Phenomenon of Electrophoretic Mobility of Cell Nuclei (EMN) as a Consequence of Biological and Physical Properties of the Cell

Zbigniew Czapla,
Department of Human Biological Development, Institute of Anthropology,
Adam Mickiewicz University, Poznań

Electrical properties of the cell

An anthropologist or auxologist studying the phenomenon of Electrophoretic Mobility of Cell Nuclei (EMN) usually neglects the biophysical aspect of the phenomena under investigation and is most often interested in the final effect that he needs to obtain in his research, namely in the percentage value of the index. In this paper I would like to describe certain physical properties of the cell and resulting electrical and electrokinetic properties directly or indirectly related with the electrophoretic mobility of cells and oscillating cell nuclei.

Electrical properties of a given substance in varying states of aggregation are conditional upon two fundamental physical values: 1) conductivity (for an anthropologist the term resistance will be more understandable[1], and 2) permittivity. These two parameters have in certain conditions values characteristic for each substance and show varying dependency on the frequency of the changes of the electromagnetic field in which they are measured, i.e. they have different dispersion values. The two above-mentioned parameters depend on temperature and usually have temperature change indices characteristic of particular substances. Here, one should point out that the resistance of a given substance is determined by the type and concentration of free charges present in this substance, and by the conditions of their movement upon the application of the electric field. Electrical permittivenes, on the other hand, depends on the spatial distribution of charges tied in atoms or particles and on the degree of their ability to shift one another in the electric field.

Taking into account the fact that it is a cell in the electrical field that is an object observed in the EMN phenomenon, it is understandable that current must flow through this cell. For the current flow to occur free electrical charges or ions must be present. Even clean water dissociates and as a result conducts electrical current. Cytoplasm in a cell is just a colloidal water solution. In water being a component of cytoplasm salts, acids and bases dissociate exceptionally strongly. Therefore, even small admixtures of these substances result in the increase of conductivity. In other words, taking into consideration certain physical characteristics the interior of a cell can be considered a multiphase colloidal system made up of a variety of protein

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[1] Resistance in this context means resistance of a developing organism against environmental factors trying to deform plastic biological structures of the organism. – definition after Cieślik J., M. Drozdowska, A. Malinowski, Zjawiska rozwoju biologicznego człowieka [in:] *Antropologia* PWN Warsaw-Poznań 1989

molecules, nucleic acids, fats, carbohydrates, water, a certain number of small-particle organic compounds and mineral salts.

In the process of the carrying of electric current cytoplasm shows characteristics of a complex electrolyte or even suspension in which certain components constitute electrolytic dispersive environment, and other ones – dispersed phase. Electric conductivity of this substance depends on the concentration of particular types of ions and on their mobility. Small ions (such as K^+, Na^+, Cl^-) play the most active part in conductivity, since they are very mobile, while macromolecules, on the one hand, are responsible for the weakening of the external field and, on the other hand, for hydrodynamic retardation of the mobility of fast ions.

With respect to electrical conductivity, cell membrane is non-conductor. These properties the membrane owes mainly to the lipid layer. Together with intra and extracellular substance the membrane forms an electrical capacitor which contributes the reactive component to the impedance of a cell, that is to the resistance of a cell with respect to the current flowing through this cell.

Due to these properties the cell undergoes so called ionic – interspatial polarisation in the electric field. In other words, the cell behaves like a huge dipole, because a great number of ions closed in the cell with its cytoplasmatic membrane cannot leave it and in this way a dipole is formed.

The above-described properties of the cell suggest certain conclusions to me in relation to the observed EMN phenomenon. Placing cells in the low frequency electric field (1-2 Hz in the EMN research) results in the flow of current. It is known from the literature [Terlecki, Kotarski 1985], that as a result of high electrical resistance of the membrane in these low frequencies ions inside of the cell undergo separation and a strong dipole is induced. For this reason the value of permittivity is very high, but all lines of current omit the cell. The increase of frequency is accompanied with gradual decline of this effect. At adequately high frequencies (approximately 20 MHz) capacitance resistance of the membrane encompasses only the resistance of the lipid layer. As a result the intracellular substance participates in the conduction of current. The ionic polarisation of the cell fades away.

Recapitulating, the effect of the oscillation of cell nuclei may result also, but not only, from the fact that though the cell as a whole is strongly resistant, at increased frequency of the electric field its resistance declines. Relating this fact to the percentage share of oscillating nuclei in the total number of nuclei observed in the ontogenesis, we may conclude that at a constant frequency of the electric field which was applied in our study cells of young individuals are more resistant, i.e. they offer higher resistance to the current in comparison with the cells belonging to older individuals. More cells have oscillating nuclei because older cells are less resistant so they offer less resistance to the electric current flowing through them. Obviously this variability of the EMN index, does not result solely from the physical properties of the cell undergoing changes in the course of ontogenesis, but mainly from changing properties of biological membranes, which will be shown further in the paper.

Electrokinetic properties of cell

Apart from water solutions of electrolytes in a cell we have to do also with colloidal solutions i.e. suspensions or emulsions. These solutions are made up of very tiny particles of solid bodies, liquids or gas bubbles dispersed in liquid medium. In an ultra-micro-non-homogeneous system, such as, for instance, our human buccal epithelium cell under observation, we distinguish at least two phases: dispersed and continuous one. On the surface of the charged particles of dispersed phase, ions of a uniform charge sign (opposite one) adsorb in the form of tight envelope. The charges of the particle itself and of the layer of ions clinging to the surface of the particle form an electrical double layer inside of which the potential has a linear course. In a certain distance from the surface ionic particles with the same sign as the adsorbed ions form the diffusion or diffuse layer. In this layer the potential does not change in a linear manner along with the distance. The value of the potential that stabilises at the borderline between the double and diffuse layers is called electrokinetic potential and is marked with letter zeta (ζ). Most often it is called simply zeta potential. The existence of the electric double layer and zeta potential is responsible for the occurrence of the so called electrokinetic phenomena, such as electroosmosis, electrophoresis, streaming potential and Dorn effect.

Due to the specific character of our research I will briefly describe the phenomenon of elecrophoresis. Typical electrophoresis consists in the movement of charged particles of diffuse phase in relation to the stationary dispersion medium, as a result of the application of the electric field. When particles are charged positively, they travel to cathode (cataphoresis), while particles with negative charge migrate to anode (anaphoresis). Electrophoresis makes it possible to determine the charge of particles and the value of electrokinetic zeta potential. The latter one is calculated form the following equation:

$$\xi = \frac{k\eta\mu}{\varepsilon E}$$

η - viscosity
ε - permittivity of dispersion medium
μ - mobility of particles
E – electric field intensity
k – coefficient dependant on particle shape; k = 4 for cylindrical particles, and k = 6 for spherical ones.

Electrokinetic zeta potential plays a significant part in biological systems and has been known for a relatively long time. Yet still there are no satisfactory results of the research on this phenomenon. Nevertheless, it is known for instance that it prevents agglutination of erythrocytes. Also, due to zeta potential erythrocytes are pushed to the lumen of a blood vessel, which reduces friction. On the basis of the above equation the parameter μ describing the mobility of particles in electrophoresis can be calculated. At this moment I would like to discuss the aspect of electrophoretic mobility with regard to cells and nuclei, that is the aspect directly describing the EMN phenomenon.

Electrophoretic mobility of cells and cell nuclei

The notion of electrophoretic mobility of cells (μ) in biology was probably introduced for the first time by Helmholtz-Smoluchowski [after MAYHEW, NORDLING 1968] which was mentioned in the article by H. A. Abramsoon, S. Moyer and M. Gorin. *Electrophoresis of Proteins, and Chemistry of Cell Surfaces.* Publ. Reinhold, New York 1942, where on page 108 the authors proposed a physical formula describing this phenomenon:

$$\mu = \frac{\zeta\Sigma}{4\pi\eta} = \frac{\sigma\chi}{\eta} \quad \text{unit: } \frac{\mu/\text{sec}}{\text{volt}/\text{cm}}$$

where:

ς= zeta potential

Σ = dielectric constant of the medium at the electrophoretic shear layer

η= viscosity of the medium at the electrophoretic shear layer

σ= charge density of the cell surface

χ= effective thickness of the ionic double layer surrounding the surface

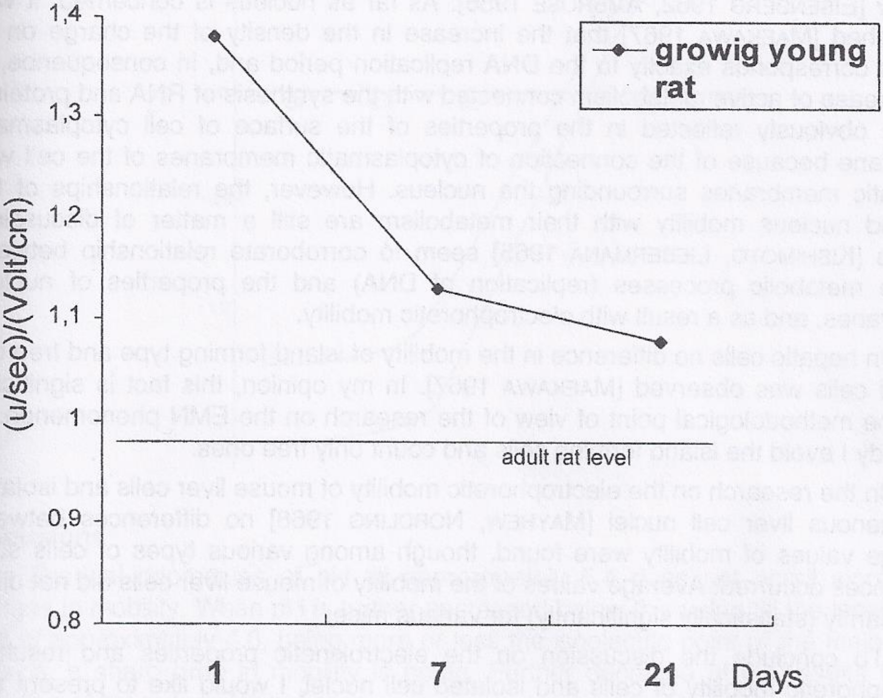
If throughout the duration of the experiment the conditions are stable resulting in the stability of parameters χ, η, Σ, then electrophoretic mobility of cells and cell nuclei is directly proportional to zeta potential and to the density of charge on the cell surface and it does not depend on the size. At the same time this means that in the cells and in isolated nuclei the charge density on their surface is the same. The work [MAYHEW, NORDLING 1968] corroborates also speculations on the increase of charge density along with the increase of the intensity of metabolic processes. This entails other consequences too, such as the fact that the density of charges increases in the cells after an operation on a part of liver. In these cases, in the regenerating cells with increased metabolism gradual increase in mobility is observed Table 1.

Similar phenomena are observed in young, developing rats metabolising with greater intensity than adult animals. The charge on their epithelium is higher and as a result they have greater cell mobility. The below diagram Fig 1 clearly confirms the dependencies observed in the EMN phenomenon.

Table 1. Changes in the electrophoretic mobility of animal liver cells after partial hepatoctomy [Eisenberg et al. 1962].

Number of animals	Period after operation [h]	Average electrophoretic mobility
20	0	0,97
3	0,5	1,11
2	2	1,20
4	6	1,25
3	12	1,26
4	24	1,22
6	48	1,28
3	72	1,17
3	21 days	0,98

Fig. 1 Changes in electrophoretic mobility of liver cells in the course of the ontogenesis of young rats [Eisenberg et al. 1962].



Conclusion

As shown in the diagram, the mobility of young cells is approximately 42% higher than the mobility of liver cells of adult rat. The diagram indicates also that the mobility of these cells decreases in the course of the post-natal growth.

Observing human buccal epithelium cells under the microscope we follow the course of specific electrophoresis, where negatively charged cells migrate in the direction of the positive end or anode (anaphoresis) [SHAKHBAZOV 1986]. It is obvious then that in this case the cell behaves like a giant macromolecule (dispersed substance) in the dispersion medium of 0,09% NaCl solution [MAKAŁOWSKA 1992]. Reading the works from the 1960s describing the EMN phenomenon one comes to think that that is where the phenomenon under discussion derives from. The very specific character of the method and the mathematical formula of the EMN index is based simply on the percentage share of cells with oscillating nuclei in relation to the share of cells with non-oscillating nuclei. Thus, the Electrophoretic Mobility of Cell Nuclei is nothing else but the migration of the human buccal epithelium cells (in our study) in the electric field. It should be noted that from the point of view of the very methodology of EMN this aspect is neglected with the whole attention focused on the effect of macro-oscillating nuclei.

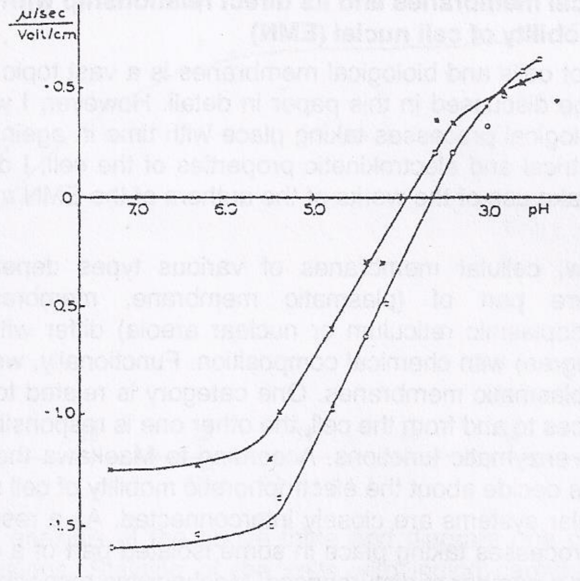
It is interesting that the surface of a cell usually has a negative charge [MAEKAWA 1967]. For this reason the research on the EMN phenomenon indicates that whole cells migrate towards the anode. It is also interesting that malignant cells have a much higher negative charge, which results in their varying electrophoretic mobility [EISENBERG 1962, AMBROSE 1956]. As far as nucleus is concerned, it was established [MAEKAWA 1967] that the increase in the density of the charge on its surface corresponds exactly to the DNA replication period and, in consequence, to the increase of active metabolism connected with the synthesis of RNA and proteins. This is obviously reflected in the properties of the surface of cell cytoplasmatic membrane because of the connection of cytoplasmatic membranes of the cell with plasmatic membranes surrounding the nucleus. However, the relationships of the cell and nucleus mobility with their metabolism are still a matter of discussion. Studies [KISHIMOTO, LIEBERMANA 1965] seem to corroborate relationship between intense metabolic processes (replication of DNA) and the properties of nuclear membranes, and as a result with electrophoretic mobility.

In hepatic cells no difference in the mobility of island forming type and free cell type of cells was observed [MAEKAWA 1967]. In my opinion, this fact is significant from the methodological point of view of the research on the EMN phenomenon. In my study I avoid the island forming cells and count only free ones.

In the research on the electrophoretic mobility of mouse liver cells and isolated homogenous liver cell nuclei [MAYHEW, NORDLING 1968] no differences between average values of mobility were found, though among various types of cells such differences occurred. Average values of the mobility of mouse liver cells did not differ significantly (statistically significantly) for various mice.

To conclude the discussion on the electrokinetic properties and resulting electrophoretic mobility of cells and isolated cell nuclei, I would like to present two diagrams explaining in what way pH and temperature affect the mobility of cells (Fig. 2 and Table 2) This, in my opinion, is directly related to the research and methodology concerning the EMN, since the change in the electrophoretic mobility of cells must entail changes in the number of oscillating nuclei.

Fig. 2 **Effect of pH on electrophoretic mobility of normal liver cells and regenerating cells of an adult rat (48 hours after the operation, crosses-normal cells, dots-regenerating) after [EISENBERG ET AL. 1962]**



Concusions

Gradual decreasing of pH till approximately 5.4 does not entail significant changes in mobility. When pH is further decreased below this value till the isoelectric point of approximately 4.0, being more or less the isoelectric point of the majority of proteins, a sharp drop in mobility is noted. Further decrease of pH results in the reversion of the value of the charge.

Table 2 Influence of temperature on electrophoretic mobility of the liver cells of an adult rat [after EISENBERG ET AL. 1962]

Number of Animals	Temperature [^{0}C]	Time of Heating [minutes]	Mean Mobility (μ/sec)/(volt/cm)	Percentag e Increase in Mobility
6	27	-	0.98	100
3	60	30	0.95	97
2	65	10	1.39	142
4	70	10	1.65	168
3	96	30	1.65	168

Conclusions

Heating to the temperature exceeding 60 °C does not cause significant changes in the electrophoretic mobility of cells. At 65 °C a 42% increase in mobility is observed. Further heating results in 68% increase of mobility, but heating to the temperature exceeding 70 °C does not cause any further changes in the electrophoretic mobility. An assumption can be made that at a certain value of temperature (between 60-70 °C) the mobility stops changing.

Ageing of biological membranes and its direct relationship with electrophoretic mobility of cell nuclei (EMN)

The ageing of cells and biological membranes is a vast topic and for the lack of room it cannot be discussed in this paper in detail. However, I will try to at least present certain biological processes taking place with time in ageing membranes in the context of electrical and electrokinetic properties of the cell, I described earlier. To this end I will make use of the works of the authors of the EMN method published in the 1990s.

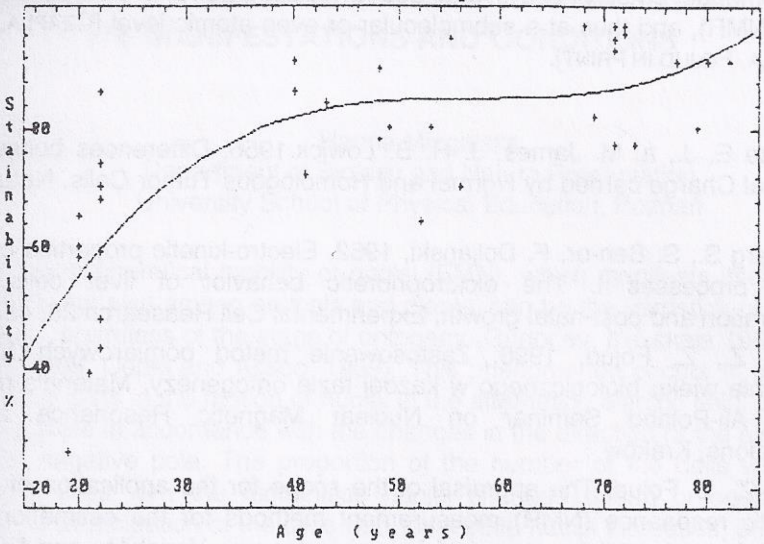
As we know, cellular membranes of various types depending on which organelle they are part of (plasmatic membrane, membrane surrounding mitochondrion, endoplasmic reticulum or nuclear areola) differ with their structure and (to a lesser degree) with chemical composition. Functionally, we can distinguish two categories of plasmatic membranes. One category is related to the permeation of various substances to and from the cell, the other one is responsible for enzymatic properties, it fulfils enzymatic functions. According to Maekawa these properties of cellular membranes decide about the electrophoretic mobility of cell nuclei [MAEKAWA 1967]. Intramolecular systems are closely interconnected. As a result, a damage to one of biological processes taking place in some isolated part of a cell affects other processes, causing a number of disturbances. Mechanisms responsible for damages to cells are sometimes difficult to describe, but most often one of the four intracellular systems becomes damaged. The four systems are: oxygen respiration system, cellular membrane system or systems maintaining the functions of cellular membranes (mainly synthesising phospholipids); system of the synthesis of enzymes and structural proteins and genetic apparatus reparation systems [Zabel 1995]. There is evidence that transporting properties of the cellular membrane, e.g. properties related to the functioning of the sodium-potassium pump change with age.

Shckorbatov [SHCKORBATOV 1995A, 1995B] and his team conducted research that clearly corroborated relationships between the changing with age EMN index and the degree of biological membranes degradation being an effect of biological ageing processes. In order to prove that, he introduced indigo carmine into buccal epithelium cells of individuals in various phases of ontogenetic development. The results of these studies are shown in the below table and diagram (Table 3 and Fig. 3).

Table 3 - Differences in the stainability of cells depending on their age. [SHCKORBATOV ET AL. 1995A*, 1995B].

Age	Number of subjects	EMN [%]	% Stainability of cells
19 - 22*			57.8 ± 4.4
40 - 56*			81.9 ± 2.6
69 - 80*			89.5 ± 2.2
19 - 24	21	66.1 ± 2.9	68.7 ± 4.2
40 - 56	12	46.3 ± 3.8	80.4 ± 2.2
69 - 82	10	40.4 ± 4.2	87.2 ± 2.8

Fig-3 Changes in the stainability of human buccal epithelium cells in the course of ontogenesis in three separate age groups [SHCKORBATOV ET AL. 1995A*].



Upon the analysis of the above table and diagram one can easily draw the following conclusions: Staining of the cells with indigo carmine in the course of ontogenesis changes differently to the changes in the EMN index. In the stable and involutional pheses of the ontogenesis the index correlates negatively with the stainability diagram. This is a clear corroboration of the fact that with age cells increase their permeability for certain substances for which they were not permeable in the earlier phases of ontogenesis. In reference to electrical properties discussed at the beginning of this paper, one can say that with age a cell transports more free ions, which means that deteriorated cellular membrane is less resistant (has lower resistivity). Properties of the EMN phenomenon with regard to cellular membranes have their origin also in the changes occurring with age in the nucleus and they refer to DNA as well as to its interactions with chromatin proteins. These interactions are electrostatic in character and their strength depends on the charge and conformation of proteins. The reason behind such reactions in the nucleus is not quite clear. There is a theory that a change in the ionic environment of nucleoplasma is the cause. It has been confirmed that quantity of some ions in the cell nucleus increases with age.

Recapitulation

The EMN phenomenon, still only partly explained, is related to the biochemical composition and physiology of cellular structures as well as to the properties of physical and chemical nature or with electrokinetic and electrostatic properties of nuclei and other cellular structures undergoing change with age. This results mainly but not only from the degradation of protein-lipid membranes. The degree of the degradation increases with age and manifests itself in their increased permeability, which can be related directly to the decreasing values of the EMN index in the stable and involutional phases of the ontogenesis. To conclude, I would like to point out to the fact that there are also studies providing explanation for the EMN phenomenon at the cytogenetical, biochemical and physiological levels which has not been referred to in this work. I would like to add that we are trying to explain the essence of the EMN phenomenon employing the methods of spectrometry of nuclear magnetic resonance (NMR), and thus at a submolecular or even atomic level [CZAPLA, FOJUD 1998, CZAPLA, FOJUD IN PRINT].

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