Membrane permeability of rabbit red blood cells biology essay

In this experiment, we look at the swelling and lyses of red blood cells (RBCs), when in a very hypotonic environment. Other key concepts include, the measure of resistance of the red blood cells to expand and lyse, due to the influx of water into the cell. We examine the permeability of the cell plasma membrane to various solutes, using haemolysis as a marker of solute penetration. We can also understand how the phenomenon of osmosis works, how it stabilizes cell volume and turgor pressure via the movement of water in and out of the cell down its aquaporins . Lastly, we can take a look how other factors determine permeability of a solute.

By principle, " Osmosis the movement of water from a region of lower solute concentration to a region of higher solute concentration across a semi-permeable membrane." Animal cells will swell when they are placed in a hypotonic solution (i. e., one in which the concentration of solutes is lower than it is in the cytosol). Water enters them by osmotic flow. Rupture of the plasma membrane by a flow of water into the cytosol is termed osmotic lysis. Immersion of all animal cells in a hypertonic solution (i. e., one in which the concentration of solutes is higher than it is in the cytosol) causes them to shrink as water leaves them by osmotic flow. Consequently, it is essential that animal cells be maintained in an isotonic medium, which has a solute concentration close to that of the cell cytosol.

For the 1st experiment, osmotic fragility is explored by suspending the RBC's in NaCl solutions of different concentrations, and observing how many cells swell, rupture and release haemoglobin which can. The concentrations of NaCl solution used were10mM 30mM, 50mM 85mM, 90mM, 100mM , 110Mm and 150 mM.

For the 2nd part, permeability of RBC's to various solutes were measured by suspending the RBCs in NaCl solution of standard concentration and measuring haemolysis time, which is the time taken from the addition of the test solute to the appearance of light. The various test solutes used were urea, methyl urea, dimethylurea, methylurea, thiourea, ethylene glycol, propylene glycol, triethylene glycol, diethylene glycol and water. The faster the solute enters the cells, the quicker the cells lyse, thus the shorter the haemolysis time. I propose that the percentage haemolysis of the RBCs will deacrease with increasing concentration of NaCl solution.

Materials and Methods

SCIE1106: Molecular Biology of the Cell LABORATORY MANUAL. Refer to pages: 44-47 for experiment 1, and pages 46-47 for experiment 2.

Results

For experiment 1, at (time zero), all the solutions were light pink and turbid. After five minutes, as illustrated by table1, the NaCl solutions of concentrations 10mM- 70Mm became clear; NaCl solutions of (80mM-150mM) still remained turbid. The solutions all intensity of the pink colour decreased with increasing concentration. Centrifugation on the tubes to clear floating cells, which would interfere with spectrophotometric measurements, was carried out for five minutes, two layers were formed. A clear pink liquid (supernatant) and reddish RBCs settled at the bottom of the tube (pellet). Table 2 indicates that the 10Mm tube did not the pellet at the bottom, while the 30mM-50mM had pellets. Absorbance of the supernatant 550nm indicated the 10Mm tube had had the highest absorbance, while the 150 mM tube had lowest absorbance. The readings had a wide range from 0. 040to 0.

548. Graphs of concentration versus absorbance, and concentration versus percentage heamolysis were plotted, with a visibly decreasing trend for both graphs. For both graphs, there was a steep drop and eventually becomes gentle.

For experiment 2, with reference to table 6,

Discussion and Conclusions

For experiment A, the turbidity of the solutions was due to creanation. Increasing the concentration of the bathing solution, resulted in the inside of the cell having a higher water potential, thus causing the influx of water out of the cell, than water moving in. This decreased the amount of the amount of RBCs that lysed, which turn decreased percentage haemolysis. Turbidity increased with increasing concentration of NaCl. The 10nm tube did not contain any pellet and it this meant that most of the RBCs had lysed, therefore the percentage lysis at 10mM concentration is 100%.

For the second experiment, we can establish that haemolysis time and molecular weight are linearly related or proportional. The greater the MW, the bigger the molecule is, making it harder to permeate the cell membrane. Conversely, the smaller molecules, can permeate the membrane easily. We can roughly deduce the size of the molecule by it’s MW. Haemolysis for urea compounds increase with lipid solubility. Non-polar molecules have greater solubility in lipids than in water. Haemolysis time for the glycol compounds increased in with increasing lipid solubility. Non-polar and hydrophibic substances can pass through the lipid bilayer easily compared to polar or hydrophilic molecules.

By reference to a similar experiment that was conducted, in which erythrocytes of different mammalian species, were immersed in solutions of different concentrations of NaCl and a graph of % haemolysis versus % indicated that % haemolysis decreases with increasing concentration of NaCl. This confirms the hypothesis proposed as conforms to experimental results

factors affecting permeability are molecular size of a solute, degree of ionization, as permeability decreases with increasing ionization.

Results: Tables And Graphs

Experiment 1

Table 1: Appearance of RBC/ NaCl mixtures at T= 5minutes

Concentration of NaCl/mM

10

30

50

70

80

85

90

100

110

150

Appearance

clear

clear

clear

clear

cloudy

cloudy

cloudy

cloudy

cloudy

cloudy

Table2: Appearance of RBC/NaCl after centrifugation for 5 minutes.

Concentration of NaCl/mM	Appearance
10	clear
30	clear, pellet
50	clear, pellet
70	clear, pellet
80	clear, pellet
85	clear, pellet
90	clear, pellet
100	clear, pellet
110	clear, pellet
150	clear, pellet

clear, pellet

clear, pellet

Table3: Absorbance of Supernatants at 550nm

Concentration of NaCl/mM

10

30

50

70

80

85

90

100

110

150

Absorbance/ ABS

0. 548

0. 422

0. 359

0. 106

0. 108

0. 109

0. 085

0. 081

0. 067

0. 040

Graph1: Absorbance/ ABS at 550nm against concentration of NaCl/ mM. Absorbance at 10 mM is 0. 548 (100%).

Table 4: Table of percentage haemolysis against concentration of NaCl solutions.

Concentration of NaCl/mM

10

30

50

70

80

85

90

100

110

150

Percentage haemolysis

100

77

65. 5

19. 3

19. 7

19. 9

15. 5

14. 8

12. 2

7. 3

Graph 2:`Percentage Haemolysis/ % VS Concentration of NaCl/mM . Percentage haemolysis at 10mM is 100%.

Class Results (Lab Group A) Experiment 1: Average

Table 5: Average Absorbance & Percentage Haemolysis obtained by combining class results.

Concentration of NaCl/mM

10

30

50

70

80

85

90

100

110

150

Absorbance/ ABS

0. 394

0. 366

0. 394

0. 222

0. 123

0. 126

0. 114

0. 096

0. 084

0. 123

Percentage Haemolysis

100

95. 4

100. 6

56. 2

32. 12

28. 5

32. 2

27. 6

24. 23

12. 21

Graph 3: This is the graph of Average Absorbance against Concentration of NaCl.

Graph4: This is the graph of Average Percentage Haemolysis against Concentration of NaCl.

Experiment 2

Table6: Molecular weights and individual results time taken for the solutes to enter and lyse the cells.

Test Solute

Molecular Weight

Haemolysis time

(s)

Urea

60

7

Methylurea

74

11

Dimethylurea

88

951

Thiourea

76

419

Ethylene Glycol

62

48

Propylene Glycol

76

59

Triethylene Glycol

150

270

Diethylene Glycol

106

91

Glycerol

92

223

Water

18

17

Graph 5: The compounds can be split into two groups. Urea Groups and Glycol groups. From which a graph of Haemolysis time / (s) vs. Molecular Weight Can be plotted. This is the graph of Haemolysis Time /(s) Vs Molecular Weight of Urea Group Compounds.

Graph 6: Graph of Haemolysis Time(s) VS Molecular Weight of Glycol Group Compounds.

Table 7: Table with corrected permeability values. Corrected P-values were obtained by taking the square root of the molecular weight and dividing it by the haemolysis time.

Test Solute

Molecular Weight

KÐμthÐμr

Haemolysis Time (s)

Correct P

Urea

60

0. 00047

7

1. 107

Methylurea

74

0. 0012

11

0. 782

Dimethylurea

88

0. 0116

951

0. 00986

Thiourea

76

0. 0063

419

0. 0208

Ethylene Glycol

62

0. 0053

48

0. 164

Propylene Glycol

76

0. 035

59

0. 148

Triethylene Glycol

150

0. 0031

270

0. 0454

Diethylene Glycol

106

0. 004

91

0. 113

Glycerol

92

0. 00066

223

0. 0430

Water

18

0. 0025

17

0. 250

Test Solute

Molecular weight

(MW)

Haemolysis Time/ s

Kether

MW1/2

MW1/2/ Haemolysis time)

Log10(MW1/2/ Haemolysis time)

Log10(Lipid solubility)

Urea

60

7

0. 00047

7. 75

1. 107

0. 044148

-3. 3279

Methylurea

74

11

0. 0012

8. 60

0. 782

-0. 10679

-2. 92082

Dimethylurea

88

951

0. 0116

9. 38

0. 00986

-2. 00612

-1. 93554

Thiourea

76

419

0. 0063

8. 72

0. 0208

-1. 68194

-2. 20066

Table 8: Table of Urea Compounds with log10 (Corrected Permeability) and log10 (Lipid Solubility). This returns the base- 10 logarithm of a number.

Graph 7: From the above values a graph of Log 10 (Corrected permeability) against log10 (Lipid Solubility) can be plotted. This is the graph for the Urea Family.

Table 8: Table of Glycol Compounds with log10 (Corrected Permeability) and log10 (Lipid Solubility). This returns the base- 10 logarithm of a number.

Test Solute	**Molecular weight (MW)**	**Haemolysis Time/ s**	**Kether MW1/2**	**MW1/2/ Haemolysis time)**	**Log10(MW1/2/ Haemolysis time)**	**Log10(Lipid solubility)**
Ethylene Glycol	62	48	0. 0053	7. 87	0. 164	-0. 78516

-2. 27572

Propylene Glycol

76

59

0. 035

8. 72

0. 148

-0. 82974

-1. 45593

Triethylene Glycol

150

270

0. 0031

12. 25

0. 0454

-1. 34294

-2. 50864

Diethylene glycol

106

91

0. 004

10. 3

0. 113

-0. 94692

-2. 39794

Glycerol

92

223

0. 00066

9. 59

0. 0430

-1. 36653

-3. 18046

Graph 8: From the above values a graph of Log 10 (Corrected permeability) against log10 (Lipid Solubility) can be plotted. This is the graph for the Glycol Family.

Class Results(Lab Group A) Experiment 2: Average

Table10: This is the table of the average obtained from the class results.

Test Solute

Molecular Weight

Average Heamolysis time

(s)

Kether

MW1/2

MW1/2/ Haemolysis time

Log10(MW1/2/ Heamolysis time)

Log10(Lipid solubility)

Urea

60

9. 45

0. 00047

7. 75

0. 820

-0. 08619

-3. 3279

Methylurea

74

12. 65

0. 0012

8. 60

0. 680

-0. 16749

-2. 92082

Dimethylurea

88

177. 4

0. 0116

9. 38

0. 0529

-1. 27654

-1. 93554

Thiourea

76

387. 85

0. 0063

8. 72

0. 0225

-1. 64782

-2. 20066

Ethylene Glycol

62

42. 6

0. 0053

7. 87

0. 185

-0. 73283

-2. 27572

Propylene Glycol

76

74. 5

0. 035

8. 72

0. 117

-0. 93181

-1. 45593

Triethylene Glycol

150

197. 3

0. 0031

25

0. 0621

-1. 20691

-2. 50864

Diethylene Glycol

106

145. 2

0. 004

10. 3

0. 0709

-1. 14935

-2. 39794

Glycerol

92

240. 55

0. 00066

9. 59

0. 0399

-1. 39903

-3. 18046

Water

18

24. 7

0. 0025

4. 24

0. 172

-0. 76447

-2. 60206