Biology 101 Laboratory Exercise 1:
Movement of Materials in Biological Systems

Pre-lab Assignment:

Read this lab exercise and the textbook reading assignment for lab, for information about water and it's importance in biological systems. There is no written pre-lab assignment for this first week of lab.

Objectives:

At the conclusion of this exercise, you will have gained skill and experience in:

- the appropriate use and understanding of the terms that describe solutions and the movement of materials
- investigating the factors that affect the movement of materials in living & non-living systems
- identifying the responses of various cell types to changes in the relative solute concentrations within the cell and the surrounding external environment
- using the de-shelled chicken egg as a cell model, anticipating experimental results based on this model, and recognizing the limitations of the model
- data collection and analysis; as well as extrapolation of the possible effects of treatments not directly tested, by constructing and using appropriate graphs
- transferring conclusions based on experimental results to explain observations related to the movement of materials in other organisms

Introduction:

Water is the universal **solvent** of living systems. Most of the materials with which the cell must deal in every day life are dissolved in this liquid, creating an aqueous (aqua, *L.* = water) **solution**. For example, nutrients from the environment such as glucose (a simple sugar), and wastes that the cell produces such as carbon dioxide are dissolved in the water inside and surrounding cells. Because the water molecules (like all molecules) are in constant random motion, the dissolved molecules and ions (**solutes**) become evenly mixed throughout the solvent. This physical process creates an opportunity to dilute or distribute materials within or between the internal and external environment of the cell; in other words, enabling the cell to efficiently exchange materials and energy with its environment, in order to support **metabolism** and maintain **homeostasis**.

The movement of materials within cells and between cells and their environment is essential to all living organisms. Since the cell has to maintain its life functions in the face of ever changing conditions, it must <u>regulate</u> the movement of materials into and out of the cell. This function, among others, is performed by the **plasma membrane**: a dynamic barrier that differentiates between materials based on their chemical nature, allowing only some materials to pass through easily, and restricting the passage of others. Since not all materials penetrate the membrane equally well, the membrane is said to be **selectively permeable**.

Materials (molecules and ions) pass through the plasma membrane due either to passive transport or active transport. The **passive transport** of materials across cell membranes is strictly a physical process, i.e. following the natural physical laws, and occurs without the expenditure of energy in both living and non-living systems. **Diffusion** is the term that describes this spontaneous process by which there is a <u>net movement</u> of molecules or ions from a region in which they are highly concentrated to a region in which their concentration is lower (down the **concentration gradient**). In contrast, **active transport** of materials across cell membranes is in a direction against natural physical forces and requires that the cell do work, using cellular energy in the form of ATP.

The experiments in this lab exercise are designed to demonstrate the phenomena found in living systems related to the movement of molecules, especially water molecules! A special kind of diffusion in which water molecules move across a selectively permeable barrier down their concentration gradient is termed **osmosis**. Recall that cells create and maintain an internal environment containing a great variety of solutes (sugars, proteins, vitamins, salts, etc.). Also recall that the environment surrounding cells may change with respect to the solutes it contains. Therefore, the relative concentration of water molecules may change accordingly, resulting in a net movement of water across the cell membrane due to osmosis. For example, if the environment suddenly becomes salty, the relative concentration of water molecules outside the cell decreases (it now contains a smaller proportion of water molecules in the solution), and there will be a net movement of water molecules out of the cell into the surrounding environment. *What do you think the cell will look like after this happens?* Solutions to this problem of net water movement in response to concentration gradients are many and varied. A rigid **cell wall**, a feature of plants and fungi for example, is an adaptation that helps maintain cell shape despite changes in water content. Alternatively, cells may capitalize on this phenomenon by purposefully adjusting solute concentrations inside the cell, in order to promote the movement of water and a corresponding change in water pressure (turgor pressure) in the cell. (For more information, see Cain et al., p. 479, and Starr, p. 87)

We use the comparative terms hypotonic, hypertonic, and isotonic to describe the potential for osmosis; i.e. the relative concentrations of solute particles in two aqueous solutions on both sides of a selectively permeable barrier.

Hypotonic: [*Gr.* hypo = under + tonos = tension] the solution that contains the lower concentration of solute is said to be hypotonic (hypoosmotic). There will be a net movement of water molecules across the cell membrane out of a hypotonic solution into the hypertonic solution.

Hypertonic: [*Gr.* hyper = above + tonos = tension] The solution that contains the higher concentration of solute is said to be hypertonic (hyperosmotic). There will be a net movement of water molecules across the cell membrane into a hypertonic solution from the hypotonic solution.

Isotonic: [*Gr.* isos = equal + tonos = tension] Having the same concentration of solute as another solution. If two isotonic (isoosmotic) solutions are separated by a selectively permeable membrane, there will be an equal movement of water molecules in both directions (no net flow). This is described as the state of **equilibrium**.

Note: The total number of solute molecules is the variable that affects the potential for osmosis, not the kind of solute (sugars, salts, amino acids, etc.). In addition, because it is the random thermal kinetic energy of molecules (their random motion) that is responsible for diffusion, changes in temperature will affect the rate of net movement, regardless of the overall direction of movement. *Discuss with your lab partner(s) why this is true.*

Materials: (Work in groups of 2)

- microscope slides and coverslips
- Carmine's dye with toothpicks
- Methylene blue & Potassium permanganate crystals, each with labeled forceps
- Petri dishes
- Filter paper
- Six potato cores
- Six 150 ml beakers labeled 0%, 10%, 20%, 30%, 40%, and “unknown”; each half-filled with the appropriate sucrose (table sugar) solution
- balance
- weighing boats
- kimwipes or paper towels

DEMONONSTRATION MATERIALS:

- 3 de-shelled chicken eggs
- 3 fingerbowls: labled and filled with “hypotonic”, “isotonic” and “hypertonic” sucrose solutions

Methods: (Start procedures 4 & 5 first; everyone completes procedure 3; only 1 person per table completes procedures1 & 2 and shares results.)

Procedure 1: **Brownian Motion**

Wet the tip of a toothpick and use it to collect a small amount of Carmine's dye. Place the dye in a small drop of water on a clean microscope slide and stir gently. Add a coverslip and observe under the microscope, eventually at 400X magnification. Look for the independent, random movement of dye particles due to the bombardment by the invisible water molecules. *Explain any <u>non-random</u> motion that you observe:*

Procedure 2: **Diffusion of a solute through a liquid**

Wet two pieces of circular filter paper with tap water. Drain each thoroughly (they should not be dripping wet) and place each in the bottom half of a petri dish. In the center of one filter paper place one crystal of potassium permanganate (mol. wt. 158) using forceps. In the other petri dish, place an approximately equal amount of methylene blue (mol. wt. 320) on the center of the moistened filter paper. Place the covers on each dish. Observe throughout the course of the lab, and record the movement of both materials by diffusion. *Suggest an explanation for the different rates of diffusion that you observe:*

Procedure 3: **Osmosis in Animal cells**

DEMONSTRATION: Your lab TA will begin this procedure with 3 de-shelled chicken eggs. The shell has been dissolved away by placing the eggs in dilute acetic acid (vinegar) for 24-36 hours. The remaining membrane (the shell membrane) is selectively permeable. ***You*** *are responsible for recording and interpreting the results of this demonstration.*

1. Weigh each egg separately to the nearest 0.1g and record the weights in Table 1 at time "0". Place each of the 3 eggs into separate bowls containing the sucrose solutions labeled “hypotonic”, “isotonic” and “hypertonic”. Record the time that each egg goes into its solution.
2. At one hour, carefully remove each egg, wipe off all excess solution, and again weigh each egg separately. Record the weights at one hour in Table 1. Construct and interpret a graph of your results as directed. *Record your general observations regarding the effect of osmosis on these eggs in the three different solutions, and explain why.*

***Procedure 4:* Effect of Solute Concentration on Osmosis in Plant cells**

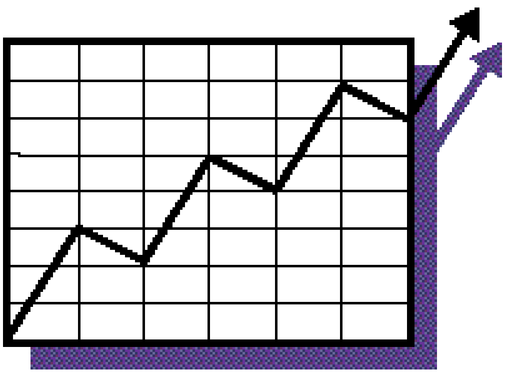
Each pair of students will be supplied with 6 undamaged cylindrical potato cores (5-6 cm long). We will assume that the cells of each core have approximately the same concentration of solute inside. Then, based on the rate of osmosis, you will attempt to determine what this concentration is, inside the potato cells.

1. Keep the potato cores covered in the large petri dish until you are ready to use them, to prevent moisture loss.
2. Weigh a potato core to the nearest 0.1g and record the weight in Table 2 at time “0”.
3. Immediately place it in one of the solutions and record the time. Repeat steps 2-3 for the remaining 5 potato cores.
4. Leave the potato cores in their solutions for 1 hour.
5. Remove the first potato cylinder and blot excess liquid from the outside of it. Weigh the cylinder to the nearest 0.1g and record its weight in Table 2. Repeat this step for the remaining 5 cores.

***Procedure 5:* Effect of Temperature on Osmosis in Plant cells**

To illustrate the effect of temperature (the average thermal kinetic energy of molecules) on the rate of osmosis, you will perform a second experiment.

1. Obtain 2 potato cores that have been held at room temperature. Weigh each core separately to the nearest 0.1g and record the weights in Table 3 at time "0" in the columns for cold and hot.
2. Place one core into a beaker containing 40% sucrose that was kept in a freezer or cold room. Place the second core into a beaker also containing 40% sucrose, which was kept in an incubator set to 37^{o} C (body temperature). Record the time that the cores enter the solutions. Return these beakers containing potato cores to their respective temperature environments.
3. At 1 hour, carefully remove each core, wipe off excess solution, and again weigh each core separately. Record the weights in Table 3.
4. Complete Table 3 by using the data obtained from the potato core in 40% sucrose at room temperature in Procedure 4.



Results:

Table 1. Weight of Eggs (g) and total weight change as a result of osmosis when placed in solutions of different sucrose concentrations. (*Procedure 3)*

Time (min.)	Hypotonic	Isotonic	Hypertonic
0 min			
60 min			
Total change			
% Total change			

Total change = weight at end of time interval - weight at start of time interval
*%Total change = (Total change/ start weight) * 100*

Table 2. Weight of Potato cores (g) and total weight change as a result of osmosis when placed in solutions of different sucrose concentrations. *(Procedure 4)*

Time (min.)	dist. Water	10% Sucrose	20% Sucrose	30% Sucrose	40% Sucrose	Unknown solution
0 min						
60 min						
Total change						
% Total change						

Table 3. Weight of Potato cores (g) and total weight change as a result of osmosis when placed in 40% sucrose solutions held at different temperatures. *(Procedure 5)*

Time (min.)	Cold	Room Temp.	Hot
0 min			
60 min			
Total change			
% Total change			

Total change = weight at end of time interval - weight at start of time interval
*%Total change = (Total change/ start weight) * 100*

Movement of Materials: **Name ______________________________**
Analysis and Post-lab Assignment **Lab TA ____________________________**

Complete the following graphs and answer the questions. This post-lab assignment serves as an introduction to writing the **Results** section of a scientific paper. Your answers and the completed figures will be graded for post-lab points. Your TA may ask that your report be submitted on separate paper, typed, and in paragraph form. Include major headings (e.g. *Osmosis in Animal Cells*) to organize your report.

Osmosis in Animal Cells
Prepare a graph using the format below in which you plot the <u>% total weight change</u> for the 3 eggs (using data from Table 1). Fill in the values on the Y-axis.

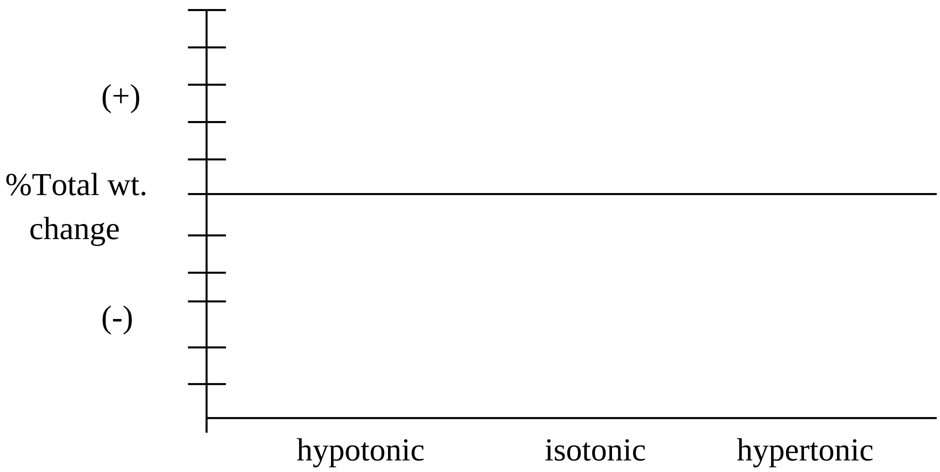


Figure 1. Effect of solute concentration on the degree of osmosis in de-shelled chicken eggs.

Answer the following questions, based on Figure 1.

1. Describe the <u>net direction</u> to the movement of water in all three eggs

2. Was our "isotonic" solution perfect (Yes or No)? <u>Explain</u> your answer.

Effect of Solute Concentration on Osmosis in Plant Cells
Prepare a graph using the format below in which you plot the % total weight change over 60 minutes for each sucrose solution (using data from Table 2). (Do not include the results for the egg in the unknown solution, since you do not know what that concentration is!) You will have one line in this figure. In effect, you will be constructing a type of standard curve. Fill in the values for the Y-axis.

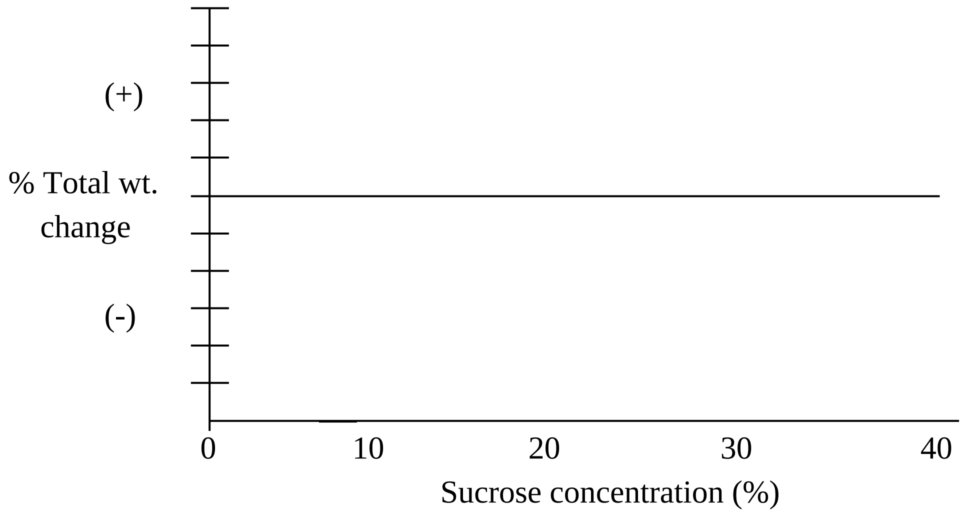


Figure 2. Effect of sucrose concentration on total weight change due to osmosis in de-shelled chicken eggs.

Answer the following questions, based on Figure 2.

3. Use Figure 2 to determine the solute concentration (%) inside the core (in other words, the concentration of the solution that would be isotonic relative to the external sucrose solution). How did you get this information from Figure 2?

4. Locate the point on Y- axis representing the % total weight change for the core in the unknown solution. Now determine the sucrose concentration (%) of the unknown solution. Explain how to get this information from the graph.

5. Predict what would happen to its weight if a core was put into a bowl containing a 35% sucrose solution. Explain how to get this information from the graph.

Effect of Temperature

Prepare a graph using the format below in which you plot the % total weight change for the 3 potato cores in which environmental temperature was manipulated (using data from Table 3). In effect, you will be constructing a type of standard curve. Fill in the values for the Y-axis.

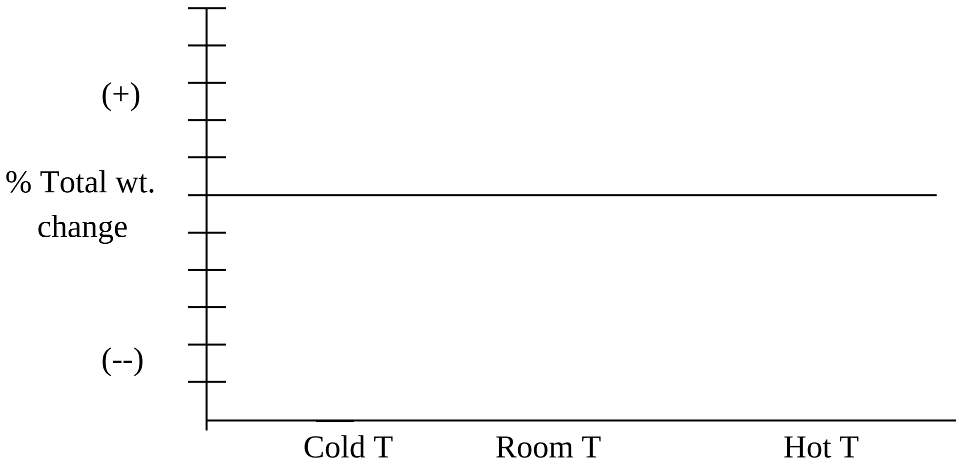


Figure 3. Effect of temperature on the degree of osmosis in potato cores held in a 40% sucrose solution.

Answer the following questions, based on Figure 3.

6. Was there any difference in the rate of osmosis for the cores placed in solutions at different temperatures? Describe.

7. Did the rate of osmosis change with temperature as expected for each of the potato cores? Explain.

8. Would increasing the temperature to 60ºC cause an even greater increase in the rate of osmosis? Why or why not?

Glossary:

Active transport

- Movement of materials across the cell membrane that does not occur unless the cell expends energy (ATP)

Cell wall

- A rigid layer of material outside the cell membrane for support of all plant cells, and most fungi and prokaryotes

Concentration gradient

- The comparative difference in the concentration of a substance in two regions, such that one region is more concentrated than the other; describes the potential direction for diffusion

Diffusion

- The random movement of small particles that leads to an even distribution either within an enclosed region or across the two sides of a permeable membrane

Equilibrium

- In diffusion, the state at which small particles are evenly distributed within an enclosed region or across the two sides of a permeable membrane, each with an equal chance of moving in either direction, resulting in no net movement

Homeostasis

- A characteristic of living things in which internal conditions are maintained within acceptable limits despite changes in the external environment

Metabolism

- The organized set of all chemical reactions that occur within an organism

Molecule

- a chemical combination of two or more atoms that form a specific chemical compound.

Osmosis

- The diffusion of water across a selectively permeable membrane as a result of a concentration difference

Passive transport

- Movement of materials across the cell membrane that will occur spontaneously, and does not require the cell to expend energy

Plasma membrane

- The selectively permeable membrane that surrounds all cells; composed primarily of phospholipids and proteins

Selectively permeable

- A barrier which allows the passage of some ions and molecule easily, and not others

Solute

- A substance dissolved in a solvent

Solution

- A mixture of solutes in a solvent, such that the dissolved substances cannot be distinguished from the fluid

Solvent

- Any fluid in which other substances dissolve

Thermal kinetic energy

- the random movement (kinetic energy) of molecules and similar particles, producing heat