Name number Study group

Theoretical part

Osmotic fragility test

The cytoplasmic membrane of red blood cells

The red blood cell cytoplasmic membrane not only represents a barrier between the interior of the erythrocyte and the outer environment, it also plays a crucial role in intercellular communication and adhesion. Some of its important features are deformability, flexibility as well as durability which allows cells to move through capillaries (the diameter of which can be as narrow as half of the diameter of the erythrocyte). Erythrocytes possess all these features thanks to the cytoskeletal proteins where spectrin plays the central role. Spectrin fibre is formed by spectrin alpha and beta and further interacts with actin filaments to create an intracytoplasmic cytoskeleton connected to the inner layer of the cytoplasmic membrane by protein 4.1 and ankyrin. The older the erythrocyte gets, the less durability, flexibility and deformability it possesses, and after approximately 120 days it is destroyed in the spleen.

Osmotic resistance

The resistance of erythrocytes to mechanical, chemical and osmotic damage is not identical in all red blood cells, and therefore some are able to resist higher stress levels than others. This is dependent on the cell's age, shape (e.g. spherical), enzyme content (e.g. genetic deficiency of glucose-6-P dehydrogenase), etc. The osmotic fragility test is a specific method used in the differential diagnosis of haemolytic anaemias.

Diffusion	Movement of particles based on the concentration gradient
Osmose	Movement of solvent molecules through the semipermeable membrane into a region with higher solute concentration
Osmotic pressure	Pressure required to stop osmosis
Osmolarity	Measured solute concentration defined as the number of osmotic active molecules per 1 litre of its solvent
Osmolality	Same as osmolarity but per 1kg; = 2 $[Na^+]$+[glc]+[urea] = 275-295mmol/kg H_2O
Tonicity	Represents the effect of osmotic activity in the relationship with a cell

Name number Study group

Pathology:

- Elevated values of minimal osmotic resistance
  - Inherent haemolytic anaemias
- Decreased values of maximal osmotic resistance
  - Polycythemia vera
  - Thalassemia
  - Sickle-cell anaemia
  - Fe^{2+} deficiency
  - Status post splenectomy

Isotonic haemolysis

The isotonic haemolysis of red blood cells occurs under two specific conditions:

- Isotonic solution of glucose – glucose is absorbed by cells until the solution becomes hypotonic and the cells die
- Isotonic solution of urea – urea freely penetrates cells following the concentration gradient and at the same time the tonicity of the solution decreases (urea does not respect the semi-permeability of the cytoplasmic membrane)

Name number Study group

Protocol
Osmotic fragility test

Methods

Equipment: Stand with 13 test tubes, solution of 1% NaCl, distilled water, physiological solution (0.9% NaCl), 2 graduated pipettes (10 ml), dropper, anti-coagulated human blood, gloves.

Procedure:

1. Twelve test tubes are filled, by using two pipettes, with decreasing amounts of 1% solution of NaCl and increasing amounts of distilled water according to the table below. The last test tube is filled with the physiological solution (0.9% NaCl). Always use a clean pipette for each test tube.

Tube number	1	2	3	4	5	6	7	8	9	10	11	12
NaCl 1% (ml)	6.3	6.0	5.7	5.4	5.1	4.8	4.5	4.2	3.9	3.6	3.3	3.0
H_2O (ml)	3.7	4.0	4.3	4.6	4.9	5.2	5.5	5.8	6.1	6.4	6.7	7.0
% NaCl	0.63	0.60	0.57	0.54	0.51	0.48	0.45	0.42	0.39	0.36	0.33	0.30

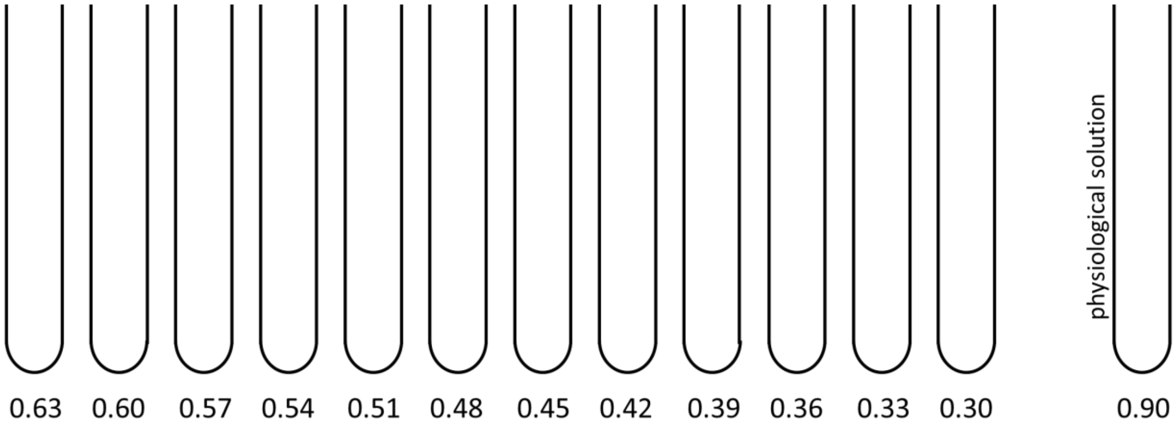
Table for preparation on NaCl solutions of decreasing osmotic pressure

2. Each test tube is carefully mixed.
3. Two drops of blood are placed into each tube and the content is again carefully mixed. After this, do not move the stand or the test tubes so as not to interrupt the sedimentation of non-haemolysed red cells.
4. After approx. 2 hours (min. after 30 minutes) of standing at room temperature, estimate the range of haemolysis by examining the colour of the supernatant solution above the sediment cells and the intensity of turbidity of the cell suspension. The tube with the normal physiological solution serves as the control since blood taken some time ago may already be partially haemolysed.

In the lowest concentrations of NaCl a complete haemolysis occurs, i.e. all red cells disintegrate. The content of the tube is quite pellucid (a text can read through the tube). At a certain concentration, however, the haemolysis is not complete, as a portion of the blood cells (i.e. those with the highest resistance) did not haemolyse, which appears as a slight turbidity in the lower part of the tube – incomplete haemolysis. The lowest concentration where a small fraction of cells remained non-haemolysed designates the s.-c. *maximal resistance*. On the other end of the concentration scale one finds a tube where the haemolysis is hardly distinguishable: the solution above the sediment is only slightly coloured by haemoglobin released from the least resistant cells. The concentration where osmotic haemolysis just begins to appear is designated as the *minimal resistance*. When non-haemolysed erythrocytes form a non-transparent sediment on the bottom of the test tube with a yellowish fully transparent NaCl solution above it, such a test is designated as no haemolysis.

The *osmotic resistance range* is the difference between the maximal and minimal resistance value.

Results



- Minimal osmotic resistance was observed in ………%NaCl (physiological: 0.4 - 0.44 %)
- Maximal osmotic resistance was observed in………%NaCl (physiological: 0.3 - 0.34 %)
- Mark osmotic resistance range.

Conclusion

Note down all the types of haemolysis known to you (there are 6 types) and explain their mechanism.

…………………………………………………………………………………………

…………………………………………………………………………………………

…………………………………………………………………………………………

…………………………………………………………………………………………

…………………………………………………………………………………………

…………………………………………………………………………………………

…………………………………………………………………………………………