Lab: Diffusion and Osmosis

Introduction

The cell membrane encloses the contents of all cells, organelles and many cytoplasmic inclusions, and regulates what gets in and out. This is called **selective permeability**. Selective permeability is necessary for the maintenance of life processes.

Diffusion

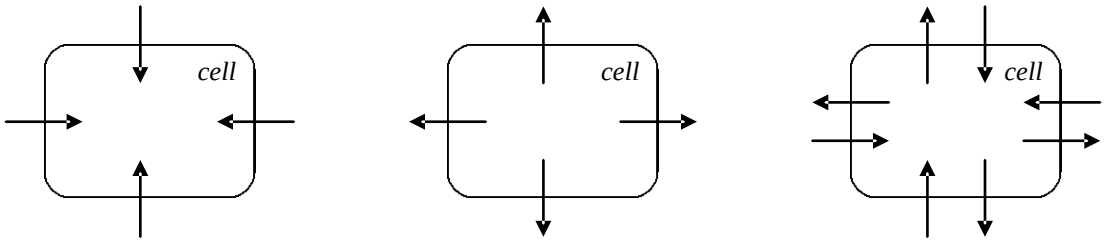
Diffusion is the movement of particles from where there are many such particles to where there are few; in more scientific language, we say that diffusion is the movement of particles from areas of *high concentration* to areas of *low concentration*. Given a cell membrane, molecules will try to diffuse from the side of the membrane where the molecules in question enjoy a high concentration to the other side where the concentration is lower. This movement depends, of course, on whether or not the molecules are able to physically pass through the membrane. Molecules may be too large to pass through the tiny channels that exist in membranes; if the cell needs the components of these molecules, the molecules have to be disassembled (digested) into smaller units that can then pass through

Osmosis

If a cell is placed into a solution that has a *higher* water concentration than the cell, water tends to flow into the cell. Similarly, if a cell is place into a solution that has a *lower* water concentration than the cell, water tends to flow out of the cell. In the first case, we say that the solution in which the cell is placed is **hypotonic**, or having a lower solute concentration than the cell. In the second case, we say that the solution is **hypertonic**, since it has a higher solute concentration than the cell. We describe a third situation, where the cell and solution have the same water concentrations, as being **isotonic**. In isotonic solutions, water still flows from one system into another, only it occurs both ways, equally. The passage of water across a membrane from an area of lower solute concentration (higher water concentration) to higher solute concentration (lower water concentration) is called **osmosis**.

Dialysis tubing is composed of a membrane synthesized of cellulose. Since it contains pores of a certain size, it allows molecules of certain size through, and those that are larger cannot get through; hence, it is semipermeable. Besides being used in medicine, dialysis tubing has many experimental applications, as we shall see in this lab.

Figure 4.1: Direction of water flow into or out of a cell placed in hypotonic, hypertonic and isotonic solutions.



hypotonic solution | hypertonic solution | isotonic solution

Iodine and Benedict's Tests

Iodine turns dark blue/purple in the presence of amylose(starch). Benedict's reagent, when mixed with monosaccharides and some disaccharides, turns orange/red when heated. These biochemical tests will be used in this lab.

Laboratory Objectives

- Define and apply the terms hypotonic, hypertonic and isotonic.
- Explain and demonstrate a technique to test whether or not glucose, iodine and amylose can pass through semipermeable membranes.
- Describe what happens when cells are placed in hypotonic, hypertonic and isotonic solutions.
- Describe and demonstrate the use of iodine and Benedict's reagent tests.

Day One: Osmosis

1. Obtain three, 250 mL beakers per lab *table*—They should be labeled 1 through 3 with tape.

2. Into beakers 1 and 2 place about 200 mL of water and into beaker 3 place about 200 mL of 50% sucrose solution.

3. Each lab group should obtain 3 lengths of dialysis tubing approximately 13 cm in length and 6 dialysis tube clips. If dialysis tube clips are not available, small paper clips will suffice.

4. Fold over about 1 cm of the end of each dialysis tube then fold it over again and clip securely closed with a tube clip (or paper clip). The other end of each tube should be left open.

5. Fill the bags with 10 mL of the following solutions, using calibrated pipettes.
    a) Fill bag 1 with 10 ml water
    b) Fill bag 2 with 10 mL 50% sucrose solution
    c) Fill bag 3 with 10 mL water

6. Squeeze the bottom of each bag as you fill it and clamp/clip it closed to eliminate any air trapped in the bag.

7. Weigh each bag as accurately as possible and record in your lab report.

8. You're going to weigh each bag after 15 minutes to determine changes in weight over time. At time zero, place each bag in the corresponding beaker (bag 1 goes into beaker 1, bag 2 goes into beaker 2, etc.).

9. After approximately 15 minutes, take the bags from the beakers, carefully pat dry with paper towels to remove excess fluid, and weigh as accurately as possible, recording the mass. After weighing, IMMEDIATELY return the bag to its corresponding beaker! DO NOT keep the bag out of a beaker while waiting to use a scale; it's okay to be a minute or two off of your time!

10. When finished, dispose of the fluids down the sink and the solids in the trash. Rinse the glassware very well and pick up any spilled sucrose solutions with lots of water to ants!

Day Two: Passive Transport of Molecules Through a Semipermeable Membrane

1. Place about 200 mL water in a 250 mL beaker. Add just enough iodine solution to distinctly color the water, but don't add so much that you can't clearly see through the solution; record the color in your lab report.

2. Obtain a 13 cm section of dialysis tubing. Fold over one end twice and clamp as before.

3. Soak the dialysis tubing in water for about a minute.

4. Open the unclamped end and add 5 mL each of 30% glucose and 10% amylose solutions.

5. Hand close the end of the bag and shake well to mix.

6. Record the color of the contents of the bag in your lab report.

7. Rinse the outside of the dialysis bag in water

8. Place the dialysis bag into the beaker with the aqueous iodine solution so that the unclosed end of the dialysis bag hangs over the edge; be careful not to allow the contents of the bag to spill out or mix with the iodine solution! To secure the bag, if needed, wrap a rubber band around the beaker and tuck the untied end of the dialysis bag under it.

9. Start a boiling water bath using a hot plate and 600 mL beaker ½ full of tap water.

10. After 20 minutes, remove the dialysis bag from the solution and note the color of its contents, as well as the color of the solution in the beaker; enter these data in your lab report.

11. Obtain four test tubes, a test tube stand, test tube clamp, grease pencil and two disposable pipettes. Label the test tubes "1," "2," "amylose + iodine" and "glucose + Benedict's." These last two test tubes will be positive controls for iodine and Benedict's tests.

12. Using a disposable pipette, into test tube #1, place 1 mL of solution from the dialysis tube.

13. Using another disposable pipette, into test tube #2, place 1 mL of solution from the beaker. Toss the disposable pipettes into the trash. From the stock solutions, place 1 mL of amylose into the "amylose + iodine" test tube and 1 mL of glucose solution into the "glucose + Benedict's" test tube.

14. Into test tubes #1, #2 and the one labeled "glucose + Benedict's," place 1 mL of Benedict's reagent. Into the test tube labeled "amylose + iodine place 1 mL of amylose and 2 drops of iodine solution. Note the color in Results and set this test tube aside.

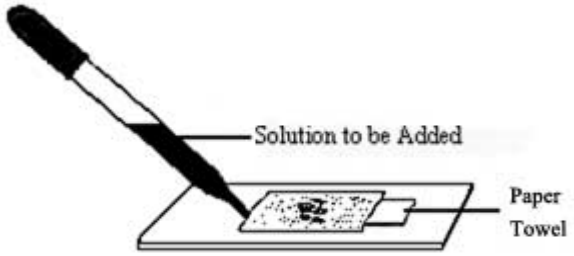
15. Place test tubes #1, #2 and the one labeled "glucose + Benedict's" into the boiling water bath for 5 minutes. After 5 minutes, using a test tube clamp, remove the test tubes from the water bath and let them cool in a test tube rack for 10 minutes. Note the resultant color of both tubes and the positive reference, and record these data in your lab report.

16. When finished with the hot plate setup, ask to see if anyone else needs it; if not, turn the hot plate off, and leave it set up for the next class.

Day Three: Biological Effects of Osmosis in plant *Cells with Cell Walls and animals cells without.*

1. Obtain a compound microscope and standard microscope slide and cover slip. Make a wet mount using a small, young *Elodea* leaflet and a drop of water; apply the cover slip.

2. Observe the *Elodea* under 400x; remember to first find the *Elodea* with 40x, increase the power to 100x, then up to 400x. Note the general shape and condition of the contents of the plant cells and record these observations in your lab report. Also make a sketch (not detailed drawing) of a representative cell showing this condition. Do your drawing as you observe your cell.
3. While watching your representative cell, add one to several drops of hypertonic solution (5% NaCl) by placing the flat edge of a small piece (~ 3 cm x 3 cm) of paper towel on one edge of the coverslip and dispensing the solution directly on the edge of the coverslip on the opposite side. Note the general shape and condition of the contents of the plant cells and write this in Results. Again, make a sketch of your representative cell showing this condition and label your cell as being in a hypertonic environment! Do your drawing as you observe your cell.



4. While you are watching your representative cell, imbibe through one to several drops of hypotonic solution (DW again). Note the general shape and condition of the contents of the plant cells and write this in Results. Once again, draw your representative cell showing this condition and label the cell as being in a hypotonic environment. Draw an arrow from your first drawing to your second to your third to indicate the progression of drawings. Do your drawing as you observe your cell; you may NOT draw the cell later from memory!

5. Have your instructor initial your three drawings for credit.

6. Repeat steps 1-5 but this time look at an animal microorganism found in pond water.

Lab: Diffusion and Osmosis
Lab Report Results

Name: _____

Date: _____ Period: _____

Osmosis

Table 1: Mass of dialysis bags over time.

	Mass of Bags (grams)		
Time	Bag 1	Bag 2	Bag 3
0			
15			
change			

Passive Transport of Molecules through a Semipermeable Membrane

Table 2: Experimental results testing ability of glucose, iodine and amylose to passively transport

	Initial		Final Color	Benedict's Test Color
	Contents	Color		
Dialysis Bag				
Beaker				

Table 3: Positive references for iodine and Benedict's test.

Reagents	Color of Positive Test
iodine + amylose	
Benedict's reagent + glucose + heat	

Biological Effects of Osmosis in Cells With Cell Walls

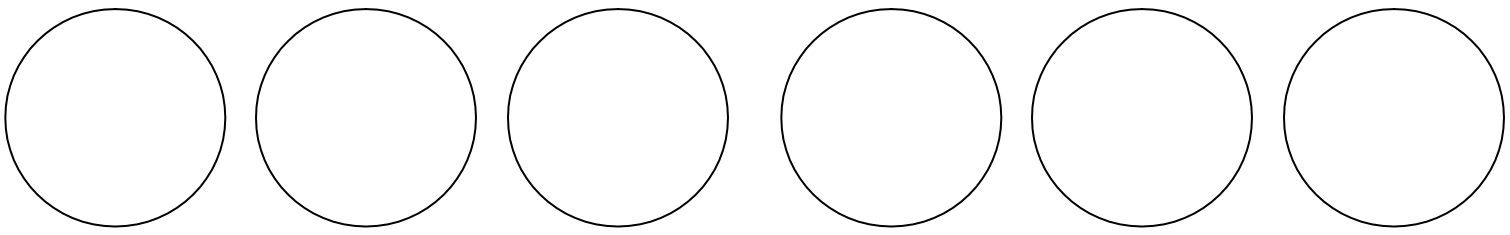
Table 4: General shape and condition of the contents of Plant and animal cells.

Tonicity	[NaCl]	General Shape & Condition	
		Plants	Animals
hypotonic original	0%		
hypertonic	5%		
hypotonic at end	0%		

Figure: Drawings

plant cells *microscopic animal cells*

hypotonic (original) hypertonic hypotonic hypotonic (original) hypertonic hypotonic



Discussion

Osmosis

1. Which bag had the greatest change in mass? Why?

2. Which bag had the least change in mass? Why?

3. Can water pass freely through the membrane of a dialysis tube? What evidence is there supporting your answer?

4. Can sucrose pass freely through the membrane of a dialysis tube? What evidence is there supporting your answer?

Passive Transport of Molecules Through a Semipermeable Membrane

1. Was there evidence of amylose being able to diffuse through the dialysis tubing membrane? What was the evidence?

2. Was there any evidence of glucose being able to diffuse through the dialysis tubing membrane? What was the evidence?

3. Based on this evidence, do you think that the sucrose in the osmosis exercise was able to diffuse across the membrane? What evidence supports your answer?

Biological Effects of Osmosis in plant and animal cells

1. Explain what happened when you placed plant and animal cells in isotonic, hypertonic and hypotonic solutions, and why what you observed happened!

2. Why does a wilted plant “crisp up” when you water it?

3. Why do you think plants need to be kept in hypotonic solutions?