

ELECTRIC FIELD IN THE CYTOMEMBRANE AS A FACTOR OF ENERGY METABOLISM INTENSIFICATION

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Abstract. This work is devoted to the evolutionary development of cellular structures and role of the electric fields in the cell membrane. We showed that the main channels of transmembrane potential generation are the output from cell of hydrogen ions, appearing under utilization of energy-intensive substrates in the cytoplasm, and different rates of metabolism of lipid mass in the internal and external layers of membrane. We considered also the mechanisms of electric field generation in the hydrocarbon part of the membrane (in the region of lipid "tails") under coordinated lean of the "heads" in one layer of bilayer. According to proposed logic, Donnan potential seems not a potential of thermodynamic equilibrium and exists on the membrane due to the process of archaic metabolism in the cells. We showed also that the asymmetry of membrane permeability arisen for molecules with dipole moment and minimum cross-section at its positive and negative ends because of the presence of the electric field in the membrane. In the evolutionary process, this asymmetry determined the selection of molecules by chiral symmetry associated with the dipole moment. It explains existence in the living world only those molecules, having the same chirality with respect to their dipole moment. In the experiment with erythrocytes we found that the stronger electric field on their membrane, the more significant redistribution of glucose molecule (having the dipole moment and minimal cross-section at the positive end of dipole) between plasma and cytoplasm in favor of the last. In the experiments with erythrocytes, we also revealed predicted effect of asymmetry of permeability for water molecules, providing preservation of erythrocyte volume under oxygenation of venous blood.

Keywords: lipid bilayer, membrane potential, flower metabolism, dipole, domain, Donnan potential, permeability asymmetry, glucose, water, osmotic particles

PACS: 87.23.Kg

Bibliography – 9 references

Received 28.05.2016

RENSIT, 2016, 8(1):91-103

DOI: 10.17725/rensit.2016.08.091

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1. INTRODUCTION

A general line of the cell world development from the very beginning is a steady intensification of energy metabolism of the cell structures. This work is devoted to the role of electric field in the membrane of the cells in this development. A transmembrane potential of the cells is known to be negative and varies in the range from 10 mV to 150 mV. Therefore, electric field intensity can reach 10-15 kV/mm, i.e. to be near the area of voltage breakdown. Membrane-associated and embedded proteins decrease the breakdown threshold of lipid layer. However, some of these proteins – molecular pumps generate and maintain transmembrane potential difference. A significant part of energy is spending for synthesis and functioning of these proteins in cell. The subject of this work is an answer to the question – why cells required increasing transmembrane potential difference up to the values preceding their electric breakdown in the process of evolutionary development

2. A TRANSMEMBRANE POTENTIAL DIFFERENCE AS ONE OF LEADING FACTOR IN THE FORMATION, EVOLUTIONARY DEVELOPMENT AND FUNCTIONING OF THE CELL

2.1. INEVITABILITY OF APPEARANCE OF THE ELECTRIC FIELD IN THE MEMBRANE LIPIDS OF LIVING CELLS

An evolutionary approach has necessarily to take into consideration the main features of living systems, which distinguish them from the structures of inanimate nature. First of all, it is maintaining of thermodynamic unstable

condition of living system, which requires continuous expenditure of free energy. Secondly, it is flow homeostasis of all system elements – their continuous degradation of substances and further their reproduction *de novo*.

Maintaining of the lipid vesicles condition (precursors of modern cell [1]) and modern cells had been provided earlier and is being provided in cells due to free energy generation in the processes of the energy-intensive substrates utilization in the cytoplasm. Dissociation of these compounds themselves and final products of their degradation caused increasing of hydrogen ions H^+ concentration. The output of the ions along with the degradation products from cells to the surrounding aqueous medium and the input of big organic compounds provided stationary conditions for exchange of cell material and flow homeostasis. Ions H^+ , ahead of all other products utilization of energy-intensive substrates, generated the transmembrane potential in the lipid layer.

Another channel of the electric field appearance in the lipids, as evolutionarily ancient as previous, was associated with a flow homeostasis of lipid mass. The channel inevitably arises in the presence of even very weak metabolism (in comparison with metabolism of modern cells). Synthesis of lipids, demanding energy expenditures, had been performed earlier and is being performed now only in the internal layer of membrane. Their spontaneous decay had been carried out and is being carried out in both layers of membrane. Therefore, equilibrium of lipid mass settled only under higher density of lipids in the inner layer. Estimations show that at the same density of lipids in the inner and outer layers, the «heads» vertically oriented to the surface of membrane provided the

potential jump of 100 mV. These jumps are equal in a magnitude for both layers; however, in the area of «heads» in outer and inner layers electric fields are directed oppositely. Therefore, in the region of lipid «tails» the potential value is unchanged and the field is absent. However, this region is at a negative potential with respect to both the internal and external aqueous environment (curve 1 in **Fig. 1a**). The fields in the «heads» overlapped with edge fields of the internal and external aqueous media charged negatively with respect to lipids in the membrane. It leads to weakening of the fields in the area of «heads» of both layers and to decreasing of the depth of a potential well in the region of lipids «tails». Besides, molecules of water invade into a space of polar «heads» under influence of thermal motion. Having big dipole moment, these molecules further reduce a strong field in the region of dipole «heads» and decrease the depth of a potential well in the area of hydrocarbon «tails» approximately to 20 mV (curve 2 in **Fig. 1b**).

The higher value of a potential jump corresponds to the inner layer of membrane due to more dense packing of lipids. Because of a different density of lipids in the inner and outer layer an electric field arising in

the area of hydrocarbon «tails» is directed towards the cytoplasm (curve 3 in **Fig. 1c**). This field is added to the field generated by the hydrogen ions exit, causing the transmembrane potential difference $\Delta\varphi$. **Fig. 1d** shows the potential course normally oriented to the membrane and the electric field values.

A total electric field in the area of the «heads» of the outer layer tends to incline polar «heads» to the surface of membrane. Electric field component caused by the hydrogen ions exit tends to straighten the «heads» of an inner layer. It should be noted [2, 3] that a close «heads» location in the both layers of membrane leads to a simultaneous inclination of all the heads within a domain, bounded by built-in proteins, and in the one direction. Besides, the direction of «heads» inclination in a domain does not depend on position of heads in another. There is an activation barrier between the two conditions of heads position (straight and incline). The distribution of domains quantity by the heads position in the each layer of membrane obeys the Boltzmann equation and it is in a strict correspondence with the depth of potential wells separated mentioned barriers [3]. Namely because of the presence of the electric fields in the membrane of vesicles

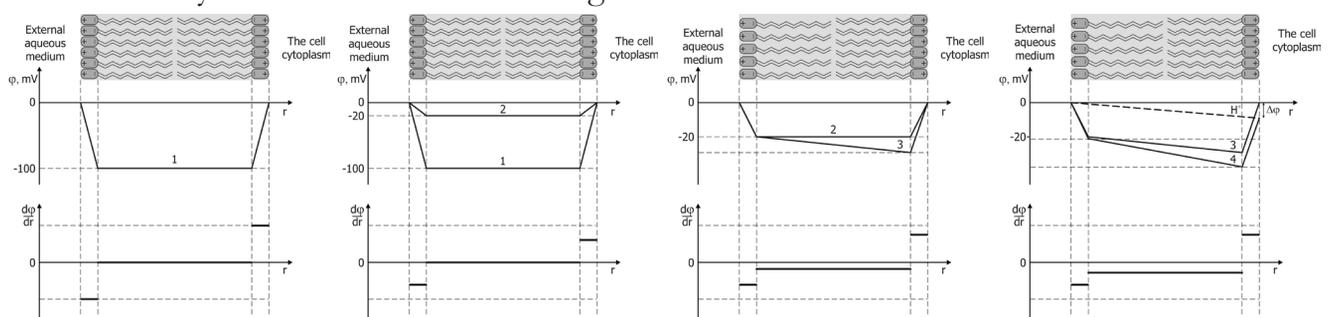


Fig. 1. Distribution of potential φ normally to the membrane surface and of electric field intensity dp/dr in dependence of the thickness of membrane r : a) provided that a dipole moment of lipid «heads» of both layers is directed normally to the membrane surface and all other factors are not taken into account; b) taking into account the edge fields of surrounding aqueous media and hydrocarbon part of membrane, and separate molecules of water penetrating between polar «heads»; c) taking into account the higher density of lipids in the inner layer of membrane in comparison with outer layer; d) taking into account hydrogen ions exit raised in the cell under metabolism presence.

and first cells, the quantity of domains with inclined «heads» in the outer layer were more than in the inner layer.

As mentioned above, energy metabolism causes an appearance of transmembrane potential difference $\Delta\varphi$ due to the hydrogen ions exit from the cytoplasm. Because of a low intensity of metabolism the value $\Delta\varphi$ was much less than 10 mV. Electric field generated is directed towards the cytoplasm and tends to incline lipids «heads» of outer layer and straighten ones in the inner layer. It leads to decrease of an activation barrier for the transition of dipole «heads» in the outer layer from vertically oriented with respect to surface of membrane to inclined position. The transition decreased the potential jump on the outer layer almost in 2 times (curve 5, Fig. 2a). For lipids of the inner layer the situation is reverse. The field in the lipids tends to hold lipids in vertically position. An activation barrier of their transition from vertically oriented lipids to inclined ones is higher, what makes this transition less possible. As a result of the impact of all the considered fields on lipid dipoles, most of domains of the outer layer were in the condition with inclined heads. The angle of inclination was 60 degrees to the normal

of the membrane surface. The «heads» of inner layer had mostly vertical position. A potential jump on the «heads» of outer layer decreased approximately in 2 times. The potential difference in the hydrocarbon part of membrane was settled ~ 10 mV. As the potential value $\Delta\varphi$ was less than 10 mV, the field intensity was ~ 1 kV/mm.

It should be noted that namely this potential difference on the hydrocarbon part of membrane mistaken for thermodynamic equilibrium potential (Donnan potential) that is settled on the membrane without energy metabolism in cells. According to described logic, there are two mistakes.

First of all, one does not pay attention to the influence of archaic metabolism processes inherited by a cell from previous evolutionary stages. Evolutionary late and complex processes of utilization of energy-intensive substrates are relatively easy to inhibit by special agents, having suppressed an activity of main enzymes catalyzing oxidation reactions. However, to inhibit the earliest and the simplest evolutionary processes of enzyme degradation of practically all organic compounds, including enzymes themselves, and accumulation of the part of released energy in macroergs,

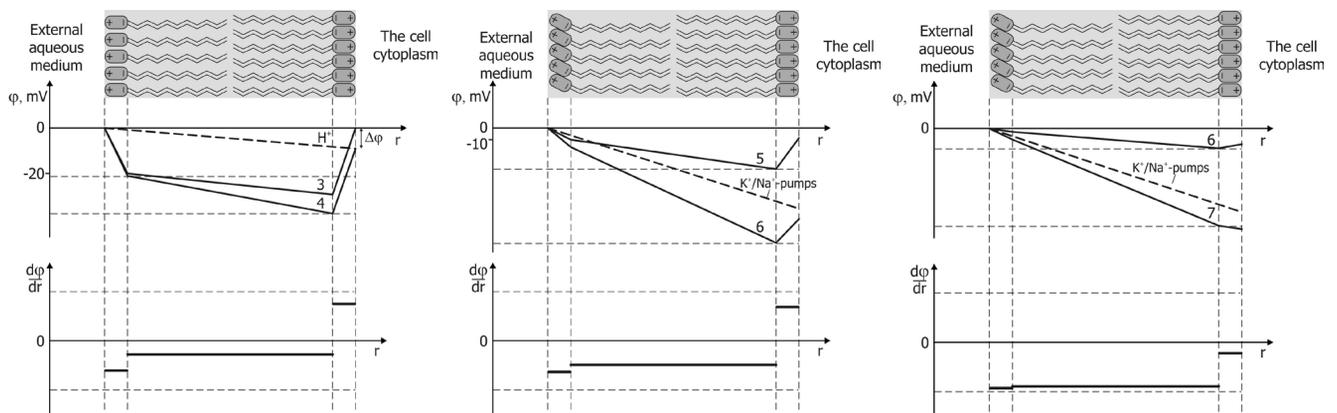


Fig. 2. Distribution of the potential φ normally to the membrane surface and of electric field intensity $d\varphi/dr$ in dependence of thickness of membrane r : a) taking into account of the inclination of the «heads» of outer layer by 60° relatively to the membrane surface normal; b) taking into account the emergence of sodium-potassium pumps in the membrane; c) the direction of evolutionary changes of the potential φ and the intensity of electric field $d\varphi/dr$ distribution as intensification of metabolism is shown.

is significantly harder. Noted, the simplest processes based on enzyme degradation of all organic compounds (all kinds of «-ase») have provided a flow homeostasis of all the organic compounds in lipids vesicles, precursors of modern cell, and is providing now in all cells [1]. Exactly in a manner, a native quality of the most organic compounds has been reliably achieved and is being achieved in a flow of substances. The higher the rate of the substance flow in the cytoplasm, the lower the level of spoiled (degenerated) organic compounds, which cannot perform their functions.

Secondly, a transmembrane potential difference is defined experimentally by the distribution of the concentration of charged ions capable transmit through lipid layers. Such distribution is set due to the potential jump in 10 mV caused by the inclination of a main part of the «heads» of the outer layer. As the contribution of an inhibited main metabolism in the fields formation is minimized ($\Delta\varphi \ll 10$ mV), it is specified potential jump that is determined the distribution of charged ions on either side of the membrane.

Summarize mentioned above arguments, one can concludes that if the cell has the remains of energy metabolism and if sodium-potassium pumps is absent or suppressed then a transmembrane potential difference is inevitably set on the hydrocarbon part of lipids. This factor causes the redistribution of charged particles capable transit through membrane. The cytoplasm occurred to be under lower potential that surrounding aqueous media. The main feature of generation of such potential (10 mV) is its independence from the cell size and from dissociation

of proteins in the cell. Assuming all the processes of energy metabolism is stopped in the cell but the lipids of its membrane is saved native, the potential difference between the cytoplasm and an external aqueous media will decrease almost till 0 mV.

To conclude this chapter, we notice that in the processes of an evolutionary development of living cells new facility is formed – this is a sodium-potassium pump. The earliest destination of pumps was to reliably provide the presence of electric field directed inward the cell (Fig. 2b and Fig. 2c) both in the lipid «heads» and «tails». Biological practicability of such innovation is presented below.

2.2. ELECTRIC FIELD IN THE CELL MEMBRANE AS A FACTOR OF SELECTION OF ORGANIC COMPOUNDS BY TYPE OF SYMMETRY IN THE FORMING CELLS (PASTEUR CURIE LAW)

The compounds formed a basis of energy-intensive and plastic material and involved in the process of energy metabolism of modern cells are multipolar. The first multipole moment is an electric dipole moment of the system of charges of molecule directed from a negative charge to positive one. An electric field in the membrane directed inward the cell turns dipole along its lines of flux. Membrane will be permeable for the molecules having a minimal electron density, and, therefore cross-section, on their dipole vector tip. The resistance of the membrane to diffusion motion of molecules will be minimal too.

Dextrorotatory molecules of sugar (D-form) and levorotatory molecules of amino acids (L-form) satisfy conditions listed above. A dipole moment of the molecules is directed to the end group

-COH with a minimal cross-section (Fig. 3b). One type of symmetry differs from another by the cross-section on the dipole vector tip. The permeability of membrane for molecules with such location of this group is higher than for molecules having this group on the opposite end of dipole (Fig. 3c). The transition of water molecules from outside through membrane is obstructed as for molecules shown on Fig. 3c. The cross-section on the dipole pin is bigger than on the dipole tail. Therefore, when an electric field is present on the membrane the entrance of water is obstructed in comparison with the exit. The molecule of water has no chiral elements and it does not rotate the plane of a polarized light. Therefore, the effect of light plane rotation does not related directly with the membrane permeability asymmetry. Determining factors are the presence of dipole moment in the molecule and the differences of the cross-sections on the pin and the end of dipole. Chiral elements, arranged to dipole moment as mentioned above, have provided and are providing now selection and storage of the biologically active compounds needed for

energy metabolism intensification. This is an explanation of Pasteur Curie law¹.

Inevitably consequence of the phenomenon considered is an inequality of equilibrium concentrations of such neutral molecules in the external aqueous medium and in the cytoplasm with energy metabolism. Organic compounds with a minimal cross-section on the dipole vector pin are accumulated in cytoplasm to higher concentration than molecules having a maximal cross-section on the pin of the dipole. This circumstance seems to play a crucial role in selection of organic compounds formed a basis for energy-intensive and plastic material in metabolism of living systems. We state that we found «a delicate mechanism capable differ one type of symmetry from another» proposed by N.N. Moiseyev.

Such selection of organic compounds perhaps was a crucial factor on the final stage of cell formation in the appropriate conditions on the cooling Earth. Without the selection processes of formation of structural precursors and first cells would be inevitably delayed. In this case cell precursors needed more time to test the benefits of each of new compound, which quantity incredibly

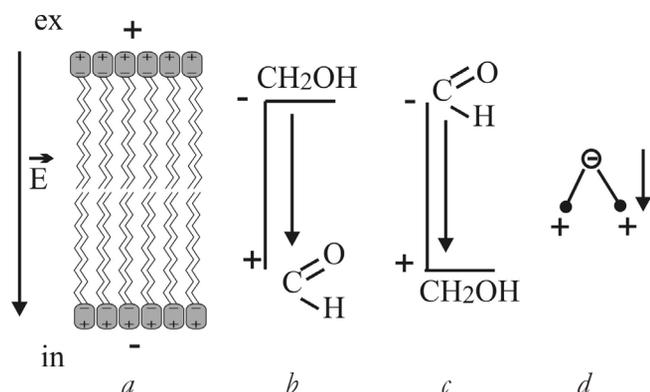


Fig. 3. Scheme of electron density distribution (cross-section) for the dipole molecules: a) cytoplasmic membrane and the direction of electric field intensity; b) dextrorotatory molecules of sugar (D-form); c) levorotatory molecules of sugar (L-form); d) the molecule of water.

¹Louis Pasteur discovered the property of bioorganic compounds to rotate the vibrations vector of transverse plane of the light wave – to polarize light, having divided all the organic compounds by two types of rotators: a clockwise or a counterclockwise. It has been showed that all living organisms use only one type of these rotators for their material synthesis and never both of them. Van't Hoff interpreted this property of the biomolecules by the presence of asymmetric carbon atom in the structure, which becomes anisotropic [4]. Pierre Curie explained this property of bioorganic compounds by the dissymmetry of the mirror symmetric biomolecules. Calvin called it a chiral symmetry [5].

increased on this evolutionary stage; and a living world would never be appear.

3. ASYMMETRY OF THE PERMEABILITY OF LIPID MEMBRANES INDUCED BY ELECTRIC FIELD FOR WATER MOLECULES (CHARAKHCHYAN EFFECT)

As mentioned above, asymmetry of the permeability of lipid membrane (one-way transition) induced by electric field takes place not only for molecules, having chiral elements and able to polarize light, but also for molecules that is not polarizing light.

Water molecule has a dipole moment equal $1.84 D$ and a maximal cross-section on the dipole vector tip (Fig. 3d). Therefore, the lipid membrane is more permeable for exit of water molecules than for entrance due to the direction of electric field. This effect of asymmetry of the membrane permeability was utterly useful for evolutionary development of the cells. General line of living world development is steady intensification of free energy generation by utilization of energy-intensive substrates. The storage of osmotic particles of energy-intensive and plastic material and increasing of enzymes concentration in the cytoplasm were inevitable consequences of the general line. Therefore, the flow of water to the cells continuously increases. It was compensated by the flow of water from cells that was provided by asymmetry of cytomembrane for these molecules.

As mentioned above, amplification of electric fields and regulation of their intensity were provided by appearance of new facilities. One of those was a sodium-potassium pump; its activity increases a

transmembrane potential difference and, therefore, intensity of electric field in the membrane lipids. Evolutionary earlier autoregulation of asymmetry of the membrane permeability for water formed on the ability of the proteins to associate with membrane and to decrease field in the membrane. The effect of a nonspecific membrane permeability increasing caused by weakening of the field in the lipids under association of big proteins with membrane is shown in work [7].

Gain control of electric fields and their intensity in lipids steel provided with new equipment. One of these molecules are K^+/Na^+ ATPase, which increases the transmembrane action potential difference and, hence, the field strength in the lipid membranes. Evolutionary earlier autoregulation asymmetry of cell membrane permeability for water formed on the ability of proteins and their complexes associated with the membrane and thereby weaken the field in lipids. The effect of increasing the non-specific membrane permeability caused by the weakening of the field in the lipid when associated with them large particles is shown in [7].

To summarize this chapter we should pay attention to history of the question of membrane permeability asymmetry for water molecules. The first scientist who revealed and experimentally investigated such effect in the thin films of organic material induced by electric field was Andrew A. Charakhchyan (LPI of the Russian Academy of the Sciences). Unfortunately, he did not publish the results of his works, but the effect of membrane permeability asymmetry induced by electric field for water we called «Charakhchyan effect».

4. EXPERIMENTAL STUDY OF THE EFFECT OF ASYMMETRY OF MEMBRANE PERMEABILITY FOR WATER AND GLUCOSE MOLECULES

For experimental part we chose erythrocytes from human whole blood as a model. We used samples of blood in volume of 5-10 ml and standard anticoagulant EDTA-K2.

4.1. ASYMMETRY OF ERYTHROCYTE MEMBRANE PERMEABILITY FOR GLUCOSE

First part of our experiments was devoted to study of the influence of intensity of electric field changes in the membrane of erythrocyte on the equilibrium distribution between blood plasma and cytoplasm.

Molecules of deoxyhemoglobin have significantly greater charge than oxyhemoglobin molecules in the biological range of the pH values [8]. Thus, first ones form complexes with 2,3-diphosphoglycerate molecules whose concentration is equal to the concentration of hemoglobin [9]. In turn, these complexes have a higher affinity to erythrocyte membrane than molecules of oxyhemoglobin. Therefore, in erythrocytes of a venous blood the layer of such complexes greater by dozens percent than on the erythrocyte membrane of an arterial blood. Our investigation shows that the inner layer of erythrocyte membrane of venous blood has 2.5 additional layers of the complexes and arterial blood – 1.5 layers of oxyhemoglobin molecules.

As sizes of hemoglobin molecules (5 nm) and thickness of erythrocyte membrane (6-10 nm) are close values, thickness of additional layers of hemoglobin significantly changes the intensity of field (under the same transmembrane potentials). Obviously, during oxygenation of venous blood the intensity of electric field increases due to decreasing of

membrane thickness (oxyhemoglobin leaves the membrane).

To test the effect of glucose redistribution between plasma and cytoplasm, next experiment was carried out. The sample of venous blood with EDTA-K2 was divided in two equal parts. One part was saturated with oxygen during 15 minutes with constant stirring. Then both samples stay during 1.5-2 hours for natural erythrocyte sedimentation. After removing the sediment we two samples of blood plasma – sample no. 1 (plasma of venous blood) and sample no. 2 (plasma of oxygenated blood – analog of arterial blood).

Further we measured the concentration of glucose in first n_1 and second sample n_2 by means of a special set-up that was developed in LPI in the Optics Department. The values of glucose concentration significantly differed in two samples and were outside the error of one measurement. If studied effect would not take place in the erythrocytes, the value $\eta = n_1/n_2$ would be equal to 1. However, the value $\eta = n_1/n_2$ was >1.1 . This means that the oxygenation of erythrocytes leads to decreasing of glucose concentration in blood plasma. According to considerations described above, such redistribution of glucose can be caused by increasing of intensity of electric field in membrane due to oxyhemoglobin dissociation from the membrane.

The next experiment was the final point in the experimental proof of the effect of asymmetry of membrane permeability in the erythrocyte. The experiment was carried out with the same donor's blood as in previous experiment. Initially we measured the glucose concentration in venous blood sample. Further this sample was saturated with oxygen (analog of arterial blood) and

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the glucose concentration was measured. Then this sample was exposed to the reverse process of deoxygenating to the level corresponding to venous blood (40 mm Hg in the blood plasma). The concentration of glucose obtained in the blood plasma was very close to initial glucose concentration in the venous blood sample, but was a little bit lower. Observed slight decreasing of glucose concentration in comparison with initial level can be explained by asymmetry of membrane permeability (the exit of glucose molecules from the cell is more difficult than entrance). The second reason of incomplete recovery of glucose concentration is the processes of utilization of glucose in cells in glycolysis.

So, the asymmetry of membrane permeability induced of electric field for glucose molecules is maintained in erythrocytes.

4.2. ASYMMETRY OF ERYTHROCYTE MEMBRANE PERMEABILITY FOR WATER

The experimental study of membrane permeability asymmetry for water was carried out also on the erythrocytes of donor's blood. The whole venous blood with anticoagulant EDTA-K2 was divided by two samples. The blood in one sample was saturated with oxygen to the level of an arterial blood. We measured hematocrit values in each sample (venous and oxygenated). In oxygenated blood we anticipate increasing of the erythrocytes volume (and hence, hematocrit) due to the osmolarity growth (~ by 5 mmol/l) caused by the dissociation of hemoglobin and 2,3-diphosphoglycerate complexes. Besides, osmolality level should increase due to the effect of redistribution of glucose under oxygenation showed above.

But we obtained decreasing of hematocrit value in oxygenated blood sample.

Estimations show that blood hematocrit measured in percent should have increased by at least 1% after oxygenation. But measured hematocrit value in the oxygenated blood turned to be less by 0.2 % than in venous. The error of mean value of hematocrit in each series of experiment was less than 0.05%.

Apparently, excess flow of water into erythrocyte cytoplasm caused by osmolarity growth during blood oxygenation is compensated by additional flow of water from erythrocyte to blood plasma. We state that such compensation is provided by increasing of membrane permeability asymmetry for water molecules caused by growing of electric field intensity in membrane lipids.

5. DISCUSSION

Evolutionary approach to analysis of biological significance of the negative potential on cell membrane allows us to elucidate inevitability of its appearance in cells and their precursors provided that they have metabolism. The most archaic metabolism is a flow homeostasis of lipids.

We showed that Donnan potential in the cell is not a thermodynamic equilibrium potential and it exists owing to the processes of energy metabolism (including archaic metabolism). If process of energy metabolism would be switched off, transmembrane potential will decrease relatively quickly. To switch off the archaic metabolism based on releasing of energy in one-act organic catalysis of the decomposition of complex compounds very hard. To reach this demands to switch off action of all enzymes. Therefore, on account of this source of free energy the generation of Donnan potential takes place in the cells. If this source would be switched

off, i.e. under thermodynamic equilibrium, potential on the cell membrane will not be generated.

Experiments with erythrocyte of preserved blood show that energy of this source of energy is enough to maintain transmembrane potential difference during extended period of time – from several weeks to 2-3 months. Despite of the glucose reserves had time to spend, erythrocyte did not lose their ability to be saturated by oxygen – during saturation the color of blood changed from dark red to carnation. Therefore, in the process of blood storage energy was enough for ferric hemoglobin reduction. There can be no question of transition of red blood cells in a state of thermodynamic equilibrium.

In the absence or shortage of energy-intensive and plastic material the cells "eat up" themselves, i.e. utilized the compounds formed a basis of their structures. All sorts of "-ase" play a leading role in the utilization of the compounds, for example, peptidases, lipases, and other. Their action provides an additional influx of free energy in the cell and a flow of organic compounds in their cytoplasm.

The presence of transmembrane potential difference and thus an electric field cause the appearance of cytomembrane permeability asymmetry for organic molecules and water molecules having a dipole moment. In the process of birth and evolution of cellular world because of this phenomenon there was selection of organic compounds by the type of symmetry (the law Pasteur-Curie). With all things being equal the type that is provided (due to the higher permeability of the membrane at the transition of such molecules from an aqueous environment to the cytoplasm compared with reverse

transition) a higher equilibrium concentration of organic compounds in the cytoplasm has been selected in the environment. For such compounds the exit out of the cell is difficult and the entry into the cell is facilitated. It should be emphasized that this also applies to basic macroergs. They seem to be "held" in the cytoplasm.

In the presence of field in the membrane water molecules easier leave the cells than penetrate there. This effect ("effect Charakhchyan") is helpful to save the integrity of the cells. It provides compensation for water osmotic flow associated with higher concentrations of the enzymes therein, which are not able to penetrate through the membrane. The higher the field of lipid membrane, the more powerful given compensatory flow of water from the cell.

It should be noted the local character of the effect. Under the same potential difference field is weakened in the places of association of large organic molecules with membrane lipids. Accordingly, a compensatory (in relation to osmotic) flux of water from the cell is less at these locations than in the regions of the membrane without the adhesion. To maintain the constancy of cell volume the entire water flow from the cell I_{in} must be exactly equal to the flow of water into the cells cytoplasm I_{ex} . According to Fick's law, these flows can be expressed by the corresponding formula. For the effluent from the cytoplasm:

$$I_{in} = (\sigma_{in}^{op} S^{op} + \sigma_{in}^{cl} S^{cl})(1 - \eta_{in})n_{in},$$

where σ_{in}^{op} – membrane permeability for water molecules from the cytoplasm into the aqueous environment of the membrane in areas without major adhesion molecules and their complexes, S^{op} – the total open area of all its free areas are adhered to large

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organic molecules and their complexes; σ_{in}^{cl} and S^{cl} – specified values for the portion of the internal surface of the membrane to adhere to these large molecules and their complexes; η_{in} – the proportion of area of the membrane from the cytoplasm occupied osmotically active particles and prevents the escape of water molecules from the cell, and n_{in} – the concentration of water molecules in the cytoplasm.

Correspondingly, for influent to the cytoplasm:

$$I_{ex} = \sigma_{ex} S_0 (1 - \eta_{ex}) n_{ex},$$

where σ_{ex} – permeability of membrane for water penetrating from surrounding water medium into cytoplasm; $S_0 = S^{op} + S^{cl}$ – area of external lipid layer; η_{ex} – external part of membrane area occupied by big osmotic particles that prevent entrance of water molecules from aqueous medium to the cell; n_{ex} – concentration of water molecules in the surrounding aqueous area.

As concentration of osmotic active particles greater in the cell than in the surrounding medium, η_{in} value is always more than η_{ex} value. The organic compounds, synthesized in cells and present in cytoplasm as an aqueous solution, occupy more proportion of volume than organics in the external medium. Hence, concentration n_{ex} is more than n_{in} value. Result flux of water through cell membrane is:

$$I_{res} = \sigma_{ex} S_0 (1 - \eta_{ex}) n_{ex} - (\sigma_{in}^{op} S^{op} + \sigma_{in}^{cl} S^{cl}) (1 - \eta_{in}) n_{in}, \quad (1)$$

where the direction chosen is toward cytoplasm.

If the membrane permeability for water does not depend on electric field in the membrane (σ_0) and it would be the same for both directions, the resulting water flow I_0 would be determined by the relation:

$$I_0 = \sigma_0 S_0 [(1 - \eta_{ex}) n_{ex} - (1 - \eta_{in}) n_{in}]. \quad (2)$$

To transform the last relation to the form: $I_0 = \sigma_0 S_0 n_{ex} [1 - \eta_{ex} - (1 - \eta_{in}) n_{in} / n_{ex}]$. As the values are $n_{in} / n_{ex} > 1$ and $\eta_{in} > \eta_{ex}$, then flow I_0 directed in the cell causes continuously swelling of the cell. Wherein, concentration of soluble substances will equalize on both sides of the membrane. If this process accompanied by continuous swelling of the cell will not stop, the cell inevitably died by necrosis way due to membrane rupture.

Therefore, increasing of an enzymatic pool as a way of steady intensification of free energy generation quickly exhausted itself. In the processes of natural selection optimal sizes of the cells were chosen, which guaranteed their resistance to mechanical stress from the external aqueous environment.

Asymmetry of membrane caused by electric field for biologically active particles appeared to be very useful for the cells. It opened the possibility of increasing of enzymatic pool as well as energy-intensive and plastic material, provided that the volume of the cell remains optimal.

The higher field in the membrane, the higher the equilibrium concentrations of the osmotic particles in the cytoplasm under constant conditions of surrounding aqueous medium. In the process of evolutionary transformations on account of increasing of transmembrane potential difference, conditions for growth of osmotically active compounds were created. To do this, the whole possible range of strengths (up to pre-breakdown) electric field in lipids was involved.

To increase transmembrane potential difference the cell began to use the large proteins and their complexes – "molecular pumps." It should be noted the particular importance of this evolutionary facility. The cells received a new agent capable to

influence on the acidity of the cytoplasm and, therefore, the activity of enzymes and metabolism at whole.

Bright example of usage of the effect of asymmetry of lipid membranes permeability by living cells is the mechanism of fresh water pumping from the cytoplasm of amoeba. Growth of its vacuoles is associated with lipid synthesis *de novo* only in the outer layer of the membrane. Presence of transmembrane potential and field in the hydrocarbon part of lipids is caused by the difference in lipid density in the outer and inner layers of vacuoles. This field is directed from the internal part of vacuoles to external, i.e. directed oppositely to the field on the amoeba cytomembrane. Since the field is directed out of vacuoles, the water flow inward vacuoles; and vacuoles grow. On the other hand, on the separate areas of the cytomembrane where no organic adhered thereon, the resulting water flow is directed out cells, while at other sites with adhesion of large molecules, in contrast, flow of water is directed inside. Both streams are exactly equal to each other, which provides a constant volume of amoeba (analysis of ratio (1) showed that such conditions are realizable by varying its member variables). Water streams formed in the cytoplasm are used for transport of vacuoles to the places not subjected to the said adhesion. Here they fuse with the membrane, taking fresh water out to the aqueous environment.

Transport of big compounds and vesicles with biologically active substances in the cytoplasm of modern cells is implemented by mechanisms based on the asymmetry of lipid membrane permeability for water. The water streams formed in the cytoplasm transfer the compounds from the place of formation to the places where their action

is more productive or they can be effectively removed.

6. CONCLUSION

In the work we considered a number of effects that directly or indirectly affect the cell metabolism. We showed how the mechanisms providing the intensification of energy metabolism were formed in the processes of evolutionary development. We pointed out the principles and useful consequences of such mechanism. Other possibilities of their using for cell metabolism regulation are also indicated.

Analysis conducted covers a huge period of time from the origin of first cell up to genesis in the cells of compounds – hormones precursors synthesized now in the multicellular organism. However, this analysis is fairly schematic and raises the questions.

It should be noted two important moments. We showed that known in literature «Donnan potential» is generated due to the energy of archaic metabolism that is not corresponding to the condition of thermodynamic equilibrium. We revealed also that the asymmetry of electric field induced by electric field is realized for molecules having a dipole moment.

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