Simulation of peritoneal sepsis and its treatment with serum in an experiment: peculiarities of morphological disorders of liver, spleen and kidney tissues

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Sepsis develops as a normal inflammatory response to various infections. It proceeds with the picture of a complex heterogeneous syndrome, which often leads to the development of multiple organ failure. The number of affected organs correlates with mortality. Organ failure is characterized by a sequence - initially pulmonary, then renal, cardiovascular, and in the terminal stage, there is a failure of the central nervous system function. The aim of the study was to model peritoneal sepsis in an experiment, develop a therapeutic serum as an analog of anti-reticular cytotoxic serum by O. O. Bogomolets (ACS), and investigate the therapeutic properties and specific morphological changes in the liver, spleen, and kidneys of septic and treated animals.

To simulate peritoneal sepsis, laboratory mice were injected intraperitoneally with a solution of 10% of filtered fecal suspension of guinea pig (from 0.05 to 3.0 ml), the level of the toxic dose at which all mice died was determined (0.35 ml of fecal suspension) after that lethal bloodletting was administered under thiopental anesthesia. Their spleens were removed, from which therapeutic serum was made. Guinea pigs of the experimental group were injected with a suspension of the spleen of mice with an increasing dose from 0.02 to 0.2 ml after the simulation of fecal peritonitis. The optimal therapeutic dose at which the ants continued to live for 14 days was determined: 0.08 ml. Under thiopental anesthesia (50 mg/kg), their liver, kidneys, and spleen were taken for histological studies. The processing of the obtained histological specimens was carried out according to generally accepted methods. Histological preparations were studied using an SEO SCAN light microscope. It was found that under conditions of peritoneal sepsis in the liver, the lobular organization of hepatocytes was dramatically disturbed. The central veins and vessels of the portal tracts were moderately dilated and filled with blood, but the lumens of the sinusoids were practically not visualized. The sizes of hepatocytes increased sharply, contours were erased, and intercellular connections were disrupted. Histological examination of the kidney revealed a drastic decrease in the blood volume in the vessels of the arterial bed, which is visualized by the structural manifestations in the cortical layer. Collaptoïd shrinkage of glomerular vessels was observed, which manifested in their sharp reduction in size. A significant part of the endothelocytes was damaged. Examination of the spleen revealed a significant increase in the area of the red pulp due to the pronounced expansion of the sinusoids and an increase in their blood supply. The white pulp exhibited small, moderately diffuse foci of lymphocyte clusters. Follicle structures were practically not visualized. Histological examination of the liver in animals with simulated peritoneal sepsis on the background of correction with an extract from the spleen of mice revealed a moderate expansion and full blood vessels of the portal tracts and central veins. The contours of the vast majority of hepatocytes grew clear, intercellular contacts were restored. Histological examination of the kidney revealed a moderate increase in the blood volume in the vessels of the arterial bed, mainly in the cortical layer. A mild expansion and fullness of blood vessels of the glomeruli was observed, which was manifested by their increase in size. An increase in macrophage-type cells was observed in the perivascular areas. Histological examination of the spleen revealed a pretty large area of red pulp, moderate
Introduction

Sepsis is a potentially life-threatening pathology caused by infection and characterized by a poorly controlled systemic inflammatory response [7]. Sepsis, severe sepsis, and septic shock pose significant problem for the global healthcare system [15]. In the United States alone, over 600,000 cases of sepsis are registered annually, with more than 200,000 people dying from severe sepsis and its complications each year [17]. It is evident that there is a need for the development of improved treatment methods to reduce the mortality rate among patients with severe sepsis.

Peritoneal sepsis is complicated by the development of multiple organ dysfunction in 25% of cases [7], characterized by structural damage to organs and tissues, which can result in death [5, 12]. Despite the wide range of intensive therapy and antibiotic treatments available, the mortality rate for severe forms of sepsis remains at 50.8% [5]. In 1925, our distinguished compatriot, O. O. Bogomolets, developed a serum (an extract from organs responsible for the immune response) and named it antireticular cytotoxic serum (ACS) [10, 13, 18]. Both in experiments and clinical applications, this serum has demonstrated significant therapeutic effects in sepsis. Over time, the use of Bogomolets' serum has lost its relevance for various reasons [10, 13, 18].

The aim of the study was to model peritoneal sepsis in an experiment, develop a therapeutic serum (analog of ACS), and investigate the therapeutic properties and specific morphological changes in the liver, spleen, and kidneys of septic and treated animals.

Materials and methods

22 white mice weighing 30.51±3.02 g and 26 guinea pigs weighing 950.4±5.0 g were used in the experimental part of the study, they were kept in the vivarium of I. Ya. Horbachevsky Ternopil National Medical University and received a standard diet [11].

During the work with laboratory animals, the rules of humane treatment of experimental animals and the requirements approved by the Bioethics Committee of Ternopil National Medical University and the International requirements for the humane handling of animals according to the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" were followed.

In the first stage of the experiment, we modeled fecal peritonitis in mice [16, 19]. For this purpose, we injected 0.05 ml to 1.0 ml of a 10% filtered fecal suspension of guinea pigs into the abdominal cavity of the mice. The suspension was obtained by mixing the feces of the animals with an isotonic solution of sodium chloride and double-filtering it through a double layer of gauze. It was administered to the mice in the abdominal cavity using a puncture method. The control group consisted of 6 white mice. At the 6th hour after the introduction of the fecal suspension, the mice became apathetic, less mobile, and sought sources of water. The body temperature increased to 43.8±2.4°C (normal range: 37.0-40°C) [1, 9]; the respiratory rate decreased to 170.0±10.0/min (normal range: 200-283/min) [1, 9]; the heart rate also decreased to 70.0±20.0/min (normal range: 600-730/min) [3, 9]. The reaction to external stimuli was inhibited, and the animals assumed a lateral position. Starting from the 6th hour, the animals that received the highest doses of toxins were began to die. By titration, we determined the volume of the extract at which half of the mice died (LD50) - 0.2 ml of fecal suspension.

A double dose (2 LD50) of the suspension was administered into the abdominal cavity of the mice, and just before the animals' death, under thiopental anesthesia, lethal bloodletting was performed. We collected the spleen, minced it by grinding in a porcelain mortar, added 5.0 ml of distilled water, centrifuged it, and extracted the liquid portion.

In the second stage of the experiment, we induced fecal peritonitis in guinea pigs. For this purpose, we injected 3.0 ml of a 10% filtered fecal suspension into their abdominal cavity, obtained by mixing feces from 2-3 intact animals with an isotonic solution of sodium chloride and filtering it twice through a double layer of gauze [2].

The control group consisted of 6 guinea pigs. Within 24 hours, the guinea pigs exhibited hypokinesia, coordination disturbances, slowed reaction to external stimuli, and an elevated body temperature of 39.5±0.2°C (normal range: 37.20±0.03°C) [21]. The respiratory rate, initially increased, gradually decreased to 65.0±7.03 breaths per minute (normal range: 89-120 breaths per minute) [1, 21]. The heart rate increased to 430.0±20.0 beats per minute (normal range: 200-283/min) [1, 9]; the heart rate also decreased to 170.0±20.0/min (normal range: 600-730/min) [3, 9]. The reaction to external stimuli was inhibited, and the animals assumed a lateral position. Starting from the 6th hour, the animals that received the highest doses of toxins were began to die. By titration, we determined the volume of the extract at which half of the mice died (LD50) - 0.2 ml of fecal suspension.

Keywords: peritoneal sepsis, morphological changes, liver, spleen, kidneys, therapeutic immune serum.
lifespan of over 14 days for guinea pigs with peritonitis, and it was determined to be 0.08 ml. After 72 hours, these guinea pigs were subjected to lethal bloodletting under thiopental anesthesia, and the liver, kidneys, and spleen were collected for histological research.

The tissues were immersed in a 10 % buffered neutral formalin solution, processed using the LogosOne histoprocessor, and embedded in paraffin blocks. Sections of 4-5 μm thickness were obtained using an AMR-400 rotary microtome and stained with hematoxylin and eosin. Histological specimens were examined under a light microscope SEO SCAN and documented using the Vision CCD Camera video camera, which has an image output system for histological slides.

The initial processing of the obtained data was conducted using descriptive statistical methods, presenting the results for quantitative variables as follows: the number of observations (n), the mean (M), and the standard error of the mean (m) [6].

**Results**

Histological examination of the liver in guinea pigs three days after inducing peritoneal sepsis revealed significant cellular degenerative changes. The lobular organization of hepatocytes was severely disrupted (Fig. 1, 2). Central veins and portal tract vessels moderately dilated and reached a full blood volume capacity, but the sinusoidal lumens were practically not visualized (see Fig. 2). The size of hepatocytes drastically increased, and their contours became blurred, while intercellular connections were disrupted. In a significant portion of cells, the cytoplasm became homogeneous (see Fig. 1), indicating pronounced eosinophilia, although large-droplet fatty dystrophy remained the dominant manifestation (see Fig. 2).

Nuclei were visualized in the majority of cells showing signs of karyopyknosis. Due to the significant presence of intracellular lipid inclusions, their localization was shifted towards the periphery (see Fig. 2). Occasional hepatocyte necrosis was observed. A small number of lymphocytes and histiocytes were visualized in the perivascular spaces of the portal tracts (see Fig. 2).

Histological examination of the kidney revealed a significant decrease in the vascular blood supply of the arterial bed, which was visualized by structural changes in the cortical layer. Collapsoid wrinkling of the glomerular vessels was observed (Fig. 3), resulting in a pronounced reduction in their size. A substantial portion of the endothelial cells was damaged. The inner leaflet of the capsule showed compression, and signs of protein dystrophy were observed. The capsule lumens were practically not visualized.

The lumens of the excretory tubules were significantly narrowed, with some of them containing inclusions in the form of hemoglobin pigment. The basal membranes remained partially intact, with some of them showing increased vascular perfusion. The epithelium of the excretory tubules exhibited various stages of protein dystrophy. The nuclei of the tubular epithelium (intensely stained) were well visualized in practically all cells, mainly located in a peri-basal position (see Fig. 3). The basal membranes partially thickened due to collagenous stromal edema.

The examination of the spleen revealed a significant increase in the area of the red pulp due to pronounced sinusoidal dilation and increased vascular perfusion (Fig. 4). The white pulp was characterized by small, moderately diffuse foci of lymphocyte clusters. Follicular structures were hardly visualized. The number of reticular cells in the germinal centers of periarteriolar areas was virtually non-visualized, while hyperplasia of T-lymphocytes

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![Fig. 1. Liver of an animal with peritoneal sepsis. Severe disruption of lobular structure, absence of lobular organization, and pronounced intracellular fatty dystrophy. Stained with hematoxylin and eosin. x200.](image1)

![Fig. 2. Liver of an animal with peritoneal sepsis. Disruption of lobular structure, pronounced macrovesicular fatty dystrophy, absence of sinusoidal lumens. Congestion of portal tract vessels. Stained with hematoxylin and eosin. x200.](image2)
was observed. Throughout the parenchyma of the spleen, a significant number of macrophages with phagocytosed lymphocytes or their fragments were visualized in the sinusoidal lumens, appearing as chromophilic bodies and debris cells, predominantly found in the mantle zones. Sinusoids in the marginal zones showed significant dilation, accompanied by an increased number of erythrocytes within their lumens.

Manifestations of spleen hyperplasia also occurred due to an increase in the number of sideroblasts and siderophages. The congestion and expansion of sinuses were accompanied by an increased presence of freely dispersed granules of hemosiderin. Swelling in the inter trabecular areas of the stroma was observed to progressively intensify.

During the study of structural changes in the liver, kidney, and spleen of guinea pigs suffering from peritonitis and treated with serum (obtained from mouse spleen), the following changes were observed.

Histological examination of the liver revealed moderate dilation and congestion of the portal tracts and central veins. Concurrently, there was expansion and congestion of the sinusoids (Fig. 5). Increased numbers of macrophages were also observed within their lumens (see Fig. 5). The lobular structure showed moderate restoration. Particularly in the periportal tract areas, partial restoration of the trabecular organization was visualized. The contours of the majority of hepatocytes became distinct, and intercellular connections were restored. The number of lipid inclusions sharply decreased. Moderate manifestations of protein hyaline droplet dystrophy were observed (see Fig. 5). The number of binucleated hepatocytes increased.

The nuclei were visualized in the vast majority of cells, with a significant portion exhibiting signs of karyopyknosis. Necrosis of hepatocytes remained sporadic. Isolated lymphocytes and histiocytes were visualized in the perivascular spaces of the periportal tracts.

Histological examination of the kidney revealed a moderate increase in the vascular congestion of the arterial vasculature, predominantly in the cortical layer. There was slight dilation and engorgement of the glomerular vessels, accompanied by an increase in their size. The majority of endothelial cells remained damaged. The nephrothelium of the inner layer of the capsule appeared flattened, with...
signs of protein dystrophy in the cytoplasm, and the capsule lumens were hardly visualized. Perivascular areas showed an increase in macrophage-like cells.

The lumens of certain collecting ducts were moderately dilated, with some of them containing proteinaceous masses. The basal membranes of the ducts remained largely intact, with a moderate increase in their vascularity (Fig. 6), and a slight reduction in perivascular edema. An increase in the number of macrophages was observed. The epithelium of the collecting ducts was at different stages of proteinaceous dystrophy. The nuclei of the epithelial cells appeared slightly brighter, well visualized in almost all cells, and predominantly located basally (see Fig. 6).

The majority of epithelial cells were in different stages of protein dystrophy, with increased cell size, resulting in significantly narrowed lumens of the tubules. However, epithelial cell necrosis was sporadic (see Fig. 6).

Histological examination of the spleen revealed a relatively large area of red pulp, moderate expansion and congestion of sinuses, and pronounced peri-sinusoidal swelling (Fig. 7). However, there was a significant increase in the area of white pulp, characterized by the formation of follicles (see Fig. 7). The white pulp exhibited marked hyperplasia, with a significant increase in the number of reticular cells in the germinal centers of peri-arteriolar regions. Additionally, throughout the parenchyma of the spleen, a considerable number of macrophages containing phagocytosed lymphocytes or their fragments in the form of chromophilic bodies and cellular debris were observed mainly in the marginal zones within the sinusoidal lumens. The sinusoids in the marginal zones remained dilated with a moderate number of erythrocytes within their lumens. The presence of sideroblasts and siderophages remained significant.

Discussion
Thus, our experimental work confirms to an extent the results obtained by our distinguished compatriot O. O. Bogomolets in his use of the developed antireticular cytotoxic serum [10, 13]. However, unlike the original source where O. O. Bogomolets used extracts from the immune organs of large cattle or horses [4, 18, 20], we used white mice as the biological material, whose metabolism occurs much faster than in the guinea pigs, on which peritonitis was modeled.

We found that modeling peritoneal sepsis in guinea pig livers led to a significant disruption of the lobular structure, with acute disturbances in the portal vein system circulation combined with pronounced fatty degeneration. In the kidneys, there was an increase in vascular spasms mainly in the cortical layer, and moderate expansion of the stromal vessel blood supply, resulting in decreased blood supply to the nephrothelium and a significant reduction in organ function. In the spleen, there was hyperplasia of the red pulp with increased sinusoidal blood flow and the development of perisinusoidal stromal edema, accompanied by an increase in the number of siderophages and excessive immune system activity (reduced area of the white pulp).

The use of therapeutic extract contributed to the involution of sepsis and a significant extension of animal life (by 6.7 times). Histological examination of the liver showed a reduction in dystrophic changes (protein and fatty degeneration), decreased venous congestion in the portal vein system, and enhanced hepatocyte regeneration. In the kidney, there was a moderate enlargement of the arterial vessel blood flow, mainly in the cortical layer, significant improvement in the condition of the basal
membranes of the collecting ducts, and a decrease in dystrophic manifestations in the epithelial structures. In the spleen, there was an increase in the area of the white pulp, indicating an enhanced immune status.

Conclusion
1. In the experiment, a therapeutic serum was developed for modeling peritoneal sepsis in white mice by extracting tissue from their spleen.

References

Modelling the Peritoneal Sepsis: The Application of Therapeutic Serum in Guinea Pigs. The application of the therapeutic serum in guinea pigs during the modeling of peritoneal sepsis yielded positive results in the histological picture of detoxification organs (liver), the immune system (spleen), and overall blood circulation (kidneys).

3. The extension of the lifespan in guinea pigs suffering from peritonitis and treated with the serum encourages further scientific research in this direction.
потім ниркова, серцево-судинна і у термінальну стадію виникає недостатність функції центральної нервової системи. Метою дослідження було змоделювати в експерименті перитонеальний сепсис; розробити лікувальну сироватку - аналог антитетапікулярної цитотоксичної сироватки О. О. Богомольця (АТС) і дослідити лікувальні властивості та особливості морфологічних змін тканин печінки, селезінки, нирок у септичних і пролікованих тварин. Для моделювання перитонеального сепсису лабораторним мишам внутрішньоочеревинно вводили розчин 10 % профільтрованої калової суспензії мурчаків (від 0,05 до 1,0 мл), встановлювали рівень токсичної дози, при якій всі миші гинули (0,35 мл калової суспензії), після чого під тіопенталовим наркозом проводили летальне кровопускання. У миші видаляли селезінку, з якої виготовляли лікувальну сироватку. Мурчакам дослідної групи після моделювання калового перитоніту вводили суспензію селезінки мишей з наростаючою дозою від 0,02 до 0,2 мл. Виявили оптимальну лікувальну дозу, при якій мурчаки продовжували жити протягом 14 днів: 0,08 мл. Під тіопенталовим наркозом (50 мг/кг) у них видаляли печінку, нирки та селезінку для гістологічних досліджень. Отримані гістологічні препарати у подальшому обробляли за загальноприйняттою методикою. Гістологічні препарати вивчали за допомогою світлового мікроскопа SEO SCAN. Встановлено, що при перитонеальному сепсисі в печінці частково організація гепатоцитів різко порушення. Центральні вени та судини портальних трактів помірно розширювалися, ставали повнокровними, проте просвіти синусоїдів практично не візуалізувалася. Розміри гепатоцитів різко збільшувалися, контури стиралася, міжклітинні зв'язки порушувався. Гістологічні дослідження нирки виявило різке зниження кровонаповнення судин артеріального русла, що візуалізувалося структурними проявами кіркового шару. Спостерігалося колаптоїдне зморщення судин клубочків, що проявлялося їх різким зменшенням у розмірах. Значна частина венозних судин клубочків збільшувалася. Дослідження селезінки виявило значне збільшення площі червоної пульпи через уражені розширення синусоїдів та збільшення їх кровонаповнення. Біла пульпа виявила помірну помірно-дифузними фокусами збільшений лімфоцит. Структури фолікулів практично не візуалізувалася. Гістологічні дослідження печінки тварин із модельованим перитонеальним сепсисом на фоні корекції екстрактом селезінки мишей виявили помірне розширення й повнокров'я судин портальних трактів та центральних вен. Контур переважної більшості лейкоцитів ставали більш чіткими, їх відновлювався міжклітинні контакти. Гістологічні дослідження нирки виявило помірне збільшення крововиталяння судин артеріального русла, переважно кіркового шару. Встановлено незначне розширення та повнокров'я судин клубочків, що проявлялося збільшенням їх розмірів. У венозних судин клубочків спостерігалось ускладнення зменшення плазми відносно крововиталяння. Гістологічні дослідження печінки виявило помірне збільшення крововиталяння судин арterіального русла, переважно кіркового шару. Встановлено незначне розширення та повнокров'я судин клубочків, що проявлялося збільшенням їх розмірів. У периваскулярних ділянках спостерігалось збільшення кількості макрофагальних клітин. Папілярні частки нирок збільшувалися, вони були вираженими перисинусоїдальними набряками. Гістологічні дослідження нирки виявило зменшення плазми відносно крововиталяння.

Ключові слова: перитонеальний сепсис, морфологічні зміни, печінка, селезінка, нирки, лікувальна імунна сироватка.