

Longevity Factors and Mountain Water of Bulgaria in Factorial Research of Longevity

Ignat Ignatov^{1*} Oleg Mosin² Borislav Velikov³

1. ScD, Professor, Scientific Research Center of Medical Biophysics (SRCMB), 32 N. Kopernik St., Sofia 1111, Bulgaria

2. PhD (Chemistry), Biotechnology Department, Moscow State University of Applied Biotechnology, 33 Talalikhina St., Moscow 109316, Russian Federation

3. PhD, Ass. Professor, Aquachim JSC, 83 Prof. Tzvetan Lazarov Blvd., Sofia, Bulgaria, St Ivan Rilski University of Mining and Geology

*E-mail of corresponding author: mbioph@dir.bg

Abstract

This paper submits data on longevity factors and mountain water in factorial research of the longevity phenomenon in mountainous and field areas in Lovech region and in municipalities of Teteven, Yablanitsa and Ugarchin, Bulgaria. A dependence was established among various internal and external factors on the phenomenon of longevity – residence area, water, air, health status, body mass, diet, stress, smoking, positive attitude toward life, physical activity, gender and heredity. The research was made with the author methodology of Ignatov. The participants of the project “NATURE, ECOLOGY, LONGEVITY” were divided of two groups. First grope was long-living people and the second one was their brothers and sisters. It is shown that mountain water is among the most important factors for longevity. Natural waters derived from various Bulgarian water springs were studied by the NES and DNES-method. As an estimation factor was researched the composition of water. Also were studied the values of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules, as well as the local maximums in the IR-spectra of various samples of water and human blood serum. For healthy people there is local maximum at -0.1387 eV and wavelength – 8.95 μm . For a group of people in critical condition of life and patients with malignant tumors the greatest values of local maximums in IR-spectra were shifted to lower energies relative to the control group with healthy people. The local maximum at -0.1387 eV and wavelength – 8.95 μm was detected in the IR-spectrum of mountain water. The report proves that water is one of the most important factors for longevity. The obtained results testify to the necessity of consumption of clean natural water whose qualities are satisfied the mountain water from Bulgarian water springs. The most favorable are the waters with a local maximum in 8.95 μm in the IR-spectrum, analogous to the same local maximum in the serum of a healthy person.

Keywords: mountain water, residence area, air, health status, body mass, diet, stress, smoking, positive attitude toward life, physical activity, gender and heredity.

1. Introduction

The question of longevity has always been an exciting one for humanity. Aging is a biological process, which leads to reduction of the vital functions of the body, limiting its adaptive capacities, and development of age-related pathologies and ultimately increasing the likelihood of death; all this 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 is given the longevity of immediate direct genetic ancestors. That is why the direct descendants of centenarians generally have the best chances for longevity. O. Burger demonstrates that life expectancy has increased substantially from the 19th to the 20th century and that this cannot be advantageously associated with the human genome (Burger *et al.*, 2012). The main factors of longevity are water quality, food and improved advancement of medicine. For example, in Bulgaria the average life expectancy from 1935 to 1939 was 51.75 years, while from 2008 to 2010 it was 73.60 years.

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

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

Water is the main substance of life. The human body is composed of 50 to 75% of water. As middle result human body contains water 48 ±6% for females and 58 ±8% for males (Watson, 1980). With aging, the percentage of water in the human body decreases. Hence, the factor of water quality is the essential factor for the research (Pocock *et al.*, 1981; Howard & Hopps, 1986). Water is present in the composition of the physiological fluids in the body and plays an important role as an inner environment in which the vital biochemical processes involving enzymes and nutrients take place. Water is the main factor for metabolic processes and aging. Earlier studies conducted by us have demonstrated the role of water, its structure, isotopic composition and physico-chemical (pH, temperature) in the growth and proliferation 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 the chemical structure of the H₂O 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 H₂O molecules, binding them into water associates (clusters) with the general formula (H₂O)_n where n varies from 3 to 60 units (Saykally, 2005; Ignatov, Mosin, 2013c). Another important indicator of water quality is its isotopic composition. Natural water consists of 99.7 mol.% of H₂¹⁶O, whose molecules are formed by ¹H and ¹⁶O 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 – HD¹⁶O, HD¹⁷O, HD¹⁸O, D₂¹⁶O, D₂¹⁷O, D₂¹⁸O, while 3 configuration are formed by isotopomers of oxygen – H₂¹⁶O, H₂¹⁷O, H₂¹⁸O.

This report studies the influence of various internal and external factors on the phenomenon of longevity – residence area, water, air, health status, body mass, diet, stress, smoking, positive attitude toward life, physical activity, gender and heredity. The research was carried out under the joint scientific project “NATURE, ECOLOGY, LONGEVITY” conducted in Bulgaria. Within the frames of this project 217 people living in the municipalities of Teteven, Yablanitsa and Ugarchin, Lovech district (Bulgaria), where the largest number of long-living people and their siblings have lived, 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 residence area, health status, body mass, smoking, positive attitude toward life, physical activity, gender and heredity.

2. Material and methods

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

For preparation of growth media we used D₂O (99.9 atom %) received from the Russian Research Centre "Isotope" (St. Petersburg, Russian Federation). Inorganic salts were preliminary crystallized in D₂O and dried in vacuum before using. D₂O distilled over KMnO₄ with the subsequent control of deuterium content in water by ¹H-NMR-spectroscopy on Bruker WM-250 device ("Bruker", Germany) (working frequency – 70 MHz, internal standard – Me₄Si) and on Bruker Vertex ("Bruker", Germany) IR spectrometer (a spectral range: average IR – 370–7800 cm⁻¹; visible – 2500–8000 cm⁻¹; the permission – 0,5 cm⁻¹; accuracy of wave number – 0,1 cm⁻¹ on 2000 cm⁻¹).

1% (v/v) solution of human blood serum was studied with the methods of IR-spectrometry, non-equilibrium (NES) and differential non-equilibrium (DNES) spectrum. The specimens were provided by Kalinka Naneva (Municipal Hospital, Bulgaria). Two groups of people between the ages of 50 to 70 were tested. The first group (control group) consisted of people in good clinical health. The second group included people in critical health or suffering from malignant diseases. The device for DNES was made from A. Antonov on an optical principle. In this study we used a hermetic camera for evaporation of water drops under stable temperature (+22–24 °C) conditions. The water drops are placed on a water-proof transparent pad, which consists of thin folio and a glass plate. The light is monochromatic with filter for yellow color with wavelength $\lambda = 580 \pm 7$ nm. The device measures the angle of evaporation of water drops from 72,3⁰ to 0⁰. The spectrum of hydrogen bonds among H₂O molecules was measured in the range of 0.08–0.1387 eV or $\lambda = 8.9$ –13.8 μ m using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H₂O molecules in human blood serum.

Interviews have been conducted with 217 Bulgarian long-living people and their siblings. Their water consumption, heredity, body weight, health status, tobacco consumption, physical activity, attitude towards life has been analyzed. With DNES method we performed a spectral analysis of 12 mountain water springs located in Teteven district (Bulgaria). The composition of the water samples was studied in the laboratory of "Eurotest Control" (Bulgaria). Statistics methods were attributed to the National Statistical Institute of Bulgaria. IR-spectra were registered on Bruker Vertex ("Bruker", 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); Non-equilibrium Spectrum (NES) and Differential Non-equilibrium Spectrum (DNES). Statistical processing of experimental data was performed using t-criterion of Student (at $p < 0.05$).

3. Results and discussions

3.1. Comparative analysis between longevity of long living people and their siblings. DNA replication and aging

54 long-living people over 90 years old have been studied together with their siblings. The average life span of long-living people is 92.1, and of their brothers and sisters it is 74.1. The difference in life expectancy of the two groups is reliable and it is $p < 0.05$, with Student's criteria at a confidence level of $t = 2.36$. There are 21 519 residents in Teteven and 142 of them have been born before 1924. Fig. 1 shows the

interrelation between the years of birth of long-living people (age) and their number, Teteven municipality, Bulgaria.

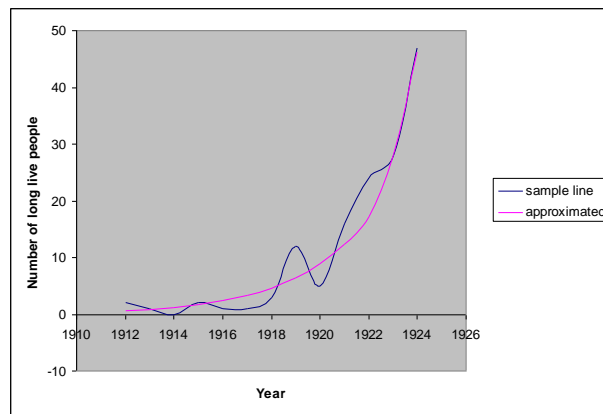


Fig. 1. Interrelation between the year of birth of long-living people (age) and their number, Teteven municipality, Bulgaria.

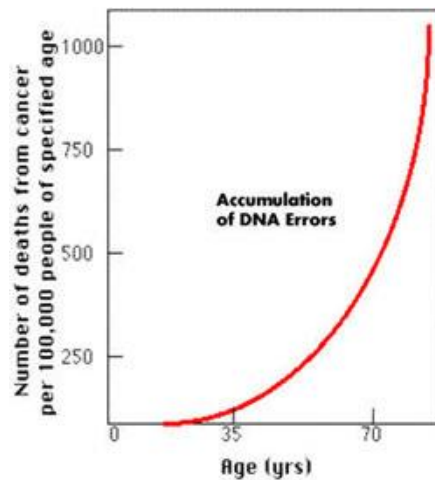


Fig. 2. Interrelation between age and number of cancer patients (Orgel, 1963)

The rate of aging increases with time. In 1963 Orgel showed that the aging process is associated with the synthesis of proteins. Fig. 2 shows Orgel’s results about the interrelation between age and number of cancer patients. The accumulation of errors in the synthesis of proteins increases exponentially with age. Cells taken from elderly people show reduced transcription or transmission of information from DNA to RNA. Therefore, the probability of cancer increases with age. The interrelation between the number of centenarians in the mountainous municipality of Teteven (Bulgaria) and their age is close to exponential.

3.2. Studying the mountain water sources of Teteven, Bulgaria

Table 1 shows the composition of the seven mountain springs in Teteven (Bulgaria). The water contains Ca^{2+} ions (40.44 – 113.6 mg/ dm³), Na^{+} ions (0.624 – 7.3 mg/ dm³), Mg^{2+} ions (2,1 – 15.99 mg/ dm³), Fe^{2+} ions (5.00 – 40.2 mg/ dm³) and SO_4^{2-} (15,9 – 142.4). The mountain sources from Zlatishko-Tetevenska Planina (Zlatishko-Tetevenska Mountain) are richer of ions than the mountain sources from Vasiliovska Planina (Vasiliovska Mountain). The mountain sources of Zlatishko-Tetevenska Planina (South part of

Teteven) are Klindiovo, Gorna cheshma, Dolna cheshma and Sonda. The mountain sources of Vasiliovska Planina are Vila Cherven, Gechovoto and Ignatov izvor. The pH of the sources is from 6.82 till 8.00.

Table 1. The composition of the seven mountain springs in Teteven (Bulgaria) and their pH values

Sources	Ca ²⁺	Na ⁺	Mg ²⁺	Fe ²⁺	SO ₄ ²⁻	pH
	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)
1. Klindiovo	89.9 ±9.0	4.1 ±0.4	6.98 ±0.7	40.2 ±4.0	17.7 ±1.8	8.0 ±0.1
2. Gorna cheshma	103.6 ±10.4	4.2 ±0.4	15.5 ±1.6	9.6 ±0.96	142.4 ±14.2	7.3 ±0.1
3. Dolna cheshma	94.4 ±0.94	2.5 ±0.3	12.10 ±1.21	9.0 ±0.9	15.99 ±1.60	7.9 ±0.1
4. Sonda	113.6 ±11.4	7.3 ±0.7	15.99 ±1.60	5.00 ±0.5	57.2 ±5.7	7.3 ±0.1
5. Vila Cherven	x	x	x	x	13.3 ±1.3	7.5 ±0.1
6. Gechovoto	66.0 ±6.0	1.46 ±0.15	2.1 ±0.2	11.4 ±1.1	15.9 ±1.6	7.94 ±0.1
7. Ignatov izvor	40.44 ±4.04	0.624 ±0.624	2.46 ±0.25	13.0 ±1.4	17.9 ±1.8	6.82 ±0.1

3.5. Heredity, stress, diet, tobacco smoking, body mass, air and longevity

The data show that the average difference between the length of life of long-living people and their brothers and sisters is 18 years. Long-living people and their brothers and sisters have common heredity. They were lived in different conditions and brothers and sisters were influence on different negative factors for longevity – smoking, stress, overweight, diseases, and low physical activity. 54 long-living people over 90 years old have been studied together with their brothers and sisters (163 people). The average life span of long-living people is 92.1, and of their siblings is 74.1. Table 2 shows the data for long-living people as way of life. The difference in life expectancy of the two groups is reliable and it is $p < 0.05$, with Student's criteria at a confidence level of $t = 2.36$. Scientific studies have shown that tobacco smoking increases the number of free radicals in the body (Pryor, 1997). The increase of free radicals leads to a deterioration of DNA replication. Adelman, Saul and Ames have shown in their research that free radical-induced DNA damage may play a central role in the aging process. Of the 54 analyzed long-living people, only 3 have smoked for several years. All interviewed centenarians have had normal body mass throughout their lives. In overweight people, the water content decreases to 45% (Guyton, 1976). Reducing the water as percentage content affects body metabolism. According to experimental data, aging can be limited if food calories are reduced by 40–55%. It has been proven on rats that obese rats live longer than slimmer ones

(Weindruch, 1986).

Table 2. Data for long-living people depending on their way of life

Number of long-living people	Health status	Body mass	Physical activity	Smoking	Gender	Heredity	Positive attitude towards life
54	In good health 48	Normal 54	54	Abstainers 51	Female 37	Parents and grandparents over 90 18	54
	With diseases 6	Above normal 0		Smokers 3	Male 17	No heredity 36	

Table 3. Distribution of long living people by gender. Distribution of parents and grandparents of based on gender

Number of centenarians	Gender 20 th and 21 st century	Parents and grandparents over 90 20 th and 21 st century
54	Female 37 Middle age 96.4	Female 15 Middle age 94,5
	Male 17 Middle age 91.8	Male 13 Middle age 95,4

Table. 4. Results of long-living people and their brothers and sisters with regard to smokers, people suffering of malignant tumors, strokes and heart attacks

Number of long-living people	Number of brothers and sisters of long-living people	Percentage of long-living people / brothers and sisters of long-living people (%)
57	163	
Smokers 3	Smokers 24	5.6/14.7
Abstainers 54	Abstainers 139	
Cancer 1	Cancer 12	1.9/7.4
Heart attacks and strokes 1	Heart attacks and strokes 6	1.9/3.7

Table 3 shows an interesting trend, but it requires additional data for statistical analysis. In 2013 and 2014 for long-living people the percentage of females was 69% and of males 21%. The percentage of parents and grandparents of long-living people is 54% for females and 46% for males. The two differentiating factors are stress and probably smoking.

Table 4 shows that the percentage proportion of long-living people smokers and their brothers who smoke is 5.6/14.7%. Among the surveyed 163 participants in the project, only males were smokers. Accordingly, the proportion of people suffering from malignant tumors is 1.9/7.4%. The ratio of people who have had a heart attack or stroke is 1.9/3.7%. These results influence the age difference between the two groups as statistically reliable at a level of $p < 0.05$ of Student criteria.

It is known that during the process of aging T-cell generation from the thymus is much reduced (Tsukamoto *et al.*, 2009). 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 for females than for males. In our study with 54 long-living people, all of them have had normal body weight throughout their lives. 48 of them are in excellent clinical health, and 6 have diseases. It is doubtful that these people would have reached longevity without being healthy. All have performed regular physical activities. They live in an ecological environment in which the combination of mountain water and air, physical activity, diet, and limited stress are optimal for longevity. A test has been created for the state of muscles, joints and tendons with prognostics for a longer life (De Brito, 2012). In the mountain areas of the municipalities Teteven, Yablanitsa and Ugarchin (Bulgaria) the air has more oxygen and negative ionization. Near the sea the air also has negative ionization. Also the basic food of the people, who are living near the sea, is fish. Sea food is assumed to have positive effect on cholesterol level – a negative factor for cardiovascular diseases. This is one of explanations for the number of centenarians of sea areas.

3.3. Empirical evidence on life duration; impact of mountain water

Statistical evidence shows that long-living people inhabit mainly high mountain areas. In the Russian North there are more centenarians compared to other parts of Russia. 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 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 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 Teteven, Yablanitsa and Ugarchin municipalities the oldest inhabitant in field areas is 94 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 main differentiating factors are mountain air and water.

Data for Bulgaria:

Varna district – centenarians 44 per 1 million, plain and sea

Pleven district – centenarians 78 per 1 million, plain

Teteven district – centenarians 279 per 1 million, hills and mountain

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 mountain water obtained after the melting of ice. Altai and Buryat water sources are known as moderately warm, with temperatures of 10–15 °C, the water is generally ice-free in winter. This phenomenon is explained by the fact that the melt water contains a low percentage of deuterium compared with ordinary tap water, that is believed to have a positive effect on the tissue cells and metabolism. Melt water in Russia is considered to be a good folk remedy for increasing physical activity of the human body, enhancing the vitality of the organism and has a beneficial effect on metabolism (Goncharuk *et al.*, 2013).

Analyses of water across the planet show that mountain water contains the smallest amounts of deuterium atoms in water molecules. In winter and early spring deuterium content is reduced. This water also contains Ca^{2+} , Mg^{2+} , Na^+ , Fe^{2+} , Fe^{3+} and SO_4^{2-} . Mountain water in springtime is the result of the melting of ice and snow from the mountains. Natural ice modification I_h (hexagonal lattice) is much cleaner than the water. The growing crystal of ice always strives to create the perfect crystal lattice and removes impurities. Melted water has a markedly ice-like structure and the clusters of water molecules are better preserved in it due to the presence not only of hydrogen, but also of covalent links. Each cluster exists for a short time and there is a continuous destruction of water clusters and the formation of new ones. Water analyses of different mountain springs from Bulgaria and Russia indicate that mountain water contains 3–5% lesser amount of deuterium atoms as HDO compared to river water. In natural waters the deuterium content is uneven: from 0,02–0,03 mol.% for river and sea water, to 0.015 mol.% for Antarctic ice water. Deuterium is a heavier isotope of hydrogen. The concentration of deuterium atoms is less than 1% in the water of human body. The study of the water spectrum in the human body can also answer the question of longevity. Some authors consider the hardness of water as a factor in cardiovascular disease. A mild correlation has been proven, but we do not consider water hardness to be decisive for human longevity.

3.4. Clinical evidence with blood serum testing

In 1983 Antonov and Yuskesselieva discovered a new physical effect. They showed experimentally that in the process of evaporation of water drops, the wetting angle θ decreases discreetly to 0, and the diameter of the drop base is only slightly altered. Based on this, through measurement of the wetting angle at equal

intervals of time, the function of distribution according to value of last $f(\theta)$ is determined. The function is called spectrum of the state of water. A theoretical research of Luzar, Svetina and Zeksh has established a relation between the surface tension of water and the energy of hydrogen bonds among the water molecules.

With the use of formula (1) and the condition for a mechanical equilibrium of the drop at the time of evaporation the following expression is found:

$$f(E) = b \times f(\theta) / (1 - (1 + bE)^2)^{1/2} \quad (1)$$

$$b = 14,33 \text{ eV}^{-1}$$

It gives the connection between the energy spectrum of water $f(E)$ and state spectrum $f(\theta)$. The relation between the wetting angle θ and the energy of the bond among water molecules E is represented in the following way:

$$\theta = \arcsin(-1-14,33E) \quad (2)$$

The energy is measured in electronvolts (eV). Because the energy spectrum of water is received as a result of non-equilibrium process of evaporation of water drops, the term non-equilibrium energy of water is used (NES). NES can help determine the average value of bond energy among the water molecules.

$$\text{The difference: } \Delta f(E) = f(\text{sample}) - f(\text{control sample}) \quad (3)$$

is called differential non-equilibrium energy of water (DNES).

Fig. 3 shows the average spectrum of deionised water. On the X-axis there are few scales. The energies of hydrogen bonds among the water molecules are calculated in eV. The function of distribution according to energies $f(E)$ in unit eV^{-1} is written on the Y-axis. For DNES the function is $\Delta f(E)$ in unit eV^{-1} .

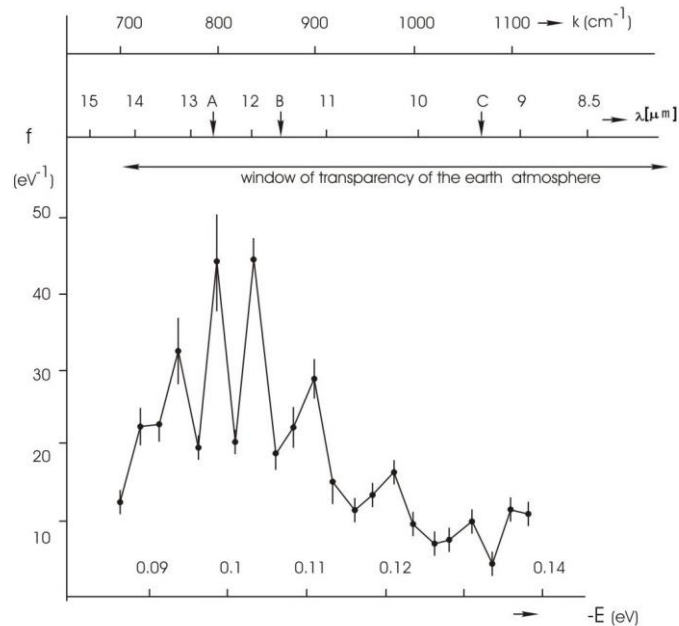


Fig. 3. NES-spectrum of deionized water (chemical purity 99.99%, pH = 6.5–7.5, total mineralization 200 mg/l, electric conductivity 10 $\mu\text{S}/\text{cm}$). The horizontal axis shows the energy of the H...O hydrogen bonds in the associates – E (eV). The vertical axis – energy distribution function – f (eV^{-1}). k – the vibration frequency of the H–O–H atoms (cm^{-1}); λ – wavelength (mm)

Arrow A designates the energy of hydrogen bond among the water molecules, which is accepted as most reliable in spectroscopy. Arrow B designates the energy of hydrogen bonds among the water molecules according to formula (1) through which the value is determined:

$$\bar{E} = -0,1067 \pm 0,0011 \text{ eV (4)}$$

Arrow C designates the energy at which the thermal radiation of the human body, considered like an absolute black body (ABB) with a temperature $36,6^{\circ}\text{C}$, is at its maximum. A horizontal arrow designates the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle infrared range of the Sun toward the Earth and from our planet toward the surrounding cosmic space. It is seen that the atmosphere window of transparency almost covers the energy spectrum of water.

Studies by Ignatov (2012) were performed on a 1% solution of blood serum with the method of spectral analysis of a Non-equilibrium energy spectrum (NES) and Differential non-equilibrium energy spectrum (DNES). Empirical blood serum samples were provided by Kalinka Naneva Municipal Hospital, Teteven, Bulgaria. The samples were divided into 2 groups of people between 50 and 70 years of age. The first group consisted of people in excellent health. The second group consisted of people in a critical state and suffering from malignant tumors. The average energy of hydrogen bonds among water molecules in the blood serum was examined as a biophysical parameter. The result was obtained as a difference between the spectrum of 1% solution of blood serum (NES) and a control sample with deionized water (NES). This spectrum is DNES. The first group obtained the result $-9.1 \pm 1,1 \text{ meV}$, and the second $-1.6 \pm 1,1 \text{ meV}$. There is a statistical difference between the two groups of results according to the t-criterion of Student at level up $<0,05$. For the control group of healthy people the value of the spectrum of the largest local maximum is at -0.1387 eV or at a wavelength of $8.95 \mu\text{m}$. For the group of people in a critical state and the patients with malignant tumors, the values of the spectrum of the largest local maximums shift to lower energies compared with the control group. A study of blood serum was performed by the method of infrared spectral analysis (Krasnov, Gordetsov, 2009). The following local maximums in the spectrum of absorption were received: $8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35$ and $9.76 \mu\text{m}$. The resulting local maximum at $8.95 \mu\text{m}$ (Ignatov, 2012) is close to the one obtained by at $8.85 \mu\text{m}$. (Dou, X. et al. 1996; Krasnov, Gordetsov, 2009) In the control group of healthy people, the function of distribution according to energy $f(E)$ at $8.95 \mu\text{m}$ has an average value of 75.3 reciprocal $\text{eV} (\text{eV}^{-1})$. In the group of people in critical condition this value is 24.1 reciprocal eV . The confidence level of the obtained results is $p < 0.05$ by the t-criterion of Student. The resulting local maximum at $8.62 \mu\text{m}$ of heavy water is close to the one obtained by Russian scientists at $8.58 \mu\text{m}$.

In 1992 Antonov performed experiments with impact on tumor cells of a mouse in water. There was a decrease of the spectrum compared with the control sample of cells from a healthy mouse. Decrease was also observed in the spectrum of the blood serum of terminally ill people to that of healthy people. With the age increase of long-living blood relatives, the function of distribution according to energies at -0.1387 eV decreases. With this group a result was obtained at DNES $-5.5 \pm 1,1 \text{ meV}$ at an age difference of 20-25 years compared with the control group.

Most of the long-living people in Bulgaria inhabit Bulgarian Mountains. Some of the mountain waters from Bulgarian Mountains have a spectrum similar to the spectrum of the blood serum of healthy people in $8.95 \mu\text{m}$. The local maximums have been pointed out at -0.11 and -0.1387 eV . The value at -0.11 eV is indicator of the presence of Ca^{2+} ions. The value at -0.1387 eV is characteristic of inhibiting the development of cancer cells. Experiments conducted by Antonov with cancer tissue of mice demonstrated a reduction of this local maximum from positive to a negative value in DNES spectrum. The sample is with cancer tissue of mice in deionized water (first NES spectrum) and control sample is the same deionized water (second NES spectrum). The difference DNES is from first and second NES spectrum. There is positive value in NES spectrum for tissue of healthy mice. There is a new parameter in the Table 5. This is the local maximum at $(-0.1362 - -0.1387 \text{ eV})$ (Ignatov, Mosin, 2012). This value is in the spectrum NES as function of distribution according to energy $f(E)$. The norm has a statistically reliable result for blood serum for the control group with people with cancer and the local maximum of $f(E)$ at $(-0.1362 - -0.1387)$ is 24.1 eV^{-1} . The norm has a statistically reliable result for blood serum for the control group with people with cancer and the local maximum of $f(E)$ at -0.11 is 24.1 eV^{-1} . The function of distribution according to energy $f(E)$

for Teteven tap water at (-0.1362 – -0.1387) eV is 23.8 ± 1.2 and at -0.11 eV is 11.8 ± 0.6 eV⁻¹.

Table. 5. Local maximums in DNES-spectra of water from different sources

Sources	Local maximum* at (-0.1362 – -0.1387) eV	Local maximum* at -0.11 eV
	eV ⁻¹ norm (>24.1)	eV ⁻¹ norm (>12.5)
1. Klindiovo	47.1 ±2.4	23.2 ±1.2
2. Gorna cheshma	20.0 ±1.0	26.7 ±1.3
3. Dolna cheshma	31.6 ±1.6	24.3 ±1.2
4. Sonda	48.8 ±2.4	29.3 ±1.5
5. Vila Cherven	44.4 ±2.2	1.4
6. Gechovoto	44.4 ±2.2	17.1 ±0.9
7. Ignatov izvor	31.6 ±1.6	10.4 ±0.5

*Notes: f(E) is function of distribution according to energy; function of distribution according to energy f(E) for tap water Teteven at (-0.1362 – -0.1387 eV) is 23.8 ± 1.2 eV⁻¹ and at -0.11 eV is 11.7 ± 0.5 eV⁻¹.

4. Modifications of ice and mountain water

14 crystalline modifications of ice are known today, each has its own structure and a character of disposition of hydrogen atoms (Table 6). Natural ice is represented by the hexagonal I_h configuration. Crystals of all ice modifications are made up of H₂O molecules, linked by hydrogen bonds into a 3D carcass, consisting of individual tetrahedrons, formed by four H₂O molecules. In the crystalline structure of natural ice I_h hydrogen bonds are oriented towards the tetrahedron apexes at strictly defined angles equal to 109°5' (in liquid water this angle is 104°5') (Fig. 4). In ice structures I_c, VII and VIII this tetrahedron is nearly identical to 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 undercrossing 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 remaining two vertices of the tetrahedron are occupied by two pairs of non-shared electrons of O-atom, not participating in the formation of molecular bonds. The carcasses of hydrogen bonds allocate H₂O molecules in the 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: H₂O 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 (anomaly of temperature, pressure, density, viscosity, fluidity, etc). According to theoretical calculations, ice melting breaks about 15% of all hydrogen bonds (Mosin & Ignatov, 2011); by further

heating to 40 °C breaks down about half of hydrogen bonds in H₂O associates. In water vapor hydrogen bonds are absent.

Table 6. Ice crystal modifications and their physical characteristics*

Modification	Crystal structure	Hydrogen bond lengths, Å	Angles H–O–H in tetragonals, °
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
XII	Tetragonal	4.01	90
XIII	Monoclinic	7.47	90–109.7
XIV	rhombic	4.08	90

*Notes:

I_h – natural hexagonal ice; I_c – cubic ice.

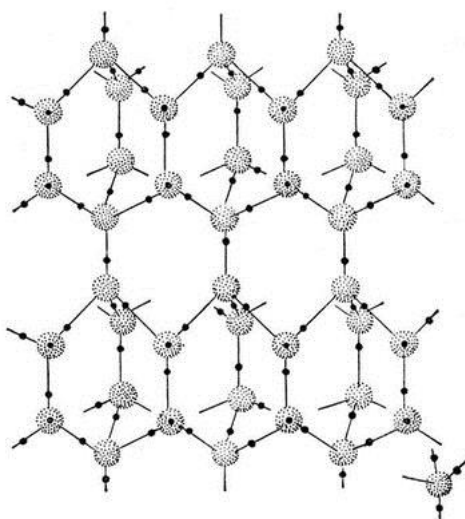


Fig. 4. Hydrogen bonding in crystalline structure of natural ice I_h under the temperature of -10 °C and normal pressure at 1 atm. Grey balls – O-atoms, black balls – H-atoms. To the right below is shown the

structural unit of crystalline ice structure.

Mountain water in springtime is the result of the melting of ice and snow accumulated in the mountains. Natural ice with I_h modification (hexagonal lattice) is usually much cleaner than water, because solubility of all substances (except NH_4F) in ice is extremely low. The growing ice crystal is always striving to create a perfect crystal lattice and therefore displaces impurities. Meltwater has a certain “ice-like” structure, because it preserves the hydrogen bonding among water molecules; as a result it forms complex intermolecular associates (clusters) – analogues of ice structures, consisting of a larger or smaller number of H_2O molecules (Fig. 5). However, unlike ice crystal, each associate has a very short time of existence; as a result there occur the constant processes of decay and formation of water associates having very complicated structure (Ignatov & Mosin, 2013e). The specificity of intermolecular interactions characteristic for the structure of ice, is kept in meltwater, as it is estimated that in the melting of ice crystals, only 15% of all hydrogen bonds in the associates are destroyed. Therefore, the ice inherent connection of each H_2O molecule with four neighboring H_2O molecules is largely disturbed, although there is observed the substantial “blurring” of oxygen lattice framework. Processes of decay and formation of clusters occur with equal probability that is probably why physical properties of meltwater 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). Further heating of fresh meltwater above $+37\text{ }^\circ\text{C}$ leads to a loss of its biological activity. Storage of meltwater at $+22\text{ }^\circ\text{C}$ 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 a 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 hold 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 meltwater varies from 4.4 at $1.5\text{ }^\circ\text{C}$ to 4.9 at $80\text{ }^\circ\text{C}$.

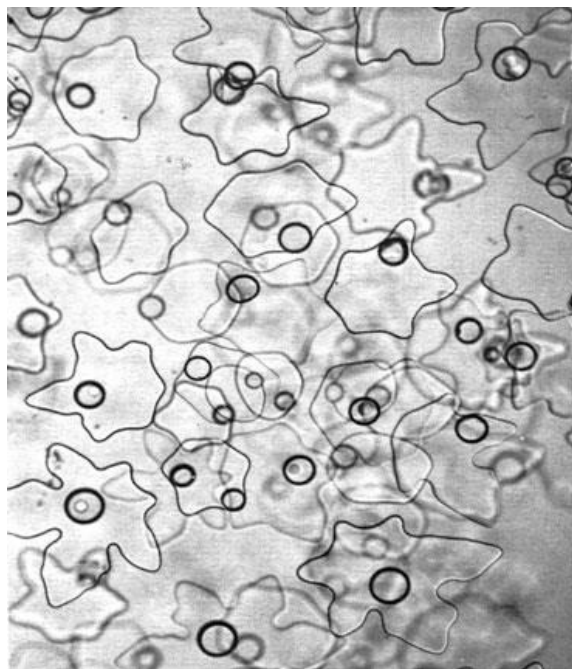


Fig. 5. Structure of meltwater according to computer simulations with “smearing” fragments of regular ice structures.

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 °C (Table 2). The degree of natural purification of melt water from impurities makes up ~50–60%. The concentration of salts of rigidity – Ca^{2+} , Mg^{2+} , Fe^{2+} , Fe^{3+} , heavy metals and organochlorine compounds, as well as heavy isotopes, including deuterium in melt water is less than 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. 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 .

Table 7. Composition of melt water

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

Analyses of water from various sources of Russia and Bulgaria show that mountain water contains on average ~2–4% less deuterium in HDO form, than river water and sea water. In natural waters the deuterium content is distributed irregularly: from 0.02–0.03 mol.% for river and sea water, to 0.015 mol.% for Antarctic ice water – the natural water most purified from deuterium containing deuterium in 1.5 times less than that of seawater. According to the international SMOW standard isotopic shifts for D and ^{18}O in sea water: $\text{D}/\text{H} = (155.76 \pm 0.05) \cdot 10^{-6}$ (155.76 ppm) and $^{18}\text{O}/^{16}\text{O} = (2005.20 \pm 0.45) \cdot 10^{-6}$ (2005 ppm) (Lis *et al.*, 2008). For SLAP standard isotopic shifts for D and ^{18}O in seawater: $\text{D}/\text{H} = 89 \cdot 10^{-6}$ (89 ppm) and for a pair of $^{18}\text{O}/^{16}\text{O} = 1894 \cdot 10^{-6}$ (1894 ppm). In surface waters, the ratio $\text{D}/\text{H} = \sim(1.32\text{--}1.51) \cdot 10^{-4}$, while in the coastal seawater – $\sim(1.55\text{--}1.56) \cdot 10^{-4}$. Waters of other underground and surface water sources contain varied amounts of deuterium (isotopic shifts) – from $\delta = +5,0$ D,%, SMOW (Mediterranean Sea) to $\delta = -105$ D,%, SMOW (Volga River). The natural waters of CIS countries are characterized by negative deviations from SMOW standard to $(1.0\text{--}1.5) \cdot 10^{-5}$, in some places up to $(6.0\text{--}6.7) \cdot 10^{-5}$, but there are observed positive deviations at $2.0 \cdot 10^{-5}$. 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.%).

5. Clinical evidence on the benefits of deuterium depleted water for health.

When biological objects are 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 bonds in the range $k_H/k_D = 5-7$ for C-H versus C-D, N-H versus N-D, and O-H versus O-D-bonds (Mosin, 1996). Our previous studies have shown that heavy water of high concentration is toxic for the organism, chemical reactions are slower in D_2O , compared with ordinary water, and the hydrogen bonds formed with participation of deuterium are somewhat stronger than those formed from hydrogen (Mosin *et al.*, 1996). In mixtures of D_2O with H_2O with high speed there occur 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 the 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 $+101.44$ °C, freezes at $+3.82$ °C, has density of 1.1053 g/cm³ at 20 °C, and the maximum density occurs not at $+4$ °C as in H_2O , but at $+11.2$ °C (1.1060 g/cm³). These effects are reflected in the chemical bond energy, kinetics, and the rate of chemical reactions in D_2O .

The chemical reactions and biochemical processes in the presence of D_2O are somehow slower compared to H_2O . D_2O is less ionized, the dissociation constant of D_2O is smaller, and the solubility of the organic and inorganic substances in D_2O is smaller compared to these ones in H_2O (Mosin, 1996). However, there are also such reactions in which rates in D_2O are higher than in H_2O . In general these reactions are catalyzed by D_3O^+ or H_3O^+ ions or OD^- and OH^- ions. According to the theory of a chemical bond, the breaking up of covalent C-H bonds can occur faster than C-D bonds, the mobility of D_3O^+ ion is lower on 28,5 % than H_3O^+ ion, and OD^- ion is lower on 39,8 % than OH^- ion, the constant of ionization of D_2O is less than that of H_2O (Lobishev & Kalinichenko, 1978). The substitution of H with D affects the stability and geometry of hydrogen bonds in an apparently rather complex way and may, through the alterations in the hydrogen bond zero-point vibration energies, alter the conformational dynamics of hydrogen (deuterium)-bonded structures of DNA and proteins in D_2O (Cleland, 1976). It may cause disturbances in the DNA-synthesis, leading to permanent changes on DNA structure and consequently on cell genotype (Thomson, 1960; Katz, 1960; Den'ko, 1970; Török *et al.*, 2000). Our experiments demonstrated that the effects of deuterium on the cell possess a complex multifactor type connected to alterations 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 malfunction, violation of hormonal regulation and 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 2–3 times larger in size in D_2O (Mosin & Ignatov, 2012a). High concentrations of D_2O suppress enzymatic reactions, cell growth, mitosis and synthesis of nucleic acids (Lamprecht *et al.*, 1989; Cioni & Strambini, 2002; Vertes, 2003). Thus, 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). The last fact allows considering adaptation to D_2O as adaptation to the nonspecific factor affecting simultaneously the functional condition of several numbers of cellular systems: metabolism, ways of assimilation of carbon substrates, biosynthetic processes, and transport function, structure and functions of macromolecules. Evidently cells are able to regulate the D/H ratios, while its changes trigger distinct molecular processes. One possibility to modify intracellular D/H ratios is the activation of the H^+ -transport system, which preferentially eliminates H^+ , resulting in increased D/H ratios within cells (Somlyai *et al.*, 2012).

We have obtained results on growth and adaptation to D₂O 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% D₂O, plants – up to 50–60% D₂O, and protozoa cells are able to live on ~90% D₂O. Further increase in the concentration of D₂O 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 the 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 strengthens 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). 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 it 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 pO₂ was ~15%, i.e., cell respiration increased 1.3 times (Kolesov & Pomytkin, 2006). A beneficial effect on the health of experimental mice was evidenced by 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). The total effects of deuterium depleted water depend 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). 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 the change of the isotopic composition of urine and Ca²⁺ content it was recorded as well. Daily consumption of deuterium depleted water allows to 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. The described effects occur at higher concentrations of heavy water and at a 25% lower concentration of deuterium in the deuterium depleted water. Estimates show that at a body weight of 70 kg, in a concentration of water from 50 to 75% or 62.5% on average, the human body contains 44 liters of water. Deuterium content in the human body is some 30 g. With the use of 2 liters of water per day, with a natural water content of 0.015 to 0.03 %,

With the use of mountain water, this contains 3-5% less deuterium atoms. this number is 28.5 g (this figure is also not correct; from 3% decrease we have 0,009 g of deuterium, while from 5% decrease we have 0,03 g of deuterium, therefore, the average decrease will be 0,0195 g, or $0,3 - 0,0195 = 0,2805$ g, the difference gives the physiological levels of deuterium). A large part of the deuterium is excreted with urine. Even at low concentrations it may cause significant fluctuations in cellular systems. Varnavski performs an independent experiment with *Drosophila melanogaster*. There were 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). According to the two authors the following relationship is valid. With the increasing of age, the percentage of water in the body decreases from 75 to 50%. Deuterium atoms are difficult to separate from the water in the human body. With the years, deuterium atoms accumulate. Thus, even in small doses they affect the human body metabolism (Mosin, Ignatov, 2014). 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 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. 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 not involved in formation of hydrogen bonds are usually given the narrow bands in IR spectrum and the associated group - 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. Comparison of IR spectra of water solutions and its deuterated analogues (D₂O, HDO) is of considerable interest 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. The IR spectrum of the water molecule is examined in detail from the microwave till the middle (4-17500 cm⁻¹) visible region and the ultraviolet region - from 2000 Å to ionization limit at 980 Å. 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 (2000 to 980 Å) 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 spectrum - from 5700 to 2000 Å corresponds to transitions to higher vibrational levels of the ground electronic state). The results are shown in Fig. 6. The result is reliable regarding the content of deuterium in natural waters from 0.015-0.03%. With measurement of deuterium content it was measured the percentage of water in human body (IAEA, 1996).

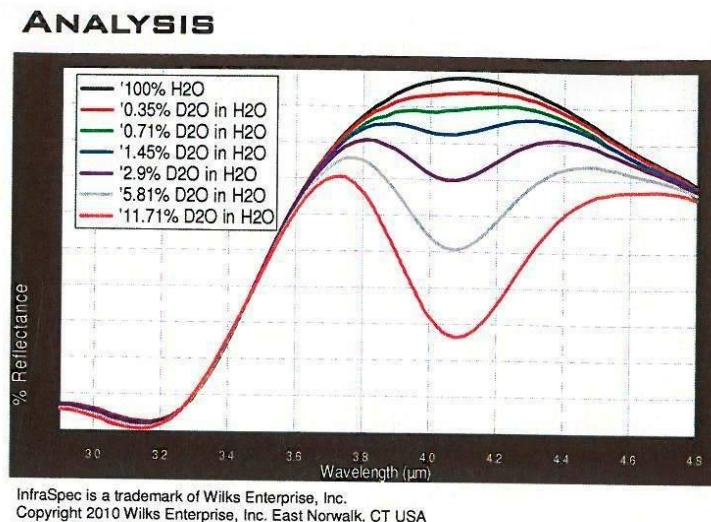


Fig. 6. IR-spectra of water with varying deuterium content

5. Conclusion

Our research shows that the composition of water and its spectrum influence the vital processes occurring in the human cells and can cause premature aging. The composition of the water, which we are drinking, and deuterium atoms affect the spectrum and hence the vital functions (Ignatov, Mosin, 2012). In our

opinion, the direct relationship of man with nature – in particular the consumption of pure mountain water, accompanied by regular physical activity and a positive attitude towards life – can explain the difference between the larger number of centenarians who live in the mountain areas and the average number. Additional beneficial factors for longevity such as clean air with increased oxygen content and vitalized food from eco-farms will need further research. In this report we show some evidence that mountain water is one of the most important factors for longevity. We examine the possibility to delay the aging process by delaying errors in transcription and replication of DNA in the process of protein synthesis depending on the water we drink. The most favorable are the waters with a local maximum in 8.95 μm in the infrared spectrum, analogous to the same local maximum in the serum of healthy persons. There are results with the following factors for longevity: health status, body mass, smoking habit, gender, heredity, nervous tension (stress), and a positive attitude towards life. The project will be continued with research in the field area (Pleven region), sea area (Varna region) and in a second mountain area (Smolyan region) in Bulgaria. We need additional data for parents and grandparents of long-living people. Also we will make additional analyses of different types of water. Additional statistical analysis for all factors will be material to draw final conclusions.

Acknowledgements

The authors express their gratitude to Assen Toshev for statistical analyses and structuring of the figures. The authors also thank Ass. Borislav Velikov (Aquachim) and assistants of the project NATURE, ECOLOGY, LONGEVITY - Lieselotte Eder, Horst Elzenbeck, Mandy Protze-Käberer, Zohra and Manfred Dome, Paul Kleindienst, Helmut Gebert, Petra Schmidt, Enrico Bauer, Pascal Boesinger for supporting of the research.

References

- Adelman, R., Saul, R. & Ames, B. (1988) Oxidative damage to DNA: relation to species metabolic rate and life span, *PNAS*, 85 (8): 2706-2708.
- Andreeva, E.L., Konstantinova, N.A., Burovkova, L.B. & Sinyak, Y.E. (2005) Effect of different isotopic composition of water on the proliferative activity of endothelial cells *in vitro*. *Aerospace and Environmental Medicine*, 39(3): 46-52.
- Antonov, A., et al. (1995) Mountain Observatory on Musalla OM2, *Bulgarian Academy of Science*, Sofia: 39.
- Avila, D.S., Somlyai, G., Somlyai, I. & Ascher, M. (2012) Anti-aging effects of deuterium depletion on Mn-induced toxicity in a *C. elegans* model. *Toxicol. Letters*, 211(3): 319-324. doi: 10.1016/j.toxlet.2012.04.014.
- Badin, V.I., Gasteva, G.N., Drobyshevskiy, Y.V. *et al.* (2004) Study the behavior of water with negative deuterium isotope shift in the body of calves. *Proceedings of the Academy of Industrial Ecology*, 3: 73-78 [in Russian].
- Berdishev, G. G. (1989) Reality and illusion of immortality longevity: *Politizdat*: 1-89. [in Russian].
- Burdeynaya, T.N. & Chernopyatko, A.S. (2012) Physiological effects of drinking water enriched with ^1H and ^{16}O . In: *2nd International Congress on Deuterium Depletion*. Budapest, Hungary, 17-18 May, 2012.
- Burger, O., Baudisch, A. & Vaupel, J. W. (2012) The Retardation of Aging in Mice by Dietary Restriction: Longevity, Cancer, Human mortality improvement in evolutionary context, *PNAS*, 109 (109) 44: 18210-18214.
- Cioni, P. & Strambini, G.B. (2002) Effect of heavy water on protein flexibility. *Biophysical J.*, 82(6): 3246–3253.
- Giguere, P. & Harvey, P. (1956) On the infrared absorption of water and heavy water in considered states, *Canadian Journal of Chemistry*: 34(6): 798-808.
- Goncharuk V.V., Kavitskaya A.A., Romanyukina I.Y. & Loboda, A (2013) Revealing water's secrets: deuterium depleted water. *Chemistry Central Journal*, 7(1):103.
- Groth, H. (2012) Megatrend “Global Demographic Change” Tackling Business and Society Challenges in

- 2030 and beyond, WDA Forum, *University of St. Galen*: 1-31.
- De Brito et al. (2012) Ability to sit and rise from the floor as a predictor of all-cause mortality, *European journal of preventative cardiology*.
- Den'ko, E.I. (1970) Influence of heavy water (D₂O) on animal, plant and microorganism's cells. *Usp. Sovrem. Biol.*, 70(4): 41-49 [in Russian].
- Dou, X., Yamaguchi, X., Yamamoto, H., Uenoyama, H., Ozaki, Y. (1996) Biological Applications of Anti-Stokes Raman Spectroscopy: Quantitative Analysis of Glucose in Plasma and Serum by a Highly Sensitive Multichannel Raman Spectrometer, *Applied Spectroscopy*, 50 (10): 1301.
- Guyton, A. (1976) Textbook of Medical Physiology (5th ed.). Philadelphia: W.B. Saunders. 1-424
- Howard, C. & Hopps (1986) Chemical qualities of water that contribute to human health in a positive way, *Science of the total environment*, 54: 207-216.
- Ignatov, I. (2005) *Energy Biomedicine*, Gea-Libris, Sofia, 1-88.
- Ignatov, I. (2010) Which Water is Optimal for the Origin (Generation) of Life? *Euromedica*, Hanover: 34-37.
- Ignatov, I. (2011) Entropy and time in living matter, *Euromedica*: 74.
- Ignatov, I., Mosin, O. V. & Naneva, K. (2012) Water in the Human Body is Information Bearer about Longevity, *Euromedica*, Hanover: 110-111.
- Ignatov, I. & Mosin, O. V (2012) Isotopic Composition of Water and its Temperature in Modeling of Primordial Hydrosphere Experiments, VIII Int. Conference Perspectives of the Development of Science and Technique, *Biochemistry and Biophysics*, 15: 41-49.
- Ignatov I. (2012) Conference on the Physics, Chemistry and Biology of Water, Water in the Human Body is Information Bearer about Longevity, NY: *Vermont Photonics*.
- Ignatov I., Mosin O.V. (2013) Possible Processes for Origin of Life and Living Matter with modeling of Physiological Processes of Bacterium *Bacillus Subtilis* in Heavy Water as Model System, *Journal of Natural Sciences Research*, 3 (9): 65-76.
- Ignatov, I., Mosin, O. V. (2013) Modeling of Possible Processes for Origin of Life and Living Matter in Hot Mineral and Seawater with Deuterium, *Journal of Environment and Earth Science*, 3(14): 103-118.
- Ignatov, I., Mosin, O. V. (2013) Structural Mathematical Models Describing Water Clusters, *Journal of Mathematical Theory and Modeling*, 3 (11): 72-87.
- Ignatov, I., Mosin, O. V. (2013) Isotopic Composition of Water and Longevity, *Naukovedenie*, 14(1):2-10.
- Ignatov, I., Mosin, O. V. (2014) The Structure and Composition of Carbonaceous Fullerene Containing Mineral Shungite and Microporous Crystalline Aluminosilicate Mineral Zeolite. Mathematical Model of Interaction of Shungite and Zeolite with Water Molecules *Advances in Physics Theories and Applications*, 28: 10-21.
- Ignatov, I., Mosin, O.V., Velikov, B., Bauer, E. & Tyminski, G. (2014) Longevity Factors and Mountain Water as a Factor. Research in Mountain and Field Areas in Bulgaria, *Civil and Environmental Research*, 6 (4): 51-60.
- Ignatov, I., Mosin, O. V., Niggli, H.&Drossinakis, Ch. (2014) Evaluating Possible Methods and Approaches for Registering of Electromagnetic Waves Emitted from the Human Body, *Advances in Physics Theories and Applications*, 30: 15-33.
- Introduction to Body Composition Assessment Using the Deuterium Dilution Technique with Analysis of Saliva Samples by Fourier Transform Infrared Spectroscopy, *Int. Atomic Energy Agency (IAEA)*, 12: 1-96.
- Katz, J.J. (1960) The biology of heavy water. *Scientific American*: 106-115.
- Katz, J.J., Crespi, H.L., Czajka, D.M. & Finkel, A.G. (1962) Course of deuteration and some physiological effects of deuterium in mice. *American Journal of Physiology*, 203: 907-913.
- Kolesov, O.E. & Pomytkin, I.A. (2006) Influence of natural concentration of heavy water isotopologues on the rate of ²H₂O generation by mitochondria. *Bulletin of Experimental Biology and Medicine*, 11: 514-516.
- Krasnov, V. V. & Gordetsov, A. S. (2009) Infrared spectral analysis of blood serum as level of disturbances of metabolic processes in infusion children pathology: 83-94.
- Lamprecht, I. Schroeter, D. & Paweletz, N. (1989) Disorganization of mitosis in HeLa cells by deuterium oxide, *European journal of cell biology*, 50 (2): 360-369.
- Lobishev, V.N. & Kalinichenko, L.P. (1978) Isotopic effects of D₂O in biological systems. Moscow, *Nauka*: 215 [in Russian].

- Mann, D. (2002) Negative Ions Create Positive Vibes, *WebMD Feature*, Article/65/72756.
- Marque, S., Jacqmin-Gadda, H., Dartigues, J. F. & Commenges D (2003) Cardiovascular mortality and calcium and magnesium in drinking water: an ecological study in elderly people, *Eur. J. Epidemiol.* 18 (4): 305–9.
- Mosin O.V. & Ignatov I. (2012) Isotope effects of deuterium in bacterial and microalgae cells at growth on heavy water (D₂O). *Water: Chemistry and Ecology*, 3, 83–94 [in Russian].
- Mosin, O. V, Ignatov, I. (2012) Studying of Isotopic Effects of Heavy Water in Biological Systems on Example of Prokaryotic and Eukaryotic Cells, *Biomedicine*. 1 (1-3): 31-50.
- Mosin O.V. & Ignatov I. (2013) Microbiological synthesis of ²H-labeled phenylalanine, alanine, valine, and leucine/isoleucine with different degrees of deuterium enrichment by the Gram-positive facultative methylotrophic bacterium *Brevibacterium methylicum*. *International Journal of Biomedicine*, 3(2), 132–138.
- Mosin, O.V., Shvets, V.I., Skladnev, D.A., & Ignatov, I. (2012) Studying of microbic synthesis of deuterium labeled L-phenylalanine by methylotrophic bacterium *Brevibacterium Methylicum* on media with different content of heavy water, *Russian Journal of Biopharmaceuticals*, 4 (1): 11–22 [in Russian].
- Mosin, O. V.& I. Ignatov, I. (2013b) The Structure and Composition of Natural Carbonaceous Fullerene Containing Mineral Shungite, *International Journal of Advanced Scientific and Technical Research*, 3, 6(11-12): 9-21
- Mosin, O. V., Ignatov, I., Skladnev, D. A & Shvets, V. I. (2013c) Use of Gram-positive Chemoheterotrophic Bacterium *Bacillus Subtilis* B-3157 with HNP-cycle of Carbon Assimilation for Microbiological Synthesis of [2H] riboxine with High Level of Deuterium Enrichment, *European Journal of Molecular Biotechnology*, 2: 63-78.
- Mosin, O. V., Shvets, V. I, Skladnev, D. A. & Ignatov, I. (2013d) Microbiological Synthesis of [2H]-inosine with High Degree of Isotopic Enrichment by Gram-positive Chemoheterotrophic Bacterium *Bacillus Subtilis*, *Applied Biochemistry and Microbiology*, 49 (3): 233-243.
- Mosin, O.V. & Ignatov, I (2014) Biosynthesis of photochrome transmembrane protein bacteriorhodopsin of *Halobacterium halobium* labeled with deuterium at aromatic amino acids residues of 2,3,4,5,6-²H₅]Phe, [3,5-²H₂]Tyr and [2,4,5,6,7 -²H₅]Trp. *Chemistry and Materials Research*, 6(3): 38-48.
- Mosin, O. V., Shvets, V. I, Skladnev, D. A. & Ignatov, I. (2013f) Microbial Synthesis of ²H-labelled L-phenylalanine with Different Levels in Isotopic Enrichment by a Facultative Methylotrophic *Brevibacterium Methylicum* with RuMP Assimilation of Carbon, *Supplement Series B: Biomedical Chemistry*, 7 (3): 247-258.
- Mosin, O.V. & Ignatov, I. (2013a) Studying of the biosynthesis of ²H-labeled inosine by a Gram-positive chemoheterotrophic bacterium *Bacillus subtilis* B-3157 on heavy water (²H₂O) medium. *Chemical and Process Engineering Research*, 15: 32–45.
- Mosin, O.V., Shvets, V.I., Skladnev, D.I. & Ignatov, I (2013) Microbial synthesis of ²H-labelled L-phenylalanine with different levels of isotopic enrichment by a facultative methylotrophic bacterium *Brevibacterium methylicum* with RuMP assimilation of carbon. *Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry*, 7(3): 249-260
- Orgel L. (1963) The Maintenance of the Accuracy of Protein Synthesis and Its Relevance to Aging, *Biochemistry*, 49: 517–521.
- Panayotova, M., Velikov, B. (2002) Kinetics of Heavy Metal Ions Removal by Use of Natural Zeolite, *Journal of Environmental Science and Health*, 37(2): 139-147.
- Pocock, S.J, Shaper, A.G & Packham R.F. (1981) Studies of water quality and cardiovascular disease in the United Kingdom, *Sci. Total Environ.* 18: 25–34.
- Pryor, W. (1997) Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity, *Environ Health Perspect.*, (105) 4: 875–882.
- Pulyavsky, A.G. (1986) Kinetics of exchange and the biological effect of heavy water (D₂O). *Hygiene and sanitation*, 3: 63-66 [in Russian].
- Rubenowitz, E., Axelsson, G., Rylander, R. (1999) Magnesium and calcium in drinking water and death from acute myocardial infarction in women, *Epidemiology*, 10 (1): 31–6. *irect*, 317 (1-2): 1-4.
- Saykally, R. et al. (2005) Unified Description of Temperature-Dependent Hydrogen Bond Rearrangements in Liquid Water, *PNAS*, 102(40): 14171–14174.

- Sergeeva, N.S., Sviridov, I.S. & Timakov, A.A. (2003) Study the effect of water with a low content of deuterium on the growth of transplantable cultures of human tumor cells in experiments *in vitro*. In: *Materials of interdisciplinary conference with international participation "New Biocybernetic and telemedicine technologies of XXI century"*, Petrozavodsk (Russia), 23-25 June 2003, 39 [in Russian].
- Sinyak, Y., Grigoriev, A., Gaydadimov, V., Gurieva, T., Levinskih, M. & Pokrovskii, B. (2003) Deuterium-free water in complex life-support systems of long-term space missions. *Acta Astronautica*, 52: 575.
- Somlyai, G., Jancso, G., Jakli, G *et al.* (2012) Deuterium depletion from tissue culture to human clinical studies. In: *2nd International Congress on Deuterium Depletion*. Budapest, Hungary.
- Stom, D.I., Ponomareva, A.K., Vyatchina, O.F. (2006) Influence of water with varying content of deuterium on red Californian hybride (*Eusenia fetida Andrei Bouche*). *Bull. RAS*, 6(52): 167–169 [in Russian].
- Thomson, J.F. (1960) Physiological effects of D₂O in mammals. Deuterium isotope effects in chemistry and biology. *Annals of the New York Academy of Sciences*, 84: 736-744.
- Török, G., Csik, M., Pintér, A. *et al.* (2000) Effects of different deuterium concentrations of the media on the bacterial growth and mutagenesis. *Egészségtudomány/Health Science*, 44: 331-338.
- Tsukamoto, H. *et al.* (2009) Age-associated increase in lifespan of naïve CD4 T cells contributes to T-cell homeostasis but facilitates development of functional defects, 106(43): 18333–18338.
- Varnavskiy, I.N., Berdyshev, G.D. & Prilipenko V.D. (2002) Healing relict water - the discovery of the third millennium. *Questions of chemistry and chemical technology*, 5: 168-174 [in Russian].
- Vertes A. (2003) *Physiological effect of heavy water. Elements and isotopes: formation, transformation, distribution*. Dordrecht: Kluwer Acad. Publ.:111–112.
- Watson, P. E. *et al.* (1980) Total body water volumes for adult males and females estimated from simple anthropometric measurements, *The American Journal for Clinical Nutrition* (33) 1: 27-39.
- Weindruch, R. (1986) The Retardation of Aging in Mice by Dietary Restriction: Longevity, Cancer, Immunity and Lifetime Energy Intake, *Journal Nutrition*, Vol. 116(4): 641–54.
- Woodhead, R. (1984) *Molecular Biology of Aging*. NY: Basic Life Science, Vol. 35: 34–37.