Lab 3 Name _____

DIFFUSION AND OSMOSIS

OBJECTIVES

--To gain a better understanding of diffusion and osmosis.
--To understand these terms: diffusion, osmosis, diffusion or concentration gradient, Brownian motion, hypotonic, hypertonic, isotonic, hyposmotic, hyperosmotic, isosmotic, selectively permeable, semipermeable.
--To further your understanding of experimental design and practice hypothesis testing.
--To understand the process of data collection and analysis.

This lab will introduce you to 1) diffusion and osmosis (especially as they relate cell membranes) and 2) the scientific method. You will actually be using the scientific method as you work through one of the activities (the "Egg" lab) in this lab.

There are numerous components to this lab exercise, so please read the entire lab BEFORE coming to class. Some of the components are demonstrations that will be performed by the instructor. For the dialysis slide demonstration you need to record both pre-experimental and post-experimental data in the charts provided, and answer the questions that follow. The demonstration on Brownian motion will be set up by your instructor, and will likely be available for viewing throughout most of the lab period. Please make sure that you observe this demonstration sometime during the lab, and remember to answer the questions regarding this demo!

The "egg lab" portion of this lab is our first experiment of the quarter. Lab 1 and Lab 2 were both examples of "Descriptive Science", whereas, the "Egg lab" is an example of "Hypothesis-Driven Science". In this lab, you will be asked to first formulate both a **hypothesis** and a **prediction** pertaining to the experiment at hand. You will be given two solutions (solution A and solution X). Your job is to determine if **Solution X** is hypotonic, isotonic, or hypertonic to a cell. You will be able to do this by using "model cells". We will model cells by using eggs. "Eggs??!" you exclaim. Yes, eggs! For further instruction, see the "Egg Lab Section of the lab" below.

We will also be investigating the effects of high-salt solutions on plant cell morphology. **Plasmolysis** is a specific term that refers to the changes in plant cell structure that we may observe during this experiment.

Finally, we will investigate the relationship between diffusion and cell size. This will be done using small agar cubes (agar is a seaweed-based, jello-like substance) and a colorimetric readout. Do you think that diffusion is a highly effective way for a cell to receive and exchange gasses, nutrients, and waste? We will work to answer this question based on data generated in the lab. The data will either support or refute the idea of diffusion as an effective mechanism for cellular transport, in different sized agar 'cells'.

MATERIALS (For demonstrations)

microscope slide of milk or India ink
iodine dialysis slide filled with starch solution
beaker of water with I_2KI (Lugol's solution) added

MATERIALS (per group) FOR THE "EGG LAB" AND ELODEA PLASMOLYSIS

two prepared eggs
two beakers (Soution A, Solution X)
tray for containing the eggs, etc.
Elodea *plant sprigs*
scale or balance
2 weigh boats, paper towels
slides and cover slips
40% NaCl solution (for *Elodea* experiment)

PROCEDURES: Note that some portions of this lab should be done simultaneously. You will need to budget your own time.

A. Demonstration of Diffusion and Osmosis (Demonstration)

1. A demonstration dialysis slide and a beaker of solution with the following contents will have been set-up before class:

Contents of Dialysis Slide	Contents of Beaker
Water	Water
Starch	Iodine

2. Based on an understanding of the dialysis membrane, what are your predictions regarding the movement of water molecules and solute particles when the dialysis slide is placed into the beaker? (Record on data sheet below)

3. Record the results of the demonstration experiment and draw conclusions regarding your predictions on the data sheet below.

B. Brownian Motion (Demonstration)

Note: Once this demonstration has been set up by your instructor, you may observe it at any time. In order to understand how substances pass through a membrane, it is important to realize that **molecules are in constant motion**. Molecular motion is a form of energy: the translational, vibrational, and rotational kinetic energies of molecules. (These are just fancy terms to describe different *types* of movement, translational means side to side, vibrational means that bonds can be 'pulled' in different directions, and rotational means that atoms may rotate about a bond. You DO NOT need to memorize these terms). Although individual atoms are impossible to see here, their existence is revealed by the jiggling--called **Brownian motion**--of minute particles suspended in water.

1. Observe the demonstration set up on the microscope. We have placed a solution with small particles (may be milk or India ink) on a slide and set it up under a microscope at high power (400X).
2. Answer the questions provided on the data sheet below.

C. Osmosis: The Egg Lab

The "Egg Lab": Determining the Relative Concentration of an unknown solution by Osmosis

In this portion of the lab, you will be given two solutions (solution A and Solution X). Your job is to determine determine if **Solution X** is hypotonic, isotonic, or hypertonic to a cell. The 'cells' we are using are actually eggs. These eggs have been soaked in vinegar for about 2 weeks. Vinegar decalcifies the egg shell, essentially dissolving the shell by removing calcium. After the vinegar treatment, what remains around the egg is **a semipermeable membrane**. This membrane is permeable to water molecules, but not to large proteins or to salts. You will be soaking one egg (Egg A) in Solution A, and the other egg (Egg X) in Solution X, and recording the weight of the eggs at three time points. (See below). From the data (the weight of the eggs at different time points), you will be able to determine if Solution X was hypertonic, hypotonic, or isotonic to Egg X when the experiment began. You will also determine the relative tonicity of Solution A.

This experiment is an example of "Hypothesis-Driven Science". Before you DO the experiment, you will **first** formulate both a **hypothesis** and a **prediction** pertaining to the **tonicity of Solution X, relative to Egg X.** Remember that a hypothesis is really an educated guess. Trust me, you are educated enough for this guess!! You know from lecture that the tonicity of a solution can be: hypertonic, hypotonic or isotonic relative to another solution (or cell, etc). So, really, your hypothesis takes a 1 in 3 shot (guess) at the tonicity of the unknown solution (Solution X). Your **prediction** is also written **BEFORE** the experiment is

started. Your prediction (written as an "If....., then...." sentence), should logically follow from your hypothesis. Remember that you are predicting whether Egg X: gains weight, loses weight, or experiences no change in weight as a direct result of soaking in Solution X.

Part 1 (For solution A only)

1. Carefully weigh one egg in its boat (Dry the egg carefully before you weigh it).
2. Record the "pre-weight" in Table 1 below for 'Egg A'. This is "Time Zero".
3. Place Egg A into its corresponding beaker (Beaker A).
4. After 15 minutes, remove the egg, dry, and weigh it.
5. Record the "15 minute-weight" in Table 1 below. Immediately place the egg back into Beaker A.
6. After another 15 minutes (or 30 minutes total), remove the egg, dry, and weigh it.
7. Record the "post-weight" in Table 1 below.

Part 2 (For solution X only)

1. Carefully weigh one egg in its boat (Dry the egg carefully before you weigh it).
2. Record the "pre-weight" in Table 1 below for 'Egg X'. This is "Time Zero".
3. Place Egg B into its corresponding beaker (Beaker X).
4. After 15 minutes, remove the egg, dry, and weigh it.
5. Record the "15 minute-weight" in Table 1 below. Immediately place the egg back into Beaker X.
6. After another 15 minutes (or 30 minutes total), remove the egg, dry, and weigh it.
7. Record the "post-weight" in Table 1 below.

Analyze your data and draw conclusions about your hypothesis and prediction based on this analysis (aka, Was your hypothesis correct? How do you know?). Based on your results, answer the questions below

Part 3-You also will GRAPH your data in Graph 1. Include the data for Egg A, and Egg X on the same graph. To do this clearly, you will need to devise a 'graph legend' for the two data sets. You could use circles and squares for the two data sets, or two different colors, etc. If you need help setting up your graph, please ask me! You will probably need to do this portion of the lab outside of lab time.

D. Plasmolysis -- Observing Osmosis in a Living System

If a plant cell is immersed in a solution that has a higher solute concentration than that of the cell, water will leave/enter (circle one) the cell. The loss of water from the cell will cause the cell to lose the pressure exerted by the fluid in the plant cell's vacuole, which is called turgor pressure. Macroscopically, you can see the effects of loss of turgor in wilted house plants or "limp" lettuce. Microscopically, increased loss of water and loss of turgor become visible as a withdrawal of the plasma membrane from the cell wall **(plasmolysis)** and as a decrease in the size of the vacuole (Figure 1).

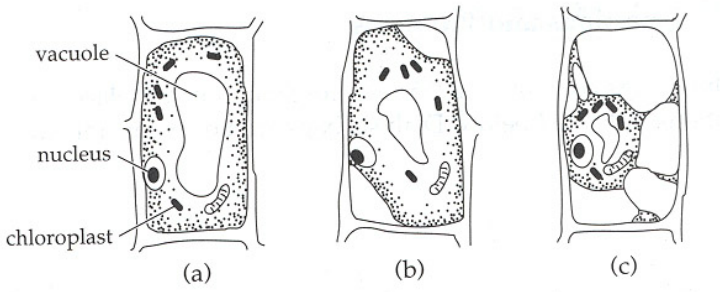


Figure 1. *Plasmolysis in an epidermal cell of a leaf: (a) Under normal conditions, the plasma membrane is pressed against the cell walls. A large vacuole occupies the center of the cell, pushing the cytoplasm and nucleus to the periphery. (b) When the cell is placed in a solution with a higher concentration of solutes than that of the cell, water passes out of the cell, and the cell contents contract. (c) In an even more concentrated solution, the cell contents contract still further.*

1. Obtain a leaf from an *Elodea* plant. Place it in a drop of water on a slide, cover it with a coverslip. Using the scanning power objective, find the sample and bring it into focus. Next examine the material at (100X) and then at high power (400X).
2. Locate a region of healthy cells and sketch a few adjacent cells in the left box of Table 4 below. Note especially the location of the chloroplasts. (Don't forget to include total magnification.) For the next step, do NOT move the slide.
3. While touching one corner of the coverslip with a piece of Kimwipe to draw off the water, add a drop of concentrated salt solution to the opposite corner of the coverslip. Be sure that the salt solution moves under the coverslip. Wait about 5 minutes, then examine as before. (This technique works sometimes, and sometimes it does not. Thus, you may be asked to make a new wet mount slide, using the high salt solution in place of water.)
4. Sketch in the right box below the same cells you sketched in step 1.

E. Diffusion in Agar Cells: (Illustration of the relationship between diffusion and cell size)

In this portion of the lab, you will be given a block of agar that you will cut into 3 sizes. We will use these 3 differently sized blocks to demonstrate **the effect of size on diffusion**. The agar has been made with phenolphthalein (a pH indicator) in it, which will act as a color indicator when it comes in contact with the **base (NaOH)** used in the experiment.

1. Each group will cut 3 agar cubes (a 3 cm cube, a 2 cm cube, and a 1 cm cube) from the block of agar available. Cut as accurately as possible.
2. Pour 200 ml of 0.1 M NaOH solution into the 400 ml beaker. Note the time and immerse the 3 blocks in the NaOH solution. Let the cubes soak for 10 minutes.
3. After the 10 minute soak, remove the agar cubes and blot them dry with a paper towel.
4. Promptly cut each cube in half and measure the depth to which the pink color has penetrated. In other words, measure from one edge how far to the center the pink color penetrated.
5. Record the results in the data table below, and answer the questions that follow.

DATA SHEET NAME_____

(YOU ONLY NEED TO TURN IN THESE PAGES)

A. Dialysis Slide Demonstration:

Pre-experiment **Prediction:**

Table 1: Your Pre- and Post- observations from the Dialysis Slide Demonstration

	Dialysis Slide	Beaker
Pre-experimental contents	Water, Starch	Water, Iodine
Pre-experimental color		
Post-experimental color		

Is their evidence of the diffusion of starch molecules? If so, in which direction did starch molecules diffuse?

Is their evidence of the diffusion of iodine molecules? If so, in which direction did iodine molecules diffuse?

What can you say about the permeability of the dialysis slide? (What particles could move through and what particles could not?) And what conclusions can you draw about your predictions? (Were you correct or incorrect with your predictions?)

B. Brownian Motion Demonstration

Do the particles seem to move randomly or in a definite path?

Can you see individual water molecules?

Is the movement of a particle due primarily to the movement of its own molecules or to bombardment by water molecules? Explain.

Would an increase in temperature increase or decrease the rate of Brownian movement? Explain.

C. Egg Experiment

Table 2. Remember, your hypothesis should be an educated guess as to the **tonicity** of **Solution X**, ***in relation*** to Egg X, at the START of the experiment! Remember, also, that your prediction should be in the form of an , "If....., then....." sentence, with some form of measurable DATA following the 'then'.

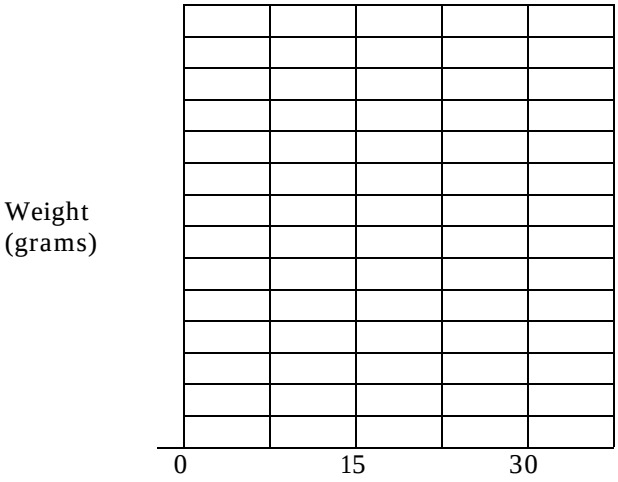
Hypothesis:
Prediction:

Table 3

	Egg A	**Egg X**
Pre-weight (grams) "Time Zero"		
15 minute weight (grams)		
Post-weight (grams) "Time 30 minutes"		

Interpretation (circle one): support reject the Hypothesis.
Reasoning (if supported, why; if rejected, why)
If rejected, a corrected hypothesis:
Reasoning (why you corrected the hypothesis the way you did):

Graph 1



What trends did you notice in the weight (gain or loss) of Egg X? Egg A?

Based on the results above, what was the tonicity of Solution X, with respect to Egg, at the start of the experiment?

Fill in the blanks:

Prior to the start of the experiment, Solution A was _____ to Egg A.

Prior to the start of the experiment, Solution X was _____ to Egg X.

*In your own words, what does it mean if a cell is **isotonic** to its environment?*

D. *Elodea* Leaf Plasmolysis Experiment

Table 4. Sketch your *Elodea* leaf cells here. Be sure to note your magnification and label your drawings!

Water	salt water added

Elodea Leaf Plasmolysis Experiment continued:

What happened to the Elodea cells placed in salt water?

Assuming that the cells have not been killed, what should happen if the salt solution were to be replaced by water?

Does cell turgor (rigidity) influence the overall turgor of the plant part (such as leaf or stem)?

Can plant cells burst due to osmosis alone? Explain.

E. Diffusion in Agar Cells

Table 5: Results of the agar cell diffusion experiment

Cube	Surface Area (cm^2)	Volume (cm^3)	Surface Area to Volume Ratio	Diffusion Depth (mm)	Diffusion Rate (mm/min)
1 cm					
2 cm					
3 cm					

What effect did size have on the depth that the NaOH reached into the cell? Did the NaOH reach the center for any of the cells?

How can these results be used to explain the limit of growth in a living cell?