Cell Biology

Membrane Permeability

Membrane Structure

A defining feature of cells is the *plasma membrane* which separates the cell from its external environment. The plasma membrane is composed of a *lipid bilayer* and a variety of proteins that are embedded in the bilayer. The lipid portion of the membrane serves a barrier function, preventing most molecules and ions from passing in or out. In order for most molecules or ions to enter or exit the cell, they must pass through a channel or carrier protein in the membrane. In this way, the plasma membrane is said to be *selectively permeable*.

Membrane Permeability

The plasma membrane is freely permeable to water. The diffusion of water across a semi-permeable membrane in response to a concentration gradient is known as *osmosis*. Cells normally occupy an environment with a particular concentration of solutes. Usually, the internal solute concentration is equal to the external solute concentration so there is no net movement of water molecules across the membrane. Such a solution is said to be *isotonic*. If the solute concentration outside the cell is greater than inside the cell, the solution is said to be *hypertonic*; if the solute concentration outside the cell is less than that inside the cell, the solution is *hypotonic*. Cells in isotonic solutions maintain a relatively constant internal volume because there is no net movement of water into or out of the cell. When a cell is placed in a hypotonic solution, water will enter the cell in response to the osmotic gradient. This influx of water will cause the cell to swell and, if the concentration gradient is steep, the cell may burst, a process known as *lysis*. When a cell is placed in a hypertonic solution, water will leave the cell causing the cell volume to decrease and the cell will shrink, a process known as *crenation*.

In plant cells, the plasma membrane is surrounded by a rigid *cell wall*. Normally, the plasma membrane is held tight against the cell wall so that there is no visible space between the two structures. When a plant cell is placed in a hypertonic solution, the plasma membrane will pull away from the cell wall as a result of the net flow of water out of the cell. This process is called *plasmolysis* (Note, there is no lysis of the cell as the plasma membrane remains intact). If the same cell is transferred to a hypotonic solution, recovery can occur as water flows back in to the cell and *deplasmolysis* occurs. These changes are easily observed with light microscopy. By observing the behavior of plant cell membranes in hypertonic and hypotonic solutions, it is possible to determine if a cell is in a hypertonic, hypotonic, or isotonic solution. By observing the extent of plasmolysis and deplasmolysis with various molecules, it is possible to determine the rate at which these molecules cross the lipid bilayer.

There are three objectives of this laboratory: (1) To examine the effects of of hypertonic, hypotonic, and isotonic solutions on cells. (2) To infer the concentration of solutes that is characteristic of an isotonic solution. (3) To investigate the rate at which various alcohols are able to penetrate the plasma membrane. The cells to be examined included mammalian red blood cells and leaf cells from the common freshwater plant *Elodea*.

Penetration of Alcohols

Among the molecules that are able to pass through the lipid bilayer without the aid of proteins are those that are soluble in lipids. In 1899, E. Overton demonstrated that the greater the lipid solubility of a molecule, the greater its rate of penetration of the lipid bilayer. This relationship is known as Overton's rule.

The lipid solubility of a compound can be specified by the ratio of its solubility in an organic solvent to its solubility in water. Such a ratio is called a *partition coefficient* and is calculated as:

P_C = (solubility in diethyl ether) / (solubility in water).

The partition coefficients of the alcohols to be tested are given below.

Alcohol	**Chemical Formula**	**Partition Coefficeint**
Methanol	CH_3OH	0.14
Ethanol	CH_3CH_2OH	0.26
Ethylene Glycol	$HOCH_2CH_2OH$	0.0053
1-Propanol	$CH_3CH_2CH_2OH$	1.9
Propylene Glycol	$CH_3CH(OH)CH_2OH$	0.018
Glycerol	$HOCH_2CH(OH)CH_2OH$	0.00066

In addition to the lipid solubility of a molecule, its overall size and its molecular structure also influence its rate of penetration of the lipid bilayer. For example, since hydroxyl (-OH) groups can form hydrogen bonds with the water surrounding the cell, hydroxyl groups in a molecule will tend to retard its rate of penetration.

Procedures

To examine red blood cells, place a single drop of each cell suspension on a slide and add a coverslip. Wet mounts for all plant cell observations are prepared as follows: Remove a leaf from a healthy plant and place the leaf on a microscope slide with the *top leaf surface facing up.* Drain the excess water and add two drops of the appropriate solution (sucrose [Section B] or alcohol [Section C]) and a coverslip. Observations should be made under high-dry magnification

A. Observations of mammalian red blood cells.

Part I

1. Place 0.5 ml of mammalian blood into each of 10 tapered-bottom glass centrifuge tubes. Centrifuge at 10,000 rpm for 5 minutes; remove the tubes carefully and decant the plasma with a pipette. The red cells will not be tightly packed; do not be concerned if you also remove a few of them.

2. Add 1 ml of one of the salt and sucrose solutions shown below* to tubes 1 - 8; add 1 ml of distilled water to tube 9. In tube 10, resuspend the cells in several ml of 0.9% NaCl and set aside for the hemolysis tests.

3. Mix the contents of tubes 1 - 9 thoroughly with a wooden applicator stick and allow to stand for a few minutes. Examine a smear of the cells under the microscope and record your observations.

   *NaCl concentrations: 0.9%, 0.05*M*, 0.15*M*, 0.4M and 0.6*M*;
   *sucrose concentrations: 5%, 10% and 15%

Part II

1. Mix the resuspended cells ("blood") from Part I, Step 2, with distilled water in the wells of a spot plate to produce a series of suspensions in 0.8%, 0.7%, 0.6%, 0.5%, 0.4% and 0.3% NaCl. **Note: These are not the same NaCl solutions you used for the preceding part of the exercise.**
   Mix the solutions as follows:

   8 drops "blood" + 1 drop distilled water = 0.8%
   7 drops "blood" + 2 drops distilled water = 0.7%

6 drops "blood" + 3 drops distilled water = 0.6%
5 drops "blood" + 4 drops distilled water = 0.5%
4 drops "blood" + 5 drops distilled water = 0.4%
3 drops "blood" + 6 drops distilled water = 0.3%

2. Allow the solutions to stand for about a minute and then fill a capillary tube from each well. Plug the end with Critocap and centrifuge in the clinical centrifuge.

3. Examine the plasma portion of the resulting centrifuged sample and compare the degree of hemolysis (pinkness or redness) from each NaCl concentration **as well as from a sample that was completely hemolyzed by mixing the cell suspension with excess distilled water.**

B. Determination of isotonic concentrations of sucrose solutions.

1. Prepare a wet mount of an *Elodea* leaf in a 0.6*M* solution of sucrose.

2. Examine the preparation for plasmolysis. On a separate piece of paper, sketch a plasmolyzed cell, labeling as many cell parts as possible.

3. Prepare wet mounts of additional leaves in 0.2*M*, 0.3*M*. 0.4*M* and 0.5*M* sucrose solutions.

4. Examine each slide periodically for 5 min. Record whether plasmolysis has occurred and the extent of plasmolysis (slight, moderate, severe).

5. To observe deplasmolysis, remove the coverslip from a slide showing plasmolysis, drain the sucrose solution, and add two drops of distilled water. After one minute, replace the coverslip and examine the slide for five minutes.

C. Measuring the rate of penetration of alcohols.

The alcohols to be tested are methanol, ethanol, ethylene glycol, 1-propanol, propylene glycol, and glycerol. The concentration of each is 0.4*M* in an isotonic sucrose solution. The alcohols should be tested one at a time on fresh *Elodea* preparations.

1. Prepare a wet mount of an individual *Elodea* leaf in two drops of the appropriate alcohol (remember to drain excess water from the leaf before adding the alcohol solution). Record the time that the slide was prepared on the Data Sheet.

2. Examine the cells under high-dry magnification and note the duration and extent of palsmolysis observed. If plasmolysis does not occur after five minutes, assume it

will not occur and enter "none" on the data sheet. If plasmolysis does occur, record its extent (slight, moderate, severe) and continue to observe for deplasmolysis.

Watch for the first movement of the cell membrane towards the cell wall, which indicates that deplasmolysis is beginning. Record the time that deplasmolysis begins on the Data Sheet. If deplasmolysis does not begin in ten minutes, assume it will not occur and note this on the Data Sheet.

Exercise adapted from Bregman, A. 1986. *Laboratory Exercises in Cell and Molecular Biology*. New York: Wiley.

Data Sheet

List the alcohols in the order in which they penetrate the plasma membrane of *Elodea* leaves.

Fastest

Slowest

Alcohol	Time wet mount prepared	Extent of plasmolysis	Time deplasmolysis begins	Elapsed time until deplasmolysis begins
Methanol				
Ethanol				
Ethylene glycol				
1-Propanol				
Propylene glycol				
Glycerol				

Points to Ponder (as you write your lab report)

Why do the erythrocytes and plant cells respond differently to the various salt and sucrose concentrations?

What is the isotonic NaCl concentration for the erythrocytes? What is the difference between isotonic solutions and isosmotic solutions?

Why do the erythrocytes hemolyze in some of the test solutions?

During plasmolysis in *Elodea*, what occupies the space between the cell membrane and the cell wall?

What approximate concentration of sucrose solution is isotonic for *Elodea* leaf cells? How do you know?

Explain what the numerator and denominator of the partition coefficient tell you about lipid solubility. Is a large partition coefficient associated with greater or lesser lipid solubility?

In the alcohol experiments, what causes deplasmolysis?

Why is it important that the alcohols be dissolved in isotonic solutions of sucrose?

Consider two molecules with similar partition coefficients. Do your results allow you to predict whether the size of each affects its rate of penetration?

Compare the results of the penetration of the alcohols ethanol and ethylene glycol. Provide an explanation for any difference in rate observed.

How do you know the alcohols are not penetrating the plasma membranes by active or passive transport?