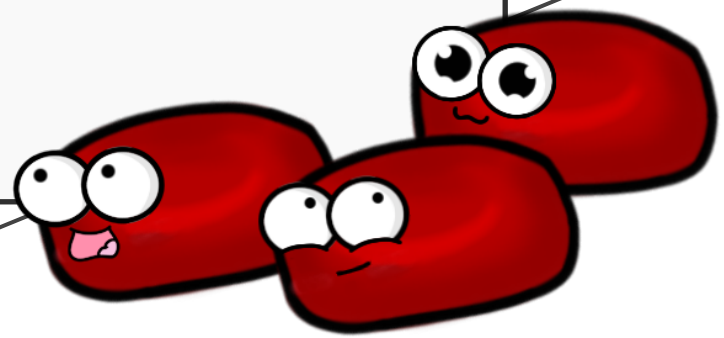
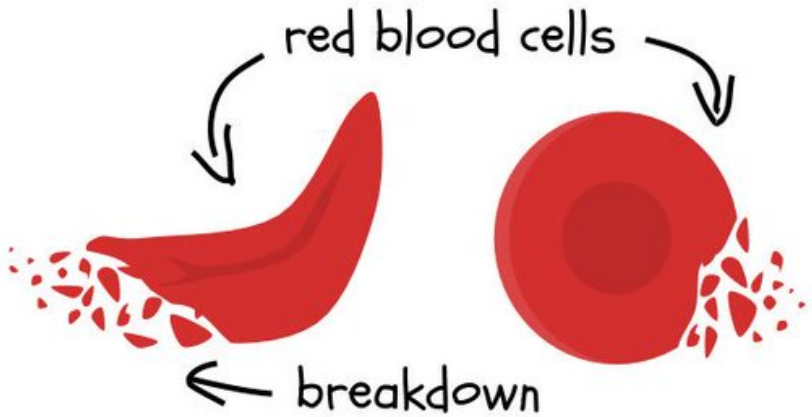
Blood Biochemistry BCH 471[Practical]

Lab (4) Hemolyzing 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.*

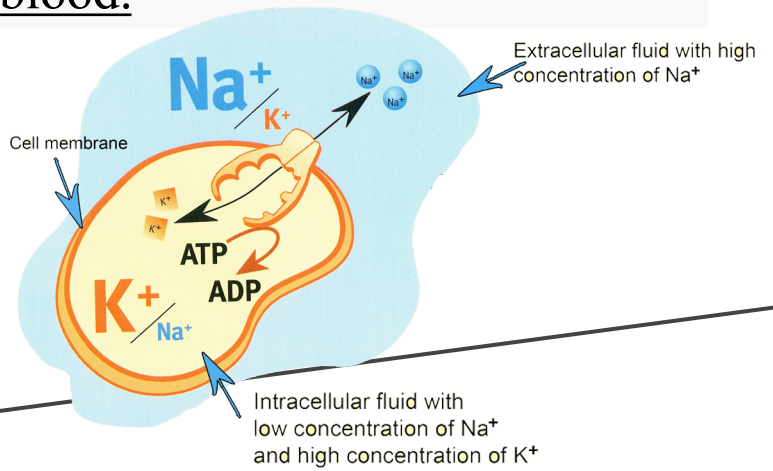


Hemolysis in Vivo

- **Conditions that can cause hemolysis include:**

1. Immune reactions
2. Infections
3. Medications
4. 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

1. Improper technique during collection (e.g. incorrect needle size, excessive suction)
2. pH imbalance (addition acid or base)
3. Placing RBCs in a hypotonic solution

Note: 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
- Estimation of hemoglobin
- To obtain erythrocyte free preparation of leukocyte and platelet

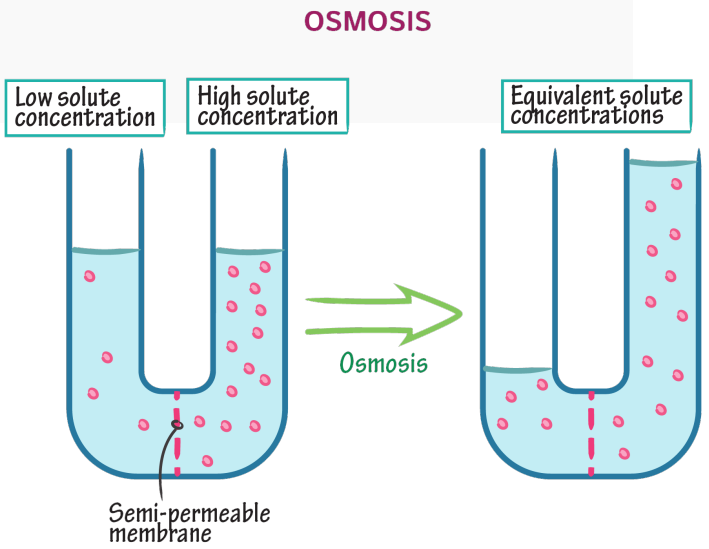
Osmosis and Osmotic Pressure

Osmosis:

- It is the diffusion of solvent molecules across a semi-preamble membrane into a region of higher solute concentration.
- Once an equilibrium is reached the flow of water stops.

Osmotic pressure: the pressure exerted by a solvent passing through a semi-permeable membrane in osmosis.

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



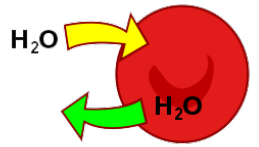
Tonicity

Types of solutions:

➢ **Isotonic**

- A solution that has the same solutes 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.

Solute inside the cell = Solute outside the cell



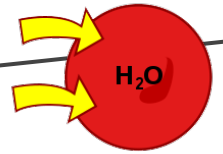
Tonicity

Types of solutions:

➢ **Hypotonic**

- 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.
- The RBCs will burst or hemolyzed.
- Any concentration of NaCl that is **lower than 0.9%,** will be considered hypotonic for cells.

Solute outside the cell < Solute inside the cell



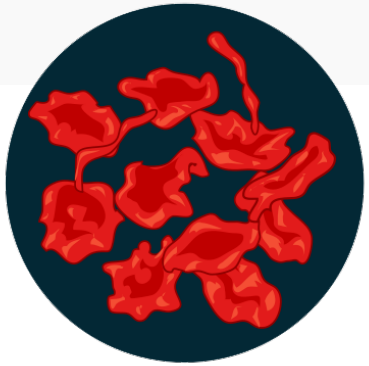
Tonicity

Types of solutions:

➢ **Hypertonic**

- 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.
- The RBCs will be shrink.
- Any concentration of NaCl that is **higher than 0.9%,** will be considered hypertonic for cells.

Solute outside the cell > Solute inside the cell



Practical Part 💉

Objectives

1. To detect the presence of hemolysis in blood sample.
2. To detect the presence of blood in a biological sample.

Calculations

How to Calculate the Concentration of an Isotonic Solution of a Specific Substance:

- For example you want to know the **grams** of NaCl that will make a **100 ml** isotonic solution , knowing that the osmolarity of RBC = 0.308 Osmolar.

First: Calculate the molarity from osmolarity equation: [1]

Osmolarity = 0.308 Osmolar

No. of dissociation particles = 2, since NaCl → $Na^+ + Cl^-$

→ $\mathbf{M} = \frac{\textbf{Osmolarity}}{n} = \frac{0.308}{2} = 0.154\ M$

[1] Osmolarity = M x n

Where:

M = molarity

n= No. of dissociation particles

Calculations

Second: Calculate the No. of moles expressed in (w/v %): [2]

To calculate in w/v % → **M= No. of moles / V (in L)**

→ **No. of moles = M x V (in L)** =

→ 0.154 (from step 1) x 0.1 (100 ml, because you want it as %)= 0.0154 moles

Third: Calculate weight in grams knowing that Mwt of NaCl = 58.5 g/mol: [3]

→ Wt (g) = No. of moles x Mwt =

→ 0.0154 (from step 2) x 58.5= 0.9 g

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

[2] Molarity $= \frac{\text{No. of moles of solute}}{\text{Volume (L)}}$

[3] No. of moles $= \frac{\text{Wt(g)}}{\text{Mwt}}$

Experiment (1): Hemolysis Test

Method

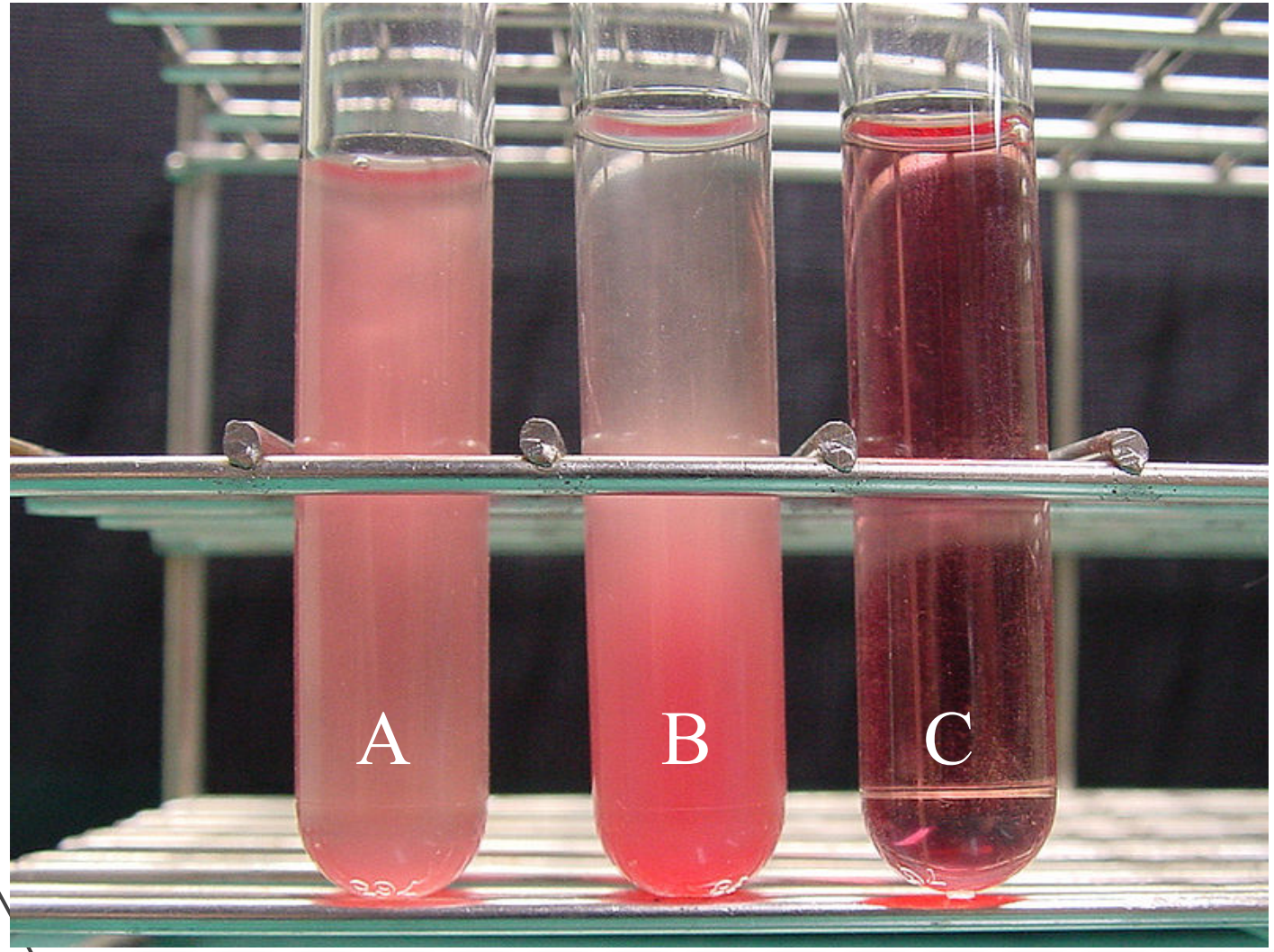
1. Label 6 tubes (A→ F). Then, add 1ml of RBCs suspended in saline into each tube

	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	

2. Wait 30 min
3. Observe wither hemolysis has taken place

💡 Pause and Think **What type of solution is distilled water considered?**

Results



A Normal, non-hemolyzed sample

B Sedimented after one hour

C Hemolyzed sample

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

Experiment (2): 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 heme group of hemoglobin possesses a peroxidase-like activity which catalyzes the breakdown of hydrogen peroxide (H_2O_2)
- The oxidizing species formed in this reaction can then react with benzidine giving blue greenish color.

Heme (hemoglobin) + H_2O_2 → H_2O + [O]

[O] + benzidine → blue greenish complex

Note: 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

Experiment (2): Detection of Blood by Benzidine Test

Method

- Place 3ml of sample in a boiling water bath for 3 minutes.
- Cool it under tap water.
- Add 2 ml Benzidine+ 1 ml H_2O_2

Results

- If the test is negative → blood is absent from sample.
- If the test is positive → blood is probably **not definitely** present in sample.

➢ For this reason these tests are often described as **"presumptive tests"** .

Positive results