

EXERCISE 5

Name _____

How are substances transported within cells and across cell membranes?

Objectives

After completing this exercise, you should be able to:

- ◆ Describe the kinetic theory of matter and explain what kinetic energy is.
- ◆ Distinguish between movement and net movement. Also, describe the factors that affect the direction and speed of molecular movement and net movement.
- ◆ Define and distinguish between: selectively permeable membrane, plasma membrane, dialysis tubing.
- ◆ For each of the following transport mechanisms, explain how the mechanism works and discuss the factors that affect the rate and direction of transport: simple diffusion, facilitated diffusion, osmosis, dialysis, active transport.
- ◆ Describe how chemical indicators can be used to test for the presence of starch and reducing sugars.
- ◆ Distinguish between isotonic, hypotonic, and hypertonic solutions. Also explain what happens when cells are placed in each type of solution.

Prelab

Before you come to lab, read this entire exercise. You must also answer all questions and complete all assignments on the first 8 pages of this exercise. Your instructor will give you directions on when and where to turn in your work.

Cell survival depends on the movement of substances (such as enzymes, and chemical messengers) within the cell. In addition, certain materials (such as nutrients) must enter the cell, and other materials (such as waste products) must leave. In order to enter or leave a cell, materials must cross the plasma membrane. The movement of substances within the cell or across the plasma membrane is called **cell transport**. Mechanisms of cell transport can be divided into 2 main categories: **passive transport** and **active transport**.

Both passive and active transport require energy. However, with passive transport, the cell itself does not expend any energy. In other words, the cell is passive. Energy for passive transport usually comes from random molecular motion. With active transport, on the other hand, the cell must expend some of its own energy, stored in molecules of ATP.

In this lab, you will study various types of passive transport. To understand how passive transport works, you need to understand the **kinetic theory of matter**. According to this theory, all atoms and molecules are in constant random motion. This gives them energy of motion, also called **kinetic energy**. The kinetic energy of atoms and molecules is detected by humans as heat. As a substance is heated, the atoms and molecules move faster, and their kinetic energy increases. Furthermore, the constant random motion of atoms and molecules causes adjacent substances to become evenly mixed together, even if the substances are undisturbed by outside forces.

As an example, suppose you place a sugar cube in a glass of water. Within a few minutes, the currents created by dropping the sugar cube into the water subside and the water is completely still - to the naked eye. Even if left undisturbed, however, each water molecule in the glass is actually moving in a straight line until it bumps into the sugar cube, the glass, or another water molecule; then it ricochets off in another direction. At the same time, individual sucrose molecules in the cube are also moving and being bombarded by water molecules from all directions. This bombardment eventually shakes individual sucrose molecules free of the sugar cube.

As each sucrose molecule dissolves in the water, it moves off in a straight line until it bumps into something and changes direction. Therefore, each sucrose molecule that separates from the sugar cube will move away from the cube sometimes, and toward it sometimes; its movement is random. However, when the cube is first placed in the water, more sucrose molecules will be moving away from the sugar cube (where the sugar concentration is higher) than are moving back toward the cube. Therefore, we say **movement** of the sucrose molecules is random, but **net movement** is from the area where the sugar concentration is higher to the area where the sugar concentration is lower (i.e. down a concentration gradient.) Likewise, the water molecules in the glass also move at random, but more water molecules will be moving toward the sugar cube (where the water concentration is lower) than are moving away from it. Therefore, **net movement** of the water molecules is toward the sugar cube. Note that each substance shows **net movement** down its own concentration gradient. As a result, net movement of water and sucrose are in opposite directions. This process continues until the sucrose and water molecules are evenly mixed – a point called **equilibrium**. At equilibrium, the sucrose and water molecules continue to move at random but there is no longer any net movement of either substance.

The **net movement** of a substance from a region where it has a higher concentration to a region where it has a lower concentration, due to random molecular motion, is called **diffusion**. Diffusion is a widespread and important process which occurs in both living and non-living systems. Because diffusion occurs under a variety of conditions, scientists have adopted the following terms to specify particular types of diffusion:

- **Dialysis** refers to the diffusion of **solutes** across a **selectively permeable membrane**. A selectively permeable membrane (also called a **semipermeable membrane**) is a membrane that allows some substances to pass through easily while other substances pass through very slowly or not at all.
- **Osmosis**, on the other hand, refers to the diffusion of the **solvent** across a selectively permeable membrane. Because water is the solvent in all living systems, biologists usually define osmosis as the diffusion of **water** across a selectively membrane.
- **Facilitated diffusion** refers to diffusion of a substance across the plasma membrane of a cell with the help of specific transport proteins.
- **Simple diffusion** refers to diffusion of a substance without the help of specific transport proteins.

Although various types of diffusion have been recognized, all share the following characteristics:

- Net movement of each substance is caused by random molecular motion.
- Net movement of each substance involves passive transport.
- Net movement of each substance is down its own concentration gradient.
- At equilibrium, random molecular motion continues but there is no longer any net movement.

In this lab, you will study several different types of diffusion using both non-living and living systems. First, you will conduct two experiments utilizing non-living systems:

- You will study the diffusion of 2 dyes, potassium permanganate and methylene blue, through a non-living, gel-like material called **agar**. Agar is prepared by boiling a complex polysaccharide (called agarose) in water until it dissolves and then allowing the solution to cool. As the solution cools, it turns into a semi-solid gel.
- You will study both dialysis and osmosis across a membrane made of **dialysis tubing**. Dialysis tubing is a commercially prepared, artificial membrane made of cellulose. The membrane contains microscopic pores that allow ions and molecules to pass through. The size of the pores in the membrane determines the size of the ions and molecules that can pass through.

Finally, you will conduct one experiment utilizing a living system:

- You will study the process of osmosis across the plasma membrane of living cells found in the leaves of an aquatic plant called *Elodea*.

How do substances move across a selectively permeable membrane?

A selectively permeable membrane allows some substances to pass through easily, while other substances pass through very slowly or not at all. The selective permeability of a membrane is related to its structure. For example, all cell membranes, including the plasma membrane, are selectively permeable. Cell membranes are composed of a lipid bilayer with embedded proteins. Because lipids are nonpolar, they attract other nonpolar molecules but repel polar molecules and ions. Proteins, on the other hand, often have large polar or charged regions that attract polar molecules and ions. These characteristics of lipids and proteins affect the ability of different types of substances to pass through the plasma membrane:

- Nonpolar molecules (such as fatty acids) can easily mix with and diffuse through the lipid bilayer of the plasma membrane. This process is called **simple diffusion**.
- Very small polar molecules (such as water) can also diffuse through the lipid bilayer. Even though nonpolar lipids tend to repel polar molecules, these molecules can pass through the lipid bilayer because of their small size. However, they pass through more slowly than nonpolar molecules.
- Larger polar molecules (such as amino acids and sugars) and ions of any size (such as Ca^{++}) cannot diffuse through the lipid bilayer. However, in some cases these substances can diffuse through the plasma membrane by passing through specific protein channels or carriers embedded in the membrane. Because these substances need the help of proteins to diffuse across the membrane, this process is called **facilitated diffusion**.

Your Turn

Assume there is a higher concentration of each of the following substances in the extracellular fluid surrounding a cell than in the cell's cytoplasm. Predict whether the substance would be more likely to enter the cell by simple diffusion or by facilitated diffusion, and explain why.

O₂ _____

Explain why: _____

Glucose _____

Explain why: _____

Na⁺ _____

Explain why: _____

Insulin (a protein) _____

Explain why: _____

Estrogen (a lipid) _____

Explain why: _____

Hydrogen ions _____

Explain why: _____

Dialysis tubing is another example of a selectively permeable membrane. However, its selectivity is much more limited than the selectivity of cell membranes. Microscopic holes, or pores, in the dialysis tubing allow substances to be separated *only* on the basis of size. Unlike with cell membranes, whether the substance is nonpolar, polar, or charged has little effect on its ability to cross the tubing. Molecules and ions that are smaller than the pores can move through the tubing, while those that are larger than the pores cannot. Dialysis tubing can be ordered from scientific supply companies in a variety of pore sizes. Dialysis is routinely used in biochemistry and molecular biology laboratories to separate and purify substances on the basis of size.

Your Turn

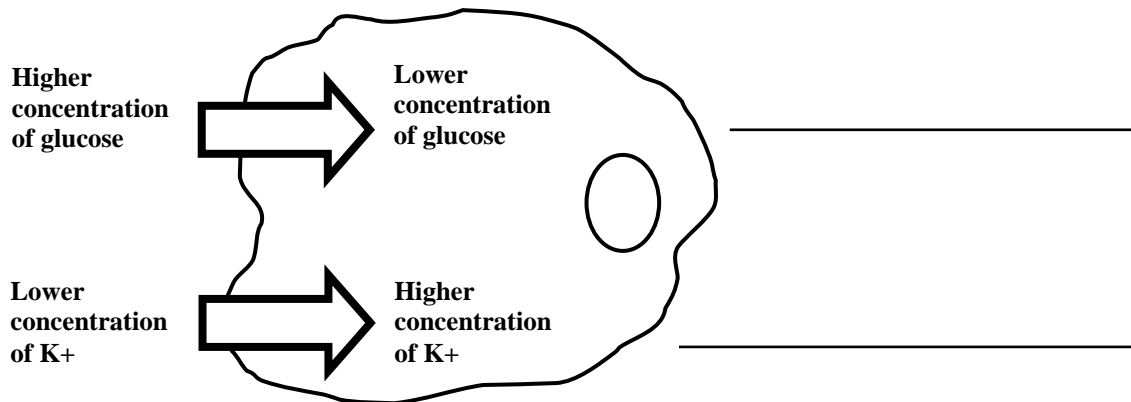
Of the substances listed in the exercise above (O₂, glucose, Na⁺, insulin, estrogen, and hydrogen ions) which would **not** pass easily through the plasma membrane by simple diffusion, but probably would pass through the pores of dialysis tubing? (Hint: There are 2 correct answers.)

Explain why: _____

Diffusion of materials through cell membranes always involves net movement of a substance down its own concentration gradient (from higher concentration to lower concentration.) However, in some cases substances must be transported across cell membranes *against* their concentration gradient (from lower concentration to higher concentration.) This requires cellular energy and, therefore, a form of **active transport**. For example, the transfer of a phosphate group from an ATP molecule to a membrane protein may change the shape of the protein, causing it to pump a substance across the membrane against its concentration gradient. Whenever a substance is being moved against its concentration gradient, a form of active transport is required.

Your turn

Simple diffusion, facilitated diffusion, and active transport allow the cell to be very selective about what is allowed to cross its membranes. On each line below, write whether the indicated substance would enter the cell by simple diffusion, facilitated diffusion, or active transport.



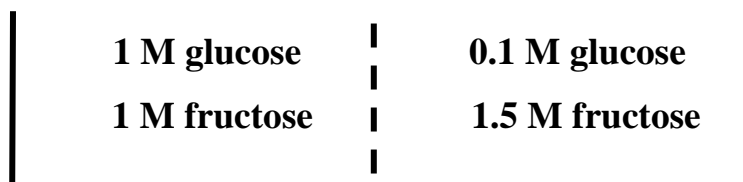
How does solute concentration affect dialysis and osmosis?

If two aqueous solutions are separated by a selectively permeable membrane, each **solute** that can pass through the membrane will show net movement down its own concentration gradient. The diffusion of solutes across a selectively permeable membrane is called **dialysis**. At the same time, if water (the solvent) can pass through the membrane, it will also show net movement down its own concentration gradient. Biologists call the diffusion of water across a selectively permeable membrane **osmosis**.

Keep in mind that when we compare 2 aqueous solutions, the one with the higher concentration of solutes will have the lower concentration of water, and vice versa. Scientists use specific terms to compare the solute concentrations of different solutions. If 2 solutions have the same total concentration of solutes, the solutions are **isotonic** (or isosmotic.) On the other hand, if 2 solutions have different concentrations of solutes, the one with the lower concentration of solutes (higher concentration of water) is **hypotonic** (or hypoosmotic) and the one with the higher concentration of solutes (lower concentration of water) is **hypertonic** (or hyperosmotic.)

Your Turn

The following diagram represents a container with two aqueous solutions separated by a selectively permeable membrane. Water and both solutes can pass through the membrane freely.



I. Before equilibrium is reached in this container:

1. Movement of glucose across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
2. Net movement of glucose across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
3. Movement of fructose across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
4. Net movement of fructose across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
5. Movement of water across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
6. Net movement of water across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
7. Compared to the solution on the right, the solution on the left is best described as
A. isotonic B. hypotonic D. hypertonic
8. Compared to the solution on the left, the solution on the right is best described as
A. isotonic B. hypotonic D. hypertonic
9. Net movement of water is
A. from the hypertonic solution to the hypotonic solution
B. from the hypotonic solution to the hypertonic solution
C. both directions
D. neither direction

II. After equilibrium is reached in this container:

1. Movement of glucose across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
2. Net movement of glucose across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
3. Movement of fructose across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
4. Net movement of fructose across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
5. Movement of water across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
6. Net movement of water across the membrane can best be described as
A. left to right B. right to left C. both directions D. neither direction
7. Compared to the solution on the right, the solution on the left is best described as
A. isotonic B. hypotonic C. hypertonic
8. Compared to the solution on the left, the solution on the right is best described as
A. isotonic B. hypotonic C. hypertonic

III. If a cell is placed in an isotonic solution:

1. Movement of water will be
A. into the cell B. out of the cell C. both in and out D. neither direction
2. Net movement of water will be
A. into the cell B. out of the cell C. both in and out D. neither direction
3. The cell will tend to
A. shrink B. swell C. both D. neither

IV. If a cell is placed in a hypotonic solution:

1. Movement of water will be
A. into the cell B. out of the cell C. both in and out D. neither direction
2. Net movement of water will be
A. into the cell B. out of the cell C. both in and out D. neither direction
3. The cell will tend to
A. shrink B. swell C. both D. neither

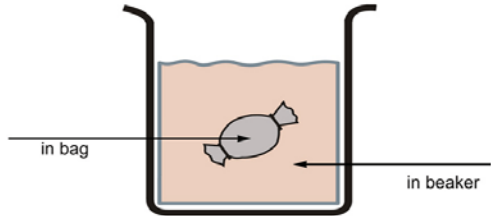
V. If a cell is placed in a hypertonic solution:

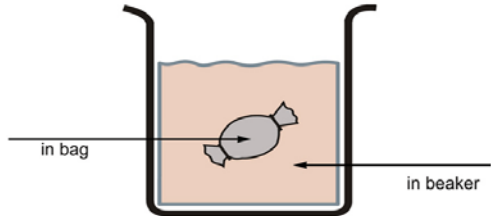
1. Movement of water will be
A. into the cell B. out of the cell C. both in and out D. neither direction
2. Net movement of water will be
A. into the cell B. out of the cell C. both in and out D. neither direction
3. The cell will tend to
A. shrink B. swell C. both D. neither

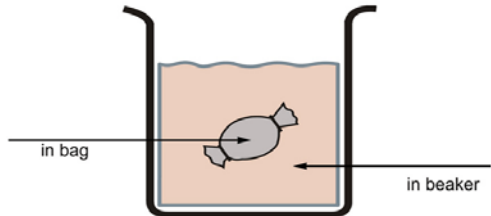
VI. Osmosis across membranes made of dialysis tubing

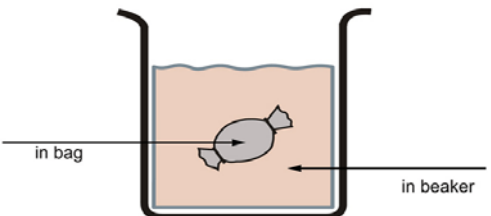
In part III of the Lab Procedures, you will set up an experiment to study both dialysis and osmosis using bags made of **dialysis tubing**. During this experiment, four bags will be filled with different solutions and then placed in beakers also filled with different solutions. Each bag will be weighed at the beginning and end of the experiment in order to determine whether net movement of water was into the bag, out of the bag, or neither. If a bag increases in weight, that means net movement of water was into the bag; if a bag decreases in weight, that means net movement of water was out of the bag; and if there is no change in weight, that means there was no net movement of water.

Read Part III of the **Lab Procedures** (pp. 10 - 11) and then label the arrows in the diagrams below to indicate the solution placed inside each bag and beaker. Then, based on your knowledge of how solute concentration affects osmosis, predict whether the weight of each dialysis bag will increase, decrease, or remain the same.

Bag #1 prediction	
Weight of the bag will A. Increase B. Decrease C. Remain the same	

Bag #2 prediction	
Weight of the bag will A. Increase B. Decrease C. Remain the same	

Bag #3 prediction	
Weight of the bag will A. Increase B. Decrease C. Remain the same	

Bag #4 prediction	
Weight of the bag will A. Increase B. Decrease C. Remain the same	

How can I determine if specific solutes are present in a solution?

It is often a challenge to determine whether or not a certain solute is present in a solution. Many different solutes have the same color (most solutions found in cells are clear), most have no odor, tasting the solution could be hazardous, etc. Sometimes we can test for the presence of a particular solute by adding a chemical indicator to the solution. An indicator is a chemical that will react with the solute being tested for and produce a visible change. This change could be a change in color, the formation of a solid material within the solution (a precipitate), or the formation of a gas. A **negative control** involves adding the indicator to a solution that does **not** have the solute being tested for. A **positive control** involves adding the indicator to a solution that has the solute being tested for. By running both negative and positive controls, you are able to rule out the possibilities that the test is defective, or that your glassware is contaminated. This will help avoid drawing the wrong conclusions from your test results.

Lab Procedures

This lab requires a clock-watcher! Begin this lab exercise by setting up Parts II and III, and noting the time when you begin each activity. Then do Parts I, IV, and V while waiting for diffusion, osmosis, and dialysis to occur. The clock-watcher will be responsible for reminding the group when it is necessary to observe and measure the dye circles for Part II and when it is time to remove the dialysis bags for part III.

I. Observe the effects of molecular motion

Begin this activity after you have set up Parts II and III.

1. Place a drop of water on a clean microscope slide. Using a pair of forceps, break off a small piece of lichen (about the size of a match head) and place it in the drop of water. Use the blunt end of the forceps to thoroughly grind the lichen in the water into grayish green powder.

NOTE: Lichens are organisms that superficially resemble plants but are not true plants. Instead, they are masses of interwoven cells from 2 different types of organisms – a fungus and an alga. The fungus is a non-photosynthetic organism and the alga is a photosynthetic protist. The fungal component and the algal component reproduce separately and remain distinct, yet interact as the lichen grows. Such a close, consistent co-existence is called **symbiosis** and the organisms involved are called **symbionts**.

2. Place a cover slip on the drop and view the ground-up lichen with the microscope. Find an area of the slide where the “lichen powder” is spread thinly, so there is plenty of light and you can see individual, separated grains of the powder.
3. Focus on low power first, and then switch to high power. Under high power, you should see tiny particles that are vibrating in place. The vibration is caused as water molecules on the slide move around and collide with the visible particles. This visible movement is called “Brownian motion” and is evidence for the kinetic theory of matter. Draw a diagram and describe your observations in your lab notebook.

II. Determine how molecular size and temperature affect the rate of diffusion

Begin this activity first.

1. Obtain the following materials:

2 room temperature agar plates, labeled A and B	Marking pen
1 refrigerated agar plate, labeled C	Ruler
Potassium permanganate solution	Micropipetters
Methylene blue solution	straw

2. Turn the agar plate labeled “A” upside down and make two small dots on the bottom of the plate with the marker. (Note: the bottom half of the agar plate is the side that contains the agar.) Each dot should be at least 2 cm from the edge of the plate and at least 3 cm away from the other dot on the bottom of the agar plate. Next to one dot, write “PP” (for potassium permanganate) and next to the other dot write “MB” (for methylene blue). Mark plates “B” and “C” the same way.
3. Remove the lid from agar plate “A.” Use the straw to make a well in the agar at each dot that you have marked. To do this, fold the straw over at one end to make a tight seal and hold it with one hand. Then, pinch the straw with your fingers to expel some air from the straw. Then, bore a well in the agar with the opposite end of the straw by pushing it into the agar. Finally, release the fingers pinching the straw. This will create a small vacuum in the straw and will pull a plug of agar up into the straw. Discard this and repeat until your agar plates each have two wells.

- Using a micropipettor, withdraw 25 μL of potassium permanganate solution and transfer it to the well that you have made directly above the correspondingly labeled dot. Do not let the well overflow. Use the same procedure to transfer 25 μL of methylene blue dye solution to your plate "A".
- Repeat this for the potassium permanganate and methylene blue wells on plates "B" and "C." Work quickly with the "C" plate so that it remains cold.
- Keep plates A and B at room temperature, and place plate C back in the refrigerator (or on ice if a refrigerator is unavailable). For the remainder of your lab period, measure the diameter of the dye circles every 15 minutes. Record your measurements in your lab notebook, using a table such as the one below:

Time (min)	Potassium permanganate			Methylene blue		
	Agar A	Agar B	Agar C	Agar A	Agar B	Agar C
0						
15						
30						
45						
60						
75						
90						

After you have prepared the agar plates with the dye solutions, begin Part III. Remember to observe your plates from this activity and record your measurements in your notebook every 15 minutes during the rest of the lab period.

III. Observe how water and solutes move across an artificial semipermeable membrane (dialysis tubing)

Begin this activity second.

- Label four 200-300 mL beakers with the numbers 1-4. Fill each beaker with about 150 mL of the solutions listed in the table below.

⇒ **Always wear gloves when handling dialysis tubing!** Oils from your fingers will plug up the pores and ruin the tubing.
- Cut 4 pieces of dialysis tubing if they haven't been provided pre-cut for you. Each piece should be approximately 20 cm long.
- Soak the dialysis tubing in a small beaker of distilled water for 1-2 minutes. Then use the following procedure to create 4 "dialysis bags" that you will fill with the solutions listed in the table below:
 - Remove a piece of tubing from the distilled water and separate the sides to form a tube (similar to the way you separate the sides of a plastic produce bag at the grocery store.) Place a clip across the bottom of the tubing or carefully tie it closed. Take care to make a tight seal without tearing the bag.
 - Fill the bag with approximately 10 mL of the appropriate solution (see the table below) using a 10 mL pipette. Be sure to use a clean pipette for each solution.
 - Carefully squeeze air out of the bag and place a clip across the top of the bag, or tie it closed. **The closed bag should not be tightly filled;** leave enough room in the bag to allow it to swell as more water enters. Use a paper towel to carefully blot dry any spilled solution on the outside of the bag. Place the bag on a paper towel. You can write on the paper towel to identify the bags 1- 4.

Fill the beakers as follows:

Beaker #1: distilled water

Beaker #2: distilled water

Beaker #3: distilled water

Beaker #4: 10% glucose solution

Fill the bags as follows:

Bag #1: 1% starch solution

Bag #2: 20% glucose solution

Bag #3: 40% glucose solution

Bag #4: 10% glucose solution

4. After you have filled all of the bags and blotted them dry, weigh them, and record their “before dialysis” weights in a clearly labeled table in your lab notebook.
5. Place all the bags in their corresponding beakers at the same time and record the time.
6. When the bags have been in the beakers for at least 90 minutes (take more time if you have it), remove all of the bags from the beakers at the same time. **Do not discard the solutions in the beakers!** Rinse the bags with tap water, carefully blot them dry with paper towels, and place them on a clean paper towel, labeling the bags by writing on the paper towel.
7. Weigh the bags and enter the data in the table in your lab notebook in order to compare this weight with the weight of the dialysis bags before incubating them in the beakers of solutions.
8. Carry out a series of tests, using the 2 indicators you studied in part IV, to determine which solute(s) crossed the dialysis membrane and which solute(s) did not. **Record the results in your lab notebook and show them to your instructor before you discard the dialysis bags and the solutions in each beaker.**

IV. Determine which indicator to use to detect the presence of glucose or starch

In this part of the lab, you will determine how to use indicators to detect the presence of two substances: starch and a reducing sugar (such as glucose).

1. a. Label three test tubes B1, B2, and B3. To tube B1 add 1.0 ml dH₂O; to tube B2 add 1.0 ml 40% glucose solution; and to tube B3 add 1.0 ml 1% starch solution.
- b. Add 5 drops of the Benedict’s solution to each test tube. Note the appearance of each tube, and then place all three tubes in the boiling water bath for about 1 minute.
- c. Retrieve your test tubes from the boiling water bath. Describe the appearance of each tube after it was removed from the boiling water bath:
Tube #1 _____ (dH₂O)
Tube #2 _____ (glucose)
Tube #3 _____ (starch)
- d. Benedict’s reagent can be used to detect the presence of which substance? _____
- e. The color for a positive test is _____
- f. The color for a negative test is _____

2. a. Label three test tubes L1, L2, and L3. To tube L1 add 1.0 ml dH₂O; to tube L2 add 1.0 ml 40% glucose solution; and to tube L3 add 1.0 ml 1% starch solution.
- b. Add 5 drops of Lugol's iodine to each test tube.
- c. Describe the appearance of each tube after the Lugol's iodine was added:
 Tube #1 _____ (dH₂O)
 Tube #2 _____ (glucose)
 Tube #3 _____ (starch)
- d. Lugol's iodine could be used to detect the presence of which substance? _____
- e. The color for a positive test is _____
- f. The color for a negative test is _____

V. Observe how osmosis affects living cells

1. Make a wet mount of an *Elodea* leaf.

You will do all of this section of the lab activity using one wet mount of an *Elodea* leaf.

Do not discard your wet mount until you have *completed* Part V!

2. Focus on the leaf using low power first, and then high power. While viewing the leaf under high power, draw a diagram of one cell labeling the chloroplasts, cytoplasm, plasma membrane, and cell wall.
3. Place a drop of the 10% NaCl solution on the right edge of the cover slip. **While looking through the microscope**, because you want to watch the cells' response *as it is occurring*, touch the corner of a Kimwipe to the left edge of the cover slip. This will draw the salt solution under the cover slip by capillary action.
4. In your lab notebook, describe what happens to the cells as the 10% salt solution flows over them. After waiting several minutes, draw another diagram of one cell, again labeling the chloroplasts, cytoplasm, plasma membrane, and cell wall. Briefly describe the difference between the 2 diagrams. **Ask your instructor to check both diagrams before proceeding to step 5.**
5. **Using the same slide**, repeat step #3 using distilled water instead of 10% NaCl. In your lab notebook, describe what happens to the cells as the distilled water flows over them.

Clean up

Dispose of your solutions in the proper waste containers.

Clean your glassware and place all equipment and solutions back where you found them. Leave your work area in the same order that you found it in.

All disposable glassware goes into the special glass disposal receptacle. Dialysis tubing may be disposed of in the waste paper baskets.

Wipe off your workspace with a damp paper towel.

Make sure everything that you have used is clean, put away, or discarded. Ask your instructor to check your work area before you leave.

Postlab

Part I

1. Describe what you observed while viewing the grains of “lichen powder” under the microscope. Were the movements of the grains random or in a specific direction? Was the rate of movement uniform or did it vary?
2. Explain what was happening at the molecular level to cause the grains of “lichen powder” to vibrate.

Part II

3. Make a scatter diagram of your data from part II, plotting diameter of the dye circles against time. **Make two separate graphs**, one for potassium permanganate and one for methylene blue. On each graph, show the data for all three plates (A, B, and C). Use a different color or symbol for each plate. Make sure that each graph has a descriptive title and that all parts are clearly labeled. Also make sure that each graph occupies a full page and that you place the independent and dependent variables on the correct axes. **Use the “Graphing Check List” on p. 18 of the Prelab for Exercise #2 to make sure you have included all necessary information on your graph.**
4. Next, for each graph prepared in question 3 (one for potassium permanganate and one for methylene blue), use linear regression to determine the equation of the best-fit straight line for the data from each plate (A, B, and C). Plot the 3 best-fit straight lines on each graph, using a different color or style for each line. Also, write down the equation of each best-fit line and clearly show which line corresponds to each equation. When you are finished, you should have 3 best-fit lines and 3 equations displayed on each graph.
5. Examine the 6 linear regression equations that you calculated in question 4. Which part of each equation is a measure of how rapidly diffusion took place?
6. Agar forms a gel when heated in water and allowed to cool. The gel consists of water “trapped” within a network of polysaccharide threads. A denser network of polysaccharide threads will slow down the diffusion of the dye through the trapped water. In your experiment, plates “A” and “C” had the same concentration of agar while plate “B” had a different concentration. In addition, plates “A” and “B” were kept at room temperature while plate “C” was kept in a refrigerator.
 - A. Which 2 plates should you compare to see the effect of agar concentration on the rate of diffusion? Based on this comparison, which plate had the higher concentration of agar? Explain your reasoning.
 - B. Which 2 plates should you compare to determine the effect of temperature on the rate of diffusion? What does this comparison show? Explain your reasoning.
7. Potassium permanganate has a molecular weight of 158.0 Daltons, and methylene blue has a molecular weight of 373.9 Daltons. Based on your results, what can you conclude about the effect of molecular weight on the rate of diffusion? Explain your reasoning.

Parts III & IV

8. For each of your dialysis set-ups, answer the following: Did the dialysis bag gain weight, lose weight, or stay about the same weight? Make a bar graph that shows weight change for each set-up. **Use the “Graphing Check List” on p. 18 of the Prelab in Exercise #2 to make sure you have included all necessary information on your graph.**
9. The change in weight of the dialysis bags during your experiment was mainly due to the net movement of water into or out of the bags. For each dialysis set-up, describe the direction of net movement of water during your experiment (into or out of the bag) and explain why this net movement occurred. **Make sure you give four separate answers—one for each dialysis set-up.**
10. Indicate which solute(s) passed through the dialysis membrane and which solute(s) did not, and explain how you were able to determine this.

11. Explain **why** the solute(s) that passed through the dialysis membrane did, and why the solute(s) that didn't pass through the membrane didn't.
12. Suppose you have a solution containing both salt (NaCl) and glucose, and dialysis tubing with a pore size that allows the passage of Na⁺ and Cl⁻ ions, but doesn't allow the passage of glucose. How could you remove essentially all of the salt from the solution?

Part V

13. When the *Elodea* cells were placed in 10% saline solution:
 - A. Describe the visible change that took place and explain why it occurred.
 - B. Describe the movement of water across the plasma membrane, and explain why it occurred.
 - C. Describe the **net** movement of water across the plasma membrane, and explain why it occurred.
14. When the *Elodea* cells were placed back in distilled water:
 - A. Describe the visible change that took place and explain why it occurred.
 - B. Describe the movement of water across the plasma membrane, and explain why it occurred.
 - C. Describe the **net** movement of water across the plasma membrane, and explain why it occurred.
15. If you repeated this experiment using animal cells instead of plant cells, what do you think you would have observed that was different from what you observed in plant cells? Explain your reasoning.