EDVOTEK®

AP BIOLOGY BIG IDEA 2 INVESTIGATION 4

EDVO-Kit: AP04

Diffusion and Osmosis

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop an understanding of the molecular basis of diffusion and osmosis and its physiological importance. Students will analyze how cell size and shape determine the rate of diffusion, how solute size and concentration affect diffusion across semi-permeable membranes and how these processes affect water potential. Students will also calculate water potential of plant cells.

Experiment Components

Store the entire experiment at room temperature.

This experiment is designed for 10 lab groups.

Investigation I : Surface Area & Cell Size

A Agar Powder
B Phenolphthalein Solution
C Sodium Hydroxide (NaOH) pellets

Investigation II: Modeling Diffusion & Osmosis

D Powdered sucrose
E NaCl
F Powdered Glucose
G Ovalbumin
- Dialysis tubing

Investigation III: Observing Osmosis in Living Cells

- Solutions from Investigation II
- Microscope slides
- Coverslips

Requirements

Investigation I

- Beaker*
- Ruler
- Razor
- Plastic spoon
- Paper towel
- Timer

Investigation II

- Scales
- 1 ml, 5 ml, and 10 ml pipets
- Graph paper
- Distilled or deionized water
- Beakers* (400 ml)

Investigation III

- Elodea tip or Moss**
- Microscope

*Beakers can be substituted with clear disposable plastic cups.
** Elodea tip can be purchased from biological supply companies (such as Connecticut Valley Biological). Moss can be obtained from a greenhouse or from the woods.

Background Information

Figure 1 - Diffusion of Molecules
Random movement of solute (dissolved particles) and solvent (water molecules) will result in an evenly distributed solution.

DIFFUSION

Diffusion is the net flow of molecules from a region of high concentration to a region of low concentration. This difference in concentration of a substance across space is called a **concentration gradient.** Diffusion is due to the random movement of particles. This phenomenon was first observed by Robert Brown in 1827 and is called **Brownian movement**. All objects in motion have **kinetic energy**, or energy of motion. Particles of matter move in straight lines until they collide with other particles. After colliding, the particles rebound, move off in straight lines until the next collision. There is no loss of energy. Diffusion will continue until there is no concentration gradient (**Figure 1**).

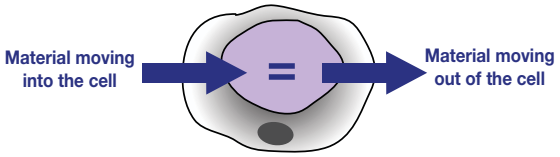


Figure 2 - Dynamic Equilibrium
Molecules are still in motion, but there is no net change when dynamic equilibrium is reached.

In diffusion, molecules move randomly colliding with one another until they become evenly distributed. For example, if one puts a teaspoon of a purple dye, potassium permanganate, into a beaker of water, then the dye molecules, or **solute** (dissolved molecules), will collide randomly with the water molecules, or **solvent.** These random collisions within the **solution** will scatter the molecules of solute and solvent until they are evenly mixed. However, the molecules will still continue to collide with each other and move about randomly. At this point, there is no overall change in concentration. This condition is known as **dynamic equilibrium.** A system is most stable when it has reached equilibrium. A system will tend to go to equilibrium (lowest, accessible energy state) in the absence of added energy (**Figure 2**).

OSMOSIS

Osmosis is a special type of diffusion. It is the diffusion of **water** molecules across a semi-permeable membrane (a membrane that allows for the diffusion of certain solutes and water) from an area of higher water concentration to one of lower water concentration. For example, if a 1 M aqueous starch solution is separated from a 0.5 M aqueous starch solution by a semi-permeable membrane, then water molecules will move from the 0.5 M aqueous starch solution (higher water molecule concentration) toward the more concentrated 1M starch solution (lower water molecule concentration) until an **equilibrium** of water molecules exists between the two solutions. Since the semi-permeable membrane did not allow for the passage of starch molecules, the 1M-starch solution will gain in volume as the water moves in (**Figure 3**).

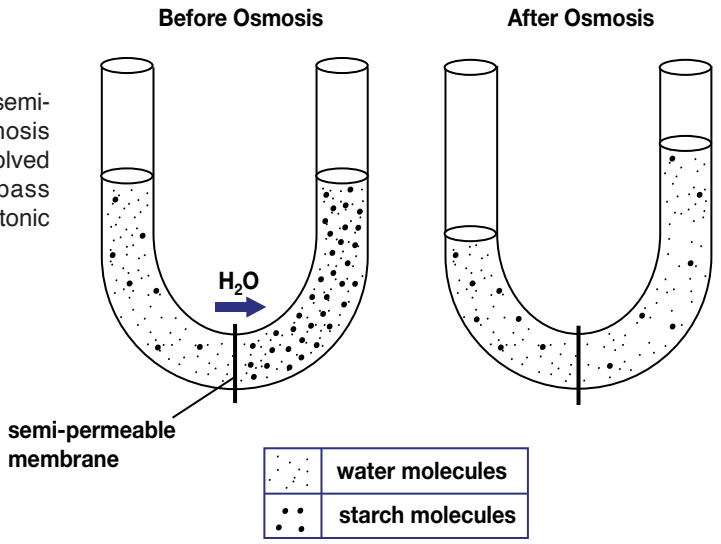
All unicellular and multicellular organisms are surrounded by water solutions. A solution in which the concentration of dissolved substances or solutes is the same as the concentration inside the cell is an **isotonic solution.** It also means that the concentration of water is the same as inside the cell. The cell is in dynamic equilibrium in an isotonic solution. These living cells will not be damaged by a gain or loss of water.

A solution in which the concentration of solutes is lower than the concentration inside the cell is called a **hypotonic solution.** However, the water concentration is higher inside the cell. A cell placed in a **hypotonic** solution will gain water by osmosis and swell in size. This results in an internal pressure. An animal cell, lacking a cell wall, will swell and may A **hypertonic solution** is a solution in which the concentration of solutes is higher than the concentration inside the cell. Therefore, the water concen-

Background Information

lyse, or burst, in a hypotonic solution. A plant cell, having a rigid cell wall will be able to resist the pressure. This increase within a plant cell is known as **turgor pressure.** Turgor pressure gives support and shape to plant cells (**Figure 4**).

Figure 3 - Osmosis
Water molecules will move across a semi-permeable membrane during osmosis to a higher concentration of a dissolved substance (solute) that cannot pass through the membrane (from hypotonic solution to a hypertonic solution).



tration is lower than inside the cell. Animal cells placed into a hypertonic solution will lose water and shrivel up due to the decreased pressure inside the cell. A plant cell placed in a hypertonic solution will lose water from its large central vacuole. The plasma membrane and cytoplasm will shrink away from the cell wall, The end result is the loss of water and a decrease in turgor pressure and is known as **plasmolysis.** This is commonly known as wilting.

PASSIVE AND ACTIVE TRANSPORT

The plasma membrane is a highly selective barrier consisting of two layers of lipid. Embedded in these layers are a wide variety of proteins, glycoproteins, and glycolipids.

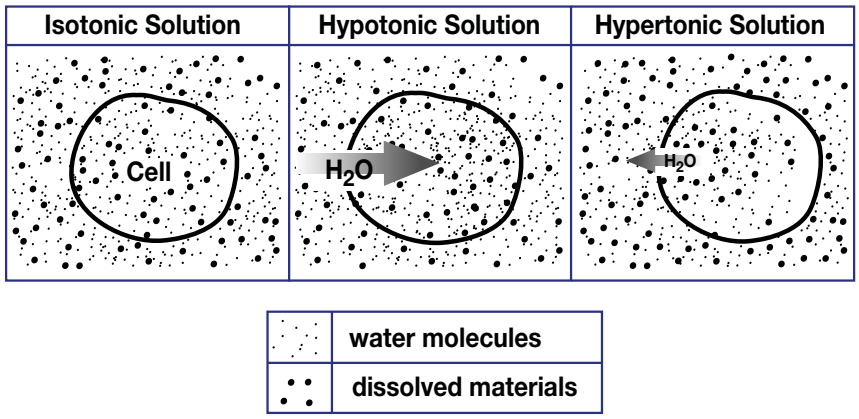


Figure 4 - Solution concentration's effect on cells.
The amount of water entering and leaving cells placed in isotonic solutions is the same. Cells will remain the same size and shape. The cells placed in hypotonic solutions will gain water and swell, while those placed in hypertonic solutions will lose water and shrink.

Background Information

The membrane components are always in a dynamic state of flux, which may create transient pores. Solutes may move through the membrane by either **passive** or **active transport.** **Passive transport** occurs when a solute molecule diffuses down a concentration gradient. There is no expenditure of energy. No ATP is used. Those molecules that are less polar (more lipid soluble) will generally penetrate the membrane more rapidly than polar molecules (more water soluble). However, small polar molecules such as water pass directly through the membrane pores (**Figure 5**).

Passive diffusion of larger molecules possessing high polarity and charge such as sugars and amino acids enter the cell via mediated transport mechanisms. The process known as **facilitated diffusion** uses a carrier protein in the plasma membrane to facilitate the speed of movement of large molecules from a region of high concentration to low concentration. A carrier protein selectively binds to a solute molecule on one side of the membrane, undergoes a conformational change, and releases the solute molecule on the other side of the membrane. Sugar molecules are transported in this manner. Other transport proteins provide passageways by which selective molecules may enter and leave a cell. Most of these dissolved biological materials would not be able to diffuse through the lipid bilayer (**Figure 5**).

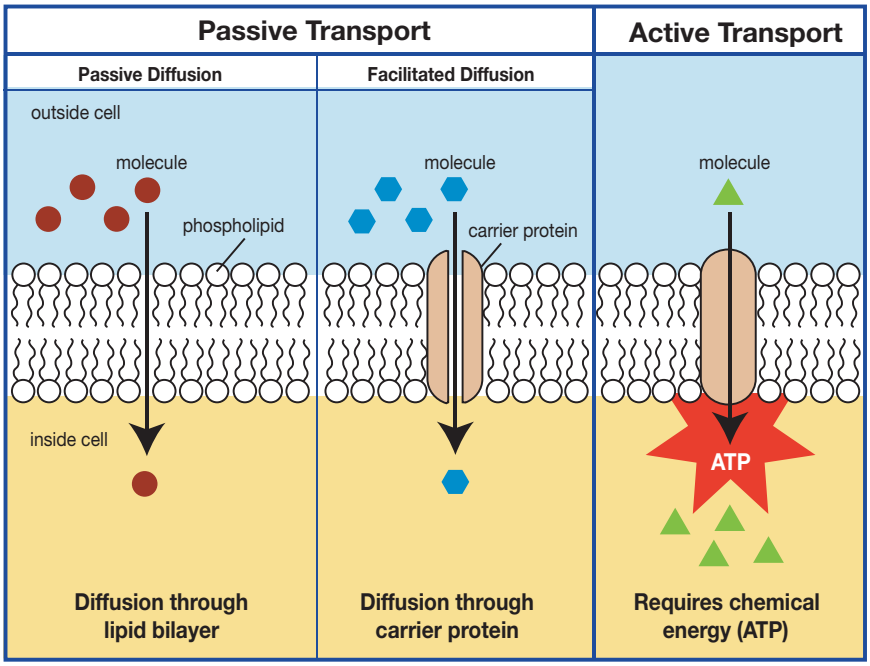


Figure 5 - Comparison of Passive Diffusion, Facilitated Diffusion, and Active Transport
In passive diffusion, hydrophobic molecules and small, uncharged molecules move down their concentration gradient directly across the membrane without the expenditure of energy. In facilitated diffusion, hydrophobic molecules diffuse through a transport protein down their concentration gradient across the membrane. Active transport move molecules up against their concentration gradient by mean of a transport protein. This requires the expenditure of ATP for energy.

Active transport occurs when a solute molecule is moved across a membrane against the concentration gradient by the utilization of chemical energy, or ATP. Active transport can create intracellular concentrations of sugars and amino acids 2 to 50 times higher than extracellular concentrations. A proton pump uses ATP to pump hydrogen ions out of the cell and produce a proton gradient with a higher concentration outside of the cell.

The net uptake or loss of water by the cell depends on which component, the extracellular or cellular fluids, has the **highest water potential .** Water potential is abbreviated by the Greek letter **psi (Ψ)**. Water potential is affected by two physical factors, that is solute concentration (solute potential, **Ψs**) and applied pressure component (pressure potential, **Ψp**). Remember water always moves across a membrane from the solution of **higher** water potential to one with **lower** water potential. The effects of pressure and solute concentration on water

Background Information

potential are represented by this equation:

Ψ		Ψp		Ψs
Water Potential	=	Pressure potential	+	Solute potential

The addition of solutes results in a higher osmotic potential and a decrease in the water potential of the system into the negative range. An increase in pressure raises the water potential of the system into the positive range. Water movement is directly proportional to the pressure on a system. The lower the water potential of a solution, the greater the tendency of water molecules to move into it by osmosis. For example, if potato cells are placed in pure water there will be a net influx of water into the cells, since pure water has a water potential of zero and the water potential in the cell is lower or more negative due to the cytoplasmic solutes. The potato cells will swell and gain in mass. There will be an increase in turgor pressure. When the water potential of the cell equals the water potential of the pure water outside the cell, a dynamic equilibrium is reached and there will be no net water movement.

Likewise, if potato cells are placed in sucrose solutions where the water potential of the cells are higher than the water potential of the sucrose solutions, there will be a flow of water out of the cells. The cells will shrink and lose mass. Therefore, the addition of sucrose to the water outside the potato cells, results in a decrease in the water potential of the solutions surrounding the cells. One can add an amount of sugar to the water, so that the water potential outside the cell is the same as the water potential inside the cell. There will be no net movement of water. However because the water potential inside the cell results from the combination of both pressure potential and solute potential, the solute concentrations inside and outside the cell will not be equal. If one continues to add sugar to the solution outside the cell, water will leave the cells as it moves from an area of higher water potential to an area of lower water potential. Plasmolysis of the cells will result.

WATER POTENTIAL

Water potential can be calculated by first calculating the solute potential of a sucrose solution using the following formula:

$$\Psi s = -iCRT$$

i = Ionization constant (since sucrose does not ionize in water, it is 1.0).

C = Molar concentration of solute

R = Pressure constant (R = 0.0831 liter bars/mole K).

T = Temperature K (°C of solution + 273)

The water potential of the solution can be calculated by knowing the solute potential of the solution and knowing that the pressure potential of the solution is zero. The water potential will be equal to the solute potential of the solution.

$$\Psi = \Psi s$$

Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

The purpose of this experiment is to understand the molecular basis of diffusion and osmosis and its physiological importance. The specific student objectives are:

1. To understand that cell size and shape are important factors in determining the rate of diffusion.
2. To understand by investigation the mechanisms and physiological importance of diffusion and osmosis.
3. To understand how solute size and concentration gradients affect diffusion across semi-permeable membranes.
4. To understand the concept of water potential and how it is affected by solute concentration and pressure potential.
5. To understand how plant cells respond to high solute concentration solutions (hypertonic solutions) and relate result in terms of plasmolysis.

There are three subparts in this experiment. Investigation I allows the students to use artificial cells to study the relationship of surface area and volume. For Investigation II, students will create models of living cells to explore osmosis and diffusion. Students complete the exercise by observing osmosis in living cells in Investigation III.

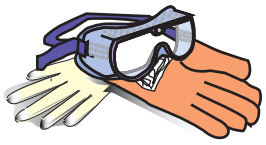
Investigation I: Surface Area and Cell Size. In Investigation I, because cell size and shape are important factors in determining the rate of diffusion, students will investigate the movement of molecules across cell membranes by exploring the relationship between surface area and volume.

Investigation II: Modeling Diffusion and Osmosis. In Investigation II, students create models of living cells using dialysis tubing. Students fill their model cells with two dyes of different molecular weights to visually demonstrate the size selectivity of membranes. The experiments will also demonstrate changes in the equilibrium of the diffusible dye as it is removed from the system.

Investigation III: Observing Osmosis in Living Cells. In Investigation III, the water potential of plant tissue will be determined. Potato cells will be placed into solutions containing different concentrations of sucrose. Some of the solutions will have lower water potentials (higher solute concentration) than the cells and are hypertonic. Others will have higher potentials (lower solute concentration) and are hypotonic relative to the cells. Changes in cell mass will occur by osmosis. Investigation III also demonstrates the effect a hypertonic solution has on plant cells.

Experiment Overview and General Instructions

LABORATORY SAFETY GUIDELINES



1. Wear gloves and goggles while working in the laboratory.
2. Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR!

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:
- Record your observations.

After the Experiment:
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Investigation I - Surface Area and Cell Size

Objective

In Investigation I, because cell size and shape are important factors in determining the rate of diffusion, students will investigate the movement of molecules across cell membranes by exploring the relationship between surface area and volume.

The phenolphthalein in the agar cubes reacts with the Sodium Hydroxide Solution (NaOH), changing the color of the cube to pink. After the cubes are exposed to NaOH, students will be able to see how far the NaOH diffused based upon the change in color that it caused. This will allow the students to determine the relationship between diffusion and the surface area and volume of the cubes.

Use extreme caution when working with razor blades!

Use extreme caution when working with NaOH. Wear safety goggles and gloves and work in a well ventilated area.

Procedure

1. Each group will cut three agar cubes - a 3 cm cube, a 2 cm cube, and a 1 cm cube. Using the provided razor, carefully cut as accurately as possible.
2. Carefully pour 100 ml of 0.1 M NaOH solution into the 200-ml beaker.
3. Note the time and immerse the three agar cubes in the NaOH solution. Fill the beaker with more NaOH, if needed, so that the cubes will be completely submerged in the solution.
4. Let the agar cubes soak for 10 minutes with periodic gentle stirring.
5. After 10 minutes, use a spoon or tongs to remove the agar cubes. Let dry on a paper towel.
6. Using the razor, carefully cut each cube in half.
7. Using the ruler, measure the depth to which the pink color has penetrated.
8. Set up and complete the following data table
    a. Calculate the surface area and volume of each agar cube and record these values in the table on page 11.
    b. Calculate the surface area and volume of this portion of the cube and record these values in the table on page 11.

Investigation I - Surface Area and Cell Size

Analysis of Results

Cube Size	Surface Area (cm^2)	Volume (cm^3)	Surface-to-Volume Ratio	Size of colored portion of cube (cm)	Surface area of colored portion (cm^2)	Volume of colored portion(cm^3)	Diffusion Rate (mm/min)
1 cm							
2 cm							
3 cm							

Table I – Surface Area and Cell Size

1. If the length of the side of a cube is increased, the surface area to volume ratio of the cube _____

2. **The rate of diffusion into the cubes is the volume of the colored area divided by the time it took.** Calculate the rate of diffusion for each of the cubes.

   Cube #1: _____
   Cube #2: _____
   Cube #3: _____

3. In which of the cubes was the rate of diffusion greatest? _____

Investigation II - Modeling Diffusion and Osmosis

Objective

In Investigation II, students create models of living cells using dialysis tubing. Students fill their model cells with two dyes of different molecular weights to visually demonstrate the size selectivity of membranes. The experiments will also demonstrate changes in the equilibrium of the diffusible dye as it is removed from the system.

Dialysis membranes are made of purified cellulose containing microscopic pores. The pore size, which is controlled during manufacture, determines the membrane's permeability to solutes of different sizes. Increasing size generally corresponds to increasing molecular weight when molecules have similar shapes. The dialysis tubing being used in this experiment will act as a cell model in the osmosis procedure. Osmosis is the net flow of water across a semi-permeable membrane due to changes in solute concentrations. Increases in solute concentrations decrease the concentration of water. Water diffuses from a region of higher concentration to a region of lower concentration. In this experiment, different solutions behave like an osmotic system. The dialysis tubing will only be in the beaker for 30 minutes. As this is not long enough for the system to come to equilibrium, a net change in water distribution should be observed.

Procedure

A. Identification of the solutions per student group

- Five different solutions are provided in the Osmosis procedure - 1 M Sucrose Solution, 1 M NaCl Solution, 1 M Glucose Solution, 5% Ovalbumin Solution, and distilled water.
- Choose up to ten pairs of different solutions for the entire lab. One solution from each pair will be inside the model cell (dialysis tubing), and the other solution will act as the liquid outside of the cell (in the beaker).
- Each group will perform the osmosis experiment using their assigned pair. Also, one group can be assigned to design the control model cell, which will have water inside and outside. Label the beakers to indicate what solution is inside the cell and inside the beaker. Record this information in Table II.A.

Group	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Soln. inside Cell										
Soln. in Beaker										

Table II.A Solution Identification

Investigation II - Modeling Diffusion and Osmosis

B. Performing the Diffusion and Osmosis procedure using the dialysis tubing

1. Tie a knot at one end of each piece of dialysis tubing. Start approximately one inch from the end. DO NOT TIE THE KNOT TOO TIGHTLY, otherwise tubing may tear or puncture. Keep tubing moist but avoid having too much water inside.

2. Fill each dialysis tubing with 10 ml of the solution you choose for inside the cell. Tie a knot at the open end of the tubing as instructed in Step 1. Remember to leave enough space for water to diffuse into the cell.

3. Weigh each cell and record the initial weight.

4. Immerse the dialysis tubing by filling each beaker with the second solution (outside cell) for that pair.

5. After 30 minutes, remove the tubing and blot dry with paper towel. Weigh each piece and record the mass as final mass in Table II.B.

6. Determine percent change between initial mass and final mass:

7. $$\frac{\text{FINAL MASS - INITIAL MASS}}{\text{INITIAL MASS}} \times 100 = \%\ \text{CHANGE}$$

8. Record these values in Table II.B.

9. Graph the % change on the Y-axis versus the class group number on the X- axis.

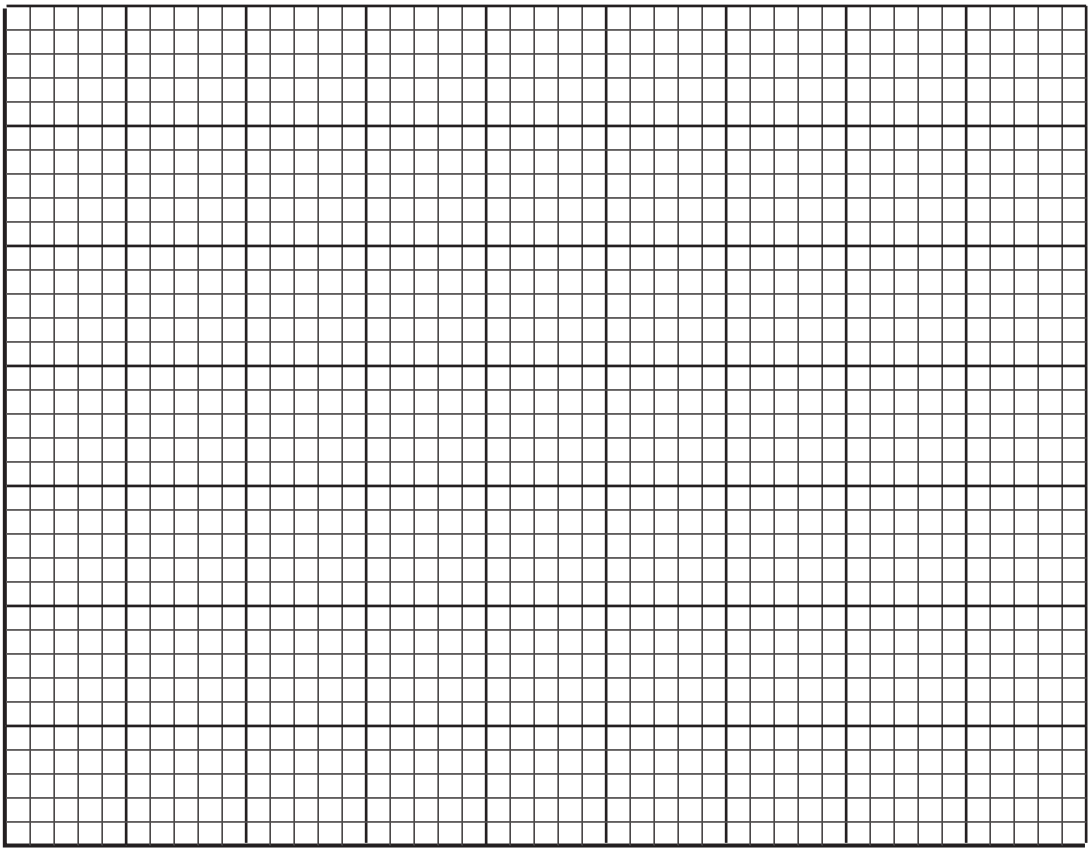
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	Group Average
Initial Mass											
Final Mass											
Mass Difference											
% Change in Mass											

Table II.B – Osmosis Results – Class Data

Investigation II - Modeling Diffusion and Osmosis

Analysis of Results

1. Graph the results from your class average.
2. Label the independent variable (horizontal x-axis).
3. Label the dependent variable (vertical y-axis).
4. Title the Graph



Investigation III - Observing Osmosis in Living Cells

Objective

In Investigation III, the water potential of plant tissue will be determined. Potato cells will be placed into solutions containing different concentrations of sucrose. Some of the solutions will have lower water potentials (higher solute concentration) than the cells and are hypertonic. Others will have higher potentials (lower solute concentration) and are hypotonic relative to the cells. Changes in cell mass will occur by osmosis. Investigation III also demonstrates the effect a hypertonic solution has on plant cells.

Procedure

1. Start by looking at the leaf-like structure from *Mnium hornum* (moss) under the light microscope.
    a. Place the moss on a microscope slide.
    b. Observe and draw the cells at 400 X total magnification.
2. Prepare a wet mount of Moss, as follows:
    a. Add one or two drops of tap water on a microscope slide.
    b. Add a cover slip. Observe and draw the cells at 430 X total magnification. Describe the appearance of the Moss cells.
3. Test the Moss with one of the four solutions prepared in Investigation II - 1 M Sucrose Solution, 1 M NaCl Solution, 1 M Glucose Solution, 5% Ovalbumin Solution.
    a. Using the wet mount of Moss prepared in step 2, add two or three drops of the solution to one edge of the cover slip.
    b. Place a piece of paper towel along the opposite edge of the coverslip. The liquid will soak into the paper towel, drawing the solution under the coverslip.
    c. Observe and draw under 430X. Describe what has occurred.

Analysis of Results:

Describe what happened to the Moss as it was exposed to the different solution.

Study Questions

Investigation I

1. What is diffusion? What is osmosis?
2. Explain how turgor pressure occurs.

Investigation II

1. Which pair(s) that you tested did not have a change in weight? How can you explain this?
2. If you compared 1 M solutions, was a 1 M NaCl solution more or less hypertonic than a 1 M sucrose solution? What is your evidence? What about 1 M NaCl and 1 M glucose and 1 M sucrose?
3. Does the protein solution have a high molarity? What is evidence for your conclusion?
4. Based on what you learned from your experiment, how could you determine the solute concentration inside a living cell?

Investigation III

1. What is plasmolysis?
2. Give some examples where plasmolysis is observed in daily life.