BCH 471

Hemolysing Agents & Detection of Blood

Blood Hemolysis

- **Hemolysis** (from the Greek Hemo: meaning blood, - lysis, meaning to break open)

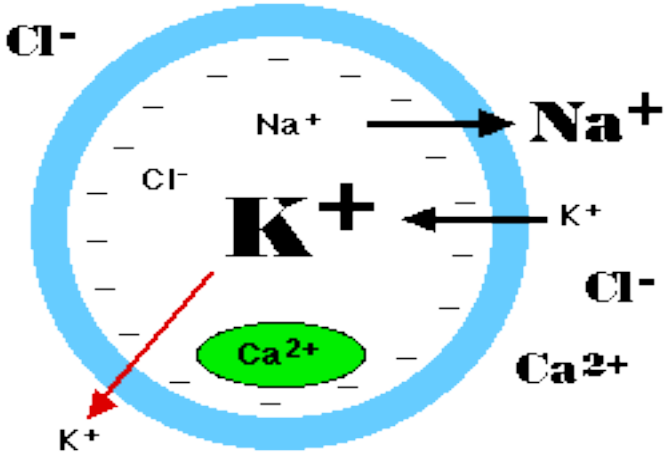
- It is the breaking open of red blood cells and the release of hemoglobin and the red cell contents into the surrounding fluid (plasma)

- Hemolysis may occur *in vivo* or *in vitro*

Hemolysis *in vivo*

- Conditions that can cause hemolysis include: Immune reactions, Infections, Medications. Toxins and poisons.

- Because 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.



Hemolysis *in vitro*

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

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

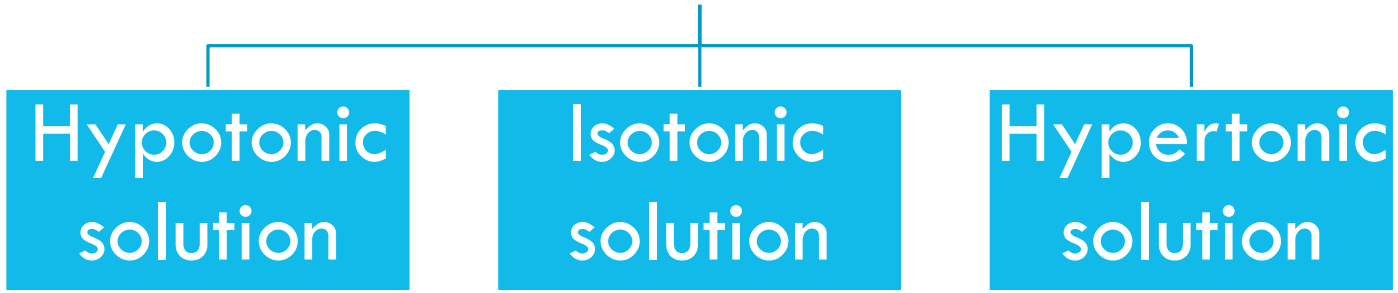
When Blood Hemolysis Should Be Done?

- Breaking down RBCs to release their content is often necessary for biochemistry
- Estimation of hemoglobin
- To obtain erythrocyte free preparation of leukocyte and platelet

Osmosis and Osmotic Pressure

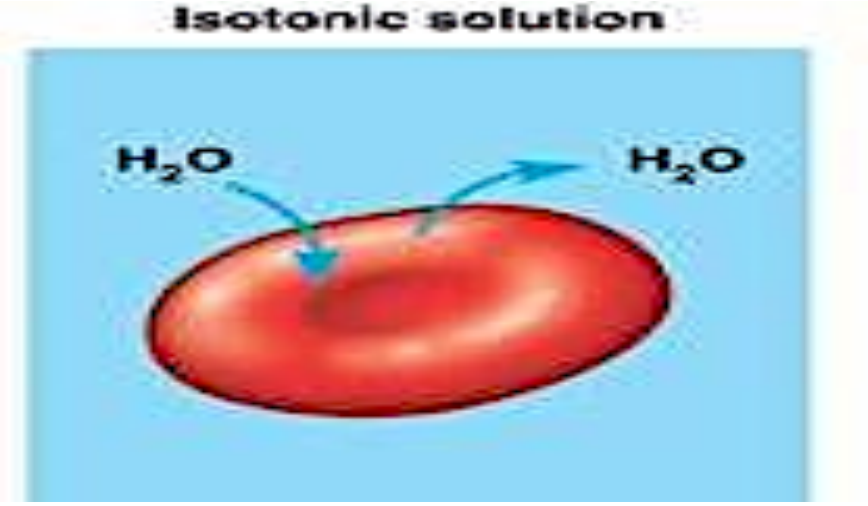
Osmosis: It is the diffusion of water across a selectivity preamble membrane into a region of higher solute conc. Once an equilibrium is reached the flow of water stops

Osmotic pressure: If the pressure that generate from osmosis



Isotonic Solution

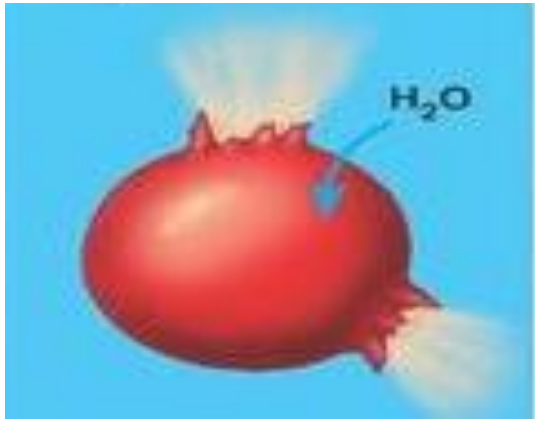
- A solution that has the same salt concentration as the normal cells of the body and the blood, having equal **osmotic pressure**.
- Example of Isotonic solution is **sodium chloride 0.9%,** have the same osmotic pressure as serum and they do not affect the membranes of the RBCs.
- In hospitals, intravenous fluids are isotonic.



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.

Solute inside the cell > Solute outside the cell



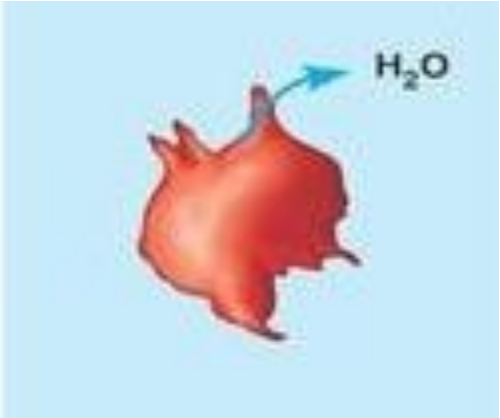
- The RBCs will burst or hemolyzed

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

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.

Solute inside the cell < Solute outside the cell



- The RBCs will be shrink

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

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

Osmolarity of RBC = 0.308 Osmolar → O=M x no.of dissociation partials

NaCl →Na+ +Cl- (no.of dissociation particles=2)

0.308 = M x 2 → M=0.154 M

-To calculate in w/v % expression → M=mole/V (in L)

No.of moles = 0.154 x 0.1 (100 ml, because you want it as %)

No.of moles = 0.0154 moles → No.of moles = Wt/Mwt

Wt= 0.0154 x 58.5=0.9 g

=0.9 % → the concentration of NaCl that will make an isotonic solution

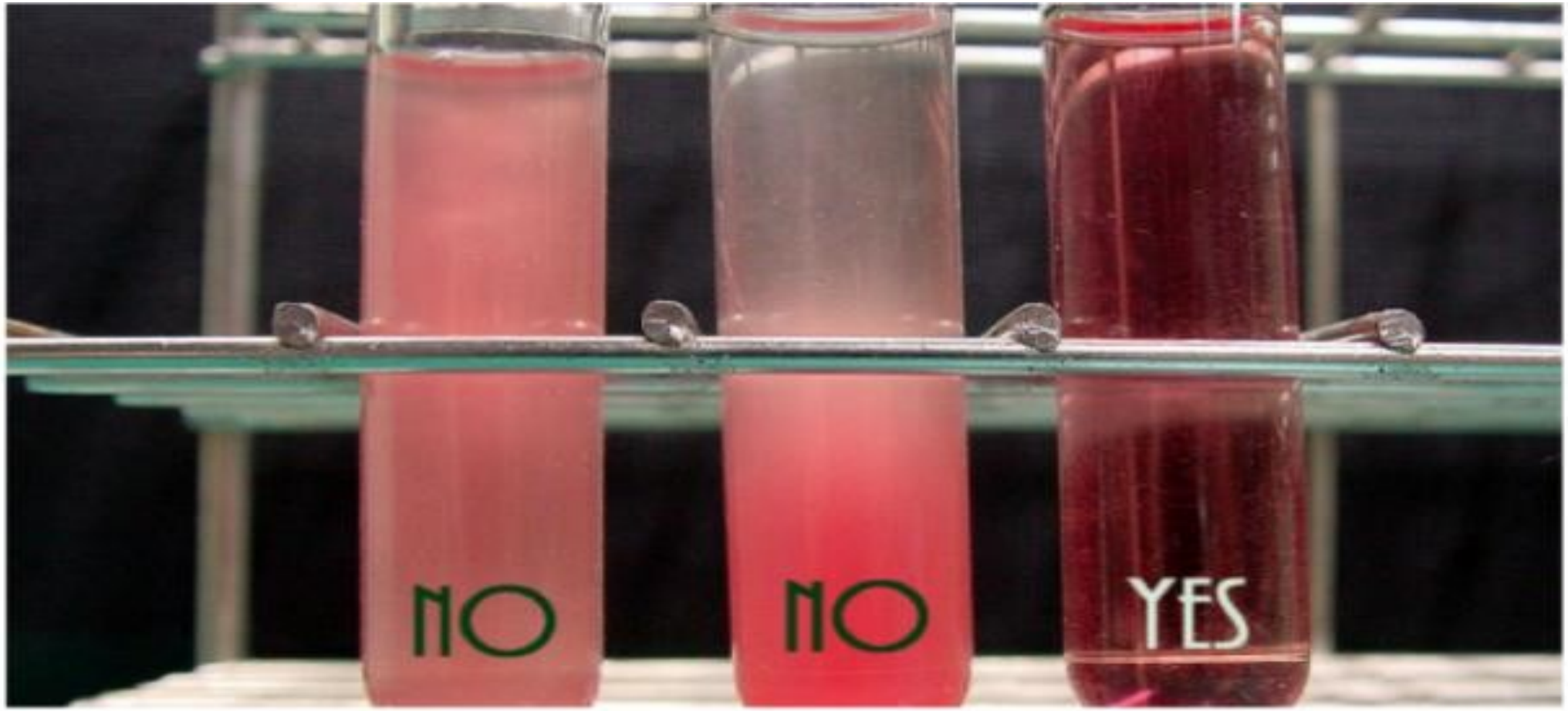
Method

	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F
NaCl 0.45%	5 ml					
NaCl 1.2%		5 ml				
Sucrose 6%			5 ml			
NaOH 0.1M				3 drops		
HCl 0.1 M					3 drops	
Dis. Water						5 ml
NaCl 0.9%				5 ml	5 ml	

- Wait 30 min
- Observe wither hemolysis has taken place

What type of solution is distilled water considered?

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, stomach contents etc.

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

Method → 3 ml of Sample + 2 ml Benzidine + 1 ml H_2O_2

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".*