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COMPLEX SYSTEMS BIOPHYSICS

Isotopic Effects of Low Concentration of Deuterium in Water on Biological Systems

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Abstract—Isotopic effects of deuterium in water are studied in a broad range of concentrations on a number of biological objects of different organization levels. The results obtained show that biological objects are sensitive to variations of isotope composition in water. A decrease or increase in deuterium concentrations in water may cause activation or inhibition of biological functions. The values of biological isotopic effects of low deuterium concentration may even be higher than those of high deuterium concentration. No regularity in response for all the objects studied failed to find out in a range of deuterium concentration in water from 4 ppm to 1%.

Keywords: isotopic effects of deuterium, deuterium depleted water, Na,K-ATPase, spermatozoa, fish spawn, mollusca, duckweed, seeds of amaranth, cress-salad, wheat

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INTRODUCTION

Investigation of biological isotopic effects of heavy water began right after obtaining enriched heavy water in 1933 [1, 2]. It became clear that concentrated heavy water D_2O inhibits all vitally important processes and an opinion formed about that heavy water is incompatible with life. The evolution of view in this direction with analysis of physicochemical mechanisms of isotopic effects can be found in monograph [3]. Along with influence of D_2O, doubtless interest is presented by investigation of the influence of small variations of deuterium content in water on living organisms and model systems, inasmuch as natural water comes to be a mixture of isotopic form of water molecules because of the presence of stable isotopes ^1H, ^2H, and also ^{16}O, ^{17}O, ^{18}O. The relative content of the most widespread isotopes deuterium and ^{18}O in natural waters constitutes on average 0.015% (150 ppm) and 0.2% and varies in the limits 0.0079–0,0195% and 0.1887–0.2083% respectively, in consequence of fractionation of water isotopes during phase transitions vapor–liquid–ice (snow), during sorption and filtration in global natural processes [4].

Experiments with water slightly enriched for deuterium were begun as far back as in the thirties of the last century [5–8], but they for a long time were not resumed and not cited. Experimentally it was shown that small changes of the natural isotopic composition of water may lead to unexpectedly large effects, often opposite to the action of concentrated D_2O. The next essential step was a series of works on investigations of melted water obtained from snow and containing a reduced amount of deuterium in consequence of natural fractionation [9, 10]. Shown was an activating influence of melted water obtained from snow on a series of biological processes, and disclosed was an increase of productivity of agricultural animals and plants. A proof of the influence of just the altered isotopic composition was served by tests with normalization of the isotopic composition of melted water by addition of a small amount of heavy water. The results of such experiments did not differ from control ones. A review of early works with small concentrations of deuterium in water is presented in [11]. These works, and also the opinion that longevity of highlanders is conditioned by intake of melted water with reduced deuterium content lay into the basis of an idealized notion about that full liberation of water from heavy

[1] *Editor's Note*: This is the closest possible equivalent of the original publication with all its practical details, statements and terminology, phrasing and style, so the reader can make sound judgment; English title, Abstract and keywords provided by authors. *A.G.*

hydrogen isotope will lead to significant positive results for health and life expectancy. However in our works, as well as in the forgotten works the 30s of the last century, shown was an activating action of small concentrations of deuterium, exceeding the natural content, on the hydrolytic activity of Na,K-ATPase from bovine brain, the rate o regeneration of hydroid polyps, growth rate of some microorganisms [12–14]. In experiments on development of unicellular algae in the summer season in arctic drifting ice, characterized by variable isotopic composition through the ice thickness, disclosed was an increase in biological activity both upon an increase and upon a decrease of deuterium concentration relative to its content in the ancient ocean water [15]. Further development of works in this field is connected with the beginning of industrial production of deuterium-lightened water. The interest in studying the biological effects of lightened water strengthened after disclosure of inhibition of the growth of a culture of tumor cells and proposition to use lightened water in therapy of oncological diseases [16, 17]. A review of works on the main effects of lightened water can be found in work [18].

The aim of the proposed work is to regard the isotopic effects of water deuterium in a broad range of concentrations on biological objects of different levels of organization with an aim of revealing possible common regularities.

EXPERIMENTAL

For tests use was made of: water with decreased content of heavy isotopes – D = 4 ± 1 ppm, 18O = 849 ± 15 ppm, ^{17}O = 222 ± 3 ppm; heavy water (99.6%, "Izotop"); chemical reagents of "ch.p." grade. The exact value of the isotopic composition of laboratory distilled water was not measured, taking its mean value equal to 145 ppm. Use was made of the following biological objects: Na,K-ATPase from duck salt glands; eggs and spermatozoa of loach *Misgurnus fossilis*; spermatozoa of man *Homo sapiens*, bull *Bos taurus* and frog *Rana temporaria*; great ramshorns *Planorbis corneus*; seeds of salad cress *Lepidium sativum*, amaranth *Amaránthus albus* and wheat *Tríticum polonicum*; duckweed *Lemna perpusilla*.

In every series of tests we conducted checking of the action of an "artificial" control on the studied objects. Under "artificial" control we understand water or medium containing a natural concentration of deuterium but prepared by mixing light and heavy water or medium. Tests with reorganized water are necessary in order to establish that the tested water does not contain unidentifiable admixtures that might influence the results of experiments.

The hydrolytic activity of protein was registered by the concentration of inorganic phosphate by the method of Rathbun and Betlach [18].

During studying the development of loach eggs use was made of Holtfretter's medium [19]. Cocoons with eggs of great ramshorns were placed into light and distilled water without addition of chemical substances. The development of embryos was evaluated by development tables [20]. Three-month snails having grown in usual water were caught and placed into mineral medium containing 1.7 mM NaCl, 0.3 mM KCl, 0.1 mM $CaCl_2$ prepared in light and distilled water.

The mobility of spermatozoa was registered with an analyzer of toxicity (ZAO "BMKInvest", Moscow) [21]. The mobility with the aid of the given device was determined as the product of cell concentration by the mean distance covered by them. The analyzer consecutively makes 25 measurements in several capillaries (one cycle of measurements). After the last measurement the device returns into the initial state and the cycle of measurements is repeated. The time of one measurement is set by the experimenter. The test was stopped when the mobility of sexual cells dropped to zero. The values of mobility obtained in one cycle over five capillaries at a definite concentration of deuterium were averages and root mean square deviation was found.

One measurement was conducted in a suspension taken into one capillary of rectangular section and representing the medium with spermatozoa. From every tube with suspension containing a definite concentration of deuterium we gathered five capillaries and with these we filled the measuring sections of the device. In this way, in one series of experiments it was possible to investigate the influence of five concentrations of deuterium on the mobility of spermatozoa (including control). Non-used tubes with suspensions were stored in a special heated base of the device. Both storage and registration was conducted at a temperature of 40 ± 0.1°C for bull spermatozoa and 37 ± 0.1°C for human spermatozoa.

Use was made of standard media for mammalian spermatozoa – medium TALP II or glucose-citrate medium. Media were prepared in light and distilled water. For obtaining intermediate concentrations of deuterium they were mixed in definite proportions. In order to obtain a medium with a deuterium concentration higher than the natural value, heavy water was added as that there would be no significant dilution. The time of one measurement constituted 20 s.

During work with spermatozoa of fish and amphibian the heating was switched off and we worked at room temperature (23°C). Inasmuch as the spermatozoa of loach and frog quickly lost mobility (already in the third cycle of measurements we observed zero values of mobility), with them we conducted only one cycle of measurements. For second and following measurements we took new capillaries, filled them with suspension and replaced used capillaries. The time of one measurement constituted 10 s.

Table 1. Value of activity of protein Na,K-ATPase at different concentration of deuterium in the medium

Deuterium content	Value of activity, nmol/(h μg). Indicated is 5% confidence interval
0.0004	181 ± 4
0.0024	158 ± 6
0.0145	179 ± 5
4.95	174 ± 4
9.9	169 ± 8

The spermatozoa of cold-blooded organisms were stored in 0.7% solution of NaCl. Before registration of mobility the suspension was diluted with water of necessary isotopic composition until the volume increase four times.

In test on safe keeping of native human ejaculate use was made of standard cultivation medium. Before experiment the ejaculate was treated with accordance with established requirements of WHO [22]. The procedure of treatment did not allow attaining a deuterium concentration in suspension less than 37 ppm. After treatment the suspension was divided into equal parts and supplemented with medium with a definite content of deuterium so that the cell concentration in the final suspension would constitute 20 ± 1 mln/mL. Tubes with ready samples were stored in a CO_2-incubator at a temperature of 36.7 ± 0,1°C. The amount of live and dead spermatozoa was determined by a Vital Screen staining method. One measurement for one sample means determination of the amount of live and dead cells out of 200 pcs.

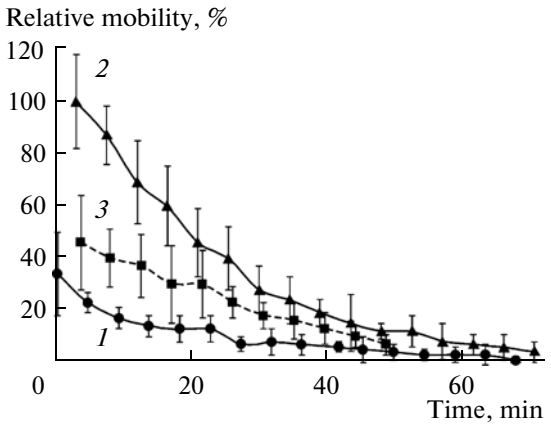

Fig. 1. Dependences of mobility of bull spermatozoa on time at different deuterium concentrations in the medium. Points are connected for illustrativity of the graph. Plotted is root mean square deviation ($N = 4$). As 100%, taken is the initial mean value in control. Designations: *1* – 10 ppm; *2* – control; *3* – 5 %.

Tests on cultivation of duckweed were conducted with the use of Kvitko medium prepared in distilled and light water.

Seeds of wheat, amaranth and salad cress were germinated in isotopically-altered water without addition of any chemical compounds. The concentration of oxygen in a sealed jar with germinating seeds of wheat ($n = 100$) was registered with a thin-film oxygen sensor for a duration of 57 h from the moment of soaking the seeds.

The number of germinated amaranth and salad cress seeds was registered one time in 2 h. In one Petri dish 50 seeds were contained for every investigated deuterium concentration.

For processing of results we used formulae and tables of mathematical statistics [23].

RESULTS AND DISCUSSION

Tests with all investigated biological objects have shown that the results obtained with "artificial" control water do not differ from the results obtained with distilled water. This signifies that light water does not contain unidentifiable admixtures that might have influenced the results of experiments.

Na,K-ATPase. The experiment has shown a nonmonotonic dependence of enzyme activity upon lowering the deuterium concentration relative to natural content (Table 1). Upon lowering the deuterium content in the medium to 30 ppm the hydrolytic activity of the enzyme Na,K-ATPase decreases by 12% relative to value in control (145 ppm). But in the medium with deuterium content of 4 ppm a significant difference with control is absent.

Sexual cells. The mobility of bull spermatozoa obtained from frozen ejaculate, as dependent on time at different deuterium concentrations is shown in Fig. 1.

In all investigated cases the mobility of bull spermatozoa was reliable higher in control. Both increasing and decreasing the deuterium concentration relative to natural content led to inhibition of the mobility of spermatozoa.

The mobility of sexual cells of the bull at a deuterium content of 30 ppm is same as at 10 ppm, while at 1% it coincided with that at 5%. At a deuterium content in the medium of 60 and 90 ppm the mobility of bull spermatozoa was same as in control or at a content of 1 or 5% deuterium. With the course of time the mobility of spermatozoa decreases, which led to a decrease of the difference of mobility in conditions of different isotopic composition with the absence of significant change in the kinetics of cell death. A significant distinction of mobilities was observed in the course of the first 10 min of experiment.

In this way, in the region of natural deuterium concentration one observes a maximum of biological activity.

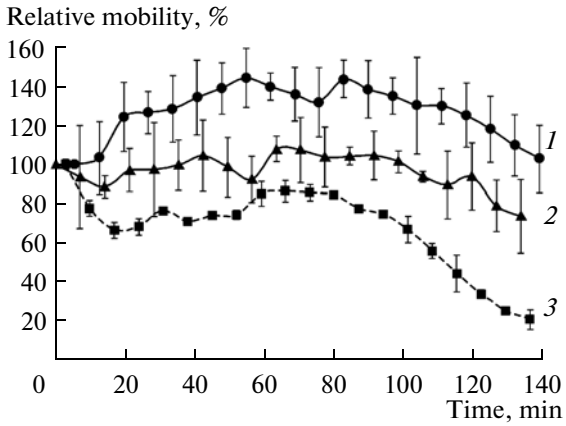

Fig. 2. Dependences of mobility of human spermatozoa on time at different deuterium concentrations in the medium. Points are connected for illustrativity of the graph. Plotted is root mean square deviation ($N = 4$). As 100%, taken is the initial mean value in control. Designations: *1* – 4 ppm; *2* – control; *3* – 60 ppm.

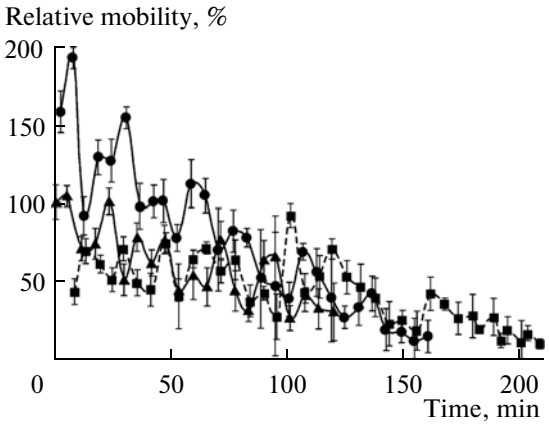

Fig. 3. Dependences of mobility of loach spermatozoa on time at different deuterium concentrations in the medium. Points are connected for illustrativity of the graph. Plotted is root mean square deviation ($N = 4$). As 100%, taken is the initial mean value in control. Designations: circles – 4 ppm; triangles – control; squares – 0.5%.

The change in mobility of human spermatozoa obtained from native ejaculate with the course of time at different deuterium concentrations in the medium is shown in Fig. 2. As distinct from the preceding experiment with frozen ejaculate, one observed different kinetics of mobility of human spermatozoa at different deuterium concentrations in the medium. In the medium prepared in light water with deuterium content of 4 ppm, one observes activation of mobility to 140% of initial control value in the course of the first hour of measurements and prolonged preservation of mobility up to 140 min. In the medium containing 60 ppm deuterium, right away one observed significant inhibition. At a deuterium content in the medium of 0.5, 1 and 5% the mobility of sexual cells (not shown in figure) did not differ from mobility in control. In case of greater deuterium concentrations, 30 è 60%, in the course of an hour one observes reduction of the mobility of spermatozoa by 40 and 60% respectively. Cells become immotile in 13 and 8 h for 30 and 60% D2O-medium respectively. The obtained data on motile activity of human spermatozoa in the medium prepared in concentrated heavy water fully coincide with data of K. Kanwar [25].

In Fig. 3, shown is the temporal dependence of the mobility of loach spermatozoa at different deuterium content. Analogously to the results of tests with human spermatozoa, in the medium with deuterium content of 4 ppm the loach spermatozoa are also more motile relative to control. But by the end of test at the 140th min the mobility of cells significantly decreases and reliable differences in water with different isotopic composition are not observed. Attracting attention is a quasiperiodic change in the mobility of spermatozoa against a background of monotonic decrease in their mobility. An unexpectedly large effect is registered in water with a deuterium content of 370 ppm, where loach spermatozoa are three–six times more motile than in control in the course of the entire experiment. Therewith a further increase of deuterium concentration to 600 ppm and 0.5% did not then lead to significant isotopic effects.

For frog spermatozoa reliable differences between mobility in control and in light water were not revealed.

Viability of human spermatozoa, obtained from native ejaculate, upon storage in the medium with different deuterium concentrations is shown in Fig. 4.

As it is seen, the longest of all the spermatozoa are preserved in the medium with usual water. The magni-

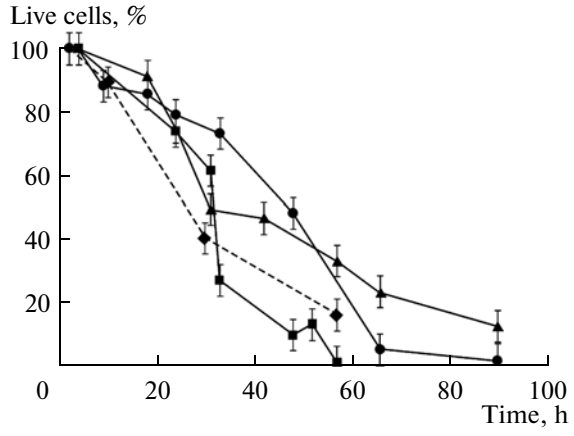

Fig. 4. Dependences of survivability of human spermatozoa at different deuterium concentrations in the medium. Points are connected for illustrativity of the graph. Indicated is 5% confidence interval. Designations: circles – 37 ppm; squares – 107 ppm; triangles – control; rhombi – 81 %.

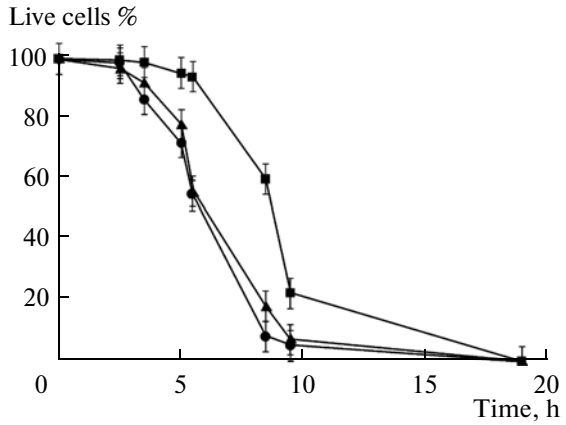

Fig. 5. Dependences of survivability of activated eggs at different deuterium concentrations in the medium. Points are connected for illustrativity of the graph. Indicated is 5% confidence interval. Designations: circles – 4 ppm; triangles – control; squares – 20%.

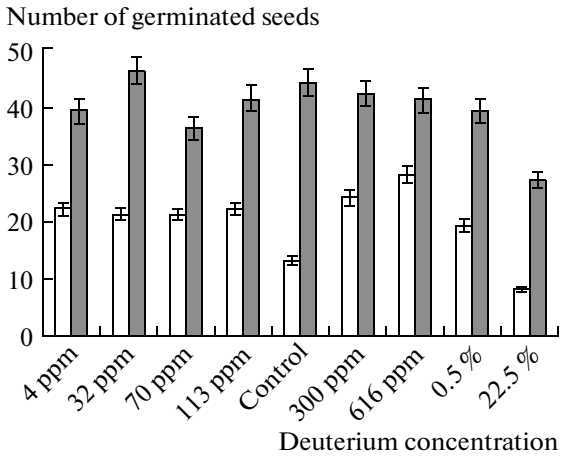

Fig. 6. Number of germinated amaranth seeds in 24 h (light bars) and 37 h (dark bars) after soaking 50 seeds. Indicated is 5% confidence interval.

tudes of cell death in the medium with deuterium content in the interval 5–40% (not shown in figure) significantly did not differ between themselves and constituted 80% elapsing a day after the beginning of experiment. Data obtained at 60 and 90 ppm insignificantly differed from control. Surprisingly, at a deuterium concentration equal to 81% the viability turned out to be even better than at 107 ppm. Comparing viability with mobility, it can be said that the complex of processes connected with mobility turns out to be more sensitive to the isotopic composition of water. On the whole, as a result of conducted experiments we have not revealed advantages of storage of native spermatozoa in the medium prepared in water with altered isotopic composition.

Dynamics of death of unfertilized loach eggs. Upon contact with water in loach eggs processes are launched as upon fertilization, i.e. they are activated. Inasmuch as fusion with a spermatozoon is none, the eggs in some time die. The dynamics of death of activated loach spawn is shown in Fig. 5. The results obtained in light water reliably (with 5% significance level) did not differ from results in control. The content in water of 20% deuterium caused retardation of death of unfertilized eggs by 3 h. By the obtained results one can judge about that in the medium containing 20% there is slow-down of biochemical processes connected with biological clock and proceeding in activated eggs.

Table 2. Influence of light water on survival of loach larvae and great ramshorns

	Loach larvae		Great ramshorns	
	initial number	final number	initial number	final number
Control	166	25	6	5
Light water	165	53	6	1

Embryonic development of eggs of loach and great ramshorns. The influence of light water on the tempos of embryonic development of eggs of loach and great ramshorns was not disclosed. The development in the medium prepared in light water took place same as in control.

Postembryonic development of larvae of loach and great ramshorns. In Table 2, presented are data on the influence of light water on survivability of loach larvae and 3-month great ramshorns. The column "final number" corresponds to the number of live tested objects on the seventh day of experiment.

The results of tests with loach larvae and great ramshorns are diametrically opposite. While the survivability of loach larvae in light water is reliably higher as compared with control (5% significance level), the survivability of snails, conversely, is significantly lower in light water relative to control (10% significance level). Despite that great ramshorns have shown a high rate of death in light water, their behavior (time of residence on vessel walls and liquid surface) in light water did not differ from behavior in control.

The obtained data also show that the response of a biological system to variations of deuterium content in water depends on the studied system and its function.

Plants. Germination of salad cress and amaranth seeds. The quantity of germinated amaranth seed in 24 and 37 h and the quantity of germinated salad cress seeds in 24 and 48 h after soaking as dependent on deuterium concentration in water is shown in Fig. 6 and Fig. 7 respectively. The amaranth seeds by the 24th hour in control germinate reliably worse than in water with different deuterium content (from 4 ppm to 0.5%). Only the deuterium concentration of 22.5% expectedly leads to reduction of the number of germi-

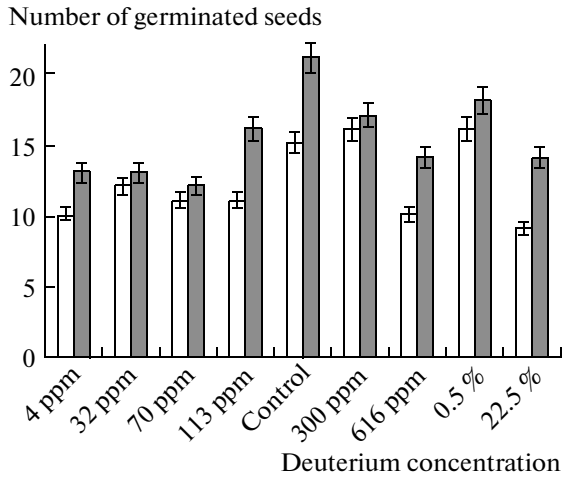

Fig. 7. Number of germinated salad cress seeds in 24 h (light bars) and 48 h (dark bars) after soaking 50 seeds. Indicated is 5% confidence interval.

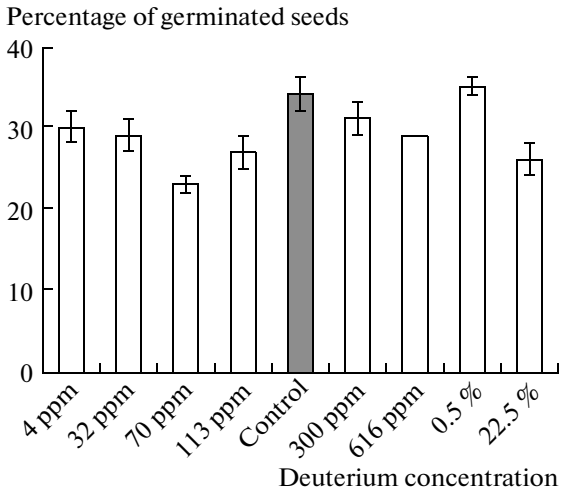

Fig. 8. Percentage of germinated salad cress seeds in 72 h after soaking. Plotted is root mean square deviation (n = 200).

nated seeds. By the 37th hour the number of germinated seeds in control becomes higher of equal to the value in test groups. In the case of experiment with salad cress the results in control group always were higher than in test groups. Tests were repeated two times for amaranth seeds and four times for salad cress seeds. The germination of amaranth seeds in 72 h in all tests constituted 100%, consequently, isotopic effects were not observed. At the same time the germination of salad cress seeds turned out to be less than 40% and depended on the isotopic composition of water, which is shown in Fig. 8. The smallest amount of germinated seeds turned out in water with a deuterium content of 70 ppm. It is noteworthy that the germination of salad cress seeds in water with deuterium content of 70 ppm is almost the same as in water with high deuterium content (22.5%), – respectively 22 and 26%.

The obtained results of tests with salad cress and amaranth seeds also show a statistically significant nonmonotonic character of the dependence of biological responses on isotopic composition of water in the region of small deuterium concentrations.

Respiration of germinating wheat seeds, lengths of shoots and roots of seedlings. Measured is the concentration of oxygen in a sealed vessel with germinating wheat seeds in the course of 57 h after soaking, and calculated is the oxygen consumption rate. If as a unit we take the value of rate in the control group, the dependence of the rate of oxygen consumption by germinating wheat seeds on deuterium concentration will look as follows (Fig. 9). One should pay attention to that the reduction of the rate at 32, 113 and 616 ppm is even more significant than in the presence of 50% D_2O. This fact is surprising, inasmuch as a change of concentrations of deuterium near its natural content leads to a greater effect than an increase of its content more than three thousand times. It is logical to suppose that the smaller the oxygen consumption rate, the greater amount of oxygen must remain in the vessel upon completion of experiment. The results confirm the given statement for all concentrations except 70, 113 and 300 ppm (Fig. 10). The obtained data give ground for supposing that only at these deuterium concentrations at some time moment there takes place growth of respiration intensity.

In 120 h, measured were the sum lengths of shoots and roots of all seedlings. Their mean values with 5% significance level are indicated in Table 3.

Observed is significant activation of the growth of shoots at 616 ppm and 0.5% and roots at 32 ppm. Strong inhibition of total growth quite expectedly is

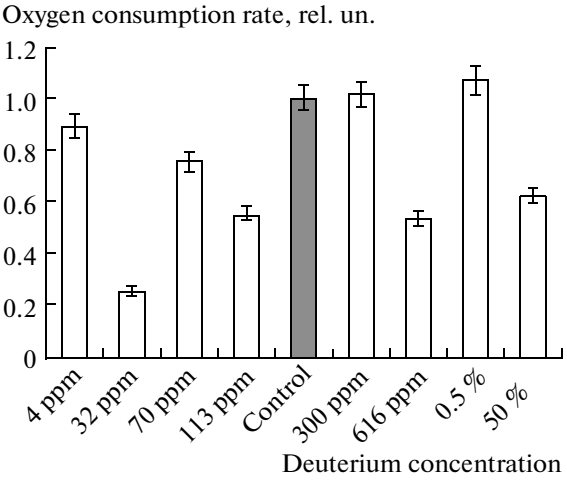

Fig. 9. Rate of oxygen consumption by germinating wheat seeds as dependent on deuterium concentration. Indicated is 5% confidence interval.

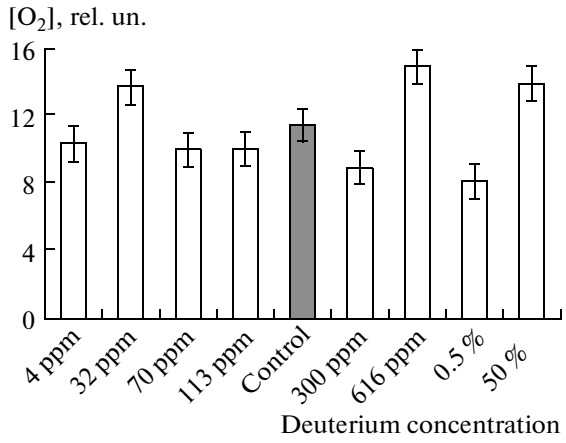

Fig. 10. Content of oxygen in the vessel at the end of test (120 h). Indicated is 5% confidence interval.

observed in 50% D_2O. At other concentrations isotopic effects are not observed.

Duckweed. No distinctions either in morphology, or in growth rate, or in the quantity of leaflets, or in the tempos of budding in light water and in control in tests with duckweed were revealed.

CONCLUSIONS

As a result of conducted investigations it is established that in a range of deuterium concentrations from 4 ppm to 1% in a majority of cases one observes a nonmonotonic dependence of responses of biological systems on the relationship D/H.

A significant reduction of measured parameters relative to control upon lowering of deuterium concentration is observed in tests on registration of the mobility of bull spermatozoa and human spermatozoa, germination of salad cress seeds, rate of respiration by germinating wheat seeds. A local maximum in biological responses at a natural content of deuterium manifests itself in tests on registration of the mobility of bull spermatozoa, germination of salad cress seeds, number of germinated amaranth seeds (37 h after soaking) and rate of respiration by germinating wheat seeds. Despite the like characteristics in responses of investigated biological system, it cannot be stated that there exists a universal response in the region of deuterium concentrations from 4 ppm to 1%.

There arises a natural question about the mechanisms of observed isotopic effects. The first question—why do we consider only the isotopic effects of deuterium? Indeed upon fractionation of water we get also an altered content of ^{17}O and ^{18}O. A simple answer consists in that in a harmonic approximation the rate of reactions is inversely proportional to the root of the masses of reacting atoms. In this case the maximal values of kinetic isotopic effects without account taken for tunneling for D, ^{17}O and ^{18}O atoms would constitute 1.4, 1.03 and 1.06 respectively. Clearly, the isotopic effects of oxygen are significantly smaller. An exception is constituted by isotope ^{17}O, possessing a magnetic moment and corresponding additional isotopic effects [26]. These effects, however, must manifest themselves at large concentrations of the isotope.

Possibly, deuterium-depleted water does possess properties strongly differing it from usual water? It is known that mixtures of usual and heavy water behave closely to ideal solutions [27, 28]. In attempts to explain the increase in the rate of the work of enzyme at small additions of deuterium into usual water we confirmed this point of view, studying the heat capacity of usual water enriched for deuterium [29]. A it is known, the greatest thermodynamic isotopic effect in heavy water manifests itself in viscosity and constitutes 20%. This characteristic is quite essential for many biophysical processes, in that number conformational changes of biopolymers, phenomena of transfer and mobility. In an approximation of ideality of solution, in order to distinguish the viscosity of usual water and water containing 4 ppm deuterium, it is necessary to conduct measurements with an accuracy on the order of 10^{-5}. Such accuracy is not available to us. Using a capillary viscometer, we did not disclose differences in viscosity of usual and light (4 ppm) water with 1% accuracy. Measurements of sound velocity in two media to an accuracy of the sixth digit also confirm ideality of water in the 4–145 ppm range. In this way, it is hard to expect a rational explanation of the observed effects at the expense of unusual thermodynamic properties of light water. It is also hard to

Table 3. Mean values of lengths of shoots and roots of wheat seedlings

[D]	4 ppm	32 ppm	70 ppm	113 ppm	Control (140 ppm)	300 ppm	616 ppm	0.5%	50%
Mean shoot length, mm	46 ± 3	53 ± 3	48 ± 2	52 ± 3	50 ± 3	48 ± 3	60 ± 4	56 ± 3	26 ± 3
Mean root length, mm	48 ± 3	55 ± 3	46 ± 2	49 ± 2	49 ± 3	44 ± 2	48 ± 3	53 ± 3	18 ± 1

explain the nonmonotony and change of sign of the magnitudes of isotopic effects in the investigated range. Clearly, for explanation of the observed effects further investigations are necessary.

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