Diffusion and Osmosis

Passive Movement of Molecules in Biological Systems

Learning Objectives

By the end of this exercise you should be able to:

1. Observe Brownian movement and understand its relationship to molecular movement.
2. Explain the factors controlling a substance's direction and rate of diffusion.
3. Determine the direction and relative rates of diffusion of molecules of different sizes.
4. Determine the direction and rate of osmosis into and out of simulated cells in hypotonic, hypertonic, and isotonic environments.
5. Describe how hypotonic, hypertonic, and isotonic solutions affect the volume and integrity of blood cells.
6. Describe how a hypertonic solution affects the volume and integrity of plant cells.

MULTI-MEDIA **Please visit www.mhhe.com/vodopich10e to review multi-media resources tailored to this lab.**

All molecules display random thermal motion, or kinetic energy; this is why a dissolved molecule tends to move around in a solution. Kinetic energy causes molecules to diffuse outward from regions of high concentration to regions of lower concentrations. This random movement is constant, but the net movement of molecules from high to low concentration continues until the distribution of molecules becomes homogenous throughout the solution. For example, when a dye dissolves in a container of water, the dye disperses as the crystal dissolves. The rate of dispersal depends on the concentration of the dye, the size of the dye molecules, the temperature of the solution, and the density of the solvent. Regardless of this rate, the dye will eventually become uniformly distributed throughout the solution. This phenomenon is easily illustrated by placing a drop or crystal of dye into a glass of water (fig. 9.1).

In this exercise you will study the diffusion of molecules in artificial and living systems.

BROWNIAN MOVEMENT

Heat causes **random motion** of molecules and passively moves molecules in biological systems. Although we cannot directly see molecules move, we can see small particles move after they collide with moving molecules. This motion was originally described in 1827 by Robert Browning as he observed dead pollen grains in water and viewed them with a microscope. The pollen grains were being jostled by collisions from water molecules. **Brownian movement** is visible using your microscope's high magnification. Carmine red dye mixed with soap produces a good suspension of small particles. The particles of red dye are small enough to vibrate when water molecules bump into them.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 9.1

Observe Brownian movement

1. Place a small drop of a carmine red suspension on a microscope slide and cover the drop with a coverslip.

Figure 9.1

Beakers of water before and after diffusion of a dye. Random movements of water and dye molecules drive diffusion, eventually resulting in a uniform distribution of the dye. Convection currents may also help distribute the dye in these solutions.

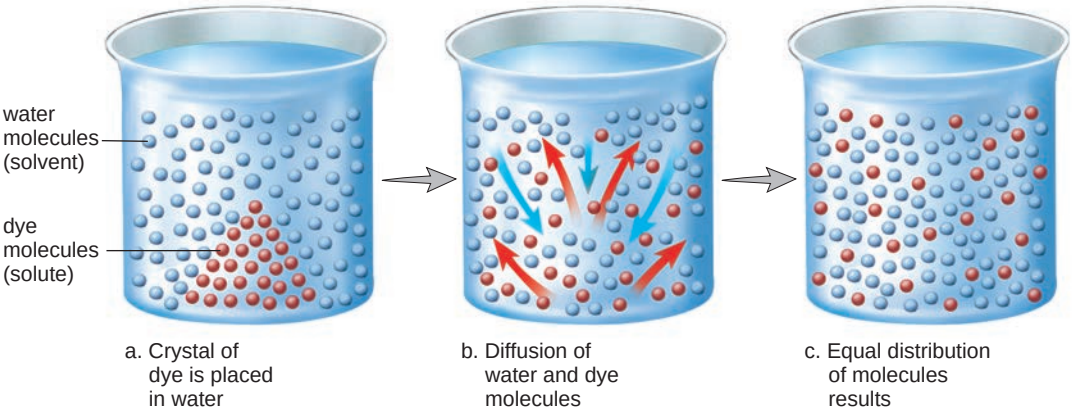


Figure 9.2

Process of diffusion. Diffusion is spontaneous, and no chemical energy is required to bring it about. (*a*) When a dye crystal is placed in water, it is concentrated in one area. (*b*) The dye dissolves in the water, and there is a net movement of dye molecules from higher to lower concentration. There is also a net movement of water molecules from a higher to a lower concentration. (*c*) Eventually the water and dye molecules are equally distributed throughout the container.

2. Focus first at low magnification; then rotate to higher power (40×). Be careful not to get dye on the objective lens.
3. Fine focus the image. At first the field of view will appear uniformly reddish gray. But with sharp focus, you will see thousands of small particles vibrating rapidly.
4. Check with your instructor to determine if your microscope has oil immersion magnification and if you need this to easily view the particles. If needed, follow their instructions for using this objective.
5. Leave the microscope light on. Observe any changes in motion with increased heat.

Question 1

a. Briefly describe your observation of the moving pigment particles.

b. Does the movement of particles change visibly with heat? If so, how?

DIFFUSION

In biological systems, substances often move through solutions and across membranes in a predictable direction. This passive, directional movement of molecules is **diffusion** (fig. 9.2). The *direction* of diffusion depends on the presence of a gradient of concentration, heat, and pressure. Specifically, molecules diffuse from an area of high concentration, heat, and pressure to an area of low concentration, heat, and pressure. The *rate* of diffusion is determined by the steepness of the gradient and other characteristics of the specific molecule in question, such as its size, polarity, or solubility.

Even though temperature, pressure, and concentration all affect diffusion, temperature and pressure are relatively constant in most biological systems. Therefore, concentration is usually the best predictor of a substance's direction of diffusion. But remember that temperature and pressure gradients may also affect diffusion.

Diffusion and Molecular Weight

Before your class meeting your instructor inoculated some petri plates containing agar with either potassium permanganate (molecular weight = 158 g $mole^{-1}$), malachite green (molecular weight = 929 g $mole^{-1}$), or methylene blue (molecular weight = 374 g $mole^{-1}$).

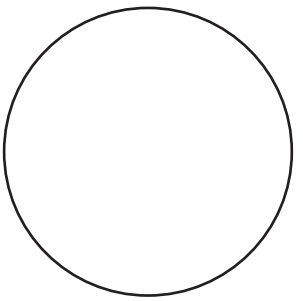
Question 2

Which would you predict would diffuse faster: a substance having a high molecular weight or a substance having a low molecular weight? Why?

Procedure 9.2

Observe diffusion as affected by molecular weight

1. Examine one of the prepared agar plates and note the three halos of color. These halos indicate that the chemicals have diffused away from the two original spots and moved through the agar.
2. Measure the halos with a ruler.
3. Record within the outline of a petri dish your observations of the size of each halo.



Question 3

a. Considering the different molecular weights of potassium permanganate, malachite green, and methylene blue, which should have the larger halo after the same amount of time? Why?

b. Do molecules stop moving when diffusion stops? Explain your answer.

DIFFUSION AND DIFFERENTIALLY PERMEABLE MEMBRANES

Membranes surround cells and organelles and organize an immense number of simultaneous reactions. However, the barrier imposed by a cellular membrane does not isolate a cell. Instead, it allows a cell to selectively communicate with its environment. Membranes are "alive" in the sense that they respond to their environment and allow some molecules to pass while retarding others. Thus, membranes are selective and **differentially permeable** (fig. 9.3). This selective permeability results from the basic structure of membranes. Membranes have a two-layered core of nonpolar lipid molecules that selects against molecules not readily soluble in lipids. You'll learn more about membrane structure in Exercise 10.

Membrane permeability to a solute depends on a combination of the solute's size, charge (ions), polarity, and lipid solubility. **Polar molecules** have postively charged areas and negatively charged areas. **Nonpolar molecules** have no local areas of charge. Small, uncharged, nonpolar, lipid-soluble molecules pass most easily through the lipid core of a membrane (see fig. 10.4).

In general, small molecules pass through a membrane more easily than do large molecules. We can demonstrate membrane selection for molecular size by using a bag made from **dialysis tubing** to model a differentially permeable membrane. **Dialysis** is the separation of dissolved substances by means of their unequal diffusion through a differentially permeable membrane. Dialysis membranes (or tubing) are good models of differentially permeable membranes because they have small pores that allow small molecules such as water molecules to pass but block large molecules such as glucose. However, remember that living cell membranes also discriminate among molecules based on charge and solubility whereas dialysis tubing does not. Dialysis tubing is only a physical model of a cell and its selectivity is based only on molecular size.

Examine some dialysis tubing. Although the dried material looks like a narrow sheet of cellophane, it is a flattened, open-ended tube.

In procedure 9.3 you will use two indicators: **phenolphthalein** and **iodine.** Phenolphthalein is a pH indicator that turns red in basic solutions (see Exercise 5). Iodine is a starch indicator that changes from yellow to dark blue in the presence of starch (see Exercise 6).

Procedure 9.3

Observe diffusion across a differentially permeable membrane

1. Obtain four pieces of string or dialysis clips and two pieces of water-soaked dialysis tubing approximately 15 cm long.
2. Seal one end of each bag by folding over 1–2 cm of the end. Then accordion-fold this end and tie it tightly with monofilament line or string (fig. 9.4). The ends of the tube must be sealed tightly to prevent leaks.

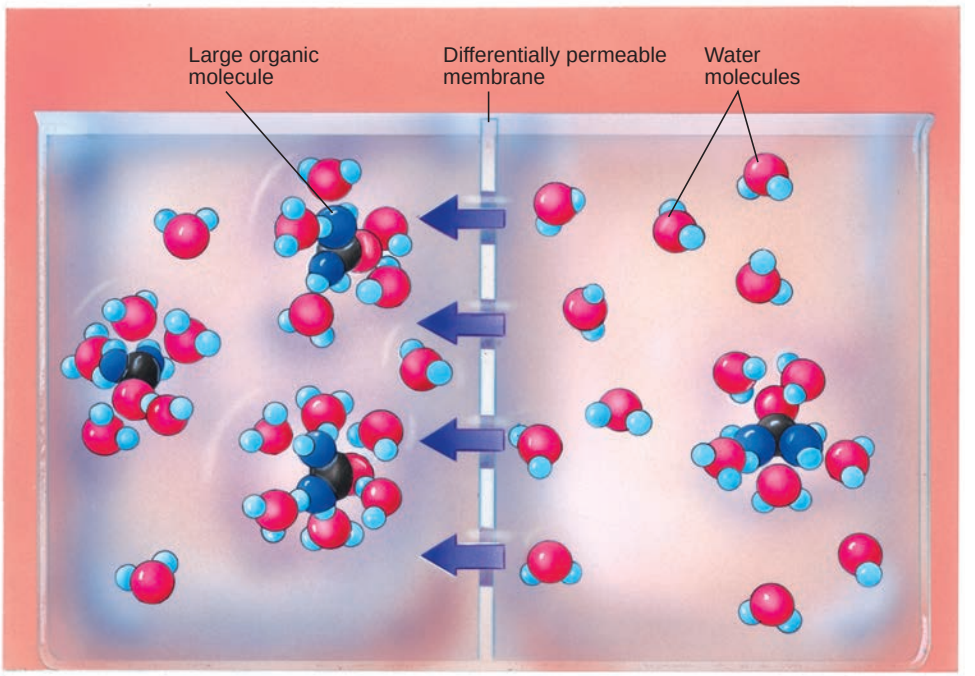


Figure 9.3
A differentially permeable membrane prevents the movements of some molecules but not others. Arrows indicate the movement of small molecules, such as water, from an area of high concentration to an area of lower concentration. The large molecules cannot pass through the membrane.

3. Roll the untied end of each tube between your thumb and finger to open it and form a bag.
4. Use either a graduated cylinder or pipet to fill one tube with 10 mL of water and add three drops of phenolphthalein. Seal the open end of the bag by folding the end and tying it securely.
5. Fill the other bag with 10 mL of starch suspension. Seal the open end of the bag by folding the end and tying it securely.
6. Gently rinse the outside of each bag in tap water.
7. Fill a beaker with 200 mL of tap water and add 10 drops of 1 M sodium hydroxide (NaOH). Submerge the dialysis bag containing phenolphthalein in the beaker.

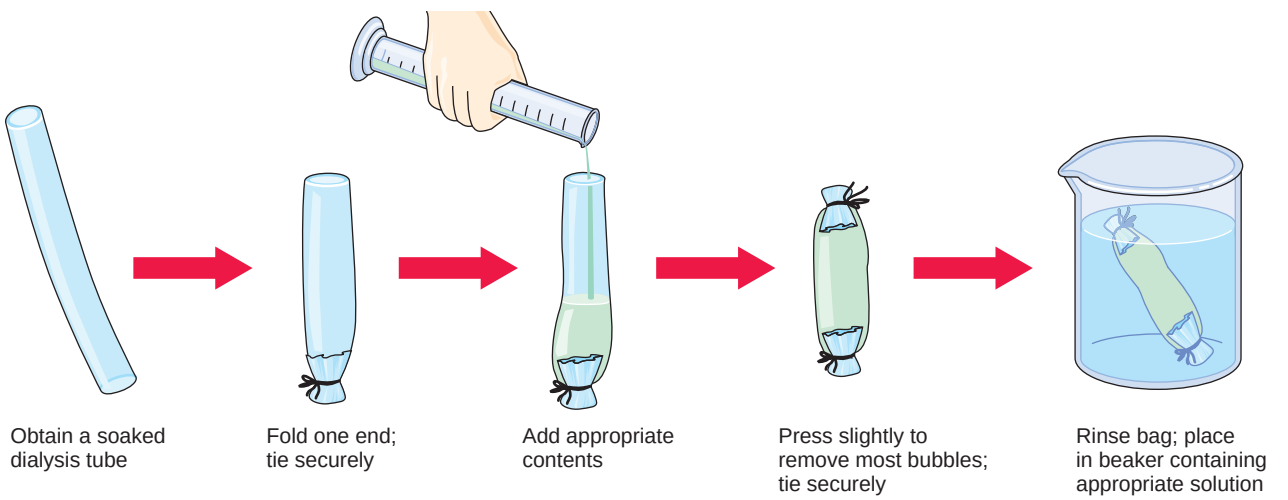


Figure 9.4
Preparation of dialysis tubing as a model of a cell surrounded by a differentially permeable membrane.

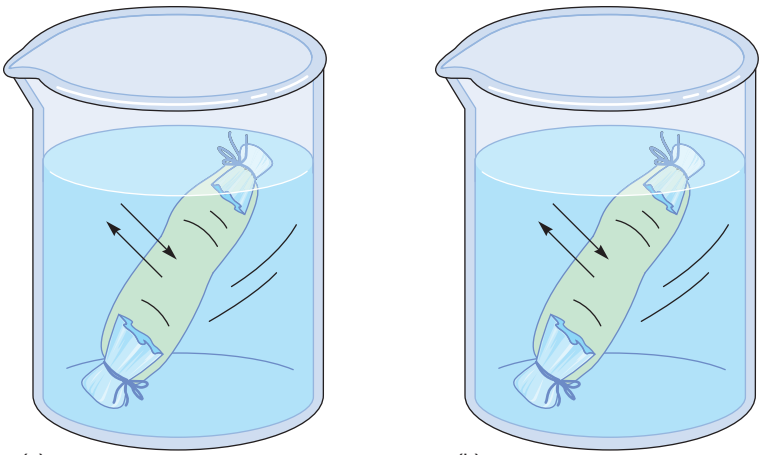


Figure 9.5

(*a*) Movements and reaction of sodium hydroxide and phenolphthalein through a differentially permeable membrane. (*b*) Movements and reaction of iodine and starch. Record the results of your experiment on this diagram.

Do not spill the NaOH. It is extremely caustic.

8. Fill a beaker with 200 mL of tap water and add 20–40 drops of iodine. Submerge the dialysis bag containing starch in the beaker.

9. Observe color changes in the two bags' contents and the surrounding solutions.

10. In this experiment some of the solutes can move through the membrane and some cannot. Water can freely move through the membrane, but the movement of water is not of interest in this experiment.

11. Record in figure 9.5 the color inside and outside the bags. Label the contents inside and outside the bags.

Question 4

a. Describe color changes in the two bags and their surrounding solutions.

b. For which molecules and ions (phenolphthalein, iodine, starch, Na^+, OH^-) does your experiment give evidence for passage through the semipermeable membrane?

c. What characteristic distinguishes those molecules and ions passing through the membrane from those that do not pass through the membrane?

OSMOSIS AND THE RATE OF DIFFUSION ALONG A CONCENTRATION GRADIENT

The speed at which a substance diffuses from one area to another depends primarily on the concentration gradient between those areas. For example, if concentrations of a diffusing substance at the two areas differ greatly, then diffusion is rapid. Conversely, when the concentration of a substance at the two areas is equal, the diffusion rate is zero and there is no net movement of the substance.

Osmosis is diffusion of water across a differentially permeable membrane. Osmosis follows the same laws as diffusion but always refers to water, the principal solvent in cells. A **solution** is a homogenous, liquid mixture of two or more kinds of molecules. A **solvent** is a fluid that dissolves substances, and a **solute** is a substance dissolved in a solution.

We can simulate osmosis by using dialysis bags to model cells under different conditions and measuring the direction and rate of osmosis. Each of the four dialysis bags in the following experiment is a model of a cell. Bag A simulates a cell with a solute concentration that is hypotonic relative to its environment. **Hypotonic** describes a solution with a lower concentration of solutes, especially those solutes that do not pass across the surrounding membrane. Water moves across semipermeable membranes out of hypotonic solutions. Conversely, the solution surrounding bag A is hypertonic relative to the cell. **Hypertonic** refers to a solution with a high concentration of solutes.

Bag B represents a cell whose solute concentration equals the concentration in the environment; this cell (bag B) is isotonic to its environment. **Isotonic** refers to two solutions that have equal concentrations of solutes. Bags C and D are both hypertonic to their environment and have higher solute concentrations than the surrounding environment. Remember that the solute (sugar) does not pass through the membrane—only the water does.

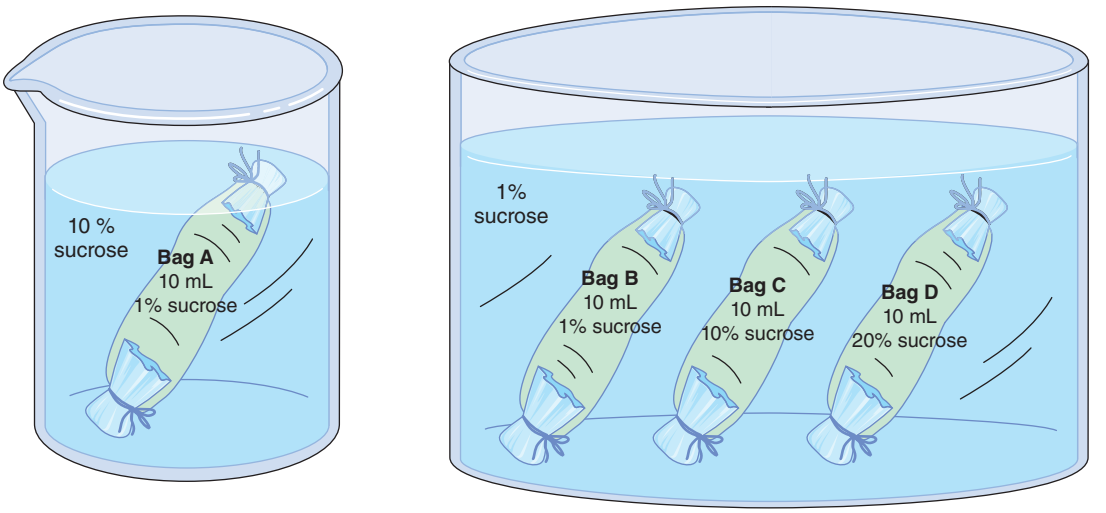


Figure 9.6
Experimental setup for four cellular models used to measure the rate of osmosis.

Table 9.1

Changes in Weight of Dialysis Bags Used as Cellular Models*

	0 Min	15 Min		30 Min		45 Min		60 Min	
	Initial Weight	Total Weight	Change in Weight	Total Weight	Change in Weight	Total Weight	Change in Weight	Total Weight	Change in Weight
Bag A									
Bag B									
Bag C									
Bag D									

*Each change in weight is only for the previous 15-min interval.

NOTE

Start this experiment at the beginning of the lab period so that you'll have enough time to see results.

Procedure 9.4

Observe osmosis across a concentration gradient

1. Obtain eight pieces of string and four pieces of water-soaked dialysis tubing each 15 cm long. Seal one end of each tube by folding and tying it tightly.
2. Open the other end of the tube by rolling it between your thumb and finger.
3. Fill the bags with the contents shown in figure 9.6. To label each bag, insert a small piece of paper with the appropriate letter (A, B, C, or D written on it in pencil).
4. For each bag, loosely fold the open end and press on the sides to push the fluid up slightly and remove most of the air bubbles. Tie the folded ends securely, rinse the bags, and check for leaks.
5. Gently blot excess water from the outside of the bags and weigh each bag to the nearest 0.1 g.
6. Record these initial weights in table 9.1 in the first column.
7. Place bags B, C, and D in three individual beakers or one large bowl filled with 1% sucrose (fig. 9.6). Record the time.
8. Place bag A in a 250-mL beaker and fill the beaker with 150 mL of 10% sucrose. Record the time.
9. Remove the bags from the beakers at 15-min intervals for the next hour (or at intervals indicated by your instructor), gently blot them dry, and weigh them to the nearest 0.1 g. Handle the bags delicately to avoid leaks, and quickly return the bags to their respective containers.
10. During the 15-min intervals, use your knowledge of osmosis to make hypotheses about the direction of water flow in each system (i.e., into or out of bag), and the extent of water flow in each system (i.e., in which system will osmosis be most rapid?).
11. For each 15-min interval record the total weight of each bag and its contents in table 9.1. Then calculate and record in table 9.1 the change in weight since the previous weighing.

Procedure 9.5

Graph osmosis

1. Use the graph paper at the end of this exercise to construct a graph with *Total Weight (g)* versus *Time (min)*. *Total Weight* changed in response to differences in the independent variable, so *Total Weight* is the **dependent variable.** The dependent variable is always graphed on the vertical axis. *Time* is the variable that you established and actively controlled and, therefore, is the **independent variable.** The independent variable is always graphed on the horizontal axis.
2. Graphs must have a title (e.g., Relationship between Time and Weight Gain), correctly labeled axes (e.g., *Total Weight*, *Time*), a label showing measurement units (e.g., g and min), and values along each axis (e.g., 0, 15, 30, 45, 60). Include these in your graph.
3. Plot the data for total weight at each time interval from table 9.1.
4. Include the data for all four bags as four separate curves on the same graph.

Question 5

a. Did water move across the membrane in all bags containing solutions of sugar?

b. In which bags did osmosis occur?

c. A concentration gradient for water must be present in cells for osmosis to occur. Which bag represented the steepest concentration gradient relative to its surrounding environment?

d. The steepest gradient should result in the highest rate of diffusion. Examine the data in table 9.1 for Change in Weight during the 15- and 30-min intervals. Did the greatest changes in weight occur in cells with the steepest concentration gradients? Why or why not?

Question 6

a. Refer to your graph. How does the slope of a segment of a curve relate to the rate of diffusion? If so, how?

b. What influence on diffusion (i.e., temperature, pressure, concentration) causes the curves for bags C and D eventually to become horizontal (i.e., have a slope = 0)?

WATER POTENTIAL

Plants need to balance water uptake and loss as it moves from one part of a plant to another and in and out of cells by osmosis. However, the concentration gradient of water and solutes doesn't solely determine the direction and rate of water movement. Physical pressure influenced by cell walls and evaporation is also important. Plant physiologists refer to the combined effects of concentration and pressure such as that from cell walls as **water potential;** water will flow from an area of high water potential to an area of low potential. Both high water concentration (low solute concentration) and high pressure increase water potential. Similarly, high solutes and low pressure decrease water potential. In simple terms, water flows through a plant from the higher water potentials of the root tissues toward the lower water potentials of leaves. These lower potentials in leaves are created by their loss of water to the atmosphere (see Exercise 33). In the following procedure you will measure the concentration of solutes in potato cells and relate this concentration to water potential.

Procedure 9.6

Determine the concentration of solutes in living plant cells

1. Locate the five beakers prepared by your instructor with five concentrations of salt (NaCl) solution.
2. The cylinders of potato that you see in the solutions were all originally the same size (i.e., the same length or weight). Check the beaker labels to determine which measure of size (length or weight) you will be using as your data.
3. Record the initial values in table 9.2.
4. Carefully remove three of the potato cylinders from each solution and measure their size.
5. Record your data in table 9.2.
6. Calculate the mean change in size and record the data in table 9.2.
7. Your instructor may ask you to graph your data (see Question 7f). Follow his or her instructions.

Question 7

a. Which potato cylinders increased in size or weight? Why?

Table 9.2

Change in Length of Potato Cylinders Surrounded by Different Salt Concentrations

Concentration of Salt Solution (%)	Initial Size of Cylinders (millimeters or grams)	Changes in Size of Three Sample Cylinders			Mean Change in Size
0	_____	_____	_____	_____	_____
0.9	_____	_____	_____	_____	_____
5	_____	_____	_____	_____	_____
10	_____	_____	_____	_____	_____
15	_____	_____	_____	_____	_____

b. Which solution(s) contained a higher concentration of solutes and therefore a lower water potential than in the potato cells? Explain your answer.

c. Which salt solution best approximated the water potential in the potato cells? How do you know this?

d. For a growing potato plant what would you predict as the water potential of the potato relative to the soil? Relative to the leaves?

e. What might be some sources of error in this experiment?

f. How could a graph of your data help you estimate the solute concentration of potato cells?

HEMOLYSIS OF BLOOD CELLS

Living red blood cells (erythrocytes) are good models for studying osmosis and diffusion in hypotonic, hypertonic, and isotonic solutions. Osmosis occurs when living cells are placed in a hypotonic or hypertonic environment and water diffuses into or out of the cell (fig. 9.7). For example, in the previous experiment, water moved into cells toward the low concentration of water. However, osmosis into animal cells increases the hydrostatic (i.e., water) pressure and may burst the cells because they lack cell walls. This destruction of a cell by the influx of water (causing the cell to burst) is called **lysis.** Such destruction of a red blood cell is called **hemolysis.** If water flows out of a cell into a hypertonic solution, the cell will shrivel and become crenate.

Detect hemolysis and crenation in blood cells in three different solutions using the following procedure.

Procedure 9.7

Observe hemolysis

1. Obtain and label three test tubes and fill them with the solutions listed in table 9.3.
2. Add four drops of fresh sheep's blood to each tube.

Wash your hands thoroughly after working with blood products. Always handle sheep blood with caution and avoid skin contact.

3. Cover each tube with Parafilm and invert the tubes to mix the contents.
4. Hold each tube in front of a printed page and determine if you can read the print through the solution (fig. 9.8). Record your results in table 9.3.
5. Obtain a microscope, slide, and coverslip.
6. Use an eyedropper or pipet to obtain one drop from each tube. Make a wet mount and examine the blood cells. Use low magnification first and then higher magnification.
7. Record in table 9.3 the cell's condition as crenate, normal, or lysed.

Question 8

a. Through which test tubes could you read the printed page? Why?

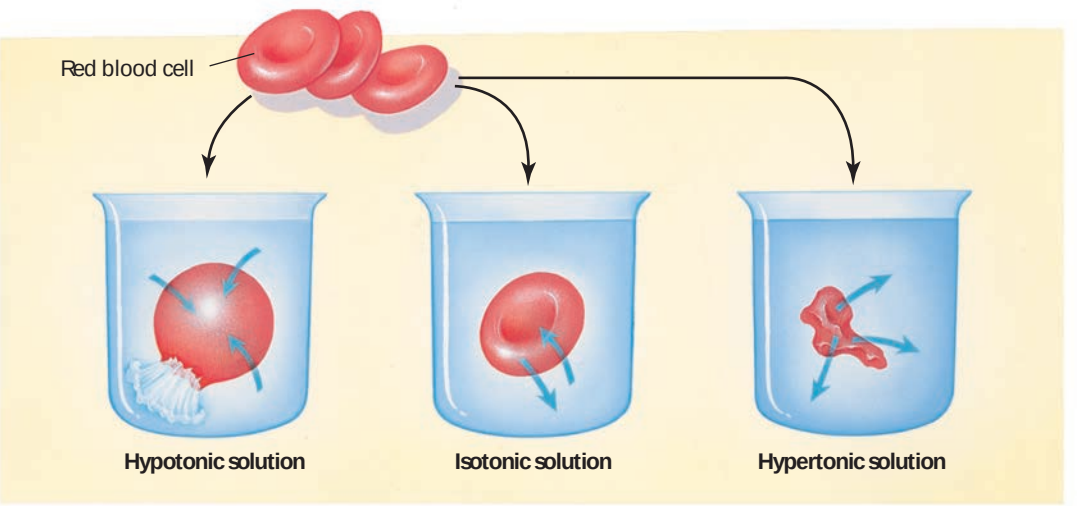


Figure 9.7
Osmosis of water surrounding animal cells. When the outer solution is hypotonic with respect to the cell, water will move into the cells and the cells will lyse; when it is hypertonic, water will move out of the cells and the cells will shrink (i.e., become crenate).

Table 9.3

Hemolysis of Red Blood Cells Exposed to Three Solutions with Different Solute Concentrations

Tube	Contents	Readable Print (yes/no)	Cell Condition (crenate/normal/ lysed)
1	5 mL 10% NaCl	_____	_____
2	5 mL 0.9% NaCl	_____	_____
3	5 mL distilled water	_____	_____

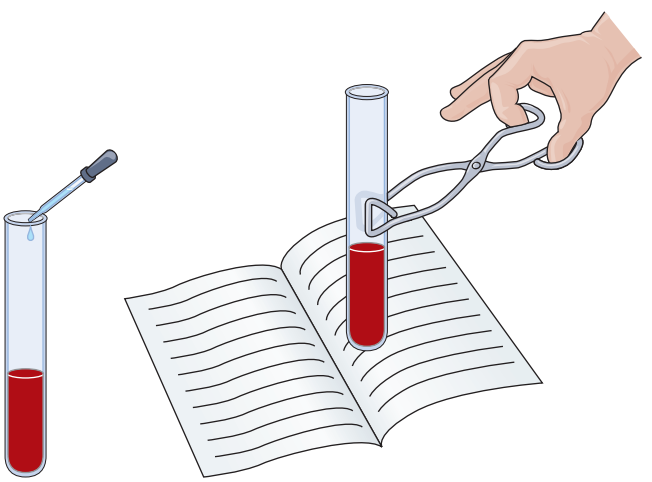


Figure 9.8
Experimental setup for determining hemolysis. Hypertonic solutions will hemolyze cells.

b. Which concentration of NaCl lysed the cells?

c. Which of the three solutions most closely approximates the solute concentration in a red blood cell? How do you know?

PLASMOLYSIS OF PLANT CELLS

Plasmolysis is the shrinking of the cytoplasm of a plant cell in response to diffusion of water out of the cell and into a hypertonic solution (high salt concentration) surrounding the cell (fig. 9.9). During plasmolysis the cellular membrane pulls away from the cell wall (fig. 9.10).

In procedure 9.8 you will examine the effects of highly concentrated solutions on osmosis and cellular contents.

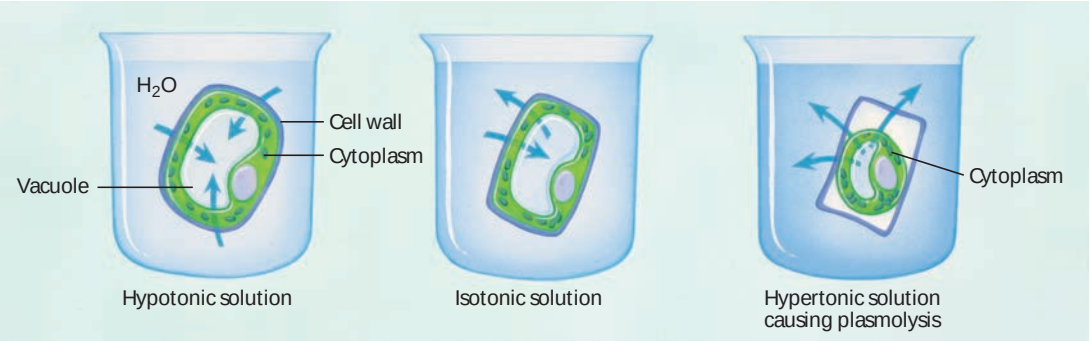
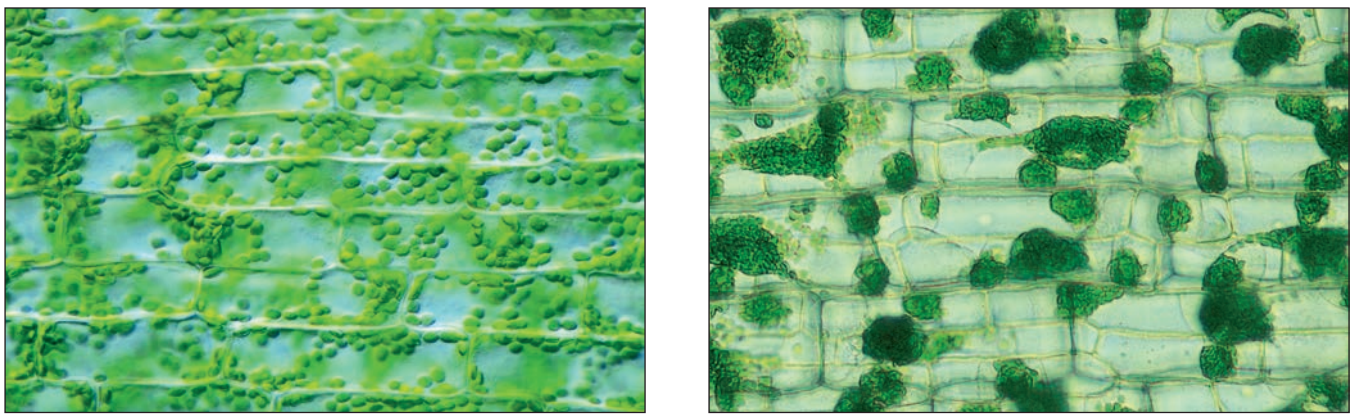


Figure 9.9

Osmosis of water into and out of plant cells. In most plant cells the large central vacuole contains a high concentration of solutes (i.e., the environment surrounding the cell is hypotonic to the cell), so water tends to diffuse into the cells, causing the cells to swell outward against their rigid cell walls. However, if a plant cell is immersed in a high-solute (hypertonic) solution, water will leave the cell, causing the cytoplasm to shrink and pull away from the cell wall.



(a) (b)

Figure 9.10

(*a*) Turgid *Elodea* cells. (*b*) Plasmolyzed *Elodea* cells showing the effects of exposure to a hypertonic solution.

Procedure 9.8

Observe plasmolysis

1. Prepare a wet mount of a thin layer of onion epidermis or *Elodea* leaf. Examine the cells.
2. Add two or three drops of 30% NaCl to one edge of the coverslip.
3. Wick this salt solution under the coverslip by touching a piece of absorbent paper towel to the fluid at the opposite edge of the coverslip.
4. Examine the cells. The cytoplasm is no longer pressed against the cell wall. This shrinkage is **plasmolysis.**

Question 9

a. Why did the plant cells plasmolyze when immersed in a hypertonic solution?

b. What can you conclude about the permeability of the cell membrane (i.e., the membrane surrounding the cytoplasm) and vacuolar membrane (the membrane surrounding the vacuole) to water?

To observe the effects of cellular plasmolysis on a larger scale, compare petioles of celery that have been immersed overnight in distilled water or in a salt solution.

Question 10

What causes crispness (i.e., firmness, crunchiness) in celery?

INVESTIGATION

Determining the Concentrations of Solutes in Plant Tissue

Observation: Water moves into and out of cells along a concentration gradient. The more solutes that are present in cells, the greater the tendency for water to move into the cells.

Question: What is the approximate concentration of solutes in a piece of apple?

a. Establish a working lab group and obtain Investigation Worksheet 9 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 9 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. Why must particles be extremely small to demonstrate Brownian movement?

2. What is the difference between molecular motion and diffusion?

3. If you immerse your hand in distilled water for 15 min, will your cells lyse? Why or why not?

4. Your data for diffusion of water across a differentially permeable membrane in response to a sucrose gradient could be graphed with *Change in Weight* on the vertical axis rather than *Total Weight*. How would you interpret the slope of the curves produced when you do this?

5. How do cells such as algae and protists avoid lysis in fresh water?

WRITING TO LEARN BIOLOGY

Where in an animal might pressure affect diffusion of a substance?