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FEATURES OF THE DYNAMICS OF THE MICROBIOCENOSIS OF THE ORAL CAVITY OF INTACTS RATS

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The experiment investigated the qualitative and quantitative composition of bacterial groups in the microbiotopes of the oral cavity of intact rats (20) for twenty-eight days. The material for microscopic and bacteriological studies was the microflora from the biotope of the oral cavity of animals, in particular, from microbiotopes of the tooth surface in the gingival margin, mucous membrane of oral vestibule and mucous membrane of oral cavity proper. During the experiment, stable microbiocenoses were formed from the gram-positive and gram-negative microorganisms of different species common to all intact animals in the studied biotope, however, they differed in quantitative composition in different microbiotopes of the oral cavity. The quantitative indicator of α-hemolytic streptococci significantly decreased in the microbiotopes of the mucous membrane of oral vestibule and of oral cavity proper, compared with the corresponding indicator of the microbiotope of the tooth surface in the gingival margin during the experiment, which testified to the initial stages of formation of dental biofilm in this nishe of the oral cavity. The qualitative and quantitative composition of normal and opportunistic microbiota in the population of rats kept under standard conditions stabilized on the twenty-eighth day of the experiment.

Key words: *oral cavity, microbiotopes, rats, bacterial groups.*

Relationship of the publication with the planned research works. The results of the article correspond to the research plan of Danylo Halytsky Lviv National Medical University and are part of the scientific work of the Normal Anatomy Department and Operative Surgery and Topographic Anatomy Department «Structural organization, angioarchitectonics and anthropometric features of organs during pre- and postnatal development under the influence of exogenous and endogenous factors» (state registration number 0115U000041), «Morphofunctional features of organs in the pre – and postnatal periods of ontogenesis, under the influence of opioids, food additives, reconstructive surgery and obesity» (state registration number 0120U002129).

Introduction. The interaction between the microbiota and the host modulates crucial aspects of normal physiology, metabolism, immunity and neurological function [1]. Oral microbiome is a well-balanced dynamic ecosystem and is an important and complex bacterial community in the body [2, 3, 4, 5, 6]. It is believed that the oral cavity is a «gateway to overall health» [7], however, the microbiocenosis of the oral cavity is relatively insufficiently studied, compared to other bacterial groups of biotopes, in particular, the intestine [8]. Significantly more bacteria are found in the oral cavity compared to other parts of the gastrointestinal tract – from 160 to 300 species [8], and according to metagenomic researchers, the number of microorganisms is more than 700 species, including bacteria, fungi and viruses [1, 5, 6, 9, 10, 11]. Obligatory and facultative microorganisms of the oral cavity exist in symbiosis with the macroorganism and act as a primary target under the influence of various exogenous and endogenous factors, which leads to dysbiotic changes and to the development of the inflammatory process in the mucous membrane of the oral cavity and periodontal tissues [7, 12, 13]. Accordingly, in the oral cavity there is a high risk of foci of chronic microbial infection, which later become a source of constant pathogenic contamination and sensitization of the digestive tract and the body as a whole [14].

Ecological theory of microbiome study and heterogeneity of bacterial groups in the oral cavity requires more careful study [15]. In view of this, it is necessary to study the microbiota of the oral cavity in the norm, in the dynamics at different times of quarantine observation, which can be achieved in the experiment. In addition, the use of experimental animal models is important for the study of the etiopathogenesis of inflammatory diseases of the oral cavity, periodontium and useful in evaluating the use of the latest therapeutic methods in clinical practice [16, 17]. However, in the available professional literature we have not found data on the peculiarities of the composition of bacterial communities in the microbiotopes of the oral cavity in normal laboratory animals under standard conditions during their long-term maintenance.

The aim – to investigate in the qualitative and quantitative composition of bacterial groups in the microbiotopes of the oral cavity of intact rats for twenty-eight days.

Object and methods of research. The study was performed on 20 white outbred adult male rats, weighing 160 grams, aged 4,5 months. Before the experiment, a thorough examination of the animals was performed, visually paying attention to the color and moisture of the mucous membrane of the oral cavity. Collection of material was carried out on the first, fourteenth and twenty-eighth days. The material for the study was the microbiota of different parts of the oral cavity of rats, namely, from microbiotopes of the tooth surface in the gingival margin (TS-GM), mucous membrane of oral vestibule (MMOV) and mucous membrane of oral cavity proper (MMOCP**)**.

All animals were quarantined in the vivarium, on a standard diet. Keeping, care, labeling and all other manipulations were carried out in compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [Strasbourg, 1985]. The Commission on Bioethics of Danylo Halytsky Lviv National Medical University established that the conducted research meets ethical requirements according to the order of the

Table 1 – Qualitative and quantitative composition of bacterial flora in microbiotopes of the oral cavity of intact rats on the first day of the study (CFU/ml)

№	Bacterial groups	Quantitative composition		
		TS-GM	MMOV	MMOCP
1.	Nonhemolytic streptococci	45.1±5.1	25.5±5.5*	15.1±4.4*
2.	Gram-positive non-spore forming rods	9.0±1.2	7.0±1.2	6.0±2.8
3.	Gram-positive spore forming rods	9.0±1.1	8.1±2.2	7.0±1.5
4.	α-hemolytic streptococci	65.4±4.3	52.4±5.2	21.3±4.1*
5.	Coagulase-negative staphylococci	10.3±3.6	8.1±2.5	9.0±1.3
6.	Enterococci	18.3±2.9	12.1±3.3	9.0±2.7*
7.	Escherichia coli	7.0±1.5	5.0±2.4	4.0±0.8

Notes: data are presented in the form of M±SD, where M is the average value, SD is the standard deviation; *p<0.05 – significant difference of the microbiotopes of MMOV and MMOCP relative to the microbiotope of the tooth surface in the area of the gingival margin (TS-GM).

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In microscopic examination the smear material was applied on a glass slide, fixed over a burner flame and Gram stained. Took into account the presence of cellular elements and their number in the field of view, on the morphology and interposition of cells, the presence of extracellular structures with the participation of different bacterial morphotypes. In order to obtain the level of microbial colonization was carried out by sampling with a calibrated loop of primary material (0.02 ml) and transferred to a test tube with isotonic solution (1 ml). This procedure was repeated 5 times from this mikrobiotope. In 0.2 ml of saline contained the number of microorganisms that were introduced in one calibrated loop – 0.02 ml. In the laboratory, 0.2 ml of material was taken from a test tube and inoculated into 5 Petri dishes with a dense nutrient medium. As a result of counting the total number of sprouted colonies, a quantitative indicator in colony-forming units was obtained per 1 ml (CFU/ml). Conventional special, differential diagnostic and selective media were used: meat-peptone agar, blood agar, Endo medium, Saburo medium, yolk-salt agar. Identification of selected cultures was performed by a set of morphotinctorial, cultural and biochemical properties. Some species were identified using standard test systems *Api system Bio Merieuox*, France. For further statistical analysis, the obtained data were tested for normality by calculating the asymmetry and excess coefficients and the results of the Shapiro-Wilk test (p<0.05). The data were presented as M±SD, where M is the mean, and SD is the standard deviation. All statistical calculations were performed using RStudio v. 1.1.442 and R Commander v.2.4-4. Diagrams and tables were created using Microsoft Office Excel.

Research results and their discussion. Microscopic examination of smears from microbiotopes of the oral cavity revealed that the microbiota was the same in all intact animals. Studies conducted at different times of the experiment (1, 14 and 28 days) indicated the dominance of gram-positive microorganisms. The smears showed elongated filamentous bacteria – *Leptotrix* (genus *Lactobacillus*). Gram-positive coccal microbiota was visualized as separate clusters. Gram-negative microorganisms, spindle-shaped or rod-shaped, were observed in some places in the field of view. Single leukocytes and epitheliocytes were also noted in the smears. Bacteriological studies in the microbiotopes of the oral cavity of intact rats on the first day of the study showed the predominance of coccal gram-positive microbiota, namely, non-hemolytic and α-hemolytic streptococci, coagulase-negative staphylococci and Enterococci. When studying the material from the biotope of the oral cavity of intact rats on the first day of the experiment, it was found that the density of microbial colonization by streptococci was significantly higher in the microbiotope TS-GM. Thus, the quantitative indicator of non-hemolytic streptococci of microbiotopes MMOV (25.5±5.5 CFU/ml) and MMOCP (15.1±4.4 CFU/ml) significantly decreased – 1.8 and 3.0 times, respectively, compared with the similar indicator of the microbiotope TS-GM (45.1±5.1 CFU/ml) (p<0.05). The quantitative indicator of α-hemolytic streptococci (bacterial species *Streptococcus mutans, Streptococcus salivarius* and others) of the MMOCP microbiotope (21.3±4.1 CFU/ml) was significantly lower – 3.1 times compared to the corresponding indicator of the microbiotope TS-GM (65.4±4.3 CFU/ml) (p<0.05). The number of enterococci (9.0±2.7 CFU/ml) of the MMOCP microbiotope significantly decreased – 2.0 times, compared with the corresponding indicator of the microbiotope TS-GM (p<0.05), and the quantitative composition of enterobacteria – Escherichia coli of the microbiotope MMOCP (4.0±0.8 CFU / ml) almost halved when compared with the corresponding quantitative composition of the microbiotope TS-GM. Quantitative indicators of gram-positive spores and non-spore rods, as well as coagulase-negative staphylococci on the first day of microbiological studies did not have significant differences in the microbiotopes of the oral cavity of intact rats **(table 1)**.

Table 2 – Qualitative and quantitative composition of bacterial flora in microbiotopes of the oral cavity of intact rats on the fourteenth day of the study (CFU/ml)

№	Bacterial groups	Quantitative composition		
		TS-GM	MMOV	MMOCP
1.	Nonhemolytic streptococci	43.4±3.2	18.2±2.5*	24.4±1.3*
2.	Gram-positive non-spore forming rods	9.1±1.3	14.1±1.8	11.2±2.1
3.	Gram-positive spore forming rods	9.2±1.2	5.0±0.9	9.3±1.7
4.	α-hemolytic streptococci	68.6±4.1	36.4±2.7*	26.1±1.6*
5.	Coagulase-negative staphylococci	11.2±1.3	13.5±1.6	21.1±2.9*
6.	Enterococci	18.4±2.6	14.7±1.9	22.3±3.1
7.	Escherichia coli	7.1±1.1	7.6±1.4	8.0±1.2

Notes: data are presented in the form of M±SD, where M is the average value, SD is the standard deviation; *p<0.05 – significant difference of the microbiotopes of MMOV and MMOCP relative to the microbiotope of the tooth surface in the area of the gingival margin (TS-GM).

The results of studies on the fourteenth day of the experiment indicated a certain stabilization of the indicators that characterize the individual microbiomes of the oral cavity of intact rats, but the general trend did not change significantly. As in studies at the beginning of the experiment, the largest number of streptococci was found in the microbiotope of TS-GM. Quantitative indicators of non-hemolytic streptococci significantly decreased in microbiotopes MMOV (18.2±2.5 CFU/ml) and MMOCP (24.4±1.3 CFU/ml) – 2.4 and 1.8 times, respectively, compared with similar indicator of the microbiotope TS-GM (43.4±3.2 CFU/ml) (p<0.05).

The quantitative indicator of α-hemolytic streptococci significantly decreased in the microbiotopes of MMOV (36.4±2.7 CFU/ml) and MMOCP (26.1±1.6 CFU/ml) – 1.9 and 2.6 times, compared with the corresponding indicator of the microbiotope TS-GM (68.6±4.1 CFU/ml) ($p<0.05$). The quantitative composition of bacilli – gram-positive spores and non-spore bacilli differed slightly in the microbiotopes of MMOV and MMOCP, however, the number of these bacteria in the microbiotope TS-GM did not change, as in the previous period of the experiment **(table 2)**.

Table 3 – Qualitative and quantitative composition of bacterial flora in microbiotopes of the oral cavity of intact rats on the twenty-eighth day of the study (CFU/ml)

№	Bacterial groups	Quantitative composition		
		TS-GM	MMOV	MMOCP
1.	Nonhemolytic streptococci	44.5±3.6	32.6±1.2	18.4±2.5*
2.	Gram-positive non-spore forming rods	9.3±1.7	12.4±2.3	10.5±1.4
3.	Gram-positive spore forming rods	9.0±1.3	9.1±1.5	6.1±1.2
4.	α-hemolytic streptococci	66.4±2.1	41.7±3.2*	24.1±2.7*
5.	Coagulase-negative staphylococci	10.6±1.2	10.4±1.1	15.7±2.5
6.	Enterococci	18.9±2.7	16.2±1.5	15.3±2.8
7.	Escherichia coli	8.2±1.3	9.3±1.5	9.4±2.1

Notes: data are presented in the form of M±SD, where M is the average value, SD is the standard deviation; *$p<0.05$ – significant difference of the microbiotopes of MMOV and MMOCP relative to the microbiotope of the tooth surface in the area of the gingival margin (TS-GM).

The quantitative indicator of coagulase-negative staphylococci (bacterial species *Staphylococcus epidermidis, Staphylococcus saprophyticus* and others) in the microbiotope of MMOCP (21.1±2.9 CFU/ml) significantly increased – 1.9 times compared with the similar indicator of TS-GM microbiotope ($p<0.05$). Significant differences in the quantitative composition of Enterococci in the microbiotopes of the oral cavity were not observed, however, the content of Enterococci in the microbiotope of MMOCP (22.3±3.1 CFU/ml) increased significantly compared to the previous stage of the experiment. The quantitative composition of Escherichia coli in the microbiotopes of the oral cavity of intact rats on the 14th day of the experiment did not differ **(table 2)**.

According to studies of the microbiocenosis of the oral cavity of intact rats on the twenty-eighth day of the experiment, it was found that the largest amounts of streptococcal microbiota. The quantitative indicator of non-hemolytic streptococci in the MMOCP microbiotope (18.4±2.5 CFU/ml) significantly decreased – 2.4 times compared with similar indicators of the microbiotope TS-GM (44.5±3.6 CFU/ml) ($p<0.05$). It should be noted that the number of non-hemolytic streptococci in MMOCP also decreased slightly compared to the previous period, however, in the microbiotope the MMOV increased significantly, in contrast to the quantitative composition of these microorganisms in the microbiotope TS-GM, which remained at the same level during the experiment. As in the previous terms of the experiment, on the twenty-eighth day there was a consistently high quantitative composition of α-hemolytic streptococci in the microbiotope TS-GM (66.4±2.1 CFU/ml). Thus, the quantitative index of α-hemolytic streptococci in microbiotopes MMOV (41.7±3.2 CFU/ml) and MMOCP (24.1±2.7 CFU/ml) significantly decreased – 1.6 and 2.7 times, respectively, compared with similar indicators of the microbiotope TS-GM ($p<0.05$) **(table 3)**.

When comparing the quantitative characteristics of the streptococcal microbiota of the microbiotope TS-GM for twenty-eight days, it was noted that the number of nonhemolytic and α-hemolytic streptococci did not change significantly during the experiment, in contrast to other studied microbiotopes of oral intact animals **(figure)**. At the same time, in comparison with the previous term (14 days) of the experiment, the number of α-hemolytic streptococci in the microbiotope of MMOV increased significantly relative to the quantitative composition of these microorganisms in the microbiotope of MMOCP. Quantitative indicators of other species of detected microorganisms (coagulase-negative staphylococci, Enterococci, Escherichia coli, gram-positive non-spore forming rods and spore forming rods) did not differ significantly in the microbiotopes of the oral cavity of rats, formed on the twenty-eighth day of the experiment. This indicated the stabilization of the microbiocenosis of the oral cavity in the quarantine of intact animals in the experimental conditions.

The study of the oral microbiome plays an important role in the diagnosis of dental pathology and has a preventive nature in the prevention, early detection and treatment of diseases of the digestive system. The microbiocenosis of the oral cavity is diverse in its composition due to continuous contact with the external environment, where there is synergy and interaction of oral microorganisms on the effects of exogenous factors [2, 6, 7, 9]. It is reported that the stability of a particular biotope is formed due to the peculiarities of the symbiotic microbiota [1].

In the study of microbiocenosis we found that under standard conditions of intact animals in one cell, in the oral cavity formed a stable microbiota common to all individuals, namely, saprophytic and opportunistic species of microorganisms. Although niches in the oral cavity, such as tooth surfaces and epithelial surfaces of mucous membranes mainly consist of the same microorganisms, however, some may be present in different proportions in both healthy individuals and at each stage of oral disease [5, 6, 8, 9, 11]. In addition, changes in the availability of oxygen, nutrients and the effect of saliva promote the

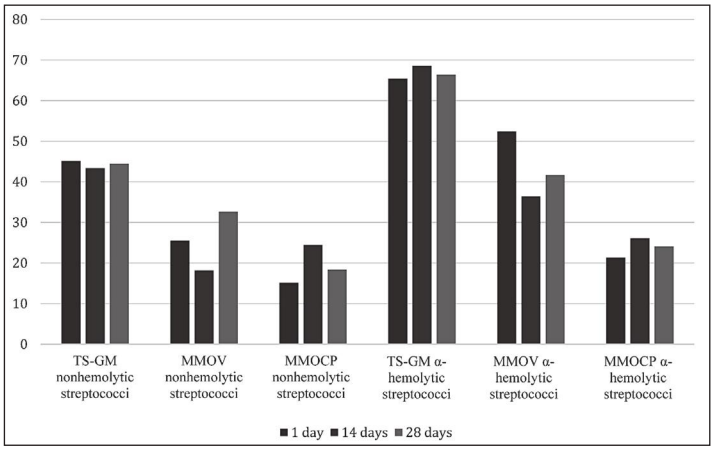

Figure – The level of microbial colonization of streptococcal microbiota in the microbiotopes of the oral cavity of intact rats for twenty-eight days.

growth of various microorganisms, and, conversely, these bacteria may be involved in the creation of their own small niche through the formation of complex communities of multispecies dental biofilm, which promotes bacterial adhesion and depends on coaggregation between bacterial associations [4, 10].

Our data indicate that a significantly high level of microbial colonization by potentially cariogenic α-hemolytic streptococci caused the formation of dental biofilm in the gingival margin and tooth surface of intact animals. In this case, the qualitative and quantitative bacterial composition of microbiotopes of the tooth surface in the gingival margin (biofilm formation) was normally the most stable with long-term observation and the place of the most pronounced contamination of microorganisms.

It should be noted that the normal microflora of the oral cavity at the level of biofilm formation reduces the colonization of the habitat by pathogenic bacterial strains and corrects the immune response at all levels [4]. The balance of the immune system is crucial for a stable response of the host. While an inadequate immune response will contribute to infection, an inflammatory response due to an overreaction of the immune system leads to tissue damage [2, 3]. Microbiome studies are far from complete and require further research, as well as greater attention to the functional component of the microbiocenosis and a clearer description of the different structures of each body microbiome in different contexts [8]. The data obtained by us on the peculiarities of the microbiocenosis of the oral cavity of rats in the norm can serve as a basis for the comparative characterization of dysbiotic changes caused by the harmful effects of various exogenous and endogenous factors.

Conclusions. During the experiment, stable microbiocenoses were formed from the gram-positive and gram-negative microorganisms of different species common to all intact animals in the studied biotope, however, they differed in quantitative composition in different microbiotopes of the oral cavity. The quantitative indicator of α-hemolytic streptococci significantly decreased in the microbiotopes of the mucous membrane of oral vestibule and of oral cavity proper, compared with the corresponding indicator of the microbiotope of the tooth surface in the gingival margin during the experiment, which testified to the initial stages of formation of dental biofilm in this nishe of the oral cavity. The qualitative and quantitative composition of normal and opportunistic microbiota in the population of rats kept under standard conditions stabilized on the twenty-eighth day of the experiment.

Prospects for further research. In the future it is planned to conduct experimental studies under the influence of various exogenous factors and compare with the obtained data of the normobiocenosis of the oral cavity of rats.

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ОСОБЛИВОСТІ ДИНАМІКИ МІКРОБІОЦЕНОЗУ РОТОВОЇ ПОРОЖНИНИ ІНТАКТНИХ ЩУРІВ

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Резюме. Мікробіом ротової порожнини являє собою добре збалансовану динамічну екосистему та є важливою і складною бактеріальною спільнотою в організмі. Дослідження проведено на 20 статевозрілих безпородних білих щурах-самцях, масою тіла 160 г, віком 4,5 місяців. Перед проведенням експерименту проводили ретельний огляд тварин, візуально звертали увагу на колір і вологість слизової оболонки ротової порожнини. Забір матеріалу здійснювали на першу, чотирнадцяту і двадцять восьму доби. Матеріалом для досліджень була мікробіота різних ділянок ротової порожнини щурів, а саме, з мікробіотопів поверхні зубів у ділянці ясенного краю, слизової оболонки присінка рота та слизової оболонки власне ротової порожнини. При мікроскопічних дослідженнях матеріал наносили на предметне скло, фіксували над полум'ям пальника і фарбували за мето-

дом Грама. При бактеріологічних дослідженнях, у результаті підрахунку загальної кількості проростих колоній одержали кількісний показник у колонієутворюючих одиницях на 1 мл (КУО / мл). Отримані дані, для подальшого статистичного аналізу, проходили перевірку на нормальність шляхом обрахування коефіцієнтів асиметрії та ексцесу, з використанням критерію Шапіро-Уілка ($p<0.05$). Центральну тенденцію для всіх даних було представлено у вигляді M±SD (середнє значення ± стандартне відхилення). При мікроскопічних дослідженнях мазків з мікробіотопів ротової порожнини встановлено, що мікробіота була однотипною у всіх інтактних тварин. Проведені дослідження у різні терміни експерименту (1, 14 і 28 доби) вказували на домінування грампозитивних мікроорганізмів. У мазках виявляли продовгастої форми ниткоподібні бактерії – *Leptotrix* (роду *Lactobacillus*). Грампозитивну кокову мікробіоту візуалізували у вигляді окремих скупчень. При порівнянні кількісної характеристики стрептококової мікробіоти мікробіотопу поверхні зубів у ділянці ясенного краю упродовж двадцяти восьми діб відмічено, що кількість негемолітичних і α-гемолітичних стрептококів в ході експерименту істотно не змінювалася, на відміну від показників у інших досліджуваних мікробіотопах ротової порожнини інтактних тварин. При цьому, при порівнянні з попереднім терміном (на 14 добу) експерименту кількість α-гемолітичних стрептококів у мікробіотопі слизової оболонки присінку рота значно зростала стосовно кількісного складу цих мікроорганізмів у мікробіотопі слизової оболонки власне ротової порожнини. Кількісні показники інших видів виявлених мікроорганізмів (коагулазонегативні стафілококи, ентерококи, кишкова паличка, грампозитивні спорові та неспорові палички) істотно не відрізнялися у мікробіотопах ротової порожнини щурів, сформованих на двадцять восьму добу досліду. Це вказувало на стабілізацію мікробіоценозу ротової порожнини при карантинному утриманні інтактних тварин в умовах експерименту.

Ключові слова: ротова порожнина, мікробіотопи, щури, бактеріальні угрупування.

FEATURES OF THE DYNAMICS OF THE MICROBIOCENOSIS OF THE ORAL CAVITY OF INTACTS RATS

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Abstract. Oral microbiome is a well-balanced dynamic ecosystem and is an important and complex bacterial community in the body. The study was performed on 20 white outbred adult male rats, weighing 160 grams, aged 4,5 months. Before the experiment, a thorough examination of the animals was performed, visually paying attention to the color and moisture of the mucous membrane of the oral cavity. Collection of material was carried out on the first, fourteenth and twenty-eighth days. The material for the study was the microbiota of different parts of the oral cavity of rats, namely, from microbiotopes of the tooth surface in the gingival margin, mucous membrane of oral vestibule and mucous membrane of oral cavity proper. In microscopic examination the smear material was applied on a glass slide, fixed over a burner flame and Gram stained. In bacteriological studies, as a result of counting the total number of sprouted colonies, a quantitative indicator in colony-forming units was obtained per 1 ml (CFU/ml). For further statistical analysis, the obtained data were tested for normality by calculating the asymmetry and excess coefficients and the results of the Shapiro-Wilk test ($p<0.05$). The data were presented as M±SD, where M is the mean, and SD is the standard deviation. Microscopic examination of smears from microbiotopes of the oral cavity revealed that the microbiota was the same in all intact animals. Studies conducted at different times of the experiment (1, 14 and 28 days) indicated the dominance of gram-positive microorganisms. The smears showed elongated filamentous bacteria – *Leptotrix* (genus *Lactobacillus*). Gram-positive coccal microbiota was visualized as separate clusters. When comparing the quantitative characteristics of the streptococcal microbiota of the microbiotope of the tooth surface in the gingival margin for twenty-eight days, it was noted that the number of nonhemolytic and α-hemolytic streptococci did not change significantly during the experiment, in contrast to other studied microbiotopes of oral intact animals. At the same time, in comparison with the previous term (14 days) of the experiment, the number of α-hemolytic streptococci in the microbiotope of mucous membrane of oral vestibule increased significantly relative to the quantitative composition of these microorganisms in the microbiotope of mucous membrane of oral cavity proper. Quantitative indicators of other species of detected microorganisms (coagulase-negative staphylococci, Enterococci, Escherichia coli, gram-positive non-spore forming rods and spore forming rods) did not differ significantly in the microbiotopes of the oral cavity of rats, formed on the twenty-eighth day of the experiment. This indicated the stabilization of the microbiocenosis of the oral cavity in the quarantine of intact animals in the experimental conditions.

Key words: oral cavity, microbiotopes, rats, bacterial groups.

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The Authors declare no conflict of interest.

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