FEATURES OF THE TRANSLOCATION CAPACITY OF BACTERIA FROM THE LARGE INTESTINE TO INTERNAL ORGANS AT DIFFERENT TIMES AFTER TIMECTOMY

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Abstract. *The immune system plays an important role in the formation of normal microflora in human biotopes. Cells of this system participate in the regulation of normal microflora activity, the quantitative composition of the microbiocenosis of human biotopes, and dosed antigenic stimulation using transient bacteraemia. Transient bacteraemia leads to constant antigenic stimulation of the immune system, ensuring its normal functioning. However, when the immune system's regulation of the quantitative and qualitative composition of the normal microflora of the large intestine is disrupted, there is an increase in the translocation of microorganisms from the intestine, which is called the 'bacterial translocation phenomenon,' leading to the appearance of signs of endogenous intoxication.*

Keywords: *thymectomy, microorganisms, translocation ability of microorganisms*

TIMEKTOMIYADAN KEYINGI TURLI MUDDATLARDA BAKTERIYALARNING YO'G'ON ICHAKDAN ICHKI ORGANLARGA TRANSLOKATSIYA QILISH QOBILIYATINING XUSUSIYATLARI

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Annotatsiya. *Immun tizimi inson biotoplarida normal mikrofloraning shakllanishida muhim rol o'ynaydi. Uning hujayralari normal mikrofloraning faolligini, inson biotoplari mikrobiotsenozining miqdoriy tarkibini va vaqtinchalik bakteremiyaning nazorat ostidagi antigenik stimulyatsiyasini tartibga solishda ishtirok etadi. Vaqtinchalik bakteremiya immun tizimining doimiy antigenik stimulyatsiyasiga olib keladi va uning normal ishlashini ta'minlaydi. Biroq, immun tizimining yo'g'on ichakdagi normal mikrofloraning miqdoriy va sifat tarkibini tartibga solish buzilishida mikroorganizmlarning ichakdan translokatsiyasi kuchayadi, bu bakterial translokatsiya deb nomlanuvchi hodisa bo'lib, endogen intoksikatsiya belgilariga olib keladi.*

Kalit so'zlar: *timektomiya, mikroorganizmlar, mikroorganizmlarning translokatsiya qobiliyati*

ОСОБЕННОСТИ ТРАНСЛОКАЦИОННОЙ СПОСОБНОСТИ БАКТЕРИЙ ИЗ ТОЛСТОГО КИШЕЧНИКА ВО ВНУТРЕННИЕ ОРГАНЫ В РАЗЛИЧНЫЕ СРОКИ ПОСЛЕ ТИМЭКТОМИИ

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Аннотация. *При формировании нормальной микрофлоры биотопов человека важное место занимает иммунная система, клетки данной системы участвуют в регуляции деятельности нормальной микрофлоры, количественного состава микробиоценоза биотопов человека, а также дозированной антигенной стимуляции с помощью транзиторной бактериемии. «Транзиторная бактериемия» приводит к постоянной антигенной стимуляции иммунной системы, обеспечивая нормальное функционирование деятельности иммунной системы. Но при нарушении регуляции иммунной системой количественного и качественного состава нормальной микрофлоры толстого кишечника происходит увеличение транслокации микроорганизмов из кишечника, которая называется «феноменом бактериальной транслокации», что приводит к появлению признаков эндогенной интоксикации*

Ключевые слова: *тимэктомия, микроорганизмы, транслокационная способность микроорганизмов*

It is known that macroorganisms and microorganisms in their various biotopes are in constant interaction and symbiosis. This interaction, as well as the quantitative and qualitative composition of the microflora of human biotopes, is regulated by the body's immune system [2;3]. Changes in the body's immune system lead to an imbalance of indigenous and facultative microorganisms in various human biotopes, including the large intestine, which manifests itself in the form of dysbiosis (dysbacteriosis) of the large intestine.

In addition, this condition leads to a disruption in the constant antigenic stimulation of the immune system, which is achieved by 'transient bacteraemia' (the entry of normal microflora into the bloodstream through the intestinal mucosa). 'Transient bacteraemia' leads to constant antigenic stimulation of the immune system, ensuring the normal functioning of the immune system. However, when the immune system's regulation of the quantitative and qualitative composition of the normal microflora of the large intestine is disrupted, there is an increase

in the translocation of microorganisms from the intestine, which is called the 'bacterial translocation phenomenon,' leading to the appearance of signs of endogenous intoxication [6;9].

Although it is known that 'transient bacteraemia' and 'bacterial translocation' are closely related to the activity of the body's immune system, and moreover, these processes are regulated by the immune system, the role of the thymus in these processes remains unknown.

Materials and methods. To study the translocation ability of microorganisms from the colon to internal organs at various times after thymectomy, experimental studies were conducted on laboratory animals (white mongrel rats). The study involved 135 white non-pedigree rats, 3 months old, weighing 160-180 g, which were divided into the following groups. The main group consisted of white non-pedigree rats that underwent thymectomy, and after 1, 3, and 6 months, the translocation ability of microorganisms from the large intestine to internal organs at various times after thymectomy was studied (n=60). The comparison group consisted of white non-pedigree rats that did not undergo surgery but had their thymus removed ('falsely-operated') and were studied after 1, 3, and 6 months for the translocation ability of microorganisms from the large intestine to internal organs (n=60). Control group – intact white mongrel rats (n=15).

The main and comparative groups, in turn, were divided into three subgroups. The results of studying the translocation ability of microorganisms from the colon to internal organs after one month (O1), three months (O2) and six months (O3) after thymectomy, one month (C1), three months (C2) and six months (C3) after a 'falsely operation'.

Results. The results showed that in intact animals, no growth of translocated microorganisms was detected in the liver and spleen – the percentage of microbial isolation (PMI) was 0%. In addition, no microorganisms were identified in the peripheral blood of white mongrel rats, i.e., in this case, the PMI was also 0%. The same results were obtained in the group of falsely - operated rats at all study time points – 1, 3, and 6 months after the falsely operation (PMI was 0%). This means that surgical intervention without thymus removal had virtually no effect on the phenomenon of bacterial translocation, as was the case in intact animals.

Different results were obtained in thymectomised experimental animals. One month after thymus removal, microorganisms that had translocated from the gastrointestinal tract were identified in the liver of the animals, accounting for 100% (20 out of 20 animals). The same results were obtained when studying the spleen of rats—PMI was 100% (20 out of 20 animals). It is interesting to note that the same results were obtained when culturing the peripheral blood of animals, with the trend and intensity of changes being the same as in bacteriological studies of the liver and spleen - PMI was 100%. This fact indicates that after thymectomy, the regulatory function of the immune system is disrupted, which led to an increase in the translocation of bacteria from the gastrointestinal tract to the internal organs in the experiment.

The same studies were conducted with a group of white mongrel animals three months after thymectomy (n=20). The results showed that the trend of changes remained the same, i.e., a high degree of microbial colonisation was observed in the liver, spleen, and peripheral blood, but the intensity of the changes differed slightly from the data obtained one month after thymectomy. In falsely-operated animals, no microbial growth was observed 3 months after surgery, and the PMI was 0% in all cases (20 out of 20 animals). However, 3 months after thymectomy, the result was positive, i.e., translocated microorganisms were identified in the liver, spleen, and blood. While the PMI in the liver was 100% (20 out of 20 animals), in the spleen and blood this result was 90.0±6.7% (n=18) in each case. The results prove that even after 3 months, increased bacterial translocation continues, reaching its maximum due to disturbances in the regulatory function of the immune system - thymectomy. Although a slight decrease in the intensity of bacterial translocation was observed during this period after thymectomy.

Similar studies were conducted six months after thymectomy in laboratory animals. In intact and falsely-operated animals, no microbial growth was detected in the liver, spleen, or peripheral blood during this study period, i.e., the PMI was 0% (0 out of 15 and 20 animals). However, the parameters of the animals six months after thymectomy differed sharply from those of the control group (intact) and the comparison group (falsely -operated). In the liver of these animals, the PMI was 85.0±7.9% (n=17), and in the spleen, this parameter was 60.0±10.9% (n=12). The decrease in PMI continued in the blood of the animals, reaching 50.0±11.2% (n=10). The different percentages of these microorganisms being found is explained by the proximity of the organ to the intestine, where translocation occurs, the massiveness of the entry and the species composition of the microorganisms, since the translocation ability of microorganisms (gram-positive cocci and gram-negative bacteria) varies and differs from one another.

A comparative analysis of PMI in thymectomised animals at different study time points shows that PMI decreases in the organs of the studied animals as the time since thymectomy increases. A gradual decrease in PMI is observed depending on the time since thymectomy (Fig.1).

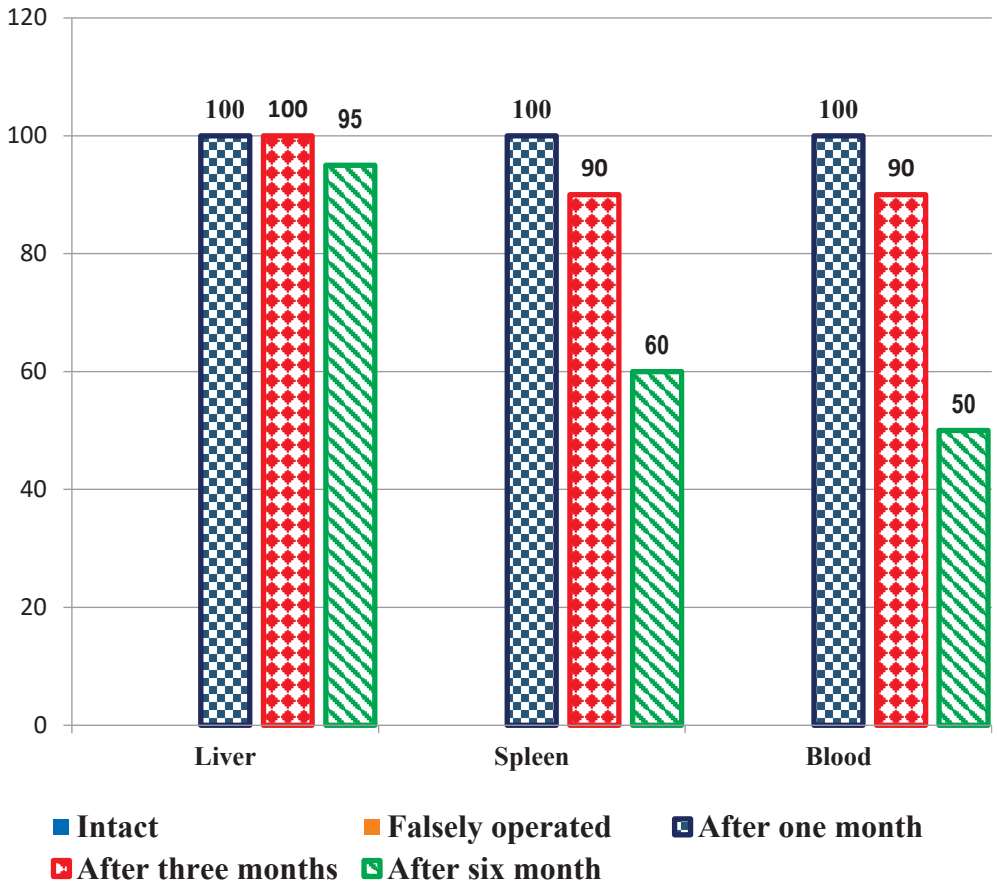


Fig. 1. **Comparative indicators of PMI in intact, falsely-operated, and thymectomised white mongrel rats at different study time points, % (no growth in intact and falsely-operated rats – 0%)**

Thus, the study and evaluation of PMI from internal organs (liver, spleen) and biological fluids (peripheral blood) of white mongrel rats at different times after thymectomy showed that after thymectomy, 100% of microorganisms were detected in the liver, spleen and blood of animals, whereas in intact and falsely-operated animals, the PMI was 0%. This fact indicates that the thymus, being the central organ of the immune system, is directly involved in the regulation of the immune system, in particular the local immunity of the mucous membranes. After thymectomy, there was a sharp increase in the number of microorganisms that translocated from the intestine to the internal organs of the animals.

The results obtained prove the involvement of the thymus in regulating the quantitative and qualitative composition of the normal microflora of the large intestine, resulting not only in dysbiotic processes in the intestine, but also in increased passage of microorganisms capable of translocation to the internal organs of animals in the experiment. The decrease in the translocation activity of microorganisms, which was expressed in a decrease in PMI from the liver, spleen, and blood 3 and 6 months after thymectomy, is explained by the gradual restoration of the regulatory function of the immune system.

Further research is devoted to deciphering microbiological studies, i.e., determining the generic composition of isolated microorganisms and their occurrence in the form of monocultures and associations of microorganisms. This is an important point, since determining the generic composition allows us to establish the degree of translocation of representatives of the normal intestinal microflora, as well as the degree of contamination of the organs under study and peripheral blood in the experiment. Determining these facts allows us to establish the degree of influence of thymectomy on individual genera of microorganisms and the massiveness of translocating microorganisms.

Studies have shown that bacteriological methods have identified five genera of microorganisms, including two Gram-positive cocci (*Enterococcus spp, Staphylococcus spp*) and three Gram-negative bacteria (*Escherichia coli, Enterobacter spp, Proteus spp*). The genus Escherichia includes only one species, so it is presented here as a species.

The results showed that three months after thymectomy, the percentage of microorganism monoculture cultureability increased slightly, reaching 25.0±9.7% in the liver (a 1.25-fold increase compared to the data after one month), in the spleen to 44.4±11.7% (a 1.27-fold increase), and in the blood to 27.8±10.6% (a 1.08-fold decrease). However, all parameters were not statistically significant (P>0.05).

However, after 6 months, the sowability of microorganisms increased, and in all organs studied, the results differed significantly by 4.12, 2.62 and 3.0 times, respectively, compared to the data one month after thymectomy (P<0.05), reaching 82.4±9.2% in the liver, 91.7±7.9% in the spleen, and 90.0±9.5% in the blood. This fact clearly shows an increase in the percentage of microbial monoculture isolation depending on the timing of thymectomy.

Analysis of the results showed that as the period after thymectomy increases, the percentage of detection of microorganisms from the internal organs and blood of laboratory animals decreases.

Thus, three months after thymectomy, there was a slight decrease in the percentage of bacteria isolated from the liver (to 75.0±9.71%) and spleen (to 55.6±11.7%) and a slight increase in blood (to 72.2±10.6%). It should be noted that all the parameters obtained did not differ significantly from each other (P>0.05).

However, six months after thymectomy, we observed a different picture, with all indicators differing significantly from each other. The percentage of microbial association spread decreased 4.55 times in the liver (from 80.0±8.9% to 17.6±9.2%, P<0.001), from the spleen by 7.83 times (from 65.0±10.7% to 8.3±7.9%, P<0.001), and from the blood by 7.0 times (from 70.0±10.2% to 10.0±9.5%, P<0.001).

Thus, a comparative study of the percentage of monoculture and microorganism association from internal organs (liver, spleen) and biological fluid (blood) showed that with an increase in the observation period after thymectomy, the studied parameters changed in different directions, the monoculture of microorganisms tended to increase, reaching a maximum 6 months after thymectomy (P<0.05), while the association of microorganisms tended to increase (P<0.001), with the intensity of changes being greater when determining the percentage of microbial association isolation (Table 1).

Table 1.

Comparative parameters of sowability of monoculture and association of microorganisms from laboratory animal organs depending on the timing of thymectomy

Terms	Monoculture			Microbial associations		
	Liver	Spleen	Blood	Liver	Spleen	Blood
After one month	4	7	6	16	13	14
	20,0±8,9	35,0±10,7	30,0±10,2	80,0±8,9* ↑	65,0±10,7*↑	70,0±10,2* ↑
After three months	5	8	5	15	10	13
	25,0±9,7	44,4±11,7	27,8±10,6	75,0±9,71* ↑	55,6±11,7 ↔	72,2±10,6* ↑
After six months	14	11	9	3	1	1
	82,4±9,2	91,7±7,9	90,0±9,5	17,6±9,2* ↓	8,3±7,9* ↓	10,0±9,5* ↓

*Note: absolute values in the numerator, relative (%) values in the denominator; * - sign of significance in relation to a monoculture of microorganisms; ↓, ↑ - direction of change; ↔ - no significant difference.*

It was established that one month after thymectomy, the inoculation rate of the association of microorganisms was 4.0 times higher than the inoculation rate of monoculture strains (P<0.001). Practically the same results were obtained for the spleen and peripheral blood of animals, with an increase of 1.9 and 2.3 times, respectively (P<0.001). It can be seen that the trend of changes was the same, but the intensity of the changes differed.

However, three months after thymectomy, although the trend of changes persisted, the intensity of differences between the compared characteristics decreased. The differences between monoculture and microial associations were 3.0 times (P<0.01) in the liver, 1.25 times (P>0.05) in the spleen, and 2.6 times (P<0.001) in the blood, in favour of microorganism association.

Six months after thymectomy, we observed a different picture: we noted a decrease in the sowing of microorganisms from the studied objects, as well as a decrease in the proportion of associations and an increase in the values of microorganism monocultures. Thus, microorganisms isolated from the liver as monocultures were 4.7 times more numerous than those isolated as associations (P<0.001). Practically the same data were obtained for the spleen (11.0 times more monoculture, P<0.001) and peripheral blood (9.0 times more monoculture, P<0.001).

The next stage of the research involved studying the sowability of individual genera of microorganisms from the internal organs and blood of laboratory animals in an experiment.

The results showed that Gram-negative bacteria have a greater translocation capacity than Gram-positive cocci, which is manifested by a higher percentage of isolation in the former (P<0.001).

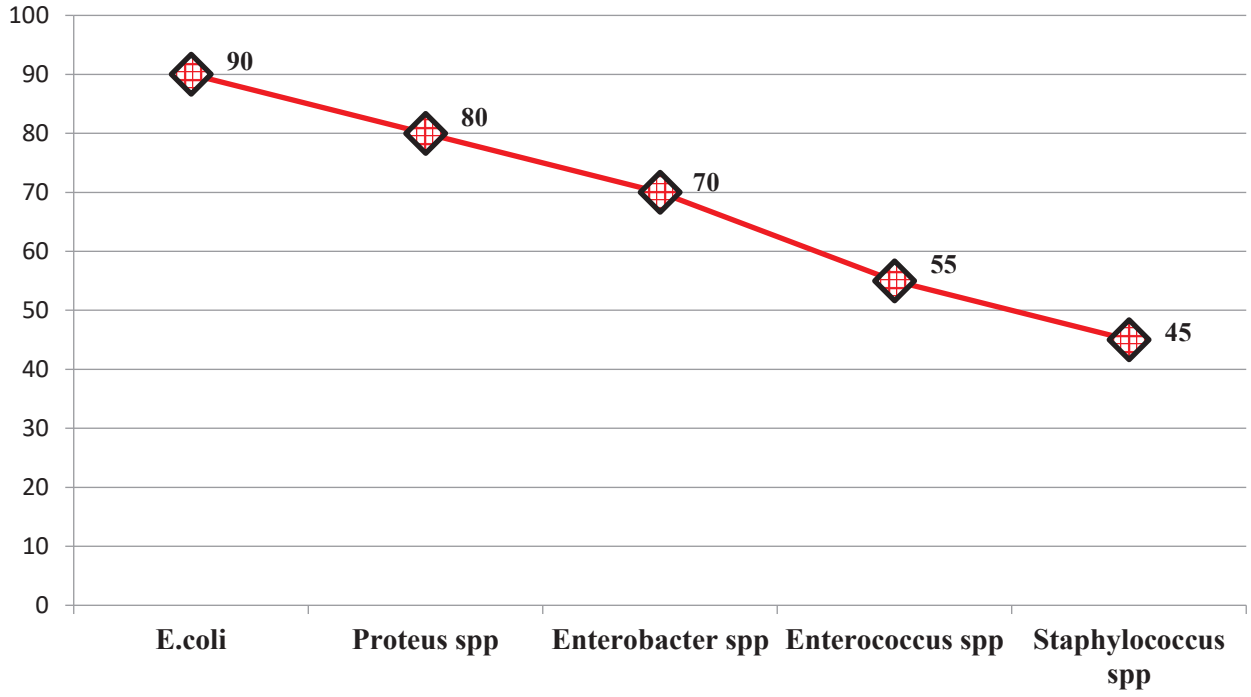


Fig. 2. **Parameters of microorganism sowability from animal organs 1 month after thymectomy, %**

If we look at the figures, we see confirmation of the above, as *Escherichia coli* was highly viable one month after thymectomy (90.0±9.5%), other representatives of Gram-negative bacteria *Enterobacter spp* and *Proteus spp* were identified in 70.0±10.2% (n=14) and 80.0±8.9% (n=16), respectively, and Gram-positive cocci – *Enterococcus spp* and *Staphylococcus spp* were isolated in 55.0±11.1% (n=11) and 45.0±11.1% (n=9) of cases, respectively – Fig. 2.

It was established that Gram-positive cocci (*Enterococcus spp, Staphylococcus spp*) were significantly less prevalent than Gram-negative bacteria (*Escherichia coli, Proteus spp, Enterobacter spp*) - P<0.001. This indicates that the translocation ability of Gram-negative bacteria is higher than that of Gram-positive cocci.

Conclusion. The results obtained suggest that the immune system regulates not only the translocation of microorganisms from the gastrointestinal tract to extraintestinal organs, but also the mass of translocating microorganisms. A comparative study of the percentage of monoculture and microbial associations from the liver, spleen, and blood showed that with an increase in the observation period after thymectomy, the studied parameters changed in different directions, monoculture microorganisms tended to increase, reaching a maximum 6 months after thymectomy, and microbial associations tended to increase, with the intensity of changes being greater when determining the percentage of microbial association isolation. It was established that the translocation ability of Gram-negative bacteria is greater than that of Gram-positive cocci, which is manifested by a higher percentage of isolation in the former. Gram-positive cocci (*Enterococcus spp, Staphylococcus spp*) were significantly less isolated than Gram-negative bacteria (*Escherichia coli, Proteus spp, Enterobacter spp*). This indicates that the translocation ability of Gram-negative bacteria is higher than that of Gram-positive cocci.

Thus, thymectomy performed on white non-pedigree rats in the experiment has a significant effect on the phenomenon of bacterial translocation, which contributes to an increase in the translocation ability of microorganisms from the intestine to the extraintestinal organs of animals, and an increase in the mass transition of representatives of the normal intestinal microflora through the mucous membranes into the internal organs. This fact indicates the involvement of the immune system and thymus in regulating the quantitative and qualitative composition of the normal microflora of the large intestine and the bacterial translocation of these microorganisms in laboratory animals in the experiment.

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