بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture 7

Regulation of Hb

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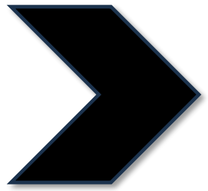
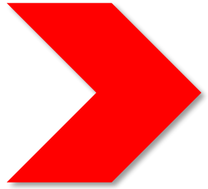
Color coding used in the modified:



Black: the original slides

Blue: the doctor’s explanation/words

Gray: additional information and explanation



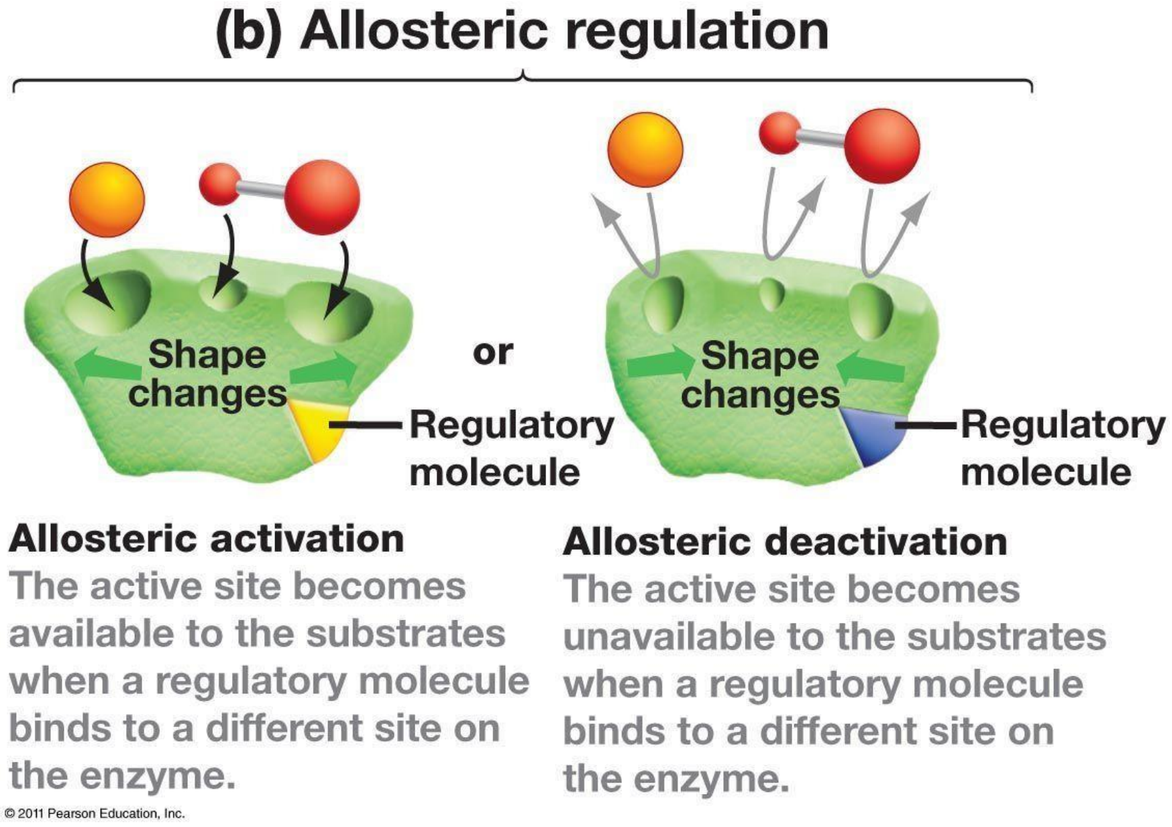
Red: important information

Regulation of hemoglobin function

Prof. Mamoun Ahram

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.
  - Heterotropic modulators are different from the ligand.



Allosteric effectors

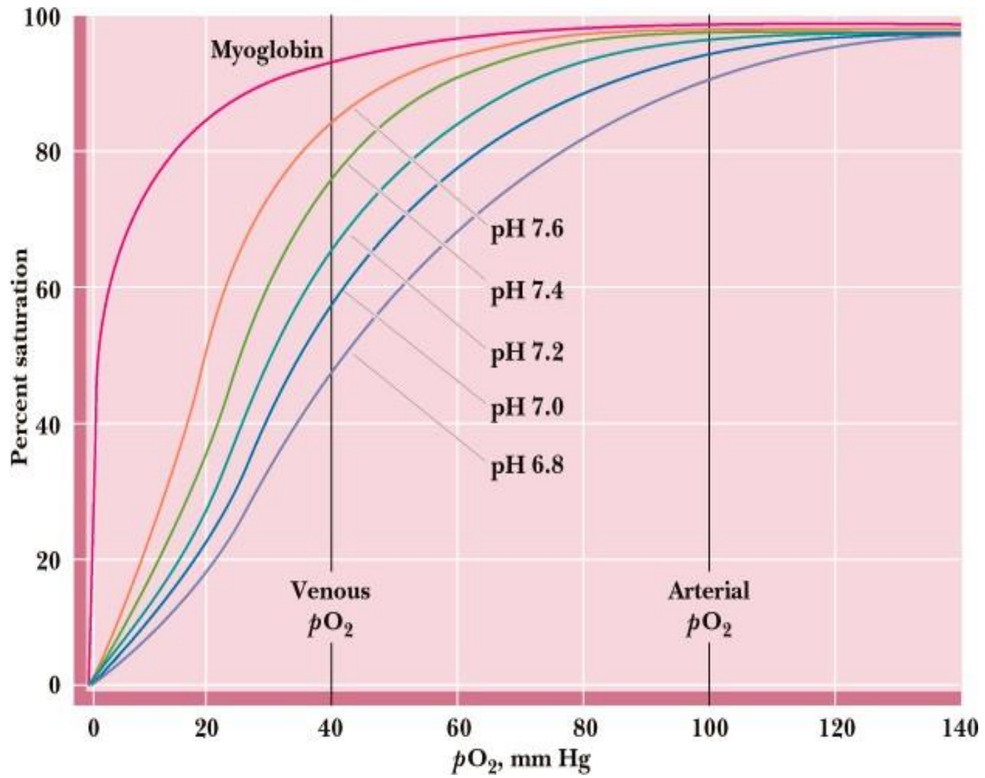
- The major heterotropic effectors of hemoglobin
  - Hydrogen ion,
  - Carbon dioxide
  - 2,3-Bisphosphoglycerate
  - Chloride ions

- A competitive inhibitor
  - Carbon monoxide

The effect of pH

The 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 binding of H+ causes the electrostatic interactions to stabilize the hemoglobin to favor the T state and as a result we have low affinity of O2

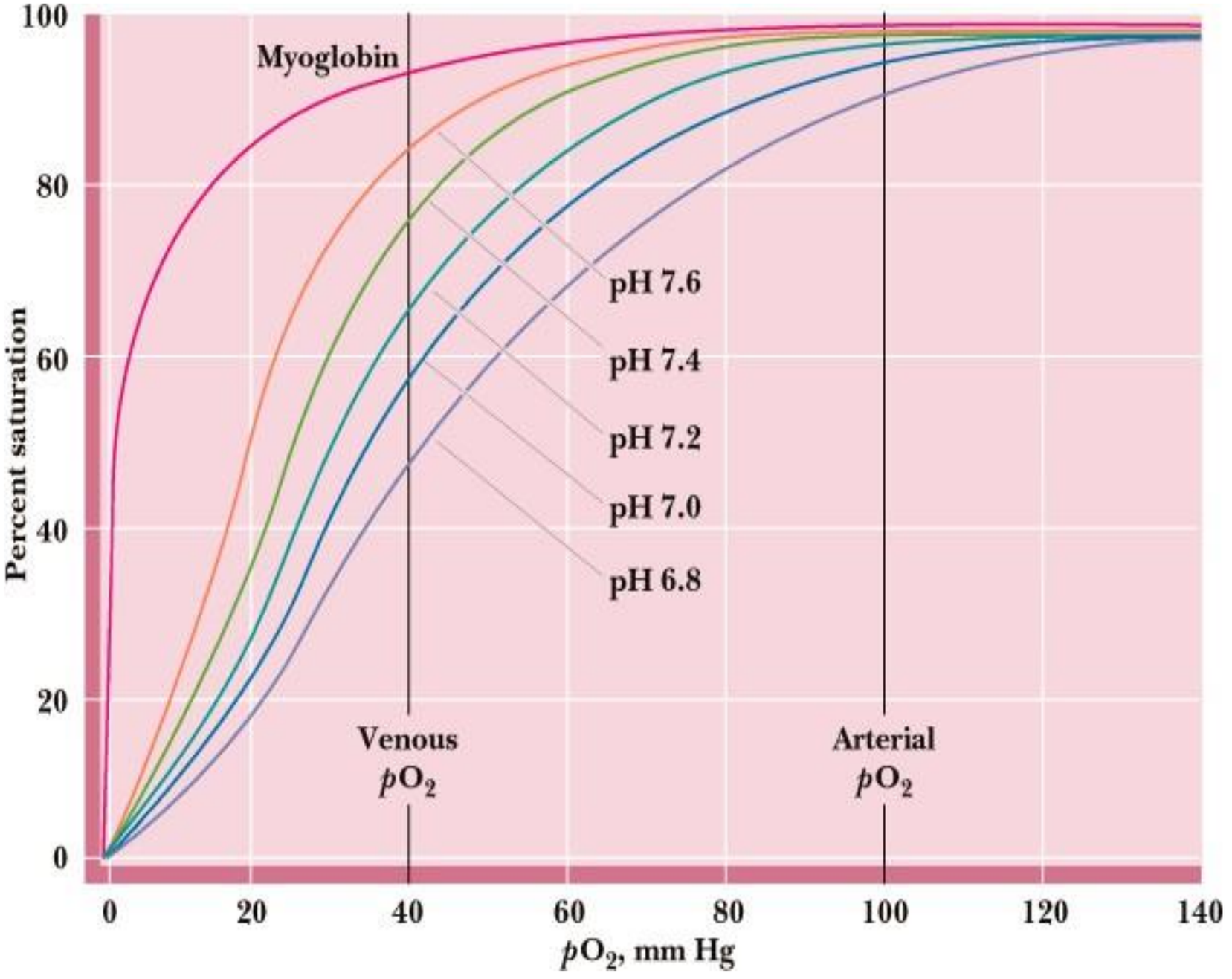
protons (H+) increase -> pH decreases -> Affinity of oxygen decreases

At pH = 7.6, the p50 is 20 mmHg

At pH = 6.8 the number of protons is higher compared to other pH levels, the p50 = 40 mmHg.

At pH = 6.8 we have lower affinity compared to the rest of the pH levels because the p50 is higher (recall that p50 means the amount needed to fill 50% of the hemoglobin with oxygen and because the p50 value is high we need a higher amount of oxygen to fill the hemoglobin with 50% oxygen)

So in conclusion:
p50 increases -> low affinity & needs more oxygen to fill the hemoglobin with 50% oxygen


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

The pH tissue is lower than the pH in lungs

Metabolism (glucose, fats, fatty acids) turn into $CO_2 + H_2O$ which then turn into acids.

Glycolysis: breaking glucose to form pyruvate (pyruvic acid)

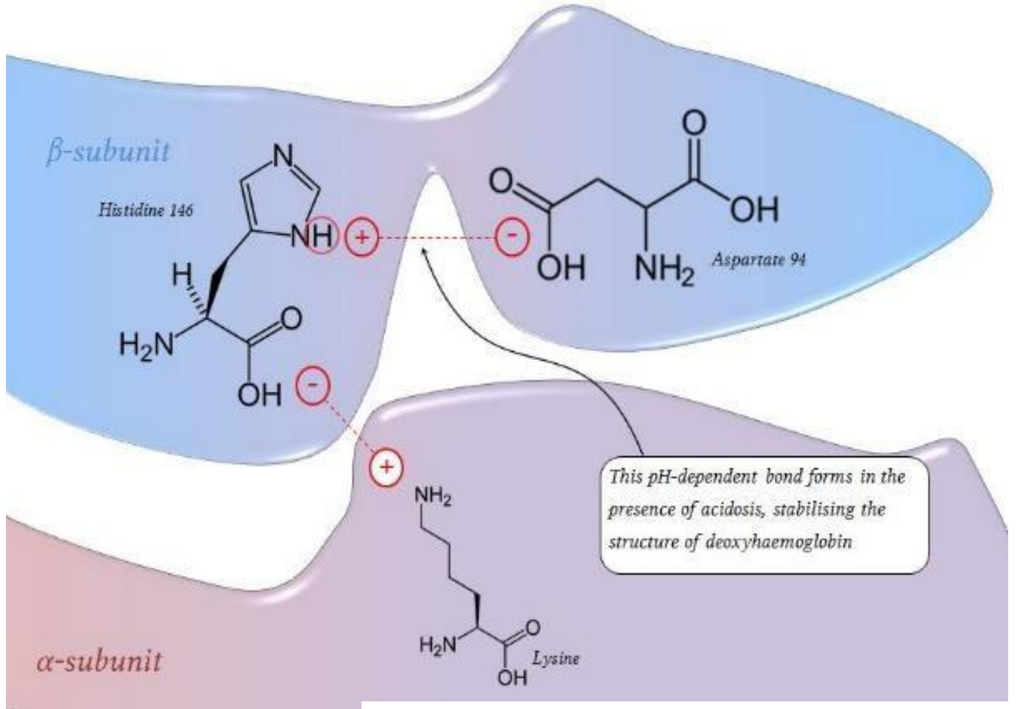
Examples of acids:

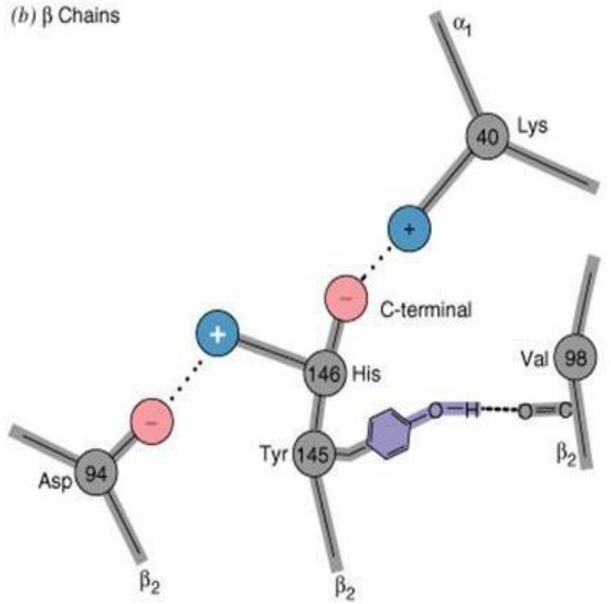
1) Pyruvate can turn into acetyl CoA that then becomes a part of the Kreb's cycle
2) Acetate (Acetic acid)
2) Alpha-Ketogluterate (Alpha-Ketoglutaric acid)
3) Oxaloacetate (Oxalic acid)

These acids decrease the pH of tissues
CO_2 production increases -> H_2CO_3 -> forming protons (decreases pH) and bicarbonate

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, stabilizing the T form of hemoglobin.
- The protonated His146 also forms a salt bridge to Asp94 within the same chain.
  - The pKa of the imidazole ring of His146 is increased from 7.3 in the R-state to 7.7 in the T-state, meaning that it is deprotonated (charged) in the R-state and protonated (charged) in the T-state, meaning that at low pH (in tissues), it is easier for hemoglobin to be in the T state.
- This favors the deoxygenated T-form of hemoglobin.





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

pKa of His (alone) = 6.0
pKa of His as a residue has another value that depends on its surroundings,
pKa of His 146 (R-state) = 7.3
pKa of His 146 (T-state) = 7.7

The affinity of histidine for protons increases slightly from the R state (pKa = 7.3) to T-state (pKa = 7.7)

pH blood = 7.40
pH tissues ≈ 7.20 (less than the blood's pH due to metabolism)

When oxygen binds to the hemoglobin, the electrostatic interactions are broken and it turns from T state to R state (pKa = 7.3) and the histidine is mainly unprotonated in that case because the pH is higher than the pKa of R state.

When the hemoglobin reaches the surrounding tissues, its pKa changes from 7.3 (R state) to 7.7 (T state) and it's mainly protonated because the pKa is higher than the pH.
Since it's protonated, it is positively charged and it has electrostatic interactions which favors the T state and as a result oxygen is released.

So remember:
Histidine is unprotonated in R state (no electrostatic interactions & pKa is lower than pH) while it's protonated in the T state (has electrostatic interactions & pKa is higher than pH)

Where do protons come from?

- Metabolism of glucose to CO_2 and lactic acid.
- *H^+'s* are produced at high levels in metabolically active tissues from CO_2 by carbonic anhydrase.
  - In the lungs, the reverse effect occurs, where the high levels of O_2 cause the release of CO_2 from hemoglobin.

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

The pH in tissues is lower than blood because of 2 factors:

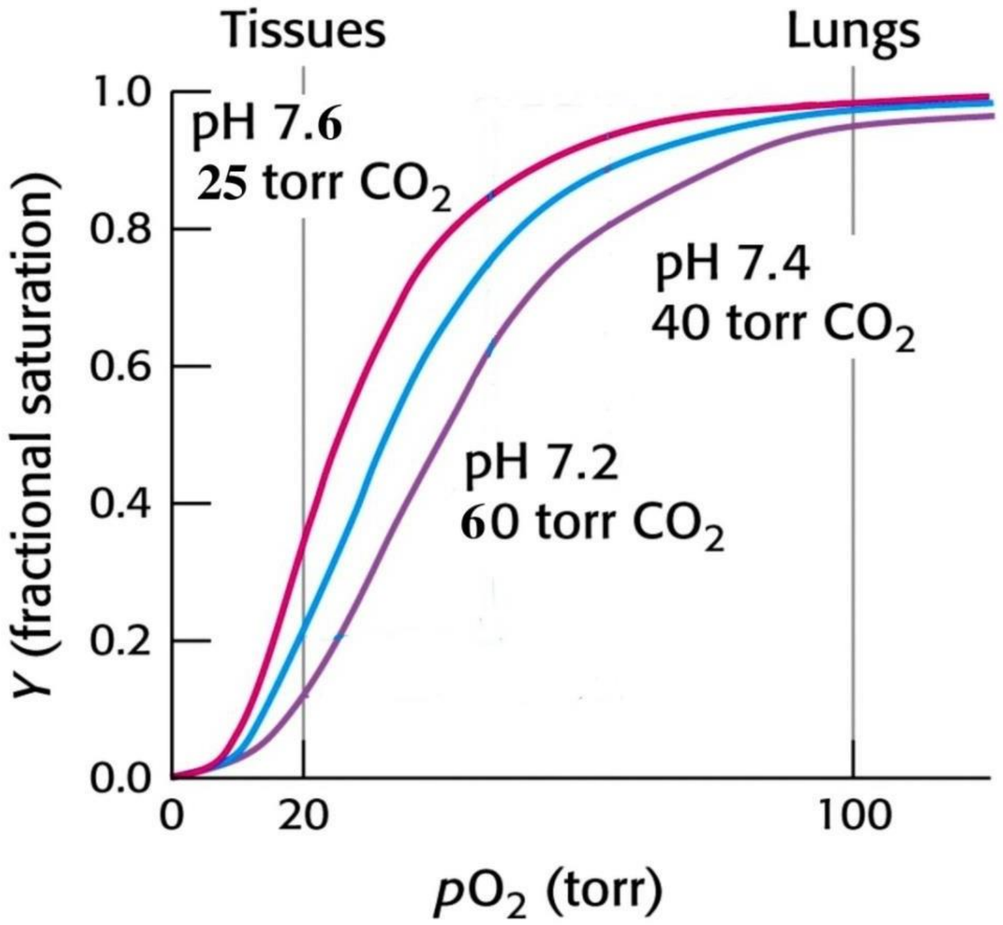
1) CO2 with water release protons (and bicarbonate)
2) lactic acid releases protons as well

As a result the pH decreases and the hemoglobin is present in the T state due to the presence of electrostatic interactions

The effect of CO_2

Mechanism #1 - production of protons

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

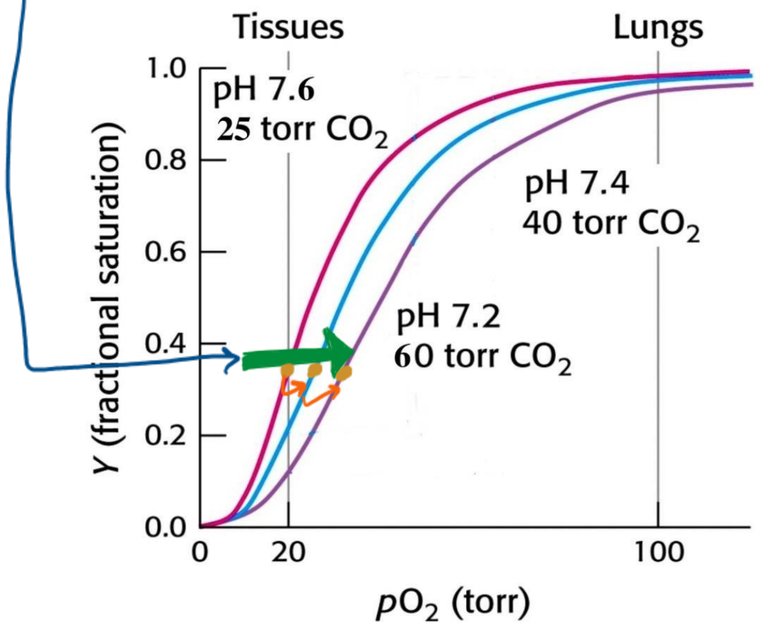


CO2 has 2 effects which affects the hemoglobin: $CO_2 + H_2O \Longleftrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$

The first effect is producing protons (H+)

As CO2 increases the number of protons increases and as a result the pH level decreases

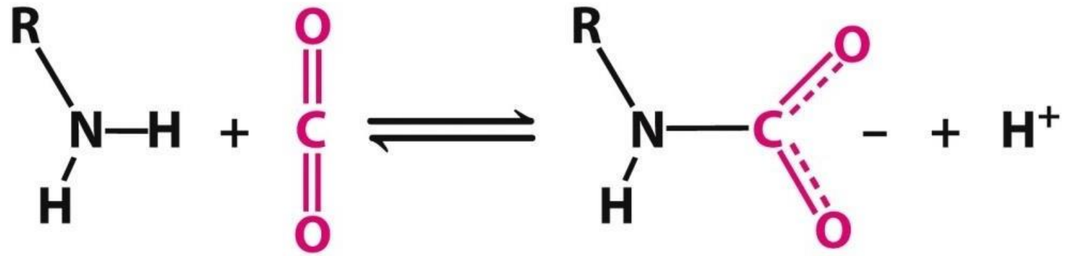
The oxygen saturation curve shifts to the right



Shifting to the right:
As the CO2 increases, the p50 increases and therefore the affinity decreases

Mechanism #2- formation of carbamates

- Hemoglobin transports some CO_2 directly.
- CO_2 combines with the free α-amino terminal groups(Of both alpha & beta chains)
- , forming 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.

CO2 is a heterotropic (molecule different from O2) allosteric modifier (specifically a **negative** allosteric modifier because it decreases the hemoglobin's affinity for oxygen), while O2 is a positive homotrophic allosteric effector (O2 is the only homotrophic effector for hemoglobin)

You may ask yourself, is the effect of the CO_2 direct (directly affecting the hemoglobin, increasing the p50 and therefore decreasing the affinity) or is it indirect by changing the pH ?

It is both, as production of protons indirectly affects hemoglobin while the carbamates (second mechnism) directly affects it.

But which one is stronger?

The next slide answers that...

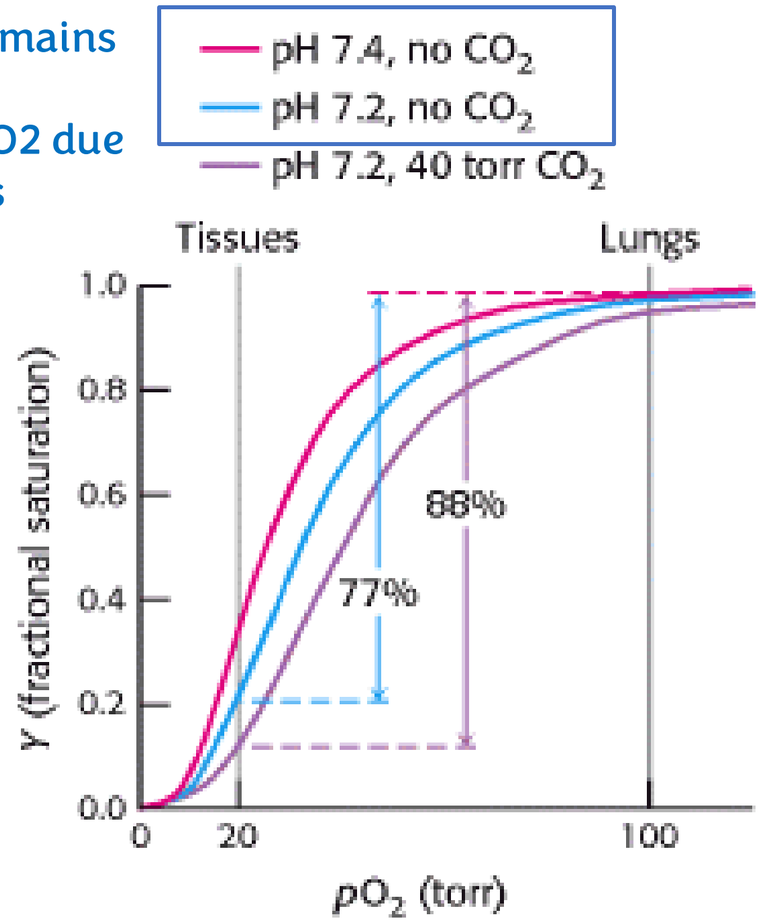
Which mechanism has a stronger effect?

- 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 shifts the oxygen dissociation curve to the right, even when the pH is held constant.

Notice that there is no CO2 meaning that this is the effect of pH

The pH remains 7.2 when adding CO2 due to buffers

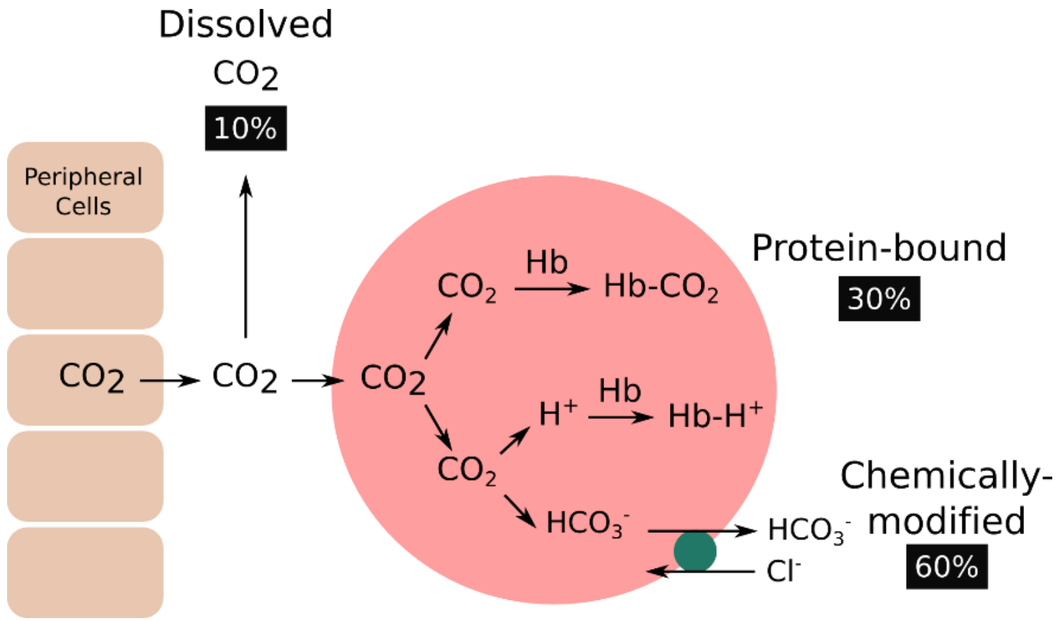


The curve shifts to the right because of the effect of CO2

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 (it changes into the R-state due to the higher pH and pressure of oxygen in the lungs) .
- A small percentage (CO2 is hydrophobic) **10%** of CO_2 is transported as a dissolved gas.

In peripheral tissues, it diffuses out to the plasma and return to the RBCs when reaching lungs to react with H+ and give CO2 and H2O via Carbonic anhydrase (it catalyzes both directions of the reaction)



Physiological buffers: The **main blood buffer is bicarbonate**. We also have proteins such as Hb which can release and bind to protons, regulating the pH in blood. CO2 produces protons but pH doesn't change because Hb acts like as a sponge

The <u>isohydric shift</u> is the movement of CO2 in/out of cells without changing pH as a result of hemoglobin acting as an effective buffer

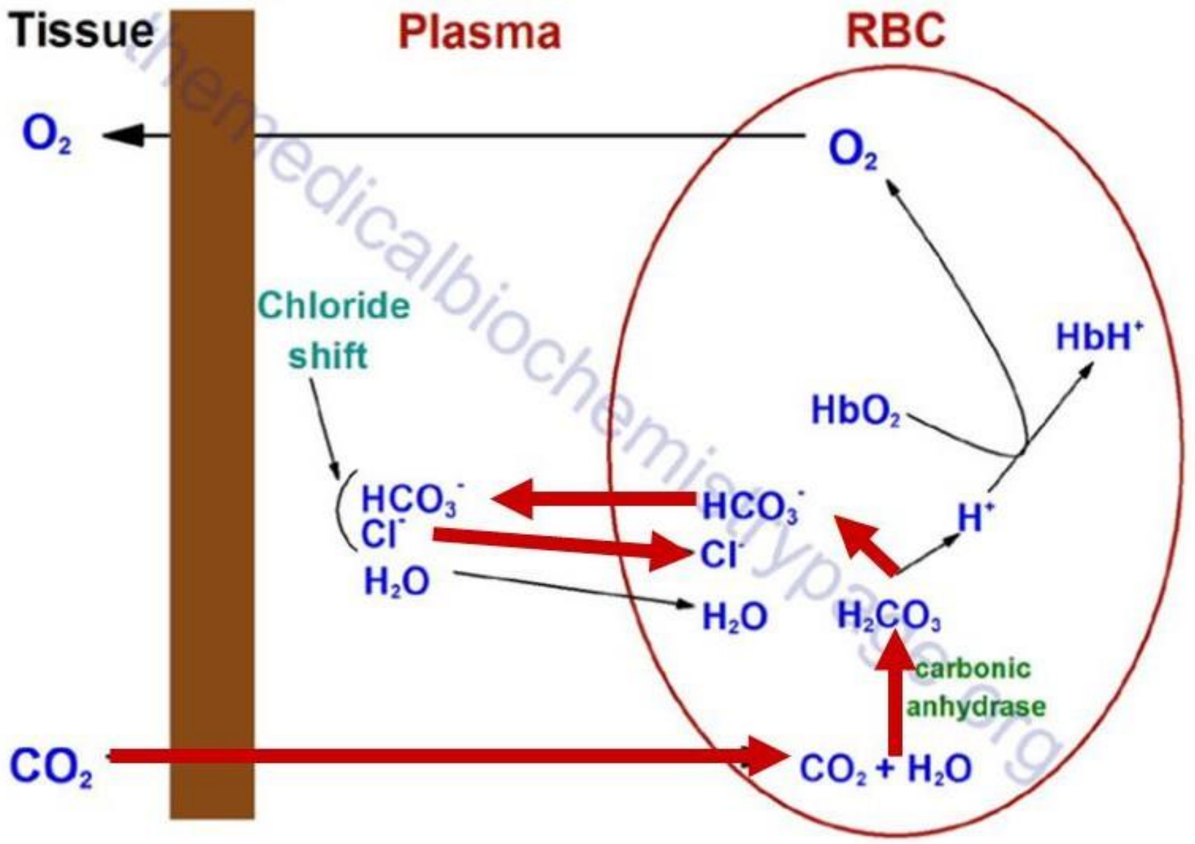
Effect of Chloride ion

(negative heterotropic allosteric effector)

Chloride shift

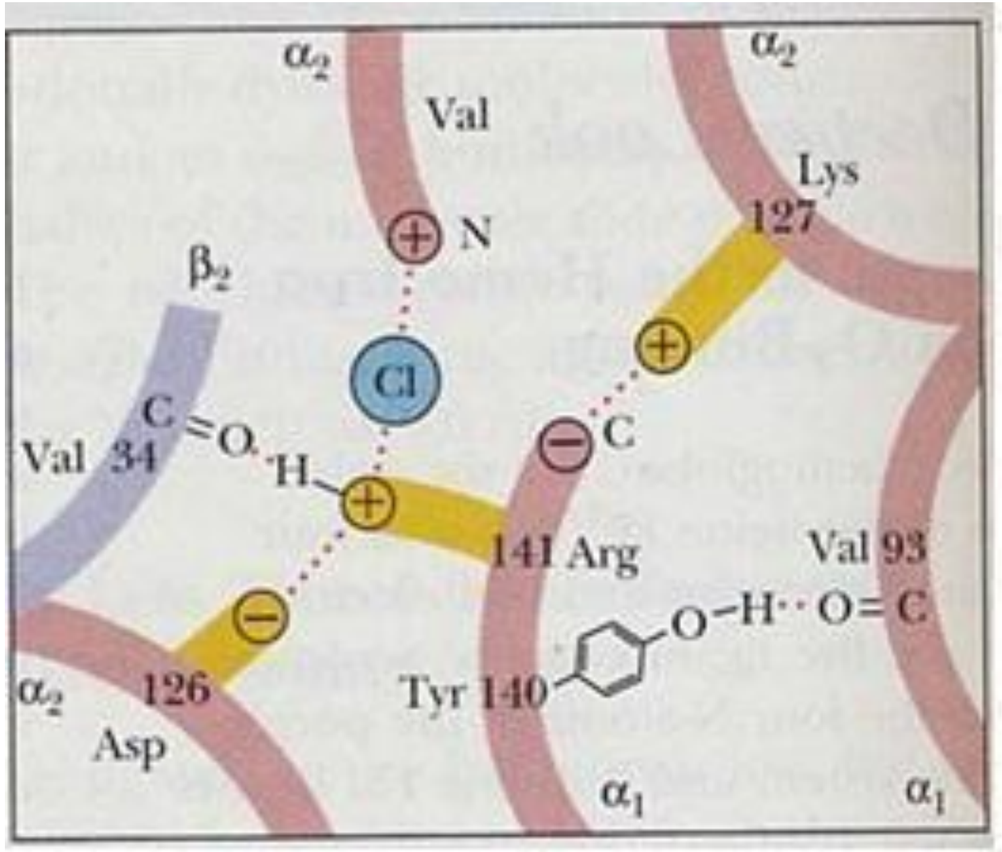
- **Bicarbonate diffuses out of the red blood cells into the plasma in venous blood and visa versa in arterial blood.**
- Chloride ion always diffuses in an opposite direction of bicarbonate ion in order to maintain a charge balance.
- This is referred to as the "chloride shift“.

- When bicarbonate ion leaves the RBC , the RBC loses a lot of negative charges, which is restored by the chloride ion.



Effect of chloride ions

- Chloride ions interact (electrostatic indeed) 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, P_{50} increases)



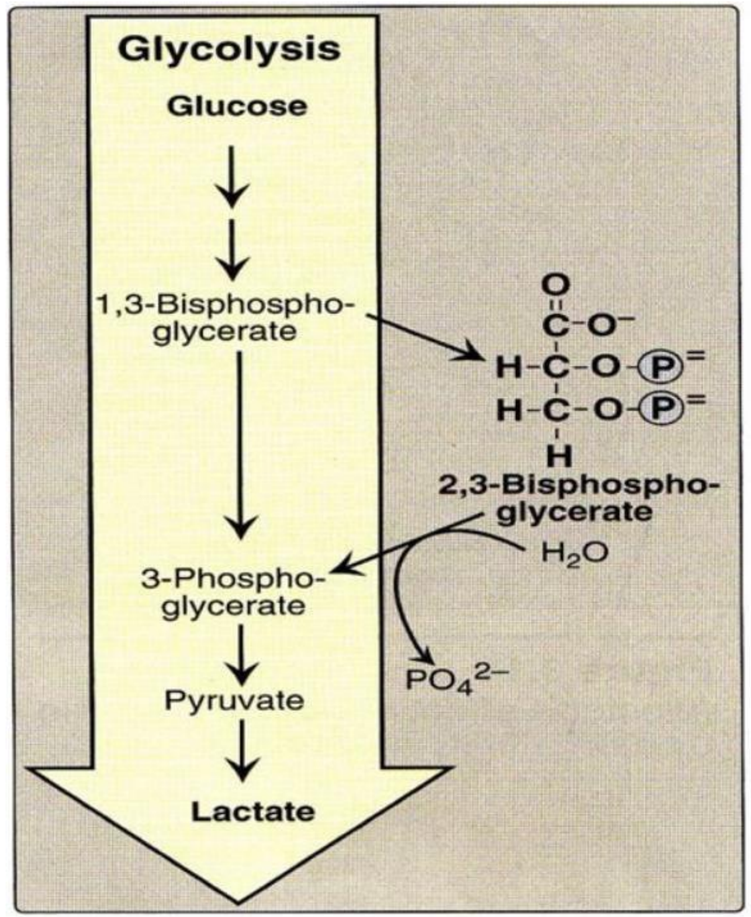
Effect of 2,3-bisphosphoglycerate

intermediate of glycosidic pathway that turns glucose to pyruvate.

The difference between “Bis-” and “Di” is that di means two phosphates linked together, while bis means there's two phosphates but linked to different carbons.

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.



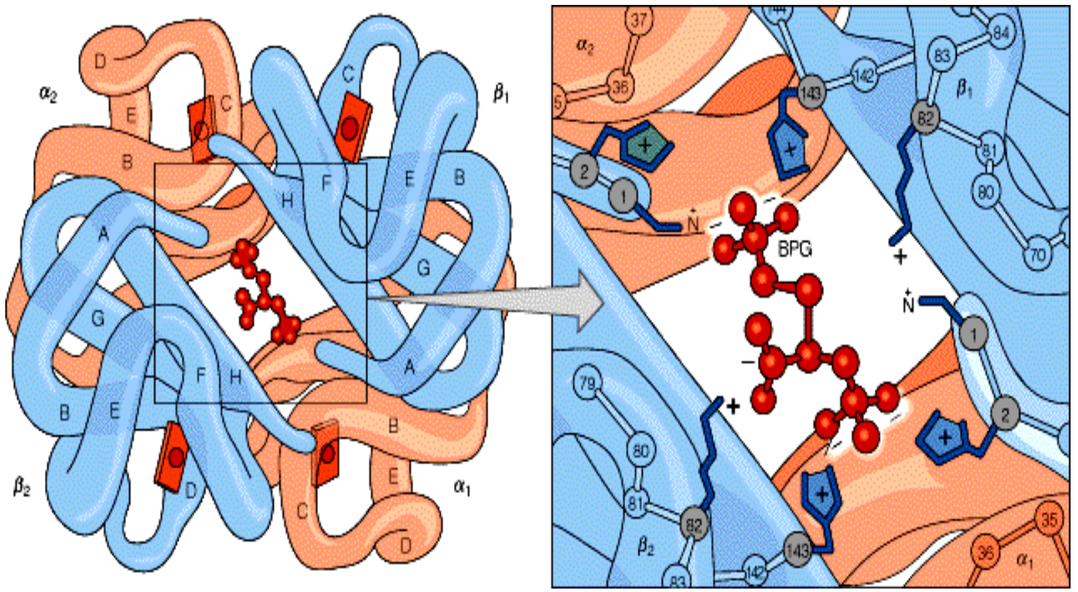
small amount of 1,3-BPG turns to 2,3-BPG
They are isomers (constitutional)

2,3-BPG –hemoglobin interaction

(the most important regulator of hemoglobin) See slide 27

- 2,3-BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 2,3-BPG/hemoglobin tetramer. (making a lot of electrostatic interactions with the amino acids residue surrounding it)
- This binding stabilizes the T-state hemoglobin reducing the binding of oxygen to hemoglobin and facilitating oxygen release.

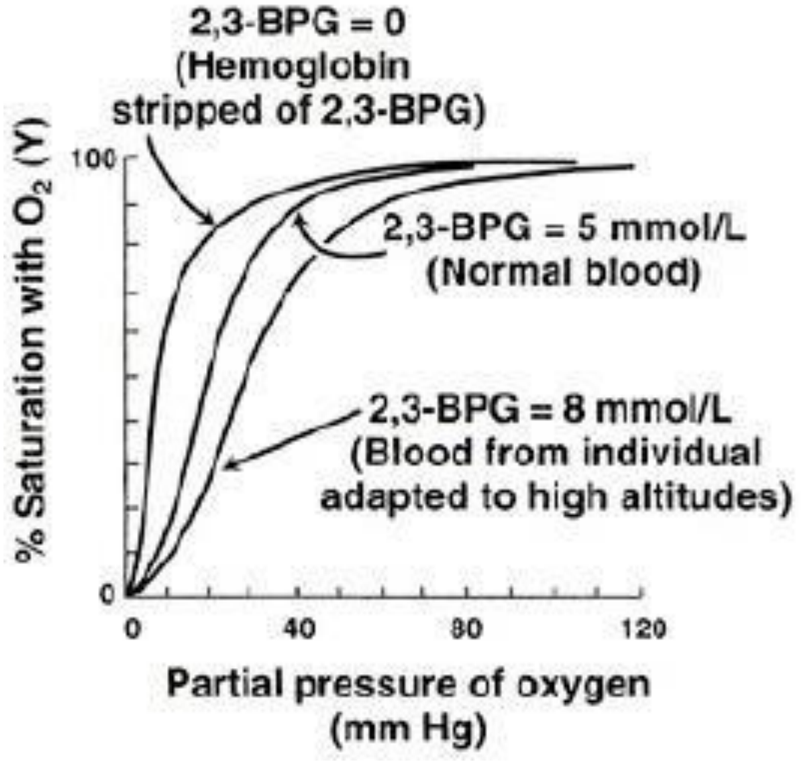
2,3-BPG forms salt bridges with the **terminal amino groups of both β chains** and with a **lysine** and **His143.**



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.



The curve is still sigmoidal not hyperbolic

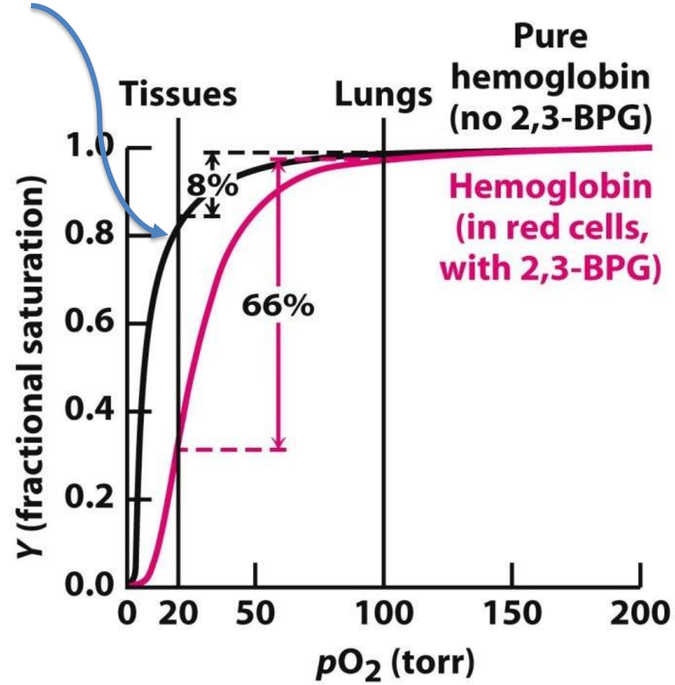
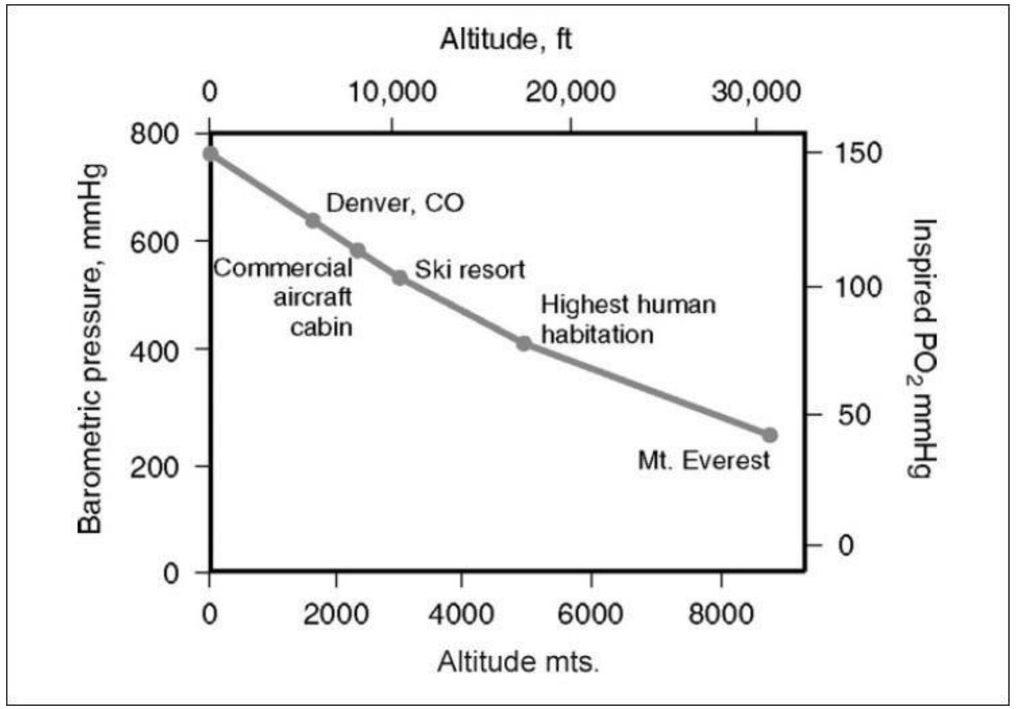


Figure 7.16
Biochemistry, Seventh Edition

Hb with 2,3-BPG (affinity decreases, P50 increases, releases more oxygen in tissues)

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

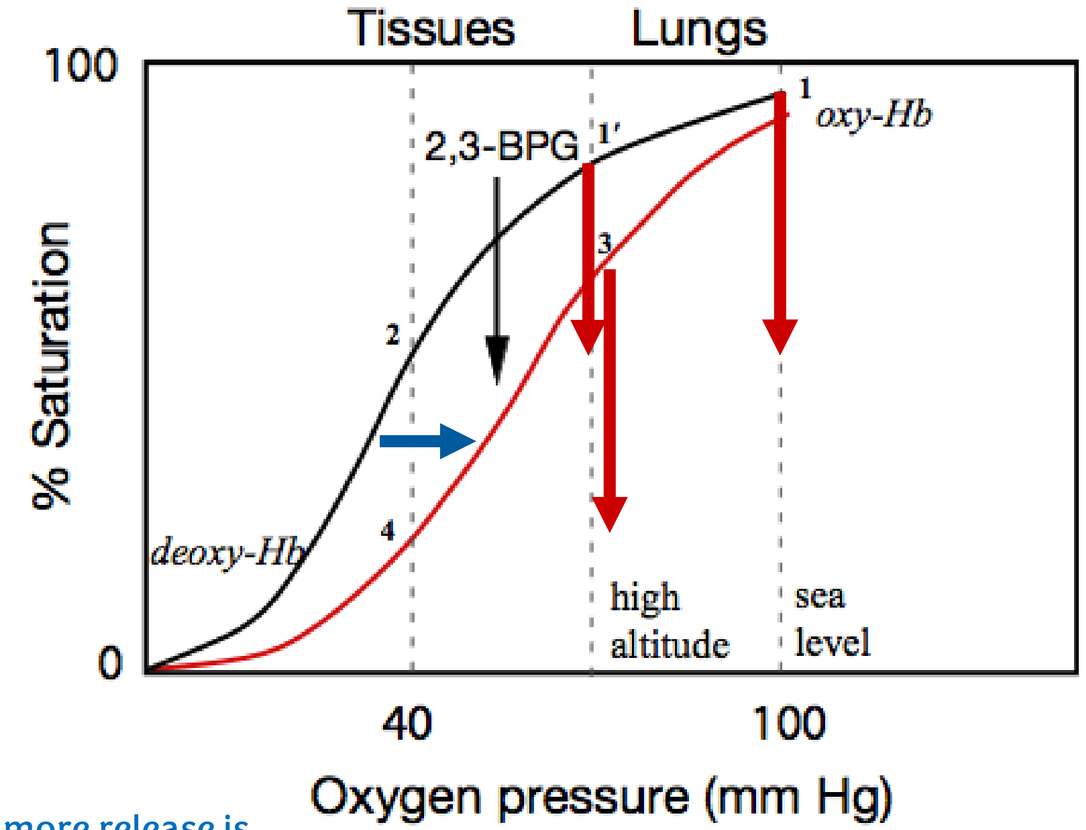
More binding
Less release

Less binding
More release

Better explanation of the role of 2,3-BPG

Important for understanding the concept

- 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 <u>no adaptation</u>), 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 <u>increased</u> 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.



So, less binding more release is better in high altitudes (when inspired air has a low % of O2)

It is not a perfect solution, but over time, there is increased production of red blood cells to provide more hemoglobin and compensate for the smaller amount of oxygen it can bind.

What the prof is trying to illustrate is ,in the case of high altitudes, BPG is added so the curve shifts to the right the affinity decreases P50 increases due to the decrease in oxygen levels so Hemoglobin molecules are less saturated in comparing with sea level position but releases much more oxygen in the tissues.

يعني بدل ما تحمل 20 تفاحة وتوصل 5 ، انت بتحمل 7 تفاحات وبتوصل 4

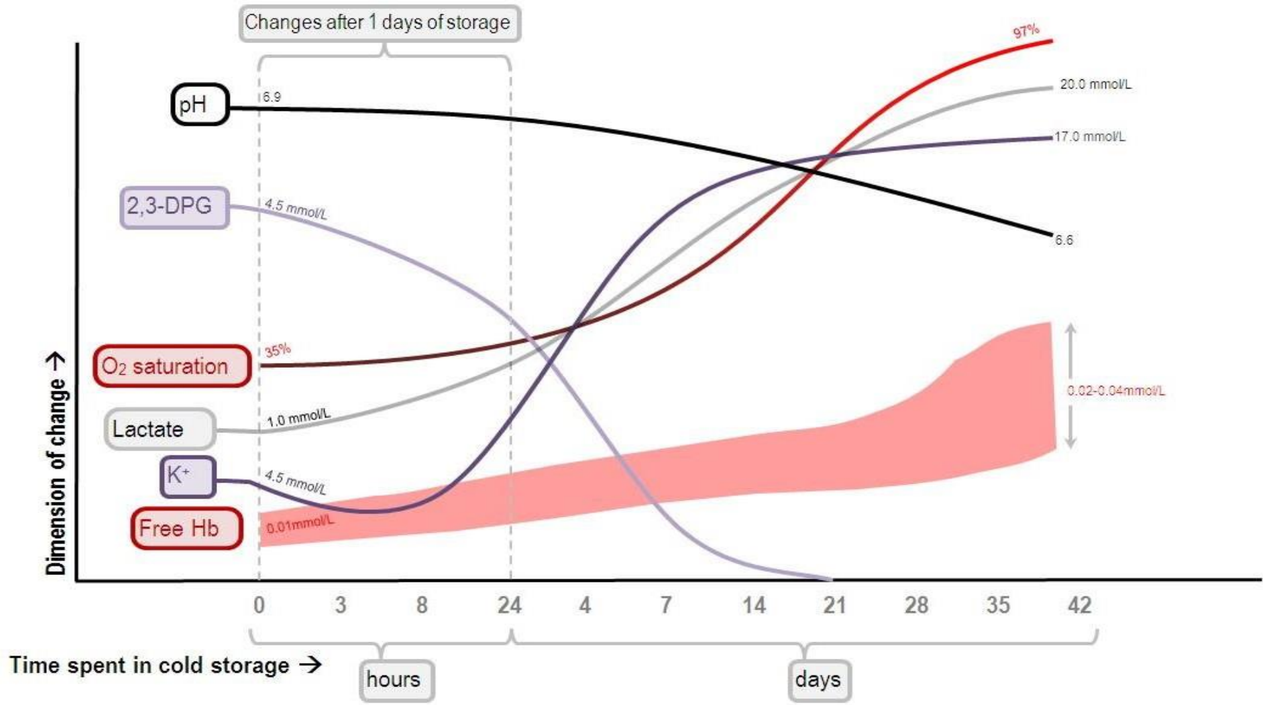
2,3-BPG in transfused blood

- Storing blood results in a decrease in 2,3-BPG (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.**

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

When we store the blood, the 2,3-BPG and ATP will be degraded with time. So, if this blood is transfused to a patient, he will not benefit from it immediately, because his body needs to synthesize 2,3-BPG first and this takes some time (6-24 hours)

That’s why blood banks add 2,3-BPG to the blood before giving it to patients in need.

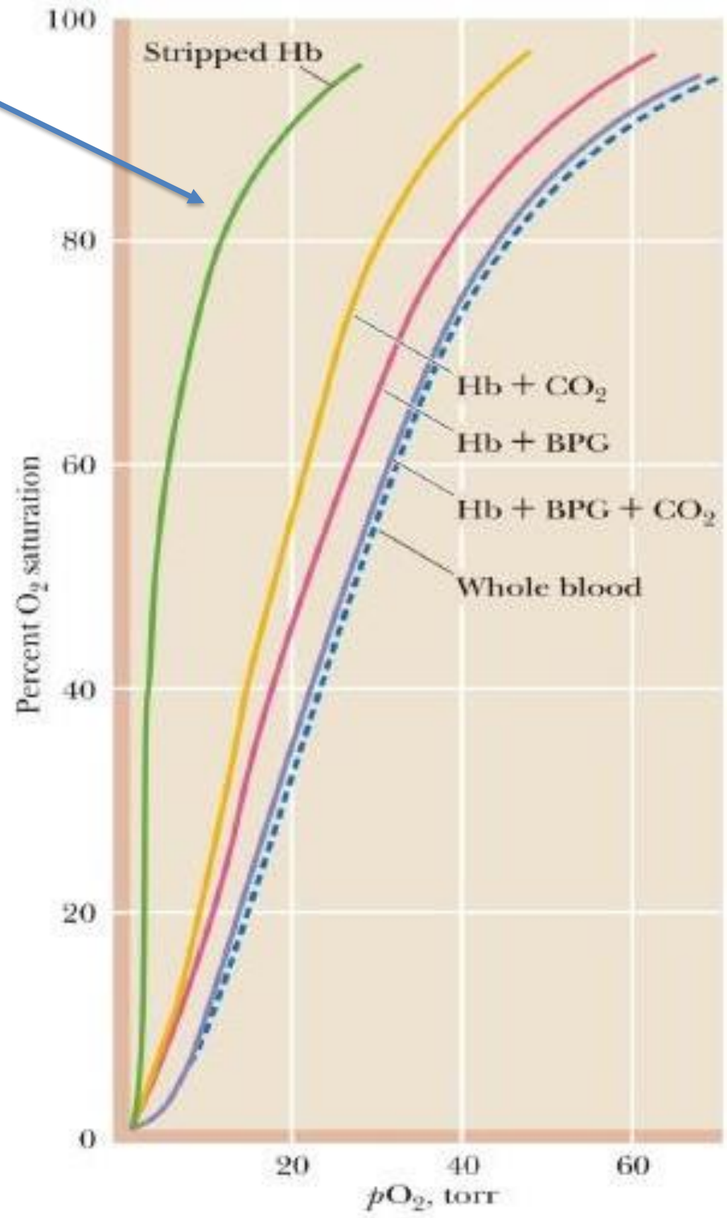


Along with 2,3-BPG and ATP, pH is decreased free Hb is increased because of the hemolysis of RBCs

2,3-BPG and CO_2 are important players

It is sigmoidal also

This graph tells us how important the CO2 and BPG are in our blood basically both of them are the key factors in determining our blood's affinity for oxygen as you can notice hemoglobin alone has very high affinity, so we reduce it by adding CO2 and BPG.



As you can see, the effect of BPG and CO2 is the most important. It is obvious that with only these 2 regulators the curve is very near to the normal Hb curve which depends on other factors also (Cl- for example).
You can also infer that BPG is more important than CO2 because its graph is more shifted than CO2 graph.

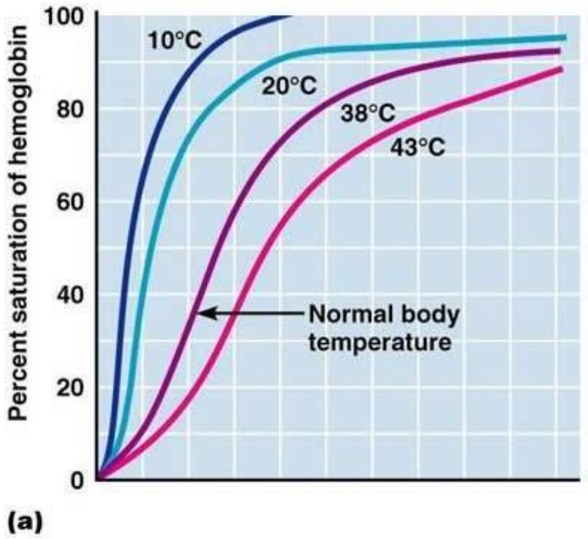
Effect of temperature

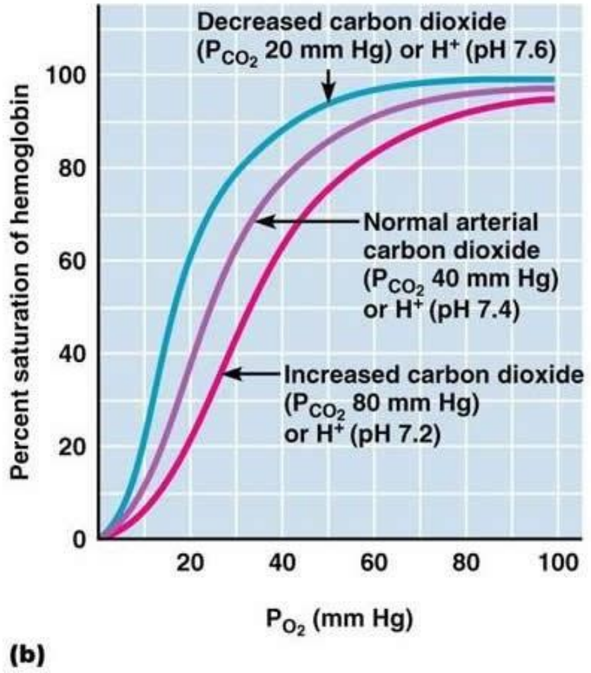
Effect of temperature

- 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.

When you practice for example, the metabolism will increase, the temperature of your body will increase, the pH will decrease, and those play a major role in releasing oxygen in the needed tissues.

People with fever need more O2 because tissues need it more (the metabolism rate is high). So, the temprature increases ---> the affinity of Hb is reduced to increase the release of O2 to tissues (that's why they breath so much).

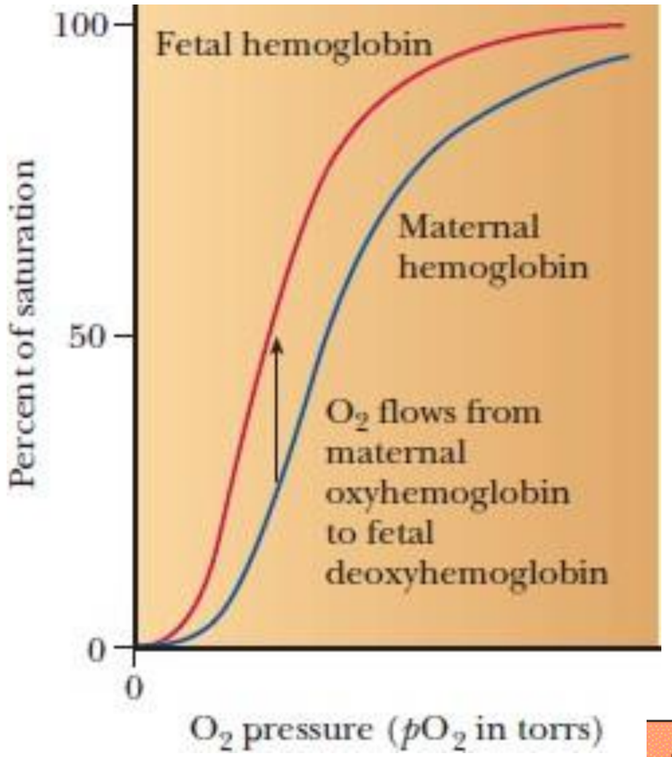


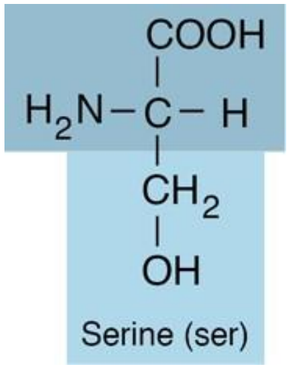


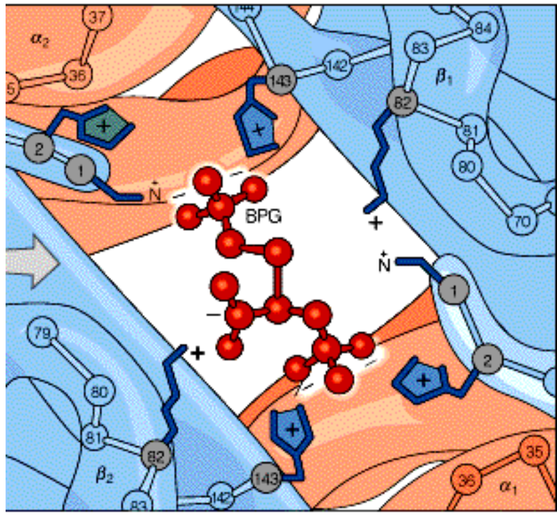
Other considerations

Fetal hemoglobin

- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HbA) Specifically, HbA1
  - 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.
  - Since serine cannot form a salt bridge with 2,3-BPG, it binds weaker to HbF than to HbA. Therefore, the affinity of HbF is higher







Some may say that that the increase in the affinity will affect the releasing of oxygen as we explained before , the doctor said he doesn't have a specific explanation in whether the high affinity affects the fetus or not . His most probable explanation was that the fetus has a very high Metabolic activity which reduces the pH and excessive divisions this may affect the release of oxygen from HbF but we don't know it exactly.

Effect of CO

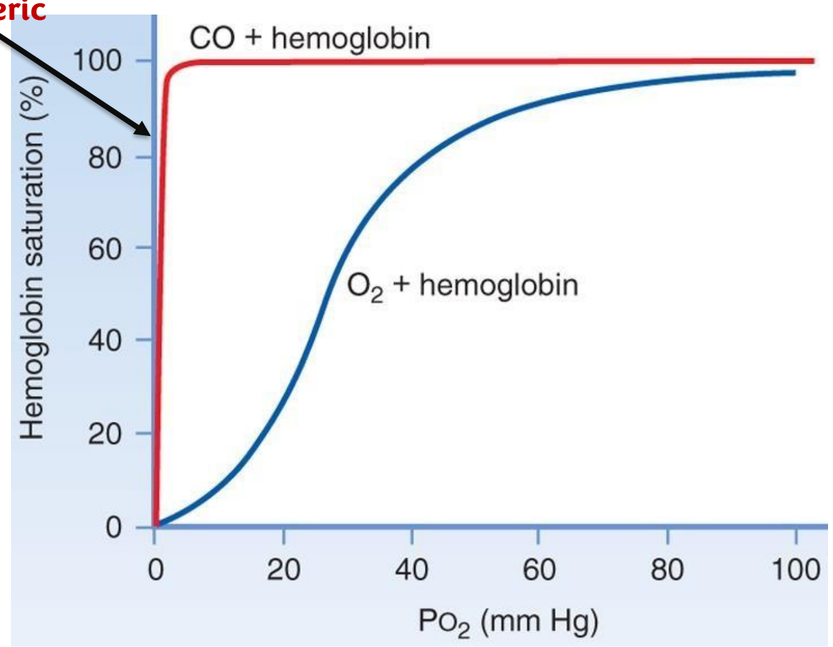
Effect of CO

There will be a repulsion between distal His and CO which makes the molecule **bent** So the binding affinity of CO will be lower (but still higher than O2) and one more advantage to oxygen that **O2 is way more abundant the 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.

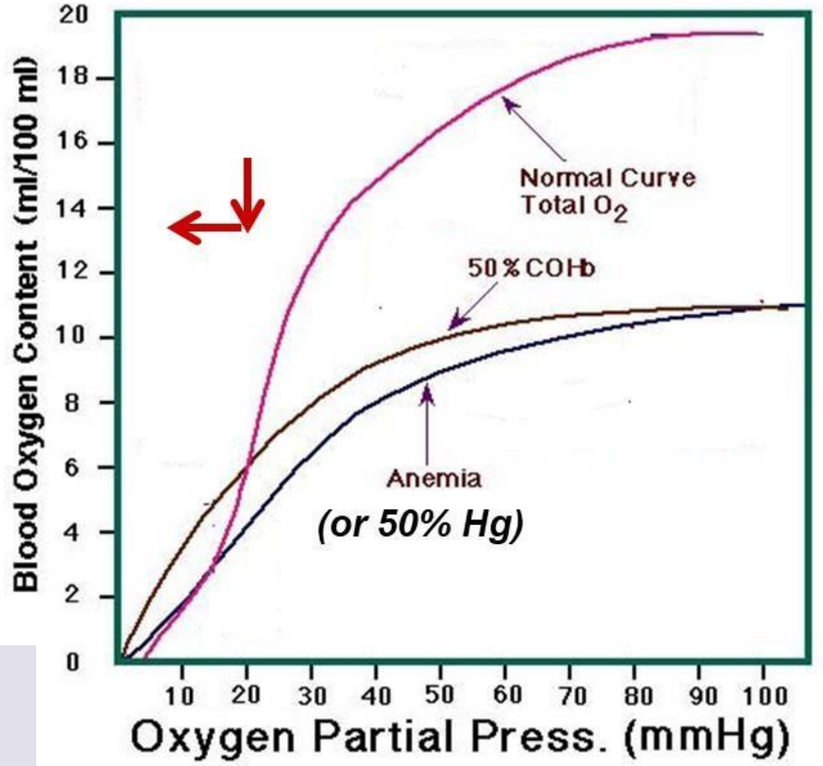
This curve is hyperbolic (only one state (R). the protein is not allosteric anymore)

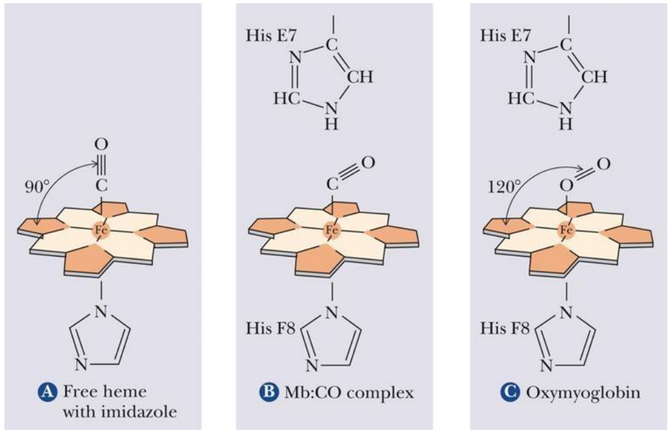
$(Hb + O_2)$ versus $(Hb + CO)$



You can notice from the curve that even with the increase affinity towards oxygen the degree of saturation is lower than Oxygen on the normal curve Because CO took some place

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





Distal His allows CO to enter because its structure is near to O2

The CO will convert the hemoglobin into R state, So the affinity to oxygen will be much higher, but there will be no advantage of this. You can see that the shape of the curve is hyperbolic, which means that the CO will increase the affinity of the hemoglobin towards O2, but the problem is that:

- CO is a competitive inhibitor. It competes with oxygen on the binding sites which lowers the amount of O2 the hemoglobin molecules can load, so it will increase the affinity but it will reduce the amount of bound oxygen .
- One more problem is when the hemoglobin goes to the tissues as it is carrying both O2 and CO it won't release oxygen because it will stay on the R state.

You can go back to slide 12 in this modified to understand more about the affinity of CO to heme: Globular Proteins pt.2

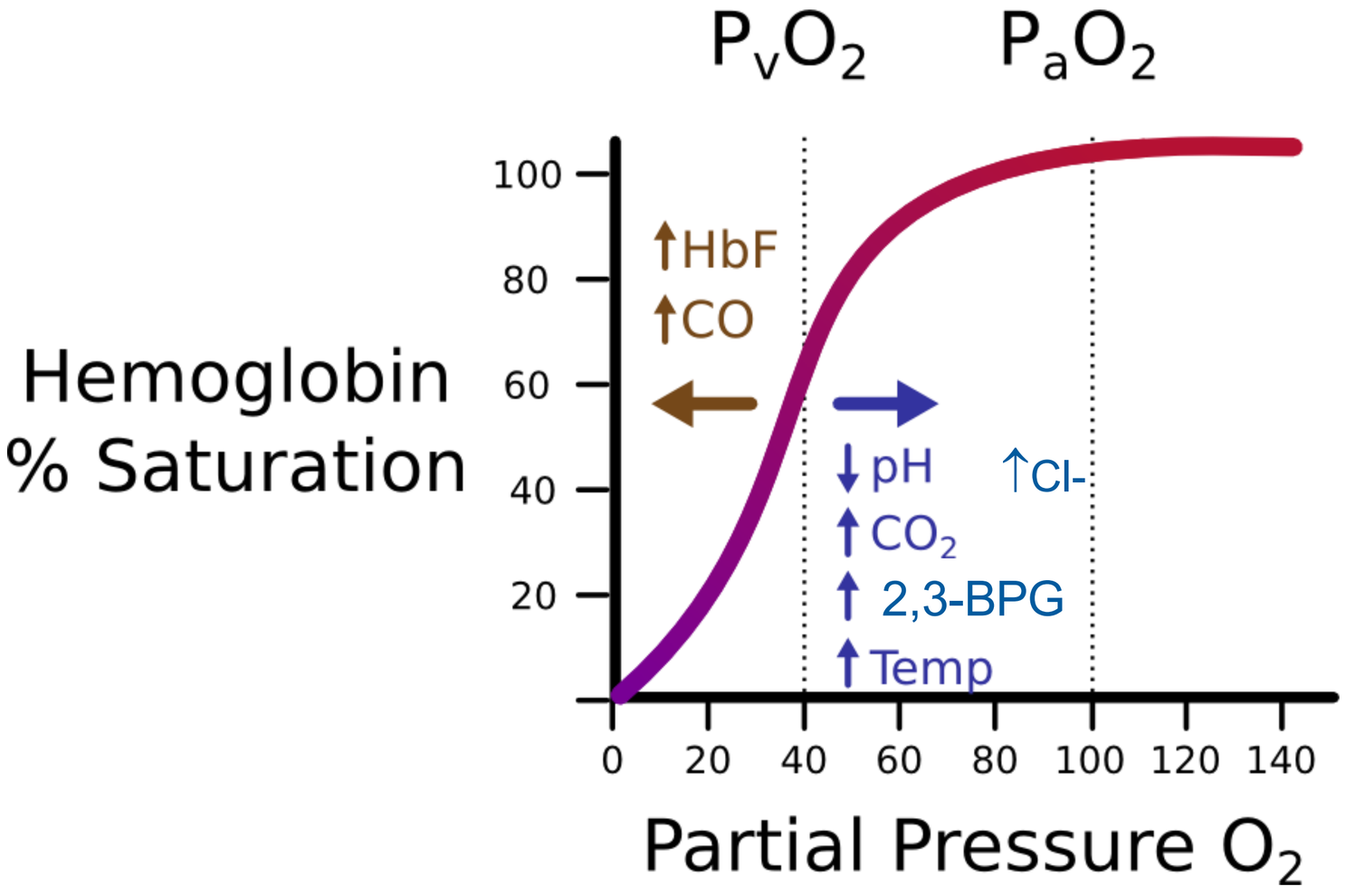
Relevant information

- 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.

"You are raw models you shouldn't smoke, it doesn't make sense, and it is against logic .
You should always remember that you are in question before Allah always and before people"

Dr. Mamoun Ahram

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

QUIZ!

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	31,39,40	Words were shuffled	Words are in order
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks’ Basic Medical Biochemistry (6th edition)