

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 osmosis across semi-permeable membranes. Students will also examine water potential in live plant cells.

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Safety Data Sheets can be found on our website:

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All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

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Experiment Components

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
- Transfer Pipets

Store the entire experiment at room temperature.

This experiment is designed for 10 lab groups.

Requirements

Investigation I

- Beaker*
- Ruler
- Dull and unserrated knife, thin strip of hard plastic, or razor
- Hot plate or microwave
- Plastic spoon
- Paper towel
- Timer
- HCl
- Shallow tray
- Distilled or deionized water

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.

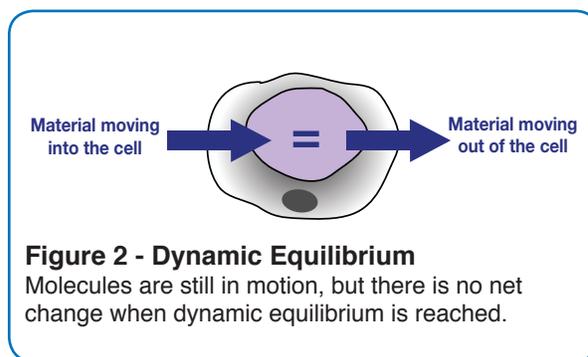
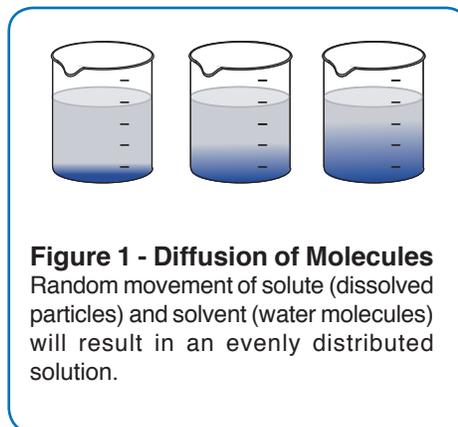
** Elodea tip can be purchased from biological supply companies (such as Connecticut Valley Biological). Moss can be obtained from a greenhouse or from the woods.

Background Information

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**).

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 solvent or water across a semi-permeable membrane (a membrane that allows for the diffusion of certain solutes and water) from an area of higher concentration to one of low 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.

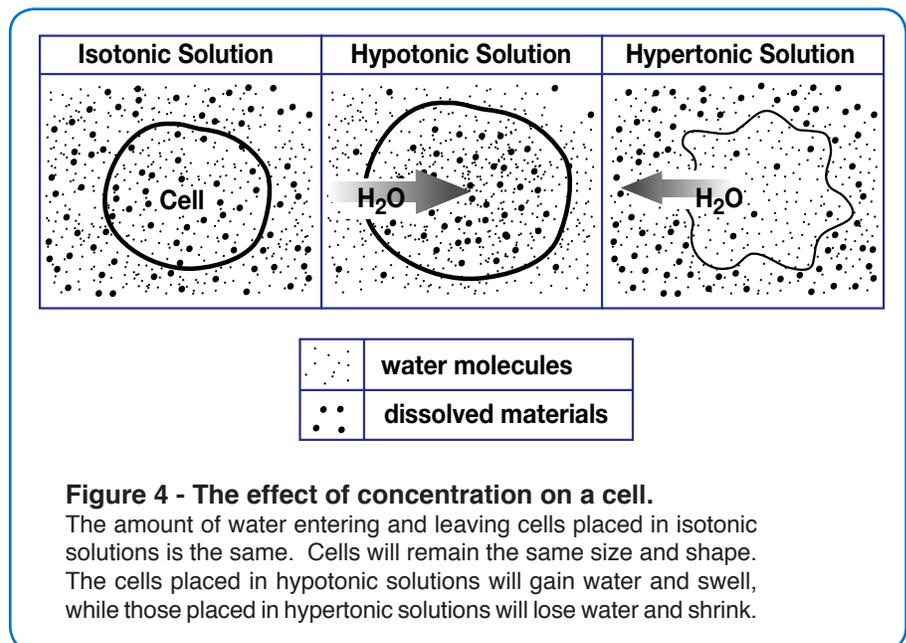
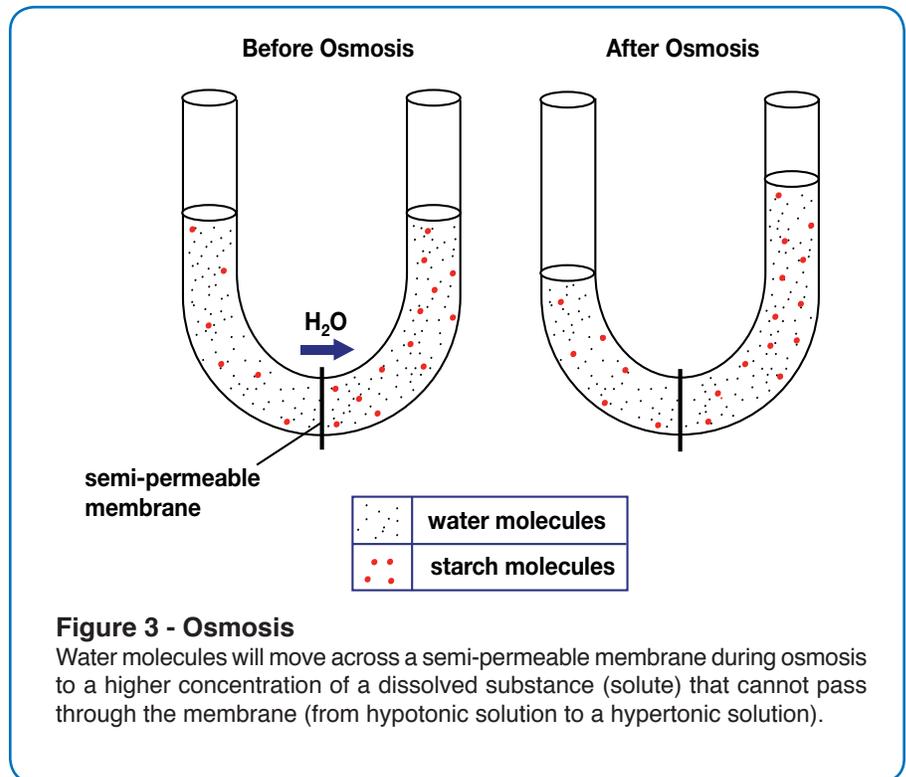
Background Information

A solution in which the concentration of solutes is lower than the concentration inside the cell is called a **hypotonic solution**. In this situation, the water concentration is lower 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 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).

A **hypertonic solution** is a solution in which the concentration of solutes is higher than the concentration inside the cell. Therefore, the water concentration 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 in plant cells 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. The membrane components are always in a dynamic state of



Background Information

flux, which may create transient pores. Solute molecules 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).

Diffusion of larger polar and/or charged molecules, like amino acids or sugars, is assisted by specific transport proteins. The process known as **facilitated diffusion** uses a carrier protein in the plasma membrane to facilitate the 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).

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.

WATER POTENTIAL

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

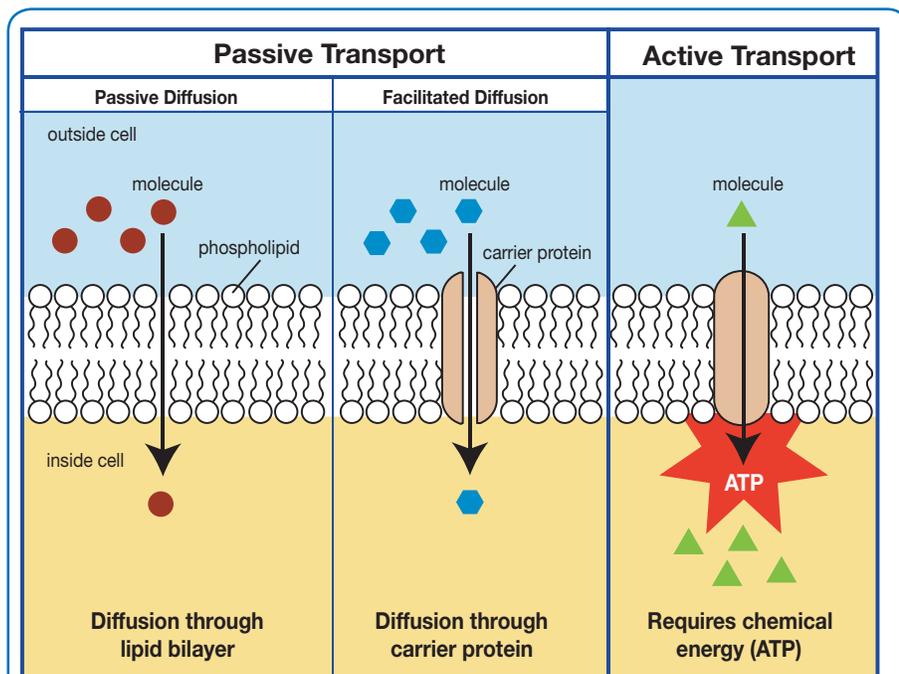


Figure 5 - Comparison of Passive Diffusion, Facilitated Diffusion, and Active Transport

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.

Background Information

factors, that is solute concentration (solute potential, Ψ_s) and applied pressure component (pressure potential, Ψ_p). 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 potential are represented by this equation:

$$\begin{array}{rcccl} \Psi & & \Psi_p & & \Psi_s \\ \text{Water} & = & \text{Pressure} & + & \text{Solute} \\ \text{Potential} & & \text{potential} & & \text{potential} \end{array}$$

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 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 to plasmolysis.

There are three subparts in this experiment. Investigation I allows the students to use artificial cells to study the relationships between surface areas, volumes, and diffusion rates. 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 this experiment, 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 this experiment, students create a model of a living cell using dialysis tubing. Microscopic pores in the dialysis tubing allow small sized solutes to pass through, mimicking the selective permeability of a cell's membrane. Students will select a unique pair of solutions to place in and outside of their cell, and then observe the change in mass of their cell over time.

Investigation III: Observing Osmosis in Living Cells. In this experiment, students observe the process of plasmolysis in live plant cells that are exposed to different solutions. By observing changes in the cell's walls and central vacuole students should be able to classify each solution as hypertonic or hypotonic relative to the plant cell.

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.

PROCEDURE

1. Each group will cut three agar cubes - a 3 cm cube, a 2 cm cube, and a 1 cm cube. 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. Carefully cut each cube in half.
7. Using a ruler, measure the edge lengths of the clear and pink portions of the cube.
8. Set up and complete the following data table
 - a. Calculate the original surface area, volume, and surface to volume ration for each cube and record these values in the table on page 11.
 - b. Calculate the volume of the clear portion of each cube using your measurement from step 7.
 - c. Calculate the volume of the pink portion of each cube by subtracting the clear portion volume from the original volumes.



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.

Investigation I - Surface Area and Cell Size

Cube Size (cm)	Surface Area (cm ²)	Volume (cm ³)	Surface-to-Volume Ratio	Edge length of clear portion (cm)	Volume of clear portion (cm ³)	Volume of pink portion (cm ³)
1						
2						
3						

ANALYSIS OF RESULTS

Table I – Surface Area and Cell Size

1. If the length of the side of a cube is increased, will the surface area to volume ratio of the cube increase or decrease?
2. **The rate of diffusion into the cubes is the volume of the pink portion 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 a model of a living cell using dialysis tubing. To explore the effects of selective membranes on water movement and osmosis they select a unique pair of solutions to place in and outside of their cell, and then observe the change in mass of their cell over time.

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.
- As a class choose up to ten pairs of different solutions to test. The first solutions in each pair will be inside the model cell (dialysis tubing) while the second solution will act as liquid outside of the cell (in the beaker). 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.

Experiment Procedure

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. Re-weigh each cell and record the final weight.
6. Determine percent change between initial weight and final weight:

$$\frac{\text{FINAL WEIGHT} - \text{INITIAL WEIGHT}}{\text{INITIAL WEIGHT}} \times 100 = \% \text{ CHANGE}$$

7. Record each group's results in Table II.B.

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

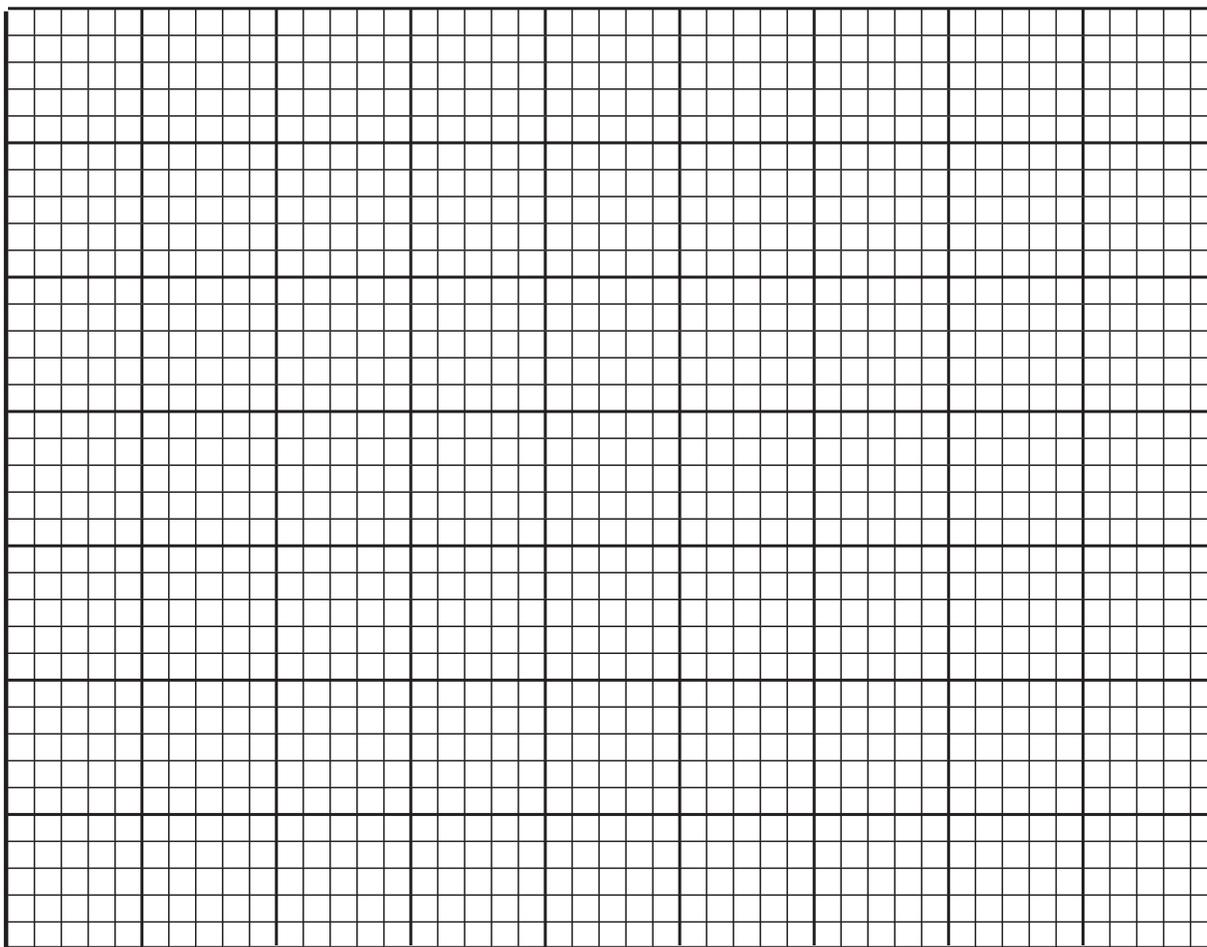
Table II.B – Osmosis Results – Class Data

Investigation II - Modeling Diffusion and Osmosis

ANALYSIS OF RESULTS

1. Graph the result from Table II.B. In this investigation, the independent variable was the group number/ solution combination and the dependent variable was the percentage weight change. Remember to label both axis and to title the graph.
2. Which tested pair(s) did not have a change in weight? How can you explain this?

Experiment Procedure



Investigation III - Observing Osmosis in Living Cells

OBJECTIVE

In Investigation III, students observe the process of plasmolysis in live plant cells that are exposed to different solutions. By observing changes in the cell's walls and central vacuole students should be able to classify each solution as hypertonic or hypotonic relative to the plant cell.

PROCEDURE

1. Start by looking at the leaf-like structure from 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 onto the moss sample.
 - b. Add a cover slip. Observe and draw the cells at 400 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 400X. Describe what has occurred.

ANALYSIS OF RESULTS

1. Which solution did you add? What did you expect would happen?
2. Describe what happened to cell when the solution was added. Pay attention to key organelles like the chloroplasts, the central vacuole, and the cell wall as well as to the overall shape, size, and appearance of the cell.
3. During step three what direction was the water moving? Does this suggest the solution you chose was hypertonic or hypotonic relative to your cells?
4. What prevented your cells from either bursting or collapsing?

Study Questions

1. What is diffusion? What is osmosis?
2. What is plasmolysis?
3. Explain the differences between hypotonic, hypertonic and isotonic in terms of water concentration inside and outside of the cell, net water movement, and changes in cell size.
4. Explain how turgor pressure occurs.

Instructor's Guide

Notes to the Instructor & Pre-Lab Preparations

Overview of Laboratory Investigations

The "hands-on" laboratory experience is a very important component of science courses. Laboratory experiment activities allow students to identify assumptions, use critical and logical thinking, and consider alternative explanations, as well as help apply themes and concepts to biological processes.

EDVOTEK experiments have been designed to provide students the opportunity to learn very important concepts and techniques used by scientists in laboratories conducting biotechnology research. Some of the experimental procedures may have been modified or adapted to minimize equipment requirements and to emphasize safety in the classroom, but do not compromise the educational experience for the student. The experiments have been tested repeatedly to maximize a successful transition from the laboratory to the classroom setting. Furthermore, the experiments allow teachers and students the flexibility to further modify and adapt procedures for laboratory extensions or alternative inquiry-based investigations.

Organizing and Implementing the Experiment

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site: www.edvotek.com

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Pre-Lab Preparations

INVESTIGATION I: SURFACE AREA AND CELL SIZE

A. Preparation of the agar/phenolphthalein solution

1. Add all of the agar content to a flask or beaker (1 L size or larger).
2. Add 500 ml distilled or deionized water to the powdered agar. Swirl and stir to dissolve the powder (a stir plate, if available may be useful).
3. Bring the agar solution to a boil. Stir frequently until solution is clear.
4. Remove from heat. As the agar mixture cools add the entire contents of Phenolphthalein Solution to the agar solution. Mix well.

Note: If the mixture is pink, add a few drops of dilute hydrochloric acid until the pink color disappears.

5. Adjust to a final volume of 750 ml with distilled or deionized water. Mix well.
6. Pour the agar/phenolphthalein solution into a shallow tray to a depth of 3 cm and allow it to set.

NOTE: A tray measuring 10 cm X 20 cm that is at least 3 cm deep will accommodate agar/phenolphthalein solution. Volume adjustments may be necessary depending on the tray used.

Save 10 ml of the Sucrose, NaCl, and Glucose solutions for Investigation III (30 ml total). The remaining volumes and solutions should be provided to students groups for in cell solutions.

7. Cut the agar into 3 cm x 3 cm x 5 cm blocks, one per lab group.

B. Preparation of NaOH Solution

1. Dissolve all of the Sodium Hydroxide (NaOH) pellets in 750 ml of distilled or deionized water.
2. Mix well and bring the final volume to 1.2 L with distilled or deionized water.
3. Each group will use 100 ml of 0.1 M NaOH solution.

For Investigation I, each student group will receive:

- Agar block
- Dull and unserrated knife, thin strip of hard plastic, or razor
- NaOH solution in beaker
- Spoon or tongs
- Paper towel
- Ruler
- Gloves

Pre-Lab Preparations

INVESTIGATION II: MODELING DIFFUSION AND OSMOSIS

A. Preparation of Dialysis Tubing

1. Cut the dialysis tubing into 7-inch sections. One to two days before the laboratory, soak the dialysis tubing in distilled water:
 - a. Place the cut dialysis tubing in a 600 ml beaker and cover with 400 ml distilled water.
 - b. Tubing should be covered by distilled water.
2. Each student will use one section of dialysis tubing to model cells.

B. Preparation of Solutions

Five different solutions (1 L each) are needed - 1 M Sucrose Solution, 1 M NaCl Solution, 1 M Glucose Solution, 5% Ovalbumin Solution, and distilled water. These five solutions yield enough material for 15 beakers (300 ml each) of the "outside cell" solution. The remaining volume of these five solutions can be shared among the student groups to use as the "inside cell" solutions.

Choose up to 10 pairs of different solutions for 10 lab groups. One solution from each pair will be in the model cell of dialysis tubing, and the other will be the outside the cell in the beaker.

Also, one control model cell, which will have water inside and outside, can be prepared for the entire class by a student group or the instructor.

Prepare the solutions as instructed below*, and carefully label the beakers to indicate what solution it contains.

1. 1M Sucrose Solution: Dissolve all of the Sucrose in 750 ml of distilled or deionized water. Mix well and bring the final volume to 1 L with distilled or deionized water.
 2. 1 M NaCl Solution: Dissolve all of the NaCl in 750 ml of distilled or deionized water. Mix well and bring the final volume to 1 L with distilled or deionized water.
 3. 1 M Glucose Solution: Dissolve all of the Glucose in 750 ml of distilled or deionized water. Mix well and bring the final volume to 1 L with distilled or deionized water.
 4. 5% Ovalbumin Solution: Dissolve all of the Ovalbumin with 750 ml of distilled or deionized water. Mix well and bring the final volume to 1 L with distilled or deionized water.
- * The 5th solution is distilled or deionized water.
5. Aliquot 300 ml of each solution into one, two, or three 400 ml sized beakers.
 6. Save 10 ml of the Sucrose, NaCl, and Glucose solutions for Investigation III (30 ml total). The remaining volumes and solutions should be provided to students groups for in cell solutions.

For Investigation II, each student group will need:

- One 400 ml beaker containing the "outside cell" solution
- 1 piece of hydrated (soaked) dialysis tubing
- 10 ml pipets
- Gloves
- Remaining volume of five solutions (Sucrose, NaCl, Glucose, Ovalbumin, water) for use as the "inside cell" solution (**to be shared among student groups**).

Pre-Lab Preparations

INVESTIGATION III - OBSERVING OSMOSIS IN LIVING CELLS

1. Locate and set up class microscopes with a 400 x magnification.
2. Distribute a moss sample, slide, cover slip, pipet, and paper towel to each student.
3. Distribute remaining solutions prepared in Investigation II - 1 M Sucrose Solution, 1 M NaCl Solution, 1 M Glucose Solution. Students will also need a small volume of tap water to prepare the wet mount.

Experiment Results and Analysis**INVESTIGATION I**

Below are representative results. Exact measurements may differ depending on additional factors such as the classroom's and the solution's temperature.

**Table I – Surface Area and Cell Size**

Cube Size (cm)	Surface Area (cm ²)	Volume (cm ³)	Surface-to-Volume Ratio	Edge length of clear portion (cm)	Volume of clear portion (cm ³)	Volume of pink portion (cm ³)
1	6	1	6 to 1	0	0	1
2	24	8	3 to 1	1.5	3.375	4.625
3	54	27	2 to 1	2.6	17.576	9.424

1. Which solution did you add? What did you expect would happen?

Answers will vary. Students should expect osmosis to occur when the cell is exposed to their solution and should expect the cell to undergo plasmolysis if they think the solution is hypertonic or to show signs of turgor pressure if they think the solution is hypotonic.

2. If the length of the side of a cube is increased, will the surface area to volume ration of the cube increase or decrease?

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

3. 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.

The rate of diffusion for cube 1 is 0.1 cm³/min.
The rate of diffusion for cube 2 is 0.4625 cm³/min.
The rate of diffusion for cube 3 is 0.9424 cm³/min.

4. In which of the cubes was the rate of diffusion greatest?

Cube #3

Experiment Results and Analysis

INVESTIGATION II

1. Graph the result from Table II.B. In this investigation, the independent variable was the group number/solution combination and the dependent variable was the percentage weight change. Remember to label box axis and to title the graph.

Results will vary.

2. Which tested pair(s) did not have a change in weight? How can you explain this?

The control pair (water-water) had no changes in weight since there is no concentration difference of fluid on both sides of the membrane. Tubes containing 5% albumin and submerged in water may also have no weight change.

INVESTIGATION III

1. Describe what happened to cell when the solution was added. Pay attention to key organelles like the chloroplasts, the central vacuole, and the cell wall as well as to the overall shape, size, and appearance of the cell.

Answers will vary. When exposed to a solution with a higher concentration of sugar or salt, plasmolysis occurs and the cell membrane shrinks away from the cell wall, the central vacuole collapses, and the chloroplasts become concentrated in the center of the cell. However the cell itself does not get smaller. When exposed to a solution with a lower concentration of sugar or salt the plant cells may appear slightly larger or bulge on the sides. This slight increase in volume may also be observed when plant cells are exposed to pure water.

2. During step three what direction was the water moving? Does this suggest the solution was hypertonic or hypotonic relative to your cells?

Answers will vary. When exposed to a solution with a higher concentration of sugar or salt, the net flow of water will be out of the cell indicating a hypertonic solution. When exposed to a solution with a lower concentration of sugar or salt or to the pure water the net flow of water is into the cell indicating a hypotonic solution.

3. What prevented your cells from either bursting or collapsing?

The cell did not burst because as the volume increased so did turgor pressure. This led to higher water potential in the cell and, consequently, a decrease of water flow into the cell. Likewise, the cell wall remained intact even when the water was leaving the cell and prevented the cell from collapsing.

**Please refer to the kit
insert for the Answers to
Study Questions**