Biochemistry - HLS

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Regulation of hemoglobin function

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Hematopoietic-lymphatic system

In this lecture, we'll be discussing the following :

1. Regulation of hemoglobin function.
2. Different allosteric effectors that regulate the switching between T and R states.

Resources

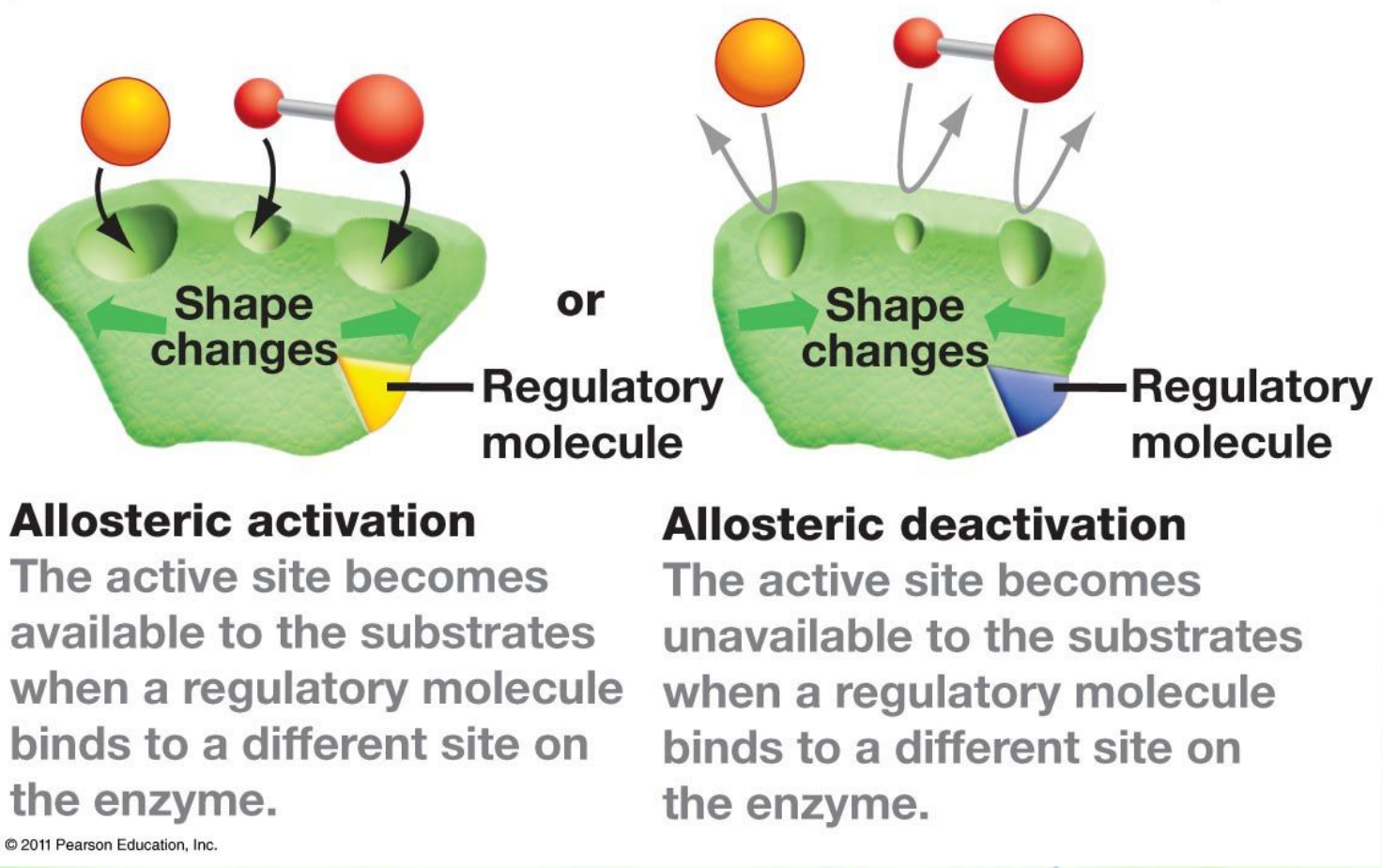
- This lecture
- Mark's Basic Medical Biochemistry
  - Check the index at the end of the book; Ch. 7

Allosteric regulation

- Remember that :
  1. hemoglobin is an allosteric protein that has 2 states.
  2. These 2 states/structures are regulated by binding to different molecules.

- These molecules don't need to bind to the same place where the substrate or the original molecule (like O2) binds to meaning that they can bind to regulatory regions rather than binding to the oxygen binding site.

- This are known as allosteric effectors.



Allosteric effectors

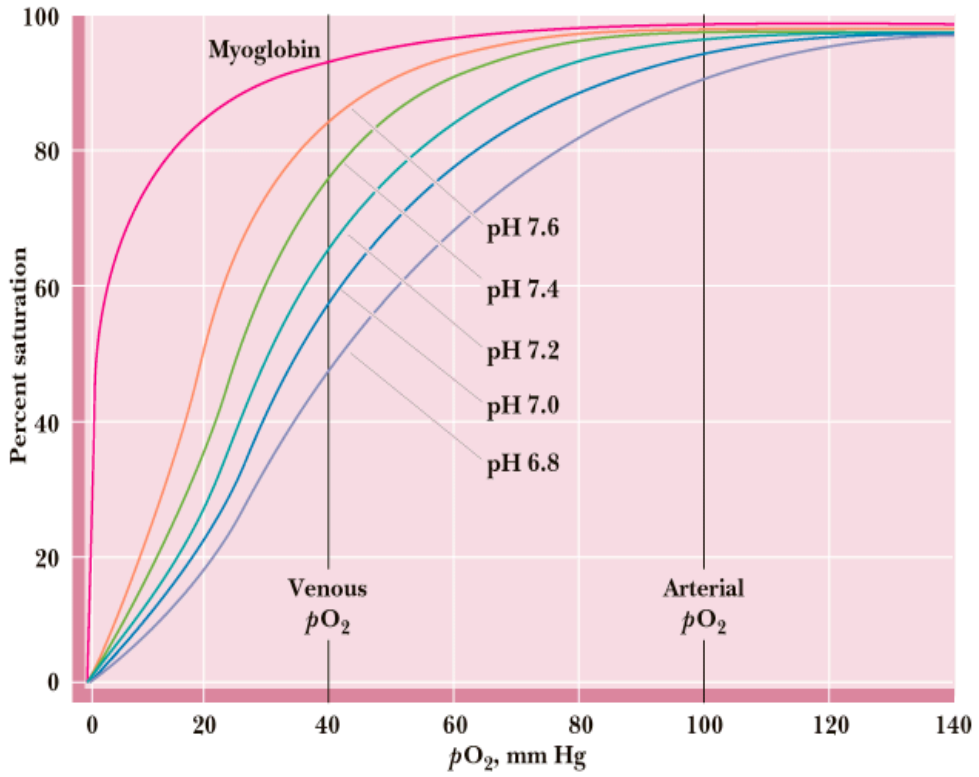
- The major heterotropic effectors of hemoglobin (binding of these molecules would affect the binding of different molecules –O2 in our case-)
  - Hydrogen ion,
  - Carbon dioxide
  - 2,3-Bisphosphoglycerate
  - Chloride ions

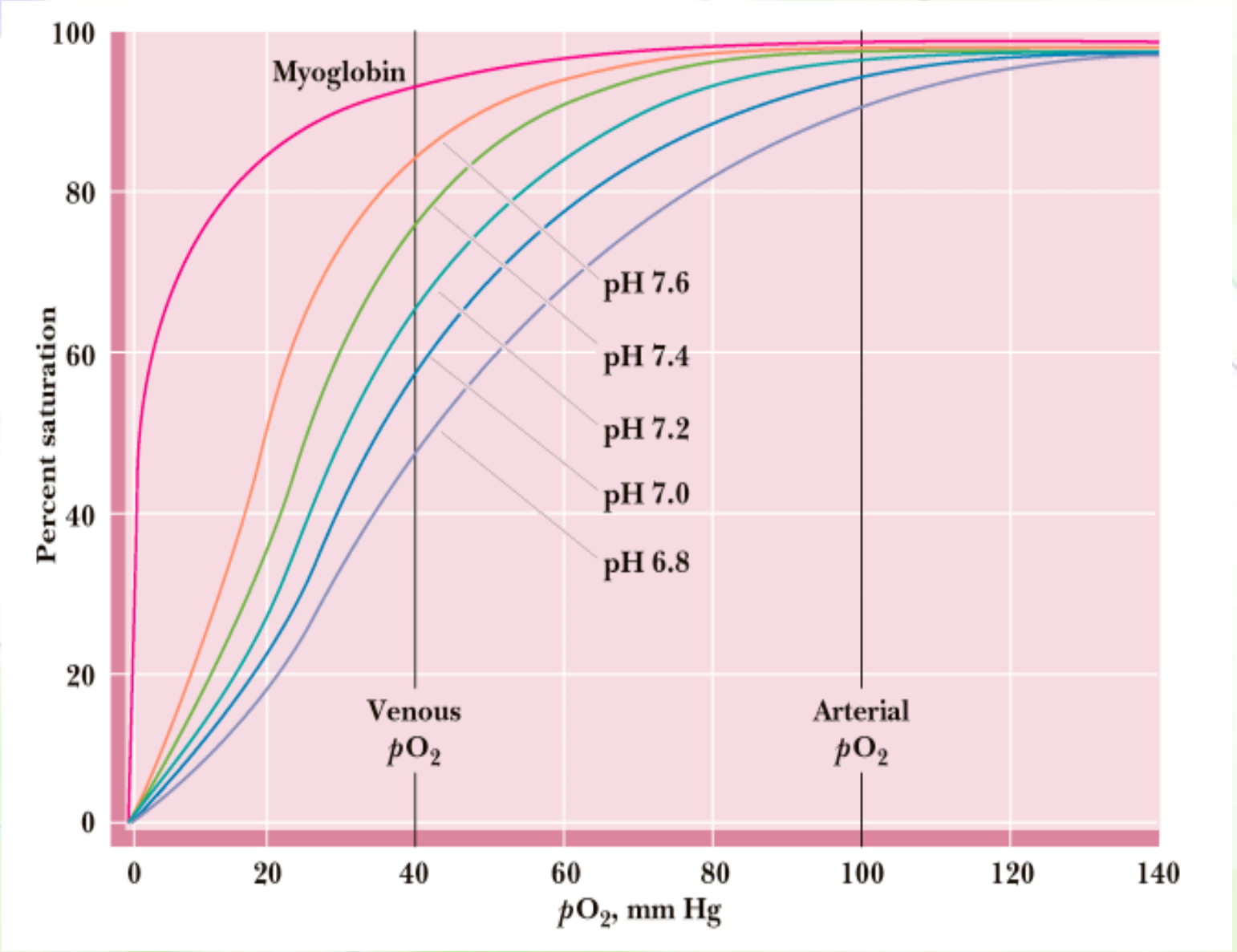
- A competitive inhibitor
  - Carbon monoxide

- Oxygen is a homotropic effector because binding of an O2 would affect the binding of another O2 (we're talking about the same molecule).

The effect of pH

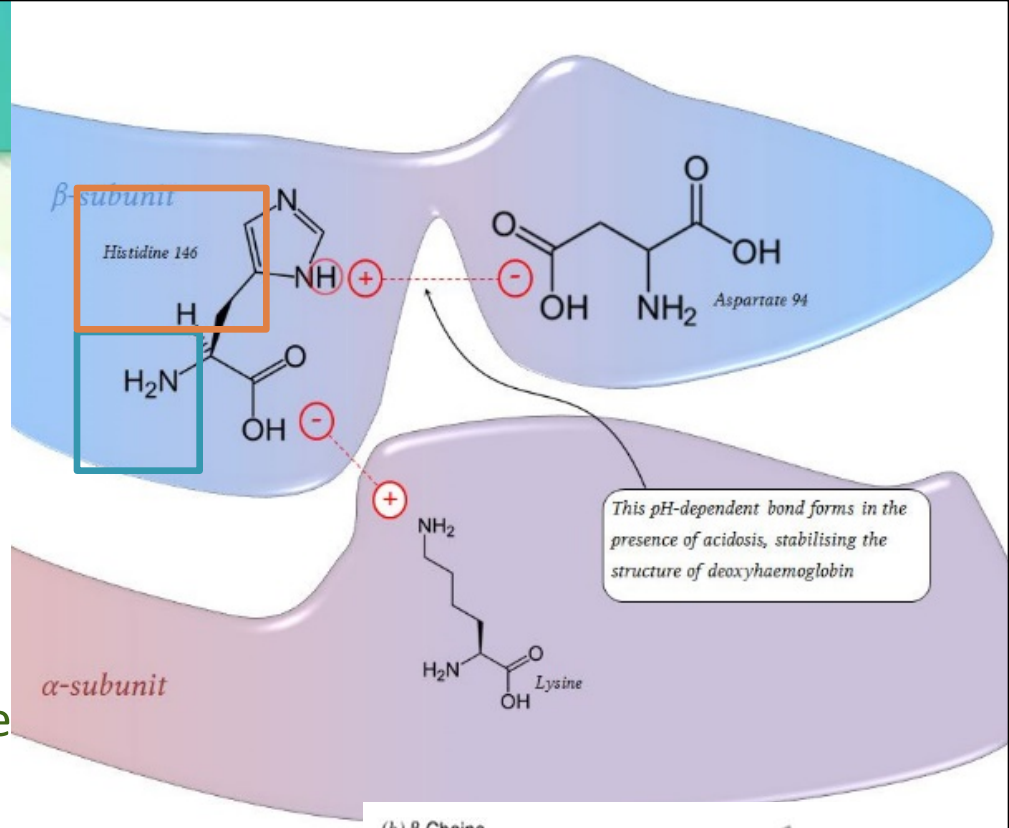
- The binding of H+ to hemoglobin promotes the release of O_2 from hemoglobin and vice versa.
- This phenomenon is known as the Bohr effect (the effect of pH on O2 affinity)
- As pH goes down, affinity goes down as well. More hydrogen (surrounding hemoglobin), less pH, less affinity.
- when pH decreases, we need more oxygen to get 50% saturation of the hemoglobin molecule/s.
- When pH increases (lowering the amount of protons), the affinity increases and the amount of oxygen needed to reach 50% saturation decreases.

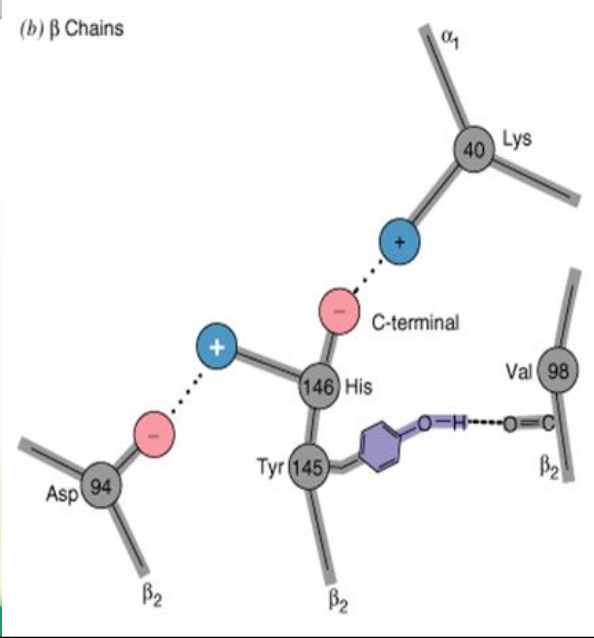



100
80
60
40
20
0
Percent saturation
Myoglobin
pH 7.6
pH 7.4
pH 7.2
pH 7.0
pH 6.8
Venous
pO_2
Arterial
pO_2
0
20
40
60
80
100
120
140
pO_2, mm Hg

Mechanism of Bohr effect

- Increasing H+ causes the protonation of key amino acids, including the last histidine residue of the β chains (His146).
- 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.
  - The reduction in pKa means that His 146 would lose the proton much easier.
- This favors the deoxygenated form of hemoglobin.
- Histidine has 2 charged groups (carboxyl group and R group). These 2 groups can form electrostatic interactions.
- The switching from T → R state causes a change in the pKa (it's the pH where the concentration of the conjugate base is equal to the concentration of the acid itself , Unprotonated / protonated).
- When the R group loses the proton → no more positive charge → no more electrostatic interaction between R group and Asp94 and the molecule becomes more relaxed (R state).





Where do protons come from?

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

CO2 combines with water (enzyme : carbonic anhydrase) forming the carbonic acid which dissociates into bicarbonate ion and protons

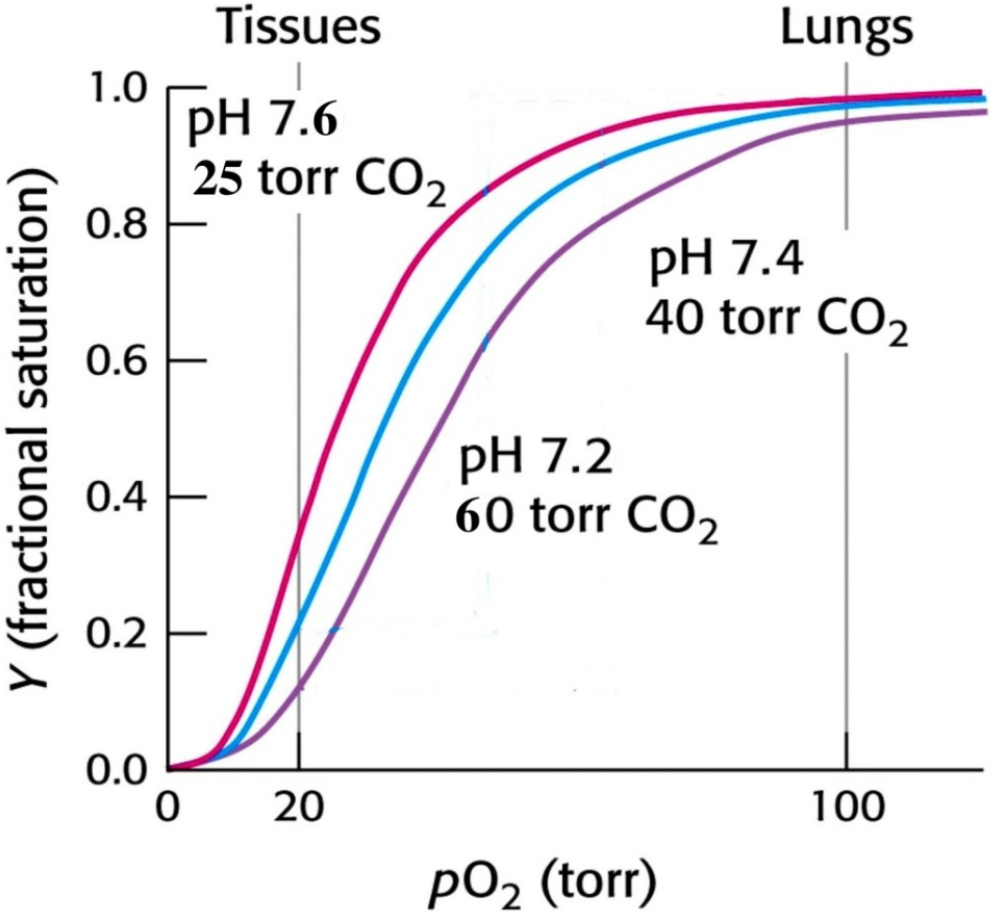
- CO_2 and H+ are produced at high levels in metabolically active tissues by carbonic anhydrase. (production can be by metabolism and Kreps Cycle)
- pH in tissues is lower than pH in the lungs and that's due to metabolism and the production of a lot of protons which will cause a protonation of His146 and formation of the T form and this makes sense because we need low affinity in tissues so that oxygen would detach and leave the hemoglobin to be releases in the tissues.
- 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)

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

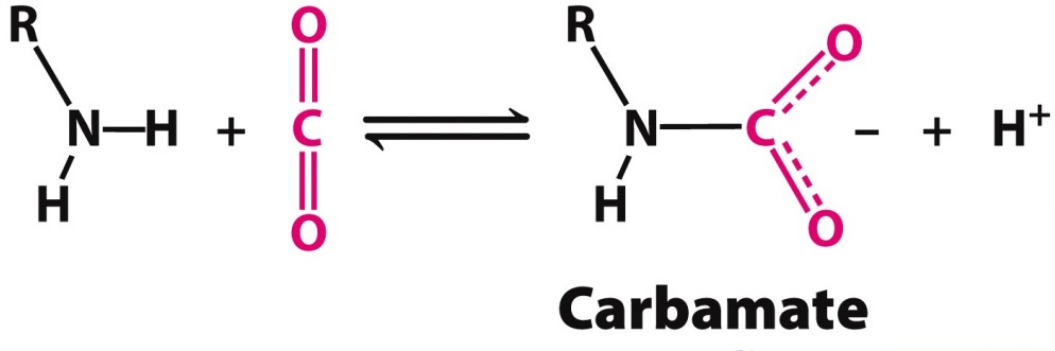
- The more CO2 you have (the more protons you produce), the lower the pH
- Note the pressure of CO2 in different situations regarding pH
  1. 25 torr → 7.6 pH
  2. 40 torr → 7.4 pH
  3. 60 torr → 7.2 pH



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

- CO_2 itself can bind to the free alpha amino terminal groups of polypeptides forming what is known as carbamate.
- This would create more negative charges → more electrostatic interactions can take place and stabilize the T state of hemoglobin molecule



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

How can we know which experiment is more important than the other ?

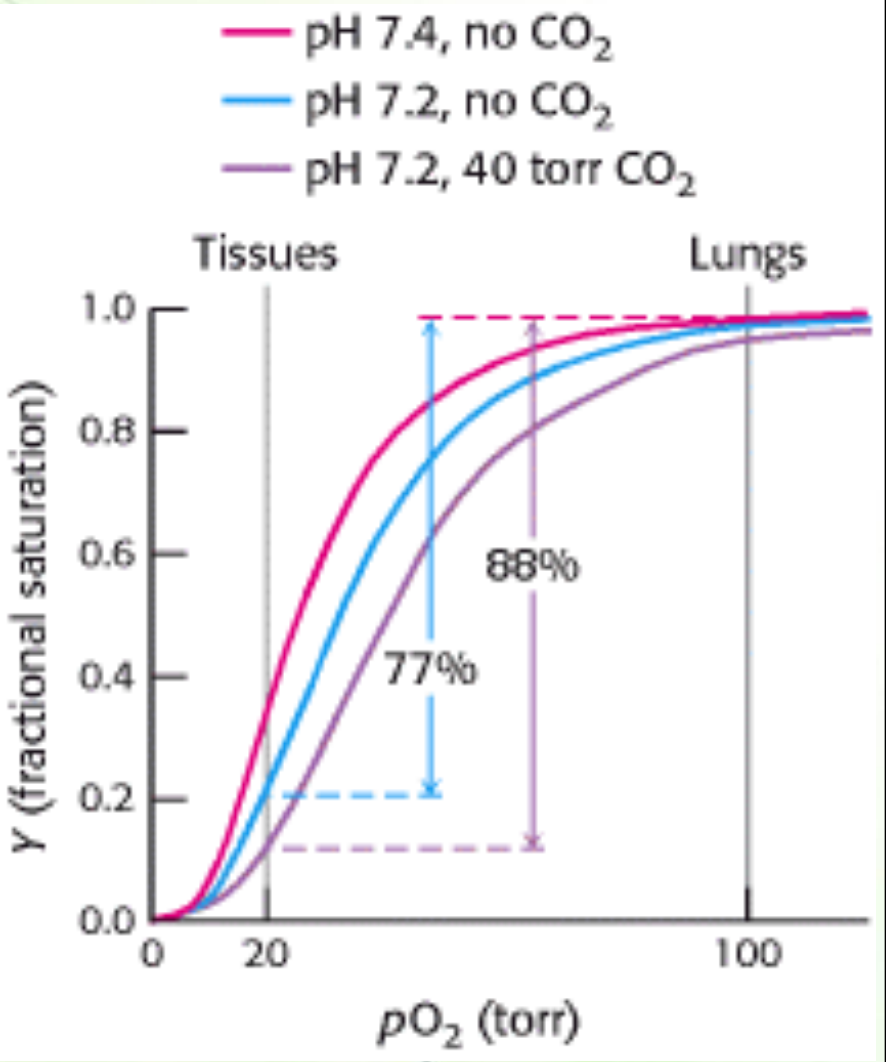
- About 75% of the shift is caused by H+.
- About 25% of the effect is due to the formation of carbamino compounds.

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.

An experiment was done where pH was stabilized using buffers (without affecting CO2)

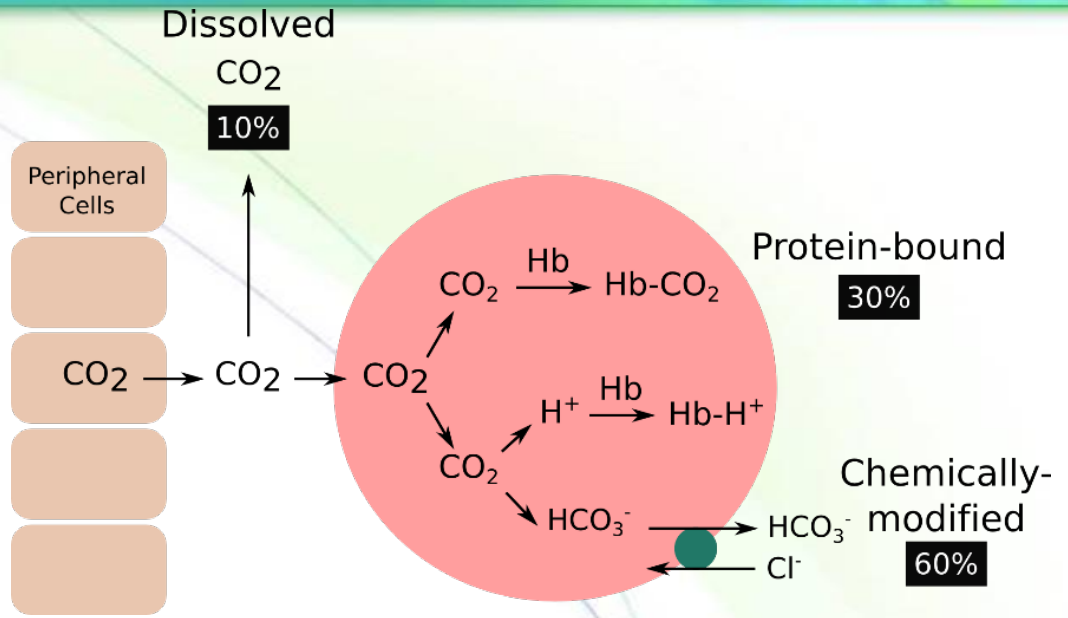
1. Pink VS blue lines can be used to compare the effect of pH (it's the only variable) → there's a shift in the saturation curve to the right (lower affinity when pH is lower)
2. Blue and purple lines can be used to compare the effect of CO2 (it's the only variable) → there's a shift in the saturation curve to the right (CO2 lowers affinity)



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.

CO2 leaves the tissues heading to the blood (red blood cells). Some of the CO2 is bound to the hemoglobin but most of it is converted to ions (bicarbonate goes out)



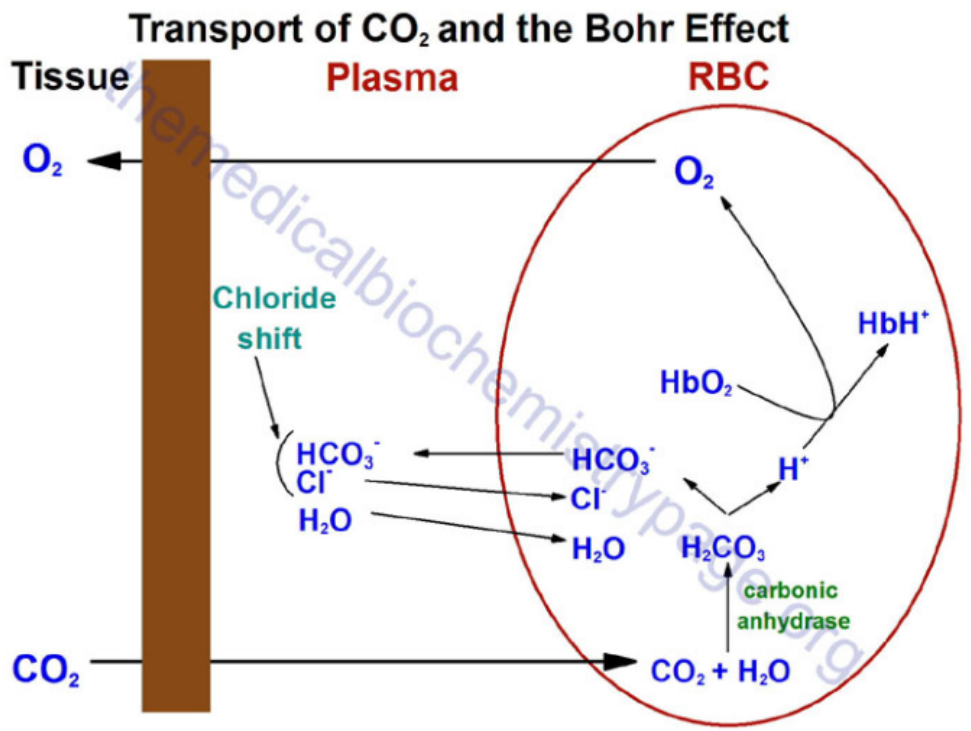
The movement of CO_2 in/out of cells does not change the pH, a phenomenon called isohydric shift, which is partially a result of hemoglobin being an effective buffer.

This means that the formation of protons won't affect the pH.

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. There's a charge moving across the plasma membrane and the difference in charges must be maintained.
- Chloride ion always diffuses in an opposite direction of bicarbonate ion in order to maintair a charge balance.
- This is referred to as the "chloride shift".



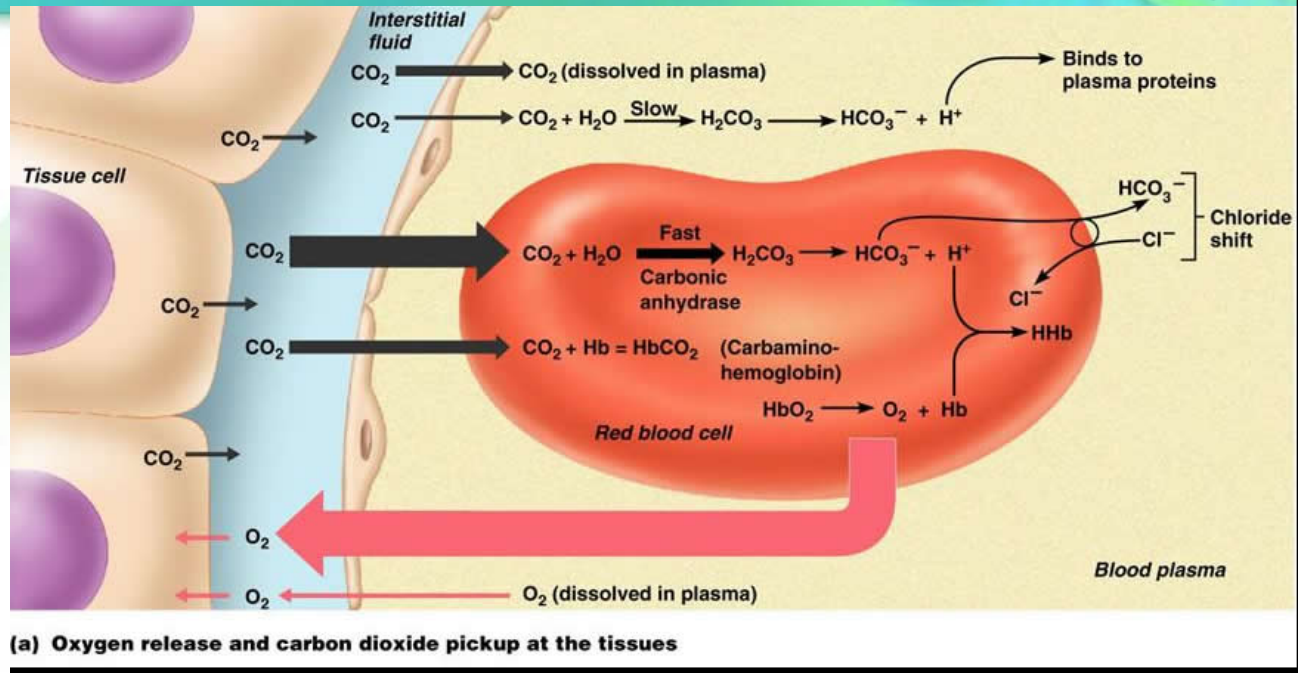
The process was mentioned earlier but go through the figure one more time just to make sure you understand everything :)

Note that :

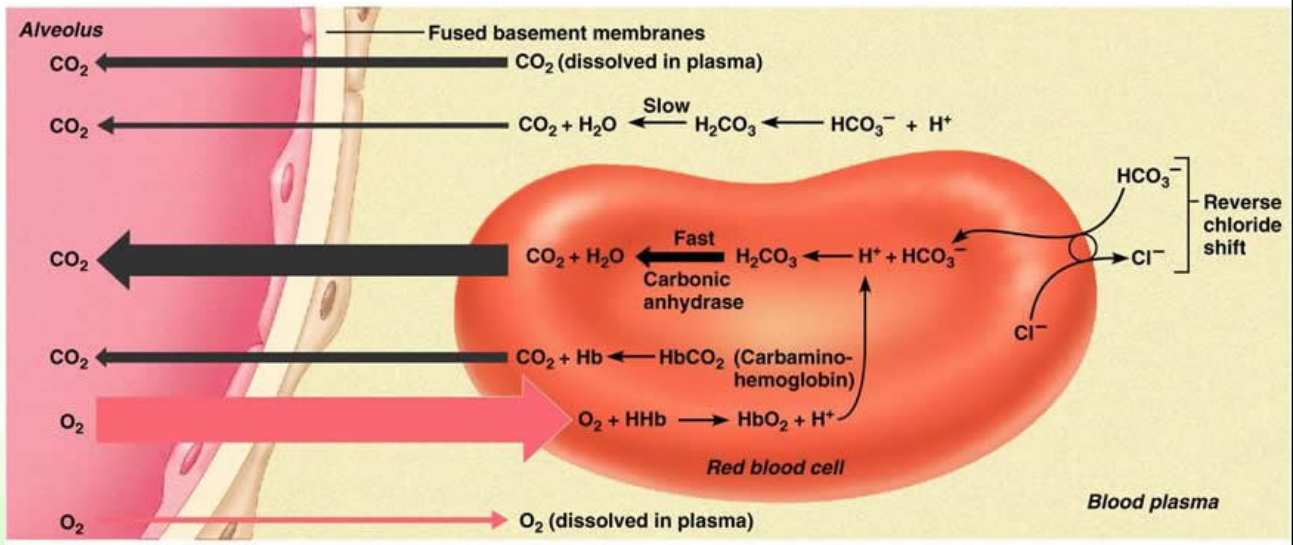
1. In this RBC (figure a) , hemoglobin is mainly in the T state. (switched from R → T) due to Bohr effect .
2. In lungs, the process is reversed and hemoglobin is mainly in the R state.

Q : Is there an effect for chloride on the transition of hemoglobin from T → R state and vice versa ?

A : YES !



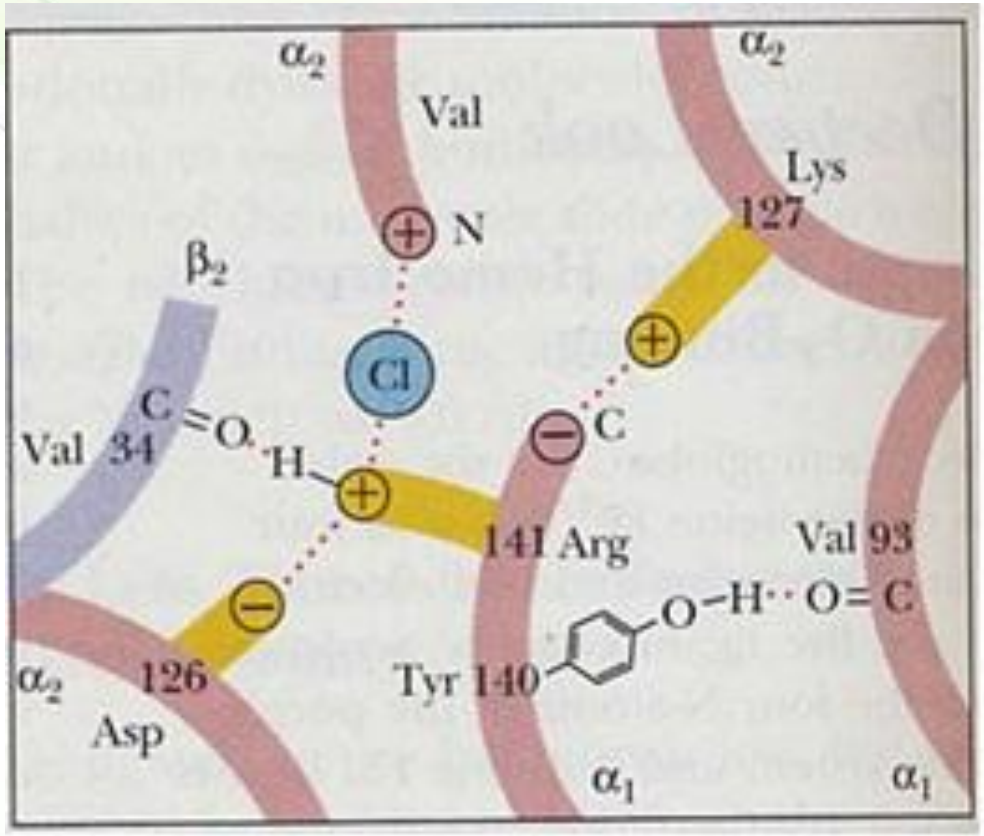
(a) Oxygen release and carbon dioxide pickup at the tissues



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

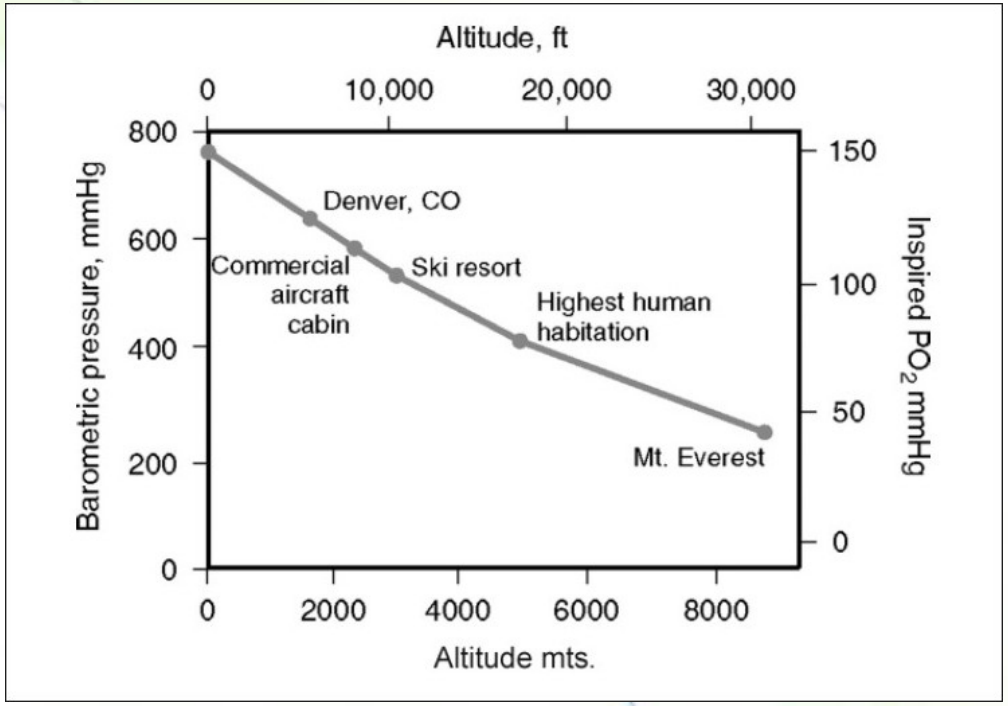
Effect of chloride ions

- Chloride ions interact with N-terminus of α2 chain and Arg141 of α1 chain (this happens once their level inside the cells increase)
- These interactions increase and they stabilize the T structure of hemoglobin lowering the affinity.
- Increasing the concentration of chloride ions (Cl-) shifts the oxygen dissociation curve to the right (lower affinity)
- The opposite takes place in lungs



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



PAO2 = pressure of oxygen in arteries (lungs)
PVO2 = pressure of oxygen in veins (tissues)
So there's a release of 60 torr of O2 in tissues.

if you go to places with high altitudes the pressure of oxygen decreases and the amount of oxygen released I tissues decreases (it's not that much compared to normal situations – sea level)

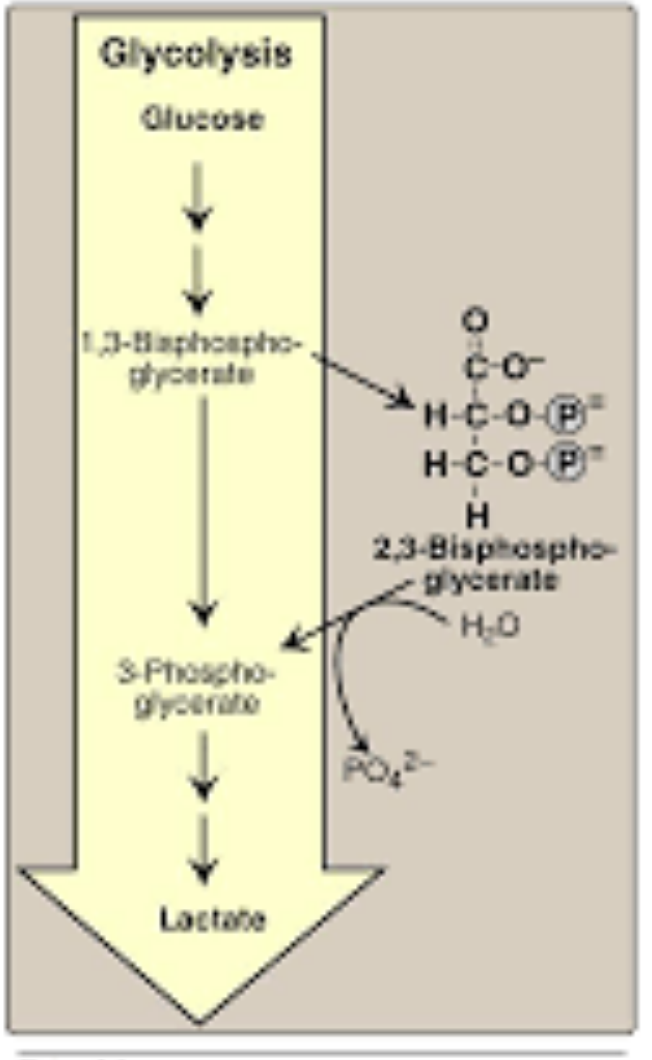
The higher you go, the lower the O2 pressure is

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

Major player

- 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.

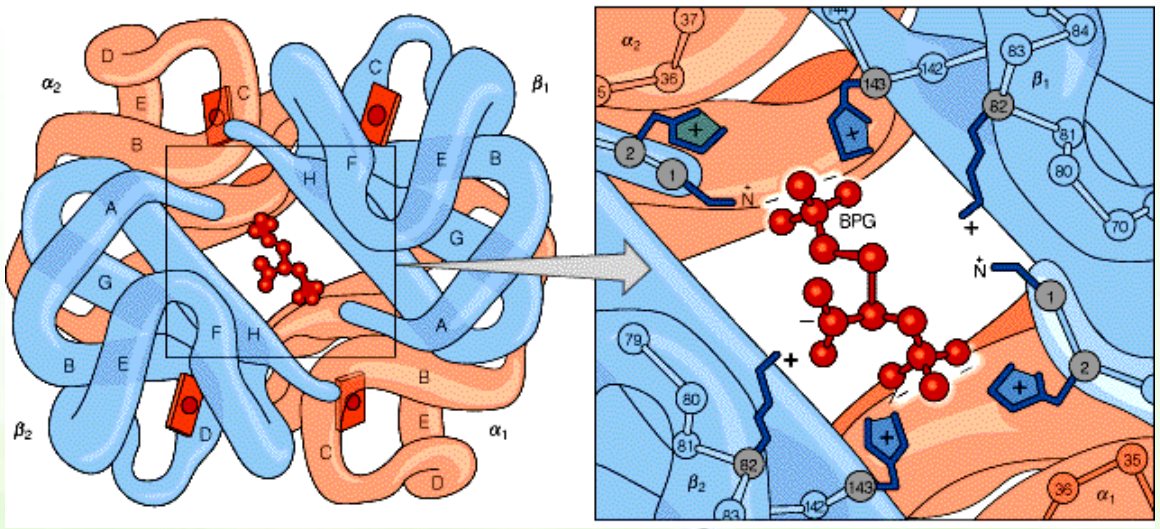
Glucose is converted to pyruvate going through an intermediate known as 1,3-Bisphosphoglycerate which in turn can be converted to an isomer known as 2,3-Bisphosphoglycerate



BPG –hemoglobin interaction

- BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 BPG/hemoglobin tetramer. 2,3 BPG binds to hemoglobin at a ratio of 1:1 mainly when it's in the T state
- This binding increases the energy needed to transform hemoglobin from the T state to R state.
- It forms a lot of electrostatic interactions with 2 lysine 2 histidine, etc. (one of them is His143) and this binding stabilizes hemoglobin in the T state preventing it from rotating around the axis to switch into the R state
- Bound, 2,3-BPG reduces binding of oxygen to hemoglobin and facilitates oxygen release.
- When O2 binds to heme, there'll be some resistance to changing hemoglobin from T → R but eventually BPG will be kicked out so that hemoglobin would change into the R state.

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.
- 2,3-BPG shifts the curve to the right reducing the affinity.
- When there's no 2,3-BPG the sigmoidal curve is shifted to the left (very high affinity, looks like myoglobin).
- If 2,3-BPG amount increases to 8mmol/L , the curve shifts to the right even more → even lower affinity than usual
- If you go to a place with high altitude, you'll have a hard time breathing in your first 2 days, but your body will adapt eventually by increasing the amount of 2,3-BPG

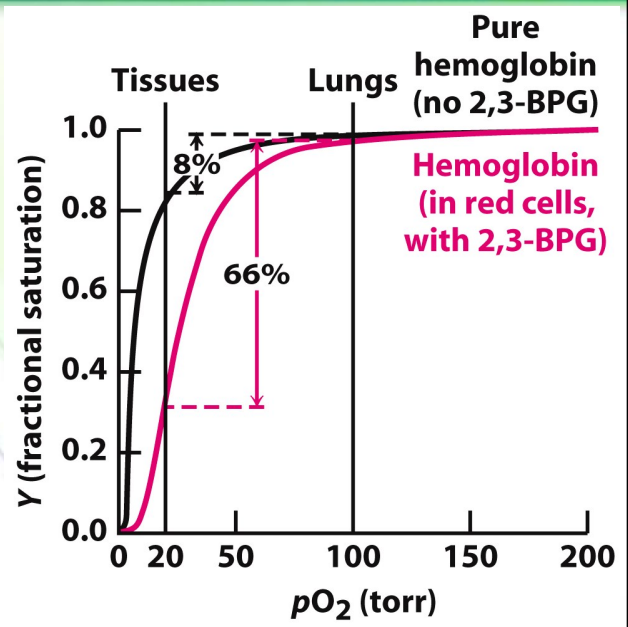
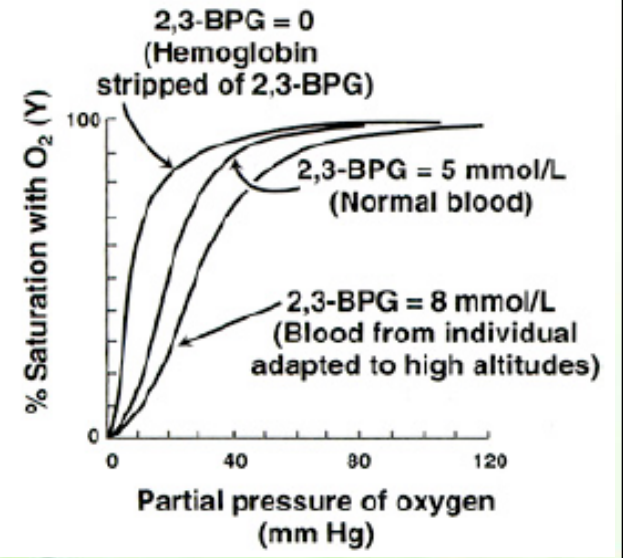
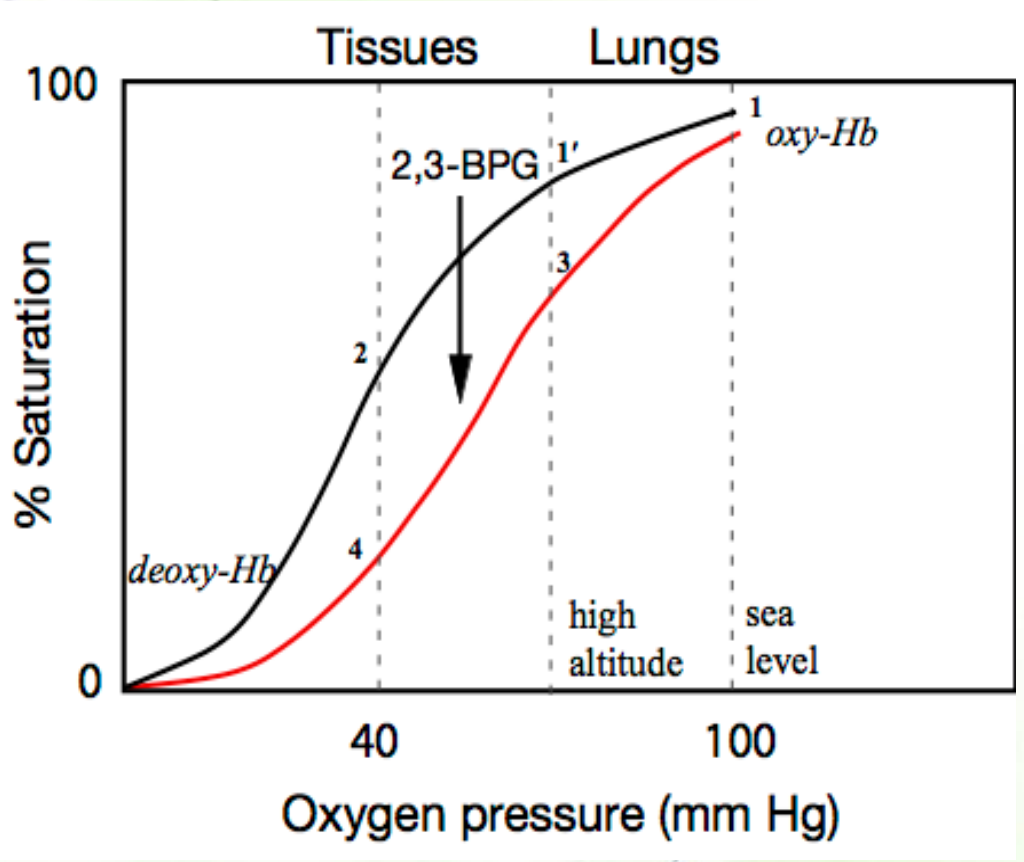


Figure 7.16
Biochemistry, Seventh Edition



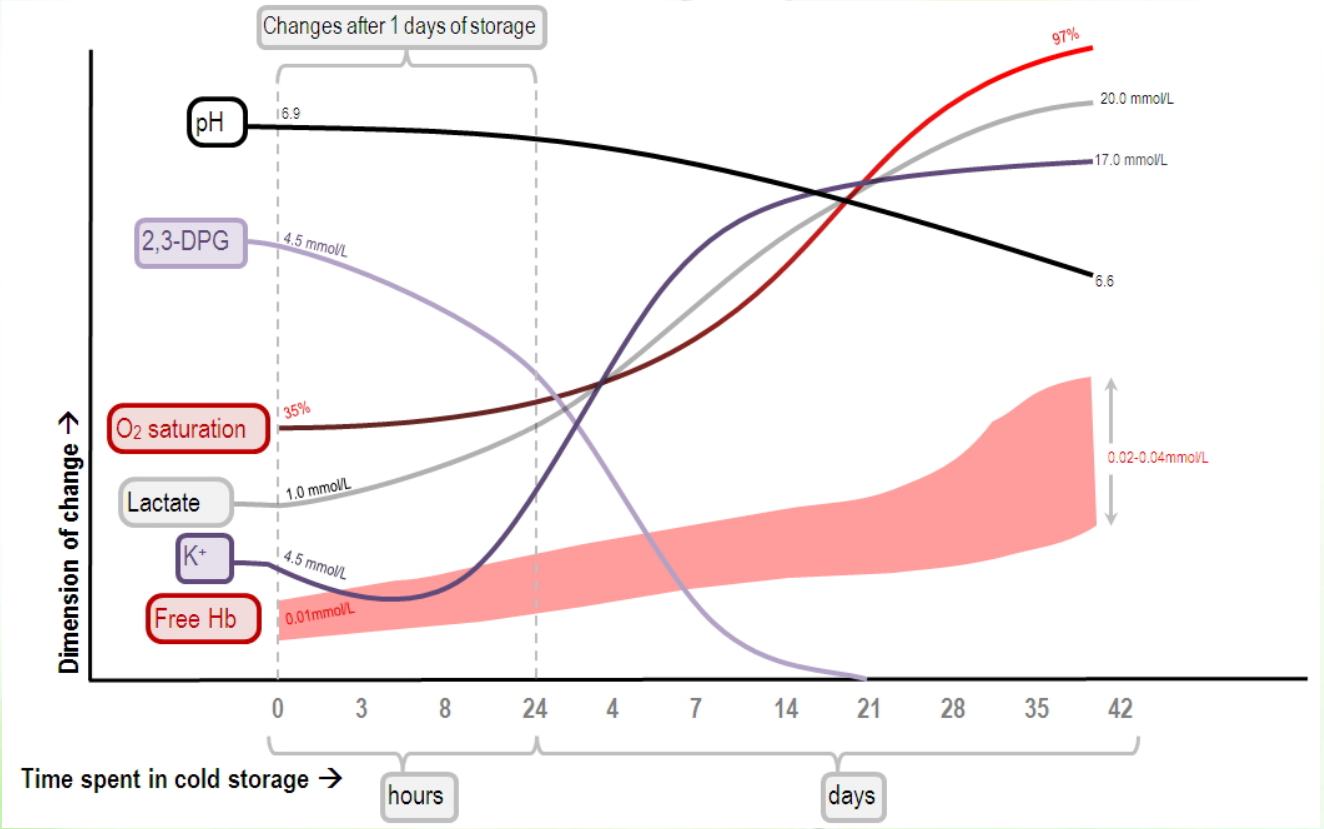
Better explanation of role of 2,3-BPG

- 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.

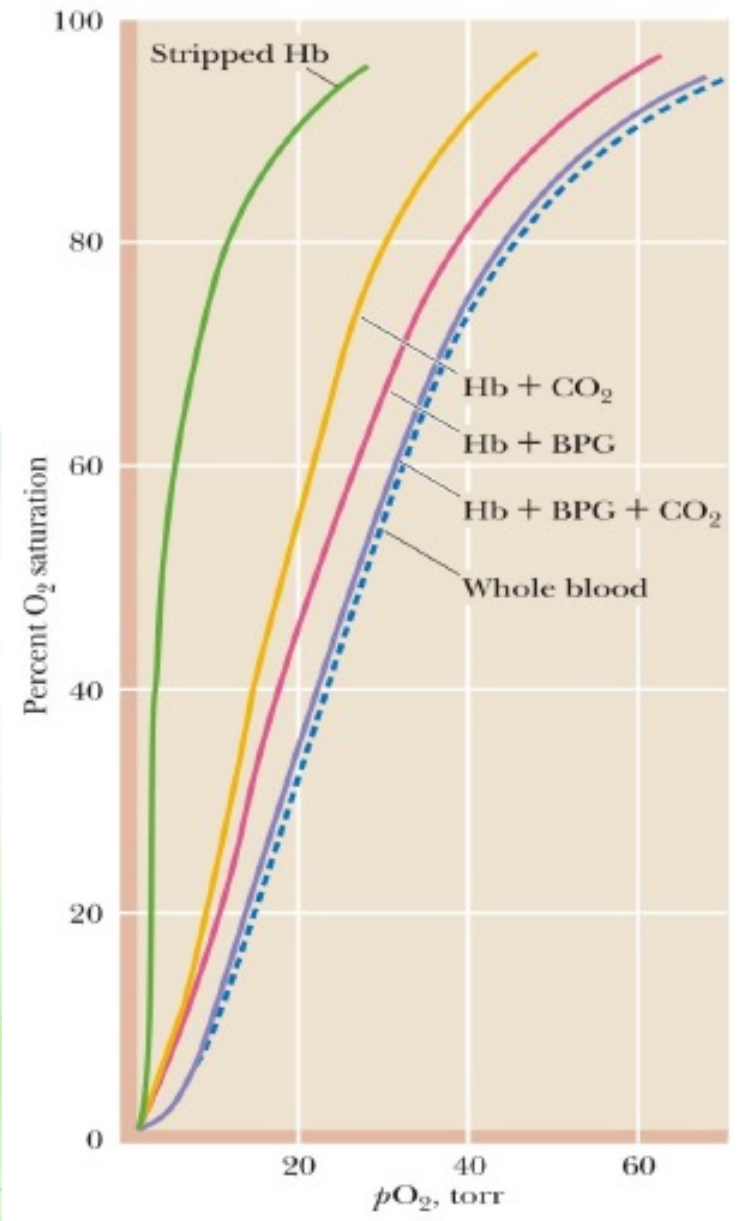


2,3-BPG in transfused blood

- Storing blood results in a decrease in 2,3-PBG (and ATP), hence hemoglobin acts as an oxygen "trap", not an oxygen transporter. If a person donated blood and this blood is stored even at 4 degrees, the level of 2,3-BPG goes down until there is no 2,3-BPG after 24 hours.
- Transfused RBCs are able to restore their depleted supplies of 2,3-BPG in 6–24 hours. In normal individuals
- Severely ill patients may be compromised because they need it in their blood to release oxygen easily.
- Both in 2,3-PBG and ATP are rejuvenated. They are added to their blood sample

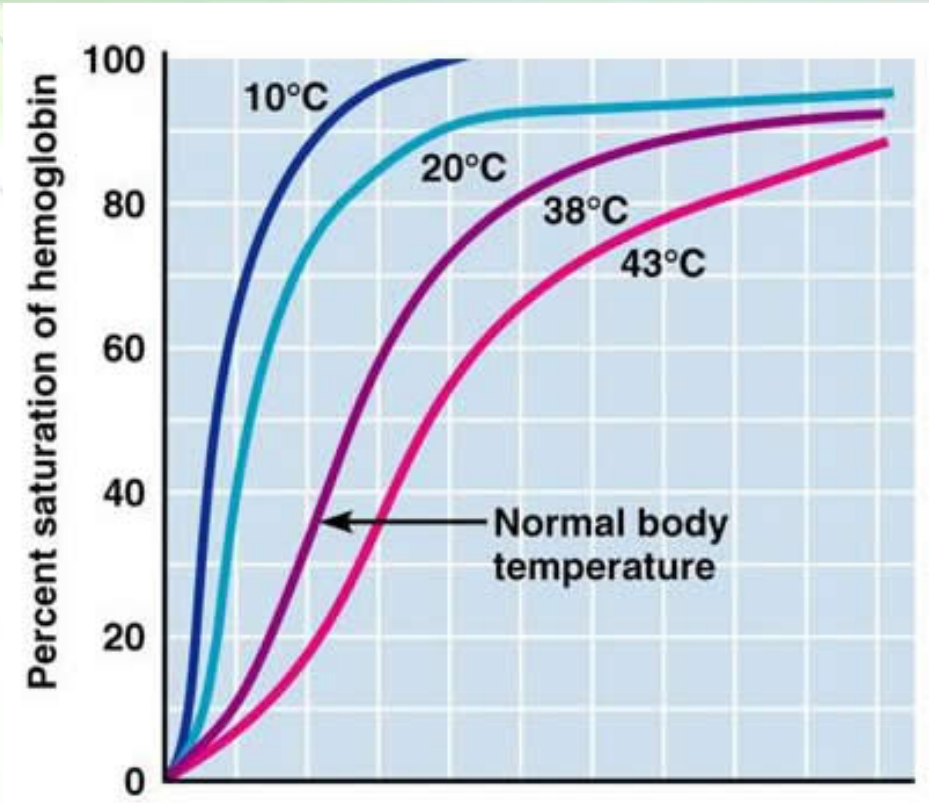


2,3-BPG and CO2 are important players



Effect of temperature

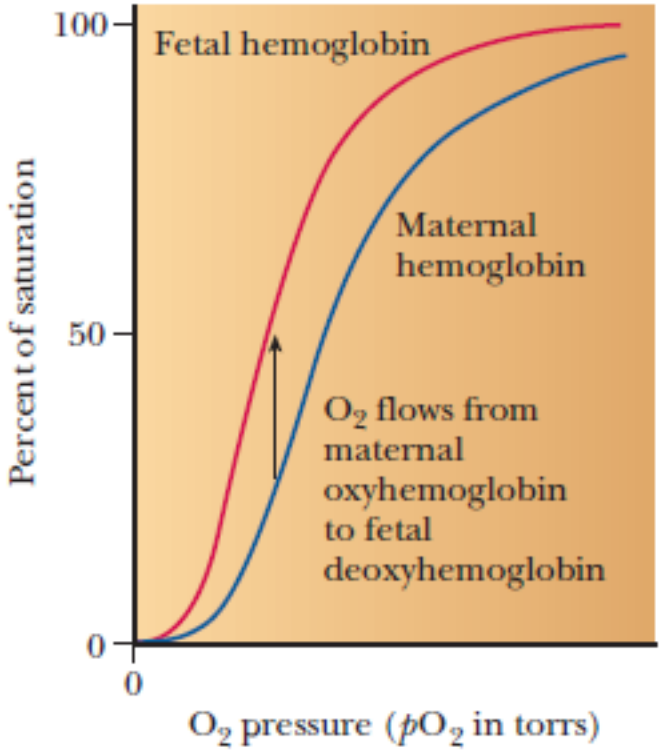
- An increase in temperature decreases oxygen affinity and therefore increases the P50. as normal body temperature goes up, the curve shifts to the right
- That's good btw, if you are exercising then your oxygen will be released into your tissue (which is exactly what you need) and if you were sick your tissue must have a high metabolic rate to fight the disease.
- 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 (release) from HbO_2.
- So, it's a DUAL effect on affinity :
  1. Direct by affecting the structure or the interactions between O2 and the iron of the heme molecule
  2. Indirect by increasing the metabolic rate of RBCs

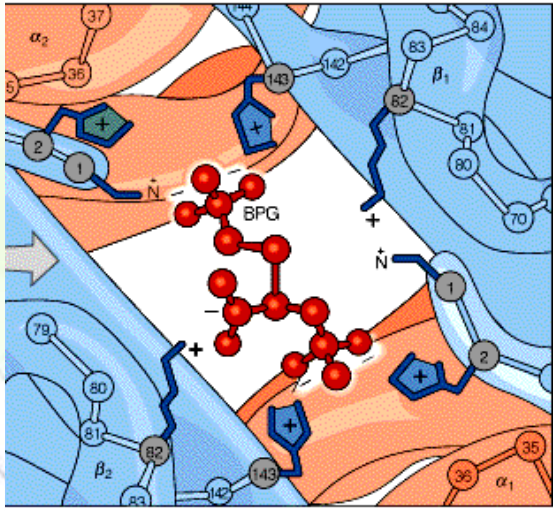


Other considerations

Fetal hemoglobin

- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HBA) – the curve shifts to the left so you need less O2 to fill 50% of the fetal hemoglobin.
  - HbA = $\alpha 2 \beta 2$
  - HbF = $\alpha 2 \gamma 2$
- His143 residue in the β subunit is replaced by a Ser in the γ subunit of HbF.
  - Since Ser cannot form a salt bridge with 2,3-BPG, it binds more weakly to HbF than to HbA.





His143 is important in forming electrostatic interactions with 2,3-BPG and since it doesn't exist in this case, we end up with weaker interactions to 2,3-BPG and high affinity to O2.

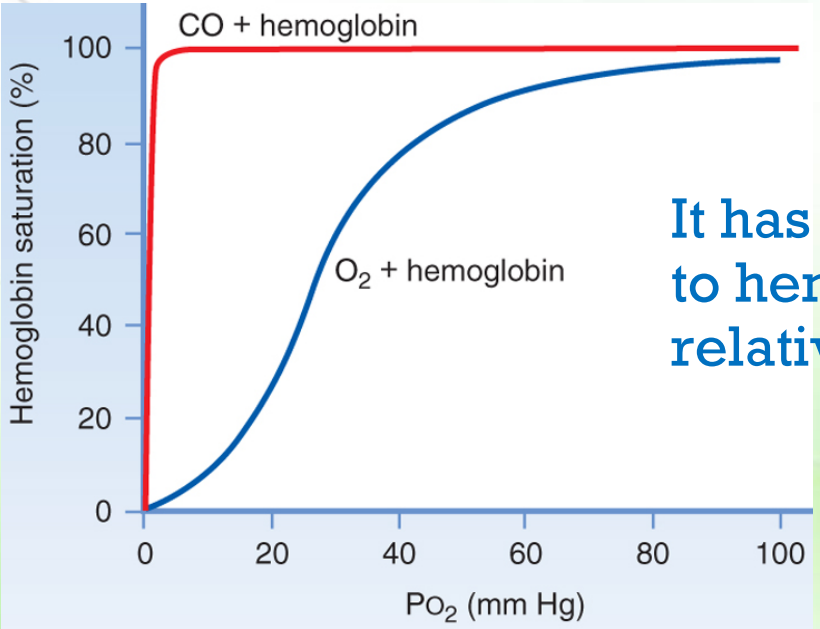
This means a mother can compensate the low level of O2 in her blood by breathing faster for example or taking deep breaths while the fetus can only steal O2 from his mother.

Effect of CO

Carbon monoxide is toxic and fatal

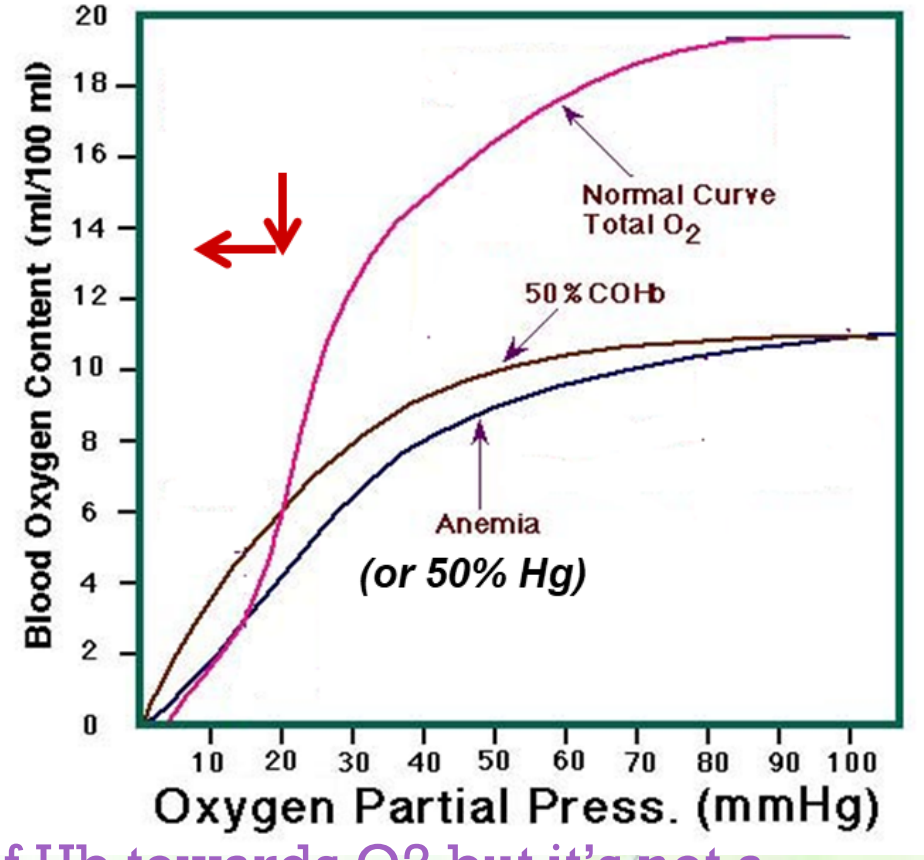
- In addition to competing with oxygen in binding to hemoglobin, affinity of Hb-CO towards oxygen increases resulting in less oxygen unloading in peripheral tissues.

(Hb + O_2) versus (Hb + CO)



It has high affinity to hemoglobin in relative to O2

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



It increases the affinity of Hb towards O2 but it's not a benefit because O2 won't be released and It keeps binding preventing O2 from unloading

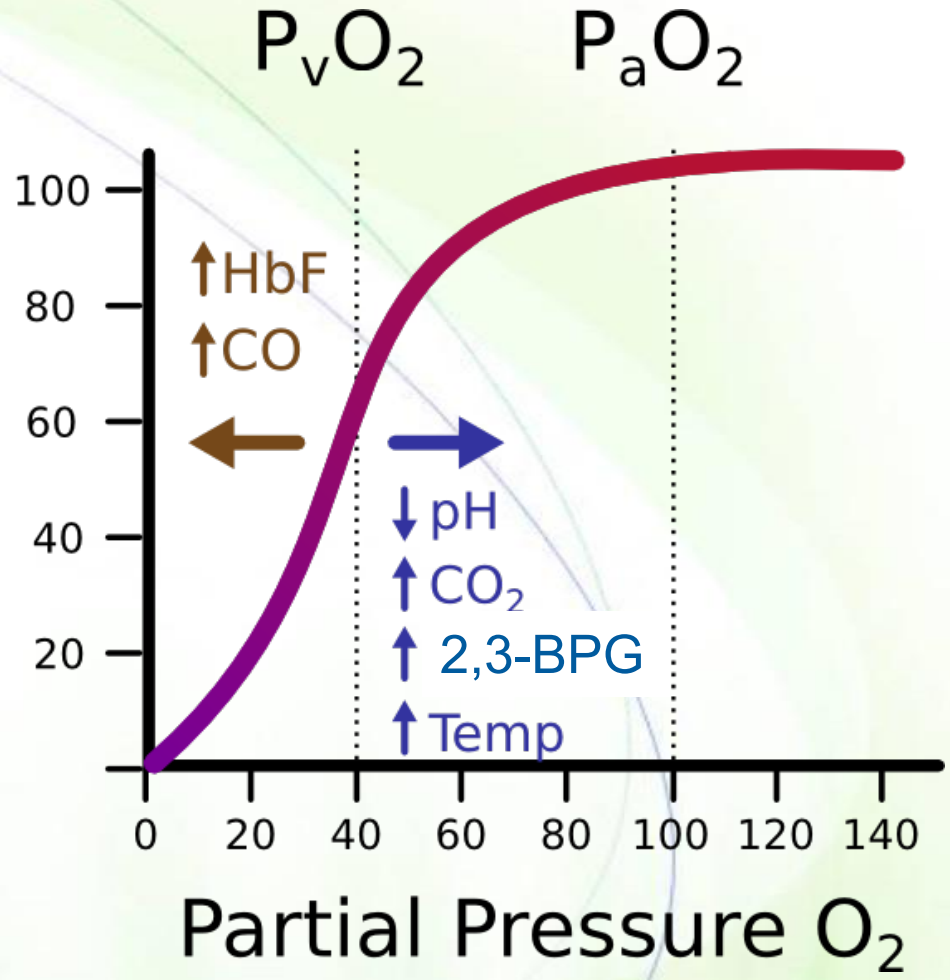
Relevant information

- 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

To sum up our lecture :

1. This Oxygen saturation curve can shift to the right (affinity decreases) , or to the left (affinity increases)



2. Affinity can be affected by different factors:
   a) Shift to the left : HbF (increases affinity, histidine changes into serine) & CO (increases affinity but it's fatal because it occupies O2 inside and prevents its release in tissues).
   b) Shift to the right : lower pH (Bohr effect), increasing CO2, increasing 2,3-BPG & increasing Temperature.