EFFECT OF VIBRATION STRESS BOTH ON THE SHIFTS OF SOLUBLE PROTEIN FRACTIONS FROM RAT LIVER AND ON THE ARGINASE I ACTIVITY

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Vibration pathology is in the second place among diseases connected to the profession. Wide spread of vibration factor in industry significantly damages the organism health, and it conditions the social importance of the problem. The paper presents the results of studies of the vibration effect both on the protein fraction shifts and on the activities of I type arginase (Arg) IA and IB from white rat liver under the conditions of 5-, 10- and 15-day vibration state. In the initial state of vibration some decrease of Arg IA and Arg IB activities was revealed as compared to the control without general proteinspectrum change of liver extract. Along with vibration effect, the expression level of Arg IA was increased with duration enhancement, which possibly is due to hyperammonemia, in the result of which ureagenesis is stimulated. Stimulation of easily available adaptation reserves were described due to protein catabolism. Analysis of shifts of general and enzyme protein fractions in the conditions of vibration effect allows to reveal their qualitative and quantitative changes and to make important the organism adaptation reactions that result from anabolic and catabolic process relations. It is possible that the revealed changes in the activity of arginase IA and IB are a biologically expedient mechanism for regulating the activity of hepatic arginase during vibration stress.

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Introduction. Vibration (mechanical oscillations) is one of the most spread environmental factors affecting living organisms, which is accepted by all cells of organs and tissues; this effect is reached to capillary level, accompanied by deviation of input and reduction processes of oxygen. Addition of amount of oxygen active forms lies at the basis of vibration effect mechanism, which contributes to tissue hypoxia formation in organism [1]. In the conditions of oxygen unsatisfactory, the biological oxidation processes are suppressed and, as a consequence, the amount of ATP and creatine-phosphate are decreased; the amount of ADP, AMP and inorganic phosphate are increased. The energy unsatisfactory is formed and due to this glycolysis stimulation, incompletely oxidized products are accumulated, and

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Acidosis is developed. Acidosis stimulates lipolysis, proteolysis and non-enzymatic hydrolysis of proteins, but re-synthesis of lipids, proteins and nucleic acids, vice versa, is suppressed. As a result of this ketosis is emerged, negative azotic equilibrium is formed [2]. Due to hypoxia, the formed metabolic disorders inevitably lead to specific and non-specific deviation of cells and in severe cases even to irreversible structural damages [3, 4].

Character of changes in organism in relation to various stress factors is possible to understand in the result of revelation of biochemical processes taking place only in these conditions. Stress syndrome is non-specific component of an urgent adaptation, which plays a role of a mobilizing factor, contributing to activation of energetic and plastic store to realize the specific adaptation transformations in organism [5].

Wide spread of vibration factor in production sufficiently damages the organism healthy state, conditioning the social importance of this problem. According to the conductive mode, vibration is divided into general and localized forms [6]. Vibration is observed as a strong stress factor, which has a negative influence on the psychic-mobile workability, emotional field and human mental activity. In the case of increasing of both oscillation frequency and its effect duration, changes are formed that in most cases lead to pathology – vibration disease formation [7, 8].

Vibration pathology is in the second place among deceases, connected to profession. Displaying stress effect, vibration stimulates pituitary-adrenaline system, contributing to activity of redox processes and particularly altering protein exchange processes that play an important role in providing of organism non-specific resistance [6]. Proteins are the most complicated structure having organic compounds, entering into organism consistence and are the most important ones from the biological point of view. Study of protein metabolism state in the conditions of influence of various stress factors is of great interest. Disorders of activity of organism different systems are maintained by many investigators, but data on the vibration effect on protein exchange are confined and it serves as a basis to examine the general vibration influence on the movement of individual protein fractions.

Taking the fact into account, that azotic exchange is a prominent ring of metabolism regulation process and the study of azotic exchange regulating mechanisms is one of important questions, in particular, the mechanism of neutralization of ammonia in the liver is the ornithine cycle, one of the key enzymes of which is arginase (Arg) I.

I type Arg (EC3.5.3.1) that is an enzyme, containing binuclear Mn$^{2+}$, cleaves L-arginine to urea and L-ornitine. Arg is an oligomer enzyme, consisted of 3 similar subunits with 35–37 kDa molecular weight, in the active center of each subunit, on the floor of crevice with 15Å depth there are Mn$^{2+}$ binuclear clusters – MnA$^{2+}$ and MnB$^{2+}$ [9].

The aim of the study was the revelation of the vibration effect on both generally soluble protein consistence of rat liver and arginase activity of I type. These data are novel and can be useful for understanding mechanisms of vibration effects on animals and human.
Materials and Methods.

Subjects and Materials. White, male rats, with 180–200 g weight, were used in experiments. Rats were kept in vivarium at 18–23°C, 12/12 h night/day relation and fed by standard diet. Animals received water and food without any confinement. Animals were exposed to decapitation that was realized after anesthesia by ester-chloroform mixture. Biological ethics principles were preserved according to European Union Instruction (86/609 EEC) and Helsinki declaration, and the methods have been approved by National Bioetics Committee (Armenia). For experimental works, the animal liver was used that was homogenized by Potter-Elvehjem type glassy homogenizator at +4°C (Potter-Elvehjem Glass, Germany) by 0.005 M Tris-HCl buffer (pH 7.4).

Homogenates were centrifuged by 20000 g, 30 min, at +4°C (CLR-1, RF).

Vibration Method. Vibration was realized on special vibrostent, having sonic-isolated cell. The animal whole organism was exposed to vibration. Vibration deviation amplitude was equal to 0.4 mm. Vibration frequency was 60 Hz, which corresponds to vibration average frequency (31.5–63.0 Hz). Effect direction was horizontal. The animal was exposed to vibration 2 h daily during 5, 10 and 15 days.

At data analysis, vibration sum dose levels were used that reflect the accumulation portion of vibrational effect. These criteria are analogous in physical meaning to international standards used for vibration.

Arginase Activity and Enzyme Fractionation Assays. Arg activity was determined by Rathner method with some changes [10]. Prepared in 5% homogenate in 0.005 M Tris-HCl buffer. Homogenates were centrifuged by 20000 g, 30 min, at +4°C (CLR-1, RF). In the experimental sample, 2.4 mL of 0.01 M NaOH-glycine buffer, 0.2 mL of MnCl₂ × 4H₂O (5 μM)-0.4 mL of L-arginine were added (50 μM). The control sample contained all reagents except L-arginine, which instead contained distilled water. Samples were incubated at 37°C for 60 min. The enzyme activity was stopped by adding 1 mL of 10% TCA.

Enzyme activity was expressed by urea μmol released from 1 g protein. For determine urea, 1 mL of an enzyme preparation, 2.5 mL of an acid mixture (1 part of concentrated H₃PO₄, 3 parts of concentrated H₂SO₄, FeCl₃ l. MnSO₄) and 0.25 mL of a 1.5% solution of diacetylmonooxime were poured into a glass test tube for the determination of urea. The tubes were shaken and the contents were boiled in the dark for 45 min in a water bath. Frozen yellow samples are color imitated with a spectrophotometer (Genesys 10S UV-VIS, Thermo Scientific, USA) at a wavelength of 478 nm.

Enzyme fractionation and determination of the molecular mass of proteins was realized by gel-filtration mode (Sephadex G-200, Pharmacia, Uppsala, Sweden). Balancing and elution were done by 0.05 M tris-HCl buffer (pH 7.4), the elution volume was equal to 4 mL (flow rate of 24 cm/h). After fraction gathering, the protein absorption was measured at 280 nm wavelength and arginase activity was determined in each sample by spectrometric mode.

To determine the V₀ value (free column volume) was used a 0.2 M blue dextran solution (Blue dextran 2000, Pharmacia, molecular weight 2×10⁶ Da). Soy urease (480,000 Da), yeast alcohol dehydrogenase (150,000 Da), human serum albumin (75,000 Da), pepsin (35,000 Da), trypsin (23,000 Da), and ribonuclease (12,000 Da)
were used as marker proteins. Calibration curves were obtained by gel-filtration mode, where the molecular weights of the marker proteins were determined by means of the elution volume (Fig. 1).

Data Processing and Reagents. Statistic treatment of data was realized using Student Fisher differences and liability method [11]. The reagents of analytical grade were used throughout.

Results and Discussion. Fractionation of extract from white rat liver was carried out by gel-filtration mode in natural conditions (control sample) and after vibration exposure of animals during 5, 10 and 15 days. The analysis data are presented in Figs. 2–5.

Fig. 1. Determination of the molecular mass of arginase of the liver of white rats by gel-filtration mode (Sephadex G-200). Marker proteins: 1. urease; 2. alcohol dehydrogenase; 3. human serum albumin; 4. Pepsin; 5. Trypsin; 6. ribonuclease.

Fig. 2. Fractionation of rat liver extract by gel-filtration mode (Sephadex G-200) before vibration stress. Protein absorption was measured at 280 nm wavelength and arginase activity was expressed by urea μmol released from 1 g protein.
As it is seen from Fig. 2, here are 3 protein fractions (I, II and III) and 2 isoforms of I type Arg (Arg IA, with molecular weight 12 kDa and Arg IB – 42 kDa).

It is seen from Fig. 3 that in the result of 5-day vibration stress maximal values of Arg IA and Arg IB activities were decreased somehow, as compared to the control variant, but the total activity was increased though, the general protein spectrum of liver extract was not changed. Presumably, in conditions of 5-day stress, biochemical adaptation processes are developed in organism that, according to literature data, it
took place by 2 phases [7]. The first phase is an urgent adaptation period, which corresponds to 5-day stress in our experiment and is characterized by mobilization of surface adaptation possibilities of organism in conditions of hypoxia. Despite the direction of biochemical processes, the first step is catabolic one, when in living organism a hypo-energetic state is formed.

Along with increasing of vibration effect, duration the fractionation scenario of rat liver proteins thoroughly was changed (Fig. 4). It was pronounced that the expression level of Arg IA was enhanced which is connected with ureagenesis stimulation and possibly results from oxidative stress emerged due to vibration [12]. Arg IB was repressed. Induction of arginase IA is possible to result from hyperammonemia that is formed by excess amount of ammonium in blood. Hyperammonemia is formed by various factor effects, among these factors the most spread ones are conditioned by liver disorders. Liver transaminates amino-acids to L-glutamine-acids, thus conditioning amino-acid metabolism, then L-glutamine-acid is transported to liver mitochondria and deaminated by glutamate dehydrogenase. This process is accompanied by free ammonium release, which immediately turns to urea and passes to cytosol by cyclic mode. Ammonium formed by this way is synthesized by liver mitochondrial metabolism and does not affect the system ammonium quantity. System ammonium is a side product of urea, glutamine and alanine exchanges. Significant accumulations of non-system free ammonium lead to urea synthesis acceleration and the latter can take participation to complex-formation with proteins and other polymers [12]. Due to the aforementioned processes, according to our data, possibly the ornithine cycle is activated, which particularly is expressed by arginase IA activity increasing.

Absence of the third piece of general proteins in conditions of 10-day vibration stress is worthy to be paid attention to; presumably it is connected to catabolism activation of proteins due to stress effect. Most probably, in the conditions of 10-day stress, the observed changes coincide with the phenomena taking place during the long-term adaptation, described by some authors [2], when catabolism of easily available reserves is realized, which permits relieving non-favorable metabolism shifts that were formed due to the stress effect. This stimulates mobilization of organism adaptation possibilities. During the intensive effects of stress factors, pronounced changes of protein metabolism occur, moreover, the revealed changes do not have to be directly connected to the acting factor, but they are only a result of non-specific adaptation mechanisms [12].

As it is seen from Fig. 5, in the 15th day of vibration effect, the adaptation possibilities were sharply decreased, and the vibration pronounced negative effect was revealed. Activity of Arg IA was significantly decreased and functional unsatisfactory of arginase is noticed. The third fraction of Arg IB and general proteins was not determined. Protein fraction shifts in animal liver, exposed to 15-day vibration, are possible to be connected to number of factors – energetic exchange decrease, unsatisfactory of cytoskeleton and protective proteins (multi-chaperon system action).
In the case of the studied stress factor – vibration, the organism adaptation reactions have an important value [3]. Along with quantitative shifts of liver protein composition possibly the qualitative changes also occur. Presumably, in stress conditions, phase changes occur, during which relation between anabolic and catabolic processes changes, deviating to direction of the latter. The described processes can be accompanied by modification of protein physicochemical properties, particularly, by protein phosphorylation [2].

**Conclusions and Significance.** Considering the fact that there are no data in the literature on the effect of vibration stress on changes in the fractional composition of liver proteins, and in particular arginase I, we can state that the data on the changes in Arg activity, we obtained for the first time, shed light on some aspects of this problem.

Thus, the character of concentration change of liver general and enzyme proteins can give information about tissue and cellular damages in vibration stress conditions and the identification of protein fractions and further analysis of data will allow to elucidate the examined process character and to open new perspectives for creation of various therapeutic measures.

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Влияние вибрационного стресса на сдвиги фракций растворимых белков и активность аргиназ I типа печени крьс

Вибрационная патология занимает второе место в ряде заболеваний, связанных с профессиональной деятельностью. Широкое распространение вибрационного фактора в промышленности наносит вред организму, чем и обусловлена социальная значимость проблемы. В работе представлены результаты исследовений воздействие общей вибрации на сдвиги белковых фракций и активность аргиназ I типа (IA и IB) печени белых крьс в условиях 5-, 10- и 15-дневной вибрации. В начальной стадии вибрационного воздействия обнаруживалось определенное снижение активности аргиназы IA и IB по сравнению с контрольной группой на фоне неизменного общего белкового спектра экстрактов печени. По мере удлинения воздействия вибрации наблюдалось увеличение уровня экспрессии аргиназы IA, что, вероятно, было обусловлено гиперамонемией и усиленiem уреагенеза. Обсуждается роль стимуляции легкодоступных адаптационных резервов за счет катаболизма белков. Анализ сдвигов общих и ферментных белков в условиях вибрации позволил обнаружить качественные и количественные изменения адаптационных реакций, которые обусловлены взаимодействиями анаболических и катаболических процессов. Не исключено, что выявленные изменения активности аргиназы IA и IB являются биологически целесообразным механизмом регуляции активности печеночной аргиназы при вибрационном стрессе.