ORAL CAVITY: MICROBIOME, LOCAL IMMUNITY, DISEASES

Learning guide

Kharkiv International Medical University

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Oral cavity: microbiome, local immunity, diseases: learning guide for applicants of higher medical education. institutions of 1V level accreditation. Kharkiv, 2026. 91 p.

The textbook on the discipline "Microbiology, Virology and Immunology" corresponds to the program of the Ministry of Health of Ukraine and is recommended for applicants to higher medical educational institutions of the IV level of accreditation in the specialties "Dentistry", "Medicine" of the educational and qualification level "Master".

The textbook contains sections on medical microbiology that study the oral microbiome and its role in the development of pathological processes. The main types of microorganisms of the normal oral microbiome, pathogenic bacteria, fungi and viruses are described, as well as the most up-to-date information on methods of microbiological research of oral diseases. The issues of local immunity of the oral cavity and its disorders in pathological conditions are considered.

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CONTENT

Preface

From a biological point of view, the human oral cavity is an ecological environment for the microbiota that lives in it. The microorganisms form a specific biofilm that plays an important role both in physiological processes and in the occurrence of pathology. The permanent microbiota of the oral cavity is associated with caries and periodontal diseases. According to modern ideas, it is microorganisms that are the cause of these diseases

It has been determined that 400-500 species of microorganisms are specific to the oral cavity. Such a rich diversity of microflora in this biotope is facilitated by very favorable conditions: optimal temperature and humidity, a sufficient amount of organic matter, the reaction of the environment, etc. It is believed that the vast majority of its representatives are resident flora, which has a stabilizing effect on the existence of the general biofilm of the oral cavity. However, a number of species, being residents, can participate in pathological processes.

Nature provides for a symbiotic relationship between the host organism and the microbiota. Moreover, the microorganisms are integrated into the homeostasis, physiology, metabolism and immune response of the host organism. The main functions of the microbiome are considered to be metabolic, protective and trophic, due to which bacteria contribute to the digestion of food, provide colonization resistance of the organism, suppress the growth of pathogenic microorganisms and stimulate the immune response. When the adaptive mechanisms of local and general immunity of the host organism are disrupted, its inadequate adhesive and colonization abilities are revealed.

Features of the manifestation of pathological conditions in the form of caries, diseases of the mucous membrane and periodontal disease are associated with changes in the relationship between the host organism and its microbiome. Resident microflora becomes pathological only if the conditions of its existence in the host organism are disturbed. These disturbances are usually associated with changes in the physiology of the host organism.

The study of the oral microbiome provides an understanding of it as a structured system with differentiation of functions between its representatives and a significant impact on the state of the biotope it inhabits and on the entire organism as a whole.

Chapter 1. CHARACTERISTICS OF THE ORAL MICROBIOME

The oral microbiota contains associations of symbiotic microorganisms that belong to different taxonomic groups and are maximally adapted to exist in the conditions of a macroorganism. Normally, the microbiocenosis is represented by bacteria, fungi, spirochetes, and protozoa. Oral microorganisms have several mechanisms of adaptation, existence, and reproduction in the oral cavity. Conventionally, the oral microflora can be divided into several types. The dominant ones are "characteristic" groups of microbes, the number of species of which is small, but the total number is more than 95% of all microbial cells. This is the so-called indigenous (obligate, autochthonous, resident) microbiota, which is specific to a given biotope.

The second group is facultative (additional, accompanying) microbiota, the number of which does not exceed 5% of the total number of bacterial cells. The third group is transient microbiota (allochthonous, accidental, residual), which accidentally enters the oral cavity, and the number of which is less than 0.1%. These are migrants from other biotopes (nasopharynx, intestines), as well as from the environment. Sometimes it may include pathogenic microorganisms that will contribute to invasion and can be the cause of the disease. Transient microorganisms are not capable of long-term survival in the body and therefore are an optional component of the microbiota.

Despite the constancy of the species composition of the microbiota, the number of microorganisms can vary significantly during the day. Significant changes occur when the body's protective functions decrease. Violation of the barrier functions of the mucous membranes can cause such changes in the microbial composition that cause autoinfectious processes.

Thus, the oral microbiota is specific, differing from other biotopes in both composition, quantity, and functions. The dominant place belongs to bacteria, which are distributed heterogeneously: the content of microorganisms in the oral cavity is from 4 to 5 billion in 1 ml, and in dental plaque - up to 1 trillion in 1 g of material.

Factors influencing the formation of the oral microbiome: 1) anatomical and physiological state of the oral mucosa (mucous membrane folds, gingival pockets, exfoliated epithelium); 2) temperature, pH, redox potential of the oral cavity; 3) saliva secretion and its composition; 4) condition of the teeth; 5) normal functions of salivation, chewing and swallowing; 6) hygienic condition of the oral cavity; 7) composition of food; 8) state of nonspecific resistance of the organism. Each of the above factors affects the quantitative and qualitative composition of the microbiota in different biotopes of the oral cavity and contributes to maintaining a balance between microbial associations.

The role of normal microbiota in the oral microbiocenosis:

1. antagonistic effect on pathogenic species of bacteria due to the synthesis of bacteriocins, hydrogen peroxide, alcohols, fatty and lactic acids, pH changes, competition for carbon and energy sources.

2. Synthesis of vitamins (group B, K, folic acid) and essential amino acids.

3. Stimulation of lymphoid tissue and immune reactions, support of the activity of nonspecific defense factors (complement, lysozyme, etc.), which provides immunological protection of human body.

4. Stimulation of secretion of mucous and salivary glands.

5. Synthesis of biologically active substances (enzymes, histamine, mediators, etc.), which stimulate various physiological processes.

6. Detoxification of endogenous and exogenous toxins.

7. Enzymatic breakdown of biopolymers, including dietary fibers (cellulose, glycans, galactosides, etc.), the breakdown products of which are involved in glucogenesis, lithogenesis, and cholesterol synthesis.

Characteristics of the main representatives of the oral microbiota. The most numerous are bacterial biocenoses, which play a major role in maintaining the constancy of this biotope (Table 1). About 75% of the bacterial flora belongs to obligate anaerobes. The ratio of bacteria with anaerobic respiration to aerobic bacteria is 10:1.

Table 1.1

The main representatives of the bacterial microbiota of the oral cavity

Type of respiration	Morphology	Gram stain	Genus
Obligate anaerobes	Cocci	Gram positive	*Peptostreptococcus* *Peptococcus*
		Gram negative	*Veillonella*
	Rod-shaped bacteria	Gram positive	*Bifidobacterium* *Propionibacterium*
		Gram negative	*Bacteroides* *Fusobacterium* *Leptotrichia* *Porphyromonas* *Prevotella*
	Spiral bacteria	-	*Borrelia* *Treponema*
Obligate aerobes and facultative anaerobes	Cocci	Gram positive	*Streptococcus,* *Staphylococcus*
		Gram negative	*Neisseria*
	Rod-shaped bacteria	Gram positive	*Lactobacillus* *Corynebacterium*

	Filamentous bacteria	Gram positive	*Actinomyces*
	Spiral bacteria	-	*Leptospira*

Genus Streptococcus.

Streptococci are one of the main inhabitants of the oral cavity, found in 100% of human saliva (up to 10^8-10^{11} cells per 1 ml).

Streptococci have a spherical or oval shape, gram-positive, non-motile, do not form spores, facultative anaerobes (Fig. 1.1). In smears from cultures grown on solid nutrient media, they are arranged in pairs or short chains, in preparations from broth cultures in the form of long chains. Media with the addition of blood, serum or ascitic fluid are used for the cultivation of streptococci.

Streptococci ferment carbohydrates with the formation of lactic acid, which is due to their antagonistic activity against many putrefactive bacteria. By forming acids, streptococci reduce the pH in the oral cavity, which contributes to the development of caries. In addition, they synthesize insoluble polysaccharides from sucrose.

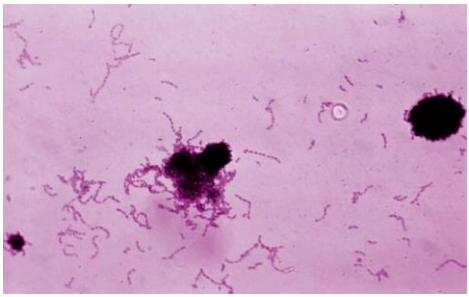

Fig 1.1. *S. mitis. Gram stain.*
CDC/ Richard Facklam. *https://phil.cdc.gov/Details.aspx?pid=1043*

Streptococci live in oxygen-rich areas of the oral cavity. The main common representatives of the *Streptococcaceae* family include *S. salivarius, S. mitis, S. sanguinis, S. milleri*. They are called "oral streptococci". *S.salivarius* lives mainly on the tongue. *S. sanguis* and *S. mutans* are primarily present in high concentrations on the teeth and are detected after their damage. *S.sanguinis*, due to the ability to directly attach to the oral mucosa, provides fixation of many other oral microorganisms that colonize the surface of the teeth, contributing to the occurrence of carious lesions and periodontal disease.

All of them are α-hemolytic (they form a green zone of hemolysis on blood agar) (Fig. 1.2) or non-hemolytic, unable to produce streptolysins or streptokinase, but are often the cause of inflammatory processes in the oral cavity.

Fig 1.2. Colonies of *S. mitis* on blood agar.
https://open.maricopa.edu/redmountainmicro/

Typical representatives of oral streptococci are the species *S. salivarius* and *S.mitis*, which are found in the oral cavity in 100% of cases.

A characteristic feature of *S. salivarius* is the ability to form a capsule as a result of the synthesis of viscous polysaccharides from sucrose. Streptococci are found in the most frequent localization of caries: in the fissure area, on the proximal surfaces of the teeth.

S. salivarius is easily determined by the shape of the colonies that form on gelatin containing 5% sucrose. They are large mucous colonies with a large number of levans. These streptococci are found in dental plaque in small quantities, but they are quite abundant on the mucous membranes and in saliva. *S. salivarius* inhibits the growth and reproduction of streptococci, but does not act on actinomycetes.

S. mitis is the dominant species of streptococci isolated from dental plaque. A heterogeneous species of greenisch streptococci with weak biochemical activity. Only a few strains of *S. mitis* are able to synthesize extracellular polysaccharides.

S. sangius is the second most abundant in dental plaque. Biochemical activity is higher than that of *S. mitis*. Many strains of *S. sangius* exhibit cariogenic activity.

The species *S.mutans* has the most pronounced cariogenic properties. The species *S.mutans* has various subspecies that differ in serological properties: *mutans, rattus, cricetus, sobrinus*. All strains of *S. mutans* ferment sucrose, fructose, glucose, sorbitol, mannitol, inulin, cellobiose, raffinose, trehalose, esculin, salicin, ribose, melibiose, α-methylglycoside. *S. mutans* forms a large amount of extracellular water-insoluble polysaccharides such as dextran and water-soluble levan. Sucrose is the only sugar that *S. mutans* uses to form extracellular dextran, which allows the formation of dental plaque. Many strains of *S. mutans* produce antagonist substances that inhibit the growth of other streptococci and actinomycetes.

Genus Staphylococcus.

Staphylococci in the oral cavity of a healthy person are found on average in 30% of cases, gram-positive, under microscopy are arranged in the form of clusters of grapes (Fig. 1.3). Facultative anaerobes. Staphylococci, like all representatives of the microbiota of the oral cavity, are chemoorganotrophs.

S. epidermidis predominates on the gums and in dental plaque of healthy people. *S.aureus* can be carried on the mucous membrane of the nasopharynx. Coagulase-positive staphylococci can cause purulent-inflammatory processes of the mucous membrane of the oral cavity and other organs and systems of the human body.

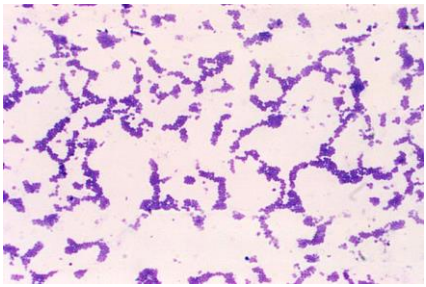

Fig 1.3. Staphylococcus. Gram stain.
CDC/ Richard Facklam. *https://phil.cdc.gov/Details.aspx?pid=2297*

Genus Neisseria.

Neisseria are aerobic cocci, arranged in pairs in the form of coffee beans (Fig. 1.4). Non-motile, gram-negative, do not form spores. They are found in various biotopes of the oral cavity, mainly on the mucous membrane, which is in contact with atmospheric air. Thus, *N. flaveckens* and *N. subflava* live on the surface of the tongue, *N. elongate* - on the surface of the teeth, *N. sicca* inhabits the palate. As aerobes, *Neisseria* use oxygen, creating conditions for the development of obligate anaerobes. They are isolated together with other bacteria in inflammatory processes of the mucous membrane.

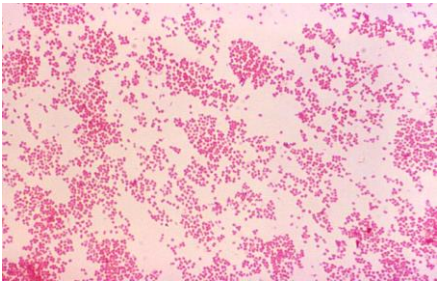

Fig. 1.4. *N. flavescens*. Gram stain.
CDC/W.A. Clark. *https://phil.cdc.gov/Details.aspx?pid=15002*

Genus Peptococcus.

Peptococci are obligate anaerobic cocci, located singly, in pairs, in the form of short chains. Gram-positive. Non-motile. Chemoorganotrophs with complex nutritional needs. Peptococci have weak saccharolytic activity, break down peptones and amino acids. Together with fusobacteria, spirochetes and peptostreptococcus, they are isolated in periodontitis, pulpitis, abscesses. The typical species is *Peptococcus niger*.

Genus Peptostreptococcus.

Peptostreptococci – obligate anaerobic cocci, arranged in pairs or chains. Gram-positive. Non-motile. Have weak saccharolytic activity. Nutrient media with the addition of blood are used for cultivation. The most common species are *P.magnus, P. anaerobius, P. micros.*

Genus Veillonella.

Veillonella are obligate anaerobic gram-negative cocci, located in pairs, singly, in small clusters (Fig. 1.5). Non-motile. They require complex nutrient media. Two species are distinguished in the oral cavity: *V. parvula* and *V. alcalescens*. In the oral cavity, *Veillonella* are mainly found in high concentrations in the ducts and secretions of the salivary glands. In the saliva of healthy people, they are present at a concentration of 10^7-10^{11} cells per 1 ml. They have an anti-caries effect by breaking down lactic acid, which is formed by greenish streptococci. At the same time, being in the community of pathogens, they produce nutrients necessary for their growth and reproduction. They cause inflammatory diseases as part of bacterial associations.

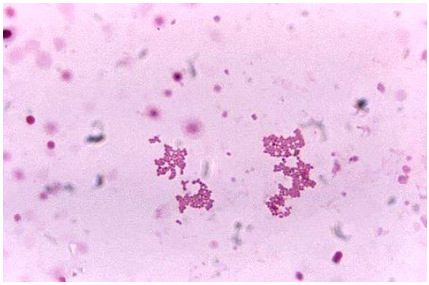

Fig 1.5. Veilonella Gram stain.
CDC/Gilda Jones. *https://phil.cdc.gov/ ID #10776*

Genus Lactobacillus.

Lactobacteria are lactic acid bacteria, gram-positive, non-motile, do not form spores and capsules.They are characterized by great polymorphism - short and long, thin and thick, filamentous and branched forms (Fig. 1.6). Facultative anaerobes. They cause lactic acid fermentation with the formation of a large number of acids. They differ

from each other in saccharolytic properties and on this basis homofermentative and heterofermentative species are distinguished. Homofermentative species (*L. casei*) cause homofermentative fermentation and form only lactic acid during the decomposition of carbohydrates. Heterofermentative species (*L. fermenti, L. brevis*) cause heterofermentative lactic acid fermentation, form lactic acid, acetic acid, alcohol, carbon dioxide. The most commonly isolated species are *L. acidophilus, L. casei, L. brevis, L. fermentum*.

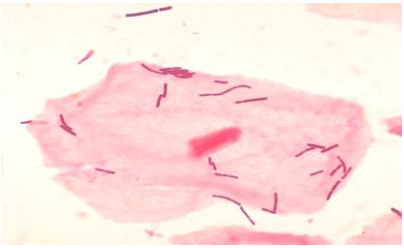

Fig 1.6. Lactobacillus. Gram stain.
https://alchetron.com/Lactobacillus

Due to the formation of a large amount of lactic acid in the process of lactobacilli's life, they inhibit the growth (are antagonists) of other microbes: staphylococci, *Escherichia coli* and dysentery bacilli. Surfactants isolated from *Lactobacillus casei* have a pronounced antioxidant and antiproliferative energy, which suppresses the growth of *Staphylococcus aureus*. In addition, lactobacilli due to their antifungal potential inhibit the reproduction of *Candida* fungi, namely *C.albicans, C. tropicalis, C. glabrata, C. parapsilosis* and *C. dubliniensis*. On the other hand, the release of a large amount of acids intensifies the carious process. The number of lactobacilli in the oral cavity increases with caries.

Genus Corynebacterium.

Corynebacteria are gram-positive straight or slightly curved rods, arranged singly or in pairs at an angle in the form of the Latin letter V (Fig. 1.7). Non-motile, do not form spores. May have club-shaped thickenings at the ends of the cell. Facultative anaerobes. Store volutin.

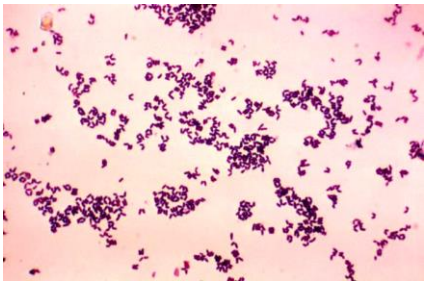

Fig. 1.7. Corynebacteria. Gram stain.
CDC/W.A. Clark. *https://phil.cdc.gov/Details.aspx?pid=19500*

Non-pathogenic corynebacteria are a permanent microbiota of the oral cavity of a healthy person. Due to the ability to synthesize vitamins B and K, necessary for the reproduction and growth of bacteria, the main purpose of *Lactobacillus*, *Corynebacterium* and *Bifidobacterium* is to participate in the creation of the oral microbiocenosis. In addition, lactobacteria inhibit the reproduction of *Candida* fungi. Also, surfactants isolated from *L. casei* have a pronounced antioxidant effect, inhibiting the growth of *Staphylococcus aureus*.

Genus Bifidobacterium.

Bifidobacteria are obligate anaerobic gram-positive, slightly curved or branched rods, arranged in the form of the Latin letters "Y" and "X". They may have thickenings at the ends of the cell (Fig. 1.8). Non-motile, do not form spores. Bifidobacteria are antagonists of pathogenic and conditionally pathogenic bacteria due to the inhibition of their growth by antimicrobial substances and the formation of biofilms that prevent colonization of the mucous membrane.

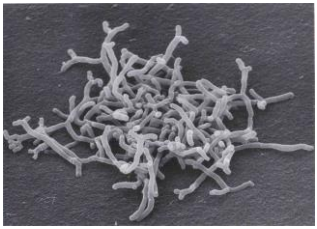

Fig. 1.8. Bifidumbarteria. SEM.
https://alchetron.com/Bifidobacterium-longum

Genus Bacteroides.

Bacteroides are obligate anaerobic, gram-negative, polymorphic, rods of different sizes. Non-motile, do not form spores. Have a capsule. The most common species are *B. melaninogenicus, B. oralis, B. fragilis, B. forsythus*. The number of bacteroids increases

in purulent-inflammatory diseases of the oral cavity (in dental granulomas, in actinomycosis, osteomyelitis, etc.), often together with other anaerobic bacteria.

Genus Fusobacterium.

Fusobacteria are obligate anaerobic, gram-negative, polymorphic spindle-shaped rods of various lengths with pointed ends. Non-motile, do not form spores and capsules. They ferment carbohydrates with the formation of acetic, lactic and propionic acids. Fusobacteria inhabit gingival pockets and carious dentin. The most common species *F. necrophorum* and *F. nucleatum* are isolated in large quantities together with other anaerobic bacteria in ulcerative-necrotic lesions of the mucous membrane.

Genus Porphyromonas.

Porphyromonads are obligate anaerobic, gram-negative short rods. Non-motile, do not form spores. They grow well on blood agar with the formation of a dark pigment. The most common species are *P. asaccharolytica, P. gingivalis* and *P.endodon*talis. They are isolated in large numbers in various purulent-inflammatory diseases of the oral cavity.

Genus Prevotella.

Prevotella are obligate anaerobic, gram-negative, polymorphic rods. Non-motile, do not form spores. The most common species: *P. melaninogenica, P.denticola, P. buccae, P. oris, P. oralis*. They are inhabitants of the gingival sulcus and pockets of the mucous membrane. They cause periodontal disease.

Genus Propionibacterium.

Propionibacteria are facultative anaerobic, gram-positive, polymorphic rods, coccoid or slightly branched, located singly, in small clusters or short chains. Non-motile, do not form spores. They are antagonists of opportunistic and pathogenic bacteria. By synthesizing enzymes of aggression, they can damage tissues, causing pulpitis, periodontitis, etc.

Genus Leptotrichia.

Leptotrichia are obligate anaerobic rods that have the shape of straight or curved rods with sharp or rounded edges, arranged in pairs or chains (Fig. 1.9). Non-motile, do not form spores and capsules. Fresh cultures can be gram-positive. With light microscopy, both gram-negative and gram-positive cells can be observed on one slide. In the oral cavity, they are found in the form of thin, segmented, filamentous structures, sometimes with branching. *L. buccalis* is most often isolated, which forms lactic acid, which affects the development of caries and the formation of tartar.

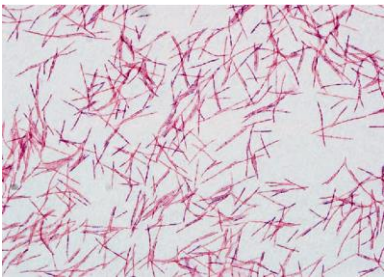

Fig. 1. 9. Leptotrichia. Gram stain.
https://pubmed.ncbi.nlm.nih.gov/26294950

Genus Actinomyces.

Actinomycetes are obligate or facultative anaerobic gram-positive rods or filamentous bacteria. They can form branched threads in tissues (druses). The threads are thin, without partitions, easily fragmented with the formation of rods and cocci, which are located singly, in pairs, in the form of the Latin letters "Y, V", or in clusters in the form of a front garden (Fig. 1.10). Non-motile, do not form spores and capsules. They ferment carbohydrates with the formation of acetic, lactic, succinic and formic acids. The most common species *A.israelii*, *A.viscosus*, *A.naeslundii* and *A.odontolyticus* inhabit the mucous membrane, salivary gland ducts, participate in the formation of tartar and plaque, the development of caries and periodontal diseases.

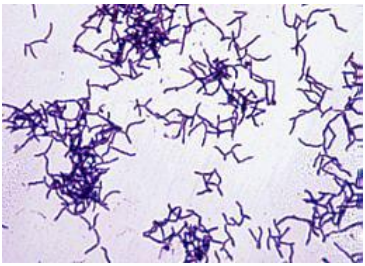

Fig. 1.10. Actonimyces. Gram stain.
https://alchetron.com/Actinomyces#actinomyces-d74fda90-48e7-4d9d-bc59-e7d977f65aa-resize-750.jpeg

Family *Spirochaetaceae.*

Spirochetes include the genera *Treponema, Leptospira, Borrelia*. Gram-negative, very motile due to endofibrils located inside the cell. They do not form spores and capsules, resistant to the action of lysozyme and lipase of saliva, poorly phagocytosed. They are localized mainly in gingival pockets.

Genus *Treponema.*

Treponemas are gram-negative, spiral bacteria that form tightly coiled spirals with equal-height whorls, which can be up to 14 (Fig. 1.11). Movements are diverse - from helical to bending. They stain well by silver impregnation, but poorly by the classical Romanovsky-Giemsa method (faint pink). Typical species - *T. denticola, T. orale, T. macrodentium.* They belong to periodontogenic bacteria. They form associations with other bacteria. Their presence in the oral cavity may indicate a generalization of the process.

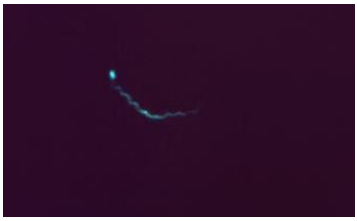

Fig. 1.11. Treponema. Immunofluorescence microscopy.
CDC/Joseph C. Lowell.*https://phil.cdc.gov/Details.aspx?pid=17893*

Genus *Leptospira.*

Leptospires are aerobic spiral bacteria that form thin spirals with 14-28 small whorls. The cells are curved in the form of the Latin letters S and C. They do not form spores and capsules. Under dark-field microscopy, they appear as thin, silvery threads with thickened and curved ends (Fig. 1.12). They stain pink according to Romanovsky-Giemsa.

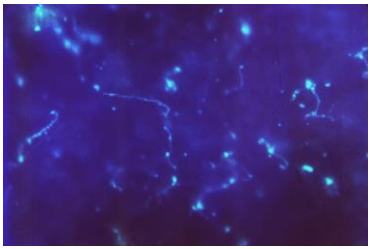

Fig. 1.12. Leptospira. Immunofluorescence microscopy.
CDC/Mildred Galton. *https://phil.cdc.gov/Details.aspx?pid=1346*

Genus *Borrelia.*

Borrelia are obligate anaerobic spiral bacteria with 3-8 irregular whorls (Fig. 1.13). They stain blue-violet according to Romanovsky-Giemsa. The most common are *B. buccalis* and *B. vincentii*, which live in gingival pockets and in the folds of the mucous membrane.

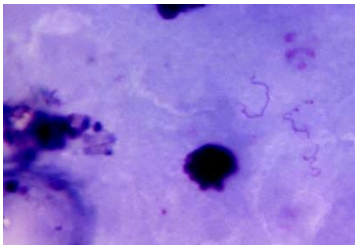

Fig. 1.13. Borrelia. Immunofluorescence microscopy.
CDC/Katherine R. Sulzer. *https://phil.cdc.gov/Details.aspx?pid=21575*

Родина *Mycoplasmataceae.* Рід *Mycoplasma*.

Spirochetes are isolated together with other bacteria in pathological processes of the oral cavity (periodontitis, caries, ulcerative necrotic lesions, etc.).

Family *Mycoplasmataceae.* Genus *Mycoplasma.*

Mycoplasmas are the smallest bacteria, which are characterized by polymorphism due to the absence of a cell wall. Facultative anaerobes, gram-negative, non-motile, do not form spores and capsules, reproduce by budding, binary fission, fragmentation of filaments. *M. salivarium* and *M. orale* are most often isolated, which cause periodontal disease.

Genus *Candida.*

Candida belongs to yeast-like fungi. Fungal cells can be round, ovoid, cylindrical in shape (Fig. 1.14). They reproduce by budding. They do not have a true mycelium, but form a pseudomycelium, which consists of chains of elongated cells. Gram-positive, but can stain unevenly: the periphery of the cell is purple, and the central part is pink. Antigens have a complex structure and cause specific sensitization in the body with the synthesis of corresponding antibodies. In the oral cavity of 70% of healthy people, *C. albicans, C. glabrata, C. tropicalis* and *C. krusei* are isolated. Pathogenic properties are most pronounced in *C. albicans*. Against the background of decreased immunity, they can cause local lesions of the mucous membrane or generalized forms of candidiasis.

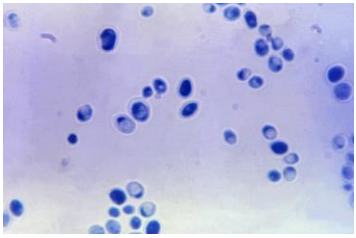

Fig. 1.14. Candida. Gram stain.
CDC/Lucille K. Georg. *https://phil.cdc.gov/Details.aspx?pid=22294*

Protozoa

The most common oral protozoa are isolated in 50% of healthy people and are represented by *Trichomonas elongata (T. tenax*) and *Entamoeba gingivalis*. They inhabit the crypts of the tonsils, dental plaque, periodontal pockets and participate in the development of gingivitis and periodontitis.

Among the above microorganisms, streptococci make up half of the permanent microbiota of the oral cavity, and the other half is represented by *Veillonella* and diphtheroids. Other species of bacteria, fungi and protozoa belong to secondary representatives and are isolated in smaller quantities. Permanent species are a stabilizing part of the microbiocenosis, and S.*mutans, Bacteroides, Actinomycetes, Lactobacilli* are aggressive part. The qualitative and quantitative composition of the microbiota is influenced by antibiotics, diet, physiological factors, somatic pathology.

Representatives of the non-permanent microflora of the oral cavity are rare and in small quantities. This is facilitated by factors of non-specific resistance, as well as the antagonistic role of streptococci and lactobacteria. In case of violations of the physiological state of the oral cavity, representatives of the non-permanent microbiota can multiply and cause pathological processes. In some pathological conditions, representatives of enterobacteria that are normally absent may be isolated, namely the genera *Escherichia, Klebsiella, Proteus, Aerobacter*. In purulent-inflammatory processes, *Pseudomonas* spp may appear. In carious cavities and root canals of teeth, obligate anaerobes *C. perfringens* may be found, which are able to break down collagen and destroy dentin in case of caries.

Questions and test tasks for self-testing for chapter 1 (the number of correct answers may vary)

1. The oral microbiocenosis is called:

a) a set of bacteria that inhabit the oral cavity as a kind of ecological niche of the human body and enter into biochemical, immunological and other types of interaction with the macroorganism;

b) a set of representatives of different taxonomic groups of microbes that inhabit the oral cavity as a kind of ecological niche of the human body and enter into biochemical, immunological and other types of interaction with the macroorganism;

c) a set of representatives of different taxonomic groups of bacteria.

2. List the functions of the normal microbiota of the oral cavity:

a) it maintains physiological inflammation in the mucous membrane and increases readiness for immune reactions;

b) it stimulates the reproduction of pathogenic species of bacteria that enter the oral cavity;

c) it is the causative agent of major dental diseases;

d) it inhibits the development of lymphoid tissue.

3. The microbiota of the oral cavity of a newborn mainly includes:

a) lactobacteria;
b) streptococci;
c) neisseria;
d) spirochetes.

4. The composition of the oral microbiota is influenced by the following factors:
a) food composition;
b) state of the immune system;
c) use of medications;
d) state of the nervous system.

5. Which residents of the oral cavity belong to gram-positive bacteria?
a) *Veillonella*;
b) *Leptotrichia*;
c) *Peptococcus*;
d) *Lactobacterium*.

6. Which residents of the oral cavity belong to gram-negative bacteria?
a) *Bifidobacterium*;
b) *Neisseria*;
c) *Leptospira*;
d) *Streptococcus*.

7. What are the characteristics of bifidobacteria?
a) gram-negative microorganisms;
b) rods with thickenings and branches at the ends;
c) anaerobes;
d) paired rods, often filamentous in shape

8. What is the species of borrelia of the oral cavity?
a) *B. recurrentis*;
b) *B. buccalis*;
c) *B. caucasica*;
d) *B. burgdorferi*.

9. Treponemas that live in the oral cavity are mainly represented by the following species:
a) *Treponema pallidum*;
b) *Treponema orale*;
c) *Treponema macrodentium*;
d) *Treponema denticola*.

10. Lactobacilli are characterized by:
a) Gram-positive staining;
b) capable of spore formation;
c) Gram-negative staining;
d) capable of forming cell chains.

Answers to test questions for chapter 1

№ of question	True answers	№ of question	True answers
1	b	6	b, c
2	a, c	7	b, c
3	a, b	8	b
4	a, b, c	9	b, c, d
5	c, d	10	a, d

Chapter 2. FACTORS OF PATHOGENICITY OF ORAL MICROORGANISMS

In the oral cavity, microorganisms are constantly exposed to adverse factors, which include: local immunity factors, such as saliva, neutrophils, Ig A, soluble microbicidal substances, the presence of food residues, antimicrobial drugs, etc. Most bacterial species have very complex and advanced mechanisms of molecular adaptations and their own defense system, represented by a complex of pathogenicity factors and an increased ability to form biofilms.

Conditionally, the pathogenicity factors of microorganisms are divided into 4 groups: the first group determines the interaction of bacteria with the epithelium and colonization of the zone of primary infection; the second group ensures the resistance of microbes to a human body's defense factors and the ability to reproduce; the third group induces the synthesis of cytokines and inflammatory mediators that contribute to immunopathology; the fourth group consists of toxins and toxic products that cause pathological changes in the organs and tissues of a human body.

The interaction of a microorganism with host cells begins with the process of adhesion of microbial cells to epithelial cells, tooth enamel and a foreign body. Adhesion is mediated by adhesins on the surface of bacteria and receptors of epithelial cells of the oral cavity, tooth enamel structures. Adhesins are special macromolecular complexes of microbial cells that are part of bacterial fimbriae or surface structures of the cell wall, with the help of which the pathogen is fixed and attached to specific surfaces.

It has been established that in the main periodontal pathogen *Porphyromonas gingivalis*, fimbriae are combined with salivary enzymes, extracellular matrix proteins, and symbiont bacteria.

The concentration of salivary glycoproteins is positively correlated with *S.mutans* adhesion. The higher is the concentration of salivary glycoproteins, the higher is the probability of *S. mutans* adhesion. Due to the production of insoluble glycans and extracellular polymeric substance, *S. mutans* belongs to the most virulent (cariesogenic) representative of the oral microbiota, which has the best adhesive ability.

Streptococcus pyogenes strains containing M protein and lipoteichoic acid are also well fixed on the epithelial cells of the human oral cavity.

The main adhesin of *Staphylococcus aureus* is fibrinogen-binding protein, localized on the surface of the bacterial cell, which mediates adhesion to the surface of endothelial matrix proteins, including fibrin, fibronectin.

Some bacteria can colonize the surface of mucous membranes or teeth by attaching to the surface structures of other bacteria, i.e., by coaggregating. Streptococci of various species coaggregate with actinomycetes; *F. nucleatum, Veillonella, F. nucleatum* binds to *Porphyromonas gingivalis* and *Treponema* spp. Coaggregation enables indirect adhesion of bacteria to epithelial cells and tooth surfaces and may be important in the development of dental plaque, as it promotes the colonization of bacteria that are unable to adhere to the pellicle.

Streptococcus oralis and *Actinomyces oris* coaggregate with each other and attach individually and in coaggregates to hyphal elements of *C. albicans*, further enhancing fungal growth on denture surfaces.

An example of coaggregation is the synthesis of extracellular polysaccharides (glucans) from sucrose by *S. mutans*. These polysaccharides promote bacterial attachment to teeth and enhance the stability of the growing plaque matrix.

Adhesion is a key factor in biofilm formation, as it largely determines the fate of the microbial population, namely whether it will remain in a planktonic state or transition to a biofilm mode of existence.

Biofilm is a community of microorganisms attached to each other and located in a matrix consisting of extracellular polymeric substances synthesized by them. This community is characterized by a change in phenotype, which is manifested by a change in growth parameters and expression of specific genes.

In the process of biofilm formation, the decisive role is played by "primary colonizers", i.e. microorganisms that form the primary biofilm, into which companion microorganisms subsequently settle.

The composition of the biofilm is mainly characterized by a variety of species of bacteria or fungi, between which synergistic relationships are formed.

The process of biofilm formation occurs in a short period of time and is characterized by maximum activity up to 24 hours.

The formation of a biofilm goes through a number of stages. At the first stage, the adhesion of microorganisms to the surface occurs, which is the basis for attachment. This stage is reversible, since the adhered cells can return to the planktonic form of existence. The second stage is fixation, which consists in the complete attachment of cells to the surface due to the formation of extracellular substances by microorganisms that create strong adhesion. At the third stage, microcolonies are formed that are separate communities of adhered microorganisms that actively divide and are held by the secreted matrix. Then the microcolonies

merge and a mature biofilm is formed, which has a complex three-dimensional structure that can rearrange its shape and change size (Fig. 2.1).

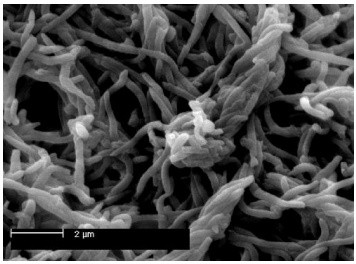

Fig. 2.1. Coaggregation of rod-shaped *F.nucleatum* and ovoid *P.gingivalis* in biofilm. *https://doi.org/10.1099/00221287-148-2-467*

Biofilm biomass is embedded in a thick mucous layer of polysaccharides, lipopolysaccharides and glycoproteins. This exopolysaccharide matrix is synthesized by the biofilm bacteria themselves after their adhesion to the surface and protects the microorganisms from external influences, such as ultraviolet light, radiation, pH changes, osmotic shock, desiccation. Exopolysaccharides adsorb metals and minerals, dissolved organic matter, concentrate nutrients, enzymes and growth factors. The extracellular matrix constitutes 80-90% of the biofilm mass, and bacteria – 10-20%.

Being in close contact, microorganisms can exchange DNA segments not only between bacteria of the same species, but also between other species that are part of the biofilm. This phenomenon of quorum signaling or network communication of bacteria (Quorum sensing) coordinates the expression of bacterial genes depending on environmental conditions. As a result, they acquire new properties that allow them to adapt to different environmental conditions, acquire multiple resistance to antibiotics. It has been established that bacteria and fungi in biofilms survive in the presence of antibiotic concentrations 500-1000 times higher than their minimum inhibitory concentration in vitro in the planktonic form. Quorum sensing (QS) functions through the indirect effect of signaling molecules. Gram-negative and gram-positive bacteria use different systems of signaling molecules. For example, *Staphylococcus* spp. synthesize cyclopeptides, and gram-negative bacteria synthesize acylhomoserine lactones (AHL).

One of the special mechanisms of biofilm resistance is the formation of persister cells in them, resistant to the influence of both aggressive environmental factors and antimicrobial agents. The phenomenon of persistence is a genetically non-inherited (phenotypic) property of a small part of the bacterial population to maintain viability even in the presence of lethal doses of antibacterial drugs. It manifests itself in the fact that, in the presence of "bacteriostatic" concentrations of antibiotics-inhibitors of protein

synthesis, the formation of some proteins, especially components of the cytoplasmic membrane of microorganisms, continues, albeit at a reduced rate. At the same time, a slow growth of the bacterial population is observed due to the growth of some cells.

Dental biofilms are represented by a complex community of many microorganisms. In the formation of dental biofilm, the primary colonizers are the bacteria *Actinomyces oris, Streptococcus gordonii* and *S. oralis*. The following are *Porphyromonas gingivalis* and *Veillonella parvula,* and the late colonizers are *Aggregatibacter actinomycetemcomitans*.

Other pathogenicity factors: the presence of hemolytic, lecithinase, DNase, plasmacoagulase, antilysozyme activities, as well as the production of toxins, enzymes that cause hemolysis of erythrocytes and exoenzymes that destroy cells and tissue fibers.

Thus, hemolysins are able to damage the plasma membrane of erythrocytes as a result of the formation of pores, causing direct lysis of erythrocytes and obtaining Fe+ from the host organism by microorganisms.

Severe hemolysis is more often observed in pathogenic microorganisms (*Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa*). The manifestation of hemolytic activity is also possible in representatives of resistant microbiota (*Streptococcus salivarius, Streptococcus mutans, Streptococcus sanguis, Streptococcus mitis, Neisseria* spp.) and even in the fungi *Candida albicans*.

Among the pathogenicity factors, a special place is occupied by the lecithinase activity of microorganisms, especially *S. aureus*. Lecithinase, destroying lecithin in the cell membranes of leukocytes and other cells, promotes the release of receptors with which microorganisms interact.

Antilysozyme activity is attributed to markers of bacterial persistence. Antilysozyme activity is exhibited by fungi of the genus *Candida*.

DNAse destroys DNA fragments in the extracellular matrix, which are neutrophil extracellular traps for the destruction of pathogens.

Plasmocoagulase, which is produced by *S. aureus*, as an aggression factor causes blood plasma to clot. The fibrin film, covering the bacterial cell, protects it from phagocytosis and bactericidal substances of serum.

Thus, an important role in the formation of resistant strains of microorganisms is due to the ability to form biofilms and secretion of pathogenicity factors, which determine the duration of their existence.

Questions and test tasks for self-testing for chapter 2 (the number of correct answers may vary)

1. The virulence of *S. aureus* is associated with the presence of:

a) hemolysin;

b) collagenase;

c) lecithinase;

d) endotoxin.

2. What structures of the bacterial cell perform the function of adhesins?

a) pili;

b) cell wall;

c) spores;

d) mesosomes.

3. What is the ability of a part of a bacterial population to remain viable even in the presence of lethal doses of antibacterial drugs called?

a) toxigenicity;

b) virulence;

c) commensalism;

d) persistence.

4. The virulence of *Streptococcus pyogenes* is associated with the presence of:

a) pili;

b) collagenase;

c) hemolysin;

d) metabolites: volatile and long-chain fatty acids.

5. What property of *S. mutans* promotes the attachment of bacteria to teeth and enhances the stability of the plaque matrix?

a) the presence of flagella;

b) synthesis of glucans;

c) endotoxin;

d) collagenase.

6. Which of the following microorganisms is the most virulent (cariesogenic) representative of the oral microflora?

a) *S. aureus*;

b) *S. pyogenes*;

c) *S. mutans*;

d) *S. sanguis*.

7. What is the name of the phenomenon of network communication of bacteria, which coordinates the expression of bacterial genes depending on environmental conditions?

a) Quorum Sensing;

b) persistence;

c) seroconversion;

d) phage conversion.

8. In the formation of dental biofilm, the primary colonizers are bacteria:

a) *S. pyogenes*;

b) *S. oralis*;

c) *Actinomyces oris*;

d) *Veillonella parvula*.

9. What part of the mass of the biofilm is made up of bacteria?

a) 1-5%;

b) 90%;

c) 50-70%;

d) 10-20%.

10. What virulence factor of *S. aureus* causes the formation of a fibrin film around the bacterial cell, which protects it from phagocytosis and bactericidal substances of serum?

a) plasmacoagulase;

b) hemolysin;

c) capsule;

d) hyaluronidase.

Answers to test tasks for chapter 2

№ of question	True answers	№ of question	True answers
1	a, c	6	c
2	a, b	7	a
3	d	8	b, c
4	c	9	d
5	b	10	a

Chapter 3. Characteristics of the main biotopes of the oral cavity

The oral cavity is a specific ecological niche of the human body, where a complex bacterial community of human residents is formed. The complexity of the oral microecosystem is due to anatomical features namely the presence of several biotopes inhabited by a certain spectrum of colonizing microorganisms. Each of these biotopes differs significantly in the pH of the environment, temperature, composition of organic and inorganic compounds, etc. At the same time, the composition of the microbiota is not constant, the quantitative and qualitative characteristics of the biotope are influenced by transit microorganisms with varying degrees of adhesion, properties of oral fluid, presence of somatic pathology, age, nature of nutrition, as well as the level of oral hygiene.

Quantitative and qualitative disturbances in the composition of symbionts of the oral cavity biotope, disturbances in their interaction with the human body are of decisive importance in the occurrence of many nosological forms, such as caries, periodontitis, gingivitis, etc.

The species composition of individual areas of the oral cavity largely depends on the oxidation-reduction potential (ORP) and pH of the environment. Many enzymatic reactions are redox reactions, in which some components are oxidized and others are reduced. Their ratio is the ORP, or redox potential (rH2) of the environment. Anaerobic

bacteria require a reduced environment (negative ORP) for growth, while aerobes require an oxidized environment (positive ORP).

In the oral cavity, different values of redox potential are determined in certain biotopes, which allow the growth of aerobes, facultative anaerobes and obligate anaerobes. The dorsum of the tongue and the mucous membranes of the cheeks and palate are aerobic environments with a positive redox potential, therefore the growth of facultative anaerobes is better supported here. The gingival crevice and adjacent tooth surfaces have the lowest ORP and, as a result, the highest concentration of obligate anaerobic bacteria.

During the formation of dental plaque, a rather rapid (within 7 days) change in ORP from a positive level on clean tooth surfaces to a negative one is observed. This drop in ORP is the result of oxygen consumption by facultative anaerobes, as well as a decrease in the ability of oxygen to diffuse through plaque. This partly explains the increase in the number of obligate anaerobes during plaque formation.

The oral cavity can be divided into several biotopes:

- oral mucosa;
- salivary gland ducts;
- gingival fluid and gingival sulcus area;
- oral fluid;
- dental plaque and dental calculus.

The mucous membrane covers various anatomical structures of the oral cavity: tongue, cheeks, gums, hard and soft palate, floor of the oral cavity and partly lips. It is the largest biotope inhabited by a diverse microbiota, which differs in its different parts: on the surface, gram-negative facultative anaerobic microorganisms and streptococci are mainly isolated. Obligate anaerobes prevail in the sublingual folds and crypts. *Streptococci, Neisseria, Corynebacteria* and yeast-like fungi are found on the mucous membrane of the soft and hard palate.

The highest density of bacteria is observed on the surface of the tongue. The surface of the tongue is covered with papillae of 5 different species, which protects bacteria from mechanical removal and promotes colonization of the biotope. The main representatives of the biotope are *S. salivarius* and *S. mitis*, peptostreptococcus, *Veillonella,* as well as bacteroides and actinomycetes. Some of these species prefer the root of the tongue, others - the lateral surfaces. The largest biomass is colonized on the root of the tongue.

The next biotope is the ducts of the salivary glands. Saliva is formed both in the acini and in the ducts of the salivary glands. Due to the bactericidal action of saliva, the ducts of the salivary glands in a healthy person remain sterile. Sometimes *Veillonella* are found in them in small quantities.

Gingival sulcus and gingival fluid. The gingival sulcus is a narrow space between the tooth surface and the adjacent part of the gum. Its depth is normally

from 1 to 2 mm. Morphological features of the structure of the epithelium, connective tissue and terminal vessels in the gingival sulcus area and the osmotic gradient ensure the formation and release of gingival fluid into the lumen of the gingival sulcus.

In a healthy gingival sulcus, the number of bacteria is insignificant, with a predominance of facultative gram-positive microorganisms. Pathogens of periodontitis, such as *Porpyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitas*, and spirochetes, may not be detected or may be detected in very small quantities.

The periodontal pocket microbiota in patients with inflammatory periodontal diseases is represented by *Streptococcus* spp. in almost 66%, which are the predominant bacteria in dental plaque and maintain biofilm homeostasis. The next most common genus is *Proteobacteria* (11%), followed by *Bacteriodes* (3%) and *Fusobacteria* (2%). Other bacteria are observed in less than 1%, including *Actinobacteria, Cyanobacteria*, and *Spirochetes*.

The amount of gingival fluid is normally 0.5-2.4 ml per day. A complex set of components is found in the gingival fluid: electrolytes, proteins, enzymes, immunoglobulins, components of the complement system, lysozyme, leukocytes, desquamated epithelial cells, as well as microorganisms and their metabolites. Obligate anaerobes are present here, namely bacteroids, porphyromonads, prevotels, fusobacteria, veillonella, as well as actinomycetes, spirochetes, mycoplasmas, protozoa and yeast fungi. The concentration of microorganisms in the gingival fluid increases with the formation of a pathological gingival pocket in periodontitis. Optimal conditions for the reproduction of obligate anaerobic flora in the gingival pocket are created as a result of food retention, detritus in the pocket, impaired fluid circulation, which leads to a drop in the redox potential. In such conditions, representatives of bacteroids prevail, namely *Prevotella melaninogenocus, Porphyromonas gingivalis*, which enhance inflammatory processes in the periodontium.

Oral fluid as a biotope of the oral cavity performs the main function of ensuring interaction between all biotopes of the oral cavity. 750-1500 ml of saliva is secreted per day. When it enters the oral cavity, flowing saliva mixes with leukocytes and microorganisms, resulting in the formation of oral fluid or mixed saliva. The physiological activity of the salivary glands ensures the maintenance of oral cavity homeostasis due to the specific composition and properties of oral fluid. Oral fluid has mineralizing, protective, digestive and cleansing functions. The pH level of oral fluid determines the parameters of the state of colloidal systems, and the concentration of ions determines the degree of mineralization of tooth enamel. In addition, immunoglobulins, proteins and enzymes of oral fluid participate in antimicrobial protection. Microorganisms that constantly enter the oral fluid actively multiply in it or attach to the mucous membrane or enamel, where they maintain their vital activity for a long time.

The microbiota of oral fluid is represented by streptococci, neisseria, veillonella, as well as motile species - spirochetes and spirilla.

The number of bacteria in oral fluid varies from 43 million to 5.5 billion per 1 ml (average - 750 million per 1 ml), in plaque and gingival sulcus - 200 billion cells per 1 g of sample, in 1 mg of dental plaque substance there are 500 billion cells.

Surface formations on teeth. In the occurrence and especially in the development of dental caries and periodontal diseases, a significant role is played by surface formations on teeth. In the development of etiopathogenetic prevention of dental diseases, it is necessary to take into account the features of these formations, their physiological and pathogenetic effect on the organs of the oral cavity.

Soft plaque and dental plaque is unmineralized dental deposit.

Soft plaque is a collection of microorganisms of various species incorporated into the matrix. 1 mg of dental plaque substance contains 500×10^{11} microbial cells. In the dental plaque microbiocenosis, the ratio of bacteria is as follows: facultative streptococci - 27%, facultative diphtheroides - 23%, anaerobic diphtheroids - 18%, peptostreptococci - 13%, veillonella - 6%, bacteroids - 4%, fusobacteria - 4%, neisseria - 3%, vibrios - 2%. There may also be six types of fungi in plaque.

Soft begins to accumulate 2 hours after brushing your teeth. During the first day, coccal flora predominates on the tooth surface, in 24 hours rod-shaped bacteria are more common. In 2 days, numerous rods and filamentous bacteria are found on the surface of the soft plaque.

Stages of soft plaque formation. There are 3 main phases in the process of soft plaque formation. The first stage lasts the first 4 hours after brushing your teeth, when the bacteria that have survived begin to multiply and spread. By the end of this period, up to 1 million different microorganisms can be found in the mouth. The second phase is considered to be the period 4-7 hours after thorough brushing, at this stage the number of bacteria has already increased 10 times. Bacteria are fixed on the surface of the teeth, contributing to the formation of soft, thin plaque. At this stage, streptococci and lactobacteria still dominate. The acids they secrete destroy the enamel, which leads to caries.

In 6-7 hours after brushing your teeth, we can already talk about 3 phases, while the soft plaque becomes visible and takes on its final structure. Its composition is now dominated by anaerobic bacteria that do not require oxygen and therefore can live in the thickness of the layers. Under the influence of saliva and microbes, the plaque gradually thickens and hardens. Mineralization and transformation of soft plaque into dental calculus occurs.

Dental calculus is formed most often near the openings of the salivary ducts. It presses on the gingival sulcus and irritates it, disrupts the metabolism between tissues and saliva. All this contributes to damage to tooth enamel and the

development of gum inflammation (gingivitis). Later, the process spreads to deeper layers.

Thus, with the development of soft plaque, its microbiota changes depending on the type of breathing of bacteria. Initially, plaque contains aerobic microorganisms, more mature ones contain aerobic and anaerobic bacteria.

The most important role in the formation of soft plaque is played by *S.mutans*, which actively form it on any surfaces. *S. salivarius* first adheres to the clean surface of the tooth, and then *S. mutans* adheres and begins to multiply. At the same time, *S. salivarius* disappears from the soft plaque very quickly. The formation of the plaque matrix is influenced by enzymes of bacterial origin, for example, neuraminidase, which is involved in the breakdown of glycoproteins to carbohydrates, as well as the polymerization of sucrose to dextran-levane.

Dental plaque is a dense formation located above the pellicle and consists of microorganisms located inside the matrix, which is formed due to proteins, polysaccharides, lipids brought there by saliva and produced by microorganisms themselves, and some inorganic substances (calcium, phosphates, magnesium, potassium, sodium, etc.). Plaques are tightly attached to the surface of the teeth (Fig. 3.1). Dental plaque is usually the result of structural changes in soft plaque - this amorphous substance, which tightly adheres to the surface of the tooth, has a porous structure, which ensures the penetration of saliva and liquid food components into it. The accumulation of end products of microbial life and mineral salts in soft plaque slows down this diffusion, since its porosity disappears. As a result, dental plaque appears, which can only be removed by force and not completely.

Formed by glycoproteins and other proteins of the oral fluid, the pellicle is the primary matrix on which microorganisms are adsorbed, which is the initial stage of the formation of dental plaque. Further colonization and active reproduction of microorganisms contributes to the growth and maturation of dental plaque, as well as modification of the species composition of microbiota.

It is in dental plaque that the active vital activity of microorganisms occurs, which is accompanied by the formation of acid, enzymatic activity and other processes of the metabolism of microorganisms. Dental plaque plays the most important role in the occurrence of caries and inflammatory periodontal diseases.

Dental plaque has a porous structure that allows nutrients to freely penetrate into its deep layers. These are carbohydrates that are easily fermented (sucrose, fructose, glucose, etc.).

Most often, dental plaque is located above the gums, in the cervical area, in the fissures. Plaque consists of 80-85% water. Calcium, phosphates, fluorides predominate among mineral components.

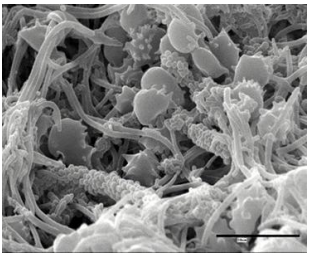

Fig. 3.1. Dental plaque.SEM.
https://www.birmingham.ac.uk/news-archive/2017/national-biofilms-innovation-centre-1

Soft plaque and dental plaque are the most complex biotopes that form on the surface of the teeth. Up to 90% of microorganisms (over 60 species) of the oral cavity are concentrated in soft plaque. The species composition of dental plaque is represented by almost all microorganisms with a predominance of streptococci. The number of bacteria is from 100 to 300 million per 1 mg. However, their number varies in different people at different periods of life.

According to modern ideas, dental plaque is a typical variant of biofilm. Dental plaque begins to form with the formation of a pellicle on the tooth surface. Its formation occurs in several stages:

1) Association (binding): bacteria attach to the pellicle due to the action of physical forces.

2) Adhesion (gluing): since bacteria have special surface molecules (adhesins) that bind to receptors of the thin film, some of the bacteria become "primary colonizers" (streptococci and actinomycetes). Later, other microorganisms join the primary colonizers.

The adhesion process is very fast: in 5 minutes, 10^5-10^6 bacterial cells per 1 cm^2 attach. Further, for about 8 hours, the adhesion rate decreases and is stable, and in next 1-2 days, the number of attached bacteria reaches 10^7-10^8 per 1 cm^2.

3) Bacterial proliferation begins.

4) Microcolonies are formed. Many streptococci secrete protective extracellular polysaccharides (e.g., dextrans, levans).

5) Biofilm is formed (“attached plaque”): microcolonies create complex groups with metabolic advantages for their components.

6) Soft plaque growth: biofilm is characterized by a primitive “circulatory system”. Mature dental plaque has a circular structure and is permeated with a system of channels for nutrients and metabolic products of the microbial community. Soft plaque begins to “behave” like a complex organism. The number of anaerobic microorganisms increases. Metabolic products and components of the walls of rejected cells (e.g., lipopolysaccharides, vesicles)

contribute to the activation of the immune response of human body. Bacteria within the biofilm are protected from phagocytic cells and from exogenous bactericidal agents.

In the dynamics of dental plaque formation, significant changes in the nature of the microbiocenosis occur. The general trend is a change in the composition of the microbiota from the dominance of aerobic and facultative anaerobic forms, mainly cocci, to obligate anaerobic gram-negative rods and spiral forms.

The 1st phase of dental plaque formation is the first 2-4 hours after thorough teeth brushing. It mainly consists of cocci (streptococci *S. mutans* and *S. sanguis, Neisseria* and *Staphylococcus*) and short rods (lactobacteria). This is the so-called "early" dental plaque.

Oral streptococci play an important role in the formation of dental plaque. *S.mutans* is of particular importance, since these microorganisms actively form dental plaque, and then plaque on any surfaces. A certain role is played by *S.sanguis*. Thus, during the first 8 hours, the number of *S. sanguis* cells in plaques is 15-35% of the total number of microbes, and by the second day - 70%; and only then their number decreases. *S. salivarius* is detected in plaques only during the first 15 minutes, its number is insignificant (1%). There is an explanation for this phenomenon (*S. salivarius, S. sanguis* are acid-sensitive streptococci).

Intensive and rapid consumption of carbohydrates leads to a sharp decrease in the pH of plaque. This creates conditions for a decrease in the proportion of acid-sensitive bacteria, such as *S. sanguis, S. mitis, S. oralis*, and an increase in the number of *S. mutans* and lactobacilli, which leads to acid production, increasing tooth demineralization.

The first two days on the tooth surface in the composition of soft plaque, gram-positive cocci prevail. The more days pass, the more facultative aerobes give way to anaerobes and move to the surface of plaque. In soft plaque, the following begin to appear: *Veillonella parvula*, gram-negative anaerobic cocci, gram-positive and gram-negative rods, *Actinobacillus actinomycetemcomitans, Capnocytophaga* spp. Then *Fusobacterium nucleatum* and *Prevotella intermedia* join. In early soft plaque, the species *Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum* dominate, in mature plaque - *Porphyromonas gingivalis*, spirochetes.

2nd phase takes 4-5 days. During this period, a mature plaque is formed, which is characterized by a decrease in the proportion of gram-positive cocci and the prevalence of gram-variable filamentous forms such as *Leptotrichia*, as well as *Fusobacteria* and *Veillonella*. The so-called dynamic plaque is formed.

Phase 3 develops in 6-7 days. Dental plaque acquires its final form in terms of symbiont composition, although quantitative shifts in it occur constantly. The number of aerobic species of *Neisseria* and facultative anaerobic streptococci sharply decreases. Gram-negative obligate anaerobic bacteria *Bacteroides, Fusobacteria*,

Veillonella and Gram-positive actinomycetes, microaerophilic streptococci and peptostreptococci dominate. On the 9-11th day, fusiform bacteria (*Bacteroides*) appear, the number of which is rapidly increasing.

The total number of bacteria in dental plaque increases from 90-100 in the 1st phase of formation to 1-10 million in the 2nd phase. In the third phase of formation, depending on many factors, the number of bacteria is calculated in tens and hundreds of billions per 1 g.

Thus, during the formation of dental plaque, aerobic and facultative anaerobic microbiota initially prevail, which sharply reduces the redox potential in this place, thereby creating conditions for the development of obligate anaerobes.

The bacterial composition of the dental biofilm may be constant and contain mainly non-pathogenic microorganisms, but the microbial balance may be disturbed due to an imbalance of adverse factors and protective mechanisms. In this case, the species composition of the biofilm is replaced, and cariogenic microorganisms prevail. A local decrease in the pH value under the plaque below the "critical" (pH = 4.5-5.0) leads to demineralization of hard tooth tissues.

It has been established that microbes have different affinity even for different tooth surfaces. In addition, the adhesion process is influenced by mechanical factors associated with the chewing process, physicochemical conditions, etc. Therefore, on different surfaces of the teeth, in pits and fissures, the composition of microbiota is somewhat different even within the same tooth.

Dental plaque is also formed on the surface of fillings, and its composition is somewhat different and depends on the nature and quality of the filling material.

The state of dental plaque is a key mechanism for the occurrence and development of dental caries.

Enzymes secreted by microorganisms contribute to the mineralization of dental plaque. As the process of plaque spreading under the gums occurs, subgingival dental deposits begin to form, where gram-positive cocci, gram-negative bacilli, and spirochetes prevail.

3 zones of colonization are distinguished:

1. Microorganisms fixed to the tooth surface.
2. Microorganisms located in the gingival sulcus and epithelial attachment.
3. Surface bacterial plaque.

Microorganisms secrete a number of substances that maintain the volume of the dental plaque matrix.

The development of dental plaque is influenced by a number of factors:

1. Bacterial attachment. Microorganisms begin to attach to each other in a certain sequence: streptococci, gram-positive bacilli, *Fusobacterium nucleatum* species, *Porphyrormonas gingivalis, Veillonella parvula, Actinobacillus actinomycetem*

comitans, Prevotella spp. Saliva, primarily Ig A, contained in it, prevents the formation of bacterial plaque.

2. Bacterial metabolism. Normal growth and vital activity of microorganisms depends on the availability of a nutrient substrate, the pH of the environment, and the oxygen saturation of enzyme systems. The metabolic products of one type of microorganisms can negatively affect another.

3. Mechanical cleaning of the tooth surface. Even despite maintaining oral hygiene and thorough cleaning of the teeth, soft plaque still does not disappear. The most pronounced accumulations of soft plaque are observed in hard-to-reach places for cleaning, for example, on approximal surfaces.

Dental plaque contains inorganic and organic substances. Inorganic substances include calcium (2.7%), magnesium (0.57%), phosphorus oxide (3.8%), nitrogen (12.6%). Organic substances include free amino acids, lipids, etc.

Supragingival dental deposits are usually light in color, have a loose or dense consistency, are easily removed compared to subgingival dental deposits. Subgingival dental deposits are tightly fixed to the surface of the tooth root. They are usually brown in color.

The composition of the dental plaque, depending on the localization (supragingal and subgingival), is different. Streptococci and lactobacilli are more common on the plaques of the teeth of the upper jaw, and *Veillonella* and filamentous bacteria are more common on the plaques of the lower jaw. Actinomycetes are isolated from the plaques on both jaws in the same amount,

From the point of view of the etiology of periodontal diseases, dental plaque is more aggressive than hard dental deposits not only due to the large number of microorganisms, but mainly due to changes in its virulence.

Microorganisms of dental plaque are divided into two groups: the first, cariogenic bacteria, consists of *Streptococcus mutans, Lactobacillus acidophyllus, Streptococcus sobrinus*. The second one is periodontogenic bacteria, which include *Streptococcus sanguis, Veionella parvulla, Streptococcus uberis, Porphyromonas gingivalis, Capnocytophaga ochracea, Rothia dentacariosa, Actinomyces viscosus, Phoprionibacterium aches, Praevotella intermedia, Fusobacteriae spirochetes, Veilonella alcoleusceus, Actinobacillus* and others.

In the etiology and pathogenesis of inflammatory periodontal diseases, the leading role belongs to mineralized dental deposits, the formation of which occurs as a result of calcification of soft plaque.

Dental calculus is formed as a result of mineralization of dental plaque. Calcium phosphate crystals, which are deposited inside the plaque, can be closely associated with the enamel surface, sometimes it is difficult to determine where the enamel ends and the calculus begins. Depending on the location on the tooth surface, supragingival and subgingival tartar are distinguished.

Supragingival calculus is located above the gingival margin, it is easy to detect on the surface of the teeth. This calculus is usually gray or white-yellow in color, hard or clay-like consistency, easily separated from the tooth surface by scraping or chipping. The color of the calculus depends on the influence of food products, nicotine, as well as iron oxides, copper and other substances. Supragingival calculus is most often localized on those surfaces of the teeth that are located with the exits of the excretory ducts of the salivary glands.

In the mechanism of supragingival calculus formation, calcium-phosphate compounds from saliva play an important role, i.e. it belongs to the salivary type. Supragingival calculus consists of 70-90% inorganic (phosphates, calcium carbonate, trace amounts of other trace elements and metals) and 10-30% organic (epithelium, leukocytes, microorganisms, food residues) parts. About 10% of the organic part of the tartar is carbohydrates (glucose, galactose, rhamnose, etc.).

Dental calculus also contains organic substances: proteins, carbohydrates, polysaccharide complexes, lipids. The inorganic composition of dental calculus is chemically identical to the inorganic components of bone, dentin and cement. The mineral component of dental calculus is represented by calcite, brushite, hydroxyapatite, monetite.

Subgingival calculus is detected only by probing. This calculus is usually dense, hard, dark brown or greenish-black in color, tightly attached to the underlying surface. The calculus covers the neck of the tooth within the gingival sulcus, can be located on the cementum of the root, in the periodontal pocket. The composition of subgingival calculus is similar to supragingival.

There are 3 stages of calculus formation:

I - accumulation of mineral components (approximately 45-60 days after the formation of plaque);

II - growth and improvement of phosphate crystals (from 45-60 to 650-700 days);

III - completion of calculus formation, the predominance of inorganic substances in it over organic ones (after 650-700 days).

All surface formations on the teeth, except for the cuticle and pellicle, are infected and play a negative role in the development of dental diseases. However, the main role in the development of inflammation in periodontal tissues belongs to the soft bacterial plaque located on the mineralized surface of calculus.

Relationships of microorganisms in the oral microbiocenosis. Colonization of the oral cavity is influenced by a number of factors: the ability of microorganisms to adhere, primarily to the epithelium and enamel, the nature of the relationship between members of the microbiocenosis, the characteristics of bacterial metabolism and its effect on other types of microorganisms.

The initial stage of colonization is adhesion to the mucous membrane or to the teeth, which ensures further reproduction (colonization) of microorganisms. Adhesion factors in gram-negative bacteria are pili or fimbriae, in gram-positive bacteria -

lipoteichoic acid. Some bacteria can use adhesins of other microorganisms in the process of coaggregation between different types of bacteria. Thus, coaggregation occurs between streptococci and actinomycetes, *Veillonella*, and fusobacteria. *S.mutans* is able to synthesize extracellular polysaccharides from sucrose, which stabilize the plaque matrix. Coaggregation is an example of commensalism between microbial species, whereby bacteria that are unable to adhere to the pellicle can have indirect adhesion and colonize the tooth surface and epithelial cells.

The relationships between members of the oral microbiota can be mutually beneficial and antagonistic. The growth and reproduction of bacteria are influenced by nutrients, vitamins, pH, and redox potential. In ecosystems, complex food chains arise between bacteria. Streptococci *S. sanguis* and *S. mutans*, as well as lactobacteria actively reproduce in the presence of sucrose, and the lactic and formic acids they synthesize are used by *Veillonella* as an energy source. Corynebacteria synthesize vitamin K, and yeasts and yeast-like fungi synthesize B vitamins, which stimulate the growth of peptostreptococcus, bacteroids, *Veillonella*, and fusobacteria.

Facultative anaerobes absorb oxygen, lowering the redox potential, thereby creating favorable conditions for obligate anaerobes.

Antagonistic relationships between microorganisms also affect the composition of the oral microbiota. *Veillonella* consume organic acids, thereby increasing the pH of the environment, which inhibits the reproduction of cariogenic streptococci and lactobacteria. Microaerophilic streptococci produce hydrogen peroxide, acidic metabolites, bacteriocins, which inhibit the growth of fuso- and corynebacteria. Bifido- and lactobacteria acidify the environment, which inhibits the reproduction of yeast and yeast-like fungi and thereby causes a decrease in the synthesis of vitamins, without which the reproduction of many species of bacteria is delayed.

Formation of the oral microbiocenosis. Normally, the fetus is sterile. Microorganisms begin to appear in the child's body during passage through the mother's birth canal (primary microbial colonization of the body). In the first 6-8 hours after birth, the child's oral cavity is colonized by aerobic and facultative anaerobic species: diphtheroides, *Neisseria* spp., lactobacilli, sarcinae, staphylococci and streptococci. Obligate anaerobic species are absent, which is due to the absence of teeth, which are necessary for the existence of these bacteria. At the 4th month of life, streptococci, mainly *S. salivarius*, bifidobacteria, lactobacilli, *Neisseria* spp., hemophilic bacteria, as well as yeast-like fungi and yeasts, predominate in the oral cavity. Fusobacteria and *Veillonella* can vegetate in the folds of the mucous membrane.

Teething contributes to a sharp change in the qualitative composition of microorganisms, which is characterized by the appearance and rapid increase in the number of obligate anaerobes. At the same time, microorganisms are distributed and "populated" by them in the oral cavity in accordance with the

features of the anatomical structure of certain regions. At the same time, numerous microsystems with stable microbial populations are formed. The microbiota of the oral cavity of preschool children is similar to the microflora of adults and consists of bifidobacteria, leptotrichia, fusobacteria, peptostreptococcus and spirilla. Spirochetes, bacteroids and protozoa appear in the oral cavity only at about 14 years of age, which is associated with age-related changes in the hormonal background of the body.

With the formation of subgingival and supragingival tartar and with the use of dentures, the total number of microorganisms in the oral cavity increases. In old age, when teeth are lost, there is a significant decrease in the content of obligate anaerobic bacteria.

S.S. Socransky and co-authors proposed a concept that explains the transition from a healthy state to clinical manifestations of oral diseases by a sequential change in microbiota: from facultative gram-positive species of bacteria to anaerobic gram-negative ones. They divided bacteria into 5 different color clusters depending on the severity of the impact on the development of the disease: "green", "yellow", "purple", "red" and "orange". Thus, the authors do not correlate the presence of bacteria of the "green" (which includes various species of *Capnocytophaga - C. ochracea, C.gingivalis, C. sputigena; Campylobacter concisus, Eubacteria nodatum* and *Streptococcus constellatus*) and "yellow" (several species of Streptococcus - *S. sanguis, S. oralis, S. intermedius, S. gordonii* and *S. mitis*) clusters with the development of periodontal diseases. At the same time, the presence of the "purple" cluster (*Veillonella parvula, Actinomyces odontolyticus, A. naeslundii, A. viscosus*) is associated with the development of gingival bleeding, which is detected by palpation or probing. The appearance of bacterial colonies of the first three clusters on the surface of the teeth is a harbinger of colonization by bacteria of the orange or red clusters.

The appearance of the "orange" (subspecies *Fusobacterium periodonticum, Prevotella intermedia, P. nigrescens, Peptostreptococcus micros, C. rectus, C. gracilis, C. showae, Eubacterium nodatum* and *S. constellatus*) and "red" (closely related species *T. fors, P. gingivalis* and *T. denticola*) clusters is closely associated with pronounced signs of the development of the clinical picture of periodontal disease, namely with an increase in the depth and bleeding of the periodontal pockets that form. Later it was found that the species *Actinomyces actinomycetemcomitans* does not belong to any of the clusters, it is determined only in aggressive (juvenile) periodontitis.

Questions and test tasks for self-testing to chapter 3 (the number of correct answers may vary)

1. What are the main representatives of microorganisms that colonize the oral mucosa?

a) *Veillonella;*

b) *Peptostreptococcus*;

c) *Lactobacillus*;

d) *Neisseria*;
e) *Streptococcus.*
2. What are the main species of streptococci that inhabit the oral cavity?
a) *S. salivarius*;
b) *S. pyogenes*;
c) *S. mutans*;
d) *S. sanguis*.
3. What are the main representatives of microorganisms that live in oral fluid?
a) *Veillonella*;
b) *Vibrio*;
c) *Lactobacilli*;
d) *Neisseria*;
e) *Streptococcus*.
4. The microcenosis of gingival pockets is characterized by the following conditions:
a) oxygen content is reduced significantly;
b) presence of favorable conditions for the habitat of strict anaerobes;
c) increased oxygen content;
d) presence of favorable conditions for the habitat of aerobes.
5. What are the main representatives of microorganisms that live in the gingival fluid and gingival sulcus?
a) fusobacteria;
b) bacteroides;
c) leptotrichia;
d) neisseria;
e) spirochetes.
6. What groups do microorganisms inhabiting the gingival fluid and gingival sulcus belong to?
a) filamentous obligate aerobic species;
b) filamentous obligate anaerobic species;
c) spiral obligate aerobic species;
d) spiral obligate anaerobic species.
7. What microorganisms colonize the salivary gland ducts?
a) *Veillonella*;
b) *Nocardia*;
c) *Lactobacterium*;
d) *Neisseria*;
e) *Streptococcus*.
8. The microcenosis of gingival pockets is characterized by the following conditions:
a) oxygen content is reduced significantly;

b) presence of favorable conditions for the habitat of strict anaerobes;
c) increased oxygen content;
d) presence of favorable conditions for the habitat of aerobes.
9. List the characteristics of *Veillonella*:
a) gram-positive microorganisms;
b) diplococci;
c) anaerobes;
d) coccus-like and rod-like forms.
10. List the characteristics of *Bifidobacteria*:
a) gram-negative microorganisms;
b) rods with thickenings and branches at the ends;
c) anaerobes;
d) rods arranged in pairs, often filamentous in shape.

Answers to test tasks for chapter 3

№ of question	True answers	№ of question	True answers
1	e	6	b, d
2	a, c, d	7	a
3	a, c, d, e	8	a, b
4	a, b	9	b, c, d
5	a, c, e	10	b, c

Chapter 4. MICROBIOLOGY OF CARIES AND ITS COMPLICATIONS AND RESEARCH OF MICROBIOTA IN THESE DISEASES

Dental caries is a pathological process that develops after teething and is manifested by demineralization and destruction of hard tissues of the teeth and the formation of a defect in the form of a carious cavity.

The occurrence of dental caries is associated with the activity of cariogenic microorganisms, nutrition with a predominance of easily digestible carbohydrates, the composition and properties of oral fluid, the level of oral hygiene, etc.

Dental plaque (Fig. 4.1) is one of the important etiological and pathogenetic links in the development of dental caries. Initial caries lesions occur in areas where favorable conditions are created for plaque accumulation (pits and fissures, on proximal surfaces and cervical areas).

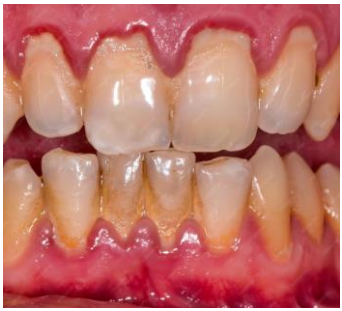

Fig. 4.1. Dental plaque.
*https://www.freepik.com/premium-photo/gum-recession-closeup-front-teeth_*

The occurrence of caries is associated with the microbiota of dental plaque, among which streptococci play a leading role. *S. mutans* has the most pronounced cariogenic potential due to the following properties:

1. Formation of colonies on teeth in the form of dental plaque. Dental plaque tightly adheres to the tooth surface due to glycoproteins, has a mesh structure filled with microorganisms and deposited carbohydrates such as levan and decabrane. Dental plaque is separated from the oral cavity by a membrane of glycopolysaccharides that are not destroyed by salivary amylase.

This form of existence of bacteria in the mouth is advisable from the standpoint of their life support, because it is easier to ensure:

a) the reproduction process;

b) protection from harmful influences;

c) accumulation and deposition of food.

2. Production of a large number of extracellular polysaccharides, which ensure the adhesion of bacteria to each other and the tooth surface, which leads to the growth and thickening of soft plaque.

Streptococci synthesize extracellular polysaccharides - soluble and insoluble glycans (dextran) and levan (fructan). Soluble polysaccharides are easily broken down by microorganisms, and insoluble glycan provides adhesion of microorganisms, causing intercellular aggregation of bacteria and fungi in plaque (*Neisseria, A. vicosus, Noccardia, C. albicans*). Insoluble glycan prevents the diffusion of lactic acid, which is formed by microbes, which causes demineralization of enamel, causing dental caries. In addition, extracellular polysaccharides prevent the entry of phosphates and calcium ions into the enamel, which complicates the process of remineralization.

3. Carbohydrate breakdown. When carbohydrates are ingested that are easily fermented, especially those with low molecular weight (glucose and sucrose), two processes occur: their rapid metabolism by glycolysis with the formation of organic acids, mainly lactic acid. A kind of "metabolic explosion" occurs, when

acid production increases 10-100 times within 5-15 minutes. At the same time, a certain amount of acids penetrates from the plaque into saliva, and the main part remains in the plaque, diffusing to the enamel surface. There is a decrease in the pH of dental plaque to 4.4-5.0, while the return to normal values occurs much more slowly, sometimes within 2 hours, especially in the area of contact between the teeth.

Such a change in the concentration of hydrogen ions is dangerous for the enamel, since at a pH value below the critical level (about 5.5), the dissolution of hydroxyapatite crystals in the least stable areas of the enamel may occur. Acids penetrate the subsurface layer of the enamel and cause its demineralization. The microspaces between the crystals increase, which leads to an increase and strengthening of the permeability of the tooth enamel. Due to this, ideal conditions are created for the penetration of microorganisms into the inter-prism spaces. That is, the source of acid formation penetrates the enamel, forming a cone-shaped lesion.

At the early stage, caries is a focal demineralization that occurs as a result of a change in pH on the enamel surface under dental plaque. At this stage, so named the "white spot", the pathological process is reversible, and remineralization of the tooth enamel is possible. In this case, the surface layer of enamel is preserved both due to the influx of mineral substances from its collapsing layers and due to the influx of substances from the environment. Thus, when the processes of de- and remineralization are in balance, caries does not occur. When the demineralization process predominates, caries occurs in the white spot stage. The process may not stop there, but serve as a starting point for the formation of a carious defect (Fig. 4.2).

The second slower process is the formation of glucose polymers (levan, dextran, other compounds), which represent a depot of carbohydrates (such as glycogen) that the microorganisms stores to ensure life in the absence of nutrients.

Thus, for the occurrence of dental caries, an etiological factor is necessary such as cariogenic microbiota of the oral cavity. Without it, dental caries cannot occur under any conditions. In the presence of cariogenic microorganisms, the development of caries can occur only under the presence of certain conditions and factors.

For this, the intake of easily fermentable carbohydrates must be ensured, and dental plaque must be formed. But even under this condition, caries does not necessarily form. As a result of the constant production of acid, the processes of demineralization must prevail over remineralization. In this case, caries can develop at a low level of resistance of tooth enamel.

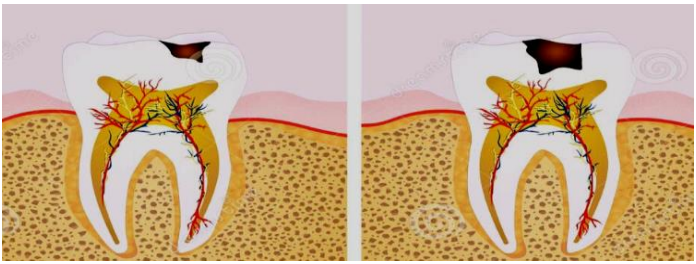

Fig. 4.2. Dental caries.
https://www.dreamstime.com/stock-illustration-tooth-decay-scheme-illustration-image58166933

Pathomorphologically, caries is characterized by five zones of damage, two of which - the zones of demineralization and infected dentin - contain a large number of microorganisms. Under the action of enzymes secreted by bacteria, further dissolution of the organic substance of dentin occurs. Deeper are layers of compacted transparent, translucent and intact dentin, in which bacteria are absent.

Cariogenic microorganisms. Dental plaque does not form without microorganisms, so its cariogenicity is associated with the cariogenic bacteria present in it, which produce a significant amount of acids. Most bacteria in dental plaque (and especially cariogenic ones) are able to synthesize iodophilic polysaccharides, which are identified as intracellular types of glycogen. During caries, bacteria that produce hyaluronidase multiply, which can actively affect the permeability of enamel. Cariogenic bacteria in dental plaque are also able to synthesize enzymes that break down glycoproteins. It has been established that the higher the rate of formation of dental plaque, the more pronounced cariogenic effect it has.

The greatest importance in the development of caries is played by oral streptococci (*S. mutans, S. sangius*), lactobacteria and some actinomycetes.

If streptococci prevail in various biotopes of the oral cavity, then the proportion of lactobacteria in plaque is about 1% of the number of microorganisms that inhabit dental plaque. The role of lactobacteria increases sharply with the increase in carious lesions. They synthesize a large amount of lactic acid and are not sensitive to low pH. They play a decisive role in the destruction of dentin after enamel destruction.

The increase in the number of *S. mutans* and lactobacilli leads to an acceleration of acid production, which increases the demineralization of teeth.

Actinomycetes affect the roots of teeth in the elderly in the process of exposing the root area of the tooth. Together with bacteroids and other

microorganisms, actinomycetes secrete proteases that destroy dentin and increase carious lesions.

Further progression of the carious process leads to the development of complicated forms of caries: pulpitis and periodontitis.

Microbial flora in pulpitis. Pulpitis is an inflammatory process that spreads to the coronal pulp or to the entire pulp diffusely (Fig. 4.3). The main etiological factor of pulpitis is microorganisms that penetrate the pulp from the carious cavity. These are mainly aerobic microorganisms or associations of obligate anaerobic microorganisms with aerobic ones.

Fig. 4.3. Pulpitis.
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Acute pulpitis initially has a limited character and has the character of serous inflammation.

In acute serous pulpitis, greenish and non-hemolytic streptococci can be detected. Usually, within a day, the serous nature of the inflammation turns into purulent.

In purulent pulpitis, golden staphylococci and β-hemolytic streptococci are of great importance. In necrotic pulp, anaerobic representatives (peptostreptococci, bacteroids, spirochetes, actinomycetes, pathogenic staphylococci) are found in large numbers, but putrefactive bacteria (Proteus, Clostridia) can also be found.

Microbial flora in periodontitis. Periodontitis is an inflammation of connective tissue located between the compact plate of the dental alveolus and the cementum of the tooth root (Fig. 4.4). The most common cause of periodontitis is microflora that penetrates the periodontium through a carious cavity, through the opening of the tooth apex or through a pathological gingival pocket. Microorganisms fill the tooth canal and spread into the periodontium. In the initial stages of inflammation, greenish and non-hemolytic streptococci are usually found.

In acute purulent periodontitis, microbial associations are found, including staphylococci, streptococci, gram-positive and gram-negative bacilli, including putrefactive bacteria. In chronic forms, the main role is played by streptococcal anaerobic flora - peptostreptococcus. Actinomycetes, spirochetes, vibrios, and bacteroids are found in apical granulomas.

Fig. 4.4. Periodontitis.
https://www.dreamstime.com/royalty-free-stock-photo-tooth-decay-image24574035

Microbiological examination of caries and its complications. The study of microbiota is based on bacterioscopic and bacteriological methods. Material for examination: material from the carious cavity, dental plaque, oral fluid.

Before the examination of dental plaque, the oral cavity is hygienically treated by mechanical methods under the control of the hygiene index. The area of dental plaque is determined by special staining solutions.

Supragingival plaque and plaque on the accessible smooth surface of the tooth (from the side of the tongue, cheeks) can be removed with a sterile scaler or excavator. To remove plaque from pits and fissures, a sharp probe is used, plaque from proximal surfaces - a sterile thread. The selected material is placed in a transport nutrient medium to preserve the vital activity of microorganisms. To increase the accuracy of the study, the plaque content is dispersed by shaking with beads in a homogenizer or using ultrasound for 10 seconds.

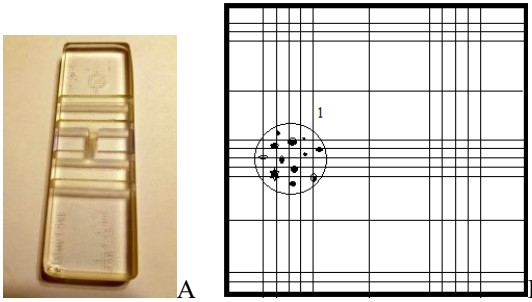
The material from the carious cavity is taken with a sterile bur, having previously removed the surface layers of softened dentin with another sterile bur. The dentin is placed in a transport nutrient medium using a sterile trowel.

Unstimulated saliva is collected in sterile tubes 2 hours after eating for 10 minutes. To obtain stimulated saliva, 1-2 drops of sterile 2% citric acid solution are applied to the back of the tongue. 0.1 ml of oral fluid is taken for examination.

The material is weighed and diluted in sterile saline (1:10, 1:20, etc.), and then inoculated onto special nutrient media.

Microscopic counting of microorganisms in clinical material is carried out in a Goryaev camera, which is designed to determine the number of cells in a certain volume of liquid (Fig. 4.5).

Determination of viable cells on nutrient media after incubation is carried out by counting the number of colonies - CFU (colony-forming units) and taking into account the dilution of the material.



А Б

Fig. 4.5. Goryaev camera: A – camera; B – counting grid.

For this purpose, the material is sown using the separating stroke method, which is based on the mechanical separation of microorganisms on the surface of a dense nutrient medium. First, a bacteriological loop with a culture is applied to the surface of the agar medium in several parallel strokes (Fig. 4.6). The loop is sterilized, cooled, touching the uninoculated part of the medium, then 40 strokes are made at an angle of 45^0 from sector A to sector B. The loop is sterilized again, cooled and 4 strokes are applied in the perpendicular direction B, and after another sterilization - 4 more strokes in the direction G. With such sowing, the material is diluted 10 times in each sector. Thus, in the last sector there will be a 10^3-fold dilution of the material.

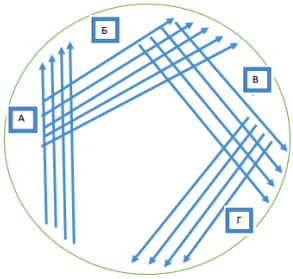


Fig. 4.6. Scheme of seeding by streak plate method.

The culture is incubated in a thermostat and after a certain time the results are recorded. Usually, individual colonies grow on the lines of sector G.

The total number of viable bacteria in 1 ml of material (CFU / ml) is calculated by the formula:

$N = n \cdot 10^3$, where N is the total number of microorganisms, n is the number of colonies in the sector.

Questions and test tasks for self-testing for chapter 4 (the number of correct answers may vary)

1. In the first hours of plaque formation, the following are mainly recorded:

a) aerobic species of microorganisms;

b) anaerobic species of microorganisms;

c) aerobic and facultative-anaerobic species of microorganisms;

d) microaerophilic species of microorganisms.

2. In the second stage of plaque formation, the following are mainly recorded:

a) aerobic species of microorganisms;

b) anaerobic species of microorganisms;

c) aerobic and facultative-anaerobic species of microorganisms;

d) microaerophilic species of microorganisms.

3. The process of plaque formation begins:

a) with the interaction of salivary glycoproteins with the tooth surface and the formation of a pellicle;

b) with the deposition of oral streptococci on the tooth enamel;

c) with the appearance of *Veillonella*, *Corynebacteria* and *Actinomycetes* on the tooth surface;

d) with a sharp increase in the number of anaerobic microorganisms.

4. In the surface layers of dental plaque, the microbiota is represented mainly by the following microorganisms:

a) *S. mutans*;

b) actinomycetes;

c) spirochetes;

d) *Bacteroides*

5. In the depths of dental plaque, the microbiota is mainly represented by the following microorganisms:

a) *S. mutans*;

b) actinomycetes;

c) spirochetes;

d) *Bacteroides*.

6. List the factors that belong to the group of general cariogenic factors:

a) malnutrition and drinking water;

b) dental plaque and bacteria;

c) heredity, which ensures the fullness of enamel;

d) carbohydrate food residues.

7. List the factors that belong to the group of local cariesogenic factors:

a) poor nutrition and drinking water;

b) dental plaque and bacteria;

c) heredity, which ensures the fullness of enamel;

d) carbohydrate food residues.
8. The main cariesogenic microorganism is:
a) *S. mutans*;
b) *S. pyogenes*;
c) *S. mitis*;
d) *S. sanguis*.
9. The initial stage of caries development is:
a) aggregation of bacterial cells;
b) adhesion of streptococci to tooth enamel;
c) formation of acidic products;
e) formation of glucans from glucose.
10. The most likely causes of caries are:
a) cariesogenic diet;
b) use of fluoride-containing drugs;
c) genetic predisposition of the organism;
d) activity of microorganisms.

Answers to test tasks for chapter 4

№ of question	True answers	№ of question	True answers
1	a	6	a, c
2	c	7	b, d
3	a	8	a, d
4	a	9	b
5	b, c, d	10	a, b, c

Chapter 5. MICROBIOLOGY OF PERIODONTAL DISEASES

The periodontium is a complex morphofunctional complex of tissues that surrounds and holds the tooth in the alveolus. All components of the periodontium (gum, periodontium, alveolar bone tissue and cementum) are closely related in development and structure, which ensures the performance of various and complex functions - barrier, trophic, plastic, support-retaining, etc.

More than 80% of the world's population is prone to periodontal diseases, which lead to tooth loss, the appearance of foci of chronic infection in the oral cavity, a decrease in the body's reactivity, microbial sensitization and other disorders. Inflammatory periodontal diseases (gingivitis and periodontitis) are widespread among the population: 60-70% after 30 years and 85-97% after 65 years, and are the leading cause of tooth loss in most adults.

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The microflora of the periodontal pocket is normal. The microflora of a healthy periodontal pocket is quite limited and is located under the gums on the surface of the teeth in a layer about 60 nm thick (from 1 to 20 cells). In the gingival groove, the microflora consists of gram-positive facultative anaerobic cocci (streptococci *S. sanguis* - 25%, *S. mitis* - 12.5%, in smaller quantities - staphylococci, peptostreptococcus) and rods (actinomycetes: *A. israelii, A. viscosus, A. naeslundii, A. odontolyticus, Propionibacteria*). They make up 90% of the population. Spirochetes are found in 1.8%. The ratio of motile to non-motile forms is 1/49.

Periodontal diseases. Periodontal diseases are various diseases of inflammatory and metabolic-dystrophic nature in terms of their origin, development mechanism and course, which are accompanied by the destruction of the complex of anatomical formations surrounding the tooth, namely the gums, the collagenous base of the periodontal ligament and the bone of the alveolar process.

Periodontal diseases include:

1. Gingivitis (inflammation of the mucous membrane of the gums).
2. Periodontitis (inflammatory destructive process accompanied by loss of tooth-gingival attachment and dystrophic lesion of periodontal tissues).
3. Idiopathic diseases (periodontal syndromes of genetic diseases with progressive lysis of periodontal tissues).
4. Periodontomas (tumor and tumor-like diseases).

Mechanism and conditions of periodontal disease. Microorganisms of dental plaque and immunopathological mechanisms are involved in the occurrence of the inflammatory process in periodontal tissues.

Normally, the oral cavity contains many microorganisms, and they do not have a pathogenic effect. In addition, there are a number of mechanisms of protection against the possible pathogenic effect of dental plaque. Saliva plays the main role, which effectively prevents excessive accumulation of microorganisms in the interdental spaces, in the area of dental-gingival junctions. Antimicrobial components of saliva (lysozyme, beta-lysine, etc.) inhibit the growth of microorganisms and thereby prevent their harmful effect on the periodontium.

Near the most vulnerable areas (dental-gingival grooves) is a dense capillary network. In the case of increased release of toxins, enzymes and other microbial pathogenic factors by microorganisms, protective blood cells (leukocytes) and their components enter these areas actively, inactivating or destroying microbial cells. Thus, the processes of microbial invasion and antimicrobial protection are normally quite balanced. Normal microbiota can cause pathological changes in the periodontium when microorganisms accumulate in very large quantities and the usual local protective mechanisms are unable to neutralize their toxic and enzymatic effects, or when the activity of local protective forces is insufficient.

In the pathogenesis of periodontal diseases, 4 stages can be distinguished.

At the first stage, colonization of bacteria occurs, mainly *S.sanguis* and *Actinomyces*, which firmly attach to the surface of the pellicle. Then other microorganisms join, which leads to an increase in the mass of dental plaque in different directions. Gingival fluid, growth factors and chemotaxis contribute to the migration of bacteria into the gingival sulcus, where they attach to the surface of the tooth, epithelium or other microorganisms and can resist the flow of gingival fluid. It is known that anaerobic microorganisms with pathogenic properties vegetate in bacterial plaque. *Actinobacillus, Actinomycetem comitans, Porphyromonas gingivalis, Bacteroidas forsythus, Streptococcus intermedius, Spirochetes, Campylobacter rectus, Eikenella corrodens*, etc. are present here.

The next stage is invasion. Oral bacteria usually do not penetrate the gum tissue, but bacterial antigens have this ability. The most active in this regard are lipopolysaccharides, dextrans and lipoteichoic acids. They penetrate the gums through the epithelium of the gingival groove to various depths up to the surface of the alveolar bone. Bacteria and their waste products cause local and generalized immune responses, while also having a destructive effect on the tissues, causing vascular damage, the development of inflammation and tissue necrosis.

Lipolysaccharides and other antigens of bacterial origin contribute to the release of collagenase-type enzymes by macrophages and leukocytes, which also damage the gum tissue.

Stage III - destruction of periodontal tissues. Microorganisms and their waste products lead to the destruction of periodontal tissues by direct toxic effects, similar to those exerted by exotoxin or histological enzymes.

Microbial enzymes are able to increase capillary permeability, cause disruption of the epithelial membrane permeability and penetrate the subepithelial base of the gingival mucosa. Collagenase, hydrolyzing collagen, is able to destroy the collagen of the periodontal ligament and bone tissue of the alveolar process. Bacterial hyaluronidase contributes to the destruction of the connective tissue epithelium, fibroblasts, dilation of microvessels, increased permeability of their walls, and enhanced migration of leukocytes.

Toxins have a direct effect on periodontal tissues. *Actinobacillus*, *Actinomycetem comitans*, *Porphyromonas gingivalis* secrete toxins, which are lipopolysaccharide-nucleic complexes that have an autolytic effect, disrupt cellular metabolism, cause vasomotor disorders, and contribute to the sensitization of the body. Cellular elements of the gums are damaged, toxins and enzymes of microorganisms penetrate into the soft gingival structures, and an acute inflammatory reaction develops. Further, the process spreads from the gums to the bone tissue. Due to increased osteoclast activity, bone resorption occurs. The pathogenic effect of microorganisms continues, and the inflammation becomes chronic.

Like any inflammation caused by an infectious agent, inflammation of periodontal tissues depends not only on the presence of microorganisms, but also on the general condition of the whole organism. The degree of prevalence of inflammatory-dystrophic processes in the periodontium, clinical and morphological features and the outcome of inflammation are determined by the level of physiological protective mechanisms. Insufficiency of immunity or violation of its individual links leads to increased virulence of microorganisms.

Against the background of altered reactivity of the body after diseases, intoxications, with vitamin deficiency, ulcerative gingivitis may develop. In this case, the gingival margin is covered with ulcers, which is accompanied by an increase in temperature, an increase in submandibular lymph nodes, and the appearance of an unpleasant odor from the mouth. In ulcerative gingivitis, along with streptococci and staphylococci, fusobacteria and spirochetes are detected in large numbers. The presence of fusospirochetosis indicates a violation of the resistance of periodontal tissues to the microflora of the oral cavity.

With a long course of catarrhal gingivitis, the inflammatory process can spread to the gums and bone tissue. The result is the destruction of the epithelial attachment and the formation of a pathological periodontal pocket, resulting in periodontitis, which is characterized by bleeding gums, deposition of subgingival plaque and tartar, tooth mobility, and the release of pus from under the gum when pressed. However, the main sign of periodontitis is a pathological periodontal pocket.

The immunopathogenesis of periodontal diseases is divided into two phases: reversible and irreversible.

During the reversible phase, a normal immune response occurs from the local tissues, the mechanism of which is associated with the active reproduction of gram-negative bacteria in gingival pockets and dental plaque. Microorganisms of the pathological periodontal pocket and their waste products significantly affect both the condition of the peri-gingival tissues and the body as a whole. The local effect is especially pronounced due to bacteria that produce histolytic enzymes (hyaluronidase, chondroitin sulfatase, collagenase and other proteinases).

During the decay of gram-negative microorganisms, endotoxin and other cytological substances are released; in addition, bacteria produce toxic metabolites (ammonia, organic acids, hydrogen sulfide). All these compounds can significantly disrupt the normal metabolism of tissues or cause inflammatory reactions that can be destructive. The histological effect of bacterial enzymes is considered the main cause of the appearance of a pathological gingival pocket. Absorbed into the blood, microbial and tissue toxins cause chronic intoxication and sensitization of the body. Thus, using skin allergy tests, patients were found to have a state of hypersensitivity to streptococci, staphylococci, neisseria, actinomycetes and other microorganisms.

When studying the contents of the periodontal pocket, IgG, IgA, IgM, complement fractions C3 and C5, leukocytes, as well as abundant infiltration of the gum tissues by plasma cells, lymphocytes, and macrophages were detected. All this suggests that many antigen-antibody reactions and manifestations of cellular immunity occur precisely in the periodontal pocket. Antigen-antibody immune complexes activate the complement system. At the same time, various biologically active substances are released from the cells. The formation of immune complexes should contribute to the cleansing of the oral mucosa from microbial antigens, which are then captured and degraded by phagocytes migrating to the focus of inflammation. That is why neutrophil chemotaxis, increased phagocytosis, and lymphocyte transformation are observed in the inflammation zone.

Clinical manifestations of the reversible phase are signs of local inflammation - gingivitis. If microbial antigens continue to enter the focus of inflammation, protective mechanisms can cause tissue destruction. Bacterial enzymes, as well as lysosomal enzymes of phagocytes (proteinases: elastase and collagenase) cause bone resorption. Damage to the ligamentous apparatus and resorption of the alveolar tissue lead to tooth mobility. Pathological gingival pockets are the entrance gates for secondary bacterial purulent infection. In this case, gingivitis turns into periodontitis.

During the irreversible, immunopathological, phase, T-lymphocytes are sensitized by autoantigens of periodontal tissues, enhanced by microbial endotoxins. Autoimmune mechanisms cause irreversible periodontitis with atrophy of osteocytes and alveolar processes of the jaw.

Thus, the spread of the process in periodontitis occurs in a descending manner, that is, the epithelium is first affected, then the deep connective tissue elements of the gums, epithelial attachment, ligamentous apparatus and finally bone tissue. Periodontitis, when the process spreads to the periosteum, can be complicated by the formation of an abscess.

Periodontal pathogens. The microflora in periodontal pockets differs in different individuals. At the beginning of the disease, facultative anaerobic and aerobic coccal flora prevails - streptococci, enterococci, neisseria. Later, they are replaced by obligate anaerobes - peptostreptococcus, veillonella, bacteroids, actinomycetes.

Periodontal pathogens are characterized by the presence of a number of pathogenicity factors that cause a prolonged inflammatory process:

- Adhesion factors contribute to the attachment of bacteria to epithelial cells, hydroxyapatite and to other bacteria in the process of co-aggregation of microorganisms.

- Invasion factors: enzymes protease, hyaluronidase, RNase, DNase and collagenase, cause tissue lysis.

- Endotoxins of gram-negative bacteria cause immunopathological processes that cause bone tissue destruction. In addition, they produce cytotoxic substances such as indole, ammonia, fatty acids, amines, etc., which also destroy periodontal tissues.

- The polysaccharide capsule and enzymes of gram-negative bacteria provide their protection against immunoglobulins and complement.

Microbiota in gingivitis. The total number of microbes in gingivitis is 10-20 times greater than in healthy periodontium. Even before the appearance of clinical symptoms, microscopy allows you to detect an increase in gram-negative anaerobic microorganisms. In this sense, the preclinical phase of inflammatory periodontal diseases can be considered as a kind of dysbacteriosis, which is caused by an unhealthy lifestyle, metabolic disorders in periodontal tissues, endocrine dysfunctions. With prolonged gingivitis, the proportion of gram-negative bacilli (fusobacteria, bacteroids, etc.) is about 45% of the total flora. Gram-positive bacilli (*Actinomyces naeslundii, A. viscosus, A. israelii*) are detected with a frequency of about 25%. Eubacteria and propionibacteria are isolated in small quantities. Gram-positive facultative anaerobic streptococci are detected in 27% of cases.

Microbiota in periodontitis. In chronic periodontitis, the microflora is extremely diverse and can consist of more than 150 different species, among which the proportion of obligate anaerobic gram-negative rods (bacteroids) and spirochetes reaches 40%.

Periodontopathogenic bacteria of the 1st order: *Porphyromonas gingivalis, Bacteroides fopsythus (Tannerella forsythia), Aggregatibacter actinomycetemcommitans*. They have high adhesiveness, produce collagenase, damage dentin. *Porphyromonas gingivalis* is a gram-negative anaerobe, has an endotoxin, which has a wide range of pathogenic properties that increase the destruction of alveolar bones.

Periodontopathogenic bacteria of the 2nd order: *Prevotella melaninogenica, Prevotella intermedia, Treponema denticola, Peptococcus niger, Fusobacterium nucleatum, Actinomyces israelii*. Prevotella secrete a strong endotoxin, the enzyme phospholipase, disrupt the integrity of epithelial cell membranes. The main factor in the pathogenicity of actinomycetes is the ability to produce leukotoxin, which causes leukocyte lysis, resulting in a decrease in the protective functions of tissues. Bacteria also produce substances that significantly improve the adhesion of the microorganism to tissues and break down collagen fibers.

Gram-positive bacteria are mainly attached to cementum, while gram-negative bacteria are able to multiply in the loose layers of subgingival plaque, which is located near the apical part of the pocket. The leading etiological factor of inflammatory periodontal diseases is the microbiota of dental plaque. The inflammatory process in periodontal tissues begins with the colonization of the surface of the gums and teeth by facultative anaerobic bacteria (*A. viscosus, S. mutans, B. melaninogenicus, F. nucleatum*). The main mechanism of microbial invasion into periodontal tissues is their translocation from the "dental" plaque and infection of the entire complex. In this case, periodontal pathogenic bacteria overcome the immunobiological barriers of the oral cavity.

Microbiological examination of periodontal diseases. Bacterioscopic and bacteriological methods are used for diagnosis. Material for examination: subgingival dental plaque, gingival fluid.

To obtain material of subgingival dental plaque from the periodontal pocket, a sharp probe is used. Gingival fluid from the pathological gingival pocket and gingival groove is collected with a scaler or curettage spoon, as well as with a micropipette, sterile threads or filter strips according to the principle of capillarity.

For microscopic examination, material from the gingival pocket is collected on celluloid narrow plates, inserting them into the pocket and pressing them to the surface of the tooth root from the gum side. Bacteria from the tooth root stick to the inner side of the plate, and microorganisms from the gingival fluid stick to the outer side.

The collected material is immediately placed in a transport nutrient medium. Further cultivation and identification of microorganisms is carried out according to the classical scheme.

Questions and test tasks for self-testing for chapter 5 (the number of correct answers may vary)

1. Periodontal diseases include:

a) a group of diseases in which demineralization and softening of hard tooth tissues occurs with subsequent cavity formation;

b) a group of diseases of an inflammatory and metabolic-dystrophic nature, accompanied by destruction of gum tissues, including the collagenous basis of the periodontium and the bone of the alveolar process;

c) a group of diseases of an inflammatory nature that are directly related to the tissues located inside and around the tooth;

d) acute or chronic inflammatory processes that occur in the coronal or root pulp.

2. Indicate the pathogenetic mechanisms of inflammatory periodontal diseases:

a) reproduction of anaerobic microflora;

b) demineralization of enamel;

c) microtraumas of the mucosa;

d) immunological reactivity of the human body.

3. Specify the groups of microorganisms to which periodontal pathogenic species of bacteria belong:

a) bacteroides;

b) actinomycetes;

c) streptococci;

d) peptostreptococcus.

4. The leading link in the development of periodontal diseases is:

a) actinomycetes;

b) bacteroides;

c) neisseria;

d) streptococci.

5. List the virulence factors of periodontal pathogenic bacteria:

a) adhesion factors;

b) invasion factors;

c) aggression factors;

d) toxigenicity factors.

6. Periodontal pathogenic species include:

a) *Treponema orale*;

b) *Streptococcus mutans*;

c) *Porphyromonas gingivalis*;

d) *Prevotella melaninogenica*.

7. In gingivitis, the following changes are observed:

a) an increase in the total number of microbes in periodontal tissues;

b) an increase in gram-negative microflora;

c) replacement of coccal flora with rod-shaped forms;

d) an increase in gram-positive microflora.

8. In prolonged gingivitis, the following are detected in the subgingival plaque:

a) fusobacteria;

b) bacteroids;

c) spirochetes;

d) actinomycetes.

9. In periodontitis, the following changes in microflora are observed:

a) an increase in the number of cocci;

b) an increase in the number of rod-shaped forms and spirochetes;

c) an increase in the ratio of motile to immobile forms to 1:1;

d) a decrease in the total number of microorganisms.

10. Specify the microorganisms that are detected in periodontitis:

a) *Neisseria meningitidis*;

b) *Porphyromonas gingivalis*;

c) *Prevotella melaninogenica*;

d) *Fusobacterium nucleatum.*

Answers to test tasks for chapter 5

№ of question	True answers	№ of question	True answers
1	b	6	c, d
2	a, d	7	a, b, c
3	a, b, d	8	a, b, d
4	b	9	b, c
5	a, b, c, d	10	b, c, d

Chapter 6. MICROBIOME IN INFLAMMATIONS OF THE ORAL MUCOSA

The oral mucosa (OM) has certain differences from other mucous membranes of the human body. It is resistant to the effects of physical and chemical stimuli, as well as to the penetration of infectious agents. Resistance to the effects of stimuli is determined by the integrity of the epithelium and the presence of keratinization areas in areas where the greatest mechanical load is noted, which ensures the barrier function of the OM.

The OM is lined with a multilayered squamous epithelium consisting of several layers of cells. The basal membrane, the mucous membrane itself, and the submucosa are located beneath it. The epithelium is directly facing the oral cavity and is constantly renewed as a result of the exfoliation of the upper layer. Regeneration in the OM is quite active. The mucous membrane, epithelial cells, and their receptors are the first line of defense against the penetration of foreign agents.

Pathological changes in the mucous membrane occur under the influence of external stimuli such as mechanical, physical, chemical ones. A feature of the oral mucosa is that any prolonged injury causes its infection.

Prolonged exposure to a traumatic factor leads to a change in the microbial balance, initially the mechanism of catarrhal inflammation is triggered, which can later turn into purulent with the participation of bacterial flora.

Depending on the localization of the inflammatory process, damage to the mucous membrane of the oral cavity has different names: stomatitis (mucous membrane of the cheeks), glossitis (tongue), gingivitis (gums), cheilitis (mucous membrane of the lips).

Inflammatory processes can be caused by specific or nonspecific pathogens.

Oral mucosa lesions caused by specific pathogens. These include:

1) viral infections (herpetic stomatitis, manifestations of herpes zoster and other viral infections);

2) bacterial infections (streptococcal stomatitis, staphylococcal granuloma);

3) venereal diseases (syphilis manifestations, gonorrheal stomatitis);

4) mycoses (candidiasis, actinomycosis);

5) infectious diseases (measles, scarlet fever, chickenpox, tuberculosis).

Viral infections of the oral mucosa. Oral mucosal lesions are observed in many viral infections.

Herpetic gingivostomatitis is caused by the herpes simplex virus type 1 (Fig. 6.). This virus also causes keratoconjunctivitis, herpetic eczema, herpetic fever, meningoencephalitis and some other diseases.

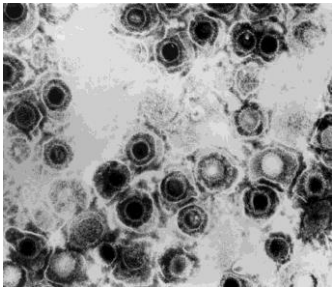

Fig. 6.1. Herpes simplex virus. Electron microscopy/
https://alchetron.com/Herpes-simplex-virus

Primary infection with the herpes virus occurs at the age of 6 months to 6 years, when children become infected from adult virus carriers. Herpetic gingivostomatitis occurs in the form of an acute infection, later activation of latent carriage is possible. Gingivostomatitis in herpes infection is characterized by fever, drooling, severe pain in the mouth. The disease is manifested by hyperemia, vesicles that quickly turn into aphthae, which are localized on the mucous membrane of the palate, lips, tongue and transitional folds. Aphthae have the appearance of oval-shaped erosions with smooth edges and a bottom, covered with a grayish-white coating, necrotic areas of the gums have a yellowish-white color. The disease may be complicated by the accession of coccal microflora.

Laboratory diagnostics is carried out by viroscopic, virological, biological and serological methods.

The causative agents of herpetic angina are Coxsackie A viruses, which belong to the picornavirus family. Clinical manifestations of the disease: general hyperemia of the oral mucosa and vesicular rashes, which quickly burst with the formation of aphthae.

Virological, serological and biological methods are used for virological diagnostics.

Acute bacterial infections. Purulent-inflammatory processes (gingivostomatitis, furuncles, chronic lip fissures, gingivitis, chronic ulcerative pyogenic granuloma) can be caused by streptococci (*S. pyogenes*) (Fig. 6.2) and staphylococci (more often *S.aureus*). Clinically, these diseases are manifested by erosions with purulent discharge. Pyogenic cocci penetrate through microtraumas. Mixed strepto- and staphylococcal

flora causes impetigo, which is characterized by the development of a pustular focus on the skin of the face, red border of the lips and oral mucosa.

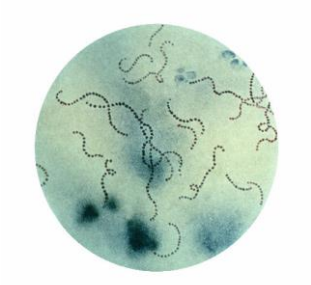

Fig. 6.2. *S. pyogenes*. Methylene blue stain.
CDC. *https://phil.cdc.gov/Details.aspx?pid=2109*

Laboratory diagnostics of coccal diseases of the oral mucosa is based on the bacteriological method.

Venereal diseases. The causative agent of gonococcal stomatitis is gonococcus (*Neisseria gonorrhoeae*) (Fig. 6..3). The disease is transmitted by contact-household and sexual routes, as well as during the passage of a child through the birth canal of an infected mother. Clinical manifestations: hyperemia, edema of the oral mucosa, erosions with a viscous mucopurulent secretion. In gonorrhea, the gums are swollen and inflamed, ulcers may appear on the lips, tongue and mucous membrane of the cheeks. The pharynx and salivary glands may be affected.

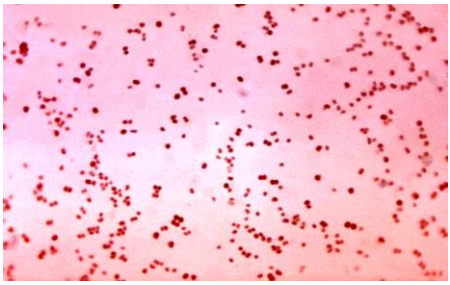

Fig. 6.3. *N. gonorrhoeae*. Gram stain.
CDC/ Renelle Woodall. *https://phil.cdc.gov/Details.aspx?pid=14855*

Syphilis is caused by *Treponema pallidum*. The bacteria resemble a thin spiral, which consists of 8-12 uniform coils (Fig. 6.4). They do not form spores and capsules. They are motile due to fibrils located inside the cell. Microaerophile or obligate aerobe. They are not stable in the external

environment. The pathogen is transmitted sexually, transplacentally and by contact-household routes.

Fig. 6.4. *T. pallidum.* Dark field microscopy.
CDC/ Richard O. Deitrick. *https://phil.cdc.gov/Details.aspx?pid=20488*

The disease has a chronic course and goes through several stages. Primary syphilis is characterized by the formation of primary syphiloma - a hard chancre, which appears in a 3-4-week incubation period. The chancre can form on the red border of the lips, tongue, oral mucosa, tonsils (chancre-amygdalitis). Oral chancres account for 55% of all extragenital localizations. The secondary period is manifested by a polymorphic rash (roseola, papules, pustules) on the mucous membranes and skin. On the mucous membrane of the oral cavity, the rash appears on the hard palate, tonsils, cheeks along the line of teeth closure and has the appearance of dense elements with a whitish smooth surface. The papules are painless. The ulcers resemble aphthae. With rashes on the larynx, the patient develops hoarseness. An increase in temperature is noted.

Tertiary syphilis is manifested by gummy lesions. In the oral cavity, single or multiple gums can be localized on the lips. They have the appearance of bluish-red dense painless tubercles. In the absence of treatment, gummy perforation of the soft and hard palate develops. Manifestations of congenital syphilis appear at 1-2 months of life and are characterized by swollen, thickened lips of yellow-red color, ulcers on the surface of the oral mucosa, which subsequently scar. Robinson-Fournier scars in the corners of the mouth are characteristic.

Late congenital syphilis develops after 2 years and is characterized by Hutchinson's triad: Hutchinson's teeth (chisel-shaped or barrel-shaped incisors, hypoplasia of the chewing surface with a crescent-shaped notch on the free edge), labyrinthine deafness and parenchymal keratitis (corneal clouding). Other manifestations of dental dystrophy: Fournier's pike tooth (change in the canine with thinning of its free end), Moon's tooth (underdevelopment of the chewing tubercles of the first molars), etc.

Bacterioscopic and serological methods are used for laboratory diagnostics.

Mycoses. Most oral mucosal mycoses are caused by yeast-like fungi of the genus *Candida*. The most pathogenic among the 150 species of fungi of the genus *Candida* is *C. albicans*, which is the causative agent of candidiasis.

By structure, *Candida* cells belong to eukaryotes. They have a yeast form, hyphae (non-septated mycelium) and pseudohyphae (pseudomycelium) - thin elongated cells arranged in the form of threads that do not have a common shell (Fig. 6.5). By type of respiration, aerobic. Fungi are quite resistant in the external environment, survive better on moist surfaces.

A characteristic form of infection for *Candida* is carriage, which can cause endogenous infection. *Candida* are representatives of the normal microbiota of the mucous membranes of the oral cavity, as well as the gastrointestinal tract, genital tract and skin. With a weakening of local immunity, the number of yeast-like fungi can increase. In addition, there are exogenous sources of infection with fungi of the genus *Candida*.

Oral candidiasis (pseudomembranous candidiasis or thrush) is characterized by the appearance of a white coating on the oral mucosa, which has the appearance of curdled milk. Exacerbation of the infectious process often occurs in premature newborns, as well as in children who are on artificial feeding. In adults, candidiasis develops against the background of secondary immunodeficiencies.

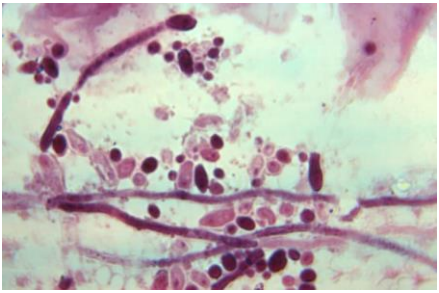

Fig. 6.5. Pseudohypha and blastoconidia of *C. albicans.*
CDC/Hardin. *https://phil.cdc.gov/Details.aspx?pid=21201*

Acute atrophic candidiasis is a consequence of acute pseudomembranous candidiasis. Characteristic signs are heartburn and dry mouth. The oral mucosa is dry, fiery red. A plaque that is difficult to remove can be observed in deep folds and contains epithelial cells and fungi of the genus *Candida* in the form of mycelium and pseudomycelium. The dorsum of the tongue is crimson red, shiny, dry, filiform papillae are atrophied.

Chronic atrophic candidiasis is often the result of wearing dentures. By localization there are: candidal cheilitis (infection of the lip areas), glossitis (infection of the tongue), canker sores (infection of the corners of the mouth).

Hyperplastic candidiasis is manifested by hyperemia of the mucous membrane, the formation of large white papules, which sometimes merge.

Mainly, the back of the tongue, the back of the palate and the mucous membrane of the cheeks near the corners of the lips are affected. A chronic course is possible. The disease is considered precancerous.

Microbiological diagnostics is based on microscopic, serological, mycological and allergological studies.

Infectious diseases. The causative agent of measles is a virus of the *Paramyxoviridae* family. The route of transmission is airborne. In the catarrhal period, the disease is characterized by hyperemia of the mucous membrane of the cheeks and the appearance of whitish-yellow round spots with a diameter of 1-2 mm, which are located near the molars, less often - on the mucous membrane of the gums or lips (Koplyk-Filatov symptom). On the 3rd-4th day, the Koplyk-Filatov spots disappear and an exanthema appears on the skin and an enanthema on the mucous membrane of the soft palate. The enanthema has the appearance of small bright red or pale red spots of the correct rounded or elongated shape.

For laboratory diagnostics, serological, virological and viroscopic methods are used.

Scarlet fever. The causative agent of scarlet fever is *Streptococcus pyogenes*. The route of transmission is airborne. Preschool children are more often affected. The manifestations of the disease are due to the pathogenicity factor - erythrogenic toxin. Clinical manifestations: bright hyperemia of the mucous membrane of the palate and tonsils ("burning throat"), bright red mushroom-shaped papillae and white coating on the tongue, bright pink or red small rash on the skin, which appears on the 2-3rd day of illness.

To confirm the diagnosis, a bacteriological method is used.

Diphtheria. The causative agents is *Corynebacterium diphtheriae*, which is capable of producing a toxin. Gram-positive rods are arranged in the form of Latin letters Y, V, L, have thickenings at the ends of the cells (Fig. 6.6). Non-motile, do not form spores. Aerobes.

The route of transmission is airborne. The insular form of diphtheria is characterized by a white or grayish-white plaque in the form of small plaques on the tonsils, tongue, palatine arches, on the mucous membrane of the cheeks, sometimes at the site of an extracted tooth. In the widespread form of diphtheria, there is a dirty-gray or yellowish-gray filmy plaque on the background of dim hyperemia. Atypical forms of diphtheria are distinguished, similar to lacunar, catarrhal or follicular angina, diphtheria of the nose and wound surfaces.

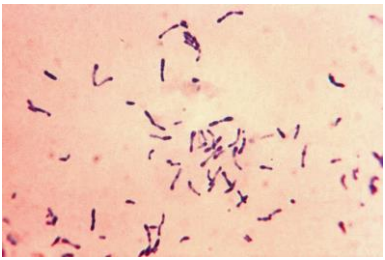

Fig. 6.6. *C. diphtheriae.* Methylene blue stain.
CDC/P.B. Smith. *https://phil.cdc.gov/Details.aspx?pid=7323*

For microbiological diagnostics of diphtheria bacteriological and bacterioscopic methods are used.

Tuberculosis. The causative agents are *Mycobacterium tuberculosis* and *M.bovis*. Gram-positive rods. Non-motile, do not form spores. They stain better according to Ziehl-Nielsen (Fig. 6.7). Obligate aerobes. Resistant in the environment.

Mechanisms of infection – aerogenic, less often – contact and alimentary. Transmission routes – airborne, airborne-dust, contact-household, with dairy products. Pathogens enter the oral cavity by hematogenous means. The disease initially manifests itself by the formation of a red tubercle of 1-3 mm on the gums and near the front teeth, on the palate and on the upper lip. Over time, an ulcer forms in the center of the tubercle. Then there is destruction of the bone tissue of the interalveolar septa, which causes mobility and tooth loss. In chronic tuberculosis, smooth, shiny scars appear at the site of the ulcers. The process is complicated by secondary bacterial or fungal infection.

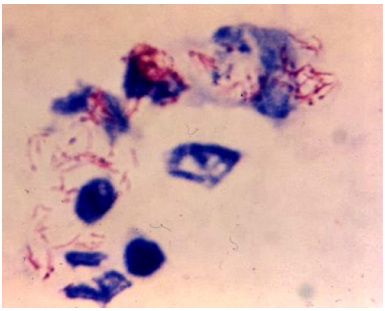

Fig. 6.7. *M. tuberculosis.* Ziehl-Neelsen stain.
CDC/George Lubica. *https://phil.cdc.gov/Details.aspx?pid=18080*

Microbiological diagnostics is based on bacterioscopic, bacteriological, biological and molecular biological studies. The allergical method is also used.

Actinomycosis. The causative agents are *Actinomyces israelii* and *A.viscosus*. Gram-positive branched bacteria with a tendency to fragmentation. Non-motile, do not form spores. Facultative or obligate anaerobes.

Actinomycosis can develop as an endogenous infection with a decrease in local immunity of the mucous membranes of the oral cavity. Exogenous infection is possible when the mucous membranes are injured and actinomycetes enter the environment (cereals, soil). The endogenous form of infection prevails. Humans are not a source of infection.

Actinomycetes are representatives of the normal microflora of the oral cavity. In inflammatory processes, they penetrate deep into the tissues - near the destroyed roots of the teeth, pathological gingival pockets in periodontitis.

Actinomycosis is characterized by the growth of granulation tissue around the lesion. Large granulomas are formed in the soft tissues and jaw bones. After necrosis of the central part of the granuloma, pus is released from it through fistulas.

Microscopy of pus reveals characteristic granulomas, which are called drusen (Fig. 6.8). They are the result of a local delayed-type hypersensitivity reaction and have the appearance of yellow granules. Gram staining reveals a plexus of thin hyphae with thickenings at the ends in the center of the drusen, which are surrounded by eosinophilic accumulations. The central part of the drusen is gram-positive, and the periphery is gram-negative. The existence of bacteria in this form protects them from phagocytosis and antibodies.

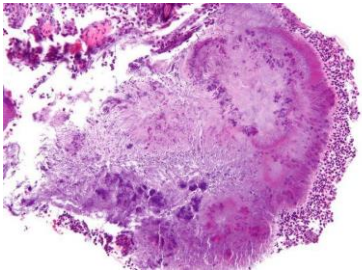

Fig. 6.8. Drusen in actinomycosis
https://alchetron.com/Actinomyces-bovis#actinomyces-bovis-45291a40-5273-4217-b49b-cf6061565ce-resize-750.jpg

The disease may be complicated by secondary infection with pyogenic microorganisms.

Bacterioscopic and allergological methods are used for diagnosis.

Nonspecific (endogenous) lesions of the oral mucosa. In superficial stomatitis, which does not have a specific pathogen, an increase in the number of

aerobic coccal flora (staphylococci, neisseria), as well as aerobic bacilli (diphtheroids) is noted. Deep stomatitis is characterized by ulcerative-necrotic manifestations of the surface of the oral mucosa. Anaerobic flora prevails here, mainly bacteroids and spirochetes, as a result of which these lesions were called "fusospirochetosis". Representatives of anaerobic microflora (*Veillonella, Peptostreptococcus, Vibrio, Actinomycetes)* are also detected.

Gingivostomatitis of Simanovsky-Plaut-Vincent (fusospirochetosis) is classified as opportunistic infections of the oral cavity. This is a mixed infection caused by two inhabitants of the oral cavity - *Treponema vincentii* (Fig. 6.9) and *Fusobacterium nucleatum* (Fig. 6.9, 6.10).

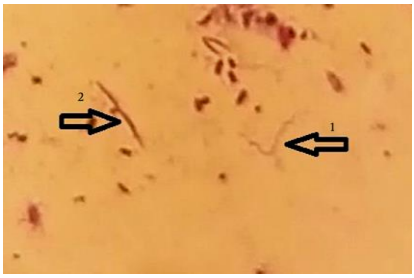

Fig. 6.9. *T. vincentii* (1) and *F. nucleatum* (2).
https://doi.org/10.5812/archcid.23292

Gingivostomatitis of Simanovsky-Plaut-Vincent is an infectious inflammatory disease that affects the tissues of the tonsils. In this case, the lymphoid tissue of the tonsils is necrotized. A gray-yellow, green or white plaque forms on the tonsils, which is easily removed, but after that the tissues bleed. Ulcers also form, which can spread to the pharynx, gums and mucous membrane of the cheeks. The disease can develop with hypovitaminosis, hypothermia, stressful conditions, weakened local immunity, as well as against the background of chronic alcoholism, smoking and chronic diseases. Gingivitis and caries can also be complicated by fusospirochetosis.

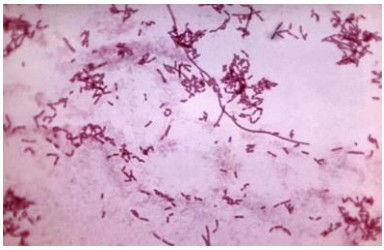

Fig. 6.10. *Fusobacteria.* Fuchsin staining.

CDC/ Holdeman. *https://phil.cdc.gov/Details.aspx?pid=16210*

Fusobacteria and spirochetes are always present in the gingival pockets and folds of the oral mucosa. The inflammatory process is initiated by coccal microflora, which is joined by other bacteria, including *Treponema vincentii* and *Fusobacterium nucleatum*. Fusobacteria are able to destroy collagen fibers of connective tissue due to the enzyme collagenase. Nitrogen-containing products of collagen breakdown are absorbed by spirochetes. Anaerobic conditions in necrotic tissues contribute to the reproduction of other anaerobes (bacteroids, peptostreptococcus and peptococcus), which damage tissues. Clinically, the disease manifests itself as filmy-ulcerative lesions on the mucous membrane of the pharynx, cheeks, gums, and tonsils.

Diagnosis of gingivostomatitis of Symanovsky-Plaut-Vincent is based on bacterioscopic examination.

Microbiological diagnostics of inflammatory diseases of the oral mucosa. Clinical material for stomatitis: plaque from the mucous membrane, tongue; pus, exudate from ulcers and erosions.

Scraping from the surface of the tongue and mucous membrane is done with a sterile spatula, a spatula or taken with a sterile cotton swab from an area of 1 cm^2. Material from erosions and ulcers is taken with a dry or moistened with a saline solution swab.

To prepare smears-imprints, dry degreased glass with polished edges is used, which is applied to the mucous membrane or elements of the lesion.

To prevent saliva from getting into the sample, it is necessary to cover the studied area with sterile cotton swabs.

Clinical material for microbiological examination should be taken before the start of antibacterial therapy or 10-12 hours after the drug is discontinued.

Material for the isolation of obligate anaerobic microorganisms is obtained by puncture with a syringe, from which air is previously removed. Pieces of tissue and swabs with the material are immediately immersed in a transport nutrient medium. When transporting such samples to the laboratory, protect them from the effects of atmospheric oxygen.

Basic nutrient media: for calculating the total microbial inoculation - 5% blood agar, for staphylococci - yolk-salt agar, for streptococci - sugar broth, for lactobacteria - vegetable-milk medium, for fungi of the genus *Candida* - Sabouraud medium, for obligate anaerobes - Wilson-Blair medium, for enterobacteria - Endo medium.

Identification of isolated cultures is carried out by morphological, cultural and biochemical properties in accordance with current regulatory documents.

Determination of the number of microorganisms is carried out by counting colony-forming units (CFU) on nutrient media and converting them to 1 ml of oral fluid, 1 g of dental plaque, 1 cm^2 of the surface of the tongue and mucous membranes.

Questions and test tasks for self-testing to chapter 6 (the number of correct answers may vary)

1. A culture of coccal bacteria was isolated from the oropharynx of a boy suffering from chronic tonsillitis. In smears, they were arranged in the form of chains. What microbes could these be?

a) streptococci;
b) staphylococci;
c) escherichia;
d) clostridia;
e) vibrios.

2. In the culture of pus from a boil, spherical microbes were found, arranged in the form of clusters of grapes. What morphological forms of microbes were found?

a) diplococci;
b) micrococci;
c) streptococci;
d) staphylococci;
e) tetracocci.

3. In a smear from the plaque on the tonsils of a patient with suspected diphtheria, blue rods with purple thickenings at the poles were found. What method of staining the smears was used?

a) Burry;
b) Neisser;
c) Leffler;
d) Gins;
e) Gram.

4. When examining mucus from the nasopharynx, the bacteriologist followed certain rules for preserving pathogens in the material. Bacterioscopic examination revealed the presence of gram-negative cocci that resemble coffee beans and are arranged in pairs. Name the pathogen that was isolated by the bacteriologist?

a) *Staphylococcus aureus*;
b) *Neisseria meningitides*;
c) *Neisseria gonorrheae*;
d) *Moraxella lacunata*;
e) *Acinetobacter calcoaceticus*.

5. The laboratory received sputum from a patient with tuberculosis. What staining method should be used to detect tuberculosis pathogens?

a) Ziehl-Nielsen
b) Gram
c) Romanovsky-Giemsa
d) Buri-Gins
e) Neisser

6. On the oral mucosa of a 20-year-old woman, a dentist noticed a rounded ulcer with a dense bottom and smooth edges, resembling a hard chancre. What diagnostic method should be used at this stage of the disease to confirm the etiology of the pathological process?

a) bacterioscopic;
b) bacteriological;
c) biological;
d) serological;
e) allergological.

7. In a micropreparation made from an ulcer on the patient's lips, stained according to Romanovsky-Giemsa, the doctor found thin microorganisms with 12-14 uniform curls of pale pink color. The causative agent of which infectious disease can we talk about in this case?

a) relapsing typhus
b) syphilis
c) leishmaniasis
d) leptospirosis
e) trypanosomiasis

8. The virulence of *Fusobacterium nucleatum* is associated with the presence of:

a) pili;
b) collagenase;
c) leukocidin;
d) metabolites: volatile and long-chain fatty acids.

9. After prolonged antibiotic therapy, a patient has whitish spots on the oral mucosa. Gram-positive oval kidney-shaped cells were found in the prepared smears. What are these pathogens?

a) *Candida* fungi;
b) staphylococci;
c) sarcinae;
d) actinomycetes;
e) tetracocci.

10. On the oral mucosa of a 20-year-old woman, a dentist noticed a round ulcer with a dense bottom and smooth edges, resembling a hard chancre. What diagnostic method should be used at this stage of the disease to confirm the etiology of the pathological process?

a) bacterioscopic;

b) bacteriological;
c) biological;
d) serological;
e) allergological.

Answers to test tasks for chapter 6

№ of question	True answers	№ of question	True answers
1	a	6	a
2	e	7	c
3	e	8	a, b c, d
4	b	9	a
5	a	10	a

Chapter 7. ODONTOGENIC INFECTION OF THE MAXILLOFACIAL REGION

Odontogenic infection is an inflammatory process associated with the tissues around and inside the tooth. Its development is influenced by the anatomical and topographic features of the entrance gate of the infection (odontogenic focus) and the surrounding tissues (soft tissues of the maxillofacial region, periosteum and bone). Favorable conditions for the rapid spread of infection are their anatomical proximity, a large number of lymphatic and blood vessels between these tissues.

The role of normal microbiome in the development of odontogenic infection. The main role in the development of inflammatory diseases of the maxillofacial region belongs to obligate anaerobes, since they predominate in the microbiocenosis of the oral cavity. The causative agents of odontogenic purulent-inflammatory processes are representatives of the genera *Peptococcus, Peptostreptococcus, Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Leptotrichia, Actinomyces*. They belong to non-spore-forming anaerobic bacteria. Their role in the pathogenesis of the disease is due to the presence of a number of pathogenicity factors, which include:

- cell surface structures (capsule, pili);
- enzymes (neuraminidase, collagenase, fibrinolysin, DNAse, beta-lactamase, heparinase);
- toxins (hemolysins, endotoxin, hemagglutinins, leukocidin);
- metabolites (fatty acids).

Associations of 3-5 or more types of obligate anaerobes or their associations with aerobes *(Pseudomonas aeruginosa* and *Neisseria*) and facultative anaerobes (more often streptococci and staphylococci) may participate in the inflammatory process. Symbiotic relationships between them ensure the synergy of their pathogenic action in the focus of inflammation.

The clinical picture of the disease is determined not by the type of pathogen, but by the affected organ.

For the development of odontogenic inflammation, several conditions must be met:

1. The spread of microorganisms beyond their natural biotope.
2. A decrease in the body's defenses.
3. The presence of favorable conditions for the reproduction of anaerobic bacteria.

Local causes of the spread of bacteria beyond their inherent ecological niche: injuries to the oral mucosa, various surgical interventions (tooth extraction), endoscopy, punctures, tissue necrosis, tumor decay, etc.

General causes of a decrease in the body's immune defense: starvation, overwork, hypothermia, local circulatory disorders, blood loss, as well as the use of certain medications.

Of great importance in the pathogenesis of odontogenic infections are chronic foci of infection in the oral cavity, namely chronic osteomyelitis, periodontitis, chronic periodontitis, chronic gangrenous pulpitis, chronic pericoronaritis.

Microbiome in inflammatory processes of the maxillofacial region. The composition of microbial associations that are detected in odontogenic diseases includes anaerobic gram-positive and gram-negative rods, peptococci, peptostreptococci, β-hemolytic streptococci, staphylococci (*S. aureus*), putrefactive bacteria.

In acute odontogenic inflammatory diseases, non-spore-forming obligate anaerobic bacteria (peptostreptococcus, peptococci, fusobacteria, bacteroids, less often actinomycetes), as well as streptococci are isolated.

For chronic odontogenic inflammations, associations of obligate anaerobes with facultative anaerobic streptococci and staphylococci are characteristic.

Periostitis is an inflammation of the periosteum. The study of pus in acute purulent periostitis most often reveals microbial associations, which include staphylococci (golden, plasmacoagulase) and β-hemolytic streptococci, in some cases - gram-positive and gram-negative bacilli, putrefactive bacteria.

Osteomyelitis is a purulent-necrotic process that develops in the bones and bone marrow. The source of infection is pathogens of diseases of hard and soft tissues of the tooth, as well as periodontal tissues. Osteomyelitis occurs as a result of penetration into the bone of microorganisms from the focus of periodontitis, is an example of a mixed infection, in which staphylococci play a leading role, much less often their combination with streptococci. In osteomyelitis, microbial associations may be detected, which include staphylococci, streptococci, gram-positive and gram-negative microorganisms, including putrefactive bacteria.

Abscess is purulent inflammation of tissues with their melting and the formation of a purulent cavity, can develop in the subcutaneous tissue, muscles, bones (localized inflammatory process). Odontogenic abscesses are caused by microbial associations,

which are dominated by staphylococci, streptococci, gram-positive and gram-negative bacilli. The leading pathogens are considered to be staphylococci resistant to antibiotics.

Phlegmon is acute diffuse purulent inflammation, which spreads to several areas of the head and neck. With phlegmonous inflammation, clostridia - pathogens of anaerobic gas infection - sometimes penetrate the tissue, which significantly aggravates the course of the disease and worsens the prognosis.

Microbiological study of microbome in odontogenic diseases. The purpose of the study: to clarify the etiology and pathogenesis of the disease, control treatment, determine the sensitivity of microorganisms to antimicrobial drugs.

The main method is bacteriological. Material for research: material from the root canal, punctates, purulent discharge, pieces of tissue.

Material from root canals is taken with root needles with cotton turunds. Punctates in abscesses, fasciitis and phlegmons are taken with a syringe, from which air is previously removed. Pieces of tissue are taken during surgical interventions from the depth of the focus with a cotton swab. Clinical material is placed in a transport medium that ensures the preservation of the vital activity of microorganisms for 6 to 12 hours due to the peculiarities of its composition.

Isolation and identification of microorganisms are carried out by standard methods in accordance with current regulatory documents.

Questions and test tasks for self-testing to chapter 7 (the number of correct answers may vary)

1. The characteristic features of odontogenic inflammation are:

a) progression of the process from local to general;

b) occur in the form of a mixed infection;

c) are easily treatable;

d) there is a sequential change in the dominance of aerobic, facultative anaerobic and obligate anaerobic species.

2. A mixed infection is:

a) a disease accompanied by a full set of characteristic symptoms;

b) a disease that occurs without clinically pronounced symptoms;

c) a disease caused by a opportunistic bacteria only;

d) a disease caused by several pathogens.

3. List the conditions necessary for the development of odontogenic inflammation:

a) the exit of microflora beyond the limits of its inherent ecological niche in the body;

b) decreased immunity;

c) the presence of conditions for the reproduction of anaerobes;

d) the presence of molecular oxygen.

4. Specify the features of the collection and study of material in odontogenic inflammation:

a) carrying out all manipulations under aerobic conditions;
b) taking material under anaerobic conditions;
c) carrying out all manipulations under anaerobic conditions;
d) cultivating the studied material under aerobic and anaerobic conditions.
5. In odontogenic inflammatory processes, microbiological methods are used:
a) to clarify the etiology and pathogenesis of the disease;
b) to control the treatment;
c) to predict the outcome of the disease;
d) to determine the sensitivity of the microbial association to antibiotics.
6. The material for research in odontogenic inflammatory processes is:
a) material from the root canal;
b) material from the carious cavity;
c) punctate;
d) purulent discharge.
7. Why is the clinical material placed in transport media?
a) they ensure the preservation of the vital activity of bacteria;
b) they ensure the convenience of transportation;
c) they prevent contamination of the environment with microbes;
d) they inhibit transit microorganisms.
8. The main method of studying microbiome in odontogenic inflammations of the maxillofacial region is:
a) bacterioscopic method;
b) bacteriological method;
c) biological method;
d) serological method.
9. Periostitis of the jaw is called:
a) the process of demineralization and softening of hard tissues of the tooth, which leads to the formation of a defect in the form of a cavity;
b) inflammation of the soft tissues of the tooth;
c) inflammation of the soft and hard tissues surrounding the tooth;
d) inflammation of the periosteum;
e) inflammation of bone tissue.
10. In acute odontogenic inflammations, the following are most often found:
a) bacteroids;
b) diphtheroids;
c) peptostreptococci;
d) fusobacteria.

Answers to test tasks for chapter 7

№ of question	True answers	№ of question	True answers

1	a, b, d	6	a, c, d
2	d	7	a
3	a, b, c	8	b
4	b, d	9	d
5	a, b, c, d	10	a, c, d

Chapter 8. LOCAL IMMUNITY OF THE ORAL CAVITY

Local immunity (colonization resistance) is a set of innate and acquired mechanisms of protection and regulation of cellular and molecular interaction. This is a complex of protective factors of various nature, formed in the process of evolutionary development, which provides protection of the mucous membrane of those organs that are in direct contact with the external environment.

Its main function is to maintain homeostasis of the internal environment of the human body, it is the first barrier to microorganisms and any antigens. From this point of view, local immunity is an integral part of general immunity, and at the same time, it is an autonomous system in its functions.

Antimicrobial defense mechanisms are divided by specificity into:

- nonspecific,
- specific.

Nonspecific protection of the oral cavity is a set of mechanical, chemical and physiological processes, the implementation of which does not depend on the recognition of the antigenic structure of microbes and is not controlled by the genes of the immune response.

Nonspecific factors of oral cavity protection can be divided into mechanical and immunobiological.

Mechanical factors are represented by:

1) the mucous membrane and submucosal layer of the oral cavity;

2) enamel, dentin and tooth pellicle.

Immunobiological factors are found in the oral fluid:

1) humoral - lysozyme, lactoferrin, peroxidase, mucin, interferon, beta-lysins, etc.;

2) cellular (phagocyte cells) - macrophages (monocytes and neutrophils) and microphages (granulocytes).

Factors of nonspecific resistance of the oral cavity. The mucous membrane, hard tissues of the tooth and the pellicle are practically impermeable to most microorganisms in the norm. When the mucous membrane is damaged, the integrity of the hard tissues of the tooth is violated, for example, in caries, favorable conditions are created for the spread of bacteria beyond the biotope and the development of the inflammatory process.

The process of mechanical cleansing of the oral cavity occurs during and outside of meals due to the constant secretion of saliva by the salivary glands. In the normal

state of the oral cavity, the rate of salivation is up to 2.5 ml/min, significantly decreasing at night. Normally, 1.5-2 liters of saliva are secreted per day. Constant saliva secretion contributes to intensive cleansing of the oral cavity, washing out food residues, microbial flora, metabolic products and fermentation.

Mixed saliva has a whole complex of functions: digestive, protective, trophic, buffer. The protective functions of saliva are determined by nonspecific factors and some indicators of specific immunity.

The main cellular factors of oral fluid that form nonspecific resistance of the oral cavity. In the local immunity of the oral cavity, connective tissue cells of the mucous membrane play a major role. The bulk of these cells are fibroblasts and tissue macrophages, which easily migrate to the focus of inflammation. Phagocytosis on the surface of the mucous membrane and in the submucosa is carried out by phagocytic cells (granulocytes and macrophages). They contribute to the cleansing of the focus from pathogenic bacteria. In addition, mast cells are located between collagen fibers and around the vessels, that ate potential participants in allergic reactions of the anaphylactic type. Plasma cells of the connective tissue provide local synthesis of antibodies, mainly immunoglobulins of the sIgA class.

Polymorphonuclear leukocytes, monocytes, lymphocytes, which enter it from the gingival pockets, are always found in the saliva of healthy people.

Phagocytic cells in huge numbers (of the order of several million per minute) enter the oral fluid through the gingival crevices. 80% of them are neutrophils and monocytes. It was assumed that such a large number of cells with phagocytic activity creates a powerful barrier to the penetration of infectious agents. However, further study of this biological phenomenon proved that as soon as leukocytes come into contact with hypotonic saliva, they largely lose their phagocytic functions. Such inactivation of phagocytes has an important biological significance. If the weakening of phagocytic function did not occur, then the entire resident microflora of the oral cavity would be destroyed within a few hours. Meanwhile, the permanent microbome of the oral cavity itself is an important biological factor of nonspecific resistance. However, the residual activity of such a large number of leukocytes is sufficient to capture food particles remaining in the oral cavity and, thus, acting synergistically with salivary enzymes, cleanse the oral cavity of possible areas of microbial development.

When foci of inflammation occur in the oral cavity, where there is an increase in osmotic pressure, the local activity of phagocytes can increase significantly. This is how their protective action is carried out, directed directly against a specific pathogen.

The main humoral factors of the oral fluid, which form nonspecific resistance of the oral cavity. The composition of saliva in different people is very individual, although almost 99% is water and only 1-1.5% is a dry residue, which contains the most important factors of nonspecific resistance of the oral cavity. Humoral factors of natural

resistance include lysozyme, lactoferrin, lactoperoxidase and other enzymes, components of the complement system, interferon and some other proteins.

Lysozyme is a mucolytic enzyme from a large group of low-molecular proteins, well soluble in water and buffer solutions at all pH values. In a human body, it is found in almost all tissues and biological secretions, among which saliva is in second place in terms of lysozyme content (200 μg/ml) after tear fluid (7000 μg/ml). The enzymatic properties of lysozyme are manifested in the ability to cleave glycosidic bonds of peptidoglycans of bacterial cell walls, which determines its antimicrobial effect. In addition, it participates in the processes of regulating the permeability of tissue barriers, regeneration and healing of oral wounds. Lysozyme enhances phagocytosis and potentiates the lytic activity of the sIgA complex with the C3 fraction of complement against gram-negative bacteria. Lysozyme enters saliva as a result of active secretion by mononuclear phagocytes, as well as the destruction of polymorphonuclear leukocytes, which contain it in large quantities.

Lactoferrin is an iron-containing transport protein, the bacteriostatic effect of which is associated with its ability to compete with bacteria for iron respiratory enzymes. Synergism of lactoferrin with antibodies has been noted. Lactoferrin is synthesized by granulocytes.

Peroxidase is a complex iron-containing enzyme. Its bactericidal effect is manifested in a complex with hydrogen peroxide. Peroxidase is synthesized in the parotid salivary glands and in blood granulocytes.

β-lysines act on the cytoplasmic membrane, causing autolysis of bacteria. They exhibit their bactericidal activity mainly against anaerobic pathogenic and opportunistic bacteria.

Nucleases (RNAse and DNAse) are involved in the cleavage of nucleic acids. In this regard, their biological role is to degrade nucleic acids (mainly viral), which can be of great importance in protecting the body from the penetration of an infectious agent through the oral cavity and the onset of an infectious process.

Tetrapeptide sialin (glycyl-glycyl-lysyl-arginine) neutralizes acidic products formed as a result of the vital activity of the oral microbiome. It has a strong anti-caries effect.

Mucins are high-molecular and low-molecular mucous glycoproteins. They constitute about 16% of all saliva proteins and determine its viscosity. Due to their high surface activity, mucins are adsorbed on all surfaces of the oral cavity, provide cohesion and anti-adhesive properties of oral fluid, and are part of dental plaque. Their function is to provide a protective barrier for oral tissues from environmental aggression factors, inactivate microorganisms, and also have a fungistatic effect on fungi.

Complement is a complex multicomponent protein system. The main functions of the complement system: lysis of bacterial cells, stimulation of phagocytosis due to opsonization and participation in anaphylactic reactions. Only the C3 fraction of the

complement system is found in small quantities in saliva. Others are absent or found in small quantities. The activity of this system is enhanced in the presence of inflammatory processes in the mucous membranes, when other complement fractions are delivered from the vascular bed and thus the protective reaction is enhanced.

Interferon is a low-molecular-weight thermostable protein produced by cells in response to the action of inducers (usually viruses, double-stranded viral RNA or mitogens). In small quantities, it is present in all biological secretions, its production increases when a virus penetrates a human body within a few hours after infection. Interferon blocks intracellular reproduction of viruses, thereby preventing damage to other cells, and also has an immunomodulatory effect. In the oral cavity, it is synthesized by macrophages, lymphocytes, and fibroblasts of the mucous membrane.

Acid glycoproteins are agglutinins of non-immunoglobulin nature, containing a lot of N-acetylneuraminic acid, which is able to block the neuraminidase of viruses, which leads to agglutination and loss of adhesive ability.

Proteins rich in proline have a bacteriostatic effect on streptococci, binding to them, and also give viscosity to saliva.

Proteins rich in histidine have a bactericidal effect, inhibiting glucose transport in bacteria, thereby blocking the glycolysis process. Some proteins rich in histidine are involved in the processes of reducing the growth of fungi of the genus *Candida*, for example, they bind on the surface of *C. albicans* with a 67 kDa protein, forming a complex that mediates the death of the fungal cell.

In total, oral fluid contains more than 50 enzymes with a diverse spectrum of action, including those that prevent the adhesion of pathogens to the mucous surface and tooth enamel.

Thus, a large number of nonspecific factors that have a mechanical, chemical and biological effect on the development of the infectious focus constantly act in the oral cavity. At the same time, the protective activity of these factors is not their main, but their secondary function, since normally (in the absence of infectious aggression) they participate in various physiological processes occurring in the oral cavity (wetting of food, crushing and digestion of its solid components, liberation from food residues, etc.).

Specific (immunological) resistance is a complex of biological mechanisms aimed at maintaining the constancy of the internal environment of the organism, through which everything genetically foreign is recognized and eliminated.

Immunological resistance is provided by tissues and cells of the immune system. Specific protection factors are divided into humoral (immunoglobulins) and cellular (T and B lymphocytes).

Specific antimicrobial resistance factors acting in the oral cavity (humoral and cellular). Immunological resistance is provided by tissues and cells of the immune system. The immune system of the oral cavity is associated with lymphoid tissue, in which a structured and diffuse component is distinguished. The first contains single

unencapsulated follicles, as well as such organized formations as tonsils. The second component is represented by single cells that infiltrate the epithelial layers of the mucous membranes (T-lymphocytes) and the lamina propria, as well as the submucosal layer (mainly B-lymphocytes).

Tonsils (pharyngeal and palatine) are accumulations of lymphoid tissue between the mucous membrane, which forms crypts, and the connective tissue layer, which is the source of blood supply to the tonsils. Lymphocytes in the tonsils are located in the follicles and interfollicular space. Here, B lymphocytes predominate, carrying IgA as a receptor.

Diffuse lymphoid tissue of the mucosa is represented by lymphocytes of the lamina propria and interepithelial lymphocytes. Lymphocytes of the lamina propria are 80% represented by B cells carrying IgA on the surface, as well as plasma cells containing IgA in their cytoplasm. Interepithelial lymphocytes are almost exclusively T cells, the bulk of which differentiates towards Th2 helpers under antigenic stimulation and ensures the development of a humoral immune response. In addition, the mucous membranes contain two types of non-lymphoid cells that perform immunological functions: dendritic cells and the epithelial cells themselves. Dendritic cells of the mucous membranes have a pronounced ability to bind antigen, but a low ability to present it to T lymphocytes. Activation of these cells occurs either after migration to the lymph nodes, or locally under conditions of inflammation. Epithelial cells in a resting state do not have signs of immunocytes, but under conditions of inflammation they are able to present antigen to T-helper cells and stimulate the proliferation of T-cytotoxic cells.

Thus, on the surface of the oral mucosa, almost all major populations of immunocompetent cells are determined: T- and B-lymphocytes, plasma cells. In the oral fluid and secretions, immunoglobulins of various classes and mediators of the immune response interleukins are detected.

At the level of the mucous membrane, submucosal layer of the oral cavity and lymphoid apparatus of the maxillofacial region, all stages and mechanisms of the immune response are implemented to one degree or another.

Processing and presentation of the antigen is carried out by phagocytic cells of the oral cavity, primarily monocyte, macrophages and fibroblasts, due to the presence of enzymes in them that break down large protein-polysaccharide molecules into separate fragments that carry specific antigenic determinants epitopes. Epitopes are fixed on MHC class 2 proteins, which are synthesized in these cells, and are displayed on the macrophage membrane. In normal gum tissue, the number of macrophages is about 2% of all cells, and in gingival fluid it reaches 18%.

The antigen recognition function, which begins the stage of immunoregulation, is implemented due to specific antigen receptors present in T-helpers and other lymphoid cells located on the mucous and submucosal layer of the oral cavity.

Regarding the effectiveness of the mechanisms of cellular immunity in the oral cavity, normal leukocytes and lymphocytes largely lose their activity upon contact with hypotonic saliva. In this regard, only sensitized cells that do not contact saliva and exit to areas of the oral mucosa that are isolated from the external environment can effectively perform cytotoxic functions in the oral cavity.

Humoral specific immunity is associated with the functioning of the B-arm of the immune system. The interaction of cell antigens with B-lymphocytes causes their proliferation and differentiation into plasma cells, which are localized in the salivary glands and the lamina propria of the mucous membrane and at the same time produce the main humoral factor such as secretory immunoglobulin A.

In the oral cavity, the primary contact with the antigen, its processing and activation of B-lymphocytes occur in organized structures (tonsils). Here, the antigen is recognized by B-lymphocytes carrying IgA on the membrane. As a result of interaction with Th2-helpers, B-cells are transformed into blastocytes and enter the recirculation. Blastocytes enter the regional lymph node, where they experience an additional stimulating effect from the Th2-cells, and again enter the recirculation.

Having reached the lamina propria, B cells mature here to the stage of plasma cells and secrete IgA antibodies - monomeric and dimeric. IgA monomers enter the bloodstream and become serum immunoglobulins, and dimers interact with receptors of epithelial cells of the mucous layer and, having bound it, penetrate into these cells. Here the receptor undergoes partial degradation, as a result of which a fragment of the receptor remains in the IgA molecule - the secretory component. In this form, secretory IgA (sIgA) is released onto the surface of the mucous membrane. Here, sIgA binds antigens on the surface of the mucous membranes and ensures activation of complement in the classical way, inhibits the adhesion of bacteria, neutralizes viruses and prevents the absorption of antigens through the mucous membrane of the oral cavity.

The most significant in the specific immunity of the oral cavity from the five classes of immunoglobulins (IgA, IgM, IgG, IgD, IgE) are class A antibodies in the secretory form (slgA). The mechanism of action of sIgA is that it indirectly, through the activation of phagocytes, leads to the lysis of bacteria, prevents their colonization of mucous membranes and the surface of the teeth, and neutralizes viruses. Secretory IgA, unlike serum IgA, is a dimer. It has two molecules of the IgA monomer, connected by a J-chain and a glycoprotein SC (secretory component), which provides resistance of slgA to proteolytic enzymes of saliva.

In healthy people, in the stroma of all exocrine glands (including salivary glands) and mucous membranes that communicate with the external environment, the vast majority of plasma cells produce IgA. The leading role in the formation of sIgA is played by submucosal accumulations of lymphoid cells

of the Peyer's patches type, covered with a special cuboidal epithelium. Antigenic stimulation leads to the selection of clones of B-lymphocyte precursors that synthesize IgA. Antigenic action simultaneously activates regular subpopulations of T cells that control the proliferation of B-lymphocytes. Then, lymphocytes can exit Peyer's patches with subsequent circulation and settlement in various mucous membranes and exocrine glands (including salivary glands), where T-helpers predominate. This ensures the functional unity of the entire immunological system of local protection of the body against infections.

According to the characteristics of the immunoglobulin content, extravascular fluids of the oral cavity are divided into internal and external secretions. Internal secretions are secretions of gingival pockets, in which the content of immunoglobulins is close to their concentration in blood serum. In external secretions, for example, saliva, the amount of IgA significantly exceeds their concentration in blood serum. At the same time, the content of IgM, lgG and IgE in saliva and serum is approximately the same. Secretory IgA is more resistant to the action of proteolytic enzymes compared to serum IgA, since the secretory component shields the hinge part of the IgA molecule that is most sensitive to the action of enzymes.

Secretory sIgA is present in the saliva of children from birth. The concentration of sIgA increases significantly in the early postnatal period. By the 6th-7th day of life, the level of sIgA in saliva increases almost 7 times. A normal level of sIgA synthesis is one of the conditions for sufficient resistance of children of the first months of life to infections affecting the oral mucosa.

Secretory IgA has the following protective functions:

1) it binds antigens and causes their lysis;

2) it inhibits the adhesion of bacteria and viruses to mucosal cells, which prevents the onset of the inflammatory process, and even their adhesion to tooth enamel (i.e. has an anti-caries effect);

3) it prevents the penetration of antigens (allergens) through the mucous membrane.

Sufficient levels of sIgA can prevent the development of some viral infections in the oral cavity, for example, herpes infection, in which the level of local IgA secretions correlates better with antiviral protection compared to serum antibodies. It is likely that sIgA antibodies. contribute to the elimination of the virus after its neutralization.

In addition, sIgA forms immune complexes with foreign antigens and allergens that have entered the oral mucosa, which are removed from the body with the participation of nonspecific factors (macrophages and the complement system). In individuals with sIgA deficiency, antigens are freely adsorbed on the mucosa and enter the blood, which can lead to severe consequences of allergization.

Due to these functions, sIgA are the leading factors of the first line of defense of the body against infectious and other foreign agents. Antibodies of this class prevent the occurrence of pathological processes on the mucous membrane, without causing its trauma, since the interaction of sIgA with the antigen, unlike antibodies of the IgG and IgM classes, does not cause activation of the complement system.

IgM is the largest immunoglobulin by molecular weight and the main factor of the early specific immune response and is the first to appear after infection. IgG and IgA, which penetrate from the bloodstream into the oral cavity secretion, are quickly inactivated by the action of salivary proteases and, thus, are unable to perform their protective function, and antibodies of classes M, E and D are found in insignificant quantities. The level of IgE reflects the allergic background of the body, increasing mainly in allergic diseases.

Lysozyme and secretory IgA are included in the functional concept of the "colonization resistance barrier" of mucous membranes, in the formation of which, on the one hand, normal microbiome participates, on the other - epithelial cells and their receptors, complementary to the adhesins of bacteria that form the microbiocenosis of a particular biotope.

Immune factors play a significant role in the formation of resistance to caries. When considering the importance of immunological disorders in the process of the onset and development of caries, it is revealed, on the one hand, the insufficiency of local protective mechanisms of the oral cavity, and on the other - damage to the immunological system of the whole organism. In caries-susceptible, as well as in caries-resistant individuals, it was found that the level of IgA and IgG in the oral fluid changes, but in the blood serum remains unchanged.

In caries-resistant individuals, a high content of sIgA was detected. With insufficient production of sIgA, as compensation, an increase in IgM synthesis occurs. In the absence of IgA and IgM in saliva or a significant decrease, a tendency to increase the intensity of caries is noted. The mechanism of the influence of secretory immunoglobulin on the susceptibility of teeth to caries is explained by its penetration into dental plaque and pellicle, as a result of which the fixation of microorganisms on the tooth surface decreases, and their phagocytosis by neutrophils is also accelerated.

Disturbances of local immunity also lead to the development of periodontal tissue diseases, oral mucosa and depend on the concentration of secretory IgA. A decrease in the level of sIgA leads to the development of pathological processes that are often repeated in the oral cavity. A high level of lysozyme is able to stimulate the synthesis of sIgA. If lysozyme is not active enough, this deficiency is compensated by a high level of immunoglobulins, enhanced production of sIgA, that is, the deficiency of one factor is compensated by another, potentiating the nonspecific protection of the oral cavity. In

inflammatory periodontal diseases, there is no such compensation. Thus, in gingivitis, the local deficiency of immune protection is associated not only with the deficiency of individual factors, but also with the inability to compensate for the deficiency of lysozyme with immunoglobulins. The activity of lysozyme increases significantly against the background of professional and rational hygiene, and oral cavity sanitation.

Cellular (cell-mediated) immune response is carried out by accumulating in the body a clone of T-lymphocytes that carry receptors specific for a given antigen and are responsible for cellular reactions of immune inflammation, delayed-type hypersensitivity, in which, in addition to T-lymphocytes, macrophages participate.

B- and T-lymphocytes, when repeatedly encountering the same antigen, multiply rapidly and give an acute response, ensuring the preservation of immunological memory. The immune response begins with the recognition of the antigen by receptors on the surface of lymphocytes. Later, these cells begin to actively proliferate, differentiate into effector cells. Together with the mechanisms of nonspecific protection (phagocytes, complement system, NK cells), they eliminate the antigen.

Immunological memory is realized by short- and long-lived subpopulations of T- and B- memory cells located in the tissues of the gums and periodontium, in the lymph nodes of the maxillofacial region. The presence of these cells ensures the rapid development of a secondary immune response of the humoral type on the mucous membrane.

Thus, the main role in the specific protection of the oral cavity belongs to humoral immune mechanisms.

Under the influence of antigens of different nature and properties, the immune response of the macroorganism has its own characteristics.

Features of immunity in bacterial infections. The immune response of a human body in response to a bacterial infection is largely determined by the pathogenicity factors of the microorganism and its ability to produce toxins.

The main factors of antibacterial protection are antibodies and phagocytes. Antibodies effectively inactivate biologically active molecules of a bacterial cell (toxins, aggression enzymes, etc.), label them, trigger the mechanism of bacteriolysis and participate in immune phagocytosis. Phagocytes carry out phagocytosis, including immune, extracellular destruction of the pathogen using ion radicals and antibody-dependent bacteriolysis.

A number of bacteria belonging to facultative representatives of the microbiome are characterized by increased resistance to the action of complement, lysozyme and phagocytes (incomplete phagocytosis). These include mycobacteria, brucellae and some others. In relation to these microbes, antibodies and phagocytes are not effective enough, and the infectious process

itself has a tendency to become chronic. In such a situation, the macroorganism involves the cellular link of immunity, which leads to allergization of the body.

Features of antiviral immunity. Immune protection of a human body in viral infections has features due to two forms of virus existence: extracellular and intracellular.

The main factors that provide antiviral immunity are specific antibodies, T-killers, natural killers, interferon and serum inhibitors of viral particles. Specific antiviral antibodies are able to interact only with extracellular virus. Antibodies neutralize the viral particle, preventing its adsorption on the target cell, infection and generalization of the process, and also bind viral proteins and nucleic acids. The formed immune complexes are eliminated by immune phagocytosis.

Interferon also has a significant antiviral effect. It does not act directly on the intracellular virus, but binds to the receptor on the cell membrane and induces enzyme systems that inhibit all biosynthetic processes in it, including virus reproduction.

Serum inhibitors nonspecifically bind to the viral particle and neutralize it, thereby preventing the adsorption of the virus on target cells.

Features of antifungal immunity. Fungal antigens have relatively low immunogenicity; they practically do not induce antibody formation, but stimulate the cellular link of immunity. The main factors of antifungal immunity are activated macrophages, which carry out antibody-dependent cytotoxicity of fungi.

Manifestations of hypersensitivity reactions in the oral cavity. Allergy is a state of pathologically increased sensitivity of the body to repeated administration of an antigen due to an inadequate response of the immune system. Immune mechanisms that provide protection of the body can lead to tissue damage, manifesting as hypersensitivity reactions.

According to the speed of manifestation and mechanism, allergic reactions can be divided into two groups - allergic reactions (or hypersensitivity) of the immediate type and delayed type.

Allergic reactions of the humoral (immediate) type are mainly due to the function of antibodies of the IgG and especially IgE classes. They are characterized by rapid development after contact with the allergen (minutes).

Immediate reactions are divided into three types:

Type 1. Anaphylactic reactions - immediate type, atopic. They are caused by the interaction of allergens coming from the outside with IgE antibodies fixed on the surface of mast cells and basophils. The reaction is accompanied by activation and degranulation of target cells with the release of allergy mediators (mainly histamine). Examples of type 1 reactions are anaphylactic shock, atopic bronchial asthma, atopic dermatitis, which develop in response to the administration of drugs.

Type 2. Cytotoxic reactions. They involve cytotoxic antibodies (IgM and IgG), which bind antigen on the cell surface, activate the complement system and phagocytosis, and lead to the development of antibody-dependent cell-mediated cytolysis and tissue damage. An example is cytotoxic reactions in drug allergies.

Type 3. Immune complex reaction. Antigen-antibody complexes are deposited in tissues (fixed immune complexes), activate the complement system, attract polymorphonuclear leukocytes to the site of fixation of immune complexes, and lead to the development of an inflammatory reaction. Bacterial, viral, and drug antigens can act as allergens. Examples include periodontal diseases (ulcerative-necrotic gingivitis, periodontitis), Arthus phenomenon (clinically manifested as acute necrotic hemorrhagic inflammation), postherpetic erythema multiforme, and serum sickness.

Delayed-type hypersensitivity (DTH) is a cell-mediated hypersensitivity or type 4 hypersensitivity associated with the presence of sensitized lymphocytes. The effector cells are T lymphocytes. They recognize foreign antigens and secrete various lymphokines, stimulating the cytotoxicity of macrophages, enhancing the T- and B-immune response, causing the onset of an inflammatory process. T-cell sensitization can be caused by agents of contact allergy (haptens), antigens of bacteria, viruses, fungi, and protozoa.

Examples: allergic reaction in brucellosis, tuberculosis, actinomycosis, candidiasis, etc.; contact allergy (drug stomatitis – acrylic resins, radiopaque substances, prostheses, etc.); periodontal diseases; ulcerative-necrotic stomatitis.

Thus, the main functions of the immune system are to protect the body from pathogenic factors. Both nonspecific defense mechanisms and specific immune response to specific infectious antigens are involved in the implementation of these functions. The specific immune response enhances nonspecific defense mechanisms, making them more targeted.

Questions and test tasks for self-testing to chapter 8 (the number of correct answers may vary)

1. The main specific factor of local immunity of the oral cavity in caries is:
a) sIg A;
b) complement;
c) Ig G;
d) interferon.
2. The mechanism of protective action of sIg A in caries is the ability to:
a) neutralize acids;
b) lyse bacteria;
c) prevent adhesion of *S. mutans*;
d) lyse *S. mutans*.
3. Saliva performs the following functions:
a) helps cleanse the oral cavity by washing out food debris;

b) participates in the processes of mineralization and demineralization of enamel;

c) ensures the normal functioning the mucous membrane;

d) participates in the implementation of nonspecific resistance mechanisms.

4. Specify the humoral factors of nonspecific protection of the body that act in the oral cavity:

a) lysozyme;

b) interferons;

c) secretory Ig A;

d) complement proteins.

5. Indicate the humoral factors of specific defense of the organism that act in the oral cavity:

a) lysozyme;

b) lactoferrin;

c) secretory Ig A;

d) complement proteins.

6. Indicate the cells that participate in the formation of nonspecific resistance of the oral cavity:

a) monocytes;

b) neutrophils;

c) fibroblasts;

d) epithelial cells.

7. Indicate the role of epithelial cells of the mucous membranes in the development of immune reactions:

a) present antigen to T-helpers;

b) bind antigen;

c) stimulate the proliferation of B-lymphocytes;

d) stimulate the proliferation of T-cytotoxic cells.

8. Indicate the following statements that are true for lysozyme:

a) cleaves peptidoglycan;

b) cleaves nucleic acids;

c) active in a weakly acidic and neutral environment;

d) active in an alkaline environment.

9. In the oral cavity, the primary contact with the antigen, its processing occurs:

a) in the regional lymph nodes;

b) in the mucous membrane;

c) in the tonsils;

d) in its lamina propria.

10. The following types of allergic reactions can occur in the oral cavity:

a) type I hypersensitivity reactions;

b) cytotoxic type hypersensitivity reactions;
c) immune complex type hypersensitivity reactions;
d) type IV hypersensitivity reactions.

Answers to test tasks for chapter 8

№ of question	True answers	№ of question	True answers
1	a, b, c, d	6	a, b
2	b, d	7	a, d
3	a, b, c, d	8	a, c
4	a, b, d	9	c
5	в	10	a, b, c, d

Chapter 9. THE RELATIONSHIP BETWEEN THE ORAL MICROBIOME AND OTHER BODY SYSTEMS

The oral and intestinal microbiomes are the most diverse in terms of the number of microorganism species and microbial cells. Microorganisms can travel from the oral cavity to the intestine via saliva and food, and intestinal microbiota can enter the oral cavity through autoinfection or from another person. Members of these two microbiocenoses mutually regulate normal physiological and pathological processes in their respective habitats. Excessive microbial invasion into another biotope can alter the ecosystem, cause dysbiosis, and lead to disease.

The oral microbiome influences the development of localized diseases such as caries, gingivitis, periodontitis, and stomatitis. Furthermore, oral microorganisms can influence the onset and progression of systemic diseases. Oral bacteria can enter the bloodstream through small microtraumas of the mucous membrane during inflammatory diseases, spreading to any organ. In this case, they actively influence physiological processes, and colonization can lead to serious illnesses.

The intestine is protected from excessive colonization by oral bacteria by chemical factors such as acid and bile, but under certain dysfunctions this barrier is disrupted, leading to interorgan translocation of bacteria.

Oral microbiota is found in various gastrointestinal diseases. Oral commensals *Haemophillus, Veilon*ella, and *Fusobacterium nucleatum* have been identified in inflammatory bowel diseases. Periodontitis, a chronic inflammatory disease of the oral cavity, can significantly influence the pathogenesis of these diseases. Oral pathogens such as *Porphyromonas gingivalis* can alter intestinal barrier function and mucosal permeability, leading to chronic inflammation and the development of severe diseases such as Crohn's disease and ulcerative colitis. Inflammatory bowel diseases are risk factors for colorectal cancer. Oral bacteria such as *Peptostreptococcus* and *Fusobacterium* have also been isolated from the intestines of patients with colorectal cancer. These bacteria induce local inflammatory

responses and intestinal cell proliferation. Thus, periodontitis is associated with an increased risk of colorectal cancer.

Intestinal dysbiosis also causes chronic liver disease. Since the oral microbiome can influence the development of intestinal dysbiosis, its role in liver pathology is also significant. The periodontogenic bacterium *P. gingivalis* is found in significant quantities in the oral cavity of patients with liver cirrhosis, meaning changes in the oral microbiome can be used to confirm intestinal dysbiosis and various gastrointestinal diseases. For example, increased levels of *Porphyromonas*, *Fusobacterium*, *Oribacterium*, and *Haemophyllus* bacteria are detected in the saliva of patients with hepatocellular carcinoma.

Periodontitis is also associated with a risk of developing pancreatic ductal adenocarcinoma. Periodontogenic bacteria *P. gingivalis* and *F. nucleatum* subsp. *vincentii* can penetrate the intestine and pancreas, contributing to the pathogenesis of this disease.

The microbiota may influence cancer development through the induction of an inflammatory response, an immune response, or DNA damage. By interacting with epithelial and immune cells, bacteria induce the production and release of metabolites that stimulate systemic responses. Thus, bacteria may influence pathogenesis in distant organs

Oral dysbiosis is associated with diseases in other systems. For example, *Porphyromonas gingivalis* can cause rheumatoid arthritis, aspiration pneumonia, esophageal cancer, and coronary heart disease. The oral commensal *Streptococcus viridans*, when introduced into the bloodstream, can trigger blood clots. Alterations in the oral microbiota are associated with a number of systemic diseases, such as diabetes, Alzheimer's disease, and cardiovascular disease. In Alzheimer's disease, an increase in the number of bacteria of the genera *Leptotrichia*, *Moraxella*, and *Sphaerochaeta* in the oral cavity is observed, which can be used as a prognostic marker for this disease. Furthermore, P. gingivalis bacteria were found in the brain tissue of a deceased patient with Alzheimer's disease. Bacterial entry into the brain causes inflammation and neurodegeneration, which exacerbates the disease.

An increased abundance of *Actinobacteria* and *Firmicutes* is observed in patients with diabetes mellitus, and bacteria of the genus *Anaeroglobus* are found in the oral cavity of patients with atherosclerosis. Furthermore, oral commensals are found in atherosclerotic plaques of patients with coronary artery disease. Odontogenic bacteria are also found in thrombus aspirates from patients with heart attacks and strokes, in coronary artery stenoses, and in thrombus aspirates from patients with venous and arterial thrombosis. Streptococcus viridans is detected in cerebral thrombi.

The oral microbiome has been shown to potentially influence the onset and progression of breast cancer. The oral anaerobic commensal *Fusobacterium nucleatum* has been detected in breast cancer tissue. This bacterium is secreted in

high numbers during gingivitis and periodontitis, increasing the risk of breast cancer development and tumor migration. Dysbiosis of the oral microbiome can trigger inflammatory reactions, genetic mutations, and changes in hormonal metabolism, potentially contributing to carcinogenesis in organ tissue.

Microbiome analysis can be used prognostically to assess cancer risk and monitor the effectiveness of treatment. Investigating the microbiota of the oral-gut axis, as well as understanding the correlation between oral dysbiosis and diseases of various organs, can be used for disease diagnosis and treatment prognosis.

Peripdontigenic bacteria increase formation of arherosclerotic plaques in blood vessels and deposition of amyloid in the brain. Porphyromonas gingivalis can penetrate bloodstream and trigger chronic inflammation in the brain and increase accumulation of amyloid.

TESTS FOR SELF-CHECK AND CONTROL OF KNOWLEDGE ASSISTING

(only one correct answer)

1. In a smear from the lesion in acute purulent periostitis, gram-positive cocci were found, which are located singly and in the form of clusters resembling clusters of grapes. Which of the listed bacteria have this morphology?

a) sarcina;

b) staphylococci;

c) streptococci;

d) diplococci;

e) fungy of genus *Candida.*

2. When examining a patient who complained of toothache, the dentist found white spots on the teeth - areas of enamel demineralization. Which of the listed bacteria are an aggressive part of the oral microflora?

a) Streptococcus salivarius;

b) Streptococcus pyogenes;

c) Streptococcus

d) Streptococcus mitis;sanguis;

e) Streptococcus mutans

3. Microscopic examination of dental plaque revealed gram-positive rods and gram-positive cocci arranged in pairs and short chains, which could probably participate in the pathogenesis of caries. Which of the following microorganisms have a pathogenetic significance in the development of caries?

a) lactobacilli and *S. mutans;*

b) enterococci and *S. salivarius;*

c) lactobacilli and *S. salivarius;*

d) bifidumbacteria and *S. mutans*;

e) lactobacilli and *S. aureus*.

4. Examination of a newborn revealed swollen and inflamed gums, hyperemia, erosions with a viscous mucopurulent secretion. Microscopy of smears from the discharge showed a large number of leukocytes, as well as gram-negative diplococci located inside the leukocytes and isolated. Which of the following diagnoses can be made?

a). toxoplasmosis;

b) staphylococcal stomatitis;

c) blenorea;

d) gonococcal stomatitis;

e) congenital syphilis.

5. During a preventive examination of schoolchildren, a dentist found white creamy plaques on the mucous membrane of the oral cavity of a student, which were easily removed, leaving bloody erosions. During microscopic examination, yeast forms with budding blastoconidia and pseudohyphae were found in smears from the lesions. What microorganisms were the leading etiological factor of the lesion?

a) S. mutans;

b) S. salivarius;

c) V. parvula;

d) T. vincentii;

e) C. albicans.

6. During examination of the patient, a solid phlegmon-like infiltrate was found in the cervical and maxillary region, the skin around which was blue-purple in color. The center of the lesion was necrotic, pus with an unpleasant odor was released from the ulcer. The doctor suspected actinomycosis and prescribed a microscopic examination of the pus. Which of the following should the bacteriologist detect to confirm the diagnosis?

a) gram-negative diplococci;

b) gram-positive diplococci;

c) druses;

d) acid fast rods;

e) gram-negative rods.

7. A patient with tonsillitis had a gray-yellow plaque that was easily removed with a spatula, and ulcers on the gums and buccal mucosa. The doctor suspected ulcerative-necrotic gingivostomatitis (Vincent's tonsillitis). Which of the following bacteria are often cultured together with fusobacteria in this disease?

a) *T. vincentii*;

b) *A. viscosus*;

c) *L. casei*;

d) *N. mucosa*;

e) *L. acidophilus*.

8. A pediatrician, while examining a 3-month-old child, noted that his tongue and oral mucosa were covered with a dense white coating. In the material taken from the lesion, the bacteriologist found gram-positive yeast-like cells. The doctor suspected mycosis. Which of the listed diseases is most common in children of this age?

a) actinomycosis;

b) cryptococcosis;

c) aspergilosis;

d) candidiasis;

e) diphtheria.

9. Gram-positive large oval cells with budding and elongated cells in the form of chains were isolated from the mucous membranes and sputum of a patient who had been taking immunosuppressants for a long time. Which of the listed pathogens was isolated?

a) Cryptococcus neoformans;

b) Candida albicans;

c) Aspergillus flavus;

d) Penicillium marneffei;

e) Pneumocystis jirovecii.

10. Bacterioscopic examination of mucus from the nasopharynx revealed the presence of gram-negative cocci resembling coffee beans and arranged in pairs or tetrads. Which of the following pathogens was isolated?

a) Neisseria meningitidis;

b) Streptococcus viridans;

c) Staphylococcus aureus;

d) Neisseria gonorrhoeae;

e) Streptococcus pneumoniae.

Answers to tests for self-testing and monitoring knowledge acquisition

№ of question	True answers	№ of question	True answers
1	b	6	c
2	d	7	a
3	a	8	d
4	b	9	b
5	e	10	a

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Educational edition

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Learning guide

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