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ANIMAL AND HUMAN PHYSIOLOGY

Correction of Metabolic Processes in Rats during Chronic Endotoxicosis Using Isotope (D/H) Exchange Reactions

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Abstract—The effect of isotope exchange reactions (deuterium/protium, D/H) on morphofunctional indices and the state of the antioxidant blood system in rats was studied under physiological conditions and during experimental chronic endotoxicosis of hepatorenal genesis. It was demonstrated that introduction of water with a decreased content of deuterium in the food rations of rats results in a decrease in its concentration in the blood plasma by 32–36% (to 98–106 ppm) and in lyophilized liver, kidney, and heart tissues by 13–17% (to 123–128 ppm). It was noted that it is accompanied by correction of metabolic processes, an increase in the functional activity of nonspecific protection system, and an increase in the body weight growth by the 42nd day in the group of animals that passed (for 14 days) the stage of preliminary adaptation with a change in the D/H ratio in the organism.

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INTRODUCTION

In recent years, many scientific papers on the study of isotope exchange reactions (which are able to modify significantly the functional activity of organs and tissues, as well as affect the nonspecific protection system) have been published. It is possible that the effect is realized through an increase in the adaptive possibilities of the organism, especially during pathological and some physiological states characterized by an imbalance in the work of prooxidant–antioxidant system (which requires the appropriate metabolic correction) (Somlyai, 1993; Cong et al., 2010; Olariu et al., 2010; Baryshev et al., 2012; Basov and Bykov, 2013; Dzhimak et al., 2014). The involvement of active oxygen forms and free radicals in nonspecific regulation of many processes is associated with energy exchange, apoptosis regulation, synthesis of biologically active substances, and some other manifestations of vital activity was proven (Fridovich, 1999; Boldyrev, 2001; Dubinina, 2001; Kohen and Nyska, 2002; Vladimirov, 2004; Men'shchikova et al., 2006; Reddy, 2008; Skulachev, 2009).

Modeling of chronic endotoxicosis (CET) and oxidative stress is used in the experimental practice for study of the nonspecific protection system of an organism and for objective assessment of the efficiency of correcting medical measures (Novochadov and Pisarev, 2005; Chesnokova et al., 2006; Basov et al., 2012). Use of the most objective criteria of estimation of the intensity of metabolic disorders is especially important during such studies. They include CET criteria such as the content of creatinine, urea, and bilirubin in the blood. The activity of aspartate aminotransferase (AsAt), alanine aminotransferase (AlAt), alkaline phosphatase (AP), total protein level, and the cholesterol content (CC) are determined for estimation of the functional state of the liver. In addition, indices characterizing the work of the antioxidant component of the nonspecific protection system (antioxidant activity of the blood plasma and number of reduced thiol groups (SH-groups) and functional status of the individual organism) are estimated for this purpose. The latter is substantially caused by the effect of external conditions, including the composition of the food ration also used in the experiments for regulation of the isotope exchange (deuterium/protium, D/H) reaction rates. Such studies became possible due to the development of modern technologies of obtaining food substances with a given ratio of light and heavy isotopes (Baryshev et al., 2012; Basov et al., 2014; Dzhimak et al., 2014).

It is known that water with a modified isotope D/H-composition with a decreased deuterium content (WMIC DDC) is a water ($^1H_2{}^{16}O$) isotopologue generated by light stable isotopes of the elements included in its composition. No such "pure" water ($^1H_2{}^{16}O$) exists under natural conditions; for the isotopologue obtained, fine multistage purification of nat-

ural waters is conducted or water from the initial $^{1}H_2$ and ^{16}O elements is synthesized (Yeh, 2010; Baryshev et al., 2013). Introduction of WMIC DDC in the food ration results in a decrease in the D/H ratio in the tissues of the organism due to isotope exchange reactions that are mainly activated due to a change in entropy in the living system. At the same time, the effect of isotope exchange reactions on biochemical processes and the state of the organism remains poorly studied due to the small rate of these reactions under physiological conditions (which is caused by the phenomenon of the matter isotope composition constancy in the natural habitat (West et al., 2006). Therefore, targeted development of the isotope D/H-gradient in an organism can be used for an increase in its adaptive properties under conditions caused by the effect of continuous nonspecific stress on the humoral and cellular protective systems (Lisicin et al., 2014).

The aim of this study is investigation of the effect of WMIC DDC on the state of the antioxidant system (AOS) and the functional indices of the organism of laboratory animals under experimental chronic endogenous intoxication.

MATERIALS AND METHODS

The experiments on animals were conducted according to requirements of the order of the Ministry of Heath of Russian Federation no. 267 from June 19, 2003, "On approval the rules of laboratory practice," orders of the Ministry of Heath of the Union of Soviet Socialist Republics no. 742 from November 13, 1984, "On approval of the rules of conducting studies using experimental animals," and no. 48 from January 23, 1985, "On control of conducting works using experimental animals," and ethical norms stated in Good Laboratory Practice (GLP), the Helsinki Declaration (2000), and European Community Directives 86/609EEC.

The experiments were conducted on 110 male Wistar rats at the age 4–6 months (body weight 240 ± 50 g, variation in the group ±10 g) obtained from Andreevka branch of the Scientific Center of Biomedical Technologies (Russian Academy of Medical Sciences). Animals were kept under standard vivarium conditions (temperature 20 ± 3°C, humidity 48 ± 2%, lighting regime day/night (from 6:00 to 18:00/from 18:00 to 6:00) with free access to water and food. No more than six rats were placed in plastic cages (TECNIPLAST type IV S). During the entire experiment, animals consumed a standard concentrated compound feed (State Standard R 50258-92) and water with a deuterium concentration of 150 or 40 ppm depending on the ad libitum group, and WMIC DDC was obtained in some groups of animals 14 days before the beginning of CET modeling.

To achieve CET of hepatorenal genesis, 55% tetrachloromethane oil solution (calculated 0.5 mL/kg per day) was daily introduced intraperitoneally to animals for 7 days; starting from the 8th day, a gentamycin solution (25 mg/kg of body weight) was introduced in the volume 0.5 mL for 7 days. Toxic hepatitis and nephropathy (resulting in hepatic and renal failure) were reproduced in this model on the 15th day from the beginning of injections (Novochadov and Pisarev, 2005).

WMIC DDC was obtained on the plant created at Kuban State University (KSU) (Baryshev et al., 2010). Mineralization of water with a deuterium concentration of 40 ppm was conducted by addition of mineral salts in it in order to obtain the physiologically full mineral composition (mineralization 314–382 mg/L: hydrocarbonates 144–180, sulfates <1, chlorides 60–76, calcium 6, magnesium 3, sodium 50–58, potassium 50–58) the same as for water with a deuterium content of 150 ppm. The distilled water produced on UPVA-5 redistiller (Livam PF, Russian Federation) was used as the initial water to obtain drinking water with a normal deuterium concentration (150 ppm). Daily water consumption by animals of all groups during the experiment was, on average, 18–27 mL per rat and did not depend on the physiological state of the animal. The experiment lasted 42 days from the beginning of CET modeling.

Six groups of animals were developed (with the food ration differing by the content of deuterium) for estimation of the protector WMIC DDC effect:

Group 1: animals with a CET model that consumed mineralized water (150 ppm) for 42 days;

Group 2: to estimate the protector effect of WMIC DDC, animals obtained this water (40 ppm) for 42 days starting from first days of CET modeling;

Group 3: to study the preventive and correcting WMIC DDC effect during prolonged use, animals started to obtained WMIC DDC (40 ppm) 14 days before CET modeling and then for 42 days of the experiment;

Group 4: to study the preventive WMIC DDC effect during a short course of use, rats obtained WMIC DDC (40 ppm) 14 days before CET modeling and then obtained mineralized water (150 ppm) for 42 days of the experiment;

Group 5: to study the effect of WMIC DDC on the rat organism under physiological conditions (control 1), these animals were kept under standard conditions throughout the experiment and consumed WMIC DDC (40 ppm);

Group 6: intact animals that consumed mineralized water (150 ppm) throughout the experiment (control 2).

During the experiment, we daily observed for the general state of animals, their physical activity, appetite, and other physiological parameters. Rats were weighted on electronic technical scales (Ohaus, Adventurer Pro, United States) with an accuracy ±0.1 g. All manipulations were performed before animal feed-

Table 1. State of the antioxidant system and biochemical blood indices in laboratory animals on the 15th day of the experiment ($M \pm m$)

Index	Group				
	1	2	3	4	6
AOA, nA s	941.6 ± 23.2*	892.8 ± 16.1*	1125.8 ± 14.5*	1153.6 ± 18.7*	1227.4 ± 24.9
SH-group, ODU	0.295 ± 0.037*	0.314 ± 0.028*	0.348 ± 0.051*	0.361 ± 0.027*	0.482 ± 0.02
Total protein, g/L	42.27 ± 1.93*	49.94 ± 1.68	54.07 ± 4.24	52.94 ± 3.19	53.31 ± 1.06
Bilirubin, μmole/L	15.89 ± 0.74*	18.48 ± 1.51*	13.6 ± 1.39*	15.13 ± 0.85*	5.62 ± 0.34
Creatinine, μmole/L	38.42 ± 2.69	43.04 ± 2.53	39.75 ± 4.72	43.13 ± 2.57	44.81 ± 1.62
AsAt, IU/L	125.3 ± 8.0*	136.9 ± 11.2	143.2 ± 23.1	125.6 ± 12.9*	163.2 ± 9.4
AlAt, IU/L	50.7 ± 4.6	44.7 ± 4.9	42.6 ± 3.5	37.9 ± 4	48.3 ± 2.1
AP, IU/L	113.6 ± 21.5*	145.4 ± 13.3*	118.43 ± 15.4*	153.1 ± 17.6*	66.4 ± 14.9
CC, mmole/L	1.72 ± 0.16*	1.48 ± 0.2	1.39 ± 0.14	1.47 ± 0.09	0.97 ± 0.15
TrG, mmole/L	0.56 ± 0.08	0.43 ± 0.06	0.51 ± 0.07	0.55 ± 0.09	0.41 ± 0.08

*, $p < 0.05$ as compared with Group 6; for Tables 1 and 2.

ing. The general state of the animals was normal before the beginning of the experiment. No differences in behavioral reactions were registered in animals of the Group 6 (control 2) throughout the experiment. The safety of the tested animals in the control and experimental groups was complete (100%).

On the 15th day, slaughtering of a part of the animals using a chamber for euthanasia (VetTech, Great Britain) was conducted and the blood from the heart ventricle was taken in rats from Groups 1–4 and 6 (10 animals in each group) for biochemical studies; on the 42nd day, it was taken from all studied groups (10 animals). Visual examination of the internal organs was conducted afterwards. In order to determine the effect of isotope exchange reactions on the state of the organism, the weight of the body, as well as the liver, spleen, kidney, and heart, was measured with an accuracy ±0.001 g (Acculab, Vicon, United States). Later, the integral chronic intoxication index (ICII) was calculated in conventional units based on the data obtained (Zapadnyuk and Zakhariya, 1983).

The deuterium concentration in water and blood plasma were determined on impulse JEOL JNM-ECA 400MHz NMR spectrometer (on the basis of the Center for Collective Use "Diagnostics of Structure and Properties of Nanomaterials," KSU, Krasnodar) (Baryshev et al., 2012).

In order to determine isotopic composition of internal organs, they were preliminarily dried by sublimation by means of an LS-1000 freeze dryer (Prointekh, Russia Federation). Organ samples with a mass from 0.5 to 3 mg were used; it is associated with the different density of the samples. The study of the isotope composition of the freeze-dried samples of the laboratory animal organs was conducted using a DELTAplus mass spectrometer provided with equipment for sample preparation for isotope hydrogen analysis H/Device (Finnigan, Germany) according to a previously published method (Zimermann and Cegla, 1973) in our own modification (Baryshev et al., 2012).

Biochemical studies were conducted on a semiautomatic biochemical BioChem SA analyzer (United States) using the reagent kits of the HighTechnology company (United States). The content of total protein, creatinine, urea, total bilirubin, AsAt, AlAt, AP, CC, and triglycerides (TrG) were determined as laboratory biochemical indices of endotoxicosis in the rat blood plasma (Kamyshnikov, 2011); de Ritis coefficient as AsAt/AlAt ratio was also calculated.

The study of the blood plasma antioxidant activity (AOA) was conducted in the amperometric way on Yauza-01-AAA antioxidant activity analyzer (NPO Chimavtomatika, Russian Federation) using the previously described method (Basov et al., 2006; Yashin, 2008), and the results were expressed in nanoamperes per s (nA s). The number of reduced SH-groups (which are a key component of the low-molecular AOS component) was determined in the blood by the spectrophotometric method (Ellman, 1959); the results obtained were expressed in optical density units (ODUs). Statistical estimation of the significance of the differences found in the mean values (M) between groups was conducted using the nonparametric Mann–Whitney U-criterion (the difference was considered significant at $p < 0.05$).

RESULTS AND DISCUSSION

A change in the number of biochemical indices in the biochemical blood analysis in tested animals of Groups 1–4 (indicating the development of toxic affection of internal organs) was detected on the 15th day of the experiment (Table 1). The most signif-

Table 2. Change in biochemical blood indices and state of the antioxidant system in laboratory animals on the 42nd day of the experiment ($M \pm m$)

Index	Group					
	1	2	3	4	5	6
AOA, nA s	1068.3 ± 30.7*	1139.4 ± 38.6*	1185.7 ± 40.8	1164.8 ± 34.2*	1194.1 ± 24.5	1216.5 ± 19.3
SH-group, ODU	0.413 ± 0.019*	0.429 ± 0.028	0.475 ± 0.012	0.462 ± 0.025	0.472 ± 0.008	0.493 ± 0.021
Total protein, g/L	51.07 ± 0.99	52.56 ± 1.49	54.53 ± 1.56	55.64 ± 1.97	56.93 ± 1.3	53.09 ± 0.84
Bilirubin, μmole/L	4.29 ± 0.42	5.11 ± 0.38	4.93 ± 0.25	5.35 ± 0.62	4.7 ± 0.83	5.48 ± 0.27
Creatinine, μmole/L	70.2 ± 4.53*	57.46 ± 5.72*	63.13 ± 5.3*	52.56 ± 4.55	48.73 ± 2.91	44.72 ± 1.58
AsAt, IU/L	233.2 ± 10.8*	202.1 ± 47.3	208.1 ± 21.8	203.6 ± 41	172.3 ± 25.2	161.4 ± 8.7
AlAt, IU/L	76.6 ± 7.6*	41.9 ± 4.4	56.3 ± 6.1	44.9 ± 5.5	39.37 ± 4.8	46.5 ± 2.9
AP, IU/L	184.1 ± 17.6*	148.8 ± 18.2*	171.6 ± 16.5*	125.1 ± 15.2	94.6 ± 3.9	67.1 ± 10.6
CC, mmole/L	1.68 ± 0.13*	1.11 ± 0.19	1.69 ± 0.08*	1.7 ± 0.14*	1.19 ± 0.23	1.05 ± 0.14
TrG, mmole/L	0.83 ± 0.06*	0.36 ± 0.04	0.53 ± 0.09	0.51 ± 0.04	0.62 ± 0.1	0.46 ± 0.07

icant changes were detected in Group 1: the amount of total bilirubin was increased by 182.7%; CC, by 77.3%; the de Ritis coefficient was decreased by 26.9%; and the AP activity was increased (+71.1%) against the background of hypoproteinemia ($p < 0.05$) as compared with the control 2. A decrease in the protein content in the blood serum was associated with general intoxication, most likely due to a decrease in its biosynthesis during toxic liver damage and an increase in proteinuria against the background of toxic nephropathy (Nazarenko and Kishkun, 2000). A decrease in the plasma AOA by 23.3% and in the amount of SH-groups by 38.7% was also detected, indicating significant violations in the work of humoral components of the nonspecific protection system of the organism. A decrease in de Ritis coefficient in animals of Group 1 from the 15th to 42nd days of the experiment (from 26.9 to 12.3%) is the most pronounced trait of inflammatory and toxic damage to the liver cells. Violation in glomerular filtration (a trait of nephropathy) manifesting as hypercreatininemia (+57%) (maintained until the 42nd day) was also registered (Table 2).

Less pronounced changes in the biochemical blood indices were registered in animals of Groups 3 and 4 that consumed WMIC DDC as a prophylactic measure (Table 1). At the same time, a relatively smaller decrease in the AOS potential and number of SH-groups (by 6 and 25.1%, respectively, $p < 0.05$) as compared with control 2 was registered in animals from Group 4 as compared with indices in rats from Group 3 in the serum. This preservation of the antioxidant system reserves is probably caused by increased functional activity of the nonspecific protection system on the 15th day of the experiment under conditions of preventive use of WMIC DDC.

The content of total bilirubin, creatinine, and the activity of AsAt and AP were insignificantly higher in animals from Group 2 than in animals from Group 1. Based on the comparative analysis of biochemical blood indices of animals from Groups 1 and 2 on the 15th day of the experiment, it is possible to conclude that introduction of WMIC DDC in the food rations of rats against the background of CET of hepatorenal genesis has no protective effect on the organism of laboratory animals. Therefore, due to the absence of differences when compared with Group 1 (which consumed water 150 ppm), it is possible to conclude that there is no protective effect of water 40 ppm in Group 2. The absence a protective effect is also confirmed by the negative dynamics of the body weight growth in the first 10 days of CET development (Fig. 1). A significant decrease in the body weight was observed in animals from Group 2 (on average, 23.1–25.9% at 7–10 days as compared with the beginning of the experiment). However, a positive tendency of the body weight growth was registered by the 15th day, although the loss of the body weight in this group as compared with the 1st day still remained rather significant (on average, by 25.8 g). A depression of behavioral reactions was found during the experiment in rats from Groups 1 and 2 on the 10th–14th days (most pronounced in animals from Group 1). Among the biochemical blood indices detected on the 15th day in rats from Group 2, a decrease in the de Ritis coefficient by 9.4 and an increase in AP activity by 118.9%, a decrease in the plasma AOA and amount of SH-groups by 27.3 and 34.9%, respectively ($p < 0.05$) as compared with animals from Group 6 (control 2) should be noted. A sharply pronounced specific smell of the skin (without signs of skin inflammation) was observed in animals from Groups 2 and 3 in the period of pathol-

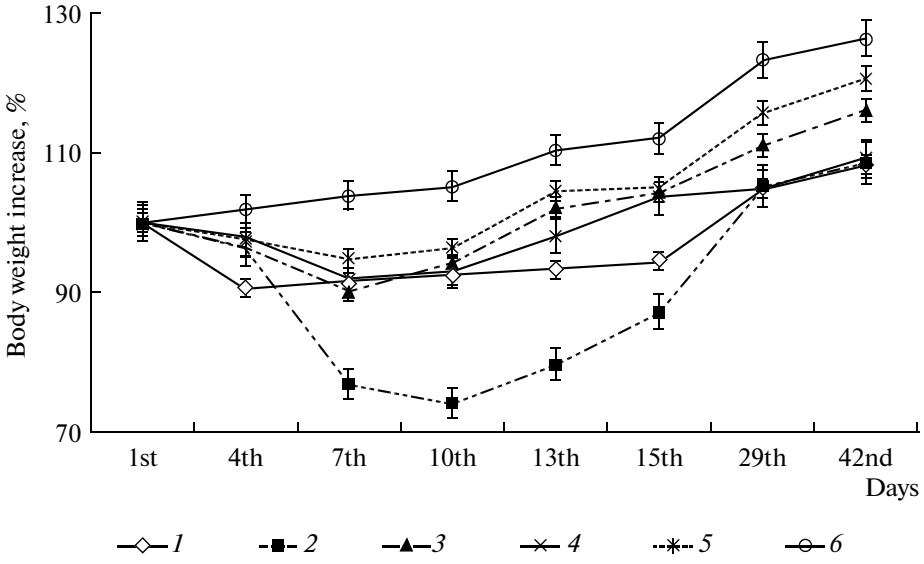

Fig. 1. Change in laboratory animal weight for 42 days of the experiment (the body weight in each group on the 1st day of the experiment is taken as 100%; $M \pm m$). (*1–6*) Groups 1–6.

ogy modeling. This is most likely caused by increased isolation of metabolism products from the organism through the skin during CET of hepatorenal genesis. Such observations were also made by other authors (Novochadov and Pisarev, 2005). In addition, such a sharp smell of the skin was not found in animals of Group 5 (control 1) also consuming WMIC DDC for 42 days.

The changes in all biochemical blood indices throughout the experimental CET modeling in all tested animals of each group had approximately the same direction and intensity. The results of the blood analysis in rats from Group 1 at the end of the experiment indicate stable violations in liver and kidney function. Hypercreatininemia, violation of lipid metabolism (an increase in CC and TrG), an increase in AlAt and AP activity, and a decrease in the blood plasma AOA by 12.2% and the amount of SH-groups by 16.2% were found in these rats on the 42nd day of the experiment (Table 2). This was accompanied by lower indices of body weight growth (+8.3%) as compared with animals from Group 6 (the growth indices of which on the 42nd day were +26.2%) (Fig. 1). Such changes are associated with a pronounced imbalance in the work of the nonspecific protection system. The same low values of the body weight growth (not significantly different from indices in Group 1) were registered in animals from Groups 2 (+8.5%) and 4 (+9.1%) (Fig. 1).

It is possible to assume that a decrease in the rat body weight on the 7th–10th days and shifts in certain biochemical blood indices are explained not only by CET modeling, but also by the ability of WMIC DDC to have some stressful effects on the organism at the largest values of the isotope gradient at the beginning of the experiment (Olariu et al., 2007). This stressful effect can be caused by a significant difference between the deuterium contents in tissues (142–149 ppm in animals in the groups 2, 3, and 5) and water (40 ppm), which results in a decrease in the deuterium level in the plasma to 98–106 ppm (32–36% lower as compared with the data in Group 6, $p < 0.05$) by more rapid D/H-exchange with the blood (153–156 ppm) (Dzhimak et al., 2014). At the same time, such changes occur significantly earlier in plasma than in tissues, which results in an additional load on the nonspecific protection system under conditions of CET development and is manifested by a pronounced decrease in the AOS capacity.

A new D/H-equilibrium was developed in lyophilized liver, kidney, and heart tissues for 15 days during CET development in animals from Groups 2, 3, and 5, and the deuterium content became smaller on average by 13–17% (122–128 ppm, $p < 0.05$) as compared with data in the control 2, resulting in a decrease in the isotope gradient (between the blood and the intracellular structures). An increase in the activity of humoral and cellular protective systems occurs against the background of such fluctuations of the isotope composition; it is probably caused by a nonspecific phenomenon of an increase in the organism resistance as a result of preadaptation (Vasil'eva et al., 2004; Busija et al., 2008), which potentiates protective mechanisms at the cellular level and is realized through secondary messengers (including active oxygen forms and free radicals). Such processes can result in an increase in the mechanisms of adaptation, including the synthesis of heat shock proteins and antioxidant enzymes (for example, superoxide dismutase and catalase), an increase in the content of anti-

nociceptive factors and low-molecular reduction AOS equivalents, change in the activity of ion channels (Na^+, K^+, Ca^{2+}, Mg^{2+}) and the ratio of energetic substrate transporters in membranes.

It is necessary to note that the dynamics of the body weight growth during the experiments in animals of Group 4 was characterized by a number of peculiarities (Fig. 1): it did not differ significantly in the first two weeks from similar indices in Group 3 ($p > 0.05$). The absence of differences between these groups is apparently associated with similar animal preparation for the experiment (introduction of WMIC DDC into the food ration for 14 days before CET modeling). Subsequently, the body weight growth in rats from Group 4 significantly slowed down and approached those in rats from Group 1, which is caused by a toxic effect on the organism associated with decreased functional activity of the nonspecific protection system. A decrease in functional activity in the nonspecific protection system was confirmed by a decrease of AOA by 4.2% and the amount of SH-groups by 6.3%, which was observed by the 15th day (Table 1) and remained until the 42nd day (Table 2).

During the comparative study of the effect of water with a decreased deuterium content on the rat organism, we established that the most significant positive changes in the biochemical blood analysis on the 42nd day were registered in animals of Group 3 (that obtained WMIC DDC throughout the experiment). A decrease in the body weight on the 7th day in animals from Groups 3 and 5 (obtained WMIC DDC throughout the experiment) by 9.9 and 5.2%, respectively, was also registered (Fig. 1). All their physiological indices were without pathological changes (as opposed to rats from Group 2).

The direct influence of WMIC DDC on the morphofunctional indices and biochemical processes of the organism can also (in addition to preadaptation phenomenon) be associated with an increase in isotope exchange (D/H) in active and allosteric enzyme centers, as well as with substitution of HDO molecules with H_2O in hydrate shell of proteins and nucleic acids (Mukhachev, 1975). This results in a change in the thermodynamic and thermokinetic indices of macromolecules (Pedersen et al., 2006). In this regard, acceleration of biochemical reactions is possible due to a decrease in the activation energy required for generation of intermediate enzyme–substrate complexes in the process of biocatalysis. Selective substitution of deuterium with protium precisely in active centers of the enzymes can be explained by means of the Brodsky theory (1957). According to this theory, such selectivity of the isotope exchange indicates that the selective effect on metabolically active compounds containing the largest amount of active atoms (that have an unshared electron pair and are able to generate hydrogen bonds) is possible during the creation of even an insignificant isotope gradient. A decrease in the content of deuterium in the hydrate shell is also accompanied by changes in the biological activity of macromolecules (which is caused by the higher frequency and amplitude of fluctuations of atomic groups developed only from light isotopes). Such reorganizations of biochemical processes in tissues with a high metabolic activity (liver, heart) are able to affect the morphofunctional indices of the whole organism (including the development of CET).

Similar macroscopic changes in the internal organs (more expressed in animals from Group 1) were found in rats from Groups 1 and 2 on the 15th day of the experiment. The liver was increased, of red color, with a flabby consistency; the stomach and intestine were overcrowded by the crop and contained insignificant amount of gases. An increase in kidneys was observed in 40% of the animals (as indicated by ICII); pronounced signs of inflammatory changes were absent in other organs. At the same time, only an insignificant increase in the liver size without signs of inflammation of the internal organs was found in rats from Groups 3 and 4 during visual survey of the internal organs.

No significant manifestations of pathological processes in the internal organs was found in animals from Groups 2–4 on the 42nd day of the experiment, while moderate hepatomegaly was registered in animals from Group 1 (based on macroscopy/autopsy and ICII calculation). At the same time, visual examination of the internal organs did not detect inflammatory pathological processes in them in rats from Groups 5 (control 1) and 6 (control 2). ICII was calculated for determination of the possible cumulative effect of WMIC DDC after measurement of the weight of the animal body and internal organs (liver, spleen, kidney, heart) (Fig. 2).

The liver and kidney ICII was simultaneously increased in animals from Groups 1 (+43.2 and +20.1%), 2 (+7.3 and +31.8%), and 4 (+6.3 and +10.2%) as compared with the intact control (Group 6) on the 42nd day of the experiment (Fig. 2). These data in combination with the results of biochemical studies allow us to conclude that violations in the work of the nonspecific protection system result in degenerate–dystrophic changes in organs of the functional system of detoxication (FSD) and worsen the adaptive possibilities of the organism during pathological states. In addition, the largest values of the heart ICII (+56.9%, $p < 0.05$) as compared with control 2 were registered in animals from Group 1, indicating a high load and possible affection of the cardiovascular system during CET modeling. It is necessary to note that only the liver ICII was slightly higher (+5.8%) in animals from Group 3 than in animals from Group 6, the heart and kidney ICII did not differ significantly, while the spleen ICII was lower by 14.8%. The cytoprotective effect of WMIC DDC during the stress effect on humoral and cellular animal protective systems (probably due to a decrease in the deuterium content in the organism) is confirmed by this. At the same time, the spleen and liver ICII in animals from Group 5 (control 1)

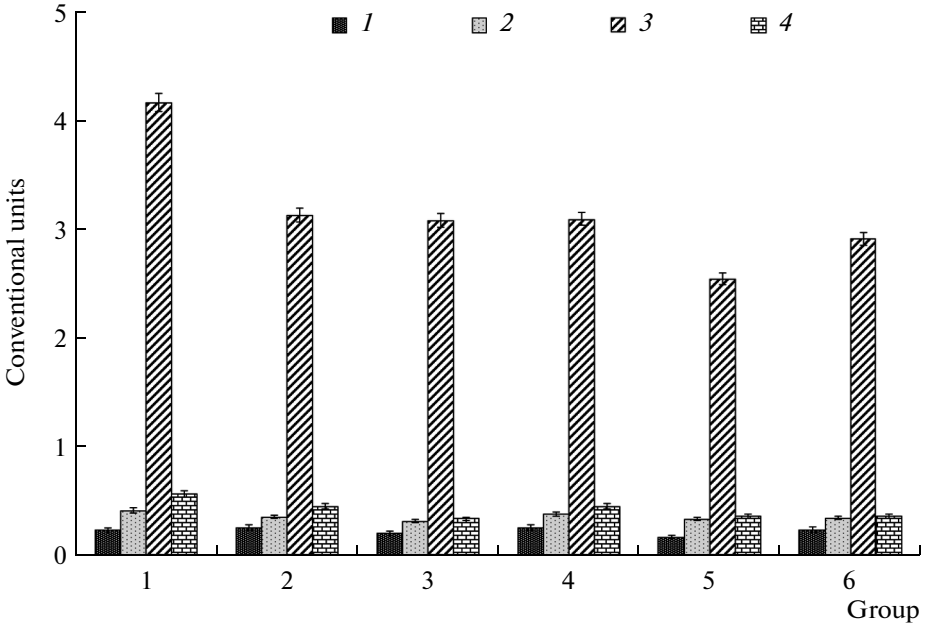

Fig. 2. Integral index of chronic intoxication in rats on the 42nd day of the experiment. *1*, spleen; *2*, kidney; *3*, liver; *4*, heart.

was lower by 30.1 and 12.7%, respectively, as compared with those in animals from Group 6, which also indicates an increase in the FSD potential in animals that obtain the food ration with a modified isotope composition under physiological conditions.

In general, the most significant shifts in the studied biochemical indices are observed in animals from Groups 1, 2, and 4, in which the lowest values of the body weight growth by the 42nd day and the most expressed increase in ICII of the internal organs were also registered. At the same time, changes in the biochemical indices were less pronounced, data on the body weight growth were significantly higher, and the spleen, kidney, and heart ICII values were lower in animals from Group 3 (which obtained WMIC DDC for a long time for estimation of its preventive and correcting effect) than the values of similar indices in animals from Groups 1, 2, and 4. No significant differences were detected during study of the obtained results of the biochemical blood analysis in animals from Groups 5 and 6; the indices of the body weight growth also did not differ, although a significant decrease in the spleen and liver ICII was detected (indicating the protective effect of WMIC DDC on FSD organs).

CONCLUSIONS

Based on the experimental data obtained, it is possible to conclude that the effect of WMIC DDC on the rat organism is caused by the development of an isotope gradient and an increase in D/H-exchange reactions (accompanied by a significant increase in the deuterium content in plasma and lyophilized liver, kidney, and heart tissues). At the same time, a decrease in the body weight was registered in clinically healthy animals from Group 5 on the 7th day, which confirms the exertion of the nonspecific protection system at the stage of the largest differences in isotope composition between the blood and tissues (preadaptation period).

The possibility of using the isotope exchange reactions (D/H) for correction of metabolic processes at different functional states of the organism (with the largest efficiency in the case of preliminary adaptation to the isotope gradient for 14 days (before CET modeling) and subsequent continuous use of WMIC DDC) was demonstrated experimentally. This causes an increase in the functional activity of the nonspecific protection system and more significant increase in the body weight on the 42nd day of the experiment. At the same time, use of WMIC DDC for a short 14-day course only in the period of preadaptation did not result in a significant increase in the adaptive possibilities of animals (indicating the absence of a prolonged effect in WMIC DDC). The absence of a protective effect of WMIC DDC during its introduction in the food ration simultaneously with CET modeling was also registered. Moreover, a mutually enhancing summation of stressor effects on the organism of laboratory animals (resulting in a pronounced negative increase in the body weight on the 10th day of endotoxicosis modeling) was found.

The ability of isotope exchange (D/H) reactions to influence the functional indices of FSD organs was established (which is confirmed by the presence of pathological macrostructural changes in the rat liver and kidneys on the 15th day of CET modeling) was

established. Such an effect of isotope exchange was found not only in animals that consumed typical water throughout the experiment, but also in animals that did not pass the preadaptation period to isotope gradient. Such changes were absent in animals of Group 3 that obtained WMIC DDC.

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