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С. Ж. Асфендияров атындағы Қазақ ұлттық медицина университеті

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ИЗВЕСТИЯ

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РЕСПУБЛИКИ КАЗАХСТАН
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**LYMPH FLOW AND CELLULAR COMPOSITION, RHEOLOGICAL PROPERTIES
OF LYMPH AND BLOOD IN ANIMALS WITH EXPERIMENTAL PERITONITIS**

Abstract. This article studies the lymph flow, the composition of the lymph and blood in acute peritonitis caused by fecal injection. Experiments have shown that acute peritonitis causes a decrease in lymph flow, shifts in the physicochemical parameters of lymph, blood plasma and urine. With fecal peritonitis in animals, an increase in the viscosity of lymph and blood in 28% and 33% in relation to the norm is characteristic. The blood clotting time in rats with peritonitis was observed within 2.79 ± 0.02 min, and in the lymph, it was 3.24 ± 0.04 min. A change in the rheological parameters of lymph and blood occurs simultaneously with a violation of the coagulation system. Clotting accelerated, which worsened the fluidity of both blood and lymph. An analysis of our results also shows that a decrease in the volumetric rate of lymph flow corresponds to a change in not only biochemical, but also physical properties of lymph. The content of electrolytes in the studied biological fluids changed. There was a tendency towards a decrease in Na^+ , K^+ , Ca^{+2} in the blood and an increase in the same elements in the lymph and in the urine. ... Probably an increase in Na^+ , K^+ , Ca^{+2} in lymph and urine is associated with a decrease in lymph flow and diuresis and with a greater concentration of these elements in a smaller volume or some depositing function of the lymphatic system. In the peritoneal fluid there have been revealed microbial associations consisting of *Escherichia coli*, staphylococci, streptococci, anaerobic microorganisms, which cause acute peritonitis and toxic infection. The data obtained by us allow that experimental peritonitis was obtained in rats. In inflammatory processes in the abdominal cavity, there is a violation of lymph and hemodynamics, as well as the physicochemical parameters of lymph and blood, expressed in a change in lymph flow in the thoracic duct flow and the color of lymph. The developed model of acute peritonitis makes it possible to objectively assess the general reactions of the body, the regularities of the onset and development of the inflammatory process in the body and, in particular, in the lymphatic system, and provides unambiguous responses from both local and general regulatory systems.

Key words: viscosity, blood, lymph, lymph flow, peritonitis, coagulability.

Introduction. In medicine, peritonitis is an important general pathological problem, the relevance of which is not decreasing at the present time. Inflammation of the abdominal cavity, manifests itself as a secondary pathological process, the most common cause of peritonitis is perforation of the genital organ of the gastrointestinal tract, as a result of which gastric or intestinal contents and microflora enter the abdominal cavity, that is, microbes and bacteria that live in the lumen of the gastrointestinal tract or traumatic destruction of the abdominal organs [1, 2].

The main causes of peritonitis are the progression of the syndrome of endogenous intoxication and the generalization of infection, leading to the decompensation of vital organs and systems [3, 4]. The main reason is significant endogenous intoxication, the main source of which is the exudate of the abdominal cavity with microbes and their exo- and endotoxins, the contents of the paretic intestine, enzymes formed during the death of cells, tissues, leukocytes and microbes themselves, even after the source of peritonitis has been eliminated [5, 6].

Massive endogenous toxemia leads to the development of systemic inflammation or systemic inflammatory response syndrome with active involvement of the immune system [7, 8]. Widespread purulent peritonitis is accompanied by excessive intake of microbial antigens and bacterial toxins from the purulent-destructive focus of the abdominal cavity, peritoneal exudate and paralytic intestine into the biological environment [9, 10].

The role of the lymphatic system in the pathology of internal organs and body systems is known; the authors found that the natural way to cleanse the focus of inflammation is regional lymphatic capillaries, vessels and lymph nodes, as well as its huge role in maintaining the constants of the internal environment [11, 12].

Modern authors define the study of the lymphatic mechanisms of detoxification of the body and metabolites as a key pathogenetic issue in the development of enteral insufficiency and the fight against toxic infection of various genesis [13, 14, 15].

However, the mechanism of the development of intestinal poly-dysfunction and the state of the lymphatic system against the background of peritonitis, in particular, the disorders of the body in the clinic or in the experiment are far from complete, there are single works on these studies. Taking into account the important role of the lymphatic system in tissue drainage and water-salt metabolism, it is of theoretical and practical interest to study the role of the lymphatic system in the development of peritonitis, lymph formation processes in experimental peritonitis. The purpose of this study is to research lymph flow, cellular composition and rheological parameters of lymph, blood in experimental peritonitis.

Material and research methods. The experiments were carried out on 35 white laboratory male rats weighing 250 ± 5 g. 2 groups of rats were formed, the 1st group - 15 control rats, the 2nd group with acute peritonitis (20 rats). We have chosen a method for modeling fecal peritonitis, which is close in etiopathogenesis, clinical manifestation and phase flow to that in humans. Acute peritonitis in rats was caused by introducing fecal suspension into the abdominal cavity at the rate of 0.5 ml of 10% solution per 100 g of animal body weight [16]. In our experiments, animals were taken for research 44-48 hours after fecal injection. The resulting suspension was injected into intact animals by the paracentetic method no later than 20 minutes after preparation. In order to avoid damage to internal organs during the introduction of fecal suspension into the abdominal cavity, the animals were placed vertically, with the caudal end up. By puncturing the ventral wall in the center along the midline of the abdomen, directing the end of the needle alternately into the right and left hypochondria, then into the right and left iliac regions, the required amount of suspension was injected.

All groups of animals were in the same conditions of feeding and keeping. All experiments with animals were carried out in strict accordance with the rules developed and approved by the local ethical commission of Kazakh National Medical University named after S.D. Asfendiyarov, as well as in accordance with the rules and requirements stipulated by the 1986 directive of the European Parliament and set out in the «Guidelines for the care and use of laboratory animals.» Drug addiction of animals was carried out by inhalation with ether through a mask in which a cotton wool with ether was placed. After drug addiction, an incision was made along the white line of the abdominal muscles, and then the thoracic lymphatic duct was dissected at the diaphragm into which a microcannula was inserted. After modeling of peritonitis in rats under ether anesthesia, lymph flow from the thoracic lymphatic duct was recorded in vivo. Lymph was collected from rats using a graduated microcannula. In the caudal part of the abdominal cavity, after collecting lymph, the abdominal aorta was dissected, and a Teflon catheter was inserted into it to collect blood. Urine samples were taken for research from the bladder.

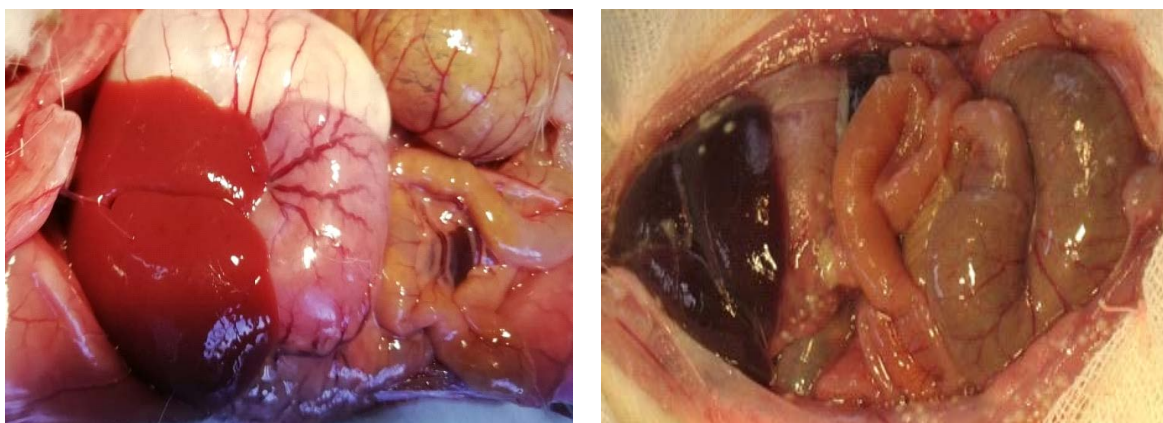
We studied the biochemical parameters of blood and lymph, glucose level using “Glucotrend-2” using test strips; the content of alanine aminotransferase (ALT) and aspartate aminotransferase (ASAT) was determined by the Reitman-Frenkel method, bilirubin was determined by the Yendrashik-Gough method, the thymol test - with thymol-veronal buffer, the total protein was determined by the birueta method, urea by a unified method according to the color reaction with diacetylmonooxime, creatinine - according to the color reaction of Jaffe with picric acid using the clinical diagnostic «Bio-Lachema-Test» (Czech Republic) [17] according to the standard method in the automatic biochemical analyzer COBOS INTEGRA 400. The physicochemical parameters of blood and lymph were determined - coagulability according to Sukharev, and viscosity using a VK-4 viscometer, hematocrit according to the generally accepted method. In animals, electrolytes were determined in blood, lymph and urine on an ABL 615/625 analyzer from Radiometer. Blood pressure was measured using strain gauges from a Dreger monitor. The analysis of the composition of the micro flora of the peritoneal fluid and the identification of microorganisms were carried out on an automatic Bacteriological analyzer «MINI API» from BIO MERIEUX. Thermometry of animals was carried out with an electronic thermometer from “Omron”. The obtained material was processed on a computer by the variational-statistical method using the Student’s test.

Results and its discussion. When modeling acute peritonitis in our experiments on rats after 48 hours, the lethality was 17% of the total number of animals. On the following days, the percentage of lethality of animals increased and by 5 days it was 57%, an accumulation of a large amount of fluid was observed in the abdominal cavity. The temperature of the animals increased to $40.6 \pm 1.20^{\circ}\text{C}$ (control $38.5 \pm 0.40^{\circ}\text{C}$). In animals with experimental fecal peritonitis, on the 5th day after the introduction of fecal suspension, we observed symptoms characteristic of acute diffuse peritonitis: weakness, loginess, hair rumpleness, rapid breathing, shortness of breath, refusal to eat, stool retention and bloating. The rats were concentrated in one of the corners of the cage. Opening the abdominal cavity revealed 2 to 5 ml of inflammatory exudate of a serous or purulent nature, sometimes with a hemorrhagic component.

Morphological changes in the abdominal cavity of rats were characterized using a visual descriptive method. In the control group of animals, the abdominal cavity of the stomach wall was normal and in all experimental groups, no significant deviations were found. In rats of the experimental group, the abdominal wall is dull, hyperemic, with purulent-fibrinous overlays on the surface and visceral surface of the liver. On the abdominal organs there are loose fibrin adhesions in the form of a «cobweb». Separate small focal hemorrhages are noted on the intestinal mesentery. The intestinal loops are swollen, filled with masses of dark color, in some places the intestine is edematous, the vascular pattern of the intestinal wall is enhanced (Figure 1). In this case, significant water-sectoral disturbances occur because of loss of total fluid, according to the authors, with a 15% decrease in the volume of the extracellular and 8% intracellular sectors [18, 19].

Obtaining a model of acute peritonitis in rats was confirmed by the results of the clinical picture, histological studies of the structure of the parietal and visceral sheets of the peritoneum and biochemical analyzes of blood and lymph, reflecting the functional state of the body. Thus, an absolute overhydration of organs develops, with an increase in the level of cellular fluid in the abdominal cavity.

According to the literature, widespread peritonitis in the toxic and terminal stage with clinical manifestations of massive polymicrobial contamination and aerobic-anaerobic endogenous microflora. When opening the abdominal cavity during the experiment, we revealed a large amount of peritoneal fluid, and purulent foci on the liver, intestines, peritoneum. We found the following microorganisms in the peritoneal fluid: *Proteus vulgaris* group – 10^6 CFU/ml; *Escherichia coli* – 10^6 CFU/ml; *Enterococcus faecalis* – 10^6 CFU/ml; *Staphylococcus vitulinus* – 10^6 CFU/ml; *Candida inconspicua/lambica* – 10^6 CFU / ml, but we found 5 microbes only in 55% of analyzes. Other samples contained 1 or 2 microbes in different combinations of *Escherichia coli* 10^3 – 10^4 cfu/ml and *Proteus vulgaris* group 10^6 cfu/ml, *Staphylococcus vitulinus* 10^2 – 10^3 cfu/ml. The study of the exudate of the peritoneal fluid revealed microbial associations consisting of *Escherichia coli*, staphylococci, streptococci, anaerobic microorganisms, which cause acute peritonitis and toxic infection.



A

B

Abdomen opening with experimental fecal peritonitis shows an inflammatory exudate from serous to purulent, sometimes with a hemorrhagic component.

Designations: A – control group, B – experimental group, after fecal peritonitis.

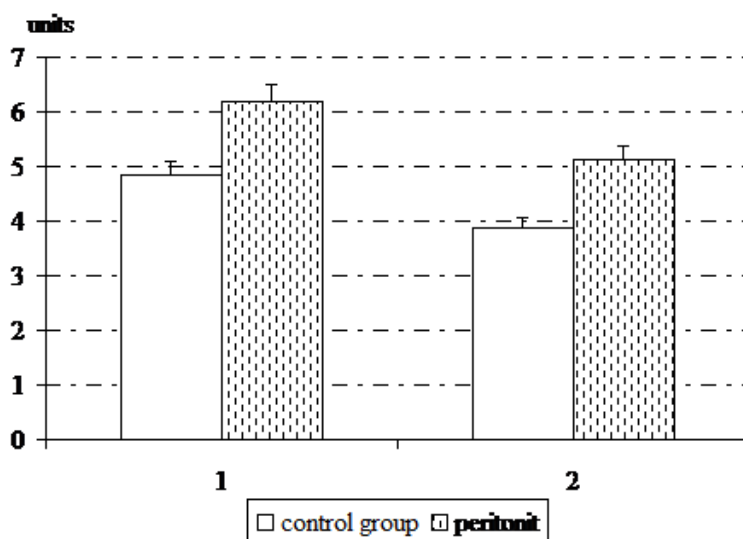
Figure 1– Abdominal cavity in control rats and after fecal peritonitis.

The results of the research showed that in animals with experimental peritonitis, destructive changes and disorders of the body have been revealed. Lymph flow in acute peritonitis decreased to $5.2 \pm 0.3 \mu\text{L}/\text{min}$ 100 g (control 7.8 ± 0.2). The observed decrease in lymph flow after experimental peritonitis was associated

with a decrease in the content of total protein in the lymph and blood plasma. Since the main property of the lymphatic system is the removal of protein released from the blood vessels into the interstitium, then a simultaneous decrease in it in the blood and lymph may indicate a violation of protein resorption by the roots of the lymphatic system, which determines the processes of lymph formation. These results indicate that with inflammation of the abdominal cavity, it significantly reduces the processes of lymph formation and lymph circulation in animals.

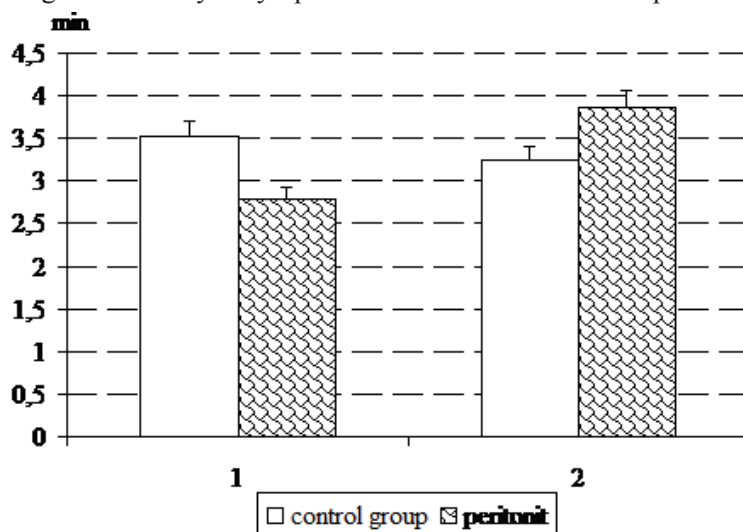
With fecal peritonitis in animals, an increase in the viscosity of lymph and blood in 28% and 33% in relation to the norm is characteristic. The blood viscosity in sick animals with peritonitis increased from 4.85 ± 0.05 to 6.20 ± 0.02 units. ($p < 0.05$ *), as well as in the lymph 3.86 ± 0.03 to 5.13 ± 0.04 units. ($p < 0.05$ *), respectively (Figure 2).

The clotting blood time in rats with peritonitis was observed within 2.79 ± 0.02 min, in control experiments 3.52 ± 0.03 min. Lymph clotting was 3.24 ± 0.04 min, in the control it was 3.86 ± 0.03 min. The clotting time of blood and lymph accelerated by 20.7% and 19%, respectively (Figure 3). A change in the rheological parameters of lymph and blood occurs simultaneously with a violation of the coagulation system. Clotting accelerated, which worsened the fluidity of both blood and lymph. An analysis of our results also shows that a decrease in the volumetric rate of lymph flow corresponds to a change in not only biochemical, but also physical properties of lymph.



Designations: Y-axis: viscosity level in units. On the abscissa: 1 – lymph, 2 – blood plasma.

Figure 2 – Change in viscosity in lymph and blood in control and experimental peritonitis.



Designations: Y-axis: coagulability in minutes. On the abscissa: 1 – lymph, 2 – blood plasma.

Figure 3 – Coagulability in lymph and blood in the control group and after experimental peritonitis.

In experimental studies, we observed that microcirculatory disorders in the focus of inflammation, along with changes in the rheological properties of blood and lymph, lead to the development of irreversible changes in cells and intercellular structures. Lymphorheological indicators indicated a change in the relative viscosity of lymph in animals with experimental fecal peritonitis, which decreased by 1.3 times. We found that a decrease in the relative viscosity of lymph contributed to a decrease in the speed of lymph movement by 1.5 times relative to the norm.

The data obtained shows that a change in the rheological parameters of lymph occurs simultaneously with a violation of the coagulation system. As noted in the experiment, the increased viscosity of lymph in acute pancreatitis, the presence of an admixture of erythrocytes in it for a tendency to thrombus formation in the thoracic duct. Other authors have shown that inflammation of the abdominal cavity activates blood coagulation processes [20], as well as a decrease in lymph flow and a change in the rheological properties of lymph in case of disorders in the body [21, 22]. All these facts indicate profound changes in the blood and lymph in experimental fecal peritonitis.

An analysis of our results also shows that a decrease in the volumetric flow rate of lymph flow corresponds to a change not only in the physical properties of lymph and blood, but also in the biochemical parameters of lymph and blood. In the lymph, the total protein content decreased more deeply by 42% than in the blood. The content of urea, creatinine, residual nitrogen increased. From these data it can be seen that the most striking changes were observed from the side of total protein, urea in the lymph and blood plasma. In animals, after the fecal suspension, the number of leukocytes in the blood already after 48 hours intensively increased from 1.0 to 1.5 times in comparison with normal values. The number of erythrocytes in the blood increased by 16% from the control values to $7.63 \times 10^6 \pm 0.5 \mu\text{l}$; the number of platelets increased by 52% to $545 \pm x10^3 \pm 11 \mu\text{l}$ ($P < 0.05$). Leukocytes increased by 36%, lymphocytes by 16%. The hemoglobin level was reduced by 12%. The hematocrit decreased slightly. Under conditions of peritonitis in the lymph, the number of monocytes increased to 6% and an increase in the number of lymphocytes by 15% was observed. With peritonitis, the concentration of Na^+ ions in the plasma increased by 5%, in the lymph by 7%, and in the urine decreased by 24%. In plasma and lymph, K^+ and Ca^+ ions slightly decreased in comparison with the control. The lymph flow after 45-48 hours from the modeling of peritonitis decreased by 41% to $4.9 \pm 0.3 \mu\text{l}/\text{min}$ per 100 g of body weight. The content of electrolytes in the studied biological fluids changed. There was a tendency towards a decrease in Na^+ , K^+ , Ca^{+2} in the blood and an increase in the same elements in the lymph and in the urine. Probably an increase in Na^+ , K^+ , Ca^+ in lymph and urine is associated with a decrease in lymph flow and diuresis and with a higher concentration of these elements in a smaller volume or some depositing function of the lymphatic system. A general analysis of blood and lymph and biochemical studies of these fluids reflect signs of a significant inflammatory process and hepatonephric insufficiency. Thus, the analysis of the data characterizing the system of blood properties showed that in all animals with the introduction of fecal suspension, clinical signs of the initial development of peritonitis were observed, which were more pronounced in sick animals with a lethal outcome. With this in mind, these changes indicated the occurrence of toxic and microbiological inflammation of the abdominal cavity in animals [23, 24].

In experimental fecal peritonitis, all sick animals showed signs of diffuse fibrinous-purulent peritonitis involving the deep layers of the peritoneum and underlying adipose and muscle tissue. The peritoneum is edematous, infiltrated, collagen fibers are swollen, homogenized with areas of decay. Dystrophic-destructive changes, edema, circulatory disorders and signs of acute exudative inflammation are determined in the underlying muscle and adipose tissue [25]. Analyzing the obtained experimental material, the following picture can be observed. We obtained an adequate model of acute peritonitis in experimental animals with an evidence base on the clinical picture by histological sections and biochemical data of blood and lymph, which significantly differed from the data of the control group of rats.

Thus, as a result of our experimental work in modeling peritonitis, it was shown that the lymphatic system is involved in the pathological process. After inflammation of the abdominal cavity, signs of widespread fibrinous-purulent peritonitis with the involvement of the deep layers of the peritoneum and underlying adipose and muscle tissue were found. Microbiological studies have shown that in most experimental animals, various aerobic and anaerobic microorganisms are found in the abdominal cavity. In experiments, the detection of conditionally pathogenic microflora during inflammation of the abdominal cavity in animals indicates a chronic bacterial infection and pronounced disorders in the immunity of the mucous membranes. Thus, the conducted studies have shown that most animals with acute peritonitis have two or more focal point of infection associated in 90-92% with *Staphylococcus aureus* [26, 27]. We received a decrease in lymph flow, an increase in the level of thrombogenic processes, an increase in the viscosity of lymph and blood, which indicates a

deterioration in the rheological properties of lymph and blood, as well as changes in the biochemical spectrum of indicators and the cellular composition of lymph.

Conclusions. The inflammatory process of the abdominal cavity simulated in an experiment on animals leads to the development of purulent-fibrinous processes in the wall of the genital organs, which may be the cause of their dysfunction, the basis of the clinical manifestations of peritoneal peritonitis. The data obtained by us allow that experimental peritonitis was obtained in rats. Thus, we obtained experimental peritonitis, which was shown in rats in the peritoneal fluid, microorganisms were found in the form of staphylococci, streptococci, etc. In inflammatory processes in the abdominal cavity, there is a violation of lymph and hemodynamics, as well as the physicochemical parameters of lymph and blood, expressed in a change in lymph flow in the thoracic lymphatic duct and the color of lymph. These results indicate that with inflammation of the abdominal cavity, it significantly reduces the processes of lymph formation and lymph circulation in animals.

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ЭКСПЕРИМЕНТТІК ПЕРИТОНИТ КЕЗІНДЕГІ ЖАНУАРЛАРДАҒЫ ЛИМФА АҒЫСЫ ЖӘНЕ ЖАСУШАЛЫҚ ҚҰРАМЫ, ЛИМФА МЕН ҚАННЫҢ РЕОЛОГИЯЛЫҚ ҚАСИЕТТЕРІ

Аннотация. Бұл мақалада нәжістік инъекциядан туындаған жедел перитонит кезінде лимфа ағысы, лимфа мен қанның құрамы зерттелген. Тәжірибелерде жедел перитонит лимфа ағысының төмендеуіне, лимфа, қан плазмасы мен несептің физикалық-химиялық көрсеткіштерінің өзгеруіне әкелетіні көрсетілген. Жануарларда нәжістік перитонит кезінде лимфа мен қанның тұтқырлығы нормаға қатысты 28% және 33% артатындығы байқалды. Егеуқұйрықтарда перитонит кезінде қанның ұю уақыты $2,79 \pm 0,02$ мин шегінде байқалды, ал лимфада $3,24 \pm 0,04$ мин болды. Лимфа мен қанның реологиялық көрсеткіштерінің өзгеруі коагуляция жүйесінің бұзылуымен бір уақытта жүреді. Қанның да, лимфаның да ағуының төмендеуі ұю уақытысының жылдамдығының артуына алып келеді. Біздің нәтижелерімізді талдаулары көрсеткендей, лимфа ағысының көлемдік жылдамдығының төмендеуі лимфаның биохимиялық көрсеткіштерімен қатар, оның физикалық қасиеттерінің де өзгеруі сәйкес келетінін көрсетеді. Зерттелетін биологиялық сұйықтықтардағы электролиттердің мөлшері өзгеретіндігі анықталды. Қандағы Na^+ , K^+ , Ca^{+2} төмендеуі, сондай-ақ бұл элементтердің лимфа мен несепте артқандығы анықталды. Лимфа мен несептегі Na^+ , K^+ , Ca^{+2} жоғарылауы лимфа ағымсы мен диурездің төмендеуімен, аз мөлшерде осы элементтердің жоғары болуы немесе лимфа жүйесінің кейбір депонирлеу қызметімен байланысты болуы мүмкін. Перитонеальды сұйықтықта өткір перитонит пен токсикоинфекцияны тудыратын *E. coli*, *Staphylococcus*, *Streptococcus*, анаэробты микроорганизмдерден тұратын микробтық қауымдастықтар анықталды. Біз алған мәліметтер егеуқұйрықтардан тәжірибелік перитонит алуға мүмкіндік береді. Құрсақ қуысындағы қабыну процестерінде лимфа және гемодинамика, сондай-ақ лимфа мен қанның физика-химиялық көрсеткіштері бұзылады, бұл кеуде лимфа жолындағы лимфа ағысы мен лимфа түсінің өзгеруімен көрінеді. Алынған жедел перитонит үлгісінен ағзаның жалпы реакцияларын, ағзадағы қабыну процесінің пайда болуы мен даму заңдылықтарын, жергілікті және жалпы реттеуші жүйелердің біркелкі жауап реакциясын қамтамасыз ететіндігі, атап айтқанда лимфа жүйесін объективті бағалауға мүмкіндік береді.

Түйінді сөздер: тұтқырлық, қан, лимфа, лимфа ағымы, перитонит, ұйығыштық.

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ЛИМФОТОК И КЛЕТОЧНЫЙ СОСТАВ, РЕОЛОГИЧЕСКИЕ СВОЙСТВА ЛИМФЫ И КРОВИ У ЖИВОТНЫХ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ПЕРИТОНИТЕ

Аннотация. В данной статье изучен лимфоток, состав лимфы и крови при остром перитоните, вызванном каловой инъекцией. В экспериментах показано, что острый перитонит вызывает уменьшение лимфотока, сдвиги физико-химических показателей лимфы, плазмы крови и мочи. При каловом перитоните у животных характерным является увеличением вязкости лимфы и крови в 28% и 33% по отношению к норме. Время свертывания крови у крыс с перитонитом наблюдалось в пределах $2,79 \pm 0,02$ мин, а в лимфы – составляла $3,24 \pm 0,04$ мин. Изменение реологических показателей лимфы и крови происходит одновременно с нарушением свертывающей системы. Свертываемость ускорилась, что ухудшало текучесть как крови, так и лимфы. Анализ наших результатов также показывает, что снижению объемной скорости лимфотока соответствует изменение не только биохимических, но и физических свойств лимфы. Изменялись содержание электролитов в изучаемых биологических жидкостях. Была выявлена тенденция к снижению в крови Na^+ , K^+ , Ca^{+2} и увеличение этих же элементов в лимфе и в моче. Вероятно, увеличение Na^+ , K^+ , Ca^{+2} в лимфе и моче связано со снижением лимфотока и диуреза и с большей концентрацией этих элементов в меньшем объеме или некоторой депонирующей функцией лимфатической системы. Выявили в перитонеальной жидкости микробные ассоциации, состоящие из кишечной палочки, стафилококков, стрептококков, анаэробных микроорганизмов, которые и вызывают острый перитонит и токсикоинфекцию. Полученные нами данные позволили получить экспериментальный перитонит у крыс. При воспалительных процессах в брюшной полости происходит нарушение лимфо- и гемодинамике, а также физико-химических параметров лимфы и крови, выражающееся в изменении лимфотока в грудном лимфатическом протоке и цвета лимфы. Разработанная модель острого перитонита позволяет объективно оценить общие реакции организма, закономерности возникновения и развития воспалительного процесса в организме и, в частности, в лимфатической системе, и обеспечивает получение однозначных ответных реакций как местных, так и общих регуляторных систем.

Ключевые слова: вязкость, кровь, лимфа, лимфоток, перитонит, свертываемость.

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