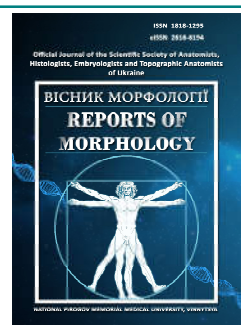




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# The role of sorbents and probiotics in prevention of structural and morphological disorders in the small intestine of animals developing in dysbiosis

**Bobyr V.V.<sup>1</sup>, Stechenko L.O.<sup>1</sup>, Shirobokov V.P.<sup>1</sup>, Nazarchuk O.A.<sup>2</sup>, Rymsha O.V.<sup>2</sup>**

<sup>1</sup>O.O.Bogomolets National Medical University, Kyiv, Ukraine

<sup>2</sup>National Pirogov Memorial Medical University, Vinnytsya, Ukraine

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### CORRESPONDING AUTHOR

e-mail: [nazarchukoa@gmail.com](mailto:nazarchukoa@gmail.com)

Nazarchuk O.A.

*The past decade is characterized by a noticeable increase in the interest of physicians in all areas of activity in the development of new and improvement of existing approaches to the correction of dysbiotic disorders. Among them, the concept of using probiotics occupies a leading position. At the same time, some enterosorbents, the mechanism of action of which is largely due to the sanitation of the intestinal lumen and due to this improvement of conditions for the vital activity of the physiological microbiota, can be attributed to the group of means of improving the normal microflora. In the context of an increase in the level of resistance to antibacterial agents, the inclusion of enterosorbents in the complex therapy of dysbiosis is an important and pathogenetically justified approach. The aim of the work was to clarify the effectiveness of the use of sorbents and probiotics for the prevention of structural and morphological disorders in the small intestine of white mice developing against the background of antibiotic-induced dysbiosis. Electron-microscopic methods showed that in the mucous membrane of the small intestine of mice after using the probiotic "Simbiter" the extinction of manifestations of cytodestructive disorders is observed. In addition, the obtained electron microscopic data, indicating the ability of probiotic drugs with the simultaneous introduction into the body of animals with a complex of antibiotics, to stimulate the body's immune response. As a result of ultrastructural analysis of the mucous membrane of the small intestine of mice, the formation of dysbiosis in which occurred against the background of the use of enterosorbents, a decrease in the severity of structural damage was found, compared with the group of animals that received only antibiotics. After using "Symbiogel", activation of plasma cells was registered, which can be an indicator of the inflammatory process and the activity of the immune response in general, as evidenced by the detection of plasma cells with dilated tubules. In general, it should be noted that the use of "Symbiogel" for the prevention of dysbiotic disorders contributes to the formation of a more pronounced immune response, compared with probiotic drugs. So, on the model of antibiotic-induced dysbiosis at the ultrastructural level, the ability of multiprobitics "Simbiter®" and sorbent "Symbiogel" to reduce cytodestructive changes in the mucous membrane of the small intestine of mice and normalize morphoimmunogenesis was proved.*

**Key words:** *microbiota, probiotics, enterosorbents.*

### Introduction

Nowadays, the interest of scientists in studying the normal human microflora is growing. In recent years, the term "normal microflora" is often replaced by the term "microbiome" [9]. It should be noted that progress in the study of the microbiome and its role in maintaining human health is considered one of the key achievements of modern biology and medicine [11].

Many convincing evidences of huge potential of action of a microbiome on various processes of functioning of a human body are received. Experts now consider the microbiome as an additional human organ, which, actively participating in digestion, management of metabolic processes, maintaining the integrity of the epithelial barrier, development and strengthening of the immune system and

a number of other physiological functions, optimizes conditions for normal life [1, 2, 4, 5, 8, 10, 14, 18].

However, recent scientific studies show that from 70 to 90% of the world's population suffers from dysbiosis of varying degrees, which, of course, indicates their significant social and environmental significance [7, 13]. Among the many causes of dysbiotic disorders in the first place is the use of chemotherapeutic antimicrobials, often broad-spectrum and with an oral mechanism of administration. Particularly dangerous in this regard is the use of antibiotics for prophylactic purposes. However, some other groups of drugs can contribute to the formation of dysbiosis by affecting the kinetics of the epithelium of mucous membranes and, accordingly, the composition of mucin (nonsteroidal anti-inflammatory drugs, laxatives, cholagogues, enveloping agents with sorbent properties and some others) [23, 25].

The past decade has been marked by increase in the interest of physicians in all areas of activity in the development of new and improvement of existing approaches to the correction of dysbiotic disorders. Among them, the concept of probiotics occupies a leading position. According to the WHO, probiotics are "living microorganisms that, when used in adequate quantities, have a beneficial effect on the host" [22]. The mechanisms responsible for various probiotic effects are usually associated with the ability of probiotics to inhibit the development of pathogenic microbes, exhibit immunomodulatory properties, stimulate the proliferation and differentiation of epithelial cells and strengthen the intestinal barrier [12, 17, 19].

Some types of enterosorbents can be referred to the group of means of improvement of normal microflora. The mechanism of their action is largely due to the rehabilitation of the intestinal lumen and the improvement due to this conditions for the functioning of the physiological microbiota. Enterosorption is a non-invasive method of efferent therapy and when choosing an adequate sorbent can help effectively cleanse the body of allergens, mediators, products of allergic or inflammatory reactions, metabolites, toxins, viruses and other components. Rehabilitation of habitats can optimize the conditions for the functioning of normal human microflora. With increasing levels of resistance to antibacterial agents, the inclusion of enterosorbents in the complex therapy of dysbiosis is an important and pathogenetically sound approach [6, 20, 21].

In recent years, much attention has been paid to enterosorbents based on clay minerals, among which bentonite clays are one of the most studied. Today, bentonites are so-called "edible" minerals with proven anti-inflammatory, antitoxic and ion exchange properties [15, 16, 24].

*Objective of work:* to determine the effectiveness of sorbents and probiotics for the prevention of structural and morphological disorders in the small intestine of mice developing on the background of antibiotic-induced dysbiosis.

## Materials and methods

White laboratory mice of the *BALB/c* line were selected as a model for studying morphofunctional changes of the small intestinal mucosa in antibiotic-induced dysbiosis and after their correction with probiotics and enterosorbents. The formation of dysbiotic conditions in animals was performed according to the previously described method [3]. In addition, these antibiotics were added to a glass of water (1 g of Ampicillin, 1 g of Metronidazole and 290 mg of Gentamicin per 1000 ml of water).

A total of 80 mice were involved in the experiment. The first group - control, formed from intact animals, was 20 individuals; group 2 - animals with established intestinal dysbiosis; group 3 - animals that in the process of modeling dysbiotic conditions orally received "Symbiter® M concentrated"; group 4 - animals that received "Symbiogel" orally during the entire period of dysbiosis modeling.

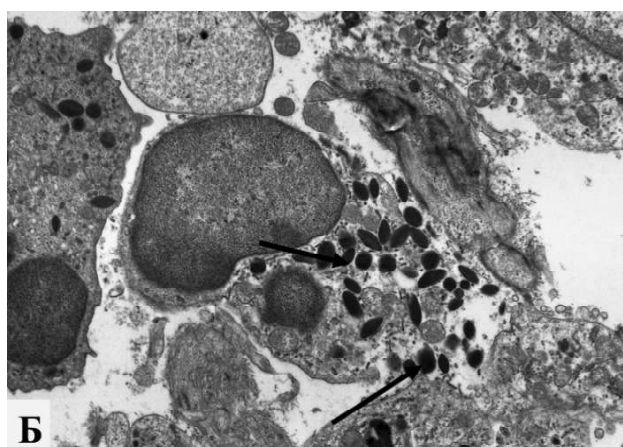
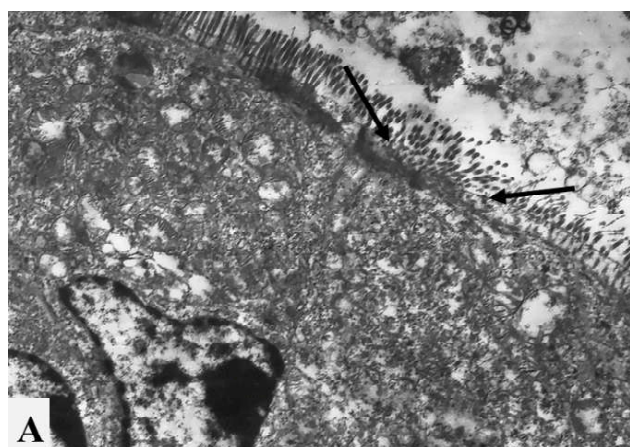
Animals were removed from the experiment 5 days after the start of modeling dysbiosis. Probiotics and sorbents were administered intragastrically to animals for 5 days in a volume of 200 µl 6 hours after receiving antimicrobial drugs to form dysbiosis.

## Results

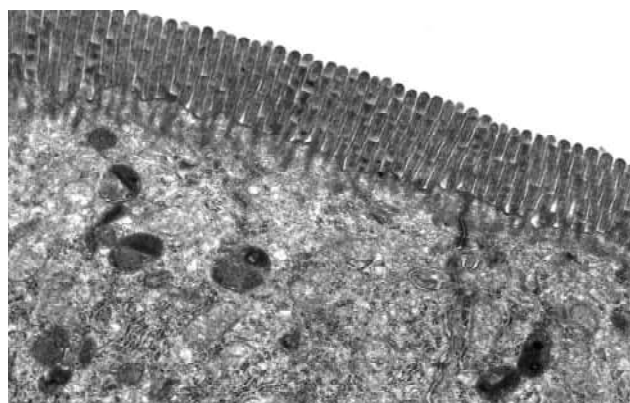
As a result of the analysis of structural and morphological changes of the small intestine of mice with antibiotic-induced dysbiosis (experimental group 2) the expressed disturbances accompanying this process are recorded: shortening of microvilli length and their partial reduction or destruction with subsequent disintegration, absence of brush border, mitochondria and the presence of autophagosomes (Fig. 1A), as well as an increase in the number of eosinophils, which are an indicator of allergic reactions, as they are directly involved in protective allergic and anaphylactic reactions of the body (Fig. 1B).

In the mucous membrane of the small intestine of mice after the use of probiotics there was a visual attenuation of cytodestructive disorders, namely a decrease in the number of desquamated microvilli, the vast majority of enterocytes retained the brush border, compared with the experimental group of animals №2 (animals with dysbiosis) (Fig. 2). The desquamation of microvilli was local in nature with a partial absence of a brush border and a slight smoothness of the plasma membrane.

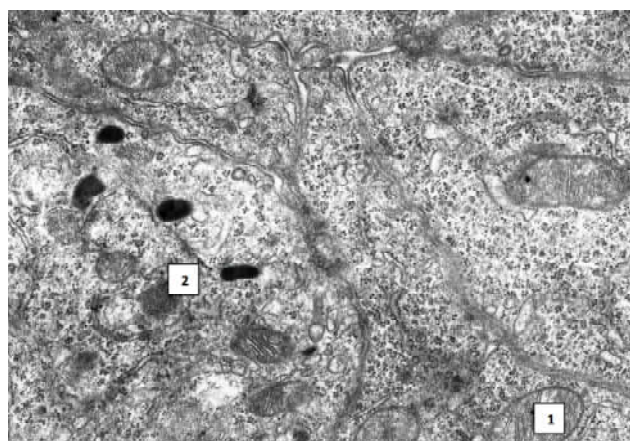
It should also be noted about the intensive formation of autophagosomes in enterocytes after the use of "Symbiter" (Fig. 3). Electron microscopic studies showed no signs of cell displacement to the basement membrane, compaction of the cytoplasm, organelles and precursors of apoptotic cells, as well as other apoptotic disorders. The number of detected Paneth cells was statistically higher, but specific changes were recorded in their granules: granules, which apparently contained defensins, gradually lost their content and transformed into structures with an electron-transparent rim. Such granules can be considered as



**Fig. 1.** Electronic microphotography. A - local reduction of the brush border of mouse small intestine enterocytes in antibiotic-induced dysbiosis (↑). x6200; B - eosinophil (1) with characteristic elongated granules with a crystalloid (↑). x8000.



**Fig. 2.** Electronic microphotography. Local desquamation, signs of partial absence of the brush border, smoothing of the plasma membrane of the microvilli of the small intestine of animals with dysbiosis after the use of probiotics. x8000.



**Fig. 3.** Electronic microphotography. Mitochondrial edema (1) and intensive formation of autophagosomes (2) in enterocytes on the background of probiotic use. x3600.

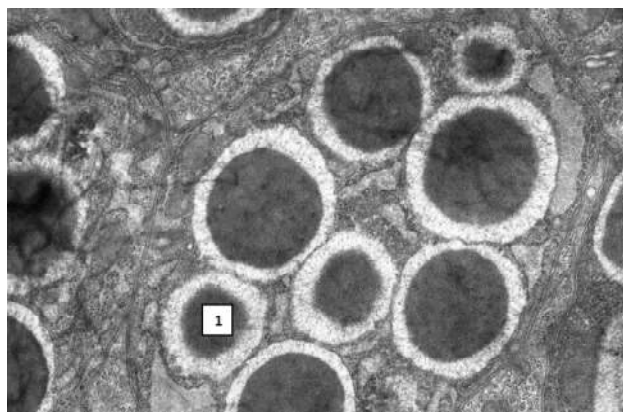
objects that are at different stages of functional activity and, obviously, are proteins (Fig. 4).

The obtained data may indicate the ability of probiotic drugs when simultaneously administered to animals with

a complex of antibiotics to stimulate the body's immune response. In addition, in contrast to the control, the expansion of the tubules of plasma cells due to their filling with antibodies was not recorded at all. Blood vessels also remained unchanged. At the same time, when using probiotics, there was a visual decrease in the number of eosinophils and basophils.

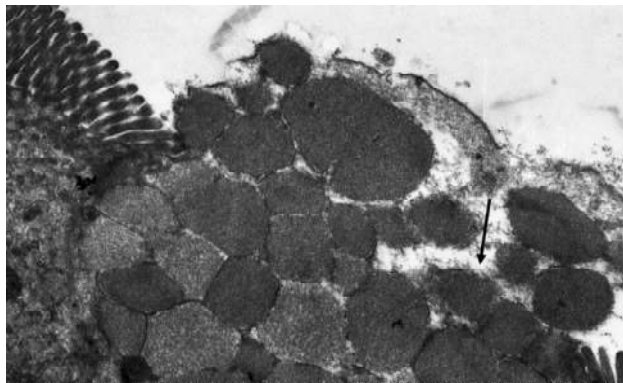
Based on the data obtained, it cannot be claimed that the probiotic strains administered to mice colonized the intestine. However, it should not be ruled out the possibility of probiotics to actively secrete metabolites when passing through the gastrointestinal tract. The latter can have a positive effect on the barrier function of the intestine. However, after the use of probiotics in the modeling of antibiotic-induced dysbiosis, microecological changes and cytodestructive manifestations in the intestines of animals were less pronounced, compared with the group where animals received only antibiotics.

As a result of ultrastructural analysis of the mucous membrane of the small intestine of mice, the formation of dysbiosis in the background of the use of enterosorbents, a decrease in the severity of structural damage, compared with the group of animals receiving only antibiotics was

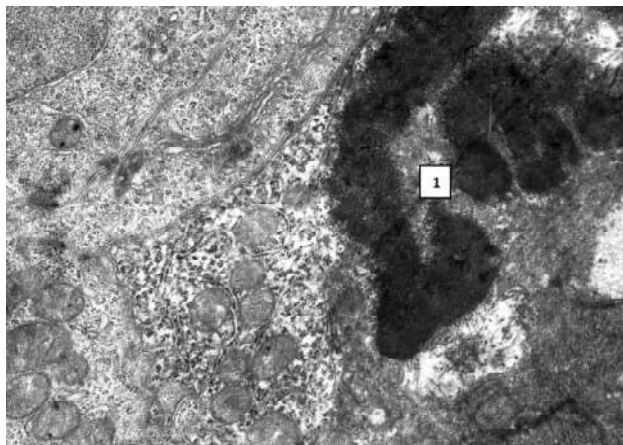


**Fig. 4.** Electronic microphotography. Transformation of Paneth cell granules (1) with the formation of cytosegresome (2). x3600.





**Fig. 5.** Electronic microphotography. Local desquamation of microvilli with partial absence of microvilli destruction (↑) and unexpressed smoothness of enterocyte plasma membrane after Symbiogel use. x8000.



**Fig. 6.** Electronic microphotography. Compacted enterocytes (1). x6000.

established. Although in some places in the mucous membrane of the small intestine was observed a visual shortening of the length of the microvilli, cases of their complete desquamation or disintegration were not recorded (Fig. 5). After using Symbiogel, we registered the activation of plasma cells, which may be an indicator of the inflammatory process and the activity of the immune response in general, as evidenced by the detection of plasma cells with dilated tubules, apparently filled with antibodies (Fig. 6).

In general, it should be noted that the use of Symbiogel for the prevention of dysbiotic disorders contributes to the formation of a more pronounced immune response, compared with probiotic drugs. At the same time, no changes were observed in the circulatory system, and it is likely that the development of antibiotic-induced dysbiosis is not reflected in the hemomicrocirculatory tract. There are no direct signs that would indicate the intensive development of apoptosis with the formation of apoptotic cells that move to the basement membrane.

It should be noted that, in general, when receiving electron microscopic sections of the mucous membrane of the small intestine, microbial cells cannot always be

registered, while after the use of Symbiogel in the intestinal lumen more often noted their presence. It should also be noted that we have recorded isolated cases of the presence of metamyelocytes and plasma cells in the own plate of the mucous membrane of the small intestine.

### Discussion

With increasing levels of resistance to antibacterial agents, the inclusion of probiotics and enterosorbents in the complex therapy of dysbiosis is an important and pathogenetically sound approach. The feasibility of correction of intestinal dysbiosis in various pathological conditions with probiotic drugs was confirmed in the Practical Recommendations of the World Gastroenterological Organization (WGO) 2002, 2008, 2011, 2012, 2014 [7, 9, 22].

An important advantage of probiotics with their inherent wide range of antagonistic activity against pathogenic and opportunistic microorganisms is that, unlike antibiotics, they do not cause the formation of resistant forms of bacteria. In addition, they have a multifaceted positive effect on the body, which consist, in particular, in ability of probiotics to reduce the permeability of tissue barriers to toxins, to have a detoxifying effect on compounds produced in the host by the pathogen. Unlike antibiotics that suppress the immune system, probiotics stimulate the production of antibodies and its non-specific factors. By producing biological substances, they promote the production of mediators by the macroorganism, which has a positive effect on the functions of the digestive tract, liver, cardiovascular, circulatory systems and metabolic processes in the body of the host; involved in the synthesis and absorption of vitamins. When interacting with immune and intestinal epithelial cells, the active signaling components of probiotics interact with antigen-recognizing receptors (TLRs - Toll-like receptors, NLRs - The NOD-like receptors or Nucleotide Oligomerization Domain receptors) and other surface receptors, and as a consequence cause - lymphocytes in Peyer's patches (CD4+ Th1, CD4+ Th2, CD8+ cytotoxic T-lymphocytes) and their production of various chemokines and cytokines [7, 23, 24].

The results of electron microscopic examination indicate that during the correction of experimental chemotherapeutic dysbiosis in laboratory animals the use of probiotic "Simbiter" allows not only to stop the destructive-dystrophic changes in the mucous membrane of the small intestine, but also stimulates repair processes.

Based on the data obtained in the study, the stimulating effect of "Symbiogel" was proved, which significantly contributes to the restoration of parietal microflora of the small intestine and eradication of translocated intestinal microflora. Under such conditions, the introduction of immobilized probiotics achieves an early therapeutic effect in comparison with therapy with free probiotic cells and enterosorbents.

## Conclusions

The results of experiments showed the property of probiotic drugs and sorbents to reduce the depth of cytodestructive changes in the mucous membrane of the small intestine in the formation of antibiotic-induced dysbiosis and normalization of immune responses that accompany the development of such disorders.

Advances in DNA sequencing and computational

technology have revolutionized the field of microbiology, but many fundamental questions remain to be answered. Obviously, future research will focus on elucidating the more precise mechanisms responsible for the interaction between the microbiome and the human body, as well as on improving the effectiveness of therapeutic approaches to the assessment and treatment of conditions associated with microbiome disorders.

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## РОЛЬ СОРБЕНТІВ ТА ПРОБІОТИКІВ У ПРОФІЛАКТИЦІ СТРУКТУРНО-МОРФОЛОГІЧНИХ ПОРУШЕНЬ У ТОНКОМУ КИШКІВНИКУ ТВАРИН, ЩО РОЗВИВАЮТЬСЯ ПРИ ДИСБІОЗІ

Бобир В.В., Стеченко Л.О., Широбоков В.П., Назарчук О.А., Римша О.В.

Минуле десятиліття характеризується помітним зростанням зацікавленості медиків усіх напрямків діяльності питаннями

розробки нових та вдосконалення існуючих підходів до корекції дисбіотичних порушень. Серед них концепція використання пробіотиків займає провідні позиції. Разом із тим, до групи засобів оздоровлення нормальної мікрофлори можна віднести і деякі ентеросорбенти, механізм дії яких в значній мірі обумовлений санацією просвіту кишки і поліпшенням за рахунок цього умов для життєдіяльності фізіологічної мікробіоти. В умовах зростання рівня резистентності до антибактеріальних засобів включення до комплексної терапії дисбіозу ентеросорбентів є важливим та патогенетично обґрунтованим підходом. Метою роботи було з'ясування ефективності використання сорбентів та пробіотиків для профілактики структурно-морфологічних порушень у тонкому кишківнику білих мишей, що розвиваються на фоні антибіотикоіндукованого дисбіозу. Електронно-мікроскопічними методами показано, що в слизовій оболонці тонкої кишки мишей після використання пробіотику "Симбітеру" спостерігається згасання проявів цитодеструктивних порушень. Крім того, отримані електронно-мікроскопічні дані, які свідчать про здатність пробіотичних препаратів при одночасному введенні в організм тварин з комплексом антибіотиків стимулювати імунну відповідь організму. В результаті ультраструктурного аналізу слизової оболонки тонкого кишківника мишей, формування дисбіозу у яких відбувалось на фоні вживання ентеросорбентів, встановлено зниження виразності структурних ушкоджень порівняно з групою тварин, які отримували лише антибіотики. Після використання "Симбіогелю" зареєстровано активізацію плазматичних клітин, які можуть бути показником запального процесу та активності імунної відповіді в цілому, про що свідчить виявлення плазматичних клітин з розширеними каналцями. В цілому слід відмітити, що використання "Симбіогелю" для профілактики дисбіотичних розладів сприяє формуванню більш вираженої імунної відповіді у порівнянні з пробіотичними препаратами. Отже, на моделі антибіотикоіндукованого дисбіозу на ультраструктурному рівні доведена здатність мультипробіотику "Симбітер®" та сорбенту "Симбіогель" зменшувати цитодеструктивні зміни в слизовій оболонці тонкого кишківника мишей та нормалізувати морфоімуногенез.

**Ключові слова:** мікробіота, пробіотики, ентеросорбенти.

#### **РОЛЬ СОРБЕНТОВ И ПРОБИОТИКОВ В ПРОФИЛАКТИКЕ СТРУКТУРНО-МОРФОЛОГИЧЕСКИХ НАРУШЕНИЙ В ТОНКОМ КИШЕЧНИКЕ ЖИВОТНЫХ, РАЗВИВАЮЩИХСЯ ПРИ ДИСБИОЗЕ**

**Бобирь В.В., Стеченко Л.А., Ширококов В.П., Назарчук А.А., Рымша Е.В.**

Прошедшее десятилетие характеризуется заметным ростом заинтересованности медиков всех направлений деятельности вопросами разработки новых и совершенствования существующих подходов к коррекции дисбиотических нарушений. Среди них концепция использования пробиотиков занимает ведущие позиции. Вместе с тем, в группу средств оздоровления нормальной микрофлоры можно отнести и некоторые энтеросорбенты, механизм действия которых в значительной степени обусловлен санацией просвета кишки и улучшением за счет этого условий для жизнедеятельности физиологической микробиоты. В условиях роста уровня резистентности к антибактериальным средствам включение в комплексную терапию дисбиоза энтеросорбентов является важным и патогенетически обоснованным подходом. Целью работы было выяснение эффективности использования сорбентов и пробиотиков для профилактики структурно-морфологических нарушений в тонком кишечнике белых мышей, развивающихся на фоне антибиотикоиндуцированного дисбиоза. Электронно-микроскопическими методами показано, что в слизистой оболочке тонкой кишки мышей после использования пробиотика "Симбитера" наблюдается угасание проявлений цитодеструктивных нарушений. Кроме того, получены электронно-микроскопические данные, которые свидетельствуют о способности пробиотических препаратов при одновременном введении в организм животных с комплексом антибиотиков стимулировать иммунный ответ организма. В результате ультраструктурного анализа слизистой оболочки тонкого кишечника мышей, формирование дисбиоза у которых происходило на фоне употребления энтеросорбентов, установлено снижение выраженности структурных повреждений по сравнению с группой животных, получавших только антибиотики. После использования "Симбиогеля" зарегистрирована активизация плазматических клеток, которые могут быть показателем воспалительного процесса и активности иммунного ответа в целом, о чем свидетельствует обнаружение плазматических клеток с расширенными каналцами. В целом следует отметить, что использование "Симбиогеля" для профилактики дисбиотических расстройств способствует формированию более выраженного иммунного ответа по сравнению с пробиотическими препаратами. Итак, на модели антибиотикоиндуцированного дисбиоза на ультраструктурном уровне доказана способность мультипробіотику "Симбітер®" и сорбента "Симбіогель" уменьшать цитодеструктивные изменения в слизистой оболочке тонкого кишечника мышей и нормализовать морфоімуногенез.

**Ключевые слова:** микробиота, пробиотики, энтеросорбенты.