EXPERIMENT #5
DIFFUSION AND OSMOSIS

Introduction
In order to live, the cell must utilize mechanisms to move essential materials into the cell and waste materials out of the cell through the cell membrane. Some of these mechanisms require an expenditure of metabolic energy, while others occur as the result of basic physical laws. This laboratory will familiarize you with the movement of molecules between the cell and its environment. Today, we will be looking at three basic movements: Diffusion, Osmosis, and Dialysis.

Goals
At the end of this laboratory, you will:
1. Be familiar with the variables governing molecular movement.
2. Be familiar with basic chemical tests to verify both positive and negative experimental results.
Experimental Procedure

A. Brownian motion

At normal environmental temperatures, all matter is in motion. Molecules in a gas or liquid are bouncing off each other, while the molecules of a solid are vibrating. The rate of motion depends upon variables such as the size of the particle, the temperature, and the concentration (amount) of the particles in a given environment.

Elodea leaf wet mount procedure.

1. Place an Elodea leaf on a slide.
2. Add a drop of water
3. Take a cover slip and draw the corner across the leaf to cut it open.
4. Place cover slip over the leaf and gently crush the leaf.
5. Place on the microscope and observe particle movement at 430X magnification.
6. Please observe the pictures on the next slide illustrating the procedure.
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Experimental Procedure
A. Brownian motion (continued):

1

2

3

4
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Experimental Procedure
A. Brownian motion (continued):

5

6

7
Experimental Procedure

B. Diffusion of Gases

Diffusion is the movement of molecules from a region of high concentration to a region of low concentration. This movement is due to the KINETIC energy that is responsible for Brownian movement. In an enclosed environment, such as a test tube or in a room, the molecules will disperse themselves until the concentration is equal throughout. The rate of diffusion is DIRECTLY proportional to the temperature of the environment and INVERSELY proportional to the molecular weight of the moving molecule. In this experiment, you will be observing the movement of two gases: Hydrochloric acid and Ammonium hydroxide in an enclosed glass tube.
Experimental Procedure

B. Diffusion of Gases (continued)

1. Place the glass tube in the ring stand as illustrated in the picture below.
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Experimental Procedure

B. Diffusion of Gases (continued)

2. Take the corks with the cotton swabs and dip them into the solutions of hydrochloric acid and ammonium hydroxide.

3. Place them immediately into the opposite ends of the glass tube.
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Experimental Procedure

B. Diffusion of Gases (continued)

4. Observe the glass tube closely. As soon as a cloudy white ring of smoke appears, take the wax pencil and mark the spot. Click on the picture below to see a video depicting the appearance of this smokey ring.

5. Measure the distance from the ends of Q-tips to the mark.

6. Answer the questions in the lab summary pertaining to this gas diffusion experiment.
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Experimental Procedures

C. Osmosis

Osmosis is the diffusion of water (or solvent) molecules through a semi-permeable membrane. The water molecules will move from a region of high water concentration to a region of low water concentration.

How can water concentration be high or low?

If you were to take 100 g of glucose and dissolve it in water. If your final solution volume is 1000 ml, you’ve just made a 10% glucose solution. Now if your do the same with 200 g of glucose and make a 20% glucose solution, which solution has the higher WATER concentration?

The 10% glucose solution contains 90% water vs. 80% in the 20% glucose solution

Hence, if the solutions were placed on opposite sides of a membrane permeable only to water, water will move across the membrane from the 10% glucose solution to the 20% glucose solution.
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Experimental Procedures

C. Osmosis - Osmosis demonstration

1. Three artificial cells will be constructed using dialysis tubing and clamps. They will be filled with either distilled water or a “pink” glucose solution. Please note their appearance.

2. Each “cell” will then be placed into a beaker containing either distilled water or the “pink” glucose solution.

3. At the end of the experiment, your lab instructor will remove the “cells” from the beaker. You will indicate whether the “cells” became flaccid, turgid, or did not change.

4. Based on your knowledge of osmosis, you will be able to determine which direction water moved to cause the observed events.

5. The next slide will show you the basic experimental set-up.
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Experimental Procedures

C. Osmosis - Osmosis demonstration

Cell

↓

Beaker

Pink = glucose solution
Clear = Distilled water
**Experimental Procedures**

C. Osmosis - Plasmolysis of an Elodea leaf

1. Prepare a wet mount of an Elodea leaf and observe it under the microscope at 430X.

2. Prepare a second Elodea leaf, but add a drop of sodium chloride (salt) solution before cover slipping it. Observe the differences between the two slides.

3. What caused the change in Elodea leaf structure?
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C. Osmosis - Dialysis experiment

In this experiment, you will be monitoring the movement of molecules across an artificial cell membrane. Due to the unique characteristics of each molecule, some will be able to cross the cell membrane while others won’t. You will be constructing an artificial cell, using dialysis tubing. Inside this cell, you will be placing a solution of protein (albumin) and sodium chloride. This cell will be “water-tight”. You will place this cell into a beaker filled with a starch and sodium sulfate solution. Notice that the cell membrane separates these distinctly different solutions. The question is: Can any of these molecules move down their concentration gradient and cross the cell membrane? At the end of this experiment, you will test for the appearance of protein and sodium chloride in the beaker solution (i.e., it must have crossed the membrane from the beaker solution into the cell solution) and the appearance of starch and sodium sulfate in the cell solution.
Experimental Procedures

C. Osmosis - Dialysis experiment

1. Construct an “artificial” cell using a piece of dialysis tubing and two orange clamps
   A. Soak the dialysis tube in distilled water for 5 minutes
   B. Fold one end over a few times and secure with one orange clamp
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Experimental Procedures
C. Osmosis - Dialysis experiment (continued)
1. “Artificial” cell procedure (continued)
   C. Fill the dialysis bag about half full with the albumin / sodium chloride solution
   D. Fold the other end and secure with the second orange clamp
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Experimental Procedures

C. Osmosis - Dialysis experiment (continued)

2. After the “cell” is constructed, rinse well with distilled water, pat to dry, and weigh to the nearest 0.01 g on the balance beam scale. Record the weight on your summary sheet.

3. After weighing the “cell”, place it in a beaker filled with 100 ml of the starch / sodium sulfate solution

4. Incubate the “cell” in the beaker for 1 - 1.5 hours.

5. Determine the contents of the beaker solution and solution inside the “cell” at the end of the experiment.

6. Please refer to your lab manual and the following slides for proper identification of molecules.
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Experimental Procedures

C. Osmosis - Dialysis experiment (continued)

7. You will be testing the presence of:
   A. Chloride ions
   B. Sulfate ions
   C. Protein
   D. Starch

   in the cell solution and beaker solution.

8. At the end of the 1 - 1.5 hour incubation period, label sixteen test tubes as followed:
   A. IB1 - IB4 = Inside the bag (cell) solution at the beginning of the experiment
   B. OB1 - OB4 = Outside the bag (beaker) solution at the beginning of the experiment
   C. IA1 - IA4 = Inside the bag (cell) solution after the incubation period
   D. OA1 - OA4 = Outside the bag (beaker) solution after the incubation period
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Experimental Procedures

C. Osmosis - Dialysis experiment (continued)

9. Into the IB tubes you will place 3 ml of the protein and sodium chloride solution.

10. Into the OB tubes you will place 3 ml of the starch and sodium sulfate solution.

11. Into the IA tubes you will divide equally the contents of your artificial cell after you have rinsed it with DI water, dried it, weighed it, and recorded its value on your summary sheet.

12. Into the OA tubes you will take 3 ml of your beaker solution that the artificial cell was incubating in.

13. You will analyze each of these tubes for either chloride ions, sulfate ions, protein, or starch...
Experimental Procedures

C. Osmosis - Dialysis experiment (continued)

14. Chloride ion analysis - Tubes IB1, OB1, IA1, OA1

   A. Into each of these tubes you will add 2 drops of AgNO3.
   
   B. If chloride is present, AgNO3 will react with it and form AgCl which is a white precipitate.
   
   C. If no chloride is present, solution will remain clear.
   
   D. See test tubes below for the “+” and “-” results.
Experimental Procedures

C. Osmosis - Dialysis experiment (continued)

14. Sulfate ion analysis - Tubes IB2, OB2, IA2, OA2

A. Into each of these tubes you will add 2 drops of 2% BaCl2.

B. If sulfate is present, BaCl2 will react with it and form BaSO4 which is a white precipitate.

C. If no sulfate is present, solution will remain clear

D. See test tubes below for the “+” and “-” results
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Experimental Procedures

C. Osmosis - Dialysis experiment (continued)

14. Protein analysis - Tubes IB3, OB3, IA3, OA3

   A. You will take protein test strips and dip them into each of the tubes
   B. If protein is present, the test strip will turn green
   C. If no protein is present, the test strip will remain yellow
   D. See strips below for the “+” and “-” results
Experimental Procedures

C. Osmosis - Dialysis experiment (continued)

14. Starch analysis - Tubes IB4, OB4, IA4, OA4

A. Into each of these tubes you will add 1 drops of Lugol’s solution.

B. If starch is present, the solution will turn blue

C. If no starch is present, solution will remain be reddish-brown

D. See test tubes below for the “+” and “-” results
Conclusions

Hopefully, this tutorial has been insightful for the upcoming laboratory. You should be prepared for the following tasks:

1. Preparing Elodea leaf wet mounts for both Brownian motion and plamolysis experiments
2. Observing the diffusion of gases within an experimental tube
3. Observing the movement of both water and dissolved solutes across an artificial cell membrane

These tasks should reinforce your basic understanding of molecules in motion and identify the forces which can influence their movement in space.