cell osmotic fragility essay sample

This experiment examines cell membrane permeability, osmosis and membrane voltages; all of which are important in understanding how cells are affected by their environment. The movement of water across membranes is important for cell volume and thus the volume of extracellular compartments. The mechanisms for solute transport are essential in maintaining cell functions and homeostasis. Furthermore, ion transport across membranes generates membrane voltages, which are important in maintaining osmotic balance. Previous related experiments have been conducted, for example de Wit and van Gastel (1969) investigated the relationships between cell age and osmotic fragility. Their findings concluded that younger cells are more resistant to lysis and older cells were comparatively more susceptible to immune haemolysis.

Naccache and Sha'afi' (1973) found that cell structure was a predominant factor in a cell's permeability to solutes. Part 1 sought to measure the movement of water in response the addition of various concentrations of NaCl. It was hypothesised that as the concentration of NaCl increases, then the amount of lysis of the red blood cells would decrease. Part 2 aimed to determine how permeable red blood cell membranes were to various solutes. It was hypothesised that as the lipid solubility of solutes (Kether) increases, as does the permeability coefficient. Part 3 of the experiment aimed to produce and measure diffusion potentials across two different membranes of semi-permeability. It was hypothesised that as the voltage increases, as does the log of the concentration gradient. Method

Methods for Part 1 and Part 2 were performed as described in the SCIE1106 Lab Manual (3). Small changes were made in Part 3 of the experiment.

Data was processed through Microsoft Excel.

Results

Various colours and stages of cell lysis was observed in Part 1. The 3 non-centrifuged test tubes (containing 0. 01M, 0. 09M and 0. 15M of NaCl respectively) were all initially cloudy. The 0. 01M tube was observed to turn almost instantly clear, whilst the 0. 09M tube only became slightly less cloudy. The 0. 15M tube remained opaque. The NaCl concentration ranged from 0. 01M to 0. 15M in the centrifuged test tubes. The tubes were observed to be transparent but showed varying intensities of colour from 0. 01-0. 07M. A diffuse pellet was observed from 0. 07-0. 15M. Figure 1 indicates that haemolysis percentage begins at above 100%, at 0 mM NaCl concentration. A slow decrease can be observed between 0 to 85 mM as the concentration of NaCl increases. A sharp negative decline of percentage haemolysis from 85 to 100 mM is shown, which eventually plateaus when NaCl concentration reaches approximately 110 mM. Part 2. The line of best fit is indicative of the directly proportional relationship between lipid solubility (Kether) and corrected membrane permeability.

Refer to Figure 2. Part 3. The initial reading of 3. 6mV was subtracted from the voltage readings. The final readings can be observed in Figure 3, where the gradients of the red and blue electrodes are near mirrors of each other. The blue gradient starts at 47. 6mV, 0. 01 log concentration, increases to 50. 5mV at 0. 03 log concentration and finally gradually declines to -21. 3mV when the log concentration reached 10. Initially the red electrode started at -56. 7mV, 0. 01 log concentration, increased to -44. 8mV at 0. 03 log concentration but then decreased slightly to -43mV at 0. 1 log concentration.

The red gradient then gradually increases until it reached 20. 8mV when the log concentration reached 10. The blue and red electrode gradients crossed paths at 1mV at log concentration of -1. 3. A trend was observed in the graph; where the higher the dilution, the longer the suspension of electrodes was needed to obtain a relatively stable reading. Discussion

It was hypothesised in Part 1 that as the concentration of NaCl increased, the amount of red blood cell lysis would decrease. The hypothesis was supported by data obtained, as shown in Figure 1. When examining the 0-85mM NaCl concentration, the decrease in percentage haemolysis can be attributed to the various ages of the red blood cells, and thus their different lysis threshold. Younger cells have a higher threshold for lysis (de Wit & van Gastel, 1969) and as the concentration increases then the number of ' younger' cells that lyse decreases as they have a higher resistance to swelling. Alternatively ' older' cells display lyse earlier, as shown in the steep drop from 85-100 mM. It should also be noted that there is no change observed at the 150 mM as the osmolarity is the same as the red blood cells. Percentage haemolysis remains low and constant towards as NaCl concentration increases, as crenation begins.

In Part 2 it was hypothesised that as the lipid solubility of solutes (Kether) increases, as does the permeability coefficient. The hypothesis was supported as shown in Figure 2, whereby both class and group data indicate a directly proportional relationship between Kether and the permeability coefficient. The observed data can be explained as lipid solubility is dependent on the molecule's structure and that molecules containing more polar groups are more lipophobic and thus are less lipid soluble. Glycerol, with a chemical formula of CH2OHCHOHCH2OH, has the lowest Kether due to the presence of three polar hydroxyl groups which give glycerol its high polarity. Propylene glycerol, with a chemical formula of HOCH2CHOHCH3 groups has the highest Kether as it is much less polar than glycerol; containing only two polar hydroxyl groups and a longer carbon chain. Diethylene glycol ((HOHCH2CH2)2O) and ethylene glycol (OHCHCH2CH2CHOH) have similar chemical formulae however the longer carbon chain in diethylene glycol makes it less polar.

There was a substantial difference in the values for the corrected permeability from group to group, which can be explained by the subjective conditions in which the haemolysis time was measured. Errors in the reaction time of experimenters, the distance from the test tube to the eye and the subjectiveness of what a solution looks like when ‘ clear’ along with the exact time at which the solution is ‘ clear’ may have all had an effect on the values obtained by each group. The use of the average value of all group’s data increased reliability however additional tests from each group should be taken in future experiments to increase the reliability of the results. In Part 3, it was hypothesised that as the voltage increases, as does the concentration of the gradient.

The hypothesis was supported as shown in Figure 3 whereby the greater the electrochemical gradient for the particular ion results in a greater rate of ion diffusion (Beilharz, 2014). With respect to their extracellular fluids, cytoplasms of cells are typically have a negative charge. Thus, the varying concentrations of solutions give rise to varying gradients that emphasise ion diffusion down the gradient. The blue electrode initially showed a positive membrane voltage value which indicated its permeability to anions. Conversely, the red electrode displayed a negative voltage value and thus was permeable to cations due to the positive ions moved out of the cell. During this part of the experiment, we were unable to obtain accurate values due to a malfunction in the laboratory equipment. More time should have been allotted as the stabilisation for each reading took a substantial chunk of time (approx. 20 minutes). Appendices

Figure 1. Osmotic fragility curve showing the percentage haemoysis of undiluted rabbit red blood cells in different concentrations of NaCl (mM) solution. It was assumed that 100% haemylosis occurred in the red blood cells of 10 mM NaCl solution. Figure 2. Demonstration of the relationship between lipid solubility (Kether) and corrected permeability values of various glycol derivative solutes for rabbit red blood plasma membranes. The permeability coefficient is graphed on a log-log scale against Kether. The corrected permeability was calculated using the mean values of the class data. A line of best fit has also been included. It is shown that there is an increase in the membrane potentials with increasing concentration gradient. Figure 3. Graph of the measurement of the corrected membrane voltages (mV) between two electrodes with differing permeability to positive and negative ions when placed in different dilutions of standard Thomson solutions. The blue electrode and red electrode diffusion potentials (mV) are plotted in their respective colour. It is shown that there is an increase in membrane potentials with increasing concentration gradient.

References

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