IONIC EQUILIBRIA AND THE RESTING POTENTIAL[1]

I. Introduction:

A. **Bioelectricity**: static or moving electrical charges created and controlled by living cells. Bioelectrical phenomena are found in all cells, however, nerve and muscle cells are especially adept at generating bioelectrical potentials (voltages).

Biopotentials: All living cells are found to exhibit some degree of **TRANSMEMBRANE POTENTIAL**; that is a voltage difference across the plasma membrane of the cell. In addition to the plasma membrane, these potentials are found across many other membranes in a cell, one good example is across the inner mitochondrial membrane. However, we will usually deal only with the so-called **Cell (transmembrane) membrane potential** that exists across the plasma membrane.

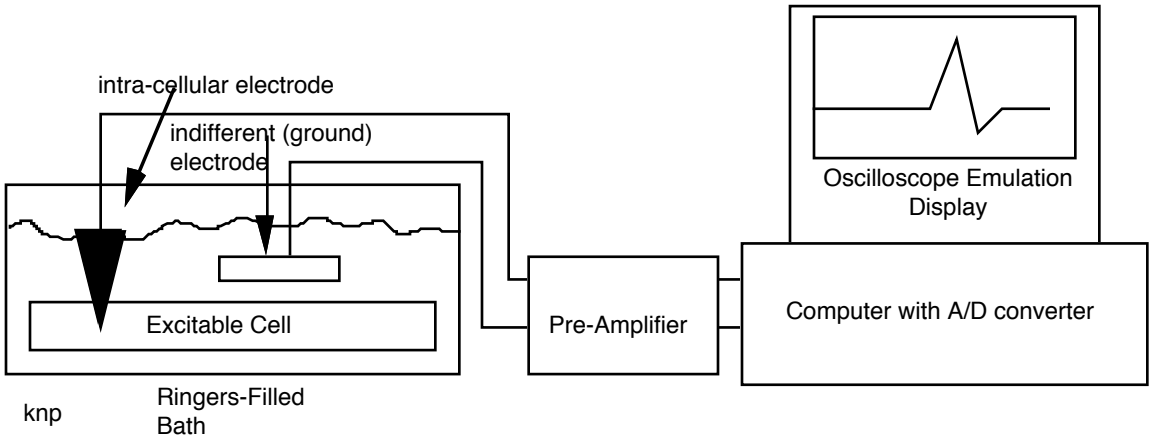
B. **Measurement of Cell Potentials**: Cell potentials are measured by comparing the electrical potential difference across the cell membrane. Two **ELECTRODES** are used:

1. the **INDIFFERENT (REFERENCE) ELECTRODE** which is placed somewhere outside of the cell in electrical contact with it (such as in the fluid bathing the cell) and
2. the **RECORDING ELECTRODE** which is placed inside the cell. The recording electrode is typically made of a piece of hollow glass drawn to a very, very fine, opened tip. This electrode is filled with an ionic solution (such as KCl) to act as a conductor.

C. **Electronics of the measurement of Transmembrane Potentials**: Since the potentials across a cell are small, (usually less than 0.1 volt i.e., 100 mV), the electrodes are hooked up to two amplifiers in series with each other:

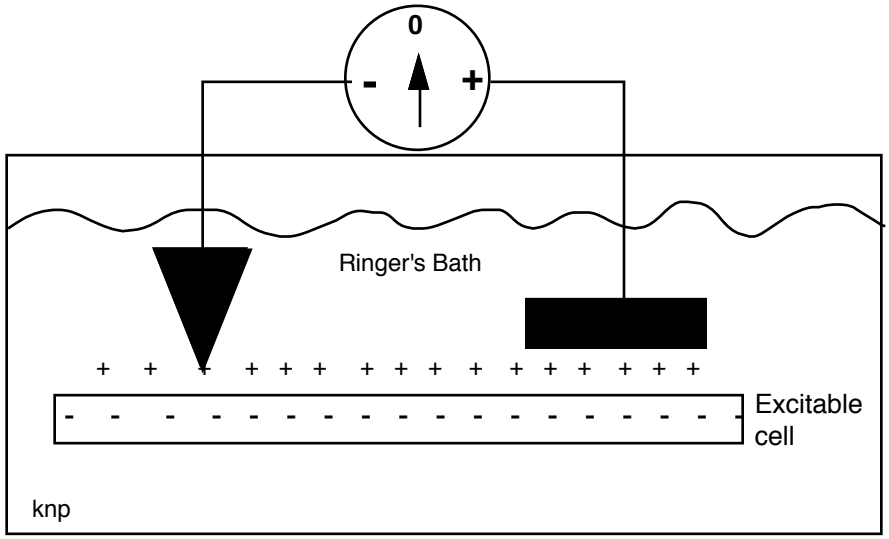
1. The first is called the **PREAMPLIFIER** and
2. the second (which is often built into the recording device) is called the **AMPLIFIER** (Realize that both are amplifiers).

The measuring device is any instrument that can act as a **voltmeter**: a simple voltmeter will do for resting potentials but in situations where voltage will change rapidly, a device that is capable of rapid response to voltage change is needed. This is done by using a **COMPUTER THAT EMULATES (= acts like) AN OSCILLOSCOPE**.



[1] Copyright ©2015 by K.N. Prestwich, Department of Biology, College of the Holy Cross, Worcester, MA 01610 USA, kprestwi@holycross.edu

3. Note that no potential is measured until the electrode is placed in the cell. Voltage of course is a measure of the difference in electrical force (voltage is more properly called ELECTROMOTIVE FORCE) between two points. When BOTH the electrodes are outside of the cell, there is obviously no (or very, very little) potential difference between them since they are in identical electrical environments:



D. Brief Preview of the Factors Responsible for Bioelectricity:

1. **Unequal concentrations of ions across a membrane**.

2. **Differential permeability of the membrane to the ions**; this can change in response to certain conditions.

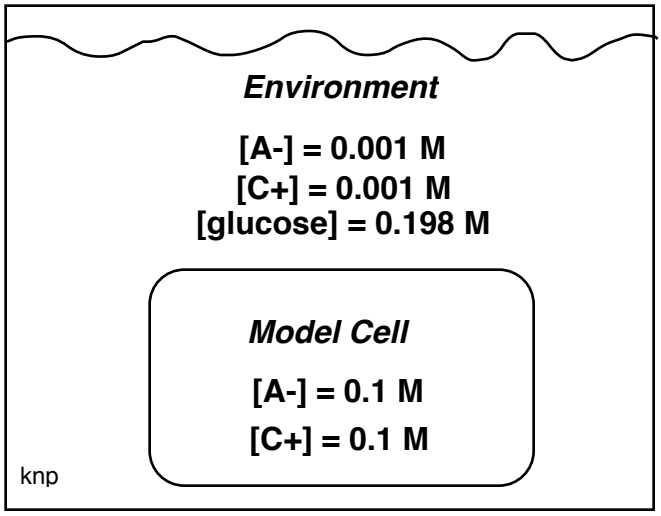
3. **Energy-requiring processes within the membrane**.

(NEXT PAGE, PLEASE)

II. THE PHYSICAL-CHEMICAL BASES OF BIO-POTENTIALS

A. The distribution of ions is the basis for all bio-potentials. Therefore we will study how their distribution determines the potentials of model systems before examining cells since the model systems are simpler and can be used to make predictions as to what is happening in the much more complex cells.

B. THE MODEL SYSTEM: the <u>initial</u> state



REQUIREMENTS FOR THE MODEL SYSTEM:

1. **NO OSMOTIC EFFECTS**: the total osmolarities of the solutions inside and in the environment are equal. Thus, there are no osmotic effects associated with this system. We will also refer to these sides (inside and out) as **COMPARTMENTS**
where a **compartment is a volume separated from other volumes by the membrane**.

? What is osmolarity? What does it affect? If a solution of NaCl is 0.2M, what is its osmolarity? How about a glucose solution of 0.2M? Or 0.2 M $MgCl_2$?

2. **<u>ESSENTIAL</u> ELECTRONEUTRALITY WITHIN A COMPARTMENT**: note that the number of (+) and (-) ions are equal within either compartment. **<u>We will do a calculation later showing that there is a very slight inequality associated with the charge across the membrane.</u>**

3. The **membrane is permeable only to the cation (C^+)**. Thus, neither the glucose nor the anion (A^-) can pass the membrane. We say that the membrane has **<u>DIFFERENTIAL PERMEABILITY</u>** with respect to the relevant ions; i.e., it is **semipermeable.**

C. WORK EQUATIONS RELATING TO IONS:

1. Any time an ion (or any other solute) exists in two different concentrations the potential for diffusion exists.

a. The movement of solute down a concentration gradient constitutes work (a force acting through distance).

b. If a concentration difference exists between two solutions and an avenue to allow diffusion (the membrane) also exists, then the POTENTIAL to do work also exists. For any ion (such as $C^{+)}$ this **DIFFUSION POTENTIAL ENERGY, W_d** (in J/mol) is:

1. $W_d = - n * R * T * \ln \frac{[C^+_{side\ 1}]}{[C^+_{side\ 2}]}$

where: **n** is the number of mols of solute that
actually diffuse,
R is the gas constant (8.314 J/(kelvins * mol))
T is absolute temperature in kelvins and the
Concentrations are in mols/L.

Note that as would be expected if the concentration ratio or/and T increases the potential energy of this system goes up since the forces responsible for the diffusion (thermal and concentration induced collisions) go up.

Notice that the sign of this work function, W_d, is given relative to whichever compartment's concentration is described in the numerator. If the other compartment (given in the denominator) is more concentrated than that given in the numerator, the value of W_d will reverse.

2. WHEN IONS ARE INVOLVED (as with our model), there is a potential for electrical work (W_e) if a separation of (+) and (-) charges has occurred between the two compartments:

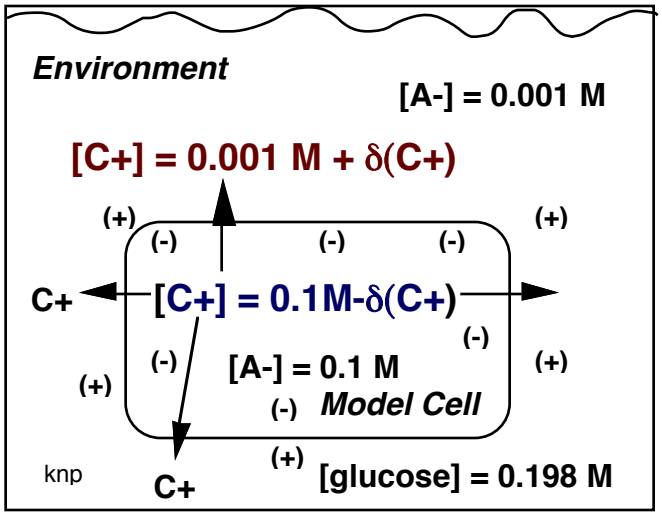
2. Work = (total # of charges moved) * electrical potential

3.. $W_e = - (n * z * F) * E$

where
W_e is the electrical potential
energy in J/(mol of charge);
n is the number of mols of charge separated;
z is the charge on each ion and the sign; ex: for calcium this is +2;
F is Faraday's constant (the number of units of (static) charge per mol of charge) it is equal to **96,500 coulombs/mol**; and
E is the electrical potential difference between the two compartments (think of it as being akin to force) in volts (V)

D. IONIC EQUILIBRIUM IN A SIMPLE SYSTEM

1. Again using the model system shown above.
2. At the start:
   a. $[C^+] = [A^-]$ within each compartment.
   b. There is a concentration difference for both of these ions across the membrane.
   c. On neither side is there a separation of charges and neither side has an excess number of (+) or (-) charges.
3. Flow of C^+ from inside to environment will begin immediately. (A^- and glucose are prevented from flowing by the membrane):



a. This flow is the consequence of diffusion as given by eq. 1, above.

b. **However, as soon as the first C^+ ions the inside for the environment, there is a relative deficiency of C^+ inside relative to the number of A^- ions on that side; likewise there is an excess of C^+ ions in the environmental compartment compared to the number of A^- ions there**.

c. **As a result, a separation of charges has developed.** It can be recorded as a voltage (potential for electrical work) across the membrane. The inside is now **ELECTRICALLY NEGATIVE** relative to the outside.

? Is there still a diffusion gradient favoring movement of C^+ from inside to outside?

d. Note that the longer flow occurs and the more C^+ ions that move inside to outside, the greater the electrical potential that exists between the two compartments (since there is more and more charge separation between the two compartments).

e. Since the outside is becoming more and more positive relative to side #1, we can also say that the inside is becoming more negative.

1. This favors the movement of the C^+ ions back inside and also "discourages" C^+ ions from leaving since there is now a net imbalance in favor of the negative anion, A^-, on this side.

2. Besides being attracted by the negative charge on the inside, C^+ ions are also being increasingly repelled by the increasing positive charge on the outer surface of the membrane.

! Notice that the "extra" + and - charges will mainly accumulate in the area just next to the membrane.
Why?

3. **Eventually an equilibrium is reached where there is no further ionic flow even though a diffusion gradient still exists. The reason is that an electrical gradient of the same magnitude exists in the opposite direction. Thus, according to simple diffusion, a C^+ ion would "like" to diffuse from outside to inside. However, an equally strong and oppositely directed electrical gradient prevents this from happening**.

(Actually, the description that I have just given is only true for the macro state -- on the micro level C^+ continues to move across the membrane but it does so at the same rate in both directions).

4. This equilibrium state is known as the **GIBBS-DONNAN EQUILIBRIUM**, usually simply known as the **DONNAN EQUILIBRIUM**. **It can be defined verbally as the state where, for some diffusible ionic species, the electrical and diffusive potential energy gradients are EQUAL AND OPPOSITE**.

Be Sure to Answer These Questions and Be Ready to Give Your Answers in Class

? Make a diagram using vectors for the (i) electrical (ii) diffusion and (iii) total force gradients with respect to C^+. Total force acting on a C+ ion is the sum of the electrical and diffusive forces. The direction of the vectors should indicate the direction of flow favored by diffusion or electrical forces and the net vector should indicate the overall force acting on an ion.

Suppose that you have a solution and "cell" like shown in the previous diagrams. You construct the cell (by filling a semipermeable bag with C^+ and A^- and then adding it to the solution of C, A and glucose as above. Qualitatively show the direction and size of these three gradients:

(a) the instant the "cell" is added to the solution

(b) a short period after

(c) when equilibrium is reached.

Describe what will happen if more C^+ and A^- are added to either side of the membrane. Make your description in terms of both diffusive and electrical gradients and in terms of equilibrium.

Use the same type of diagram you made above to illustrate what happens if more C^+ and A^- are added. Can these ions ever be added without other ions (i.e. how does one add C+)? Can these ions be added without an equal number of ions of the opposite charge?

E. THE NERNST EQUATION:

1. The Nernst equation is a useful expression that will allow us to find, **FOR A SYSTEM AT EQUILIBRIUM**:

a. the transmembrane voltage

or

b. the ratio of the concentrations of a diffusible ion in the two different compartments.

2. **Derivation of the Nernst equation:**

a. Remember that this equation is used only for a system that is at equilibrium. Recall that the Donnan equilibrium was defined as the situation where there are electrical and chemical gradients that are equal and opposite. Mathematically:

4. $W_d + W_e = 0$ or

5. $W_d = - W_e$

We can now substitute eqs. #1 and 3 into expression #5:

6. $$n * R * T * \ln \frac{[C^+]_{in}}{[C^+]_{out}} = - (n * Z * F * E)$$

and then solving for the potential, E, we find:

7. $$E = -\frac{R * T}{Z * F} * \ln \frac{[C+]in}{[C+]out}$$

This is the familiar Nernst equation. Note that many of the components of this equation are constants for a given ion and set of temperature conditions (R, T, z, and F). If for simplicity, we agree to usually use the equation for a standard temperature (20^{O} C is the one usually used), then we can lump all of these numbers into one constant. Before we do that, we will also make one other convention: since we most commonly deal experimentally with concentrations that differ by factors of 10, then we will also convert the calculation to $\log_{10}$ (which I will simply call log). Thus, our specialized Nernst equation becomes:

8. $$E = -58 * \log \frac{[C+]in}{[C+]out}$$

FOR 20^{O} C AND UNIVALENT IONS and where E is given in <u>MILLIVOLTS.</u>

TWO IMPORTANT NOTES:

i) BE SURE THAT YOU CAN CALCULATE THE CONSTANT -- COME TO CLASS WITH THIS CALCULATION DONE. Notice that we are using a rounded value that is easy to remember.

ii) The equation above GIVES THE VOLTAGE OF THE <u>INSIDE</u> RELATIVE TO THE OUTSIDE. NOTICE THAT, AS IN OUR EXAMPLE ABOVE, THE VOLTAGE ON THE INSIDE IS NEGATIVE RELATIVE TO OTHER SIDE IF $[C^+]_{IN} > [C^+]_{OUT}$. As we mentioned earlier, + ions have left the inside via diffusion making it more negative and likewise making the outside more positive. **BE CERTAIN THAT YOU UNDERSTAND THIS POINT OR YOU ARE A "LOST SOUL" AS FAR AS BIOELECTRICITY IS CONCERNED.**

F. Have our assumptions (given mid-page 3) held up?

1. There would appear to be a problem with rules #1 (equimolarity across the membrane) and #2 (electroneutrality within a compartment). Since some ions have obviously moved to set up the electrical potential between the two compartments, we have obviously violated these two assumptions.

? How big is the violation?
? Is it significant?

Or, phrased another way, how many ions really moved to set up the gradient? Most of us naively want to believe that it is a very significant number.

2. For the moment, I want you to accept at face value the fact that it is such a small number that it in no way affects the calculations of the Nernst equilibrium potential. You will have the chance to make the calculation yourself in two more classes, but first you need to learn about the concept of capacitance. Once you have learned that it will be possible for you to do a calculation showing that the number of ions that move in the

establishment of a Donnan equilibrium is about one out of 10,000,000 (1 in 10^7) that are present when for instance the potential is about 0.058V.

III. IONIC DISTRIBUTION AND RESTING POTENTIALS IN REAL CELLS

A. There are a large number of ions in the body, those of special importance to studies of bioelectricity are **K^+, Na^+, Cl^-, and proteins.** Ca^{2+} will also be important in some applications, especially in muscles. For the present, we will ignore Ca^{2+}.

1. **Proteins** are of interest because: (i) they are much more concentrated inside cells than outside and (ii) they cannot pass across the membrane (except under special circumstances that have nothing to do with the study of bioelectricity. Thus, they **are regarded as NON-DIFFUSIBLE**.

2. Proteins are all different in terms of their degree of charge, but the tendency for most is to be **NEGATIVELY CHARGED AT PHYSIOLOGICAL pH (near 7)**. The charged areas of proteins are principally on the side groups of the amino acids.

! (They are (-) charged at pH 7 since that pH is considerably above the pKa of most of COOH groups. Above the pKa, COOH groups exist mainly as COO-.)

B. The membrane of a resting cell has been found to be essentially impermeable to Na+ and proteins.

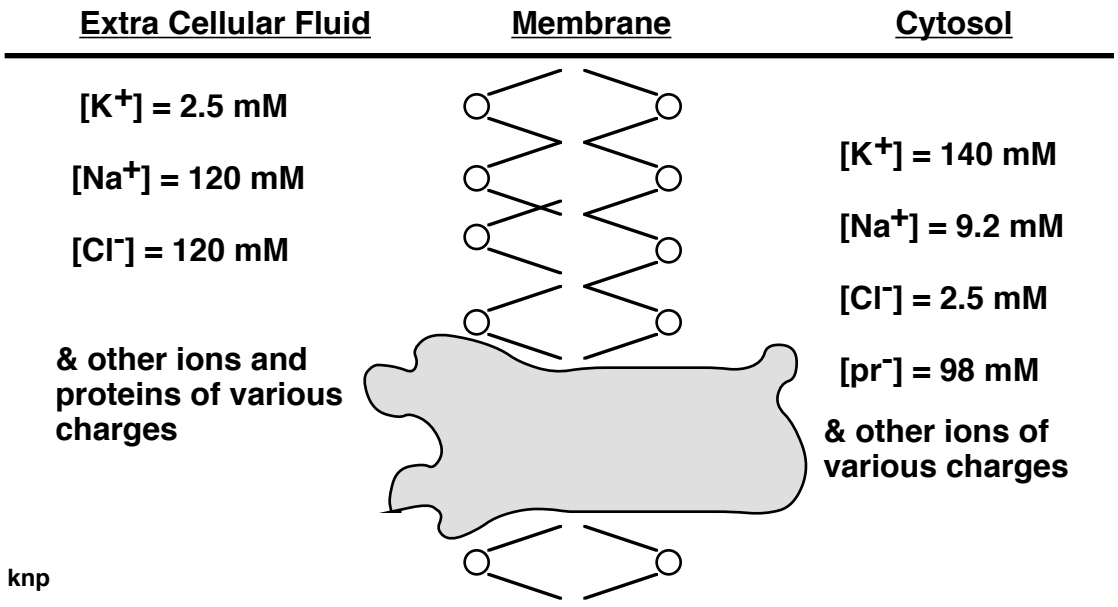
1. Both of these ions will be seen later to have important effects on the membrane potential. However, the effects are either indirect or transient. When the effects are indirect, they occur by influencing the distribution of other ions. More on this later.

2. **Since both pr- and Na^+ can be regarded as Non-diffusible, we will ignore them in our calculations of resting potentials.**

? WHY?

However, we will include them in our model because they do have an important effect on the distribution of diffusible ions due to their charge.

C. The normal distribution of ions across the membrane is such that we find the following:



(Please realize that this is just a simplified example -- cells are quite variable in terms of the ion species actually found!)

1. **Both K^+ and Cl^- can freely move down their ELECTRICAL OR DIFFUSION GRADIENTS since the membrane is permeable to them. Thus, in principle, either could be used to calculate E_m. More about this later.**

   a. In most cells, K^+ is more concentrated inside than outside while:

   b. Cl^- is normally more concentrated outside of the cell than inside.

2. Proteins are in higher concentration inside cells than outside. The net negative charge on the proteins is enough to discourage some of the K^+ from leaving and some of the Cl- from entering. Thus, the charge on intracellular proteins plays a role similar to that played by the non-diffusible A^- in our model system discussed above. Proteins also contribute an important osmotic effect to the cell.

? What would happen to all of the ions in the cell depicted above if we added more K^+ to the outside? What would happen to the electrical potential? What would happen if the pr^- was removed? What if Na^+ entered the cell?

Note: the overall picture of the relationships between different ions in a cell is a very complex and confusing topic. If you reflect on it for a moment, you will quickly see that what has been presented above is a great simplification. However, try to use this simplification for the time being and later we will try to look at the whole system with little more detail and understanding.

D. Derivation of the <u>DONNAN RULE</u>:

1. As mentioned earlier, it is possible to derive a mathematical expression from the Nernst equation that will allow us to:

a. determine whether or not a Gibbs-Donnan equilibrium exists

b. find the value of ionic concentrations needed at equilibrium (if equilibrium does not exist).

2. The derivation:

a. **ASSUMPTIONS**: both Cl^- and K^+ are free to diffuse down their electrochemical gradient. Thus, as mentioned above, either can be used to calculate the **TRANSMEMBRANE POTENTIAL, E_m**.

NOTE: Generally, when we calculate E_m we usually give the value in terms of the ions used to calculate it, for example E_{K+} or E_{Cl-}.

Since both of K^+ and Cl^- are univalent ions the appropriate versions of the Nernst eq. are:

9a. $$E_{K+} = -58 \log \frac{[K^+_{in}]}{[K^+_{out}]}$$

9b. $$E_{Cl-} = -58 \log \frac{[Cl^-_{out}]}{[Cl^-_{in}]}$$

Note that compartments used in the numerator and denominator are reversed for K^+ and Cl^-.

? WHY are compartments reversed?
What is another way to write each of these equations?

b. Since both E_{K+} and E_{Cl-} predict the same value for E_m at equilibrium, then we can set eq. 9a and 9b equal to each other.

$$-58 \log \frac{[Cl^-_{out}]}{[Cl^-_{in}]} = -58 \log \frac{[K^+_{in}]}{[K^+_{out}]}$$

solving:

10. $$\frac{[Cl^-_{out}]}{[Cl^-_{in}]} = \frac{[K^+_{in}]}{[K^+_{out}]}$$

or more generally:

11a. $$\frac{[anion_{out}]}{[anion_{in}]} = \frac{[cation_{in}]}{[cation_{out}]}$$

11b. $[A^-]_o * [C^+]_o = [A^-]_i * [C^+]_i$

This is the **Donnan rule** (also sometimes called the **Gibbs-Donnan equation**).

c. **Since we know that the expressions for the two ions are valid only at equilibrium, we can solve this equation to find whether a given set of pairs of C^+ and A^- distributions actually are in equilibrium. On substituting in the values for A^- and C^+, if both sides of the equation equal each other there is an equilibrium. If not, the system is not at equilibrium**.

? Do the handout problems dealing with the resting potential.
Describe what will happen to a cell system at equilibrium when either the voltage or the [ ] of an ion is changed.

IV. AN EXAMINATION OF HOW CLOSELY THE MEASURED DISTRIBUTIONS OF K^+ AND Cl^- FIT MEASURED E_m VALUES

A. It is possible to determine the internal and external concentrations of different ions and see if the values of E_m calculated from these concentrations actually agree with measured values of E_m.

1. The importance of this is to test our ideas about how E_m is determined based on our model system and the Nernst equation.

2. Any deviation from the predicted E_m will need to be explained.

B. A set of real ionic concentration data are contained in the figure given earlier in these notes. As a reminder, the values for Cl- and K^+ were:

ion	outside	inside
K^+	2.5 mM	140 mM
Cl^-	120 mM	2.5 mM

Obviously these are not going to give the same values for E_m.

The actual measured value of E_m is -97.5 mV.

? Calculate E_{K+} and E_{Cl-}. Given that the measured E_m is -97.5 mV what would you conclude? Is either ion very far from the predicted equilibrium distribution (as reflected by the voltage)?

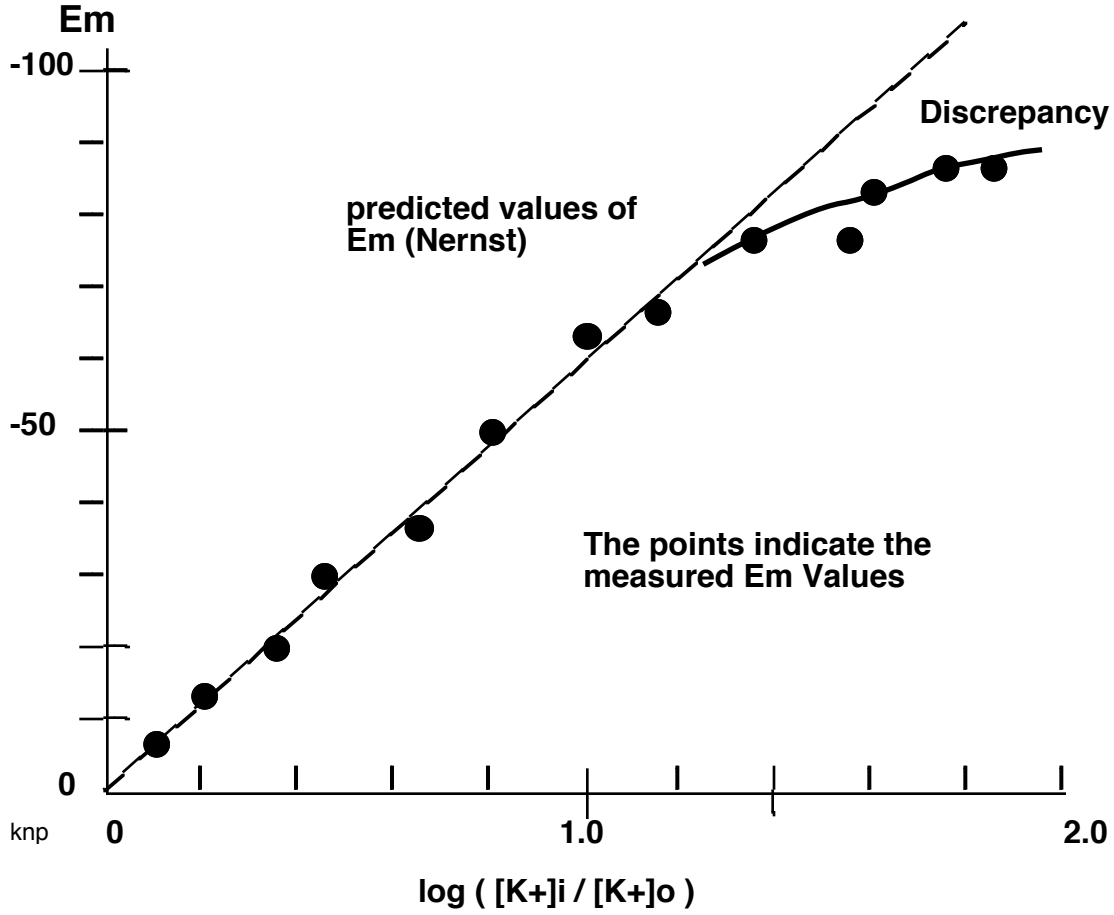
D. Now that we have settled the relative roles of K^+ and Cl^-, let's see how well the real data of E_m and $[K^+]$ conform to our predictions E_m based on the Nernst eq.

1. For our independent variable, we will plot the log $([K^+]_i/[K^+]_o)$ since this ratio is predictor of E_m.

2. For the dependent variable, we will use E_m. One plot will represent the E_m values predicted by various K^+ ratios (THE MODEL CURVE) and the other will represent the actual measured values (EXPERIMENTAL CURVE).

? Why is E_m the dependent variable?

3. The data for a squid axon are shown on the next page:



3. Note that the model fits the data very well until we get to very high K^+ ratios (that were obtained by making the external K^+ very low). At values starting near -60 mV, the deviation begins and it becomes more and more marked as the ratio is increased further, such that in a real squid axon, increases in the ratio do not cause further polarization of the membrane after -80 mV.

a. IT IS OBVIOUS THAT PASSIVE DISTRIBUTION OF K^+ IS NOT THE ONLY CAUSE OF THE E_m SINCE THE ACTUAL MEASURED E_m VALUES DO NOT ALWAYS FIT THE PREDICTED VALUES (E_{K^+}).

b. What is (are) the other causes?

1. So far, we have treated the membrane as if it were only permeable to K^+ and Cl^-. That was fine when you were a neophyte neurophysiologist, but, now that you are becoming sophisticated it is important to realize that the membrane is permeable to Na^+ also. It cannot exclude all Na^+, some will leak in. So it's best to think of the membrane as being **RELATIVELY**

IMPERMEABLE TO Na+ (WHEN COMPARED TO K^+ AND Cl^-), BUT NOT ABSOLUTELY IMPERMEABLE TO Na^+.

2. Go back to the diagram of the cell on the top of page 2. Given the distribution of Na^+, (i) Calculate the equilibrium Em would be if Na^+ alone controlled the E_m .
Be sure to use the correct version of the Nernst equation. You should get a value of (+) 62 mV.

? What do we mean by **EQUILIBRIUM POTENTIAL**?
Given the distribution of Na^+ ions given on page 9, which way would the Na^+ ions "like" to go?
Given the value of E_{K^+} (= E_m) which way would the Na^+ "like" to go?
If some Na^+ leaks into the cell, what effect will it have on the E_m ?

3. If Na^+ is able to enter the cell (and it is encouraged to do this by both electrical and diffusion gradients), it will bring with it a positive charge. The result is to make the inside of the cell less (-), that is, to move the potential towards 0. The term applied to moving the cell's potential in a positive direction is to **DEPOLARIZE THE CELL.**

a. Thus, if any Na^+ enters the cell it will affect the value of E_m .

1. The ease with which a given ion can enter a cell is a function of the **PERMEABILITY (P_{ion})** of the cell to that ion.

2. Permeability is normally defined in terms of concentration and it ignores any effects electrical attraction or repulsion may have on the ease of ion entry into a cell. More about this later.

-- Some Permeabilities of the Resting Squid Axon Membrane --

ion	Mass ($\frac{g}{mol}$), (approximate)	permeability [$\frac{mols}{(cm^2 * s * cm)}$]
K^+	39	0.00000100
Na^+	23	0.00000002
Cl^-	35	0.00000100

b. So, we can now explain the deviation of the experimental graph from expected at high $[K^+]_{in}/[K^+]_{out}$ ratios. The low rate of leakage of Na^+ starts to have a significant effect at these values since any Na^+ tends to depolarize the membrane. Further, the more negative the inside of the membrane becomes, the more the entry of Na^+ is favored and, so, for a given permeability, the greater the effect of Na^+. This is what we see in our model at E_m values more negative than -60 mV. As is usual, we will see that things are even more complicated than this since an ion pump is also involved. More on this at the end of the notes.

? Would you expect all cells to show identical curves of E_m vs. K^+ ratios? Why?

E. A BETTER MOUSETRAP: A MORE ACCURATE WAY TO PREDICT E_m

1. From the arguments above, it should be obvious that the E_m is determined by several ions.

2. A modified version of the Nernst Eq. exists that will take all ions into effect. It is called the **GOLDMAN-FIELD EQUATION** or for short the **GOLDMAN EQ.**

12. $$E_m = -58 * \log \{ \frac{P_{K+}[K^+]_i + P_{Cl-}[Cl^-]_o + P_{Na+}[Na^+]_i}{P_{K+}[K^+]_o + P_{Cl-}[Cl^-]_i + P_{Na+}[Na^+]_o} \}$$

3. Note that if you assume Na^+ permeability to be zero and if you choose to ignore Cl^- (since it follows K^+), the Goldman eq. reduces to the Nernst Equation.

? If we choose to leave both K^+ and Cl^-_{in}, compare the predicted E_m with the measured E_m. Use the ionic concentrations given on p.2 of these notes and the permeabilities given on page 10.

V. THE ROLE OF ACTIVE TRANSPORT IN THE MEMBRANE POTENTIAL

A. We'll start this section with a question: If membranes are permeable to Na^+ in any degree, what will happen to the E_m if there is no way to remove Na^+ from the cell?

Ans.: The cells will depolarize towards the Na^+ equilibrium potential. (WHY?)

B. We know that in fact cells remain polarized. Since we know that cells leak Na^+ and since it is impossible for Na^+ to spontaneously leave the cell (this would involve uphill movement against both a concentration and electrical gradient), we must postulate an active (energy-requiring) mechanism to remove the Na^+.

C. In fact, such a mechanism does exist, it is the **Na^+/K^+ ATPase (or pump).**

1. This pump uses energy contained in ATP to move Na^+ out of the cell and K^+ inward.

2. The ratio is for every 3 Na^+ removed, 2 K^+ enter (the two movements are tied together -- Na^+ cannot be moved without K^+ also being moved). Since unequal numbers of charges are moved the pump is said to be **ELECTROGENIC** -- it tends to create an electro-chemical gradient.

! In fact, Na^+pumps exist in a variety of electrogenic types and some are none electrogenic. The type depends on the type of cell and there is variation in different excitable cells, even of the same general type (e.g., neurons).

3. Even given the leakiness of membranes to Na^+, the measured E_m values do not deviate as much from the predicted values of the model as one would expect from calculations made by the Goldman equation. The reason for this smaller than predicted discrepancy is in large part due to the activity of the pump.

VITAL CONCEPT:

D. We have shown earlier that if:

1. The concentration of a diffusible ion is disturbed, or if

2. The value of E_m is changed artificially (by using an electrode) the cell will tend to resist the change. **The E_m on a moment-to-moment basis is determined by the Donnan Equilibrium**.

However, **the creation and long-term maintenance of this equilibrium is mainly due to the activity of the Na^+/K^+ pump.**

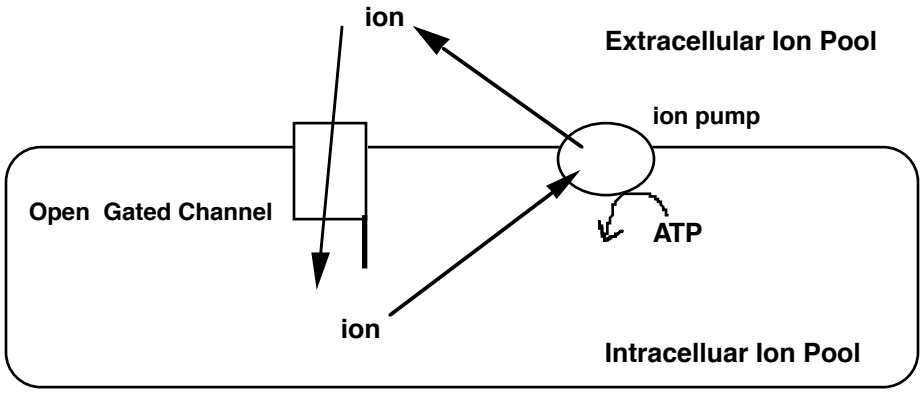
E. **OUABAIN** (pronounced wah-bain) is a drug derived from plants that INHIBITS the Na^+/K^+ ATPase. (Note is similar to a drug used to treat certain heart diseases called **DIGITALIS**. Together they belong to a class of drugs called **CARDIAC GLYCOSIDES**).

? What charge does the membrane tend to gain via the action of the pump?
If ouabain is added to the medium around a cell, what will happen eventually to the E_m? Explain.

F. In summary, we can see that the pump is vital to the existence of the E_m: without some mechanism to keep Na^+ out of the cell, very much lower (near 0) E_m values would exist. Thus, both the pump (and therefore Na^+) and the internal pr^- are vital in helping to determine (for the long run) the value of E_m.

? Try to explain in your own words the roles of passive and active processes in maintenance of the E_m.
Why do we commonly use K^+ only to calculate E_m ?

A final diagram: the role of the pump in establishing ionic and therefore electrical gradients and the role of channels in running them down. But never forget -- on a short term basis the pump can be turned off and except in the smallest of excitable cells, there will be no immediate effect on the cell's operation.



knp