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^{o} 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.

	(Concentration in mM)		
Location	**Na^+**	**K^+**	**Cl^-**
cytoplasm	**14.0**	**119.0**	**65.0**
stream/culture	**1.0**	**0.1**	**1.3**

Logs of Concentration Ratios

Na_i/Na_o = **1.15**
Na_o/Na_i = **-1.15**
K_o/K_i = **-3.08**
K_i/K_o = **3.08**
Cl_i/Cl_o = **1.70**
Cl_o/Cl_i = **-1.70**

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?

A.

B.

C.

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

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

ANSWER	COMMENT
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).	Interesting answer and logical test, **but wrong!** Pumping in solute would make osmotic gradient steeper, causing more water to diffuse inwards by osmosis.
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.	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 H_2O according to the activity gradient into the cell by osmosis, *Nitella* must have a system for regulating H_2O flow. Diffusion is determined by polarity and molecular size. Although H_2O 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 H_2O. This solutions would prevent excess H_2O from entering the cell. Another possible explanation is that *Nitella* has developed a pump system to remove excess H_2O 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 H_2O could be developed by labeling H_2O within the cell by means of a dye and observing to see if this H_2O is removed from the cell to the surrounding medium.

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

Much of second and third sentences is irrelevant.

Relatively impermeable membrane would only affect rate of H_2O entry, but cell would **eventually** swell and burst.

There are **no** known H_2O 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	COMMENTARY
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.	**Where's the Fick equation?** Comparison of the permeability coefficient of H_2O 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_2O concentration by the time it took. Then plug in for $[H_2O_o]$ and $[H_2O_i]$--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_2O 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 dermine 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	COMMENTARY
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 H_2O 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 H_2O uptake and loss and regain the concentration of solute/H_2O which is necessary for its survival. This ability to regulate the flow of H_2O allows *Nitella* to exist in solutions of various concentrations.	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*.	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 H_2O 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^{o} 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.

	(Concentration in mM)			Logs
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				Cl_o/Cl_i = -1.70

Answer the question below and then continue on to examine other answers.

ANSWERS	COMMENTARY
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.	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 electro chemical gradient that is maintained by the cell. It takes into account the changed ions of Na+, K+, Cl-. The electro chemical gradient is determined by the Nerst equation. $v = \frac{RT}{ZF}\frac{\ln[x]_o}{\ln[x]_i}$. In determining the resting potential the permeability of each ion must also be considered and factored into the equation.	Accurate answer but **no** calculations shown; therefore, answer is incomplete.
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.	Good test, but . . . . . . how specifically would voltage change under condition described?