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How Erythrocyte Volume Is Regulated, or What Mathematical Models Can and Cannot Do for Biology[1]

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Abstract—Modern concepts of the red blood cell (RBC) volume regulation are considered. It is shown that the system of ion pumps and channels in the cell membrane ensures the physiological value of volume with a precision of about 10% even at 5- to 7-fold variations of passive membrane permeability for ions. Particular attention is paid to mathematical models for evaluation of the role of different molecular mechanisms in the RBC volume control. It is shown that many questions, for example, 'why the Na^+,K^+-ATPase pumps the ions in opposite directions' or 'what is the physiological role of Ca^{2+}-activated K^+-channels', cannot be answered without adequate mathematical models of such complex control systems as cell volume control.

Key words: red blood cell, Na^+,K^+-pump, Ca^{2+}-activated K^+-channels, cell volume, mathematical models.

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The ionic asymmetry of cells, i.e., the fact that the concentrations of Na^+, K^+, Ca^{2+} ions inside the cells are strongly different from their concentrations in extracellular liquids, is known for a long time [1]. This phenomenon has been and is explained in different ways [2]. It seems quite natural to assume that such asymmetry provides the sensitivity of cells to damage [3–5]. Another supposition, indisputable with respect to excitable cells, is the statement that the ionic asymmetry is needed for electric potential generation and for ensuring the electric activity of cells [6–9]. One more version is that the presence of ion gradient permits active transport of various useful substances into a cell [10–12]. Each of these assumptions is reasonable in relation to the same types of cells and may be disputed in relation to other types. However, ionic asymmetry exists in all cells without exception. Moreover, the concentrations of univalent cations are practically the same in the cells of all types [1].

Such surprising uniformity compels us to think that the ionic asymmetry is an element of one of the most basic cell systems. In recent years is has become clear that it is really so, because any cell consists of a great number of molecules, which are absent in the environment, and these molecules generate an excess osmotic pressure in a cell, so that cells must resist osmosis and control their volume. This is the same basic problem for any cell as, for example, energy supply. For the first time, the idea of association of ionic asymmetry with osmoregulation was apparently suggested in the work of Tosteson published in 1959 [13]; it was described in more details by Tosteson and Hoffman in 1960 [14]. Nearly half a century has passed since that time, and we finally begin to understand that the necessity of maintaining constant cell volume appears simultaneously with the very first cells in the course of evolution. For this purpose, nature has invented ion pumps, special channels, and a number of other notable systems, which provide the cell with fantastic stability and autonomy. We are still far from complete understanding of the whole picture; however, what we know anyway opens up a harmonious and beautiful multilevel system of cell volume control. This regulation has been studied best in the red blood cells (RBCs) of mammals; hence, volume control in a human RBC will be in the focus of attention in this paper.

Mathematical models played a key role in the study of cell volume control [15–21]. The models provided answer to quite a number of questions, which were either very difficult or impossible to answer experimentally, predicted a series of unexpected connections in cell metabolism, and helped us to understand why a cell needs some senseless (on the face of it) biochemical reactions.

In recent years, mathematical modeling has become popular. At the same time, the overwhelming majority

[1] The article is translated by the authors.

Table 1. Electrolyte composition (mg-equ/l H_2O) of oxygenated RBC and blood plasma

Electrolyte	RBC	Blood serum	Blood plasma
Cations			
Na^+	23.0	152	152.9
K^+	143.7	4.7	5.7
Mg^{2+}	8.4	2.5	3.3
Ca^{2+}	6×10^{-5}	4.7	5.6
Cations total	175.1	163.9	167.5
Anions			
HCO_3^-	16.0	27.4	31.9
Cl^-	71.0	110.7	108.9
HPO_4^{2-}	1.0	2.2	2.2
SO_4^{2-}	–	–	1.3
2,3-DPG	34.2	0	0
Organic phosphates without 2,3-DPG	11.7	0	–
Organic acids	–	–	5.7
Proteins	35.0	19.0	17.5
Not identified	6.2	5.6	–
Anions total	175.1	163.9	167.5

of serious biologists are scornful of mathematical models. Both attitudes have their own reasons. On the one hand, a great number of mathematical descriptions of different biological systems have just translated the facts already known to biologists into mathematical language without giving any new knowledge. On the other hand, from general considerations and by analogy with the development of physics and chemistry it is clear that the deep insight into the complex systems is hardly possible without using serious mathematics. Biology also has a number of impressive examples of effective application of mathematical models. It would be enough to remember the currently famous Hodgkin—Huxley model describing the mechanisms of electric excitation of nervous cell that has had a great effect on scientific development [22]. But the most important fact is that various fields of biology have already accumulated noticeable positive experience of using mathematical models, which leads to some general conclusions. It gives rise to considering, by the example of the study of ion homeostasis and cell volume control, the question about what mathematical models can give in principle for understanding of the work of a complex biological system, where they are indispensable, and for what tasks they are practically useless.

In 1957, J.C. Skou discovered a Na^+,K^+-ATPase [23]. This discovery provided a natural biochemical basis for understanding of the nature of electric excitation of cells. By that time, it was already clear that the electric potential difference across cell membrane is necessary for the formation and distribution of nerve impulse and for contraction of muscle cells [8, 11]. Since Na^+,K^+-ATPase transfers univalent cations across the membrane, ion gradients develop [24, 25]. In its turn, this may lead to the appearance of potential difference across cellular membrane [26]. This biological function of Na^+,K^+-ATPase is so natural and clear that for many years it has shielded the main function of this transmembrane pump. Until recently, practically all papers devoted to this protein started from the statement that it is needed for creation of ionic asymmetry and electric potential on the cell membrane. However, very soon after the discovery of this enzyme it became clear that all animal cells possess this protein. It is also present in the cells that have no electric activity or have a very low transmembrane potential. Red blood cells are among them.

All animal cells, irrespective of their electric properties, have been shown to possess actually the same intracellular cation composition, considerably different from the extracellular one [1]. As one can see from Table 1, the sodium to potassium ratio in a cell is inverted relative to the extracellular environment: there is much sodium and little potassium outside the cell and vice versa inside the cell. Thus, it has been shown that the presence of Na^+,K^+-ATPase and ion gradients is a necessary but not sufficient condition for the high transmembrane potential. Special ion channels are also needed to provide electric excitation. But what do other cells need Na^+,K^+-ATPase and ion gradients for?

The studies of red blood cells have given an answer to this question. As a matter of fact, the works of D. Tosteson [13, 14, 27, 28] on red blood cells initiated the understanding of the fact that the main biological role of asymmetric distribution of cations between intra- and extracellular space is osmoregulation: all cells contain numerous substances that are needed for their vital activity and should not be dissipated in the space. Cell evolution proper began only after the appearance of an envelope separating the cell from the environment [29]. However, the appearance of the substances that cannot pass through the membrane results in an increase of osmotic pressure inside the cell. The bilayer phopsholipid membrane (natural cell wall) sustains the pressure of no more than 2 kPa. It means that the cell with the membrane area of 100 μm^2 can mechanically resist an excess pressure of 1–2 mOsm and then the membrane must break off [30]. The membrane area cannot change quickly because the rates of transport and de novo synthesis of membrane lipids are incommensurably slower than the rate of volume variation at osmotic imbalance [31].

How many osmotically active molecules are present in a cell? Crude estimations show that the concentration of high-molecular substances, i.e. proteins, nucleic

acids and polysaccharides, is no more than 5–10 mM. Low-molecular metabolites, which the cell seeks not to let out, such as intermediates of different metabolic systems, cofactors, and master molecules of ATP kind comprise 20–25 mM more [32–34]. It means that inside the cell there must appear such excess pressure that can be resisted either by creation of additional rigid walls or by reduction of the concentration of some substances, which are abundant both inside and outside. For animal cells, the nature has chosen the second way. According to "eyewitnesses", life arose in the ocean. There were a lot of sodium ions around. Therefore, the appearance of a pump draining sodium out of a cell and thus normalizing the intracellular pressure was quite natural. Ion pumps are present in all eukaryotic cells, even in those with a rigid wall that could undertake the entire load so that there would be no need of pumps. Hence it follows that the main function of the pump draining sodium out of the cells is its involvement in the function of osmoregulating systems. It leads to the appearance of ion gradients, which in the course of further evolution are adapted by the cell for solution of other problems, such as electric excitation, ability to react to damage, etc.

For compensation of the excess osmotic pressure of proteins and other useful molecules, it would be enough for the cell to reduce Na^+ concentration by 30–40 mM. Actually, Na^+ concentration in the cell is much less. At the same time, the cell performs a seemingly meaningless work: pumps in the K^+ ions in nearly the same amount. Why perform unnecessary work on ion transfer against two gradients with great energy expenses? Below we will try to answer this question.

One more question associated with the ion fluxes control concerns cell volume. It is closely related to peculiarities of the mechanics of cell membranes: the bilayer phospholipid membrane, which is a basis of plasma membranes of all cells, is practically inextensible but very flexible [35, 36]. Therefore, it limits the maximal cell volume. The more intensively the cell pumps out ions, the less is its volume. We will try also to answer the following questions: what exactly must this volume be and what is it determined by?

The answer to the latter question leads, as we will see below, to a series of new unexpected questions. But first let us consider what a red blood cell is.

RED BLOOD CELL

The major task of RBC in an organism is to provide organs and tissues with oxygen and to remove carbon dioxide. Such cells must have a high intracellular concentration of hemoglobin, which actually transfers oxygen, binding it in the lungs and releasing in tissues, and be able to easily and quickly pass through all capillary regions to supply oxygen to all cells of an organism. The evolution of mammalian RBC was aimed to develop these properties. As a result, RBC lost nearly all systems not associated with the major task of a cell:

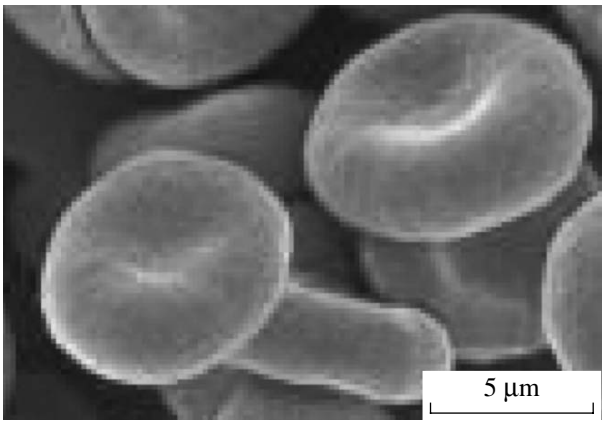

Fig. 1. Human red blood cell. Electron microphotograph has been kindly provided by Prof. G.I. Kozinets.

they have no nucleus, mitochondria, ribosomes, reticulum, nor other cell organelles [37].

The main metabolic systems of RBC are the systems of energy production and protection from oxidation and closely related systems of metabolism of the major cofactors such as adenylates, pyridine nucleotides, and glutathione [38, 39]. The only ATP source in RBC is glycolysis [37, 39]. Protection from oxidation is provided by traditional enzymes: superoxide dismutase, catalase, and peroxidase [38, 39]. The main source of electrons for redox defense is a pentose phosphate pathway [40].

Hemoglobin is the major protein of the cell. Its concentration is approximately 5 mM, which makes 95% of total cell proteins. The binding of oxygen to hemoglobin and its release in tissues do not need energy [32]. Besides the oxygen transport to tissues, RBCs transport CO_2 from tissues to the lungs. The role of hemoglobin in this process is minor; CO_2 is transported to the lungs mainly as bicarbonate anion. For accelerating the equilibration between CO_2 and H_2CO_3, RBCs possess a highly active carboanhydrase [32, 41].

The RBC membrane contains 10–12 major proteins and many tens of other proteins, the total content of which is relatively low. Integral membrane proteins are bound to the lipid bilayer and are glycoproteins. Peripheral proteins form a cytoskeleton of RBC [42, 43]. The lipid bilayer contains approximately equal quantities of cholesterol and phospholipids.

In the normal state, RBC is shaped as a biconcave disc (Fig. 1), 7.5–8.7 μm in diameter [44–46], ~1 μm and ~2 μm in the center and in the thickest part, respectively [44, 47]. RBC has a surface area of 120–155 μm^2 [47–50] and a volume of 84–107 μm^3 [48, 49, 51–54]. RBC volume is about 60% of the maximally possible value at the given surface area. This very fact determines its capacity for severe deformation, which is necessary for passing through the narrowest capillaries of 2–3 μm in diameter. The RBC volume is a dynamic

variable and depends on the balance of many concentrations on both sides of the membrane, which, in their turn, are determined by substance fluxes across the cell membrane. The permeability of RBC membrane for water is very high [55, 56]. Table 1 [16, 57] presents the concentrations of substances making the major contribution to the osmotic balance within RBC and outside, in blood plasma. One can see that the main contribution is made by cations (sodium and potassium) and anions (chloride and HCO_3^-).

Mature RBC lives in the bloodstream for 100–120 days [1]. Normally, most RBCs die in the spleen [58]: they are pushed through the slits of 0.2–0.5 μm in width [59] and the stuck cells are eliminated by macrophages [58]. Thus, the ability for volume control and, as a consequence, the ability to be easily deformed and pass through narrow capillaries is the main criteria of RBC viability and therefore strictly controlled in an organism.

MATHEMATICAL MODEL OF RBC VOLUME CONTROL

Now let us consider the concepts of volume control, describing successively all new control elements. At the same time, we will confine only to the ion homeostasis and its role in cell volume control without considering the involvement of metabolic systems in this process. It would be convenient to examine the whole aggregate of factors that influence cell volume and ion concentrations using appropriate equations. In 1960, D. Tosteson and P. Hoffman published the first mathematical estimates of how the sodium and potassium ions in red blood cells of two varieties of sheep, different in the content of cations, were associated with osmoregulation of these cells [14]. They obtained the formula linking RBC volume, *V*, with sodium and potassium concentrations on both sides of the membrane:

$$V = \frac{W}{([\mathrm{Na}]_e + [\mathrm{K}]_e + [\mathrm{Cl}]_e) - ([\mathrm{Na}]_i + [\mathrm{K}]_i + [\mathrm{Cl}]_i)},$$

where indices *i* and *e* were intra- and extracellular concentrations, respectively, *V* was RBC volume, and *W* was the total concentration of all osmotically nonpenetrating molecules.

The model of D. Tosteson described well his experimental data. However, the inaccuracy of measurements led the authors to an incorrect conclusion. They obtained the ratio of Na^+ and K^+ fluxes for Na^+,K^+-ATPase as equal to 1. In spite of this fact, the work proved to be a turning point. The authors have shown for the first time that the osmotic balance is important for volume control and this balance is determined by the fluxes of mainly two ions: passive fluxes of sodium and potassium ions through the membrane and an active flux of the same ions generated by Na^+,K^+-ATPase. At the same time, the membrane permeability for water and penetrating anions is so high that their concentrations on both sides of the membrane are close to thermodynamically equilibrium values.

Though the works of D. Tosteson and his team appeared in the early 60s of the last century, only in 1980 E. Jacobsson published a mathematical model [15] considering the K^+ and Na^+ homeostasis in RBC and its association with volume in a comparatively modern form. In 1983, our team published a related but more complete model of these processes. These two models will be used as a basis of our further statement [16, 21]. The principal variables in both models were intracellular concentrations of Na^+ and K^+ cations and major anions (chloride and HCO_3^-), cell volume, and transmembrane potential. For simplicity, let us take the concentrations of all ions outside the cell as constant. The changes in ion concentrations are determined by the changes in the volume and balance of passive fluxes across the membrane and the active fluxes generated by ion pumps.

As has been shown in the work of E. Jacobsson, the passive ion fluxes in red blood cells are well described by the Goldman equation [60, 61]. For K^+ and Na^+, we have:

$$J_\mathrm{K} = P_\mathrm{K}\frac{\frac{\varphi F}{RT}}{\exp\left(\frac{\varphi F}{RT}\right)-1}\left([\mathrm{K}^+]_e - [\mathrm{K}^+]_i\exp\left(\frac{\varphi F}{RT}\right)\right)$$

$$J_\mathrm{Na} = P_\mathrm{Na}\frac{\frac{\varphi F}{RT}}{\exp\left(\frac{\varphi F}{RT}\right)-1}\left([\mathrm{Na}^+]_e - [\mathrm{Na}^+]_i\exp\left(\frac{\varphi F}{RT}\right)\right).$$

The fluxes determine variations in the content of ions in the cell, which is a product of a given ion concentration and a variable cell volume:

$$\frac{d}{dt}\left([\mathrm{K}^+]_i\frac{V}{V^0}\right) = \mu\nu_{\mathrm{Na,K}-ATPase} + J_\mathrm{K} \quad (1)$$

$$\frac{d}{dt}\left([\mathrm{Na}^+]_i\frac{V}{V^0}\right) = -\eta\nu_{\mathrm{Na,K}-ATPase} + J_\mathrm{Na}, \quad (2)$$

where J_K and J_Na are passive fluxes of K^+ and Na^+ through RBC membrane;

P_K and P_Na are passive membrane permeability for K^+ and Na^+, respectively;

F is Faraday constant;

R is thermodynamic gas constant;

T is absolute temperature;

φ is transmembrane potential difference;

indices *i* and *e* denote intra- and extracellular concentrations, respectively.

$\nu_{\mathrm{Na,K}\text{-}ATPase}$ is the rate of ATP hydrolysis by the Na^+,K^+ pump;

μ and η are the quantities of K^+ and Na^+ ions, respectively, transferred by the pump per one act of ATP hydrolysis;

V is RBC volume;

V^0 is physiologically normal value of RBC volume.

These equations included both the active fluxes generated by the Na^+,K^+ pump and the passive fluxes of these ions through the membrane. Let us choose the parameters in these equations in accordance with the work [21]:

$$P_K = 1.24 \times 10^{-2}\ h^{-1}; \quad P_{Na} = 1.22 \times 10^{-2}\ h^{-1};$$
$$[K^+]_e = 5\ mM; \quad [Na^+]_e = 145\ mM.$$

Distribution of anions is determined only by the passive fluxes of these ions through the membrane. It is known that the RBC membrane is highly permeable for anionis: according to the data of [62, 63], the membrane permeability for anions is about 2 h^{-1}, i.e. exceeds by two orders of magnitude the permeability values for univalent cations. Generally, it is conditioned by the high protein concentration of band III. This channel is nonselective for the two major anions, Cl^- and HCO_3^- [64, 65]; therefore, it is convenient to introduce a new variable, $[A^-]$, which is the total concentration of anions penetrating through the membrane. The high membrane permeability for anions suggests that the anion concentrations are close to equilibrium:

$$\frac{[A^-]_e}{[A^-]_i} = \exp\left(-\frac{\varphi F}{RT}\right), \tag{3}$$

where $[A^-]$ is the sum of concentrations of penetrating anions (Cl^- and HCO_3^-), $[A^-]_e = 150$ mM [21]. As follows from this equation, the anions between the intra- and extracellular space are distributed in accordance with the transmembrane potential.

It is also necessary to take into account the equations describing the electrical neutrality of intra- and extracellular contents and the osmotic balance between the cell and the ambient space:

$$[K^+]_i + [Na^+]_i - [A^-]_i + ZW = [K^+]_e + [Na^+]_e - [A^-]_e = 0 \tag{4}$$

$$[K^+]_i + [Na^+]_i + [A^-]_i + W = [K^+]_e + [Na^+]_e + [A^-]_e = 2L = 300\ mOsm. \tag{5}$$

These equations take into account the presence in the cell of proteins, first of all hemoglobin, and low-molecular metabolic intermediates, which make their contributions both to electrical neutrality and to osmosis. For simplicity, these equations assume that the total concentration of all osmotically nonpenetrating molecules is W and that all these molecules have the same average charge Z. The analysis shows that more exact account for the fact that different nonpenetrating molecules possess different charges has little influence on the result. According to our estimates [16, 21],

$$W = 45\ mM; \quad Z = -0.7.$$

The system of equations (1)–(5) describes the changes in ion concentrations and cell volume as a function of system parameters such as membrane permeability for different ions, parameters of the Na^+,K^+-pump, concentrations of nonpenetrating ions, and osmolarity of the extracellular medium. These equations can be used to obtain an equation describing cell volume variation. From Eqs. (1)–(2) it follows that:

$$\frac{d}{dt}\left([K^+]_i\frac{V}{V^0} + [Na^+]_i\frac{V}{V^0}\right) = J_K + J_{Na} - (\eta - \mu)v_{Na,\,K-ATPase} \tag{6}$$

The sum of Eqs. (4) and (5) gives:

$$[K^+]_i + [Na^+]_i = L - \frac{1}{2}W(1+Z). \tag{7}$$

Multiplication of Eq. (7) by V/V^0 and differentiation by time gives:

$$\frac{d}{dt}\left([K^+]_i\frac{V}{V^0} + [Na^+]_i\frac{V}{V^0}\right) = L\frac{d}{dt}\left(\frac{V}{V^0}\right) - \frac{1}{2}(1+Z)\frac{d}{dt}\left(W\frac{V}{V^0}\right) = L\frac{d}{dt}\left(\frac{V}{V^0}\right). \tag{8}$$

On conversion of Eq. (8) using (6), we can see that the kinetics of cell volume variation is determined only by the balance of passive and active fluxes of sodium and potassium ions through the membrane:

$$\frac{d}{dt}\left(\frac{V}{V^0}\right) = \frac{1}{L}[J_K + J_{Na} - (\eta - \mu)v_{Na,\,K-ATPase}]. \tag{9}$$

The rate of the Na^+,K^+-pump increases with the growth of intracellular Na^+ concentration almost linearly [66], because the constant of binding of these ions on the intracellular side is equal to 5–13 mM [67, 68] and their concentration is equal to 10 mM (see Table 1). This rate also depends on the concentrations of ATP and extracellular K^+ [69, 70], but these values are constant in the framework of the present consideration. Hence,

$$v_{Na,K-ATPase} = \alpha[Na^+][ATP],$$

where $\alpha = 0.045\ h^{-1}$ mM, [ATP] = 1.43 mM [71].

According to the data of the work [72], the rate of the ATP uptake by Na^+,K^+-ATPase in the normal physiological state is 0.6–1 mmol/h l cells.

The performed calculations have shown that at selected parameters the model predicts the correct stationary concentrations of all ions and the correct cell volume (Table 2). This fact led E. Jacobsson to the conclusions that cell volume control is possible only at membrane potential values below a certain level and that the internal nonpenetrating ions must bear a nega-

Table 2. Steady-state values of intracellular ion concentrations and volume for the normal physiological values of model parameters

Variable	Theory	Experiment	Units of measurement	Sources
Na^+	10	16.6 ± 1.6	μmole/l cellular water	[24, 102]
K^+	130	135 ± 5.0	μmole/l cellular water	[24, 102, 103]
$Cl^- + HCO_3^-$	110	103–107	μmole/l cells	[23, 102]
Intracellular concentration of non-penetrating anions	50	17	μmole/l cells	[23, 42]
Volume	76	84–107	μm^3	[50–52, 55–57, 66]

tive charge. Besides, it was concluded that the active removal of Na^+ from the cell is sufficient for cell volume maintenance. The regulation was shown to depend on passive membrane permeability for K^+ and active removal of Na^+ from the cell. The basic conclusion of Jacobsson, which is fundamentally important for further development of this view, is that the ratio of passive and active transport is crucial for cell volume and membrane potential.

In spite of the fundamental significance of the Jacobsson's qualitative conclusion, the quantitative conclusions do not seem very surprising. These results weakly depend on any particular type of Na^+ dependence of the Na^+,K^+-ATPase rate [20] and on other parameters, because in the range of experimentally observed spread of parameters it is always possible to select a set that would correctly describe the stationary state of the cell. At this stage, we have simply translated the known facts into a new language and selected parameters so that these facts could be described quantitatively. The accuracy of measurement of the constants and parameters of this biological system is so low that a minor alteration of these constants within the existing spread gives twice as many different volume values. The range of volume variations permitted by RBC construction is small. If the volume increases more than 1.6-fold, RBC will break: it is the maximal volume that can be enclosed by an almost inextensible cell membrane with the surface area of normal RBC. The double decrease of the volume is also practically impossible, because in this case protein concentrations in the cell will increase so much that hemoglobin will become rigid and RBC will lose the ability for deformation and passage through the narrow capillaries of the spleen [73].

This reasoning shows that RBC volume has to be controlled quite precisely, i.e., the values of parameters influencing the volume should be in close correlation with each other. Otherwise, one has to assume that there is an additional adjustment mechanism that makes the volume not so sensitive to system parameters. The RBC volume in an organism must be predetermined with yet higher accuracy than it results from the approximate physical limitations pointed out above; this fact follows from examination of the RBC movement in the blood vascular system.

RBC MOVEMENT IN NARROW CAPILLARIES

The main physiological task of a red blood cell, i.e., oxygen transport, demands high deformability of this cell [74, 75]. Hemoglobin increases the oxygen concentration in the blood of animals nearly 50-fold. At the same time, blood viscosity must not be very high. It seriously constrains the rheology of RBC. For complete oxygen exchange between tissues and red blood cells, our organism has narrow capillaries; the narrowest of them are 2.5–3 μm in diameter [1]. RBC has to change its shape substantially in order to squeeze through such a capillary [76, 77]. Therefore, the rate of RBC movement in capillary depends a lot on its viscosity/elasticity characteristics. Figure 2 shows the dependence of viscosity of the intracellular contents of an "average" RBC on the RBC volume-to-surface ratio. The diameter of the minimal capillary, through which this RBC can pass through, was calculated. We can see that at a high cell volume RBC has "not enough" surface for changing its shape significantly so that to be drawn in a narrow capillary. At a lower volume this problem diminishes but the intracellular viscosity begins to grow (first of all, due to the very high hemoglobin concentration).

Such RBC can pass through a narrow capillary but needs very much time for changing its shape. As a result, the dependence of the rate of RBC movement in the narrow capillary on its volume has an explicit maximum (Fig. 3). We can see that the volume decrease by only 20% reduces the rate of filtration of red blood cells through narrow capillaries more than twice. As a result of volume increase by the same 20%, RBC gets stuck in a capillary of 4–5 μm in diameter (Fig. 3), whereas RBC with the optimal ratio can pass through a capillary that is almost two times narrower. These data clearly show that RBC volume has to be optimal and should not change much either upon variation of blood plasma parameters or during the cell lifetime. May perhaps the

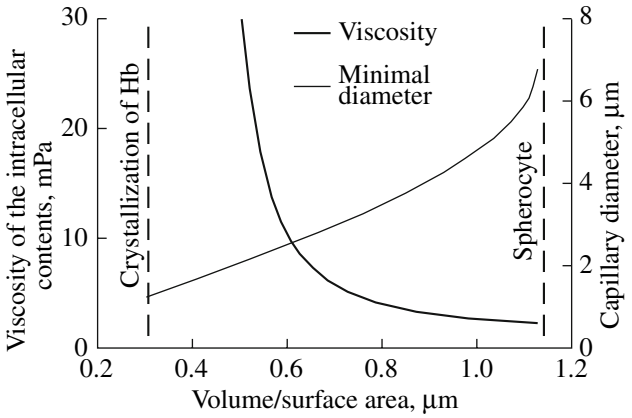

Fig. 2. Dependence of viscosity of the intracellular contents of RBC and the minimal diameter of capillary, through which it can pass, on the ratio of cell volume to cell surface area. The descending curve shows how the viscosity of intracellular contents varies at a change of this ratio. The ascending curve shows the increase of the minimal diameter of capillary, through which RBC with such ratio is able to pass.

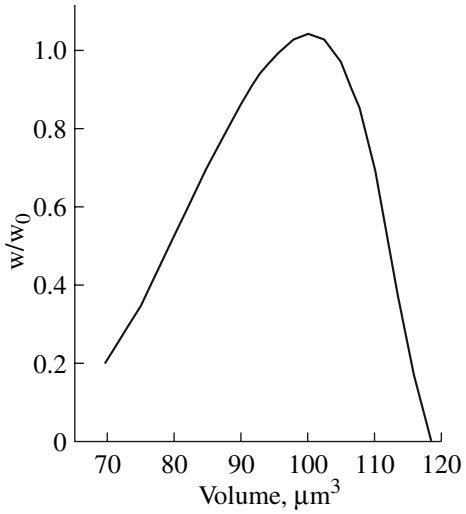

Fig. 3. Dependence of the relative rate (w/w_0) of RBC passing through a 3-mm capillary on RBC volume.

parameters of "habitat", like the parameters of RBC itself, be extremely stable? Indeed, for example, red blood cells are very sensitive to osmolarity of the environment. This is in good agreement with the data of model (1)–(5). However, osmolarity of the plasma is very stable [1]. The same is the case with other parameters of the medium but not RBC itself.

CELL MEMBRANE PASSIVE PERMEABILITY FOR CATIONS

The "Achilles heel" of a cell (not only RBC) is permeability of its membrane for small molecules, primarily cations [78]. Modification of passive membrane permeability influences the volume as much as the change in osmolarity. However, in contrast to osmolarity, it seems impossible to stabilize membrane permeability. The major cause of significant changes in membrane permeability is oxidative processes. Considerable oxygen concentration in blood, the free-radical character of oxidation/reduction processes, the presence in an organism of numerous substances, which are strong oxidation agents, all this may result in variations of the oxidation rate of cell wall proteins and lipids hundreds of times. Oxidized lipids enhance a good deal the membrane permeability for charged molecules [79, 80]; therefore, the ion permeability of RBC membrane in the normal organism may also vary 5–10 times depending on nutrition, various stresses, minor inflammation foci, and other factors [81, 82]. As a result, the cell must not only have a definite volume but also carefully maintain it, i.e. the cell needs to stabilize its volume withstanding the variations of membrane permeability.

ADVANTAGE OF Na^+,K^+-PUMP AS COMPARED WITH Na^+-PUMP IN CELL VOLUME STABILIZATION. WHY DOES Na^+,K^+-ATPase EXCHANGE THREE Na^+ FOR TWO K^+?

In the early 80s we wondered: what is the advantage of the pump that carries three sodium ions out in exchange for two potassium ions [16], so that the total transfer is one ion per ATP molecule? Would not it be simpler just to extract one sodium ion from the cell? Mathematical models are very suitable for solution of such problems, because they calculate the situations that cannot be created experimentally but may help us to elucidate the regularities in the system functioning. For example, we can easily find out what will happen if the pump drains sodium only [20, 21]. It turns out that in this case it is easy to obtain a physiologically normal value of cell volume, like in the case of pumping the ions towards each other. No advantages of the counter transfer are observed. However, the situation changes cardinally if we assume that each of these pumps stabilizes the volume. Figure 4 presents the dependences of volume changes on the passive membrane permeability for Na^+ and K^+ ions. Figure 4 examines the dependence of volume on the nonspecific change of permeability for both cations P, i.e.

$$P_{Na} = P^0_{Na}(1 + P);$$

$$P_K = P^0_K[1 + (P_{Na}/P_K)P].$$

In the normal RBC, P_{Na} and P_K are approximately equal [83, 84], therefore parameter P characterizes the nonspecific change of permeability for both ions. This is the most typical influence on RBC in an organism, because the increase of membrane permeability at oxidative stresses generates nonspecific "drains". Below we will consider how the changes in permeability for

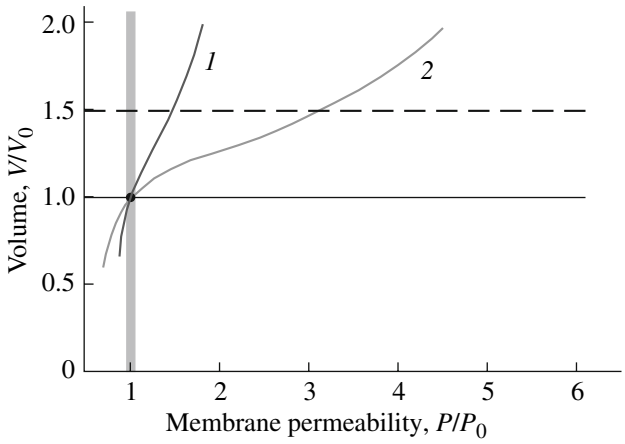

Fig. 4. Dependence of the relative change of RBC volume on the relative change of passive membrane permeability for Na^+ and K^+. Curve *1* is obtained with the assumption that the volume is stabilized by a hypothetical pump that drains only Na^+ ions from the cell. Curve *2* is obtained with the assumption that the volume is stabilized by the Na^+,K^+-pump. Here and further, V_0 and P_0 designate respectively the volume and membrane permeability of RBC at physiological values of parameters.

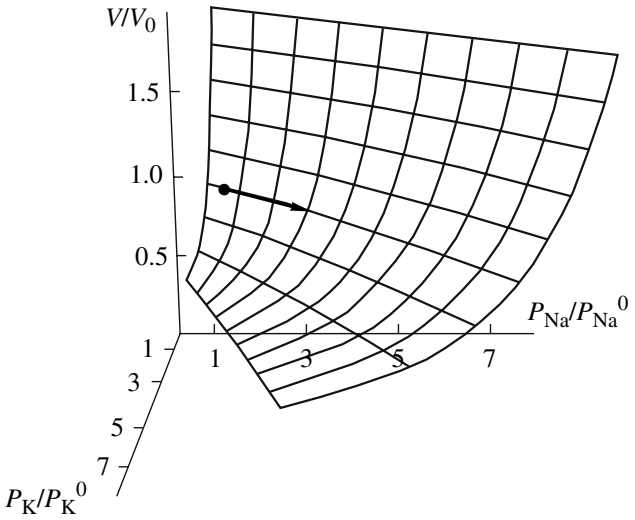

Fig. 5. The change of RBC volume at independent variations of membrane permeability for Na^+ and K^+.

each cation separately affect volume stabilization. The volume is normalized by its physiological value. The dotted line shows the critical increase of the volume, at which RBC bursts. We can see that the Na^+-pump poorly stabilizes the cell volume [85]. No more than a 1.5-fold increase of membrane permeability results in RBC death [21]. This fact obviously contradicts the experimental data, according to which the permeability of RBC may change 5–10 times without its lysis [78].

For the quantitative characterization of volume stabilization it would be convenient to introduce two dimensionless indices. The first one, stabilization coefficient, is a value reciprocal to the relative derivative

$$\vartheta = \frac{V_0}{P_0}\frac{1}{\dfrac{\partial V}{\partial P}} = -\frac{\partial \ln V}{\partial \ln P}\bigg|_{P_0,\,V_0}.$$

For Fig. 4, where both variables are laid off in relative values, it is simply the value reciprocal to the slope of the curve in a given point. The less is the curve slope at a given, e.g. physiological, parameter value, the better is stabilization. The second parameter (the dynamic range of stabilization),

$$\Omega = P_{max}/P_{min},$$

shows how many times the parameter can vary before the cell volume goes beyond the preset limits (see Fig. 4, dotted line), e.g., change by no more than 50%.

Curve *1* in Fig. 4 illustrates the best variant of volume stabilization by the pump draining sodium only, which we have succeeded to obtain by varying practically all system parameters. It corresponds to stabilization coefficient ϑ = 1.0 and to the dynamic range of 1.5. Volume stabilization noticeably improves, if the pump generates two gradients simultaneously (Fig. 4, curve *2*). Stabilization coefficient increased to 3.5 and the dynamic range increased 2.2 times. The parameters for this calculation were the same as for the pump working with a single ion. The observed considerable gain was due to the fact that Na^+ concentration in the cell was significantly lower in the presence of two gradients. In response to the prescribed change in membrane permeability, sodium concentration varies greater in the model with the two gradients. It means that the rate of ions pumping changes much greater and, as a result, the volume changes less. Sodium concentration is the main sensor of the volume, which "indicates" that the pump must change its speed. In the case of Na^+ pump, sodium concentration in the cell cannot decrease significantly, because it is limited by the osmotic balance on both sides of the membrane. If the pump carries two ions towards each other, then this limitation is superimposed on the total Na^+ and K^+ concentration in the cell. At the same time, the sodium concentration may be reduced as much as is desired.

The model not only gives an insight of the advantage of opposite pumping of the ions but also explains why the pump drains three Na^+ ions in exchange for two K^+ ions. Figure 5 shows the variation of cell volume, when the permeabilities for sodium and potassium vary independently. We can see that these permeabilities have adverse effects on the volume: the increase of permeability for sodium enhances the volume, whereas the increase of permeability for potassium decreases it. It is clear because the sodium gradient is directed inside the cell and the passive sodium flux increases the content of substance in the cell and, consequently, the volume. The situation with potassium is the opposite. Besides, there is a direction of joint variation of permeabilities when the volume change is the minimal (Fig. 5, arrow). At physiological value of

the volume, this direction coincides with the synchronous variation of permeabilities for both cations, i.e. volume stabilization is the best at nonspecific permeability variation. We have considered the analogous surfaces for the pumps with different ratios of the transferred ions [20, 21]. At the same time, all surfaces are of similar appearance, but the direction of the optimal stabilization already does not coincide with the nonspecific permeability variation. The higher is the Na^+/K^+ ratio, the more the relative variation of permeability for Na^+ corresponds to the best stabilization [20, 21].

Thus, the model shows that the ion pump is perfectly optimized for cell volume stabilization just against the damaging nonspecific increase of its permeability. This fact determines both the creation of two gradients by the pump and the transfer of just three sodium ions in exchange for two potassium ions.

SECONDARY APPLICATION OF POTASSIUM GRADIENT FOR VOLUME STABILIZATION IMPROVEMENT. Ca^{2+}-ACTIVATED K^+-CHANNELS

The occurrence of high potassium gradient directed outwards opens a number of new possibilities for a cell. Different cells use these gradients for different purposes: some cells generate high membrane potential and become electrically excitable [7–9], while other cells form systems that react to diverse external signals, including those causing damage to a cell [3–5]. Among numerous useful applications of potassium gradient, we will discuss the one used in red blood cell for still greater improvement of the quality of volume stabilization in response to nonspecific damage of the membrane. As has already been mentioned, the selective increase of membrane permeability for potassium only results in cell volume decrease. Hence, in the case of a special channel selectively permeable for potassium only, cell volume can be regulated by its opening or closing. Red blood cells possess such a channel. The increase of potassium efflux in response to the increase of Ca^{2+} concentration in the cell conditioned by this channel was discovered by Gardos as far back as in the 1950s [86, 87]. For several decades it was unclear why the cell did it. The properties of this channel were studied in detail [88, 89]; it was shown that the channel was opened by the increase of Ca^{2+} concentration in the cell only several times [90] (the half-maximal activation of K^+ channels is achieved at the intracellular concentration of potassium ions of 0.1–10 μM [91]). These channels named as Ca^{2+}-activated K^+-channels, being maximally open, increase the K^+ efflux from the cell hundreds of times (from 2 to 100–1000 μmol/h l cells) [90, 92, 93]. The insight of what it gives to a cell was got only after the models had shown that such channel could greatly improve the cell volume stabilization [20, 21].

Incidentally, by the way, we have found the answer to one more mysterious question: why is Ca^{2+} concentration in red blood cells lower by four orders of magnitude [94] as compared with the concentration in blood plasma? An enormous Ca^{2+} gradient exists in all cells and is generated due to the function of Ca^{2+}-pump, which takes up ATP and actively drains Ca^{2+} from the cell against the gradient. The emerging gradient is used by many cells for generation of intracellular signal in response to various excitations. Intracellular calcium is rightfully considered as one of the main intracellular secondary messengers that transmit information from outside to intracellular systems. Thus, here we can also see the universal aspiration of scientists for ascribing signal functions to any gradients on the cell membrane. However, for a long time it was unclear what signals exactly and to where this gradient transmitted in red blood cells.

Let us consider in more detail in what way the Ca^{2+} gradient and Ca^{2+}-activated K^+-channels affect the control of RBC volume. For taking into account the potassium flux through these channels, we must include one more flux into Eq. (1):

$$J_{CH} = P_{CH}\frac{\frac{\varphi F}{RT}}{\exp\left(\frac{\varphi F}{RT}\right)-1}\left([K^+]_e - [K^+]_i\exp\left(\frac{\varphi F}{RT}\right)\right),$$

where $P_{CH} = P_{\max}\left(\frac{[Ca^{2+}]}{[Ca^{2+}]+K_{CH}}\right)^N$,

$P_{\max}$ is maximal permeability of K^+-channels,

$P_{\max} = 1.7\ h^{-1}$ [104–106],

N is cooperativity coefficient, $N = 4$,

K_{CH} is binding constant for Ca^{2+} with the channels, $K_{CH} = 0.25$ μM.

This is a passive flux of potassium ions, which naturally depends on their concentration gradient and membrane potential. The main difference of this flux from the previously considered passive potassium flux is dependence on Ca^{2+} concentration. As a result of this dependence, our equations have one more independent variable: Ca^{2+} concentration in the cell. This concentration is usually so low that actually has no effect on balance equations (4), (5). For the changes of Ca^{2+} concentration, we can write an equation analogous to Eqs. (1) and (2), taking into account that the calcium ion is bivalent.

$$\frac{d}{dt}\left([Ca^{2+}]_i\frac{V}{V_0}\right) = -2\nu_{Ca-ATPase} + P_{Ca}\frac{2\frac{\varphi F}{RT}}{\exp\left(2\frac{\varphi F}{RT}\right)} \times\left([Ca^{2+}]_e - [Ca^{2+}]_i\exp\left(2\frac{\varphi F}{RT}\right)\right), \quad (10)$$

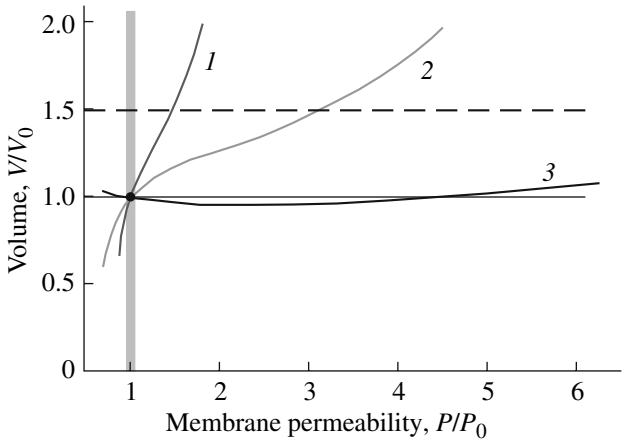

Fig. 6. Dependence of the relative change of RBC volume on the relative changes of passive membrane permeability for Na^+ and K^+. Curve *1* is obtained with the assumption that the volume is stabilized by the Na^+ pump only. Curve *2* is obtained with the assumption that the volume is stabilized by the Na^+,K^+-pump. Curve *3* is obtained with the assumption that the volume is stabilized by the Na^+ and K^+ gradients generated by the Na^+,K^+-ATPase as well as Ca^{2+}-dependent K^+-channels.

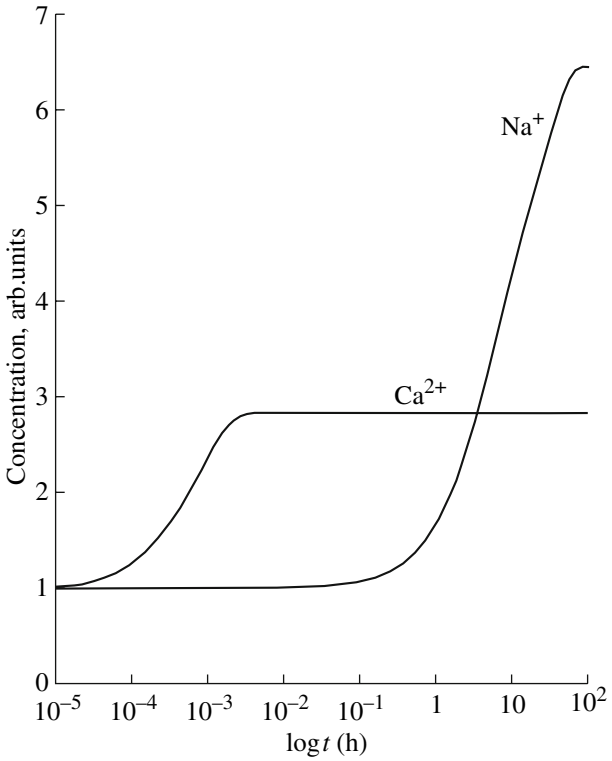

Fig. 7. The kinetics of variation of Ca^{2+} and Na^+ concentrations in red blood cells after a stepwise change of membrane permeability. The permeability for all ions was changed 5-fold in the initial moment.

where $v_{Ca-ATPase} = \alpha_{Ca-ATPase}\left(\frac{[Ca^{2+}]}{[Ca^{2+}] + K_{Ca-ATPase}}\right)^2$,

$P_{Ca} = 0.76 \times 10^{-2}$ h^{-1} [21].

The equation for intracellular Ca^{2+} (10) weakly depends on other equations of our system. This effect passes through membrane potential φ, but this potential is usually low and varies weakly. Therefore, Ca^{2+} concentration in the cell weakly depends on the medium osmolarity, protein content in the cell, etc. Calcium in this model behaves rather autonomously and has little influence on anything. However, the situation drastically changes when we consider the nonspecific changes in membrane permeability, the damaging osmotic effect of which is counteracted by Na^+,K^+-ATPase. It is reasonable to suppose that such nonspecific effects increase the membrane permeability not only for univalent but also for bivalent cations. Figure 6 (curve *3*) shows the way in which volume stabilization is varied. One can see that the efficiency of stabilization sharply increases. Close to the physiological value of permeability, the stabilization coefficient changes its sign, as if crossing the infinite value. It implies over-regulation: at increasing permeability, cell volume may even decrease at first. A number of experimental data show [95–97] that, indeed, RBC volume decreases at minor variations of permeability. At more significant variations, the volume surely begins to grow but remains in the admitted region at permeability variations three times as much as in the absence of potassium channels. The dynamic range of stabilization comes up to 7–8, which is in good agreement with the experimental data [79–82]. In this case, all gradients of cations change in response to permeability increase, i.e. in response to the variations, which are counteracted by all of the control systems described above [21]. However, the relative Ca^{2+} concentration varies most rapidly and most significantly (Fig. 7). This is just Ca^{2+} that proves to be the major sensor, which is most sensitive to damage. The presence of such sensitive sensor makes it possible to drastically increase the quality of cell volume stabilization.

In the work [18], Jacobsson's model [15] was extended due to a more detailed description of ion exchange. The expressions describing K^+,Cl^- and K^+,Na^+,$2Cl^-$-co-transporters were included into the equations for ion fluxes. Among the nonpenetrating ions, Mg^{2+} and hemoglobin were singled out separately. The non-ideal osmotic behavior of hemoglobin was taken into consideration and the equation for the proton exchange between RBC and the environment was added. The equation for Na^+,K^+-ATPase took into account the competitive inhibition of the enzyme by extracellular Na^+ and intracellular K^+, but the extremely important dependence of its rate on the concentration of intracellular Na^+ was not excluded. The correct (3 : 2) stoichiometric ratio of the transferred ions was used in the work. This model, like the previous ones, did not include such an important element of volume regulation as Ca^{2+}-dependent K^+-channels (the Gardos effect). This work has shown that the K^+,Cl^--

and K^+,Na^+,$2Cl^-$-co-transporters do not play a key role in the regulation of RBC volume, and the account of non-ideal osmotic behavior of hemoglobin and dependence of its charge on pH also little changes the basic conceptions.

CONCLUSIONS

The studies of ion homeostasis in different cells, first of all in red blood cells, have given insight into the fact that osmosis control is not confined to only pumping out of some part of osmotically active ions from the cell at an appropriate rate [20, 21]. For the cell, one of the main parameter is the constancy of volume at significant variations of disturbing impacts. These disturbing impacts include practically all cell parameters, but the strongest of them is the damage of cell membrane and the related disturbance of ion homeostasis in the cell. In this work, we have concentrated our attention just on this disturbance. However, the analysis shows that the natural mechanisms rather effectively compensate for other variations as well. For example, the enzyme activities in the cell may vary both due to the action of damaging factors and due to genetic peculiarities [71, 98]. Red blood cells may differ in the concentrations of ion pumps in the membrane [99]. Based on Eqs. (1)–(10), one can say that the decrease of the Na^+,K^+-pump activity is absolutely equivalent to the increase of cell membrane permeability. Consequently, all of the described mechanisms will compensate for the decrease of the main pump activity just like the increase of membrane permeability. Close ratios are observed also for the Ca^{2+}-pump.

The mechanisms described in this work do not exhaust all facilities used by RBC for the improvement of volume stabilization [100–104]. It has been assumed above that the function of pumps is provided by the constant ATP level in the cell, and this level does not react in any way to the changing load, which accompanies the enhancement of fluxes. In [71, 100, 105] we considered the mutual influence of energy and ion flux regulation systems. This analysis led us to the concepts of the existence of a complicated and beautiful system that controls the volume due to metabolism of adenylate nucleotides, which can effectively interfere into volume control, bringing it to perfectness, in a wide range of variation of the parameters.

The main indicator of cell volume constancy is the quality of volume stabilization. With the purpose to provide the high coefficient of volume stabilization in the wide range of variation of the parameters, the cell uses several mechanisms for controlling the ion fluxes through cell membrane. It results in such a complex pattern of joint action of diverse mechanisms that the interpretation of results becomes absolutely ineffective without mathematical models of the process. So, a certain "differentiation of labor" takes place. Mathematical models cannot open or even predict the existence of the Na^+,K^+-pump, because nature could select other molecules as substances used for osmosis control. But, in turn, it is very difficult to understand without the models why this pump is arranged in this but not any other way. Why does it pumps off two opposite gradients and why is the ratio of transferred ions two to three but not another?

In different animal cells the mechanisms of volume stabilization are similar in many respects [106–114]. All of them rest upon active ion transport by the Na^+,K^+-pump. However, they often have a number of peculiar features and use other additional mechanisms of volume stabilization.

The pumps and channels described in this work do not exhaust the entire diversity of these facilities in different cells. Even in red blood cells, a few more types of different channels and exchangers are known [101–104]. Of course, they are not always implicated in the cell volume control system. They participate in realization of other physiological mechanisms as well. And, in many cases, the most complex and disputable is the question what is the physiological function of these membrane structures. We think that often the finding of answers is impeded by the absence of appropriate model works, the role of which in this field, like in biology as a whole, has been underestimated for a long time.

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REFERENCES

1. Human Physiology, Schmidt, R. and Tews, G., Eds., Moscow, Mir, 1996, vol. 1.
2. Boldyrev, A.A. and Tverdislov, V.A., Molecular Organization and Mechanism of the Functioning of Na-Pump, Blumenfel'd, L.A., Ed., Moscow, VINITI, 1978.
3. Boldyrev, A.A., *Biologicheskie membrany i ionnyi transport* (Biological Membranes and Ion Transport), Moscow, Izd. MGU, 1985.
4. Boldyrev, A.A., Lopina, O.D., Rubtsov, A M., and Svinukhova, I.A., *Biokhimiya aktivnogo ionnogo transporta i transportnye ATFazy* (Biochemistry of Active Ion Transport and Transport ATPases), Moscow, Izd. MGU, 1983.
5. Boldyrev, A.A., Kotelevtsev, S.V., Lanio, M., Alvares, K., and Peres, P., *Vvedenie v membranologiyu* (Introduction to Membranology), Moscow, Izd. MGU, 1990.
6. Hodgkin, A.L. and Huxley, A.F., Action Potentials Recorded from Inside a Nerve Fiber, *Nature*, 1939, vol. 144, pp. 710–711.
7. Hodgkin, A.L. and Huxley, A.F., Currents Carried by Sodium and Potassium Ions through the Membrane of the Giant Axon of *Loligo*, *J. Physiol.*, 1952, vol. 116, no. 4, pp. 449–472.

8. Hodgkin, A.L., Ionic Movements and Electrical Activity in Giant Fibers, *Proc. Royal Soc. (London)*, 1958, vol. B148, pp. 1–37.
9. Hodgkin, A., Nervous Impulse, Moscow, Mir, 1965.
10. Kahlenberg, A., Urman, B., and Dolansky, D., Preferential Uptake of *D*-Glucose by Isolated Human Erythrocyte Membranes, *Biochemistry*, 1971, vol. 10, no. 16, pp. 3154–3162.
11. Jacobs, R.L., Stead, L.M., Brosnan, M.E., and Brosnan, J.T., Hyperglucagonemia in Rats Results in Decreased Plasma Homocysteine and Increased Flux through the Transsulfuration Pathway in Liver, *J. Biol. Chem.*, 2001, vol. 276, no. 47, pp. 43740–43747.
12. Kilberg, S.M., Handlogten, M.E., and Christensen, H.N., Characteristics of an Amino Acid Transport System in Rat Liver for Glutamine, Asparagines, Histidine, and Closely Related Analogs, *J. Biol. Chem.*, 1980, vol. 255, no. 9, pp. 4011–4019.
13. Tosteson, D.C., Halide Transport in Red Blood Cells, *Acta Physiol. Scand.*, 1959, vol. 46, pp. 19–41.
14. Tosteson, D.C. and Hoffman, J.F., Regulation of Cell Volume by Active Cation Transport in High and Low Potassium Sheep Red Cells, *J. Gen. Physiol.*, 1960, vol. 44, pp. 169–194.
15. Jakobsson, E., Interactions of Cell Volume, Membrane Potential, and Membrane Transport Parameters, *Amer. J. Physiol.*, 1980, vol. 238, no. 5, pp. C196–C206.
16. Ataullakhanov, F.I., Regulation of Metabolism in Red Blood Cells, *Doctoral (Biology) Dissertation*, Moscow, Institute of Biophysics, Ac. Sci. USSR, 1983.
17. Werner, A. and Heinrich, R., A Kinetic Model for the Interaction of Energy Metabolism and Osmotic States of Human Erythrocytes. Analysis of the Stationary "in vivo" State and of Time Dependent Variations under Blood Preservation Conditions, *Biomed. Biochim. Acta*, 1985, vol. 44, no. 2, pp. 185–212.
18. Lew, V.L. and Bookchin, R.M., Volume, pH, and Ion-Content Regulation in Human Red Cells: Analysis of Transient Behavior with an Integrated Model, *J. Membr. Biol.*, 1986, vol. 92, no. 1, pp. 57–74.
19. Moroz, I.A., Ataullakhanov, F.I., Pichugin, A.V., Kiyatkin, A.B., and Vitvitsky, V.M., Mathematical Model of Regulation of Red Blood Cell Volume, *Biol. Membrany* (Rus.), 1989, vol. 6, pp. 409–419.
20. Ataullakhanov, F.I., Vitvitsky, V.M., Kiyatkin, A.B., and Pichugin, A.V., Regulation of the Volume of Human Red Blood Cells. The Role of Calcium-Activated Potassium Channels, *Biofizika* (Rus.), 1993, vol. 38, pp. 809–821.
21. Martinov, M.V., Vitvitsky, V.M., and Ataullakhanov, F.I., Volume Stabilization in Human Erythrocytes: Combined Effects of Ca^{2+}-Dependent Potassium Channels and Adenylate Metabolism, *Biophys. Chem.*, 1999, vol. 80, no. 3, pp. 199–215.
22. Hodgkin, A.L. and Huxley, A.F., A Quantitative Description of Membrane Current and Its Application to Conduction and Excitation in Nerve, *J. Physiol.*, 1952, vol. 117, no.4, pp. 500–544.
23. Skou, J.C., The Influence of Some Cations on an Adenosine Triphosphatase from Peripheral Nerves, *Biochim. Biophys. Acta*, 1957, vol. 23, no. 2, pp. 394–401.
24. Kotyk, A. and Yanachek, K., *Membrannyi transport* (Membrane Transport), Moscow, Mir, 1980.
25. Post, R.L., Merritte, C.R., Kinsolving, C.R., and Albright, C.D., Membrane Adenosine Triphosphatase As a Participant in the Active Transport of Sodium and Potassium in the Human Erythrocyte, *J. Biol. Chem.*, 1960, vol. 235, pp. 1796–1802.
26. Marchesi, V.T., Furthmayr, H., and Tomita, M., The Red Cell Membrane, *Annu. Rev. Biochem.*, 1976, vol. 45, pp. 667–698.
27. Tosteson, D.C., The Cellular Functions of Active Transport of K and Na, *Physiol. Pharmacol. Physicians*, 1965, vol. 3, no. 10, pp. 1–6.
28. Hoffman, P.G. and Tosteson, D.C., Active Sodium and Potassium Transport in High Potassium and Low Potassium Sheep Red Cells, *J. Gen. Physiol.*, 1971, vol. 58, no. 4, pp. 438–466.
29. Tosteson, D.C., Active Transport, Genetics, and Cellular Evolution, *Fed. Proc.*, 1963, vol. 22, pp. 19–26.
30. Guharay, F. and Sachs, F., Stretch-Activated Single Ion Channel Currents in Tissue-Cultured Embryonic Chick Skeletal Muscle, *J. Physiol.*, 1984, vol. 352, pp. 685–701.
31. Gennis, R., *Biomembrany. Molekuliarnaya struktura i funktsii* (Biomembranes. Molecular Structure and Functions), Moscow, Mir, 1997.
32. White, A., Handler, F., Smith, E., Hill, R., and Lehman, I., *Osnovy biokhimii* (Bases of Biochemistry), Moscow, Mir, 1981.
33. Beutler, E., *Red Cell Metabolism*, N.Y., Grune & Stratton, 1975.
34. Jacobash, G.S., Minakami, S., and Rapoport, S.M., *Cellular and Molecular Biology of Erythrocytes*, Yoshikawa, J. and Rapoport, S.M., Eds., Tokyo, University of Tokyo Press, 1974, pp. 55–142.
35. Evans, E.A. and Skalak, R., *Mechanics and the Thermodynamics of Biomembranes*, Boca Raton, Fl., Crc Press, 1980.
36. Markin, V.S., Membrane Organization in the Layer Plane and Cell Shape. Biological Consequences of the Theory, *Biofizika* (Rus.), 1981, vol. 26, no. 1, pp. 158–167.
37. Rapoport, S.M., The Regulation of Glycolysis in Mammalian Erythrocytes, *Essays Biochem.*, 1968, vol. 4, pp. 69–103.
38. Rapoport, S.M. and Muller, M., *Cellular and Molecular Biology of Erythrocytes*, Yoshikawa, J. and Rapoport, S.M., Eds., Tokyo, University of Tokyo Press, 1974, pp. 167–179.
39. Grimes, A.G., *Human Red Cell Metabolism*, Oxford, Blackwell, 1980.
40. Lionetti, F.J., *Cellular and Molecular Biology of Erythrocytes*, Yoshikawa, J. and Rapoport, S.M., Eds., Tokyo, University of Tokyo Press, 1974, pp. 143–166.
41. Kilmartin, J.V. and Rossi-Barnardi, L., Interaction of Hemoglobin with Hydrogen Ions, Carbon Dioxide, and Organic Phosphates, *Physiol. Rev.*, 1973, vol. 53, pp. 836–890.
42. Mohandas, N., Chasis, J.A. and Shohet, S.B., The Influence of Membrane Skeleton on Red Cell Deformability,

Membrane Material Properties, and Shape, *Semin. Hematol.*, 1983, vol. 20, pp. 225–242.

43. Mueller, T.J., Jackson, C.W., Dokter, M.E., and Morrison, M., Membrane Skeletal Alterations during in vivo Mouse Red Cell Aging. Increase in the Band 4.1a:4.1b Ratio, *J. Clin. Invest.*, 1987, vol. 79, pp. 492–499.
44. Markin, V.S., Lateral Organization of Membranes and Cell Shapes, *Biophys. J.*, 1981, vol. 36, no. 1, pp. 1–19.
45. Glaser, R. and Leitmannova, A., Mathematical Modelling of Shape-Transformations of Human Erythrocytes, *Acta. Biol. Med. Ger.*, 1977, vol. 36, nos. 5–6, pp. 859–869.
46. Leitmannova, A. and Glaser, R., Mathematical Modelling of Human Echinocytes and the Membrane Bending of Discocytes Stomatocytes and Echinocytes, *Studia Biophysica*, 1977, vol. 64, no. 2, pp. 123–141.
47. Fung, Y.C., Tsang, W.C., and Patitucci, P., High-Resolution Data on the Geometry of Red Blood Cells., *Biorheology*, 1981, vol. 18, nos. 3–6, pp. 369–385.
48. Canham, P.B. and Burton, A.C., Distribution of Size and Shape in Populations of Normal Human Red Cells, *Circ. Res.*, 1968, vol. 22, no. 3, pp. 405–422.
49. Waugh, R.E. and Sarelius, I.H., Effects of Lost Surface Area on Red Blood Cells and Red Blood Cell Survival in Mice, *Am. J. Physiol.*, 1996, vol. 271, no. 6, Pt 1, pp. C1847–C1852.
50. Clark, M.R., Computation of the Average Shear-Induced Deformation of Red Blood Cells As a Function of Osmolality, *Blood Cells*, 1989, vol. 15, no. 2, pp. 427–439.
51. Linderkamp, O. and Meiselman, H.J., Geometric, Osmotic, and Membrane Mechanical Properties of Density-Separated Human Red Cells, *Blood*, 1982, vol. 59, no. 6, pp. 1121–1127.
52. Nash, G.B., O'Brien, E., Gordon-Smith, E.C., and Dormandy, J.A., Abnormalities in the Mechanical Properties of Red Blood Cells Caused by *Plasmodium falciparum*, *Blood*, 1989, vol. 74, no. 2, pp. 855–861.
53. Marvel, J.S., Sutera, S.P., Krogstad, D.J., Zarkowsky, H.S., and Williamson, J.R., Accurate Determination of Mean Cell Volume by Isotope Dilution in Erythrocyte Populations with Variable Deformability, *Blood Cells*, 1991, vol. 17, no. 3, pp. 497–512.
54. Paulitschke, M. and Nash, G.B., Micropipette Methods for Analyzing Blood Cell Rheology and Their Application to Clinical Research, *Clin. Hemorheol.*, 1993, vol. 13, pp. 407–434.
55. Weed, R.J. and Reed, C.F., Metabolic Dependence of Red Cell Deformability, *Amer. J. Med.*, 1966, vol. 41, pp. 681–698.
56. Whittam, R. and Ager, M.E., Vectorial Aspects of Adenosine-Triphosphatase Activity in Erythrocyte Membranes, *Biochem. J.*, 1964, vol. 93, pp. 337–348.
57. Gamble, J.L., Chemical Anatomy, Physiology and Extracellular Fluid, 6th Ed. Cambridge, Mass., Harvard University Press, 1954.
58. Klausner, M.A., Hirsch, L.J., Leblond, P.F., Chamberlain, J.K., Klemperer, M.R., and Segel, G.B., Contrasting Splenic Mechanisms in the Blood Clearance of Red Blood Cells and Colloidal Particles, *Blood*, 1975, vol. 46, no. 6, pp. 965–976.
59. Chen, L.T. and Weiss, L., The Role of the Sinus Wall in the Passage of Erythrocytes through the Spleen, *Blood*, 1973, vol. 41, no. 4, pp. 529–537.
60. Markin, V.S. and Chizmadzhev, Yu.A., *Indutsirovannyi ionnyi transport* (Induced Ion Transport), Moscow, Nauka, 1974, pp. 38–45.
61. Vereninov, A.A. and Marakhova, I.I., *Transport ionov u kletok v kul'ture* (Ion Transport in the Cell in Culture), Leningrad, Nauka, 1986.
62. Knauf, P.A., Fuhrmann, G.F., Rothstein, S., and Rothstein, A., The Relationship between Anion Exchange and Net Anion Flow across the Human Red Blood Cell Membrane, *J. Gen. Physiol.*, 1977, vol. 69, no. 3, pp. 363–386.
63. Raftos, J.E. and Lew, V.L., Effect of Intracellular Magnesium on Calcium Extrusion by the Plasma Membrane Calcium Pump of Intact Human Red Cells, *J. Physiol.*, 1995, vol. 489, pt. 1, pp. 63–72.
64. Rothstein, A., Cabantchik, Z.I., and Knauf, P., Mechanism of Anion Transport in Red Blood Cells: Role of Membrane Proteins, *Fed. Proc.*, 1976, vol. 35, no. 1, pp. 3–10.
65. Cabantchik, Z.I., Knauf, P.A., and Rothstein, A., The Anion Transport System of the Red Blood Cell. The Role of Membrane Protein Evaluated by the Use of 'Probes', *Biochim. Biophys. Acta*, 1978, vol. 515, no. 3, pp. 239–302.
66. Sen, A.K. and Post, R.L., Stoichiometry and Localization of Adenosine Triphosphate-Dependent Sodium and Potassium Transport in the Erythrocyte, *J. Biol. Chem.*, 1964, vol. 239, pp. 345–352.
67. Bonting, S.L., *Membranes and Ion Transport*, Bittar, E.E., Ed., London, Wiley Interscience, 1970, vol. 1, pp. 257–363.
68. Robinson, J.D., Free Mg^{2+} and Proposed Isomerizations of the (Na^+ Plus K^+)-Dependent ATPase, *FEBS Lett.*, 1974, vol. 47, no. 2, pp. 352–355.
69. Kennedy, B.G., Lunn, G., and Hoffman, J.F., Effects of Altering the ATP/ADP Ratio on Pump-Mediated Na/K and Na/Na Exchanges in Resealed Human Red Blood Cell Ghosts, *J. Gen. Physiol.*, 1986, vol. 87, no. 1, pp. 47–72.
70. Ataullakhanov, F.I., Buravtsev, V.N., Vitvitsky, V.M., Dibrov, B.F., Zhabotinsky, A.M., Pichugin, A.V., Kholodenko, B.N., and Ehrlich, L.I., The Association between the Rate of ATP-Consuming Processes and ATP Concentration in Intact Red Blood Cells, *Biokhimiya* (Rus.), 1980, vol. 45, pp. 1075–1079.
71. Martinov, M.V., Plotnikov, A.G., Vitvitsky, V.M., and Ataullakhanov, F.I., Deficiencies of Glycolytic Enzymes As a Possible Cause of Hemolytic Anemia, *Biochim. Biophys. Acta*, 2000, vol. 1474, no. 1, pp. 75–87.
72. Segel, G.B., Feig, S.A., Glader, B.E., Muller, A., Dutcher, P., and Nathan, D.G., Energy Metabolism in Human Erythrocytes: The Role of Phosphoglycerate Kinase in Cation Transport, *Blood*, 1975, vol. 46, no. 2, pp. 271–278.
73. Ross, P.D. and Minton, A.P., Hard Quasispherical Model for the Viscosity of Hemoglobin Solutions, *Biochem. Biophys. Res. Commun.*, 1977, vol. 76, no. 4, pp. 971–976.

74. Levtov, V.A., Regirer, S.A., and Shadrina, N.Kh., *Reologiya krovi* (Rheology of Blood), Moscow, Meditsina, 1982.

75. Baskurt, O.K. and Meiselman, H.J., Blood Rheology and Hemodynamics, *Semin. Thromb. Hemost.*, 2003, vol. 29, no. 5, pp. 435–450.

76. Ataullakhanov, F.I., Vitvitsky, V.M., Lisovskaya, I.L., and Tuzhilova, E.G., The Analysis of Geometrical Parameters and Mechanical Properties of Red Blood Cells by the Method of Filtration through Nuclear Membrane Filters. I. Mathematical Model, *Biofizika* (Rus.), 1994, vol. 39, pp. 672–680.

77. Lisovskaya, I.L., Ataullakhanov, F.I., Tuzhilova, E.G., and Vitvitsky, V.M., The Analysis of Geometrical Parameters and Mechanical Properties of Red Blood Cells by the Method of Filtration through Nuclear Membrane Filters. II. Experimental Verification of the Mathematical Model, *Biofizika* (Rus.), 1994, vol. 39, pp. 864–871.

78. Orlov, S.N., Pokudin, N.I., El-Rabi, L.S., Brusovanik, V.I., and Kubatiyev, A.A., The Transport of Ions to Human Red Blood Cells at Different Forms of Hemolytic Anemia: Correlation Analysis, *Biokhimiya* (Rus.), 1993, vol. 58, no. 6, pp. 866–873.

79. Hebbel, R.P. and Mohandas, N., Reversible Deformation-Dependent Erythrocyte Cation Leak. Extreme Sensitivity Conferred by Minimal Peroxidation, *Biophys. J.*, 1991, vol. 60, no. 3, pp. 712–715.

80. Deuticke, B., Heller, K.B., and Haest, C.W., Leak Formation in Human Erythrocytes by the Radical-Forming Oxidant T-Butylhydroperoxide, *Biochim. Biophys. Acta*, 1986, vol. 854, no. 2, pp. 169–183.

81. Kramer, H.J., Gospodinov, D., and Kruck, F., Functional and Metabolic Studies on Red Blood Cell Sodium Transport in Chronic Uremia, *Nephron*, 1976, vol. 16, no. 5, pp. 344–358.

82. Illner, H. and Shires, G.T., Changes in Sodium, Potassium, and Adenosine Triphosphate Contents of Red Blood Cells in Sepsis and Septic Shock, *Circ. Shock*, 1982, vol. 9, no. 3, pp. 259–267.

83. Brumen, M. and Heinrich, R., A Metabolic Osmotic Model of Human Erythrocytes, *Biosystems*, 1984, vol. 17, no. 2, pp. 155–169.

84. Beauge, L. and Lew, V.L., *Membrane Transport in Red Cells*, Ellory, J.C. and Lew, V.L., Eds., Academic Press, 1977, p. 39.

85. Castranova, V., Weise, M.J., and Hoffman, J.F., Anion Transport in Dog, Cat, and Human Red Cells. Effects of Varying Cell Volume and Donnan Ratio, *J. Gen. Physiol.*, 1979, vol. 74, no. 3, pp. 319–334.

86. Gardos, G., The Function of Calcium in the Potassium Permeability of Human Erythrocytes, *Biochim. Biophys. Acta*, 1958, vol. 30, no. 3, pp. 653–654.

87. Gardos, G., The Role of Calcium in the Potassium Permeability of Human Erythrocytes, *Acta Physiol. Hung.*, 1959, vol. 15, no. 2, pp. 121–125.

88. Hille, B. and Schwarz, W., Potassium Channels As Multi-Ion Single-File Pores, *J. Gen. Physiol.*, 1978, vol. 72, no. 4, pp. 409–442.

89. Maher, A.D. and Kuchel, P.W., The Gardos Channel: A Review of the Ca^{2+}-Activated K^+ Channel in Human Erythrocytes, *Int. J. Biochem. Cell. Biol.*, 2003, vol. 35, no. 8, pp. 1182—1197 [Erratum in: *Int. J. Biochem. Cell. Biol.*, 2003, vol. 35, no. 12, pp. 1682.]

90. Leinders, T., van Kleef, R.G.D.M., and Vijverberg, H.P.M., Single Ca^{2+}-Activated K^+ Channels in Human Erythrocytes: Ca^{2+}-Dependence of Opening Frequency but Not of Open Lifetimes, *Biochim. Biophys. Acta*, 1992, vol. 1112, no. 1, pp. 67–74.

91. Leinders, T., van Kleef, R.G., and Vijverberg, H.P., Distinct Metal Ion Binding Sites on Ca^{2+}-Activated K^+ Channels in Inside-Out Patches of Human Erythrocytes, *Biochim. Biophys. Acta*, 1992, vol. 1112, no. 1, pp. 75–82.

92. Simons, T.J., Calcium-Dependent Potassium Exchange in Human Red Cell Ghosts, *J. Physiol.*, 1976, vol. 256, no. 1, pp. 227–244.

93. Lew, V.L. and Ferreira, H.G., Variable Ca Sensitivity of a K-Selective Channel in Intact Red-Cell Membranes, *Nature*, 1976, vol. 263, no. 5575, pp. 336–338.

94. Wiley, J.S. and McCulloch, K.E., Calcium Ions, Drug Action and the Red Cell Membrane, *Pharmacol. Ther.*, 1982, vol. 18, no. 2, pp. 271–792.

95. Li, Q., Jungmann, V., Kiyatkin, A., and Low, P.S., Prostaglandin E2 Stimulates a Ca^{2+}-Dependent K^+ Channel in Human Erythrocytes and Alters Cell Volume and Filterability, *J. Biol. Chem.*, 1996, vol. 271, no. 31, pp. 18651–18656.

96. Rivera, A., Rotter, M.A., and Brugnara, C., Modulation of Gardos Channel Activity by Cytokines in Sickle Erythrocytes, *Am. J. Physiol.*, 1999, vol. 277, no. 4, pt. 1, pp. 746–754.

97. Rivera, A., Jarolim, P., and Brugnara, C., Modulation of Gardos Channel Activity by Cytokines in Sickle Erythrocytes, *Blood*, 2002, vol. 99, pp. 357–603.

98. Boytler, E., *Narusheniya metabolizma eritrotsytov i gemoliticheskaya anemiya* (Disturbance of the Metabolism of Red Blood Cells and Hemolytic Anemia), Moscow, Meditsina, 1981.

99. Halperin, J.A., Brugnara, C., Kopin, A.S., Ingwall, J., and Tosteson, D.C., Properties of the Na^+-K^+ Pump in Human Red Cells with Increased Number of Pump Sites, *J. Clin. Invest.*, 1987, vol. 80, no. 1, pp. 128–137.

100. Ataullakhanov, F.I., Komarova, S.V., Martinov, M.V., and Vitvitsky, V.M., A Possible Role of Adenylate Metabolism in Human Erythrocytes. 2. Adenylate Metabolism Is Able to Improve the Erythrocyte Volume Stabilization, *J. Theor. Biol.*, 1996, vol. 183, pp. 75–86.

101. Haas, M., Schmidt, W.F., 3rd, and McManus, T.J., Catecholamine-Stimulated Ion Transport in Duck Red Cells. Gradient Effects in Electrically Neutral [Na + K + 2Cl] Co-Transport, *J. Gen. Physiol.*, 1982, vol. 80, no. 1, pp. 125–147.

102. Duhm, J. and Gobel, B.O., Role of the Furosemide-Sensitive Na^+/K^+ Transport System in Determining the Steady-State Na^+ and K^+ Content and Volume of Human Erythrocytes in vitro and in vivo, *J. Membr. Biol.*, 1984, vol. 77, no. 3, pp. 243–254.

103. Lauf, P.K., Bauer, J., Adragna, N.C., Fujise, H., Zade-Oppen, A.M., Ryu, K.H., and Delpire, E., Erythrocyte K-Cl Cotransport: Properties and Regulation, *Am. J. Physiol.*, 1992, vol. 263, no. 5, pt. 1, pp. C917–C932.

104. Duhm, J. and Becker, B.F., Studies on Lithium Transport across the Red Cell Membrane. V. On the Nature of

the Na^+-Dependent Li^+ Countertransport System of Mammalian Erythrocytes, *J. Membr. Biol.*, 1979, vol. 51, nos. 3–4, pp. 263–286.

105. Komarova, S.V., Mosharov, E.V., Vitvitsky, V.M., and Ataullakhanov, F.I., Adenine Nucleotide Synthesis in Human Erythrocytes Depends on the Mode of Supplementation of Cell Suspension with Adenosine, *Blood Cells Mol. Dis.*, 1999, vol. 25, nos. 3–4, pp. 170–179.
106. Sardini, A., Amey, J.S., Weylandt, K.H., Nobles, M., Valverde, M.A., and Higgins, C.F., Cell Volume Regulation and Swelling-Activated Chloride Channels, *Biochim. Biophys. Acta*, 2003, vol. 1618, no. 2, pp. 153–162.
107. Lang, F., Lang, K.S., Wieder, T., Myssina, S., Birka, C., Lang, P.A., Kaiser, S., Kempe, D., Duranton, C., and Huber, S.M., Cation Channels, Cell Volume and the Death of an Erythrocyte, *Pflügers Arch.*, 2003, vol. 447, no. 2, pp. 121–125.
108. Lang, F., Busch, G.L., Ritter, M., Volkl, H., Waldegger, S., Gulbins, E., and Haussinger, D., Functional Significance of Cell Volume Regulatory Mechanisms, *Physiol. Rev.*, 1998, vol. 78, no. 1, pp. 247–306.
109. Lew, V.L. and Hockaday, A.R., The Effects of Transport Perturbations on the Homeostasis of Erythrocytes, *Novartis Found Symp.*, 1999, vol. 226, pp. 37—50, Discussion, pp. 50–54.
110. Fraser, J.A. and Huang, C.L., A Quantitative Analysis of Cell Volume and Resting Potential Determination and Regulation in Excitable Cells, *J. Physiol.*, 2004, vol. 559, no. 2, pp. 459–478.
111. Pannicke, T., Iandiev, I., Uckermann, O., Biedermann, B., Kutzera, F., Wiedemann, P., Wolburg, H., Reichenbach, A., and Bringmann, A., A Potassium Channel-Linked Mechanism of Glial Cell Swelling in the Postischemic Retina, *Mol. Cell. Neurosci.*, 2004, vol. 26, no. 4, pp. 493–502.
112. Weinstein, A.M., Modeling Epithelial Cell Homeostasis: Assessing Recovery and Control Mechanisms, *Bull. Math. Biol.*, 2004, vol. 66, no. 5, pp. 1201–1240.
113. Ehrenfeld, J., Raschi, C., and Brochiero, E., Basolateral Potassium Membrane Permeability of A6 Cells and Cell Volume Regulation, *J. Membr. Biol.*, 1994, vol. 138, no. 3, pp. 181–195.