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RESEARCH OF BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS OF BLOOD OF CASPIAN SEALS

Results of studying of biochemical and hematologic indicators of the Caspian seals for the last five years (2012-2016) are presented in the article. Biochemical indicators of blood were determinedusinga biochemical BioChem SA analyzer and hematologic indicators on the automatic Siemens ADVIA 2120 analyzer. By results of a research it has been revealed, lowering of the level of urea in the first group for 65%, in the second group for 73%, in the third group for 72%, in the fourth group for 30%, Decrease in concentration of urea at intoxication indicates the pathological changes in a liver leading to urea synthesis violation. Decrease in level of urea can be caused by both a hypoproteinemia, and a hypophosphatemia. Activities of ALT, AST by 1,3 times in comparison with normal amount of transferases in blood has increased by 79%, and. Increase in activity hepatic transaminase speaks about processes of death of hepatocytes owing to chronic toxic defeat and increase in blood of level of the studied enzymes. On a hemocytogrammof animals increase of total number of leukoytes, decrease the percentage of neutrophils by 2,5 times in the fourth group, growth of percentage of lymphocytes by 30 times is revealed. In the third group decrease in neutrophils and increase of lymphocytes is noted. Increase in percentage of eosinophils in the second group up to 4,89±0,18% is revealed, at the same time their absolute contents didn't increase. In the second group the percentage and absolute maintenance of lymphocytes increases, also increase in level of monocytes and eosinophils indicates influences an organism of animals toxic or of the allergic substances.

Thus, on biochemical and hematologic indicators of blood of the Caspian seals, it is possible to draw a conclusion on toxic damage of a liver that is shown in malfunction of this body.

Keywords: Caspian seal, biochemical analysis, hematologic analysis, experiment.

Introduction. In recent years there is a problem of relationship of marine mammals and the person in connection with strengthening of anthropogenic impact on the environment more and more sharply. Pollutants cause oppression of many major functions. By scientists it is established that the Caspian seal, as well as other hydrobionts standing on the highest steps of food chains of the seas and oceans more accumulate organochlorine compounds, heavy metals, hydrocarbons of oil (Zaharova N. A., 2006:214). It is known that toxicant directly or indirectly influences mammals. Pathologies register not only at influence of the increased concentration of harmful chemicals, but also at the level of maximum-permissible (maximum allowable concentrations) even at short-term contact with them (Tetel'min, V.V., 2009:330; Belov P.S., 2011:220). Pollutants make mainly damaging impact on structural elements of the liver participating in a detoxication of xenobiotics(Aryamkina O.L., 2005:27). This body plays an important role in ensuring exchange processes and protective reactions.

The biochemical approach in studying of natural populations consisting in comparison of biochemical features of different populations and their detailed studying in same (Karmoliev P. X., 2007:27; Ezdakova I.Y., 2016:14) is widely used already more half a century. Most often this approach is associated with studying of biochemical polymorphism by method of the electrophoretical analysis of proteins allowing to divide the proteinaceous molecules coded by various alleles of one locus. These literature data on biochemical polymorphism at different types of marine mammals aren't numerous and, are generally devoted to questions of systematization whereas features and dynamics of intra population structure are studied still insufficiently.

Biochemical approach in studying of natural populations of animals isn't limited to a polymorphism research. The characteristic of biochemical variability creates the actual basis for understanding of biochemical features, their essences and expressivenesses at systematically close types, and different populations, different in ecology, within one look. Accumulation of data on basic elements of the biochemical status of animals creates a basis for development of methodology of ecological-biochemical monitoring, search of sensitive indicators of ecological trouble of this or that population, specific and nonspecific changes under the influence of environment factors.

The activity of aminotransferases of serum of blood is the sensitive indicator of the damage of liver cells caused by hepatotoxical substances and at heart failure and various infections. Organospecifical enzymes of hepatocytes are released at damage or full destruction of cages. Now for diagnosis of diseases of a liver the determination of catalytic activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) is most widely used. ALT contains in the basic in hepatocytes and in, smaller a degree, in muscle cells. Increase in activity of ALT is observed at destruction of cells of a liver, and is resistant and expressed. The indicator of ALT returns to normal in case of interruption of a cytolysis, data of biochemistry on him are restored in 2 weeks. AST contains in cells of various fabrics, and the biggest activity of AST is observed in a liver, in cardiac and skeletal muscles, in kidneys. It is mainly mitochondrial enzyme. Increase in concentration of AST demonstrates damage of a liver.

One of early indicators of violation of a homeostasis of an organism at intoxication of any genesis is a change of the structural organization of proteinaceous molecules. Albumine - one of the main proteinaceous fractions of blood. Concentration of albumine in serum is higher, than concentration of other proteins. Albumine makes the main contribution to intra vascular colloidal and osmotic pressure. Besides, it is a molecule carrier of biologically important substances (GryzunovG.E, 2003:827). In this regard albumine, acting as a detoxicant, is the homeostasis regulator at a stage of transport biologically active low - and middlemolecular substances (ligands) blood. He transports endogenous physiologically active agents (hormones, vitamins, fatty acids, bilirubin, medicines, etc.) and endogenous connections of pathogenic character (Lopuhina A.I., 1993:645; Rodoman G.B., 1994:25; Koch-Wester S., 1986:311). It is possible to carry the circulating immune complexes (CIC), molecules of average weight (MCM), products of peroxide oxidation, poisons to the last.

The special attention is deserved by the characteristic of the modified albumine as his content in serum of blood serves as a nonspecific indicator of a physiological condition of an animal (Bueverov A.O., 2014:7). The connecting function of serumalalbumine is caused by the features of a structure of this protein including elements of recognition, structural adaptation and reversible fixing of connections (Ruol R.G., 2015: 4578).

The violation of conformation of a molecule of albumine caused by her irreversible linking with membranotropny endotoxins, peroxide damage of protein or his glikilirovaniye is led to decrease or full loss of his functions. Albumine represents difficult, multifunctional, antioxidant prooxidation the system reacting to change of the biochemical environment surrounding it that is expressed in change of structure of a molecule, change of its transport properties, redistribution of the centers of binding for molecules ekzo-and the endogenous nature.

Exchange of proteins – the process proceeding in an organism it is constant. One part of substance changes a form, and another breaks up. There is a release of ammonia which kidneys quickly turn into urea. This substance – an important component of residual nitrogen of blood, one of the final products of proteinaceous disintegration. Urea comes out an organism generally together with urine. Depending on the speed of such removal and from concentration of substance it is possible to estimate at blood previously, how effectively kidneys cope with secretory function. Loss by a protein organism through kidneys and intestines – inevitable and quite natural phenomenon. The main thing that in course of such process the balance was observed. Normal level of urea – confirmation of respect for balance in proteinaceous

exchange. Reduction of concentration of urea meets rather seldom. The state can speak the reasons: physiological; pathological. Considerable falling of level of urea is fixed at development of heavy illnesses and states, life-threatening, for example, at toxic damage of a liver (they are followed by decrease in synthesis of proteins and metabolism of amino acids – urea sources).

In this regard, studying of mechanisms of impact of toxic substances of exogenous and endogenous origin on blood plasma proteins, is a relevant task.

The purpose of work was studying of biochemical and hematologic indicators of blood of the Caspian seals for the last 5 years (the 2012-2016th.).

Materials and Methodas. Served as material blood of seals. Animals were examined in nature during the expeditions. Blood was taken from an ekstraduralny vein, according to the description in work (Geraci J.R., 1975:2559). Biochemical indicators of blood defined on the biochemical BioChem SA analyzer. Determined activity of enzymes - alanine aminotransferase (ALT), aspartate aminotransferase (AST), α – amylases, the content of the general protein and albumine, glucose, creatinine and urea, glucose, phosphorus and a kaltion in blood serum. For assessment of hematologic indicators Complete blood count - the general blood test on the automatic hematologic Siemens ADVIA 2120 analyzer (Germany) have moved.

Used the following indicators: WBC - Leukocytes (absolute quantity), RBC - Erythrocytes (absolute quantity), HGB - Gemoglobin (concentration), HCT - Gematokrit (percent), MCV - the Average volume of erythrocytes, MCH - the Average content of hemoglobin in a separate erythrocyte, MCHC - Average concentration of hemoglobin in the eritrotsitarny weight, RDW - Width of distribution of erythrocytes, PLT - Platelets (absolute), MPV - the Average volume of platelets, % NEUTRO - Neutrophils (relative a stake.), NEUTRO abs - Neutrophils (absolute a stake.), % LYMPHO - Lymphocytes (relative a stake.), LYMPHO abs - Lymphocytes (absolute a stake.), MONO of % - Monocytes (relative a stake.), MONO abs - Monocytes (absolute a stake.), BASO of % - Basophiles (relative a stake.), BASO abs - Basophiles (absolute quantity), EOS of % - Eosinophils (relative a stake.), EOS abs - Eosinophils (absolute quantity).

Statistical data processing was carried out with determination of average value, a mean square and standard deviation, statistical error of average and percent of distinctions. In determining the reliability of the difference between the indices of the compared groups, the reliability t-criterion was calculated, the value of P was determined from the Student's test, the changes were considered reliable at $p \le 0,001$. All data have been calculated in the software package of MS Offis Excel.

Results and Discussion. For studying of biochemical indicators of blood of the Caspian seals are analyzed for the last 5 years (2012-2016) which are presented in in the figure 1.

Content of the general protein in the fifth group has gone down for 11% of norm, and the level of albumin has gone down in the first group for 85%, in the second group for 45%, in the third group for 50%, in the fourth group for 46% and in the fifth group for 91% in comparison with the normal content of albumin in blood.

Decrease in protein content in plasma of blood (hypoproteinemia) comes to light usually at pathophysiological syndromes, the expressed decrease in biosynthesis, catabolism strengthening, and pathological distribution of protein between certain sectors of an organism (Larionova T.K., 2012:30; Kavcevich H.H., 2009:3; McConnel L.C., 2013:9). Decrease in content of protein in plasma of blood is noted also at dysfunction of digestive tract, at the long inflammatory processes, which are followed by deterioration in digestion and absorption of proteins. Development of a syndrome of hepatocellular insufficiency is characterized by decrease in proteinaceous and synthetic function that is shown by decrease in maintenance of fraction of albumin. At a liver failure - it is broken synthesis by hepatocytes of albumin and the hypoalbuminemia is shown. Hypoalbuminemia contributes to the development of hypostases and formation of ascites (in the conditions of increase in pressure of blood in vessels of a portal vein).

Level of urea has gone down in the first group for 65%, in the second group for 73%, in the third group for 72%, in the fourth group for 30%, and in the fifth group for 54% in comparison with the normal level of urea.

Decrease in concentration of urea at intoxication indicates the pathological changes in a liver leading to urea synthesis violation. As urea (residual nitrogen) is formed mainly in a liver, urea level in blood decreases at her crushing defeats (Lakin G.F., 2010:340; Aubin D.J., 2009:569; Eisner R., 2008:142). Decrease in level of urea can be caused as a hypoproteinemia (synthesis of urea is closely connected with disintegration of nucleotides), and a hypophosphatemia.

Activities of ALT has increased in the second group by 24%, in the fourth group for 79%, in the 5th group for 38%, and AST has increased in the second group by 10%, in the fourth group by 1,3 times, in the fifth group by 1,2 times in comparison with normal amount of transferases in blood.



Figure 1 – Biochemical indicators of albumin, urea and transferases for 2012-2016.

In all skilled groups the level of activity of ALT and AST in comparison with norm increases that speaks about processes of death of hepatocytes owing to chronic toxic defeat and increase in blood of level of the studied enzymes (Arroyo V., 2014:287; Frouin H., 2010:160). Important sign of toxic injury of a liver, in this case the syndrome of a cytolysis, which is shown, as we know, increase in activity of ALT and AST enzymes is. These enzymes are localized in various compartment of cages therefore conditions of their diffusion in blood differ. ALT functions in cytoplasm and AST mainly inmitochondria and therefore eliminated from a liver with a smaller speed. Owing to violation of

permeability of a cellular membrane the hyperfermentemia develops that is a marker of a syndrome of a cytolysis(Bacon B., 2002:179; Biggins S., 2005:32).

From in the figure 1 it is also visible that in serum of blood of the studied animals contents a phosphoditch has been reduced to 0,87 mmol/L. Hypophosphatemia it is caused by toxic damage of a liver (poisoning with salts of heavy metals) (Argunov M.N., 2013:31; Kennedy S., 1989:97).

In all five tests, the content of creatinine, glucose, and calcium and activity level of α – α -amylase remained within physiological norm. For studying hematologic indicators of seals for 2012-2016 have been analysedhemocytograms of animals.

The analysis of the results presented in the figure 2 reflects indicators of a hemocytograms of seals for 2011-2016. So, in dynamics increase in total number of leukocytes with 2,49±0,24 to 8,22±0,23 was observed, the total number of erythrocytes increased to 9,79±0,20, hemoglobin up to 151,12±2,3%, along with it the value of the hematocrit didn't change. Erythrocytes coefficients didn't change significantly, including the average volume of an erythrocyte, average content of hemoglobin in a separate erythrocyte, average concentration of hemoglobin in erythrocytes weight, the settlement width of distribution of erythrocytes on volume. Total number of platelets statistically authentically changed towards decrease and increase, but nevertheless remained within physiological norm.

The percentage of neutrophils decreased in the fourth group by 2,5 times of $6,16\pm0,26\%$, the percentage of lymphocytes from $0,34\pm0,19$ at the same time increased up to $18,74\pm0,50\%$ and up to $10,64\pm0,50\%$. Also there was an increase percentage the maintenance of monocytes up to $69,98\pm0,35\%$ in the third group, in the absence of changes of their absolute quantity.

The percentage and absolute maintenance of lymphocytes has increased in the second group to $72,54\pm0,50\%$ and $7,75\pm0,18\times109/L$, respectively. Reliable changes in the level of monocytes, eosinophils were noted.



Figure 2 - Hematologic indicators of serum of blood of seals

Thus, increase of total number of leukocytes, decrease the percentage of neutrophils by 2,5 times in the 4th group, growth of percentage of lymphocytes by 30 times is revealed. In the third group decrease in neutrophils and increase of lymphocytes is noted. There was an increase in percentage of eosinophils in the second group up to 4,89±0,18%, at the same time their absolute contents didn't increase. In the fifth group the percentage and absolute maintenance of lymphocytes increases, also increase in level of monocytes and eosinophils indicates influences an organism of animals toxic or of the allergic substances.

Conclusion. As a result of the conducted scientific researches the following conclusions have been drawn:

- content of the general protein in the fifth group has gone down for 11% of norm, and the level of albumine has gone down in the first group for 85%, in the second group for 45%, and in the third group for 50%, in the fourth group for 46% and in the fifth group for 91% in comparison with the normal content of albumine in blood. At a liver failure it is broken synthesis by hepatocytes of albumine and the hypoalbuminemia is shown. Hypoalbuminemia contributes to the development of hypostases and formation of ascites (in the conditions of increase in pressure of blood in vessels of a vorotny vein);

- level of urea has gone down in the first group for 65%, in the second group for 73%, in the third group for 72%, in the fourth group for 30%, in the fifth group for 54% in comparison with the normal level of urea. Decrease in concentration of urea at intoxication indicates the pathological changes in a liver leading to urea synthesis violation. Decrease in level of urea can be caused as a hypoproteinemia (synthesis of urea is closely connected with disintegration of nucleotides), and a hypophosphataemia;

- activities of ALT has increased in the second group by 24%, in the fourth group for 79%, in the fifth group for 38%, and AST has increased in the 2nd group by 10%, in the fourth group by 1,3 times and also in the fifth group by 1,2 times in comparison with normal amount of transferases in blood. Increase in activity hepatic transaminase speaks about processes of death of hepatocytes owing to chronic toxic defeat and increase in blood of level of the studied enzymes;

- ahypophosphataemia (contents a phosphoditch has decreased to 0,87 mmol/l) it is caused by toxic damage of a liver (poisoning with salts of heavy metals);

- to a hemocytogramm of animals increase of total number of leukocytes, decrease the percentage of neutrophils by 2,5 times in the fourth group, growth of percentage of lymphocytes by 30 times is revealed. In the third group decrease in neutrophils and increase of lymphocytes is noted. There was an increase in percentage of eosinophils in the second up to 4,89±0,18%, at the same time their absolute contents didn't increase. In the second group the percentage and absolute maintenance of lymphocytes increases, also increase in level of monocytes and eosinophils indicates influences an organism of animals toxic or of the allergic substances.

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КАСПИЙ ИТБАЛЫҚТАР ҚАНЫНЫҢ БИОХИМИЯЛЫҚ ЖӘНЕ ГЕМАТОЛОГИЯЛЫҚКӨРСЕТКІШТЕРІН ЗЕРТТЕУ

Түйін: Мақалада соңғы бес жылда (2012-2016 жж.) каспий итбалықтардың биохимиялық және гематологиялық параметрлерін зерттеу нәтижелері келтірілген. Биохимиялық қан көрсеткіштері BioChem SA биохимиялық анализаторында және гематологиялық көрсеткіштер Siemens ADVIA 2120 автоматты анализаторында анықталды. Зерттеу нәтижелері бойынша бірінші топтағы итбалықтар қанындағы мочевина деңгейінің 65% -ға, ал екінші топта 73% -ға, үшінші топта 72% -ға, төртінші топта 30% -ға төмендеуімен сипатталды. Қан құрамындағы мочевина концентрациясының төмендеуілері, бауырдағы патологиялық өзгерістердің нәтижесінен мочевина синтезінің бұзылуын байқатады. Мочевина деңгейін төмендеуі гипопротеинемиямен және гипофосфатемиямен байланысты болуы мүмкін. АлАТ белсенділігі 79% -ға өсті, ал АсАТ қандағы трансферттердің қалыпты мөлшерінен 1,3 есе жоғары болды. Бауырдың трансаминаз белсенділігі 79% -ға өсті, ал АсАТ қандағы трансферттердің қалыпты сәйкес, лейкоциттердің қалы салдарынан гепатоциттердің өлімін көрсетеді. Жануарлардың гемоцитограммасына сәйкес, лейкоциттердің жалы салдарынан гепатоциттердің қалы байқалады. Екінші топта ғы зақымдандағы байқалады, ал олардың асолюттік мөлшері қалыпты шама көлемінде болды. Екінші топта лимфоциттердің қалыпты шама көлемінде болды. Екінші топта лимфоциттердің қалыпты шама көлемінде болды. Екінші топта акалы артты, сондай-ақ моноциттер мен зозинофилдер деңгейінің жоғарылауы, жануарлар ағазаларына улы немесе аллергиялық заттардың әсерлерін көрсетеді.

Сонымен, каспий итбалықтары қанының биохимиялық және гематологиялық көрсеткіштері бойынша бауырдың улы зақымдануларының байқалуы, оның қызметінің бұзылуымен сипатталады деп қорытынды жасауға болады. **Түйінді сөздер:** каспий итбалықтары, биохимиялық анализ, гематологиялық анализ, эксперимент.

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ИССЛЕДОВАНИЕ БИОХИМИЧЕСКИХ И ГЕМАТОЛОГИЧЕСКИХ ПОКАЗАТЕЛЕЙ КРОВИ КАСПИЙСКИХ ТЮЛЕНЕЙ

Резюме: В статье представлены результаты изучения биохимических и гематологических показателей каспийских тюленей за последний пять лет (2012-2016 г.г.). Биохимические показатели крови определяли на биохимическом анализаторе BioChemSA, а гематологические показатели на автоматическом анализаторе SiemensADVIA 2120. По результатам исследования было выявлено, понижениеуровня мочевины в первой группе на 65%, во второй группе на 73%, в третьей группе на 72%, в четвертой группе на 30%, Понижение концентрации мочевины при интоксикации указывает на патологические изменения в печени, приводящие к нарушению синтеза мочевины. Снижение уровня мочевины может быть обусловлено как гипопротеинемией, так и гипофосфатемией. Активности АлАТ повысилось на 79%, а АсАТ в 1,3 раза по сравнению с нормальным количеством трансфераз в крови. Повышение активности печеночных трансаминаз говорит о процессах гибели гепатоцитов вследствие хронического токсического поражения и повышении в крови уровня исследуемых ферментов. По гемоцитограмме животных выявлено возрастание общего числа лейкоцитов, снижение процентное содержание нейтрофилов в 2,5 раза в четвертой группе рост процентного содержания лимфоцитов в 30 раз. В третьей группе отмечается снижение нейтрофилов и возрастание лимфоцитов. Выявлено повышение процентного содержания эозинофилов во второй группе до 4,89±0,18 %, при этом их абсолютное содержание не повышалось. Во второй группе процентное и абсолютное содержание лимфоцитов увеличивается, также повышение уровня моноцитов и эозинофилов указывает на влияния организм животных токсических или аллергизирующих веществ.

Таким образом, по биохимическим и гематологическим показателям крови каспийских тюленей, можно сделать вывод о токсическом поражении печени, что проявляется в нарушении функции этого органа.

Ключевые слова: каспийский тюлень, биохимический анализ, гематологический анализ, эксперимент.