

## OBJECTIVES

- Demonstrate diffusion across a semi-permeable membrane
- Measure the effects of various concentrations of solute on the process of osmosis
- Differentiate between hypotonic, isotonic, and hypertonic environments
- Measure and calculate water potential
- Examine the effects of osmosis on plant cells

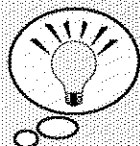
## MATERIALS

### MATERIALS NEEDED PER GROUP

- 4 Glucose indicator strips
- 1 Graduated cylinder
- 1 Plastic cup, 250 ml dialysis tubing, 7 ft.
- 6 Plastic cups, 250 ml
- Glucose starch solution, 15 ml
- IKI (potassium iodide) solution, 1 ml
- Sucrose solution, 0.2 M, 175 ml
- Sucrose solution, 0.4 M, 175 ml
- Sucrose solution, 0.6 M, 175 ml
- Sucrose solution, 0.8 M, 175 ml
- Sucrose solution, 1.0 M, 175 ml
- Distilled water, 175 ml
- Paper towels
- Potato
- Plastic wrap
- Microscope slide
- Coverslip
- Compound microscope
- Scalpel
- Forceps

### SHARED MATERIALS

- Balance
- Compound microscope
- Cork borer with plunger
- NaCl solution, 15%
- Onion epidermis



#### DID YOU KNOW?

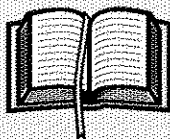
Frogs never drink water. They absorb water from their environment through the process of osmosis.

## PROCEDURE



As a general laboratory practice, it is recommended that students wear proper protective equipment such as gloves, safety goggles, and a lab apron to avoid staining any clothing or skin.

### A. Diffusion



#### **Solute:**

The constituent of a solution that is dissolved in the solvent.

#### **Solvent:**

The constituent of a solution that dissolves the solute.

#### **Concentration:**

The ratio of the mass or volume of a solute to the mass or volume of the solvent.

1. Pour 15 ml of the prepared glucose/starch solution into a graduated cylinder.



The pores in the dialysis tubing are extremely small, and can be easily clogged by any oil or dirt on your fingers and hands. Wash your hands before handling the dialysis tubing, and keep physical contact with the tubing to a minimum.

2. Obtain a piece of dialysis tubing that has been soaking in water. Tie a tight knot in one end of the tubing, or use a piece of string to tie off the end.



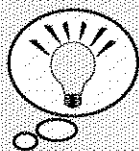
If you choose to tie off the end of the dialysis tubing with string, tie two knots, about 1/4" apart, to prevent leaking.

3. Open the tubing by rubbing the untied end between your fingers. Pour 15 ml of the glucose/starch solution into the tubing.
4. Note the color of the solution in the bag. Record the color in Table 1 in the Analysis section.
5. Determine if glucose is present in the tubing by dipping a glucose indicator strip into the solution. Record the data in Table 1.
6. Carefully tie a knot in the open end to form a bag. Be sure to leave enough space in the bag for expansion.
7. Fill a 250 ml beaker or a provided plastic cup approximately 2/3 full with distilled water. Add 1 ml of potassium iodide (IKI) to the beaker.



The IKI solution is an irritant; it affects skin and eyes, and can stain clothing. Handle the solution with caution. Wash off spills and splashes with water.

8. Note the color of the solution. Record the color in Table 1.



#### DID YOU KNOW?

Several sea-going birds, such as penguins and sea gulls, can drink salt water. They have special glands in their heads that remove and excrete salt, making it useful for consumption.

- Determine if glucose is present in the beaker by dipping a glucose indicator strip into the solution in the beaker. Record the data in Table 1.
- Completely immerse the dialysis bag in the solution in the beaker.
- Wait 30 minutes.



*Though a color change may become apparent after a few minutes, be sure to allow the tubing to remain in the beaker for 30 minutes to allow the glucose to diffuse out into the medium.*

- Remove the dialysis bag from the beaker. Record the final color of the solutions in the bag and the beaker in Table 1.
- Determine the glucose content in the beaker and in the dialysis bag using glucose indicator strips. To test the solution in the bag, make a small cut in the bag with a pair of scissors, and insert the indicator strip through this hole. Record the presence or absence of glucose in Table 1.



*Be sure to wash your hands thoroughly before leaving the laboratory.*

#### B. Osmosis

- Obtain six plastic cups and label them as follows: water, 0.2 M, 0.4 M, 0.6 M, 0.8 M, and 1.0 M.

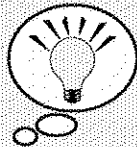


*The pores in the dialysis tubing are extremely small, and can be easily clogged by any oil or dirt on your fingers and hands. Wash your hands before handling the dialysis tubing, and keep physical contact with the tubing to a minimum.*

- Obtain six pieces of dialysis tubing from the beaker of water. Tie a tight knot in one end of the tubing, or use a piece of string to tie off the end.
- Open one piece of tubing by rubbing the untied end between your fingers. Pour 25 ml of distilled water into the tubing and carefully tie a knot in the open end to form a bag. Be sure to leave enough space in the bag for expansion. Blot the sealed tubing dry and place it in the cup labeled "water".



*If at any time the dialysis tubing becomes too dry during the procedure, simply rewet the tubing by placing in water for several seconds and continue setting up your experiment.*



#### DID YOU KNOW?

Osmosis is a reversible thermodynamic process. In other words, the direction of water flow through a membrane can be reversed at any time by proper control of the external pressure on the solution.

- Repeat this procedure with the remaining five pieces of dialysis tubing, adding a different sucrose solution to each bag: 0.2 M, 0.4 M, 0.6 M, 0.8 M, and 1.0 M. Be sure to blot each piece of tubing dry and place it in the proper cup before proceeding with the next concentration.



*Be sure to tie off each of the bags so that they are all approximately the same length.*

- Carefully blot the bags dry and weigh each one. Record each bag's initial mass in Table 2 in the Analysis section.
- Fill the six plastic cups approximately 3/4 full of distilled water. Immerse one bag in each of the cups. Be sure each bag is in the properly labeled cup.
- Wait 30 minutes. Remove the bags from the cups. Blot them dry, and weigh each one again.



*To ensure accurate readings, the bags must be as dry as possible.*

- Record the final mass of each bag in Table 2.
- Calculate the percent change in mass for each of the dialysis bags using the following formula:

$$\% \text{ Change} = (\text{Final Mass} - \text{Initial Mass}) / \text{Initial Mass} \times 100$$

Record this data in Table 2.

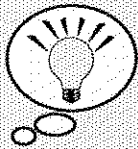
- Graph both your individual and class average results on the graph paper in the Analysis section.

### C. Water Potential



*Your instructor will assign you one or more sucrose solutions of varied concentrations and/or distilled water with which to perform the experiment. Your instructor may have prepared the potato cylinders in advance. If this is the case, begin with Step 2.*

- With the cork borer, cut four cylinders from a potato for each solution you will be using. Cut each cylinder to a length of 3 cm for greater accuracy; remove any skin from the cylinders. Place the cylinders in a covered cup or beaker.



#### DID YOU KNOW?

In 1885, Jacobus Henricus van't Hoff developed a formula to prove that thermodynamic laws are not only valid for gases, but also for dilute solutions. His pressure laws are considered the most comprehensive and important in the realm of natural science.

2. Weigh four cylinders together. Record the initial mass of the cylinders in Table 3 in the Analysis section.
3. Pour 100 ml of one solution you have been assigned into a beaker or plastic cup. Take the initial temperatures and record in Table 3. Insert four potato cylinders and cover the beaker or cup with plastic wrap.
4. Repeat the procedure for the rest of the solutions you will be using, if any. Let the beaker(s) stand overnight.
5. On the following day, measure the final temperature of the liquid in the beaker(s). Record the temperature(s) in Table 3.
6. Remove the four cylinders from each beaker. Blot them carefully with a paper towel and weigh them. Record the final mass of the four cylinders in Table 3.
7. Calculate the percent change in mass for the four potato cylinders using the following formula:

$$\% \text{ Change} = (\text{Final Mass} - \text{Initial Mass}) / \text{Initial Mass} \times 100$$

Record this data in Table 3.

8. Graph both your individual and class average results. Place the percent change in mass on the Y-axis and sucrose molarity on the X-axis. Using the graph, determine the molar concentration of the potato cylinders. This is equivalent to the sucrose molarity in which the potato core mass is constant.

#### D. Water Potential Calculation

Osmotic potential can be calculated using the following formula:

$$\psi\pi = -iCRT$$

i = ionization constant (1 for sucrose)

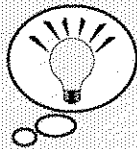
C = osmotic molar concentration (determined in part C)

R = pressure constant (R = .0831 liter bars/mole °Kelvin)

T = temperature (°Kelvin)

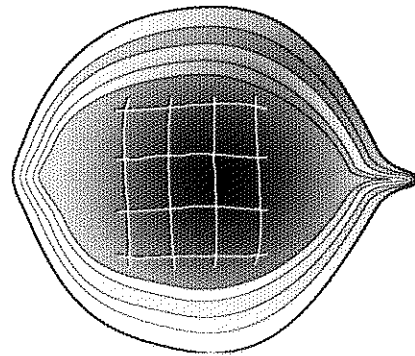
#### E. Plant Cell Plasmolysis

1. Cut an onion in half lengthwise, from top to bottom, and remove the center. Note the layers of the scale leaves in the onion. The epidermal layer, only one cell thick, between each scale is the layer that will be used in the experiment.



#### DID YOU KNOW?

In 1901, Jacobus Henricus van't Hoff received the first Nobel Prize in Chemistry for his work in chemical dynamics and osmotic electrical conductivity.



2. Make several shallow cuts approximately 1 cm apart on one of the scale leaves. Make more cuts perpendicular to the first set, resulting in 1 x 1 cm squares.
3. Using forceps, carefully remove the epidermal layer from one of the squares. If the layer tears while being removed, take another layer from one of the other squares.
4. Place the layer in two to three drops of distilled water on a microscope slide. Place a coverslip on top.
5. Examine the cell layer under 100X magnification and note the characteristics of the cells. Draw several representative cells in the proper space in the Analysis section.
6. Remove the slide from the microscope and add two to three drops of 15% NaCl solution to one side of the coverslip.
7. Carefully touch the edge of a paper towel to the other side of the coverslip in order to draw the NaCl solution across the sample.
8. Allow the slide to sit for two to three minutes in the salt solution and re-examine the sample under the microscope.
9. Note the appearance of the cells. Draw several representative cells in the proper space in the Analysis section.

**WARD'S**  
**AP Biology Lab 1**  
**Osmosis and Diffusion**  
**Lab Activity**

Name: \_\_\_\_\_  
 Group: \_\_\_\_\_  
 Date: \_\_\_\_\_

**ANALYSIS**

**Table 1**  
**Diffusion**

Time	Color		Glucose Content	
	Dialysis Bag	Beaker	Dialysis Bag	Beaker
Start				
30 minutes				

**Table 2**  
**Osmosis Investigation**

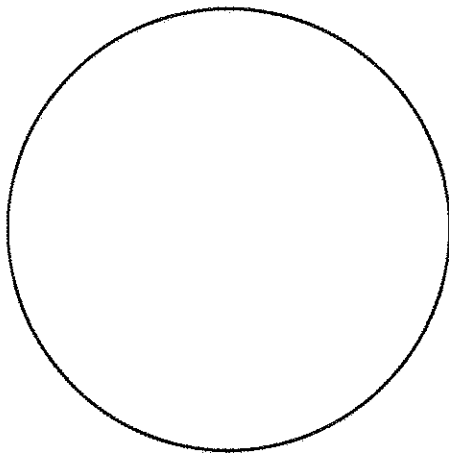
Solution	Dialysis Bag Initial Mass (g)	Dialysis Bag Final Mass (g)	Change in Mass (g)	% Change in Mass
Water				
0.2 M				
0.4 M				
0.6 M				
0.8 M				
1.0 M				

**Table 3**  
**Potato Cell Water Potential**

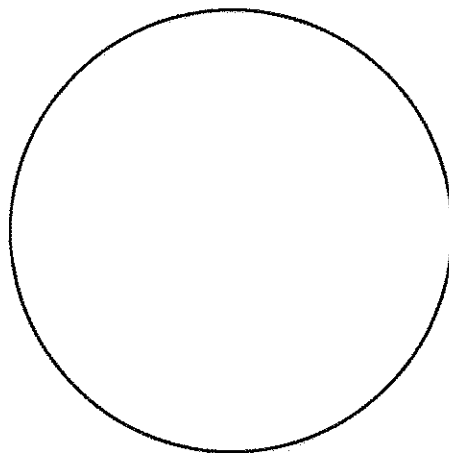
Solution	Solution Temperature °C		Potato Cylinders			
	Initial Temp	Final Temp	Initial Mass (g)	Final Mass (g)	Change in Mass (g)	% Change in Mass
Water						
0.2 M						
0.4 M						
0.6 M						
0.8 M						
1.0M						

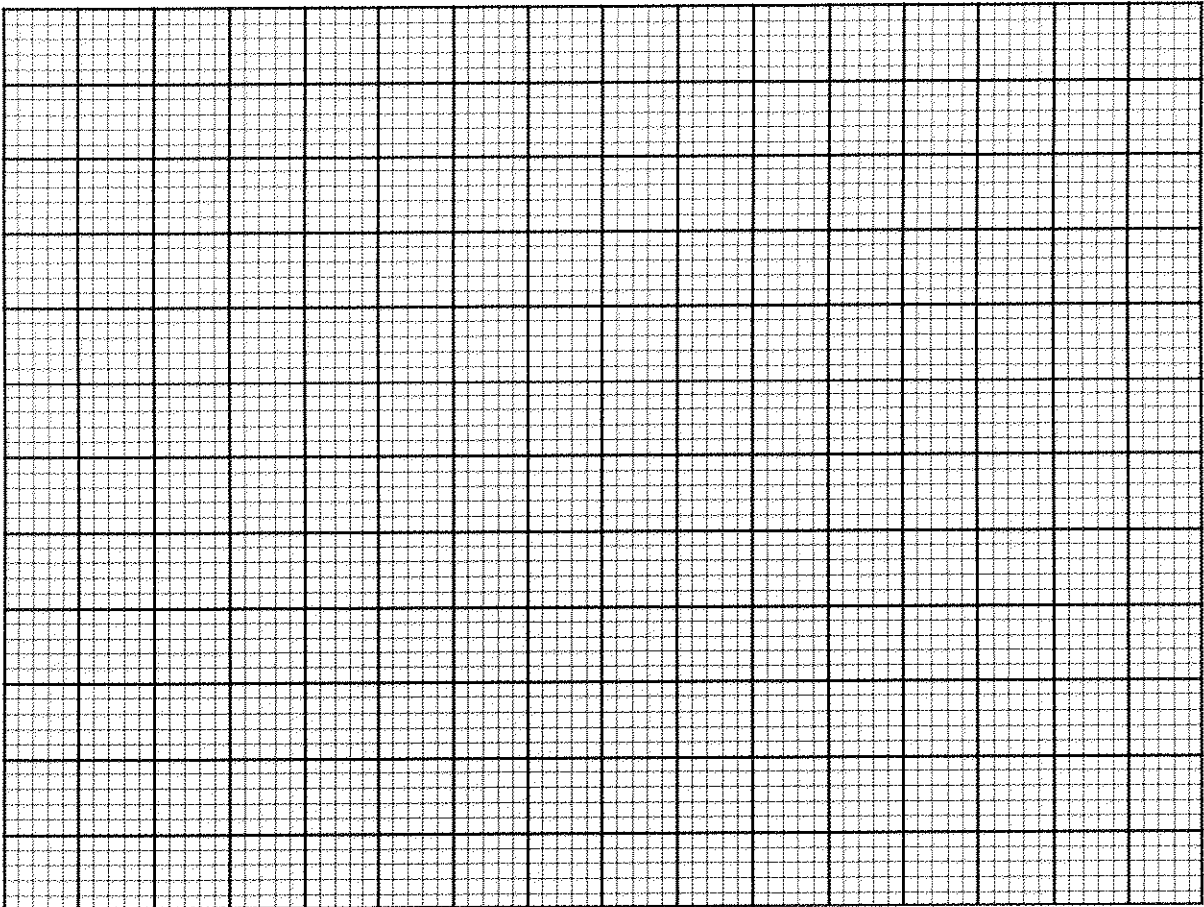
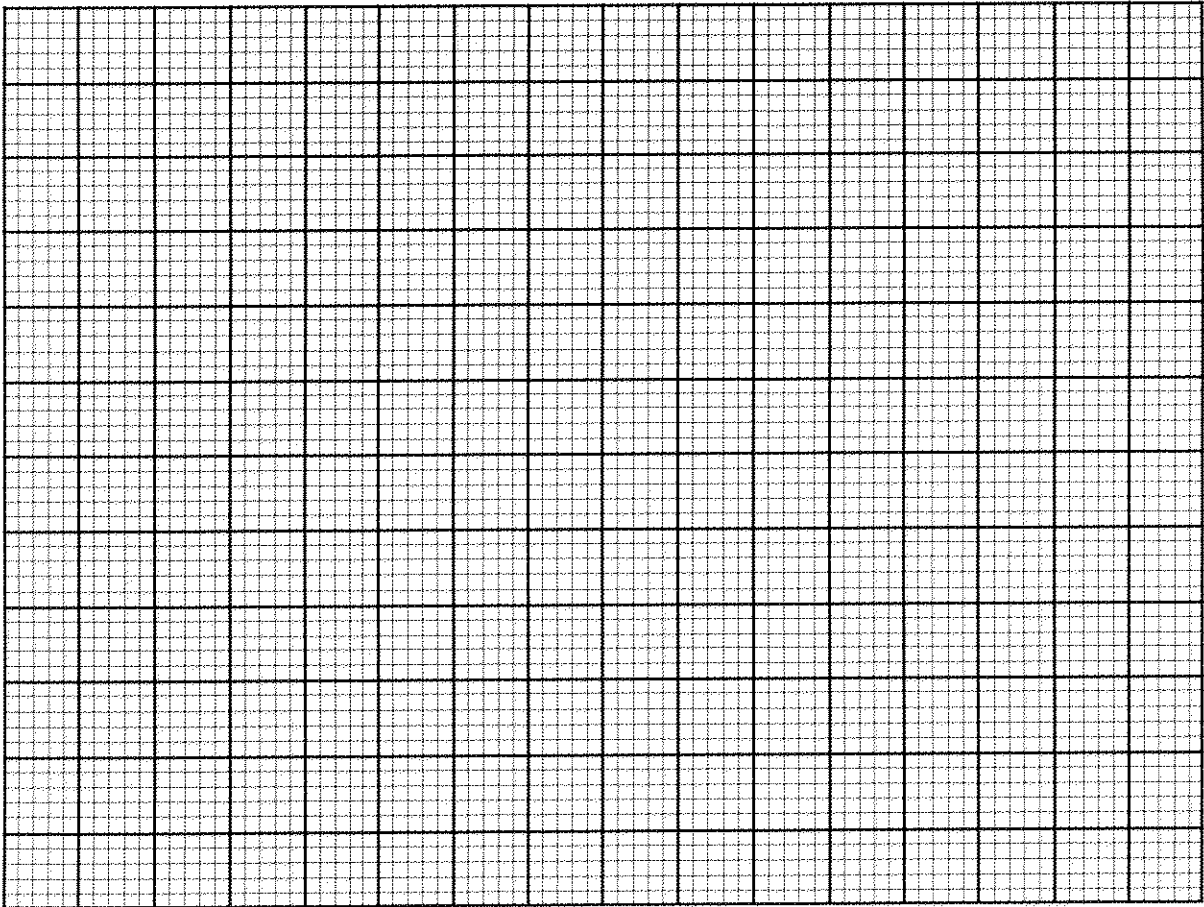
**Plant Cell Plasmolysis**

Cells in Distilled Water



Cells in 15% NaCl





**WARD'S**  
**AP Biology Lab 1**  
**Osmosis and Diffusion**  
**Lab Activity**

Name: \_\_\_\_\_  
Group: \_\_\_\_\_  
Date: \_\_\_\_\_

**ASSESSMENT**

1. Create a Venn diagram comparing osmosis and diffusion.

2. Part A of the experiment was a demonstration of diffusion. Give an example of diffusion occurring in the setup. Do you think osmosis occurred in this part of the experiment? If you answered yes, explain why you believe this to be.

3. Did the dialysis tubing serve as a selectively permeable membrane? Explain your answer.

4. In part B, what caused the mass of the dialysis bags to change? Was there more or less water in the dialysis bags at the conclusion of the experiment? Explain.

5. Was the distilled water in the beakers hypertonic or hypotonic in relation to the sucrose solutions found in the dialysis bags?

6. Suppose the dialysis bags were placed in beakers containing a 0.6 M sucrose solution as opposed to distilled water. How do you think your results would change? Sketch a graph below to show how the mass of each of the bags would be affected.

7. Study the graph you have plotted for part C of the experiment. What is important about the point where the best fit line crosses the X-axis? What is the concentration of sucrose in your potato?

8. Fill in the blanks in the statement below using the following list:  
net gain, low, hypertonic, exit, osmotic, enter, less, hypotonic, high, more, net loss, isotonic  
(not all words are used)

If a cell is placed in a \_\_\_\_\_ solution, it has \_\_\_\_\_ solute in solution than the surrounding fluid, and will therefore experience a \_\_\_\_\_ of water to its surroundings. This cell has \_\_\_\_\_ water potential since there is a great deal of \_\_\_\_\_ pressure causing water to leave the cell. Conversely, a cell sitting in a \_\_\_\_\_ solution has a \_\_\_\_\_ water potential, and since it will experience a \_\_\_\_\_ of water, there will be little osmotic pressure causing water to \_\_\_\_\_ the cell.

9. Using the data from part C of this activity and the formula for water potential from part D, calculate the osmotic potential of the sucrose solution in bars.

10. The water potential ( $\psi$ ) of a solution is equal to the osmotic potential ( $\psi\pi$ ) plus the pressure potential ( $\psi p$ ). Since there is no differential pressure acting on the solution, the pressure potential is equal to zero, making the water potential equal to the osmotic potential. If the equilibrium point between the solutions and the potato cylinders indicates the point where the two water potentials are equal, what is the water potential of the potato cells?

11. Would the water potential of the potato cells change if the cylinders were allowed to dry out? In what way?

12. What are the effects on cells when they are placed in a hypotonic solution, a hypertonic solution, and an isotonic solution?

13. Why can't humans drink salt water for hydration?