Name number Study group

Theoretical part

Red blood cell count

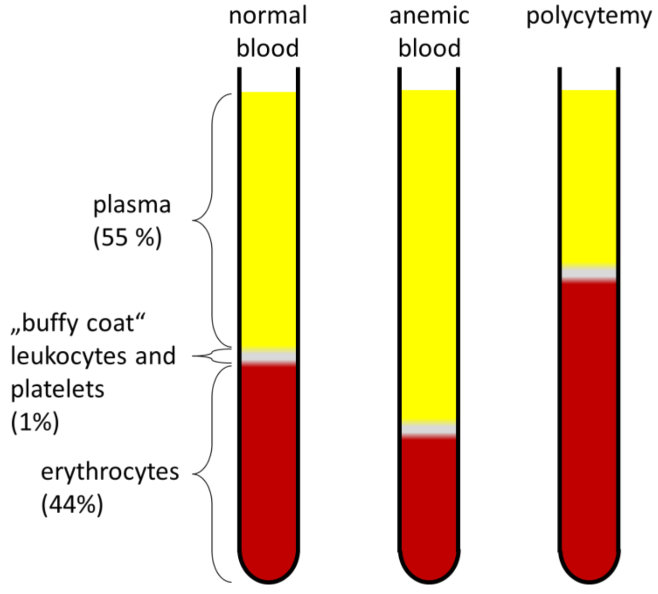
Blood and its components

Blood, one of the bodily fluids, consists of blood elements (so-called formed elements or corpuscles) suspended in blood plasma. Blood is highly specialised tissue, representing approximately 7–8% of body mass. The volume of blood circulating within the cardiovascular system in an average adult is roughly 4–6 litres, depending on gender, weight, body consistency, etc.

Blood carries out a wide range of essential functions. It represents the main transporting system supplying oxygen and nutrients throughout the body. Furthermore, blood removes metabolic waste products such as lactic acid, carbon dioxide and so forth. Blood also has an essential role in maintaining homeostasis, thermoregulation, immune reactions as well as hormonal signalling and regulation.

Blood plasma is composed of water and organic (proteins, lipids, carbohydrates, etc.) and inorganic substances (electrolytes) dissolved in the water. Blood plasma is obtained by centrifugation of anticoagulated blood with consequent separation of its components, separated based on their molecular weight. Anticoagulated blood is obtained by addition of an anticoagulant agent into the blood which must be used in the production of blood plasma. Inversely, blood serum lacking clotting factors is produced by centrifugation of blood without any anticoagulation agent (clotted blood). The anticoagulant agents prevent a coagulation cascade in any of its steps. Sodium citrate, EDTA and sodium oxalate bind calcium ions, crucial reactants in the coagulation cascade and hence are used *in vitro*, in contrast to heparin, which is used only *in vivo*. Heparin promotes the action of the anticoagulation systems, especially antitrombin III.

Blood is ranked among the non-Newtonian fluids and thus its viscosity (the measure of a fluid's ability to resist gradual deformation by shear or tensile stresses) is dependent on the shear rate as well as its content, which highly influences its flow. The haematocrit represents the volume percentage of red blood cells in blood and is examined by centrifugation or sedimentation. The physiological range is normally 39–51% in men and 35–46% in women.



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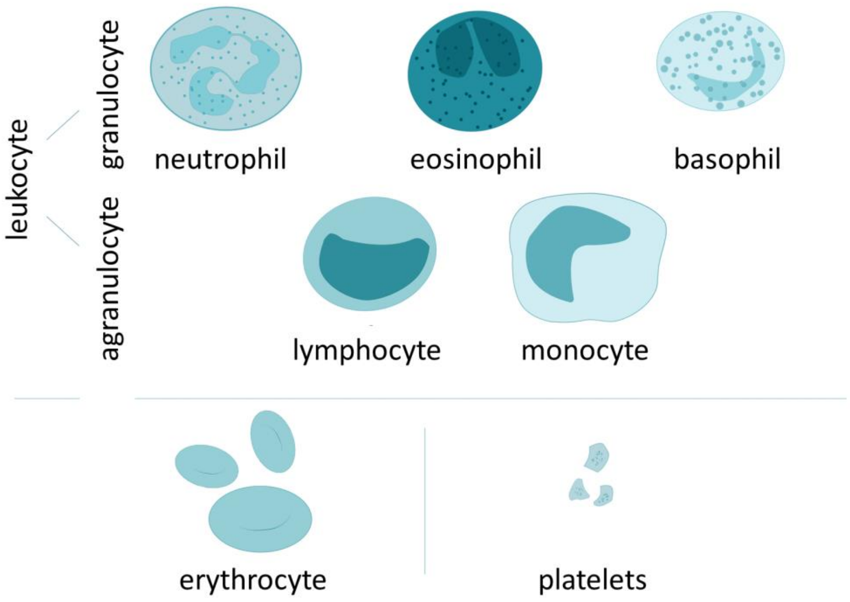
Name number Study group

Leukocytes

Leukocytes, also called white blood cells, are blood elements with a nucleus providing the body's immune response. The white cell count ranges under physiological conditions between 4000 and 10,000 leukocytes per 1 µl of blood, far less than the number of erythrocytes. Commonly white blood cells are divided into two major groups, granulocytes and agranulocytes. Granulocytes are further subdivided into neutrophils, eosinophils and basophils, all representing the cellular subsystem of non-specific immunity. The agranulocytes group comprises lymphocytes and macrophages.

Thrombocytes

Platelets (thrombocytes) are nuclei lacking discoid blood elements. They circulate in the blood in the range of 150,000 to 450,000 per 1 µl of blood. Thrombocytes are essential in haemostasis, a sophisticated mechanism preventing blood loss during injury. Moreover, platelets play a crucial role also in coagulation. Platelets are produced in the bone narrow as fragments of the megacaryocyte's cytoplasm into circulation and after 7–10 days are eliminated.



Erythrocytes

Erythrocytes, also called red blood cells, are blood elements produced in the bone marrow. As they mature, erythrocytes lose their nucleus and take on a specific shape: oval biconcave discs. Under the microscope one observes a brighter middle disc surrounded by a reddish oval. This colour discrepancy is based on differences in haemoglobin concentration within the erythrocyte. The bright central part of the erythrocyte is thinner (0.8 µm) and contains far less haemoglobin in comparison to the periphery (2.2 µm). A red blood cell (RBC) with a 7.2 µm diameter is called a normocyte. Larger RBCs are called macrocytes and smaller ones are termed microcytes. The condition when there are a lot of RBCs of differing diameter is known as anisocytosis. Poikilocytosis, on the other hand, stands for a condition where there is an excessive amount of improperly shaped RBCs. 1 µl of blood contains 4.3–5.3 * 10^6

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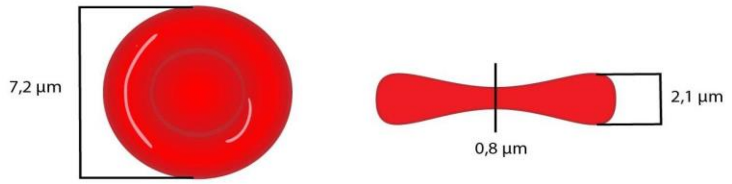
RBCs in healthy men and 3.8–4.8 * 10^6 RBCs in women. A decreased number of RBCs is called anaemia while an increased number is known as erythrocytosis or polyglobulia.



Erythropoiesis

Erythropoiesis is the specific process taking place within the bone marrow of adults which leads to production of erythrocytes. It starts with a pluripotent stem cell that, under the effect of erythropoietin, the red blood cell growth factor produced mainly in kidneys, differentiates into a reticulocyte. The reticulocyte is eventually washed out into the blood, where 48 hours later it develops into a mature erythrocyte. Physiologically the level of reticulocytes in the blood is 0.5–1.5%, but this might be increased after an injury or other blood loss (reticulocytosis). The normal life span of an erythrocyte is approximately 120 days. An old or damaged erythrocyte is eliminated in the spleen.

Erythropoiesis requires several absolutely essential substances, such as:

1. Amino acids – an essential part of the globin part of haemoglobin as well as of porphyrin
2. Iron – invaluable for hem production; a lack of it leads to iron deficiency anaemia
3. Vitamin B12 and folic acid – both crucial for nuclide acid synthesis and a lack of them causes pernicious anaemia

Haemoglobin

Haemoglobin is a protein compounded from four heme molecules (a porphyrin ring containing Fe^{2+} ion in the centre) and four globin molecules (two alpha and two beta chains). Haemoglobin not only represents the most essential transport mechanism for oxygen, but it also participates in CO_2 transport. Each globin molecule creates a hydrophobic pocket protecting a Fe^{2+} ion against its oxidation into a Fe^{3+} ion that would be incapable of further transporting oxygen. Haemoglobin is produced during the evolution of an erythrocyte from its precursors in the bone marrow from glycine and succinyl-CoA.

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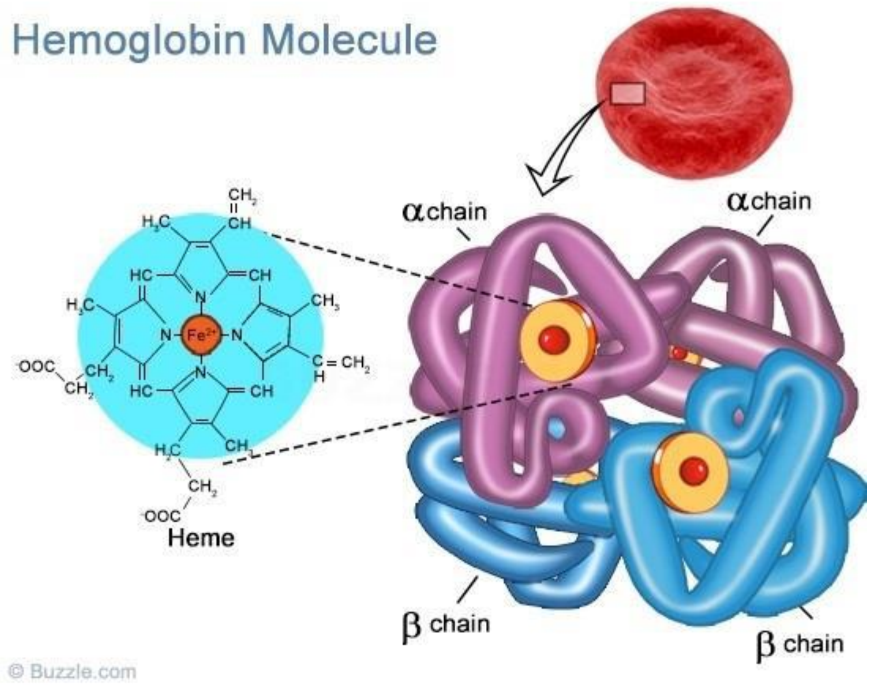

Haemoglobin forms	*Bound molecule*
Oxyhaemoglobin	O_2
Carbaminohaemoglobin	CO_2
Methaemoglobin	Heme contains Fe^{3+} instead of Fe^{2+}
Carboxyhaemoglobin	CO

The initial phase of haemoglobin degradation is located within macrophages following phagocytosis of a disrupted erythrocyte or the haemoglobin molecule leaking into plasma and bound to haptoglobin, a protein binding free haemoglobin molecules floating in plasma. Degradation of haemoglobin is caused by its oxidation into verdoglobin. Verdoglobin is transformed to green biliverdin by splitting the globin molecule off, which is eventually chopped off into amine acids. Biliverdin is enzymatically modified and, as bilirubin, it is released into the blood stream. Bilirubin as a lipophilic molecule is bound to albumin, transporting it to the liver where it is further modified by conjugation with glucuronic acid. Conjugated bilirubin, so-called direct, is excreted into the gall and consequently mixed with chymus. An increased bilirubin concentration in plasma is known as jaundice or icterus and typically first appears as yellowish discolouration of sclera and eventually mucosa and skin accompanied by itching.

Sex differences

	Men	Women
total red blood cells	4.3–5.3 x 10^{12}/l	3.8–4.8 x 10^{12}/l
hemoglobin	140–180 g/l	120–160 g/l
hematocrit	0.39–0.51	0.35–0.46

Sex differences are caused by sex hormones. Testosterone stimulates erythropoiesis by increasing erythropoietin production in kidneys, while oestrogen decreases its production.

Anaemia

Anaemia is a medical condition where haemoglobin concentration is decreased, causing insufficient oxygen transport throughout the body. Anaemia develops when the production of red blood cells is impaired or loss of them increases. Anaemia might present as pale and cold

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skin, shortness of breath, tiredness and poor physical endurance. The body compensates for anaemia by increasing the heart rate.

Iron deficiency anaemia

Iron deficiency anaemia is the most common anaemia in humans, predominantly occurring in women and caused by a lack of iron necessary for haemoglobin production. Hypoxemia caused by anaemia stimulates massive erythropoietin production and thus the erythrocytes released from the bone marrow are small and have a decreased haemoglobin content (microcytic hypochromic anaemia).

Pernicious anaemia

Pernicious anaemia is a medical condition that develops due to a lack of vitamin B12 or folic acid. Both of these vitamins are crucial for the nuclide acid production, namely thymine. Because there is not enough thymine for DNA synthesis, erythrocytes produced in the bone marrow are great and contain a lot of haemoglobin (macrocytic, hyperchromic anaemia).

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Name number Study group

Theoretical part

Determination of blood groups by slide method

Introduction

The erythrocyte cytoplasmic membrane serves as a barrier between the interior and blood under physiological conditions. Furthermore, it has a profound impact on intercellular communication and interaction. The cytoplasmic membrane is typically organised into a phospholipid bilayer with hydrophobic regions closed in on each other and hydrophilic regions interacting with the inner and outer environment. Within the phospholipid bilayer there are embedded proteins as well as lipids (mainly cholesterol) which reinforce the whole structure. A sort of plasma proteins, carbohydrates, glycolipids and glycoproteins stretches out beyond the membrane into the outer environment as antigens, based on which the wide range of blood groups is determined, such as AB0, Th, MNs, Kell, Levis, and so on. A blood group is inherited co-dominantly and remains unchanged over one's entire lifetime.

AB0 system

The AB0 system represents the most examined blood group, and is determined by the presence of the antigens A and B. These antigens are chemically glycoproteins and differ in their terminal carbohydrate chains, so-called agglutinogens. Four blood types are distinguished:

A – only agglutinogen A is present on the erythrocyte's surface
B – only agglutinogen B is present on the erythrocyte's surface
AB – agglutinogens A and B are present on the erythrocyte's surface
0 – neither agglutinogen A nor B is present on the erythrocyte's surface

Blood circulating throughout the body contains immunoglobulins IgM (anti-A and anti-B) known as agglutinins. Production of these antibodies is triggered by one's first intake of food and lasts throughout one's entire lifetime.

Group A – produces only anti-B agglutinin
Group B – produces only anti-A agglutinin
Group AB – no agglutinins are produced
Group 0 – produces both anti-A as well as anti-B agglutinin

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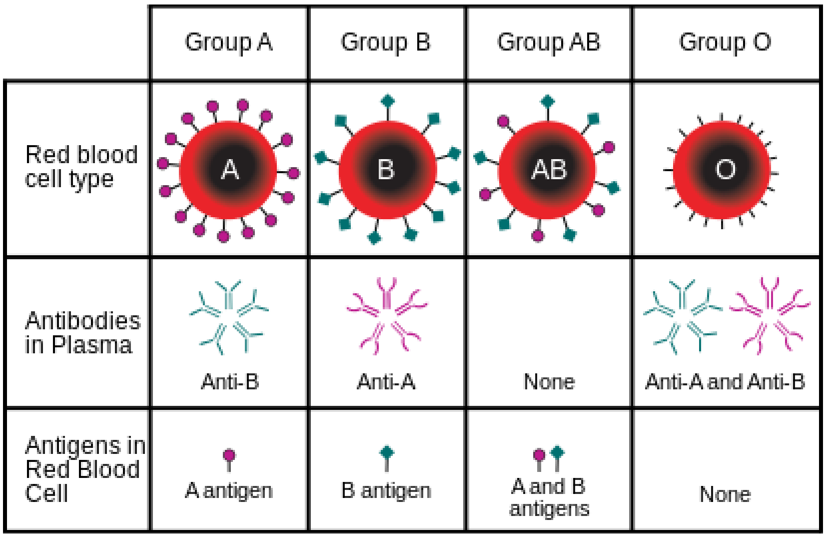
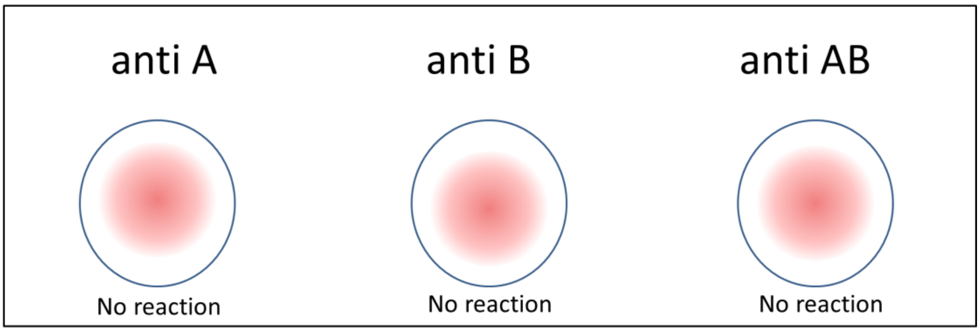


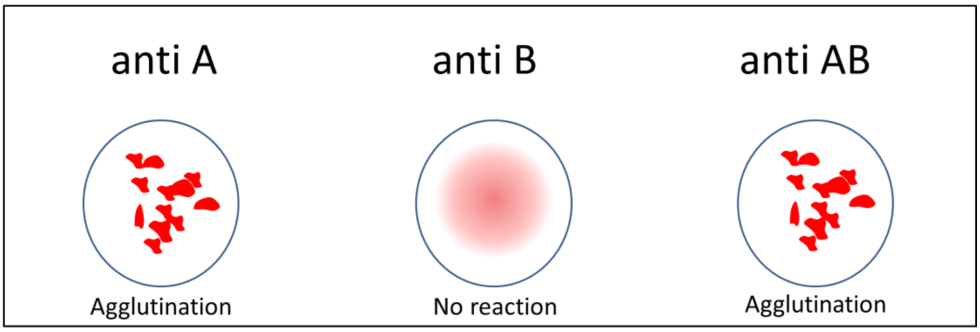
The interaction of agglutinogen and agglutinin leads to agglutination, production of floating flakes accompanied by subsequent destruction of erythrocytes by the complement system.

Explanation of slide method

Group 0



Group A



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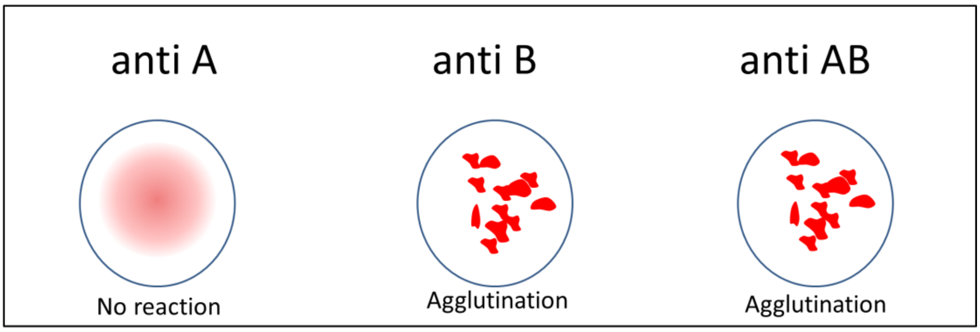
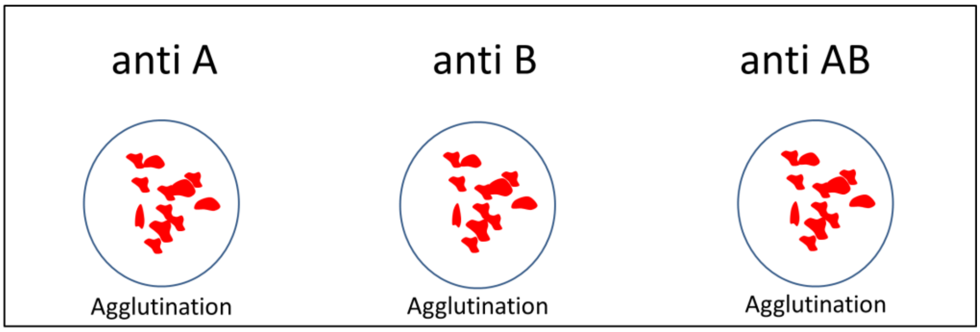
Group B



Group AB



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As an example to explain the slide method, we will look at the group A. In the blue drop containing anti-A agglutinins, there is an obvious reaction of anti-A agglutinins and red blood cells holding A agglutinogens on their surface lead to the production of large erythrocyte-immunoglobulin complexes. This reaction cannot be observed in the yellow drop owing to the lack of agglutinin anti-B which does not have its agglutinogen B counterpart on the erythrocyte's surface. In the last grey drop agglutination is displayed due to the presence of both anti-A and anti-B agglutinins, but only anti-A agglutinins take part in this reaction.

Hereditability of the AB0 system

Blood groups are inherited from both parents. The AB0 blood type is determined by a single gene producing three alleles – allele A, B and i. A specific dominance relationship occurs called codominance where allele i is recessive to the dominant A and B alleles.

Phenotype – blood group	Alleles
A	AA, Ai
B	BB, Bi
AB	AB
0	Ii

Rh factor

The Rh factor represents another blood group determined by antigens C, c, D, d, E, e. Nevertheless, the Rh factor is defined by the presence or absence of the antigen D. A Rh positive subject is a carrier of antigen D on the surface of red blood cells and vice versa. Antigen D is dominantly inherited and thus a Rh negative subject has inherited the recessive allele from the both parents. Contrary to the AB0 system, antibodies against antigen D are produced just after the Rh negative subject's first encounter with antigen D – this reaction is called immunisation. Moreover, anti-Rh antibodies are produced as IgG type, and thus are capable of being transported through the placental barrier.

Haemolytic disease of newborns (Erythroblastosis fetalis)

This disorder occurs in Rh negative mothers immunised against the Rh factor (i.e. there are anti-Rh factor antibodies circulating in her blood) and carrying a Rh positive baby. The mother's immunisation might be result of either a previous pregnancy when the mother's and a Rh positive baby's blood were mixed or by administration of an incompatible blood type. The subsequently created anti-D antibodies can be transported through the placental barrier and lead to destruction of red blood cells in the newborn. An elegant method of preventing the production of anti-D antibodies has been introduced: after each delivery of a Rh positive baby, anti-D immunoglobulin is administered to the Rh negative mother in order to destroy all Rh positive erythrocytes leaked during the labour.

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Name number Study group

Theoretical part

Erythrocyte sedimentation rate

Blood plasma

Blood plasma is an amber solution containing dissolved organic as well as inorganic compounds. Plasma represents 5% of the body mass, which stands for 3.5l in an average man weighing 70 kg. Blood plasma is obtained by the centrifugation of anticoagulated blood, thus it contains clotting proteins in comparison to the blood serum.

The organic compound of blood plasma is made up from proteins, glucose, hormones, vitamins, enzymes etc. The plasmatic proteins can be further divided into an albumin, globulin and fibrinogen fraction. The physiological concentration range of the plasma proteins is 65–85 g/l, but the vast majority is represented by albumin (25–51 g/l). The capillary wall is, under physiological conditions, a completely impenetrable barrier for the proteins dissolved in plasma and hence an osmotic pressure of around 25 mmHg is created. Furthermore, the proteins represent a valuable buffer system maintaining the pH of blood plasma in the very narrow range 7.36-7.44, a fast source of amino acids, they play an important role in immune reactions, coagulation, transportation of substances such as fatty acids, steroid hormones, bilirubin, ions, metals, drugs and so forth.

The inorganic compound of blood plasma consists of dissolved ions.

Ion	Concentration
Sodium	135–145 mmol/l
Potassium	3.7–5.1 mmol/l
Chloride	96–108 mmol/l
Calcium	2–2.6 mmol/l
Calcium ionized	1.16–1.32 mmol/l
Inorganic phosphates	0.65–1.61 mmol/l
Magnesium	0.78–1.03 mmol/l

Erythrocyte sedimentation rate (FW)

The erythrocyte sedimentation rate (Fahraeus-Westergren) is a nonspecific laboratory method providing information for the caregiver that something is going on in the patient's body. Sedimentation is a process of unsolvable particles settling in the suspension. Its counterpart is suspension stability, which is represented by zeta potential, the negative charge on the surface of erythrocytes determined by sialic acids bound on glycophorin which repel erythrocytes away from each other. This procedure is traditionally performed as anticoagulated blood placed in an upright tube and the erythrocyte sedimentation rate is measured after one hour.

Factors influencing the erythrocyte sedimentation rate:

- Red blood cell diameter – the bigger the red blood cells are, the faster sedimentation is
- The number of erythrocytes – more erythrocytes means greater zeta potential and higher suspension stability

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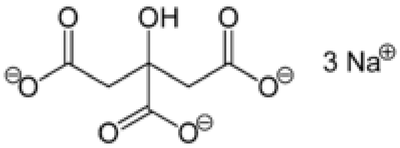
- The plasmatic proteins
    - Albumin – possess negative charge, and this leads to higher suspension stability
    - Fibrinogen – is negatively as well as positively charged and so suspension stability is decreased
    - Immunoglobulins – also hold negative and positive charge and hence lower suspension stability

Factors disrupting the zeta potential lead to rouleaux production. Roleaux sediments faster because of increased ratio of volume/surface area.
Erythrocyte sedimentation rate is measured in anticoagulating blood produced by addition of:

- Natrium citrate – binding Ca^{2+}



- EDTA - Ethylenediaminetetraacetic acid – this also acts as a chelating agent
- Natrium oxalate – the same as EDTA
- Heparin – activation of antitrombin III

Physiologic values:

- Man – 2–8 mm/h.
- Woman – 7–12 mm/h.
- Newborn – 2 mm/h
- Toddler – 4–8 mm/h.

→ the gender differences are caused by sex hormones; testosterone increases the erythropoietin production in kidneys → more erythrocytes

Pathologies:

- Increased erythrocyte sedimentation rate:
    - Pregnancy
    - Macrocytosis
    - Infection
    - Tumours – myeloma
    - Inflammations
    - Necrosis (infarction, trauma)
    - Relative/absolute albumin loss (nephrotic syndrome)
- Decreased erythrocyte sedimentation rate
    - Misshaped erythrocytes – spherocytosis
    - Polycythemia vera
    - Leucocytosis
    - Dysproteinaemia – hypofibrinogenaemia, hypogammaglobinaemia
    - Dehydration

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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

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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)

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