Cell Structures: Membrane Permeability

Intact membranes are essential for the health, well-being and the very existence of cells. What conditions affect membranes and the permeability of membranes? In this activity, you will use various solutions and observe their impact on membrane permeability. The model organism used in this activity is the beetroot. Beetroots have distinctive colors with the most commonly available beets having a dark red color. The color is produced by pigments called betalains. These pigments are housed within the central vacuole of each beetroot cell. If the plasma membrane is damaged these pigments can leak out of the cell. The more damaged the cell, the more pigment is released.

In this activity you will investigate the effect of temperature, alcohol and detergent on membrane permeability. Consider this activity and write a hypothesis for your experiment here: _____

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Materials

Beetroot (*Beta vulgaris*)
Cork borer (3-4 mm internal diameter)
Ruler
Razorblade or scalpel
Test tubes – 10
Test tube rack
Beaker – 1; 250 mL
Beaker – 2; 500 mL with boiling beads
Marker
Pipette
Ice bath
Hot plate for boiling water bath
Hot plate for 60^0 C water bath
Distilled water
Thermometer
10 % Ethanol solution
20 % Ethanol solution
90 % Ethanol solution
Dish detergent
Graduated cylinder

1. Use the cork borer to take several cores from the beetroot. Cut the beetroot core sample into 1 cm lengths. You will need 10, 1 cm pieces.
2. Add the 10, 1 cm pieces to the beaker and fill the beaker half full with distilled water. Leave the beetroot pieces in the beaker until you are ready to use them.
3. Identify your temperature incubators. The 0^0 C incubator is the freezer or ice bath; the 25^0 C incubator is the benchtop, the 60^0 C incubator is either a water bath or incubator, and the 100^0 C incubator is a boiling beaker. Turn on the hotplates. Place a beaker (500 mL) half filled with water on each hotplate. Turn one hotplate on high; turn the second hot plate about 1/3 of the way on. When the water in the beaker on the first hotplate begins to boil, turn the hotplate down. After 15 minutes use the thermometer to measure the temperature of the water on the second hotplate. If the temperature is greater than 60^0 C, then turn down the temperature and add DI water to bring down the temperature of the water in the beaker. Maintain the 60^0 C by increasing and decreasing the temperature control knob.
4. Label the test tubes as follows: 0^0 C, 25^0 C, 60^0 C, 100^0 C, 10% EtOH, 20% EtOH, 90% EtOH, 1 drop, 5 drops, and 10 drops.
5. Mark each tube 2.5 cm from the bottom.
6. Carefully remove 4 pieces of beetroot from the beaker containing beetroot and distilled water. Blot the pieces gently with a paper towel.

7. Place 1 piece of beetroot in the test tube labeled 0^0 C. Place 1 piece of beetroot in the test tube labeled 25^0 C. Place 1 piece of beetroot in the test tube labeled 60^0 C. Place 1 piece of beetroot in the test tube labeled 100^0 C.
8. Fill each of these test tubes up to the 2.5 cm mark with distilled water and then place each test tube in the appropriate incubator (0^0 C, 25^0 C, 60^0 C, 100^0 C).
9. Incubate the tubes for 30 minutes at the designated temperature. After 30 minutes remove the tubes from the incubators and record the results below.

Temperature	Color
0^0 C	
25^0 C	
60^0 C	
100^0 C	

10. What can you conclude with regard to membrane damage and temperature? Which temperature damaged beetroot membranes the most? Which temperature caused the least damage to beetroot membranes? How do you know?

11. Was your hypothesis with regard to temperature supported?

12. Membranes are composed of lipids and proteins. How is temperature affecting either or both of these components?

13. Carefully remove 3 pieces of beetroot from the beaker containing beetroot and distilled water. Blot the pieces gently with a paper towel.
14. Place 1 piece of beetroot in the test tube labeled 10 % EtOH. Place 1 piece of beetroot in the test tube labeled 20 % EtOH. Place 1 piece of beetroot in the test tube labeled 90 % EtOH.
15. Use the graduated cylinder to get ~3 mL of 10 % EtOH from the supply table. Add 10 % EtOH to the 2.5 cm mark on the test tube labeled 10 % EtOH. Dispose of any EtOH remaining in the graduated cylinder.
16. Use the graduated cylinder to get ~3 mL of 20 % EtOH from the supply table. Add 20 % EtOH to the 2.5 cm mark on the test tube labeled 20 % EtOH. Dispose of any EtOH remaining in the graduated cylinder.

17. Use the graduated cylinder to get ~3 mL of 90 % EtOH from the supply table. Add 90 % EtOH to the 2.5 cm mark on the test tube labeled 90 % EtOH. Dispose of any EtOH remaining in the graduated cylinder.
18. Incubate the tubes for 30 minutes on the benchtop. Swirl the tubes gently every 10 minutes. After 30 minutes record the results below.
19. Use the 25^0 C test tube from the temperature experiment as the control.

Ethanol Concentration	Color
0% EtOH (use the 25^0 C tube from the previous temperature activity)	
10 % EtOH	
20 % EtOH	
90 % EtOH	

20. What can you conclude with regard to membrane damage and ethanol concentration?

21. Was your hypothesis with regard to ethanol exposure supported?

22. Membranes are composed of lipids and proteins. How is ethanol affecting either or both components? Why is ethanol used as an antiseptic on skin?

23. Carefully remove 3 pieces of beetroot from the beaker containing beetroot and distilled water. Blot the pieces gently with a paper towel.
24. Place 1 piece of beetroot in the test tube labeled 1 drop. Place 1 piece of beetroot in the test tube labeled 5 drops. Place 1 piece of beetroot in the test tube labeled 10 drops.
25. Fill each of these test tubes up to the 2.5 cm mark with distilled water.
26. Add the appropriate number of drops of detergent to each tube using either a dropper bottle or pipette. Gently swirl the tube to mix the detergent into the water.
27. Incubate the tubes on the benchtop for 30 minutes. Gently swirl the tubes every 10 minutes.
28. Use the 25^0 C test tube from the temperature experiment as the control.

Detergent	Color
0 drops (use the 25^0 C tube from the temperature activity)	
1 drop	
5 drops	
10 drops	

29. What can you conclude with regard to membrane damage and detergent concentration?

30. Was your hypothesis with regard to detergent-induced damage supported?

31. Name the pigment released from beets.

32. Membranes are composed of lipids and proteins. How is detergent affecting either or both of these components?