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Isotopic Composition of Water in Research into Phenomenon of Longevity in Mountainous and Field Areas of Bulgaria

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Abstract

In this paper are submitted data on longevity factors and mountain water in factorial research of phenomenon of longevity in mountain and field areas of Bulgaria. The dependence was established among various internal and external factors on a phenomenon of longevity – residence area, health status, gender and heredity. It was shown that water is among the most important factors for longevity. Natural waters derived from various Bulgarian water springs were investigated by IR-spectroscopy, NES-, and DNES-method. It was shown, that the increased content of deuterium leads to physiological, morphological and cytology alterations of the cell, and also renders negative influence on cellular metabolism, while deuterium depleted water with decreased deuterium content on 20-30% has beneficial effects on health. By using IR-spectroscopy (NES and DENS-method) were investigated various samples of water with varying contents of deuterium, received from Bulgarian water springs and blood serum of cancer patients as well. As estimation factor was measured the values of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules, as well as local maxima in the IR-spectra of various samples of water and human blood serum at -0.1387 eV and wavelength – 8.95 μm. For a group of people in critical condition of life and patients with malignant tumors the greatest values of local maxima in IR-spectra are shifted to lower energies relative to the control group. The obtained results testify to necessity of consumption of clean natural water which quality satisfies mountain water from Bulgarian water springs.

Keywords: deuterium, heavy water, deuterium depleted water, longevity, mountain water, IR spectroscopy, NES, DNES

1. Introduction

The question of longevity has always been an exciting one for humanity. Aging is a biological process, which leads to reduction of the vital functions of the body, limiting its adaptive capacities, and development of age-related pathologies and ultimately increasing the likelihood of death, is a part of the normal ontogeny and is caused by the same processes that lead to increased functional activity of various body systems in earlier periods of life. It is possible that these processes along with other processes (growth and development of the organism, etc.) are programmed in the human genome and biological mechanism of regulation. The question to what extent aging is dependent on heredity is not sufficiently proven in modern science.

Like other biological processes, aging is accelerated under the influence of certain exogenous and endogenous factors and occurs in different individuals with different speed, which depends on genetic differences and environmental factors. The best chance for longevity gives the longevity of immediate direct genetic ancestors. That is why the direct descendants of centenarians generally have the best chances for longevity. O. Burger demonstrated that life expectancy has increased substantially from the 19th to the 20th century and that this cannot be advantageously associated with the human genome (Burger *et al*., 2012). The main factors of longevity are water quality, food and improved advancement of medicine. For example, in Bulgaria the average life expectancy from 1935 to 1939 was 51.75 years, while from 2008 to 2010 it was 73.60 years. In Russia, the average life expectancy in 2012 has reached 69 years.

From the standpoint of genetics, the process of aging is associated with disruption of the genetic program of the organism and gradual accumulation of errors during the process of DNA replication. Aging may be associated with the accumulation of somatic mutations in the genome and be influenced by free radicals (mainly oxygen and primary products of oxidative metabolism) and ionizing radiation on DNA molecules as well (Woodhead, 1984; Adelman *et al*., 1988; Pryor, 1997). Such mutations can reduce the ability of cells to the normal growth and division and be a cause of a large number of various cell responses: inhibition of replication and transcription, impaired cell cycle division, transcriptional mutagenesis, cell aging that finally results in cell death. Cells taken from the elderly people show a reduction in transcription when transferring information from DNA to RNA.

From the standpoint of dynamics, aging is a non-linear biological process, which increases over time. Accordingly, the rate of aging increases with time. The accumulation of errors in the human genome increases exponentially with time and reaches a certain stationary maximum at the end of life. L. Orgel shows that, for this reason, the probability of cancer occurrence increases with age (Orgel, 1963). According to thermodynamics, the process of aging is the process of alignment of the entropy by the human body with that of the environment (Ignatov, Mosin, 2011).

Water is the main substance of life. The human body is composed from 50 to 75% of water (Watson, 1980). With aging, the percentage of water in the human body decreases. Hence, the factor of water quality is the essential factor for the research (Pocock *et al.*, 1981; Howard & Hopps, 1986). Water is present in the composition of the physiological fluids in the body and plays an important role as an inner environment in which the vital biochemical processes involving enzymes and nutrients take place. Water is the maing factor for metabolic processes and aging (Ignatov, 2012). Earlier studies conducted by us have demonstrated the role of water, its structure, isotopic composition and physico-chemical (pH, temperature) in the growth and prolifiration of prokaryotes and eukaryotes in water with different isotopic content (Mosin & Ignatov, 2012a; Ignatov & Mosin, 2013a; Ignatov & Mosin, 2013b). These factors and the structure of water are of great importance in biophysical studies. The peculiarities of chemical structure of H_2O molecule create favorable conditions for formation of electrostatic intermolecular Van-der-Waals, dipole-dipole forces and donor-acceptor interaction with transfer of charges between H-atom and O-atoms in H_2O molecules, binding them into water associates (clusters) with the general formula $(H_2O)_n$ where n varries from 3 to 60 units (Saykally, 2005; Ignatov, Mosin, 2013c). Other important indicator of water quality is its isotopic composition. The natural water consists on 99.7 mol.% of $H_2{}^{16}O$, which molecules are formed by ^1H and ^{16}O atoms (Mosin & Ignatov, 2012b). The remaining 0.3 mol.% is represented by isotope varieties (isotopomers) of water molecules, wherein deuterium forms 6 configurations of isotopomers – $HD^{16}O$, $HD^{17}O$, $HD^{18}O$, $D_2{}^{16}O$, $D_2{}^{17}O$, $D_2{}^{18}O$, while 3 configuration are formed by isotopomers of oxygen – $H_2{}^{16}O$, $H_2{}^{17}O$, $H_2{}^{18}O$.

This paper studies the influence of various internal and external factors on a phenomenon of longevity – residence area, health status, gender, heredity, isotopic composition of water with using non-equilibrium (NES) and differential non-equilibrium (DNES) spectrum of water. The research was carried out under the joint scientific project “NATURE, ECOLOGY AND LONGEVITY” conducted in Bulgaria. In frames of this project 217 people living in the municipalities of Teteven, Yablanitza and Ugarchin, Lovech district (Bulgaria), where is lived the most number of long living people and their siblings, were studied. They have the same heredity, but have lived under different conditions. In all three municipalities there is a mountainous and a field part. Mountain and tap water is used for drinking. Statistical analysis has been conducted for heredity, body weight, food, diseases, positive attitude towards life.

2. Material and Methods

The objects of the study were various prokaryotic and eukaryotic cells obtained from the State Research Institute of Genetics and Selection of Industrial Microorganisms (Moscow, Russia). Experiments were also carried out with the samples of natural mountain water from various Bulgarian springs and human blood serum.

For preparation of growth media we used D_2O (99.9 atom%) received from the Russian Research Centre “Isotope” (St. Petersburg, Russian Federation). Inorganic salts were preliminary crystallized in D_2O and dried in vacuum before using. D_2O distilled over $KMnO_4$ with the subsequent control of deuterium content in water by ^1H-NMR-spectroscopy on Brucker WM-250 device (“Brucker”, Germany) (working frequency – 70 MHz, internal standard – Me_4Si) and on Brucker Vertex (“Brucker”, Germany) IR spectrometer (a 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}).

1% (v/v) solution of human blood serum was studied with the methods of IR-spectrrometry, non-equilibrium (NES) and differential non-equilibrium (DNES) spectrum. The specimens were provided by Kalinka Naneva (Municipal Hospital, Bulgaria). Two groups of people between the ages of 50 to 70 were tested. The first group (control group) consisted of people in good clinical health. The second group included people in critical health or suffering from malignant diseases. The device for DNES was made from A. Antonov on an optical principle. In this study was used a hermetic camera for evaporation of water drops under stable temperature (+22–24 ^{0}C) conditions. The water drops are placed on a water-proof transparent pad, which consists of thin maylar folio and a glass plate. The light is monochromatic with filter for yellow color with wavelength λ = 580±7 nm. The device measures the angle of evaporation of water drops from 72,3 ^{0} to 0 ^{0}. The spectrum of hydrogen bonds among H_2O molecules was measured in the range of 0.08–0.1387 eV or λ = 8.9–13.8 μm using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in human blood serum.

Interviews have been conducted with 217 Bulgarian centenarians and their siblings. Their heredity, body weight, health status, tobacco consumption, physical activity, attitude towards life has been analyzed. With using DNES method was performed a spectral analysis of 12 mountain water springs located in Teteven district (Bulgaria). The composition of water samples was studied in the laboratory of “Eurotest Control” (Bulgaria).

Statistics methods were attributed to the National Statistical Institute of Bulgaria.

IR-spectra were registered on Brucker Vertex ("Brucker", Germany) IR spectrometer (a 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}); Thermo Nicolet Avatar 360 Fourier-transform IR (M. Chakarova); Differential Non-equilibrium Spectrum (DNES).

Statistical processing of experimental data was performed using the statistical package STATISTISA 6 using Student's *t*- criterion (at $p < 0.05$).

3. Results and Discussions

3.1. Comparative Analysis between Longevity of Centenarians And Their Siblings.

In frames of the research 54 long living people from Bulgaria over 90 years of age have been studied together with their siblings. The average lifespan of Bulgarian centenarians is 89,1 years, and for their brothers and sisters the average lifespan is 87,8 years. The difference in life expectancy of the two groups of people is reliable and is at $p < 0,05$, *t*-Student's criteria at a confidence level of t = 2,36 years.

There have been 21519 residents in Teteven and 142 of them were born before 1924. Figure 1 shows the interrelation between the year of birth of long living people (age) and their number (Teteven municipality, Bulgaria).

It was shown in Figure 1 that the rate of aging increases with time. In 1963 L. Orgel showed that the aging process is associated with the synthesis of abnormal proteins (Orgel, 1963). Figure 2 shows L. Orgel's results on the interrelation between age and number of cancer patients. The accumulation of errors in synthesis of abnormal proteins increases exponentially over time with age. Cells taken from elderly people show the reduced levels of transcription or transmission of information from DNA to RNA. Therefore, the probability of cancer increases with age. The interrelation between the number of Bulgarian centenarians in the mountainous municipality of Teteven and their age is close to exponential.

Human experience shows that long-living people inhabit mainly high mountainous areas where mountain rivers flow being feed by mountain springs. In Russia most number of centenarians lived in Russian North and Dagestan region. One explanation for this is that water in those places contains less deuterium than ordinary drinking water (Berdishev, 1960; Varnavskiy, 2000).

In 1960-s G. Berdishev from Medical Institute in Tomsk (Russia) studied the phenomenon of longevity of centenarians in Yakutia and Altai regions (G. Berdishev, 1960). He linked the longevity of the Yakuts and the Altaians with the consumption of melt water from glaciers formed earlier in Yakutia's mountains than those ones of Greenland. According to the State's statistics most of the Russian centenarians live in Dagestan and Yakutia – 353 and 324 persons per 1 million of inhabitants. This number for all Russia is only 8 people for 1 million of inhabitants. In Bulgaria the average number of centenarians makes up 47 per 1 million, while in Teteven Municipality – 139 centenarians per 1 million of inhabitants. In the Bulgarian municipalities the oldest inhabitant in field areas is 93 years old, and the oldest inhabitant in mountainious areas is 102 years old. There are distances of no more than 50 km between these places and the only difference is mountain water and air.

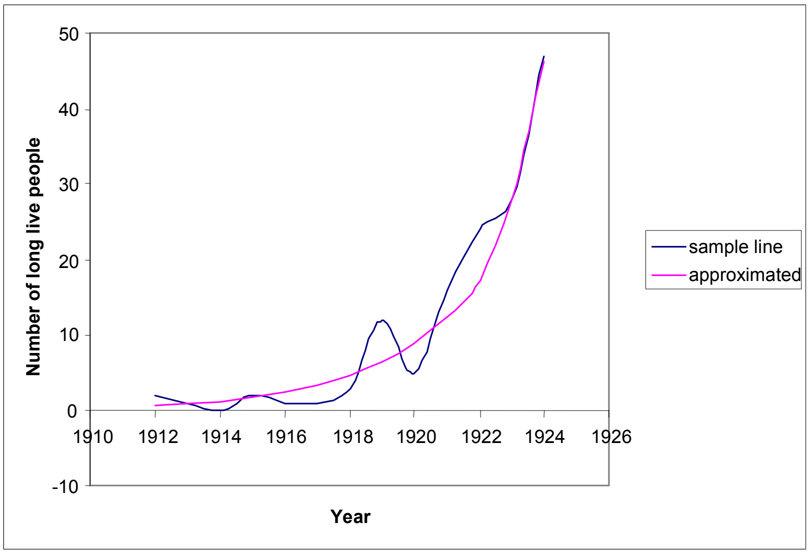

Figure 1: Interrelation between the year of birth of long-living people (age) and their number in Teteven municipality, Bulgaria.

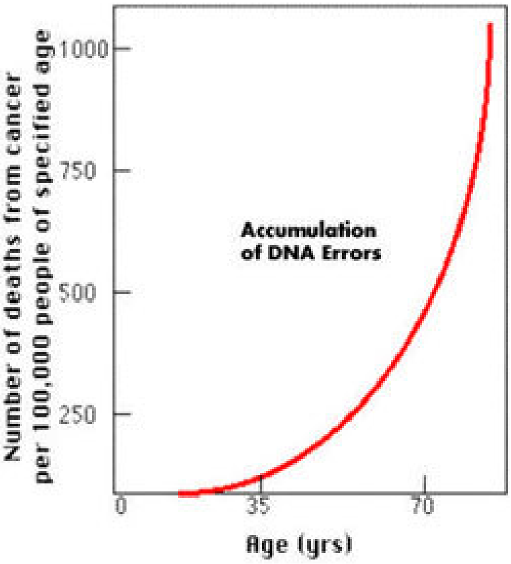

Figure 2: Interrelation between age and the number of cancer patients (Orgel, 1963).

Here are submitted the data for Bulgaria:
Varna district – centenarians 44 per 1 million of inhabitants, plain and sea regions;
Pleven district – centenarians 78 per 1 million of inhabitants, plain regions;
Teteven district – centenarians 279 per 1 million of inhabitants, hills and mountainious regions;
Bulgaria – centenarians 47 per 1 million of inhabitants.

Analogious situation is observed in the Russian North. According to G. Berdishev, people inhabiting the Russian North – the Yakuts and the Altaians as well as the Buryats, drink mountain water obtained after the melting of ice. Altai and Buryat water sources are known as moderately warm, with temperatures of 8–10 ^{0}C, the water is generally ice-free in winter. This phenomenon is explained by the fact that the melt water contains a low percentage of deuterium compared with ordinary tap water that is believed to have a positive effect on the tissue cells and metabolism. Melt water in Russia is considered to be a good folk remedy for increasing physical activity of the human body, enhancing the vitality of the organism and has a beneficial effect on metabolism (Goncharuk *et al*., 2013).

There are known 14 crystalline modifications of ice each having its own structure and a character of disposition of hydrogen atoms (Table 1). The natural ice is represented by the hexagonal I_h configuration. Crystals of all ice modifications are made up from H_2O molecules, linked by hydrogen bonds into a 3D carcass, consisting of individual tetrahedrons, formed by four H_2O molecules. In the crystalline structure of natural ice I_h hydrogen bonds are oriented towards the tetrahedron apexes at strictly defined angles equal to 109°5 (in liquid water this angle is 104^05) (Figure 3). In ice structures I_c, VII and VIII this tetrahedron is nearly the same as a regular 4 triangular tetrahedron. In ice structures II, III, V and VI the tetrahedrons are noticeably distorted. In ice structures VI, VII and VIII two intercrossing systems of hydrogen bonds are distinguished. In the centre of the tetrahedron is located an oxygen atom, at each of the two vertices – H-atom, which electron take part in formation of covalent bond with an electron pair of O-atom. The rest two vertices of the tetrahedron are occupied by two pairs of non-shared electrons of O-atom not participating in formation of molecular bonds. The carcasses of hydrogen bonds allocate H_2O molecules in form of a spatial hexagon network with internal hollow hexagonal channels inside. In the nodes of this network O-atoms are orderly organized (crystalline state), forming regular hexagons, while H-atoms have various positions along the bonds (amorphous state). When ice melts, its network structure is destroyed: H_2O molecules begin to fall down into the network hollows, resulting in a denser structure of the liquid – this explains why water is heavier than ice. The hydrogen bonding explains other anomalies of water (anomality of temperature, pressure, density, viscosity, fluidity, etc). According to theoretical calculations, at the melting of the ice breaks about 15% of all hydrogen bonds (Mosin & Ignatov, 2011); by further heating to 40 ^{0}C breaks down about half of hydrogen bonds in H_2O associates. In water vapor hydrogen bonds are absent.

Table 1: Ice crystal modifications and their physical characteristics

Modification	Crystal structure	Hydrogen bond lengths, Å	Angles H–O–H in tetragonals, ^{0}
I_h	Hexagonal	2.76	109.5
I_c	Cubic	2.76	109.5
II	Trigonal	2.75–2.84	80–128
III	Tetragonal	2.76–2.8	87–141
IV	Rhombic	2.78–2.88	70.1–109
V	Monoclinic	2.76–2,87	84–135
VI	Tetragonal	2.79–2.82	76–128
VII	Cubic	2.86	109.5
VIII	Cubic	2.86	109.5
IX	Tetragonal	2.76–2.8	87–141
X	Cubic	2.78	109.5
XI	Hexagonal	4.50	90.0
XII	Tetragonal	4.01	90.0
XIII	Monoclinic	7.47	90–109.7
XIV	rhombic	4.08	90.0

Notes:
I_h – natural hexagonal ice; I_c – cubic ice.

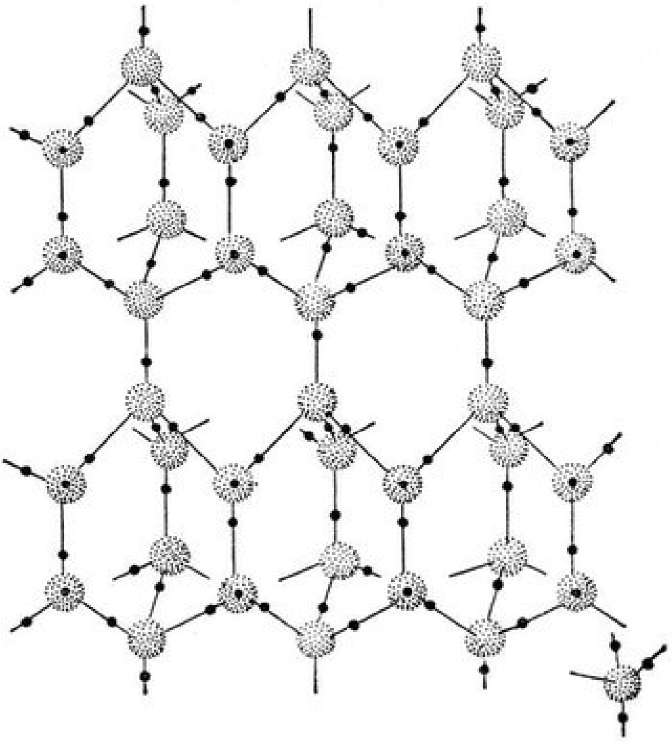

Figure 3. Hydrogen bonding in crystalline structure of natural ice I_h under the temperature at -10 ^{0}C and normal pressure at 1 atm. Grey balls – O-atoms, black balls – H-atoms. On the right below is shown the structural unit of crystalline ice structure.

Mountain water in springtime is the result of the melting of ice and snow accumulated in the mountains. Natural ice with I_h modification (hexagonal lattice) is usually much cleaner than water, because solubility of all substances (except NH_4F) in ice is extremely low. The growing ice crystal is always striving to create a perfect crystal lattice and therefore displaces impurities. Melt water has a certain “ice-like” structure, because it preserves the hydrogen bonding between water molecules; as a result it is formed complex intermolecular associates (clusters) – analogues of ice structures, consisting of a larger or smaller number of H_2O molecules (Figure 4). However, unlike ice crystal, each associate has a very short time of existence as a result there occurs the constant processes of decay and formation of water associates having very complicated structure (Ignatov & Mosin, 2013e). The specificity of intermolecular interactions characteristic for the structure of ice, is kept in melt water, as it is estimated that in the melting of ice crystal is destroyed only 15% of all hydrogen bonds in the associates. Therefore, the inherent to ice connection of each H_2O molecule with four neighboring H_2O molecules is largely disturbed, although there is observed the substantially "blurring" of oxygen lattice framework. Processes of decay and formation of clusters occur with equal probability that is probably why physical properties of melt water are changed over time, i.e. dielectric permittivity comes to its equilibrium state after 15–20 min, viscosity – in 3–6 days (Ignatov & Mosin, 2013f). Further heating of fresh melt water above +37 ^{0}C leads to a loss of its biological activity. Storage of meltwater at +22 ^{0}C is also accompanied by a gradual decrease in its biological activity; within 16–18 hours it is reduced by 50%. The main difference between the structure of ice and water is more diffuse arrangement of the atoms in the lattice and disturbance of long-range order. Thermal oscillations (fluctuations) lead to bending and breaking down of hydrogen bonds. H_2O molecules being out of equilibrium positions begin to fall down into the adjacent structural voids and for a time held up there, as cavities correspond to the relative minimum of potential energy. This leads to an increase in the coordination number, and the formation of lattice defects. The coordination number (the number of nearest neighbors) during the transition from ice to melt water varies from 4.4 at 1.5 ^{0}C to 4.9 at 80 ^{0}C.

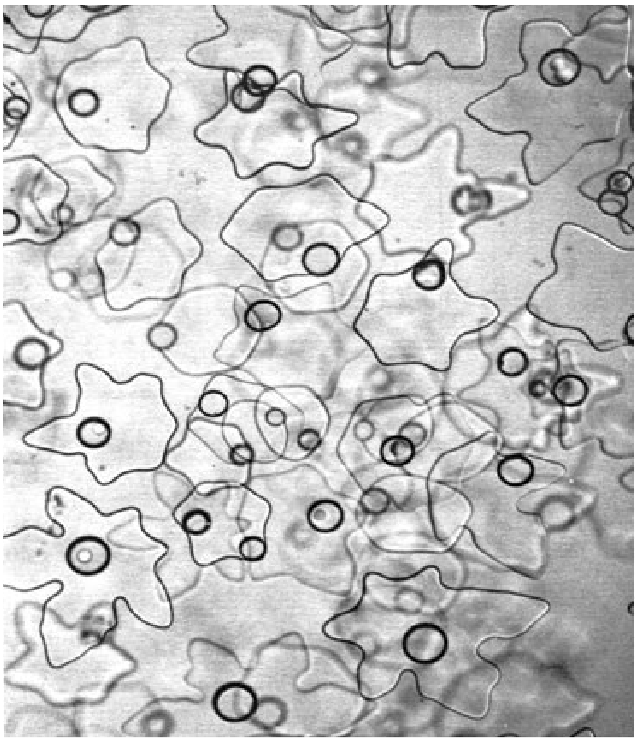

Figure 4: Structure of melt water with "smearing" fragments of regular ice structures according to computer simulations.

Preliminary analyses of water from various water sources show that mountain water as the result of natural isotope purification contains less amount of deuterium. This water also contains ions of Ca^{2+}, Mg^{2+}, Na^{+}, HCO_3^- and SO_4^{2+}. The content of K^+ and N^+ cations in the melt water is approximately 20–30 mg/l, Mg^{2+} – 5–10 mg/l, Ca^{2+} – 25–35 mg/l, the content of SO_4^{2-} – <100 g/l, HCO_3^- 50–100 mg/l, Cl^- – less than 70 mg/l, total rigidity ≤ 5 mEq/l, the total mineralization ≤ 0.3 g/l, pH – 6.5–7.0 at 25 ^{0}C (Table 2). The degree of natural purification of melt water from impurities makes up ~50–60%. The concentration of salts of rigidity – Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, heavy metals and organochlorine compounds, as well as heavy isotopes, including deuterium in melt water is less that that of ordinary portable water. This fact is important because some authors consider the hardness of the water to be among the main factors in cardiovascular diseases (Pocock *et al*., 1981; Rubenowitz et al., 1999; Marque et al., 2003). However, mild correlation was further proven that water hardness could not be a decisive factor for human longevity.

Table 2: Composition of melt water

Cations, mg/l	
$K^+ + Na^+$	20–30
Mg^{2+}	5–10
Ca^{2+}	25–35
Anions, mg/l	
SO_4^{2-}	<100
HCO_3^-	50–100
Cl^-	<70
Total rigidity, mEq/l	≤5
Total mineralization, g/l	≤0.3
pH at 25 ^{0}C	6.5–7.0

Analyses of water from various sources of Russia and Bulgaria show that the mountain water contains on average ~2–4% less deuterium in form of HDO, than river water and sea water. In natural waters, the deuterium content is distributed irregularly: from 0.02–0.03 mol.% for river and sea water, to 0.015 mol.% for water of Antarctic ice – the most purified from deuterium natural water containing deuterium in 1.5 times less than that of seawater. According to the international SMOW standard isotopic shifts for D and ^{18}O in sea water: D/H = $(155.76\pm0.05)\cdot10^{-6}$ (155.76 ppm) and $^{18}O/^{16}O = (2005.20\pm0.45)\cdot10^{-6}$ (2005 ppm) (Lis *et al*., 2008). For SLAP standard isotopic shifts for D and ^{18}O in seawater: D/H = $89\cdot10^{-6}$ (89 ppm) and for a pair of $^{18}O/^{16}O = 1894\cdot10^{-6}$ (1894 ppm). In surface waters, the ratio D/H = $\sim(1.32–1.51)\cdot10^{-4}$, while in the coastal seawater – $\sim(1.55–1.56)\cdot10^{-4}$. Waters of other underground and surface water sources contain varried amounts of deuterium (isotopic shifts) – from δ = +5,0 D,%, SMOW (Mediterranean Sea) to to δ = -105 D,%, SMOW (Volga River). 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 there are observed positive deviations at $2.0\cdot10^{-5}$. Content of the lightest isotopomer – $H_2{}^{16}O$ in water corresponding to SMOW standard is 997.0325 g/kg (99.73 mol.%), and for SLAP standard – 997.3179 g/kg (99.76 mol.%).

Thawed snow and glacial water in the mountains and some other regions of the Earth also contain less deuterium than ordinary drinking water. On average, 1 ton of river water contains 150–200 g deuterium. The average ratio of H/D in nature makes up approximately 1:5700. According to the calculations, the human body throughout life receives about 80 tons of water containing in its composition 10–12 kg of deuterium and associated amount of heavy isotope ^{18}O. That is why it is so important to purify water from heavy isotopes of D and ^{18}O.

3.2. Clinical Evidence about Influence of Deuterium Depleted Water on Organism

When biological objects being exposed to water with different deuterium content, their reaction varies depending on the isotopic composition of water and magnitude of isotope effects determined by the difference of constants of chemical reactions rates k_H/k_D in H_2O and D_2O. The maximum kinetic isotopic effect observed at ordinary temperatures in chemical reactions leading to rupture of bonds involving hydrogen and deuterium lies in the range $k_H/k_D = 5–7$ for C–H versus C–D, N–D versus N–D, and O–H versus O–D-bonds (Mosin, 1996).

Our previous studies have shown that heavy water of high concentration is toxic for the organism, chemical reactions are slower in D_2O, compared with ordinary water, the hydrogen bonds formed with participation of deuterium are somewhat more stronger that those ones formed from hydrogen (Mosin *et al*., 1996). In mixtures of D_2O with H_2O with high speed occurs dissociation reactions and isotopic (H-D) exchange resulting in formation of semi-heavy water (HDO): $D_2O + H_2O = HDO$. For this reason deuterium presents in smaller content in aqueous solutions in form of HDO, while in the higher content – in form of D_2O. The chemical structure of D_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. D_2O boils at +101.44 ^{0}C, freezes at +3,82 ^{0}C, has density of 1.1053 g/cm^3 at 20 ^{0}C, and the maximum density occurs not at +4 ^{0}C as in H_2O, but at +11.2 ^{0}C (1.1060 g/cm^3). These effects are reflected in the chemical bond energy, kinetics, and the rate of chemical reactions in D_2O.

The chemical reactions and biochemical processes in the presence of D_2O are somehow slower compared to H_2O. D_2O is less ionized, the dissociation constant of D_2O is smaller, and the solubility of the organic and inorganic substances in D_2O is smaller compared to these ones in H_2O (Mosin, 1996). However, there are also such reactions which rates in D_2O are higher than in H_2O. In general these reactions are catalyzed by D_3O^+ or H_3O^+ ions or OD^- and OH^- ions. According to the theory of a chemical bond the breaking up of covalent C–H bonds can occur faster than C–D bonds, the mobility of D_3O^+ ion is lower on 28,5 % than H_3O^+ ion, and OD^- ion is lower on 39,8 % than OH^- ion, the constant of ionization of D_2O is less than that of H_2O (Lobishev & Kalinichenko, 1978).

The comparative analysis of IR-spectra of H_2O solutions and its deuterated analogues (D_2O, HDO) 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 IR spectra of water usually contain three absorption bands, which can be identified as 1 – absorption band of the stretching vibration of OH^- group; 2 – absorption band of the first overtone of the bending vibration of the molecule HDO; 3 – absorption band of stretching vibration of OD^- group. OH^- group 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 not involved in formation of hydrogen bonds are usually given the narrow bands in IR spectrum and the associated groups – broad intense absorption 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, a manifestation of overtones and combination frequencies of OH^- groups in hydrogen bonding, as well as the proton tunneling effect (on the relay mechanism. Such complexity makes it difficult to interpret the IR spectrum and partly explains the discrepancy in the literature available on this subject.

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 appear 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 D_2O molecule these ratio compiles 2723.7, 1403.5 and 3707.5 cm^{-1}, while for HDO molecule – 2671.6, 1178.4 and 2787.7 cm^{-1}. HDO (50 mole% H_2O + 50 mole% ^2H_2O; ~50 % HDO, ~25 % H_2O, ~25 % D_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, OD^- -stretch, as well as combination of bending and libration and HDO 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 (Ignatov & Mosin, 2013*b*). The temperature dependence of individual spectral bands of liquid water is very complex (Zelsmann, 1995). 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) (Yukhnevitch, 1973). Such complexity makes it difficult to interpret the spectrum and partly explains the discrepancy in the literature available on this subject.

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 (Brubach et al., 2005). 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 OD^- group (Max & Chapados, 2009). 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 Table 4. 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 ionization limit at 98 nm^{-1} (Walrafen, 1972). In the middle visible region at 4–7500 cm^{-1} are located rotational spectrum and the bands corresponding to the vibrational-rotational transitions in the ground electronic state. In the ultraviolet region (200 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.

Results of IR-spectroscopy with device Infra Spec VFA-IR show that at 4.1 µm, even at low concentrations of deuterium of 0.35 and 0.71%, there is observed a decline in the local maximums relative to the local maximum of 100% pure water (the local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state because at changing the atomic mass of hydrogen and deuterium atoms in the water molecule their interaction will also change, although the electronic structure of the molecule and its ability to form H-bonds, however, remains the same; with the substitution with deuterium the vibrational-rotational transitions are changed, that is why it appears other local maximums in IR-spectra. These results are shown in Figure 5. The result is reliable regarding the content of deuterium in natural waters from 0.015–0.03%.

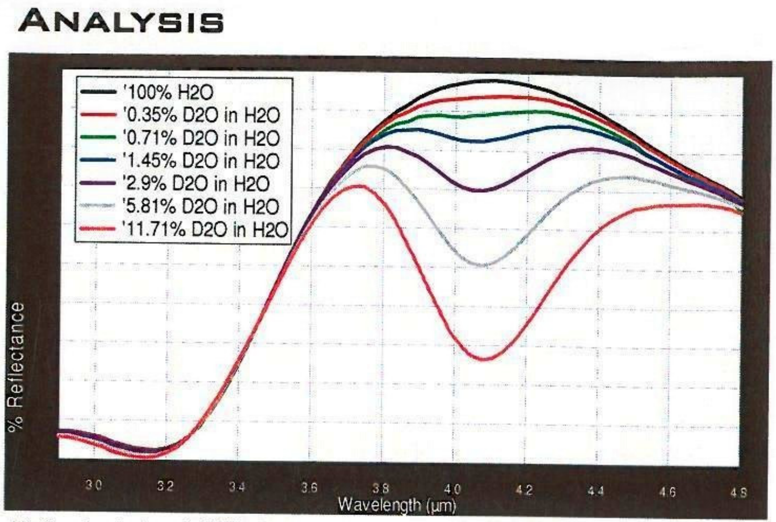

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Figure 5: The typical IR-spectra of water with varying content of deuterium

At further transition from H_2O monomers to H_4O_2 dimmer and H_6O_3 trimmer absorption maximum of valent stretching vibrations of the O-H bond is shifted toward lower frequencies (ν_3 = 3490 cm^{-1} and ν_1 = 3280 cm^{-1}) (Eisenberg & Kauzmann, 1969) and the bending frequency increased (ν_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 dimmer [$(H_2O)_2$]. These frequencies are significantly lower than the valence modes of ν_1 and ν_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 dimmer. 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 state is attributed by the appearance of additional force, preventing O-H bond bending. Deformation absorption band in IR-spectrum of water has a frequency at 1645 cm^{-1} and very 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.e. 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.

Table 4: The assignment of main frequencies in IR-spectra of liquid water H_2O and D_2O

Main vibrations of liquid H_2O and ^2H_2O				
Vibration(s)	H_2O (t = 25 ^{0}C)		D_2O (t = 25 ^{0}C)	
	ν, cm^{-1}	E_0, M^{-1} cm^{-1}	ν, cm^{-1}	E_0, M^{-1} cm^{-1}
Spinning ν_1 + deformation ν_2	780-1645	21.65	1210	17.10
Composite $\nu_1 + \nu_2$	2150	3.46	1555	1.88
Valence symmetrical ν_1, valence asymmetrical ν_3, and overtone $2\nu_2$	3290-3450	100.65	2510	69.70

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. T**he substitution of H with D 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 D_2O (Cleland, 1976). It may cause disturbances in the DNA-synthesis, leading to permanent changes on DNA structure and consequently on cell genotype (**Thomson, 1960; Katz, 1960; Den'ko, 1970; Török *et al*., 2000)**.**

Our experiments demonstrated that the effects of deuterium on the cell possess a complex multifactor character connected to changes of physiological parameters – magnitude of the *lag*-period, time of cellular generation, outputs of biomass, a ratio of amino acids, protein, carbohydrates and fatty acids synthesized in D_2O, and with an evolutionary level of organization of investigated object as well (Mosin *et al*., 2012; Mosin *et al*., 2013; Mosin & Ignatov, 2012; Mosin & Ignatov, 2013a). The cell evidently implements the special adaptive mechanisms promoting functional reorganization of work of the vital systems in the presence of D_2O.

D_2O can cause metabolic disorders, kidney's malfunction, violation of hormonal regulation and coused immunosuppression (Katz, 1960; Katz *et al*., 1962; Thomson, 1960; Czajka *et al*., 1961), notwithstanding the strong radioprotective effect of D_2O (Laeng et al., 1991; Michel et al., 1988) . Also deuterium induces physiological, morphological and cytological alterations on the cell with forming cells more 2–3 times larger in size in D_2O (Mosin & Ignatov, 2012a). 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 (Figure 6). At high concentrations of D_2O are suppressed enzymatic reactions, cell growth, mitosis and synthesis of nucleic acids (Lamprecht *et al*., 1989; Cioni & Strambini, 2002; Vertes, 2003). Thus, the most sensitive to replacement of H on D 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 (Mosin *et al*., 1999). Last fact allows to consider adaptation to D_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 macromolecules. Evidently cells are able to regulate the D/H ratios, while its changes trigger distinct molecular processes. One possibility to modify intracellular D/H ratios is the activation of the H^+-transport system, which preferentially eliminates H^+, resulting in increased D/H ratios within cells (Somlyai *et al*., 2012).

We have obtained results on growth and adaptation to D_2O of various cells of prokaryotic and eukaryotic organisms (Mosin & Ignatov, 2012; Mosin & Ignatov, 2013d). Our studies have shown that animal cells are able to withstand up to 25–30% D_2O, plants – up to 50–60% D_2O, and protozoa cells are able to live on ~90% D_2O. Further increase in the concentration of D_2O for these groups of organisms leads to cellular death. On the contrary, deuterium depleted water with 25–30% decreased deuterium content has benefitial effects on organism. Experiments on animals and plants demonstrated that after consumption of water with reduced on ~25–30% deuterium pigs, rats and mice produced a larger number of offspring, upkeep of poultry with 6-day old to puberty on deuterium depleted water leads to accelerated development of the genital organs (size and weight), and strengthen the process of spermatogenesis, wheat ripens earlier and gives higher yields, etc. (Sinyak *et al*., 1998; Syniak *et al*., 2000; Sergeeva *et al*., 2003; Badin *et al*., 2004; Stom *et al*., 2006). In addition, deuterium depleted water delays the appearance of the first metastasis nodules on the spot inoculation of cervical cancer and exerts immunomodulatory and radioprotective effect (Rakov *et al*., 2006; Turusov *et al*., 2005).

Radioprotective effects of deuterium depleted water were studied by W. Bild (Bild *et al*., 1999), V.S. Turusov (Turusov *et al*., 2005) and D.V. Rakov (Rakov *et al*., 2006; Rakov, 2007) at irradiation of mice's cells by γ-radiation at semimortal dose LD50. Survival level of animals treated with deuterium depleted water for 15 days prior to γ-radiation, was 2.5-fold higher than in control group (dose of 850 R). The surviving experimental group of mice has the number of leukocytes and erythrocytes in the blood remained within the normal range, while in the control group the number of leukocytes and erythrocytes was significantly decreased (Rakov *et al*., 2006; Rakov, 2007).

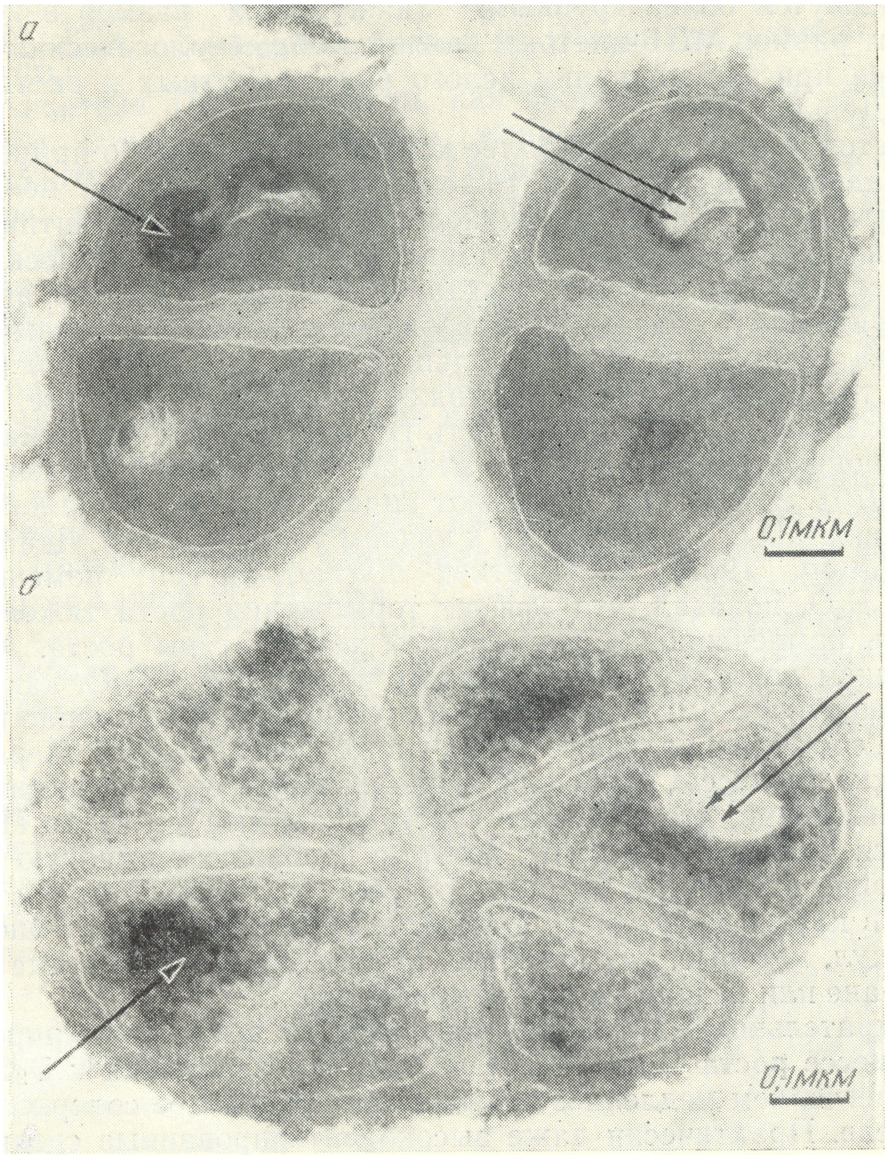

Figure 6. Electron micrographs of *Micrococcus lysodeikticus* cells obtained by SEM method: *a*) – protonated cells obtained on H_2O-medium; *b*) – deuterated cells obtained on ^2H_2O-medium. The arrows indicate the tightly-packed portions of the membranes

Consumption of deuterium depleted water by cancer patients during or after radiation therapy treatments allows restore the composition of blood and relieve nausea (Olariu *et al*., 2010). According to G. Shomlai, the results of clinical trials conducted in 1998–2010 in Hungary showed that the survival rate for patients drinking deuterium depleted water in combination with traditional therapies or after are significantly higher than for patients who only used the chemotherapy or radiation therapy (Somlyai *et al*., 1998; Somlyai, 2001; Somlyai, 2002; Somlyai *et al*., 2010).

Biological experiments with deuterium depleted water carried out in Moscow Research Oncological Institute after P.A. Herzen and N.N. Blokhin with Institute of Biomedical Problems (Sinyak *et al.,* 2003; Grigoriev *et al*., 2005; Turusov *et al*., 2006), confirmed the inhibitory effects of deuterium depleted water on the process of growth of various tumors, i.g. division of breast adenocarcinoma MCF-7 tumor cells being placed in deuterium depleted water started with a delay of ~5–10 hours (Grigoriev *et al*., 2005; Turusov *et al*., 2006; Gyöngyi & Somlyai, 2000). In 60% of mice with immunosuppressed immunity and transplanted human breast tumor MDA and MCF-7 consumption of deuterium depleted water caused tumor regression. A group of mice with transplanted human prostate tumor PC-3 consumed deuterium depleted water showed the increase in the survival rate by ~40%; the ratio number of dividing cells in tumors of dead animals in experimental group was 1.5:3.0, and in control group – 3.6:1.0 (Grigoriev *et al*., 2005; Turusov *et al*., 2006). In this regard special attention deserves two indicators: the delay of metastasis and loss of animal's weight during experiments. Stimulating action of deuterium depleted water on the immune system of animals has led to delay of development of metastasis by 40% in comparison with the control group, and weight loss in animals that consumed deuterium depleted water at the end of the experiment was 2 times less (Grigoriev *et al*., 2005; Turusov *et al*., 2006). It was also reported that deuterium depleted water may delay the progression of prostate cancer (Kovács *et al*., 2011) and inhibit human lung carcinoma cell growth by apoptosis (Cong *et al*., 2010).

Preliminary experimental results on motility of human sperm performed by V.I. Lobyshev and A.A. Kirkina (Lobyshev & Kirkina, 2012) indicates that in deuterium depleted water water (4 ppm) motility is on 40% higher during 5 hours of the registration. However, the effect depends on the initial properties of a sperm sample. These dasta indicated that deuterium content variation in water including deep deuterium depletion produce various nonlinear isotopic effect on key processes in a cell as enzyme action of Na, K-ATPase, regeneration, motility, fertilizing effectiveness and embryo developing. It should be noted that for any deuterium concentration dependence there should be an optimal condition for the best result.

One prominent effect of deuterium depletion is to inhibit fatty synthesis, chain elongation and desaturation. These anabolic reactions utilize acetyl-CoA, as well as hydrogen of water for new fatty acid pools (Boros & Somlyai, 2012). Fatty acids then are used for new membrane formation in the rapidly proliferating cell. The complex structure and molecular organization of the mammalian fatty acid synthase offer remarkable opportunities with altered morphology and flux handling properties.

The positive influence of drinking deuterium depleted water on blood chemistry included a significant reduction of glucose, cholesterol, erythrocyte sedimentation rates, leukocyte counts and cortisol (stress hormone) levels, while also revealed an increase in antioxidant capacities (Andreeva *et al*., 2005; Olariu *et al*., 2010; Burdeynaya & Chernopyatko, 2012) These data evidence the significance of deuterium depleted water to increase energy resources even in a healthy cohort, while decreasing risks of psycho-emotional stress, which is known to pose a negative influence on blood biochemistries that often lead to psychosomatic diseases and shorten life. It was also noted the positive impact of water on indicators of saturation the liver tissue by oxygen: the observed increase in pO_2 was ~15%, i.e., cell respiration increased 1.3 times (Kolesov & Pomytkin, 2006). On beneficial effect on health of experimental mice evidenced the increased resistance and weight increase compared with the control group (Bild *et al.,* 2004). It was also indicated that deuterium depleted water increases the rate of metabolic reactions (Avila *et al*., 2012). It was observed geroprotective (anti-aging), anti-mutagenic and radioprotective effects of deuterium depleted water with reduced on 5% deuterium content on the development cycle of fruit fly *Drosophila melanogaster* (Varnavski *et al*., 2002).

The total effects of deuterium depleted water depends on the following parameters – total body mass, total body water, the amount of daily consumption of deuterium depleted water and the degree of its isotope purification. The main impact of deuterium depleted water on the organism is explained by gradual reduction of the deuterium content in the physiological fluids of the body by reactions of isotopic (H-D) exchange (Pulyavsky, 1986; Mosin & Ignatov, 2012b). These results indicate that regular using of deuterium depleted water helps improve the functioning of some vital systems (Turova *et al*., 2003). With regular consumption of deuterium depleted water occurs the cleaning of organism from HDO due to the reaction of isotopic (H-D) exchange in physiological fluids, and it was recorded the change of the isotopic composition of urine and Ca^{2+} content as well. Daily consumption of deuterium depleted water allows naturally reduce the content of HDO in the human body due to isotopic (H-D) exchange (Ignatov & Mosin, 2013f). It is believed that this process is accompanied by an increase in the functional activity of cells, cell tissues and organs. It was reported about the normalization of metabolic processes, increasing of resistance of organism to adverse external influences (Krempels *et al*., 2008). Thus regular consumption of deuterium depleted water provides a natural way to reduce the content of HDO in the human body to a lower values. It has beneficial effects on metabolism, invigorates the body, and also promotes the rapid recovery after strenuous physical exercise. This testifies the usage of deuterium depleted water for residents of large cities and megapolices.

Clinical trials of deuterium depleted water with a residual content of deuterium 60–100 ppm, showed that it can be recommended as an adjunct in the treatment of patients having metabolic syndrome (hypertension, obesity, impaired glucose metabolism) and diabetes. In addition, it was shown that deuterium depleted water improves the quality of life for patients having renal stone disease (nephrolithiasis) and various disorders in the gastrointestinal tract (colitis and gastritis), cleanses the body of toxins, enhances the action of drugs, promotes weight correction, protects cells from radiation (Burdeynaya & Chernopyatko, 2012). Deuterium depleted water can be recommended for fast and deep cleaning of the human body from deuterium that is essential for metabolic disturbances. Taking into consideration the dynamics of the distribution of water in the human body, the reaction of isotopic (H/D and $^{16}O/^{18}O$) exchange and the results obtained with deuterium depleted water, it can be expected that the greatest effect the isotopic purification of water will have on the regulatory system and metabolism (Mosin, 2012).

The effectiveness of the influense of deuterium depleted water depends on the following parameters – total body mass, total body water, the amount of daily consumption of deuterium depleted water and the isotopic content of deuterium. The results on the gradual increasing of deuterium content in the human body at regular consumption of deuterium depleted water with varried residual deuterium content are shown in Table 5. This table shows that the content of deuterium in the human body decreases while drinking the deuterium depleted water. Thus, at the consumption of water with a residual deuterium content of 60 ppm deuterium content in the body decreases after 45 days to 117.3 ppm, and at the consumption of water with a residual content of deuterium 100 ppm – to 131 ppm at 1 liter of water consumption per a day, to 122.6 ppm at water consumption of 1.5 liters of water a day. Hence, the regular drinking of deuterium depleted water provides a natural way to reduce the content of HDO in the human body to a value of ~117 ppm.

Table 5. *Gradual decreasing of deuterium content in the human body over time, with regular consumption of deuterium depleted water**

Number of days	The residual content of deuterium in water, ppm		
	60	100	100
	Daily consumption of deuterium depleted water, liters		
0	1	1	1.5
1	150.5	150.7	150.8
2	145.5	147.9	146.9
7	136.5	143.6	140.5
14	130.6	138.3	134.7
21	120.8	135.6	129.6
28	120.0	133.9	126.6
35	119.6	132.6	124.5
45	117.3	131.5	122.6

*Notes:

The calculation was performed based on the following data:

- daily consumption of deuterium depleted water – 1 or 1.5 liter;
- daily water exchange rate – 2.5 liters;
- deuterium content in the body corresponds to its content in natural water ~ 150 ppm;
- the average volume of water in the body – 45 liters (average body weight ~ 75 kg).

3.3. Clinical Evidence with Human Blood Serum Testing

It was established experimentally that the process of evaporation of water drops, the wetting angle θ decreases discreetly to 0, and the diameter of water drop basis is only slightly altered, that is a new physical effect (Antonov, 1995a; Antonov & Yuskesselieva, 1983). Based on this effect, by means of measurement of the wetting angle within equal intervals of time is determined the function of distribution of H_2O molecules according to the value of f(θ). The distribution function is denoted as the energy spectrum of the water state. Theoretical research established the dependence between the surface tension of water and the energy of hydrogen bonds among individual H_2O-molecules (Antonov, 1995). The hydrogen bonding results from interaction between electron-deficient H-atom of one H_2O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen acceptor) on the neighboring H_2O molecule; the structure of hydrogen bonding may be defined as $O\cdots H^{\delta+}–O^{\delta-}$.

For calculation of the function f(E) represented the energy spectrum of water, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = \frac{14{,}33 f(\theta)}{[1-(1+bE)^2]^2}, \qquad (1)$$

where $b = 14.33$ eV^{-1}

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H_2O molecules is calculated by the formula:

$\theta = arcos\ (-1 - 14.33E)$, (2)

The energy spectrum of water is characterized by a non-equilibrium process of water droplets evaporation, therefore, the term non-equilibrium spectrum (NES) of water is used.

The difference $\Delta f(E) = f\,(samples\ of\ water) - f\,(control\ sample\ of\ water)$

– is called the "differential non-equilibrium energy spectrum of water" (DNES).

Thus, DNES spectrum is an indicator of structural changes of water as a result of various external factors. The cumulative effect of these factors is not the same for the control sample of water and the water sample being under the influence of this factor.

Figure 7 shows the average NES-spectrum of deionised water. On the X-axis are sshown three scales. The energies of hydrogen bonds among H_2O molecules are calculated in eV. On the Y-axis is depicted the function of distribution of H_2O molecules according to energies f(E), measured in unit eV^{-1}. For DNES spectrum the function is Δf(E) in unit eV^{-1}. Arrow A designates the energy of hydrogen bonds among H_2O molecules, which is accepted as most reliable in spectroscopy. Arrow B designates the energy of hydrogen bonds among H_2O molecules the value of which is calculated:

$$\bar{E} = -\,0{,}1067 \pm 0{,}0011 \text{ eV} \qquad (3)$$

Arrow C designates the energy at which the thermal radiation of the human body, considered like an absolute black body (ABB) with a temperature 36.6 ^{0}C, is at its maximum. A horizontal arrow designates the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle infrared range of the Sun toward the Earth and from the Earth toward the surrounding cosmic space. It is seen that the atmosphere window of transparency almost covers the energy spectrum of water.

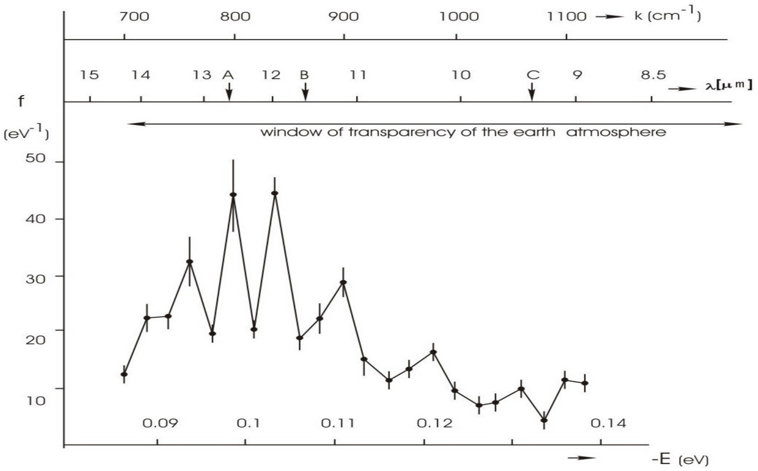

Figure 7. Non-equilibrium energy spectrum (NES) of water as a result of measurement for 1 year: on the X-axis is shown the average energy of H…O-bonds between H_2O measured in eV; on the Y-axis is shown the energy distribution function f(E) of H_2O molecules measured in eV^{-1}; λ – wavelength (μm), *k* – wave number, (cm^{-1}).

The study of the IR spectrum of water in the composition of physiologic fluids (urine, blood, serum) can also provide data on metabolic processes in the human body and longevity, because the IR-spectrum reflects the metabolic processes. Authors have conducted studies of a 1% (v/v) solution of blood serum by spectral analysis of non-equilibrium energy (NES) spectrum and differential equilibrium energy (DNES) spectrum on two groups of people between 50 and 70 years of age. The first group consisted of people in excellent health. The second group consisted of people in a critical state and patients with malignant tumors. As the main biophysical parameter was investigated the average energy of hydrogen bonds ($\Delta E_{H...O}$) between H_2O molecules in the blood serum. The result was obtained as a difference between the NES-spectrum of 1% solution of blood serum and NES-spectrum of deionized water control sample – DNES-spectrum, measured as the difference $\Delta f(E)$ = f (samples of water) – f (control sample of water). The DNES-spectrum obtained from the first group has a local maximum energy ($\Delta E_{H...O}$) at -9.1±1.1 meV and from the second group -1.6±1.1 meV. Results between the two groups have a statistical difference in Student's criterion at $p < 0.05$. For the control group of healthy people the value of the largest local maximum in the DNES-spectrum was detected at -0.1387 eV, or at a wavelength of 8.95 μm. For the group of people in a critical state and the patients with malignant tumors, the analogous values of the largest local maximums of the DNES-spectrum shifted to lower energies compared with the control group of people.

Water in the human body posses IR-spectrum that reflects the structure of water and metabolic processes in the organism. It can be demonstrated by analysis of human bllod serum by IR-spectrometry. The magnitude of the largest local maximum in IR-pectrum of blood serum from healthy people of control group observed at -0.1387 eV at a wavelength –8.95 μm. For a group of people in critical health condition and patients with malignant tumors the greatest values of local maxima in the IR-spectrum are shifted to lower energies relative to the control group. In IR-spectrum of human blood serum are detected local maxima at λ = 8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35 and 9.76 μm (Krasnov, Gordetsov, 2009). The resulting peak at λ = 8.95 μm in IR-spectrum (Ignatov, 2012) approaching the peak at λ = 8.85 μm monitored by Russian researchers. In the control group of healthy people the average value of the energy distribution function f(E) at λ = 8.95 μm compiles 75.3 eV, and in a group of people in critical condition – 24.1 eV. The level of reliability of the results is < 0.05 according to Student's t-test.

In 1995 A. Antonov performed DNES-experiments with impact on tumor mice cells in water (Antonov, 1995b). There was a decrease of the spectrum compared with the control sample of cells from a healthy mouse. The decrease was also observed in the spectrum of human blood serum of terminally ill people relative to that of healthy people. With increasing of age of long-living blood relatives, the function of distribution of H_2O molecules according to energies at -0.1387 eV decreases. In this group of tested people the result was obtained by DNES at -5.5±1.1 meV, the difference in age was of 20–25 years in relation to the control group. It should be noted that most of Bulgarian centenarians inhabit the Rhodope Mountains areas. Among to the DNES-spectrum of mountain waters similar to the DNES-spectrum of blood serum of healthy people at $\lambda = 8.95$ µm, was the DNES-spectrum of water in the Rhodopes. The mountain waters from Teteven, Boyana and other Bulgarian provinces have similar parameters.

Table 5 shows the composition of the seven mountain springs in Teteven (Bulgaria) and local maximums in DNES-spectra of water. The local maximums were detected at -0.11 eV and -0.1387 eV. The value at 0.11 eV is characteristic for the presence of Ca^{2+}. The value at 0.1387 eV is characteristic for inhibiting the growth of cancer cells. Experiments conducted by A. Antonov with cancer cells of mice demonstrated a reduction of this local maximum to a negative value. Analysis by the DNES-method of aqueous solutions of natural mineral sorbents – shungite (carbonaceous mineral from Zazhoginskoe deposit in Karelia, Russia) and zeolite (microporous crystalline aluminosilicate mineral from Most village, Bulgaria) showed the presence of a local maximum at -0.1387 eV for shungite and -0.11 eV for zeolite (Mosin & Ignatov, 2013b, Ignatov & Mosin, 2014). These results sugest the restructuring of energy values among H$_2$O molecules with a statistically reliable increase of local maximums in DNES-spectra. It should be noted that owing to the unique porous structures both the natural minerals shungite and zeolite are ideal natural water adsorbers effectively removing from water organochlorine compounds, phenols, dioxins, heavy metals, radionuclides, and color, and gives the water a good organoleptic qualities, additionally saturating it with micro-and macro-elements (Mosin & Ignatov, 2013c). It is worth to note that in Bulgaria the main mineral deposits of Bulgarian zeolites are located in the Rhodope Mountains, whereat has lived the greatest number of Bulgarian centenarians. It is thought that water in these area is cleared in a natural way by zeolite. Therefore, a new parameter is entered into Table 5 – a local maximum of energy at (-0.1362– -0.1387 eV). This value was determined by the NES-spectrum as function of distribution of individual H$_2$O molecules according to energy f(E). The norm has statistically reliable result for human blood serum for the control group of people having cancer at the local maximum of f(E) ~24.1 eV^{-1}. The function of distribution according to energy f(E) for tap water in Teteven is 11.8±0.6 eV^{-1} (Table 5).

Table 6: The composition of mountain water springs in Teteven (Bulgaria) and local maximums in DNES-spectra of water

Water sourses	Ca^{2+}	Na^{+}	Mg^{2+}	Fe^{2+}	SO$_4^{2-}$	pH	local maximum* at (-0.1362–-0.1387)
	mg/dm^3 norm (<150)	mg/dm^3 norm (<200)	mg/dm^3 norm (<80)	mg/dm^3 norm (<200)	mg/dm^3 norm (<250)	norm (6.5-9.5)	eV^{-1} norm (>24.1)
1. Klindiovo	89.9 ±9.0	4.1±0.4	6.98±0.7	40.2±4.0	17.7±1.8	8.0±0.1	47.1±2.4
2.Gorna cheshma	103.6±10.4	4.2±0.4	15.5±1.6	9.6±0.96	89.9±9.0	7.9±0.1	20.0±1.0
3.Dolna cheshma	94.4±0.94	2.5±0.3	12.10±1.21	9.0±0.9	15.99±1.60	7.9±0.1	31.6±1.6
4. Sonda	113.6±11.4	7.3±0.7	15.99±1.60	5.00±0.5	57.2± 5.7	7.3±0.1	48.8±2.4
5.Vila Cherven	x	x	x	x	13.3±1.3	7.5±0.1	44.4±2.2
6. Gechovoto	66,0±6.0	1.46±0.15	2,1±0.2	11,4±1.1	15,9±1.6	7.94±0.1	44.4±2.2
7.Ignatov izvor	40.44±4.04	0.62±0.62	2.46±0.25	13.0±1.4	17.9±1.8	6.82±0.1	31.6±1.6

Notes:
*Function of distribution of H$_2$O molecules according to energy f(E).

Another important physical parameter was calculated with using NES and DNES methods – the average energy ($\Delta E_{H\ldots O}$) of H…O-bonds between H$_2$O compiled -0.1067±0.0011 eV. The most remarkable peculiarity of H…O-bond consists in its relatively low strength; it is 5–10 times weaker than chemical covalent bond. In respect of energy hydrogen bond has an intermediate position between covalent bonds and intermolecular van der Waals forces, based on dipole-dipole interactions, holding the neutral molecules together in gasses or liquefied or solidified gasses. Hydrogen bonding produces interatomic distances shorter than the sum of van der Waals radii, and usually involves a limited number of interaction partners. These characteristics become more substantial when acceptors bind H atoms from more electronegative donors. Hydrogen bonds hold H$_2$O molecules on 15% closer than if water was a simple liquid with van der Waals interactions. The hydrogen bond energy compiles 5–10 kcal/mole, while the energy of covalent O–H-bonds in H_2O molecule – 109 kcal/mole. With fluctuations of water temperature the average energy of hydrogen H...O-bonds in H_2O molecule associates changes. That is why hydrogen bonds in liquid state are relatively weak and unstable: it is thought that they can easily form and disappear as the result of temperature fluctuations. The next conclusion that can be drawn from our research is that there is the distribution of energies among individual H_2O molecules.

4. Conclusion

Research conducted by us shows that the direct relationship of man and nature – clean air, natural food from eco-farms and physical activity explains the difference between the larger number of centenarians who live in the mountain regions of Bulgaria and Russia and their high average number. Natural water with increased content of deuterium seems to be one of the most important factors for longevity. In Bulgaria, most centenarians live in the Rhodope Mountains, while in Russia – in Dagestan and Yakutia. It worth to note that IR-spectrum of mountain water is most similar to the IR-spectrum of blood serum of healthy group of people with a local maximum at λ = 8,95 μm. Similar spectral characteristics possess mountain water from Teteven, Bojana and other Bulgarian sources. Thus, the phenomenon of longevity is a complex phenomenon involving both genetic and phenotypic characteristics of the organism to external factors and environment – free radicals, radiation, heavy isotopes, as well as the structure and the isotopic composition of drink water. Other longevity factors are living area, health status, body mass, gender and heredity. Studying of human blood serum by NES and DNES-methods show that by measuring the average energy of hydrogen bonds among H_2O molecules and the distribution function of H_2O molecules on energies it is possible to drow a vital state statusof a person and associated life expectancy. These data indicate that water in the human body has the IR-spectrum resembling IR-spectrum of human blood serum. On the characteristics of the IR-spectrum of water exerts an influence also the presence of deuterium. Thus, the phenomenon of longevity is a complex multifactorial phenomenon involving both genetic (internal) and phenotypic (external) characteristics of the organism in its adaptation the environment. Further we are planning to continue this project for the research of phenomenon of human longevity in field area (Pleven region), sea area (Varna region) and mountainious area (Smolyan region) in Bulgaria. Although additional data for parents and grandparents of long-living people are needed, total statistical analysis for all these summary factors will be essential for further scrutinized conclusions.

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