
# Regulation of hemoglobin function

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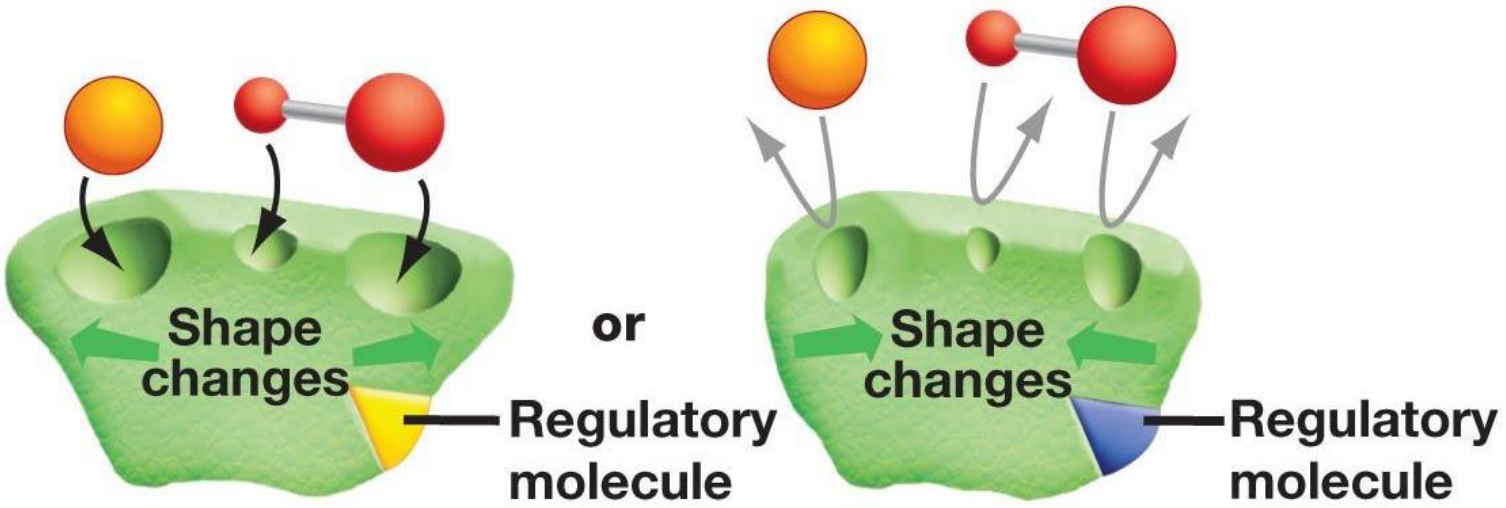
Blood module

# Allosteric regulation




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

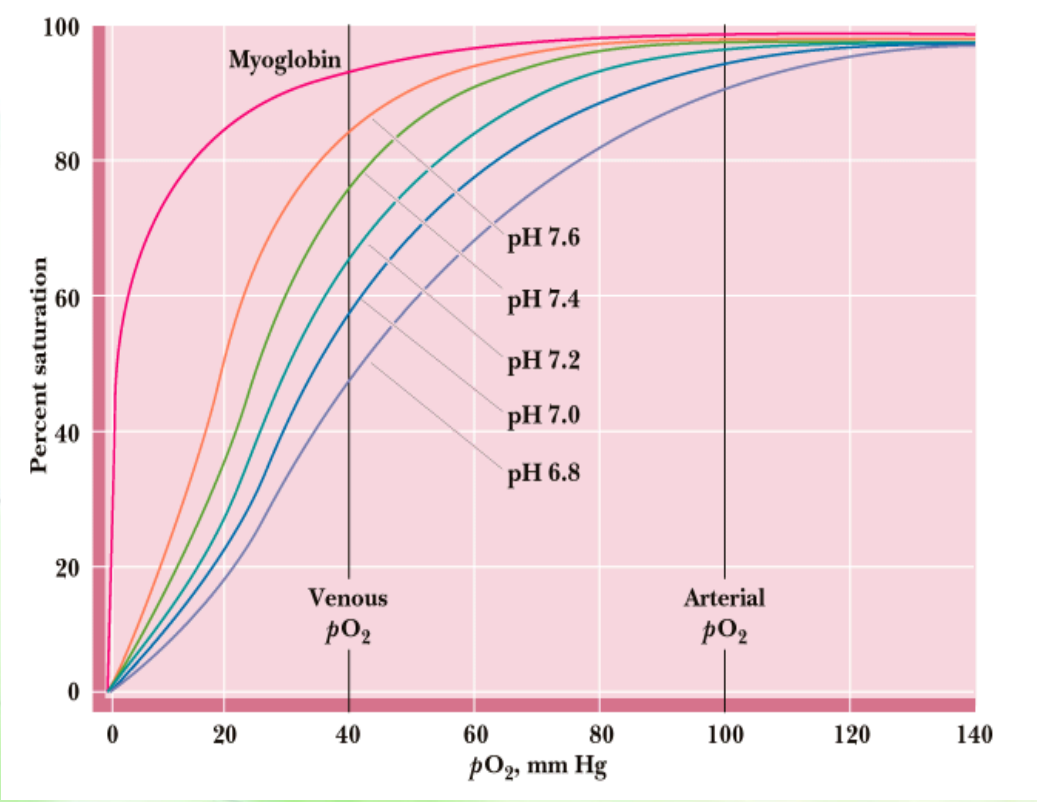
**Allosteric deactivation**
The active site becomes unavailable to the substrates when a regulatory molecule binds to a different site on the enzyme.

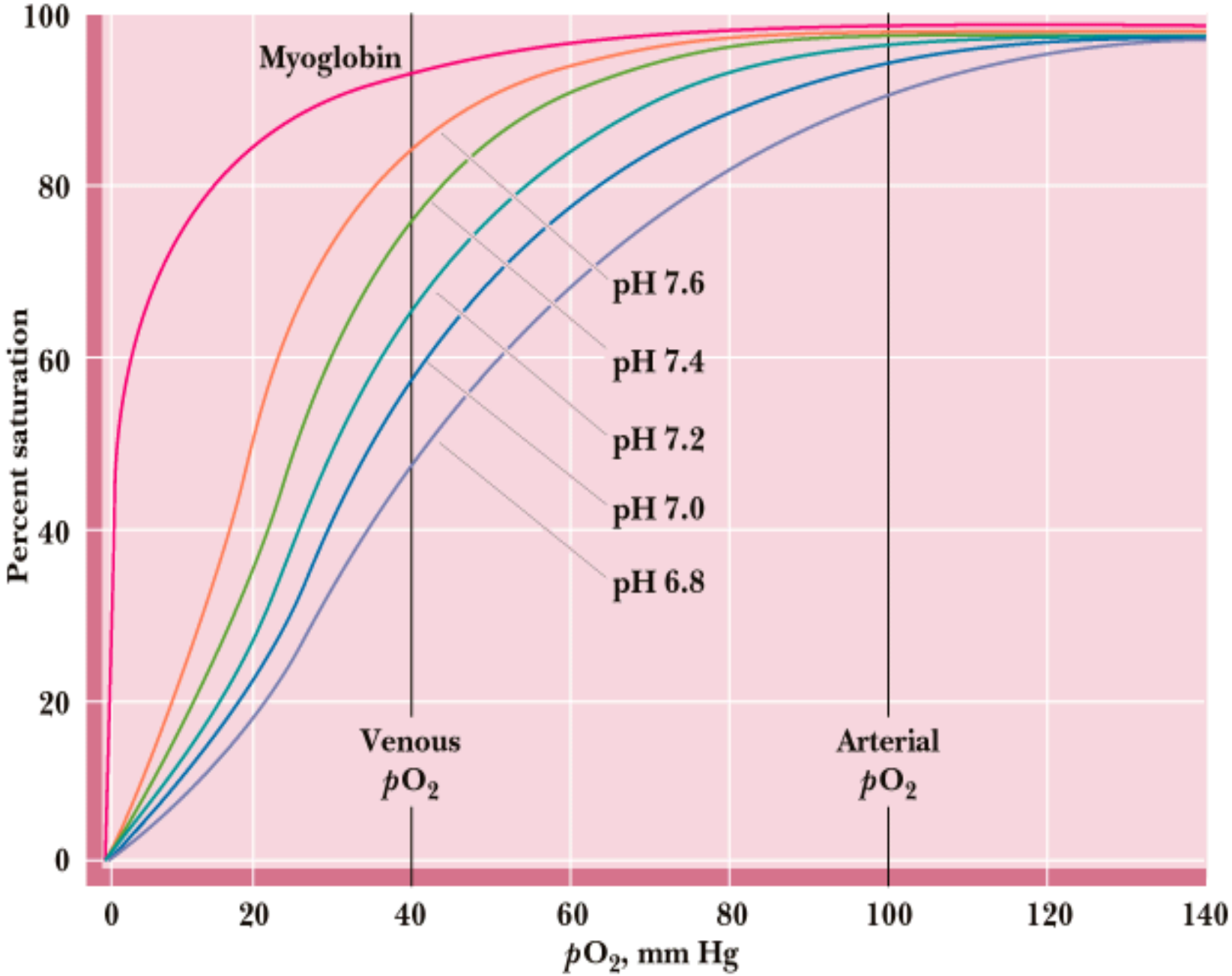
# Allosteric effectors

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

# The effect of pH

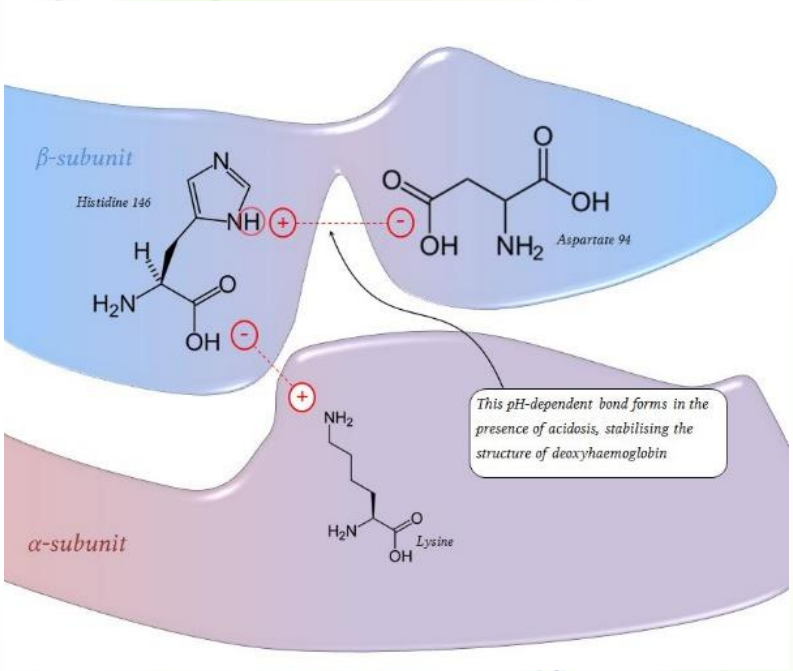
- The binding of $H^+$ to hemoglobin promotes the release of $O_2$ from hemoglobin and vice versa.
- This phenomenon is known as the Bohr effect.




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.
- The protonated histidine is attracted to, and stabilized by, a salt bridge to an aspartate.
  - The pKa of his 146 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 his 146 and lys of the α chain.
- This favors the deoxygenated form of hemoglobin.



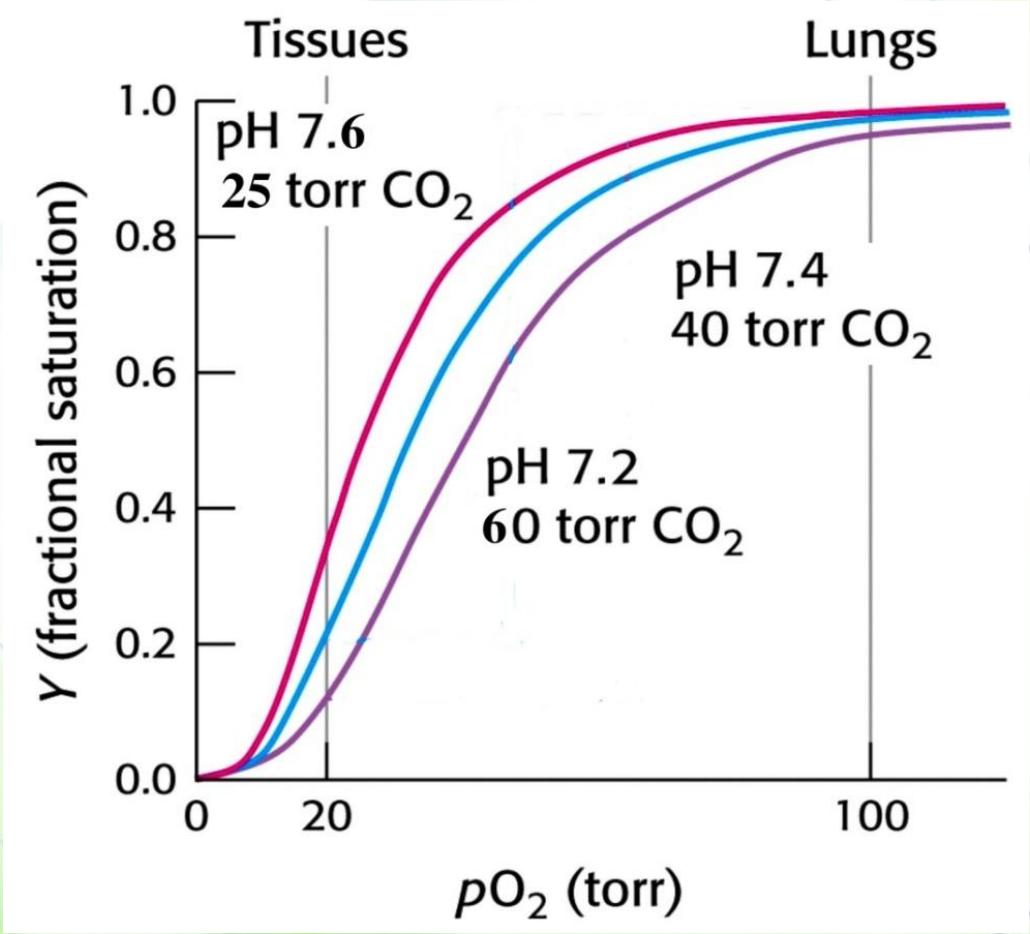
# Where do protons come from?


- $CO_2$ 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 $O_2$ cause the release of $CO_2$ from hemoglobin.

# Effect of $CO_2$

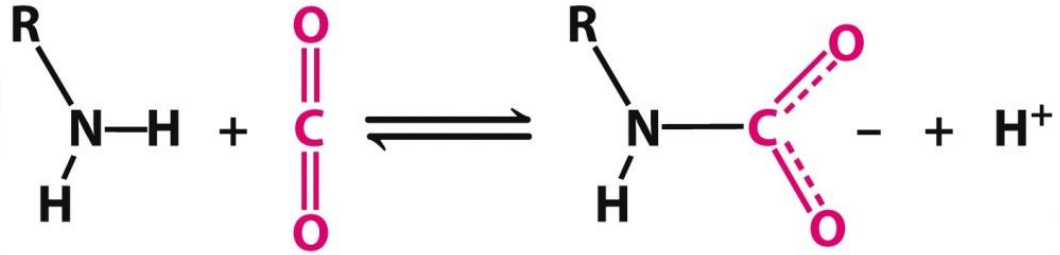
## (Mechanism #1 - production of protons)

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



# Mechanism #2- formation of carbamates

- Hemoglobin transports some $CO_2$ directly.
- When the $CO_2$ concentration is high, it combines with the free $\alpha$-amino terminal groups to form carbamate and producing negatively-charged groups



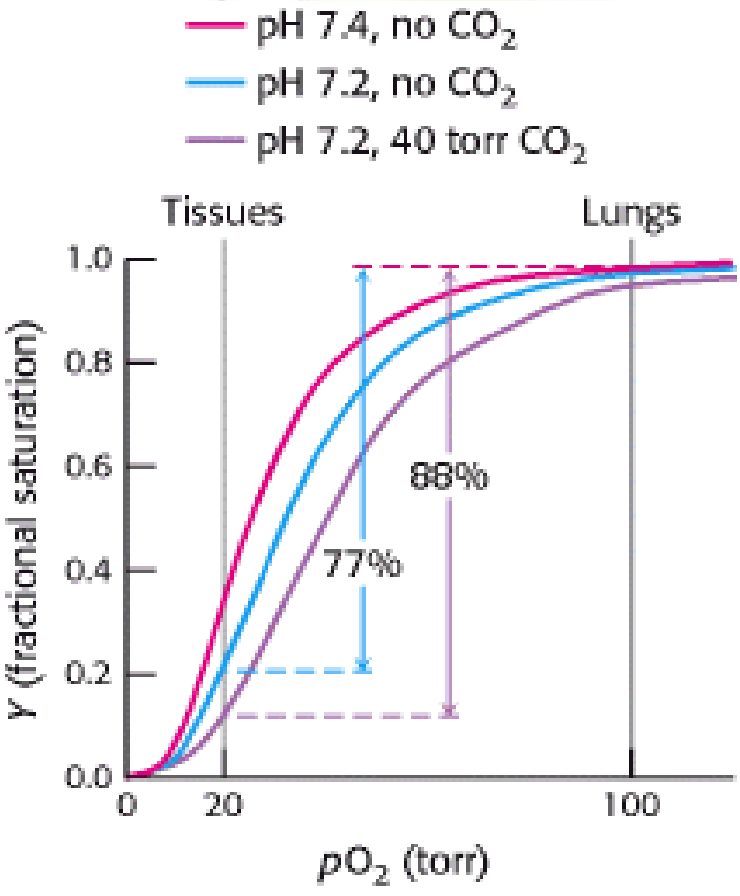

**Carbamate**

- 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.

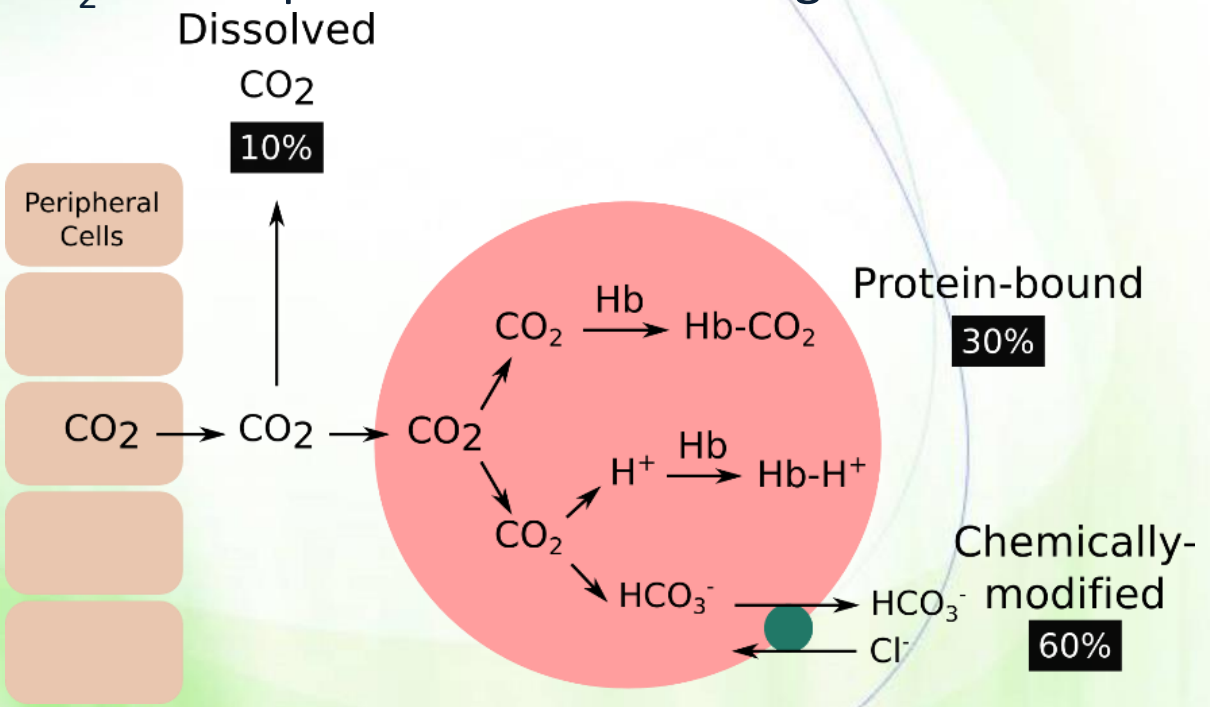
**How do we know that?**
**An increase in $CO_2$ tension will shift the oxygen dissociation curve to the right, even when the pH is held constant.**



# 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.

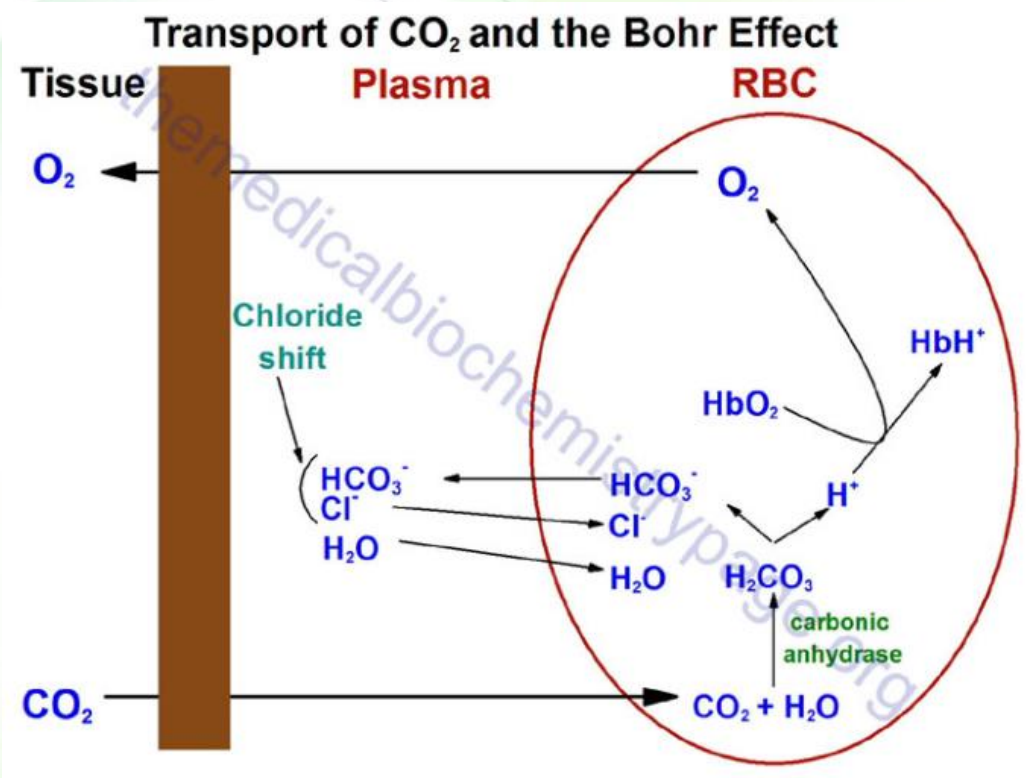
**The movement of $CO_2$ and $CO_2$ in/out of cells does not change the pH, a phenomenon called <u>isohydric shift</u>, which is partially a result of hemoglobin being an effective buffer.**

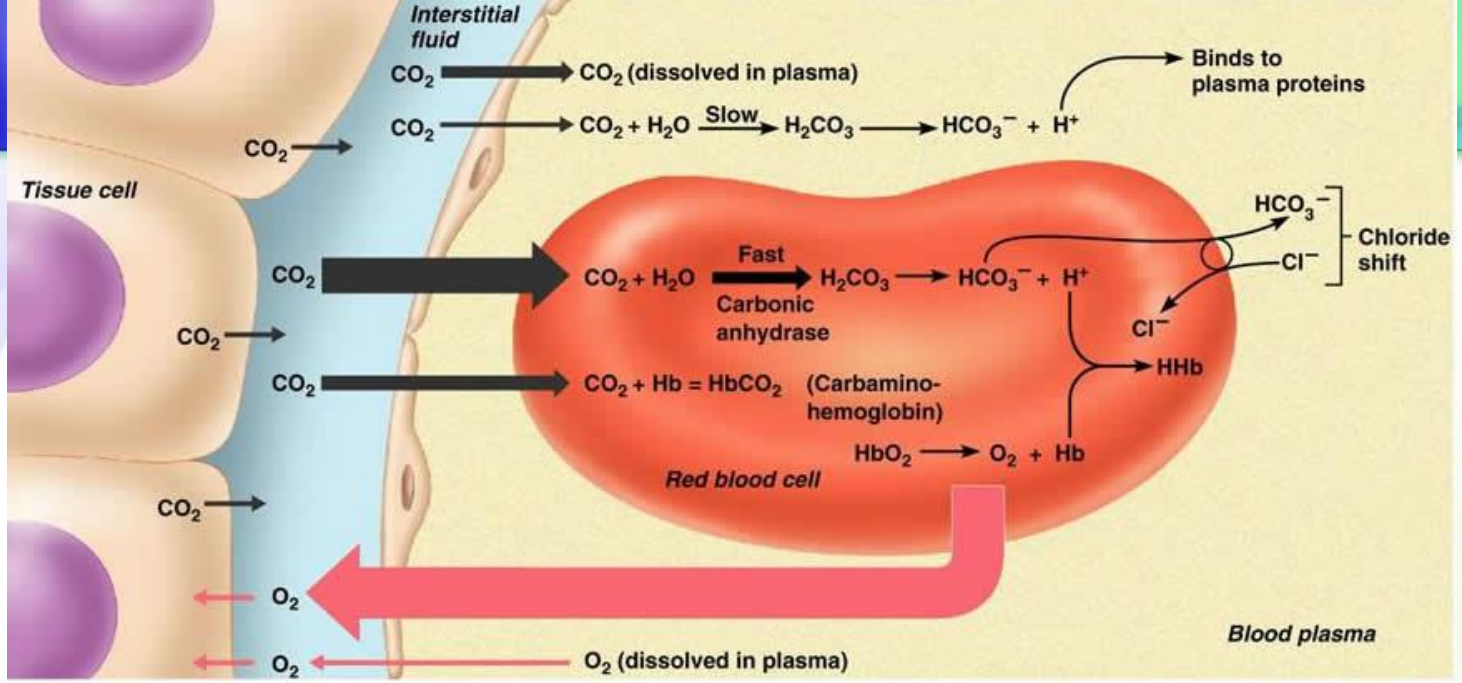


# Other allosteric effectors

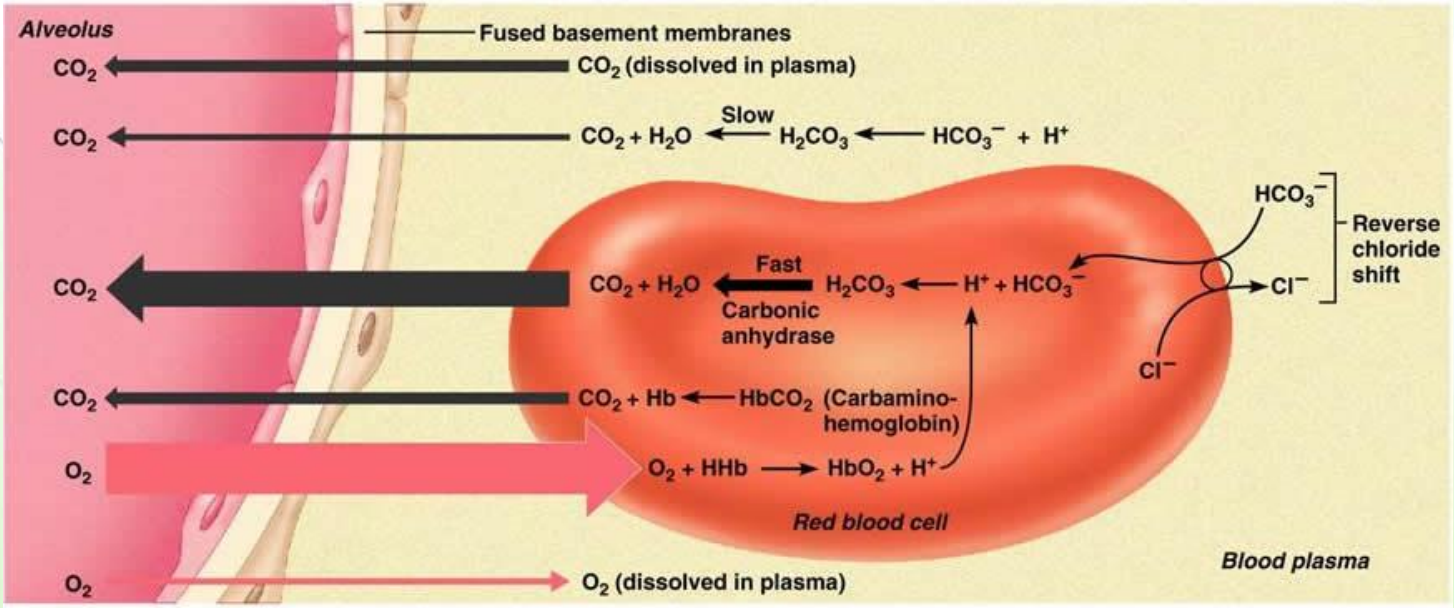
# Chloride shift

- 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“.





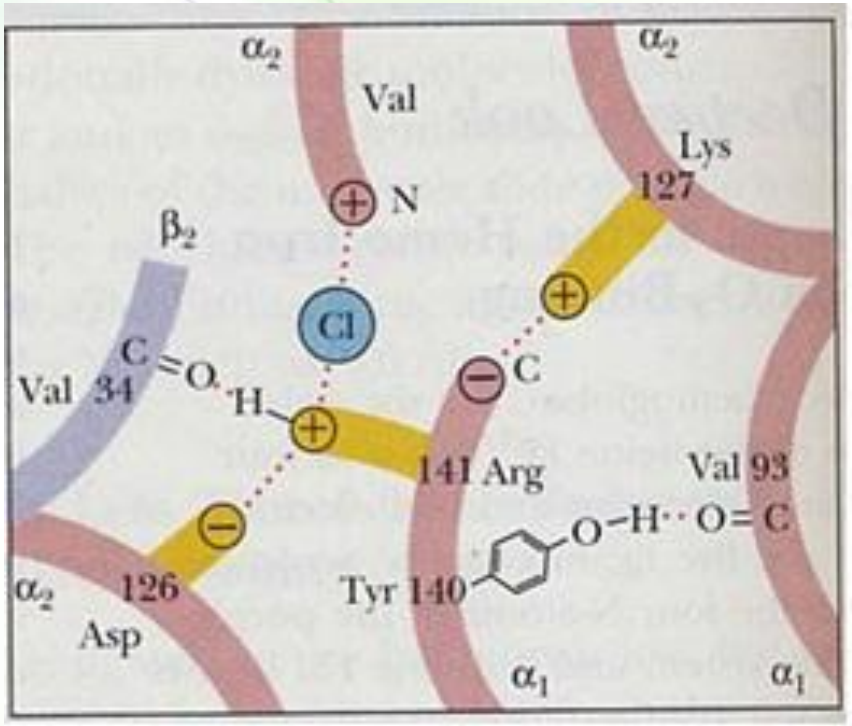

**(a) Oxygen release and carbon dioxide pickup at the tissues**




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

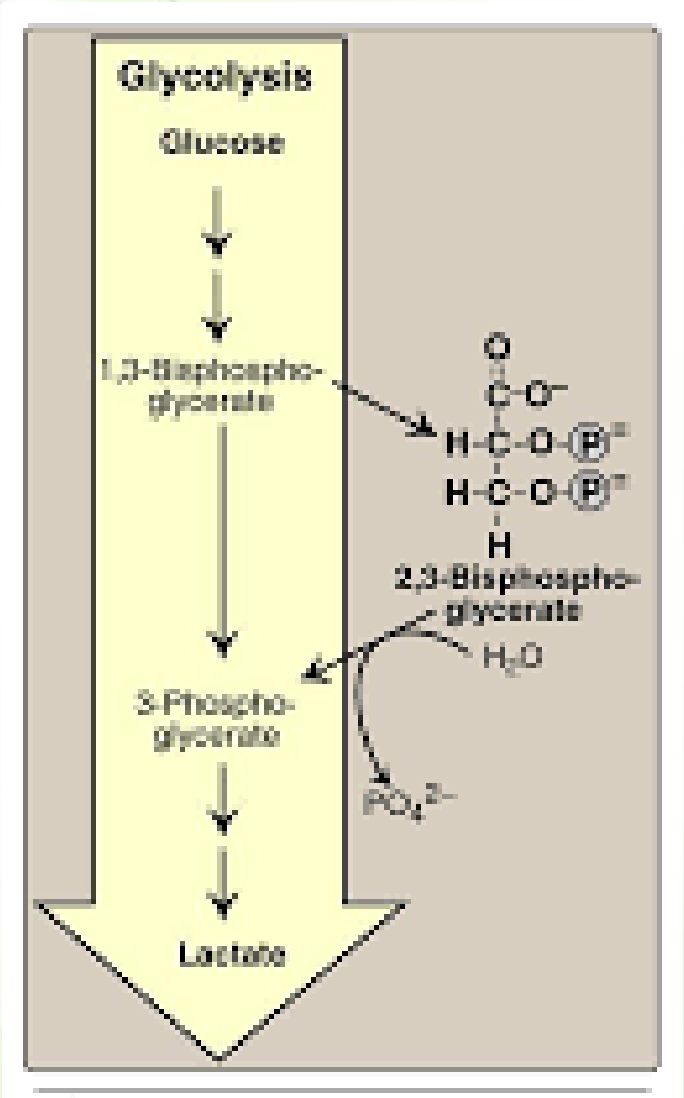
# Effect of chloride ions

- Increasing the concentration of chloride ions ($Cl^-$) shifts the oxygen dissociation curve to the right (lower affinity)
- This chloride shift may also regulate the affinity of hemoglobin for oxygen through the chloride ion acting as an allosteric effector.



# 2,3-bisphosphoglycerate (BPG)

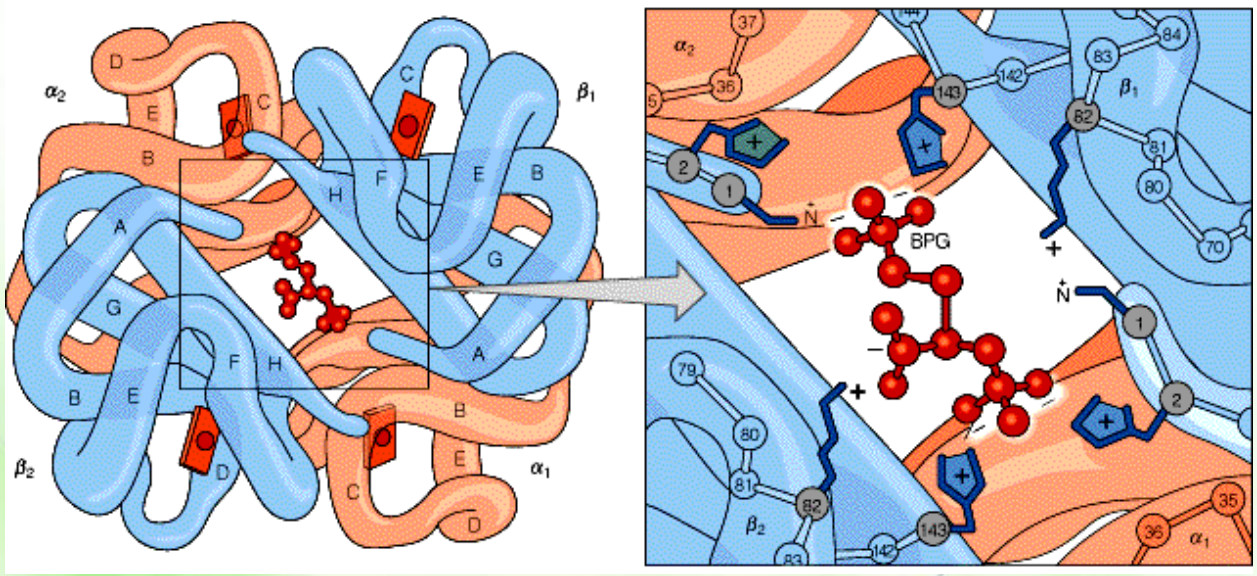
- 2,3-Bisphosphoglycerate (BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- BPG binds to hemoglobin and reduces its affinity towards oxygen.



# 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 BPG were not present p50 is close to 1 torr.

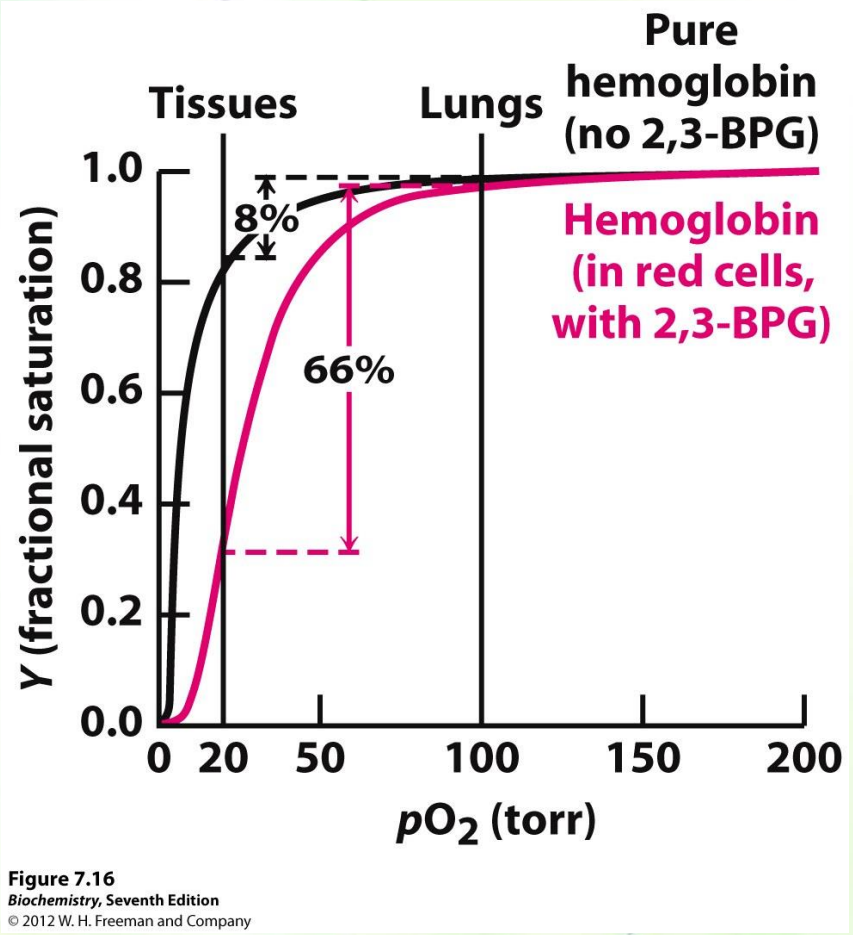
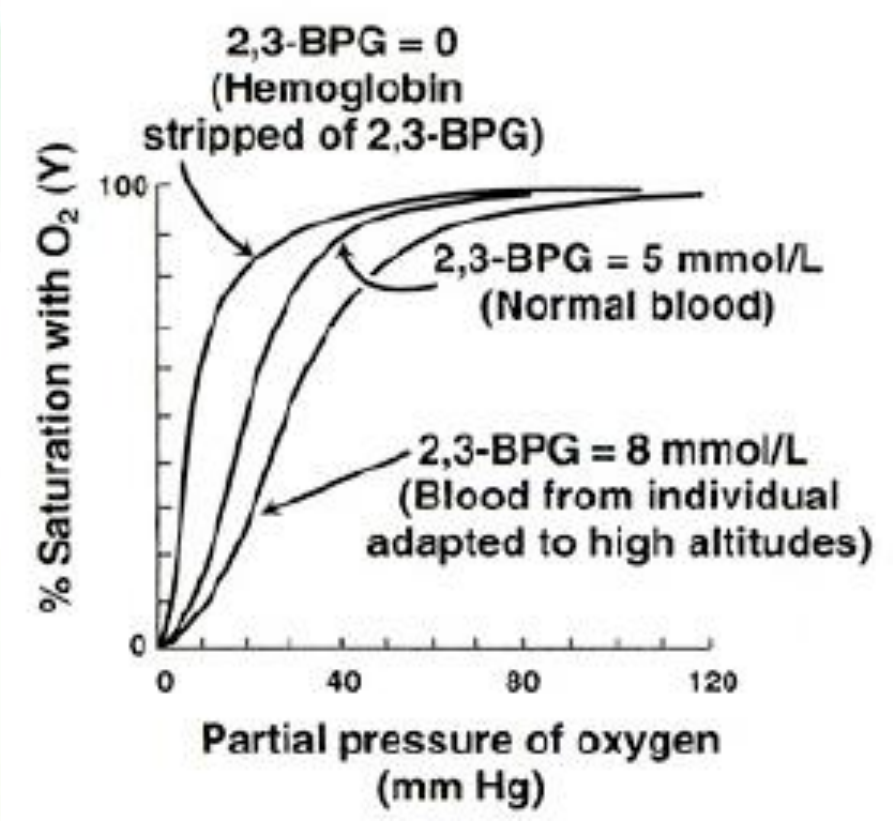



**Figure 7.16**
***Biochemistry*, Seventh Edition**

# Physiological significance

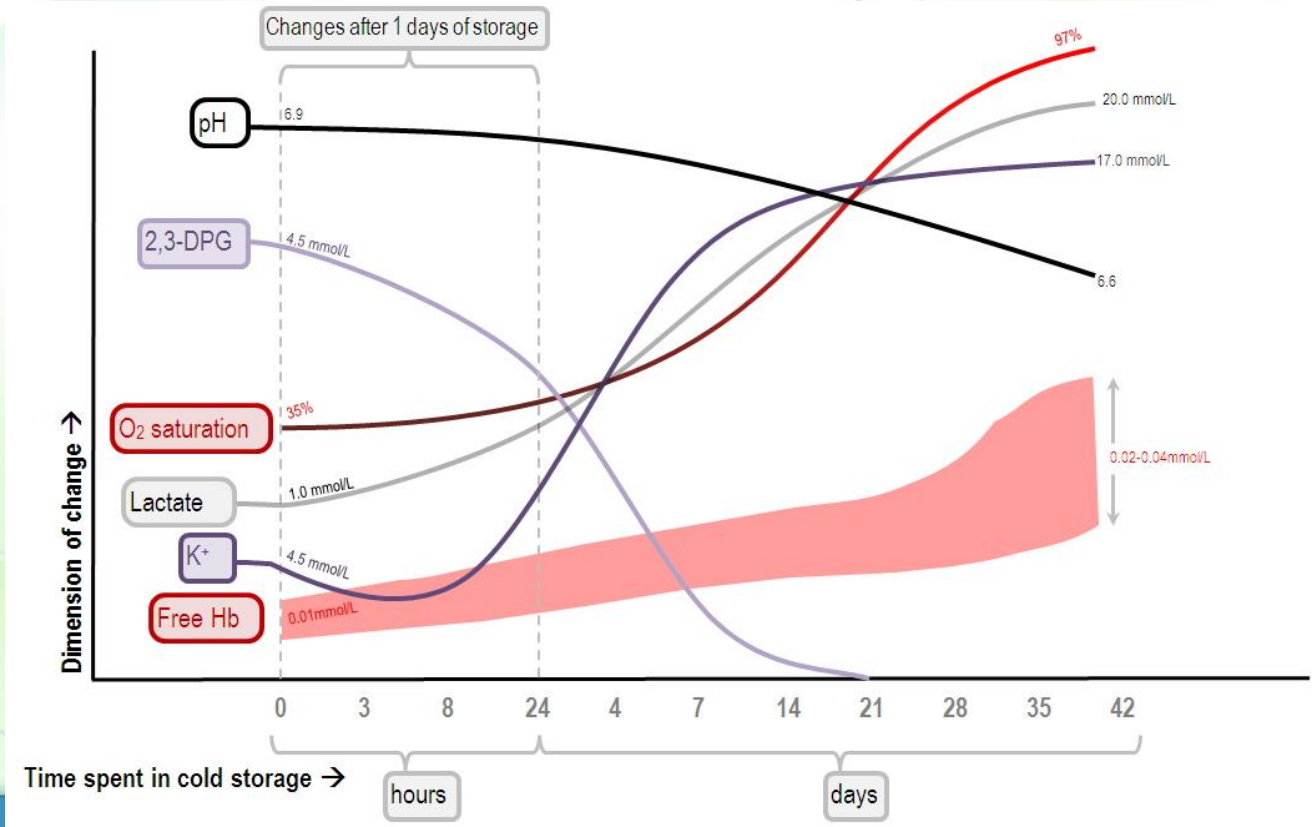
- The concentration of 2,3-BPG increases at high altitudes (low $O_2$) and in certain metabolic conditions making hemoglobin more efficient at delivering oxygen to tissues.



# 2,3-BPG in transfused blood

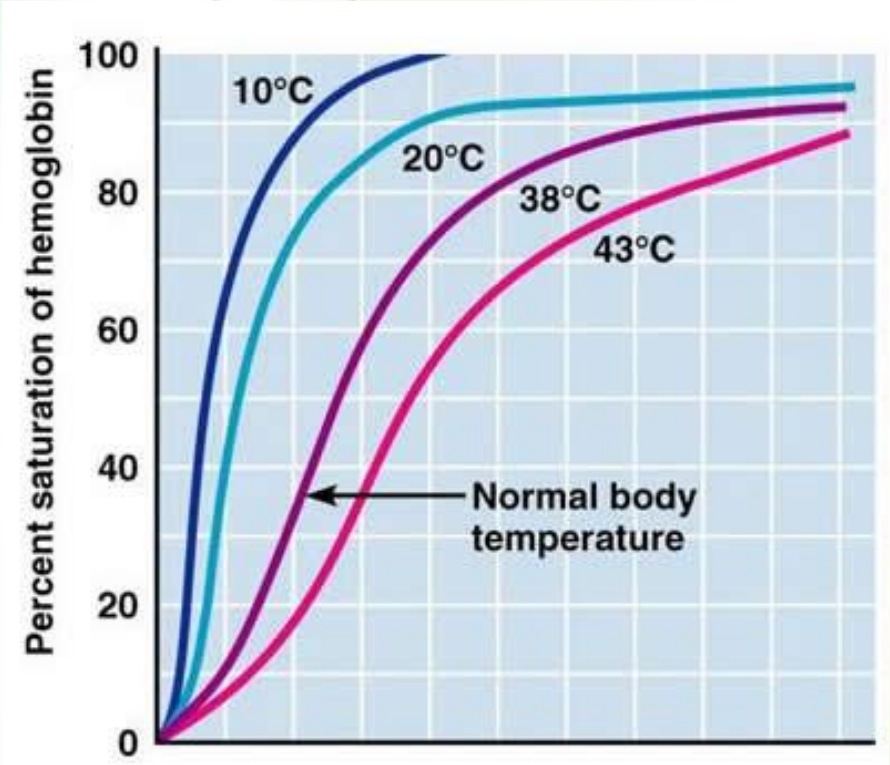
- Storing blood results in a decrease in 2,3-PBG (and ATP), hence hemoglobin acts as an oxygen “trap”, not an oxygen transporter.
- Transfused RBCs are able to restore their depleted supplies of 2,3-BPG in 6–24 hours.

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



# Effect of temperature

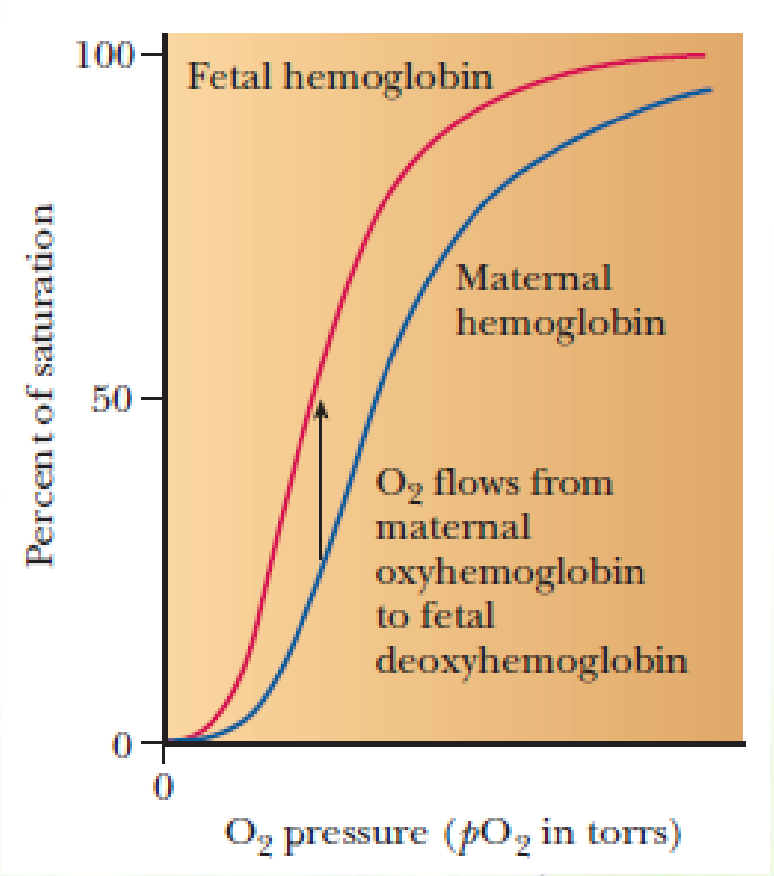
- An increase in temperature decreases oxygen affinity and therefore increases the P50.
- Temperature affects the $O_2$ binding of both myoglobin and hemoglobin.
- Increased temperature also increases the metabolic rate of RBCs, increasing the production of BPG, which also facilitates oxygen unloading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



# Other considerations

# Fetal hemoglobin

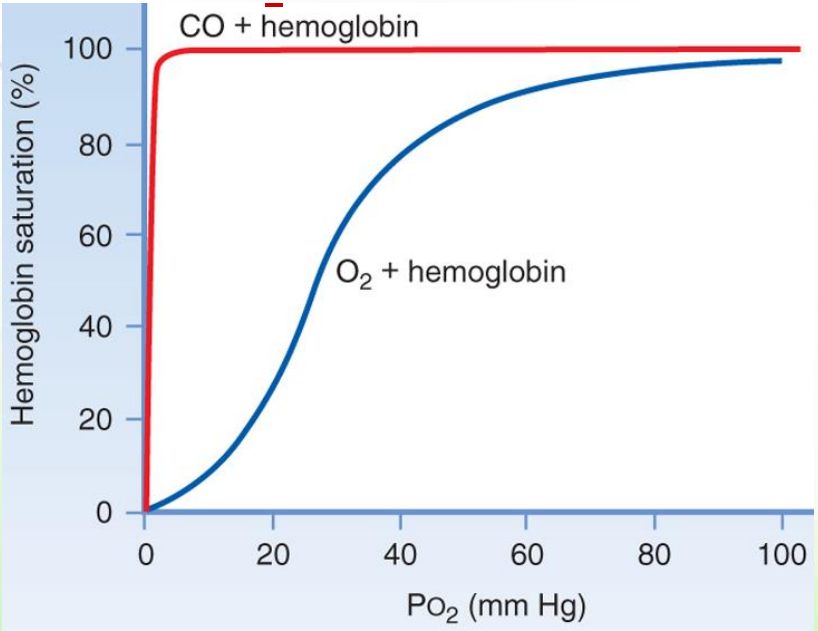
- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HBA).
  - HbA = $\alpha 2 \beta 2$
  - HbF = $\alpha 2 \gamma 2$
- A His residue in the $\beta$ subunit is replaced by a Ser in the $\gamma$ subunit of HbF.
  - Since Ser cannot form a salt bridge, BPG binds more weakly to HbF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



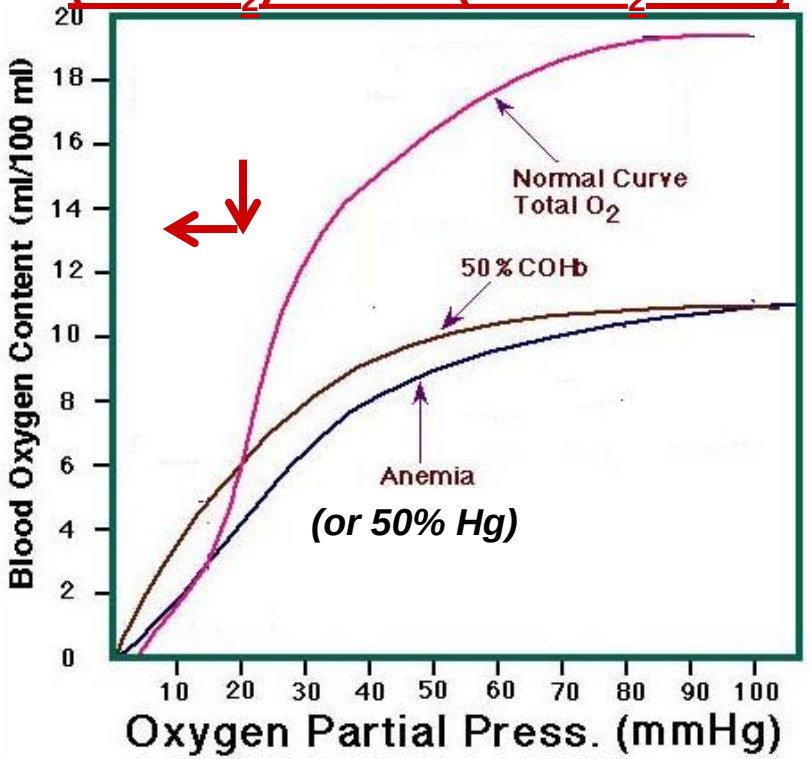
# Effect of CO

- 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)**



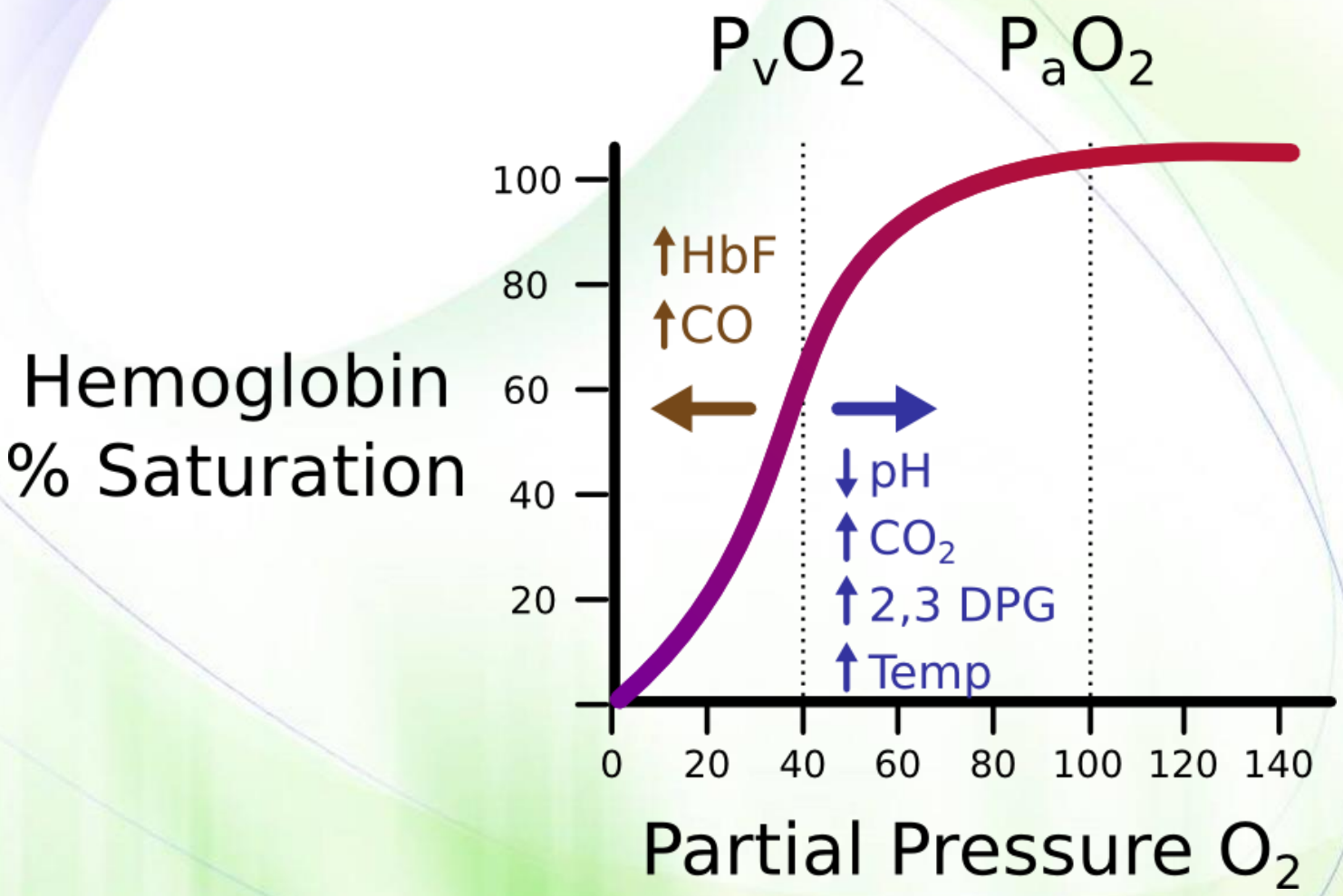

**(Hb + $O_2$) versus (Hb + $O_2$ + CO)**



# 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 smokkers, 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


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