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NEW APPROACH TO ASSESSMENT OF AGING PROCESSES AT THE CELLULAR LEVEL BY NMR SPECTROSCOPY

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Abstract: The paper presents results of the proton relaxation time T_1 measurements by ^{1}H NMR for oral epithelium samples taken from 320 subjects, men and women, in different age, from 19 to 95 year old. The times of nuclear magnetic relaxation T_1 were found to be correlated with the age of the subjects.

1. INTRODUCTION

The process of biological ageing of human organism is a multifaceted phenomenon, taking place on many levels of organisation of biological structures. The current studies of the ageing phenomena undertaken by gerontologists and geriatrists concern different aspects of the problem [1, 2] and the hypotheses or theories proposed are ambiguous and incoherent [3, 4]. It is known that the biological processes of ageing observed on the level of individual organisms and populations, are the results of those occurring on the cellular and molecular levels. Taking this fact into regard, the aim of the study reported in this paper was to check if the nuclear magnetic resonance technique was suitable to detect the process of ageing of a biological tissue in its natural environment, on the molecular level. The results of ^{1}H NMR measurements of T_1 relaxation times were proved to be correlated with the age of the subjects. Moreover, the results suggest that the biological changes in the tissue (changes of different origin e. g. lipid oxidation, cancerous processes) occurring as a function of time, impose changes in the chemical composition of the solution studied and consequently, the values of T_1 time.

2. MATERIALS AND METHODS

The substance studied was the oral epithelium tissue placed in a 0.09% solution of NaCl. The material was collected from 320 subjects aged from 19 to 95, including 222 women (from 19 to 95 years of age) and 98 men (from 21 to 71 years of age). The ^{1}H relaxation times T_1 were measured at the frequency of 200 MHZ using a Bruker CXP 200 MHZ pulse spectrometer, using the standard saturation-recovery pulse sequence $n \cdot \pi_{+x} - \tau - \pi/2_{+x}$. The $\pi/2$ pulse was 4.6 μs, $\tau = 5$ μs ÷ 10 s, $TD = 4096$, $NS = 16$. Because of a large number of samples, a typically used solenoid B_1 field coil was replaced by a simple saddle collinear coil. All measurements were carried out at 310 K, which was stabilised using a gas-flow temperature control unit with an accuracy of ±0.5 K.

3. RESULTS AND DISCUSSION

The simple regression lines proved a negative correlation of the T_1 relaxation time values with age, which was statistically significant both for men and for women. The correlation coefficient

values are 0.25 and 0.22, for women and for men, respectively and are statistically significant at the level $p < 0.05$ (Fig. 1). The degree of damage to lipid-protein membranes [5] increases with age and one of the reasons explaining this phenomenon is peroxidation of lipids under the influence of reactive forms of oxygen [6, 7]. The increase in the amount of the reactive forms of oxygen with age is responsible for decreasing molecular dynamics of the membranes, increasing amount of cholesterol in them as well as increasing number of fatty acid chains in phospholipids. The amount of the products of decomposition of glycoproteids and glycolipids deposited in the membranes increases and the enzymes in the membranes get damaged, which results in a decrease in the rate of metabolism and in a general increase in the rigidity of the membranes. Since hydrogen is the main element in the molecular structure of lipid-protein membranes, it has been assumed a reliable indicator of molecular dynamics of the membranes, suitable for assessment of the degree of their stiffening appearing as a result of the ageing.

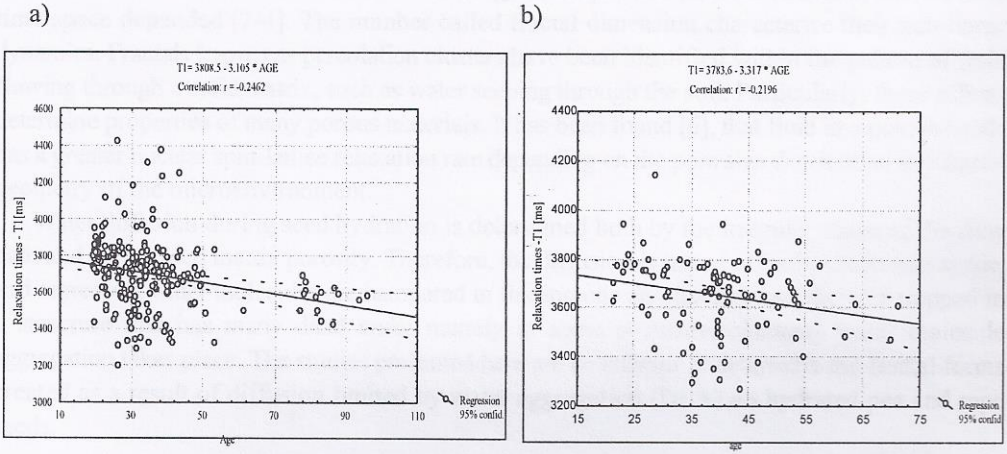

Fig. 1. Linear regression lines of T_1 relaxation time measured in oral epithelium tissue and the age of the subjects for a) men and b) women

4. CONCLUSIONS

The results confirm the indications following from the preliminary studies [8], performed for the stable and involution phases of ontogenesis, about the negative correlation between the T_1 relaxation times measured by ^{1}H NMR for oral epithelium samples and the age of the sample donors. Farther studies are expected to verify the thesis that the measurements of T_1 relaxation time may provide the information about the rate of the ageing processes (e. g. by allowing a determination of the degree of degradation of the lipid compounds).

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