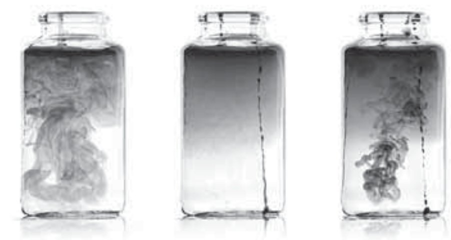
Name

Date

Section

Cell Membrane Transport: Diffusion and Osmosis



Lab 4

Movement of molecules through a space is one of the most common processes observed in the natural world. Molecular movement, especially of food or water, is vital to the life of a cell. A cell must rely on transport of products into and out of the cell in order to continue normal functioning. There are two major mechanisms of molecular movement: **passive transport** and **active transport**. Passive transport includes diffusion, osmosis, and facilitated diffusion. All are characterized by the fact that they don't require cellular energy (ATP) for molecular movement; rather, they depend on kinetic energy, which moves molecules in a direction of high concentration to low concentration. Active transport differs from the passive transport because it depends upon energy to move molecules against the concentration gradient, from an area of low concentration to high concentration. Examples of active transport are ion pumps (Na^+/K^+ along nerve membranes), endocytosis, and exocytosis. In this lab we will be investigating passive transport mechanisms; both diffusion and osmosis (the diffusion of H_2O).

EXERCISE I. Diffusion Through a Semisolid

Diffusion, a form of passive transport, moves a molecule from high molecular concentration to low molecular concentration. In this experiment, we will be investigating the influence of molecule size on diffusion through a semisolid. In addition, we will be investigating the influence of type of media on rate of diffusion.

Predictions (to help formulate hypothesis):

If placing 1 drop of different colored dye onto the Petri dish semisolid, what could be measured to compare the diffusion rates of different sized dye molecules?

How would size of the molecule change the above measurement?

HYPOTHESIS: Based on information provided in the lab handout, lecture, and textbook, write a general hypothesis(es) relating the size of the molecule to the rate of diffusion.

PROCEDURE

1. Obtain a Petri dish with 4 pre-placed holes in the media. One Petri dish per lab table.
2. Place 1 drop of each dye into an individual hole. Each dye has a different molecular weight (molecule size).
   a. Safranin
   b. Methylene Blue
   c. Malachite Green
   d. Congo Red
3. Replace the lid.
4. In five minutes measure the Congo Red to the nearest mm. ____________________

   (This value will be used for comparison in the diffusion through the liquid portion of this lab.)
5. In 1 hour measure the rate of diffusion of each color (to the nearest mm).
   a. Safranin ____________________ mm
   b. Methylene Blue ____________________ mm
   c. Malachite Green ____________________ mm
   d. Congo Red ____________________ mm

Questions

1. Rank the dye molecule size from smallest to largest.

Dye Molecule Size Smallest to Largest	Measurement in mm

2. Name at least 3 other factors that could influence diffusion rate:

3. Explain how these factors are important to living organisms.

EXERCISE II. Diffusion Through a Liquid

HYPOTHESIS: Based on information provided in the lab handout, lecture, and textbook, write a general hypothesis(es) how the media (semisolid vs. liquid) will affect diffusion.

PROCEDURE

Do not move this experiment once set up.

1. Obtain an empty Petri dish. One Petri dish per lab table.
2. Place 20 mL of d-H_2O into the dish.
3. Place 1 drop of Congo Red dye into the center of the dish. Replace lid and don't move!
4. After 5 minutes measure to the nearest mm the rate of diffusion (over closed lid).

   _____________ mm

5. Transfer the measurement of Congo Red diffusion in semisolid, Step 4, p.26 of the Semisolid Diffusion Lab.

   _____________ mm

6. Compare the rate of diffusion of the Congo Red dye in the semisolid versus the liquid. Which was faster?

Questions

1. In which media, semisolid or liquid, was the rate of diffusion the fastest?

2. What would account for the difference in the rate of diffusion?

3. Was your original hypothesis correct?

EXERCISE III. Osmosis in the Largest Living Cell…the Egg

Water molecules pass through the cell membrane in either direction. This movement of water molecules through the membrane is called **osmosis**. The direction of water movement through the membrane is dependent on a diffusion gradient. The **diffusion gradient** is the relationship between the external environment on one side of the membrane and the internal environment on the other side. If the concentrations of water are the same, the external environment is said to be **isotonic** with the internal environment. If the external environment has a high concentration of water molecules and a low concentration of solute, the water will move toward the internal environment. This condition is called **hypotonic**. Animal cells will swell or burst in a hypotonic environment. Plant cells will develop **turgor** pressure in a hypotonic environment. If the external environment has a high concentration of solute and a low concentration of water, water in the cell will diffuse out into the external environment. A condition of this sort is called a **hypertonic** environment. Plant cells in a hypertonic environment will undergo a condition called **plasmolysis**. This condition causes the plant to exhibit "wilting." In red blood cells the condition of cell shrinkage is called **crenation**.

In this lab exercise, we will be investigating the influence of different external environments on egg cells. We will try to determine which environments are hypotonic, isotonic, and hypertonic.

Prediction Table. (Circle your predictions based upon the hypothesis(es))

	distilled H_2O	1.5 M Sucrose
Osmotic Environment	hypotonic/hypertonic or isotonic	hypotonic/hypertonic or isotonic
Physical Change to Egg	swell/shrink or stay the same	swell/shrink or stay the same
Weight Changed	Positive/negative/"0"	Positive/negative/"0"

HYPOTHESIS: Based on information provided in the lab handout, lecture, and textbook, write a general hypothesis(es) for this experiment.

PROCEDURE

1. Your eggs have been decalcified by soaking in a vinegar solution for 48–72 hours, and then placed in isotonic saline solution. Select two eggs, blot dry with a towel, and weigh to nearest 0.1 gram. *Use a weigh boat whenever weighing objects on the balance. Record weight in Table 4.1.
2. Place one egg in a 250 mL beaker filled with 125 mL of distilled water and the other in a 250 mL beaker containing 125 mL of 1.5 M sucrose solution. Leave the eggs submerged for 60 minutes, then dry and reweigh. Record weight in Table 4.1. Calculate the percent weight change for each egg.
3. **Clean up**
   - Return eggs to saline solution
   - Discard sucrose and distilled water solutions
   - Rinse the beakers and place in equipment tray
   - Clean trays and the electronic balances

Before leaving the lab; thoroughly wash your hands with soap and water. Lab tabletops should be cleaned with laboratory disinfectant, and all materials returned properly to the location they came from.

Table 4.1

	Eggs	
	Distilled H_2O	1.5 M Sucrose
Initial weight		
Final weight		
Percent change		

$$\text{percent change} = \frac{(\text{final weight} - \text{initial weight})}{\text{initial weight}} \times 100$$

Questions

1. What does the egg's weight change indicate about osmosis in the two beakers?

2. Do the experimental results support all your predictions?

3. If your results differ from the predictions, why might this be so?

EXERCISE IV: Effects of Tonicity and Osmosis on a Red Blood Cell

Determination of Isotonic point in Red Blood Cells

Red blood cells were placed in the following saline (NaCl) solutions: 0.2%, 0.4%, 0.6%, 0.8%, 1.0% and 1.2%. The red blood cells' % change in weight (due to osmosis) are shown in the table below. Using the data, construct a graph to determine the isotonic point of red blood cells. (LINE OF BEST FIT.)

Concentration of saline (NaCl)	0.2%	0.4%	0.6%	0.8%	1.0%	1.2%
Percent change in weight	5.0%	3.25%	2.2%	1.2%	–1.5%	–2.5%

Use the Comment Tools and select the drawing/ pencil/add text tools to complete the graph.

1. What is the isotonic point as determined by your graph (be precise)?

2. Why would this be important to know if you were going to administer a saline IV to a patient?

3. Which saline solutions were hypertonic to the blood cells?

4. Did water move in or out of the cells in hypertonic solutions?

5. Which saline solutions were hypotonic to the blood cells?

6. Did water move in or out of the cells in hypotonic solutions?