

Diffusion and Cell Membranes – II

Objectives

1. Define the following terms: *hypotonic*, *isotonic* and *hypertonic*.
2. To determine if osmosis and diffusion both occur through a selectively permeable membrane.
3. To observe the effects of hypotonic, isotonic and hypertonic solutions on plant cells and animal cells.
4. Given any two solutions of differing osmotic potentials and separated by a selectively permeable membrane, state which solution is hypertonic and in which direction the net flow of water will occur.

Introduction

The term tonicity describes the relative concentration of solvent to solute in two solutions. A solution with the lower solute concentration is said to be hypotonic relative to the other solution. Conversely, the more concentrated solution is hypertonic relative to the first. If the solute concentrations of each solution are equal the solutions are isotonic with respect to each other. It is important to remember that these terms are relative terms, that is, the description of a solution as being hypertonic, hypotonic or isotonic depends on the solution it is being compared to. Traditionally, in biology, the cell is the frame of reference. An isotonic solution has the same solute concentration (and water concentration) as the cell; a hypertonic solution has a higher solute (and lower water) concentration than the cell; a hypotonic solution has a lower solute (and higher water) concentration than the cell.

If a cell in a hypotonic solution (low solute concentration) is enclosed in a rigid box, for example a plant cell surrounded by the rigid cell wall, the increasing water pressure inside the cell would cause water to flow back out of the cell towards the area of lower pressure. Eventually, equilibrium would be reached when the flow of water into the cell, due to the concentration differences, equals the flow of water out of the cell, caused by pressure differences. The pressure at equilibrium is called the osmotic pressure.

Since all cells contain molecules that cannot cross the plasma membrane, osmosis always occurs when cells are placed in dilute aqueous solutions. It is important, then, for cells to be able to regulate the flow of water into, and out of the cell, a process known as osmoregulation. In plant cells and bacterial cells, the cell wall prevents the cell from bursting by providing a rigid casing that helps regulate the osmotic pressure in the cell. In animals and many microorganisms, osmoregulatory organs or organelles are found. In animals the kidney adjusts the concentration of substances in the body fluids that bathe the cells. In microorganisms, like *Paramecium*, which live in freshwater, special organelles, called contractile vacuoles, accumulate and actively pump out water that flows into the cell by osmosis.

In this week's lab you will model a semi-permeable membrane and investigate tonicity by looking at the behavior of different types of cells in hypotonic, hypertonic and isotonic solutions.

Diffusion Through a Selectively Permeable Membrane

The plasma membrane of a cell is selectively permeable because it allows the diffusion of some substances and not others. Small, uncharged molecules diffuse freely across the plasma membrane, but charged molecules and large molecules cannot cross the membrane. The dialysis membrane used in this experiment simulates the activity of the plasma membrane.

Procedure

1. Obtain a piece of dialysis tubing and make a tight knot in one end with thread.
2. Fill the bag with solution A, a simulated "liquid" meal containing 10% glucose, 1% starch, 0.5% egg albumin, and 1% sodium chloride.
3. Tie the top of the tube with thread while expelling as much air as possible. The bag should be limp (flaccid).
4. Rinse the outside of the dialysis tube with distilled water.

5. Place the dialysis tube in a culture dish and add enough solution B to cover it. Solution B contains 0.5% sodium sulfate dissolved in water. Let the dish stand undisturbed for about 1½ hours.
6. Based on the recipes for solutions A and B, fill in the “Before” columns of table 1. Use a + to represent the presence and a – to represent the absence of a substance.

Table 1.

Substance	Inside Dialysis Tubing		Outside Dialysis Tubing	
	Before	After	Before	After
1. Starch				
2. Chloride Ion				
3. Sulfate ion				
4. Glucose				
5. Albumin				

7. After about 1½ hours, remove the dialysis tubing from the culture dish. Gently agitate the contents of the tubing and note any change in the tubing (Hint: is it more or less flaccid than when you started?)
8. Rinse the dialysis tubing with distilled water and carefully open the tubing. Empty the contents into a 100ml beaker. You can now test which ions and molecules crossed the membrane.
9. Obtain eight test tubes and prepare them as follows:
 - a. Into each of the first four test tubes, place 10 drops of the solution from inside the dialysis tubing. Label these I-1 to I-4.
 - b. Into the second set of four tubes, place 10 drops of the solution from outside the dialysis tubing. Label these O-1 to O-4.
10. Test for the presence of starch, albumin, glucose, sulfate ions, and chloride ions in the two sets of test tubes using the following test:
 - a. To the first test tube of each set, add 3 drops of IKI to test for starch. A blue-black color indicates a positive result.
 - b. To the second test tube of each set, add 1 drop of silver nitrate (AgNO_3) to test for chloride ions. A white precipitate indicates a positive result.
 - c. To the third test tube of each set, add 3 drops of 1% barium chloride (BaCl_2) to test for sulfate ions. A white precipitate indicates a positive result.
 - d. To test for the presence of glucose, dip a Clinistix into the fourth test tube of each set. Compare the results to the color chart on the side of the container.
 - e. To test for the presence of albumin, dip an Albustix into the fourth test tube of each set. Compare the results with the color chart on the side of the container.
11. Record your results in the “After” columns of table 1. Use a + to represent a positive test and a – to represent a negative test. Use these results to answer the following questions.
 1. At the start of the exercise, which solution (A or B) was hypertonic compared to the other (that is, which had the higher concentration of solutes)?
 2. Which solution gained water in the course of the exercise (A or B)?
 3. Which of the substances (starch, chloride ions, sulfate ions, glucose, albumin, and water) were able to pass through the membrane (in either direction)?

4. Which substance(s) moved out through the membrane?
5. Which substance(s) moved in through the membrane?
6. Why did each substance move in the direction it did?
7. By what process did the substances move across the membrane?
8. Why did some substances fail to pass through the membrane?
9. Would you expect all of the molecules of a diffusible substance to move across the membrane? Why?
10. Which of the following statements best describes the situation at equilibrium if you let the system stand for a long time?
 - a. No molecules move across the membrane.
 - b. All molecules cross the membrane equally often in either direction.
 - c. Molecules to which the membrane is permeable cross equally often in either direction.
 - d. Only water molecules cross the membrane equally often in either direction.
 - e. Molecules to which the membrane is permeable move across the membrane from a region of high concentration to a region of low concentration.
11. Did water move across the membrane? What is your evidence?
12. What is misleading about trying to equate the results of this exercise with how the cell membrane regulates passage of material?
13. Dialysis membrane is permeable to iodine (IKI). What result would you expect to see if you put IKI in solution B at the start of the exercise?

Osmosis and Tonicity

As you discovered last week, osmosis (the diffusion of water) occurs whenever two solutions of different solute concentration are separated by a selectively permeable membrane. The difference in solute concentration between the two solutions determines both the direction and rate of water flow. Water always diffuses from a hypotonic solution to a hypertonic solution; consequently, a cell placed in a hypotonic solution will gain water and a cell placed in a hypertonic solution will lose water.

The next three experiments explore tonicity (the solute concentration of a solution) using potato strips, red blood cells and *Elodea* cells.

Procedure: Potato strips

1. Using the provided cork borer cut 6 tubes of potato, each approximately 3 cm in length. Use a razor blade to cut the tubes to length; remove any skin from the ends of the tubes.
2. Label five test tubes 0, 0.1, 0.2, 0.3, 0.4, and 0.5. Place one potato tube to each test tube.
3. Fill the test tube labeled "0" with distilled water to cover the potato tube, and fill the remaining test tubes with sodium chloride solutions of the appropriate concentration to cover the potato tubes.
4. After at least 1 hour, observe the potato tubes for limpness (water loss) or stiffness (water gain) and answer the following questions.
 1. In which tube(s) has the potato become limp? Why did the water diffuse out of the potato? How would you describe the relationship between the solution and the potato (use the correct scientific term)?
 2. In which tube(s) has the potato become stiff? Why did the water diffuse into the potato? How would you describe the relationship between the solution and the potato (use the correct scientific term)?
 3. Is there any tube(s) in which the potato appears to have neither gained nor lost water? How would you describe the relationship between the solution and the potato (use the correct scientific term)?
 4. Based on these results, what is the approximate concentration of solutes in potato cells?

The Effect of Solute Concentration on Red Blood Cells

A solution of 0.9% sodium chloride is isotonic to red blood cells. In this solution red blood cells maintain their typical biconcave appearance. A solution greater than 0.9% NaCl is hypertonic to red blood cells. In such a solution, the cells shrivel, a process known as **crenation**. A solution of less than 0.9% NaCl is hypotonic to red blood cells. In such a solution, the red blood cells swell and burst, a process called **hemolysis** (**lysis** is the general term for cell bursting). These changes in appearance are visible in the light

microscope. The lysis of a red blood cell can also be monitored in the test tube. A suspension of normal or crenated red blood cells will appear cloudy; as red blood cells lyse, however, the solution changes to a clear pink.

In this exercise, you will test the effect of four solutions with differing NaCl concentrations on red blood cells.

Procedure

1. Obtain four test tubes and mark each at 2 cm above the bottom with a wax pencil. Label the tubes 1, 2, 3, and 4, respectively.
2. Fill to the mark with the appropriate saline (salt) solution: Tube 1 - 1% NaCl; Tube 2 10% NaCl; Tube 3- 0.5% NaCl; Tube 4- 0.05% NaCl.
3. Add one drop of blood to each tube. Immediately cover the tube with Parafilm and shake gently. Place the tube in front of your laboratory manual. Notice for tubes 1 and 2 that you cannot see the type on the page through the tube. However, in tubes 3 and 4 you should see the solution change from cloudy to clear. Record the time that is required for the type to appear.

Time required for clearing: Tube 3 Tube 4

4. Make a slide with a drop from each tube. Examine the appearance of the cells microscopically using the 40X objective lens.
5. Record the appearance of the cells and the correct scientific term to describe it in Table 2.

Table 2. Observations of Red Blood Cells

Tube	Solute Concentration (in test tube)	Drawing of Cells	Scientific Term
1	1%		
2	10%		
3	0.5%		
4	0.05%		

Use the results to answer the following questions.

1. What is the approximate solute concentration in a red blood cell?

2. Solutions with a solute concentration greater than 1% are _____ compared to a red blood cell. Solutions with a solute concentration less than 1% are _____ compared to a red blood cell. Solutions with a solute concentration _____ 1% are isotonic compared to a red blood cell.
3. What was the direction of water movement across the red blood cells in tube 1 (1% NaCl), tube 2 (10% NaCl), tube 3 (0.5% NaCl), and tube 4 (0.05% NaCl)?
4. By what process did the water move across the membrane?
5. Does this process require cellular energy?
6. Would you expect a red blood cell placed in a solution of 5% solute to be crenated, lysed, or normal? In a solution of 0.1% solute?
7. Why did the solutions in tube 3 and 4 become clear?
8. Which tube cleared more quickly (3 or 4)? Why?

The Effect of Tonicity on *Elodea* Plant Cells

Elodea is a plant that lives in fresh water. In such a hypotonic solution, the large, central vacuole of each *Elodea* cell gains water and exerts pressure (called **turgor pressure**), which compresses the cytoplasm and plasma membrane against the cell wall. In this situation the chloroplasts appear to be either evenly distributed throughout the cytoplasm or around the cell perimeter, with the central vacuole visible as a transparent region in the center, depending on the plane of the cell being observed.

When an *Elodea* leaf is placed in 10% NaCl, a hypertonic solution, water moves out of the central vacuole and the cytoplasm into the surrounding solution. The cell volume is reduced, and the plasma membrane visibly pulls away from the cell wall, a process known as **plasmolysis**. In this situation, water concentration inside the cell is higher than water concentration outside the cell and the net flow of water is out of the cell.

In this exercise, you will place an *Elodea* leaves in solutions of 0% and 10% solute. Fill out the first four columns of table 3 to predict the outcome of the experiment, then follow the procedure below.

Procedure

1. Make a wet mount slide of an *Elodea* leaf in water, wait 5 minutes, then observe the leaf in the microscope.
2. Use the fine focus to view the top surface of the cells, then focus downward to view the central portion of the cells.
3. Draw a diagram of the cell's appearance in the appropriate column of table 3.
4. Prepare a wet mount slide of an *Elodea* leaf in 10% NaCl, wait 2-3 minutes, then observe the leaf

- in the microscope.
5. Draw a diagram of the cell's appearance in the appropriate box in table 3.

Table 3. Effects of tonicity on *Elodea* cells

Solute Concentration (in test tube)	Water Concentration (in test tube)	Tonicity of Solution	Direction of Water Movement	Scientific Term	Drawing of Cell
0%					
10%					

Answer the following questions.

1. Did your results support your hypotheses for the exercise with the *Elodea* cells?

2. What appearance would you expect an *Elodea* cell to have in a solution of 5% solute?

Of 0.5% solute?

The following questions will help you think about the differences in the responses of plant and animal cells with respect to tonicity.

1. Do plant cells ever lyse (burst) in hypotonic solutions?
2. Do animal cells (such as red blood cells) lyse in hypotonic solutions?
3. What structural difference in plant cells compared to animal cells accounts for this difference?

Complete table 4 to compare the responses of red blood cells and plant cells to solutions of different solute concentration, or tonicity.

Table 4. Summary of Observations

Tonicity of Solution	Red Blood Cell		Plant Cell	
	Physical Change	Scientific Term	Physical Change	Scientific Term
Hypertonic				
Isotonic				
Hypotonic				