CELL TRANSPORT LAB

Objectives

1. Explain the basis of Brownian movement.
2. Describe the movement of solutes by diffusion.
3. Explain how concentration, molecular weight, and temperature affect the rate of diffusion.
4. Explain the movement of water by osmosis.
5. Explain the movement of water among isotonic, hypertonic, and hypotonic solutions and their effect on cells (plant and animal cells).

Activity 1: Brownian Movement and Diffusion of Solutes

Background

Solutes, gases, and heat all move from an area of high concentration to an area of low concentration, or until they reach equilibrium, equal net movement. This is referred to as **diffusion**, or moving "down a **concentration gradient**." This movement occurs due to **Brownian motion**, or the random movement of particles caused by their inherent kinetic energy. This causes particles to collide with one another. Colliding particles will then move away from each other (**Figure 1**).

When solutes are concentrated in one area, there will be more collisions pushing the particles away from one another to areas where there are fewer solutes. When solutes are evenly distributed in an area, there will still be movement and collisions, but there will be no net movement of solutes in a particular direction (equilibrium). In this experiment you will explore the effect of temperature and concentration on diffusion.

View the instructor's Demo of India dye under the microscope. In the space below describe the movement of the dye particles:

Procedure: (Work together as a table)

As a table, you will create three identical agar plates, one using the cold agar plate and dye from the refrigerator, one using the room temperature agar plate and dye at room temperature, and one using the warm agar plate and dye form the incubator. We will investigate not only the role of temperature on the rate of diffusion, but also the role of concentration on the rate of diffusion. To do this, we will use three different concentrations of potassium permanganate

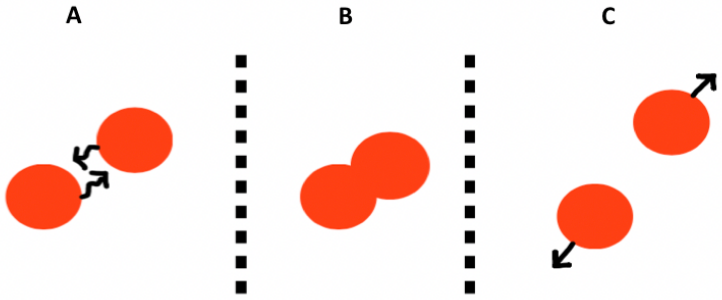


Figure 1: Solute particles display random, Brownian movement due to inherent kinetic energy (A). When the solute particles collide with one another (B), then the force of the collision will cause the molecules to now move away from one another (C).

($KMnO_4$) dye in three different petri dishes held at three different temperatures.

1. Using a wax pencil, label the top of the plate with your table number and your initials. On the cold plate write 'cold,' warm plate 'warm,' and room temperature plate 'room.'

2. Use a straw to make 3 wells in each agar dish spaced evenly in a triangle shape on the well (**Figure 2**). Poke a hole in the agar with one end of the straw. Lift the straw straight out to remove the plug and place it on a paper towel. A well should be created in the agar plate. Repeat for all three wells.

3. Discard the paper towel with the three plugs in the trash.

4. Using a wax pencil, label the three wells on each plate (on the bottom of the plate just above each well) 'A', 'B' and 'C' (**Figure 2**).

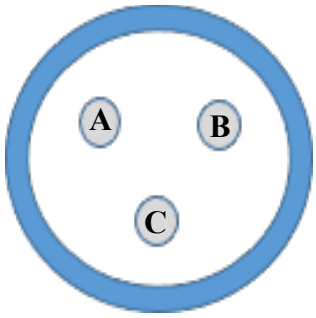


Figure 2: Example of the spacing for the 3 holes in the agar plate. The grey dots represent the holes in the agar.

5. Make the room temperature plate by using a dropper bottle of dye solution from your tray and add only 2 drops of each dye solution into the wells:

Well A = 2 drops 0.1M potassium permanganate
Well B = 2 drops 0.01M potassium permanganate
Well C = 2 drops 0.001M potassium permanganate

6. Repeat this process for the cold agar plate, using the dropper bottles of dye in the fridge, and the warm agar plate using the dropper bottles of dye in the incubator.

7. Place the cold plate in the refrigerator (4^0 C), the warm plate in the incubator (50^0 C), and the room temperature plate on your lab bench (20^0 C) and return the potassium permanganate solutions to their appropriate locations.

8. Make predictions for what you think will happen and get your instructor to initial your work. **(Circle the correct answer.)**
   a. As concentration increases, the rate of diffusion will (**increase or decrease**). Thus, the diameter of the (**.1 M, 0.01M, or 0.001M**) potassium permanganate concentration will be the largest.
   b. As temperatures increases, the rate of diffusion will (**increase or decrease**). Thus, the (**hot or cold**) agar plate will have greater diameter than the other plate.

Instructor initials: _____

9. Set a timer for 45 minutes.

10. Remove the plates from the fridge and the incubator.

11. Place a piece of white paper below each plate.

12. Using a metric ruler, measure the diameter (distance across), for the spread of dye in each well in mm. (**Figure 3)**

13. Record your results in the table below.

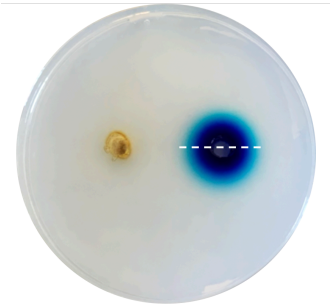
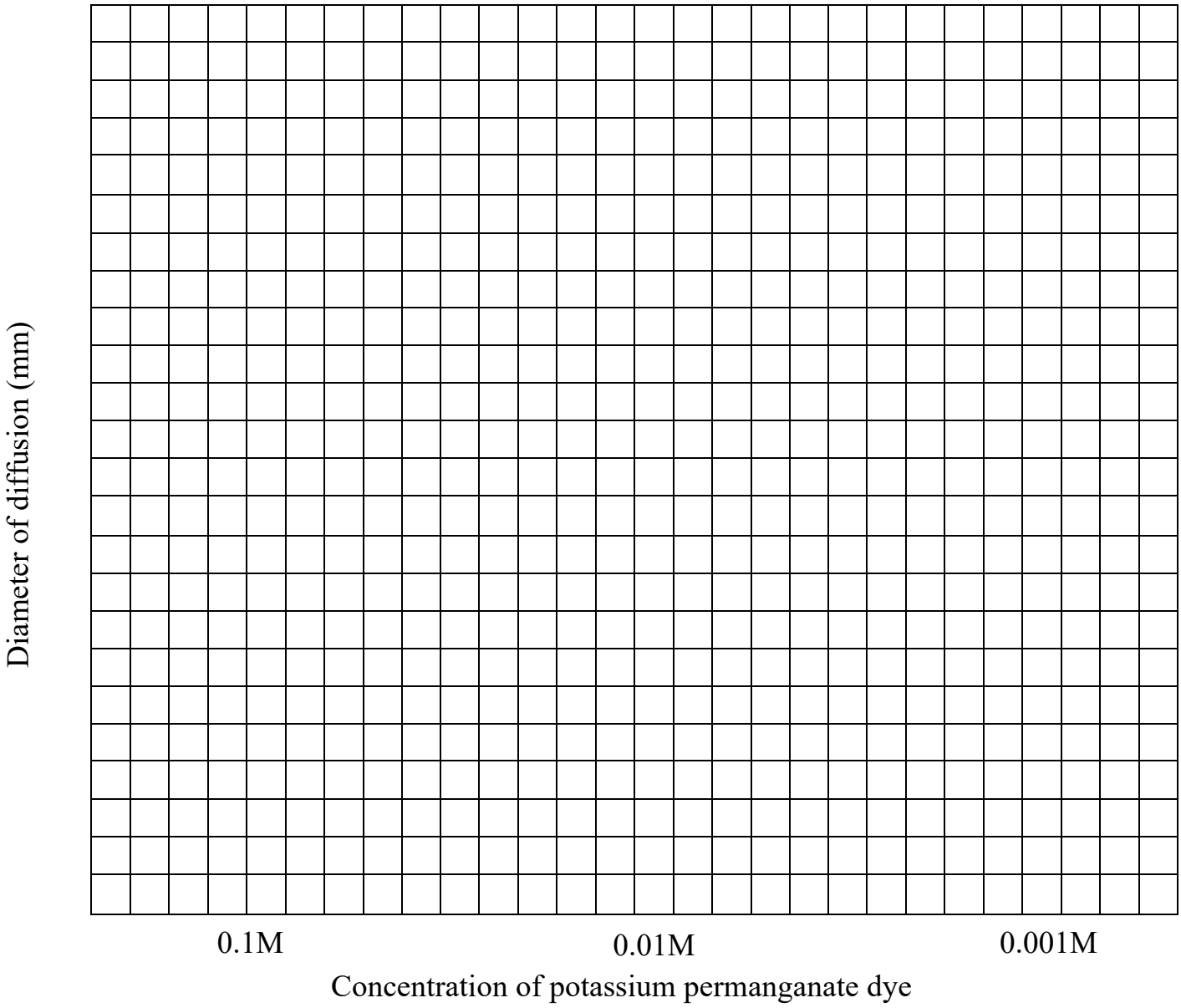


Figure 3: Agar plate with white dashed arrow representing the diameter of dye spread.

	Warm plate Diameter (mm) after 45 min.	**Cold plate Diameter (mm) after 45 min.**	**Room plate Diameter (mm) after 45 min.**
0.1 M potassium permanganate			
0.01 M potassium permanganate			
0.001 M potassium permanganate			

14. Discard the plate in the trash and return all the materials neatly to the tray.

15. Graph your results below.
    a. Add a descriptive title for your graph.
    b. Using your results from the table above, determine and write an even scale for your y axis on the graph paper below. Be sure to start a 0.
    c. Graph the results for the warm plate by putting a dot on the graph for the diameter at each concentration.
    d. Using a ruler, draw a line that best represents the data.
    e. Label the line ‘warm’.
    f. Repeat for the cold and room plates (be sure to label all of your lines clearly).



16. Using the graph above, which concentration of dye had the greatest diffusion? Use the data to justify your response.

17. Using the graph above, which temperature had the greatest diffusion? Use the data to justify your response.

18. Do your results for temperature and concentration support your prediction?

Activity 2: Measuring Osmosis in Plant Cells

Background:
Due to the selectively permeable nature of cell membranes, water can move freely across the cell membrane but large, polar, and nonpolar solutes cannot. The passive movement of water is called **osmosis** and it is driven by differences in solute content of liquids. The sum of all of the solutes (both polar and nonpolar) in a liquid is referred to as its **osmolarity**. When two liquid compartments, such as the extracellular fluid and intracellular fluid have different osmolarities, more water molecules will move toward the solution with the greater osmolarity. This is due to the polar nature of water giving it the ability to readily dissolve polar and ionic solutes. The more solutes that are present in the solution, the more water "sticks" to those solutes, leaving fewer freely moving water molecules. A **hypertonic** solution thus has greater osmolarity—a greater number of solutes compared to another solution. Similarly, the solution with a lower osmolarity, and lower concentration of solutes, is referred to as the **hypotonic** solution.
Remember, these solutions must be connected by a semipermeable barrier to allow water to freely flow between them. **Figure 4** shows the movement of water between hypotonic and hypertonic solutions that are separated by a semi permeable membrane. Notice that the net movement of water is towards the hypertonic solution (to the left in this example). If the two solutions have equal osmolarities water will still move back and forth across the cell membrane but there will be no net movement of water (meaning just as many water molecules move into one solution as to the other). When this happens, the solutions are said to be **isotonic** to one another and the two solutions have reached equilibrium.

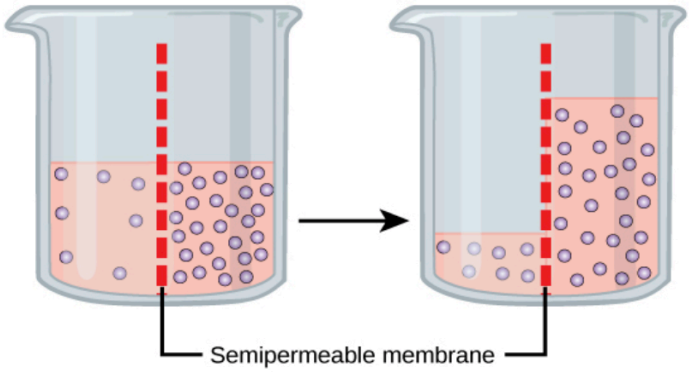


Figure 4: **Osmosis image. The left side is the hypotonic solution with fewer solutes and the right side is the hypertonic solution with more freely moveable water molecules. Water moved towards the hypertonic solution.** ***(Openstax)***

In the next two activities, we will observe osmosis in plant and then animal cells. There are some important differences in osmosis between plant and animal cells. While water will always move from the hypotonic to the hypertonic solution, an animal cell placed in a hypertonic solution will **crenate** (shrivel) as it loses water while a plant cell will undergo **plasmolysis** (the plasma membrane will pull away from the cell wall). An animal cell placed in a hypotonic solution will expand and if too much water enters, it will **lyse** (rupture). Plant cells do not rupture in hypotonic solutions. Instead, the plasma membrane pushes against the rigid cell wall to create **turgor pressure**. This helps a plant to withstand wilting. You will view plant cells in hypertonic and hypotonic solutions.

Procedure: (Work together as a table)

1. Label three beakers and three weigh boats “0.9%”, “0%” and “20%” using a wax pencil.

2. Pour 50mL of 0.9% salt solution in one beaker, 50mL of 20% salt solution in a second beaker, and 50 mL of 0% salt solution (DI water) in a third beaker.

3. Using a potato and potato corer, take three long cylinder cores (allow enough space to remove 3 cores in a row from one potato).

4. Place the cores on a cutting board and using a knife, cut all of the ends off each core (removing the peel).

5. Using a centimeter ruler and a knife, cut each cylinder to 3cm in length. put the tips of each.

6. Pat each cylinder dry with a paper towel.

7. Using a wax pencil, label three weigh boats “0.9%”, “0%” and “20%.”

8. Place the first weigh boat on the scale and push “Zero” to tare the scale.
    a. Add the core to the weigh boat.
    b. Record the weight in the table below.
    c. Remove the weigh boat and core.

9. Repeat this process for all three cores. You must now carefully keep track of which cores are going into (and then coming out of) each solution.

10. Place each core in the appropriate solution for a total of 45 minutes. You will weigh the cores at 15-minute intervals (15 min, 30 min, and 45 minutes).

11. At each time point, remove each core and pat it dry with a paper towel.
    a. Weigh each core by first taring the corresponding weigh boat. It is essential you know which core came from which solution.
    b. Record the results in the table below.
    c. Place each core back in its solution.

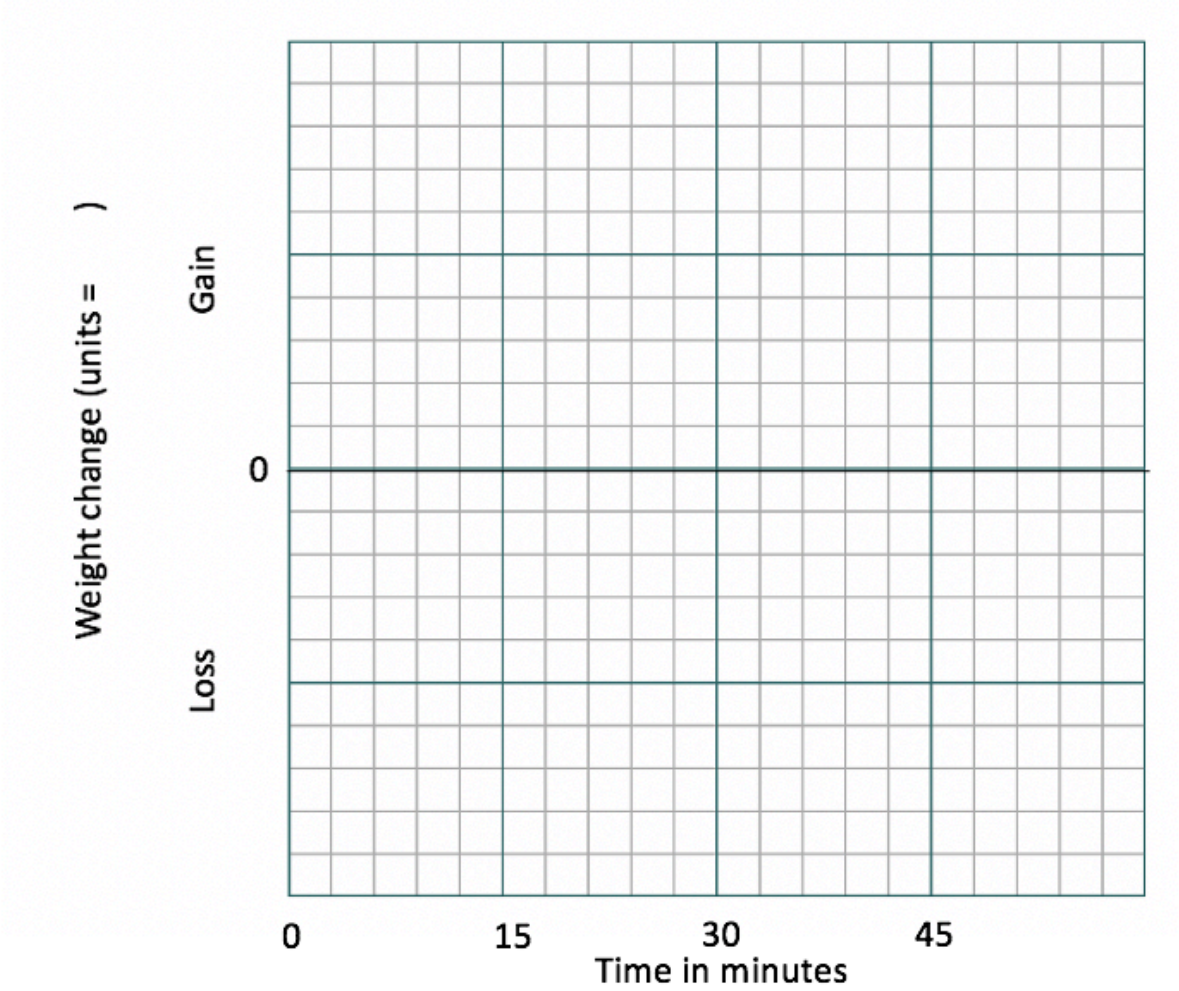
12. Repeat the procedure for 30 and 45 minutes and record your results in the table below.

	Weight of Core			
Solution	**0 minutes**	**15 minutes**	**30 minutes**	**45 minutes**
0% NaCl				
0.9% NaCl				
20% NaCl				

13. Now convert each core weight to a change in weight using the table below.

		Weight Change			
Solution	**Starting weight**	**0 minutes**	**15 minutes**	**30 minutes**	**45 minutes**
0% NaCl		0	Weight at 15 min - starting weight	Weight at 30 min – starting weight	Weight at 45 min – starting weight
0.9% NaCl		0			
20% NaCl		0			

14. Graph the weight change of the potato core for each of the three solutions on the paper below. Using your results from the table above, determine and write an even scale for your y-axis of weight change on the graph paper below (+ is above 0 line and - is below 0 line). Each solution starts with a weight change of 0 at time 0. Don't forget to generate a key so you know which solution corresponds to each line.



Activity 3: Osmosis in Animal Cells

Background:
In this activity we will observe osmosis in red blood cells under the microscope. Remember the animal cells lack a cell wall. Therefore, these cells will crenate (shrivel) when water leaves the cell and swell when water enters. If too much water enters, the cells can lyse (rupture). When this happens, the cell contents are released into the surrounding solution. The cell membrane will sometimes reform to create an empty, or “ghost” cell. These are difficult to see since they lack the red pigmentation that have spilled out of the cell. You will view red blood cells under the microscope in three different salt solutions, 0.9% NaCl, 0% NaCl and 20% NaCl. The salt solution of 0.9% is considered isotonic for red blood cells.

Procedure: (Work together as a table)

Gloves and lab coat must be worn for this activity.

1. Take 3 clean, dry slides from the slide box and lay them out in front of the solution dropper bottles “0.9% NaCl,” “0% NaCl,” and “20% NaCl.”

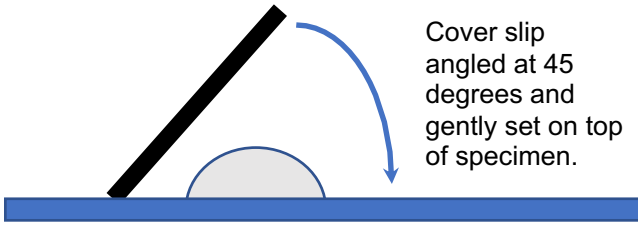
2. Label each slide with a wax pencil 0.9% NaCl,” “0% NaCl, “20% NaCl.”

3. When ready, ask your instructor for a drop of horse blood for each slide.

4. Place one drop of the appropriate NaCl solution on top of the drop of blood on each slide taking care not to touch the tip of the dropper bottle to the blood on the slide.

5. Place a plastic coverslip at a 45-degree angle over the drop and gently lower it onto the slide. You have just created a wet mount (**Figure 5**).

6. Each student in your group will use a compound microscope to find the tiny cells under the scanning objective. Move to the edge of the coverslip where the cells are less concentrated (called the feathered edge) (**Figure 6**) and then switch to low power, and finally to high power to view the red blood cells (RBCs) (**Figure 7**). Since the cells are small and transparent, increase the contrast by moving the iris diaphragm lever approximately halfway to the right as you increase the magnification.



Wet Mount

Figure 5: Proper technique for creating a wet mount.

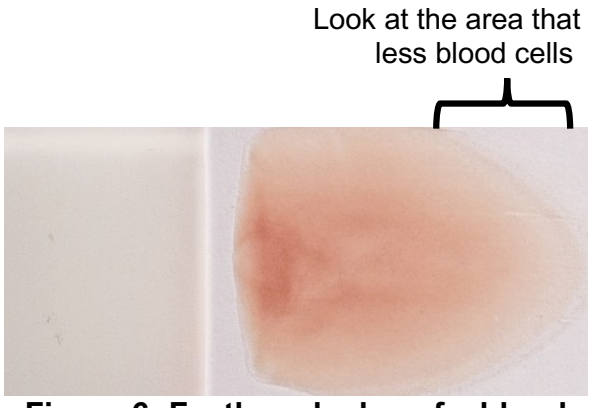


Figure 6: Feathered edge of a blood slide

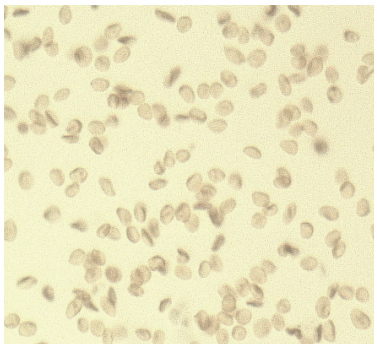
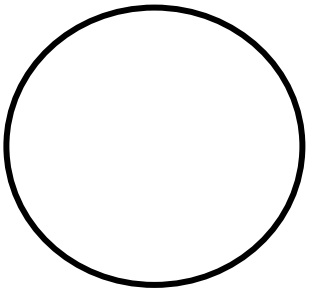
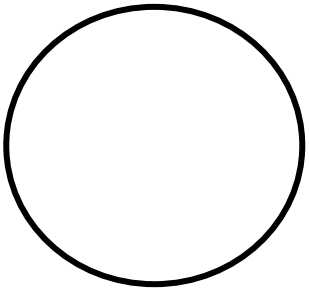
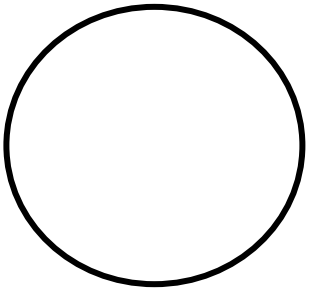


Figure 7: Healthy Red blood cells in isotonic solution. (400x)

7. Make a proper scientific drawing (with labels) below. Be sure to include the total magnification (TM) and a brief description of the shape and appearance of the RBCs. Use the following labels: **cell membrane,** and **cytoplasm.**

0.9% NaCl	**0% NaCl**	**20% NaCl**
		
TM: _____	TM: _____	TM: _____
Description of cells:	Description of cells:	Description of cells:
Water moved **into, out of, or no net change**? (Circle one)	Water moved **into, out of, or no net change**? (Circle one)	Water moved **into, out of, or no net change**? (Circle one)
Was the extracellular solution as either **hypertonic, isotonic or hypotonic?** (Circle one)	Was the extracellular solution as either **hypertonic, isotonic or hypotonic?** (Circle one	Was the extracellular solution as either **hypertonic, isotonic or hypotonic?** (Circle one

8. Could you find any cells in the 0% NaCl solution? If so, describe what they look like, compared to the cells in the 0.9% NaCl. If not, why do you think it would be difficult to view cells in this solution.

9. Why do you think dehydrated patients in the hospital are not given pure water in their IV to help rehydrate them? **Explain** your reasoning using your data from this experiment.

10. Place the used microscope slides and coverslips in the bleach solution by the sink.

11. Place any material contaminated with blood in the biohazardous container under the fume hood.

Activity 4: Plasmolysis in Plant Cells

Background
There are some important differences in osmosis between plant and animal cells due to plant cells having a cell wall in addition to a plasma membrane. While net water movement will always be from the hypotonic solution and into the hypertonic solution, an animal cell placed in a hypertonic solution will **crenate** (shrivel) as it loses water while a plant cell will undergo **plasmolysis** (the plasma membrane will pull away from the cell wall). An animal cell placed in a hypotonic solution will expand and if too much water enters, it will **lyse** (rupture). Plant cells do not rupture in hypotonic solutions. Instead, the plasma membrane pushes against the rigid cell wall to create **turgor pressure**. This helps a plant stand upright (prevents wilting). Given what you learned about osmosis in activities 2 and 3 above, generate a hypothesis for which solution will cause plasmolysis in a plant cell, 20% NaCl or 0% NaCl.

Hypothesis:

Procedure: (Work in pairs)

1. Take 2 clean slides from the slide box and lay them in front of the solution dropper bottles "0%NaCl" and "20% NaCl."

2. Label the slides with a wax pencil.

3. Using the forceps, gently remove two leaves from the Elodea plant. Place them on a paper towel to remove the moisture.

4. Place one leaf flat on each slide. Add one drop of appropriate solution onto each slide.

5. Place a coverslip at a 45-degree angle over the drop and gently lower it onto the slide. You have just created a wet mount. Repeat for the second slide.

6. Wait 1-2 minutes before observing the slide.

7. Use a compound microscope to find the cells under the scanning objective, then switch to low power, and finally to high power.

8. Observe both slides in turn. Notice the box shaped cells and locate the multiple green, circular **chloroplasts** within each cell.

9. Which slide showed plasmolysis? 0% or 20% Describe your observation in 1-2 sentences below.

10. Was your hypothesis supported?

Name: _____ **Date:** _____

Lab Checkout: When you finish the lab, please clean up your lab space and put away your materials neatly in the tray. Please get your instructor's initials to check-out of lab.

- ❑ Lab bench clean, washed, and dried
- ❑ Agar plates, straws, and potatoes have been thrown away in the trash cans
- ❑ Potato corer, knife, weigh boats have been rinsed and dried
- ❑ The wet mount slides (horse blood) have been placed into the bleach/soap solution container
- ❑ The dyes are placed in the refrigerator and incubator, respectively
- ❑ Trays neatly put away

Instructor initials:_____ **% Completion of activities:** _____

Correct Incorrect