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ZBIGNIEW CZAPLA[1], ZBIGNIEW FOJUD[2],
[1]Institute of Anthropology
Department of Human Developmental Biology, Poznań, Poland
[2]Institute of Physics
Department of Macromolecular Physics, Poznań, Poland

# THE APPRAISAL OF THE SCOPE FOR THE APPLICATION OF NUCLEAR MAGNETIC RESONANCE (NMR) MEASUREMENT METHODS FOR THE ESTIMATION OF THE BIOLOGICAL AGE IN VARIOUS STAGES OF HUMAN ONTOGENY

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**Abstract.** The main objective of the study is to find a new physical parameter meeting the criterion of the biological age estimation in various stages of human ontogeny, and thus to find a new research methodology for this field of research. For this purpose an NMR Bruker 200 MHz spectrometer was used. Two physical parameters: relaxation time ($T_1$) and spectrum shape were determined. The research material was oral mucosa epithelium sampled in a group of young people of both sexes (31 subjects) and in a group of men (18 subjects). Relaxation time ($T_1$) and spectrum shape were recorded for hydrogen nuclei ($H^1$). The physical phenomena were analysed statistically (regression analysis) and their biological interpretation was attempted.

**Key words:** *EMN - Electrophoretical Mobility of Nuclei, stage of ontogeny, biological age, ageing, free radical theory of ageing, NMR - Nuclear Magnetic Resonance, chemical shift, spin-lattice relaxation time*

## Introduction

The Electrophoretical Mobility of Cell Nuclei method used to estimate the biological age in various stages of human ontogeny, confirmed a common character of ontogenetic processes (Shakhbazov et al. 1985, 1986, Makałowska 1992, Cieślik et al. 1994, Cieślik 1995, Shakhbazov et al. 1996, Czapla 1996). Changes of the index of Electrophoretical Mobility of Nuclei (EMN) in the course of development confirm the phasic character of ontogeny characteristic of a population, with the preservation of varying levels of value of this index for a single individual. The EMN phenomenon – not fully explained as yet – is related to the biochemical makeup of cellular structures and their physiology and to physical and chemical or biodynamical properties of these structures.

In relation to the above we undertook interdisciplinary research, in order to find a physical parameter which would not be of strictly biological nature and which could become a new criterion for the estimation of the biological age in various stages of ontogeny. Finding a new physical parameter which would confirm a specific reaction of an individual within the scope of his response norm in the course of development does not seem easy. Taking the above problems into consideration we defined the following research objectives:

1. Determination of the usefulness of spectroscopy methods (NMR) for the estimation of the biological age and finding of physical parameters which could serve this purpose – working out of a new methodology of research.
2. Discussion on the differences in the development of the two physical parameters in the course of ontogeny and the appraisal of their usefulness:
   a) relaxation times ($T_1$),
   b) spectrum shape.
3. Attempt of biological interpretation of the occurring physical phenomena.
4. Attempt to answer the question: whether spectroscopy methods (NMR) and distinguished physical parameters may in future become a new criterion for the estimation of the biological age in various stages of human ontogeny.

## Material and Methods

The study covered a group of young people between 13 and 21 years of age of both sexes (31 subjects) and a group of men aged from 26 to 54 years (18 subjects). Oral mucosa epithelium was the research material. Sampling method was compatible with the method used in EMN studies (after Makałowska 1992), but due to certain technical problems, material awaiting measurement was kept in Ependorf test tubes in the temperature of $-18^{\circ}C$.

In order to determine the degree of usefulness of NMR measurement methods and to find out which physical parameters may be useful for the estimation of the biological age, measurements of spin-lattice relaxation times ($T_1$), spectrum shape and chemical shift ($H^1$), that is of hydrogen nuclei (protons) were taken

(Hausser, Kalbitzer 1993, Ślósarek 1996). For the study of relaxation times ($T_1$) of hydrogen nuclei in epithelium cells dissolved in 0.09% NaCl solution, a NMR Bruker 200 MHz pulse spectrometer was used as well as a standard inverse-recovery pulse sequence (Sobol 1981). Free precession signal values were analysed off peak at the 2/3 of the signal height. A non-standard construction of the $B_1$ field coil was used. For the duration of the experiment the co-linear solenoid coil was replaced with a simple saddle coil (Hayes et al. 1985). All the measurements were taken in the temperature of 37°C, stabilised with the accuracy of 0.1°C.

The phenomenon of the nuclear magnetic resonance consists in the resonance absorption of the energy of $B_1$ field electromagnetic wave (radiofrequency pulse) travelling through the coil and subsequently through nuclear spins in the presence of the external magnetic field $B_0$ generated in our experiment with a superconducting magnet. The radio pulse frequency was 200 MHz, while field induction in the superconducting magnet – 4.7 Teslas. Atomic nuclei (of hydrogen atoms) with non--zero magnetic moment become polarised in relation to the magnet field. We refer to such state as to the thermodynamic equilibrium of nuclear spins. Measuring of any physical values in such a system is possible only when the thermodynamic equilibrium of the system is disturbed (there is some external factor influencing it). This can be achieved through the generation of a radio pulse with resonance frequency ($\omega_0$). When the resonance condition (described with Larmor equation $\omega_0 = \gamma B_0$) is met, a strictly determined quantity of energy necessary to disturb the equilibrium of the system is absorbed (nuclear spins move to a higher energetic level). Such a system emits the earlier absorbed energy (returning to the lower energetic level) in the form of the free nuclear spin precession signal (in other words the system returns to the state of equilibrium after the time called spin-lattice relaxation time or $T_1$), (Ślósarek 1996). The $T_1$ parameter is an ideal tool for the determination of the nuclear dynamics of molecular structures and their metabolic and functional properties (Kowalski et al. 1991, Sequin et al. 1992). The following correlation occurs: the shorter relaxation time ($T_1$) the more rigid (more stable) the system, and conversely, the longer ($T_1$) the more mobile (prone to changes) the system.

The shape of the spectrum was the other parameter we studied. We focused our attention in particular on the signal bandwidth and its chemical shift in relation to water signal. The observed spectrum results from the mathematical analysis (fast Fourier transformation) of the free precession signal (Bracewell 1968).

## Analysis of results

The material was divided into 8 age categories (Fig. 1). In the group of females only 3 age categories were distinguished. The material is relatively scarce in particular categories, which is true for its entire body. Mean values of relaxation times ($T_1$) in particular age categories are as follows: in the male group mean relaxation

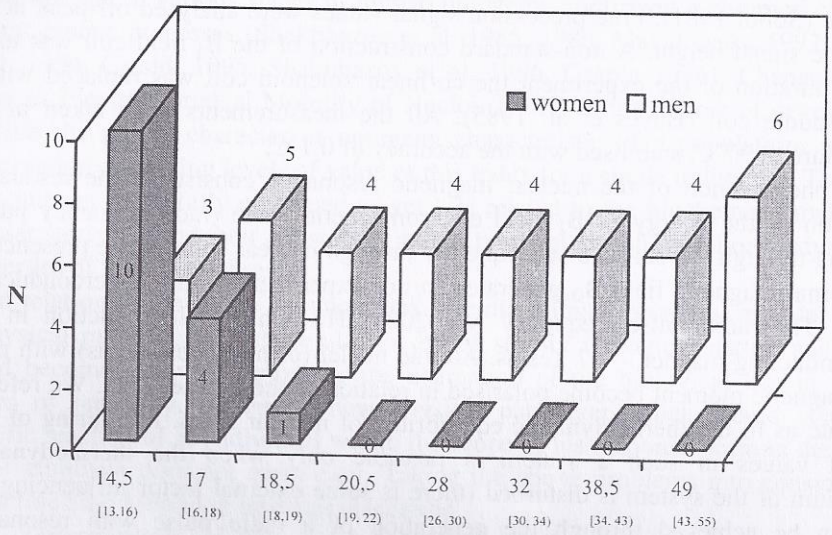


Fig. 1. Number of subjects in particular categories for the whole body of the studied material

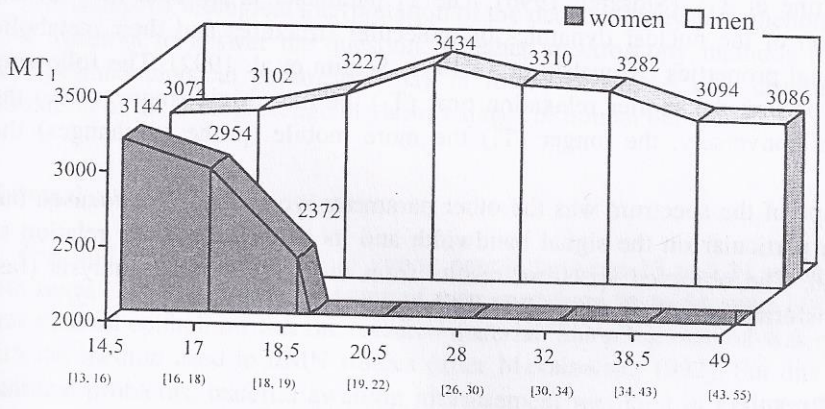


Fig. 2. Mean values of relaxation times in particular age categories

times grow in the progressive stage of ontogeny, then they gradually decrease up to the age of 55 marked with the class mid-value of 49 years of age, while in the group of girls mean values drop along with the progress of age (Fig. 2).

For the studied material regression lines of relaxation times ($T_1$) with age were determined. These confirmed the observed changes of mean values. Within the male group a group of boys aged between 15 and 22 years and a group of males over 25 years of age were distinguished. A growing tendency was observed in the group of boys. Here the growing value of the correlation coefficient $r = 0.44$, (for $N = 16$ $\alpha = 0.0863$), is significantly different from zero at the level of $\alpha = 0.1$ (Fig. 3).

In the group of males a negative tendency was observed, correlation coefficient $R = -0.55$ (for $N = 18$ $\alpha = 0.0188$). It is significantly different from zero at the significance level $\alpha = 0.05$ (Fig. 4). For girls the tendency with regard to the time ($T_1$) is negative, correlation coefficient $r = -0.42$ (for $N = 15$ $\alpha = 0.1232$), thus it is not significantly different from zero when $\alpha = 0.05$ (Fig. 5).

The other parameter we focused upon was the shape of the spectrum. In this case the material was divided by the year of birth. Five typical spectra representing particular year groups (1940, 1950, 1960, 1970 and 1980) were distinguished. All spectra (Figs. 6, 7, 8, 9, 10) are characterised with the narrow line derived from water. Wide components of the central signal are probably related to the occurrence of hydrogen nuclei ($H^1$) in the structures of chemical compounds close to or in direct chemical relation to water. Our observations indicate that in the area of 1–2 ppm of chemical shift in particular year groups a signal of a certain bandwidth characteristic of a given year group occurs. For the individuals born in 1980 and 1970 year groups (Fig. 6 and 7) from 2 to several week separable signals of relatively small bandwidth occur in this area. For individuals from 1960 year group (Fig. 8) we observe widening of spectrum lines in this area and a slight increase of their amplitude. The effects grow clearly stronger for older individuals born in 1950 and 1940 (Fig. 9 and 10), where in the area from 1 to 2 ppm chemical shift there is only one wide line of a relatively high amplitude in relation to water signal.

## Biological interpretation

A question arises: How can one interpret the changes of the distinguished parameters with age?

With regard to the metabolic processes occurring at the cellular and tissular level in the course of ontogenetic development, three principal developmental stages are distinguished: 1) progressive stage (anaplasis-evolution) with anabolic processes prevailing over catabolic processes, 2) stable stage (metaplasis-transvolution) with a balanced course of these processes, and 3) regressive stage (cataplasis-involution) with catabolic processes prevailing over anabolic ones. This is the "model" course of the ontogenetic development. Since the observed relaxation times ($T_1$) and

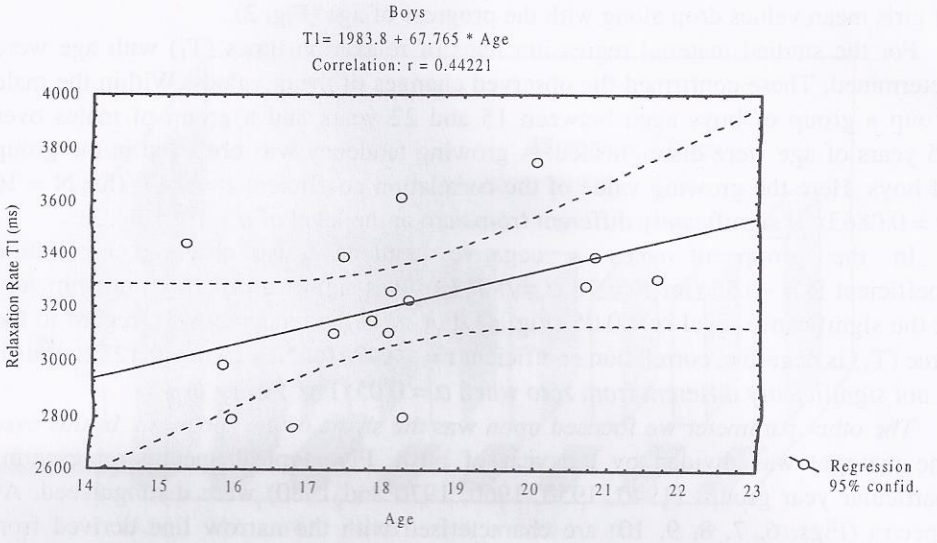


Fig. 3. Regression line of relaxation time $T_1$ with age – boys

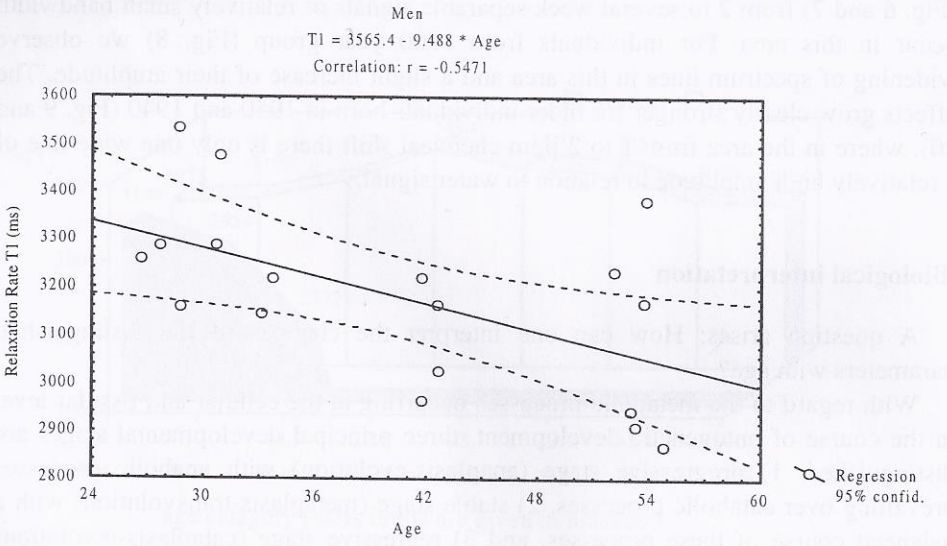


Fig. 4. Regression line of relaxation time $T_1$ with age – men

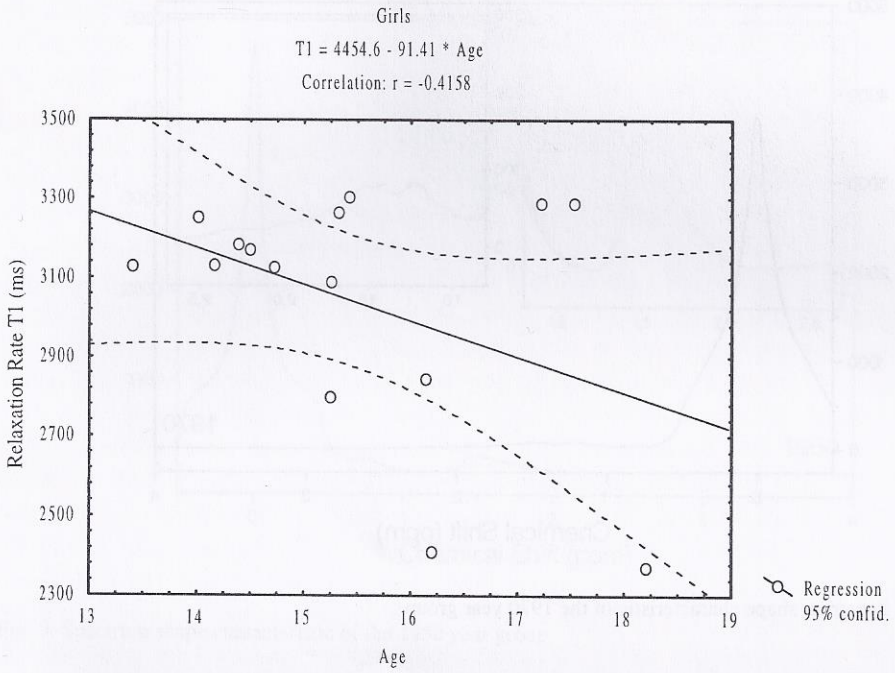


Fig. 5. Regression line of relaxation time $T_1$ with age – girls

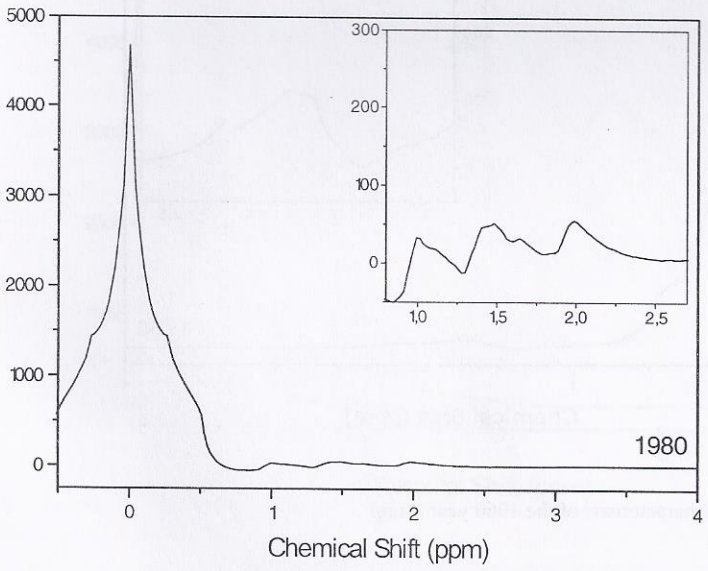


Fig. 6. Spectrum shape characteristic of the 1980 year group

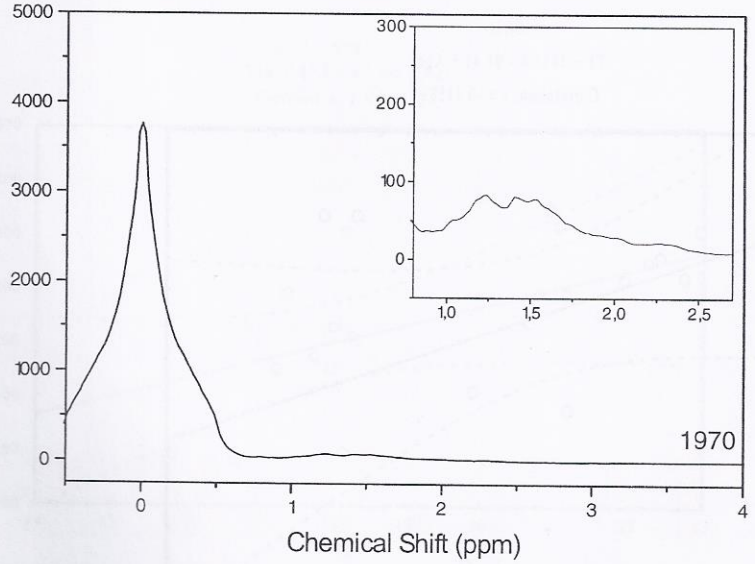


Fig. 7. Spectrum shape characteristic of the 1970 year group

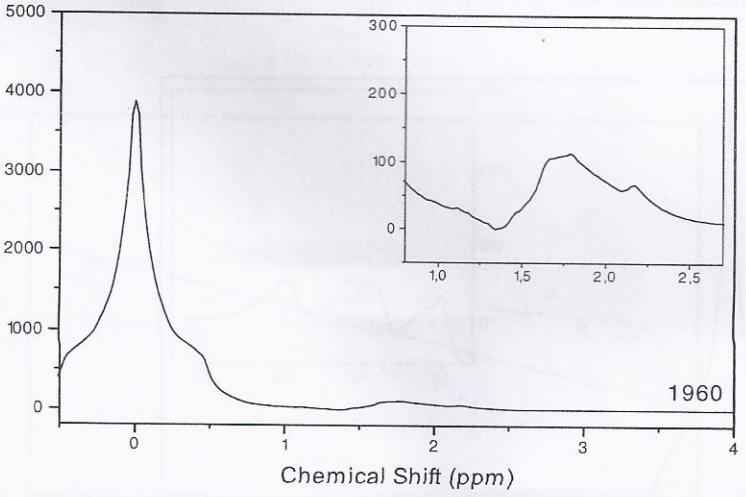


Fig. 8. Spectrum shape characteristic of the 1960 year group

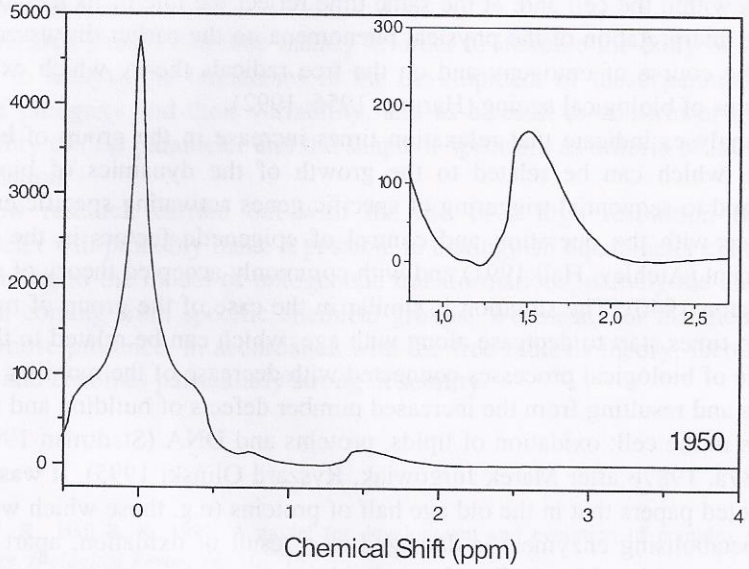


Fig. 9. Spectrum shape characteristic of the 1950 year group

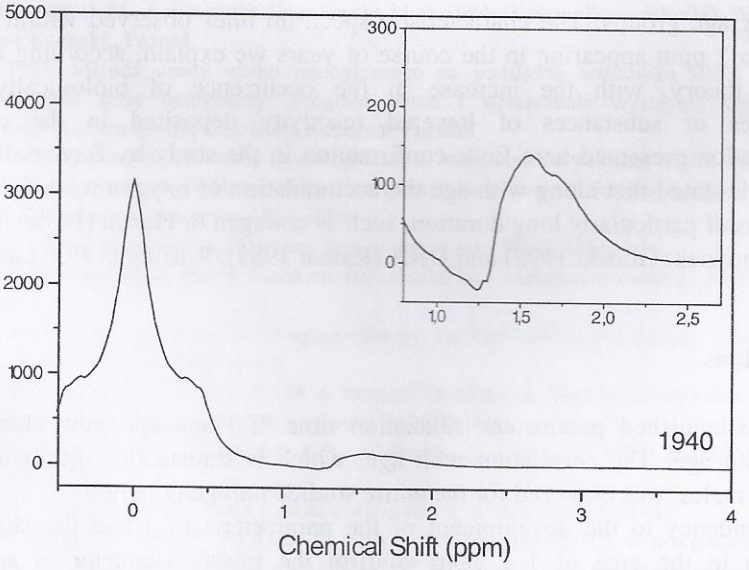


Fig. 10. Spectrum shape characteristic of the 1980 year group

spectra shapes are good indicators of the dynamics of the changes in biomolecular structures within the cell and, at the same time reflect the rate of its metabolism, we based the interpretation of the physical phenomena on the earlier discussed "model" way of the course of ontogeny and on the free radicals theory which explains the mechanisms of biological ageing (Harman 1956, 1992).

Our analyses indicate that relaxation times increase in the group of boys along with age, which can be related to the growth of the dynamics of biomolecular systems and to sequential triggering of specific genes activating specific enzymes in accordance with the operation and control of epigenetic factors in the course of development (Atchley, Hall 1991) and with commonly accepted theory of epigenesis (Waddington 1940). The situation is similar in the case of the group of men, where relaxation times start to decrease along with age, which can be related to the decline of the rate of biological processes connected with decrease of the mobility of tissular structures and resulting from the increased number defects of building and enzymatic structures in the cell: oxidation of lipids, proteins and DNA (Stadtman 1992, Oliver et al. 1987a, 1987b after Marek Jurgowiak, Ryszard Oliński 1995). It was indicated in the quoted papers that in the old age half of proteins (e.g. those which were earlier active, metabolising enzymes) is damaged as a result of oxidation, apart from that migration in rigid oxidised albuminous-lipidous membranes is also made difficult (Jurgowiak, Oliński 1995), (Oliński, Jurgowiak 1996). Such structures are more rigid by their nature and hence shorter relaxation times are observed. The above interpretation fails in the case of the female group. This probably results from the scarcity of the material and uneven distribution of the numbers of subjects in particular age groups. The characteristic spectrum lines observed within the range from 1 to 2 ppm appearing in the course of years we explain, according to the free radicals theory, with the increase in the occurrence of biologically inactive substances or substances of lowered reactivity deposited in the cells. The interpretation presented here finds confirmation in the study by Baynes from 1991, where it is stated that along with age the accumulation of oxygen related damages in molecules of particularly long duration, such as collagen or elastin (Bailey et al. 1990 after Jurgowiak, Oliński 1995) and DNA (Rattan 1989), Rusting (1993) takes place.

## Conclusions

1. Distinguished parameters: relaxation time ($T_1$) and spectrum shape change along with age. The correlation with age, which is statistically significant for the group of males, was observed for the entire studied ontogeny period..

2. Tendency to the development of the parameters: ($T_1$) and the shape of the spectrum in the area of 1–2 ppm confirm the phasic character of ontogenetic processes characteristic of the population. Within the distinguished age groups individual differentiation of the values of the mentioned parameters for an individual was observed.

3. Spectroscopy (NMR) methods – next to other methods used in auxology – proved to be useful.

4. Research should continue mainly in order to increase the body of the studied material, to analyse the tendencies in the development of these parameters in the course of ontogeny and their variability, and as a result to confirm or exclude the applicability of ($T_1$) parameter and the shape of spectrum as criteria of the biological age estimation.

5. New research carried out with the use of a high resolution (HR) pulse spectrometer will probably make it possible to distinguish a parameter changing with age according to the model of ontogenetic transformations, namely the amplitude of the signal coming from specific chemical groups. We mean, for instance, carbonyl groups whose presence, in accordance with the free radicals theory, increases along with age and becomes particularly strong in senility.

## References


Atchley W. R., Hall B. K. 1991. A model for development and evolution of complex morfological structure. *Biological Revue*, 66: 101–157.

Bailey A. J., Ranta M. H., Nicholls A. C., Partridge S. M., Elsden D. F. 1977. Isolation of alfa-amino adipic acid from mature dermal collagen and elastin: evidence for an oxidative pathway in the maturation of collagen and elastin. *Biochem. Biophys. Res. Commun.*, 78: 1403–1410.

Baynes J. W. 1991. Role of oxidative stress in development of complications in diabetes. *Diabetes*, 40: 405–412.

Cieślik J., Kaczmarek M., Kaliszewska-Drozdowska M. D. 1994. Dziecko Poznańskie 90. Wydawnictwo Naukowe Bogucki., Poznań.

Cieślik J. 1995. Metoda oceny wieku biologicznego na podstawie wskaźnika EMN w stabilnej i inwolucyjnej fazie ontogenezy. Program obrad i streszczenia wystąpień Ogólnopolskiej Konferencji Naukowej, Człowiek istota nieznana? Poznań.

Czapla Z. 1996. Poziom rozwoju cechy morfologicznej w ontogenezie a wskaźnik EMN. Program obrad i streszczenia wystąpień Ogólnopolskiej Konferencji Naukowej: Człowiek wczoraj, dziś, jutro. Agencja Wydawniczo-Handlowa AD, Lublin.

Bracewell R. 1968. Przekształcenie Fouriera i jego zastosowania. WNT, Warszawa .

Harman D. 1956. Ageing: a theory based on free radical and radiation chemistry. *J. Gerontol.*, 11: 298–300.

Harman D. 1992. Free radical theory of aging: history. In: Free radical and ageing, (eds.) Emerit I., Chance B. Boirkahauser. Basel. 1–10.

Hausser K. H., Kalbitzer H. R. 1993. NMR w biologii i medycynie. Badania strukturalne, tomografia, spektroskopia in vivo. Wydawnictwo Naukowe UAM, Poznań.

Hayes C. E., Edelstein W. A., Schenck J. F., Muller O. M., Eash M. 1985. *Journal of Magnetic Resonance*, 63: 622.

Jurgowiak M., Oliński R. 1995. Wolne rodniki a starzenie się. *Kosmos*, 44(1): 71–88.

Kowalski P., Jurga K., Miral J., Woźniak A., Sowier J. 1991. Pomiar $T_{1\rho}$ w nowotworach żołądka. Materiały XXIII ogólnopolskiego seminarium nt. Magnetycznego rezonansu jądrowego i jego zastosowań. Raport Nr 1534/PL Kraków.

Makałowska I. 1992. Przydatność i zakres stosowalności kryteriów oceny wieku biologicznego w badaniach ontogenetycznych na przykładzie metody EMN i metody analizy morfologicznej. Maszynopis pracy doktorskiej. Poznań.

Nohl H., Kramer J. 1980. Molecular basis of age-dependent changes in the activity of adenine nukleotide tranlocase. *Mech. Ageing Dev.* 14: 137–144.

Oliński O., Jurgowiak M. 1996. Reaktywne formy tlenu – uniwersalny czynnik patogenny? [ In: ] *Nowe tendencje w biologii molekularnej i inżynierii genetycznej oraz medycynie.* Barciszewski J., Łastowski K., Twardowski T. (eds.) Wydawnictwo Sorus, Poznań, pp 373–400.

Oliver C. N., Leviner R. L., Stadtman E. R. 1987a. A role of mixed function oxidation reactions in the accumulation of altered enzyme forms during ageing. *J. Am. Geriat. Soc*, 35: 947–956.

Oliver C. N., Alin B. W., Moerman E. J., Goldstein S., Stadtman E. R. 1987b. Age-related changes in oxidised proteins. *J. Biol. Chem*, 262: 5488–5491.

Pacifici R. E., Davies K. J. 1991. Protein, lipid and DNA repair systems in oxidative stress: the free radical theory of ageing revisited. *Gerontology*, 37: 166–180.

Przygocki W. 1990. Metody fizyczne badań polimerów. PWN, Warszawa.

Rieger R., Michaelis A., Green M. M. 1974. Słownik terminów genetycznych, PWRiL, Warszawa.

Rusting R. L. 1993. Dlaczego się starzejemy? *Świat Nauki*, II. 85–95.

Shakhbazov V. G., Nabokov A. L., Kolupaeva T. V. 1985. Avtorskoje svidetelstvo Nr 11696114. MKI A 61 V 10/00. Sposob opredelenija biologiceskovo vozrasta celoveka.

Shakhbazov V. G., Kolupaeva T. V. Nabokov A. L. 1986. Novyj metod opredelenija biologiceskogo vozrasta celoveka. *Laboratornoe Delo*, 7: 404–406.

Shakhbazov V. G., Grygoreva N. N., Kolupaeva T. V. 1996. Novyj cito-biofizyceskij pokazatel biologiceskovo sostajanija organizma celoveka. *Fizjologija Celoveka*, 22(6): 71–75.

Sobol W. 1981. Fizyka – Molekularna pracownia specjalistyczna. Metody impulsowe. 110–151. Uniwersytet Śląski, Katowice.

Stadman E. R. 1992. Protein oxidation and ageing. *Science*, 257: 1220–1224.

Sequin F., Pape A., Wiliams S. R. 1992. MRS of Cells. In: Magnetic Resonance Spectroscopy in Biology and Medicine, (eds.) Certaines J.D.D.E., Bovée W.M.M.J., Podo F. Pergamon Press.

Ślósarek G. 1996. Analiza strukturalna białek przy wykorzystaniu wielowymiarowej spektroskopii magnetycznego rezonansu jądrowego [ In: ] Nowe tendencje w biologii molekularnej i inżynierii genetycznej oraz medycynie. Barciszewski J., Łastowski K., Twardowski T. (eds.), Wydawnictwo Sorus, Poznań, p. 567–589.

Waddington C. H. 1940. The genetic control of wing development in Drosophila. *J. Genet.*, 41: 75.