Lab#4
220 BCH

Hemolysing Agents & Detection of Blood

Objective

1. Study the effect of different tonicity solutions on RBCs.

2. To detect the presence of blood in a sample.

Blood Hemolysis

- Hemolysis, (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)

- Hemolysis may occur in vivo or in vitro.

Hemolysis in Vivo

- Conditions that can cause hemolysis include :

- Immune reactions ،
- Infections ،
- Medications .
- Toxins and poisons.

Hemolysis in vitro,

- Placing RBCs in a hypotonic solution.
- Improper technique during blood collection (eg.incorrect needle size)
- pH imbalance (addition acid or base).

- In this lab blood hemolysis will be done by using hypotonic solutions and pH imbalance.

When blood Hemolysis should be done?

Breaking down RBCs to release their content is often necessary for biochemistry,

- For Estimation of hemoglobin.
- To obtain erythrocyte-free preparation of leukocyte and platelet.

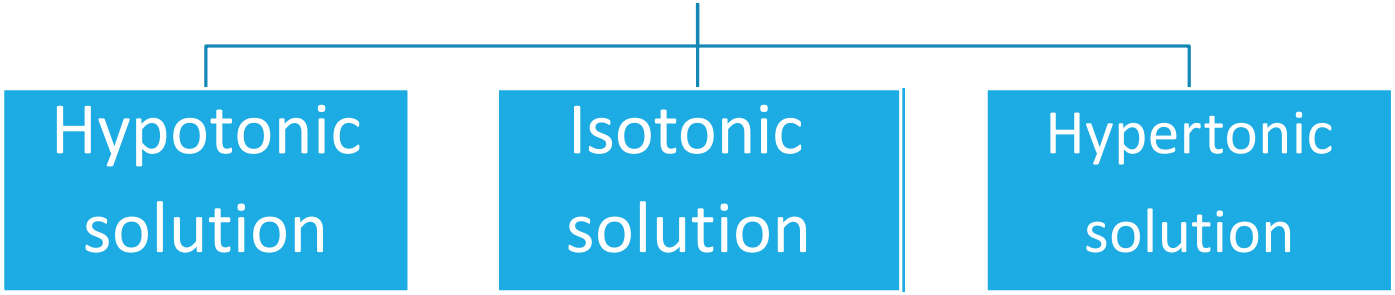
Osmosis and Osmotic Pressure

Osmosis: It is the diffusion of water across a selectivity preamble membrane into a region of higher solute concentration. Once an equilibrium is reached the flow of water stops.(to equalize the solute concentrations on the both sides).

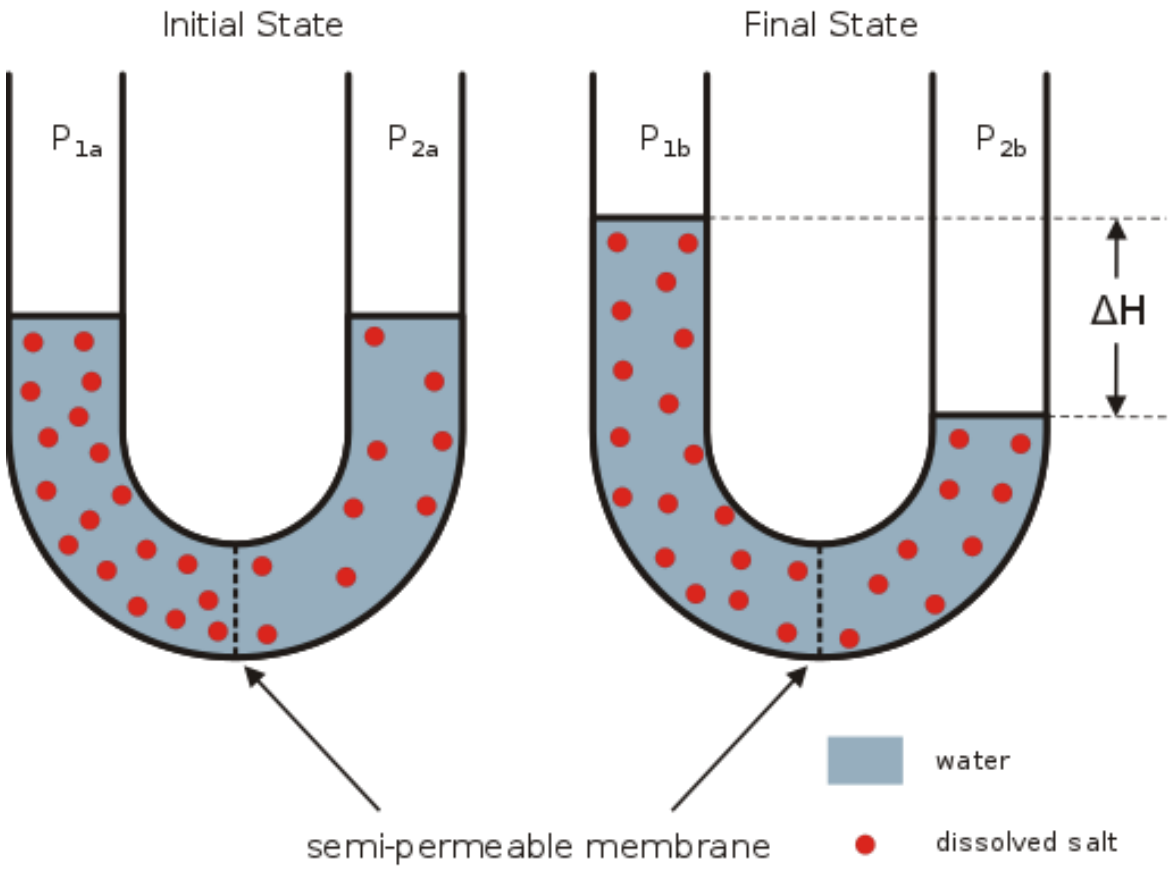
Osmotic pressure: Diffusion of water across a membrane – osmosis – generates a pressure called osmotic pressure.

Tonicity: the concentration of a solution as compared to another solution.

There are three main classifications of tonicity:

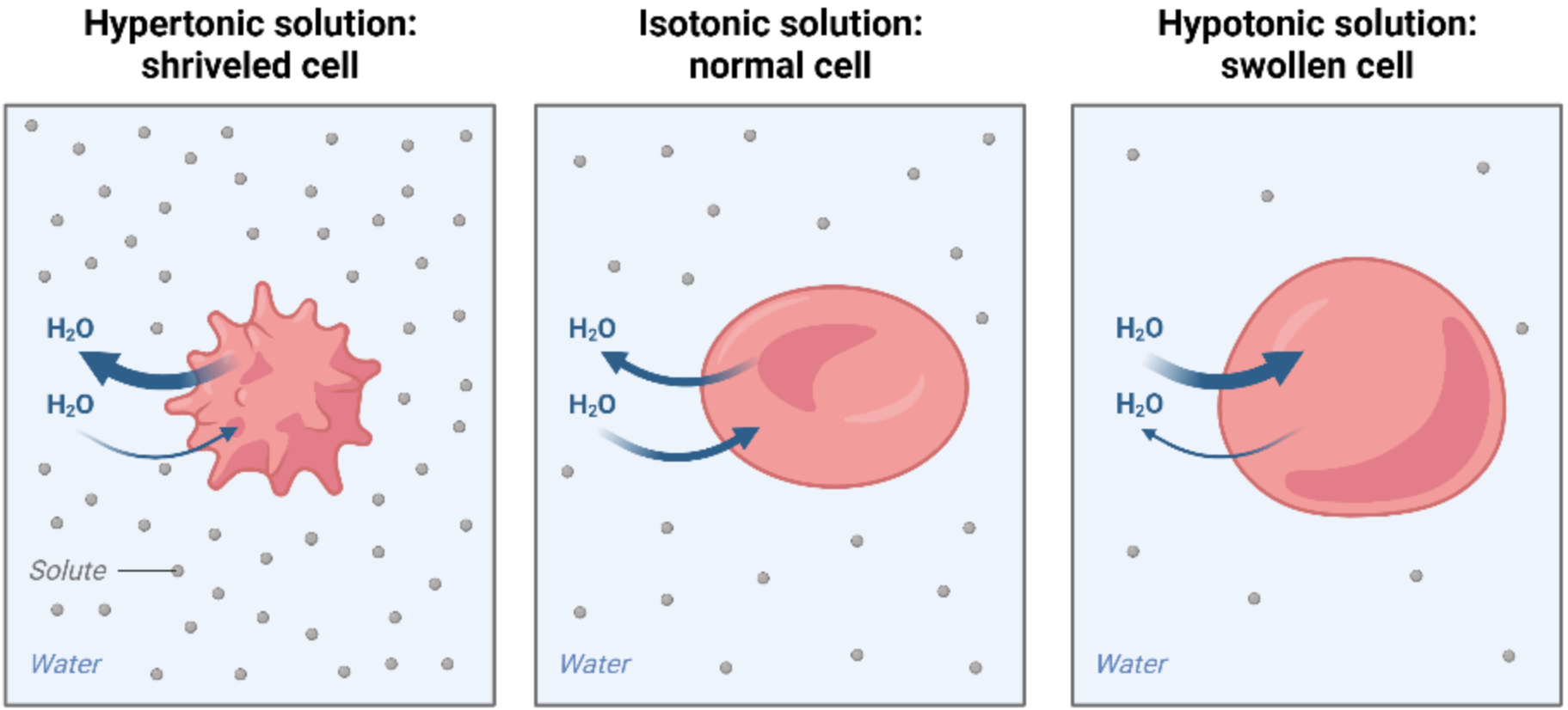


Water passing through a semi-permeable membrane



https://en.wikipedia.org/wiki/Osmosis#/media/File:Osmose_en.svg

Osmoregulation in Red Blood Cells

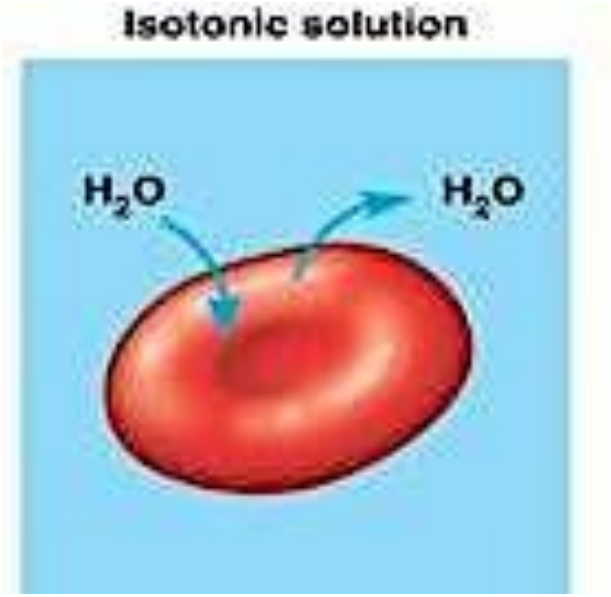


Cell in Isotonic solution

-in this solution, same solute concentration inside and outside the cell. Nothing will affect the cell.

-Example of Isotonic solution is **sodium chloride 0.9%,** have the same osmotic pressure as serum and they do not affect the membranes of the RBCs.

-In hospitals, intravenous fluids are isotonic.

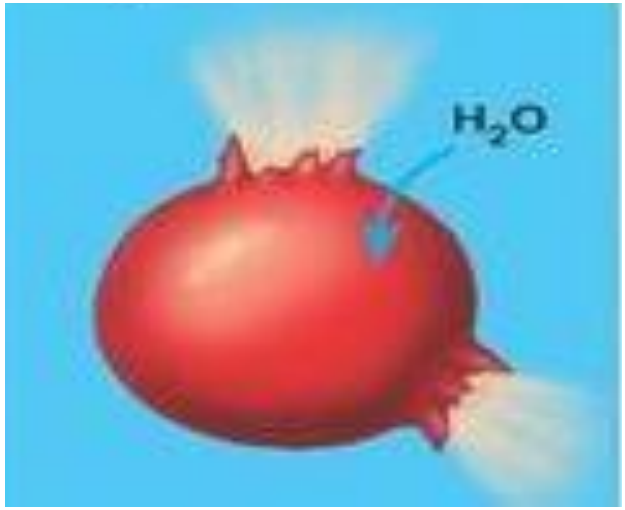


Solute inside the cell = Solute outside the cell

Cell in Hypotonic solution

- In a hypotonic solution, there is a lower concentration of solute outside a cell, than in the inside, causing the flow of water molecules to inside the cell leading to its burst.

- The RBCs will burst or hemolyzed.

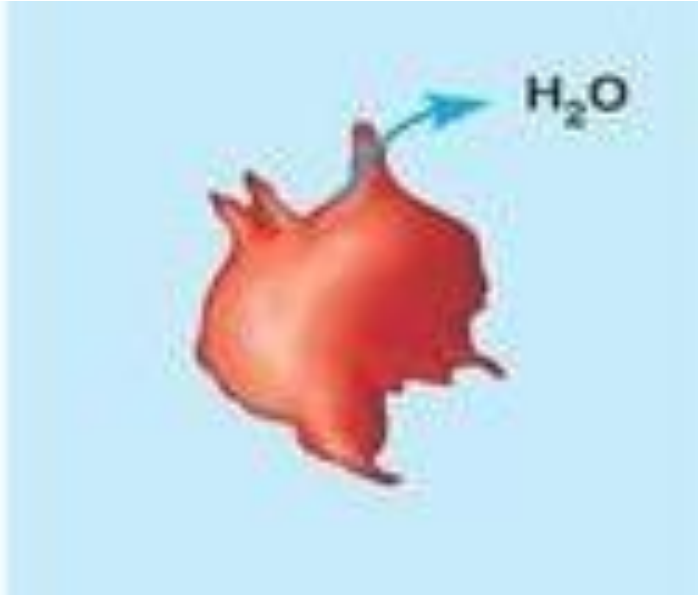


- Any concentration of NaCl that is lower than 0.9%, will be considered hypotonic for cells.

Solute inside the cell > Solute outside the cell

Cell in Hypertonic solution

In a hypertonic solution, there is a higher concentration of solute outside a cell, than in the inside, causing the flow of water molecules to outside of the cell leading to its shrink.



- The RBCs will shrink.

- Any concentration of NaCl that is higher than 0.9%, will be considered hypertonic for cells

Solute inside the cell < Solute outside the cell

How to calculate the concentration of an isotonic solution of a specific substance:

For example, you want to know the concentration of NaCl that will make an isotonic solution.

Knowing the osmolarity of RBC = 0.308 Osmolar

First: calculation of Molarity [M] of NaCl, which will give the isotonic solution:

Osmolarity of the blood = [M] x no. of dissociation partials

dissociation partials of NaCl → $Na^+ + Cl^-$ (no.of dissociation particles=2) so,

$$0.308 = M \times 2 \rightarrow$$

$$M = 0.154\ M$$

Second: calculation of the wight of NaCl in grams needed to prepare the isotonic solution in 100 ml total volume (because you want it as %), after determine the no. of moles.

A. Finding the no. of moles:

[M] = mole / Volume in L

No.of moles = 0.154 x 0.1

No.of moles = 0.0154 moles

B. Wight (Wt) of NaCl in grams :

No.of moles = Wt in g / molecular weight

Wt in g = 0.0154 moles x 58.5 = 0.9 g

So, 0.9 g of NaCl dissolved in 100 ml total volume
Is what will make an isotonic solution.

Method:

Into six dry clean test tubes (A, B, C, D, E, F), add 1 drop of blood sample, after adding the following to each tube:

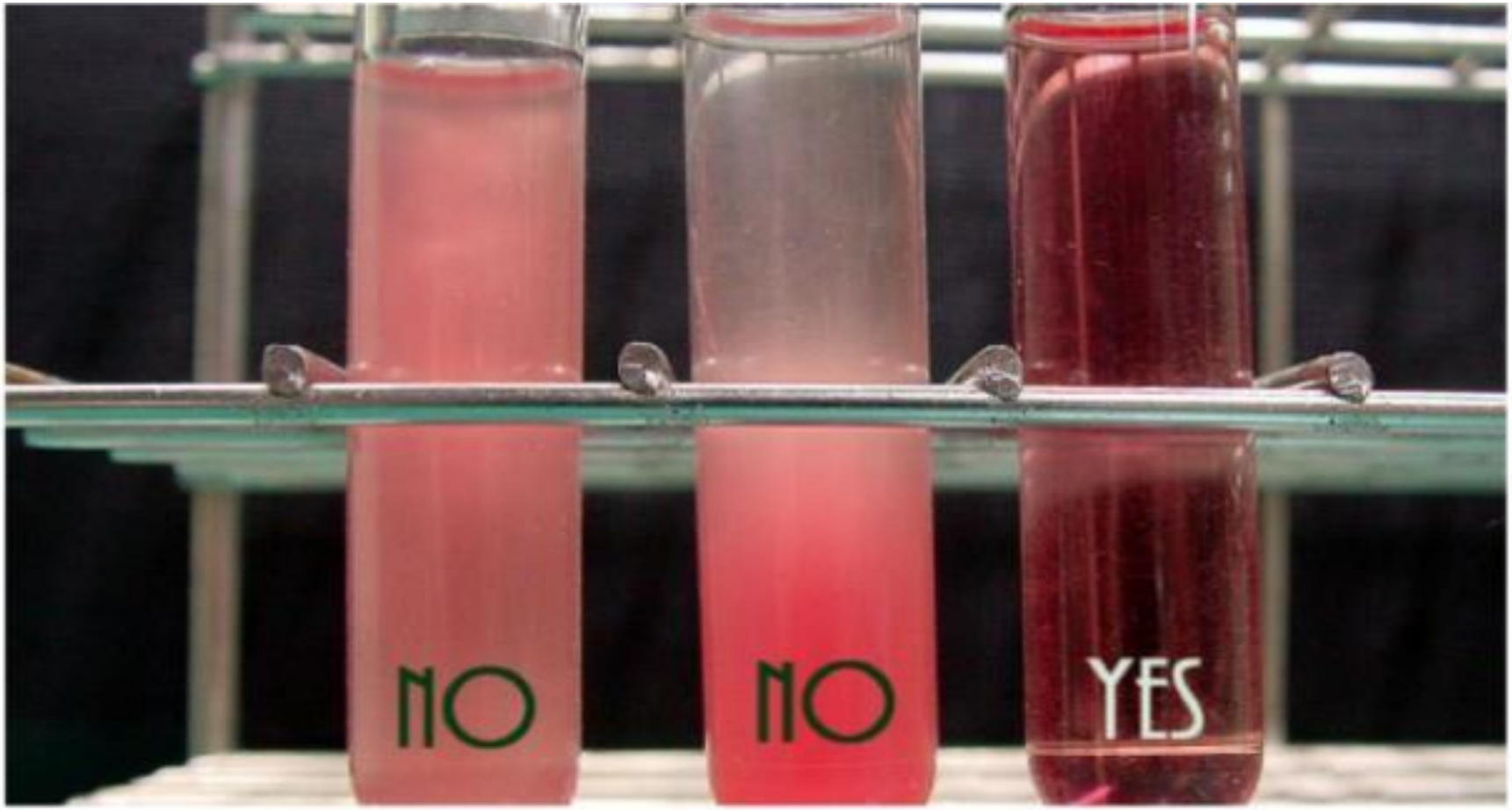
	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F
NaCl 0.45%	5ml					
NaCl 1.2%		5ml				
Sucrose 6%			5ml			
NaOH 0.1M (5 ml 0.9% saline +3 drops of base)				5ml		
HCl 0.1M (5 ml 0.9% saline +3 drops of acid)					5ml	
Dis.H_2O						5ml

Wait 10 min and observe wither hemolysis has taken place or not.

Results:

	A	B	C	D	E	F
Observation (Transparent or Opaque)						
conclusion						

RESULTS



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

Detection of blood by benzidine test:

It is often necessary to detect the presence of small quantities of blood in urine.

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 → blue greenish color

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

Results

- 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"*.