Regulation of hemoglobin function

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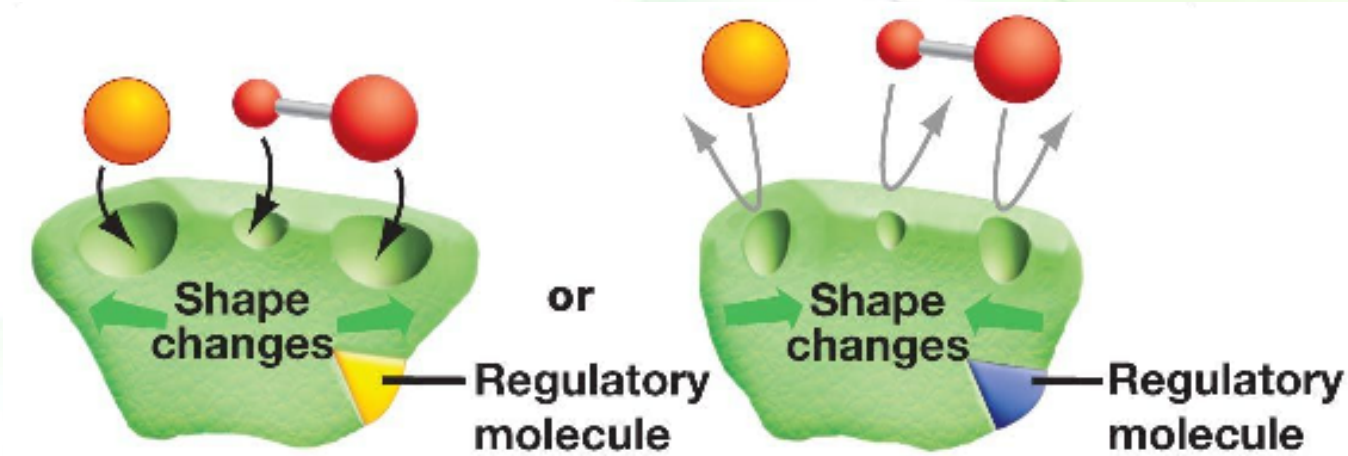
Hematopoietic-lymphatic system
2020

Allosteric regulation



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

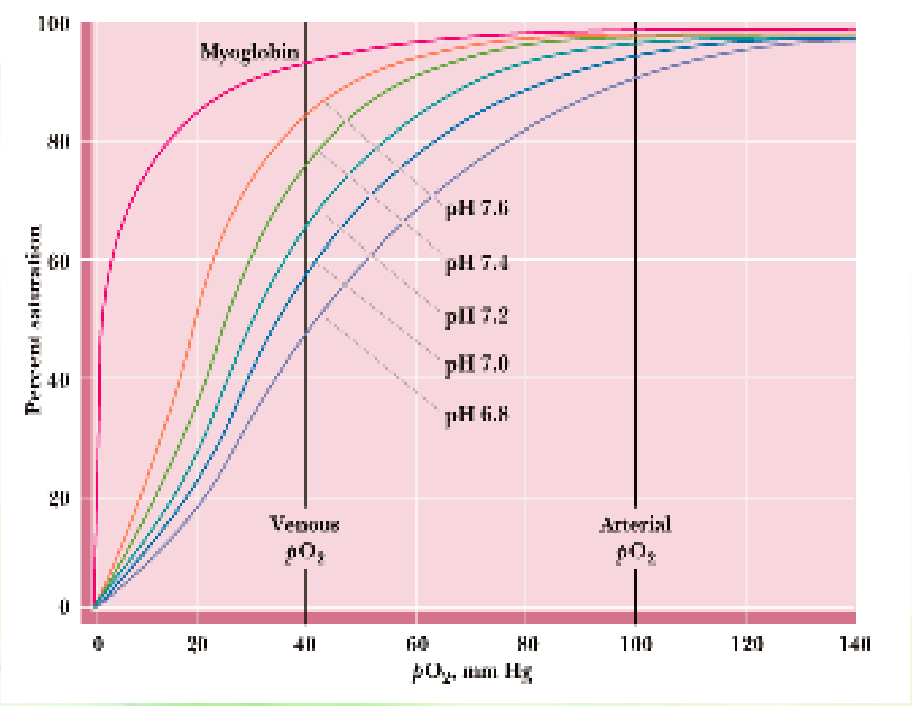
Allosteric deactivation
The active site becomes unavailable to the substrates when a regulatory molecule binds to a different site on the enzyme.

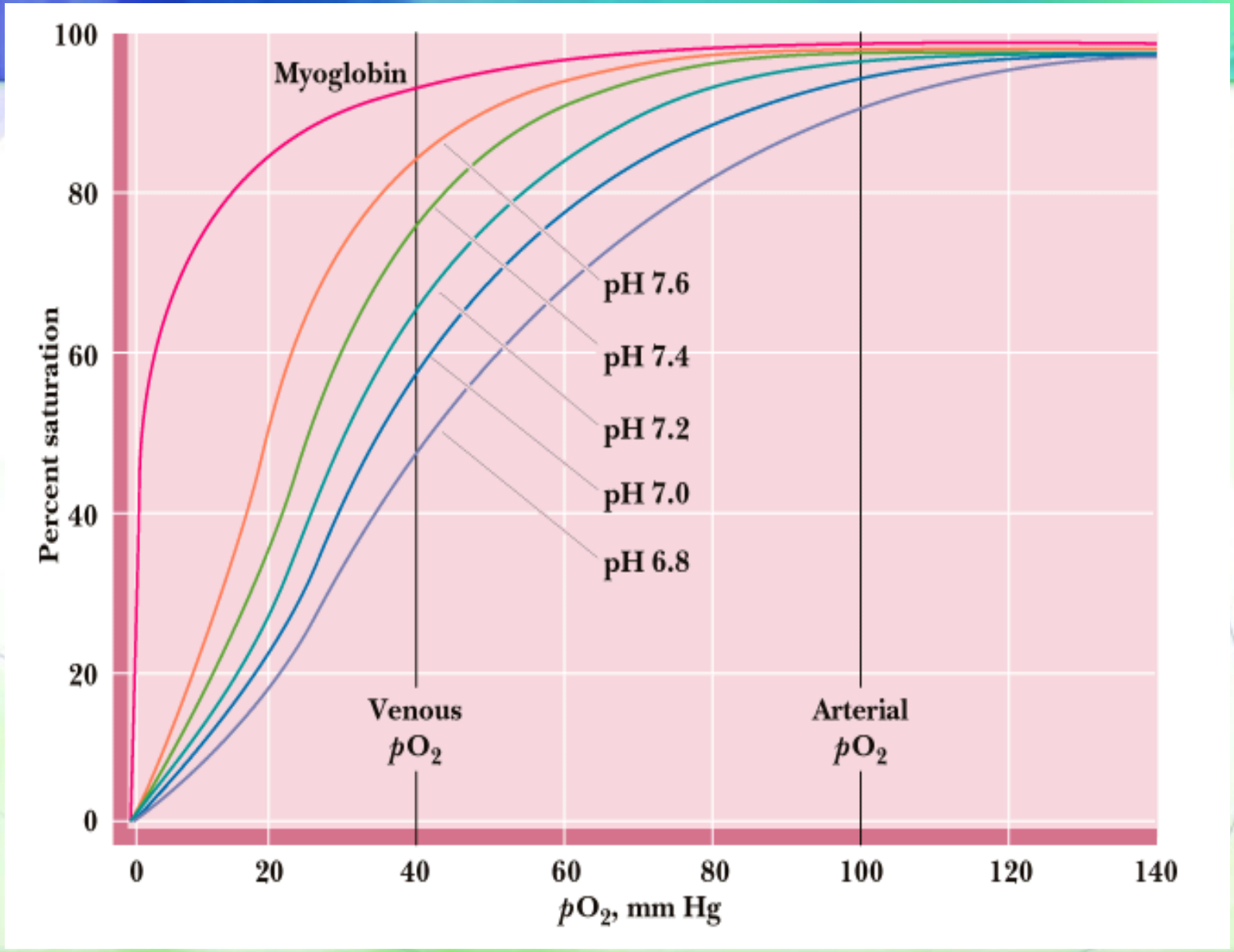
Allosteric effectors

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

The effect of pH

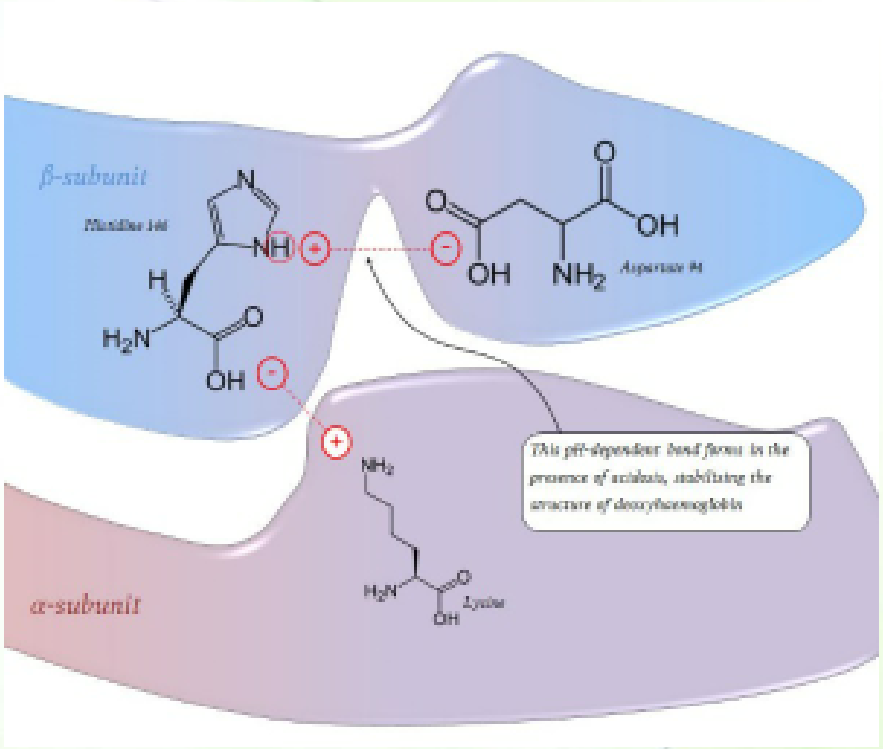
- The binding of H+ to hemoglobin promotes the release of O_2 from hemoglobin and vice versa.
- This phenomenon is known as the Bohr effect.




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+ causes the protonation of key amino acids, including the last histidine residue of the β chains (His146).
- The protonated histidine is attracted to, and stabilized by, a salt bridge to an aspartate within the same chain.
  - The pKa of His146 is reduced from 7.7 in the R state to 7.3 in the T state allowing for protonation.
- Electrostatic interaction also occurs between the carboxylic group of His146 and a lysine of the α chain.
- This favors the deoxygenated form of hemoglobin.



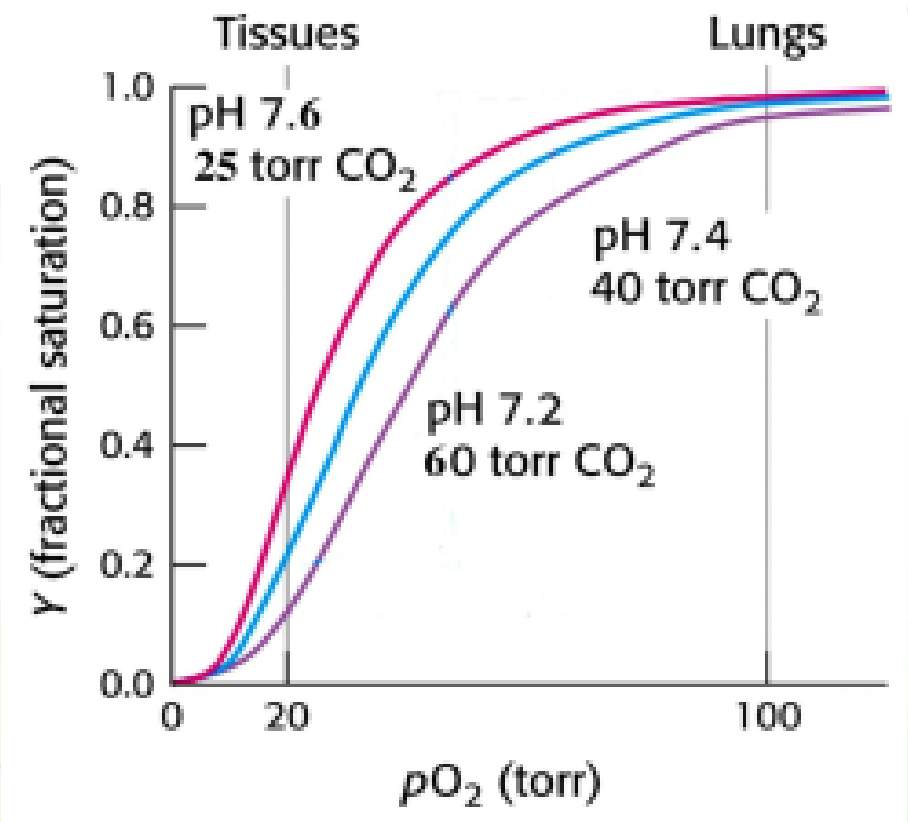
Where do protons come from?

- 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

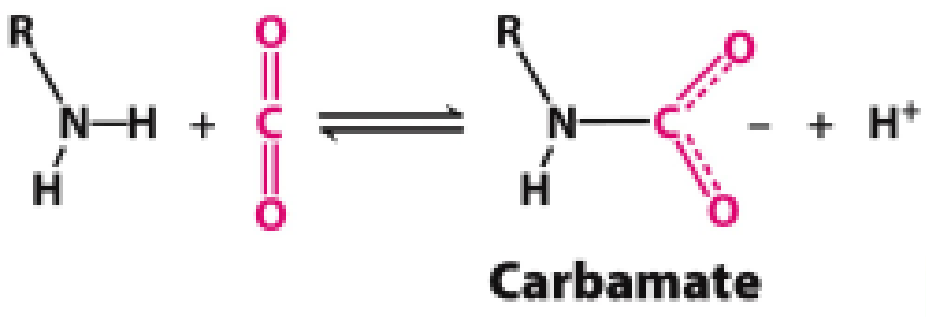
(Mechanism #1 - production of protons)

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



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



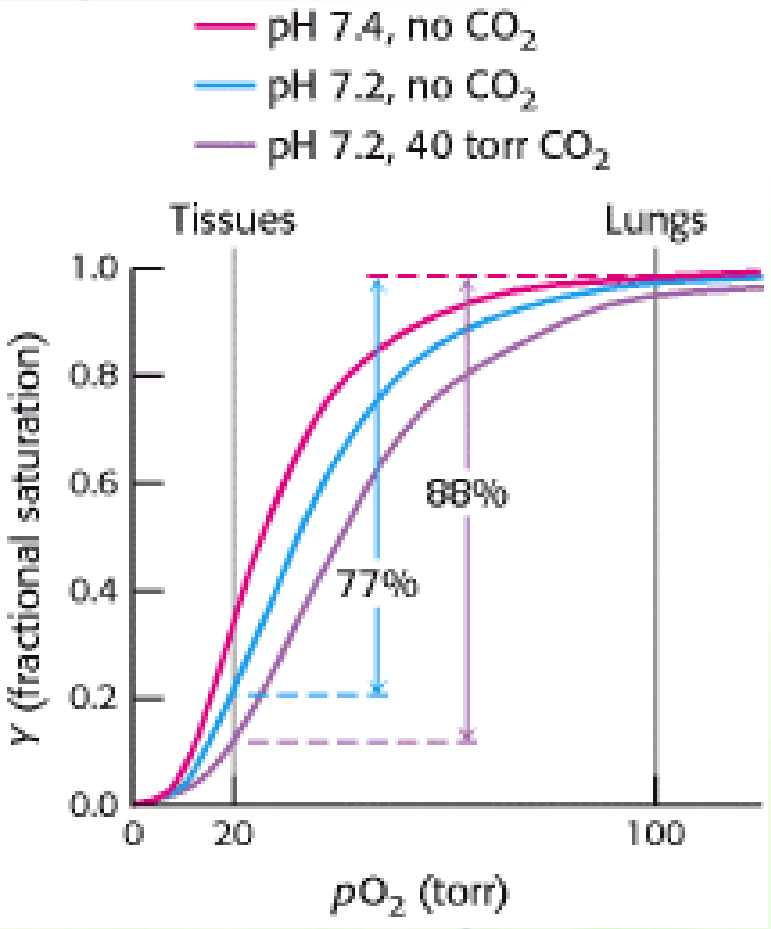
Carbamate

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

Contribution of both mechanisms

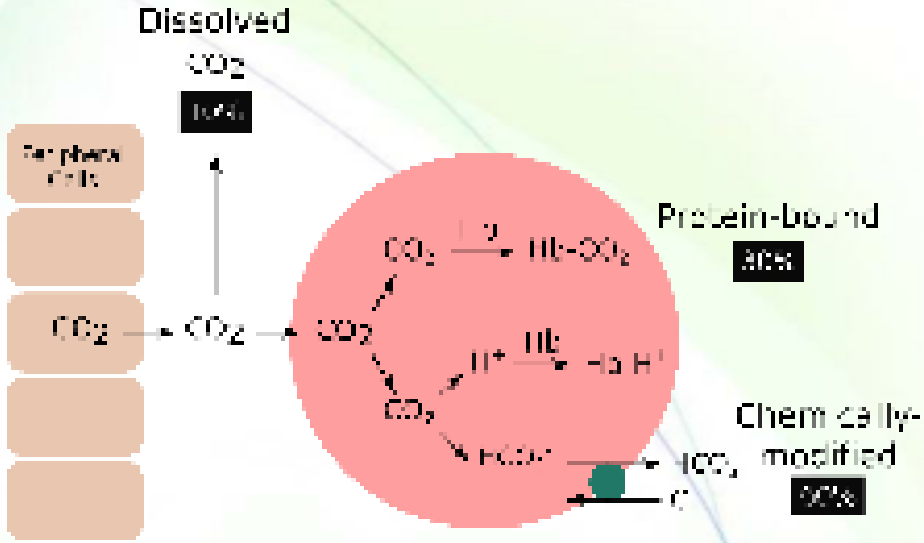
- About 75% of the shift is caused by H+.
- About 25% of the effect is due to the formation of the carbamino compounds.

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.

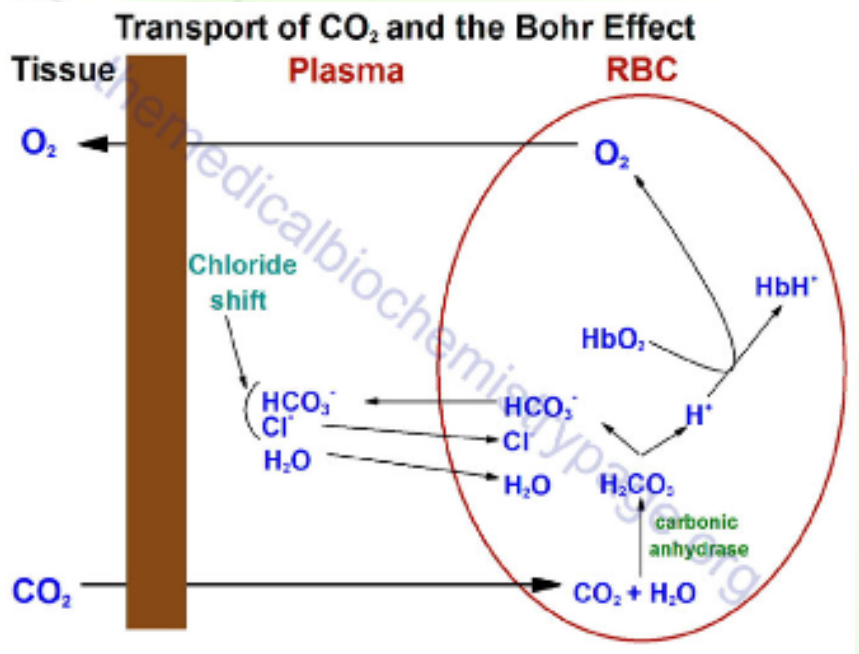


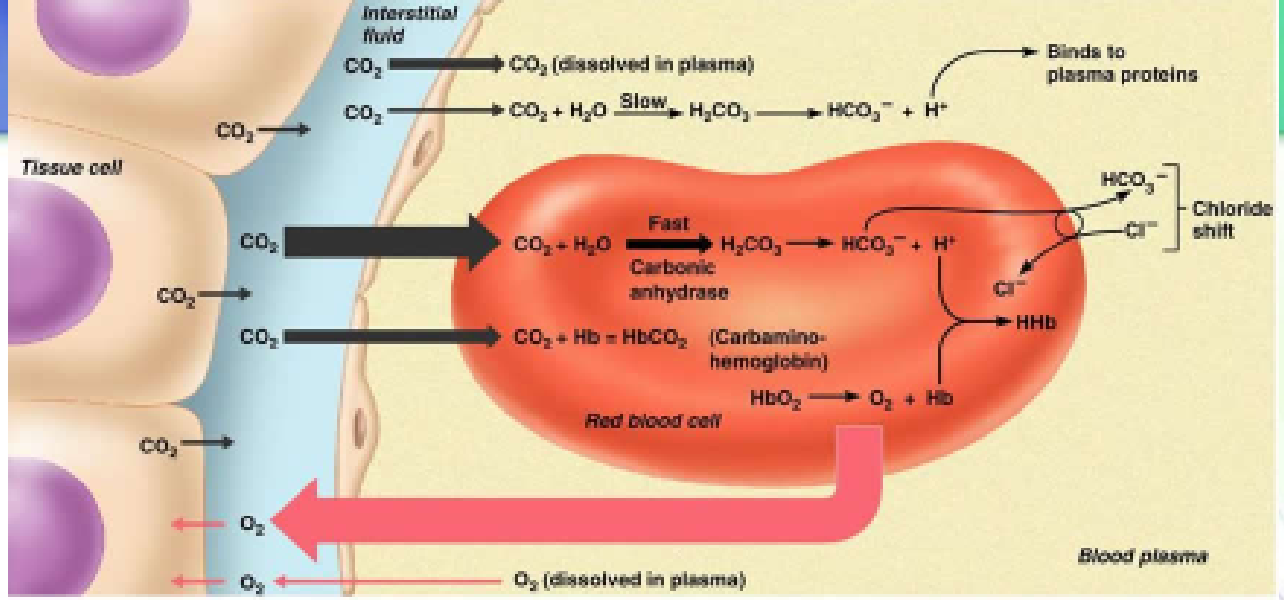
The movement of CO_2 in/out of cells does not change the pH, a phenomenon called isohydric shift, which is partially a result of hemoglobin being an effective buffer.

Other allosteric effectors

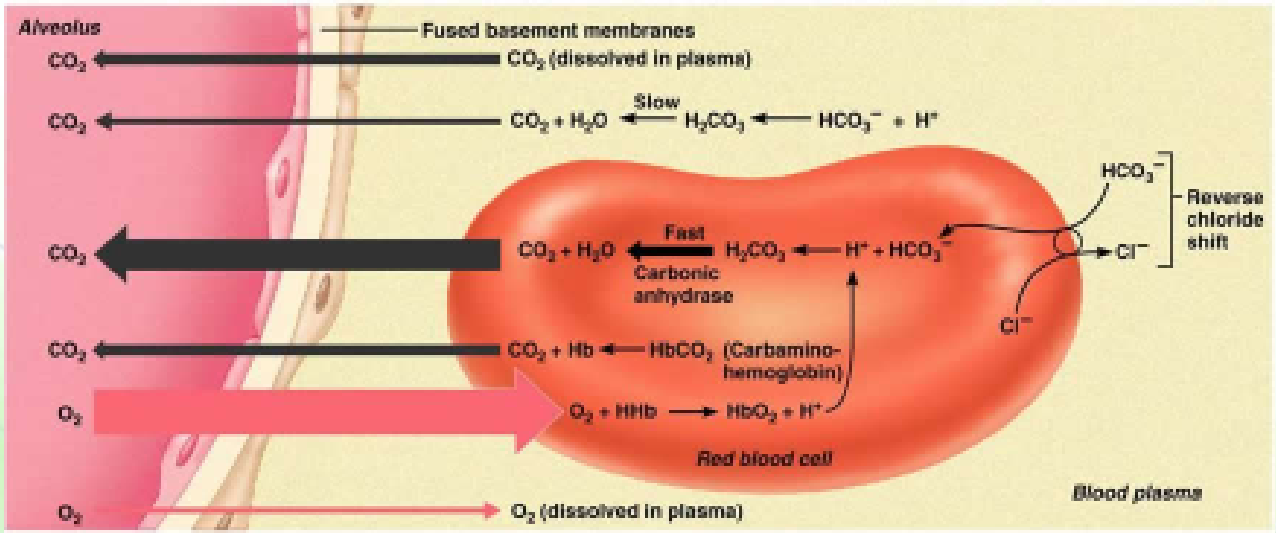
Chloride shift

- 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“.





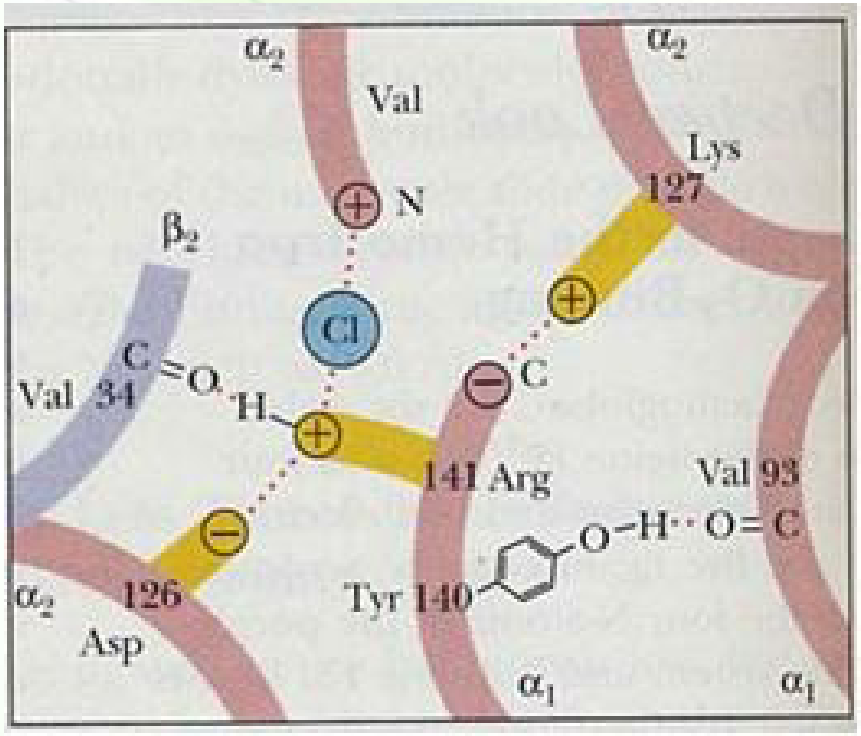
(a) Oxygen release and carbon dioxide pickup at the tissues



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

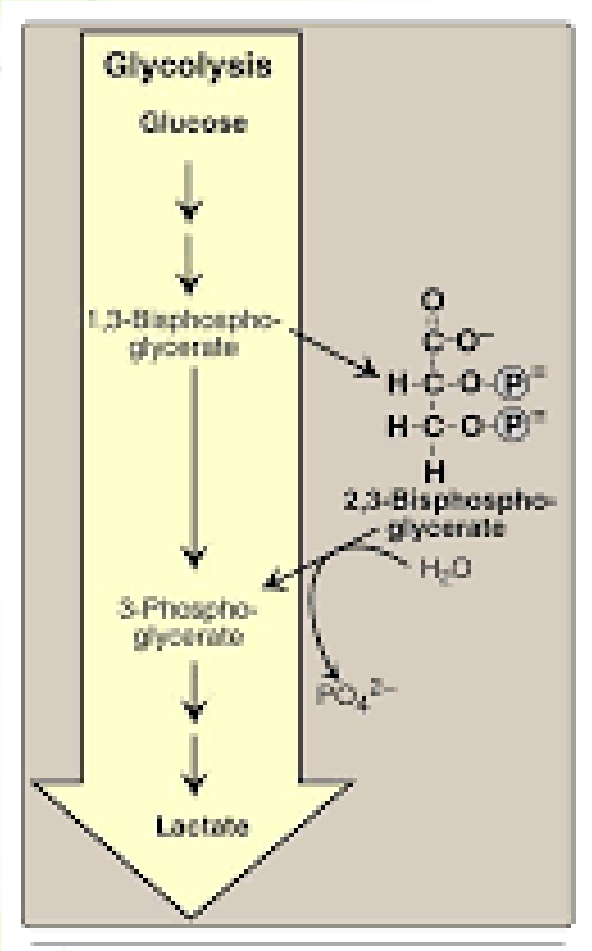
Effect of chloride ions

- 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)



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

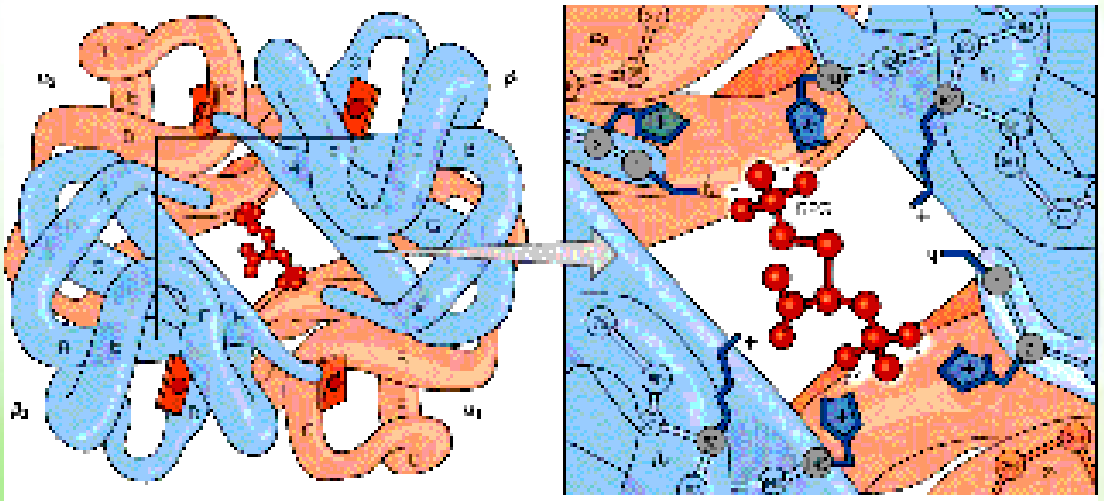
- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- BPG binds to hemoglobin and reduces its affinity towards oxygen.



BPG –hemoglobin interaction

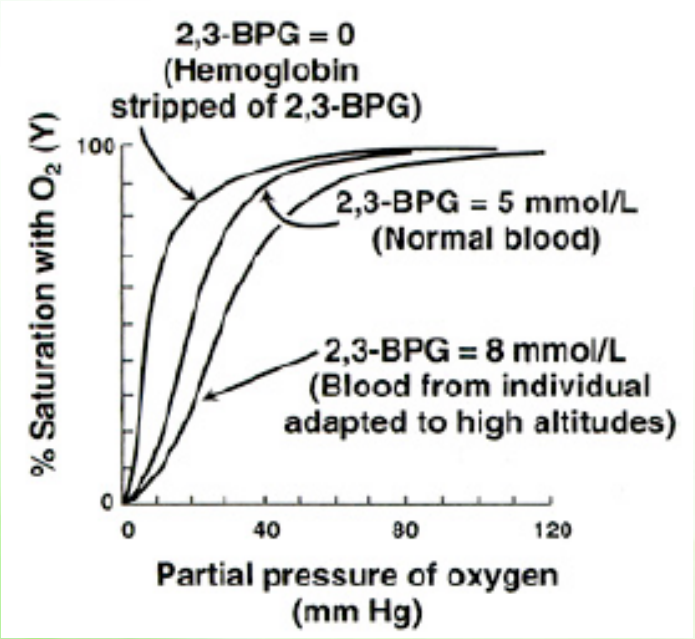
- BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 BPG/hemoglobin tetramer.
- This binding increases the energy needed to transform hemoglobin from the T state to R state.
- Bound, 2,3-BPG reduces binding of oxygen to hemoglobin and facilitates oxygen release.

BPG forms salt bridges with the terminal amino groups of both β chains and with a lysine and a histidine.



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.



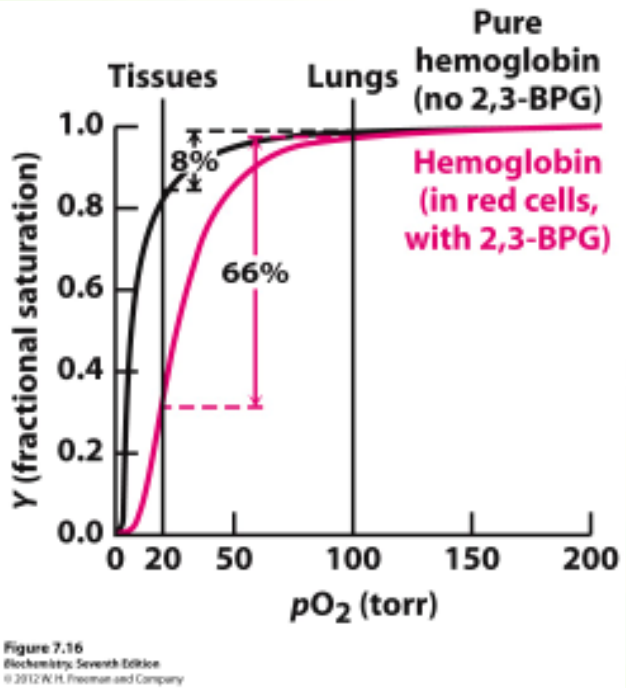


Figure 7.16
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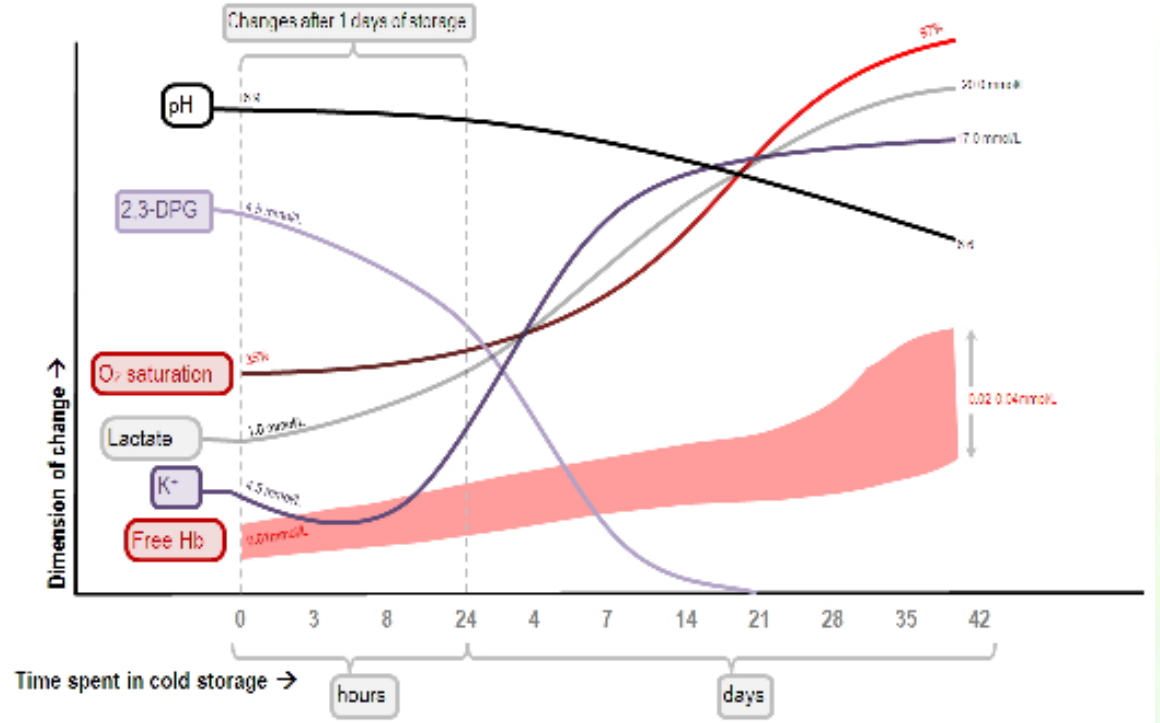
2,3-BPG in transfused blood

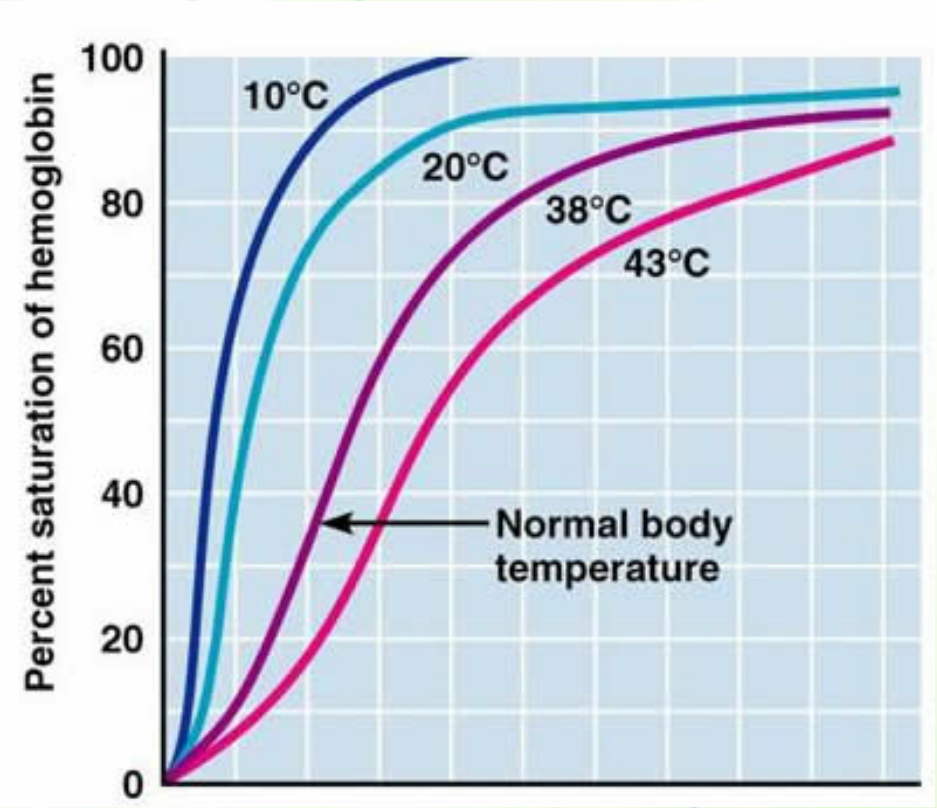
- Storing blood results in a decrease in 2,3-PBG (and ATP), hence hemoglobin acts as an oxygen “trap”, not an oxygen transporter.
- Transfused RBCs are able to restore their depleted supplies of 2,3-BPG in 6–24 hours.

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



Effect of temperature

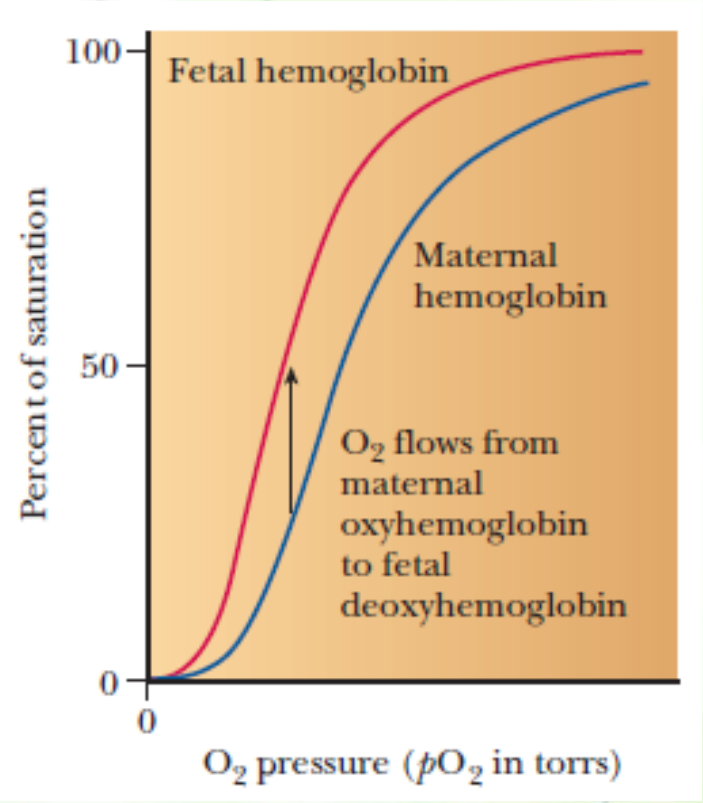
- An increase in temperature decreases oxygen affinity and therefore increases the P50.
- Temperature affects the O_2 binding of both myoglobin and hemoglobin.
- Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading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



Other considerations

Fetal hemoglobin

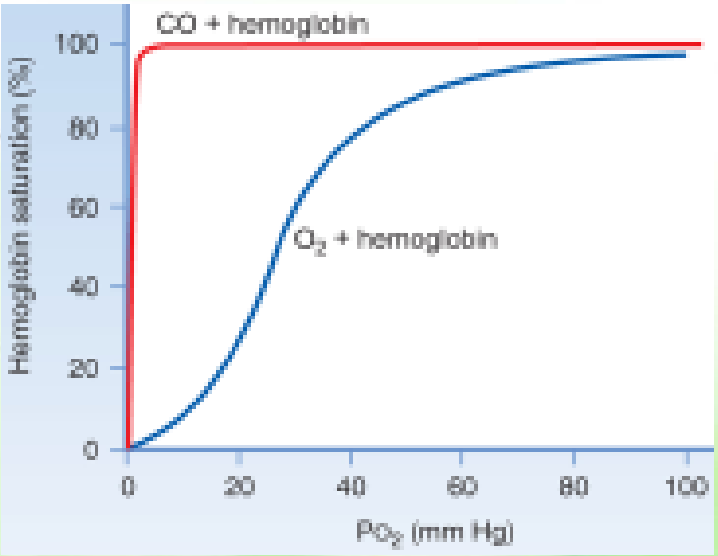
- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HBA).
  - HbA = $\alpha 2 \beta 2$
  - HbF = $\alpha 2 \gamma 2$
- A His residue in the β subunit is replaced by a Ser in the γ subunit of HbF.
  - Sibridge, 2,3-BPG nce Ser cannot form a salt binds more weakly to HbF than to HbA.



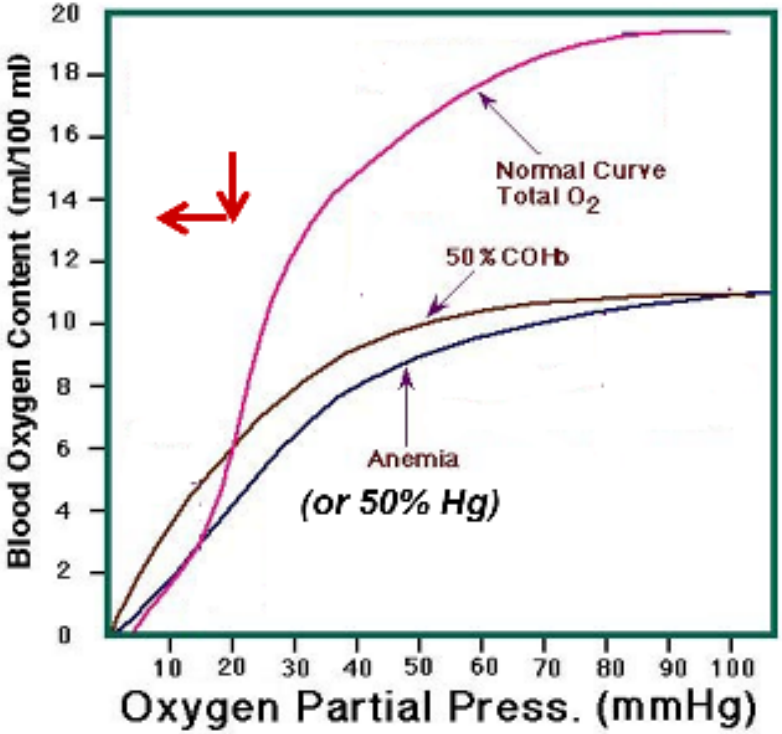
Effect of CO

- 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 $(Hb + CO)$

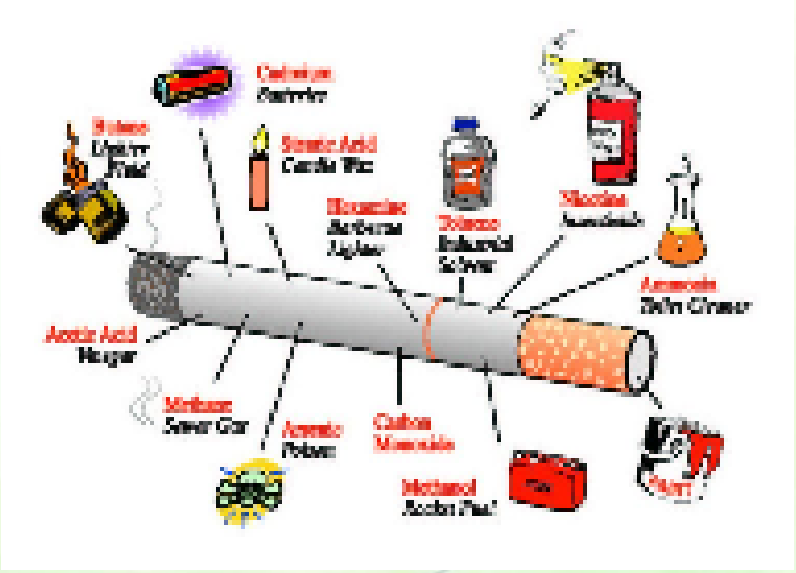


$(Hb + O_2)$ versus $(Hb + O_2 + CO)$

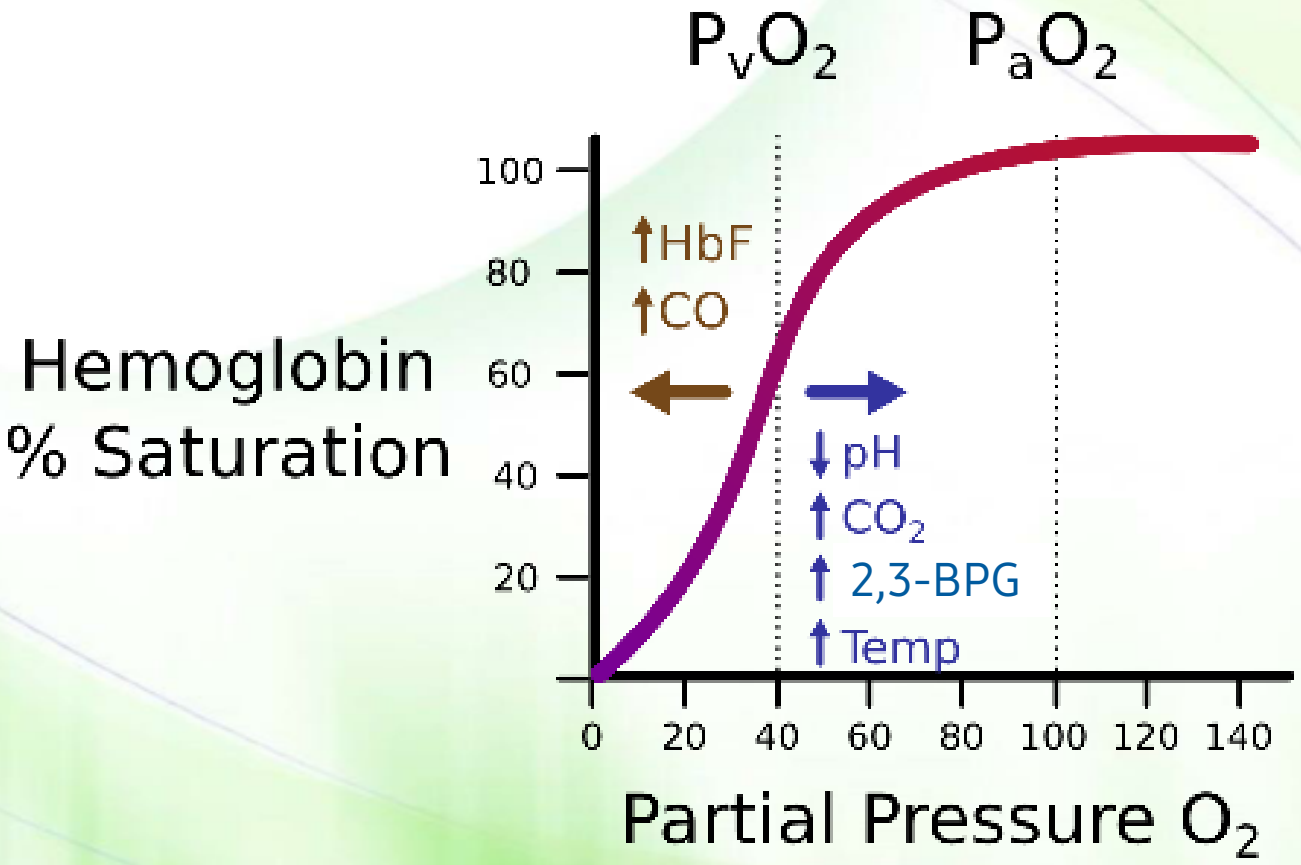


Relevant information

- 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


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