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# BILE PRODUCTION FEATURES IN CASE OF ISCHEMIC-REPERFUSION SYNDROME OF LIMBS, ABDOMINAL TRAUMA COMPLICATED WITH MASSIVE BLOOD LOSS

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## Abstract

The level of total bile acids in bile decreased in the groups of experimental animals in comparison with the control group. In the group of animals with simulated ischemic-reperfusion injury level of total bile acids in bile reached the minimal values in on the 3rd day, increasing was showed after on the 7th day, however level still remained lower than in control group. In EG-2 and EG-3 there was a unidirectional decreasing of the index by the seventh day, but in EG-3 these changes were more significant. Consequently, the level of cholesterol in the bile due to simulated injuries decreased in EG-1 and reached the minimal values in on the 3rd day and slightly increased to the seventh day of observation. In EG-2 the index gradually increased to the seventh day of observation. In EG-3 the maximum increasing was observed until the third day, after which it was at the same level. The analysis of the obtained indices testifies to the negative influence of ischemic-reperfusion syndrome on the level of cholesterol in the bile. Described changes influenced the cholato-cholesterol ratio, which was decreasing in all experimental groups. The most significant changes were in EG-3

in which abdominal trauma and hypovolemic shock were combined with ischemic-reperfusion limb syndrome.

In the conditions of an experimental trauma with an ischemic-reperfusion injury of the lower limbs, there is a violation of the indices of the biliary function of the liver, which manifest themselves as a significant reduction of the level of total bile acids in the bile with increasing cholesterol concentration.

**Keywords**: **biliary production, trauma, bleeding, reperfusion syndrome, experiment.**

**Introduction.** Ischemic-reperfusion injury is defined as a pathological process in which cell damage caused by hypoxia becomes paradoxically more acute after restoring oxygen delivery [1]. This is a dynamic process that involves two interconnected phases: local ischemic injury and inflammatory reperfusion injury, i.e. systemic phenomenon [2]. In severe cases, an inflammatory reaction due to an ischemic-reperfusion syndrome can lead to a syndrome of a systemic inflammatory response or a syndrome of multiple organ failure [3]. Ischemic-reperfusion injury of the liver is a frequent and serious complication in clinical practice, which affects its function and significantly prolongs postoperative and rehabilitation periods, increases mortality and generally worsens the overall outcome of treatment of the affected [4]. The foregoing is explained by the fact that the liver, being an organ with high energy requirements, strongly depends on the delivery of oxygen and is sensitive to hypoxic or anoxic conditions, which is often accompanied by a combined trauma, which is complicated by massive blood loss [5, 6].

Scientific research were made by a number of authors who in their studies used a model of development of multiple organ dysfunction in severe experimental trauma [7, 8]. Particular attention was paid to the study of the functional state of the liver as the central organ of detoxification of the organism [9, 10]. Its specific bile-forming and biliary excretory function are sensitive index of hepatic insufficiency and are closely linked to the variations in the key markers of traumatic illness [2, 3, 6, 9]. However, as shown by the analysis of sources of scientific literature, biliary function in conditions of abdomen injury combined with ischemic-reperfusion syndrome studied insufficiently.

**The aim of the work is** to study the features of the dynamics of bile-forming function index of the liver in the early post-traumatic period in response to blunt abdominal injury complicated with hypovolemic shock and ischemic-reperfusion syndrome.

**Materials and methods.** The working hypothesis of the experimental study is the assumption that, in terms of safe use duration of the haemostatic tourniquet, reperfusion of ischemic tissues leads to excessive formation of active oxygen species, activation of neutrophils and macrophages, hyperproduction of toxic metabolites, signaling molecules of the cytokine series and other inflammatory mediators that have a systemic effect on the body with a disruption of functions of the internal organs in case of abdominal trauma with hypovolemic shock.

The experimental study was performed on 80 nonlinear male rats (b.w. 190-220 g) with strict adherence to the "European Convention for the Protection of Vertebrate Animals, Used for Experimental and Other Scientific Purposes" (European Convention, 1984). All animals were divided into groups: 1 control and 3 experimental (for 8 animals in each group). Haemostatic tourniquets were applied for 120 minutes proximal to the lower paws of animals of the experimental groups under the thiopental-sodium anesthesia (40 mg kg-1). Elastic strips of tourniquet SWAT-T (USA) width of 10 mm were used as a tourniquets, which corresponds to the width of the tourniquet for human thigh. Tourniquets were tightened in accordance with the effective pressure index on it. Animals of experimental groups under thiopental sodium anesthesia (60 mg • kg-1) were withdrawn from the experiment by the method of total blood-flow from the heart after 1, 3 and 7 days after reperfusion. In the control group (CG), animals were only inducted into anesthesia using an equivalent dose of thiopental sodium, and subsequently withdrawn from the experiment in 2 hours.

In experimental groups blunt abdominal injury combined with ischemic-reperfusion syndrome was simulated in such way: after anesthesia by thiopental sodium, the animals were subjected to a dose-induced strike to the abdomen using a special device; by performing operative access and cutting the femur arteries blood loss was achieved in a volume from 20 to 22% of the circulating blood volume of the animal for 1 minute (acute blood loss); Ischemic-reperfusion syndrome was caused by the overlapping of the two lower limbs of animals by haemostatic tourniquets for two hours. Animals of the experimental groups were divided into 3 subgroups of research (EG): EG-1 - simulated reperfusion limb syndrome without bleeding and blunt abdomen trauma; EG-2 - severe blunt abdomen trauma and massive blood loss were modeled; EG-3 - severe blunt abdomen trauma and massive blood loss and reperfusion syndrome were modeled. The obtained data were compared with the CG.

In experimental groups animals the bile excretion function of the liver was examined after 1, 3 and 7 days after injury. Under the thiopental-sodium anesthesia (60 mg • kg-1) the general bile duct was catheterized and bile was collected for 1 hour. In the received bile,

according to the recommendations [4], the concentration of total bile acids, cholesterol, cholato-cholesterol ratio was calculated. The concentration of total, direct and indirect bilirubin of the bile also was determined by the van den Berg method in the modification of M.P. Skakun. Based on these data, the degree of conjugation of bilirubin was calculated based on the ratio: direct bilirubin×100/total bilirubin (%).

The obtained digital data were processed statistically. Reliability of index differences between experimental and control groups was estimated by using the STATISTICA program (StatSoft, Inc., USA) based on Mann-Whitney non-parametric criterion.

**Results and discussion.** As can be seen from Table 1, the level of total bile acids in bile, when tourniquets were applied on the limbs of animals, was decreased and in the the 1st day it was 9.5% lower than in the control group ($p > 0.05$). After the 3rd day, the level of bile acids was 37.5% lower than in the control group ($p<0.001$), and slightly increased to the 7th day, but still remained 18.5% lower than in the control group ($p<0.05$). In EG-2 and EG-3 similarly level of bile acids in bile was decreased for the 1st day by 10.4% ($p > 0.05$) and by 27.1% ($p<0.001$) respectively. On the 3rd day the index continued to decrease in both groups by 32.1% ($p<0.001$) and 48.9% ($p<0.001$) respectively. The index reached its minimal at the 7th day, in EG-2 it was 35.3% ($p<0.001$), while in EG-3 it was 68.8% ($p<0.001$) lower than control.

**Table 1. The level of total bile acids in bile ($g \cdot L^{-1}$) in experimental groups, Me (LQ;UQ) – median (lower and upper quartile)**

| Experimental groups | Control group | Observation points | | |
|---|---|---|---|---|
| | | the 1st day | the 3rd day | the 7th day |
| EG – 1 | 2,21 (2,06;2,48) (n=8) | 1,99 (1,89;2,19) (n=8) | 1,38*** (1,33;1,44) (n=8) | 1,80* (1,65;2,04) (n=8) |
| EG – 2 | | 1,98 (1,91;2,12) (n=8) | 1,50*** (1,37; 1,59) (n=8) | 1,43*** (1,35;1,52) (n=8) |
| EG – 3 | | 1,61*** (1,54;1,69) (n=8) | 1,16*** (1,05;1,29) (n=8) | 0,69*** (0,66;0,77) (n=8) |
| $p_{1-2}$ | | >0,05 | >0,05 | <0,01 |
| $p_{1-3}$ | | <0,01 | <0,05 | <0,001 |
| $p_{2-3}$ | | <0,01 | <0,05 | <0,001 |

Notes: here and in all tables:

1. * Reliability of index differences compare to control group ( * – $p<0,05$; ** – $p<0,01$; *** – $p<0,001$);

2. $p_{1-2}$ – reliability of index differences between EG –1 and EG – 2; $p_{1-3}$ – between EG – 1 and EG – 3; $p_{2-3}$ – between EG – 2 and EG – 3.

Comparing the experimental groups among themselves, it was found that after the 1st day with a general tendency to decrease the bile acid level in bile, the minimal values were observed in EG-3 (on average 23.3% relative to EG-1ta EG-2, $p_{1-3}<0.01$, $p_{2-3}<0.01$). There was no significant difference between the index in EG-1 and EG-2. After the 3rd day, the general tendency is maintained at which the lowest index was in EG-3, while it was 18.9% lower ($p_{1-3}<0.05$) than in EG-1 and 29.3% ($p_{2-3}<0.05$) lower than in EG-2. When comparing the 7th day in the EG-3, the decreasing in the formation of bile acids was noted at 160.9% relative to EG-1 and 107.2% relative to EG-2; the level of bile acid level in EG-2 was lower than 20.6% relative to EG-1 ($p_{1-2}>0.01$, $p_{1-3}<0.001$, $p_{2-3}<0.001$).

The analysis of the dynamics of the researched index in the experimental groups during the observation time showed the following results (Fig. 1): in the 3rd day the index in EG-1 was 44,2% lower than the index after the the 1st day ($p<0,001$), in the 7th day the index exceeded the level after the the 3rd day by 30,4% ($p<0,01$); In EG-2 was a general tendency to gradually decrease the index to the 7th day of observation, when it got the minimal index and it was 38.5% lower than index in the the 1st day ($p<0.001$); In EG-3 decreasing of index was the most significant: in the 3rd day the index was lower by 37.6% than the the 1st day and on the the 7th day index was lower by 56.0% than in the the 3rd, which was statistically significant ($p<0.001$).

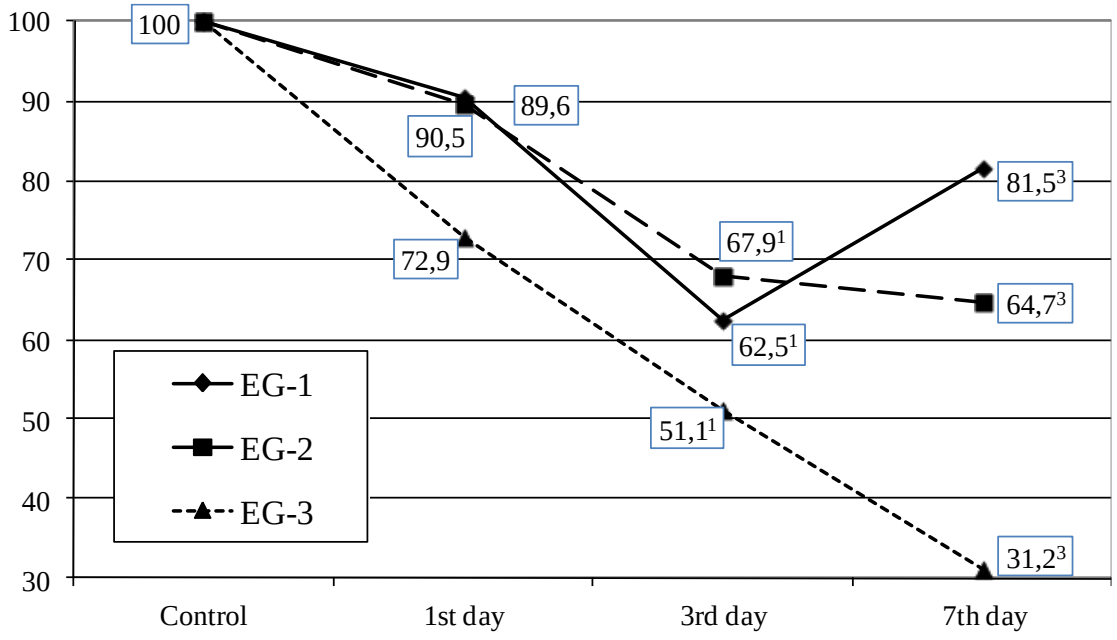


**Figure 1. Dynamics of the level of total bile acids in bile (as a percentage of the control group level) in the experimental groups. (Here and in other chapters: 1, 3 - the differences regarding the 1st and the 3rd days of the post-traumatic period are statistically reliable, p <0.05)**

However, the level of cholesterol in bile in the experimental groups was oscillatory changing (Table 2). Thus, in EG-1 after the 1st day the index decreased by 12.5% (p> 0.05) relatively to CG, after the 3rd day it continued to decrease slightly and was 20.8% less than CG, which was statistically significant (p<0,05), in the 7th day the index grew and actually reached the index in the control group. In EG-2, the index exceeded the control group values on the 1st,the 3rd and the 7th day by 12,5% (p> 0,05), 8,3% (p> 0,05) and by 12,5% (p> 0,05) respectively. In EG-3 observed a similar trend as in the EG-2. The index in EG-3 exceeded level of CG on the 1st, the 3rd and the 7th day by 25.0% (p <0.05), 54.2% (p <0.001) and 50.0% (p <0.001) respectively.

**Table 2. Cholesterol level in bile ($g \cdot I^{-1}$) in experimental groups, Me (LQ; UQ) - median (lower and upper quartile)**

| Experimental groups | Control group | Observation points | | |
|---|---|---|---|---|
| | | the 1st day | the 3rd day | the 7th day |
| EG – 1 | 0,24 (0,22;0,26) (n=8) | 0,21 (0,18;0,25) (n=8) | 0,19* (0,17;0,24) (n=8) | 0,23 (0,21;0,26) (n=8) |
| EG – 2 | | 0,27 (0,25;0,29) (n=8) | 0,26 (0,23; 0,29) (n=8) | 0,27 (0,24;0,30) (n=8) |
| EG – 3 | | 0,30* (0,27;0,33) (n=8) | 0,37*** (0,35;0,38) (n=8) | 0,36*** (0,35;0,39) (n=8) |
| $p_{1-2}$ | | <0,01 | <0,001 | >0,05 |
| $p_{1-3}$ | | <0,01 | <0,001 | <0,001 |
| $p_{2-3}$ | | >0,05 | <0,001 | <0,001 |

While comparing the experimental groups to each other for the observation time it was found that after the 1st day the minimal level was in EG-1. It was 28,8% less ($p_{1-2}$<0,01) comparison with EG-2 and 42,9% less comparison with EG-3 ($p_{1-3}$<0,01). There was no significant difference between EG-2 and EG-3 ($p_{2-3}$>0,05). For the 3rd day the maximum level was observed in EG-3: it exceeded the index in EG-1 ($p_{1-3}$<0,001) by 94,7% and by 42,3% ($p_{2-3}$<0,001) in EG-2. However the index in the EG-1 was less than in EG-2 by 36.8% ($p_{1-2}$<0,001). On the the 7th day, the highest index of cholesterol level was observed in EG-3, which exceeded the index in EG-1 and EG-2 by 56.5% ($p_{1-3}$<0,01)and by 33.3% ($p_{2-3}$<0,001) respectively.

Analyzing of dynamics of the researched index in the experimental groups showed (Fig. 2) that in EG-1 on the 3rd day the index was less by 10,5% (p> 0,05) than at the 1st day, later on the 7th day the index slightly increased in comparison with level of the the 3rd day by

5.3% (p> 0.05). In EG-2 the index was constantly decreasing and reaching the minimal level on the 7th days: the index on the 3rd day was lower by 10.0% (p> 0.05) than at the 1st day; on the 7th day it was lower by 25.0% (p <0, 05) in comparison with the 3rd day. In EG-3 the index has reached minimal level on the 7th day, levels at the 1st and the 3rd days were reliably higher 111.1% and 77.8% (p <0.001) respectively than level on the 7th day.

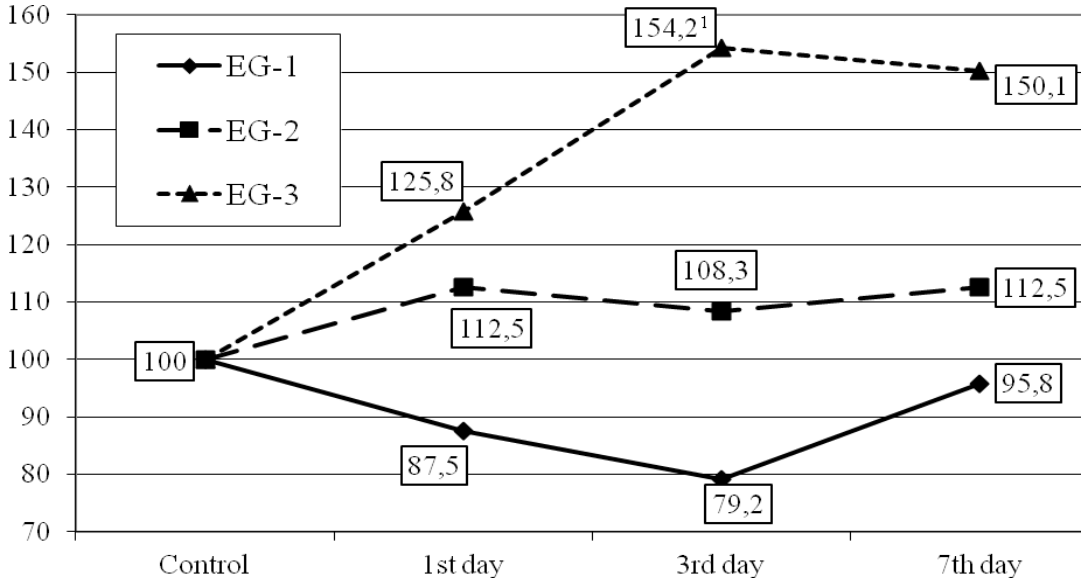


**Figure 2. Dynamics of cholesterol level in bile (percentage to level of control group) in experimental groups**

Consequently, the content of cholesterol in the bile due to simulated injuries diminished in EG-1, where it reached the minimal values in on the 3rd day and slightly increased to the seventh day of observation. In EG-2 the index gradually increased to the seventh day of observation. In EG-3 until the third day, the maximum growth was observed, after which it was at the same level. The analysis of the obtained indices testifies the negative influence of ischemic-reperfusion syndrome on the level of cholesterol in the bile.

Described changes of the level of total bile acids and cholesterol also led to cholato-cholesterol ratio disturbance (Table 3). Thus, in EG-1at the 1st day the ratio decreased by 5.8% (p> 0.05) relatively CG, after the the 3rd day ratio was 31.4% (p <0.05) and on the 7th day 20.9% (p> 0.05) less than in CG. In EG-2 ratio was by 27.2% (p <0.05), 42.9% (p <0.01) and 47.4% (p <0.01) less than in CG respectively observation points. In EG-3 the ratio was lower than in CG by 44.2% (p <0.01), 68.5% (p <0.001) and 81.5% (p <0.001) respectively observation points.

**Table 3. Cholato-cholesterol ratio in bile (relative units) in experimental groups**

**Me (LQ; UQ) - median (lower and upper quartile)**

| Experimental groups | Control group | Observation points | | |
|---|---|---|---|---|
| | | the 1st day | the 3rd day | the 7th day |
| EG – 1 | 9,94 (8,94;10,25) (n=8) | 9,36 (7,43;11,18) (n=8) | 6,82* (5,73;8,38) (n=8) | 7,86 (6,64;8,67) (n=8) |
| EG – 2 | | 7,24* (6,65;8,36) (n=8) | 5,67** (4,36; 7,04) (n=8) | 5,23** (4,68;5,59) (n=8) |
| EG – 3 | | 5,55** (4,67;6,18) (n=8) | 3,13** (2,74;3,78) (n=8) | 1,84** (1,75;2,11) (n=8) |
| $p_{1-2}$ | | <0,05 | >0,05 | <0,01 |
| $p_{1-3}$ | | <0,01 | <0,001 | <0,001 |
| $p_{2-3}$ | | <0,01 | <0,01 | <0,001 |

Comparison of the experimental groups among themselves during the experiments has shown that after the 1st day the ratio was minimal in EG-3. Accordingly, the ratios in the EG-1 and EG-2 exceeded the EG-3 by 68.6% and 30.5% respectively ($p_{1–3}$<0,01, $p_{2–3}$<0,01). On the 3rd day a similar tendency was observed, the ratios of EG-1 and EG-2 exceeded by 117.9% and by 81.2% ratio in EG-3 ($p_{1–3}$<0,001, $p_{2–3}$<0,01). At the same time, there was no significant difference between the ratio in EG-1 and EG-2 ($p_{1–2}$>0,05). On the the 7th day in EG-3 again proved to be significantly lower compared with those in other experimental groups ($p_{1–3}$<0,001, $p_{2–3}$<0,001).

Analysis of the dynamics of the ratio in the experimental groups showed (Fig. 3) that in EG-1 it reached the minimal level after the 3rd day and lightly increased to the the 7th day of observation, although there was no statistically significant difference between the indices at the observation points.

In EG-2 the ratio gradually decreased over the observation time and at the 1st day it was 21.7% (p<0.05) less than index of CG, the minimal ratio was recorded on the 7th day, although there was no significant difference with the ratio on the 3rd day. In EG-3 the cholato-cholesterol ratio reached a minimal level on the 7th day, but sharp decreasing was observed over all observation points: on the 3rd day the ratio was less by 43.6% (p<0.001) than at the 1st day; on the 7th day the ratio was by 41,2% (p<0,01) less compare to the the 3rd day ratio.

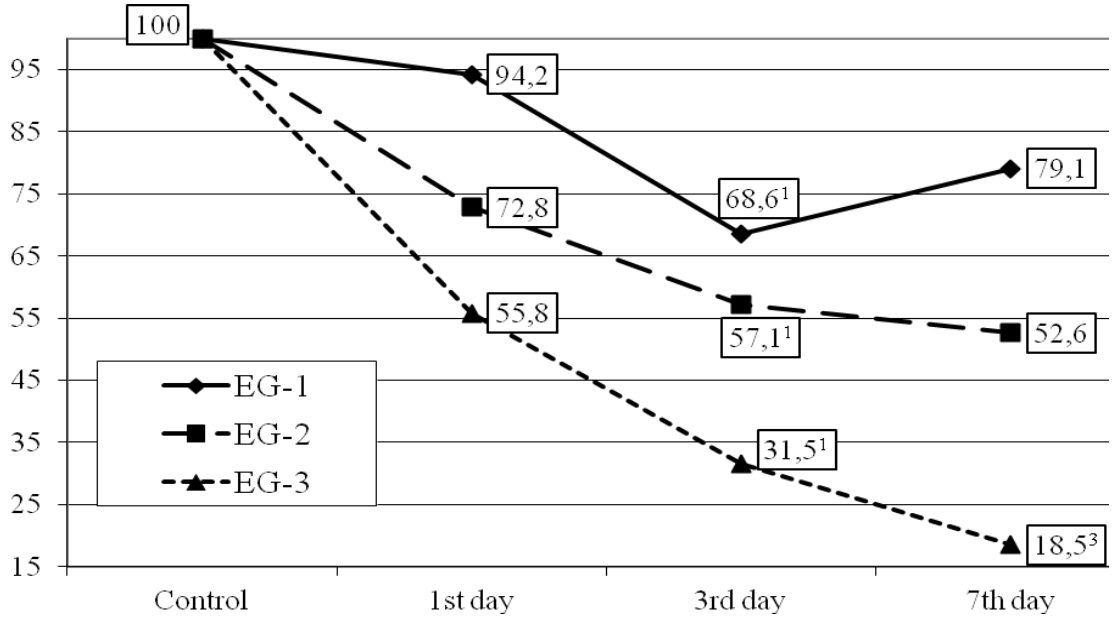


**Figure 3. Dynamics of cholato-cholesterol ratio in bile (percentage to level of control group) in experimental groups**

Thus, in response to various mechanical injuries, there are significant violations of the bile-forming function of the liver. The total bile acid level in bile decreased from the the 1st to the 7th day in EG-2 and EG-3 and it was significantly lower than the control group level for the entire observation time. In EG-1 the index was statistically lower than in the control group on the 3rd and the 7th day. The level of cholesterol in the bile increases, which was particularly noticeable on the 3rd day in the group of rats with blunt abdominal trauma, massive bleeding and ischemic-reperfusion syndrome. This leads to a significant reduction in the cholato-cholesterol ratio from the 1st to the 7th day in all experimental groups, with the largest violations occurring on the 7th day of experiment in the case of blunt abdominal trauma, massive bleeding and ischemic-reperfusion syndrome.

**Conclusion.** In the conditions of an experimental trauma with an ischemic-reperfusion injury of the lower limbs, there is a violation of the indices of the biliary function of the liver, which manifest themselves as a significant reduction of the level of total bile acids in the bile with increasing cholesterol concentration.

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