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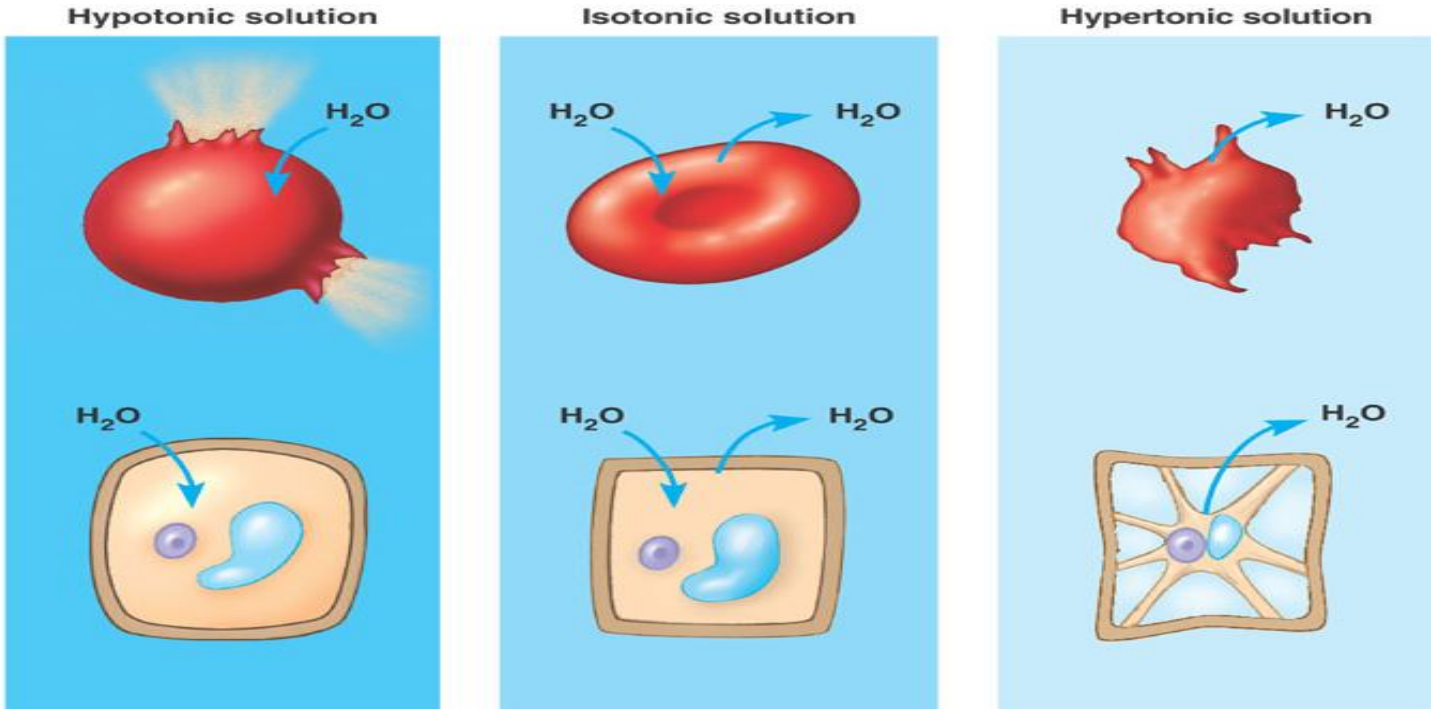
Experiment 3

Haemolysing Agents & Detection of blood



Objectives

1. To find the nature of substances that causes haemolysis of RBCs.
2. To detect the presence of blood.

Haemolysis

- Hemo: meaning blood.
- - lysis: meaning to break open.
- **Haemolysis** :
- 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 .

so elevated potassium is usually found in biochemistry tests of **hemolysed blood**.

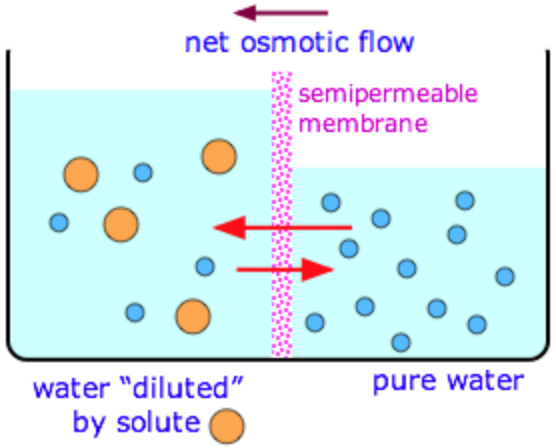
Conditions that can cause hemolysis include:

Immune reactions, Infections, Medications. Toxins and poisons.

Osmotic Pressure

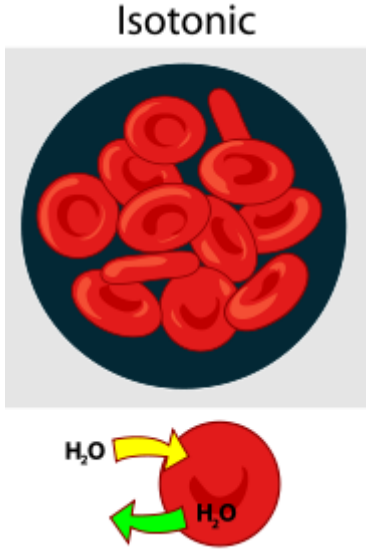
Diffusion of water across a membrane – osmosis – generates a pressure called osmotic pressure

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.



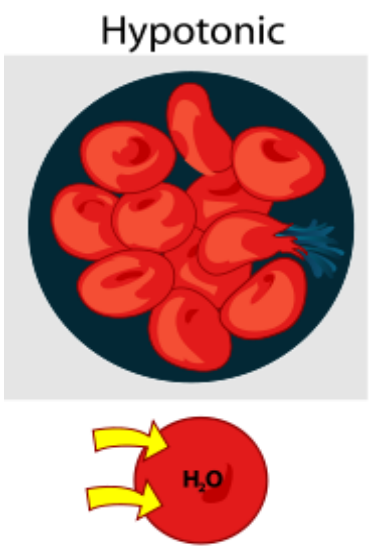
Semipermeable membranes and osmotic flow

Isotonic Solution



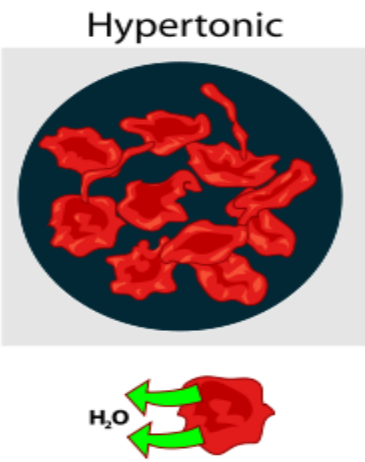
- A solution that has the same **salt concentration** as the normal cells of the body and the blood, having equal osmotic pressure.
- Solutions which are isotonic with blood, such as **sodium chloride 0.9%,** have the same osmotic pressure as serum and they do not affect the membranes of the red blood cells.
- In hospitals, **intravenous fluids** are isotonic (iso = equal or even, and tonic = tonicity).

Hypotonic Solution



- 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
- **If an IV solution was hypotonic**, 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 of solutes and solvents.
- As a result, the red blood cells would hemolyze or burst.

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.

- **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.

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.

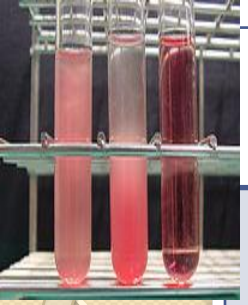
7- Water bath (variable temperature).

8- Dry clean test tubes.

9- Centrifuge.

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		5 ml 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. Note that the hemolyzed sample is transparent, because there are no cells to scatter light.							
Observation							

How to calculate the osmolarity of the Sol.

- Blood plasma is = 0.308 Osmolar
- For 0.9% NaCl
- **O = M ×(no. of dissociation particles)**

M= no. of moles/ volume (L)

no. of moles=weight/ Molecular weight

So,

weight= M x V x Molecular weight=

0.9= M x 0.1 x 58.4 =

M= 0.154 M

O = 0.154 x 2 = 0.308 Osmolar (isotonic: same osmolarity of plasma)

Detection of Blood by BENZIDINE TEST

- These methods depend on the fact that the haem group of haemoglobin possesses a peroxidase-like activity which catalyses the breakdown of hydrogen peroxide.
- The oxidising species formed in this reaction can then react with a variety of substrates to produce a visible colour change.
- Among substrates in common use are benzidine

- It is often necessary to detect the presence of small quantities of blood in urine, stomach contents etc.
- Minute amounts of blood in presence of peroxide catalyze the oxidation of benzidine giving a blue colour.
- However, the test is not specific for blood as peroxidases present in milk, potatoes and pus, as well as the ions of Fe+3, Cu+2 and K+1 will give false positive results.

Procedure

- Materials:

1- Red Blood cells suspended in saline solution.

2- Benzidine solution 3% in glacial acetic acid (freshly prepared).

3- Hydrogen peroxide solution 6% (freshly prepared).

4- Boiling water bath.

- 3ml of suspended blood cells solution is boiled in water bath for 3 minutes and then cool it under tap water.
- Add 2 ml of benzidine solution, followed by 1 ml of hydrogen peroxide solution. **A blue color is obtained.**

- The general principle is that:
- if the test is negative, blood is absent.

But

- if the test is positive, blood is probably, not definitely present.
- For this reason the tests are often described as "**presumptive**" tests.

Thank you