THE LIMITS IMPOSED BY DIFFUSION IN RESPIRATION AND STRATEGIES TO OVERCOME THESE LIMITS[1]

Summary: In this class we consider how to get around the constraints imposed by diffusion. We start with a model that shows how a spherical aerobic organism with no special adaptations is limited by diffusion and then move to a consideration of the sorts of adaptations that have evolved to get around these constraints. This gives a chance to survey respiratory systems, including some very weird ones. In particular, we look at the operation of tracheal systems and of two methods for tracheal animals to breathe under water. In addition, we examine the general features of respiration in animals that circulate O_2 and CO_2 in blood and examine the counter-current gill. Finally, the basic ventilation patterns of different types of vertebrate lungs are discussed.

I. A Model of the Constraints of Diffusion on Aerobic Metabolism:

A. In understanding respiration, one of the most useful exercises one can perform is to consider how large an animal with a given rate of metabolism could be if it had to rely on diffusion only.

1. Thus, the organism's geometry, the environmental gas tensions, and Fick's Law would determine whether or not it could obtain sufficient O_2.

? Notice that we consider only O_2. In light of the previous discussion, why do we ignore CO_2?

2. The cell that we will consider will not resemble any real cell -- it will be a simplified model cell. We will make several un-realistic assumptions to make our model simple and also because, as you will see, they will ultimately tell us a lot about the reasons that real cells are not as simple as the one we visualize here. This model was first constructed by E.N**. Harvey** in 1929 and then used somewhat more broadly by perhaps the most famous comparative respiratory physiologist of this century, August Krogh:

3. Again, our question: Let's see how big a cell can be and have a given shape:

a. Since we are interested in O_2 diffusion:

1. $$\dot{V}_{O_2} = K * A * \frac{\Delta P_{O_2}}{\Delta X}$$

Notice that this is simply Fick's Law where we are using *K* and $\dot{V}_{O_2} = \frac{\Delta Q}{\Delta t}$ since it is the rate of O_2 consumption.

b. let's assume that our cell is a sphere with a radius ***r***:

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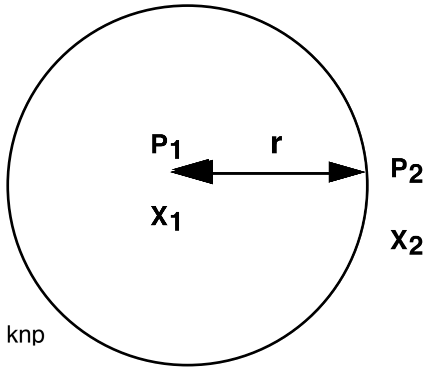
Harvey's simple Model to Find the Size of a Cell Limited by Diffusion



P_1 is the P_{O2} at the center of the cell
P_2 is the P_{O2} at the surface of the cell
X_1 is the center of the cell
X_2 is the surface of the cell
r is X_1-X_2, the radius of the cell

4. To find $\dot{V}_{O_2}$ for a sphere of any radius ***r***, we must multiply the unit (weight-specific) $\dot{V}_{O_2}$ by the weight. If we are looking at a series of animals of different size but the same composition, then weight is proportional to volume. Therefore, the total metabolism for our generalized spherical animal is:

2. Metabolism = unit $\dot{V}_{O_2}$ * # of units

--or-- M = weight-specific $\dot{V}_{O_2}$ * volume for a spherical organism. Now since volume is 4/3 * Π * r^3, then:

3. M = weight-specific $\dot{V}_{O_2}$ * $(4 / 3 * \Pi * r^3)$

5. Next, we need the surface area, ***A***, of our organism since that is where all gas is exchanged.

4. $A = 4 * \Pi * r^2$

3. By substitution of eq. #3 and 4 into #1 (Fick's Law)

5. $\dot{V}_{O_2}$ (mass specific) * $(\frac{4}{3} * \Pi * r^3) = K * (4 * \Pi * r^2) * \frac{P_2 - P_1}{X_2 - X_1}$

where $\frac{P_2 - P_1}{X_2 - X_1}$ is an expression of the partial pressure gradient from the surface of the cell (P_2 at X_2) to the center of the cell (P_1 at X_1).

If we now say that $\mathbf{X_2 - X_1 = r}$ (see fig. above) and if we assume that the P_{O2} at X_1 (the center) = 0 torr, then we can solve the equation to find the lowest P_{O2} on the surface of the cell that is consistent with the cell having adequate O_2:

6. $$P_2 = \frac{\dot{V}_{O_2} * r^2}{K * c}$$

where ***c*** is a constant, ***r*** is the radius of the cell. Note that ***c*** will <u>not</u> be equal to 3 as you might predict, there is also an integration involved in this model that I have not included -- let's ignore it and just realize that it makes ***c*** twice the value that we would have predicted.

B. <u>**Solving the Model**</u>.

1. If we assume a certain metabolic rate and environmental P_{O2} , <u>this equation will tell us the largest radius that the cell can be *if two conditions are met*</u>:

a. **O_2 is used equally by all of the cytosol** of this cell; that is, the P_{O2} will decrease uniformly as we approach the center of the cell.

b. **Only the exact center of the cell can have a P_{O2} of 0 torr**. In other words, for the whole cell to be alive, we will assume that all cytoplasm uses O2 and anywhere there is no O_2 is dead. Thus, we want to find the radius such that for a given environmental PO_2 the exact center of the cell is the only place with a P_{O2} of 0.

2. Thus, we can re-arrange eq. 6 to solve for this radius:

7. $$r = \sqrt{\frac{P_2 * K * c}{\dot{V}_{O_2}}}$$

where c is a constant, r is the radius of the cell. Note that c will <u>not</u> be equal to 3 as you might predict from the information above. There is also an integration involved in this model. I have not included this for simplicity and so let's ignore it and just realize that the integration makes c twice the value (6) that we would have predicted.

3. I<u>f we know a reasonable value for $\dot{V}_{O_2}$ and further assuming a maximum possible P_{O2} of 160 torr</u> (sea level), we can calculate the largest possible size for this organism:

a. We will assume a $\dot{V}_{O_2}$ = 1.3 ml/gh. This is a typical value for an average sized heterotrophic protist.

b. For ***K***, we will use a value of 1.8421×10^{-8} (cm^2/(min * torr)): this is the value that Krogh found for typical tissues such as muscle.

c. Finally, we will assume that the P_{O2} on the surface of the organism (P_2) is 160 torr: this would represent the value for water that is in equilibrium with the sea level atmosphere.

Solving eq. 7:

8. $$r = \sqrt{\frac{P_2 * K * c}{\dot{V}_{O_2}}}$$

$$r = \sqrt{\frac{160 * 1.8421X10^{-8} * 6}{1.3}}$$

r = 0.0036 cm; or 36 μm

This radius is **<u>much smaller than the radius of the typical protist from which we picked the metabolic rate data.</u>** For instance, *Paramecium caudatum* has a metabolic rate of about 1.3 $mLO_2/(gh)$ and has a minimal radius of 530 μm (15X larger than the predicted):

4. Unicellular Organisms and the Model. Most unicellular organisms are larger than the predicted maximum radius we have just found. How is this possible -- these are aerobic organisms, i.e., they are not getting around the problem by being anaerobic.

a. One of our assumptions is very much in error. Aerobic metabolism is not the result of reactions that are spread all over the cell. Instead, aerobic metabolism is located only in mitochondria that are located in a relatively small part of the cell. It is perfectly possible and common for these organelles to be primarily located near the periphery of the cell where the diffusion distances are short. Thus, the PO_2 at the center of the cell may have nothing at all to do with the ability of the cell to aerobic. One of the advantages of having mitochondria is the ability to concentrate all aerobic reactions in places where the PO_2 is highest.

b. Cells rely not only on diffusion to transport respiratory gases and other materials but **CONVECTIVE or BULK FLOW PROCESSES** also are important on the cellular level. Essentially, these processes enable large amounts of the medium containing the respiratory gas to be:

1. Quickly moved from one region of the cell to another (**INTERNAL CONVECTION or CIRCULATION**) -- in unicellular organisms the most obvious manifestation of this process is **CYTOPLASMIC STREAMING.**

2. External convective processes that constantly "STIR THE MEDIUM" assure that no areas of depleted PO_2 develop on the surface of the cell. Convection of the external medium is called (in respiration) **VENTILATION**. Movement of cilia would stir the medium and would be one form of ventilation in protists.

Although ultimately diffusion is the final step in movement of the respiratory gases, however, convective processes **INCREASE THE DIFFUSION GRADIENT, $\frac{\Delta P}{\Delta X}$, BY MINIMIZING DISTANCE AND MAXIMIZING THE PARTIAL PRESSURE DIFFERENCE**.

<u>Animals can also change their geometry</u>. Spheres have the lowest S/V: if an animal wants to get bigger and maintain a relatively high $\dot{V}_{O_2}$, it could change to some kind of a more flattened shape such as a cylinder or rectangular box. In addition to r, this would primarily affect the ***A*** variable in Fick's Law.

In short, there are a number of avenues opened to an organism such as unicellular protist to allow it to get bigger without lowering its metabolic rate. Every one of these allows the organism to get around some constraint of Fick's Law by maximizing one of the terms in the equation in order to maximize $\dot{V}_{O_2}$ (within the constraints of the energy available in the environment).

? Using the body shapes and habits of Poriferans, Cnidarians and Platyhelminthes, explain how each of these organisms has increased the possibilities for respiratory gas diffusion over a sessile, solid, living sphere that lives on the bottom of the ocean. Be sure to relate each adaptation to Fick's Law -- state what is maximized and how it is done.

Where are mitochondria placed in muscle cells? Is distance between mitochondria and the source of O_2 (O_2 diffusion distance) the only thing that matters in terms of placement of mitochondria?

d. Finally, we must realize that it is also a legitimate option for an animal to simply change its metabolism to a lower value if other evolutionary constraints do not permit the adaptations mentioned above.

1. Notice that this happens to a certain extent in all organisms. The 0.75 power predicts a slower rise in metabolism than expected from an isometric model.

2. Remember that animals' respiratory systems are designed for some maximal, not resting, rate of metabolism. If an animal is getting in O_2 delivery trouble when at rest, then the animal must either avoid activity or it must be good at getting ~P anaerobically.

C. TRACHEA AS A MEANS TO MINIMIZE THE DIFFUSION OF CONVECTION OF A GAS THROUGH WATER:

1. These are probably the most effective systems for getting large amounts of oxygen to cells at a low energy cost -- tissues with the highest metabolic rate in the animal kingdom are generally served by trachea.

2. They have evolved many times -- in at least three major arthropod lines and in many separate instances within these lines: insect-myriad (centipede and millipede) line, in some spiders and other arachnids (such as daddy long-legs), and in certain semi-terrestrial crustaceans.

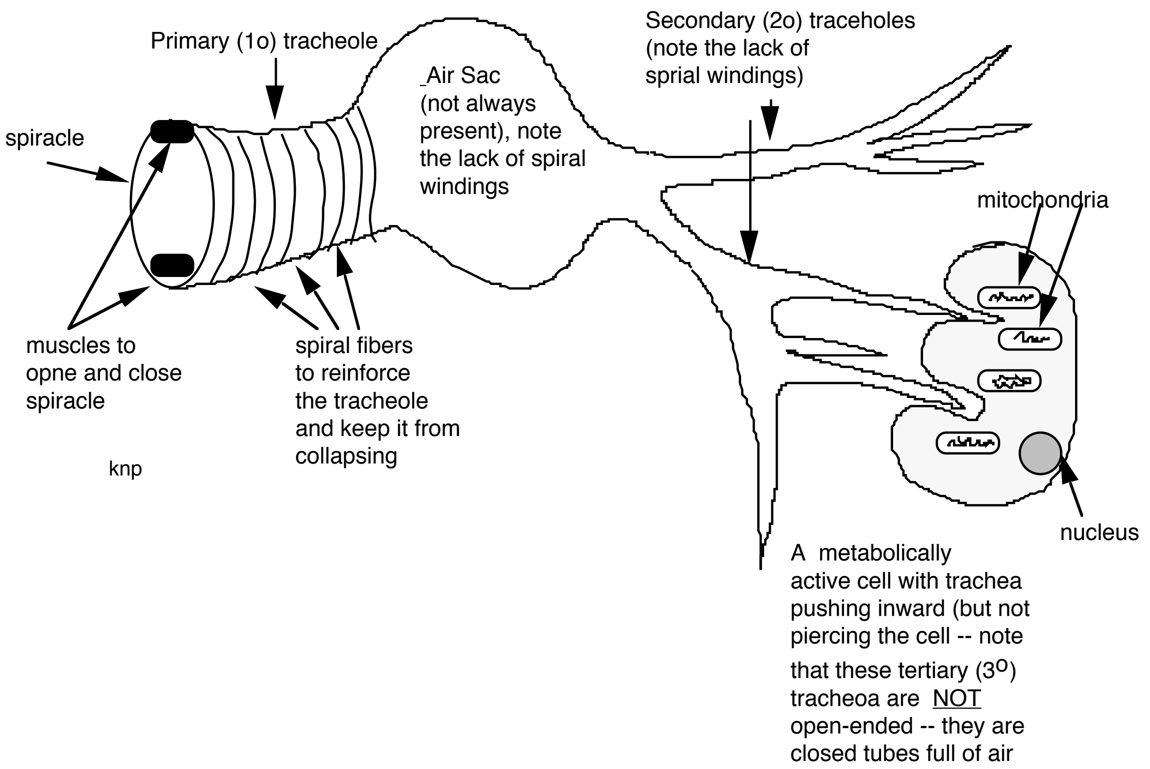
3. They have all evolved as inward extensions of the exoskeleton -- in some cases associated with the original respiratory system , in others, as *de novo* breathing tubes.

a. Trachea are simply air tubes that lead into the animal and end in the proximity of the cells to be served.

1. The openings to the trachea are called **SPIRACLES** and they are opened or closed according to muscular action.

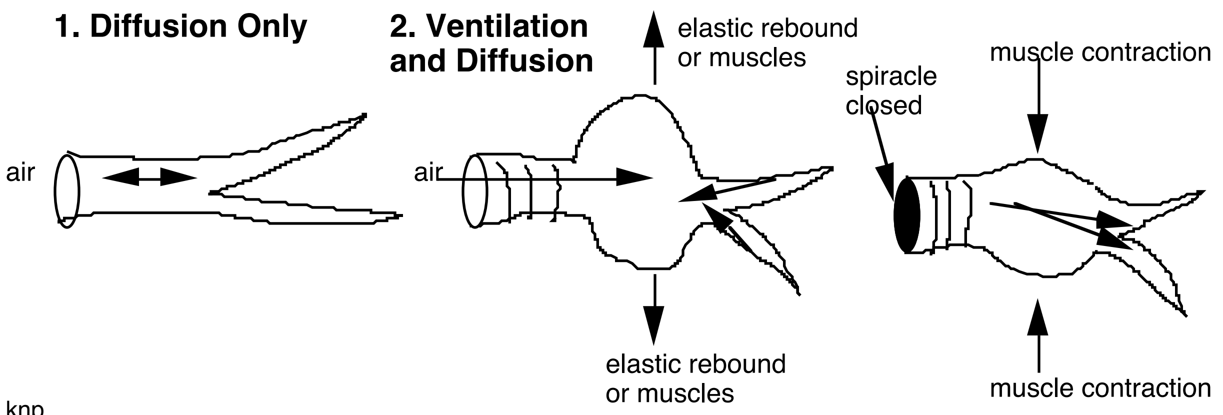
2. Much of the trachea are non-compressible tubes for airflow either by diffusion or ventilation. These areas are called the **PRIMARY TRACHEOLES** and they may be connected to air sacks that can ventilate the system by acting as a type of bellows.

3. Eventually, the trachea **ANASTOMATIZE** into a series of very fine **TRACHEOLES** that are the actual exchange sites for resp. gases. In the cases of very active muscles such as flight muscles, these push into the actual cells that use the O_2 and end next to or pushing into the mitochondria. (Note: these are closed-ended tubes; air does not bubble from their ends but instead diffuses out through a thin wall)



b. Since gases diffuse much faster in air than solution, large volumes can be enter by diffusion.

c. Further, trachea also can be ventilated. Since the density and viscosity of air is much less than water, tracheal ventilation is much less expensive energetically than the combined ventilation - circulation system of respiratory gases seen in other animals. Thus, extremely high rates of O_2 delivery and CO_2 removal can be obtained very cheaply. The movement of gas in the trachea should be thought of as a two step process: active ventilation to move large amounts of fresh air from the outside of the animal through most of the tracheal system and then diffusion down the last bit of the trachea. In small insects, there is no active ventilation and diffusion alone suffices since the distances involved are small:



(a) Elastic rebound with spiracles open -- air sac fills and 3^o tracheoles are emptied.

(b) Muscle contraction with closed spiracle moves air towards the cells, diffusion does the rest. Contraction with an opened spiracle empties the spiracle.

Active Ventilation can easily be observed in a large insect: the abdomen and thorax can be seen as being compressed and expanded.

? Compare the values of *D* for CO_2 and O_2 diffusion in air and water (go back to the last packet to find these values. Explain why trachea are so good at gas exchange when compared to systems that rely on the diffusion of these gases through water.

D. Aquatic respiration in animals that use trachea.

1. The problem here is obvious: trachea are obviously superbly designed for aerial respiration. Have they been a constraint that has prevented their possessors from living in the water? Obviously they haven't completely prevented such invasions and we will now consider some interesting adaptations that insects use in this regard:

2. **BUBBLE BREATHERS**: consider this data for certain types of water bugs (*Notonecta*) -- "back swimmers":

a. These animals take a bubble of O_2 below the surface with it.

b. Data:

1. if no O_2 in water, it dies in 5 min.
2. If the water is saturated with AIR, it lives 6 hrs.
3. If submerged in water equilibrated with 100% O_2 and if it takes a pure O_2 bubble down with it, it lives only 35 min.

? Explain the meaning of the first set of results in light of the other data.
HOW DOES THE ANIMAL LIVE SO LONG IN SITUATION B BUT NOT IN C? Obviously, the bubble in B is doing something that is allowing the animal to remain submerged for a long time - it is acting as a gill. If it were simply a store of O_2 then the animal with the pure O_2 bubble would be able to stay down longer:

c. When the water is saturated with air and the bubble is made of air the animal possesses:

1. A relatively stable bubble that will not change much in size.
2. This results in a stable, large diffusion area from water to the bubble.

d. Here's how it works:

1. When the animal first dives, the bubble has the same P_{O2} and P_{N2} as the surface air. Notice that for our purposes, the water is also in equilibrium with the air.

2. As the animal dives, two things happen, both of which shrink the bubble somewhat:

a. It uses up some of the O_2

b. The hydrostatic pressure of the water compresses the bubble and raises the P_{O2} and P_{N2} according to the total pressure (since these gases are still making up roughly the same % as on the surface). Generally, in freshwater, Pressure increases 1 ATM for each 11 meters depth. Our bugs stay in the top meter of the water.

3. The bubble has shrunken slightly (reducing its SA) and there is less O_2 in it since it has been used for:

a. metabolism and

b. some of it has diffused down its partial pressure gradient into the water. **REMEMBER: the PO_2 of the water does not increase with depth. However, not much O_2 will diffuse out of the bubble because O_2 is not as soluble in water as it is in air**.

-- SO FAR SO BAD --

4. However, soon, the PO_2 in the bubble is somewhat lower in the air bubble than the water. Now O_2 begins entering the bubble from the water according to Fick's Law. The bubble's size is stabilized in part by this inflow and in addition by the fact that N_2 is not very soluble in water. Therefore, it leaks out of the bubble slowly. The SA of the bubble is the main factor in determining how much O_2 will enter:

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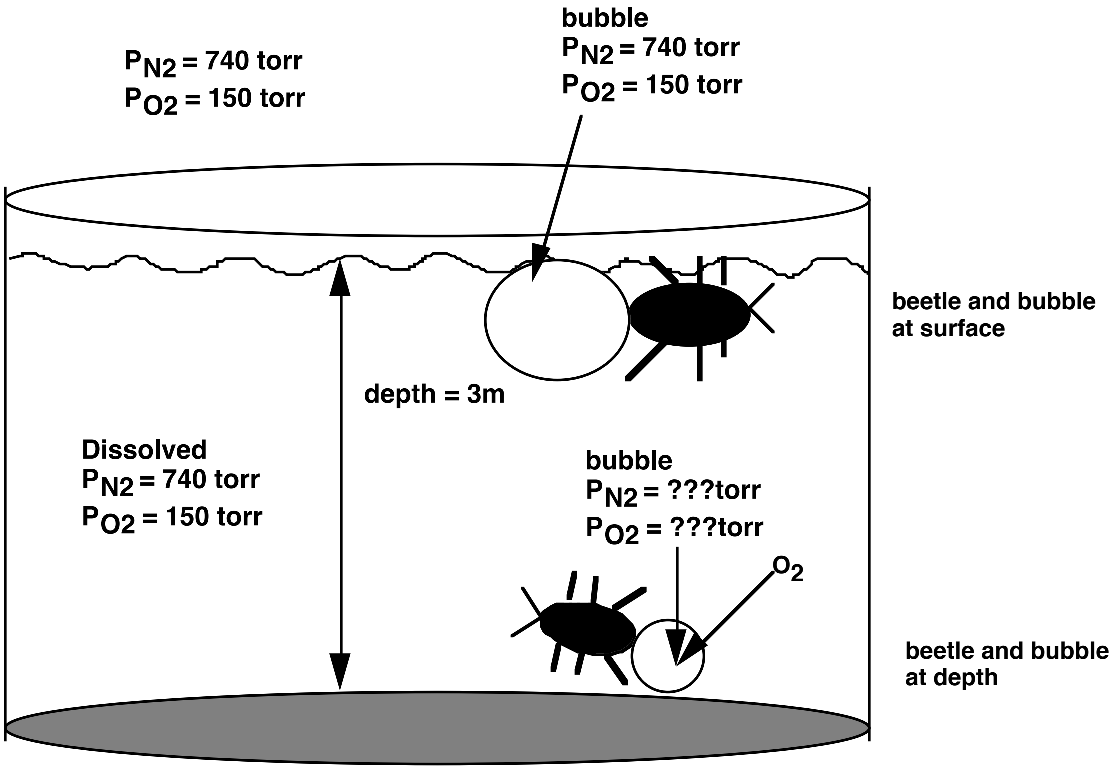

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5. The only thing that determines how long the bubble gill will work is the length of time that the bubble remains large. Notice that with time the bubble will shrink as the PO_2 drops slightly and N_2 leaks away or the animal goes deeper. Notice that once the bubble gets smaller it cannot increase in size (unless the animal comes closer to the surface --but even then the bubble is not as large as the last time the animal was at that depth). Eventually, the animal will have to surface to get a new bubble.

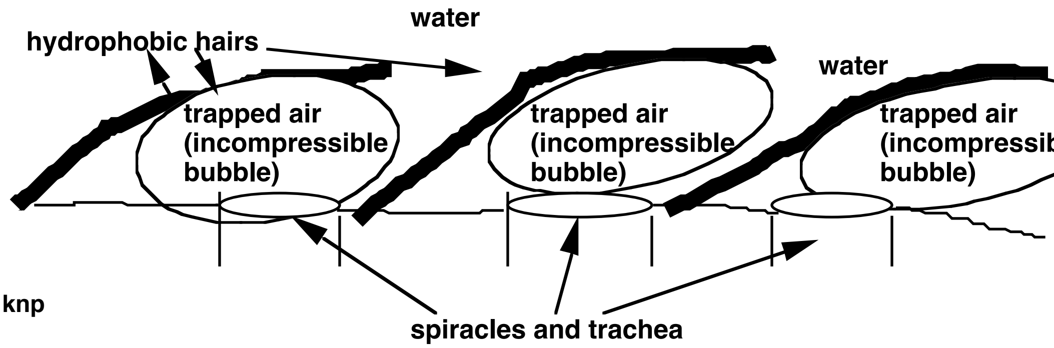
? Why have we not mentioned CO_2 in maintenance of bubble stability? What do we know about the comparable rates of diffusion of O_2 and CO_2?

Why does the animal not stay under as long with a pure O_2 bubble? Explain in detail.

3. **Plastron breathing** One solution to the problem of bubble breathing is to use a bubble that cannot change in volume:

a. In the PLASTRON GILL, air is trapped next to the body under millions of hydrophobic hairs. These hairs arch over the air bubble and the water cannot penetrate.

b. The result is a non-compressible air space and gill. The gill will last forever and is immune to being knocked off by currents.



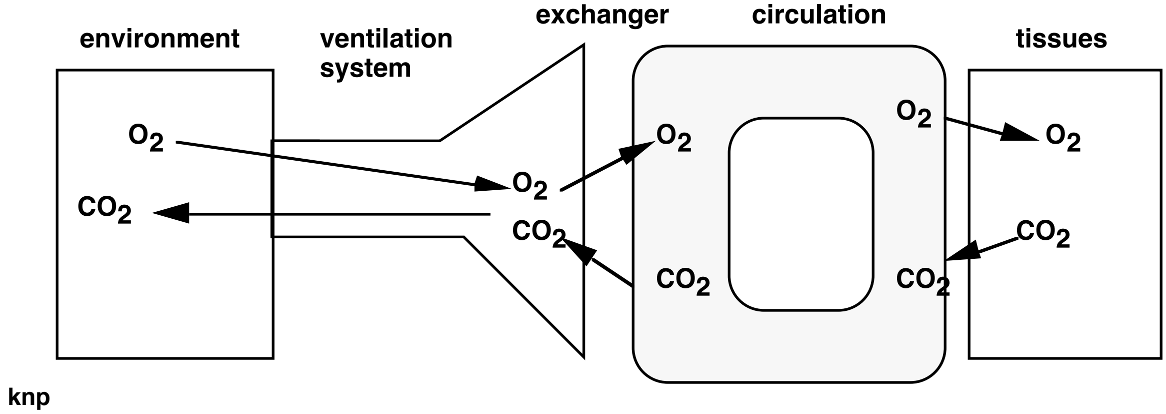
II. A GENERAL MODEL OF RESPIRATION FOR ALL LARGE ANIMALS THAT DO NOT USE TRACHEA:

A. In these animals, convective processes are important and unlike insects, two convective systems are used:

1. The ventilatory systems with moves the external medium across the respiratory surface (gill, skin or lung)

2. An internal blood or hemolymph circulation that moves fluid between the exchanger and the tissues:

The Model:



We will be using this model as the basis of the next few lectures, so know the values associated with it.

3. The purpose of these systems is to <u>maximize the diffusion gradient, $\Delta P/\Delta X$, by decreasing the diffusion distance and by maximizing the partial pressure gradients</u> for the two respiratory gases.

4. The <u>energy needed to work these systems</u> depends upon several factors:

a. the **density of the medium** -- this generally relates to viscosity and therefore to the amount of resistance to flow. We will say much more about this when we get to the circulation.

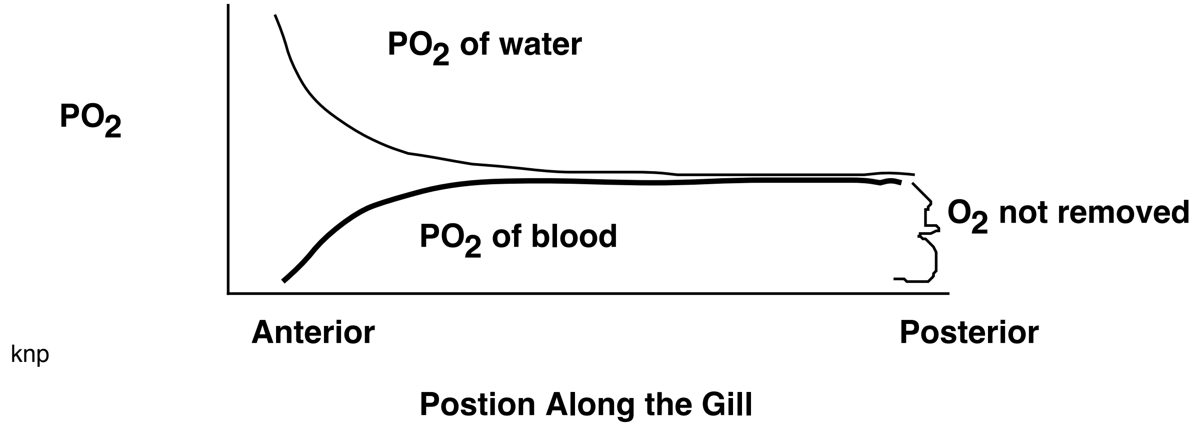
b. **Relative availability of O_2 compared to the animal's needs**: obviously, if the environment has relatively little O_2 compared to what the beast needs, it will need to convect more both on the inside and outside to get enough O_2 to pass by the tissues per unit time (both the respiratory and active tissues). Internal and external ventilation must both be considered, and the amount of work done by each is largely a function of he medium involved, the tubing through which it must pass, and the amount of O_2 that must be carried.

B. Let's consider **water-breathers** first:

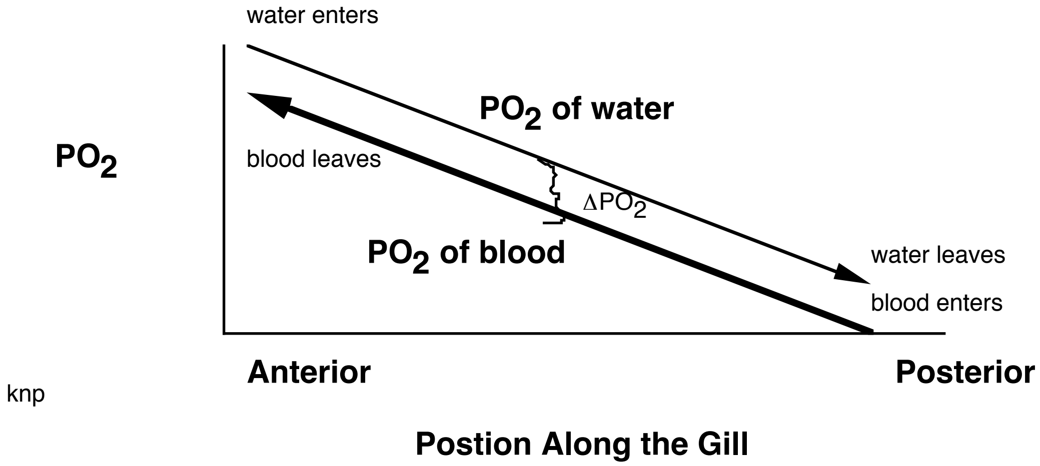
1. O_2 availability, even in saturated waters is low.
2. the medium is dense, therefore external ventilation is going to be expensive.
3. CO_2 is not too much of a problem because of the high ability of the water to absorb this gas.
4. What can a fish do to maximize its uptake of O_2?

Let's imagine two possible types of gill, **CONCURRENT AND COUNTER-CURRENT:**

Con-current:



Counter-Current



5. The **principle feature of the counter-current exchange gill** is that **a constant diffusion gradient is maintained** between blood and water; the water P_{O2} is always higher than in the blood. As a result, the gills remove nearly all of the available O_2 (90%).

-- The only type of gill found in active animals is the counter-current gill.

? In what ways are the gas situations similar in a human diver and a beetle bubble breather? Different? Is a counter-current gill required in a sluggish marine animal? Explain.
Compare this counter-current exchanger with the one we considered earlier for heat. Is separating two flows the main goal of the respiratory exchanger? Explain.

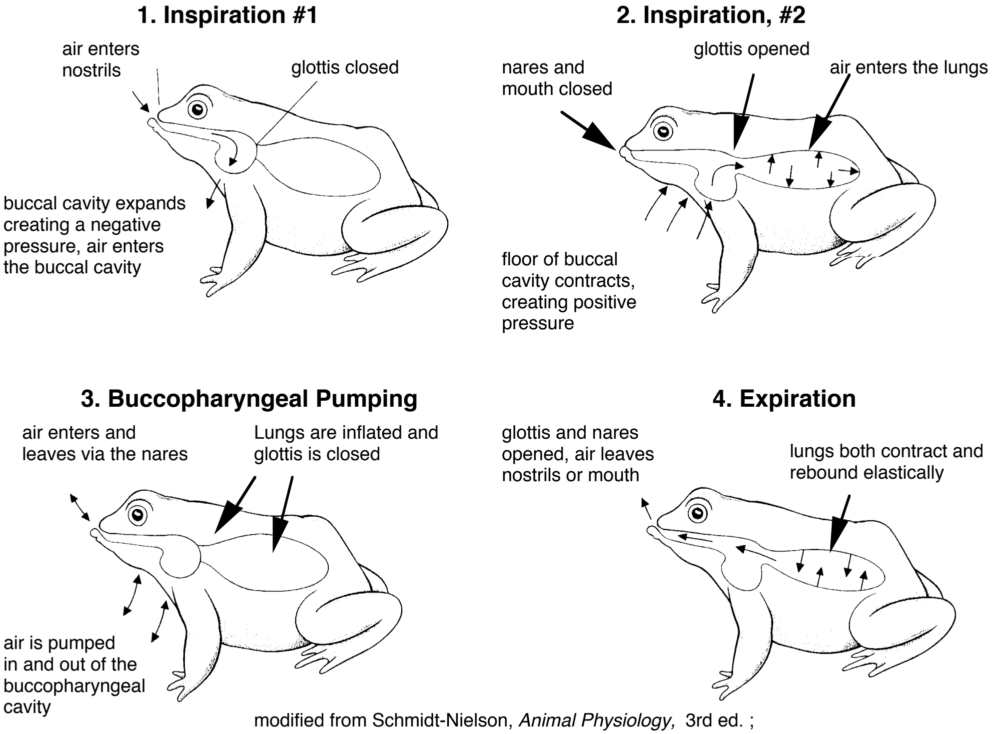
GENERAL NOTES ON THE OPERATION OF VERTEBRATE LUNGS:

A. Most vertebrate lungs operate on a **TIDAL PRINCIPAL** -- air is drawn in and out of the lung, like a wave on a beach

1. The forces required to move the air into the lungs can be either due to **"NEGATIVE" (SUBATMOSPHERIC**) pressures produced by a diaphragm (as in mammals), or more commonly, due to **POSITIVE** pressures produced in the pharyngeal region:

a. In "**POSITIVE PRESSURE**" breathers, air is first "swallowed" when the nostrils of mouth is opened and the floor of the throat (pharynx) is lowered while the glottis is closed. This creates a low pressure in the throat and air flows in.

b. Next, the nostrils are closed, the glottis is opened and the floor of the pharynx moves upward, creating a positive pressure that forces air into the lungs.



Breathing In Frogs

modified from Schmidt-Nielson, *Animal Physiology,* 3rd ed. ;
reference: C. Gans, Evolution 24:723-734, 1970

2. **Expiration** can be either **passive** or **active**. In mammals, it is usually passive and as we will see simply involves relaxation of the diaphragm and elastic recoil of the tissues in the thorax. In positive pressure ventilators, the air is expelled via the elasticity of the lungs.

a. **Active expulsion** of air occurs when muscles are involved.

1. In mammals and many other air-breathing vertebrates, one important means of forced expiration is to contract the abdominal wall muscles and thereby force the viscera against the lungs, causing them to empty.

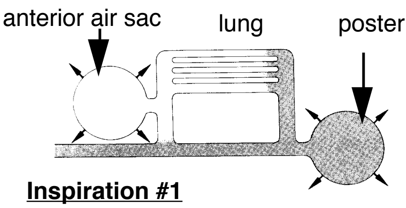
2. In many positive pressure breathers, air is also expelled by contractions of muscle fibers that are located in the lungs.

b. Active expiration is very important during exercise and also during certain specialized activities such as in production of sounds.

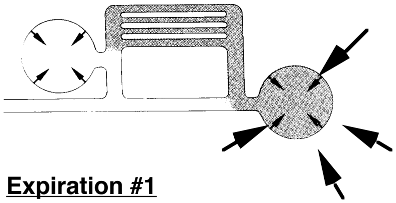
B. Birds are a special case to themselves. Unlike other vertebrates, they do not use a tidal lung. Instead, all birds use a **FLOW THROUGH LUNG**. Gas continuously flows through the lung in one direction.

1. In this system, the gas tensions remain very constant.
2. Flow of gas through the lung continues even when the bird is inspiring air. This is made possible by a series of **AIR SACS** that store much of the inspired air and then route it through the lung continuously.
3. The principal advantage of this system is that very high rates of O_2 exchange are possible -- much higher rates than are found in other vertebrates. These are very important in any animal that flies (and therefore has a high O_2 demand) and that also commonly spends time at very extreme altitude.

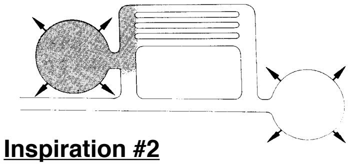
Unidirectional AIr Flow in Bird Lungs



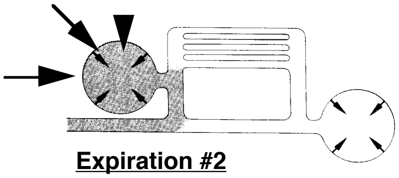
Inspiration #1
1. Anterior air sac expands, pulling old air from lungs and also pulling some air from outside into lungs
2. Posterior air sac expands pulling in air from trachea.



Expiration #1
3. Contraction of anterior and posterior air sacs. This forces old air out of the cranial sac and expells it from the animal. It also forces air from the posterior sac into the lung.



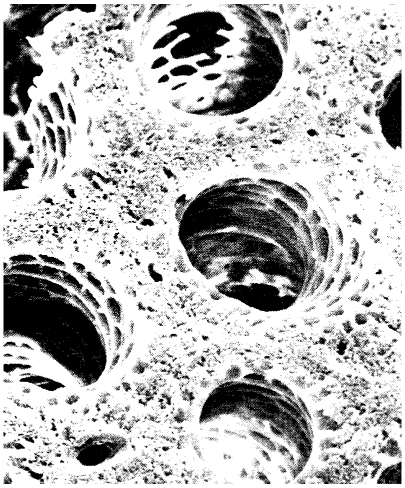
Inspiration #2
4. Expansion of the anterior sac draws air from the posterior sac through the lungs.
5. Posterior sac expands draws in air from trachea.



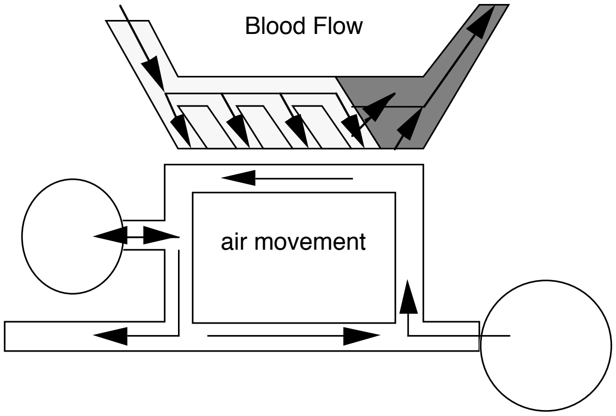
Expiration #2
6. Contraction of posterior sac pushes air forward through the lungs, anterior sac expansion pushes air out of the lungs.

Breathing birds require two respiratory cycles to move a single volume of air (shown in black) through the system; note that each cycle is identical to the next. By contrast, most animals need only one cycle to move air through lungs. The important thing to note here is that the flow is **unidirectional** -- this allows for a greater extraction of oxygen than is possible in a tidal (**bi-directional**) lung. The reason for this is that something akin to a counter-current exchanger operates -- in the case of the bird lung it called a cross-current exchanger and will be covered below. (Diagrams modified slightly from Schmidt-Nielson, *Animal Physiology*, 4th ed. -- reference Bretz and Schmidt-Nielson, 1972, J Exp Biol. 56: 57-65)

Avian Lung Structure and Cross-Current Exchange



A micrograph of the **parabronchii** (exchange areas) of the avian lung. Air flows trhough these tubes while blood flows rougly perpendicular to each parabronchus (see diagram at right).



Cross Current Exchange: Unidirectional movement of air through the lung while the blood moves at an angle to the air. The key thing is that some of the blood is only exposed to relatively high P_{O2} air (vessels to the right) while other blood is only exposed to relatively low P_{O2} air (to the left). The blood from each of these sources then mixes. The result is a much better extraction than in a concurrent exchanger or in alveolar lung, but less than in a counter-current lung.
(reference: Scheid and Piper, 1972, Respir Physiol. 16:304-312; figure after Schmidt-Nielsen, *Animal Physiology*, 4th ed. Cambridge.)