Investigating the effect of temperature on permeability of membranes

The investigation will look into the effect of temperature on membrane permeability using beetroot tissue.

Background

Cells of the beetroot contain a red dye called anthocyanin. Under normal conditions the dye is held within the cell by the cell membrane. However in an experiment to show the effect of various reagents (such as alcohol and detergent) on membrane permeability, the structure of the membrane is altered and the red dye leaks out of the cells. The reagents alter the phospholipid component of the membrane.

Prior knowledge:-

Plant cell walls contain cellulose. Cellulose is a polymer of beta glucose. Cellulose fibres have a high tensile strength; this helps the cell to withstand large pressures that develop within it as a result of osmosis. Without this the wall would burst when in a dilute solution. Cellulose fibres are freely permeable allowing water and solutes to reach the plasma membrane.

Cytoplasm is an aqueous material varying from a fluid to a jelly like consistency. The cytoplasm contains proteins. Proteins are enzymes, which can be denatured by high temperatures. Most of the protein molecules in the cytoplasm float in the phospholipid layers. Some of the proteins are embedded in the outer layer; some in the inner layer and some span the whole membrane. They stay in the membrane because they have hydrophobic portions (made from hydrophobic amino acids), which sit among the hydrophobic phospholipid tails. Hydrophilic portions (made from hydrophilic amino acids) face out wards.

***N.B. Information in the prior knowledge was obtained from biology notes, s-cool website, biology 1 and experimental work in biology by D G mackean.**

Prediction:-

I predict that the higher the temperature of water the more the red dye anthocyanin will be released from the beetroot. I think that this is because of the proteins in the cytoplasm. Proteins like all other enzymes are denatured at high temperatures. Most enzymes denature at temperatures above 50 degrees Celsius. When the proteins are denatured the phospholipid layer of the cell membrane alters and has gaps in it. The red pigments probably escape through these gaps. If temperature has no effect on the permeability then the red pigments should escape at room temperature water.

The effect of the high temperatures can cause physical damage to the cell wall or cytoplasm, for example by expansion and rupture of cells. In this case the cell sap would subsequently leek out.
I predict that temperatures of above 50 degrees Celsius would have the most effect on the membrane and most anthocyanin would be escape. Temperatures below this would have very little or no effect because the proteins in the cytoplasm and nucleus would work in very favourable conditions.

Apparatus:-

The following apparatus will be needed to carry out this experiment,

1) 6 test tubes and rack
2) Bunsen burner/electronic water bath
3) Thermometer (0-100 degrees Celsius)- to measure the water bath temperature
4) Scalpel- to cut the beetroot
5) Beaker for water bath
6) Cutting board/tile- for cutting beetroot
7) Beaker or jar- for washing dishes
8) Tongs- to handle hot test tubes
9) Colorimeter- to measure the colour transmission.
10) 5-centimetre cube syringe- to measure 5 cm cubed accurately.

Living material:-

1) Beetroot

Method for pilot study:-

Take 3 clean testubes and put them in a rack.
Cut three 1-centimetre cubes of beetroot tissue.
Set up water bath temperatures of the extreme and middle values of the temperature of your experiment. In this case they are 20, 50 and 80 degrees Celsius.
Place 5 centimetre cubes of water in the test tubes
Place the beetroot tissue in the water bath.
Make sure that the colorimeter reads 100% transmission when measuring water in a test tube.
Take a reading after every 4 minutes.
Take about 5 readings.

My pilot study results were as following,

Temperature degrees Celsius

	20	50	80
4 minutes	50	65	25
8 minutes	50	10	1
12 minutes	60	8	0
16 minutes	80	25	0
20 minutes	75	18	0

Percentage transmission

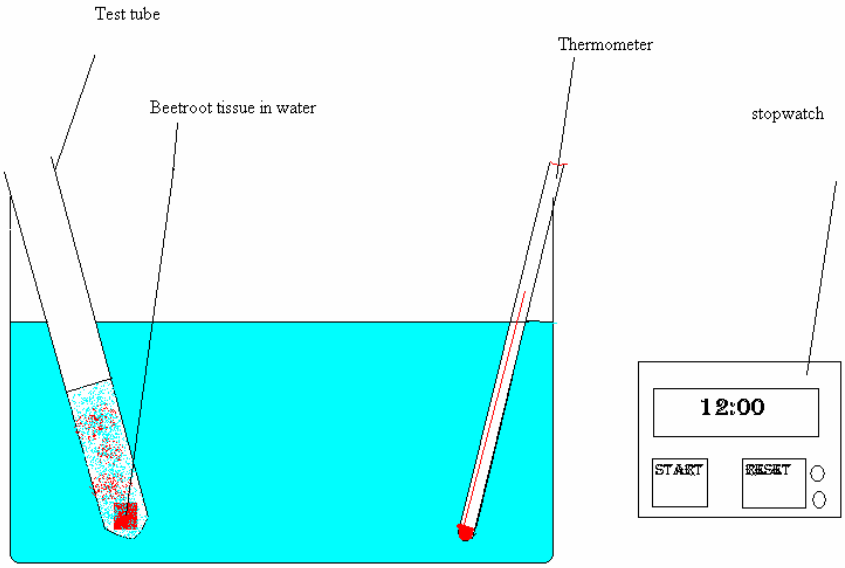
Changes to method after pilot study:-

After doing my pilot study I realised a few mistakes n my experiment.
The beetroot tissue size was too big to fit in the test tubes. For my final experiment I will change the size to about 0.5 centimetre cubed. The timing was too slow and there were some big changes in my results so I will reduce the timing to 1 minute after I take the result. There were some messed up results in my pilot study where the percentage transmission increased when it was supposed to be decreasing. I think this was due to the beetroot tissue still in the beaker when noting the result from the colorimeter. In my final experiment I will take the beetroot tissue out before taking the result. The beetroot tissues were not exactly equal. In my final experiment I will make sure they are equal by using a ruler so that I can be sure that it is a fair test. The water in the test tubes will be left in the water baths a couple of minutes before the beetroot tissues are added so that the required temperature is gained inside the tube.

Method:-

Take 7 clean test tubes and put them in a test tube rack.
Cut 7 x 0.5 centimetre cubes of beetroot tissue.
Put 5 centimetre cubes of water in each test tube.
I will use 7 different values for my input variable.
The range of temperature in my experiment will be from 20-80 degrees Celsius.
Set up water baths with temperatures 20, 30, 40, 50, 60, 70, and 80 degrees Celsius.
Put 1 test tube in each of the water baths and leave for about three minutes so that the required temperature can be gained inside the tube.
Set up the colorimeter so that there is 100% transmission when measuring water in a test tube.
After 3 minutes place the beetroot tissue in the test tubes.
Stop the stopwatch after 2 minutes and take beetroot tissue out of the test tube and place the test tube in the colorimeter and take a reading.
Take 5 readings using the same process.
Before taking the readings mix the solution gently so that there is equal colour density all around the beaker.
The experiment will be repeated a further two times after all the readings have been taken so that an average result can be obtained which will be more precise.

The apparatus will be set up as follows;



Risk assessment:-

Make sure you do not leave bags, coats or other unnecessary stuff around your experiment because it can cause trips falls and spillages etc.
Scalpels and knifes should be handled with care and do not carry them around the lab if not required.
Use a cutting board when cutting and cut with care so that fingers are not cut.
Lab coats must be worn at all times during the experiment. Lab coats should be always fastened when worn.
Safety goggles should be worn when handling hot water or anything else, which may get in contact with your eyes.
Use tongs to carry test tubes placed in high temperatures.
Precaution should be taken against splashing of water as it may be hot and could burn your skin.

Fair test:-

To make this experiment a fair test the size of the beetroot tissue must be the same and equal volumes of water should be added to each test tube. Take the readings on the colorimeter precisely after 2 minutes. Stir the anthocyanin solutions for exactly 5 seconds before putting them in the colorimeter.

How results will be analysed:-

My results would be recorded in a table with temperature against percentage transmission. The average will be taken of all the experiments and will be used as my final result to make it more precise. I will draw a line graph of my data.

Variables:-

The independent variable in my experiment is the temperature, which will be measured in degrees Celsius.
The dependent variable in this experiment is the amount of red dye anthocyanin, which leaks out of the cell membrane. This will be measured in percentage transmission.
Other variables, which may affect my experiment, are
The stirring of the red dye before measuring the percentage transmission.
The pressure will be kept constant (room temperature and pressure).
The amount of water in the test tubes will also influence the results of my experiment and will be kept the same.