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# ВІРУЛІЦИДНИЙ І БАКТЕРИЦИДНИЙ ЕФЕКТИ ЕЛЕКТРОХІМІЧНИХ АКТИВОВАНИХ РОЗЧИНІВ КАТОЛІТУ І АНОЛІТУ НА КЛАСИЧНИЙ ВІРУС СВИНЯЧОГО ГРИПУ І БАКТЕРІЮ *E. COLI DH5*

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У статті описуються результати антимікробної дії електрохімічно активованих водних розчинів (аноліт/католіт), отриманих в анодній і катодній камері електролітичної комірки. У лабораторних умовах культура клітин суспензії вірусу свинячого грипу була оброблена анолітом. Після щеплення їх з культурами клітин присутність вірусу (наявність вірусного антигену) було виміряно з використанням імунопероксидазного методу. Було виявлено, що аноліт не впливає на ріст культури клітин РК-15; вірусне зростання при зараженні клітинного моношару сповільнювалося вірусом найбільшою мірою при розведенні аноліта в пропорції 1:1 і менше в інших розведеннях; в той час як вірусне зростання при інфекції клітинної суспензії з вірусом сповільнювалося анолітом найбільшою мірою в розведенні 1:1, і менше в інших розведеннях; вірусне зростання при інфекції вірусом суспензії клітин моношару залежало від присутності аноліту у всіх розведеннях. Несподівано сильний біоцидний ефект католіту спостерігався при обробці штаму *E. coli DH5* анолітом і католітом відповідно. Для отримання додаткових даних щодо противірусної активності електроактивованих розчинів води, а також про структурні зміни, були виміряні нерівноважний енергетичний спектр (НЕС) і диференційний нерівноважний енергетичний спектр (ДНЕС) аноліту і католіту.

**Ключові слова**: аноліт, католіт, *E. coli DH5*, вірус свинячого грипу, НЕС, ДНЕС.

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# STUDYING THE BIOLOGICAL INFLUENCE OF HEAVY WATER ($^2H_2O$) ON PROCARYOTIC AND EUCARYOTIC CELLS

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## Introduction

The most interesting biological phenomenon is the ability of some microorganisms to grow on heavy water ($^2H_2O$) media in which all hydrogen atoms are replaced with deuterium [1, 2]. $^2H_2O$ has high environmental potential in biomedical studies due to the absence of radioactivity and poccebility of detecting the deuterium label in the molecule by high-resolution methods as NMR, IR, and mass spectrometry that facilitates its use as a tracer in biochemical and biomedical research [3].

The average ratio of $^1H/^2H$ in nature makes up approximately 1:5700 [4]. In natural waters, the deuterium is distributed irregularly: from 0,02–0,03 mol.% for river water and sea water, to 0,015 mol.% for water of Antarctic ice – the most purified from deuterium natural water containing in 1,5 times less deuterium than that of seawater. According to the international SMOW standard isotopic shifts for $^2H$ and $^{18}O$ in sea water: $^2H/^1H = (155,76\pm0,05).10^{-6}$ (155,76 ppm) and $^{18}O/^{16}O = (2005,20\pm0,45).10^{-6}$ (2005 ppm). For SLAP standard isotopic shifts for $^2H$ and $^{18}O$ in seawater make up $^2H/^1H = 89.10^{-6}$ (89 ppm) and for a pair of $^{18}O/^{16}O = 1894.10^{-6}$ (1894 ppm). In surface waters, the ratio $^2H/^1H = \sim (1,32–1,51)\cdot10^{-4}$, while in the coastal seawater – $\sim(1,55–1,56)\cdot10^{-4}$. The natural waters of CIS countries are characterized by negative deviations from SMOW standard to $(1,0–1,5)\cdot10^{-5}$, in some places up to $(6,0–6,7)\cdot10^{-5}$, but however there are also observed positive deviations at $2,0\cdot10^{-5}$.

The chemical structure of $^2H_2O$ molecule is analogous to that one for $H_2O$, with small differences in the length of the covalent H–O-bonds and the angles between them. The molecular mass of $^2H_2O$ exceeds on 10% that one for $H_2O$. The difference in nuclear masses stipulates the isotopic effects, which may be sufficiently essential for the $^1H/^2H$ pair [5]. As a result, physical-chemical properties of $^2H_2O$ differ from $H_2O$: $^2H_2O$ boils at +101,44 $^0$C, freezes at +3,82 $^0$C, has maximal density at +11,2 $^0$C (1,106 g/cm$^3$) [6]. In mixtures of $^2H_2O$ with $H_2O$ the isotopic exchange occurs with high speed with the formation of semi-heavy water ($^1H^2HO$): $^2H_2O + H_2O = {}^1H^2HO$. For this reason deuterium presents in smaller content in aqueous solutions in form of $^1H^2HO$, while in the higher content – in form of $^2H_2O$. The chemical reactions in $^2H_2O$ are somehow slower compared to $H_2O$. $^2H_2O$ is less ionized, the dissociation constant of $^2H_2O$ is smaller, and the

solubility of the organic and inorganic substances in $^2H_2O$ is smaller compared to these ones in $H_2O$ [7]. Due to isotopic effects the hydrogen bonds with the participation of deuterium are slightly stronger than those ones formed of hydrogen.

For a long time it was considered that heavy water was incompatible with life. Experiments with growing of cells of different organisms in $^2H_2O$ show toxic influence of deuterium. The high concentrations of $^2H_2O$ lead to the slowing down the cellular metabolism, mitotic inhibition of the prophase and in some cases – somatic mutations [8]. This is observed even while using natural water with an increased content of $^2H_2O$ or $^1H^2HO$ [9]. Bacteria can endure up to 90 % (v/v) $^2H_2O$, plant cells can develop normally in up to 75 % (v/v) $^2H_2O$, while animal cells – up to not more than 30 % (v/v) $^2H_2O$ [10]. Further increase in the concentration of $^2H_2O$ for these groups of organisms leads to the cellular death [11], although isolated cell's cultures suspended in pure $^2H_2O$ exert a strong radioprotective effect in $^2H_2O$-solutions towards γ–radiation [12, 13]. On the contrary, deuterium depleted water with decreased deuterium content has benefitial effects on organism and stimulates the cellular metabolism [14].

With the development of new microbiological approaches, there appears an opportunity to use adapted to deuterium cells for preparation of deuterated natural compounds. The traditional method for production of deuterium labelled compounds consists in the growth on media containing maximal concentrations of $^2H_2O$ and deuterated substrates as [$^2$H] methanol, [$^2$H]glucose etc. [15, 16]. During growth of cells on $^2H_2O$ are synthesized molecules of biologically important natural compounds (DNA, proteins, amino acids, nucleosides, carbohydrates, fatty acids), which hydrogen atoms at the carbon backbones are completely substituted with deuterium. They are isolated from deuterated biomass obtained on growth media with high $^2H_2O$ content and deuterated substrates with using a combination of physico-chemical methods of separation – hydrolysis, precipitation, extraction with organic solvents and chromatographic purification by column chromatography on different adsorbents. These deuterated molecules evidently undergo structural adaptational modifications necessary for the normal functioning in $^2H_2O$.

The adaptation to $^2H_2O$ is interested not only from scientific point, but allows obtain the unique biological material for the studying of molecular structure by $^1$H-NMR [17]. Trend towards the use of deuterium as an isotopic label are stipulated by the absence of radioactivity and possebility of determination the deuterium localization in the molecule by high resolution NMR spectroscopy [18], IR spectroscopy [19] and mass spectrometry [20]. The recent advances in technical and computing capabilities of these analytical methods have allowed to considerable increase the efficiency of *de novo* biological studies, as well as to carry out structural-functional biophysical studies with deuterated molecules on a molecular level.

This study is a continuation of our research into the adaptation to deuterium of various procaryotic and eucaryotic organisms. The purpose of our research was studying the influence of deuterium on the cells of different taxonomic groups of microorganisms and microalgae realizing methylotrophic, chemoheterotrophic, photo-organotrophic and photosynthetic pathways of carbon assimilation.

## Experimental Part

### *Material and Methods*

*Biological objects*

The objects of the study were various microorganisms, realizing methylotrophic, chemoheterotrophic, photo-organotrophic, and photosynthetic ways of assimilation of carbon substrates. The initial strains were obtained from the State Research Institute of Genetics and Selection of Industrial Microorganisms (Moscow, Russia):

1. *Brevibacterium methylicum B-5652*, a leucine auxotroph Gram-positive strain of facultative methylotrophic bacterium, L-phenylalanine producer, assimilating methanol via the $NAD^+$ dependent methanol dehydrogenase variant of ribulose-5-monophosphate cycle (RuMP) of carbon fixation.

2. *Bacillus subtilis B-3157*, a polyauxotrophic for histidine, tyrosine, adenine, and uracil spore-forming aerobic Gram-positive chemoheterotrophic bacterium, inosine producer, realizing hexose-6-mono-phosphate (GMP) cycle of carbohydrates assimilation.

3. *Halobacterium halobium ET-1001*, photo-organotrophic carotenoid-containing strain of extreme halobacteria, synthesizing the photochrome transmembrane protein bacteriorhodopsin.

4. *Chlorella vulgaris B-8765*, photosynthesizing single-cell blue-green alga.

*Chemicals*

For preparation of growth media was used $^2H_2O$ (99,9 atom.%), $^2$HCl (95,5 atom.%) and [$^2$H]methanol (97,5 atom% $^2$H), purchased from the "Isotope" Russian Research Centre (St. Petersburg, Russian Federation). Inorganic salts and D- and L-glucose ("Reanal", Hungary) were preliminary crystallized in $^2H_2O$ and dried in vacuum before using. $^2H_2O$ was distilled over $KMnO_4$ with the subsequent control of isotope enrichment by $^1$H-NMR-spectroscopy on a Brucker WM-250 device ("Brucker", Germany) (working frequency: 70 MHz, internal standard: $Me_4Si$). According to $^1$H-NMR, the level of isotopic purity of growth media usually was by ~8–10 atom% lower than the isotope purity of the initial $^2H_2O$.

*Growth media*

The following growth media were used (concentratioin of components are given in g/l):

1. Minimal salt medium M9 for growth of the facultative methanol assimilating methylotrophic bacterium *B. methylicum B-5662*, supplemented with 2 % (v/v) [$^2$H]methanol and increasing gradient of $^2H_2O$ concentration from 0; 24,5; 49,0; 73,5 up to 98 % (v/v) $^2H_2O$: $KH_2PO_4$ – 3; $Na_2HPO_4$ – 6; NaCl – 0,5; $NH_4Cl$ – 1,0; L-leucine – 0,01.

2. Hydrolysated medium HM1 for growth of the aerobic Gram-positive chemoheterotrophic bacterium *B. subtilis B-3157*, based on $^2H_2O$ (89–90 atom% $^2$H) and 2 % (w/v) hydrolysate of deuterated biomass of *B. methylicum B-5662* as a source of $^2$H-labeled growth substrates: L-glucose – 120; hydrolysate of deuterated biomass of *B. methylicum* – 20, $NH_4NO_3$ – 20; $MgSO_4 \cdot 7H_2O$ – 10; $CaCO_3$

– 20; adenine – 0,01; uracil – 0,01. As a control was used protonated medium containing 2 % (w/v) yeast protein–vitamin concentrate (PVC).

3. Hydrolysated medium HM2 for the growth of the extreme aerobic halobacterium *Halobacterium halobium ET-1001* (based on 99,9 atom% $^2H_2O$): NaCl – 250; $MgSO_4 \cdot 7H_2O$ – 20; KCl – 2; $CaCl_2 \cdot 6H_2O$ – 0,065; sodium citrate – 0,5; hydrolyzate of deuterated biomass of *B. methylicum B-5662* – 20; biotin – $1 \cdot 10^{-4}$; folic acid – $1,5 \cdot 10^{-4}$, vitamin $B_{12}$ – $2 \cdot 10^{-5}$.

4. Tamiya medium for the growth of the photosynthetic green microalgae *C. vulgaris B-8765* (based on 99,9 atom% $^2H_2O$): $KNO_3$ – 5,0; $MgSO_4 \cdot 7H_2O$ – 2,5; $KH_2PO_4$ – 1,25; $FeSO_4$ – 0,003; $MnSO_4 \cdot 2H_2O$ – $3 \cdot 10^{-4}$; $CaCl_2 \cdot 6H_2O$ – 0,065; $ZnSO_4 \cdot 7H_2O$ – $4 \cdot 10^{-5}$; $CuSO_4 \cdot 5H_2O$ – $5 \cdot 10^{-5}$, $CoCl_2 \cdot 6H_2O$ – $5 \cdot 10^{-6}$).

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*Growth conditions*

The cells were grown in 500 ml Erlenmeyer flasks containing 100 ml of the growth medium at +34 0C and vigorously aerated on an orbital shaker Biorad ("Biorad Labs", Poland). Photo-organotrophic bacteria and blue-green algae were grown at illumination by fluorescent monochromatic lamps LDS-40-2 (40 W) („Alfa-Electro", Russia). Growing of microalgae C. vulgaris was carried out at +34 0C in a photoreactor with CO2 bubbling. The bacterial growth was monitored on the ability to form individual colonies on the surface of solid 2 % (w/v) agarose media, as well as on the optical density of the cell suspension measured on a Beckman DU-6 spectrophotometer ("Beckman Coulter", USA) at λ = 620 nm. After 6–7 days the cells were harvested and separated by centrifugation (10000 g, 20 min) on T-24 centrifuge («Heracules», Germany). The biomass was washed with 2H2O and extracted with a mixure of organic solvents: chloroform–methanol–acetone = 2:1:1, % (v/v) for isolating lipids and pigments. The resulting precipitate (10–12 mg) was dried in vacuum and used as a protein fraction, while the liquid extract – as a lipid fraction. The exogenious deuterated amino acids and ribonucleosides were isolated from culture liquids (CL) of appropriate strain-producers. Inosine was isolated from the CL of B. subtilis by adsorption/desorption on activated carbon as adsorbent with following extraction with 0,3 M NH4-formate buffer (pH = 8,9), subsequent crystallization in 80 % (v/v) ethanol, and ion exchange chromatography (IEC) on a column with cation exchange resin AG50WX 4 equilibrated with 0,3 M $NH_4$-formate buffer and 0,045 M $NH_4Cl$ (output – 3,1 g/l (80 %); $[\alpha]_D^{20}$ = 1,61 (ethanol)). Bacteriorhodopsin was isolated from the purple membranes of photo-organotrophic halobacterium *H. halobium* by the method of D. Osterhelt, modificated by the authors, with using SDS as a detergent [21].

*Protein hydrolysis*

Dry biomass (10 g) was treated with a chloroform–methanol–acetone mixture (2:1:1, % (v/v)) and supplemented with 5 ml of 6 N $^2$HCl (in $^2H_2O$). The ampules were kept at 110 $^0$C for ~24 h. Then the reaction mixture was suspended in hot $^2H_2O$ and filtered. The hydrolysate was evaporated at 10 mm Hg. Residual $^2$HCl was removed in an exsiccator over solid NaOH.

*Hydrolysis of intracellular policarbohydrates*

Dry biomass (50 mg) was placed into a 250 ml round bottomed flask, supplemented with 50 ml distilled $^2H_2O$ and 1,6 ml of 25 % (v/v) $H_2SO_4$ (in $^2H_2O$), and boiled in a reflux water evaporator for ~90 min. After cooling, the reaction mixture was suspended in one volume of hot distilled $^2H_2O$ and neutralized with 1 N $Ba(OH)_2$ (in $^2H_2O$) to pH = 7,0. $BaSO_4$ was separated by centrifugation (1500 g, 5 min); the supernatant was decanted and evaporated at 10 mm Hg.

*Aminoacid analysis*

The amino acids of the hydrolyzed biomass were analyzed on a Biotronic LC-5001 (230x3,2) column ("Eppendorf–Nethleler–Hinz", Germany) with a UR-30 sulfonated styrene resin ("Beckman–Spinco", USA) as a stationary phase; the temperature – 20±25 $^0$C; the mobile phase – 0,2 N sodium–citrate buffer (pH = 2,5); the granule diameter – 25 μm; working pressure – 50–60 atm; the eluent input rate – 18,5 ml/h; the ninhydrin input rate – 9,25 ml/h; detection at λ = 570 and λ = 440 nm (for proline).

*Analysis of carbohydrates*

Carbohydrates were analyzed on a Knauer Smartline chromatograph ("Knauer", Germany) equipped with a Gilson pump ("Gilson Inc.", USA) and a Waters K 401 refractometer ("Water Associates", USA) using Ultrasorb CN column

(250x10 mm) as a stationary phase; the temperature – 20±25 $^0$C; the mobile phase – acetonitrile–water (75:25, % (w/w); the granule diameter – 10 μm; the input rate – 0,6 ml/min.

*Analysis of fatty acids*

Fatty acids were analyzed on a Beckman Gold System (USA) chromatograph (250x4,6 mm), equiped with Model 126 UV-Detector (USA), 20±25 $^0$C . Stationary phase – Ultrasphere ODS 5 μm; mobile phase – linear gradient of 5 mM $KH_2PO_4$–acetonitrile; elution rate – 0,5 ml/min, detection at λ = 210 nm.

*Mass spectrometry*

For evaluation of deuterium enrichment levels EI and FAB mass spectrometry was used. EI mass spectra were recorded on MB-80A device ("Hitachi", Japan) with double focusing (the energy of ionizing electrons – 70 eV; the accelerating voltage – 8 kV; the cathode temperature – 180–200 $^0$C) after amino acid modification into methyl esters of N-5-dimethylamino(naphthalene)-1-sulfonyl (dansyl) amino acid derivatives according to an earlier elaborated protocol. FAB-mass spectra were recorded on a VG-70 SEQ chromatograph ("Fisons VG Analytical", USA) equipped with a cesium $Cs^+$ source on a glycerol matrix with accelerating voltage 5 kV and ion current 0,6–0,8 mA. Calculation of deuterium enrichment of the molecules was carried out using the ratio of contributions of molecular ion peaks of deuterated compounds extracted on $^2H_2O$-media relative to the control obtained on $H_2O$.

*Scanning electron microscopy (SEM)*

SEM was carried out on JSM 35 CF (JEOL Ltd., Korea) device, equiped with SE detector, thermomolecular pump, and tungsten electron gun (Harpin type W filament, DC heating); working pressure – $10^{-4}$ Pa ($10^{-6}$ Torr); magnification – ×150,000, resolution – 3,0 nm, accelerating voltage – 1–30 kV; sample size – 60–130 mm.

*IR-spectroscopy*

IR-spectroscopy was performed on Brucker Vertex spectrometer ("Brucker", Germany) (spectral range: average IR – 370–7800 $cm^{-1}$; visible – 2500–8000 $cm^{-1}$; the permission – 0,5 $cm^{-1}$; accuracy of wave number – 0,1 $cm^{-1}$ on 2000 $cm^{-1}$).

## Results and Discussion

Numerous studies with various biological objects in $^2H_2O$ proved that when biological objects are exposed to water with different deuterium content, their reaction varies depending on the isotopic composition of water (the content of deuterium in water) and magnitude of isotope effects determined by the difference of constants of chemical reactions rates $k_H/k_D$ in $H_2O$ and $^2H_2O$. The maximum kinetic isotopic effect observed at ordinary temperatures in chemical reactions leading to rupture of bonds involving hydrogen and deuterium atoms lies in the range $k_H/k_D$ = 5–8 for C–H versus C–$^2$H, N–$^2$H versus N–$^2$H, and O–$^2$H versus O–$^2$H-bonds [22]. Isotopic effects have an impact not only on the physical and chemical properties of deuterated macromolecules in which H atoms are substituted with $^2$H atoms, but also on the biological behaviour of biological objects in $^2H_2O$. Experiments with $^2H_2O$ have shown, that green algae is capable to grow on 70 % (v/v) $^2H_2O$, methylotrophic bacteria – 75 % (v/v) $^2H_2O$, chemoheterotrophic bacteria – 82 % (v/v) $^2H_2O$, and photo-organotrophic halobacteria – 95 % (v/v) $^2H_2O$ (Fig. 1).

In the course of the experiment were obtained adapted to the maximum concentration of $^2H_2O$ cells belonging to different taxonomic groups of microorganisms, realizing methylotrophic, chemoheterotrophic, photo-organotrophic and photosynthetic pathways of assimilation of carbon substrata, as facultative methylotrophic bacterium *B. methylicum*, chemoheterotrophic bacterium *B. subtilis*, halobacterium *H. halobium* and green algae *C. vulgaris*.

Selection of methanol-assimilating facultative methylotrophic bacterium *B. methylicum* was connected with the development of new microbiological strategies for preparation of deuterated biomass via bioconversion of [$^2$H]methanol and $^2H_2O$ and its further use as a source of deuterated growth substrates for the growing other strains-producers in $^2H_2O$.

Choosing of photo photo-organotrophic halobacterium *H. halobium* was stipulated by the prospects of further isolation of retinal containing transmembrane protein bacteriorhodopsin (BR) – chromoprotein of 248 amino acid residues,

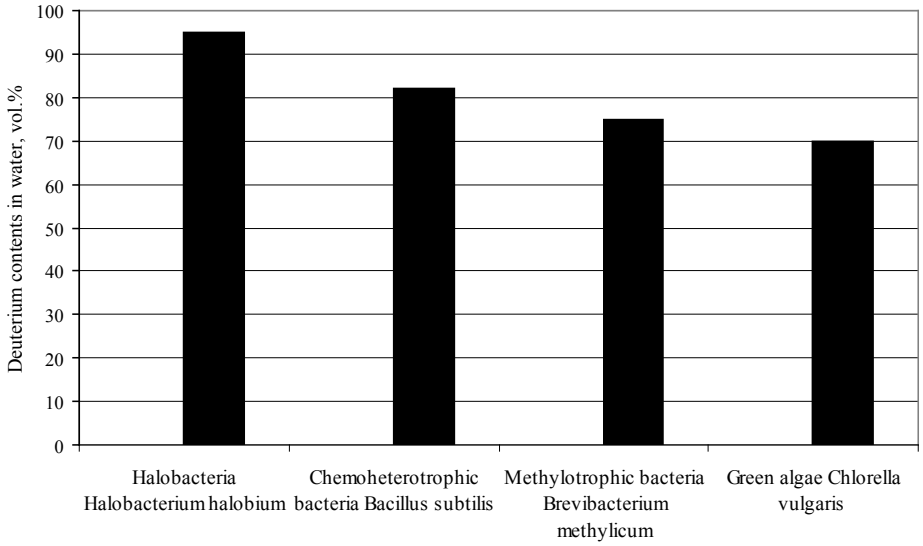


**Fig. 1.** Cell survival of various microorganisms in water with different deuterium content (%, v/v)

containing as a chromophore an equimolar mixture of 13-*cis*- and 13-*trans* C20 carotenoid associated with a protein part of the molecule via a Lys-216 residue [23]. BR performs in the cells of halobacteria the role of ATP-dependent translocase, which creates an electrochemical gradient of $H^+$ on the surface of the cell membrane, which energy is used by the cell for the synthesis of ATP in the anaerobic photosynthetic phosphorylation.

Using chemoheterotrophic bacterium *B. subtilis* was determined by preparative isolation produced by this bacterium deuterated ribonucleoside – inosine (total deuteration level 65,5 atom.% $^2H$) for biomedical use [24], and the use of photosynthetic blue-green *C. vulgaris* was stipulated by the study of biosynthesis of deuterated chlorophyll and carotenoid pigments (deuteration level 95–97 atom.% $^2H$) on growth media with high $^2H_2O$-content.

Our studies indicated that the ability of adaptation to $^2H_2O$ for different taxonomic groups of microorganisms is different, and stipulated by taxonomic affiliation, metabolic characteristics, pathways of assimilation of substrates, as well as by evolutionary niche occupied by the object. Thus, the lower the level of evolutionary organization of the organism, the easier it adapted to the presence of deuterium in growth media. Thus, most primitive in evolutionary terms (cell membrane structure, cell organization, resistance to environmental factors) of the studied objects are photo-organotrophic halobacteria related to archaebacteria, standing apart from both prokaryotic and eukaryotic microorganisms, exhibiting increased resistance to $^2H_2O$ and practically needed no adaptation to $^2H_2O$, contrary to blue-green algae, which, being eukaryotes, are the more difficult adapted to $^2H_2O$ and, therefore, exhibit inhibition of growth at 70–75 % (v/v) $^2H_2O$.

The composition of growth media evidently also plays an important role in process of adaptation to $^2H_2O$, because the reason of inhibition of cell growth and cell death can be changes of the parity ratio of synthesized metabolites in $^2H_2O$-media: amino acids, proteins and carbohydrates. It is noted that adaptation to $^2H_2O$ occures easier on complex growth media than on the minimal growth media with full substrates at a gradual increasing of deuterium content in the growth media, as the sensitivity to $^2H_2O$ of different vital systems is different. As a rule, even highly deuterated growth media contain remaining protons ~0,2–10,0 atom.%. These remaining protons facilitate the restructuring to the changed conditions during the adaptation to $^2H_2O$, presumably integrating into those sites, which are the most sensitive to the replacement of hydrogen by deuterium. The evidence has been obtained that cells evidently are able to regulate the $^2H/^1H$ ratios, while its changes trigger distinct molecular processes. One possibility to modify intracellular $^2H/^1H$ ratios is the activation of the $H^+$-transport system, which preferentially eliminates $H^+$, resulting in increased $^2H/^1H$ ratios within cells. Furthermore deuterium induces physiological, morphological and cytological alterations on the cell. There were marked the significant differences in the morphology of the protonated and deuterated cells of green algae *C. vulgaris*. Cells grown on $^2H_2O$-media were ~2–3 times larger in size and had thicker cell walls, than the control cells grown on a conventional protonated growth media with ordinary water, the distribution of DNA in them was non-uniform. In some cases on on the surface of cell membranes may be observed areas consisting of tightly packed pleats of a cytoplasmic membrane resembling mezosoms – intracytoplasmic bacterial membrane of vesicular structure and tubular form formed by the invasion of cytoplasmic membrane into the cytoplasm (Fig. 2). It is assumed that mezosoms involved in the formation of cell walls, replication and segregation of DNA, nucleotides and other processes. There is also evidence that the majority number of mezosoms being absent in normal cells is formed by a chemical action of some external factors – low and high temperatures, fluctuation of pH and and other factors. Furthermore, deuterated cells of *C. vulgaris* were also characterized by a drastic change in cell form and direction of their division. The observed cell division cytodieresis did not end by the usual divergence of the daughter cells, but led to the formation of abnormal cells, as described by other authors [25]. The observed morphological changes associated with the inhibition of growth of deuterated cells were stipulated by the cell restructuring during the process of adaptation to $^2H_2O$. The fact that the deuterated cells are larger in size (apparent size was of ~2–4 times larger than the size of the protonated cells), apparently is a general biological phenomenn proved by growing a number of other adapted to $^2H_2O$ prokaryotic and eukaryotic cells.

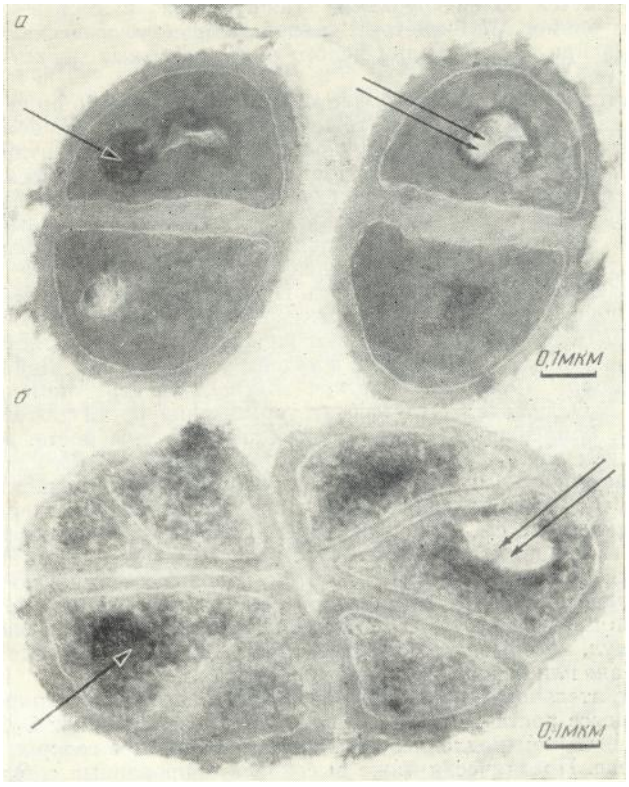


**Fig. 2.** Electron micrographs of *Micrococcus lysodeikticus* cells obtained by SEM method: *a*) – protonated cells obtained on $H_2O$-medium; *b*) – deuterated cells obtained on $^2H_2O$-medium. The arrows indicate the tightly-packed portions of the membranes

Our data generally confirm a stable notion that adaptation to $^2H_2O$ is a phenotypic phenomenon as the adapted cells eventually return back to the normal growth after some lag-period after their replacement back onto $H_2O$-medium. However, the effect of reversion of growth on $H_2O/^2H_2O$ media does not exclude an opportunity that a certain genotype determines the manifistation of the same phenotypic attribute in $^2H_2O$-media with high deuterium content. At placing a cell onto $^2H_2O$-media lacking protons, not only $^2H_2O$ is removed from a cell due to isotopic ($^1H–^2H$) exchange, but also there are occurred a rapid isotopic ($^1H–^2H$) exchange in hydroxyl (-OH), sulfohydryl (-SH) and amino (-$NH_2$) groups in all molecules of organic substances, including proteins, nucleic acids, carbohydrates and lipids. It is known, that in these conditions only covalent C–H bond is not exposed to isotopic ($^1H–^2H$) exchange and, thereof only molecules with bonds such as C–$^2H$ can be synthesized de novo. Depending on the position of the deuterium atom in the molecule, there are distinguished primary and secondary isotopic effects mediated by intermolecular interactions. In this aspect, the most important for the structure of macromolecules are dynamic short-lived hydrogen (deuterium) bonds formed between the electron deficient $^1H(^2H)$ atoms and adjacent electronegative O,C,N,S- heteroatoms in the molecules, acting as acceptors of H-bond. The hydrogen bond, based on weak electrostatic forces, donor-acceptor interactions with charge-transfer and intermolecular van der Waals forces, is of the vital importance in the chemistry of intermolecular interactions and maintaining the spatial structure of macromolecules in aqueous solutions. Another important property is defined by the three-dimensional structure of $^2H_2O$ molecule having the tendency to pull together hydrophobic groups of macromolecules to minimize their disruptive effect on the hydrogen (deuterium)-bonded network in $^2H_2O$. This leads to stabilization of the structure of protein and nucleic acid macromolecules in the presence of $^2H_2O$. That is why, the structure of macromolecules of proteins and nucleic acids in the presence of $^2H_2O$ stabilized [26].

Evidently the cell implements special adaptive mechanisms promoting the functional reorganization of vital systems in $^2H_2O$. Thus, for the normal synthesis and function in $^2H_2O$ of such vital compounds as nucleic acids and proteins contributes to the maintenance of their structure by forming hydrogen (deuterium) bonds in the molecules. The bonds formed by deuterium atoms are differed in strength and energy from similar bonds formed by hydrogen. Somewhat greater strength of $^2H–O$ bond compared to $^1H–O$ bond causes the differences in the kinetics of reactions in $H_2O$ and $^2H_2O$. Thus, according to the theory of a chemical bond the breaking up of covalent $^1H–C$ bonds can occur faster than C–$^2H$ bonds, the mobility of $^2H_3O^+$ ion is lower on 28,5 % than $H_3O^+$ ion, and $O^2H^-$ ion is lower on 39,8 % than $OH^-$ ion, the constant of ionization of $^2H_2O$ is less than that of $H_2O$. These chemical-physical factors lead to slowing down in the rates of enzymatic reactions in $^2H_2O$ [27]. However, there are also such reactions which rates in $^2H_2O$ are higher than in $H_2O$. In general these reactions are catalyzed by $^2H_3O^+$ or $H_3O^+$ ions or $O^2H^-$ and $OH^-$ ions. The substitution of $^1H$ with $^2H$ affects the stability and geometry of hydrogen bonds in an apparently rather complex way and may through the changes in the hydrogen bond zero-point vibration energies, alter the conformational dynamics of hydrogen (deuterium)-bonded structures of DNA and proteins in $^2H_2O$. It may cause disturbances in the DNA-synthesis during mitosis, leading to permanent changes on DNA structure and consequently on cell genotype [28]. Isotopic effects of deuterium, which would occur in macromolecules of even a small difference between hydrogen and deuterium, would certainly have the effect upon the structure. The sensitivity of enzyme function to the structure and the sensitivity of nucleic acid function (genetic and mitotic) would lead to a noticeable effect on the metabolic pathways and reproductive behaviour of an organism in the presence of $^2H_2O$. And next, the changes in dissociation constants of DNA and protein ionizable groups when transferring the macromolecule from $H_2O$ into $^2H_2O$ may perturb the charge state of the DNA and protein molecules. All this can cause variations in nucleic acid synthesis, which can lead to structural changes and functional differences in the cell and its organelles. Hence, the structural and dynamic properties of the cell membrane, which depends on qualitative and quantitative composition of membrane's fatty acids, can also be modified in the presence of $^2H_2O$. The cellular membrane is one of the most important organelles in the bacteria for metabolic regulation, combining apparatus of biosynthesis of polysaccharides, transformation of energy, supplying cells with nutrients and involvement in the biosynthesis of proteins, nucleic acids and fatty acids. Obviously, the cell membrane plays an important role in the adaptation to $^2H_2O$. But it has been not clearly known what occurs with the membranes – how they react to the replacement of protium to deuterium and how it concerns the survival of cells in $^2H_2O$-media devoid of protons.

Comparative analysis of the fatty acid composition of deuterated cells of chemoheterotrophic bacteria *B. subtilis*, obtained on the maximum deuterated medium with 99,9 atom.% $^2H_2O$, carried out by HPLC method, revealed significant quantitative differences in the fatty acid composition compared to the control obtained in ordinary water (Fig. 3a, b). Characteristically, in a deuterated sample fatty acids having retention times at 33,38; 33,74; 33,26 and 36,03 min are not detected in HPLC-chromatogram (Fig. 3b).

This result is apparently due to the fact that the cell membrane is one of the first cell organelles, sensitive to the effects of $^2H_2O$, and thus compensates the changes in rheological properties of a membrane (viscosity, fluidity, structuredness) not only by quantitative but also by qualitative composition of membrane fatty acids. Similar situation was observed with the separation of other natural compounds (proteins, amino acids, carbohydrates) extracted from deuterobiomass obtained from maximally deuterated $^2H_2O$-medium.

Amino acid analysis of protein hydrolysates isolated from deuterated cells of *B. subtilis* also revealed the differences in quantitative composition of amino acids synthesized in $^2H_2O$-medium (Fig. 4). Protein hydrolyzates contains fifteen

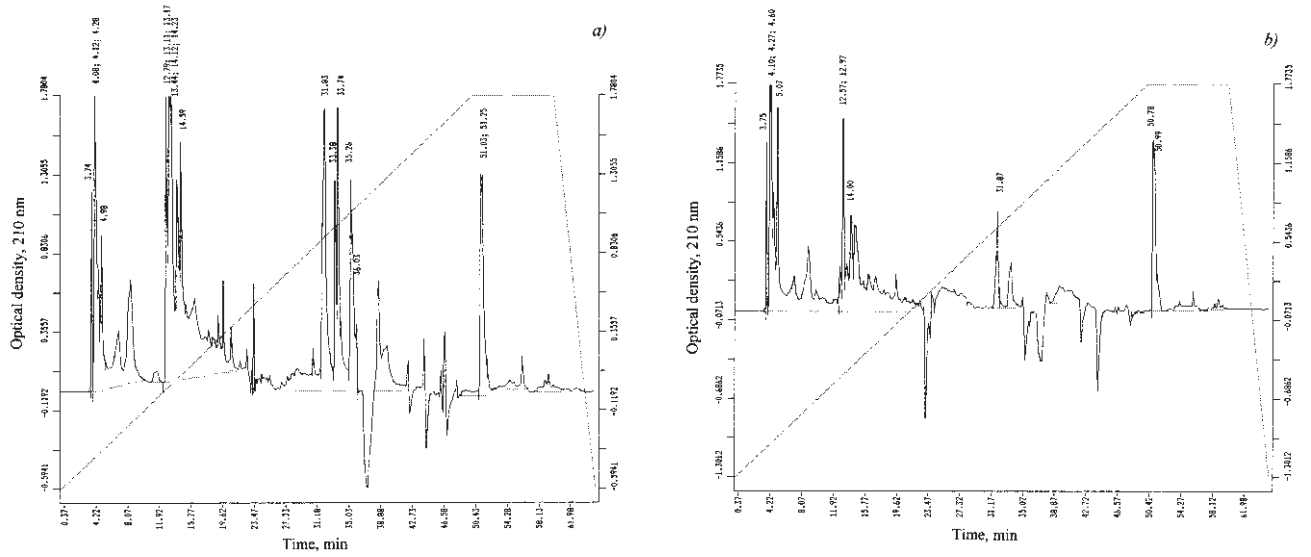


**Fig. 3.** HPLC-chromatograms of fatty acids obtained from protonated (*a*) and deuterated (*b*) cells of *B. subtilis* on the maximally deuterated $^2H_2O$-medium: Beckman Gold System (Beckman, USA) chromatograph (4,6x250 mm); stationary phase: Ultrasphere ODS, 5 μm; mobile phase: linear gradient 5 mM $KH_2PO_4$–acetonitrile (shown in phantom), elution rate: 0,5 ml/min, detection at λ = 210 nm. The peaks with retention time 3,75 min (instead of 3,74 minutes in the control); 4,10; 4,27; 4,60 (instead of 4,08; 4,12; 4,28 in the control), 5,07 (instead of 4,98 in control) 12,57; 12,97 (instead of 12,79; 13,11; 13,17 in control); 14,00 (instead of 14,59 in the control); 31,87 (instead of 31,83 in the control); 33,38; 33,74; 33,26; 36,03; 50,78; 50,99 (instead of 51,03; 51,25 for control) correspond to individual intracellular fatty acids

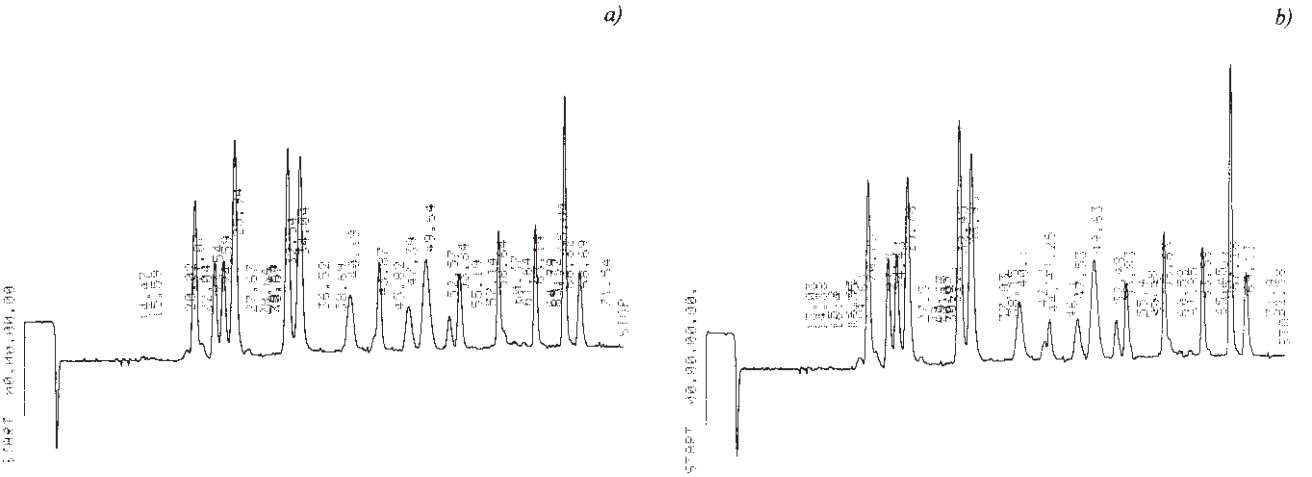


**Fig. 4.** Ion exchange chromatograms of amino acids obtained from hydrolizates of protonated (a) and deuterated (b) cells of *B. subtilis* on the maximally deuterated $^2H_2O$-medium: Biotronic LC-5001 (230□**3,2 mm) column** (“Eppendorf–Nethleler–Hinz”, Germany); stationary phase: UR-30 sulfonated styrene resin (“Beckman–Spinco”, USA); 25 μm; 50–60 atm; mobile phase: 0,2 N sodium–citrate buffer (pH = 2,5); the eluent input rate: 18,5 ml/h; the ninhydrin input rate: 9,25 ml/h; detection at λ = 570 and λ = 440 nm (for proline).

identified amino acids (except proline, which was detected at λ = 440 nm) (Table 1). An indicator that determines a high efficiency of deuterium inclusion into amino acid molecules of protein hydrolyzates are high levels of deuterium enrichment of amino acid molecules, which are varied from 50 atom.% for leucine/isoleucine to 97,5 atom.% for alanine.

Qualitative and quantitative composition of the intracellular carbohydrates of *B. subtilis* obtained on maximally deuterated $^2H_2O$-medium is shown in Table 2 (the numbering is given to the sequence of their elution from the column) contained monosaccharides (glucose, fructose, rhamnose, arabinose), disaccharides (maltose, sucrose), and four other unidentified carbohydrates with retention time 3,08 min (15,63 %); 4,26 min (7,46 %); 7,23 min (11,72 %) and 9,14 min (7,95 %) (not shown) (Fig. 5). Yield of glucose in deuterated sample makes up 21,4 % by dry weight, i.e. higher than for fructose (6,82 %), rhamnose (3,47 %), arabinose (3,69 %), and maltose (11,62 %). Their outputs are not significantly different from the control in $H_2O$ except for sucrose in deuterated sample that was not detected (Table 2). The deuterium enrichment levels of carbohydrates were varied from 90,7 atom.% for arabinose to 80,6 atom.% for glucose.

In conclusion it should be noted that comparative analysis of IR-spectra of $H_2O$ solutions and its deuterated analogues ($^2H_2O$, $H^2HO$) is of considerable interest for biophysical studies, because at changing of the atomic mass of hydrogen by deuterium atoms in $H_2O$ molecule their interaction will also change, although the electronic structure of the molecule and its ability to form H-bonds, however, remains the same. The local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state; the substitution with deuterium changes the vibrational-rotational transitions in $H_2O$ molecule, that is why it apear other local maximums in IR-spectra. In the water vapor state, the vibrations involve combinations of symmetric stretch ($v_1$), asymmetric stretch ($v_3$) and bending ($v_2$) of the covalent bonds with absorption intensity ($H_2O$) $v_1;v_2;v_3$ = 2671; 1178,4; 2787,7 $cm^{-1}$. For liquid water absorption bands are observed in other regions of the IR-spectrum, the most intense of which are located at 2100, $cm^{-1}$ and 710-645 $cm^{-1}$. For $^2H_2O$ molecule these ratio compiles 2723,7; 1403,5 and 3707,5 $cm^{-1}$, while for $H^2HO$ molecule – 2671,6; 1178,4 and 2787,7 $cm^{-1}$. $H^2HO$ (50 mole% $H_2O$ + 50 mole% $^2H_2O$; ~50 % $H^2HO$, ~25 % $H_2O$, ~25 % $^2H_2O$) has local maxima in IR-spectra at 3415 $cm^{-1}$, 2495 $cm^{-1}$ 1850 $cm^{-1}$ and 1450 $cm^{-1}$ assigned to $OH^-$-stretch, $O^2H^-$-stretch, as well as combination of bending and libration and $H^2HO$ bending respectively.

In the IR-spectrum of liquid water absorbance band considerably broadened and shifted relative to the corresponding bands in the spectrum of water vapor. Their position depends on the temperature [29]. The temperature dependence of individual spectral bands of liquid water is very complex [30]. Furthermore, the complexity of the IR-spectrum in the area of $OH^-$ stretching vibration can be explained by the existence of different types of $H_2O$ associations, manifestation of overtones and composite frequencies of $OH^-$ groups in the hydrogen bonds, and the tunneling effect of the proton (for relay mechanism) [31]. Such complexity makes it difficult to interpret the spectrum and partly explains the discrepancy in the literature available on this subject.

**Table 1**

Amino acid composition of the protein hydrolysates of *B. subtilis*, obtained on the maximum deuterated medium and levels of deuterium enrichment of molecules*

| Amino acid | Yield, % (w/w) dry weight per 1 gram of biomass | | Number of deuterium atoms incorporated into the carbon backbone of a molecule** | Level of deuterium enrichment of molecules, % of the total number of hydrogen atoms*** |
|---|---|---|---|---|
| | Protonated sample (control) | The sample obtained in 99,9 atom.% $^2H_2O$ | | |
| Glycine | 8,03 | 9,69 | 2 | 90,0 |
| Alanine | 12,95 | 13,98 | 4 | 97,5 |
| Valine | 3,54 | 3,74 | 4 | 50,0 |
| Leucine | 8,62 | 7,33 | 5 | 50,0 |
| Isoleucine | 4,14 | 3,64 | 5 | 50,0 |
| Phenylalanine | 3,88 | 3,94 | 8 | 95,0 |
| Tyrosine | 1,56 | 1,83 | 7 | 92,8 |
| Serine | 4,18 | 4,90 | 3 | 86,6 |
| Threonine | 4,81 | 5,51 | – | – |
| Methionine | 4,94 | 2,25 | – | – |
| Asparagine | 7,88 | 9,59 | 2 | 66,6 |
| Glutamic acid | 11,68 | 10,38 | 4 | 70,0 |
| Lysine | 4,34 | 3,98 | 5 | 58,9 |
| Arginine | 4,63 | 5,28 | – | – |
| Histidine | 3,43 | 3,73 | – | – |

Notes:

* The data obtained by mass spectrometry for the methyl esters of N-5-(dimethylamino) naphthalene-1-sulfonyl chloride (dansyl) amino acid derivatives.

** While calculating the level of deuterium enrichment protons (deuterons) at the carboxyl (COOH-) and $NH_2$-groups of amino acid molecules are not taken into account because of their easy dissociation in $H_2O/^2H_2O$

*** A dash means absence of data.

**Table 2**

Qualitative and quantitative composition of intracellular carbohydrates of *B. subtilis* obtained on the maximally deuterated medium and levels of deuterium enrichment of molecules*

| Carbohydrate | Content in the biomass, % of the dry weight of 1 g biomass | | Level of deuterium enrichment, % of the total number of hydrogen atoms*** |
|---|---|---|---|
| | Protonated sample (control) | The sample obtained in 99,9 atom.% $^2H_2O$** | |
| Glucose | 20,01 | 21,40 | 80,6 |
| Fructose | 6,12 | 6,82 | 85,5 |
| Rhamnose | 2,91 | 3,47 | 90,3 |
| Arabinose | 3,26 | 3,69 | 90,7 |
| Maltose | 15,30 | 11,62 | – |
| Sucrose | 8,62 | ND | – |

Notes:
* The data were obtained by IR-spectroscopy
** ND – not detected
** A dash means the absence of data.

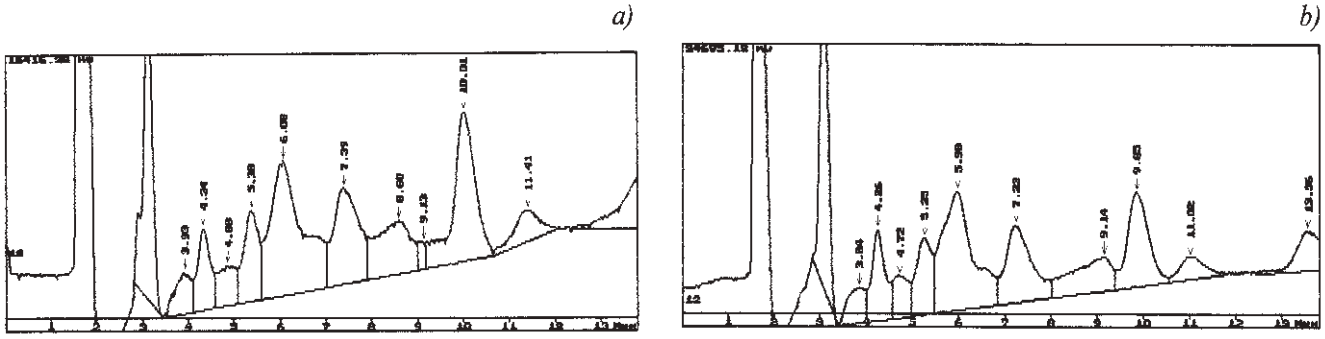


**Fig. 5.** HPLC-chromatograms of intracellular carbohydrates obtained from protonated (*a*) and deuterated (*b*) cells of *B. subtilis* on the maximally deuterated $^2H_2O$-medium: Knauer Smartline chromatograph (250x10 mm) ("Knauer", Germany); stationary phase: Ultrasorb CN; 10 μm; mobile phase: acetonitrile–water (75:25, % (w/w); the input rate: 0,6 ml/min

**Table 3**

The assignment of main frequencies in IR-spectra of liquid $H_2O$ and $^2H_2O$

| Main vibrations of liquid $H_2O$ and $^2H_2O$ | | | | |
|---|---|---|---|---|
| Vibration(s) | $H_2O$ (t = +25 $^0$C) | | $^2H_2O$ (t = +25 $^0$C) | |
| | *v*, $cm^{-1}$ | $E_0$, $M^{-1}$ $cm^{-1}$ | *v*, $cm^{-1}$ | $E_0$, $M^{-1}$ $cm^{-1}$ |
| Spinning $v_1$ + deformation $v_2$ | 780-1645 | 21,65 | 1210 | 17,10 |
| Composite $v_1 + v_2$ | 2150 | 3,46 | 1555 | 1,88 |
| Valence symmetrical $v_1$, valence asymmetrical $v_3$, and overtone $2v_2$ | 3290-3450 | 100,65 | 2510 | 69,70 |

In liquid water and ice the IR-spectra are far more complex than those ones of the vapor due to vibrational overtones and combinations with librations (restricted rotations, i.g. rocking motions). These librations are due to the restrictions imposed by hydrogen bonding (minor $L_1$ band at 395,5 $cm^{-1}$; major $L_2$ band at 686,3 $cm^{-1}$; for liquid water at 0 $^0C$, the absorbance of $L_1$ increasing with increasing temperature, while $L_2$ absorbance decreases but broadens with reduced wave number with increasing temperature [32]. The IR spectra of liquid water usually contain three absorbance bands, which can be identified on absorption band of the stretching vibration of $OH^-$ group; absorption band of the first overtone of the bending vibration of the molecule $H^2HO$ and absorption band of stretching vibration of $O^2H^-$ group. Hydroxyl group $OH^-$ is able to absorb much infrared radiation in the infrared region of the IR-spectrum. Because of its polarity, these groups typically react with each other or with other polar groups to form intra-and intermolecular hydrogen bonds. The hydroxyl groups, which are not involved in formation of hydrogen bonds, usually produce the narrow bands in IR spectrum, while the associated groups – broad intense absorbance bands at lower frequencies. The magnitude of the frequency shift is determined by the strength of the hydrogen bond. Complication of the IR spectrum in the area of $OH^-$ stretching vibrations can be explained by the existence of different types of associations of $H_2O$ molecules, a manifestation of overtones and combination frequencies of $OH^-$ groups in hydrogen bonding, as well as the proton tunneling effect (on the relay mechanism).

Assignment of main absorbtion bands in the IR-spectrum of liquid water is given in the Table 3. The IR spectrum of $H_2O$ molecule was examined in detail from the microwave till the middle (4–17500 $cm^{-1}$) visible region and the ultraviolet region – from 200 $nm^{-1}$ to the ionization limit 98 $nm^{-1}$. In the middle visible region at 4–7500 $cm^{-1}$ are located the rotational spectrum and the bands corresponding to the vibrational-rotational transitions in the ground electronic state. In the ultraviolet region (200 $nm^{-1}$ to 98 $nm^{-1}$) are located bands corresponding to transitions from the excited electronic states close to the ionization limit in the electronic ground state. The intermediate region of the IR-spectrum – from 570 nm to 200 nm corresponds to transitions to higher vibrational levels of the ground electronic state.

At the transition from $H_2O$ monomers to $H_2O$ dimmer and $H_3O$ trimmer absorption maximum of valent stretching vibrations of the O–H bond is shifted toward lower frequencies ($v_3$ = 3490 $cm^{-1}$ and $v_1$ = 3280 $cm^{-1}$) and the bending frequency is increased ($v_2$ = 1644 $cm^{-1}$) because of hydrogen bonding. The increased strength of hydrogen bonding typically shifts the stretch vibration to lower frequencies (red-shift) with greatly increased intensity in the infrared due to the increased dipoles. In contrast, for the deformation vibrations of the H–O–H, it is observed a shift towards higher frequencies. Absorption bands at 3546 and 3691 $cm^{-1}$ were attributed to the stretching modes of the dimer $[(H_2O)_2]$. These frequencies are significantly lower than the valence modes of $v_1$ and $v_3$ vibrations of isolated $H_2O$ molecules at 3657 and 3756 $cm^{-1}$ respectively). The absorbtion band at 3250 $cm^{-1}$ represents overtones of deformation vibrations. Among frequencies between 3250 and 3420 $cm^{-1}$ is possible Fermi resonance (this resonance is a single substitution of intensity of one fluctuation by another fluctuation when they accidentally overlap each other). The absorption band at 1620 $cm^{-1}$ is attributed to the deformation mode of the dimer. This frequency is slightly higher than the deformation mode of the isolated $H_2O$ molecule (1596 $cm^{-1}$). A shift of the band of deformation vibration of water in the direction of high frequencies at the transition from a liquid to a solid atate is attributed by the appearance of additional force, preventing O–H bond bending. Deformation absorption band in IR-spectum of water has a frequency at 1645 $cm^{-1}$ and weak temperature dependence. It changes little in the transition to the individual $H_2O$ molecule at a frequency of 1595 $cm^{-1}$. This frequency is found to be sufficiently stable, while all other frequencies are greatly affected by temperature changes, the dissolution of the salts and phase transitions. It is believed that the persistence of deformation oscillations is stipulated by processes of intermolecular interactions, i.g. by the change in bond angle as a result of interaction of $H_2O$ molecules with each other, as well as with cations and anions.

Thus the study of the characteristics of the IR spectrum of water allows to answer the question not only on the physical parameters of the molecule and the covalent bonds at isotopic substitution with deuterium, but also to make a certain conclusion on associative environment in water. The latter fact is important in the study of structural and functional properties of water assotiates and its isotopomers at the isotopic substitution with deuterium.

## Conclusions

The experimental data demonstrated that the effects observed at the cellular growth on $^2H_2O$-media possess a complex multifactor character stipulated by changes of morpholodical, cytological and physiological parameters, a ratio of amino acids, protein, carbohydrates and fatty acids synthesized in $^2H_2O$, and with an evolutionary level of organization of investigated object as well. The cell evidently implements the special adaptive mechanisms promoting functional reorganization of work of the vital systems in the presence of $^2H_2O$. Thus, the most sensitive to replacement of $^1H$ on $^2H$ are the apparatus of biosynthesis of macromolecules and a respiratory chain, i.e., those cellular systems using high mobility of protons and high speed of breaking up of hydrogen bonds. Last fact allows the consideration of adaptation to $^2H_2O$ as adaptation to the nonspecific factor affecting simultaneously the functional condition of several numbers of cellular systems: metabolism, ways of assimilation of carbon substrates, biosynthetic processes, and transport function, structure and functions of deuterated macromolecules.

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# STUDYING THE BIOLOGICAL INFLUENCE OF HEAVY WATER ($^2H_2O$) ON PROCARYOTIC AND EUCARYOTIC CELLS

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Biological influence of heavy water on cells of various taxonomic groups of prokaryotic and eucaryotic microorganisms realizing methylotrophic, chemoheterotrophic, photo-organotrophic, and photosynthetic ways of assimilation of carbon substrates (methylotrophic bacteria *Brevibacterium methylicum*, chemoheterotrophic bacteria *Bacillus subtilis*, photo-organotrophic halobacteria *Halobacterium halobium*, and green algae *Chlorella vulgaris*) was studied at the growth on media with $^2H_2O$. The qualitative and quantitative composition of intra- and endocellular amino acids, proteins, carbohydrates and fatty acids in conditions of adaptation to $^2H_2O$ is investigated. It is shown, that the effects observed at adaptation to $^2H_2O$, possess a complex multifactorial character and connected to cytological, morphological and physiological changes, a parity ratio of synthesized amino acids, proteins, carbohydrates and lipids, and also with an evolutionary level of the organization of the investigated object and the pathways of assimilation of substrates as well.

**Key words**: deuterium, heavy water, adaptation, isotopic effects, bacteria, micro algae.

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# ИССЛЕДОВАНИЕ БИОЛОГИЧЕСКОГО ВЛИЯНИЯ ТЯЖЕЛОЙ ВОДЫ ($^2H_2O$) НА КЛЕТКИ ПРОКАРИОТ И ЭУКАРИОТ

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Исследовано биологическое влияние тяжелой воды на клетки различных таксономических групп прокариотических и эукариотических микроорганизмов, реализующих метилотрофный, хемогетеротрофный, фотоорганотрофный и фотосинтетический способы ассимиляции углеродных субстратов (метилотрофные бактерии *Brevibacterium methylicum*, хемогетеротрофные бактерии *Bacillus subtilis*, фотоорганотрофные галобактерии *Halobacterium halobium* и зеленая микроводоросль *Chlorella vulgaris*) при росте на питательных средах с $^2H_2O$. Приведен качественный и количественный состав внутри- и межклеточных аминокислот, белков, углеводов и жирных кислот в условиях адаптации к $^2H_2O$. Показано, что эффекты, наблюдаемые при адаптации к $^2H_2O$, имеют сложный многофакторный характер и связаны с цитологическими, морфологическими и физиологическими изменениями в клетке, соотношением синтезированных аминокислот, белков, углеводов и липидов, а также с эволюционным уровнем организации исследуемого объекта и путями ассимиляции субстратов.

**Ключевые слова:** дейтерий, тяжелая вода, адаптация, изотопные эффекты, бактерии, микроводоросли.

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# ДОСЛІДЖЕННЯ БІОЛОГІЧНОГО ВПЛИВУ ВАЖКОЇ ВОДИ ($^2H_2O$) НА КЛІТИНИ ПРОКАРІОТ І ЕУКАРІОТ

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Досліджено біологічний вплив важкої води на клітини різних таксономічних груп прокаріотичних і еукаріотичних мікроорганізмів, що реалізують метилотрофний, хемогетеротрофний, фотоорганотрофний і фотосинтетичний способи асиміляції вуглецевих субстратів (метилотрофні бактерії *Brevibacterium methylicum*, хемогетеротрофні бактерії *Bacillus subtilis*, фотоорганотрофні галобактерії *Halobacterium halobium* і зелена мікроводорость *Chlorella vulgaris*) при вирощуванні на поживних середовищах з $^2H_2O$. Наведено якісний і кількісний склад внутрішньо- і міжклітинних амінокислот, білків, вуглеводів і жирних кислот в умовах адаптації до $^2H_2O$. Показано, що ефекти, які спостерігаються при адаптації до $^2H_2O$, мають складний багатофакторний характер і пов'язані з цитологічними, морфологічними і фізіологічними змінами в клітині, співвідношенням синтезованих амінокислот, білків, вуглеводів і ліпідів, а також з еволюційним рівнем організації досліджуваного об'єкта та шляхами асиміляції субстратів.

**Ключові слова:** дейтерій, важка вода, адаптація, ізотопні ефекти, бактерії, мікроводорості.

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