

## **Lymph flow and biochemical composition of lymph in case of experimental peritonitis**

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*The article studies lymph flow and lymph composition in case of acute peritonitis, caused by fecal injection. The experiments show that acute peritonitis causes decrease in lymph flow, shifts in biochemical composition and physical-chemical indexes as well as electrolyte lymph exchange, blood plasma and urine. Microbial associations that consist of colibacillus, staphylococcus, streptococcus, anaerobic microorganisms that cause acute peritonitis and toxic infection, were revealed in peritoneal liquid.*

**Key words:** peritonitis, lymph flow, lymph, ions, ALT, AST.

In medicine peritonitis is an important general-pathological problem, urgency of which does not decrease nowadays. The most frequent cause of peritonitis is perforation in hollow organ of gastrointestinal tract in result of which gastric or bowel contents and microflora, in other words, microorganisms and bacterias that exist in clear space of gastrointestinal tract, is injected into stomach cavity [1].

The part of lymphatic system in pathologies of internal organs and systems of organism is well-known as well as its huge part in supporting constants of internal environment [2]. All links of lymphatic chain take part in these processes – capillaries, vessels, lymphatic nodes. Our previous researches studied condition of lymphatic system in case of such pathologies in stomach cavity as toxic hepatitis, sugar diabetes, acute and chronic pancreatitis, these researches show the part of lymphatic system in development of pathological and adaptive processes [3, 4, 5]. It has been established that natural way of cleansing the inflammation point are regional lymphatic capillaries, vessels, and lymphatic nodes [6]. Studying lymphatic mechanisms of tissue detoxication, transportation of interstitial liquid, and metabolites is defined by modern authors as a key problem of fighting toxic infections of various genesis [7, 8, 9].

However, the data on lymphatic system condition at the background of peritonitis in clinic or experiment is far not sufficient, a short list of works was revealed. Considering the important part of lymphatic system in tissue drainage, metabolism, water-salt exchange, and its protective-compensatory and immune function, investigating part of lymphatic system within peritonitis development, processes of lymph formation in case of experimental peritonitis proves to bear theoretical and practical interest.

The objective of this research is to study and receive experimental model of peritonitis as well as lymph flow, cellular composition, biochemical and physical-chemical rheological characteristics of lymph, blood, and urine in case of experimental peritonitis.

**Methods and materials.** The tests were held upon 55 white laboratory male rats with body mass 220-250g. Two groups of rats were formed: group 1 – control, 15 rats; group 3 – with acute peritonitis, 40 rats. We selected method of modeling fecal peritonitis that is similar to ethiopathogenesis, clinical displays, and phase nature of flow to such of human disease. Acute peritonitis amongst rats was caused by introducing fecal suspension into stomach cavity in calculation 0,5ml of 10% solution per 100g of animal body mass [10]. In our experiments animals were taken to examination in 44-48 hours after fecal injection. Narcotization of animals was done with ether via inhaling method through cotton mask, dumped in ether. After narcotization cut was made along white line of stomach muscle, then chest lymphatic channel was prepared near diaphragm, and microcannula was introduced into it. After collection of lymph stomach aorta was prepares in caudal part of stomach cavity, teflon catheter was introduced into it for blood collection. Urine was obtained from bladder.

Acute experiments were taken with implementation of basic principles of Helsinki convention on humane attitude towards laboratory animals. In two days after modeling of peritonitis lymph flow was registered from chest lymphatic channel, samples of lymph, blood, and urine were collected for the future research among rats in their life time. Samples of blood and lymph were studied to define level of general amylase, glucose, contents of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), bilirubin, thymol sample, general protein, urea, creatine with automatized biochemical analyzer COBOS INTEGRA 400. Physical-mechanical indicators of blood and lymph – coagulability according to Sukharev, as well as viscosity with viscosimeter VK-4, hematocrit – according to general methodic were established. Electrolytes were defined in blood, lymph, and urine of animals with analyzer ABL 615/625, produced by Radiometer. Arterial pressure was defined with tension sensors of monitor Dreger.

Analysis of peritoneal liquid microflora and identification of microorganisms, as well as definition of their resistance against medical preparation was done at automatized Bacteriological analyzed “MINI API”, produced by BIO MERIEUX that has expert system, constructed upon international standards (NCCLS). Thermometry of animals was carried out with digital thermometer, produced by “Omron”. The received material was processed with computer via variative-statistical method with criterion of Student.

**Research results and discussion.** Under modeling of acute peritonitis within the experiment death rate equaled 48% of total number of animals within 48 hours after intoxication. During the following days rate of lethality increased and by day-5 equaled 57%. The research was held in 44-48

hours after introduction of fecal mixture (toxic stage of peritonitis), when accumulation of large amount of liquid was observed in stomach cavity. Temperature of animals grew up to  $40,6 \pm 1,2^{\circ} \text{C}$  (control -  $38,5 \pm 0,4^{\circ} \text{C}$ ). Clinical picture of acute diffused peritonitis displayed in 10-12 hours among the rats and continued to develop until the moment of surgical intervention by hours 45-48. It can be characterized by rejection of food and water, unsteady walk, hyperthermia, tachycardia and polypnea, ruffled fur, and low mobility.

During the opening of stomach cavity within the experiment we revealed a great amount of peritoneal liquid, and upon liver, bowels, and stomach walls – pus inflammations. The following microorganisms were revealed in peritoneal liquid:

<i>Proteus vulgaris group</i>	$10^6$ CFU/ml
<i>Escherichia coli</i>	$10^6$ CFU/ml
<i>Enterococcus faecalis</i>	$10^6$ CFU/ml
<i>Staphylococcus vitulinus</i>	$10^6$ CFU/ml
<i>Candida inconspicua/lambica</i>	$10^6$ CFU/ml,

but 5 microbes were registered only in 55% of all analysis. 1 or 2 microbes in different combinations were found in other samples: *Escherichia coli*  $10^3$ – $10^4$  CFU/ml and *Proteus vulgaris group*  $10^6$  CFU/ml, *Staphylococcus vitulinus*  $10^2$ – $10^3$  CFU/ml.

Under acute peritonitis lymph flow decreased down to  $5,2 \pm 0,3$  ml/min 100 g (control -  $7,9 \pm 0,2$ ). General protein in lymph decreased down to  $31,6 \pm 0,2$  g/l (control -  $42,2 \pm 0,3$ ). Changes in advantage to acute peritonitis are also proved by biochemical indexes of lymph. We revealed increase in  $\alpha$ -amylase activity in lymph up to  $980 \pm 76$  units, for the control group it equaled  $410 \pm 32$  units. ( $p < 0,01$ ).

Increase in lymph activity was registered among all rats ALT  $0,59 \pm 0,09$  and AST  $0,55 \pm 0,11$  mckat/l in comparison to control group ( $0,15$ – $0,19$  mckat/l). These ferments describe functions of liver and pancreas, therefore, the observation can be considered as a display of cytolytic syndrome. Index of thymol sample was within the limits of norm and equaled 4 units, but still was much higher than the same index of control rats (0-1 units) (table 1).

We have underlined that increase in concentration of glucose in lymph of the tested rats was much greater than in blood plasma, and equaled  $10,4 \pm 2,2$  mmole/l (control -  $4,4 \pm 1,5$  mmole/l) (table 1). A significant decrease in general protein in lymph and growth in bilirubin in blood plasm of the tested rats up to  $8,70 \pm 0,85$  mcole/l in comparison to the control group of animals ( $3,32 \pm 0,94$  mcmole/l). Also, glucose concentration in blood increased up to  $8,4 \pm 1,25$  mmole/l (control group -  $4,6 \pm 0,8$  mmole/l), as well as glucose concentration in lymph.

Plasma volume according to hematocrit decreased among the tested rats and equaled an average of  $44,0 \pm 3,0$ . Blood coagulability within the tested group was registered within  $2,91 \pm 0,2$

minutes, in control tests -  $3,50 \pm 0,2$  minutes. Lymph coagulability equaled  $3,21 \pm 0,1$  minutes, in control group -  $3,83 \pm 0,3$  minutes. Blood viscosity under acute peritonitis increase from  $5,00 \pm 0,5$  to  $6,05 \pm 0,21$  ( $P < 0,05^*$ ), and lymph – from  $3,9 \pm 0,3$  to  $5,24 \pm 0,4$  ( $P < 0,05^*$ ) ps.

Examination of blood has showed that in case of peritonitis number of erythrocytes and leucocytes increased by 13% and 79% among the rats (control -  $7,3 \times 10^6 / \mu\text{L}$  and  $4,9 \times 10^3 / \mu\text{L}$ ) correspondingly. Number of lymphocytes grew as well  $2,8 \pm 0,40 \times 10^3 / \mu\text{L}$  (control -  $2,6 \pm 0,2 \times 10^3 / \mu\text{L}$ ). Number of thrombocytes in blood increases  $581,3 \pm 14 \times 10^3 / \mu\text{L}$  (control -  $405 \pm 14 \times 10^3 / \mu\text{L}$ ). Under peritonitis leucocytes in lymph grew by 24%, and lymphocytes – by 20% (control -  $13,6 \pm 0,3 \times 10^3 / \mu\text{L}$  and  $82,1 \pm 0,8 \times 10^3 / \mu\text{L}$ ).

Table 1 – Biochemical indexes of lymph in case of experimental acute peritonitis

Index	Control group	Tested group
Glucose, mmole/l	$4,4 \pm 1,5$	$10,4 \pm 2,2^*$
General protein, g/l	$42,2 \pm 0,3$	$31,6 \pm 0,3^*$
$\alpha$ -amylase, units/l	$410 \pm 32$	$980 \pm 76^{**}$
Lymph ALT, mckat/l	$0,15 \pm 0,07$	$0,59 \pm 0,09^{**}$
Lymph AST, mckat/l	$0,19 \pm 0,06$	$0,55 \pm 0,11^{**}$
Notion: reliable in comparison to control, * - $p < 0,05$ , ** - $p < 0,01$		

Among experimental animals in lymph and urine concentration of  $\text{Na}^+$  ions increased by 10% (in control concentration of  $\text{Na}^+$  ions equaled  $138 \pm 4,1$  Mm/l in lymph and  $17,7 \pm 1,5$  Mm/l in urine), and in blood it decreased by 22%. In lymph, blood serum, and urine  $\text{K}^+$  ions grew insignificantly, and  $\text{Ca}^{+2}$  ions slightly decreased in comparison to initial data (table 2).

The results of our research show that amongst the tested rats with experimental peritonitis content of natrium, potassium, and calcium ions in blood serum decreased at the background of their increase in lymph. Thus, while amongst the control rats content of natrium in blood serum equaled  $142,0 \pm 5,3$  mmole/l, potassium contents -  $3,72 \pm 0,3$  mmole/l, calcium contents -  $0,56 \pm 0,02$  mmole/l; amongst the tested rats with experimental peritonitis these indexes in blood serum were lower by 16, 12, 27% correspondingly in comparison to control (table 2).

In comparison to the control, increase in natrium ions by 10%, potassium ions by 16%, and calcium ions by 7% was registered in lymph of the tested rats. Relative concentration of electrolytes in urine among rats with acute peritonitis in comparison to the control grows as well. However, the total amount of electrolytes discharge with urine decreases among the tested rats, as a stable and significant decrease in diuresis is registered in 92% of tests. Thus, for control group diuresis equals

1,3±0,05 mcl/min per 100g m/t, and among rats with peritonitis it equals 9,8±0,04 mcl/min per 100g m/t (p<0,05).

Table 2 – Concentration of ions in blood, lymph, and urine in case of experimental acute peritonitis

Index	Blood	Lymph	Urine
Natrium ions, mmole/l			
Control group	142,0±5,3	138,13 ±4,11	17,7±1,5
Tested group	121,9±4,48*	151,7±4,4*	19,5±2,1
Potassium ions, mmole/l			
Control group	3,72±0,3	3,57±0,20	3,25±0,10
Tested group	3,65±0,31	3,87±0,25*	3,57±0,12*
Calcium ions, mmole/l			
Control group	0,56±0,02	0,407 ±0,03	–
Tested group	0,439±0,08	0,423±0,05	0,23±0,02**
Notation: reliable in comparison to the control, *- p<0,05, **- p<0,01			

Thus, according to all indications, signs of spread fecal peritonitis are revealed among all animals, and wide inflammatory process is registered in their organism. Our experiments have established that model of peritonitis was achieved among the tested animals. Activation of blood coagulation processes takes place in stomach cavity during the inflammatory processes that obviously is a link of pathogenesis in case of acute inflammatory process [11, 12]. Biochemical indexes of blood, lymph, and urine testify for alterations in advantage of acute peritonitis and massive inflammatory process.

Investigation of peritoneal liquid exudate revealed microbial associations that consisted of colibacillus, staphilococcus, streptococcus, anaerobic microorganisms that cause acute peritonitis and infection.

The developed model of acute peritonitis allowed us to objectively evaluate general reactions of an organism, legislations of emergence and development of inflammatory process in organism and particularly lymphatic system, establishes equal response reactions in local and general regulative systems. While analyzing the received experimental material, we can observe the following image. An adequate model of acute peritonitis was received among the tested animals with conclusive foundation of clinical picture, biochemical data of blood and lymph of these animals differed significantly from the data of control rat group (general amylase, alkaline phosphatase, ALT, AST, urea, bilirubin, thymol sample, creatine, glucose, general protein, and blood cells). In case of acute

peritonitis we observed changes in rheological indexes of blood and lymph – viscosity increased, coagulability accelerated, and it degraded fluidity of blood as well as lymph.

Concentration of electrolytes in the studied biological liquids changed as well. A trend to decrease of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{+2}$  in blood and increase of the same elements in lymph and urine was registered. Probably, increase of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{+2}$  in lymph and urine is related to a decrease in lymph flow and diuresis as well as greater concentration of these elements in smaller volume or certain depositing function of lymphatic system. General analysis of blood and lymph and biochemical research of these liquids reflects certain signs of expressed inflammatory process and liver and kidney insufficiency.

Thus, development of acute peritonitis is attended by decrease in lymph flow, increase in blood and lymph viscosity, shortening in period of coagulation for these liquids, increase in number of thrombocytes leucocytes, lymphocytes, and also erythrocytes in blood, all these factors degrades fluidity of blood and lymph.

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## Summary

### **Lymph Flow and the biochemical Lymph composition in the experimental peritonitis**

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*In this article the lymph flow and composition of lymph at the acute peritonitis caused by a fecal injection is studied. It is shown in experiments, in acute peritonitis causes reduction of a lymph flow, the changes of biochemical composition and physical and chemical and electrolyte exchange of lymph, plasma of blood and urine. Revealed the microbic associations consisting of Escherichia coli, Staphylococcus, Streptococcus, anaerobic microorganisms which cause sharp peritonitis and a toxicoinfection in peritoneal fluid.*

**Keywords:** peritonitis, lymph flow, lymph, ions, ALT, AST.