Objectives

• Use dialysis tubing to model diffusion across the cell membrane
• Investigate the influence of solute concentration on osmosis
• Investigate the concept of water potential in relation to water movement into or out of plant cells

Background to Activities A and B

The cell membrane is a cell's interface with its surroundings. In one sense, this membrane must function as a barrier: it must keep together in one bundle the enzymes, DNA, and metabolic pathways that make life possible. The cell membrane must also function as a gateway: waste products must be discharged through it and essential materials (oxygen, water, etc.) must enter through it. A membrane that allows some molecules to pass through while blocking the passage of others is said to be semipermeable. Molecules pass through the cell membrane either through processes that require the cell to expend energy (active transport), or through processes driven by the kinetic (thermal) energy of molecules (passive transport).

In these lab activities, you will investigate the passage of materials through a semipermeable membrane by passive transport. The membrane you will use, dialysis tubing, is semipermeable because it has submicroscopic holes through it. Molecules are in constant random motion. By chance, a molecule’s motion may move it toward the membrane (Figure 1). If it collides with the membrane wall, it rebounds. If its motion takes it toward a pore, it may either pass through the pore, or it may rebound, depending upon the size of the molecule relative to the diameter of the pore. Molecules that are small enough to pass through the pores can pass through in either direction. Notice that on one side of the membrane solute molecules have displaced some of the water molecules. Thus, there is a higher concentration of water molecules on the opposite side of the membrane. More water molecules are available to collide with the membrane on the side having the higher concentration of water. Thus, although water molecules will move in both directions across the membrane, more will move from the side having the higher concentration to the side having the lower concentration. The movement of molecules from areas of higher concentration to areas of lower concentration is called diffusion.

The diffusion of water molecules across a semipermeable membrane is termed osmosis. A process that depends upon random motion might seem inefficient, but so many water molecules are involved and they move so fast, that it is estimated that a red blood cell floating in blood plasma gains an amount of water equal to 125 times its own volume every second. It also loses the same amount of water each second, all by osmosis. This occurs because the concentration of solutes in the blood plasma is the same as the concentration of solutes in red blood cells. Solutions that have the same solute concentration are isotonic. If we took a sample of whole blood and added salt to the plasma, increasing its solute concentration, the plasma becomes hypertonic to the solution in the red blood cells, and the cells lose water and shrink. If we add water to the blood plasma, decreasing its solute concentration, the plasma becomes hypotonic to the solution in the red blood cells. The cells gain water, swell, and may even burst.
Activity A: Diffusion

Materials
Dialysis tubing, plastic cup, glucose/starch solution, distilled water, iodine-potassium iodide (IKI) solution, dropping pipet, glucose test strips, funnel.

Caution: IKI solution can irritate the skin, mouth, and eyes, and can stain skin or clothing.

Introduction
In Activity A, you will explore the diffusion of different molecules through dialysis tubing, a semipermeable membrane. You will use glucose test strips to check for the presence of glucose and IKI solution to test for the presence of starch. As you probably know, IKI reacts with starch to give a dark blue, almost black color. When IKI reacts with starch, it becomes part of the starch molecule and is removed from solution.

Procedure
1. Pour 160–170 mL of distilled water into a plastic cup. Add approximately 4 mL of IKI solution to the water and mix well. Record the initial solution color in Table 1 on page S-4. Dip a glucose test strip into the solution and record the initial glucose test results in Table 1. Use the + symbol to indicate a positive test result for glucose and the – symbol to indicate a negative result. Discard the used glucose test strip.
2. Dip a fresh glucose test strip into the glucose/starch solution. Record the initial results in Table 1. Discard the used glucose test strip.
3. Obtain a piece of dialysis tubing that has been soaked in water. The tubing should be soft and pliable. Roll the tubing between your thumb and index finger to open it. Close one end of the tube by knotting it or tying it off with string. This will form a bag.
4. Using a small funnel, pour 15 mL of glucose/starch solution in the dialysis bag. Smooth out the top of the bag, running it between your thumb and index finger to expel the air. Tie off the open end of
5. Immerse the dialysis bag in the solution in the cup. Make sure that the portion of the bag that contains the glucose/starch solution is completely covered by the solution in the cup at all times.

6. Wait 30 minutes. While waiting, complete the following exercise.

Indicate on Figure 2 the initial locations (inside or outside of the bag) of all the kinds of molecules that are available for diffusion through the dialysis membrane.

For each of the molecules you list on Figure 2, predict in the space below their direction of net (overall) diffusion: into the bag, out of the bag, both into and out of the bag equally, or none (will not diffuse across the dialysis membrane). Give a reason for each prediction.

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7. After 30 minutes, remove the bag from the cup, blot it on paper towel, and cut a slit in the bag large enough to insert a glucose test strip. Fill in the final columns of Table 1.

<table>
<thead>
<tr>
<th>Location</th>
<th>Solution</th>
<th>Solution Color Initial</th>
<th>Solution Color Final</th>
<th>Glucose Test Results Initial</th>
<th>Glucose Test Results Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysis Bag</td>
<td>Glucose/Starch</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cup</td>
<td>IKI</td>
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</table>

Analysis of Results, Activity A: Diffusion

1. Compare your results with your predictions. Do you find any conflicts that would cause you to revise your predictions? If so, explain.

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2. Does this activity account for the diffusion of all the molecules that you listed on Figure 2 and in your predictions? If not, what data could have been collected to show the net direction of diffusion of this molecule or molecules?

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3. What does your data tell you about the sizes of the molecules relative to the pore size of the dialysis tubing?

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Activity B: Osmosis

Materials
Dialysis tubing, plastic cups, distilled water, funnel, sucrose solutions, paper towels, balance, calculator (optional).

Introduction
In Activity B, you will investigate the influence (if any) of solute concentration on the net movement of water molecules through a semipermeable membrane. The solute you will use is sucrose (cane or table sugar) in the following molar concentrations:

0.0 M (distilled water)
0.2 M
0.4 M
0.6 M
0.8 M
1.0 M
Procedure
Complete the following steps for each sucrose solution that you are assigned to test.

1. Pour 160–170 mL of distilled water into a plastic cup. Label the cup with the concentration of sucrose that you will test.

2. Obtain a piece of dialysis tubing that has been soaked in water. The tubing should be soft and pliable. Roll the tubing between your thumb and index finger to open it. Close one end of the tube by knotting it or tying it off with string. This will form a bag.

3. Using a small funnel, pour 25 mL of sucrose solution into the dialysis bag. Smooth out the top of the bag, running it between your thumb and index finger to expel the air. Tie off the open end of the bag. Leave enough room in the bag to allow for expansion.

4. Dry the bag on paper towels and then determine its mass. Record this as the initial mass in Table 2.

5. Immerse the dialysis bag in the water in the cup. Make sure that the portion of the bag that contains the sucrose solution is completely covered by the water in the cup at all times. Wait 30 minutes before continuing to the next step.

6. After 30 minutes, remove the bag from the cup and dry it on paper towels. Mass the bag and record the final mass in Table 2. Finally, determine the change in the mass of the bag and record this data in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Contents in Dialysis Bag</th>
<th>Initial Mass</th>
<th>Final Mass</th>
<th>Change in Mass</th>
<th>Percent Change in Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 M sucrose (distilled water)</td>
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<tr>
<td>0.2 M sucrose</td>
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<tr>
<td>0.4 M sucrose</td>
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<tr>
<td>0.6 M sucrose</td>
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<tr>
<td>0.8 M sucrose</td>
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<tr>
<td>1.0 M sucrose</td>
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</tbody>
</table>

Analysis of Results, Activity B: Osmosis

1. Calculate the percent change in mass. Use the following formula. Record the results in Table 2.

   \[
   \% \text{ Change in Mass} = \frac{\text{Change in Mass}}{\text{Initial Mass}} \times 100\%
   \]

2. Obtain and record the class averages for change in mass and percent change in mass.
3. What does the change in mass indicate?
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4. Write a hypothesis that this experiment is designed to test.
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5. What variable is being tested in this experiment?
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6. List at least three variables (other than the one listed in your answer to #5) that could influence the outcome of this experiment. Briefly describe the method of control used for each of these variables.
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7. Graph the percent change in mass for your group data and for the class averages. Title the graph and supply the following information:
   a. The independent variable is ____________________________________________.
   b. The dependent variable is ____________________________________________.
   Plot the independent variable on the x-axis, and the dependent variable on the y-axis.
8. Explain any differences between the graph for your group and the graph of class averages.

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9. On the basis of your data and graph, has this experiment adequately tested the variable you listed under #5?

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10. Do your results support your hypothesis, refute it, or require that you modify it?

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11. On the basis of your results, write a statement that expresses the relationship of solute concentration and direction of net movement of water molecules in osmosis.

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12. In which, if any, of the experimental setups were the solutions in the bag and outside of the bag isotonic to each other?

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13. If the experimental setup specified that only distilled water be used to fill the dialysis bag and that the sucrose solutions be used to fill the cup, how would that change your results?

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14. When you drink a glass of water, most of it is absorbed by osmosis through cells lining your small intestine. Drinking seawater can actually dehydrate the body. How?

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Background to Activity C

As you have seen, in animal cells, movement of H\textsubscript{2}O into and out of a cell is influenced by the relative concentration of solute on either side of the cell membrane. If water moves out of the cell, the cell will shrink or crenate. If water moves into the cell it will swell and may even burst, or cytolze. In plants, the situation is complicated by the presence of a rigid or semi-rigid cell wall. Consider a potato cell suspended in pure water. As water enters the cell, the cell membrane is pressed against the cell wall; thus, a physical or hydrostatic pressure develops that opposes the entrance of additional water. When the pressure inside the cell becomes great enough, no additional water can accumulate in the cell, even though the cell still has a higher solute concentration than does pure water. Thus, movement of water through plant tissue cannot be predicted simply by knowing the relative solute concentrations on either side of the plant cell membrane. It is necessary to also account for any differences in pressure between the two sides. The concept of water potential is used to combine the differences in solute concentration and pressure to predict the direction in which water will diffuse through living plant tissues.

In a general sense, water potential is a quantification of the tendency of water to diffuse from one area to another under a given set of perimeters. Traditionally, water potential is expressed in bars, a metric unit of pressure equal to 10 newtons per cm\textsuperscript{2} or about 1 atmosphere. Water potential is abbreviated by the Greek letter psi (\(\psi\)) and has two major components: solute potential (\(\psi_S\)), which is dependent on solute concentration, and pressure potential (\(\psi_p\)), which results from the exertion of pressure—either positive or negative—on a solution. Thus:

\[
\psi = \psi_p + \psi_S
\]

The water potential of pure water at 1 atmosphere is by definition zero. If we dissolve a substance in the water, the water potential drops below zero. This may seem a bit counterintuitive, but remember that we are talking about the tendency of water molecules to diffuse. This is driven by the kinetic energy of free water molecules. As solute concentration goes up, water concentration goes down, there are fewer water molecules available to diffuse, so water potential must go down. Notice that solute potential must be either zero (for pure water), or negative, if the water contains a solute. (As solute concentration increases, solute potential decreases.) Pressure potential can be positive, negative, or zero. Although water diffuses in all directions, the net movement of water will always be from an area of higher water potential to an area of lower water potential.

Returning to our example of a potato cell suspended in pure water: the system is open to the atmosphere, so \(\psi_p = 0\). We will assign a value of \(-9\) for \(\psi_S\). The initial conditions can then be described as follows:

Pure Water
\[
\psi = \psi_p + \psi_S \quad \text{or} \quad \psi = 0 + 0 = 0
\]

Potato Cell
\[
\psi = \psi_p + \psi_S \quad \text{or} \quad \psi = 0 + (-9) = -9
\]

Since \(-9\) is less than 0, water will diffuse into the cell.
At equilibrium, the following condition exists:

**Pure Water**
\[ \psi = \psi_p + \psi_S \quad \text{or} \quad \psi = 0 + 0 = 0 \]

**Potato Cell**
\[ \psi = \psi_p + \psi_S \quad \text{or} \quad \psi = 9 + (-9) = 0 \]

Since 0 = 0, there will be no net movement of water into or out of the cell.

Suppose we add solute to the water in the cup until we produce the following initial conditions:

**Water + Solute**
\[ \psi = \psi_p + \psi_S \quad \text{or} \quad \psi = 0 + (-15) = -15 \]

**Potato Cell**
\[ \psi = \psi_p + \psi_S \quad \text{or} \quad \psi = 0 + (-9) = -9 \]

Since -15 is less than -9, water will diffuse out of the cell. If this continues, the cell will shrink and the cell membrane may pull away from the cell wall, a condition known as **plasmolysis**. This often injures the cell and may kill it.

If we know the molarity of a sucrose solution that will produce equilibrium between the solution and the contents of the potato cell, we can determine the solute potential by using the following formula.

\[ \psi_S = -iCRT \]

where

- **i** = ionization constant (for sucrose, this value is 1)
- **C** = molar concentration of sucrose per liter at equilibrium (must be determined experimentally)
- **R** = pressure constant (0.0831 liter bar/mole K)
- **T** = temperature of solution in kelvins (K = °Celsius + 273)

Example: If C is experimentally determined to be 0.300 and T is 293 K, then

\[ \psi_S = -(1)(0.300 \text{ mole/liter})(0.0831 \text{ liter bar/mole K})(293 \text{ K}) \]

\[ \psi_S = -7.304 \text{ bars} \]
Activity C: Plant Cells and Water Potential

Materials
Plastic cups, distilled water, sucrose solutions, potato cores (or cork borer, scalpel, and potato), plastic wrap, paper towels, balance, thermometer (optional), calculator (optional).

Introduction
In this activity, you will investigate water potential by immersing potato cores in sucrose solutions and determining the change in mass, if any, of the cores. You will graph your data and use the graph to determine a value for C. Using the experimentally determined value for C, you will then calculate a value for \( \psi_S \).

Your group will be assigned one or two sucrose solutions to test. For each solution you test, complete the following procedure.

Procedure
1. Label a cup with the concentration of sucrose that you will test.
2. Use a cork borer to cut four cylinders of potato tissue from the potato. Do not stab your hand with the cork borer. Trim both ends of each cylinder to remove the skin. Cut each cylinder into sections that are approximately 3 cm in length. Use caution when slicing the cylinders so you do not injure yourself or others.
3. If you cannot immediately use a balance to mass your cores, place them in a beaker or cup and cover them with a lid or with plastic wrap. Why do you think this is necessary?
4. Use a balance to determine the total mass of all the potato sections. Record this as the initial mass in Table 3 on page S-11.
5. Place all of the potato sections in the labeled cup. Pour 100 mL of the assigned sucrose solution into the cup.
6. Cover the cup with plastic wrap. Why do you think this step is necessary?
7. If you have been assigned a second concentration of sucrose to test, repeat steps 1–6 using that solution.
8. Stop. Allow the potato sections to remain in the sucrose solution overnight. Then, proceed to Step 9.

Continue with Step 9 only after the potato sections have been in the sucrose solution overnight. Perform these steps for each solution you are testing.
9. Record the temperature as given by your teacher.
   Ambient temperature = ________ °Celsius
10. Remove the potato sections from the sucrose solution. Blot them on paper towels to remove excess solution. If you cannot immediately use a balance to mass your cores, cover them as before to prevent evaporation.
11. Use a balance to determine the final mass. Determine the change in mass of the potato sections. Record this data in Table 3 on page S-11.
Analysis of Results, Activity C: Plant Cells and Water Potential

1. Calculate the percent change in mass of the potato sections and record this data in Table 3. Obtain and record the class averages and record those as well.

2. Graph the class averages for percent change in mass. Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

3. On your completed graph, find the point where the line of your data crosses the 0 line (x-axis) of the grid. This is the equilibrium point; at this point there is no net gain or loss of water from the potato cells. Read the corresponding value of sucrose molarity for this point. This is the molar concentration of sucrose that produces equilibrium. Below, record this concentration of sucrose as your experimentally determined value for C. Convert ambient temperature from °C to K.

   \[ C = \text{______ mole/liter} \]
   \[ T = \text{______ K} \]

4. Using the formula \( \psi_s = -iCRT \), calculate the solute potential at equilibrium. Show your calculations in the space below.

   \( \psi_s = \text{______ bars} \)

5. Using the formula \( \psi = \psi_h + \psi_s \), give the following:
   a. The water potential of the solution at equilibrium ______________
   b. The water potential of the potato cells at equilibrium ____________

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**Table 3: Water Potential**

<table>
<thead>
<tr>
<th>Contents in Cup</th>
<th>Temp.</th>
<th>Initial Mass</th>
<th>Final Mass</th>
<th>Change in Mass</th>
<th>% Change in Mass</th>
<th>% Change in Mass (Class Average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 M sucrose (distilled water)</td>
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<tr>
<td>0.2 M sucrose</td>
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<td>0.4 M sucrose</td>
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<td>0.6 M sucrose</td>
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<tr>
<td>0.8 M sucrose</td>
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<tr>
<td>1.0 M sucrose</td>
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</tbody>
</table>
6. A chef chops vegetables into a bowl of water. Would you expect the vegetable slices to gain or lose water? Explain your answer in terms of water potential.

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7. Imagine that you are an agri-science consultant to a large corporate farm that raises 7,000 acres of wheat on desert land adjoining the Mediterranean Sea. Just before the wheat matures, all the wells used for irrigation water run dry. The farm manager wants to irrigate the fields with water drawn from the Mediterranean. From previous tests, you know that the average solute potential of root tissue taken from the wheat fields is –11.13 bars. You test the seawater and determine its solute potential to be –24.26 bars. What will you advise the farm manager and why?

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8. A marine clam is mistakenly added to a freshwater aquarium. What will happen to the clam and why?

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9. A 1 cm diameter, 6 cm long core is removed from a carrot (see Figure 3). The resulting hole is filled with corn syrup (highly concentrated sugar), and a glass tube is inserted into the hole to make a watertight seal. The carrot is suspended in a cup of pure water. Beginning with the carrot cells adjoining the hole, and going out to the carrot epidermal cells exposed to the water, describe what will happen to the carrot cells. Also, what will happen to the level of liquid in the glass tube and why?

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Figure 3