

Mass-spectrometry – a method for determining deuterium’s distribution in the body of the mammals under the effect of deuterium’s depleting medium

R. TAMAIAI*, I. ȘTEFĂNESCU, GH. TIȚESCU

National R & D Institute for Cryogenics and Isotopic Separation – I.C.I.T. Râmnicu Vâlcea
 Uzinei Street No. 4, PO 4, BOX 10, Zip Code 240050, Rm. Vâlcea, Romania

The general aspect of modifications induced by the administration of deuterium depleted water (light water) was the stimulation of cellular reactivity, reduction of harmful effects of different noxious agents, noticing also an inhibitor effect on several types of cancer. Referring at the prolonged administration of deuterium’s depleting medium it was pursued, in the present research, the mode and the grade in which deuterium is exchanged with hydrogen in the organism of mammals. The results shows that in natural conditions, the organism has the tendency to accumulate deuterium (bioaccumulation process). On the other hand, the grade of deuterium depletion seems to be under influence both of specimen genotype and behavior, and also of deuterium’s depleting medium concentration (whose effect is cumulated in time).

(Received November 15, 2006; accepted December 21, 2006)

Keywords: Deuterium exchange, Mass spectrometry, Deuterium variations

1. Introduction: deuterium (hydrogen’s constant isotope)

Hydrogen is the lightest element in nature and with a large distribution, especially as a hydrogen oxide (water) (H₂O) [1].

The main deuterium source in nature is the water. Concentration of deuterium in water varies according to the water’s geographical position of the source, decreasing gradually from oceans and seas towards the interior of the continents and reaching the minimum of concentration at the poles. Deuterium’s accepted average value in natural water is 144 ppm D/(D+H) [2]. Variations of these values are due to water’s circuit in nature and to the amount of precipitation, from the Earth’s three climatic regions (Fig. 1) [3, 4, 5]. Also, a small variation is registered with altitude.

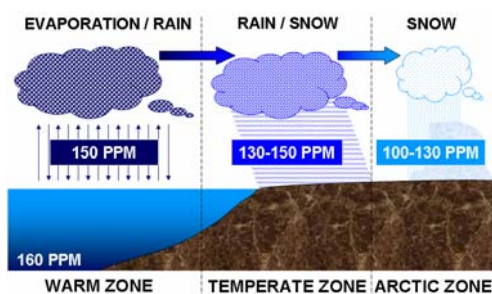


Fig. 1. The mechanism of deuterium’s variations in natural water.

As a result of water’s circuit in nature under the influence of zonal characteristics, the isotopic content in deuterium presents the following variations [6, 7, 8]:

- in oceanic waters: 150-160 ppm D/(D+H);
- in the waters from the ocean and sea coasts: 150-155 ppm D/(D+H);

- in continental waters: 130-150 ppm D/(D+H);
- in the ice bank and the polar cap: 100-130 ppm D/(D+H);
- in methane gas: 110-135 ppm D/(D+H);
- in hydrocarbons (petroleum): 125-135 ppm D/(D+H).

In over 70 years that passed since the deuterium was discovered in natural water [9] there were a lot of experiments that aimed to specify it’s effects on living matter. It was shown that while the normal quantities didn’t have harmful effects, the deuterium’s concentration increase (in culture mediums, in development mediums, alimentation, etc) respective the increase of deuterium’s intrinsic concentration through several methods with over 15-20% determines structural, metabolic, functional alterations in different grades, which can lead finally to organism’s death [1, 10, 11, 12].

Until the 90’s didn’t exist the possibility to reduce the quantity of deuterium in external or internal medium of living organisms. With the apparition of deuterium depleted water (DDW), it was obtained a rapid and easy way to reduce deuterium in biological systems by the simple administration in the development medium or in drinkable water of deuterium depleted water, also called light water.

Researches which were made have distinguished the fact that the water with reduced content in deuterium presents remarkable biological qualities with favorable effects on the state of health of animals.

In vitro, the obtained results with deuterium-depleted water (DDW) have emphasized the inhibitor effect of cellular proliferation at different cellular lines cultivated *in vitro* [13, 14, 15]. In this way, deuterium depleted water inhibits the development of the fibroblastic cells cultivated in nourishing mediums in which is present the water with low concentration of deuterium, and the cultivated cells are synchronized in G₀/G₁ phase. DDW inhibits the

synthesis of DNA at cellular lines M14 (human melanoma) which have been proved to be the most sensitive cellular line, 6 hours, toward the PC3 cellular line (prostate cancer), 24 hours and MCF7, (breast cancer) 48 hours. Also at those lines the inhibition was proved to be bigger when the synchronization was produced in G₀/G₁ phase and the concentration of deuterium in culture medium is lower.

In vivo, the research made on spontaneous experimental tumours or xenotransplanted tumours have emphasized the fact that the deuterium depleted water causes partial or complete regresses of xenotransplanted tumours [14, 16, 17]. In this way the xenotransplanted rats with the PC3 cellular line (prostate cancer) treated with DDW 90 ppm has determined a slowing of tumour's development and an increase of survival of cancerized animals with 40%. The treatment with DDW 30 ppm has begun with cellular line's MCF7 (breast cancer) xenotransplanted 24 hours post graft at immunosupressed rats determined a tumour regress with 60% comparative with the control lot. The therapy with DDW used in the veterinary clinic of oncology has induced a partial or complete regression. Thus, at dog and cat, with different spontaneous tumours, after the administration of DDW it was obtained an increase of survival and even the cure of animals, which have been treated. The treatment with DDW in human clinic on patients with malignant tumours has favorable effects. The best visible results after 1-2 months, since the beginning of the treatment, were obtained in the case when DDW came in direct contact with the tumour (tongue, esophagus, stomach, etc).

In the case of association of the cytostatic treatment with DDW, it was established not only that the therapeutically effect is more marked, but it was registered also a reduction of secondary effects of the cytostatic [18]. As action mechanism, deuterium could have a key role in the regulation of the processes with exchange of protons (H⁺), which adjusts the D/H report. In this case, the cells take notice of the modification of this report, the decrease of the deuterium's concentration produces an environmental stress, hereupon only the healthy cells can accustom quickly. From this meaning, the adjunct therapy with DDW must be continued a longer time [17, 18].

2. Experimental

Isotopic analyses of deuterium are effectuated through mass-spectrometry on water samples. For this reason, regardless of the nature or the form of biological sample, this must be processed so that the water from the sample to be extracted or to make a conversion of the sample so as the conversion's final product to be the water. For this the water was extracted through the *in vacuo* distillation method. Content determination of deuterium on the extracted water from the biological samples was made with a VARIAN MAT 250 mass-spectrometer. Water's extraction from the biological samples was made on samples resulted from the Wistar adult rats (males and females) hydrated with DDW for 60 days.

Healthy adult Wistar rats, weighting 120 grams, were raised and maintained in standard conditions in the biobase.

Lots taken in study:

- Witness lot: nourished with standard sustenance and drinkable water (60 days)
- Lot I (Experimental): nourished with standard sustenance and DDW 30 ppm (60 days);
- Lot II (Experimental): nourished with standard sustenance and DDW 60 ppm (60 days);
- Lot III (Experimental): nourished with standard sustenance and DDW 60 ppm (60 days).

Biological samples which are considered to be representative and resigned to necessary procedures for the deuterium's isotopic analysis through mass spectrometry were of two categories: liquid samples (blood) and solid samples (hepatic tissue and muscular tissue).

3. Results

Isotopic analysis results of biological samples resulted from the animals taken in study are presented in Table 1.

Table 1. Isotopic analysis results from the biological samples resulted from Wistar rats.

Species Individual (M = male / F = female)	Biological / (nourishment) sample's conc. in deuterium
R01 Rat Lot I – 1 (M) Blood	94.15 (30) ppm
R02 Rat Lot I – 2 (M) Blood	94.75 (30) ppm
R03 Rat Lot I – 3 (M) Blood	92.4 (30) ppm
R04 Rat Lot I – 1 (M) Liver	97.45 (30) ppm
R05 Rat Lot I – 2 (M) Liver	97.45 (30) ppm
R06 Rat Lot I – 3 (M) Liver	95.3 (30) ppm
R07 Rat Lot I – 1 (M) Muscle	94.65 (30) ppm
R08 Rat Lot I – 2 (M) Muscle	98.05 (30) ppm
R09 Rat Lot I – 3 (M) Muscle	96.15 (30) ppm
R10 Rat Lot II – 1 (M) Blood	112.05 (60) ppm
R11 Rat Lot II – 2 (M) Blood	110.85 (60) ppm
R12 Rat Lot II – 3 (M) Blood	108.05 (60) ppm
R13 Rat Lot II – 1 (M) Liver	112.75 (60) ppm
R14 Rat Lot II – 2 (M) Liver	110.3 (60) ppm
R15 Rat Lot II – 3 (M) Liver	108.9 (60) ppm
R16 Rat Lot III – 1 (F) Liver	117.35 (60) ppm
R17 Rat Lot III – 2 (F) Liver	116.35 (60) ppm
R18 Rat Lot III – 1 (F) Muscle	117.55 (60) ppm
R19 Rat Lot III – 2 (F) Muscle	115.6 (60) ppm
R20 Witness rat – 01 (M) Blood	148.6 (144) ppm
R21 Witness rat – 02 (M) Blood	148.65 (144) ppm
R22 Witness rat – 03 (M) Blood	149.35 (144) ppm
R23 Witness rat – 04 (F) Liver	146.8 (144) ppm
R24 Witness rat – 05 (F) Liver	147.6 (144) ppm
R25 Witness rat – 04 (F) Muscle	148.8 (144) ppm
R26 Witness rat – 05 (F) Muscle	148.35 (144) ppm

4. Discussion

Based on Table 1, Fig. 2 was composed and the isotopic analysis interpretation was made.

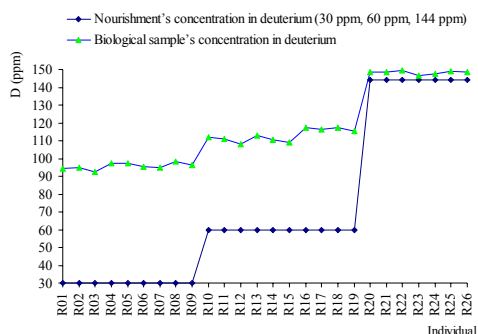


Fig. 2. Variations of deuterium concentration at rats.

From the analysis of Table 1 and Fig. 2, it can be observed that during the life (in normal conditions of development – 144 ppm) animal organism has the tendency to accumulate deuterium (the process of bioaccumulation). Also it can be observed that:

- at administration of DDW appear small variations concerning deuterium isotopic content of biological samples, probably because of the existent behavioral relations within the conspecific individuals from a group/lot and of individual and of individual's appetite (water and nourishment necessary), dictated by its genotype and phenotype;
- it was observed, a progressive decrease of deuterium content from the body of individuals which were raised with DDW 30 ppm comparatively with the body of the individuals which were raised with DDW 60 ppm;
- also it was ascertained that deuterium's level from different categories of samples (blood, muscle and liver) it's relatively balanced, without being an evident and constant correlation between tissue, its function and the localization in the organism.

5. Conclusions

As a result of previous analysis arise that the following conclusions that the incorporation grades of deuterium in animal's organism respectively the progressive capacity of elimination of deuterium from the tissues would be under the control of the interactions of several factors:

- endogenous factors.
- exogenous factors.

Endogenous factors: the specific characteristics of an individual dictated by the genotype and phenotype (water and nourishment necessary) and the behavioral relations established between the members of each lot (access at water and nourishment).

Exogenous factors: isotopic content in deuterium of DDW has a major weight in deuterium's grade of elimination from the organism and perhaps the time of administration of depleting medium.

Also it was observed that spreading of deuterium in the body of mammals is relatively uniform without being an evident and constant correlation between tissue, its function and the localization in the organism.

In future it is necessary the continuation of the experiments in progress to completely understand the mechanism and factors of bioaccumulation and depletion of deuterium from animal organism. Extremely useful would be an extension of the research also on bioaccumulation and on deuterium's distribution in organism at other mammal species, but also at other classes: birds, reptiles, amphibians, fish, and also at invertebrates, for creating an extended and useful data basis.

References

- [1] D. Kritchevsky. Ann. of The N.Y. Acad. of Sci. **84**(16), 575 (1960).
- [2] IAEA, World survey of isotope concentration in precipitation. Tech. Reports Series **96, 117, 129, 147, 165, 192, 226, 264, 311** (1969, 1970, 1971, 1973, 1975, 1979, 1983, 1986, 1990).
- [3] W. Dansgaard. Tellus **16**, 436-438 (1964).
- [4] I. Friedman, I. Redfield, A. C. Shoem, B. Harris, J. Harris. Rev. Geophys. **2**, 177-224 (1964)
- [5] IAEA, Deuterium and oxygen-18 in the water cycle. J. R. Gat and R. Gonfiantini (Eds.). Stable Isotope Hydrology. Tech. Reports Series **210, 337** (1981)
- [6] I. Friedman. Geochim. et Cosmochim. Acta **4**, 89-103 (1953).
- [7] H. Craig, L. Gordon. Ed. E. Tongiorgi, Stable Isotopes in Oceanographic Studies and Paleotemperatures, Spoleto, 9-130 (1965).
- [8] C. Kendall, J. J. McDonnell, Isotope tracers in catchments hydrology, Elsevier Science B. V., Amsterdam (1998).
- [9] H. C. Urey, F. G. Brickwedde, G. M. Murphy. Physics Review **39**, 164 (1932).
- [10] V. Ia. Aleksandrov. Tsitologia **28**(8), 790-795 (1986).
- [11] I. Hăulică, M. Peculea, I. Ștefănescu, Gh. Țițescu, M. Todiraș, W. Bild. Rom. J. of Phys. (Phys. Sci.), **35**(1-2), 25-32 (1998).
- [12] J. F. Thomson. Ann. of The N.Y. Acad. of Sci. **84**(16), 736-744 (1960).
- [13] G. Somlyai, G. Jancso, G. Jackli, K. Vass, B. Barna, V. Lakics, T. Graal. FEBS Letters **317**(1-2), 1-4 (1993).
- [14] G. Somlyai, G. Laskay, T. Berkényi, Z. Galbács, G. Galbács, S. A. Kiss, Gy. Jákli, G. Jancsó. Z. Onkologie / J. of Oncology **30**(4), 91-94 (1998).
- [15] G. Somlyai, Z. Gyöngyi. In vivo **14**, 437-440 (2000).
- [16] G. Somlyai, G. Laskay, T. Berkényi, Gy. Jákli, G. Jancsó. Ed. John Wiley & Sons Ltd. Paper **24**, 137-141 (1998).
- [17] G. Somlyai, G. Jancsó, Gy. Jákli, T. Berkényi, Z. Gyöngyi, I. Ember. Anticancer Research **21**, 16-17 (2001).
- [18] G. Somlyai, Să învingem cancerul. Ed. CONPHYS, Rm. Vâlcea (2001).