# Regulation of hemoglobin function

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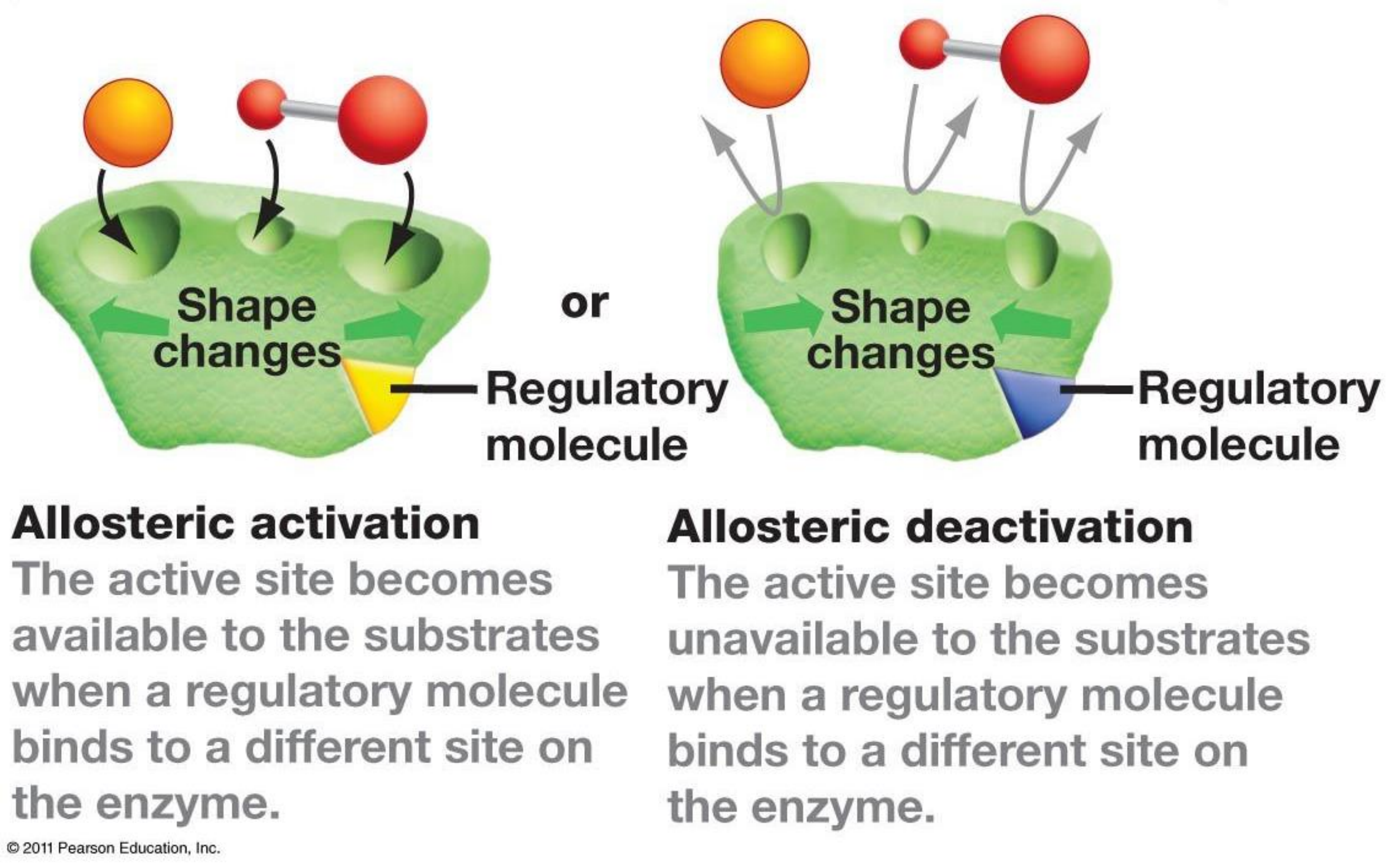
Hematopoietic-lymphatic system

# Allosteric regulation



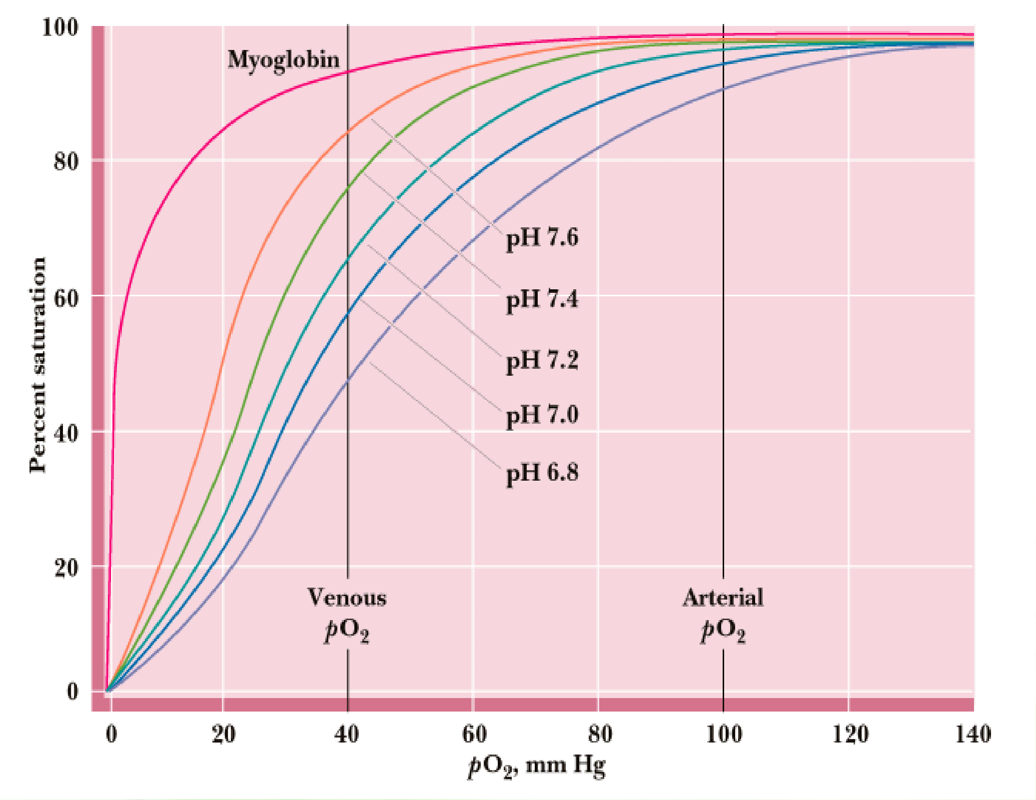
# Allosteric effectors

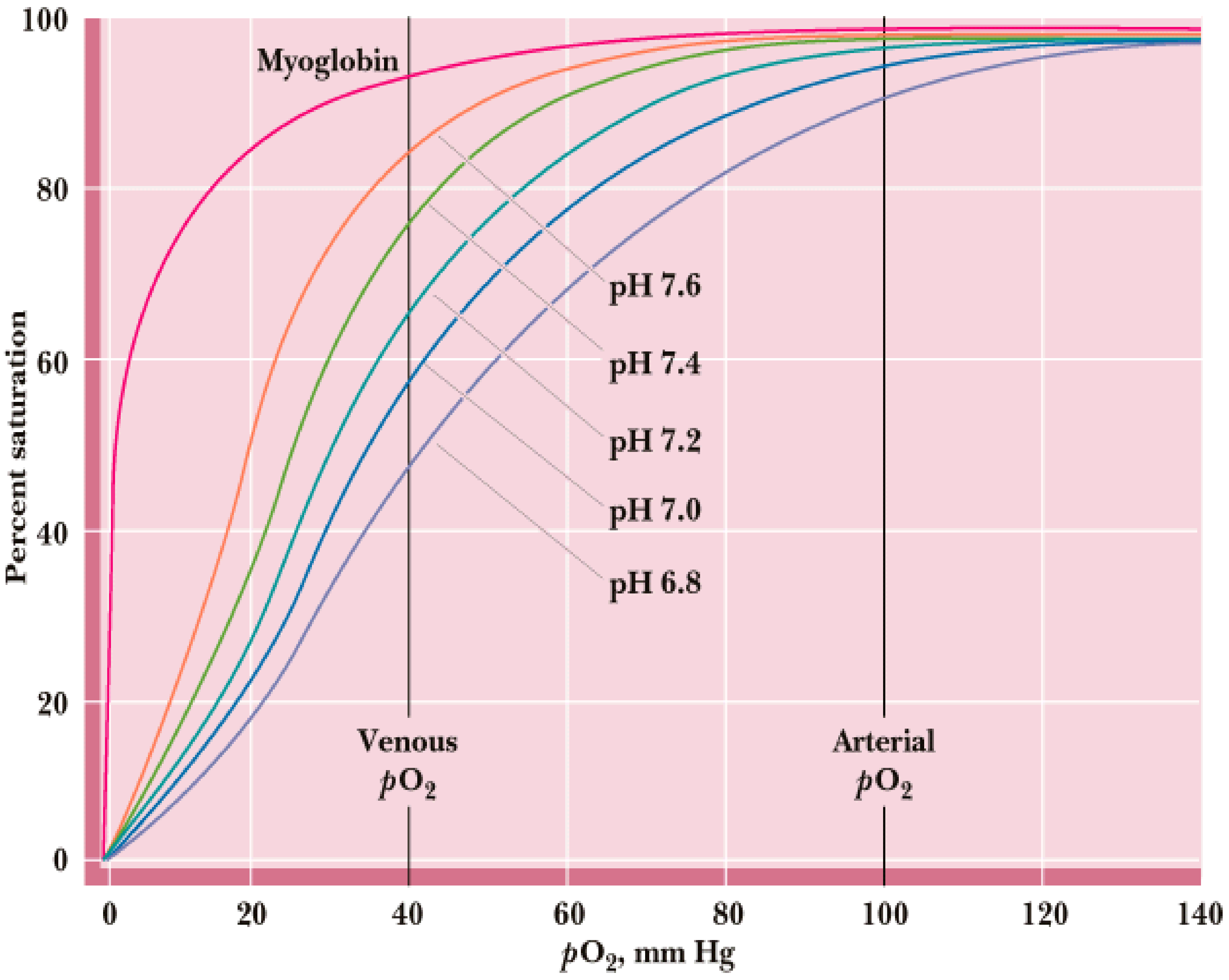
- The major heterotropic effectors of hemoglobin
  - Hydrogen ion,
  - Carbon dioxide
  - 2,3-Bisphosphoglycerate
  - Chloride ions

- 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.

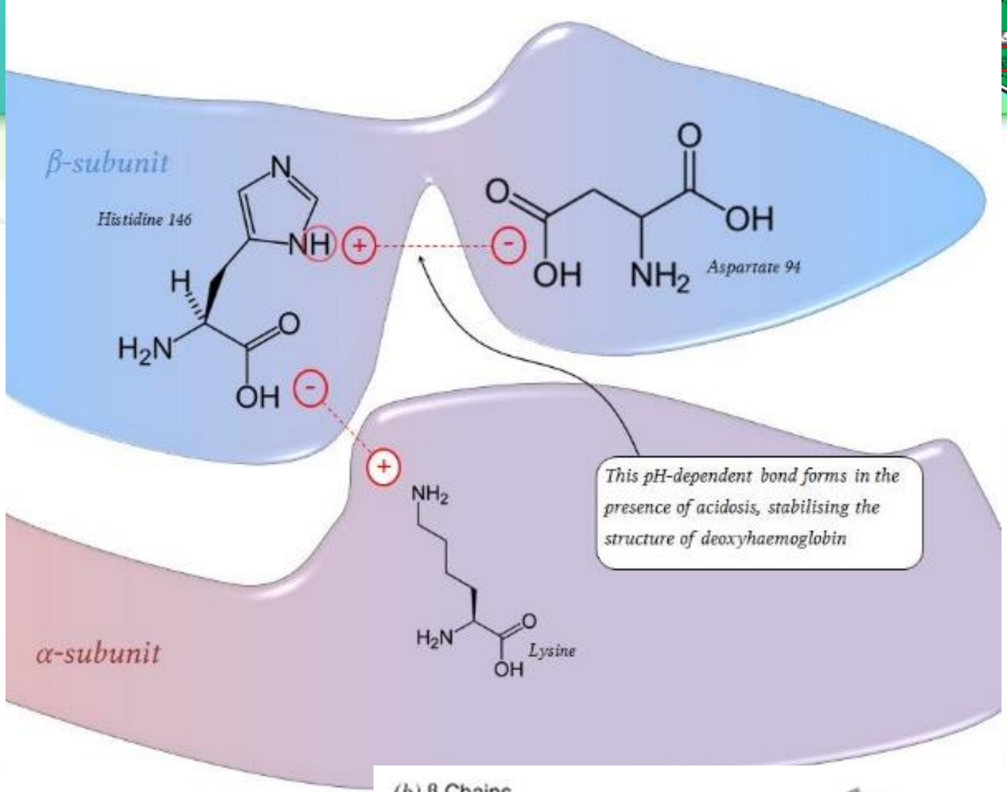


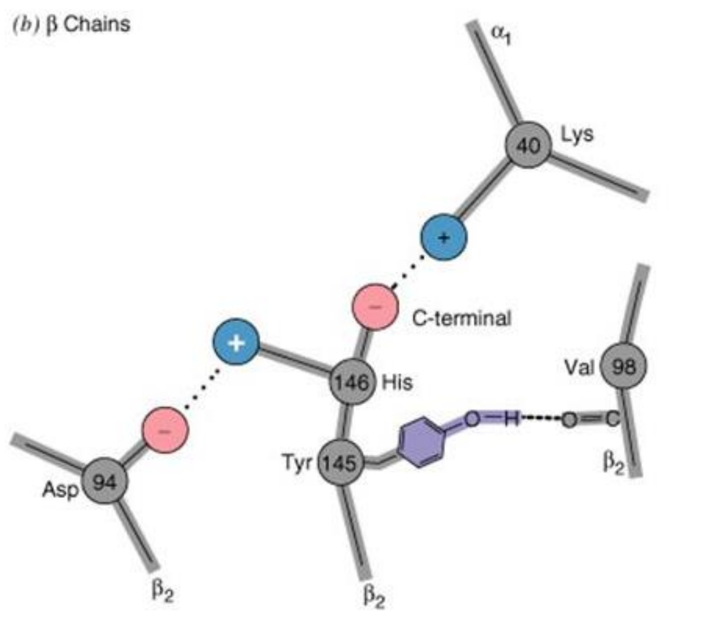


Myoglobin
pH 7.6
pH 7.4
pH 7.2
pH 7.0
pH 6.8
Venous
$pO_2$
Arterial
$pO_2$
Percent saturation
100
80
60
40
20
0
0
20
40
60
80
100
120
140
$pO_2$, mm Hg

# 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 form of hemoglobin.



# Where do protons come from?

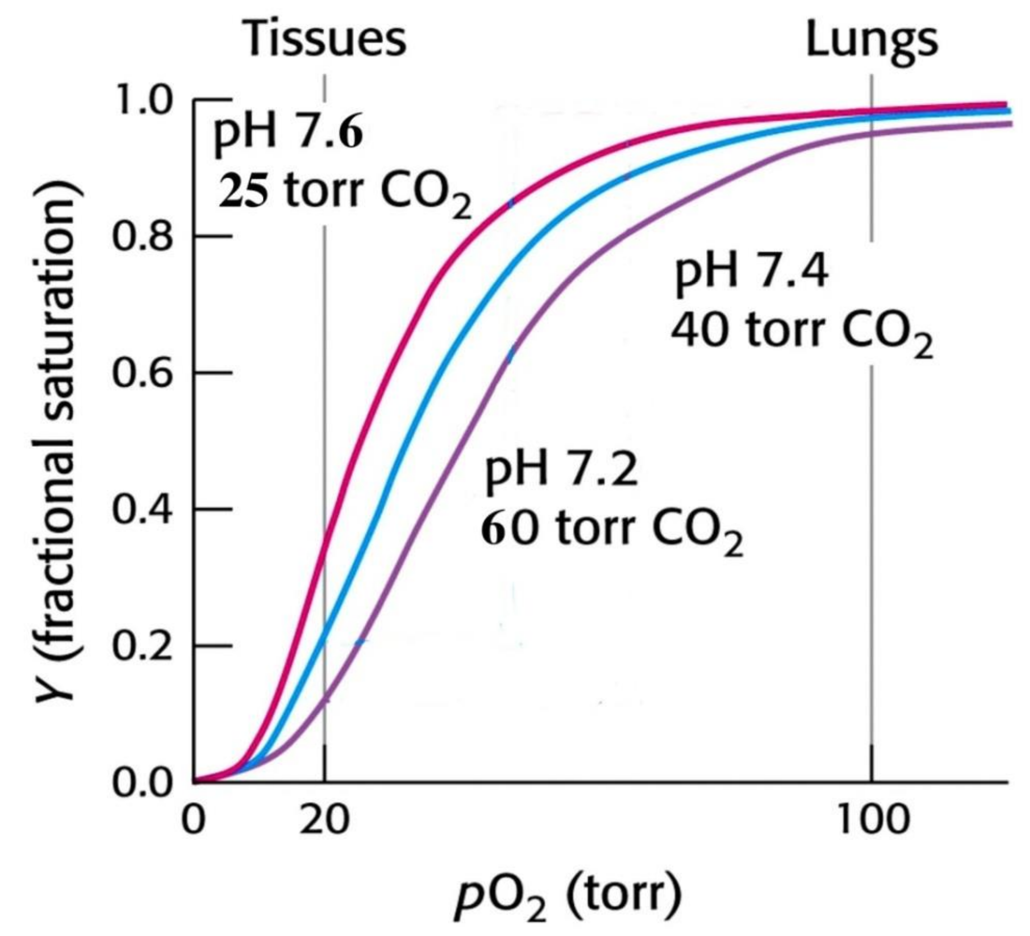
$$CO_2 + H_2O \Longleftrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$$

- $CO_2$ 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

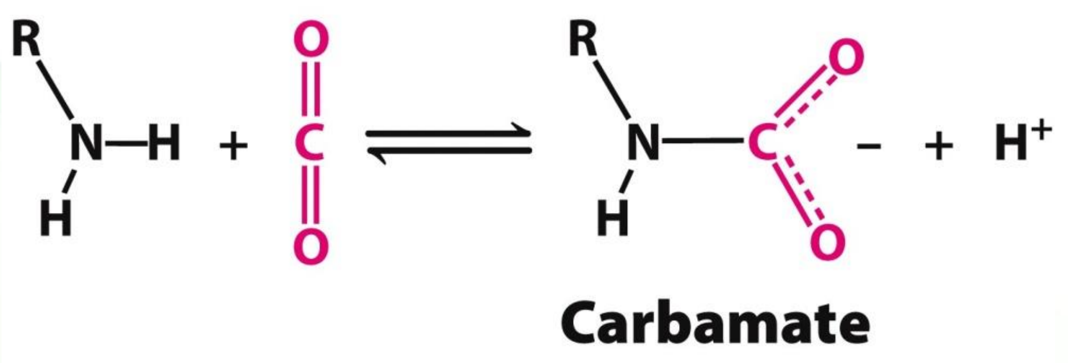
## (Mechanism #1 - production of protons)

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



# 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

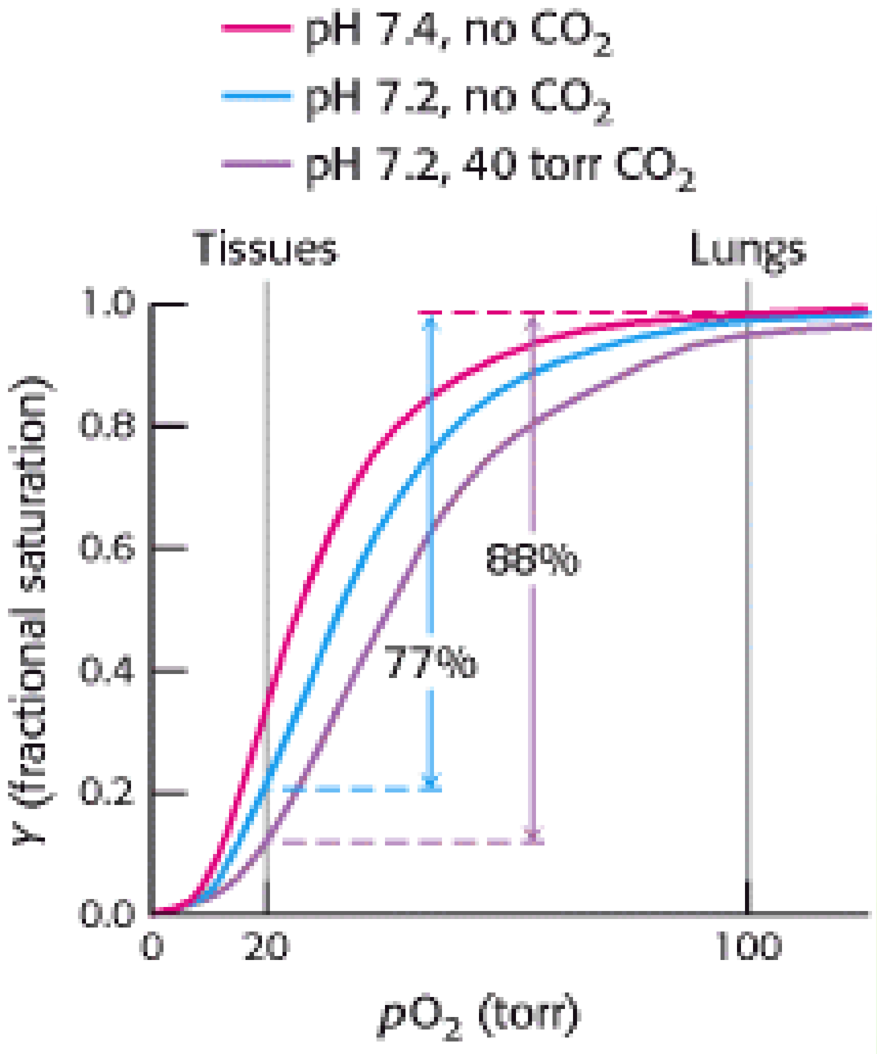



- The increased number of negatively-charged residues increases the number of electrostatic interactions that stabilize the T-state of hemoglobin.

# Contribution of both mechanisms

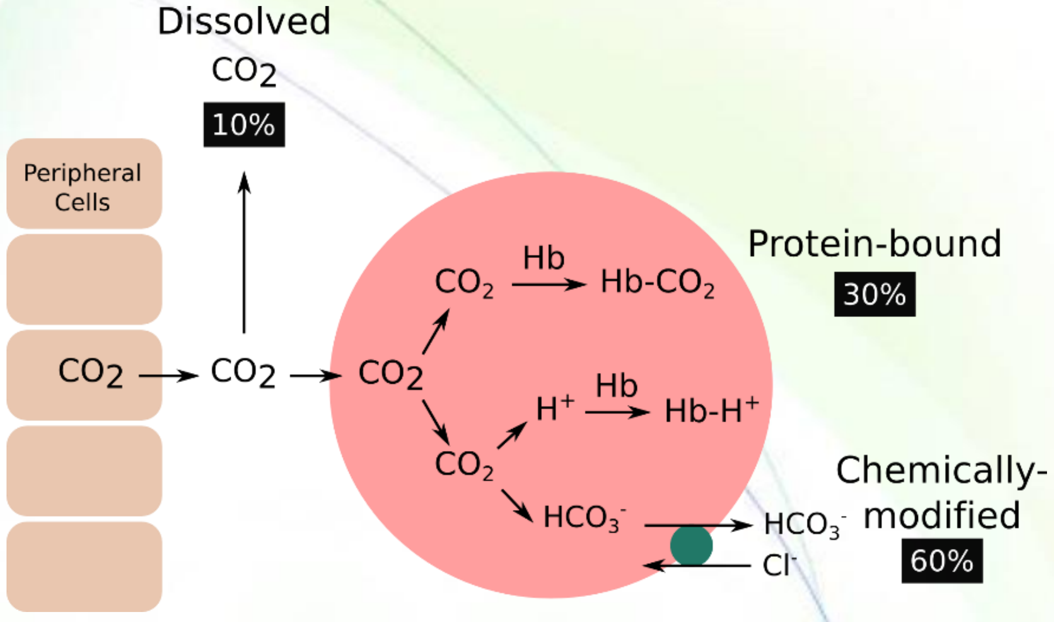
- About 75% of the shift is caused by H+.
- About 25% of the effect is due to the formation of the carbamino compounds.

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.

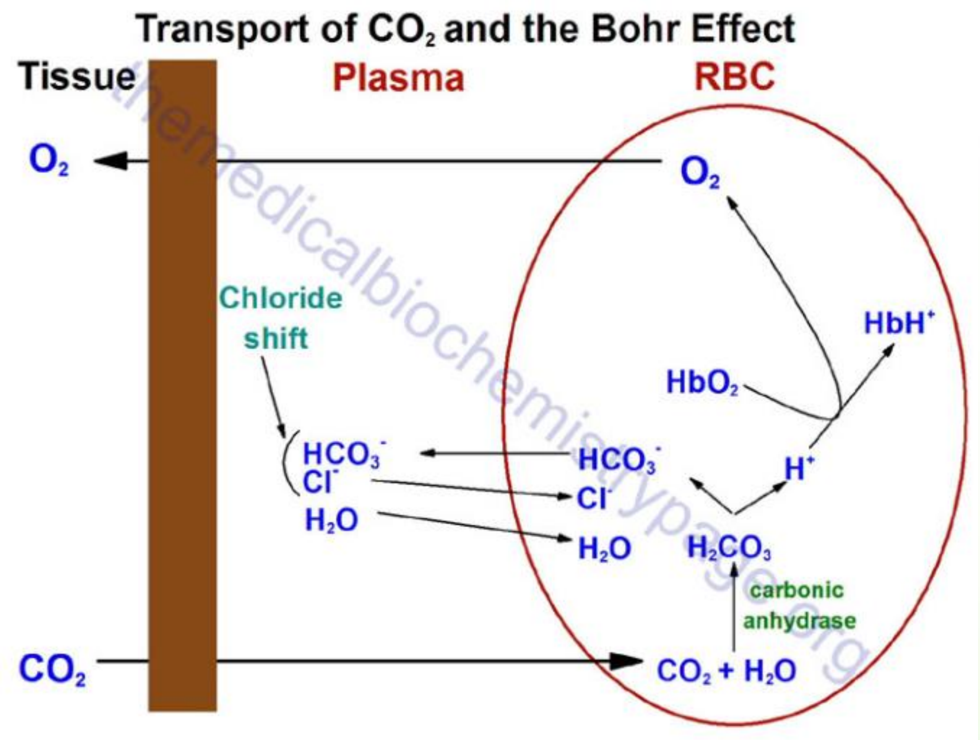


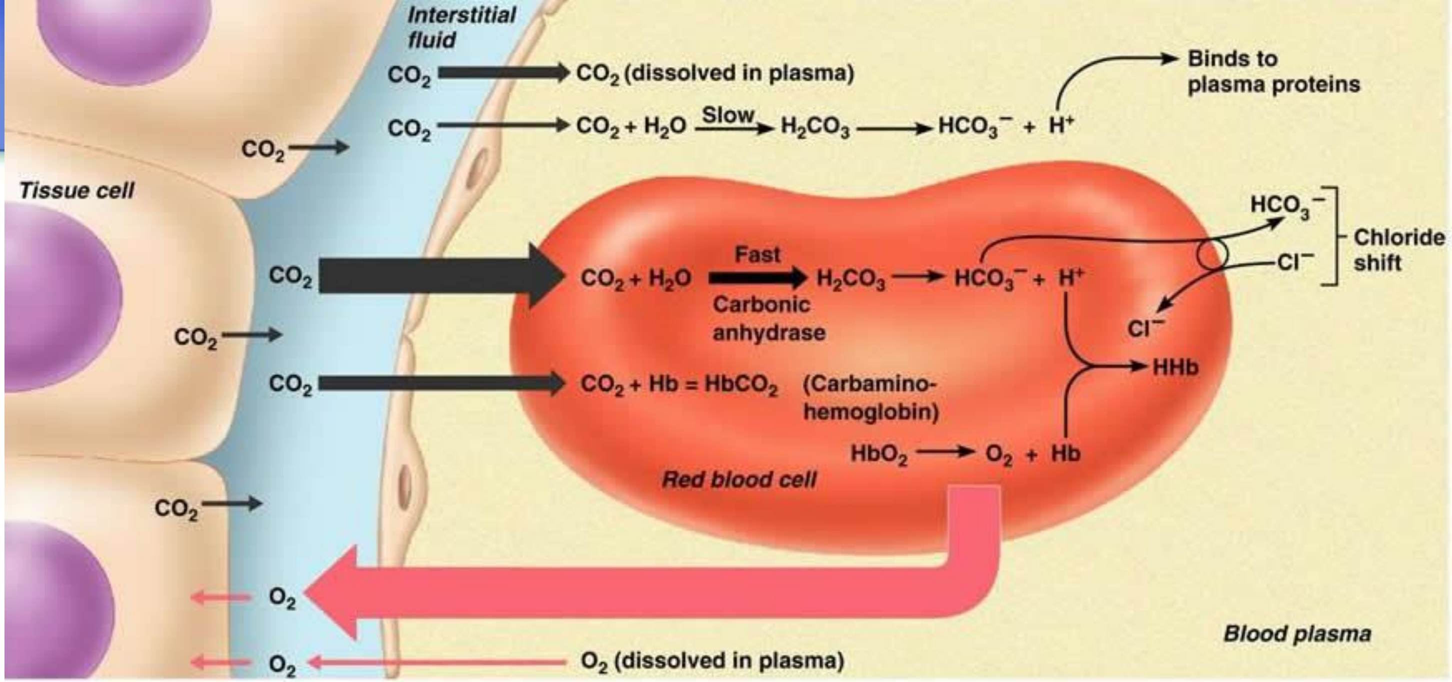

**The movement of $CO_2$ in/out of cells does not change the pH, a phenomenon called <u>isohydric shift</u>, which is partially a result of hemoglobin being an effective buffer.**

# Other allosteric effectors

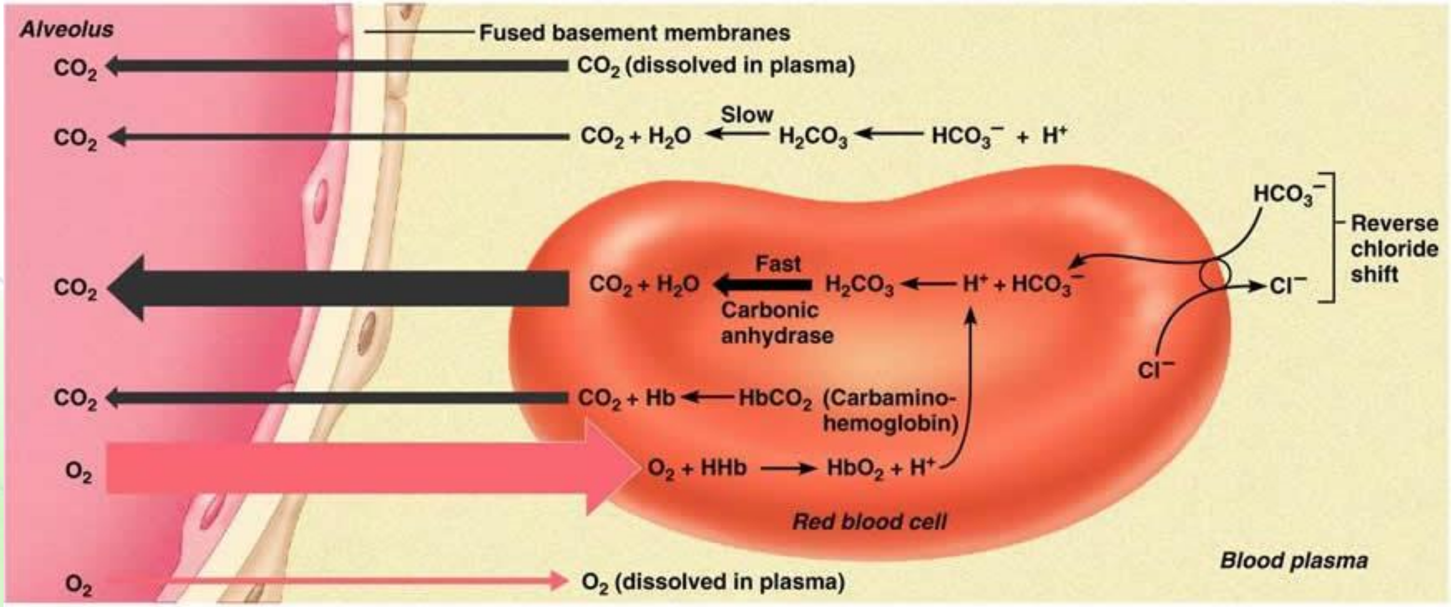
# Chloride shift

- 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".





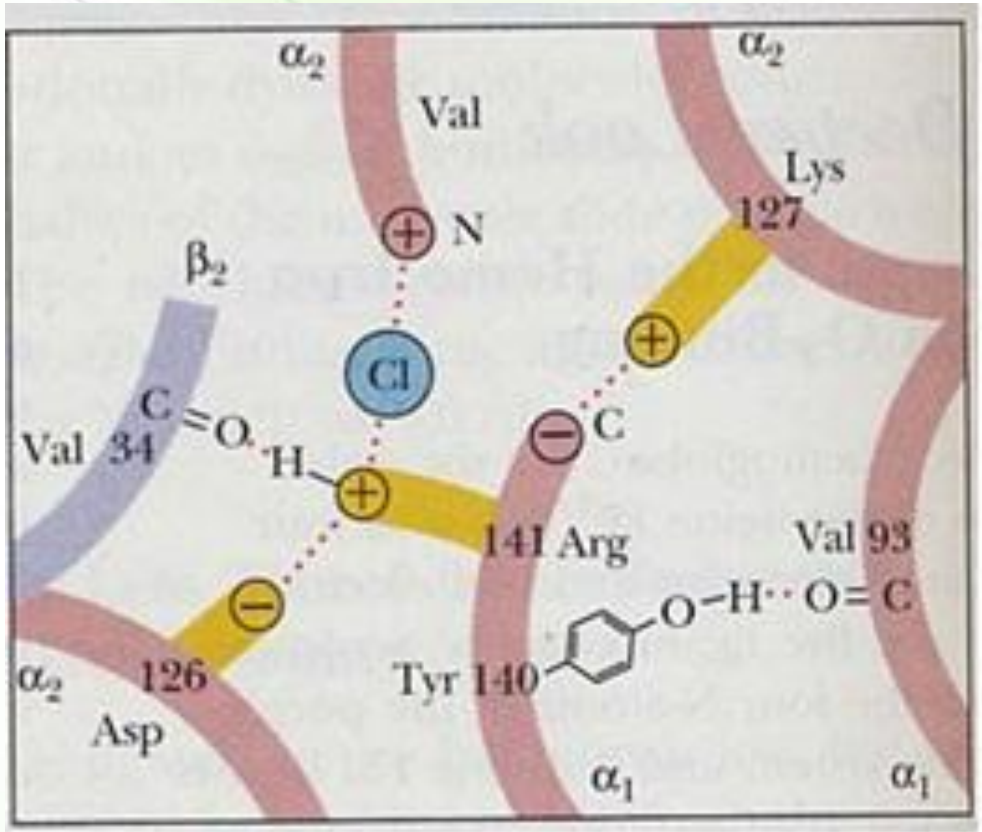

(a) Oxygen release and carbon dioxide pickup at the tissues




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

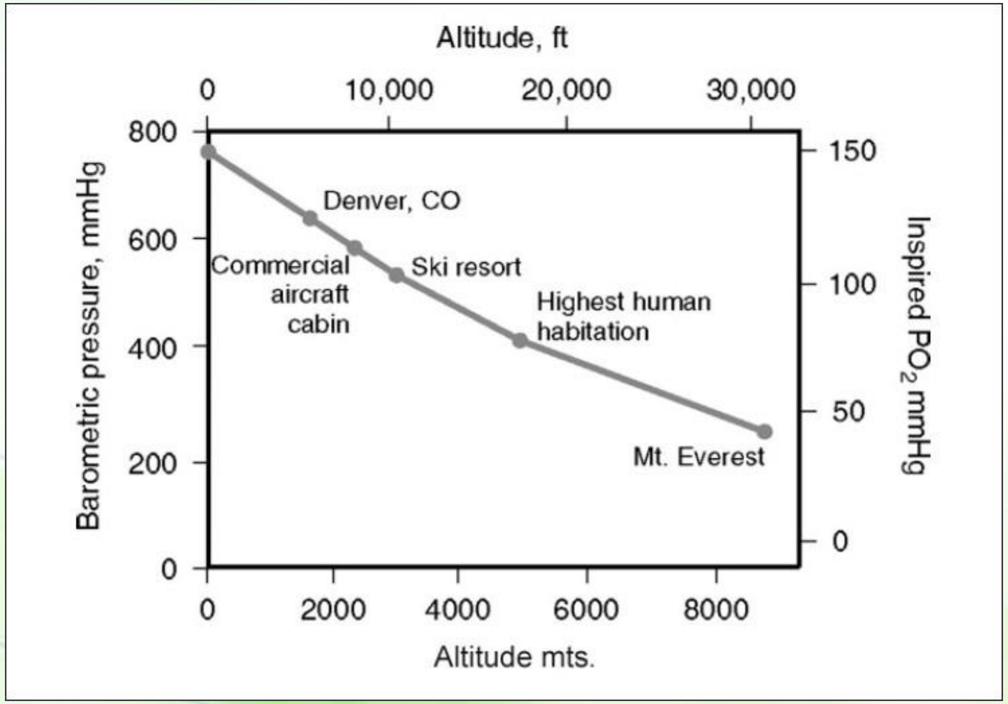
# Effect of chloride ions

- 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)



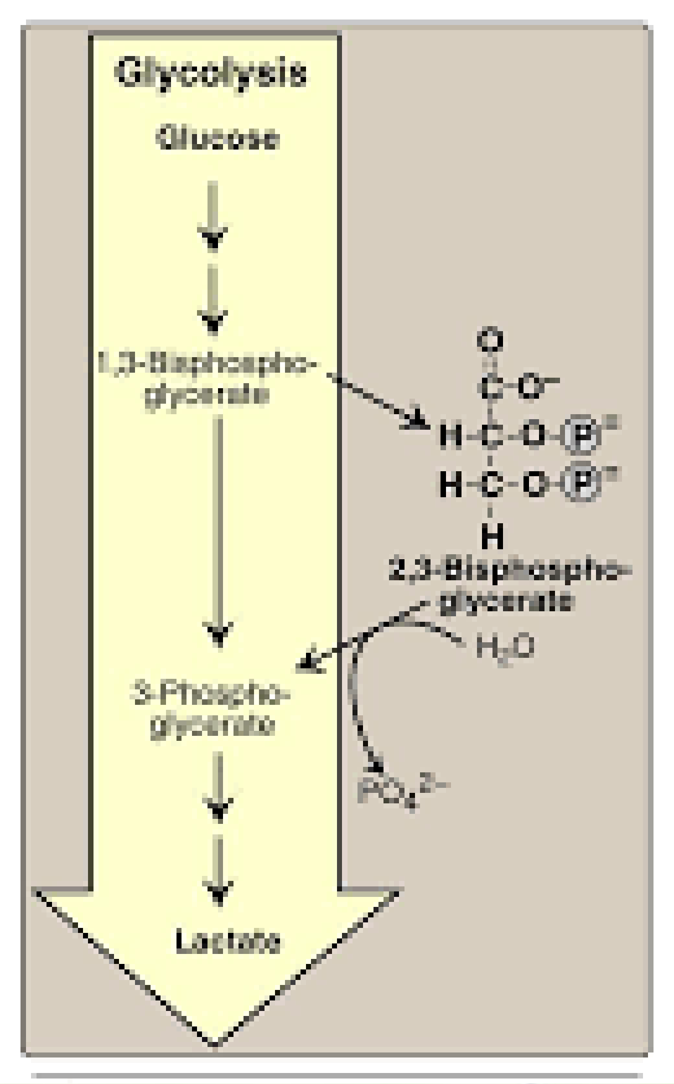
# $pO_2$ at different altitudes

| 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)

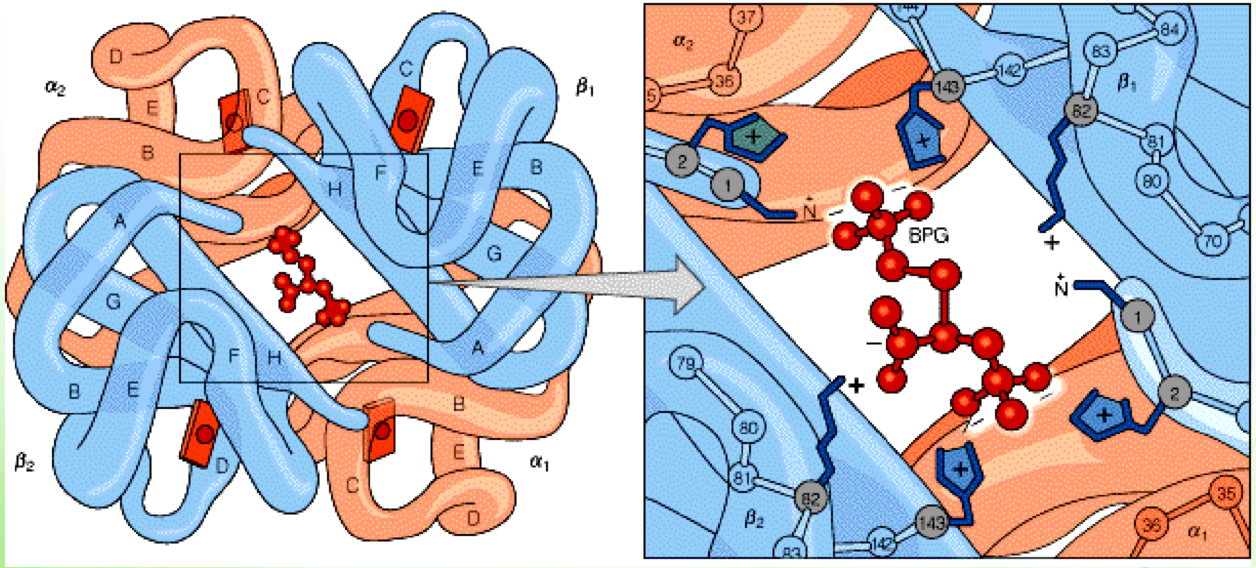
- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- BPG binds to hemoglobin and reduces its affinity towards oxygen.



# BPG –hemoglobin interaction

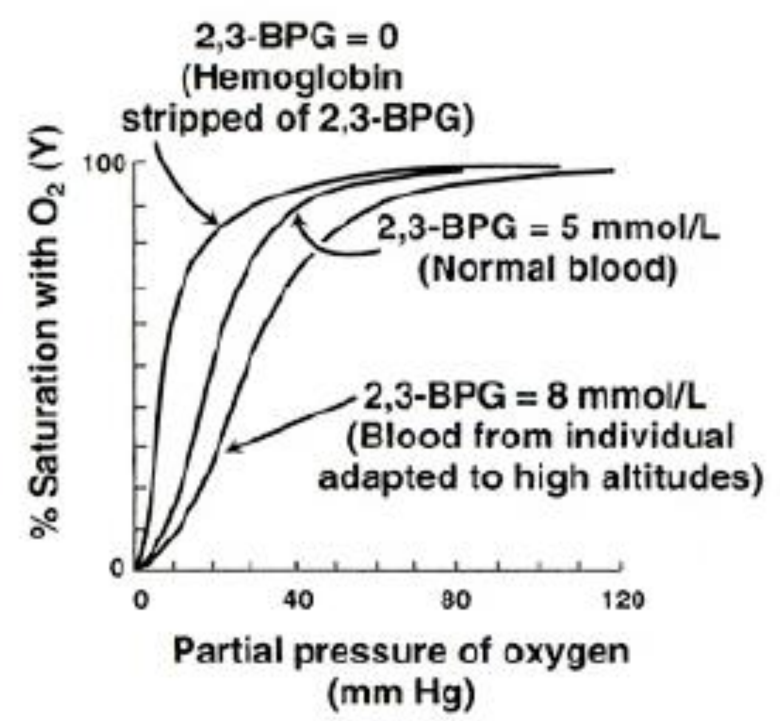
- BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 BPG/hemoglobin tetramer.
- This binding increases the energy needed to transform hemoglobin from the T state to R state.
- Bound, 2,3-BPG reduces binding of oxygen to hemoglobin and facilitates oxygen release.

**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.



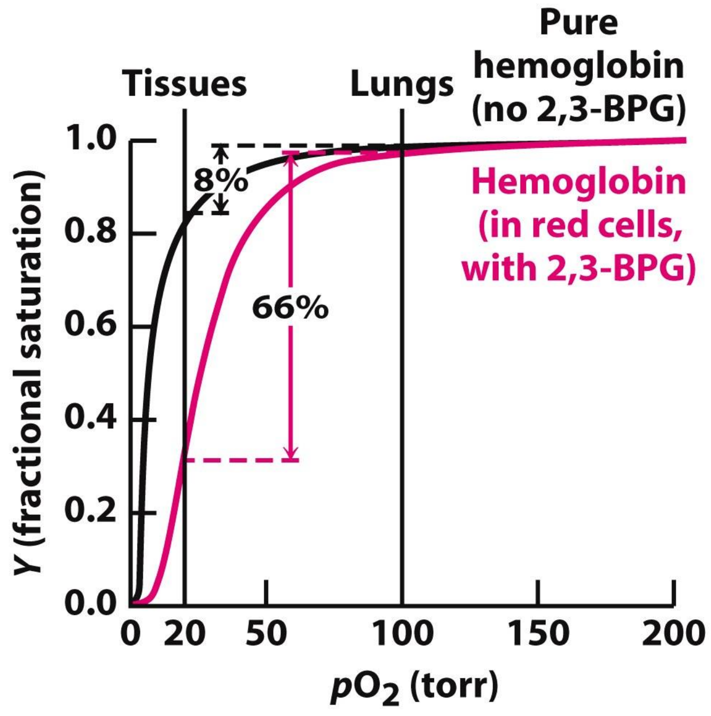
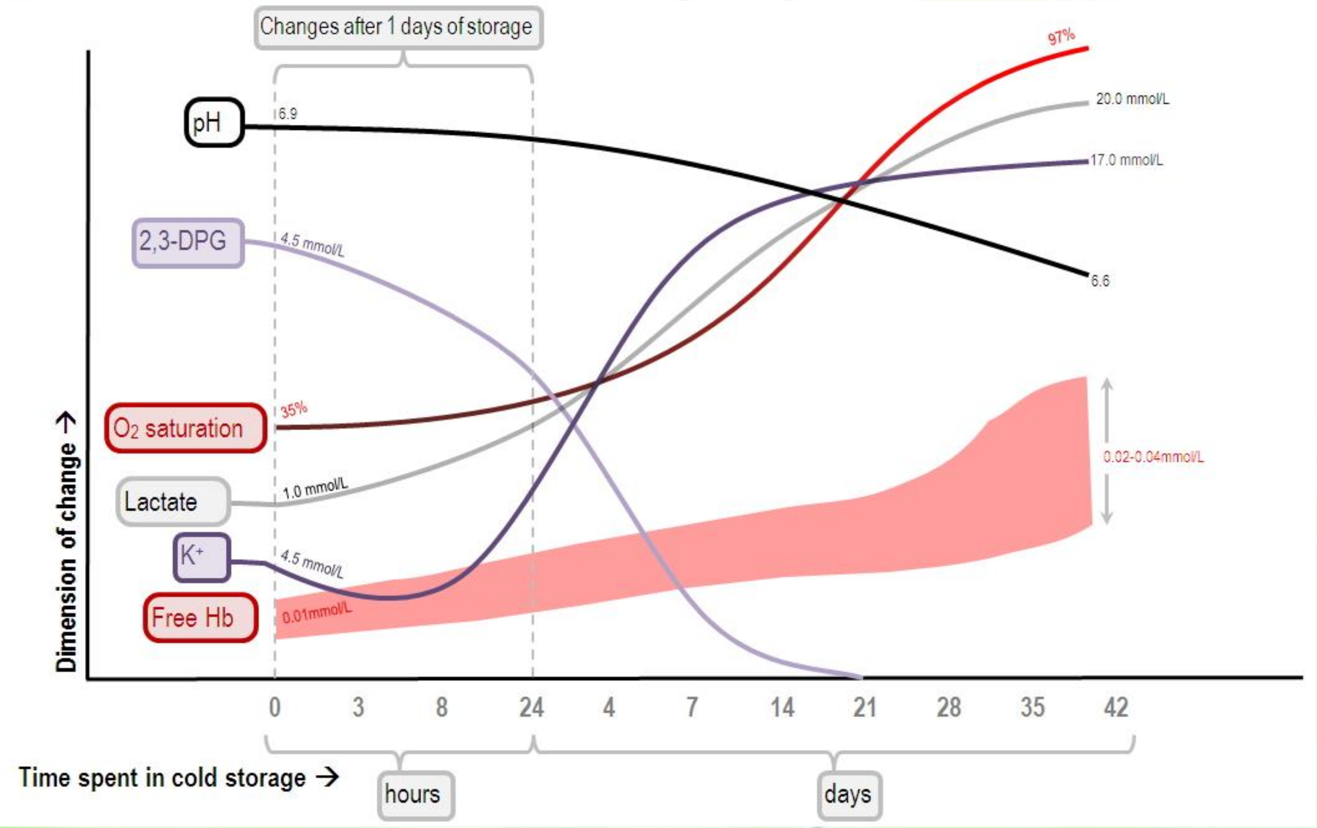




Figure 7.16
*Biochemistry*, Seventh Edition

# 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.

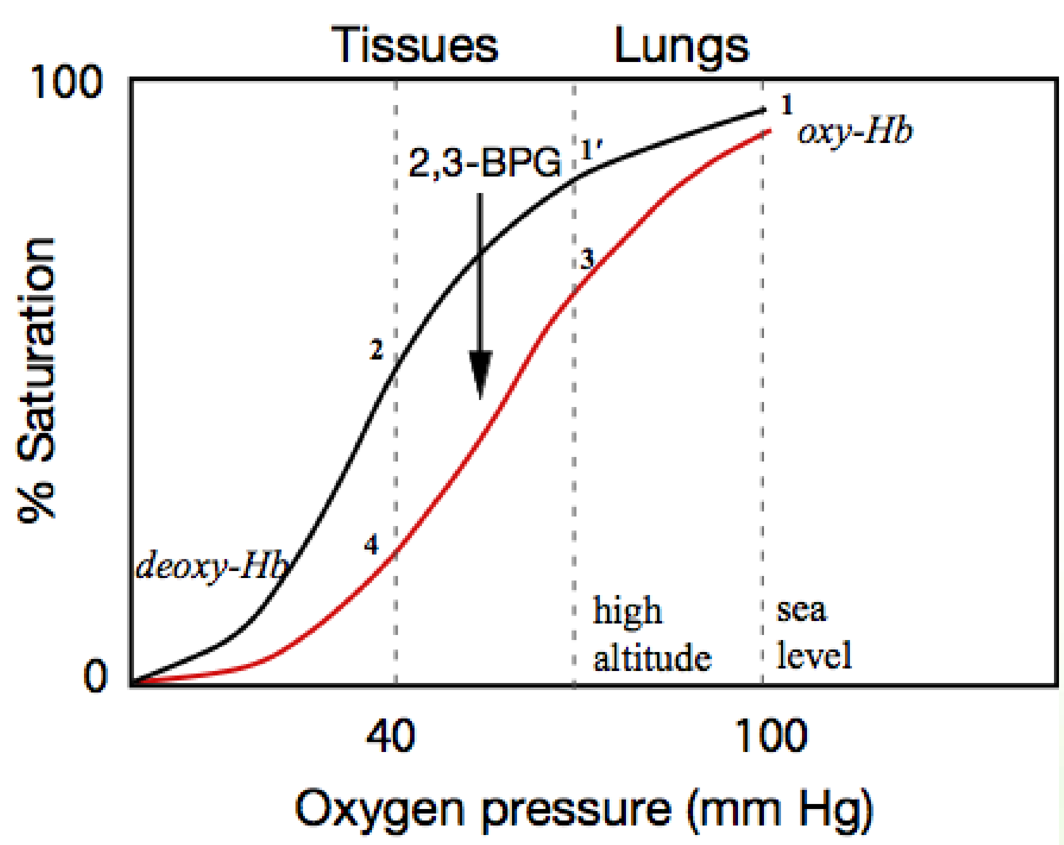
- Severely ill patients may be compromised.
- Both in 2,3-PBG and ATP are rejuvenated.



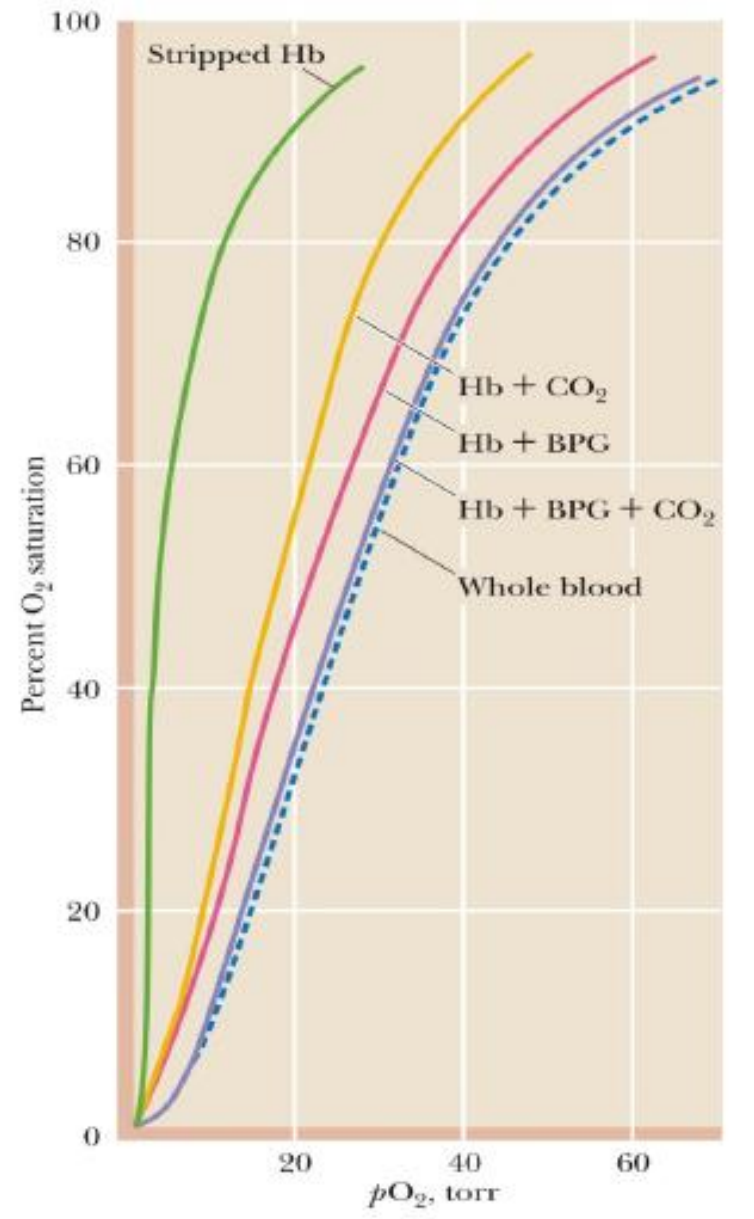
# Better explanation of role of 2,3-BPG

- 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.

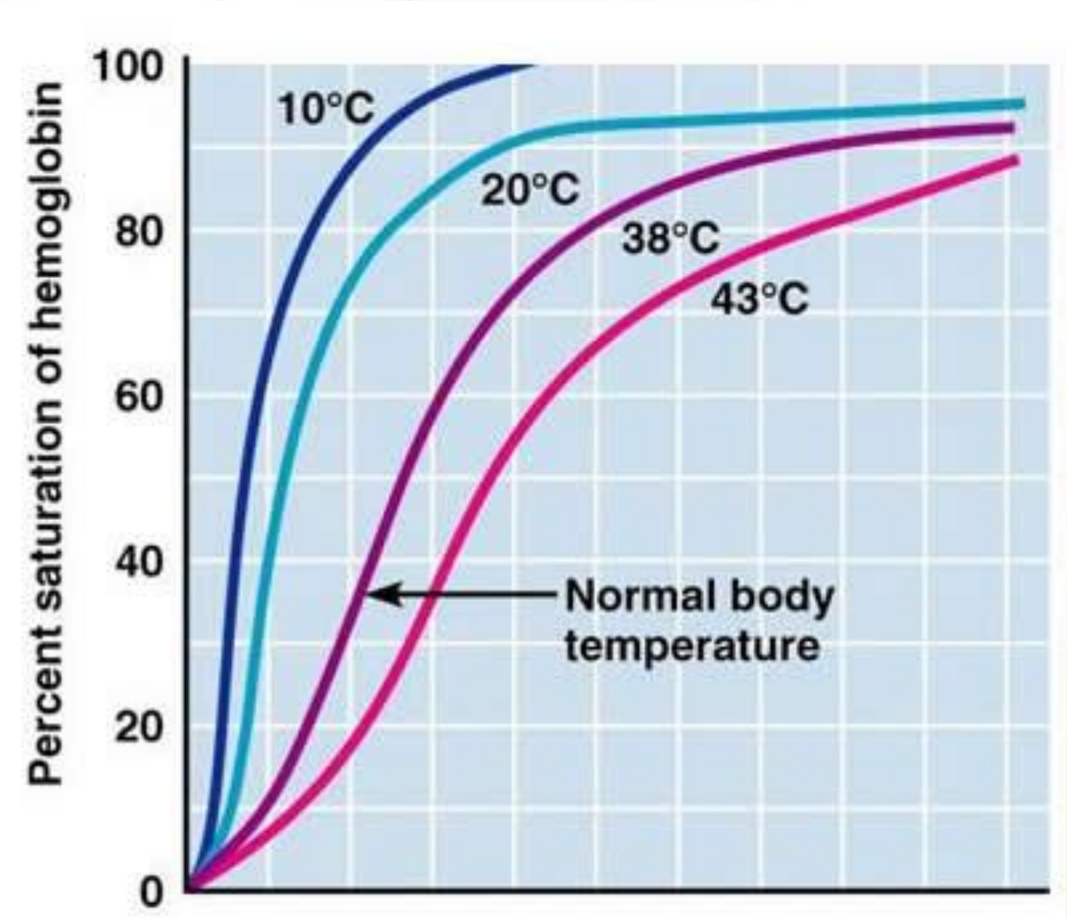


# 2,3-BPG and CO2 are important players



# Effect of temperature

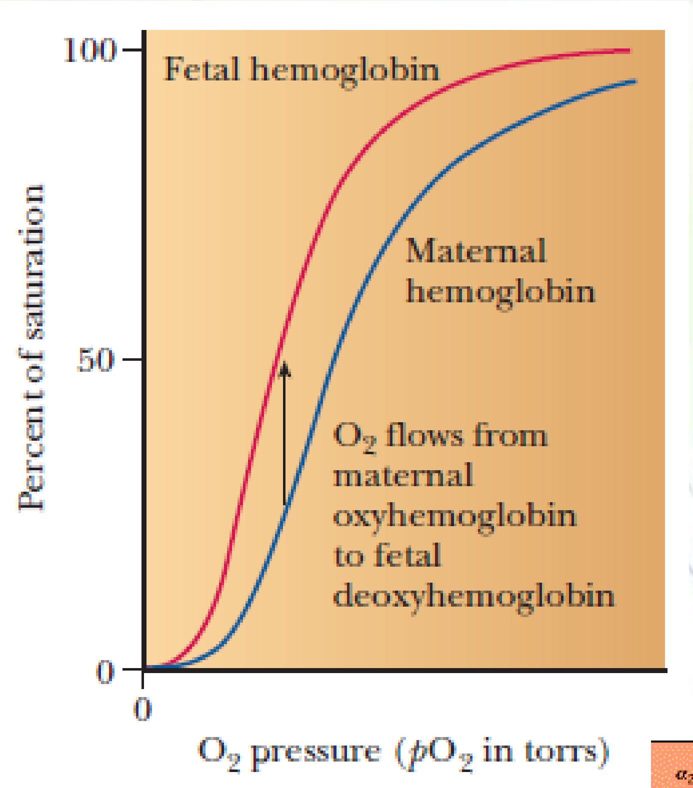
- An increase in temperature decreases oxygen affinity and therefore increases the P50.
- Temperature affects the $O_2$ binding of both myoglobin and hemoglobin.
- Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading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

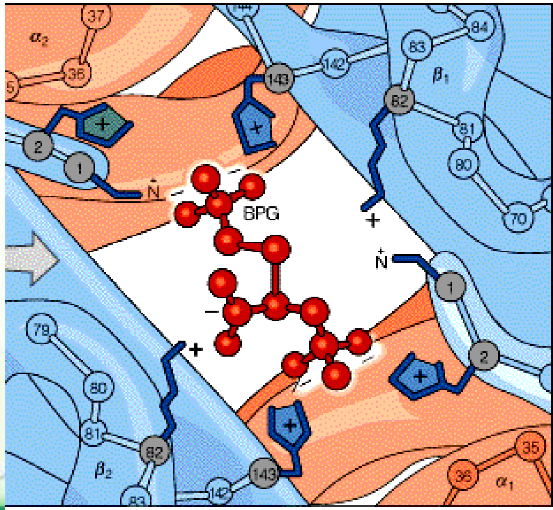


# Other considerations

# Fetal hemoglobin

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

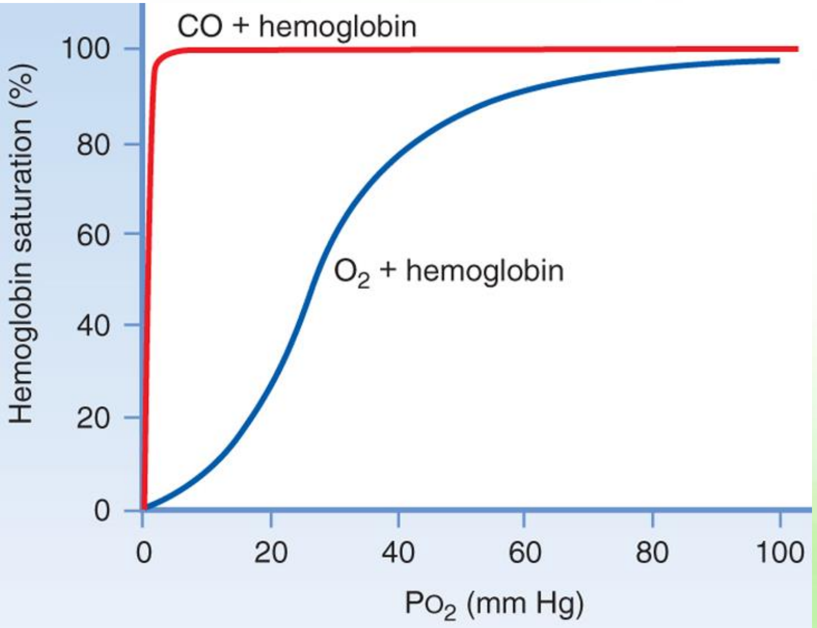


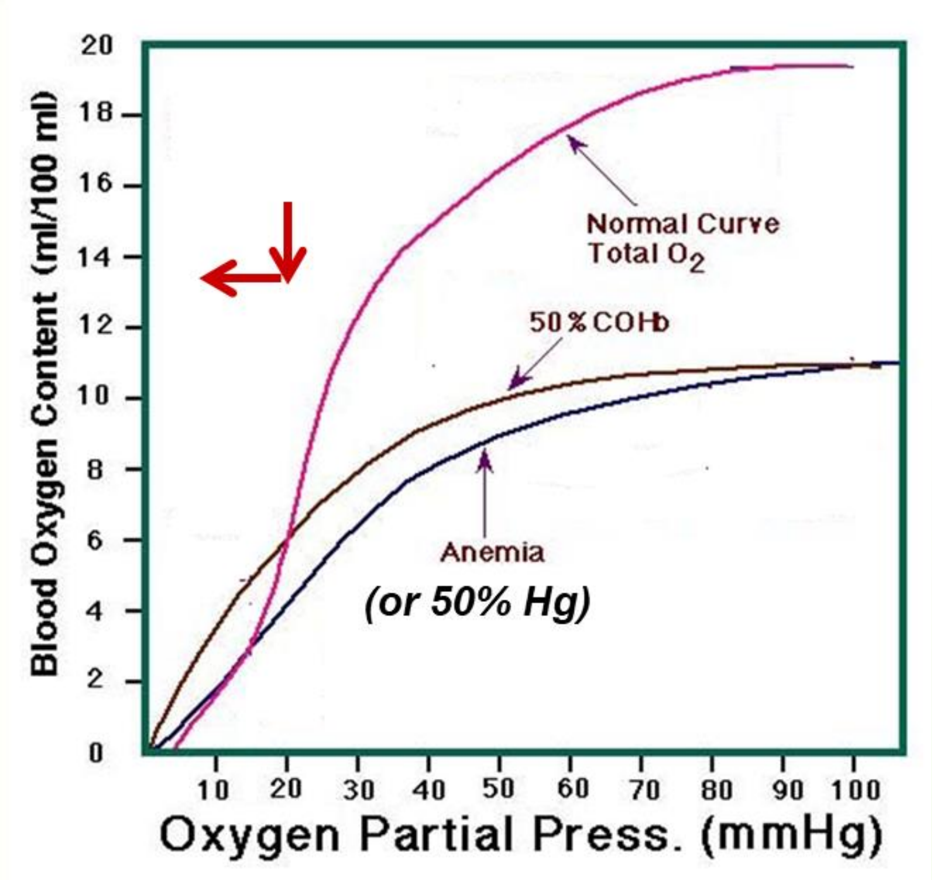
# Effect of CO

- 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 ($Hb + CO$)**



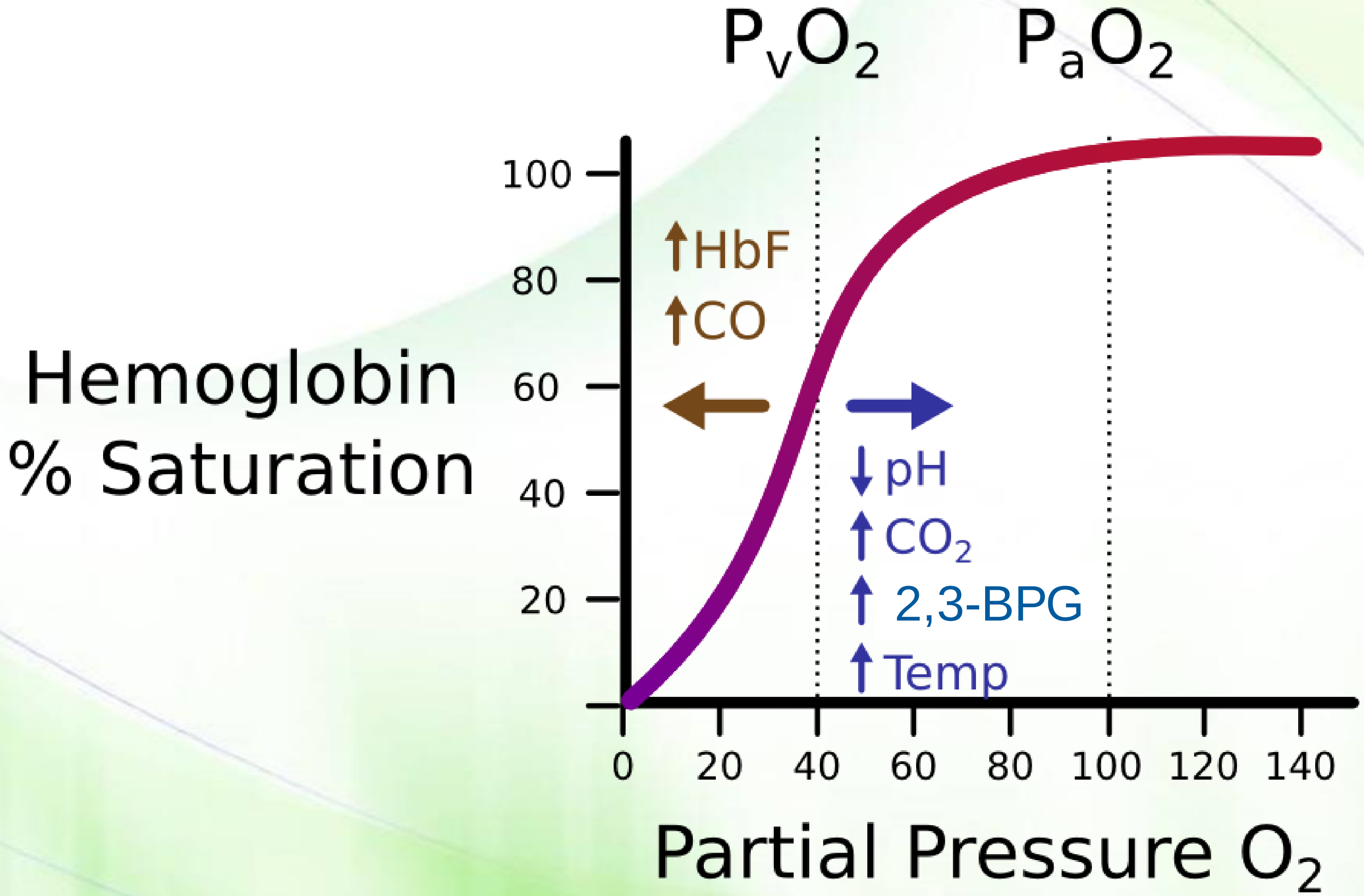

**($Hb + O_2$) versus ($Hb + O_2 + CO$)**



# 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 $O_2$