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IMMOBILIZATION STRESS INFLUENCE ON LEUKOPOIESIS INDEX AND ON ACTIVITY CHANGES OF PROLINE BIOSYNTHESIS ENZYMES

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In the article immobilization stress influence on leukopoiesis index, as well as on activity changes of proline biosynthesis enzymes on rabbit are studied. At the first stages of immobilization stress action increase of quantities of left bent neutrophilose nucleus and lymphocytes was observed, due to their quick access from bone marrow to peripheral blood: granulocytes bone marrow reserve, granulocytes bone marrow reserve, spleen and thymus lymphocytic cells stressor mobilization. Neutrophenia, eosynophenia and basophenia were also observed, during immobilization stress action duration increasing. Young and mature neutrophils quantity decrease has been observed in myelogram, that is the consequence of pressure proliferative and maturation processes of leukoblastic spire.

The highest enzyme activity in natural conditions was registered in liver and adrenal glands, whereas in conditions of immobilization stress the enzymes' activities were changed and were detected in rabbit brain.

***Keywords*:** immobilization stress, leukopoies, ornithine transaminase, proline-5-carboxylase reductase.

Introduction. Normal vital activity, is possible only with sufficient background mobility, which has a favorable effect on the natural physiological functions of the organism [1, 2]. Abrupt changes in modern lifestyle of a human have restricted mobility activities. Relative immobility and mental tension have caused various stress conditions that became the reason of many diseases. At the initial stage of any stress functional impact the system of hypothalamus––hypophysis–adrenal gland is involved, the quantity of biologically active substances in organism increases, the level of oxidation reduction processes are changed, which have a great role in providing the organism lifestyle and adaptation to exchanged environmental conditions. That is why the study of emotional stresses became very important. The immobilization stress is in the row of these stresses. The last one almost cannot be appeared without physical action. A lot of studies describe the standards of non-specific reaction regularities of hemopoietic organs during the action of various stresses, which is expressed at 3 *h* after the stressor action end and is revealed by neutrophile leukocytosis, lympho-eosinophile,

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decreasing the mature granulocytes in bone marrow and by increasing of lymphoidal cells [3–5]. Dinamic investigation of stress action on periphery blood cell composition and bone marrow has an important role in understanding the mechanisms of organism adaptation to stressor infection and for developing stress limited arrangements. Not only physiological, but biochemical changes arise in stress situations. That's why the study of aminoacids metabolism and the activity of enzymes taking place in this metabolism, are important. It is known that amino acids are the precursors of numerous biologically active substances that regulate the physiological functions of organism and provide the hystological stability of organ system. The importance of the role that amino acid of proline is playing in organism adaptation processes to stress factors is suggested. This amino acid help to increase organism adaptation to stresses and can improve human's health, but it action mechanism is not completely revealed yet.

The aim of this paper was to study the character of peripheric white blood index and cell changes in bone marrow leukoblastic shoot at dinamycs of immobilization action, as well as proline quantities changes, especially the activity of enzymes ornithine transaminase and proline-5-carboxylate reductase, taking part in proline biosynthesis in different organs (kidney, liver, adrenal gland, brain, heart) of animals submitted to stress.

Materials and Methods. Investigations were carried out on rabbits with the same sex, weight 2.5–3 *kg* and at the same care. To make the animals immobile during 30 days (3 *h* daily) they were closely fixed to the board.

The total number of leukocytes in 1 mm^3 blood, leukocytes formula and profile according to Maskovski, cell composition of leukoblastic spire, marrow indexes of neutrophile maturation were studied according to standards during the dynamics (5, 10, 15, 20, 25, 30 days) of immobilization stress action. Blood smear has been prepared, which has been fixed by ethyl alcohol for ten minutes, then was painted with Azur-eosyne for 40 *min* (by Romanovski method). To estimate the absolute quantity of various leukocyte types in 1 mm^3 blood, the relative percent of the mentioned type was multiplied by the quantity of leukocytes being in 1 mm^3 blood of the same day and divided into one hundred. To estimate the cell composition of leukoblastic spire the marrow has been taken out of animal thighbone epiphysis sector (10, 20, 30 days) with A. Kassirski needle, smear has been prepared, fixed and painted by Romanowski method. 400 cells have been counted, where the leukoblastic spire cells have been distinguished. The marrow index of neutrophile maturation was deduced, which is the ratio of young granulocytes to mature neutrophiles.

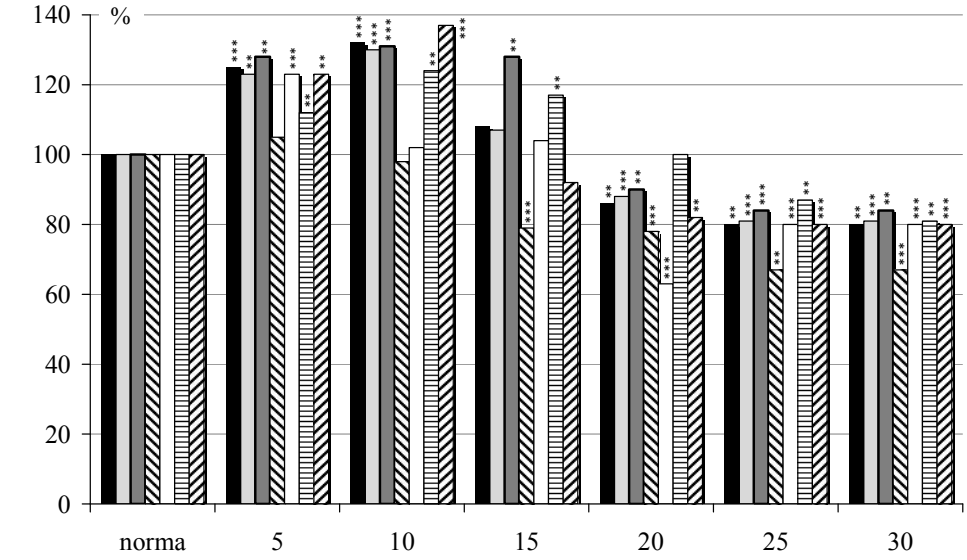
To estimate the activity of enzymes of proline biosynthesis, the 5% homogenate has been prepared. The homogenization was carried out in potassium––sodium–phosphate buffer (pH 7.4). The incubation mixture, which contained 50 *mM* ornithine, 50 *mM* α-ketoglutarate, 100 *mM* potassium–sodium–phosphate buffer (pH 7.4), 1 *mM* pyrodoxal-5-phosphate reductase, 0.5 *mL* homogenate has been prepared. The incubation was carried out at 37°*C* during an hour. This period the environment ornitine under transaminase action was transformed into proline-5-carbonoacid, then was incubated again for 15 *min* and proline-5-carbono-acid under pyroline-5-carboxilase action was transformed into proline. The reaction was stopped by three chlorine acetic acid. The patterns were centrifuged at 8000 *g*

for 10 *min*. Enzyme activity was determined according to the formatted proline quantity. Proline quantity was estimated by chemical method. 1 *mL* of ninhydrin reagent (3 *g* ninhydrin dissolved in 180 *mL* of glacial acetic acid and 20 *mL* of formalin) was added to 1 *mL* of pattern. The mixture was heated at 100ºC for 1 *min* or at 75ºC for 4 *min* in water bath, frozen and the red color has been measured by photoelectric calorimeter at 490 *nm* wavelengths. Proline was used as a standard.

The obtained results were subjected to statistical analysis by "Biostat" program. The veracity was estimated according to Student *t*-standard.

Results and Discussions. The investigation has shown that the real location leukocytosis is observed on the 5th day of immobilization stress action. The total number of leukocytes are increased by 26% from initial level ($p<0.001$). The left bent of neutrophilose nucleus has been observed in leukocyte formula. The quantity of segmented neutrophiles was 3718±133 (129%, $p<0.001$), and the quantity of stab neutrophiles was 126% ($p<0.01$) (see Figure). At the mentioned period basophiles, monocytes and lymphocytes quantities increase has been observed: 127% ($p<0.001$); 126% ($p<0.02$); 126% ($p<0.01$) respectively, but eosynophile quantity was in initial level limits 234 ± 8, at standard 225 ± 7.

The increase of total number of white blood cells in condition of 10 days of immobilization stress action has been continued and was 136% ($p<0.001$). The left bent of neutrophilose nucleus 136% ($p<0.001$), the high level of monocytes 126% ($p<0.01$) and lymphocytes 139% ($p<0.001$) is preserved in leukocyte formula.



The immobilization stress action on the character index of the periphery white blood:
** – $p < 0.01$, $p < 0.02$; *** – $p < 0.001$; others – $p < 0.05$.

The quantity of leukocytes in 1 mm^3 blood; stab neutrophiles; segmented neutrophiles; eosynophiles; basophiles; monocytes; lymphocytes.

It should be noted that eosynophiles quantity was not significantly changed, but basophiles quantity has decreased by 24% and was 102% comparing to basophile quantity of the previous day. Polymorph nuclear types of lymphocytes have

been found in blood smear, particularly narrow cytoplasm forms are increased and wide cytoplasm forms are decreased. The last one is evidence of lymphocytic system activation, which is directed to maintaining homeostasis and reflects the adaptive processes tension. At the mentioned time in myelogram the quantity of young neutrophiles is moderately reduced and lymphocyte quantity significantly increases by 63%, 18 ± 0.24 ($p<0.001$) comparing to standard 11 ± 0.21 (Tab. 1).

Table 1

Immobilization stress action on cells of the leukoblastic shoot (neutrophiles and lymphocyte)

Indexes	Initial data level	The days of investigations		
		10	20	30
young neutrophiles (promielocytes, myelocyte, metamyelocyte)	25 ± 0.82	23 ± 0.81	20 ± 0.36 p<0.001	20 ± 0.31 p<0.01
mature neutrophiles (stab segmented neutrophiles)	31 ± 0.89	30 ± 0.76	27 ± 0.74 p<0.02	28 ± 0.76 p<0.05
marrow index of neutrophiles maturation	0.8	0.7	0.6	0.7
lymphocytes	11 ± 0.21	18 ± 0.24 p<0.001	20 ± 0.19 p<0.001	17 ± 0.21 p<0.01

In peripheral blood the increase of neutrophiles and lymphocytes in the case of 10 days action of immobilization stress is due to their quick access from bone marrow to peripheral blood, apparently: granulocytes bone marrow reserve and spleen and thymus lymphocytic cells stressor mobilization in case of stress provide cell composition of peripheral blood [6]. On the 15th day of investigation the total number of lymphocytes comparing to previous days decreased and became 109%. The reliable decrease 80% ($p<0.01$) of eosynophile quantity is found in leukocyte formula. The quantity of stab neutrophiles, basophiles and lymphocytes comparing to previous days, decreased and became 109, 106% respectively, the high quality of mature neutrophiles and monocytes respectively is preserved (see Figure).

Concomitant to immobilization stress action length increase the gradual lowering of leulocytes quantity is observed. So, on the 20th day of investigation the total number of leukocytes comparing to baseline level decreases by 14% ($p<0.01$). Neutrophenia (young as well as mature neutrophiles quantity decreases), eosynophenia and basophenia: 73% ($p<0.001$), 64% ($p<0.001$) respectively were viewed in leukocyte formula. Monocytes quantity comparing to 15th day decreased and was in initial level sets.

Both young and mature neutrophiles quantity decrease has been observed in myelogram, that is the consequence of pressure proliferative and maturation processes of leukoblastic spire. The marrow index of neutrophils maturation comparing to standard 0.8 was 0.6. During the following days (25–30) of investigation the leukocytes quantity stability on low level 82% ($p<0.01$) was observed. At mentioned days the expressed lympho-eosinophenya was observed. Lymphocytes quantity became low than initial level by 20%, and eosinophiles

quantity by 33%. Basophiles quantity also were at low level 81% ($p<0.001$). The important role of basophiles in local blood flow and in processes of capillary transparency regulation is well. In case of immobilization stress basophile pressure brings to micro-vessels function regulation local mechanisms disruption. On the 30^{th} day of investigation the high level of lymphocytes and low quantity of young and mature neutrophiles were preserved in bone marrow (17 ± 0.21 ($p<0.001$), 20 ± 0.31 ($p<0.01$), 28 ± 0.76 ($p<0.05$)). The marrow index of neutrophiles maturation was 0.7. These results correspond to literature data [7, 8]. It has been shown that immobilization as stress factor decreases organism resistance, the quantity of lysozyme, complement, causes movement in immume system [9]. At the same time neutrophile and lymphocyte activity decreases, hydrolytic enzymes activity increases in neutrophiles, which can cause cytolitic processes development. Such diverse reconstructions in neutrophiles are estimated as organism natural protective forces depression [10].

Proline quantitative changes, especially, activities of enzymes: ornithine transaminase and proline-5-carboxilate reductase, which take part in proline biosynthesis, were studied in various organs: liver, heart, kidney, adrenal gland and brain of animals, that were exposed by immobilization stress in the next variant of experiments. Investigations have shown (Tab. 2), that proline biosynthesis carried out intensively in natural conditions in liver and adrenal gland, correspondingly being 7.5 and 4.5 *mM* proline in 1 *g* of fresh tissue, but the synthesis intensivity in kidney and heart comparing to liver was less respectively 9 and 3. In conditions of immobilization stress transaminase and praline-5-carboxilate reductase enzymes activities were exposed to changes, especially it was observed in rabbit brain, as if in standard conditions activity was not revealed in nervous tissue, but the mentioned enzymes activity was revealed under stress action being 1.255 *mM* proline in 1 *g* tissue. Proline biosynthesis comparing to standard conditions activates by 221% in kidney under stress action, in heart by 68%, but in adrenal gland and liver activity is observed, in liver by 52% and in adrenal gland significantly.

Table 2

Immobilization stress action on activity of proline biosynthesis enzymes in the different organs of rabit (1 μM proline per 1 g tissue, n=5, p < 0.05)

Organs	Activity of enzymes	
	control animals	animals under stress action
kidneys	0.78 ± 0.05	2.51 ± 0.22
liver	7.5 ± 0.4	3.6 ± 0.26
brain	0	1.255 ± 0.12
heart	2.5 ± 0.2	4.2 ± 0.23
adrenal gland	4.5 ± 0.25	0.31 ± 0.05

The results are obtained can confirm the data of other investigators that increase of proline amino acid in stress conditions can have adaptive meaning. So, it is shown that at stress praline, probably, makes three main functions: appear as metal binding compound, antioxidant molecule and take part in signal transformation, promoting formation of active oxygen forms in mitochondrium

electron transfer chain [11]. It is suggested that multifunctional enzyme prolidase can be very important in proline accumulation mechanism it splits proline or oxiproline residues from C-end of proteins. Prolidase enzyme has been isolated from different bacteria, it is in cytoplasm in Mammalia, and the main role is to isolate proline, especially during collagen decay. Polidase enzyme deficiency can bring to a lot of clinical symptoms [12].

So, the results were obtained in present work, as well as the discussion of literature data have shown that at the initial phase of immobilization stress action the compensatory defense mechanisms of the organism are mobilized, which provide the vital activity of organ systems by means of using the functional reserves, but the prolonged impact brings to strain of regulatory mechanisms and consequently brings to the decrease of capacity of organism reserves.

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