An Investigation to determine the effect of temperature on cell membrane permeability

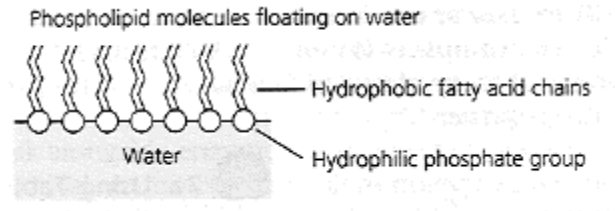
Aim:

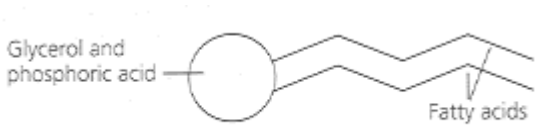
This investigation involves the estimation of the extent to which a red pigment present in the cell sap of beetroot storage tissue, passes out in defined circumstances, when the tissue is immersed for a given time in water, at various temperatures.

Background Knowledge:

Molecular Structure of the Cell (Plasma) Membrane:

The cell membrane is actually a double membrane; as appears in a mitochondrial membrane or an endoplasmic reticulum membrane. The double membrane is made up out of two layers of phospholipid molecules. The phosphates sit on the surface of the cell plasma because they are hydrophilic; while the fatty acid chains point inwards (away from the cell plasma) because they are hydrophobic.





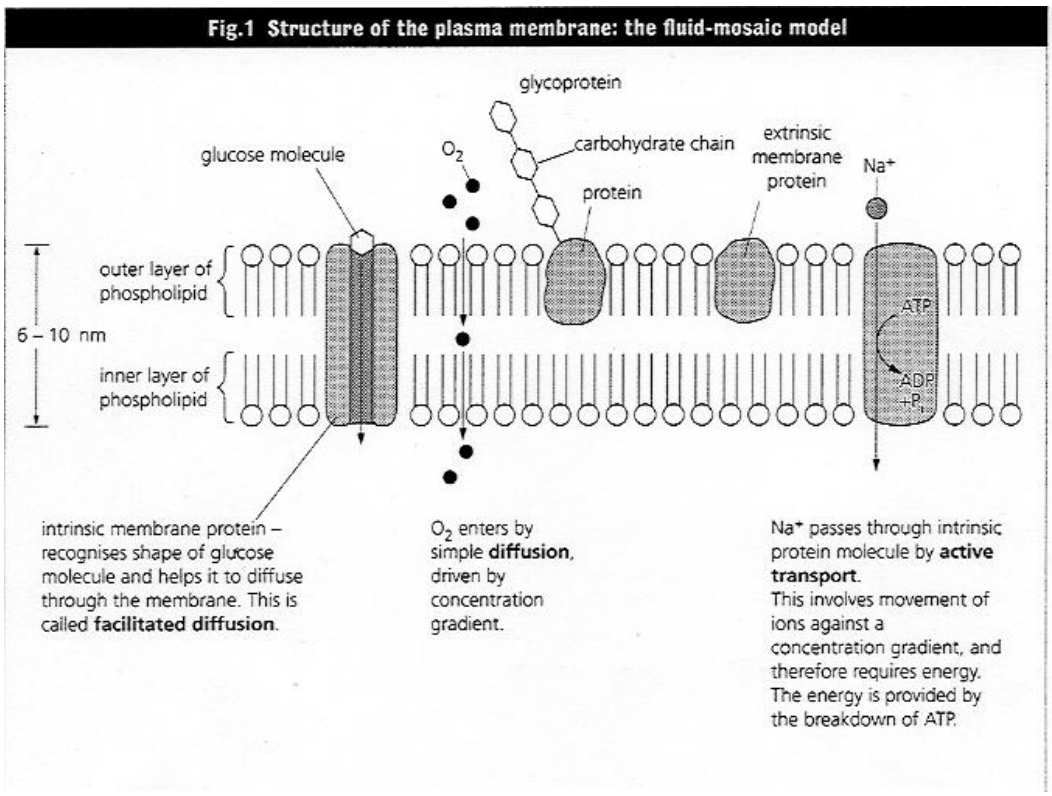
Phospholipid membranes have two main functions:

- They control the transport of materials from one side to the other.
- In chloroplasts and mitochondrion, they keep pigments and carrier molecules in fixed positions.

It was in the late 1930's that J.F. Danielli and H. Davson put forward a theory of bimolecular lipid layer. They also theorised a protein layer between the bimolecular lipid layer. However, in 1972 J. J. Singer and G. L. Nicholson put forward their fluid-mosaic model; that the acid fatty chains are in fact slightly flexible (not totally solid); and that the protein was not a continuos membrane but scattered throughout the cell in globules.

The cell membrane, or plasma membrane, surrounds the cell. It is usually between 6-10nm thick, and is only clearly visible through an electron microscope. It is made of protein and phospholipid. The structure of the plasma membrane consists of two layers of phospholipid, embedded with protein molecules (Fig. 1).

The cell membrane is described as a fluid mosaic for two reasons. First, the protein part of the cell membrane was once thought to be an even layer spread over the outside and inside of the phospholipid. It is now thought to be unevenly distributed, more like a mosaic than a layer. Second, the phospholipid part is fluid, with its molecules constantly moving about.



Some proteins on the outer edge of the membrane have carbohydrate molecules attached, usually short sugar chains. These are called glycoproteins (sugar + protein). The carbohydrate part of the glycoproteins is important in cell recognition - the ability of cells in the body to tell whether or not a cell is from another individual or another organism (e.g. in immune response). The plasma membrane also provides a boundary to the cell cytoplasm - separating it from other cells. The plasma membrane acts as a selectively permeable barrier that allows the entry and exit of certain substances. Movement of substances into and out of cells occurs through the membrane by diffusion, osmosis, active transport, phagocytosis and pinocytosis.

Fluid-mosaic membranes are found around a number of cell organelles, such as the nucleus, mitochondria, chloroplasts and cell vacuoles. The endoplasmic reticulum and the golgi apparatus are also composed of fluid mosaic membranes.

Predictions:

As a protein is an enzyme, it will be affected by temperature. Since the intrinsic membrane proteins are involved with facilitated diffusion, I hypothesise that as the temperature is increased up to its optimum temperature, the rate of diffusion will increase up to that point. This is because as more heat energy is available, more can be turned into kinetic energy and used by the protein. Once however the protein has passed its optimum temperature, (for enzyme-catalyst reactions this is 40ºC/313ºK) I hypothesise that the protein will become denatured and therefore unable to continue with facilitated diffusion.

In terms of the red pigment in the cell sap of the beetroot storage tissue, as the temperature is increased up to its optimum temperature, I hypothesise an increase in the amount of red pigment diffusing out of the cell. The red pigment should diffuse out of the cell, because there is a high concentration of red pigment in the cell and no concentration of red pigment in the water. Since diffusion always happens along a concentration gradient, the red pigment will diffuse out into the cell. However, when the protein has passed its optimum temperature and become denatured, the diffusion will stop, resulting in little or no red pigment diffusing into the water.

Relating this to graphical form, I would have expected to see a slow increase in the amount of diffusion from x = 0, altering to a steady increase, then a sudden increase and a steep gradient to the line as it approaches the optimum temperature. After the optimum temperature has been passed, I would expect to see a steady decrease in the amount of diffusion, until it finally ceased all together at the value y = 0. (This can be seen clearly in graphical form on the page following the graph of the results obtained from this experiment.)

Theory of Implementation:

The beetroot discs were prepared overnight, by immersing them in running tap water and then washing them in distilled water, to remove cell debris and any cell contents, as well as any excess red pigment.

The idea of the experiment is to immerse the beetroot discs in distilled water of varying temperatures. This is allow the proteins to begin diffusing at the temperature of the water. They are then removed from the heated water and placed in a test tube containing distilled water at room temperature. Immersing them in the distilled water allows the red pigment to diffuse out of the storage tissue and into the water. This is because distilled water is pure water, and will naturally have a higher concentration of water molecules than the beetroot disc. Therefore water will diffuse into the beetroot disc, and the red pigment will diffuse out. (As described above.)

All the test tubes will then be left for 40 minutes, to allow diffusion of the red pigment to occur and then the solution will be shaken. This is to ensure that when a sample is taken and poured into a cuvette for use in the colorimeter, the spread of red pigment will be even throughout the whole solution. The machine is then used to measure the transmission of light passing through the sample, having first been blanked at 100% for distilled water.

Implementation:

Firstly the colorimeter was plugged in and switched on. This was to allow the machine to warm up and be ready for when the 40 minutes had expired. (It takes 30 minutes for the machine to warm up.) Next the blue filter was placed in the colorimeter for this experiment.

Secondly the pre-prepared samples were obtained from their jar, and were placed in a small beaker. Then 200cm3 of distilled water was poured into a 250ml beaker and heated to an approximate temperature of 75ºC (348ºK) using a bunsen burner. A beetroot disc was then placed in the heated distilled water for exactly 90 seconds. As the temperature began to drop form 75ºC, the bunsen burner was reapplied to the beaker, to try and maintain a constant temperature of 75ºC. After exactly 90 seconds the beetroot disc was removed and placed in a test tube filled with distilled water. The test tube was then placed in a test tube rack to allow the red pigment to diffuse out of the beetroot disc.

After the 40 minutes had expired, the sample was shaken, (to ensure even distribution of the red pigment - which could affect the colorimeter reading) and taking great care not to touch the sides of the cuvette, (which could also affect the colorimeter reading,) a sample was placed in a cuvette. (A cuvette is a square tube, with two adjoining sides transparent and two adjoining sides frosted.) The cuvette was the lowered into a cuvette rack.

These exact steps were repeated at varying temperatures, recording the time started, so that 40 minutes could then be added to this, in order that the experiments were all a fair test.
After all the cuvette samples were obtained, and placed in the rack, a cuvette containing distilled water was placed in the machine. It was switched on, and the needle was blanked against 100% transmission. (Since distilled water is pure water, it will let all the light through.) The cuvette samples in order of highest temperature to lowest temperature, were then lowered onto the platform of the colorimeter. The platform was then lowered and the machine energised and a reading recorded for each of the cuvette samples.
(See page at back for a diagram of the experiment.)

Results:

Below is the results obtained from the five experiments measuring the transmission of light produced by varying temperatures; shown in tabular form. On the next page follows the same results in graphical form.

Temperature		Time Started	Time Finished	Overall Time (m)	Transmission of light (%)	Intensity (1/Transmission)
ºC	ºK					
75	348	09:50	10:30	40	25.0	0.04
70	343	09:52	10:32	40	23.5	0.042553191
65	338	09:54	10:34	40	35.0	0.028571429
60	333	10:00	10:40	40	19.0	0.052631579
55	328	10:04	10:44	40	98.0	0.010204082

Interpretation of Results:

As can be seen by the graph, there are two lines present on the graph, the yellow line, starting after the 60ºC (333ºK) mark. This is because I believe the reading circled in green to be an anomalous reading, or a discrepancy in the results obtained. I say this, because I hypothesised the graph to appear as it does in the yellow line. As stated earlier, I hypothesised that up to the optimum temperature, the diffusion of red pigment into the distilled water would increase steadily. After it had passed the optimum temperature, I hypothesised that the enzyme would become denatured and therefore as the protein becomes more denatured, the rate of diffusion will decrease and therefore there will be less red pigment present in the distilled water. This is until the protein becomes totally denatured and is unable to facilitate diffusion of the red pigment into the distilled water.

In terms of relating this to graphical form, I would have expected to see a slow increase in the amount of diffusion from x = 0, altering to a steady increase, then a sudden increase and a steep gradient to the line as it approaches the optimum temperature. After the optimum temperature has been passed, I would expect to see a steady decrease in the amount of diffusion, until it finally ceased all together at the value y = 0. (This can be seen clearly in graphical form on the page following the graph of the results obtained from this experiment.)

Conclusion:

Even though this investigation is titled, 'An Investigation to determine the effect of temperature on the permeability of plant cell membranes', I cannot draw any conclusions linking temperature with the permeability of plant cell membranes, as I have an anomalous result and there is doubt as to whether the other results obtained are in fact accurate and correct.

The graph would indicate that at 55ºC (328ºK) the cell membrane became more permeable and so allowed a greater diffusion of red pigment into the distilled water. It would also indicate that 60ºC (333ºK) is the optimum temperature for proteins in beetroot storage tissue cell membranes to facilitate diffusion, as this is the highest point the line rises to, after which it begins to fall back towards x = 0. After this temperature it would indicate that the permeability of the cell membrane began to decrease as the protein became denatured. This however, is my own theory behind the results obtained. This is not fact or a conclusion. In order to draw any conclusion, the experiment would need to be repeated and accurate result obtained. (See below.)

As I did not have a chance to compare my results with my colleagues, (who also carried out the same experiment,) I could not check whether my results were in any way similar to theirs, or calculate any class averages or class percentage errors. It would also have given me the opportunity to see if any of my colleagues attained the same results as me, and whether or not they had any discrepancies in their results.

However, I believe my results to contain one anomalous reading or discrepancy. In saying this, I should have redone the individual sample that caused a discrepancy in the results, or even repeated the entire experiment, to ensure a fair test ensued. I did want to repeat the experiment again, in order to disprove or prove if the reading was in fact anomalous, however, I did not have any time available.

I am not sure exactly why I did get an anomalous reading. In order to draw any sort of conclusion on why I attained a discrepancy in my results, further experiments would need to be conducted, considering all the possible affecting factors, as well as repeating the experiment itself. I however do not have any more time available and so cannot conduct any more experiments. I can therefore only suggest what may have caused the discrepancy in my results.

Below is a list of the contributing factors which may have caused the discrepancy in the results obtained. They are set out as a number of bullet points, in order to make logical reading and make them easy to see at a glance: (The facts are given in normal text, while the reasoning and explanations are given in italics.)

- While I was conducting the experiment, on several occasions I had trouble picking up the beetroot disc with the tweezers. Consequently this meant that the beetroot disc was in the water for longer than the specified 90 seconds. For example, on one experiment the beetroot disc may have actually remained in the water for approximately 115 seconds, instead of 90 seconds.
  This will have meant that not all the beetroot discs were in the heating water for the same time. This means that the experiment is no longer fair, as more then one variable is being changed at any one time. In terms of the rate of diffusion, this could have meant that the beetroot disc began to diffuse into the heating water, rather than into the test tube, and so this would affect the light intensity reading, since not all the red pigment diffused into the test tube.
  It could affect the temperature at which it was exposed. In those extra seconds that the beetroot disc was still in the heating water, the temperature could have changed off the intended mark, so causing a discrepancy in the results.
- Even though the specimens of beetroot disc were prepared very skilfully and accurately by our Scientific Technicians, there could be a discrepancy in the sizes of the beetroot discs. I.e. not all the beetroot discs were the same size.
  This would affect the surface area of the beetroot disc, which would directly influence the rate of diffusion. This could mean that over the 40 minutes, depending on the exact size of the beetroot discs, some beetroot disc could diffuse at an increased or decreased rate, which would consequently alter the amount of red pigment diffused into the water; and so affect the final results.
- When the samples had been left for 40 minutes in distilled water to allow the diffusion of the red pigment to occur, they had begun to settle. This meant that the test tubes had to be shaken before a

sample could be taken.
Even though the test tubes were shaken, it is possible that they were not all shaken properly, and so a sample was place in the cuvette which contained red pigment which had settled out and so causing differing readings.
Also there was no standard shake for each of the test tubes, which meant that some test tubes could have been shaken more vigorously than others, introducing another variable, causing an unfair test and encouraging misleading results.

- When the test tubes were filled with distilled water, there was no specific measurement to adhere to. Therefore it is highly probable that unequal volumes of distilled water were placed in the test tubes.
If there were unequal volumes of distilled water in the test tubes, this could have affected the rate of diffusion or the spread of the red pigment throughout the distilled water, causing inaccurate readings.
- When the cuvettes were picked up, prior to being placed in the cuvette rack and the colorimeter, even though great care was taken not to touch the sides of the cuvette, it is possible that the sides of cuvette did in fact come into contact with skin or any other debris in the air.
If the cuvette rack was dirty or the cuvettes did come into contact with the skin or any dirt particles in the air, these particles could adhere to the side of the cuvette and so affect and alter the amount of light transmitting through the cuvette, thus altering the reading.
- Prior to the samples being tested, pure water (distilled water) was blanked at 100% transmission. However, it was not blanked on 100% for each of the five samples, with the exception of the anomalous result, as I wanted to be sure the equipment was working properly.
After the each sample had been recorded, the colorimeter should have been blanked onto 100% transmission for distilled (pure) water, to ensure that the results obtained were in fact accurate for each sample, and that the dial had not moved off 100% transmission. (As it had for sample number 3, when I recalibrated the colorimeter.)
- Although it is highly improbable, there could have been a fault in the colorimeter itself, as prior to the experiment when it was energised and tested, it was producing inaccurate readings for another solution which should have read 100%.
As stated, a sample of another solution which should have produced a 100% transmission reading, after being blanked with distilled water at 100% transmission, produced inaccurate and incorrect readings. This could be attributed to the machine not being ready, as it takes 30 minutes to warm up. There were however initial errors, before this problem was finally overcome, so it can not be ruled out, however improbable, that the colorimeter could have in some way affected the readings.
- Finally there is also the chance, that the cuvette were scratched or in some way marked, so that they impaired the beam of light, thus altering the readings.
If the cuvettes were scratched or marked in the slightest manner, and even though such imperfections are not visible to the naked eye, these imperfections could restrict and alter the amount of light transmitting through the sample.

Evaluation:

As stated above in the conclusion there were several inaccuracies in the design of the experiment and at one point the experiment was no longer a fair test. (Size of beetroot discs and vigorousity of shake.) I would therefore given the time and the equipment, repeat this experiment, yet ensuring that at all times the experiment is a fair test and that are variables are constant. (Except the one under investigation - temperature.)

I would therefore try and ensure that the beetroot discs were the same size, (although with limited equipment, this is very difficult to achieve,) by measuring them with a microgauge. I would also measure out solutions using a pipette measured at 25cm3 or 20cm3. Although there will be inaccuracies with the pipette technique, I believe this would still be far more accurate than the methods employed in this experiment. I would also use a water bath to ensure that a specific temperature is adhered to, even though it will mean a delay between adjusting the temperatures. (This could be used to take an average of readings for a sample.) I would have to use tweezers, as I cannot think of another way of retrieving the beetroot discs. I would however work on the principle that if the beetroot disc was in longer than 90 seconds it would be disregarded and another used instead. Finally, in order to ensure the samples were shaken at exactly the same level, I would spin them in a centrifuge at a very low setting.