BIOLOGY 1101
LAB 1: OSMOSIS & DIFFUSION

READING: Please read pages 27-31 & 83-86 in your text prior to lab.

INTRODUCTION: All living things depend on water. A water molecule is made up of an oxygen atom bonded with covalent bonds to two hydrogen atoms. Because oxygen has a higher affinity for the shared electrons that comprise the bonds, water is a **polar molecule** (meaning that it has a positive and a negative end). This property makes water an excellent **solvent** for many chemical compounds (**solutes**) necessary in the growth and maintenance of cells. Maintaining a proper balance of water and solutes is critical for cell function. This balance is maintained by the **cell membrane** (also called the **plasma membrane**), which surrounds every cell and controls the flow of water and solutes into and out of the cell. Cell membranes are **selectively permeable**, meaning that while some substances (usually small ones) can move freely in and out of the cell, other molecules (usually large ones) cannot. **Transport proteins** embedded in the cell membrane often help to transport solutes that cannot move freely across. If this process requires energy, then it is called **active transport**. It is considered **passive transport** if no energy is expended by the cell.

Molecules of any substance are naturally in constant motion (you should have learned this in chemistry), and molecules that are closely packed together will collide more often than those that are farther apart. As molecules collide, they tend to fill up available space by pushing molecules away from densely packed areas to more sparsely packed areas. While the movement of an individual molecule is best described as random, the movement of a population of molecules can be directional. **Diffusion** is the process that describes the movement of molecules from an area of high concentration (lots of them) to an area of low concentration (where there are fewer of them). Diffusion across a cell membrane is called passive transport since the cell does not expend energy in the process. When the substance has reached **equilibrium**, the molecules are evenly distributed across the available space and diffusion is no longer directional.

Osmosis is a type of diffusion that is used to describe the movement of *water molecules* across a selectively permeable membrane, and should not be used to describe the movement of any other substance. Imagine a membrane separating two solutions with different concentrations of a solute like the sugar glucose. The solution with the higher concentration of solute is called the **hypertonic** solution. The solution with the lower concentration of solute is **hypotonic**. The hypotonic solution has a lower concentration of solute and a higher concentration of water. Therefore, water molecules will diffuse (by osmosis) across the membrane to the hypertonic side, i.e., along the concentration gradient, changing the volume of solution on each side of the membrane (refer to figures 5.12 & 5.13 in your text). When the concentration is the same on each side of the membrane, i.e., at equilibrium, the two solutions are said to be **isotonic** and water molecules will diffuse across the membrane at the same rate in both directions.

LABORATORY OBJECTIVES: The purpose of this set of laboratory exercises is to provide you with a better understanding of the process of diffusion. In this lab, and from your readings, you should learn the difference between osmosis and diffusion, and you should be able to describe what is meant by selective permeability.

EXERCISES: In this lab, we will test for evidence of both osmosis and diffusion of solutes across a membrane using artificial cells. We will use **dialysis tubing** as a model of a selectively permeable cell membrane. **Dialysis** is another term for the diffusion of solutes across a membrane. We will fill our cell models with a solution of water and several different solutes. Then we will soak our cell models in pure distilled water and test for diffusion (of solutes across the membrane) and osmosis (of water through the membrane). You will make predictions about the movement of these substances across our cell model then test these predictions.

A. Osmosis

Materials:

- 1 piece of presoaked dialysis tubing (DON'T LET IT DRY OUT!)
- 2 pieces of presoaked string/floss (DON'T LET THEM DRY OUT!)
- 1 large beaker 2/3 filled with distilled water
- 1 large beaker of tap water
- 1 graduated cylinder (25 ml)
- Paper towels
- Balance
- Hot plate
- Squirt bottle of distilled water

Procedure: (Read the entire section before you start.)

1. Work in groups of 3-4 students.

2. **Set your hot plate to high and place the large beaker full of *tap water* (not distilled water) on top to get ready for part B of this lab. Please be careful not to touch your skin, clothing, or hair to the hot plate! Seriously, do not boil the distilled water!**

3. Carefully fold over and tie off one end of your dialysis tubing with a piece of string, wrapping the thread several times around the tubing to prevent leaks. Get some help from your instructor if you need it. It is *critical* that it does not leak.

4. Using your graduated cylinder, measure 10ml of stock solution. This solution should consist of distilled water with a mixture of dissolved salt (NaCl), sugar (glucose), and starch.

5. Carefully pour the stock solution into your dialysis bag. Fold the end over and use the other piece of string to tie it off, as before. (Hint: remove most of the air from the tubing before tying and make sure to leave some extra room in the bag. It should look slightly deflated, not look like a balloon.) Again, get some help if you need it.

6. This is an important step for the success of your experiment! Thoroughly rinse the filled bag with distilled water from a squirt bottle and gently pat dry with a paper towel. Make sure the strings are fairly dry as well and that there are no apparent leaks. If hanging ends of your string are very long, you may want to trim them at this time. If the bag seems to be leaking, tie another string on the leaking end and repeat the rinsing/drying process.

7. Weigh the bag and record its mass: *Initial mass* = _____.

8. Immerse the dialysis bag in your beaker of *distilled water* (not the tap water, and not on the hotplate!) and set your timer for 20 minutes.

9. While you wait, write a hypothesis. *What will happen to the mass of the bag over the 20 minutes that it sits in distilled water?* Refer to pages 14-18 of your text if you are not sure how to correctly write a hypothesis.
   _____
   _____
   _____
   _____

10. While your dialysis bag continues to soak in distilled water, check your comprehension below.

Comprehension check

1. A 20% glucose solution actually contains (fill in the blanks)

_____% glucose and _____ % water

2. Refer to your model cell (dialysis tubing bag) that has just been put into a bath of distilled water.

(Circle the correct answer)
The highest concentration of salt is inside/outside the bag.

The highest concentration of sugar is inside/outside the bag.

The highest concentration of starch is inside/outside the bag.

The highest concentration of water is inside/outside the bag.

11. When the timer goes off (or 20min have passed), remove the bag from the water bath and set the water bath aside for part B below (DO NOT discard it). *Gently* pat the bag and strings dry with a clean paper towel and weigh the bag again. *Final mass = _____.*

12. You will test for osmosis by comparing the initial mass of your tubing to the final mass.

What is the difference between the initial and final mass? _____

Did your bag gain or lose weight? _____

Was your hypothesis regarding mass change correct? _____

13. Calculate the percent change in mass and answer the questions.

Percent change in mass = [(final mass – initial mass) / initial mass] X 100

a. *Percent change in mass* = _____. Please write your answer on the board in the space provided for your group.

b. *How do your results compare to those of other groups (ask around)?*

_____
_____
_____
_____

c. *Why do you think it is important to calculate the percent change in mass rather than just looking at the absolute change in mass? What do you think caused any change in mass of the bags observed by your group or other groups?*

_____
_____
_____
_____

B. Diffusion - You will perform 3 different chemical tests to determine the results of the diffusion experiment, but first you have to create some "controls". These are tests for the presence of salt, sugar and starch in the stock solution, which you do not necessarily know contains all three. Later you will test to see if these compounds have diffused through the membrane into the beaker of distilled water. You will compare these tests to the results of the controls.

The tests you will conduct involve **indicator chemicals** that change color if the target substance (sugar, starch, or salt) is present in solution.

- **Silver nitrate** tests for the presence of chloride (Cl^-) ions (remember that salt dissolves in water to form Na^+ and Cl^- ions). When added to a solution that contains chloride ions, the silver nitrate will change color from clear to cloudy white. Because your stock solution is also cloudy, look for a precipitate (like little snowflakes) in the bottom of your beaker. That also indicates Cl^- ions. Check with your instructor if you are not sure if you have a positive result.

- **Iodine** tests for starch. Iodine starts out red/brown, but when added to a solution where starch is present, it will turn dark blue or black. If no starch is present, the iodine will remain reddish-brown.

- **Benedict's solution** tests for the presence of sugar. It is blue and when added to a solution it will immediately turn the solution blue. After heating, though, if the solution contains glucose it will change to orange/red (looks like pumpkin). A light green or yellow color indicates a very slight amount of sugar, which probably reflects a little bit of leakage from your bag, and is not a positive test.

Materials:

- 6 test tubes in a test tube rack
- Test tube holder
- Ruler
- Marking pencil

Procedure: (Read the entire section before you start.)

1. Make sure you have 6 clean test tubes! Wash them yourself to make sure and rinse several times with distilled water. Dirty test tubes will result in a false positive in several of these tests. Label two of the test tubes "Salt", two "Sugar", and two "Starch". Now label one set of 3 (1 Salt, 1 Sugar, and 1 Starch) with a small letter "c" or the word "control" to denote the control tubes.

2. Using the ruler, place a line on each tube 1cm from the bottom of the tube. Fill the *control set* of test tubes to the 1cm line with *stock solution*. Fill the other tubes with water from the *distilled water bath* you saved from above. **Do not add stock solution to the second set of test tubes, only water from the beaker that held the tubing bag.** Be careful not to confuse which tubes are which. Go to table 1 and fill in the column marked "initial color of indicator solution".

3. To the test tubes labeled "Salt" and "Salt control," add one dropper full of silver nitrate. (**AVOID GETTING THIS ON YOUR SKIN**) Swirl the tubes gently, then observe the color of the solutions. Record your results in Table 1.

4. To the test tubes labeled "Starch" and "Starch control," add one dropper full of iodine solution. Swirl the tubes gently and observe the color of the solutions. Record your results in Table 1.

5. To the test tubes labeled “Sugar” and “Sugar control,” add one dropper full of Benedict’s solution. Swirl the tubes gently to mix the contents. Carefully lower these two test tubes into the boiling water bath and boil for 5min. Use the test tube holder to remove the tubes from the boiling water and place in the test tube rack. Observe the color of the solutions. Record your results in Table 1.

Table 1. Results of the Chemical tests

To show presence of	Tube	Indicator solution	Initial color of indicator solution	Final Color in tube	Result (+ or -)
Salt	Control	Silver Nitrate			
Salt	Experimental Test	Silver Nitrate			
Starch	Control	Iodine			
Starch	Experimental Test	Iodine			
Sugar	Control	Benedict’s			
Sugar	Experimental test	Benedict’s			

7. *According to your results, which molecules were able to diffuse through the dialysis bag? How do your results compare to the expected results (Table 3)?*

   _____
   _____
   _____
   _____
   _____

8. *If molecules are expected to diffuse from areas of high concentration to areas of low concentration, why didn’t all types of solute molecules leave the dialysis bag?*

   _____
   _____
   _____
   _____
   _____

C. Work with osmosis data set - In this activity you will learn to manipulate and interpret a scientific data set from an experiment on osmosis conducted by Drs. Goodell and Roberts. They investigated the process of osmosis and diffusion in a simple model cell similar to yours made out of a selectively permeable dialysis tubing bag. The purpose of this experiment was to test the effect of solute concentration on osmosis. They were interested in the consequences of osmosis for living plant and animal cells.

Methods:

1. They filled dialysis tubing bags with 20ml of one of the following solutions:

- Distilled Water
- 10% sucrose
- 20% sucrose
- 30% sucrose
- 40% sucrose
- 50% sucrose

2. The bags were sealed, weighed and placed in individual baths of distilled water. They left the bags for 30 minutes.

3. They removed the bags from the water and reweighed them. The initial and final masses are recorded in Table 2.

4. *Calculate the difference in mass and the percent change in mass for each bag. Then calculate the average for the two replicates at each concentration. What is the benefit of using an average of two or more replicates?*

5. *Graph your results on a separate piece of paper (You lab instructor may have graph paper for you). The **dependent variable** (average % change in mass) is usually placed on the y-axis, the **independent variable** (sugar concentration) on the x-axis. Before starting, please think about what sort of graph is most appropriate. Remember to label all axes clearly and provide units.*

Table 2. Initial and final masses of dialysis tubing bags (2 replicates per concentration) in solute concentration experiment.

Contents of bag	Initial mass (g)	Final mass (g)	Difference in mass (g)	% change in mass	Average % change in mass
Distilled water	14.83	14.61			
	14.36	14.29			
10 % sucrose	12.25	13.44			
	10.60	11.74			
20% sucrose	12.94	15.00			
	13.88	16.18			
30% sucrose	13.43	15.50			
	13.91	17.22			
40% sucrose	15.63	19.04			
	12.53	15.63			
50% sucrose	14.09	18.15			
	15.50	19.44			

6. Based on the results of this experiment:

   a. *Describe the relationship between the change in mass and the concentration of sucrose in the dialysis tubing bag.* (Hint: look at your graph)

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

   b. *Based on the results of Drs. Goodell and Roberts' experiment, what change in mass would you expect for a bag filled with 25% glucose solution? Explain how you came up with this prediction.*

_____
_____
_____
_____
_____
_____
_____
_____

Table 3: Check your results – this is what Drs. Goodell and Roberts found

To show presence of	Tube	Indicator solution	Initial color of indicator solution	Final Color	Result (+ or -)
Salt	Control	Silver Nitrate	Clear	White Precipitate	+
Salt	Experimental Test	Silver Nitrate	Clear	White Precipitate	+
Starch	Control	Iodine	Rusty Red	Black or Dark Blue	+
Starch	Experimental Test	Iodine	Rusty Red	Rusty Red	-
Sugar	Control	Benedict's	Blue	Orange or Dark Red	+
Sugar	Experimental test	Benedict's	Blue	Blue or Green	-