Variability and Evolution, 2000, Vol. 8: 135–141

ZBIGNIEW CZAPLA

Adam Mickiewicz University
Institute of Anthropology, Department of Human Developmental Biology, Poznań, Poland

THE PHASENESS OF HUMAN BIOLOGICAL DEVELOPMENT ASSESSED WITH THE USE OF SELECTED PHYSICOCHEMICAL METHODS[1]

CZAPLA Z. 2000. The phaseness of human biological development assessed with the use of selected physicochemical methods. *Variability and Evolution,* Vol. 8: 135–141, Adam Mickiewicz University, Faculty of Biology, Institute of Anthropology, Poznań.

The principal idea of the work was to show a new, original method of description of the phenomena of human biological development with the use of non-standard research methods so far unused in the ontogenetic studies in regard to the stable and involutional phases of ontogenesis. The main purpose of the work was to assess – with selected methods – individual reactions of a human organism in ontogenesis against the development of the population. Two research methods were selected. They were subsequently used to show individual reactions of a single human organism against the phenotypic variability characterising the population. The research methods employed are based on the physical and chemical properties of biological cells (the Electrophoretic Mobility of Cell Nuclei (EMN) method) as well as on the physical properties of selected physical parameters describing the properties of biological structures (the Nuclear Magnetic Resonance (NMR) spectroscopy method). One of the objectives of the study was to corroborate the applicability of the EMN index method as an adequate criterion

[1] A summary of the thesis submitted for the degree of Doctor of Sciences in Adam Mickiewicz University, Poznań 1999. Professor conferring the degree: Joachim Cieślik. The title of the thesis: "The phaseness of human biological development assessed with the use of selected physicochemical methods".

of biological age estimation in each phase of ontogenesis. High sensitivity of the EMN index made it possible to check to what extent a reaction of an individual, resulting from the operation of selected factors increasing the risk of premature death (nicotinism, alcoholism), would permit to assess the deviation of this individual organism from the theoretical course of its ontogenesis determined with the EMN method. Another task under the study involved checking to what degree the ontogenetic development curve in selected subject groups (alcoholics, smokers and non-smokers) determined with the EMN method deviated from the "control" curve.

The principal research tasks regarding the methods of the Nuclear Magnetic Resonance spectroscopy involved attempts to find a new physical parameter, which could serve as a measure of the advancement of certain biological processes accompanying human ontogenesis. In other words an attempt was made to find a physical parameter which could become a new criterion of human biological age estimation regardless of the phase of ontogenesis.

Material and Methods

The research material used in the work was obtained in a cross-sectional study. Since two methods (EMN and NMR) were in use, the material was divided in two separate parts. The research material studied with the EMN method was buccal epithelium from the mucous membrane of the oral cavity collected from 1966 live subjects, including 1299 women aged from 18 to 98 years and 667 men aged from 19 to 93 years. In the case of the NMR spectroscopy method the material was buccal epithelium from the mucous membrane of the oral cavity collected from 320 live subjects, including 222 women aged from 19 to 95 years and 98 men aged from 21 to 71 years. In the framework of partial studies researches were carried out on material of various size, involving individuals, groups of subjects and the entire body of material. This was done in order to show directional changes in the selected properties characterising the population. Furthermore, body mass and height measurements of the subjects under study were taken.

As mentioned before the basis of the research was the application of two methods used for the description of the developmental processes taking place in the material under study in the stable and involutional phases of ontogenesis. The first of the methods, the Electrophoretic Mobility of Cell Nuclei (EMN) method, which was first used in the estimation of human biological age by the researchers from the Department of Genetics and Cytology of Kharkov University, Ukraine (Shakhbazov et al. 1985, 1986a, 1986b). This interesting physicochemical phenomenon on the fact of the mobility of cell nuclei in the variable electrical field as well as the fact that in the course of ontogenesis a change is observed in the proportions between the number of the cells with mobile nuclei and the number of cells with immobile nuclei (Czapla, Cieślik 1998).

The percentage value of the EMN index is determined observing the ratio of the number of cells with mobile nuclei to those with immobile nuclei (per 100 cells taken into consideration, maintaining a proper ratio of the size and colour of the nucleus to the size of its cytoplasm). The other method is based on nuclear magnetic resonance (NMR) spectroscopy (Sequin et al. 1992; Hausser, Kalbitzer 1993; Ślósarek 1996), a phenomenon consisting in the resonance absorption of the energy of the electromagnetic wave of field B1 (radioimpulse) travelling through a coil and then through nuclear spins in the presence of the external magnetic field BO, generated in the experiment by a superconducting magnet. The frequency of the radioimpulse was 200 MHz, with the field in the superconducting magnet of 4.7 Tesla. Atomic nuclei (of hydrogen) with non-zero magnetic moment acquire a certain uniform orientation in relation to the field of the magnet. This state is referred to as thermodynamic equilibrium of nuclear spins. Measuring of any physical parameters in such a system is possible only when the system's thermodynamic equilibrium is disturbed (if the system is thrown out of balance due to some external influence). This can be achieved by means of a radioimpulse with a resonance frequency (ω_0). If the resonance condition (described with Larmor equation $\omega_0 = \gamma B_0$) is satisfied, a strictly determined amount of energy necessary to unbalance the system will be absorbed (nuclear spins jump to the higher energy level). Such a system gives back the earlier absorbed energy (reverting to the lower energy level) in the form of a signal of free precession of nuclear spins (which means it returns to the state of equilibrium after a period of time called relaxation time T1). Parameter T1 is an ideal instrument for describing the nuclear dynamics of molecular structures and their metabolic and functional properties. The following relation is observed: the shorter relaxation time (T1), the more stable the system, and vice versa – if time (T1) is longer, the system is more mobile and more prone to change. With these properties in mind, the author attempted to find out by means of population research how the relaxation time parameter T1 changed with age in the stable and involutional phases of ontogenesis (Czapla, Fojud 1999).

The EMN index values are measured with the Biotest apparatus specially constructed for this type of tests. The physical parameter T1 was measured with an NMR Bruker 200 MHz impulse spectrometer.

Results

The selected physicochemical methods applied in the study have made it possible to describe the course of ontogenetic processes in the stable and involutional phases in a manner different to that known from the widely used methods based on the examination of morphological traits. A distinct regression of the parameters under study has been observed in the stable and involutional phases of the ontogenesis, which is not recorded in the case of parameters such as body mass. As regards parameter such as body height, the nature of the changes is similar, but is not as distinct as in the case of the parameters under study.

The first part of the analysis related to the EMN method consisted in the assessment of the reliability of the applied method, and in the calculation of measuring errors of the intra- and intersubjective error. A small intrasubjective error and the high repeatability of the measurements testify to high reliability of the EMN index measurements.

Research carried out on selected individuals over periods of time covering 24 hours, one week, 2 months, and 7 months revealed that the EMN index is very sensitive to the action of various environmental factors, which is reflected in the values of individual deviations of this index in daily tests. Operation of factors commonly regarded as having adverse effect on the state of health, e.g., alcohol, resulted in the negative direction of the EMN index deviations, while the absence of such factors or the action of the factors regarded as beneficial to health such as rest, healthy diet, had effect in the form of the positive direction of the EMN index deviations. This property of the EMN index makes it possible to use it as an indicator of an organism's individual reactions to a selected environmental factor. This means that the EMN index could serve as an indicator of the physiological state of the organism. Such an application of the EMN index provides us with information on the daily reactions of the human system in response to an environmental factor.

An analysis of the mean values of the EMN index over the span of 2 months made it possible to draw conclusions on the overall biological condition of the system in the period under study. This has been confirmed by the fact of obtaining higher mean EMN index values for individuals leading a hygienic life compared to individuals who do not care for their health and neglect the need to rest. As a result of the research the author has gathered information on the environmental conditions in which a human organism under study functions as well as on the circumstances in which this organism is affected by environmental factors (not necessarily adverse ones), which is reflected in the lines of regression between the values of the EMN index and the age of the subjects determined for such individual organisms. Regression lines determined for short periods of ontogenesis (2 months) have shown that even such a brief period of time is sufficient for the EMN index to reflect the changes that have taken place in the life style declared by a subject at the start of the research. The changes of the mean values of the EMN index with age are stable and directional. The clear downward direction of the changes reflects the phaseness of biological development, a property evolutionally attributed to human populations.

The EMN index adequately describes the tendency in the changes in the ontogenetic phases under study, since it reflects variations in the advancement of ontogenetic development independently of morphological values. Mean values of the EMN index in groups isolated according to the influence of environmental factors commonly recognised as factors disturbing to the physiological condition of a human system (eg., nicotinism, alcoholism), have reflected another quality of ontogenesis. This state has been confirmed by subsequent studies comparing mean values of the EMN index according to a selected factor. The comparison of arithmetic means in the isolated groups of subjects (alcoholics, smokers and non-smokers) has revealed

the occurrence of system's individual reactions. It has shown that the greater the health hazard resulting from the use of substances (harmful environmental factors) such as alcohol and tobacco, the lower the mean EMN index values compared to the means describing the model.

The selected groups described with mean values of the EMN index, depending on the strength of operation of the factor, show correspondingly lower values against the system of reference. This corroborates the high sensitivity of the EMN index reflecting an average biological condition of individuals within isolated groups. At the same time, individuals in such groups are characterised by a lower level of the index values in biological development. In other words, regardless of morphological similarities or differences of these individuals the EMN index reflects differences in the level of advancement of their ontogenesis, resulting from the circumstances of its progress. The values of the EMN index in groups exposed to the action of a given factor are accordingly lower in relation to the reference system, while in groups characterised by the absence of these factors they are accordingly higher. However, the direction of changes is always negative in terms of regression of the intensity of the EMN index with age in the stable and involutional phases of ontogenesis.

The EMN index reflects the degree of a human organism's advancement in ontogenesis, and its deviations from an adopted model observed in particular groups of subjects result from a modifying effect of environmental factors. Similarly, the index value shows regression in the stable and involutional phases of ontogenesis. The lines of simple regression between the index and subject's age have confirmed the observed tendency with regard to the level of the mean values of the EMN index in particular age categories in the stable and involutional phases of ontogenesis. This tendency is a downward and statistically significant one in all the groups selected according to a factor affecting the subjects' health (drinking of alcohol, smoking of cigarettes). All the correlation coefficients calculated in all the categories isolated for both sexes according to an external environment factor assume relatively high values and are statistically significant. The fitted lines of curvilinear regression have shown the actual course of changes in the variables under study. Both simple regression lines and curvilinear regression lines have shown a distinct throwback in the ontogenetic phenomena described with the EMN index. The shape of the curvilinear regressions indicates that harmful environmental factors such as smoking of cigarettes and drinking of alcohol have adverse effect on the picture of ontogenetic changes and strongly disturb the development of the human system. Of the two factors, alcohol is the one, which has a stronger disturbing effect on ontogenetic development (the curve is more parallel to the time axis). One-way analysis of variance has confirmed the above results. It has shown that the EMN index reveals human system's individual reactions under the influence of harmful environmental factors and their intensity in time (with regard to the fact of smoking and duration of smoking habit). Similar results of variance analysis have been obtained for the male group.

The EMN index has revealed that organisms being in different biological condition due to the differences in the type of harmful factor operating and the strength of its

action in time, tend to return to their "normal states" over a different period of time. The pace of this recovery depends on the type of the previously acting stimulus and the time of its influence on the system. To give an example, the EMN index has shown the effect of duration of abstinence in alcoholics on the biological condition of .the system. A distinct increase in the mean values of the EMN index has been noted along with the growth of the length of the period of abstinence. This is another advantage of the EMN index application.

On comparison of the results obtained with the use of the EMN index method and the NMR method the author has found out that the direction of changes observed in the parameter T1 (relaxation times) in the course of development is similar to the course of changes of the EMN index in ontogenesis. A decrease is observed in the mean values of the parameter T1 both for women and for men. Simple regression lines determined for parameter T1 and subjects' age have corroborated a downward tendency in the level of the values of relaxation times T1 in the stable and involutional phases of ontogenesis, for both men and women. Parameter T1 correlates with time in the stable and involutional phases of ontogenesis, and the correlation is statistically significant, which is an argument in favour of the usefulness of this parameter in the description of developmental phenomena in these phases of ontogenesis. The direction of changes of the T1 parameter is consistent with the direction of changes occurring in the stable and involutional phases of ontogenesis.

Generalising, we can say that the EMN index provides us with information on the general biological or physiological state of a human organism being an individual response of this particular organism to the action of an environmental factor, while the physical properties of T1, will perhaps – assuming that a widened scope of research techniques is used – enable us to describe biological quality on the cellular and molecular level, and subsequently to translate it into individual and population level.

Conlusion

The selected physicochemical methods, namely the electrophoretic mobility of cell nuclei (EMN) method and the methods of nuclear magnetic resonance (NMR) spectroscopy describe and reflect ontogenetic phenomena on the population and individual levels. The EMN index and relaxation times T1 describe the phaseness of the ontogenetic development with a distinct throwback in their values in the stable and involutional phases of ontogenesis. The direction of changes of the EMN index and relaxation times T1 is consistent with the direction of changes taking place in the course of entire ontogenesis, which has made it possible to describe the ontogenetic phaseness. The changes in the selected traits in the ontogenetic phases under study occur independently of the changes in morphological traits, at the same time the EMN index and parameter T1 have shown different stages of ontogenetic advancement, which results from differences in the circumstances of its progress. Variability of the EMN index resulting from its high sensitivity to environmental factors has made it

possible to describe individual reaction of a subject, its level and the deviations from a theoretical course of ontogenesis. Due to the nature of the changes in the mean values of the EMN index, tendencies and the correlation over the entire period of ontogenesis, it can be used as a criterion in the estimation of biological age. The EMN index reflects the "biological quality of an organism", which changes regularly with age, it may become a marker of the pace of the biological ageing of an organism or an indicator of its state of health and biological condition. Both the EMN index and parameter T1 may enrich gerontological and geriatric sciences with a new way of explaining the ageing processes on the cell and organism levels.

Skilful application of the EMN index method may be useful in the assessment of the biological age of a developing organism as well as it can serve as a measure or an indicator of the biological condition of the organism, meaning that the index may provide us information on the state of health of an individual under examination. This state will be a result of the organism's individual reaction to the life style or the surrounding environment. Furthermore, it will be a consequence of the advancement of the processes of biological ageing observed on the individual and population levels and reflected in what is going on at the cellular level. In the case of the NMR spectroscopy method and the measurement of parameter T1 it has turned out that the attempt to find a new criterion of biological age estimation regardless of the stage of ontogenesis requires carrying out further research which needs to be extended to cover a greater number of subjects. Notwithstanding that, the methods have shed light on the new possibilities with regard to the penetration of typically biological areas such as auxology or gerontology with purely physical methods.

Selected references

Czapla Z., Cieślik J. 1998. *The Electrophoretic Mobility of Cell Nuclei (EMN) index – relation to biological and physical properties of the cell, Antropological Review.* Nakom Publishing House, Poznań.

Czapla Z., Fojud Z. 1999. *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. Variability and Evolution*. Adam Mickiewicz University, Faculty of Biology, Poznań.

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

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

Shakhbazov V.G., Nabokov A.L., Colupaeva T.V., 1985. Avtorskoe svidetelstvo Nr 1169614. MKI A 61 B 10/00. Sposob opredelenija biologiceskogo vozrasta celoveka, Nr 3355951. Bjul. Nr 28.

Shakhbazov V.G., Colupaeva T.V., Nabokov A.L., Kononenko A.A., 1986a. Avtorskoe svidetelstvo Nr 1235493. MKI A 61 B 5/16. Sposob opredelenija utomlenija celoveka, Nr 2954977. Bjul. Nr 21.

Shakhbazov V.G., Colupaeva T.V., Nabokov A.L., 1986b. Novyj metod opredelenija biologiceskogo vozrosta celoveka. *Laboratornoe Delo*, 7: 404–407.

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