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Multifactorial Research of Longevity Phenomenon in Mountainous and Field Areas of Bulgaria

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Abstract

The article outlines the data on longevity factors and mountain water in factorial research of phenomenon of longevity in mountainous and field areas of Bulgaria. It was established the dependence among various internal and external factors on a phenomenon of longevity – the residence area, health status, gender and heredity. Natural waters derived from various Bulgarian water springs as well as water with varying deuterium content and the human blood serum of cancer patients were investigated by IR, NES and DNES methods. 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, 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 reduced deuterium content until the deep removal of deuterium (60–100 ppm) has beneficial effects on human 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.

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

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 [1]. 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 the 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 [2]. 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 result 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. This is most possible that, for this reason, the probability of cancer occurrence increases with age. According to thermodynamics, the process of aging is the process of alignment of the entropy by the human body with that of the environment [3].

Water is the main substance of life. The human body is composed from 50 to 75 % of water. With aging, the percentage of water in the human body decreases. Hence, the factor of water quality is the essential factor for the research. 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 main factor for metabolic processes and aging. Earlier studies conducted by us, have demonstrated the role of water, its structure, isotopic composition and physical-chemical (pH, temperature) in the growth and proliferation of prokaryotes and eukaryotes in water with different isotopic content [4, 5]. These factors and the structure of water are of great importance for biomedical studies. The peculiarities of chemical structure of H_2O molecule create the favorable conditions for formation of electrostatic intermolecular Van-der-Waals forces, 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 [6]. 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 [7]. 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 report 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.

Material and methods

Objects of studying

The objects of the study were various prokaryotic and eukaryotic cells of microorganizms 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.

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 was 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). 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 ^{0}C 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).

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.

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.

DNES spectral analysis

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 Mylar 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 was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in human blood serum.

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).

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 $p < 0,05$).

Results and discussions

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

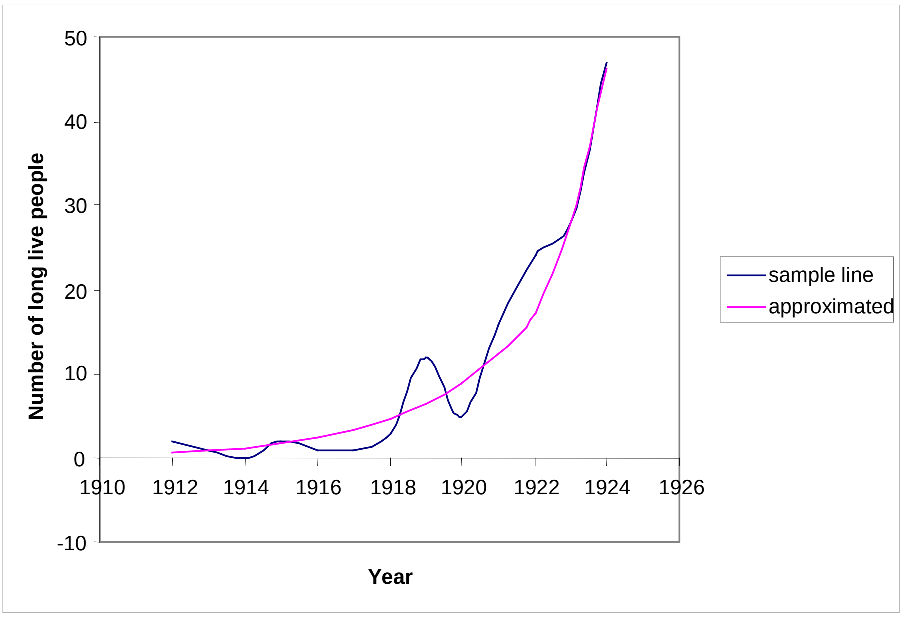

Figure 1: Interrelation between the year of birth of long-living people (age) and their number in Teteven municipality, Bulgaria.

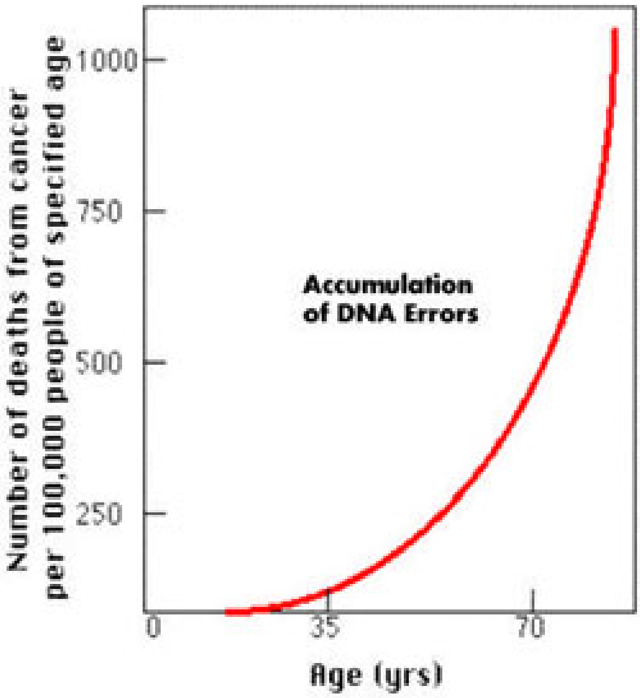

Figure 2: Interrelation between age and the number of cancer patients.

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 [8]. 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.

Empirical evidence on life duration

Human experience shows that long-living people inhabit mainly high mountainous areas where flow the rivers feed by mountain springs. In Russia the most number of centenarians live in Russian North and Dagestan region (Russian Federation). One explanation for this is that water in those places contains less deuterium than ordinary drinking water [9].

In 1989 G. Berdishev studied the phenomenon of longevity of centenarians in Yakutia and Altai regions (Russian Federation) [10]. 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 these 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 inhabitants. This number for all Russia is only 8 people for 1 million. In Bulgaria the average number of centenarians makes up 47 per 1 million, while in Teteven Municipality – 139 centenarians per 1 million. In the Bulgarian municipalities the oldest inhabitant in field areas is 93 years old, and the oldest inhabitant in mountainous 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:

- Varna district – centenarians 44 per 1 million, plain and sea regions;
- Pleven district – centenarians 78 per 1 million, plain regions;
- Teteven district – centenarians 279 per 1 million, hills and mountainous regions;
- Bulgaria – centenarians 47 per 1 million.

Analogous 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 predominantly the mountain water obtained after the melting of ice (melt-water). 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. The melted 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 [11].

The mountain water in springtime is the result of the melting of ice and snow accumulated in the mountains. Natural ice (ice I_h, 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. The melt water obtained after the thawing of ice 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) – the analogues of ice structures, consisting of a different number of H_2O molecules (Fig. 3). However, unlike the 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 [13]. 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, e.g. dielectric permittivity comes to its equilibrium state after 15–20 min, viscosity – in 3–6 days [14]. The heating of fresh melt water above t = +37 ^{0}C leads to a loss of the biological activity. The storage of melted water 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. The thermal oscillations (fluctuations) lead to the 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.

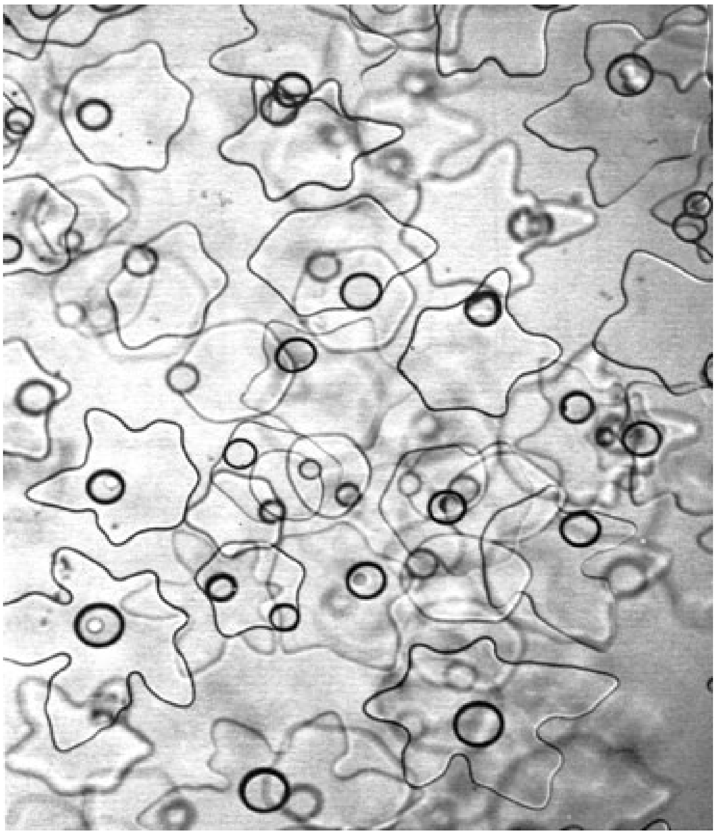

Figure 3: Structure of melt water containing "smearing" fragments of regular hexagonal ice structures according to computer simulations.

Preliminary analyses of water from various water sources show that the melt water obtained by the freeze-thaw method as well as mountain water contain less amount of deuterium as the result of natural isotope purification. The melt 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 20–25 mg/l, Mg^{2+} – 5–10 mg/l, Ca^{2+} – 25–30 mg/l, the content of SO_4^{2-} – <90 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 t = +25 ^{0}C (Table 1). The degree of natural purification of melt water from impurities makes up ~55–60%. 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 [15]. However, the mild correlation was further proven that water hardness could not be a decisive factor for human longevity.

Table 1: 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	≤o,2
pH at t =+25 ^{0}C	6,5–7,0
Deuterium content, ppm	~132,5

Analysis of water samples from various sources of Russia and Bulgaria show that the mountain water contains on average ~4–5 % 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, ^{17}O and ^{18}O, in natural water varies within the limits laid down in the basic standards of the isotopic composition of the hydrosphere SMOW and SLAP (Table 2). According to the international SMOW the standard (the oceanic water) the isotopic shifts for D and ^{18}O in seawater: D/H = (155,76±0,05)$\cdot 10^{-6}$ (155,76 ppm) and ^{18}O/^{16}O = (2005,20±0,45)$\cdot 10^{-6}$ (2005 ppm) [16]. For the SLAP standard (the Atlantic oceanic water) the isotopic shifts for D and ^{18}O in seawater: D/H = 89$\cdot 10^{-6}$ (89 ppm) and for a pair of ^{18}O/^{16}O = 1894$\cdot 10^{-6}$ (1894 ppm). In surface waters, the ratio D/H = ~(1,32–1,51)$\cdot 10^{-4}$, while in the coastal seawater – ~(1,55–1,56)$\cdot 10^{-4}$. Waters of other underground and surface water sources contain varried amounts of deuterium (isotopic shifts) – from δ = +5,o 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)$\cdot 10^{-5}$, in some places up to (6,0–6,7)$\cdot 10^{-5}$, but there are also observed positive deviations at 2,0$\cdot 10^{-5}$. The content of the lightest isotopomer – $H_2{}^{16}O$ in water corresponding to the SMOW standard is 997,0325 g/kg (99,73 mol.%), and for the SLAP standard – 997,3179 g/kg (99,76 mol.%).

Table 2: 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
$^{1}H_2{}^{16}O$	18,01056470	997,032536356	997,317982662
$^{1}HD^{16}O$	19,01684144	0,328000097	0,187668379
$D_2{}^{16}O$	20,02311819	0,000026900	0,000008804
$^{1}H_2{}^{17}O$	19,01478127	0,411509070	0,388988825
$^{1}HD^{17}O$	20,02105801	0,000134998	0,000072993
$D_2{}^{17}O$	21,02733476	0,000000011	0,000000003
$^{1}H_2{}^{18}O$	20,01481037	2,227063738	2,104884332
$^{1}HD^{18}O$	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 of 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 the drinking water from heavy isotopes of D and ^{18}O.

Clinical evidence on the benefits of deuterium depleted water (DDW) for health

When biological objects 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 [17].

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 [18]. 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 (Table 3). 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 [19]. 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. It may cause disturbances in the DNA-synthesis, leading to permanent changes on DNA structure and consequently on cell genotype.

Table 3: Changes in the physical properties of water with the 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

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 [20]. It may cause disturbances in the DNA-synthesis, leading to permanent changes on DNA structure and consequently on cell genotype [21].

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. 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 the metabolic disorders, kidney's malfunction, the violation of hormonal regulation and caused the immunosuppression [22], notwithstanding the strong radioprotective effect of D_2O [23]. Also deuterium induces physiological, morphological and cytological alterations on the cell with forming cells more 2–3 times larger in size in D_2O. At high concentrations of D_2O are suppressed enzymatic reactions, cell growth, mitosis and synthesis of nucleic acids [24]. 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. 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. 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 [25].

We have obtained results on growth and adaptation to D_2O of various cells of prokaryotic and eukaryotic organisms. 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, DDW with the decreased deuterium content until the deep removal of deuterium (60–100 ppm) has beneficial effects on organism. Experiments on animals and plants demonstrated that after the consumption of DDW with the 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 DDW leads to accelerated development of the genital organs (size and weight), and strengthen the process of spermatogenesis, wheat ripens earlier and gives higher yields [26]. In addition, DDW delays the appearance of the first metastasis nodules on the spot inoculation of cervical cancer and exerts immunomodulatory and radioprotective effect [27].

Radioprotective effects of DDW were studied in reports [28] at irradiation of mice's cells by γ-radiation at semi-mortal dose LD_{50}. Survival level of animals treated with DDW 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 [29].

The consumption of DDW by cancer patients during or after radiation therapy treatments allows restore the composition of blood and relieve nausea [30]. According to G. Shomlai, the results of clinical trials conducted in 1998–2010 in Hungary showed that the survival rate for patients drinking DDW in combination with traditional therapies or after are significantly higher than for patients who only used the chemotherapy or radiation therapy [31].

The biological experiments with deuterium DDW with residual deuterium content of 60 ppm carried out in Moscow Research Oncological Institute after P.A. Herzen and N.N. Blokhin and Institute of Biomedical Problems, was confirmed the inhibitory effects of DDW on the process of growth of various tumors, e.g. division of the breast adenocarcinoma MCF-7 tumor cells placed in DDW started with a delay of ~5–10 hours [32]. In 60% of mice with the immunosuppressed immunity and transplanted human breast tumor MDA and MCF-7 consumption of DDW caused tumor regression. A group of mice with transplanted human prostate tumor PC-3 consumed DDW 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. In this regard special attention deserves two indicators: the delay of metastasis and loss of animal's weight during experiments. Stimulating action of DDW 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 DDW at the end of the experiment was 2 times less. It was also reported that DDW may delay the progression of prostate cancer [33] and inhibit the human lung carcinoma cell growth by apoptosis [34].

The preliminary experimental results on motility of human sperm, performed by V.I. Lobyshev and A.A. Kirkina [35], indicates that in DDW (4 ppm) the motility is on 40% higher during 5 hours of the registration. However, the effect depends on the initial properties of a sperm sample. These data 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 [36]. 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 DDW on the 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 [37]. These data evidence the significance of DDW 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 DDW 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 [38]. On beneficial effect on health of experimental mice evidenced the increased resistance and weight increase compared with the control group [39]. It was also indicated that DDW increases the rate of metabolic reactions. It was observed the geroprotective (anti-aging), anti-mutagenic and radioprotective effects of DDW with reduced on 5% deuterium content on the development cycle of fruit fly *Drosophila melanogaster*.

The total effects of DDW depend on the following parameters – the total body mass, total body water, the amount of daily consumption of DDW and the degree of its isotope purification. The main impact of DDW 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. These results indicate that the regular drinking of DDW helps improve the function of some vital systems [40]. With regular consumption of DDW there 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 DDW allows naturally reduce the content of HDO in the human body due to isotopic (H–D) exchange. It is believed that this process is accompanied by an increase in the functional activity of cells, cell tissues and organs. Thus regular consumption of DDW 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 DDW for residents of large cities.

Clinical trials of DDW (Langway Water Inc., Moscow) 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 DDW 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. DDW 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 DDW, it can be expected that the greatest effect the isotopic purification of water will have on the regulatory system and metabolism.

The effectiveness of the influence of DDW depends on the following parameters – the total body mass, total body water, the amount of daily consumption of DDW and the isotopic content of deuterium. The results on the gradual increasing of deuterium content in the human body at regular consumption of DDW (Langway Water Inc., Moscow) with varied residual deuterium content of 60–100 ppm are shown in Table 4. This table shows that the content of deuterium in the human body decreases while drinking DDW. 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 DDW provides a natural way to reduce the content of HDO in the human body to a value of ~117 ppm.

Table 4: Gradual decreasing of deuterium content in the human body over time, with regular consumption of DDW (Langway Water Inc., Moscow)*

Number of days	**The residual content of deuterium in water, ppm**		
	60	**100**	**100**
	Daily consumption of DDW, 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 DDEW – 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).

Clinical evidence with human blood serum testing

A convenient method for studying of liquids is non-equilibrium differential spectrum. It was established experimentally that at 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. 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 (ESWS). The theoretical research established the dependence between the surface tension of water and the energy of hydrogen bonds among individual H_2O-molecules.

For calculation of the function f(E) represented the ESWS, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds between H_2O molecules (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 evaporation of water droplets 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 of two groups of people between 50 and 70 years of age by 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 $\Delta f(E)$ = 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. The results between the two groups have a statistical difference in *t*-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 λ = 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. The norm has statistically reliable result for samples of human blood serum for the control group of people having cancer as the local maximum of function of distribution of H_2O molecules according to energy f(E) (eV^{-1}) in samples, which equals ~24,1 eV^{-1}.

In 1995 A. Antonov performed DNES-experiments with impact on tumor mice cells in water [41]. 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 (the control group). 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 *E* = –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 Rhodope Mountains areas. Among to the DNES-spectrum of mountain waters 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 waters from Teteven, Boyana and other Bulgarian provinces have similar physical-chemical 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 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) 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 < 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 5 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 E = –0,11 eV and E = –0,1387 eV. The value at E = -0,11 eV is characteristic for the presence of Ca^{2+} in water. The value at E = -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 in water solutions containing Ca^{2+} (0,05 g/l). 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 [42]. These results sugest the restructuring of energy values among H_2O 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 adsorbents effectively removing from water organo-chlorine compounds, phenols, dioxins, heavy metals, radionuclides, and color, and gives the water a good organoleptic qualities, additionally saturating it with micro-and macro-elements. 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 believed that water in these areas is cleared in a natural way by zeolite. Therefore, a new parameter is entered into Table 5 – a local maximum of energy at E = (-0,1362±0,1387 eV). This value is determined by the NES-spectrum as function of distribution of individual H_2O molecules according to energy f(E). The function of distribution of H_2O molecules according to energy f(E) (eV^{-1}) for tap water in Teteven is 11,8±0,6 eV^{-1}.

Table 5: 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/dm³ norm (<150)	mg/dm³ norm (<200)	mg/dm³ norm (<80)	mg/dm³ norm (<200)	mg/dm³ 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 in water samples according to energy f(E).

Heredity, stress, diet, smoking, body mass as additional longevity factors

The research showed that tobacco smoking increases the number of free radicals in the body [43]. The accumulation of free radicals leads to distortion of DNA replication. Evidently free radical-induced damage of DNA molecule plays an essential role in the process of aging. These data show that the average difference between the length of life of centenarians and their brothers is 10 of the 54 studied centenarians only 3 were long-time smokers (Table 6).

Table 6: Distribution of long living people and their sublings by gender

Number of centenarians	Health status	Body mass	Smoking	Gender	Heredity	Positive attitude towards life
54	In good health 48	Normal 54	Abstainers 51	Females 37	Parents and grandparents over 90 y.a. 18	54
0	With diseases 6	Above normal 0	Smokers 3	Males 17	No heredity 36	0

Table 7: Data for centenarians depending on their way of life

Number of centenarians	**Gender 20th and 21st century**	**Parents and grandparents over 20th and 21st century**
54	Females 37 Middle age 0	Females 15 Middle age 94,5
0	Male 17 Middle age 0	Male 13 Middle age 95,4

Table 7 shows an interesting trend, which, however, requires additional data for statistical analysis. In 2013 and 2014 the number of females was 69% and males – 21%. The number of parents and grandparents of long living people was 54% for females and 46% for males. The only two different factors were stress and probably smoking.

It is known that during the process of aging T-cell generation from the thymus is much reduced [44]. The decline rate of most T-cell and B-cell lymphocytes, which are crucial for the immune system, is faster in males than in the females. Furthermore, males showed a quicker decline in the two cytokines, IL-6 and IL-10 in relation to age. Two types of immune system cells, which annihilate external attackers, CD4 T-cells and natural killer (NK) cells are increased in number with age. The increase rate is higher in females than in males.

It should be noted that the process of aging can be limited if food caloricity of diet is being restricted on 40–55 %. In studies with 54 Bulgarian centenarians, all of them have had normal body mass throughout their lives; 48 of them were in excellent health condition, while 6 have various diseases. It is doubtful that these people would have reached longevity without being healthy. All of these studied people have had great physical activity. They live in friendly ecological environment in which the combination of mountain water, physical activity, diet and less stress are optimal for longevity. Further test has been created for the state of muscles, joints and tendons with prognostics for a longer life.

Conclusion

The experimental data 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. Other longevity factors are living area, health status, body mass, gender and heredity. Studying the human blood serum by IR, 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 according to energies it is possible to draw a vital state status of a person and the associated life expectancy. The IR-spectrum of the human blood serum of healthy group of people with a local maximum at λ = 8,95 μm is most similar to the IR-spectrum of the mountain water. The similar spectral characteristics possess mountain water from Teteven, Bojana and other Bulgarian sources. On the character of the IR-spectrum exerts an influence also the presence of deuterium. Thus, the phenomenon of longevity is a complex multi-factorial phenomenon involving both genetic (internal) and phenotypic (external) characteristics of the organism in its adaptation to the environment. 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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Многофакторное исследование феномена долголетия в горных и равнинных областях Болгарии

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Аннотация. В работе представлены данные о факторах долголетия и горной воде как главном факторе долголетия в горных и равнинных районах Болгарии. Авторами установлена зависимость между различными внутренними и внешними факторами на феномен долголетия болгарских долгожителей, в том числе качеством потребляемой воды, изотопным составом воды, места жительства, состоянии здоровья, пола и наследственности. Природные воды из различных болгарских родников, а также вода с пониженным содержанием дейтерия и образцы сыворотки крови больных раком были исследованы методами ИК-спектроскопии, неравновесного энергетического (НЭС) и дифференциального неравновесного энергетического (ДНЭС) спектрального анализа. В качестве основного оценочного фактора использовали значения средней энергии водородных связей ($\Delta E_{H...O}$) между молекулами H_2O, а также локальные максимумы в ИК и ДНЭС-спектрах различных образцов воды и сыворотки крови человека при $\Delta E_{H...O}$ = -0,1387 эВ (ДНЭС-метод) и λ = 8,95 мкм (ИК-метод). Повышенное содержание дейтерия в воде приводит к физиологическим, морфологическим и цитологическим изменениям в клетках, а также оказывает негативное влияние на клеточный метаболизм, а вода с пониженным содержанием дейтерия, вплоть до глубокого удаления дейтерия (60–100 ppm) благотворно влияет на здоровье. Показано, что для группы людей в критическом состоянии жизни и больных со злокачественными опухолями наибольшие значения локальных максимумов в ДНЭС-спектрах смещаются в сторону меньших энергий по отношению к контрольной группе.

Ключевые слова: дейтерий, тяжелая вода, бездейтериевая вода, долголетие, горная вода, ИК, НЭС, ДНЭС.