THE PLASMA MEMBRANE

EXERCISE

Transport Across the Plasma Membrane

Objectives

After completing this exercise, you should be able to:

1. Describe diffusion and osmosis
2. Distinguish between and define hypotonic, hypertonic, and isotonic solutions

Materials

- Diffusion: 2 agar Petri dishes per group, small millimeter rulers, forceps, methylene blue crystals, potassium permanganate crystals
- Diffusion and Osmosis Across a Dialysis Membrane (per group): dialysis tubing 12 cm long, 2 dialysis clips, scissors, Congo red or red food coloring, 40% sucrose solution, 500-mL beakers, distilled water, gram scale
- Osmosis in Living Red Blood Cells: animal blood (uncoagulated), disposable gloves, clean microscope slides and coverslips, 4 medicine droppers per group, compound microscope, filter paper

A. Passive Transport—Diffusion

Diffusion can occur in solids, liquids, or gas, and across the plasma membrane. The net movement of substances from a region of their greater concentration to a region of their lesser concentration is called moving substances “down the concentration gradient.” A concentration gradient indicates there is a difference in the concentration of molecules or ions inside the cell (intracellular) compared to outside the cell (extracellular).

In the following activity, diffusion of two crystals through a solid (agar) will be studied. Methylene blue has a molecular weight of 320, and potassium permanganate has a molecular weight of 158. The two crystals move at different rates through the agar, which is made up mostly of water.

The plasma membrane with its unique design is responsible for discriminatingly allowing substances into and out of a living cell. Active processes require cellular energy (ATP) to transport substances against their concentration gradients across the plasma membrane. Passive processes do not require the cell to expend energy because the kinetic energy of the particles causes them to move from an area of their higher concentration to an area of their lower concentration. In this exercise, we will look at the passive processes of diffusion and osmosis.
**ACTIVITY 1 Experiment: Diffusion**

**Lab Activity**

1 **Prediction:** With your lab group, predict which crystal will move faster. *(Hint: Use the molecular weights.)* Circle your answer: methylene blue or potassium permanganate.

2 **Materials:** Obtain the materials for diffusion.

3 **Data Collection:** Measure the diffusion rates of methylene blue and potassium permanganate.
   - Decide who will set up the experiment, who will time, who will measure, and who will record.
   - Carefully using forceps, place a large crystal of methylene blue on the surface of an agar Petri dish (Figure 5.1). Be careful not to drop any extra crystals on the agar surface.
   - Using the same technique, place a similar sized crystal of potassium permanganate on the other side of the Petri dish.
   - Using a millimeter (mm) ruler, measure the crystals’ diameter of diffusion at 15-minute intervals for at least 1 hour (or longer if desired).
   - After each observation, record the diffusion diameter of each crystal in millimeters (mm) in Table 5.1.

4 Clean up as directed by your instructor.

5 **Data Analysis:** Calculate the diffusion rates for each time period for the two crystals using Table 5.1.

6 Complete the Experimental Report with your lab group.

![Diffusion in agar plate setup.](image)

**TABLE 5.1 Diffusion Results**

<table>
<thead>
<tr>
<th>TIME (MIN)</th>
<th>DIFFUSION OF METHYLENE BLUE (DIFFUSION DIAMETER IN MM)</th>
<th>DIFFUSION OF POTASSIUM PERMANGANATE (DIFFUSION DIAMETER IN MM)</th>
<th>DIFFUSION RATE MM/MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
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<td></td>
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<tr>
<td>45</td>
<td></td>
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<td>60</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EXPERIMENTAL REPORT: DIFFUSION**

**Results:** Which substance moved faster?

**Discussion:** Discuss why the diffusion rates of the two substances differed.

**Conclusion:** State how molecular weight affects diffusion rate.
**B. Diffusion and Osmosis Across a Dialysis Membrane**

Osmosis is the diffusion of a solvent (dissolving medium which is water in living organisms) across a selectively permeable membrane that occurs in response to differences in solute (substance dissolved in solvent) concentrations. Water diffuses from an area of higher water concentration (lower solute concentration) to an area of lower water concentration (higher solute concentration). Osmosis is a passive process.

In the following activity, a sucrose solution is added to a dialysis membrane bag (semipermeable membrane) which is placed in distilled water. Dialysis membranes contain small pores that allow water and small solutes to cross. If water enters the bag, the weight of the bag will increase and if water leaves the bag, its weight will decrease.

The dialysis membrane used in this experiment contains pores that are large enough to allow diffusion of sucrose and red dye molecules into and out of the dialysis membrane bag.

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**ACTIVITY 2**  
**Experiment: Osmosis and Diffusion Across a Dialysis Membrane**

**NOTE:** This experiment should be set up at the beginning of class, so that changes can be observed and recorded throughout the lab time. The results become more dramatic the longer this experiment runs.

**Pre-Lab Activity**

1. Label Figure 5.2.

**Lab Activity**

1. **Prediction:** With your lab group, predict the direction of water and sucrose movement in Figure 5.2 by circling the correct italicized choice.
   - The net movement of water (measurable by weight) will be into or out of the dialysis bag (see Figure 5.2).
   - The net movement of sucrose will be into or out of the dialysis bag (see Figure 5.2).

2. **Materials:** Obtain materials for osmosis and diffusion across a dialysis membrane.

3. **Data Collection:** Observe osmosis and diffusion through a dialysis membrane and record your results in Table 5.2.
   - Decide who will set up the experiment, who will time, who will weigh and observe the color, and who will record.
   - Cut a 12-cm piece of dialysis tubing and *soak the bag in water* for 3 minutes to make it easier to open.
   - Fold over one end of the dialysis tubing and secure it with a dialysis clip.
   - Rub the open end of the dialysis tubing between your thumb and finger to separate the sides, and fill the dialysis tubing more than three-quarters full with the red 40% sucrose solution.

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**TABLE 5.2**  
**Dialysis Bag Results**

<table>
<thead>
<tr>
<th>TIME (MIN)</th>
<th>COLOR OF BEAKER WATER</th>
<th>CHANGE IN WEIGHT OF SUCROSE SOLUTION (GRAMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td></td>
<td>Start weight:</td>
</tr>
<tr>
<td>15 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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4. **Clean Up:** Clean up as directed by your instructor.

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**FIGURE 5.2** Dialysis bag setup.
EXPERIMENTAL REPORT: DIALYSIS BAG

Results:
1. Describe how the weight of the bag changed over time.

2. Describe how the color of the beaker water changed over time.

Discussion:
1. Identify the solutes and solvent in this experiment.

2. Why was the red dye added to the sucrose solution?

3. Which direction did osmosis occur?

4. Which direction did diffusion of solutes occur?

5. Did net osmosis and diffusion of solutes stop? Did the two solutions become isotonic? Explain.

6. In this experiment, what represented the plasma membrane, intracellular fluid, and extracellular fluid?

C. Osmosis Across a Living Plasma Membrane

Osmosis results in movement of water through a selectively permeable membrane from a solution with a lower solute concentration (hypotonic) into a solution with a higher solute concentration (hypertonic). The terms hypotonic (hypo- = deficient; -tonos = stretching) solutions or hypertonic (hyper- = excessive) solutions are used only when two solutions are compared. A solution’s tonicity is a measure of the solution’s ability to change the volume of a cell by changing water content. Water will move into the hypertonic solution until the solute concentrations of the two solutions equalize to become isotonic solutions (iso- = same) or if enough pressure is applied to stop the flow of water.

Red blood cells (RBCs) are good examples to use for this osmosis experiment because their shape changes dramatically when they are exposed to hypotonic or hypertonic solutions. Their normal shape is a round biconcave disc with a smooth plasma membrane. The plasma membrane does not allow salts in the RBC cytoplasm to leave the cell, but water can freely enter or leave the cell through the plasma membrane.

Under the microscope, RBCs look two-toned, with the center being lighter because of its concavity and thinness. If the cell loses most of its water by osmosis when put in a hypertonic solution, it becomes crenated or shriveled with spiked edges. If the cell gains a significant amount of water by being placed in a hypotonic solution, it swells and may eventually burst—a process called hemolysis (hemo- = blood; -lysis = break down). As a basis for comparing various solutions to blood, the salt content (NaCl) of blood is 0.9% and is the same salt content as a physiologic saline solution.

NOTE: Remember…salt shrivels and water swells (a cell).

SAFETY NOTE: Wear safety glasses and disposable gloves when handling body fluids or animal blood.
**Exercise 5 Transport Across the Plasma Membrane**

**Activity 3 Experiment: Osmosis in Living RBCs**

1. With your lab group, discuss and identify which solutions for this experiment are hypotonic, hypertonic, or isotonic to the RBCs (Figure 5.3). The isotonic solution is also called physiologic saline. The arrows in Figure 5.3 indicate the direction of water movement.
   - 0.9% saline solution ____________
   - 5% saline solution ____________
   - Distilled water ____________

2. In Figure 5.3, identify the shape of the RBC (normal, swollen, or crenated) and the type of solution into which the RBC is immersed (isotonic, hypertonic, or hypotonic).

3. **Prediction:** With your lab group, predict the net movement of water in all three types of solutions. Circle your answer, from the italicized choices, for all three situations:
   - In the isotonic solution, the RBCs will **swell**, crenate, or not change shape.
   - In the hypotonic solution, the RBCs will **swell**, crenate, or not change shape.
   - In the hypertonic solution, the RBCs will **swell**, crenate, or not change shape.

4. **Materials:** Obtain materials to observe osmosis in RBCs.

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**Figure 5.3 Osmosis in RBCs.**
5 Data Collection:
- Decide who will set up and initially observe each slide. Every member of your group should observe each slide.

Slide #1: 0.9% saline solution
- Place a drop of animal blood on a slide with a medicine dropper and cover with a coverslip. With a different medicine dropper, add a drop of 0.9% saline solution to the blood. Tilt the slide to intermix the two solutions, and cover with a coverslip.
- Using high power, observe the slide for changes in cell shape. Blood cells are very tiny and difficult to see under low power.
- Record shape of RBCs and type of solution into which they were placed. shape ____________ type of solution ____________

Slide #2: 5% saline solution
- Place a drop of animal blood on a second slide and cover with a coverslip. Observe the RBCs using high power. On one side of the coverslip, add one drop of 5% saline solution with a clean medicine dropper. Place filter paper or a small piece of paper towel on the opposite side of the coverslip to absorb the liquid and pull the 5% saline solution into the RBCs.
- Immediately observe the second slide under high power and watch for changes in cell shape. You may have to wait a few minutes for cell changes to occur.
- Record shape of RBCs and type of the solution into which they were placed. shape ____________ type of solution ____________

Slide #2 again: distilled water
- Using slide #2 again, add one drop of distilled water to the same edge of the coverslip that was used for the saline solution.
- Hold a piece of filter paper at the opposite edge of the coverslip, observe the paper absorb the saline solution, and watch as the distilled water is drawn under the coverslip and into the RBCs.
- Immediately observe the slide under high power and watch if the RBCs change shape.
- Record shape of RBCs and type of solution into which they were placed. shape ____________ type of solution ____________

6 Clean Up:
- Place blood-stained items (slides, coverslips, and droppers) in a 10% bleach solution or as directed by your instructor.
- Place gloves in an autoclavable bag or location indicated by your instructor.
- Wash down the lab counters with 10% bleach solution in a squirt bottle and wipe with paper towels wet with 10% bleach solution. Allow to air dry.
- Wash your hands with soap and water before leaving the lab area.

EXPERIMENTAL REPORT: OSMOSIS IN LIVING RBCS

Results: Describe how the RBC shape changes when placed in each solution.
- 0.9% saline solution __________________________
- 5% saline solution __________________________
- distilled water __________________________

Discussion:
1. Describe how extracellular solute concentration affects osmosis across the plasma membrane.

2. Describe how water movement affects the cell shape.

Conclusion: State which solution(s) caused osmosis in RBCs.
A. Transport Across the Plasma Membrane

Match the definition with the term.

   a. to shrink or shrivel
   b. water moving through selectively permeable membrane
   c. substance dissolved in a solution
   d. to burst a red blood cell
   e. difference between solute concentrations across a membrane
   f. same solute concentration on both sides of membrane
   g. lower concentration of solutes than in cytosol of cell
   h. a fluid that can contain dissolved substances
   i. higher concentration of solutes than in cytosol of cell
   j. random movement of particles from their greater concentration to their lesser concentration

____ 1. concentration gradient
____ 2. crenate
____ 3. diffusion
____ 4. hemolysis
____ 5. hypertonic solution
____ 6. hypotonic solution
____ 7. isotonic solution
____ 8. osmosis
____ 9. solute
____ 10. solvent
EXERCISE 5 TRANSPORT ACROSS THE PLASMA MEMBRANE

B. Diffusion

Select the correct lettered answer.

1. In the dialysis bag experiment, sucrose and red dye molecules are:
   (a) solutes  (b) solvents

2. If there is no concentration gradient, a substance will not have net movement.
   (a) true  (b) false

   (a) ATP  (b) kinetic energy

C. Osmosis

Select the correct lettered answer.

1. An isotonic solution will ____ an RBC.
   (a) crenate  (b) hemolyze  (c) cause no change to

2. A hypertonic solution will ____ an RBC.
   (a) crenate  (b) hemolyze  (c) cause no change to

3. A hypotonic solution will ____ an RBC.
   (a) crenate  (b) hemolyze  (c) cause no change to
A. Diffusion

Select the correct lettered answer in questions 1–3.

___ 1. White blood cells engulf bacteria. This is an example of diffusion.
   (a) true   (b) false

___ 2. Food cooking on the stove in the kitchen can be smelled in the living room. This is an example of diffusion.
   (a) true   (b) false

___ 3. Oxygen in the lungs moves into the bloodstream and carbon dioxide moves in the opposite direction. This is an example of diffusion.
   (a) true   (b) false

B. Osmosis

Select the correct lettered answer in questions 4–8.

___ 4. A test tube with blood in it has a particular solution added to it. After several minutes, the solution is not clear anymore, but becomes red. Which solution was added to the blood to obtain this result?
   (a) 0.9% saline   (b) 5% saline   (c) distilled water

___ 5. A 0.8% saline solution would be ____ to the cytosol of a cell.
   (a) hypotonic   (b) hypertonic   (c) isotonic

___ 6. If a 50% sugar solution had been used in the dialysis bag in Activity 2, there would be a faster rate of osmosis.
   (a) true   (b) false

___ 7. If you placed a peeled apple or potato in a 5% salt solution, it would ____
   (a) gain weight   (b) lose weight   (c) stay the same weight
8. A person’s hands become wrinkled after spending a long, relaxing time in the tub. Tub water does not have as
many solutes in it compared with the human body. The hands look wrinkled because ____.

(a) the tub water is hypotonic to body cells and water enters the cells
(b) the tub water is hypotonic to body cells and water leaves the cells
(c) the tub water is hypertonic to body cells and water enters the cells
(d) the tub water is hypertonic to body cells and water leaves the cells

C. Application

9. When a person becomes dehydrated, the amount of water in extracellular fluids such as blood decreases, causing the
solute concentration of these fluids to increase. State whether osmosis results in water entering or leaving cells.

10. Severe vomiting and diarrhea causes a loss of water and solutes from extracellular fluids. If a person was only given
water, what effect would this have on the solute concentration of extracellular fluids? Would osmosis result in water
entering or leaving cells?