

# Biogenic Chemical Elements Isotope Ratios in Living Organism as a New Potential Indicator of Physiological State

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## Abstract

This article considers theoretical questions regarding the role of intramolecular isotopes ratios in living organism functioning, human body in particular. Special attention is given to isotopes fractioning of carbon in human body as the most studied. The aim of this investigation is to identify and to study strategic connection between features of metabolic transformations in human body, its functional status and intramolecular isotopes ratios, peculiar to this organism. The relation between intramolecular isotopes ratios values of human body separate tissues and human organism functional status is observed.

**Keywords:** Organogenic element; Intramolecular isotopes ratios; Fractioning of isotopes; Pyruvate; Metabolism

## Introduction

It is well known that all living organisms on the Earth consist of identified set of chemical elements that is genetically strictly controlled and handed down from generation to generation in the same ratios, i.e., the chemical composition of any substance is an important systemic characteristic [1]. However, two questions have been raised:

- whether the transfer is absolutely constant from generation to generation, or it changes in the process of geochemical evolution of the Earth's surface, but at a rate that is less apprehensible to human;
- Whether a chemical element location in the Mendeleev's Periodic Table corresponds to its role in the living material.

As of today, it has been proved that the ratio of biogenic elements isotopes is significantly different from natural concentrations and inhibits the metabolic processes until death. The literature tells about of therapeutic effect of monoisotopic (or close to monoisotopic) substances on the human body. As for the light monoisotopic substances there is a mention about their impact on biological systems. Regarding the impact of natural isotopes concentrations on the living organisms' activity, including human beings, this point is still debating. The impact of daily biological rhythms on the human exhalation isotopic composition and the impact of pathologies of various etiologies on carbon isotopes composition in the blood can be mentioned [2-7]. Almost all the systems of the living body contain constant and natural radioactive isotopes, the nucleuses of which are different according to the number of criteria (weight, magnetic moment, quadrupole moment, spin). Fractioning of isotopes in chemical and biochemical reaction, induced by isotopic effects, is based on two fundamental properties of nuclear atoms - the weight and magnetic moment. Mass-dependent isotopic effects divide isotopes nucleuses according to their weight; magnetic isotopic effect fractionates nucleuses as for their magnetic moments [8]. Systematic study of isotopic composition of nature-occurring compounds allowed to show in general terms the isotopic biochemical behaviour pattern and as a result it was found that the natural distribution of isotopes between biomolecules and inside them is peculiar to living bodies [9]. In fact, the above postulates that, at first, isotopes biologic fractioning is characterized not only by isotopes displacement between various isotopes of the body and environment, but also by deep differentiation of isotopic composition

between biochemical fractions, individual compounds that form part of fractions, and even inside of biomolecules. Secondly, lack of systematic differences of isotopic composition between higher and lower plants, between autotrophs and heterotrophs, or, in another words, lack of dependence of isotopic composition on supracellular system shows that those fractioning of biological isotops is on cellular level. Complicated processes of substance's transport and intercellular metabolism that correspond to higher organisms are of minor importance for fractioning of biological isotopes. Thus, numerous researchers made attempts to investigate the reasons of fractioning of biological isotopes in physical-chemical processes accompanying biosynthesis of organic compounds [9]. Fractioning of isotopes is a result of isotopes physical-chemical inequality and it may be reflected on the chemical reaction rate and energy state and system magnetics as well. Isotopic ratios of biogenic chemical elements are compound part of many processes, thus it could be considered as potential indicators of the organism functional state.

## Chemical Composition of Living Organisms

Chemical composition of modern organisms has been formed under two processes: on the one side it is composition of atmosphere, hydrosphere, lithosphere and biosphere; on the other side it is vital for the organism concentration of already existing elements ratio inside it. Bodies are able to absorb and accumulate some elements from environment. First of all it refers to the light chemical elements since the content of chemical elements in the bodies with element atomic weight increasing is decreased most of all. However, the presence of some chemical elements (even at the rate of some atoms per cell) has great impact on the intercellular metabolism processes. From these positions the attempts to create classification of so-termed "vital" chemical

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elements, the lack or overplus of which inhibits body's life activity, have been made. According to different researchers the list of chemical elements referring to the "vital" ones varies within rather extensive boundaries. However, the majority of authors consider 20 elements as constantly repeated in "vital" column. The following elements P, S, Na, K, Mg, Ca, Cl, Fe, Cu, Zn, Mn, Cr, Se, Mo, I, Co are added to the four organogenic elements (O, C, H, N) [10]. Nevertheless, the question what chemical elements identified in living material are really vital and what get into body accidentally by means of food, water and air (and are not very harmful) is still debating.

## Stable isotopes

The scope of isotopes created by nature and use by people refers to both organic and inorganic world. The isotopes ratio in living and non-living bodies reflects their previous history, i.e., according to Buchachenko "isotopes are storage media for information about the birth and transformations of molecules, and fractioning of isotopes is the chemical history. Isotopes have two memory functions. First, they take part in memory creation, storing it (through isotopic effects in the acts of birth and transformation of molecules). Second, isotopes are the heirs and keepers of memory as witnesses of chemical events (both current and ancient which took place millions of years ago). The above has been proved by numerous Earth sciences using isotopes measurements as methods for studying of essential and unaddressed issues." [8]. One can say, that stable isotopes could be used as isotopes indicators in two cases:

1. their use as "outer label" if getting into body with food, water, air or medicines that lets to describe physiological state, predict geographical region of origin and food intake by living bodies and other;
2. While identifying of so called "inner label", i.e., while using body's own isotopic ratios; "Inner label" is often used for identifying and explanation of the processes in living bodies, human in particular. The aim of "inner label" use is to study the consequence of biochemical transformations, to identify competing reaction and to measure rate of transition products formation.

## Natural isotopic effects

For successful use of isotopes it is important to understand the peculiarities and differences of isotopes and the same compounds of various isotopic compositions as well. It is known that physical and physical-chemical features of substances in case of isotopic composition change are the bigger as number of chemical element is lower (under the same difference in atomic numbers of elements).

As a result of differences in physical-chemical properties of isotopes molecules, their physical transformations are usually accompanied by fractioning of isotopes, thus, their distribution between two fractions of the substance with different isotopes ratios [11-14]. Or to put it another way, fractioning of isotopes is in that the content of one of the isotopes in the given compound is increased at the expense of decreasing of its content in another one, therefore fractioning of isotopes is a result of physical-chemical inequality that could influence the processes rate, energy state and system nuclear magnetism. The process of fractioning of isotopes (their distribution between two fractions) in non-living material is rather studied issue as of today. In order to get comprehensive explanation of identified natural isotopic effects (Table 1), the authors deem it advisable to analyse the main isotopic effects that induce fractioning of isotopes in biological systems. Thus, describing isotopic elements, the authors pointed out only elements presence in the Biosystems. As the Table 1 shows, from the kind I isotopic effects (or mass-dependant effects) in Biosystems, the phase and chemical (kinetic and thermodynamic) isotopic effects exhibit their influence. Isotopic effects of the II kind or mass-independent effects were identified in radical and ion-radical chemical reactions. Since some biochemical processes in living bodies run due to ion-radical mechanism, the presence of isotopic effects of the II kind could be expected.

**Phase isotopic effect:** The main contribution in formation of atmospheric precipitation isotopic composition and correspondingly of the continents surface waters is made by fractioning of isotopes with steam condensation; fractioning during vapour has less impact. It should be noted that correlation dependence between  $\delta D$  and  $\delta^{18}O$ , that is the same for iced water, is usually observed for atmospheric precipitation and continents surface waters. The process runs under conditions close to equilibrium ones and leads to ice enrichment of D and  $^{18}O$ . Phase effect could be expected in living bodies in "expired air - blood" system on the vapour-liquid boundary in lungs. Such prediction has been confirmed by series of experiments on investigation of hydrogen isotopic composition: expired moisture by human, atmospheric moisture, biological liquids, human metabolism tissues and products [15].

**Chemical isotopic effect:** Consists of thermodynamic and kinetic effects. Thermodynamic isotopic effect is based on tendency of isotopes molecules system to energy minimum. In fact, quantum-statistical calculations of thermodynamic states of system consisting of two atoms show that such system energy is changed during isotopes re-arrangement in it and is directed towards minimum. Kinetic isotopic effect (KIE) is based on deference of isotopes molecules reactions absolute rates. In a

Physical features. Differences providing isotopic effect (I.e.)	Isotopic effect		
	Type	Kind	Presence in Biosystems
Mass. I. e. of the 1 kind	Inertial	Gravitational	Not identified
		Diffusive	Identified in photosynthesizing plants
		Electro-magnetic (isotope ions distribution in electric and magnetic field)	Effect availability needs further investigations
	Corpuscular-chemical	Phase	Identified by the authors in "expired air - blood" system
		Absorption	No evidences
	Chemical	Thermodynamic	Intramolecular isotopic effect is identified. Presence of intramolecular isotopic effect is disputable issue
		Kinetic	Well-studied for photosynthesizing plants
		Photochemical	No evidences
		Tunnel	No evidences
	Spin, magnetic moment. I. e. of the II kind	Chemo nuclear	Nuclear-spin (magnetic)

Table 1: Isotopic effects classification.

number of physical-chemical processes in nonequilibrium conditions the fractioning of isotopes could be at the expense of kinetic factors. Share of heavy molecules with rate conditioning chemical reaction execution is less than share of light molecules that is why the most of light molecules will take part in reaction. Thus, chemical kinetic effect could cause fractioning regularity violation in isotopes triads. For hydrogen isotopes (tritium, deuterium and protium) the difference in rates of reactions with isotopes molecules is caused by difference of molecules mass and activation energy change. If two molecules are heavy the isotope composition does not have much effect on their mass. Thereof, hydrogen isotopes should be synchronic during chemical isotopic effects and conceptual difference in protium, deuterium and tritium behaviour in Biosystems cannot be explained by presence of isotopic chemical effect.

**Diffusive isotopic effect:** In case of hydrogen photosynthetic fixation by higher plants, seaweeds and autotrophic bacteria, one of the processes in charge of hydrogen functioning is CO<sub>2</sub> diffusion inside and outside plants tissue.

In a similar way to hydrogen photosynthetic fixation, one can expect impact of diffusion on light elements isotope composition and in intercellular and intracellular processes including in heterotrophic organisms. The above effect has kinetic character and should not change direction of processes for deuterium and tritium.

**Nuclear-spin (magnetic) isotopic effect (MIE):** Some chemical reactions, radical and ion-radical in particular, are connected to the change of total electron spin of reactive system or to the change of spin multiplicity, for example, to its transition from triplet to singlet state. Energy characterizing this transition depends on interaction of electrons spins and nucleus. As a result, the possibility of chemical reactions depends on nuclear spin presence and the rate of its interaction with electron spin - so called hyperfine interaction. Since in common case one element isotopes can be different due to nuclear spin size, reaction rate for isotopic compounds can be different that provides for isotopic effect presence. As distinct from KIE the MIE size depends on magnetic field, temperature, molecular and chemical dynamics and reagent spin state. MIE causes fractioning of magnetic and nonmagnetic isotopes in chemical, biochemical, geochemical and space processes. MIE mechanism can be implemented in chemical radical reactions. As is known, electrons in molecules are paired and electrons spins are compensated, i.e., total electronic spin is equal to zero. Such state is called singlet (S). When molecule decays into radicals the electrons disconnection takes place and further radicals recombination can be hindered by spins compensation necessity. Therefore, MIE and isotope fractioning depends mainly on magnetic, electron-nuclear energy, on the size of outer magnetic field, on partners' rotational and translational diffusion, on viscosity and volume closeness, on radicals' lifetime. Mass-dependent effect and MIE may exist for H/D pair and they are even commensurable. However, for heavier nucleus, starting from hydrogen, MIE is a sequence higher than mass-dependent isotopic effect. In classical (mass-dependent) isotopy all the principles are acceptable in a view of nuclear mass. There are chemically more active molecules with light nucleus in it. In magnetic isotopy, as a rule, molecules with magnetic nucleus are more active.

### Data as for fractioning of stable isotopes of biogenic elements in human body as a new kind of information about its physiological state

It is generally believed that human health is determined by external causes and environmental conditions through the all periods of its

ontogenetic development in particular. Reception of external causes is performed with the help of appropriate information formation in the form of changes in composition of compounds involved in metabolism process. There are two clear chemical information sources in human body - macromolecules (nucleic acid and proteins) and macromolecules (amino acid, lipids, sugars). They are well-studied and form the basis for contemporary investigations in biochemistry, molecular biology, chemical biology and recently in genomics, proteomics and bioinformatics. The relations between them are well-defined and in the most cases it is not difficult to identify the source of particular information part. The majority of biochemical issues are relevant to macro- and micro molecules and total metabolism has great impact on qualitative and quantitative characteristics of chemical information of these sources [16,17]. It's common knowledge that properties of genome state, having organism development complete programme, play and will play a significant part in diseases diagnostics. However, in the genome structure there is no indicated environmental factors impact on the body in which genetic information processes are implemented by means of complicated signalling system. For modern biology, the possibility of characterising the way by means of which environment interferes with genetic information, resulting in a series of phenotypic modifications, is still unaddressed issue. Investigations of proteome, that is a collection of synthesized at a time proteins, can help with identification of recent events in the environment and their impact on human health. It could be said that proteome is a short-term reflexion of physiological state and there is, probably, a short time for metabolically active proteins. Thus, neither genome nor proteome provide for long-term recording of body physiological status. That is why, researches of new sources of information that would reflect different environmental conditions impact on human body during the whole lifetime, have always been timely and actual. Apart from the above two sources of chemical information there are reasons to assume the existence of the third very essential one. This is isotopic ratios of biogenic elements that belong to micro- and macro molecules and have a lot of common characteristics. Metabolism processes impact on these ratios is still uninvestigated, although Vernadskiy mentioned their importance for life activity of living beings: "...in all cases, medical and veterinary, should be a question - what impact do calcium, magnesium, zinc salts, etc. have on the organism. Is the action of those made of ordinary elements and of elements that moved through the body the same?" [18]. Intramolecular isotope ratios have information (memory) that was put in molecule when it "was born", i.e., this is memory about chemical evolution of a substance as about collection of a great number of chemical reactions. Owing to this memory and isotope anomalies one can reconstruct the ways of chemical evolution and trace substances origin in nature [8,17]. Isotopic ratios of biogenic elements are components of many biochemical processes in the body, and then they are potential indicators of its functional state. Thus in 1969 Degens demonstrated that the difference in fractioning of isotopes in diverse Biosystems can be explained by metabolic processes. Adaptation to unfavourable conditions is accompanied by inner resources mobilization that can modulate biological fractioning of isotopes. It was shown that:

- such changes can be used as integrated index characterizing state of biochemical processes in the body;
- Isotopic intermolecular distributions can be sensitive to any deviations of biosynthesis from standard.

Nevertheless, such deviations in natural conditions cannot be explained by isotopic effects only. The phenomenon of biological

fractioning of isotopes was studied enough for H, C, O, N, Mg, Si, Se, Ca, Fe, Cu, Zn, Sc etc. Obtained results of numerous investigations have proved by Vernadskiy's anticipation [18] that living bodies can selectively use specific isotopes. In particular, Vernadskiy made an assumption that different isotopes of chemical elements can influence biota in a different ways: "... I made sure that there is a possibility of life phenomena influence on isotopic mix composition i.e., on change of chemical elements atomic weight within life process due to the organism peculiarity to choose between isotopes and change isotopic mix composition" [14]. This is an assumption, but assumption that is based on powerful observational generalization that is resulted from great number of specified facts of geochemistry. Such generalization is a statement about atoms abilities demonstration within life processes and not only their aggregations. "Life in chemical aspect is so extended phenomenon that its investigation makes us to have a new attitude to the greatest statements, on which our understanding of nature is based, and the atom and spatium in particular" [18]. Nevertheless, the most of issues in this sphere are still unaddressed.

### Intramolecular fractioning of organogenic elements

Among total amount of available evidences on biological fractioning of biogenic isotopes, the most frequent are the works on studying of intramolecular fractioning of organogenic elements (C, H, N, O), due to their majority in all Earth's living bodies composition. Such occurrence is connected with their ability to create covalent links by electron pairing and to react with each other filling their outermost shells. Apart from this, they are the lightest among elements that are able to create covalent links. Since the strength of covalent link is inversely proportional to the atomic weights of bound with its help atoms, they are structural elements of all biogenic molecules. Each stereo chemical unique position of C, H, N and O in all molecular entities has isotope ratio reflecting chemical and physical processes of molecules anabolism and catabolism as well as information about element. Thus, while investigation of 21 amino acids coded by triplet it was demonstrated that there are 104 chemical unique positions of C, 10 positions of N and 72 positions of H (taking into account inner ones  $-CH_2-$  both symmetric and those that have identical isotope ratio). In living body physically different compartments (for instance, plasma and organs) can contain amino acids with different isotope composition. Proteins, synthesized in particular organs but present in the same physical pool (plasma, for example) can also perform compartments with different intramolecular isotope ratios pointing at their origin [17]. Since 2000, the Institute of Environmental Geochemistry NAS of Ukraine jointly with the Institute of Gerontology and Research Centre for Radiation Medicine, AMS of Ukraine had started research on the study and identification of ratios of the inherent (internal) isotopes of certain nutrients (such as carbon, magnesium, hydrogen, iron) in human body tissues. The ultimate goal of our research is to identify and study the relationship between ratios of inherent (internal) stable isotopes of these elements in human tissues and its functional state [17,19-22].

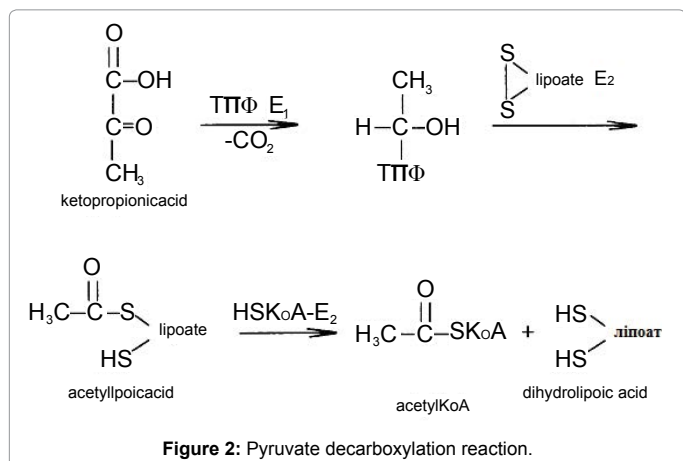
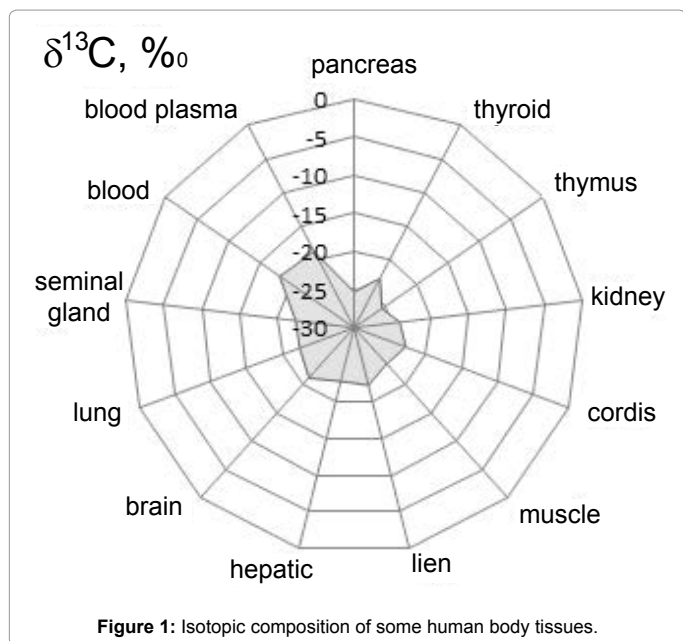
**Fractioning of carbon isotopes in biological systems of living organisms:** First intramolecular inhomogeneity of carbon in biological systems was found by Abelson and Hoering in 1961 [23]. Studying the isotopic composition of *Chlorella*, *Euglena* amino acids and others they found some patterns in the distribution of isotopes of carbon, namely that in most amino acids the carbon of carboxyl group is enriched by  $^{13}C$  relatively to the carbon of decarboxylated residue. As for heterotrophs, for a long period of time it has been thought that they fully inherit food isotopic composition and there is no fractioning of isotopes in their organism. However, lipids and most proteins of

mammals are generated in their body and are not derived from the food. Further investigations demonstrated that isotope composition of different heterotrophs tissues has different composition that is pointed at isotope fractioning with metabolism. Investigations made by De Niro and Epstein [24], as well as Ivlev et al. [3,4,25,26] were the most significant and informational for explanation of intramolecular fractioning of isotopes of carbon processes in heterotrophs organisms. Based on the undertaken studies, they made a conclusion: in every moment the cell is in particular functional state that corresponds to the given level of its energetic and biosynthetic needs that are controlled by regulatory connections system. Within certain limits this level can vary and change the ways of metabolic transformations and competition for pyruvate fund resulted in change of pyruvate fund parts ratios that are used for cell energy and fusion of needed metabolite. As a result - there are appropriate isotope variations. In 1977 De Niro and Epstein demonstrated the main isotope impact on pyruvate transformation into acetic aldehyde in yeast with the help of pyruvate dehydrogenase that later leads to the changes of  $^{13}C/^{12}C$  in yeast fatty acids [27]. A year later Lyon and Baxter [28] presented initial data as for different carbon isotope composition in different tissues of human body. The work results indicate that venous blood is best enriched in  $^{13}C$ , and thymus is the poorest (the difference is about 7%). The authors came to a conclusion that carbon isotopes ratios in tissues are not constant. They presented the first comprehensive data set on carbon isotope composition of the human body tissues (Figure 1). It may be said that different tissues of human body are characterized by heterogeneous carbon isotope ratios: blood is the mostly enriched in  $^{13}C$ , while thymus is the poorest (the difference is about 7%). The bone (carbonate) is enriched in  $^{13}C$  by approximately 10‰ in comparison to soft tissues. Hence it follows that isotopes ratio in this or that tissue can be named "isotope map". However, it should be stressed that these isotope ratios can display variability within different time scale. This variability can be referred to biorhythms, rate of endogenous and exogenous processes in the organism and environmental state. Different tissues are characterized by different dynamics of isotope ratios due to the differing metabolic rate. Having analysed head hair and nails samples as for content of  $\delta D$ ,  $\delta^{13}C$ ,  $\delta^{15}N$  i  $\delta^{18}O$  Fraser, etc. [29] every two weeks during eight months period in 2006 relatively slight fluctuations of  $\delta^{13}C$  and  $\delta^{15}N$  in hair ( $20.59 \pm 0.59\%$  and  $9.90 \pm 0.71\%$  correspondingly) and nails ( $21.14 \pm 0.56\%$  and  $10.06 \pm 1.04\%$ , correspondingly) were identified. Wide fluctuations in content of  $\delta D$  and  $\delta^{18}O$  were identified while hair investigation ( $66.2 \pm 4.1\%$  and  $14.7 \pm 1.7\%$  correspondingly) and nails ( $60.7 \pm 7.6\%$  and  $13.1 \pm 1, 5\%$  correspondingly).

Back in 2001 O'Connell et al. [30] revealed that:

- bone collagen is enriched on 1.4‰ ( $\delta^{13}C$ ) and 0.86‰ ( $\delta^{15}N$ ) compared with hair keratin;
- there is no significant difference between  $\delta^{13}C$  content in hair keratin and nails;
- nail keratin is enriched in  $\delta^{15}N$  in comparison to hair keratin on 0.65‰.

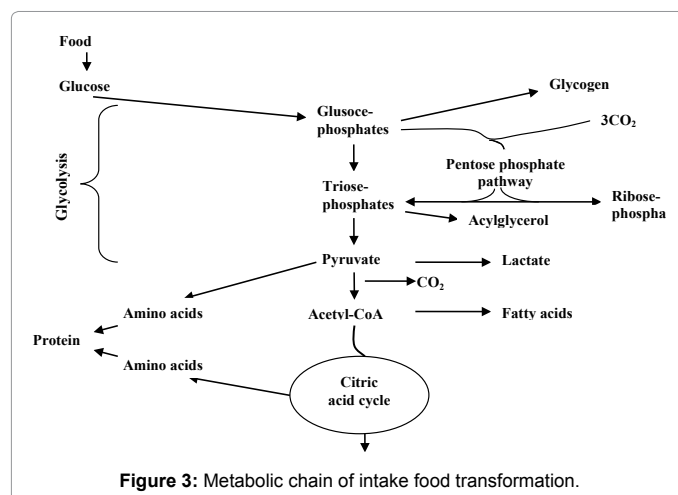
Thus, the assumption was made that differences in  $\delta^{13}C$  content can be determined by differences in hair keratin and bone collagen amino acid composition. Carbon isotope fractioning in living bodies is determined by intracellular processes and by pyruvate decarboxylation reaction (Figure 2). The character of this fractioning depends on human dietary regime since almost all intake carbohydrates are transformed into glucose the main part of which decomposes up to ketopropionic acid by means of glycolysis and generates so called pyruvate fund (Figure 3). Fractionating of carbon isotopes occurs exactly at the stage



of pyruvate fund parts division on the body's vital needs. The part of pyruvate fund fermentatively decarboxylates resulting in generation of acetyl-CoA and CO<sub>2</sub>. In this process the lighter part of pyruvate fund is lost where C<sub>2</sub> and C<sub>3</sub> atoms do not contain heavy isotope. This is due to the fact that energy of heavy isotopes bond exceeds energy of light isotopes bond and correspondingly decarboxylation reaction is faster for light molecules of ketopropionic acid. Freed during the reaction carbon dioxide contributes to the intake CO<sub>2</sub>. Acetyl-CoA can enter citric acid cycle (Krebs cycle). Another part of pyruvate fund enriched in <sup>13</sup>C (as a result of substrate fund exhausting), is spent on biosynthetic needs. In presented mechanism of carbon fractioning of isotopes the influence of <sup>13</sup>C nucleus magnetism on this process is constrained since the main stage of fractioning of isotopes, pyruvate decarboxylation occurs by means of coenzyme A and ketopropionic acid condensation reaction. Necessary condition for MIE occurring is availability of unpaired electrons on boundary molecular orbitals, i.e., MIE is typical for reactions involving radicals or radical ion. The emphasis on isotopic ratios of carbon in human body was made by Ivlev [31]. He investigated the character of daily curves of carbon isotope composition (CIC) of expired air by healthy people and people with diabetes and obesity; dependence of hair CIC on the health status of

the people under survey; dependence of blood serum CIC on endocrine disease character; variations of CO<sub>2</sub> CIC of expired air and urea of healthy people, people with diabetes and obesity. Range of daily δ<sup>13</sup>C variations presented some differences in isotope dislocating in different hormonal metabolic states. In 2008-2009 we made surveys focused on identification of dependence between intramolecular isotopic ratios of carbon in venous blood and body functional state. Several groups of people of different age categories were examined. Among them there were healthy people and those who suffered from particular diseases. Figure 4 presents venous blood carbon isotope composition measurements results.

δ<sup>13</sup>C isotope shift in the blood of healthy young people is in the range -23.1 - -23.7‰, and as for aged people this value is in the range between -22.9 - -23.7‰. As for ill people of different age these values are correspondingly -21.6 - -22.9‰ and -21.2 - -22.9‰. Obtained results indicate the absence of age changes of δ<sup>13</sup>C level in blood (at least based on this survey) of healthy people and nonspecific impact of pathology on light and heavy carbon isotopes ratio in blood. It is obviously, that enrichment of ill people blood with <sup>13</sup>C heavy isotopes is caused by increasing of cells need for energy determined by change of functional state of the body; upon that the ratio of pyruvate fund use in cells for ATP synthesis is increased sharply causing perceptible accumulation of heavy isotope in the remaining part. One can assume that due to particular number of heavy isotopes present in metabolic processes, a lot of cell proteins do not exchange their amino acids (probably due to destruction of cellular metabolism energetic compound) with "metabolic fund" of amino acids involving into biosynthesis of this or that protein. Described mechanism of carbon fractioning of isotopes is completely feasible. The main factor of fractioning of isotopes is the difference in <sup>12</sup>C and <sup>13</sup>C nuclear mass. However, discovering of magnetic isotopic effect for <sup>13</sup>C in chemical reactions put a question: whether the differences of <sup>12</sup>C and <sup>13</sup>C magnetic features can be "taken into account" by biological systems throughout their life. Signified differences of reactive abilities of magnetic and non-magnetic carbon nucleus occur under free radical polymerisation since magnetic isotopic effect is "multiplies" in chain reaction. Thus, Turro et al. [32] investigated styrole emulsion polymerisation initiated by photolysis of two chemically identical isotope forms of dibenzyl ketone PhCH<sub>2</sub>COCH<sub>2</sub>Ph and Ph<sup>13</sup>CH<sub>2</sub>CO<sup>13</sup>CH<sub>2</sub>Ph. Initiators of polymerisation are benzyl radicals generated mostly in secondary radical pair (Ph<sup>12</sup>CH<sub>2</sub> and <sup>13</sup>CH<sub>2</sub>Ph) that appears as a result of fast decarboxylation of the primary triplet pair of benzoyl and acyl radicals.



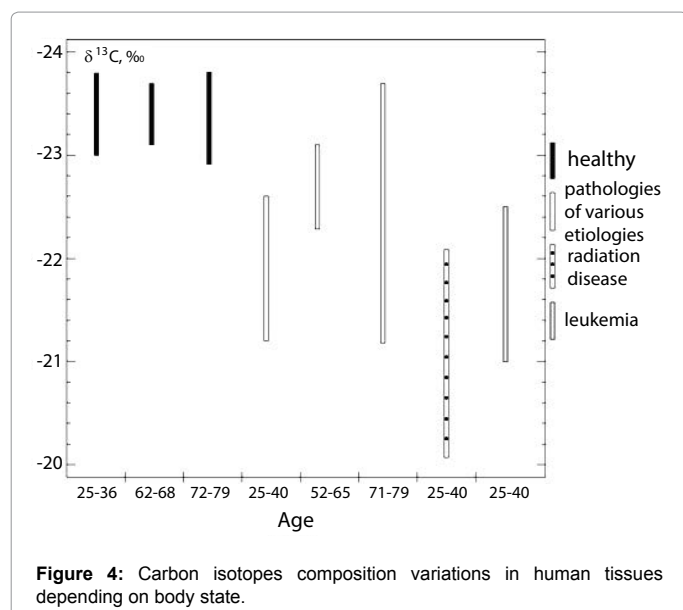


Figure 4: Carbon isotopes composition variations in human tissues depending on body state.

According to the experiment, substitution of  $^{12}\text{C}$  non-magnetic nucleus for its magnetic "twin"  $^{13}\text{C}$  changes radicals' chemical activity on 30-40%. In the first case, where active centre of polymerisation initiation is  $^{12}\text{C}$ , the rate and degree of polymerisation is much higher than in the second one, where active centre of polymerisation initiation is  $^{13}\text{C}$ . This is due to the fact that in the second case superfine interaction induces fast spin conversion of radicals with active  $^{13}\text{C}$  from triplet state to singlet state and they mostly recombine.

**Intramolecular fractioning of hydrogen isotopes:** Hydrogen has three isotopes: protium, deuterium and tritium with mass numbers 1, 2, 3. Protium and deuterium are stable isotopes, tritium is radioactive (half-life periods 12.26 years). Deuterium and protium in natural compounds are contained in the ratio of 1/6400 (according to atoms number). Tritium is contained in nature in a small quantity  $1.3 \times 10^{18}$  Bq. Deuterium magnetic moment is  $0.86 \mu_B$ , and protium  $-2.79 \mu_B$ . Protium spin is  $\frac{1}{2}$ , and deuterium is  $-1$  and therefore the energy of hyperfine electron-nuclear interaction with protium proton is 6.5 times bigger than with deuterium proton. Consequently, spin conversion of radical protic pairs is far exceeds the rate of deuterium pairs from  $6.5^2$  times for short-lived pairs to  $6\frac{1}{2}$  times for long-lived pairs [8]. Such differences in nucleus characteristics can be the reason for peculiar behaviour of any isotopes in chemical or biochemical reactions. Main theoretic and experimental investigations as for hydrogen fractioning in human body were performed by Demikhov - head of isotope chemistry laboratory. Hydrogen is found in different natural compounds in the form of  $\text{H}_2$ , (-OH), (-CH), (-SH) i (-NH); in the earth conditions its bulk is found in water. Relative difference in hydrogen isotopes masses is maximum; therefore natural variations of its isotope composition reach 700‰ in the earth samples that has made hydrogen isotopic effects well-studied. It is known that hydrogen isotopes enter the human body along with portable water and food. Being inside the organism, water becomes a participant of various biochemical processes and as a result atoms can be recurring units of different compounds synthesized by the organism. Available example of that how water isotope composition reflects on protein synthesized by the organism is presented in paper [33]. The authors have demonstrated direct dependence between human hair isotope composition (H, O) (that is mostly consists of  $\alpha$ -keratin protein) and

intake drinking water. Water in cells is in special structured state, intermediate between liquid water and ice. Layers of oriented water molecules surround all the hydrophilic macromolecules in protoplasm (including proteins and nucleic acid molecules). It is obvious that one should better speak about correspondence of peculiar features not in the same macromolecules, but in the macromolecules surrounded by the layers of structured water. Such correspondence can be significantly disrupted in case of unequal isotope composition of structured water in the cell [34,35]. According to the data from scientific surveys made in 1930s as for heavy water ( $\text{D}_2\text{O}$ ) impact on organisms life activity there appeared an idea about their anomalously high sensitivity to substitution of protium ( $^1\text{H}$ ) for deuterium (D). As a result the conclusion was made that heavy water suspends a lot of intracellular processes and cell fission is impossible when the content of deuterium in the environment is more than 50%. However, as a result of  $\text{D}_2\text{O}$  larger availability it was possible to carry out regular quantitative studies of the deuterium impact on living body [36,37]. Thus, Katz determined that a lot of unicellular organisms (weed and bacteria) under specific conditions can be reproduced in case of almost full substitution of  $\text{H}_2\text{O}$  for  $\text{D}_2\text{O}$  in the environment [38]. But in case of direct transfer of some weed species or bacteria with natural isotope composition from ordinary water into water with high concentration of  $\text{D}_2\text{O}$  (70-100%), a peculiar full or partial "anabiosis" is observed. In the first case the cell growth and fission is completely stopped. In the second case large part of cells during "anabiotic period" continue to grow without fission and can reach enormous size; some of them are ten times bigger than normal cells. After some particular period of time, that is different for various organisms, an adaptation to unfamiliar environment takes place and survived cells start to grow and fission normally. The same anabiotic phenomena are observed during return of adapted to isotope clear  $\text{D}_2\text{O}$  cells to  $\text{H}_2\text{O}$ . It should be stated that such adaptation was absent in the organisms of higher organizational level [39]. Strong antimetabolic action of  $\text{D}_2\text{O}$  was identified during the first experiments. Thus, in 1938 Barbour and Allen [40] described slowdown in growth and involution of transplanted lymphosarcoma and carcinoma of the breasts of mice receiving 40%  $\text{D}_2\text{O}$  as drinking water. However, the total lifetime of affected by tumour mice under  $\text{D}_2\text{O}$  action was less than in the group under control. The same issue is considered in other papers [41,42]. Among recent works one can outline a study that shows that pancreatic cancer activity in cell culture AsPC-1, BxPC-3, i PANC-1 is significantly reduced in case of systematic consumption of 10-30%  $\text{D}_2\text{O}$  and difluorodeoxycytidine. In addition, the authors showed that ingestion of water containing 10-30%  $\text{D}_2\text{O}$  does not influence much on the level of mononuclear cells in peripheral blood that is the evidence of limited adverse  $\text{D}_2\text{O}$  effect on bone marrow cells [43]. On the contrary, the other works emphasise positive (in addition to traditional forms of treatment) effect of the lighter (as for deuterium) water during oncological diseases therapy. Antitumor effect and safety of "light water" for humans were proved by clinic investigations along with prostate cancer treatment [44]. Practically identical antitumor impact of heavy and light water as well can testify that deuterium content in tissues within particular limits is very important for body healthy functioning. At first glance it seems possible to explain "isotopic anabiosis" by disturbance of geometric conformity of DNA and RNA macromolecules or protein during isotope hydrogen substitution. Structure of the above macromolecules is fixed by hydrogen bonds. That is why it could be assumed that small diversities in numerous hydrogen bonds sizes in macromolecule can essentially alter three-dimensional structures and, thus, make interaction of protium macromolecules with deuterium ones impossible [14]. However, significant reason against explanation of anomalous  $\text{D}_2\text{O}$

effects just by hydrogen bonds deformation is the results of survey performed by Borek and Rittenberg [45]. These authors observed anabios similar to deuterium one for *E. coli* bacteria during their transfer from  $H_2^{16}O$  to 92%  $H_2^{18}O$ . Change of hydrogen bonds size and toughness along with substitution of  $^{16}O$  for  $^{18}O$  is much less than when H is substituted for D, however,  $D_2O$  and  $H_2^{18}O$  suppression level of cells growth and fission was of one and the same series. Authors made a conclusion that change of geometric conformity of just macromolecules, in case of environmental water substitution, cannot be fully responsible for observed phenomena complex. Several papers on nucleic acid special magnetic properties studies were published in 1960s [46,47]. The authors believed that changes of the above properties are the most evident in case of change of isotopic composition of water between macromolecules taking part in reduplication. The appearance of the areas with anomalous D/H ratio as a result of abrupt change of the water isotopic composition in the chromosomes or DNA strands can lead to the changes in their magnetic properties that cause the disruption of information transmission and macromolecules movement coordination necessary for normal mitosis [46,47]. Example of different hydrogen isotopes magnetic properties impact on reaction path can be photolysis of two dibenzyl ketones -  $PhCH_2COCH_2Ph$  and  $PhCH_2COCD_2Ph$  [32]. After photolysis it was measured the output of  $PhCH_2CH_2Ph$  and  $PhCD_2CD_2Ph$  got by means of recombination of secondary radical pairs generated after decarboxylation of the primary pairs. It turned out, that rate of spin conversion of radical protium containing pairs significantly exceeds deuterium pairs' conversion rate. As a result, the output of protium dibenzyl was 33%, and deuterium - only 28% [32]. Almost no one had studied the hydrogen isotopic composition of the human body up to the beginning of the XXI century. We can recall only a verbal message by Krouse as for determination of  $\delta D$  in human urine. He found that human urine is weighted about 30‰ compared with the isotopic composition of local tap water. In 2005, Institute of Environmental Geochemistry NAS of Ukraine Demikhov carried out a series of experiments as for studying of isotopic composition of hydrogen in tissues, liquids and products of human metabolism [48]. Water of human blood, saliva, sweat, urine is characterized by similar hydrogen isotopic composition within measurements accuracy. These substances are enriched in deuterium on ~30‰ in comparison to local drinking water ( $\delta D=74‰$ ). Increasing of  $\delta D$  in human blood, saliva, sweat, urine and comparing to local drinking water should be compensated by protium appropriate amount discharge from human body. The most possible way can be protium clearance by means of sebaceous glands secretion that was experimentally proved as an example of ear wax (Table 2) [48]. Recently the papers where isotopes  $^{13}C$  and D are considered as potential instrument for protection from oxidants destructive effect in human body have been appeared [49,50]. Particular action of oxygen free radicals, that are co-products of some biochemical reactions, is connected to destructive processes causing its insensescence. Oxygen free radicals, that are aggressive electron acceptors, destroy fragile DNA chain and other proteins mostly by C-C and C-H links, taking off electrons from one of the atoms. Substitution of macromolecule H and  $^{12}C$  for D and  $^{13}C$ , according to the authors, should significantly increase links energy (in case of substitution of H for D - by a factor of 80), that will contribute to less weakness of macromolecules under free radicals action. Selective substitution of H and  $^{12}C$  for D and  $^{13}C$  in components that are the most fragile for oxidants action significantly increases strength of macromolecules in general [50]. However, the authors do not consider the issue about change of energetic and conformational intermolecular correlation (as a result of macromolecules three-dimensional structure damage), as well as

electromagnetic properties of macromolecule in case of substitution of H and  $^{12}C$  for D and  $^{13}C$ .

#### Intramolecular fractioning of nitrogen and oxygen isotopes:

Interesting data about nitrogen and oxygen isotopes were obtained by Metges and Petzke [51]. They have identified  $\delta^{15}N$  thirteen free amino acids in human plasma (Figure 5). Phenylalanine and threonine were the most exhausted  $^{15}N$ . Apart from this, slight variations of  $\delta^{15}N$  (from 10 up to 15‰) were identified in alanine, leucine, proline and ornithine. In terms of metabolism phenylalanine and tyrosine are different in  $\delta^{15}N$  on ~15‰. Later Petzkethors [52] have measured  $\delta^{15}N$  and  $\delta^{13}C$  for fourteen amino acids of human hair (Figure 6). The difference between lower and upper value of peculiar amino acids in  $\delta^{13}C$  was ~30‰ (leucine and glycine), and in  $\delta^{15}N$  - ~25‰ (threonine and proline). It follows from the data, presented in Figure 5, that the most minimal values of  $\delta^{13}C$  have so called essential amino acids (histidine-valine). As it is known, these amino acids do not synthesise in eukaryotic organism. Their isotopic composition (as for carbon) is constant, since carbon chain does not change during biochemical transformations. Isotopic changes in such amino acids can perceive hydrogen and nitrogen atoms. In case with hydrogen, this is related to the low energy of H-C, thus, isotopic exchange with the "environment" is possible. In case with nitrogen - some part of essential amino acids depending on body biosynthetic needs can reanimate causing other amino acids production. Another dependence is observed for substitutional and partially substitutional amino acids. Such amino acids like alanine and asparatic acid are significantly enriched in  $^{13}C$  compared to amino acids synthesized from Krebs cycle metabolites and have similar  $\delta^{13}C$  values. On our opinion, this is connected to that

No	Sample	$\delta D$ , ‰
1	Tap water	-74
2	Expiration moisture	-83
3	Human saliva (water)	-49
4	Human blood (water)	-48
5	Human sudor (water)	-45
6	Human urine	-44.5
7	Human hair	-78
8	Human nails	-82
9	Pork	-79
10	Human earwax	-161

Table 2: Hydrogen isotopic composition of human tissues and liquids.

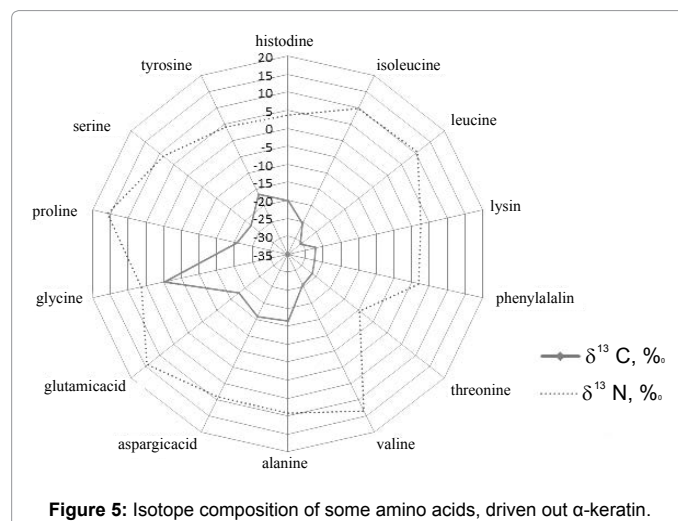
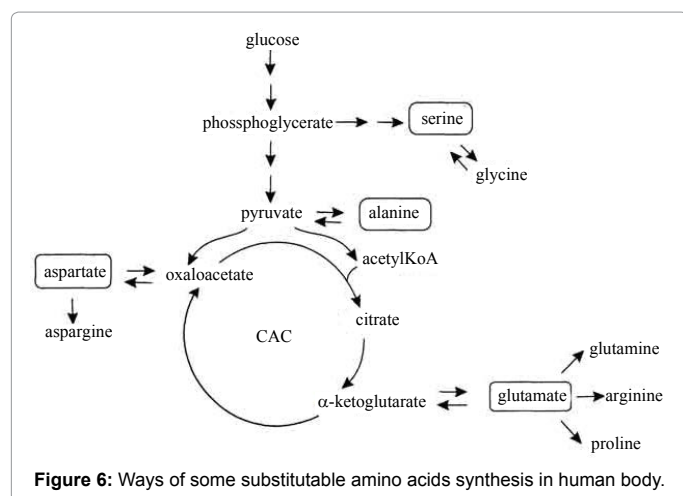


Figure 5: Isotope composition of some amino acids, driven out  $\alpha$ -keratin.



these two amino acids are synthesized directly from pyruvate fund (Figure 6). Such amino acid like tyrosine is not directly synthesized in organism. It is generated in the body due to fermentation catalysis or along with food intake. As it is shown on Figure 6, serine and proline amino acids are not synthesized from ketopropionic acid. The most enriched in  $^{13}\text{C}$  amino acid is glycine. It can be synthesized in human body in two ways: by means of fermentation catalysis from serine or by means of direct synthesis from carbon dioxide and ammonia. Since serine is one of the most  $^{13}\text{C}$  depleted amino acids, it is presumably that great contribution to glycine synthesis is made by reaction of its generation from carbon dioxide and ammonia. Based on the above it may be concluded that carbon fractioning of isotopes in human body (and not only human) is an integral part of metabolic processes, so-called "Isotope metabolism". Isotope metabolism - is intramolecular fractioning of isotopes on particular stages of biochemical reactions (cleavage, synthesis and interconversion of complex compounds), caused by differences in fundamental properties of isotopes atom nucleus - mass number and magnetic moment. The above notion has been introduced by the authors in 2000 for explaining of fractioning of isotopes processes in case of organism functional state change. The metabolic processes state is an effective instrument for body functional state diagnostics and it could be assessed by deviations in isotope ratios. Fuller et al. [53] have demonstrated that except for diet the  $\delta^{15}\text{N}$  value is being influenced by the nitrogen balance level in the organism. During investigations, hair samples were taken from eight pregnant women stressed out due to morning vomiting and checked for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Content of  $\delta^{13}\text{C}$  in the process of morning ailment during pregnancy did not changed in comparison to the period before pregnancy. At the same time,  $\delta^{15}\text{N}$  value increased in the period of weight loss caused by morning ailment. A tendency to  $\delta^{15}\text{N}$  reducing in hair was noted according to body weight gain, cure from experienced stress and delivery date coming. The above data indicate that human tissues are under influence of deviation in nitrogen homeostasis: catastatic state causes increasing of  $\delta^{15}\text{N}$  content while anabolic one leads to its reducing. As for hydrogen isotopes biological fractioning, the majority of available evidences suggest the impact of direct and/or mediated isotope ratio of oxygen in consumable water on human tissues  $\delta^{18}\text{O}$  [33]. Thus, the database of C, H, N isotopes ratios and other light elements in human body depending on health and age is being created as a result of detailed study of available scientific literature on the above issue and own investigations. We believe that change of ratios of typical (inner) stable isotopes in human body depending on pathological changes caused by metabolism disturbance may be potential base for

creation and development of early diagnostics criteria. The authors also think that for diagnostics accurate methods development it is necessary to analyse isotopic composition of representative set of vital elements complex. As of today there is rather large number of works referring vital elements intramolecular fractioning (among them there are our works on Fe and Mg intramolecular fractioning in human body). They demonstrate that the organism selects particular isotopes of microelements for biosynthetic needs; and the selection criterion is not the mass, but presence of magnetic moment in atomic nucleus [17].

### Isotopes variations of some biogenic elements in living bodies' biosystems

Starting from the first half of XX century, a lot of researchers have been dealing with the issue of biogenic elements fractioning of isotopes. It was proved that isotopes distribution between and inside biomolecules is typical for organisms; that isotopes behaviour naturally controlled by the processes in biosphere, thus geochemical investigations includes detailed study of isotopic composition of biological objects. Buchachenko et al. [8] for the first time described possible mechanism of Mg fractioning of isotopes in mammalian body. They demonstrated that in the process of enzymatic reaction of adenosine triphosphate from creatine phosphate and adenosine diphosphate, the rate of phosphorylation reaction is increased twice with the presence of  $^{25}\text{Mg}^{2+}$  ion. For isotopic forms of  $^{24}\text{Mg}$  and  $^{26}\text{Mg}$  there are no any differences in reaction rate. Such behaviour of  $^{25}\text{Mg}$  was explained by its magnetic properties. Similar effects were obtained by Black [54] during magnesium isotopic composition investigation in chlorophyll-*a* in the process of photosynthesis. Undertaken in 2009 experimental works on magnesium isotopic composition in human body demonstrated that blood of the patients with distinct pathology is enriched in  $^{25}\text{Mg}$ .  $^{24}\text{Mg}/^{25}\text{Mg}$  ratios variations range for examined patients with diseases of blood-vascular and hematopoietic systems was 7.36-7.50, while for healthy people this interval was within 7.56-7.76. Walczyk and von Blanckenburg [55,56] demonstrated that isotopic composition of human intestinal tract mostly has light ferrum isotopes. They have discovered that human blood and tissues have similar isotopic composition in terms of ferrum (average  $\delta^{56}\text{Fe}$  is 2.74‰ and 2.58‰ correspondingly); hair enriched in  $^{54}\text{Fe}$  ( $\delta^{56}\text{Fe}=3.8\%$ ), and liver is enriched in  $^{56}\text{Fe}$  (at average is 1.37‰  $\delta^{56}\text{Fe}$ ). Average content of  $\delta^{57}\text{Fe}$  in human blood is estimated at y 3.8‰. Ohno et al. [57] identified content of  $\delta^{56}\text{Fe}$  and  $\delta^{57}\text{Fe}$  in human red blood cells as about 3‰ i 4.5‰ accordingly. The tendency for enrichment of human blood in  $^{54}\text{Fe}$  and exhaustion in  $^{56}\text{Fe}$  and  $^{57}\text{Fe}$  was also supported by other observations. Mareschal et al. [58] for the first time assessed cooper fractioning in human blood; content of  $\delta^{65}\text{Cu}=0.30\%$ . There is also evidence in favour of zinc fractioning of isotopes in human body. The same authors informed that content of  $\delta^{66}\text{Zn}$  in whole blood is 0.41‰. Stenberg et al. [6] measured content of  $\delta^{66}\text{Zn}$  for human hair and whole blood (0.60‰ and 0.56‰ accordingly). Later Ohno et al. assessed content of  $\delta^{66}\text{Zn}$  and  $\delta^{68}\text{Zn}$  for human hair as 0.16‰ and 0.31‰, accordingly, while the content of  $\delta^{66}\text{Zn}$  and  $\delta^{68}\text{Zn}$  in human red blood cells is 0.43‰ and 0.83‰ accordingly. Season fluctuations of the above values were not identified. There are relatively few evidences of gender differences availability in the processes of stable fractioning of isotopes. Data by Walczyk and von Blanckenburg [55] demonstrate that content of  $\delta^{56}\text{Fe}$  and  $\delta^{57}\text{Fe}$  in men's blood is  $\sim 0.3\%$  lower than in women's. The most comprehensive information about biogenic elements isotopes variations in Biosystems of living bodies is presented in Man and Geosphere monograph released in 2010 edited by Florynskiy, D.Sc. in engineering [59]. In comparison to the investigations of isotopic variations in plants and animals, the processes of fractioning of isotopes



in human body are still difficult to understand. However, it could be stated that natural internal isotopic ratios of some biogenic elements could be a holder of additional information as for metabolic processes state in human body (under normal and pathological conditions).

### Behavioural features of different pairing isotopes in biological systems

The most of chemical elements, as a rule, are isotopic mixture. The similarity of their chemical features determines the electron shell structure. Isotopes are sort of one and the same chemical element atoms the nucleuses of which contain the same neutrons number. Accordingly, possible further variations of protons and neutrons ratio in nucleus: nonpaired-nonpaired ( ${}^7_7\text{N}$ ), nonpaired-paired ( ${}^8_7\text{N}$ ), paired-nonpaired ( ${}^7_6\text{C}$ ,  ${}^9_8\text{O}$ ) and paired-paired ( ${}^6_6\text{C}$ ,  ${}^8_8\text{O}$ ). Differences in fundamental isotopes nucleuses properties can form distinctive features of behaviour of isotopes of different pairing in chemical and especially in biochemical reactions. While studying of stable and radioactive isotopes behaviour we have identified discrepancy in their behaviour. Identified deviation from mass-dependent linear isotopic effect that is in proportional change in behaviour of all the isotopes with the same chemical composition is appropriate to isotopes mass change. Such peculiarities in behaviour of radioactive isotopes are very interesting in terms of importance of the issues related to the annual increasing of tritium and radioactive carbon emissions into environment caused by anthropogenic factors (NNPs, nuclear fuel reprocessing facilities, etc.). In spite of rather large number of works as for radiobiological effect of tritium and radioactive carbon to the living bodies, the reasons (mechanisms) of their accumulation in molecular structures are still unclarified. Behavioural differences can be caused by differences of physical fundamental characteristics of paired and nonpaired isotopes nucleuses (paired - nucleus mass, nonpaired - mass and spin). As a result, the probability of chemical reactions is dependent on nucleus spin presence and the rate of its interconnection with electron spin - so-called hyperfine interaction that may have an impact on reaction rate for isotopic molecules, on energy state and on nuclear magnetism of the system. Another possible reason for stable radioactive isotopes behavioural differences may be the difference in concentration of the above isotopes that is influence of super-low concentrations of chemical agents on Biosystems. Investigations of hydrogen and carbon triads' isotopic composition in Biosystems (vegetation, mammals and human being) have been carrying out by the Institute of Environmental Geochemistry NAS of Ukraine for a long period of time. Contradictory behavioural peculiarities of stable and radioactive components of the above triads have been identified in the process of the obtained results systematization. The first concerning fact was that accumulation of tritium and  ${}^{14}\text{C}$  in trophic chains has been recorded in a number of works [60]. These facts were inconsistent with stable isotopes behaviour in trophic chains. Actually, no one has discovered deuterium or  ${}^{13}\text{C}$  accumulation in food chain. We have not found any literature data as for different behaviour of H and C isotopes in their triads (H-D-T and  ${}^{12}\text{C}$ - ${}^{13}\text{C}$ - ${}^{14}\text{C}$ ) in Biosystems and, moreover, scientific explanation of this difference. The most of investigations are limited by studying of either stable or radioactive components without their behavioural characteristics comparison. First of all our investigations refer to studying of isotopes natural concentrations in biological systems. At the same time, the method of marked atoms, using stable and radioactive isotopes as well, is widely used in medical and biological practice. This method allows tracing of mechanisms of consequent biochemical processes in organism by means of overseeing the marked isotope destiny in metabolic products, biological liquids and tissues. However, introduction of marked atoms in biological systems without

precise understanding of their behavioural peculiarities could lead to wrong interpretation of the results. Earlier it has been demonstrated that isotopic dislocation of  $\delta\text{D}$  in human body tissues, biological liquids and metabolic products is close to balance with environment and there is no D concentration in trophic chains [48]. There is a balance of hydrogen isotopes distribution in organism, filling of biological liquids water is compensated by lipids lightening. Tritium behaviour investigation (T) reveals opposite tendency to its accumulation in trophic chains, moreover, there is the most T accumulation in plants lipid fraction. With the aim of H-D-T isotopes triad behavioural differences identification, we have carried out investigations as for their content measurement in a tap water, human urine and expiratory air liquid. As it can be seen from Table 3, the activity of consumed water and expiratory air liquid is appropriately higher than activity of human metabolic products that is an evidence of different behaviour of D and T in human body (measurement error is:  $\delta\text{D} - \pm 4\%$ ;  $\delta^{13}\text{C} - \pm 0.5\%$ ; activity (A): T - 5%,  ${}^{14}\text{C} - 0.6\%$ ). Obviously tritium, in contrast to deuterium, is concentrated in molecular structures of the body that causes its accumulation in trophic chains. Behavioural differences in Biosystems have been identified for deuterium and tritium as well. The contrary behaviour of hydrogen isotopes pleiad components in biological systems, to our mind, may refer to the presence of dipole moments of  $\text{H}_2\text{O}$ , HDO and HTO molecules and to various magnetic properties of hydrogen isotopes nucleuses. In case of hydrogen, expected decreasing of  $\delta^{13}\text{C}$  values was identified in lipid fractions of Biosystems. As for radioactive carbon ( $\delta^{14}\text{C}$ ), value of its fractioning in Biosystems should be more significant (in comparison to  $\delta^{13}\text{C}$ ), in accordance to mass-dependent isotopic effect, than it was in practice. However, literature data suggesting the presence of  ${}^{14}\text{C}$  concentrating, as in case with T, in trophic chains of plants, animals and human beings in relation to its content in ground air.  ${}^{14}\text{C}$  content in plants (green grass, wheat) is twice higher than in ground air in case of  ${}^{13}\text{C}$  concentrating absence in trophic chains. However, there are literature data indicating that there is  ${}^{14}\text{C}$  concentrating in plants, animals and human trophic chains in relation to its content in ground air. There are data where content of  ${}^{14}\text{C}$  in plants (green grass, wheat) is twice higher than  ${}^{14}\text{C}$  content in ground air. Our data on isotopic composition of  $\delta^{13}\text{C}$  indicate the absence of  ${}^{13}\text{C}$  concentrating in trophic chains. As Table 4 shows, there is a decreasing of  ${}^{13}\text{C}$  concentration in investigated objects in comparison to atmospheric air (-7‰). Fact of  ${}^{14}\text{C}$  accumulation in food chains points to different behaviour of  ${}^{13}\text{C}$  and  ${}^{14}\text{C}$ . Identified behavioural differences of stable and radioactive isotopes

Sample	$\delta\text{D}\%$	Activity T, Bq $\times$ dm <sup>-3</sup>
Tap water	-74	14.5
Atmospheric moisture	-79	12.7
Expiration moisture	-121	8.5
Urine	-58	10.8

Table 3: Content of hydrogen isotopes in investigated objects.

Sample	$\delta^{13}\text{C}$ , ‰	A ${}^{14}\text{C}$ , Bq $\times$ g <sup>-1</sup>
Meat	-22.9	0.239
Skin	-21.5	0.239
Hair	-22.5	0.238
Fat	-26.0	0.241
Albumen (mixed diet)	-19.6	0.236
Egg-yolk (mixed diet)	-22.4	0.241
Egg shell (mixed diet)	-3.9	0.246
Albumen (diet: C <sub>4</sub> plants, corn)	-14.1	0.242
Egg-yolk (diet: C <sub>4</sub> plants, corn)	-17.2	0.241
Egg shell (diet: C <sub>4</sub> plants, corn)	+0.5	0.247

Table 4: Hydrogen isotopes content in investigated objects.

are likely to be referred to nucleuses pairing or to super low concentration of radioactive isotopes that requires more strict approach to modelling of the processes of fractioning of isotopes with different pairing but one and the same chemical element. Data obtained for isotopes with the one pairing may be non-representative for isotopes with the other pairing. Our investigations, first of all, refer to studying of variations of natural concentrations of intramolecular isotopes in biological liquids, tissues and products. Isotopic changes may be potential source of information about metabolic processes in human body in normal and pathological states as well. Method of isotopic indicators (marked atoms), using stable and radioactive isotopes as well, is widely used in medical and biological investigations practice. This method allows tracing the mechanisms of consequent biochemical transformations in organism by means of overseeing the marked isotope behaviour in metabolic products, biological liquids and tissues. However, introduction of marked atoms of some chemical elements that are not coincide with the pairing of similar atoms in the organism without precise understanding of their behavioural peculiarities in various Biosystems could lead to wrong interpretation of obtained results and accordingly to wrong diagnostics. It has been known about nuclear spin and its magnetic moment for 40 years and about nuclear spin effects in chemical reactions (for radical or ion-radical) for 30 years. Such effects have been studying mostly by means of strong magnetic field outside source. Magnetic field induces spin conversion from singlet state into triplet energetic state where back electron transfer is forbidden. As a result it influences the rate of chemical transformations and their results. Similar effect could be also achieved without outer magnetic field but with magnetic nuclear atoms that in such case are magnetic field sources themselves. In the process of reaction there is a sorting on magnetic and nonmagnetic nucleuses causing fractioning of isotopes between initial reagent and reaction products. Existence of magnetic isotopic effect has already been proved for isotopes of such elements like H, C, O, Mg, Si, S, Ge, Sn, Hg. Magnetic isotopic effect for all the above mentioned elements has been presented in laboratory conditions. There is no reliable evidence of its influence on observed isotopic variations in natural objects. In spite of carrying out of numerous analysis of the most studied carbon and oxygen isotopic effects, there are difficulties with kinetic and magnetic effects division in different natural objects. This is due to that in exchange reservoir - biosphere,  $^{13}\text{C}$  kinetic retardation is compensated by its ability to react at the expense of magnetic properties. Moreover,  $^{13}\text{C}$  behaviour comes under influence of the Earth magnetic field. And the Earth itself is particular solenoid that should sort isotopes. And the magnetic poles change their location up to "reverse polarity". It is quite possible that observed wide range of  $\delta^{13}\text{C}$  in biogenic carbonates could be explained by means of taking into consideration not only kinetic but also magnetic mechanism of these objects generation. May be it will be possible to address this issue in case of more detailed studying of such isotopic triads like H-D-T,  $^{12}\text{C}$ - $^{13}\text{C}$ - $^{14}\text{C}$ ,  $^{16}\text{O}$ - $^{17}\text{O}$ - $^{18}\text{O}$ ,  $^{24}\text{Mg}$ - $^{25}\text{Mg}$ - $^{26}\text{Mg}$ .

## Conclusions

It is very important to understand natural features of isotopes and differences in features of compounds with different isotopic composition for successful use of isotopes in human economic activity. It is the more so in the process of investigation of complex biological systems. All the living organisms from bacteria to human being as well as their biochemical systems are characterized at least by two isotopic parameters:

- Particular isotopic composition that might be named "isotopic map" of the organism, biosystem, etc. This parameter like body's element composition is clearly attached to the environment and is interdependent and interrelated.

- Presence of clear interrelations between stable and natural radioactive isotopes of those biogenic elements that take part in living bodies life activity.

Stable and natural radioactive isotopes play the main role in physical and chemical properties of the element itself as well as in all natural processes as a whole. As of today the understanding of isotopes like atoms of some chemical elements, which nucleuses are different in mass numbers, has been added by the differences in their quantal characteristics. Accordingly, classical kinetic isotopic effects, referred to nuclear mass, have been extended by the effects based on the difference of their magnetic moments. All the processes of fractioning of isotopes including intramolecular fractioning of isotopes in living bodies are related to the above two fundamentally different characteristics of atoms of the same chemical elements. Differences in fundamental nuclear properties may influence the rate of chemical reactions and energetic state and nuclear magnetism of reactive systems. The above statements are the further development of Vernadskiy's idea about isotopes division related to the phenomena where chemical affinity does not reduce physical strength displaying. Experiments carried out by the Institute of Environmental Geochemistry NAS of Ukraine facilitated development of the new direction for investigations - nuclear chemistry based on studying of fundamental characteristics of nuclear isotopes with different pairing and on their behavioural peculiarities in living and non-living nature systems as well. The above direction is becoming important in view of annual increasing of tritium and radioactive carbon emissions caused by anthropogenic factors. For medical and biological issues such investigations may give new knowledge about mechanisms of metabolic transformations in living bodies. In radiological sphere intramolecular natural isotopic ratios of organogenic and some biogenic elements may be the sources of information about physiological state of the organism and may be used with diagnostic aim. Modern level of biology sciences and sciences about the Earth and living substance development has formed single powerful complex of sciences about various life manifestations and its diversity. Transformation of ideas of Vernadskiy, who at the beginning of XX century formed the basis of their interdependence, creates a platform for new ideas, notions and new sciences directions at the present and future as well.

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