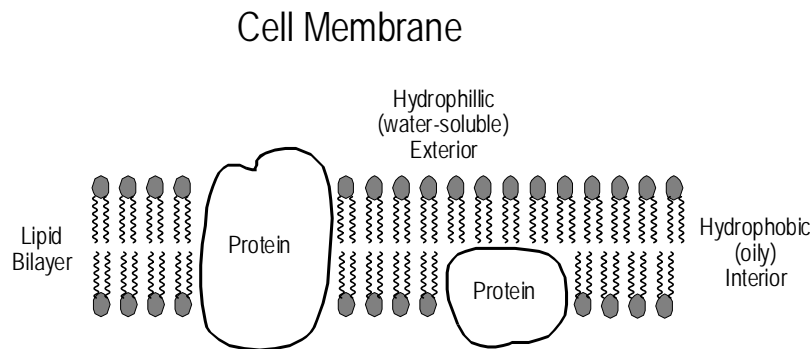


EFFECT OF SALT ON CELL MEMBRANES

LAB CELL 2

INTRODUCTION

A eukaryotic cell, a cell with a nucleus, not only has a plasma membrane as its external boundary, but it also has a variety of membranes that divide the internal space into discrete compartments, separating processes and cell components. The complex and varied design of cell membranes gives them the remarkable properties that allow them to serve the variety of specific functions required by different types of cells. One of the most significant properties of membranes is selective permeability. This permits some molecules and ions to pass freely through the membrane, but excludes others from doing so. Understanding the structure and properties of cell membranes is important to understand how cells function.



Although the details of cell membrane composition and structure vary according to its specific function, all of them are composed of mainly lipids and proteins in a structure known as a lipid bilayer. This is a fluid structure that allows for the flexibility required for cell growth and movement in addition to the selective permeability that is so central to membrane function.

One component of plant cells that usually contains water is the *vacuole*, which is surrounded by a membrane known as the *tonoplast*. In beet plants, the vacuole also contains a water-soluble red pigment, *betacyanin*, which gives the beet its characteristic color. If the tonoplast is damaged, however, the contents of the vacuole will spill out into the surrounding environment. In the case of beets, when the membrane is damaged, the red pigment will leak out into the surrounding environment. The intensity of color in the environment should be proportional to the amount of cellular damage sustained by the beet.

References:

- Biology with Calculators*, Vernier Software & Technology, 2000.
Kirby, J., Mortellaro, J., Prockup, J. "Effects of Temperature and Solvents on the Cell Membrane, SOTM LAB B14." <http://library.marist.edu/sotm/html/b14.html> (29 September 2002).

The presence of certain salts is essential for most plant growth, but too much salt can kill plants. Even salts that are not transported across cell membranes can affect plants—by altering the osmotic balance. Osmosis is the movement of water across a semi-permeable membrane from a region of low solute concentration to a region of higher solute concentration. It can greatly affect a cell's water content when the concentration of solute outside the cell is different from the concentration inside the cell. You will test to see how this osmotic stress affects the cellular membrane integrity.

The salt solutions used in this experiment are clear and colorless. If the beet pigment leaks into the solution, it will turn the solution red. The intensity of color in the solution should be proportional to the amount of cellular damage sustained by the beet. As the concentration of pigment in the solution increases, it absorbs more light. In this experiment, the absorbance of light at 460 nm will be used to monitor the extent of cell membrane damage.

PURPOSE

The purpose of this experiment is to study the effect that osmotic balance has on the integrity of cell membranes by visible spectroscopy.

EQUIPMENT/MATERIALS

6 test tubes and test tube rack	Kimwipes
cuvettes	cork borer with 4-mm inside diameter
Spectronic 20 Genesys	small dowel rods
beets	10 mL graduated cylinders
ruler	scalpel or razor blade
marker	apron
wash bottle with deionized water	gloves

FOR ENTIRE CLASS:

Salt solutions (5 mL/group): 0%, 3%, 6%, 9%, 12%, 15%

10 mL graduated cylinders labeled: 0%, 3%, 6%, 9%, 12%, 15%

SAFETY

- Always wear an apron and goggles in the lab.
- Beets will stain hands and clothing. Gloves should be worn, in addition to aprons, to prevent staining.

PROCEDURE

1. Cut 6 uniform cylinders of beet using a cork borer with a 4-mm inside diameter. Line up the pieces, cut off the ends and make another cut to obtain 15-mm long pieces (all pieces must be the same size).
2. Rinse each piece for 2 minutes in a large beaker of tap water to remove the excess red dye that leaked during the cutting procedure.
3. Number each of your six test tubes 1 – 6.
4. Using the table below as a guide, pour 5 mL of salt solution into each of the test tubes:

Test Tube Number	Salt Concentration %
1	0
2	3
3	6
4	9
5	12
6	15

5. Add one piece of beet to each of the 6 test tubes and begin timing for 15 minutes. During the 15 minutes, use the procedure shown by your instructor to gently mix the contents of each of your six test tubes *every minute*. **Note:** During this time your teacher may also have you zero the Spec 20. See steps 7 - 12.
6. When the 15 minutes is up, carefully pour the solution from each of the six test tubes into six different cuvettes until each is about $\frac{3}{4}$ full. Be carefully to keep track of which test tube was used to fill each of the cuvettes!
7. Ensure that the spectrophotometer has warmed up for at least 20 minutes. Use the same instrument for the entire experiment.
8. Set the spectrophotometer to 460 nm.
9. Fill a cuvette about $\frac{3}{4}$ full with deionized water. This is the "blank cuvette".

10. Place the blank cuvette into the sample compartment of the spectrophotometer with the triangle on the cuvette facing the front of the instrument.

Note: Before inserting a cuvette into the spectrophotometer, wipe it clean and dry with a kimwipe, and make sure that the solution is free of bubbles. Do not touch the clear sides of the cuvette.

11. Press **0 ABS 100%T**.
12. Remove the blank cuvette from the instrument.
13. Place the cuvette containing the solution from test tube “1” into the spectrophotometer. Make sure that the triangle on the cuvette is facing the front of the instrument. **Do not press 0 ABS 100%T.**
14. Record the absorbance of the solution from test tube “1” in the Data Table.
15. Repeat steps 13 and 14, for the solutions from test tubes 2 – 6.

PROCESSING THE DATA

1. Using a spreadsheet or graph paper, make a graph of absorbance vs. salt concentration. Absorbance should be plotted on the y-axis, and salt concentration on the x-axis.

DATA SHEET

Name _____

Name _____

Period _____ Class _____

Date _____

EFFECT OF SALT ON CELL MEMBRANES

DATA TABLE

Test Tube	Salt Concentration %	Absorbance
1	0	
2	3	
3	6	
4	9	
5	12	
6	15	

QUESTIONS

1. Which concentration of salt produced the most intensely red solution? The least intensely red solution?
2. Which salt concentration(s) had the least effect on the beet membrane? How did you arrive at this conclusion?
3. Did more damage occur at high or low salt concentrations? Explain why this might be so.
4. An effective way to kill a plant is to pour salt onto the ground where it grows. How might the salt prevent the plant's growth? Is this consistent with your data?

DISCUSSION

In a class discussion, compare your group's results with those of other groups. Are your data and graphs similar? What conclusions regarding salt concentration and cell membranes can you make from your investigation?