EXPERIMENT (2)

2. Haemolysing Agents& Detection of blood

2.1 Introduction

2.1.1 Haemolysis (from the Greek Hemo: meaning blood, - lysis, meaning to break open):

It is the breaking open of red blood cells and the release of hemoglobin and the red cell contents into the surrounding fluid (plasma). The concentration of potassium inside red blood cells is much higher than in the plasma and so elevated potassium is usually found in biochemistry tests of hemolysed blood. Conditions that can cause hemolysis include: Immune reactions, Infections, Medications. Toxins and poisons.

2.1.2 Osmotic Pressure

Diffusion of water across a membrane – osmosis – generates a pressure called osmotic pressure.

If the pressure in the compartment into which water is flowing is raised to the equivalent of the osmotic pressure, movement of water will stop.

2.1.3 Isotonic Solution

A solution that has the same salt concentration as the normal cells of the body and the blood, having equal osmotic pressure.As opposed to a hypertonic solution or a hypotonic solution. Solutions which are isotonic with blood, such as sodium chloride 0.9%, have the same osmotic pressure as serum and they do not affect the membranes of the red blood cells. In hospitals, intravenous fluids are isotonic (iso = equal or even, and tonic = tonicity). Since the cell membranes of red blood cells are selectively permeable (allowing for diffusion of solvent, when the concentration of solvent is greater on one side), equilibrium allows the red blood cells to retain their shape.

2.1.4 Hypotonic Solution

It has less than normal tension, the concentration of solute. In a hypotonic solution, there is a lower concentration of solute outside a cell, creating an environment with lower osmotic pressure than what is contained within the cell. For example, a hypotonic sodium chloride solution is less concentrated that isotonic or hypertonic solutions. If an IV solution was hypotonic (less solvent = more dilute), there would be less pressure on the red blood cells. The red blood cell would actually swell, in an attempt to equalize the concentration or tension (known as osmotic pressure) of solutes and solvents. As a result, the red blood cells would hemolyze or burst.

2.1.5 Examples of Hypotonic Solutions

1- 0.45% NaCl (half – normal saline solution); since normal saline is 0.9% NaCl, any solution less than 0.9% is hypotonic.
2- Dextrose 2.5% in water.
3- Dextrose 2% in water.

2.1.6 Hypertonic Solution

A solution that has a lower water potential and a correspondingly higher osmotic pressure than another solution. In a hypertonic solution, the plasma membrane of a red blood cell would separate and pull away from the cell membrane.

2.1.7 Examples of Hypertonic Solutions

1.2% NaCl (more than concentration in normal saline solution); since normal saline is 0.9% NaCl, any solution higher than 0.9% is hypertonic.

The next table indicates the changes on the nature of the red blood cells.

No.	Type of Solution	Changes on Red Blood Cell
1	**Isotonic Solution**	the cells were diluted in serum: Note the beautiful biconcave shape of the cells as they circulate in blood.
2	**Hypotonic Solution**	Most have swollen so much that they have ruptured, leaving what are called red blood cell ghosts. In a hypotonic solution, water rushes into cells.
3	**Hypertonic Solution**	A concentrated solution of NaCl was mixed with the cells and serum to increase osmolarity; water has flowed out of the cells, causing them to collapse.

2.2 Procedure

2.2.1 Materials

The packed Red Blood cells prepared from part 1 suspended in saline solution.

1- Saline Solution (0.9% sodium chloride NaCl) as an isotonic solution.
2- Sodium Chloride Solution 0.45%, as a hypotonic solution.
3- Sodium Chloride Solution 1.2% as a hypertonic solution.
4- Sucrose Solution 6%.
5- Sodium Hydroxide Solution 0.1 M.
6- Hydrochloric Acid Solution 0.1 M.
7- Water bath (variable temperature).
8- Dry clean test tubes.
9- Centrifuge.

2.2.2 Method

Into seven dry clean test tubes (A, B, C, D, E, F, G), pipette 3 drops of the suspended RBC"s in Saline solution, and add to each tube as indicated the following table:

	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F	Tube G
NaCl 0.45%	5 ml						
NaCl 1.2%		5 ml					
Sucrose 6%			5 ml				
NaOH 0.1 M				3 Drops			
HCl 0.1 M					3 Drops		
Dis. Water						5 ml	
NaCl 0.9%				5 ml	5 ml		5ml Heat slowly in the water bath and note the temperature at which haemolysis started.
Wait 30 minutes. Observe whether Haemolysis has taken place, i.e. whether the colour of the solution is changed or Centrifugation may be of help.							
Observation							
Conclusion							