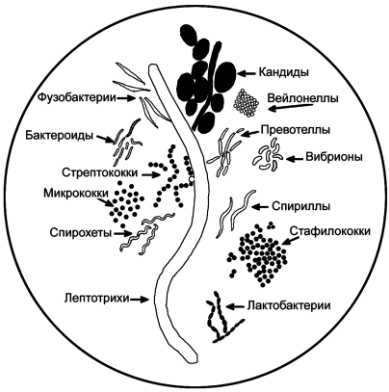
ODESSA NATIONAL
MEDICAL UNIVERSITY

***Chair of microbiology, virology and immunology***

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# MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY OF ORAL CAVITY

*Educational materials for students*

Odessa- 2011

**CONTENS**

# 1. IMMUNITY AND NONSPECIFIC FACTORS OF RESISTANCE IN THE ORAL CAVITY

## 1.1. Structure and function of oral cavity

Students know enough about anatomical and physiological features of human oral cavity. Notify only some questions that are relevant to its protective mechanisms.

Oral cavity in closed condition is a narrow horizontal slit formed by hard palate and tongue. It is topographically divided by tooth into two divisions: frontal and proper oral cavity.

The oral cavity is covered with mucous membrane, where salivary glands are located. Innervation of the cavity wall, its blood and lymph flow are closely related to vascular and nervous system of the jaws. Slit space between the cervical tooth surface and the edge of the gums is called a periodontal or gingival pocket. The tooth surface and oral mucosa are covered with pellicle - an insoluble mucous membrane which works as a barrier that protects the mucous membrane from drying, controls its permeability. Pellicle is also a barrier from microorganisms penetration.

The main component of the pellicle is mucin, which is present in the saliva. This substance is highly viscose, elastic, adhesive. Protective properties of the pellicle depend primarily on the size of mucoid gel. The pellicle also include acidic proteins and the cationic glycoproteins.

The oral cavity is involved in breathing, voice formation, articulation, modulation. One of its main functions is to ensure the first stages of digestion. This step means the mechanical crushing and fermentation, which is provided by saliva enzymes. Saliva is the secret of the three pairs of major salivary glands (parotid, sublingual and submandibular). In addition to enzymes, saliva also contains mucins, immunoglobulins, and other organic and inorganic substances, food residuals. Enzyme diastase, which is contained in saliva, converts starch, hard splittable by microbes, into dextrose, which is easily digested by them.

## 1.2. Cellular factors of the oral cavity nonspecific resistance

In the world around us there is always a huge number of microorganisms that come into our body mostly through the mouth and can have devastating effects. A number of protective mechanisms, including in the oral cavity, works against them.

To a great extent constitutional factors ensure the protection, that make one species of adverse to certain infections that can affect other species. In addition, there is, firstly, a number of nonspecific antimicrobial systems, whose activity is independent of previous exposure to the antigen, and, secondly, specific acquired immunity. Both components are involved in protection from infectious agents in

the oral cavity on the mucous membrane surface, as well as on the surface of the tooth.

**The barrier function.** The easiest way to avoid infection is to prevent the introduction of the pathogen in tissues of the body. The main line of defense in the oral cavity is the mucous membrane and the enamel, which are impermeable to most infectious agents. When the integrity of these integuments is compromised by external factors, or as a result of metabolic disorders in the body, the infection becomes a major problem.

So, a result of trauma or jaw surgery may have complications of purulent-inflammatory nature. These processes are invoked and are the result of microorganisms affect.

Epithelium of the oral cavity has the ability to keratinize. It is the most noticeable in the epithelium of the anterior hard palate. Somewhat less is noticed the phenomenon of keratinization in the epithelium of the gums. It is believed that the process of keratinization in the epithelium of the oral cavity has a protective nature.

If the tooth enamel is affected by corrosive substances (acids) that are formed by microorganisms, it can be corrupted. This in turn leads to the development of caries. Enamel is also a reliable barrier between saliva and dental pulp, is rich in blood vessels and cellular elements.

Microorganisms and remnants of food are constantly removed from the mouth with saliva, are removed by an act of swallowing - excretory function.

**Inflammation**. Acute inflammatory is the reaction characterized by dilated capillaries (hyperemia), exudation of plasma proteins and other fluids as a result of changes in hydrostatic and osmotic pressure (swelling) and the accumulation of polymorphonuclear leukocytes (PNL). It arises in the oral mucosa and periodontal tissues under the influence of various stimuli, particularly as a result of infection.

The sequence of events that happens is visible in Figure 1.1. This reaction is the first response to the body's defenses to the stimulus. It is not only a direct opposition to the microorganisms, but the trigger of the other protection stages, as well as specific one's.

**Phagocytosis** - the absorption and digestion of particles with specialized cells, phagocytes. Phagocytosis was opened by I.I. Mechnikov. Opening date is associated with 1882, when Mechnikov firstly unveiled his phagocytic theory of immunity at a meeting of the Odessa Society of Physicians and Naturalists. That is why the $100^{th}$ anniversary of the phagocytic theory the whole scientific world celebrated in Odessa, in Odessa-based research institute of epidemiology and virology named by I.I. Mechnikov – Mechnikov's heir created bacteriological station.

Phagocytosis in the oral cavity soft tissues, as in the whole body, is carried out by two types of cells that Mechnikov defined as micro-and macrophages.

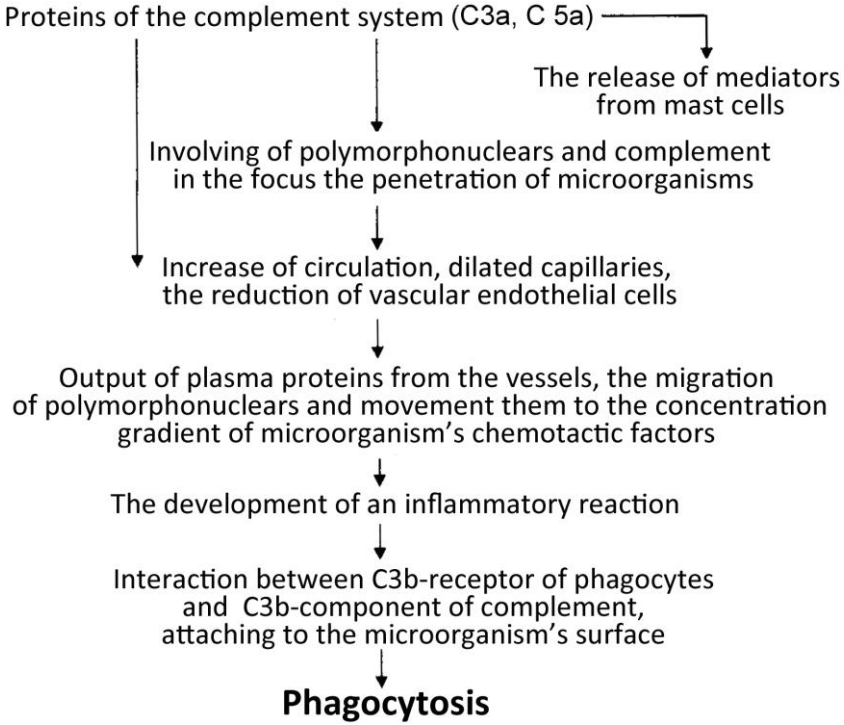



Fig. 1.1. The development of the inflammatory reaction (according to I.Royt, 1991)

The process of phagocytosis involves several stages:

1. **Chemotaxis** (zoom) - purposeful phagocyte movement to the object of phagocytosis through the action of chemicals in the environment that stimulate directed movement of phagocytes. Ability to chemotaxis is caused by specific receptors in the phagocyte membrane.

2. **Adhesion** (attachment) is carried out either by non specific physic-chemical interaction of the phagocyte membrane with an object of phagocytosis or by receptor interaction of phagocytes and microorganism body.

Cell membranes, including membrane of phagocytes, have the total negative charge, which ensures their isolation and inhibits auto-phagocytosis (phagocytosis of macroorganism's cells by its phagocytes). Hydrophilicity of some bacterial cell wall components prevents them from passing through the hydrophobic phagocytes membrane. To overcome this, on the surface of phagocytes receptors are located, which provide effective binding of particles coated with appropriate ligands (molecules capable to bind to receptors, from lat. Ligo - binding); phago-

cytes have receptors for the Fc-fragment of some immunoglobulin isotypes, as well as for the C3 complement component and other factors. The presence on the surface of microbial cell immunoglobulin and complement, which act as opsonins (opso- in translation from the latin - cooking), markedly increases the absorption process and, in some cases, the digestion. Interaction of opsonising factors with receptors on the phagocyte membrane has the nature of regular communication and resembles the action of the "zipper".

3. **Endocytosis** – diving of phagocyted particle into the phagocyte. This process goes against the inert particles and non-pathogenic microorganisms without the involvement of additional factors. Pathogens are usually phagocytized only after opsonization factors stimulating phagocytosis.

Associated with specific membrane receptors, phagocytes, or not, foreign particles or microbial cell is surrounded by a membrane of phagocytes. It forms phagocytic vacuole, or phagosomes as a result of phagocyte intussusception.

4. **Intracellular digestion** occurs in the phagolyzosomes, which are formed by the merger of phagosome with the cell lyzosomes, which are located inside the phagocyte bactericidal factors.

This bordering of potentially toxic molecules is necessary to protect the cells from self-destruction and to create an environment in which the bactericidal molecules can function effectively. In phagolyzosomes the object of phagocytosis is killed and digested by different enzyme systems. Microbocidic phagocytes mechanisms are diverse.

**Oxygen-dependent microbocidic mechanisms**. Phagocytosis is accompanied by increased glycolysis and increase in protein synthesis and membrane phospholipids. After absorption there occurs respiratory burst, which means sharp increase in oxygen consumption. This is accompanied by increased activity of many enzymes and leads to the restoration of molecular oxygen in a variety of highly reactive intermediates, such as superoxide anion ($O_2$ ●), hydrogen peroxide ($H_2O_2$), singlet oxygen (O ●), hydroxyl radical (OH ●). They have a bactericidal activity and form the oxygen-dependent bactericidal mechanisms. Superoxide anion - a free radical which is formed by reduction of molecular oxygen by one electron restoration, highly active and highly toxic to both animal cells and microorganisms. It is also a substrate for superoxide dismutase, which produces hydrogen peroxide for the subsequent microbes killing.

Myeloperoxidase uses hydrogen peroxide and ions of iodine and chlorine to form at least two bactericidal systems. One of them, halogenation (inclusion of iodine or chlorine) of bacterial cell wall, which causes the death of the microorganism. In the second mechanism of how myeloperoxidase and hydrogen peroxide damage the cell wall, is converting amino acids into aldehydes, which possess antimicrobial activity.

In phagocytes there are also oxygen-independent mechanisms, which are also capable for the destruction of the absorbed material. Some of these enzymes may damage the membrane. For example, lysozyme and elastase act on the pepti-

doglycan of bacterial cell walls, and then hydrolytic enzymes provide a complete digestion of the inactivated microorganisms. Cationic proteins of lysosomes damage the cell walls of bacteria and some viruses that have supercapsid, for example, herpes simplex virus. Antimicrobial action is also in a protein lactoferrin. It binds with iron, making it inaccessible for those bacteria that require iron for multiplication. High acidity in phagolizosomed (pH 3.5 - 4,0) can have a bactericidal effect: this is probably due to the formation of lactic acid during glycolysis. Furthermore, many lyzosomal enzymes have pH optima in the acidic environment.

After killing, which lasts about 15 minutes, most of the microorganisms are digested and soluble by lysosomal enzymes. Degradation products are thrown out by exocytosis.

However, phagocytosis may be incomplete. Incompleteness of phagocytosis may be due to the high protective properties of the microorganism, the presence of a capsule, dense cell wall, aggressins production, the damaging effect of microbes on the phagocytes, the ability of bacteria to intracellular parasitism. In this case opsonizing action of antibodies and complement can lead to the completion of phagocytosis.

On the other hand, the incompleteness of phagocytosis may be a consequence of the phagocyte defect - the lack of its bactericidal mechanisms.

In some cases, due to incomplete phagocytosis bacteria can even multiply inside phagocytes, and phagocytizing cells may die, while their decay is the release into the tissues of living organisms. Mechnikov identified two forms of phagocytes - macrophages and microphages, identified the main difference between macrophages - the ability to phagocyte not only microorganisms, but also the body's cells, in contrast to microphages, which are mainly active against microorganisms.

Microphages are the polymorphonuclear leukocytes (PNL), mainly, neutrophilic granulocytes, neutrophils. PNL have a common hematopoietic precursor with other blood cells, in peripheral blood there are more of them than other leukocytes.

Polymorphonuclear neutrophils - short-lived non-dividing cell with a segmented nucleus and a set of beads that are not stained by hematoxylin and eosin, unlike similar structures in eosinophils and basophils. In neutrophils there are known three types of granules: primary azurophilic granules, containing myeloperoxidase, a small amount of lysozyme and a set of cationic proteins, secondary "specific" granules that contain lactoferrin, lysozyme and the protein that binds vitamin $B_{12}$; tertiary granules, similar to conventional lysosomes, they contain acid hydrolases.

Significant reserves of glycogen, which can be used during glycolisis, allow these cells to exist in anaerobic conditions.

Polymorphonuclear neutrophils provide a primary defense against pyogenic bacteria.

In the local immunity of the oral mucosa connective tissue cells of the mucous membrane play a significant role. The bulk of these cells are fibroblasts and macrophages cell.

**Macrophages** are derived from bone narrow promonocytes when, after differentiation into blood monocytes they are finally trapped in the tissues as mature macrophages, which form a system of mononuclear phagocytes. They are everywhere - in the connective tissues and basal membranes surrounding small blood vessels. It is also the osteoclasts of bone tissue. In addition, macrophages line the sinusoids of spleen and medullar sinuses of lymph nodes, where their main function is to filter out the foreign agents. Being actively moving cells, they migrate to foreign substances, tumor cells and subjected them to lysis. Unlike neutrophils, macrophages are long-lived cells with well-developed mitochondria and endoplasmic reticulum. The function of macrophages is reduced mostly to fight against those bacteria, viruses and protozoa that can exist within a host cell.

There known more than 100 biologically active substances which are produced by macrophages. Among them there are many which carry out their protective functions outside the macrophages (e.g., lysozyme, proteins of the complement system, alpha-interferon, etc.) or are the immunoregulatory, that is, indirectly affect the immune response (interleukin-1, gamma-interferon, and others).

It is necessary to focus on the role of eosinophils. According to modern notions eosinophils are involved in the extracellular destruction of large objects of phagocytosis (protozoa, worms). Eosinophils around the parasites and release toxic substances that kill them, and macrophages absorb and digest the body of already dead parasites.

Oral mucosa serves not only as the mechanical barrier function, but also has a pronounced microbocidic effect. Fibroblasts and tissue macrophages play an important role, which can easily migrate into the inflammatory focus. Phagocytosis on the surface of the mucosa and submucosal connective tissue is carried by granulocytes and macrophages. They contribute to the clarification from the pathogenic bacteria source. In addition, between the collagen fibers around vessels there located mast cells - potential participants in allergic reactions of anaphylactic type.

**Natural killer cells**. In the human body there is functioning a population of lymphocytes-like cells with natural cytotoxicity against the "target-cells ". They are called natural killer cells or natural killers (NK - natural killer). NK are the effector cells with antitumor, antiviral and anti-parasitic activity. They are capable spontaneously, without prior contact with the antigen, to kill tumor cells and cells infected with certain viruses or parasites. Apparently, the main function of the NK is an anti-tumor "oversight". This system of nonspecific cellular defense is probably phylogenetically more ancient than the specific T-cell immune mechanisms.

Morphologically, the NK are great granules-containing lymphocytes - large granular leukocytes - BGL. Their characteristic azurophilic cytoplasmic granules

are analogues of phagocytic cells lyzosomes. However, NK does not possess phagocytic function. Nonspecific nature of their cytotoxic activity distinguishes these cells from antigen-specific T-killers and the K-cells, mediated antibody-dependent cytotoxicity (show killer effect only after the activation of antigen-antibody complexes). Among the human white blood cells NK are ranged from 2 to 12%.

It is believed that NK are able to recognize certain structures of high-glycoproteins, which appear on the membrane of virus-infected cells. Viral glycoproteins in the NK membrane give the possibility to distinguish virus-infected cells from the normal. Recognition of target cells and closing to it is carried due to NK receptor. As a result, NK are activated and the contents of their granules released into the extracellular space. Extracellular lysis of target cells occurs. Leukocytes enter the saliva through the gingival physiological channel. Normally, 1 $cm^3$ of saliva contains about 4000 white blood cells, and in an hour about 500 migrate into the oral cavity.

Thus, in the mouth constantly have strong cellular factors of nonspecific protection, providing the first barrier on one of the most common pathways of infectious agents in humans.

### 1.3. Humoral factors of the oral cavity nonspecific protection

Bactericidal action of the mucous membranes. Saliva, salivary glands, acts as a protective barrier that prevents the attachment of bacteria to epithelial cells and has antimicrobial properties. During the day, the salivary glands produce from 0,5 to 2 liters of saliva, which has bacteriostatic and bactericidal properties because of humoral factors: lysozyme, lactoferrin, lactoperoxidase, components of the complement system, immunoglobulins

Lysozyme (enzyme muramidase) - an important antibacterial component of saliva, which breaks down the cell wall peptidoglycans of sensitive bacteria).

In 1909 P.L. Lashchenko posted a message about the bactericidal white of egg action on the Bacillus, Megatherium, Proteus bacteria, and suggested the existence of a specific enzyme protein, rendering these effects. In 1922, Alexander Fleming found that indeed such enzyme is found in egg white, tears, and secretions from the nose and is called lysozyme. Like other enzymes in the future lysozyme was named by its specific substrate and is currently in the literature known as muramidase, because it splits the main substance of the bacterial cell wall - murein (murus - wall) by disrupting communications between the first carbon atom of the N-acetylmuramic acid and fourth carbon atom of N-acetylglucosamine, which is part of the bacterial cell wall.

Lysozyme is found in many human secretions, all warm-blooded and cold-blooded animals: the white of the egg (titre 1: 600 000), saliva (1: 300), tears (1: 40 000), milk, intestinal mucus and tissues of various internal organs, as well as

in skeletal muscle, brain, leukocytes. High concentrations of lysozyme detected in amniotic membranes, and the waters of the fetus.

Content in different tissues varies widely. The plasma concentration of lysozyme is maintained at a certain level and is about 8.5 mg / ml. The content of lysozyme in the serum is determined by receipt of lysozyme in the blood from the cells and tissues that synthesize lysozyme, and its degradation rate in tissues and catabolism in kidneys. Suggest that the most of lysozyme is synthesized by tissue macrophages and young neutrophils. Macrophages synthesize and secrete lysozyme continuously and more intensively than other cells, although the highest content of lysozyme is detected in neutrophils, where it is contained in the lysosomes and is released only due to the degranulation of neutrophils. In humans about 3 / 4 of lysozyme, contained in plasma is catabolised per hour. Muramidaza is also synthesized in the synovial membranes, cartilage tissue, lacrimal, salivary and mammary glands. However, all of this lysozyme is only a small portion of the total amount synthesized in the body. Daily production of lysozyme, arriving in the plasma of healthy human is about 150 mg a day, every day productions by disrupted neutrophils - 300 mgr per hour per kilogram of body weight. There is a direct relationship between the velocity of neutrophils circulation and content of lysozyme in plasma. The level of lysozyme is reduced in diseases of the blood system, accompanied by neutropenia, and increases in diseases accompanied by neutrophilia. Destruction of neutrophils leads to an increase of lysozyme in the plasma.

According to the chemical nature of lysozymes are low molecular weight proteins, primary structure of which consists of a single chain containing 130-160 amino acids residues. Lysozyme is soluble in acid medium, inactivated by heavy metal salts, is resistant to trypsin, but is split by pepsin. The molecule has the shape of an ellipsoid sized 3 x 2 x 4.5 nm. On one side of the molecule there is a deep crevice. The optimum enzyme activity due to pH 5,0-7. The maximum activity is seen at 60 ° C. Lysozyme performs in the animal a number of important biological functions.

Muramidase decreased concentration in saliva was accompanied by increase of oral cavity inflammatory diseases frequency, and blocked tear ducts leads to infection of the eye cornea. Lysozyme stimulates phagocytosis of neutrophils and macrophages both in vivo, and in vitro. This is due to changes that occur on the surface of microbial cells under the influence of lysozyme, and possibly due to their surface structures integrity violation.

Lysozyme stimulates the synthesis of antibodies. This increases the duration of higher antibody titers preservation. Lysozyme selectively stimulates response to specific antigen. Removal of lysozyme from the blood causes a complement, properdin and β-lysine decrease in serum levels.

In newborn blood serum lysozyme was found usually in higher concentrations than in maternal serum, respectively, 13.5 and 9.5 mg / ml. Later lysozyme titer decreases and rises again in young and middle age (1,6 - 3,8 mg / ml). In

elderly people lysozyme titers in the blood drops to 2-3 ug / ml. Lysozyme exhibits bactericidal and bacteriostatic action against many types of bacteria. Certain strains of the same microbes species exhibit a large range of sensitivity. To high concentration (10 mg / ml) are usually sensitive 70-97 and even 100% of all strains. As for the medium (10-15 mg / ml) and low-dose (0,3-0,075 ug / ml), most strains are not sensitive to them. With multi-pass lysozyme passages can hundreds or thousands of times increase the concentration resistance and even get lisozyme-dependent strains.

Lysozyme in vitro potentiates the lytic action hydrolytic enzymes on bacteria. Lysozyme do not cause self-lysis of the bacteria.

Muramidaza in low concentrations increases the permeability of the bacteria cell wall for DNA than stimulates the processes of transformation. This mechanism is apparently important in processes of bacteria variability.

Lysozyme titer in serum is decreased during acute infectious diseases, pneumonia and salmonellosis. The degree of reduction, depends on the condition severity. Dropping to very low levels is an unfavorable clinical course. In the recovery period content of lysozyme in blood serum comes to normal. In chronic inflammatory diseases titers decreased during the exacerbation of the process. Reduced titers of lysozyme in rheumatism indicates about the activation of the ground process or intercurrent infections accession. In nose mucus lysozyme content decreases with atrophic processes in the upper respiratory tract epithelium, but increases sharply in mucus cell hypertrophy. Muramidaza has pronounced antibacterial properties against Micrococcus lysodeicticus, Staphylococcus aureua, Corynebacteria diphtheria, salmonella, shigella, escherichia, influenza virus, arboviruses.

Protective effect of lysozyme is due to bactericidic influence, stimulating effect on phagocytosis, the ability to neutralize some microbial toxins, as well as anti-inflammatory effect. It potentiates the specific activity of some antibiotics. In connection with this property is its applied in the clinic for therapeutic purposes.

In the oral cavity lysozyme falls from the parotid ducts and submandibular glands, where is produced by leukocytes. Titer of lysozyme in saliva of healthy people according to recent data can range from 0 to 1: 64. The important role of lysozyme in the local immunity of the oral cavity may indicate to increased frequency of infectious and inflammatory processes, developing in the mouth with a decrease in its activity in the saliva.

To determine the titers of lysozyme in saliva there prepared a number of sequence dilutions of saliva in test tubes, each contribute to 1 ml suspension of 1 billion microbial cells / ml Micrococcus lysodeiticus and incubated at 37 ° C for 3 h. The titer of lysozyme is determined by the last dilution in which is observed complete lysis of the bacteria - enlightenment. Accounting for the reaction can be carried out visually or nephelometrically by photoelectrocolorimeter or nephelometer.

Concentration of individual blood plasma proteins, which are generally termed as "acute phase proteins, increases dramatically in response to infection or tissue damage, as well as in tumor-genesis and pregnancy. This is a large group of proteins that have antimicrobial activity, feasibility to phagocytosis, complement activation, the formation and elimination of the inflammatory focus. Acute phase proteins are produced in the liver under the action of cytokines, mainly IL-1, IL-6, tumor necrosis factor TNF-a. The bulk of acute phase proteins are C-reactive protein and serum amyloid A and P. Other groups of acute phase proteins are blood coagulation factors, metal-binding proteins, protease inhibitors, complement components, and others. Due to inflammation blood levels of most proteins increases many times, and the definition of C-reactive protein is one of the accepted methods for inflammatory processes diagnosis.

C-reactive protein was named due to the ability to access and precipitate Str. pneumoniae C-polysaccharide. Further, it was the established that C-reactive protein (CRP) is attached to phosphatidilcholin - a the cell membrane component of any cells. It is capable to bind to the microorganisms, activated lymphocytes, damaged cells of different tissues, and activate complement. Accessing to neutrophilic phagocytes, CRP enhances phagocytosis and elimination of phagocytosis objects. However, the CRP suppresses the superoxide production and the release of enzymes from the phagocytes granules, thus protecting the tissue from damage.

Serum amyloid P is close to the CRP structure, has ability to activate complement.

Serum amyloid A - lipoprotein, has the ability to neutrophils, monocytes and lymphocytes chemattraction. Increased level of this protein in the blood is observed in tuberculosis and rheumatoid arthritis.

Another group of acute phase proteins are iron binding proteins - haptoglobin, hemopexin, transferrin - and impede a feasibility microorganisms reproduction which need this element.

The level of protease inhibitors in the blood increases 2-3 times during inflammation. Antitrypsin, macroglobulin anti-chimotripsin hinder the tissues destruction by proteases of neutrophils in the foci of inflammation.

In the course of infection, such microbial products like endotoxin, stimulate the production of macrophage interleukin-1 (IL-1), which is an endogenous pyrogen. It causes fever, which increases the effectiveness of many defense mechanisms. In addition, IL-1 affects on the liver, increasing the CRP synthesis and secretion. IL-1 is a monokines - regulatory protein, produced by monocytes, which acts as a nonspecific stimulator of T-helper cells subpopulation. This is extremely important in the mechanism of the immune response to antigenic stimulus.

**Interferon system** - α, β, and γ - interferon (a group of non specific protection factors). Anti-infective activity is characterized primarily by alpha-interferon (leukocytic), which is produced by leukocytes and virus-infected cells. Cells of

the oral mucosa is a target for some viruses, and the mechanism interferon-alpha anti-viral action is essential for oral cavity tissues. The fact that interferon, synthesized by infected cells, is released into the intercellular space, where it binds to specific receptors of neighboring uninfected cells. It is believed that the cell to which the interferon binds is disturbed protein synthesis by reducing the synthesis and degradation of RNA, as well as the virus in case of its penetration. Thus, the cells become unsuitable for virus reproduction. The end result is the formation of the barrier from uninfected cells around the outbreak of viral infection that prevents its dissemination.

**Lactoferrin** - iron-transport protein, bacteriostatic effect of which is linked to its ability to compete with bacteria for iron. There marked lactoferrin synergistic action with antibodies. His role in oral cavity local immunity clearly manifest in a breast-feeding conditions, when the newborn receive mother's milk, high concentrations of this protein in combination with secretory immunoglobulins (SIgA). Laktoferrin is synthesized in granulocytes.

**Lactoperoxidase** is a thermostable enzyme that exhibits bactericidal activity in the complex with thiocyanate and hydrogen peroxide. It is resistant to the action of enzymes that carry food, cooking, active at pH from 3,0 to 7,0. In the oral cavity blocks the adhesion of S.mutans. Lactoperoxidase appears in the saliva of children from the first months of life.

**Sialin** contained in the saliva neutralizes acidic foods, the generators as a result of vital activity of dental plaque microflora, which carries a strong anti-caries action.

**Complement system**. Complement is called a complex of proteins (about 20) that form a cascade system (the products of one-phase reaction are catalysts for the next). For such systems is typical rapid formation of multiple amplified response to the primary signal.

Complement system has several biological functions. This is, firstly, the role of the adhesion reactions. Phagocytes have receptors for certain complement system proteins, and therefore effectively takes place the process of microorganisms phagocytosis, which joins the protein. Such complement action is called a opsonizing.

Secondly, the formation of biologically active enzymes C3a and C5a. These are small peptides that are cleaved during the complement activation from molecular precursors. They operate on phagocytes, particularly neutrophils, causing the activation of respiration process, and are also anafilatoxins and can cause the mediators output from mast cells and blood basophils.

Third, C5a is a potent chemotaxic agent for neutrophils and can affect the endothelial cells of capillaries, increasing their permeability.

Fourth, this is a function of microorganism membranes destruction.

Complement is characterized by the fact that this complex of proteins is normally found in an inactive state and has no noticeable effect. The action of the complement system is associated with the cascade activation of its components.

There are two main ways of complement activation - the classic and alternative pathway.

**The classic way of complement activation by antigen-antibody complex**. When an antibody reacts with the antigen, which is contained in the microorganism membrane, there is activated the first complement component (it is noted as C1), activation products C1 activates C4, then there activated C2 → C3 → C5 → C6 → C7 → C8 → C9. The last component, C9, appears as a result of two molecules activation in the membrane-attack complex "in the form of a transmembrane channel in the cells membrane. This channel completely tights water and electrolytes, cell receives Na ions that water, which leads to a cell lysis - Fig. 1.2.

In such a way ossurs microorganisms, erythrocytes and other cells death and lysis. This case requires mandatory action of antibodies, which belong to immunoglobulin classes G and M (other classes of immunoglobulins do not activate complement by the classical way).

**Alternative pathway of complement activation** occurs without the antibodies participation. Polysaccharides of many bacteria (mostly - nonpathogenic, pathogenic bacteria resistant to the action of complement and may even inactivate it) bind and activate C3, properdine joins and stabilize C3 - another factor of nonspecific humoral defense.

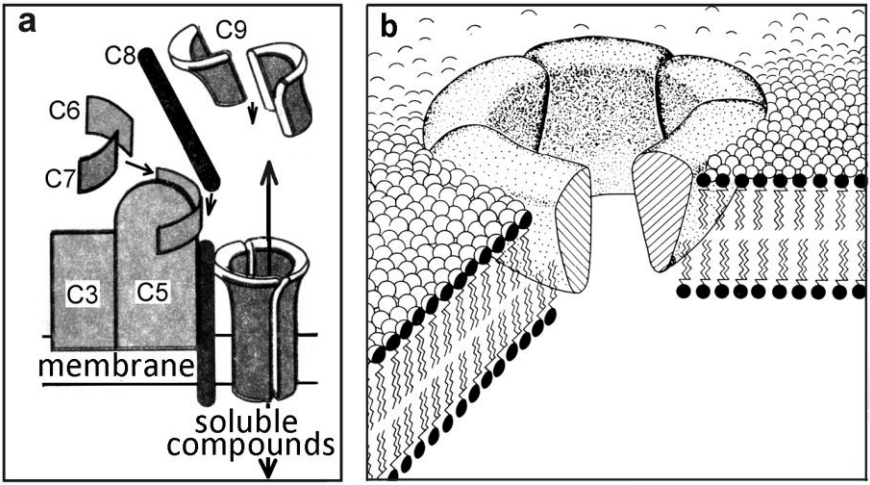



Fig. 1.2. SCHEME OF ACTIVATED COMPLEMENT

a - Molecular organization of membrane-attack complex

(By I. Royt).

b - Pore nodel in the cell membrane, formed as a result of complement activation (by M. Mayer).

Further activation of the complement cascade is similar to the classic route of activation: C3 → C5 → C6 → C7 → C8 → C9, with the formation of membrane-attack complex.

Thus, complement performs an important protective role in organizing. As noted above, complement is also involved in phagocytosis activation. Therefore, we define the content of complement in serum of people to assess the body's resistance.

It is believed that the conditions for complement lytic action activation in the oral cavity are less favorable than in the bloodstream, however, in the salivary glands detected C3 complement system fraction.

### 1.4. Factors of specific protection in the oral cavity

Besides, cellular and humoral factors of nonspecific resistance in oral cavity there act mechanisms of specific, immunological protection. Immunological mechanisms are provided by imunne system and its cells – antigen-reactive lymphocytes.

First of all, protection from infection is carried by subepithelial accumulations of lymphoid tissue are not limited by connective tissue capsule. It may be diffuse accumulations of lymphocytes, plasmatic cells and phagocytes, or more organized tissue with follicles (lingual, palatine and pharyngeal tonsils).

**Cellular factors of immunological defense** are the antigen-reactive effector T-cells, which provide the development of cellular immunity.

The term **"cellular immunity"**, or "cell-mediated immunity" refers to the specific immune responses in which the antibodies do not participate, and play the main role of antigen-reactive lymphocytes. For a long time the only phenomenon known about cellular immunity has been delayed hypersensitivity-type (DHT), which resulted often in greater damage to system, than protection.

For the first time this type of reaction was described by Robert Koch in the 1890. R. Koch revealed that as a result of the tuberculosis micobacteria intradermal injection or protein extract from them (tuberculin) into infected with tuberculosis, but not in healthy guinea pigs, there appears growing skin reaction. After that, tuberculin skin test became an example of, DHT and this type of reaction was called "tuberculin-like reaction. The term "delayed hypersensitivity" due to the fact that the most severe skin reaction develops within 48 - 72 hours after antigen injection. The reaction is expressed in the creation of a dense nodule with infiltration of mononuclear cells.

DHT is immunologically specific, but not caused by passive serum antibodies and is not transmitted. It has been proven that DHT and other types of cellular immunity is determined by T - lymphocytes.

Cellular immunity is involved in these immunological functions:

**Delayed hypersensitivity-type - DHT.**

Immunity in infectious diseases that are caused by obligate and facultative intracellular parasites. These include bacterial infections (e.g. tuberculosis, leprosy, listeriosis, brucellosis), fungal (e.g. histoplasmosis, coccidioidomycosis, blast fungus disease), protozoa (such as leishmaniasis, trypanosomosis) and viral (e.g. measles, mumps).

**Transplant immunity and the reaction of "graft-versus-host".**

Immunological surveillance.

Anti-tumor immunity.

**The mechanism of cellular immunity**. For the development of cellular immunity antigenic stimulus character is very important. It is best-developing is when infected by intracellular parasites. Dead vaccines and other non-living antigens do not stimulate cellular immunity, if not enter together with the stimulant of Freund's adjuvant type. Only the T-dependent antigens cause the cellular immunity development. Deposited on the skin of certain chemicals (for example - dinitrophthorbenzol) regularly cause delayed hypersensitivity.

Each T-cell has on its surface a receptor specific for one antigenic determinant (epitope) and is connected only with antigens that bear this epitope. Due to contact with the corresponding antigen of T – lymphocytes there exposed blasttransformation, proliferation, differentiation and function of memory cells and effector-cells that provide cellular immunity.

With the development of the cellular immune response T-helper cells react with antigens that are presented on the surface of macrophages or other cells in a complex with molecules of class II corticosteroids. In this case they relief the biological mediators (lymphokines), which activate the macrophages and contribute death.

**Humoral factors of immunological defense** are the antibodies - immunoglobulins.

IgG and IgM antibodies provide complement activation by the classic way through C1 → C3 → C5 → C9 → membrane-attack complex, providing antimicrobial action. In addition, they neutralize toxic microorganism substances, activate phagocytosis through interaction between Fc-antibody fragment forming a complex with antigen, the receptor of phagocytes, as well as through the respective connection with phagocyte receptor joined to the complex AG-AB component C3 complement component.

There is a perception that lymphoid tissue, associated with the mucus membranes, forms a special secretory system in which there circulate cells, synthesizing IgA and IgE.

One of the major immunological protective factors that determines the immunity of oral cavity, are antibodies. Protective effect of antibody is explained by their penetration into dental plaque and pellicle, and interaction with surface antigens of microorganisms. This prevents the fixation and adhesion of microorgan-

isms on the tooth surface, but also accelerates their phagocytosis PNL (opsonizing action of antibodies). Antibodies are also capable to neutralize the bacteria enzymatic activity.

Crucial role in local mucosal immunity providing in the oral cavity are class A antibodies, particularly in their secretory form-SIgA. In healthy people in the all external secretion glands stroma (including salivary glands) and mucous membranes, communicating with the external medium, the vast majority of plasma cells produces IgA.

By the content of immunoglobulins differs inside and outside secrets of the oral cavity. Inner secrets are the gum pockets secrete, in which immunoglobulins content is close to their blood serum concentration. External secrets are the saliva and mucus. In external secrets, such as saliva, the number of IgA far exceeds from the concentration in their blood serum, while IgM, IgG and IgE contain in saliva and serum is approximately the same. Ratio of IgA, IgG and IgM in saliva is 20:3:1. The IgA is accounted as 85% of all saliva immunoglobulins. sIgA is found in the saliva, beginning from 2 months after birth. At the age of 6 months (early teething period) increases dramatically the number of bacteria in the mouth and, consequently, increases the concentration of immunoglobulin. At 6-7-th year of life its level in saliva is increased by almost 7 times. Normal SIgA synthesis is one of the conditions of sufficient sustainability of newborns to infections, affecting the oral mucosa.

Secretory immunoglobulin SIgA is more resistant to the proteolytic enzymes action in comparison with serum IgA.

Secretory immunoglobulins SIgA can perform multiple degence functions. They inhibit the adhesion of bacteria, neutralize viruses, and prevents the antigens absorption (allergens) through the mucosa of the shell. For example, SIgA-antibodies inhibit adhesion of cariogenic streptococcus S. mutans to the tooth enamel, which hinder the development of caries. Sufficient level of SIgA-antibody is able to prevent some viral infections development in the oral cavity, for example herpetic infection. In individuals with SIgA deficiency antigen is freely adsorbed on the oral mucosa and enter the blood, which can lead to serious allergization consequences. Antibodies of this class prevent the pathological processes occurrence in the oral cavity without causing her injury, as the interaction of SIgA-antibody with antigen, in contrast to Ig G and M does not cause complement system activation. However, aggregated SIgA can activate complement by alternative pathway through C3. Among non-specific factors that can stimulate the SIgA synthesis, it should be noted vitamin A. Immunoglobulins, which are found in saliva, are synthesized in their own plate of mucous membrane and salivary glands. Experiments with fluorescent antiglobulin sera showed that the IgA molecule, overcoming a tight epithelial layer lining the mucosa, may pass this way both through the intercellular space, and through the epithelial cells cytoplasm.

IgA enters saliva as a result of serum extravasation across the zone of inflammation, as well as through the damaged mucosa. Plain epithelium lining the oral mucosa, acts as a passive molecular sieve. This is the most effective way of IgG come.

There is a mechanism of selective IgM transport through the epithelial barrier. With a sIgA deficit IgM concentration increases in oral fluid.

## 2. METHODS OF MICROBIOLOGICAL RESEARCH IN DENTISTRY

The value of microbiological studies in the dental clinic is determined not only by the fact that they can be used to diagnose infections caused by specific pathogens (fungal, tuberculosis, etc.). Such studies are also needed to clarify many etiology and pathogenesis questions, dental caries prevention, treatment and prognosis and its complications, periodontal disease and other pathological processes in the occurrence or development which involve a variety of the oral cavity microbial flora representatives.

Microbiological investigation of oral cavity may be carried out for the diagnosis of microbial processes in the oral cavity, determine the pathogens drugs sensitivity, treatment efficacy monitoring.

Most of the bacteria that live in the oral cavity are unable to induce monoinfection. Frequently observed lesions are caused by microbial associations. Accordingly, to identify the specific etiologic agent it is necessary to establish its dominance and to determine the correlation with other microflora throughout the mucosa, or in a separate sector.

Microbiological examination should begin with a proper collection and transport of the material in the laboratory. This stage of microbiological studies has the main interest for the future dentist, so we'll consider it in detail.

### 2.1. Conditions and methods of material sampling

In the selection of groups for research should always be taken into account prior infection with antibiotics and dental caries.

Any local application, oral or parenteral antibiotics may be more or less significantly change, particularly, the oral cavity microbial flora, although usually these changes are short-lived. Therefore, if the research task differs from finding the antibiotics effects it is not recommended to examine those persons who took antibiotics during the preceding 3 weeks.

Technique of any material sampling except saliva, are basically the same. Material is taken in most cases (exceptions are specified) by thin cotton bristles at the root needles or tightly curled wool balls with a diameter about 2 to 5 mm. To investigate the inflamed pulp in situ can be used narrow strips of dense lanceolate

and absorbent filter paper length about 1,5 cm These materials must be sterilized in a Petri dish or in paper bags. Sterilization is performed by dry-air method - in the dry heat cabinet. Standard boundary conditions of sterilization are: 165-170 ° (not higher than, because paper and cotton wool layers can become brittle) within 1 hour or 155-160 ° for 2 hours. Turundas to the root needle or cotton ball just before taking the sample is slightly moistened with a sterile saline touch in a test tube. Moistened paper strip is not needed. Immediately after sampling, turundas, removed by tweezers at the root tip, cotton ball or paper strip (as well as material taken in other ways) is immersed in a test tube with nutrient medium. In this case the normal bacteriological techniques crops are followed - above the burner flame.

**The choice of medium,** in which the material is placed depends on the objectives of further research.

1. When they mean the microbial flora fullest characterize, the medium must provide the best preservation of all microorganisms in the material until the primary crops in other environments. In such cases, the recommended liver broth, preferably blood serum (rabbit or horse). Preparation: to 1 l of meat peptone broth (MPB) is added liver broth 200 ml, the pH is set in the range 7,2-7,4, poured into 3 ml of a centrifugal or vasserman tubes and sterilized at 115 °. In test-tube there added 2 ml of serum in aseptic conditions with the subsequent sterility control. For liver broth preparation 500 g liver (cattle), passed through a meat grinder, poured 1 liter of distilled water, boiled for 20 min. Filtered and sterilized at 115 °.

A material sample is stored in liver broth until the crops, no longer than 4 hours in a cool room (10-16 °). Cold storage can lead to some asporogenic anaerobes cell death.

2. If the objective of the microbial flora study is the certain bacteria types detection, the material is placed immediately in the elective medium. Tube as soon as possible is put in a thermostat. Prior to this test tube with the corn liquid media stored in the refrigerator, and a semi-liquid - at 10-16 °.

Conditions and material sampling technical details with a particular pathological process are defined by features of the microbial flora localization study. Saliva samples are taken at all the same pathological processes.

**The saliva microbial flora study.** Studies are carried out at dental caries, parodontopathies, dysbacterioses to detect shifts in the oral cavity microbial flora content. Determine only the microbial cells presence or amount of different microorganisms species. Most often is needed to determine the lactic acid bacteria amount in dental caries.

The role of saliva is taken to the attention, chewing and swallowing in the processes of oral cavity self-purification of the microorganisms abundance, should be taken samples of saliva always in the same period in between meals: an empty stomach or 3-4 hours after eating. In the day of sampling examinee should abstained from brushing and mouthwash.

For microorganisms washout from the saliva of those areas where they mostly accumulate (interdental space, the surface of the tongue, gingival pockets, etc.), can be used two ways. Choosing one or the other is associated with contingent subjects.

On the examination of individuals or adults and adolescents small groups should be given preference to stimulate saliva flow by chewing pieces (about 1.5 cm 3) sterile wax or paraffin. Sterilization is performed in an autoclave, large portions in flasks. Before use paraffin or wax is melted in a water bath, poured into sterile Petri dishes and cut into pieces with a sterile, slightly heated scalpel. The stimulation duration should always be the same (note the clock), but not less than 3 minutes. It is enough to collect 5-6 ml of saliva, which is achieved easily. Preferably, to gather saliva gradually during stimulation, but not after it. In this case, care must be taken to not swallow the saliva. Immediately before stimulation surveyed rinsed his mouth with boiled water.

The mass screening, especially children, is feasible by only the second method. Surveyed prerinsed his mouth with 50 or 100 ml of sterile saline to remove food debris and stimulate salivation. After 10-15 min. the surveyed is give onrinse his mouth with 8 or 10 ml of sterile saline and take saliva, diluted in this way - slop liquid. It should be monitored closely to rinse it strong and long enough (up to 3 min.) And to slop fluid to be collected in full. If part of it will be swallowed it is not suitable to determine the microorganisms number, but can be used for other studies (with a corresponding note.)

In both methods saliva is taken in the wide-tubes or count barrels, sterilized under double paper caps, which ensures the sterility of the glass edges, which the examinee can touch by his lips. The same precaution is necessary for test tubes sterilization with saline mouthwash. When the sample is taken, a paper cap is replaced by bacteriological cotton stopper, sterilized in a paper bag or in a Petri's dish.

Samples of saliva are kept up to research date in the refrigerator (not freezing) not more than 4 hours.

**The study of microbial flora in dental caries**. Aimed to make those microorganisms characteristics, which play the greatest role in the carious defect onset and progression, affecting the products of their metabolism directly on dental hard tissue, examined dental plaque and dentin.

Dental plaques are formed from the plaque on the teeth and are non-calcified accumulation of microorganisms adherent to the tooth surface due to the mucous dense substances deposition - mucoid and polysaccharides, mainly dextran. If the enamel is not altered by plaque caries process, the conglomerates of microorganisms are isolated from the enamel surface with a thin layer of these substances that make up the plaque matrix. The dental plaque formation is disturbed by meals and most brushing; time after teeth cleaning is called plaque age. Within age, the plaques thickness and their adhesion strength to the tooth surface increase. However, there are significant changes in their microbial flora. Salivation,

chewing and brushing influence the plaque formation in a greater or lesser extent depending on its location. Difference in the plaque microbial flora on different teeth and even on different surfaces of the same tooth is associated with this.

This summary explains the main conditions for the dental plaque microbial flora study in a single person or in several people parallely: to get comparable results material should be taken from the same or symmetrically placed patches (if it is not possible, the study is not carried out) of the same age and at one and same time after a meal. A number of studies have investigated the plaque (one way or another location), aged from several hours to 2 weeks of fasting and 1-4 hours after eating.

Immediately before taking the material surveyed rinses his mouth with boiled water2-3 times. If plaque is precervical, the gum edge is shield, as in fillings, from saliva. Plaque is removed by curettage sterile instruments. The material is taken from the tool collected by cotton balls.

**Dentine** is examined after careful detritus removal from the cavity. It is necessary to avoid accidental microbial "discoveries": the detritus microbial flora can contain any microbial species that shortly inhabit in the mouth, i.e., introduced from the outside. Microbial flora of dentinal tubules varies considerably in direction from surface to depth. Therefore, samples are taken due to the dentin layer removal during cavity processing. If the study objectives include comparative microbial flora characterization of various dentin layers, after taking samples from each layer burrs are replaced. Dentine chips are gathered by cotton balls or bristles on the root needles.

**The study of microbial flora in parodontopathies**. To characterize the microbial flora in periodontal disease inflammatory-dystrophic form is taken material from the surface of the gums with cotton balls and from the gum pockets by cotton bristles at the root needles. If there is an abundant purulent discharge study is limited by pus analysis taken from a depth of periodontal pockets after a careful removing it from the gums and teeth neck surface with cotton balls. In the examination day the patient should refrain from tooth brushing, rubbing the gums and use mouthwash solutions, elixirs, or drinking water. Immediately prior to sampling the material patient rinses the mouth with boiled water. To study microorganisms properties, involved in the inflammatory process genesis and development, cervical dental plaques are explored the boundary in mild gingivitis (see above).

**The study of microbial flora in the pulpit**. Methods of investigation and results evaluation are determined by the inflammation nature and therapeutic measures.

If you use a biological method of pulpitis treatment and pulp horn is not opened, then the pulp microbial flora properties must be judged merely suspected – according to the study results of dentin taken from the caries cavity bottom the after its machining. There remains, of course, unclear which of the microbial species found in the dentine, are penetrated into the pulp.

If in biological acute pulpitis treatment method pulp horn is detected, exudate on its surface is explored. After softened dentine removing by an excavator, pulp is carefully touched by thin cotton bristles, or the tip of the filter paper strip. This study may show how organisms predominate in the inflamed pulp, but does not detect all microbial species present in it, especially in cases of pulp tissue slight contamination. Note that when sampling is often not possible to avoid contamination of the dentin microbial flora.

When research is conducted in pulp amputation or extirpation of teeth, affected by dental caries, before surgery it is necessary to make a thorough mechanical treatment cavity to avoid pulp contamination during its removal. Extracted tissues are immediately removed from pulpextractor and placed in a nutrient medium. With the help of bristles on the root needles there taken samples from the pulp surface and the tooth cavity walls. In the case of the root pulp removal the material is taken as far as possible from the root canals depth.

**On examination of the teeth pulp affected by caries**, microorganisms can be detected with the greatest reason be regarded as inflammation causative agents in cases where the cavity is opened during surgery. If the set of tooth cavity is more or less destroyed by the caries process, the coronal pulp contamination from the cavity side is inevitable. Therefore, in all pulpitis forms with an open pulp horn can be found various microbial flora representatives of the detritus cavity. Contamination of the pulp with microorganisms in chronic pulpitis is more than in acute and maximal at gangrenous pulpitis. To judge about all these microorganisms involvement in the pulpitis development is not possible. For the presumptive judgment about the periodontal microbial flora in cases of pulpitis complication by apical periodontitis can be used a study of root canals in all pulpits, except gangrenous.

**The study of microbial flora in apical periodontitis**. To determine the content and microbial flora properties of apical periodontitis can be achieved through the exudates study at the time of his admission into the root canal, after apical hole opening. Before taking the sample the machining and channel expansion should be made without the antiseptics and antibiotics use: sterile saline and, as usual, alcohol and ether are used. After apical hole opening, the root exudate is taken by turundas on the needle.

In addition, due to the conservative periodontitis treatment microorganisms which are present in inflamed tissues, can be detected in cases of fistula formation. Pus in the fistula holes is carefully removed. After processing the gum with alcohol and iodine tincture sample is taken from the fistula depth turundas to the root tip.

Terms of the microbial flora study are optimal for radiectomy, especially because during the surgery it is always possible to prevent apical periodontal contamination. Technique of sampling is usual (use bristles).

**In tooth extraction** as a contamination of its surface and the alveoli walls by the gums edge and gingival pocket microbial flora can not be removed. It is ap-

propriate to examine the tooth roots, but not the alveoli contents, as it is possible to remove this "accidental" flora only from the root surface. Conditions are the most favorable research if there are pararadical cysts, cystogranulomas or granulomas, not separated from the root of a tooth during its removal. To slow the extracted tooth surface drying, it is immediately placed in a sterile vial with a moistened cotton stopper under a rubber cap or a sterile Petri dish with the cotton balls in it. Keep the tooth at room temperature, but not longer than 2 hours. Before sampling, the roots of the teeth are moistened with alcohol and carried out once through the flame (flaming). The contents of the cyst and cystogranulomas is taken after dissection or puncture with a Pasteur pipette or bacteriological loop and contribute on the growth medium. Large granulomas are cut from the tooth root, is transferred to another Petri dish and crushed with sterile scissors or a scalpel and mixed with 1 ml of culture medium. Material is put in a test tube with the same medium, gathering it with a rubber balloon Pasteur pipette with a wide opening. Large particles contribute to the tissue environment tweezers. In a small amount or absence of granulomas extend the root canal with a sterile bur from the apical hole. Thee sawdust cement is taken by the cotton balls.

**The study of microbial flora in the jaw osteomyelitis, abscess and phlegmon**. For the pus taking, in addition to turundas and cotton balls, swabs are also used, intended for the study of pharynx microbial flora. Swab after sampling pus is immersed in a culture medium and left in her. Tube, in which the wire is inserted with a tampon should tightly close the tube with the medium (pick up tube of suitable diameter).

In cases of osteomyelitis the samples are taken from the tooth alveoli bottom. In subacute and chronic jaw osteomyelitis stages the pus is taken from a depth of the fistula (as in periodontitis). In sequestrectomy pus, small sequestra and granulation tissue are examined. In the cases of abscesses and phlegmon there examined exudate, obtained from the outer sections and from the oral cavity. Thus it is necessary to take measures that prevent material contamination (fencing operation field from saliva, mucous membranes and processing of skin with alcohol and ether).

In conclusion, we reiterate that the results corresponding to the objectives study, and the correct evaluation depends on compliance with the conditions and all the material sampling technical details. The basic principle of any research is the consistent methodology. From the foregoing it is evident that this principle while taking the material for the oral cavity microbial flora study can be achieved only by careful management and technical training.

### 2.2. Microscopic examination

Microorganisms of the oral cavity normal microflora can be observed in preparations made from dental plaque, saliva and Gram-stained, or by other methods. Microscopy should be conducted using immersion microscope. In prep-

arations there detected bacteria of various shapes - cocci, rod-shaped, twisted and filamentous. Direct normal microflora microscopic examination is not effective because of the significant polymorphism that is characteristic for many species, as well as the fact that some species are morphological counterparts, or have significant similarity. For example, the similarity between a different species of the Streptococcus genus, which are part of the oral cavity microflora.

Bacterioscopic method for diagnosis can be used for initial wound infection diagnosis. So, in preparations made from pathological material in gas anaerobic infection and Gram-stained, there found a large number of gram-positive rod-shaped polymorphic microorganisms. On staining these preparations by the Hins-Burri method some sticks can be detected by the capsule. Around the bacteria red capsule is observed as a transparent bezel on a dark background. Capsule-forming are, particularly, Clostridium perfringens.

Most of the bacteria causing the oral cavity infection is morphologically similar to organisms that are present in the normal microflora (staphylococci, streptococci), or are representatives of the microflora (pathogens of Vincent stomatitis) This reduces the preliminary microscopy diagnosis reliability. Note, that, in some cases, the microscopic method can be effective.

In oral mucosa candidal lesions in the preparation of the plaque, Gram-stained, there revealed numerous round and ovoid cells of Candida genus fungi, located in the disordered clusters. Sizes are variable enough cells as a result of reproduction by budding. The complexity of the candidiasis microscopic diagnosis is, firstly, that the fungi of Candida genus can be present transiently in the healthy human oral microflora, so you need to pay attention to the number of these microorganisms in the area of vision. Secondly, there is a defined complexity of differentiation in conditionally pathogenic fungi of the Candida genus from non-pathogenic yeasts, which are constantly present in the content of the oral cavity microflora. Differential symptom of yeast-like fungi is pseudo-mycelium - cells elongated or filamentous. For yeast pseudo-mycelium is not typical.

Representatives of the Neisseria genus are in preparations Gram-stained with or methylene blue stained, and stand out among normal microflora due to their inherent intracellular location in leukocytes, where they breed (the incomplete phagocytosis phenomenon), and also due to the pair location. However, to differentiate meningococci and gonococci is not possible.

Treponema pallidum – syphilis causative agent - can be detected microscopically in the localization of lesion in the oral cavity. Apply dark-field microscopy technique. In preparation "hanging" or "crushed" drop can be watched live spirochetes - extremely mobile with 8-12 primary uniform curls. Due to the fact that the normal microflora content non-pathogenic species of spirochetes, it may be appropriate to take on the study of regional lymph node punctate. Identification of spirochetes in the pathological material is evidence of a pathogen and, thus, that they are not normal microflora representatives.

Transmission of tuberculosis (Mycobacterium tuberculosis, M. bovis) may also affect the oral cavity mucous membrane (tuberculous lupus, and ulcerative miliary and colicvational tuberculosis). For the Mycobacterium detection preparations should be painted according to the Tsill-Nielsen method. In this case, rod-shaped acid-resistant TB germs are colored in red, whereas non-acid-resistant accompanying flora is painted in blue. Bacteria are located single and as disordered clusters.

Tsill-Nielsen method can also be used for leprosy agents coloring (M. leprae) in preparations made from scraping the oral mucosa ulcers bottom and edges. M. leprae is found in the epithelial cells cytoplasm. They are rod-shaped, located wives mainly parallel to each other. To differentiate M. leprae and M. tuberculosis should be used Semenovich-Martsinovskiy's coloring method, where more acid-resistant M. leprae are colored red, and other mycobacteria types - in blue.

For the microscopic actinomycosis diagnosis there made preparations from pus, can be studied in the preparation of "crushed" drop unpainted or in a fixed and Gram-stained preparation. There discovered the mycelium (thin branched cells intertwined) and druzes (granular education).

Microscopic method of infectious diseases microbiological diagnosis (particularly those in which the infectious process localized in the oral cavity) has a modified current version, which can be related to a particular group of microbiological diagnosis methods – express-methods. Common features of express diagnostic methods are reducing the time needed for diagnosis, as well as improving the data accuracy.

True, the results obtained using rapid methods sometimes require classical methods subsequent confirmation.

In this case, it is a serological immune-fluorescence test. To perform this reaction with study material (saliva, pus, etc.) that contain pathogens (studied antigens), process with specific to one or another kind of standard diagnostic serum antibody tentatively linked to a fluorescent substance (e.g., fluoresceinisotiocionate). In the case of relevant antigens and antibodies, they are connected and on preparation microscopy using a fluorescence microscope in the area of view will be observed microorganisms stained with fluorochrome - they will glow. This is so-called direct immune-fluorescence test.

There can be used the reaction of indirect immune-fluorescence. For this reaction it is needed non-luminescent standard diagnostic serum with antibodies to pathogens, as well as standard anti-species fluorescent serum with antibodies to that animals species immunoglobulins, the serum of which was used as a standard diagnostic antimicrobial (for example, it may be fluorescent anti-rabbit antiglobulin serum).

Reactions of direct and indirect immune-fluorescence are also used in scientific oral cavity microflora research.

There can be used as highly sensitive serological reactions with other labels - an enzyme (enzyme immunoassay - EIA), radioactive (radioimmunoassay - RIA).

The principle of these reactions is very similar to the immune-fluorescence reactions formulation, but did not record the reactions carried out microscopically and by using a spectrophotometer (EIA) or a scintillation counter (RIA).

### 2.3. Bacteriological examination

The microbiological studies results are heavily dependent on the pathological material type, time and manner of its capture. These factors are often decisive for the correct results interpretation. Microorganisms evolve and die, they are sensitive to many chemicals and can be found in various body fluids and tissues. Therefore, the collected material should be processed so as to maximize the preservation of the pathogens viability and multiplication.

Irregularity of collection and material delivery timeliness in the laboratory can contribute to the low frequency of its causative agent isolation. It should also be noted that microorganisms can behave differently in the presence of another species individuals. For example, streptococcus "viridans" group (viridans streptococci), which are normal human oral cavity inhabitants, inhibit the Candida genus fungi growth.

In gathering material for microbiological research is needed regardless of the sample to observe the following general requirements:

-enough material;

-material compliance with the infectious process (e.g., saliva, rather than sputum, discharge from the wound depth, not from its surface);

-material collection only in sterile containers;

-strict adherence to aseptic technique by a fence material;

-material delivery in the laboratory as soon as possible;

-material for research is taken prior to antimicrobial treatment.

**Transportation** of any material in the microbiology laboratory for the study should be undertaken as soon as possible (in the hospital - almost immediately after the material collection). Delivery of material in the hospital performed only by medical personnel (patients in any case not be involved in this process!). Material should be transported in specially designed for this purpose the container (e.g., bix) with accompanying documents, filled by the attending physician in accordance to particular facility accepted rules.

In dental practice, bacteriological and mycological studies are carried out:

1) when it is necessary to clarify the mucous membrane infectious lesions nature;

2) for specific diseases (such as tuberculosis, gonococcal stomatitis);

3) in case of purulent processes;

4) to determine the pathological focus microflora sensitivity to antibiotics and other chemotherapeutic drugs (in case of ulcers that are not healed for a long time, chronic inflammation).

Bacteriological examination of clinical material contains three stages: sampling for a study, cropping on nutrient media, pure culture isolation, identification (defining the causative agent type), the results analysis.

Collecting material for bacteriological examination in dentistry should follow these rules: before taking the material does not use any mouthwashes, do not brush teeth before taking the material, wash the mouth with warm water, clean the surface of the ulcer, the material is taken from the ulcer depth and immediately send to a laboratory. Taking the material and the planting on special mediums can be carried out in the dentist's office.

Material for bacteriological studies to identify Candida genus fungi is a plaque on a mucous membrane. For microscopic examination of pathological material is collected from the lesions by heated and cooled platinum loop or a dental knife.

Study of microflora in different ecological niches, particularly, the oral cavity, often includes the integration of not only the quality of the (species) microorganisms that inhabit the study area, but its composition and quantity.

It is may be important to determine the microbial species percentage, i.e. the proportion of each selected species in the microbial association.

Accordingly, the first requirement is dimensional fence material, followed by per volume unit (1 ml of saliva) or area (1 cm2 of the oral mucosa, the tooth or prosthetic material).

For example, the candidiasis diagnosis can be put only on the yeasts identification basis in the material from the patient. These mushrooms may also be present in healthy people. When the Candida fungus concentration is high, it indicates their involvement in the pathological process. This also refers to opportunistic bacteria, which are detected in the oral cavity.

To reveal the greatest microorganisms species number the material should be studied simultaneously spread on a variety of differential diagnostic and selective medium.

For example, to reveal the diphtheroids is used Buccini's medium; hemolytic pathogenic microflora (E. coli, staphylococcus, streptococcus, Neisseria revealed in 5% blood agar. Vitelline-salt Chistovich's agar can distinguish staphylococci by lecithinase activity. Saburo medium is intended to reveal the fungi cultures, in including Candida genus yeast-like fungi. Endo medium - for E. coli, and the Ploskirev's medium - to reveal the other Enterobacteriaceae family members (e.g. Klebsiella, Proteus). Planting material on sloping meat-peptone agar by the Shukevich's method (the condensing fluid slant agar) reveals the creeping Proteus and Pseudomonas aeruginosa growth. Blaurokk's medium is used to reveal bifidobacteria, MRS-2-lactobacills, KAB - for Bacteroides, and anaerobic cocci fusobacteries.

To reveal gonococci there used culture media with human proteins - a blood or serum agar.

Tubercle bacillus was cultivated on Lowenstein-Jensen's medium.

Some pathogens do not grow on nutrient media (e.g., Mycobacterium leprae), or can not be identified in pure cultures due to variability (Treponema pallidum). In such cases, the selection of pure cultures is not performed.

Kitt-Tarocci's medium, blood-sugar agar, an iron-sulfite agar are used for pure clostridia cultures isolation - gas gangrene pathogens (Clostridium perfringens, C. novyi, C. septicum, and others). In this disease the pathogen pure culture selection is necessary not only for diagnosis but also for the treatment purpose, which will be effective due to the application specific antitoxic sera in combination with other ethiotropic and pathogenic agents.

For the odontogenic sepsis diagnosis microbiologic blood is performed study, blood is taken by vein puncture.

Prior to vein puncture the skin is processed by 70% alcohol (exposition is not less than 30 sec.) apply 1-2% tincture of iodine in the form of a circle diameter 1,5-2 cm (the exposure is not less than 30 sec.). For patients allergic to iodine you can only use 70% alcohol (the exposure is not less than 60 sec.). On vein puncture it is taken a sterile syringe in an adult - 10-20 ml, a child - 1 - 5 ml. It is correct to make an immediate culture inoculation in the medium in the vial in a ratio of 1:5 to 1:10. If you can not – it is preferable the immediate delivery of the material in the syringe, with which puncture was performed to reduce the risk of contamination (pollution).

Sowing is carried out at least twice a day; immediate delivery of samples to the laboratory

Content of special nutrient media is described in the references on bacteriology.

For the many infectious lesions effective treatment it should be determined the causative agent pure culture sensitivity to the chemotherapeutic agent, particularly antibiotics. Variability of bacteria is the reason that the same bacteria species strains, revealed in different patients may be at varying degree susceptible to the same antibiotic.

**Sensitivity to antibiotics** in practice is most often determined by the indicator discs method. This method is called the agar diffusion method. The principle of the method is that on the medium in a Petri dish the pure culture is inoculated like a lawn (so as to obtain confluent growth on the medium surface), seed is dried in an incubator and on its surface are placed standard size paper discs, impregnated with an antibiotic solution of certain concentration. Samples are incubated for 18-24 hours. During this time, it is performed the antibiotic diffusion in the medium and culture growth. The greater is culture sensitivity of to the chemotherapeutic agent action, the greater is the "no growth zone" diameter (including the disk diameter). Culture is generally defined as sensitive to the drug, if the zone diameter of 15-25 mm, high sensitivity, if the diameter of the no growth zone is greater than 25 mm.

### 2.4. Biological examination

Animals infection with pathological material is appropriate when, firstly, there are animals susceptible to this pathogen, and they may be observed the characteristic pathogenetic features (tuberculosis) or property agent (pneumococci capsule-formation) are important for microorganisms identification and, secondly, there is no other more humane ways to solve the diagnosis problem. In dental practice, the biological method is not widely used.

At the same time to study the dental caries and chronic periodontal disease etiology and pathogenesis it may be useful to create these diseases models, using animals as a model of influence.

### 2.5. Serological examination

Diagnosis by identifying antibodies diagnostic titer in a serum, or the fact of the serum antibody titer rise at least 4 times in the course of the disease with specific diseases such as gonococcal infection, tuberculosis, syphilis, influenza, measles, etc . (disease manifestations can be detected by a dentist) is carried out conventional serological methods. Thus, regardless to the infectious process localization, in syphilis there used serological tests complex that contains a reaction of micro-precipitation (MPR), complement-binding (CBR, or Wasserman's) and immune-fluorescence reaction. Also used EIA. Such unified approach by the fact that immune system has a range of the same organism protection reactions type, regardless of the infection gates.

Quantitative determination of the local immunity strength (titer sIgA) in saliva as accurate quantification of the IgG antibodies titer to pathogens in blood serum. Therefore it is more convenient to study the serum.

Serological caries and chronic periodontal disease diagnosis is not effective due to the fact that these lesions are caused by weak immunogenic agents. This means that the antibody titer in serum to the caries and periodontitis causative agent is not significantly higher titers, corresponding to antibodies in healthy people. By the way, a weak immune response to these infectious agents is a cause of chronic stroke and complications ethiotropic caries and periodontal disease treatment.

At the same time it should be noted that the tendency to such diseases, along with other factors, depends on the organism immune status as a whole. The protection contributes by nonspecific and specific mechanisms. Most effective in terms of possible disease recurrence may be dental care aimed not only to the affected oral tissues, but also provides an integrated approach to improving the organism state as a whole. In this regard, an important assessment of the protective body mechanisms (immune status) in order to effectively immune-modulating effects on the body. In this regard, the introduction in a dental prac-

tice of methods for assessing immune status is relevant, as well as immune-modulating therapy of chronic diseases such as caries and periodontitis.

### 2.6. Allergy testing

Allergy testing in the specific diseases microbiological diagnosis with the infection or allergic reactions localization in the oral cavity are as universal as the serological methods for diagnosis of these diseases and has no difference from allergy testing to diagnose these diseases under the conditions of the other manifestations localization. Thus, in the tuberculosis diagnosis there is the intradermal tuberculin test (Mantoux), in the syphilis diagnosis - thes is the intradermal test with luetin, etc. It should be noted that in the infectious diseases microbiological diagnosis through the allergy testing it is usually conducted the delayed-type responsiveness hypersensitivity (48-72 hours after allergen administration). Diagnostic value of redness zone diameter and induration at the allergen injection site. Delayed reaction detects the cellular immunity (T-effectors) reactivity against the pathogen.

In the periodontitis diagnosis to identify the induction etiological factor of the process allergic tests are inappropriate to use for the same reasons as the serologic method (weak immune response).

## 3. Oral cavity microflora

One of the most informative indicators of state of the organism as a whole, and also an oral cavity – an oral cavity microflora and the state of its nonspecific and specific resistance. These components determine the development of caries, periodontitis, lesions of the oral cavity mucosa. According to WHO, these diseases affect nearly 100% of the adult population of the world.

Microflora of the oral cavity is a complex and resistant to external factors system that can not be ignored as a factor in the positive influences on the one hand, and as a source of infectious diseases on the other. Only a physician who understands the laws of the formation and functioning of this system can effectively correct the processes of microflora influence on the tooth and periodontal patients who come to him.

### 3.1. Relevance and the history of the issue

Human is in close interaction with the environment, this fully applies also to the microcosm that surrounds him. The human body - is an ecological system, which for a considerable period of time there were certain relationships between the different microorganisms groups and kinds. The role of microorganisms in the body's vital functions is so significant that researchers are inclined to think about

the physiological significance of the human body microflora, comparable to the value of any organ system.

A. Levehuk was the first who saw that the scraping of the tooth germs has more microbes "than the people in the whole kingdom." He believed that these organisms are not harmful if they are in the body of healthy person.

The first solid scientific research in this area was made by Mechnikov. At the beginning of XX century II Mechnikov and G.D. Belonovsky actually already formulated general ideas on the human body microflora, whereby the normal microflora - a collection of microorganisms that inhabit different parts of the healthy person body. Mechnikov was considering the positive role of normal microflora, but also prevent the possibility of slow poisoning microorganism products of microbes vital activity as the premature aging cause. In this case, it was a phenomenon of general pathologic scale rather than on specific diseases, which may also be associated with a normal microflora. Today, after 100 years of Mechnikov research, the doctors of different specialties, but not least dentists think about the normal microflora of the human body as a whole and to the oral cavity microflora in particular, with great attention. Take into account its positive role and it is regarded as a potential source of pathogenic factors. Thus, the modern doctrine of the normal microflora has completely specific clinical and pathological significance as the basis for a new section - Clinical Microbiology, which is the scientific foundation for developing methods of pathogenetic treatment, in particular, and dental diseases, biological agents.

### 3.2. Oral cavity microflora general characteristics

Oral cavity contains a large number of microorganisms.

This is facilitated precisely the location of the mouth at the beginning of the digestive system, its relationship with the respiratory system. With meals and air in the mouth there gets a large number of microbes from the environment. Cigarettes, utensils, dirty hands promote the entry of microorganisms and its contact with different objects.

Microbes can get into your mouth, not only from the environment, but also from the organism itself. For example, with saliva in the mouth germs get rabies and mumps, the blood can be included syphilis and tuberculosis.

Oral microorganisms are extremely diverse. The species composition is a big part of the normal human body microflora. Most of them are in a state of symbiosis with each other and, crucially, with the body.

Symbiosis is a close interaction between two or more organisms of different species together.

During the symbiotic nature of such release of its varieties: neutralism, mutualism, commensalism, antagonism, parasitism.

Neutralism - the absence of any interaction between the human body and the microorganisms that enter the skin or mucous membranes. Such relationships are

added to the microbes that get into your mouth, but are representatives of the water or soil microflora and can not find in the mouth of conditions for their existence.

Mutualism - a form of symbiosis, where both participants benefit from the interaction. Most of the permanent representatives of the microflora are mutualists (e.g., E. coli E. coli, bacteria of the genus Lactobacillus and others in the large intestine). Mutualistic interaction of the human body with oral cavity microflora representatives are studied enough, but some species of the genera Lactobacillus and Streptococcus, perhaps, take part in the digestive process, which begins in the mouth.

Commensalism is such an interaction, when the microorganisms find themselves the appropriate conditions for the existence (food, moisture, body temperature, pH), but their vitality is not harmful to the body, either it is not useful. Oral microflora is an example of what a commensal one or another kind of microorganisms can be attributed due to insufficient study of the issue. This contributes to a "weakness" of interaction, long time required for the manifestation of the results of interaction. So, a result of research years has proved the involvement of S. mutans in the process of dental plaque formation, which precedes the caries process.

It should be remembered that the beneficial or harmful to the human body can be not only a direct interaction of microorganisms with the human body, and the interaction of microbes with each other. Thus, the antagonists of S. mutans may be used as biological agents for the prevention and treatment of dental caries, following the example of how the composition of the colon microflora resumes eubiotics - drugs that contain live microorganisms (characteristic of the normal intestinal flora) with antagonistic effects on pathogenic bacteria. These are drugs like colibacteria that contains E. coli specific biovars and other similar preparations. In this case, different types of bacteria enter into a competitive relationship, characterized that one population suppresses another.

Parasitism - is another form of symbiosis, which is characterized by the fact that the interaction is for the benefit of one and injury to the other cooperating parties. Often this term describe the interaction of the human body to cause infectious diseases.

A number of microorganisms is an etiological factor in dental disease (stomatitis, gingivitis, and others). Pathogens of these diseases come into parasitic interactions with the body, affecting certain structures of the mouth.

Oral cavity is the main way for the penetration of many microorganisms. At the same time it is the natural environment of existence for many groups of bacteria, fungi. There are favorable conditions for breeding bacteria: always a uniform moisture content, a fairly constant temperature (near 37 ° C), slightly alkaline (pH of about 6,9-7,0) leftovers as a nutrient medium, the epithelium, which peels off, the specific structure of gum (pockets, crypts, gaps between teeth, a hollow carious teeth). The total concentration of bacteria in saliva ranges from $1x10^7$ to

$1x10^{10}$ microbial cells in 1 ml in plaque are many more - from $1x10^{11}$ $1x10^{13}$ up to 1 g of material.

Due to the fact that the oxygen content in different parts of the mouth is different, there are conditions for the existence of both aerobes and anaerobes.

It should be noted that since the first days after the birth oral microflora does not coincide with the oral cavity microflora composition of adults who surround him. Transient species that are stepping with the food and for which no ecological niches are quickly removed. Such selectivity is preserved in adults. In particular, even among men and women do not necessarily reflect the composition of the oral cavity microflora.

Oral cavity microflora is a complex natural dynamic biocaenosis fixed and variable populations.

distinguished between permanent (resident, obligate, autochthonous) and temporary (casual, transient, optionally, allochthonous) oral cavity microflora.

Permanent microflora is a collection of different microorganisms species populations, which has developed in the course of evolution, is resistant to the oral cavity factors, and is a binding biological system in the oral cavity. Among the resident microflora there are described near the 30 species of microorganisms.

Temporary microflora is not required for the oral cavity and represents microbes that come from other parts of the microorganism and the environment with food, water and air. Duration of their stay in the mouth is limited.

The formation of peculiar to human "physiological" microflora is a consequence of the mutual organism and microbes adaptation. In human in the process of its development as a species has been formed and entrenched hereditary high immunity level to the microbial species that could repeatedly enter the body from the surrounding environment. However, the selection took place slightly viral microflora representatives, which in the process of variability gained sufficient strength to the organism protective factors.

Microbial flora of the oral cavity as part of the microflora as a whole, as a biological and physiology of the body's healthy person, illustrates a fact of the huge biological importance of the unity of the organism and the environment. Mutual adaptive changes cause biological "balance" not only between the body and adapt to the microbial flora, but also between species that are in this microflora. Phylogenetically predetermined equilibrium relations are characterized by considerable stability, and violations occur only in the case of body protective functions reduction. Under this condition may occur auto-infectious processes and disbacterioses. Even disinfecting rinses, pastes and partial antibiotics packs only briefly reduce the number of microorganisms in the mouth.

Thus, the normal oral microflora has a double meaning: the biological barrier and the potential autoinfection tank.

### 3.3. The main representatives of the permanent oral cavity microflora

Microbial flora of the oral cavity is extremely diverse and consists of representatives of all microorganisms groups: bacteria, actinomycetes, fungi, protozoa, spirochetes, viruses. A significant portion of microbial oral adult before anaerobic species - see Fig. 3.1.

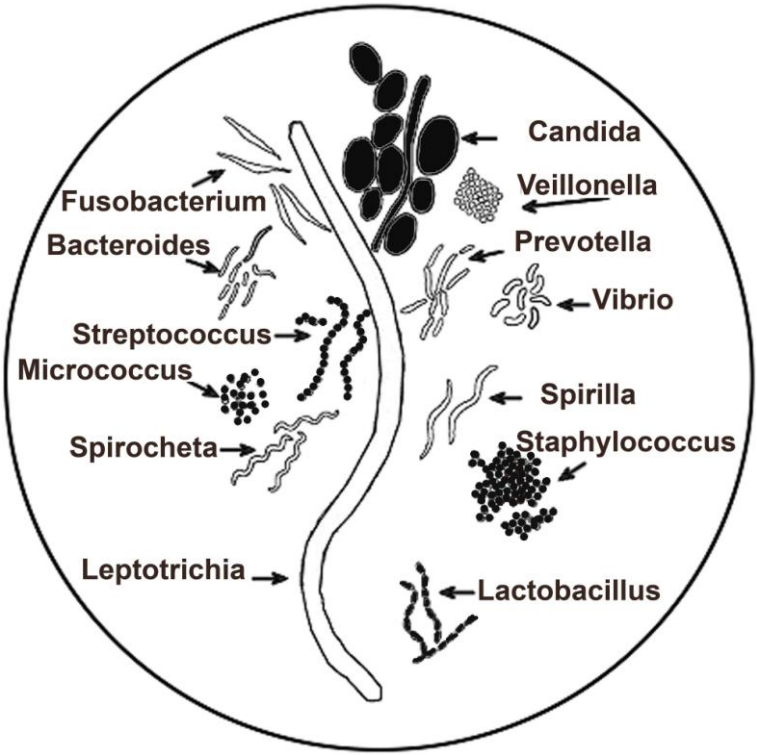



Fig. 3.1. The main representatives of the permanent oral cavity microflora ( Gram + bacteria colored black)

More than a half of permanent resident microflora is represented by veylonella and diphtheroids.

Staphylococci, lactobacilli, spirochaetes, Leptospira, fuzobakteria, bacteroides, neisseria, yeast and other fungi, protozoa are found in the mouth in much smaller quantities. Although these organisms are constantly in the mouth, they never are as numerous as streptococci, and diphtheroids, veylonella.

Consider the major groups of microorganisms - representatives of normal oral cavity microflora - see Table. 3.1.

**Table. 3.1. Bacteria belonging to the plaque**

| Form | Gram-positive | | Gram-negative | |
|---|---|---|---|---|
| | Aerobes, facultative anaerobes | Anaerobes | aerobes, facultative anaerobes | Anaerobes |
| Cocci | Streptococci | Peptococci Streptococci | Neisseria | Veylonella |
| Rod-shaped | Actinomycetes Lactobacilli Corynebacteria | Bifidobacteria Propionibakteria | | Bacteroids Prevotella Fuzobakteria Leptotrihia |
| Spirochetes | | | Leptospira | Treponema, Borrelia |

### 3.3.1. Aerobic and facultative anaerobic bacteria

The main representatives of the oral cavity microflora is a heterogeneous group of slightly viral streptococci. The cells from spherical to oval, up to 2 microns in diameter, non-spores, located in pairs or in chains when grown in liquid media. Gram-positive. Among the residents is an alpha-and beta-hemolytic and gamma non-hemolitic form.

S. mutans, S. sanguis adhere well to the enamel and dominate in the dental plaque. With a significant enzymatic activity, streptococci ferment carbohydrates to form lactic and other organic acids. Acids, which are formed by the streptococci enzymatic activity, inhibit the reproduction of some putrefactive microbes that can get into the mouth from the external environment. At the same time, the shift of pH in the acid side leads to decalcification of the tooth enamel.

Of great importance is the ability of streptococci to synthesize polysaccharides from sucrose. In this part of the glucose molecule is converted into dextran and the glucan, and fructose - a levan. Insoluble dextran is a factor in adhesion and promotes the dental plaques formation, and soluble glucan and levan is a source of further acid production even in the absence of receipt of carbohydrates from the outside.

Pneumococci / S. pneumoniae / are in the upper airways and oral cavity. There are opportunistic and pathogenic biovars.

Staphylococci are found in the mouth less than streptococci, they emit about 30% of the people. The most frequently encountered Staphylococcus epidermidis, S.saprophyticus, much less S.aureus. Staphs enter the mouth shortly after the birth of the person, because that is widely distributed in nature, existing in the air and on the skin of people. Staphylococcus is not found in the oral environment

for the continued existence, but it may be the cause of various inflammatory processes, tongue, mucous membranes and gums. Staph is involved in the development of pulpitis, it is found in the hollows of decayed teeth, between teeth, the mucous membrane of the lips.

It should be noted that in the oral staphylococcal infection is much less likely to accompany multiple injuries (even the wounds of the jaw with a crushing bones) than in the skin. This is due to the fact that the saliva of a bactericidal effect on staphylococci. Saliva is a lysozyme and other factors of natural immunity, effective with respect to staphylococci.

Among the Gram-negative cocci were found Neisseria - Neisseria sicca, N. musosa, as well as branhamellas - Branchamella catarrhalis. It is diplococci, do not form spores. Have a microcapsule, polymorphic. In contrast to the pathogenic form (Neisseria meningitidis, N. gonorrhoeae) these grow on ordinary nutrient media.

Rod-shaped bacteria. The genus Lactobacillus has in its composition not less than 25 species. Bacteria have the form of sticks from the long and thin to short coccobacillus. Often form chains. More often have some peritrihia. Spores are not formed, in young cultures - gram-positive in old cultures and increased acidity - Gram-negative. Some strains appears grainy (including bipolar) or streaking. Among the lactobacilli are as optional as well as strict anaerobes. One of the most important features - the ability to ferment carbohydrates to form lactic acid.

Lactobacilli is constantly grows in a healthy oral cavity. The most frequently isolated are Lactobacillus casei, L. acidophilis, L. fermentum. In connection with the formation of a large amount of lactic acid in the carbohydrates splitting they delay reproduction (they are antagonistic-hundred) other microbes: Staphylococcus, Escherichia coli and others. At the same time lowering pH in the oral cavity is one of the factors that lead to the caries development.

Bacteria have the form of sticks from the long and thin to short type of coccobacillus. Often form chains. As a rule, are immobile, rare to meet mobile (peritrichia). Some strains are found grainy, including bipolar or banding when stained with Gram or methylene blue. They haven enzyme metabolism, but can grow in air, and some are strict anaerobes. One of the most important properties - the ability to ferment sugar to form lactic acid, lactate, but not fermented. Additional fermentation products - acetic acid, succinic acid, $CO_2$ and ethanol. Gelatin is not liquefied, indole and hydrogen sulfide are not formed, katalase-negative. Nitrate is reduced rarely and only at pH above 6.0. Rarely form a pigment from yellow or orange to rust or brick red. Have complex nutritional needs specific to each species. Acidophilic - optimum pH 5,5-5,8. Usually grow at pH 5.0 and below. Found in various dairy products, water, sewage, beer and wine at the fruits and fruit juices, in salt, marinades, leaven dough. Are the oral cavity inhabitants, gastrointestinal tract and vagina of many warm-blooded animals and humans.

Lactobacillus casei. - short or long sticks with a diameter less than 1.5 microns, often have flattened ends and form a chain, are fixed. Colonies on solid

medium are smooth, have the shape of lentils, or diamond, whitish or pale yellow. Growth in broth is accompanied by haze. Usually enzyme sorbits maltose and sucrose are often ferments more slowly, glycogen and starch are not split. The main product of fermentation - lactic acid. Ribose ferments with the formation of lactic and acetic acid without gas. For the growth needs of riboflavin, folic acid, calcium pantothenate and niacin.

The genus Corynebacterium submitted by S. pseudodiphtheriae (diphtheroid morphologically identical tinctorial and S. diptheriae). Differ from pathogenic corynebacteria diphtheria arrangement of cells in parallel, biochemical and antigenic properties, do not produce toxins that grow on ordinary nutrient media.

A characteristic feature of corynebacteria is their ability to reduce the redox-potential, creating conditions for the anaerobes life.

In the mouth there are Haemophulus influenzae, H. parainfluenzae. This is a small rod-shaped Gram-negative bacteria without controversy, concerning the mechanism in organic form is sometimes a delicate capsule.

Curved bacteria. Vibrio buccalis gram-negative bacteria, morphologically correspond to the characteristic of Vibrionaceae family members.

In the mouth there detected Mycoplasma - Mycoplasma orale, M.salivarium. These are gram-negative, highly polymorphic microorganisms (cocci and from grains to filamentous forms). They do not have a cell wall that determines the polymorphism. Have a three-layer membrane, sometimes – capsule-like layer.

Actinomycetes - a small gram-positive filamentous, often branched microorganisms. In the oral cavity are generally available: Actinomyces viscosus, A. odontolyticus, A. bovis. Are predominantly in the plaque due to adhesion to enamel and aggregation with other microorganisms. Constitute the stroma of tartar. It also turns on the oral mucosa, in cavities, pathological gingival pockets, in the straits of salivary glands.

### 3.3.2. Anaerobic microorganisms

Microflora of the oral cavity is rich for anaerobic organisms, which represent different taxonomic groups.

#### Anaerobic cocci

**Peptostreptococcus**. This genus includes five species: P.anaerobius, P.productus, P.lanceolatus, P.micros and P.parvulus. Peptostreptococci - gram-positive, have the form of spherical or oval cells with a diameter 0,7-1,0 mm; sometimes - 0,3-0,5 mm. Arranged in pairs in the form of short or long chains, the flagella are not, the dispute is not, strictly anaerobic bacteria. Ferment carbohydrates to form acid or gas or both. Some species form a gas in peptone water with no carbohydrates. The fermentation of carbohydrate and peptone form, either individually or in combinations of acetic, formic, propionic, butyric, isobu-

tyric, valeric, nylon, succinic acid. Nitrates are not reduced, do not have catalase, typically do not form indole, gelatin is not dissolving. Rarely have hemolytic properties. Do not form a lecithinase, lipase. Can be isolated also from normal and infected female genital tract, the respiratory tract and intestines of healthy humans and animals from septic wounds, and appendicitis, as well as from the oral cavity with purulent infections, maxillofacial area abscesses, periodontitis, pulpitis, dental caries. Frequently there are encountered peptostreptococci in association with Fuso-bacteria and spirochetes.

**Veillonella**. The genus Veillonella (family Veillonellaceae) are gram-negative small (0,3-0,5 mm in diameter), fixed, do not form spores anaerobic cocci. In light microscopy they appear as diplococci, clusters of cells or in short chains. Carbohydrates and polyols are not fermented. Gelatin is not liquefied, indole is not formed, did not possess hemolytic properties, restore nitrate to nitrite to form hydrogen sulfide from sulfur-containing amino acids. Of the lactate formed acetate, propionate, $CO_2$ and H2. To separate cultures using agar medium containing lactate and vancomycin (7.5 mg / ml) to which veyllonella are resistant at a concentration of 500 mg / ml. Colonies on solid medium, small (1-3 mm), smooth, lenticular. A typical representative is V. alcalescens.

Veylonella important feature is the ability to break down lactic acid, which formed as a result of the enzymatic activity of others (including - cariogenic) bacteria. Due to this, they show anti-caries action. Veylonella colonize oral mucosa, a large number of them contained in the saliva.

**Anaerobic rod-shaped bacteria.**

Most anaerobic rod-shaped microorganisms of oral cavity belong to the family of Bacteroidaceae. That representatives of the Bacteroides, Prevotella, Fusobacterium, Leptotrichia. They are all Gram-negative, non-sporic. Different enzymatic characteristics, the ability to form black pigment, nutritional needs and the ability of growth in the presence of 20% bile.

Bacteroides genus includes more than 45 species. Of them, B. fragilis. B. ureolyticum, B. thetaiotaomieron most often cause disease in humans.

This gram-negative, do not form spores, mobile (peritrihia) or sticks, strict anaerobes. The main difference from the genus Bacteroides of fuzobacteria lies in the fact that they form glucose and peptone as one of the main butyric acid products (without isobutyric and isovaleric acids). In the metabolism of hydrocarbons transitions or peptone fermentation products, except for butyric acid, are also a combination of succinic, lactic, acetic, formic and propionic acids. Catalase, lecithinase and lipase are usually not formed. Reproduction is stimulated by hemin and vitamin K, do not form pigments. Bacteroides antigens differ by variability and hardly used for their identification and differentiation.

Bacteroids belong to conditionally pathogenic bacteria. Type species - B. fragilis is the most numerous form of human colon, sometimes taking up to 99%

of the microflora. In immune-deficient individuals is involved in the occurrence of chronic inflammatory processes in association with aerobic bacteria. Virulence factors are the capsule, outer membrane proteins that are involved in adhesion. Capsular polysaccharide as a factor of aggression, protect the bacteria from phagocytosis. However, the above mentioned types of Bacteroides produce a number of enzymes: neuraminidase, fibrinolysin, heparinase involved in invasion, as well as products of metabolism - fatty acids with short chain, biogenic amines, violating the functional activity of macrophages and leukocytes. LPS is involved in suppressing the phagocytic cells activity of. These species are found in Bacteroides peritonitis, abdominal abscess in the cavity, lung, and chronic inflammatory processes at other sites.

Bacteroids are predominant inhabitants of human colon. In the mouth there is the most frequent B. cappillosus.

Diagnosis is made by bacteriological examination in order to isolate a pure culture and its subsequent identification, for treatment there used broad-spectrum antibiotics.

The genus Prevotella species includes P. melaninogenicus, P.oralis, P.oris. and P. intemedius. This polymorphic fixed asporogenic Gram-negative rod-shaped bacteria. Close to the bacteroids (previously they were not distinguished). Form a capsule. Have moderate saccharolytic properties. P. melaninogenicus form pigments of black or dark brown.

Virulence factors are the same as that of bacteroids. In addition, they produce a protease that breaks down the IgA, and form a very toxic LPS, which can cause septic shock. Occur in the lung abscess, pleurisy and periodontitis.

Ecological niche - the mouth and upper respiratory tract. For treatment are used modern penicillins.

P. melaninogenicus is isolated from the oral cavity, feces, soft tissue, genitourinary system, and usually only in the pathogenic microbial associations. Hallmarks of P. melaninogenicus: form a black pigment on agar varnished with blood. Colonies on this medium after 2-3 days incubation, gray, or brown, or black with a diameter of 0,5-3,0 mm, lightening with longer incubation (5-14 days). Broth with glucose during the growth form diffuse turbidity and sediment. Most strains needs hemin for the growth (1mkg/ml)

According to the degree of enzymatic activity P.melaninogenicus is divided into three subspecies: strongly fermenting, slightly fermented and non-fermentative. Subtypes differ in saccharolytic, proteolytic activity and acid formation from glucose:

1. Strongly saccharolytic, non-proteolitic P.melaninogenicus subsp. melaninogenicus - from glucose to form acids, no proteolytic properties.

2. Moderately saccharolytic, moderately proteolytic P. Melaninogenicus sub sp. intermedius.

3. Not fermenting glucose P. Melaninogenicus subsp. asaccharolyticus.

P.oralis isolated from periodontal pockets in humans and infections in the mouth cavity, upper respiratory and genital tract, and usually only in pathogenic microbial associations. Hallmarks of P.oralis: black pigment does not form; ferments carbohydrates intensively, one of the main fermentation products - succinic acid. Hemin for growth is not required. Does not form arabinose acid. Long and thin cells are not formed. Colonies on solid medium after two days round, smooth, convex, translucent, with a diameter 0,5-2,0 mm. Hemolytic properties, as a rule, does not possess. On sugar broth growth is accompanied by haze, no precipitate or the formation of sludge. Decarboxylases of lysine, ornithine, arginine and glutamic acid, as well as urease and hydrogen sulfide are not formed.

P.oris is isolated from periodontal pockets, as well as from blood, abscesses in the face, neck, and wound infection. Antibiotic treatment is carried out taking into account antibioticogram, good results are obtained with a combination of antibiotics with metronidasol.

The genus Fusobacterium includes 16 species, among which for Medical Microbiology greatest interest F. plauti, F. nucleatum and F. necroforum. Fuzobacteria are localized in gingival pockets in association with the spirochaete and are found in the oral cavity of people in 100% of cases.

Fuzobacteria - polymorphic, mostly spindle-shaped, gram-negative bacillus. In pure culture can be met filiforms to 80-100 microns, and branching forms. Obligate anaerobes. Spores and capsules are not formed. Hemoorganotrophes, possess saccharolytic properties, proteolytic activity is weakly expressed. The main product of fermentation - butyric acid, which is produced in large quantities.

Fuzobakteria enter the body through the oral mucosa. At the site of entry forms there is an ulcer. Released large quantities of butyric acid inhibits phagocytosis. Fracture fuzobakteria released endotoxin (LPS). F.necroforum produces exotoxin, causing hemolysis and damaging leukocytes. Apparently it pertains to membranotoxins. In conjunction with the spirochaete is Vincent's disease, is characterized by necrotic tonsils.

Fuzobakteria occur mainly in the sulcus of oral cavity, intestines and genital tract, also identified from the blood and purulent lesions of various organs, as well as from trophic ulcers.

Laboratory diagnosis is carried out by bacteriological tests, difficulties arise in identifying fuzobakteria and bacteroids and other bacteria differentiation.

For treatment using phosphomycin, which fuzobakteria very sensitive, as well as combine antibiotics with metronidazole (metrogilom).

**Leptotrihii**, members of the genus Leptotrichia - have the kind of straight or slightly curved Gram-negative rods with a diameter 1,0-1,5 mm and a length 5,0-15,0 mm, with one or two rounded or, more likely, pointed ends. Two or more cells are combined in septate, filaments of different lengths, which in old cultures reach a length of 200 m and more intertwined with each other. On lysis of cells in the filaments appear spherical or onion-round swellings. Do not form branching and clavate cells. Immobile. Strict anaerobes. Heterotrophs with complex nutri-

tional needs. Glucose is fermented with the formation of acid without gas. The main products of fermentation - lactic and acetic acid, butyric acid is not formed. Gelatin is not liquefied, nitrates usually do not recover. Indole, $H_2S$ and catalase are not formed. Habitat - human oral cavity.

Leptotrichia buccalis - a typical species of the genus. Distinguishing marks: in the column of agar form lobed, convoluted, meandering colonies that resemble the head of Medusa, on medium containing crystal violet, colonies have an iridescent look. Surface colonies consistency ranges from buttery to fragile.

L.buccalis grows better in an atmosphere containing 5% $CO_2$, and medium supplemented with serum, ascites liquid, or starch. Growth in the depth of the agar column usually starts at 1 cm below the surface. Growth does not occur in the absence of 2% NaCl. It grows well in medium containing 0.001% crystal violet or 10mkg/ml streptomycin. Ferments glucose, fructose, maltose, mannose, and sucrose with the formation of acid without gas. Usually ferments as salicin, trehalose, galactose, lactose, raffinose and starch. Leptotrihia are a part of the dental plaque, pigmented spots of enamel and carious teeth dentin tubules. L. buccalis - centre for the deposition of plaque and tartar. Proved their involvement in the development of caries due to significant acid formation, and L. buccalis is a synergist of lactobacilli and is involved in tissues demineralization. Together with the actinomycetes leptotrihia make up the bulk of the dental plaque organic matrix.

**Bifidobacteria (Bifidobacterium bifidum)** - these are Gram-positive, obligate anaerobic bacteria of the Actinomycetaceae family. Differ by pronounced polymorphism. Fresh bacteria have the form of straight or slightly curved rods, which are chains. Cells often can be either branching or thickened at the ends. Important enzymatic properties - the splitting of carbohydrates to lactic and acetic acids. Found in the mouth and gastrointestinal tract of most people.

**Curved-shape anaerobes**. Campylobacter - types of Campylobacter concisus, C.sputorum considered as a commensal of oral cavity. Perhaps they play a role in the pathogenesis of periodontitis. Campylobacter - Gram-negative, slender, spirally curved rods, have one or more turns of the spiral. When connecting two cells in the chain resemble the wings of seagulls. Polymorphic, spores and capsules do not form. Microaerophilic. Carbohydrates are not fermented.

**Family Spirochaetaceae**. Since the eruption of deciduous teeth in a child they are permanent members of the oral cavity microflora. Typical feautres: filamentous forms, the lack of controversy and capsule, twisting, actively moving through the pronounced flexibility of the body. Represented by the genera Treponema and Vorrelia. Treponema oral species are T.denticola (usually appears in the joints of teeth with gums), T.macrodentium and T.orale are identified in the gingival pockets.

Treponema denticola has the form of thin spiral cell length 6,0-16,0 mm and a diameter of 0,10-0,25 mm. The ends of the cells are slightly bent. Two axial filaments (fibrils) are attached to each end of the cell. Very few of the axial fila-

ment, as it were stretched between the ends of the cells. Mobile. Young cells rapidly rotate around its axis. Grow well on medium with peptone, yeast extract and serum, under anaerobic conditions. Colonies after two weeks of cultivation white, diffuse, the size of 0,3-1,0 mm. Growth is inhibited in the presence of 3% or more NaCl or 1% bile. For growth necessarily requires the addition of animals serum. Adding of cocarboxylase stimulates the growth. Carbohydrates are not enzymated. Hydrolyze starch, glycogen, dextrin, esculin, gelatin, but not hippurate. Most strains form indole and $H_2S$. Detected in the oral cavity of humans and chimpanzees, usually at the attachment of teeth with gums.

Treponema orale - thin spiral cell length of 6-16 mm and diameter 0,10-0,25 mm. Usually form chains. One of the axial filament is attached to each end of the cell. In broth culture of the late cells are often grainy. Have active mobility. Grow on medium containing peptone and yeast extract, as well as on the medium used for cultivation of mycoplasmas without crystal violet. Each of these media contains glucose, cysteine, nicotinamide, cocarboxilase, tetrahydrochlorid spermine, sodium isobutyrate, and 10% inactivated rabbit serum. In liquid medium gives a uniform turbidity. Carbohydrates are not fermented, but amino acids do, forms indole and $H_2S$. Hydrolyze gelatin, but not starch. Found in periodontal pockets in humans.

Treponema macrodentium - thin spiral cell length of 5-16 microns and a diameter of 0.10 -0.25 mm. The ends of the cells are sharpened. One of the axial filament is attached to each end of the cell. Highly mobile, young cells have a rapid rotary motion. Grow on medium containing peptone, yeast extract, 10% serum or ascites, cocarboxilase, glucose and cysteine. For growth it necessarily requires the addition of animal serum, which can be replaced by isobutyrate, spermine and nicotinamide. Carbohydrates are fermented and used as energy sources. Ferments form acid without gas, glucose, fructose, maltose, xylose, sucrose, galactose and ribose. Do not ferments mannose, rhamnose, lactose, arabinose, mannitol, inulin, sorbitol and salicin. Does not hydrolyze starch, but hydrolyzes gelatin. Indole is not formed, forming hydrogen sulfide. Found in periodontal pockets in humans.

Borrelia buccalis - convoluted cells with 7-20 m and a diameter of 0,3-0,4 mm. This is one of the largest oral spirochetes, sluggishly moving. It has a meandering, weak flexion and rotational mobility. This kind of Borrelia is poorly understood, but it was isolated from the oral cavity of human.

### 3.3.3. Other oral cavity microorganisms.

**Fungi**. On the oral mucosa of the majority of people there are fungi of the genus Candida (C. albicans, C. tropicalis). The intensity of the fungi reproduction depends on the physiological state of microbial associations, relationships, antagonists and synergists in these systems.

**Protozoa**. In the mouth of 50% healthy people can vegetate the simplest, namely: Entamoeba gingivalis, Trichomonas elongata, T.tenax. Found predominantly in the plaque, tonsils crypts, periodontal pockets purulent content. Trichomonas more than an amoeba, are in the healthy people oral cavity.

Increasing the number of protozoa in the oral cavity is observed due to non-compliance of the oral hygiene rules. In very large quantities, they are identified in gingivitis and periodontitis.

**Viruses**. The constant presence of virus in the oral cavity is a single point of view there. However, the permanent presence of herpes simplex virus, cytomegaly shows the identification of specific antibodies to the mentioned viruses in the blood serum in 80-90% of people.

### 3.4. Temporary oral cavity microflora

Oral cavity, connecting with the external environment, is a gateway for pathogenic, opportunistic and saprophytic microorganisms. These organisms are temporary (casual, transit) microflora.

The composition of aerobes and facultative anaerobes random oral microflora includes bacteria: gram-negative rod of the genera Escherichia, Klebsiella, Aerobacter, Proteus, Pseudomonas, Gram-positive bacilli (Bacillus subtilis), as well as tetracocci.

Obligate anaerobic bacteria that are transiently located in the mouth, this gram-positive spore-forming bacillus Clostridium putrificum and S. perfringes.

## 4. Microbial colonization in oral cavity

### 4.1. The oral microflora role in the human body

The most studied is the role of microflora in the human colon, because it is the most numerous (in its composition has up to 500 species, 40% of the mass of faeces are living and the dead mikroorganisms). But the second highest number of species in the human body is oral microflora. Oral mucosa is always "dirty" by a large number of microorganisms that are stepping in air, food, water, etc. Despite this, the composition of oral cavity microflora in healthy person is fairly constant and it is evidenced the importance of its role for the human body as a whole and for the functioning of the digestive system in particular.

You can select multiple areas of positive impact of oral cavity microflora on the life of the human body.

1. Stimulation of nonspecific resistance factors that act in the oral cavity:

- Stimulation of oral cavity mucous membranes physiological inflammation;
- Stimulation of macrophage activity;

- Maintaining the humoral factors level of nonspecific resistance (in particular - lysozyme) in saliva and blood;

- Stimulation of normal antibodies.

2. Antagonism to some pathogenic bacteria.

Suppression of some other species is due to higher biological potential (proliferation rate, the rate of uses of food substrates), resulting in production of toxic metabolites ($H_2O_2$, spirits, dairy products and fatty acids) by the synthesis and release into the environment of exogenous control of interspecies - bakteriocines.

3. Participate in the processes of digestion.

These processes start already in the mouth. Expressed as a normal microflora representatives enzymatic activity of detoxic nature.

4. Forming the ability to immune reactions.

The monobiontes (microflora -free animal) most characteristic feature is underdeveloped lymphoid tissue. Monobionted that fall within the normal microbial environment, die of infectious processes caused by such kinds of microorganisms, to which animals are usually not sensitive. This is due to the dependence of lymphoid tissue forming from the antigenic stimulus, especially a large range of antigenic stimuli, which are provided by microflora. Oral cavity - part of the digestive tract mucous membrane, which is the first contact with microbial antigens, that enter the body with food and water. Analysis of antigenic stimuli that come this way, occurs primarily in pharyngeal ring lymph nodes.

Immune responses to antigens are not only local, though they are important for the protection of the oral mucosa (mainly due to secretory immunoglobulin A - sIgA). In forming an answer there are involved the immune system organic mechanism in general and in response to a certain extent, manifested at the level of organic mechanism.

It should be noted also the oral cavity normal microflora negative impact on the life of the human body.

First, the vast majority of its members under certain conditions can cause self-infection. Most typical in this respect are fungi of the genus Candida - they cause candidiasis, which is characterized by lesions of the oral mucosa; S. mutans, which is involved in the formation of dental plaque and in the following lesions. If you violate the integrity of the cavity mucous of the mouth, jaws, as a result of trauma or surgeries you may have acute purulent-inflammatory processes caused by microorganisms, which were in the oral cavity.

Secondly, it is halitosis (from lat. Halitus - breath, osis - an unhealthy state) - an unpleasant smell from the mouth. He is one indicator of the bad health indicators.

The reason for halitosis may be lesions of respiratory tract different parts by infection or digestive system, but it is believed that in most cases it is caused by disruption of oral cavity normal microflora: an increase in the bacteria number that are involved in decay, accompanied by the hydrogen sulphide, mercaptans

and other compounds formation which have an unpleasant odor. It is believed that in the emergence of halitosis are involved obligate anaerobic bacteria Bacteroides melaninogenicus, Fusobacterium nucleatum, Peptostreptococcus anaerobius, Peptostreptococcus productus, Peptostreptococcus lanceolatus, Veilonella alcalescens and facultative anaerobe Klebsiella pneumoniae.

Third, the normal microflora - the source of the horizontal genetic information propagation in the world of microorganisms, which ensures rapid drug resistance development. If the first bacteria "learned" to defend against certain types of antibiotics, but today we are talking about multiple resistance to drugs, which is controlled by R-plasmids or R-factors, which can be transmitted not only one bacteria form as a result of conjugation, and bacteria of other species by other recombination types (transformation, transduction).

Some studies suggest a carcinogenic homogeneity of certain metabolic microorganisms products.

Chromogenic bacteria studies are small, but bacteria chromogenic properties, i.e. the ability to synthesize pigments, lead to the pigment spots formation in the cavities.

Acidophilic bacteria is an oral cavity bacteria group that can grow in media with acidic pH, for example, in medium supplemented with tomato juice. Often acidophilic bacteria have also acidogenic, i.e. the ability to ferment carbohydrates to form acidic products of organic and inorganic acids (lactic acid, $CO_2$, etc.). This is primarily lactobacillus, yeasts, staphylococci and some streptococci. In the caries process absence, this group of bacteria is 0.05% of the total number of microorganisms. Due to the dental caries conditions of their number increases by 8-10 times. Reduction of the same oral fluid pH to values lower than the critical (less than 6,0-6,2) leads to the appearance of her demineralizating properties: it becomes capable to dissolve calcium and phosphate forms, which are part of the tooth enamel .

Bacteria capable of splitting proteins (exhibit proteolytic activity), can destroy enamel and dentin protein (it is considered that bacteria proteolytic action is not a leading factor in the development of caries).

### 4.2. Microbial colonization of different oral cavity parts

To colonize the human oral cavity microorganisms must attach to the mucosal surface or the tooth. The first stage of adhesion occurs efficiently in bacteria with increased hydrophobicity. In particular, oral streptococci are adsorbed on the surface of the teeth and on the epithelial cells of the mucosa. Significant role in the adhesion process is played by fimbriae, available in many oral cavity microorganisms. Features of the structure of adhesins largely determine the microbes localization in the oral cavity. So, Streptococcus sanguis is firmly fixed on the tooth surface, and Streptococcus salivarium - on the surface of the mucous membrane epithelial cells.

Attaching the bacteria to the tooth surface occurs very quickly. Many of the microbial cells are not able to bind directly to the tooth enamel, but may settle on the surface of other bacteria have adhered to form a connection like "cell to cell." Subsidence of cocci along the perimeter of filamentous bacteria leads to the so-called "corn cob" formation. The microbial associations formation in different oral cavity areas is determined by the biological characteristics of species that live here, among which there are both synergistic and antagonistic relationship. For example, lactic acid, formed from the metabolism of oral streptococci and lactobacilli, used as an energy resource veyllonellami, which leads to an increase in environment pH and may provide anti-cariogenic action. Corynebacterium form vitamin K - a growth factor of many other bacteria, and yeast genus Candida are able to synthesize vitamins needed for growth of lactobacilli. Latest in the process of exchange of producing lactic acid, which are acidified environment, hinders the adhesion and colonization of yeast, which in turn reduces the amount of vitamins needed for many organisms, and delay their growth.

Oral streptococci are antagonists of fuzobacteria, Corynebacterium, etc. This antagonism is associated with the formation of lactic acid, hydrogen peroxide, bacteriocins. Lactic acid is formed by oral streptococci, inhibits the growth of many microorganisms, thereby contributing to the proliferation of lactobacilli. Corynebacterium, reducing the value of the redox potential and create conditions for the growth of facultative and strict anaerobes under aerobic conditions. In periodontal pockets, the folds of mucosal crypts of the oxygen level is much reduced. This creates favorable conditions for the development of strict anaerobes - fuzobacteria, Bacteroides, leptotrichia, spirochetes. 1 ml of saliva may contain up to 100 million anaerobic microorganisms.

Stated determine differences of microflora in different parts of the mouth - Figure 3.2.

Besides saliva, bacteria are locared mainly in three areas:

1) in dental plaque on tooth crowns and the presence of caries – in a cavity;

2) in the gingival sulcus;

3) on the back of the tongue, especially in his back parts.

The number of bacteria in saliva is an average of 750 million in 1 ml. The plaque and gingival sulcus - nearly 100 times higher - about 200 billion cells per 1 g of sample.

On those parts of teeth that are close to the mucosa and on the chewing surfaces of microbes is greater thanon the dental surface free sites. The number of microorganisms is greatly increased if the teeth are covered by stone. Germs are not only on the surface of the teeth, but can also penetrate into dentinal canal. On smooth and shiny surfaces of the mucous membrane are mainly gram-positive cocci (diplococci, streptococci) and veylonella.

Most bacteria are permanent flora in the natural folds of the oral mucosa, gingival pockets, interdental gaps.

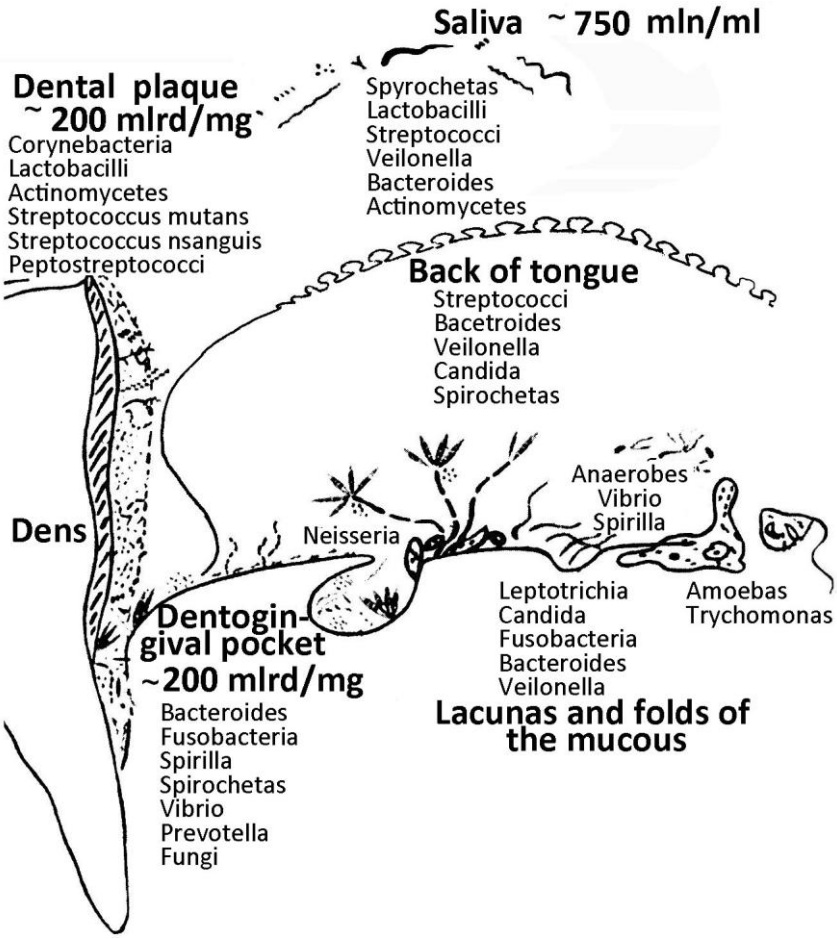



Figure 3.2. Microflora in different parts of the mouth

Spirochetes, Prevotella melaninogenicus are typical for gingival gap, where their number reaches 1 - 5%. Are here as diphtheroids, fuzobacteria, vibrios. It turns out these bacteria in plaque.

Quantitative and qualitative composition of microflora in the oral cavity as a whole and its individual parts in particular, is determined not only by physical and chemical conditions. Have the receptor interaction of shells of a microorganism with a definite structure of the surface mucosa and dental enamel. This is the basis of the so-called "colonization" (settlement), the essence of which lies in the fact that representatives of the microflora of the breed for the most part throughout the volume of the oral cavity and on the surface of certain structures, where

they are attached (this phenomenon is called "adhesion") . Adhesion may be due to the synthesis of microbial extracellular polymers such as dextran, hyaluronic acid. Some bacteria can carry out the adhesion of polymers with saliva, such as mucin. Some organisms have specialized structures on the surface - they drank, they are able to specifically interact with specific structures in the company, that is, the interaction is the receptor nature. Organisms that are not factors of adhesion, can linger in the mouth mechanically, it is possible that not all the existing mechanisms of adhesion of microorganisms are known today and they will still be investigated in the future. The ability of microorganisms to attach to the surface in different parts of the oral cavity is one factor that determines its ecological niche.

Reproduction leads to the formation of clusters - the colonies. Thus, numerous bacteria from the normal microflora of the mouth form a film that shields the sensitive surface structures from the invasion of pathogenic bacteria. This protection is not just mechanical. Antagonism of the normal microflora is also implemented at the level of interspecies fight. This phenomenon is called "colonization resistance".

On quantitative and qualitative composition of oral microflora is largely affected by the composition of food: an increased amount of sucrose leads to an increase in the proportion of streptococci and lactobacilli, while glucose has no such action. The collapse of food contributes to the accumulation of saliva and gingival fluid of carbohydrates, amino acids, vitamins and other substances used by microorganisms as a nutrient substrate. However, organisms do not disappear from the mouth, even when feeding a person through a tube. The composition of the oral cavity microflora and other habitats largely affects the immune, endocrine, nervous and other systems, the use of certain medications, particularly antibiotics, which violate the stability of the oral cavity microflora. A role in altering the composition of microbial associations is oral hygiene.

### 4.3. Microflora of plaque

Plaque, or plaque - a cluster of bacteria in the matrix of organic substances, mainly proteins and polysaccharides, an offering there and saliva produced by the microorganisms. Their relationships within the plaques are complex and are both in bacterial commensalism and competition and antagonism, which play an important role in determining the composition and properties of plaque. Distinguish between supra-and subgingival plaque. The former have pathogenetic importance in the development of dental caries, the second - during the development of pathological processes in the periodontium. Plaque formation is a polymicrobial, as it revealed more than 60 species of microorganisms, and 1 mg of plaque can contain more than 800 million microorganisms.

All of them are divided into 2 groups: acidophilic microorganisms capable to develop in an acidic environment, and proteolytic microorganisms that produce proteases.

The first group includes the lactic acid streptococci, lactobacilli, actinomycetes, leptotrihia and Corynebacterium. Among acidophilic bacteria there are acidogenic ones, able to synthesize sucrose from a large number of lactic acid.

The presence of streptococci in the oral cavity was shown by Miller in 1898. - and γ-hemolytic streptococci, and rarely - β-form. Later they were found in plaque. Guggenheim (1968) divided the plaque streptococci into 4 groups: Streptococcus salivarius, S. mitis, S. sanguis, S. mutans.

S.salivarius is easily defined morphologically in the form of colonies formed on meat-peptone gelatin containing 5% sucrose. Their diameter can be 5 mm. These streptococci are found in dental plaque in small amounts, but they are pretty much on the mucous membranes and saliva.

**Table. 4.1. Microflora percentage content**

**Five-day plaque content**

| **The bacteria name** | **Bacteria, %** | |
|---|---|---|
| | **Non-caries teeth** | **Caries teeth** |
| **Acidophilic bacteria** | | |
| Streptococcus | 45 | 55 |
| Actinomyces | 5 | 7 |
| Lactobacillus | 4 | 12 |
| Leptotrichia | 2 | 7 |
| Corynebacterium | 6 | 2 |
| Totally: | **62** | **83** |
| **Proteolotic bacteria** | | |
| Peptostreptococcus | 3 | 3 |
| Ristella | 7 | 6 |
| Fusobacterium | 5 | 5 |
| Vibrio | 6 | 2 |
| Veillonella, | 10 | 1 |
| Neisseria | 5 | 0 |
| Ramibacterium, | 2 | 0 |
| Catenabacterium | | |
| Spirochetes | | |
| Total: | **38** | **17** |

S. mitis make up the bulk of streptococci isolated from dental plaque. They are very heterogeneous and have low biochemical activity. Only certain strains of S. mitis are able to synthesize extracellular polysaccharides.

S. sanguis ranks second on the quantitative content in the plaque. According to its biochemical activity it exceeds S.mitis. Among S.sanguis encountered quite a lot of strains possessing cariogenic activity.

Among acidophilic bacteria of dental plaque are a large number of filamentous forms (actinomyces, lactobacillus, leptotrihi). Actinomycetes form levan, lactobacilli do not form extracellular polysaccharides, except for Lactobacillus casei, which may form some of the capsular polysaccharides; leptotrihia do not form polysaccharides.

The second group of plaque bacteria are anaerobic, which possess proteolytic properties and use the food proteins and amino acids. Number of anaerobes in dental plaque is reduced on using sucrose and increases in the use of maltose. All anaerobes can degrade collagen and cause periodontal diseases. This group includes peptostreptococci, ristelly, fuzobacteria, vibrios, veylonella, Neisseria, and remibacteria katenobacteria, as well as spirochetes.

In addition to the mentioned microorganisms in dental plaque were found, and other kinds of microorganisms, particularly yeast-like fungi, diphtheroids, staphylococci.

With the immunofluorescence method revealed Nockardia that a large number were in the one-day dental plaque. Nockardia are aerobes. They can play an important role in initiating the dental plaque formation. Furthermore Nockardia, at an early stage of dental plaque formation in it, there are Neisseria. Unlike streptococci Neisseria inherent slow growth. In the plaque in 86% of cases occur N. sicca, producing polysaccharides, and N. subflava, which does not produce them. In the rapidly developing plaque is dominated by N. sicca, with its slow development in most cases, reveals N. subflava.

At all stages of development of dental plaque streptococci predominate in it. In the early stages of dental plaque development streptococci are found in association with aerobic and facultative anaerobic cocci and short rods. At later stages of plaque, this association is preserved only in the surface layer, whereas in deeper layers than streptococci reveals a variety of anaerobic microorganisms, many of which have a strand-like.

### 4.4. The plaque formation mechanism

Mandatory condition for the plaque formation is the presence of oral cavity microorganisms. It is experimentally proved that gnotobiotic (amicrobic) beings never forme plaque and tartar. Saliva is involved in the dental plaque formation. This is influenced by some features of the surface.

Stage I - the formation of cell-free organic film on the surface of the tooth enamel. This film different authors call or acquired cuticle, or pellicle. The film thickness ranges from 1 to 10 microns. It is formed quite rapidly during contact with saliva from the tooth surface. Usually, the formation of the pellicle takes several minutes to several hours. The chemical composition of pellicle is a glycoprotein complex. The 65% of total amount of protein-binded carbohydrates are hexoses. As part of pellicle there also found ketosacchar and uronic acid. However, the pellicle of glycoproteins contain almost no sialic acid and fructose, although a significant amount of both carbohydrates found in saliva glycoproteins.

Pellicle formation mechanism is not fully understood. Some authors believe that pelicle is formed by spontaneous deposition of saliva proteins, which is greatly enhanced by acidification, as well as the presence of calcium ions and phosphates. These ions can help to precipitate proteins from solution and to enhance the adhesion of deposited protein to the surface of hydroxyapathite.

Calcium ions are involved in the early phase of bacterial attachment to the enamel organic shell, but did not affect the microorganisms binding to each other during the dental plaque formation.

In the saliva there found macromolecular glycoproteins that have the ability to agglutinate bacteria. Such agglutinins are part of the tooth pellicle and may determine the initial adhesion of certain oral microorganisms on tooth surfaces. Agglutinins exhibit their activity only in the presence of calcium ions. The total content of agglutinins in the saliva is about 1% of the total protein concentration.

Stage II plaque formation begins in a few minutes after the pellicle formation and consists of nearly simultaneous adsorption on the surface of the pellicle proteins, microorganisms, and epithelial cells.

Glycoproteins, which are precipitated from the saliva, are the first important component of the matrix of the dental plaque future. The second component of the matrix polysaccharides are sticky type of dextran, which are produced by certain streptococci strains. These two components - a chemically modified glycoproteins of saliva and extracellular polysaccharides - form the basic medium (matrix), dental plaque, which is colonized by oral microorganisms.

In the early stages of plaque major extracellular component is coccal microflora, which was later replaced by a rod-and thread-like forms. In addition to microorganisms, tooth-dimensional plaque meet the epithelial cells, which, after adsorption on the surface of the pellicle can be expanded. One of the factors contributing to the bacteria deposition on the pellicle surface, is the presence of saliva proteins, which cause microorganisms agglutination. According to Peret, organizing the necessary elements of soft plaque are the microbial enzymes. The author divides them into two groups depending on the interaction mechanism with components of dental plaque. The first group consists of hydrolases, in particular, the neuraminidase and other mucopolysaccharides, which in the presence of saliva glycoproteins and calcium ions stimulate the bacteria agglutination. The second group of enzymes that promote the plaque formation, is represented by

synthetases, in particular, dextransaccharose, which promotes the sucrose formation, dextran, having the sticky properties and thus amplifying microorganisms agglutination.

A few days later, plaque turns into its Stage III - the mature dental plaque stage and is a structurally complex polymicrobial formation thickness of 200 microns. At this stage the plaque is most harmful to tooth enamel, as they are formed by pathogenic factors such as hydrolytic enzymes (protease, hyaluronidase, etc.) and various organic acids (lactic, propionic, acetic and others) may cause the pellicle dissolution, chemical tooth enamel destruction and dissolution. This plaque is removed together with the particles rejected by the enamel. But in cases where a mature plaque are anaerobic conditions, there is a change of microorganisms (the change of aerobes anaerobes), decrease acid production and an increase in pH, calcium accumulation and deposition in the form of phosphate salts, a transition of plaque in his stage IV, which is called tartar.

According to tartar formation theory, plaque microorganisms are capable to produce the saliva components (at the point of stagnation), ammonia, resulting in alkalify environment and precipitate components of tartar.

### 4.5. Changes in the microflora, depending on age, human health and other factors

Before the birth the oral cavity is sterile. Microflora gets there during the passage through the birth of the child path and as a result of eating. Different microorganisms are identified in the study of newborns in the dynamics:

7 hours after birth - 7 species;

24 hours - 12 species;

10 days - 21 species;

12 days constitutes of a normal microflora.

Predominantly aerobic nature of the microflora remains to cutting of the first tooth. In the future there are anaerobic bacteria. Characteristic of the toothless mouths is a decrease in anaerobic flora. The total number of microorganisms increases protezonositeley.

Under normal conditions (do not use antiseptic toothpaste, antibiotics and other drugs), the species composition of oral cavity microbial flora rather constant, but the number of microbes can vary considerably.

Number of microbial flora depends on factors such as morphology cavity company, the composition of saliva and the intensity of her formation, diet, hygienic condition of the oral cavity, the presence of somatic diseases and other factors.

Of all the factors that determine the state of the microflora of the mouth, saliva is the principal. The most important factors in this regard is the intensity of its formation, viscosity, content of mineral components, buffering properties, pH, major metabolites, the organic compound (amino acid, polysaccharide, vitamin,

purine and thymidine), anti-bacterial properties (content of lysozyme, secretory antibodies, leukocytes).

Disorders of salivation, chewing and swallowing always lead to an increase in the number of microorganisms in the mouth. Various anomalies and defects that complicate the passage of saliva washing away germs (carious lesions, dental implants, and other non-removable) lead to an increase in the number of microorganisms in the mouth.

Changes in the state of oral microflora and dental diseases caused by pathogens (stomatitis, glossitis, gingivitis, and others) are observed in the pathology of the digestive organs, hematopoietic, endocrine, cardiovascular, renal system.

Microflora composition depends on the food. For example, an excess of carbohydrates in food contributes to acid-breeding fauna, abundance of proteins gives the opposite effect. Application of the large sucrose amount increases the amount of dental plaque and the prevalence of their S. mutans and S. sanguis, yeast-like fungi.

Microorganisms need certain vitamins, because the change of their content is changing the composition of microflora.

Significant impact on the quantity, but to a certain extent and species composition have hygienic activities (eating fruits, mouth rinse after eating, brushing teeth), with non-compliance with the rules of oral hygiene increases dramatically the number of bacteria, particularly anaerobic bacteria and putrefactive bacteria.

The number of microorganisms in the oral cavity varies throughout the day, the leading role played by production of saliva, which is significantly reduced during the night.

The factors that cause changes in the microflora should include the elimination of all lesions, removal of decayed teeth.

Antibiotics also affect the composition of microflora, with each antibiotic effect on certain groups of microbes, resulting in dysbiosis.

Loss of teeth involves reducing the number of dental flora. So, S. mutans and S. sanguis, yeast, lactobacillus, and spirochetes disappear completely or their numbers greatly reduced in a "toothless" period and the number of S. salivarius increases.

During the first two weeks after the prosthesis remains a high streptococci level, while the number of lactobacilli and yeasts significantly reduced. After 3-5 weeks the contents of lactobacilli and yeasts increased and decreased levels of streptococci to the original.

On the composition and quantity of microflora somatic diseases affect. For example, C.albicans is significantly more common in diabetic patients (80%) than in healthy people (50%).

Any form of missing teeth replacement is always accompanied by the introduction into the mouth of a foreign body, which can lead to various complications. Under the prosthesis is almost always an inflammation of the mucous

membrane. Chronic inflammation is observed in all areas and in the prosthetic bed. Contribute to this dysfunction of salivary secretion and irrigation of the mucous membrane with saliva, changes the properties of saliva (pH and ionic composition), a temperature increase of 1-2 ° C on the surface of the mucous membrane, etc.

Given that removable dentures serving mainly elderly people with low immunobiological reactivity and concomitant diseases (hypertension, diabetes, etc.), changes in the composition of oral microflora are completely natural. All this creates conditions for the development of prosthetic stomatitis. As a result of various causes under the dentures, the conditions for the appearance of plaques, similar to sub-and supragingival. They represent an accumulation of microorganisms in an organic matrix, which is also the accumulation of acid, reducing the pH to a critical level of 5.0. This contributes to enhanced proliferation of yeast genus Candida, which plays an important role in the prosthetic stomatitis etiology. They are found in 98% of cases at a location of the prostheses surface. In 68-94% of persons using dentures, candidiasis occurs. Colonization of the oral mucosa yeast-like fungi may cause the "corners" of mouth.

Microorganisms from the oral mucosa can infect the gastrointestinal tract and respiratory tract.

In addition to yeast-like fungi in patients with removable dentures in the oral cavity exhibit a large number of other bacteria: Escherichia coli, staphylococci, enterococci, etc.

## 5. Dental caries microbiology and immunology

### 5.1. The microorganisms' role in dental caries etiology and pathogenesis

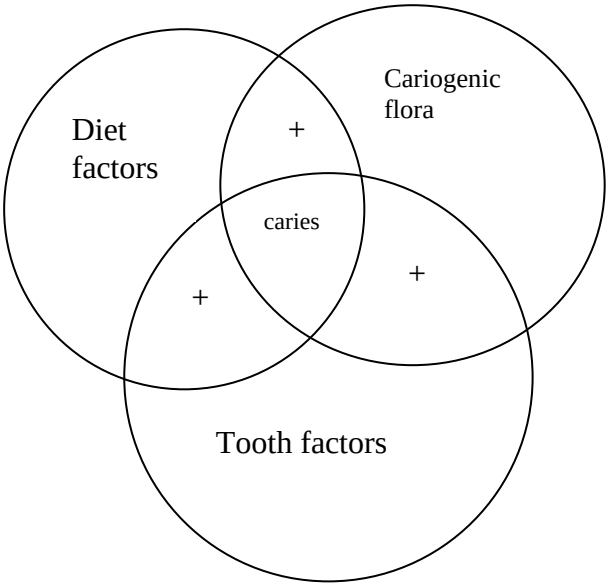
Caries - a pathological process due to which occurs demineralization and softening of dental hard tissues with the subsequent cavities formation. Normally, the tooth enamel is in a state of dynamic equilibrium between the ever-flowing de-and remineralization processes. Demineralization is caused by free hydrogen ions, which are the main source of organic acids - products of oral microorganisms metabolism. The destruction rate of enamel significantly increases with decreasing pH below 5. Great importance for the caries process development has acidic foods contact duration with dental enamel. Caries develops on the tooth surface, which are in prolonged contact with the formed acids. This leads to a gradual increase microspaces between the enamel prisms crystals. In the formed tiniest defects there penetrate microorganisms and damage the enamel on the sites located along the outer and inner surfaces. The long demineralization process is completed by dissolving the sustainable surface layer and formation of a cavity in the tooth.

Dental caries is the most common human disease. Epidemiological surveys indicate a significant distribution of this disease, affecting 80-90% of the population of the globe.

Despite significant differences in the evaluation of the enamel demineralization mechanism in dental caries, given by different authors, it is undeniable that the dental caries etiologic agent are oral microorganisms. Greatest importance in the caries development are oral streptococci S.mutans, S. sanguis, lactobacillus, actinomyces (A. viscosus).

If there are certain cariogenic factors (a significant sucrose amount in the food, the specific proteins presence in the saliva that cause microorganisms agglutination and their adhesion to the enamel surface), these microbes form the enamel plaque on the surface. Waste products of the dental plaque microflora - it's organic acids, chelators (peptides, amino acids) enzymes. The cariogenic factors formation in substantial numbers are stimulated by the carbohydrates presence in the diet that are easy to ferment.

There is a scheme of plaque participation in the dental caries pathogenesis, which takes into account recent data microbiology, physiology and biochemistry of the teeth and oral cavity - Fig. 5.1, 5.2, 5.3.



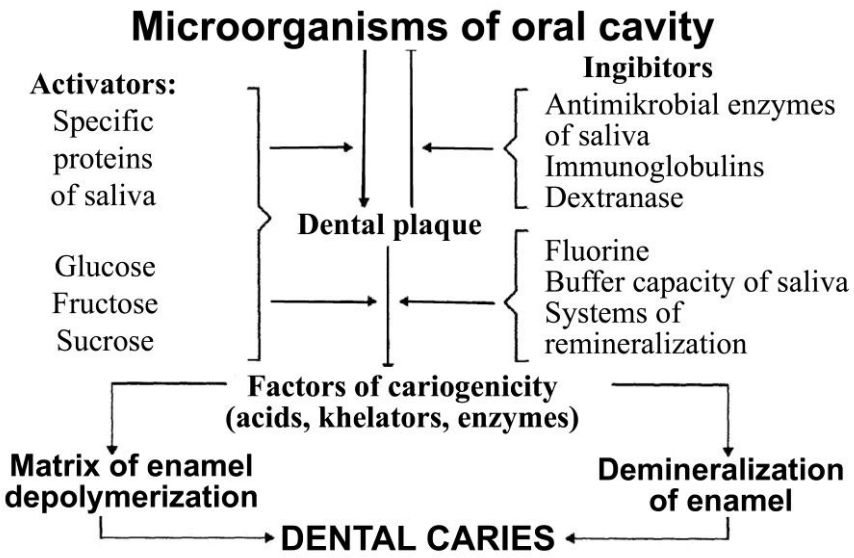



**Fig. 1. Scheme of participation of oral cavity microorganisms in the cariogenesis**

The leading role belongs to the oral cariogenic streptococci of Streptococcus mutans. For the first time S. mutans was isolated by J. Clarke patient caries in 1924. This streptococcus detected in dental plaque, in saliva, in feces and blood. S.mutans differs from other streptococci, the morphology of the colonies, the ability to ferment mannitol, sorbitol, several other biochemical properties (enzyme-induces rhamnose, salicin and inulin, does not form hydrogen peroxide, gives Voges-Proskauers' reaction), the ability of cells to adhere to smooth surfaces with the sucrose and antigenic properties presence. Study of antigenic structure revealed eight serotypes of S. mutans: a, b, c, d, e, f, g, h. In human dental plaque most often occurs with serovar. Described and other types of cariogenic streptococci.

Pathogenicity S.mutans relates primarily to its ability to bind to the smooth surface of the teeth and form cariogenic plaque. This property is due to the polymers synthesis from sucrose, which is present in food. In S. mutans detected glucoziltransferase enzyme that converts sucrose into glucan polymer (dextran), contributing to streptococci attachment to the tooth surface and the dental plaque formation. In addition to glucan S. mutans synthesizes fructanes from sucrose

using a special enzyme fructoziltransferase. Fructanes as glucans are involved in the dental plaque formation.

Under the cariogenic factors influence in tooth enamel demineralization processes there occured depolymerization, resulting in an irreversible lesions.

The process of dental caries development at all stages of the deterrent effect caries inhibiting factors, which include: saliva antimicrobial system, the micronutrient presence, especially fluorine, saliva high buffering properties can neutralize acids formed during fermentation, the proteolytic enzymes inhibitors presence, intensity of the enamel remineralization and its high caries resistance, increasing pulp trophic function and the increasing organism resistance. One fairly effective caries inhibiting factors is oral hygiene.

As shown by experimental studies on gnotobiotic animals for the development of caries optional feature is the microorganisms presence. The per os streptococci introduction in sterile animals leads to the typical teeth lesions. But not all streptococci are equally possess the ability to form plaque. Shown that only some strains of these organisms have an increased ability to form plaque and cause tooth loss. Main biochemical difference between cariogenic streptococci is their ability to synthesize large amount of gelatinous extracellular polysaccharides, particularly dextran, as well as the ability to ferment sucrose and other carbohydrates, to form lactic acid.

Cariogenic properties are all strains of S. mutans. Cariogenic S. sanguis is very high. S. salivarius did not cause lesions.

In the dental caries pathogenesis the crucial role played by carbohydrates fermentation by the oral cavity bacteria with the acids formation, the latter dissolved calcium phosphate enamel or dentin, which leads to cavities.

Formation of different acids in plaque associated with certain microorganisms kinds. Streptococci, which are the most powerful acid-forming plaque to form almost exclusively lactic acid, lactobacilli, along with the lactic acid can form a propionic, acetic and butyric acid, Neisseria and veylonella convert lactic acid into propionic and acetic acid, and carbon dioxide and water - in the pyruvic and acetic acid.

Lactic acid, except streptococci is formed by Corynebacteria, actinomycetes and leptotrihia. Certain that the dental plaque cariogenic properties are associated with lactic acid that can dissolve the calcium, which is part of the saliva or tooth, even in periods when the pH rises above the critical value.

In the plaque there revealed more than 50 different enzymes, many of which are destructive to tooth tissue. Of these enzymes, the greatest attention is drawn to proteolytic, produced by various microorganisms. Studies Gottlieb allowed to come to conclusions, that the primary stage of carious lesions development is the microorganisms' proteolytic enzymes effect, resulting in broken lamellae, organic shell enamel prisms and beams. The proteases action facilitates access and deep microorganisms' penetration into the enamel depths with a subsequent dissolution of hydroxylapathite under the acids influence.

Carious process occurring independently, quickly leads to pulp inflammation.

Complications caries process can also occur when untimely and improper dental caries treatment.

Pulpitis is the most common carious process complication. The most common cause inflammation of the pulp is infected by microorganisms and their toxins action. Contribute to the pulpitis emergence: carious cavity bottom traumatic treatment, overheating dentin layers in the processing cavity boron without cooling and with excess pressure at its bottom, treatment cavity strong antiseptics, which are irritating to the tooth pulp, the use of filling materials without insulating gasket or poor insulation of the carious cavity bottom, and, consequently, the pulp.

Microflora of the pulp in a state of inflammation is very close to oral microflora: streptococci (particularly serogroup D, less serogroups C, A, F, G, etc.), lactobacilli and their association with streptococci, staphylococci, spirillum, fuzobakterii and others.

Pathways of infection may be different:

1) through the tooth crown, from the dentinal tubules cavity, or directly into the naked pulp;

2) through cracks in the enamel is intact caries tooth crown;

3) less infection penetrates into the pulp through profound pockets in alveolar pyorhhea;

4) rarely seen pulp inflammation as a result of infection introduction through hematogenic pathway in common diseases: typhus, malaria, influenza and other common infections.

In response to a damaging factor there raises difficult biochemical, histochemical, ultrastructural vascular tissue reactions. Degree occurring disorders depends on the virulence of the bacteria that cause inflammation, their toxins actions, cell metabolism products, as well as the reactivity of pulp and body. Acute pulpitis is characterized by the exudative processes development, as inflammation proceeds according to the hyperergic reaction type that leads to a dramatic swelling of the pulp tissue. Its volume increases and this causes pain. A few hours from the acute inflammation beginning it acquires the character of a purulent process formed infiltrates and abscesses. The acute pulpitis result is pulp necrosis, or the process becomes a form of chronic pulpitis proceeding with peaking.

### 5.2. Immunity factors of dental caries

Modern research has proven that the dental caries emergence and development depends on the overall condition of the body. Significant caries development is observed in people who have had acute infections or other chronic diseases. In this case, the damage degree of dental caries and especially its manifestations are not so much with the disease nature, but with his seriousness.

Long-term clinical observations made it possible to formulate a position on dental caries prevalence depending on the nonspecific resistance state (G.D. Ovrutskiy, 1991).

Most researchers believe that the content and lysozyme activity of blood in dental caries conditions is reduced. In particular, the decrease in lysozyme activity, prevents the carious process progress. This gives grounds to conclude that low lysozyme levels in the blood may be indicative of the human propensity to dental caries.

For the nonspecific resistance evaluation in dental caries was determined as the phagocytic neutrophils' activity. Indicators of the peripheral blood neutrophils functional activity leukocytes in humans with a single uncomplicated dental caries close to those in healthy people. In multiple dental caries leucocytes phagocytic activity is reduced.

Most authors noted reduction in the total protein in dental caries multiple lesions there observed hypoalbuminemia, combined with hypergammaglobulinemia.

Thus, we can assume that the nonspecific defense suppression factors of the organism plays a role in the dental caries pathogenesis.

Accumulated data do not suggest that the caries development is associated with serum Ig A, Ig G, Ig M. In people with significant dental caries lesion-specific antibodies to S. mutans detected more often than people who are resistant to decay.

**Oral cavity local immunity in caries**. Local immunity - an immune system link that protects tissues directly in contact with the microbial environment. The local immunity system in addition to nonspecific factors is specific, which primarily include sIgA. Secretory IgA is produced by salivary glands plasma cells. In the oral mucosa ratio of the IgA, IgG and IgM content is 20:3:1. In saliva the highest content of sIgA, which accounts for 85% of the immunoglobulins amount.

The IgA content in saliva is significantly increased at the age of 6 months, which coincides with the beginning of the primary teeth eruption. This is due to a sharp increase in the number of oral cavity microorganisms. sIgA has the ability to form complexes with other proteins and is very resistant to digestive enzyme action. Biological sIgA activity is relative to dental caries associated with both enzymatic activity cariogenic streptococci inhibition, and the peculiarities of the microorganisms' localization on the teeth surface.

sIgA has anti-adhesive ability relative to bacteria that are already attached. Optimal conditions for the sIgA action are created only when the conditions of its cooperation with lysozyme, sIgA activator.

According to the literature, with heavy defeat dental caries sIgA content is low.

One of the important factors for local protection, is lysozyme. In the presence of lysozyme enhanced lytic activity sIgA. It was found that the carious process enhancement is accompanied by a decrease in the lysozyme content in saliva.

**Characteristics of tooth enamel as an antigenic substance**. It is given that the typical decay starts with the tooth enamel defeat, the question arises about the immunological competence of this tissue.

Organic matter is a required enamel component. By their nature, proteins that make up tooth enamel, similar to collagen.

F.M. Burnet named the organs and tissues, which are characterized by relatively immune "privilege", defense. Introduction emulsions such "defense" organs in complete Freund's adjuvant can induce autoimmune reactions.

Immunogenicity of enamel proteins is conserved only in the initial enamel period. Immunogenicity of the protein formed enamel is not proven. There are virtually unknown diseases of other body systems caused by enamel proteins.

Apparently, the enamel should not be regarded as "defense" tissue because it is a proper barrier, which provides relative dentin layers isolation. Enamel is isolated from the blood, it is impermeable to large molecules, in particular immunoglobulins.

Lack of tooth enamel cellular lymph and blood vessels elements excludes the relation of this tissue to the immune system. The enamel is permeable to relatively small size molecules.

Enamel can be regarded as a reliable barrier between the saliva, which washes it, and dental pulp, rich in blood vessels and cellular elements.

**Dental caries in immune-deficient states**. When immunodeficiency dental caries is characterized by intensive development and an aggressive leak. For a short time, there are expressed different teeth groups, including teeth that are resistant to this disease type - the lower incisors and canines. In this case, caries is localized on the smooth teeth surfaces, which are not typical for caries lesion.

### 5.3. Anti-caries vaccination

Recognition of the dental caries infectious nature opens up possibilities for this disease prevention by active immunization. Researches in the field of caries vaccine were begun in the 40-ies years. It was found that the most promising is the development of a vaccine against S.mutans, which has the most pronounced cariogenic. Of the 8 serovars S. mutans (a, b, c, d, e, f, g, h) is the most cariogenic with serovar.

First was used to immunize vaccine prepared from S. mutans cell bodies. However, after the anti-caries vaccine introduction from killed microbial S.mutans bodies or from the cell walls of this organism may be pathological reactions of the heart, kidneys and other organs on the antigenic streptococcus substrate.

It is known that endocarditis can be caused by hemolytic streptococcus, often - S. sanguis, S. bovis, S. mutans. Some of these streptococcal antigens have an influence on the synthesis of antibodies capable for endocarditis. These bacteria have surface receptors, makes it that returns them to become fixated on the tissues. Antigen S. mutans exhibits cross-reactivity with heart tissue.

With a view to renouncing the use of whole-cell vaccine for S. mutans have been made attempts to identify the main agents responsible for these organisms antigenicity. Among the antigens that play a role in the dental caries development is an enzyme glucosyltransferase.

S.mutans virulence is associated with the extracellular glucose polymers synthesis, which allow these microorganisms to adhere to solid surfaces and take part in the soft plaque formation. Glucosyltransferase - an enzyme that carries out the dextran synthesis and promotes the plaque formation. Use for anti-caries immunization glucosyl showed high efficiency.

There was successful as an attempt to use as anti-caries a ribosomal vaccine preparation consisting of 55% of the IgA and 45% - of protein.

Thus, many aspects of anti-caries vaccine can be considered nearly solved. This primarily concerns the anti-caries immunization effectiveness. It is certainly effective, but it should be emphasized that dental caries is not a non-mediocre threat to the patient life. At the same time after vaccination there are possible heart diseases. It needs special attention to the human streptococcal antigens introduction.

The main anti-caries vaccines action mechanism is associated with secretory immunity activity stimulation. Selective sIgA formation after vaccination prevents sticking cariogenic microorganisms to the surface of the teeth crowns and prevents plaque and retards the streptococci multiplication contained in it.

Have now been received purified protein antigens from the shells of microbe bodies, after the introduction of which pathological changes in heart are not revealed. However, anti-caries vaccine - is an extra burden on the immune system. It is proved that the anti-caries vaccine can lead to temporary but significant nonspecific defense mechanisms weakening.

Recognizing the critical importance of dental caries prevention, especially in childhood, when a local oral immunity has not yet formed, we should still be wary of mass anti-caries vaccination. Fluoridation of drinking water (1-5 mg / L) is sufficiently reliable alternative to vaccination against dental caries, which is advisable to use only as a means to combat dental caries severe forms. It can also be used as a dental caries preventing means in certain congenital and secondary immunodeficiency states.

## 6. ROLE OF MICROORGANISMS IN THE PERIODONTAL DISEASE DEVELOPMENT AND IMMUNITY CONDITION IN PERIODONTAL DISEASES

### 6.1. Involvement of oral microorganisms in the periodontitis pathogenesis

Periodontal disease, according to WHO (1978), is observed in 80% of children and almost 100% of the most countries adult population.

Participation of microorganisms in the periodontal tissue inflammation development is recognized by both national and foreign dentists.

Principal place of microorganisms residence in the oral cavity are dental pockets, as well as dental plaque and bacterial plaque on gingival shells and other oral cavity organs. For example, if in saliva (oral fluid) contains no more than 5 million microorganisms per 1 ml, 1 ml of dental pocket contains several billion of them. More microorganisms are found in dental plaque.

Development of periodontitis is directly dependent on the plaque amount and total microbial oral cavity contamination, and in the back - on the hygienic measures effectiveness.

Most authors believe that the main role in the periodontitis etiology plays Streptococcus mutans, Actinomices viscosus and Bacteroides melaninogenicus association.

With the gingivitis and periodontitis development, as a rule, tissue invasion by periodontal microorganisms is accompanied by a temporary bacteremia. The bacteria presence in the intercellular substance, as well as in epithelial cells and connective tissue gum is proved by bacteriological, histological, immunological and ultrastructural methods, and most profoundly in the periodontium penetrate spirillum and protozoa.

Microorganisms of dental plaque, dental pocket and gingival fluid are capable to generate a pathogens variety, which are concentrated in the dental pockets liquid penetrate, gums and negatively affect the periodontium. Consider these factors.

The waste microbes' products, toxins affect the periodontal tissues state. Exotoxins - gram-positive microflora derivatives - are common to the mouth and do not have a significant pathogenic potential. Endotoxins - gram-negative derivatives - has often aggressive action at the bacterial application site, stimulate the antibodies formation that cause vasomotor disturbances, violate the cell metabolism, lead to hemorrhagic necrosis.

Anaerobe Bacteroides melaninogenicus forms a potent endotoxin, which is easily absorbed cement root canal. Bacterial endotoxins are synthesized by microorganisms of lipopolisaccharid nature - Veulonella Fusobacteria, Leptotrichia, Spirochaeta, and a few others. Bacterial liposaccharides are contained in the den-

tal pockets liquid and their number correlates with the inflammation clinical stage.

Proved that microbial endotoxins can easily penetrate through the dental pocket epithelium and cause a number of pathological changes in periodontium (increased extravasation, plasmin-kinin system activation, the collagenase secretion, lysosomal acid hydrolases from macrophages and in polymorphonuclear leukocytes). Bacteria endotoxins cause inflammation and tissue necrosis.

Low-molecular metabolic toxins contain a variety of amines, ammonia, indole, skatole, hydrogen sulfide, volatile fatty acids, phospholipids, and more. Most of these compounds are produced by S. mutans. Using histological methods, it was proved that these endotoxins primarily affect the nerves, disrupting nerve trophic processes in the periodontium.

Inflammatory and toxic effects on periodontal tissue are carried by enzymes that are produced by dental plaque microorganisms. Microbial enzymes are easily penetrate the gum tissue, which promotes bacterial hyaluronidase (spreading factor).

Bacterial enzymes have such pathogenic properties as neuraminidase, phospholipase C, DNA-ase. Bacterial neuraminidase ACT-proper pathogens dissemination by increasing tissue permeability and immune cells inhibition. In addition, neuraminidase drastically alters the antigens properties. Treatment of cells with this enzyme contributes to the autoantibodies development in the body and complement.

Phospholipase C lyses the leukocytes membrane and inhibits their protective phagocytic response. Particularly strong effect on periodontal tissue is carried by bacterial proteolytic enzymes. They are all in varying degrees, have anti-inflammatory action. Collagenase, which hydrolyze gums and alveolar bone collagen, cause the stroma protein destruction of these tissues. The only microorganisms capable to cleave not only the denatured, but native gum collagen is Prevotella melaninogenicus.

Plaque bacteria produce elastase, capable to destroy the vascular wall elastic tissue structure and thus cause increased bleeding.

S. sanguis produces a protease which cleaves saliva IgA.

Microorganisms of dental plaque and dental pocket produce a large number of substances that stimulate leukocytes chemotaxis. Positive granulocytes chemotaxis is caused by Corynebacteria, E. coli, staphylococcus, fungi of the genus Candida, as well as actinomycetes.

Pathogens in dental plaque microorganisms have the ability to stimulate the lysosomal enzymes secretion by polymorphonuclears, and this property is directly dependent on the ability to synthesize dextran from sucrose.

Plaque bacterial antigens cause lymphocytes sensitization and thus contribute to the blast transformation reaction implementation. Ability to activate the blast transformation reaction have such microorganisms: Actinomyces viscosus, Actinomyces naeslundii, Streptococcus mutans, Streptococcus sanguis, Bacteroides

melaninogenicus, Veillonella alcalescens, Leptotrichia buccalis, Lactobacillus acidophilus, and others.

Microorganisms of dental plaque significantly stimulate the lymphokines secretion by activated T-lymphocytes. Lymphokines have the ability to activate procollagenase, amplify polymorfphnuclears and monocytes chemotaxis, stimulate the osteoclasts activity, and increase vascular permeability.

In summary, Fig. 6.1. The basic stages of oral microorganisms participation in the periodontitis pathogenesis

development is the microbial plaque formation on teeth and gums, as well as the microorganisms multiplication in dental pocket. Toxic substances that are produced by microorganisms, combined the term "pathogenic factors" and consist of various inflammatory mediators (amines, kinins), hydrolytic enzymes (protease, hyaluronidase, neuraminidase), chemotaxis stimulants, cytotoxic agents and bacterial antigens. Primary response to the gums effects of these factors and, primarily, inflammatory mediators, is the gingivitis development. Pathological changes in gingivitis are reversible, but long-term inflammation maintenance leads to increased hystogematic barriers permeability, a significant increase in leukocyte migration and periodontal tissues infiltration, the bacterial antigens interaction with antibodies, increased leukocytes lysosomal enzymes secretion. The following lymphocytes blast transformation, which leads to the plasma-energy cells and tissue basophils formation, stimulating the lymphokines secretion and osteoclasts activation, determines the destructive processes development in hard and soft periodontal tissues.

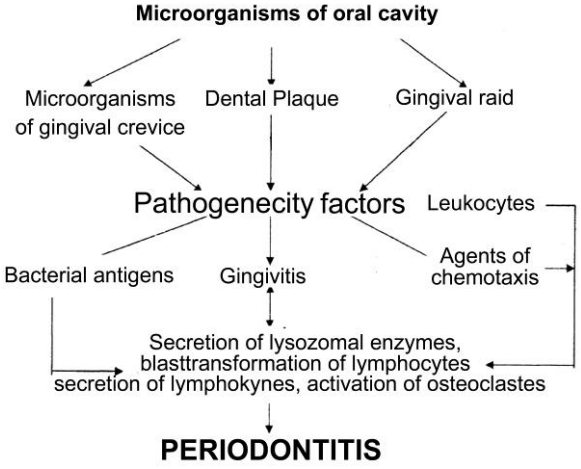



Fig. 6.1. The scheme of oral microorganisms participation in the periodontitis pathogenesis (by A.P. Levitsky, I.K. Mizin)

As can be seen from the diagram, the causal factor for the periodontitis

### 6.2. Nonspecific organism resistance in periodontitis

Study of the organism reactivity makes it possible to identify biological constancy patterns of the organism, the protective-compensatory factors importance in disease development and affect the very essence of the disease process with a view to prevention, treatment and prognosis.

The study of properdin content in blood serum showed that patients with mild periodontitis before treatment, it was lower than in healthy people. Decrease of this index is particularly significant (8-fold lower than normal) was observed in patients with periodontitis moderate to severe forms. Thus, the decline in properdin characterizes the degree of inflammatory-destructive process in the periodontium.

The serum lysozyme level in patients with inflammatory and destructive periodontal disease forms are 5-7 times lower than in healthy people.

In patients with periodontitis showed a reduction in leukocytes phagocytic activity, which depended on the periodontium pathological process severity, it was particularly significant in severe disease.

Patients with generalized periodontitis revealed a significant macrophage activity inhibition and reduced reactivity. Installed neutrophils increased damage in contact with the vascular and gums antigen in patients with periodontal disease. They, unlike healthy people neutrophil damage index was significantly increased. The damage intensity to neutrophils was dependent on disease stage. The most significant figures were observed in patients with severe disease stage compared with patients in gingivitis. This figure was significantly higher in contact with vascular antigen than with the gum one. There also identified high leukergial indexes or leukocytes sintering reaction in patients with severe periodontitis compared with the control group. Bandwidth of leukergic test depended on the disease stage. Average agglomeration of leukocytes in the periodontitis advanced stages usually exceeds that in gingivitis.

Evaluating the obtained data, we can speak about the presence in periodontal tissues diseases of cytoallergic and agglomeration effects, due to the sensitized neutrophils interaction with vascular and gums antigen.

Inhibition of leukocyte migration in moderate severity periodontitis was observed more frequently. In this case, a vascular antigen cause a significant leukocyte migration inhibition. Revealed facts raise white blood elements sensitization to the gums and vascular antigens indicate that changes of cellular immunity factors in catarrhal generalized periodontitis and heavier inflammatory-destructive changes in periodontal tissues (periodontitis).

Thus, in periodontitis there is observed reduction in nonspecific reactivity, respectively to the pathological process severity.

### 6.3. Participation of the immune system in the periodontitis development

**Primary lesion**. 2-4 days after the plaque accumulation there begins an acute inflammatory reaction that is localized within the gingival sulcus and in the nearby epithelium and connective tissue areas. Through gingival pocket goes fluid exudation with polymorphonuclear leukocytes (PNL) and extravascular IgG-complement deposition, fibrin and PNL. Individual cells and macrophages can be identified. This histological picture is consistent with the reaction caused by immune complexes or type III allergic reaction. The primary lesion is apparently a reaction to the chemotactic agents formation induced by plague antigens (Fig. 6.2. ).

At this stage, patients develop antibodies to various bacteria plaque, which creates conditions for the immune complexes formation. Recent call by the classical complement activation, and LPS and other plaques components can activate complement by the alternative pathway.

During the immune complexes reaction in the complement system activation, biologically active peptides accumulate in the tissue. Among these fractions C3a and C3a (anaphylaxins) have a significant vasoactivity, which is manifested in the ability to cause vessels extension and vascular wall permeability violation.

In an inflammatory reaction induced by immune complexes are involved granulocytes, platelets, proteins, blood clotting, kinin-formation. This raises the periodontal tissue primary lesion.

**Early stage lesions**. At this stage, there is dense infiltrate forming, predominantly lymphocytic but also containing some macrophages and plasma cells. Also, fibroblasts degeneration and a collagen loss. Exudation IgG-complement, fibrinogen, and PNL in the connective tissue proceeds, and there is an increased fluid inflow, containing PNL to periodontal pockets. These signs indicate that at this process stage plays the main role in cell-mediated immunity allergic type IV or delayed-type hypersensitivity reaction.

Lymphoid cells, especially the young blood stimulated blasts have increased affinity for nonspecific inflammatory tissue, so they can get into the gum inflammation area on the primary lesion stage. At the early lesion stage cells are capable to allocate some neurotransmitters, such as a factor inhibiting macrophages and mitogenic factors migration that contribute to the leukocytes localization into tissues and lymphocyte proliferation.

**Persistent failure**. At this stage, growing for 2-3 weeks after the plaque infiltrate accumulation, composed mostly of plasma cells. Lesions covering most of areas that are adjacent to the boundary epithelium layer and begin to spread in the tooth root direction, is the subsequent collagen loss, and slit-like gingival pocket becomes an abnormally enlarged cavity. In his own plate epithelium delayed complement, and continues to flow from the pocket of fluid containing the PNL. At this process stage can maintain for many years, with histological features of the type IV immune response.

It is also possible the type III reaction joining, since the pathological process is characterized by plasma cells tissue infiltration.

After 14-21 days after the plaque accumulation there begins proliferative response of lymphocytes to plaque antigens. This process may take part specific plaque bacterial antigens, but more active stimulation may be caused by polyclonal B-cell mitogens (LPS, dextran and levan). Because the plaques antigens affect both T-and B-cells, and the young-stimulated blasts occupy most of tissue inflammation areas, with persistent lesions in the presence of these cells continuous supply in the lesion. Plasma cells secreting cells are the final differentiation stage, in this order to maintain stable lesions for the duration, often calculated in years or decades, a constant lymphocytes flow.

**Advanced lesion**. This stage shows the pathological gingival pockets formation, periodontal ligament collagen ulceration and subsequent bone destruction, which leads to loosening and ultimate teeth loss. Vasculitis develops, and cellular infiltration consists of lymphocytes, plasma cells and macrophages. There is continued gingival exudate accumulation, containing immunoglobulins, complement components, and PNL, as well as separate mononuclear cells. There is a reason to believe that IV and III type reactions are responsible for the tissue destruction reaction, but does not exclude the type II reaction mechanisms participation (cytotoxic responses).

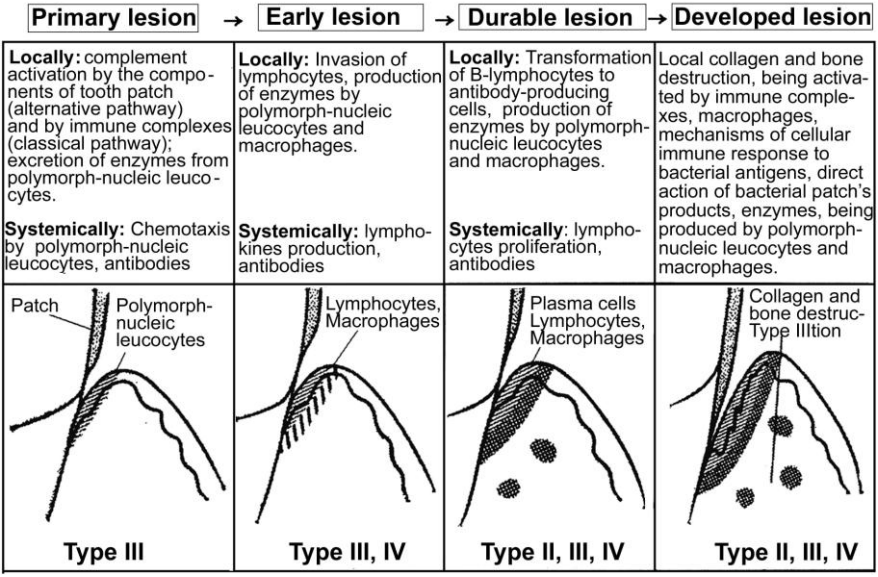



Fig. 6.2. The scheme of oral cavity microorganisms participation in parodontitis pathogeny

## 7. ORAL CAVITY MUCOSA INFECTIOUS LESION

### 7.1. Bacterial stomatitis

***Gonococcal stomatitis.*** Gonococcal lesion of the oral cavity mucosa - gonococcal stomatitis - is rare. The disease occurs in newborns due to the impact of gonococci (Neisseria gonorrhoeae) in the oral cavity of the child during childbirth provided passage through an infected mother's maternity ways. Possible drift of infection from staff and other patients. Typically, the simultaneous loss of the gonococcus oral cavity mucosa, nose and conjunctiva.

Gonococcal stomatitis is also found in adults. At the same time often affects throat and tonsils, less common stomatitis, gingivitis, laryngitis. The defeat of the oral mucosa is found primarily in male homosexuals and people who have orogenital contacts.

The first gonococcal stomatitis symptoms - redness, swelling, minor erosion on the mucosa and viscous puromucous secret. In more severe cases if left untreated can spread process, there is a large number of erosions and ulcers on the mucous membrane of cheeks, tongue, gums and throat, tonsils, larynx. Mucosa flushed, covered with a large number of gray purulent plaque with an unpleasant odor.

Progress gonococcal stomatitis, usually asymptomatic. Adults are rarely seen a sore throat, body temperature rises.

Diagnosis is confirmed by microscopic finding gonococci purulent exudate. Smears stained by Gram and methylene blue. Gonococci are defined by three characteristic features: Gram-coloring, bean-shaped diplococci, intracellular location. For the detection of gonococci in the smear is also used direct and indirect IFA.

***Scarlatinal stomatitis***. The causative agent of scarlet fever is a hemolytic streptococcus group A, able to produce erythrogenic toxin.

One day before the rash shows signs of mucous catarrhal stomatitis. The mucous membrane of the tonsils and soft palate is flushed, bright-red, appears on it small-dotted bright red rash. At the same time due to erythematous skin appears small-dotted red rash. The skin around the chin and mouth remains pale, forming a so-called nasolabial Filatov's triangle.

Observed the development of catarrhal sore throats. Dorsum of tongue is covered by whitish-gray coating. Starting from 3-4 days, the back tongue cleared of plaque, it becomes bright red, dry, brilliant. Across the back of the tongue can be seen enlarged fungiformic papillae, resembling grains raspberries, which gave grounds to refer to the language of scarlet fever "raspberry."

***Dysenteric stomatitis***. Dysentery oral cavity mucosa changes is a reflection of high intoxication, high fever and dehydration. Oral mucosa is pale, swollen, her teeth marks observed on the cheeks and the lateral surface of the tongue.

Tongue is pale, dry with hyperplastic filiformic papillae. With a favorable development of the disease mucosa gradually becomes more humid, gets a pink coloring. In the protracted course of dysentery oral mucosa retains pallor, but becomes cyanotic hue. In the area of the gingival margin is a thin, transparent, well-translucent vessels.

**Tuberculosis.** The causative agent of tuberculosis is a human Mycobacterium tuberculosis and M. bovis. Tuberculosis of the oral cavity mucosa and lips is often a secondary, much less develops primary tuberculosis of the mucous membrane in the form of a primary tuberculous complex. Tubercle bacilli can enter the oral mucosa as an endogenous way (hematogenous, lymphogenous, per continuetatum), and exogenous.

Oral cavity mucosa is a poor breeding ground for tuberculosis mycobacteria: in the mucosa of the majority of patients with tuberculosis, they die. If, however, there is its defeat, the clinical disease depends on several factors, primarily on the general course of tuberculosis and the immunological state of the organism.

Form of secondary tuberculosis in lesions of the oral cavity mucosa may occur tuberculous lupus, ulcerative miliary tuberculosis and collicvative.

Primary tuberculosis, or primary tuberculous complex on the lips and oral mucosa is rare, mostly in children. It occurs as a result of infection occurring airborne, less nutritional means. After an incubation period, whose duration is 8-30 days, the site of the entrance gate of infection occurs painful ulcer diameter 1-1,5 cm, jagged edges and gray bottom. 2-4 weeks after the formation of ulcers increases and compacted submandibular lymph nodes.

During this form of TB can be very difficult, especially in infants because of possible generalization of the process.

Tuberculous lupus is the most frequent form of secondary tuberculous lesions maxillofacial region.

Localized lesions of tuberculous lupus primarily on the face (in the form of "butterfly"), spreading to his upper lip, red border, at least - the mucous membrane of the gums and alveolar process of maxilla in anterior teeth, hard and soft palate, upper lip and cheeks. Sometimes the process is localized only in the red border.

The clinical course, characterized by slow, tuberculous lupus is infiltrative, lumpy, ulcer and scar stage.

The main primary element in tuberculous lupus is “lupoma” - specific tuberculous tubercle of red and yellow-red color, soft texture, with a diameter of 1-3 mm. Posted lupoma groups: fresh formed on the periphery, and those that are centrally located, tend to sulphurous decay and merge with neighboring tubercles. In this form there are shallow ulcers with soft, ragged, little painful edges.

Ulcers in lupus of the oral cavity mucous membranes and red fringe lips may be 1-10% of cases undergo malignant degeneration.

Regional lymph nodes are increased, becoming thick, welded to the hilly packages. Mantoux test is usually positive. Mycobacterium tuberculosis detected very rarely.

Histopathological study reveals typical tubercles with epithelioid cells, Pirogov-Langans's giant cells and lymphoma at the periphery. Tubercle bacilli are found in small amounts.

Miliary tuberculosis, ulcerative – variant of secondary tuberculous lesions of the oral mucosa that develops because of reduced reactivity.

Mycobacterium tuberculosis, standing out in large numbers of sputum, with severe progressive course of the process as a result of inoculation of the open hearths of infection (often from the cavity of the lungs) are taking root in the mucosa (usually in the field of injury) through the cheeks of closing the teeth, back and sides tongue, soft palate. This gives rise to typical tubercles, the subsequent development that accompanied the collapse in the center and the formation of a shallow, at first small, with rough soft edges, very painful sores, which has a creepy character and grows on the periphery, sometimes reaching large sizes. In the case of the prolonged existence of ulcers associated secondary infection. Lymph nodes at the start of the existence of ulcers may not be palpated, and subsequently increased, becoming densely-elastic, painful.

With the help of material from cytological examination the ulcer detected among the elements of inflammation and a mixed microflora giant Pirogov-Langans' cells and epithelioid cells. Sometimes (when stained by Ziehl-Neelsen) can be detected Mycobacterium tuberculosis. In most of these patients there is reduced reactivity, the Mantoux test is often negative.

Collicvative tuberculosis (skrofuloderma) - a rare form of secondary tuberculosis occurs mainly in children. A typical feature of this form is in deep mucosal sites layers, which will eventually degrade. This gives rise to ulcers of irregular shape, soft consistency, with jagged edges and podrytymi sluggish granulations on the bottom. Small-painful ulcer, when it formed irregular wound, so-called ragged scars.

Leprosy is caused by Mycobacterium leprae (Mycobacterium leprae), is a chronic Generalized infection and is characterized by times of the various clinical manifestations of granulomatous lesions of the skin, mucous membrane of upper respiratory tract and oral cavity, the peripheral nervous system, eyes, and in case of late diagnosis - the internal organs and bone -muscular system.

Isolated tuberculoid and lepromatous leprosy types. The defeat of the oral cavity mucosa may occur only when lepromatous type that develops in people with drastically reduced reactivity (negative leprominic test). In these patients the skin, oral mucosa, internal organs, along nerve trunks occurs lepromatous infiltrates with cells that are not capable (in contrast to tuberculoid type) to destroy phagocytized Mycobacterium leprae, so they are free to multiply in these cells.

As the skin leprosy manifestations in the oral cavity mucosa are diverse and are characterized by the development stages: infiltration - hump-ulcer - a scar.

Lepromatous infiltration of grayish-white, sometimes with a dark-blue areas, slightly raised above the surrounding mucosa. Subsequently, on a background infiltrate irregularly appear small size of ulcer with tuberous base and irregular, slightly raised edges, that is the bottom of the ulcer shows structureless necrotic masses, which pass into the granulation tissue. Ulcers leave round or radiant smooth shiny white scars that may cause deformation of the soft palate, tongue, while there are new infiltrates, bumps and sores.

Depending on the localization of leprous lesions of them have certain characteristics. So, on the mucous membrane of lips first appears diffuse redness, cyanotic spots, thickening of the epithelium. Leprous tubercles, clearly shown in the red portion of the lips, less likely to happen on its inner surface, are located in the submucosa. Tubercles for a long time remain unchanged, but then may be formed on the surface are painless ulcers, discharge of which it dries, forming a light-yellow rind. Cicatrization of ulcers causes deformation.

Lesions on the gums lepromatous begin with infiltration. Swollen gums become lush, red, sometimes cyanotic, bleeding, painless. On the mucosal surface of erosion formed, which due to scar formation cause wrinkling gingival margin. Retraction of the gums occurs, exposure of tooth roots.

For the leprosy diagnosis there is used direct microscopic study of smears from the mucous membranes of the nose and the bottom and ulcers edges of the oral mucosa, which were stained by Tsiell-Nielsen. Mycobacterium and leprosy are both in deep and in superficial layers of the epithelium in the cytoplasm of epithelial cells.

**Syphilis**. Syphilis is caused by pale treponema (Treponema rallidum). Affects the skin, mucous membranes, internal organs and nervous system. Distinguish between acquired and congenital syphilis.

Infection, but genital way, can occur through an infected common goods, including a dental instrument, if the agent gets from it on broken skin or mucous membranes. Some authors believe that the pale treponema can penetrate through intact mucous membranes.

In connection with the undulating syphilis course, the different nature of the clinical and morphological changes that occur at different stages of disease, distinguished incubation, primary, secondary and tertiary period of acquired syphilis.

Oral mucosa and lips red border are affected at all stages of the disease, but the incubation period. The incubation period for syphilis is generally 3-4 weeks, with no clinical symptoms.

Primary syphilis is the period begins with the appearance on the site of entry of pale treponema chancre or primary syphiloma, which is mainly localized on the lips, gums, tongue, tonsils. The development of the chancre begins with the

appearance of limited redness in the center of which two or three days comes at the expense of sealing infiltration. In the central part cartilaginoid palpation infiltrate developed necrosis and erosion formed a painless bright red color. The bottom of the erosion is meat-red, dense, shiny, sclerosal, later grayish-white, with a "greasy" on the fly.

If a secondary infection joins, the erosion deepens, becoming an ulcer covered with dirty gray necrotic plaque.

5-7 days after the appearance of the chancre is an increase in regional lymph nodes. Over the last week of the primary period of syphilis develops poliadenitis all or most of the lymph nodes, which reach the size of a pea. Nodes have a tight elastic consistency, painless, movable, skin over them is not changed. Primary syphilis is the period of 6-7 weeks.

If you suspect a chancre should be repeated studies in the dark field of discharge from the chancre on the pale treponema. Can be used an indirect IFA staining according to Romanowsky-Giemsa. Detection of the pathogen is crucial in the diagnosis of primary syphilis, because serological tests become positive only after 3 weeks after the chancre. In the absence of secretions in primary syphiloma pale treponema it can be detected in the lymph node punctate.

The secondary period of syphilis is manifested in the oral mucosa in the form of roseola and papules.

Secondary syphiloderm have the common symptoms:

- Rash does not violate the integrity of the mucous membrane;
- As a rule, it is not accompanied by subjective sensations;
- When a rash of secondary syphiloderm almost 10% of the cases observed concomitant positive serological reaction (Wasserman, sedimentary, immobilization of pale treponemas);
- Simultaneously with the defeat of the oral mucosa occur roseolous, papular and pustular rash on the skin;
- Secondary syphiloderm is accompanied by poliadenitis.

Roseolous (blotchy) rash mainly occurs symmetrically on the palatine arches, soft palate, tonsills, its elements can be collected in separate areas (erythematous angina). They have a stagnant-red, sometimes with copper highlights, color and clear boundaries. Mucous membrane is a little swollen.

The most frequent manifestations in the oral mucosa secondary, especially recurrent, syphilis is a papular rash. Basically it is localized on the tonsils, the palatine arches, the soft palate, where the papules coalesce into solid lesions (papular angina), as well as the language of the mucous membrane of cheeks, especially through the closing of the teeth and gums.

First papule is sharply limited, dark-red lesions with a diameter of 1 mm, with a small infiltrate at the base. Subsequently, this defeat is a dense, round, size 3-10 mm, painless. It has distanced himself from the normal mucous membrane of a simple hyperemic infiltrated a whisk, gently rising above the level of the

mucous membrane. Due to the maceration of the epithelium on the surface they become whitish papules coloring, and around them remains an inflammatory halo that resembles aphthae. On scraping the surface of these papules covers by macerative epithelium scrapes, and then formed the erosion of red colour. In the study of emissions from these erosions have been detected a pale treponema.

In non-sanated oral cavity, in the case of the poor state of the secondary infection accession, papules can ulcerate. There is considerable soreness and redness around the area of induration is expanding.

On the mucous membrane there often formed multiple papules, which are at different stages of development. First papules are focal, but due to constant stimulation, they acquire a tendency to peripheral growth and coalescence in the plaque, which dominate the mucosa. Patches of secondary syphilis are the most dangerous source of transmission to asexual.

The duration of secondary syphilis lasts from 4-6 weeks to 6 months. Even without any special treatment at the end of this period the changes in the mouth disappear.

The diagnosis of secondary syphilis based on clinical symptoms and laboratory tests (detection of lesions in the elements pale treponema, positive Wasserman reaction, sedimentary, immobilization of pale treponemes).

Tertiary syphilis begins 4-6 years after onset and can last for decades. The defeat of the oral cavity mucosa characterized by the development of inflammatory infiltrates gum and tubercles), which tend to decay. Tertiary syphilis rash is less contaminative, because have almost no pale treponemas.

Gummy syphilis can be localized in any part of the oral mucosa, but more often - on the soft and hard palate, tongue. Most gum single. First, in the thickness of the mucous membrane forms a painless joint (node), which is gradually increasing. Subsequently, necrotizing node in the center, after a discharge of gummy stem there is a deep, painless ulcer. Heal ulcers gradually with the formation of stellate scar drawn. When you localize gum in the sky in its place there is often a perforation.

Gummy defeat of language can be for as separate gum (knotty glossitis), at least - in the form of diffuse gummy sclerosis (diffuse sclerosing glossitis).

Regional lymph nodes with tertiary syphilis can not be changed.

The diagnosis of tertiary syphilis is established on the basis of clinical and laboratory data (reaction immune-fluorescence and immobilization of pale treponemas positive in 100% of cases; Wasserman and sedimentary responses are positive in 50-80%). For suspected syphilis patients should be referred to venereal diseases.

In congenital syphilis, the first symptoms appear even at 1-2 months of life. Lips become swollen, thickened, yellow-red color. On the surface of the affected oral mucosa appear ulcers, which are further scarring. Especially, characteristic scars are in the corners of the mouth (scars Robinson-Fournier). The appearance

of congenital syphilis at a later date on the oral mucosa, changes resemble gummy. Serological tests are usually positive.

***Necrotizing ulcerative Vincent's stomatitis.***

Necrotizing ulcerative Vincent's stomatitis (synonym: fuzospirochetous oral cavity trench, Botkin Simanovsky-plaut-Vincent's stomatitis) - an infectious disease that occurs due to a reduced reactivity in the presence of adverse conditions in the mouth, develops as an immune reaction in response to the sensitization of tissue oral mucosa fuzospirillar anaerobic microflora and is characterized by necrosis and ulceration.

The disease occurs under the influence of fuzospirillar disease - borrelia and fuzobacteria symbiosis. This symbiosis under normal conditions is a saprophyte of the mouth and in the gaps between the teeth, periodontal pockets, deep cavities, root canals and the crypts of tonsils.

Vincent stomatitis often develops in the presence of hypothermia, stress, trauma, surgical interventions. By provoking factors include malnutrition, vitamin deficiencies, smoking, alcohol abuse, overwork. The main factor that leads to the development of the disease is unhygienic maintenance of the oral cavity. Development of the disease is strongly associated with local irritants, like sharp protrusions decayed teeth, sunken artificial crowns, severe teething third molars, especially in the lower jaw.

When poor hygienic oral cavity condition - density plaque proliferate fuzobacteria, bacteroides, spirochetes and other microorganisms. It comes amid a general decline of immunity and barrier function of oral mucosa. Microorganisms and their toxins are slowly penetrate into the connective tissue cavities and gum company, where the parasite multiplies. In the case where such a state lasts for weeks or months, will develop chronic stomatitis or gingivitis, which is immune lesion of the oral mucosa of delayed type. This leads to a reduction of blood stasis, thrombosis, and regional necrosis.

It is believed that fuzospirochetes arises because of the previous inflammatory process caused by staphylococcus and streptococcus. Then there is an active breeding fusiform bacteria and spirochetes associated with the presence in them of the enzyme collagenase, which participates in the destruction of collagen fibers of connective tissue. In this case, nitrogen-containing low molecular weight products resulting from the collagen collapse, can be assimilated by the spirochetes.

Anaerobic conditions are created in the necrotic tissue, preventing a rapid recovery and lead to subsequent damage to system tissue. Fusiformic bacteria are developing, together with other anaerobes.

The clinical picture is characterized by the formation of ulcers covered by necrotic masses, which are fairly easy to removed, then, there's formed a bleeding bottom. The edges of the ulcer are rough, red, without significant compaction. The ulcer may reach 2-4 cm in diameter. Near the main ulcer may develop small

sores. Mucosa around the ulcer is swollen and hyperemic. Constant symptom is an unpleasant foul breath.

In addition to ulcers, occurs pseudo-membranous or diphtheroid membrane, a form of Vincent's disease. The process usually occurs only in the tonsils, which produce a yellowish-gray film. Inflammatory effects on the periphery are almost nonexistent. Film form of Vincent's disease is less common than peptic ulcer.

The diagnosis of angina Vincent put on clinical grounds and detection fuzospirillar symbiosis. Differential diagnosis must be made with diphtheria. Detection of symbiosis does not yet allow discard the diagnosis of diphtheria. Only in the absence of diphtheria bacilli can be excluded the diagnosis of mixed infections.

Prognosis of angina and stomatitis Vincent's favorable, although in some cases, in the absence of rational therapy, the disease is prolonged and may last several months.

**Actinomycosis** - a chronic granulomatous suppurative lesion of different systems and organs with a characteristic infiltration of tissues, abscesses and fistulae, dense granules (friends) in pus, which is caused by actinomycetes.

For a long time actinomycetes were classified as fungi, but the study of their morphology and biological properties allowed scientists to refer them to the bacteria. Unlike fungi, actinomycetes do not have a cell wall chitin or cellulose, and she is like a bacteria, are not capable of photosynthesis, the mycelium, they form a fairly primitive. They reproduce only asexually. Since the bacteria actinomyces combines the absence of the kernel (have a nucleoid), sensitivity to bacteriophages and antibiotics (in the presence of resistance to antifungal agents), as well as the ability for good growth in a slightly alkaline (not acidic). The most common causative agents of human actinomycosis is Actinomyces israelii, at least - A.albus, A.bovis, A.naeslundii and others.

Actinomycetes are constantly in the mouth, forming part of plaque, stone microflora of periodontal pockets and cavities. The development of actinomycosis is most often associated with endogenous on-the causative agent of falling into the tissue, but the source of infection may also be actinomycetes, which vegetates on grasses, soil and enter the body through broken skin or mucous membranes. Some importance in the occurrence of actinomycosis have injury mucosa company and banal inflammation. A crucial role in the pathogenesis of actinomycosis is the reactivity of the organism. Importance in this case has peculiar (under the conditions of re-invasion of actinomycetes) and nonspecific sensitization, a predetermined purulent inflammatory processes.

The primary symptom of the disease is the formation of specific actinomicous granuloma - a dense infiltration of the surface, formed around the mycelium and Druze's actinomycetes. Skin as a mucus-pack wrapper over hyperemic infiltrate, in some areas becoming cyanotic. After some time in the granuloma cells formation and softening occur ulcers. Of the latter stands on the skin surface or in

the mouth a small amount of pus, in which can be detected druses of actinomycetes. If the point of entry of the pathogen is oral cavity mucosa, the lesion is localized often in the language, gums, wound extractions.

Actinomycosis is distinguished as primary and secondary. Development of primary actinomycosis of the oral cavity mucosa is often associated with traumatic injuries of the mucous membrane stalks and ears of wheat cereals, the edges of the teeth, fish bone, or other sharp objects. The clinical course is slow, quiet, without the temperature reaction, with little pain. Depending on the location of infiltration it has its own specific characteristics: the lower lip or cheek, he clearly is limited, often circular, welded together with the underlying tissues, the sublingual area and on the bottom and sides of the tongue infiltrate a wide but superficial.

Secondary lesions of the oral cavity mucosa actinomycosis occur as a result of the spread of actinomicouse process of the subject of deep tissue layers. Inflammatory effects in the mucous membrane are more frequently observed during exacerbation of the process, when the mucous membrane pressure welded with the subject-specific infiltration and formation of fistulas, which, unlike those in the skin, quickly scars. In the surrounding mucosa appear sclerotic education.

Clinical diagnosis of actinomycosis of the oral mucosa should confirm the results of microbiological and immunological studies. Microscopic examination of pus friends or thin non-septal branched mycelium can be detected as a "crushed" the drop, and in preparations stained by Gram, Tsille-Neelsen or Romanowsky-Giemsa. The great importance has the immunological study (skin-allergic test with actinolisate, leukocyte migration inhibition test with actinolisate) and cytology biopsy of diseased tissue.

### 7.2. Viral stomatitis

Influenza is an acute respiratory infection, which are the causative agent of influenza viruses A, B, C, relating to the family Orthomyxoviridae. Lesion of the oral mucosa nonspecific and depend on the reactivity and tropism of the virus with respect to certain tissues.

Since the beginning of flu develops catarrhal stomatitis with flame hyperemia, paresthesias, burning of the oral cavity mucosa. The most striking changes are observed in the mucosa of the soft palate, palatal arches, tongue, pharynx. At first - the second day of the disease on the background of catarrhal changes in the soft palate appears millet grain red rash, which is formed by hyperplasia of the epithelium of excretory ducts of salivary glands. The emergence of such a rash in healthy people during a flu epidemic may be an early symptom of the disease. Early symptoms of influenza in the oral mucosa at the site of the cheeks, tongue, lips can be deskvamative and even degenerative-necrotic process, which manifests itself strongly hyperemia, enhanced epithelial desquamation, petechiae, the emergence of numerous small papules with hemorrhagic exudate, which quickly

burst to form painful erosion of bright red color or the AFL. At 3-4 day hyperemia and granularity of the soft palate injection, vascular changes, the appearance of petechiae, which are for 7-8 day illness disappear.

Sometimes the transition to the soft palate and the mucosa of the cheeks at the end of the disease produces large thin-walled blisters that contain hemorrhagic exudate and persist from several hours to 1,5-2 days, and then burst, forming a large, cleared of plaque erosion . With a low resistance to the oral mucosa and erosion of the body and aphthae with the accession of a secondary infection may ulcerate and then there is canker-ulcerative or ulcerative-necrotizing stomatitis. In this period there are frequent periodontal diseases exacerbation, rashes, recurrent herpes, sometimes there neuritis trigeminal and facial nerves. At the end of the disease as a manifestation of immunodeficiency can possibly develop an acute herpetic stomatitis, or candidiasis.

In the diagnosis of influenza, as well as other oral cavity mucosa viral lesions, we must rely on the data of the epidemiological situation, history, clinical signs of disease. Among the laboratory methods used virological (infection of chicken embryos and identification of the virus using RGGA), serology (detection of increasing titers of specific antibodies by RGGA, CLS, ELISA), immunofluorescence (allows you to detect viral antigen in the smears, imprints from the mucous membrane) method.

**Herpetic stomatitis**. Herpesvirus infection is one of the most widespread and uncontrolled viral human infections.

Herpes simplex virus, entering the body through the oral mucosa and the nasal pharynx in early childhood, it remains to persist in the body mainly in latent form, without causing clinical symptoms. Under the influence of provoking factors in adverse conditions (reduction of immune-reactivity), the virus can move into an active state and cause the defeat of the oral mucosa. These factors include the flu, tuberculosis, pneumonia, periodontal lesion, sinuses, hypothermia, long-term insolation, stressful situations, surgical intervention, intoxication, and others. For a man who previously had undergone an acute herpetic stomatitis, these factors often cause relapse.

**Acute herpetic stomatitis**. When the primary form of acute herpetic stomatitis is the primary contact of lips and oral mucosa with herpes.

Primary herpes usually occurs in children aged 6 months-W, when disappearing specific to the herpes virus antibodies obtained from the mother's blood. In most cases, primary herpes has a subclinical course and only 1-10% of infected people develop clinically severe symptoms of acute herpetic stomatitis, which are the primary immune response to invading virus.

The incubation period of acute herpetic stomatitis is generally 6-8 days. The disease begins sharply - from general malaise, headache, increase in body temperature to 37-40 ° C. These symptoms for 24-48 hours associated pain in the mouth, which is enhanced during conversation and eating. The mucous membrane becomes hyperemic, swollen. In the area of the lips, cheeks, tongue, floor

of mouth, palatal arches appear small papules that are placed in groups. They are filled with clear liquid, which subsequently becomes cloudy. Papules may coalesce and burst in 2-3 days, forming a widespread erosion of the bright red color, covered with bloom. Saliva volume increases, it becomes viscous. However, often affects the red border of lips and skin, which borders it.

HSV infection can have a professional nature (loss of facial skin and finger brushes dentists), lead to serious damages, herpetic keratitis, encephalitis, and hepatitis.

Herpes simplex, simple herpes has a primary element of defeat - a bubble. Cell localization of the eruption of bubbles is a limit: mucosa - the skin near the orifices (red border of lips, passing into the skin, wings, nose, nasolabial groove, eyes, reproductive organs, skin).

**Chronic recurrent herpes**. After undergoing a primary herpetic infection of the virus remains in the body, obviously, throughout his life, and the disease goes into a latent phase, prolonged virus infection, which is often accompanied by relapses.

Chronic recurrent herpes is mainly for adults and one in ten children with acute herpetic stomatitis. Development of the disease is a testament to reduce the overall immune system and reactivity of the oral mucosa, it can appear on the skin and mucous membranes.

Quite often, relapse occurs after trauma (biting, separation and processing of the tooth with an orthopedic purpose), infections, hypothermia or a clear link with the menstrual cycle or exacerbation of chronic diseases of the digestive canal.

Clinical manifestations of herpes infection as the primary and recurrent forms of the skin may be the same. Recurrent herpes of the oral mucosa more often localized on the hard palate, cheeks, tongue and occurs mostly as a result of the above-mentioned triggering factors. In most cases of recurrent herpetic stomatitis, rash limited to the typical dynamics of the process: the bubbles appear group merge and burst, forming erosion with polycyclic contours. In the first few days of existence of erosion, they are very painful, this results in pain reaction of regional lymph nodes.

**Chicken-pox**. The virus is varicella-zoster (the virus VZ) causes, mostly in children, chickenpox, which is accompanied by the development vezikuleznoy rash on the skin and mucous membranes. In adults, the same virus causes herpes zoster.

The disease begins with acute fever and rash appears on the skin. Simultaneously, a rash in the mouth: the tongue, hard palate, pharyngeal mucosa. Vesicles in the oral cavity unstable and quickly burst, forming a circular erosion of small size and shallow ulcers grayish-pink, bordered by red inflammatory rim.

The rash may not appear at the same time, because of what elements of the rash are at different stages: papules, vesicles, crusts. Each following a rash ac-

companied by an increase in body temperature to 38 ° C and above. At 3-4 days of illness the rash dries up, the body temperature drops, the overall condition of the patient improves.

In the typical clinical diagnosis of the disease is not a hardship. In order to confirm the diagnosis used virus-scopic, virological and serological methods as well as in other herpes lesions.

Shingles is caused by herpes viruses (virus varicella-zoster, the virus VZ).

Development of the disease is considered as the result of reactivation of the virus VZ, existing in latent form in sensory ganglia of the people who chickenpox chicken-pox.

Disease occurs predominantly in the cold season, mostly in middle-aged and older. For risk groups include patients with immune deficiencies, disease, malignant growth and drug addicts. Development of herpes zoster also contribute to traumatic injuries in the area of the rash.

Throat is sharply hyperemic, sometimes with a cyanotic hue, as a manifestation of adenopathy have hyperplasia of the tonsils. Angina is a persistent long-term course and did not respond to antibiotics. On W-D-fourth day of the disease at the border of hard and soft palate petechiae appear.

Depending on the severity of the infectious mononucleosis may develop catarrh, herpes, or necrotizing ulcerative stomatitis, which is often accompanied by petechial hemorrhages on the mucous membrane and skin. Tongue is coated gray-white film, a marked hyperplasia of fungiformic papillae and the lingual tonsil. Patients face swells, nasal breathing is difficult, there may be nosebleeds.

In addition to clinical symptoms, the importance in the diagnosis of infectious mononucleosis has a haemogramm. Observed leukocytosis, one of the most characteristic signs of the disease is the appearance in blood mononuclear cells of atypical medium and large sizes with a wide basophilic cytoplasm - the atypical mononuclear cells and lymphocytes, their number is 10-15% or more. Eosinophils are almost nonexistent. Hemoglobin and red blood cells count is close to normal, ESR was 20-30 mm / hour.

Measles - an acute viral disease of most of childhood, characterized by the general intoxication, fever, catarrh of the mucous membranes of the respiratory tract and papular rash. The measles virus belongs to the family Paramyxoviridae, genus Morbillivirus. Transmission occurs through airborne droplets, infectious patient from the last day of the incubation period and up to 4-5 days after the rash appears.

Pathognomonic for measles is the appearance in the catarrhal period (1-2 days before the rash) on the mucosa of cheek Bielski-Filatov-Koplik's stains as dots, whitish, 1-2 mm in diameter, surrounded by a rim of hyperemia. These elements are located mostly in the transitional fold or mucous membrane of cheeks in the molar area, somewhat less frequently - on the mucosa of the gums or lips. They resemble a spray of lime, sprinkled on the surface hyperemic spots that are

slightly above the level of the mucosa and never merge with each other. Pathological Bielski-Filatov-Koplik's stains represent areas of necrotic epithelium. In the same period are often raided grayish-white color of the mucous membrane of gums, which are a consequence of necrosis and desquamation of the epithelium affected virus. With the emergence of skin rash (on the fourth day after raising the temperature) Bielski-Filatov-Koplik's stains disappear.

At the same time with a rash on the skin (appears first on the face and behind the ears), and sometimes just before, on the mucous membrane of the soft and hard palate occurs cortical exanthema: rash of small red spots that are irregular in shape.

Picture of the disease is so characteristic that the diagnosis is easily determined clinically. If necessary, apply methods of laboratory diagnosis: detection of antigen of the pathogen in mucus from the nose and throat (RIF), virus isolation in cell cultures and its identification by IFA, HAI, RN, revealing increase in antibody titer sera from patients (RCC, HAI, RN).

**Foot and mouth disease**. The causative agent of FMD is a virus belonging to the family Picornaviridae, genus Aphtoviras and causes severe epidemics in cattle and pigs.

Infection of man occurs as a result of dairy products and meat or during contact with sick animals FMD. The virus enters the body through damaged skin and mucous membranes.

The incubation period is 2-18 days, the disease begins acutely, it is typical signs of the temperature to 38-39 ° C, headache, pain in joints and muscles. There is a sensation of burning in the mouth and excessive salivation.

After 1-2 days in the hyperemic and swollen mucosa appear small size bubbles that burst, and in their place are formed aphthous elements. The simultaneous loss of the mucous membrane of larynx, nose, eyes and genitals. The defeat of the oral mucosa is often accompanied him like skin lesions near the nose wings, as well as interdigital folds, base of nails, soles. It may result in infarction of parenchymal organs.

Laboratory diagnosis is to allocate the agent in cell cultures and detection of specific antibodies in paired sera RN and CLS.

### 7.3. The defeat of the oral cavity mucosa due to HIV infection

Acquired immunodeficiency syndrome (AIDS, AIDS from the English. Acquired Immune Deficience Syndrome) was first isolated as a separate disease in 1981. The causative agent of AIDS is human immunodeficiency virus (HIV), which belongs to the family Retroviridae, subfamily Lentivirinae.

HIV infection in Ukraine was first documented in 1987, when the mass screening of the population to HIV infection. Most affected by HIV Odessa, Mykolayiv, Donetsk Region and the Autonomous Republic of Crimea.

The source of infection is the only person - the patient or a virus carrier. The virus is found in all body fluids, on transmission, parenteral, sexual, transplacental, through biological fluids.

In the body, the virus infects cells that are specific to its receptor CD4. This receptor has a large number of T-helper cells (T4 lymphocytes), in a smaller - macrophages, monocytes, astrocytes (cells of the CNS). Most sensitive to the virus of T-helper cells, because they are mass death, which is a major cause of immunodeficiency in HIV infection. For people affected by HIV infection, characterized by 3 groups of diseases - opportunistic infections, cancer and CNS.

Manifestations of HIV infection in the mouth is very polymorphic. They include: 1) fungal, bacterial, viral lesions, 2) neurological disorders, 3) disorders of unknown etiology (recurrent aphthous ulcers, progressive necrotic ulcers, toxic Epidermolysis, trophic and dekubitalnye ulcers, salivary gland enlargement, dry complex, submandibular lymphadenopathy, hyperpigmentation ).

In August 1990, in Amsterdam working group of leading dentists from across Europe has put forward a classification of oral manifestations of HIV infection. It was suggested to distinguish three groups of pro-phenomena based on the reliability of HIV infection.

The first group - the defeat of the oral mucosa, which is most closely related to HIV:

1. Candidiasis (erythematous, hyperplastic, pseudomembranous).
2. The hair leukoplakia (Epstein-Barr virus).
3. HIV-gingivitis.
4. Necrotizing ulcerative gingivitis.
5. HIV-periodontitis.
6. Kaposi's sarcoma.
7. Non-Hodgkin's lymphoma.

The second group - the defeat, to a lesser extent associated with HIV infection:

1. Atypical ulcers (oropharyngeal).
2. Idiopathic thrombocytopenic purpura.
3. Diseases of the salivary glands (xerostomia, one-or two-sided increase in salivary glands).
4. Viral infection (which differs from the predetermined Epstein-Barr virus): caused by cytomegalovirus, a virus herpes simplex, human papilomavirus (warty lesion - condyloma acuminate, focal epithelial hyperplasia, a common wart), varicella-zoster virus (herpes zoster, varicella).

The third group - lesions that may be associated with HIV infection:

1. Bacterial infections (eg gingivitis, periodontitis), actinomycosis, and also due to Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Mycobacterium avium.
3. Exacerbation of apical periodontitis.

4. Fungal infection of non-candidous etiology (cryptococcosis, histoplasmosis, mucoromicosis).

5. Osteomyelitis.

6. Sinusitis.

7. Abscess, cellulitis

In frequency the most common disease of oral mucosa are distributed as follows: Candida (88%), herpetic lesions (11 - 17%), xerostomia (19 - 28%), exfoliative cheilitis (9%), ulcers (7%), desquamative glossitis (6%), hair leukoplakia (5%), sarcoma galoshes (4 to 50%), hemorrhage.

Candidiasis of the oral mucosa in patients with AIDS has several forms of the course: pseudomembranous, at which a significant amount of soft white lesions that resemble curdled milk and cereal are easily removed by scraping; hyperplastic - like leukoplakia, accompanied by the presence of dense white lesions, soldered to the surface mucous membrane of the mouth, and atrophic (erythematous). The latter form is characterized by the appearance of erythematous patches, against which there are pseudo-membranous changes without flying in places - spots hyperkeratosis.

From bacterial infections have fuzospirochetosis that determines the appearance of acute necrotizing ulcerative gingivitis in the frontal area. It happens in AIDS patients infected with HIV.

Secondary viral agents causing hairy leukoplakia. The hair resembles a soft form of leukoplakia and leukoplakia is a whitish hair growths, which are not removed by scraping. They are located mostly along the edge of language and are somewhat similar to chronic hyperplastic candidiasis.

Ulcers and aphthae in the oral cavity in AIDS patients are more often localized on the sky and caused by Cryptococcus neoformans (yeast, which the kidneys and does not form a mycelium).

Manifestation of AIDS in the oral cavity may be the appearance of painful ulcers on the mucous membrane of the sky and the language associated with cytomegalovirus infection (group of herpes viruses), with a characteristic slow, protracted, recurrences, lack of efficacy of treatment by conventional methods. Observed simultaneously ulcerative herpetic lesions in the mouth zone of the Klein and the nasal mucosa. Established that all the early manifestations of AIDS in the oral cavity are characterized by a constant flow, and their treatment is ineffective.

Reported HIV-defining opportunistic infections considered markers of specific stages of AIDS. Thus, Candida albicans is diagnosed in most patients in stage and lymphadenopathy in the midst of disease. Individuals at risk infection Candida albicans can be considered as an initial symptom of AIDS.

The combination of oral candidiasis and infection caused by different viruses suggests incompetence immune response of the epithelium of the oral mucosa and is regarded as the initial manifestation of AIDS.

Vascular tumors - sarcoma galoshes - vector-borne disease characterized by many pigmented lesions. In patients with AIDS is found primarily in the oral cavity (76%) in the sky and is characterized by exophytic growth, painless, lush texture (in the form of soft bluish nodule), a high frequency of malignancy. Soft tissues are a coloring from brown to bluish-red.

Burkitt lymphoma is localized in the lower jaw. Before its formation patients complain of tooth pain, ulceration of the mucous membrane, the increase in submandibular lymph nodes. X-ray examination at this time reveal bone resorption.

Individuals who abuse smoking, watching epidermoid carcinoma, which is located on the tongue or on the bottom of the mouth. For AIDS to develop other tumors - lymphoreticular sarcoma, squamous cell carcinoma and the like. Radiation and chemotherapy are tagged with tumors in patients with AIDS are ineffective.

Laboratory diagnosis of HIV infection is based on three approaches: the detection of antiviral antibodies, detection of HIV in the test material, identification of specific changes in the immune system.

The most common are techniques that are based on the identification of antiviral antibodies. The most widely used ELISA, by which detected antibodies to HIV. Due to the possibility lozhnopozitivnyh results, all positively reacting sera to be further analyzed by immunoblotting. Virologic method little used in connection with the complexity of virus cultivation. The study of the immune system is used mostly for staging and prognosis, as well as for selecting appropriate therapy.

**7.4. Mycotic stomatitis**

Among the diseases of the oral cavity mucosa caused by pathogenic fungi, dominated by the defeat of fungi Candida. For the most part the agent is C.albicans, much less - C.tropicalis and other species.

**Candidiasis**. Fungi of the genus Candida are widespread in nature. Normally, they are saprophytes and occur in small numbers in the oral cavity in 50% of the population. In the case of reducing the body's defenses and barrier function of oral mucosa and development of dysbiosis these fungi are pathogenic.

State of reduced immune reactivity may be caused by the action of antibiotics, corticosteroids, cytostatics, irradiation, development of malignant tumors, blood diseases, disorders of digestive system, metabolic disorders (diabetes, hypovitaminosis), AIDS and the like.

An important prerequisite of candidiasis of the oral mucosa is also certain oral health: moisture, temperature, aeration, and especially the pH shift toward acidity increase, unhygienic maintenance of the oral cavity, sucking sugary products, predominantly carbohydrate diet, a violation of the rules to use removable dentures, non-sanated oral cavity, the presence of cavities, periodontal pockets and chronic diseases of the oral mucosa.

Downstream candidiasis of the oral mucosa is divided into acute (pseudomembranous and atrophic) and chronic (hyperplastic and atrophic).

Acute pseudo-membranous candidiasis of the oral mucosa develops in infants who are weakened by infectious diseases, bronchitis, indigestion, as well as in preterm infants. In some older children, it is observed with rickets, exudative diathesis, hypovitaminosis and so on. Pathogen is a Candida albicans. Furthermore autoinfection, infection can occur through mother's teat and teat contaminated utensils. In children, mothers pay attention to the appearance of the mucous membrane of cheeks, lips, tongue, palate, white spots or white cheese-like plaque, which is largely accumulated in the retention areas in the form of plaque or film. If it is caused by Candida pseudotropicalis, plaque has a yeasty character.

In the case of light flow freely Candida plaques are removed, while in their place remains cell flushing. In severe plaque cell layers are merged into continuous film surface, eventually thicken and spread to all parts of the mouth. Peel off such a raid is not easy, after scraping underneath reveal erythema and erosions that bleed.

Acute atrophic candidiasis develops oral mucosa hypersensitivity to the fungi genus Candida.

Clinic of acute atrophic candidiasis is characterized by xerostomia, bright red mucous membranes and a sense of burning, the plaque absence. Sometimes the red border of lips there is a very significant amount of non-scabs.

If acute candidiasis is not treated, there develops chronic candidiasis.

Chronic hyperplastic candidiasis develops in people who consume cytostatics, antibiotics, tuberculosis patients, blood diseases, and AIDS. It is characterized by the appearance of white patches that can merge into a solid cheese-like layers, which eventually thicken and become yellowish. Depending on the topography of lesions are distinguished candidal glossitis, cheilitis, cheilitis angularly, palatine. Most often this disease occurs in the mucosa near the corners of the mouth, on the back of the tongue, soft palate. The course of chronic hyperplastic candidiasis accompanied by dryness, hyperemia and edema of the oral mucosa, in some cases the hypertrophy of the papillae of the language.

Chronic atrophic candidiasis often occurs in people who use removable dentures laminar, and is characterized by redness, swelling, dryness and burning of the mucous membrane, emitting a viscous stringy saliva.

Sufficiently frequent form of chronic atrophic candidiasis is Candida cheilitis - defeat the entire surface of the lips or corner portion (Candida Zayed). The disease is characterized by edema, hyperemia, thinning, dryness of the red border, some deepening of cross-cut grooves and the formation and separation from the surface of the lips of scales of different sizes.

The characteristic features of Candida pick a maceration of the skin in areas of the angle of the mouth, cracks, covered with white coating, as well as burning and soreness, which are observed during the opening of the mouth.

Diagnosis of the oral mucosa candidiasis is set according to history, clinical course of disease and the results of microbiological investigations. We must bear

in mind that the mycosis are regarded as markers of different stages of HIV infection.

Important to establish a definitive diagnosis of candidiasis are the data of cytological examination raid, in which apart from desquamated epithelial cells, leukocytes, food residue and various associations of micro-organisms exhibit considerable number pseudo-micelium or cells of the fungus. In acute candidiasis a considerable number of cells of the fungus, which are in the process of division, the chronic - is dominated by filaments pseudo-micelium.

## 8. **Immune-pathological processes in oral cavity**

To the immune-pathological processes occurring in the oral cavity are related hypersensitivity reactions (allergy, hyperfunction of immune system), autoimmune diseases and immunodeficiency states. Immunological status may be congenital, genetically determined and acquired during the life of the individual. By the origin immune-pathological states are divided into endogenous, caused by auto-antigens, lymph proliferation, neurohormonal regulation disorders, and exogenous, which may form due to infectious agents, medications and other factors influence.

*8.1. Hypersensitivity reactions*

In the oral cavity there are all the types of immune-pathological processes. These include hypersensitivity reactions of the generalized I (anaphylactic) type and the generalized II (cytotoxic) type, which are found in cases of drug-hypersensitivity. For example, due to local anesthesia with novocaine.

The most dangerous form of type I allergic reaction is Quinke's edema, which extend to the throat and threatens with suffocation. The mechanism of disease is associated with the antigen - antibody reaction, which occurs on the membrane of mast cells with homocytotropic IgE antibodies class, and accompanied by a massive release of histamine and histamine-like substances to the blood.

Type III hypersensitivity reaction (immune complex) on the oral mucosa is associated with the immune complexes formation. It can be caused by either bacterial or medication antigens. These reactions may occur during periodontitis, necrotizing ulcerative gingivitis, post-herpetic polymorph erythema. They lead to necrosis, arising as a result of vessels wall damage by the immune complex, which are formed inside the blood vessels and deposited on the basal membrane. For example, in necrotizing ulcerative gingivitis plasma cells of gingival connective tissue produce IgG and IgM antibodies, which form immune complexes with incoming microbial antigens. This leads to the complement system activation by the classical path and causes immune damage like Arthus' phenomenon, which appear as the superficial vasculitis, thrombosis and necrosis.

Type IV hypersensitive reaction (cell-type) in the oral cavity arise under the influence of infectious agents. Classical example of such influences is the development of infection allergy due to tuberculosis. HRT also develops due to many

other infections, as well as in contact dermatitis and drug stomatitis. Drug stomatitis is often observed in association with the widespread use of acrylic resins, arsenic, ryvanol, X-ray contrast substances and antibiotics in dentistry. The skin-allergic tests showed that artificial substances that prosthesis are made from, cause allergy in 0,5-5% of cases. On the early stages of ulcerative gingivitis the immune inflammation in the cellular nature is predominant featuring sensible T-lymphocytes and lymphokines-attracted macrophages (Table 8.1).

In stomatitis different types of immune-pathology can be met. They can develop due to sensitization against microbial and drug allergens, as well as autoallergens. Due to presence of hyperergic type inflammatory reaction and increased capillaries permeability is observed the prevalence of alterative inflammation forms on the exsudative and necrotic changes of the oral mucosa areas due to organism sensitization. Depending on this fact there divided serous and ulceratively-necrotic stomatitis.

**Table 8.1. DISEASES OF HYPERSENSITIVITY**

| Allergy Type | Immune-pathologic reactions mechanism | Manifestations in the oral cavity |
|---|---|---|
| Type I anaphylactic shock | Generation of cytotropic IgE. Antigen – antibody reaction with the release of mediators such as histamine (Hi) | Anaphylactic shock (medicamentous), Quinke's edema, atopic dermatitis, urticaria |
| Type II cytotoxic | IgG production against antigens included in the cell membrane, antigen - antibody reaction through complement activation (C) | Cytotoxic reactions due to drug allergy, recurrent aphthous stomatitis |
| Type III immune-complex | Generation of IgM and IgG precipitating antibodies, antigen excess, pathogen response, initiated by immune complexes (IC) through complement and leukocyte activation | Recurrent aphthous stomatitis. Infectious diseases, Arthus' reaction, parodontopathies (necrotizing ulcerative gingivitis, periodontitis), post-herpetic polyphorm erythema |
| Type IV, cellular | Accumulation of sensitized T-lymphocytes (TL), the reaction between antigen and sensitized TL-effectors HRST, the lymphokines production, and cytotoxic reactions with the participation of attracted macrophages | Allergic manifestations of infectious diseases (tuberculosis, actinomycosis, candidiasis, etc.) and autoimmune diseases, contact allergy (medicamentous stomatitis), parodontopathies, necrotizing ulcerative stomatitis, recurrent aphthous stomatitis |

To the immune-pathological conditions with mixed allergy type is related an aphthous stomatitis, due to which are observed II, III and IV types hypersensitivity reactions with the presence of autoimmune process. In the etiology and pathogenesis of aphthous stomatitis a significant importance have the manifestations of HRT to a number of bacterial antigens, and especially to those, occuring in the oral cavity. Particularly, due to making skin allergic probes the HRT reaction may develop to streptococci, staphylococci, E. coli and other antigens or simultaneously to several bacterial antigens. In the blood of people suffering from this disease, there are corresponding antibodies. From the aphths in patients with recurrent aphthous stomatitis, except bacteria, can be extracted herpes simplex viruses and adenovirus type I, which also causes the state of hypersensitivity. Aphth itself is a cellular infiltration containing lymphocytes, which corresponds to allergic HRT reaction immune-morphology, induced to antigens of oral cavity microorganisms.

In the development of recurrent aphthous stomatitis a special role is played by autoantigens which accumulate in the tissues of the oral cavity mucosa in certain conditions. To the antigens of pathologically altered cheek mucosa are added so-called N-antigen. The presence of this antigen in patients confirm the autoimmune concept of the recurrent aphthous stomatitis origin. The disease differs with chronic course and is characterized by periodic remissions and exacerbations. On the oral cavity mucosa appears the ulcerating aphths. Usually, this disease continues throughout the life of the patient, with the most typical manifestation in the age of 20-40 years.

In the occurrence of recurrent aphthous stomatitis a definite role is played by hereditary and constitutional factors: is observed a congenital genetic predisposition to this disease, resulting in rather frequent familiar manifestations of the disease.

### *8.2. The role of immunodeficiency in the oral cavity diseases*

Protection of the oral mucosa is carried out in a high degree with immunoglobulins class A, that is why this class of immunoglobulins deficiency has the greatest interest for dentists. In the defects of T-lymphocytes patients are threatened to be firstly affected with viral and fungal infections. The first signs of immunodeficiency is often a candidiasis stomatitis (yeast infection) or severe long flow of herpetic stomatitis. Congenital defect of cellular immunity with the immune-regulation mechanisms disorder as a result of the T-suppressor genes predominance may be associated with chronic mucocutaneous candidiasis (chronic granulomatous candidiasis).

Immunodeficiency states may be either a cause or a consequence of hypersensitivity disease or autoimmune diseases. For example, IgA deficiency promotes the artificial antigens absorption through the mucous membrane which cause sensitization of the organism.

As clinical manifestations of immunodeficiency conditions can serve the infections caused by opportunistic microorganisms, autoimmune diseases, allergic reactions, tumors. In an oral cavity are often manifested diseases of microbe etiology (stomatitis, gingivostomatitis, etc.) which are the consequences of the primary and secondary immunodeficiency conditions. Thus, in children with immune failure, the oral mucosa is often affected by bacteria, viruses or fungi as a result of the sharp decline of cellular or humoral protection. Such complications occur in children with primary defects - congenital dysplasia of the thymus, acquired Hodgkin, leukemias, selective IgA, lysozyme or interferon deficit. Similar complications are characteristic for patients with secondary immunodeficiency induced by medicinal therapy (glucocorticosteroids, etc.). In the pathogenesis of oral mucosa candidiasis endogenous causes dominate. The chief-one among them is the secondary immunodeficiency that arises in patients with diabetes, leukemia, chronic infections (tuberculosis and others), in the post-surgery period. Indicative is a particularly high susceptibility to candida infection in infants and old people, i.e. in individuals who have age immunodeficiency conditions. Due to the the absence of candidiasis in patients with specific antibodies and a simultaneous decrease of specific skin allergic reaction is possible to predict an unfavorable course of the disease.

9***. Nosocomial infection (NI) in dental clinics***

Dental care for the population is one of the mass medical care types. At the same time to the dentist can come patients suffering from acute and chronic forms of septic diseases, viral hepatitis, HIV-positive. Often, patients are unaware, whether they have some form of disease or are the carriers of nosocomial infections among patients and staff.

**Therefore, all patients should be considered as potentially infected people, including HIV infection.**

**Nosocomial infection** - NI (by WHO) - is any clinically expressed disease of microbial origin, which affects a patient during to his admission to hospital or treatment, as well as an infectious disease of hospital staff (department), as a result of his work in this institution, regardless of the disease symptoms during their stay in hospital or after discharge.

Consequently, the concept of "NI" includes:
- diseases of in-patient department patients;
- diseases of patients, receiving care in polyclinics;
- nosocomial infection of medical staff.

A place of infection- medical institution - gather all 3 types of NI.

All nosocomial infections of bacterial and viral etiology can be divided into two groups:

1. Nosocomial infections caused by pathogenic agents (conventional infectious diseases) as a result of their introduction to the hospital bacterial carriers or people in the incubation period. This group of patients is only 15% of all nosocomial infections.

2. Septic-purulent nosocomial infections caused by numerous group of opportunistic microorganisms. This group accounts 85% of all nosocomial infections of exogenous or endogenous nature.

The source of pathogen infection are patients with severe forms of the disease, as well as carriers of infectious agents virulent strains.

Epidemiologically important sources of nosocomial infections are primarily the infection carriers.

In dental practice, the problem of NI is the greatest. This is due to the fact that different kinds of microorganisms, found in the mouth, are not only of the resident mouth microflora, but also the agents of various infectious diseases. Particularly, in human saliva there are potentially causative agents of diseases such as tuberculosis, diphtheria, influenza, herpes, fungal infections, HIV-infection etc.

It is generally accepted to provide 4 types of infection transmission mechanism:

- fecal-oral;
- airborne;
- domestic
- transmissible

With the health care development and modern treatment technologies introduction has formed a new, powerful, artificially created (unnatural) mechanism of nosocomial infections transmission, associated with medical procedures (injections, surgeries, associated with invasive diagnostic procedures, transfusion, invasive curative procedures). The flow of medical procedures continuously rises. According to WHO, about 30% of medical procedures are not proved by necessity. Accordingly, it should be noted the lack of one-time curves in the dental outpatient practice, particularly, saliva ejectors, burs, all kinds of endodontic instruments, etc.

Some significance has the injection pathway of transmission. Post-injection abscesses may virtually develop after the any drug introduction.

Factors of transmission in the dental practice:

- the hands of health care worker;
- tools, instruments, equipment;
- towels, door knobs, taps;

• medicines;
• air.

The medical staff of dental clinics with insufficient protection and infringement of sanitary-epidemiological regimen expose themselves to a risk of getting infections, with consequent consequences - Table. 9.1.

This is not a complete list of infectious diseases that can be transmitted due to treatment of diseases of the maxillofacial area.

Worldwide, hepatitis B, C, D are considered to be professional diseases of medical workers who deal with patients blood: each year over the world 30 thousand of doctors are infected with hepatitis B, each day one of them dies.

Infectionists indicate that official statistics do not give correct information because some dentists are treated anonymously by hiding the fact of hepatitis B and C, because of fear to remain without patients.

Factors contributing to the growth of NI:

1. Pandemic Viral Hepatitis Group B and C, HIV, spread drug in-take in society;
2. Significant increase of nosocomial strains (resistance to new generation antibiotics);
3. Creation of large hospital complexes with its special ecology;
4. Raising of modern health care "aggressiveness", due to the spectrum of invasive diagnostic and treatment methods extension;
5. Widespread use in public health care practice of expensive medical equipment and thus the complexity of its disinfection and sterilization;
6. Unfavorable ecologic conditions;
7. The increase in people with reduced resistance.

**Table. 9. 1 Possible nosocomial infections consequences**

| INFECTIONS | POSSIBLE CONSEQUENCES |
|---|---|
| AIDS | lethal outcome |
| Candidiasis | systemic organ failure |
| Varicella | Herpes zoster |
| Tuberculosis | disability, lethal outcome |
| Gonorrhea | infertility, arthritis |
| Hepatitis A | virus carrier |
| Hepatitis B, C, D, G | virus carriage, fatal |
| Herpetic conjunctivitis | virus carriage, blindness |
| Herpetic panaritium | virus carriage |
| Infectious mononucleosis | virus carriage |
| Influenza | temporary disability, virus carriage |

| | |
|---|---|
| Legionnaires' disease (Legionellosis) | lethal outcome |
| Measles | disability, encephalitis |
| Rubella | congenital defects |
| Diphtheria | virus carriage, in severe cases lethal outcome |
| Mumps | virus carriage |
| Staphylococcal infection | skin lesions, virus carriage, lethal outcome |
| Streptococcal infection | rheumatic heart disease, lethal outcome |
| Syphilis | CNS disease, lethal outcome |
| Tetanus | often disability, lethal outcome |
| Respiratory infections | temporary disability, virus carriage |

**Nosocomial diseases prevention can be only based on a set of preventive measures, ensuring effective disinfection-sterilization regimen in dental clinics**

### 10. Disinfection and sterilization in dentistry

Disinfection is the process of microorganisms elimination from the infected tools, clothes, surrounding objects with the physical or chemical effects - disinfectants. Disinfection is the removal of known pathogens, except bacterial spores, from objects and surfaces.

Disinfected shall be performed to all tools that do not have contact with the wound, blood or injecting medicaments.

Disinfection is used in cases where there is a need to remove the dirt that contains infectious agent from the surface, which can not be sterilized (hands, floors, walls, equipment). At the same time there is no guarantee of full infection disposal (possibly preserved spores of microorganisms resistant to the effects of the environment). Therefore, all equipment and tools for invasive intervention should be sterilized.

Products used during septic operations or surgical manipulations in infectious patients undergo disinfection before presterilization cleaning and sterilization. In addition, tools for medical purposes after surgery, injections, etc., in people suffered from hepatitis B or hepatitis with unspecified diagnosis (viral hepatitis), as well as carriers of HBS-antigen should be disinfected.

Sterilization is the destruction of all microorganisms, including spores, by exposure to heat, radiation, chemical substances, filtration. Sterilization should be performed for all products that come into contact with a wounded surface, in con-

tact with blood or commuting with injectable medications, and certain types of metal tools that during operation in contact with mucous membranes and can cause its damage.

### 10.1. The main traditional sterilization methods in dentistry

Characteristics of sterilization methods is presented in Table. 10.1.

**Table. 1.10 BASIC METHODS OF STERILIZATION**

| | **Steam** | **Air** | **Chemical** |
|---|---|---|---|
| Temperature | 111 - 132 ˚ C | 160-180 ˚ C | 18-50 ˚ C |
| Time | 5-20 min | 30 min | 240 min |
| Full cycle | 6-30 min | 75 - 120 min | 300 min |
| Sterilizing agent | saturated steam under excessive pressure, temperature | dry heated air temperature | Chem. disinfectants: $H_2O_2$ dezoxon alaminol, persept etc. |
| Equipment | autoclave | dry heat cabinet | no special equipment |
| Applicability | metal, glass, textiles, rubber, PVC, latex | metal, glass | metal, glass, polymers, rubber |
| Advantages: | Short full cycle, the ability to sterilize heat-sensitive products | Low cost of equipment | No special equipment |
| Disadvantages: | Higher cost of equipment | Long cycle risk of damage by high temperatures, high power | Long cycle-processing, the cost of disinfectants in continuous operation above the cost of equipment |

The large advantage over the others has a steam sterilization method, or autoclaving. Short cycle and low temperature allow the use of this method for sterilization of almost all instruments and consumables used in dentistry.

To ensure smooth and quality sterilization process function requires:

1) **Proper preparation of instruments for sterilization**. It is very important to carry out thorough cleaning of instruments before sterilization, remove small particles remaining after surgery.

2) **Good water quality**. For steam sterilization has to be applied only high quality water that will avoid damage the autoclave and sterilized material. It can be distilled or demineralized water.

3) **Sterilization quality control**. The effectiveness of sterilization process sheck is carried out by pressure, temperature, and duration of the cycle parameters monitoring. The results are recorded in the daily observations journal, which are automatically carried by an autoclave. The ratio also requires a bacteriological monitoring every six months.

4) **Proper loading**. One of the main conditions for an adequate sterilization degree is proper equipment loading. This means that heavy and bulky items should be on the bottom and not interfere with the free circulation of steam, condensate removal.

### 10.2. Other methods of sterilization in dentistry

Currently in dentistry other methods of sterilization are becoming more spread: glasperlen sterilization and low-temperature methods - gas, plasma, ozone and radiation.

Glasperlen method is intended for quick sterilization of small metal tools with no cavities, channels, and interlocking parts. Method is very simple - the tool is immersed in the medium of small glass beads, heated to 190 ° - 290 ˚C (so that over the working surface of the tool remained layer of balls at least 10 mm) at 20 - 180 seconds, depending on the size and weight of the tool. This method is used mainly by dentists for fast sterilization of small instruments - hog pulpoextractors, root needles, diamond heads, etc., as well as larger working parts - probes, floats, shovels, spatulas, etc. You can also sterilize acupuncture needles. Method advantages are the short sterilization time and lack of supplies.

For thermolabile medical devices (endoscopes and tools to them, dialyzers, catheters, etc.) gas sterilization is the most acceptable method. For this purpose are used chemical compounds, which have an unconditional sporocide action: Ethylene oxide, methyl bromide, a mixture of ethylene oxide and methyl bromide (EM mixture) and formaldehyde.

Despite the fact that ethylene oxide is a toxic substance (single exposure manifests itself as a low-hazard substance 4-hazard class, with constant exposure - a substance the 2nd class of danger), it is extremely popular as a sterilizing agent. However, its toxicity makes to conduct the degasation of sterile tools (with afterburning of released ethylene oxide - it is a very burning).

Gas sterilization is much more complicated method than traditional methods of sterilization by steam and hot air. In this case, it is necessary to maintain a certain level of temperature strictly, humidity, concentration of sterile gas, pressure and exposure.

The most famous ethylene oxide sterilizer is "Kombimat" MMM (Munich Medical Manual). Sterilization is carried out at a temperature of 42 - 55 ˚ C for 60 - 90 minutes. The results of practical usage shows a significant superiority of ethylenoxid sterilization method over alternative in versatility, efficiency, maintainability and technical support. At the conclusion of experts, the use of ethylene oxide sterilization allows sterilization to ensure that the total volume of thermolabile instruments and tools available in the health facilities to reduce capital equipment costs, operating costs for the purchase of supplies, to increase equipment productivity, turnover of sterilized prodcts and to prolong their operation .

Sterilization of thermolabile products with formaldehyde is at second place after the ethylene oxide. The optimum temperature range for formaldehyde sterilization should be 60 - 800 ° C, pressure - from 0,25 to 0.475 bar, at a concentration of formaldehyde from 8 to 15 mg / liter. Actually formaldehyde is used at a concentration about 30 mg / L, exposure to 60 minutes for a total cycle time is 3.5 hours (including degassing of sterilized products (aeration)).

Not all products, sterilized with ethylene oxide, can be sterilized with formaldehyde. Recommended with the exception of optical instruments, implantable products, endoscopic equipment.

The most popular device for formaldehyde sterilization is the Euro-Formomat " produced by the same concern," MMM ". So-called plasma sterilization, acting with streams of hydrogen peroxide in combination with low-temperature plasma, which is the decay products of hydrogen peroxide (hydroxyl groups OH, OOH), formed under the influence of electromagnetic radiation, emitting visible and ultraviolet radiation, is currently in its infancy. Hydrogen peroxide and plasma does not possess the penetrative abilities as ethylene oxide, but they have great advantages - breaks down into nontoxic products - water and oxygen, having no harmful effects on the environment. Sterilization is conducted at the temperature - 46 - $50^0$C for 54 - 72 minutes. Currently, there are no generally accepted international standards for this method. There are certain restrictions on sterilization materials containing cellulose and rubber. The high cost of equipment and supplies narrows the spectrum of this method application. In addition, sterilization of hollow multi-product requires the use of additional consumable devices, further increase in the cost of the sterilization cycle.

Currently, the most well known system of plasma sterilization - STERRAD produced by ASP (USA), consisting of three devices of different sizes: 50, 100 and 200 liters. Over 95% of medical devices may be subjected to sterilization in these devices.

Not be sterilized by plasma products from polyamide, some sulfides, surgical linens, dressings, articles of pulp, powders, liquids.

Thus, apparently using the plasma method is the most appropriate for the sterilization of thermolabile unique products available in a single item and used repeatedly during the day, while the daily routine sterilization still need more accessible and cheaper method. One of the highest oxidation potentials is ozone. That's why he has long attracted the attention of specialists concerned with the degree of sterilization. For many years, ozone is used to disinfect drinking water and air, and only recently it has been suggested for sterilization in medicine. Sterilization is an ozone-air mixture produced by ozone generator from air. However, oxidizing ability of ozone limits its range of applicability. In contact with them can be damaged steel products, copper, rubber etc. In addition, ozone is toxic, and devices available today do not allow us to protect staff from contact with it. An important circumstance is that the repeatability of the method is still discussed. To monitor the process, there are only the first class indicators (witnesses).

Sterilizing agent due to radiation sterilization is penetrating gamma- or beta-radiation. The most widely used gamma-emitting isotope, cobalt-60 isotope is less cesium-137, due to its low-energy radiation. Beta-emitting isotopes of general-use are extremely rare, as the beta radiation has a much less penetrating power.

Efficiency of radiation sterilization depends on the total dose of radiation and does not depend on time. Average lethal dose for microorganisms is always the same, whether there was exposure at low intensity for long periods of time or for long at a high intensity. Dose of 25 kGr (2.5 Mrad) effectively guarantees the destruction of high-resistant spore forms of microorganisms.

Radiation sterilization has a number of technological advantages: high level of microbial inactivation, the possibility of sterilization of large quantities of materials, process automation, the possibility of sterilization materials in any sealed packaging (except pa-dioneprozrachnoy). The important fact is that the temperature of sterilized products during sterilization does not increase.

The radiation technique is used for industrial sterilization of disposable products from polymeric materials, cutting tools, sutures and bandages, and some medications.

In hospitals radiation sterilization does not applied in connection with a large expensive plants and by considerations of safety technique.

## 10.3. Methods for the sterilization effectiveness monitoring

### 10.3.1. **Presterilization cleaning control**

Control of presterilization cleaning quality of dentistry tools is spend due to plan for 1 time in 2 years and epidemic indications by setting of azopiramic or amidopirin samples, as well as by raising the phenolphthalein test. Self-control in health facilities is carried out every day, in offices - at least 1 time a week old by the shay nurse.

Presterilization cleaning quality control is carried out prior to sterilization.

**Azopiram probe**. Azopiram detects the presence of blood traces, pyroxidases of plant origin, chlorine, washing powder with bleach and rust. Azopiram contains 10% Amidopyrine, 0,1-0,15% aniline hydrochloride and 95 ° ethyl alcohol, is storaged in a tightly closed bottle at room temperature (10-23 ° C) not more than 1 month. Before staging azopiram sample there mixed azopiram and 3% hydrogen peroxide solution in equal volume amounts of this reagent and work for 1-2 hours. Test with azopiram is 10 times more sensitive than amidopirin.

**Amidopirin probe**. Working solution for the amidopirin test is a mixture of equal amounts of 5% amidopirin alcohol solution, 30% acetic acid and 3% peroxide solution. This test determines the quality of cleaning tools from the blood remnants.

**Phenolphthalein test** reveals the presence of alkaline cleaning agents residuals. For the reaction there is used 1% solution of phenolphthalein.

On a controlled product is applied 2-3 drops of reagent and rub it with tampon. With a positive test occurs green-violet staining, quickly, within a few seconds, turning into pink-lilac and brownish. Staining, coming later than 1 minute is not considered. Brownish color appears in the presence of rust, and chlorine-containing oxidants, in other cases, staining is pink-violet.

With a positive amidopirin test the staining is blue-green. Phenolphthalein test due to not washed components of synthetic detergents gives a pink coloration.

For positive samples, the batch controlled items under-lying re-treatment until negative results.

Controlled : in a central sterilization - 1% of each product names processed per shift, in departments - 1% while processed products, but not less than 3 units (before each loading of medical devices for sterilization).

**10.3.2. Control the sterilization completeness**

In the complex of medical products sterilization measures the organization and conduct of monitoring effectiveness values a lot. Used current methods and means of control is not always possible to identify defects of sterilization, which entails raising the level of nosocomial infections.

Monitoring of the sterilization effectiveness equipment is carried out by physical, chemical and biological (bacteriological-logical) methods. The reliability of these methods varies. Physical and chemical methods are used for operational control and allow you to monitor compliance with the parameters of modes steam, gas, and of the air sterilization, temperature, pressure, exposure. The disadvantage of these methods is that they can not serve as proof of effective sterilization. Reliable for determining the efficiency is only a bacteriological method.

**Physical methods**

Physical methods of control are carried out by means of measuring temperature (thermometers, thermocouples), pressure (manometers, manovacuumetry) and time (timers). Modern sterilizers are equipped by recording devices, fixing some parameters of each sterilization cycle.

**Chemical methods**

Based on the use of indicators to monitor critical parameters of sterilization process. Critical parameters are: for the steam sterilization method - temperature, time of the given temperature impact, water-saturated steam, for air sterilization method - the temperature and exposure time of the temperature, for gas sterilization methods - the concentration of gas used, temperature, time exposure, relative humidity, for radiation sterilization - a complete absorbed dose.

Chemical indicators divided into six classes.

Indicators of **1st class** are indicators (“witness”) of the process. An example of such indicator is termoindicator tape, glued prior to sterilization or sterilizing textile packaging box. Change the color of tape indicates that the package has

been exposed to the sterilization process. The same indicators may be placed in sets of surgical instruments or the surgical linen.

**2nd class** indicators do not control the parameters of sterilization, he assesses the effectiveness of air removal from the chamber steam sterilization of the torus.

Indicators for **the 3rd class** are indicators of a single parameter. They assess the maximum temperature, but do not show the time of its impact (benzoic acid to control the steam sterilization, sucrose, hydroquinone, and a number of other substances - to control air sterilization).

Class 4 - a multiparameter indicators. They contain stains, which change color with combination of several parameters of sterilization, most often - the temperature and time. Examples of such indicators are termo-time indicators for air sterilization monitoring.

**5th class** - integrating indicators. These indicators respond to all critical parameters of sterilization method. Characteristic of this class indicators is compared with the high-resistant microorganisms inactivation.

**Class 6** - indicators-emulators. These indicators should respond on all control critical parameters of sterilization method.

**Biological method**

Along with the physical and chemical there used bacteriological monitoring method of sterilization. It is designed to monitor the effectiveness of equipment sterilization. Currently, control of air and steam sterilization used samples of land containing microorganisms highly resistant to sterilizing factors effect. However, the resistance of microorganisms in different samples varies, which does not standardize the results of control.

Currently, for the bacteriological control studies are used bioassays with metered amount of controversy test culture. Monitoring the effectiveness of sterilization using bioassays recommended 1 time in 2 weeks. In foreign practice there used biological testing at least 1 time a week.

In some cases, there is a need for control with the help of bioassays each sterilizer load. First of all, we are talking about the sterilization of instruments used to perform complex surgical interventions that require the use of highly powersterile materials. Each download of implantable products should also be subjected to bacteriological monitoring. In this case, utilization of the sterilized material is delayed until the results of negative control. The same principles in determining the periodicity of control are encouraged to adhere of gas sterilization, which is more complicated in comparison with other methods.

### 10. 4. Maintenance of anti-epidemic regime in the dental office

**Processing facilities dental office**

1. To keep the temperature and humidity in the office, use the air filters.

2. Before taking patients to conduct wet cleaning with the use of various disinfectants. Wipe clean with a cloth 2 times with an interval of 15 minutes with disinfectant solutions (3% chloramine, 6% hydrogen peroxide, 70% alcohol, etc.) of the surface of all objects in order to destroy vegetative forms of bacteria.

3. Then, switch on an ultraviolet device for annihilation of airborne and surface bacteria. (Calculation of bactericidal lamps in 2.5 W at 1 m for 1 hour).

**II. Disinfection, sterilization** in the dental office

1. Disinfection should be subjected on all of the equipment, not having the contact with the wound or the blood (using a solution of 6% hydrogen peroxide, 3% bleach, 70% alcohol, etc.)

2. There needed a complete sterilization of the material. Sterilization shall be subjected all the objects in contact with the wound surface, blood, and certain types of medical instruments that are used in contact with mucous membranes and may cause the damage.

3. Before sterilization all the tools, burs etc. should be soaked in detergent solution for 40-50 minutes to clear them from protein, fat, mechanical contaminants and pharmaceuticals. Cleaning should be done jet, rotary methods with the use of ultrasonic baths, which are placed in 6% solution of hydrogen peroxide and detergent substance (powder, "Lotus", "Progress", etc,).

4. Depending on the sterilized material can be used thermal, chemical or gas sterilization methods. Preference should be given to thermal methods as the most reliable. However products from rubber, polymers, optical equipment, some tools, heart-lung machine, artificial kidney can not undergo thermal processing.

5. Sterilization by steam under pressure is carried out in autoclaves. Mode of sterilization can destroy not only bacteria, spores, and viruses such as hepatitis B virus (serum hepatitis) and HIV. Pressure of 2 atm. (132 ° C) for 1 hour. Sterilization is carried out in sterilization boxes, slut, marked bags of wet strength paper. This method is recommended for products from metal, glass syringes, rubber, textiles, some of the polymers.

6. Some tools (especially cutting) is recommended to sterilize in glass-perlen sterilizer at 240 ° C for 5 - 10 seconds.

7. Sterilization by dry heat in a dry heat oven is held at a temperature of 180 ° C for 150 minutes (2.5 hours). Duration of exposure can also destroy the hepatitis B and HIV. Sterilization is the dry product in a paper package (shelf life 20 days). Sterilization is possible without the packaging, but then the product should be used immediately after sterilization.

8. Chemical method of sterilization means that the products are expressed immersed in a solution of 6% hydrogen peroxide for 6 hours or in a camera with 40% formaldehyde steams in ethanol for several hours, depending on the sterilized material.

9. Recently, due to the advent of new instrumentation, has become more widely used gas sterilization method. It is performed in special chambers or gas

sterilizers desktop, where there are ethylene oxide or a mixture of ethylene with methyl bromide. Sterilization occurs at a temperature of 35 ° C to 42 ° C for several hours or days in special packages marked:

-if contact with blood, tissue was less than 30 minutes, then metal products are sterilized for 4 hours, rubber, plastics - 24 hours.

- if contact with blood, tissue was more than 30 minutes, metal products are sterilized within 24 hours, rubber, plastics - one week, the light-heart-kidney unit within 2 weeks. Such prolonged sterilization is associated with HIV and viral hepatitis.

10. In order to prevent the serum hepatitis B and HIV is recommended to use single-use items (syringes, injection needles, blood transfusion systems, etc.)

**10.5. Asepsis and antisepsis in dentistry**

In dentistry more than in other areas of medicine, strict observance of the aseptic and antiseptic rules is needed, as any dentistry procedure if conducted on infected tissues. Not only the removal of carious tooth or root canal treatment, but a simple examination of the patient oral cavity is associated with infection of used for this purpose tools. To exclude the transfer of microorganisms from one patient to another, as well as to prevent infection of healthy tissues located deeper, the work is allowed only with sterile instruments.

All medical equipment and care facilities, depending on the degree of patients infection risk, associated with the use of these items can be divided into three categories: 1) "critical " tools and objects of care, 2) "half-critical" tools and objects of care, and 3) "non-critical" tools and objects of care.

"Critical" items - the tools,which enter the bloodstream and normally sterile body tissue. They include, for example, surgical instruments, cardiac catheters and implants. In the case of contamination of any microorganisms, there is a significant risk of patients infection. Thus, the tools and objects relative to this category must be sterile.

Particular problem is the thermolabile instruments, which can not be sterilized, such as laparoscopes. Sterilization by ethylene oxide gas or liquid chemical agents requires a long time, so in many hospitals for this type of tool is used high-level disinfection. However, this procedure does not eliminate all bacterial spores, which increases the risk of patients infection.

"Half-critical" consider the objects in contact with the mucous membranes of simple or damaged skin (such as inhalers, bronchiscopes and endoscopes). Half-critical instruments should be subjected thorough cleaning followed by disinfection, which removes all bacteria and spores of most bacteria.

"Non-critical" items come into contact only with intact skin (e.g., blood pressure monitor cuff, stethoscopes, bedpans). These items should be sterile and may of them contain bacterial spores on its surface.

Depending on the type of medical supplies and purpose of its use, disinfect high-level (THL), intermediate (DIL) and low levels (DLL).

In carrying out the DHL there killed all microorganisms, except bacterial spores. This disinfection method should be used for all “half-critic” objects. For the DHL there used glutaraldehyde, chlorine dioxide, 6% hydrogen peroxide solution and the means by peracetic acid. These chemicals can be used for sterilization function, but the exposure is greatly increased.

During the DIL there killed vegetative forms of bacteria, including mycobacteria, most viruses and fungi (except for bacteria spores). Small non-lipid viruses (e.g. enteroviruses, rhinoviruses) are more resistant to bactericide substances, while a large lipid viruses such as adenoviruses, hepatitis B and HIV are usually killed during the DIL.

DIL should be used for "non-critical" items. This method can also be used for disinfection of some "half-critical" items, such as hydrotherapy baths for patients with damaged skin. To DIL there referred compounds based on 70% and 90% ethanol or isopropyl alcohol, chlorine, some tools and phenol-containing substances and iodophors.

During DLL there killed vegetative forms of the most bacteria transitions form, viruses and fungi. Bacterial spores, mycobacteria, and small non-lipid viruses are not killed. DLL can be used only for "non-critical' tools. Low-level disinfectants include preparations based on quaternary ammonium compounds, some iodophors and phenol-containing medicaments.

In some cases, the hospital may need to be iterated in the use of disposable medical supplies. These situations include: interruption of the hospital supply, the use of "noncritical" items instead of the "critical", or when savings need.

However, with repeated use of disposable equipment, a number of unanswered questions concerning the toxicity of residuals, pyrogens formation, the functional reliability of equipment and its structural integrity, legal and ethical issues, as well as the risk of patients infection. It should be avoided the multiple use of disposable equipment and limited only to cases provided by the manufacturer, respecting the special instructions for reuse, quality control and application time limit.

In dentistry at the current work, as in general surgery, is applied high-temperature sterilization: a) boiling in water (the majority metal and glass instruments), b) processing with steam under pressure in an autoclave (bandages, linen, gloves), c) flowing steam sterilization (underwear, gloves), d) tool sterilization in the oven or special dry-air sterilization analyzer, d) burning (flambing). In addition, there used cold sterilization using chemical agents that provide bactericide action.

As in the therapeutic and prosthetic dentistry office basic dressings are cotton balls and rollers. Cotton balls manufactures by nurses rolling small pieces of cotton wool between the palms, moisted with glycerin. Harvested enough cotton balls are placed loosely in a special drum (Bix) for sterilization, which is made in autoclave for 30 minutes at 120 ° C and 1 atm. Cotton rolls are made of cotton wool, winding spread-eagled on his left hand a piece of wool on the handle of the

probe. Pen probe having a faces (to prevent slipping wool), is kept in right hand and rotates until the roller forms. Preparation of cotton bristles at the root tip exercise I and II fingers of his left hand. To do this, take some floss and plain them to obtain a thin layer. Received cotton bristles with a brush on the end then sterilized in an autoclave.

Sterilized cotton and gauze balls, cushions, napkins, and cotton turundas before use are stored in closed slut. Sterility of dressing material is difficult to maintain because of frequent slut opening during the day, so some authors suggest packaging cotton balls, rollers and cotton bristles into paper bags and sterilize them in an autoclave. Paper bags with 5-6 cotton balls, cotton 4.6 turunds and 6-7 cotton swabs are wrapped, as powders. Each pack after sterilization is intended for one patient, the rest of the package is remain in slut. Despite the everyday multiple opens up of slut, dressing in such conditions, retains its sterility within a week.

Utensils used for preparation of an anesthetic solution before the boiling is washed with hydrochloric acid to neutralize the alkali, which can get to the dishes. Beakers, applied for patients mouthwash is also processed with (inside and outside) the concentrated solution of this acid, and then washed them with hot water in the sink and rinse in hot water (using forceps). Then treated glasses put on the big clean tray upside down.

Used glass plates for medicaments are cleaned with knife from cement and artificial dentin, then washed in the hot water, wiped with a solution of hydrochloric acid, rinsing in hot water and wiped with a clean towel. Ren-form basins for a set of tools are sterilized by boiling in sterilizers of large size. Method of firing with alcohol is expedient to apply only to sterilize large basins, used for hand washing (flambing method).

Some difficulty is associated with **tips sterilization**. Dental handpieces in contact with saliva and blood of patients, and therefore should receive the most careful handling. They can not be sterilized by boiling-water. Sterilization of tips in a dry land enclosure is not provided, so that their sterilization by autoclaving is carried out optimally - in the apparatus with the function of predvacuum pulsing and vacuum drying, which allows you to remove contaminated air not only from the sterilizer chamber, but also from all the cavities of sterilized instruments, providing the subsequent flow in these hot steam. Microorganisms are destroyed only in contact with hot steam, and if in lumina of tools or tips will be infected contaminated air, the sterility is not assured.

Used in burrs are recommended for 2 hours to be put in a 2% chloramine solution, or 6% hydrogen peroxide solution. They were then purified using a metal brush on the dentine chips, put into a tray for tools and washed with hot water and soap under the faucet. After washing under the hot running water, add 1% solution of soda and boil for 20-30 minutes in a tray. Then the water is drained from the tray, at one end of a sterile towels there are spilled the burrs, and with the other one, dipped in ether, wipe them. In order to dry the acceptable short-

term burning them in a tray over the flame of a gas burner, and then transmit them to unfolding in sterile Petri dishes.

**Dental mirror** after a few quick boiling are fading, so they are made out and only the handle is boiled. The mirror itself is sterilized by cold processing in an alcohol solution or hydrogen peroxide. After use, thoroughly wash the mirror with soap, dipped to 1-2 sec. in the boiling water sterilizer, after 'which is placed in a glass with alcohol or 6% hydrogen peroxide solution. In alcohol-mirror should be at least for l 1/2-2 hours, and in a solution of hydrogen peroxide - at least 40 minutes.

In recent years, dentistry is increasingly used method of dry-air instruments sterilization. After washing with a brush, we scrap it in warm water tray with a set of instruments installed in dry-air sterilizer. Typically, sterilization is made at 160-180 ° C for 30-40 min. However, syringes can not be sterilized, as can unsolder the metal end of a glass cylinder.

### 10. 6. HIV infection prevention in dentistry.

Given the clinical features of AIDS dentist may be the first physician to suspect this disease. Although the saliva of infected AIDS, HIV is a slightly quantitative, the dentist should be aware that he (like other professionals, who contact with body fluids of AIDS patients) is a member of a professional risk group.

For dentists there is a risk of HIV contracting through AIDS patients or carriers casual bites, from entering their saliva on broken skin or mucous membrane of the physician, as a result of injury by the tools used to treat patients. In addition, the use of dental drill turbine may cause nosocomial infections such as AIDS and hepatitis B.

Because of the possibility of dentists contact with AIDS patients or virus carriers, they recommend such measures to prevent it:

- Receiving information about possible risk factors in patients;
- Antiseptic skin and work in rubber gloves;
- The application of (if possible) tools, materials, and single-use needles; perfect sterilization and disinfection materials, which are used repeatedly.

Specialists suggest that adequate prophylaxis gives the opportunity to avoid HIV infection, even in a risk group. Because personal protection should be carried out by all medical personnel (use rubber gloves, special glasses, plastic masks, special gowns and hats).

Necessary to avoid minor tools injuries that come into contact with blood and saliva of patients. For a significant decrease of aerosol formation is not recommended to use a turbine boron-machine, it is better to use terminals with fewer turnovers. Significantly reduces the aerosol pollution of the working area by using kopherdam. Gloves and masks are desirable to be changed after each patient, at least - every hour. Hands should be washed under running water after the examination or treatment for each patient and processed the 4% chlorhexidine.

Gowns should have as little as possible sutures ammounts and tightly close the chest. It is advisable to use disposable gowns made of synthetic fabrics.

**Safety instructions when working with biomaterials, potentially infected with HIV**

**I. General Provisions**

AIDS is a disease with a fatal outcome, developing as a result of abnormalities in the immune system. The incubation period of disease is 5-10 years. Cases of spontaneous recovery or cure for AIDS are not mentioned. Pathogens are the T-lymphotropic retroviruses HTLV-3 (HIV-1) and HTLV-4 (HIV-2). Transmission - with the blood (cells, serum), sex, from mother to child through breast milk. Non-resistant viruses are killed after a 30-min exposure to 20% solution of ethanol. Therefore, all the measures for the prevention of lesions, hepatitis viruses, and are sufficient to protect against HIV infection. When working with infectious material must comply with three basic rules: to change a dressing gown, work in gloves and wash hands frequently.

**II. Work Regulation**

1. Work in the department should be performed in specifical lab coats. Keep them in a wardrobe at the entrance to the department, put on before work and take off when leaving the office.

2. All furniture and equipment in the department should have a plastic or metal cover that can be easily disinfected. The tables should be tanked with a disinfectant solution (70% solution of ethyl alcohol).

3. Tubes of biomaterial must be marked, carefully covered up (plugs, wax, plaster) and delivered in the unbreakable containers that can be easily disinfected.

4. All work associated with acceptance of biomaterial and the procedure, you must perform in disposable gloves. During operation, all injury of the hands must be closed (plaster).

5. Centrifugation tubes with a biomaterial should be carried out in a centrifuge having a separate cover for each glass.

6. While working with biomaterial there should be used the means of the eyes degence from the liquid (protective glass shield, glasses).

All disposable materials, contacted with the biomaterials (tubes, adhesive paper, gloves) should be immediately after use threw into a special container with des. solution (70% ethyl alcohol). After the completion of all work to wipe the surface (tables, equipment) with swab dipped des. solution. All used in the formulation of disposable materials (test tubes, gloves, caps, tubes, boards, etc.) have o be soaked in des. solution.

Methods and means of various objects disinfection, medical and other appointments after a survey of HIV-infected individuals are presented in Table. 10.2.

**Table. 10.2. METHODS AND TOOLS DISINFECTION AFTER SURVEYS IN HIV-INFECTED PERSONS**

| Product names | Disinfectant Agent | Solution conc. | Exposure | Method of processing |
|---|---|---|---|---|
| The surface of the laboratory tables | chloramine<br>hydrogen peroxide,<br>ethyl alcohol,<br>sulfohlorantin | 3%<br>6%<br>70%<br>0,5% | | In case of blood contamination of the table surface - immediately double-wipe cloth with one of the solutions. |
| Pipettes, test tubes, melanzhers, kukov's plates, glasses and other laboratory glassware | chloramine<br>hydrogen peroxide and detergent | 3%<br>6% | 60 min<br>60 min | full immersion, followed by washing under running water |
| Syringes, needles, probes, catheters | chloramine B,<br>boiling | 3% | 30 min<br>30 min | preference instruments single use, before disposal disinfected in 3% solution of chloramine |
| Mirrors (dental, guttural, nasopharyngeal) | Hydrogen Peroxide | 6% | 60 min | Total immersion, followed by washing with running water |
| Blood residuals (Clotted blood, serum) | dry bleach, two-thirds-base calcium salt hypochlorite | 1:5<br>1:5 | 60 minutes<br>60 min | collected in a bowl, poured one of these medicaments and mix thoroughly |
| Spatulas, wooden, metal, | chloramines,<br>boiling | 3% | 60 min<br>30 min | Total immersion |