards oxygen.



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.

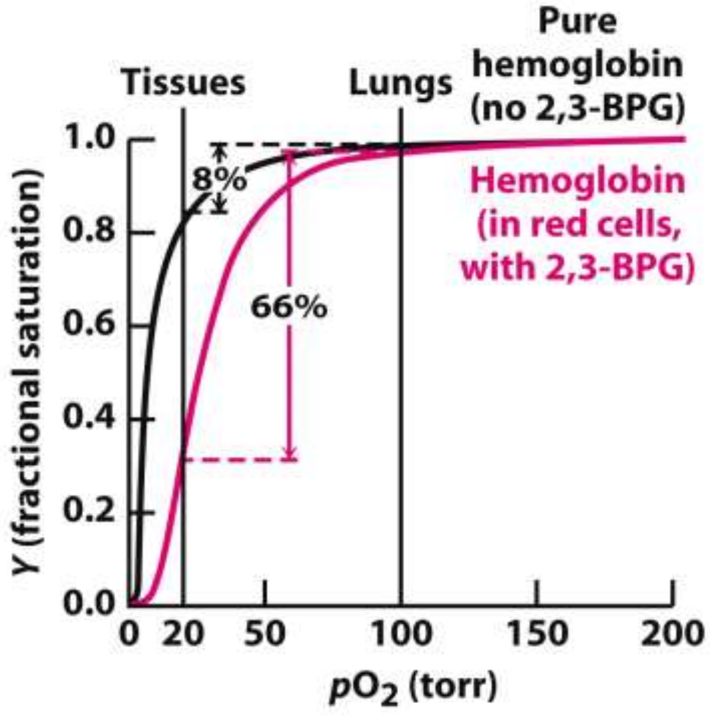


Figure 7.16
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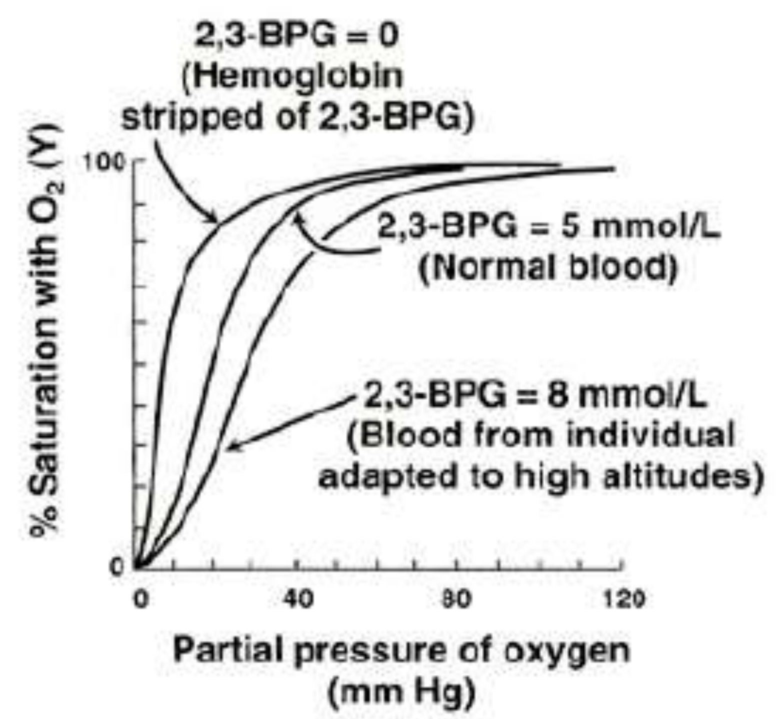
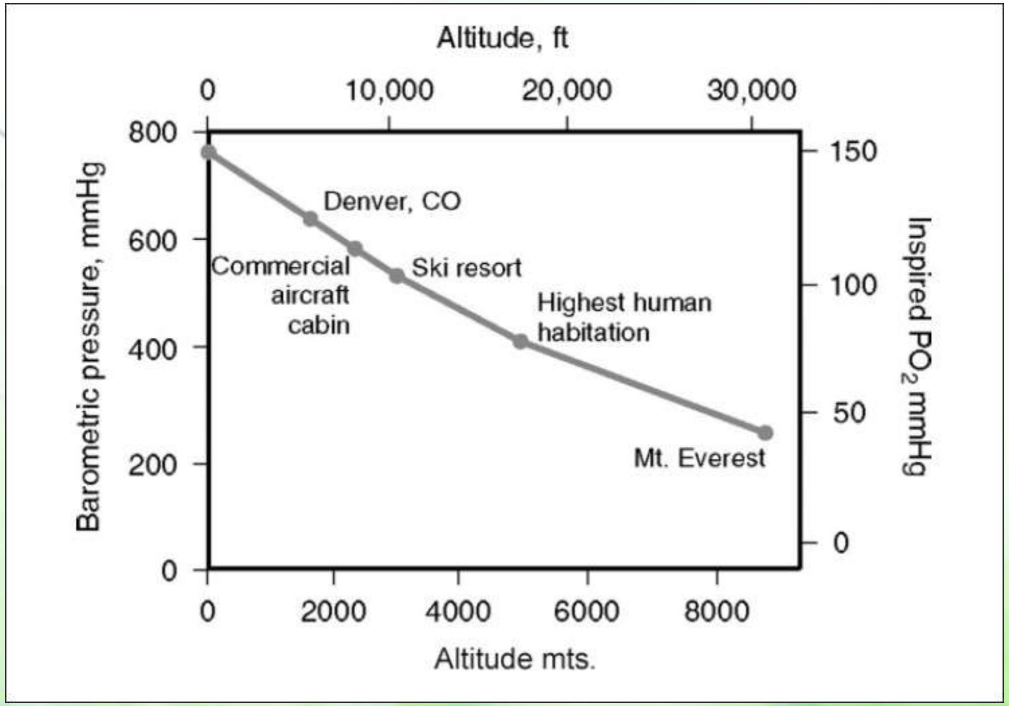
- The concentration of 2,3-BPG increases at high altitudes (low O_2) and in certain metabolic conditions making hemoglobin more efficient at delivering oxygen to tissues.



But pO_2 is low at high altitudes!!!

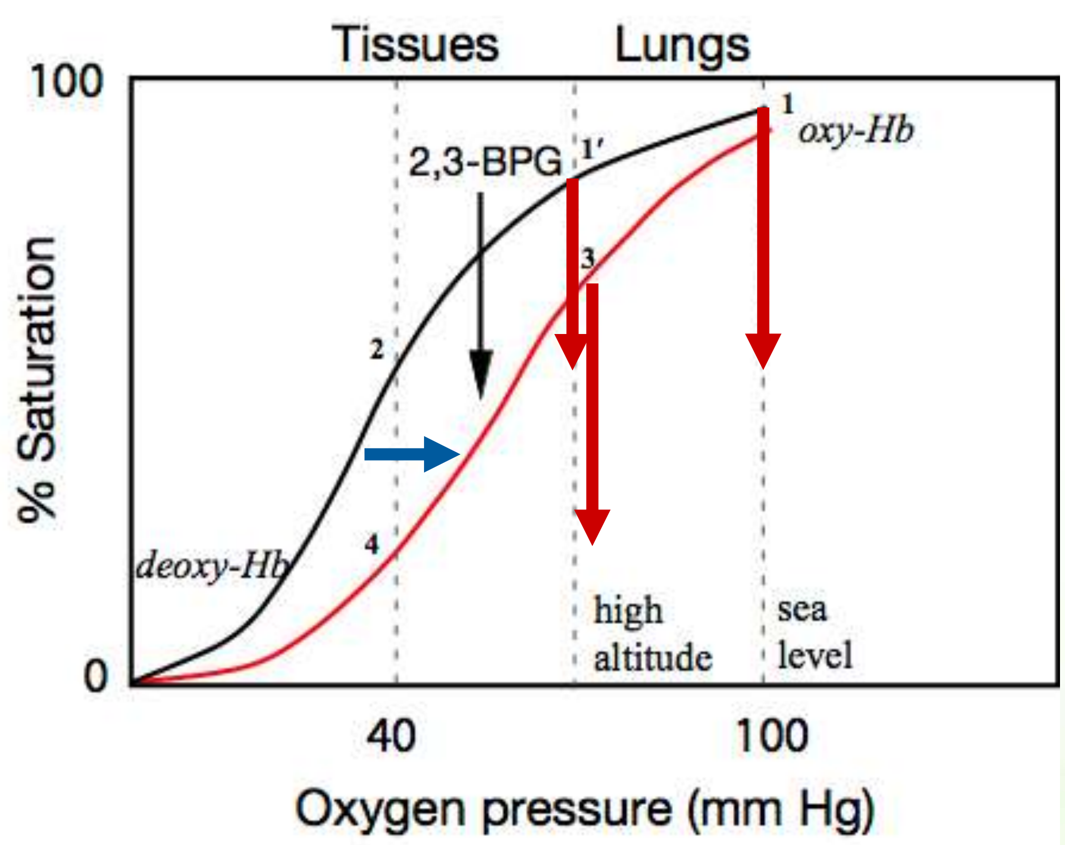
Altitude (feet)	Atmospheric Pressure (mm/Hg)	PAO_2 (mm/Hg)	PVO_2 (mm/Hg)	Pressure Differential (mm/Hg)	Blood Saturation (%)
Sea Level	760	100	40	60	98
10,000	523	60	31	29	87
18,000	380	38	26	12	72
22,000	321	30	22	8	60
25,000	282	7	4	3	9
35,000	179	0	0	0	0



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.

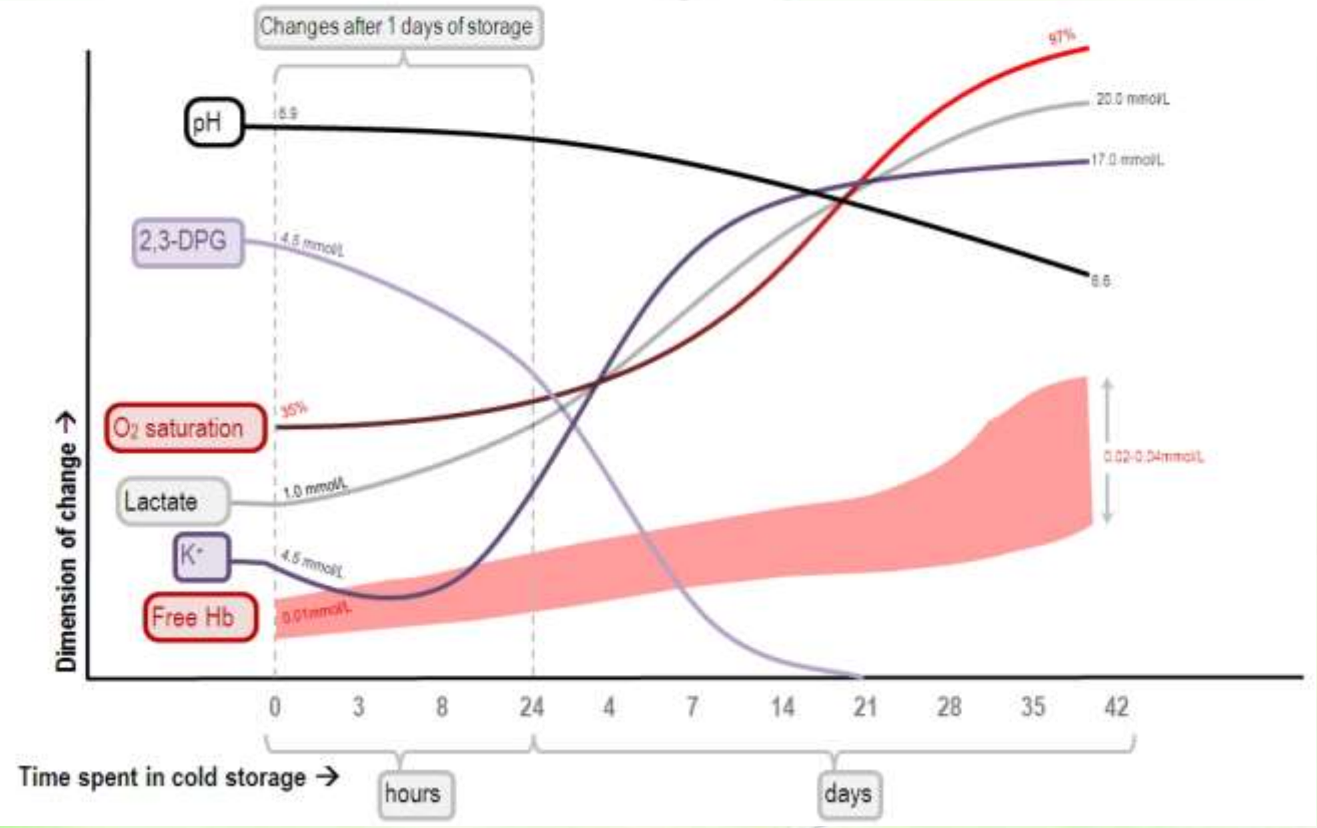
- This is not a perfect solution, but over time there is increased production of red blood cells to provide more hemoglobin to compensate for the smaller amount of oxygen it can bind.



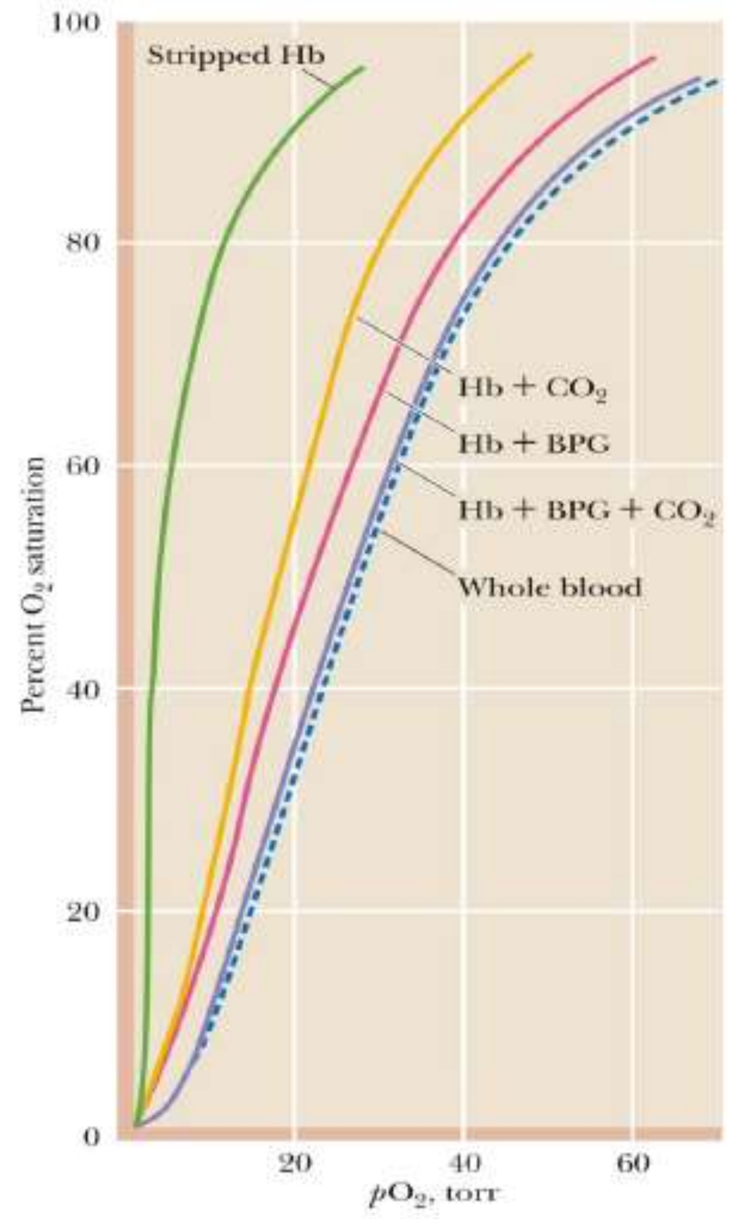
2,3-BPG in transfused blood

- 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.



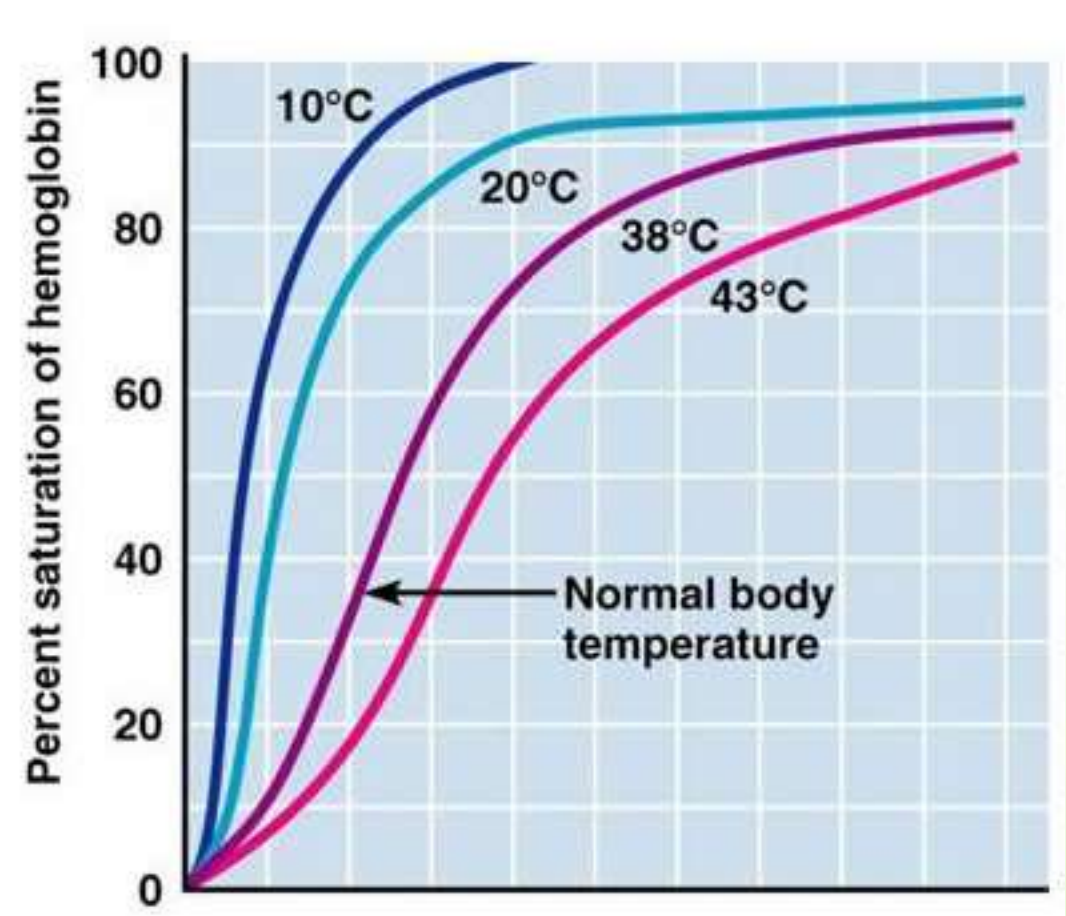
2,3-BPG and CO2 are important players



Effect of temperature

Effect of temperature

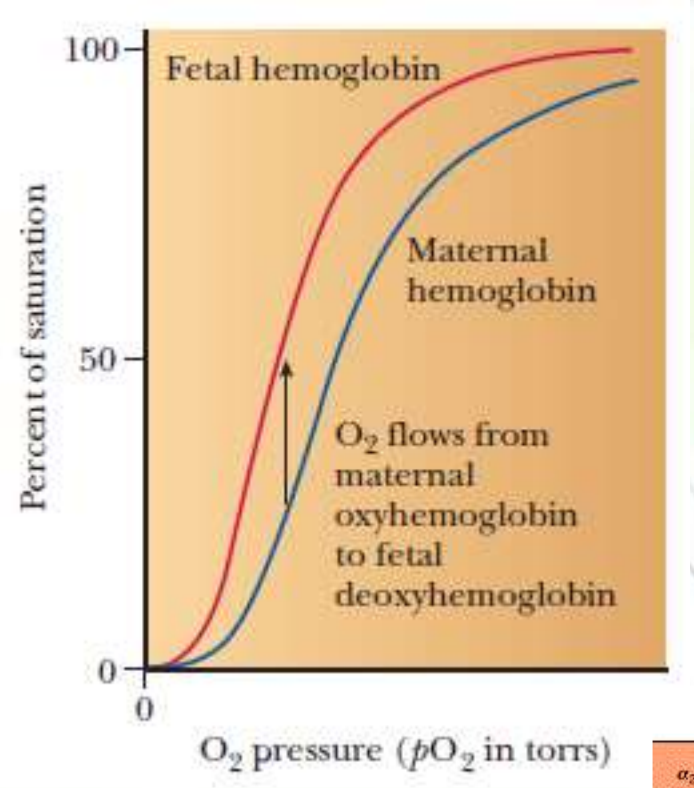
- An increase in temperature decreases oxygen affinity and therefore increases the P50.
- Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading from HbO_2.

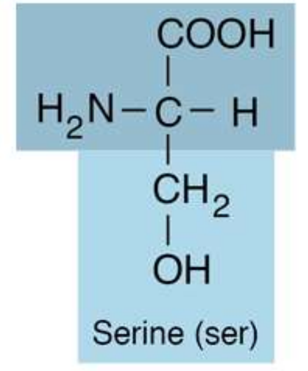


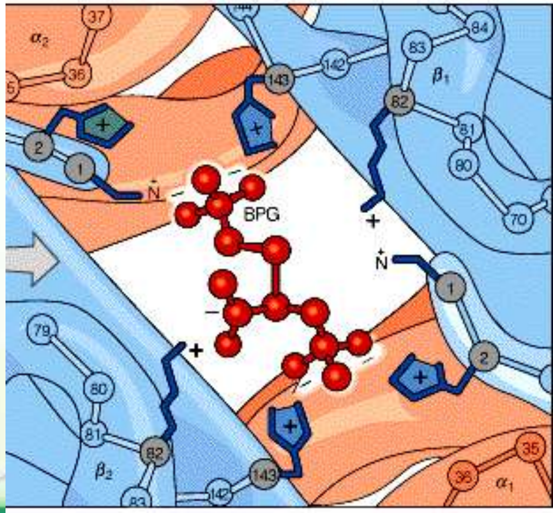
Other considerations

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.





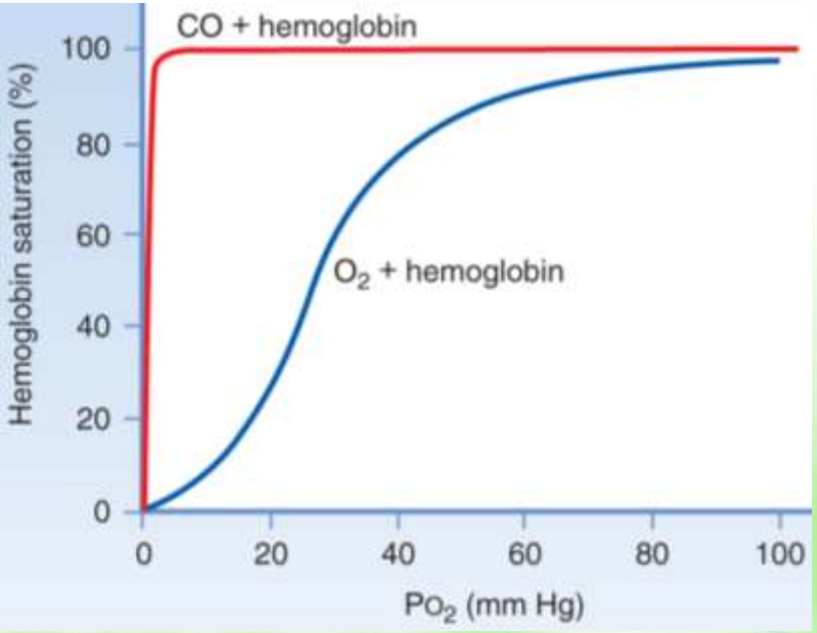


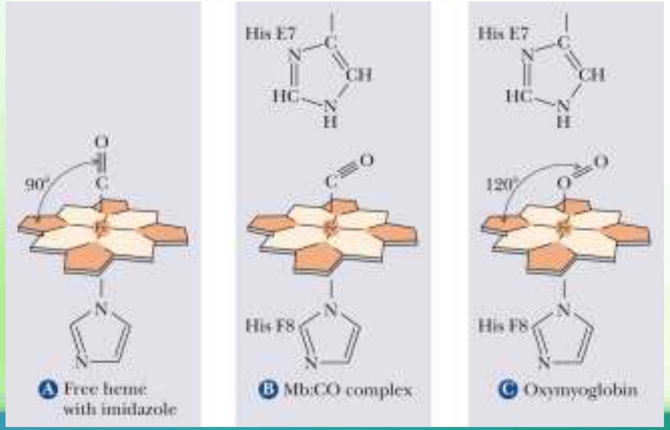
Effect of CO

Effect of CO

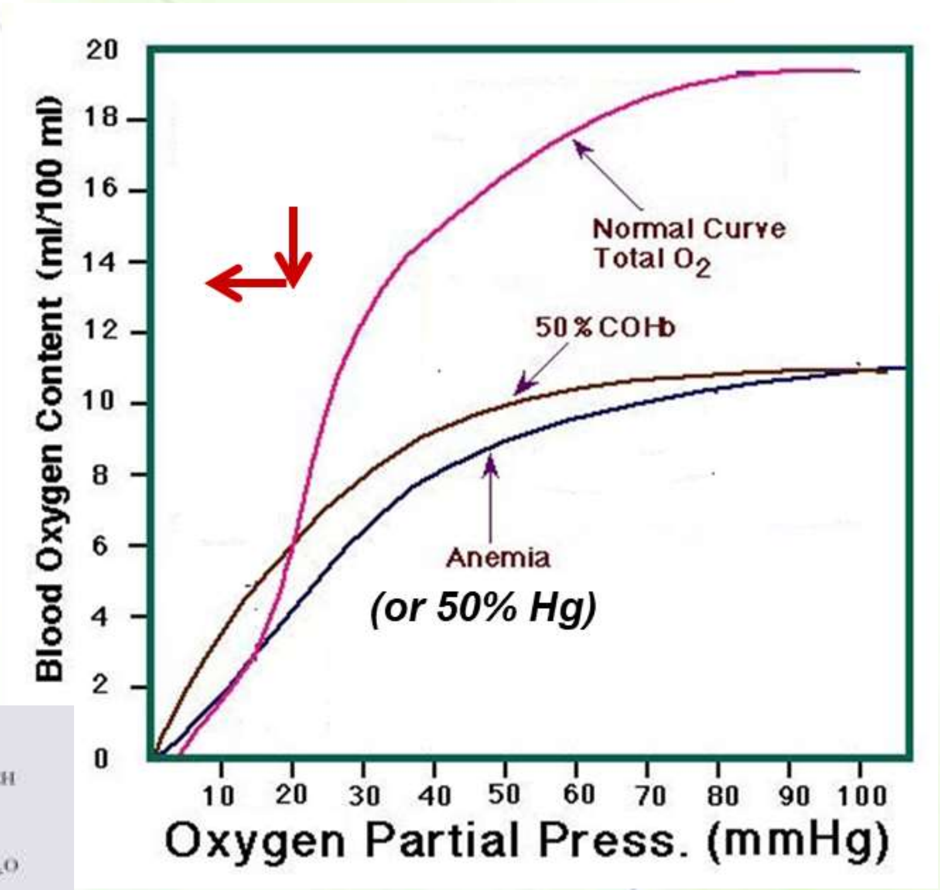
- In addition to competing with oxygen in binding to hemoglobin, the affinity of Hb-CO towards oxygen <u>increases</u> resulting in less oxygen <u>unloading</u> in peripheral tissues.

<u>($Hb + O_2$) versus ($Hb + CO$)</u>





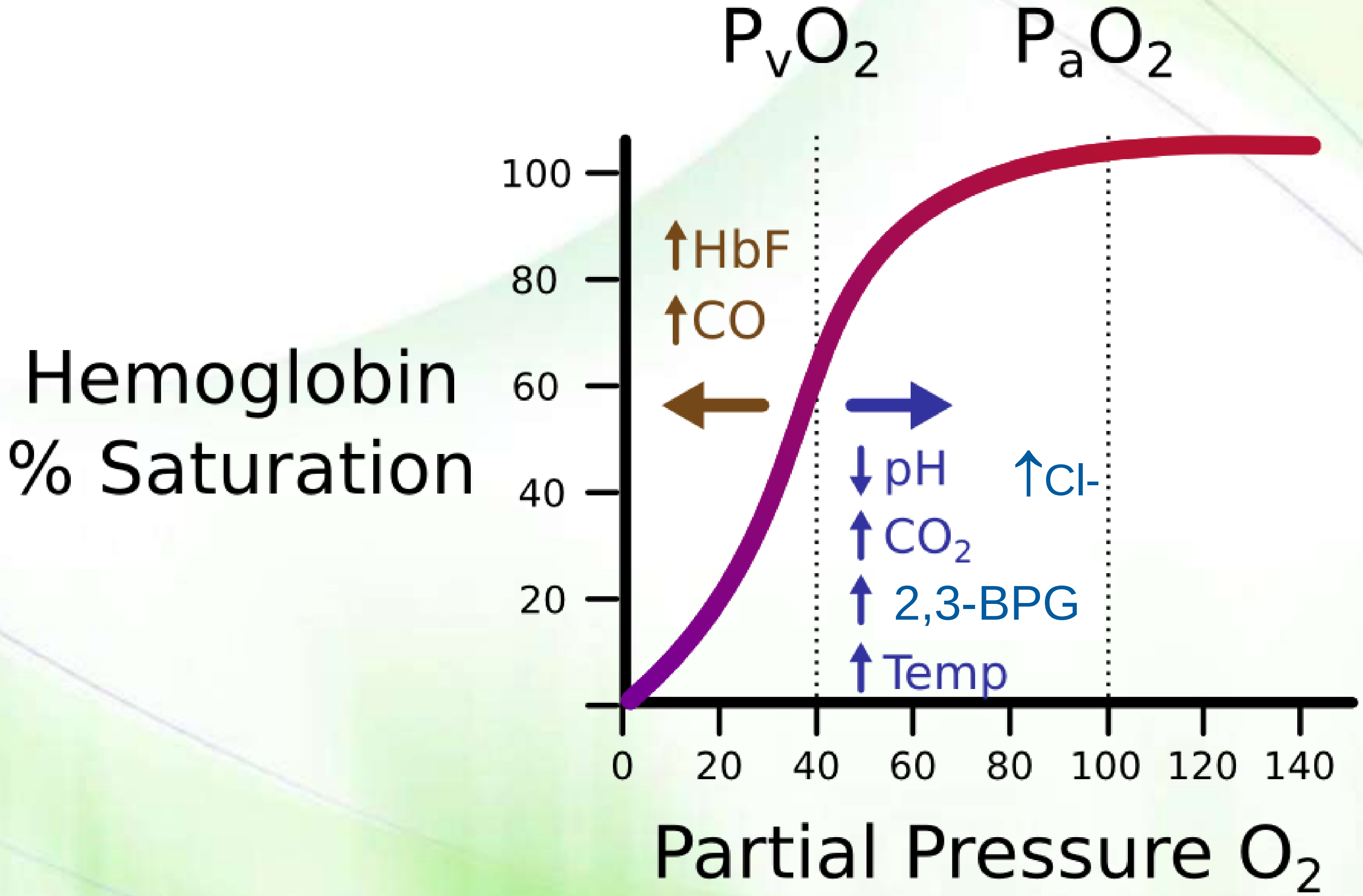
<u>($Hb + O_2$) versus ($Hb + O_2 + CO$)</u>



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



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