ФИЗИОЛОГИЯ КРОВИ.
МАТЕРИАЛЫ ЛЕКЦИЙ

PHYSIOLOGY OF BLOOD.
LECTURE NOTES

Рекомендовано Учебно-методическим объединением по медицинскому образованию Республики Беларусь в качестве пособия для студентов учреждений высшего образования по специальностям 1-79 01 01 «Лечебное дело» и 1-79 01 07 «Стоматология», обучающихся на английском языке

2-е издание

Минск БГМУ 2017
УДК 612.1(075.8)-054.6
ББК 28.707я73
С28

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Издание содержит материалы лекционного курса нормальной физиологии, включенные в раздел «Физиология крови», а также тему «Химическая сигнализация». Изложены важнейшие понятия физиологии крови, необходимые для подготовки к практическим занятиям, коллоквиуму по данному разделу и экзамену. Подробное объяснение, значительное количество иллюстраций и схем облегчает понимание материала и способствует его глубокому усвоению. Первое издание вышло в 2014 году.

Предназначено для студентов 1-2-го курсов медицинского факультета иностранных учащихся, обучающихся на английском языке по специальностям «Лечебное дело» и «Стоматология».

УДК 612.1(075.8)-054.6
ББК 28.707я73

Учебное издание

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ФИЗИОЛОГИЯ КРОВИ. МАТЕРИАЛЫ ЛЕКЦИЙ
PHYSIOLOGY OF BLOOD. LECTURE NOTES
Пособие
На английском языке
2-е издание

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Подписано в печать 21.06.17. Формат 60×84/8. Бумага писчая «Снегурочка».
Ризография. Гарнитура «Times».
Усл. печ. л. 6,04. Уч.-изд. л. 4,0. Тираж 60 экз. Заказ 501.

Издатель и полиграфическое исполнение: учреждение образования
«Белорусский государственный медицинский университет».
Свидетельство о государственной регистрации издателя, изготовителя,
распространителя печатных изданий № 1/187 от 18.02.2014.
Ул. Ленинградская, 6, 220006, Минск.


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Theme 1
INTERNAL ENVIRONMENT OF THE BODY.
HOMEOSTASIS

The basic unit of the living organism is a cell. Cells of different tissues of the body may differ significantly from each other but all of them share some common features. One of these features is the way of deriving energy from biological oxidation (oxidative phosphorylation) reactions (fig. 1.1):

![Fig. 1.1. The substances the cell uses to obtain energy and the main end products formed in biological oxidation reactions in the cell](image)

Thus cells need a **constant supply of oxygen** and **nutrients** and a **constant elimination (excretion)** of waste products.

Simple unicellular organisms are able to get necessary substances transported through their membrane from the **external environment** and remove exchange products into it. But multicellular organisms do not have this possibility. Thus it became necessary to develop the “delivery systems” to all the cells:

- firstly — **blood**, the fluid able to **transport** all the necessary substances;
- the **circulation system** (**cardiovascular** system), to provide blood movement and delivery of transported oxygen and nutrients to each cell;
- the system of **O₂ supply** to the circulating blood (**respiratory system**);
- the system of **nutrients supply** to the circulation (**gastrointestinal tract**);
- the waste products **excretion** system (**kidneys** mainly).

Due to the constant functioning of these systems necessary substances are constantly supplied to and some substances constantly removed from the extracellular fluid. This results in the relative constancy of the extracellular fluid composition and therefore the constancy of the conditions for cells existence. As the extracellular fluid is the “place of living” of cells, it is the **internal environment of the organism**.

**Homeostasis** is the constancy of the internal environment of the organism as well as the mechanisms of its maintaining.

**THE MAIN FLUID COMPARTMENTS OF THE BODY**

The total amount of water in adults constitutes about 60 % of body weight. The percentage of total body fluid decreases with age: in newborns the total body water ranges from 70 to 75 % of body weight, and in older persons it becomes less than 60 % due to the increase of fat tissue content and loss of tissue water.
About 2/3 of the whole body fluid is the intracellular fluid, whereas 1/3 is the extracellular fluid. The extracellular fluid, in turn, consists of few compartments, the main of which are the interstitial (intercellular) fluid and the fluid part of blood (without blood cells), blood plasma (fig. 1.2). The volume of blood plasma is about one fourth of the whole extracellular fluid volume; that means that the interstitial fluid comprises the larger part of the extracellular compartment. The interstitial fluid and blood are separated by the vascular wall which is easily permeable to all blood substances except proteins.

<table>
<thead>
<tr>
<th>Intracellular compartment</th>
<th>Extracellular compartment:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial fluid</td>
<td>Blood plasma</td>
</tr>
</tbody>
</table>

Fig. 1.2. The main compartments of the body

Another small part of extracellular fluid is referred to as transcellular fluid. It includes the fluid in the synovial, peritoneal, pericardial, and intraocular spaces, and the cerebrospinal fluid, as well.

**Differences in the compartment composition**

The intracellular and extracellular compartments are divided by a cell membrane that is highly permeable to water but not permeable to electrolytes. Therefore the compositions of the two large compartments differ from each other (table 1.1). The main ions of the intracellular fluid are potassium, $K^+$ ions. The extracellular fluid main ions are sodium, $Na^+$, and chlorine, $Cl^-$ ions.

**Table 1.1**

<table>
<thead>
<tr>
<th>Intracellular Fluid (ICF):</th>
<th>Extracellular Fluid (ECF):</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K^+$, Mg$^{2+}$, HPO$_4^{2-}$ ions, proteins</td>
<td>$Na^+$, $Cl^-$, HCO$_3^-$ ions</td>
</tr>
</tbody>
</table>

The main parts of the extracellular fluid are divided by a vessel wall that is highly permeable not only to water but to all electrolytes and other substances excluding proteins. So, the only difference in composition of the interstitial fluid and blood plasma is the higher protein content of blood. The concentrations of all ions are practically similar in the extracellular fluid as a whole, including blood and the interstitial fluid. If any difference of low-molecular substances concentration arises between blood plasma and the interstitial fluid, this difference immediately disappears due to the transition of substances through the blood vessel wall down the gradients, thus balancing the concentrations.

Maintenance of the constancy of the internal environment for the cell requires keeping the composition of the extracellular fluid relatively constant. There exist many indices of the body fluids that must be kept at a constant level. It includes ion concentrations, protein and glucose blood levels, and many others. But there are a few most essential constants among all these indices. These constants have very narrow normal limits of variations so they are considered to be the strict constants of homeostasis.
The main physical and chemical constants of homeostasis

The main body fluids *strict constants* of *physical and chemical nature* include the osmotic blood pressure, the oncotic blood pressure and pH of blood. The other important constants are the arterial blood oxygen level, body temperature, blood glucose level and others.

**OSMOTIC PRESSURE**

The osmotic pressure of a solution is created by all dissolved particles present in this solution. The force of osmotic pressure is applied to the solvent molecules which are attracted by the dissolved particles — solutes. The universal solvent of all the solutions of the body is water. If there are two compartments divided by a semi-permeable membrane (permeable for water but not permeable for the dissolved substances), water moves to the compartment that contains the solution having *higher* concentration of the dissolved substances (and thus the lower water content). Thus, water moves through the membrane down its own gradient — from the area of higher water molecules concentration to the area of lower water concentration. This water transition through the membrane is referred to as *osmosis* (fig. 1.3).

![Fig. 1.3. Osmosis: the movement of water through semi-permeable membrane to the side of the solution having higher concentration (C₂) of NaCl](image)

As shown in the fig. 1.4, an osmotic pressure difference that creates water movement through a semi-permeable membrane can be measured as the hydrostatic pressure difference which opposes further water movement into the column of connected vessels that contains non-diffusible solute. As these non-diffusible particles attract water, the level of fluid in the left column of the connected vessels in the picture becomes elevated, while the other column level decreases thus creating the pressure hydrostatic difference between the two columns. This hydrostatic pressure difference is equal to the osmotic pressure of the solution in the left column of the connected vessels.

According to the given characteristics of osmotic pressure, the extracellular fluid osmotic pressure (as well as blood plasma osmotic pressure) depends on
the **total amount** of particles of **all dissolved substances** (electrolytes and non-electrolytes). The total number of particles in the solution is measured in **osmoles**. When 1 mole of a substance is added to 1 liter of water (1 mol/L), it creates the osmotic pressure of 1 Osm/L. But if the substance molecule dissolution in water produces two particles, it creates osmotic pressure 2 Osm/L. For example, the water solution of NaCl 1 mol/L has the osmotic pressure 2 Osm/L as it dissociates into Na\(^+\) and Cl\(^-\) ions thus creating the two particles from one molecule.

There are the two terms, **osmolality** and **osmolarity**. Osmolality is expressed as osmoles per kilogram of water; osmolarity is expressed as osmoles per liter of solution. For the body fluids the difference is negligible; mostly the osmolarity of blood is measured.

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![Fig. 1.4](image)

**Fig. 1.4.** Water transition through a semi-permeable membrane in the connected vessels which contain non-diffusible solute particles in one column: the initial state (a) and the resulting balance of the osmotic and hydrostatic pressures (b)

The substances of the extracellular fluid are present in concentrations much less than 1 mol/L; their concentration is expressed in millimoles per liter (mol \(\times\) 10\(^{-3}\)), and less. Therefore the total amount of the dissolved particles, or the osmotic pressure of the extracellular fluid is expressed in **milliosmoles per liter**, mOsm/L.

The two main fluids of the extracellular compartment, the interstitial fluid and blood plasma, as it was mentioned above, differ from each other only by their protein content. This composition difference does not create a marked difference in the osmotic pressure. The osmotic pressures of the interstitial fluid and blood plasma are practically similar because of a very large molecular weight of proteins and therefore their small concentration expressed in moles (mmol) per liter. The protein content in blood is few times higher than that of the interstitial fluid and it amounts to 60–85 g/L. But as the protein particles (molecules) are very large, their millimolar concentration is less than 1 millimole per liter, and so their contribution to the osmotic pressure is less than 1 mOsm/L.
The value of **blood plasma osmotic pressure** in a healthy individual is about $290 \pm 10 \text{ mOsm/L}$. The main contribution to the blood plasma osmotic pressure is made by electrolytes — ions, $Na^+$ and $Cl^-$ ions in the first place.

**Table 1.2**

<table>
<thead>
<tr>
<th>Blood plasma substance</th>
<th>Concentration (mean values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Na^+$</td>
<td>145 mmol/L</td>
</tr>
<tr>
<td>$Cl^-$</td>
<td>105 mmol/L</td>
</tr>
<tr>
<td>$HCO_3^-$</td>
<td>25 mmol/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>5 mmol/L</td>
</tr>
<tr>
<td>$K^+$</td>
<td>4.5 mmol/L</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>$\leq 1 \text{ mmol/L}$</td>
</tr>
<tr>
<td><strong>Total concentration of all substances</strong></td>
<td>$290 \pm 10 \text{ mOsm/L}$ $(280–300)$ — the osmotic pressure</td>
</tr>
</tbody>
</table>

As seen from the table 1.2, sodium and chlorine ions together create the osmotic pressure of 250 mOsm/L, whereas all the rest substances of blood plasma create just the rest 40 mOsm/L of the total osmotic blood pressure.

As the osmotic pressure influences water shifts through the semipermeable membrane (permeable for water only) such as the cell membrane, it determines **water distribution** between the cells and the extracellular compartment of the body. There is a constant exchange of water between cells and extracellular fluid but the amount of water entering the cells is precisely balanced with the amount of water going out of the cell. This balance is maintained due to the **equality** of the osmotic pressures of the intracellular and extracellular fluids. The composition of the intracellular fluid differs from that of the extracellular one, but the total concentration of all substances in milliosmoses per liter is exactly the same in both fluids.

The extracellular fluid composition and its osmotic pressure can be changed due to the excessive gain (or loss) of water or the excessive gain (or loss) of ions and other low-molecular substances. We can drink water or have salty food normally within wide limits; only the persons having some diseases (mainly kidney diseases) are limited in their abilities to drink fluids and eat salty food. All this is possible due to the very strict regulation of the extracellular fluid osmotic pressure in order to keep it at the same level as the osmotic pressure of the intracellular fluid. But if there is a deviation of the osmotic pressure level of the extracellular fluid, the osmotic pressure **difference** arises that causes water movement to the compartment having a higher value of the osmotic pressure.

Thus, in case of the **increase** of the osmotic pressure of the whole extracellular fluid (including the blood plasma osmotic pressure) the water transition occurs from the cells to the extracellular compartment until the balance of the two osmotic pressures is achieved. That results in the **cellular dehydration** (the cells volume decreases). In case of the **decrease** of the extracellular fluid osmotic pressure the water transition occurs from the extracellular compartment to the cells, until the balance of the two osmotic pressures is achieved. That results in a **cellular edema** (the cells volume increases) (fig. 1.5). Both states are unfavorable for the cells and
the organism as a whole but in the first place it is dangerous to the central nervous system cells, neurons. They are highly sensitive to the changes of their volume, and any of such changes causes serious disorders in their functioning. Cellular dehydration may cause such symptoms of the CNS disorder as headache, apathy, and disorientation. What is much more dangerous is the rapid development of a cellular edema that includes brain edema and results in neurological symptoms such as nausea, lethargy, and may lead to seizures, coma, permanent brain damage, and death.

Therefore the osmotic pressure is one of the strict homeostatic constants and its regulation is so precise.

**Fig. 1.5.** The consequences of the extracellular fluid osmotic pressure changes

Many types of solutions are administered intravenously to provide the transfusion of some medicines or nutrients for patients. One of the basic requirements to such solutions is that they must have the same osmotic pressure as the extracellular fluid and blood plasma.

Solutions that have the same osmotic pressure as the blood plasma are called **isotonic solutions**.

- 0.9 % (0.85–0.9 %) NaCl solution is isotonic (the other term for this solution is physiological);
- 5 % glucose solution is isotonic.

These solutions are used for transfusion of various drugs.

Solutions having higher concentration and therefore higher osmotic pressure (Posm) are hypertonic, and those having lower concentration and Posm — hypotonic.

When the cells are surrounded by fluid having the same Posm as inside the cell, i.e. in isotonic solution, the water influx and efflux are balanced because there is no difference of Posm. But when there is a difference of Posm water goes to the compartment that has a higher concentration of solutes:
- in hypertonic solutions water goes from the cells to the surrounding fluid; it results in cell shrinkage (dehydration of the cells);
• in hypotonic solutions water goes to the cells; it increases the cell volume and intracellular pressure and results in cell swelling (cellular edema).

**Oncotic Pressure**

Colloid osmotic (oncotic) blood pressure is the part of osmotic pressure created only by blood plasma proteins. The main contribution to the oncotic pressure is made by albumins, relatively small proteins which make up the largest fraction of blood plasma proteins. The number of particles (molecules) of these proteins is much higher as compared with the other blood proteins. Therefore the contribution of larger proteins, globulins and fibrinogen, is much less marked.

Oncotic pressure is mostly measured in mm Hg. The normal value of blood plasma oncotic pressure is about 25–30 mm Hg.

Oncotic pressure (P onc) is the important homeostatic constant which determines water distribution within the extracellular compartment, between the interstitial fluid and intravascular fluid — blood plasma. The vessel wall is practically not permeable for proteins and more or less permeable for other plasma components so the only difference between the interstitial fluid and plasma is a higher protein content of plasma. Thus, the oncotic pressure of blood is higher than that of the interstitial fluid which surrounds vessels. It holds a certain amount of water inside the vessels. The increase of plasma oncotic pressure results in the shift of water into the vessel. In case of protein loss the oncotic pressure is lowered. It results in the shift of water from vessels to the interstitial space. This type of water shifting not to the cells but to the interstitial space is called an interstitial edema.

The main reasons for the oncotic pressure increase can be water loss. It causes a relative increase of the oncotic blood pressure which attracts some water from the interstitial space into the vessels. This actually contributes to the restoration of the circulating blood volume and therefore to the restoration of blood pressure.

As for the decrease of the oncotic pressure, it is the result of blood protein loss. It occurs in case of severe diseases of the liver, in prolonged starvation, but most often as a result of kidney diseases when the kidney filter is damaged and blood proteins are lost with urine. All these cases lead to the interstitial edema development. This is the most frequent type of edema in medical practice. The interstitial edema may develop due to other reasons, e.g., due to the increase of the hydrostatic blood pressure.

**pH as the index of H+ ions concentration**

The concentration of H+ ions in the body fluids is strictly regulated and maintained at a constant level. Most of the enzyme systems of the body can work only in the narrow range of H+ ions concentrations, and even small deviations from the norm may affect enzymes activity and disturb body functions.

To evaluate H+ ions level the logarithm index of hydrogen is used, pH. Chemically neutral solutions have pH 7.0. As pH is inversely related to the H+ concentration, the higher level of pH corresponds to a low H+ concentration, and lower pH values correspond to a high H+ ions concentration.
The arterial blood plasma pH is about $7.4$ ($7.35–7.45$). The mean venous blood pH is about $7.35$ due to the higher amount of $\text{CO}_2$ taken from tissues and transported mostly as $\text{HCO}_3^-$ ions. Such values actually are slightly alkaline. As the end products of metabolism are acidic, it enables us to oppose the acidification of body fluids. So for the pH of blood the value $7.4$ ($7.35$) is considered to be neutral. When the arterial blood plasma pH value falls below the lower normal limit ($< 7.35$) it is referred to as acidosis, and when pH rises above the higher limit ($> 7.45$) it is referred to as alkalosis (fig. 1.6).

![Fig. 1.6. The scale of arterial blood pH: normal values and values corresponding to the states of acidosis and alkalosis.](image)

To regulate $\text{H}^+$ ions concentration of the body fluids and prevent acidosis or alkalosis we have three lines of defense.

The first line of defense is a set of acid-base buffer systems of body fluids. The components of buffer systems can rapidly bind excess of $\text{H}^+$ or $\text{OH}^-$ ions in order to maintain $\text{H}^+$ ions concentration relatively constant. There are 4 such systems:

1. **Bicarbonate** buffer system.
2. **Phosphate** buffer system.
3. **Hemoglobin** buffer system.
4. **Protein** buffer system.

1. The bicarbonate buffer system is one of the most powerful. It consists of two substances present in blood plasma as well as in the whole extracellular fluid: carbonic acid $\text{H}_2\text{CO}_3$ and salt $\text{NaHCO}_3$. The weak acid $\text{H}_2\text{CO}_3$ partially dissociates into $\text{H}^+$ and $\text{HCO}_3^-$ ions. The salt $\text{NaHCO}_3$ almost entirely dissociates into $\text{Na}^+$ and $\text{HCO}_3^-$ ions. In case of the increase of $\text{H}^+$ ions concentration these ions become bound by $\text{HCO}_3^-$-ions:

$$\text{H}^+ + \text{HCO}_3^- \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{CO}_2 + \text{H}_2\text{O}$$

Due to this reaction the additional amount of $\text{H}_2\text{CO}_3$ is formed that can be excreted by the lungs as $\text{CO}_2$; the concentration of hydrogen ions remains practically unchanged.

In case of the increase of $\text{OH}^-$ ions concentration, e.g., due to adding a strong base $\text{NaOH}$, the base reacts with the acid $\text{H}_2\text{CO}_3$:

$$\text{NaOH} + \text{H}_2\text{CO}_3 \rightleftharpoons \text{NaHCO}_3 + \text{H}_2\text{O}$$
The basic salt formed in this reaction is a much weaker base as compared to NaOH. Thus the resulting pH level again is maintained almost the same.

2. The phosphate buffer system is important in buffering intracellular fluid because of a much higher concentration of phosphate in this fluid. The phosphate buffer system, as well, plays an important role in buffering renal tubular fluid due to a high concentration of phosphate in the kidney tubules. This buffer system consists of the two salts, NaH$_2$PO$_4$ and Na$_2$HPO$_4$. Thus, the key elements of this buffer system are the two ions: H$_2$PO$_4^-$ and HPO$_4^{2-}$. One of these ions, H$_2$PO$_4^-$, is more acidic, while the other, HPO$_4^{2-}$, is more basic (alkaline). Adding the strong acid such as HCl causes its reaction with the basic salt, and adding the strong base such as NaOH causes the reaction with the acidic salt as follows:

\[
\text{HCl} + \text{Na}_2\text{HPO}_4 \rightleftharpoons \text{NaH}_2\text{PO}_4 + \text{NaCl}
\]

\[
\text{NaOH} + \text{NaH}_2\text{PO}_4 \rightleftharpoons \text{Na}_2\text{HPO}_4 + \text{H}_2\text{O}
\]

These reactions change the ratio of the two buffer system components but pH level change is insignificant. Sodium chloride and water formed in the reactions are neutral substances.

3. The hemoglobin buffer system is powerful due a high amount of hemoglobin in blood. Hemoglobin is able to bind H$^+$ ions. It occurs in the capillaries of the systemic circulation when CO$_2$ formed in tissues enters blood where it gets converted into its main transport form, HCO$_3^-$ ions:

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-
\]

This reaction results in the formation of numerous H$^+$ ions. It could decrease the pH level of venous blood significantly, but really pH of venous blood with respect to arterial blood decreases from the initial value 7.4 to 7.35 only. This occurs due to the binding of H$^+$ ions by hemoglobin. The reaction of CO$_2$ conversion into H$^+$ and HCO$_3^-$ ions takes place in red blood cells where hemoglobin (Hb) immediately binds most of formed hydrogen ions:

\[
\text{H}^+ + \text{Hb} \rightleftharpoons \text{HHb}
\]

The opposite reaction occurs in the lungs capillaries where hydrogen ions are released from hemoglobin and converted back to the carbonic acid which breaks up into CO$_2$ and H$_2$O.

4. The protein buffer system operates mainly within cells where the protein concentration is very high. As proteins are amphoteric compounds due to the presence of both acidic (carboxylic, COOH) and basic (amino group, NH$_2$) groups, they are able to act as acids or as bases under certain conditions. Therefore proteins form the buffer system that helps maintain a constant pH level in the cells.

All the conversions performed by buffer systems occur with the rate of chemical reactions, or within seconds. But their buffer capacity is limited as the amount of substance that reacts to the acid or base is limited.

The second line of defense from the deviation of H$^+$ ions concentration is respiration which provides the removal of CO$_2$ from blood and thus the decrease of
carbonic acid $\text{H}_2\text{CO}_3$. The higher $\text{CO}_2$ level in blood is, the higher the lung ventilation becomes. It results in the increase of $\text{CO}_2$ elimination with the expired air and in the decrease (back to normal) of $\text{H}^+$ ions concentration. In accordance with this, the decrease of $\text{CO}_2$ level in blood decreases lung ventilation, which results in $\text{CO}_2$ accumulation. It helps to restore the normal level of blood plasma $\text{H}^+$ ions concentration.

These changes of respiration provide an effective regulation of blood pH level within few minutes.

And the third line of defense is the function of kidneys which are able to excrete more acidic or more alkaline urine thereby maintaining the constancy of blood $\text{H}^+$ ions concentration. The process of acid or alkaline compounds excretion is relatively slow as it requires hours or even days. But kidney functioning is the most powerful way of regulation of the acid-base state in our organism.
Theme 2
BASES OF INFORMATION EXCHANGE OF THE CELL
WITH THE ENVIRONMENT: CHEMICAL SIGNALING

Every cell of a multicellular organism maintains the exchange with the environment of 3 basic types: exchange of substance, energy and information. This is necessary for cell survival and for the integration of cells functioning in the organism, which finally determines the survival of the organism as a whole.

Information can be transferred by means of various substances as well as by energy. There are different types of signals carrying information:

- signals of physical nature — light, sound, pressure, temperature;
- signals of chemical nature — hormones, neurotransmitters, cytokines, growth factors, molecules of odorants and flavouring substances;
- signals of physicochemical nature — pH, osmotic pressure, colloid osmotic (oncotic) pressure, partial pressures of gases, electric potentials;
- complex signals — combinations of sounds, colors, odors; word as a special kind of signal.

To be a true signal it must be perceived by receptors. There are two basic types of receptors.

I. Molecular, or cellular receptors — complex molecules of the plasma membrane of the cell (or intracellular membranes) that serve for perceiving chemical signals and changing cell functioning.

II. Sensory receptors — complex cells designed for sensory signals perceiving and transforming them to nerve impulses which can be transmitted to the central nervous system.

Types of intercellular communication

Intercellular communication involves molecular receptors.

The basic types of intercellular communication between cells are auto- and paracrinia, endocrinia (and neuroendocrinia), communication through synapses, and communication through gap junctions.

Both auto- and paracrinia are the type of communication by releasing substances into interstitial fluid where these substances are available for nearly located cells of one organ or tissue. In autocrinia the cell releases substances which act on the same cell receptors and may change its functioning. In paracrinia these substances act on the neighboring cells.

The endocrine way of communication is accomplished by releasing biologically active substances into the internal environment of cells — blood. Then these substances circulating in the blood can influence any distant cell of the organism which has receptors to it. The neuroendocrine way of communication is a special subtype of the endocrine one which differs by releasing substances from the neuronal cells possessing the endocrine function (neuroendocrine cells).

In the synaptic type of communication the cells of the nervous system send nervous impulses down its axons. The axons form special contacts with the target cell that are named synapses through which impulses are transferred by special chemical substances — neurotransmitters.
The **gap junctions** are special channels that pass through the membranes of the two neighboring cells and connect their cytoplasms. Gap junctions enable the adjacent cells to exchange substances including electrolytes, and therefore to pass the excitation from cell to cell as it occurs in cardiac or smooth muscle.

The schematic representation of all types of intercellular communication described above is given in fig. 2.1.

![Diagram of various types of intercellular communication](image)

*Fig. 2.1. The types of intercellular communication*

Chemical signaling is a universal way of cells functioning regulation. Electric signaling by synapses developed later in evolution. This way of communication is the fastest. It also involves molecular receptors interaction with chemical substances as does the chemical signaling, but it results in changing the membrane electric potentials which can be transmitted rapidly.

**Classification of molecular receptors**

I. Plasma membrane receptors
   1. 1 transmembrane segment (1-TMS) receptors
   2. 7 transmembrane segments (7-TMS) receptors
   3. Ligand-gated ion channels

II. Intracellular receptors
   1. Nuclear receptors
   2. Cytosolic receptors
The signal molecule able to bind to receptors is named a ligand. Molecular receptors recognize and bind with specific ligands; this binding results in signal transmission. Ligands include hormones, neurotransmitters and local chemical factors (growth factors, cytokines etc.). Ligands can be divided by their hydro- or lipophilic nature. This division is important because only lipophilic ligands can enter the cell through the plasma membrane, so they can bind to intracellular receptors directly. Hydrophilic ligands are lipid-insoluble and cannot cross plasma membrane. So they exert their action on the cell through binding to the cell external receptors. Thus there is a general rule for various ligands: lipophilic substances (steroid and thyroid hormones) bind to intracellular receptors; hydrophilic proteins and peptides (as the most of hormones are) bind to the cell plasma membrane receptors (fig. 2.2). As hydrophilic ligands cannot enter the cell they may need second messengers to transfer their influence to the cell interior.

![Diagram](image)

**Fig. 2.2.** Binding of ligands to their receptors depending on the type of ligand

**THE BASIC WAYS OF SIGNAL TRANSMISSION INVOLVING MEMBRANE RECEPTORS**

1. 1-TMS receptors are integral membrane proteins that possess an extracellular domain for recognizing and binding with ligands and an intracellular domain having catalytic activity (mostly tyrosine kinase). Binding with ligand results in activation of the intracellular protein portion (tyrosine kinase), which starts to perform phosphorylation of various protein substrates. The adding of phosphate groups to proteins leads to the change of their properties: activation of enzymes, structural changes and so on.

- **Kinases** accomplish phosphorylation of proteins by **phosphate** group transfer from ATP to a substrate protein molecule. Phosphorylation by **kinases** and dephosphorylation by **phosphatases** are the main ways of cell proteins functioning regulation.

  *Examples* of 1-TMS receptors ligands:
  - insulin and various growth factors.

2. 7-TMS receptors are integral membrane proteins too. They have an extracellular domain for ligand binding, as well. Inside the membrane their chains pass the membrane through 7 times (7 transmembrane segments). They are coupled with special **G-proteins** (GTP-binding proteins) at the inner side of the cell membrane. G-proteins consist of 3 subunits: α, β, and γ. When the ligand binds to the extracellular part of the receptor, the G-protein becomes activated: its subunit α binds GTP and gets detached from the other subunits. Then it binds to the effector
enzyme located at the inner side of the cell membrane and activates it (fig. 2.4). Effector enzymes form second messengers — intracellular mediators of the ligand (hormone) action. Thus the hydrophilic hormone itself is the first messenger; it cannot enter the cell and therefore it requires the second messenger formation inside the cell. The function of second messengers is the activation of intracellular protein kinases phosphorylating various substrate proteins. Thus in case of the stimulation of 7-TMS receptors (7-TMS R) protein phosphorylation also takes place as in case of 1-TMS R. But in this case one ligand molecule initiates activation of many kinase molecules.

Compare the structure of 1-TMS and 7-TMS receptors in fig. 2.3. The mechanism of functioning of 7-TMS receptors is shown in fig. 2.4.

**Fig. 2.3.** The structure of 1-TMS and 7-TMS receptors

**Fig. 2.4.** The general mechanism of the functioning of 7-TMS receptors
(Modified from Guyton & Hall, 11 ed., 2006)

The most important effector enzymes are adenylate cyclase (AC), guanylate cyclase (GC), and phospholipase C (PhL C). AC forms the second messenger cyclic adenosine monophosphate (cAMP) from ATP, GC — cyclic guanosine monophosphate (cGMP) from GTP, and PhL C forms two second messengers at
Once, inositol triphosphate (IP$_3$) and diacylglycerol (DAG), from the membrane phospholipid, phosphatidyl inositol. The fifth second messenger is Ca$^{2+}$ ions.

Second messengers activate special protein kinases (table 2.1): cAMP activates protein kinase A (PK A), cGMP activates protein kinase G (PK G), and DAG activates protein kinase C (PK C). As to inositol triphosphate, it serves as a ligand for the ligand-gated Ca channels in the membrane of endoplasmic reticulum (EPR) in smooth muscle cells. When IP$_3$ opens these Ca$^{2+}$ ion channels it results in Ca$^{2+}$ ions release to the cytoplasm. The increase of intracellular concentration of Ca$^{2+}$ ions leads to their binding with intracellular protein calmodulin. The resulting Ca$^{2+}$-calmodulin complex activates a special kinase necessary for initiating the contraction of smooth muscles. Thus, IP$_3$ is involved into the smooth muscle contraction.

So, there are 5 classical second messengers:
1. cAMP (cyclic adenosine monophosphate).
2. cGMP (cyclic guanosine monophosphate).
3. IP$_3$ (inositol triphosphate).
4. DAG (diacylglycerol).
5. Ca$^{2+}$ ions.

Second messengers, their formation and functions

<table>
<thead>
<tr>
<th>G-protein activated effector enzyme</th>
<th>Substrate for second messenger formation</th>
<th>Second messenger</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylate cyclase</td>
<td>ATP</td>
<td>cAMP</td>
<td>Protein kinase A activation</td>
</tr>
<tr>
<td>Guanilate cyclase</td>
<td>GTP</td>
<td>cGMP</td>
<td>Protein kinase G activation; channels opening as ligand</td>
</tr>
<tr>
<td>Phospholipase C</td>
<td>Phosphatidyl inositol diphosphate</td>
<td>Inositol triphosphate</td>
<td>Ca$^{2+}$ ions release from EPR to the cell cytoplasm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diacylglycerol</td>
<td>Protein kinase C activation</td>
</tr>
</tbody>
</table>

The basic sequences of signal transmission involving 7-TMS receptors:
- Adenylate Cyclase — cAMP pathway (fig. 2.5)
  Ligand + 7-TMS R $\rightarrow$ G$_s$-protein activation $\rightarrow$ Activation of adenylate cyclase $\rightarrow$ cAMP formation $\rightarrow$ Activation of protein kinase A $\rightarrow$ Certain proteins phosphorylation (enzymes, channel structural proteins etc.) $\rightarrow$ Change of cell functioning.
- Phospholipase C — Inositol Triphosphate pathway (fig. 2.6)
  Ligand + 7-TMS R $\rightarrow$ G$_q$-protein activation $\rightarrow$ Activation of phospholipase C $\rightarrow$ inositol triphosphate (IP$_3$) & diacylglycerol (DAG) formation
  $\downarrow$ Ca$^{2+}$ ions release from EPR $\downarrow$
  $\downarrow$ Ca$^{2+}$-calmodulin complex formation $\downarrow$
  Activation of protein kinase C $\downarrow$
  Certain proteins phosphorylation $\downarrow$
  Change of the cell functioning
Fig. 2.5. Adenylate cyclase — cAMP pathway (PP<sub>i</sub> — inorganic phosphate groups)
(modified from S. I. Fox, 12 ed., 2011)

Examples of 7-TMS receptors using the adenylate cyclase pathway:
β-adrenergic receptors for adrenaline (epinephrine) and noradrenaline (norepinephrine);
glucagon receptors, TSH (thyroid-stimulating hormone),
ACTH (adrenocorticotropic hormone) receptors, ADH (antidiuretic hormone) receptors, type V<sub>2</sub>.

Fig. 2.6. Phospholipase C — IP<sub>3</sub> pathway (PIP<sub>2</sub> — phosphatidyl inositol diphosphate)
(modified from S. I. Fox, 12 ed., 2011)

Examples of 7-TMS receptors using the phospholipase C pathway:
α-adrenergic receptors for norepinephrine; angiotensin II receptors,
oxytocin receptors, ADH (antidiuretic hormone) receptors, type V<sub>1</sub>.
3. **Ligand-gated ion channels** are another kind of plasma membrane receptors. Ligand binding to the extracellular part of channel forming proteins changes protein molecules conformation and it results in channel opening allowing ions influx or efflux according to their concentration gradients and charges. Thus, channels opening results in the change of intracellular ion concentrations as well as of the membrane potential, i.e. the change of cell functioning. And this change occurs rapidly in comparison with slower processes of 1-TMS and 7-TMS receptors activation.

*Examples* of **ligand-gated ion channel** receptors and their ligands: *nicotinic cholinergic receptors* (n-AChR) for *acetylcholine* (ACh); *glutamate* receptors etc.

**Signal transmission involving intracellular receptors**

Intracellular receptors have a ligand-binding domain and a DNA-binding domain. Lipophilic (lipid-soluble) hormones *diffuse* across the cell membrane and bind to either a *cytosolic* or *nuclear* intracellular receptor. Binding to the receptor causes a conformational change in it, which exposes the DNA-binding domain. Then this domain interacts with the nuclear DNA and initiates transcription resulting in production of a *mRNA*. The mRNA is *translated* in the cytoplasm, and *specific proteins* are produced. These proteins can be enzymes, structural proteins and other molecules changing cell functioning. Thus, intracellular receptors activation influences the cell by *new proteins synthesis* (Fig. 2.7). It requires time and therefore the action of this type of receptors is slow enough. It may take hours and even days to affect body functions by intracellular receptors activation.

*Fig. 2.7. The mechanism of signal transmission involving intracellular receptors*  
(from Guyton & Hall, 12 ed., 2011)

*Examples* of intracellular receptors and their ligands: *steroid* and *thyroid* hormones receptors; receptors of *vitamin D. Steroid hormones* include
the following groups of hormones: glucocorticoids (cortisol), mineralocorticoids (aldosterone), sex hormones (androgens — testosterone, and estrogens — estradiol), and progesterone. Thyroid hormones include triiodothyronine (T<sub>3</sub>) and thyroxine (tetraiodothyronine, T<sub>4</sub>). All these hormones as well as vitamin D are lipid-soluble, so they diffuse across the cell membrane and bind to intracellular receptors.

**EXAMPLES OF THE FINAL EFFECTS OF LIGAND-RECEPTOR INTERACTION**

1-TMS R

**Insulin** binds to its receptors and activates the intracellular part of the receptor protein having tyrosine kinase activity. The activated tyrosine kinase phosphorylates a number of protein enzymes, and it results in the increased uptake of glucose to the target cells by directing the insertion of glucose transporters into cell membranes. Due to the activation of certain enzymes insulin promotes glycogen formation from glucose in the liver and muscle cells. Thus due to the action of insulin tissues can use glucose and the blood glucose level is decreased.

7-TMS R

**Epinephrine** (adrenaline) binding to β-adrenergic receptors in heart muscle cells activates G<sub>s</sub>-protein, which, in turn, activates adenylate cyclase. The adenylate cyclase increases cAMP concentration in the cell. Each 4 molecules of cAMP activate one protein kinase A molecule; so many PK A molecules start to phosphorylate proteins. Some of these substrate proteins are structural proteins of the cell membrane which form Ca<sup>2+</sup> channels; their phosphorylation changes protein conformation and opens the channels through which Ca<sup>2+</sup> ions enter the cell. **Increase** of intracellular Ca<sup>2+</sup> concentration results in the increase of heart muscle contraction force. That is why epinephrine makes heart to beat strongly.

**Ligand-gated ion channels**

**Acetylcholine** binding to nicotinic receptors of neuromuscular junction (synapse) results in Na<sup>+</sup>-K<sup>+</sup> channel opening. It causes the depolarization of the postsynaptic membrane and the generation of action potential at the adjacent parts of the muscle cell membrane. Action potential propagation along the membrane of the muscle cell causes Ca<sup>2+</sup> ions release from the sarcoplasmic reticulum to the cell cytoplasm and finally results in the contraction of the skeletal muscle.

**Intracellular receptors**

The active form of vitamin D, 1,25-dihydroxycholecalciferol, induces a DNA transcription and synthesis of Ca<sup>2+</sup>-binding protein. So vitamin D is necessary for Ca<sup>2+</sup> ions absorption from the gastrointestinal tract.

**Aldosterone** induces a DNA transcription and synthesis of Na<sup>+</sup>-K<sup>+</sup>-ATPase (pump) in the cells of kidney tubules, thus increasing Na<sup>+</sup> reabsorption to blood and K<sup>+</sup> secretion to the tubular lumen; it results in the increased Na retention in the body and K ions excretion. Thus aldosterone causes the increase of Na<sup>+</sup> concentration and the decrease of K<sup>+</sup> concentration in blood.
Theme 3
FUNCTIONS OF BLOOD.
BLOOD COMPOSITION. RED BLOOD CELLS

BLOOD primarily is a medium for transporting various substances in the organism. Thus, the main blood function is a transportation function, and accomplishing this basic function blood carries out many other interconnected functions:

- transport of the gases (O\(_2\) and CO\(_2\)) in a chemically bound state mainly: O\(_2\) is transported from the lungs to tissues, and CO\(_2\) — in the opposite direction;
- transport of nutrients from their absorption or storage sites to the cells where they are utilized;
- transport of the exchange products from cells to the excretory organs;
- transport of hormones, vitamins and enzymes from the sites of their formation to target cells;
- homeostasis maintenance (pH, osmotic pressure, ion concentrations etc.);
- heat distribution (heat transfer) in the organism: blood takes heat from the organs having higher temperature and passes it on to the organs that have lower temperature;
- defensive function of blood consists in the specific and non-specific mechanisms of body resistance to infection, due to the ability of blood cells to destroy invading agents by the process of phagocytosis or by forming antibodies;
- blood clots formation to prevent bleeding in case of vessel damage.

A total blood amount in the organism is 6–8 % of the body mass (thus in an average person having a body mass of about 60–70 kg the volume of circulating blood is about 4–5 liters).

BLOOD COMPOSITION

Blood is a fluid, but it is not a classical fluid as it consists of particles — blood cells, and a fluid part — plasma. That is why blood properties may be variable. Blood viscosity may vary from 4.5 to 5 units (versus H\(_2\)O viscosity, taken for 1). Plasma viscosity is about 2 (1.8–2.2). Thus, blood viscosity depends on the cells number and primarily on the red blood cells content as they are the most numerous blood cells.

The blood cells share in a total blood volume is called hematocrit (or packed cell volume, PCV). When the total blood volume is taken for 100 %, hematocrit amounts in men 40–49 %, and in women 36–42 %. The rest is the plasma volume. So, the cells constitute slightly less than half of blood volume. Mainly
PCV depends on the red blood cells count. The hematocrit is evaluated by blood centrifugation. Gravity makes blood cells settle to the bottom, so blood is separated into the cells and plasma, and the share of cells can be measured (fig. 3.1).

**Blood plasma** contains the following **main components** (dissolved in water):
- **Ions**: Na⁺, Cl⁻, HCO₃⁻ and others.
- **Proteins**.
- **Nutrients, vitamins** and **trace elements** (Fe, Cu, Co, J and others).
- **Hormones** and **enzymes**.
- **Intermediate exchange product** (lactic acid, pyruvic acid).
- **End exchange products** to be eliminated, including CO₂ (excreted by the lungs), urea, uric acid, creatinine, ammonia (excreted by kidneys), bilirubin (excreted by the liver) and others.

**Blood plasma proteins**

The total amount of proteins in blood plasma is 60–85 g/l. **Proteins** are divided into the following groups: **Albumins** (38–50 g/l) **Globulins** (20–36 g/l) **Fibrinogen** (2–4 g/l).

Blood proteins basic **functions** are:
- **transportation** of various substances in blood;
- **oncotic** (colloid osmotic) blood pressure creation;
- **buffer function** (as proteins are amphoteric);
- **nutritional**.

**Albumins** are the most numerous and the smallest among blood proteins (their molecular weight is about 69 000; for proteins it is not much), so the amount of albumins molecules is much higher than that of the other blood proteins. Therefore they make the main contribution to the creation of oncotic blood pressure which in turn affects the circulating blood volume and blood pressure. They may also transport many substances (lipids, hormones etc.) at their surface and may serve as a source of amino acids (nutritional function).

**Globulins** are much larger proteins; they are divided (by electrophoresis) into fractions of α₁, α₂, β and γ-globulins. The first three fractions mainly carry out transportation of various ions (Cu, Fe), hormones and metabolites. As for γ-globulins fraction, it is composed of antibodies (immunoglobulins) produced by lymphocytes, so γ-globulins have the **defensive function**.

Most of blood plasma proteins are produced by the liver.

The separation of blood plasma proteins into fraction can be performed by means of electrophoresis. When a plasma sample is placed into the electric field near a cathode, the negatively charged plasma proteins start to migrate away from the negative pole (cathode) and toward the positive pole (anode) at different rates which are influenced by their charge, size and shape. The smallest proteins, albumins, move the longest distance, the other proteins follow at less speed and form a whole sequence of fractions (fig. 3.2).
The other blood plasma protein, fibrinogen, takes part in the process of blood coagulation. Fibrinogen molecules are very large; they have the shape of long sticks and are able to turn into fibrin (under thrombin action), forming threads necessary for red blood clot formation.

Blood cells are red blood cells (erythrocytes), white blood cells (leucocytes) and platelets (thrombocytes).

**Blood Cells: Red Blood Cells (RBC)**

Red blood cells, or erythrocytes, got their name for their scarlet color that is due to oxygen-binding protein hemoglobin. This protein is packed into the cells to prevent its leakage from vessels to the tissues or, in particular, from glomerular filter to kidney tubules. Normal red blood cells are biconcave discs having a mean diameter approximately 7.5 μm and thickness at the thickest point of 2.5 μm and in the center of 1 μm or less (fig. 3.3). The average volume of red blood cell is about 80–100 fl, or femtoliters (×10⁻¹⁵ l), (MCV, Mean Corpuscular Volume). As erythrocytes do not contain nuclei, it enables hemoglobin to fill the whole space inside the cells.

The specific shape of red blood cells permits them to change their shape remarkably as they pass through capillaries (which have a diameter about 4–9 μm). Due to a great excess of cell membrane surface in respect to the volume, a moderate deformation does not stretch the RBC membrane, and consequently does not rupture the cell, as would occur for many other cells. Thus, red blood cells have the ability to reversible deformation.

Due to their shape red blood cells are, as well, distinguished by high osmotic resistance. Osmotic resistance is evaluated in hypotonic solutions where the osmotic pressure gradient makes water go to the cell (fig. 3.4). Due to
the excess of a membrane surface area red blood cells can withstand (to a certain degree) the transition of water into it. Normal RBC begin to rupture in NaCl solutions having concentration as low as 0.45 % on the average (this is about a half of normal isotonic solution concentration, 0.9 %; such a decrease of ion concentrations and therefore of osmotic pressure never occurs in a living organism as much smaller decrease may become lethal). As older red cells are more fragile they are destroyed first, then at lower concentrations younger cells begin to rupture. The youngest RBCs are destroyed at NaCl solution concentrations of about 0.35 % (fig. 3.5). Thus, normal osmotic resistance of RBC is characterized by the following limits:

- **minimal** — 0.46–0.48 % NaCl (at this concentration older RBC start to rupture).
- **maximal** — 0.32–0.34 % NaCl (at this concentration all RBC are ruptured).

\[ \text{Fig. 3.5. Changes of RBC in a range of NaCl solution concentrations. The limits of RBC normal osmotic resistance} \]

Respectively, at concentrations higher than the minimal limit (but lower than that of isotonic solution) normal RBC do not rupture; cells just become swollen. At concentrations between the minimal and maximal limits partial hemolysis occurs; it means that a part of cells is ruptured, and a part is just swollen, but not destroyed. At concentrations lower than the maximal limit all RBC are destroyed. This type of RBC destruction due to the increase of the volume and rupture of cell membrane by water that enters the cell is called osmotic hemolysis.

The test of RBC osmotic resistance is performed in the test tubes with hypotonic NaCl solutions having concentrations in the range from 0.6 % to 0.25 %. Blood drops are added to all test tubes and the results are evaluated after 1 hour when red cells which haven’t been ruptured form the sediment at the bottom of test tubes. If a solution above the sediment remains colorless, there is no hemolysis. In case of a partial hemolysis a pink color appears in the solution above the sediment (fig. 3.5). The presence of a pink color is due to hemoglobin released from ruptured red cells. Complete hemolysis does not leave sediment of red cells, and all hemoglobin is released into the solution which becomes pink-red in color and transparent due to the absence of particles as large as cells.
The evaluation of RBC osmotic resistance is helpful in diagnosis of anemia types, as different kinds of pathological RBC may have various deviations of their osmotic resistance. Too large or spherical RBC have much lower osmotic resistance, therefore the limits of resistance (NaCl solution concentrations) become higher than normal. Some types of pathological RBC may have higher than normal osmotic resistance.

RBC hemolysis may also be mechanical, chemical, thermal or biological. The latter type of hemolysis, the biological one, occurs under the action of biological substances, such as enzymes which destroy membranes of red blood cells or antibodies which bind to the receptors of red cells and bind the cells together; the binding of red blood cells finally results in their destruction.

The normal number of RBC differs in men and women. Due to androgens the organism of men has higher oxygen requirements, and androgens stimulate higher RBC production in men as compared to women. Norms for RBC number per one liter of blood are:

- (3.9–5.1) × 10^12/1 — in men
- (3.7–4.9) × 10^12/1 — in women

If the number of RBC (erythrocytes) is lower than normal, it is called *erythrocytopenia*. Erythrocytopenia is a general sign of various anemias. If the number is higher than normal, it is called *erythrocytosis*.

RBC counting is not sufficient for the evaluation of the blood oxygen-carrying function. It is important to assess the blood hemoglobin content. Hemoglobin content norms are the following (in grams per liter of blood):
- 130–170 g/l — in men
- 120–150 g/l — in women

Hemoglobin of an adult person (HbA) consists of iron-containing heme groups and the protein part, globin, having 4 polypeptide chains: 2 α chains and 2 β chains (fig. 3.6, 3.7). All types of hemoglobin, including fetal hemoglobin, HbF, and adult hemoglobin, HbA, differ from each other by their polypeptide chains, which determine the binding affinity of Hb for O₂. Heme groups are similar in all the types of hemoglobin. Each hemoglobin molecule has 4 heme groups and therefore can bind 4 molecules of O₂. Oxygen binding that occurs in the lungs is easily reversible and permits oxygen release in tissues. Oxygen binding by hemoglobin is referred to as hemoglobin oxygenation:

\[
\text{Hb} + \text{O}_2 \rightleftharpoons \text{HbO}_2
\]

Hb oxygenation does not change the state of heme iron atom (ferrous iron, Fe²⁺) to the ferric state (Fe³⁺), as it happens under oxidation. In the presence of strong oxidizing agents Hb is oxidized to the methemoglobin (or ferrihemoglobin) which cannot transport oxygen. This is a dangerous state of poisoning resulting in hypoxia. Another type of Hb pathological state is carboxyhemoglobin formation in case of carbon monoxide (CO) poisoning. The affinity of Hb for CO is much higher than for O₂, so the presence of a minute CO quantity in the air is enough to produce dangerous poisoning resulting in hypoxia that could be lethal.
Normal states of hemoglobin include oxygenated form (oxyhemoglobin — Hb reversibly bound with O₂), deoxygenated (reduced) Hb (after O₂ release in tissue capillaries), and carbohemoglobin (bound with CO₂, which is connected to the globin NH₂ groups).

Along with O₂ transportation hemoglobin constitutes one of the major buffer systems of the organism. Hb is able to bind H⁺ ions and therefore it prevents marked pH shift to the acid state in the venous blood after adding CO₂ from the tissues.

As hemoglobin is packed in RBC, it is clear that there should be the relationship between the number of cells per liter of blood and the amount of hemoglobin per liter of blood. Actually, there are few indices for the evaluation of average hemoglobin content in one red blood cell (table 3.1):

<table>
<thead>
<tr>
<th>Index</th>
<th>Normal values</th>
<th>Index description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCH (Mean Corpuscular Hemoglobin)</td>
<td>25.4–34.6 pg (g × 10⁻¹²)</td>
<td>Absolute hemoglobin content in one RBC. It is calculated by dividing Hb content per 1 liter of blood by the total RBC number per 1 liter of blood.</td>
</tr>
<tr>
<td>Color index (CI)</td>
<td>0.8 – 1.05</td>
<td>Relative hemoglobin content in one RBC. It is calculated according to the formula: ( CI = \frac{3 \times Hb (g/l)}{Red\ Blood\ Cells \times 10^{-10}} ) or simply ( 3 \times \frac{Hb}{1st\ digits\ of\ RBC} )</td>
</tr>
</tbody>
</table>

Actually both indices are the same, but the second one is additionally reduced to the simple value close to 1.0 (which is considered optimal) by multiplication by \( 3 \times 10^{10} \).

If MCH index is lower than 25.4 pg (and Color Index is lower than 0.8) RBC are considered hypochromic (which means less colored — because of low hemoglobin content). If MCH index is higher than 34.6 pg (and CI is higher than 1.05) RBC are considered hyperchromic (having high hemoglobin content).

As the concentration of hemoglobin in the cell (Mean Corpuscular Hemoglobin Concentration, MCHC) is usually constant (about 32–36 %), hypochromic RBC as a rule are smaller than normal in size (microcytosis), and the hyperchromic RBC are larger in size (macrocytosis).
RED BLOOD CELLS FORMATION — ERYTHROPOIESIS

In the early few weeks of embryonic life primitive red blood cells are produced in the yolk sac. During the middle trimester of gestation RBC are produced mainly in the liver (as well as in the spleen and lymph nodes). Then, during the last part of gestation and after birth RBC are produced only by the bone marrow.

Until a person is 5 years old RBC are produced by bone marrow of practically all bones. Then bones become less productive, and after 20 years of age blood cells are produced only in the vertebrae, the sternum, the ribs and the ilia.

Red blood cells (and all blood cells) are derived from the bone marrow pluripotential hemopoietic stem cells. Their growth and step-by-step differentiation results in the formation of all types of blood cells. These processes are controlled by special growth and differentiation inducers produced by the surrounding cells in the bone marrow, or the microenvironment of stem cells. Hemopoiesis-inducing microenvironment is constituted by such cells as macrophages, T lymphocytes, fibroblasts, endothelial cells and other cells present in the bone marrow. The main growth inducers are interleukins. The main differentiation inducing factor for RBC production is erythropoietin. This substance is formed mainly in the kidneys under the decrease of tissue oxygenation. If hypoxia of any origin develops (including blood loss, anemia, cardiac failure or pulmonary diseases, as well as moving to the high altitudes where the atmospheric pressure is low) it causes increased erythropoietin formation and stem cells differentiation resulting in increased RBC production. Thus, the main parameter that is regulated is not the RBC amount in blood but the result of RBC functioning, the level of oxygen supply to the tissues. So, the main factor stimulating erythropoiesis is hypoxia which increases erythropoietin production.

During the successive steps of red blood cells formation in a bone marrow their immature precursors, erythroblasts, lose nucleus and other organelles, and become fully filled with hemoglobin. The last precursors of mature RBC are called reticulocytes due to the presence of reticular remnants of organelles. Normally reticulocytes make up about 0.5–1.2% of RBC in the blood. The increase of this index is a sign of increased erythropoiesis. For example, the increase of reticulocytes could be found after blood loss.

A number of substances is necessary for the normal process of erythropoiesis. These substances are vitamins B₁₂ (cyanocobalamin), B₂ (riboflavin), B₆ (pyridoxine), C (ascorbic acid), folic acid, pantothenic acid; and trace elements: iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), and selenium (Se). Vitamins B₁₂ and folic acid are particularly important for the final maturation of RBC. Both of them are essential for DNA synthesis. Therefore, the lack of either vit B₁₂ or folic acid causes failure of nuclear division and maturation resulting in the formation of large but immature red cells — megaloblasts. Due to their odd shape they are fragile and have a short life span; as a result megaloblastic anemia develops. The common cause of such anemia is poor vitamin B₁₂ absorption which develops in persons with gastric mucosa atrophy. Normal gastric mucosa produces special glycoprotein — an intrinsic factor necessary for vit B₁₂ absorption. This factor binds the vitamin in the stomach thus
preventing from vitamin destruction, and then the complex of vitamin (the extrinsic factor) and the intrinsic factor moves to the intestine and finally is absorbed in the ileum. The lack of this factor causes failure of vit B₁₂ absorption and results in anemia development. The daily requirement of this vitamin is about 2–5 micrograms.

**DESTRUCTION OF RED BLOOD CELLS**

The average life span of normal RBC is about 100-120 days. During their life RBC use glycolysis reactions to receive energy by metabolizing glucose and forming small amounts of ATP used for membrane elasticity maintaining and for preventing oxidation of Hb and of other proteins in the red cells. However these metabolic systems of RBC become progressively less active. RBC membranes lose their elasticity, and the cells become more and more fragile. The old fragile red cells rupture during passage through capillaries. Many of the RBC are fragmented in the spleen, where they have to squeeze through narrow capillaries about 3 μm wide. Therefore the spleen is called “the red blood cells cemetery”. Not only the old RBC are destroyed but even the newly formed cells having some deviations that make them fragile are discarded in the same way.

The process of the destroyed old or abnormal red blood cells utilization is shown in fig. 3.8. The hemoglobin released from the cells is immediately phagocytized by macrophages located around the vessels, particularly in the spleen, liver, and bone marrow. Protein parts of Hb molecules (globin) as well as RBC membrane proteins are split into amino acids and re-used. Iron is extracted from heme and then released from macrophages back to the blood where it is transported by plasma globulin transferrin to the bone marrow for new RBC production or to the liver for storage in the form of ferritin. The main source of iron for new RBC formation is re-used iron derived from the aged RBC destruction. Due to this the daily requirement in iron received with food is only about 10–15 mg.

The rest part of heme (porphyrin) is converted by macrophages, through a series of stages, into the bile pigment bilirubin. Then it is released into the blood, taken in by the liver cells and secreted into the bile. With the bile bilirubin is released into the intestine and thereby is excreted. Bilirubin gives the bile its yellow color; in case its concentration in the blood is increased, it can give yellowish color to the whites of the eye and to the skin. As bilirubin is formed due to RBC destruction, one of the reasons for the blood bilirubin increase is increased hemolysis. The increased level of blood bilirubin has a toxic effect.

**ERYTHROCYTES SEDIMENTATION RATE (ESR)**

The Erythrocytes Sedimentation Rate (ESR) is an important clinical index which in the first place allows evaluation of the inflammatory process intensity or of infectious disease severity. The normal values or ESR differ for men and women:

1–10 mm/h — in men
2–15 mm/h — in women

As red blood cells are heavier than blood plasma they slowly settle to the bottom of a thin test tube (capillary). To determine the value of ESR blood is mixed with a 5% solution of sodium citrate to prevent blood clotting, than
the resulting mix is taken to the capillary having graduations in millimeters. The capillary is set up vertically for 1 hour. After 1 hour when the result is evaluated, the red portion of a column of fluid in the capillary comes down for a certain number of millimeters. In the fig. 3.9, e. g., the value of ESR is 7 mm/hour.

![Fig. 3.8. Recycling of iron from hemoglobin](from G. Pocock, C. D. Richards, 3 ed., 2006)

![Fig. 3.9. Determination of ESR](from G. Pocock, C. D. Richards, 3 ed., 2006)

The ESR value mostly depends on the properties of blood plasma rather than on the properties of red blood cells. The main factor which causes changes of ESR value is the content of globulins and fibrinogen in blood plasma. The red blood cells membranes are negatively charged. Therefore normally they are repelled from each other, and their sedimentation is slow. But large protein molecules decrease their negative charge thus decreasing RBC mutual repulsion. Due to this RBC tend to form the so-called “coin columns” – stacks of red cells, one above another. The stacks of red cells settle down faster than single red cells, and the ESR increases.

In case of inflammation or infectious disease the immune response of the body includes antibodies formation. Antibodies belong to the fraction of globulins; so when the blood globulin content increases the level of ESR becomes higher.

Another factor which influences the ESR level is the amount of RBC. The less RBC number is, the higher ESR becomes, as it decreases mutual repulsion of red cells and their settling down becomes faster. Thus erythrocytopenia results in the increase of ESR. Also the lower number of RBC in women explains as well their higher normal values of ESR.

The thickening of blood in case of dehydration, increase of albumins content, and erythrocytosis may result in the decrease of ESR.
Theme 4
WHITE BLOOD CELLS. PLATELETS.
BASES OF HEMOSTASIS

The total number of white blood cells (WBC), or leucocytes, is $4–9 \times 10^9/l$. Their number is rather constant but they are not the same cells every time as they perform their functions not only in the blood (like red blood cells) but in almost all the tissues of the body. While moving in the blood stream they are ready to leave the blood vessel if their function is necessary at various points of tissues. White blood cells are the mobile units of the body’s protective system. Leucocytes circulate in blood for a rather short time and then enter the tissues where they may live sometimes for a long time; sometimes they may die in a few hours combating the invading agents. Their main function is protection of the body from infectious and toxic agents by their direct destruction (phagocytosis) or by forming antibodies which subsequently help to destroy them.

Leucocytes are divided into 2 different groups, granular (granulocytes) and agranular (agranulocytes) leucocytes (fig. 4.1).

<table>
<thead>
<tr>
<th>Granulocytes</th>
<th>Agranulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basophils</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Monocytes</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4.1. Types of white blood cells: schematic and histological view (from C. Junqueira, 12 ed., 2010)

Granular white cells received their name due to the presence of granules in their cytoplasm. There are 3 types of granular cells which are differentiated by staining with different agents. Cells with granules colored in blue by basic stains are called basophils. Basophils constitute only 0–1 % of all blood leucocytes. Cells with granules colored in pink-red by acidic stain eosin are called eosinophils (1–5 %). Cells with granules that have little affinity for either stain are neutrophils. Neutrophils in turn are divided into few types which differ from each other by the degree of their maturity. Most neutrophils are mature cells, segmentonuclear neutrophils. They constitute the majority of all leucocytes as well (46–68 %). But there is a certain amount of immature neutrophils in the circulating blood (fig. 4.2). Most immature cells, myelocytes, should not be present in the circulating blood (0 %). Their name implies that these cells are located in bone marrow (myelos).
The next stage cells are *metamyelocytes*, or *young* (juvenile) neutrophils, which normally constitute no more than 1% of all WBC (0–1%). The last precursors of mature neutrophils are *band neutrophils*, having *rod-like* nuclei. These cells make about 1–5% of all WBC; soon they turn into mature segmentonuclear neutrophils. The nucleus of mature neutrophil has a *segmented* appearance; another name of the cell is a *polymorphonuclear* neutrophil.

![Diagram](image)

*Fig. 4.2. Types of neutrophils according to their maturity*

Agranulocytes include monocytes and lymphocytes. **Monocytes** make up 2–9% of WBC. **Lymphocytes** are the second most numerous group of WBC after neutrophils, as lymphocytes constitute 18–40% of all WBC. These cells are divided into 2 groups — **T lymphocytes** and **B lymphocytes**. They cannot be differentiated by their appearance but have different functions, **T lymphocytes** are responsible for *cell-mediated immunity*, and **B lymphocytes** are responsible for the formation of *antibodies* that provide *humoral immunity*.

The percentage of all leucocytes types is referred to as **leucocyte formula** (table 4.1).

**Table 4.1**

<table>
<thead>
<tr>
<th>Granulocytes</th>
<th>Agranulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basophils</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Monocytes</td>
</tr>
<tr>
<td>Myelocytes</td>
<td></td>
</tr>
<tr>
<td>Young neutrophils</td>
<td></td>
</tr>
<tr>
<td>Band neutrophils</td>
<td></td>
</tr>
<tr>
<td>Segmentonuclear neutrophil</td>
<td></td>
</tr>
<tr>
<td>0–1%</td>
<td>18–40%</td>
</tr>
<tr>
<td>1–5%</td>
<td>2–9%</td>
</tr>
<tr>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>0–1%</td>
<td></td>
</tr>
<tr>
<td>1–5%</td>
<td></td>
</tr>
<tr>
<td>46–68%</td>
<td></td>
</tr>
</tbody>
</table>

Almost all leucocytes are formed in the **bone marrow** as well as the other blood cells, and only *lymphocytes* are formed in the lymph nodes, spleen and thymus as well.

**Properties and functions of WBC.**

**Phagocytosis: neutrophils & monocytes**

One of the most important leucocytes functions that provide the defense of the organism from infectious and other invading agents is **phagocytosis**. The function of immediate migration to the focus of inflammation or injury and subsequent phagocytosis is performed by mature **segmentonuclear neutrophils**. **Neutrophils** possess **receptors** to various antigens, bacterial endotoxins, cytokines and other factors secreted in the focus of infection or tissue injury. It makes them **sensitive** to the increased
concentration of these factors. In case these substances bind to the cells’ receptors, neutrophils stop their movement in the blood stream, attach themselves to the vessel wall and begin to squeeze through the pores between endothelial cells in order to leave the vessel. This process of extravasation (movement out of the vessel) is referred to as **diapedesis**. Having got outside the vessel neutrophils start to move to the focus of injury, in the direction of the highest concentration of toxins and cytokines. This process of active migration directed to the focus of inflammation or injury is called **chemotaxis**. The velocity of neutrophils movement may reach 40 μm/min. The most important chemoattractants for leucocytes are Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF).

Diapedesis and chemotaxis of leucocytes to the focus of injury is shown in fig. 4.3.

*Fig. 4.3. Diapedesis and chemotaxis of leucocytes (modified from Guyton & Hall, 12 ed., 2011)*

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Diapedesis and chemotaxis of leucocytes to the focus of infection is shown in fig. 4.4.

*Fig. 4.4. The stages of phagocytosis (from S. I. Fox, 12 ed., 2011)
On approaching the particle to be phagocytized, the neutrophil first attaches itself to the particle, then projects pseudopodia in all directions around the particle, so that the pseudopodia meet each other on the particle’s opposite side and fuse (fig. 4.4). The phagocytized particle turns inside the cell, in the phagosome. Then inside the neutrophils the phagosome fuses with lysosomes. Lysosomes contain a set of enzymes necessary for digesting various biological substances. Having digested bacteria or other particle the neutrophil releases remnants of undigested substances out from the cytoplasm. Neutrophils are considered microphages due to their smaller size. One neutrophil can usually phagocytize from 5 to 20 bacteria before it becomes inactivated and dies. But more and more neutrophils come to the focus of injury, and together they constitute an important part of the defensive system. In addition they are able to secrete lysosomal cation proteins and interferons, which help to destroy invading agents and take part in the other leucocytes attraction.

The life span of neutrophils is about 4–7 days. After entering the blood they circulate in the vessels on the average for 4–8 hours, and then leave the vessels to combat the invading bacteria and other particles.

The other phagocytes are monocytes. Blood monocytes have a very small ability to fight infectious agents. They usually circulate in the blood about 10–20 hours, and then leave for the tissues. However, once they enter the tissues, they begin to grow up, sometimes increasing their size as much as 5-fold, up to 80 μm (= 0.08 mm). The process of growth and maturation takes about 8 hours. During this period monocytes develop inside a great number of lysosomes and peroxisomes. While lysosomes contain enzymes for biological substances degradation, peroxisomes contain powerful oxidizing agents such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl ion (–OH). These substances are highly bactericidal. All these properties along with their large size make tissue monocytes very powerful phagocytes, or macrophages. Indeed, tissue macrophages are capable of phagocytizing as many as 100 bacteria. Macrophages can phagocytize particles much larger than bacteria. After the completion of phagocytosis they may survive and keep on functioning for many months. Their life span in the tissues is months or years.

Another function of macrophages is to perform the presentation of antigens to other immune cells (fig. 4.5). Having digested bacteria the macrophage passes bacterial antigenic substances (mostly some components of the bacterial wall, proteins or large polysaccharides) to the immune cells — T or B lymphocytes — and
activates these cells. This transfer occurs through a direct cell-to-cell contact when
the antigen located on the macrophage membrane surface binds to the receptors of
a lymphocyte so that the macrophage “presents” the antigen to the lymphocyte.

While some macrophages are mobile cells, a large portion of macrophages
becomes attached to the tissues and settle there. In case of invasion these settled tissue
macrophages are the first to begin phagocytosis. But their main task is to secrete interleukin-1 and other cytokines attracting neutrophils to the focus of invasion.

Some tissues or organs where the possibility of invasion is higher are extremely rich in macrophages. For example, skin and subcutaneous tissues have a large amount of tissue macrophages called histiocytes. In case of inflammation these cells can divide in situ (at the place) and form more macrophages. One of the routes by which bacteria invade the body is through the gastrointestinal tract, and large numbers of bacteria constantly pass into the portal blood. However, before entering the general circulation, this blood must pass through the liver. The liver sinuses are lined with powerful tissue macrophages called Kupffer cells, which are able to destroy bacteria immediately. Another route for invasion is the respiratory system, and a large number of alveolar macrophages provide defense there. Also there are many macrophages in the lymph nodes, the spleen and bone marrow.

**BASOPHILS**

Basophils of the circulating blood form large mast cells in the tissues. Their life span is from months to years. The granules of basophils and mast cells contain a number of vasoactive substances — histamine, heparin, bradykinin, serotonin, slowly reacting substance of anaphylaxis (SRSA) and others. To perform their normal function mast cells secrete these substances in small quantities which is sufficient for regulating the local permeability of capillaries and maintaining the fluidity of blood by preventing its coagulation (the latter by heparin mainly). Mast cells and basophils play an important role in allergic reactions because antibodies of the type that causes allergic reactions, immunoglobulins of IgE type, possess the property to become attached to mast cells and basophils. A single mast cell or basophil can bind as many as half a million molecules of IgE antibodies. Then, when the specific antigen reacts with the antibody, it causes the degranulation of mast cells — release of large quantities of histamine and other substances into the interstitial fluid (fig. 4.6).

Histamine together with the other substances produce the dilation of local blood vessels, attraction of eosinophils and neutrophils, increased permeability of capillaries and transition of fluid from capillaries to the tissues with local edema formation. Therefore, most of the symptoms of allergy develop due to the action of histamine and other substances released from mast cells. Various types of allergic diseases including asthma, urticaria, hay fever and anaphylaxis are based on mast cells abnormal reactions.

**EOSINOPHILS**

Eosinophils are weak phagocytes. But they have a specific ability to detoxify some toxins of protein origin, so they are very important in defense against
parasites and their products. They can attach to the parasite and release substances which are able to kill it. Another important function is performed by eosinophils in case of allergic reactions. But in contrast to basophils which are the main participants of tissue damage in allergy, eosinophils help to eliminate the damage caused by allergic reaction. Eosinophils are attracted by basophils chemoattractants to the tissues where allergic reactions have occurred, and alleviate the tissue damage due to their ability to destroy antigen-antibody complexes and to produce enzyme histaminase which causes degradation of histamine released from mast cells. Eosinophils life span is 10–12 days.

![Fig. 4.6. The role of mast cell in allergy: mast cells degranulation and histamine release in response to allergen (from S. I. Fox, 12 ed., 2011)](image)

**Lymphocytes**

Lymphocytes take a constant circuit from lymph tissue to the blood: they continually enter the circulation along with lymph drainage from the lymph nodes; then after a few hours they pass back into the tissues by diapedesis, then re-enter the lymph and so on. It enables them to perform a constant immune surveillance in the organism. Their important ability is to distinguish the own and foreign substances. Immune system cells recognize the body own antigens and do not attack them; in case of exposure to the invading agent the cellular or humoral immunity is activated.

**T-lymphocytes**

Lymphocytes of this type are differentiated in the thymus (hence they are T-lymphocytes). They are subdivided into a few groups according to their division of functions: T-helpers, T-suppressors, T-killers, T-amplifiers, T-memory cells. Their cooperation in immune reactions provides the cell-mediated immunity. The direct destruction of foreign cells is performed by T-killers, or cytotoxic T-cells. At first they attach themselves to foreign cells. Then they release specific hole-forming proteins, called perforins, which make large holes in the attacked cell.
membrane. After this the T-killer cell releases **cytotoxic substances** (through the holes) directly into their cytoplasm (fig. 4.7). The attacked cell dies and soon entirely dissolves.

**Fig. 4.7.** Direct destruction of a foreign cell by T-killers (cytotoxic T cells) (from Guyton & Hall, 12 ed., 2011)

As transplanted cells are foreign for the organism, the **transplant is rejected** by T lymphocytes if the immune cells function is not inhibited. In case of formation of **own mutant cells** T lymphocytes destroy them in the same way.

**B lymphocytes**

B lymphocytes are differentiated in bone marrow, the *bursa* analog in mammals (*B*-cells were first discovered in birds which have a special structure — *bursa* of Fabricius). They are responsible for **humoral immunity** which is the immunity mediated by **antibodies** — specific protein molecules transported by blood (*humor* or fluid). After the entry of the **foreign antigen**, the lymphoid tissue **macrophages** phagocytize it and then perform **presentation of antigen** to the adjacent B lymphocyte (as well as to T-helper). This causes **activation** of B lymphocyte and its conversion into the **plasma cell**. These cells start to divide rapidly producing more than 100 cells from the one in a day. Every plasma cell synthesizes antibodies (**γ-globulins**) against a certain antigen at extremely high rate (fig. 4.8). Antibodies are secreted into the lymph and are carried to the circulating blood.

**Fig. 4.8.** Activation of B cells, their cloning and antibodies formation (from Guyton & Hall, 12 ed., 2011)
Antibodies can inactivate the invading antigens by **agglutination** (bonding together), **precipitation**, and **lysis** (cell destruction). Together with the **complement system** (the system of about 20 blood plasma proteins taking part in immune reactions) antibodies ensure subsequent effective phagocytosis of the invading agent. Some of the activated B-cells do not turn to plasma cells but form an additional quantity of the same B-cell (B-memory). Due to these memory cells, in case of **repeated entry** of the same antigen the **secondary response** will be much more potent and rapid.

**LEUCOCYTOSIS AND LEUCOPENIA**

The increase of white blood cells number is called **leucocytosis**. It can be **physiological** (1) or **absolute (reactive or true)** leucocytosis (2). **Physiological** leucocytosis is usually a small increase of WBC number caused by food intake, strenuous exercise, or emotional stress. It is produced by WBC **redistribution** among tissues. **Absolute** leucocytosis is caused by the increase of **WBC formation** in bone marrow, which is stimulated by **interleukins** and other cytokines released by macrophages and other cells in case of foreign agents invading (fig. 4.9). The main stimulating factors for the increase of white blood cells production by red bone marrow are given in table 4.2.

**Table 4.2**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full name</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte-monocyte colony-stimulating factor</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte colony-stimulating factor</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Monocyte colony-stimulating factor</td>
</tr>
</tbody>
</table>

**Fig. 4.9.** Increased formation of granulocytes and monocytes by bone marrow under stimulation from macrophages and inflamed tissue cells (From Guyton & Hall, 12 ed., 2011)

The diagnosing of absolute leucocytosis is based on the presence of the signs of leucocytes increased formation — the increase of the **immature** cells percentage. In case of inflammation or infectious disease along with the **total WBC number increase** there is usually an increase of immature neutrophils share (percentage) — **band neutrophils** in the first place (>5 %) and sometimes **young neutrophils**, or metamyelocytes, (>1 %) as well. In severe infections there may appear **myelocytes** in the peripheral circulating blood (>0 %).
<table>
<thead>
<tr>
<th>WBC types</th>
<th>%</th>
<th>Properties and functions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Granulocytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myelocytes</td>
<td>0 %</td>
<td>Phagocytosis <em>(microphages)</em></td>
</tr>
<tr>
<td>metamyelocytes</td>
<td>0–1 %</td>
<td>Ability to the <em>diapedesis</em> and <em>active migration</em> to the focus of inflammation or injury <em>(chemotaxis)</em></td>
</tr>
<tr>
<td>(young neutrophils)</td>
<td></td>
<td>Secretion of lysosomal cation proteins and <em>interferons</em></td>
</tr>
<tr>
<td>band neutrophils</td>
<td>1–5 %</td>
<td>Presence of <em>receptors</em> to antigens, bacterial endotoxins and other factors</td>
</tr>
<tr>
<td>segmentonuclear neutrophils</td>
<td>46–68 %</td>
<td>Life span — 4–7 days (after 4–8 hours of circulation in blood)</td>
</tr>
<tr>
<td><strong>Basophils</strong></td>
<td>0–1 %</td>
<td>Contain in their granules <em>histamine</em>, heparin, bradykinin, serotonin, SRSA and others</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regulation of capillary <em>permeability</em> and fluidity of the blood <em>(preventing its coagulation by heparin release)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Releasing substances contained in their granules <em>(degranulation) in allergic reactions</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secretion of <em>chemoattractants</em> for neutrophils and eosinophils</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Life span — <em>months</em> and <em>years</em></td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td>1–5 %</td>
<td><em>Detoxification</em> and breakdown of protein toxins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Histaminase production and <em>degradation of histamine</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Destruction of <em>antigen-antibody complexes</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Antiparasitic immunity</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weak ability to phagocytosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Life span — 10–12 days</td>
</tr>
<tr>
<td><strong>Monocytes</strong></td>
<td>2–9 %</td>
<td>Phagocytosis <em>(macrophages)</em>: they are able to engulf particles much larger than bacteria, and survive after that Chemotaxis</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Formation of Interleukin-1</em> and other <em>cytokines</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Antigen presentation</em> to the lymphocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abundance of <em>lysosomes</em> and <em>peroxisomes</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Life span — <em>months</em> and <em>years</em></td>
</tr>
<tr>
<td><strong>Lymphocytes</strong></td>
<td>18–40 %</td>
<td>Life span — <em>months</em> and <em>years</em></td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>(60–80 % lymph.)</td>
<td>Differentiation in thymus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Responsible for the <em>cell-mediated immunity</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Destruction of foreign cells by direct contact</em> with them (T-killers attach to foreign cells and release perforins and cytotoxic substances to their cytoplasm)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Participation in the <em>transplant rejection</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Destruction of <em>own mutant cells</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Cytokines</em> secretion</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>(15–20 % lymph.)</td>
<td>Differentiation in <em>bone marrow</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Responsible for the <em>humoral immunity</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Antibody synthesis</em> against foreign antigens</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After the contact to antigen B-cell turns to the <em>plasma cell</em> synthesizing antibodies</td>
</tr>
<tr>
<td>Zero cells</td>
<td>(about 10 % lymph.)</td>
<td>T and B lymphocytes <em>precursors</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Destruction of the cells <em>bound</em> to antibodies, <em>cancer</em> cells and cells <em>infected with viruses</em></td>
</tr>
</tbody>
</table>
This type of changes in the leucocyte formula is called a shift to the left, as in writing we start listing cells, from the youngest to mature ones, from the left to the right, and the normal balance is predomination of mature cells; so any increase of immature young cells percentage shifts the balance to the left (table 4.4).

| Table 4.4
| Shift to the left in the leucocyte formula

<table>
<thead>
<tr>
<th>Neutrophils</th>
<th>Mature neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myelocytes</td>
<td>Young neutrophils</td>
</tr>
<tr>
<td>0 %</td>
<td>0–1 %</td>
</tr>
<tr>
<td>Band neutrophils</td>
<td>1–5 %</td>
</tr>
<tr>
<td>Segmentonuclear neutrophils</td>
<td>46–68 %</td>
</tr>
</tbody>
</table>

Increased % = Shift to the left

Thus, the presence of a shift to the left in the leucocyte formula along with the total WBC number increase (leucocytosis) testifies for the inflammatory process or infectious disease.

In case of physiological leucocytosis this shift is not present in blood analysis. Therefore the increase of WBC number without a shift to the left in leucocyte formula is not a sign of infection or inflammation.

Leucopenia is the decrease of WBC number. It may be caused by various factors inhibiting bone marrow functioning, such as X-rays, radiation, some chemicals and drugs. Leucopenia results in poor immunity.

The functions of all the types of leucocytes are presented in table 4.3.

**PLATELETS**

Platelets, or thrombocytes, are formed in bone marrow from large cells, megakaryocytes. These cells, in the bone marrow or while squeezing through the capillaries, fragment into pieces and form about 3–4 thousand platelets from each cell. So platelets do not have nuclei as well as erythrocytes. Their life span is about 10 days. The normal amount of platelets in the blood is 150–450 × 10⁹/l.

Platelets are round or oval discs 1–4 micrometers in diameter. The main function of platelets is providing the reactions of hemostasis necessary to prevent hemorrhage from damaged vessels. Thrombocytes membrane contains glycoproteins which are able to bind to the injured endothelial cells and exposed collagen fibers in the vessels wall; also it contains factors necessary to activate the sequential stages of the blood clotting process.

In small vessels (less than 0.2 mm in diameter) platelets are capable of repairing the damage without blood clotting (coagulation) initiation. The activated platelets adhere to the injured areas of blood vessels; they become sticky and release factors activating other platelets. Finally many platelets attached to each other close a hole in the damaged vessel and form the platelet plug, or white thrombus.

In larger vessels platelets are necessary to activate blood coagulation, and then they participate in the formation of a blood clot, or red thrombus, together with other blood cells.
In case of the decreased number of platelets, or *thrombocytopenia*, the person has the tendency of developing numerous small hemorrhages. These hemorrhages can be seen on the skin, but they develop throughout the body. The less the platelet number is, the more expressed hemorrhagic disturbances are. The decrease of platelets number below $50 \times 10^9/l$ results in spontaneous bleedings development, and the level about $10 \times 10^9/l$ and less may become lethal.

**BASES OF HEMOSTASIS**

**Primary** hemostasis means a fast (within several minutes) formation of a platelet plug at the site of vessel injury which is very important for the termination of bleeding from small vessels having low blood pressure. The components of primary hemostasis are the vascular wall, platelets and their special factors. The primary hemostasis stages are:

1. **Spasm** (constriction) of vessels.
2. **Platelets adhesion** and **aggregation** with the formation of a platelets plug.
3. **Retraction** (constriction and consolidation) of the platelets plug.

**Secondary** hemostasis starts as a rule on the basis of the primary one and follows it. It provides arrest of bleeding from larger vessels with high blood pressure. Secondary hemostasis starts from the formation of prothrombinase. This stage is rate-limiting as the next stages occur rapidly. The prothrombinase is not a single substance but a complex of activated substances necessary to convert prothrombin ($\alpha_2$ globulin) into thrombin in the presence of Ca$^{2+}$ ions. Active thrombin in turn converts blood protein fibrinogen into fibrin. That results in the formation of a network of sticky threads which entrap blood cells to form a red blood clot. The secondary hemostasis stages are:

1. **Prothrombinase** (prothrombin activator) formation.
2. **Conversion** of prothrombin into thrombin.
3. **Conversion of fibrinogen** into fibrin, formation of fibrin threads and eventually the red blood clot formation after the entrapment of blood cells (fig. 4.10).

![Fig. 4.10. Red blood cells entrapped into fibrin threads (red blood clot)](image-url)
Theme 5

BLOOD TYPES.
ABO SYSTEM. RHESUS SYSTEM

There is a variety of the complex glycolipid and other molecules on the surface of red blood cells membrane, as well as on other cells. Each of these glycolipid molecules is an antigen. The total number of known erythrocyte antigens at present exceeds 400, and these antigens may be found in any combination. Therefore the total complex of these antigens is individual and unique for every organism.

About 30 of such antigens are found very often; and some of them are present in all (or almost all) people. These systems of antigens are of high importance in medical practice. The major system of antigens is known as ABO system.

Only in case of foreign antigen invasion into the organism the immune system usually starts to produce antibodies against this antigen. This is true for all the other systems of red blood cell antigens, but ABO system possesses antibodies without additional specific stimulation of their production. This is one of the most important differences of ABO system from all the other blood cells antigen systems, along with the fact that its antigens are present in all people.

Blood types (groups) were discovered in 1900 by the Austrian immunologist K. Landsteiner. He had found the first three blood types, and a few years later the forth blood type was discovered. Blood types are discriminated by the presence (or absence) of the two antigens — A and B. There is one more antigen, H, in ABO system. But H antigen is present in all blood types so it does not make difference in blood types. There are 4 variants of antigens presence — A, B, both A and B, or none of them; so there are 4 blood types, named according to the antigen(s), present on the red blood cell surface. A person may have blood type A (having only A antigens), type B (having only B antigens), type AB (both A and B antigens), or type O (neither A nor B antigens). Blood types are also designated by Roman numerals: I (O), II (A), III (B), and IV (AB).

Antigens of ABO system are glycolipid molecules located on the surface of red blood cells membrane (fig. 5.1). These antigens are encoded by the genes located on the 9th chromosome. Each person inherits two genes (one from each parent) that control the production of antigens of ABO system. The genes for A or B antigens are dominant to the gene for O, since O actually means the absence of A or B. Therefore persons having blood type A may have genotypes AA (I^A I^A) or AO; type B — genotypes BB (I^B I^B) or BO. Persons having blood type O can have only O genes and genotype OO, and blood type AB — only genotype AB.

Antibodies of ABO system are blood plasma proteins (γ-globulins). Most of them are IgG and IgM immunoglobulin molecules which are produced by B lymphocytes (plasma cells). Right after birth, the antibodies of ABO system are practically absent in blood plasma. Their production starts during the first year of life. A few months after birth an infant begins to produce antibodies — anti-A (α) antibodies when type A antigens are not present in red blood cells, and anti-B (β) antibodies when type B antigens are not present in the cells. The maximal blood level
(titer) of antibodies is usually reached at 8 to 10 years of age, and then it gradually declines throughout the whole life.

![Image](image.png)

**Fig. 5.1.** The structure of ABO system antigens on the surface of red blood cells membrane (from W. F. Ganong, 22 ed., 2005)

It is believed that the reason of antibody formation against the antigens of ABO system which are not present in blood of a person is that after birth a small amount of antigens similar to A and B antigens enters the body with food, in some common bacteria (cross-reacting antigens). So the immune system of a baby starts to produce antibodies against those antigens which are foreign for the organism.

Thus, the following combinations of ABO system antigen/antibody exist:

**Table 5.1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Blood types</th>
<th>Antigen(s) of erythrocytes</th>
<th>Antibody(s) in plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>OO</td>
<td>I (O)</td>
<td>–</td>
<td>α, β</td>
</tr>
<tr>
<td>AA, AO</td>
<td>II (A)</td>
<td>A</td>
<td>β</td>
</tr>
<tr>
<td>BB, BO</td>
<td>III (B)</td>
<td>B</td>
<td>α</td>
</tr>
<tr>
<td>AB</td>
<td>IV (AB)</td>
<td>AB</td>
<td>–</td>
</tr>
</tbody>
</table>

The most frequent are types O and A, type B is less frequent, and type AB is rare. The average percentage of blood types may differ in different countries and continents, but individuals having blood types O and A approximately constitute more than 40% of all people each, type B may constitute up to 10%, and type AB — only a few percent.
TRANSFUSION REACTIONS RESULTING FROM MISMATCHED BLOOD TRANSFUSION

In case of a transfusion of mismatched donor blood to the recipient (a patient who takes the donor blood, from Latin *recipe* — take) the antigens of red blood cells become exposed to the antibodies able to bind these antigens. Binding of the antibodies to the corresponding antigens on the red blood cells surface causes the agglutination of these red blood cells. Red blood cells become attached to each other through antigens on their surface. Each antibody has at least two binding sites for antigens (as it is for IgG, shown in fig. 5.2), so it can cause bonding of the two cells via their antigens. Each red cell has many of such antigens, so it becomes bound to many other cells. Thus, aggregates or flocks of cells are formed (agglutination, fig. 5.3, 5.4). For this reason antigens are called agglutinogens, and antibodies are called agglutinins. Immunoglobulins M (IgM) are powerful agglutinins as they have 10 binding sites. As all the other antibodies, agglutinins of ABO system are produced by B lymphocytes.

![Fig. 5.2. Structure of the typical IgG antibody (from Guyton & Hall, 12 ed., 2011)](image)

The ability of antibodies to produce agglutination of red blood cells depends on the concentration of antibodies (antibodies titre, or titer) in blood plasma. After the transfusion antibodies titer of the donor blood becomes lower as the antibodies are diluted in the blood of the recipient. Relatively smaller volume of the donor blood added to the recipient body does not significantly dilute the recipient’s blood. So the recipient antibodies titer remains relatively high. Therefore the highest possibility of agglutination is for the donor’s red blood cells. Agglutination of red blood cells of the recipient occurs to a much lesser extent.

On the basis of the higher possibility of donor’s erythrocytes agglutination by recipient’s antibodies the concepts existed previously of a *universal donor* and a *universal recipient*. Persons having blood type O were considered universal donors because their red blood cells cannot be agglutinated by ABO system antibodies due to the absence of antigens A or B on the cell’s surface. Thus it was considered possible to transfuse blood type O (in limited volume) to a person having any blood type.
Accordingly persons having blood type AB were considered universal recipients due to the absence of ABO system antibodies in their blood, so that the transfusion of any blood (red blood cells having any ABO system antigens) would not cause agglutination of donor’s red cells. But in both these transfusion cases the agglutination occurs to the certain extent due to binding of donor’s antibodies to recipient’s red blood cells. At present such concepts as a universal donor and a universal recipient are not used, and it is allowed to transfuse blood (or blood preparations) of the same ABO blood type only. All other cases are considered the mismatched blood transfusion.

<table>
<thead>
<tr>
<th></th>
<th>Type A</th>
<th>Type B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigens of red blood cells</td>
<td><img src="image1" alt="Type A antigens" /></td>
<td><img src="image2" alt="Type B antigens" /></td>
</tr>
<tr>
<td>Antibodies in plasma</td>
<td><img src="image3" alt="Type A antibodies" /></td>
<td><img src="image4" alt="Type B antibodies" /></td>
</tr>
<tr>
<td>Agglutination reactions</td>
<td><img src="image5" alt="Type A agglutination" /></td>
<td><img src="image6" alt="Type B agglutination" /></td>
</tr>
</tbody>
</table>

*Fig. 5.3. Scheme of agglutination of red blood cells by corresponding antigens (from S. I. Fox, 12 ed., 2011)*

*Fig. 5.4. Formation of red blood cells aggregates — agglutination*

The clamps of agglutinated red blood cells which are formed due to mismatched blood transfusion then plug small blood vessels throughout the circulatory system, impairing the blood and oxygen supply to the tissues. This dangerous state is referred
to as a posttransfusion shock. After a few hours (in some cases — instantly) hemolysis of the agglutinated red blood cells may develop, releasing hemoglobin from the cells. In case of massive hemolysis hemoglobin released from destroyed red blood cells leaks through the glomerular filter into renal tubules and then can block many tubules. Along with the greatly decreased arterial blood pressure and therefore decreased renal blood supply it causes severe kidney damage — the acute renal failure. Also massive hemolysis often results in activation of intravascular blood clotting (the disseminated intravascular coagulation of the blood, DIC). Normal activation of blood coagulation occurs in case of the vessel wall damage, and the blood clot formed as a result blocks the damaged part of the vessel to prevent from further hemorrhage. But intravascular activation of blood coagulation results in the formation of small blood clots inside the microcirculatory vessels, in the arterioles, capillaries and venules throughout the body, so that the blood supply to the tissues through these vessels becomes partially or totally blocked. Thus, the development of disseminated intravascular coagulation is the most dangerous consequence of mismatched blood transfusion, which can become lethal within a few hours.

**BLOOD TYPING IN ABO SYSTEM**

To prevent the mismatched blood transfusion and its consequences it is necessary to determine the blood type precisely using accurate methods of blood typing. There are two methods to determine the blood type of a person.

**Blood typing using standard sera**

One method uses the standard sera of different blood types (O, A, B or all four types). Standard sera are obtained from the donor blood. The serum is a defibrinated blood plasma (plasma without fibrinogen that becomes converted into fibrin and precipitates). Except for the fibrin it contains all the rest blood proteins including antibodies of ABO system.

At first the drops of standard sera are prepared on the test plate, and then much smaller drops of the examined blood are added into each serum. Mixing the examined blood of a patient with these sera containing the known antibodies one can see agglutination in certain sera and its absence in the others (fig. 5.5). The combination of results is different for all 4 blood types (table 5.2). The general rule of the tests using sera is the ratio of the serum and blood volumes of about 10 : 1 (the drop of serum should be approximately 10 times bigger than the drop of the examined blood). It provides predomination of antibodies that is necessary to produce agglutination of practically all red blood cells of the added drop of examined blood so that the result is clearly visible.

Agglutination usually occurs within the first minute or so, but sometimes a few minutes are necessary; the final result is evaluated in 5 minutes.

When examined blood is added to a drop of serum, the serum antibodies can bind only the antigens of the examined blood and do not react to the antibodies of the examined blood. Thus, blood typing is the identification of the antigens present on the membrane of red blood cells of the examined blood.
Red blood cells of blood type O do not have antigens A or B, so these cells do not become agglutinated in any standard serum. Red blood cells of any other blood types have at least one antigen and therefore do become agglutinated in certain sera. Thus, the absence of agglutination in all sera certifies that the examined blood belongs to type I (O).

Red blood cells of blood type A possess antigen A, therefore these cells should be agglutinated in all sera having antibody binding A antigen, antibody α. These antibodies are present in the standard sera of types O and B. So when agglutination occurs in the sera of types I (O) and III (B) (fig. 5.6, a), the examined blood is blood type II (A).

As the erythrocytes of blood type B have antigen B, these cells should be agglutinated in all sera having antibody β. These antibodies are present in the standard sera of types O and A. Thus, if agglutination occurs in the sera of types I (O) and II (A) (fig. 5.6, b), the examined blood is blood type III (B).

Finally, the erythrocytes of blood type AB have both antigens A and B, so the presence of even one antibody, α or β, in the serum is sufficient to cause agglutination of added erythrocytes. Agglutination does not occur only in the serum of blood type AB, as this serum does not have any antibodies. Therefore if agglutination of red blood cells of the examined blood is observed in the sera of types I (O), II (A), and III (B), the examined blood is blood type IV (AB).

**Table 5.2**

<table>
<thead>
<tr>
<th>Examined blood</th>
<th>Standard sera and its antibodies</th>
<th>I (α, β)</th>
<th>II (β)</th>
<th>III (α)</th>
<th>IV (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (O)</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II (A)</td>
<td></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>III (B)</td>
<td></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IV (AB)</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

– means the absence of agglutination; + means agglutination

**Fig. 5.5.** Test plate for blood typing

**Fig. 5.6.** Examples of blood typing using standard sera
As one can see, agglutination should never occur in the serum of blood type IV (AB) since this serum does not contain antibodies of ABO system, α or β.

**Blood typing using monoclonal sera**

Another method uses monoclonal sera containing the antibodies produced by a single clone of cells grown in culture. To obtain monoclonal serum animals (e. g., mice) are immunized with a particular antigen. Then antibody-producing B lymphocytes (plasma cells) are taken from their spleen and fused with the tumor myeloma cells. Such fusion results in formation of antibody-producing immortal hybridoma cells which are able to grow and reproduce rapidly. Every such cell growing in culture forms the single clone of cells that produce only one specific antibody. These pure and specific antibodies are produced in high concentration; that makes monoclonal sera highly active and reliable.

There are two types of monoclonal reagents necessary for blood typing in ABO system — anti-A and anti-B. Anti-A serum contains anti-A antibodies which bind A antigen (like natural α antibody), anti-B serum binds B antigen (like natural β antibody). The test is performed similarly to using standard sera: a small drop of blood is added to a drop of serum and mixed, the ratio of the serum and blood drop volumes is about 10 : 1. The combination of agglutination differs for all 4 blood types (table 5.3).

<table>
<thead>
<tr>
<th>Examined blood</th>
<th>Monoclonal reagents</th>
<th>Anti-A</th>
<th>Anti-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (O)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II (A)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>III (B)</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>IV (AB)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

As one can see, **anti-A** serum acts like type III (B) standard serum containing α antibody, and **anti-B** serum acts like type II (A) serum containing β antibody.

Thus, if there is no agglutination in both monoclonal sera, it means that examined blood is type O. If agglutination is present in both sera, the blood type is AB. Blood type A is determined by the presence of agglutination in anti-A serum only, and blood type B can be determined by the presence of agglutination only in anti-B serum (fig. 5.7). It is evident that the method using monoclonal sera is easier, reliable and does not require the use of donor blood in order to obtain sera.

![Fig. 5.7. Examples of blood typing using monoclonal sera](image)
RHESUS SYSTEM

Antigens of the Rhesus system were discovered in 1940, by K. Landsteiner and A. Wiener. The Rhesus antigen was first discovered in rhesus monkey; therefore it was named Rhesus factor (Rhesus antigen, Rh). The major difference between ABO system and Rh system is the absence of innate antibodies in Rh system whereas in ABO system antibodies are determined by genotype and develop spontaneously.

There are six common types of Rh antigens, designated \( C, D, E, c, d, \) and \( e \). But the major antigen of Rhesus system is \( D \) antigen, which is much more antigenic. A person having this antigen in his red blood cells is called Rh-positive, whereas a person who does not have type \( D \) antigen is said to be Rh-negative. About 85\% of all white people are Rh positive and 15\% – Rh negative. In American blacks, the percentage of Rh-positives is about 95\%, whereas in African blacks it is practically 100\%.

Either Rh positive or Rh negative people do not have antibodies against Rh antigen. Anti-rhesus antibody may develop only in Rh negative persons in two situations when Rh antigen enters their blood.

1. The first case is mismatched blood transfusion of Rh-positive blood to the Rh-negative recipient. In this case the foreign Rh antigen (D-antigen) enters the organism. In response to this antigen the antibody formation process is initiated. This is a slow process; the maximal antibodies concentration is reached in 2–4 months after the transfusion, when most of the transfused cells have already finished their life. But since then the antibody concentration may remain at a rather high level for a long time, at least for a few years. So, while the first mismatched blood transfusion of Rh-positive blood to the Rh-negative person does not have visible consequences (it causes the initiation of anti-Rh antibodies formation), the second and any other repeated similar transfusion do result in the immediate agglutination of Rh-positive donor red cells. In case of repeated transfusion of Rh-positive blood to such a person who is already immunized against the Rh antigen by mismatched transfusion in the past, the transfusion reaction can be as severe as a transfusion reaction caused by mismatched types of blood of ABO system.

2. Another case is a pregnancy of Rh-negative mother with Rh-positive fetus. During pregnancy normally mother is not exposed to the Rh antigen of a fetus since the mother’s blood is separated from the fetus blood by the placenta. At the time of birth, however, a certain amount of the newborn baby’s blood may enter the mother’s circulation, and the mother’s immune system may begin to produce antibodies against the Rh antigen. It does not always occur since the amount of infant’s red blood cells that enter mother’s circulation may be minimal. But if the woman starts to produce antibodies against the Rh factor and their level remains high for at least a few years, in the second and subsequent pregnancies these antibodies could cross the placenta and cause agglutination and hemolysis of the Rh-positive red blood cells of the fetus. Therefore, the second and other next babies could be born with a condition called erythroblastosis fetalis, or hemolytic disease of the newborn.
Due to an intensive hemolysis baby has a decreased RBC number (anemia). Hypoxia that develops due to the hemolysis of red blood cells and the products of hemolysis stimulate the hematopoietic tissues of the fetus, and later of the neonate, in order to replace the hemolyzed red blood cells. The liver and spleen become greatly enlarged and continue to produce red blood cells even after birth. Due to the rapid production of red cells, many premature forms of red blood cells, including nucleated blastic forms, are released into baby’s peripheral blood. It is because of the presence of these nucleated blastic red blood cells that the disease is called erythroblastosis fetalis.

Another dangerous symptom of hemolytic disease of the newborn is a high level of bilirubin in blood. It develops because the hemoglobin released from the hemolyzed red blood cells becomes converted by the fetal macrophages into bilirubin. Abnormally high level of bilirubin causes yellow coloring of the baby’s skin (jaundice) and exhibits high toxicity. A high level of bilirubin may result in permanent mental impairment or damage to motor areas of the brain due to accumulation of lipid-soluble bilirubin in lipid-rich neuronal cells of some brain nuclei, causing their destruction (kernicterus, what means nuclear jaundice).

Without the appropriate treatment, the anti-Rh agglutinins from the mother may circulate in the infant’s blood for another 1–2 months after birth, destroying more and more red blood cells.

In case of intensive hemolysis and high bilirubin level in the neonate the best treatment is to replace the neonate’s blood with Rh-negative blood. About 400 milliliters of Rh-negative blood is infused over a period of a few hours while the neonate’s own Rh-positive blood is being removed (replacement transfusion). The Rh-negative red blood cells would not be agglutinated by anti-rhesus antibodies of mother, if any would remain in neonate’s blood after the replacement transfusion.

**Prophylaxis** of the hemolytic disease of the newborn

Erythroblastosis fetalis can be prevented by injecting the Rh-negative mother with an antibody preparation against the Rh antigen within 72 hours after birth of each Rh-positive baby. This is the type of passive immunization in which the injected anti-D antibodies inactivate the Rh antigens of fetal red blood cells which entered the mother’s circulation and thus prevent the mother from production of own anti-Rhesus antibodies. Due to the implementation of this method of prophylaxis the incidence of the hemolytic disease of the newborn has become greatly decreased.

**Blood typing in the Rh system**

Blood typing in the Rh system is done using anti-Rhesus sera containing anti-Rh antibodies. Such serum can be prepared from the blood of animal (or Rh-negative human) immunized by Rh antigen (the universal anti-Rhesus serum) or it can be produced as monoclonal serum.

The difference of the two methods of Rh blood typing is that the determination of blood Rh antigen using the universal anti-Rh serum is carried out in the test tube,
and the test using the monoclonal serum is carried out on the test plate. The ratio of serum and blood volumes is as usual approximately 10 : 1.

Regardless of the type of anti-rhesus serum the evaluation of the test result is the same since the agglutination of red blood cells by anti-Rh antibodies may occur only if red blood cells have Rh antigen on their surface.

In case of red blood cells **agglutination** the examined blood is **Rh-positive**. If agglutination of red blood cells in anti-Rh serum does not occur, the examined blood has no Rh-antigens and therefore is Rh-negative (fig. 5.8).

**Fig. 5.8. Examples of blood typing using monoclonal anti-Rhesus serum**

### BLOOD TRANSFUSION TESTS

Blood transfusion tests are carried out before the transfusion of blood (or its components) to make sure that donor blood is of the same type in both ABO and Rh system that the blood of a recipient and to prevent the development of mismatched blood transfusion reactions.

Before blood transfusion the following **tests** should be carried out:

1. Blood typing of the **recipient** and **donor** blood in the **ABO** system.
2. Blood typing of the **recipient** and **donor** blood in the **Rhesus** system.
3. Determination of the **individual compatibility** in the **ABO** system.

To carry out the test the **serum of the recipient** is prepared from the recipient’s blood. A drop of the donor’s blood is added to the serum placed on the test plate. If agglutination does not occur, the donor’s blood is compatible with the recipient’s blood.

4. Determination of the **individual compatibility** in the **Rhesus** system.

The test is carried out **the same way** as the previous one except the **temperature**: serum and blood drops are mixed on the **Petri dish** placed on the **water bath** at $t = 46–48 \, ^\circ C$. If agglutination does not occur, the donor’s blood is compatible with the recipient’s blood.

5. **Biological test**. This test is a preliminary transfusion of **small amounts** of blood in order to observe possible signs of disturbances and prevent severe damages in case of incompatibility not detected by other tests.

The donor blood already checked for the compatibility by all the tests described above is transfused intravenously in the amount about **10–15 ml** and then the state of the recipient is watched for **3–5 minutes**. If the signs of
disturbances do not appear, the same blood volume is transfused repeatedly. After close observation of the patient for 3–5 minutes the transfusion is done for the third time. If during next few minutes the state of the patient has not impaired, the blood can be transfused safely.

The signs of possible blood incompatibility include any significant changes of the heart and respiration rates, blood pressure level, changes of the skin color (paleness or redness), complaints about headache, dizziness and others. Complaints about the lumbar pain are more specific and evidence the damage of kidneys. In case of any of these signs presence blood transfusion is not carried out.

At present the whole blood transfusions are carried out rarely. In case of necessity donor blood preparations or blood substituting solutions can be used.

DONOR BLOOD PREPARATIONS are erythrocyte mass (red blood cells separated from plasma), thrombocyte mass, blood plasma and others. The type of blood preparation is chosen in accordance with indications.

BLOOD SUBSTITUTING SOLUTIONS are preparations able to substitute to some degree one or several blood functions. The simplest solutions are the physiological solution (0.9 % NaCl solution) and 5 % glucose solution. More complex solutions are used to maintain the circulating blood volume and arterial blood pressure (hemodynamic solutions). Such solutions contain high-molecular substances which create colloid-osmotic (oncotic) pressure and therefore contribute to the blood volume maintaining by attracting fluid to vessels and holding it within the circulation system. As high-molecular substances cannot cross the vessel wall, they remain in the circulation for a certain time and help to maintain blood volume and arterial pressure (for example, after blood loss or a shock). Other important functions of blood substituting solutions are detoxication of the organism by breakdown or elimination of various toxins; providing parenteral nutrition; facilitation of diuresis and others. Many blood substituting solutions are polyfunctional.

There are some basic requirements to blood substituting solutions. One of the most important requirements is that the main physical and chemical properties of blood substituting solutions such as osmolarity, pH, and viscosity must be close to that of blood plasma. The substances of blood substituting solutions must be able to become subsequently completely eliminated from the organism without injury to the tissue and impairment of the function of organs, or be metabolized by enzyme systems of the organism.
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