Quantitative Physiology I / Molecular and Cellular Systems, BMEN E4001x
Notes 03 - Oxygen and Carbon Dioxide transport in blood and Hemoglobin
Chapter 28,29 of B & B; Chapter 1.2 of K & S

A major role of blood is oxygen and carbon dioxide transport between lungs and tissues. While this isn't completely in the framework of understanding cellular physiology, it is a key example of how chemical kinetics and equilibria provide a functional, elegant system.

We will assume the following conditions describe normal, resting tissues:

gas	solubility, s (M/mmHg)	atmosphere (mmHg)	alveolar (mmHg)	tissues (mmHg)
O_2	1.4E-6	150	100	40
CO_2	3.3E-5	<1	40	46

Solubility, denoted k, s, and sometimes σ, reflects the ability of a solution at equilibrium with a gas or mixes of gases to hold a particular **dissolved** gas.

$$[X]=s_X*P_X.$$

This is Henry's Law. Solubility is dependent on a wide range of factors, including temperature, solvent, and gas.

Dissolved gas is simply not enough to handle this transport.

A typical number for oxygen demand for a 70 kg person at rest is ~ 11 mmol/min of O_2.

This was originally cast as 250 ml/min, but let's just focus on the mole version. If you want this conversion, assume standard conditions; 1 atm, 273 K. Using the ideal gas law, 1 mole of gas takes up 22.4 L.

Now, taking the numbers above and assuming a 5 L/min cardiac output:

dissolved gas capacity = cardiac output * change in partial pressure * solubility

= 5 L/min * 60 mmHg * 1.4E-6 M/mmHg

= 0.4 mmol/min O_2.

A similar situation exists for dissolved carbon dioxide.

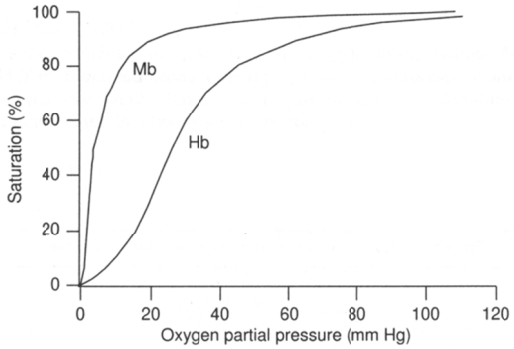
Additional capacity comes from tying up O_2 and CO_2 in complexes in blood.

This section, based on parts of B&B Ch. 28, deals with how our treatment of multimolecular binding makes up this capacity, and is presented as examples of how physiology results from the elaboration of simple concepts into complex systems. We will deal with the equilibrium conditions of gas transport, assuming these gases reach equilibrium with various forms in the lungs and tissues. Further elaboration of these concepts, will be taken up in Ch. 29 in QPII, and in the final module of AQPI.

Myoglobin and Hemoglobin serve as carriers and storage of oxygen.

Hemoglobin is a major oxygen carrier in blood.

- Tetramer protein; each unit somewhat similar to myoglobin, present in muscle tissue.
- Binds four molecules of oxygen per molecule of Hb.
- Typical blood value of Hb is 2.3 mM.
- K_D~25 mmHg
- Myoglobin has one heme group and binds one oxygen. Michaelis-Menten-type binding
- They exhibit very different shaped curves



Cooperativity

Build upon the enzyme kinetics framework with the following adaptations:

- Leave out the product generation step. This is identical to Keener & Sneyd, with the assumption that k2=0.
- Simplification in that we will derive the Hill equation for two binding site, and just state the general case, which can handle more binding sites.
- Thus, to unite with hemoglobin, think of S as O_2, C_0 as hemoglobin with no oxygen bound, C_1 as hemoglobin with 1 oxygen bound, C_2 as hemoglobin with two bound, with the understanding that C_3 and C_4 will be envisioned by extension of this model. Note the similarity of $C_{0,1,2}$ with E.

$$S + C_0 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1$$

$$S + C_1 \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} C_2$$

$$C_0 + C_1 + C_2 = C_T$$

Differential equations for two intermediates

$$\frac{dc_1}{dt} = k_1 s c_0 - k_{-1} c_1 - k_2 s c_1 + k_{-2} c_2$$

$$\frac{dc_2}{dt} = k_2 s c_1 - k_{-2} c_2$$

$$c_0 + c_1 + c_2 = c_T$$

Now, using the quasi-steady-state approximation

$$c_1 = \frac{K_2 c_T s}{K_1 K_2 + K_2 s + s^2}$$

$$c_2 = \frac{c_T s^2}{K_1 K_2 + K_2 s + s^2}$$

$$K_1 = \frac{k_{-1}}{k_1}; K_2 = \frac{k_{-2}}{k_2}$$

The number of ligands bound is

$$\text{Ligands} = 0 * c_0 + c_1 + 2c_2 = \frac{(K_2 + 2s) c_T s}{K_1 K_2 + K_2 s + s^2}$$

Examine two extreme cases:

First: independent active sites

Take the forward and reverse reaction rates for a single site to be k_+ and k_-, and noting that for this two binding situation, there are thus two ways to have a single ligand in the complex but only one way to have either zero or two ligand bound,

$$k_1 = 2k_+ ; k_2 = k_+$$
$$k_{-1} = k_- ; \; k_{-2} = 2k_-$$

Then, as

$$K_1 = \frac{k_-}{2k_+}; K_2 = \frac{2k_-}{k_+}$$

or....

$$K = \frac{k_-}{k_+}; K_1 = K/2; K_2 = 2K$$

$$\text{Ligands} = \frac{2c_T (K + s) s}{K^2 + 2Ks + s^2} = 2\frac{c_T s}{K + s}$$

Second: extreme (or infinite) cooperativity

Assume that the first ligand is hard to attach, but the next ones are easy. At the same time, preserve the overall association constant; that is, keep $K_1 K_2$ = constant.

This is achieved by increasing k_2 (letting it go to infinity) while decreasing k_1 (letting it go to 0).

Then, as $K_1 \to \infty$, $K_2 \to 0$

$$\text{Ligands} = 0 * c_0 + c_1 + 2c_2 = \frac{2c_T s^2}{K_1 K_2 + s^2} = \frac{2c_T s^2}{K_m^2 + s^2}$$

$$K_m^2 = K_1 K_2$$

In general, for n locations on a protein with cooperative binding,

$$\text{Ligands} = \frac{n c_T s^n}{K_m^n + s^n}$$

$$K_m^n = \Pi_{i=1}^n K_i$$

Examine a more realistic cases – somewhere between independent binding and infinite cooperativity.

The last equation only applies to the case of extreme, complete cooperativity. Ligand binding may in fact exhibit a behavior that is somewhere between independent and complete cooperativity, reflecting a degree of coordination between binding sites. Consider the hypothetical situation in which Hemoglobin is comprised of two sets of two-member subunits (that much is true) that don't communicate with each other (not quite true). In that case, one would expect binding to look like infinite cooperativity between two units, rather than four.

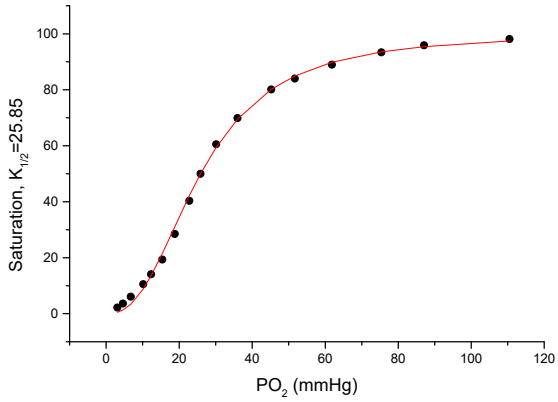
A general, empirical approach to this is to let the exponential terms of the above equation be anything between 1 and the number of subunits in question.

The above expression can be rewritten as follows, for a protein with m binding sites, an exponential coefficient n, and present at a concentration c_T:

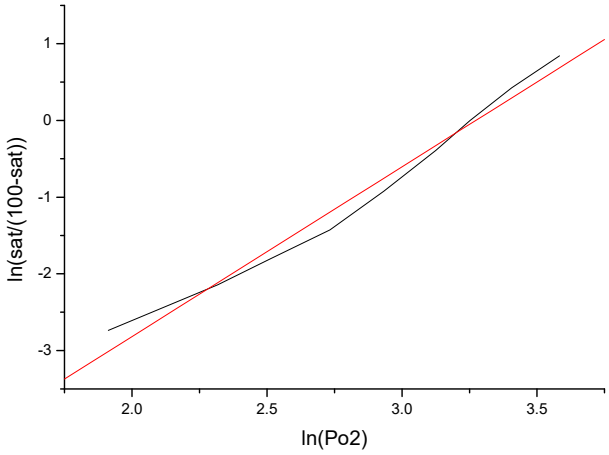
$$\text{Ligands} = (m * c_T) * \frac{s^n}{K_m^n + s^n}$$

- The term "n" can vary between 1 and m, including non-integer terms, and reflects the degree of cooperativity between binding sites:
    - n=1: independent binding
    - n=m: extreme cooperativity
- This "n" term is called the Hill coefficient
- A plot of ln(L/(Lmax-L)) vs ln(s), a Hill plot, should be a straight line of slope n.

What does this mean for hemoglobin?



Using n=2.45, K_m=25.85, rather good fit. However, there are some deviations, and the Hill plot suggests something systematic is going on.



Cooperativity Models

Where does cooperativity come from? Most models, which took root in the mid 1900s, invoke a few principles:

- Cooperative binding proteins are composed of several identical subunits, occupying equivalent positions on the proteins.
- Each subunit has one binding site, and all binding sites are equivalent
- These subunits somehow interact, based on binding of each subunit to its ligand.
- Each subunit has specific conformational states, relating to the binding affinity of each subunit.
- In a typical two-state system, these are denoted R and T, a relaxed and tense state.

For hemoglobin, the R state corresponds to a protein conformation containing O_2 (or CO or CO_2) and, because the protein is configured to accept the ligand, it is a high-affinity form. Conversely, the T state corresponds to a ligand-free conformation of low affinity.

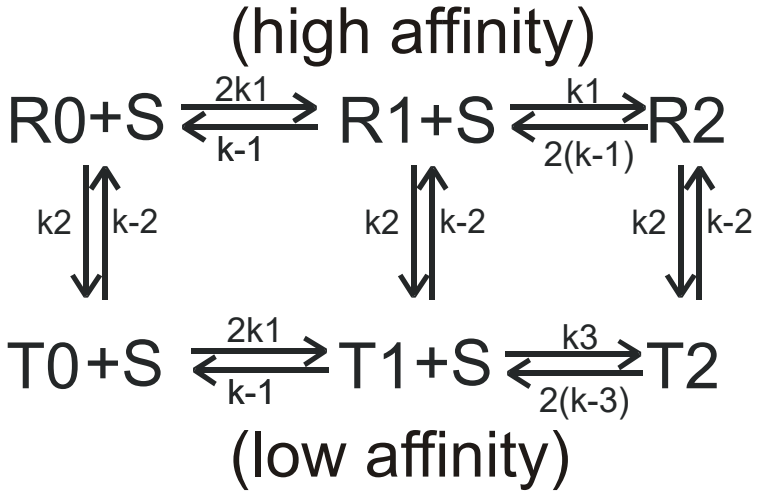
In the absence of ligand (O_2), subunits are in the T state, and binding of a little oxygen drives a conformational change to the R state.

In the Monod-Wyman-Changeux (MWC) model, the four subunits are so tightly locked that they must all be in the same state at any given time. A change in one subunit induces a change in all the others.

In the Koshland-Nemethy-Filmer (KNF) model, the four units have a degree of independence, this is called a sequential model.

Both models give rise to sigmoidal profiles, and probably represent two ends of the spectrum of what is really going on.

This is a basic MWC model, coding a 2-subunit protein rather than a 4-subunit one. We're not going to go into the quantitative aspects of this in any more detail in this class....



There is a physical basis for the idea of there being two forms of the protein. This is from Boron & Boulpaep, Chapter 28, and is only included here as perspective; the structural basis for this change is not formally part of this course. Also, see Nelson, Section 9.6 for a discussion.

CO_2 transport is heavily dependent on bicarbonate buffering.

One of the most important buffering systems is the carbonic acid/bicarbonate buffering system; dissolved carbon dioxide reacts with water to produce carbonic acid. Dissociation of this acid yields bicarbonate and a hydrogen ion.

$$CO_2 + H_2O \xleftrightarrow{slow} \underset{(\text{carbonic acid})}{H_2CO_3} \xleftrightarrow{fast} \underset{(\text{bicarbonate})}{HCO_3^-} + H^+$$

- Formation of carbonic acid, bicarbonate, and carbonate provide a way of tucking CO_2 into the solution, increasing capacity for a given change in P_{CO2}.
- In blood at "equilibrium", about 90% of total CO_2 (as accounted through this mechanism) is in the form of bicarbonate.
- The first reaction is very slow, too slow for useful exchange. Carbonic anhydrases (B&B page 637) accelerate this reaction, brining the effective rates to useful numbers.
- This capacity does come at the cost of a change in pH as CO_2 is taken up.

Blood, in conjunction with exchange of gases with the atmosphere, offers an effective means for dealing with these changes. Moreover, changes in pH due to CO_2 and metabolism-related acids are used as a control signal to balance various physiological processes.

We're going to cover three aspects of bicarbonate buffering that are rather surprising:

- Exchange of CO_2 with air enhances buffering
- Analysis of interplay between bicarbonate & non-bicarbonate buffering – Davenport diagrams.
- Interactions of CO_2 and O_2 with hemoglobin.

Review: Buffers

Start with the Brønsted treatment of acids and bases, in which an acid can donate a proton and a base can accept a proton.

$$pH=-\log([H^+])$$

Solution	**pH**
gastric secretions	0.7
Soda	2
Cytosol of a cell	7.2
Pancreatic fluid	8.1

Clearly, control over pH is important for protein/system function

General Theme:

$$HA \leftrightarrow H^+ + A^-$$

For strong acids and bases, this dissociation is essentially complete. For weak acids and bases at equilibrium, the balance between dissociated and associated states follows the LeChatlier Principle: a system at equilibrium reacts to an input by minimizing the effect of that input.

Note: this look like the reverse of the binding reaction we just discussed, largely because this is cast in terms of a dissociation reaction, rather than binding. Need to be able to go between these by looking at the system.

Consider being at low pH, at which weak acid is protonated ([HA]). As one adds a strong base, pH rises, removing H^+ from the solution (by binding to OH^-). On balance, H^+ is replenished by action of HA dissociation. Same thing happens in reverse.

In short, if you are in a realm where a weak acid/base is present in both protonated and deprotonated forms in significant fractions, addition of strong base or strong acid will result in a change of pH change of much less than the unbuffered case.

Quite often buffer salts have multiple dissociations, and may salts are multivalent, but leave this complication out.

Quantitatively:

$$HA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} H^+ + A^-$$

at equilibrium,

.

$$\frac{d[HA]}{dt} = 0 \Rightarrow k_1[HA] = k_{-1}[H^+][A^-]$$

$$\frac{[H^+][A^-]}{[HA]} = \frac{k_1}{k_{-1}} = Ka = \text{dissociation constant}$$

Note that Ka is a dissociation constants; the "a" indicates that it is specific for an acid.

Henderson-Hasselbach equation:

$$pKa = -\log\left(\frac{[H^+][A^-]}{[HA]}\right) = -\log[H^+] - \log\left(\frac{[A^-]}{[HA]}\right)$$

switch left and right sides

$$pH = pKa + \log\frac{[A^-]}{[HA]}$$

Buffer characteristics

Buffer strength defined as the concentration of protonated and deprotonated forms of weak acid.

Buffer power (β) defined as the amount of strong base needed to produce a given change in pH.

$$\beta \equiv \frac{\Delta|\text{strong base}|}{\Delta\text{pH}} = -\frac{\Delta|\text{strong acid}|}{\Delta\text{pH}}\text{; units of conc./pH unit}$$

Example of pH calculation

Consider a buffer solution containing 10 mM of the monovalent salt HEPES, pKa = 7.55.

HEPES: 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)

.1 ml of 1 M HCl is added to **100 ml of this buffer which has an initial pH of 7.2**. What is the pH of this solution after it has come to equilibrium (long enough to come close)? Compare this to the change in pH in the absence of a buffer salt. What is the buffer strength?

So:

- Use two conservation equations: total $[H^+]_T = [H^+]+[HA] = 10^{(-pH)}+[HA]$
- Use total acid $[A]_0 = [HA]+[A^-]$ to get to [HA]
- Since $Ka=[H^+][A^-]/[HA]$, $[A^-]=Ka*[HA]/[H^+]$
- $[A]_0=[HA]*(1+Ka/[H^+])$, thus $[HA]=[A]_0/(1+Ka/[H^+])$
- Put that into conservation eqn for $[H^+]$
- to get $[H^+]_T = [H^+]+[A]_0/(1+Ka/[H^+])$; $[H^+]=10^{(-pH)}$ <= Core equation
- at pH 7.2, 10 mM buffer, $[H]_T$ = 6.91 mM.
- Added acid brings conc. to (6.91mM*100ml + 1000mM*0.1ml)/(100.1ml)=7.90 mM.
- Working backwards $[H^+]^2+(Ka+A_0-[H]_T)[H^+]-[H]_T *Ka=0$; use quadratic eqn.
- to get pH=6.97, so drop of 0.23 pH units.
- In absence of buffer, pH=3
- Buffer strength = -1E-3 M / (6.97-7.2)=4.3 mM/pH.

Bicarbonate buffering is an open system.

The equilibrium between dissolved CO_2 in liquid versus that in the atmosphere (Henry's law) raises the buffering power of this system.

Where do things stand for bicarbonate? For following discussion, pKa=6.1.

With body pH around 7.4, $[HCO_3^-]/[CO_2] \sim 20$, mostly in bicarbonate, a bit far from the max buffering zone for bicarbonate.

Buffer strength for a closed system

General solution

Major points (slides work through a key example)

- Buffer is present at relatively high concentrations in typical solutions
- To solve for a new pH of a system following addition of strong acid or base, note that:
  - A^- + HA will remain constant.
  - H^+ + HA will be changed by the addition of strong acid or base. Safe to assume that addition of strong base will use up H^+ (given K_w is so small)

In this case, the buffering power can be approximated by the equation in B&B (Equation 27-20)

$$\beta_{closed} = 2.3[\mathrm{A}]_0 \frac{[H^+] * Ka}{([H^+] + Ka)^2}$$

- As a function of pH, or $[H^+]$, this is a bump with a peak around Ka.

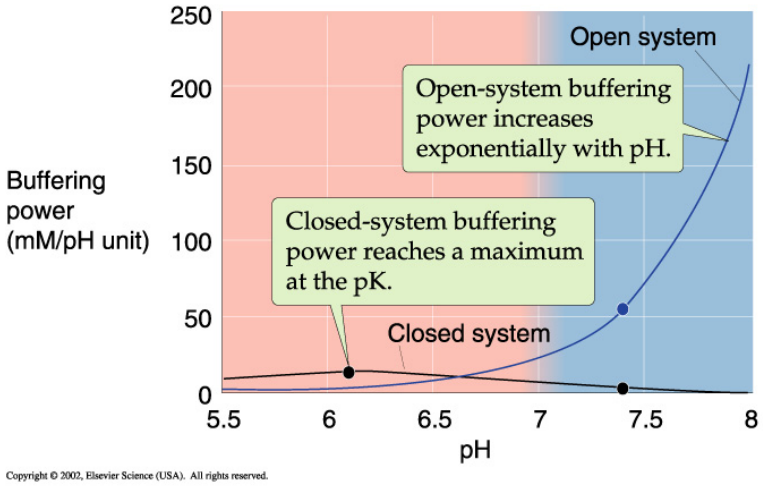
How does an open system behave?

Key thing is that the conjugate base is in communication with a big reservoir, which can replenish/absorb stuff. In short: $[CO_2]=s_{CO2}*P_{CO2}$.

When the buffer is allowed to equilibrate with the environment, the total amount of buffer is not a constant. This is an ***open system***, and, going through the same process for getting buffer power:

$$\beta_{open} = 2.3 * [HCO_3^-]$$

What does this mean?



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Pretty cool, buffer power increases with increasing pH!

Behavior of blood

typical conditions:

P_{CO2}= 40 mm Hg; $[CO_2] \sim 1mM$

pH=7.4, $[H^+] \sim 40$ nM

$[HCO_3^-] \sim 26$ mM

With the relation $Ka=\frac{[HCO_3^-]*[H^+]}{[CO_2]}$; any increase in $[CO_2]$ is met with a proportional increase in the product $[HCO_3^-]*[H^+]$. However, the stoichiometry of this single buffer system indicates that equal amounts of $[HCO_3^-]$ and $[H^+]$ get evolved from any converted $[CO_2]$. $[HCO_3^-]$ being so much more present than $[H^+]$ implies two rough results:

- A change in $[CO_2]$, by changing P_{CO2}, for example, induces a proportional change in $[H^+]$, but no change in $[HCO_3^-]$
- A change in $[HCO_3^-]$ induces a proportional change in $[H^+]$ (not a proportional change in pH), at constant $[CO_2]$

In short, this system is interesting, but complex. Also, what happens in the context of additional buffers?
Look at Davenport diagrams and pH regulation.

Davenport diagrams and pH regulation:

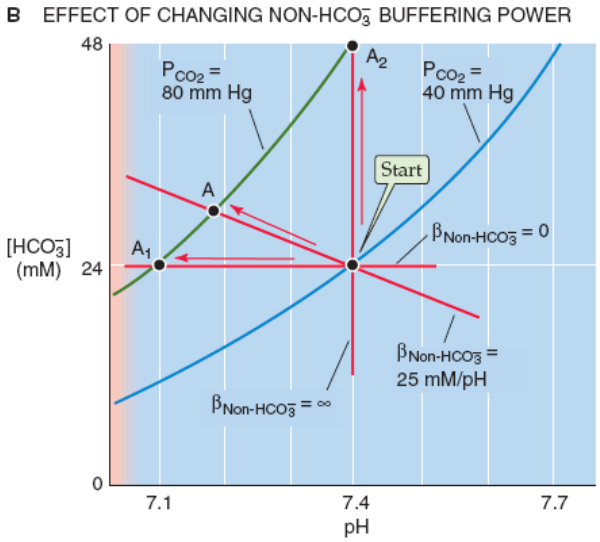
Davenport diagrams are a convenient way to analyze what is going on in blood in response to changes in P_{CO2} and metabolic activity.

This section follows closely the discussion in \

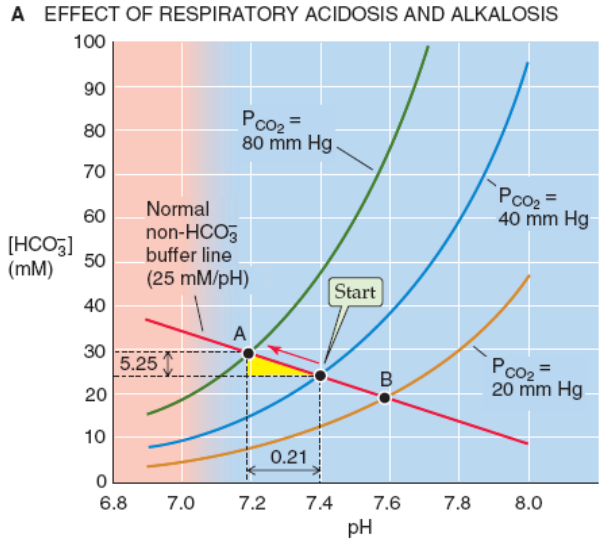
B&B.

Blood has lots more buffers, with varying pKa. This yields a relatively flat buffering power of strength 25mM/pH

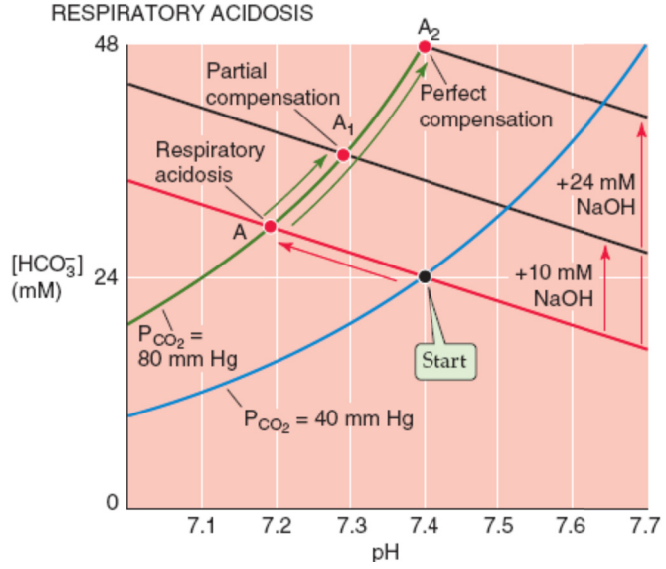
Isopleths relate P_{CO2} and HCO_3^-, buffer line represents compensation by buffer.



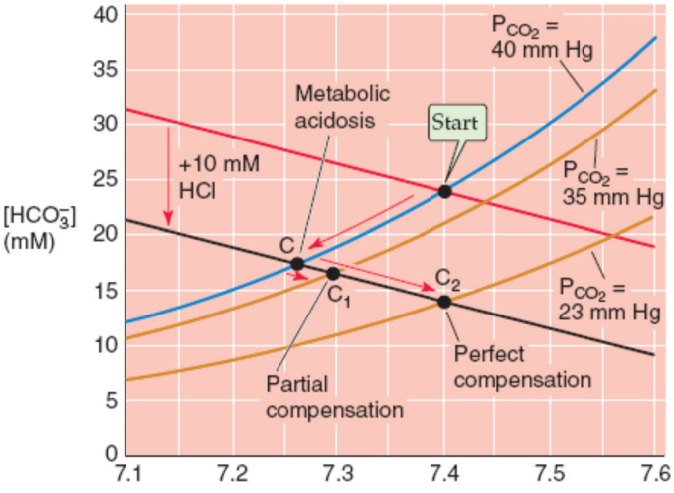
Then, how do respiratory and metabolic processes act to balance a shift?



A METABOLIC COMPENSATION TO RESPIRATORY ACIDOSIS



A RESPIRATORY COMPENSATION TO METABOLIC ACIDOSIS



The Bohr Effect, pH, and CO_2

a host of physiological conditions, such as exercise, lower the pH of blood, through production of both acid and CO_2, which, as we discussed earlier, produces acid.

A lowering in pH by addition of acid shifts the hemoglobin saturation curve right, *i.e.*, K_M increases. Based on figure 28.5a, K_M can vary from 20-35 mmHg as pH goes from 7.6 to 7.2. The effect is to allow dumping of more oxygen at lower pH.

There are titratable groups on the Hb surface; several amino acids have pKa's in the 6-8 range (histidine, serine, etc.). Protonation of these groups can cause changes in protein conformation. It is believable that these changes translate into the changes in K_M, potentially by changing the balance between R and T forms to the T form.

The response of hemoglobin to pH itself is the Bohr effect.

In addition to generating H^+, CO_2 can also bind directly to amine groups on the hemoglobin surface. These carbamino compounds lower the affinity of hemoglobin to O_2. So, a rise in CO_2 initiates a dump of oxygen.

CONVERSELY, a rise in O_2 causes a virtual dump of CO_2, encouraging release of CO_2 in the lungs. Hemoglobin plays a minor role in CO_2 transport.

Temperature:

Activity generates heat. Following a similar reasoning as above, one would think that increased temperature raises K_M. It does, as is illustrated in Figure 28-4.