How-To-Do-It

Demonstrating the Effects of Stress on Cellular Membranes

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Living beet cells are excellent models for some simple experiments involving cellular membranes. Membranes are functionally important because they separate and organize chemicals and reactions within cells by allowing selective passage of materials across their boundaries. As in all biology, a membrane's structure relates to its function, and an understanding of membrane function is fundamental for introductory biology students.

Unfortunately, most laboratory experiments investigating characteristics of membranes are prohibitively complex for introductory biology courses or include artificial rather than living membranes. This paper describes two simple procedures allowing students (grades 7-12) to experiment with living membranes and to relate their results to fundamental membrane structure.

The membranes of living eukaryotic cells, including beet cells, are composed of a bilayer of phospholipid molecules interspersed with protein molecules. A phospholipid molecule is a combination of a phosphate group and two fatty acids bonded to a three-carbon glycerol chain (Figure 1). The resulting phospholipid molecule is polarized. The polar (charged) phosphate group is hydrophilic (water-loving) and the nonpolar fatty-acid groups are hydrophobic (water-fearing).

Polarized phospholipids will innately self-assemble into a double-layered sheet of molecules forming a membrane. The hydrophobic tails of the lipids form the core of the membrane and hydrophilic groups line both surfaces (Figure 1). This elegant assembly is stable and allows selective penetration by small lipid-soluble, hydrophobic molecules. The lipid bilayer resists penetration by most large, hydrophilic molecules.

Roots of beets (Beta vulgaris) contain an abundant red pigment called betacyanin, which is localized almost entirely in the large central vacuoles of the beet cells. These vacuoles are surrounded by a vacuolar membrane (i.e., tonoplast) and the entire beet cell is further surrounded by a cellular or plasma membrane. As long as the cells and their membranes are intact, the betacyanin will remain inside the vacuoles. However, if the membranes are stressed or damaged, betacyanin will leak through the tonoplast and plasma membrane and produce a red color in the water surrounding the stressed beet. This red color allows a student to easily assess damage to living membranes by monitoring the intensity of color produced by stressful, experimental treatments such as extreme temperatures or lipid-dissolving solvents.

The Effect of Temperature Stress on Membranes

Extreme temperatures provide a good set of treatments for student experimentation because high or low temperatures can physically destroy a membrane. In addition, temperatures can be easily and accurately measured. To prepare for such an experiment, you'll need:

fresh beets	beaker
a thermometer	metric ruler
refrigerator	razor blade
freezer	six test tubes
corkborer	a test tube rack
forceps	

Use the cork borer and razor blade to cut six sections of beet tissue into cylinders 15 mm long and 5 mm in diameter. Rinse the beet sections to remove pigment from the damaged cells. Each of the sections will be subjected to one of the temperatures listed in Table 1.

For the two coldest treatments, place two beet sections in two labeled test tubes and place one tube in a freezer (−5 degrees Celsius) and one tube in a refrigerator (5 degrees Celsius) for 30 minutes. Then add 10 ml of water to each test tube and place them in a rack at room temperature.

Table 1. The color intensity of betacyanin leaked from damaged membranes treated at six temperatures.

Tube Number	*Treatment (°C)*	*Color Intensity (0-10)*	*Absorbance (460 nm)*
1	70		
2	55		
3	40		
4	20		
5	5		
6	−5		

For the warmer treatments (i.e., 20, 40, 55, 70 degrees Celsius), heat a beaker of water to 70 degrees Celsius and submerge a beet section in the water for one minute. Place the section in a labeled test tube with 10 ml of water at room temperature. Cool the beaker of water using ice or tap water to 55 degrees Celsius and

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submerge another section for one minute. Place this section in a labeled test tube with 10 ml of water at room temperature. Repeat the procedure of cooling and submersion for each of the remaining temperature treatments. After completing all the treatments you should have a rack of six labeled test tubes, each with 10 ml of water at room temperature and a beet section which has been subjected to a different temperature. Shake the tubes occasionally and allow 30 minutes for the pigment to leak out of the stressed cells. Then remove the beet sections from the tubes.

While students wait for the experiments to proceed, you might discuss the construction of graphs to display the results or consider the implications of all membranes having similar structure.

The water surrounding the stressed beets will contain various amounts of betacyanin (Figure 2). You can assess the relative damage or stress caused by each temperature treatment by comparing the intensity of color in each tube. Although a spectrophotometer will provide the most accurate color readings, middle school instructors may wish to have the students estimate the color using a subjective scale. We suggest using values 1-10 as a subjective scale measuring color intensity. The darkest solution would have a value of 10 and the lightest a value of 1. Record your results in Table 1.

Questions for students

1. Which temperatures stressed and damaged the membranes the most?
2. Exactly how could high tempertures tear a membrane?
3. Did low temperatures stress the membranes by the same mechanism as high temperatures?

Spectrophotometric Analysis

Students can use spectrophotometers to objectively assess the relative amounts of betacyanin resulting from membrane damage. Inexpensive spectrophotometers (colorimeters) can easily measure the absorbance of 460 nm light by betacyanin. This light absorbance is a direct measure of the concentration of betacyanin and an indirect measure of membrane damage. Although you can assess the results of the above treatments without electronic equipment, use of a spectrophotometer enhances the experiments by quantifying the results.

After making and recording the absorbance readings for each temperature, these data can easily be plotted on X-Y axes. Plot temperature, the independent variable, on the X axis and absorbance on the Y axis.

Questions for students

1. Did any two treatments produce solutions of similar color intensity?
2. What is the advantage of using a machine rather than your eyesight to measure color intensity?

The Effect of Organic Solvent Stress on Membranes

The lipid structure of membranes can be altered by organic solvents which dissolve a membrane's lipid component. Acetone and alcohol are readily available solvents that severely stress membranes. We suggest an experiment that compares the membrane disruption (lipid solubility) of acetone with that of alcohol and tests the effects of various concentrations of each solvent. Be sure to warn students of the hazards of organic solvents. Most solvents, such as acetone, are flammable and volatile. Students must avoid breathing the fumes and avoid any skin contact with the solvents.

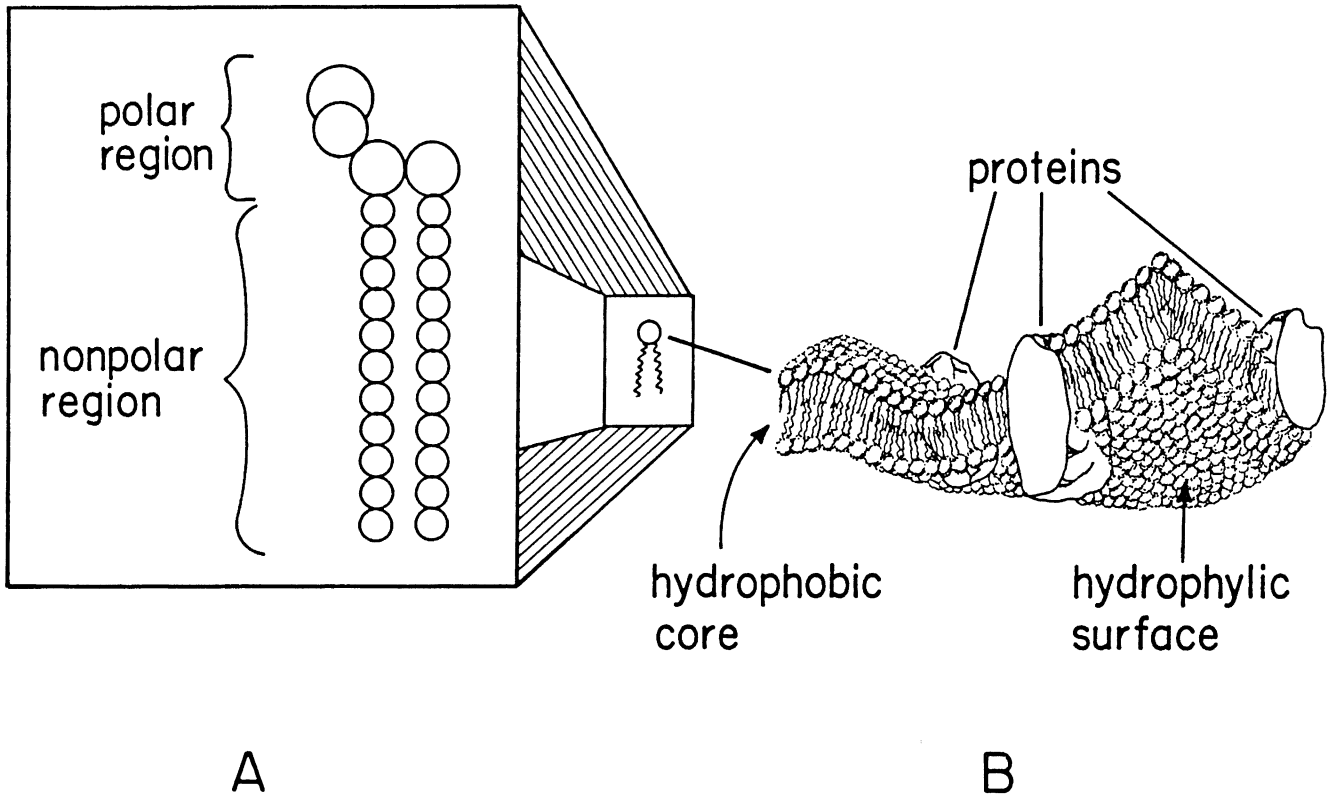

Figure 1. A. A phospholipid molecule. B. Model of a bilayer membrane.

Table 2. The color intensity of betacyanin leaked from damaged membranes treated with three concentrations of two organic solvents.

Tube Number	*Treatment*	*Color Intensity (0-10)*	*Absorbance (460 nm)*
1	1% Acetone		
2	25% Acetone		
3	50% Acetone		
4	1% Methanol		
5	25% Methanol		
6	50% Methanol		

To prepare for an experiment on the effects of solvents on membranes, you'll need:

fresh beets
a cork borer
razor blade
metric ruler
graduated cylinder
10 ml of acetone
10 ml of methanol or ethanol
six test tubes
a test tube rack

Prepare 1 percent, 25 percent, and 50 percent (v/v) solutions of acetone in water, and three more solutions of the same concentrations using methanol in water. Cut and rinse six beet sections as described in the experiment on temperature stress. Place one section in each of six labeled test tubes and add 10 ml of one of the six solvents to each test tube. Seal the test tubes with corks to avoid escaping fumes. After 30 minutes, remove the beet sections and compare the red color of each solution. Record the color intensity of each tube in Table 2, using a subjective scale of 1-10 as described in the experiment on temperature stress.

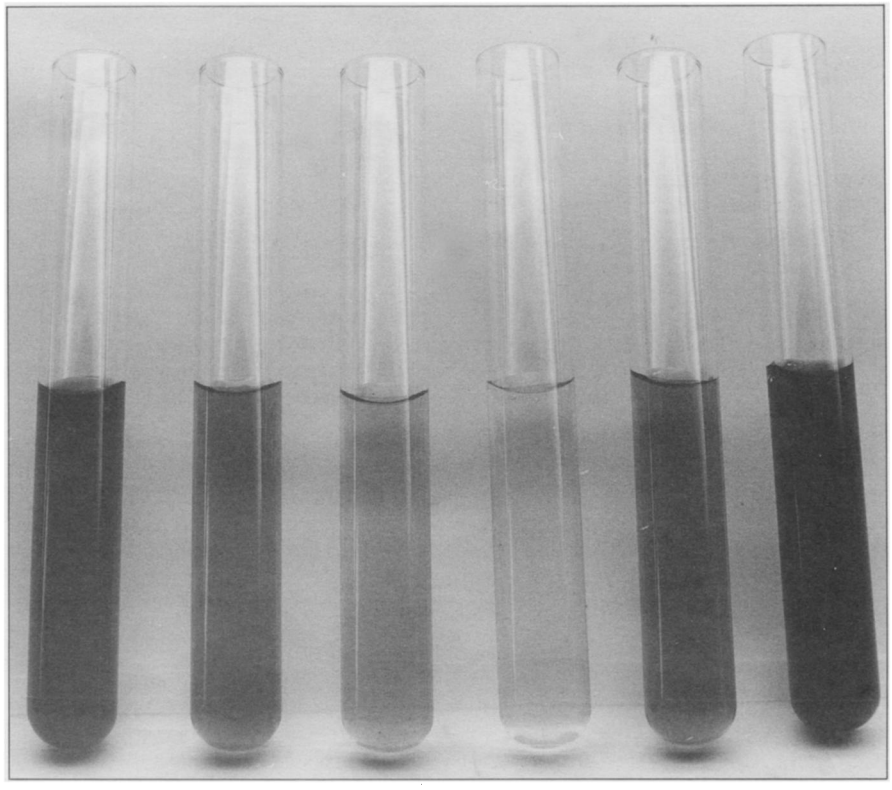

Figure 2. Six solutions of betacyanin from a beet tissue treated at six temperatures. From left to right, treatments were −5, 5, 20, 40, 55, and 70 degrees Celsius.

Questions for students

1. Which solvent stressed the membranes more?
2. Did higher concentrations of the solvents cause more damage?
3. Are lipids soluble in both acetone and alcohol?
4. Which solvent would you conclude has the greatest lipid solubility?