BMEN E4001x: Quantitative Physiology I / Molecular and Cellular Systems

Notes 09 - Pump-leak model & resting potential

K&S Chapter 2

Some typical numbers for concentrations.

Ion	concentration (mM)		
	interstitial space (ext)	cell ("typical") (int)	V^{nernst} (mV)
Na^+,mammalian cell	145	15	+58
K^+, mammalian cell	4.5	120	-84
Cl^-, mammalian cell	116	20	-45
Na^+, squid giant axon	440	50	+54
K^+, squid giant axon	20	400	-75
Cl^-, squid giant axon	560	52	-59

$\Delta V = V_{\text{int}} - V_{ext} = V^{Nernst} \equiv -\frac{k_B T}{ne} \ln\left(\frac{c_{\text{int}}}{c_{ext}}\right)$;where n = valency. Also shown as z. In short, bottom term should be charge of an individual ion.

In the last lectures, we looked at the diffusion of a single species through a membrane, most likely through channels or carriers, and came up with the Nernst potential. Now, look at what underlies the situation in which we have multiple species. Clearly, something is wrong. First, with the defined internal and external concentrations, how can we have different "resting" potentials; our discussion until now suggests that resting is that flow through channels is zero. The system seems to be overspecified. Moreover, the resting potential of cells (-50 to -80 mV) is not near any of these. Cl^- and K^+ are about right, but not exact. Nernst potentials for Na^+ is way off; this is in fact referred to as the sodium anomaly.

In this section, we're going after a "resting" state of the cell, which, except as noted, will describe as a situation in which the cell may require energy to maintain, but the membrane voltage and ion concentrations both externally and internally are maintained. Note that in the GHK model, maintenance of ion concentrations is not guaranteed.

Gibbs-Donnan equilibrium

Important equilibrium state, based on the assumptions:

- Concentrations of each permeant species adjusts until V^{Nernst} is identical for all permeant species, and equal to the resting voltage.
    - Since the external concentration is held constant, internal concentrations should change to meet the Nernst potential
- Charged species add up to provide electroneutrality both inside and outside cell
    - This works for the external solution, but internally, there seems to be a problem.
    - Extra internal charge is provided by proteins and DNA inside cell, on the order of 125 mM excess single electron charge.
    - Electroneutrality can be violated at small scales, but not "macroscopic" ones.

Effectively, this pushes for:

$$\frac{[Na^+]_e}{[Na^+]_i} = \frac{[K^+]_e}{[K^+]_i} = \frac{[Cl^-]_i}{[Cl^-]_e}$$

$[Na^+]_e+[K^+]_e-[Cl^-]_e=0$; roughly done.

Mammalian cell: 145 mM (Na^+) + 4.5 mM (K^+) – 116 mM (Cl^-) = 34 mM excess positive.

$[Na^+]_i+[K^+]_i-[Cl^-]_i-125mM=0$; these conc. to be determined.

Solving for internal concentrations,
$[Na^+]_i$= 202 mM, $[K^+]_i$= 6.3 mM, $[Cl^-]_i$=83 mM, V_R=-9 mV

Clearly, these are not describing the "resting" cell properly.

Goldman-Hodgkin-Katz

We started from

$$j(x) = -D\frac{dC(x)}{dx} + D\frac{q\varepsilon}{k_B T}C(x)$$

and got to

$$j = \frac{D}{L}\frac{qV}{k_B T}\frac{C_i - C_e \exp\left(-\frac{qV}{k_B T}\right)}{1-\exp\left(-\frac{qV}{k_B T}\right)} = P\frac{qV}{k_B T}\frac{C_i - C_e \exp\left(-\frac{qV}{k_B T}\right)}{1-\exp\left(-\frac{qV}{k_B T}\right)}; P = \frac{D}{L}$$

Note that B&B also allows for partition coefficient in this equation, and applies this only for simple diffusion, so P=β*D/L

For a membrane permeable to several ions, the potential at which no *net* ionic flow happens is the Goldman-Hodgkin-Katz potential. Note that it is not no net flux of each ion species, but no net flow of charges.

Using the GHK electrodiffusion equations from last time, this has to come from:

$$V_R = -\frac{RT}{F}\ln\left(\frac{P_{Na}[Na]_i + P_K[K]_i + P_{Cl}[Cl]_e}{P_{Na}[Na]_e + P_K[K]_e + P_{Cl}[Cl]_i}\right)$$

Again, in the context of diffusion through a slab, P=βD/L. To approximately generalize to the case of channels/pores, anything that looks like a small, limited slabs of water connecting either side of the membrane, consider this to be a term expressing propensity for diffusing across this structure. Moreover, consider that this might be a term measured rather than calculated from knowledge of the structures and solutes.

For the moment, consider $P_K = 25\ P_{Na} = 2\ P_{Cl}$; $P_{Cl}=12.5\ P_{Na}$ This gives V_R=-56 mV. Pretty darn reasonable.

In concept, GHK will work within a simple pore or channel, any time we can consider transport *through* a defined slab, which is essentially what the pore is. Note that additional handling of how molecules get to the slab will be needed.

Pump-leak model

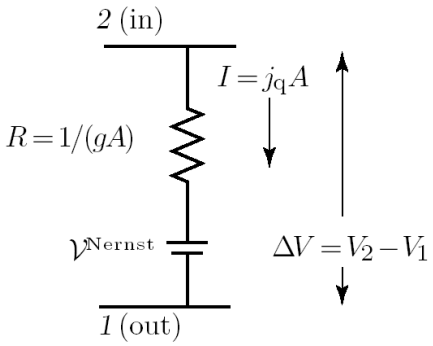
This last model suggests that "resting" is an interesting condition in which things are flowing. Cells are far from Donnan equilibrium. Now, something has to work to keep the gradients up; after all, there are net fluxes of ions!

As discussed earlier, the Na/K pump is a main source for these gradients. A short history is below, but more important that we get to the pump-leak model, which combines electrodiffusion with pumps.

- 1951: Ussing and Zehran demonstrated pumping of Na ions by frog skin, even when $\Delta V=0$ and Na^+ conc. same on both sides. Na^+ must be actively pumped.
- 1955: Hodgkin and Keynes showed that K^+ is also pumped, and is needed to get Na^+ transport. Metabolic inhibitors interrupted Na/K pumping, so energy is needed.
- 1957: Skou isolated a Na/K transporter, an active transporter, sodium pump. Subsequent studies showed that the flux of K^+ was 2/3 that of Na^+

Leak - Ohmic hypothesis:

Model electro-driven transport through a small patch of membrane, with pores/channels but no carriers or active transportes, as the following:



That is, given a Nernst potential dictated by the differences in concentration and an actual, MEASURED difference in voltage, the flux through the membrane is linearly related to the difference in voltage.

$$j_{q,i}^{\text{ohmic}} = z_i q j_i^{\text{ohmic}} = \left(\Delta V - V^{\text{Nernst}}\right) g_i$$

$j_{q,i}$=charge flux of species i
j_i=concentration flux of species i
z_i=number of charges for species
q=fundamental charge of electron
g_i=conductance per area; squid axon: 5 $m^{-2}\Omega^{-1}$

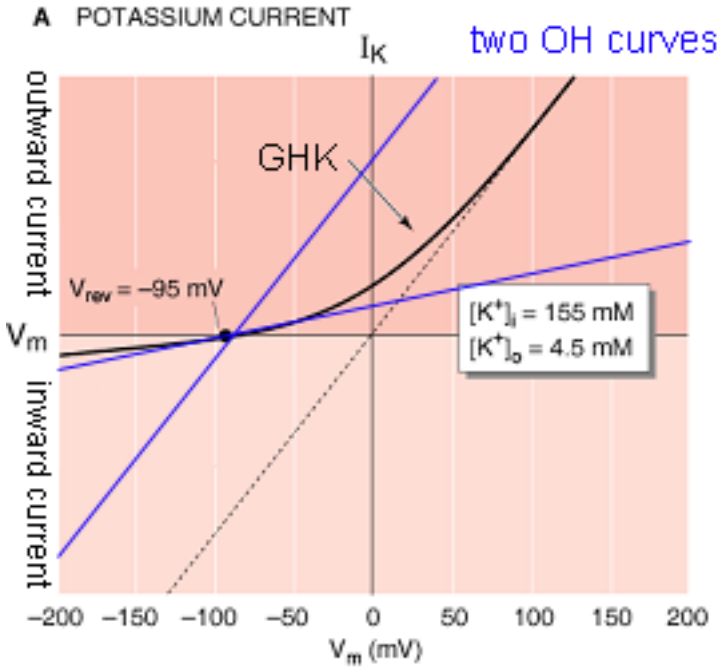
Notes:

- This is our first casting of the cell membrane as an electrical circuit, but our second encounter as a analog of chemical diffusion. More to come.
- Outward flow of positive ions is a positive flux; ΔV greater than V^{Nernst}; i.e., ΔV not as negative as predicted, so more positive charges can flow out.
- g's are a similar idea to P's in GHK, simply a proportionality constant. These are not interchangeable, though.
- g's are measured under very specific conditions, including membrane potential and ion concentrations.
- **g's will always be in reference to electric flow, thus siemens per area.**
- **In contrast, j's can be either molecular flux or current, depending on the situation. Include the charge per molecule as appropriate.**

- What are the underlying assumptions to get a linear g? These operate in a non-saturating configuration. Also small differences in voltage. In short, this is linear theory. How does this compare to GHK electrodiffusion?
- Different ions have different g's. Those that are impermeant are important in maintaining electroneutrality. In particular:

$$g_{K^+} \approx 25 g_{Na^+} \approx 2 g_{Cl^-}$$

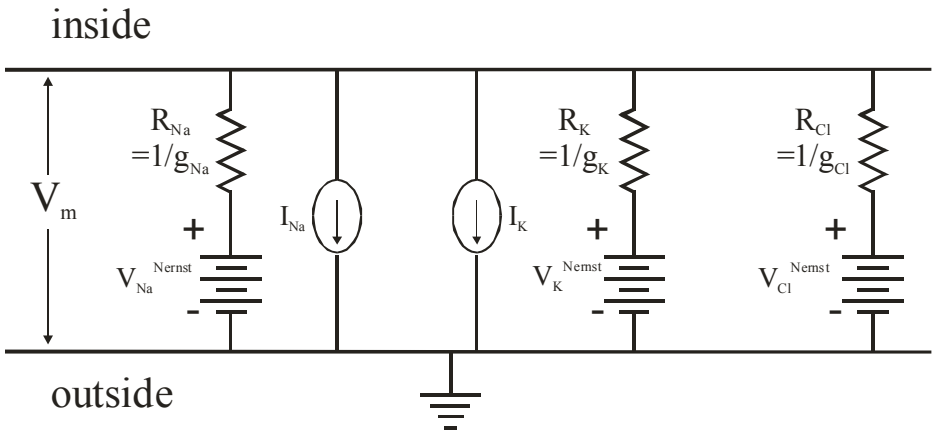
  This is why we looked at KCl rather than NaCl in bioelectric potentials.
- Comparing ohmic hypothesis and GHK:



  - Both approaches combine chemical and electromotive gradients
  - Both approaches yield no net flux when membrane potential is at V^{Nernst}
  - However, Ohmic is linear from that point, while GHK is more complicated.
  - How well Ohmic and GHK agree depends on how g is measured
  - Remember that both are approximations, anyway....

Pump-leak

Now, apply the leak model for each of the three species, Na^+, K^+, Cl^-. Also, incorporate the presence of an Na/K pump.



We're going to follow the concentrations of the three species separately, imposing the idea that net flux of EACH species is zero at steady state. Moreover, the membrane voltage across each one has to be equal. This can be represented in the following equivalent circuit, with the condition that to achieve steady state, we're going to impose restrictions not only on total current being zero (as was done for GHK, although GHK didn't include pumps) but the flux of each species needs to be zero.

pump-leak for sodium/potassium:

$$j_{Na^+} = \frac{g_{Na^+}}{q}\left(\Delta V - V_{Na^+}^{\ Nernst}\right) + j_{Na^+}^{pump} = j_{Na^+}^{ohmic} + j_{Na^+}^{pump}$$

$$j_{K^+} = \frac{g_{K^+}}{q}\left(\Delta V - V_{K^+}^{\ Nernst}\right) + j_{K^+}^{pump} = j_{K^+}^{ohmic} + j_{K^+}^{pump}$$

$$j_{Cl^-} = \frac{g_{Cl^-}}{q}\left(\Delta V - V_{Cl^-}^{\ Nernst}\right)$$

- Now, note the relation between pumped sodium and potassium fluxes:

$$j_{Na^+}^{pump} = -\frac{3}{2} j_{K^+}^{pump}$$

We're at steady state, so for EACH species, the ohmic and pump fluxes must also be equal, leading to:

$$-j_{Na^+}^{ohmic} = j_{Na^+}^{pump} = -\frac{3}{2} j_{K^+}^{pump} = \frac{3}{2} j_{K^+}^{ohmic}$$

In this model, Cl^-, while more permeant than Na^+, is not pumped, so the Nernst potential must govern.

Okay, taking the relations between ohmic flows, which will get us back to the Nernst and membrane potentials:

$$\left(\Delta V - V_{Na^+}^{Nernst}\right) g_{Na^+} = -\frac{3}{2}\left(\Delta V - V_{K^+}^{Nernst}\right) g_{K^+}$$

Which, when solved for ΔV, becomes:

$$\Delta V\left(2g_{Na^+} + 3g_{K^+}\right) = 3V_{K^+}^{Nernst} g_{K^+} + 2V_{Na^+}^{Nernst} g_{Na^+}$$

or.....

$$\Delta V = \frac{3V_{K^+}^{Nernst} g_{K^+} + 2V_{Na^+}^{Nernst} g_{Na^+}}{\left(2g_{Na^+} + 3g_{K^+}\right)}$$

Has this gotten us anywhere?

Going back to the original table, and assuming $g_{K^+} \approx 25 g_{Na^+}$,

- for the squid giant axon, predicted resting potential = -72 mV, close to that for K+
- for mammalian cell, predicted resting potential = -80 mV, again, close to that for K^+
- When conductance for an ion dominates, that ion will dominate determination of resting potential
- While pure GHK approach gave a better predictor of resting potential, pump action is critically important to cell function. We'll get to this in the next lecture. GHK is a good estimate of resting potential, though.

Before going further, some important relations to help relate the different notations you see for GHK electrodiffusion:

N_A= Avogadro's number = 6.023E23 molecules/mol

k_B = Boltzman constant = 1.38E-23 J/K

R = universal gas constant = 8.31 J/(gmol*K) = k_B*N_A

q or e = electron charge = 1.602E-19 C

F = Faraday's constant = 9.65E4 C/gmol = charge on a mole of electrons = q*N_A

With these two relations, note that:

$$-\frac{k_B T}{e = q} = -\frac{RT}{F}$$

At this point, I'm not going to straighten out the various notations, which are consistent within but different between B&B, K&S, and Nelson, best to be able to go between these.

Important notes, discussion

In the discussion of the ohmic hypothesis:

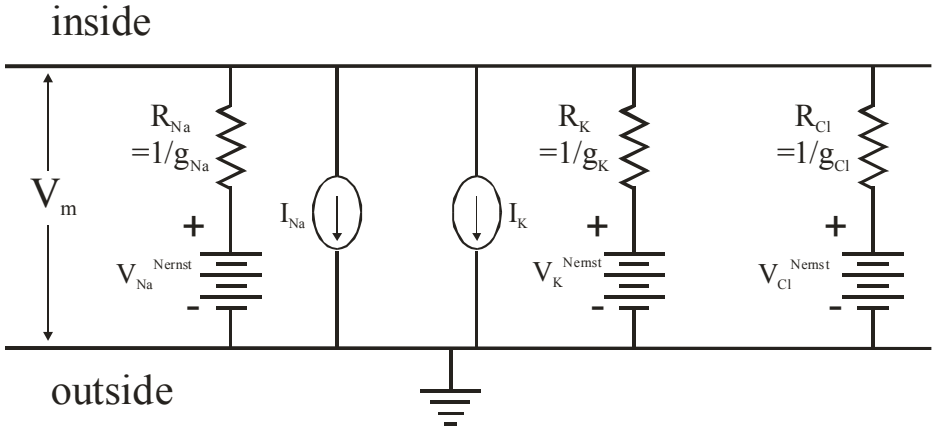
- g's will always be in reference to electric flow, thus siemens per area.
- In contrast, j's can be either molecular flux or current, depending on the situation. Include the charge per molecule as appropriate.

The three "resting" models:

- Gibbs-Donnan: accounts for a selectively-permeable membrane, and the presence of charges species that cannot cross the membrane. Nernst potential for all permeable species must be equal. Not good representation of resting state for many reasons, but captures important forces.
- Goldman-Hodgkin-Katz: Builds upon GHK electrodiffusion. For specified internal and external concentrations of permeable species, in conjunction with a potentially complex parameter P, the GHK potential is that for which total ion flux is zero. We've turned this around and specified that this voltage represents equilibrium. At this state, individual species may have net flux, so gradients can run down.
- Pump-leak: incorporates the source of concentration gradients. Builds upon GHK in that the net flux of each species must be zero. Importantly, the presence of pumps is explicity included. Also, our implementation uses the Ohmic hypothesis to simplify analysis.
- We will build upon the pump-leak system. The forces underlying Gibbs-Donnan equilibrium are important, and that concept will come back to us. GHK is important in that it gives a tractable, but more complicated, model of diffusive transport, but doesn't explicitly incorporate the pumps.

Electric circuit diagrams

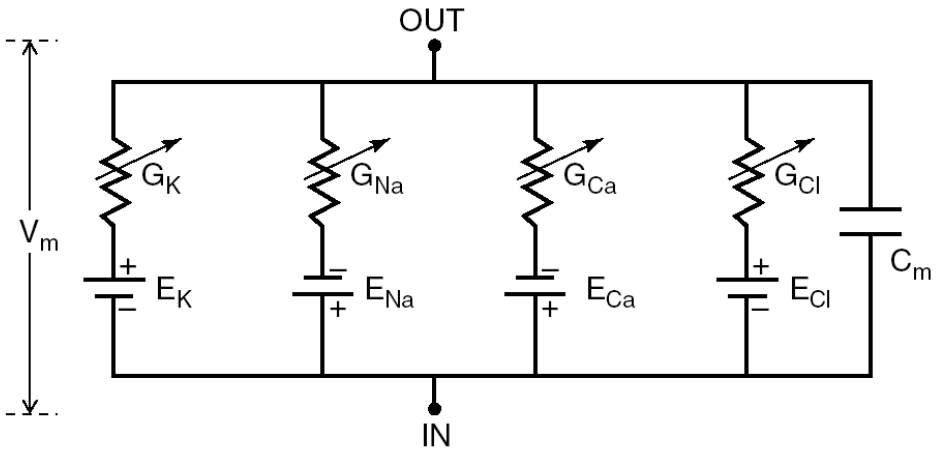
The representation of the Na/K/Cl pump-leak system was drawn schematically as:



Others draw it in an alternative form (this one from B&B). In this scheme note the following differences:

- inside & outside, vs. top & bottom of the drawing.
- direction of the Nernst potential sources
- the capacitor comes in next time

These diagrams are ultimately equivalent, but it is important to be able to orient yourself to the conventions being followed in a specific system.


OUT
V_m
G_K
G_{Na}
G_{Ca}
G_{Cl}
C_m
E_K
E_{Na}
E_{Ca}
E_{Cl}
IN