ACID BASE AND THE REGULATION OF RESPIRATION [*]

Summary: The avenues of CO_2 transport are discussed followed by a detailed look at blood acid-base balance and the role of CO_2 in this balance. The Davenport diagram is introduced as a means of visualizing acid base disturbances and compensations.

I. THE ACID-BASE BALANCE OF THE BLOOD AND ITS REGULATION:

A. Introduction to and Review of pH Buffers:

1. Plasma pH is tightly regulated; we want to consider some of the factors that are involved in the determination and regulation of blood-acid base balance.

2. Let's remind ourselves that <u>buffers exist</u> when we have a <u>solution of a</u> <u>**weak acid**</u> <u>and its</u> <u>**conjugate base**</u> and that they <u>**RESIST BUT THEY DO NOT PREVENT**</u> **pH CHANGES**.

3. In the body, we generally deal only with weak acids (the stomach being one big exception). We can describe the state of a weak acid in terms of the degree of dissociation of the weak acid:

1. $HA \longleftrightarrow H^+ + A^-$

where HA is the acid and A^- is its conjugate base.
The mass-action ratio or **DISSOCIATION CONSTANT (*K*)** for this reaction is:

2. $K = \frac{[H^+]*[A^-]}{[HA]}$

by re-arranging equation #2, we can write:

3. $[H^+] = K * \frac{[HA]}{[A^-]}$

we can convert this into an equation with a log form:

4. $\log [H^+] = \log K + \log (\frac{[HA]}{[A^-]})$

This can be simplified: since **pH = - log [H+]**; thus we can multiply the equation #4 by -1 and then substitute *p*H for - log[H+]:

5. $- \log [H^+] = - \log K - \log (\frac{[HA]}{[A^-]})$

or (next page)

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6. $pH = -\log K + \log\left(\frac{[\text{conjugate base}]}{[\text{acid}]}\right)$

If we now define the **<u>negative log of the dissociation constant</u> as being the $\mathbf{pK_a}$**, we can re-write eq.6 as:

7. $pH = pK_a + \log\left(\frac{[\text{conjugate base}]}{[\text{acid}]}\right)$

This equation is known as the **<u>HENDERSON-HASSELBALCH EQUATION</u>**

Now let's consider an important special case that explains what the pK_a is and what it means. **Suppose we wish to know <u>the pH where buffer is most effective</u>. Obviously, this will be the pH where the concentrations of the conjugate base and acid are equal since a disturbance in either direction can be resisted**. If we substitute equal values for these two species into the Henderson-Hasselbalch equation:

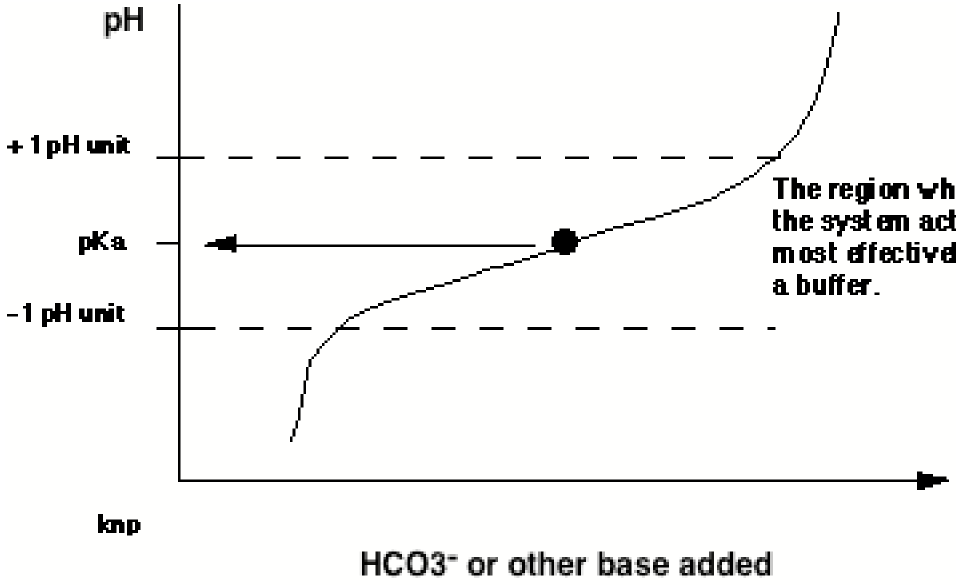
8. $pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$

and since $[A^-] = [HA]$, then

9. $pH = pK_a$

Here are a few common misconceptions to <u>get over now</u> -- before we go on further in our discussions of acid base:
First and foremost, **the only thing that matters in determining *p*H is the$[H^+]$**.
Notice that it is determined by the pKa, concentrations of the weak acid and conjugate base -- not by just one of these factors.
So -- the [base] is not a primary concern. Base only matters to the extent that the base <u>helps</u> to determine the amount of H^+ in the solution. The same thing can be said for the concentration of the weak acid.
Notice that if more acid dissociates, the *p*H goes down as expected. But **what commonly surprises folks is that the concentration of the base increases.** <u>Free yourself of the notion that every base molecule somehow finds every H^+ and reacts with and neutralizes it</u>. An instant of thought shows this to be total bunk, yet it is a very common notion for people to have. Finally, notice that the pKa says something about the relative mix of acid and base at a given $[H^+]$ -- as it should since it is the negative log of the dissociation constant. More about this below, just think about it for the moment.

Thus, the pK_a gives the pH where a buffer is most effective. We can graph the behavior of a buffer system with respect to how *p*H changes as the system is titrated:



Note that for approximately +/- 1 *p*H unit around the pK_a the buffer is very effective at resisting the effects of adding or removing H^+. Thus, the value of the pK_a when compared to the actual *p*H of the system is an indication of the buffering ability or contribution of the given buffer.

? Suppose that the pKa (see above) of an acid is 6.1 (e.g., carbonic acid) as we will treat it in the body). It is now placed in a chemical environment such that other compounds add or remove H^+ to the solution (i.e., it is placed in a solution where it can act as a pH buffer).
(i) At a *p*H of 7.4, in what form is most of this acid-base system -- acid or conjugate base? (ii) How about at *p*H 6.1? (iii) At *p*H = 5.0? (iv) Which of these solutions has the most free H^+? (v)The least?

ANS: (i) conjugate base, (ii) equal, (iii) acid, (iv) *p*H 5.0 (v) *p*H 7.4. Be able to explain all these answers in some detail.

There is one other factor that is important in understanding the effectiveness of a buffer system: the **AMOUNT (usually the equivalent of the CONCENTRATION) of the buffer**. Thus, even if a buffer is operating far from its pK_a, it can still be an important buffer if enough of it is present. The reason is that the ability to release or absorb a given number of H^+ ions is not only related to the pKa vs. *p*H but also to the total number of molecules available to react.

4. The <u>important buffers in the blood</u> are the **bicarbonate buffer system**, the **phosphate ($H_2PO_4^- \leftrightarrow HPO_4^{2-}$) buffer** , and **hemoglobin.**

a. Phosphate and Hb are important since their pK_a's (meaning the overall pK_a for Hb) are close to the physiological range.

b. <u>Hb is especially important since there is so much</u>

c. <u>Bicarbonate</u> <u>is important due to its **abundance**</u>. Its ***pK_a* is 6.1** (**<u>remember</u> <u>this</u> <u>number</u>**).

5. In organisms, we must deal with several acid-base systems that are in action simultaneously. As a reminder, the primary ones that we need to consider are:

(i) $H_2CO_3 \leftrightarrow HCO_3^-$ (bicarbonate buffer system)

(ii) $H_2PO_4^- \leftrightarrow HPO_4^{2-}$ (phosphate buffer system)

> **!** Note: The strength of the phosphate buffer system is partially controlled hormonally via the action of **CALCITONIN and PARTHORMONE** and their actions on the phosphate pool in the bones -- both can affect phosphate levels by changing the net addition of subtraction of Ca^{++} salts in the bones.

(iii) $R\text{-}NH_3^+ \leftrightarrow R\text{-}NH_2$ (proteins and a. acids, Hb is most important in the plasma).

6. **In a given body "compartment"** (such as the plasma or the intracellular fluid, etc.), **the *p*H is the same for all of these acid-base systems** since they are **all in equilibrium with the same pool of H^+**.

a. We can state a rule called the **<u>ISOHYDRIC PRINCIPLE</u>**: **if we know the ratio of $K * \frac{[HA]}{[A^-]}$ for any one buffer of a complex set of buffers, we know it for all of them since we can write the following relationship (eq. #4) for each buffer system**:

$$[H^+] = K_1 * \frac{[HA_1]}{[A_1^-]}; \qquad \textbf{and} \qquad [H^+] = K_2 * \frac{[HA_2]}{[A_2^-]}$$

or more completely:

$$[H^+] = K_1\frac{[HA_1]}{[A_1^-]} = K_2\frac{[HA_2]}{[A_2^-]} = K_3\frac{[HA_3]}{[A_3^-]} = K_4\frac{[HA_4]}{[A_4^-]}etc., \qquad \textbf{etc.}$$

b. Thus, although **all of the buffer systems help to determine the plasma pH, <u>we only need to study one of them</u> in order to understand the overall action of the PLASMA BUFFER SYSTEM**.

? If you know that plasma pH is 7.4 and if you know the *pK_a* of different buffer systems, can you determine the ratio of acid to conjugate base for each of those systems?

7. The buffer system that we study and monitor the most is the bicarbonate buffer system. By doing this, we know much of what is happening in the other systems, since a disturbance in any one buffer affects all of the systems.

8. In addition, the bicarbonate buffer system is of interest since it is the one that can be changed the most rapidly -- since the system is in equilibrium with CO_2, any change in respiration will change this system. Thus, we know that most short-term regulation of plasma pH is achieved through the regulation of the bicarb. system.

9. We can re-write the Henderson-Hasselbalch eq. (# 7) for the bicarb system:

10. $$pH = pK_a + \log \frac{[HCO_3^-]}{\text{acid}}$$

Now, recall that the acid, H_2CO_3, is in equilibrium with dissolved CO_2. This means that in effect the CO_2 acts as if it were part of the acid pool. Thus, the amount of acid available for this buffer system is more like the sum of the H_2CO_3 and dissolved CO_2 than it is the H_2CO_3 by itself. Since the equilibrium of the reaction $CO_2 + H_2O$ ---> carbonic acid is far to the left (there are about 500 molecules of CO_2 per molecule of H_2CO_3 (acid) at equilibrium), and since $CO_{2(d)}$ is easy for us to measure, we can use dissolved CO_2 as a stand-in for carbonic acid. To do this we re-write equation #10 as follows:

11. $$pH = pK_a + \log \frac{[HCO_3^-]}{\alpha_{CO_2} P_{CO_2}}$$

We are able to do this since $\alpha\, CO_2 * PCO_2$ determines the amount of CO_2 that is in solution. Please see the note in your textbook about the value of the *p*Ka for the carbonic acid/bicarbonate system.

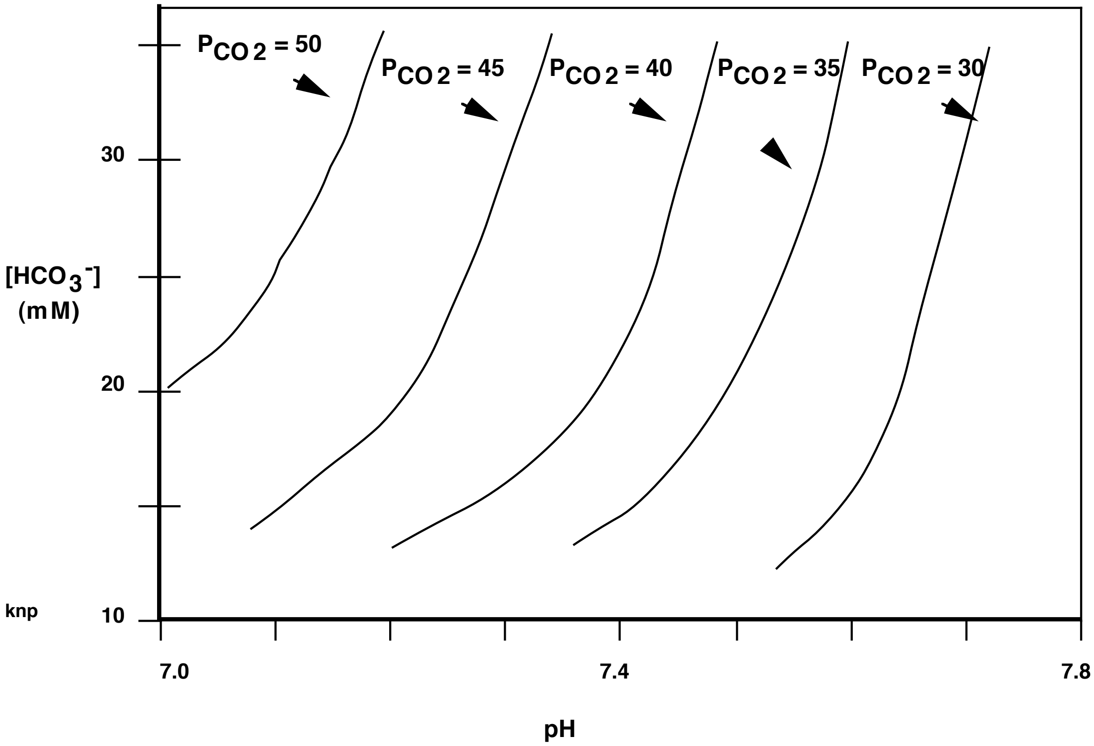
! In human plasma at 37°C the value of α CO_2 is given as $\mathbf{0.03 \frac{m\ mols CO_2}{L\ solution * torr}}$

B. To describe the behavior of acids and bases in the body, physiologists have developed a very useful but complicated graph known as the **Davenport Diagram**.

1. Since we are studying acid - base via the bicarbonate buffer system, we will be interested in inter-relating the following variables: *p*H, [bicarbonate], and P_{CO2} . Notice that these are all variables in the Henderson-Hasselbalch eq. for bicarb buffer given above (#12)

2. We will start by plotting a graph of $[HCO_3^-]$ vs. *p*H. These two are selected because they are <u>easily measurable</u> attributes of a plasma sample.

3. Next, we will use the Henderson-Hasselbalch eq. to plot a number of lines called **ISOPLETHS**. These are lines that are calculated for a constant value of P_{CO2} with the amount of bicarbonate being varied. Typically these are plotted for reasonable values of P_{CO2} in normal and abnormal states. A good range is +/- 20 torr from the "normal" arterial P_{CO2} of 40 torr. Our Davenport diagram now looks like this:

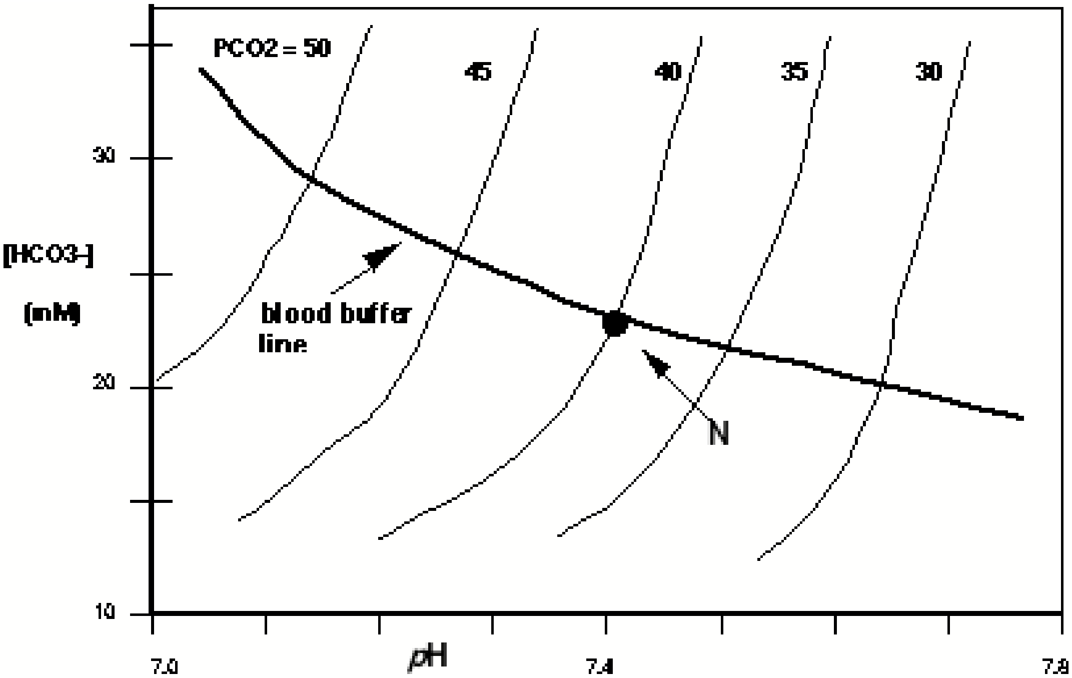


Plesae note that these isopleths are only approximately of the correct form -- the correct ones can be easily calculated using the Henderson-Hassalbach equation.

4. Next, we can put in some data that is obtained empirically: the **BLOOD BUFFER LINE**. This is simply the buffer line for [bicarb] vs. *p*H when a sample of blood is titrated with acid or base. The line has a distinctive shape that is determined by the following:

(a) Obviously, as more base is added the pH will increase since H^+ ions are removed by reaction <u>with some of the added base</u>.

(b) In addition, since we are dealing with a buffer system, as we add OH^- (base) and H^+ in the blood is consumed, the $HCO3^-$ will also decrease as some of the HCO_3^- loses a H^+ to become CO_3^{2-}. The Davenport diagram with a blood buffer line will look like the graph below:



Again plesae note that these isopleths and the blood buffer are only approximately of the correct form — the correct isopleths can be calculated using the Henderson-Hassalbach equation and the blood buffer line must be determined empirically.

On the plot, **N (which should exactly intersect the 40 isolpeth and blood buffer line) represents the** **<u>normal point for arterial blood</u>: *p*H 7.4, P_{CO2} = 40 torr, and [HCO_3^-] = 24 mM. Thus (when the solubility of CO_2, 0.03 mmols/(l sol'n * torr), is considered) the ratio of bicarbonate to CO_2 is 20 to 1**. This will always be the case when a pH of 7.4 is obtained with the bicarb. buffer. Know this point! (It is valid for humans and some other mammals).

C. Now let's use the graph to explore <u>what happens when various disturbances occur</u>.

1. Suppose that we place the subject on a respirator and turn it up such that the V_E is much too great. Obviously, the subject is **<u>HYPERVENTILATING</u>** (or I should say, we are forcing him/her to hyperventilate). What will happen?

a. As we will see in the next class, P_{ACO2} will decrease in hyperventilation. The result is that the P_{aCO2} also decreases since arterial gas is in equilibrium with the alveolar value. Thus the bicarb/CO_2 increases from 20:1 and the blood becomes more alkaline.

b. How will the blood change with respect to *p*H and [HCO_3^-]? Note that essentially <u>we are titrating the blood by removing acid</u>. Thus, the change is described <u>shifts along the blood-buffer curve, with the final resting point being equal to the intersection of the blood line and the P_{CO2} isopleth of the hyperventilating lung</u>. (See the following graph).

? What will happen when the subject (starting from normal) HYPOVENTILATES?

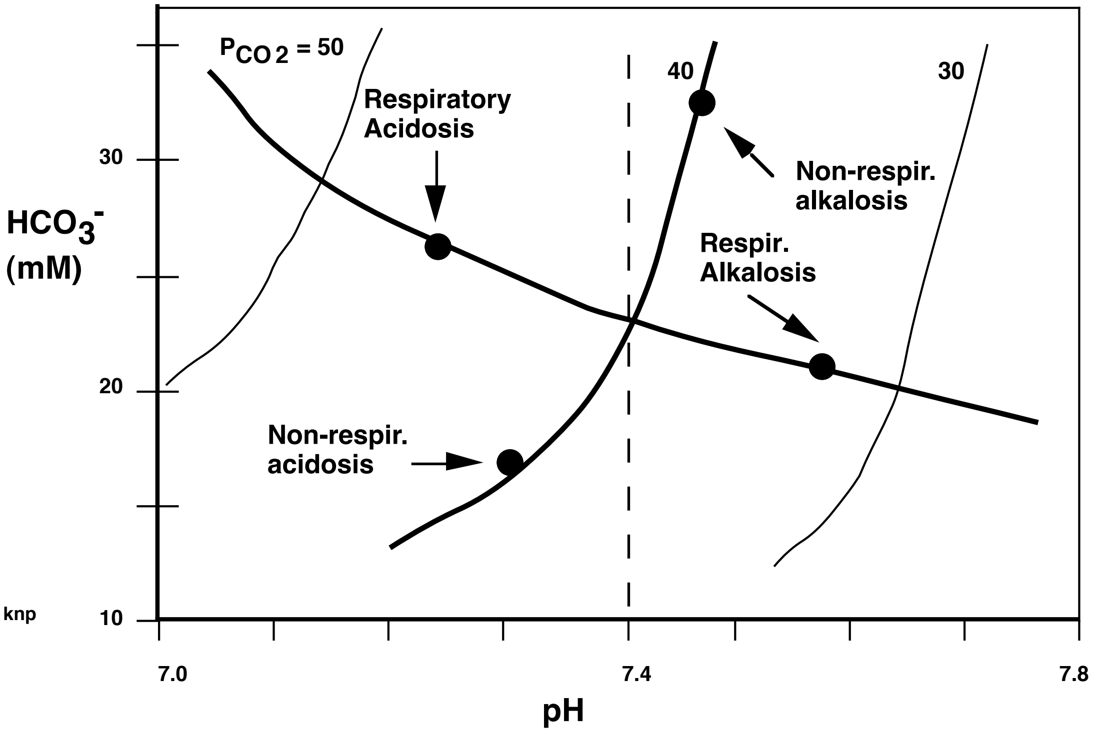
We call disturbances of acid base balance that are caused by mismatches in ventilation compared to demand as being **RESPIRATORY in nature**, they result in either a pH that is higher than normal **(RESPIRATORY ALKALOSIS)** or lower **(RESPIRATORY ACIDOSIS)**.

2. Likewise suppose that the subject consumed or produced a large amount of acid. What would happen? The acid would react with HCO_3^-, thus we know this parameter would drop. In addition, the pH would also fall since not all the H^+ that is being added to the blood would be removed. This would be seen as the arterial Davenport value leaving the blood buffer line and traveling down the P_{CO2} = 40 isopleth a distance determined by the amount of bicarbonate consumed. Notice that once again the bicarb./CO_2 drops below 20:1 and the pH falls. (See the following graph)

? Describe what happens when a large amount of base is consumed.

3. When excess acid or base is introduced through either diet or via metabolic (including disease states) processes, we term the condition **NON-RESPIRATORY ACIDOSIS or ALKALOSIS.** An older alternative term that we will also use is **Metabolic alkalosis or acidosis.**

? Based on the location of someone's blood gas point, how would you distinguish between Non-respiratory and Respiratory Blood gas disturbances? Between Acidosis and Alkalosis? Use the blood buffer line and the P_{CO2} isopleths as your reference points.



Different types of alkalosis and acidosis can be easily identified on this graph by their position. Any disturbance along the P_{CO2} = 40 isopleth such that the [HCO_3^-] differs from 24 mM is considered to have a non-respiratory cause since normal P_{CO2} is maintained. If the [HCO_3^-] is greater than 24 mM, the condition is termed non-respiratory alkalosis; if below 24 mM it is termed non-respiratory acidosis. By contrast, conditions which involve P_{CO2} values that are different from 40 are said to be respiratory in cause. In their pure sense they are essentially the titration of blood with different amounts of CO_2 from 40 -- if more, it is respiratory acidosis (due to extra carbonic acid); if less it is respiratory alkalosis.

Finally, note that it is possible to have more than one condition at a time. For instance, non-respiratory acidosis and respiratory acidosis. Locate such a point.
Again plesae note that these isopleths and the blood buffer are only approximately of the correct form -- the correct isopleths can be calculated using the Henderson-Hassalbach equation and the blood buffer line must be determined empirically.

D. COMPENSATION FOR ACID BASE DISTURBANCES.

1. <u>*p*H is a tightly regulated variable -- disturbances are potentially life threatening</u>.

2. If an acid base disturbance occurs, there are several things that can be done to restore the body to normal pH. In the following discussion, we will assume that the disturbance (such as hyperventilation) cannot simply be reversed. What can be done then?

a. **<u>Irreversible respiratory disturbances</u>**. Here the P_{CO2} is either too high or low. To return to normal *p*H when P_{CO2} cannot be changed requires that bicarb be either lost or gained -- thereby restoring the 20:1 ratio of bicarbonate to CO_2.

1. The **kidneys** are a major avenue of **bicarbonate and H^+ removal and conservation.**

2. The **level of bicarb can be adjusted by either increasing or decreasing bicarb. elimination in the urine or by doing the opposite with H^+ elimination**.

b. **Irreversible metabolic (non-respiratory) disturbances**: the P_{CO2} is normal but bicarb. levels are off the normal blood buffer line intersection for the given P_{CO2}.

1. Compensation is via the respiratory system where the P_{CO2} can be changed.

2. This of course will also indirectly affect the bicarb system.

? Show how (using the Davenport diagram) compensation would occur to NON-RESPIRATORY ALKALOSIS. To RESPIRATORY ACIDOSIS.

3. To remind ourselves of the roles of the kidney and lung in the regulation of acid-base, we often restate the Henderson-Hasselbalch relationship as:

12. $pH = pK_a + \log \frac{\text{kidney}}{\text{lung}}$

(note that this relationship is only useful for animals such as mammals that regulate P_{CO2} in the respiratory exchanger and H^+ and HCO_3^- in the kidney.

II. The Regulation of Breathing:

A. Introduction:

1. In the first set of notes on respiration, we overviewed general models of respiratory gas exchange that contained two convective steps (ventilation and circulation) and two diffusive steps (tissue/blood and blood/alveolar space). We also considered how O_2 and CO_2 are carried in the blood and we have just finished discussing how CO_2 relates to acid/base. We are now in a position to look at the overall regulation of this exchange process.

2. The first point is that this regulation is achieved in the convective processes since they are under the control of the nervous system. Diffusive changes tend to follow changes in blood flow and ventilation. For the moment then, we will focus on ventilation. We also should mention that **both the respiration and circulation must be regulated together** in order to deliver adequate oxygen and remove CO_2 so as to not prevent acid-base disturbances. Here is a very important summary of the ways that convection controls diffusion and that circulation and ventilation inter-act:

(a) As you should recall from the general respiration model in the first set of notes, the main places regulation can occur are in the two convective steps -- **ventilation of the lungs** and **circulation (which is essentially ventilation of the tissues)**.

(i) Notice that **we cannot really make regulatory changes in the diffusion between blood and lungs except as a result of changes in circulation or ventilation**. For instance, **deeper ventilation (larger tidal volume) will result in:**

a) **increased area as more alveoli are opened** in different parts of the lungs. Thus area is really determined by ventilation depth. It will also increase the average diffusion distance somewhat but not as much as you might think since the main effect of a larger tidal volume is to open more alveoli until very large lung volumes are reached.

(b) **increased partial pressure gradient as the P_{O2} is increased and the P_{CO2} is decreased in the alveoli**. Changes in circulation also affect this gradient since for a given tissue metabolism, the lower rate of circulation the lower the blood P_{O2} and higher the blood P_{CO2}. To make things simple, we will simply assume that the blood arrives in the lungs with constant values of CO_2 and O_2.

(ii) Similarly, increases in circulation have the effect of:

(a) **keeping average partial pressure gradients of the two respiratory gases higher in a capillary for a given metabolic rate** and thereby increasing movement of the gases;

(b) they are **usually accompanied by opening of more blood vessels to a particular tissue** as we will see when we examine the regulation of microcirculation. The result of this is to **increase area** (more vessels opened per unit volume of tissue) and **decrease average diffusion distance**.

(iii) **for this packet, we will simply assume that the circulation increases or decreases to match tissue need. Therefore, we will only be concerned with how the ventilation system also changes to match need** .

(iv) We will also need to keep in mind what we learned in the last packet dealing with CO_2 -- **respiration is used not only to provide oxygen but also as a primary means to regulate the overall acid-base balance of the body!!!**

B. General Regulation of Ventilation in Mammals; Since the goals of breathing and circulation is to (i) maintain adequate oxygen delivery and (ii) to help maintain pH by adjusting CO_2 elimination it would make sense to monitor the concentration of these two gases and make regulatory adjustments accordingly.

1. P_{O2}: Under most conditions **at the altitude a species evolved** at (we will take this to be near sea level (i.e., below 5000 feet for the purposes of this discussion) the **P_{AO2} (Alveolar P_{O2}) is never so low that the blood Hb**

isn't saturated. This is **due to the position of the loading region of the O_2-Hb dissociation curve.** Thus, under these conditions, arterial O_2 is very poor potential regulator of respiration since changes in arterial P_{O2} make little difference in the amount of oxygen contained in the blood.

a. The major O_2 sensors are located in the **CAROTID BODIES** that are in a sinus of the internal carotid artery. They measure oxygen content and therefore, they simply would not respond to a slight under-ventilation that results in a drop in P_{O2} so long as the blood is still essentially saturated. Put another way, such a small change makes no difference to the tissue's ability to obtain oxygen and so it is ignored (in fact, it is not even registered!).

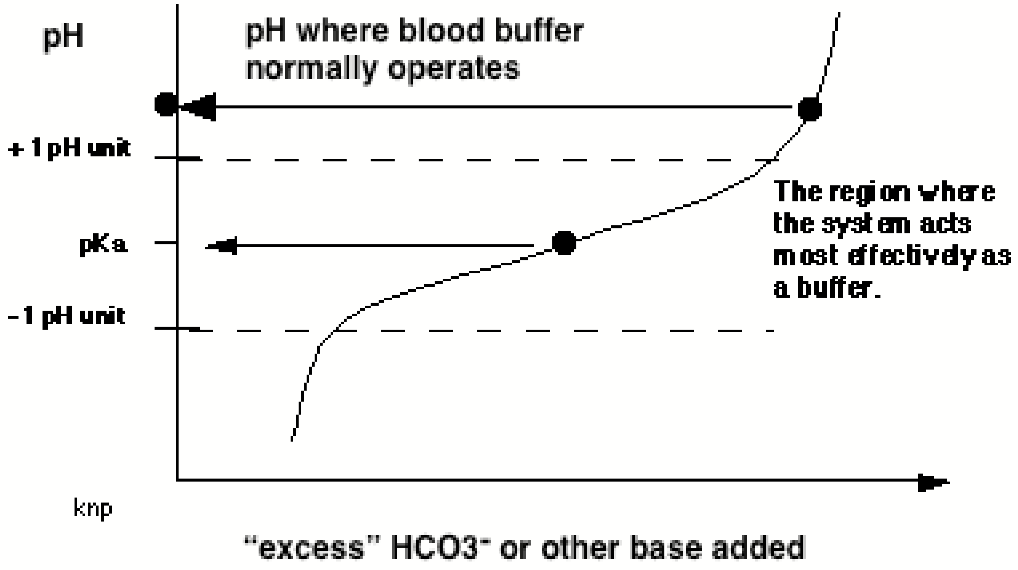
b. On the other hand, if P_{O2} gets low enough so that blood is no longer saturated, then P_{O2} is a very potent stimulus of respiration. The relationship is summarized later on a graph on the next page.

c. These receptors are ancient. As you will recall from the textbook, their action produces what is normally the critical signal in the regulation of ventilation by fishes. We have maintained them, but as we will shortly see, under normal atmospheric conditions, they are not too important to us. However, under conditions of low ambient oxygen -- as are often faced by fishes, they become very important.

Summary: At this points one may wonder so what?-- as long as adequate oxygen is delivered to the tissues, then there is no reason to worry if the P_{O_2} changes somewhat due to fluctuations in demand and ventilation.

- **Under these conditions, one might assume that it is not important to regulate ventilation very precisely**. In fact, if adequate oxygen were all that mattered, imprecise regulation would be OK under these conditions.
- But there is **another problem** and that is that **if CO_2 is not eliminated at the same rate as it is produced there will be changes in overall body acid/base balance.**
- As we have seen, the bicarbonate CO_2 buffer system is the most powerful one in the body since it is in such high concentration, since CO_2 is produced in all tissues and since CO_2 can easily enter or leave any tissue via diffusion.
- And, we have seen since the start of this course that **CHANGES IN PH HAVE THE POTENTIAL TO CHANGE EVERY PHYSIOLOGICAL PROCESS IN THE ORGANISM** since it often has potent effects on protein conformation.

2. P_{CO2}: Thus, by contrast, we are very sensitive to slight changes in CO_2 from set point. Recall that this sensitivity is possible because the ***p*H of the body (generally a bit above 7) is maintained in a region of the CO_2-bicarb titration curve where a slight change in bicarb. or CO_2 will cause a large change in *p*H:**



Note that this is **due to operation of the system a long way from its *pKa*.**

(a) CO_2 is sensed in certain **central receptors in the hypothalamus**.

(i) In fact, **these receptors sense *p*H** which shouldn't surprise you too much since it is very easy to make to a protein that is sensitive to change in pH.

(ii) But a bit of deeper understanding is called for. **They are still referred to as CO_2 receptors since the H^+ they sense is from CO_2 that diffuses into the fluid that bathes the receptors.** Once it arrives, it produces carbonic acid.

(iii) It is important to realize that this is H^+ not from exactly the same pool of H^+ ions that are found in the blood since **highly charged H^+ will not readily pass the "blood/brain barrier"**. CO_2 on the other hand, has no trouble passing this barrier.

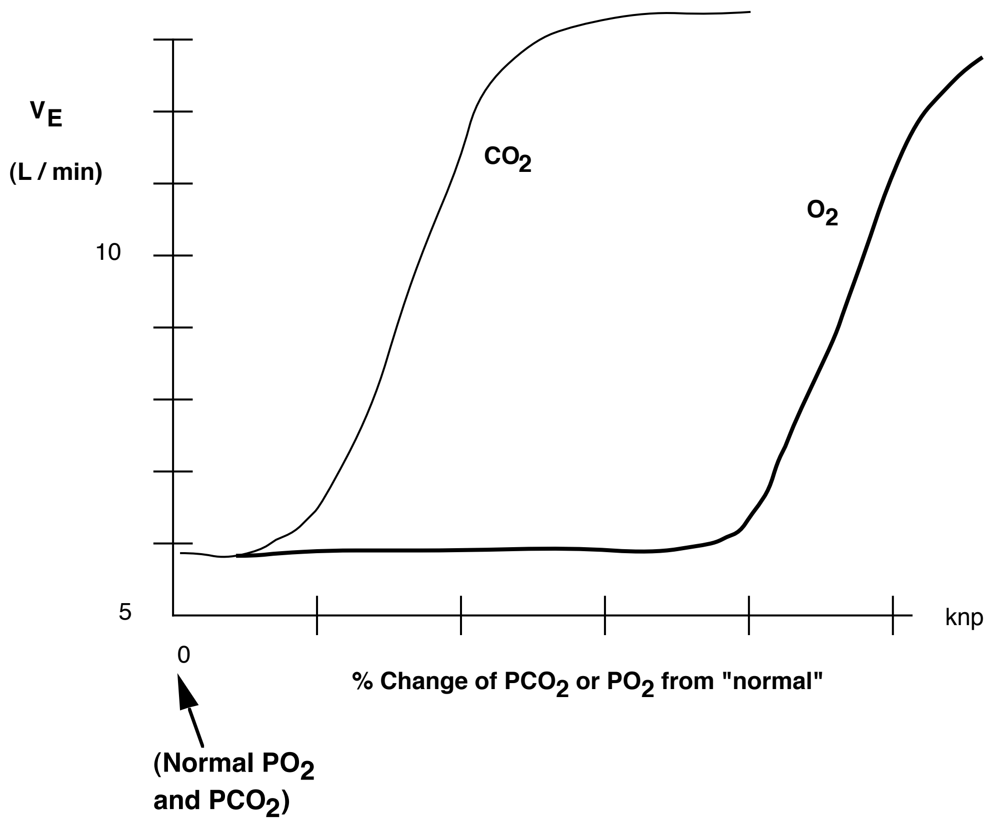
(iv) These central receptors are therefore more sensitive to changes in CO_2 and are **only indirectly influenced by other things (e.g., lactate) that change pH of the blood**. They are indirectly affected because a change in pH does **influence the amount of free carbon dioxide**.

? How is it that a change in blood pH will change the amount of dissolved CO_2? What affect will an increase in lactic acid have on the concentration of CO_2 around central CO_2 receptors?
What will be the effect on breathing?
On arterial *p*H?

On arterial P_{O2}?

? How is it possible that if this system can actually be so far away from its *pKa*? In other words, discuss the negative feedback regulation of the CO_2 bicarbonate system at a *p*H well away from its pKa (which is the pH it would tend to have in an inorganic system). Discuss the negative feedback system you learned in the first packet with respect to CO_2. What is the regulated variable? What is the set point? **What about this system is genetically controlled**? What are environmentally measured variables?

b. Here's a diagram of the effects of CO_2 and O_2 on the regulation of respiration:



From this graph you should see that if oxygen does change enough, it can become a potent stimulus for breathing. Also notice that at very large departures of CO_2 from normal that no further increase in minute volume occurs.

<u>Questions on the next page</u>

? Do you think the lack of further response in minute ventilation to extreme departures of P_{CO2} from normal is adaptive? Explain. Why (mechanistically) should the response change at these departures?

? Since breathing is regulated by negative feedback, explain why use of arterial CO_2 content for regulatory purposes will result in regulation of P_{aO2}.

? Why should arterial blood gas content be monitored? Why not venous? Is the use of arterial blood an accident or is it adaptive?

-- Just for fun --

? Should it be possible to make a system that would regulate using oxygen instead of CO_2 that would be just a sensitive to change in gas content? (Do you think it would be easier to design through evolution a protein that would respond to oxygen or CO_2 ?) If so, what would be different about this new oxygen based system as compared to the oxygen regulated system we possess? What would be different about the set point, and regulated variable?

The CO_2-regulated system seems to respond more or less incrementally (within limits) as the level of CO_2 changes. How is such an analog response generated and why might CO_2 be especially good for producing such a response. Would it be easy to do with oxygen? What would be a good ancestral protein from which to evolve an oxygen sensor?