LAB TOPIC 3

Diffusion and Osmosis

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Describe the mechanism of diffusion at the molecular level.
2. List several factors that influence the rate of diffusion.
3. Describe a selectively permeable membrane, and explain its role in osmosis.
4. Define *hypotonic, hypertonic,* and *isotonic* in terms of relative concentrations of water and solute (dissolved substance).
5. Discuss the influence of the cell wall on osmotic behavior in cells.
6. Explain how incubating plant tissues in a series of dilutions of sucrose can give an approximate measurement of osmolarity of tissue cells.
7. Explain why diffusion and osmosis are important to cells.
8. Apply principles of osmotic activity to medical, domestic, and environmental activities.
9. Discuss the scientific process, propose questions and hypotheses, and make predictions based on experiments to test hypotheses.
10. Practice scientific persuasion and communication by constructing and interpreting graphs.

Introduction

Maintaining the steady state of a cell is achieved only through regulated movement of materials through cytoplasm, across organelle membranes, and across the plasma membrane. This regulated movement facilitates communication within the cell and between cytoplasm and the external environment. The cytoplasm and extracellular environment of the cell are aqueous solutions. They are composed of water, which is the **solvent,** or dissolving agent, and numerous organic and inorganic molecules, which are the **solutes,** or dissolved substances. Organelle membranes and the plasma membrane are **selectively permeable,** allowing water to freely pass through but regulating the movement of solutes.

The cell actively moves some dissolved substances across membranes, expending adenosine triphosphate (ATP) (biological energy) to accomplish the movement. Other substances move passively, without expenditure of ATP from the cell, but only if the cell membrane is permeable to those substances. Water and selected solutes move passively through the cell and cell membranes by **diffusion,** a physical process in which molecules move from an area where they are in high concentration to one where their

concentration is lower. The energy driving diffusion comes only from the intrinsic kinetic energy (energy of motion) in all atoms and molecules. If nothing hinders the movement, a solute will diffuse until it reaches equilibrium.

Osmosis is a type of diffusion; in cells it is the diffusion of water through a selectively permeable membrane from a region where it is highly concentrated to a region where its concentration is lower. The difference in concentration of water occurs if there is an unequal distribution of at least one dissolved substance on either side of a membrane and the membrane is impermeable to that substance. For example, if a membrane that is impermeable to sucrose separates a solution of sucrose from distilled water, water will move from the distilled water, where it is in higher concentration, through the membrane into the sucrose solution, where it is in lower concentration.

Three terms, **hypertonic, hypotonic,** and **isotonic,** are used when referring to two solutions separated by a selectively permeable membrane. Figure 3.1 represents a U-shaped glass tube with a selectively permeable membrane separating two solutions. In Figure 3.1a, the solution on the right side of the membrane is hypertonic; that is, it has a greater concentration of solute molecules that cannot cross the membrane than the solution on the other side of the membrane. It is described, therefore, as having a greater osmolarity (solute concentration expressed as molarity). The hypotonic solution on the left side of the membrane has a lower concentration of solute molecules, or a lower osmolarity, than the solution on the right. When the two solutions are in equilibrium, the concentration of nonpenetrating solute molecules relative to water molecules being equal on both sides of the membrane, the osmolarities are equal and the solutions are said to be isotonic (Figure 3.1b). The *net flow* of water is from the hypotonic to the hypertonic solution. When the solutions are isotonic, there is no net flow of water across the membrane.

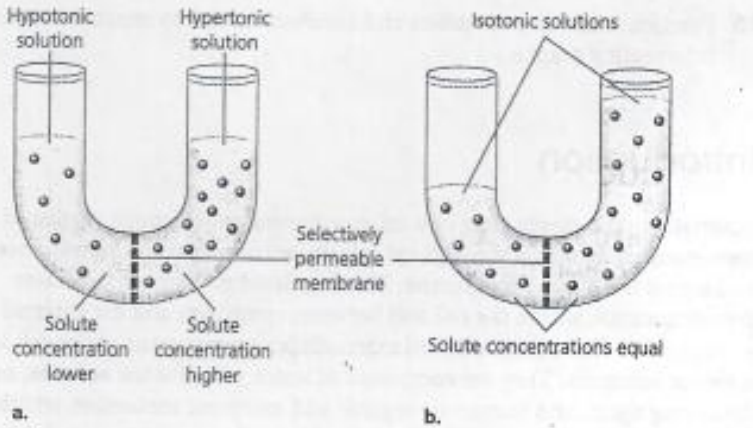

FIGURE 3.1

Osmosis. a. Two solutions separated by a selectively permeable membrane. Water molecules (the solvent) can pass through the membrane pores, but molecules of the solute (dissolved substance) cannot. The solution on the left of the membrane has a *lower* concentration of solute molecules and is said to be *hypotonic*. The solution on the right of the membrane has a *higher* concentration of solute molecules and is said to be *hypertonic*.

b. Isotonic solutions *after the net flow* of water molecules from the hypotonic solution to the hypertonic solution. As water molecules diffuse through the membrane, eventually the concentration of the solute will be equal on both sides of the membrane. These two solutions are now *isotonic*.

EXERCISE 3.1
Diffusion of Molecules

In this exercise you will investigate characteristics of molecules that facilitate diffusion, factors that influence diffusion rates, and diffusion of solutes through a selectively permeable membrane.

Experiment A. Kinetic Energy of Molecules

Materials

dropper bottle of water
carmine powder
dissecting needle
slide and coverslip
compound microscope

Introduction

Molecules of a liquid or gas are constantly in motion because of the intrinsic kinetic energy in all atoms and molecules. In 1827, Robert Brown, a Scottish botanist, noticed that pollen grains suspended in water on a slide appeared to move by a force that he was unable to explain. In 1905, Albert Einstein, searching for evidence that would prove the existence of atoms and molecules, predicted that the motion observed by Brown must exist, although he did not realize that it had been studied for many years. Only after the kinetic energy of molecules was understood did scientists ask if the motion observed by Brown and predicted by Einstein could be the result of molecular kinetic energy being passed to larger particles. We now know that intrinsic molecular kinetic energy is the driving force of diffusion. In this experiment, you will observe large particles suspended in water in motion similar to that observed by Brown, traditionally called **Brownian movement.** You will relate the motion observed to the forces that bring about diffusion.

Procedure

Work in pairs. One person should set up the microscope while the other person makes a slide as follows:

1. Place a drop of water on the slide.
2. Touch the tip of a dissecting needle to the drop of water and then into the dry carmine.
3. Add the carmine on the needle to the drop of water on the slide, mix, cover with a coverslip, and observe under the compound microscope.
4. Observe on low power and then high power. Focus as much as possible on one particle of carmine.
5. Record your findings in the Results section, and draw conclusions based on your results in the Discussion section.

Results

Describe the movement of single carmine particles.

1. Is the movement random or directional?
2. Does the movement ever stop?

3. Do smaller particles move more rapidly than larger particles? Other observations?

Discussion

1. Are you actually observing molecular movement? Explain.

2. How can molecular movement bring about diffusion?

3. List several processes in cell metabolism where diffusion is important.

Experiment B. Diffusion of Molecules Through a Selectively Permeable Membrane

Materials

string or rubber band
wax pencil
30% glucose solution
starch solution
I_2KI solution
Benedict's reagent
hot plate
500-mL beaker one-third filled with water
handheld test tube holder
3 standard test tubes
disposable transfer pipettes
2 400-mL beakers to hold dialysis bag
30-cm strip of moist dialysis tubing

Introduction

Dialysis tubing is a membrane made of regenerated cellulose fibers formed into a flat tube. If two solutions containing dissolved substances of different molecular weights are separated by this membrane, some substances may readily pass through the pores of the membrane, but others may be excluded.

Working in teams of four students, you will investigate the selective permeability of dialysis tubing. You will test the permeability of the tubing to the reducing sugar, glucose (molecular weight 180), starch (a variable-length polymer of glucose), and iodine potassium iodide (I_2KI). You will place a solution of glucose and starch into a dialysis tubing bag and then place this bag into a solution of I_2KI. Sketch and label the design of this experiment in the margin of your lab manual or on separate paper to help you develop your hypotheses.

You will use two tests in your experiment:

1. *I_2KI test for presence of starch*

   When I_2KI is added to the unknown solution, the solution turns purple or black if starch is present. If no starch is present, the solution remains a pale yellow-amber color.

2. *Benedict's test for reducing sugar*

   When Benedict's reagent is added to the unknown solution and the solution is heated, the solution turns green, orange, or orange-red if a reducing sugar is present (the color indicates the sugar concentration). If no reducing sugar is present, the solution remains the color of Benedict's reagent (blue).

Question

Remember that every experiment begins with a question. Review the design of this experiment in the Introduction. Formulate a question about the permeability of dialysis tubing. The question may be broad, but it must propose an idea that has measurable and controllable elements.

Hypothesis

Hypothesize about the selective permeability of dialysis tubing to the substances being tested.

Prediction

Predict the results of the I_2KI and Benedict's tests based on your hypothesis (if/then).

Procedure

1. Prepare the dialysis bag with the initial solutions.
   a. Fold over 3 cm at the end of a 25- to 30-cm piece of dialysis tubing that has been soaking in water for a few minutes, pleat the folded end "accordion style," and close the end of the tube with the string or a rubber band, forming a bag. This procedure must secure the end of the bag so that no solution can seep through.
   b. Roll the opposite end of the bag between your fingers until it opens, and add 4 pipettesful of 30% glucose into the bag. Then add 4 pipettesful of starch solution to the glucose in the bag.
   c. Hold the bag closed and mix its contents. Record its color in Table 3.1 in the Results section. Carefully rinse the outside of the bag in tap water.
   d. Add 200 mL of water to a 400- to 500-mL beaker. Add several dropperfuls of I_2KI solution to the water until it is visibly yellow-amber. Record the color of the H_2O-plus-I_2KI solution in Table 3.1.

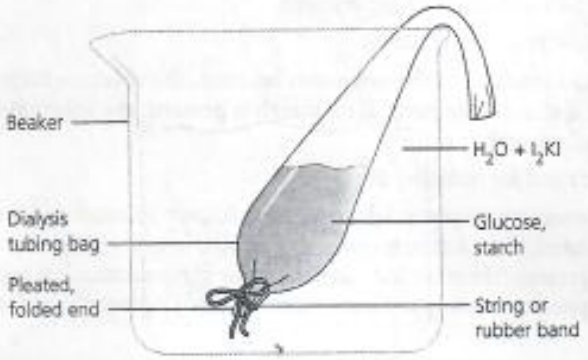

FIGURE 3.2
Setup for Exercise 3.1, Experiment B. The dialysis tubing bag, securely closed at one end, is placed in the beaker of water and I_2KI. The open end of the bag should drape over the edge of the beaker.

   e. Place the bag in the beaker so that the untied end of the bag hangs over the edge of the beaker (Figure 3.2). *Do not allow the liquid to spill out of the bag!* If the bag is too full, remove some of the liquid and rinse the outside of the bag again. If needed, place a rubber band around the beaker, holding the bag securely in place. If some of the liquid spills into the beaker, dispose of the beaker water, rinse, and fill again.

2. Leave the bag in the beaker for about 30 minutes. (You should go to another lab activity and then return to check your setup periodically.)
3. After 30 minutes, carefully remove the bag and stand it in a dry beaker.
4. Record in Table 3.1 the final color of the solution in the bag and the final color of the solution in the beaker.
5. Perform the Benedict's test for the presence of sugar in the solutions.
   a. Label three clean test tubes: control, bag, and beaker.
   b. Put 2 pipettesful of water in the control tube.
   c. Put 2 pipettesful of the bag solution in the bag tube.
   d. Put 2 pipettesful of the beaker solution in the beaker tube.
   e. Add 1 dropperful of Benedict's reagent to each tube.
   f. Heat the test tubes in a boiling water bath for about 3 minutes.
   g. Record your results in Table 3.1.
6. Review your results in Table 3.1 and draw your conclusions in the Discussion section.

Results

Complete Table 3.1 as you observe the results of Experiment B.

TABLE 3.1 Results of Experiment Investigating the Permeability of Dialysis Tubing to Glucose, I_2KI, and Starch

Solution Source	Original Contents	Original Color	Final Color	Color After Benedict's Test
Bag				
Beaker				
Control				

Discussion

1. What is the significance of the final colors and the colors after the Benedict's tests? Did the results support your hypothesis? Explain, giving evidence from the results of your tests.

2. How can you explain your results?

3. From your results, predict the size of I_2KI molecules relative to glucose and starch.

4. What colors would you expect if the experiment started with glucose and I_2KI inside the bag and starch in the beaker? Explain.

EXERCISE 3.2
Osmotic Activity in Cells

All organisms must maintain an optimum internal osmotic environment. Terrestrial vertebrates must take in and eliminate water using internal regulatory systems to ensure that the environment of tissues and organs remains in osmotic balance. Exchange of waste and nutrients between blood and tissues depends on the maintenance of this condition. Plants and animals living in fresh water must control the osmotic uptake of water into their hypertonic cells.

In this exercise, you will investigate the osmotic behavior of plant and animal cells placed in different molar solutions. What happens to these cells when they are placed in hypotonic or hypertonic solutions? This question will be investigated in the following experiments.

Experiment A. Osmotic Behavior of Animal Cells

Materials

On demonstration:

test tube rack
3 test tubes with screw caps, each containing one of the three solutions of unknown osmolarity
ox blood
newspaper or other printed page

For microscopic observations:

4 clean microscope slides and coverslips
wax pencil
dropper bottle of ox blood
dropper bottles with three solutions of unknown osmolarity

Introduction

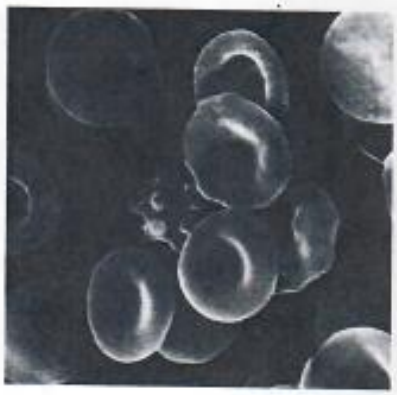

FIGURE 3.3
Human red blood cells. The star-shaped cell in the center has lost water and crenated.

Mature red blood cells (erythrocytes) are little more than packages of hemoglobin bound by a plasma membrane permeable to small molecules, such as oxygen and carbon dioxide, but impermeable to larger molecules, such as proteins, sodium chloride, and sucrose. In mammals these cells even lack nuclei when mature, and as they float in isotonic blood plasma, their shape is flattened and pinched inward into a biconcave disk. Oxygen and carbon dioxide diffuse across the membrane, allowing the cell to carry out its primary function, gas transport, which is enhanced by the increased surface area created by the shape of the cell. Scientists questioning what happens to red blood cells in different molar solutions observed that the cells respond dramatically if they are not in an isotonic environment. When water moves into red blood cells placed in a hypotonic solution, the cells swell and the membranes burst, or undergo **lysis.** When water moves out of red blood cells placed in a hypertonic solution, the cells shrivel and appear bumpy, or **crenate.** In this experiment, you will investigate the behavior of red blood cells when the osmolarity of the environment changes from isotonic to hypertonic or hypotonic. (See Figure 3.3.)

Hypothesis

Hypothesize about the behavior of red blood cells when they are placed in hypertonic or hypotonic environments.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Observe the three test tubes containing unknown solutions and blood on demonstration. These tubes have been prepared in the following way.

   Test tube 1: 15 mL of unknown solution A

   Test tube 2: 15 mL of unknown solution B

   Test tube 3: 15 mL of unknown solution C

   Your instructor has added 5 drops of ox blood to each test tube.

   Observe the appearance of each test tube. Is it opaque? Is it translucent? Describe your observations in Table 3.2 in the Results section.
2. Be sure each test tube cap is securely tightened, then hold each test tube flat against the printed newspaper article or page of text.
3. Attempt to read the print. Describe in Table 3.2 in the Results section.

   Continue your investigation of the osmotic behavior of animal cells by performing microscopic observations of cells in the three unknown solutions.

Have your microscope ready, and observe slides immediately after you have prepared them. Do one slide at a time.

4. Label four clean microscope slides A, B, C, and D.
5. Place a drop of blood on slide D, cover with a coverslip, and observe the shape of the red blood cells with no treatment. Record your observations in Table 3.3 in the Results section.
6. Locate the three dropper bottles (A, B, C) containing solutions of unknown osmolarity. Put a drop of solution A on slide A and add a coverslip. Place the slide on the microscope stage and carefully add a small drop of blood to the edge of the coverslip. The blood cells will be drawn under the coverslip by capillary action.
7. As you view through the microscope, carefully watch the cells as they come into contact with solution A; record your observations in Table 3.3.
8. Repeat steps 6 and 7 with solutions B and C.
9. Record your observations in Table 3.3. Draw your conclusions in the Discussion section.

TABLE 3.2 Appearance of Unknown Solutions A, B, and C

	Appearance of the Solution	Can You Read the Print?
Test tube 1 (unknown A)		
Test tube 2 (unknown B)		
Test tube 3 (unknown C)		

Results

1. Record your observations of the demonstration test tubes in Table 3.2.
2. Record your microscopic observations of red blood cell behavior in Table 3.3.

Discussion

Explain your results in terms of your hypothesis.

1. Explain the appearance of the three test tubes on demonstration.

2. Based on the demonstration and your microscopic investigation, which of the three solutions is hypotonic to the red blood cells?

   Hypertonic?

   Isotonic?

   Verify your conclusions with the laboratory instructor.

TABLE 3.3 Appearance of Red Blood Cells in Test Solutions

Solution	Appearance/Condition of Cells
D (blood only)	
A	
B	
C	

3. What conditions might lead to results other than those expected?

Experiment B. Osmotic Behavior in Cells with a Cell Wall

Materials

On demonstration:

2 compound microscopes labeled A and B
1 slide of *Elodea* in a hypertonic salt solution
1 slide of *Elodea* in distilled water

Introduction

In their natural environment, cells of freshwater plants and algae are bathed in water containing only dilute concentrations of solvents. The net flow of water is from the surrounding medium into the cells. To understand this process, review the structure of *Elodea* cells from Lab Topic 2.

The presence of a cell wall and a large fluid-filled central vacuole in a plant or algal cell will affect the cell's response to solutions of differing molarities. When a plant cell is placed in a hypertonic solution, water moves out of the cell; the protoplast shrinks and may pull away from the cell wall. This process is called **plasmolysis,** and the cell is described as **plasmolyzed** (Figure 3.4). In a hypotonic solution, as water moves into the cell and ultimately into the cell's central vacuole, the cell's **protoplast** (the plant cell exclusive of the cell wall—the cytoplasm enclosed by plasma membrane) expands. The cell wall, however, restricts the expansion, resulting in **turgor pressure** (pressure of the protoplast on the cell wall owing to uptake of water). A high turgor pressure will prevent further movement of water into the cell. This process is a good example of the interaction between pressure and osmolarity in determining the direction of the net movement of water. The hypertonic condition in the cell draws water into the cell until the membrane-enclosed cytoplasm presses against the cell wall. Turgor pressure begins to force water through the membrane and out of the cell, changing the direction of net flow of water (Figure 3.5).

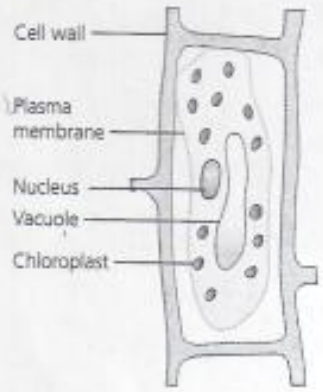

FIGURE 3.4
Plant cell placed in a hypertonic solution. Water leaves the central vacuole and the cytoplasm shrinks, a process called plasmolysis.

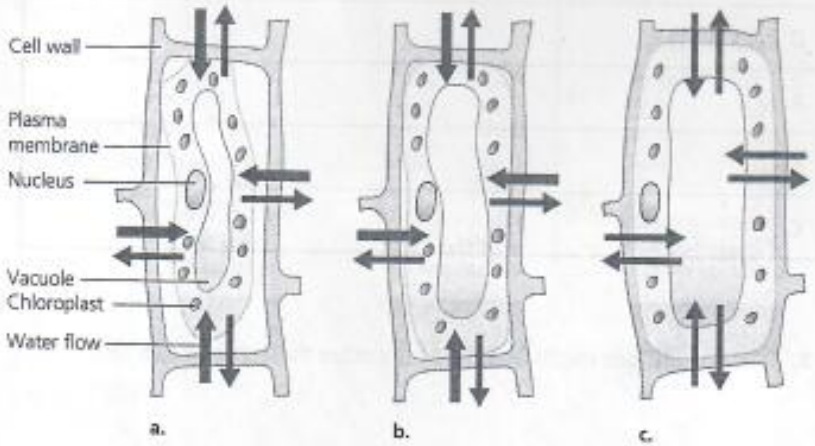

FIGURE 3.5
The effect of turgor pressure on the cell wall and the direction of net flow of water in a plant cell. A plant cell undergoes changes in a hypotonic solution. (a) Low turgor pressure. The net flow of water comes into the cell from the surrounding hypotonic medium. (b) Turgor pressure increases. The protoplast begins to press on the cell wall. (c) Greatest turgor pressure. The tendency to take up water is ultimately restricted by the cell wall, creating a back pressure on the protoplast. Water enters and leaves the cell at the same rate.

Scientists call the combined force created by solute concentration and physical pressure **water potential.** For a detailed explanation of water potential, see a discussion of plant transport mechanisms in your text (e.g., Chapter 36 in *Campbell Biology,* 10th ed.). In contrast to an animal cell, the ideal state for a plant cell is turgidity. When a plant cell is turgid, it is not isotonic with its surroundings but is hypertonic, having a higher solute concentration than its surroundings. In this state, the plant cell protoplast presses on the cell wall. The pressure of the protoplast on the cell wall is an important force in plant activity. For example, it may cause young cells to "grow" as the elastic cell wall expands.

For this experiment, two slides have been set up on demonstration microscopes. On each slide, *Elodea* has been placed in a different molar solution: One is hypotonic (distilled water) and one is hypertonic (concentrated salt solution).

Question

Propose a question about the movement of water in *Elodea* leaves placed in different molar solutions.

Hypothesis

Hypothesize about the movement of water in cells with a cell wall when they are placed in hypertonic or hypotonic environments.

Prediction

Predict the appearance of *Elodea* cells placed in the two solutions (if/then).

Procedure

1. Observe the two demonstration microscopes with *Elodea* in solutions A and B.
2. Record your observations in Table 3.4 in the Results section, and draw your conclusions in the Discussion section.

Results

In the margin of your manual, sketch the appearance of the *Elodea* leaves and describe the appearance of the *Elodea* cells in Table 3.4.

TABLE 3.4 Appearance of *Elodea* Cells in Unknown Solutions A and B

Solution	Appearance/Condition of Cells
A	
B	

Discussion

1. Based on your predictions and observations, which solution is hypertonic?

   Hypotonic?

2. Which solution has the greatest osmolarity?

3. Would you expect pond water to be isotonic, hypertonic, or hypotonic to *Elodea* cells? Explain.

4. Verify your conclusions with your laboratory instructor.

MB Student Media Videos—Ch. 7: Turgid *Elodea*; Plasmolysis

EXERCISE 3.3
Investigating Osmolarity of Plant Cells

Knowing the solute concentration of cells has both medical and agricultural applications. In plants, scientists know that for normal activities to take place, the amount of water relative to solute concentration in cells must be maintained within a reasonable range. If plant cells have a reduced water content, all vital functions slow down.

In the following experiments, you will estimate the osmolarity (solute concentration) of potato tuber cells using two methods, change in weight and change in volume. You will incubate pieces of potato tuber in sucrose solutions of known molarity. The object is to find the molarity at which weight or volume of the potato tuber tissue does not change, indicating that there has been no net loss or gain of water. This molarity is an indirect measure of the solute concentration of the potato tuber. This measure is indirect because water movement in plant cells is also affected by the presence of cell walls (see Figure 3.5c).

Work in teams of four. Each team will measure either weight change or volume change. Time will be available near the end of the laboratory period for each team to present its results to the class for discussion and conclusions.

Experiment A. Estimating Osmolarity by Change in Weight

Materials

1 large potato tuber
7 250-mL beakers (disposable cups may be substituted)
wax marking pencil
forceps
balance that weighs to the nearest 0.01 g
aluminum foil
petri dish
sucrose solutions: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 molar (*M*)
razor blade
cork borer
deionized (DI) water (0 molar)
paper towels
metric ruler
calculator

Introduction

In this experiment, you will determine the weight of several potato tuber cylinders and incubate them in a series of sucrose solutions. After the cylinders have incubated, you will weigh them and determine if they have gained or lost weight. This information will enable you to estimate the osmolarity of the potato tuber tissue.

Question

What question is being investigated in this experiment?

Hypothesis

Hypothesize about the osmolarity of potato tuber tissue in relation to the sucrose solutions.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Obtain 100 mL of DI water and 100 mL of each of the sucrose solutions. Put each solution in a separate, appropriately labeled 250-mL beaker or paper cup.

Cork borers and razor blades can cut! Use them with extreme care! To use the cork borer, hold the potato in such a way that the borer will not push through the potato into your hand.

2. Use a sharp cork borer to obtain seven cylinders of potato. Push the borer through the length of the potato, twisting it back and forth. When the borer is filled, remove from the potato and push the potato cylinder out of the borer. You must have seven complete, undamaged cylinders at least 5 cm long.
3. Line up the potato cylinders and, using a sharp razor blade, cut all cylinders to a uniform length, about 5 cm, removing the peel from the ends.
4. Place all seven potato samples in a petri dish, and keep them covered to prevent their drying out.

In subsequent steps, treat each sample individually. Work quickly. To provide consistency, each person should do one task to all cylinders (one person wipe, another weigh, another slice, another record data).

5. Remove a cylinder from the petri dish, and place it between the folds of a paper towel to blot sides and ends.
6. Weigh it to the nearest 0.01 g on the aluminum sheet on the balance. Record the weight in Table 3.5 in the Results section.
7. Immediately cut the cylinder lengthwise into two long halves.
8. Transfer the potato pieces to the water beaker.
9. Note what time the potato pieces are placed in the water beaker. Time: _____.
10. Repeat steps 5 to 8 with each cylinder, placing potato pieces in the appropriate incubating solution from 0.1 to 0.6 *M*.

Be sure that the initial weight of the cylinder placed in each test solution is accurately recorded.

11. Incubate 1.5 to 2 hours. (As this takes place, you will be performing other lab activities.)
12. Swirl each beaker every 10 to 15 minutes as the potato pieces incubate.
13. At the end of the incubation period, record the time when the potato pieces are removed. Time: _____.

    Calculate the approximate incubation time in Table 3.5.
14. Remove the potato pieces from the first sample. Blot the pieces on a paper towel, removing excess solution only.
15. Weigh the potato pieces and record the final weight in Table 3.5.

16. Repeat this procedure until all samples have been weighed in the chronological order in which they were initially placed in the test solutions.
17. Record your data in the Results section, and complete the questions in the Discussion section.

Results

1. Complete Table 3.5. To calculate percentage change in weight, use this formula:

$$\text{Percentage change in weight} = \frac{\text{weight change}}{\text{initial weight}} \times 100$$

If the sample gained in weight, the value should be positive. If it lost in weight, the value should be negative.

2. Plot percentage change in weight as a function of the sucrose molarity in Figure 3.6.
   a. Place a 0 in the middle of the *y*-axis. Choose appropriate scales.
   b. Label the axes of the graph: Determine dependent and independent variables, and place each on the appropriate axis (see Lab Topic 1 for assistance in graphing).
   c. Graph your results. Weight increase (positive values) should be above the zero change line on the "percentage change in weight" axis. Weight decrease should be below the zero change line.
   d. Construct a curve that best fits the data points. Use this curve to estimate the osmolarity of the potato tuber.
   e. Compose an appropriate figure title.

TABLE 3.5 Data for Experiment Estimating Osmolarity by Change in Weight

Download an Excel version from www.masteringbiology.com in the Study Area under Lab Media

Approximate time in solutions: _____

	Sucrose Molarity						
	0.0	0.1	0.2	0.3	0.4	0.5	0.6
Final weight (g)							
Initial weight (g)							
Weight change (g)							
% change in weight							

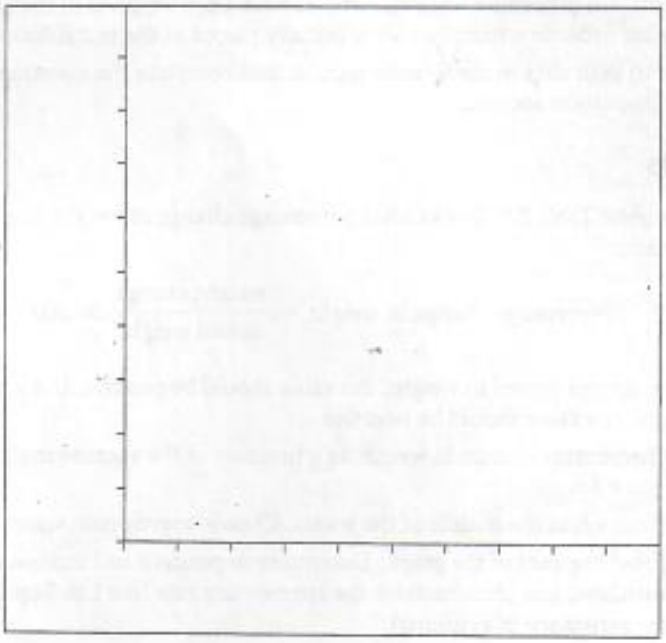

FIGURE 3.6

Discussion

1. At what sucrose molarity does the curve cross the zero change line on the graph?

2. Explain how this information can be used to determine the osmolarity of the potato tuber tissue.

3. In more dilute concentrations of sucrose, the weight of the potato pieces _____ (increases/decreases) after incubation. What forces other than solute concentration will have an impact on the amount of water taken up by the potato pieces (see Figure 3.5c)?

4. Estimate the osmolarity of the potato tuber tissue.

Experiment B. Estimating Osmolarity by Change in Volume

Materials

1 large potato tuber
vernier caliper
7 250-mL beakers (disposable cups may be substituted)
wax marking pencil
forceps
petri dish
razor blade
cork borer (0.5-cm diameter)
sucrose solutions: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 *M*
DI water (0 *M*)
metric ruler
paper towels
calculator

Introduction

In this experiment, you will determine the volume of several potato tuber cylinders by measuring the length and diameter of each. To make these measurements you will use a vernier caliper, a tool that is used by scientists, engineers, and precision woodworkers to measure the distance between two opposite sides of an object. The caliper you will use can accurately measure to 0.1 mm. You will then incubate the potato cylinders in a series of sucrose solutions. After the cylinders have incubated, you will again measure their length and diameter and determine if they have increased or decreased in size. This information will enable you to estimate the osmolarity (solute concentration) of the potato tuber tissue.

Question

What question is being investigated in this experiment?

Hypothesis

Hypothesize about the osmolarity of potato tuber tissue.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Practice measuring with the vernier caliper (Figure 3.7a, b).
   a. Identify the following parts of the caliper and add these labels on Figure 3.7a: *stationary arm, movable arm, ruler, vernier scale.* Notice that the numbers on the bottom ruler scale are centimeters; each graduated line is 1 mm.
   b. Choose a small object (a coin will work) and place it between the two arms, adjusting the movable arm until both arms just touch the object.

FIGURE 3.7a
Vernier caliper. Identify the stationary arm, movable arm, ruler, and vernier scale.

c. Note the 0 mark on the vernier scale (Figure 3.7b). The graduated line on the ruler just to the left of the 0 mark is the distance between the caliper arms measured in whole millimeters. In Figure 3.7b, that number is 22 mm. Write that number for your object as the answer in blank (1) below.

d. Look at the graduated lines between 0 and 10 on the vernier scale. Note the line on the vernier scale that exactly matches with a line on the ruler. That line on the vernier scale is the measurement in tenths of a millimeter, which should be added to the whole-millimeter reading. In Figure 3.7b, that number is 4. Write the measurement in tenths of a millimeter for your object as the answer in blank (2) below.

What is the size of your object?

1. _____

2. _____

Total measurement: _____

When you know how to measure using the caliper, proceed to the next step.

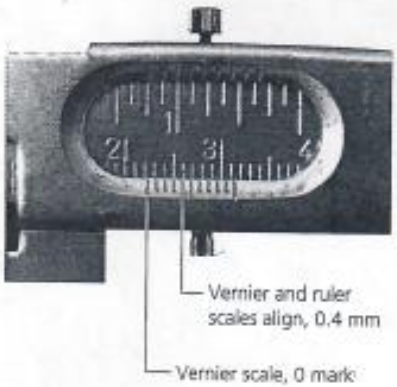

FIGURE 3.7b
Enlarged vernier scale. The correct measurement is 22.4 mm.

2. Obtain 100 mL of DI water and 100 mL of each of the sucrose solutions. Put each solution in a separate, appropriately labeled 250-mL beaker or paper cup.

Use cork borers and razor blades with extreme care! To use the cork borer, hold the potato in such a way that the borer will not push through the potato into your hand.

3. Use a sharp cork borer to obtain seven cylinders of potato. Push the borer through the length of the potato, twisting it back and forth. When the borer is filled, remove it from the potato and push the potato cylinder out of the borer. You must have seven complete, undamaged cylinders at least 5 cm long.

4. Line up the potato cylinders and, using a sharp razor blade, cut all cylinders to a uniform length, about 5 cm, removing the peel from the ends.

5. Place all seven potato samples in a petri dish, and keep them covered to prevent their drying out.

In subsequent steps, treat each sample individually. Work quickly. To provide consistency, each person should do one task to all cylinders (one person wipe, another measure, another record data).

6. Remove a cylinder from the petri dish, and place it between the folds of a paper towel to blot sides and ends.
7. Using the caliper, measure the length and diameter of the cylinder to the nearest 0.1 mm, and record these measurements in Table 3.6 in the Results section. To measure, both arms of the caliper should touch but not compress the cylinder.
8. Transfer the cylinder to the 0 *M* (water) beaker.
9. Note the time the cylinder is placed in the 0 *M* beaker. Time: _____.
10. Repeat steps 6 to 8 with each cylinder, placing the cylinders in the appropriate incubating solution from 0.1 to 0.6 *M*.

Be sure that the initial length and diameter of the cylinder placed in each test solution are accurately recorded.

11. Incubate from 1.5 to 2 hours. (During this time period, you will be performing other lab activities.)
12. Swirl each beaker every 10 to 15 minutes as the cylinders incubate.
13. At the end of the incubation period, record the time each cylinder is removed from a solution. Time: _____.

    Calculate the approximate incubation time in Table 3.6.
14. Remove the cylinders in the chronological order in which they were initially placed in the test solutions.
15. Blot each cylinder as it is removed (sides and ends), and use the vernier caliper to measure the length and diameter to the nearest 0.1 mm.
16. Finish recording your data in the Results section, and answer the questions in the Discussion section.

Results

1. Complete Table 3.6. To calculate the volume of a cylinder, use this formula:

$$\text{Volume of a cylinder } (\text{mm}^3) = \pi\,(\text{diameter}/2)^2 \times \text{length}$$
$$(\pi = 3.14)$$

To calculate percentage change in volume, use this formula:

$$\text{Percentage change in volume} = \frac{\text{change in volume}}{\text{initial volume}} \times 100$$

If the sample increases in volume, the value will be positive. If it decreases in volume, the value will be negative.

TABLE 3.6 Data for Experiment Estimating Osmolarity by Change in Volume

Download an Excel version from www.masteringbiology.com in the Study Area under Lab Media

Approximate time in solutions: _____

	Sucrose Molarity						
	0.0	0.1	0.2	0.3	0.4	0.5	0.6
Final diameter (mm)							
Final length (mm)							
Final volume (mm^3)							
Initial diameter (mm)							
Initial length (mm)							
Initial volume (mm^3)							
Change in volume (mm^3)							
% change in volume							

2. Plot percentage change in volume as a function of the sucrose molarity in Figure 3.8.
   a. Place a 0 in the middle of the *y*-axis. Choose appropriate scales.
   b. Label the axes of the graph: Determine dependent and independent variables, and place each on the appropriate axis (see Lab Topic 1).

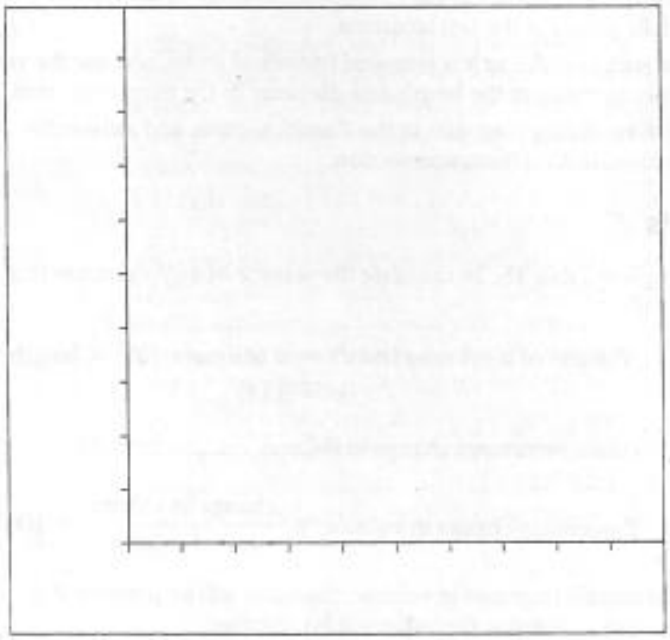

FIGURE 3.8

_____

_____

_____

c. Graph your results. Volume increase should be above the zero change line on the "percentage change in volume" axis. Volume decrease should be below the zero change line.

d. Construct a curve that best fits the data points. Use this curve to estimate the osmolarity of the potato tuber.

e. Compose an appropriate figure title.

Discussion

1. At what sucrose molarity does the curve cross the zero change line on the graph?

2. Explain how this information can be used to determine the osmolarity of the potato tuber tissue.

3. In more dilute concentrations of sucrose, the volume of the potato pieces _____ (increases/decreases) after incubation. What forces other than solute concentration will have an impact on the amount of water taken up by the pieces?

4. Estimate the osmolarity of the potato tuber tissue.

Reviewing Your Knowledge

1. Once you complete this lab topic, you should be able to define and use the following terms. Provide examples if appropriate.
selectively permeable, solvent, solute, diffusion, osmosis, hypotonic, hypertonic, isotonic, turgor pressure, osmolarity, water potential, Brownian movement, lysis, crenate, plasmolysis, plasmolyzed, turgid
2. Compare the response of plant and animal cells placed in hypertonic, isotonic, and hypotonic solutions.
3. Students conduct the following experiment by placing a selectively permeable dialysis bag in a beaker containing a 5% solution of fructose (monosaccharide). The dialysis bag contains a 10% solution of albumin (large protein). Sketch the experimental design in the margin of your lab manual. Answer the following questions:
At the beginning of the experiment is the solution in the beaker hypertonic, hypotonic, or isotonic?

After 30 minutes the students test the solutions for the presence of albumin and fructose. Was albumin present in the bag? In the beaker?
Was fructose present in the bag? In the beaker?
Did the dialysis bag increase or decrease in volume?