HLS

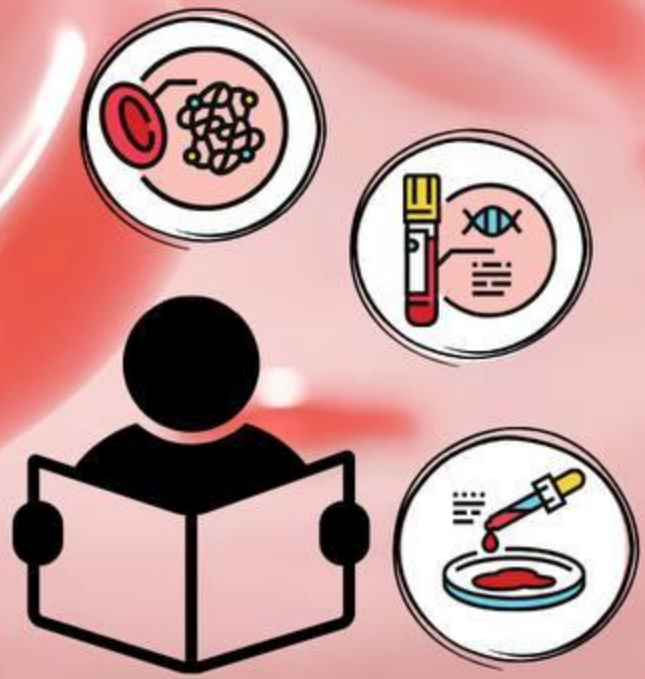
MODIFIED NO. 2

BIOCHEMISTRY

كتابة: عمر الصمادي ، عمر النعانعة

تدقيق: مريم مناصرة

الدكتور: مأمون أهرام



Regulation of hemoglobin function

Prof. Mamoun Ahram
Hematopoietic-lymphatic system

Color code

- Slides
- Doctor
- Additional info
- Important

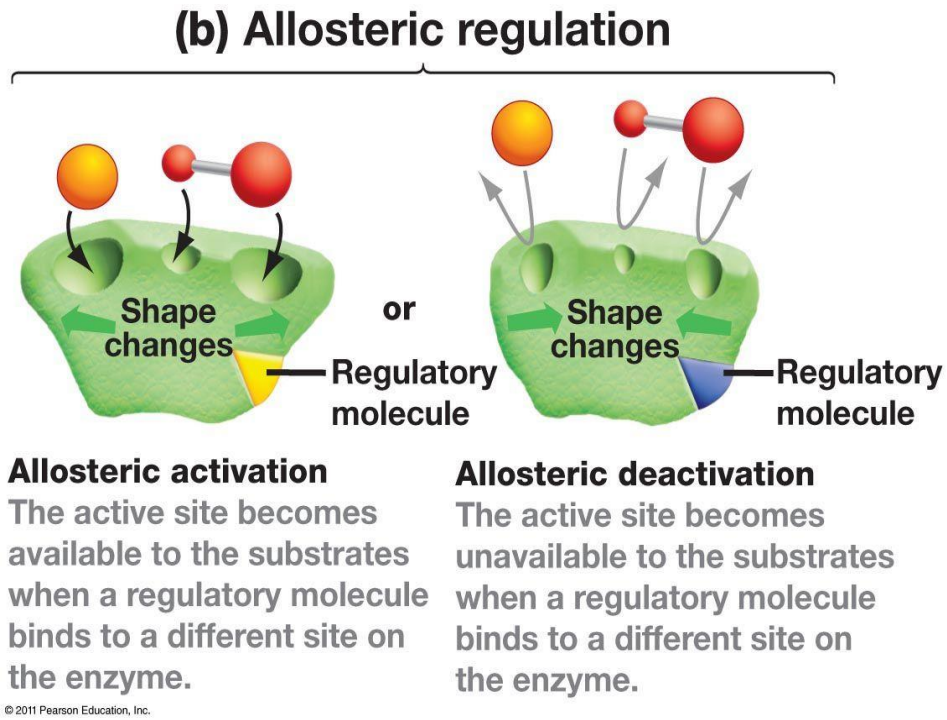
Allosteric regulation

- 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.

We previously mentioned that, in the case of hemoglobin, oxygen acts as a homotropic positive allosteric effector.

  - Heterotropic modulators are different from the ligand.

In this lecture, we will discuss the heterotropic modulators of hemoglobin.



Allosteric effectors

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

They are negative allosteric effectors

- A competitive inhibitor
  - Carbon monoxide

The effect of pH and H^+

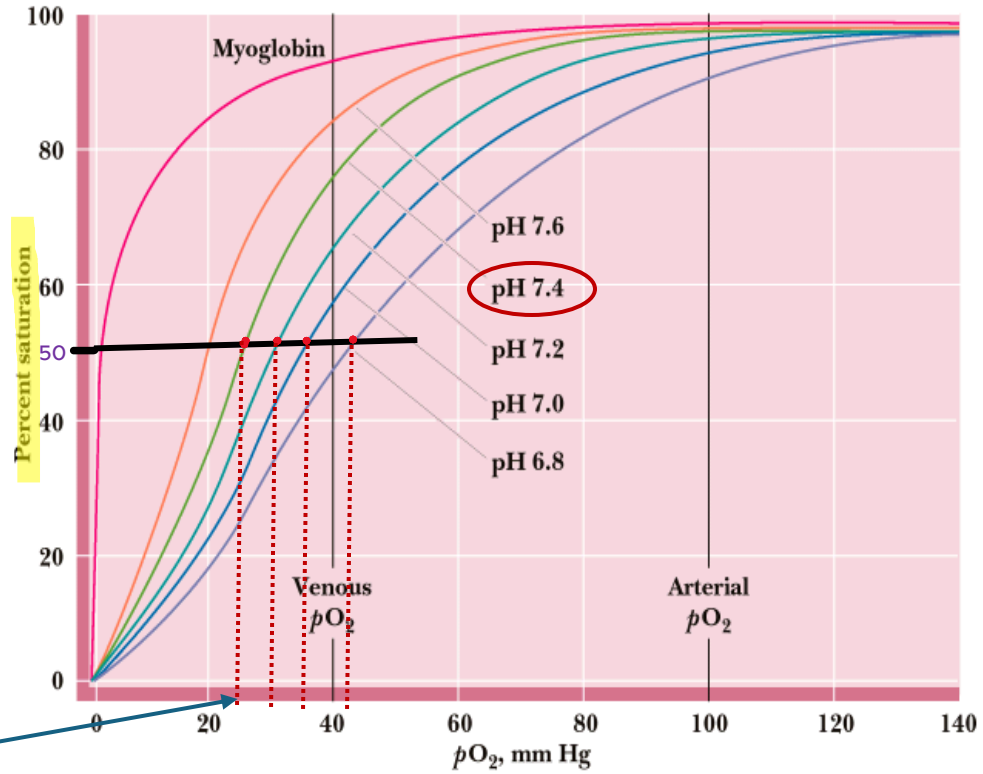
The effect of pH

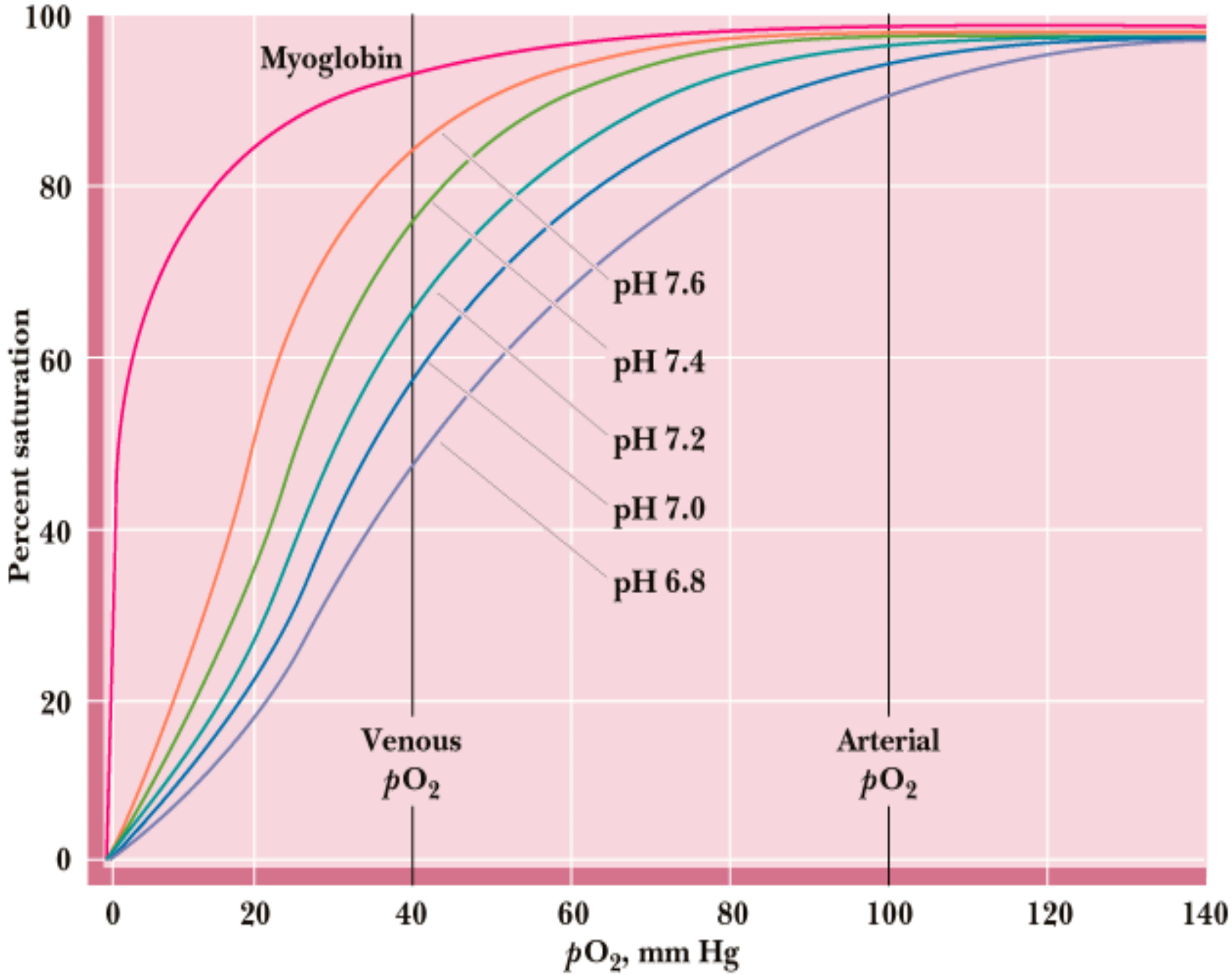
- The binding of H^+ to hemoglobin promotes the release of O_2 from hemoglobin and vice versa.
- This phenomenon is known as the **Bohr effect**.

pH of 7.4 is blood's normal pH, now notice ! the lower the pH (more hydrogen ions) the higher the P50 (the lower the affinity), this is called Bohr effect (a scientist called Bohr first described this phenomenon).

The p50 value indicates hemoglobin's affinity for oxygen, representing the (pO2) at which hemoglobin is 50% saturated with oxygen.

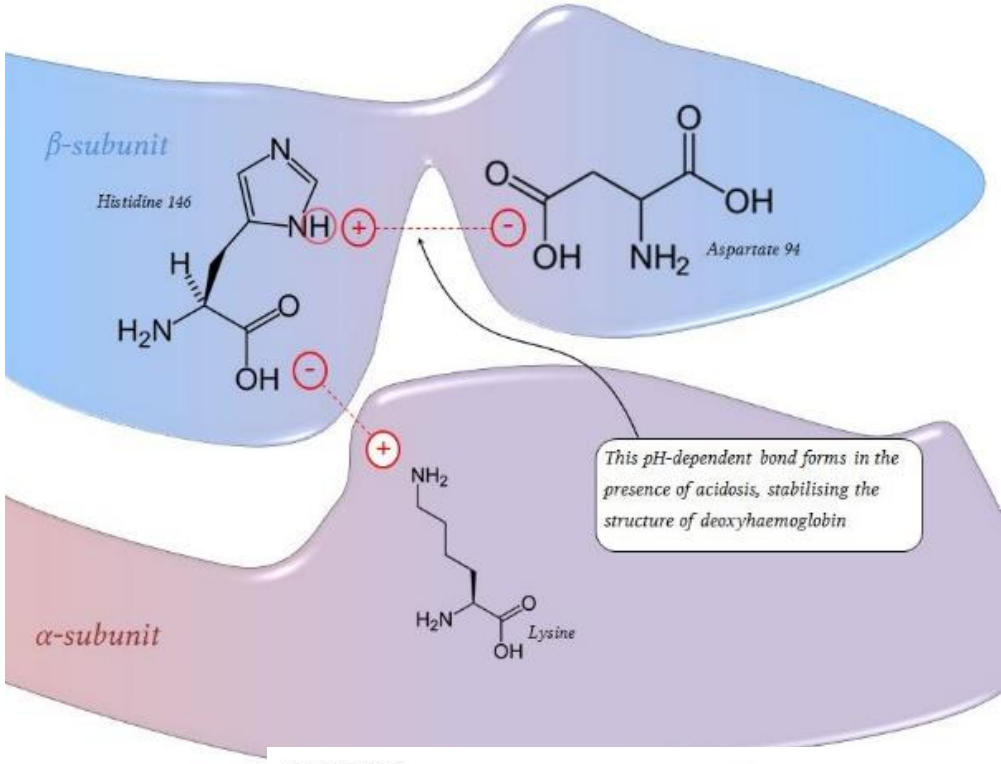
A lower p50 value indicates a **higher affinity**

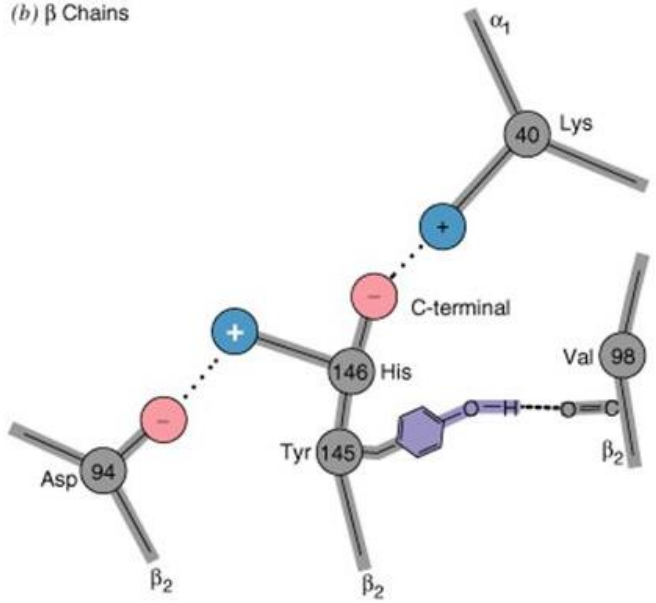



100
80
60
40
20
0
Percent saturation
Myoglobin
pH 7.6
pH 7.4
pH 7.2
pH 7.0
pH 6.8
Venous
pO_2
Arterial
pO_2
0
20
40
60
80
100
120
140
pO_2, mm Hg

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.





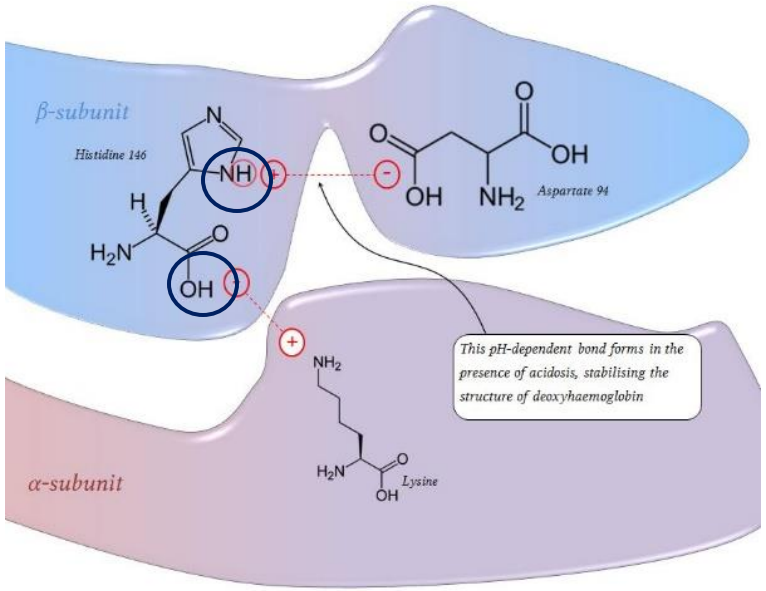
Note
- When pH> pKa, the group is deprotonated.
- When pH < pKa, the group is protonated.

Hb = Hemoglobin

- In tissues with active metabolism, H+ levels are elevated (making the environment slightly more acidic than the normal pH of 7.4) due to the increased production of H+ and CO2, which are byproducts of the metabolic activity. (the contribution of CO2 to H+ levels will be explained soon)
- This will cause the Bohr effect —> lowering the affinity of Hb for O2 —> increasing the release of O2 in the tissues.

- How does this happen?
- The His146 on Hb beta chain molecule plays the main role in this effect!
- In normal H+ levels (in lungs) —> the imidazole ring of His146 has pKa of 7.3 —> His146 is deprotonated (losses protons circled in the pic) —> His 146 losses electrostatic interactions —>Hb is more likely to be in R state

- In high H+ levels (in tissues) —> the imidazole ring of His146 has pKa of 7.7 —> His146 is protonated —> electrostatic interactions with Hb alpha chain —> Hb is more likely to be in T state releasing the oxygen.



The effect of CO_2

Where do protons come from?

- 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 sources of H+ in blood are:

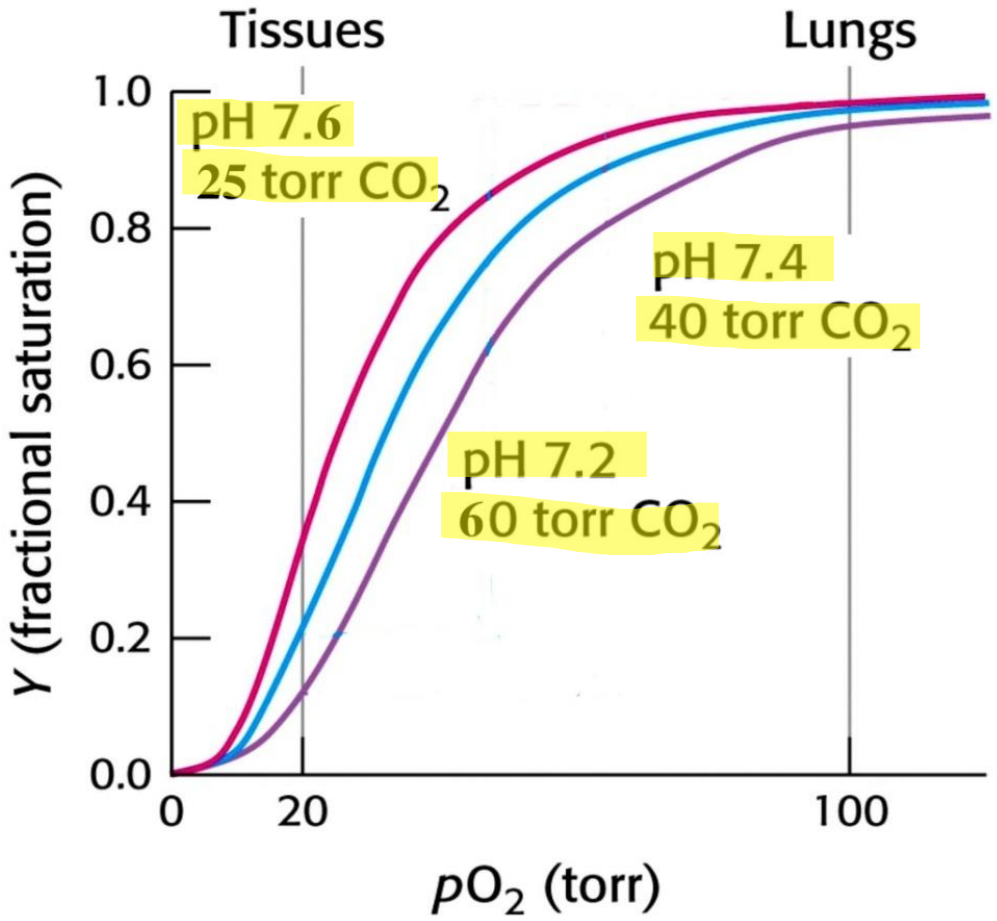
1. Metabolism
2. Carbonic acid (H2CO3) coming from CO2 in the reaction above, it needs an enzyme called **carbonic anhydrase**.

Mechanism #1 - production of protons

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

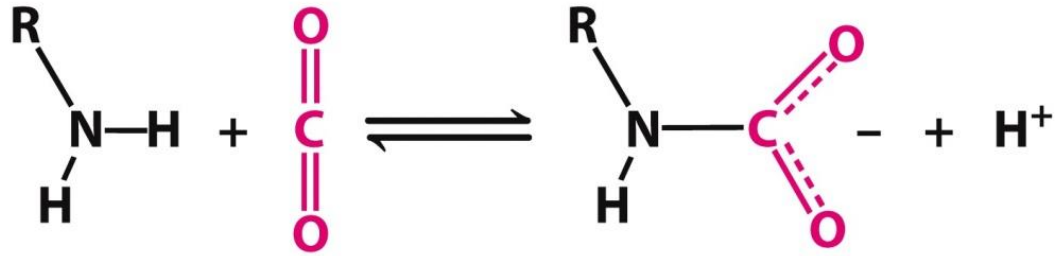
Notice how pH reduces when CO2
pressure increase

P50 increases when pH is reduced (affinity of Hb-O_2 is decreased)



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

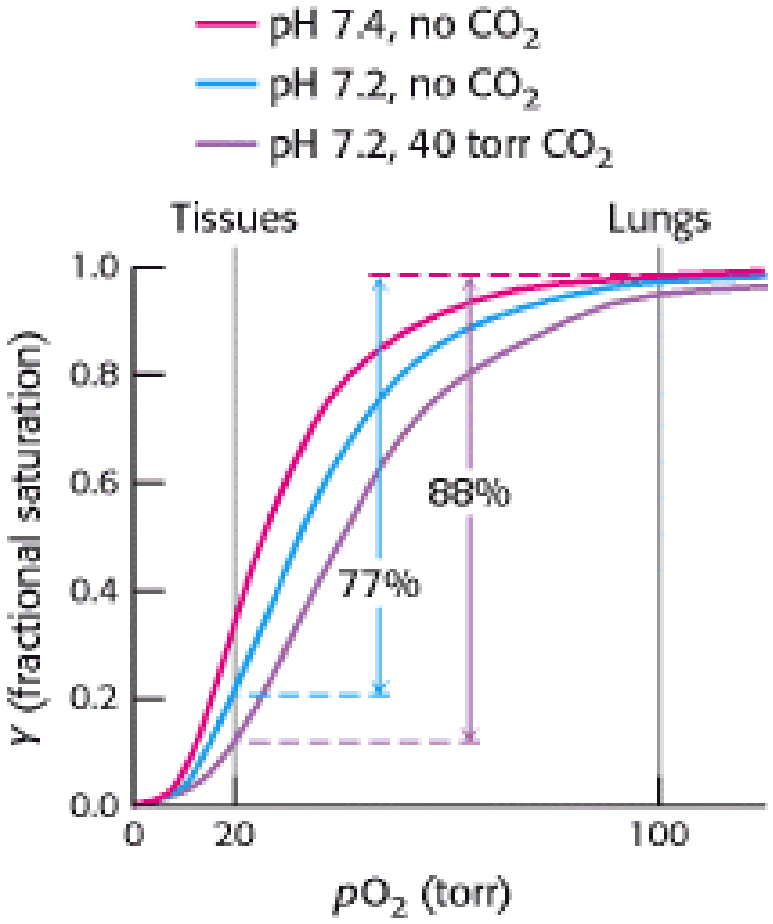
- The increased number of negatively-charged residues increases the number of electrostatic interactions that stabilize the T-state of hemoglobin.

- CO_2 binds on Hb, but not on the heme group.
- The N-terminus of the Hb subunits are normally positively charged and do not make any electrostatic interactions.
- CO_2 binds on the free alpha N-terminus of the subunit making it a negatively charged carbamate, which does make electrostatic interactions, favoring the stability of the T state of Hb.

Which mechanism has a stronger effect?

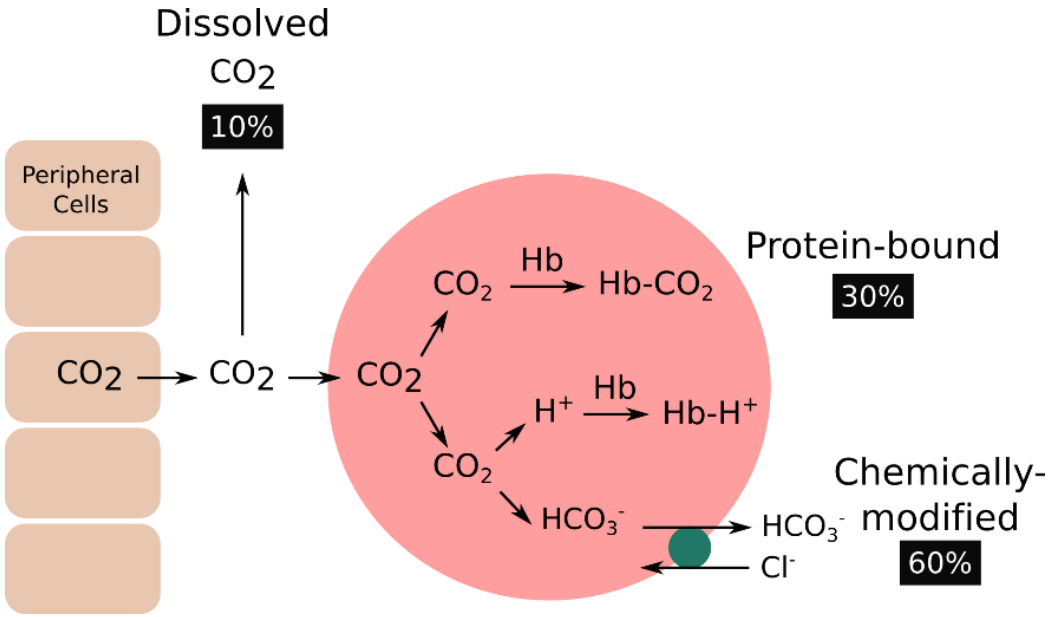
- About 75% of the shift is caused by H^+.
- About 25% of the effect is due to the formation of the carbamino compounds.

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.



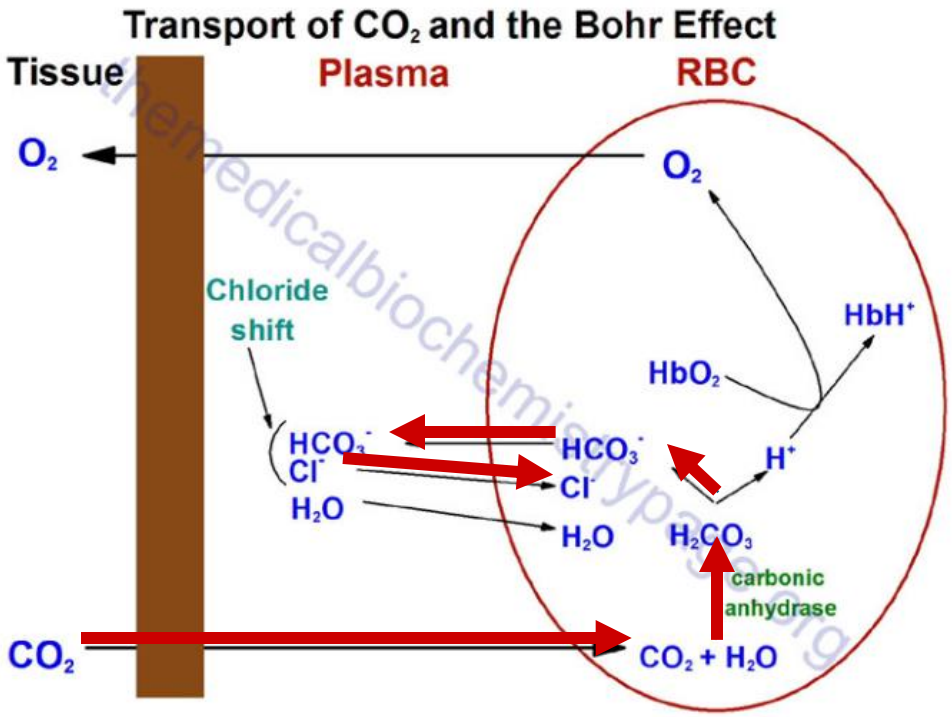
The movement of CO_2 in/out of cells does not change the pH, a phenomenon called isohydric shift, which is partially a result of hemoglobin being an effective buffer.

- Hb binding ability for H+ gives it a very good buffer effect, the Hb buffer effect is called isohydric shift.
- The high amounts of the negative bicarbonate (HCO3-) leaving the RBC makes some electric instability, therefore Cl- comes in the RBC in exchange of HCO3- to maintain the electric balance.
- The dissolved CO2 in plasma is only 10% because CO2 is hydrophobic.

Effect of Chloride ion

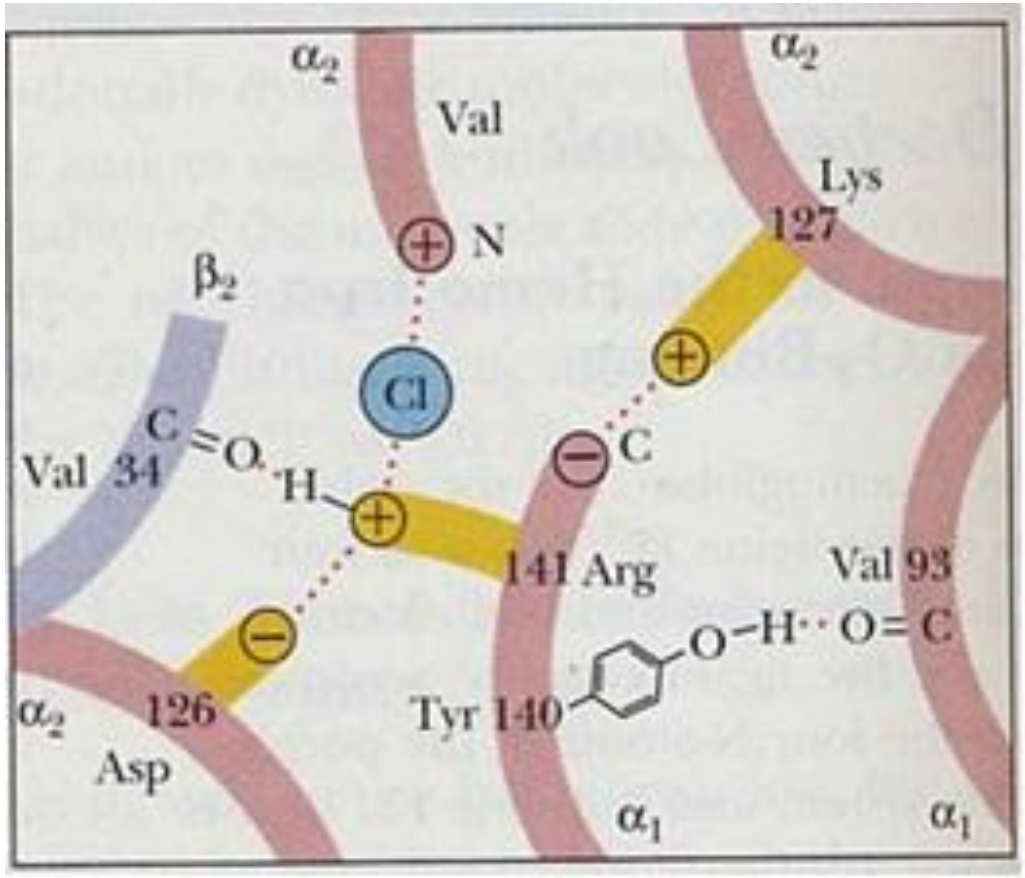
Chloride shift

- 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)



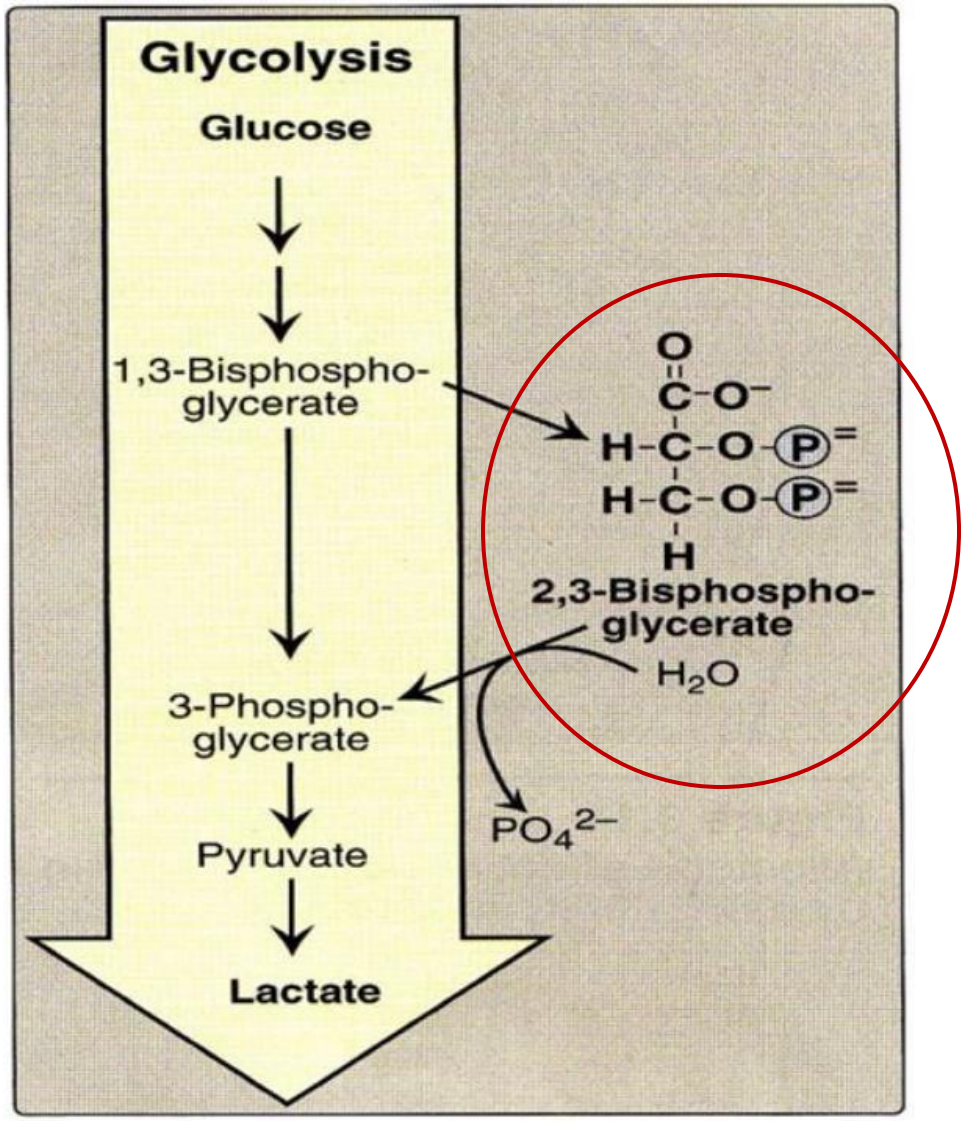
Cl- also has a stabilizing effect on the T state of Hb lowering the affinity for O2.
So when HCO3- leaves the RBC and Cl- enters, it also contributes to the whole O2 affinity-lowering mechanisms we talked about before, and everything works in harmony.

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.

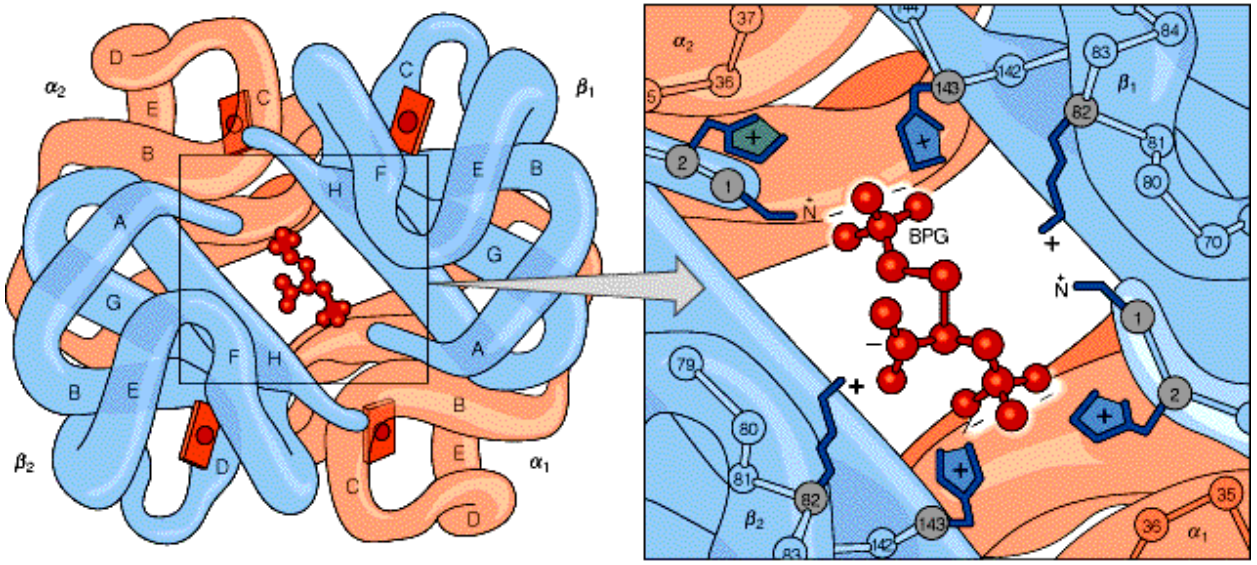
- The difference between bisphospho and diphospho is that in diphospho the 2 phosphates are on the same group on the molecule, while in bisphospho the 2 phosphates are on different groups.



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.



- When Hb is in the T state, it has a space in its center.
- This space gets occupied by a molecule called 2,3- bisphosphoglycerate (2,3 BPG), holding the Hb in the T state, decreasing the affinity for O2.
- 2,3BPG is a normal intermediate of glycolysis which takes place in the cytosol
- No more than 1 (2,3BPG molecule) can bind on 1Hb molecule, so the binding ratio is 1:1.

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.

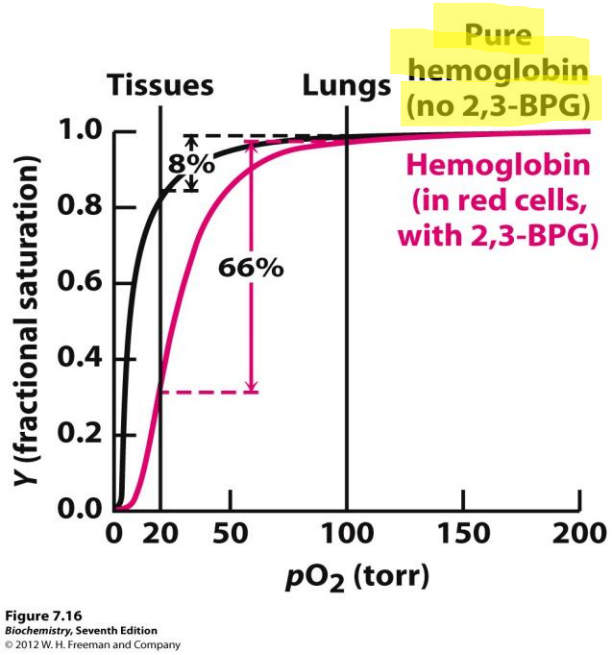
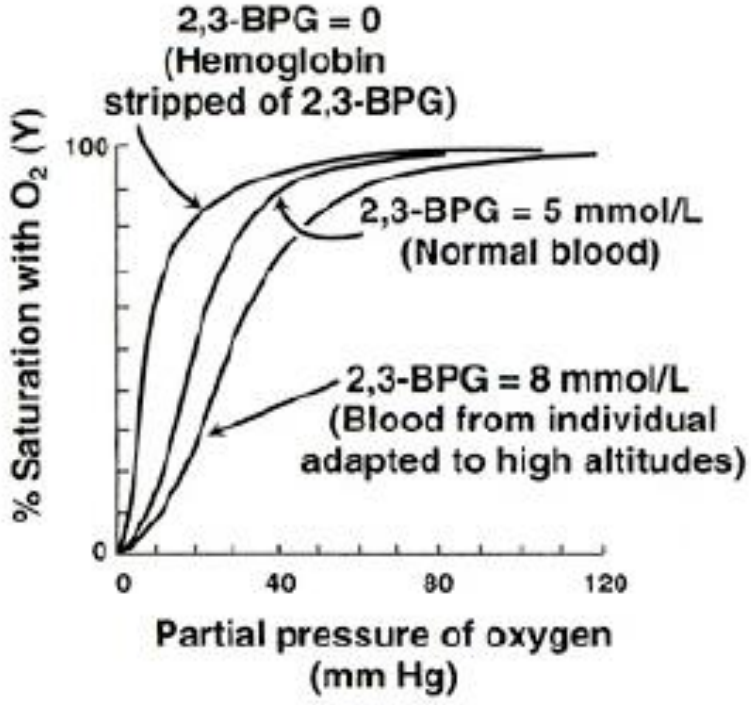


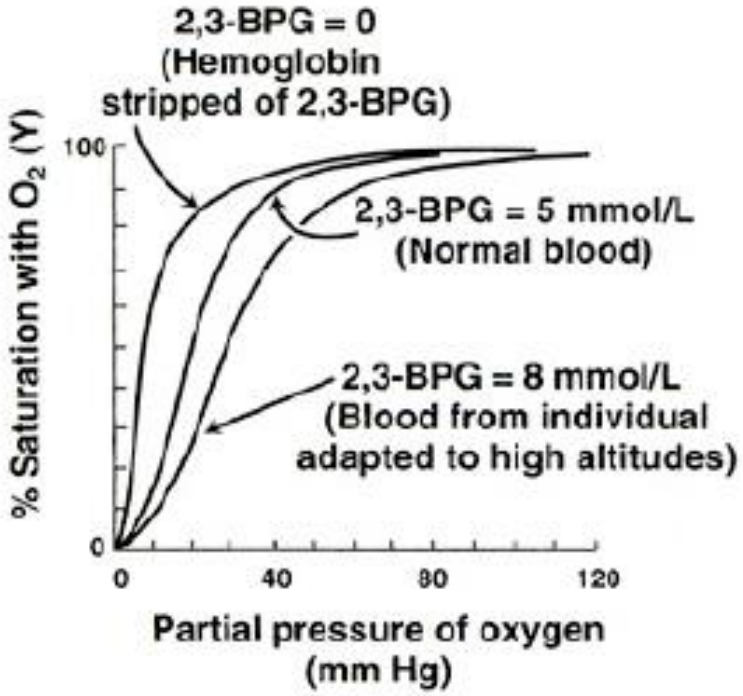
Figure 7.16
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

- 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.



This graph illustrates the effect of 2,3-BPG; without it, the graph resembles that of myoglobin (although it remains sigmoidal), demonstrating how 2,3-BPG reduces oxygen affinity.

At high altitude your body will need time to readjust and adapt the low O2 by increasing the 2,3-BPG in the blood so promotes the release of more oxygen =T-state



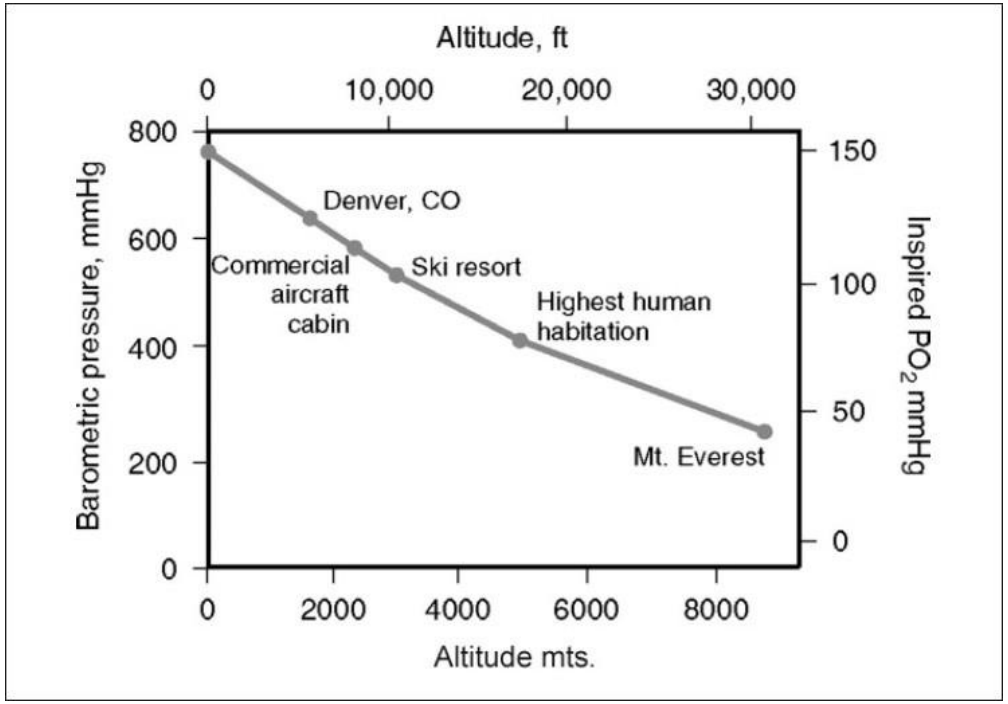
Alright, so if O2 levels are already low, how will it become saturated when it reaches the lungs? Will it arrive at the tissues completely depleted?

Absolutely not! refer back to the first slide.

Hemoglobin is allosteric.

But pO_2 is low at high altitudes!!!

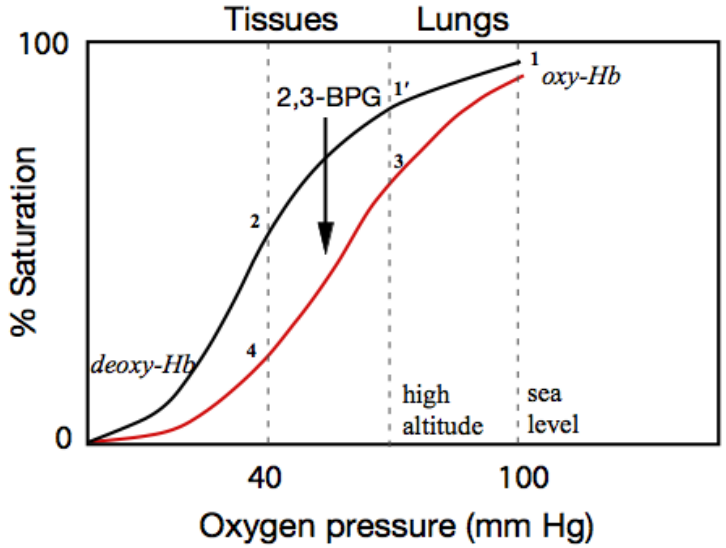
Altitude (feet)	Atmospheric Pressure (mm/Hg)	PAO_2 (mm/Hg)	PVO_2 (mm/Hg)	Pressure Differential (mm/Hg)	Blood Saturation (%)
Sea Level	760	100	40	60	98
10,000	523	60	31	29	87
18,000	380	38	26	12	72
22,000	321	30	22	8	60
25,000	282	7	4	3	9
35,000	179	0	0	0	0



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.

More release compensates less binding !



Under normal conditions, the release of O2 is around 40%. At high altitudes, saturation decreases; however, the presence of 2,3-BPG (causing a rightward shift) allows the release to remain approximately 40%. Without 2,3-BPG, saturation will be higher, but the release will be lower, which is worse.

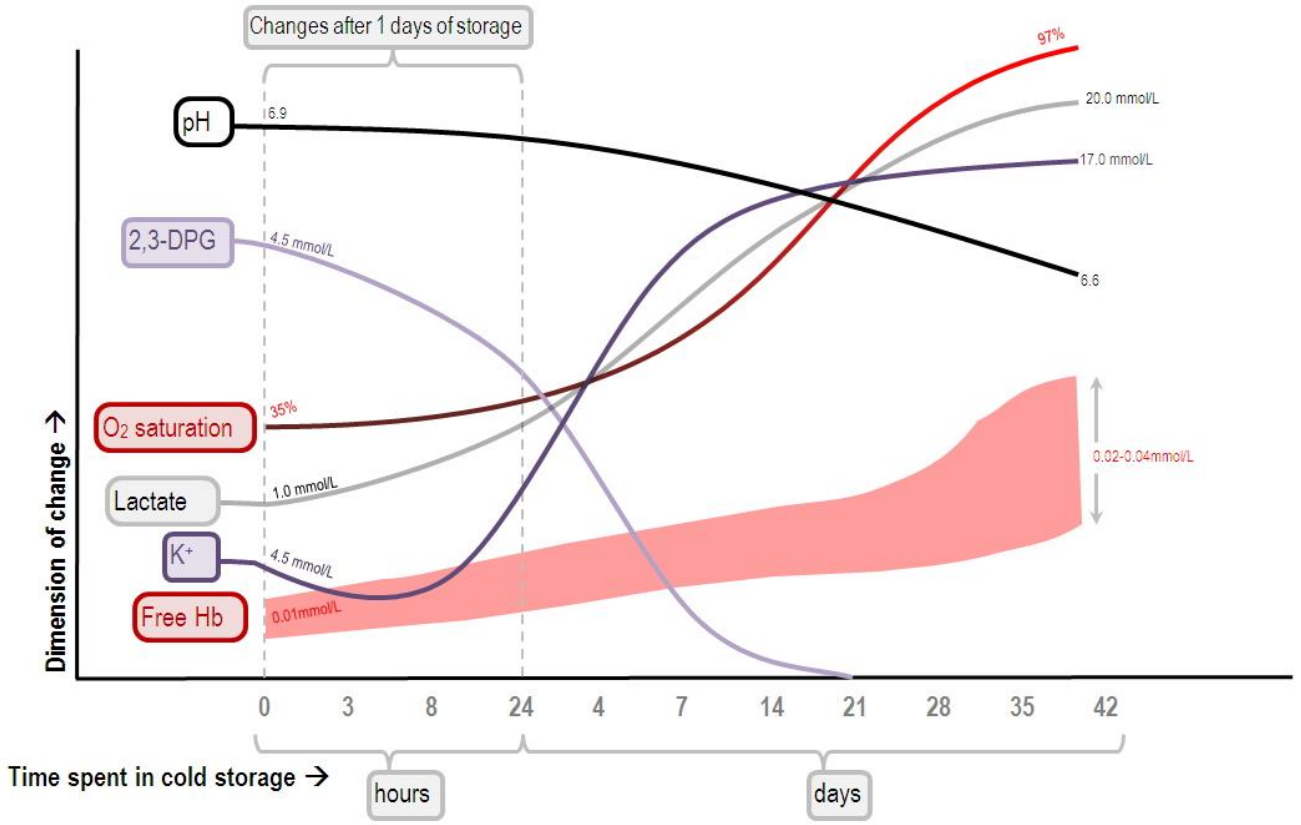
2,3-BPG in transfused blood

- 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.

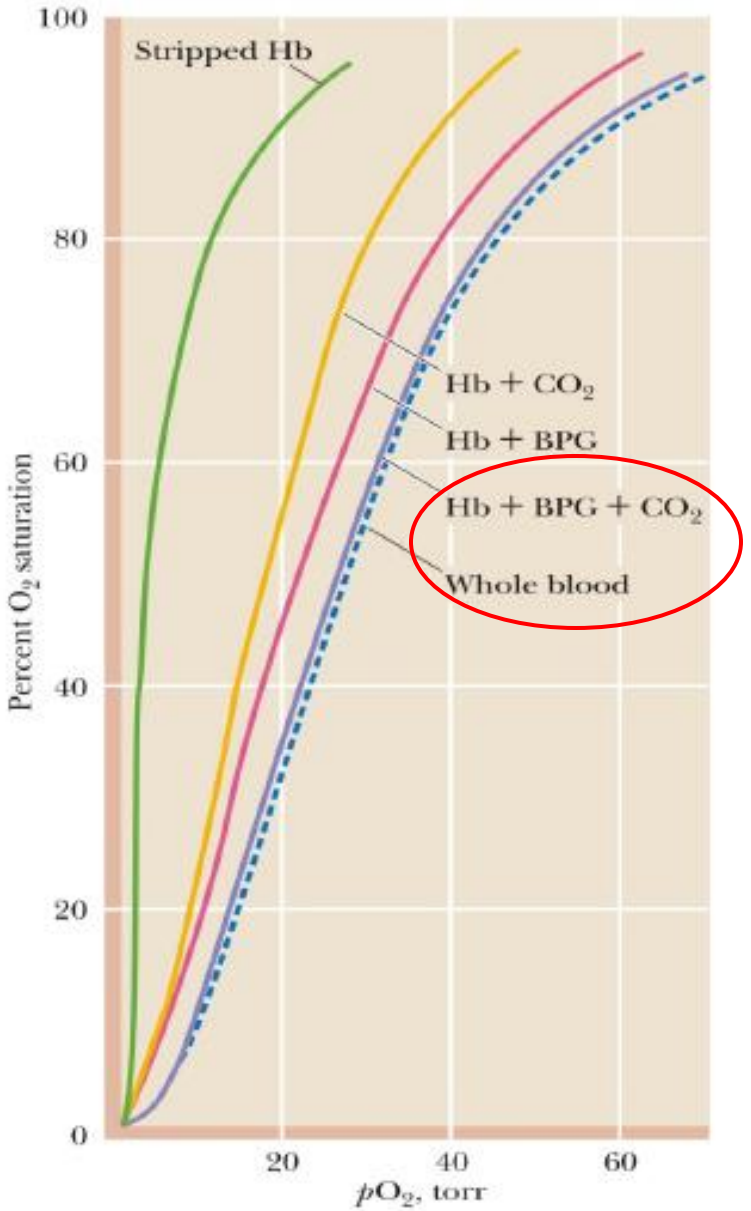
This is considered a long duration, particularly in emergency situations where patients will not benefit from blood lacking 2,3-BPG. Therefore, we inject it to them.

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

With time,2,3-BPG is degraded so the blood Hb can’t release the O2



2,3-BPG and CO2 are important players

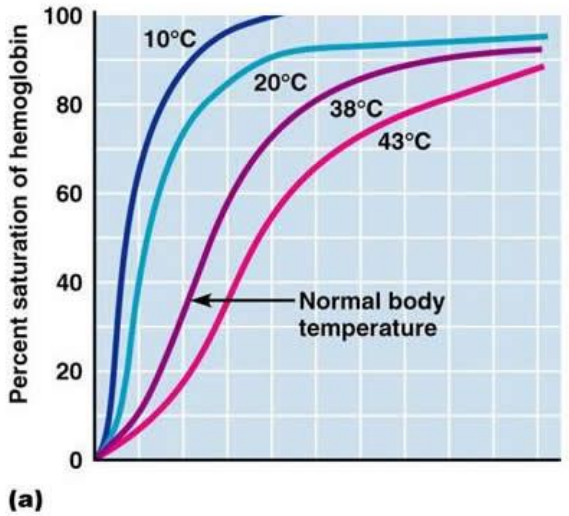


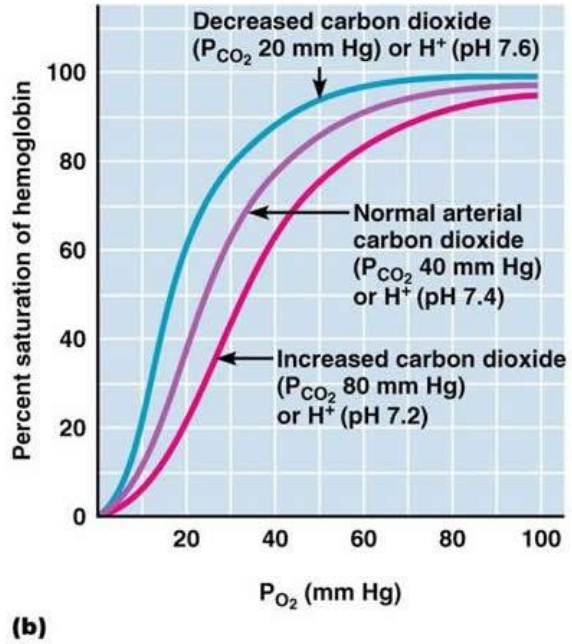
Just notice how these factors all work together.

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.





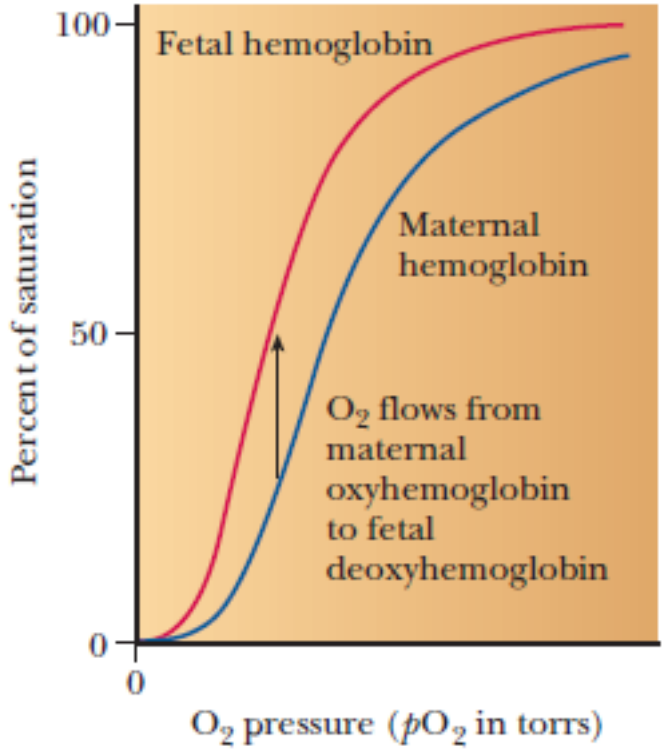
If you're exercising or have a fever, your temperature rises. This shift causes the graph to move to the right, resulting in lower affinity, higher p50, increased O2 release, and a greater demand for O2. Overall, higher temperatures enhance the release of oxygen.

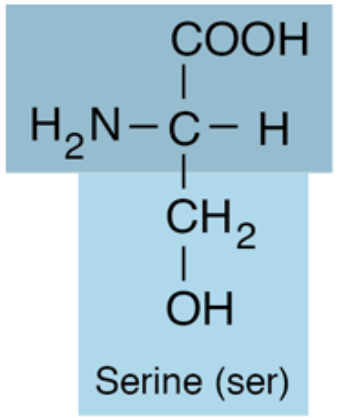
Other considerations

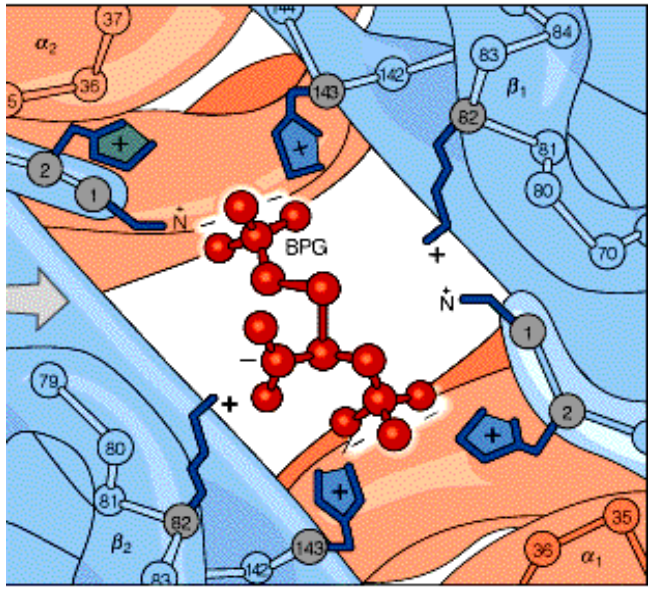
Fetal hemoglobin

- Fetal Hb (HbF) has higher(present in
- R-state- it takes/steals O2 from the mother cuz of the fewer interaction with 2,3-BPG)
- 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. (SER is the major change but we have more changes in amino acids)
  - Since serine cannot form a salt bridge with 2,3-BPG, it binds weaker to HbF than to HbA.

Since serine cannot form a salt bridge with 2,3-BPG like His143 does in adults, this weakens the binding of 2,3-BPG to hemoglobin, resulting in less stabilizing of Hb at T-state a lower p50 and higher affinity.





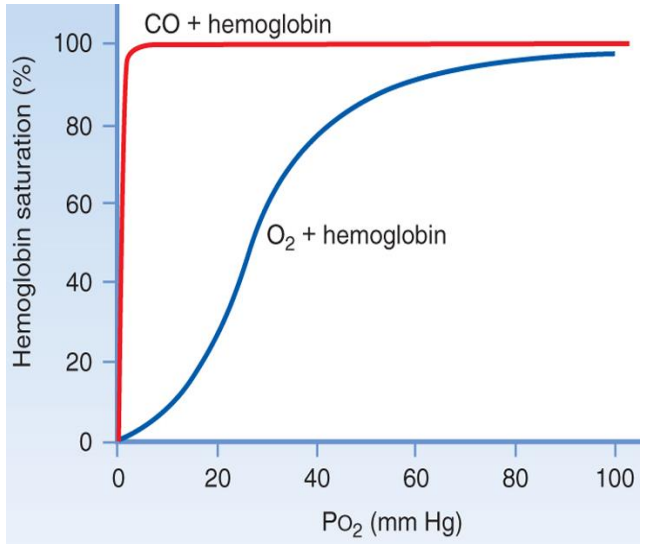


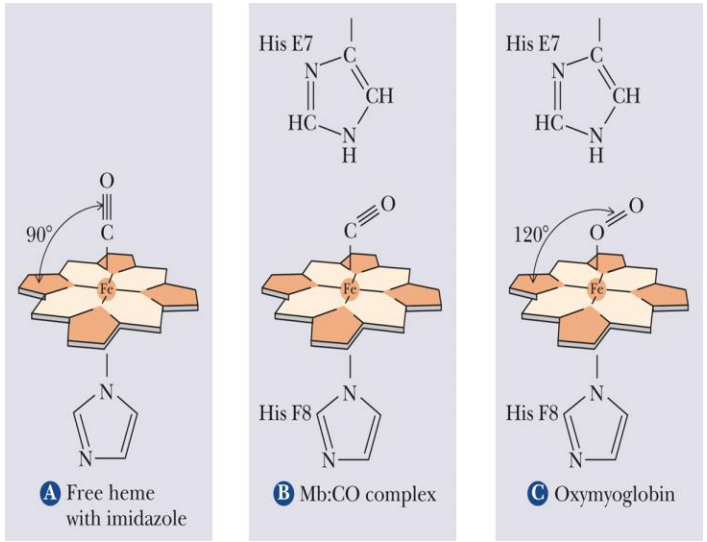
Effect of CO

Effect of CO

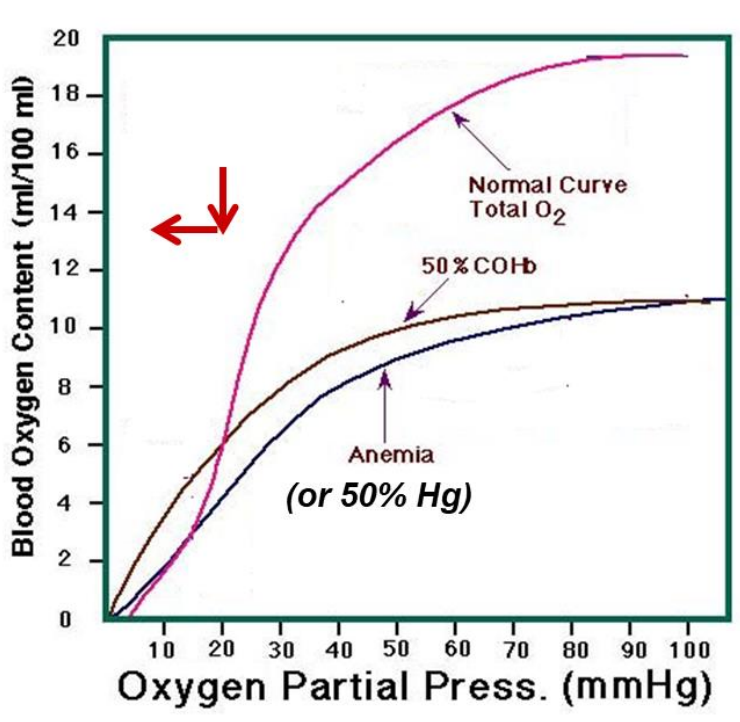
- In addition to competing with oxygen in binding to hemoglobin, the affinity of Hb-CO towards oxygen increases resulting in less oxygen unloading in peripheral tissues.

(Hb + O_2) versus (Hb + CO)





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



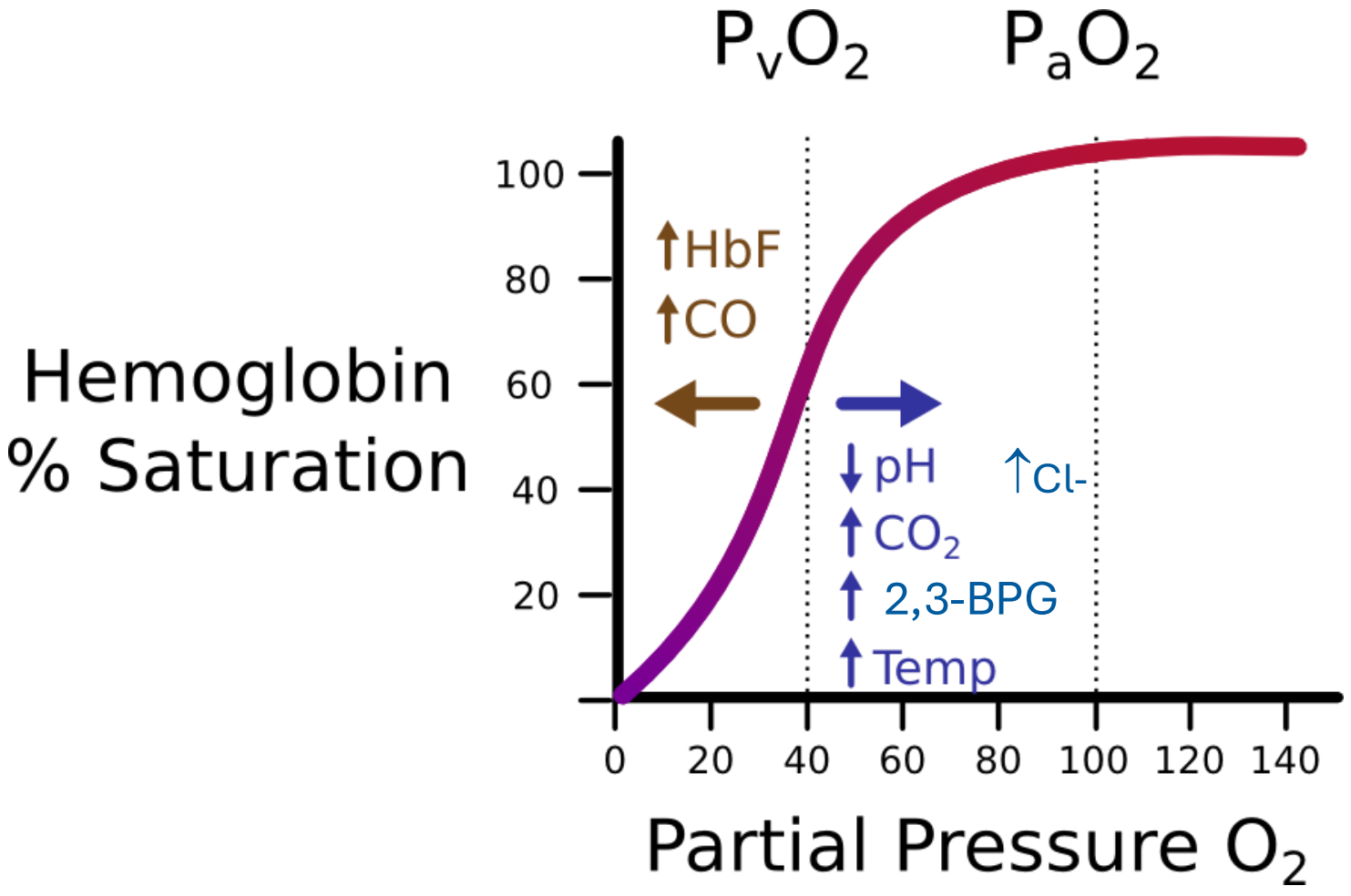
It's compared to anemia because the binding of CO is irreversible (it does not shift from the R to the T state), resulting in a permanent loss of binding sites for O2 on hemoglobin.

When carbon monoxide (CO) binds to the heme, it keeps hemoglobin in the R-state continuously, significantly reducing the number of available oxygen binding sites. Additionally, CO shifts the oxygen already bound to hemoglobin from the T-state to the R-state, preventing its release in the tissues. Thus, there are two key factors at play: an increase in affinity and a decrease in binding sites.

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

عن أبي مالك الأشجعي عن أبيه أنه سمع النبي صلى الله عليه وسلم وأتاه رجل فقال يا رسول الله كيف أقول حين أسأل ربي قال قل اللهم اغفر لي وارحمني وعافني وارزقني وجمع أصابعه الأربع إلا الإبهام ، فإن هؤلاء يجمعن لك دينك ودنياك

VERSIONS	SLIDE #	BEFORE CORRECTION	AFTER CORRECTION
V1→ V2			
V2→V3			

امسح الرمز و شاركنا بأفكارك لتحسين أدائنا !!