**RUSSIAN NATIONAL RESEARCH MEDICAL
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Department of pathophysiology and clinical pathophysiology

LECTURES ON THE COURSE
OF PATHOPHYSIOLOGY

PART 1

GENERAL PATHOPHYSIOLOGY

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Lecture 1
Introduction in course of pathophysiology

Pathology literally is the study of suffering. It likes a bridging between theoretical disciplines: anatomy, physiology, biochemistry, histology and clinical practice. In course of pathophysiology you will study the functions of an organism in state of the disease. There are different definitions of disease, one of them, nowadays, is widely acceptable and very essential for disease understanding.

According this definition the disease is a complex of organism injury and its responses which usually result in limited adaptation of an organism to every time changing life conditions. For example, a patient with serious heart disease isn't tolerant to physical exercises or a patient with chronic renal failure can't take excess of water. The responses of organism to injuring factors, which adapt them to the surrounding are named the compensatory processes but, sometimes, they may play not only protective role but to be a source of injury.

The disease isn't chaotic process but there is a new order in the life of a patient, and the life is going to its own, but new way, according to other laws then in normal. But it is life! And your future goal is to learn and understand these laws and use them in your clinical practice, moreover, this knowledge in future will help you to provide the rational clinical care and therapy of your patients.

Traditionally, the study of pathology is divided into general and clinical pathology. The former is concerned with the basic reactions of cells and tissues to abnormal stimuli that are responsible for many diseases. The latter examines specific responses of different organs and tissues to an injury in course of disease. But general pathology, except mentioned above problems, includes the problems of common nosology (nosos- "Greek" disease). Nosology is a science of etiology, pathogenesis, classification, periods, and outcomes of a disease. Each disease has the cause and its own mechanism (pathogenesis), site in classification, periods, and outcome. The cause is determined as etiology, mechanism- as pathogenesis, and they both are the backbone of the pathophysiology.

The problem of etiology

Etiology is a science of the causes and conditions when disease can be realized. According to modern conception of etiology of disease all factors provoking disease may be divided into intrinsic (hereditary, constitution) and extrinsic (physical, chemical, and biological factors).They are determined as causative factors.

Some diseases are of unknown etiology (idiopathic). But there is no disease without a cause. Another deal, that nowadays we don't know the causes of some diseases, for example cancer. But experimental and clinical data every year bring us reliable information about the role of some viruses, especially retroviruses, chemicals, radiation, and our hereditary in outstanding and development of this "horror of humanity". The other example is an allergen as the cause of allergic disease. But there is no disease when a patient avoids the contact with allergen, and for this reason the doctors strongly recommend their patients not to be in contact with provoking disease allergen. But it ought to be said, that before the treatment it is necessary to find out the allergen which evokes the disease. It may be pollen of

the grass or blooming trees, home or library dust or, eventually, dandruff of the pets. There is no doubt that the knowledge and understanding of the role of causative factor as the trigger of a disease is too important for successful treatment.

Classification of disease causes

Intrinsic factors:

Inherited	Degenerative	Nutritional	Congenital
Metabolic	Neoplastic	Psychogenic	Immunologic

Extrinsic factors

Inanimate:	Animate
Physical agents:	Infections:
force, temperature, radiation,	viruses, bacteria
atmosphere pressure,electricity,	worms, fungi, protozoa
chemicals agents	protozoa, insects

The next feature of the cause of the disease is its specificity. There is a close connection between etiologic factor and the clinical features or symptoms of the disease. That connection helps a doctor to diagnose the disease. For example, such child infections as mumps, measles, rubella or scarlet fever possess by very specific symptoms in form of characteristic skin rash. Why? Now we don't have the answer to this question but, nevertheless, we use it in our pediatric practice. Distinctive features of radiation factor action are the following: depression of hematopoiesis, as an early symptom, chromosomal and genes abnormalities, as postponed effects, plus associated with them inborn malformations, oncologic disease, and shortened life span of patient.

But etiology minds not only the role of cause but also the importance of conditions in outstanding of the disease. Conditions rather play role the modifying factors, and usually they change "a face of the disease".

For example, age, sex, race, complexion, ethnic, climate and, eventually, geographic factors influence the outstanding and development of the disease. So, acute form of lympholeukemia is mostly characteristic of the children but, on the contrary, chronic lympholeukemia more often occurs in the people in their after 55-60years. Next example; the antibodies to infectious agents that have given from their mother, protect children during the first year of their life (passive innate immunity). The men prone more to a peptic ulcer of stomach or duodenum than women, but such endocrine disease as hyperthyroidism is more characteristic of women after 50year. It's too important to keep in mind the factors which influence the pattern of a disease. So, accidents, including poisoning and infection are mostly characteristic of a childhood but, as for the premature babies, they are very characteristic of respiratory failure due to congenital lack of surfactant and development of an acute respiratory distress syndrome (surfactant decreases surface tension in alveolus and facilitates the respiration).The aging process is an atrophy (wasting effects of the age), and for these reason specific senile alterations are the diseases of eye, movement disorder, and psychical changes in old people.

About influence of geography the disease development. As an example of ethnic group influence may be the following evidences: carcinoma of penis virtual-

ly unknown among Jews and Moslems who practice circumcision in early age, preserving concentration of smegma about the glans penis. Sickle cell anemia more often occurs in African population, whereas, pernicious anemia has a much higher rate among the Scandinavians and rare in black population.

It must be said that extrinsic factors influence depends on many constituencies. For example if it concerns with electric trauma; the voltage of the current, time of its action, kind of current, and current power postulate the severity of injury. The other example: the outcome of ischemia (blood deprivation of tissue) depends on the time of low tissue blood perfusion and type of the organ which was under ischemic condition. So, the brain, heart and kidney, no having quite enough collateral circulation, are very much predisposed to the ischemic injury, and infarction development as unfavorable outcome.

Pathogenesis

It is determined as a sequence of the events in response to the causative factors which provoke pathologic process or disease. Term pathogenesis reflects the development and evolution of the disease which has to run its course,passing through several stages. Start of a disease, in whole, is associated with the cell injury, and then it is spreading over the surrounding tissue involving more and more cellular space and, at last, the whole organ. But, simultaneously, in response to the injury to restitute homeostasis protective compensatory mechanisms are activated. Mostly, they are the output of our natural adaptation created during the process of biological evolution. The bright example of such kind of local protective reactions to any injury is an inflammation. In course of inflammation any pathogen of infectious or non-infectious nature is localized, destroyed, eliminated and, eventually, there is a start of the tissue repair with total or partial tissue recovery. If the injury is serious and threatens to an organism, not only local reaction in form of inflammation, but whole organism becomes involved in the struggle against a pathogen. Non-specific reaction of organism, in whole, is named an acute phase response. Four life-important systems take part in an acute phase response. They are the following: CNS, endocrine, immune, and hematopoietic systems. The synthesis of the special proteins by the liver is a very essential characteristic feature of the acute phase response. On the one hand, these proteins enhance the protective mechanisms of an organism but, on the other hand, they can limit tissue destruction.

Russian pathologist Ippolit Davidovsky suggested an image our organism as a black box. In some cases we know but, sometimes, don't know which causative factor is "at the entry", but, nevertheless, we can see the clinical features at "the exit" of the box. Lasts are the symptoms of the disease. But what is inside the box? We can't see the processes which are running inside the organism that equal the content of the box in our mind. But by which way could we uncover a secret inside? Experimental pathology can shed light on the secrets hidden in "a black box" using experiments "in vivo" and "in vitro". They help to reproduce some elements of pathology in form of separated pathogenetic chains of the processes that are running in a" black box". Moreover, pathologists make use the methods of clinical observations, and on their base create clinical conception of the disease

pathogenesis. We many times check up the conception, and then mentally build the analogous between the processes which are going in organism of the animals and humans with the lot of corrections.

To understand all said above, let consider some examples of "black box".

A lack of oxygen due to lower atmosphere pressure is the cause of the altitude disease. The symptoms of the disease are going step by step when an individual is climbing the mountain are the following: tachycardia, dyspnea, and euphoria, and they may be followed by severe disturbances in respiration and cardiovascular activity. How can we imagine the pathogenesis of tachycardia and dyspnea as the symptoms of the altitude disease? Let us combine the pathogenetic chain, corresponding to the events, proceeding in the organisms found themselves in the mountain of 3000-3500m high (table 1).

Pathogenesis of tachycardia and dyspnea symptoms in patient with an altitude disease

Decreasing in atmosphere pressure leads to diminished oxygen content in inspirated air and decreases partial pressure of oxygen in the alveolar air

↓

Low oxygen level in the lung capillaries and systemic circulation

↓

Exitation of chemoreceptors in the lung, sinocarotid and aortal zone stimulates corresponding centers in CNS

↓

Tachycardia, deep and frequent breathing

Besides, direct activation of sympathetic nerve system by catecholamines stimulates ionotropic and chronotropic influences the heart, resulting in tachycardia and increased stroke volume.

Next example is an edema as a classic sign or symptom of an acute inflammation. The causative factors of inflammation, as was said, may be different, including infectious or non-infectious factors.

Pathogenesis of edema in acute inflammation

Both kinds of etiologic factors provoke local injury of tissue with alteration of microcirculation directly and under biological active substances, preformed in the cells such as histamine, and formed "de novo".
Among the lasts must be called bradykinin from plasma and arachidonic acid derived from lipids of cell membranes

↓

All these substances, histamine, bradykinin and PgE-2 are very vasoactive. They increase local vascular permeability in the site of their action and promote passage of plasma proteins and leukocytes through the capillary wall

↓

Edema is a result of exudation of blood plasma, rich of the proteins and emigrated leukocytes due to inflammatory mediators activity (increased vessels permeabilty and leukocytes emigration)

In conclusion must be said:

1. edema in the site of an acute inflammation in any case is a "deal with hands" of biological active substances, ultimately appearing there due to tissue injury.

2 there is no inflammatory edema without tissue injury, and for this reason, nowadays, the anti-mediatory therapy with non-steroid anti-inflammatory drugs blocking cyclooxigenase activity (NSAIDs) and glucocorticoids inhibiting phospholipase A-2) are in our arsenal to cope with inflammation.

The other example is pathogenesis of renal hypertension.

Pathogenesis of renal hypertension
Injury of kidney of different causes

↓

Hypo-perfusion of the kidney

↓

*Activation of RAAS with high activities of AT-II, aldosterone,
and ADH in the blood*

↓

*Increased resistance of arterioles under angiotensin II action and increased ECFV
via aldosterone and ADH high production.*

Resume: Two major factors which increase AP in course of renal hypertension are the following: one of them is increased peripheral blood vessels resistance as a result of high level of AT-II, but another one, is increased ECFV due to high blood level of aldosterone and ADH.

As for pathogenetic therapy, it's become clear that ACE (anti-converting enzyme) blockers are the most important as a rational therapy in case of any hypertension associated with RAAS activation, including renal hypertension.

The other example of pathogenetic chain is ketoacidosis, characteristic of an acute complication of diabetes mellitus type I

Pathogenesis of ketoacidosis in case of diabetes mellitus type I
*Lack of insulin, mostly due to the autoimmune processes in the endocrine pancreas
with significant loss of beta-cells*

↓

Energetic starvation of insulin-dependent tissue

↓

*Metabolic disturbances in form of lipolysis predominance when instead of glucose
an organism use of fat acids as a source of energy.*

↓

Excessive formation of the ketone bodies

↓

*Flooding of an organism with the ketone bodies due to their accumulation and
problems in their oxidation in liver*

↓

*Disturbances in acid base and water- electrolyte balances (metabolic acidosis and
hyperosmomolar dehydration of an organism)*

↓

Disturbances in central neurotransmission

↓

Ketoacidotic coma

The main chain of the pathogenesis and the role of vicious circles in the disease pathogenesis

The main chain of the pathogenesis of any disease may be determined as a point or position in pathogenesis chain, which forms the center or base of pathology (background) with its all unfavourable consequences. It is a lack of insulin when diabetes mellitus type I and lack of receptors to insulin (insulin-resistance) in case of diabetes mellitus type II. Increased resistance of medium and small bronchioles to airflow in case of bronchial asthma and decreased myocardial contractility in heart insufficiency of different causes. Direct inhibition of the respiratory center in case of morphine poisoning and uncontrolled excessive cell growth in tumors are the others examples of the main chains of disease pathogenesis.

Vicious circle in pathology is a condition when the cause provokes the consequence which, in turn, capable to exaggerate (enhance) the effect of initial own causative factor. For example, in acute renal failure provoked by toxic substances, RAAS is activated because of low kidney perfusion but, in turn, as a response to improve the glomerular filtrate rate, the afferent kidney arterioles spasm leads to secondary restriction of kidney blood supplying. The last triggers an additional ischemic injury of the kidney which doesn't improve the kidney circulation, but rather impairs it. The other example: one of the most compensatory mechanisms in the hyperthermia is a massive sweating and lung hyperventilation that can provide a heat dissipation. But due to organism dehydration, as a unfavorable consequence, the hemoconcentration is possible which, in turn, results in disturbances in brain microcirculation. Hypothalamic failure to maintain the heat balance of an organism may lead to breakdown of thermoregulation. Thermoregulation homeostasis is shifted to heat accumulation, and the temperature of an organism tends to be an equal to the temperature of surrounding. Mentioned above clinical examples show how important for a doctor to break down any vicious circle in pathology, and such way improve a patient's condition. Nowadays, revealing of the vicious circle in pathogenetic chain of disease with its erradication is the best method of pathogenetic therapy.

Methods of disease therapy

An optimal method is a preventive therapy when there is no contact of an organism with causative factor. This variant may be illustrated by an isolation of the healthy people from people with infectious disease, excluding the allergens from the food when food allergy, or contact with professional hazards. The next optimal method is the erradication or weakening the influence the cause of the disease; last it is classified as etiologic therapy. The best example of such kind of treatment is antibiotic therapy when the drug kills animate factors (microbes, protozoa). But more often we use pathogenetic therapy, which give us a possibility to incline into some chains of disease pathogenesis, abrupt some its points, and such way, to provide the correction of pathology. In endocrine disease, when the lack of corresponding hormone, for example, lack of insulin in the patients with diabetes mellitus type I (insulin dependent form) it is replacement therapy with insulin injections, but hyperthyroidism is treated with the drugs which decrease , either the produc-

tion of thyroid hormones or their end-effects to sensible to the hormone tissues (target tissues). Immunosuppressive, anti-inflammatory, antihypertensive, diuretics, and chemotherapy are the examples of the drugs that can provide the pathogenetic and symptomatic therapy. As symptomatic treatment, may be used non-narcotic or narcotic analgesic drugs, especially they are necessary in postoperative conditions and in the patient with oncologic disease to cope with unbearable pain. Antipyretic drugs get used to avoid of complications in form of cramps in the pediatric practice, but in old people they can decrease the heart overload provoked by severe tachycardia. Diuretics are capable to decrease the intracranial pressure and, such way, to avoid the complications in form of brain edema and its possible negative outcomes in form of the disturbances in CNS functions.

Periods of the disease

Any disease has some consecutive periods following one by one in a strong order. The first is a latent period when the signs of the disease very often may be revealed only by laboratory examinations. The latent period is a time interval between the exposure of the cause of the disease (pathogens) and the appearance of its symptoms. For example, chronic renal failure is going through three stages: latent, azotemia, and uremia. Latent period is asymptomatic and may be revealed only by the functional kidney tests.

Latent period of an iron-deficiency anemia passes without decreased hemoglobin and erythrocytes number in blood unit, but total iron-binding capacity of the blood serum (TIBC) at that time usually is increased. In case of infectious disease latent period is designed as incubation period. Next period of the disease is a period of clinical features (symptoms). Their appearance helps the doctor to recognize the disease. As for liver pathology, it is a jaundice, decreased Hb and the number of the erythrocytes in anemia, and hemorrhage in form of hematomas in the patients with hemophilia.

During this long standing period may be the decline in severity or exacerbation. Decline in severity named a remission. In case of leukemia, that time the blood film is improved and may look like near normal. The blast cells disappear and immature cells are decreased in number. Another example: in rheumatic fever during remission there are no fever and swelling of the joints, and at the same time, a pain becomes lesser. Period of exacerbation in the patients with leukemia is manifested by flooding of the blood with immature white cells, blasts appearance and severe anemia with thrombocytopenia. Clinically, septic complications and hemorrhagic syndrome accompany these blood changes. But in rheumatic fever there are the signs of an acute phase response and pain with swelling of the joints are characteristic.

All diseases accordingly to the length of their course, clinically are divided into acute and chronic. Acute forms usually last not more than 4-6 weeks. Chronic diseases tend to be accompanied by the relapses, when clinically they become similar to an acute disease. All chronic diseases start with acute phase.

The outcomes of the disease

The possible outcomes of the disease may be the following: complete recovery or incomplete, when disease transforms into chronic form and may result in death. Many factors influence the outcome of the disease, and one of them is early diagnosis and rational therapy. And in conclusion must be said, that the more early diagnosis is determined, the more successful treatment, and better prognosis and favourable the outcome of disease.

Lecture 2
HEMORHEOLOGY AND MICROCIRCULATION
Rheological properties of blood: norm and pathology

Rheology is the study of deformations and flow of liquids. The units of measurement are shear force and shear rate, their ratio is called viscosity and is expressed in hundredth fractions of a poise – *centipoises (cP)*. Thus, viscosity is internal friction, or the property of a fluid to exert resistance to shearing layers. The lower viscosity, the greater fluidity and vice versa.

1. Viscosity of blood depends on the velocity of blood flow (in a true solution viscosity does not change at any velocity or at rest if the temperature and pressure are constant).
2. Blood flows along the tubes –blood vessels with various diameters. Blood viscosity depends on the diameter of a vessel – it is the lowest in capillaries (1.7 – 2.0 cP).
3. Blood viscosity depends on the ratio of the formed elements (blood cells) and blood plasma which is different in different parts of the vascular network (local hematocrit) and can change with alterations of blood flow velocity (Fig.1), as well as in some pathological conditions.
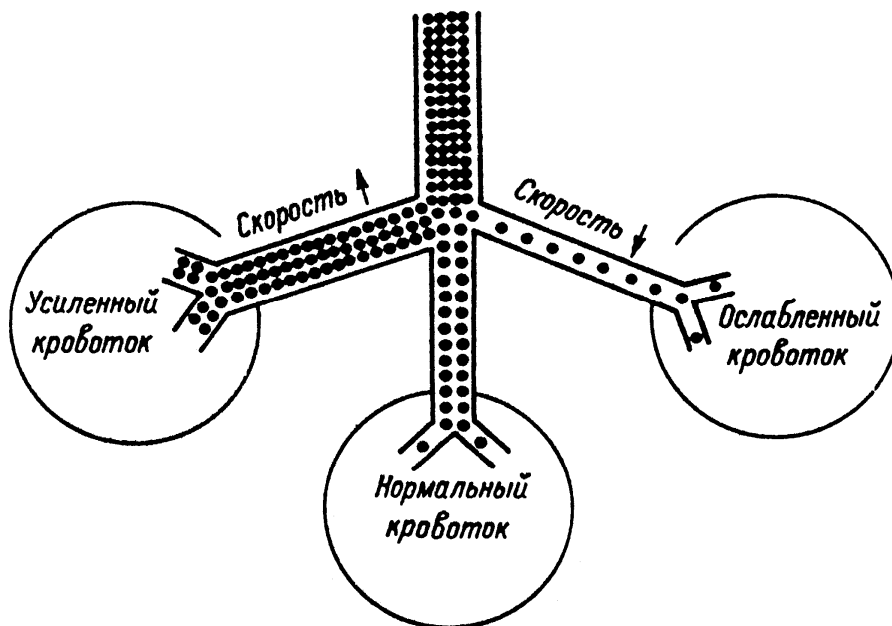
Fig.1 Changes of local hematocrit in microcirculatory channels depending on blood velocity in the supplying arteries.

Microcirculatory blood flow is determined primarily by rheological properties of blood which change in various pathologies and, especially, at terminal states. Viscosity of the whole blood is normally about 4-5cP (1.5 times as high as that of plasma), and in pathological conditions it fluctuates between 1.7-22.6 cP.

Main factors affecting rheological properties of blood:

1. Viscosity of blood is in inverse dependence on the velocity of blood flow: the lower velocity, the greater viscosity and the worse fluidity. Venous blood is more viscous because the speed of blood flow in veins is lower. When microcirculation is disturbed, blood viscosity increases: at prestasis it is 100-200 times as high as normal, and at stasis fluidity is lost.

2. Viscosity is in direct linear dependence on hematocrit (hematocrit is a ratio of blood cell volume to total blood volume; normally it is about 0.45 L/L). As the total volume of erythrocytes (red blood cells) in the blood is 160 times as large as the volume of leucocytes and platelets, blood can be regarded as a two-phase system: suspension of erythrocytes in blood plasma. When hematocrit rises from 0.40 to 0.50 L/L, there is a twofold increase in blood viscosity. There are local fluctuations of hematocrit in blood channels: in capillaries it is 0.12 L/L, in the mixed arterial and venous blood it is 0.45L/L.

3. Erythrocytes (if hematocrit is normal) contribute to reduction of blood viscosity in capillaries, because blood acquires non-Newtonian properties due to erythrocytes. Fluidity of blood in capillaries is high because fluidity of erythrocytes is high: red blood cells act in microvessels as droplets of fluid. While in relatively large vessels viscosity of the whole blood is 1.5 times as high as viscosity of hemoglobin solution of the same concentration, in capillaries the whole blood viscosity is reduced by half as compared with that of hemoglobin solution. The decisive factor in the ability of red blood cells to reduce viscosity is their deformability. Plasma viscosity depends on deformability of red blood cells: the less deformability, the higher viscosity. Rigid, undeformable cells increase viscosity. Decreased deformability may be caused by higher concentrations of calcium ions in the membranes of erythrocytes which occurs, for instance, at hypoxia. Viscosity also increases when the blood contains larger amounts of abnormal red blood cells (e.g. spherocytes) or red blood cells containing hemoglobin with an altered structure.

4. Viscosity depends on the degree of aggregation of blood cells, mainly erythrocytes. Moderate reversible aggregation of erythrocytes with formation of rouleaux (resembling stacks of coins) in large blood vessels is considered to be physiologically normal. This aggregation provides a more effective transfer of erythrocytes in the blood flow, and thus, contributes to tissue oxygenation. Marked irreversible aggregation of erythrocytes is pathological, it increases blood viscosity. High viscosity, in turn, stimulates aggregation of blood cells.

5. Viscosity depends on protein and lipid composition of blood plasma. Increased concentration of large protein molecules leads to increased viscosity. Thus, increase in globulin level up to 1-2% is accompanied by twofold increase in viscosity. Thrombin, and especially fibrinogen, raise viscosity (viscosity of plasma is 20%

higher than that of serum). Products of fibrinogen breakdown also increase viscosity. Albumin, on the contrary, reduces viscosity because it acts as a disaggregating agent. Lipid composition of plasma also plays an important role. Higher viscosity is observed with increased ratio cholesterol/ phospholipids, increased concentration of lipoproteins of low and very low density as well as products of peroxidative oxidation of lipids.

6. Acidosis, hypercapnia in particular, is a factor elevating viscosity. It decreases blood circulation rate and increases viscosity of venous blood.

7. Disturbance of blood flow arrangement in microvessels. Normally, in the middle of the blood flow there are erythrocytes, oriented longitudinally. When the blood flow decreases, or condition of erythrocytes becomes worse, they arrange themselves across the vessel approaching the vessel walls, which impedes the blood flow even more.
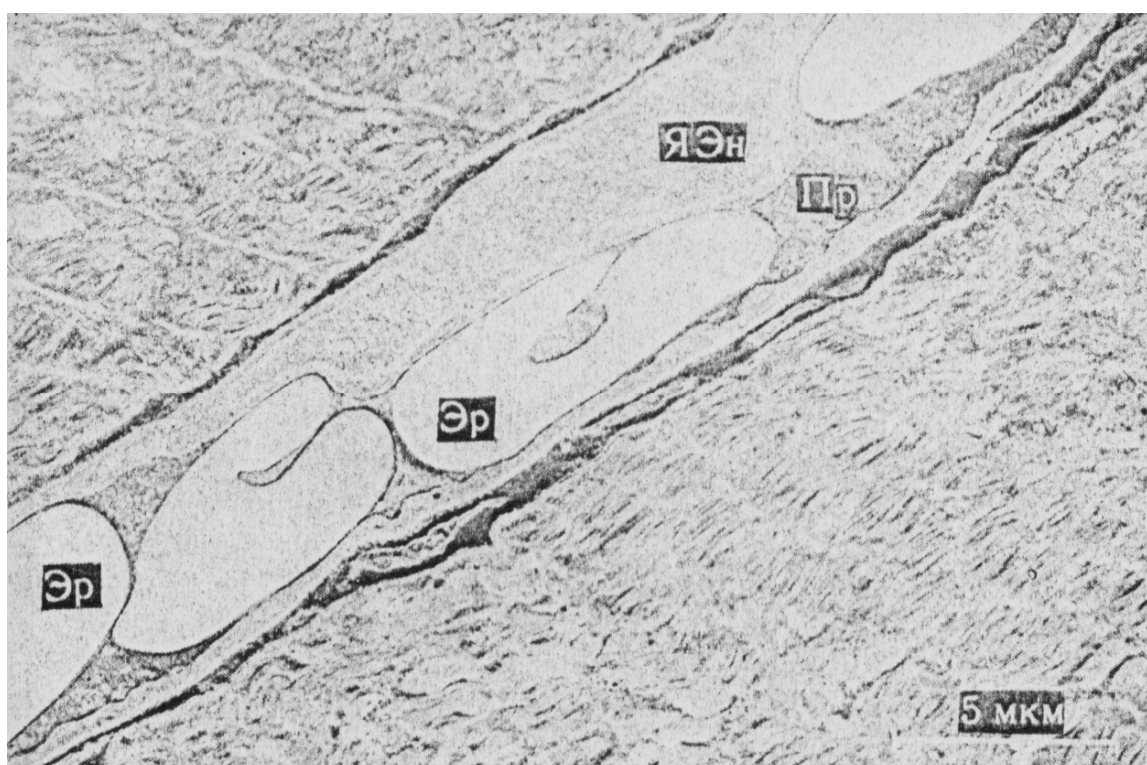
Fig.2. Erythrocytes passing along a capillary, the lumen of which is less then the diameter of erythrocytes. The gastrocnemius of a rat. 20 days after denervation.

Correction principles of disturbances of rheological properties of blood.
1. Normalization of hemodynamics (restoration of the velocity of peripheral blood flow.
2. Controlled hemodilution (thinning of blood and decreasing viscosity).
3. Injection of disaggregating agents and anticoagulants (prevention of thrombi formation).
4. Use of drugs decreasing rigidity of erythrocyte membranes.
5. Normalization of acid-base balance of blood.
6. Normalization of protein composition of blood (injection of albumin solutions).
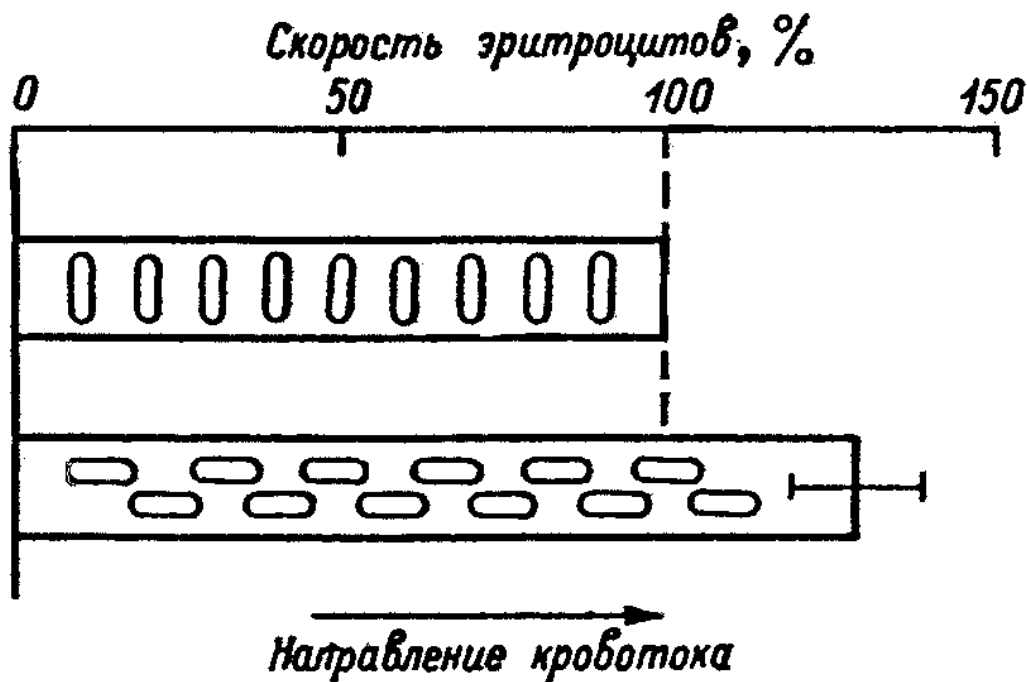
Fig. 3 Velocity of erythrocytes, oriented along the vessel axis as compared with the velocity of adjacent erythrocytes with transverse orientation.

Hemodilution and disaggregation are achieved by using Haemodes (medication) and low-molecular dextrans which increase electrostatic repulsion between blood cells, decrease viscosity attracting water, cover the endothelium and vessels with a separating film, form complex compounds with fibrinogen, decrease lipid concentration.

DISORDERS OF MICROCIRCULATION

Blood circulation system is divided into macrocirculation – the cardiac pump, buffer- vessels (arteries) and capacity- vessels (veins) - and microcirculation. The aim of microcirculation is to join the blood circulation system and systemic circulation of fluids in the body and to distribute the cardiac output among the organs according to their needs. That is why each organ has its own system of microcirculation corresponding to its function. Nevertheless, three basic structural types of the terminal vascular channels have been identified and described: classic, bridge-like and net-like.

The system of microcirculation is composed of the following microvessels:
1. arterioles (diameter 100 μm and less);
2. precapillary arterioles, or precapillaries, or metarterioles (diameter 25-10 μm);
3. capillaries (diameter 2-20 μm);
4. postcapillary venules, or postcapillaries (diameter 15-20 μm);
5. venules (diameter up to100 μm);

Besides there are arteriole-venule anastomoses –direct junctions between arterioles/arteries and venules/veins. Their diameter is from 30 to 500 μm, they are found in most organs.
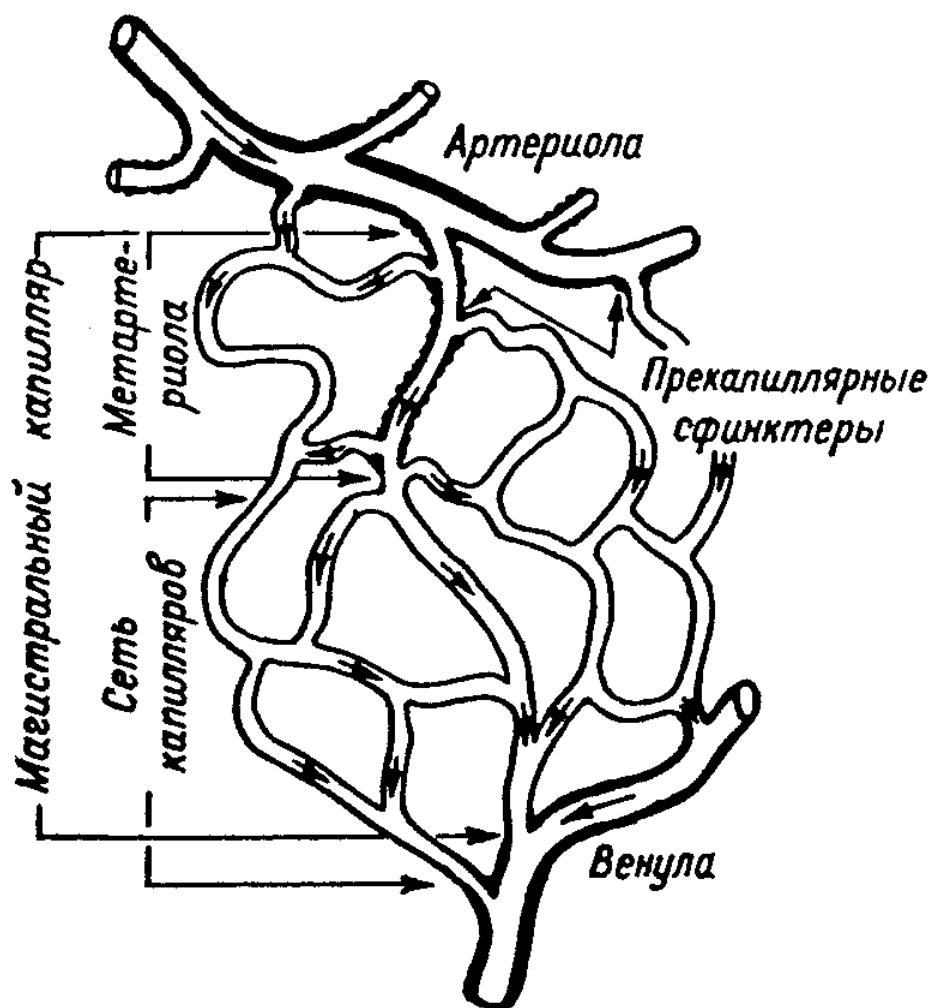
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Fig.4 The scheme of the microcirculatory channels.
    main(magistral) capillary; capillary network; metarteriole; arteriole,
    precapillary sphincter; venules.

  The driving force in the microcirculatory system is perfusion pressure or arterio-venous pressure difference. This pressure depends on systemic arterial and venous pressure and is influenced by the activity of the heart, total blood volume and systemic peripheral vascular resistance. The relationship between the central and peripheral blood circulation is expressed by the formula $\mathbf{Q = \delta P/R}$ (Q – intensity [volume velocity] of blood flow in the microcirculatory system; δP - arterio-venous pressure difference; R –peripheral [hydrodynamic] resistance in the given vascular bed). Changes in δP and R play the major role in disorders of the peripheral circulation. The less peripheral resistance, the greater blood flow intensity and vice versa. The regulation of the peripheral circulation and microcirculation in all organs is provided by changes of resistance in their vascular system. Increased blood viscosity increases hydrodynamic resistance and, thus, decreases the intensity of blood flow. Even more hydrodynamic resistance depends on the radius of vessels: hydrodynamic resistance is inversely proportional to *the forth power of the vessel radius (r^4).* Hence, changes in the area of the vessel lumen (constriction or

dilation) influence the blood flow more significantly than such factors as viscosity or pressure changes.

The main regulators of microcirculation are small supplying arteries and arterioles and arterio-venous anastomoses. When the supplying arterioles dilate: 1) the velocity of blood flow increases; 2) intracapillary pressure increases; 3) the number of the functional capillaries increases. The latter will also depend on the opening of the precapillary sphincters – relaxation of two or more smooth muscle cells at the beginning of a capillary.
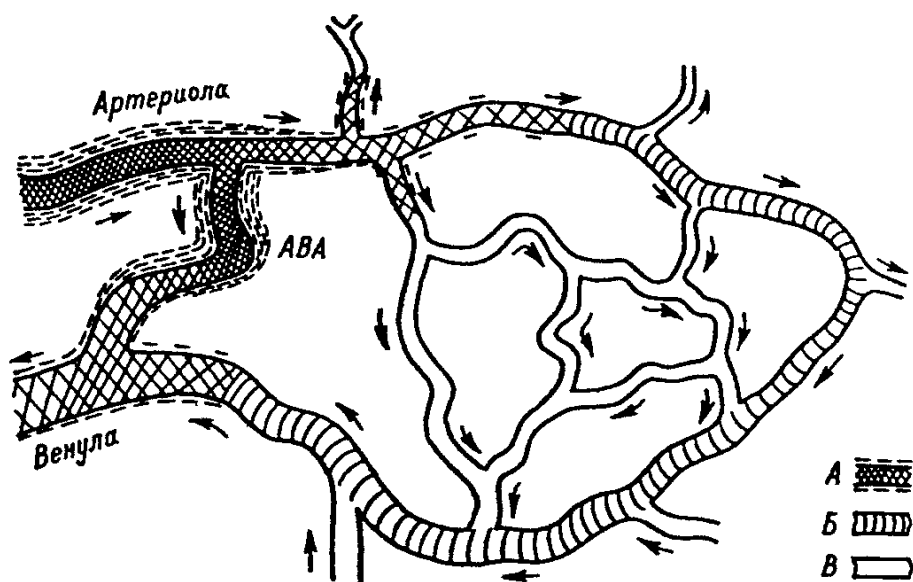
Fig.5 The scheme of the basic vessels of the microcirculatory bed.
A – smooth muscle cells of microvessels with vasomotor innervation;
B – main (magistral) capillary; C- network capillaries; AVA – arterio-venous anastomoses.

The lumen of microvessels can change actively only if there are smooth muscle elements in its structure. Fig. 5 shows the types of vessels which contain these elements (hatched). So, vegetative nerves innervate all blood vessels except capillaries. Changes in the tone of vessels and vascular sphincters may be caused by nervous, humoral and local regulatory mechanisms.

Table 1. Regulation of microcirculatory channels

Type of microvessel	Diameter (μm)	Wall thickness (μm)	Regulation*	
			nervous	humoral
Arteriole	20-25	5-6	+++	+
Small arteriole	18-20	3-4	++	+
Metarteriole	15	2	+	++
Precapillary sphincter	10-12	2	-	+++
True capillary	8-10	1	-	+
Venule	20-30	4-5	-	+
Small vein	30-50	6-8	+	+

*Number of crosses shows degree of regulation

Nervous regulation is provided by the vegetative nervous system. Vasomotor nerves mainly belong to its sympathetic division (more rarely – to parasympathetic one) and abundantly innervate arterioles of the skin, kidneys and splanchnic area. In the brain and skeletal muscles these vessels are innervated relatively poorly. The mediator in the synapses is noradrenalin which always causes muscle contractions. The degree of contraction of the vessel muscles depends on the frequency of nerve impulses. Resting vascular tone is maintained by constant incoming impulses with the frequency of 1-3 impulses per second (so called tonic impulsation). At impulse frequency of 10 per second the constriction of vessels is maximal. So, *increased impulsation of vasomotor nerves leads to vasoconstricton, decreased impulsation – to vasodilation*, the latter being limited to the basal vascular tone (the tone which is observed in the absence of impulses in the vasoconstrictor nerves, or when these nerves are cut).

Parasympathetic cholinergic vasodilating fibers innervate the vessels of the external genitals, small arteries of the pia mater of the brain. The nervous mechanism is also responsible for dilation of skin vessels in response to mechanical or chemical stimuli. This is the axon-reflex, provided by nociceptor (pain sensing) fibers and neuropeptides.

Sensitivity of muscle cells to vasoactive substances varies. Microvessels are 10-100 as sensitive as large vessels, the most sensitive to vasoconstricting and vasodilating agents are precapillary sphincters. It was found that similar reactivity is shown in response to electrical stimulation (Table 2). When a pathology is present, sensitivity of microvessels to vasoactive substances changes.

Table 2.

Reactivity gradient of microcirculatory channels of the mesentery of rats.

Type of vessel	Diameter (μm)	*Stimulation*	
		adrenalin(μg)	*electrical (V)*
Artery	50	0.01	4.0
Arteriole	20	0.005	3.5
Precapillary sphincter	12	0.001	2.0
Venule	40	0.05	5.0

Vessel reactivity differs in various organs and tissues, especially in relation to adrenalin (Table 3). The most sensitive to adrenalin are microvessels of the skin.

Table 3.

Reactivity of microvessels of rats to threshold concentration of adrenalin.

Microvessels	*Adrenalin*
Skin	0.002-0.004
Skeletal muscle	0.02-030
Omentum	0.1-0.2
Mesentery	0.2-0.4
Intestinal wall	0.75-1.00
Urinary bladder	0.005-0.020

Humoral regulation is provided by hormones and chemical substances secreted in the body. Vasopressin (antidiuretic hormone -ADH) and angiotensin II cause vasoconstriction, kallidin and bradykinin - vasodilation. Adrenalin (epinephrine) secreted by the adrenal glands can exert vasoconstricting as well as vasodilating effect. The effect is determined by the number of α- and β- adrenergic receptors on the membrane of vascular muscles. If α-receptors predominate, the result is vasoconstriction, if β-receptors – the result is vasodilation.

Local regulatory mechanisms provide metabolic autoregulation of the peripheral blood circulation. They adjust the local blood flow to specific needs of the organ. *In this case, the metabolic vasodilating effects dominate the nervous vasoconstricting effects, and sometimes completely inhibit them.* Microvessels are dilated due to shortage of oxygen and effects of some metabolic products – carbon dioxide, increased amounts of H-ions, lactate, pyruvate, ADP, AMP and adenosine, many mediators of damage or inflammation – histamine, bradykinin, prostaglandins A and E and substance P. It is considered that some mediators cause endothelial cells to secrete nitric oxide which leads to relaxation of smooth muscles and vasodilation. Mediators of damage –serotonin, prostaglandins F, thromboxan and endothelins – cause constriction of microvessels.

As for the ability of capillaries to constrict, the answer is rather negative than positive because they have no smooth muscle cells. Those researchers who observe *active* constriction of capillary lumen explain it by contraction of endotheliocytes in response to irritation and bulging of their nuclei into the lumen. *Passive* constriction and even complete closure of capillaries occurs when the strain of their walls exceeds the intravascular pressure. This condition develops when the blood flow in the supplying arteriole is reduced. Significant dilation of capillaries is also difficult because 95% of their wall resilience is provided by the surrounding connective substance. Only if this substance is destroyed, for example, by inflammatory exudation, the increased intracapillary pressure can cause extension of the capillary walls and their significant dilation.

Changes in the capillary blood flow are likely to depend also on leucocytes. Unlike erythrocytes which are disc-shaped, leucocytes have a spherical shape and the diameter of 6-8 μm, so their volume is 2-3 times as great as that of erythrocytes. When a leucocyte enters a capillary, it is "stuck" in its ostium for a while. This can take from 0.05 sec. to several seconds, and the blood flow in the capillary stops. After the leucocyte "slips" into the capillary, the blood flow is restored.

The basic types of disturbances of the peripheral bloodflow and microcirculation are:
1) arterial hyperemia;
2) venous hyperemia;
3) ischemia;
4) stasis.

Thrombosis and embolism are not regarded as independent pathologies of microcirculation though they cause its serious disturbances.

1. ARTERIAL HYPEREMIA

Arterial hyperemia is the increase of blood flow to an organ or a tissue. The blood flow increases due to dilation of small arteries and arterioles (active hyperemia). In arterial hyperemia hydrodynamic resistance drops, volumetric (Q) and linear (V) velocity of blood flow (Q=VS) and the number of functioning capillaries increase (the increase in pressure gradient, linear velocity and hematocrit causes the closed capillaries to become functional ones). As the outflow of blood is not disturbed, and edema develops seldom, the resilience of the capillary wall does not change and dilation of capillaries is not significant. However, the total area of the cross section of the whole microcirculatory bed extends because there are more functioning capillaries. The blood flow is laminar, it means that it is clearly separated into the axial (blood cells) and the outer/parietal (plasma) flow; the absence of the internal friction contributes to faster blood circulation. The tissue produces more tissue fluid and, consequently, the lymph outflow increases.

Acceleration of blood flow reduces the time of contact of blood with the tissue and decreases the time of oxygen diffusion to the tissue. This does not lead to worsening of metabolism because is compensated for by a greater number of functioning capillaries for a tissue unit; on the contrary, the metabolism even increases. As a result, the level of oxygen in the venous blood is higher, the arteriole-venule oxygen difference is lower and the venous blood has a more scarlet color.

Thus, changes in the microcirculation in arterial hyperemia are characterized by dilation of arterioles, increased number of functioning capillaries, increased velocity of the blood flow, a clear separation of the blood flow into the axial and plasmatic/ parietal flows and bright scarlet color of blood in the vessels.

External signs and symptoms of arterial hyperemia are: bright scarlet color of the organ (dilation of the blood vessels, greater number of functioning capillaries, increased hematocrit, arterialization of the venous blood), increased turgor (tension) of the tissues (engorgement of the vessels and increased production of tissue fluid), increased temperature of the superficial tissues (flush of warm arterial blood). The causes of arterial hyperemia may be physiological irritators – external (heat, mechanical irritation, sun rays) and internal (reflex, metabolites). This type of hyperemia is called physiological. Examples of physiological hyperemia: working (intensification of an organ function), reflex (exposure to adequate doses of physiological irritators); emotions – shame, anger.

Hyperemia caused by disease-producing irritating agents – mechanical, physical, biological, chemical, both external and internal, is called pathological. Examples of pathological hyperemia: *inflammatory*, *collateral* (intensification of blood flow in collateral vessels due to obstruction of the blood flow in the main vessels, *postanemic* – hyperemia after quick restoration of arterial blood flow (removal of the pressing factor – ligature, tumor, accumulation of fluid), *vacant* – caused by a decrease in barometric pressure (local, under the influence of cupping-glasses), *angioneurotic* – hyperemia of the face in lupus erythematosus, *infectious skin rash, hyperemia of one half of the face in neuralgia.*

Any type of arterial hyperemia arises from dilation of supplying arteries or arterioles. There are three mechanisms causing such dilation:

1. Myoparalytic mechanism is the most frequent because it is connected with relaxation of the muscular elements of arterioles and precapillaries caused by the action of local factors – metabolites and damage mediators.
2. Neuroparalytic mechanism develops when vasoconstricting fibers of the nerves and their centers are cut, paralyzed or damaged in other ways (by bacterial toxins and pharmacological substances).
3. Neurotonic mechanism. Hyperemia develops as a result of a decrease in the tone of vasoconstrictors, an increase in the tone of vasodilators, or axon-reflex. Sympathetic vasodilators cause development of arterial hyperemia in the pancreas, salivary glands, tongue, cavernous bodies. This mechanism causes flushing of cheeks with shame or anger, reddening of the skin to remove excessive heat from the body. Axon-reflex takes part in inflammatory hyperemia.

The significance of arterial hyperemia is linked with its consequences. The short-term consequence is an increase in functional abilities of an organ or a tissue. Prolonged hyperemia can lead to hypertrophy, hyperplasia and even accelerated development of organs and tissues. Arterial hyperemia of the organs enclosed in a limited space is sometimes accompanied by unpleasant sensations – headaches, tinnitus (hyperemia in cerebral vessels), aches in joints. If there is a defect in the vessel wall, the vessel can rupture under the influence of high pressure and cause hemorrhage.

2. VENOUS HYPEREMIA

Venous hyperemia is the increased blood supply to an organ or tissue because of the difficulties of blood outflow. The necessary condition of this type of hyperemia is insufficient outflow of blood through the roundabout veins.

Venous blood stasis develops as a result of mechanical obstruction to the blood outflow from the microcirculatory bed to the venous system (local venous hyperemia) or due to elevated pressure in large veins – it can occur in such conditions as left or right ventricular cardiac insufficiency, increased pressure in the portal vein due to liver cirrhosis. In this case venous stasis occurs in multiple venous networks.

Blood pressure in the veins increases right before the obstacle to the blood flow. As a result: 1) arterio-venous pressure difference decreases which leads to slowing down of the flow of blood, linear velocity of the blood flow drops; 2) increased intravascular pressure extends the vessels and causes their dilation: all functioning veins and capillaries widen, nonfunctioning venous vessels open; 3) elasticity of the connective tissue inside the vessel walls and around them increases; 4) filtration of fluid from venules and capillaries to the surrounding tissue increases.

Venous hyperemia causes not only slowing down of the blood flow, but other changes: pulsating or pendulum-like flow. If the pressure before the obstacle reaches the diastolic pressure, the blood flow will stop during the diastole and will renew during each systole, i.e. it will be pulsating. If the pressure before the obsta-

cle exceeds the diastolic pressure in the supplying arteries, the orthograde (direct) blood flow will be observed only during the systole, and during the diastole the blood flow will become retrograde (reverse) due to the distortion of the pressure gradient. Such blood flow is called pendulum-like.

Slowing down of the blood flow prolongs the time of contact of blood with the tissue and, and thus, the time of diffusion of oxygen to this tissue. Arteriole-venule oxygen difference increases, the level of reduced hemoglobin in the venous blood grows up and cyanosis (bluish color of the skin) develops. The percentage of the reduced hemoglobin in the venous blood is more than 5-6%. The partial pressure of oxygen in the tissue and its pH moderately decreases and the local partial pressure of carbon dioxide increase.

So, venous hyperemia is characterized by considerable dilation of veins and capillaries, slowing down of blood flow and dark red color of blood in the vessels. The number of functional capillaries does not usually change.

External signs and symptoms of venous hyperemia are: dark red or purple color of the organ (dilation and increase in number of venous vessels, increased hematocrit because of transudation and increased percentage of reduced hemoglobin in the venous blood); distension of the organ or tissue area (engorgement of vessels, edema); decreased temperature of the superficial tissues (decreased flush of warm arterial blood).

The causes of venous hyperemia: mechanical obstacle in a vein – thrombosis, pressure by a tumor or extended pregnant uterus, increased pressure in large veins.

Short-term consequences of venous hyperemia: moderate hypoxia of an organ or tissue, congestive stasis and edema or dropsy. Chronic venous hyperemia is accompanied by diapedetic hemorrhages into organs and tissues, atrophy and even necrosis of the most aerobic parenchymatous cells and overliving and proliferation of the stromal cells which leads to sclerotic changes in the organ.

3. STASIS

Stasis is a complete stop of blood flow in a vessel. There are several types of stasis: 1) congestive – the result of venous hyperemia causing elevation of venous pressure and equalizing it with arterial pressure (difficulty in blood outflow); 2) post-ischemic – the result of ischemia causing lowering of arterial pressure to the level of venous pressure (difficulty in influx); 3) true, or capillary stasis –the result of changes in blood viscosity and fluidity in capillaries. In real pathological processes these mechanisms can combine, generating mixed stasis. Arteriole- venule pressure difference as the driving force of blood flow is lost in congestive and post-ischemic stasis, but is preserved in true stasis. Congestive and post-ischemic stases are principally reversible, true stasis is reversible only at the beginning because as it develops, changes in blood cells and plasma occur which consolidate obstruction of capillaries and venules.

True stasis is characterized by an increase in capillary resistance to blood flow. According to Poiseuille's law the pressure gradient is $\delta P = 8L\eta Q/\pi r^4$, where η – blood viscosity, Q – volumetric blood flow velocity, r – radius of the vessel, L – length of the vessel. If the flow is unchanged, the main role in vascular resistance

will be played by blood viscosity. Blood <u>viscosity increases</u>, firstly, due to <u>slowing down of blood flow</u> in microvessels which often precedes stasis (for example, in inflammation, stasis develops under conditions of venous hyperemia), and secondly, as a result of <u>enhanced intravascular aggregation (clumping) of erythrocytes</u>. Sometimes erythrocyte aggregation precedes low blood flow, sometimes these processes come in parallel.

Intensification of erythrocyte aggregation is caused by damage to erythrocytes and loss of their deformity. The surface of erythrocytes during aggregation becomes rough or "fluffy". It looks like erythrocytes have a viscous cover. Erythrocytes stick together forming rouleaux and move in a vessel not in a longitudinal, but in a transverse position , and these conglomerates can "cork" the vessel.

Stasis develops more readily at low blood flow, higher hematocrit (for example, due to transudation), at vessel flexures, in the presence of adhesion molecules on leucocytes and endothelium, at increased blood concentrations of globulins and fibrinogen. At first blood stops in response to the action of damaging agents causing stasis. Once the stasis has developed, the blood flow stops in the whole capillary – below and above the focuses of the initial stasis. As the stasis is progressing, there appears "sludge". The sludged blood differs from normal. It contains aggregations of erythrocytes, leucocytes, platelets. There is no clear borderline between the surface of blood cells and their plasma, the rouleaux become homogeneous. Homogeneity of the blood flow is lost, aggregations become more marked, then they are sedimentated . The liquid fraction of blood leaks through the venule walls to the surrounding tissue and the blood becomes more viscous. The vessel walls do not receive sufficient nutrition and begin to lose their normal shape.

The causes of true capillary stasis are damage to tissues by both external (chemical, mechanical, biological, etc) and internal factors, slowing down of blood flow, increased hematocrit in polycythemias, changes in red blood cell properties in anemias, etc.

Pathogenic significance of blood stasis largely depends on the organ in which it develops. Stasis is especially dangerous in microvesssels of the brain, myocardium and kidneys. Besides, stasis contributes to thrombi formation.

4. ISCHEMIA

<u>Ischemia is a decrease in blood supply of an organ or tissue due to insufficient blood inflow. It is caused by blockage or narrowing of the supplying artery and absence (or insufficiency) of collateral blood supply to the given area.</u>

In ischemia the inflow of blood is decreased and the outflow remains equal to the inflow. The pressure markedly drops in the arterioles to the periphery of the narrowing, less significantly – in the venules. Pressure gradient is decreased, which leads to reduction in the linear and (more markedly) volumetric blood velocity. Separation of the blood flow into axial and plasmatic layers disappears. In the ischemic area the blood flow is slow and erythrocytes are redistributed in the microvessels – the blood going to the capillaries is poor in erythrocytes and has low hematocrit, so functional capillaries turn into plasmatic. Then, because of the low intracapillary pressure some capillaries close and the number of functional ca-

pillaries decreases. Due to the low intravascular pressure filtration of fluid from vessels to the tissue also decreases. The amount of tissue fluid reduces and the lymph outflow becomes weaker and can stop. Ischemia leads to deprivation of oxygen and energy-producing substances and accumulation of metabolic products. There is hypoxia, hypercapnia and local acidosis.

Depending on the cause, there are three types of ischemia: obturational, compression and neurospastic. It has been found that after severe ischemia production of nitric oxide in endothelial cells decreases, which causes massive adhesion of neutrophils to the walls of microvessels and worsens their patency. The erythrocytes remaining in the vessels are mostly damaged by excess of metabolic products and have less ATP; they begin to aggregate which is very dangerous when the blood flow has stopped. In this case, even an increase in longitudinal pressure gradient in microvessels as a result of restored blood flow from the arteries (for example, through collateral vessels) is not sufficient to restore the blood flow in these microvessels and the blood column in the vessels of the ischemic area is static. It may explain the causes of no-reflow effect which makes impossible quick restoration of blood flow after prolonged ischemia.

So, changes of microcirculation in ischemia are characterized by significant narrowing of microvessels, slowing down of blood flow, absence of separation of blood flow into axial and plasmatic currents and paleness of the tissue area. The number of functional capillaries decreases.

External signs and symptoms of ischemia are: paleness of the organ (due to narrowing and decreased number of functional vessels, increased portion of plasmatic capillaries), slight shrinkage of an organ or tissue (due to decreased number of functional vessels, decreased volume of tissue fluid), lower temperature of superficial tissues (decrease in influx of warm arterial blood). Symptoms such as pain, tingling, numbness, creepy feeling may develop.

The causes of ischemia are: 1) complete or partial blockage of the supplying peripheral artery by a thrombus or embolus; 2) pathological vasoconstriction (angiospasm); 3) sclerotic or inflammatory changes of arterial walls; 4) squeezing of arteries by outside forces. A specific situation leading to ischemia is collateral ischemia which develops with quick redistribution of blood, for example, in the brain vessels in collapse.

The consequence of short-term ischemia is a decrease in functional characteristics of the organ, the consequence of long-term ischemia is hypoxic necrobiosis. The local necrosis of tissues caused by acute disturbance of its blood supply is called *infarction*. Over time, postnecrotic sclerosis develops at the site of infarction.

Ischemia of a magistral arterial vessel causes collateral arterial hyperemia through myoparalytic mechanism (effect of accumulated metabolites). If collateral vessels are absolutely sufficient (their total diameter is not less than the diameter of the blocked magistral artery), infarction does not develop (in the limbs, liver, intestines, lungs). In the kidneys, retina, spleen, the basin of the medial cerebral artery collaterals are absolutely insufficient, therefore, in these organs infarctions occur

rather often. Some organs have relatively sufficient collaterals, for example, the heart. In this case myocardial infarction is caused by atherosclerotic damage to the vessels. As a result, the functions of the endothelium and monocytes are impaired. It promotes thrombosis and angiospasm and can cause significant narrowing of the coronary blood vessels (decrease in their diameter up to 75%).

5. THROMBOSIS.

Thrombosis is intravital formation of a clot of stabilized fibrin and formed elements of blood on the internal surface of blood vessels with partial or complete obturation of their lumen.

Thrombi can form both in arterial and in venous vessels which differ in blood flow velocity. The common causal factor in both cases is a lesion of a vessel wall. In arterial vessels rapid blood flow decreases concentration of plasma coagulation factors and the main role in thrombosis is played by platelets (thrombocytes). They are activated, stick to the lesion site and together with leucocytes form a white thrombus within 2-3 minutes. Later fibrin builds up in the thrombus, fibrin filaments "catch" erythrocytes and the white thrombus turns into a red one. In the venous vessels with slow blood flow activation of platelets is accompanied by activation of coagulation hemostasis with fibrin formation, that is why a red thrombus forms (within 4-9 minutes).

The consequences of thrombosis in arteries are ischemia, post-ischemic stasis and necrosis, in veins – venous hyperemia and congestion stasis.

6. EMBOLISM.

Embolism is blockage of arteries by circulating particles and conglomerates (emboli) not typical of the normal blood flow. Emboli move in the blood until they reach a blood vessel too narrow for them to pass, stick there and block the blood flow. It causes ischemia and post-ischemic stasis at the periphery of the embolus and can cause infarction if there is a lack of collateral vessels.

Emboli causing infarction of the lungs are brought from the venous system of the systemic circulation and the "right heart"; emboli causing myocardial infarction, infarction of the brain, internal organs and limbs come from the "left heart" or pulmonary veins; emboli causing infarctions of the liver come from unpaired abdominal organs.

According to the origin of emboli all embolisms are divided into two groups. Endogenous embolisms: 1) thromboembolism (> 90% of all cases) – the embolism is caused by a thrombus (or its parts) which comes from the site of its formation; 2) fat embolism – the embolism is caused by fat droplets which appear after fractures of long bones or destruction of fatty tissue; by the products of aggregation of blood chylomicrons; 3) tissue embolism – caused by amniotic fluid, particles of injured tissues; 4) tumor embolism – caused by a tumor particles (at tumor disintegration) or by tumor cells (at metastasis). Exogenous embolisms: 1) microbial or parasitic embolism - at sepsis, bacteremia and blood parasite invasion; 2) air embolism – caused by air bubbles coming from the atmospheric air into large veins where the pressure is lower than atmospheric; 3) gas embolism – caused by gas bubbles

formed in the blood when barometric pressure rapidly decreases, for example, when a diver quickly rises from a depth, or in aircraft decompression.

Lecture 3
Acute Inflammation. Microcirculation and inflammatory mediators

It's impossible to imagine a man who never had any injury of the body. Not at once, we had mechanical trauma in form of the cut of the skin, burns, and infection but every time the win was on our side due to protective local and systemic reactions of our organism to injury. Inflammation belongs to such local protective reaction, but acute phase response is presented by reactions of whole organism to severe injury.

Definition of inflammation

It is local protective response of an organism to injury in which the blood, connective tissue cells, and local nerve elements are involved.

Must be added that it is provided by:
1. Isolation and damage of pathogen by its killing and lysing,
2. Attraction of the leukocytes to the site of injury,
3. Repair of the wound by proliferation of connective tissue elements, and sometimes, scar formation.

As was said, the response includes the reactions of local circulation, connective tissue, blood and nervous elements. Inflammation is a typical pathologic process elaborated by complex organisms during their evolution, and despite the different causes provoked the inflammation it is going by common way and possesses by very similar local symptoms. These signs are the following: rubor-redness, calor-warmth, dolor-pain, and tumor-swelling. These symptoms had been described many centuries ago by Roman doctor Caelcus, and later, Greece scientist Galen added the fifth sign "functio laesa" that means disturbances in functions of injured tissue.

Etiology and pathogenesis of inflammation

Different causes may provoke an inflammation, both extrinsic or intrinsic. Extrinsic factors are in form of infectious or non-infectious ones. Examples of intrinsic factors may be such as immune complexes, malignant cells or aggressive gastric juice in case of stomach ulcer, bile appearance in inappropriate sites, or urea acids accumulation in the joints. At the same time, pathogenesis of inflammation may be represented by the chain of events which starts with a cause action, when each cause provokes the consequence in form of the event which, in turn, starts to play causative role to attitude the next consequence. Such way a pathogenetic chain is combined.

Inflammatory process includes not only an injury of the tissue but universal responses of tissue to that injury. The complex of injury manifestations and reactions of tissue to them is defined as pathogenesis of inflammation. There are several phenomena characteristic of the pathogenesis of an acute inflammation. First, immediately after tissue injury there are the disturbances in the microcirculation can be observed. They are the following in their sequence of events:

- spasm of the small vessels, short-termed (from seconds to 1-2 minutes) but not obligatory, usually only if severe injury, mechanical or burns
- active hyperemia,
- congestion,
- stasis

Spasm seems to be a result of direct influence of injuring factor the smooth muscle of the microcirculatory vessels and release and influence the catecholamines from catecholamine nerve endings. Active hyperemia may be explained by axon-reflex due to the participation of nociceptive fibres of C group which send their collateral branches and supply by the nerve endings the vessels of the microvasculature. Active hyperemia clinically results in redness of tissue due to the dilation of the microcirculatory vessels (arterioles, metarterioles, capillaries, and small venules). Moreover, it is characteristic of increasing in number of working capillaries. Under the biological active substances, such as histamine, NO, PgI-2 and kinins small vessels, including capillaries, are dilated and an influx of arterial blood to the tissue or organ makes them scarlet or brightly red and warm. Dilation of the small vessels, acceleration of blood flow and increased tissue tension are very characteristic of inflammatory active hyperemia. Active hyperemia followed by congestion. Why? Very soon after start of cell injury lot of biological active substances are released and formed "de novo" (newly synthesized) in the site of injury. They, in turn, increase vessels permeability to the small proteins, especially in postcapillary venules and capillaries. As a result, there is an exudate formation in site of injury (exudate is an inflammatory fluid in contrast to the transudate-non-inflammatory one). Exudate, in turn, pushing on the vessels wall from outside creates the problems in venous blood circulation, and decreases the pressure gradient between arterial and venous areas of microcirculation. Small thrombi and destruction of connective fibers, which support the small veins, complete a slowdown of blood flow. Capillaries and veins are passively dilated and the organ, which is full of venous blood, becomes cyanotic. Naturally, the temperature of organ (not internal but skin) drops and organ becomes increased in size due to its swelling. Eventually, it comes to stasis signed as a full stop of microcirculation. It is irreversible capillary stasis. On the one hand, it can be explained by neuroparalysis of the blood vessels wall, but on the other hand, by the hemolysis of the erythrocytes and other blood elements death, both make the stop of microcirculation the irreversible.

But the most prominent feature of acute inflammation which can't be visible in the light microscope is increased permeability of the capillaries and postcapillary venules. Its value may be measured either by the number of the particles which can pass through the vessels wall for the certain time or the time, when

certain number of the particles can leak through. This phenomenon usually occurs in the small vessels, where the lack of smooth muscle weakens wall dense, and it sets when active hyperemia begins to transform into congestion. The intensity of the vessels leakage depends on severity of injury and its target tissue. Mostly, it is a result not only pathogen influence on the vessels wall but associated with biological active substances properties. There are three stages are known in high vessels permeability:

1. First, early stage is associated with release of histamine in course of mast cells degranulation provoked either by primary injuring factor or secondary. Secondary mast cell degranulation with histamine release associated with such biological active factors as anaphilatoxins (C3a and C5a activated complement fractions), different proteolytic enzymes, which appear in injured tissue later. It's short-termed period that usually lasts lesser than 30-40 minutes. Histamine initiates an appearance of the gaps between endothelial cells which in normal are cemented in vast majority of the capillaries. Acting through the special histamine H-1 receptors, it transforms the oval shapes of the endothelial cells into the round ones, that ultimately leads to appearance of the small spaces (gaps) between the endothelial cells. The matter of the fact, that stimulation of H1- receptors provokes accumulation of intracellular Ca ++ and then interaction of contractile structures inside the endothelium. As was said, this phenomenon is very short in time and permits only liquor and small proteins reversibly to pass through the small vessels wall. It is a start of inflammatory exudation. If we inject histamine in a volunteer intracutaneously we can observe so called Lewis triad of skin inflammatory symptoms: redness, swelling, and pain in form of itching. These symptoms last lesser than 30-40 minutes and imitate classical inflammatory signs. Histamine is the first vasoactive substance which dilates (via NO action) the microcirculatory vessels and increases their permeability. The matter of the fact, that histamine triggers the synthesizing of NO in endothelium and the last, in turn, acting to the nearby situated smooth muscle cells, provokes their relaxation and arterioles dilation

2. Second stage is known as the late and prolong because, not at once, but for a long time (hours and days) can support high vessels permeability and their dilation. It is mostly associated with bradykinin formation from the kininogens of plasma. In addition must be said that bradykinin is responsible for nociceptive receptors stimulation, and such way it provokes severe pain in the site of inflammation. It's a very strong algesiogenic substance.

3. Third stage is called postponed component of late response. It seems to be supported by PgE-2

Some words must be said about PgE-2. As it's known, it supports and prolongs an any symptom of inflammation, including redness and swelling due to prolongation of other mediators actions. .As for a pain, PgE2 sensitizes the nociceptive receptors to algesiogenic stimuli.

In serious injury of the blood vessels there is a desintegration of the basal membrane which accompanied by exfoliation of lamina propria from the endothelial cells. Moreover, may be destruction of collagen and elastic fibres, embedded

into a basal membrane. Very often, the mucopolysaccharides lose their charge and become more permeable. If not only endothelial cells, but a basal membrane is injured completely, there is no possibility for tissue to be restituted, and it usually results in a scar formation.

Increased blood vessels permeability is the most important factor of an exudate formation. But the other two factors of exudate appearance are very important too. They are the following: one of them is increased hydrostatic pressure in the microcirculatory vessels, but the other one is increased oncotic pressure in the surrounding connective tissue. There are several types of the exudates are known: serous, catharrhal, pyogenic, hemorrhagic, fibrinous, and purulent. Sometimes, it may be mixed. The type of exudate mostly depends on the nature of causative factor and site of inflammation.

The role of biological active substances in acute inflammation

First of all, it must be said that biological active substances (we call them the mediators of inflammation) play the role of conductors in inflammation and, as soon as they appear on the stage of inflammation scenery, all phenomena become are in progress, both the vessels and cellular ones. They possess by different structures, sources and mechanisms of action to the target cells. Besides, they may be in the preformed shape, for example, histamine in the granules of mast cells or basophils, or synthesized "de novo", such as the derivates of the arachidonic acid or kinins. They all are responsible for each event occurring in course of inflammatory process and reflect its symptoms.

The functions of inflammatory mediators

- act as vasoactive substances (increase small vessels permeability, modulate the diameter of these vessels, and number of acting capillaries)
- attract the phagocytes, accumulating them in site of inflammation
- kill and degrade the pathogens
- take part in reparation of the injured tissue

Histamine It's a biogenic amine, the product of aminoacid histidine decarboxilation. The main tissue storage of histamine is the mast cells of the connective tissue, but in the blood it is served in basophils. Any kind of injury, mechanical, chemical, radiation or some biological active substances and toxins, including bacterial toxins, can provoke mast cell degranulation and histamine release from their granules. In turn, via H-1 receptors histamine acts to the endothelial cells that provokes the fist short-termed phase of vessels permeability and vasodilation. Vasodilation isn't direct property of histamine, but is it mediated by NO synthesizing in the endothelial cells. It must be added, that only NO and PGI-2 are the real vasodilators, but other biological active substances act to the H-1 receptors to synthesize NO with it's following vasoactive effect of vasodilation. Histamine supports an axon reflex due to mast cells degranulation. Last may be triggered by substance P-releasing from nociceptive endings when they are

exitated by injuring factors. Moreover, histamine is a strong spasmogenic and algesiogenic substance, and it can provoke a pain, rather in form of itching.

Serotonin (5-hydroxytriptamine) It's biogenic amine too, but it is mostly elaborated by the platelates from aminoacid tryptophan. Its role in inflammation isn't such clear as a role of histamine, the universal mediator of injury , but serotonin increases the vessels permeability and provokes spasm of veinules, and such way, predisposes a tissue to edema formation . It's realized through the weakness of exflux of the blood from the site of inflammation that increases hydrostatic pressure in microcirculatory bed. Serotonin also can provoke pain.

Bradykinin By other words is slowly acting. It originates from activated during injury plasma proteins, but precisely when factor XII Hageman's is converted from XII into XII-a active form. Such conversion leads to activation of four plasma systems: clotting, fibrinolytic, complement and kallikrein-kinin system.

Mechanisms of bradykinin formation

Injury

↓

Activation of Hageman' factor

↓

Conversion of pre-kallikrein into kallikrein

↓

Splitting of alpha-2 plasma globulins (kininogens) up to bradykinin, of 9 chains polypeptide
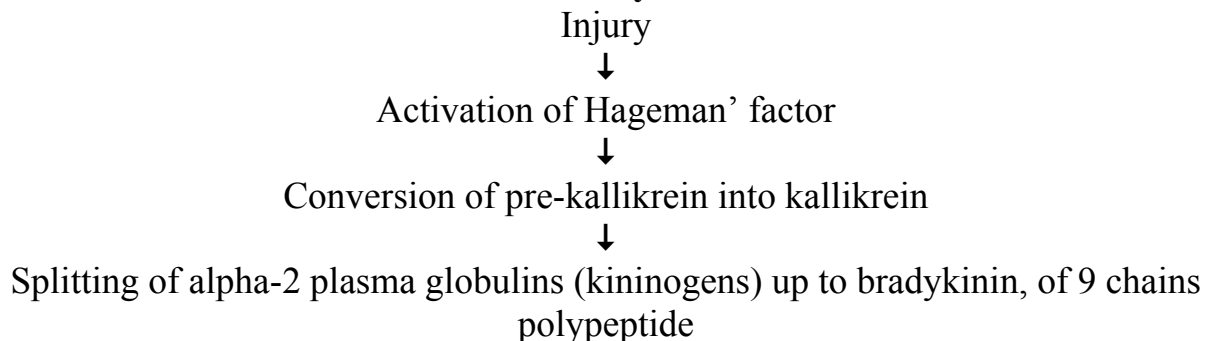
Bradykinin possesses by multiple mechanisms of action. It increases vessels permeability, provokes their dilation and a very prolong spasm of smooth muscle; also it is responsible for severe local pain.

Derivates of the phospholipids

When cell injury, different types of phospholipases, embedded into the cell and organell membranes are activated with corresponding consequences. So, phospholipase type C activation in mast cell membrane leads to mast cells degranulation with histamine release. At the same time, phospholipase A-2 of multiple cell membranes, especially of leukocytes and macrophages ones which are present in the site of inflammation in a big quantity, takes part in phospholipids degradation with arachidonic acid formation. Then, arachidonic acid under special enzymes is metabolized according to the two pathways: cyclooxigenase and lypooxigenase. But phospholipase D is responsible for synthesis of PAF from all membrane lipids (PAF –platelet activating factor).

PAF (platelet-activating factor

It has a very complex structure of phospholipids origin and many cells seem to be its source. PAF influences the course of inflammation, and its functions are very strong and multiple.

Role of PAF in an acute inflammation

- Increases the vessels permeability

- Provokes leukocyte aggregation and adhesion to the vessels wall through molecules adhesion expression on both the leukocytes and endothelium,
- Activates platelets that makes them more sticky to the vessels wall and active in the primary and secondary hemostasis,
- Plays role of chemattractant for the phagocytes,
- Stimulates release and formation not only inflammatory mediators, but synthesized "de novo" mediators of an acute phase response (IL-1, IL-6 and TNF) by the cells of inflammation

Derives of arachidonic acid

Prostaglandins and leukotriens belong to them. Metabolism of arachidonic acid, as was said above, may be realized through the two pathways: cyclooxygenase or lipooxygenase. It depends on the activity of the embedded into cell membrane corresponding enzymes. The start of the cyclooxygenase pathway of metabolism is initiated with PgG-series formation in many cells and, in turn, PgG is converted into PgH and then, there are particular conversions occur in different inflammatory cells. For example, various cells are capable for elaboration of PgE_2 and $PgF_{2\alpha}$ alpha, but the endothelial cells elaborate PgI_2, mast cells- PgD_2. At the same time, the platelets produce TXA-thromboxanes of different lines. It seems to be the supply with specialization of described cells in the site of injury.

The mechanism of PgE_2 line of prostaglandins action is the following: first of all, they support many inflammatory phenomena: vasodilation, high permeability of microcirculatory vessels and sensitize nociceptive endings to the algesiogenic factors, such as histamine, bradykinin, hyperkalemia, hyperacidemia, so as to P substance action. For this reason we use the NSAIDs (non-steroid anti-inflammatory drugs) for inflammation treatment and analgesia of other reasons, because they are the cycloxygenase blockers. Moreover, PgE_2 also has spasmolytic properties which can facilitate a pain too. The $PgF_{2\alpha}$ line possesses by opposite properties, and they are known as strong spasmogenic substances.

PgI_2(prostacycline), elaborating by the endothelial cells acts as real vasodilator. It also is the antagonist of TXA_2 and supports blood vessels thromboresistance. In opposite to PgI_2, TXA_2 is very thrombogenic and may provoke spasm of the small vessels. As was said, TXA_2 line is mostly produced by the platelets.

Leukotrien series are the products of arachidonic acid metabolism according to its lipooxigenase pathway. The process starts with unstable hydroperoxides formation- HETE (hydroperoxide eucosotetraen acid).Then leukotriens A series step by step are converted in B,C,D and E series. Some of them are very active in acute inflammatory response. So, LTB_4 is known as a strong polyvalent attractant which responsible for leukocyte emigration with tissue infiltrate formation, but complex CDE_4 named the " slow reacting substance" because provokes some postponed but prolong spasmogenic effect to smooth muscle .It increases vessels permeability and provokes bronchospasm in the patients with bronchial asthma.

The products of complement system activation

As was mentioned above, one of the activated plasma system which becomes the source of the inflammatory mediators is the complement system. Mostly, this system is activated according two pathways: classical and alternative. The trigger of the former is antigen- antibody complex but the latter can be activated by different enzymes from plasma, including XII Hageman's clotting factor, or leukocytes granules enzymes. The common pathway of complement system activation starts with C3a. The following active complement fractions are mostly involved in inflammatory process, acute and chronic, moreover. an immune or non-immune origin:

- C3b together with IgG and IgM are opsonins and assist in phagocytosis

-C3a and C5a are anaphylatoxins. C3a and C5a provoke mast cell degranulation and, hence, they are known as anaphylatoxins due to release of histamine from the mast cells which is the major mediator of anaphylaxis. They are strong chemattractants and may express the adhesion molecules on both leukocytes and endothelial cell.

- auto-assembling on different cell membranes macromolecular complexes consists of C5b6789 fractions; it is responsible for cell death due to appearance under them of multiple wholes in the cell membranes as a result of membrane drilling with next osmotic cell lyzing.

Lecture 4

Cellular events in an acute inflammation

Last time we discussed the problem of alteration in microcirculation in the site of injury as a start of inflammation. Then we discussed the role of some biological active substances that usually called the conductors of the process. It was the first stage of inflammation plot. Now we are going on our conversation and discussion on the problem of next cellular events.

When active hyperemia turns into congestion in light microscope we can observe the rolling of the leukocytes, rather along the capillary and postcapillary wall. Initially they come over the vessels wall only for a short time and then are carried away by the blood stream, but then they become more stick and fixed to the vessels wall. At that moment the leukocytes "try to catch something" on the vessels wall but can't stay nearby longer. Later, they acquire an ability to be more stick and to cover the vessels wall in pavement manner. At last, they are ready to leave a blood stream for surrounding tissue where they must fight against any pathogen.

The chain of the events touching on the leukocyte behavior at that moment may be presented in such consequence:

margination—rolling—activation—adhesion—transmigration

Mechanisms of leukocyte adhesion

In leukocyte adhesion both leukocytes and vessels wall are involved. Such substances as microbial polysaccharides and some mediators of inflammation, but later the mediators of an acute phase response are capable to express the adhesion molecules both on the leukocytes and endothelium membranes. As for inflammatory mediators, the following must be called: LTB-4, PAF, C3a and C5a-anaphylatoxins. At the same time, IL-1 and TNF-alpha also become responsible for these phenomena but some later, when the macrophages appear on the "field of the battle".

There are three classes of adhesion molecules are known: selectins, integrins and superfamily of the globulins. Some selectins are expressed on the endothelial cells, they named P-selectins or Weible-Paladies bodies. The lasts exist in pre-formed shape inside the cells in special vesicles, and after stimulation by histamine or thrombin such selectins immediately appear on the surface of the endothelial cells. Next form are the E-selectins, and they both, (E- and P- selectins) are responsible only for the rolling of leukocytes along the vessels wall, but not a firm adhesion which must precede to leukocyte transmigration. At the same time, L-selectins are expressed on the leukocyte membrane, and they are responsile only for the rolling of the leukocytes.like endothelial selectins. More firm adhesion needs elaboration of beta-integrins by the leukocytes and ICAM1 and ICAM-2 molecules by the endothelial cells with their expression on the corresponding cell membrane. Needless to say, that they are producing later, and their appearance precedes a leukocyte transmigration. Both are synthesized "de novo".

The common features of leukocyte emigration

This phenomenon was described by Russian microbiologist Ilya Mechnikov, the Noble laureate at the beginning of the last century. He shared his Noble prize with German scientist Paul Erlich for the discovery in immunology. Mechnikov created cellular theory of immunity describing the phagocytosis. At the same time, Erlich insisted on priority of the humoral factors (antibodies) in immunity.

The law of Mechnikov sounds so:

1. Firstly, polynuclear phagocytes emigrate, mostly neutrophils and then mononuclear forms-monocytes.

2. There are three steps in leukocyte emigration: margination and adhesion, transmigration, and residence of the leukocytes in tissue.

What is the mechanism of different types of leukocytes emigration? It's possible to observe it only in electron microscope, but rolling and adhesion might be seen in light microscope too. You'll have this possibility in practice (experiment of Congheim).

The fact, that adhesion molecules are very important for the first step of emigration must be proved by the inhibition of leukocyte emigration when get used the antibodies to adhesion molecules. These monoclonal antibodies against the adhesion molecules retard an acute inflammation and, such way, can predispose an or-

ganism to chronic inflammation. There are some hereditary deficiencies in their synthesizing. The adhesion molecules deficiencies named DAL-1 and DAL-2 (defect adhesion of leukocytes). DAL-1- corresponds to deficiency of b-chains of integrins, but when DAL-2- results to the deficiency in the receptors to L-selectins on the leukocytes.

Through the vessels wall neutrophil moves by extending its pseudopods that pull the remainder of the cell in the direction of extension likes automobile with its wheels. Locomotion is a genetic property of the leukocyte. It was estimated that neutrophil can't be in the rest even in normal and persists in form of "chaotic dance" I when it throws out the pseudopods. But emigrated leukocyte has difficulties in navigation problem to arrive to correct extravascular location, and both chemattractants and their receptors on the leukocyte membrane are engaged in this moving. Chemokins or chemattractants in step by step manner call the leukocytes in the site of their high concentration via stimulation leukocyte locomotion. At first, the neutrophil enters the gap between the endothelial cells and then, being activated before by the chemattractants, releases some hydrolyzes on the basal membranes. These are the elastase, collagenase, depolarizing the mucopolysaccharides of the vessels walls. The proteolytic enzymes make vessels wall more permeable, and such way, facilitate a leakage of the leukocytes through the small vessels. If you try to observe the vessels wall in electron microscope before and after leukocyte passage, you hardly ever find any defect in the vessels wall. Monocytes can emigrate by the same way or by pinocytosis using their glycolytic reserve. Obviously, the antagonists of glycolysis can arrest a pinocytosis due to creating the lack of energy for monocyte active transport. In contrast to the neutrophils and monocytes, the lymphocytes emigrate only in small venules with special tall type of endothelium. Such endothelial cells possess by so called "homming" receptors that are necessary for lymphocyte transmigration. It must be added, that in opposite to the phagocytes, which have got "one way ticket " wandering only from the blood to the interstitium, lymphocytes possess by so called " ticket return" and can traverse the vessel there and back. Being the first in emigration, neutrophils predominate in the tissue and infiltrate it during first 6-24 hours but then, on 2-4-day they partially are replaced by the monocytes. Later the lasts are transformed into inflammatory tissue macrophages. In this conversion they change not only the size, becoming lager, but the shape of the nucleus. The macrophages have a long- standing course in the tissue and dominate as the cellular unit in the site of chronic inflammation.

Phagocytosis

The steps of phagocytosis

1st.step. Recognition and attachment of the foreign particles to be ingested by the leukocyte

2nd.Its engulfment with subsequent formation of the phagocytic vacuole
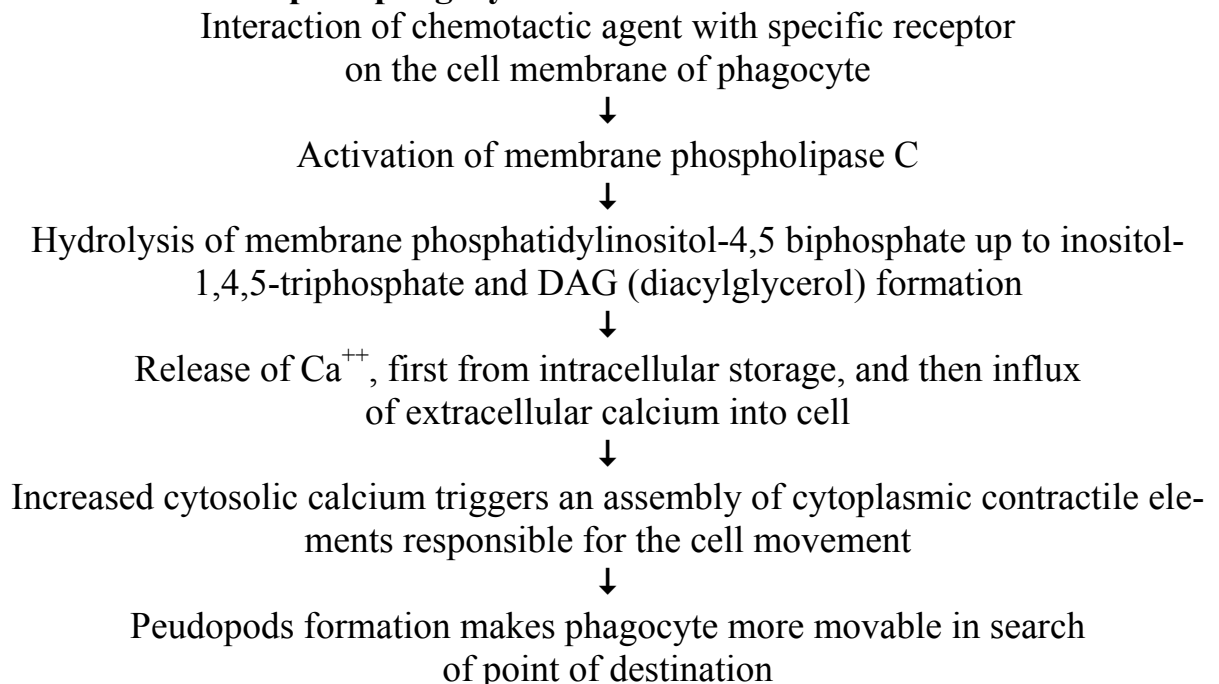
3rd. Killing and degradation of the ingested material

Recognition

The moving of the leukocyte toward an injured tissue named chemotaxis. Its locomotion activity may be explained by positive chemical gradient of biological active substances named the chemattractantts. Both exogenous and endogenous substances can act as chemattractants. Mostly, exogenous agents are the bacterial products; lipopolysaccharides and proteins. Endogenous chemical mediators include:

- Activated complement system components, especially C5a and C3a - anaphylatoxins
- Products of lipooxygenase pathway metabolism, mainly LTB-4
- PAF
- Interleukin 1 and TNF-alpha as the late chemattractants
- All listed above substances are polyvalent chemattractants because they attract all types of obligatory phagocytes, including the neutrophils, eosinophils, monocytes and macrophages
- As for neutrophils, IL-8 and neutrophil chemotactic factors, released from the mast cells, are the special or monovalent ones. Histamine, eosinophil chemotactic factor from the mast cells and eotaxin from endothelium are the monovalent chemattractants for the eosinophils. Special monocytic chemotactic factor is working for the monocytes, but lymphotaxin for lymphocytes. These substances let phagocyte to "see or smell" a pathogen.

The steps of phagocyte movement in certain direction
Interaction of chemotactic agent with specific receptor
on the cell membrane of phagocyte
↓
Activation of membrane phospholipase C
↓
Hydrolysis of membrane phosphatidylinositol-4,5 biphosphate up to inositol-1,4,5-triphosphate and DAG (diacylglycerol) formation
↓
Release of Ca^{++}, first from intracellular storage, and then influx
of extracellular calcium into cell
↓
Increased cytosolic calcium triggers an assembly of cytoplasmic contractile elements responsible for the cell movement
↓
Peudopods formation makes phagocyte more movable in search
of point of destination

Attachment of the particles to be ingested by the leukocyte
It's better if the opsonins are present. The role of opsonins fulfil such substances as activated C3b component of complement system, IgGs, IgMs, and C-reactive plasma protein. Via specific receptors on the phagocytes these substances opsonize them acting like a clay, gathering the foreign particles on the surface of

phagocyte. For this reason, in the immunized organisms which reach of the immunoglobulins, phagocytosis is more active and, moreover, the treatment with specific serum immunoglobulins is strongly recommended in case of a pyogenic infection, especially in children.

Ingulfment

A pseudopod flows around (hugs) the object to be engulfed and enclosed completely a particle within a phagosome created by a phagocyte membrane. Then the lysosomes become fused with limited wall of phagosome, resulting in release of granules content with following formation of phagolysosome. Degranulation of activated inflammatory neutrophils, monocytes and other obliged phagocytes leads to digestion of foreign particles, and very often to death of the phagocytes. Many of them are found in the pus (special purulent exudate consisted of died leukocytes and tissue debris).

Killing and degradation

Killing may be realized by two pathways: oxygen dependent and oxygen non-dependent. Phagocytes are not the "cannibals", and before digestion of alive particles the lasts must be killed.

The steps of oxygen dependent killing proceeding in obligate phagocytes

1. Chemattractants initiate the respiratory burst of a leukocyte, when it uptakes greedy an oxygen. It must be noted that in normal, not being activated, any leukocyte supports its energetic needs by the glycogenolysis with participation of hexosomonophosphate shunt.
2. After phagosome is arranged, the external membrane of the leukocyte contacts with cell cytoplasm due to cytoplasm invagination. That time, two components of NADPH- system (cytoplasm and membrane) meet each other, and it results in activation of NADPH -ase.
3. Activated NADPH-ase, in turn, transfers a single electron on an oxygen molecule with following superoxide radical formation.
4. Then part of the superoxide dismutases with such end product as hydrogen peroxide formation
5. Eventually, in Fentone's reaction when transient metals iron and cooper are involved, hydroxil radical OH' is formed.
6. Reduction of NADP up to NADPH is realized through hexoso- mono-phosphate shunt activity

Described above reactions, result in the three primary radicals or active oxygen species formation. They possess by very strong bactericidal properties. These reactions are going in any type of the obliged phagocytes, but the granulocytes possess by stronger weapon in their fighting against a pathogen. It is myeloperoxidase system.

The granulocytes: neutrophils and eosinophils, except the primary oxidants, produce the secondary oxidants, because they possess by the enzyme myeloperoxidase in their azurophilic granules. It's their marker, and yet in the bone marrow,

they get it during maturation. As was said, granulocytes possess by myeloperoxidase system. It consists of enzyme myeloperoxidase, hydrogen peroxide and halids (chlorides and bromines); the system doesn't possess by specify, acting mortally to any pathogen. Assembly of hydrogen peroxide, myeloperoxidase and chlorides results in hypochloric anion (OCL-) and hypochloric acid (HOCL-) formation; those in further interact with the aminogroups of proteins and form chloramines, very strong oxidants.

Hydroperoxide-myeloperoxidase-halide system is not specific of organism, but very effective in the struggle against such pathogens as worms, protozoa, fungi, and cells infected by the viruses.

Oxygen non-dependent mechanisms of killing

It includes both, enzymatic and non-enzymatic factors containing in neutrophil granules: cationic protein, granzymes, BIP-factor (bacterial permeability increasing protein), besides, the lysozomal content and lactic acid in their vacuoles. Major basic protein in eosinophil granules is the best bactericidal weapon in the fight against the worms and protozoa . Some substances, releasing from neutrophil granules, possess by bacteriostatic properties. That is non- less important as the protective mechanisms due to retarding the growth of animate pathogens. So, lactoferrin, binding iron, inhibits bacterial respiration, but protein, binding vitamin B12, interferes with proper bacterial growth. And, eventually, the leukocyte hydrolyses complete a process of foreign bodies destruction by lysing of dead particles; the lasts are presented by the tissue debris and dead phagocytes.

As for phagocytosis, it may be non-completed when bacteria or other particles remained inside the phagocytic vacuole as the residents, for example, meningococci or gonococci. It's a very danger for an organism, because one day the weakness of itsr immunity may result in dissemination of infection all over the organism. The other variant is interrupted phagocytosis when the object is too large to be ingested by phagocyte. In this case the phagocyte acts as a "valiant", attacking the enemy by releasing of the oxygen species and enzymes via bombarding an object. This fighting may injury not only a pathogen but normal surrounding tissue too. Such kind of injury may be observed in chronic bacterial or immune inflammation: allergic dermatitis, psoriasis, and skin lesions in lupus erythematosus. For this reason the drugs, including the antioxidants, may become very useful in case of these diseases treatment.

Defects in leukocyte function

Leukocytes play a cardinal role in a host defense against any pathogen. Defects in leukocyte functions, both genetic and acquired ultimately lead to increased vulnerability at face of infection. To sum up, the defects include both inherited and acquired variants.
 1. Defects of leukocyte adhesion,
 2. Defects of phagocytosis.

The formers inherited were mentioned above, but the example of the latter is Chediak-Higashi syndrome. This is autosomal recessive condition characterized by neutropenia, defective granulocyte degranulation and delayed microbial killing. In this syndrome the granules of neutrophils are very huge due to aberrant organelles fusion, moreover, the transport of lysosomal enzymes to phagocytic vacuoles is slowdown. The next is chronic granulomatous disease when defective synthesizing of several components of NADP-oxidase which is necessary for superoxide generation.

Acquired deficiency may be represented by myeloid leukemia when the blood of patient is fool of immature granulocytes with a very weak myeloperoxidase activity. The matter of the fact, that the more mature granulocytes, the more the cells which rich of the myeloperoxidase activity. Besides, there is a low adhesion properties of both, leukocytes and endothelium are in the patients suffering of diabetes mellitus and chronic kidney insufficiency, so as, thermal injury and malnutrition.

Role of different cellular elements in acute inflammation
Mast cells and basophils

They are the main source of histamine and together form so called mastocyto-basophil system. During acute inflammation mast cells completely disappear of the site of injury, but a repair of tissue is manifested by the renewal of their population. There are two variants of mast cell degranulation: primary and secondary. The primary is under injuring agent but secondary is provoked by the mediators of acute inflammation, such as anaphylatoxins, PAF, cationic proteins of the neutrophils, oxidants, and different proteolytic activities released from the variety of cells involved in injury. Besides histamine, heparin and other mucopolysaccharides, possessing by opposite anti -inflammatory properties, are present in mast cell granules. These cells, also content chymase and tryptase activities and the factors for neutrophil and eosinophil chemotaxis (NCF-chemotactic factor and ECF- eosinophl chemotactic factors). They provide with these cell-recruits an infiltration of tissue especially if they are of allergic or parasitic origin. Moreover, mast cells can be a source of such mediators as the eucosanoids, PAF and, at last, produce the mediators of an acute phase response. It mast be added, that the mast cells of connective tissue possess by the lot of receptors to Fc- fragments of IgE. High level of serum IgE, which characteristic of immune status of patient with allergy, contributes an interaction of antigen with fixed on the mast cells surface IgEs, that resulting in mast cells degranulation and flooding of tissue with histamine.

Eosinophils

In a large quantity they are found in the tissue or exudates in course of allergy, parasitic disease, especially, in the patient with ascariasis. Histamine is the most important factor for their attraction to the site of inflammation. There are the lot of the receptors to the Fc-fragment of IgE also are situated on eosinophil membrane. IgE-antibodies in very high titer revealed in the patients with described pathology. Antigen- antibody reaction on the eosinophil membrane may result in

their degranulation with release of their content. It includes the oxidants, main basic protein and such enzymes as histaminase and arylsulfotase. The lasts take part in inactivation correspondingly histamine and leukotriens. So, degranulation of the eosinophils has a double meaning. On the on hand, it leads to an injury of the pathogen, but the surrounding tissue may suffer too (positive and negative components of inflammatory response) but, on the other hand, it protects tissue from flooding of the site of inflammaton with an excess of the proinflammatory mediators (positive effect).

Macrophages

If the neutrophils are seemed to be the cells of the first line of defense in inflammation, the macrophages provide the second line. Why? Neutrophils appear first on the stage of inflammatory "performance" because they are very sensible to chemattractants and mobile, moreover, their life span is not longer than 1-3 days, and the population is replenished by hectic bone morrow granulocytopoiesis. They are characterized by early emigration to the tissue, but if the wound isn't infected in 2-3 days the macrophages appear on the "field of the buttle".

Then the macrophages turn into the inflammatory macrophages, very active in their function in compare with "quiet" tissue macrophages. In the site of inflammation they tend to be fused with giant cell formation.

The functions of macrophages in the site of inflammation

1. They are the obliged phagocytes and, like neutrophils, can elaborate free radicals, but in form of only primary oxidants; release hydrolytic enzymes and ingest the tissue unwilling elements with their following killing and degradation
2. They called scavengers because of anability the tissue cleaning of the debris. Produce collagenase, elastase and plasminogen activator providing the lthrombi lysis and reconstitution of microcirculation in the site of inflammation
3. Take part in the rebuilding of the connective tissue, producing angiogenetic factor of small vessels renewal, FGF (fibroblast growth factor) and fibronectin. Last plays role the railway for the replenish of tissue with mast cells population via moving them from the blood to the connective tissue
4. Fulfill an antigen-presenting function. Wearing on their surface MHC class II molecules, they present the information about the "non-self" to the lymphocytes, and such way, realize the connection between inflammation and immunity
5. Activated macrophages elaborate and produce the mediators of an acute phase response. Lasts are very important for connection between the site of injury and whole organism
6. They take part in so called granulomatous inflammaton that is a variant of chronic inflammation, for example, intuberculosis, syphilis, leprosis, or brucellosis.

The outcomes of inflammation

In optimal condition when action of the causative factors is withdrawn and unfavorable consequences stop to act, we usually say about complete recovery. Which events that time occur in the site of inflammation?
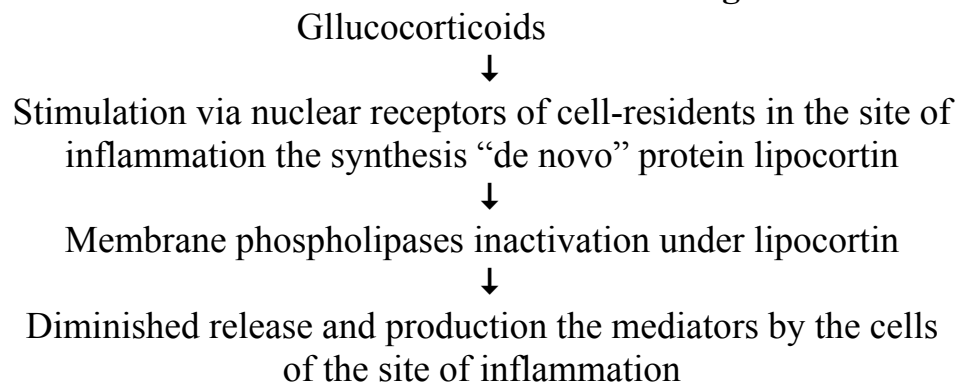
1. Decline in mediators formation

2. Reparation of the blood vessels permeability
3. Cut off a leukocyte emigration
4. Elimination of exudate consisting of the proteins and cell debris

The mediators with the rest of destroyed tissue and blood elements are drainaged via the newly organized blood and lymphatic vessels, however, only when vessels basal membrane is served, complete reconstruction of the tissue is possible.

Some principles of the acute inflammation therapy

Because an inflammation very often is associated with serious negative complications it needs the anti-mediators therapy. For this reason, in case of allergic inflammation antihistamine therapy is shown. Also, in any case of inflammation the treatment with NSAIDs which block cyclooxigenase and decrease prostaglandins formation is recommended. But the most effective are the glucocorticoids . The mechanism of treatment with glucocorticoids may be presented by the following step by step developing events:

Mechanisms of inflammation treatment with glucocorticoids

Gllucocorticoids

↓

Stimulation via nuclear receptors of cell-residents in the site of inflammation the synthesis "de novo" protein lipocortin

↓

Membrane phospholipases inactivation under lipocortin

↓

Diminished release and production the mediators by the cells of the site of inflammation

Besides, glucocorticoids inhibit the proliferation of connective tissue and immune responses of an organism. As known, the glucocorticoids diminish cortisol-dependent T-lymphocyte cell population and decrease the small vessels permeability which, in turn, creates the obstacles in lymphocytes function and their recirculation. These facts can be used to explanation a positive influence of glucocorticoids the chronic excessive proliferative inflammation, so as, the immune diseases outstanding and development. For this reason, we call glucocorticoids therapy as "not a therapy of our choic but rather therapy of despair", that means, in a very problematical case only glucocorticoids but, not other drugs, may stop severe inflammation of different reason.

Lecture 5
ACUTE PHASE RESPONSE
In complex organisms the response to injury includes both local and systemic reactions. As for the local ones, it is an inflammation but systemic reaction called

an acute phase response (APR) .First of all, APR is represented by the fever, and seems to be the higher rise in temperature the more active pathologic process.

Definition: APR is a non-specific reaction of whole organism to severe injury with activation of four life-important systems: nervous, endocrine, hematopoietic, and immune system. To this characteristic must be added destructive catabolic processes in form of proteolysis and lipolysis. Special role in APR belongs to changed metabolism in the liver, when the last actively synthesizes so called acute phase response proteins detriment to other proteins, for example the albumens and transferrin.

APR is initiated by the action of various stimuli, infectious and noninfectious, exogenous or endogenous injuring factors. So, infection, severe trauma, including surgery, burns, x-ray disease, tumors, and autoimmune disease may become a cause of APR. But the clinical signs of APR depend a little on the provoking it factor. Why? The matter of the fact, that all kinds of injury result in synthesis by the cells of injured organism the same mediators of acute phase response. They act locally and for a distance when are spreading via the blood all over organism, but they are the same, and have much in common in their activity. For this reason, despite the diversion in initial factors provoking pathological process, we can find the similar, non-specific responses of an organism in form of clinical symptoms and laboratory data in its course, and fever is a good evidence of them. We are known, the higher rise in temperature (fever), the more severe injury.

An acute phase response mediators control a disease in whole, like inflammatory mediators control local inflammatory process. You are known the mediators of inflammation, but in case of APR the most important, moreover, pivotal factors, playing the role of mediators are the following synthesized "de novo" cytokines: IL-1, TNF and IL- 6.

Biological significance of APR

Being the "alarm" substances, they make an organism capable to build a defense against the pathogen. It is doubtless, the biological significance of APR is a protection of an organism against the pathogen via mobilizing of the most important for our life systems. However, this response may create the lot of problems for own organism, because the high concentration of the mediators can provoke overwhelming destructions on any area of our organism with serious further complications in form of organopathology (heart, liver, or kidney insufficiency). By other words, we must remember that APR carries a double sense, positive and negative.

The connection between inflammation and APR

There is no doubt that local injury stimulates attraction of blood cells in the site of injury, and they all together with cells- tissue residents become the sources not only inflammatory but APR mediators too. Contact of the cells with injuring factors and some biological active substances, that are in excess in site of injury, ultimately results in "de novo" synthesizing by these cells of APR mediators.

The systems involved in APR with their responses

Systems	Responses
CNS	Fever, loss of appetite and weight, drowsiness, depression
Endocrine	High ACTH and cortisol production
Hematopoietic	Neutrophil leukocytosis with regenerative left shift
Immune	Activation of T-and B-lymphocytes

Characteristics of the mediators of an acute phase response
IL-I

Among them, IL-1 was discovered by the first, and for its ability to initiate a fever was named endogenous pyrogen. Il-1 is produced by all nucleus-containing cells, but mainly, by the macrophages and tumor cells. Its production is very high by the cells of monoblastic leukemia. Also IL-I was named "interleukin" for its ability of lymphocyte activation.

IL-1 characteristic features:

- isn't preformed mediator and synthesized "de novo" by the cells, predominately after injury
- M-17,5 KD
- produced in two forms: IL-1 alpha and IL-1 beta
- IL-1 receptors- antagonist produced by the liver found in the blood
- Both forms of IL-1 have got the similar receptors on the surface of different cells, and for this reason they possess by the same effects
- They are the products of the different genes on the chromosome 2
- IL-1 alpha is associated with the cell membrane, but beta-form circulates in the blood and can pass through hemocranial barrier in certain regions of the brain
- In normal the only traces of IL1 can be revealed in the blood. Its level in normal may arise during ovulation or severe physical exercises

Which factors can stimulate an elaboration of IL-1? These are the microorganisms and their products such as mucopolysaccharides, or polysaccharides of artificial pyrogens. In addition, as initiators of its synthesis, must be called such biological active substances as leukotriens, FAT and other mediators of inflammation, also TNF and IL-6 which as its partners in APR. Moreover, urates and phosphates in the kidney and joints, bile salts on the peritoneum may be responsible for its synthesis too. Some substances, such as PgE-2 in high concentration, histamine and glucocorticoids inhibit an action of IL-1 to the target cells. In opposite, IL-I itself, stimulates an elaboration of PGE-2 by such cells as brain neurons, fibroblasts, bones, and strip muscle cells.

Biological effects of IL-1 to the target cells
Local effects of IL-1

- Macrophages: stimulation of chemotaxis and phagocytosis, superoxide, TNF and IL-6 production and expression of MHC class II on the macrophages surface
- Neutrophils: activates chemotaxis, phagocytosis, superoxides production, enhances adhesive properties of endothelium and forces leukocyte emigration; stimulates of neutrophil degranulation
- T- lymphocytes: increase synthesis of IL-2 and the receptors to IL-2 to their mitogen activity
- B- lymphocytes; amplifies (makes effort) IL-2 synthesis and expression of its receptors on the cell surface , so as IL-4 and MHC-class II. Increases proliferation of pre-activated B-cells
- NK+ cells: makes effort cell cytotoxicity, increases IF-gamma and IL-2 production by the cells
- Fibroblasts: activates prolferation, induction of IL-6, interferon gamma, CSF (colony stimulating factors), and PgE-2 secretion
- Mast cells and basophils: increases histamine release
- Endothelium:: stimulation of proliferation, increases adhesion and thrombogenic potential, and secretion of NO

Distant effects of IL-1

- Bone marrow: stimulation of polypotent cells proliferation and acts as early cytoclone
- Bone and cartilage: stimulation of collagenase activity, osteoclasts and PGE-2 production, destruction of the bones and cartilage lysis. Enhances proliferation of sinovial fibroblasts and chondrocytes
- Strip muscles:proteolysis, loss of water and increases prostaglandins synthesis
- Liver: increases synthesis of acute phase response proteins via IL-6 synthesis
- CNS: via increased PgE-2 content in the brain provokes fever and inhibits the centers of appetite that leads to serious loss of weight. Destructive processes in the bones and wasting strip muscles may to be inserted in the list of weight loss causes

Clinical features of IL-1 influences an organism

Fever has a double meaning, positive and negative. Positive sites may be explained by bacteriostatic influenses due to an activation of blood circulation, metabolism, and increasing in desintoxication function of the liver and kidneys. Negative ones, in form of the cramps in little children and heat shock; serious disturbances in cardiovascular system in old people

Positive influences an organism of IL-1

- Being an endogeneous pyrogen, stimulates activity of cardiovascular system, and fever is very destructive to the microorganism
- Activation both specific and non-specific immunity via stimulation of the neutrophils function, macrophages and lymphocytes

- Via colony stimulation factors replenishes the obligate phagocyte population
- Via IL-6 synthesis, increases synthesis of acute phase response proteins in the liver that play role of anti-proteinases that restrict proteolytic activity of the phagocytes.
- Stimulating hemostasis, makes up the obstacles to spreading of any pathogen all over an organism. It also takes part in the recovery of tissue after injury.

The following consequences seem to be negative:
- Disturbances in a sleep, up to lethargy, non-adequate behavior, myalgia, pain in the joints and severe headache, loss of appetite up to cachexia, and other gastrointestinal complications. Sometimes, destruction of the cells of incretory pancreas may occur
- Very special negative role IL-1 plays in endotoxin shock .It provokes severe hypotension due to direct influence the vessels smooth muscle, besides it stimulates elaboration of NO by the endothelial cells. Last substance relaxes the smooth muscle of resistive vessels, that can result in acute hypotension
- High concentration of IL-1 and PGE-2 is revealed in synovial fluid of the patients with rhematoid arthritis. The matter of the fact, that IL-1 stimulates elaboration of PGE by some cells which, in turn, may destroy the bones with their consequent remodeling. The cells of the joints also produce some phospholipases under IL-1. From here, becomes understood the using of cyclooxigenase blockers and glucocorticoids in the treatment for different types of pathologic conditions in the joints (inflammation, arthrosis)
- The blood level IL-1 arises in gastrointestinal disease and food poisoning, Crone's disease, acute and chronic leukemia, but especially significant its increasing is found in monoblastic leukemia
- IL-1 plays the role of growth factor for smooth muscle of the vessels in atherosclerosis. A large quantity of IL-1 is produced by the foam cells forming the atherosclerotic patches

Therapy of IL-1 dependent conditions

Which way IL-1 can be neutralized in clinic to avoid of serious disturbances in hemodynamic in case of septic shock? Theoretically, using the antibodies to the IL-1.But these antibodies may do the harm to an organism, and nowadays, the receptors to IL-1 are cloned. They are the recombinant proteins obtained by the method of gene engineering, which doesn't provoke any unfavorable complications in form of disturbances in immunity, moreover, these receptors don't possess by specious reactivity.

They usually injected intravenously and catch solved form of IL-beta in the blood. This method of septic shock treatment diminishes the level of patients mortality from 100 to 60%.

Tumor necrosis factor (TNF)

There are two forms of TNF: TNF-alpha or cachectin and TNF-beta or lymphotoxin. The former mainly produced by macrophages, but the latter- by the lymphocytes. TNF-beta was named so for its ability to provoke significant loss of weight up to chachexia (a very strong loss of weight and physical exhausting of the patient). How can be explained this name TNF? As was estimated in the experiments, TNF can lyse a tumor, and clinical observations get us an evidence of spontaneous disappearance of tumors in some patients. The history of its discovery backs in time to the 3d decade of last century when English doctor William Colin described the regression of the stomach tumor in the patient with tuberculosis. Evidently, it wasn't direct effect of bacteria to the tumor, but rather, bacteria of tuberculosis provoked an APR with high level of the TNF in the blood of that patient .If the mice with transplanted tumor are treated with BCG-vaccine the tumor is lysed too, due to TNF elaboration in their organisms, as a response to the BCG treatment.

The genes which are responsible for the production of both form of TNF are carted on the same chromosome 6. molecular weight of TNF-alpha is about 17 kD. It is not so large protein consists of 157 aminoacids. TNF has a key role in such pathological conditions as inflammation and immunopathology. Multiple effects of TNF outset when on the "field of the battle" appear the macrophages. On the one hand, TNF possesses by many properties of the IL-1 but, on the other hand, local effects of IL are 1000 times stronger than the same of TNf, but must be added that TNF enhances the effect of IL-1.

However, they both have much in common:

- Stimulate adhesion molecules expression on the leukocytes, endothelial cells and chemotaxis of the leukocytes
- Provoke elaboration of the oxidants in the leukocytes
- Make lymphocytes to produce such growth factor for lymphocytes as IL-2
- Enhance production of lymphotoxin by the lymphocytes, stimulate production of IF-gamma by the macrophages, and antibodies by B-lymphocytes
- Stimulate many cells of the site of inflammation (macrophages, endothelium and fibroblasts) to produce G- and GM- CSF (granulocytic an granulocytic-monocytic colony-stimulating factors).
- They both are responsible for such symptom as fever and, (not directly but via IL-6),
- stimulate production of the acute phase response proteins by the liver.

The difference between TNF and IL-1

TNF is synthesized by the lesser number of the cells, and first of all, by the macrophages and leukocytes. Moreover, it has more cytotoxic effect, increases HLA II and I classes of molecules on the corresponding cells and, such way, provides better immune recognition of the foreign material, mostly, the neoplastic cells. Can't activate T-cells directly but possesses by antiviral effect "in vitro".

Increased blood level of TNF-alpha is revealed in the following normal and pathological conditions

- Pregnancy
- Malaria and leushmaniosis
- In 50% of the patients with malignant tumors
- Meningitis
- Transplant rejection,
- In restitution of the hematopoiesis after radiation

Positive and negative features of TNF

In the experiments on the mice a large dose of TNF provokes their shock and death. At the same time, low doses of TNF save the animals of lethal doses of X-ray and parasiic infection. Treatment of the volunteers with TNF provokes a fever shivering, sometimes, lethargy and drop of AP, moreover, all symptoms of severe flu. Very high concentration of it is revealed in the patients with severe course of meningitis and in the patients with malignant tumors.

As was said, one of the most pathogenic effects of TNF in high concentration is cachexia. Last may be observed in the patients with tuberculosis, cancer, severe autoimmune disease. How it might be explained? One of the possible mechanisms of cachexia is inhibition by TNF-alpha the lipoproteinlipase activity, arranging in normal the lipid stores in a fat tissue. Another one explanation is associated with an inhibition of the centers of appetite in the hypothalamus. The third mechanism is connected to myolysis and loss of water by the muscles. Some properties of TNF protect an organism against malignant tumor ;

Anti-tumor effects of TNF may be explained by the next its properties
- Activation of the lymphocytes and, as a result, stimulation of organism's immunity
- Providing necrosis of the vessels supplying tumor
- Inhibition of broken DNA reparation of tumor cells after their injury
- Stimulation of terminal differentiation in the neoplastic cells facilitating their immune recognition by antigen –presenting cells

Interleukin 6

It is the next cytokine of an acute phase response mediators family. IL-6 mostly takes part in immune response regulation, hematopoiesis and inflammation. The main sources of the mediator are the following cells: activated endothelium, macrophages, fibroblasts, and lymphocytes. IL-6 was discovered in the year 1989 and has molecular weight 26 kD. Like previous cytokines, it is a newly synthesized substance. The following factors mostly stimulate its formation: viruses, bacteria, their products, and such cytokines as IL-1 and TNF, platelet growth factor and many others, but each through the cell genome activation. The lot of receptors to IL-6 are found in the liver, additionally must be said that cortisol manages IL-6 action in the liver to synthesis of the acute phase response proteins, but opposite PgE2 and histamine it inhibit. Synthesis of acute phase response proteins in the liver is a very prominent feature of IL-6.

The main effects or IL-6

- Responsible for end-staged differentiation of T- and B-lymphocytes,
- Possesses by strong antiviral properties via expression of MHC class I on the surface of cells invaded by the viruses; the last makes them more recognizable by the immune system
- Strong mitogen for actively proliferating cells, including the granulocytes and monocytes
- Strong mitogen for the tumor cells, and for this reason it is found in a large quantity in some growing malignant tumors, for example, plasmacytoma, osteosarcoma, glioblastoma, and carcinoma of the bladder
- Early marker of disease severity, because its level in the blood increases too early, in
- 4- 6 hours after start of a disease
- Aggravates inflammation of the bones due to osteoclasts activation and destruction of bones, but its major effect is the acute phase response proteins synthesis in the liver
- (80% of IL-6 receptors are in the liver), moreover, the complex of IL-6 with its receptor enhances its possibility to amplificate the effect of IL-6 on the target cells

Acute phase response proteins

Synthesis of these proteins, conducting by IL-6 in the liver, as was said, is cortisol-dependent process. Acute phase response, accompanying by CNS activation, evokes increase the ACTH production by pituitary gland and, in turn, cortisol secretion by the adrenal cortex. One of the most important cortisol effects is a proteins synthesis in the liver, when their destruction on the periphery and driving the aminoacids into the liver.

On the one hand, all these proteins according to concentration in the blood, may be divided into positive reactants with increased blood concentration and negative ones, if their blood content is diminished. It mast be said, that only transferrin and albumens belong to negative reactants.

Some of these proteins are capable to aggravate the effects of APR, for example, C-reactive protein or activated complement fractions, but the other ones can restrict them. The bright examples of such forms of limitation are anti-proteinases and fibrin effects.

Acute phase response proteins and their effects

Acute phase response	Effects
C-reactive protein	Activator of complement system and acting as opsonin
Serum amyloid A	Possesses by anti-toxic function and takes part in pathogen clearance

They are practically absent in the blood in normal but their concentration in the blood during acute phase response is increased 1000-10.000 times

| Fibrinogen | Activates hemostasis, restricting spreading an in- |

	jury. Its level in the blood is increased 2-10 folds in 2-4 hours after injury
Alpha-1 anti-plasmin	Restricts plasmin activity
Alpha-1 acid glycoprotein	Blockers proteinase action
Haptoglobin	Plays role of anti-proteinase, blockers cystin proteinases
Apha-1 antitrypsin	Blocks connective tissue destruction (main anti-proteinase of blood serum)
Alpha-1 anti-chemotrypsin	Inhibits collagenase activity and catepsin G
Cerulloplasmin	Transports cooper and plays anti-oxidant role
Transferrin	Transport iron to the point of its destination (bone marrow)
Albumens	Support blood oncotic pressure, metabolic function as the constituent of important metabolites, including the enzymes and plastic materials

Active fractions of complement system play role of pro-inflammatory mediators.

Mostly, these proteins are the positive reactants of an acute phase response, but transferrin and albumens are the negative reactants,that means decreasing in their concentration in the blood during an acute phase response. Drop of transferring during APR may lead to iron-deficiency anemia, that isn't rare in the patient with severe disease, moreover, sometimes, together with significant loss of weight it may be the first manifestation of the disease.

Acute phase response and hemostasis

Increased coagulability of the blood and thrombogeinc properties of the blood vessels wall are very characteristic of an acute phase response. Last may result in thrombi formation in the small and large vessels. DIC-syndrome may complicate severe form of an APR. Such activation of hemostasis can be explained by the next events:

- Under IL-1 ,TNF-alpha thromboxane A-2, and PAF synthesis become increased, however, these substances not only increase a vessels permeability which influence viscosity of the blood, but can aggregate the platelets, predisposing to slowdown of the blood flow and, finally, to stasis and thrombi formation
- At the same time, IL-1 and TNF increase not only procoagulant activity of plasma, but provoke synthesis of procoagulants by the endotheliocytes
- Eventually, IL-6 stimulates fibrinogen synthesis by the liver

Negative reactants of an acute phase response

These are the proteins which blood level becomes diminished. The albumens and transferring belong to them. Lack of transferring, as was said above, explains us a very characteristic of the patients an iron deficiency anemia. This anemia is

refractory to the treatment with iron-containing drugs due to lack of protein-transporting media. Also may be revealed the decreased serum zinc but increased cooper.   In conclusion may be added that, of biological position, APR is a strong protective reaction, likes an inflammation, but its storming form may be very destructive, up to mortal for the individuals.

Lecture 6
IMMUNODEFICIENCIES

What is a biological role of an immune system?   It is believed that immune system protects us against the disturbances in tissue homeostasis. The last potentially may be broken by both types of injurious factors, external and internal. As for the external ones, may be named infectious or non-infectious pathogenic factors of our surrounding (infects, some drugs, poison and allergens), but the internal insults may be presented, for example, in form of immune complexes and tumor cells. They all are the foreign substances, and their elimination from the organism is a target for the immune cells activity. It is doubtless, an immune system keeps safe our life but its protective reactions like a "sword of two ends". On the on hand, it is in positive persistent struggle againstan infection but, on the other hand, the reactions to allergens to rid off them, or autoimmune processes may be very destructive.   Lasts, in biological sense are the useful, because clean us of the "non-self" but, as forsome individuals, they may have a disease as outcome, and moreover, a death.

Both, weak and strong immune responses   may do the harm but, sometimes, as was said, be mortal to an organism. All immune responses may be divided into two categories: immunodeficiencies, when immune response is too weak and "blesses'' the infections, especially opportunistic ones, and the hypersensitivity reactions. In this case an immune response isn't adequate and exceeds all expectations (allergy or autoimmune disease). In both cases we say about pathological immune responses.

We are known the two variants of immunological reactivity: congenital and acquired. The factors of the congenital immunity are the following: phagocytosis and complement system, which are very close to each other, because some activated fractions of complement system such as C3b–opsonins and anaphylatoxins C5a and C5 stimulate phagocytosis . The cytiokines like IL-1, TNF, and IL-6 are strongly associated with native immunity too. Biological liquors, mucosal lines and skin barriers can complete the list.

Is the congenital immunity specific reaction of an organism or not? It seems to be "yes" in sense of its possibility to differentiate the "self" from "non-self".

The main characteristic features of acquired immunity
- Realized by T- and B-lymphocytes
- Possesses by specification

- Possesses by immune memory

In accordance with the different factors of immunity all immunodeficiencies may be divided into   the next groups listed in classification::

Classification of immunodeficiencies
- Deficiency on phagocytes
- Deficiency in complement system
- B-cell deficiency
- T-cell deficiency
- Combined B-and T-cells deficiency

 To some up, all listed below variants may be congenital as well as acquired

Common features of congenital immunodeficiencies

Rare enough, but they all are the consequences of genes defects or abnormalities in organism's development. Very often they occur in childhood and together with other malformations in the internal organs and/or soft tissues.

The most clinical features of both congenital and acquired immunodeficiencies
- Recurrent infection of the gastrointestinal tract and respiratory system
- High risk of a tumor disease
- Disturbances in hematopoietic system
- Congenital abnormalities of different organs and soft tissues
- Shortened life span of patient

The causes of acquired immunodeficiencies
- Infections, first of all AIDS, rubella, cytomegalovirus
- Tumors of lymphoid system (leukemia or lymphomas)
- Immuno-supressive drugs, glucocorticoids or chemotherapeutic ones
- X-ray therapy, often before the organ transplantation
- Burn disease
- Uremia and associated with it hemodialysis
- In aged people
- Starvation
- Spleenectomy and chronic hemolysis, injuring spleen
- Chronic infections with assumption of the lot of immuno- protective factors

Opportunistic infection is a hallmark of an immunodeficiency condition. Moreover, due to the character of infection associated with the causative factor, we can estimate the deficiency of immunological factor which is involved in it.

Immunodeficiencies and associated with them infections

Immunodeficiency	Infection
T-cell form	Opportunistic infection (bacteria, viruses, fungi, pneumocystis carinii)
B-cell form	Streptococci, staphillococci,

	enteroviruses, (septic chronic meningitis, encephalitis)
Granulocyte defects	Staphillococci, pneumococci, candida albicans, aspergolla
Complement system deficits	Piogenic bacteria (incapsulated)

Characteristics of immunodeficiencies of different forms
T-cells deficiency
Common characteristics of T-cell population

Mostly, this population of the lymphocytes belongs to the T-forms (70%) and their favourite places for their residence are the following: thymic gland, lymph nodes, and some areas of the spleen. It is believed, that this population is the "brain" of an immune system, because the functions of the B-cells strongly depend on the T-cells influence, mainly, through the elaborated by T-cell special cytokines. The struggle against the intracellular pathogens (viruses, protozoa) and tumor cells is the most important function of T-cells. They possess by specific TCR (T-cell receptors) with abilities not only recognize the foreign agents, but in complex with CD+3 receptors on their membrane, to trigger the reactions of proliferation and cloning so named cells-effectors. Last cells, in turn, can interact with "non-self" substrates, carrying specific antigen and destroy them. For this reason, decreasing in T-cell blood-composition or lack of their activity may lead to opportunistic infection or low anti-tumor resistance.

Which way T-cells influence the immunity? There are two populations of T-cells: T-helpers with the surface marker $CD4^+$, T-suppressors or cytotoxic lymphocytes with the marker $CD8^+$. Their ratio in the blood in normal 2:1

All T-helpers, in turn, are divided into two populations:T-helpers type 1 and type 2. As to type 1 population, it is responsible for the triggering of the cellular immunologic reactions, but opposite, type 2 helpers, they mostly are involved in the immune humoral reactions. Via Th type 2 cytokines: IL4, IL-3.IL-5 and IL-13 they stimulate antibodies formation. At the same time, T-helpers type1 produce another cytokines, such as IL-12,IL-2, TNF, and IF-gamma which realize not humoral, but rather cellular immunity.

In intra-uterus period T-helpers type 2 predominate, however, just after birth, when a contact with infection starts, the helpers type 1 become gradually prevailed. This phenomenon is known as a phenomenon of immune deviation. The matter of the fact, that the first contacts of the newborns with any infection need an activation of T-helpers type1. Lasts realize the immunological guardianship of the children in early age, when humoral anti-infection protection isn't so strong. The number of T-helpers type 2 is growing with activation of humoral immunity, especially in allergic patients, when we can see the shift of isotype IgG in use of IgE-antibodies. It is known, that in the families having many children (often infections), the events of allergic disease more rare than in the families with few children (rare infections); by other words, any infection really inhibits the synthesis of

IGEs which are responsible for an allergy. Simultaniously, IgG-synthesis is growing, and hence the "infectious theory" of an allergic disease.

The causes of acquired T-cell-deficiencies:
- Chronic candidiasis. It is infection provoked by yeasts like fungi, generally, candida albicans as a part of the normal flora of the mouth, skin, intestinal tract or vagina. These are favourable sites if local injury but, sometimes, the systemic reactions may occur.
- Sarcoidosis - chronic progressive generalized granulomatous reticulosis, involving almost any organ or tissue with presence of tubercles consisting of epythelioid cells. It is the example of T-cells loss of unclear nature, and granulomas in the lymphoid organs are very similar to those in tuberculosis
- Leprosy - chronic communicable disease characterized by production of granulomatous lesions of the skin, mucous membranes, and peripheral nervous system. The severity of leprosy is directionally proportional to loss of T-cells but, sometimes, they completely disappear from the blood and the growth of correspondence bacteria can't be controlled

Congenital T-cell immunodeficiencies
- Syndrome Di-Georgi is a classical example of this form of immunodeficiency. It possesses by the following characteristics:
- Combine congenital malformations of the thymus, parathyroid glands and some cells of the thyroid gland, but the main target cells are the cells of the immune system, predominately T-cells
- Very often disturbances in the immunity combine with the heart, large blood vessels, possible anomaly with face (micrognathia - unusual smallness of the jaw, especially the lower jaw) and wide set of eyes with low set of the ears (hamster's appearance)
- Low level of T-cells in the lymph nodes and spleen
- Content of IG depends on the severity of the disease; may be normal or decreased
- More often it is chromosomal disease, because in 90% of cases deletion of the unknown genes located on the 22q position is revealed
- The patients are prone to the virus and fungi infection, which can be provoked so easy when T- cells immunity is weakened
- The severity of the disease depends on the level of thymus hypoplasia, and when full aplasia only transplantation of thymus can preserve the life of the child. Bone marrow transplantation is strongly recommended in this case too.

The functions of the B-cells and characteristics of B- cell deficits:
- B-lymphocytes occupy 10-20% of blood cell lymphocyte population
- Mostly, they are found in the bone marrow, in lymph node cortex in form of the follicles, spleen-white pulp, tonsils, elsewhere in the gastrointestinal tract as solitary follicles, and appendix

- After antigen stimulation they are transformed into plasma cells with further antibodies production by these cells
- Of 5 isotypes only 4 are found in the blood: IgG,IgM, IgA, and IgE. The last IgD is associated with cell membrane, and likes IgM, may fulfill the role of the receptors
- Like the receptors of T-cells, B-cell-receptors possess by the specificity. This specificity is associated with the rearrangement of the genome of B-cells (immune response genes) under antigen influence. That time, each B-cell is transformed into plasma cell producing, in turn, the certain class of the immunoglobulin named monoclonal antibodies
- Switching on one class of the immunoglobulin into another named a switching of the isotypes of the antibodies
- Some markers of B-cells, as CD19, CD21 are characteristic only for mature B-lymphocytes we use in the leukemia diagnosis as a test on B-cell immunophenotyping
- CD 21 on the B-cell membrane play role of the receptors to complement, and bind to the Epstein-Barr virus receptors. For this reason, B-cells are the targets to these viruses invading
- CD40 membrane receptors are necessary for switching the IgM isotype into IgG isotype
- Maturation of B-lymphocytes and their conversion into plasma cells regulated by the following cytokines: I L-4,IL-11,IL-13 producing by activated Th-lymphocytes type 2 and inhibited by IL-12 synthesizing by Th-type 1 lymphocytes

Hereditary forms of B-deficits

X-linked agammaglobulinemia Bruton's type

Characteristic features:

- Male pathology
- Absence or significant diminish of all classes of immunoglobulins in the blood
- Absence of circulating B-lymphocytes, plasma cells or germinal centers in lymphoid tissues
- Susceptibility to bacterial infection which appears very early after disappeared maternal antibodies from the blood
- Replacement therapy and bone marrow transplantation may be recommended as treatment
- Cellular response to infection isn't disturbed (rubella, infectious parotitis, or smallpox

The base of the pathogenesis is failure of pre-B-cells to differentiate into mature B-cells and then, into plasma cells due to hereditary lack of enzyme btk-Bruton's tyrosine kinase. The matter of the fact, that btk is necessary for complete assembly of the immunoglobulin molecule. In case of its genetic lack only heavy chains of immuniglogulin are synthesized, but not the light ones, and, as a result, there is a very low content of the antibodies in the blood and infection .

Partial IgA deficit and its characteristic features:
- Frequency among the European people-1:600
- IgA secreted by the mucosal lines of different organs, so lack of them may lead to weakening of these natural barriers, and the patients with such kind of deficiency are prone to allergic reactions and respiratory infection
- Sometimes, it may be latent without clinical manifestation and not always is diagnosed
- Replacement therapy may be recommended in case of the complications

Usual variable immunodeficiency   Common features:
- Not rear and has late onset
- Low level of IgG is revealed
- B-cells usually are normal, but there are disturbances in their maturation due to obvious impaired cooperation of T- and B cells via lack of IL-4 or IL-13 production, or other mechanisms. It seems to be a primary T-cell deficiency.

Syndrome of IgM hyperproduction Common features:
- Production of IgM is increased and correspondingly blood level of IgG decreased
- Base of the pathology is abnormality in the isotype switching from IgM into IgG
- Pathology can be explained by disturbances in ligand-receptor interaction, ligand CD40 on the T-lymphocytes but receptors CD 154 on the B-lymphocytes . For this reason, the processes of transcription in the immune response genes in B-cells is absent (no stimulation), and isotype IgM can't be switched into IgG-isotype
- Complication in form of recurrent pyogenic infection, so as IgGs play role of opsonins
- Risk of opportunistic infection
- If level of IgM is very high hemolytic anemia may be as complication
- Proliferation of plasma cells in elder people may lead to plasmacytoma formation tumor which more often occurs in the colon with possible hemorrhages as life threatening complications

Acquired B-cell or immunoglobulin deficits Common features:
- Revealed in some tumors: lymphomas and myelomas when Ig-secreting cells are replaced by tumor cells
- Infections with incapsulated bacteria are characteristic, especially in myelomas
- After splenectomy, because lack an antibody-independent immune response is realized in the spleen; the cases of hyposplenism associated also with sickle cell anemia when spleen can't fulfill its function due to its sequestration. For this reason, before splenectomy prophilactic treatment with antibiotics and injections of anti-meningoccial or anti-pneumococcal serum are strongly recommended. These procedures decrease a risk of infection in the patients.

Combined immunodeficiencies

There are several types of combined immunodeficiencies, and among them the "Severe Combined Immunodeficiency" due to hereditary lack of the enzyme adenosindeaminase is.

Characteristic features:

- ADA (adenosine deaminase deficiency) results in accumulation of deoxyadenosine and its derivates purins in the bone marrow cells which are the precursors of both T- and B-lymphocytes. These derivates of purine are very toxic for lymphoid cells, especially for the cells of T-line. Hence, the drop of T-cells in the blood is more significant than the same of B-cells
- Oral candidias as opportunistic infection in children is characteristic, and rush when swaddling is observed very often
- Very often occur an ability to further development and early death
- The patients are very prone to such infections as: candida albicans, pneumocysta carinii, pseudomonas, cytomegalovirus, and other opportunistic infection
- Sometimes, just after birth, the threatening to the newborn rush on the skin appears, because that time T-cells of the maternal organism enter the newborn and provoke the reaction of transplant against the host

Swiss type of the combined immunodeficiency

The base of this pathology isn't clear, but seems to be the result of disturbances in T-and B-cells cooperation with lack of immunoglobulins production. Clinical features are in form of fever, diarrhea, and loss of weight. It is X-linked disease.

Acquired immunodeficiency syndrome-AIDS

Definition It is severe retroviral disease that characterized by profound immuno-depression which ultimately leads to opportunistic infection, secondary neoplasms, and severe injury of nervous system. Mortality of a disease is 100%
Mid age of the patients is about 22-44 years, and one third of them are the women but the rest are the men. This disease is revealed in 193 countries but at first was diagnosed in the USA.

Epidemiology of AIDS

- Homosexuals and bisexuals account for 57% of cases
- 6% of individuals received narcotics via the blood (drug-abused individuals)
- Heterosexual contacts -10%
- 2% are the children and among them 90% acquired a virus from the maternal organism via the liquor or virus-infected cells (through placenta or blood in delivery and later, a milk feeding)
- Hemophiliacs 0,8% of recipient's blood or blood components-1,2%, and in 6% the risk factors are unknown (can't be determined)

The ways of transmission

- Sexual contacts-75%

- Parenteral –via the blood transfusion
- From mother to the newborn
- The sources of virus are the seminal liquor and, lymphocytes when sexual contact via rectum

Etiology

The retrovirus of human deficiency HIV is non-transforming belonging to the lentivurus family. Two groups of the viruses were isolated from the patients, and they called HIV-1 and HIV-2. HIV-1 is the most common type associated with AIDS. In practice the antibodies to P-24, the main virus antigen, must be discovered in the blood of the patient.

The main features of immunogenesis

Via different pathways a virus founds itself in the blood where its receptor gp-120 binds to membrane high affinity receptors of CD4+ lymphocytes. It likes "the mortal handshake" because leads to CD4 cells death. Such interaction between the virus and CD4+ triggers CD8+ cytotoxic lymphocytes synthesis, and the lasts kill CD4+ helpers. As a result the ratio of CD4+ / CD8+ shifts a lymphocyte population to the side of cytotoxic forms to the determent of CD4+ forms. Such alteration in lymphocyte family makes our immune system a very weak in the struggle against any infection, especially, opportunistic infections.

Keeping in mind a leading role of T-helpers in their influence the B-cells, macrophages and NK+ activity, we can estimate the fact of the profound failure of immunity in case of significant decreasing T- helpers in their number.

Special coreceptors CCR-5 on the T-helpers are necessary for interaction of the virus with CD4+receptor with all unfovaurable to an organism consequences, and if T cell CCR-5 are absent, an invasion of the lymphocyte is impossible. For this reason, the individuals who don't possess by these coreceptors are not susceptible to the HIV and can't be infected.

Common features of AIDS pathogenesis:
- Receptors for gp-120 also are found on the macrophages and dendritic cells
- In the macrophages a virus only replicated, and this replication occurs not in the blood but in the organs which are rich of the macrophages. Macrophages are the reservoirs for the viruses
- Dendritic cells, together with the viruses on their branches, especially in the spleen, postpone the time of organism infecting
- There is a severe struggle between the CD8+ and CD-4 + lymphocytes with a very sad outcome when CD4+ lymphocytes number is progressively diminished and a time of complications comes
- All chains of the immunity become very weak and disable, and among the following:

a. B-cell line and associated with it antibodies formation, including antivirus antibodies

b. Cytotoxic function of the macrophages due to decreased production of IF-gamma

c. Cytotoxic function of CD8+T- cells due to low synthesis of TNF-beta

d. NK+-cells activity due to low production of TNF-alpha

e. ADCC reactions become very weak too because diminished antibodies production and low cell phagocytic activity

Significant decline in all immune cell functions ultimatetely leads to the blessing of opportunistic infection and tumor growth.

The stages of AIDS with corresponding to them absolute content of CD4$^+$cells in the blood

1st. an early or acute	800<the number of T helper lymphocytes>500
2nd.mid stage	499< T helper lymphocytes >200
3rd. final or crisis	Less than 200 cells in blood microL

This decline is lasting usually for 6-10 years, and all that time the macrophages carry on the viruses to the CNS

The main features of the first stage:

- High level of virus production
- CD8+ control viremia in course of auto-limitation of infection
- Non-specific symptoms of the infection: fever, myalgia, loss of weight, night sweating, anorexia, and sore a throat
- Appearance of the symptoms in 3-6 weeks after invasion and disappearance in 2-4 weeks after
- On the second month virus becomes a resident of the lymphoid tissue and begins its replication in the macrophages, but not enters the blood
- When CD8+ lymphocytes are activated the decline in the CD4+ forms are temporary retarded (viruse titre begins to fall)
- Virus is in a "prison" (spleen and lymph nodes, but not in the blood), and a development of infection is postponed for some time
- It is a start of temporary balance between infection and immune resistance of an infected organism

The mid stage or chronic. Characteristic features:

- Destruction of the CD4+ infected lymphocytes and flooding of the blood with viruses, invading new lymphocytes
- In a "step by step" manner there is a decline in number of CD4+ lymphocytes and formation of the mutant forms of the virus, which make them unrecognizable and addssome problems in immune protection by CD8+ lymphocytes
- The number of CD4+ decreased up to 200 in blood microliter
- With macrophages virus is delivered to the brain and other organs and infects them
- Dendritic cells of the spleen play role of the traps for the viruses

The final stage or crisis is manifested by:

- Fever, loss in weight, diarrhea, and all symptoms of an acute phase response
- Opportunistic infection (accounts for 80% of the death)

- Neuropathology (brain injury with toxoplasma gondi in 50-60% of the cases)
- Among infections: candidias, micobacteria tuberculosis, herpes, and cytomegaloviral infection.
- Candidias injury in form of stomatitis and eosophagitis is a very characteristic
- Sometimes, infection may last 10 and more years. Such prolong course can be explained by special type of patient's immunity when content of CD4+ in the blood supported for enough long time and relatively stable. On the other hand, it can be associated with special virus factor. Virus factor minds deletion in one of the virus gene (nef). It is believed that the protein product of this gene plays crucial role in virus progression. In these relatively resistant patients a level of CD4+ lymphocytes is served enough for a long time. Appearance of some specific complications of AIDS in form of malignant tumors seriously aggravates a course of disease

The secondary tumors in patients with AIDS

1. Kaposi sarcoma   2. Non-Hodgkin lymphoma   3. Cervical cancer in women

The basic points of such risk of neoplasm seem to be connected to the following positions in viral infection:

a. Profound inhibition of cellular immunity in form of decline in T-helpers has negative influence an anti-tumor resistance

b. Prolong viral infection may lead to change in the genome of patent's cells and result in conversion of the protooncogenes into oncogenes with further synthesis of oncoproteins as cell growth factors.

Immunodeficits as a result of the disturbances in granulocytes

The granulocytes and, especially, the neutrophils are the first line of our defense against the pathogens of infectious and non-infectious nature (non-specific immunity), which was given us from the birth. DAL-1 and DAL-2 are the congenital types of leukocyte adhesion molecules deficiency. Lack of beta 2-integrins is a base of DAL-1 but, at the same time, low expression of L- selectins molecules on leukocyte membrane is associated with DAL-2. Disturbances in the synthesis of both type of adhesion molecules ultimately lead to impaired leukocyte adhesion with following lack of their emigration and phagocytosis. That time an acute inflammation tends to be converted in chronic one which can lead to exhaustion of the immune factors due to their excessive assumption. The next similar example is loss by granulocyte bactericidal properties due to impaired NADPH system. The third example may be presented by the Chediac-Higasi syndrome when degranulation of the granulocytes is disturbed, and we name this condition as granulomatous inflammation.

Acquired types of granulocyte deficiency may be illustrated by such pathology as:

- Diabetes mellitus and termal injury-defect of adhesion and chemotaxis
- Hemodialysis (the same defects)
- Leukemia, aplastic anemia, sepsis, starvation, treatment with cortisol, and X-ray disease are accompanied by failed phagocytosis and also lack of granulocytic microbicidity.

Lecture 7
IMMUNOPATHOLOGY
ALLERGY

Analysis of allergic disease epidemiology points to the wide spreading of the pathology among the people and increasing in frequency in the year; by other words, the staircase of these diseases becomes steeper and steeper. What kind of pathology an allergy belongs to? It is immune pathology, but opposite to immunodeficiencies when the base of pathology is directly associated with a lack of protective factors of immunity and an organism becomes open to different type on infection , in case of allergic reactions the inmmune reactions show an extreme response which, sometimes, may turn out the organism in ruin. These reactions are going with the real injury of tissue and belong to the hypersensitivity reaction type I according to Gell and Coombs' classification.

Term "allergy" was introduced in medicine by Austrian pediatrician Von Pirquet . "Allos"-another, "ergon"- action (Greek). It is really unusual group of disease, because they provoked by normal factors of our surrounding. More than 100 years ago Von Pirquet observed a very unusual reaction of the child to the injection of anti-diphteric serum, and he named it allergic reaction. Nowadays, the list of allergic reactions and diseases is widened and tends to be added. They all may be divided into general reaction-anaphylactic shock and local ones which we call atopic reactions as the reactions without a place-"topos" in a system of disease classification. Among the atopic diseases the widely- spreading: atopic bronchial asthma, hay fever, food and drug allergy. We determine this pathological condition as high hypersensitivity of an organism to the substances of antigen or hapten (partial antigen) nature. These factors called the allergens and, of course, they are the only extrinsic factors and may be found in our surrounding. Mostly, molecular mass of the allergens is about 10-70 kD. When allergen's molecular mass is lesser it can't trigger an immune response. On the other hand, if the molecular mass is much more, it can't pass through the natural barriers, including the mucosal lines. Among them must be called home dust which mostly responsible for such disease as bronchial asthma. In connection with, it must be added that ticks, as component of the beddings (feather pillows and bed), play not last role in etiology of atopic bronchial asthma. As for hay fever, different types of pollen of blowing grass and trees are involved in course of this disease. The disease clinically appears in different forms: conjunctivitis, rhinitis, nasopharingitis, and moreover, dermatitis. Mostly, they are the season diseases and belong to well diagnosed. Food allergy is more characteristic of children, and in early childhood allergy to milk proteins is the most actual. It creates many problems in children feeding. Also, is necessary to say some words about the stings of the bees or wasps. The matter of the fact, that their venoms content a very complex protein which seems to be the strong antigen responsible for anaphylactic shock.

Notable genetic, environmental factors and triggers connected
to allergic disease development

Genetic factors
- Presence of specific HLA alleles
- Polymorphism of the genes for beta-chain of high affinity receptors to IgE(Fc-RI-beta)
- Polymorphism of IL-4 family of cytokines genes

Environmental factors
- Receipt of antibiotics in first two years of life
- Vaccination to prevent of disease
- Having few siblings
- Excessive hygiene

Triggers
- Viral infection
- Very often exposure of allergen
- Tobacco smoke
- Indoor and outdoor pollutants
- Defect of respiratory tract epithelium, skin and gut also may predispose to atopic disease.

One of nowadays accepted classification is that which takes in mind the ways via different allergens penetrate into an organism. According this classification we differentiate: inhaled, food contacting, injected allergens; the last includes bee sting venom. Finally, must be said that only allergens are the causes of allergy, and for this reason, in any case of allergy treatment we strongly recommend our patients to avoid a contact with an allergen. Nevertheless, in atopic disease, hereditary predisposition of the patient isn't completely fatal but plays a very essential role. Environmental factors and triggers are very essential too in allergic diseases outstanding and further development too.

Common characteristic feature of allergic reactio
- Named anaphylactic or IgE-dependent reactions due to pivotal role of IgE in their pathogenesis
- May appear in form of systemic reaction-anaphylaxis or local anaphylaxis as atopic disease
- According to the time of clinical features appearance they may be divided into initial early response (within some minutes and usually tend to subsided for 60 minutes) and late phase response (in 2--8 hours and sustained without additional allergen exposure)
- In opposite to anaphylactic shock, hereditary predisposition plays a very important role in atopic diseases
- Prolong contact of an organism with specific allergen leads to its sensitization-acquisition of high sensitivity to allergen, and the base of the phenomena is elaboration of specific IgE against the allergen

- Th 2 type are confidential with the process of IgE synthesizing, because they secrete the instructive cytokines:IL-4,IL-3,IL-5,Il-10,IL-13
- Among the cells which are involved in anaphylactic reaction mast cells and basophils play the most important role. First of all, they possess by lot of receptors (from 300 to 3000) to Fc-fragment of IgE ,and when a titre of IgE becomes enough high, the antibodies set on the mast cell and basophil membranes, by other words, these cells become "armed with IgE in the face of specific allergen"
- Mast cells and basophils are the sources of the primary and secondary mediators of allergy. Among the primary mediators, first of all, histamine must be named; other primary or preformed, such as NCF (neutrophil chemotactic factor), ECF (eosinophil chemotactic factor), chimases and tryptases, Derives of the arachidonic acid: prostaglandins, leukotriens, and PAF belong to the secondary ones.
- Besides, mast cells can produce various cytokines, supporting and prolonging an allergic inflammation
- Eosinophils seem to be "are very interested" in allergic reactions. According to the last data, they wear the IgE receptors on their membrane like mast cells but in less number. Such way, via an interaction of these receptors with specific antigen, eosinophils can realize their response to specific allergen
- Releasing by eosinophils their content plays a double role in atopy. On the one hand, such enzymes as histaminase and arylsulfotase may correspondingly inactivate histamine and SRS (slow reacting substance) but, on the other hand, their oxidants and basic protein seem to be are very strong destructive substances, and not only to pathogen but for the host tissue too. It must be said that eosinophilia is strongly corresponds to allergic and parasitic diseases, and such cytokines as IL-3, IL-5 and CSF-granulocytic factor play not a last role in phenomenon eosinophilia
- In some cases diagnosis of atopic reactions minds the skin tests to reveal specific IgE fixed on the skin mast cells
- The treatment of atopic disease includes specific immunotherapy and non-specific, mostly, with antihistamine drugs

Common pathways of anaphylactic reaction pathogenesis

By the convention, a course of anaphylactic reaction passes three stages:
- 1st stage-immunological or sensitization
- 2nd stage -pathochemical
- 3rd stage- of clinical features

Immune stage of type I hypersensitivity

An immune stage clinically isn't manifested and, likes the others types of immunization, characteristic of antibodies to specific allergen elaboration. Significant family shift is observed in the ratio IgE/IgG in use of the firsts in the blood of patient, as a result of relative hyper-IgE globulinemia. Fc-fragments of IgE are responsible for interaction with high- affinity receptors for the IgE Fc receptors on

the mast cell membrane. But many events are going between a primary contact of an organism with allergen and appearance of excess of IgE on the mast cell surface.

In a long and complex chain of events in organism sensitization minds not a single exposure to allergen. The start sets via primary contacts of an allergen with the triad of antigen-presenting cells, wearing MHC class II on their membrane surface: dendritic cells, macrophages, and B-lymphocytes. After processing of antigen within these cells, including digestion and release of antigen determinants or epitops, they present the products of antigen transformation, mostly, to CD+4 cells type II. Why type 2? It may be explained by high level of heterogenicity of an antigen which is so far from our own tissue antigenicity. Then processed antigen forms membrane complex with antigen-recognizing cells T-2 helpers-lymphocytes via specific T cell receptors and CD3+surface antigen. Last,, in turn, triggers these lymphocytes activation in form of proliferation, synthesis and production of the most important for further reactions cytokines. They are the following: IL-4,IL-3,IL-13.and IL-4 as a decisive factor to switching the IgG isotype of B-cells into IgE one. As for IL-13, it is a very similar in its function to IL-4 and influences mostly to increase the B-lymphocyte activity. IL-5 and IL-3 help to maturation of eosinophils in the bone marrow, as well, increase their chemotactic activity.

CD 40^+ cellular membrane receptor of B-cells seem to be an additional costimulating factor which is necessary for expression of mature RNA with the following IgE synthesis. Additionally must be said that both IF-gamma and IL-12 inhibit the humoral reactions, shifting immunity to the cellular pathway. This phenomenon is realized due to an increasing in Th-1 cells number and their activity. Congenital lack of IF-gamma was described in one of the newborn boy with severe atopic dermatitis that had occurred to him in age of 12 months. The phenomenon may be explained by derepression of IgE synthesizing when IF-gamma failure was.

Prevalence of Th-type 2 helpers and their activation with following Il-4 and IL-13 secretion results in synthesizing of IgE and their exposition on the B-cell membrane. Next moment in a chain of sensitization is transformation IgE-carrying B-cells on their membrane into plasma cells with secreting monoclonal antibodies specific to the allergen. As soon as the level of IgE becomes sufficient to saturate with them the mast cells, the lasts are "ready to meet" an allergen. Repeated exposures to specific allergen result in antigen-antibodies formation on the mast cell membrane with their following activation, degranulation, and blood flooding with histamine.

Pathochemical stage of type I hypersensitivity

When antigen (allergen) interacts with the IgE -antibodies, attached previously to the mast cells membrane, multivalent antigen binds to more than one IgE molecule and cause cross-linkage of adjacent IgE antibodies. The bridging of IgE molecules with antigen-antibody formation activates the multiple phospholipases, imbedded in cell membrane and initiates some independent processes in the mast cells:

1. Degranulation with release of preformed mediators
2. Synthesizing of the mediators "de novo"
3. Synthesizing and release some cytokines

Mediators are responsible for the symptoms of allergy
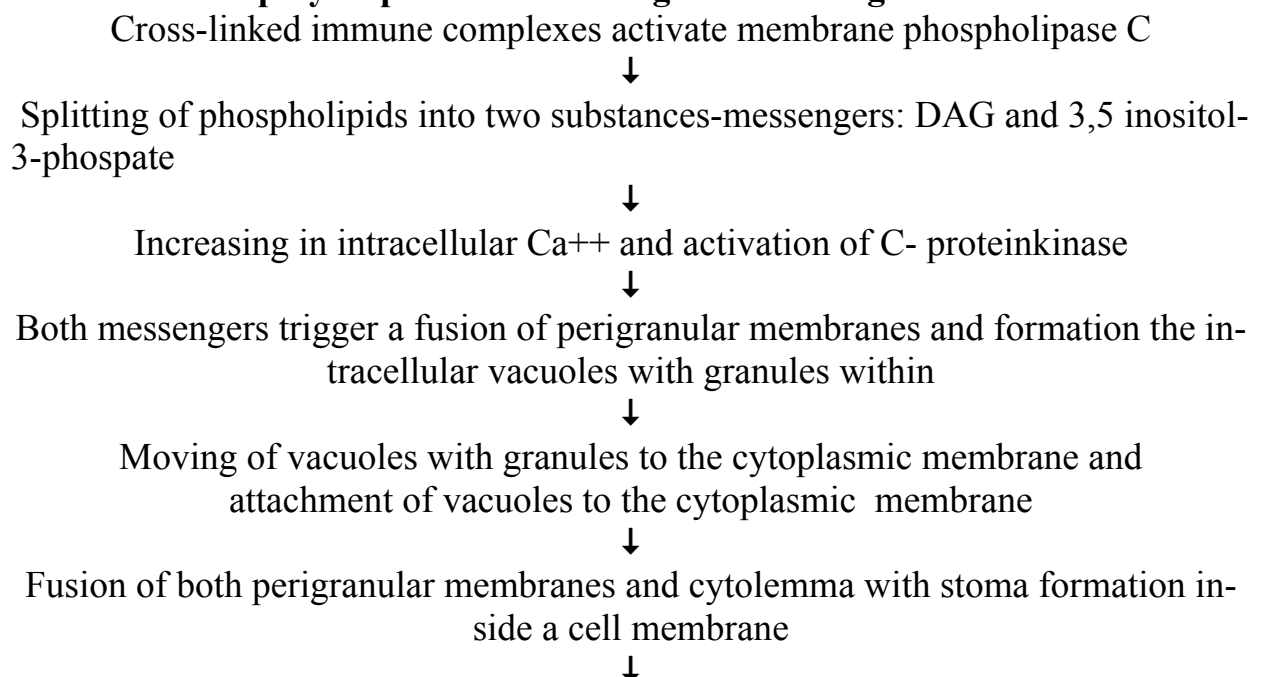
Primary mediators

The role of mast cells and basophils in anaphylactic reactions and the mechanisms of mast cell degranulation. These cells have much common in structure and seem to be the function, that directly associated with histamine of their granules.

Histamine is a biologic amine, the product of aminoacid histidine decarboxilation. Antigen- antibody complexes trigger mast cell and basophil degranulation with histamine release in surrounding tissue. The cyclic nucleotides play a special role in mast cells degranulation. First, immune complexes, activating membrane adenilatecyclase for a very short time, increase intracellular concentration of cAMP and then steady diminish its concentration, but at the same time, cGMP intracellular concentration becomes lesser. For these reason, the substances which are capable to increase concentration cAMP in the mast cells can prevent their degranulation. That's why, the sodium chromglycate(intal) and acting, like it drugs, steady increasing the cAMP in the mast cells, have success in allergy treatment.

Mast cell degranulation is manifested by histamine-content granules extrusion in surrounding space.

Parallel with described above energetic processes, which are necessary for mast cell degranulation after antigen-antibody interaction on the mast cell membrane, the next events are in process. Some of them may be observed in light or electron microscope.

Step by step events reflecting mast cell degranulation

Cross-linked immune complexes activate membrane phospholipase C
↓
Splitting of phospholipids into two substances-messengers: DAG and 3,5 inositol-3-phospate
↓
Increasing in intracellular Ca^{++} and activation of C- proteinkinase
↓
Both messengers trigger a fusion of perigranular membranes and formation the intracellular vacuoles with granules within
↓
Moving of vacuoles with granules to the cytoplasmic membrane and attachment of vacuoles to the cytoplasmic membrane
↓
Fusion of both perigranular membranes and cytolemma with stoma formation inside a cell membrane
↓

But the matter of the fact, that weekly acid pH inside the mast cell is very appropriate to perigranular membrane stability, but pH of extragranular space is a neutral, that provokes a lysis of granules with histamine release into tissue.

Role of mast cell receptors in their degranulation and its drug-induced inhibition

The following receptors are found on the mast cell membrane:

- H-2 receptors to histamine
- Alpha- and beta-adrenoreceptors
- M-cholinoreceptors,
- Purine or adenosine-receptors
- PGE and PgF-2-alpha receptors

Stimulation of M-cholinoreceptors, alpha-adrenoreceptors, adenosine and PgF-2 alpha ones under antigen-antibody reaction results in mast cell degranulation with histamine release. This process is accompanied by steady decreasing in cAMP and increasing cGMP in a mast cell cytoplasm. For inhibition of histamine release from mast cells these receptors must be blocked in course of allergy treatment by appropriate antagonists.

By contrast, stimulation of beta-adrenoreceptors, H-2 histamine, and PgE-receptors by their agonists inhibits mast cell degranulation, that's why these agonists, especially beta-receptor agonists, are widely used as antihistamines.

Histamine as the universal mediator of an allergy

Effects of histamine includes: increased vascular permeability, increased secretion of nasal, bronchial and gastric glands but, as for bronchochonstrictor's effect, it is very effective in some animals, for example, guinea pig but not in a man. For this reason, the role of histamine in an accident of bronchial asthma is limited by the edema of bronchial submucosal line and mucus hypersecretion. Both provoke the narrowing of the small bronchi which, in turn, causes an increase in resistance of low airways to an air during expiration. As for bronchospasm, the other mediators of allergy are responsible for the phenomenon in people.

Except histamine, which is associated with granule proteogycans, in mast cell granules anticoagulant heparin, chondroitin sulfate and special chemotactic substances are found. The lasts include eosinophil chemotactic factor and neutrophil chemotactic factor; both are the most important, moreover, play a pivotal role in scenario of a late phase response in atopic reactions. Enzymes of granule matrix include proteases- chymase, tryptase, and several acid hydrolases. These enzymes, when released, may lead to kinin and complement system activation with a very pro-inflammatoty properties of their end-staged products..

Secondary mediators

PAF

Secondary mediators include the derives of mast cell membrane phospholipids: PAF (platelet-activating factor), metabolites of arachidonic acid (prostaglandins and leukotriens), and the cytokines.

PAF is formed due to activation of D-2 phospholipase of mast cell membrane by immune complexes. PAF is the complex of lipid substances which causes activation and aggregation of the platelets, release of histamine, bronchospasm, increased vascular permeability, and vasodilation. It mast be added that, being a strong polyvalent chemattractant, PAF expresses the adhesion molecules on the endothelial cells, eosinophils and neutrophils, providing their emigration and moving towards an epycenter of acute immune inflammation. These newly elicited cells mostly are responsible for the late phase response in atopy.

Leukotrienes

Leukotriens play extremely important role in type I hypersensitivity reactions.

They all are the products of lipooxigenase pathway of arachidonic acid metabolism.

Leukotrienes complex of fractions (CDE)-4 named slow reacting substance of anaphylaxis (SRS-a). It possesses by a very strong spasmogenic effect, especially to smooth muscle of small bronchi. From here, it is evident a leading role of SRS-a in pathogenesis of bronchial asthma accident, moreover, LTC-4 and LTD-4 fractions are the vasoactive substances, and together with histamine can provoke an edema of bronchi submucosa. Also LTB-4 is known as polyvalent chemattractant and influences a mobility of neutrophils, eosinophils, and monocytes as phagocytes..

Prostaglandins

Mast cell is a source of PGD-2, one of the end-products of cyclooxigenase pathway of arachidonic acid metabolism. It causes intense bronchospasm, as well as, increased mucus secretion.

Cytokines

As believed, mast cells produce a variety of cytokines which support and prolong immune stage of anaphylactic reaction. They also are responsible for systemic reactions in form of an acute phase response in course of allergy. Among the cytokines, elaborated by mast cells must be named TNF-alpha, IL-1,IL-3,IL-4, IL-5,IL-6 and GM-CSF. Many of these cytokines also are produced by Th-2 cells. It must be added, that some of them, for example IL-1 and TNF-alpha, possess by local effects, aggravating an allergic inflammation, but at the same time, they work for a distance, provoking fever or loss of an appetite). The other ones- IL-3, IL-5, gm-CSF actively increase a number of granulocytes, especially, eosinophils by the bone marrow. Being the cytoclones, they fulfill these cells populations in the blood (hence- eosinophilia).with following cell recruitment to the site of allergic inflammation. As was mentioned above, IL4 is a crucial factor for IgE synthesizing.

The stage of clinical features

The basic moments of the stage are the responses of the target cells to biological active substances. They include the vasoactive reactions resulting mostly in local edema, spasm of smooth-muscle organs in form of broncospasm, laryn-

gospasm or colic, and mucus hypersecretion in allergic rhinitis or bronchial asthma.

Mechanisms of allergic inflammation

As was said, atopic reactions in form of allergic rhinitis, accident of bronchial asthma or urticaria according to their time of appearance may be divided into two forms: early and late response. As for early reactions, their mechanism predominately associated with realize and formation of preformed and newly synthesizing mediators. Mast cells are the pivotal cells in these reactions, because precisely they become an area where immune complexes are assembling and triggering flooding of a tissue with primary and secondary mediators, but histamine plays the central role in such type of reaction,

Reactions of late phase which start not earlier then in 2-3hours without repeated an allergen exposure. The matter of the fact, that such chemical mediators as eosinophil and neutrophil chemotactic factors, histamine, eotaxin, leukotriens, PAF, and neuropeptides, as well as TNF and IL-1, being early and late chemattractants, provoke the recruitment of allergic proinflammatory cells. As a result, eosinophils, neutrophils, and monocytes, infiltrate flooded with them tissue. Once these cells are in the tissue, they induce local release of a variety of the enzymes, oxidants and chemattractants which additionally recruit the leukocytes from the blood.
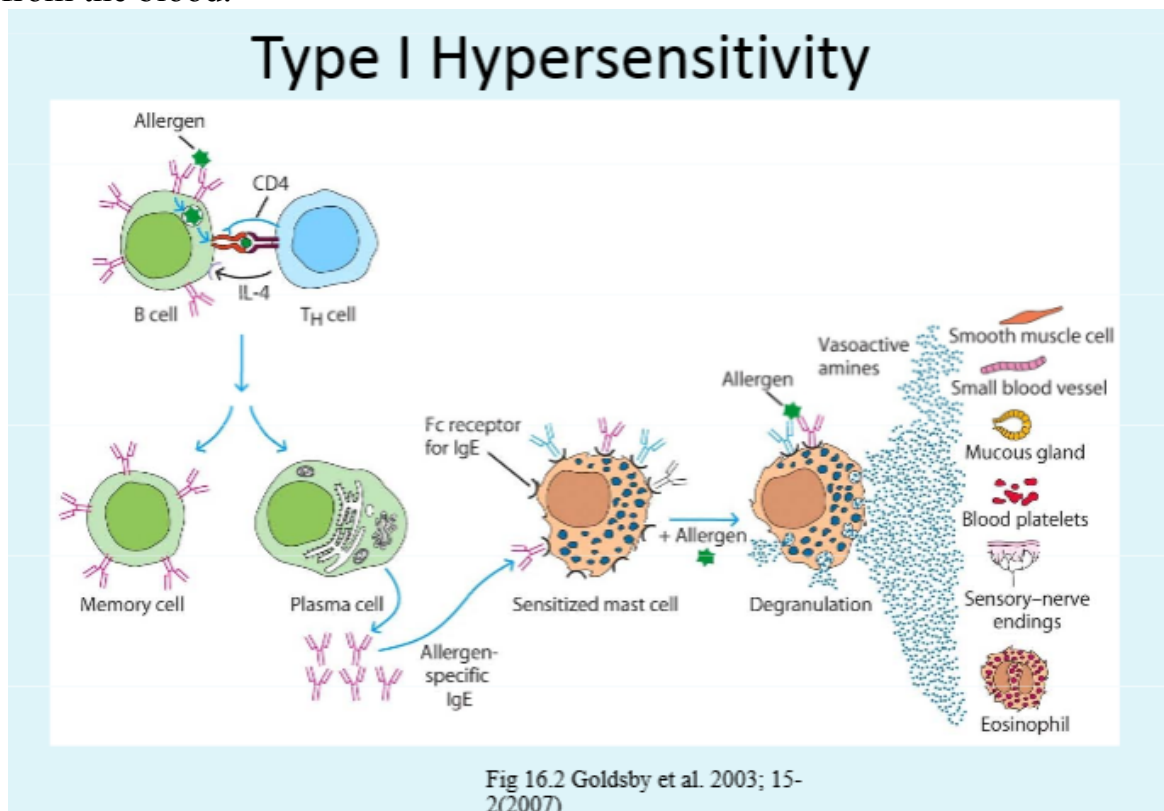
PICTURE 1. Pathogenesis of type I of hypersensitivity reaction

 In humans systemic anaphylaxis may occur after administration of some drugs, rather penicillin and others antibiotics, foreign serum (e.g. antiserum), hormones and enzymes, as well as, a stinging of the insects. Food components (shellfish,

peanuts, chocolate, oranges and strawberry) may be responsible for anaphylactic shock too, especially in children.

These reactions occur within minutes because an antigen reacts with pre-formed antibodies on the surface of mast cells. Death may occur within minutes too. As for the special characteristics of systemic anaphylactic reaction, must be named the following:

- Role of hereditary insignificant
- Method of provoking more often artificial
- Shock organ is a single for the species
- Type I of hypersensitivity is involved
- A severity of disorder varies with the level of sensitization
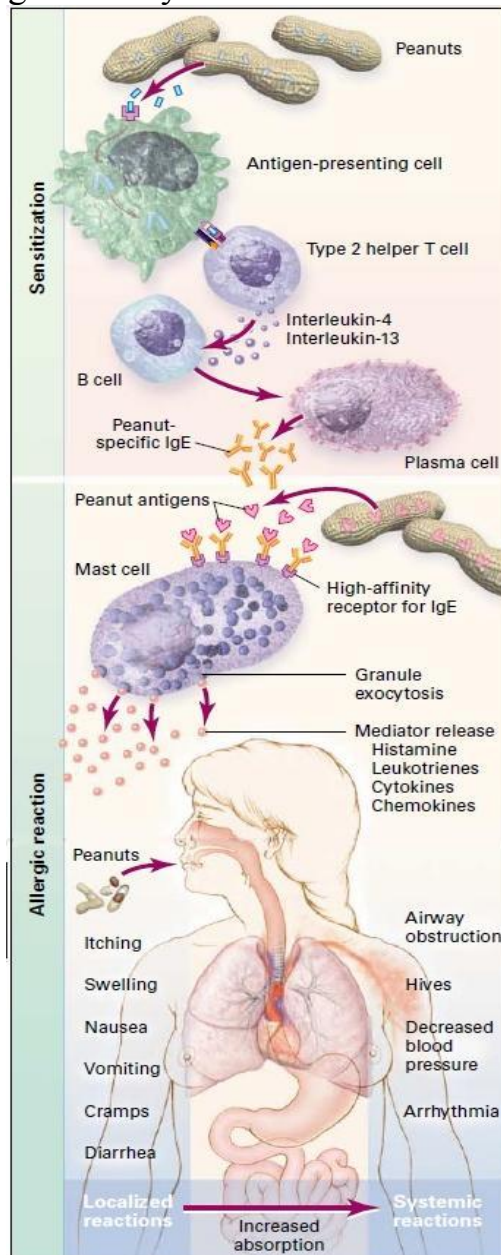- Shock dose of antigen usually isn't known

Figure 1. The Mechanism of Sensitization and Allergic Reaction.

PICTURE 2   Anaphylactic shock is the most severe allergic reaction.

Possible clinical features of anaphylactic shock:

- Skin itching, burning and redness with vesicles-urticaria
- Spasm of small bronchioles, asphyxia, and possible acute respiratory syndrome
- Edema of the larynx and hoarseness
- Vomiting, diarrhea, cramps, colics, and abdominal pain
- Decreased blood pressure and arrhythmia

Cardiac acute insufficiency and collapse as systemic reactions, may be fatal to a patient.

Diagnosis and treatment of allergic atopic reactions

We set a treatment of a disease after study of patient's family history, in some cases skin testing, RAST (radio-allergosorbent) or RIST (radio-immunosorbent) which are used to precisely determine specific allergen which is responsible for patient immunization. After such identification two methods to minimize allergen influence an organism may be used: specific and non-specific therapy, but very often they are combined.

Specific one minds the immunotherapy because it modifies an immune response via switching a synthesis of IgE into relative preference of IgG production in the patient over IgE content. It minds, for enough a long time, twice in a week, subcutaneously injections of very small doses of specific allergen which injections must be progressively increasing in time.

Non-specific therapy mostly is realized by antihistamines treatment, symptomatic therapy, for example, the broncholytic drugs in bronchial asthma, moreover, nowadays the inhibitors of mast cell degranulation are very popular. They all reduce allergic symptoms, as well as, the glucocorticoids. Lasts via synthesis of protein lipocortin within the inflammatory cells inhibit all cell phospholipases, and such way, arrest the last is known as pathogenesis of an allergy.

Lecture 8
IMMUNOPATHLOGY
Type II, III and IV of hypersensitivity
<u>Type II hypersensitivity reactions</u>

These reactions are named cytopenic or cytotoxic due to the cell population death or injury. Very often its type of immunopathology is accompanied by whole organ injury which is manifested by its hypofunction or hyperfunction. Nowadays, as an example of organ hyperfunction is only the Graves disease may be called. All reactions of type II hypersensitivity named disregulatory, because in both cases, hypo-or hyperfunction the disturbances in organ arrangement are observed.

The common features of type II hypersensitivity:

- Antigen is associated with cell membrane
- Antibodies bind to antigen "in situ" on the cell membrane by their Fab-fragments when

- Fc-fragments are free
- Antigen-antibody complexes are situated on cell membrane
- Antibodies belong to IgG-1,2,3 subclasses and IgM
- Whole destruction or partial injury of the target cells are realized by complement-
- dependent or complement non-dependent pathways
- Complement dependent way includes: complement-dependent cell lysing or
- phagocytosis
- Non- complement-dependent pathway is presented by ADDC (antibody-dependent cell-
- mediated cytotoxicity)
- The following mediators are involved in it: activated complement fraction:C3b,C3a,C5a
- and C56789 macromolecular complex, reactive oxygen species, cell lyzosomal
- enzymes, and lesser bradykinin
- The nature of an antigen may be the following:
  a. different drugs and chemicals resulting in heteroimmune reactions
  b. of infectious nature: bacteria, viruses or parasites
  c. non-changed structures of the cells in case of loss of immune tolerance when autoimmune disease or transfusion of incompatible groups(ABO) blood

Clinical examples of type II hypersensitivity with their antigens
The examples of immune blood cell cytopenia

- Immune hemolytic anemia
- Immune agranulocytosis
- Immune thrombocytopenia

Cytotoxic reactions seem to be the base of multiple autoimmune diseases, but now of unknown reasons and with not a good prognosis.

The examples of some autoimmune diseases and their antigens

| Autoimmune disease	Antigen
Autoimmune glomerulonephritis	Basement membrane of kidney glomerule
Myasthenia gravis	Postsynaptic membrane receptors in peripheral cholinergic synapses
Eaton-Lamberth syndrome	Presynaptic terminates in peripheral cholinergic synapses
Diabetes mellitus type I	Antigens of insulin-producing beta-cells of Langerhans' islets
Pernicious anemia (of B_{12} deficiency)	Antigens of stomach parietal cells
Graves disease	Antigens of thyroid cells, the retroorbital and pretibial tissues

As was said above, there are two variants of cell injury are involved: complement-dependent reactions and complement non-dependent ones.

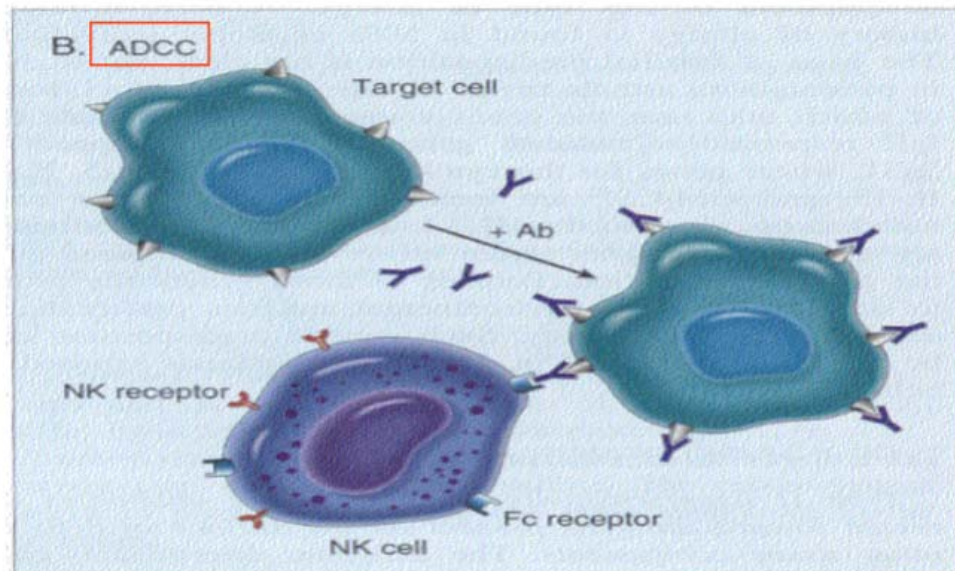*Complement-dependent reactions*

These reactions include direct lysis of cell by membrane-attacking complex and phagocyte opsonization with following phagocytosis.

In first case, antibodies in optimal titer cover the membrane located antigen with their Fab-fragments. That time formed on the cell membrane immune complexes activate complement system according to the classical pathway. It ultimately leads to the cascade of reactions, resulting in assembly of the membrane attack complexes C5b6789, which disrupt cell membrane via the "drilling" holes formation. Osmotic lysis completes a cell death. Activated C3b complement fraction, as opsonin, also mediates complement-dependent target-cells phagocytosis. The matter of the fact, that all obliged phagocytes possess by the receptors to the opsonins including C3b-fraction of complement. Together with antibodies (IgG and IgM), C3b opsonizes the phagocytes that make them more active in their phagocyting function.

This form of type II hypersensitivity mostly involves blood cells and seems to be responsible for such pathology as immune forms of hemolytic anemia, agranulocytosis, and thrombocytopenia. Nevertheless, antibodies also may be directed against extracellular tissue elements (glomerular basement membrane in cytotoxicl form of glomerulonephritis).
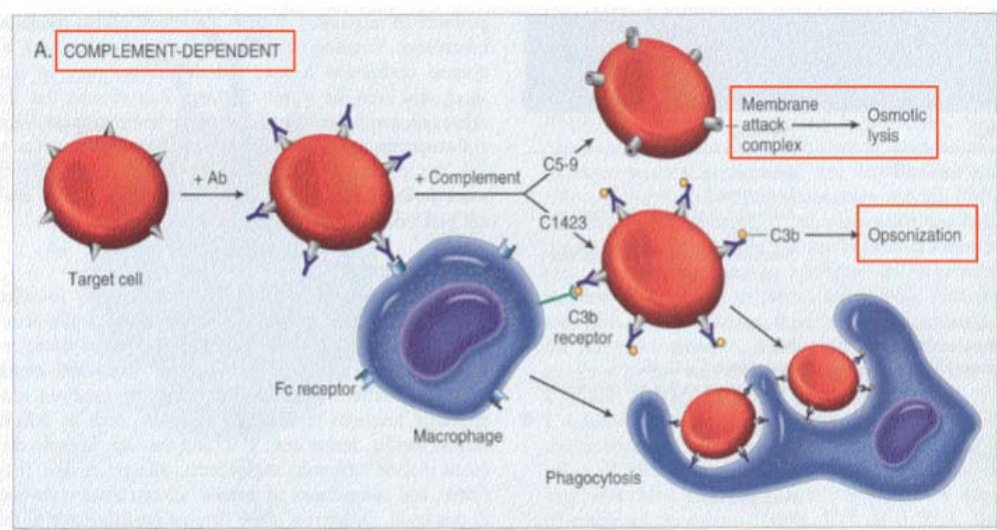
Picture 3. Mechanisms of cell injury in type II hypersensitivity

PICTURE 4. Antibody-dependent cell-mediated cytotoxicity (ADCC)

Complement non-dependent reactions

As in described above pattern, antibodies cover an antigen associated with a cell membrane by their Fab- fragments. At the same time, Fc-fragments attach to the phagocytes and NK-cells via their receptors to them. This bind forms a "bridging" of target cell with phagocyte or NK-cells resulting in activation of these cells and releasing of their content (enzymes and oxidants). Lasts attack and injury the target cells. ADCC is working very effectively in a struggle of an organism against the intracellular parasites and tumors.

Type III hypersensitivity (immuno-complex mediated)

It must be said that, mostly, these reactions are represented by autoimmune diseases, but serum sickness disease (not autoimmune) belongs to the immuno-complex reactions too.

Classification of immuno-complex reactions

1. Common reaction of an organism in form of acute serum sickness disease
2. Systemic autoimmune diseases:

a. rheumatoid arthritis	c. dermatomyositis
b. systemic lupus erythematosus	d. polyarteritis nodosa

3. Local immune -complex diseases (like Arthus-reactions):

a. immune vasculitis	d. farmer's lung
b. autoimmune glomerulonephritis	e. autoimmune orchitis
c. autoimmune serositis (pericarditis or pleuritis)	f. sympathetic ophtalmopathy

P.S. This list may be prolonged and widened

Some antigens associated with immune complex disorders may be qualified as exogenous and endogenous, but at the same time, infectious or non-infectious origin.

Antigens of autoimmune disease and disease manifestations

| Antigens	Clinical manifestations
Exogenous infectious agents:	
Yersinia enterocolitica	Glomerulonephritis, infectious endocarditis
Bacteria streptococci	Arthritis
Treponema pallidum	Glomerulonephritis
Viruses:	
hepatitis B, cytomegalovirus	Polyarteritis nodosa
Parasites	
plasmodium sp, schistosoma sp.	Glomerulonephritis
Fungi: actinomycetes	Farmer's lung
Drugs or chemicals:	
foreign serum	Serum sickness disease
heroin	Glomerulonephritis
Endogenous:	
Nuclear antigens	Systemic lupus erythematosus
mmunoglobulins	Rheumatoid arthritis

Common features of immune complex diseases:

- Antigen-antibody complexes are formed in the blood, interstitium, and in the site of primary antigen contact with tissue
- Immune complexes activate four protein systems of the blood: complement, kallikrein-kinin, coagulation, and fibrinolytic with corresponding end-products formation
- Slight excess of soluble antigen, persisting in organism for enough long time is characteristic of antigen-antibody reaction, and both, exogenous and endogenous antigens may trigger an immune reaction. In opposite situation, when the large complexes are formed in a great antibody excess, they can be rapidly removed from the circulation by the monocytes and macrophages but, in contrast, small or mid- weight complexes in slight antigen excess are poorly recognized by the phagocytes
- Reminders of antigen inside the phagocytes support a process of organism immunization.
- Small vessels, pericardium and pleura, skin, kidneys and joints are the favourite sites of complex antigen-antibody accumulation, because these organs predominately take part in immune complexe filtration
- Deposits of immune complexes in tissue provoke an inflammation, that may be morphologically proved by tissue infiltration with mononuclear cells. These are the monocytes and lymphocytes, but by the end of disease remission, when chronic process transforms into an acute one, a neutrophil population hands over
- The following mediators are responsible for acute and chronic immune inflammation: activated complement fractions-C3b,C3a,C5a, lyzosomal enzymes of cells, neutrophil cationic proteins, oxidants, and such product of kallikrein-kinin activation as bradykinin

- Active platelet aggregation and formation of fibrinoid deposits in small vessels with their microthrombosis. They provoke ischemic injury with possible local tissue necrosis
- Due to persisting of immune complex, such form of inflammatory reaction may be characterized as non-fading

It is widely accepted that the reaction passes through 3 phases:
1. Immune- complex formation, mostly in the blood
2. Immune- complex deposition in tissue
3. Immune-complex-mediated inflammation

Primary contact with antigen activates predominately B-lymphocytes with following selection of specific antibodies on their surface, amplification and proliferation of plasma cells with their monoclonal IgG and IgM synthesizing. And the same time, a clone of B- memory cell is formed, and it facilitates the following processes of immunization after repeated antigen exposures. The secondary immune response occurs in the blood and interstitium after repeated contacts with antigen. Formed immune complexes are circulated in the blood and can be accumulated in the site of antigen residence in tissue.
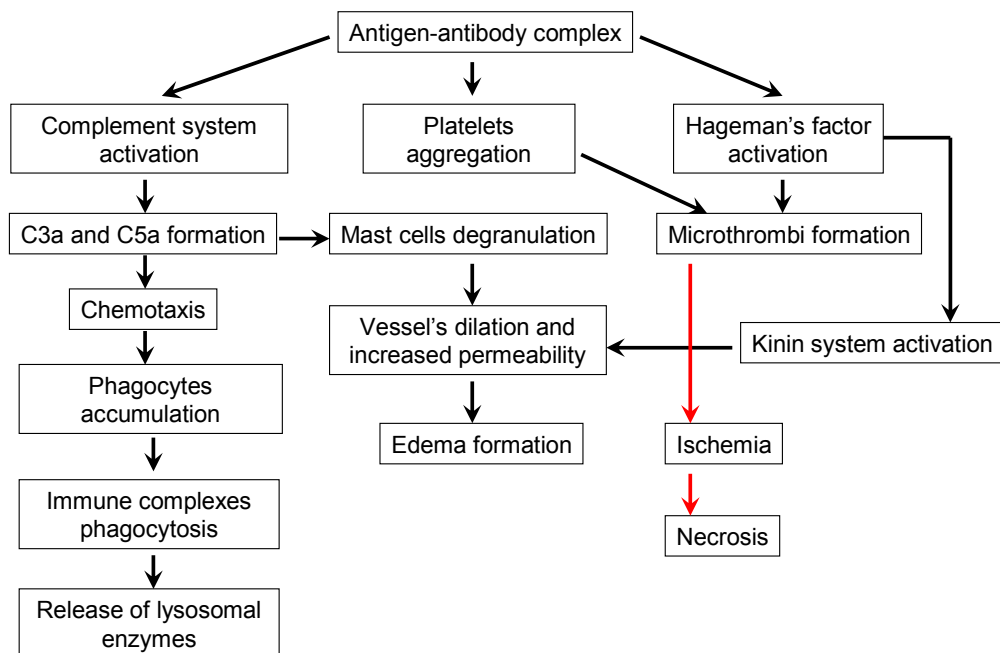
Picture 5. Pathogenesis of type III hypersensitivity

As was said above, they are deposited in so named "filtrating organs": small vessels, kidney, skin, joints, and serosa (pericardium and pleura) with the following immune inflammation. These are the favourite sites of autoimmune inflammation.

According to their sources, all mediators of immune inflammation may be divided into two groups: cellular and originated from plasma. As for cellular origin, the following must be named: the lyzosomal enzymes and oxidants; but from

plasma; activated complement fractions. C3b which together with immunoglobulins acts as opsonins; C3a and C5a s chemattractants to phagocytes ,and at last, macromolecular attacking complex C5b6789 injuring some cells via drilling their membranes. In addition must be said that ADCC also takes part in an inflammation. Activated by immune complexes kallikrein-kinin system becomes a source of bradikinin, a very strong vasoactive substance and possessing by algesive properties. Besides, activated clotting cascade supplies the blood by fibrinogen in high concentration. Its deposits may be found in the small vessels and interstitium. Block of the microcirculation in different organs and tissue is a distinctive feature of immune complex inflammation.

Serum sickness disease

Originally, the name of disease was proposed due to possible pathological reaction of man to injection of foreign serum but, nowadays, some drugs, being in complex with our normal blood proteins (e.g., penicillin) are capable to initiate the disease too.

Serum sickness disease usually is a result of a single injection of antigen appearing in 7-14 days after. By that time the titer of circulating antibodies riches a maximal level but antigen yet doesn't disappear from the blood completely. As a result, antigen-antibody reaction occurs on the different area of an organism, and it is reflected by clinical manifestation of disease. Fever, urticaria, arthralgia, lymph nodes enlargement and proteinuria are the most characteristic. In some cases may be changes in ECG pattern. All these symptoms may be explained by immune complexes deposition in corresponding tissues and organs with following their injury.

Local immune- complex injury (Arthus reaction)

It is definited as a local area of tissue necrosis resulting from acute immune complex vasculitis, usually elicited in the skin. This reaction can be produced in experiment by intracutaneous challenged injection of antigen in an immune animal, having circulating antibodies against the antigen (sensitized previously). Due to the excess of injected antigen and pre-existing in immunized organism antibodies, antigen-antibody complexes are formed and pass through the small vessels wall. Here they locally precipitated and trigger an inflammatory reaction. In contrast to type I reactions (IgE-mediated) which appear immediately, the Arthus' lesion develops in a few hours and reaches its peak 10 hours after such injection. It can be seen as visible edema with local hemorrhages followed occasionally by ulceration and necrosis. The last is classified by morphologist as fibrinoid necrosis of the vessels. It mast be added, that similar processes may occur in other organs, for example in kidney, and for this reason such pathology is qualified as the Arthus -likes reaction.

The types of antigens involved in selected autoimmune diseases

1. Nuclear- double stranded DNA which penetrate the intact cells (SLE-systemic lupus erythrematosus)
2. Cytoplasmic –mitochondria (primary billiary circhosis)

3. Cells – parietal cells and intrinsic factor (pernicious anemia), blood cells, beta-cells of pancreas9 diabetes mellitus type I)
4. Proteins – immunoglobulin (rheumatoid arthritis, thyroglobulin (Hashimoto's thyroiditis)
5. Structural antigens – glomerular basement membrane of kidney (glomerulonephritis)
6. Receptors – thyroid receptors (Grave's disease), acetylcholine receptors (myasthenia gravis)

Genetic factors in autoimmunization
Connection of some autoimmune disease with type of HLA class

Autoimmune disease	Type of HLA class	Risk in%
Rheumatoid arthritis	DR3-4	5.8
Insulin-dependent diabetes mellitus	DR3/D4	14.3
Systemic lupus erythematosus	DR2/DR3	

General factors of autoimmunization and common features
of autoimmune diseases

- Loss of immune tolerance (inability to evoke an immune response to self-antigens) due to disturbances in clone deletion. Clone deletion minds the elimination T- and B-lymphocytes with high affinity receptors to the self-antigens in thymus
- Modifications of self-antigens by viruses, bacteria, parasites, chemical substances, including the drugs (molecular mimicry)
- Disarrangements in genes that code MHC molecules class I and class II, following by failed normal immune cell recognition
- Failure of immune cell apoptosis of hereditary or acquired nature
- Role of cross-reacting antigens is an evident (streptococcal M-proteins with cardiac glycoprotein or basement membrane in kidney)
- Releasing of sequestrated antigens. Some tissue completely sequestrated during their development (spermatozoa, ocular antigen-lens, colloid of thyroid gland, and myelin). Post-traumatic process and infection may lead to immune system contact with them , and set an elaboration of the auto-antibodies and auto-reactive lymphocytes. For example, post-traumatic uveitis or orchitis may result to sympathetic process (occurs in other of pair organ), because their antigens encounter the immune cells in the blood and trigger an immune response
- Autoimmune diseases mostly belong to type II and III hypersensitivity

In conclusion must be said that the most important the three points of autoimmune disease pathogenesis, and we can pick them out:
1. Disturbances in proper immune mechanisms
2 Infection and associated with infection cross-linked antigens
3. Genetic factors

Autoimmune disease treatment includes prescription of:
- immunosuppressive drugs

- glucocorticoids
- anti-thymic globulin
- plasmopheresis that eliminates immune complexes out of blood
- symptomatic therapy directed against an inflammation and associated with it pain

Type IV of hypersensitivity

According to its immunologic mechanisms this type may be divided into two variants: delayed type or cytotoxic one.

Classification of type IV hypersensitivity

I. Delayed type when CD4+ lymphocytes predominately are involved:
- tuberculin skin test (Mantoux)
- lepromatin skin test
- contact dermatitis
- skin hypersensitivity to ivy and oak and granulomatous inflammation

II. Cytotoxic type when CD8+ lymphocytes predominately are involved:
- graft rejection
- lysis of infected with viruses, bacteria and parasites cells
- anti-tumor immunity

The antigens of type IV hypersensitivity reactions are very multiple and include both infectious and non-infectious substances, moreover foreign proteins and cells.

The causative factors of type IV hypersensitivity reactions

- Simple chemical agents (haptens) which react with the proteins (as carriers) of the host
- Infections: viruses, bacteria, and parasites
- Transplants
- Modified under different causes tissue elements of the host

THEY ALL ARE THE ENDOGENEOUS SUBSTANCES!

Last fact can explain, why this type of hypersensitivity reactions rather challenges the cellular immunity. Additionally must be said that described above antigens possess much in common with the tissue elements of the host.

The common features of type IV:

- It is cell-mediated response
- Delayed type is associated with Th1 cells and MHC class II, but cell-mediated toxicity- with $CD8^+$ and MHC class I involving
- Memory pool of Th-1 cells is a very important to supply with information about antigen and supporting an immune response
- The main cytokines are involved in these reactions:IL-2, IL-12, IF-gamma, and TNF (alpha and beta forms)

Overview of the DTH response

(a) Sensitization phase

Intracellular bacteria

APC

CD4+ TH

T_H1 cells (generally)

Antigen-presenting cells: Macrophages Langerhans cells

DTH-mediating cells: T_H1 cells generally CD8 cells occasionally

(b) Effector phase

Secreted IFN-γ

Sensitized T_H1

Membrane TNF-β

Resting macrophage

Class II MHC

TNF receptor

Activated macrophage

T_H1 secretions: Cytokines: IFN-γ, TNF-β, IL-2, IL-3, GM-CSF Chemokines: IL-8, MCAF, MIF

Effects of macrophage activation: ↑ Class II MHC molecules ↑ TNF receptors ↑ Oxygen radicals ↑ Nitric oxide

Kuby J et al., Immunology 2003

Picture 6. Pathogenesis of delayed type

Upon the first exposure to an antigen, macrophages, dendritic cells an B-lymphocytes take a contact with antigen together with MHC class II, and after processing of the antigen (release of the epytops or antigen determinants) these antigen-presenting cells pass an information about antigen to the Th type 1 but, in any case, together with MHC class I (transformed cellular peptide). Processed antigen in complex with cell peptide triggers proliferation and differentiation of Th type, but only after interaction with T-lymphocyte membrane receptors TCR+CD3 and some costimulating receptors. One part of such T-helpers forms a memory cell pool to keep an information about antigen for enough a long time. the last facilitates repeated immune responses to specific antigen.

Upon re-exposure, the pull activated cells and memory cells secrete the lymphokines listed above. These lymphokines ,on the one hand, provoke proper reaction in form of specific inflammation, but on the other hand, during repeated antigen re-exposer a clone of memory cells make delayed reaction of hypersensitivity non-fading and long termed. It must be added, that term "delay" underlines a time of this type manifestation, which occurs not an earlier then in 24-48 hours after re-exposure of an antigen.

Clinical manifestation of delayed type hypersensitivity ;
- Mantoux reaction (tuberculin skin test) is characteristic of local skin area and erythema with induration peaks at about 48 hours following intradermal injection of tuberculin
- Contact dermatitis and granulomatous inflammation, in contrast to skin tuberculin test, characteristic of aggregation of epithelioid cells

- In conclusion must be reminded that cell-mediated cytotoxic hypersensitivity is important in viral infection, tumors, and graft rejection but delayed type can support cytotixic type too.

Each immune injury is mediated by special type of immune cells with their cytokines. As for delayed type of hypersensitivity, Th 4+ lymphocytes type 1 and associated with them cytokines must be called, at the same time, cytotoxic reactions are associated with predominately activation of cytototoxic CD8+ lymphocytes with their cytokins.

Mediators of delayed type of hypersensitivity with their effects

Mediators	Effects
IL-12	Drives differentiation of native CD4+ to CDh1 cells Provokes IF-gamma secretion by T-cells and NK+ cells Inhibits humoral reactions of hypersensitivity
IF-gamma	Activates Th1 cell differentiation and phagocytosis Forces an expression of MHC class I and II molecules to help an immune recognition of an antigen
IL-2	Provokes T-cell proliferation (strong mitogen)
TNF-alpha	Stimulates adhesive properties both, the leukocytes and endothelium, leukocyte emigration (especially, monocytes and lymphocytes) Stimulates IL-8 secretion and, such way, chemotaxis of neutrophils Stimulates blood flow in the site of inflammation through PGE-2 synthesis
TNF-beta	mainly produced by T-lymphocytes and possesses by strong cytotoxic or (lymphotoxin) cytolytic influences the surrounding cells and, such way, provokes their injury

Transplant rejection

Transplant rejection involves several types of hypersensitivity reactions and, nowadays, it attracts the interests of both, the scientists and clinical specialists of many medical branches to solve the problems of tissue incompatibility. Last is the first problem on the way to overcome an immunological rejection of tissues or organs, however, the transplantation of skin, kidneys, heart, lungs, liver, spleen, bone marrow and, endocrine glands are in the surgery practice.

Common features and pathogenesis of a graft rejection:

- Antigens (transplants) possessing by MHC class I are different to the recipient
- Graft rejection is a complex process in which both, cell-mediated immune reactions and circulating antibodies play role
- As for cell-mediated reactions, both via activated CD8+ cytotoxic lymphocytes and Th cells type I trigger and support the rejection, however, CD8+ cells play leading role in a lyzing of graft. At the same time, Th type1 lymphocytes through their cytokines for enough long time support the reaction via attraction and proliferation the mononuclear cells, moreover, these lymphokines increase vascular permeability

- Cells that are responsible for the onset of reaction seem to be the T cells of the transplant; they recognize the allogenic MHC molecules class I on the surface of antigen-presenting cells of the donor
- Antibody-mediated reactions play not such important role as cellular responses
- Hyperacute rejection occurs when preformed anti-donor antibodies are present in the blood of recipient, for example, when prior blood transfusion from HLA-non identical donor In such circumstances rejection occurs immediately after transplantation
- Antibodies usually injury the small blood vessels of the graft with complement involving, but CD8+ lymphocytes mostly struggle against the parenchymatous cells

Blood deprivation of the transplant ultimately leads to its acute vasculitis following by organ necrosis and rejection. However, graft surviving me may be prolonged using the following measures:

1. minimization of HLA disparity between the donor and the recipient matching all possible histocompatibility antigens
2. immunosuppressive therapy with azatoprene, corticosteroids, cyclosporine, and anti-lymphocytic globulins. However,.such therapy may lead to opportunistic infection and tumor disease
3. before the transplantation of allogenic hematopoietic cells, usually in leukemia, not only immunosuppressive drugs but local irradiation can be used

Lecture 9
Disturbances in hemostasis

Introduction

Definition Hemostasis is a complex of physiological events which supports blood in fluid, but in case of bleeding provides thrombi formation.

In normal hemostasis is maintained due to the steady state of the following parameters:

1. Properties of the vessels wall, including endothelium, subendothelial matrix, and collagen fibres
2. Quantity and quality of the blood elements, rather platelets and erythrocytes
3. Cooperation of four plasma systems: clotting, plasmin, kallikrein-kinin, and system of complement.

All disturbances in hemostasis principally may be divided into hypo-or hypercoagulation. The former belongs to the disease of hemorrhage (hemorrhagic diathesis) but the later, to thrombotic conditions. It must be added, that hemorrhagic diathesis more characteristic of the children, but as for thrombotic conditions, they more often occur in the adults, and their risk increases in aging.

The process of thrombus formation passes through two stages. The first is named the primary hemostasis, and the blood vessels and platelets are involved in it. The second one is named coagulation hemostasis, when the clotting cascade not only completes thrombi formation but forms very solid thrombotic plug. It must be

added, that simultaneously blood plasmin system realizes obligatory control of the coagulation process.

Mechanisms of primary hemostasis

Spasm of small blood vessels immediately follows any trauma. In this process the next factors take part:

- Axon reflex and spasmogenic biological active substances such as catecholamines, releasing from sympathetic endings of the corresponding fibres
- Endothelins from endothelial cell
- Serotonin from the platelets

Primary hemostasis mechanism

Injury of endothelium

↓

Platelets adhesion

↓

Platelets activation with their degranulation and release of biological active substances plus the changes in thrombocyte shape (viscosity metamorphosis)

↓

Platelets aggregation and formation of primary non-stable plug

All above described mechanisms may provide a stop of bleeding without involving more complex mechanisms of hemostasis regulation and control. If these mechanisms are not efficient enough, some later, the mechanisms of secondary hemostasis start to work. They are more complex and form a very solid hemorrhagic plug. It must be said, that primary hemostatic plug, as a result of primary hemostasis activation isn't stable, and only secondary hemostasis can provide a real solid hemostatic plug due to intensive thrombin and fibrinogen formation, but it needs a time. As a result, local thrombi are organized with simultaneously plasmin system activation. The last limits excessive thrombosis and, moreover, spreading thrombi over an organism via blood circulation.

In normal blood vessels wall is thromboresistive that provides its oppose to local thrombosis after injury, more often observing in aging people

Thromboresistive factors in hemostasis

- Secretion of NO and PgI-2 by endothelial cells
- Presence of heparin-like molecules on the endothelial membrane
- Binding thrombin with thrombomodulin
- Plasminogen activation by tPA (tissue plasminogen activator)
- Activation of C-protein blood system with its cofactor S
- Fibrinolysis activation

Just after vessels injury collagen becomes naked, and subendothelial matrix is ready to contact with platelets. Collagen is a main adhesion stimulator of any blood cells, including the platelets, and the wider a surface of naked collagen, the more intensive its adhesion.

Another important factor of adhesion is Von Willebrand's factor, which synthesized by the liver and endothelium. Expression of this factor is increased many times when vessels injury with following its exposure on endothelial surface.

Parallel with its expression the receptors to Von Willebrand's factor on the platelets are growing; they named the glycoprotein Ib. Combining with Von Willebrand's factor, gp-Ib forms the lot of bridges between endothelial cells and platelets.

Hence, decreasing in vessels thromboresistance or decrease in number of the platelets and their adhesive properties (defective platelet function) may lead to the disturbances in primary hemostasis in form of hemorrhagic diatheses

The disorders related to disturbances in primary hemostasis

Like the other diseases, they all may be divided in hereditary or acquired.

The following hereditary syndromes and diseases may be illustrated by the disturbances in primary hemostasis associated with abnormalities in adhesion and aggregation of the thrombocytes :
1. Bernard- Soulier syndrome
2. Von Willebrand's disease
3. Glanzman's thrombasthenia

Bernard-Soulier syndrome as a variant of thrombocytopathy

It is autosomal-recessive disease when synthesizing of gp-Ib and its expression on the platelet membrane are disturbed. In this case the adhesion abilities of the platelets are decreased, that ultimately results in primary hemostasis alteration in form of bleeding.

Von Willebrand's disease

Yet the other basic mechanism is a pivotal factor in pathogenesis of Von Willebrand's disease. It is hereditary lack of vW-factor synthesized by the endothelial cells and liver. It is a very complex disease of hemostasis, which includes both abnormalities: in primary and secondary hemostasis. As for primary hemostasis, deficiency of vW-factor leads to abnormal platelet adhesion but, as for secondary hemostasis, we must keep in mind that this factor is known as a carrier of factor VIII - one of the members of the clotting cascade assembly. In such form of deficiency coagulation hemostasis works inappropriately. The last means inability of solid plug formation in case of vessels injury. The disease named pseudohemophilia, because the base of hemophilia concerns with the lack of VIII factor too. Nevertheless, the basic mechanism of hemophilia is only X-linked hereditary lack or weak activity of factor VIII of clotting system. The disease is X-linked and for this reason occurs only in men. Finally, must be said that both diseases have much in common, clinical sounds and laboratory indices.

Platelet aggregation in normal

Such substances as ADP and TXA-2 set the reaction of platelet aggregation, and new portions of thrombin and fibrinogen prolong them. PGI-2 (prostacyclin) is a strong antagonist of such type reaction because it provokes platelet disaggrega-

tion. From here becomes clear that the blocker of cyclooxygenase aspirin must inhibit TXA-2 formation, but at the same time, it activates PGI-2 synthesis that helps to decrease a risk of thrombi formation.

Glanzman's thrombastenia

It is a disease of primary hemostasis associated with hereditary lack of gp IIb IIIa receptors to fibrinogen on the platelet membrane. The disease is characteristic of enough severe and prolong hemorrhages due to failed platelet aggregation.

Platelet pathology

Before the deep analyzing of platelet pathology it's necessary to list their functions.

Platelet functions

- Play role of bread winners of blood vessels wall, secreting the growth factor which provides nutrition and renewal of the epithelial cells and basal membrane
- Provide a primary plug formation
- Serotonin secretion contributes to early spasm of small blood vessels and, such way, to stop bleeding, moreover, their ADP and TXA-2 take part in further platelet aggregation and vessels spasm
- Provide the secondary hemostasis via secreting clotting factors and supply organizing thrombotic mass with the phospholipid matrix
- * They are the sources of Ca++ and ADP which are the potential accelerators of platelets aggregation

Generally, the platelets contain alpha-granules and dense bodies as the storages of multiple biological active substances. It's doubtless, that decrease platelets in number or disturbances in their storage and secretion function may result in pathology of hemostasis. Such kind of pathology, as usually, may be divided into hereditary and acquired forms. Hereditary forms are known as the diseases of thrombocytic pool storage, but the acquired examples are the following:

- Unfavourable consequences of treatment with aspirin which inhibits cyclooxygenase activity, and such way decreases TXA-2 synthesis
- Uremia when urine toxins alter aggregation and adhesion of the platelets
- DIC syndrome when not only number of the platelets is decreased, but there are the serious disturbances in platelets adhesion and aggregation

As for clinical manifestation, cutaneous bleeding is seen in form of pinpoint hemorrhages (minute red pots due to escape of a small amount of blood). Petechiae especially are prominent in the dependent areas where the capillary pressure is higher. They may become confluent and give rise to ecchimoses (larger than peteciae up to 1-2cm in size) Often there is a long history of easy bruising and not rare nosebleeds.

Laboratory indices used in thrombocytopathy revealing

- Prolong bleeding time (in normal 2-9 minutes)
- Platelet counts usually in normal

- Prothrombin time - PT normal
- Partial thromboplastin time - PTT normal
- Some platelet function tests can reveal abnormalities in platelet adhesion, aggregation, and secretion

Hemorrhagic diatheses related to thrombocytopenia

The causes of thrombocytopenia may be represented by both acquired and congenital factors, besides, of immune or autoimmune origin

The causes of thrombocytopenia

1. Diminished production of thrombocytes by the bone marrow:
   - Aplastic anemia of different reasons
   - Leukemia
   - Megaloblastic anemia
   - Drug-induced chemotherapy
   - Alcohol
   - Infections: HIV, measles
2. Shortened life-span of the platelets on the periphery (in the blood):
   a. autoimmune injury of the platelets:
   - idiopathic thrombocytopenic purpura
   - systemic lupus erythematosus
   b. isoimmune: post-transfusion and neonatal states
   c. heteroimmune:drug-associated (guanidine, heparin, and sulfa- compounds)
3. thrombotic microangiopathies in form of thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome
4. infections: infectious mononucleosis, HIV, and cytomegalovirus

Idiopathic thrombocytopenic purpura

It is essential or idiopathic form of autoimmune origin. Immune thrombocy-topenic purpura (ITP) is characteristic of purpura (redness) when there is a special type of skin or mucosa hemorrhages in form of petechiae or ecchimoses.

We are known the two variants of the disease: acute and chronic. Acute form is very characteristic of children and, sometimes, may occur in self-limited form.

The common features of ITP

- Both sexes are involved in the disease
- Sudden treacherous onset, very often in 14-21 days after infection, especially a viral which supports an autoimmune origin of the disease.
- Anti-platelet antibodies belong to IgG class
- Death of the platelets corresponds to reaction type II hypersensitivity (cytotopenic)
- In 75-80% of the patients splenectomy is very beneficial
- Activation of megakariocytopoiesis in the bone marrow
- As for the symptoms, petechiae and echimoses, especially provoking by trauma that are often symmetrically .The favourite sites of hemorrhages are

the nose, mouth flour, and soft tissues. Melena, hematuria, and menstrual bleeding are characteristic too

Characteristic laboratory findings include:

- Accelerated thrombocytopoiesis with formation of abnormally large megakariocytes
- Prolong bleeding time (in normal 2-8min)
- Decreased number of platelets (in normal 180-450x10.9 per liter)
- All indices characteristic of secondary hemostasis disease, such as PT (prothrombin time) and PTT (partial thromboplastin time) are in normal
- Tests to anti-platelet antibodies presence are not widely available

Hemorrhagic diatheses related to abnormalities in blood vessels wall

They also may be qualified as hereditary or acquired and a very characteristic of aged people; but other causes also may provoke such type of pathology

Causes of hemorrhagic diathesis due to abnormalities in small blood vessels wall

- Multiple infections including meningococcemia, sepsis and severe forms of ricketsiosis; they can provoke the vasculitis and DIC-syndrome
- Drug-dependent diseases associated with immune reactions and complex antigen – antibody depositions in the vessels wall.
- Scorbutus (severe vitamin C avitaminosis) and Euler-Danlos syndrome, both related to different types to collagen abnormalities (collagen supports elastic component of the vessels wall)
- Cushing syndrome with its protein- wasting effect leading to increase in vascular permeability
- Henoch- Shonlein purpura or capillary toxicosis (hemorrhagic vasculitis). It is systemic reaction of hypersensitivity initiated by immune complex deposition in the small blood vessels wall all over the body, especially the kidneys (the organs of elimination or filtration of waste products)
- Hereditary teleangioectasia. It is autosomal dominant disease characteristic of an appearance of narrow and tortuous very thin blood vessels prone to bleeding

Clinical features and laboratory data

More often bleedings aren't significant (petechiae or purpura), visible on the skin and mucosal line, but may be bleeding into joints and subperiostal region or soft tissue. Rare, but hematuria, metrorrhagia or nosebleeding occur too.

As for serum laboratory findings, usually only blood vessels resistance is decreased, but others indices revealing hemostasis abnormalities are negative.

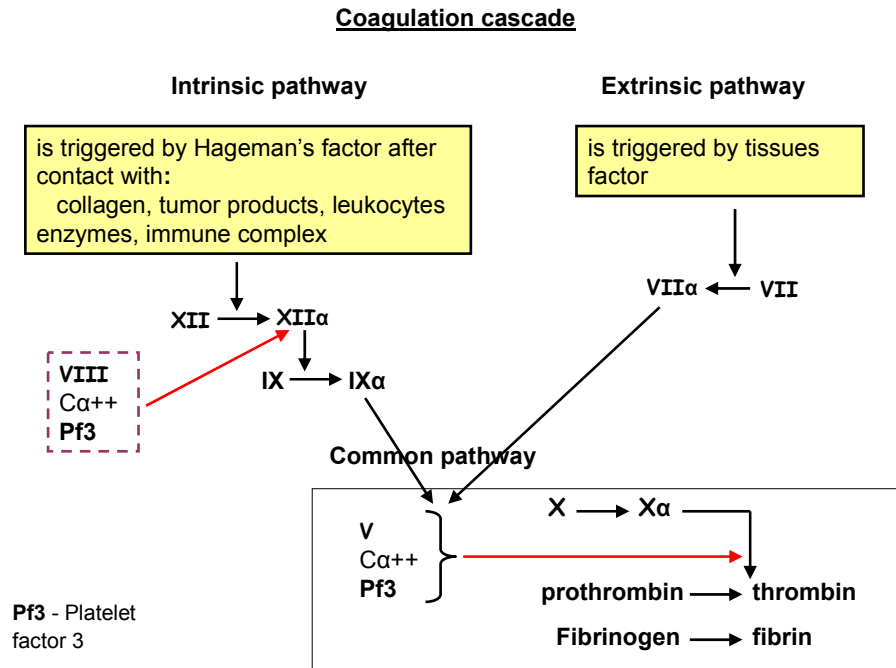*Pathology of secondary hemostasis*

Coagulation cascade seems to be the third component (after endothelial cells and platelets) of hemostasis, and it is the major component, because only it can support the firm thrombotic plug formation.

Due to its nature, it is the cascade of reaction, when each non-active component (pro-enzyme) is converted into active form (enzyme) in presence of its accel-

erators; Ca++ -ions mostly play role of those accelerators. The cascade includes 4 dynamic steps:
1. Prothrombinase formation
2. Thrombin formation via prothrombin convertion into thrombin
3. Under thrombin fibrinogen converts into fibrin
4. Clot retraction (high density clot without serum) formation, and then- lysis of the clot

Coagulation cascade

Intrinsic pathway

is triggered by Hageman's factor after contact with**:**
    collagen, tumor products, leukocytes enzymes, immune complex

Extrinsic pathway

is triggered by tissues factor

XII ⟶ **XIIα**

VIIα ⟵ **VII**

VIII
Cα++
Pf3

IX ⟶ **IXα**

Common pathway

Pf3 - Platelet factor 3

X ⟶ Xα

V
Cα++
Pf3

prothrombin ⟶ thrombin

Fibrinogen ⟶ fibrin

Picture 7. Picture of two pathways of blood coagulation

This assembly occurs on the surface of erythrocytes, predominately, on their phospholipid matrix, and all components stand together due to the Ca++ claying role. The clot is held by the endothelial cells or platelets.

It must be said, that thrombin is a central figure not only of blood coagulation, but nflammatory response too, because it restricts further spreading of pathogenic factors via blood vessels.

However, simultaneously, through cooperation with thrombin receptors and formation of complex thrombin-antithrombin, excess of blood coagulation is inhibited. This complex is activated by heparin- like molecules on the endothelial surface, and hence, it is become clear a very beneficial role of heparin in thrombotic conditions treatment.

Traditionally, the pathways of coagulation are divided into two forms: intrinsic and extrinsic. The former is activated by factor XII-Hageman's, the latter-by tissue factor.

Intrinsic pathway is triggered by XII factor activation that previously was in contact with naked collagen, proteases from leukocytes and altered plasma. Then the factor VIII, Ca++ and platelet factor 3 are combined together in form of complex, converting IX non-active factor into IXa (active) one.

Extrinsic pathway is shorter, when tissue factor, as initiator in case of massive tissue trauma, activates factor VII converting the last into VIIa. But both converge at the point when X factor turns into Xa. The following factors are necessary to transform X-factor into Xa; V factor, Ca++ and platelets factor 3.

Due to the necessity of certain factors (the participants), either extrinsic or intrinsic pathways to be involved in coagulation, we can estimate or differentiate the type of disturbances (developing according to intrinsic or extrinsic pathway) via special tests, involving the special clotting factors.

Tests to reveal the abnormalities in clotting cascade activity

1. Test to reveal the disturbances in intrinsic pathways-PPT is partial thromboplastin time- PTT
2. For extrinsic pathway disturbances -PT-prothrombin time must be assayed

Anticoagulant system

It includes:
1. Fibrinolysis under tissue plasminogen activator (predominately containing in macrophages). The system of plasmin is challenged by XII factor, triggering simultaneously by clotting cascade activation
2. Endogenous anticoagulants support this effect. They are: heparin, anti-thrombin II, and protein C with cofactor S
3. All described above are the primary anticoagulants.
The following substances belong to secondary anticoagulants: the products of fibrin degradation which are formed in process of strong of coagulation (DIC-syndrome) and antibodies formation against the clotting factors. Treatment with poor-cleaned heparin may lead to such type of pathology too.

The diseases of the secondary hemostasis

In practice, deficiency of any clotting factor, hereditary or acquired origin, may lead to such type of disturbances, and as a result- to bleeding disorder.

Hemophilia is a bright example of such kind of pathology. Hereditary or acquired lack of any clotting factor, synthesized by the liver, belong to the diseases of secondary hemostasis too. It must be added that DIC-syndrome includes both type of hemostasis disturbances, primary and secondary.

Hemophilia Etiology and pathogenesis

There are three variants of hemophilia: A, B and C, but hemophilia A is the most spreaded variant and accounts for 80% cases of a disease. Both types of hemophilia, A and B are x-linked diseases. There is a deficiency or abnormality of factor VIII is a base of hemophilia A, but in case of hemophilia B it is factor IX (Christmas factor). It must be said that in any case a severity of disease is directly proportional to the rest concentration of named clotting factor in the blood or its activity. In hemophilia A, when disease is very severe, the blood level of factor VIII is near 1% of normal activity, in moderate form its activity is about 2-5%, but in the mild form-6-30%.

Common features of hemophilia

- Practically only men suffer from the disease due to x-linked hereditary form, but the women are the carriers of the pathological gene situated on the x-chromosome
- Hematomas type of bleeding or appearance of ecchimoses, but not just after trauma (postponed type); at the same time, petechiaes as a variant of bleeding are absent
- There is no solid hemorrhagic plug because secondary hemostasis doesn't follow the primary one
- Spontaneous bleeding is possible, but more often after trauma (extraction of tooth or injury of another reason), especially in children
- Hemarthrosis is very characteristic and, sometimes, the patient needs an operative surgery
- Hematomas may be formed in soft tissue
- The most danger is the hematoma in the brain

Laboratory findings characteristic of hemophilia
- Time of bleeding isn't changed
- Content of the platelets usually in normal
- PT-in normal
- Vessels wall resistance in normal
- PTT is prolonged
- Decreased factor VIII or IX in the blood correspondingly to type A or B hemophilia

The treatment minds replacement therapy in form of fresh blood or freezing;transfusion of lack factor is the best. Nowadays, recombinant protein (factor VIII is synthesized), but it is very expensive

DIC-syndrome (dissiminated intravascular coagulation syndrome)

There are some clinical variants of the syndrome: acute, subacute, and chronic one. An acute form is often a life-threatening complication of such pathology as sepsis or severe obstetrics situations. It is not disease, as nosologic unit, but rather non-specific dreadful complication with possible mortal outcome.

DIC-syndrome etiology includes both, infectious and non-infectious factors, but it is necessary to keep in mind that usually it is life-threatening condition with high level of mortality

ETIOLOGY OF DIS syndrome

I. Obstetric complications
2. Infections:
   a. gramm-negative sepsis   b. meningococci   c. malaria
3. Malignant tumors:
   a. carcinoma of pancreas   b. lung and stomach carcinoma
   c. acute promyelocytic leukemia
4. Massive tissue injury:
   a. trauma   b. burns   c. intensive surgery
5..Miscellaneous:

a. acute intravascular hemolysis   b. snake bits   c. vasculitis
d. liver disease   e.   heat shock

Due to a non-specific response to any trauma, just after injury the two pathways of clotting cascade are activated. The DIC-syndrome passes three stages.
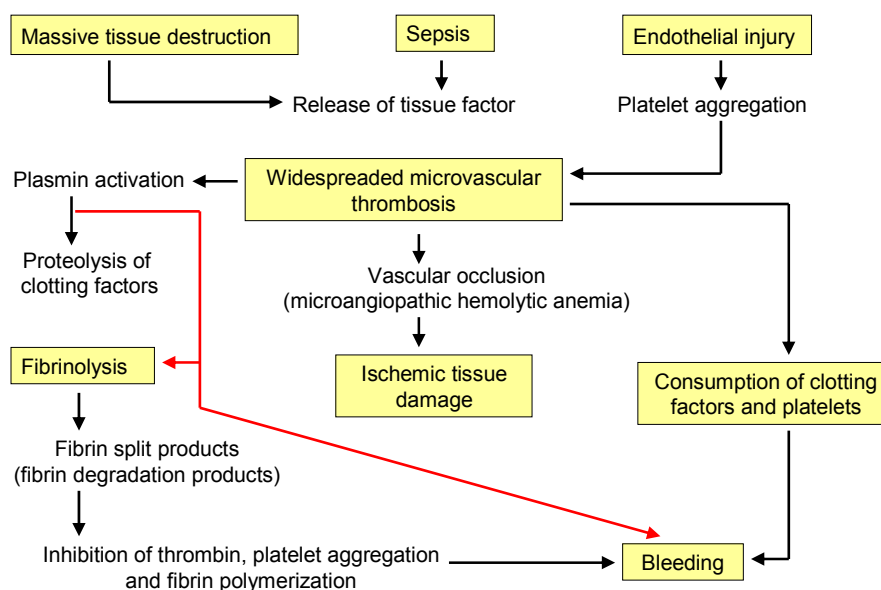
The stages of DIC-syndrome
1. Activation of coagulation with hypercoagulation
2. Assumption of the blood elements and clotting factors with their deficiency
3. Activation of fibrinolysis
4. Recovery stage

Pathogenetic features of DIC-syndrome

Hypercoagulation is provoked by severe injury due to release of tissue factors and activation of Hageman's factor in a contact of plasma with different surfaces. As a result, there are the spreading thrombosis of the microcirculatory vessels and ischemic tissue injury. A blockade of microcirculation in life-important organ: brain, lung, kidney may result in death. The next stage is manifested by the consumption of the platelets and clotting factors when blood is exhausted the factors which are necessary for normal coagulation. The second   stage of DIC-syndrome starts, and it ultimately leads to fibrinolysis activation. That time, Hugeman' factor, except triggering the coagulation cascade, activates the three other plasma systems: plasmin, kallikrein-kinin, and complement systems. High plasmin system activity may result in conversion of the first stage of pathology into third-fibrinolysis. It must be added that formed thrombi are very small and not enough solid. They easy degraded under proteolytic enzymes with outcome of the secondary anticoagulants appearance in the blood. The lasts seriously complicate pathologic condition. These substances, named fibrin degradation products, play a very negative role provoking, sometimes, not-faded profuse bleeding. Simultaneously, activated kallikrein-kinin plasma system, mostly via bradikinin, increases small vessels permeability with its all negative consequences. As for active fractions of complement system, their effects are the pro-inflammatory and cell- injuring.

Pathophysiology of DIC-syndrome

Picture 8 Pathophysiology of DIC-syndrom

Principles of DIC-syndrome treatment

- Etiotropic treatment
- Replacement the cellular constituents of the blood
- Hemostasis correction
- Inhibition of plasma anti-protease activity
- Plasmapheresis
- Anti-aggregants
- Replacement of lost blood
- Normalizing of ABB and electrolyte balances
- Treatment of organopathology

Lecture 10
STRESS AND GAS ROLE IN PATHOLOGY

Conception of stress was introduced into medicine by Canadian physiologist Hans Selye in the year 1936, and as we are known he borrowed this term from the mechanical sciences. Under this terminology he minded a pressure of different extreme factors to an organism These factors can be either of external or internal origin, infectious or non-infectious nature; for example, intensive surgery including massive blood loss, mechanical or barotrauma, burn, severe infection, autoimmune disease, and malignant tumors. Stress may be acute or chronic, and even of psychological nature.

Definition: it is a non-specific complex of psycho-physiological reactions of organism as a response to the life-threatening factors.

Experimental study of stress

In process of research of stress on the animals (white rats) Selye came to the conclusion that stress passed through three stages. The first stage was called him as alarm reaction, and it usually lasted for 24 - 48 hours. At this stage all reserved defensive possibilities of an organism usually become mobilized to struggle against a pathogen. The next second step named the phase of resistance when due to the long-standing compensatory mechanisms, very steady, an organism becomes adapted to stress and copes with the pathogen for enough a long time. In this case we say about a favourable course of stress. The third stage named the exhausting and its name can explain everything. The conversion of resistance stage into exhausting one is associated with an insufficiency of adaptive mechanisms to oppose a pathogen and destructive processes in the organism, provoked by stressor. The stage is very danger and may lead to a death.

Analyzing the different tissues and organs of the experimental animals under stress provoked multiple pathogens(formalin injection, exposition of the animals to loud sound or immobile condition during 24 hours), Selye came to conclusion that non-specific triads of the symptoms was very characteristic of the stress. The triad described by Selye consists of:

- -hypertrophy of the adrenal cortex
- thymic gland and lymph nodes involution
- -in 30%-40% of cases there were the ulcerative processes in the gastrointestinal tract.

Selye described morphologic and functional changes in adrenal gland of the rats, endured stress, according to three stress stages. At the first stage adrenal cortex was very rich of the secretion granules with their high activity, accompanied by an excessive cortisol production. At the second stage there was a very steady hypertrophy and hyperplasia of the adrenal cortex, those were provided by the lot of mitosis in this area, naturally, with increased cortisol secretion. But at the third stage an adrenal cortex became very thin and exhausted of secretion granules. Clinically it sounds that stress seems to be is one of the reasons of an acute adrenal insufficiency. It must be added, that study of Selye came us to an idea of replacement treatment of patients with short-termed, high doses of cortisol in case of extreme situation, associated with severe stress. Besides, excess of cortisol in stress also can explain an involution of thymus and lymph nodes as a variant of immunosuppression. Such cortisol ability get used in the hypersensitivity conditions treatment. But cortisol in a large dose may provoke the ulcers in gastrointestinal tract. So, according to Selye's observations, stress seems to be the condition of hypercortisolism which possesses by double meaning, positive and negative.

General adaptation syndrome (GAS)

The term GAS introduced by Selye minds a non-specific reaction of whole organism to the stress situation. Now we consider it as a part of an acute phase response, however, GAS seems to be the only the part of an acute phase response

when hypothalamo-pituitary-adrenal glands system plays a pivotal role. There is no doubtful, that Selye didn't study precisely the behavior of immune and hematopoietic systems during the GAS, but hypothalamo-pituitaty-adrenal axis activation precisely was in his field of view.   GAS seems to be only a neuroendocrine part of an acute phase response. It must be added, that in difference to acute phase, response, GAS can be provoked not only by infectious and noninfectious factors (material substunses) but psychological factors too, moreover, we can single out a psychological stress.

The ways of GAS realizing may be represented by the following chain of the events:
- *Stressors activate different receptors of an organism: exteroreceptors – nociceptors which   are found in the skin, mucosal lines, interoreceptors in the blood vessels wall, and sleeping nociceptors in the internal organs, moreover, analyzers, such as visual and hearing receptors
- Next step is activation of monoaminergic neurons in CNS which control the peptidergic neurons of hypothalamus. It mast be added, that monoaminoergic mediators include such very important neurotransmitters as catecholamines, serotonin and GABA. They are responsible for a balance between releasing and statin- factors secreting by the hypothalamus
- CRF, in turn, stimulates elaboration and secretion of ACTH by basophil cells of the pituitary gland
- The last event in described above chain is synthesizing and release of glucocorticoids by the adrenal cortex

All these events are accompanied by high level of blood catecholamines, secreting by the sympathetic endings of corresponding fibres, adrenal medulla, and other variants of chromaffin tissue sources. Such reactions result in flooding of the blood with catecholamines and glucocorticoids

So, the stress axis, according to Selye's theory, may be represented in form of the following sequence of the events depicted below:

Hypothalamo-pituitary-adrenal gland axis activation as a base of stress-reaction
Stressor influence the different receptors including analyzers
↓
Activation of monoaminergic synapses in CNS
↓
Hypothalamus activation and CRF secretion
↓
Secretion of ACTH by pituitary gland
↓
Increase of synthesis and production of glucocorticoids by adrenal cortex

It is not doubt that sympathetic nerve system plays role of an initiator in stress reaction, but at the same time, the response in form of high cortisol production supports and prolongs the endocrine pattern of GAS.

Hence, we have two major hormones of stress, catecholamines and glucocorticoids. However, effects of catecholamines is shorter in comparison with glucocorticoid ones. The lasts can support a long- standing adaptation of an organism to stress because, on the one hand, glucocorticoids enhance and prolong sympathetic nerve system effects but, on the other hand, high level of blood glucose is maintained not only by the catecholamines but by such effect of cortisol as gluconeogenesis. Both activate lypolisis for energetic needs and, eventually, catecholamines accelerate systemic blood flow. Nowadays, serious surgery with postoperative trauma, burns, massive blood loss and accident of bronchial asthma in form of status asthmaticus need the glucocorticoids for treatment. It is forced to life safe measures in form of hormonal replacement of failed cortex glucocorticoid function during a stress.

Protective role of stress hormones
Catecholamines

At the start of XX century French physiologist Claude Bernard, studying the level of blood catecholamines in the animals under life critical situations, signed them as the hormones of the "fight and fly."

These hormones can help not only the animals but the man too to overcome physical and psychical extreme situations. Being the first line of a defense in these life-threatening conditions, catecholamines mobilize all reserves of an organism. They possess by positive physiological and psychological effects. On the one hand, these hormones influence positively the metabolism of tissue and organs but, on the other hand, activate psychical abilities, and such way they postulate an appropriate behavior to organism surviving in extreme situation.

What are the mechanisms of defensive influence of catecholamines during a stress?

1. Support a proper level of glucose in the blood via activation of glycogenolysis in the liver.

Fat tissue is one of the most important target to catecholamines, and via beta-1 adrenoreceptors they activate lipolysis when free fatty acids can be used as a high energetic substances. It is especially important for a supporting of the heart and strip muscle activity during "the fight and fly"

2. Influence the cardiovascular system. Possessing by positive chronotropic and ionotropic effects, they correspondingly promote tachycardia and increase in a heart stroke volume, and, such way, increase minute cardiac volume. This, in turn, results in adequate organs blood perfusion when metabolic request is higher than in normal. They also maintain an appropriate arterial pressure, both systolic and diastolic

3. Redistribution of the blood flow in use of the most life-important organs (brain and heart) is a function of the catecholamines too. Via activation of alpha receptors of the small vessels of the peripheral organs they drive the blood into central organs and increase end- diastolic presser in the heart that, in turn, increases heart stroke volume. Moreover, via beta-adrenoreceptors epinephrine dilates the brain and coronary heart vessels which so necessary for their high activity.

4. Increase in rate and depth of breathing to support an organism with oxygen supplying.
5. Mobilize a psychical activity to rational behavior and avoiding of the mistakes in that not easy to organism situation.

Catecholamine adaptation of the newborns, altering their delivery is the bright example of positive influence these hormones a stress- reaction.

Possible causes of their stress may be evoked by an inevitable hypoxia under which newborns have occurred with during the passing through the delivery ways. However, the pediatricians believe the stress is useful for the newborns due to a very high level of the blood protective catecholamines in their organism that time.

Comparison of the analyses of blood catecholamine content in the newborns with the men content during physical exercises, in sauna and in women during delivery, moreover, in a patient with pheochromacytoma (benign tumor arised from adrenal medulla and producing cathecholamines)) revealed that the most concentration of these hormones is really in the blood of the newborns. The catecholamines protect the newborns, make them more adaptive to the new life. It was estimated that the children born "per vias naturalis" are more resistant to possible unfavourable life conditions than those, who beared by Caesar's section. Protective effects, except described above, includes reabsorbtion of the water from the surface of the lung just after delivery and synthesizing of surfoctant which decreases surface tension in alveoles to avoid of their collapse in respiration.

Catecholamine response of the newborns

The content of catecholamines in blood in Nm/l (pcg)
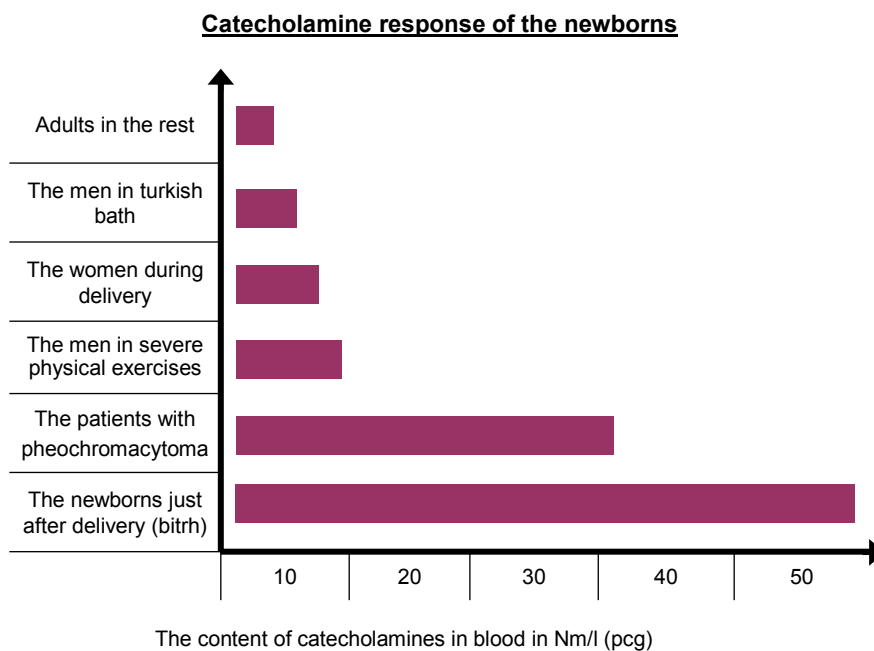
Picture 9. Catecholamine response of the newborns in comparison with the same of other groups of people

Besides, activation of sympathetic nerve system results in dilation of the pupils, and possibly, takes part in initial sense attachment (love) between mother and her child. According to the photos of the newborn's faces they looked happy when enter our world.

Glucocorticoids

They provide long-termed protection of an organism against the pathogens and unfavourable consequences associated with provoked by them organism destruction. Positive role of glucocorticoids may be illustrated by the following effects to the metabolism and their influence some organs and tissues:

- Like cathecholamines they maintain blood glucose but via active gluconeogenesis and lipolysis
- Support proper blood circulation, acting as permissive substances in relation to the catecholamines
- They are considered as indirect modulators of CNS functions, because a high concentration of glucocorticoids is a result of active secretion of CRF. At the same time, our behavior and, moreover, our immunity depend on the production of CRF by the hypothalamus.

Anti-inflammatory properties of glucocoricoids

Glucocorticoids inhibit release and production of pro-inflammatory mediators via suppression of an activity of different membrane phospholipases. This effect is associated with synthesis of protein lipocortin by the cells in the site of inflammation. Such mediators as histamine, derivates of arachidonic acids, PAF, and different intracellular hydrolases are responsible for multiple phenomena of inflammation. Among them are increased permeability, activation of microcirculation, leukocyte emigration and chemotaxis; in any case become evident that inhibition of their activity is equal to anti-inflammatory effects. In addition must be said that glucocorticoids can inhibit an immune response, especially, cellular, and such way act as immunosuppressive substances.

The most important shifts in blood cell population under glucocorticoids

During a stress the following hematological indices usually observed: leukocytosis with absolute neutrophilia and regenerative left shift of neutrophil nucleus, but lymphopenia and eosinopenia. Glucocorticoids seem to be the cytoclones to the neutrophils but, at the same time, they redistribute and destroy the lymphocytes decreasing in their quantity.

Stress-limiting systems and their role in a stress reaction

As was described above, stress triggers activation of sympathetic nerve system which, in turn, leads to hypersecretion of ACTH and cortisol. But non-limited catecholamine effects   may result in overwhelming destruction of various systems and organs, and go an organism to the ruin. In prolong time of biological evolution some stress-limiting system were developed to protect an organism against its destruction. These systems via their corresponding mediators can limit both, central and peripheral exceed responses to the stress factors.

Among them, the most important are the central opioid, serotonin and GABA systems. It seems they realize a training of any organism to repeated insults that occur in normal too, but at the same time stress-limiting reactions become more active in GAS. As for peripheral stress-limiting system, anti-oxidant, prostaglandin and adenine- nucleotides ones may be mentioned. But the central mechanisms are

the most important for limiting very stormy effects of the catecholamines. The opiate system become involved in a classical response to the stress and realized due to such opioid peptides as endorphins and enkephalins. It must be said that both, ACTH and beta-endorphins have the same source in form of propiomelanocortin which is synthesized in the pituitary gland. However, during the stress they are produced simultaneously in a very high concentration. The other opioid peptides of this family are synthesized in the multiple regions of the brain and by the adrenal medulla enterochromaffin cells.

Effects of opioid peptides

They increase a threshold of pain sensitivity of the nociceptive receptors and such way inhibit a sense of pain during the stress. This, in turn, helps an organism to concentrate all physical and psychical reactions (severe pain restricts these possibilities). The mechanism of pain inhibition is the following: beta-enkephalins play the role of neurotransmitters which are working in serotoninergic antinociceptive system, where they suppress release of substance P by the presynaptic terminates in the secondary neurons, situated in the posterior horns of the spinal cords. The conduction of pain through the secondary nociceptive neurons is inhibited or suppressed completely.

Suppressed activity of sympathetic nerves system under opiates seems to be a result of restriction of catecholamines release by specific presynaptic terminates or catecholamines influence the postsynaptic nerve endings in corresponding synapses. Taking in mind a very important triggering effect of sympathetic nerve system in stress, it's become possible to understand an anti-stressor effect of the opiates.

The next stress-limiting system is serotoninergic. During a stress elaboration of serotonin in CNS, especially, in locus coeruleus and in the regions closely related to limbic system is increased. Undoubtedly, that serotonin influences the behavior of the man realized not only for a stress. However, during the stress, when concentration of serotonin in the blood extremely high, activation of descending anti-nociceptive central pathways and associated with it modulation of the behavior, may help the people to pass through the unfavourable stress situation, especially when it is acute stress.

Moreover, stress-reaction which can be provoked by different factors is accompanied by GABA-system activation. In experiments on the animals GABA-system stimulation inhibited an appearance of stomach ulcer, heart fibrillations, and arrest of an extreme heart activity as the examples stress negative consequences.

All in all, analyzing stress-limiting reactions must be said that glucocorticoids, themselves, are an important line of defense during the stress. Except mobilization of our energetic resources, glucocorticoids possess by strong anti-inflammatory properties, moreover, via immunologic mechanisms they can eliminate foreign tissue substances which can support inflammation. Cortisol inhibits both, release of preformed mediators of inflammation and their synthesis "de novo", including acute phase response cytokines, besides, it seems to be not a very strong but an immunosuppressor.

From stress to the disease

This problem is closely associated with the targets which "are chosen" by stress to injury. It may be supposed some factors which the certain diseases depend on. Among them are the following conditions:

- Genetic predisposition (age, sex, etc) of an organ which is involved
- Intrinsic factors, such as nutrition, possible infection, and psychical condition of an organism at that moment
- Frequency and heaviness of the stress factor action to chosen organ

Nowadays, a very popular the theory of hypokinesia according that lack of physical activity and prolong internal organ's hypofunction may lead to a disease.

Next is, so named, psychosomatic diseases. By psychosomatic disease we call pathology when the start and basic mechanisms of primary disturbances underlie in a psychic (psycho-) but, secondary, concerns with a special organ (somatic).

Now as psychosomatic diseases we mind the following: ischemic heart disease and its life-threatening complication as myocardial infarction, hypertonic disease, and stomach ulcer. Other diseases, for example, neurodermatitis, migrene in form of hemicrania are under our suspicion too. Sometimes, they are named as the diseases of adaptation but rather, according to Hans Selye, they are diseases of crashed adaptation.

Stress and cardiovascular diseases

Cardiovascular system is the main target to stress factors. The patients with ischemic heart disease and essential hypertension are very sensitive to the strong emotions due to a high level of genetic responsiveness of the adrenoreceptors. At the same time, very often such responses are accompanied by activation of the heart and small vessels functions. It needs a lot of catecholamines and correspondingly of cortisol. The hectic function of these organs constantly is under prolonged activation of stressor axis. It leads to periodically or for a long time to flooding an organism with catecholamines and glucocorticois. However, during a long stress-reaction adrenal cortex becomes exhausted. Moreover, sometimes, adaptive mechanisms, being prolonged and excessive, turn into disadaptive, and it is a real way to pathology.

There is the lot of evidences which support this theory. Analysis of the family history of patients with myocardium infarction, severe arrythmias and hypertonic disease revealed a connection of disease with a stress. It must be said that more often the disease usually occurred in some months later an insult. It is necessary to list the basic pathogenetic features which supposed to be were involved in .

The role of the stress in pathogenesis of hypertensive conditions

There are the following neurohumoral factors are suspected:
- Vasoconstrictive effect of catecholamines
- Periodicaly exitation of the baroreceptors of sinocarotid and aortal zones under stress leads to growing of their base tone; the receptors become accustomed to abnormal high arterial pressure (for this reason a patient doesn't have unpleas-

ant sense). As a result, baroreceptors begin to accept increased blood pressure as a normal one. So, prolong activation of sympathetic nerve system in stress an organism needs the higher figures of AP in comparison with normal

- Increased level of blood cortisol possesses by permissive influence the sympathetic tone on the one hand, but on the other hand, in a high dose glucocorticoids act like mineralocorticoid aldosteron ; the last retains sodium in the blood, and such way increases ECFV (extracellular flow volume)
- Activation of RAAS via direct sympathetic effect to kidney blood flow leads not only to increase blood circulation volume but increases peripheral blood vessels resistance

Stress, ischemic heart disease and myocardial infarction

Together with hereditary factors, smoking, hypo-adynamia and overweight stress is the one of the most important among the risk factors. The following pathogenetic factors seem to be involved in these diseases outstanding:

Prolong coronarospasm under increased blood level of the catecholamines with the following ischemic injury

- Increased level of intracytoplasmic myocardiocyte Ca^{++} and its results:
- a.activation of different membrane phospholipases in cardiomyocytes with increased lipid peroxidation
- c. derives of arachidonic acid formation with prostaglandines and leukotriens negative pro-inflammatory effects
- d weakness of CPR activity and accumulation of Ca++ in cytoplasm of cardiomyocytes
- e. weakness of energy elaboration due to overload of mitochondria with Ca++

All listed above factors lead to disturbances in systolic and diastolic function of the heart and myocardium insufficiency.

Catecholaminemia, in turn, activates coagulation system and such way increases risk of thrombi formation in coronary arteries. Redistribution of the blood flow during a stress diminishes perfusion of the heart. Stimulation of sympathetic nerve system may provoke different types of arrythmias which negatively influence the coronary flow. It can be explained by significantly decreased time of diastole, but coronary perfusion realizes namely that time.

Stress is associated with lipolysis, but hyperlipidemia is one of the most important factors of atherosclerosis formation, including coronary artery atherosclerosis.

Role of stress in stomach ulcer formation

Stress is one of the risk factors which responsible for the ulcers in gastrointestinal tract together with hereditary factors and abnormality in patient's diet. Stress encourages an activation of both, sympathetic and parasympathetic nervous system. Their high activity may lead to imbalance in stomach glands secretion which can result in production of very aggressive gastric juice with possible peptic ulcer formation as autodigestion. Simultaneously, catecholamines may provoke spasm of the vessels, supplying the stomach. Ischemia and ischemic injury with a deep de-

fect in the stomach wall seem to be the unfavourable consequenses of these processes. It must be added, that hypercortisolism which accompanies stress, plays a "vicious "role in this   case. First of all, glucocorticoids enhance the protein catabolism and such way retard a regeneration of the epithelium of gastrointestinal tract, weakening the connective junctions between the epithelial cells. Lasts let the protons, secreting by the parietal stomach cells to be reinfused and injury a stomach wall.

Glucocorticoids increase stomach juice secretion but depress mucose, bicarbonates and prostaglandins production, and such way they decrease protective mechanisms that in normal, in sum, form gastroprotection against the different insults. It must be added, that prostaglandins influence positively the local blood flow and mucose plays role a mechanical barrier. Besides, mucose contents the factors of immune protection (IgA, T-lymphocytes) and, eventually, bicarbonates that diminish an acidity of stomach juice. In a resume must be said that stress provokes an injury of stomach wall but, at the same time, weakens the forces that it watching and surviving.

Lecture 11
PATHOPHYSIOLOGY OF CARBOHYDRATE METABOLISM. DIABETES MELLITUS
1. General pathogenesis of carbohydrate metabolism disturbances.

The human body contains significantly less carbohydrates (not more than 2% of dry body mass) than proteins and lipids. Carbohydrates have various functions, the most important of which are energy-producing (the main source of energy for cells) and structural (indispensible component of most intracellular structures). In addition, carbohydrates are involved in the synthesis of nucleic acids (ribose, desoxyribose) and form compounds with proteins (glycoproteins, proteoglycans), lipids (glycolipids) and other substances (heteromonosaccharides). Carbohydrates are components of many enzymes and regulatory systems, providing numerous specific functions.

Chemically carbohydrates are aldehydes and ketones of polyatomic alcohols. Monosaccharides are linked together by glycosidic bonds forming disaccharides, oligosaccharides (3 to 6 monosaccharide units) and polysaccharides (glycogen, starch). The most common monosaccharides in the body are pentoses (component parts of nucleic acids and many co-enzymes, e.g NADPH) and hexoses (glucose, fructose, galactose). Glucose plays the most important role in energy metabolism. First, it is the only source of energy for the central nervous system (CNS) which has no energy stores and does not use other sources of energy, such as proteins and fats (except ketone bodies in starvation). Second, the organism makes a glucose reserve in the form of glycogen which readily breaks down and releases glucose into the blood. Third, complete oxidation of one glucose molecule (to CO_2 and

H_2O which are easily removed from the organism) requires less energy than oxidation of a fatty acid, and gives a significant energy output – 38 molecules of ATP.

Carbohydrate metabolism has the following stages:

- *digestion and absorption of carbohydrates in the gastrointestinal tract;*
- *synthesis and breakdown of glycogen;*
- *intermediate metabolism of carbohydrates and their utilization in tissues.*

Factors causing carbohydrate metabolism disturbances can appear at each of these stages.

1.2 *Disturbance of glycogen synthesis and breakdown, glycogenoses*

Inside cells, glucose coming from the blood is phosphorylated to form glucose-6-phosphate (G6P) in a hexokinase reaction. Glycogen, a polymer molecule which can contain up to a million of monosaccharides, is synthesized from G6P due to a combined action of glycogen synthase and the branching enzyme. In this process glycogen undergoes a sort of crystallization as a result of which it has no osmotic effect. Such form of glycogen is suitable for storage inside cells (if the same amount of glucose molecules were simply dissolved in the cell cytoplasm, the cell would be inevitably destroyed by osmotic forces).

Glycogen is contained in the cells of all tissues. The largest amounts of glycogen are found in the liver and muscles, whereas in the cells of the nervous system its content is minimal. The rate of glycogen breakdown is determined by the organism needs. Under normal conditions, glycogen breakdown releases into the bloodstream from 1.9 to 2.1 mg of glucose per each kilogram of body mass. The main "supplier" of glucose is the liver, because its cells, unlike muscle cells, can hydrolyze glucose-6-phosphate to form glucose.

Intensification of glycogen breakdown. In muscles intensive glycogenolysis takes place during physical exertion. Glucose is partly metabolized to CO_2 and H_2O to yield maximal amount of ATP, and partly – to lactic acid which comes to the blood and to the liver where it can be resynthesized to form glucose. In the liver glycogenolysis is activated in response to a decrease in the blood serum concentration of glucose or as a component of stress reaction. The main hormones activating glycogenolysis are glucagon, adrenalin (epinephrine) and cortisol. To a lesser degree, glycogenolysis is activated by some conditions accompanied by hyperproduction of STH (somatotropic hormone) and thyroid hormones. Activation of the sympathetic nervous system also promotes glycogenolysis. Activation of glycogenolysis leads to an increase in the blood glucose level.

Decrease in glycogen synthesis occurs at hypoxia because the production of ATP necessary for glycogen formation is impaired. As the liver is the main site of glycogen synthesis and storage, a severe damage to this organ accompanied by inhibition of its glycogen-producing function results in depletion of the general glycogen reserves. Insufficient glycogen reserve in its main depot – the liver – interferes with correction of hypoglycemia which develops when not enough glucose is taken with food (starvation, disorders of the gastrointestinal tract) or when glucose is actively consumed (physical exertion, stress).

Deficit of exogenous glucose and depletion of its endogenous stores deposited as glycogen leads to energy metabolism being provided at the expense of proteins and fats. This is accompanied by a loss of plastic material and accumulation of ketone bodies which cause acidosis and intoxication.

Under hypoxic conditions (general insufficiency of blood circulation and respiration, severe anemias, etc), when anaerobic respiration is predominant, lactic and pyruvic acids are excessively accumulated, which promotes tissue acidosis. Excessive mobilization of glycogen as a source of glucose under the conditions of its ineffective anaerobic utilization at chronic hypoxia leads to depletion of glycogen stores which enhances hypoglycemia.

Blocking acetyl-CoA production leads to disturbances of mutual transformations of carbohydrates, lipids and proteins because all such transformations have an intermediate acetyl-CoA stage. Acetyl-CoA is produced in mitochondria as a result of oxidative decarboxylation of pyruvic acid. Hypoxia, arsenic intoxication, some hypovitaminoses (e.g. deficiency of vitamin B_1 - thiamine) damage the pyruvate-dehydrogenase system and reduce acetyl-CoA synthesis. Due to the universal role of acetyl-CoA, it affects a variety of cells, tissues and organs – from erythrocytes to the CNS.

Changes in gluconeogenesis activity have a marked influence on the level of glucose in the body. This process is an additional source of endogenous glucose owing to its synthesis from glycogenic amino acids (alanine, glycine, serine, etc), lactic and pyruvic acids, glycerol and some other compounds in the liver and kidney cells.

Gluconeogenesis is largely activated when glycogen utilization is insufficient to maintain the blood glucose level adequate to meet the body needs. It occurs during long periods of starvation or exhaustive physical work.

Basic hormonal stimulators of gluconeogenesis are glucocorticoids and glucagon. Activation of gluconeogenesis is also promoted by adrenalin, STH and thyroid hormones because they enhance lipolysis, i.e. increase the level of fatty substrates which turn into carbohydrates. Increased production of these hormones is accompanied by activation of gluconeogenesis and, as a result, by hyperglycemia. The reverse side of this process is catabolism of fats and proteins (in the lymphoid tissue, skin, muscles) which provides substrates for glucose synthesis.

Inhibition of gluconeogenesis under hypoglycemic conditions occurs when there is a deficit of the contra-insulin hormones, excessive production of insulin (insulinoma) and in severe liver diseases.

II. Pathological changes of glucose concentrations in blood (hypo- and hyperglycemia) and urine (glucosuria)

II.1 Types of physiological regulation of carbohydrate metabolism

In order to provide normal energy exchange, glucose must be constantly delivered in adequate amounts to all body tissues. Disturbance of the mechanisms controlling glucose metabolism at any stage manifests itself in deviations of the blood glucose concentration (hypo- or hyperglycemia). Fasting glucose concentration in the whole blood is 3,3 − 5,5 mmol/l. Deviations from this level which are

not connected with organic pathology are insignificant (within $\pm$ 30% of the given interval) and temporary. They are linked to food intake (postprandial hyperglycemia), physical and emotional stress (stress hyperglycemia), and relatively short periods of fasting (fasting hypoglycemia).

Stabilization of the blood glucose level is achieved by adequate regulation of the carbohydrate metabolism with the participation of the CNS, pancreas, liver, adrenal glands, intestines, kidneys and tissues actively consuming glucose (muscles, adipose tissue).

Renal regulation. In the kidney glomeruli plasma glucose is actively filtered and then completely reabsorbed in the proximal tubules through an energy-dependent mechanism. As a result, the secondary (final) urine does not contain glucose. When the transport systems are overloaded (plasma glucose level exceeds its renal threshold – about 9 mmol/l) glucosuria develops, and the excess glucose is removed.

Hormonal regulation. The level of glucose is influenced by a wide range of hormones, but only insulin decreases glucose concentration in the blood. Insulin is a polypeptide consisting of 51 amino acids arranged in two chains. It is produced as a larger, physiologically inactive molecule, called proinsulun only in the secretory granules of β-cells of the islets of Langerhans in the pancreas. After the activation of glucose receptors of β-cells, a partial proteolysis of proinsulin molecules takes place resulting in the formation of insulin and C-peptide (connecting peptide).

Table 1.

Insulin concentrations in blood plasma of healthy individuals

Period of measuring insulin in the blood	Insulin concentration
12-hour fasting (in the morning, before meal)	6 – 25 mkIU/ml (36 – 150 pmol/L)
Prolonged fasting with glucose decrease to < 3,3 mmol/L	< 6 mkIU/ml (<36 pmol/L)
Maximum level after stimulation with glucose or glucagon	> 200 mkIU/ml (> 1200 pmol/L)

Basal insulin concentration determined radioimmunologically is 6 – 25 mkIU/ml (36 – 150 pmol/L). After oral stimulation with glucose, its level increases 5 – 10 times in 1 hour as compared to the baseline.

The most important effect of insulin is providing transmembrane transport of glucose into the cells of insulin-dependent tissues, such as muscles and adipose tissue. Various tissues are supplied with special glucose transporters. The brain, peripheral nerves, erythrocytes, vessel walls, liver, kidneys, intestines have such transporters on their membranes and their glucose uptake is determined by its concentration in the blood or intestine lumen. These are *noninsulin-dependent tissues*. In muscle and adipose tissue, which compete for glucose with the CNS, glucose transporters are located inside the cells, adjacent to the membrane. Taking into consideration large amounts of muscle and adipose tissue in the body, the ability of

these tissues to use glucose for muscle contraction (muscles) or for making glucose stores (glycogen in muscles, triglycerides in adipose tissue), availability of glucose for these tissues could quickly lead to hypoglycemia and brain death. However, there are very few glucose transporters on the membrane surface of these tissues.

After a meal and increase in glucose concentration in the blood, the secretion of insulin begins. Insulin molecules interact with a special glycoproteid receptor (INSR) of the membranes of muscle and adipose cells. After that, glucose transporters previously hidden inside the cells (type GLUT-4) are quickly expressed on the cell surface providing glucose transport into the cells.

Between the meals, when blood glucose concentration is normal, insulin is not secreted and glucose cannot get into muscle and adipose tissue in significant amounts. That is why these tissues are called *insulin-dependent*. The liver, to a certain degree, can also be regarded as insulin-dependent. Its membrane is permeable for glucose, but whether glucose remains inside or not, depends on insulin. It activates the enzymes of glycogen synthesis (correspondingly, glycogen breakdown decreases) and suppresses the enzymes of gluconeogenesis.

Some hormones counterregulate the action of insulin, raising blood glucose level. These are glucagon, adrenalin (epinephrine), glucocorticoids, somatotropic hormone (STH), thyroid hormones. These hormones are called *hyperglycemic*. The effects of insulin and contra-insulin hormones normally provide a relatively stable level of glucose in the blood. In low concentration of insulin (e.g. during fasting), hyperglycemic effects of insulin are enhanced.

II.2 Hypoglycemia, hypoglycemic coma.

Hypoglycemia is a condition in which blood glucose concentration is less than 2,75 mmol/L. Blood glucose concentrations ranging from 2,75 to 3,3 mmol/L, though decreased, do not cause clinical symptoms (asymptomatic hypoglycemia) or cause minor symptoms.

Causes of hypoglycemia. Hypoglycemia is divided into physiological and pathological.

Physiological hypoglycemia occurs in healthy hungry people. It disappears after a meal.

Pathological hypoglycemia may be associated with internal causes or initiated by exogenous factors (e.g. medications or alcohol). Most often such type of hypoglycemia occurs during fasting (when the stomach is empty).

Fasting hypoglycemia is caused by:
- severe lesions of the liver and kidneys;
- endocrine pathologies causing insufficient production of contra-insulin hormones (insufficiency of adrenal hormones, STH, hypothyroidism or excessive insulin production (insulinoma);
- intake of exogenous insulin and medications with hypoglycemic effect (especially, sulfonylurea derivatives);
- cachexia with depletion of muscle and adipose tissue;

- mesenchymal tumors (such tumors actively consume glucose and often cause metastatic destruction of the glands producing contra-insulin hormones, for example, adrenal glands);
- continuous physical activity without adequate alimentary compensation for energy losses;
- prolonged fever (especially in cachectic/ malnourished patients);
- starvation (at nervous anorexia or due to the absence of appetite in chronic diseases accompanied by acute phase response)

Clinical symptoms of acute hypoglycemia

1) <u>Symptoms associated with CNS disturbances</u>: fatigue, headache, dizziness, vision disturbances, dormancy (stupor, confusion), amnesia, paresthesia, hemipareses, nausea, vomiting, convulsions.

2) <u>Symptoms associated with the activation of the vegetative nervous system:</u>

a) *adrenergic symptoms* (reflect the compensatory reaction of the sympathetic system to hypoglycemia): hunger, palpitation, tachycardia, tremor, ataxia, paleness, tingling sensation in the lips and fingers;

b) *cholinergic symptoms* (result from the imbalance in the vegetative nervous system on the background of progressing CNS disturbances): sweating, nausea, vomiting.

Severity of the symptoms of acute hypoglycemia depends on the speed and degree of the blood glucose level drop. When the level of glucose drops to less than 2.5 mmol/L, hypoglycemic coma may develop.

Hypoglycemic coma is the extreme degree of acute hypoglycemia; it is manifested in the loss of consciousness accompanied by disturbances of the regulation of vital functions of respiration and blood circulation on the background of deep suppression of the CNS.

II.3 Hyperglycemia

Hyperglycemia is an increase in blood glucose concentration above 5.5 mmol/L. Depending on etiological factors, there are several types of hyperglycemia:

1) *Alimentary (postprandial, physiological) hyperglycemia* develops after consuming large amounts of readily digestible carbohydrates with food.

2) *Stress hyperglycemia* is caused by the effects of catecholamines and glucocorticoids, intensively produced in stress conditions due to the activation of the sympathetic and pituitary-adrenal systems, on carbohydrate metabolism.

3) *Hyperglycemia associated with pathological hyperproduction of contra-insulin hormones.* The cause is pathological hyperfunction or a tumor of the endocrine glands producing contra-insulin hormones (glucagonoma → glucagon; pheochromocytoma → adrenalin; eosinophilic adenoma of the pituitary gland → STH; thyrotoxicosis → T_3, T_4; a tumor of zona fasciculata of the adrenal cortex → cortisol).

4) *Hyperglycemia in diabetes mellitus*. The cause is absolute and/ or relative insulin deficiency.

III. Diabetes Mellitus
III.2 Modern definition and classification of forms of diabetes mellitus

Diabetes mellitus (DM) is a group of metabolic diseases characterized by persistent hyperglycemia due to absolute or relative insulin deficiency. Insulin deficiency and prolonged hyperglycemia result in the disturbance of all metabolic processes and development of acute and chronic (late) specific complications of DM.

In absolute insulin deficiency blood concentration of this hormone is less than normal.

In relative insulin deficiency its blood concentration may be normal or even elevated, and a decrease in insulin efficiency is due to insulin resistance of the insulin-dependent tissues.

According to the latest classification (WHO, 1999) (Table 4) there are four forms of DM:

1) **Type 1 diabetes mellitus** (formerly, insulin-dependent diabetes mellitus): -
   - autoimmune;
   - idiopathic;
2) **Type 2 diabetes mellitus** (formerly, insulin-independent diabetes mellitus);
3) **Symptomatic, or secondary diabetes**;
4) **Gestational diabetes mellitus**

In this WHO classification, Groups 1and 2 (Type 1 and Type 2 diabetes mellitus) are regarded as essential diabetes, i.e. as a disease with many predisposing factors and unknown etiology. (The difference between Type 1 and Type 2 DM is given below).

Group 3 (symptomatic DM) includes the forms of the secondary diabetes, caused by some definite factors: endocrinopathies (Cushing disease and Cushing syndrome, acromegaly, pheochromocytoma, etc), diseases of the pancreas (pancreatitis), genetic defects of insulin structure, defects of insulin receptors, impaired function of beta-cells, some medications (cyclosporine, thiazide diuretics, etc), genetic syndromes (Down's, Kleinfelter's, Turner's, etc) and other causes.

Group 4 includes gestational DM developing during pregnancy. It usually disappears after delivery, though in some patients the disease persists, or temporary regresses with subsequent reappearance of clinical symptoms.

The proportion of patients with DM1 is 10-12%, with DM2 – 85-90%, with other types –less than 1%. Gestational diabetes develops on average in 2-4% of pregnant women. In 2/3s of patients with gestational diabetes it is manifested as DM2 (it is corrected with diet, physical exercise), in 1/3 – as DM1 (requires administration of insulin).

III.3 Pathogenesis of insulin deficiency in Type 1 diabetes mellitus.

The main factor of DM1 pathogenesis is destruction of the pancreatic beta-cells and, hence, absolute insulin deficiency. Clinical manifestations of diabetes develop when 85-90% of beta-cells are destroyed. Depending of the mechanism of the pancreatic cell death, DM1 is divided into idiopathic and autoimmune, the latter being ten times as frequent as the former.

Autoimmune DM1

This form of diabetes is associated with internal (genetic) and external (triggering) factors which together initiate immune reactions damaging the cells of the islets of Langerhans.

The probability of autoimmune DM1 development is conditioned by certain types and combinations of HLA genes located on chromosome 6 (diabetogenic alleles of HLA-DP, -DQ, -DR groups) and other diabetogenic genes: nowadays, about 20 such genes are known.

Etiology of autoimmune DM1 is poorly understood. According to modern concepts, the pathogenetic mechanism of beta-cell destruction represents a sequence of interactions of many external triggering factors. In genetically predisposed people activation of immunocompetent cells occurs on the background of increased production of various cytokines (interleukin-1, tumor necrosis factor, γ-interferon, etc), anti-inflammatory prostaglandins, nitric oxide and others, combined action of which leads to destruction, apoptosis and decrease in amounts of beta-cells, and thus, to clinical picture of diabetes. It is believed that the most important triggering factors for DM1 are viruses of innate rubella, epidemic parotitis, adenoviruses, coxsackie viruses.

Infiltration of the islets by lymphocytes (Tx1, CTL, CD8), NK-cells and macrophages (insulinitis) occurs at very early stages of autoimmune DM1 development and indicates the involvement of cell immunity in the pathological processes.

Autoantibodies to various types of beta-cells are found in the blood serum of most patients having autoimmune DM1 at the preclinical stage and practically all patients at the early clinical stages. The role of autoantibodies in the pathogenesis of DM1 remains unclear.

Nevertheless, these antibodies indicate the ongoing process of beta-cell destruction, regardless of the presence or absence of the clinical symptoms of DM. That's why the presence of the antibodies to the islet cells allows to diagnose autoimmune DM1 at the latent stage (when only a small percentage of the islet cells is affected and carbohydrate metabolism is unchanged).

There several periods of DM1 development:

I – is characterized by genetic predisposition. There may be some contributory factors: infection or intoxication triggering autoimmune destruction of beta-cells. It lasts from 3-4 to 10-12 years.

II – autoimmune destruction of beta-cells begins, but insulin production by the remaining cells is sufficient to meet the body's needs.

III – latent diabetes: fasting glucose level is still normal but the blood sugar curve after glucose load is pathological which indicates significant decrease in the number of beta-cells.

IV – symptomatic diabetes: nearly 90% of beta-cells is destroyed, fasting hyperglycemia and clinical symptoms. On average, the age of patients by this stage is about 20 years old.

V – terminal diabetes with severe complications.

III.4 Pathogenesis of insulin deficiency in Type 2 diabetes mellitus.

The main factor of the pathogenesis of DM2 is *insulin resistance* (poor sensitivity of insulin-dependent tissues to insulin) accompanied by *relative insulin deficiency* even with compensatory hyperinsulinemia.

Table 2

Main features of Type1 and Type2 diabetes mellitus

feature	DM1	DM2
population incidence	**0.2 – 0.5%**	**2 – 4%**
age at the onset	**children, young people**	**people over 40**
development of symptoms	**acute**	**gradual (months, years)**
physique(constitution)	**slim**	**often obese**
blood insulin level	**decreased**	**normal or increased**
urine	**glucosuria and often acetonuria**	**glucosuria**
tendency to ketoacidosis	**yes**	**no**
antibodies to islet cells	**yes**	**no**
heredity	**<10% of 1st line relatives affected, concordance among twins -30-50%**	**> 20% of 1st line relatives affected, concordance among twins -80-90%**
HLA-association	**yes**	**no**
late complications	**predominantly microangiopathy**	**predominantly macroangiopathy**

There is a hypothesis that genetic predisposition to Type2 DM is caused not by mutations, but by changes in the expression levels of the genes coding insulin secretion, its interaction with insulin receptors in the target tissues and processes responsible for functional state of insulin receptors in insulin-dependent tissues.

Whatever the nature of hereditary predisposition to DM2 may be, for the disease to develop there must be non-genetic triggering factors. These include obesity, older age, hypodynamia, pregnancy, stress. Insulin resistance is supposed to be caused either by a decrease in the number of insulin receptors in the target tissues (muscles, adipose tissue, liver) or disturbances in the post-receptor interactions in insulin-dependent tissues (internalization of the hormone-receptor complex, autophosphorylation of the receptor β-subunit or phosphorylation of other protein substrates of intracellular signaling pathways).

The islets of Langerhans respond to insulin resistance by compensatory increase in insulin secretion, which allows to overcome insulin resistance for some

time, preventing the development of persisting hyperglycemia. Prolonged compensatory functioning of beta-cells is accompanied by their decompensation which at the later stages of DM2 results in transformation of relative insulin deficiency into absolute insulin deficiency and requires the use of insulin therapy (as in DM1).

↓ *Utilization of glucose by muscle and adipose tissue*
↓
↑Contra-insulin hormones (glucagon)
↓
↑Breakdown of glycogen in the liver and muscles
↓
Hyperglycemia + ↑lactate
↓   ↘

Glucosuria, polyuria   Acidosis
↓
Loss of water, electrolytes + bicarbonate
↓
Dehydration   ⟶   thirst
↓
Blood thickening, hypovolemia
↓
Disturbance of peripheral blood circulation

↓BP → ↓blood flow in the kidneys → anuria → coma → death

Fig. 2 Pathogenesis of carbohydrate metabolism disturbance

To counteract tissue energy deficiency the organism activates the processes directed to elevating glucose level in the blood:
1) Increased secretion of glucagon, which blocks sugar-lowering effects of insulin. In a marked ketoacidosis (which means severely compromised carbohydrate metabolism) secretion of the other contra-insulin hormones (catecholamines, cortisol, STH) also increases.
2) Active breakdown of glycogen in the liver and muscles.
3) Increased activity of glucose-6-phosphatase in the intestines accompanied by increased absorption of nutritional glucose into the blood.
4) Increased gluconeogenesis in the liver and, to a lesser extent, in the kidneys. Processes of glycogenolysis (in the liver and muscles), proteolysis (mainly in muscles) and lipolysis (in adipose tissue) are activated.

The result of all these processes is hyperglycemia leading to acute as well as chronic (late) complications of DM.

Protein metabolism. Excessive protein catabolism interferes with normal plastic (regenerative) processes. This accounts for bad healing of tissues after inju-

ries in diabetic patients. Disturbances of protein metabolism also negatively influence the functions of the immune system, specifically, the production of protein mediators regulating immune response, and antibodies. This explains poor resistance to infections in patients with DM.

Lipid metabolism. Increase in lipolysis and suppression of lipogenesis resulting from insulin deficiency and excess of contra-insulin hormones (mainly, glucagon) mobilize free fatty acids (FFA) from their depots in adipose tissue. This is accompanied by hyperlipidemia and excessive transport of FFA into the liver which leads to its fatty infiltration. The liver switches the metabolism of FFA from re-etherification to oxidation, in order to maintain energy exchange under the conditions of intracellular glucose deficiency. This leads to production of large amounts of acetyl-CoA which is actively transformed into ketone bodies (acetoacetic acid, β-oxybutyric acid and acetone) under the conditions of suppressed lipogenesis.

If overproduction of ketone bodies in the liver (ketogenesis) exceeds the ability of the body to utilize and excrete them, it results in ketonemia, metabolic acidosis and intoxication. This mechanism underlies one of the most severe acute diabetic complications – ketoacidotic coma.

The excess of acetoacetic acid increases the synthesis of cholesterol, low density and very low density lipoproteins (LDL and VLDL) and leads to atherosclerosis of blood vessels.

Water-electrolyte and acid-base balance. Hyperglycemia increases plasma osmolality which leads to polyuria (more than 2 L of urine daily) and polydipsia (intense thirst accompanied by intake of large amounts of liquid). Polyuria is caused by osmotic diuresis when high osmotic pressure of primary urine (because of glucosuria) prevents water reabsorption in renal tubules.

Hyperosmolar hypohydration leads to the subsequent important factors of the pathogenesis – hypovolemia, decreased blood volume, and hypoxia.

Hyperketonemia causes ketonuria – acetone in the urine. The kidneys excrete excessive ketone bodies as sodium and potassium salts, i.e. there is a substantial electrolyte loss.

III.6 Pathogenesis of acute (diabetic comas) and chronic (late) complications of diabetes mellitus.

There are two groups of complications of diabetes mellitus: acute and chronic. Acute complications develop within some hours or days, chronic – over months, but more often –over years and even decades. That's why chronic complications are called "late".

Acute complications

Acute complications include ketoacidotic, hyperosmolar (hyperglycemic), and lactacidotic comas. Hypoglycemic coma which can complicate sugar-lowering therapy is discussed separately.

Ketoacidotic coma is the most common acute complication of endocrine diseases and is typical of DM. Mortality reaches 6-10%, and in children with DM1it

is the most frequent cause of death. This type of coma results from rapidly progressing insulin deficiency.

Predisposing factors are***:***

- too small doses of insulin;
- non-compliance with the treatment regimen (missing insulin injections, insulin with expired date);
- sharply increased insulin demand (in infectious diseases, traumas and operations, stress, accompanying endocrine disorders with overproduction of contra-insulin hormones (thyrotoxicosis, acromegaly, pheochromocytoma, Cushing disease), pregnancy.

Damaging mechanisms at ketoacidotic coma are linked to intoxication with ketone bodies, metabolic acidosis, hypovolemia, hypoxia and cell dehydration.

Ketone bodies, especially acetone, actively interact with lipid components of cell membranes and suppress normal functioning of many intracellular enzymes, lipid-rich structures of the CNS suffering most of all.

In severe cases hypovolemia leads to a decrease in renal blood flow, which is accompanied by weakening of glomerular filtration and oliguria (small amount of urine). This leads to azotemia and worsening of acidosis due to a decreased excretion of nitrous waste and hydrogen ions by the kidneys. Azotemia and acidosis lead to disturbances in all organ systems with a life threat, mainly due to suppression of the CNS functions which regulate circulation and breathing.

The symptoms of ketoacidosis are loss of appetite, nausea and vomiting, abdominal pains, vision deterioration, confusion and loss of consciousness, suppression of reflexes, decreased blood pressure, Kussmaul's breathing (rare, deep, noisy), symptoms of dehydration (decreased tissue turgor, soft eyeballs, fruity smell of exhaled air.

The cause of hyperosmolar coma is a relative insulin deficiency due to insulin resistance. The amount of insulin in the organism is sufficient to prevent active lipolysis and ketogenesis, but not sufficient to counteract the growing hyperglycemia. Most often coma arises because of increased insulin demand due to the enhanced action of contra-insulin hormones in the development of acute phase response (infectious diseases, mechanical injuries and surgical operations, burns and frostbite, acute pancreatitis, myocardial infarction, etc) or in concomitant endocrine disorders (thyrotoxicosis, acromegaly, pheochromocytoma, Cushing disease).

Damaging mechanisms at hyperosmolar coma are linked to dehydration of all tissues because of plasma hyperosmolality (>350 mosmol/kg), marked hyperglycemia (>40 mmol/L) and decreased blood volume.

Dehydration of the brain structures with a sharp drop of intracranial pressure results in general depression of the CNS manifested in neurological disturbances, growing confusion, loss of consciousness and, ultimately, coma. Hypovolemia leads to disturbances of hemocoagulation which causes development of DIC-syndrome, arterial (infarction, stroke) and venous (especially in the basin of the inferior vena cava) thromboses.

Table 3

Laboratory findings in diabetic comas

Findings	Ketoacidotic coma	Hyperosmolar coma	Lactacidotic coma
Glucose, mmol/L (normal 3.3-5.5)	up to 19-33	up to 55	normal or slightly increased
Ketone bodies, mmol/L (normal - up to 1,7)	up to 17	normal	normal or slightly increased
Lactate, mmol/L (normal 0.4-1.4)	up to 10	normal	up to 2-7
pH	<7.3	normal	<7.3
Osmolarity, mosmol/L (normal 185-300)	slightly increased	350-500	normal

Late complications of diabetes mellitus

Late complications of DM include:

- macroangiopathy (obliterating atherosclerosis of the aorta, coronary, cerebral and peripheral arteries; diabetic foot);
- microangiopathy (retinopathy, nephropathy);
- diabetic neuropathy;
- diabetic cataract.

Microangiopathy is typical of DM1, whereas macroangiopathy – of DM2. The latter is associated with the age factor, because DM2 patients are usually elderly people with gradual progression of systemic atherosclerosis, potentiating the effect of chronic hyperglycemia on arterial vessels.

Pathogenesis of macroangiopathies. Macrovascular diabetic complications are caused by atherosclerosis, the risk of which in diabetic patients is 4-5 times as high as in general population. Diabetic macroangiopathy is characterized by damage to the vessels of the arterial network of the brain, heart and limbs (lower legs and feet in particular).

The causes of increased frequency of systemic atherosclerosis and thrombotic complications in diabetic patients include:

-*Impaired lipid metabolism.* It is manifested as general lipemia with increased levels of LDLs and VLDLs and decreased levels of HDLs. This causes lipid buildup in the arterial intima and rheological disturbances, such as higher blood viscosity and tendency to thrombosis.

- *Endothelial dysfunction*. Patients with DM have decreased levels of nitric oxide which contributes to constantly high vascular tone and more active formation of adhesion molecules (ICAM-1, E-selectins). Increased adhesion of platelets, macrophages and monocytes to the endothelium stimulates secretion of biologically active substances causing local inflammation and formation of thrombi.

- Changes in the hemostatic system. In DM there is a tendency to lower fibrinolytic activity, higher levels of many coagulation factors and increased vascular-thrombocytic hemostasis.

- Proliferation of smooth muscle cells of arteries. It is stimulated by excessive STH production and growth factors secreted by activated platelets and macrophages accumulated at the sites of a marked endothelial dysfunction.

- Oxidative stress. It results from glucose oxidation in prolonged hyperglycemia. This process yields such products as protein carbonils, lipid peroxides and others, damaging directly or indirectly vessel walls.

Pathogenesis of microangiopathies, neuropathy and cataract

Poorly controlled glycemia is the main, though not the only, etiological factor of all chronic complication s of DM. Prolonged and uncontrolled effect of glucose on various structures of cells, tissues and organs is called glucosotoxicity.

There are several ways of realization of this phenomenon:

- Glycation of proteins. Glucose can react with proteins to form glycated products without participation of enzymes. In these interactions, at first, early products such as Schiff bases and fructosamines are formed, which later become stable products of glycation. The degree of glycation is the highest in long-living proteins which leads to dysfunction of blood serum proteins, cell membranes, peripheral nerves, collagen, elastin, lens, LDLs, hemoglobin. Changes in protein conformation due to glycation not only disrupt their function but promote production of autoantibodies to these proteins and their subsequent destruction.

The end products of glycation take part in expression of various genes involved in development of pathological reactions and morphological structures.

The results of these processes are different pathological conditions including nephropathy, neuropathy, retinopathy, cardiomyopathy, impaired transport of oxygen and subsequent tissue ischemia.

- Accumulation of sorbitol. In hyperglycemia glucose is accumulated in insulin-independent tissues (nervous system, retinal pericytes, lens, vessel walls, pancreas) to which it goes down a concentration gradient. Under the influence of aldosoreductase glucose is transformed into cyclic alcohol – sorbit (normally, practically all glucose must be metabolized inside the cells in the hexokinase reaction to form glucose-6-phosphate which is then used in various metabolic reactions). When sorbitol is accumulated, intracellular osmotic pressure increases which causes cell hyperhydration (osmotic edema). Besides, sorbitol turns into fructose which is more active than glucose in stimulating glycation of intracellular proteins contributing to disruption of cell metabolism.

- Autooxidation of glucose. Highly reactive free radicals are formed in cells, especially those of the endothelium and nervous tissue.

Pathogenesis of nephropathy in DM is connected with disturbances of the synthesis and metabolism of glycosaminoglycans which are part of the glomerular basement membrane, and retinopathy – with neovascularization due to enhanced synthesis of various growth factors.

III.7 Diagnosis of diabetes mellitus.

Clinical presentation of a "full-blown" diabetes mellitus consists of typical symptoms and complaints which include:

- thirst accompanied by intake of large amounts of liquid (<u>polydipsia</u>); increase in daily diuresis (<u>polyuria</u>); weight loss (in DM1) or obesity (DM2) in combination with increased appetite (<u>polyphagia</u>) (3 poly-). Besides DM can be accompanied by fatigue, weakness, skin itching, furunculosis, urogenital disorders (chronic pyelonephritis, chronic cystitis, in women – vaginitis, in men – balanitis and decreased sexual potency); vascular disorders (coronary heart disease, disorders of cerebral circulation, damage to peripheral arteries, trophic ulcers of feet); peripheral neuropathy (disturbance of sensitivity, pain, reduction of reflexes); signs of nephropathy (proteinuria, renal edemas, arterial hypertension); vision disturbances (due to progressing retinopathy).

To confirm the diagnosis of DM any two of the following laboratory findings are sufficient:

- fasting plasma glucose level is > 7.0 mmol/L;
- two hours after glucose tolerance test with 75g of glucose, its serum level is > 11 mmol/L
- glucosuria (with polyuria)

III.8 General principles of DM therapy.

Type 1 diabetes mellitus:

- replacement therapy – lifelong, daily single or, more often, multiple insulin injections;
- nutrition therapy (restriction of food rich in carbohydrates) as obligatory background for insulin therapy;
- moderate physical activity along with nutrition changes and changes in insulin doses.

Type 2 diabetes mellitus:

- *Stage 1 of treatment*:
  - for people with normal body mass – nutrition therapy (carbohydrate restriction)
  - for obese people – treatment of obesity
- *Stage 2 of treatment*:
  - sugar-lowering medications in tablets
- *Stage 3 of treatment*:
  - insulin therapy

III.9 Principles of therapy of acute conditions in DM

Ketoacidotic coma. Replacement therapy with insulin plus glucose, restoration of fluid volume, electrolytes and pH.

Hyperosmolar coma. Restoration of fluid volume and osmotic pressure of blood by infusion of hypotonic solution (0.45%) of sodium chloride (2-3L) and electrolytes plus short acting insulin preparations in small doses (5-10U/h).

WATER AND ELECTROLYTES IMBALANCE

Water is the main constituent of the body. The fluids of the body are distributed among functional compartments or spaces. They are: intracellular and extracellular; extracellular divided into smaller compartments – interstitial and intravascular. Water moves freely among body compartments and is distributed by osmotic, oncotic and hydrostatic forces. The sum of fluids within all compartments constitutes the total body water (TBW).

Distribution of body water.

	% of body water	Volume (liters)
Intracellular fluid	40	28
Extracellular fluid	20	14
intrastitial	15	11
intravascular	5	3
Total body water	60	42

TBW depends on weight, ages, sex.
TBW in relation to body weight.

Body build	adult male	adult female	infant
normal	60	50	70
lean	70	60	80
obese	50	42	60

The proportion of TBW varies with the amount of body fat and age. Because fat is hydrophobic, very little water is contained in adipose cells. Individuals with more body fat have proportionally less TBW and tend to be more susceptible to fluid imbalances that cause dehydration. Although daily fluid intake may fluctuate widely, the body regulates water volume within relatively narrow range. The primary sources of body water are drinking, ingestion of water in food, water derived from oxidative metabolism. Normally, the largest amount of water are lost through renal excretion, lesser amount are eliminated through the stool and vaporization from the skin and lungs. Water and electrolytes balance is regulated by kidney, lungs, circulating system, skin, muscles, intestine, bones and hormons: ADH, aldosteron.

Osmotic forces determine the movement of water through all three compartments. Osmosis is the movement of water "down" a concentration gradient. That is, across a semipermiable membrane from a region of higher water concentration to a lower. Osmosis directly related to both hydrostatic pressure and solute concentration but not to particle size and weight. For example, particles of plasma albumins are small but more concentrated in body fluids than the larger and heavier particles of globulins. Therefore albumin exerts a greater osmotic force than globulin.

The main osmotically active particles of the extracellular space are Na, CL,HCO3. IN the intracellular compartment they are: K, Mg< PO2 (phosphates).

A molar solution of undisociated molecules has an osmolality of one osmole/kg and would exert an osmotic pressure of 22,4 atmospheres (1 milliosmole exerts an osmotic pressure of about 17 mm Hg). Osmolality or the total concentration of dissolved or colloidal particles is the most important determinant of water movement. The normal osmolality of body fluids is 280 to 294 mosm/kg.

If water is added to the extracellular space it will dilute all compartments until the osmolality on either side of membrane become equal. If on the other hand , isotonic NaCl is administrated intravenously the volume expansion is confined to the extracellular fluid. Water moves freely between plasma and interstitial fluid. When blood glucose is raised, it attracts water from the intracellular compartment and produces a lowering of serum Na concentration (for every 100 mg/dl decreased 1,6 mEq/l Na).

The distribution of water and movement of nutrients and waste products among capillary, plasma and interstitial spaces occur as a result of changes in hydrostatic pressure and osmotic forces at the arterial and venous ends of capillary.Because water, Na, and glucose readily move across the capillary membranr, the plasma proteins maintain the effective osmolality by generating plasma oncotic pressure. Osmotic forces within the capillary are balanced by hydrostatic pressure which arises from cardiac contraction. The movement of fluid back and forth across the capillary wall is called <u>NET FILTRATION</u> and is described by Starling hypothesis   (low).
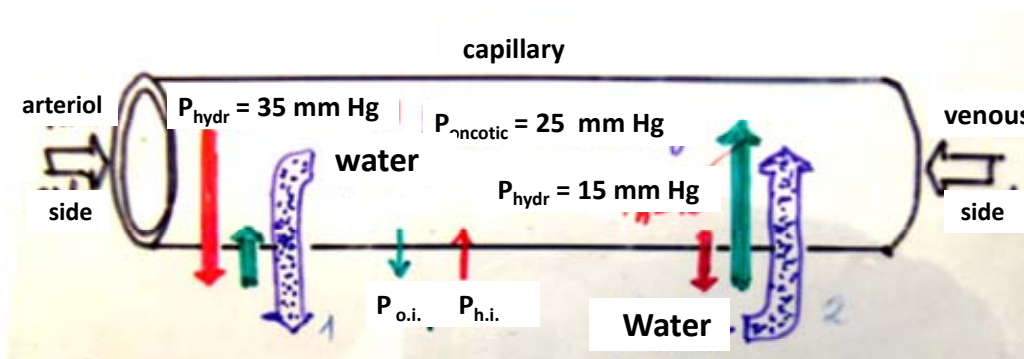
CAPILLARY NET FILTRATION FORCES

Net filtration = (Forces favoring filtration) - (Forces opposing filtration)
1. Net filtration = 35-25 = 10
2. Net filtration = 15 – 25 = -10

Net filtration = forces favoring filtration – forces opposing filtration

The forces favoring filtration or movement water out of the capillary into interstitial space include: the capillary hydrostatic pressure and oncotic interstitial .pressure. The forces opposing filtration are the plasma oncotic pressure and inter-
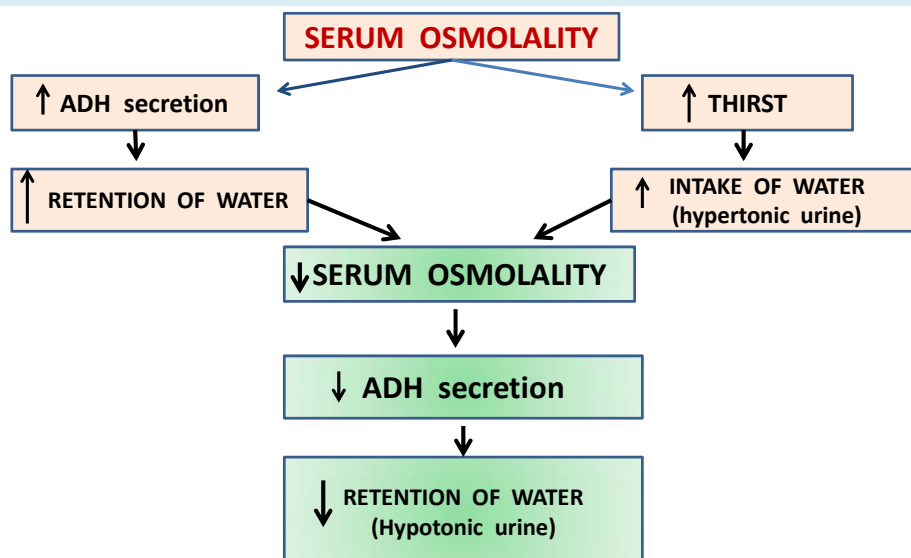
stitial hydrostatic pressure. Normally, the interstitial forces are negligible because only a very small percentage of plasma proteins crosses the capillary membrane. Thus the major forces for filtration are within the capillary. As the plasma moves from arterial to the venous end of the capillary, changes in the forces of hydrostatic pressure facilitate the movement of water across the membrane. 1) Oncotic pressure remains constant (25-28 mm Hg) because proteins do not cross the capillary membrane. 2) At the arterial end hydrostatic pressure is grater the capillary oncotic pressure and water filters into interstitial space. 3) At the venous end of the capillary oncotic pressure exceeds hydrostatic pressure. Fluids then are attracted back into circulation, balancing the movement of fluids between the plasma and interstitial space. The overall effect is filtration at the arterial end and reabsorption at the venous end.

Arteriol end of capillary: hydrostatic pressure exceeds oncotic pressure → water filters into interstitial space.

Venous end of capillary: Oncotic pressure exceeds hydrostatic pressure → water reabsorbs inside of capillary

Mechanisms that maintain normal serum osmolality (280 mOsm/kg) are thirst and effect of antidiuretic hormone (ADH). When serum osmolality increases, central osmoreceptors in hypothalamus become activated. This leads to thirst behavior and increased secretion of ADH. Both increases water in serum and decreases serum osmolality, then the negative back loop switches on and ADH secretion decreases.

Mechanisms that maintain normal serum osmolality

SERUM OSMOLALITY

↑ ADH secretion → ↑ RETENTION OF WATER

↑ THIRST → ↑ INTAKE OF WATER (hypertonic urine)

↓SERUM OSMOLALITY

↓ ADH secretion

↓ RETENTION OF WATER (Hypotonic urine)

ADH is synthesized in supraoptic and paraventricular nuclei of pituitary. The secretion of ADH responds to both osmotic and nonosmotic stimuli.

Regulation of ADH secretion
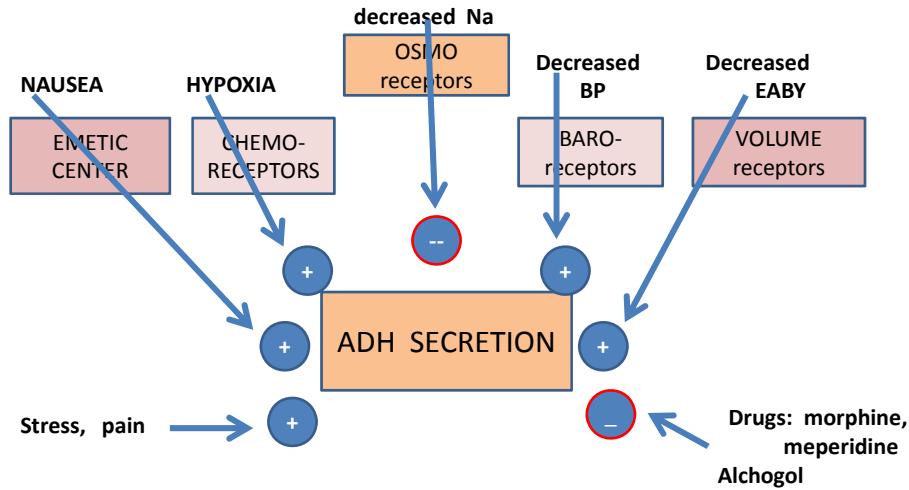
ADH secretion increases by stress, trauma, pain, exercise, nausea, nicotine exposure to heat, and drug such as chloroform and morphine, apparently activating cholinergic neurotransmitters in hypothalamus.

ADH secretion decreases with a decrease in plasma osmolality (decreased concentration of Na, glucose), an increase in intravascular volume, hypertension, alcohol ingestion.

ADH binds to its receptor on epithelial cells of collecting duct, activates G protein, adenilate ciclase, cAMP dependent protein kinase and increases permeability for water of epithelial cell membrane. This increased permeability leads to increase in water reabsorption and production of more concentrated urine.
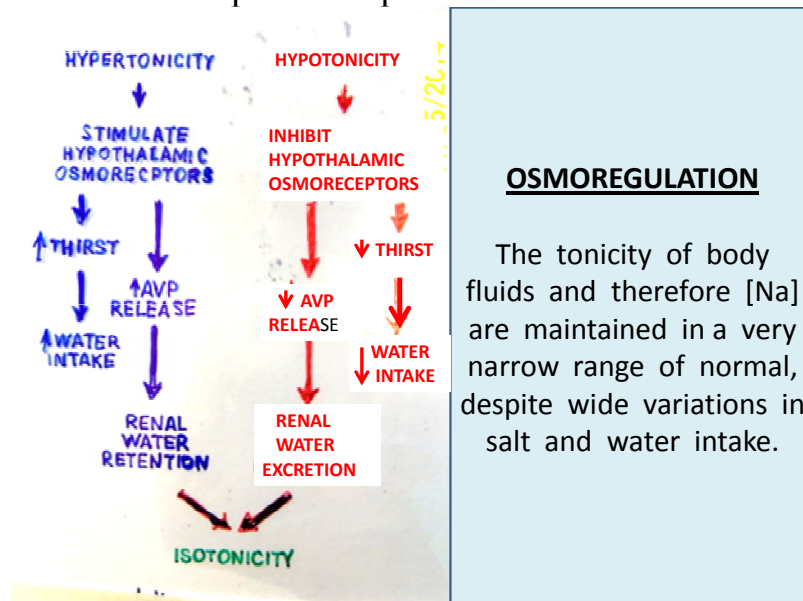
OSMOREGULATION

The tonicity of body fluids and therefore [Na] are maintained in a very narrow range of normal, despite wide variations in salt and water intake.

Water depletion – water loss exceeds its intake (negative water balance).

Causes: loss of fluid from skin (sweating, burns),
gastrointestinal tract (vomiting, diarrhea)
kidneys (diuretic therapy, renal diseases, adrenal insufficiency)

Symptoms: thirst, weight loss.

Signs: soft, sunken eyeballs, dry tongue and mucous membranes, loss of skin turgor, tachycardia, hypotension, oliguria with high urine specific gravity.

Laboratory: elevated Hb and hematocrit (haemoconcentration)
increased serum osmolality, increased serum [Na]

Treatment: - water by mouth / isotonic solution i/v + 5% glucose solution
replacement therapy with ADH

Water excess or hyperhydration. To this condition may lead 1) exxcesive water intake, 2) insufficiency of water excretion.

Causes: - renal failure, - cardiac failure, - hypoproteinemia (liver diseases, nephrotic syndrome, malnutrition, protein losing enteropathy), - iatrogenic (excessive i/v fluid administration), - endocrine diseases (hyperaldosteronism, Cushing's syndrome). – toxaemia of pregnancy.

Symptoms: swelling of ancles, dyspnoea, periorbital swelling.

Signs: - edema, - pleural, pericardial afflusion, - ascites.

Laboratory: decreased serum [Na], haemodilution.

Therapy: 1) Treat the cause. 2) Adjunct to therapy: - salt and fluid restriction, diuretics.

Alteration in water movement lead to edema.

Edema is the accumulation of fluid within interstitial spaces. It is a problem of fluid distribution and does not indicate a fluid excess. In some condition sequestered fluids can cause both edema and dehydration.

There are the four most common mechanisms of edema:

1. - increased hydrostatic pressure,
2. - decreased plasma oncotic pressure,
3 - increased capillary permeability,
4 - lymphatic obstruction.

Causes of edema:

1. Increased capillary pressure: arterial dilation (inflammation),
venous obstruction,
hepatic obstruction,
heart failure
thrombophlebitis
increased vascular volume:
heart failure,
increased level of ADH,
pregnancy.

2. Decreased plasma oncotic pressure: liver diseases, starvation, protein-lossing kidney diseases, extensive burns.

3. Increased capillary permeability: inflammation, neoplastic diseases, tissue injuries.

Examples of edematous mechanisms during some pathological processes:

MECHANISM OF EDEMA FORMATION DURING INFLAMMATION

Increased capillary permeability

Decreased production of Plasma proteins

Loss of plasma proteins

Decreased capillary oncotic pressure

Increased tissue oncotic pressure

EDEMA

Decreased transport of Capillary filterd proteins

$\uparrow Na + H_2O$ renal retention

Lymph obstruction

Fluid movements into tissue

Decreased absorption of Interstitial fluid

Increased capillary hydrostatic pressure

Pathogenesis of nephrotic syndrome edema

Altered glomerular permeability

Increased filtration of plasma proteins, Decreased reabsorption in tubular

PROTEINURIA

HYPOALBUMINEMIA

EDEMA

Hepatic synthesis of lipoproteins

Hyperlipoproteinemia

LIPIDURIA
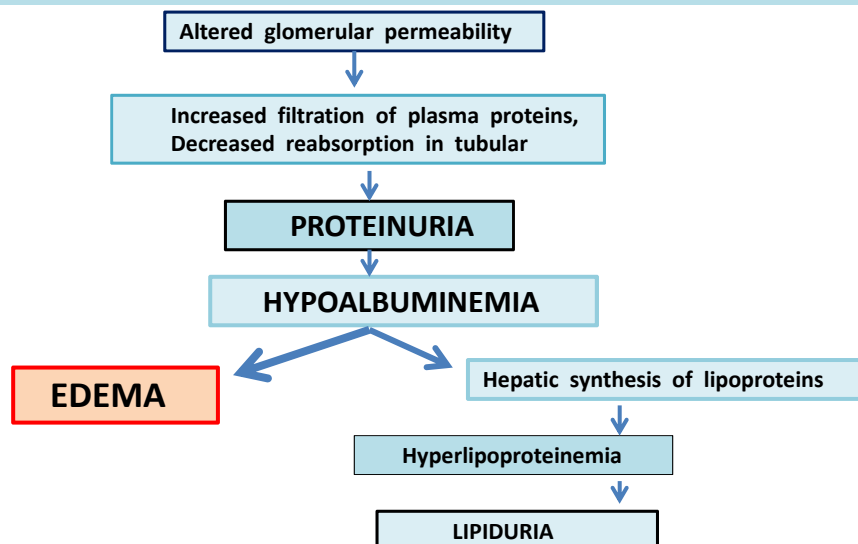
ROLE OF SODIUM.

Na account for 90% of ECF cations (positively charged ions). The concentration of Na is maintained within a narrow range 136 to 145 mEq/l. Milliequivalen per liter indicate the number of electrical charges per volume of fluid.

Sodium has many important body functions, including:

-regulation of osmolality,
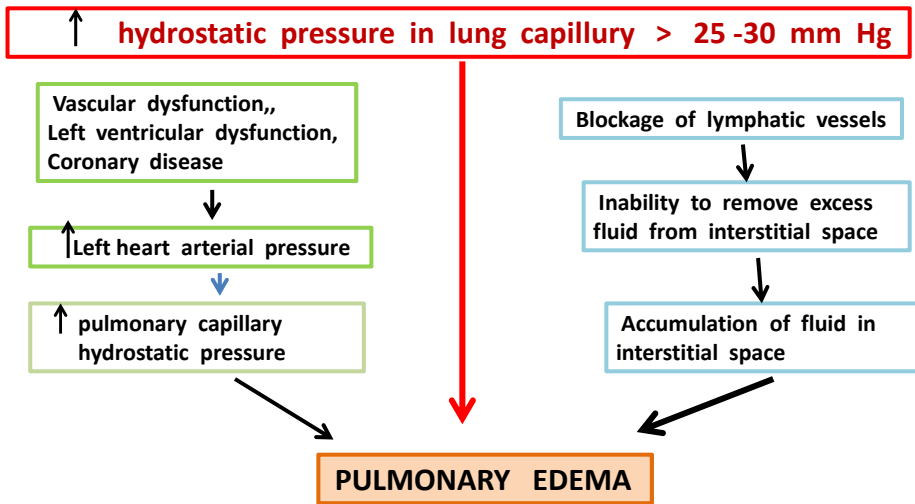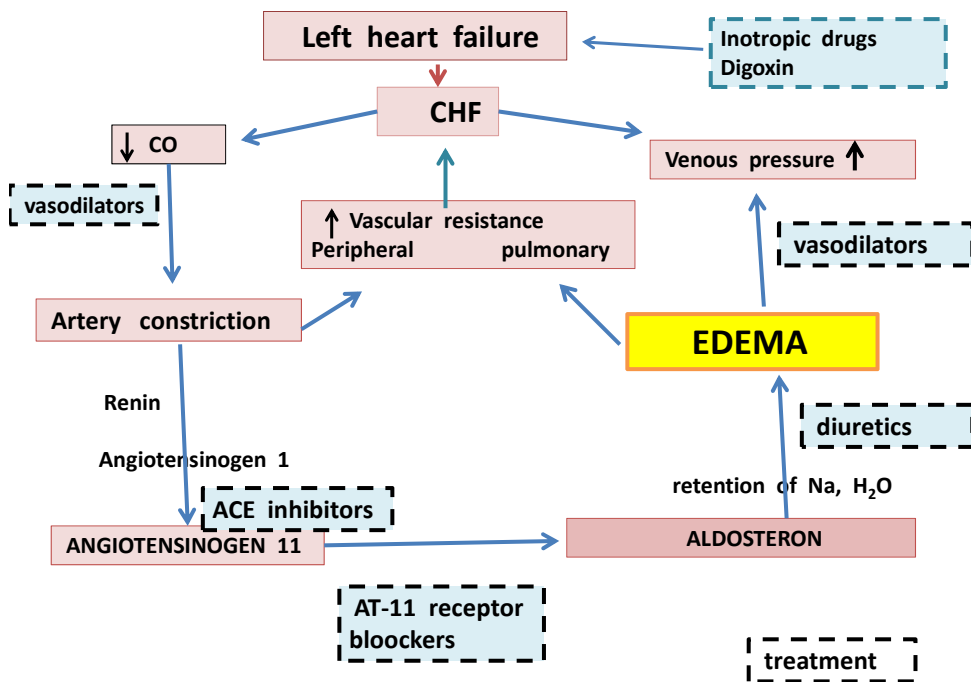- working with potassion and calcium to maintain neuromuscular irritability for conduction of nerve impulses,
- Na is a base for sodium bicarbonate and regulates acid – base balance,
- Na participates in cellular chemical reactions and membrane transport.

The average dietary intake of Na ranges from 5-6 g/day, the minimal daily requirement of sodium is 500 mg. Sweating depletes sodium and water volume and increases the body's sodium requirement.

Pathogenesis of pulmonary edema

↑ hydrostatic pressure in lung capillury > 25 -30 mm Hg

Vascular dysfunction,,
Left ventricular dysfunction,
Coronary disease

↓

↑ Left heart arterial pressure

↓

↑ pulmonary capillary hydrostatic pressure

Blockage of lymphatic vessels

↓

Inability to remove excess fluid from interstitial space

↓

Accumulation of fluid in interstitial space

PULMONARY EDEMA

Mechanism of cardiac edema:

Left heart failure ← Inotropic drugs Digoxin

CHF

↓ CO

vasodilators

↑ Vascular resistance
Peripheral   pulmonary

Venous pressure ↑

vasodilators

Artery constriction

EDEMA

diuretics

Renin

Angiotensinogen 1

retention of Na, H_2O

ACE inhibitors

ANGIOTENSINOGEN 11 → ALDOSTERON

AT-11 receptor bloockers

treatment

The kidney – the main regulator of Na

BLOOD VOLUME

V_2 ← V_0 → V_1

Decrease in vascular volume

Increase in vascular volume

Na retention

Na excretion

CONTROL MECHANISMS:
Sympathetic nervous system
RAAS
Atrial natriuretic peptide

Control mechanisms

Sympathetic nervous system

SNS adjusts GFR to changes in blood volume

↑ vascular volume → ↓ SNS activity → ↑ GFR
↓ vascular volume → ↑ SNS activity → ↓ GFR

RAAS (renin-angiotensin-aldosterone system)

Changes in BP; RBF; GFR; [Na⁺] in tubular fluid

JUXTAGLOMERULAR APPARATUS

Atrial natriuretic peptide ANP

↑atrilal volume → ↑ ANP

The kidney regulates sodium balance through renal tubular and Henle loop reabsorption. With an excess or deficit of sodium in relation to water, a combination of hormonal, neural, renal mechanisms acts synergistically to control sodium balance.

Control mechanisms: 1. Sympathetic nervous system (SNS),
2. Renin–angiotensin-aldosterone system (RAAS)
3. Atrial natriuretic peptide (ANP)

SNS adjusts glomerular filtration rate (GFR) to changes in blood volume. Increased vascular volume leads to decreased SNS activity and to increase GFR.
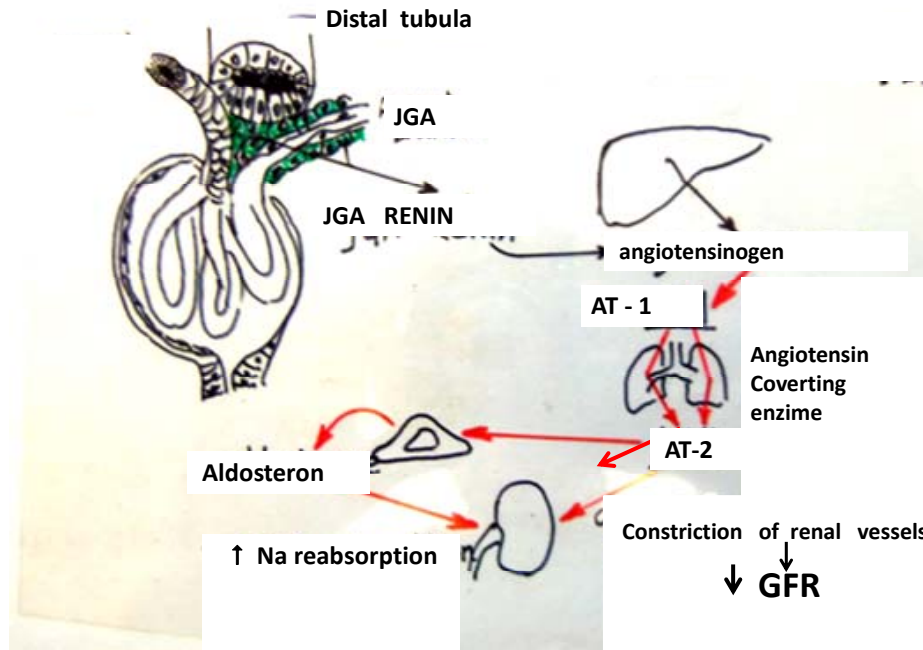
Decreased vascular volume leads to increase SNS activity and to decrease GFR.

RAAS: changes in blood pressure, blood flow, GFR, [Na] in tubular fluid activates of uxtaglomerular apparatus. It releases renin, which secrets to the blood and break down angiotensinogen to form angiotensin-1 (AT-1). AT-1 comes to the lung, where under the effect of ACE, turns into AT-2.

Effects of AT-2: increase secretion of aldosterone, which increases reabsorption of [Na] in kidney, constriction of renal vessels, increase heart rate and power, constriction of arteriols, effects on CNS: thirst, activation of SNS, increase secretion of antidiuretic hormone. All this leads to increase blood volume and blood pressure.

Atrial natriuretic peptide (ANP) releases from right atrium, when right atrium volume increases. ANP has following effects: antagonist of AT-2 peripheral effects, in CNS it inhibits secretion of ADH and thirst.

Distal tubula

JGA

JGA RENIN

angiotensinogen

AT - 1

Angiotensin Coverting enzime

AT-2

Aldosteron

↑ Na reabsorption

Constriction of renal vessels

↓ GFR

Alteration in sodium balance.

HYPONATREIMIA (less than 135 mM/l) leads to decrease serum osmolality less then 280 mosm/l and causes the movement of water into cells with cell swelling.

Causes: a) delutional hyponatriemia: water retention in excess of sodium, total body sodium normal or elevated:

-congestive cardiac failure, cirrhosis with portal hypertension ascites, iatrogenic: intravenous fluids with [Na] less 130 mM/l, increased ADH secretion, increased serum glucose, psychogenical polydipsia.

b) sodium depletion: GIT loss, excessive sweating, diuretics, solt-lossing nephropathy, adrenal insufficient (Addison's disease).

In additional, replacement of fluid loss with i/v 5% dextrose in water can cause a dilutional hyponatiemia. Excessive sweating may stimulate thirst and intake of large amount of water, which dilute sodium.

Hyponatiemia also may be hypoosmolar or hypertonic.

Hypooamolar hyponatriemia is produced during acute oliguric renal failure, severe congestive heart failure, cirrhosis, impaired renal excretion of water.

Both total body water and sodium level are increased but TBW exceds the increased sodium, producing a hypoosmolar hyponatriemia.

Hypertonic hyponatriemia developes with hyperlipidemia, hyperproteinemia, hyperglycemia. Increased in plasma lipids and proteins displace water volume and decrease sodium concentration. Hyperglycemia increases extracellular fluid osmolality and attracts water from the intercellular compartment. The osmotic fluid shift to the ECF in turn dilutes the concentration of sodium and other electrolytes.

Clinical manifestation.

Deficit of sodium alters the ability of cells to depolarize and repolarize normally. Behavioral and neurological changes, which characterize hyponatriemia include:

lethargy, dizziness, headache, anorexia, nausea, vomiting, confusion, seizure, coma. Pure sodium losses may be accompanied by loss of extracellular fluid causing an isotonic hypovolemia with symptoms of hypotension, tachycardia, decreased urine output. Weight gain, edema ascitis and jugular vein distension are characteristic of dilutional hyponatriemia.

Treatment. When symptoms is caused by water intoxicaton, decrease water intake, saline solution orally, furosemide (edema), increase sodium intake (dietary salt), physiological solution i/v ,when there is sodium depletion.

Hypernatriemia. ([Na] exceed 147 mEq/l)

Mechanisms: -1. Water loss is excess of sodium (total body Na – normal, extracellular fluid volume low). -2. Excessive Na intake (total body Na and ECFV increased)Sodium gains cause intracellular dehydration. Movement of water to ECF may cause hypervolemia. With accompanying water loss, both ICF and ESF dehydration occur.

Hyperosmolality is common result of hypernatriemia.

Causes: Low ECFV: -osmotic diuresis during diabetes melitus (Ketoacidosis),

-severe water deprivation (diabetes insipidus – excess production of ADH), -fever or respuratory infections, which increase respiratory rate and enhance water loss with lungs, - excessive sweating, - watery diarrhea, - poliuria.

High ECFV: - iatrogenic: i/v NaHCO3 for treatment of acidosis, hypertonic saline solution, - high [Na] as a result of over secretion of aldosterone, - Cushing syndrome, caused by excess secretion of glucocorticoids, which also causes increased secretion of aldosterone.

Clinical manifestations: Water is redistributed to extracellular space and intracellular dehydration ensues.

- increased serum osmolality, - increased serum [Na], - increased Ht, - increased BUN.

- thirst, oliguria, increased urine specific gravity,
- intracellular dehydration (dry skin, mucous membrane)
- CNS: headache, agitation, restlesness, decreased reflexes, seizures, coma
- cardiovascular system: tachycardia, decreased blood pressure, weak thready pulse.

Treatment: Low ECFV – Oral water + electrolytes, that had been lost, 5% glucose + electrolytes replacement solution i/v.

High ECFV – diuretics.

ROLE OF POTASSIUM.

Potassium is the major intracellular electrolyte and contributes to many important cellular functions. Total body potassium content is about 4000 mEq/l, with most of it locates in the cells. Daily dietary intake of potassium is 40 to 150 meq/d,
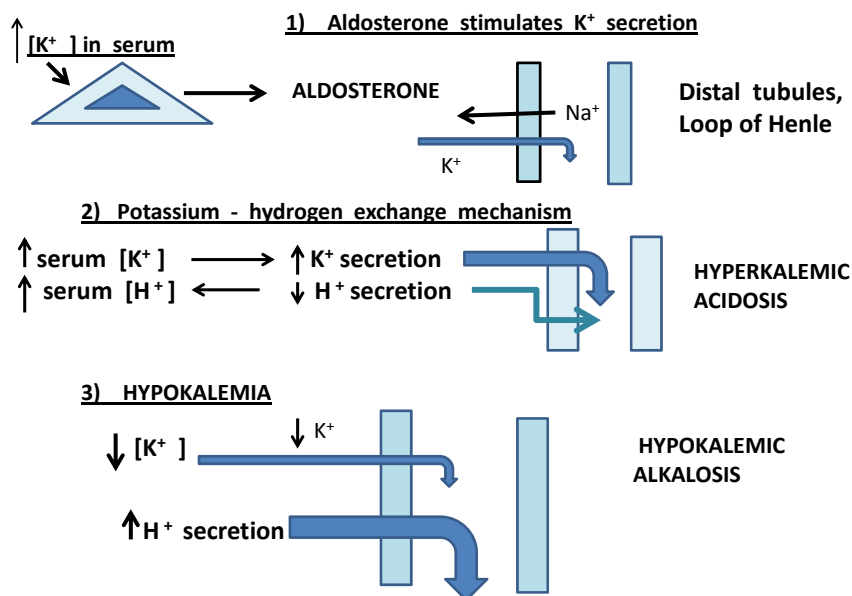
with an average 1,5 mEq/kg body weight. The intracellular fluid concentration of [K]=150-160 mEq/l. The extracellular fluid concentration of [K]=3.5-4.5 mEq/l.

The difference in concentration is maintained by a sodium-potassium active transport system (Na/K ATF-ase pump).The ratio of ECF to ICF [K] is the major determinant of the resting potential which is necessary for transmission of nerve impulses. Potassium is necessary for: - is required for glycogen deposition in liver and skeletal muscle cells; - maintain the resting potential; - maintain the normal cardiac rhythm; - maintain skeletal and smooth muscle contraction.

Regulation of potassium balance

The Kidney provides the most effective regulation of potassium balance. Potassium – hydrogen exchange mechanism.

Renal regulation of K⁺ balance

1) Aldosterone stimulates K⁺ secretion

↑ [K⁺] in serum → ALDOSTERONE → Na⁺ ← / K⁺ — Distal tubules, Loop of Henle

2) Potassium - hydrogen exchange mechanism

↑ serum [K⁺] → ↑ K⁺ secretion
↑ serum [H⁺] ← ↓ H⁺ secretion — HYPERKALEMIC ACIDOSIS

3) HYPOKALEMIA

↓ [K⁺]   ↓ K⁺ — HYPOKALEMIC ALKALOSIS
↑ H⁺ secretion

H-ions accumulate in the ICF during acidosis. During acidosis K shifts out of the cell to the ECF to maintain a balance of cations across the cell membrane. The decreased ICF K results in decreased secretion of K into the urine by distal tubular cells, contributing to hyperkaliemia. in contrast, state of alkalosis causes potassium shift into the cells, so the distal tubular cells increase their secretion of K into urine, contributing to hypokaliemia.
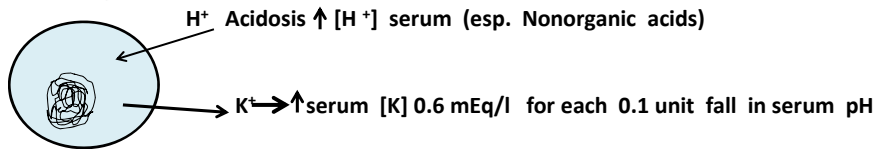
Aldosterone stimulates K secretion.

Aldosterone, besides acting to conserve sodium, can secrets potassium, when its concentration is increased. Aldosterone also increases the secretion of K from the sweat glands.

INSULIN contributes to the regulation of plasma K level by promoting the movement of K into liver and muscle cells. Insulin therefor can be used to treat hyperkaliemia. Dangerously low level of plasma K can result from the administration of insulin, when potassium level is depresed.Potassium balance is especially significant in treatment of insulin dependent diabetes.

CATECHOLAMINES also influence K concentration in ECF. β-2 adrenergics stimulate the movement of K into cells. α-adrenergics shift K out of the cells.

Regulation of potassium balance

H$^+$ Acidosis ↑ [H $^+$] serum (esp. Nonorganic acids)

K $\leftharpoondown$ → ↑serum [K] 0.6 mEq/l for each 0.1 unit fall in serum pH

K$^+$ - H$^+$ exchange:

↑ [K$^+$] serum ⟶ ↑K secretion /↓ H$^+$ secretion ⟶ **metabolic acidosis**

↓ [K] serum ⟶ ↓ K secretion / ↑secretion of H $^±$ ⟶ **metabolic alkalosis**

ALDOSTERONE ⟶ ↑ K losses
↑ K$^+$ in serum ⟶ ↑ ↑ aldosteron secretion

HYPOKALIEMIA develops ,when [K] less then 3.5 mEq/l
Causes: -Inadequate intake; Excessive renal losses: diuretic therapy, diuretic phase of renal failuure, hyperaldosteronism, Cushing's syndrome; -Excessive loss from GIT: vomiting, diarrhea; Excessive skin loss; -Treatment of diabetic ketoaci-dosis, - prolonged iv therapy without K, -.Metabolic alkalosis.
Clinical manifestations: - increased thirst, - impaired ability to concentrate urine,
- poliuria, - decreased urine osmolality, - nocturia, - anorexia
- nausea, - paralytic ileus
- hypotension, - cardiac disrhythmias,
- when K$^+$ in serum < 2,5 mM/l – stoppage of heart in systole
- muscle tenderness, muscle cramps
- confusion, depression
- metabolic alckalosis.
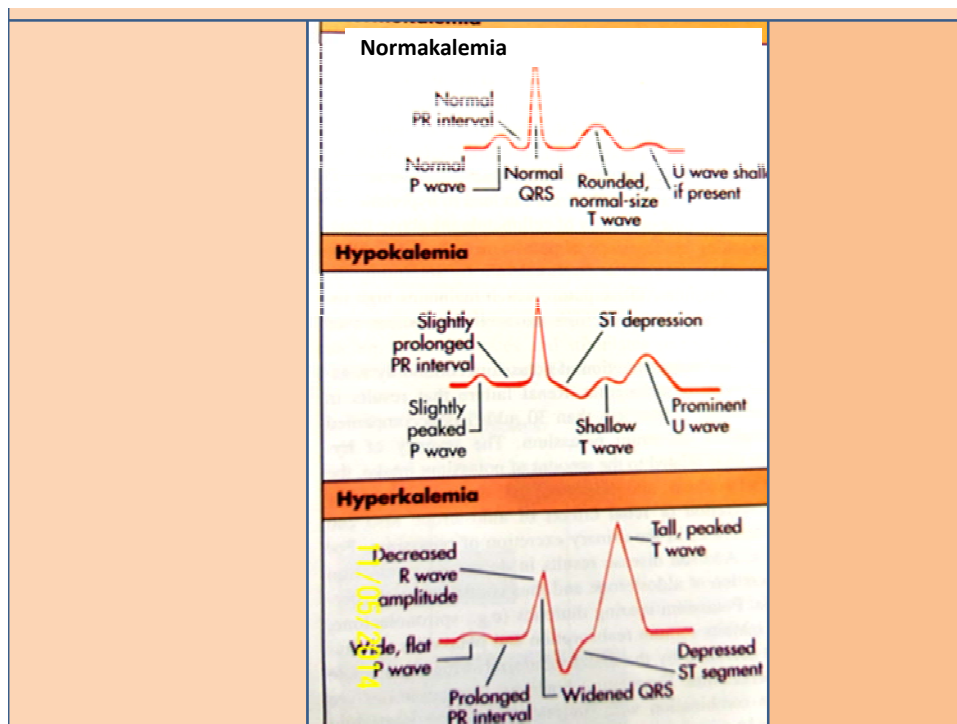**Hyperkalemia:** when serum K$^+$ > 5,0 mM/l
Causes: Excess intake and gain; increased oral intake;
Excess or rapid infusion of K-containing fluids; tissue trauma; burns, crashing in-juries; Inadequate renal losses: renal failure, adrenal insufficiency, potassium – sparing diuretics, angiotensin-converting enzyme inhibitors.
Clinical manifestation: - paresthesias, weakness and dizziness, muscle cramps;
nausea, vomiting, diarrhea, intestinal colic;
ECG changes: tall peaked T-waves, depressed ST segment, widening QRS, asystolia, cardiac arrest – when K$^+$> 7 mM/l in serum –stoppage of heart in diastole.

Normakalemia

Normal PR interval

Normal P wave — Normal QRS — Rounded, normal-size T wave — U wave shall if present

Hypokalemia

Slightly prolonged PR interval — ST depression

Slightly peaked P wave — Shallow T wave — Prominent U wave

Hyperkalemia

Decreased R wave amplitude — Tall, peaked T wave

Wide, flat P wave — Depressed ST segment

Prolonged PR interval — Widened QRS

Management: - Calcium gluconate 10 ml of 10% solution iv;

         40% glucose + insuline iv,

          correction of acidos

Lecture 14
ACID – BASE IMBALANCE

Hydrogen ions concentration must be regulated within a narrow range for the body to function normally.

Slight changes in amounts of hydrogen can significantly alter biological processes in cells and tissue. Hydrogen ion is necessary to maintain membrane integrity and the speed of enzymatic reactions. Most pathologic conditions disturb acid-base balance and the degree of severity may be more harmful than the disease process.

The hydrogen ion concentration is commonly expressed as pH – The negative logarithm of hydrogen ions in solution.

$$pH = Log \frac{1}{[H]} \quad or \quad pH = - Log [H]$$

Normal pH of blood is equal 7.35 – 7.45.

Acid – base homeostasis

Normally, body acids and bases are regulated within a narrow range so that membrane excitability, enzyme systems, and chemical reactions can function in an OPTIMAL WAY.

Acid – base status of the body is regulated by 3 major mechanisms:
1) Buffers

2) The respiratory system
3) The renal system

Normal laboratory values for acid-base parameters:

PaCO₂ (arterial blood)	**36 – 44 mm Hg (adult)**
	30 – 34 mmHg (infants)
HCO₃⁻ (serum)	**22 – 26 mEq/l (adult)**
	19 – 23 mEq/l (infants)
pH (arterial blood)	**7.35 – 7.45 (adult)**
	7.36 – 7.41 (infants)

Laboratory measurements such as arterial blood gas values are useful indicators of acid-base status of extracellular fluids.

1. The partial pressure of carbon dioxide in arterial blood (PaCO₂) is an indicator of respiratory component of acid-base balance.

2. The plasma bicarbonate ion (HCO₃⁻) concentration is an indicator of the renal (metabolic) component of acid-base balance.

The pH of the blood indicates the net result of normal acid –base regulation, or any acid –base imbalance and the body's compensatory responses

It is important to remember that pH measured clinically is that of the blood, but not reflects the pH inside of the cells or in cerebrospinal fluid.

The sources of hydrogen ion: a) metabolism, b) diet.

The serum pH can be calculated using formula of Henderson – Hasselbach equation:

$$pH = pK_a + \log \frac{[HCO3]}{[CO2]}; \text{ when this ratio is} = 20:1 \rightarrow pH = 7,4$$

Acids are continuously generated. Body acids exist in two forms:
1) volatile – can be eliminated as carbon dioxide gas [CO₂]
2) nonvolatile – can be eliminated by kidney (carbonic acids lactic, acetic, dissociated fatty acids)

Nonvolatile acids:
Strong nonorganic acids: - Sulfuric ← S–containing amino acids
(methianine, cysteine, cystine)
- Hydrochloric ← arginine, lysine
- Phosphoric ← nucleic acids
Organic acids: - Lactic acid ← incomplete oxidation of glucose
-Ketoacids ← incomplete oxidation of fat

Buffers.
Buffers are the chemicals that help to control the pH of body fluids.
Each buffer system consists of weak acid, which releases hydrogen ions when the fluid is too alkaline, and a base, which takes up the hydrogen ions when the fluid is too acidic. In this way changes in pH are neutralized immediately by the action of buffers. The main among them are:
- **in extracellular fluid:** -bicarbonate buffer. -protein buffer,
-phosphate buffer, -ammonia buffer, -bone tissue
- **in intracellular fluid:** -hemoglobin buffer inside the erythrocytes,

-protein buffer, - phosphate buffer.

These buffers are the first line of defense against pH disorders.

1. Bicarbonate ions (HCO_3^-) and carbonic acid (H_2CO_3) - the two components of **bicarbonate buffer system** – are in chemical equilibrium in extracellular fluid.

If too much acid (lactic acid) is present, the HCO_3^- takes up the H^+ and becomes carbonic acid. Through the action of enzyme - carbonic anhydrase – carbonic acid is then exerted through the respiratory system in form of CO_2 and H_2O. Thus, the excess acids is neutralized:

$$HCO_3^- + H^+ \leftrightarrows H_2CO_3 \xrightarrow{carb.anhydraze} CO_2 \uparrow + H_2O \uparrow$$
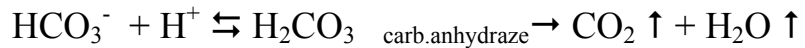
If too little acid is present in extracellular fluid, the carbonic acid releases hydrogen ions: $H_2CO_3 \leftrightarrows HCO_3 + H^+$

For pH of blood to be within the normal range the ratio of HCO_3^- to H_2CO_3 must be 20 : 1: means that 20 bicarbonate ions must be present for every carbonic acid molecule. Any deviation from this ratio alters the pH of blood.

2. **Hemoglobin-buffer system**

CO_2 is produced in tissue cells and diffuses in plasma, where it is transported as dissolved carbon dioxide (5%), or it combines with water (90%) to form carbonic acid , or it combines with proteins, from which hydrogen ions has been released. Most of CO_2 diffuses into erythrocytes and combines with water. This lead to appearance of carbonic acid, which immediately dissociates into bicarbonate (HCO_3^-) and hydrogen ions (H^+). The bicarbonates shift into plasma, but chloride ions (Cl^-) shift into red cells to maintain electronutrality. With the help of enzyme – carbonic unhydrase- hydrogen ions react with hemoglobin, which releases his oxygen, to form restored hemoglobin.

3. **Respiratory contribution**

The respiratory system is the defense system against acid-base disorders. Body cells are continuously producing carbon dioxide. Together CO_2 and H_2O make carbonic acid. The lungs excrete CO_2 and H_2O from the body .Therefore, during the process of exhalation the lungs are effectively excreting carbonic acid. Thus, the respiratory system adjusts the amount of carbonic acid that remains in the body.

The rate and depth of respiration are strongly influenced by activity of chemoreceptors that sense the partial pressure of CO_2 ($PaCO_2$) and pH of the blood. $PaCO_2$ indicates how effectively the respiratory system is excreting carbonic acid. Carbonic acid is known as the only volatiile acid, because it can be excreted as gases. The other acids are nonvolatille, they are organic acids. If the nonvolatile acid (such as lactic acid) accumulates in the blood, the rate and depth of respiration will increase (hyperventilation). Because the excess hydrogen ions stimulating the chemoreceptors. Hyperventilation does not excrete lactic acid from the blood, but does remove carbonic acid. This process will decrease pH, but keep pH of dropping too low.

Respiratory response to imbalance of any acid, except carbonic acid, is called **compensation**. The compensatory response does not correct pH disorder, but it

does compensate for it by adjusting pH back toward normal, even through other blood chemistry values are made abnormal in the process.

The compensatory response to deficit of any acid, except carbonic acid, is **hypoventilation.** By decrease the rate and depth of respiration the body retains carbonic acid. This accumulation of H_2CO_3 helps to keep the pH of the blood from rising to fatal level when another acid is deficient.

Thus, hypoventilation allows CO_2 to accumulate and increases the amount of carbonic acid in blood. Hyperventilation leads to decrease CO_2 in blood and decreases the amount of carbonic acid in blood.

Respiratory compensation from acid – base imbalance requires at least several hours.

Renal contribution

The kidneys are the other defense system against acid-base disorders.

The kidneys can excrete any acid from the body (except carbonic acid). Cells continuously produce metabolic acids during metabolism and kidneys excrete these metabolic acids. If metabolic acids begin accumulate in blood, kidneys increase their acid excretion mechanisms to correct the problem.

The body's ability to correct an excess or deficit of metabolic acids depend on normal function of renal system. Infants excrete more bicarbonates in their urine then older children or adults do; their kidneys are less effective in excreting acids.

The kidneys have several mechanisms that accomplish acid excretion.

Bicarbonate ions (HCO_3^-) are contained in the fluid filtered from the blood at the glomerulus. They become part of tubular fluid. As this fluid moves through the renal tubules, hydrogen ions are secreted into the fluid. Secretion of H^+ occurs in the proximal tubules by the process of Na^+/H^+ exchange, and in the collecting duct by means of H^+-ATPase. The processes in renal tubular cells that generate H^+ for secretion into renal tubular fluid simultaneously cause the generation of HCO_3^-, which is returned to the blood. Thus: for every hydrogen ion, which secret into renal tubular fluid, one molecule of bicarbonate is reabsorbed (is returned to the blood).

Most of H^+ in renal tubular fluid combines with other chemicals:
  -urine buffers (phosphate and creatinine, filtered at the glomerulus)
  -ammonia (NH_3) , which is produced by renal tubular cells
  -bicarbonate ions, that were filtered at the glomerulus

Thus net H^+ excretion is accomplished in a form of buffered H^+ (called titratably acidity) and H^+ attached to ammonia. $NH_3 + H^+ \rightarrow NH_4^+$

When kidneys need to excrete more hydrogen ions, the renal tubular cells increase the production of urinary ammonium ions. Ammonium ions (NH_4^+) are net lipid soluble, so they do not easily cross the renal tubular fluid back to the blood.

The concentration of HCO_3^- in plasma is a reflection of the effectiveness of renal regulation of metabolic acids. If metabolic acids are accumulating in the blood, they will be buffered by HCO_3^- and the concentration will drop below normal.

Thus: a decreased concentration of HCO_3^- in plasma indicates a relative excess of metabolic acids. An increased concentration of HCO_3^- leads to deficits of metabolic acids.

Normal laboratory parameters for acid - base balance

Actual pH = 7.35 – 7.45

Actual pCO_2 ; $PaCO_2$ = 40 – 45 mm Hg $PvCO_2$ > 46mm Hg

BB (Buffer Base) = 48 (42 - 52) mmol/l

BE (Base Excess) BE = -2.5 + 2.5 mmol/l   A measure of the non-respiratory component of acid - base system

Positive BE results from the addition of base to the blood or the removal of H

Negative BE - base deficit - accumulation of non-volatile acids in the blood

SB (Standart Bicarbonate) = 25 – 28 mmol/l

AB (actual Bicarbonate) = 24 (19 - 25) mmol/l

AP (anion gap) = Anions - Kations = 12 mEq/l

Acidosis (pH < 7.35)

The pH of the blood is a function of the concentration of bicarbonate ions and carbon dioxide. An acidosis is caused by too high a concentration of CO_2 or too low a concentration of HCO_3^- in the blood.

Metabolic acidosis is the most frequent and severe acid-base imbalance.

In metabolic acidosis, a noncarbonic acids increase or bicarbonate is lost from extracellular fluid. This can occur quickly, as in lactic acidosis from poor perfusion, or more slowly as in renal failure or diabetic ketoacidosis.

Causes: Increased acids: -Ketoacidosis (diabetes mellitus, starvation)

-Severe infection,   - Fever,   -Shock,

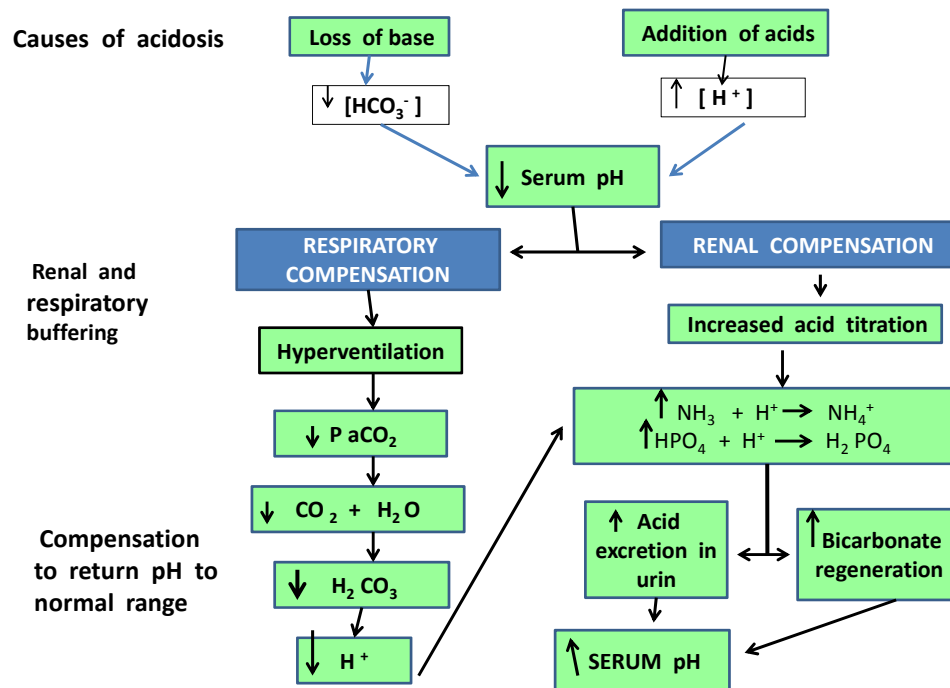-Renal failure,   - Burns,   - Hypoxia

-          Decreased bases: -Diarrhea,   Intestinal decompression

-Gastro-intestinal fistula

In metabolic acidosis noncarbonic acids increase or bicarbonate is lost from the extracellular fluid. This can occur quickly, as in lactic acidosis from poor perfusion or more slowly as in renal failure or diabetic ketoacidosis.

Causes of acidosis

Loss of base → $\downarrow$ [HCO$_3^-$]

Addition of acids → $\uparrow$ [H$^+$]

$\downarrow$ Serum pH

Renal and respiratory buffering

RESPIRATORY COMPENSATION ↔ RENAL COMPENSATION

Hyperventilation

Increased acid titration

Compensation to return pH to normal range

$\downarrow$ PaCO$_2$

$\downarrow$ CO$_2$ + H$_2$O

$\downarrow$ H$_2$CO$_3$

$\downarrow$ H$^+$

$\uparrow$ NH$_3$ + H$^+$ → NH$_4^+$
$\uparrow$ HPO$_4$ + H$^+$ → H$_2$PO$_4$

$\uparrow$ Acid excretion in urin

$\uparrow$ Bicarbonate regeneration

$\uparrow$ SERUM pH

The calculation of the anion gap can be helpful to distinguish different types of metabolic acidosis.

The Anion Gap in differential diagnosis of metabolic acidosis.

Decreased Anion Gap (<8 mEq/l):
Hypoalbuminemia ($\downarrow$ unmeasured anions)
Multiple myeloma ($\uparrow$ unmeasured cationic IgG)
Increased unmeasured cations (K$^+$, Ca^{+2}, Mg^{+2})

Increased Anion Gap (> 12 mEq/l): *Presence of unmeasured metabolic anion*:
Diabetic ketoacidosis,   Alcoholic ketoacidosis   Lactic acidosis
Starvation,   Renal insufficiency
                     Presence of drug chemical anion:
Salicylate poisoning   Methanol poisoning
Ethylene glycol poisoning

Normal Anion Gap (8 – 12 mEq/l)
Loss of bicarbonate: - Diarrhea,   Pancreatic fluid loss,   Ileostomy
Chloride retention: Renal tubular acidosis,   Ileal loop bladder
                  Parentheral nutrition (arginin, lysine)

Clinical manifestations:
Blood: $\downarrow$ pH, $\downarrow$ HCO$_3^-$ (primary), $\downarrow$ pCO$_2$ (compensatory)
Gastro-intestinal function: -anorexia: - nausea, vomiting, - abdominal pain
Neural function: - weakness, - depression, - lethargy, - stupor, - coma,
                  $\downarrow$ pH in cerebrospinal and intestinal fluid in brain
Cardio-vascular function: - peripheral vasodilation, - decreased heart rate, - cardiac dysrhythmia
Skin: - warm and flashed
Skeletal system: - bone diseases
Signs of compensation: -Kussmaul breathing, - hyperkalemia, - acidic urine,
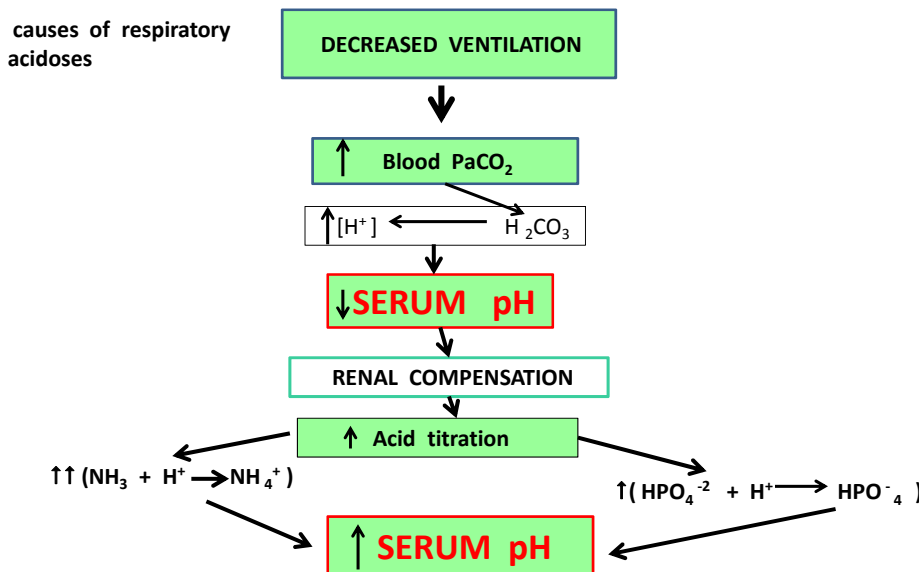   -increased concentration of NH$_4$ in urine

Treatment: Sodium bicarbonate administration is required to elevate pH to a safe level, particularly if there is renal failure. Accompanying sodium and water deficit must also be corrected.

Respiratory acidosis.

Causes:

1. Impaired gas exchange : decreased ventilation, hypercapnia,
   restrictive pulmonary diseases: pneumonia, disorder of lung paranchima,
   pulmonary edema, obstructive pulmonary diseases,
   acute respiratory distress syndrome.

2. Impaired neuromuscular function ; disorders of chest wall (injury or sur-
   gery\0,
   respiratory muscle fatigue, paralysis. Kyphoscoliosis,
   hypokalemia

3. Impaired respiratory brain control:
   trauma or depression of respiratory centre,
   respiratory depressant drugs (barbiturates, narcotics)
   central sleep apnea

Respiratory acidosis

causes of respiratory acidoses

DECREASED VENTILATION

$\uparrow$ Blood $PaCO_2$

$\uparrow[H^+] \longleftarrow H_2CO_3$

$\downarrow$SERUM pH

RENAL COMPENSATION

$\uparrow$ Acid titration

$\uparrow\uparrow (NH_3 + H^+ \longrightarrow NH_4^+)$

$\uparrow(HPO_4^{-2} + H^+ \longrightarrow HPO_4^-)$

$\uparrow$ SERUM pH

Clinical manifestation : -headache,-tachycardia, -cardiac arrhythmia, -neurological symptoms, -cyanosis, -hypoxic syndrome.

Treatment of respiratory acidosis The restoration of adequate alveolar ventilation removes excess CO_2. If alveolar ventilation cannot be maintained spontaneously because of drug overdose or neuromuscular disorders, mechanical ventilation is required. The arterial pH, pCO_2, pO_2, HCO_3^- must be carefully monitored. Renal reduction of pCO_2 can cause respiratory alkalosis with seizures and death.

Renal buffering is usually effective in compensating uncomplicated chronic respiratory acidosis. In presence of hypoxemia and hypercapnia oxygen can function as a respiratory depressant, when the respiratory centre is no longer stimulated by the lower pH and elevated pCO_2 . Therefore oxygen should be given cautiously.

Metabolic alkalosis pH > 7.40, increased level of HCO_3
Metabolic alkalosis is common and occurs when bicarbonate is increased due to excessive loss of metabolic acids.
Causes: 1) increased bases: - intake of bicarbonate,
$\qquad\qquad\qquad\qquad$ -massive transfusion with citrated blood
$\qquad\qquad\qquad\qquad$ -extracellular fluid volume depletion
$\qquad\qquad$ 2). decreased acids:-emesis, -gastric suction, -vomiting, -hypokalemia,
$\qquad\qquad\qquad\qquad$ -extracellular fluid volume depletion,
$\qquad\qquad\qquad\qquad$ -hyperaldosteronism, -diuretic therapy.
Clinical manifestation:
The symptoms vary due to many causes of metabolic alkalosis. Some common symptoms, such as weakness, muscle cramps and hyperactive reflexes are related to volume depletion and electrolyte losses. Decreased extracellular fluid volume leads to hypotension. Hypokalemia is the cause of muscle weakness and polyuria. Because alkalosis causes a decrease in ionized Ca, tetany may develop. Initial excitation of CNS changes to depression, lethargy and coma. Respiration is slow and shallow to increase content of CO_2. Confusion and convulsions occur during severe alkalosis. The oxyhemoglobin curve shifts to the left, decreasing dissociation of oxyhemoglobin and increasing risk of arrhithmia. Death occurs when pH≈ 7.8
Compensatory response is hypoventilation, to retain carbonic acid within the body.
Treatment.
With hypocloremic alkalosis and volume depletion a sodium chloride solution is required for correction. The administration of potassium corrects alkalosis caused by hyperaldosteronism or hypokalemia. The potassium causes hydrogen to move back to the extracellular fluid and decreases loss of hydrogen from distal tubular.
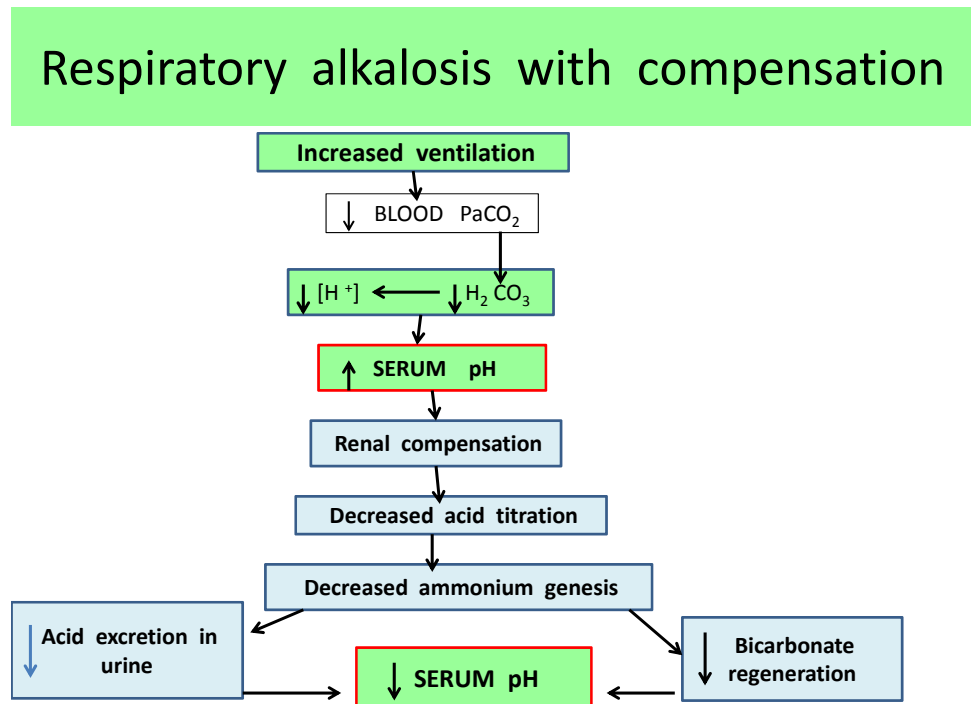**Respiratory alkalosis pH>7.4 , ↓$PaCO_2$**
Respiratory alkalosis occurs when there is alveolar hyperventilation and excessive reduction of carbon dioxide (hypocapnia).
Causes : by hyperventilation H_2CO_3 excreted during expiration (rapid and deep)
$\qquad$ -gram-negative sepsis, meningitis, head injury can stimulate respiratory
center
$\qquad$ in the brainstem $\qquad$ -prolonged sobbing, $\qquad$ -hysteria,
$\qquad$ -pulmonary diseases, $\qquad$ hypoxemia, $\qquad$ -alcohol withdrawal,
$\qquad$ -congestive heart failure, $\quad$ -thyrotoxicosis, $\qquad$ -hypermetabolic states
$\qquad$ -anemia, $\qquad\qquad\qquad$ -early salicylate.
$\qquad$ Respiratory alkalosis occurs within minutes of hyperventilation. Cellular buffer provide immediate compensation with shift H^+ from intracellular to extracellular fluid. If there is a significant deficit of CO_2 , this H^+ shifts are not very ef-

fective. During chronic respiratory alkalosis renal compensation restores pH by decreasing H^+ excretion or/and
becarbonate absorption.

Respiratory alkalosis with compensation

Increased ventilation

↓ BLOOD $PaCO_2$

↓ $[H^+]$ ← ↓ H_2CO_3

↑ SERUM pH

Renal compensation

Decreased acid titration

Decreased ammonium genesis

Acid excretion in urine

↓ SERUM pH

Bicarbonate regeneration

.

Clinical manifestation. Respiratory alkalosis, like metabolic alkalosis, is irritating to central and peripheral nervous system.
Symptoms: dizziness, confusion, convulsions, coma, paresthesia in fingers, increased neuromuscular irritability, cerebral vasoconstriction, carpopedul spasm and other symptoms of hypocalcemia. Deep and rapid respirations are the primary symptoms of respiratory alkalosis.
Treatment
The underlying disturbance must be identified and treat. Hypoxemia must be corrected.
-Inhalation of carbogen, -anticonvulsant therapy. Water-electrolyte imbalance must be corrected.
Nomogramm by Ziggard – Andersen will help to find disturbances of acid-base balance using pH and $PaCO_2$ in blood.

Lecture 15
NEOPLASIA

Definition neoplasia literally means "new growth", and the new growth is a neoplasm.

The common properties of neoplastic cells are the following:

- Non-limited in the growth
- Tend to be immature-anaplasia
- Don't fulfill their function
- Threaten to a surrounding tissue and very often to whole organism

The problem of neoplasm growth is very close to the problem of biological growth in whole. Why usual replenish of cell population after partial its loss doesn't exceed the initial mass? What are the mechanisms which control a process of renewal cells population after their death (regeneration)? Why neoplasm doesn't need such control? Only after solving the problem of normal cells growth we will obtaint a key to explanation of neoplasia development when the cells growth becomes uncontrolled, relatively independent, unlimited, and accompanied by disturbances in cell maturation.

Actuality of the problem

It is one of the most important problems of humanity because this disease is characteristic of a very high rate of mortality. After heart disease, a cause of the death of neoplasm occupies the second place .It is the greatest problem of theoretical medicine and practice. It isn't a secret that every year the disease takes away the millions people lives who suffer of unwearable pain. It is a problem of families and states; the lasts every year spend a lot of money to treat such a horrible disease. There is the " Institute of Cancer" practicall in each developed State, and the most Noble's prices have the scientists which are working on the oncologic problems. However, now the clinical needs overtake scientific discovery.

The theory of tumor progression

The theory was introduced in oncology in 60th by Foulds. According his theory tumor is a cell mass which is increasing in time, and during the growth acquires the new properties which make it more and more malignant. Its new abilities don't disappear over time and become irreversible. These are the morphological atypia with growing anaplasia; last means that the cells tend to be more and more immature.

The most prominent signs of tumor progression

- Growing anaplasia
- Biochemical atypia
- Metastazing
- Autonomal growth

Classifications of tumors

According to the character of growth and its influence an organism there are two variants of tumor are differentiated: benign and malignant.

Benign tumor-versus malignant one

Characteristics	Benign	Malignant
Histology	Typical of tissue origin Few mitosis size and shape	Anaplastic: with abnormal cell. Many mitosis
Growth rate	Slow, often they have a capsule	Rapid, havn't a capsule.
Location/metastases	Strictly local/ no metastases	Infiltrative growth/ frequent metastases
Tumor necrosis	Rare (have little vasculature)	Common (rich vasculature)
Recurrence after	Rare	Common
Prognosis	Good	Poor if infiltrative growth Surgery is the best

P.S. Anaplasia-lack of cell differentiation. Metastasis- distant site of tumor spreading

The other type of classification is according to the tissue which a tumor arised from. The following types of them may be called: epithelial –cancer, connective tissue-sarcomas, hematopoietic-leukemia and set. Biological properties of malignant tumor and given them advantages in growth

Special biological properties of malignant tumors may be explained by an alteration of their genome, and must be said, that last supplies them by the lot of advantages in their growth and possibilities to occupy whole organism.

They are the following:

1. among them and the most important is production by the own cells of the growth factors that contribute to tumor independent growth
2. loss of contact inhibition in the culture of tissue growth. The normal cells cross their growth when contact to each other in culture; they are growing in form of monolay, however, the malignant ones grow in form of warts. They climb each other, by another words, they possess by "antisocial behavior" being deaf to the extrinsic request
3. high mobility and moving easy from one site to another become possible due to changes in their genome, letting them to express the adhesion molecules to extracellular matrix and produce the lot of proteolytic enzymes. The lasts facilitate a lysis of matrix and their permeation through a capillary wall. In normal only the phagocytes and, lesser, the lymphocytes may realize this function
4. high double energetic supplying, aerobic and anaerobic when Pasteur's effect is inhibited. It means that anaerobic pathway of respiration is realizing despite an oxygen tissue supplying. The phenomena brings a gain in the growth too

Morphological anaplasia

It is cardinal sign to estimate a tumor grading by its biopsy. Last lets a pathologist to diagnose for a disease and its prognosis. The lower grade of cell maturation the more malignant tumor and the worst prognosis to a patient. The

growth rate of tumor is correlated with its level of maturation; anaplasia is a loss of cell ability to be differentiated, and it presence gives a key to the pathologist to diagnose the grading and staging of a disease. These indices are very important to prediction of cancer clinical behavior, estimation of anaplasia degree, and tactic in plane of urgent surgery.

Grading refers to morphological characteristic; the higher degree (grading of anaplasia), the more malignant tumor potential. Staging reflects the location and pattern of tumor spreading within a host.

In connection with it must be considered the followings

- Tumor size
- Extent of local growth
- Lymph nodes and organs involvement
- Presence of distant metastases

TNM classification minds: tumor—nodes—metastases. As to prognosis of the tumor disease and following treatment, it is life-important for pathologist to estimate the stage of malignant tumor progression. Neoplastic cells possess by abnormal histology.

Morphological signs of cell anaplasia include the following:

- Disorganization of cells when they look unlike to each other, and are very different in size and shape
- Abnormal location of mitosis
- Nuclei are darker than normal (hyperchromatic) as a sign of an active protein synthesis and high rate of proliferation.
- Very often can be found disposition in microtubules and microfilaments and appearance of special microfila

About microfilaments, they are specific: desmin in the tumors originated from the connective and muscle tissue, keratin in the epithelial origin tumors, vimentin in sarcomas, lymphomas and some carcinomas, and other markers of altered proteins. These proteins revealing is a very important in diagnosis of tumor disease, and nowadays these tumor markers are used in clinic

- Appearance of gigantic round and very ugly mitochondria says about energetic anomalies in tumor cells
- Surface of the tumor cell is very folded and characterized by the numerous vesicles, microvillisies and branches. The lasts increase a square of the cell surface and possibilities to interact with the other cells. The changes in the receptor apparatus of the tumor cells is one of the key problems of the tumor growth

Biochemical atypia

The most characteristic feature of biochemical atypia is the changes in synthesis of multiple proteins. Among them, loss of the MHC class I molecules that interfere with immune recognition of tumor cells. The matter of the fact that, on the on hand, these cells become some foreign to an organism because they possess by special tumor –specific antigens; but, on the other hand, they arised from own

cell population with normal organospecific antigens. So, the tumor cells seem to belong to the "self" and, at the same time, to non-self "that can explain the problems in their immune recognition.

Tumor cells are capable to synthesize very unusual, not characteristic of mature cells substances. These substances can be used in diagnostic practice. Some tumor cells acquire a property to secrete such chemical substances as the hormones, oncofetaproteins, enzymes and glycoproteins. This phenomen called the paraneoplastic syndrome, because it isn't cardinal sign of tumor growth, but the only other consequence of changed cell genome.

Paraneoplastic syndrome

Some tumors, not possessing by endocrine origin are capable to synthesize and secrete the hormones, for example, ACTH, ADH, insulin, parathyrin and others. Ectopic elaboration of the hormones (by inappropriate tissue) is the base of the paraneoplastic syndrome. The name of the syndrome, as was said above, isn't connected directly to the main property of tumor cell to grow without limit, but it is associated with the abnormality of the tumor cells due to derepression of some genome.This abnormality permits the cell to synthesize forbidden substances which are not corresponded to their normal function. More often, paraneoplastic syndrome accompanies such tumors as small-cell carcinoma of the lung, breast carcinoma in women and colon. Besides, such autoimmune diseases as dermatomyositis, myasthenia gravis, and Eaton-Lamberth's syndrome seem to be the paraneoplastic syndromes too.

Paraneoplastic syndrome may result in some complications of tumor disease in form of thrombosis, anemia, destruction of vessels wall and hemorrhages, up to a death of patient. On the one hand, they may be as the first manifestation of disease and can help to recognize a disease, but on the other hand, to mask a disease and point to the wrong way of the treatment.

Invasion and metastasizing

Invasion and metastasizing are cardinal signs of a malignant tumor. They are the main causes of very high mortality of the patients. The base of the metastasizing includes the following points:

1. Infiltrating cell growth and cell permeation through the small blood and lymph vessels
2. Very weak connection with the adjacent tumor cells
3. Favourable situation for their life strategy in place of residence

Cascade of metastasizing consists of next four stages
Stages of metastazing

1. Separation of the tumor cells from the main tumor node and inclining into a cellular matrix
2. Permeation into the lumen of small blood and lymphatic vessels and aggregation with other blood cells, especially the platelets with spreading them over an organism; their conglomerates may provoke possible embolism as complication

3. Attachment to the vessels intima in capillaries with possible small thrombi formation
4. Accommodation in the surrounding tissue

Then they take root in the new place of settling and set an extravascular proliferation, forming the daughter's nodes

The basic mechanisms of metastasizing

It is known that when the cells are growing in culture they stop their growth being in contact to each other. For this reason its phenomena is called the contact inhibition.

But tumor cells don't have this ability, and loss of contact inhibition is one of the main biological characteristics of these cells. Their behavior, as was said, is very "unsocial' and in the culture these cells climb each other and their growth reminds the wart growth. This phenomena also can be explained by the loss of some form of the adhesion molecules on the tumor cell surface, for example, E-kadgerins (E-epithelial). However, the density of another types of adhesion molecules on their membrane which are responsible for the attachment to the molecules of extracellular matrix is increased, and such receptor-dependent association lets the tumor cells move without obstacles through the connective tissue; takes them not long time to rich the vessels wall. The matter of the fact, that only phagocytes possess by high motility and ability to move through the connective tissue and permeate the blood and lymphatic vessels, lesser-lymphocytes. It is their genetic property and recorded in their genome. But the tumor cells are the cells with changed genome which give them a lot of advances to enhance their growth and adaptation up to be the immortal.

After adhesion to the ECM molecules by special receptors tumor cells, due to possibility to synthesize plasmin, clean their way off small thrombi and secrete different types of proteases to facilitate a spreading. So, collagenase type IV, lyzing collagen, is responsible for easy permeation of tumor cells through a vessels wall. Association of tumor cells with other cells survives them and facilitates their implantation in the tissue. Moreover, the platelets supply them by the special growth factor. Being in the blood stream, tumor cells are in danger because the immune cells can attack them, especially natural killers, and for these reason the patients with lower immunological protection may be are in a risk of tumor disease. The factors of risk in this situation are the following: aging and primary and secondary immunodeficiencies. In addition must be said; if a tumor disease is in progress our organism can't cope with the increasing mass of these cells which can avoid an immune recognition. A failure of immunological barrier ultimately leads to decreasing in tumor cells elimination from an organism, and settle them with the start of malignant growth.

Autonomous tumor growth

In process of tumor progression its sensitivity to the chemotherapy and X-ray influence become lesser and lesser. Such changed behavior is characteristic of the hormonal-active tumor too when we treat our patient with the hormones of opposite sex. Mostly, the phenomena can be explained by the fact that on the first stage

of the tumor progression tumort has a monoclonal origin that means, the tumor cells are presented by the progeny of only one cell with particular characteristic. When tumor cells proliferate actively the risk of additional gene mutation becomes increased, and monoclonal tumor growth is turned into polyclonal one. It means the formation of new generations of the cells with high strategy of surviving and low sensitivity to the external influences. New progeny acquires particular properties due to the following factors:

- Self-supplying production of the growth factors, receptors to them, and other growth stimulators
- Disappearance of the growth factor inhibitors in the tissue which named keylons
- Secretion of the angiogenetic factor that helps tumor to organize an appropriate microsurrounding vasculature
- Antigenicity becomes very weak due to loss of MHC molecules class I. Last, hardly ever facilitates an immune recognition of tumor cells
- Adaptation to the chemotherapy via synthesis of the substances which destroy the chemotherapeutic drugs
- Acquirement of an ability to repair own cell DNA after X-ray and chemotherapy injury in course of tumor treatmen or spontaneous destructiont

Systemic influences tumor an organism and symptoms of tumor disease

The symptoms of malignant tumor disease are associated both, local and systemic influences of tumor and, first of all, there are the symptoms of an acute phase response. The lasts include: easy fatigue, fever, serious loss of weight and poor appetite, anemia and others non-specific signs.

However, the following complications occur more often:

- Compression of surrounding tissue by the tumor mass with the disturbances in an organ function
- Necrosis of the tumor tissue with absorption of necrotic products by the blood and intoxication development
- Production by the tumor some biological active substances including the hormones, so called paraneoplastic syndrome development
- Destruction of enough large vessels and surrounding tissue that may lead to massive hemorrhage and chronic or acute anemia with all their unfavourable consequences
- Decreasing in patients immunity predisposes them to various types of opportunistic infection which very often become a threatening to the life
- Malignant tumor, especially acute promyelocytic leukemia and carcinoma of prostatic gland, more often than other types of malignant tumors can be complicated by the DIC syndrome

Cancer cachexia

The patients with malignant tumor who ever don't have the gastrointestinal forms suffer of severe loss of weight up to cachexia. Last term means significant

loss of weight predominately due to decreasing in fat and then, muscle tissue. As usual, it is accompanied by a very poor appetite or full refuse of food by patient and an expressed fatigue. Nowadays, we prone to explain this phenomena by the elaboration of some acute phase response cytokines, and among them TNF-alpha (cachectin) and IL-I play the most important role. They inhibit the appetite centers in CNS and lipoproteinlipase activity in a fat tissue, and such way, decrease the bulk of the fat. It must be added, that low insulin secreting can provoke muscle hypotrophy with its flabbiness and weakness.

The theories of cancerogenesis and the common mechanisms of cell neoplastic cell transformation. Nowadays, there the four theories of cell neoplastic transformation are accepted, and they are based on supposed them provoking. factors.

Modern theories of cancerogenesis

- Virus theory
- Of chemical cancerogenesis
- Radiation theory
- Hereditary theory

Hereditary tumors which more often occur in a childhood are responsible for relatively small group of diseases.

According to the data of experimental pathology the process of the malignant tumor formation is passing some stages. At first, cancerigenic factor of named above infectious or non-infectious origin, provokes unfavourable mutation in the genome of certain somatic cell, but in case of hereditary tumor in germ cell. Then mutant cell is proliferating and its progeny is represented by the monoclonal pull of the transformed cells. The following genes become the target in such types of mutation:

- genes of growth or protooncogenes. They are normal genes that become switched on in the periods of an organism growth, reparation of the tissue after injury, and physiological loss of hormonal dependent tissue. Under cancerigenic factor the protooncogene is converted into oncogene which, in turn, becomes responsible for oncoprotein synthesis. It must be added that oncogenes are the dominant genes, so far as for monoclonal tumor proliferation, enough an only single mutant protooncogene appearance
- Genes which inhibit the growth or tumor suppressing genes. Malignant tumor appears in case of inactivation or hereditary loss of one or both alleles. The example of such kind of gene is Rb-gene (Rb- retinoblastoma). Loss of two copies of this gene ultimately leads to the malignant eye tumor- retinoblastoma. The tumor arises from retinoblasts- precursors of mature retinocytes, and it predominately is found in children. If there is only one defective gene, its healthy counterpart protects an organism against a disease, but when such shield disappears the risk of the disease becomes very high
- Genes which regulate apoptosis may be as dominant as recessive. Bcl-2 , bax and p-53 belong to this group of genes. Imbalance in such genes expression may result in the weakness of apoptosis and increasing in amplification of

the protooncogenes with cell overproduction, and such way make the cells immortal.

- Genes of DNA reparation. They provide the reparation of DNA in case of injury and stabilize genome. For this reason, any mutation in a genome when these systems can't cope with reparation may lead to conversion of protooncogene into oncogene with all described above unfavourable consequences.

Mechanisms of virus cancerogenesis

The theory of virus cancerogenesis, in common, was created due to experiments on the animals, mostly mice. There are many experimental evidences of oncogenic influences   some viruses of different biological organization level, beginning with the plants, however, we know so little about the human oncogenic viruses. Classification of the oncoviruses and tumors which they provoke. Now all oncoviruses are divided into two categories: DNA and RNA- containing forms.

To DNA containing viruses belong adenoviruses and polyoma virus that provoke the tumors in the animals but only in experimental conditions. The other viruses, for example, the buffalo papilloma virus can provoke both benign and malignant tumor in their natural hosts. Human DNA containing viruses that capable to provoke a malignant tumor may be divided into 4 groups.

DNA-containing viruses of man

1. Papillomas viruses
2. Epstein-Barr virus provokes the Berkitt's lymphoma
3. Virus of hepatitis B can provoke the liver cancer
4. Herpes virus – Caposhi sarcoma

To RNA-viruses, provoking the different tumors in the animals, belong sarcoma virus of hen and leukemic viruses (Moloni and Graffy) of the mice. However, we are known only one human RNA-content oncovirus, and this is HTLV-I of lympoleukemia/lymphoma in the adults.

Hereditary mechanisms of virus cancerogenesis

Before the virus cancerogenesis description it is necessary to give some definitions which accepted by modern experimental oncology:

Nowadays, two theories of virus neoplastic transformation of a normal cell into tumor one are accepted. The first theory is associated with integration of virus genome with protooncogen with next oncogene formation. The variants of mutation of protooncogene may be different and interfere with the other targets too: antioncogenes, apoptic genes, or DNA reparation genes. It's so called insert mechanism. Mutated gene becomes oncogene, but in case of RNA-content viruses the last must synthesize DNA on the matrix RNA with enzyme reverse transcriptase involving. The secondary mechanism realized via insertion or attachment of very strong virus promoter to protooncogene .That promotor virus used to get for own amplification, but being attached to cell protooncogenes, it creates many copies of those genes. The promoter virus region named the long terminal repeat unit. For example, such promoter virus gene, attached to the gene of heavy chain of the im-

munoglobulin may lead to the tumor pathology in form of plasmacytoma. At the same time, being fixed on the gene of CSF (colony-stimulating factor), it becomes responsible for chronic myeloleukemia which is characterized by uncontrolled proliferation of the granulocytic cells.

Chemical cancerogenesis

The start of the study of chemical cancerogenesis on the experimental animals coincided with a discovering of the polycyclic carbohydrates in the beginning of 20 century. They all were the products of coal resin processing. The first 3,4 benzpyren was, but later DMBA, (dymethyl-benzantracen) synthetic analogous showed stronger cancerogenic properties; it usually get used in an experimental oncology. These substances are capable of both, local and resorbtion activity; last means that a substance may act not only in the place of rubbing into a skin but for a distance. The other group is represented by the aromatic amines and some dyes. They possess by organotropic effects because can provoke the tumor of the specific organs. So, amineacetofluoren provokes in experiment the liver tumor in rats, but beta-naphtylamine can initiate cancer of the bladder, but not only on the experimental animals but in a man too. From history we are known that in twenties of last century German workers, who were engaged in the manufacturing such synthetic dyes as naphtylamines, not rare suffered of bladder cancer. To natural cancerogens which possess by potential ability to provoke a tumor belong aphlotoxin B-1 producing by particular kind of the fungi (aspergillus flavis). Nitrosamines, asbest, vinilchloride, and some metals may be added to this list. Another group is named endogenous cancerogens because they are elaborated by own organism. They are our natural metabolites or steroids. The derivates of the aminoacid tryptophane 5-oxyindolacetate in the experiment can provoke leukemia in high leukemic line of the mice. The next substance, oxyantranyl acid is the derivate of the tyrosin. Cholesterol after X- ray treatment "in vitro", bile acids, and estrogens after their application on the mucosal line of mice uterum may be involved in the process too.

He stages of chemical cancerogenesis

We have experimental improvements that chemical cancerogenesis passes the two stages: initiation and promotion. Initiation is the result of the direct influence of exogenous cancerogen, when cell DNA is a target in the process. The chemical cancerogens very often possess by mutagen properties, but sometimes, the mutagenic and cancerogenic abilities don't coincide. However, a contact with only initiator isn't enough for tumor appearance. Later, the tumor cells must proliferate to increase in mass, and this process named promotion. The substances which aggravate a proliferation of previously genetically changed cells are the following: in the experiments it is croton oil but in our organism these are the substances which play role the triggers in the growth. As the promotion factors non-saturated food fat acids are suspicious when breast and colon cancer; an imbalance in sex hormones when tumor of hormonal dependent tissue and, at last, chronic inflammation accompanied by an active cell renewal. We have experimental data, that only the consequence of use first initiator and then promoter, leads to tumor formation in an

animal. If during the interval between initiator and promoter treatment oleum therpentinum to take as applicator on the skin, the result will be negative, because oleum therpentinum kills the initiated cells. In conclusion must be said that only using both substanses in right order we can achieve a positive result.

Chemical cancerogenes with provoked by them tumors

Initiators

- Tobacco smoke – many tumors
- Benzene – leukemias
- Vinyl-chloride –angiosarcomas of the liver
- Beta-naphtylamine – cancer of the urinary bladder
- Azodyes – tumor of the liver
- Aflotoxin –mesotheliomas and lung tumors
- Arsenic – skin cancer

Promoters

- Croton oil -skin cancer in rats
- Hormones (estrogens)
- Chronic inflammation

Experiments illustrating of two steps chemical cancerogenesis

DMBA → Crotone oil → **Tumo**r

DMBA → Therpentine → **No tumor**

DMBA → Crotone oil → Therpentine → **Tumor**

DMBA → Therpentine → Crotone oil → **No tumor**

Radiation cancerogenesis

Nowadays is estimated exactly that any form of radiation energy, such as ultraviolet or ionizing radiation in form of alpha, beta or gamma-particles, have got an ability to provoke a tumor in the animals and people.

There is a connection between an x-ray exposition and a skin cancer in the radiologistsand the individuals who were working at the atomic power stations was revealed. A tragedies in Japan and, later in Chernobyle, provoked an increasing in cancer morbidity among the corresponding populations, and estimated a close connection between the radiation and tumor disease. The following types of the malignant tumors seem to be the radiation origin: leukemia, cancer of thyroid gland and breast in women when there is common radiation was, but in case of local radiation these are the skin or bones cancer.

Pathogenic influence of ultraviolet radiation

Ultraviolet radiation seems to be a risk factor for different types of skin cancer, including melanoma as the worst variant. The severity of an injury depends on the length of the wave, time of exposition, square under radiation, and constitution of radiated organism.

So, European population, especially red-light haired persons with a light gentle skinis more sensible to prolong pathogenic influence of the UV than the people with darkcomplexion.

At the cell level UV inhibits cell division, inactivates cell enzymes and provokes chromosome and gene mutations, moreover, in corresponding high dose UV can provoke cell death. Formation of pyrimidine dimers in DNA is the base of the gene mutation. In normal such type of DNA injury is corrected by the enzymes of DNA reparation, but very intensive UV radiation can overtake these reparation abilities. That' why, in the individuals with hereditary anomalies in DNA reparation (pigmen xeroderma or Fanconi'syndrome) there is a high risk of skin tumor under long exposition to ultraviolet.

Oncoproteins and their abilities

Transformation of normal cells genome into tumor one leads to the synthesis of the oncoproteins. Oncoproteins are the products of oncogenes! They play role of the factors which by different ways are involved in cell growth. According to their function all oncoproteins may be classified into some groups.

Variants of oncoprotein functions
- Growth factors
- Receptors to growth factors
- Membrane G-proteins which are involved into transduction of the growth signals from the membrane to cell cytoplasm
- Secondary messengers conducting the signals from cytoplasm to the nucleus
- DNA-binding proteins which involve DNA in process of duplication with a next enter the cell in cycle

Some selected oncogenes, their mode of activation and associated with them tumor

Category	Protooncogene	Mechanism	Associated human tumor
Growth factors (fibroblast GF)	hst-1	Overexpression	Stomach cancer
Growth factor receptor (CSF-receptor)	fms	Point mutation	Leukemia
Proteins involved in signal transduction	ras	Point mutation	A variety of human cancers, including lung, colon, pancreas; many leukemias
Nuclear regulatory proteins	myc	Translocation	Burkitt's lymphoma

P.S Name "ras" protooncogene corresponds to viral oncogene, provoking rat sarcoma (r and s); fms: f and s –felina sarcoma; myc: m –mice and c- carcinoma

Protective cell reactions against a tumor formation

In our organism these protective mechanisms are represented by the row of biological phenomena which had been formed in our evolution. They include both, specific and non-specific reactions that prevent appearance and development of a tumor in the organism. Antioncogenes seem to be the first line of the anti-tumor

protection those weregiven us by the evolution as the factors working against the oncogenes. The second protective factors are the enzymes which DNA repair. The Fanconi's syndrome is a bright clinical example of loss those enzymes accompanied by the multiple malformations in the patient. At the same time, such patients are very prone to tumor formation. Their mid life span usually is about 21-22 years, not more, and a malignant tumor is a main cause of their death. The tumor formation also is characteristic of pigment xeroderma and Bloom's syndrome, when the abnormalities in DNA repair are found too.

Next very important protective against tumor mechanisms are anti-tumor immune reactions both, specific and non-specific. The most evidence of this fact is a high morbidity of the patients with immunodeficiency, primary and secondary, of malignant disease. Among these tumors lymphoma is leading.

Tumor and immunity

Before the discussion on the problem it's necessary to pick out some positions concerned the specific features of immunity of the patient with tumor disease. They are listed below:

- In course of tumor progression an antigen-negative or antigen-low subclones are selected; such cells try to avoid of immune recognition in the blood. It may be explained by loss of MHC-class I by the tumor cells which are so important for T-lymphocytes recognizing activity of the "non-self". It must be added that T-cells more than B-cells specialize in the recognition of the tumor cells with changed MHC-class I.Moreover, the difficulties in recognition are also associated with the fact that a tumor cell, one the one hand, is the "self" but, on the other hand, the "non-self".

- Some tumor cells, like melanoma and hepatoma are capable to provoke an apoptosis of CD 8^+ lymphocytes ,and such way to weak the killing of the tumor cells

- At last, some kind of tumor cells don't elaborate the costimulating molecules which are necessary for the anti-tumor immune response, and such way to inhibit T-cell immune protection.

In conclusion must be said that, on the one hand, the tumor appears more often in the individuals with some form of immunodeficiency but, on the other hand, there is no doubt that a weakness of immune response seems to be a result of systemic influence of the tumor a patient with the tumor disease.

The role of cytotoxic lymphocytes in ant-tumor resistance

Anti-tumor immune protection includes both, cellular and humoral immunity, but the first one plays the more important role. CD4+ and CD8+ lymphocytes both are involved in the immune reactions directed against the tumor-specific and tumor cell-associated antigens.

Such mediators as TNF-alpha and TNF-beta (lymphotoxin) enhance the local destructive effects of anti-tumor reactions. TNF-alpha mostly released from the macrophages, but lymphotoxin - from T-lymphocytes. IL-2, being a strong mytogenic substance enhances the lumphocytes proliferation. Nowadays, IL-2

used for activation "in vitro" the T-lymphocytes of the patients to treat some tumors (melanoma and hypernephroid cancer). As the matter of fact, that IL-2 accelerates proliferation of T- lymphocytes in culture and activates synthesis of cytotoxic substances by them. Such method of treatment is called an adoptive method because it minds adoption of own lymphocytes by the patient.These T-lymphocytes named LAC-activated (leukine-activated). Being taken from the patient and then returned to them after stimulated by IL-2 (transfected back) the lymphocytes are working more effectively.

The role of native killers in anti-tumor resistance

Natural killers (NK+ cells) are the lymphocytes capable to destroy the tumor cells without their previously sensitization by the tumor antigen. Their role is the most important in the non-specific immune struggle against a tumor. The matter of the fact, that the tumor cells, especially with the most malignant properties, lose their membrane HLA molecules; it leads to the difficulties in their immune recognition by the cytotoxic lymphocytes. The natural killers can correct this situation. Not possessing by the ability to recognize an antigen, they find the lectin receptors which so characteristic of the cells with high rate of proliferation. It must be added, that tumor cells belong to such kind of cells, moreover, it is one of their main characteristic features. When NK+ cells contact with the tumor cells the special proteins perforins of the NK+ cells arrange the channels in the tumor cells membrane, and the granzymes, in turn, provoke tumor cell death, often via apoptosis. Such phenomena, as cell lysis and apoptosis, are inhibited due to special receptors in case of the meeting NK+ cells with the normal cells bearing on their surface MHC class I as their "passport". It's useful to remind that the tumor cells are so poor of these molecules.

In immune anti-tumor protection humoral immunity plays only secondary role. Nevertheless, the antibodies take part in ADCC (antibody-dependent cell cytotoxicity), phagocytosis, and complement-dependent lysis of the tumor cells.

TNF and its role in the immune protection

As for TNF –alpha, it is one of the mediators of an acute phase response, and it is working on the systemic reaction of the organism, but TNF-beta-lymphotoxin mostly possesses by local cytopathogenic effects

Some mechanism of TNFalpha anti-tumor reactions are listed below:

- Provokes hemorrhagic necrosis of tumor (from here the name). It seems to be associated with stimulation and increased synthesis by the tumor cells of factor VII as a part of the way of prothrombinase synthesis with further thrombosis of the supplying by the blood tumor vessels
- Activates cellular and humoral immunity
- Inhibits DNA reparation after its injury under chemo- and X-ray therapy

In conclusion may be said, that nowadays immunotherapy, including adoptive immunotherapy and local treatment of tumor with low concentration of TNF is in-

cluded in the protocol of anti-tumor treatment some patients, for example, melanoma or kidney cancer.