Mathematical Model of Melt Water and Mountain Water from Bulgaria obtained by IR, NES and DNES-Methods

Ignat Ignatov[1*]   Oleg Mosin[2]   Enrico Bauer[3]

1. DSc, Professor, Scientific Research Center of Medical Biophysics (SRCMB),

N. Kopernik Street, 32, Sofia 1111, Bulgaria

2. PhD (Chemistry), Biotechnology Department, Moscow State University of Applied Biotechnology,

Talalikhina Street, 33, Moscow 109316, Russian Federation

3. Dipl. Eng., World Demographic and Aging Forum, Sankt Gallen, Switzerland,
St. Gallen, Kanton, 9030, Switzerland

Abstract

Natural waters derived from various Bulgarian water springs as well as mountain water, melt water and water with varying deuterium content and the human blood serum of cancer patients were investigated by IR, NES and DNES methods to establish the structural mathematical models of water. 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 IR- and DNES-spectra of various samples of water and human blood serum at $\Delta E_{H...O}$ = -0,1387 eV (the DNES-method) and λ = 8,95 μm (the IR-method). The increased content of deuterium in water leads to physiological, morphological and cytology alterations of the cell, and also renders negative influence on cellular metabolism, while deuterium depleted water with the residual 60–100% ppm deuterium content has beneficial effects on health. For a group of people in critical condition of life and patients with malignant tumors the greatest values of local maxima in DNES-spectra are shifted to lower energies relative to the control group. In frames of the research was carried out the computer calculation of elemental polyhedral nanoclusters with a formula $(H_2O)_n$, where n = 3–20. Based on this data some important physical characteristics of water were obtained, e.g. the average energy of hydrogen bonding between H_2O molecules in the process of cluster formation was measured by the DNES method compiles -0.1067 ± 0.0011 eV.

Key words: deuterium, heavy water, deuterium depleted water, mountain water, IR, NES, DNES

1. Introduction

Water is the main substance of life. The human body is composed from 50 to 75% of water (Watson, 1980). 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. 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 the main factor for metabolic processes and aging (Ignatov, 2012). It performs a vital role in numerous biochemical and metabolic processes occurring in cells with participation of water, being a universal polar solvent for hydrophilic molecules having an affinity for water. Hydroxyl groups (-OH) in H_2O molecule, are polar and therefore hydrophilic. Moreover water acts as a reagent for a big number of chemical reactions (hydrolysis, oxidation-reduction reactions). In chemical processes water due to its high ionizing ability possesses strong amphoteric properties, and can act both as an acid and a base in reactions of chemical exchange.

Modern science has confirmed the role of water as a universal life sustaining component, which defines the structure and properties of inorganic and organic objects, consisted from water. The recent development of molecular and structural-chemical concepts has enabled to clarify an explanation of ability of water molecules to form the short-lived hydrogen bonds with neighboring molecules and many other chemical substances to bond them into intermolecular associates. It has also become clear the role of bounded water in forming hydrated substances and their physicochemical conduct in aqueous solutions.

Earlier studies conducted by us have demonstrated the role of water, its structure, isotopic composition and physical-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 varies from 3 to 50 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 – $H_2^{16}O$, $H_2^{17}O$, $H_2^{18}O$.

The aim of this research was studying the composition of melt and mountain water obtained from different water sources of Russia and Bulgaria.

2. Material and Methods

2.1. Objects of studying
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.

2.2. Studying the Bulgarian centenarians
Interviews have been conducted with 415 Bulgarian centenarians and long lived people 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 15 mountain water springs located in municipalities Teteven and Kuklen (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.

2.3. Preparation of water samples with varying deuterium content
For preparation of water samples with varying deuterium content 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).

2.4. Preparation of melt water
The melt water was obtained from Moscow tap water by the freeze-thaw method in a standard procedure: 1,5 l of Moscow tap water was placed in a glass jar with a lid and placed in the refrigerator freezer at -14 ^0C for 4–5 hours. Then, the first ice crystals were mechanically removed from the mixture, and the jar again was placed in the freezer additionally for 8–10 hours before ¾ of liquid freezes. Thereafter, the liquid brine is decanted and the remaining ice was thawed at room temperature and used for further experiments. The melt water was stored in a glass container in refrigerator. Other experiments were carried out with deuterium depleted water (DDW) with residual deuterium content of 60–100 ppm, purchased from Langway Water Inc. (Moscow).

2.5. Studying the human blood serum
1 % (v/v) solution of human blood serum was studied with the methods of IR-spectroscopy, 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 years 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.

2.6. DNES spectral analysis
The device for DNES was made from A. Antonov on an optical principle. For this was used a hermetic camera for evaporation of water drops under stable temperature (22±24 ^0C) conditions. The water drops were placed on a water-proof transparent pad, which consists of thin Mylar folio and a glass plate. The light is monochromatic with filter for yellow color with wavelength $\lambda = 580\pm7$ 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 was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in human blood serum.

2.7. IR-spectroscopy

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}) and on Thermo Nicolet Avatar 360 Fourier-transform IR (M. Chakarova).

2.8. Statistical processing of experimental data

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

3. Results and Discussion

3.1. Properties of Melt Water

Human experience shows that long-living people inhabit mainly high mountainous areas where the 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 mountain 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.

Here are submitted the data for Bulgaria:

1) Varna district – 44 centenarians per 1 million of inhabitants, plain and sea regions;

2) Pleven district – 78 centenarians per 1 million of inhabitants, plain regions;

3) Teteven district – 279 centenarians per 1 million of inhabitants, hills and mountainous regions;

4) Bulgaria – 47 centenarians per 1 million of inhabitants.

The analogous situation is observed in the Russian North. According to G. Berdishev (Berdishev, 1989), people inhabiting the Russian North – the Yakuts and the Altaians as well as the Buryats, drink the mountain water obtained after the melting of ice. Altai and Buryat as well as Kavkaz 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 an ordinary tap water that is believed to have a positive effect on the tissue cells and metabolism. The 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 thought to have a beneficial effect on metabolism (Goncharuk et al., 2013). In the world are also popular the water sources containing the melt water from Canada, Norway, Island and Alaska.

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 the 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 1). 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 covalent bonds, but participating in formation of two intermolecular hydrogen bonds with two neighbor H_2O molecules. 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 t = +40 ^{0}C breaks down about half of hydrogen bonds in H_2O associates. In water vapor hydrogen bonds are absent.
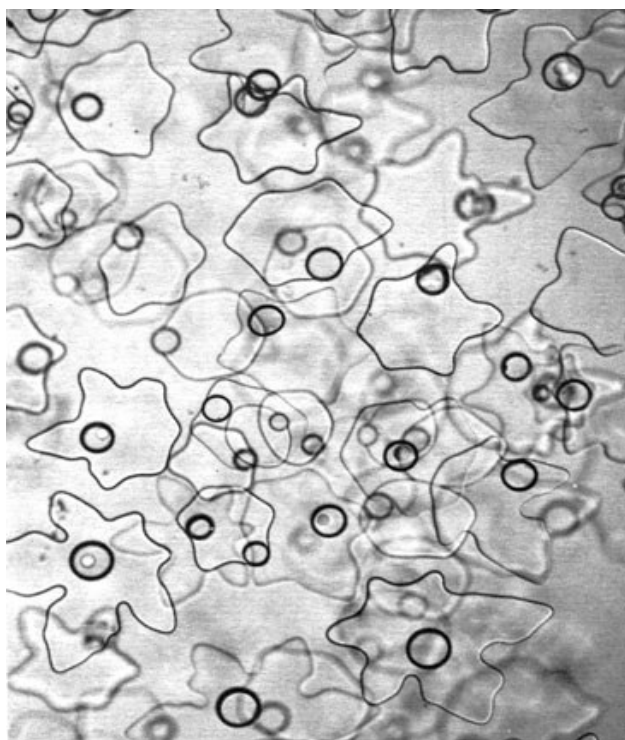

Figure 1: Structure of melt water with "smearing" fragments of regular hexagonal ice structures according to computer simulations

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.

Table 2: Physical properties of ice I_h

Property	Value	Note
Heat capacity, cal/(g·^{0}C)	0.51 (0 ^{0}C)	Significantly decreases with decreasing temperature
Heat of melting, cal/g	79.69	–
Heat of vaporization, cal/g	677	–
The coefficient of thermal expansion, 1/^{0}C	$9.1 \cdot 10^{-5}$ (0 ^{0}C)	Polycrystalline ice
Thermal conductivity, cal/(cm·sec·^{0}C)	$4.99 \cdot 10^{-3}$	Polycrystalline ice
Refractive index	1.309 (-3 ^{0}C)	Polycrystalline ice
Specific electrical conductivity, Ohm^{-1}·см$^{-1}$	10^{-9} (0 ^{0}C)	The apparent activation energy of 11 kcal/mol
Surface conductivity, Ohm^{-1}	10^{-10} (-11 ^{0}C)	The apparent activation energy of 32 kcal/mol
Modulus of elasticity Young's, 1 g·cm/s^2/cm^2	$9 \cdot 10^{10}$ (-5 ^{0}C)	Polycrystalline ice
Resistance, МН/м^2:		
crushing	2.5	Polycrystalline ice
rupture	1.11	Polycrystalline ice
shear	0.57	Polycrystalline ice
Dynamic viscosity, Poise	10^{14}	Polycrystalline ice
The activation energy during deformation and mechanical relaxation kcal/mol	11.44-21.3	Increases linearly at 0.0361 kcal/(mol·^{0}C) from 0 to 273.16 K

Notes: 1 cal/(g·^{0}C) = 4.186 kJ/(kg·K); 1 Ohm^{-1}·cm^{-1} = 100 Sim/m; 1 dyn = 10^{-5} N; 1 N = 1 kg·m/s^2; 1 dyn/cm=10^{-7} H/м; 1 cal/(cm·s·^{0}C) = 418.68 W/(м·K); 1 Poise = g/cm·s = 10^{-1} N·s/м^2.

High pressure ice of trigonal II and tetragonal III modifications is formed by hollow combs, formed by the hexagonal corrugated elements shifted from each other by 1/3 (Fig. 2 b,c). These ice structures are stabilized in the presence of the noble gases as helium and argon. In the monoclinic structure of ice V the angles between the neighboring O-atoms are varied from 86^0 to 132^0, which is very different from the valence angle in the H_2O molecule makes up $105^0$47'. Ice of VI tetragonal modification consists of two inserted into each other scaffolds, between which there are no H-bonds, resulting in a body-centered crystal lattice (Fig. 2 e). The basis of the structure of ice VI constitutes the hexamers - blocks of six H_2O molecules. Their configuration is exactly the same as the structure of a stable cluster of water, which gave computer calculations. A similar structure with frames of ice I, inserted into each other, have ice of cubic modification of VII and VIII, which are low temperature ordered forms of ice VII. Upon the pressure increasing the distance between O-atoms in the crystal lattice of ice VII and ice VIII will decreases, as a result is formed the ice structure X, in which O-atoms are arranged in a regular grid with arranged protons.

Ice XI is formed by deep freezing of ice I_h with addition of alkali below 72 K at normal pressure. Under these conditions, the hydroxyl defects of the crystal are formed, allowing to the growing ice crystals change their structure. Ice XI has a rhombic crystal lattice with an ordered arrangement of protons and is formed simultaneously in many centers of crystallization around the hydroxyl crystal defects.

Among the ice there and metastable forms of IV and XII, which lifetimes are seconds (Fig. 2 d,f). For obtaining the structures of the metastable ice, the ice I_h is to be compressed to a pressure of 1.8 GPa at a temperature of liquid N_2. These ice structures are formed much easier and especially stable if supercooled heavy water is subjected to pressure. Another modification of the metastable ice IX is formed by supercooling of ice III and, therefore, is its low temperature form.

The last two modifications of ice – the monoclinic XIII and rhombic XIV configuration were discovered by scientists from Oxford (UK) in 2006. On Earth, such modifications of ice cannot be formed, but they can exist on the cooled frozen planets and comets. The calculation of density and the heat flows from the surface of the moons of Jupiter and Saturn suggests that Ganymede and Callisto has to be the ice shells, in which alternate ice I, III, V and VI. At the Titan's the ice does not form a crust shell, but the mantle, the inner layer of which consists of ice VI and other high-pressure ice modifications and clathrate hydrates, on the top of which is located hexagonal ice I_h.

In ice I_h the carcasses of hydrogen bonds allocate H_2O molecules in form of a spatial hexagon network with internal hollow hexagonal channels. 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 up to +40 ^{0}C breaks down about half of hydrogen bonds in water associates. In the water vapor hydrogen bonds are absent. The physical properties of ice I_h are shown in Table 2.

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 of hydrogen bonds. Those out of the equilibrium positions H_2O molecules fall 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) varies from 4.4 at +1.5 ^{0}C to 4.9 at +80 ^{0}C.
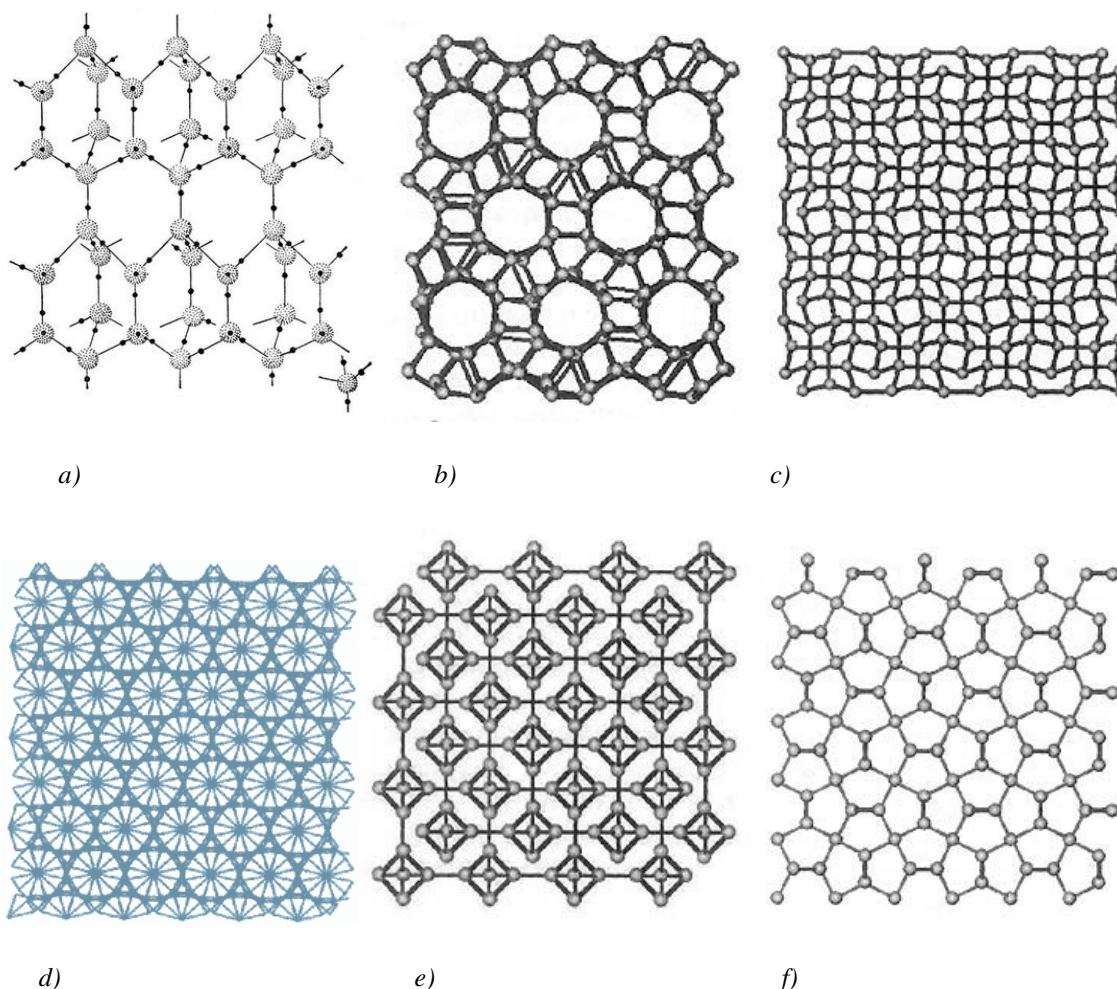

Figure 2: Ice crystalline modifications: *a*) - natural hexagonal ice I_h; *b*) – ice of II configuration; *c*) – ice of III configuration; *d*) – ice of IV configuration; *e*) – ice of VI configuration; *f*) – ice of XII configuration.

The reports about the evidence on the existence of analogs of crystal structures in water have become more frequent in scientific literature nowadays (Michaelides & Morgenstern, 2007). As computer modeling showed, H_2O molecules after being placed in carbon nanotubes under high pressure and low temperatures formed the crystalline nanostructures like DNA double helix (Bai *et al*., 2006). In modeling experiments water got frozen inside the carbon nanotubes with the diameter 1.35–1.90 nm, with pressure 10–40000 atm and temperature -23 ^{0}C. The hydrogen bonds in an ice crystal got distorted leading to the formation of a double-walled helix. The inner wall of this structure represents a four–fold twisted helix, while the outer consists of four double helixes, resembling a helix of DNA molecule (Figure 3). While being frozen at 17 K on hydrophobic surfaces of Cu, Ag

and their salts, water became crystallized into two-dimensional ice hexamer-nanocrystal, consisting of six hexagonally attached H_2O molecules (Figure 4).

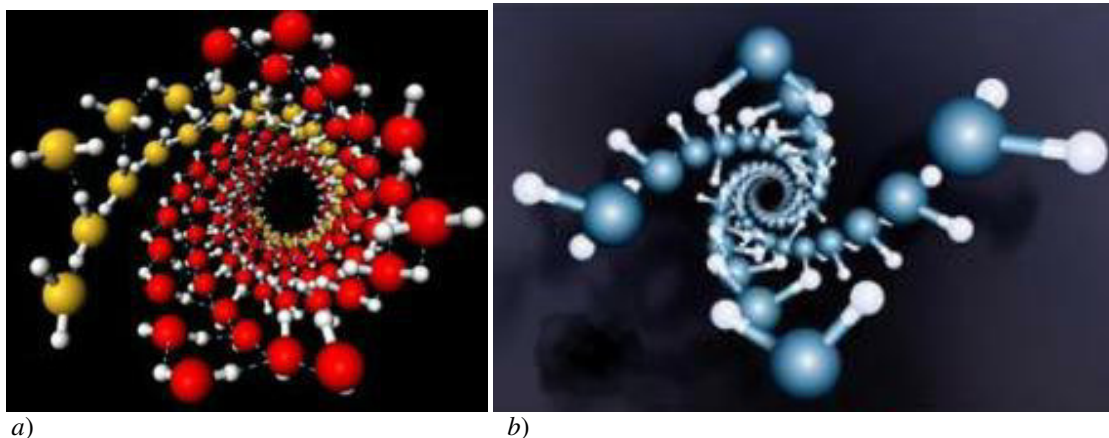*a)*   *b)*

Figure 3: Structures of water crystals in carbon nanotubes according to computer simulations (Bai *et al.*, 2006) (diameter of nanotubes 1.35–1.90 nm; preassure 10–40000 atm; temperature -23°C): *a*) – a general view of the crystal structure of water in nanotubes, *b*) – the inner wall of the structure of water.
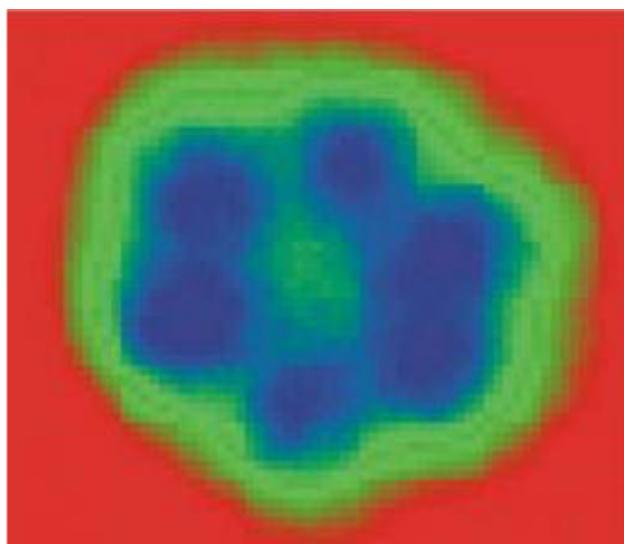

Figure 4: Image of hexamer nanocrystal of ice water (average size ~1 nm) by a scanning tunneling microscope after freezing water to 17 K on the hydrophobic metallic plates of Cu and Ag (Michaelides & Morgenstern, 2007).

The 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 the 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 H_2O 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 3). 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).

In 2005 R. Saykally (USA) calculated the possible number of hydrogen bonds and the stability of water clusters depending on the number of H_2O molecules (Figure 5) (Saykally, 2005). It was also estimated the possible number of hydrogen bonds (100) depending on the number of H_2O molecules (250) in clusters (Sykes, 2007). O. Loboda and O.V. Goncharuk provided data about the existence of icosahedral water clusters consisting of 280 H_2O molecules with the average size up to 3 nm (Loboda & Goncharuk, 2010). The ordering of H_2O molecules into associates corresponds to a decrease in the entropy (randomness), or decrease in the overall Gibbs energy (G = ΔH – TΔS). This means that the change in enthalpy ΔH minus the change in entropy ΔS (multiplied by the

absolute temperature T) is a negative value. These results are consistent with our results on the research of DNES spectra of water on which it is possible to may make the conclusion about the number of H_2O molecules in water clusters. The DNES spectrum of water has energy ranges from -0.08 to -0.14 eV. The spectral range lies in the middle infrared range from 8 to 14 μm ("window" of the atmosphere transparency to electromagnetic radiation). Under these conditions, the relative stability of water clusters depends on external factors, primarily on the temperature. We demonstrated that H_2O molecules change their position in clusters depending on the energy of intermolecular H…O hydrogen bonds. The values of the average energy ($E_{H…O}$) of hydrogen bonds between H_2O molecules in the formation of cluster associates with the formula $(H_2O)_n$ compile -0.1067 ± 0.0011 eV. As the energy of hydrogen bonds between H_2O molecules increases up to -0.14 eV, the cluster formation of water becomes "restructuring". In this case, the redistribution of energies among the individual H_2O molecules occurs.
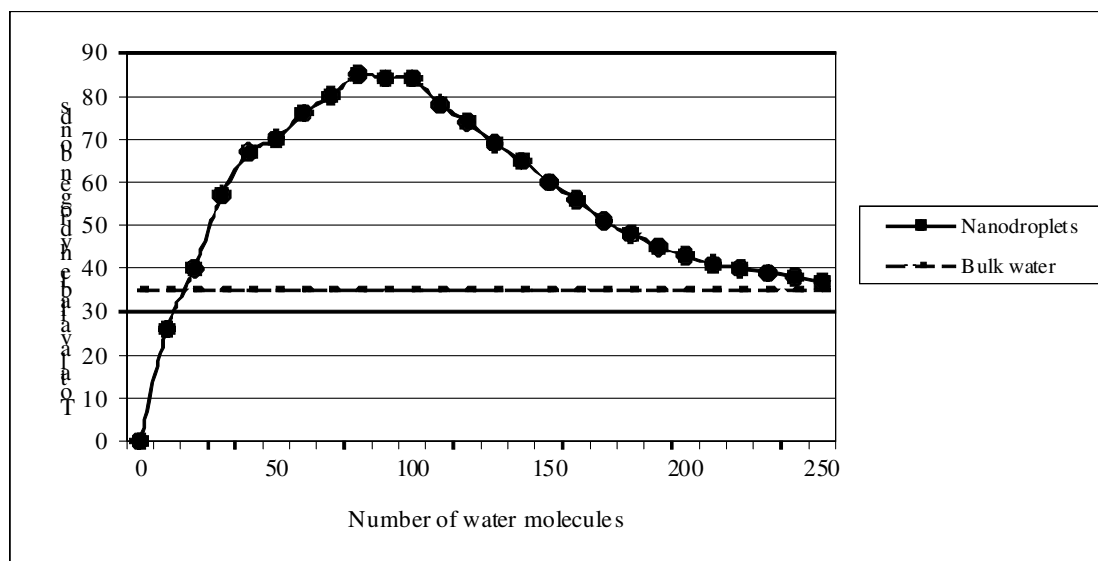

Figure 5: The total number of hydrogen bonds depending on the number of H_2O molecules in clusters.

All these experimental data including our data indicate that water is a complex associated non-equilibrium liquid consisting of associative groups containing according to the present data, from 3 to 20 individual H_2O molecules (Tokmachev *et al*., 2010). Associates can be perceived as unstable groups (dimmers, trimmers, tetramers, pentamers, hexamers etc.) in which H_2O molecules are linked by van der Waals forces, dipole-dipole and other charge-transfer interactions, including hydrogen bonding. At room temperature, the degree of association of H_2O molecules may vary from 2 to 6. In 2000 it was deciphered the structure of the water trimmer, and in 2003 – tetramer, pentamer and the hexamer (Wang & Jordan, 2003). In 1993 K. Jordan (USA) (Tsai & Jordan, 1993) calculated the possible structural modifications of small water clusters consisting of six H_2O molecules (Figure 6a–c). Subsequently, it was shown that H_2O molecules capable of hydrogen bonding by forming the structures representing topological 1D rings and 2D chains composed from numerous H_2O molecules. Interpreting the experimental data, they are considered as pretty stable elements of the structure. According to computer simulations, elemental clusters are able to interact with each other through the exposed protons on the outer surfaces of hydrogen bonds to form more complicated clusters of more complex structure.

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 the physical properties of melt water are changed over time, e.g. dielectric permittivity comes to its equilibrium state after 15–20 min, viscosity – in 3–6 days (Ignatov & Mosin, 2013f). The heating of fresh melt water above t = +37 [0]C leads to a loss of the biological activity. The storage of melt water at t = +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 t = +1.5 ^{0}C to 4.9 at t = 80 ^{0}C.

a)   *b*)   *c*)
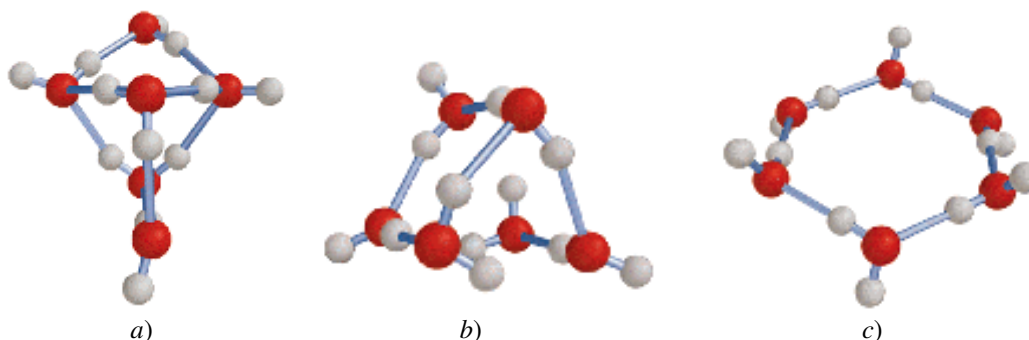
Figure 6: Computer calculations of small water cluster structures (*a–c*) with general formula $(H_2O)_n$, where n = 6 (Tsai & Jordan, 1993).

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 3). 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 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 3: Chemical composition of melt water obtained from tap water by the freeze-thaw method

Cations, mg/l	
$K^+ + Na^+$	20–25
Mg^{2+}	5–10
Ca^{2+}	25–30
Anions, mg/l	
SO_4^{2-}	<90
HCO_3^-	50–100
Cl^-	<50
Other physical characteristics	
Total rigidity, mEq/l	≤ 5
Total mineralization, g/l	$\leq 0,2$
pH at t =+25 ^{0}C	6,5–7,0
Deuterium content, ppm	~129,5

Table 4 shows the optimal chemical composition of water, hardness, the local extremum eV^{-1} at (-0.1362–-0.1387 eV), and total mineralization of water as the middle result of different studies. The water samples were taken from areas between 600 and 1300 m attitude in Bulgaria and from Kavkaz, Russia. It is worth to note that in these areas are living long lived people.

Table 4: The optical chemical composition of water, hardness, local extremum eV^{-1} at (-0.1362–-0.1387 eV) and total mineralization of water

Indicators	Results of mountain water (Bulgaria) (mg/l)	Results of melt water (Russia) (mg/l)
Sodium(Na)+Potassium (K)	6.1	< 30
Calcium (Ca)	29.5	< 50
Magnesium (Mg)	1.5	< 10
Iron (Fe)	0.083	–
Manganese (Mn)	0.0017	–
Zinc (Zn)	0.007	–
Sulfates (SO_4)	21.9	< 100
Chlorides (Cl)	3.2	< 70
Carbonates (CO_3)	< 2.0	–
Hydrocarbonates (HCO_3)	69.7	< 100
Other values	Results	
Active reaction (pH)	6.7 normal	6.5–7.0
Electroconductivity	410.9 µS/cm	< 2000
Hardness of water	4.65 dH Moderately soft	<33.7
Total mineralization (g/l)	0.132	< 0.3
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	49.7	>24.1

Analysis of water samples from various sources of Russia and Bulgaria show that the mountain water contains on average ~2–4% less deuterium in form of HDO than the 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. The concentration of water molecules containing heavy isotopes of D, 17O and 18O, in natural water varies within the limits laid down in the basic standards of the isotopic composition of the hydrosphere SNOW and SLAP (Table 5). According to the international SMOW standard (Oceanic water)) the isotopic the 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 the SLAP standard (the Atlantic oceanic water) the 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 varied 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.%).

Table 5: The calculated mass concentrations of isotopologues in natural water corresponding to international standards of SMOW* and SLAP**

Isotopologue	Molecular mass, u	Isotopic content, g/kg	
		SMOW	SLAP
$^1H_2^{16}O$	18.01056470	997.032536356	997.317982662
$^1HD^{16}O$	19.01684144	0.328000097	0.187668379
$D_2^{16}O$	20.02311819	0.000026900	0.000008804
$^1H_2^{17}O$	19.01478127	0.411509070	0.388988825
$^1HD^{17}O$	20.02105801	0.000134998	0.000072993
$D_2^{17}O$	21.02733476	0.000000011	0.000000003
$^1H_2^{18}O$	20.01481037	2.227063738	2.104884332
$^1HD^{18}O$	21.02108711	0.000728769	0.000393984
$D_2^{18}O$	22.02736386	0.000000059	0.000000018

Notes:

*SMOW (average molecular weight = 18.01528873 u)

**SLAP (average molecular weight = 18.01491202 u)

The 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 (DDW) on Organism

The 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).

Isotopic effects of deuterium, which occur in macromolecules of even a small difference between hydrogen and deuterium, have the effect upon the structure. The sensitivity of enzyme function to the structure and the sensitivity of nucleic acid function (genetic and mitotic) may lead to a noticeable effect on the metabolic pathways and reproductive behaviour of an organism in the presence of D_2O (Cleland *et al*., 1976). The changes in dissociation constants of DNA and protein ionizable groups when transferring the macromolecule from H_2O to D_2O may perturb the charge state of the DNA and protein molecules (Cioni & Strambini, 2002). All this can cause variations in nucleic acid synthesis, which can lead to structural changes and functional differences in the cell and its organelles.

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 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 t = +101.44 ^0C, freezes at t = +3,82 ^0C, has the density of 1.1053 g/cm^3 at t = +20 ^0C, and the maximum density occurs not at t = +4 ^0C as in H_2O, but at t = +11.2 ^0C (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 (Table 6). 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 (Mosin, 1996). 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).

Table 6: Changes in the physical properties of water with isotopic substitution

Physical properties	$H_2^{16}O$	$D_2^{16}O$	$H_2^{18}O$
Density at 20 ^{0}C, g/cm^3	0.997	1.105	1.111
Temperature of maximum density, ^{0}C	3.98	11.24	4.30
Melting point under 1 atm, ^{0}C	0	3.81	0.28
Boiling point temperature at 1 atm, ^{0}C	100.00	101.42	100.14
The vapor pressure at 100 ^{0}C, mm Hg	760.00	721.60	758.10
Viscosity at 20 ^{0}C, cP	1.002	1.47	1.056

At replaicing changing of hydrogen by deuterium 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 appears 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, e.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 ^{0}C, 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^{2}HO 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 absorption bands in the IR-spectrum of liquid water is given in Table 7. 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. The result is reliable regarding the content of deuterium in natural waters from 0.015–0.03%.

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 (v_3 = 3490 cm⁻¹ and v_1 = 3280 cm⁻¹) (Eisenberg & Kauzmann, 1969) and the bending frequency 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 dimmer [$(H_2O)_2$]. 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 absorption 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, e.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.

Table 7: The assignment of main frequencies in IR-spectra of H_2O and D_2O

Main vibrations of liquid H_2O and ^2H_2O				
Vibration(s)	H_2O (t = +25 ⁰C)		D_2O (t = +25 ⁰C)	
	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

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. Thus the 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. 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 7). 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 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. 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. 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).
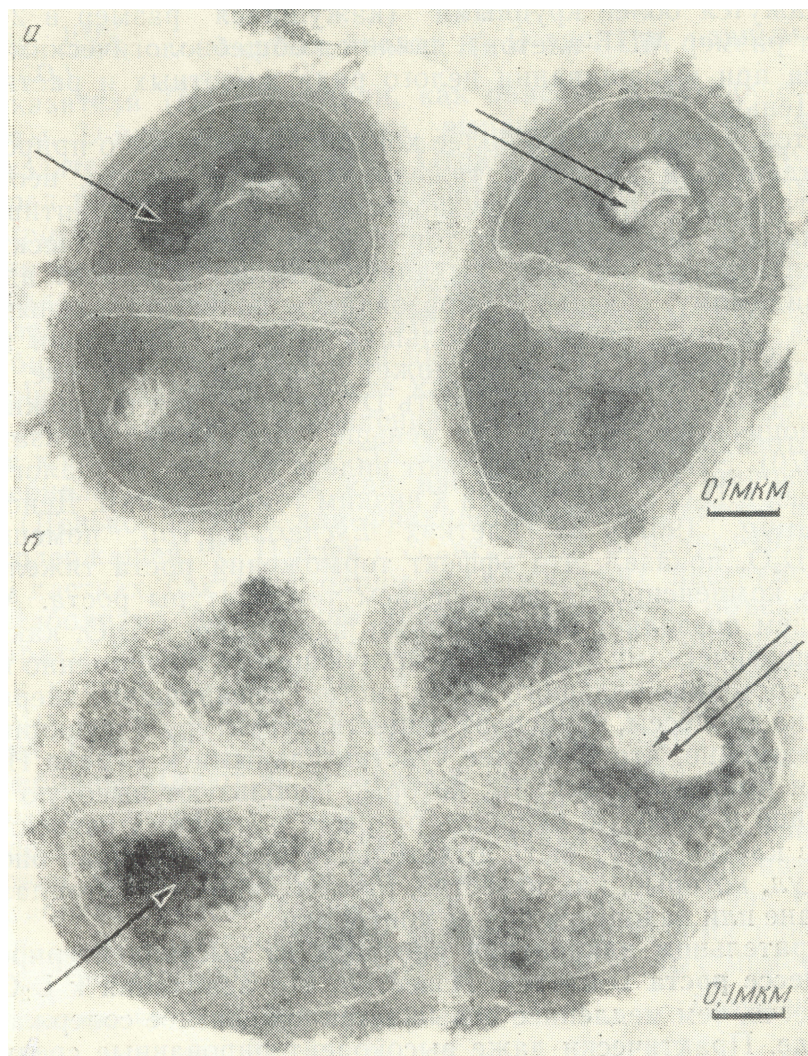
Figure 7: Electron micrographs of *Micrococcus lysodeikticus* cells obtained by SEM method: *a*) – protonated cells obtained on H_2O-medium; *b*) – deuterated cells obtained on ^2H_2O-medium. The arrows indicate the tightly-packed portions of the membranes

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 beneficial 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).

The 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 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.

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 influence 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 varied residual deuterium content are shown in Table 8. 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 8: 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

A convenient method for studying of liquids is non-equilibrium differential spectrum. 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).

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:

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

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 Δf(E) = f (samples of water) – f (control sample of water)

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

Thus, the DNES spectrum is an indicator of structural changes in water, because the energy of hydrogen bonds in water samples differ due to the different number of hydrogen bonds in water samples, which may result from the fact that different waters have different structures and composition and various intermolecular interactions – various associative elements etc. The redistribution of H_2O molecules in water samples according to the energy is a statistical process of dynamics.

We have conducted studies of 1 % (v/v) solution of human blood serum taken from two groups of people between 50 and 70 years of age by IR, NES and DNES spectral analysis. The first group consisted of people in excellent health. The second group consisted of people in a critical state and patients with malignant tumors. The average energy of hydrogen bonds ($\Delta E_{H...O}$) between H_2O molecules in the blood serum was investigated as the main biophysical parameter. The result was registered 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 Δf(E) = f (samples of water) – f (control sample of water). The DNES-spectrum obtained from the first group has a local extremum energy ($\Delta E_{H...O}$) at E = –9.1±1.1 meV and from the second group at E = –1.6±1.1 meV. The 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 E = -0.1387 eV, or at a wavelength λ = 8.95 μm. For the group of people in a critical health 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. For a group of people in critical health condition and patients with malignant tumors the greatest values of local extremum in the IR-spectrum are shifted to lower energies relative to the control group of people.

In 1995 were performed DNES-experiments with impact on tumor mice cells in water containing Ca^{2+} (Antonov, 1995). There was a decrease in the DNES-spectrum compared with the control sample of cells from a healthy mouse. The decrease was also observed in the DNES-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-method 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 many of Bulgarian centenarians inhabit the Rhodopes Mountains areas. Among to the DNES-spectrum of mountain waters the similar to the DNES-spectrum of blood serum of healthy people at λ = 8.95 μm, was the DNES-spectrum of water in the Rhodopes. The mountain water from Teteven, Boyana and other Bulgarian provinces has similar parameters.

The study the physiologic fluids (urine, blood, serum) by IR- and DNES-spectroscopy can also provide data on metabolic processes in the human body and longevity, because the IR- and DNES-spectra reflect the metabolic processes. It was demonstrated by the analysis of human blood serum by IR- and DNES-spectroscopy. The magnitude of the largest local maximum in IR-, DNES-spectra of human blood serum from healthy people of control group was observed at E = –0,1387 eV (the DNES-method) and at λ = 8,95 μm (the IR-method). 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 8 main local maxima at λ = 8,55; 8,58; 8,70; 8,77; 8,85; 9,10; 9,35 and 9,76 μm. The resulting peak at λ = 8,95 μm in the IR-spectrum of human blood serum (Ignatov, 2012) approaching the peak at λ = 8,85 μm monitored by Russian researchers (Krasnov, Gordetsov, 2009). In the control group of healthy people the average value of the energy distribution function f(E) measured by the DNES-method at λ = 8,95 μm compiles to 75,3 eV, and in a group of people in critical condition – 24,1 eV. The level of reliability of the results is p < 0,05 according to the Student's *t*-criterion.

Comparatively, we studied the water samples of various Bulgarian water springs by the DNES-method. Table 9 shows the composition of the seven mountain springs in Teteven (Bulgaria) and local maximums in DNES-

spectra of water samples. 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+} in water.

Table 9: The composition of mountain water springs in Teteven (Bulgaria) and local maximums in DNES-spectra of water

Sources	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	6.9±0.7	40.2±4.0	17.7±1.8	8.0±0.1	47.1±2.4
2.Gorna cheshma	103.6±10.1	4.2±0.4	15.5±1.6	9.6±0.96	89.9±9.0	7.8±0.2	20.0±1.0
3.Dolna cheshma	94.4±0.94	2.5±0.3	1.10±1.2	9.0±0.9	15.9±1.6	7.9±0.1	31.6±1.6
4. Sonda	113.6±11.4	7.3±0.7	15.9±1.6	5.00±0.5	57.2±5.7	7.3±0.1	48.8±2.4
5. Vila Cherven	–	–	–	–	13.3±1.3	7.5±0.1	44.4±2.2
6. Gechovoto	66.0±6.0	1.4±0.15	2.1±0.2	11.4±1.1	15.9±1.6	7.9±0.1	44.4±2.2
7. Ignatov izvor	40.4±3.1	0.6±0.1	2.46±0.2	13.0±1.4	17,9±1,8	6.8±0.1	31.6±1.6

Notes:

*Function of distribution of H_2O molecules according to energy f(E).

Another important physical parameter was calculated with using NES and DNES methods – the average energy ($\Delta E_{H...O}$) of H…O-bonds between H_2O 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_2O 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

The research conducted by us shows that the melt water has more diffuse arrangement of O- and H-atoms in the lattice and disturbance of long-range order. Thermal oscillations (fluctuations) lead to bending and breaking of hydrogen bonds. The average energy of hydrogen bonding between H_2O molecules in the process of cluster formation was measured by the DNES method compiles -0.1067 ± 0.0011 eV. It was also shown that water clusters formed from D_2O were more stable, than those from H_2O due to isotopic effects of deuterium. The 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. Deuterium depleted water with 25–30% decreased deuterium content has beneficial effects on organism. The 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. 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 draw a vital state status of 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. These data may help establish the mathematical model of melt water and its structure.

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