# 5 CHAPTER Blood physiology

## 1. Physical and chemical properties of the blood

### 1.1. General characteristics of the blood. Blood functions.

Total blood volume is about 6-8% of the body weight. Take into account, that the blood viscosity is close to 1 g/cm$^3$, and weight of averaged person is 70 kg, the blood volume will be in the range of 4-6 liters (of about 5 liters in average). Such volume of blood in the body is called ***normovolemia***. Accordingly, decreasing in blood volume (for example, in blood loss) is called ***hypovolemia***, and its increasing (for example, after excessive ingestion of beer) - ***hypervolemia***.

Blood is composed of a liquid matrix (***blood plasma***) and formed elements of the blood: ***erythrocytes***, ***leukocytes*** and ***platelets***, which are present in plasma in form of suspension. The ***hematocrit*** is the percentage of erythrocytes in the total blood volume. It ranges from 40 to 48% (an average - 44%). Hematocrit is a relatively constant homeostatic parameter and it is steady increased in healthy people only if they live in high altitudes. Essential decreasing of hematocrit level can be caused by hematopoiesis disturbance (for example, anemia). However, it should be remembered, that hematocrit is very sensitive to changes in water balance of the body. So, hematocrit can be increased in state of dehydration (for example, after staying in the Finnish bath) and significantly reduced after taking a large amount of fluid, or after transfusion of blood substitution solutions. All possible physiological and pathological causes, which lead to changes in hematocrit, should be taking into account. Normal range of hematocrit level is called ***normocythemia***, its increasing - ***polycythemia***, and decreasing - ***oligocythemia***.

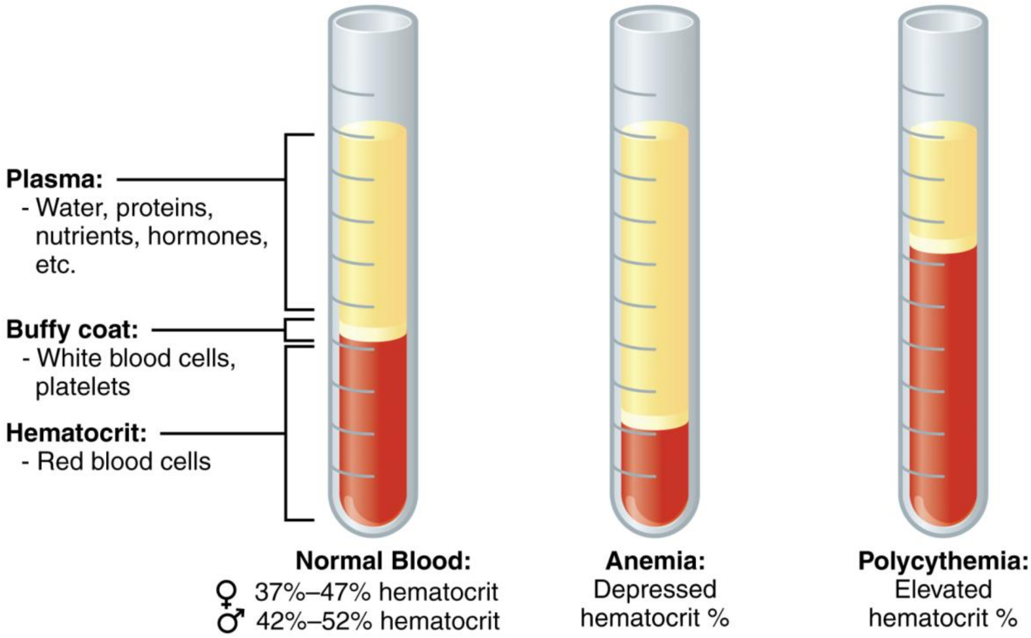


Fig.5.1. Composition of blood.


Blood is an integral part of many functional systems, so it performs a variety of functions within these systems. But they all relate to its overall transport function, which is ensured by the circulation of blood through the vascular system.

The main functions of the blood are:

• **respiratory** – consists in binding and transferring of oxygen from the lungs to the tissues, and carbon dioxide from the tissues to the lungs;

• **trophic** – consists in providing all cells of the body with nutrients and metabolites;

• **excretory** – consists in transporting of metabolism by-products to the excretory organs;

• **thermoregulation** – consists in transferring of heat from organs with higher heat production to other organs;

• **integrative and regulatory** – consists in providing humoral regulation of metabolism;

• **protective** - is associated with the presence of specific and non-specific immunity factors in the blood.

In addition, blood is the source of all liquids, secrets and excretions of the body. Lots of organs and tissues have a significant effect on the blood composition, because blood is involved in the implementation of many functions. Therefore, the functional state of the body organs can be evaluated by analyzing of the blood composition.

## 1.2. The chemical composition of the blood plasma.

The main component of blood plasma is water. The percentage of water in plasma is normally close to 91%. The rest 9% (dry residual) is composed of dissolved substances: electrolytes, carbohydrates, lipids, organic acids and bases, intermediate products of metabolism of nitrogen and non-nitrogenous origin, vitamins and proteins. Some of these components are constant parameters (most electrolytes), but the content of others ones varies greatly depending on the state of the organism (for example, nutrients and waste products).

The major component of the plasma dry residual is proteins – about 7%. Protein content ranges from 66 to 87 g/l. The main plasma proteins are ***albumins*** (35 – 52 g/l), ***globulins*** (20 – 35 g/l), and ***fibrinogen*** (2 – 4 g/l). The ***albumin-globulin coefficient*** (percentage ratio of albumins to globulins content) is important for evaluation of the plasma protein content in doctor practice, and it is in range of 1.3-2.2 for the healthy adult population. Plasma proteins can be divided into fractions using electrophoresis. (Fig.5.2). The percentage ratio of protein fractions is very important for the diagnosis a lot of pathological conditions, for example, some types of anemia, acute inflammatory processes and autoimmune diseases. Increasing of β-globulin fraction is observed in case of iron deficiency anemia due to the increase of the protein ***transferrin,*** that transports iron. The other example is increased percentage ratio of the γ-globulin fraction in patients with myeloma, whose plasmocytes produce a large number of Bence Jones proteins.

***Albumins*** are the most homogeneous fraction of plasma proteins and make the largest contribution to maintaining ***oncotic pressure***. Oncotic pressure is a part of the total plasma osmotic pressure, which determines the fluid movement between the blood and the intercellular fluid. In addition, albumins serve as a reserve of amino acids for protein synthesis and therefore perform a plastic function. The large surface of the albumin molecule and its high negative charge provide maintenance of colloidal plasma stability and suspension properties of the blood. Albumins adsorb and carry out a lot of substances of exogenous and endogenous origin, including bilirubin, steroid hormones, thyroxin, $Ca^{+2}$ ions,

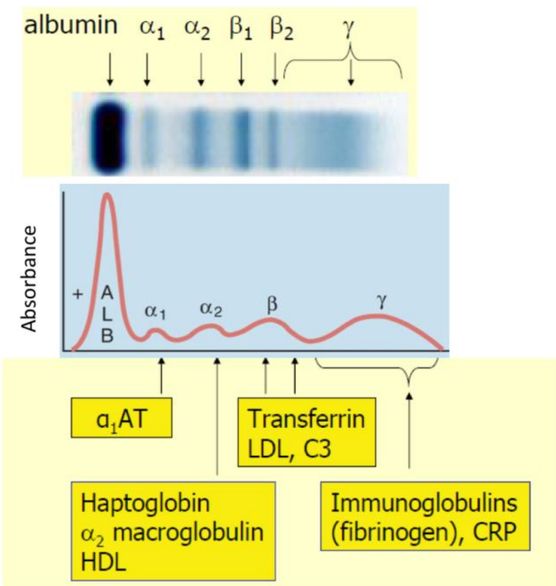


Fig.5.2. Main components of plasma proteins.

penicillin, sulfanilamide on their surface.

***Alpha-globulins*** are glycoproteins, what means that proteins are bound to carbohydrates. So, 2/3 of all blood glucose circulates in form of glycoproteins. These proteins transport hormones, vitamins, microelements. Unlike albumins, globulins are specific to the substrate they transport. Proteins, that transport iron and iodized thyroid hormones, can be a good example. This fraction contains a number of proteolytic enzyme inhibitors, as well as ***prothrombin*** (precursor of thrombin)– one of the blood clotting factors and ***angiotensinogen*** – precursor of active components of RAAS.

Table 5.1.

Major blood components of healthy people.

| Component and % of blood | Subcomponent and % of component | Type and % (where appropriate) | Site of production | Major function(s) |
|---|---|---|---|---|
| Plasma 46–63 percent | Water 92 percent | Fluid | Absorbed by intestinal tract or produced by metabolism | Transport medium |
| | Plasma proteins 7 percent | Albumin 54–60 percent | Liver | Maintain osmotic concentration, transport lipid molecules |
| | | Globulins 35–38 percent | Alpha globulins—liver | Transport, maintain osmotic concentration |
| | | | Beta globulins—liver | Transport, maintain osmotic concentration |
| | | | Gamma globulins (immunoglobulins) —plasma cells | Immune responses |
| | | Fibrinogen 4–7 percent | Liver | Blood clotting in hemostasis |
| | Regulatory proteins <1 percent | Hormones and enzymes | Various sources | Regulate various body functions |
| | Other solutes 1 percent | Nutrients, gases, and wastes | Absorbed by intestinal tract, exchanged in respiratory system, or produced by cells | Numerous and varied |
| Formed elements 37–54 percent | Erythrocytes 99 percent | Erythrocytes | Red bone marrow | Transport gases, primarily oxygen and some carbon dioxide |
| | Leukocytes <1 percent Platelets <1 percent | Granular leukocytes: neutrophils eosinophils basophils | Red bone marrow | Nonspecific immunity |
| | | Agranular leukocytes: lymphocytes monocytes | Lymphocytes: bone marrow and lymphatic tissue | Lymphocytes: specific immunity |
| | | | Monocytes: red bone marrow | Monocytes: nonspecific immunity |
| | Platelets <1 percent | | Megakaryocytes: red bone marrow | Hemostasis |

***Beta-globulins*** are plasma protein fraction, that is the richest in lipids and it transports 75% of all plasma lipids. In addition, this fraction contains the protein ***transferrin***, which transports iron, most of the complement system proteins, many of blood clotting factors.

***Gamma-globulins*** are called immunoglobulins, since this fraction includes antibodies.

***Fibrinogen*** is the main protein, responsible for the blood clotting.

Summarizing the above, it can be noted, that the functions of plasma proteins are:

- maintenance of plasma oncotic pressure;
- providing a suspension state of blood and its rheological properties;
- participation in the maintenance of acid-base balance;
- hemostasis (stopping of bleeding);
- protective;
- transport;
- nutritious (as a reserve of amino acids).

***Plasma electrolytes*** are represented by the $Na^{+}$, $K^{+}$, $Ca^{+2}$, $Mg^{+2}$, $Cl^{-}$, $HCO_{3-}$, $HPO_4^{-2}/H_2PO_4^{-}$, $SO_4^{-2}$ ions. Sodium and chlorine ions determine the osmotic pressure of plasma, volume of circulating blood and are related to the level of blood pressure. Bicarbonates and phosphates determine the buffer reserves of blood plasma. That's why, the state of acid-base balance is evaluated depending on these ions. Potassium and calcium ions play an important role in regulation of myocardium and skeletal muscles contractile function and in a variety of other physiological processes. Moreover, deviation of these ions concentration, both going beyond the upper and lower normal ranges, is dangerous.

The content of some ions in the plasma is very low. Therefore, they are called ***microelements*** (copper, cobalt, iron, manganese, zinc, chromium, strontium). Microelements play an important role in the processes of cell metabolism, as they form part of the prosthetic groups of enzymes, that catalyze biochemical reactions. The concentration of most plasma electrolytes remains relatively constant due to special regulatory systems. In general, the mineral content is about 0,9% of the total plasma volume.

<u>Substances of organic origin</u> make up the rest of the dry plasma residual. These include:

- ***nitrogen-containing products of protein catabolism*** (urea, uric acid, creatine, creatinine), which are generally called ***residual nitrogen***, normally ranged of 14-28 mmol/l. In most cases, the violation of the kidneys excretory function (uremia) can be diagnosed by increasing in the content of residual nitrogen. But this indicator loses its informative value regarding the kidneys function in case of the liver pathology. In such situation, ***creatinine*** (the by-product of metabolism in the skeletal muscle) is more informative indicator, because its concentration in the plasma is inversely proportional to the excretory function of the kidneys.

• ***Plasma lipids*** are evaluated by level of total cholesterol, low density lipoproteins (LDL), very low density lipoproteins (VLDL), high density lipoproteins (HDL), and triglycerides. The fact is, that lipids in plasma are predominantly bound to beta-globulins and form lipoproteins that include cholesterol, triglycerides, and phospholipids. However, high levels of VLDL and LDL suggest violation of the lipids transport inside the cells. At the same time, HDL carry excess of cholesterol, that is released by the cells, into liver. Therefore, low content of HDL indicates a violation of lipid metabolism in tissues or reduced biosynthesis of β-globulins in the liver. The displacements in the ratio of different lipoprotein fractions are considered as a risk factor in the development of vascular atherosclerosis, even in case, when the content of total cholesterol doesn`t go beyond the normal level. Conversely, if total cholesterol is increased, while the VLDL and LDL are in the normal ranges, because of increased HDL content, then it isn`t considering as a serious lipid metabolism disorder. The ***coefficient of atherogenicity (CA)*** can be calculated by the next equation: ***CA = (LDL + VLDL) / HDL***. This coefficient is very important for estimation of atherosclerosis risk development. The dangerous value of CA is > 3.0 units. Increasing in the content of triglycerides also is considered as the risk factor for the development of cardiovascular disease and diabetes mellitus.

• ***Pigment metabolism*** is evaluated according to the indicators of total bilirubin and its fractions: direct and indirect. Increasing in these indices can be observed in case of blood system (hemolytic anemia), liver or bile-excretory system pathology. The fractions of bilirubin allow to distinguish these violations. For example, the increase in total bilirubin occurs due to direct (conjugated) bilirubin in case of bile excretion blockage from the gall bladder (calculous cholecystitis). Such state is called "mechanical jaundice". The indirect (free) bilirubin is predominantly increased in parenchymal lesions of the liver (hepatitis).

• ***Enzymes***– alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and G-glutamyltransferase (GGT) – are using for evaluation of the hepato-biliary system function. Thus, ALP increases with hepatitis, ALT - often increases with blockage of biliary tract, an increase in AST reflects the overall liver damage, GGT is a marker of alcoholic liver injury.

• ***Glucose*** is a compulsory component of the biochemical blood sample, which is important in diagnosis of diabetes mellitus (its concentration on an empty stomach in healthy people ranges from 4,1 to 5,9 mmol/L).

• ***Hormones and vitamins*** – are useful in assessing the functional state of the endocrine glands and to diagnose avitaminosis.

## 1.3. Physical and chemical properties of blood and plasma.

The blood functions are significantly dependent on its physical and chemical properties, among which the most important are osmotic pressure, viscosity, colloidal stability, suspension properties and acid-base balance of blood.

***Osmotic blood pressure*** is created by the substance dissolved in it, and its value is determined by the concentration of all dissolved molecules, and not by their size. Since most of the plasma ions are inorganic ions, the osmotic plasma pressure to a large extent depends on their concentration. In norm, it is about 7,5 atmospheres or 280-300 mosmol/l (1 mosmol / liter is the osmotic pressure of the solution, where 1 mmol of any substance is dissolved in 1 liter of this solution). The part of proteins in osmotic pressure is only 0,03-0,04 atm. or 25-30 mmHg. This part of osmotic pressure is called ***oncotic pressure***. Despite such a small part, oncotic pressure plays an important role in redistribution of the fluid between blood and tissues. The fact is, that the walls of most capillaries are practically impermeable to proteins. The concentration gradient of proteins on both sides of the vascular wall is provided by the low concentration of proteins in the intercellular space. As for inorganic ion, their concentration in the circulatory system and intercellular fluid is almost equal. That`s why oncotic pressure keeps a portion of water in the circulatory system, and its fluctuations are reflected in the redistribution of fluid between blood and tissues.

If the internal fluid or artificially prepared dilutions have the same osmotic pressure as the blood plasma they are called ***isotonic***. The examples of isotonic solution are 0.9% solution of NaCl or 5% glucose solution. Solution with higher osmotic pressure, then isotonic one, is called ***hypertonic***, and with the lower – ***hypotonic***.

Osmotic pressure provides the movement of a solvent through a semipermeable membrane from a solution with a lower concentration to a solution with a high concentration of dissolved substances. Such transition can be observed in the presence of erythrocytes in hyper- or hypotonic solutions. In a hypertonic solution, water from erythrocytes passes into a solution, what explains why they are wrinkled. In the presence of erythrocytes in hypotonic solutions,

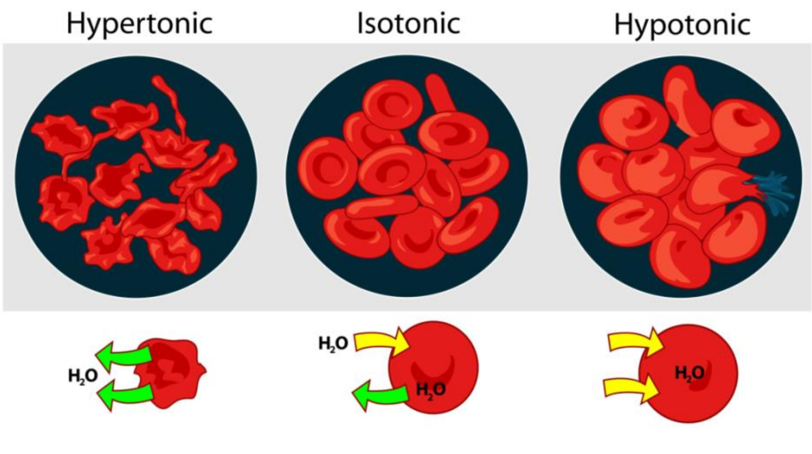


Fig.5.3. Influence of the osmotic pressure on blood cells.
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water on the contrary passes from the solution into erythrocyte, causing its swelling and subsequent hemolysis. Such hemolysis is called ***osmotic***. The practical significance has to determine the ***osmotic resistance of erythrocytes***– that is, their resistance to the action of hypotonic solutions. The index of this resistivity is the concentration of hypotonic solution, in which erythrocytes are not yet hemolyzed. The ***minimal osmotic resistance*** in the norm corresponds to 0,46% NaCl solution. Only the weakest erythrocytes are hemolyzed in such solution. ***Maximal osmotic resistance*** corresponds to 0,33% NaCl concentration, which causes complete hemolysis of all erythrocytes.

The ***blood viscosity*** is its ability to resist the flow of fluid particles moving relative to one another. In other words, this is the parameter that characterizes the internal friction of the liquid. The blood viscosity is determined in relation to the viscosity of water, which is taken as 1. The viscosity of whole blood and plasma is significantly different, since the formed elements substantially increase the internal friction of the liquid. Thus, the viscosity of the plasma is 1,5-1,8, and the whole blood viscosity is 3,5-5,5 units.

The viscosity of whole blood is dependent on the concentration of large-molecular proteins, lipoproteins and on the erythrocytes number. The viscosity raises, when the concentration of proteins and erythrocytes are increased. The viscosity of the blood is directly proportional to the peripheral resistance of the blood vessels; therefore, it affects the heart work essentially. That is, why during dehydration, the temporary increase in hematocrit causes a significant increase in blood viscosity and can lead to heart failure as result. Decrease in blood viscosity is also dangerous (for example, during anemia and hypoproteinemia), because it changes of blood flow in vessels from laminar to turbulent, what in turn can increase the load on the heart.

***Colloid plasma stability*** is associated with a superficial negative charge of proteins, which is called ***φ-potential***. Due to the same charge, the proteins mutually repel and promote the maintenance of large molecular aggregates and formed elements in the suspended state. As the plasma albumin concentration is high, it is preferable to determine plasma's colloidal stability. Immediately decrease in colloid plasma stability and aggregation of erythrocytes can be caused by decrease in concentration of albumins and increase in concentration of

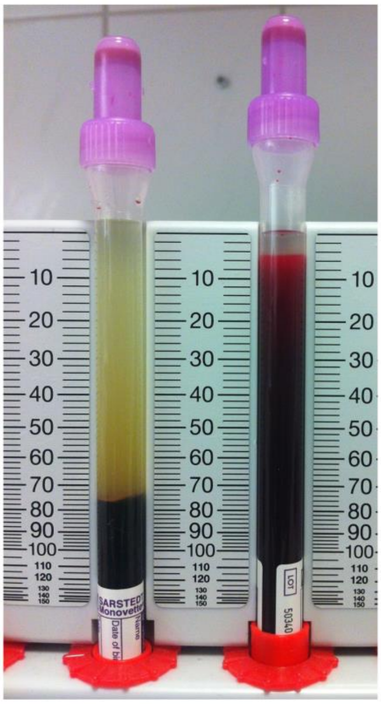


Fig.5.4. Determination of erythrocytes sedimentation rate (ESR).

By Abdullah Sarhan (Own work) [CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0)], via Wikimedia Commons globulins. The measure of the suspension properties of the blood is ***erythrocyte sedimentation rate (ESR)***. In norm it makes: for men 1-10 mm / hour. and for women - 2-15 mm / hour. ESR is an important diagnostic criterion for inflammatory processes and autoimmune diseases, which usually reflects changes in the normal ratio of plasma protein fractions in favor of globulins. There are states of physiological increase in ESR, such as after eating, after heavy physical work, in the last third of the period of pregnancy. Interestingly, in some diseases, the ESR may even be lower than its normal value. For example, it occurs by sickle cell anemia and diabetes mellitus with severe hyperglycemia.

# 2. Functions of erythrocytes

## 2.1. General characteristics of erythrocytes.

Erythrocyte is a great example of highly specialized cells. Its specialization is transport of respiratory gases by the blood. All the morphological features of erythrocyte and its metabolic substances are subordinated to this function.

Fig.5.5. Shape of normal red blood cells.
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Erythrocytes are flexible, biconcave, anucleated cells. This shape provides the most advantageous interrelation between the diffusion surface area and cell volume, what reduces the distance between hemoglobin and outer membrane (diffusion distance) and as result contributes to the rapid gas exchange. In case some pathology states, erythrocytes with altered form can be found, for example, ***spherocytes*** and ***sickle-shaped erythrocytes.***They aren`t able to provide efficient transport of gases and have been quickly destroyed in the reticulo-endothelial system organs. The diameter of the erythrocyte is about 7,5 µm, what is slightly higher than the diameter of the true capillaries. Due to the erythrocytes flexibility they can easily change the shape and pass

Fig.5.6. Sickle red blood cells.
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through capillaries with narrower diameter. Such ability is associated with specific structural proteins (spectrin), presented in the erythrocytes. The mature erythrocyte doesn`t have nucleus, most organelles, in particular - mitochondria. Therefore, it receives the energy necessary to maintain its livelihoods, through anaerobic glycolysis. Glucose is also oxidized by pentose pathway, which results in the formation of ***2,3-diphosphoglycerate***. This substance changes the affinity of hemoglobin to oxygen. The energy that is generated in the form of ATP in erythrocyte is used for the work of active ion pumps, in particular Na-K, which transports sodium out from erythrocyte and potassium into erythrocyte/ Simultaneously calcium pumps that transport calcium out from the cell. Anions, mainly, can freely penetrate through membrane of erythrocytes. In addition, the energy is used to restore the components of erythrocyte, which are gradually destroying. Glycoprotein membranes contain ***sialic acid***, which dissociates and provides a negative charge on the surface of the erythrocyte, which, in turn, contributes to the maintenance of erythrocytes in the suspended state. A very important enzyme of erythrocyte is ***carbonic anhydrase***, which catalyzes the formation

Table 5.2.

Possible causes of anemia.

| Categories of Anemia | Causes or Examples |
|---|---|
| ***Inadequate erythropoiesis*** | |
| Iron-deficiency anemia | Dietary iron deficiency |
| Other nutritional anemias | Dietary folic acid, vitamin $B_{12}$, or vitamin C deficiency |
| Anemia due to renal insufficiency | Deficiency of EPO secretion |
| Pernicious anemia | Deficiency of intrinsic factor leading to inadequate vitamin $B_{12}$ absorption |
| Hypoplastic and aplastic anemia | Destruction of myeloid tissue by radiation, viruses, some drugs and poisons (arsenic, benzene, mustard gas), or autoimmune disease |
| Anemia of old age | Declining erythropoiesis due to nutritional deficiencies, reduced physical activity, gastric atrophy (reduced intrinsic factor secretion), or renal atrophy (depressed EPO secretion) |
| ***Blood loss (hemorrhagic anemia)*** | Trauma, hemophilia, menstruation, ulcer, ruptured aneurysm, etc. |
| ***RBC destruction (hemolytic anemia)*** | |
| Drug reactions | Penicillin allergy |
| Poisoning | Mushroom toxins, snake and spider venoms |
| Parasitic infection | RBC destruction by malaria parasites |
| Hereditary hemoglobin defects | Sickle cell disease, thalassemia |
| Blood type incompatabilities | Hemolytic disease of the newborn, transfusion reactions |

of carbonic acid from carbon dioxide and water, and reverse reaction. Both these reactions are basic for the transport of carbon dioxide by blood.

The normal range of erythrocytes in peripheral blood is aout 4,0-5,0 × $10^{12}$/liter in males and 3,7-4,7 × $10^{12}$/liter in females. Such gender difference is determined by a higher concentration of androgens in men that are responsible for the stimulation of erythropoiesis, as well as with periodical blood loss in women with menstruation. In addition, women have significantly higher percentages of adipose tissue, which is relatively less vascularized, compared to skeletal muscle tissue that prevails in men. Decrease in number of erythrocytes is called ***anemia*** and can be caused by a variety of causes (Table 5.2). Normal range of reticulocytes in peripheral blood ranges from 0,5 to 1,5%. Reticulocytes are the precursor of mature erythrocytes, which have received their name due to the special cell structures constructed by clusters of ribosomes. An increase in the content of reticulocytes may be associated with an accelerated erythropoiesis after bleeding or, with pathological reasons (for example, bone marrow tumors).

## 2.2. Hemoglobin functions.

Complex protein ***hemoglobin*** constitutes almost 90% of the dry mass of the erythrocyte. Its molecular weight is about 60,000 daltons. Each molecule of hemoglobin is formed by the protein part - globin and nonprotein part, consisted from 4 subunits, each of which are represented by ***heme*** (iron-containing porphyrin derivates). The protein part consists of two alpha- and two beta-polypeptide chains. Hemoglobin with a such structure is called ***type A hemoglobin*** ( A - means adult). It forms the bulk of hemoglobin in healthy adults. Fetal blood contains mainly ***type F hemoglobin*** (F - means fetus). The protein part of type F-hemoglobin is represented by two alpha - and two gamma-polypeptide chains. This hemoglobin has a higher affinity to oxygen, but red blood cells with this type of hemoglobin are less flexible and more resistant to deformation. After child birth, type F hemoglobin is gradually replacing by type A-hemoglobin. The S, C and other types of

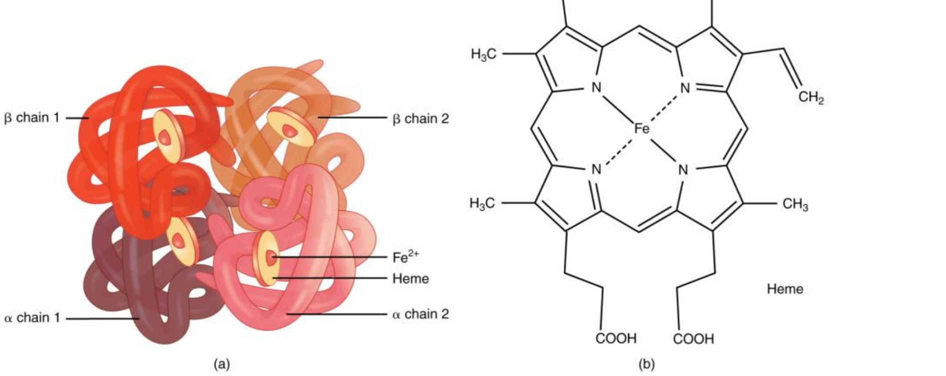



Fig.5.7. Structure of hemoglobine.

hemoglobin can be found in the blood in case of hereditary pathology. These types are differ in the structure of their polypeptide chains and cause red blood cells to have low osmotic and mechanical resistance. The average range of hemoglobin in healthy men is about of **130-160 g/l** and in healthy women – **120-150 g/l**.

Hemoglobin has the ability to bind and easily give up oxygen. This ability can be quantitatively characterized by such an indicator as the ***oxygen capacity of hemoglobin***– the maximal volume of oxygen, which 1 g of hemoglobin can bind. This value is equal to **1,34 ml of $O_2$ per 1 g** of hemoglobin. Oxygen inside hemoglobin molecule is bound to hem and the electrical valence of iron +2 doesn`t change. The saturation of hemoglobin by oxygen significantly depends on the tension of oxygen and carbon dioxide in the blood, the pH of the blood, and the concentration of ***2,3-diphosphoglycerate*** in erythrocytes. The compound of oxygen with hemoglobin is called ***oxyhemoglobin***. It is unstable and dissociates into oxygen and ***deoxyhemoglobin*** in tissues, due to low oxygen and high carbon dioxide tension,. The protein part of hemoglobin has the ability to bind carbon dioxide inside the working tissues, forming a ***carbhemoglobin***. So, about 20% of total $CO_2$ is transported by blood in this form. Deoxyhemoglobin has a significantly greater ability to bind $CO_2$ than oxyhemoglobin. Therefore, in the lungs, the deoxyhemoglobin is converting into oxyhemoglobin, loses the affinity to carbon dioxide and releases it into plasma. These transformations are called the ***Holden`s effect***.

***Carboxyhemoglobin*** is a compound of hemoglobin with carbon monoxide (CO) and it is formed in case of poisoning by CO. The iron valences in this compound are blocked by carbon monoxide and carboxyhemoglobin loses the ability to transport oxygen. This compound is very persistent and difficult in dissociate.

***Methemoglobin*** is formed by the influence of strong oxidants (such as potassium permanganate, cyanides, etc.), which chemically transfer iron from two-valence state to trivalent. Hemoglobin also loses the ability to transport oxygen to tissues, since it is irreversibly binding to heme.

It is known, that hemoglobin binds small amount of ***nitric oxide*** from alveolar air in lungs capillary system and transports it to tissues, where it is cleaved and causes vasodilator effect.

Hemoglobin is an important buffer compound, that determines up to 35% of the total buffer capacity of the blood. Therefore, decrease in hemoglobin content in the blood negatively affects the compensation acid – base balance disturbances. The buffering effect of hemoglobin depends on the cyclic transformation of the deoxyhemoglobin into the oxyhemoglobin during the respiratory process.

## 2.3. Erythropoiesis and its regulation.

Erythropoiesis is the process of red blood cells formation in the red bone marrow. The ***polypotent steam cell*** is the precursor of erythrocytes and it turns into erythrocyte through a series of stages lasting 3-5 days (Fig.5.8). The immediate precursors of mature erythrocytes are ***reticulocytes*** (cells,

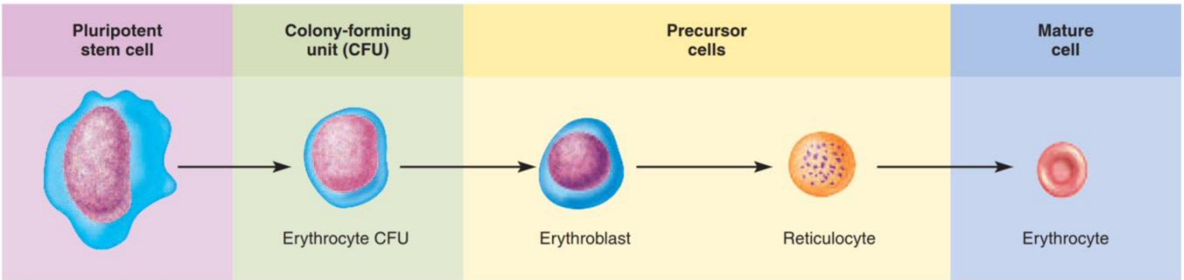



Fig.5.8. Main stages of erythropoiesis.

that have granular or filamentous formations inside the cytoplasm).

***Erythropoiesis*** is controlled by the renal hormone erythropoietin. Small amount of this hormone is also produced by macrophages of peripheral blood and liver. In the kidneys, erythropoietin is synthesized by peritubular cells of renal tubules, that are hypoxia-sensitive. Kidney hypoxia can occur, for example, when a person temporarily stays in the mountains (where the partial pressure of oxygen is low), or in case of blood loss, when hematocrit decreases, etc. In all these cases, the secretion of erythropoietin increases. Decreases in erythropoietin secretion can be caused by increase in the oxygen partial pressure in the blood. Erythropoietin intensifies the proliferation of precursor cells of the erythrocytes and stimulates the synthesis of hemoglobin in these cells. The mechanism of erythropoietin action is realized through the influence on the cell nucleus and stimulation of the m-RNA synthesis necessary for the formation of enzymes, catalyzing the synthesis of hemoglobin.

***Androgens,*** that have a reactogenic action in relation to erythropoietin, and ***catecholamine,*** that stimulate proliferation of cells in the erythroid series are non-specific stimulators of erythropoiesis. Female sex hormones affect the erythropoiesis in the opposite way.

Normal erythropoiesis requires a number of biologically active substances in the bone marrow, that should be transported to bone marrow. First of all, it is ***vitamin*** $B_{12}$ and ***folic acid (vitamin*** $B_9$***)***. These substances take an active part in the in the dividing and maturing of the erythrocyte precursors cells. If there is a deficiency of these substances in the body, giant nuclear slow maturated cells –***megaloblasts*** are formed instead of normal erythrocytes. Anemia, which occurs in this case, is called ***pernicious anemia*** (malignant). Very often, inadequate production of gastromucoprotein (***internal Castle factor***) by parietal cells of the gastric mucosa causes the deficiency of vitamin $B_{12}$. The $B_{12}$ itself is called the ***external Castle factor***. The role of the internal factor consists in binding $B_{12}$, what prevents its destroying by gastric juice. For example, the pernicious anemia occurs due to the deficiency of the internal factor of the Castle in patients with gastritis and peptic ulcer.

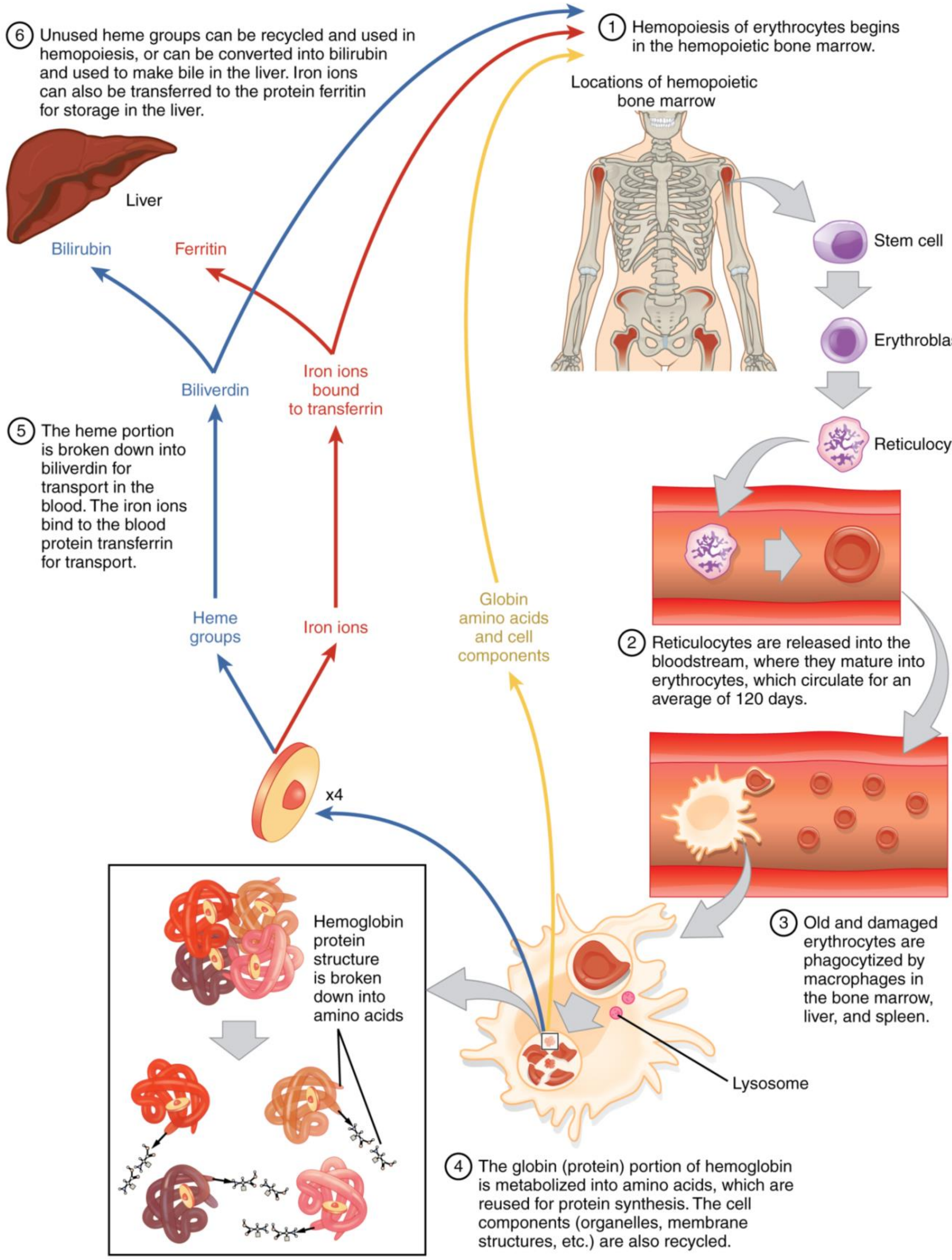



Fig.5.9. Erythrocyte life cycle.

Vitamins $B_2$, $B_6$, C, E, PP; microelements: copper, nickel, cobalt, which take part in the synthesis of heme as cofactors of enzymes, are also necessary for normal erythropoiesis.

Erythropoiesis requires daily delivery of about 25 mg iron into the bone marrow. Almost all this amount of the iron is coming into the red bone marrow for reuse after the destroying of erythrocytes in the spleen and the reticuloendothelial system, Only about 1 mg of iron per day is absorbed in the small intestine, what replaces its loss with urine, feces, and menstrual blood.

The iron is consumed in the form of $Fe^{+2}$ and $Fe^{+3}$ ions. However, only bivalent iron ions can be absorbed by the intestinal epithelium. Most $Fe^{+3}$ ions are converted into $Fe^{+2}$ ions by the hydrochloric acid, presented in the gastric juice. Therefore, the iron absorption is disturbed in patients with low acidity of the gastric juice. In the stomach, bivalent iron ions bind to the protein ***gastroferritin***, which is produced by the gastric glands, and transported in this way to the small intestine. Then it is absorbing into the blood, where it binds to the transport plasma protein, called ***transferrin*** - one of the components of the beta-globulin fraction. The concentration of this protein in the plasma ranges from 1.8 to 2.6 mg/L and it transports approximately 3 mg of iron. Transferrin can be binding to the erythroblast membrane receptors. Mature erythrocytes don`t have such receptors, therefore they are unable to absorb iron. After transferrin breakdown, iron enters the erythroblast, where it is used for the synthesis of heme, and its excess will be stored in form of protein, called ***ferritin***. Ferritin, in turn, is absorbed by macrophages and forms amorphous insoluble aggregates – called ***hemosiderin,*** inside the lysosomes. This process most actively occurs in the liver due to synthesis of the main component of ferritin – protein apoferritin - by hepatocytes. In this case, ferritin releases iron ions, which are using for erythropoiesis, biosynthesis of myoglobin and cytochromes. The removal of iron ions from the depot is regulated by the hormone of the liver, called ***hepsidin***, which blocks the membrane protein, responsible for iron ions transport from the cell.

The typical duration of the erythrocyte life is on average 120 days. Aging of erythrocyte results in the destruction of membrane proteins and the release of its contents to the extracellular space. Particularly, intensive removal of aged red blood cells occurs when blood flows through the microcirculatory vessels of the spleen. Erythrocytes with the weakest cell membrane are destroyed here and their content is absorbed by macrophages and metabolized to the initial components. At first, the heme is cleaved from globin, and the iron from heme is released into the plasma, where it binds to transferrin, and is transported to the red bone marrow for reusing. The rest of the heme is converted into a pigment of ***biliverdine***, and then - into a pigment ***bilirubin*** by macrophage enzymes, which are secreted in the blood, and binds to albumins. The liver removes it from albumin and includes into the bile, that will be released into the digestive tract. Bilirubin, as a part of bile, moves into intestine, where microflora converts it into ***urobilinogen***, which gives the characteristic brown color to the feces. A part of bilirubin is converted into ***urobilin*** (urochrome), which causes a yellow color of urine. Thus, high level of bilirubin in the blood may be due to various causes, such as excessive hemolysis of erythrocytes, liver diseases, or pathology of the biliary system. It is possible to distinguish these conditions using a biochemical blood analysis data, which provide information about various bilirubin fractions (direct and indirect).

## 2.4. Blood groups according to the ABO system and the Rh factor.

There are about 300 antigens on the membrane of erythrocytes, which cause the formation of antibodies against themselves, entering inside the body of other people. Erythrocytes of each person contain an individual set of specific antigens. Depending on the presence of certain antigens in erythrocytes, blood is classified into groups according to different systems. The classification of blood into groups by the ABO system and the Rh factor is the most practical.

The ***ABO system*** was proposed in 1900 by Carl Landsteiner, who received the Nobel Prize in 1930 for his study. This system is based on the presence antigens H, A and B inside erythrocytes, which are called ***agglutinogens***. However, Landsteiner believed that only 2 of these agglutinogens exist: A and B. The blood, erythrocytes of which don't contain neither A nor B agglutinogens, was designated as O. Nowadays it is shown that agglutinogens A and B are formed by transformation of the antigen H, which is contained in erythrocytes of group O. The main special feature of this system is, that blood plasma of each person contains antibodies to agglutinogens A and B in different combinations. These antibodes are called as ***agglutinins anti-A and anti-B***. In case of presence in blood the agglutinogens and agglutinins with the same designation ( A and anti-A. or B and anti-B) simultaneously, erythrocytes are destroying by hemolysis. For example, it can occur during incorrect hemotransfusion.

<u>Therefore, simultaneous circulation in the blood of the same agglutinogen and agglutinin is impossible.</u>

The system ABO distinguishes 4 blood groups (Fig.5.10):

- ***Group I*** includes red blood cells with agglutinogen H and agglutinins anti-A and anti-B in plasma;
- ***Group II*** includes red blood cells with agglutinogen A and agglutinin anti-B in plasma;
- ***Group III*** includes red blood cells with agglutinogen B and agglutinin anti-A in plasma;
- ***Group IV*** includes red blood cells with agglutinogens A, B; agglutinins are absent in plasma.

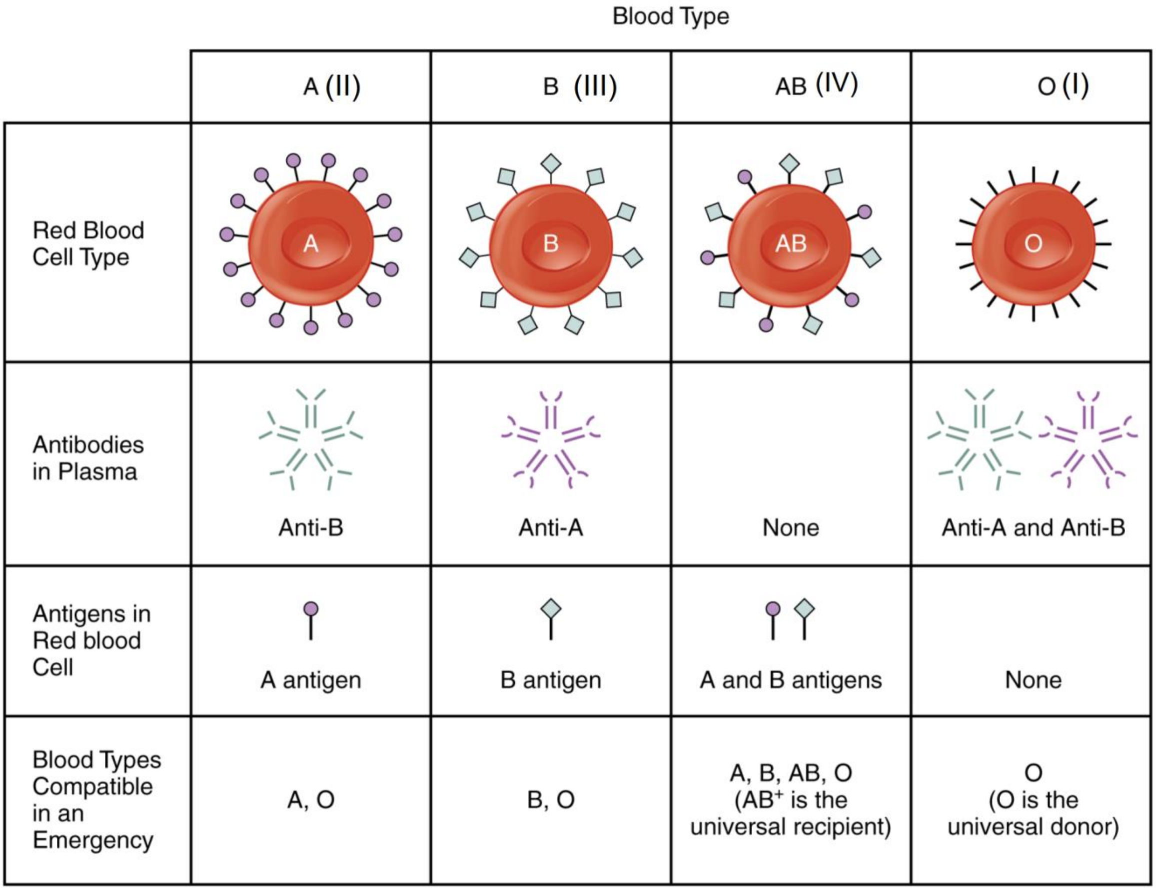



Fig.5.10. Blood types according to ABO system.


To exclude the possibility of group conflicts in transfusions, blood must be transfused only of the same group, although theoretically there are combinations of donor and recipient groups, that do not cause transfusion complications. In particular, erythrocytes of the first group are not agglutinated by serum of other groups, since they do not contain antibodies to the H-antigen. Therefore, a person from group I sometimes is called as an absolute donor. However, the plasma of the blood group I contains agglutinins anti-A and anti-B, which agglutinate erythrocytes of other groups (the recipient), especially when large volumes of blood are transfused.

In the case of incompatible blood transfusion into the recipient's body, the immune agglutination reactions of the donor erythrocytes with the agglutinins of the recipient occur. Accumulation of agglutinated erythrocytes clogged the small vessels and causes the microcirculatory disturbances in the recipient's organs, which may lead to pathient’s death.

***Blood groups according to the Rh factor.***

Depending on the presence of Rh-factor in erythrocytes, the blood is divided into *Rh-positive* and ***Rh-negative***. Rh-antigen is a complex antigen that includes 3 components, denoted as antigens C, E, D. The most potent antigen, that determines the Rh activity of the blood, is the D-antigen.

Erythrocytes of Rh-positive blood contain D-antigen. Accordingly, it is absent in the erythrocytes of Rh-negative blood. Normally, the blood does not contain antibodies to the D-antigen, which distinguishes this classification from the ABO system. If Rh-positive blood enters to the body of the Rh-negative recipient, his immune system begins to produce anti-rhesus antibodies and becomes sensitized to the Rh-factor. Repeated transfusion of such blood to the previous sensitized recipient is very dangerous, because it causes transfusion complications, which are similar to the transfusion of incompatible blood according to ABO system.

The such the situation is clinically important, when the pregnant woman carrying child with inherited Rh-positive blood from the father (Fig. 5.7. Fetal erythrocytes may enter to the mother's blood during later stages of pregnancy and at birth, what leads to production of anti-rhesus antibodies by the mother immune system (sensitization). In case of repeated pregnancy,

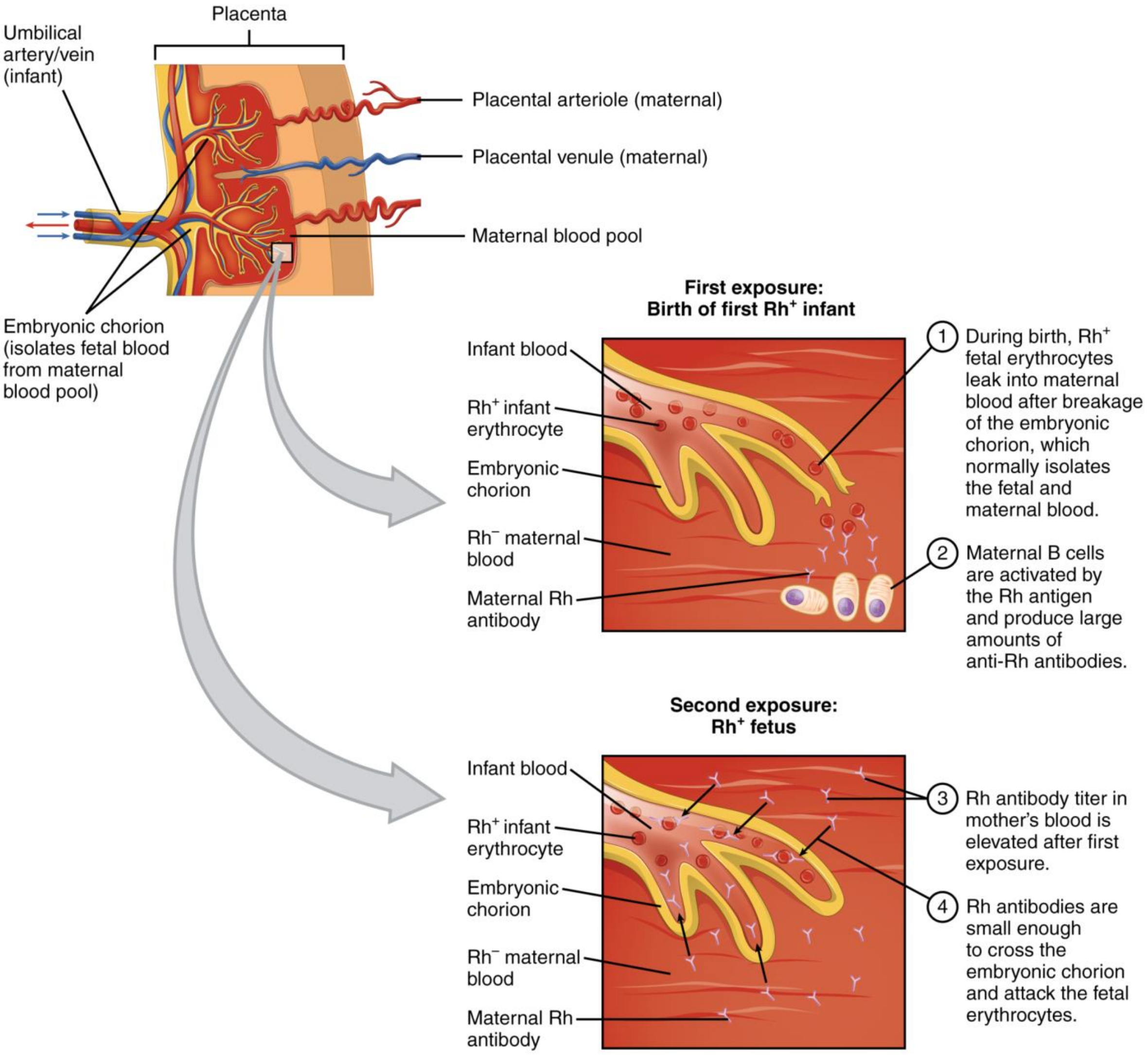



Fig.5.11. Erythroblastosis fetalis.

when the child inherits blood Rh-group from the father again, the antibodies of the sensitized mother immune system will hemolyze child erythrocytes, which, as a rule, causes the abortion and the birth of the dead fetus. If the fetus survives, then he will be born with hemolytic disease of the newborn or ***fetal erythroblastosis***. Fetal erythrocyte hemolysis stimulates fetus hematopoietic tissue to release the large quantity of immature erythrocytes precursors (erythroblasts) into the peripheral blood. Radical prevention of such situation for Rh-negative women is achieved by choosing Rh-negative sexual partner. But since there are only few Rh-negative males (about 15% of the European population), another way of Rh-incompatibility prevention, called ***D-prevention***, has been developed. The essence of this method consists in the injection of anti-D-serum to the mother’s body in the late stages of pregnancy (28-32 weeks), when the fetus blood can contact with the mother's blood. Such serum contains the D-antibodies in high concentration and rapidly hemolyzes the fetus erythrocytes, not allowing them to stimulate the mother immune system to produce her own D-antibodies. This serum is obtained by immunization of guinea pig with Rh-positive blood.

Along with the classification of blood into groups by the ABO system and the Rh-factor, there are many others classifications, (Duffy, Kell, Kidd, Lewis, and others), which take into account about 500 different antigens and their combinations on the erythrocyte surface. Sometimes, the incompatibility of these groups can cause transfusion complications and hemolytic disease of newborn, even if there is blood compatibility by the ABO system and the Rh factor. However, the consideration of these groups is very important for criminology, anthropology and population genetics.

# 3. Leukocytes and their functions

## 3.1. General characteristics of leukocytes.

The number of white blood cells in adults is normally 4–10x$10^9$/l. Increasing in the number of leukocytes is called ***leukocytosis***, and decreasing – ***leukopenia***. The main function of leukocytes is protective one. Unlike erythrocytes, leukocytes perform most of their functions in the connective tissue of the skin and internal organs, and to a lesser extent -- in the blood. Leukocytes enter the bloodstream from the places of their formation (bone marrow, thymus, lymphoid tissue), circulate in the blood for several hours and migrate to the tissue. The average lifetime of leukocytes (except immune memory lymphocytes) is 3-5 days. White blood cells are able to move independently from bloodstream using their contractile proteins. Unlike erythrocytes, they have nucleus and other organelles. Depending on the presence of granules in the cytoplasm, the leukocytes are divided into two groups: ***granulocytes*** (containing granules) and ***agranulocytes*** (without granules). The main types of granulocytes are neutrophils, basophils and eosinophils. Agranulocytes are divided into lymphocytes and monocytes (table.5.3).

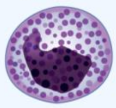
*Neutrophils* make up the majority of all leukocytes in the blood (in average 40-70%). Their granules have an affinity to neutral dyes, what gives them the name. Neutrophil diameter is in average 9-12 microns. They are a non uniform cells and consist of young, rod-nuclear and segmental-nuclear neutrophils. So, in young forms the nucleus is round; in rod-nucleus - elongated, and in segmented-nuclear - has 2-3 projections. After exiting the bone marrow, neutrophils are remaining in the blood for only 6-8 hours and migrate rapidly to the tissues. The main function of neutrophils is to phagocyte the foreign microorganisms and tissue debris. Sometime, they are called ***microphages*** for their ability to phagocytosis and relatively small size. The lysosome enzymes (proteases, peroxidases, DNA acids, lipase) are responsible for digestion of microorganisms, which were previously phagocyted. Neutrophils can produce energy, like erythrocytes, by anaerobic glycolysis, so they are able to perform their functions in tissues with low oxygen content (by edema, or inside of inflammation places). So, the pus, formed during inflammatory processes, consists mainly of neutrophils and their residues. It is believed, that the gender identity of a person can be determined using cytological investigation of neutrophils. Thus, at least 7 of the 500 neutrophils in female have specific structures, called ***the drum sticks***. Actually, they are round-shaped chromatin granules, connected to the nucleus with a thin connections.

*Eosinophils*- have such name due to the tendency of their granules to acidic dyes. Their number makes on average 2-4% of all leukocytes. This quantity undergoes pronounced daily fluctuations. Their number is 20% less than the average daily during the second half of the day and in the morning, and 30% more – at the night. These deviations are inversely related to the level of glucocorticoid secretion by the adrenal cortex. Eosinophil diameter is about of 10-14 microns. These cells also are considered as microphages, but their phagocytic activity is lesser compared to the neutrophils. Eosinophils perform a number of specific functions, related to the inactivation of factors, responsible for the development of allergic and autoimmune reactions, and they also have a toxic effect on helminthes and their larvae.

***Basophils***– are the cells with diameter about of 8-10 microns and they have a segmented nucleus. Their granules are prone to absorb the alkaline dyes. The amount of basophils in blood is in range 0.5-1% of the

Table 5.3.

Morphological and functional characteristics of leukocytes.

| | | | | | |
|---|---|---|---|---|---|
| **Leukocytes (white blood cells)** | | 7000 (5000–10,000) per 1 $mm^3$ | Obvious dark-staining nucleus | All function in body defenses | Exit capillaries and move into tissues; lifespan of usually a few hours or days |
| | **Granulocytes including neutrophils, eosinophils, and basophils** | 4360 (1800–9950) | Abundant granules in cytoplasm; nucleus normally lobed | Nonspecific (innate) resistance to disease | Classified according to membrane-bound granules in cytoplasm |
| | Neutrophils  | 4150 (1800–7300) | Nuclear lobes increase with age; pale lilac granules | Phagocytic; particularly effective against bacteria. Release cytotoxic chemicals from granules | Most common leukocyte; lifespan of minutes to days |
| | Eosinophils  | 165 (0–700) | Nucleus generally two-lobed; bright red-orange granules | Phagocytic cells; particularly effective with antigen- antibody complexes. Release antihistamines. Increase in allergies and parasitic infections | Lifespan of minutes to days |
| | Basophils  | 44 (0–150) | Nucleus generally two-lobed but difficult to see due to presence of heavy, dense, dark purple granules | Promotes inflammation | Least common leukocyte; lifespan unknown |
| | **Agranulocytes including lymphocytes and monocytes** | 2640 (1700–4950) | Lack abundant granules in cytoplasm; have a simple-shaped nucleus that may be indented | Body defenses | Group consists of two major cell types from different lineages |
| | Lymphocytes  | 2185 (1500–4000) | Spherical cells with a single often large nucleus occupying much of the cell's volume; stains purple; seen in large (natural killer cells) and small (B and T cells) variants | Primarily specific (adaptive) immunity: T cells directly attack other cells (cellular immunity); B cells release antibodies (humoral immunity); natural killer cells are similar to T cells but nonspecific | Initial cells originate in bone marrow, but secondary production occurs in lymphatic tissue; several distinct subtypes; memory cells form after exposure to a pathogen and rapidly increase responses to subsequent exposure; lifespan of many years |
| | Monocytes  | 455 (200–950) | Largest leukocyte with an indented or horseshoe-shaped nucleus | Very effective phagocytic cells engulfing pathogens or worn out cells; also serve as antigen-presenting cells (APCs) for other components of the immune system | Produced in red bone marrow; referred to as macrophages after leaving circulation |

total number of leukocytes. Basophile granules contain such biologically active substances as heparin and histamine. Heparin is a substance, that prevents blood clotting (an anticoagulant), and histamine has powerful vasodilation effect. Gamma-globulin receptors are located on the surface of the basophils, to which IgE fraction of immunoglobulines can bind. This fraction is responsible for allergic reactions. After binding, the complex antigen-antibody is fixed on the receptors of the basophile membrane and causes release of histamine from granules, what results in typical allergic manifestations – skin redness, itching, and, sometimes, bronchial spasm. Therefore, the increase in the number of basophils indicates predisposition to allergic reactions.

***Lymphocytes***– make up 20-40% of all leukocytes. Their cytoplasm does not contain granules, and the nucleus has round shape. Unlike granulocytes, these cells, after being released into the blood, retain the ability to proliferate and differentiate. There are two large fractions of lymphocytes: T-lymphocytes and B-lymphocytes. T-lymphocytes are formed in the thymus, and B-lymphocytes – in the bone marrow. After leaving the places of maturation, they migrate into the secondary lymphoid organs – lymphoid nodes, spleen, lymphoid tissue of the stomach and intestine, pharyngeal tonsils. When foreign bodies (antigens) enter the macroorganism, lymphocytes, that are sensitive to them, are intensively multiplying and provide the formation of so-called cellular and humoral immunity.

***T-lymphocytes*** are responsible for the cellular immunity. Depending on the specialization in the implementation of the immune response, there are three subtypes of T-lymphocytes: T-helper cells, cytotoxic T-lymphocytes (T-killers), and T-suppressors (cells suppressing the cellular and humoral immunity interactions). T-lymphocytes make up 70-80% of all lymphocytes; the remaining part - 20-30% -are ***B-lymphocytes,*** responsible for the humoral immunity. After antigenic stimulation, they are multiplying and mature into plasmocytes, that are capable of producing a large number of antigen specific antibodies.

1-5% lymphocytes do not have the properties of neither T- nor B-lymphocytes. They are considered as special fraction – "***zero lymphocytes***." Their most probable function is the destruction of cancer cells and infected by viruses cells.

A special type of lymphocytes –***natural killers (NK cells)*** specialize in the destruction of cancer cells and play an important role in the innate immunity. They are somewhat larger, than normal lymphocytes, and contain granules with enzymes, that damage the membranes of their target cells. Their content in a healthy person is on average 100-200x10*$^{6}$ cells/liter.

***Monocytes***– make up 2-10% of all leukocytes. They have the largest size comparing to all other leukocyte types – 16-20 microns in diameter. They are circulating in the blood after exiting the bone marrow for up to 3 days. Then monocytes migrate to the tissues, proliferate and turn into fixed, histiocytes, which can also be multiplied in the inflammation areas. They are transformed into osteoclasts in the bone tissue The main function of monocytes is the phagocytosis of bacteria and injured cells. Therefore, they be considered as macrophages. Monocytes co-operate with lymphocytes in realization of the immune response. In particular, they provide the presentation of antigens to other immunocompetent cells. Macrophages synthesize a number of biologically active substances – components of the complement system, interferon, as well as ***endogenous pyrogen***– a protein that enters the bloodstream, shifts the thermoregulatory center of the hypothalamus toward the higher fixed point and causes an increase in body temperature during inflammatory processes.

## 3.2. Leukogram, its age related and pathological changes.

Percentage ratio of different forms of leukocytes in peripheral blood is called leukocyte formula or leukogram.

Table 5.4

**Leukogram of the healthy adult**

| Leuko-cytes` type | Neutrophils | | | Eosino-phils | Baso-phils | Lympho-cytes | Mono-cytes |
|---|---|---|---|---|---|---|---|
| | juvenile | immature | segmented | | | | |
| Percent | 0 – 1 | 1 – 5 | 45 – 70 | 1 – 5 | 0 – 1 | 20 – 40 | 2 – 10 |

An increase in the number of juvenile and immature forms of neutrophils is called ***shift to the left***. Such shift is often due to increased leukopoiesis in myeloid leukemia. However, it can also be detected after a considerable amount of blood loss and reflects reparation processes in the red bone marrow. ***A shift to the right*** means an increase in the percentage of segmented forms of neutrophils above the norm and occurs much less frequently than the shift to the left.

The leukocyte formula has pronounced age-specific features. Thus, a child is born with a ratio of neutrophils to lymphocytes like adult. But followed next days it begins to increase the percentage of lymphocytes and decrease the percentage of neutrophils. Their numbers become equal to 5-6 days of life. This equalization is called the ***first crossing of the leukocyte formula***. The number of lymphocytes increases after first crossing until 5 years of life and exceeds the relative content of neutrophils. But then at age of 5-6 years the ***second crossing of the leukocyte formula*** occurs, when the percentage of neutrophils and lymphocytes become equal again. After second crossing the leukocyte formula gradually acquires an appearance, typical for adult.

The leukocyte formula also changes significantly in some pathological conditions. Thus, acute bacterial infections, as a rule, are accompanied by ***neutrophilic leukocytosis*** and a decreasing in the number of eosinophils and lymphocytes. The appearance of ***monocytosis*** indicates a favorable course of the infectious process. Chronic infections are characterized by ***lymphocytosis***. ***Eosinophilia*** is observed in autoimmune diseases and intestinal invasions by helminthes. An allergy, as a rule, is accompanied by an increase in the percentage of basophils. When evaluating a leukocyte formula, it is necessary to take into account that it is informative in the absence of significant leukocytosis. Therefore, the more reliable diagnostic index is the absolute value of different types of leukocytes in blood

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## 3.3. Immunity. Its types and basic mechanisms.

Our body is constantly attacked by viruses, bacteria, Protozoa and their waste products . These microorganisms and molecules are carriers of foreign genetic information and threaten our biological identity. Even our own cells can become genetically foreign as a result of mutations when they are affected by various factors (viruses, microorganisms, radiation),.There is a special system in the body, that identifies and destroys such genetically alien cells and macromolecules. This system is called ***immune system***. Its main components are bone marrow, thymus, lymph nodes and lymphoid formations of the stomach and intestines, as well as lymphoid tissue of internal organs and leukocytes in blood and tissues. The immune system provides two types of protective mechanisms: ***nonspecific***– directed against all factors, regardless of their origin; and ***specific*** ones - directed specifically against a certain alien factor. Each of these types is realized in two ways: through ***cellular*** and ***humoral*** immunity. The cellular immunity is related to the influence of leukocytes on the alien factor, and the humoral– destroying the invaders by dissolved in the blood and intercellular fluid protective substances of protein origin and antibodies (immunoglobulins). The division of protective mechanisms into specific and nonspecific is conditional, because the inclusion of nonspecific mechanisms requires the prior recognition of the foreign factor by specific mechanisms. That is, why both types of mechanisms interact during the immune response. As result, four types of immunity exist, depending on their mechanisms:

1. ***Non-specific cellular immunity*** includes the inflammatory response and phagocytosis, which are realized by micro- and macrophages, dendritic and NK-cells.
2. ***Non-specific humoral immunity***, which is presented by:
   • Lysozyme – protein, contained in saliva, lacrimal fluid, blood, airway mucus and other body fluids.
   • Complement system – consists of 11 plasma proteins, which are denoted by the letters C 1 ... 11. In physiological conditions, these

proteins are inactive, but can be activated directly by foreign agent, as well as through the specific cellular mechanisms. Sequential cascade activation of these proteins leads to the lysis of foreign cells membranes and death of infected cells.

• Properdine system – consists of three components: peptide P, factor B - glycoprotein and proteolytic enzyme D. This system also affects foreign antigens.

• Leukins and beta-lysine – substances, released by white blood cells;

• Plakins – substances, secreted by platelets.

• Interferon – a protein, secreted by B- and T-lymphocytes, macrophages and fibroblasts, which has antiviral activity.

3. *Specific cellular immunity* are realized by T-lymphocytes, macrophages and dendritic cells. During the entire life, the bone marrow is delivered to the bloodstream, and from there into the thymus, small amounts of precursors of T-lymphocytes. They acquire superficial receptors for various antigens in the thymus and go out into the blood, migrating to the secondary lymphoid organs. If these organs are affected by the antigen, those lymphocytes, that have receptors for this antigen, are intensively proliferated and differentiated into effector T-lymphocytes. T-lymphocytes make up 60-80% of all lymphocytes. Approximately 10% of this amount circulate in the blood for a very long time (up to 10 years), without dividing. These are the cells of the immune memory. If antigen enters the bloodstream, it activates these cells, and they immediately stimulate the proliferation of effector T-lymphocytes, that destroy the antigen. Among effector T-lymphocytes the following classes are distinguished:
    - T-helper cells – stimulate differentiation of B-lymphocytes and proliferation of cytotoxic T-lymphocytes;
    - T-killers –provide cytotoxic effect on the antigens;
    - T-suppressors – suppress the immune response to a specific antigen, controlling its intensity.
4. *Specific humoral immunity* is providing by B-lymphocytes, which are formed in the red bone marrow. But their differentiation is carried out in the lymph nodes, the spleen, and the Payer`s plaques of the intestine. B-cells have receptors to antigens on their surface, which are represented by immunoglobulins M type. The small quantity of B-lymphocytes continues to circulate in the blood, providing the immune memory. Most of B-limphocytes migrate into tissues and are converted to plasma cells, that secrete antibodies.

## 3.4. Cooperation of immunocompetent cells in the immune response.

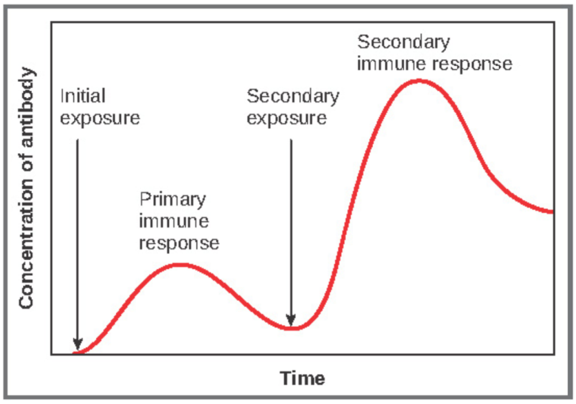



Fig.5.12. Primery and secondary immune responce.


The first exposure of the antigen to the body causes proliferation of immune memory lymphocytes and the formation of a small number of effector T-lymphocytes and antibodies after a certain latent period, that lasts from a few days to several weeks. This reaction of the immune system is called the ***primary immune response***. It unfolds, usually, within 3-4 weeks. In case of the next exposure of this antigen, a high concentration of antibodies to it is produced quickly without any latent

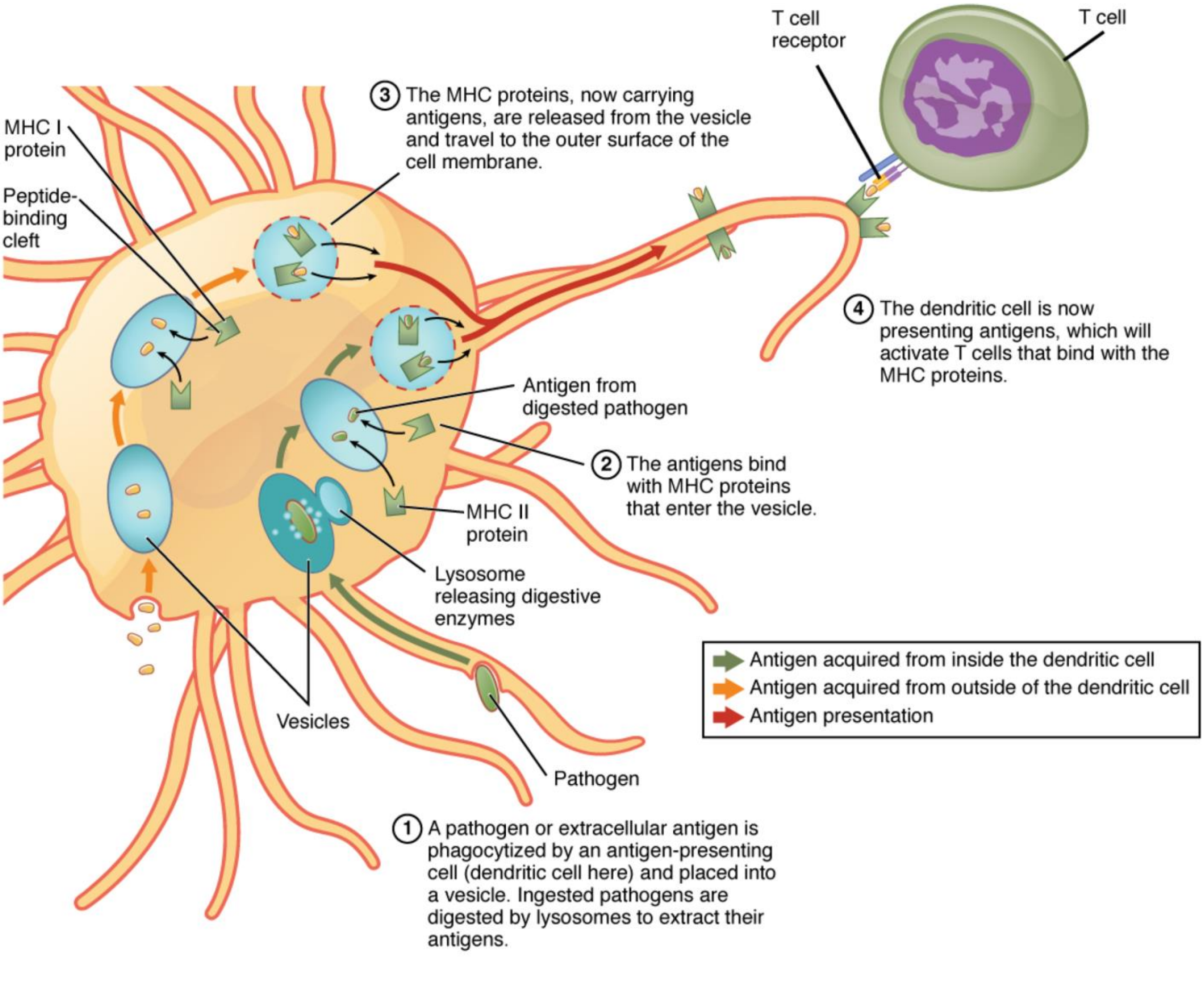



Fig.5.13. Antigen processing and presentation.

period. Beside of this, it is occurred a significant increase in the number of effector T-lymphocytes, specifically sensitive to this antigen in the blood. Described events are called the ***secondary immune response***, which is maintaining for a long time: months and even years.

The implementation of the immune response requires the cooperation of various immunocompetent cells. Only antigen-expressing cells, which include macrophages, dendritic cells and B-lymphocytes, can react directly with antigens. After meeting antigen (viruses, bacteria, foreign proteins), they are absorbed by antigen-presenting cells (Fig. 5.13). In these cells, antigenic determinants are isolated from foreign objects using lysosomal enzymes,

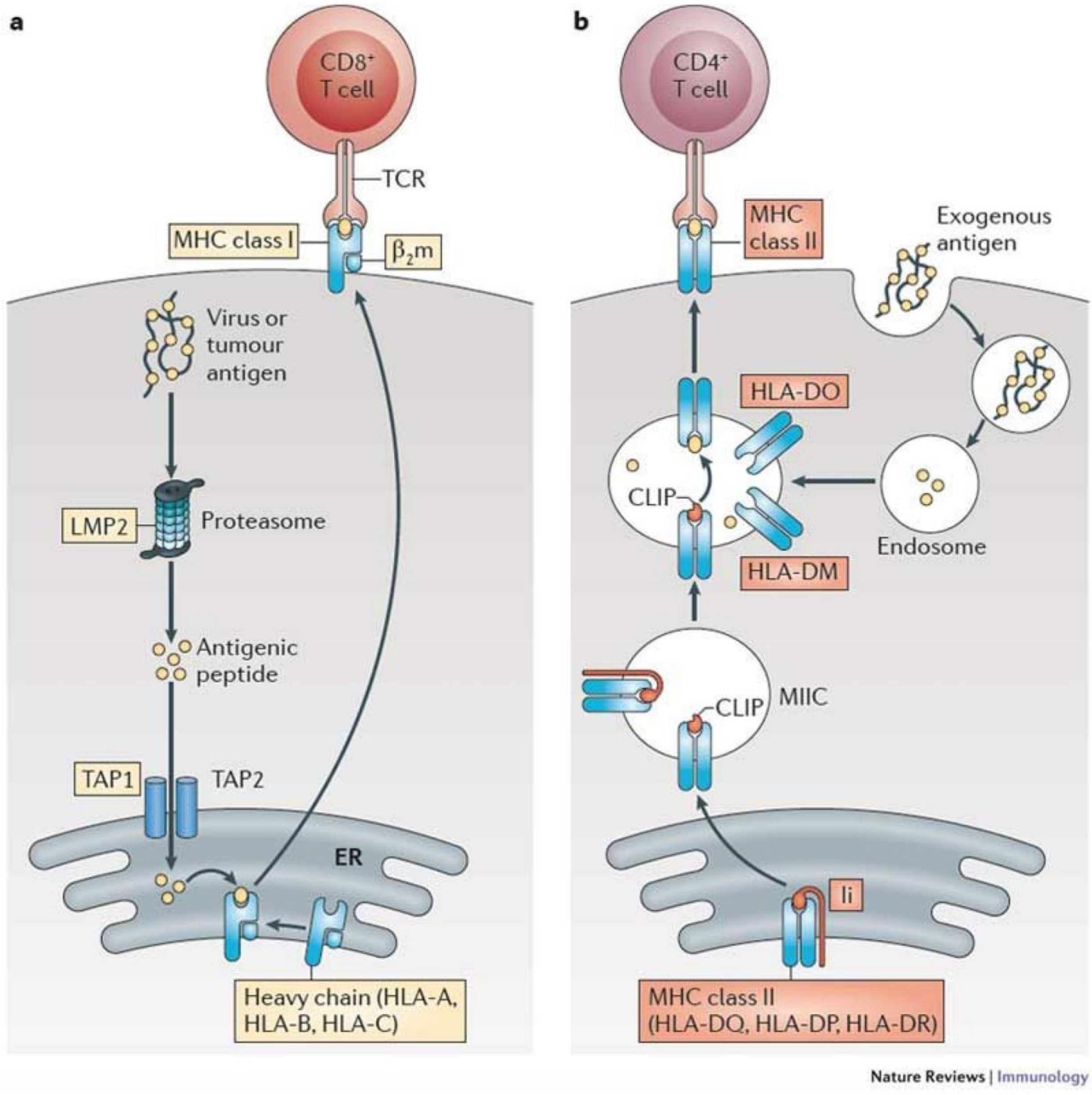



Fig.5.14. The difference between MHC I (a) and MHC II (b).

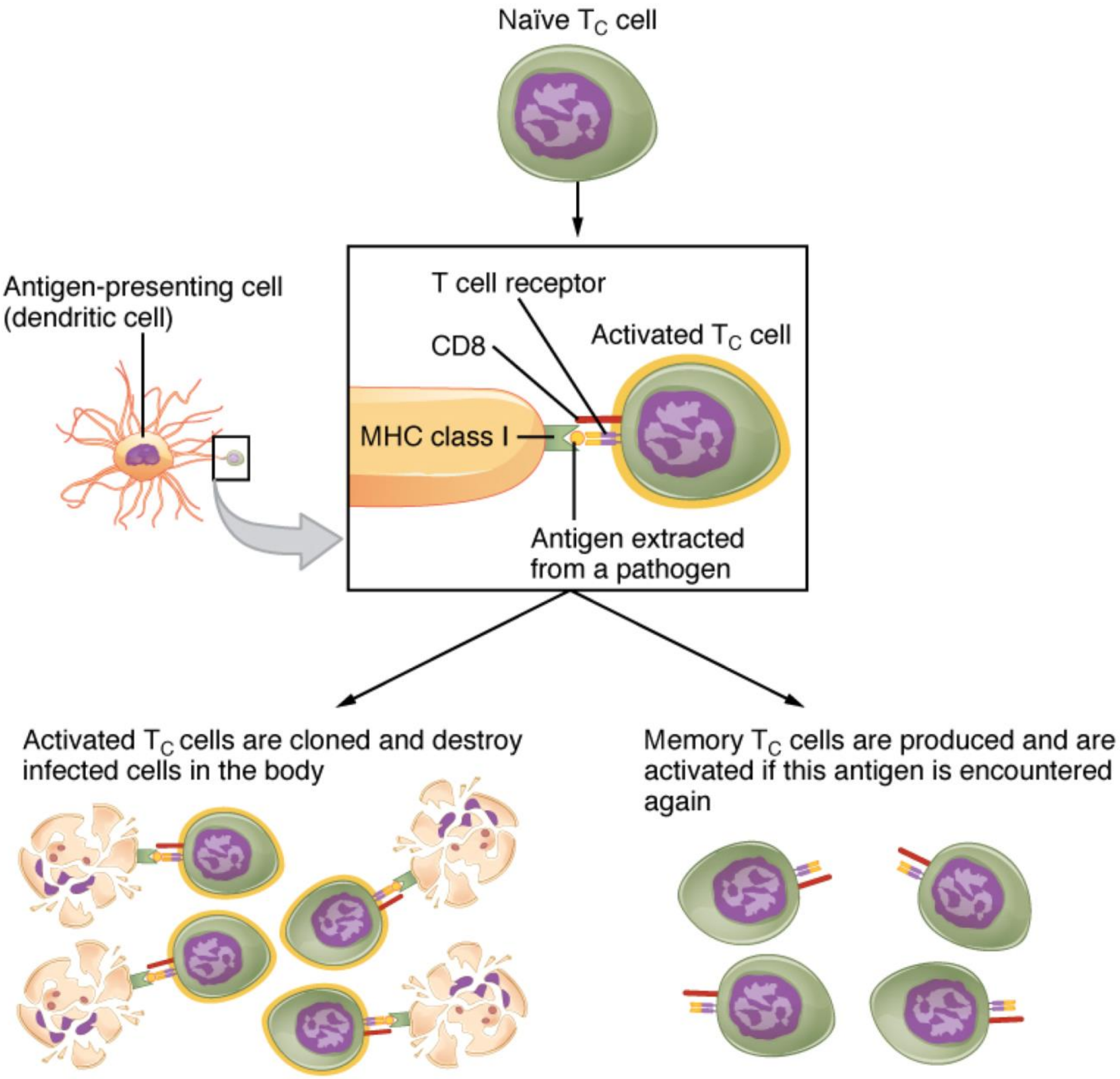



Fig.5.15. Clonal selection of T-lymphocytes.


combined with glycoproteins of the large ***complex of histocompatibility (MHC)*** and transported to the cell surface. The term "MHC" refers to a family of genes, encoding own membrane proteins, which are a marker, that allows cells of the immune system to distinguish their "own" from "foreign".

There is MHC of the 1st and 2nd types (Fig. 5.14). Virtually all cells of the body (with the exception of erythrocytes) have MHC of I type. However, only the $CD8^+$ receptors of cytotoxic T-lymphocytes, which destroy the cell infected with antigen, can bind to MHC-I molecules. At the same time antigen-presenting cells along with MHC type I contain also MHC of II type. Only T-helper cells have CD4 + receptors, which can bind to MHC of II type . So, T-helper cells join the antigen-MHC II complex on the surface of the antigen-expressing cell and begin to secrete cytokines, that stimulate proliferation of the corresponding clone of T-helper and T-killer. Additional stimulation of T-helper is provided by interleukins, which are produced by antigen-presenting cells. Activated T-helper cells, thanks to their $CD4^+$ receptors, bind to B-lymphocytes and stimulate their transformation into plasmocytes, that start secreting specific immunoglobulins. Antibodies, in turn, involve non-specific protective mechanisms (phagocytosis, complement system, interferon) to the immune response. The part of the activated B-lymphocytes continue to circulate in the blood, providing immune memory. The main stages of the immune response are presented in Fig. 5.15 and 5.16.

According to the ***clonal selection theory***, in the human body there are cells of immune memory, that have receptors to any potentially possible antigen, which can enter the internal environment. When encountered with such antigen, it is absorbed by antigen-presenting cells and is broken down inside the lysosomes. Then antigenic determinants are transported to the surface of the cell and give impulse to the powerful protective immune reactions described above. If during the period of embryonic development,

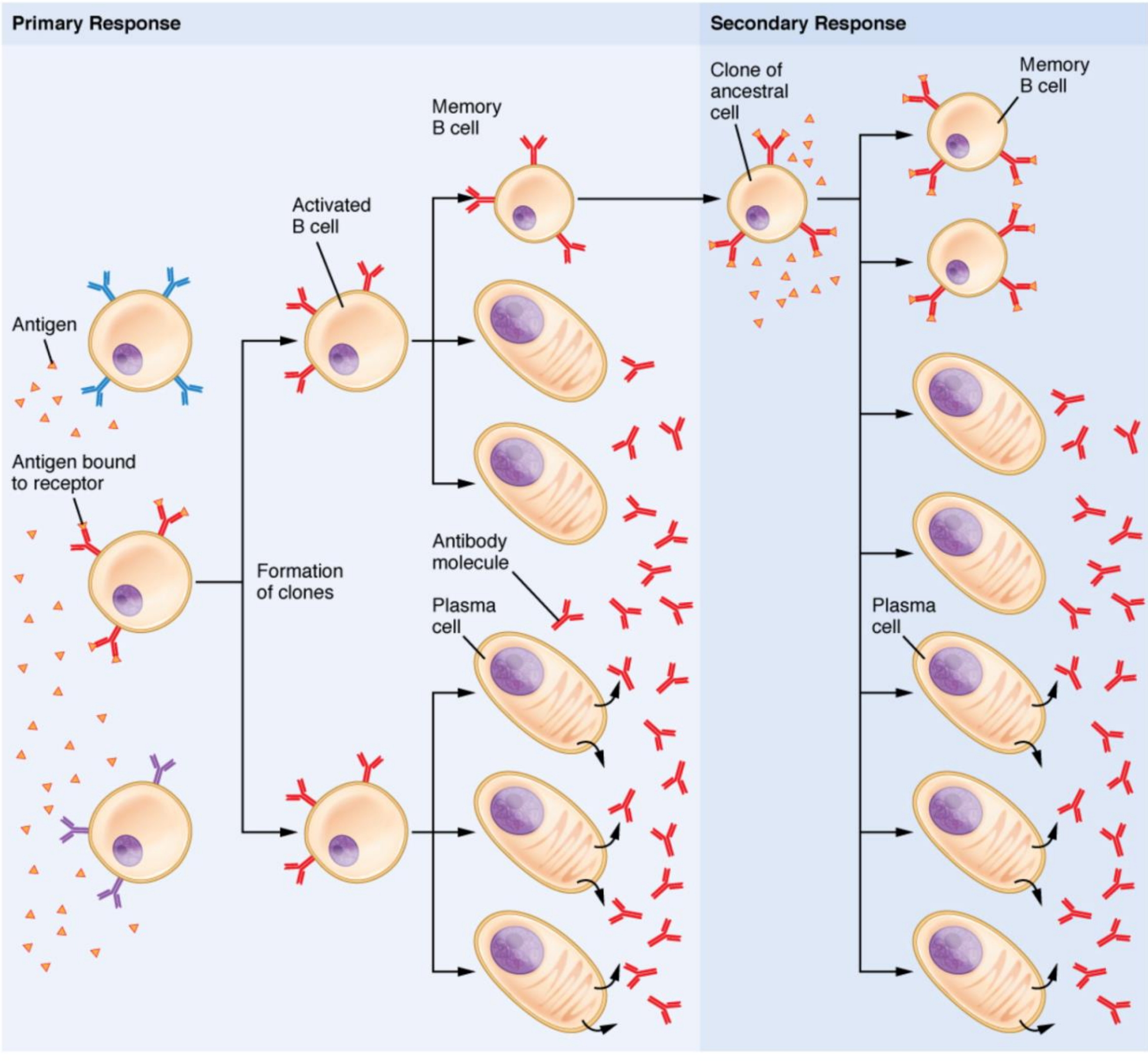



Fig.5.16. Clonal selection of B-lymphocytes.

when the immune system is formed, the fetus is contacted with a certain antigen, then it forms ***immunological tolerance*** to this antigen in adulthood.

# 4. Functions of platelets. Coagulation and anticoagulation system

## 4.1. Blood platelets and their functions.

**Platelets** are small subcellular fragments that are released from megakaryocytes. They are composed of a concentrate of megakaryocyte membrane, cytoplasm, granules, and organelles They circulate throughout blood vessels and survey the integrity of the vascular system. The number of platelets in peripheral blood is in range 200-400 × $10^9$/l. Their diameter is about 1,5-4 microns, thickness is about 0,5-0,75 microns. Platelets are incapable of dividing. They circulate in blood for 5-11 days, and then are destroying in the liver, lungs and spleen. The functions of platelets are quite diverse. The most important from them is participation in the hemostasis system. The platelet membrane and its internal granules contain a large number of biologically active substances, which are considered as platelet clotting factors. These are phospholipids of membranes, thrombostenin, thromboxane A2, and others. One of the important biologically active substances of platelet is the ***vascular growth factor***, which affects the proliferation of endothelial and smooth muscle cells in blood vessels wall.

The ability of the platelets to continuously change their size and shape is most significant for hemostasis. During hemorrhage platelets are forming numerous processes (***pseudopodia***), using which they can be fixed to the damaged surface of the vascular wall. This ability is due to the contractile proteins actin and myosin, presented in the platelets.

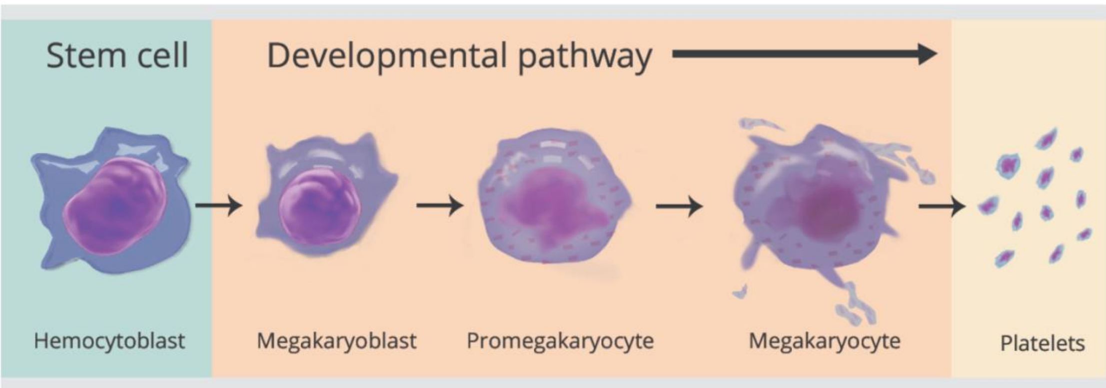



Fig.5.17. Main stages of thrombopoiesis.

Thrombocytes also perform a protective function due to their ability to phagocytosis of bacteria, viruses and immune complexes.

Platelets are formed in the red bone marrow like other blood cells by differentiating of polypotent steam cells. Their direct precursor is giant nuclear cells - megakaryocytes, from which cytoplasm bits are wiped off, clamped with a plasma membrane and become platelets. Each megacarocyte produces an average of about 1000 platelets. The process of thrombocyte formation is stimulating by the hormone ***thrombopoietin***, which is secreted by cells of the bone marrow, spleen and liver in response to a decrease of platelet count in peripheral blood.

## 4.2. General characteristics of the hemostasis mechanisms.

Mechanisms of bleeding stoppage (hemostasis) are divided into ***primary (vascular-platelet) hemostasis*** and ***secondary (coagulation) hemostasis***. Primary hemostasis is realized during the first few minutes after the onset of bleeding. However, it is able to stop bleeding from small vessels only. In most cases, it only gives an impulse to the starting of cascade mechanism of coagulation hemostasis.

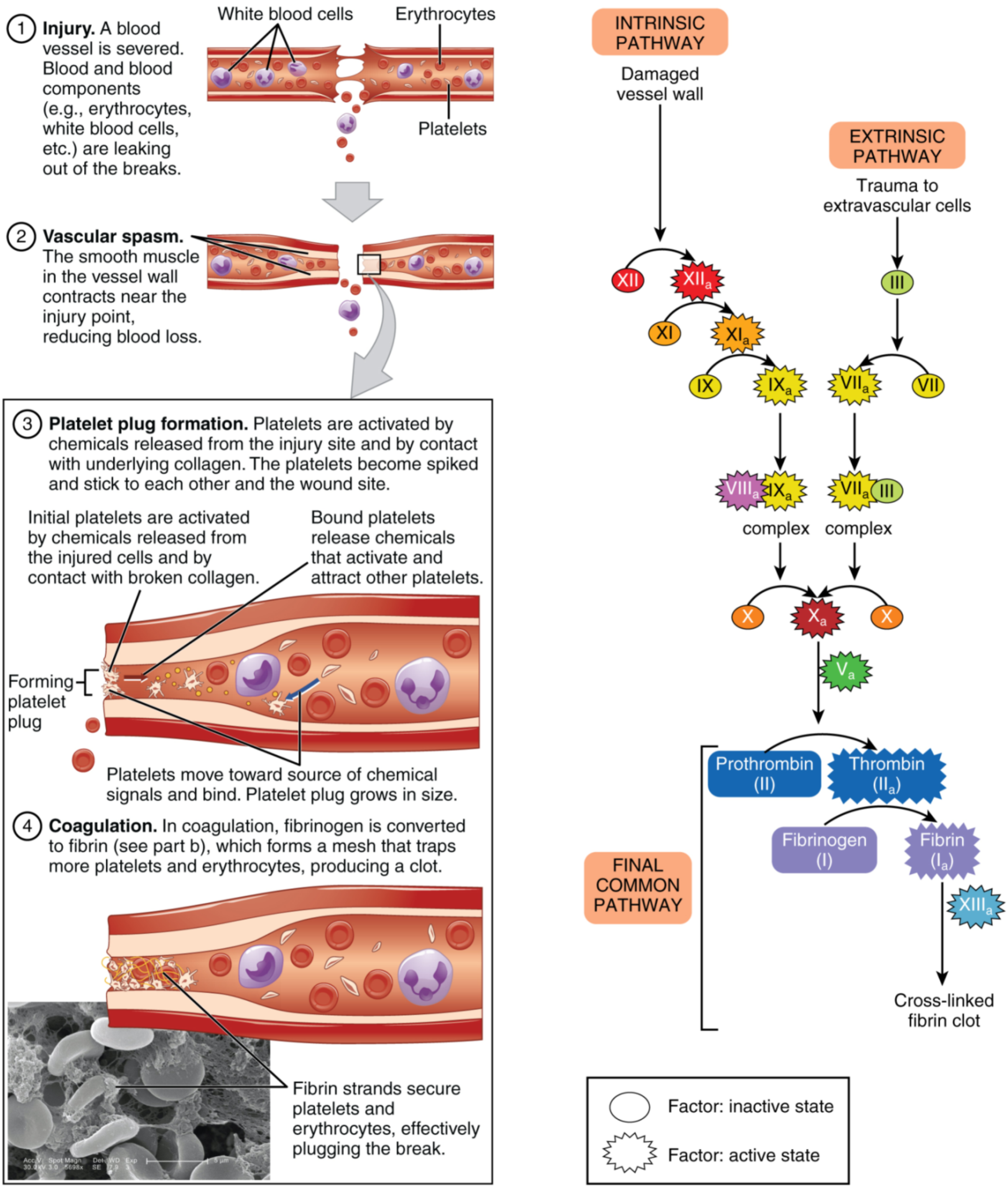
***Primary hemostasis includes:***

- ***reflectory constriction of vessels*** immediately after their damage. This constriction is supported by vasoactive substances, released from platelets during their activation. These are serotonin, adrenalin, thromboxane A2;
- ***platelet adhesion*** - gluing to the site of vessel damage, which is achieved through the reaction of collagen threads with a platelet membrane. The activated platelets form pseudopodia, by which they are fixed to the walls of the vessel;
- ***reverse aggregation of platelets*** - consists in the adhesion of platelets to each other and the formation of the so-called "white blood clot", that covers the damaged area of the vascular wall.

***Coagulation hemostasis*** consists in the conversion of the fibrinogen (dissolved protein) to the insoluble fibers of fibrin, which forms a dense net, fixing the blood cells (mainly –red blood cells). In this way, a "red thrombus" is formed, that reliably covers the damaged place of the vessel and prevents the blood from entering the neighboring tissues. The main stages of this process were described by Moravits in 1915. Despite the fact, that a lot of previously unknown biologically active substances, involved in coagulation hemostasis are discovered nowadays, basic ideas about the mechanisms of this process hadn’t changed significantly.

The process of coagulation begins with the formation of ***prothrombinase complex*** (thromboplastin) as a result of consistently acting enzymatic processes. It is formed by the interaction of the lipid factor with the plasma confinement factors. Depending on the origin of the lipid factor, ***extrinsic*** and ***intrinsic*** pathway of coagulation hemostasis are distinguished.

The extrinsic pathway is related to the activating lipid factor, released from the damaged vascular and tissue cells, and the intrinsic pathway - to




(a) The general steps of clotting (b) Fibrin synthesis cascade

Fig.5.18. The mechanisms of blood clotting: a) primery hemostasis (steps 1-3); b) coagulation hemostasis (step 4)

the activating lipid factor, released from the platelets.

Next stages are common to both pathways. ***Prothrombin*** (plasma protein) is converted into ***thrombin*** due to the action the prothrombinase complex in the presence of calcium ions, and the latter one stimulates the transformation of ***fibrinogen*** into ***fibrin***.

## 4.3. Mechanisms of primary hemostasis.

The platelets are gluing on inner surface of the vascular wall at the place of damage, due to electrostatic forces of attraction to collagen fibers.

At the same time, the platelets are activating and release the biologically active substances, that enhance the reflectory vasoconstriction of the damaged vessel (serotonin, adrenaline) and activate the adjacent blood circulating platelets (***adenosine diphosphate (ADP)*** and ***thromboxane*** $A_2$). These substances facilitate platelets adhesion to the first layer, formed at the place of the damage. An important role in the mechanism of platelet adhesion plays a specific protein, the ***Willebrand factor (WF)***, which is secreted by activated platelets and circulating in the plasma in small concentration. This protein has receptors for collagen and platelets, so it is able to create special bridges between platelets and collagen fibers, promoting their adhesion to the damaged area of the vessel. Activated platelets release additional portions of ADP and thromboxane A2 and, because of the positive feedback, platelets aggregation occurs. As result, a "white blood clot" (white thrombus) is forming. Plasma fibrinogen stimulates the platelet aggregation. At the same time, ADP stimulates the release of ***prostacyclin*** (a tissue hormone from the prostaglandin group) and ***nitric oxide*** by the intact endothelium, which prevent the adhesion and aggregation of thrombocytes to intact vessels wall. In general, the formation of white thrombus takes 2-4 minutes. It doesn’t only close physically the defect of the vascular wall, but also brings together damage edges through the actin-myozin complexes of activated platelets. The sealing of the white thrombus is facilitated by ***thrombostenin***, one of the factors of blood clotting, which is secreted by platelets. In addition, these platelets also release other cellular factors that trigger coagulation hemostasis.

## 4.4. Mechanisms of coagulation hemostasis.

***Coagulation hemostasis*** is the most effective protective mechanism in case of intense internal or external bleeding. However, if large vessels (especially the arteries) are damaged, even this mechanism is not able to stop the bleeding by itself. On the other hand, coagulation hemostasis can cause thrombosis even in the absence of bleeding. Therefore, in the body, in addition to the system of coagulation hemostasis, an ***anticoagulation system*** operates, which counteracts blood clotting. Normally, these systems are finely balanced. The physician needs to know the basic stages of both these processes to understand the mechanisms of their interaction and drug correction, if necessary.

A large number of biologically active substances are involved in the implementation of coagulation hemostasis. Some of them circulate in plasma in the form of inactive precursors (***plasma factors***), and the other are secreted by platelets (***cellular factors***). Key clotting factors, their standard notation and functions are listed in Table 5.5.

According to the international nomenclature, the plasma factors are indicated by the numbers of the Roman alphabet. The factor number reflects not the sequence of its inclusion into the process of coagulation, but - the opening chronology. Some factors include the names of the sick people in which they were first detected. For example, XII is the Hageman factor. Platelets factors are commonly used to denote by the PF abbreviation with the Arabic numerical index. For example, $PF_3$ is a platelet factor 3. Most of the clotting factors are inactive in the blood. To indicate their active status, a small letter "a" is used.

According to modern views, there are ***5 consecutive stages*** in a coagulation hemostasis:

Table 5.5.

Clotting plasma factors.

| Factor number | Name | Type of molecule | Source | Pathway(s) |
|---|---|---|---|---|
| I | Fibrinogen | Plasma protein | Liver | Common; converted into fibrin |
| II | Prothrombin | Plasma protein | Liver* | Common; converted into thrombin |
| III | Tissue thromboplastin or tissue factor | Lipoprotein mixture | Damaged cells and platelets | Extrinsic |
| IV | Calcium ions | Inorganic ions in plasma | Diet, platelets, bone matrix | Entire process |
| V | Proaccelerin | Plasma protein | Liver, platelets | Extrinsic and intrinsic |
| VI | Not used | Not used | Not used | Not used |
| VII | Proconvertin | Plasma protein | Liver * | Extrinsic |
| VIII | Antihemolytic factor A | Plasma protein factor | Platelets and endothelial cells | Intrinsic; deficiency results in hemophilia A |
| IX | Antihemolytic factor B (plasma thromboplastin component) | Plasma protein | Liver* | Intrinsic; deficiency results in hemophilia B |
| X | Stuart–Prower factor (thrombokinase) | Protein | Liver* | Extrinsic and intrinsic |
| XI | Antihemolytic factor C (plasma thromboplastin antecedent) | Plasma protein | Liver | Intrinsic; deficiency results in hemophilia C |
| XII | Hageman factor | Plasma protein | Liver | Intrinsic; initiates clotting in vitro also activates plasmin |
| XIII | Fibrin-stabilizing factor | Plasma protein | Liver, platelets | Stabilizes fibrin; slows fibrinolysis |

1. Formation of prothrombin activator (prothrombinase complex);
2. Formation of thrombin;
3. Formation of fibrin;
4. Retraction of the blood clot;
5. Fibrinolysis.

Each of the stage is a cascade of enzymatic reactions, in which the products of the previous reaction catalyze the following. In addition, some of them provide a positive feedback with other stages, significantly accelerating the whole process. In particular, it is thrombin, which accelerates the activation of factors VIII, IX, X, XI, XII and platelet aggregation. This means that the formation of even small amount of thrombin significantly accelerates the formation of the thrombus.

***The first stage*** of coagulation hemostasis is the formation of a ***prothrombin activator***, which is a complex of Xa, V, III factors and $PF_3$. This stage can be realized in two pathways: extrinsic and intrinsic ones, which differ in the trigger mechanism. But both of them lead ultimately to the activation of the X factor. When damage of the vascular wall (bleeding) occurs, both pathways are proceeding in parallel way, mutually reinforcing each other. In this case, the extrinsic pathway proceeds much faster (10-15 seconds), comparing to the intrinsic one (3-6 minutes). However, in the absence of bleeding, the first stage of coagulation can only be realized by intrinsic way. Such a situation often occurs in patients with atherosclerotic lesions of the vascular wall and causes thrombosis inside the coronary and cranial blood vessels.

***The extrinsic pathway*** begins with the releasing of phospholipids from the damaged vascular wall and adjacent tissues, having proteolytic properties (factor III). This factor is combined with the factor VII, and they together activate the X factor in the presence of $Ca^{2+}$ ions (Fig.5.18).

***The intrinsic pathway*** begins with the releasing of phospholipids from activated platelets ($PF_3$) during their adhesion and aggregation. At the same time, XII factor is activating due to contact with the collagen of the damaged vascular wall. XIIa factor initiates sequential cascade activation of factors XI, IX and VIII, resulting in activation of factor X. Starting from this moment, both pathways are united in a common pathway, that ends with formation of prothrombin activator. The key factor of common pathway is the factor V. It is activated by the Xa factor and by the newly formed thrombin. The positive feedback accelerates this process. Combining with the III factor, $PF_3$ factor and $Ca^{2+}$ ions, become prothrombin activator (or by other terminology - prothrombinase complex).

***The second stage*** of coagulation hemostasis is the conversion of prothrombin into thrombin, stimulated by the prothrombin activator in the presence of $Ca^{2+}$ ions. Prothrombin is a protein from $\alpha_2$-globulin fraction with a molecular weight of 68700, which is synthesized in the liver with the participation of vitamin K. Its average concentration in the blood is about

0,15 g / l. Reducing the prothrombin concentration in the blood (caused by liver diseases or vitamin K deficiency) leads to hypocoagulation and may be the cause of internal bleeding. This stage is very fast (it lasts a few seconds) and stimulates the previous stage by mean of positive feedback.

***The third stage*** of coagulation hemostasis is the transformation of the dissolved plasma protein of fibrinogen into an insoluble fibrin protein, which forms a reticular structure at the place of vessel damage. It serves as a kind of framework for the future thrombus, which reliably prevents further bleeding. The catalyst of this stage is the proteolytic enzyme ***thrombin***, formed as a result of the previous stage. The fibrinogen molecules are spliting into monomers that are polymerizable. This process is accelerated by the influence of a fibrin-stabilizing (XIII) factor, which is inactive in plasma, and partly secreted by activated platelets. Activator of the XIII factor is thrombin. This stage lasts for several minutes and finishes with the formation of the so-called "red blood clot". It is named due to the fact, that its base, in the contrast to the "white" (platelet) thrombus, is build from erythrocytes, that are fixed into the fibrin network.

***The fourth stage*** - a blood clot retraction – occurs during the next 20-60 minutes and consists on the sealing the blood clot due to the loss of water and its transformation into an elastic bark, which reliably covers the defect of the vascular wall. This stage is stimulated by ***thrombostenin***, which is secreted by thrombocytes, contained inside the red blood clot.

***The fifth stage*** of coagulation hemostasis – ***fibrinolysis of the blood clot*** - begins a few days after stopping of bleeding (Fig.5.19). Simultaneously, repairing processes of the damaged vessel area occur. The driving force of fibrinolysis is the formation of a proteolytic enzyme ***plasmin*** from its precursor ***plasminogen***, which is synthesized by the liver cells and circulates in the blood in an inactive form. The activator of plasminogen is the enzyme ***kalikrein***, which, in turn, is a product of conversion of its precursor prekalikrein. This conversation, basically, occurs under the influence of the XII factor (Hageman). In addition, damaged perivascular tissues release ***tissue plasminogen activator (t-PA),*** which also stimulates the formation of plasmin. It has been established, that t-PA can also be formed in some organs (in particular, in the lungs, uterus) and stimulates the fibrinolysis of microemboli, when blood clotting becomes excessive.

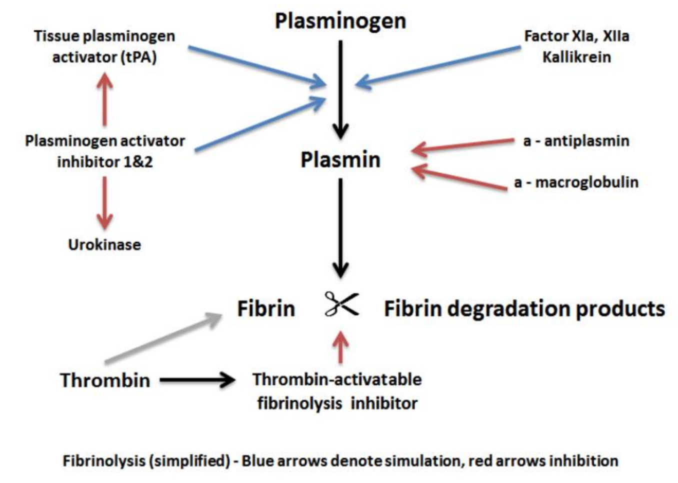



Fig.5.19. Fibrinolysis pathway.

Endogenous plasminogen activators include also the enzyme ***urokinase***, that is synthesized in the kidneys by the tubules epithelium. Plasmin not only efficiently cleaves polymer chains of fibrin, but also stimulates the formation of kalikrein on the principle of positive feedback, significantly accelerating the process of fibrinolysis.

In some infectious processes, fibrinolysis may be stimulated by ***exogenous plasminogen activators*** of bacterial origin, called exogenous fibrin kinases (for example, hemolytic streptococcus secretes such fibrin kinase). The fibrin digestion products are absorbed by macrophages, which provide gradual resorption of the clot. The reparative processes with the participation of fibroblasts occur at the damaged vessel area, that lead to the restoration of the integrity of the vascular wall. These processes are stimulated by the ***platelet-derived vascular growth factor***, which secretes thrombocytes, that are part of the thrombus.

## 4.5. Anticoagulation system of blood.

The maintenance of blood in a liquid state is achieved due to the fine balance between the coagulation system and the factors that counteract the formation of blood clots. The last factors make up the ***anticoagulation system*** of blood. It is represented by:

1. Protein ***thrombomodulin***, which is part of the endothelial membrane of the vascular wall. This protein binds thrombin, decreasing the activity of coagulation hemostasis.
2. ***C-protein of the plasma***. The complex thrombomodulin-thrombin activates the C-protein of plasma, which, in turn, inactivates factors V and VIII. If the integrity and smoothness of the vascular wall is violated, thrombomodulin is less resistant to coagulation. On the contrary, factor XII is activating by collagen of the damaged vascular wall and triggers the intrinsic pathway of coagulation hemostasis.
3. ***Fibrin fibers,*** that absorb 85-90% of thrombin and prevent the activation of the following stages of coagulation hemostasis.
4. Plasma protein - ***antithrombin III*** (from a fraction of α-globulins), which binds the rest thrombin.
5. Polysaccharide ***heparin***, secreted by platelets and some other cells (basophils, mast cells). The anticoagulation effect of heparin is manifested only, if it forms the complex with antithrombin III. This complex increases the effectiveness of antithrombin III in hundreds of times. In addition, the given complex inactivates a number of plasma clotting factors (XII, XI, X, IX). Normally, the concentration of heparin in the blood is too low for the manifestation of its anticoagulation effect. However, in some pathological conditions accompanied by degradation of basophils and mast cells, the concentration of heparin is increasing and the anticoagulation power of the blood is substantially increased too.

6. ***Plasmin*** - the final player of fibrinolysis, which not only dissolves thrombi, but also hydrolyzes such clotting factors as factor V, VIII, XII and prothrombin.

## 4.6. Typical abnormal conditions accompanied by insufficiency of coagulation hemostasis.

Disturbance of the balance between the coagulation and anticoagulation system may be manifested as excessive bleeding due to insufficiency of coagulation hemostasis, or – as excessive thrombosis with increased activity of the coagulation system. Excessive bleeding most often is associated with 3 reasons: 1) Vitamin K deficiency and liver disease; 2) hemophilia; and 3) thrombocytopenia.

1. ***Vitamin K deficiency*** affects biosynthesis a lot of blood-clotting factors in the liver - in particular, prothrombin, VII, IX and X factors. Vitamin K in the human body is producing by the microflora of the large intestine and is delivered with consumed food products (especially of animal origin). However, the necessary condition for its absorption into the blood is sufficient production of bile by the liver and its transporting into the duodenum. Therefore, the deficiency of vitamin K is observed in diseases of the liver (hepatitis, cirrhosis, fatty dystrophy, etc.), as well as in the pathology of biliary tract (gallstone disease, dyskinesia of the biliary tract, etc.), and is associated with the bleeding. In addition, even in the absence of vitamin K deficiency, the parenchymal lesions of the liver lead to the decreased synthesis of proteins by hepatocytes (including the factors of aggregation) and contribute to internal and external bleeding.

2. ***Hemophilia*** - a violation of blood clotting, which is diagnosed almost exclusively by men and has a genetic origin. There are several variants of hemophilia encountered in clinical practice. Male patients most often (in 85% of cases) have a defect in the X-chromosome, that leads to the absence of VIII clotting factor in their blood. This variant of the disease is called ***classical hemophilia (or hemophilia A)***. There is other variant of this disease, by which biosynthesis factor VIII is partially affected, what results in lack only its high molecular weight component. This variant is called **Willebrand disease**, which has some clinical specificity comparing to the classical hemophilia A. The remaining 15% of hemophilia cases are associated with a deficiency of the IX clotting factor. Such variant of disease is called ***hemophilia B***. In all cases of hemophilia, damage of the vascular wall (cuts, injuries, nosebleeds, etc.) threatens the patient's life, because the bleeding can be stopped itself due to insufficiency the 1st stage of coagulation hemostasis.

3. ***Thrombocytopenia*** - pathological condition, by which there is a decrease in the number of platelets in peripheral blood ($<180x10^9$ / l). Unlike hemophilia, bleeding in the patient can be induced not only by the damage of large vessels, but occurs predominantly at the level of the

microcirculatory bed and shallow veins, causing characteristic changes in the skin called ***thrombocytopenic purpura***.

## 4.7. Typical abnormal conditions accompanied by excessive activity of coagulation hemostasis.

Excessive coagulation hemostasis is a cause of thrombosis, which can lead to death-threatening complications, such as myocardial infarction, or ischemic stroke. The formation of embolus in the bloodstream is facilitated by 1) damage of the vessel's epithelium (for example, atherosclerotic lesions), and 2) decrease in speed of blood flow in the vascular system. Below some typical hypercoagulation pathological conditions are listed.

1.***Thrombosis of the lower extremities veins*** often threatens the patients with low physical activity, especially, if they are staying for long time in the upright position. This is also true for long-term immobilized neurological patients. Stagnation of venous blood creates favorable conditions for the formation of blood clots, which can enter into the pulmonary circulation through the vena cava inferior and cause ***thromboembolism of the pulmonary artery*** or its branches. Depending on the ischemic area size the outcomes for patients can be quite different: or immediate death or relatively long course of the disease. In last case, timely anticoagulation therapy can save the patient life. Recently, synthetic drug t-PA (tissue plasminogen activator) is used for treatment of such complication.

2. ***The syndrome of disseminated intravascular coagulation*** occurs if lots of injured or dead tissues enter simultaneously in the vascular bad, release the lipid factor and stimulate the development of coagulation hemostasis. Typically, formed in this way blood clots have a small size, but they are circulating in different parts of the vascular system and sharply worsen the microcirculation in important regions of the body. Such situation occurs, for example, by sepsis of bacterial origin. This condition is characterized by the presence in the blood of both bacteria and their toxins, as well as residues of necrotic tissues (septicemia, pyemia), which stimulate the microthrombosis and may be complicated by ***septic hemodynamic shock***. A paradoxical symptom in this condition is the patient's predisposition to bleeding, which can be explained by absorption of plasma clotting factors on numerous thrombi.

## 4.8. Methods of clinical assessment of the blood coagulation system.

Several tests are used to characterize the various stages of coagulation hemostasis for the purpose of clinical evaluation of the functional state of the coagulation system:

1. ***Time of bleeding.*** This index characterizes the total effectiveness of coagulation hemostasis and is measured in minutes. In healthy people, its normal value ranges from 1 to 6 minutes. This time is significantly increased in the absence of one or more plasma clotting factors, as well as by thrombocytopenia of different origins. However, it does not answer the question about the mechanism of coagulation hemostasis disturbance, but only fixes its presence and severity.

2. ***Prothrombin time*** is an indicator of the prothrombin concentration in the blood. Its normal value is 12-18 seconds. To determine this indicator, the taken from the patient fresh blood sample is immediately oxalated (adding of oxalic acetic acid or citric acid sodium) in order to bind of $Ca^{2+}$ ions and to avoid the transformation of prothrombin into thrombin. Then, it needs to get plasma from the blood (by centrifuging), and add calcium ions as well as thromboplastin (factor III) to it. This procedure results in the activation of the extrinsic pathway of coagulation hemostasis and the blood clot formation. The prothrombin time of the tested blood sample is compared with a similar parameter of a blood sample taken from a healthy person and obtained under the same conditions. The results of the study can be expressed either in the units of blood clotting time for the tested and control blood sample (for example, 12s / 13s), or in the form of a ***normalized prothrombin index***, which is the ratio of these two time intervals. In this case, the prothrombin index is considered normal if it is in the range of 0.9-1.1. In Ukraine, the ***prothrombin index (PTI)*** is often used, which is reversed to the ***normalized prothrombin index*** and expressed as a percentage. (PTI = (control prothrombin time / prothrombin time of the patient) * 100). The normal value of the PTI lies in the range of 90-110%. Because of this indicator characterizes the extrinsic pathway of coagulation hemostasis, it provides information about the concentration in the blood, preferably II, VII and X factors, which are primarily dependent on vitamin K. Therefore, it is often used to control the treatment of patients by indirect anticoagulants (neodicumarin, pelentan, sinkumar, phenylene, etc.). At the same time, this index does not allow to judge about the deficit other clotting factors, involved in intrinsic pathway (XII, XI, IX, VIII) and can remain normal, even by hemophilia.

3. ***Activated partial thromboplastin*** time is an indicator, that is obtained similarly to prothrombin time with the small difference. A special reagent - an activator of the XII factor - is adding to the plasma instead the thromboplastin. As a result, the fibrin formation occurs by the intrinsic pathway and time of blood clot forming characterizes the activity of the relevant factors. In particular, with this method it is possible to diagnose hemophilia and control anticoagulant therapy with heparin. The normal value of this indicator is within 25-38 seconds.

# 5. Physicochemical and physiological mechanisms of maintenance of acid-base balance

## 5.1. Concept about pH and buffer properties of solutions.

The acid or alkaline reaction of the solution depends on the ratio the concentrations of free hydrogen ions to hydroxyl ions. The product of the concentration of hydrogen and hydroxyl ions in water solutions is a constant number, called the ***dissociation constant of water*** , which is equal to $10^{-14}$:

$$[H^+] \times [OH^-] = 10^{-14}.$$

In practice, the reaction of the solution is evaluated by the concentration of hydrogen ions in it. However, absolute levels of this ion concentration in biological solutions usually are very low. For example, the concentration of $H^+$ ions in chemically pure water at a temperature of 22 ° C is $10^{-7}$mol/liter. It is more convenient to estimate this concentration on a logarithmic scale using the special index, which is called hydrogen index(pH).

***pH is the negative decimal logarithm of the molar concentration of hydrogen ions***:

$$pH = - \lg [H^+].$$

Calculation for chemically pure water gives a pH value equal to 7:

$$pH = - \lg [10^{-7}] = - (- 7) = 7.$$

In fact, water is a ***neutral*** solution, because it has the same concentration of hydrogen and hydroxyl ions. If the concentration of hydrogen ions in the solution is greater than in water, then its pH<7. Such solution is called ***acidic***. If the concentration of hydrogen ions in solution is less than in water, then its pH> 7. Such solution is called ***alkaline***.

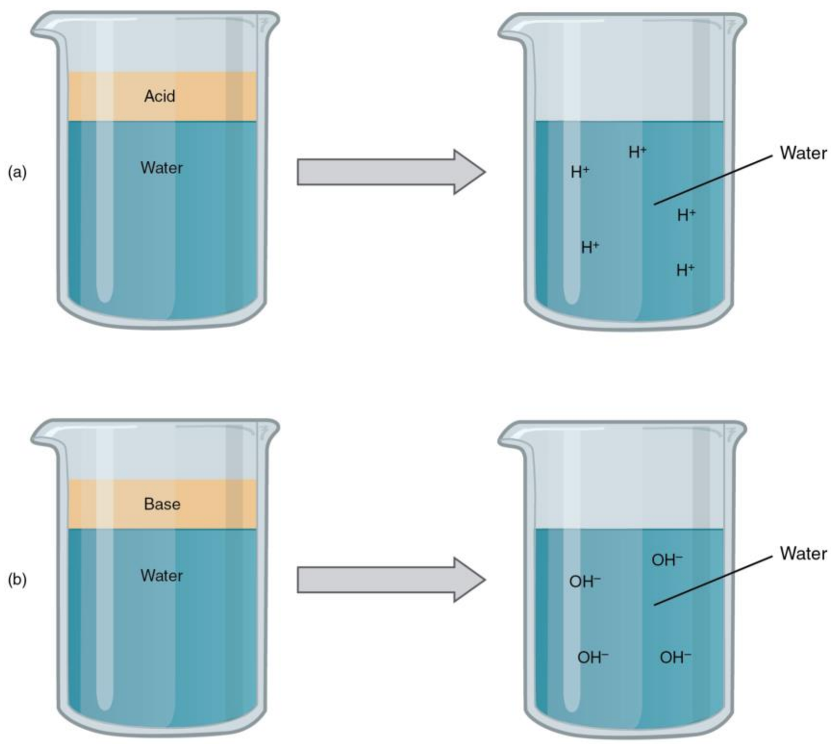



Fig.5.20. Acids (a) and Bases (b) in solution.


The intercellular fluid and intracellular content have an average pH close to 7.4. It means, that they are weakly alkaline solutions.

Most biological fluids in the body have the ability to maintain a relatively stable pH, despite the constant exposure of endogenous and exogenous acids and bases to their composition. This ability is called ***buffer properties***. The buffer properties of water solutions are due to the presence of weak acids, which only slightly dissociate. By Bronsted definition, ***acids*** are substances, that release hydrogen ions (proton donors) in solutions, and ***bases*** (***alkalines***) - substances that bind these protons. Let's consider the dissociation reaction of an acid from these point of view: **$HA \leftrightarrow H^+ + A^-$** (1)

If this reaction occurs from the left to the right (dissociation), the substance HA can be considered as is an acid. If the reaction proceeds in the opposite direction (association), then substance A can be considered as a base. It is called a ***conjugated base***.

The equilibrium in the dissociation reaction is shifted to the right for strong acids such as HCl, but weak acids are dissociated to a small extent. It means, that biggest part of weak acid molecules aren't dissociated on ions. The most of the acids involved in the metabolism (carbonic, lactic, pyruvic acid, etc.) actually are the weak acids and have the buffer properties. There

$$\frac{[H]^+ \times [A]^-}{[HA]} = K$$

is a dynamic equilibrium between the dissociation and association reactions of such acids, which is described by the equation:
where the square brackets contain molar concentrations of substances, and K is the dissociation constant.

(2)

Thus, when an additional amount of $H^+$ is added to the solution, the equilibrium in equation (1) is shifted to the left due to the binding of a part of the hydrogen ions to the conjugated base, which leads to an increase in the concentration of the non-dissociated acid. When excess hydroxyl ions is added to the solution, they are neutralized by free hydrogen ions, what leads to the additional dissociation of acid and the increasing of the conjugated base concentration. In both cases, the concentration of hydrogen ions (and hence - pH) will remain at the previous level, but the concentration of the non-dissociated acid and the conjugated base is changing according to the principle of Le Chatelier.

After logarithming the equation (2) can be represented as follows:

$$pH = pK + \lg\frac{[A]^-}{[HA]}$$

The last equation is called the ***Henderson-Hasselbalch equation***. pK is a constant that characterizes the buffer zone of the solution. Thus, at equilibrium of conjugated base and acid concentrations, lg [$A^-$] / [HA] = lg1 =

0. This means, that the pK of the buffer system would be equal to the pH of the solution, in which half of the acid is dissociated.

## 5.2. pH of blood and its significance for homeostasis.

The pH of arterial blood in healthy people at 37°C ranges from 7.35 to 7.45 (average 7.4). It needs to be clarified, that this value is related to plasma. It has been experimentally determined, that the pH inside the erythrocyte is 7.28 - 7.29, but it is technically more difficult to measure than a similar plasma index. Therefore, pH of the plasma is used as a pH indicator of whole blood. Venous blood has a more acid reaction, its pH is about of 7.34-7.36. In healthy humans, the weakly alkaline blood reaction is maintaining at the indicated limits, despite the fact, that the acid products of the metabolism constantly enter the bloodstream. The pH stability is especially important for metabolism, since the activity of most enzymes is optimal only, if pH corresponds these limits. The pathological pH deviation in acid (<7.35) or alkaline (> 7.45) direction are called ***acidosis*** and ***alkalosis,*** respectively. Both of these conditions adversely affect all metabolic processes because they violate the spatial configuration of the active centers of enzymes and cause metabolic disorders in organs and tissues. Most often pH disturbance in the clinic is acidosis, which requires medical treatment, ascertaining and, if possible, eliminating its causes.

Another important aspect of the negative effects of significant pH deviations from the optimal level on the body metabolism is the change in excitability of the nervous and muscular tissue cells. So, in acidosis, positively charged hydrogen ions are diffused from the blood into cells according to their concentration gradient. In the cytoplasm, they bind to intracellular proteins due to their buffering properties. In order to maintain the electrical balance, an additional amount of $K^+$ ions comes from the cell and creates hyperpolarization of the cell membrane. As you know, the hyperpolarized membrane is less excitable, then usual. Decreased excitability of nervous and muscular tissues is clinically manifested by such symptoms as muscle weakness, mental function disorders, and in severe cases, even a coma. Opposite excitability changes are observed by alkalosis. In this case, $H^+$ ions exit from the cell into the intercellular space, where their concentration is relatively less. It results in increasing of $K^+$ ions concentration inside the cell, what allows to maintain the electrical balance of cations between the extracellular and intracellular environments. Reducing the $K^+$ ions diffusion on the outer surface of the cell membrane causes its depolarization, which, in turn, leads to increased excitability in the central nervous system and muscle tissue. The binding of calcium ions by plasma proteins makes some contribution to increasing the excitability of tissues in the alkalosis because it increases the sensitivity of voltage gated sodium channels in cell membranes. Clinical manifestations of such changes are spastic contractions of skeletal muscles, convulsions, mental alterations.

The largest source of hydrogen ions in the human body is the $CO_2$ , produced during aerobic respiration. Converting carbon dioxide into carbonic acid in tissues and its subsequent dissociation into hydrogen and hydrocarbonat ions creates the preconditions for acidosis, if the lungs can't cope with the timely exhaling of carbon dioxide into the atmosphere. The accumulation of hydrogen ions in the body also occurs because of excessive producing of non-volatile intermediate metabolism products, such as lactic acid, citric acid components, ketone bodies, etc. The source of acid excess in the body may be some of the components of our diet, in particular, meat and dairy food. At the same time, some vegetables and fruits generate alkaline metabolites in the human body ($HCO_3^-$). These include, in particular, leafy vegetables, cereals, potatoes, pumpkins, melons, etc (Fig.5.21).

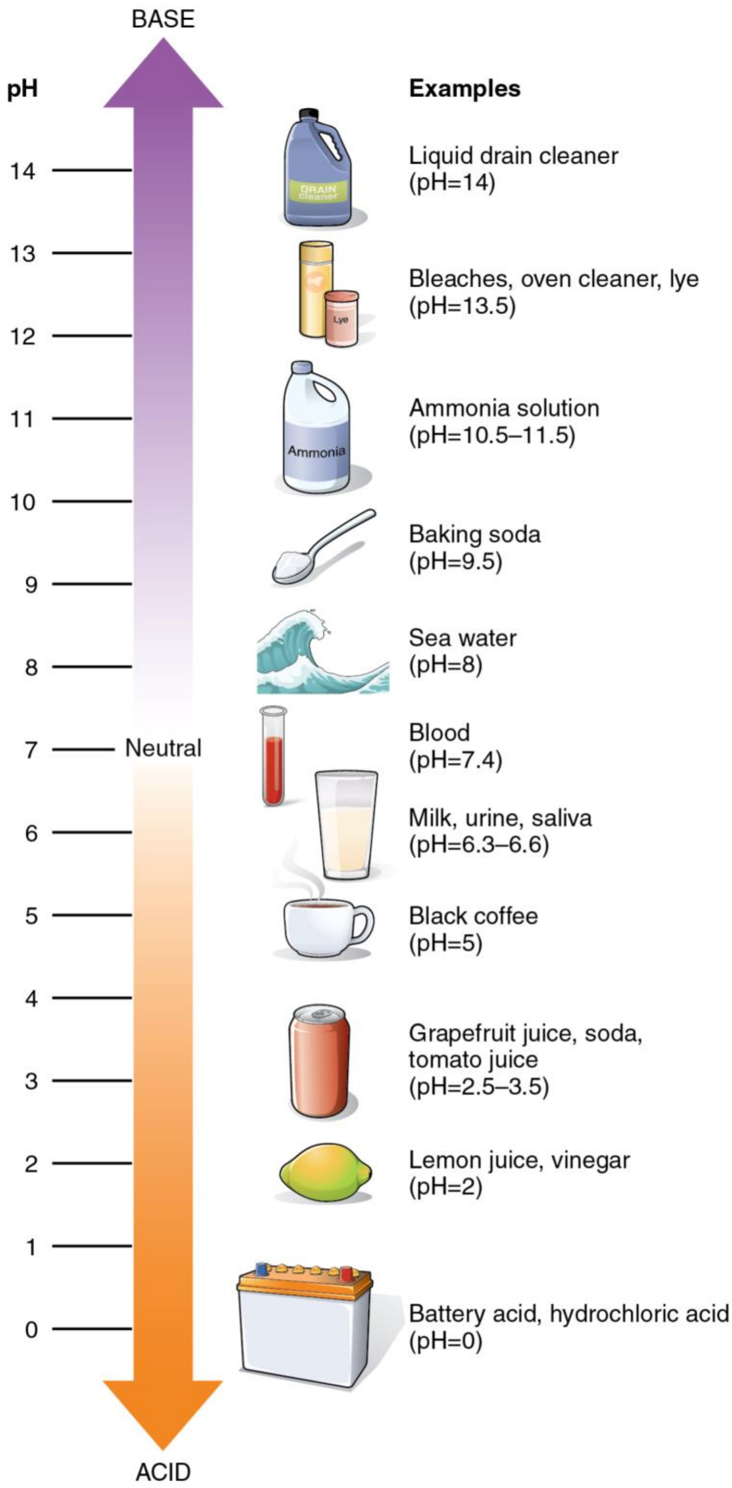



Fig.5.21. pH of different objects on pH scale.


Several mechanisms are involved in the regulation of the acid-base equilibrium, which interact with each other and do not allow significant deviations in pH from its optimal level to occur. The first line of defense against violations of acid-base balance (ABB) is the buffer blood system. The second line of defense is the respiratory mechanisms of pH control, which are implemented by changing the lungs ventilation. Finally, the most powerful control mechanisms (third line of defense) is regulation through changing of the excretory renal function.

## 5.3. Blood buffer systems and their physiological characteristics.

There are four buffer systems in the blood: ***bicarbonate, phosphate, hemoglobin and protein***. In the process of the acid-base regulation, the buffer systems are connected with the corresponding physiological systems, organs and tissues: bicarbonate - with the system of external respiration, kidneys, blood and tissues; phosphate - with blood and kidneys; protein - with blood and tissues; hemoglobin - with blood, lungs ventilation and tissues.

***Bicarbonate buffer system.***

This system consists of a weak carbonic acid ($H_2CO_3$) and its conjugated base – $HCO_3^-$, which are in an appropriate proportion in blood. With the appearance of acidic products, which release ion $H^+$, they react with the bicarbonate to form $H_2CO_3$. The carbonic acid dissosiate in erythrocytes using the enzyme ***carbonic anhydrase*** to water and carbon dioxide, which is exhaling by the lungs.

When additional hydroxyl ions enter into the blood, they bind with hydrogen ions, what causes additional dissociation of $H_2CO_3$ to hydrogen ions and bicarbonate, whose concentration in the blood increases.

$$CO_2 + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+$$

In this case, the lungs decrease the releasing of carbon dioxide into atmosphere to replenish the content of $H_2CO_3$in the blood, and the kidneys excrete an additional amount of bicarbonate ions with urine.

The Henderson-Hasselbalch equation for a bicarbonate system can be represented in such form:

$$pH = pK' + \lg\frac{[HCO_3]^-}{[H_2CO_3]}$$

Since the concentration of carbon dioxide in the plasma is 400 times greater than the concentration of carbonic acid, in practice the $CO_2$ pressure, which is in equilibrium with the blood plasma, is more accessible measurement parameter, then carbonic acid. Taking this into account, Henderson-Hasselbalch equation for the bicarbonate system will have the form:

$$pH = 6,1 + \lg \frac{[HCO_3^-]}{0,03 \times Pco_2}$$

where 0,03 (mmol/l/mmHg) - the coefficient for the transition from units of carbon dioxide pressure to the concentration of carbonic acid. In this equation, pK = 6,1; and the ratio of the concentration of bicarbonate to the corrected carbon dioxide pressure is about 20: 1.

***Phosphate buffer system.***

The phosphate buffer system is formed by dihydrophosphate ($H_2 PO_4^-$), which acts like an acid, and hydrophosphate ($HPO_4^{2-}$), which acts as a conjugated base, according to equation:

$$H_2PO_4^- \leftrightarrows HPO_4^{2-} + H^+$$

This buffer system operates in such a way: when there are excess hydrogen ions (acidosis), they are bounding by hydrophosphate, forming a low dissosiated dihydrophosphate. In case of hydrogen ions deficiency (alkalosis), dihydrophosphate additionally dissociates, releasing hydrogen ions into the solution and forming an additional amount of conjugated base - hydrophosphate. The pK phosphate buffer equals to 6.8 and is quite close to the pH of the blood. But the capacity of this buffer system is relatively small due to the low concentration of inorganic phosphates in the blood. At the same time, the phosphate buffer system plays a very significant role in the renal tubules and in the intracellular environment, where the concentration of phosphates is relatively high.

***Protein buffer system.***

The buffering properties of proteins are due to the amphoteric properties of amino acids. Thus, a carboxyl group behaves like an acid, and the amino group – like a base. In the acidic environment, proteins behave like bases, binding hydrogen ions with an amino group, and in alkaline – like acids, releasing into solution a proton, which together with the hydroxyl group forms water, according to these equations:

$$R\text{-}NH_2 + H^+ = R\text{-}NH_3^+$$

$$R\text{-}COOH + OH^- = R\text{-}COO^- + H_2O.$$

The protein buffer system is, particularly, important as an intracellular buffer. Its contribution to the overall buffer capacity of the blood is relatively low (without taking into account the hemoglobin buffer).

***Hemoglobin buffer system.***

A bigger part of the protein buffer system (about 75% its buffer capacity) is represented by hemoglobin system. It is due to high content of hemoglobin in the blood (approximately 4 times larger than other proteins) and high content of amino acids histidine in it The buffer role of hemoglobin is closely related to its respiratory function. Oxyhemoglobin is a stronger acid than deoxyhemoglobin. It acquires alkaline properties and acts like an acceptor of hydrogen ions in the tissues, getting rid of oxygen, and becomes a donor of hydrogen ions in the lungs, joining oxygen. Released hydrogen ions neutralize alkaline metabolites. Thus, the exchange of oxygen potentiates the buffering properties of hemoglobin. In addition, deoxyhemoglobin directly binds $CO_2$ in tissues capillaries due to amino groups, forming a ***carbhemoglobin*** (***Holden effect***).

The buffer capacity of the plasma consists of 43% of the total buffer capacity of the blood, of which the part of bicarbonate buffer is - 35%; protein - 7%; phosphate - 1%. The buffer capacity of erythrocytes is 57% of the total buffer capacity of blood, of which the part of bicarbonate buffer is 18%; hemoglobin - 35%; phosphate - 4%.

## 5.4. Participation of the respiratory system in the regulation of acid base balance.

The processes of gas exchange between blood and atmospheric air, that occur in the lungs, are very closely related to the regulation of the acid-base balance of the human body. This connection is realized through the

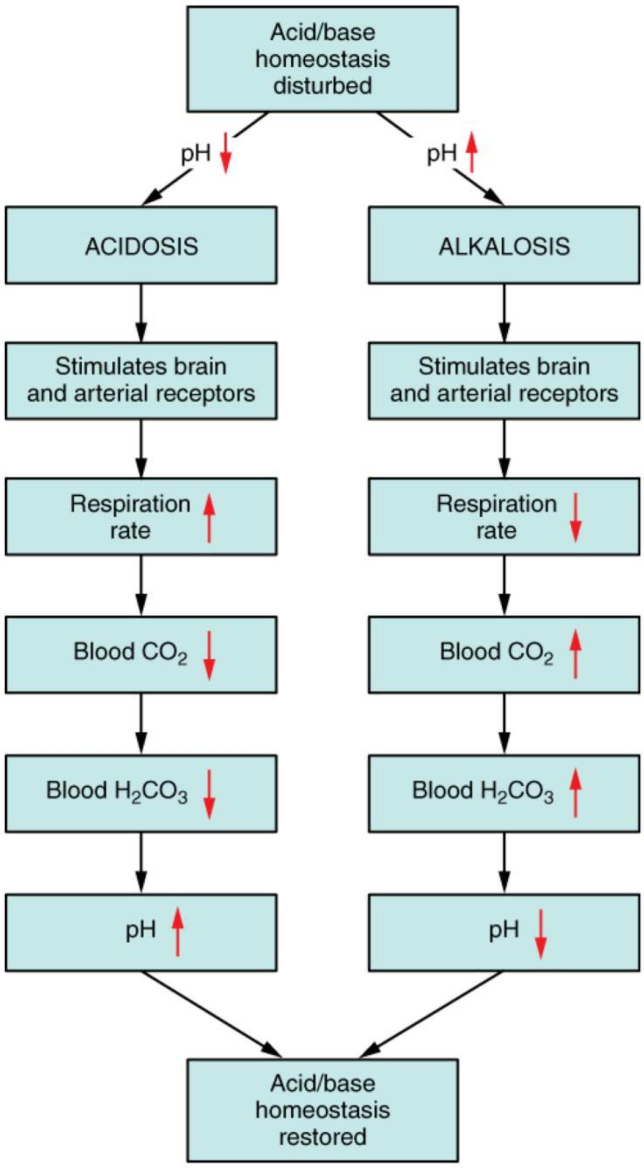



Fig.5.22. The role of breathing in compensation of acid-base disturbances.

bicarbonate buffer system of the blood, which is ventilatory opened. One of the components of this system - carbon dioxide - can more or less be exhaled into the atmosphere, what affects the concentration of the acidic or alkaline part of the buffer. It is very clearly illustrated by the Henderson-Hasselbalch equation for a bicarbonate buffer system. If in this equation the denominator in expression $HCO_3^-$/ 0,03x$CO_2$ decreases, then the pH value tends to increase. This situation occurs to compensate metabolic (not associated with the pathology of external respiration) acidosis. Decrease in carbon dioxide pressure is achieved by hyperventilation of the lungs. If the denominator increases, then the pH tends to decrease. Such a changes are achieved by hypoventilation of the lungs and contributes to the carbon dioxide delay in the body and result in the compensation of metabolic alkalosis.

Thus, compensation for pathological changes in the acid-base balance is closely related to the regulation of respiration. So, when the pH of the blood is decreased (acidosis), inspiratory neurons of the respiratory center are reflectory stimulated. As a result, ventilation of the lungs increases, what leads to increased elimination of $CO_2$ to the atmosphere. This gas is a product of the decomposition of carbonic acid, which, in turn, was formed by binding bicarbonate to hydrogen ions. With an increase in the concentration of alkaline ions in the blood, the activity of the respiratory center decreases, this causes the decrease in ventilation of the lungs. In this way, $CO_2$ is retained in the blood. It is converting to carbonic acid, which dissociates on additional bicarbonate ions and hydrogen.

Consequently, the lungs operate as a physiological homeostatic system, that can produce an excess of volatile carbonic acid, released into the atmosphere in form of $CO_2$, or to restore the buffer capacity of the bicarbonate buffer by retaining the carbon dioxide in the body because of a negative feedback between the $CO_2$ pressure of the arterial blood and the functional activity of the respiratory center.

## 5.5 The involvement of the kidneys in the regulation of acid base balance.

Excretory renal function is the third, most powerful defense line of the body against significant changes in the acid base balance (ABB). Compensatory influences of the kidney are realized through 4 interconnected mechanisms:

1. ***Antiport*** - secretion of $H^+$ ions into the urine in exchange for reabsorption of $HCO_3$ ions - into blood;
2. ***Regulation of reabsorption and secretion of $HCO_3$ in renal tubules*** depending on the presence of acidosis or alkalosis;
3. ***Secretion of ammonium ions ($NH_4$ +)*** into urine;

4. ***Excretion of dihydrophosphates or hydrophosphates*** depending on the presence of acidosis or alkalosis.

Renal compensation of ABB disturbances develops relatively slowly (within several hours), but it is capable to sustain the vital body functions for a long time, even i n severe cases of ABB. The tubular epithelium of the kidneys contains special enzymes, such as ***carbonic anhydrase*** (like erythrocytes), and special transport proteins of the membrane, that provide interconnected transport of hydrogen ions and bicarbonate as well as sodium and potassium ions in opposite directions ***(antiport)***. Fig. 5.23 Illustrates the processes occurring in the epithelium of the renal tubules by excess and deficiency of hydrogen ions in the blood, related to the bicarbonate transport.

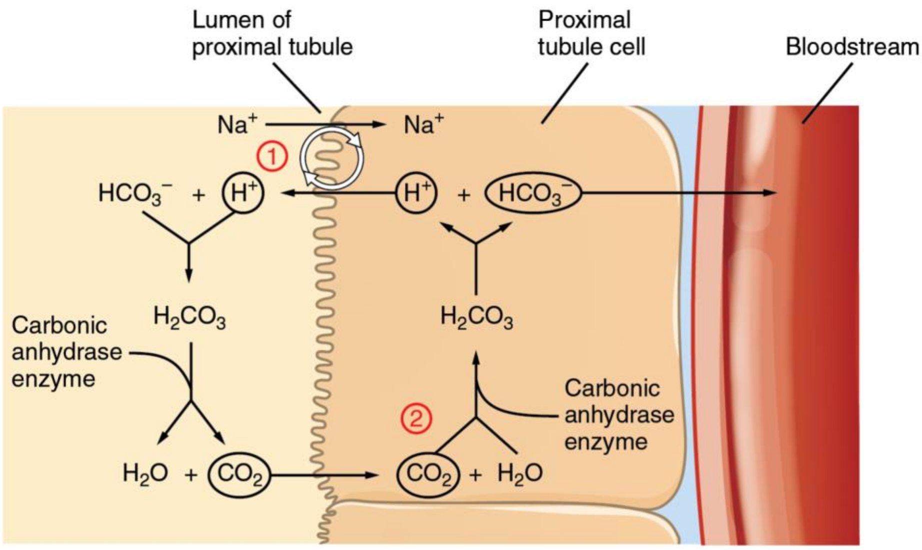



Fig.5.23. Conservation of bicarbonate in Kidney.



Normally, the kidneys produce weak acid urine with a pH of 5-7. But in case of compensation of acidosis and alkalosis, urine may be very acidic (pH < 4) or weakly alkaline (pH > 8). If the content of non-volatile acids in the blood increases, then the kidneys produce an excess of hydrogen ions in the form of dihydrophosphate and ammonium ion. The formation of these compounds in the urine is due to secretion of hydrogen ions by the renal tubules epithelium in exchange for the reabsorption of $Na^+$ ions (***antiport***). The processes of tubular hydrogen ion secretion are associated with bicarbonate reabcorbtion into the blood. In alkalosis, the processes of tubular secretion of hydrogen ions, and, therefore, of $Na^+$ and bicarbonate reabsorption are suppressed, what leads to producing of alkaline urine by kidney.

## 5.6. The role of the gastrointestinal tract and the liver in the displacement of acid base balance.

The state of acid-base balance significantly depends on the nature of human nutrition. Thus, plant food contains lots of organic acid salts, which are a source of conjugated bases, that are accumulated in the blood and create the alkaline reaction of the internal environment (as a rule, it is compensated alkalosis.) Similar changes in the ABB occur in case of long alkaline mineral waters drinking. Protein food of animal origin promotes the acidification of the internal environment, since amino acids formed by the metabolism are converted to non-volatile inorganic acids (phosphoric and sulfuric).

The gastrointestinal tract plays a relatively small role in the regulation of ABB. However, the amount of alkaline components in the blood increases in the pathology (for example, peptic ulcer and gastritis with hypersecretion of hydrochloric acid). Disorders of the bowel function also may be accompanied with changes in the ABB. For example, when intestinal obstruction occurs, frequent vomiting leads to loss of hydrogen and chloride ions, what requires external correction.

The role of the liver in maintaining of the normal ABB is manifested in the fact that it is place, where utilization of lactic acid with the formation of glycogen, as well as the oxidation of ketone bodies occur. The secret of the liver, bile, has, as a rule, a weakly alkaline reaction. But depending on the metabolic needs, the amount of alkaline components in the bile may be higher or lower. By liver disease, all types of metabolism, in particular, lipid metabolism, which can lead to severe metabolic acidosis, are disturbed. However, the liver can also play a role in compensation of ABB disturbances. So, in acidosis, the liver enhances the formation of glutamic acid, and in alkalosis - the conversion of ammonia into urea.

## 5.7. Laboratory diagnosis of acid base disturbances.

The state of acid-base equilibrium can be estimated by analyzing several physicochemical parameters of the plasma related to the bicarbonate buffer system. The fact is, that this system, unlike the other, is simultaneously ventilatory open and associated with the excretory function of the kidneys. Therefore, it responds most quickly to the deviations of the ABB, providing compensation, which can be either full or partial. In severe cases, decompensated disorders of the ABB occur, when the pH goes beyond homeostasis. The main indicators of the ABB include:

- ***pH of the blood***. By the value of pH it can be judged whether the concentration in the blood of hydrogen ions is normal, or it is changed in one direction or another. At the same time, the normal value of pH (7.35 -7.45) still does not provide grounds to establish with certainty the absence of the ABB disturbances, since it does not allow to exclude compensated acidosis or alkalosis. The criteria for partial compensation (***partly compensated***) of acidosis is the range of pH from 7.35 to 7.2; alkalosis - from 7.45 to 7.6. The criteria for

***decompensated*** acidosis is the pH value of <7,2; for decompensated alkalosis - pH> 7.6.

• ***$PaCO_2$***. Deviation of $CO_2$ tension in the arterial blood from the norm (35-45 mm Hg) is a sign of respiratory disturbance of acid-base balance or it points to lung compensations of metabolic ABB disturbances.

• *[$HCO3^-$]*. Concentration of bicarbonate ions in arterial blood (normally 22-28 mmol/l) indicates either the primary metabolic type ABB disorder or the involvement of the kidneys in compensation of respiratory acidosis or alkalosis.

• *excess of bases (BE - base excess)* - the deviation of the total concentration of all buffer bases from the normal level. In the norm, BE varies from -4.0 mmol/l to +4.0 mmol/liter. This is the most informative ***metabolic*** parameter of the acid-base balance, which shows the difference between the actual concentration of conjugated bases in the tested blood sample and the reference (control) value of this concentration under standard conditions. This parameter can be obtained by titration of blood. It shows how much mmoL of acid or base should be added to 1 liter of arterial blood to bring its pH to 7.4 at standard conditions: t = 38 ° C, $PCO_2$ = 40 mm Hg, 100% blood oxygen saturation, hemoglobin content of 150 g/l and plasma proteins level of 70 g/l. Negative values of BE denotes a lack of bases (to bring the pH to 7.4, the tested blood should be to be titrated with base), and positive - an excess of conjugated bases (to bring the pH to 7.4, the test blood should be titrated with acid). The zero value of BE indicates that under standard conditions, the pH of the trsted blood is 7.4 without titration. The informativeness of BE concerning the metabolic disorders or their compensation by buffer bases is not much higher than the concentration of plasma bicarbonates, since they are the largest component of the alkaline buffer reserve of blood.

In clinical practice, 4 typical ABB disorders are distinguished:

*1. Respiratory acidosis*
*2. Respiratory alkalosis*
*3. Metabolic acidosis*
*4. Metabolic alkalosis*

For the purpose of rapid diagnosis of these disorders, it is enough to analyze only 3 following parameters (pH, $PaCO_2$ and [$HCO_3^-$]) using a certain algorithm, illustrated by Fig. 5.24. The degree of compensation for the disorder (compensated, partly compensated or decompensated condition) is judged by the pH level (see above).

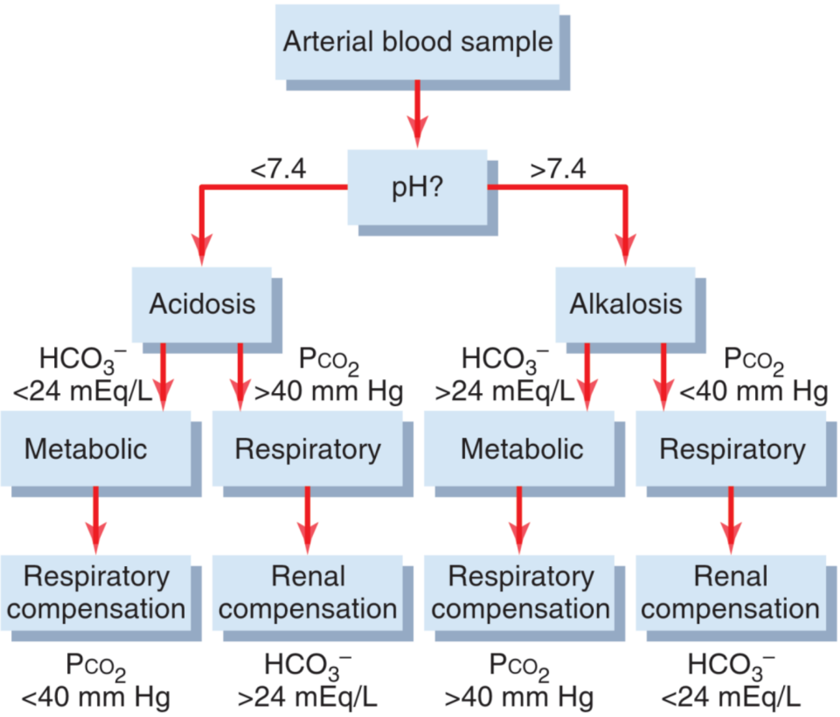



Fig.5.24. Diagnostic algorithm for acid-base disturbances.
By Guyton and Hall Textbook of Medical Physiology.- 13 ed. - 424.

For example, according to such patient data: pH = 7.33; $PaCO_2$ = 64 mm Hg; [$HCO_3^-$] = 38 mmol/l, it is possible to establish a preliminary diagnosis of simple disorder of ABB - "Partly compensated respiratory acidosis". In some cases, if patient data is not consistent with the presented algorithm, it is possible to assume that, he has a ***mixed disorder:*** the metabolic and respiratory acidosis simultaneously or the metabolic and respiratory alkalosis simultaneously. These disorders are more severe, than simple ones, since there is no compensation for the etiological factor. The following patient data can be a good example of a mixed respiratory and metabolic acidosis: pH = 7.25; $PaCO_2$ = 55 mm Hg; [$HCO_3^-$ = 12 mmol/L.

For the diagnosis of mixed disorders, the graphical empirical scheme was proposed by Cogan MG and Rector FC Jr. (Cogan MG, Rector FC Jr.: Acid-Base Disorders in the Kidney, 3rd ed Philadelphia: WB Saunders, 1986). This scheme was elaborated by analyzing all theoretically possible relationships between pH, $PaCO_2$ and the concentration of $HCO_3^-$ in arterial blood of the patient, taking into account the dynamics of the compensation mechanisms. Oval in the center of the scheme means no violation of the ABB. If the point, that reflects the patient's data, falls into the shaded area, then it is a simple disorder of the ABB, and if it is projected into a non-shaded area, then a mixed disorder is diagnosed.

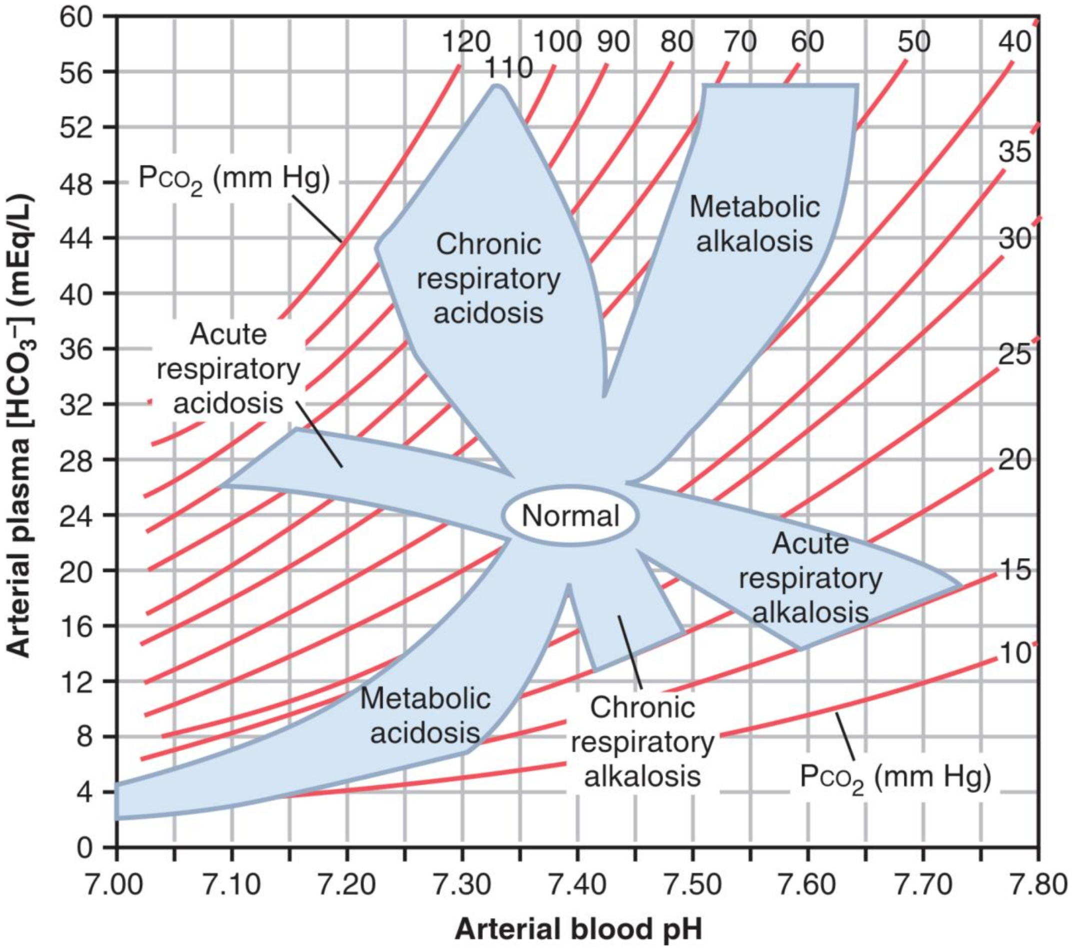



Fig.5.25. Nomogram for determining of acid-base disturbance types.
By Guyton and Hall Textbook of Medical Physiology.- 13 ed. - 425.

## 5.8. Clinical and physiological characteristics of typical acid base disorders.

***Respiratory acidosis.***

The main cause of respiratory acidosis is alveolar hypoventilation, which leads to slow withdrawal of $CO_2$ from the blood and increase in $PaCO_2$ to a level higher than 45 mm Hg. (***hypercapnia***). This situation can occur in the case of breathing inhibition by some drugs (eg, barbiturates, alcohol), in the onset of bronchial asthma (due to increased resistance of the respiratory tract), in violation of respiratory gases diffusion (fibrosis of the lungs, severe pneumonia), muscular dystrophy and others diseases of the skeletal system (due to decreased lung ventilation). The most common cause of respiratory acidosis is chronic obstructive pulmonary disease (COPD), including ***emphysema***. The parameters of oxygen pressure in arterial blood $PaO_2$ (norm 80-100 mm Hg) and saturation of $HbO_2$ (norm -95-100%).are an additional diagnostic sign and criterion of respiratory acidosis severity in such states. Compensation for respiratory acidosis is possible only with the involvement of renal mechanisms - secretion of $H^+$ into the urine and increased reabsorption of bicarbonates into the blood. However, these mechanisms are not turn on immediately, but several hours after the onset of respiratory acidosis. Therefore, acute respiratory acidosis is characterized by a drop in pH level with a simultaneous increase in $PaCO_2$ , but normal bicarbonate and BE level. After the inclusion of renal compensation, the pH is shifted towards normalization, and the concentration of bicarbonates and BE becomes higher, than normal. If the mechanisms of renal compensation are insufficient (kidney disease), the ABB disorder may remain partially compensated , and in severe cases - even transformed into an uncompensated one. Treatment of respiratory acidosis includes, if possible, elimination of its causes. if necessary, mechanical ventilation of the lungs is using and also - intravenous administration of lactate, which is converted by the liver into bicarbonate. All therapeutic measures are carried out with the obligatory control of pH, blood gases and electrolytes.

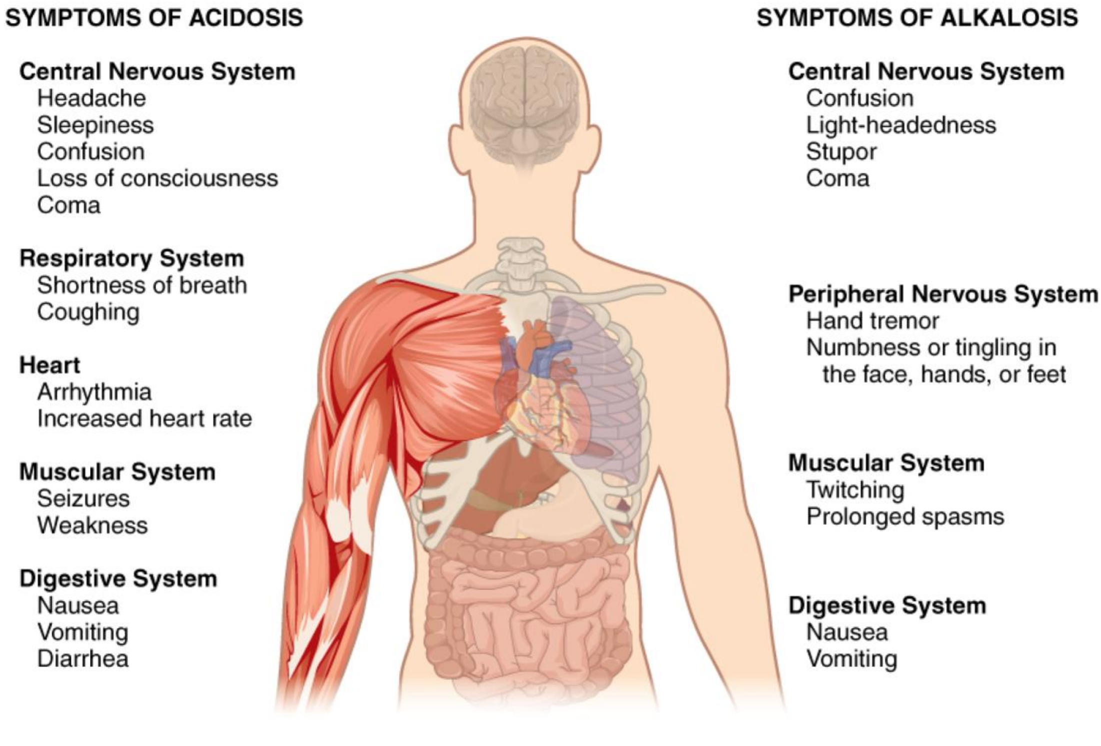



Fig.5.26. Clinical symptoms of acidosis and alkalosis.

*Respiratory alkalosis*.

This ABB disturbance is much less frequent than respiratory acidosis. Its cause is, as a rule, an increase in alveolar ventilation, accompanied by inadequate metabolic carbon dioxide production. As a result, $PaCO_2$ in the blood decreases to levels below 35 mm Hg. (hypocapnia) and pH of the blood increases. Renal compensation is achieved by increased releasing of bicarbonates with urine and simultaneous inhibition of hydrogen ions secretion by tubular epithelium.

In clinical practice, respiratory alkalosis is most often encountered in the case of inadequate artificial ventilation of the lungs and in patients with mental overexcitation (hysterical conditions), which are accompanied with hyperventilation of the lungs. In the last case, for the correction of the state, the patient is offered to breathe in a paper bag for a while, what results in increase of $CO_2$ tension in the blood.

Respiratory alkalosis can occur with prolonged staying of an untrained person in high altitudes, where the overall atmospheric pressure decreases, and as a result - the carbon dioxide and oxygen tension in the arterial blood are reduced. The danger of this condition is that, the respiratory system does not have sufficient regulatory stimulus to increase ventilation despite of tissue hypoxia. The $CO_2$ tension in the blood isn't sufficient for the respiratory center's excitation.

***Metabolic acidosis***.

This is the most common type of ABB disorder in clinical practice. It arises in that cases, where the delivery into the body of $H^+$ ions of exogenous (food, medicine, drinks) or endogenous (increased muscular activity, disease of internal organs) origin exceeds its excretion from the body. The metabolic sources of $H^+$ ions include increased amount of lactic acid, as a result of enhanced anaerobic oxidation of nutrients (lactic acidosis) and increasing level of ketone bodies as a result of enhanced decomposition of fatty acids and some amino acids (ketoacidosis). An example of ketoacidosis is a disturbance of ABB in diabetes mellitus and during low-carbohydrate diets. A common feature of all metabolic acidoses is decreased concentration of bicarbonates and BE, that are responsible for the tendency to decrease in pH level due to the equilibrium shift in the bicarbonate buffer system. This trend is countered by respiratory compensation, which, unlike respiratory acidosis, unfolds immediately. It is revealing by lungs hyperventilation with followed decrease in the carbon dioxide tension of the arterial blood. The kidneys also take part in compensation. They are able to secrete hydrogen with urine and increase reabsorption of bicarbonates into the bloodstream. If the compensatory capacity of the patient's lungs and kidneys is preserved, then full or partial compensation comes fairly quickly with close to the norm pH values and relatively low levels of the bicarbonates and $PaCO_2$ in the arterial blood. Another clinical cause of the metabolic acidosis is the excess loss of $HCO_3^-$. For example, such state is observed in prolonged profuse diarrhea, when a large amount of fecal masses with alkaline reaction is lost through the digestive tract. To distinguish this type from other types of metabolic acidosis the concept of "anion gap" is used. Anion gap is the difference between the concentration of the main plasma cation Na+ and the two main anions: $Cl^-$ and $HCO_3^-$:

$$\text{"anion gap"} = [Na^+] - [Cl^-] - [HCO_3^-]$$

In healthy people, the "anion gap" is within the range of 8-14 mmol/l. If bicarbonates are spent on binding with hydrogen ions (for example, by lactic acidosis), their concentration decreases, and the concentration of chlorine ions does not change. As a result, anion gap becomes higher than normal. In the case of metabolic acidosis associated with the loss of bicarbonates through the digestive tract, its concentration in the blood is also reduced, but the concentration of chlorine ions increases compensatory and the "anion gap" remains within normal limits.

***Metabolic alkalosis.***

This ABB disturbance is associated with increased levels of alkaline metabolites (antacids, excessive consumption of bicarbonate mineral waters) or excessive loss of non-volatile organic acids (eg frequent vomiting with loss of hydrochloric acid in gastric juice). In both cases, the result is the same: a decrease in the concentration of hydrogen ions and a tendency to shift the pH to higher values. There are compensatory mechanisms, that counteract this trend. Lungs participation in the compensation of metabolic alkalosis is realized through the hypoventilation of the alveoli and accumulation of carbon dioxide, which is converted by the bicarbonate buffer system into an additional amount of hydrogen ions. However, such shifts increase the concentration of bicarbonates, what reduces the efficiency of compensation. In addition, hypoventilation leads to an undesirable effect - reducing the oxygen supply of the body with the subsequent hypoxia of tissues and organs. Therefore, renal compensatory mechanisms are more important. These are the increased excretion of bicarbonates with urine and inhibition of hydrogen ions antiport in kidney.