بسم الله الرحمن الرحيم

Biochemistry

﴿ وَقُل رَّبِّ أَدْخِلْنِي مُدْخَلَ صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَل لِّي مِن لَّدُنكَ سُلْطَانًا نَّصِيرًا ﴾

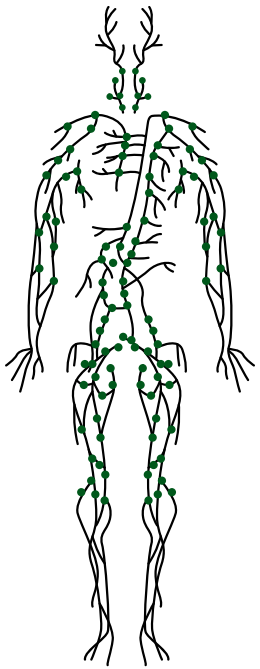
ربنا آتنا من لدنك رحمة وهيئ لنا من أمرنا رشدًا

MID | Lecture #2

Regulation of Hemoglobin Function

Written by: Mais alrahahleh
Ghena Nusair

Reviewed by: Leen Mamoon



اللهم اغفر لصالح وارحمه رحمةً واسعةً، واجعل قبره روضةً من رياض الجنة.

اللهم ثبّت قلبه على الإيمان، واجعل عمله مقبولاً، ونوِّر له قبره واجعل مثواه فسيح الجنان.

اللهم ارحم شهداء غزة ومن سبقهم من الشهداء، واجعل دماءهم شفيعةً لهم وانتصارًا لدينك، واجمعهم مع النبيين والصديقين والشهداء والصالحين.

اللهم اغفر لهم ما تقدّم من ذنبهم وما تأخّر، واملأ قبورهم نورًا ورضوانًا

اللهم ألطف بحزن أهاليهم وذويهم، وارحم تراب بلادهم، وانصر المظلومين وأعِزّ الحق.

Regulation of hemoglobin function

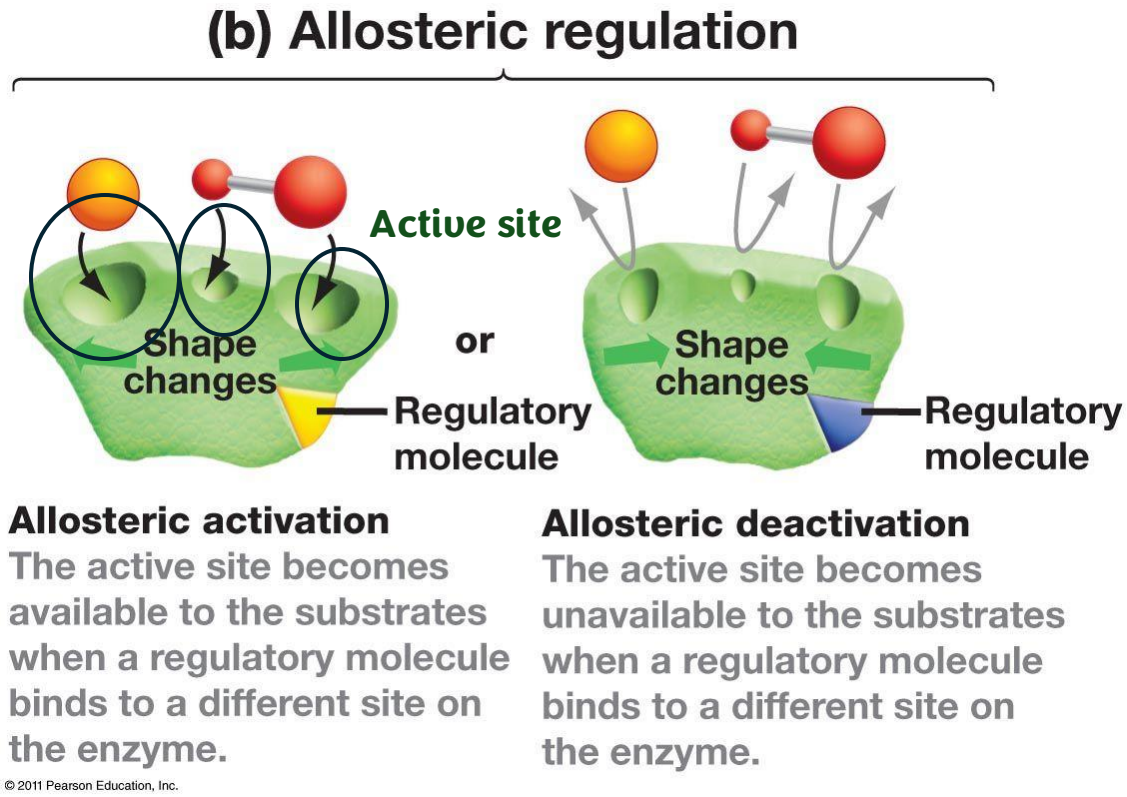
Prof. Mamoun Ahram
Hematopoietic-lymphatic system

اللهم إني أسألك فهم النبيين، وحفظ المرسلين، وإلهام الملائكة المقربين، اللهم اجعل لساني عامرًا بذكرك،
وقلبي بخشيتك، وسري بطاعتك، إنك على كل شيء قدير.

Allosteric regulation

➢ Is the modulation of the protein funtion when a regulatory molecule binds to it in a site other than the active site.

- Ligands that induce conformational changes in *allosteric* proteins are referred to as allosteric modulators or effectors.
- Allosteric modulators may be inhibitors (decrease activity) or activators(increase activity).
- Allosteric regulators can be either:
- Homotropic modulators: are the same as the ligand itself, such as O_2
- Heterotropic modulators: are different from the ligand (next slide)



✓ **Oxygen** is both the **ligand** in one subunit and an **allosteric regulator** for the other subunits!

Allosteric effectors

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

✓ Almost all of these heterotropic effectors ,when their **concentrations increase, induce a conformational shift** of hemoglobin from the **(R) state** to the **(T) state**, thereby facilitating **enhanced oxygen delivery.**

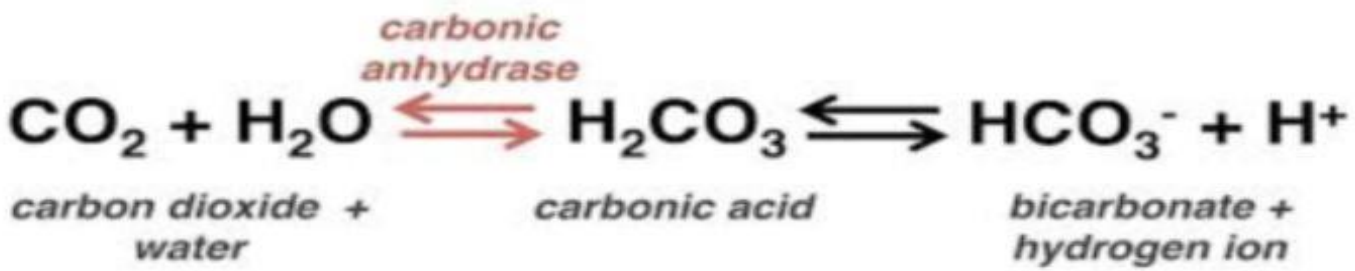
- A competitive inhibitor
  - Carbon monoxide

1- The effect of pH and H^+

- These PH changes (decreased due to H+ increase)occur near active tissues, primary because of Co2 .

The effect of pH

- The binding of H^+ to hemoglobin promotes the release of O_2 from hemoglobin to the metabolically active cells, and the binding of O_2 at the lungs releases the H+.
- This phenomenon is known as the **Bohr effect**.



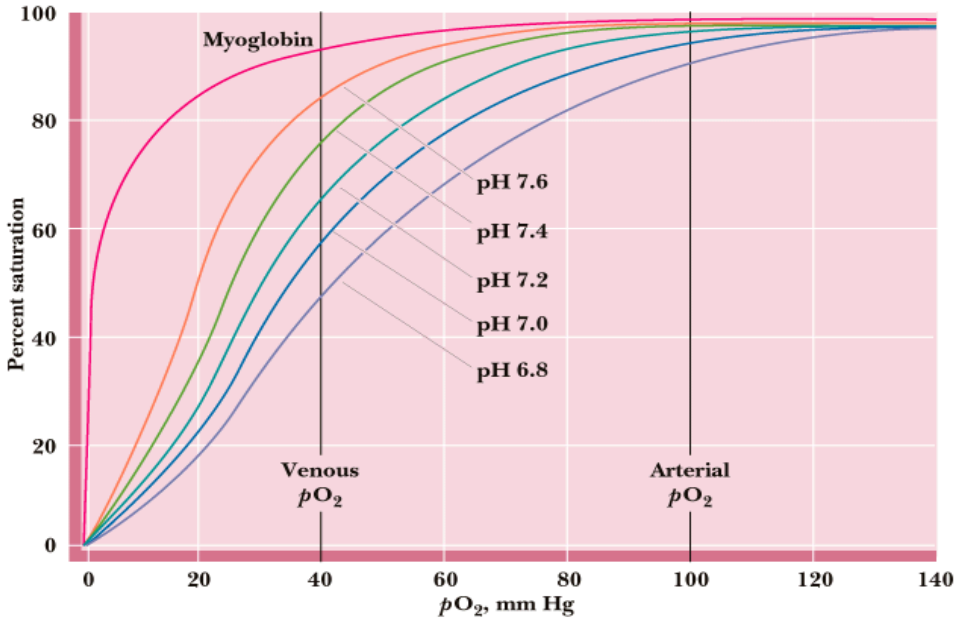
- Bicarbonate (HCO_3^-) → transported in the plasma to the lungs to be exhaled.
- Protons (H^+) → bind to hemoglobin, stabilizing the T-state, which promotes oxygen release to the active tissues.

- **The reaction between CO_2 and H_2O to form carbonic acid (H_2CO_3)** can occur **spontaneously** in **plasma**, but the rate is **extremely slow**.
- **Within red blood cells**, this reaction is catalyzed by the enzyme ***carbonic anhydrase***, which greatly accelerates the conversion of CO_2 to H_2CO_3.
- The resulting carbonic acid rapidly dissociates into bicarbonate (HCO_3^-) and hydrogen ions (H^+),These H^+ ions constitute the primary source of intracellular protons in erythrocytes.

Effects of pH on the oxygen Dissociation Curve of hemoglobin

- As **pH decreases** (↑[H^+]), **hemoglobin's affinity for O2 decreases**→more oxygen is needed to saturate hemoglobin and facilitate oxygen release to tissues. (Bohr effect), the sigmoid curve shifts to the right and P50 increases .
  - ✓ ***Po2* refers to the partial pressure of oxygen at which hemoglobin is 50% saturated.**

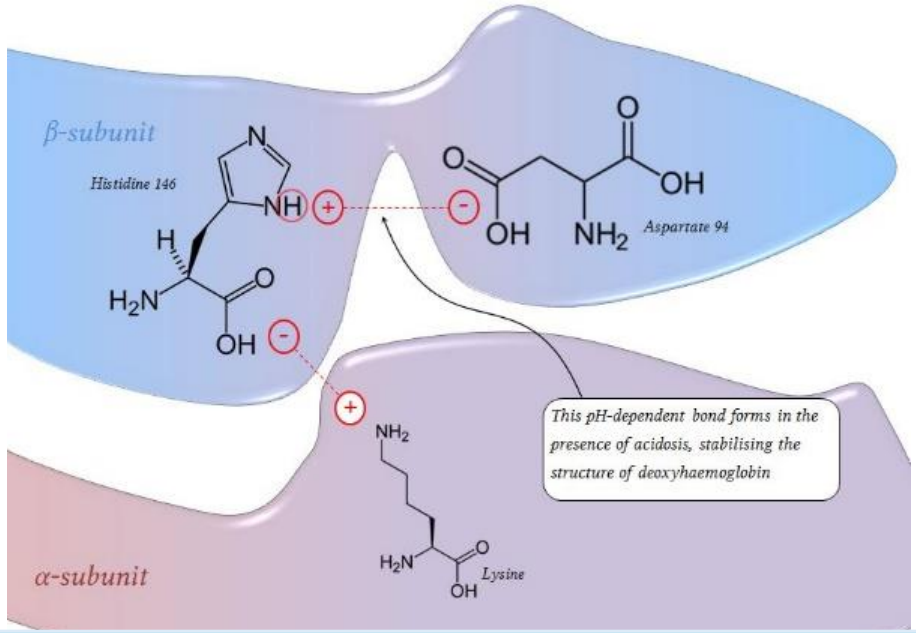
- High proton concentration–>Low pH–>low oxygen affinity , stabilization of the T state of hemoglobin more ,higher P50, shifting the sigmoid curve to the right.
- Low protons concentration –>higher pH–> higher oxygen affinity, stabilizing the R state of hemoglobin more, lower Po2 ,shifting the sigmoid curve to the left.



Mechanism of Bohr effect

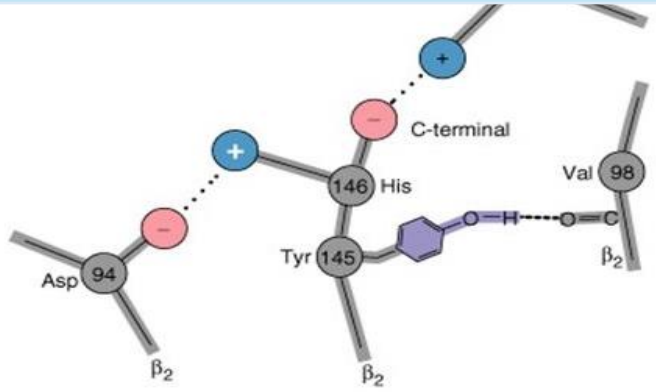
!! Go to the next slide then come back

- Increasing H^+ (in tissues) causes the protonation of key amino acids, including the last histidine residue of the β chains (**His146**).
- Once the Hemoglobin is at the tissues, it starts losing the Oxygen and starts changing to T state. The protonation of His146 speeds up the process as electrostatic interaction occur between the carboxylic group of His146 and a lysine of the α and a salt bridge forms between the same His and Asp94 within the same chain. These interactions stabilize the T state and keep His in an environment that increases its pKa leading to further stabilizing of the salt bridges.
- The reason behind the increase in the pKa is the movement of the His to a more negative environment. Although this should decrease its pKa because it would be more likely to lose the H+, nonetheless, it seems that there are multiple negative charges that bind to this H+ so none can pull it away from the rest. Furthermore, pulling this H+ away would add more negative charge to the environment, thus increasing the repulsion.
- When hemoglobin reaches lungs, Oxygen binds to it changing it from the T state to the R state, causing a destabilization in the whole structure which leads to breaking of the electrostatic bonds and changing to the position of the His to a place where its pKa becomes lower which causes it to loose the H+.



Note

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



****Pka** is the pH in which half of molecules of an acid/base are protonated and the other half is not ,AAs and buffers have different Pka , and it tells in which pH zone this buffer acts the best (pka- 1_pka +1) , so as an example an buffer with pka=4 , its best buffering zone is between 3 and 5

Mechanism of Bohr effect

❖ **Let's organize the information**

In active tissues:

- ↑ H^+ → protonation of His146 in β-chain
- Protonated His146 forms electrostatic interactions:
  - ✓ With *Lys* in α-chain
  - ✓ With *Asp94* in same β-chain → forms salt bridge
- These interactions stabilize the T-state → promotes O_2 release

In the lungs:

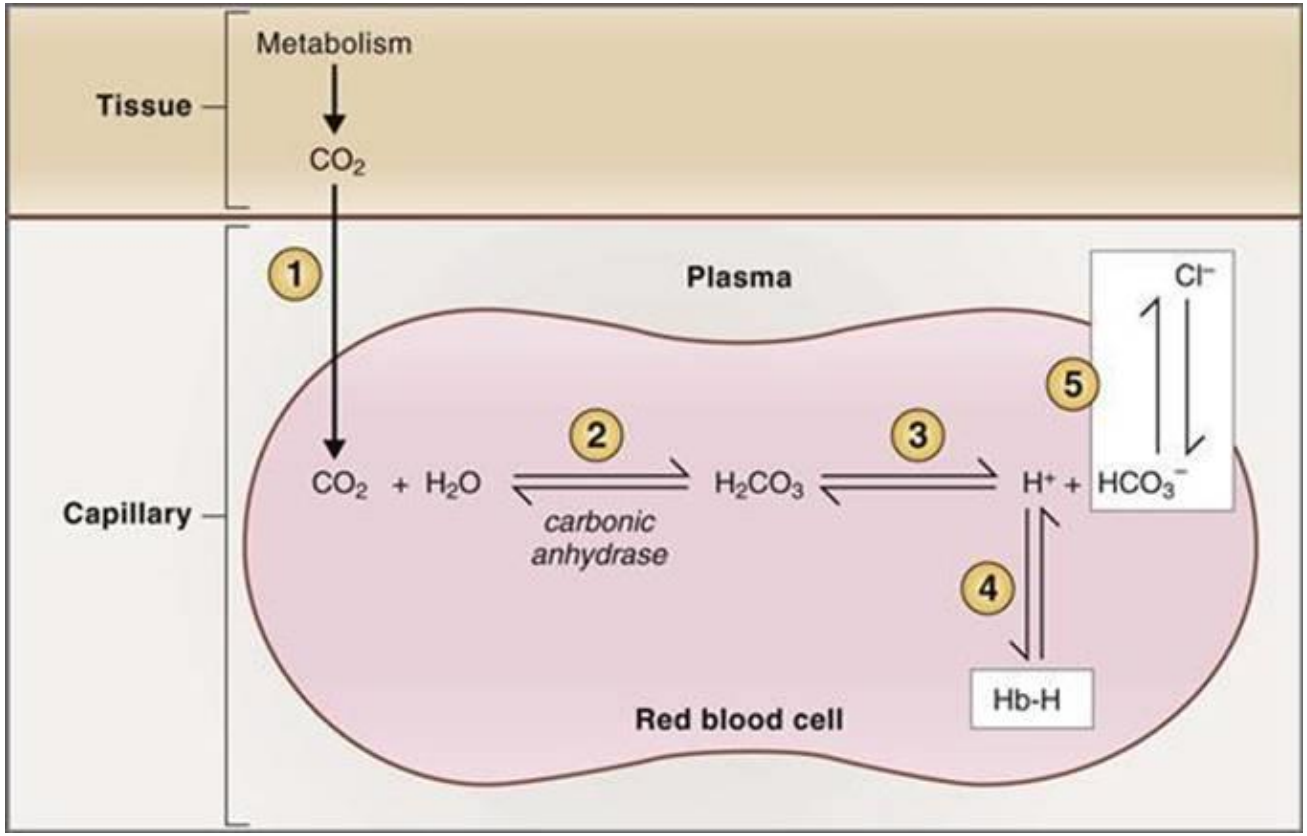
- O_2 binds → Hb transitions from T-state → R-state
- Electrostatic interactions break
- His146 loses proton → R-state stabilized → O_2 affinity increases → Hb becomes saturated with O_2

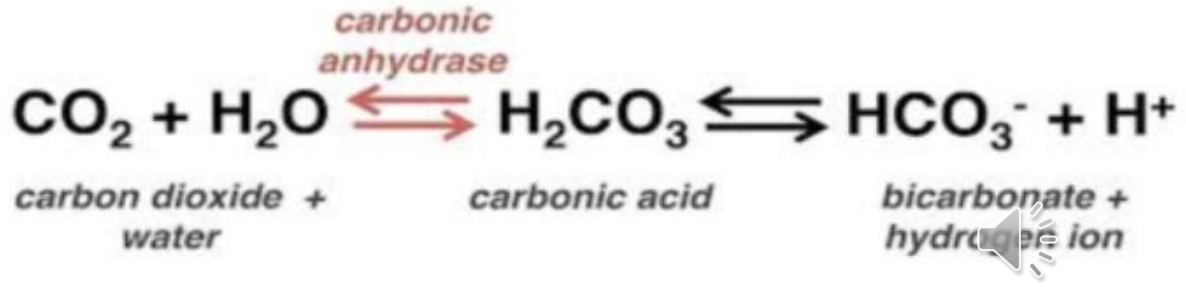
- Why does His146 pKa increase?
  - The negative environment would normally favor proton loss.
  - Multiple negative charges surround the proton → prevent its release → further stabilize T-state.
  - the molecule holds on to its proton more tightly → pKa increases

	pH	$[H^+]$	O_2 affinity	P50	Curve shift	State
Active tissues	Low	High	Low	High	Right	T
Lungs	High	Low	High	Low	Left	R

Where do protons come from?

- Co2 is produced at high levels in metabolically active tissues. Then it enters the RBCs and reacts with H2O through carbonic anhydrase as shown in the figure and the equation to the right to produce H2CO3 (carbonic acid) which breaks to H+ which binds to hemoglobinand HCO3- that exite the RBC un exchange with chloride (bicarbonate).
- In the lungs, the binding of O2 to the hemoglobin causes the H+ to be released as described in the previous slide.

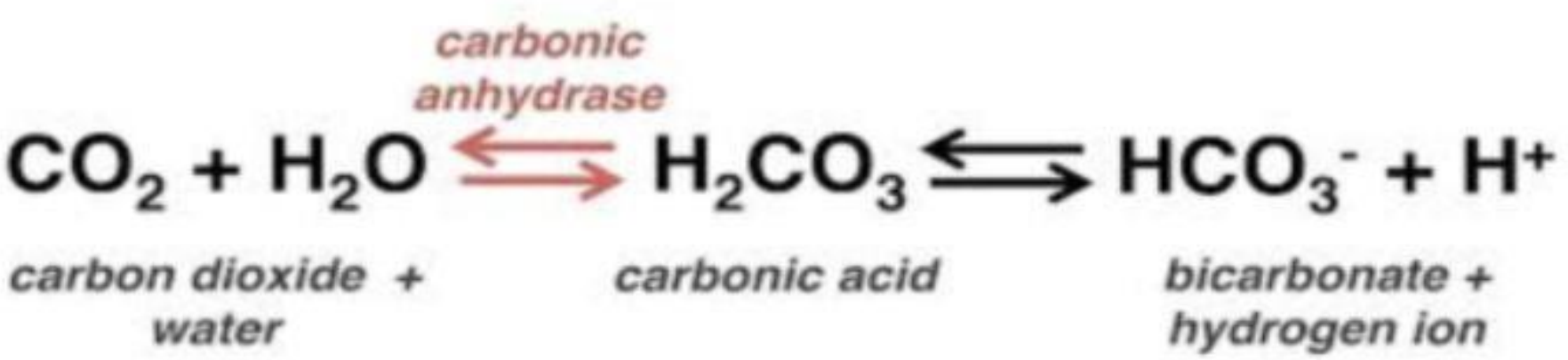




2- The effect of CO_2

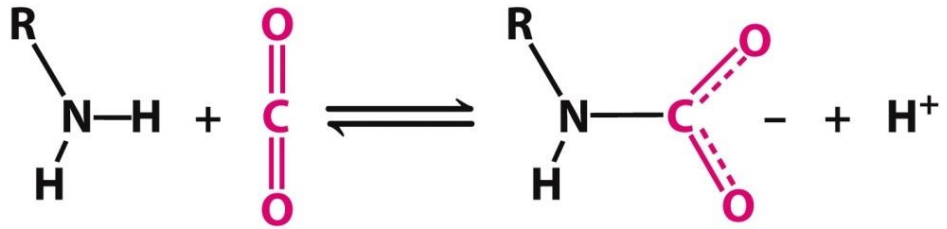
Mechanism #1 - production of protons

- The primary way of Co2 transportation:



Mechanism #2- formation of carbamates

- **Co2 could be transported by many ways such as:**
- Hemoglobin transports some CO_2 directly ,**only 30%** .
- When the CO_2 concentration is high, it combines with the free α-amino terminal groups to form carbamate and producing negatively-charged groups



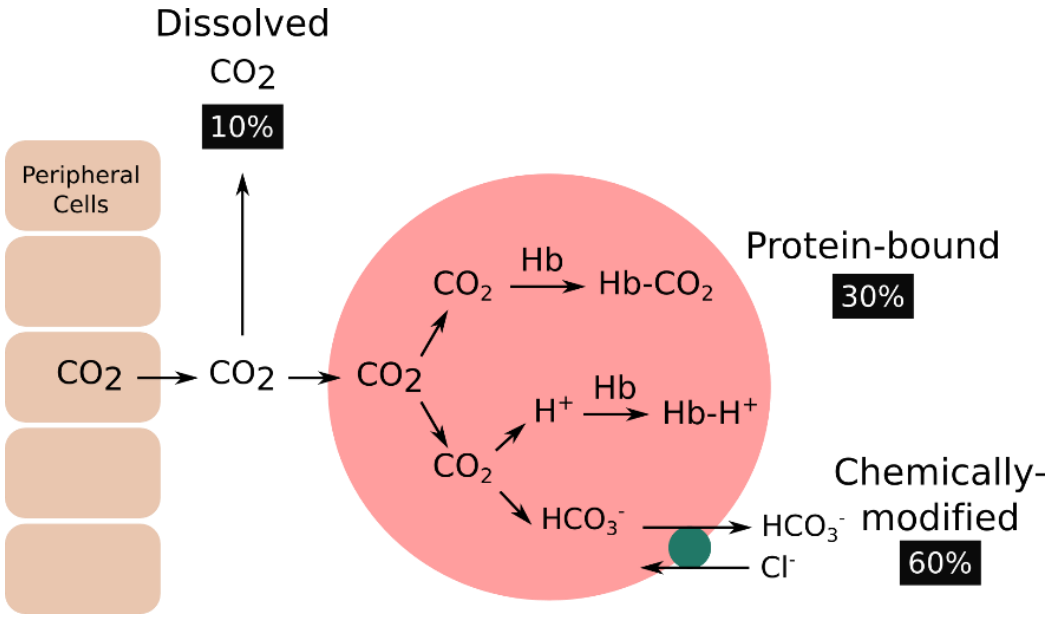
Carbamate

- This by product (protons) in this rxn does not participate significantly in the low pH inside the RBCs as the protons that are produced from the breakdown of carbonic acid.

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

Transport of CO_2 into lungs

- 1) Approximately 60% of CO_2 is transported as bicarbonate ion, which diffuses out of the RBC in exchange with Chloride to stabilize the net negative charge in the cell.
- 2) About 30% of CO_2 is transported bound to N-terminal amino groups of the T form of hemoglobin .
- 3) A small percentage of CO_2 is transported as a dissolved gas.



The movement of CO_2 in/out of cells (the level of protons inside the RBCs is high) does not change the pH, a phenomenon called isohydric shift, which is partially a result of hemoglobin being an effective buffer in binding to the H+.

Which mechanism has a stronger effect in lowering hemoglobin's affinity to O2?

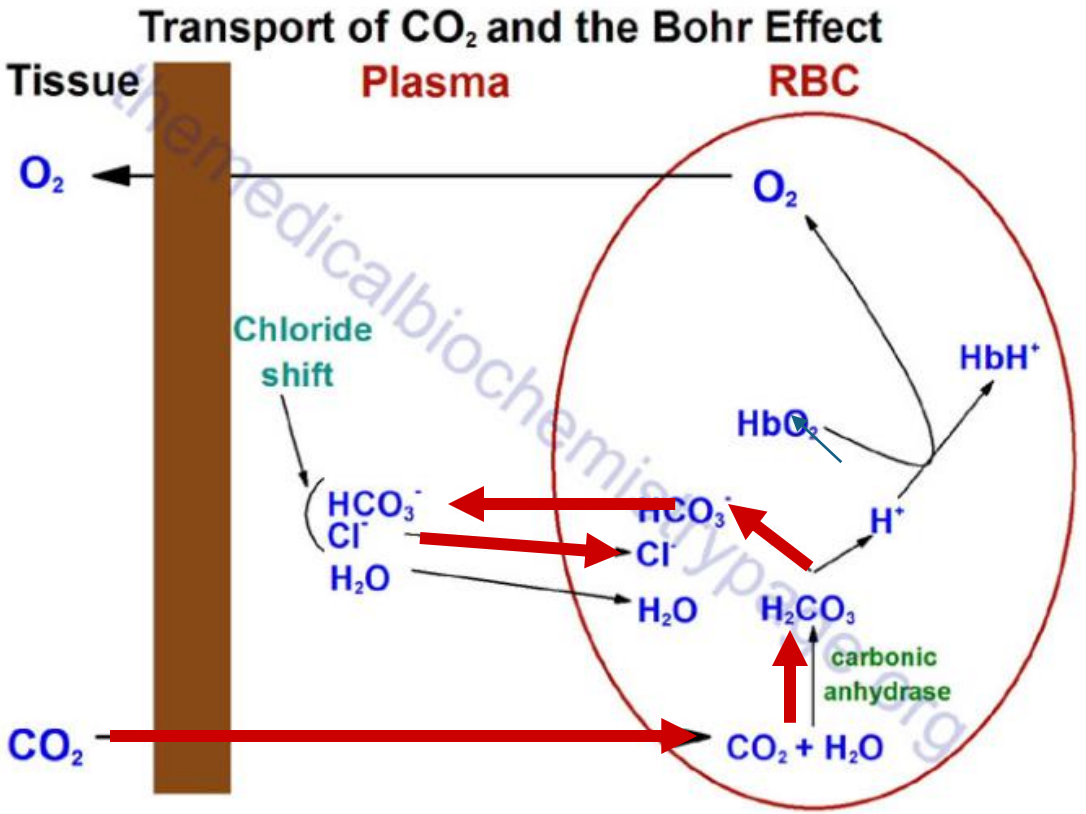
- About 75% of the shift is caused by H^+.
- About 25% of the effect is due to the formation of the carbamino compounds.
- How do we know that? By changing one factor at a time and keeping the others constant. We saturate the hemoglobins with O_2, then we start reducing it (the O_2) without any effector (control) and we record the p50. Then we saturate the hemoglobin again and we start reducing the Oxygen in the same rate as above but now we perform the test at low pH (different pHs can be measured). Then we do the same but with varying CO_2 concentrations instead of pH, then we compare which causes bigger shift to the right **with each one's independent curve.**

For better understanding of the Oxygen Dissociation Curve watch this short video at the end of this lecture. Click here

3- Effect of Chloride ion

Chloride shift

- Bicarbonate diffuses out of the red blood cells into the plasma when the RBCs are near active tissues, but enters into the RBC near the lungs in order to be converted to CO_2 then released into the lungs.
- 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“.



Regarding previous slide

✓**In tissues:**

- Bicarbonate diffuses out from RBC to tissues and chloride enters the RBC

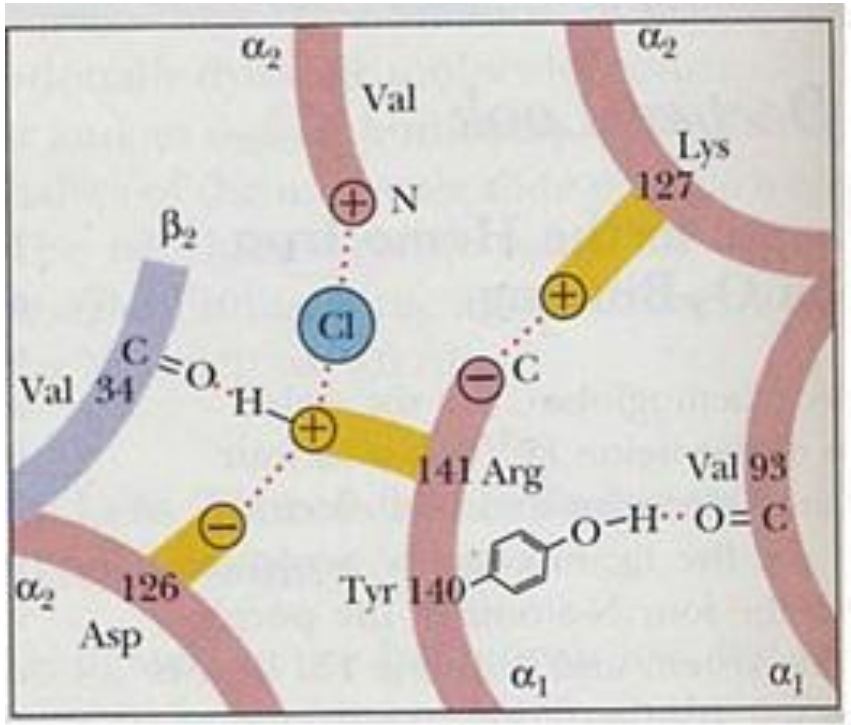
✓**In the lung:**

- Chloride diffuses out and bicarbonate enter the RBC, then bicarbonate will bind to H+(source:O2 binding to Hb will cause H+ dissociation) leading to H2CO3 formation, then H2CO3 will dissociate into H2O and CO2, CO2 will be released .

Effect of chloride ions

- The –ve charge of chloride facilitates the formation of electrostatic interaction (between α2, β2 for example) ,it also stabilizes the T state.

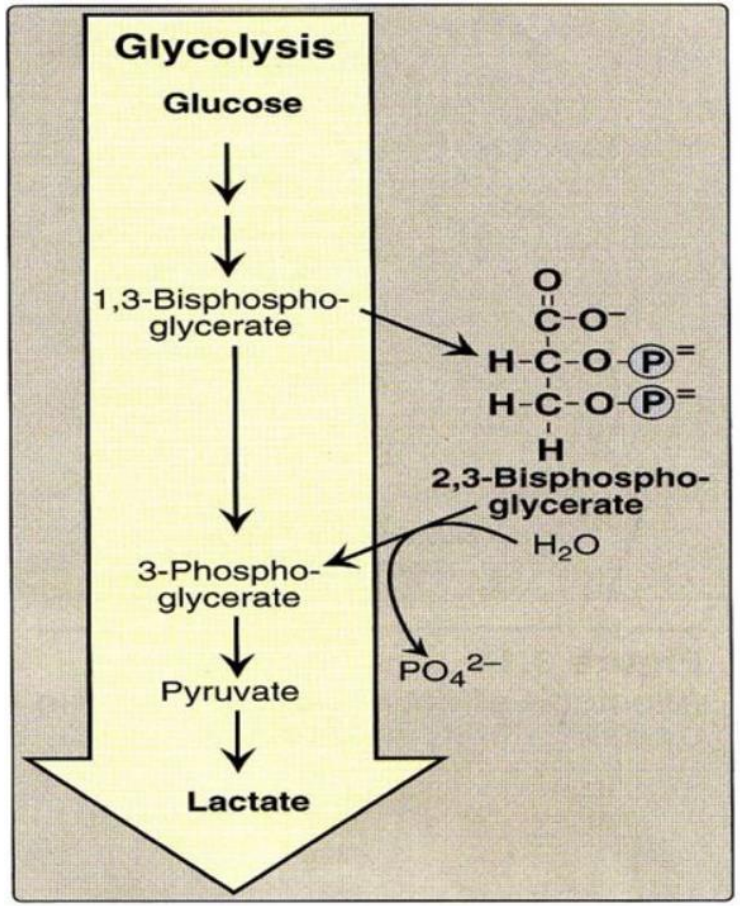
- Chloride ions interact with both the N-terminus of α2 chain and Arg141 of α1 chain stabilizing the T-state of hemoglobin.
- Increasing the concentration of chloride ions (Cl^-) shifts the oxygen dissociation curve to the right (lower affinity).



4- Effect of 2,3-bisphosphoglycerate

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

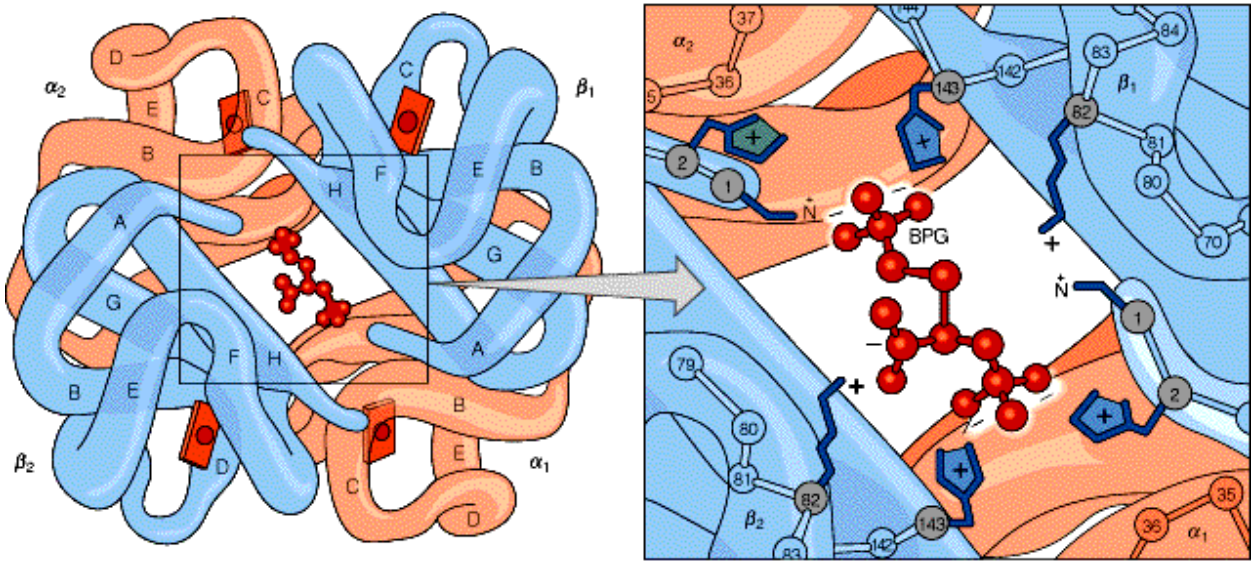
- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- It binds to hemoglobin and reduces its affinity towards oxygen.



2,3-BPG –hemoglobin interaction

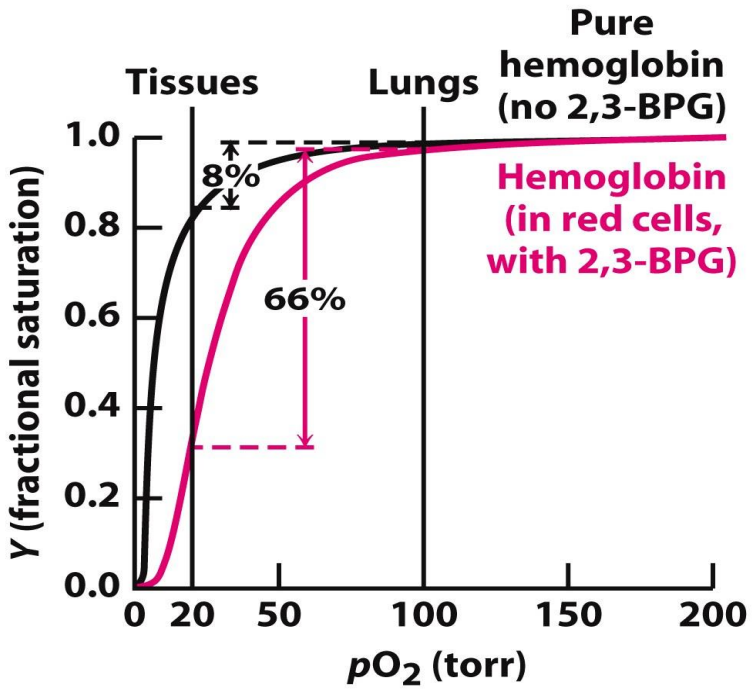
- 2,3-BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 2,3-BPG/hemoglobin tetramer.
- This binding stabilizes the T-state hemoglobin reducing the binding of oxygen to hemoglobin and facilitating oxygen release.

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

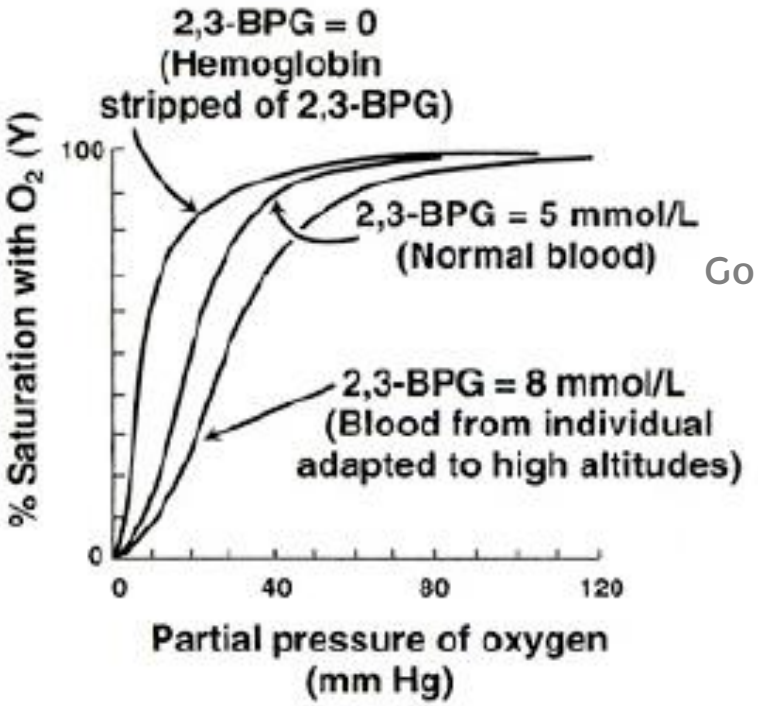


Effect of 2,3-BPG on oxygen binding

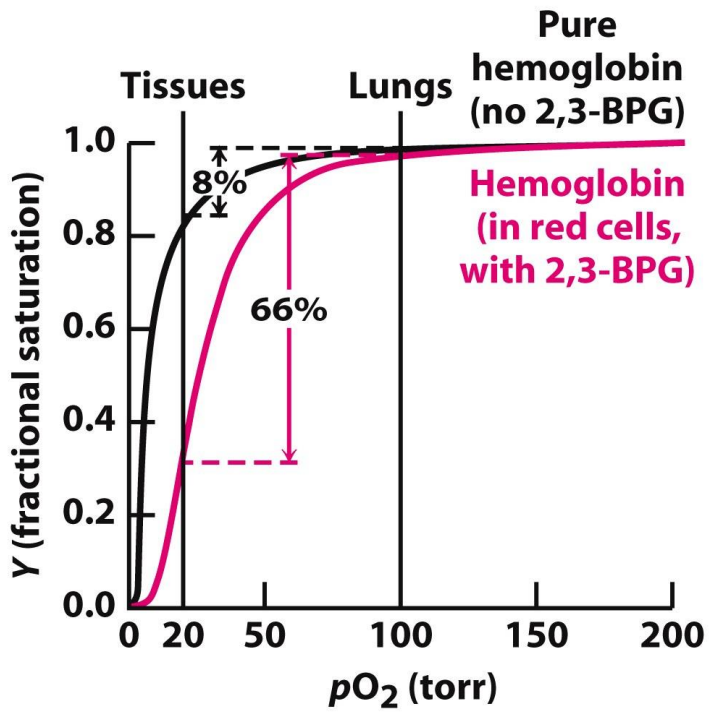
- In the presence of 2,3-BPG, the p50 of oxyhemoglobin is 26 torr.
- If 2,3-BPG were not present, p50 is close to 1 torr.
- Notice the big drop of saturation with and without oxygen at the tissues
- The concentration of 2,3-BPG increases at high altitudes (low O_2) and in certain metabolic conditions making hemoglobin more efficient at delivering oxygen to tissues.

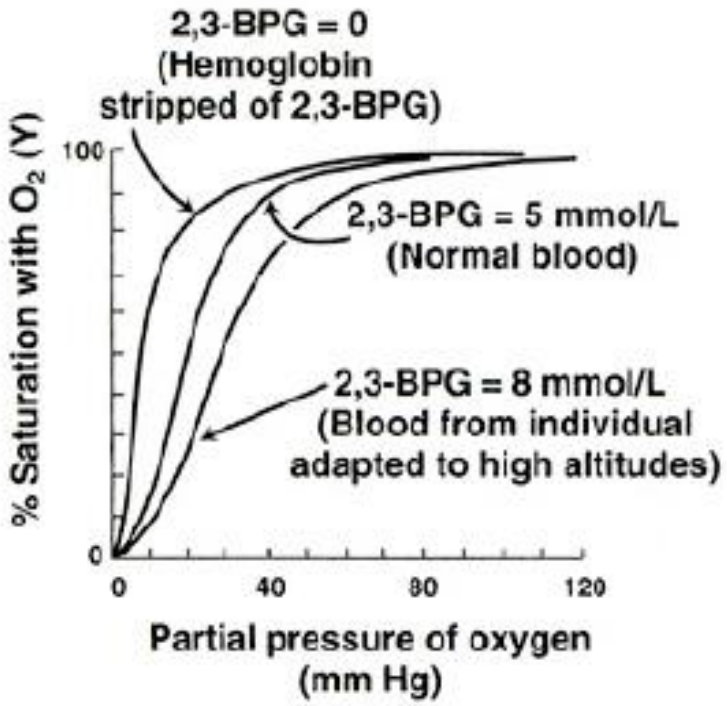


h Edition
and Company



Go to the next slide





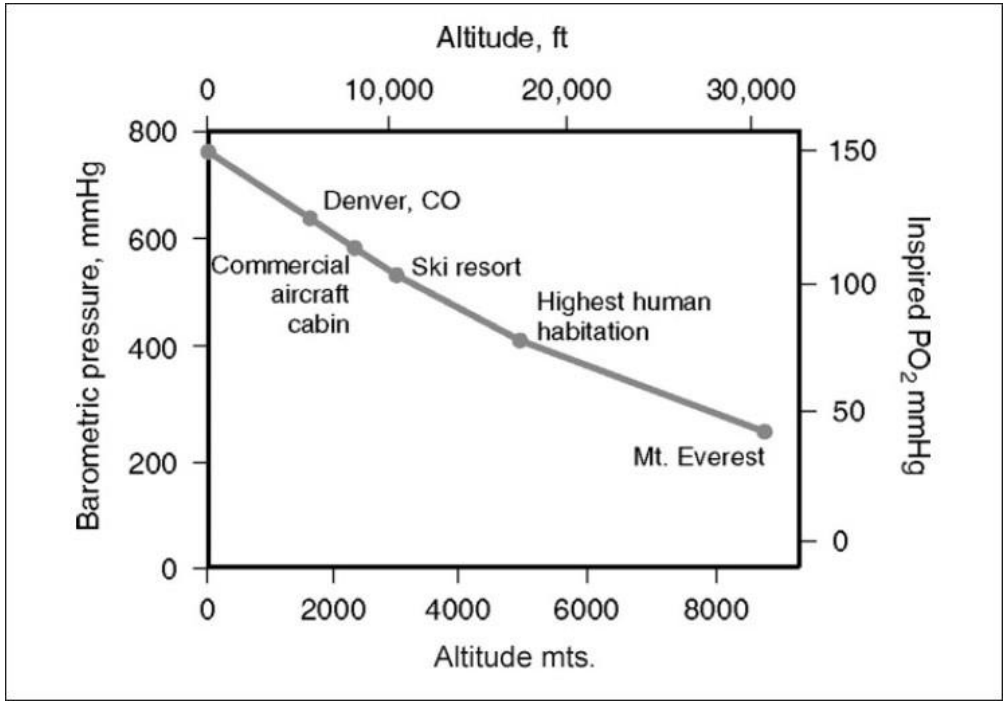
- In case of pure Hb (no 2,3BPG):
- ✓ Hb needs much more lower O2 levels to start releasing O2 to tissues

- In the presence of 2,3BPG:
- ✓ Affinity of O2 decreases releasing O2 more easily at the same pressure

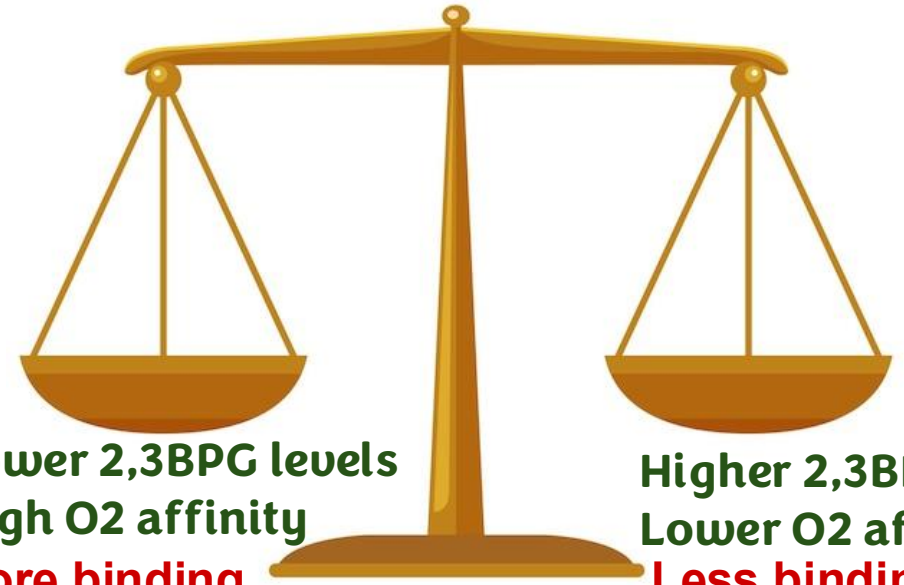
- look at second figure and notice how the curve shifts to the right when 2,3BPG levels increase.

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



- ✓ Note that in the presence of 2,3BPG Hb requires higher oxygen pressure to become saturated in lung, but it releases oxygen more readily in the tissues.



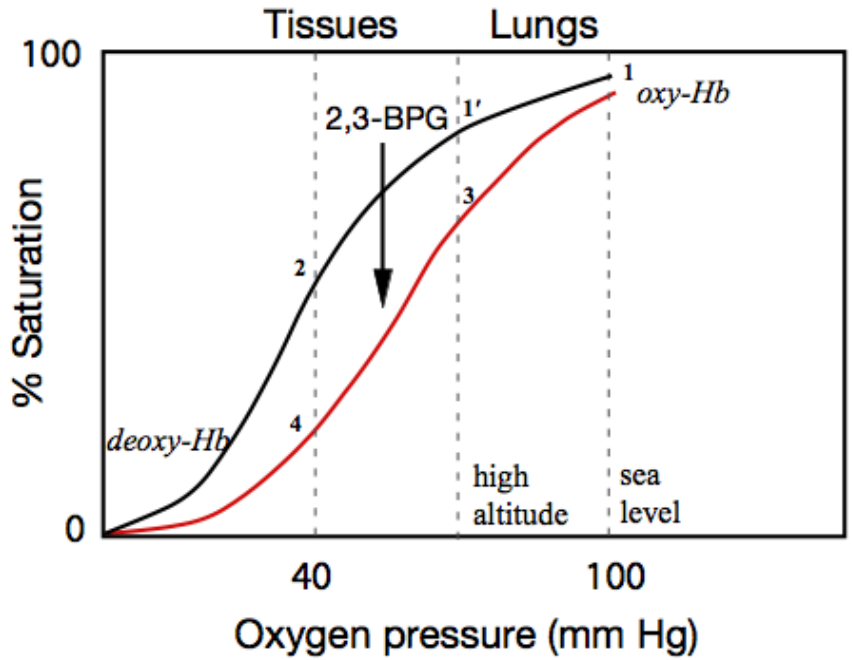
Lower 2,3BPG levels
High O2 affinity
More binding
Less release

Higher 2,3BPG
Lower O2 affinity
Less binding
More release

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.

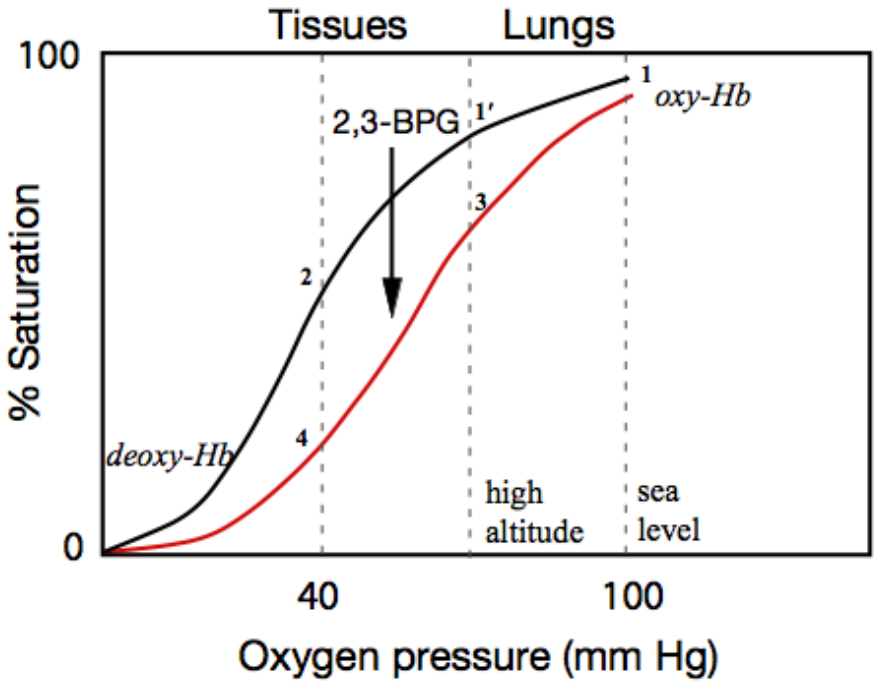


Better explanation of the role of 2,3-BPG

- 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.

  In other words : 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.

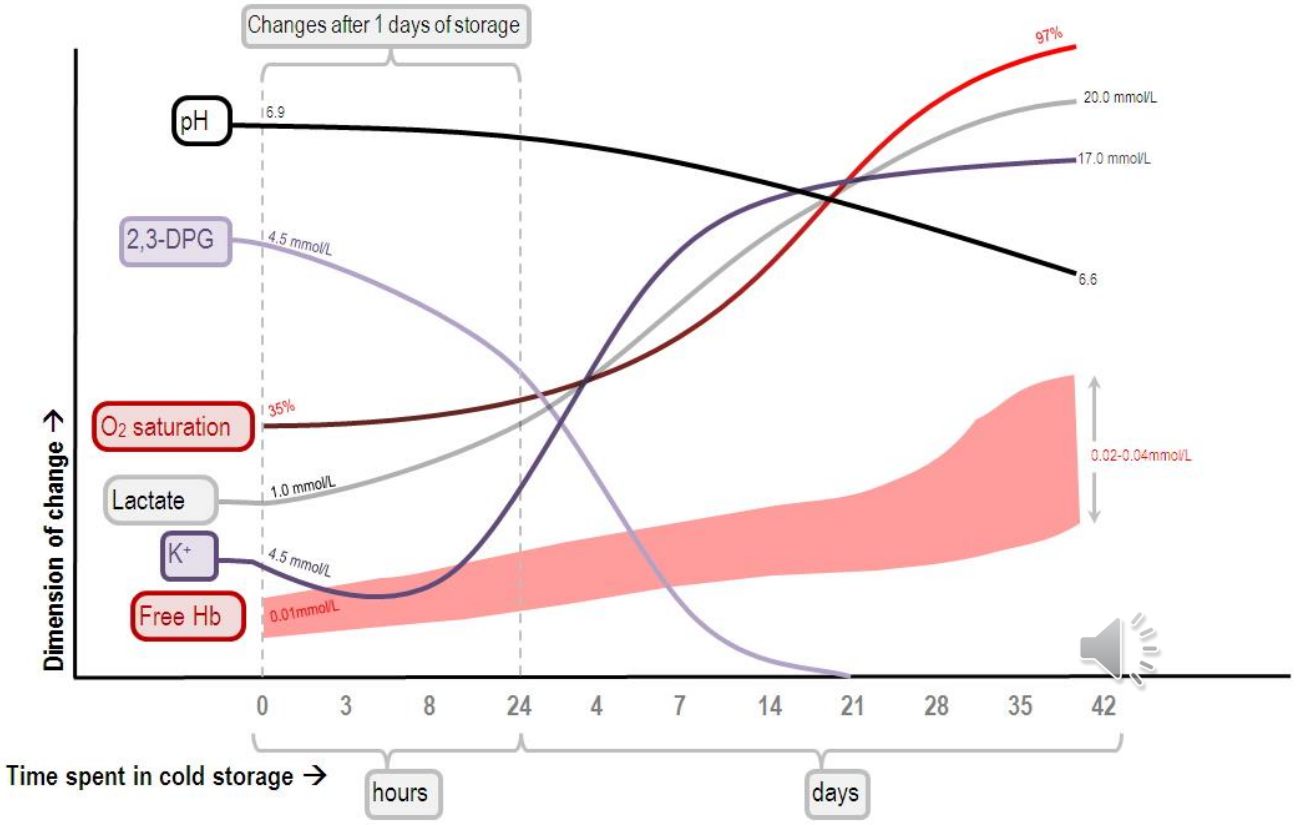
- This is not a perfect solution, but over time there is increased production of red blood cells to provide more hemoglobin to compensate for the smaller amount of oxygen it can bind.



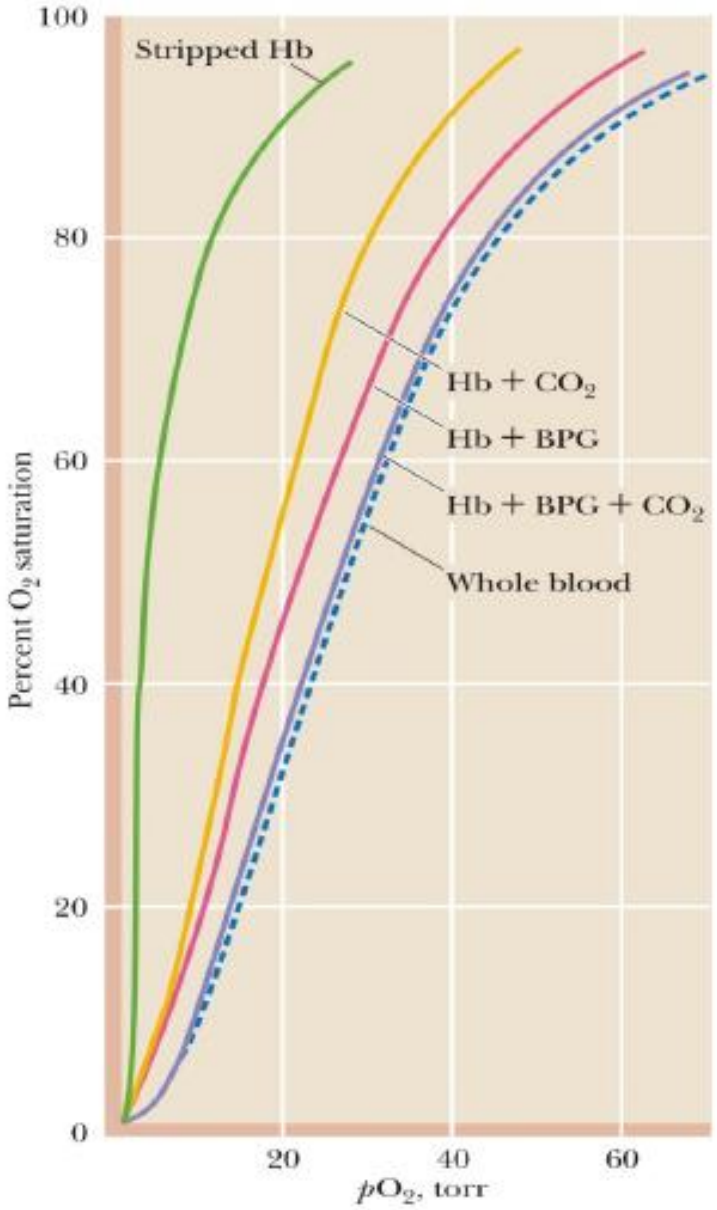
2,3-BPG in transfused blood

- Storing blood results in a decrease in 2,3-PBG (and ATP **which facilitates O2 release**), 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.

- **You can transfuse this blood to competent patients but** Severely ill patients may be compromised.
- Both 2,3-PBG and ATP are rejuvenated.



2,3-BPG and CO2 are important players

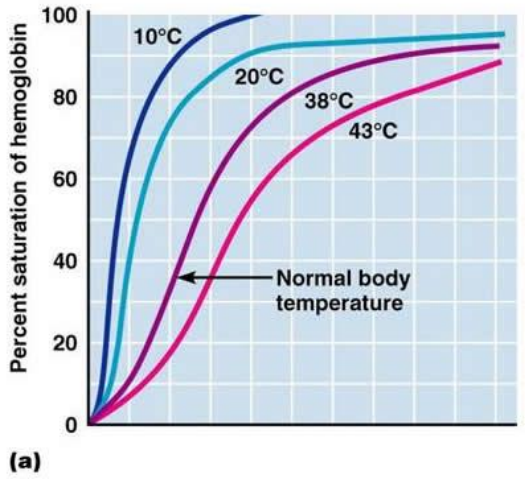


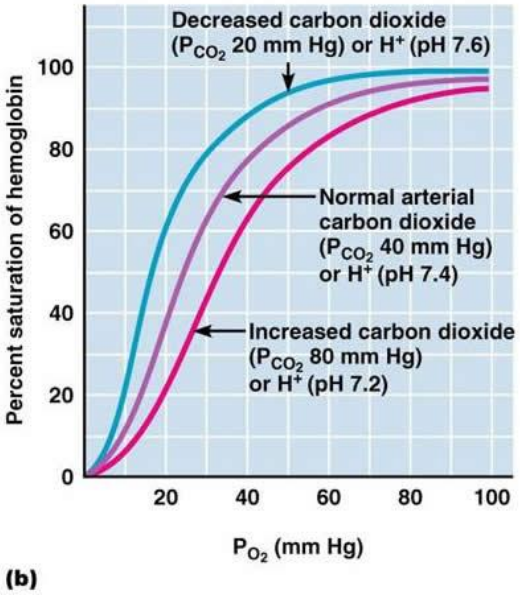
- ✓ **Notice how factors affect the oxygen release**

Other factors: A-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.





Other factors: B- Effect of CO

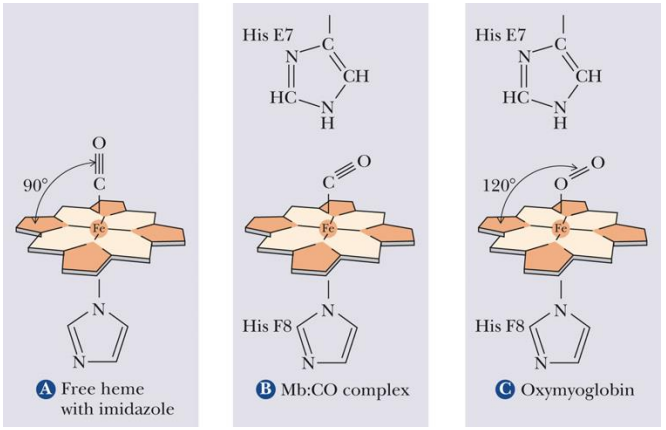
- CO is considered a competitive inhibitor(not allosteric regulator).

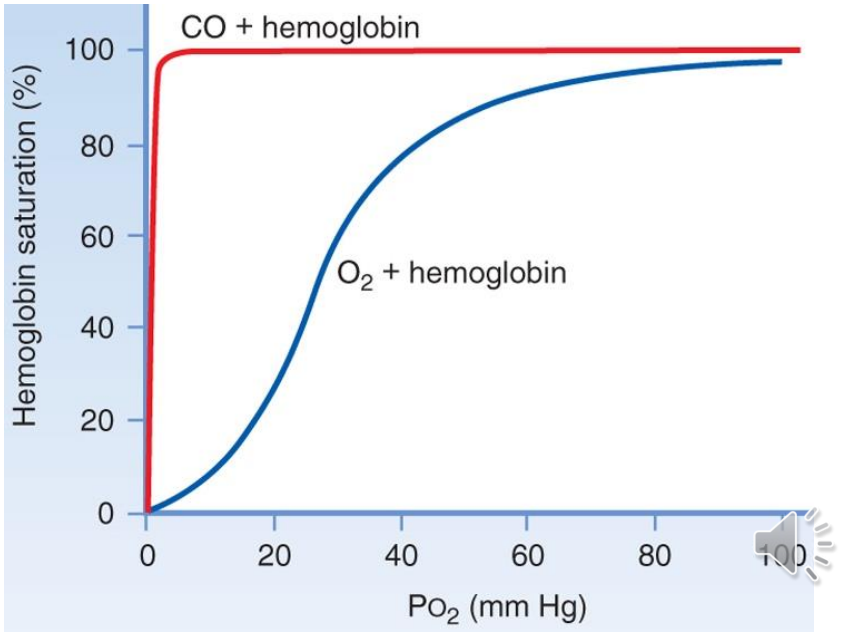
Effect of CO

- CO has higher affinity to Fe (stronger bonds with Hb) than O2.

- 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 ,impeding O2 releasing from Hb.

(Hb + O_2) versus (Hb + CO)→ the curve illustrates the release of oxygen in the presence and in the absence of CO.





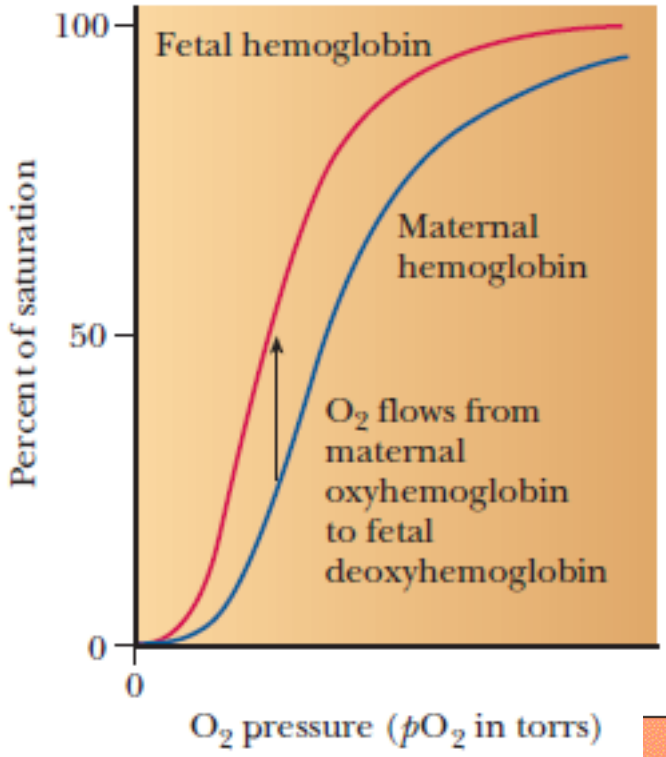
Relevant information

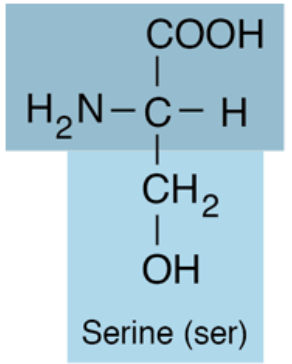
- Due to pollutants, the concentration of CO-Hb in the blood is usually 1% in a non-smoker.
- In smokers, CO-Hb can reach up to 10% in smokers.
- If this concentration of CO-Hb in the blood reaches 40% it would cause unconsciousness initially, followed by death.
- Increasing the amount of CO in inspired air to 1% and above would be fatal in minutes as that would increase the CO-Hb in blood to 40%

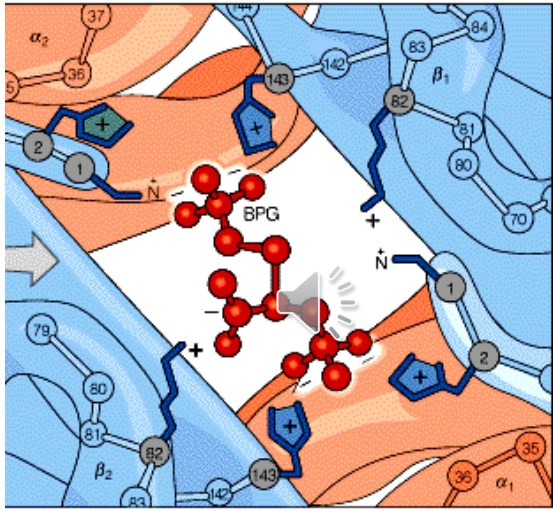
Other considerations

Fetal hemoglobin

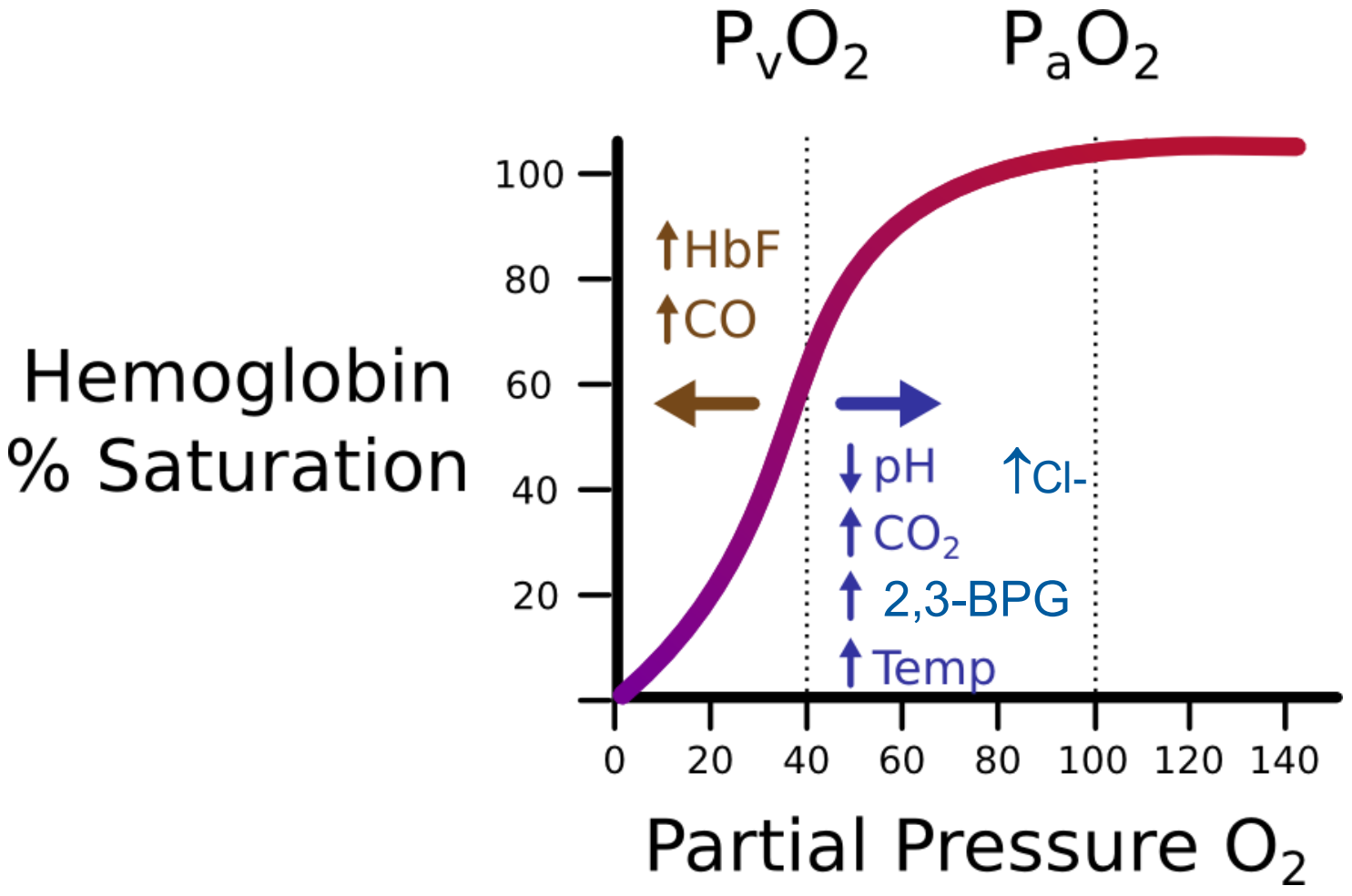
- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HBA).
  - HbA = $\alpha 2\beta 2$
  - HbF = $\alpha 2\gamma 2$
- His143 residue in the β subunit is replaced by a serine residue in the γ subunit of HbF.
  - Since serine cannot form a salt bridge with 2,3-BPG, it binds weaker to HbF than to HbA, **so Hb remain in the R state rather T state, thus higher affinity to O2.**







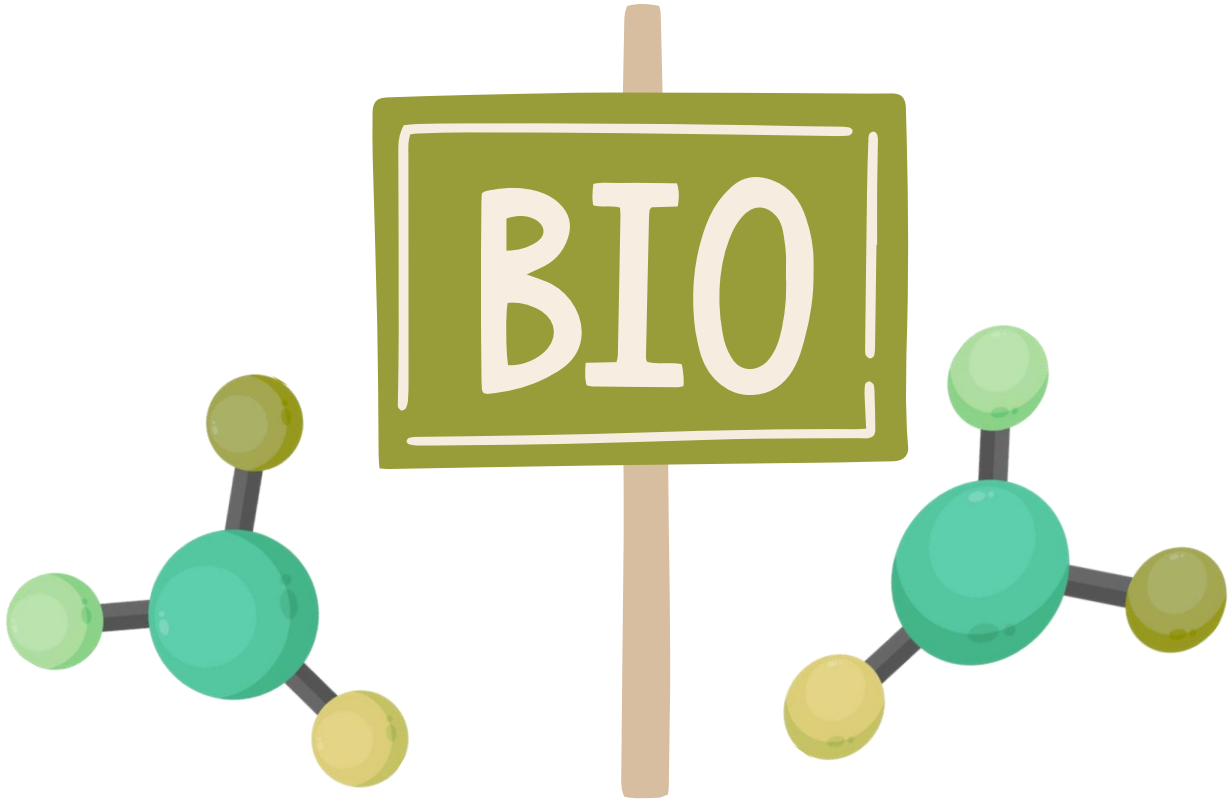
Summary


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

Summary

Effector	Primary mechanism	Effect on Hb O_2 affinity	Curve / P50 change	Net physiological consequence
Hydrogen ion (H^+) — Bohr effect	Protonation of key residues (e.g. His146 β) → formation of salt-bridges/electrostatic interactions that stabilize the T (tense) state	Decreases O_2 affinity	Right shift of O_2–Hb curve; P50 increases	Promotes O_2 release in metabolically active (acidic) tissues. Major contributor (~75%) to rightward shift.
Carbon dioxide (CO_2)	(A) Converted to $H^+ + HCO_3^-$ (via carbonic anhydrase) → increases H^+ (Bohr effect) ; (B) directly forms carbamates at N-termini of Hb → adds negative charges	Decreases O_2 affinity (both indirectly via H^+ and directly via carbamates)	Right shift; part of the shift due to CO_2 is independent of pH (carbamate effect).	Facilitates CO_2 transport and O_2 release in tissues. Carbamate formation contributes ~25% of the effect.
2,3-Bisphosphoglycerate (2,3-BPG)	Binds to central cavity of deoxy-Hb (between β chains), stabilizes T state by electrostatic interactions	Decreases O_2 affinity substantially	Right shift of curve; P50 increases	Increases O_2 delivery to tissues (especially important in chronic hypoxia/altitude). Helps maintain O_2 unloading when arterial saturation falls.
Chloride ion (Cl^-)	Binds near N-termini / interacts electrostatically with subunits (part of chloride shift)	Decreases O_2 affinity (stabilizes T state)	Right shift (modest)	Helps stabilize T-state and supports O_2 release; Cl^- movement (chloride shift) balances HCO_3^- transport.

Biochemistry Quiz #2



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	Slide 11 First line	H+ is produced at high levels in metabolically active cells Note: This mistake is from the doctor's slides.	Co2 is produced at high levels in metabolically active cells
V1 → V2			