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BCH 471

Experiment (4)

Haemolysing Agents & Detection of blood

OBJECTIVES

- ✓ To find the nature of substances that cause haemolysis of RBCs.
- ✓ To detect the presence of blood.

HAEMOLYSIS

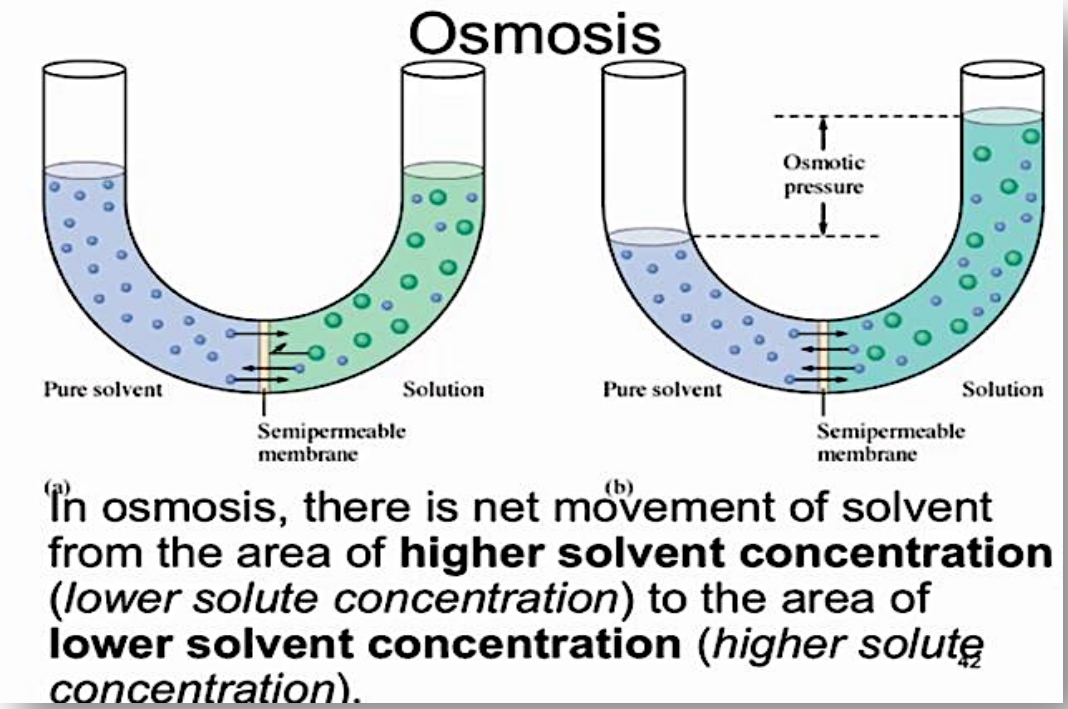
- It is the breaking open of RBCs and the release of hemoglobin and the red cell contents into the surrounding fluid (plasma).
- The concentration of potassium inside red blood cells is much higher than in the plasma → so elevated potassium is usually found in biochemistry tests of hemolysed blood.
- Haemolysis may occur in:
- Vivo:
  - *Causes:* Immune reactions, Infections, Medications, Toxins and poisons, or by a defect in the RBC memebrane.
- Vitro:
  - *Causes:* Placing RBCs in a hypotonic solution.

IMPORTANCE OF HAEMOLYSIS PRACTICALLY

- **Breaking down RBCs to release their content is often necessary in biochemistry for:**
  - Estimation of heamoglobin.
  - To obtain erythrocytes-free preparation of leukocytes and platelets.

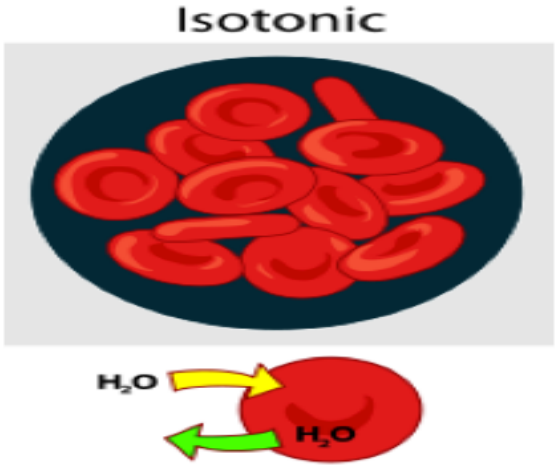
OSMOTIC PRESSURE

- Osmosis : It is the diffusion of water across a selectively preamble membrane into a region of higher solute conc., once an equilibrium is reached the flow of water stops .
- Osmotic pressure : It is the pressure that generates from osmosis .



ISOTONIC SOLUTION

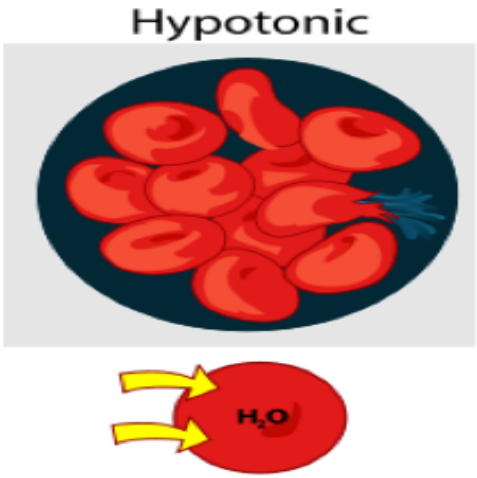
- It is a solution that has the same salt concentration as the normal cells of the body and the blood, having equal osmotic pressure.
- For example: 0.9% NaCl solution, have the same osmotic pressure as serum → it does not affect the membranes of the RBCs.
- In hospitals, intravenous fluids are isotonic.



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.
- As a result, the RBCs would hemolyze or burst.

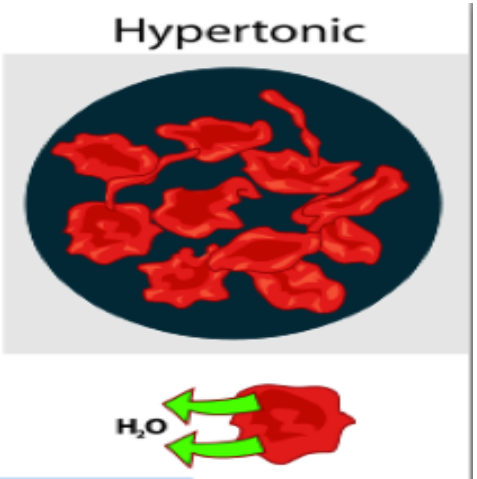
- Examples of Hypertonic Solutions
  - 0.45% NaCl (Lower concentration than normal saline 0.9%NaCl)
  - Distilled water
  - Dextrose 2 or 2.5% in water.



HYPERTONIC SOLUTION

- In a hypertonic solution, there is a higher concentration of solute outside a cell, creating an environment with higher osmotic pressure than what is contained within the cell.
- As a result, the RBCs would shrink.

- Example of Hypertonic Solutions
  - 1.2% NaCl (higher concentration than normal saline 0.9%NaCl)



HOW TO CALCULATE THE OSMOLARITY OF A SOLUTION

For example: Calculate the osmolarity of NaCl ?

Osmolality of RBC = 0.308 Osmolar

O = M × no. of dissociation particles

$NaCl \rightarrow Na^+ + Cl^-$ (no. of dissociation particles = 2)

M= no. of moles/ volume (L)

0.308 = M x 2 → M = 0.154 M

**To calculate in w/w% expression

M = no. of moles / V (in L)

no. of moles= weight/ Molecular weight So,

weight= M x Molecular weight (in 1000 ml)

= 0.154 x 58.5 = 9 g

9 g → 1000 ml

? g → 100 ml

w/w% of NaCl = (9 x 100) / 1000 = 0.9% → isotonic

METHOD

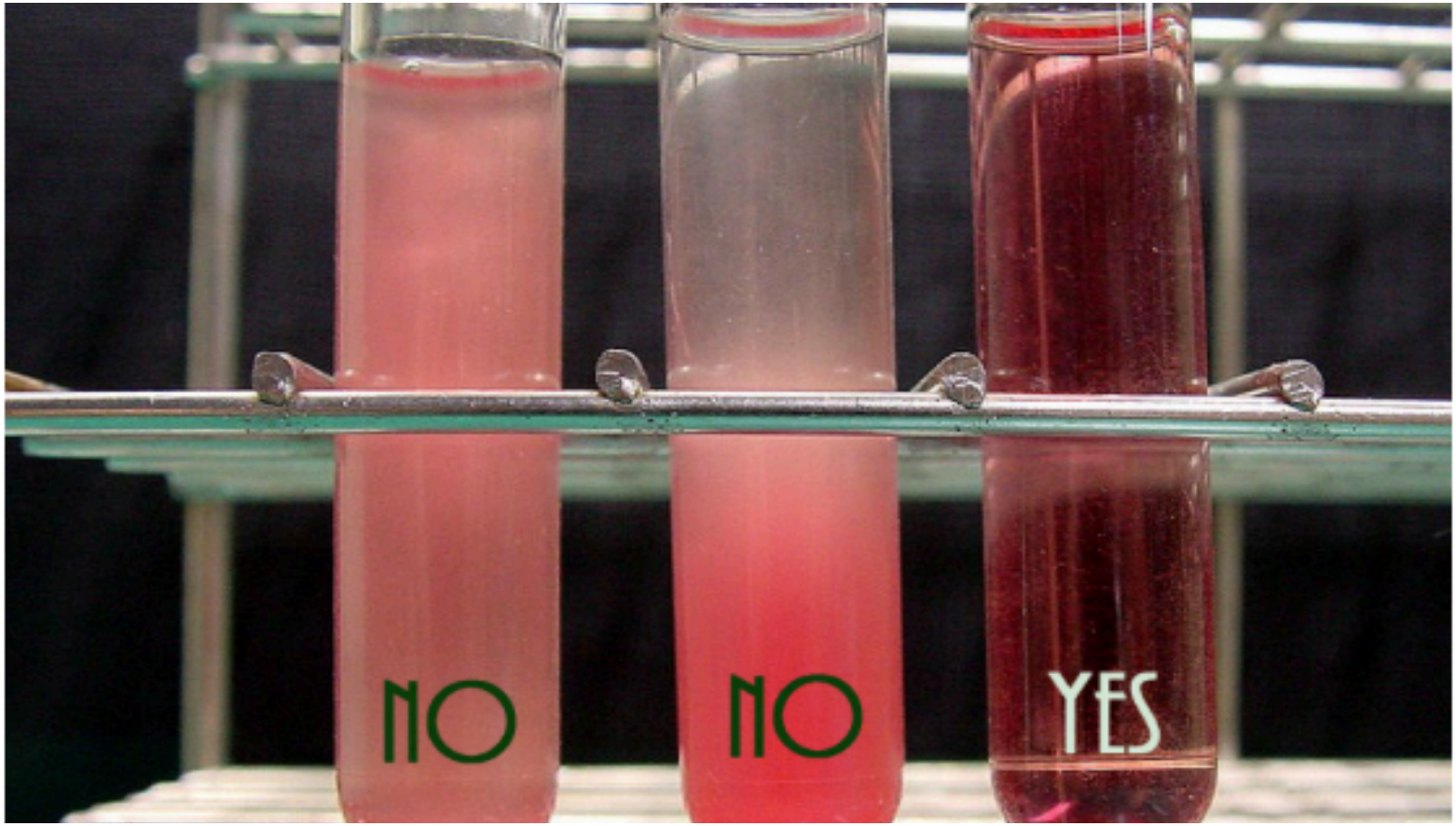
- Into 6 dry clean test tubes (A, B, C, D, E, F), pipette 3 drops of the suspended RBC's in saline solution, and add to each tube as indicated the following table:

	A	B	C	D	E	F
NaCl 0.45%	5ml					
NaCl 1.2%		5ml				
Sucrose 6%			5ml			
NaOH 0.1M				3drops		
HCl 0.1M					3drops	
Dis. H2O						5ml
NaCl0.9%				5ml	5ml	

- Wait 30 minutes.
- Observe whether Haemolysis has taken place

Mwt: NaCl= 58.5 Sucrose= 342.3

RESULTS



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

DETECTION OF BLOOD BY BENZIDINE TEST

- Principle:
  - This method depend on the fact that the haem group of haemoglobin possesses a peroxidase-like activity which catalyses the breakdown of hydrogen peroxide (H_2O_2).
  - The oxidising species formed in this reaction can then react with benzidine giving a blue color.

- It is often necessary to detect the presence of small quantities of blood in urine, stomach contents etc.
- The test is not specific for blood because peroxidases are present in milk, potatoes and pus, as well as the ions of Fe^{+3}, Cu^{+2} and K^{+} will give false positive results..

METHOD

- 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.

NOTE

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".*