Determination of Deuterium Concentration in Biological Fluids by NMR Spectroscopy

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\textbf{Abstract}—In the work by means of NMR spectroscopy received data of isotope (D/H) composition of women biological fluids during lactation. It is found that under the natural conditions concentration of deuterium in the various biological fluids is significantly different, that is accompanied by formation of isotope (D/H) gradient (oral fluid $\gg$ blood plasma $> \text{human breast milk}$). The high efficiency of methods for determining the concentration of deuterium in body fluids by means of NMR spectroscopy using trifluoromethanesulfonate europium (III) showed.

\textbf{Keywords}—Deuterium depleted water, oral fluid, blood plasma, lanthanide shift reagent.

\section{I. INTRODUCTION}

The isotopic composition of biogenic elements varies in objects of different nature because of geographical factors and for the reasons of radioactive transformations, kinetic and thermodynamic isotope effects. The widest range of variations has hydrogen. Thus, natural deuterium concentration in water of various objects ranging between approximately 100 ppm (80 to 180 ppm) \cite{1}. Water is one of the most active biological compounds, affecting virtually all biochemical and biophysical processes in the organism. In particular, according to the literature, the water with artificially modified deuterium content has a significant effect on metabolic processes in living systems. Moreover, it is typical not only for water with increased level of this isotope, but with a lowered by 2-3 times compared to the world average ($\approx 155$ ppm) \cite{2}–\cite{6}.

A number of studies indicate that light water has immune modulating properties \cite{7}–\cite{9}.

Thus, a few works of various research groups were dedicated to the study of onco-cell growth in nutrient solution prepared on water with a low deuterium content \cite{10}–\cite{12}.

One of the latest research directions in this field become the study of water with a low deuterium content (deuterium depleted water) effect on condition of prooxidant-antioxidant system, which is a unit of nonspecific defense of organism \cite{13}–\cite{16}.

One of the main methods of measuring deuterium concentration in biological fluids is quantitative NMR spectroscopy \cite{17}, which is due to the simplicity of sample preparation procedures and analytical process can be used as a rapid method in experimental and clinical practice, such as monitoring processes of isotope (D/H) metabolism in individuals consuming dietary products with modified isotopic composition for prophylactic or therapeutic purposes.

The purpose of this study was to investigate the isotope (D/H) content of various biological fluids using NMR spectroscopy for developing non-invasive methods of monitoring of heavy isotopes content in the body, including consuming of water with low deuterium content.

\section{II. MATERIALS \& METHODS}

In this study we used NMR spectrometer JNM-ECA 400, which had the following characteristics: induction static magnetic field equal to 9.389766 T; a frequency range from 10 MHz to 400 increments of 0.01 Hz; the resonance frequency of the nuclei $^2\text{D}$: 61.371 MHz

For the sample we used a calibrated basic NMR ampoule 4.97 $\pm$ 0.013 mm in diameter and 178 mm length in which was placed the substance, investigated on isotopic composition. The inner ampoule (coaxial external standard) with a length of 32 mm and volume of 40 ul was inset in the basic NMR ampoule and contained water with a known isotopic composition. Internal NMR ampoule contained additives dissolved trifluoromethanesulfonate europium (III), due to $^2\text{H}$ NMR signal of water inside the ampoule was shifted relative to the signal of test substance from the basic ampoule. This allowed us to make the integration of individual signals of spectrum relative to each other. We used a number of calibration samples of pure water (impurity content not more than 0.01\% by weight) with a known content of deuterium, in accordance with international standards of IAEA, VSMOW and SLAP.
To determine \(^{2}\text{H}/^{1}\text{H}\) ratio to the inner NMR ampoule we placed sample of water with (CF$_3$SO$_2$)$_3$Eu equal to (0.045 ± 0.005) mol/l, while the deuterium content of water in the solution conformed to the investigated deuterium concentration range in measuring object.

The content of other impurities was not more than 0.01% by weight. For preparation of solutions we used dispensing micropipette 1 ml increments of 0.010 mL with disposable tips and laboratory analytical balance with the maximum permissible error of single weighing ± 0.0005 g. All experiments in a series of samples, including calibration, were performed under identical conditions of NMR measurements and with the same settings of device.

For measurements on nuclei \(^{2}\text{H}\), we found the optimal values: gain 60; shift 5 ppm; scan 10 ppm; observation time of free induction decay 6 sec; number of scans 256; relaxation delay of 10 T$_1$ (D$_2$O) ≥ 7 sec; temperature inside resonator 25°C. The results are expressed in ppm (hereinafter referred by the content of deuterium).

Parallel determination of the deuterium concentration in these samples was performed in the “Precision methods of analysis” Center in People's friendship university of Russia (Moscow) on spectrometer JEOL JNM ECA 600 by the standard method of measuring absolute intensities without using of external standard.

\(^{3}\text{H}\) content was measured in biological substrates: oral fluid, blood plasma, human breast milk in two groups of maternity patients in the Municipal Budgetary Health-care Institution "Rodlinyi dom" (Krasnodar). Group 1 (n=14) consisted of women receiving ordinary diet (which includes drinking water, in which the deuterium concentration was 150 ppm), group 2 (n=10) consisted of women receiving water with reduced deuterium content, besides the usual diet (60 ppm, “Langvey” produced by "MTC Iceberg", Moscow, Russia certificate of state registration RU.77.99.19.006.E028090.07.11 from 20.07.2011) in the amount of up to 1.5 liters per day for 25-30 days before the test.

Before the measurement of isotopic composition of biological fluids we produced preliminary sample preparation, including centrifugation (centrifuge minispin Eppendorf) at 1500 rpm for 15 minutes. Under these centrifugation conditions in biological fluids cellular components deposited (e.g. erythrocytes and leucocytes in blood plasma, epithelial cells and leucocytes in the oral fluid and human breast milk), but retained the basic biomolecules (proteins, lipids, carbohydrates). It eliminates the fluctuations of the isotopic composition due to cellular structures lysis in the research process [18]. The impurity content of organic substances in supernatant took into account by recording the \(^{1}\text{H}\) NMR spectra of all samples and adjusting to them \(^{1}\text{H}\) NMR results. Statistical processing was performed using the R Development Core Team, (2008), the difference was considered significant at p<0.05. For evaluation of correlations used Pearson's coefficient (r$_{\text{Pearson}}$).

### III. RESULTS & DISCUSSION

The studies found that under physiological conditions, there is a gradient of the deuterium content (oral fluid >> blood plasma > human breast milk) and its level in biological fluids differ significantly (Table 1). Differences in bioliquids isotopic composition may be due to various causes. Perhaps it is due to the biochemical composition of these bioliquids.

It is known that deuterium concentration in vivo in water, proteins, lipids, carbohydrates differ greatly, due to differing rates of isotope (D/H) exchange reactions from different chemical bonds of biomolecules. That the isotopic exchange in vivo is most pronounced in the links -OH, -SH, -NH$_2$ (=N-H), but virtually absent in the links -R$_3$C-H.

Therefore, to clarify the causes of isotopic D/H gradient we performed correlation analysis of the deuterium content in biological fluids and biochemical composition of blood plasma, oral fluid, and human breast milk.

#### TABLE 1

<table>
<thead>
<tr>
<th>Biological fluid</th>
<th>Blood plasma, ppm</th>
<th>Oral fluid, ppm</th>
<th>Human breast milk, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>144.3±0.6</td>
<td>159.8±0.4*</td>
<td>141.6±0.4*</td>
</tr>
<tr>
<td>Group 2</td>
<td>128.5±0.8^</td>
<td>134.9±1.2^</td>
<td>127.2±0.7^</td>
</tr>
</tbody>
</table>

* - p<0.05 compared to blood plasma parameters, ^ - p<0.05 in comparison with indicators of group 1.

According to the scientific literature [19], [20], content of proteins in blood plasma is from 6.5% to 8.5%, lipids - about 0.5 - 1.0%, carbohydrates - about 0.1%, whereas in oral fluids and human breast milk this figures differ several times from them.

Most of the lipids among the studied objects contained human breast milk (approximately 4%), whereas in oral fluid lipids are virtually absent (0.006% - 0.007%).

The content of proteins in blood plasma is significantly higher than in human breast milk (approximately 1.0%) and oral fluid (approximately 0.1%). At the same time, the content of carbohydrate in human breast milk exceeds ten times those found in plasma and is about 6.9%, whereas in oral fluid these rates are two orders of magnitude lower (about 0.03%). As a result of correlation analysis, it was found that there was a direct correlation between the content of water in biological fluids, and the content of deuterium in dependence on values of the reference range (maximum and minimum) r$_{\text{Pearson}}$ ranged from 0.85 to 0.99 (p<0.05). Wherein was found an inverse relationship between the content of organic compounds in biological fluids and figures of deuterium: proteins r$_{\text{Pearson}}$ ranged from -0.45 to -0.50, for carbohydrates r$_{\text{Pearson}}$ was from -0.61 to -0.62, lipids r$_{\text{Pearson}}$ ranged from -0.69 to -0.77 (p<0.05). It is indicating primarily the dependency of deuterium concentration on drinking diet and less on the content of organic molecules except molecules of lipids, characterized by high rates of r$_{\text{Pearson}}$. It can be explained with the lowest intensity of isotope (D/H) exchange in the hydrophobic (non-polar) lipids radicals that make a sustainable contribution to a final concentration of deuterium even under condition of water with different isotopic composition consumption. In addition, this relationship between the content of biomolecules and deuterium must be considered when elaborating algorithms of noninvasive assessment of heavy
non-radioactive isotope in the body, including deuterium, since the biochemical composition of biological fluids can be quite variable depending on the person's lifestyle (diet, physical activity, and other factors). However, it is impossible to explain completely the differences of isotopic composition by only peculiarities of biochemical composition in these bioliquids. Apparently, there are additional mechanisms of regulation of isotope exchange in living beings. Also, the difference of isotopic composition of blood plasma, oral fluid and human breast milk may be due to changes in speed of metabolic processes in the body, when under conditions of high energy production within the cells increased formation of water from hydrogen isotopes comprising the biological substrates of oxidation (i.e., the different classes of organic compounds). At the same time, water, which is formed during the oxidative processes inside cells, may significantly differ on deuterium content from extracellular water replenished in the body, mainly from the diet. Another probable cause of the physiological isotopic gradient is the possible presence of selective mechanisms of heavy isotopes entry cross histomorphatic interfaces such as gematosalivary and blood-lactation barriers.

The main function of blood-lactation barrier is regulation of permeability to physiologically important substances in the formation of breast milk, moreover, it is one of the important mechanisms of the infant protection in case of toxic substances enter the mother's blood.

In group 2, consumed water with decreased deuterium content, there was a significant decrease in deuterium (Table 1): blood plasma – 10.9% (p<0.05 compared with those in group 1), oral fluid – 15.6% (p<0.05), human breast milk – 10.2% (p<0.05). It should be noted that although group 2 remained isotope (D/H) gradient (oral fluid> blood plasma ≥ human breast milk) absolute differences between the deuterium content of biological fluids decreased by more than 2 times. The data in the blood plasma and human breast milk were not significantly different. Such dynamics of deuterium concentration may also indicate a decrease in the content of deuterium in water which is part of studied bioliquids; at the same time its content almost does not change in organic substrates. It can also indicate a lower fluctuation of deuterium concentration, primarily in cells that synthesize breast milk, due to the preferential formation of water from organic substrates in milk-secreting cells, and not entering it from blood [18], [21]. The percentage of intracellular water, which is formed directly in the cell, e.g., as a result of oxygen reduction processes in the mitochondria, can vary significantly in different tissues (due to their different metabolic activity) and in the same tissue at an intensity change of metabolic reactions in the process of its functioning.

During results verification of deuterium content measurements in biological fluids by the method of measuring the absolute intensities, we obtained similar rates isotopic gradient. Differences in the results obtained for similar bioliquids amounted to less than 3 ppm, which indicates accuracy of the proposed method. In addition, due to use of new methodological approaches using lanthanide shift reagents we were able to reduce significantly the time of experiment.

IV. CONCLUSION

Thus, on the basis of studies done we found that the developed methods for the isotopic ratios measuring by NMR using probes containing shift reagents can be also directed to the solution of ecological problems and screening of the human condition, specifically for the express control of isotopic composition of liquid of natural origin, as well as for monitoring the concentration of deuterium in the body when used the products with modified isotopic composition.

By virtue of work performed we found reference material, which has been selected with the same molecular structure as the tested. We also added lanthanide shift reagents: trifluoromethanesulfonate europium (III) to the standard.

Also this study demonstrated existence of isotopic D/H gradient (oral fluid > blood plasma > human breast milk) in human biological fluids under physiological conditions also due to the water content and biochemical composition of these bioliquids. The highest negative correlation among the organic components (r_{Pearson, max} = -0.77) noted between deuterium concentration and lipid content in particular biological fluids.

In condition of consuming water with a reduced content of deuterium, the greatest change in the deuterium concentration was observed in the oral fluid and blood plasma, whereas fluctuations in the deuterium concentration in breast milk were significantly less. At the same time reduced the absolute values of gradient (D/H): oral fluid> blood plasma ≥ human breast milk.

REFERENCES


