I. TEACHER NOTES & GUIDELINES

TITLE OF LAB: Effects of Temperature and Solvents on the Cell Membrane

DEVELOPERS OF LAB: Adapted by James Kirby JD726, Jennifer Mortellaro JD449, and James Prockup JD575 from a publication by the Department of Biological Sciences at Western Michigan University, Kalamazoo, MI

OVERVIEW OF LAB:
DESCRIPTION:
The purpose of this lab is to illustrate the effects that temperature and solvents have on the cell membrane.

Red beet tissue contains large amounts of betacyanin, a red pigment, located in the large internal membrane vacuoles. When the membrane is damaged, this pigment can cross the vacuole membrane and cell (plasma Membrane). In this experiment we will take pieces of beet root and test what types of environmental stress disrupts the membrane. This should give students an insight into the composition of the membrane.

CURRICULUM CONSIDERATIONS:
This lab is intended to be used in a Regents Biology Curriculum. This lab can be used when studying the topic of cell membrane structure and function. This lab is expected to be completed in two, 40-minute periods.

SAFETY CONSIDERATIONS:
Solvents/household liquids, which the students want to use in Part IV, must be approved by teacher before use.

BACKGROUND INFORMATION

A. SCIENTIFIC VIEWPOINT: A major breakthrough in cell biology was the fluid mosaic model hypothesis of membrane design. In this model proteins are an integral part of the membrane. The fluid mosaic model proposed by Singer and Nicolson is a mixture of proteins and lipids.

The lipids are in the form of a bilayer. The hydrophobic parts of the lipid molecule face the inside while the hydrophilic portions of each lipid face either the cytoplasmic or extracellular environment. The lipids are primarily phospholipids.

Proteins in the membranes are of two types: Integral and Peripheral. Integral proteins are embedded within the bilayer. These proteins are very difficult to remove from the membrane and to study them one has to use detergents to extract them. Some of these integral proteins work as channels for the movement of ions and other molecules through
the membrane. Peripheral proteins are attached to the surface of the membrane usually by a charge and are much easier to extract.

The membrane is described as fluid because the bilayer proteins and lipids can move laterally within the membrane. To maintain the fluid nature of the membrane, animal cells require Cholesterol. This steroid acts as antifreeze to keep the membrane fluid even though animal cell lipids have fatty acids that might keep the membrane solid at body temperature. Most plant cells do not require cholesterol in their membranes because of the higher polyunsaturated content of their membrane lipids.

B. MISCONCEPTIONS AND ACCEPTED SCIENTIFIC EXPLANATIONS
1. All cell membranes are not the same.
   * All cell membranes share the same phospholipid structure, although the lipids vary in different types of cells. The membrane proteins and associated carbohydrates vary extensively from one cell type to another.

Membranes are porous.
   * Membranes are selectively-permeable. Only certain substances will pass through them. Permeability differs with the cell type.

Membranes are rigid.
   * Membranes are flexible due to the properties of the phospholipid bilayer and cholesterol.

OBJECTIVES
2. Become familiar with the use of a spectrophotometer.
3. Design and carry out an experiment using knowledge obtained from completing Part III of this lab.

EQUIPMENT AND MATERIALS
PROVIDED BY SOTM:
- Spectrophotometers 20D+
- Two (2) Hot Water Baths
- Cuvettes six (6) per group
- Cork Borer with a 6-mm inside diameter

PROVIDED LOCALLY:
- Beets
- Six (6) test tubes per group
- Test tube rack
- Freezer set below zero (0) degrees Celsius
- Ice
- Thermometer
- Various solvents that are suggested by the students
- Rulers
- Scalpels or Razor Blades

*Optional, by request

*Laptop Computers
*Interfaces and cables
*Surge Protectors
ADVANCE PREPARATION AND PROCEDURAL HINTS
1. Turn on the water baths 20 minutes prior to use. One water bath is to be set for 55C, while the other bath is to be set for 80C.
2. Freeze rinsed samples of the beet so that each group can have one.
3. Turn spectrophotometer on at least 20 minutes prior to use.
4. After 20 minutes, the spec will be ready for calibration.
   a. Set wavelength 460nm.
   b. Set to transmission mode.
   c. With the chamber empty, turn the left knob until the display reads 0.0
   d. Set to absorbance mode (You should now see 1.999 blinking).
   e. Place a blank into the chamber and turn the right knob until the display reads 0.0

II. PRE-LAB

PRE-LAB EXERCISE TO ELICIT STUDENTS’ PRIOR KNOWLEDGE AND MISCONCEPTIONS.
Divide class into groups of three or four students. In a five to ten minute time frame, have each group answer the following questions:
1) Draw and label the Fluid Mosaic Model of a membrane.
2) Explain how substances travel through a membrane.
3) Would the membrane model look different at different temperatures? Explain
4) How would the ingesting of alcoholic and non-alcoholic drinks affect the membrane? Be specific.

DISCUSSION OF PRECONCEPTIONS
Have one person, from each group, discuss with the class the group’s answer to each question.

III. EXPLORATION OF SCIENTIFIC PRINCIPLE & INTRODUCTION OF EXPERIMENTAL PROTOCOL

PROBLEM
What effect will temperature changes have on a membrane?

EXPERIMENT AND TECHNICAL OPERATION OF EQUIPMENT
PROCEDURE
1. Cut 5 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-min in a large beaker of tap water to remove the excess red dye that leaked during the cutting procedure.
3. Place one piece into each of the 6 test tubes and write a number on the tube/lid with a marker.
   a) Frozen (from teacher)
   b) On ice for 15-mins
   c) 25C (room temp) for 15-mins
   d) 37C (hold in your hand) for 15-mins
4. After temperature treatments, add 5-mL of distilled water, cover with parafilm and allow to stand for 15-mins. During this time the teacher will preview with you the operation of the spectrophotometer. Invert the tubes gently several times during the 15-mins.
5. Pour the liquid from test tube (a) into a clean cuvette. Wipe down the outside of the tube, and measure the absorbency of the liquid at 460-nm on the spectrophotometer.
6. Repeat step #5 for test tubes (b) through (f).
7. Record the values on an excel spreadsheet and construct a graph that best illustrates your results.
8. Clean up.

DISCUSSION
In a class discussion compare your team results with those of other teams. Are your data and graphs similar? What conclusions regarding temperature and cell membranes can you make as a result of your investigation?

IV. ELABORATION OF SCIENTIFIC PRINCIPLE: INQUIRY-BASED STUDENT INVESTIGATION

PROBLEM: Will solvents or household liquids have an affect a membrane?

HYPOTHESIS OR PREDICTION
Guiding questions:
Which solvents or household liquids will have no affect on a membrane? Why?
Which solvents or household liquids will have an affect on a membrane? Why?
Be sure that your hypothesis/prediction is a statement and not a question.

EXPERIMENTAL DESIGN
Guiding questions:
What are you going to measure?
What equipment will you need to test your hypothesis?
What procedures could you use to test your hypothesis?
Can you give a rationale for every step of your experiment?
Checkpoint (Teacher checks students’ experimental design for feasibility.)

PLAN FOR DATA COLLECTION & ANALYSIS
Guiding questions:
How will your data be organized?
Do you need a data table to record your data?
Will you need to graph your data?
How much data will you need to make a conclusion?
Checkpoint (Teacher checks students’ plan for feasibility.)
CONDUCTING THE EXPERIMENT

Checkpoint (Teacher monitors students’ investigations in progress)

ANALYSIS OF DATA

Guiding questions:
- Is your data organized properly?
- Were there any surprises with your results?
- What new questions were formed because of this experiment?
- Does your data support your hypothesis/prediction

Checkpoint (Teacher checks students’ analysis)

DISCUSSION OF RESULTS:

V. Evaluation

TRADITIONAL
Have students re-answer the questions from the pre-lab.

ALTERNATIVE
Have students design an experiment to further study the structure of the membrane. This experiment should **not** be about temperature or solvents.

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