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COMPLEX SYSTEMS BIOPHYSICS

# Influence of Deuterium Depleted Water on Freeze-Dried Tissue Isotopic Composition and Morphofunctional Body Performance in Rats of Different Generations

**S. S. Dzhimak[a], M. G. Barishev[a], A. A. Basov[b], and A. A. Timakov[c]**

[a] *Kuban State University, Krasnodar, 350040 Russia*
*e-mail: jimack@mail.ru*
[b] *Kuban State Medical University, Krasnodar, 350063 Russia*
*e-mail: son_sunytch79@mail.ru*
[c] *MTK "Iceberg" Ltd, Moscow, 125424 Russia*
*e-mail: sig1@rambler.ru*

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**Abstract**—The influence of deuterium depleted water on the body of different rat generations was investigated in physiological conditions. As a result of this study it was established that the most significant and rapid reduction in D/H equilibrium was observed in plasma (by 36.2%), and lyophilized kidney tissues (by 15.8%). Less pronounced deuterium decrease was characteristic of liver tissue (9.3%) and heart (8.5%). Stabilization of the isotopic exchange reaction rate was fixed in the blood and tissues of rats, starting from the second generation. At the same time when deuterium depleted water (40 ppm) was used in dietary intake, the change in morphological and functional parameters in laboratory animals associated with the processes of adaptation to the effects of substress isotopic D/H gradient was also noted. The study shows that modification of only drinking water intake regime can't significantly change the deuterium content in tissues of metabolically active organs, because of the concurrent deuterium receipt in feed substances of plant and animal origin.

*Keywords*: deuterium depleted water, deuterium, tissue isotopic composition, deuterium in blood plasma, NMR spectroscopy, mass spectroscopy

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## INTRODUCTION

In domestic and foreign scientific literature one encounters sufficiently many works in which a study is made of the influence of water with reduced (relative to natural) content of deuterium (light water) on physiological activity of living organisms [1–3]. Among them one may isolate several directions: study of antitumor effects, investigations of the impact of light water on the endogenous antioxidant system and nonspecific resistance of the organism; study of biological effects of light water on living objects of varied level.

A sufficiently large quantity of investigations on studying antitumor effects of light water have been conducted, at that in some works it was shown that deuterium-depleted water, for example, inhibits the growth of a xenoplansplanted tumor in mice [4]. Somewhat later the same scientific-research group executed a series of clinical trial and demonstrated the availability of antitumor effects of light water in patients with oncological diseases [5, 6]. A series of works of the last decade is devoted to studying the growth of various cellular oncocultures in nutrient media prepared in deuterium-depleted water [7, 8]. In these works, shown was the ability of light water to change the rate of tumor cell growth, which, possibly, will be used also in clinical practice.

In investigations of Russian and foreign scientists, also demonstrated was the availability of radioprotector properties in light water [9–12], by which one may explain its genoprotective effect in some low-dose toxic impacts on the organism.

Influence of deuterium-depleted water on indices of the endogenous prooxidant-antioxidant system in blood and homogenates of lyophilized tissues is being studied in the main from the middle of 2000s [13, 14] and continues at the present time [15, 16]. In these works it is shown that prolonged introduction in the feed ration of deuterium-depleted water leads to elevation of the potential of a system of nonspecific defense of the organism of laboratory animals and promotes attenuation of manifestations of disbalance of the prooxidant-antioxidant system in pathological states, connected, first of all, with intensification of processes of free-radical oxidation.

Quite a number of works are devoted also to vivid demonstration of biological effects of light water. Thus

for example, it is established that light water is capable of more quickly taking the therein dissolved dye (methylene blue) out of olfactory pits of *X. laevis* larvae [17]. In their experiments D.I. Stom with coauthors have shown that earthworms chose wells with light water (13 ppm) and avoided deuterium-enriched water, also it was shown that in light water the mass of earthworms increases faster than in water containing heavy hydrogen isotopes [18]. Apart from that, demonstrated is the ability of light water to activize the growth of algae *Dunaliella tertiolecta* on an exponential phase [19]. In works of V.I. Lobyshev with coauthors upon investigations of the growth of cultures of bacteria with various lipid composition of cell membranes in liquid medium with alternating isotopic composition of water it is indicated that some bacteria enriched in lecithin (*M. organophilum*) are activized by small concentrations of deuterium, while in others (*R. vacuolatum*) the interval of stimulating response is shifted to the side of elevated concentrations of deuterium in water (to 1%) and weakly expressed. The reaction of bacteria containing sphingolipids in the entire interval of small concentrations of deuterium was absent. In bacteria containing in membranes glycolipids instead of phospholipids (*M. jannaschiana* and *C. creseentus*), a response to light water turned out to be, just as in the lecithin cluster, not of the same type [20].

Despite such broad attention of scientists of different countries to unique properties of water with modified isotopic composition, heretofore there is no unified opinion about the mechanisms of influence of light water on biological objects. Some authors explain the mechanism of influence of light water on activation of the immune system of the organism by means of its impact on the kinetics of the reaction of $H_2O_2$ generation by isolated mitochondria [21], therewith they have established that reduction of deuterium concentration, relative to natural level, leads to reliable acceleration of the investigated reaction. In other scientific investigations it is shown that various metabolic processes lead to fractionation of hydrogen and carbon isotopes in different degree [22]. From ancient times (Archaean era) in oceanic water the concentration of oxygen isotopes has practically not changes, while the concentration of hydrogen isotopes was subject to a change by 25 ± 5‰. Therefore a hypothesis was expressed that in the period of evolutionary development of living organisms in natural water it was the content of deuterium that was lower than in our times, and in this connection light water comes to be more physiologic for the organism [23].

Along with that the influence of water on the isotopic composition of tissues and morphofunctional indices in multicellular organisms in various generations is investigated insufficiently, which presents special interest, because study of the morphofunctional status presents as one of the main informative indices of individual development of the organism, state of its health, while introduction in the feed ration of light water may exert influence on indices of the system of nonspecific defense and adaptational possibilities of the organism, especially in young of various generations.

In connection with the above-expounded, the aim of the present investigation was a study of the isotopic composition of blood and lyophilized tissues in rats of various generations upon introduction in their feed ration of light water, and also investigation of the influence of reactions of isotopic exchange (D/H) on morphofunctional indices of the organism in laboratory animals.

## EXPERIMENTAL

The work was executed on rats of the Wistar line, 20 animals each in every studied group, which were formed on the basis of 4–6 males (30–40%) and 14–16 females (60–70%). The first generation was composed of rats at an age of 4–6 months (body mass 240 ± 50 g, oscillation of body mass over group ±10 g), which were divided into two groups: $A_1$ ($n = 20$) and $B_1$ ($n = 20$) – as dependent on the isotopic composition of water in their feed ration. In group A the animals of all generations (groups $A_{1-5}$) through the duration of the entire experiment received a usual feed ration and light water (40 ppm, here and further the content in deuterium is indicated). In group the animals of all generations (groups $B_{1-5}$) through the duration of the entire experiment received a usual feed ration and mineralized water (150 ppm). Subsequently the animals born in groups $A_1$ and $B_1$ constituted a second generation of rats – groups $A_2$ ($n = 20$) and $B_2$ ($n = 20$) respectively, the issue of which then constituted groups $A_3$ and $B_3$ and so on. In groups $A_1$ and $B_1$ the investigation of isotopic composition of blood plasma and organ tissues was conducted in four weeks after the beginning of experiment. In all groups of 2–5 generations the study of isotopic composition of blood and lyophilized tissues (liver, kidney, heart) was executed on the 4–6 month of development. Apart from that, in groups $A_1$, $A_3$ and $B_1$, $B_3$ we conducted weighing of animals in the course of three weeks with an aim of evaluating the influence of reactions of isotopic exchange (D/H) on the gain in body mass of adults (group $A_1$) after introduction in their feed ration of light water and of newborn rats (group $A_3$) in the first three weeks after birth. Therewith the parents of the latter (group $A_2$) throughout the duration of entire ontogeny also received deuterium-depleted water (40 ppm).

All animals were kept in vivarium under similar conditions in respect of temperature, humidity, illumination, and also received and identical feed ration. Laboratory rats resided in vivarium under air temperature from 20 to 22°C, humidity – not greater than 50%, in a light regime – day-night. Animals were accommodated in identical plastic cages and kept on a standard ration (groats, meat and vegetables).

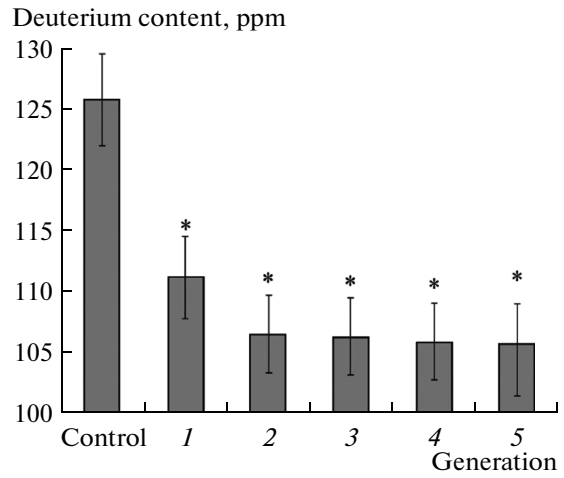


**Fig. 1.** Deuterium content in lyophilized tissues of kidney in rats of five generations. * $p < 0.05$ as compared with indices of control group B, *1–5* – respective generations of rats from group A.

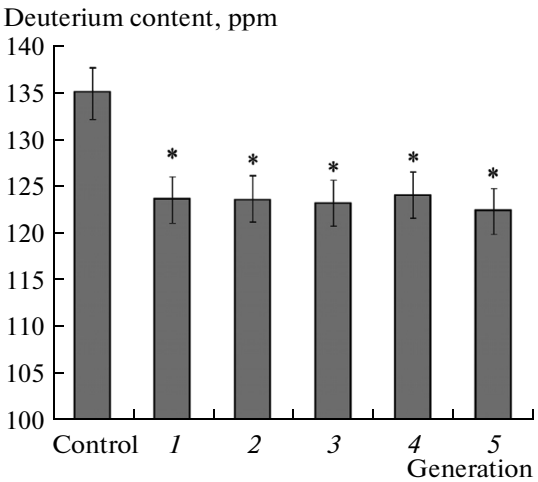


**Fig. 2.** Deuterium content in lyophilized tissues of liver in rats of five generations. * $p < 0.05$ as compared with indices of control group B, *1–5* – respective generations of rats from group A.

Deuterium-depleted water was obtained on an installation elaborated in the Kuban State University [24, 25]. The initial concentration of deuterium in the obtained water constituted 40 ppm. Mineralization of the obtained water was executed by means of adding mineral salts for obtaining a physiologically full-fledged mineral composition (mineralization 314–382 mg/L: hydrocarbonates 144–180 mg, sulfates less than 1 mg, chlorides 60–76 mg, calcium 6 mg, magnesium 3 mg, sodium 50–58 mg, potassium 50–58 mg), which was identical for water with deuterium content 40 ppm and 150 ppm.

Apart from that, over the duration of the entire experiment we conducted observation of physical activity of animals, their appetite, character of stools. Also daily we executed clinical examination, weighing of animals and accounting of consumption of light water (recalculated per one individual). All investigations were conducted roughly at one and the same time before feeding animals. The safety of test animals in control and experimental groups $A_1$ and $B_1$ was complete (100%) over the duration of the entire experiment, while in groups $A_{2-5}$ and $B_{2-5}$ constituted 90–95% and 80–95% respectively.

Determination of deuterium concentration in the obtained water was conducted on a pulse NMR spectrometer JEOL JNM-ECA 400 MHz by procedure FR.1.31.1999.00073 "Procedure of executing measurements of the content of deuterium in water, water-organic and organic solutions by the method of spectroscopy of nuclear magnetic resonance".

For determination of the isotopic composition of lyophilized organs of laboratory animals use was made of a mass-spectrometer DELTAplus (Finnigan, Germany) [26]. Oscillations of the level of deuterium in blood and lyophilized tissues of animals of the control group in all generations ($B_{1-5}$) did not exceed 1%, which has allowed deducing a mean control magnitude of deuterium content for every investigated organ and blood plasma.

For determination of the isotopic composition of nutrition products each organic samples of mass from 0.5 to 3.0 mg was fried by sublimation in accordance with method [27].

Statistical treatment of the obtained data was actualized by methods of variational statistics with the use of free software – system of statistical analysis R (R Development Core Team, 2008), as reliable we deemed a difference at $p < 0.05$.

## RESULTS AND DISCUSSION

From the results of executed investigations it follows that upon changing the isotopic composition of the feed ration, in the given case deuterium-depleted water, in the organism of laboratory animals we also observe a decrease in the concentration of this isotope. As regards rats of group A from 1st to 5th generation, then, as evident from Figs. 1 and 4, the content of deuterium in kidneys and blood plasma is roughly identical with the exception of the first generation, where the deuterium concentration is somewhat elevated relative the subsequent generations of animals in groups $A_{2-5}$ by 13–19% and 4–5% respectively ($p < 0.05$). The quicker, though less substantial in absolute values, attainment of D/H-equilibrium in the first generation was observed in metabolically active tissues of liver and heart (Figs. 2 and 3 respectively). The dynamics of reduction of deuterium concentration in organs of different generations is nonidentical, thus in lyophilized tissues of kidney of group $A_5$ we observe a reliable reduction of deuterium concentration by 15.8% as compared with the control group of animals. At the

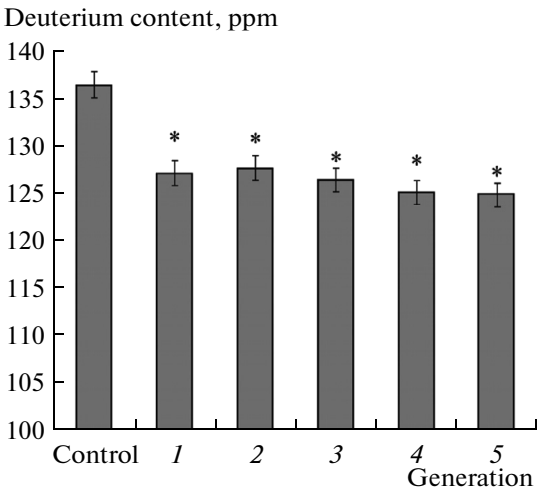


**Fig. 3.** Deuterium content in lyophilized tissues of heart in rats of five generations. * $p < 0.05$ as compared with indices of control group B, *1–5* – respective generations of rats from group A.

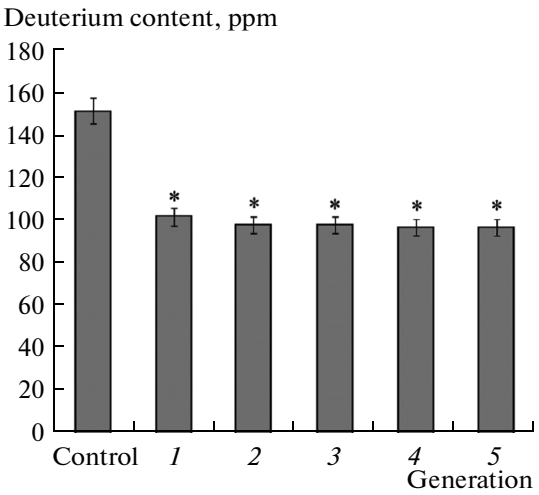


**Fig. 4.** Deuterium content in blood plasma in rats of five generations. * $p < 0.05$ as compared with indices of control group B, *1–5* – respective generations of rats from group A.

same time in lyophilized tissues of liver in group $A_5$ the reduction of deuterium content constituted 9.3% ($p < 0.05$), while in lyophilized tissues of heart 8.5% ($p < 0.05$). At that the most significant reduction of deuterium concentration took place in blood plasma (by 36.2%, $p < 0.05$), which positively correlates with data of D.M. O'Brien with coauthors, which have shown that upon changing the isotopic composition of the diet of Chironomidae (Diptera) only 30.8 ± 2.6% of heavy water may be replaced in the organism with light water [28].

Such nonuniform reduction of deuterium concentration in organs of laboratory animals may be connected with their different metabolic activity, and also peculiarities of change in the morphofunctional state of cellular structures, which reflects a process of adaptation of the organism in the changed conditions of vita activity upon formation of an isotopic gradient against a background of quicker reduction of deuterium in blood. It is known that most actively the reaction of isotopic exchange D/H go in compounds having atoms with an unshared electron pair and capable of forming intermediate reaction complexes upon participation of hydrogen bonds, in which synchronous transition of ($H^+$) and deuterons ($D^+$) from one molecule to another is realized. Therefore more often such exchange is observed in compounds having hydroxyl (–OH), rarer thiol groups (–S–H), primary and secondary amino groups ($-NH_2$ and =N–H), whereas particularly in hydrocarbon bonds ($R_3C-H(D)$) in natural conditions such exchange is practically impossible, which partly explains the fact of incomplete isotopic D/H exchange in biological objects, where a majority of hydrogen atoms is bound with carbon atoms. An alternative and quicker path of exchange of deuterium for protium in tissues and blood includes exchange of HDO and $H_2O$ molecules upon primary and secondary solvation of various macromolecules. Oscillations of the isotopic composition of tissues may lead to an increase in activity of nonspecific defense systems, which is explained by a phenomenon of preadaptation [29, 30], potentiating the defense mechanism in cells in conditions of substress impact of various factors (for example, temperature, hypoxia etc.), in that number it is by an analogous mechanisms that a nonspecific defense system may react in conditions of created D/H gradient, which may be connected with a response reaction of cellular regulatory systems, taking into account the tendency of living matter to constancy of isotopic composition. Adaptive reactions are realized prevalently at the expense of more active exchange of deuterium in biologically active molecules: in active centers of enzymes or sites of allosteric regulation, in transcription factors, and also in the hydration shells of proteins and nucleic acids, which may change their thermodynamic, and consequently also thermokinetic characteristics, elevating the adaptational possibilities of the organism evenà upon a relatively insignificant (from 10 to 30%) exchange of heavy isotopes in tissues. Insignificantly expressed and slower D/H exchange in organs is possible for the reason of supply of additional amounts of deuterium in the composition of nutrients. In this connection we conducted evaluation of the content of deuterium in nutrition products of the test and control groups of animals (carrot, wheat grain, meat), the indices of which constituted from 137.22 to 142.52 ppm (table).

From the data presented in the table it is seen that a higher content of deuterium is revealed in products of plant origin, it was 2.4–3.9% greater in comparison with meat products. The obtained results directly correlate with data of work [31], which confirms the pos-

Content of deuterium in some nutrient products ($M \pm m$)

| | Nutrients of plant origin | | | Nutrients of animal origin | | |
|---|---|---|---|---|---|---|
| Product | Carrot | Wheat grain | Barley grain | Pork meat | Beef meat | Chicken meat |
| [D], ppm | 142.21 ± 1.06 | 142.52 ± 0.73 | 141.43 ± 0.94 | 138.16 ± 0.44 | 137.85 ± 1.13 | 137.22 ± 0.81 |

sibility of participation of deuterium-containing food substances in replenishment of the pool of heavy hydrogen isotopes, especially in tissues with a high rate of exchange processes (liver, heart), where constants supply of new biosubstrates for plastic and energetic exchange takes place.

Upon evaluation of the influence of reactions of isotopic exchange on the indices of increment in laboratory animals it was established that introduction in the feed ration of water with a deuterium content of 40 ppm leads to a phenomenon of pre-adaptation in the main in the first two weeks of experiment. At that in the first generation (group $A_1$) in the course of a week we observed reduction of body mass as compared with initial indices by 5.2%. Further there occurred an increase of the positive gain in body mass, which returned by dynamics in control values on the third week of experiment and reflected a period of adaptation of the organism to a formed isotopic D/H gradient (Fig. 5). However even after the third week the mean body mass of animals in group A1 remained reduced by 7.7% as compared with indices of the control group ($B_1$, $p < 0.05$).

Another dynamics of gain in body mass was noted in the third generation (group $A_3$), when in the first week after birth the mass of rats also was smaller as compared with control group by 26.9%. However in three weeks after birth we noted elevation of the adaptational possibilities of the organism in rats receiving light water, which was accompanied by an increase of the gain in body mass, which even exceeded the analogous indices of control (group $B_3$) by 32.1% (Fig. 6).

We should note a smaller dispersion of the indices of body mass, and also higher survivability in rats in group $A_3$ ($\sigma = 1.77$ g, $n_{21} = 95\%$) as compared with group $B_3$ ($\sigma = 3.20$ g, $n_{21} = 90\%$), which points to elevation of adaptational possibilities upon intake of light water. Such a fact may be tied with activation of mitogenic factors, leading to expression of genes of the enzymes of antiradical defense and heat shock proteins, upon impact of isotopic D/H gradient on component of the system of nonspecific defense of the organism, i.e. more fast and full-fledged realization of mechanisms of long-term adaptation in animals whose parents consumed light water in the period of entire ontogeny. Apart from that, we noted faster sexual maturation in rats receiving deuterium-depleted water, it was by 1.0–1.5 months faster and in animals of the control group. At that the first litter was distinguished by smaller numbers and greater homogeneity of body mass indices, which, possibly, confirms a substress impact of isotopic D/H gradient on the organism of a female.

In this way, in the conducted investigations we have demonstrated a possibility of modification of the isotopic composition of blood and organ tissues upon

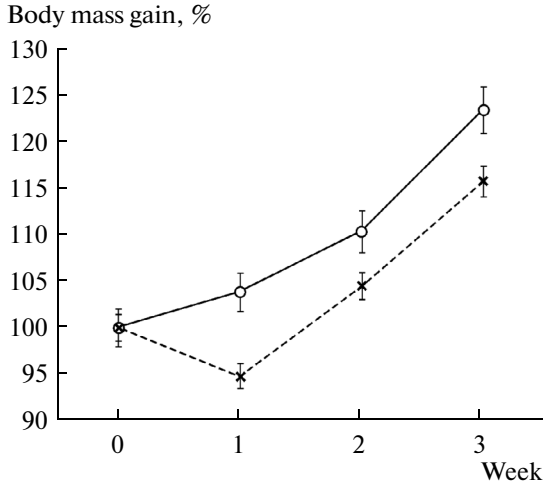


**Fig. 5.** Change in mass of laboratory animals of first generation in the course of three weeks of experiment (body mass in each group in the beginning of experiment taken as 100%, $M \pm m$). *1* – group A1, *2* – group B1.

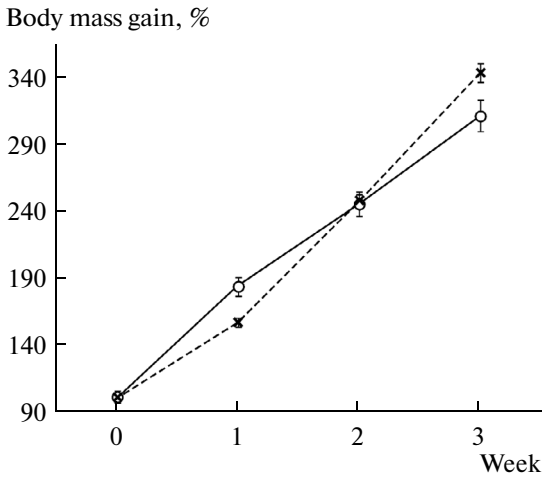


**Fig. 6.** Change in mass of laboratory animals of third generation in the course of three weeks after birth (body mass in each group in the beginning of experiment taken as 100%, $M \pm m$). *1* – group A1, *2* – group B1.

introduction in the feed ration of deuterium-depleted water, and also the influence of reactions of isotopic exchange on realization of adaptational processes in the organism.

## CONCLUSION

On the basis of conducted investigations it is established that introduction in the feed ration of light water leads to formation of an isotopic D/H gradient in the first three weeks between blood plasma and organ tissues. There takes place reduction of deuterium concentration in blood by 36.2% and in tissues of liver, kidney and heart by 9.3%, 15.8% and 8.5% respectively, which is accompanied by a change of the adaptational possibilities of the organism. It is shown that changing only the drinking regimen does not allow essentially changing the content of deuterium in tissues of metabolically active organs, this is conditioned by parallel supply of deuterium in the composition of nutrient substances of plant and animal origin. A stable D/H-equilibrium in blood plasma of tissues of liver, kidney and heart commences in animals in the second generation and is preserved over the duration of life of five generations without reliable changes. Against a background of isotopic D/H gradient there develops a negative gain in body mass in rats of the first generation, reaching a maximum by the end of third week. In the following on the third week the dynamic of body mass gain is restored in rats of the first generation, but the absolute values of their morphofunctional indices do not reach control values. Reactions of isotopic D/H exchange in rats of the third generation lead to deceleration of body mass gain by 26.9% on the first week after birth. However subsequently on the third week the gain dynamics in third-generation rats exceeds the indices of control group by 32.1%, which reflects faster elevation of long-term adaptational possibilities of the organism upon reduction of deuterium content over the duration of entire ontogeny, including the intrauterine period. The obtained results allow speaking of the ability of deuterium-depleted water to change the isotopic (D/H) composition of blood and tissues and elevate, in this way, the potential of defense systems of the organism upon preparation thereof to subsequent stressful impact or upon possible development of alternative pathological processes.

## ACKNOWLEDGMENTS

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