Membrane Function

1. The giant, single-cell alga *Nitella* lives in fresh water and is readily cultured in the laboratory. For this reason and also because of its large size and favorable optical properties, this alga has been a favorite object of study by cell biologists and cell physiologists.

Consider the following experiments and answer all of the following questions concerning the *Nitella* plasma membrane.

A. (5 pts) Why doesn't this alga swell osmotically and burst in its natural environment? How could you test the validity of your explanation?

B. (5 pts) Using the Fick equation, how would you determine the permeability coefficient of the *Nitella* plasma membrane to water?

C. (5 pts) If you place *Nitella* in a solution of 10% glycerol and observe it under a phase contrast microscope, you would note first its shrinkage followed by a gradual return to its normal shape. What is the simplest accurate explanation of these results?

D. (8 pts) Like other cells *Nitella* exhibits a membrane, or "resting", potential whereby its cytoplasm is negative with respect to its environment. The actual value of this potential (at 20°C) is -138 mV. How is this potential likely generated and how could you test your hypothesis? (Your reasoning must be explicit and detailed and all relevant calculations must be shown.) The intracellular and extracellular concentrations of the major inorganic ions, as well as log values for their concentration ratios, are indicated below.

<table>
<thead>
<tr>
<th>Location</th>
<th>(Concentration in mM)</th>
<th>Logs of Concentration Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td>cytoplasm</td>
<td>14.0</td>
<td>119.0</td>
</tr>
<tr>
<td>stream/culture</td>
<td>1.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The first three questions may be answered on the following page; the last question later. Remember! What useful information is provided by the question, what must you remember from text and reading, and what questions are (and are not) being asked?
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A.

B.

C.

Having answered the questions yourself, now consider the following answers others provided.

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A. Why doesn't this alga swell osmotically and burst in its natural environment? How could you test the validity of your explanation?

**ANSWER**

**Example 1.** Most likely, this organism has a number of "pumps" to take in solutes against their gradients, keeping the cell from hemolysing. There are a number of ways to test this hypothesis. One way would be to introduce an inhibitor that binds to the "pump" sites and restricts pumping of solutes (e.g., ouabain).

**Example 2.** Osmosis occurs only on the tail of another ion which is diffusing, as in binding to a polar molecule or ion (ex. Na⁺). If these concentrations are kept at a certain level in the cell, the water will not be admitted. To test this, change the concentration of the water carrier in or out of the cell and monitor the rate of osmosis.

**COMMENT**

Interesting answer and logical test, **but wrong!** Pumping in solute would make osmotic gradient steeper, causing more water to diffuse inwards by osmosis.

Principle is partially correct, but **very** unlikely that solute concentration in cytoplasm is equal to solute in fresh water.

It is unclear what test is showing.
Example 3. Due to high concentrations of intercellular ions and other substances the flow of H2O according to the activity gradient into the cell by osmosis, *Nitella* must have a system for regulating H2O flow. Diffusion is determined by polarity and molecular size. Although H2O is polar and therefore not readily admissible it is so small it usually passes through the membrane uninhibited. *Nitella* may have developed a membrane which is relatively impermeable to H2O. This solutions would prevent excess H2O from entering the cell. Another possible explanation is that *Nitella* has developed a pump system to remove excess H2O from its interior. The probable explanation contains both these elements.

One way to test this hypothesis is through an examination of *Nitella*’s permeability according to the Fick equation. A method to test the possibility of a system for removing excess H2O could be developed by labeling H2O within the cell by means of a dye and observing to see if this H2O is removed from the cell to the surrounding medium.

Good opening sentence but answer is very **wordy**.

Much of second and third sentences is irrelevant.

**Relatively** impermeable membrane would only affect rate of H2O entry, but cell would **eventually** swell and burst.

There are **no** known H2O pumps! Creative, but must first rule out simpler explanations.

**Excellent** test of weak (unlikely) hypothesis.
B. Using the Fick equation, how would you determine the permeability coefficient of the *Nitella* plasma membrane to water?

**ANSWER**

**Example 1.** Taking the Fick equation, I would test the rate of diffusion across the *Nitella* plasma membrane and compare it to the other substances of known permeability coefficients. By creating a solution where only water was moved across the membrane the rate of diffusion could be calculated comparing the rate of diffusion to other substances of known permeability coefficients the permeability coefficient of *Nitella* could be obtained.

**COMMENTARY**

Where's the Fick equation? Comparison of the permeability coefficient of H$_2$O with that of other substances irrelevant here.

How is the rate of diffusion to measured?

Weak answer!

**Example 2.** To determine the permeability, you would want to hemolyze cells. It would be calculated by dividing the change in the H$_2$O concentration by the time it took. Then plug in for [H$_2$O$_0$] and [H$_2$O$_i$]-this will give you K. It's really a pointless calculation because everything happens so fast that the permeability coefficient is huge and meaningless.

Where's the Fick equation?

Nitella won't "hemolyze"--see answer to Question A.

How is the concentration of H$_2$O measured?

What does it mean to talk about the concentration of a solvent?

Don’t “fight” the question! The student here doesn’t know enough to draw this conclusion.

**Example 3.** We can measure in laboratory the rate of diffusion of particles and we also can control the solute concentrations inside and outside of the cell. Therefore we can determine the coefficient of permeability.

Good start at an answer! Variables need to be related, with constants, to rate.

State the Fick equation!

All three answers suffer from the same weakness. If the questions requires the use of an equation to determine a solution, the equation *must* be used.
C. If you place *Nitella* in a solution of 10% glycerol and observe it under a phase contrast microscope, you would note first, an immediate shrinkage (of both the cell and its central vacuole) and then a gradual return to its normal shape. What is the simplest, accurate explanation of these results?

**ANSWER**

**Example 1.** *Nitella*, in its natural environment exists in a situation in which the outside environment contains much less solute than its interior. Therefore if it is immediately immersed in a 10% glycerol solution, it will atrophy due to loss of H₂O moving out of the cell according to the activity gradient--the higher concentration of solute outside this cell. Gradually the cell would adjust to the situation by regulation of its H₂O uptake and loss and regain the concentration of solute/H₂O which is necessary for its survival. This ability to regulate the flow of H₂O allows *Nitella* to exist in solutions of various concentrations.

**COMMENTARY**

Good answer so far! although “atrophy” is the wrong verb.

Vague—how does water regulation work and how would it affect outcome?

Last sentence interesting but irrelevant.

**Example 2.** Phase contrast gives good contrast observation so that substances are dark enough to see instead of the faintness you might get under bright field. However, what is lost is the little details (physically) that you would have been able to note under bright field. That may account for the shrinkage at first of the cell and vacuole. Perhaps it was losing detail in favor of a sharper, more focused albeit smaller image. The other explanation is that glycerol is an alcohol with a polar -OH end (from the electronegative oxygen). It is more difficult for polar molecules to pass through membranes so their rate of passage is considerably slower. So perhaps this "gradual return" could be attributed to the time it may take for the glycerol to move into *Nitella*.

**COMMENTARY**

First part of answer suggests initial change is an optical artefact. Accept the details provided at face value: **Don't side-step the question.**

Second part of answer changes tack and is essentially correct, but doesn't address initial shrinking (due to H₂O loss) in an explicit manner.

(Focus changes, resulting in an incomplete answer!)
Example 3. This would suggest that the glycerol is some what hyperosmotic solution which causes the slight crenation, however, the restoration of the cell’s shape and the fact that this is at room temperature indicate that a dynamic equilibrium has been restored.

How does crenation result from a hyperosmotic environment? What is a "dynamic equilibrium"? Answer is much too general. It may reflect complete understanding, but answer lacks sufficient detail.

D. Like other cells *Nitella* exhibits a membrane, or "resting", potential whereby its cytoplasm is negative with respect to its environment. The actual value of this potential (at 18° C) is -138 mV. How is this potential likely to be generated and how could you test your hypothesis? *(Your reasoning must be explicit and detailed and all relevant calculations must be shown.)* The intracellular and extracellular concentrations of the major inorganic ions, as well as log values for their concentration ratios, are indicated below.

<table>
<thead>
<tr>
<th>Location</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cl⁻</th>
<th>Logs</th>
</tr>
</thead>
<tbody>
<tr>
<td>cytoplasm</td>
<td>14.0</td>
<td>119.0</td>
<td>65.0</td>
<td>Naᵢ/Naₒ = 1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Naₒ/Naᵢ = -1.15</td>
</tr>
<tr>
<td>stream/culture</td>
<td>1.0</td>
<td>0.1</td>
<td>1.3</td>
<td>Kₒ/Kᵢ = -3.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kᵢ/Kₒ = 3.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clᵢ/Clₒ = 1.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clₒ/Clᵢ = -1.70</td>
</tr>
</tbody>
</table>

Answer the question below and then continue on to examine other answers.
**Example 1.** This resting potential is generated because ions are moving in and out of the cell. It is negative because ions like Na+ and K+ are leaving the making the cytoplasm negative. The log of Na outside/Na inside is negative so that is why the resting potential is negative. The hypothesis could be tested by changing the concentrations outside and inside and determining the change difference in the membrane potential.

**COMMENTARY**

Generally true answer, but lacking calculations, it’s much too vague.

Test is correct, but what exactly would results show?

**Example 2.** The resting potential is determined by the electrochemical gradient that is maintained by the cell. It takes into account the changed ions of Na+, K+, Cl-. The electrochemical gradient is determined by the Nerst equation: $v = \frac{RT}{ZF} \ln\left(\frac{[x]_0}{[x]_i}\right)$. In determining the resting potential the permeability of each ion must also be considered and factored into the equation.

To test the hypothesis to see if it is these ions that make up the resting potential, one could place the cell in a test solution that is higher in concentration of the ions than the cell. A voltage meter may be inserted into the cell and the solution, to see if the resting potential is changed.

**COMMENTARY**

Accurate answer but no calculations shown; therefore, answer is incomplete.

Good test, but . . .

. . . how specifically would voltage change under condition described?
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2. Like many other animal cells, erythrocytes change their shape and volume osmotically when placed in solutions that are not isotonic. For mammalian cells these changes are more or less permanent as long as the cells are kept in the non-isotonic media. Duck erythrocytes, however, gradually regain their original volume with prolonged incubation in many non-isotonic media. The following two-part experiment illustrates this behavior.

In part A, four populations of identical duck RBC's were suspended in an isotonic solution containing 130 mM NaCl, 10 mM KCl, 10 mM hydrogen ion buffer and 5 mM glucose. The volumes of these cells were then monitored for 8 min. Then, the tonicities of two of the four suspensions were changed: the cellular volumes in those suspensions immediately increased about 25%, as illustrated below. (The media surrounding the other two populations were not changed.) At the same time, ouabain was added to one of the treated and to one of the untreated populations. In part B of the experiment, the volumes of all four populations were monitored for an additional 90 minutes, as illustrated below.

Given these results and your knowledge of osmosis and the membrane biology of mammalian erythrocytes, answer all of the following questions

A. (4 pts) What was probably done to change the tonicities of the two suspensions?
B. (4 pts) What is the probable osmotic basis of the cells' immediate response in “A” to the change in tonicity?

C. (4 pts) What is ouabain and what do the ouabain data indicate?

D. (8 pts) Describe explicitly how the treated cells gradually regain their normal volume. Postulate one possible mechanism and explain clearly how it would work.

E. (5 pts) Describe one additional experiment, and the expected results, that would test the validity of your hypothesis.
3. Typically, the cytoplasm of most animal cells is rich in potassium and poor in sodium, a condition opposite to that found in the blood and extracellular fluids surrounding these cells. Examine closely the following set of data, obtained by precise analysis of the ionic composition of blood from 3 mammalian species.

<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Intracellular</th>
<th>Extracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td>1. Human</td>
<td>19</td>
<td>136</td>
<td>78</td>
</tr>
<tr>
<td>2. Dog</td>
<td>135</td>
<td>10</td>
<td>87</td>
</tr>
<tr>
<td>3. Cat</td>
<td>142</td>
<td>8</td>
<td>84</td>
</tr>
</tbody>
</table>

A) (6 pts) Assuming these data are valid, what mechanism(s) could be proposed to account for the non-equilibrium condition that apparently exists between a human erythrocyte and its environment, with respect to these ions?

B) (8 pts) How can the interspecific differences be explained? (Note: your reasoning must be explicit!)

C) (6 pts) Discuss succinctly experimental procedures for testing your hypothesis(es) and the expected results.
4. (32pts) In addition to the Na\(^+\) and K\(^+\) pump, the plasma membranes of many animal cells contain a Ca\(^+\) pump that helps maintain cytoplasmic levels of Ca\(^+\) (100 nM) four orders of magnitude lower than that present in the extracellular environment (1 mM). It’s possible to study this pump using inside-out vesicles (ISO microsomes) that have been prepared from plasma membranes of erythrocyte ghosts or the homogenates of other cell preparations. In the following experiment, an aliquot of ISO microsomes containing 2.0 mg protein was suspended in 1 ml (final vol) of a test medium containing 4 mM Mg\(^{2+}\)-ATP and 0.12 M KCl (at pH 7.4), and the hydrolysis of ATP was measured over time. After 1 min, 2.0 µMol of Ca\(^{2+}\) was added to the reaction mixture; 2 min later a Ca\(^{2+}\) ionophore (A23187) was added to the mixture. Non of the additions changed the final volume of the reaction mixture. The additions occurred at the times indicated by arrows.

Consider these data and answer all the following questions. All calculations must be shown to receive full credit, your reasoning must be explicit, and your explanations detailed.

A. (4pts) Why must ISO (in contrast to right-side-out) microsomes be used to study this particular calcium pump?

B. (4 pts) What is the specific activity of the pump ATPase, expressed in micromoles of ATP hydrolyzed per mg protein per min?
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C. (7 pts) Why does ATP hydrolysis increase rapidly and then reach a plateau, following the addition of Ca$^{2+}$?

D. (6 pts) How does the addition of A23187 stimulate ATPase activity? Would you expect a second plateau? Why or why not?

E. (5 pts) Propose a test of your hypothesis for either question C. or D. and indicate clearly what the results would show.

F. (6 pts) How could you determine which membrane protein is the Ca$^{2+}$ pump and estimate its molecular weight? (Note: some methods are better than others, involving fewer assumptions.)
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5. (12 pts) During her adventures through the Looking Glass, Alice kept a scientific journal in which she described many of the creatures she encountered. One of these, which fortunately neither sang nor danced, was a giant squid. Alice noted the squid were rapidly moving, voracious predators, and astutely inferred their position at the apex of the aquatic food chain in the Looking Glass World (LGW). (So, too, the Red Queen in the terrestrial realm, but that’s another problem!) Not surprisingly, Alice also recorded their complex muscular and nervous systems, and found their cells (and extracellular fluids) exhibited the ionic concentrations (in mM) listed in the Table below; also listed in the Table are log values for the ratios of these ions. The monovalent ions trans Looking Glass, however, differ from those on this side, and the common ones in squid are represented in the Table below as "M+", "N+", and "O-".

<table>
<thead>
<tr>
<th>Ion:</th>
<th>M+</th>
<th>N+</th>
<th>O-</th>
<th>Logs of Concentration Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside cells</td>
<td>180</td>
<td>6</td>
<td>34</td>
<td>([M+]<em>{\text{in}}/[M+]</em>{\text{out}} = -0.93)</td>
</tr>
<tr>
<td>Outside cells</td>
<td>21</td>
<td>244</td>
<td>300</td>
<td>([M+]<em>{\text{out}}/[M+]</em>{\text{in}} = 0.93)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([N+]<em>{\text{in}}/[N+]</em>{\text{out}} = 1.60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([N+]<em>{\text{out}}/[N+]</em>{\text{in}} = -1.60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([O-]<em>{\text{in}}/[O-]</em>{\text{out}} = -0.95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([O-]<em>{\text{out}}/[O-]</em>{\text{in}} = 0.95)</td>
</tr>
</tbody>
</table>

Patch-clamping measurements by LGW scientists indicate the resting membrane potentials of nerve and muscle cells in these extraterrestrial creatures is +88 mV, with cytoplasm positive relative to the external environment. LGW has a uniform aquatic temperature of 298 0K (a sultry 25 0C). Consider these data and answer all the following questions, showing all relevant calculations.

a. (4 pts) Which ion is closest to equilibrium in these creatures?

B. (4 pts) To which ion is the LGW squid cell membrane most permeable? Why?

C. (4 pts) How is the resting potential likely generated in these cells? Briefly explain the basis for your answer.
6. In addition to transporting oxygen red cells also remove CO₂ in the form of [HCO₃⁻], from actively metabolizing cells. As erythrocytes circulate through tissue capillaries, HCO₃⁻ is picked up and cytoplasmic Cl⁻ is lost. The reverse processes - HCO₃⁻ efflux and Cl⁻ uptake - occur in the small capillaries of the lungs where the concentration of CO₂ is naturally quite low. Consider the membrane mechanism(s) possibly responsible for these transport phenomena and answer the following questions.

A. (6 pts) Are Cl⁻ and HCO₃⁻ transport likely mediated by active or passive mechanism(s)? Which is the more reasonable hypothesis? Why?

B. (24 pts) Describe how any three of the following determinations could be made, indicating clearly what the results would show.

A. whether Cl⁻ and HCO₃⁻ transports are carrier-mediated?
B. whether a single antiport or two uniport carriers are involved?
C. whether Band 3 or another membrane constituent(s) are involved?
D. whether the transport processes are active or passive?
7. Hereditary spherocytosis (HS), a rare form of anemia in humans, is characterized by slightly spherical red blood cells. Affected individuals have fewer red cells than do normal individuals, possibly because HS cells are more susceptible to osmotic lysis than are normal cells (and because they are more readily trapped by the spleen): when placed in a slightly hypotonic saline solution that would cause little or no hemolysis in normal cells, significant numbers of HS cells burst, as illustrated in the following Table:

<table>
<thead>
<tr>
<th>Material</th>
<th>% Cells Hemolysing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal RBC</td>
<td>5</td>
</tr>
<tr>
<td>HS RBC</td>
<td>50</td>
</tr>
</tbody>
</table>

To examine the mechanism responsible for HS, investigators suspended samples of normal and HS erythrocytes in identical physiological saline media and measured the uptake of radioactive sodium ($^{22}$Na) over time. The data are presented in the figure below, as average fluxes +/- 1 S.E.

Consider these data, what you know about plasma membrane structure and function, and answer the following questions.

A. (4 pts) Why are HS human erythrocytes more susceptible to hypotonic perturbations than normal cells?

B. (4 pts) Would you expect the Na/K pump to exhibit an activity in HS cells different than what is exhibited in normal cells, suspended in identical media? Why or why not?
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C. (6 pts) What do these data suggest is the basis for the HS condition? Propose a physiological mechanism to account for the data.

D. (6 pts) Propose a correlative structural mechanism to account for the data, using a diagram as necessary.

E. (6 pts) Describe a test of either hypothesis and indicate clearly how the possible results would test the validity of your hypothesis.