

Bobyry V. V., Nazarchuk O. A. The role of sorbents and probiotics in the prevention of structural-morphological disorders in mice with dysbiosis on the background of virus-bacterial infection. Journal of Education, Health and Sport. 2020;10(8):549-558. eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2020.10.08.067>
<https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2020.10.08.067>
<https://zenodo.org/record/4292447>

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8.2) and § 12.1.2) 22.02.2019.

© The Authors 2020;

This article is published with open access at Licensee Open Journal Systems of Nicolaus Copernicus University in Torun, Poland

Open Access. This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited. This is an open access article licensed under the terms of the Creative Commons Attribution Non commercial license Share alike.

(<http://creativecommons.org/licenses/by-nc-sa/4.0/>) which permits unrestricted, non commercial use, distribution and reproduction in any medium, provided the work is properly cited.

The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 04.08.2020. Revised: 14.08.2020. Accepted: 31.08.2020.

UDC: 616.34:615.211.099

THE ROLE OF SORBENTS AND PROBIOTICS IN THE PREVENTION OF STRUCTURAL-MORPHOLOGICAL DISORDERS IN MICE WITH DYSBIOSIS ON THE BACKGROUND OF VIRUS-BACTERIAL INFECTION

V. V. Bobyr¹, O. A. Nazarchuk²

¹Bogomolets National Medical University, Kyiv

²Vinnitsa National Pirogov Memorial Medical University

03057, Kyiv-57, Peremogy Avenue, 34, e-mail: vitalibobyr@ukr.net

Abstract

Interest in the study of probiotics has grown significantly in recent years. One of the reasons for such increased attention to this problem is the results of numerous experimental studies, which show the important role of individual representatives of the normal microbiota in the aetiology and pathogenesis of many infections and even somatic human diseases. However, the violation of microbial ecology, as a rule, is accompanied by pollution of the internal environment of the body with toxic compounds of both exogenous and endogenous nature, so enterosorbents are among main approaches of improving the normal microbiota.

The experiments allowed to establish a pronounced hepatoprotective function of the sorbent “Smektovit”, which is manifested by a decrease in destructive changes that accompany the process of modelling viral and bacterial infections on the background of dysbiosis. In addition, the results of the presented studies showed the ability of probiotic “multiprobiotic Symbiter[®]” and sorbent “Smektovit” to reduce deep cytodestructive messages

in the simulation of viral and bacterial infections at the rate of dysbiosis and normalization of immune responses, as they accompany such processes. At the same time, it is noted that the preventive effect of probiotics is more pronounced in the intestine, and sorbents – in the liver.

Key words: dysbiosis; infection; probiotics; sorbents; viruses.

Introduction. Today, scientists often define dysbiosis as a condition of the microbial ecological system in which there is a simultaneous violation of the functions and mechanisms of interaction of its key components: the macro-organism and indigenous microbiota associated with the mucous membranes of cavities and skin [1]. However, obviously, at the heart of all these processes are changes in the structural components of the intestine, which are caused by qualitative and quantitative changes in the normobiota [2, 3]. It is important that the human intestinal microbiota differs little from the intestinal one of other mammals, which facilitates the methodology of its study.

It is possible that the viral factor is a key element that determines the depth of ultrastructural disorders with pronounced apoptotic changes. The obtained results prompted us to develop algorithms aimed at the prevention of such disorders in animals, primarily through the use of sorbents and probiotics.

Materials and methods. A total of 150 mice were used in the experiment, which were divided into 5 groups (30 animals in each group). Modelling of dysbiotic states was carried out according to the method developed by us [4]. 1 group – animals, the formation of dysbiosis in which was carried out simultaneously using the “multiprobiotic Symbiter[®]” manufactured by LLC “O.D. Prolisok” Ukraine, infected with Coxsackie B3 virus, the 2nd group – animals, the formation of dysbiosis in which was carried out simultaneously with the use of “Smectovit” – bentonite gel with controlled cationic substitution (LLC “O.D. Prolisok”, Ukraine) infected with Coxsackie B3 virus, the 3rd group – animals, the formation of dysbiosis in which was carried out simultaneously using the probiotic “multiprobiotic Symbiter[®]”, infected Coxsackie B3 and Salmonella typhimurium, the 4th group – animals, the formation of dysbiosis in which was carried out simultaneously using the sorbent “Smectovit”, infected Coxsackie B3 and Salmonella typhimurium, the 5th group – control. Experimental salmonellosis was modelled by infecting mice with a pathogenic strain of Salmonella typhimurium isolated and identified at Kiev City Clinical Hospital No 4 (clinical isolate No 7683). Animal infection with bacteria was carried out intraperitoneally in the volume of 0,5 ml (1 billion microbial bodies) per individual. Probiotics and enterosorbents were administered intragastrically to animals in a volume of 200 µl in 6 hours after

administration of antimicrobial drugs. The observation period for the animals was 21 days. Based on publications that refer to the maximum time of accumulation of pathogens in the relevant organs and systems of the body and the duration of their circulation, the animals were removed from the experiment on the third day after infection [5].

Results and discussion

It was found that mice after the use of Symbiogel, compared with the control, slightly normalized intestinal ultrastructure – decreased the number of desquamated microvilli and smoothness of the plasma membrane, observed less pronounced dystrophic changes in the lamina propria and submucosal submucosal base of the intestines. However, some enterocytes developed apoptotic changes with displacement of cells to the basement membrane, compaction of the cytoplasm and organelles, as well as the formation of pre-apoptotic and apoptotic cells.

Prophylactic use of Symbiter in the process of modeling dysbiotic conditions in mice infected with virus Coxsackie B3, allowed to obtain positive results. After analysis of electrograms apoptotic changes, in comparison with control, were not revealed, only insignificant signs of dysbiosis with visual shortening of length of microvilli of mucous and not expressed smoothness of a plasma membrane of enterocytes were observed. Moreover, total desquamation of microvilli with destruction and subsequent decay was not recorded, as well as oedema of mitochondria, pronounced enlightenment of the matrix, expansion of intercellular contacts and other pathomorphological features inherent in dysbiosis (Fig. 1).

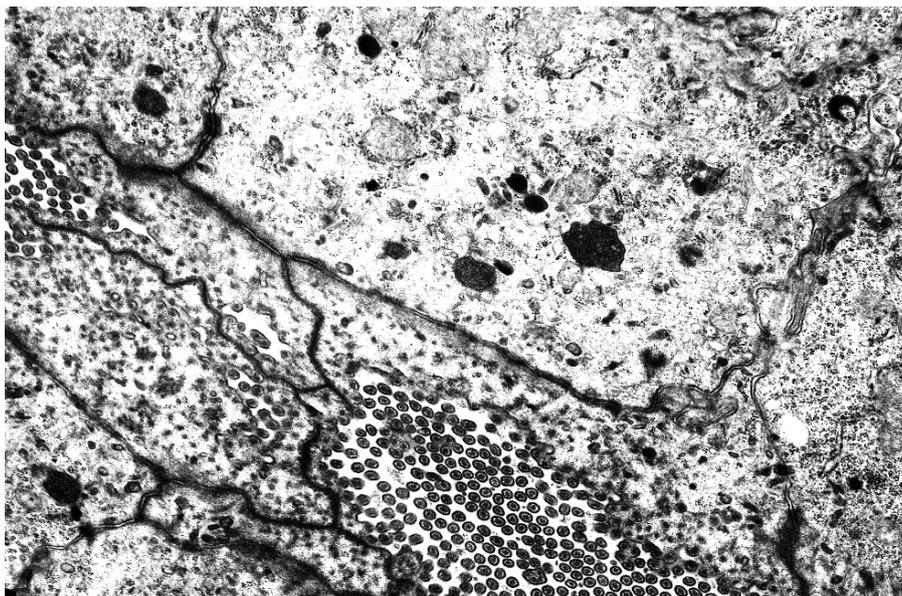


Fig. 1. Electronic microphotography. No signs of apoptosis in the intestine of mice with dysbiosis infected with Coxsackie B after using Symbiter. Magnification 12800 ×.

Nowadays there is known that the course of viral and bacterial infections may be accompanied by the development of degenerative processes in the internal organs, as well as the development of necrotic changes in the liver, which may be generalized. With this in mind, we studied the effectiveness of the use of sorbents and probiotics to prevent cytomorphological disorders in mice with dysbiosis infected with mixed (Coxsackie B and salmonellosis) infection.

Analysis of micrographs of sections of the intestine showed a pronounced prophylactic effect of probiotic drugs, compared with the control, which is manifested by a decrease in cytodestructive signs of dysbiosis in the intestine of animals infected with Coxsackie B3 and *Salmonella typhimurium*, in particular the lack of total microvilli desquamation, plasma membrane smoothness, mitochondrial oedema and autophagosome formation. No apoptotic nuclei and other pathomorphological signs of apoptosis were detected, as well as dystrophic changes in the intestinal mucosa and submucosal base of the intestine. There was only migration of neutrophils, monocytes and lymphocytes in the intestinal interstitium, oedema of the interstitial space and increased infiltration of the mucous membrane by macrophages (Fig. 2).

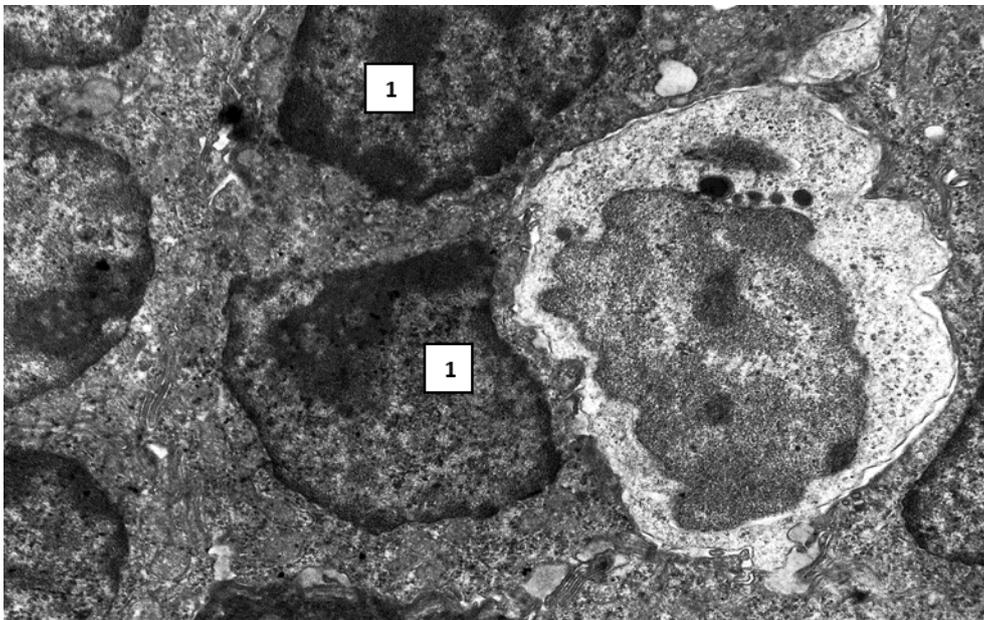


Fig. 2. Electronic microphotography. Migration of lymphocytes (1) in the intestinal interstitium, oedema of the interstitial space after the use of Symbiter in animals with dysbiosis infected with Coxsackie B and *Salmonella typhimurium*. Magnification 8000 ×

The use of probiotics in the modelling of viral and bacterial infections on the background of dysbiosis did not significantly affect the structure of the spleen: white pulp is represented by its characteristic cells: B-lymphoblasts, lymphocytes, plasma cells, macrophages, a small number of T - lymphocytes, dendritic, interdigitant and reticular cells. Among them, altered after apoptosis cells and apoptotic bodies were sometimes registered (Fig. 3). For the spleen, this is the norm, because it is constantly renewed cells of the lymphocyte lineage. Residual bodies are absorbed by macrophages with their subsequent cleavage.

After the use of "Symbiter" in the liver cells of animals with dysbiosis infected with enteroviruses and salmonella, in comparison with the control, some normalization was noted, but nonspecific changes were observed, which testified to the activation of the processes occurring in this organ (Fig. 4 (A-D)). In the preparations we found hepatocytes that had large rounded nuclei, in which euchromatin predominated, so they were in an active state, in the cytoplasm of these cells there was a large number of both free ribosomes and attached to the tubules of the granular endoplasmic reticulum (Fig. 10.14B). The increase in the number of lymphocytes in the space of Disse, apparently, is the result of immune activation, and the increase in the number of fat granules in Ito cells, which included fat-soluble vitamins, is evidence of activation of liver metabolism.

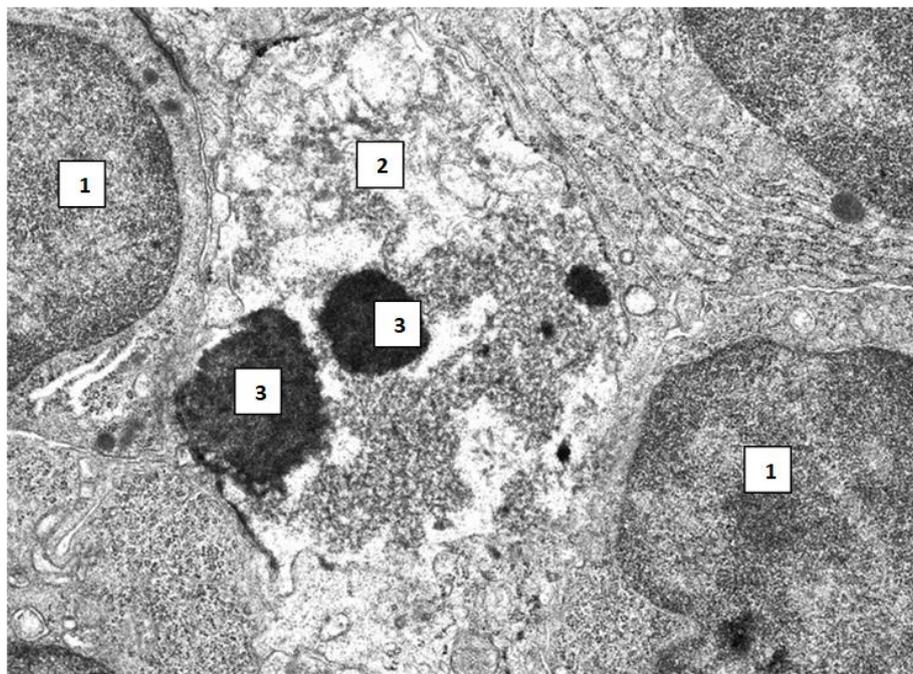


Fig. 3. Electronic microphotography. Spleen of mice with dysbiosis infected with Coxsackie B and Salmonella typhimurium, after using Symbiter: lymphocytes (1), plasma cells (2), apoptotic bodies (3). Magnification 16000 ×

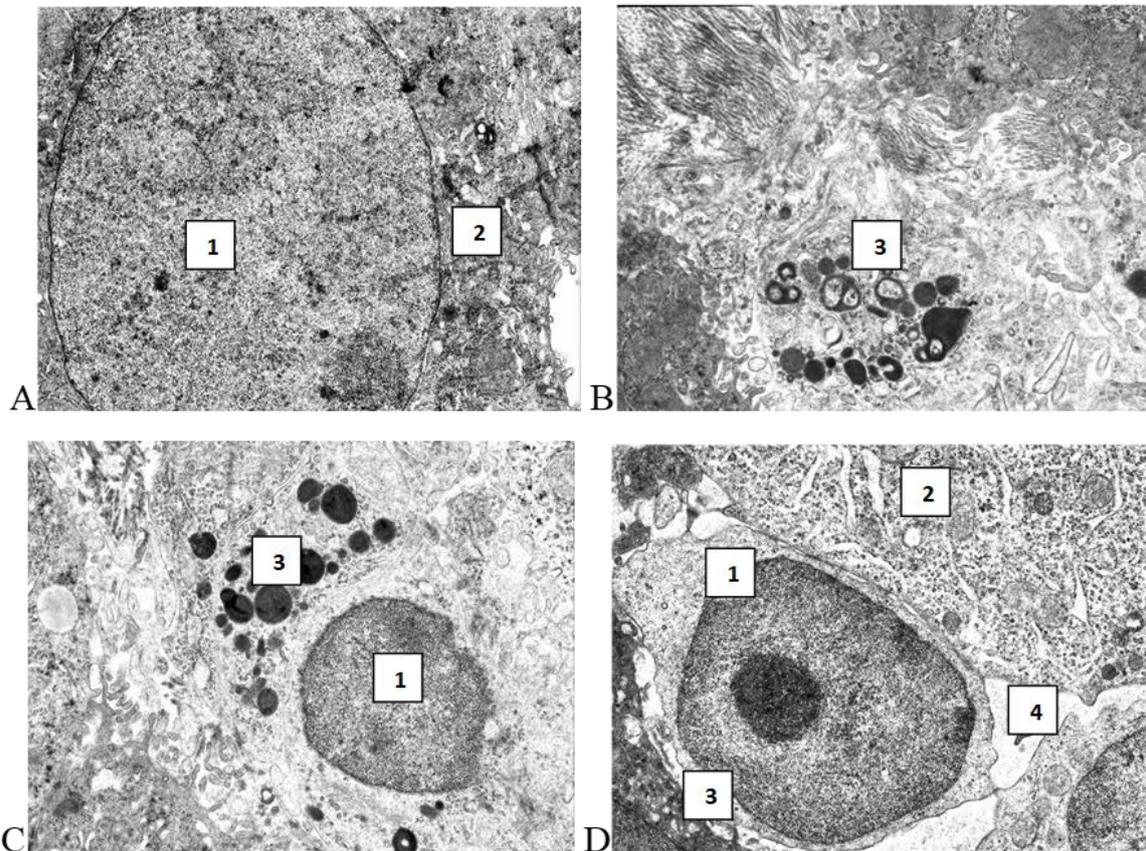


Fig. 4 - Electronic microphotography. Liver of mice with dysbiosis infected with Coxsackie B and Salmonella typhimurium after using Symbiter. A.B. Hepatocyte nucleus (1), hepatocyte contact (2); C. Ito cell nucleus (1), hepatocyte (2), lipid granules (3), Disse space (4); D. Hepatocyte (1), granular endoplasmic reticulum (2), lymphocytes (3), Disse space (4). Magnification 14000 ×

When using Symbiogel in the process of modeling dysbiosis in mice, further infected with a viral-bacterial mixture, in comparison with the control, there was a decrease in the depth of violations at the level of ultrastructural organization of the intestine, however, they were less pronounced compared to the prophylactic effect of probiotics. Occasionally, apoptotic changes were recorded, visual shortening of the length of mucosal microvilli was observed, and in some places their total desquamation, swelling of mitochondria, formation of autophagosomes, expansion of intercellular space and other morphological signs of dysbiosis. It should be noted that local apoptotic processes, which were accompanied by compaction of the nuclei, also affected plasma cells (Fig. 5).

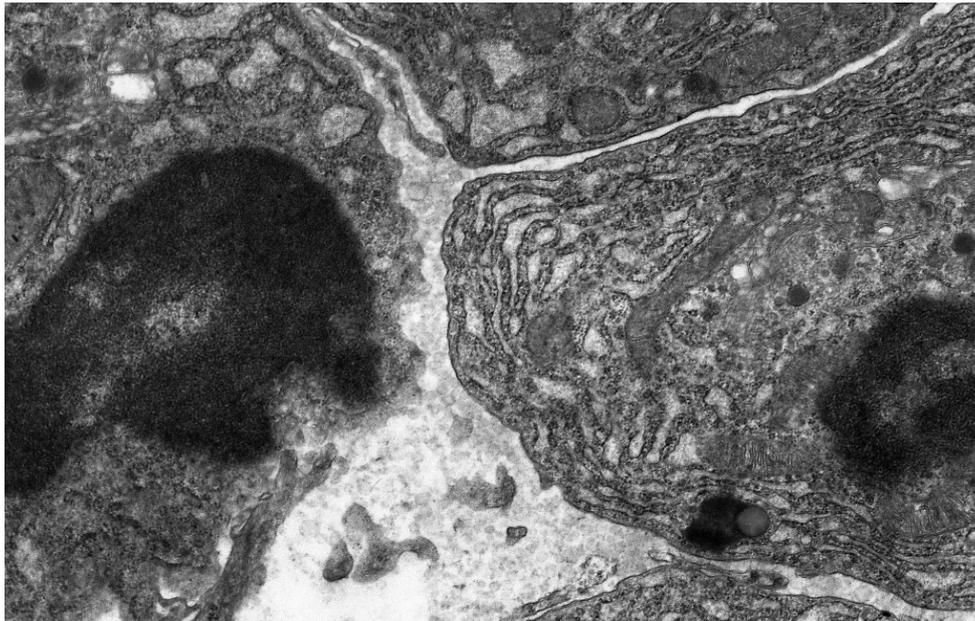


Fig. 5. Electronic microphotography. Local compaction of plasma cell nuclei in mice with dysbiosis, infected with Coxsackie B and *Salmonella typhimurium*, after using Symbiogel. Magnification 8000 ×

Electron microscopic studies of the spleen of mice under the conditions of administration of “Symbiogel” showed that in general the ultrastructural organization of this organ has not changed - in a spleen the cellular structure inherent in these animals in norm is presented: leukocytes, among them lymphocytes, plasma cells, macrophages, sometimes there are apoptotic little bodies. Such structures were observed in the control group of animals as a result of self-purification from spent cells. Perivascular also found thrombocytes, apparently, it is connected both with a difference of contacts of endothelial cells, and with obturation of a gleam of capillaries by erythrocytes (fig. 6 A-D).

Electron microscopy showed that in the liver of mice infected with the viral-bacterial mixture, after the use of sorbents in the modeling of antibiotic-induced dysbiosis, compared with the control, minimal changes in ultrastructures were detected, indicating a slight violation of metabolism of this organ. The vast majority of bile capillaries had an enlarged lumen, in almost all mitochondria crypts, on which ATP synthesis actually takes place, were not structurally expressed (Fig. 7 A-B).

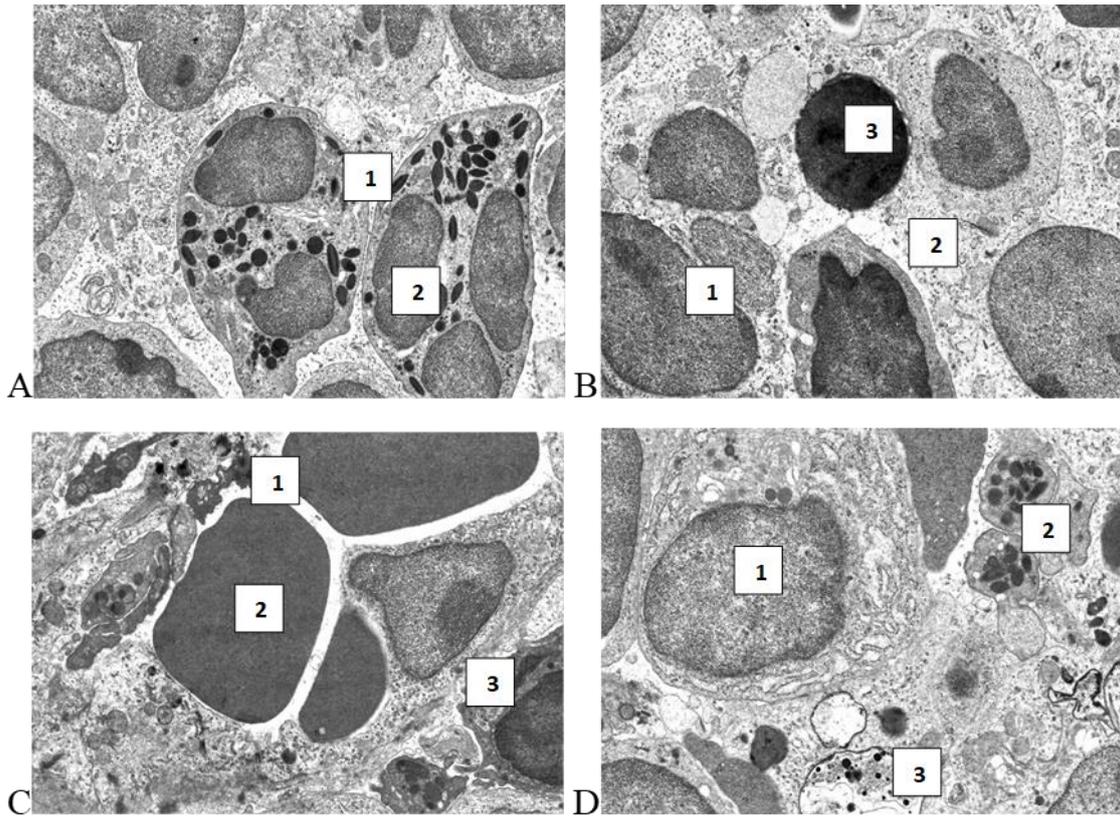


Fig. 6. Electronic microphotography. Spleen of mice with dysbiosis infected with Coxsackie B and *Salmonella typhimurium* after using Symbiogel. A. Eosinophils (1) surrounded by lymphocytes (2). Magnification 8400 ×. B. Lymphocytes (1), cellular detritus (2) and apoptotic body (3). Magnification 8400 ×. C. Electronic microphotography. The spleen of mice under the conditions of sorbents. Vessel lumen (1), erythrocytes (2), endotheliocytes (3). Magnification 11200 ×. D. Plasmacytes (1), platelets (2), macrophage residues (3). Magnification 11200 ×

Slightly expanded zones of the Disse space were found (Fig. 7 D). However, in comparison with animals that did not receive sorbents, after the use of "Symbiogel" no necrotic changes were recorded, there was no cytolysis of hepatocytes, mass death of organelles, in particular mitochondria, which in control group were compacted and subjected to lysis, as well as destructive changes in the tubules of the granular and agranular endoplasmic reticulum and the loss of ribosomes. In general, the structural organization of hepatocytes and blood vessels was preserved.

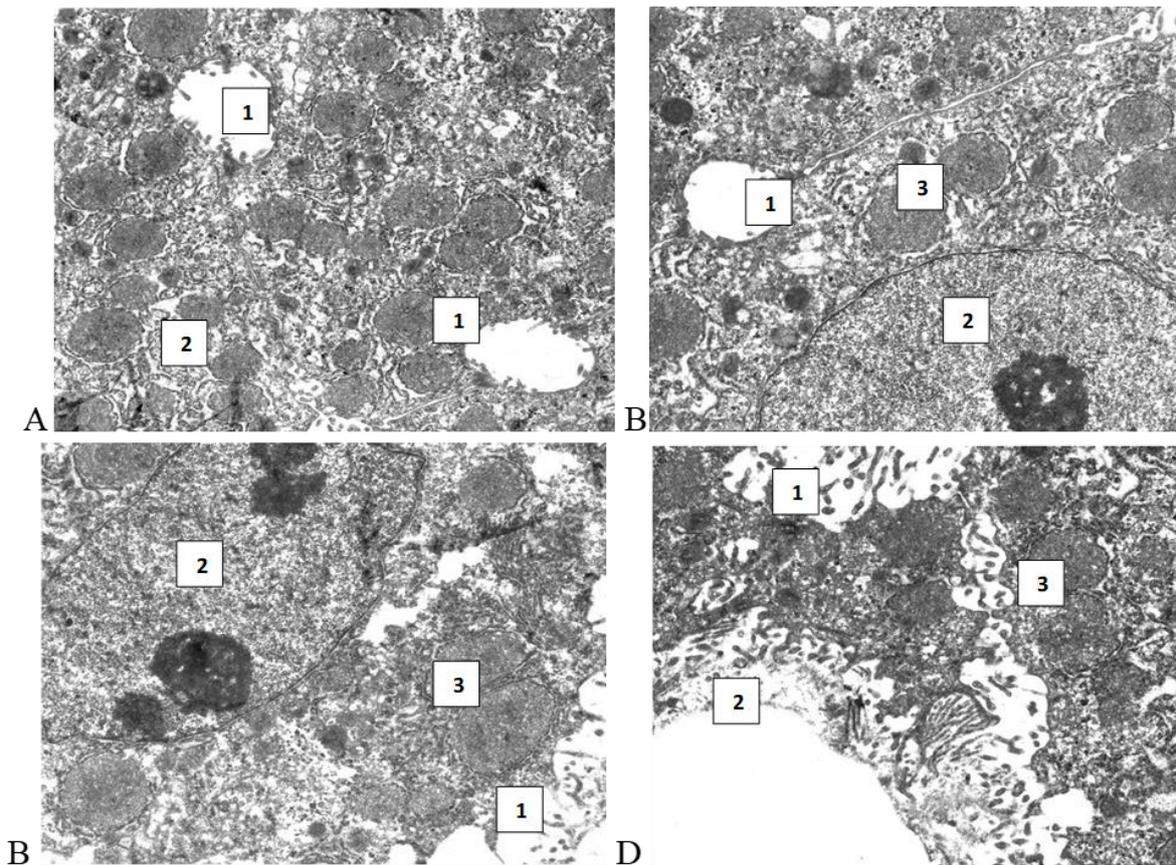


Fig. 7. Electronic microphotography. Liver of mice with dysbiosis infected with Coxsackie B and *Salmonella typhimurium* after using Symbiogel.

A - Lumen of bile capillaries (1), contact of hepatocytes (2), mitochondria (3). Magnification 14000 ×. Lumen of bile capillaries (1), hepatocyte nucleus (2), mitochondria (3). B - Magnification 14000 ×. C - Magnification of 14000 ×. D -Disse space (1), capillary lumen (2), hepatocyte mitochondria (3). Magnification 16000 ×

Conclusion. Despite the fact that the use of Symbiogel in the process of modelling dysbiotic conditions in mice had little effect on microbiological parameters of dysbiosis, experiments allowed to establish a pronounced hepatoprotective function in these drugs, which is manifested by a decrease in destructive changes accompanying the process of modelling viral and bacterial infections. Obviously, enterosorbents promote the extraction, fixation and removal from the gastrointestinal tract the bacterial toxins, products of natural metabolism in high concentrations, activated enzymes, inflammatory mediators, biologically active substances, opportunistic pathogens, viruses and etc.

In general, the results of the presented studies showed the ability of probiotic drugs and sorbents to reduce the depth of cytodestructive disorders in the simulation of viral and

bacterial infections on the background of dysbiosis and normalization of immune responses that accompany the development of such processes.

References

1. Shirobokov V. P., Yankovskiy D. S., Dyiment G. S. Microbes in biogeochemical processes, evolution of the biosphere and the existence of mankind (2014).. Kyiv: Veres O.I.; 464 p..
2. Kravchuk R.I., Shejbak V.M., Zhmakin A.I., Goreckij M.V., Egorov A.S. (2007). Characteristics of ultrastructural changes in the mucosa of the jejunum after acetaminophen-induced dysbacteriosis in rats. *Journal of the Grodno State Medical University*, (1), 106-109.
3. Johnson C.D., Kudsk K.A. (1999). Nutritional and intestinal mucosal immunity. *Clin. Nutr.*, 18 (6), 337-344.
4. Bobyr V.V., Ponyatovsky V.A., Djugikowa E.M., Shyrobokov V.P. (2015). Modeling of dysbiotic disorders with laboratory animals. *Biomedical and Biosocial Anthropology*, 26, 230-233.
5. Yevtushenko O.I. Enteroviruses in pathology of the fetus and newborn : Dissertation for the degree of Doctor of Medicine : 03.00.06. Bogomolets National Medical University, Kyiv, 2007. 302 p.