Diffusion and Osmosis
Passive Movement of Molecules in Biological Systems

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.

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 collisions 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. 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.
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.
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.

![Caution: 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; that is, 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.

**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 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.
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** |
| **Bag A** | **Bag B** | **Bag C** | **Bag D** |
| **Initial Weight** | **Total Weight** | **Change in Weight** | **Total Weight** | **Change in Weight** | **Total Weight** | **Change in Weight** | **Total Weight** | **Change in Weight** |
| | | | | | | | | |

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

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 25% 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?

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?

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?

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?
### TABLE 9.2

<table>
<thead>
<tr>
<th>Concentration of Salt Solution (%)</th>
<th>Initial Size of Cylinders (millimeters or grams)</th>
<th>Changes in Size of Three Sample Cylinders</th>
<th>Mean Change in Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 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?

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### 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 bursts 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.

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**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.

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**Question 8**

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

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?
**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**

<table>
<thead>
<tr>
<th>Tube</th>
<th>Contents</th>
<th>Readable Print (yes/no)</th>
<th>Cell Condition (crenate/normal/lysed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 mL 10% NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5 mL 0.9% NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5 mL distilled water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 9.8**

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

**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.

**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.
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.

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 tissue?

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?