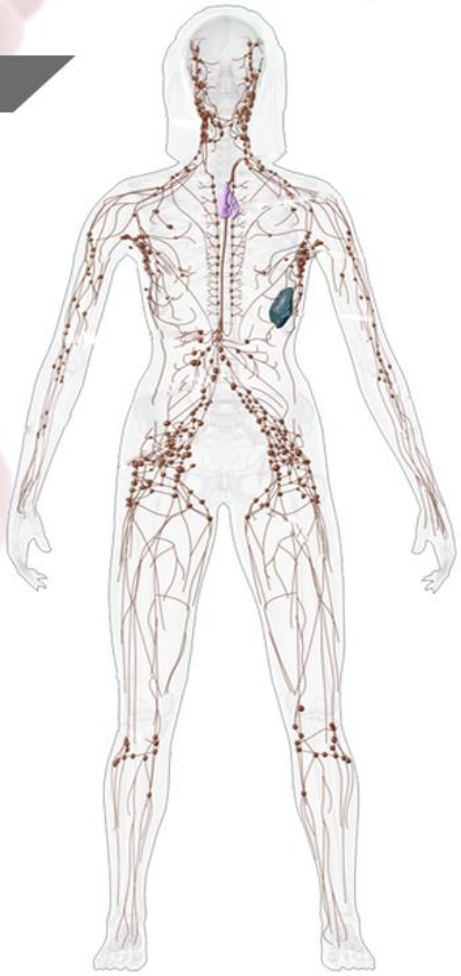
Sheet 2

Hematology and Lymphatic system

Subject | Biochemistry



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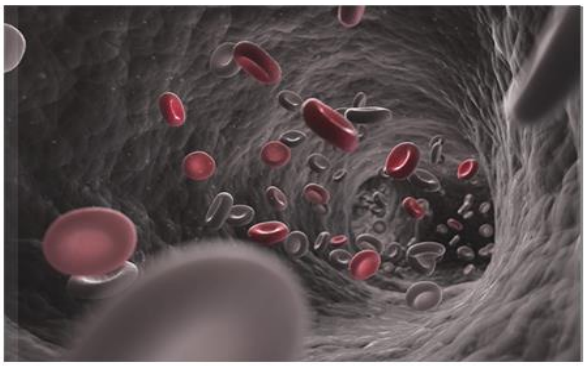
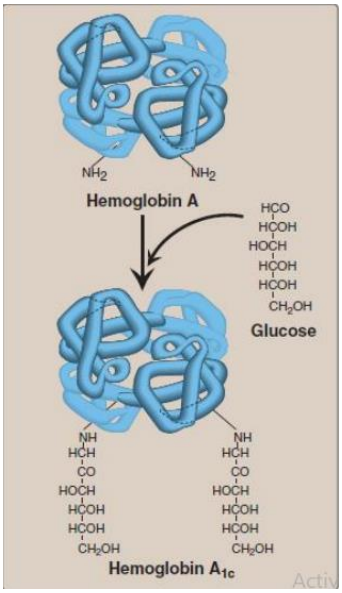




HbA can be glycosylated with a hexose and is designated as HbAc. The major form (HbA1c) is attached to glucose attached to valines of β chains. This happens particularly when the level of glucose in blood is high. HbA1c is present at higher levels in patients with diabetes mellitus. This has been used as a diagnostic tool for diabetes or as a follow-up for the treatment of diabetic patients.

There are 2 tests for indicating diabetes or prediabetes:

1. Total cholesterol level in fasting individuals (I think the doctor meant Blood fasting glucose level). Fast overnight then make a blood test first thing in the morning. This is used to see glucose level right at that moment, and it should be between 80 and 120, this is the normal range. The range for prediabetic is between 120 and 140.
2. Glycosylated Hb (HbA1c): Provides a longer-term trend, similar to an average, of how high your blood sugar levels have been over a period of time (2-3 months), that is because it takes time for Hb to be glycosylated and stays like that for a long time (2-3 months) which makes it a good long term indicator of glucose level.

Limitations of HbA1c test is that it does not capture short-term variations in blood glucose, exposure to hypoglycemia and hyperglycemia, or the impact of blood glucose variations on individuals' quality of life.

HbA1c can be expressed as a percentage (DCCT unit) or as a value in mmol/mol (IFCC unit). IFCC is a newly recommended measurement. The percentages are the ratio of glycosylated Hb to the total Hb molecules in the patients. Normal range is between 5-6%, and 6-7% in prediabetes. Above 7% it's considered diabetes and the higher the percentage the higher the stage.

The new IFCC is measured in millimolar of HbA1c per mole of total Hb, and it should be between 31 and 42. IFCC also gives a little more room/larger range than using percentages and it is also more accurate than using smaller numbers such as 5 and 6.

BLOOD GLUCOSE		STATUS	HbA1c	
mmol/L	mg/dL		%	mmol/mol
5.4	97	Normal	5	31
7.0	126		6	42
8.6	155	Pre-Diabetes	7	53
10.2	184	Diabetes	8	64
11.8	212	Diabetes	9	75
13.4	241		10	86
14.9	268	Diabetes	11	97
16.5	297		12	108

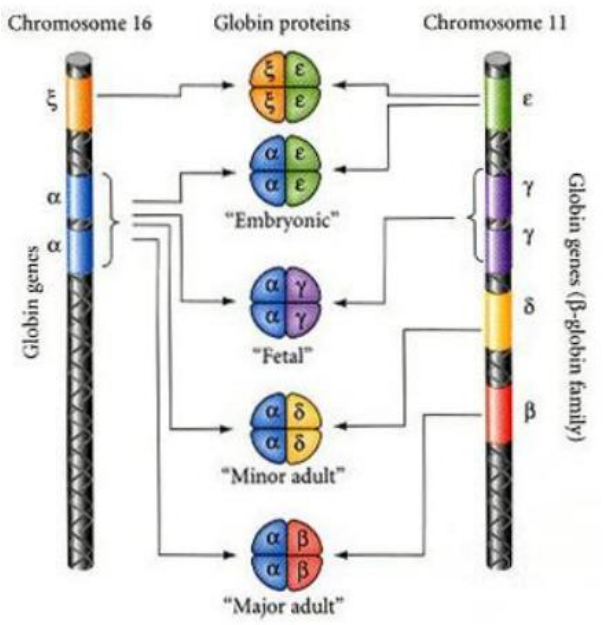
Genetics of globin synthesis:

There are multiple types of globin chains, these types are:

1. Alpha
2. Beta
3. Zeta
4. Epsilon
5. Gamma

These are different populations of globin proteins and they exist as clusters.

On chromosome 16 we have the α gene cluster which contains two α genes (α1 α2) and ξ gene, alpha comes after the zeta. Alpha has 2 identical genes, alpha 1 and alpha 2, on each chromosome, these are important when we study hemoglobinopathies because an individual in this case will have 4 genes/alleles for alpha, 2 on each chromosome.



On chromosome 11 we have the β gene cluster which contains ε gene, two γ genes, δ gene, and β gene in that order respectively. There is only 1 beta, so every individual has 2 genes/alleles for beta, 1 on each chromosome but for alpha there are 4, 2 on each chromosome. This makes a difference in thalassemia diseases.

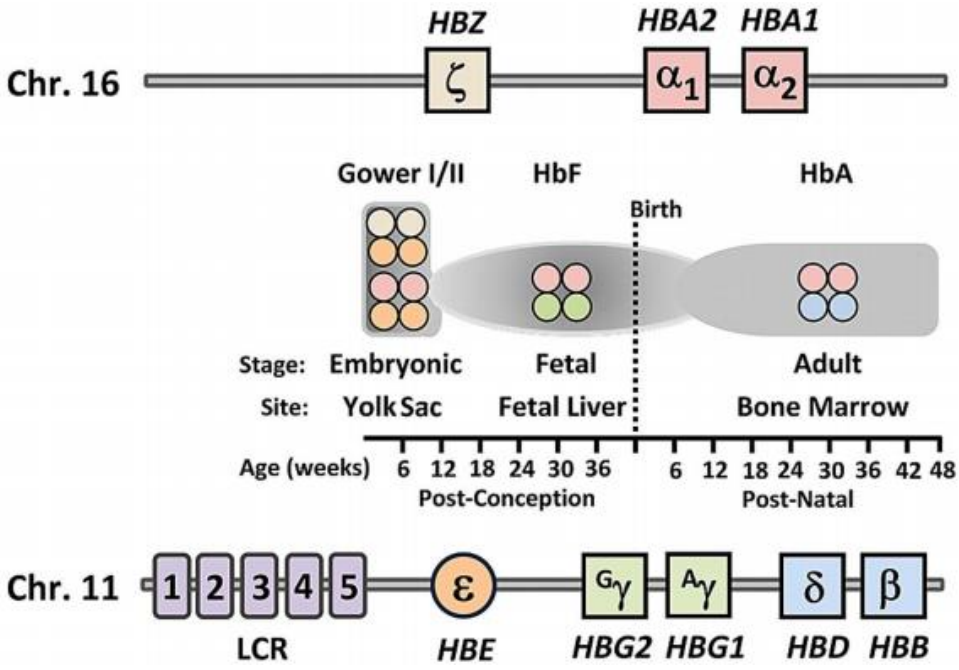
The order of the genes reflects the order of expression, it means that genetic switching is controlled by a transcription factor-dependent developmental clock, independent of the environment, E.g. In embryonic stage we have expression of zeta followed by alpha and in the beta globin there's expression of epsilon then gamma then beta and delta depending on the developmental stage (embryo, fetus, or an adult).

NOTE: Premature newborns follow their gestational age.

Premature neonates (7months) still have HbF (fetal Hb) and the switch happens according to their gestational stage which is at 9 months. They follow the same timing as normal born babies.

When globin gene expression was studied, it was found that each gene has its own promoter and regulatory sequences (activators, silencers) right before where transcription starts.

The β-globin cluster is controlled by a master enhancer called locus control region (LCR), which is upstream of epsilon. This LCR region is an enhancer that controls the expression of all chromosome 11 clusters.

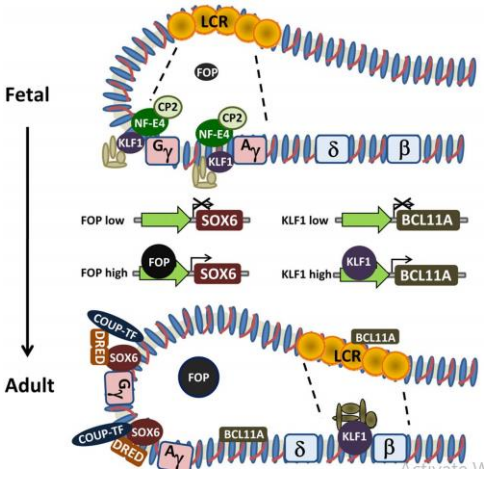


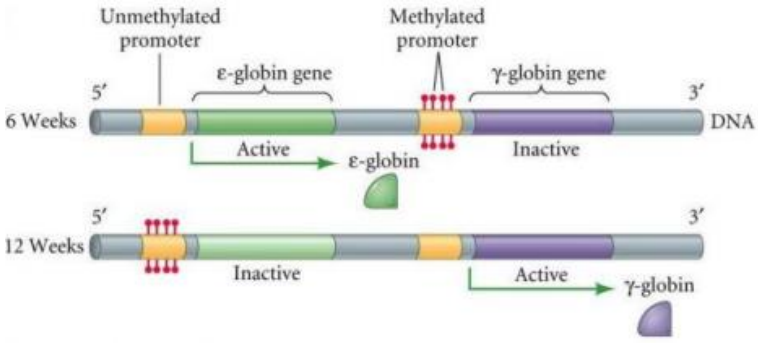
The mechanism of regulation:

Regulation follows different mechanisms. Each gene has its own promoter and regulatory element and they are all controlled by the LCR. In fetal stage they have specific transcription factors that bind to promoter region of gamma and they interact with the enhancer to induce expression of gamma globin. Later, there's a switch where we have transcription factors sitting on the promoter region of beta and the proteins that bind to the enhancer region interact with the transcription factors regulating the transcription of beta. The whole process is timed and programmed.

So, control happens in three ways:

1. By enhancer
2. Chromatin looping, which is different in different stages
3. Epigenetics, methylation of the promoter region which makes it inactive, so no expression of the gene.



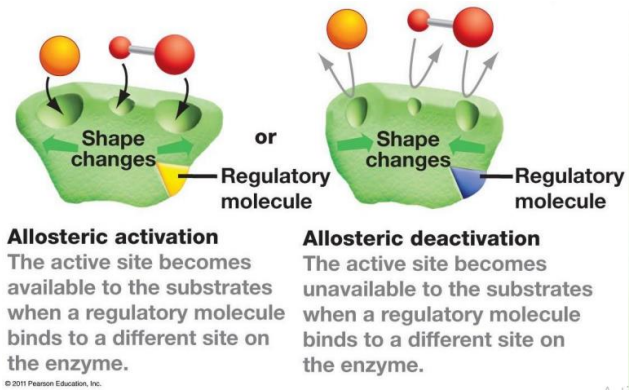


These mechanisms of regulation can be used in treatment of medical conditions such as beta thalassemia, meaning that if someone can't synthesize beta globin, we can rescue this patient by inducing the expression of gamma globin to compensate for the missing beta globin.

Delta globin is not expressed as much as other globin molecules because there is something wrong with its promoter which makes it inactive, this could be due to a mutation in the previous human generations.

Regulation of hemoglobin function

Hemoglobin is an allosteric protein that is regulated by allosteric effectors. These regulatory molecules bid to the protein altering its structure thus facilitating its transition from the T-state to the R-state and vice versa. The allosteric effectors could be inducers or inhibitors.



Oxygen is a homotropic allosteric effector, meaning that it affects the binding of another oxygen molecules (similar molecule). There are other allosteric effectors known as heterotropic allosteric effector that affect the binding of different molecule, such as oxygen.

Usually, these allosteric effectors bind at a different site than where the ligand binds, but sometimes, like oxygen for example, it binds to the "active site", which is the same site where the ligand binds.

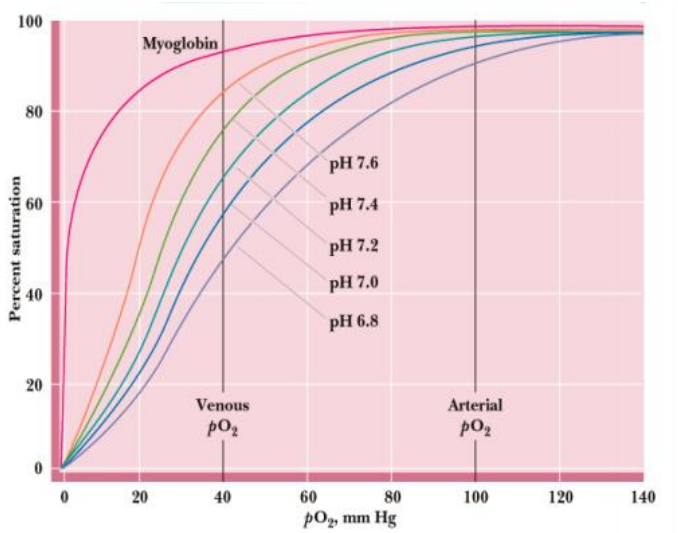
The major heterotropic effectors of hemoglobin are:

1. Hydrogen ion
2. Carbon dioxide
3. 2,3-Bisphosphoglycerate
4. Chloride ions
5. Carbon monoxide

Let's start talking about pH (Hydrogen ions).

The affinity of hemoglobin towards oxygen is influenced by pH.

The normal pH is 7.4 (green graph), as you shift to the right, affinity is reduced (higher p50). Shifting to the left, affinity increases (lower p50). P50 gives an indication of the affinity of hemoglobin toward oxygen, if the p50 is high this means that you need more oxygen to saturate 50% of the hemoglobin molecules which means that the affinity is low, and vice versa.



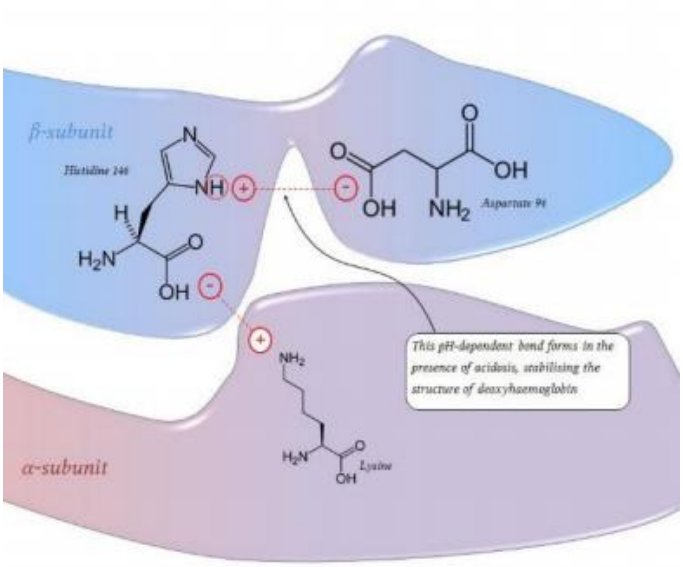
The shape of the graph is sigmoidal, and as the pH decreases (higher acidity) affinity of hemoglobin towards oxygen decreases. The graph shifts to the right.

By that we conclude that the binding of H+ to hemoglobin promotes the release of O2 from hemoglobin and vice versa. This phenomenon is known as the Bohr effect.

If you think about it logically, the pH in tissues is lower than in the lungs, and this is because the tissues are more active and as a result, protons (H+) and CO2 will be released both causing a decrease in the pH. This means that the hemoglobin's affinity is much higher in the lungs than in the tissues which allows it to collect oxygen from the lungs and release them in the tissues.

The mechanism of the Bohr effect:

It all has to do with the electrostatic interactions. So, what happens is that increasing H+ causes the protonation of key amino acids, including the last histidine residue (146) of the β chains (different than the proximal and distal histidines). The protonated (+) histidine is attracted to, and stabilized by, a salt bridge to an aspartate (-) on the same beta chain. So, the positive histidine forms an electrostatic interaction with negatively charged a.a, this enforces the electrostatic interactions within the same peptide (beta chain). Electrostatic interaction also occurs between the carboxylic group of this histidine 146 and lysine of the α chain. This facilitates hemoglobin to switch from the R-state to the T-state, the deoxygenated form of hemoglobin. This is how the affinity of hemoglobin towards oxygen is reduced and oxygen is released.



Now the question is, where do protons come from?

1. They are byproducts of metabolism
2. From CO2

CO2 and H+ are produced at high levels in metabolically active tissues by carbonic anhydrase. Krebs cycle in tissues releases large volumes of CO2 which can combine with water by the action of carbonic anhydrase enzyme to produce carbonic acid. Carbonic acid dissociates immediately into bicarbonate ions (HCO3-) as well as protons (H+). This is the main source of H+ ions in our body. This generation of H+ facilitates the release of O2.

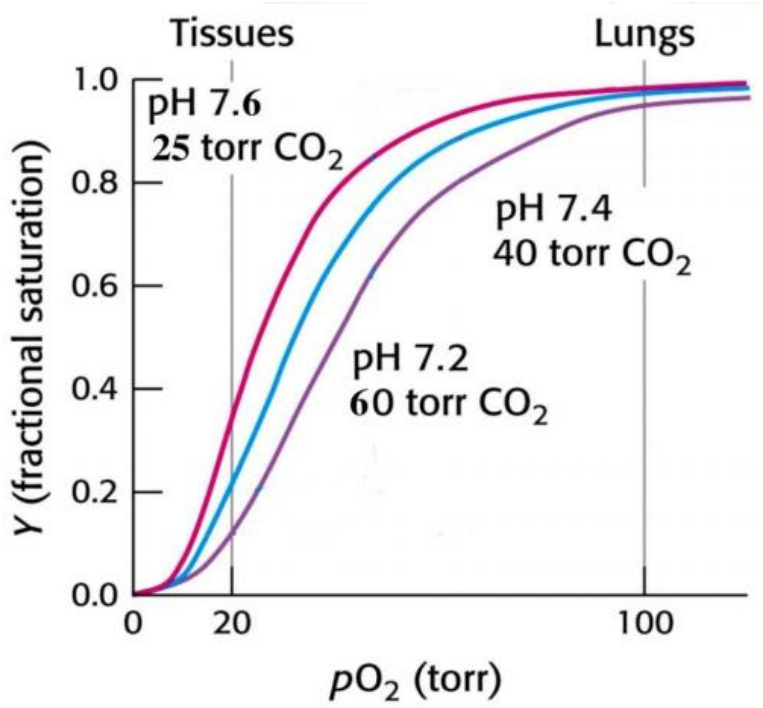
In the lungs, the reverse effect occurs and high levels of O2 cause the release of CO2 from hemoglobin.

CO2 affects the affinity of hemoglobin towards oxygen by two mechanisms:

1. Production of protons (indirect effect)
2. Formation of carbamates (direct effect)

Mechanism #1 – Formation of protons:

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



As the graph shows, when the pressure of CO2 increased from 25 torr to 40 torr, pH decreased from 7.6 to 7.4, this decreases the affinity towards oxygen which is indicated by the shift of the graph to the right. The same happened when pressure of CO2 increased from 40 torr to 60 torr. This happens because more H+ will be

produced when the pressure of CO2 is increased, which leads to decreased pH and so decreased affinity.

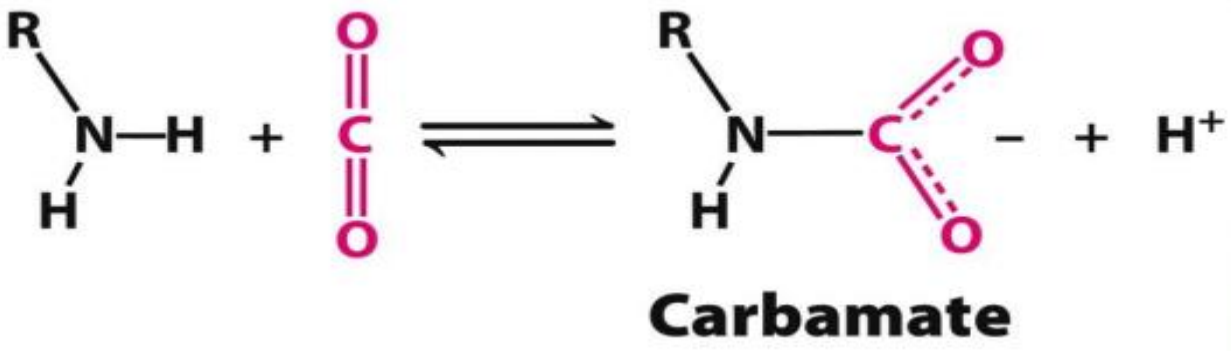
Mechanism #2 – Formation of carbamates:

In this mechanism, CO2 affects affinity directly by interacting with the hemoglobin molecule.

The primary function of hemoglobin is to transport oxygen, while the other function is to directly transport CO2 from tissues to lungs. This happens by binding directly with CO2.

When the concentration of CO2 is high, as in tissues, it diffuses into RBCs where it combines with the free α-amino terminal groups to form carbamate and producing negatively-charged groups, this means that each hemoglobin molecule will bind to 4 CO2 molecules in the form of carbamate.

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



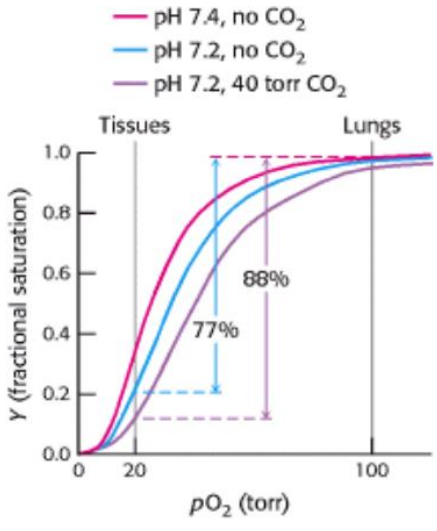
Contribution of both mechanisms

Which mechanism is the predominant reason behind the CO2 effect?

It turned out that about 75% of the shift is caused by H+, while only about 25% of the effect is due to the formation of the carbamate compounds.

This was proven by the fact that an increase in CO2 tension will shift the oxygen dissociation curve to the right, even when the pH is held constant.

If you notice in the graph to the right, the first shift to the right (pink to blue) was due to the decrease in pH. The second shift (blue to purple) was due to increase in the concentration of CO2 which reflects the direct effect of CO2 on affinity. Remember: shifting to the right means lower affinity.

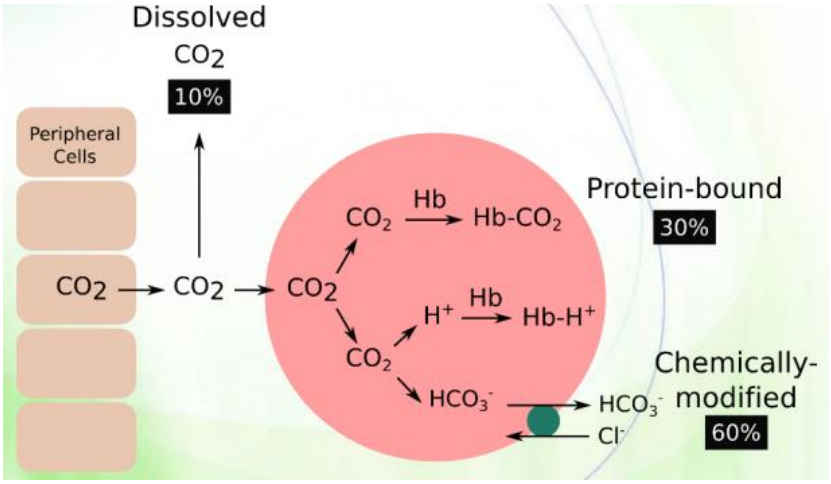


Transport of CO2 into lungs

Approximately 60% of CO2 is transported as bicarbonate ion, which diffuses out of the RBC.

About 30% of CO2 is transported bound to N-terminal amino groups of the T-state of hemoglobin.

A small percentage of CO2 is transported as a dissolved gas.



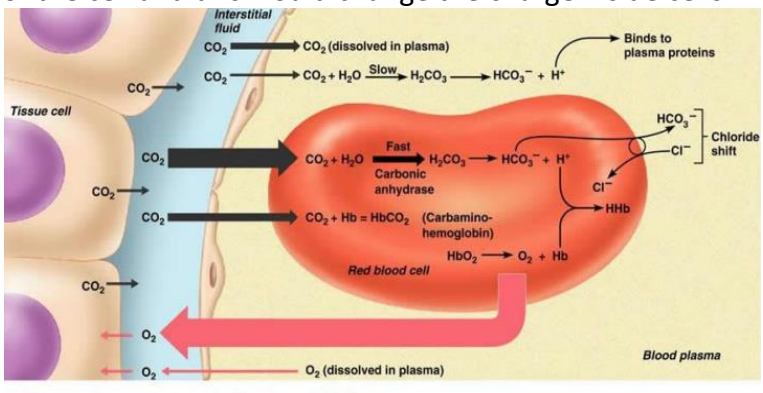
The movement of CO2 in/out of cells does not change the pH, a phenomenon called isohydric shift, which is partially a result of hemoglobin being an effective buffer. Hemoglobin can bind to excess protons or CO2, helping the bicarbonate system in buffering the blood.

Other allosteric effectors:

1. Chloride shift (chloride ions)

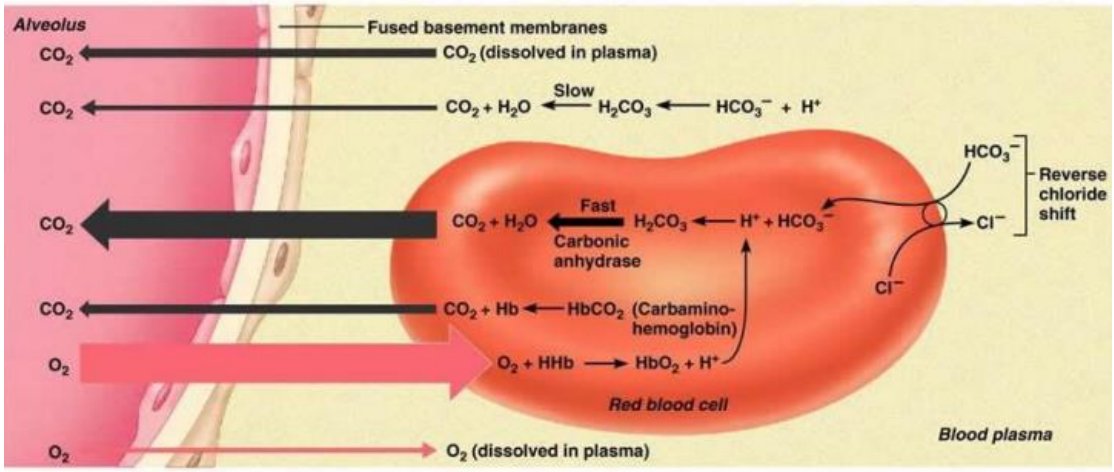
Bicarbonate diffuses out of the red blood cells into the plasma in venous blood and vice versa in arterial blood. Chloride ion always diffuses in an opposite direction of bicarbonate ion in order to maintain a charge balance This is referred to as the "chloride shift."

What happens is when CO2 diffuses out form tissues and into RBC it gets converted to carbonic acid and dissociates into bicarbonate ions and protons, some bicarbonate ions leaks out of the cell and this would change the charge inside cells. This charge must be balanced, so as a result, chloride ions will go into the RBCs, bicarbonate ions leave while chloride ions enter, this maintains the charge inside cells.



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

The opposite is also true, so when RBCs reach lungs, bicarbonate ions enters the RBCs where they get converted into carbonic acid which then dissociates to give CO2 and water. As bicarbonate ions goes inside the RBCs, the chloride ions will go out of RBCs, again balancing the charge inside cells. This is known as "Reverse chloride shift."



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

Effect of chloride ions:

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

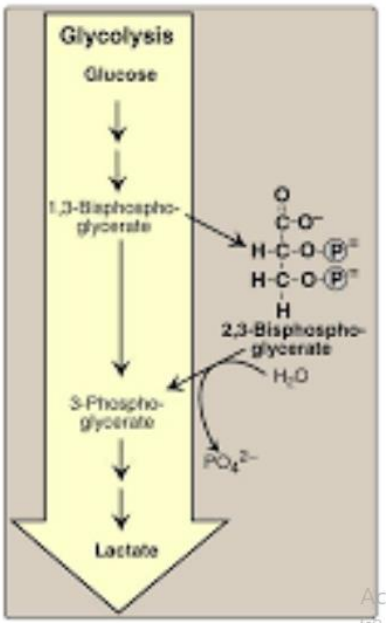
Once chloride ions get into the cell, they would will make electrostatic interactions with the a.a of hemoglobin. By that it induces hemoglobin to become in the T-state, thus facilitating the release of oxygen. This is what happens in tissues as it is shown in the picture above.

On the other hand, if chloride ions leave the cell, hemoglobin will shift into the R-state, this would increase the affinity. This what happens in the lungs as shown in the picture above.

2. 2,3 bisphosphoglycerate (2,3 BPG)

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

Quick recap: In glycolysis, glucose gets converted into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate which is then converted to 1,3 bisphosphoglycerate then into 3-phosphoglycerate and the final product in pyruvate.



1,3 bisphosphoglycerate gets isomerized into 2,3 bisphosphoglycerate which is a byproduct of the glycolysis pathway.

2,3 bisphosphoglycerate can be converted to 3-phosphoglycerate, which is a molecule in the glycolysis pathway, yet this conversion does not produce ATP, unlike conversion of 1,3-BPG to 3-PG. This is considered a waste.

This conversion is still advantageous, why?

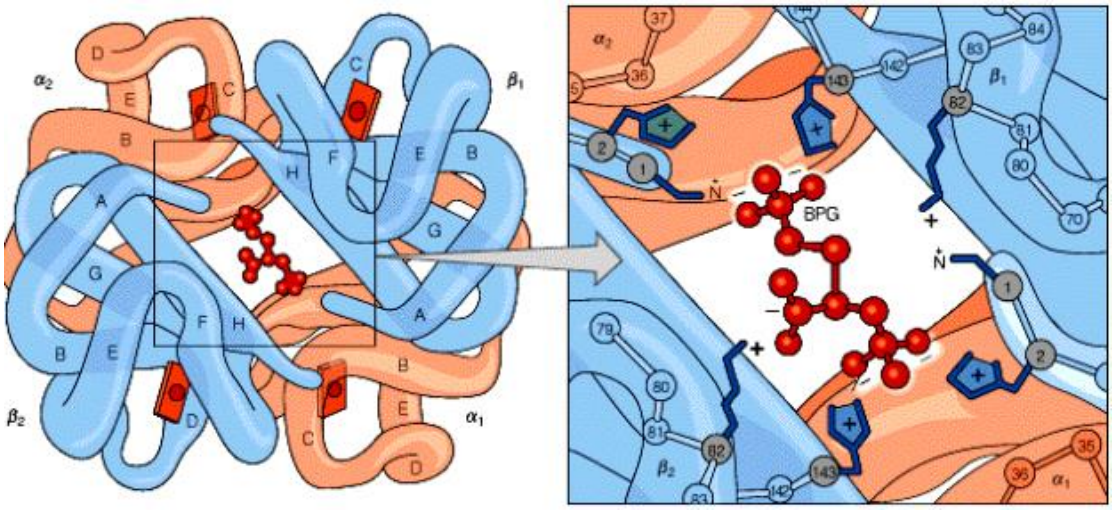
Because BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 BPG/hemoglobin tetramer. 2,3-BPG has negative charges creating more electrostatic interactions as it binds to hemoglobin, so this binding increases the energy needed to transform hemoglobin from the T-state to R-state.

Imagine you have an opened empty box and you put your hand inside it and start moving your hand from side to side, the box will actually wiggle, but if you have a stick placed diagonally inside this box, you won't be able to move your hand and the box wont wiggle. The same thing happens here, 2,3-BPG acts as a stick in the middle of the hemoglobin molecule so that it increases its stability and prevents it form movement, makes it more tense (T-state).

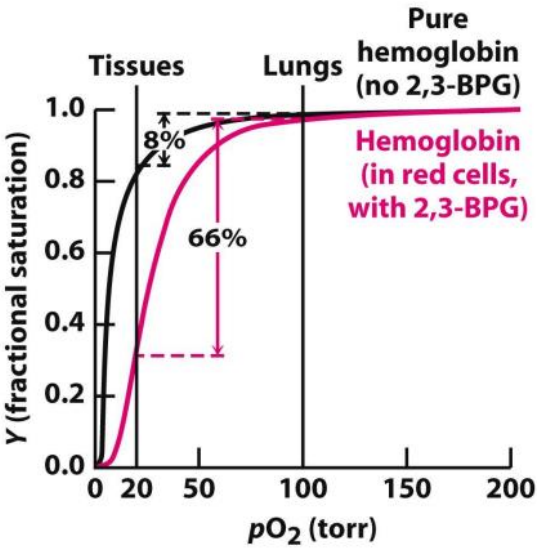
Bound, 2,3-BPG stabilized hemoglobin in the T-state, this reduces binding of oxygen to hemoglobin and facilitates oxygen release.

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

NOTE: 2,3-BPG is considered one of the most important allosteric effectors of hemoglobin.



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 was not present p50 is close to 1 torr. This makes hemoglobin lacking 2,3-BPG very similar to myoglobin.

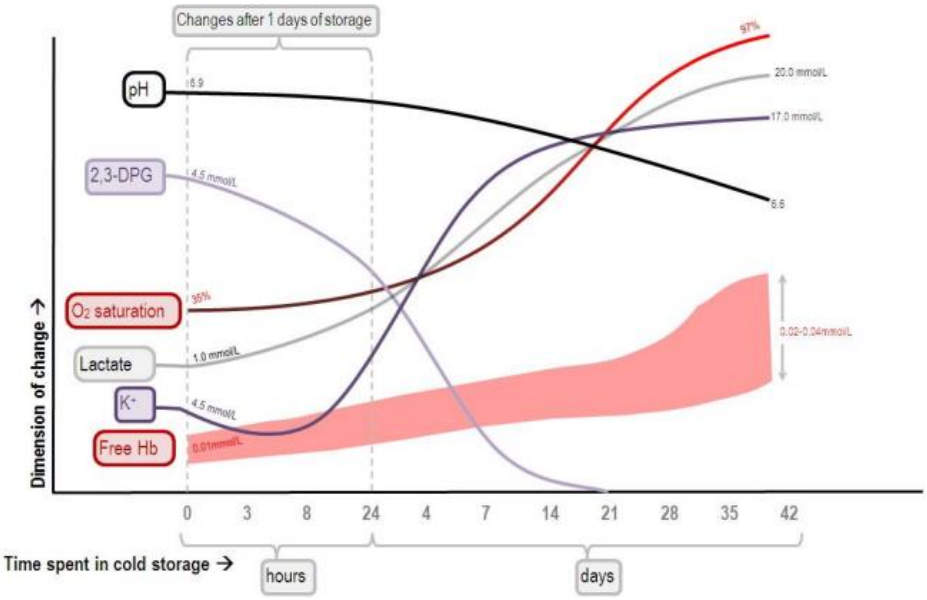
2,3-BPG stimulates the sigmoidal shape of the hemoglobin saturation curve, and it induces the formation of the R-state and the T-state.

What 2,3-BPG does is that it stimulates the transition of hemoglobin into the T-state, the oxygen releasing state. This happens in tissues which makes sense because in tissues we have metabolism, such as glycolysis which produces 2,3-BPG as a byproduct which then diffuses inside RBCs making them release the bound oxygen into the tissues.

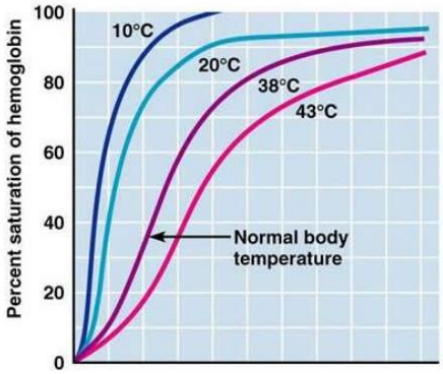
2,3-BPG has a physiological significance in high altitudes. The concentration of 2,3-BPG increases at high altitudes (low O2) and in certain metabolic conditions making hemoglobin more efficient at delivering oxygen to tissues. If you go to a mountain or any high-altitude area, at first it would be difficult to breath but after a couple of days the body adapts by producing more 2,3-BPG.

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 can restore their depleted supplies of 2,3- BPG in 6–24 hours. RBCs should be rejuvenated before they are given to patients, this happens by adding a special solution to them which contains both 2,3-BPG and ATP to compensate for the loss of 2,3-BPG. Severely ill patients may be compromised.



3. Temperature

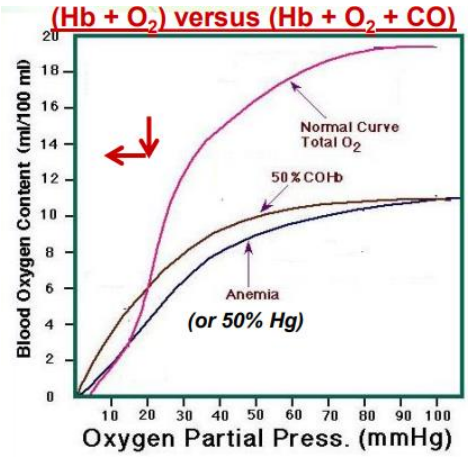


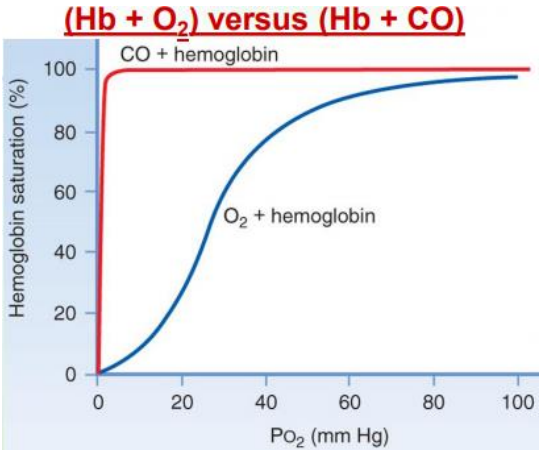
An increase in temperature decreases oxygen affinity and therefore increases the P50 (shifts the curve to the right). Temperature affects the O2 binding of both myoglobin and hemoglobin. Increased temperature means increased metabolic rate of RBCs, increasing the production of BPG, which also facilitates oxygen unloading from HbO2 because more oxygen is needed in tissues. Same thing with exercise, hemoglobin should have lower affinity, so it releases oxygen. Exercising increases the body temperature and that increases the release of oxygen into tissues.

4. Carbon monoxide (CO)

CO has higher affinity towards heme than oxygen. In addition to competing with oxygen in binding to hemoglobin, affinity of Hb-CO towards oxygen increases. So basically, CO increases the affinity of hemoglobin towards oxygen, meaning that oxygen is bound more tightly to hemoglobin (trapped in the R-state), and this is not good because it results in less oxygen unloading in peripheral tissues.

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 nonsmoker. In smokers, COHb can reach up to 10% in smokers, this is not good because it means that more hemoglobin is bound to CO making it non-functional. 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.

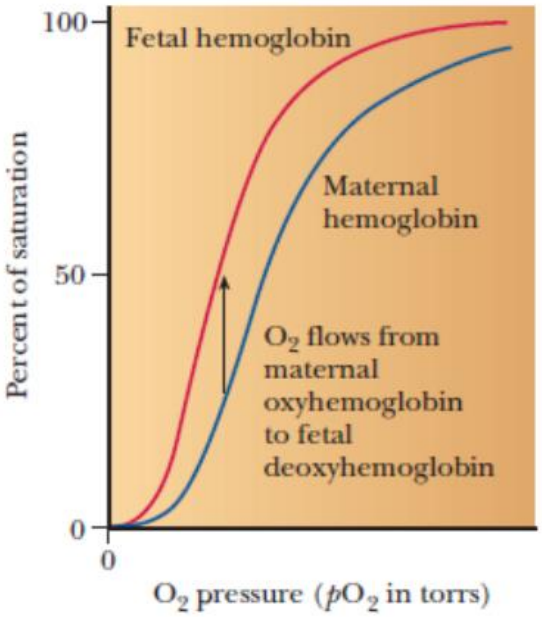




Fetal Hemoglobin

There is a difference between HbF and HbA, not only in the structure (HbA = $\alpha 2\beta 2$, HbF = $\alpha 2\gamma 2$) but also in function. One of these differences is that a His residue in the β subunit is replaced by a Ser in the γ subunit of HbF. So, the positive charge of His is gone which leads to a smaller number of electrostatic interactions. Since Ser cannot form a salt bridge, BPG has a weaker binding to HbF than to HbA. As a result, fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HbA).

The fetus needs to take more oxygen than his mother, and this is of the reasons behind the fast and hard breathing of the mother during pregnancy. It's all logical.



Summary