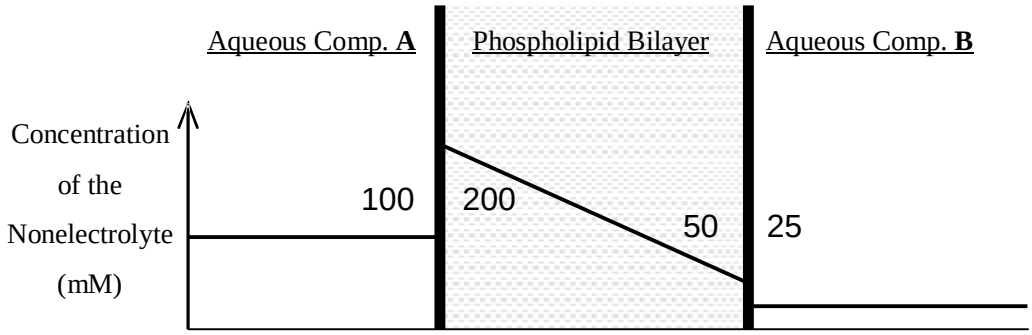
PID: 71034738

Pledge: I have not given or received aid on this exam. _____

Q1.

$b = \dfrac{\text{solubility of solute in lipid bilayer}}{\text{solubility of solute in water}}$, so the concentrations of the nonelectrolyte in the bilayer are $[\text{Non.}]_{\text{A-bilayer}} = 200$ mM, $[\text{Non.}]_{\text{B-bilayer}} = 50$ mM .

I draw the diagram below:

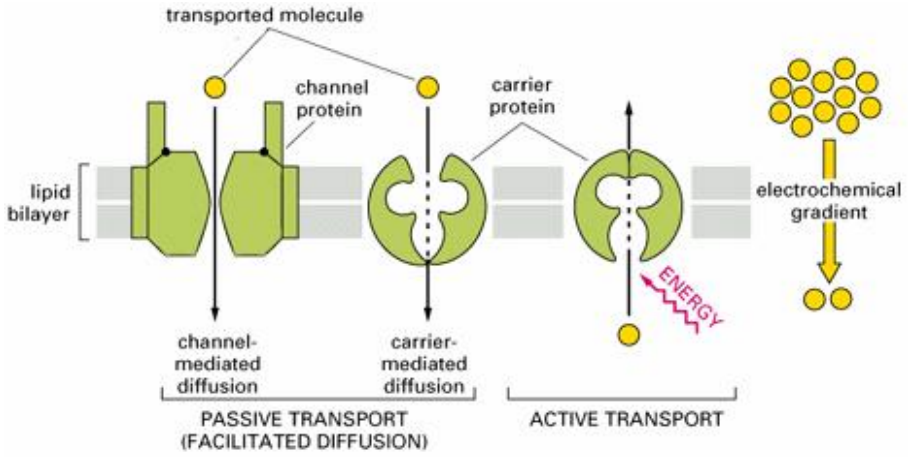


The light solid line shows the concentration profile of the nonelectrolyte in this system, and the specific concentrations are labeled in millimole. [The heavy solid lines represent the two layers of the phospholipid bilayer.]

Q2.

Passive transport: transport of a solute across a membrane down its concentration gradient or its electrochemical gradient, using only the energy stored in the gradient.

Active transport: movement of a molecule across a membrane or other barrier driven by energy other than that stored in the electrochemical gradient of the transported molecular.



[1]

This diagram from MOC4 compares the passive and active transports.

Similarities between the passive and active transports:

1. Both are protein complexes mediated in the transporting. These transports, constituted with different subunits, have both hydrophobic and hydrophilic parts.
2. D-glucose transport, a carrier-mediated passive transport, needs to alter the conformation during transporting. Glucose binds to the stereospecific site of the transport, and the binding intensity changes with different conformations. Na^+-K^+ pump, a primary active transport, has at least two subunits. And it also changes conformation during transporting, and Na^+/K^+ ions bind to specific sites on the complex.

Differences between the passive and active transports:

1. These two transports transport different substrates. One transports D-glucose, the other Na^+/K^+ ions.
2. Passive transport depends on the substrate's concentration or electrochemical gradient to transport substrate. Here, D-glucose transport follows the D-glucose concentration gradient to transport the D-glucose. Primary active transport is couple with the ATP hydrolysis (Na^+-K^+ pump *etc.*). And secondary active transport needs a preformed gradient (generally Na^+ or H^+), which is different from the substrate, to drive the transport. So active transports need "outside" energy to work.
3. Transporting by a passive transport is reversible, but an active transport usually follows a direction to transports the substrates.

[1] *MCOB4*, pp.618

4. Channel-mediated passive transports do not need to change the conformation greatly to transport the substrate, while active transports and carrier-mediated passive transports have to.
5. Different transports have different functions. Na^+-K^+ pump is used to control cell volume, provide electrochemical gradient for driving other transports, and establish the resting potential. D-glucose transport supplies the cell with glucose, while is the energy resource.

Q3.

In the derivation of the Nernst Equation, we do not assume that the membrane potential is negative inside. The equilibrium potential (internal potential minus external potential) of the ion in *marsi polaris* still can be calculated based on the Nernst Equation.

$$E_{Li^+} = -\frac{RT}{ZF}\ln\frac{[Li^+]_{in}}{[Li^+]_{out}} = -\frac{8.31\times300}{1\times9.65\times10^4}\ln\frac{10}{120}\,V \approx 64.1\,mV$$

$$E_{Cs^+} = -\frac{RT}{ZF}\ln\frac{[Cs^+]_{in}}{[Cs^+]_{out}} = -\frac{8.31\times300}{1\times9.65\times10^4}\ln\frac{140}{5}\,V \approx -85.9\,mV$$

$$E_{Br^-} = \frac{RT}{-ZF}\ln\frac{[Br^-]_{in}}{[Br^-]_{out}} = \frac{8.31\times300}{1\times9.65\times10^4}\ln\frac{120}{10}\,V \approx 64.1\,mV$$

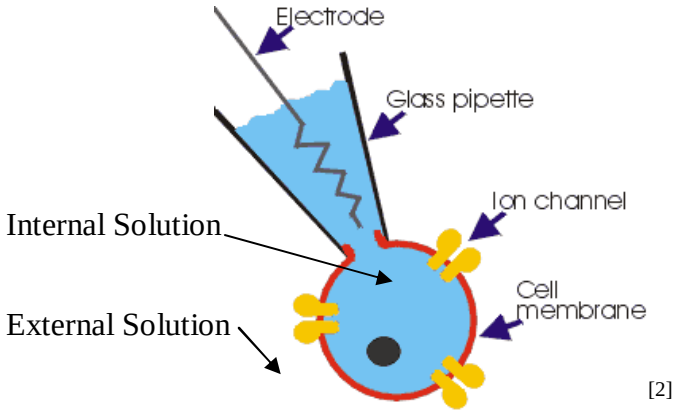
a. When the membrane is at its resting potential (E_r=+65 mV), Cs^+ is furthest from its equilibrium potential.

b. The equilibrium potential of Cs^+ is -85.9 mV.

Q4.

I read some papers and following their strategies design the methods to test the new protein is a Ca^{2+}-activated Cl^- channel or $2Na^+$:$1Cl^+$ coupled co-transporter, and the expected results are apart from related papers.

As the endogenous monovalent ion conductances are few, we could ignore the endogenous currents.



[2]

A diagram shows the whole cell current patch clamp.

4.1 Test dependence of current on intracellular [Ca^{2+}] (apart from Dalton S, 2003)

Methods:

Cells are washed twice with appropriate solution then resuspended and allowed to settle on a glass coverslip for 10 min; the unsettled cells were removed by washing the coverslip twice.

Recording is conducted by using a patch clamp amplifier. Pipettes are pulled from capillaries, and fire-polished to produce a tip resistance of 3-5 MΩ in the external solution. Internal solution (pipette solution) contains (in mM) KCl 145, TES 10, and EGTA 5 plus $CaCl_2$ 0.41, 1.14, 3.74, to yield free [Ca^{2+}] = 10, 150, and 300 nM. The external solution contains (in mM) NaCl 140, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1.2, TES 10, glucose 10, pH 7.4. The capacitive current was compensated.

After whole-cell configuration is achieved, the cells are held at -80 mV, and a series of voltage steps from -180 to +40 mV with a 20 mV increment are applied to the cell each 200-ms to measure the current. Currents are sampled and analyzed.

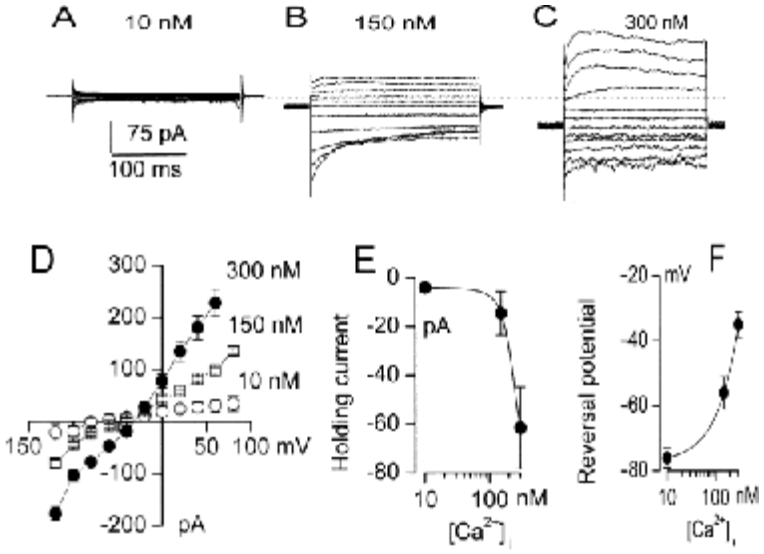
Expected Results:

Figs A, B, C are the recorded membrane currents, obtained with an internal solution containing 10, 150, and 300 nM free [Ca^{2+}] as indicated before. Figs D, E, F are the analysis parts of Fig A, B, C.

Fig D is the plot of current (mean ± SE) vs. membrane potential, measured during step pulses in cells studied with 10 nM (empty circles), 150 nM (empty squares), and 300 nM (filled circles) free [Ca^{2+}] in the internal solution. Fig E and F are plots of holding current

[2] www.iac-usnc.org/Methods/ wholecell/equipment.html

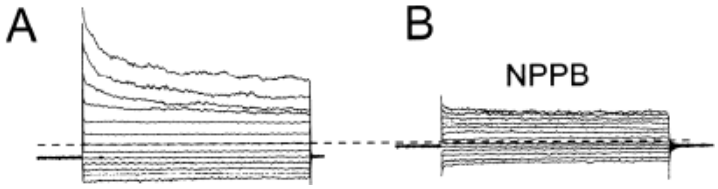
(E; mean ± SE) measured at the holding potential -80 mV, and plots of reversal potential (F) for the data in D.



If we get almost the same repeatable patterns of Dalton's, in which the currents become larger with increasing [Ca^{2+}], we could conclude that the new protein is a Ca^{2+}-activated Cl^- channel.

4.2 Test Cl^- channel specific blockers

Use the current results from the internal solution containing 150 nM free [Ca^{2+}] as control (Fig A). Add Cl^- channel specific blockers 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) to the internal solution containing 150 nM free [Ca^{2+}], and record the membrane currents by whole cell patch clamp (Fig B). If we see the repeatable decreases of currents after add of NPPB in the internal solution, we could conclude that the new protein is a Cl^- channel, not a $2Na^+$:$1Cl^+$ coupled co-transporter.



4.3 Analysis Na^+ and Cl^+ coupling ratios

The $2Na^+$:$1Cl^+$ coupled co-transporter is an absolutely novel transporter, and there is no related study reported before. I can not figure out detailed parameters, which need many real experiments to obtain, and just write down an analysis strategy here.

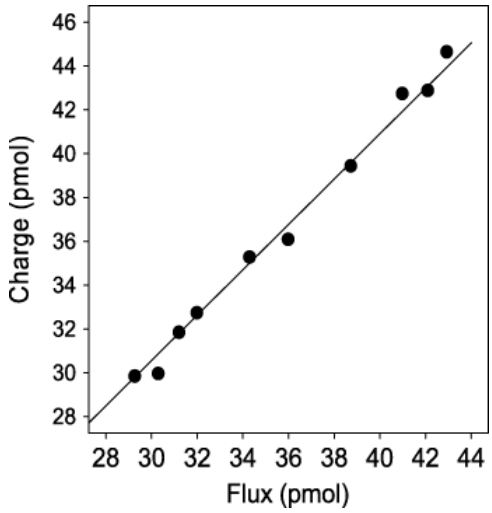
We use radiotracer transport-induced current measurements under whole cell

voltage-clamp conditions. Individual cells are placed in a perfusion chamber and clamped at an appropriate potential in external solution (free of Na^+ and Cl^-) for a while to measure baseline currents.

Then change the external solution with a medium containing radioactive Cl^- and current is measured for a while followed immediately by reperfusion with Na^+/Cl^--free medium until current returns to baseline.

The cell is recovered from the chamber and solubilized with SDS for liquid scintillation counting.

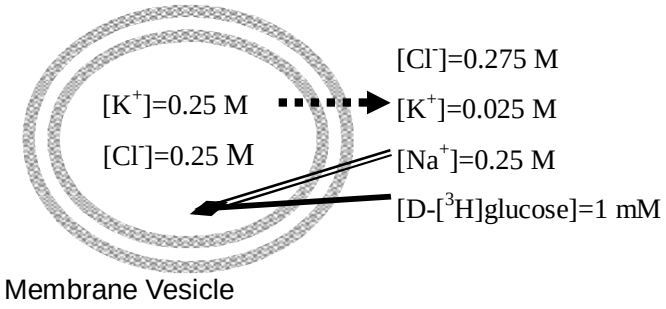
The total movement of charge across the membrane can be calculated from the current-time integral, and correlated with the measured radioactive Cl^- flux for each cell to calculate the charge:flux ratio. If the protein is a $2Na^+$:$1Cl^-$ coupled co-transporter, we should get a diagram just as the one below (The net charge should be the same as the radioactive Cl^- flux). If it is a Ca^{2+}-activated Cl^- channel, there will be no linear relationship between charge and flux.



Q5.

In Na^+-dependent D-glucose co-transporter system, incoming two Na^+ couples with one D-glucose. And the influx velocity, which is small, is determined by the character of the Na^+-dependent D-glucose co-transporter and the concentration gradient across the membranes. The Na^+ gradient enhances the uptake of the sugar (Murer, H., 1974), and the D-glucose also stimulates the flux of Na^+ (Beck, J.C., 1978). As we have constant relatively D-glucose concentration here, we do not have to think about the influence of D-glucose to the Na^+.

Valinomycin, a compound that binds metal ions and diffuses across membranes carrying the bound ion, transports potassium.



Here is a figure show the concentrations of different substrates in the membrane vesicle system. Na^+-dependent D-glucose co-transport stimulates the influx of (2 Na^+ + 1 D-glucose). In order to keep the overall electro neutrality, K^+ flows out passively following the concentration gradient of potassium.

Add of valinomycin into the system increases the discharge of K^+' following the K^+-gradient (vesicle > media), as valinomycin is an ionophore which specifically transports potassium. More outflow of K^+ makes the inside of the membrane vesicle more negative. The efflux of K^+ must be coupled to the flux of another ion because of the requirement of the overall electro neutrality. In this system, increase the influx of Na^+ is the only choice. As Na^+-dependent D-glucose co-transport couples the influx of Na^+ with [^{3}H]D-glucose. So we observed that the uptake of [^{3}H]D-glucose was greatly increased when valinomycin was added. As the normal velocity of Na^+/D-glucose influx is small, we found that the vesicle took up only small amount of [^{3}H]D-glucose before the addition of valinomycin.

And we could predict that after enough time, when the potassium concentrations across the membranes become equal, the uptake velocity of [^{3}H]D-glucose should drops down (Fig 2, Murer, H., 1974).

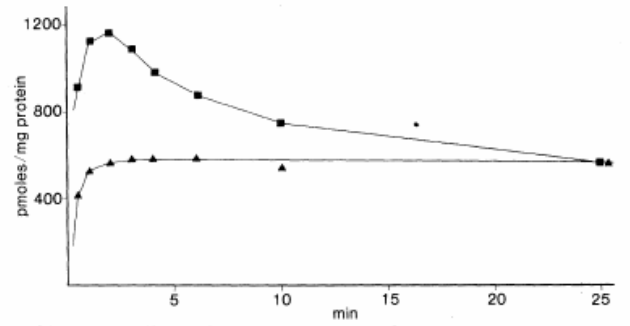


FIG. 2. Effect of valinomycin on the D-glucose uptake of K^+-preloaded vesicles: D-Glucose uptake in the presence of valinomycin (■——■) or in the absence of valinomycin (▲——▲).

REFERENCE:

Beak, J.C., and Sacktor, B. (1978) *J Biol. Chem.* 253, 7158-7162

Dalton S, Gerzanich V, Chen M, *etc*. (2003) *Glia.* 42, 325-339

Murer, H., and Hopfer, U. (1974) *Proc. Natl. Acad. Sci. U.S.A.* 71, 484-488