AP INVESTIGATION #4

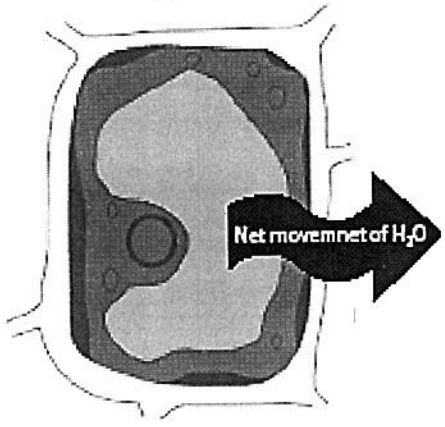
DIFFUSION & OSMOSIS: GUIDED-INQUIRY LAB ACTIVITY KIT #36-7404

LAB CONCEPTS

In this lab, you will learn about:

- Diffusion
- Osmosis
- Water Potenial
- Hypertonic, hypotonic, isotonic solutions
- Cellular membrane structure and function
- Maintaing homeostasis
- Cellular growth and regulation

Figure 1
Plant cell in Hypertonic Solution.



The cell is plasmolyzed.

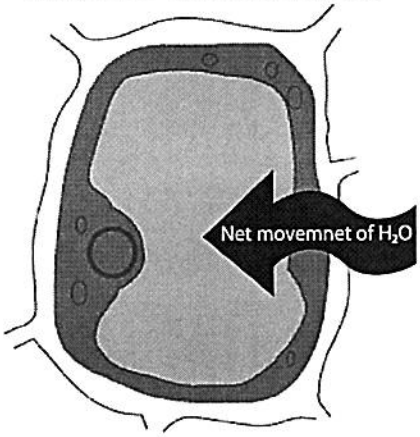
BACKGROUND

The absorption of nutrients, excretion of cellular wastes, and the exchange of respiratory gases are life processes which depend upon efficient transport of substances into, out of, and throughout living cells. Diffusion is one of the most common and efficient means by which substances are passively transported between cells and their aqueous environment. Diffusion is the movement of a substance (liquid or gas) through a concentration gradient from high to low concentration. Diffusion is vital to many life functions of a cell. Diffusion allows the transport of vitally important nutrients and compounds without the expenditure of excess metabolic energy. Diffusion is responsible for the transport and exchange of oxygen and carbon dioxide in the lungs. During respiratory gas exchange, oxygen is broken down and carbon dioxide produced. As a result, the concentration of oxygen is always lower inside the cell than outside, while the concentration of carbon dioxide is higher inside. The gases travel down their respective concentration gradients through the body's intercellular fluid. Oxygen is picked up by the oxygen-poor blood as it passes through the lungs and diffuses into the body cells as carbon dioxide diffuses out.

The cell membrane is the selectively permeable barrier whose total surface area is important to regulating the substances that diffuse into or out of the cell. Osmosis is a special kind of diffusion that occurs as water is separated by a selectively permeable membrane with different solute concentrations on either side of the membrane. In osmosis, water moves from regions of low solute concentration to regions of high solute concentration. Small, neutrally charged molecules such as oxygen, carbon dioxide, and glucose can pass freely through the membrane, while the diffusion of other materials is restricted. Such restricted substances or more efficiently transported substances, such as water, are passed through the membrane by channel proteins, also known as aquaporins. Water can travel through aquaporins at a rate of three billion water molecules per second, far faster and efficient than osmosis.

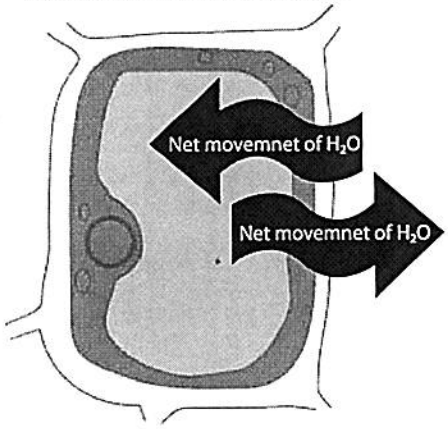
Osmosis also has a major impact on living cells. Organisms rarely exist in environments with solute concentrations that match their cytoplasm; there are usually more or fewer dissolved particles in one of two compared solutions separated by a membrane, such as a cell and the media in which it exists.

Figure 2
Plant cell in Hypotonic Solution.



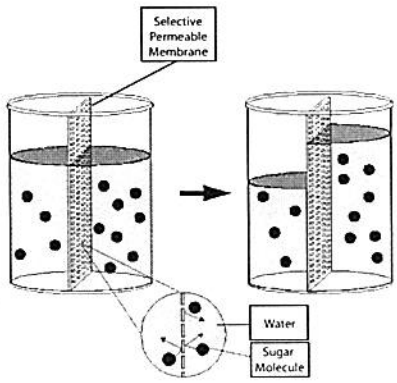
The cell is turgid.

Figure 3
Plant cell in Isotonic Solution.



The cell is flaccid.

Figure 4
Water Potential in a Tube.



BACKGROUND (continued...)

A hypertonic solution is a solution in which the solute concentration is higher outside of the cell and low water potential, therefore, water will flow to the external environment, whereas, hypotonic solutions consist of a low concentration of solutes and high water potential, therefore, water will flow at a greater rate into the cell. Isotonic solutions on the other hand are solutions in which the solute and solvent concentrations are at equilibrium, there is no net flow of movement across the selectively permeable membrane. Only a solute's relative concentration, or water potential, affects the rate of osmosis; the size of the solute molecules does not matter. Thus, higher the concentration of solutes, the faster water will flow through the membrane to equalize the concentration; this is due to the higher water potential of the solution.

Rates of diffusion and osmosis, and the water potential of a solution, can all be determined through experiments and mathematical calculations. The rate at which a cell can diffuse substances across the cell membrane can be measured by the rate of diffusion.

The rate of diffusion, derived from Fick's law, is dependent on the amount of surface area in which diffusion can occur. The rate of diffusion can be calculated using the formula on the following page.

$$\textbf{Rate of diffusion} = \text{Surface area} \times \frac{\text{Concentration difference}}{\text{Distance}}$$

The osmotic potential is calculated using known concentrations of solutions. A solution of sugar and water is placed in a tube and the tube is covered by a selectively permeable membrane. If this tube is placed in a beaker of pure water, the water will diffuse into the tube and the level of the water will rise. Determining how high the water rises helps ascertain the water potential of the solution. Water potential determines the direction and rate of osmosis; it consists of two components—pressure potential, the exertion of pressure on a solution; and osmotic potential, the relative concentration of solutes within the two solutions and is measured in bars.

For example, in Figure __ the water initially enters the tube because there is a negative osmotic potential in the sugar–water solution. However, the force of gravity begins to exert pressure on the rising column; when the force of gravity, pressure potential, equals the osmotic potential, the sugar-water solution in the column stops rising. The water potential is at zero and dynamic equilibrium has been established. The pressure potential can

be determined from the height of the column.

BACKGROUND (continued...)

With the water potential and the pressure known, osmotic potential can be easily determined.

Water Potential (ψ) = Pressure Potential (ψ p) + Osmotic Potential ($\psi\ \pi$)

The water potential of a solution is determined by calculating the osmotic potential when the pressure potential is zero.

$$\psi = \psi\ p + \psi\ \pi;\ \text{if}\ \psi\ p = 0, \text{then}\ \psi = \psi\ \pi$$

SAFETY PRECAUTIONS

- Read all instructions before starting the lab activities.
- Wear chemical splash goggles at all times during this experiment.
- Follow all classroom safety procedures (tie back long hair, remove dangling jewelry, know where emergency equipment is located, etc.).
- Wash your hands with soap and water before leaving the laboratory.

PART I: CELL SIZE & DIFFUSION

INTRODUCTION

Why are cells so small? Most cells grow, but upon reaching a certain size, a cell will divide becoming two smaller cells. This is how multicellular organisms, like humans, grow. But why do cells stop growing once they reach a certain size? Why does a cell divide and multiply rather than simply growing bigger? One possible answer can be found in the relationship between cell size and the diffusion of substances across the cell membrane.

In this lab activity, students will investigate the relationship between cell size and diffusion which will help to explain why cells remain small. The students will create the model cells of three different sizes and measure the extent and rate of diffusion into each cell. In addition, the students will also calculate the surface to volume ratio for each model cell.

- Why do cells elongated length wise versus width?

- Cell expansion in plants require enzymes to loosen the existing cell wall and produce new material. Can you think of a mechanism which causes the loosening of the cell wall?

MATERIALS LIST PER LAB GROUP

- ❑ 3 Phenolphthalein agar cubes: 3x3 cm, 2x2 cm, and 1x1 cm
- ❑ 1 Plastic knife
- ❑ 1 Plastic spoon
- ❑ 1 Plastic cup
- ❑ 1 Vinylite white plastic ruler, 6" metric system
- ❑ 1 White vinegar, 100 mL
- ❑ 1 Timer

PROCEDURE

When performing this lab activity all data should be recorded in a legal scientific notebook or on the laboratory sheet provided. Students will need to construct their own data tables, where appropriate, in order to neatly and accurately capture the data from their investigations.

The agar cubes have been prepared with 1% phenolphthalein, which is a pH dye indicator. The chart on the following page indicates a color scale of pH for phenolphthalein.

PROCEDURE TIPS

- Record all data **IMMEDIATELY** in your laboratory notebook or the provided laboratory sheet.

FORMULAS

All formulas for calculations are provided and are listed below:

- Surface Area =

  length x weight x # of sides

- Volume =

  length x weight x height

- Extent of Diffusion =

$$\frac{\text{Total Cube Volume} - \text{Volume of Cube which has NOT Changed Color}}{\text{Total Cube Volume}} \times 100$$

- Surface Area: Volmume Ratio =

PROCEDURE (continued)

1. Using the metric ruler, measure the dimensions of each agar cube and record the measurements in your laboratory sheet.

2. Place the three cubes carefully into a plastic cup. Add white vinegar until the cubes are submerged. Using a plastic spoon, keep the cubes submerged for 10 minutes turning them frequently.

NOTE *Be careful not to scratch any surface of the cubes.*

3. As the cubes soak, calculate the surface area, volume, and surface area to volume ratio for each agar cube.

4. After 10 minutes, use the spoon to remove the agar cubes and carefully blot them on dry paper towel. Then, cut the cubes in half, rinsing and drying the knife between each cut.

Did you observe the color change? What does the change in color indicate?

5. Using a metric ruler, measure the distance in centimeters that the white vineagar diffused into each cube.

6. Calculate the rate of diffusion for each cube in centimeter per minute (cm/min.).

7. Calculate the volume of the portion of each cube which has not changed color.

8. Calculate the extent of diffusion into each cube as a percent of the total volume.

REFLECTION QUESTIONS

1. Looking at your data, what conclusion can you draw about cell size and the rate of diffusion? Should you accept or reject your hypothesis for this investigation? Explain.

2. The phenolphthalein agar cubes were prepared with 1% phenolphthalein and 2% Sodium Hydroxide (NaOH). Explain the role of the NaOH and the vinegar in the experiment? Which solution was acidic, basic, or neutral? Explain.

EXPERIMENTAL DESIGN TIPS

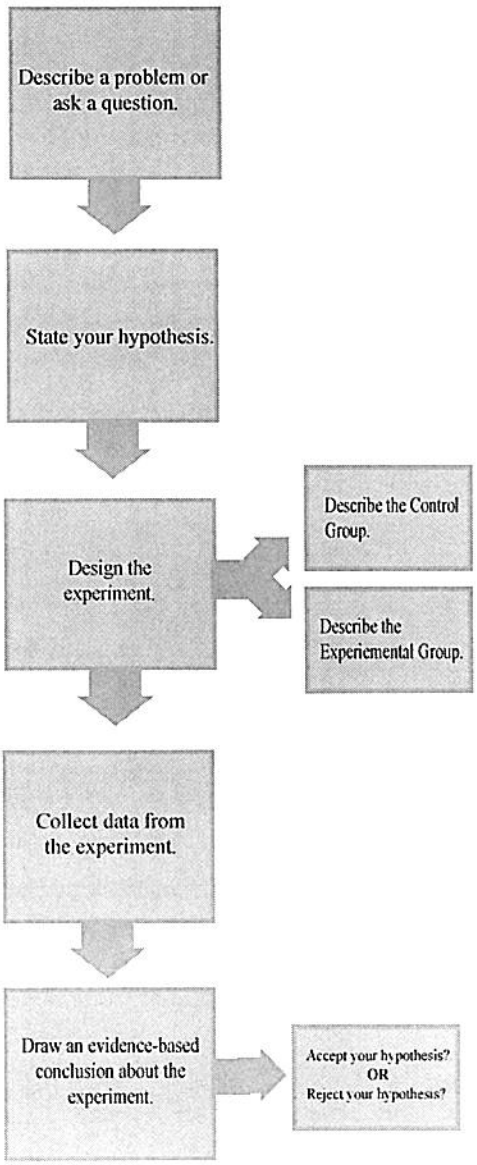
- Consider the following questions when designing your experiment:
  - The entire experiment will be designed to provide an answer for the described problem or the question asked.
  - When describing the experimental group, identify the dependent and independent variable within the experimental group.
  - Record ALL observations and any questions generated during the experiment in your laboratory notebook or the provided laboratory sheet.
  - When drawing conclusions, use specific data from your experiment to support your conclusions.

EXPERIMENTAL DESIGN AND INVESTIGATION

Design a experiment to investigate your hypothesis about the efficiency of diffusion by the designing different cell sizes using the materials previously listed. Have your teacher check over your experiment design and initial your design for approval. Once your design is approved, investigate your hypothesis. Be sure to record all observations and data in your laboratory sheet or notebook.

NOTE *Use the Scientific Method when designing you experiment. Be sure to include the following in your design:*

Figure 5
Scientific Method



PART II: MODELING OSMOSIS IN LIVING CELLS

INTRODUCTION

Imagine you are nurse and a patient is admitted to the hospital for dehydration. The attending doctor prescribes intravenous fluids (IV) for you to administer to the patient. You go to the medical supply area and you notice the label on the bags lists various solutes in water. One of the solutes that stand out is 0.9% sodium chloride (NaCl).

- Why would sodium chloride be an important solute to have in intravenous fluids?
- Would you want to use an IV fluid with a high concentration of sodium chloride on your dehydrated patient? Why or why not?
- Explain how you would determine which concentration of IV fluid would be the most efficient in rehydrating your patient?

In this lab activity, students will investigate osmosis occurs as it occurs living things, such as plant and animal cells. The students will construct and simulate model cells in an external environment, to relate solutes passing through a semi-permeable membrane in hypertonic, hypotonic, and isotonic solutions.

MATERIALS LIST PER LAB GROUP

❑	1	Roll of String
❑	1	Balance
❑	1	Graduated Cylinder
❑	5	Plastic Cup, 250 mL
❑	7 ft.	Piece of Dialysis Tubing, 20 cm.
❑	250 mL	1M Sucrose Solution
❑	250 mL	1M Sodium Chloride
❑	250 mL	1M Glucose Starch Solution
❑	250 mL	5% Ovalbumin Solution
❑	500 mL	Distilled OR Tap Water

PROCEDURE TIPS

- Record all data IMMEDIATELY in your laboratory notebook or the provided laboratory sheet.
- Wash your hands before handling the dialysis tubing, and keep physical contact with the tubing to a minimum.
- Remember to label your model cells. Record the pairs in your laboratory notebook in their respective order of your lab set-up.

PROCEDURE

NOTE

When performing this lab activity all data should be recorded in a legal scientific notebook or on the laboratory sheet provided. Students will need to construct their own data tables, where appropriate, in order to neatly and accurately capture the data from their experiments and investigations.

1. Your teacher has prepared four different solutions. You need to pair four combinations of solutions to test in your model cells. Within each pair, one of the solutions will act as the external environment (interstitial fluid) of the cell and the other solution will act as the intercellular fluid (cytoplasm) in your model cell. There should be a subtotal of four model cells, and your fifth model cell will be your control, consisting of water on both sides of the semi-permeable membrane, for a total of 5 model cells.

2. Prior to starting the lab, hypothesize if water will diffuse into or out of each model cell. Record your hypotheses in your laboratory notebook or the provided laboratory sheet.

HINT

Use your knowledge about solution gradients to help you construct your hypotheses.

NOTE

The pores in the dialysis tubing are extremely small, and can be easily clogged by any oil or dirt on your fingers and hands. Wash your hands before handling the dialysis tubing, and keep physical contact with the tubing to a minimum.

3. Obtain five pieces of dialysis tubing from the beaker of water. Tie a tight knot in one end of each piece of tubing, or use a piece of string to tie off the end.

4. Measure and pour 10 mL of one of the prepared solutions into a graduated cylinder.

5. Open the tubing by rubbing the untied end between your fingers. Pour 10 mL of prepared solution into the tubing. Carefully tie a knot in the open end to form a bag. Be sure to leave enough space in the bag for expansion. Fill a beaker with 250 mL or a provided plastic cup 2/3 of the way full with the pairing solution.

PROCEDURE TIPS

- Record all data IMMEDIATELY in your laboratory notebook or the provided laboratory sheet.
- If you choose to tie off the end of the dialysis tubing with string, tie two knots, about 1/4" apart, to prevent leaking. (Step 5)
- Remember to clean the graduated cylinder in between solutions. (Step 6)

FORMULAS

- % Change in mass =

$$\frac{\text{Final Mass - Intial Mass}}{\text{Initial Mass}} \times 100$$

PROCEDURE (continued...)

6. Repeat Steps 4 and 5 for the remaining four cells.

NOTE *Remember to clean the graduated cylinder in between solutions.*

7. Determine the initial weight of each cell and record the results in your laboratory notebook or the provided laboratory sheet.

8. Completely immerse the model cells in their pairing solutions in the beaker or cup.

9. Wait 30 minutes and record any observations in your laboratory notebook or the provided laboratory sheet. When 30 minutes has passed, remove the model cells from the solution and determine the final weight of each of the model cells. Record the final weights in your laboratory notebook or the provided laboratory sheet.

10. Calculate the percent change in weight and record your results in your laboratory notebook or the provided laboratory sheet.

11. Graph the change in mass (g) for each of the model cells.

STOP *Do* NOT *discard any of your solutions from this part of the lab activity as they will be used in Part III as well.*

PROCEDURE TIPS

- Record all data IMMEDIATELY in your laboratory notebook or the provided laboratory sheet.
- If you choose to tie off the end of the dialysis tubing with string, tie two knots, about 1/4" apart, to prevent leaking. (Step 5)
- Remember to clean the graduated cylinder in between solutions. (Step 6)

EXPERIMENTAL DESIGN AND INVESTIGATION

Design a experiment to investigate ~~experiment to investigate~~ any lingering questions you have about osmosis. Have your teacher check over your experiment design and initial your design for approval. Once your design is approved, investigate your hypothesis. Be sure to record all observations and data in your laboratory sheet or notebook.

NOTE *Use the Scientific Method when designing you experiment. Refer to Figure 5 on page 6 of this lab for elements of design.*

REFELCTIONS

1. Examine the initial and final weights of the model cells. What caused the mass of the dialysis bags to change? Was there more or less water in the dialysis bags at the conclusion of the experiment? Explain.

2. Of the prepared solutions, which one is a protein solution? Using supporting evidence, explain whether or not the protein solution has a high molarity.

3. Compare the tonicity of the solutions inside the model cells to determine which solution was more or less hypertonic or hypotonic than another. Then determine which solution in the beakers was more or less hypertonic. Which combination of solutions, if any, would give you an isotonic environment? Explain.

PART III: OSMOSIS IN LIVING PLANT CELLS

INTRODUCTION

Osmosis has a major impact on living cells. The interactions between the plasma membrane, solutes, and water are essential to plant and animal cellular function and maintenance of homeostasis. In plants, diffusion aids in the transport of water and nutrients in the plasma membrane of the xylem. A plant's root cells expend energy to maintain high solute concentration in the xylem, which forms a concentration gradient within the roots for water; a higher solute concentration equals lower water concentration and high water potential. Water flows down the concentrations gradient, causing it to diffuse into the roots. Water pressure builds up within the xylem, forcing it up the stem. The force exerted on the cell wall by the water is known as Turgor pressure; it is responsible for supporting the stems and leave of plants. Osmosis allows for the absorption and early transport of water into the root system of plants, with transpirational pull, and helps transport water into the xylem.

In this lab activity, students will microscopically observe an *Elodea densa* plant leaf and explore the effects of different solution concentrations on the cells. Students will then design an experiment, with the provided materials, to identify the concentrations of different sucrose solutions and use the solutions to determine the water potential of plant tissues, white or sweet potatoes.

MATERIALS LIST PER LAB GROUP

❑	1	Thermometer	❑	1	Microscope Slide
❑	1	Graduated Cylinder	❑	5	Paper Towels
❑	6	Plastic Cups	❑	1	Pair of Forceps
❑	150 mL.	Red Mystery Solution	❑	1	Compound Microscope
❑	150 mL	Orange Mystery Solution	❑	1	Scalpel
❑	150 mL	Yellow Mystery Solution	❑	1	Coverslip
❑	150 mL	Green Mystery Solution	❑	6	Potato tubers
❑	150 mL	Blue Mystery Solution	❑	1	Balance
❑	175 mL	Distilled Water			

PLANT CELL PLASMOLYSIS

Prior to starting this activity, consider the following questions:

- What do changes do you expect to see in the appearance of the cells when exposed to differenct concentrations of solutions?
- The cell wall allows the plant cells to be more resilient to the changes of solute concentrations of their external environment. Define turgor pressure and predict if there is an increase or decrase of tugor pressure within cells treated by different concentrations of solutions.

PROCEDURE TIPS

- Record all data IMMEDIATELY in your laboratory notebook or the provided laboratory sheet.
- To speed up the reaction to the cells in solution, wick your solution through the cells using a paper towel from the opposite end of the coverslip (Step 5)

PROCEDURE

1. Using the forceps, remove an *Elodea densa* leaf from its stem and place it gently on a clean microscope slide.
2. Add two to three drops of distilled water to the slide and cover with a coverslip.
3. Examine the cell at 40X maginification and note the characteristics of the cells. Draw several representative cells of your observations.
4. Remove the mircoscope slide and add two to three drops of one of your solutions, from Part II of this lab, across the leaf sample.
5. Allow the slide to sit for two to three minutes in the solution and re-examine the sample under the microscope.
6. Note the appearance of the cells. Draw several representative cells of your observations.

NOTE *Label all visible structures and organelles in your drawings.*

REFELCTIONS

1. What are the effects on animal cells when they are placed in isotonic, hypotonic, and hypertonic solutions?
2. Look at the previous experiments and investigations you performed. Determine which methods would be necessary to determine the rate of osmosis, calculating water potential, the comparison water potential between plants, and determining solute concentrations in solutions.

PROCEDURE TIPS

- Record all data **IMMEDIATELY** in your laboratory notebook or the provided laboratory sheet.

EXPERIMENTAL DESIGN AND INVESTIGATION

Design a experiment to investigate ~~experiment to investigate~~ identify the concentrations of your color-coded sucrose solutions and then use that data to determine the water potential of plant tissue, the potato tubers. Have your teacher check over your experiment design and initial your design for approval. Once your design is approved, investigate your hypothesis. Be sure to record all observations and data in your laboratory sheet or notebook.

NOTE *Use the Scientific Method when designing you experiment. Refer to Figure 5 on page 6 of this lab for elements of experimental design.*

REFLECTIONS

1. If you have not done so, graph your results from your investigation. Study the graph and determine what is important about the point where the best fit line crosses the X-axis? What is the concentration of the solution in your potato?

2. Using the data from your investigation, calculated the water potential of the solutions in bars.

3. The water potential of a solution is equal to the osmotic potential plus the pressure. Since there is no differential pressure acting on the solutions, the pressure potential is equal to zero, making the water potential equal to the osmotic potential. If the equilibrium point between the solutions and the potato cylinders indicates the point where the two water potentials are equal, what is the water potential of the potato cells?

ADDITIONAL QUESTIONS (optional)

Your instructor may assign the following quesions as a homework assignment. Answer the questions, based on your laboratory data collected during the performed experiments.

1. Examine your data and describe the characteristics of cell size, surface area, and surface area to volume ratio which best meet the diffusion needs of living cells.

2. The size of some human cells is 0.01 mm. Using the formulas in Part I this activity, calculate the surface areat to volume ratio of such a cell (assume the cell is a 0.01 mm cube). Describe the extent and rate of diffusion into this living cells in comparison to the smallest agar cube. Explain.

3. Based on your knowledge and experience of this series of lab activities, describe how you can test and verify the diffusion of glucose?

4. Use the words in the word back to fill in the blanks of the following statement:

net gain	low	hypertonic	exit
osmotic	enter	less	hypotonic
high	more	net loss	isotonic

If a cell in a _____ solution, it has _____ solute in solution thatn the surrounding fluid, and will therefore experience a _____ of water to its surroundings. This cell has a _____ water potential since there is a great deal of _____ pressure causing water to leave the cell. Conversly, a cell sitting in a _____ solution has a _____ water potential, and since it will experience a _____ net gain of water, there will be little osmotic pressure causing water to _____ the cell.

FURTHER INQUIRY INVESTIGATIONS

1. Research specific examples of cells in the human body and describe how they have adapted their cell membrane surface to become more efficient for absorption or secretion.

2. Protists such as the paramecium and amoeba, egg cells, and slime molds are examples of exceptionally large cells. Investigate how each has adapted to overcome the surface area to volume ratio problem.

3. Apply your understanding about your osmosis and your designed experiment to explain how you would determine the solute concentration inside a living cell.

Name	Lab Course/Section	Date

Experiment Title & Number	Lab Partner(s)

Signature	Date	Teacher's Initials