Procedure 1: Surface Area and Cell Size

Cell size and shape are important factors in determining the rate of diffusion. Think about cells with specialized functions, such as the epithelial cells that line the small intestine or plant root hairs.

Prelab Research Questions:

- What is the shape of these cells?
- What size are the cells?
- How do small intestinal epithelial and root hair cells function in nutrient procurement?

Acid/Base Prelab Questions

- Which solution is an acid? Hydrochloric Acid [HCl]
- Which solution is a base? Sodium Hydroxide [NaOH]
- What color is the dye in the base? In the acid? Base – Pink | Acid - Colorless
- What color is the dye when mixed with the base? Pink

These three blocks will be your models for cells.

- What is the surface area of each of your three cells?
- What is the total volume of each of your cells?
- If you put each of the blocks into a solution, into which block would that solution diffuse throughout the entire block fastest? Slowest? How do you explain the difference?

Procedure 2: Modeling Diffusion and Osmosis

Background:

The term **tonicity** describes the relative concentration of solvent to solute in two solutions. A solution with the lower solute concentration is said to be **hypotonic** relative to the other solution. Conversely, the more concentrated solution is said to be **hypertonic** relative to the first. If the solute concentrations of each solution are equal the solutions are **isotonic** with respect to each other. It is important to remember that these terms are relative terms, that is, the description of a solution as being hypertonic, hypotonic, or isotonic depends on the solution it is being compared to. Traditionally, in biology, the cell is the frame of reference. An isotonic solution has the same solute concentration (and water concentration) as the cell; a **hyper**tonic solution has a **higher** solute (lower water) concentration than the cell; a **hypo**tonic solution has a **lower** solute (higher water) concentration than the cell.

Since all cells contain some molecules that cannot cross the cell membrane, osmosis always occurs when cells are placed in dilute aqueous solutions. It is important, then, for cells to be able to regulate the flow of water into and out of the cell, a process known as **osmoregulation**. In plant cells and bacterial cells the cell wall prevents the cell from bursting by providing a rigid casing that helps regulate the osmotic pressure in the cell. In animals and many microorganisms, osmoregulatory organs or organelles are found. In animals the kidneys adjust the concentration of substances in the body fluids that bathe the cells. In microorganisms, like Paramecium, which live in freshwater, special organelles called contractile vacuoles accumulate and actively pump out water that flows into the cells by osmosis.

In this experiment, you will create models of living cells using dialysis tubing. The plasma membrane of a cell is selectively permeable because it allows the diffusion of some substances and not others. **Small uncharged molecules diffuse freely across the plasma membrane, but charged molecules and large molecules cannot cross the membrane.** Like cell membranes, dialysis tubing is made from a material that is selectively permeable to water and some solutes. You will fill your model cells with different solutions and determine the rate of diffusion.

Big idea 2: Cellular Processes: Energy and Communication

• How can you use weights of the filled cell models to determine the rate and direction of diffusion?
• Suppose you could test other things besides weights of the dialysis tubes. How could you determine the rates and directions of diffusion of water, sucrose, NaCl, glucose, and ovalbumin?
• Will protein diffuse? Will it affect the rate of diffusion of other molecules?

Materials

• Distilled or tap water
• 1 M sucrose
• 1 M NaCl
• 1 M glucose
• 5% ovalbumin (egg white protein)
• 20 cm-long dialysis tubing
• Cups
• Balances

Procedure:

1. Obtain materials listed above

2. With the deionized water wet the dialysis tubing and then roll the tubing back and forth between your fingers until it opens. Use the squeeze bottle of water to add a little bit of water to the inside of the dialysis tubing to keep it open.

3. With one strip of dental floss make a tight knot in one end of the dialysis tube.

4. Fill the bag with Solution A, a simulated "liquid meal" containing 10% glucose, 1% starch, 0.5% egg albumin, and 1% sodium chloride.
   a) Using the graduated cylinder to measure out ~10 mL of solution A and use the funnel to pour the solution into the dialysis tube. (You may not need all 10mL to fill it)
   b) Leave space at the top to tie another knot

5. Tie the top of the tube with dental floss while expelling as much air as possible. The bag should be limp.

6. Rinse the outside of the dialysis tube with distilled water.

7. Place the dialysis tube in a beaker with the help of the plastic dialysis tube holder (see picture for reference) add enough solution B to cover it. Solution B contains 0.5% sodium sulfate (Na_2SO_4) dissolved in water.

   a) Label the outside of the beaker with the names of your group members and your class period.

Let the beaker stand undisturbed for ~40 minutes.

8. Clean your work area. Rinse all materials and place them back on their treys on the head table.

9. Based on the recipes for solutions A and B, fill in the "Before" columns of **table 1.** Use a **+** to represent the presence and a **-** to represent the absence of a substance.

Materials: Part 2

- Beaker, dialysis tubing, solution
- Well plate
- Squeeze bottle of deionized water
- Scissors
- Small beaker
- 2 plastic pipettes
- Printer paper and a writing utensil

10. After ~40 minutes, remove the dialysis tubing from the beaker. Gently agitate the contents of the tubing and note any changes (is it more or less rigor than when you started)?

11. Rinse the dialysis tubing with distilled water and carefully open the tubing. Empty the content into a clean beaker. You can now test which ions and molecules have crossed the membrane.

12. Obtain a well plate and prepare as follows.
    a) Into each of the first three wells, place 10 drops of the solution from inside the dialysis tubing. Label these I-1 to I-3. (place a piece of paper next to it to keep track)
    b) Into the second three wells, place 10 drops of the solution from the outside of the dialysis tubing. Label these O-1 to O-3.

13. Test for the presence of starch, albumin, glucose, sulfate ions, and chloride ions in the two sets of solutions using the following tests: Add 3 drops of Iodine into I-1 and O-1 to test for starch. A blue-black or darker color indicates a positive result.

a. Add 1 drop of silver nitrate ($AgNO_3$) into I-2 and O-2 to test for chloride ions. A white precipitate or cloudy appearance indicates a positive result.

b. Add 3 drops of 1% barium chloride ($BaCl_2$) into I-3 and O-3 to test for sulfate ions. A white precipitate or cloudy appearance indicates a positive result.

14. Dip a Uristix into the beaker from outside the tubing and into the beaker with the contents from inside the tubing to test for the presence of glucose and albumin (protein). Wait 10 min for the Uristix to fully develop. Compare the results to the color chart on the side of the container.

15. Record your results in the "After" column of table 1. Use a **+** to represent the presence and a **-** to represent the absence of a substance.

Table 1

Substance	Inside Dialysis Tubing		Outside Dialysis Tubing	
	Before	After	Before	After
Starch				
Chloride Ion				
Sulfate Ion				
Glucose				
Albumin				

Analysis: Use these results to answer the following questions.

1. At the start of the exercise, which solution (A or B) was hypertonic compared to the other (which had the higher concentration of solutes)?

2. Which solution gained water in the course of the exercise (A or B)? How do you know?

3. Which of the substances (starch, chloride ions, sulfate ions, glucose, albumin, and water) were able to pass through the membrane (in either direction)?

4. Which substance(s) moved out through the membrane?

5. Which substance(s) moved in through the membrane?

6. In general why did the substances move in the direction they did?

7. By what process did the substances move across the membrane?

8. Why did some substances fail to pass through the membrane?

9. Would you expect **all** of the molecules of a diffusible substance to move across the membrane? Why?

Alternate Investigation

Step 1 Choose up to four pairs of different solutions. One solution from each pair will be in the model cell of dialysis tubing, and the other will be outside the cell in the cup. Your fifth model cell will have water inside and outside; this is your control. Before starting, use your knowledge about solute gradients to predict whether the water will diffuse into or out of the cell. Make sure you label the cups to indicate what solution is inside the cell and inside the cup.

Step 2 Make dialysis tubing cells by tying a knot in one end of five pieces of dialysis tubing. Fill each "cell" with 10 mL of the solution you chose for the inside, and knot the other end, leaving enough space for water to diffuse into the cell.

Step 3 Weigh each cell, record the initial weight, and then place it into a cup filled with the second solution for that pair. Weigh the cell after 30 minutes and record the final weight.

Step 4 Calculate the percent change in weight using the following formula:
(final – initial)/initial X 100. Record your results.

- Which pair(s) that you tested did not have a change in weight? How can you explain this?
- If you compared 1 M solutions, was a 1 M NaCl solution more or less hypertonic than a 1 M sucrose solution? What is your evidence? What about 1 M NaCl and 1 M glucose and 1 M sucrose?
- Does the protein solution have a high molarity? What is evidence for your conclusion?
- How could you test for the diffusion of glucose?
- Based on what you learned from your experiment, how could you determine the solute concentration inside a living cell?
- What factors determine the rate and direction of osmosis?
- What would you predict if you used a starch solution instead of the protein?
- Can you diagram the flow of water based upon the contents of your model cell and the surrounding solution?
- When will the net osmosis rate equal zero in your model cells? Will it ever truly be zero?
- Based upon your observations, can you predict the direction of osmosis in living cells when the cells are placed in various solutions?
- How is the dialysis tubing functionally different from a cellular membrane?

Application Questions:
You are in the hospital and need intravenous fluids. You read the label on the IV bag, which lists all of the solutes in the water.

- Why is it important for an IV solution to have salts in it?
- What would happen if you were given pure water in an IV?