BLOOD-GAS TRANSPORT OF O_2 AND CO_2*

Summary: This set of notes examines the problems faced by animals that use bloods to circulate O_2 and CO2 and also the mechanisms animals have evolved to overcome some of the problems associated with using blood to transfer respiratory gases. We will also closely examine the interrelationship between O_2 and CO_2 transport.

I. INTRODUCTION

A. In air breathers, large amounts of O_2 and CO_2 are always resident in the lung. (We will see how large these reserves are a bit later). These reserves assure:

1. A relatively constant flow of oxygen between the lung and the bloodstream.

-- and, as we will see,

2. The high CO_2 in the lung will have important consequences in terms of how acid-base relationships work in the body.

B. In this class, we will deal with the movement of gases via the circulation:

1. how the gases are carried, chemically and physically and
2. how O_2 and CO_2 affect the transport of each other.

II. THE CIRCULATION OF RESPIRATORY GASES:

A. General and Comparative Aspects of Circulation of Respiratory Gases:

1. In insects, this step is essentially deleted since the O_2 is directly piped to the cells. The gas reaching the cells is close to 20% O_2 and thus is as highly concentrated as is possible in our atmosphere. Also, air is very light and easily moved. Thus, pumping large volumes of O_2 is metabolically inexpensive in trachea-possessing animals.

2. Most animal groups utilize a circulating liquid (called **BLOOD** or **HEMOLYMPH**, depending on whether it moves in a closed circulation (**blood**) or opened circulation (**hemolymph**) -- which do arthropods have?). Three things must be considered about circulations:

a. Compared to trachea, **the use of blood has the advantage that the water loss from the organism is easier to manage**. This is because most water loss is limited to one area (the internal respiratory system -- the lungs) and it is relatively easy to devise methods to minimize water loss. An example of such a water-conserving mechanism is the intermittent counter current heat exchanger we considered earlier.

However, there are many disadvantages:

b. **Blood is heavier, denser and more viscous than air**. Thus, the energy costs associated with moving gases through blood are very high compared to air.

c. **Aqueous solutions do not hold much O_2 since the solubility of oxygen is low**. Thus, unless the organism does something to enhance the ability of blood to carry oxygen, the animal's metabolic rate will need to be low and/or the circulatory rate very high (high circulation rates will bring more volumes of low O_2 blood per unit volume to the tissues per unit time).

3. *IN SUMMARY*, IF THE ANIMAL DOES NOTHING TO AVOID THE SIMPLE CONSTRAINTS OF THE LOW SOLUBILITY OF OXYGEN IN AQUEOUS SOLUTIONS, IT WILL FACE TWO IMPORTANT CONSEQUENCES:

a. **Little O_2 can be transported to the tissues per unit of blood pumped** (low O_2-delivery efficiency in terms of vol. O_2 per vol. of blood pumped) -- Thus, little O_2 is pumped in comparison to the amount of work done to pump the blood. To deliver a large amount of oxygen, if it is only carried in solution, means that a viscous, heavy liquid must be pumped at a very high rate!

kprestwich@holycross.edu

b. The low solubility means that there is relatively little storage of oxygen in solution. **In humans, the solubility of oxygen in blood is:**

0.003 ml O_2 /(dl blood * torr)

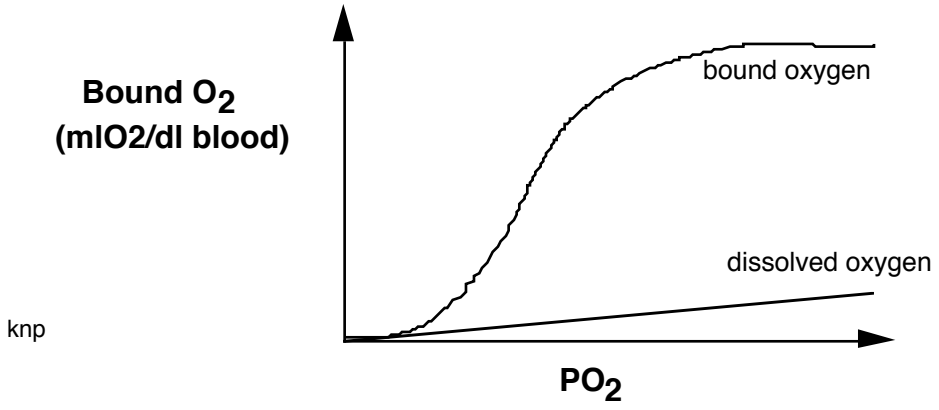
This will affect transition periods between different levels of metabolism and times when the animal might need to hold its breath -- in both cases, low body stores of oxygen mean more reliance on anaerobic metabolism or an inability to rapidly change metabolism or go without a constant source of oxygen.

4. The only animals whose bloods that transport oxygen only in physical solution have:
   a. A low rate of metabolism and
   b. They live in very cold waters: (Remember that a for most gases increases at low temps. and therefore an aqueous solution will carry more dissolved O_2).

The foremost examples of animals that carry O_2 only in a dissolved form are the **ANTARCTIC ICE FISH** (which we will meet again later in the course).

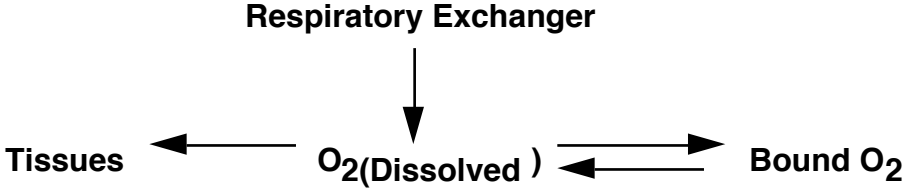
B. For most animals, what is needed is a method to increase the amount of oxygen that can be carried by blood.

1. This can be done through the addition of some type of compound that reversibly binds O_2 in the range of P_{O2} that are seen in the body.



? Give an example of at least one compound that we have considered previously that performed an analogous task.

2. Let's consider the role and general operation of these compounds:



Notice that as O_2 enters the blood from the lung via diffusion, first it dissolves in the blood. Some of it may then bind to the carrier and be removed from solution. This is exactly analogous to the process that we already considered with CALSEQUESTRIN.

a. The O_2 that binds to the carrier is removed from solution and serves to **buffer the value of the dissolved** O_2. Put another way, a large amount of oxygen can enter or leave the blood without the P_{O_2} changing very much.

b. The tissues (like the lung) exchange gas directly with the dissolved O_2 -- not with the carrier. The carrier simply buffers changes in P_{O_2} and acts as an O_2 reserve.

c. Thus, oxygen carriers really have two overlapping functions --

(i) storage of potentially large amounts of oxygen and

(ii) buffering the P_{O_2} of plasma and therefore tissue and thereby keeping diffusion rates high.

? For a given increase in P_{O_2} which type of blood will have more O_2 enter it -- one with a carrier or without? Or will they be the same? Explain.

Explain what happens in both cases to the P_{O_2} when they unload a given volume of oxygen at the tissues.

? It is easy to see why increased storage and therefore carrying capacity of oxygen might be advantageous. Would this always be so? Should all animals have similar abilities to carry oxygen in their blood/hemolymph? Explain.

? Why should buffering a value a P_{O_2} be considered an important function independent of increased storage? To answer this, you should consider the function of biochemical processes in the mitochondria that we considered early in the course.

3. **All oxygen carriers are proteins**. There are two things you should know about:

a. **Hemoglobin**: Structurally, **Fe^{++}** is bound to a **porphyrin ring** (the **HEME**) that is itself bound to a protein. **One Fe^{++} binds one** O_2.

1. In vertebrates, **hemoglobins are quaternary proteins made of 4 subunits each with its own heme.** The act of hemoglobin binding to one subunit affects the ability of the other subunits to bind O_2. This is called **COOPERATIVITY**.

a. In adult humans, there are two each of two different types of subunits to make up the tetramer: two a subunits and two b. They differ in terms of amino acid sequence and in certain aspects of their chemistry.

b. Different forms of these subunits exist; in particular, for example, **the γ subunit is found in fetal hemoglobin** and greatly changes the binding characteristics of the Hb in the unborn child as compared to an adult (more on this later).

2. Hemoglobins are found in nearly all vertebrates and in many different types of invertebrates such as many annelid worms (including *Lumbricus*).

3. A hemoglobin-like compound is found in muscle -- it is a monomer (and therefore shows no cooperativity) and is called **MYOGLOBIN**. It functions to increase O_2 transport into muscle via facilitated diffusion (see Scholander's work cited in Schmidt-Nielson's text) and also acts as an O_2 store in the muscle.

b. **Hemocyanin**: This contains **Cu^{++} bound directly to a protein**; each bound O_2 is coordinated to two Cu^{++}. This is found in many invertebrates including nearly all arthropods that do not rely on trachea for O_2 transport and also in most mollusks. It is blue-colored when oxidized, thus showing for once and for all that arthropods are the only true blue bloods in the animal kingdom.

c. Examples of two other O_2 -carriers are:

1. **Hemerythrin**: **No porphyrin, uses Fe^{++}**; otherwise like Hb. Found in some types of marine worms. Violet colored.

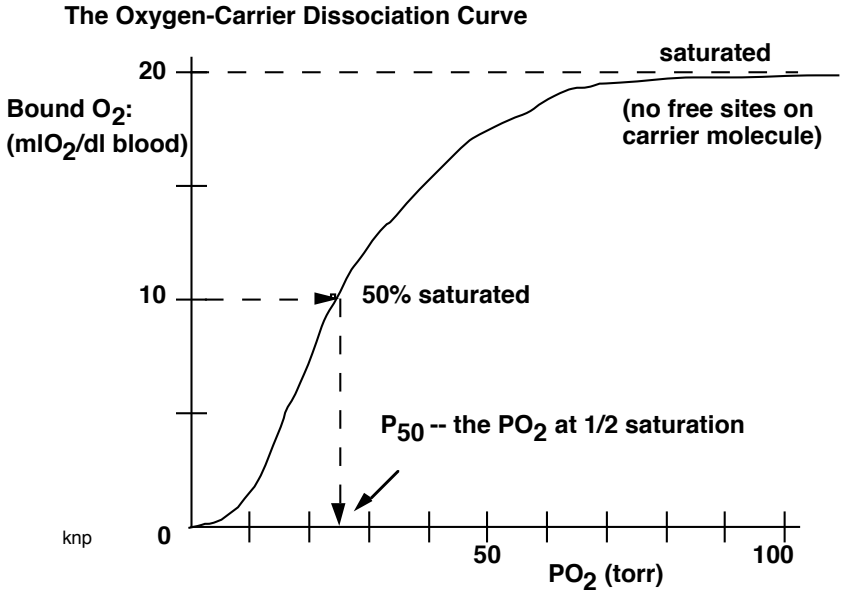
2. **Chlorocuroin**: Fe^{++} is bound to a porphyrin that is bound to a protein very different from Hb. It is green colored.

? Most evidence from modern molecular techniques suggests that these various carriers are descended from mitochondrial cytochromes. Make an argument in favor of this line of reasoning based on when the cytochromes appeared relative to O_2 -carriers and also on the function of each type of molecule.

C. **When these carrier compounds are present in appreciable amounts, for all intents their characteristics define the features of the blood as a carrier of oxygen**. It is because **they often carry the vast majority of the oxygen transported in the blood**.

1. The features that are important in describing blood and its ability of carry O_2 are:
   a. the **shape and position of the O_2 -carrier dissociation curve**,
   b. the **oxygen capacity** of the solution; and
   c. the **effects of various ligands**.

Features a and b are closely related: the O_2 dissociation curve describes the oxygen carrying characteristics of a solution at any P_{O2} . We can show these effects by plotting an O_2 **-CARRIER DISSOCIATION CURVE** (or since this for Hb and O_2 , the O_2 **-Hb DISSOCIATION CURVE**)



D. Note the relationship between the amount of oxygen carried by blood and the P_{O2} is **sigmoidal**. This **indicates the cooperative nature of the binding of O_2 to Hb** as a result of the interaction of different subgroups of the Hb.

? Since Hb is a tetrameric quaternary protein -- are the hemes directly connected to each other?

E. Keep in mind that binding is **REVERSIBLE**.

F. The point where the blood will carry no more O_2 bound to the Hb is called **SATURATION**.

1. Note that even at saturation, blood can still carry more O_2. However, the only available means to do so is **in solution**.

2. Thus, since human blood is generally saturated at low P_{O2}s (below 80 torr), breathing pure O_2 only increases the total O_2 in the blood marginally by increasing the dissolved O_2.

G. In the curve above, we have plotted the total amount of oxygen carried in the blood vs. the P_{O2}. The problem with this is that **different samples of blood can often carry different amounts of O_2.**

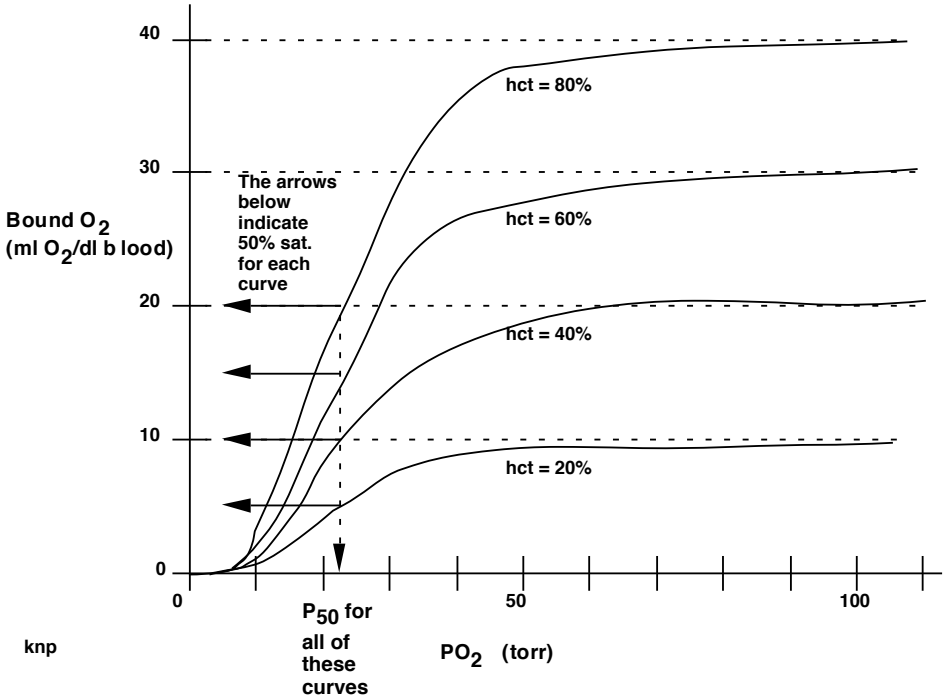
1. In general, **the amount of O_2 that can potentially be carried is largely determined by the amount of carrier present** in the blood. **In humans, this is a function of the total amount of Hb present**. Total Hb in turn is a **function of the total number of erythrocytes**. Erythrocyte content can be measured by centrifugation of blood and determining what proportion of the blood is red cells. This is termed the **HEMATOCRIT (hct)**.

2. Typical mammalian hematocrits are between 35 and 48%. For our purposes, we will assume that "normal" human hematocrit is 40% and that this corresponds to an **ability to bind 20.9 ml O_2 /dl blood when the blood is equilibrated to a P_{O2} of 100 torr.** Of this, nearly all of the O_2 is bound to Hb.

Thus: If hct = 40%, the O_2 **CAPACITY** (the STPD volume of O_2 carried when the Hb in this sample of blood is saturated with O_2, this would be true at ca. 80 torr) is **20.9 ml O_2 /dl blood.**

? What is the O_2 capacity for someone with an hct of 20%? Of 60%? What are anemia, polycythemia, and blood doping and how do they relate to O_2 capacity? Would the same general relationships be true with hemocyanin?

3. Since samples of blood do not always have the same hct, it is difficult to compare them using the plot of O_2 content vs. P_{O2}:



? What is the equivalent of O_2 carrying capacity when one is discussing and enzyme instead of an oxygen carrying protein? We discussed it early in the course.

4. To allow us to compare the BINDING PROPERTIES OF DIFFERENT SAMPLES OF BLOOD, we use another measure of the amount of oxygen in the blood. We call this measure the **PERCENT SATURATION (% SAT)** and it is given by:

1. $$\%\ \text{SAT} = \frac{(\text{vol. } O_2 \text{present in blood} * 100)}{(O_2 \text{ capacity})}$$

Thus, a sample of blood where all of the sites for O_2 transport are occupied is referred to as **100% saturated** (rather redundantly).

5. O_2 -Hb Dissociation curves based on % SAT vs. P_{O_2} are useful in that they describe the characteristics of the blood O_2 binding independent of the hct. One important measure used in characterizing different bloods is the **P_{50}**, defined as **the P_{O_2} where the blood is 50% saturated.**

? What other measure of "protein performance" have we encountered with a definition similar to P_{50}? Explain.
What mathematical procedure is eq. #1 an example of? -- (We have often used this procedure to compare data from different individuals or the same individual at different times).

6. The shape of the dissociation curve and the location of the P_{50} are in themselves quite important. Note that the **curve can be divided into two regions**. One shows very little change in % sat with a change in P_{O_2} the other, a rapid change with P_{O_2} .

a. **LOADING REGION**: This is the FLAT part of the curve **where the % SAT changes very little with a large change of P_{O_2}** .

1. The P_{O_2} values found in this region are typical of the P_{O_2} values commonly seen in the lungs of an animal at typical altitudes for that species.

2. Over a relatively wide range of P_{O_2} values, a constant and complete saturation is reached.

b. **UNLOADING REGION**: here the **%SAT changes rapidly with a small change in P_{O_2}** . Thus, we can say that there is great sensitivity here to P_{O_2} compared to the loading region.

1. Tissue P_{O_2}s are found in this region.

2. Slight changes in P_{O_2} will result in large changes in %saturation and large amounts of O_2 being delivered to the tissues.

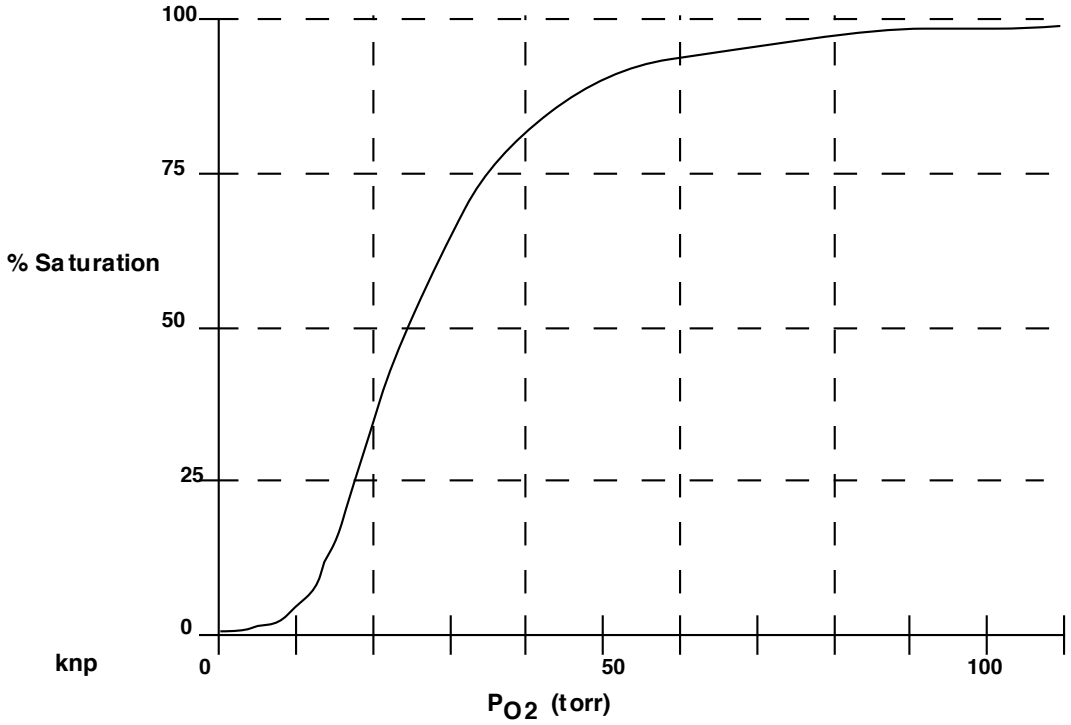
NOTE: REALIZE THAT THE TERMS LOADING AND UNLOADING REGIONS REFER TO THE P_{O_2} CONDITIONS FOUND IN THE TISSUES OF THE LOADING (LUNG) AND UNLOADING (REST OF BODY AREAS. THE SHAPE OF THE DISSOCIATION CURVE ASSURES THAT DIFFERENT EFFECTS WILL OCCU EACH PLACE.

7. By now you should have the basics down. So, let's examine a **simple (= unreal)** model of O_2 transport by the blood. Assume that for the person whose O_2 dissociation curve is shown below:

solubility of oxygen in blood = 0.003 ml O_2 /(dl blood * torr)
the hct = 40% and therefore bound O_2 capacity = 20.9 ml O_2 /dl blood

the arterial P_{O2} (Pa O_2) = alveolar P_{O2} ($P_A O_2$) = 100 torr

P_{O2} in the tissues = 25 torr



1. Draw a second Y- axis (on the right) that shows the volume of bound oxygen as a function of P_{O2} .
2. Draw a transport plot for dissolved oxygen (amount of oxygen on the right Y-axis (instead of amount bound) vs. P_{O2}
3. Calculate the amount of oxygen in the arterial blood. Your figure should include both bound and dissolved. What is the % saturation of the Hb in the arteries?
4. Calculate bound, dissolved, and total oxygen in the capillary blood.
5. Calculate how much oxygen was delivered to the tissues. What percentage of this came from bound? From dissolved?
6. For questions 2 and 3, label points on the dissolved and bound O_2 curves for arterial and capillary blood. Graphically show how much of the available bound and dissolved oxygen went to the tissues.
7. How much more O_2 would be delivered to the tissue if the P_{O2} dropped to 20 torr?
8. How much less O_2 would be delivered if the $P_a O_2$ dropped to 70 torr?
9. In regards to questions 7 and 8, what do these questions indicate about the "loading" and "unloading" regions of the O_2 -Hb dissociation curve?

BRING THE ANSWERS TO ALL OF THESE QUESTIONS TO CLASS

We'll return to this in a minute. Now, let's examine the effects of substances and physical factors that interact or affect the shape of the Hb molecule.

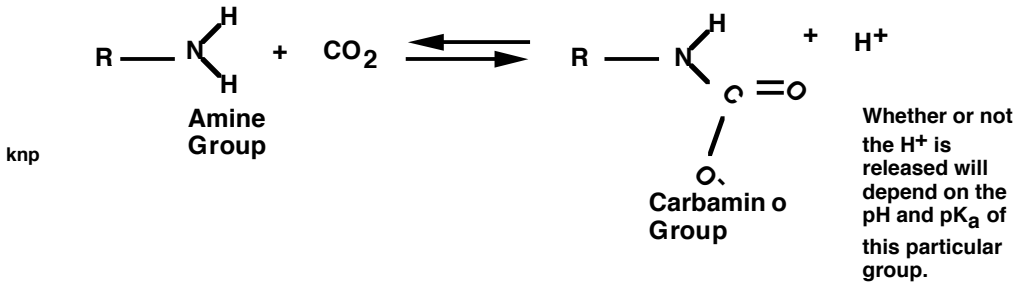
8. Various **factors that can affect the characteristics of the dissociation curve**:

a. **Temperature**: shifts P_{50} to a greater value as T goes up. In other words, higher temperatures lower the affinity of O_2 for Hb.

b. **Phosphate ligands to the Hb: 2,3 DPG, ATP, and Pi** all **reduce the affinity of Hb for** O_2 via allosteric effects.

c. CO_2 **-- Two effects**:

1. CO_2 directly binds to the Hb molecule to **form Carbamino compounds**. In Hb these typically form on the α-amino groups of each of the four subunits of the Hb quaternary protein. Thus, one Hb protein can carry up to four CO_2 molecules as carbaminos – but note that these are NOT carried in the same place as the O_2 molecules (those are on the hemes) and they do not directly compete to bind



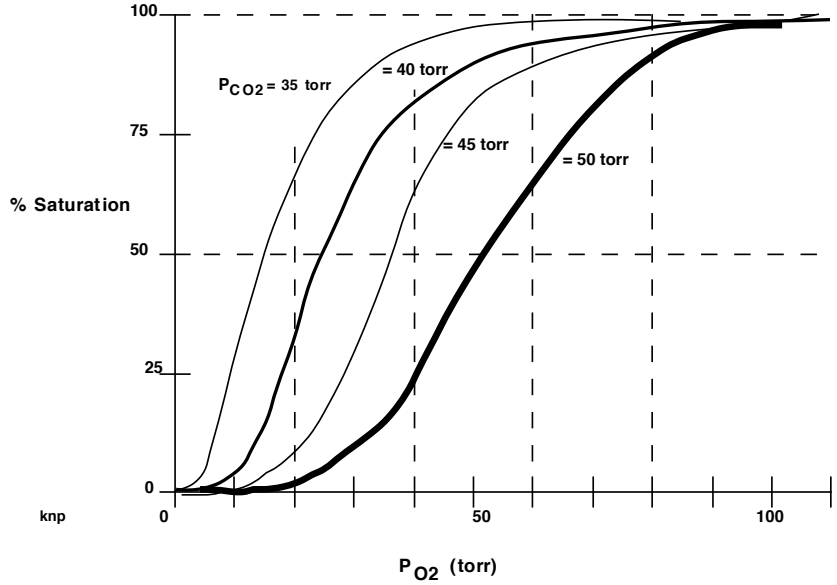
the result of the formation of these compounds is to **reduce the affinity of Hb for** O_2 -- again via allosteric effects.

2. CO_2 **concentration** indirectly affects the pH via the **production of carbonic acid -- lower pH decreases the affinity of Hb for** O_2 **. Note that lactic acid will also have this effect or will potentiate the pH effect of** CO_2 .

9. When exercise is not involved, the effects of CO_2 are the most important to consider since the concentration of CO_2 is different in arterialized vs. venous blood. The net result of this difference in CO_2 is called the **BOHR EFFECT** after the famous Danish Physiologist Christian Bohr (father of Nils)

a. The Bohr effect can best be described as the lowering of O_2 **affinity of Hb by increases in** CO_2 **. In other words, as [** CO_2 **] increases, the** P_{50} **increases.**

b. Graphically, there is an entire family of shifted curves depending on the amount of CO_2 in the blood:



10. **What is the functional significance of the Bohr effect? Since it only applies to oxygen that is transported by Hb, you will ignore dissolved oxygen in this problem.**

(a) -- What if there was no Bohr effect? Assume that **the P CO_2 throughout the body remains constant at 40 torr**. Assume HCT = 40% to find the actual amount of oxygen bound to Hb (see earlier in these notes). Find the amount of O_2 delivered to the tissues if the $P_a O_2$ is 100 torr and the tissue P_{O_2} is 25 torr.

(note: this problem uses an unrealistic circumstance (constant P CO_2) to help to illustrate a point about the Bohr effect).

(b) Compare your results in (a) with what happens if:

(i) the P_A CO_2 is 40 torr. Assume that the arterial blood comes to equilibrium with this value - by the time blood leaves the lungs P_a CO_2 is also 40 torr.

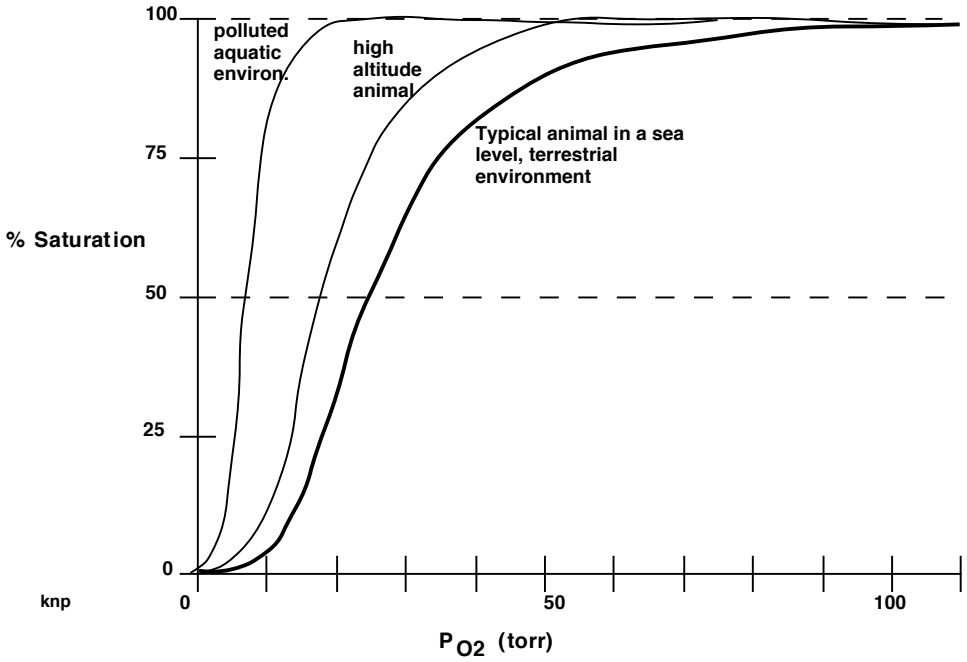
(ii) the P_t CO_2 (P CO_2 in the tissues) is 50 torr (more often referred to as the P_V CO_2 (partial pressure of venous CO_2 since the venous blood is equilibrated with the tissue CO_2). **Use the same P_{O_2} values as in a above.**

? What does this last problem reveal about the function of the Bohr effect?

We must realize that the O_2 -dissociation curve for a single P CO_2 is a static entity and only represents the relationship between P_{O_2} and saturation of the blood at one moment or place. If the P CO_2 conditions change, the characteristics of the blood change and you should envision the properties of the blood sliding from one curve to another depending on conditions.

III. ADAPTATIONS FOR O_2 TRANSPORT:

A. In low O_2 environments (such as polluted waters or high altitudes), animals often evolve O_2 carriers with extremely low P_{50}s.



? What are the relative advantages and disadvantages of shifting the curve to the left?

1. In many extreme cases the curve may be shifted so far to the left that the bound O_2 is used only in emergencies and is not normally used in gas exchange.

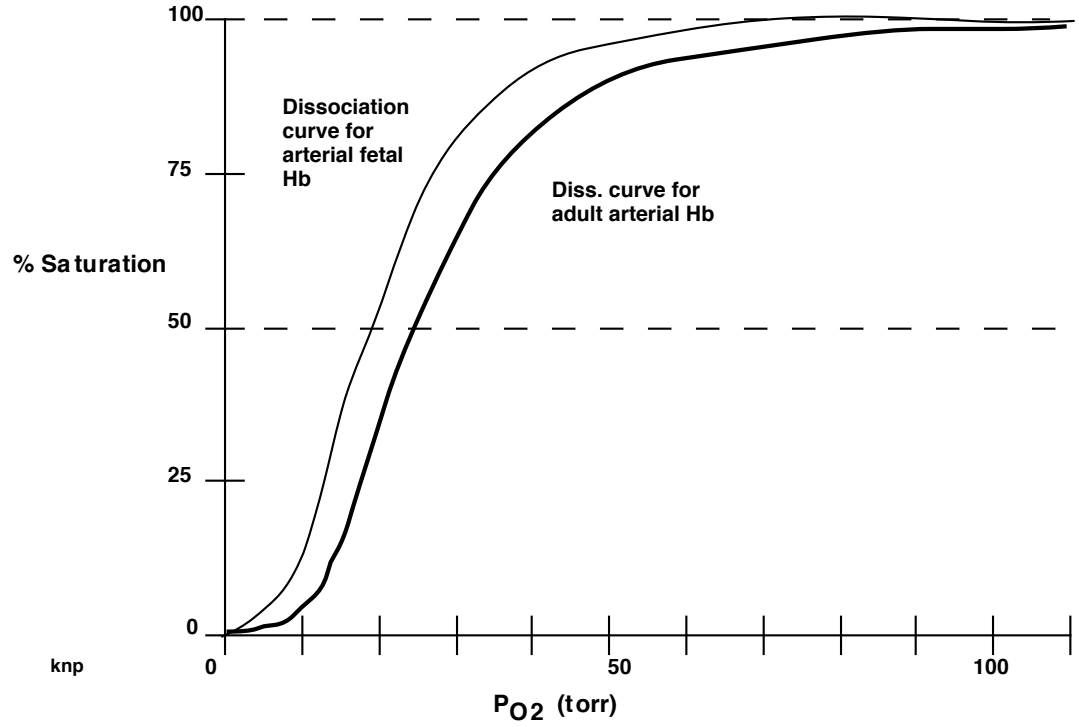
? Where does the O_2 for gas exchange come from in this case?

2. In high altitude mammals, the curve is somewhat shifted to the left. This allows the animal to fully load its blood with O_2 at the low P_{O2} s found at altitude. However, the tissues of these animals must be more tolerant to low P_{O2} s (called **HYPOXIA**) than their relatives living at sea level.

? WHY? What types of biochemical changes must accompany such changes in tolerance to hypoxic conditions?

We will cover human adaptations to high altitude in a later class -- they are quite different from what we have just covered for mammals that evolved at high altitude.

B. **Fetal Hb**: the curve is shifted to the left in most placental mammals:



? Explain the advantage to the fetus.
Would you expect this adaptation in a marsupial or monotreme? Why?

IV. The Transport of Carbon Dioxide:

A. At a common tension (partial pressure), CO_2 is much more soluble in blood than is O_2, thus there will be much more dissolved CO_2 present than there will be dissolved O_2.

B. Avenues of CO_2 transport:

1. <u>Dissolved</u> CO_2: at 37^{o} C the solubility coefficient for CO_2 in blood is *ca*. 0.06 ml/(dl*torr)

2. <u>BOUND</u> FORMS:

a. **<u>Carbaminos</u> on the proteins, especially Hb** since it is the most abundant protein in the blood.

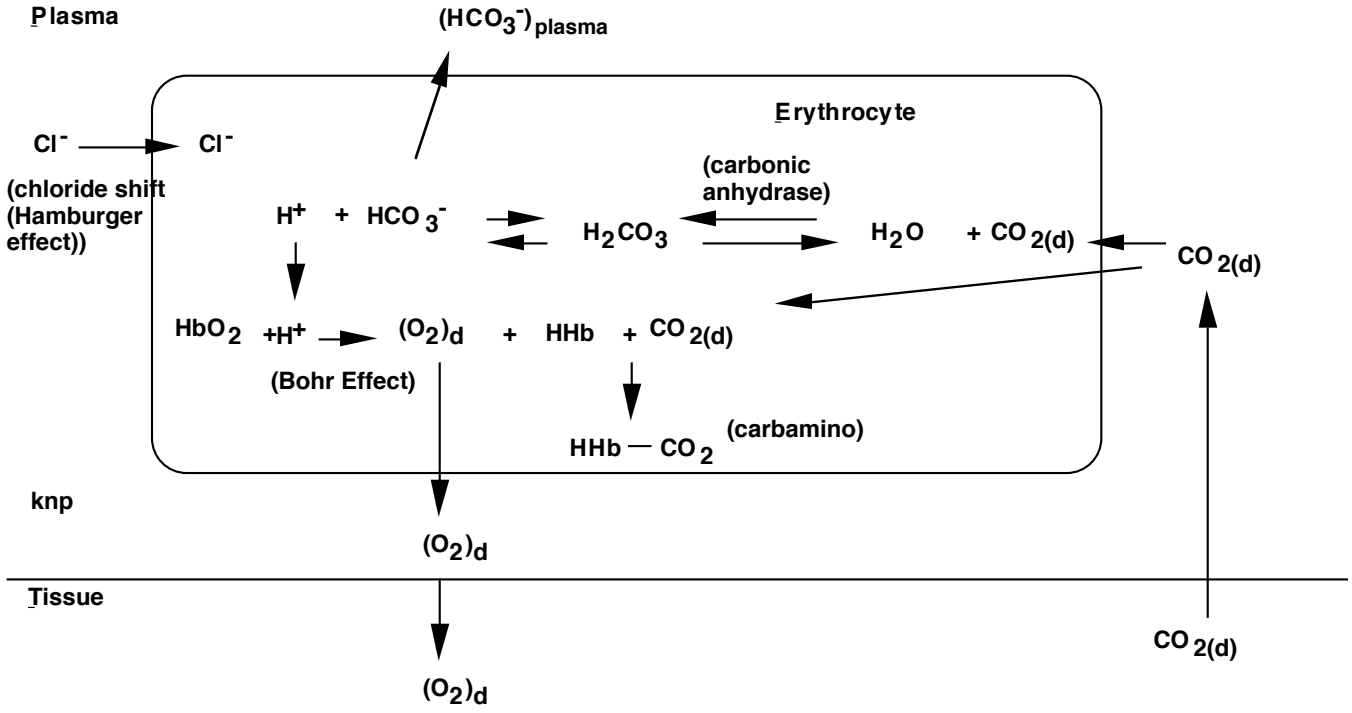
b. **<u>Bicarbonate</u> and Carbonic acid**:

Carbonic anhydrase

1. $H_2O + CO_2$ <----------> H_2C0_3 <----> $H^+ + HCO_3^-$

this is one of the few reactions in the body that actually takes place at a reasonable rate even without a catalyst. However, there is an enzyme, **carbonic anhydrase**, which is present inside the RBCs and it rapidly increases the rate of this reaction.

The Exchange of CO_2 and O_2 Between the Blood and Tissue



EXPLANATION of diagram above:

- CO_2 enters the rbc
- Some of it remains as dissolved CO_2. However most of it either:
- Binds to Hb to make a carbamino as we saw in the last set of notes. This results in an allosteric change in the Hb molecule: it lowers the affinity of Hb for O_2.

  H+ + CO_2 + HbO_2 <--> H Hb-carbamino + O_2

- Most CO_2 is quickly converted to H2CO3 with the help of <u>carbonic anhydrase</u>; the H_2CO_3 quickly dissociates into HCO_3^- and H^+. The **<u>H^+ ions are quickly picked up by histidine side chains on the Hb molecules</u>** (the Hb is an important buffer in the blood). This is part of the Bohr effect from the last set of notes.

- The **HCO_3** does one of three things:

  (a) remains dissolved in the rbc intracellular fluid

  (b) flows out of the cell into the plasma (in the process, it is exchanged for Cl^-, this exchange of negative for negative ion is called the **Hamburger effect** or **Chloride shift.** The bicarbonate that has left the rbc can now:

  (i) remain dissolved in the plasma or

  (ii) bind to plasma proteins as carbaminos (R-NHCOO + H+) with the H+ produced by this reaction buffered by plasma buffers (see below).

? Erythrocytes change size as they pass through the lung and tissue capillaries. In one case they swell and in the other , they shrink. Which occurs where and why?

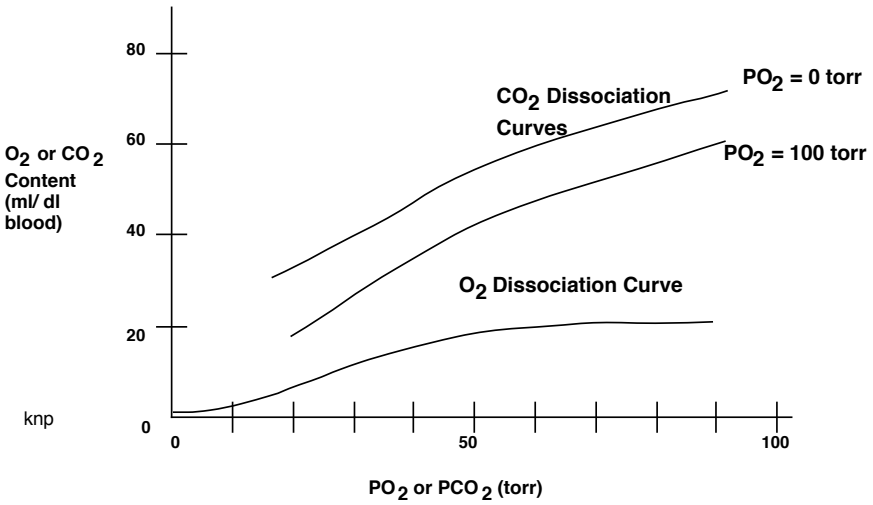
3. We can now ask how much CO_2 moves via each of these various pathways:

Source and Amount (vol%)	venous	arterial	(V-A) difference (vol%)
Dissolved	**3.1**	**2.7**	**0.4**
HCO_3^-	**47.0**	**43.9**	**3.1**
carbamino	**3.9**	**2.4**	**1.5**
total	**54.0**	**49.0**	**5.0**

a. We can see from the above table that **the majority of the CO_2 that is exchanged (5.0 vol%) comes from the bicarbonate pool** (3.1vol%, i.e. 62%)

b. Most of the remainder (30%) comes from carbamino compounds.

4. From the diagram on the last page, we saw that **Hb forms carbamino compounds**. Since there are only so many sites where these can form, then, like the O_2 - Hb dissociation curve, there is also a CO_2 **- Hb dissociation curve:**



a. Notice that **the degree of oxygenation affects the amount of CO_2 carried. This is called the Haldane effect** which is defined as the lowering of Hb affinity for CO_2 at high P_{O2} -- i.e., when Hb also binds O2.

b. Thus, the Haldane and Bohr effects are seen in the same tissues at the same times -- **high CO_2 allosterically lowers** O2 **affinity whereas high** O2 **allosterically lowers CO_2 binding and vice versa**. Since CO_2 tends to be highest in places where O2 is lowest and vice versa, we get different effects in different tissues --

(i) Tissues: raised CO_2 (positive Bohr) and lowered O2 (negative Haldane) cause affinities for O2 to drop and CO_2 to increase in tissues while

(ii) Lungs raised O2 (positive Haldane) and lowered CO_2 (neg. Bohr) in the lungs causes more CO_2 to be released (Haldane) and under conditions of low P_{O2} may actually increase the amount of oxygen bound (Bohr). Keep in mind that although different sites are involved for the two gases, the Hb molecule intimately links their transports.