Membrane Permeability

Background Information

A defining feature of all cells is the plasma membrane that separates the interior of the cell from its external environment. The plasma membrane is composed of a lipid bilayer and a variety of proteins that are embedded in that 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.

The plasma membrane of most cells 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.

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. This rule states that 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:

\[ P_c = \frac{\text{solubility in diethyl ether}}{\text{solubility in water}}. \]

The partition coefficients of the alcohols to be tested are given below.
<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Chemical Formula</th>
<th>Partition Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>CH₃OH</td>
<td>0.14</td>
</tr>
<tr>
<td>Ethanol</td>
<td>CH₃CH₂OH</td>
<td>0.26</td>
</tr>
<tr>
<td>Ethylene Glycol</td>
<td>HOCH₂CH₂OH</td>
<td>0.0053</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>CH₃CH₂CH₂OH</td>
<td>1.9</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>CH₃CH(OH)CH₂OH</td>
<td>0.018</td>
</tr>
<tr>
<td>Glycerol</td>
<td>HOCH₂CH(OH)CH₂OH</td>
<td>0.00066</td>
</tr>
</tbody>
</table>

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.

There are three objectives of this laboratory: (1) to examine the effects of hypertonic, hypotonic, and isotonic solutions on cells, (2) to infer the concentration of solutes that is characteristic of an isotonic solution, and (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*.

**Experimental Procedures:**

To examine red blood cells, place a single drop of each cell suspension on a slide and add a coverslip.

1. Observe red blood cells in the presence of varying tonicities. To do this, 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 remove 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.
   1.) Resuspend the packed cells in tube #10 in several mls of 0.9% NaCl. This tube will be used for the hemolysis tests described in part 2 of these procedures (“blood”). Add 1 ml of one of the solutions listed below* to each of the tubes labeled 1 – 8. Add 1 ml of distilled water to the 9th tube. The solutions to be used are *NaCl concentrations: 0.9% (tube #1), 0.05M (tube #2), 0.15M (tube #3), 0.4M (tube #4) and 0.6M (tube #5); *sucrose concentrations: 5% (tube #6), 10% (tube #7) and 15% (tube #8).
   2.) 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.

2. Prepare a wet mount of an *Elodea* leaf in a 0.6M solution of sucrose 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 and a coverslip. Observations should be made using 400X magnification.
1.) Examine the preparation for plasmolysis.

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

3.) Examine each slide periodically for 5 minutes, recording whether plasmolysis has occurred and, if so, the extent of plasmolysis (slight, moderate, severe).

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

3. Measure the membrane penetration of a series of alcohols. The alcohols to be tested are methanol, ethanol, ethylene glycol, 1-propanol, propylene glycol, and glycerol. The concentration of each is 0.4M 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 each alcohol (remember to drain excess water from the leaf before adding the alcohol solution). Record the time that the slide was prepared.

2.) Examine the cells under 400X magnification and note the duration and extent of plasmolysis observed. If plasmolysis does not occur after five minutes, assume it will not occur and record it as such. If plasmolysis does occur, record its extent (slight, moderate, severe) and continue to observe for an additional 10 minutes, watching for deplasmolysis.

3.) Deplasmolysis will be indicated when there is first movement of the cell membrane towards the cell wall. Record the time deplasmolysis begins. If deplasmolysis does not begin within ten minutes, assume it will not occur and record it as such.