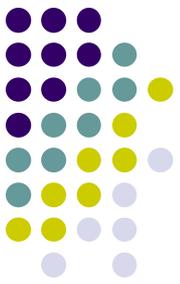




# Osmotic Fragility Test

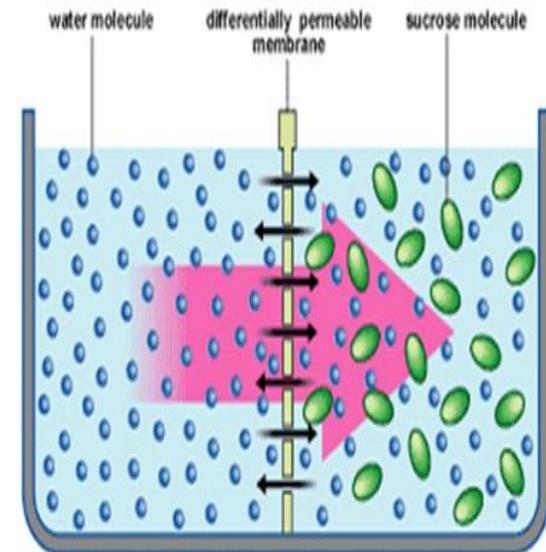
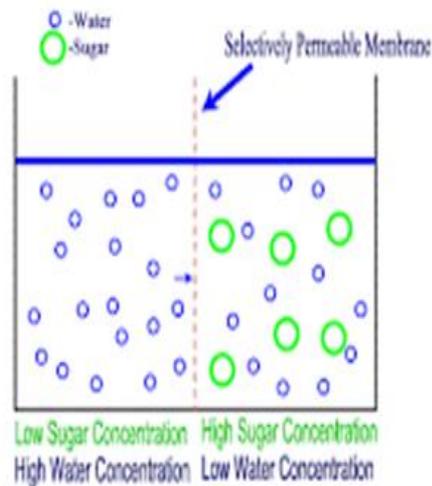
October 2018  
Physiology Lab-5

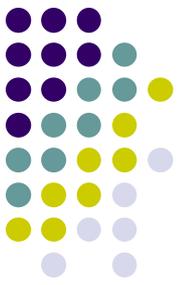
Asst. Lec. Zakariya A. Mahdi  
MSc Pharmacology



# Osmosis

- **Osmosis** is the spontaneous net movement of solvent molecules through a semi-permeable membrane into a region of higher solute concentration, in the direction that tends to equalize the solute concentrations on the two sides.





# Erythrocyte fragility

- **Erythrocyte fragility** refers to the tendency of erythrocytes (red blood cells, RBC) to hemolyse (rupture) under stress (commonly osmosis).

# Osmotic fragility

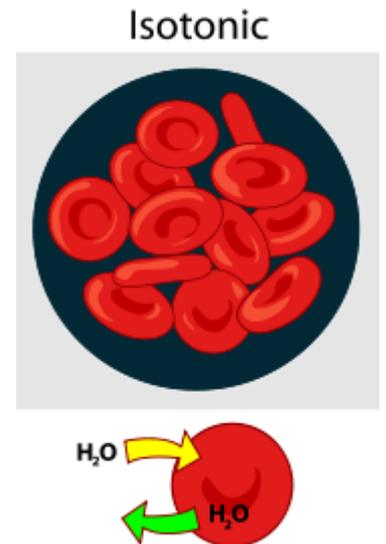


- **Osmotic fragility:-** is a test to measure red blood cell (RBC) resistance to hemolysis when exposed to a series of increasingly dilute saline solutions. This term refers to the susceptibility of red cells to being broken down by osmotic stress.
- **Hemolysis:-** This term refers to the breaking down (bursting) of red cells resulting in release of Hb into the surrounding fluid.
- **The sooner hemolysis occurs, the greater the osmotic fragility of the cells.**

# Isotonic solution



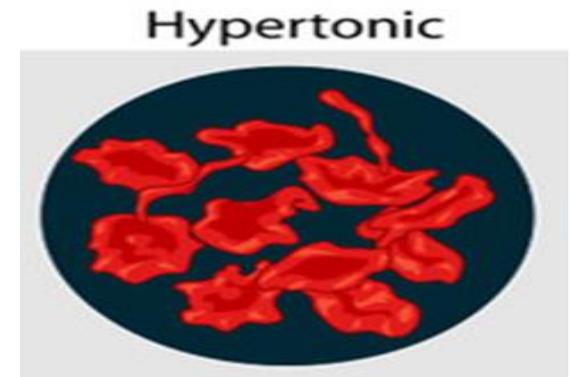
- **Isotonic solution:** A solution that has the same salt concentration as cells and blood.
- When cells are in isotonic solution, movement of water out of the cell is exactly balanced by movement of water into the cell. A 0.9% solution of NaCl (saline) is isotonic to animal cells.



# Hypertonic solution



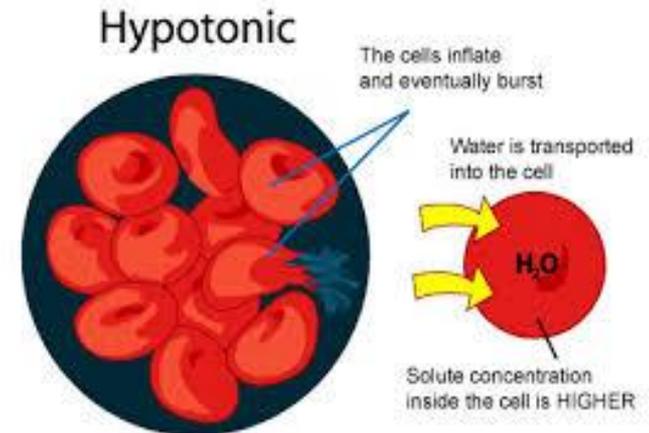
- In a hypertonic solution the total molar concentration of all dissolved solute particles is greater than the concentration in a cell.
- If concentrations of dissolved solutes are greater outside the cell, the concentration of water outside is correspondingly lower. As a result, water inside the cell will flow outwards to attain equilibrium, causing the cell to shrink.



# Hypotonic solution



- In a hypotonic solution the total molar concentration of all dissolved solute particles is less than that of a cell.
- If concentrations of dissolved solutes are less outside the cell than inside, the concentration of water outside is correspondingly greater. When a cell is exposed to such hypotonic conditions, there is net water movement into the cell.

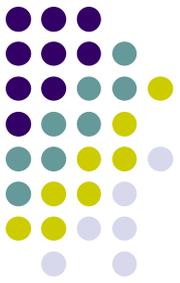


# PRINCIPLE



- The normal red cells can remain suspended in normal saline (0.9% NaCl solution) for hours without rupturing or any change in their size or shape.
- But when they are placed in decreasing strengths of hypotonic saline, they absorb water (due to osmosis) and finally burst. The ability of RBCs to resist this type of hemolysis can be determined quantitatively.

# Osmotic Fragility Test



## Notes:

- When RBC's are placed in distilled water they will swell, burst & the hemoglobin will be released and the hemoglobin will color the plasma {haemolysis}
- If the RBC's are placed in 0.9%NaCl nothing happens to them because it is isotonic with the cells
- If we put the RBC's in a hypertonic solution they will shrink

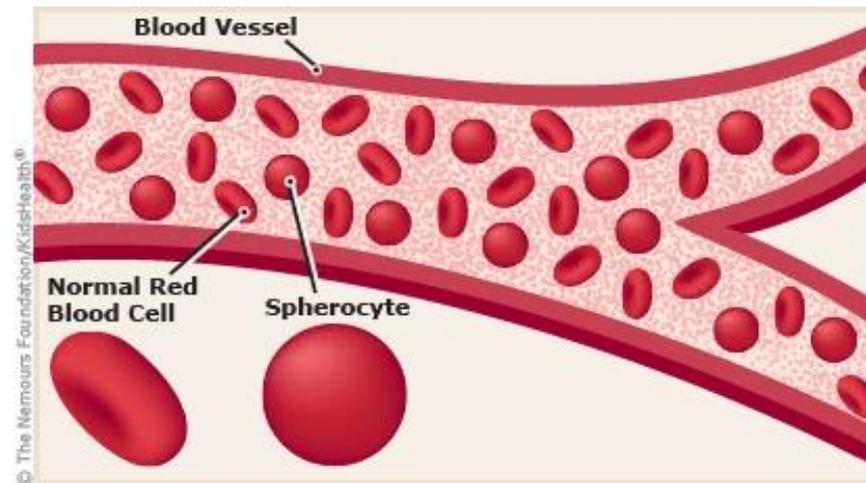
## Factors affect the osmotic fragility:

- The main factors affecting the osmotic fragility test is the shape of the RBC's which in turn is dependent on the
  1. Cell membrane permeability.
  2. Surface-to – volume ratio

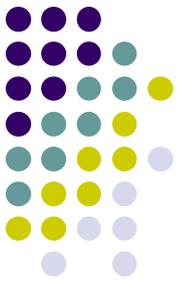
# Why the Test is performed?



- This test is performed to detect thalassemia and hereditary spherocytosis .
- Hereditary spherocytosis is a common disorder in which red blood cells are defective because of their round, ball-like (spherical) shape. These cells are more fragile than normal because they are less likely to expand.



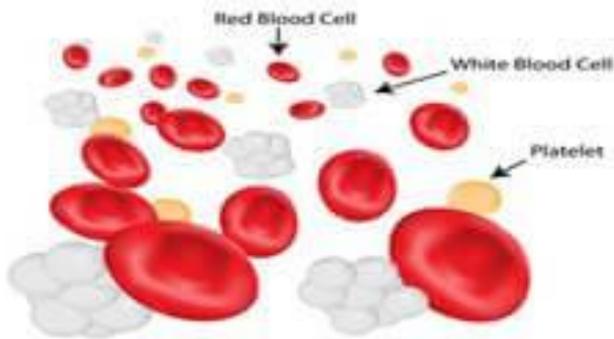
# Why the Test is performed?



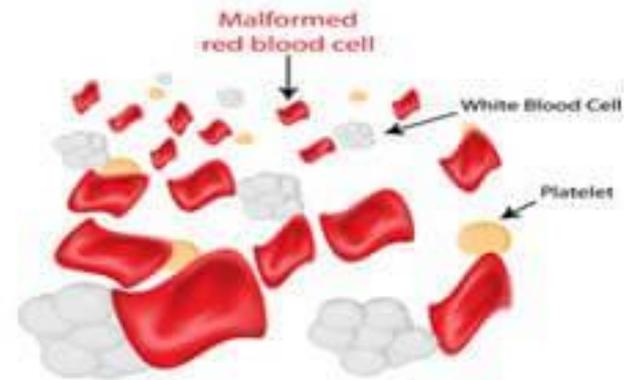
- Cells that are flatter than normal are more likely to expand, and thus have decreased osmotic fragility .
- **Thalassemia** is an inherited condition that affects the portion of blood (hemoglobin) that carries oxygen.

## Thalassemia

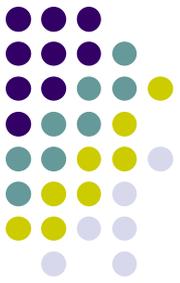
Normal



Thalassemia

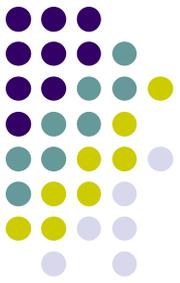


# Why the Test is performed?



- The test only indicates that a proportion of the red cells have decreased surface-to-volume ratios and are more susceptible to lysis in hypo- osmotic solutions.
- Cells with increased surface-to-volume ratios, such as occur in thalassemia and iron deficiency, may show decreased osmotic fragility.

# Osmotic Fragility Test



## Purpose:

- 1- To aid diagnosis of hereditary spherocytosis & Thalassemia.

## Method

Manual osmotic fragility test

## Material & instruments

- 1. Test tubes
- 2. NaCl with different concentrations
- 3. Heparinized venous blood
- 4. Distilled water

# Procedure

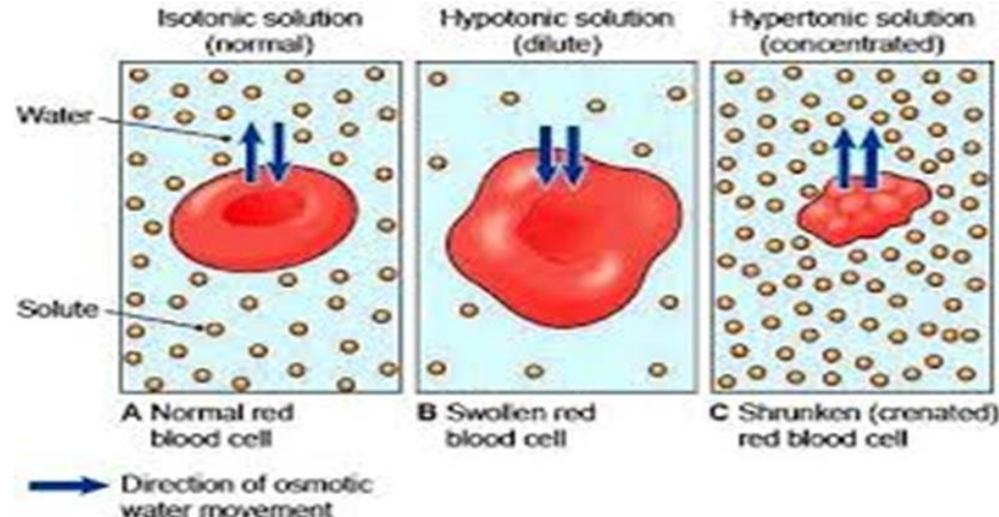
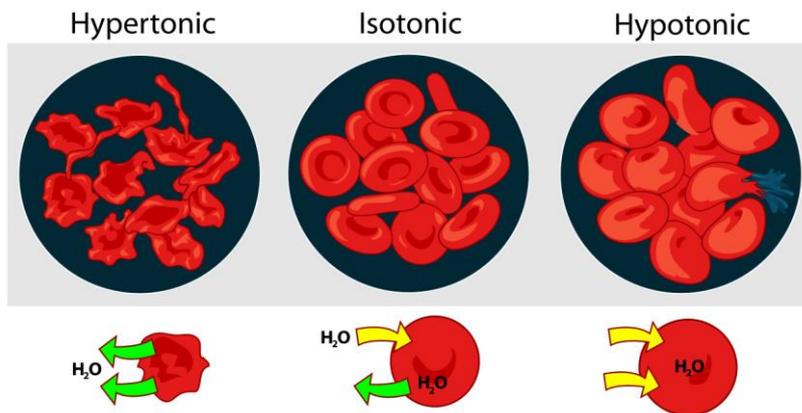


- 1-** Prepare different concentrations of NaCl start with 0 concentration i.e. put only D.W. then put in the rest of the test tubes the following concentrations of NaCl 0.3, 0.35 0.4, 0.45, 0.5, 0.9 %
- 2-** Put a few drops of heparinized blood in each test tube and read the results visually
- 3-** Carefully observe each tube for depth of red color of the supernatant and the mass of red cells at the bottom.
- 4 -** Place a small drop of each of the following solutions on a separate, clean microscope slides: 5% NaCl, 0.9% NaCl, 0.4% NaCl, and distilled water.
- 5-** Observe each slide and note the appearance of the blood cells. What has happened to the cells?

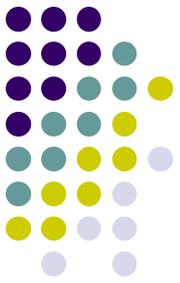
# Observation and Results :



- **Red cells in hypertonic saline.** In hypertonic solutions, the RBCs , like other body cells, shrink (crenate) due to movement of water out of the cells (**exosmosis**).
- **Red cells in hypotonic saline.** In hypotonic saline, water moves into the red cells (**endosmosis**). They swell up and lose their biconcave shape, becoming smaller and thicker. When they swell and become completely spherical, further increase in volume is not possible without an increase in their surface area.

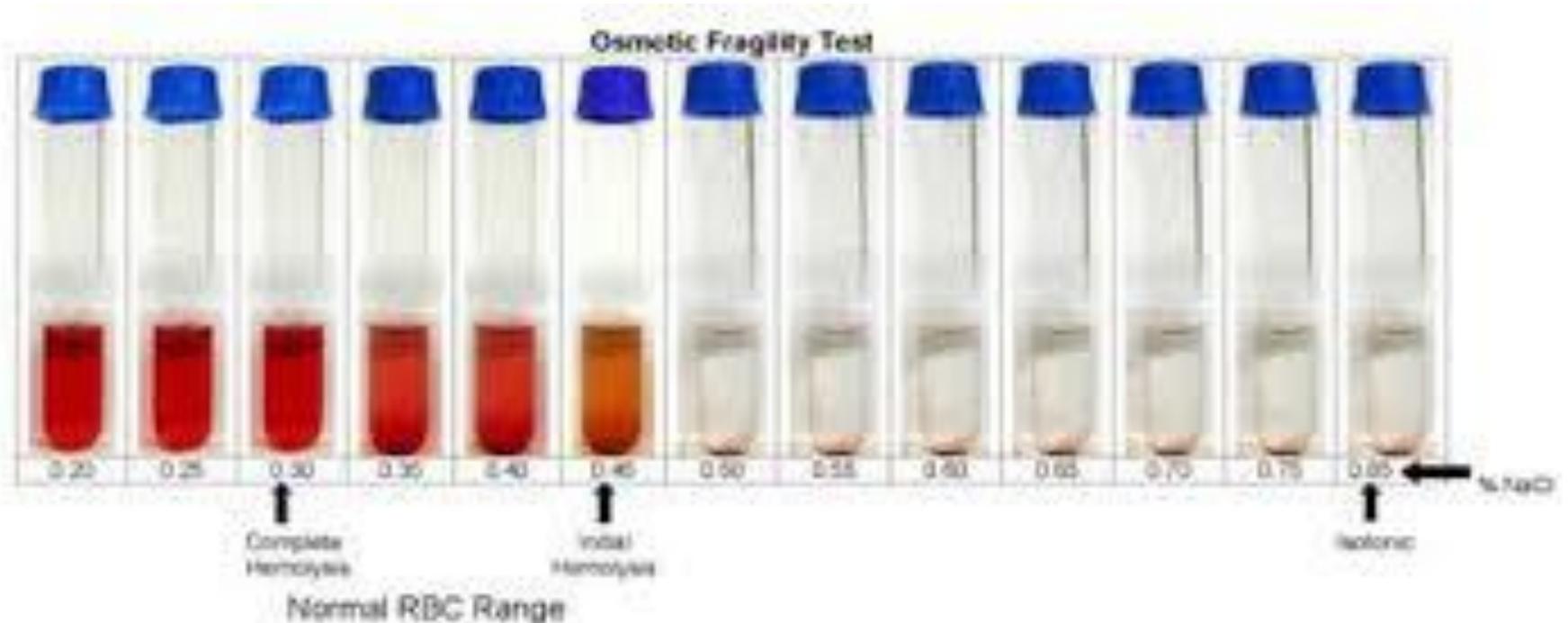


# Discussion

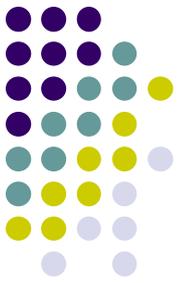


## Normal Range of Fragility

- Normally, hemolysis begins in about( 0.45%) saline.
- Hemolysis will be complete at 0.3 % NaCl.
- No cells hemolyze in solutions of 0.5% saline and above.



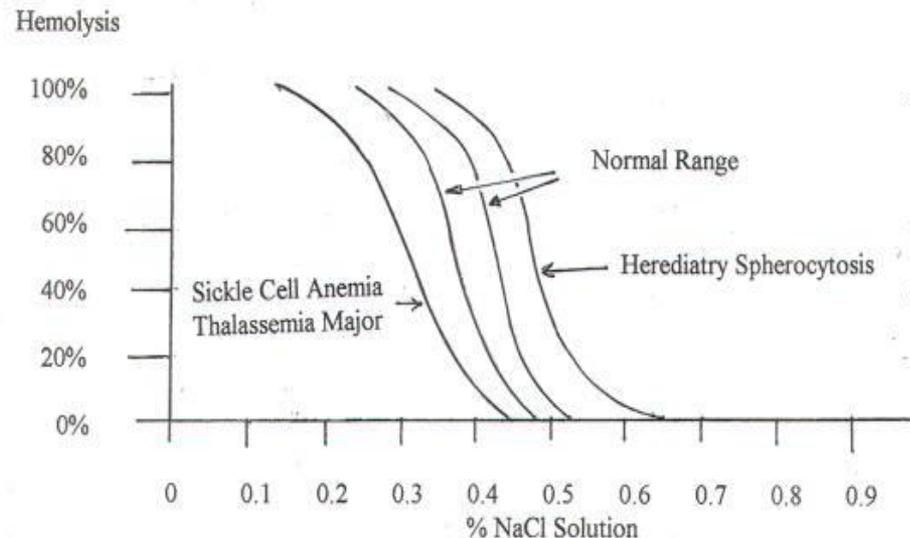
# Medical applications



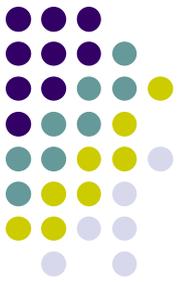
**A ) Increased red cell fragility (increased tendency to hemolysis) :** It is seen in the following conditions:

- 1. Hereditary spherocytosis.**
- 2. Autoimmune hemolytic anemia.**
- 3. Toxic chemicals, poisons, infections, and some drugs (aspirin).**
- 4. Deficiency of glucose 6-phosphate dehydrogenase (G6D).**

Typical Graphs for RBC Osmotic Fragility



# Medical applications



- **B. Decreased red cell fragility (increased resistance to hemolysis):**  
**Osmotic fragility decreased in:**
  - Thalassemia.
  - Iron deficiency anemia.
  - Sickle cell anaemia

