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A Possible Role of Adenylate Metabolism in Human Erythrocytes

2. Adenylate Metabolism Is Able to Improve the Erythrocyte Volume Stabilization

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We constructed and studied a mathematical model that describes the control of cell volume, ion balance, energy and adenylate metabolism in human erythrocytes. According to the model, adenylate metabolism can provide an effective stabilization of the cell volume over relatively large changes of cell parameters. For example, the steady-state value of the cell volume remains almost unchanged when the cell membrane permeability increases by 15-fold. The cell volume also changes only slightly over large changes in the parameters of energy metabolism. The relaxation time for the cell volume changes is about 100 hr over changes in these parameters. In other words, the volume stabilization operates most effectively against long-term slow perturbations.

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1. Introduction

Circulating erythrocytes have to pass through capillary diameters which are smaller than their own diameter. They can achieve this by their ability to bear large deformations. In order for the erythrocyte to have high deformability it must maintain physiological value of cell volume. This is provided by the operation of ionic pumps. The dynamic equilibrium in this system may be readily perturbed by changes in various parameters of the cell. For example, the erythrocyte in the organism is subjected to severe oxidative stresses that may lead to the increase in the cell membrane permeability to cations and, as a result, to osmotic imbalance and the increased cell volume (Ataullakhanov *et al.*, 1986). An increase in cell membrane permeability of the erythrocyte and perturbations of ion balance are observed in some pathologies (Illner & Shires, 1982; Kramer *et al.*, 1976; Mir & Bobiuski, 1975). It is natural to assume that the erythrocyte has special mechanisms for stabilization of the cell volume under variations in cell parameters.

We previously examined the hypothesis that adenylate metabolism contributes to the stabilization of the cell volume (Ataullakhanov *et al.*, 1996). According to this hypothesis, adenylate metabolism, increased or decreased adenylate pool and intracellular ATP can control ion pumping. A simple mathematical model was used to analyse the effect of changes in the cell membrane permeability to ions. Analysis of the model showed that the perfect stabilization of ion concentrations (and the cell volume as a consequence) can be achieved over a broad range of cell membrane permeabilities if the intracellular level of ATP could be made properly adjustable. To provide this, the model assumes that AMP degradation in the erythrocyte is activated by ATP and inhibited by AMP. An allosteric control of the enzymes of adenylate metabolism was experimentally demonstrated (Ascari & Rao, 1968; Bontemps *et al.*, 1986, Itoh, 1981; Itoh *et al.*, 1986; Van den Berghe *et al.*, 1988; Yung & Suelter, 1978; Kyd & Bagnara, 1980). Nevertheless, none of the known models of erythrocyte metabolism takes into account

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the existence of such control (Shauer *et al.*, 1981; Joshi & Palsson, 1989, 1990).

The simple model was convenient for analytical and numerical analysis but volume of the erythrocyte was not described directly. Moreover, the balance of the concentrations of only one ion and only one challenging effect were examined. This prompted us to develop a more detailed model based on the relations that appeared to be effective in the simple model. The new model describes the balance of the concentrations of Na^+, K^+ and anions between the erythrocyte and its environment; osmotic control of the cell volume; glycolysis as the process of ATP resynthesis from ADP, and adenylate metabolism. Our description of ion homeostasis and its relation to the cell volume is based on the results of the study by Moroz *et al.* (1989). The interaction of the key enzymes, hexokinase and phosphofructokinase, is thought to govern the glycolysis rate (Ataullakhanov *et al.*, 1977, 1980, 1981*a*; Rapoport, T. *et al.*, 1977b; Otto *et al.*, 1977). Adenylate metabolism, similar to the previous model (Ataullakhanov *et al.*, 1996), is given by terms describing AMP synthesis and degradation, and the exchange of adenylate nucleotides through adenylate kinase reaction. Using the model, we studied how the erythrocyte volume, intracellular Na^+, K^+, and adenine nucleotide concentrations change when the cell membrane permeability and parameters of the energy metabolism vary.

2. Description of the Mathematical Model

2.1. Ion Balance and the Erythrocyte Volume

Jacobsson's model (Jacobsson, 1980) was taken as a basis for the description of ion balance and erythrocyte volume:

$$\frac{d}{dt}\left([K^+]_i \frac{V}{V_0}\right) = 2v_{ATPase} + P_K \frac{\frac{\Delta\varphi F}{RT}}{\exp\left(\frac{\Delta\varphi F}{RT}\right) - 1} \times \left([K^+]_e - [K^+]_i \exp\left(\frac{\Delta\varphi F}{RT}\right)\right) \quad (1)$$

$$\frac{d}{dt}\left([Na^+]_i \frac{V}{V_0}\right) = -3v_{ATPase} + P_{Na} \frac{\frac{\Delta\varphi F}{RT}}{\exp\left(\frac{\Delta\varphi F}{RT}\right) - 1} \times \left([Na^+]_e - [Na^+]_i \times \exp\left(\frac{\Delta\varphi F}{RT}\right)\right) \quad (2)$$

$$[K^+]_i + [Na^+]_i + [A_p^-]_i + [A_n^{Z-}]_i = [K^+]_e + [Na^+]_e + [A_p^-]_e + [A_n^{Z-}]_e \quad (3)$$

$$[K^+]_i + [Na^+]_i - [A_p^-]_i - Z \cdot [A_n^{Z-}]_i = 0 \quad (4)$$

$$\frac{[A_p^-]_i}{[A_p^-]_e} = \exp\left(\frac{\Delta\varphi F}{RT}\right) \quad (5)$$

where V and V_0 are the current and the normal physiological cell volumes; $\Delta\varphi$ is the transmembrane potential; F is the Faraday constant; R is the universal gas constant; T denotes absolute temperature; P_K and P_{Na} are the passive permeability of the erythrocyte membrane for K^+ and Na^+, respectively; v_{ATPase} denotes the rate of transport Na^+/K^+ ATPase (the ion pump); $[A_p^-]$ and $[A_n^{Z-}]$ are the concentrations of permeable (Chloride and HCO_3^-) and impermeable anions, respectively; subscripts i and e denote intra- and extracellular variables and parameters, respectively; Z is the average charge of the impermeable anions.

Equations (1) and (2) describe active and passive K^+ and Na^+ fluxes across the erythrocyte membrane. The rate of Na^+/K^+ ATPase can be written as:

$$v_{ATPase} = \alpha_{ATPase}[Na^+]_i[ATP] \quad (6)$$

where α_{ATPase} is the activity of transport ATPase. Expression (6) does not include any dependence on the extracellular K^+ concentration which is assumed to be constant. Passive ion fluxes are described using Goldman's approach (Kotyk & Janacek, 1975).

Equation (3) describes the osmotic balance in the system. The erythrocyte is considered as an ideal osmometer (Freedman & Hoffman, 1977; Savitz *et al.*, 1964), i.e., the intra- and extracellular concentrations of osmotically active components are equal. Equation (4) describes the electroneutrality of the cell content and takes into account the

contributions of sodium and potassium ions, anions like HCO_3^- and Cl^- that can penetrate the membrane, and polyvalent impermeable anions (e.g., haemoglobin and 2,3-diphosphoglycerate). Equation (5) describes the equilibrium distribution of permeable anions over the intracellular and extracellular compartments in the presence of the transmembrane potential. Cell membrane permeability to Cl^-, HCO_3^- and water is several order of magnitude higher than that for K^+ and Na^+ (Fortes, 1977; Sha'afi, 1977). The characteristic times to reach (1) osmotic equilibrium between cells and their milieu and (2) equilibrium in the distribution of permeate anions are small (approximately 0.1 s) (Tosteson, 1959; Moronne *et al.*, 1990). Electroneutrality is achieved even faster. Therefore, it is justifiable to use steady-state equations (3–5) for describing the partitioning of osmotically active components, charges, and permeate anions at times of the order of one second and longer.

An assumption is made that the osmolarity of the medium is determined only by the extracellular concentrations of the permeable ions ($[A_n^{z-}]_e = 0$). The following values of the ion concentrations (Beiling *et al.*, 1966; Funder & Wieth, 1966; Halperin *et al.*, 1987; Mir & Bobiuski, 1975; Quintanilla *et al.*, 1988), normal rate of transport Na^+/K^+ ATPase (Brumen & Heinrich, 1984; Halperin *et al.*, 1987; Mir & Bobiuski, 1975; Segel *et al.*, 1975), and intracellular ATP concentration (Ataullakhanov *et al.*, 1981*a*; Halperin *et al.*, 1987; Kramer *et al.*, 1976; Rapoport, I., 1977b) were estimated from the literature: $[K^+]_e = 5$ mM, $[Na^+]_e = 145$ mM, $[A_p^-]_e = 150$ mM, $[K^+]_i = 130$ mM, $[Na^+]_i = 10$ mM, $[A_p^-]_i = 110$ mM, $v_{ATPase} = 2.1 \cdot 10^{-4}$ mM s^{-1}, [ATP] = 1 mM. Normal physiological values of the remaining steady-state parameters are readily determined from equations (1–5): $[A_n^{z-}]_i = 50$ mM, $Z = 0.6$, $P_K = P_{K0} = 4.0 \cdot 10^{-6}$ l s^{-1}, $P_{Na} = P_{Na0} = 3.9 \cdot 10^{-6}$ l s^{-1}, $\Delta\varphi = -8.4$ mV, $\alpha_{ATPase} = 2.1 \cdot 10^{-5}$ l/(mM·s).

We earlier used the set of equations (1–5) to analyse the effect of changes in passive permeability of the membrane to cations on the erythrocyte volume and showed that this set well describes the volume as a function of various parameters of ion homeostasis of the erythrocyte (Moroz *et al.*, 1989). The model predicts that the erythrocyte is best protected from non-selective (with respect to Na^+ and K^+) changes in the membrane permeability. Such non-selective changes in the membrane permeability seem most likely to occur naturally in the organism (e.g., perturbations of non-selective permeability of the erythrocyte membrane caused by oxidative stress). Therefore, it is reasonable to focus attention to the non-specific alterations in the membrane permeability. We will describe such alterations using the following expressions: $P_K = q \cdot P_{K0}$; $P_{Na} = q \cdot P_{Na0}$, where q is a variable parameter.

2.2. ENERGY AND ADENYLATE METABOLISM

ATP consumed by transport Na^+/K^+ ATPase and other ATP-consuming systems is replenished in the erythrocyte by glycolysis. If adenylate metabolism is not taken into account, glycolysis may be described by the following set of equations:

$$\frac{d}{dt}\left([G6P]\frac{V}{V_0}\right) = v_{HK} - v_{PHI} \tag{7}$$

$$\frac{d}{dt}\left([F6P]\frac{V}{V_0}\right) = v_{PHI} - v_{PFK} \tag{8}$$

$$\frac{d}{dt}\left([FDP]\frac{V}{V_0}\right) = v_{PFK} - v_{ALD} \tag{9}$$

$$\frac{d}{dt}\left([DHAP]\frac{V}{V_0}\right) = v_{ALD} - v_{TPI} \tag{10}$$

$$\frac{d}{dt}\left([GAP]\frac{V}{V_0}\right) = v_{ALD} + v_{TPI} - v_{GAPDH} \tag{11}$$

$$\frac{d}{dt}\left([1,3DPG]\frac{V}{V_0}\right) = v_{GAPDH} - v_{PGK} \tag{12}$$

$$\frac{d}{dt}\left([3PG]\frac{V}{V_0}\right) = v_{PGK} - v_{PGM} \tag{13}$$

$$\frac{d}{dt}\left([2PG]\frac{V}{V_0}\right) = v_{PGM} - v_{ENO} \tag{14}$$

$$\frac{d}{dt}\left([PEP]\frac{V}{V_0}\right) = v_{ENO} - v_{PK} \tag{15}$$

$$([ATP] + [ADP] + [AMP])\frac{V}{V_0} = a \tag{16}$$

$$\frac{[ADP]^2}{[AMP][ATP]} = 1 \tag{17}$$

where G6P, F6P, FDP, DHAP, GAP, 1,3DPG, 3PG, 2PG, PEP, correspond to the intracellular concentrations of glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, dihydroxyacetone phosphate, glyceraldehyde-3-phosphate, 1,3-diphosphoglycerate, 3-phosphoglycerate, 2-phosphoglycerate, and phosphoenolpyruvate, respectively. Symbol a corresponds to total adenylate content of the cell.

v_{HK}, v_{PHI}, v_{PFK}, v_{ALD}, v_{TPI}, v_{GAPDH}, v_{PGK}, v_{PGM}, v_{ENO}, v_{PK} denote rates of the hexokinase, phosphohexoseisomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, and pyruvate kinase reactions, respectively. The adenylate kinase reaction is believed to be in equilibrium because the activity of adenylate kinase is high in the erythrocyte (Mohrenweiser *et al.*, 1981). Equation (17) describes the adenylate kinase equilibrium, with the equilibrium constant equal to one.

Equations (7–17) do not contain such variables as the concentrations of glucose, pyruvate, lactate; lactate dehydrogenase reaction was also excluded from our consideration. Normally, *in vivo* erythrocyte glycolysis is always saturated in glucose. The concentrations of lactate and pyruvate, being controlled at a whole-body level, do not depend on the erythrocyte glycolysis. Contributions of hexosemonophosphate and 2,3-diphosphoglycerate bypass pathways are small under normal conditions (Ataullakhanov *et al.*, 1981*b*; Rapoport, I. *et al.*, 1977a) and may be neglected. The rate of ATP resynthesis is proportional to the glycolysis flux that is controlled by two key glycolytic enzymes (hexokinase and phosphofructokinase) which interact through a feedback mechanism of inhibition of hexokinase by glucose-6-phosphate (Ataullakhanov *et al.*, 1977, 1980, 1981*a*; Otto *et al.*, 1977; Rapoport *et al.*, 1977b). As the phosphofructokinase reaction is irreversible, the glycolytic flux is not affected by metabolites of the subsequent reactions of glycolysis. The fact that the activity of hexokinase in human lerythrocytes is well below that of the activities of all other enzymes of glycolysis (Beutler, 1978; Lestas *et al.*, 1982; Mohrenweisser *et al.*, 1981) allows a quasi steady-state approach to describe phosphohexoseisomerase reaction and all the reactions following the phosphofructokinase reaction. This means that the set of eqns (7–17) may be reduced to give the following set:

$$\frac{d}{dt}\left([G6P]\frac{V}{V_0}\right) = \frac{v_{HK} - v_{PFK}}{1 + 1/K_{PHI}} \tag{18}$$

$$([ATP] + [ADP] + [AMP])\frac{V}{V_0} = a \tag{19}$$

$$\frac{[ADP]^2}{[AMP][ATP]} = 1. \tag{20}$$

We exclude fructose-6-phosphate concentration using the equilibrium relation $[G6P] = K_{PHI}[F6P]$ for the phosphohexoseisomerase reaction. The explicit expressions for the rates of hexokinase and phosphofructokinase reactions were constructed earlier using the results of experimental studies of kinetic properties of the isolated enzymes and the regulatory properties of glycolysis in the whole erythrocyte (Ataullakhanov *et al.*, 1977, 1980, 1981a; Gerber *et al.*, 1974; Kuhn *et al.*, 1974):

$$v_{HK} = \alpha_{HK}\frac{[ATP]/K_{11}}{1 + [ATP]/K_{11} + [G6P]/K_{12}} \tag{21}$$

$$v_{PFK} = \alpha_{PFK}\frac{[ATP][G6P]/K_{PHI}}{(K_{33} + [ATP])(K_{32} + [G6P]/K_{PHI})} \times \frac{\left[1/(1 + [AMP]/K_{34}) + 2[AMP]/K_{34} + [AMP]\right]}{\left[1 + L\frac{(1 + [ATP]/K_{35})^4}{(1 + [AMP]/K_{34})^4(1 + [G6P]/K_{PHI}K_{36})^4}\right]} \tag{22}$$

where α_{HK}, α_{PFK} are the activities of hexokinase and phosphofructokinase, respectively. We use the following normal physiological values of the parameters in these expressions: $\alpha_{HK} = \alpha_{HK0} = 2.0\cdot10^{-3}$ mM s^{-1}, $K_{11} = 1$ mM, $K_{12} = 1.2\cdot10^{-2}$ mM, $\alpha_{PFK} = \alpha_{PFK0} = 61.3\cdot10^{-3}$ mM s^{-1}, $L = 10^8$, $K_{31} = 10$ mM, $K_{32} = 0.1$ mM, $K_{33} = 2$ mM, $K_{34} = 10^{-2}$ mM, $K_{35} = 0.1$ mM, $K_{36} = 3.7\cdot10^{-4}$ mM, $K_{PHI} = 3$.

The set of equations (18–20), taken together with expressions (21) and (22), describe the glycolysis rate as a function of the concentration of ATP (if ATP concentration is considered as a parameter) (Fig. 1). The dependence, presented in Fig. 1, is an important characteristic of glycolysis, i.e., of its capacity to adjust the rate of ATP production in response to changes in the rate of ATP consumption by the cell (Ataullakhanov *et al.*, 1977, 1980, 1981a; Atkinson, 1968, Reich *et al.*, 1976). Figure 1 demonstrates a good agreement between the results of calculations and experimental data reported for human erythrocytes (Ataullakhanov *et al.*, 1981a; Blum *et al.*, 1969; Halperin *et al.*, 1987; Segel *et al.*, 1975).

In conclusion, the use of expressions (21) and (22) is adequate for the description of the hexokinase and phosphofructokinase reactions: an excellent coinci-

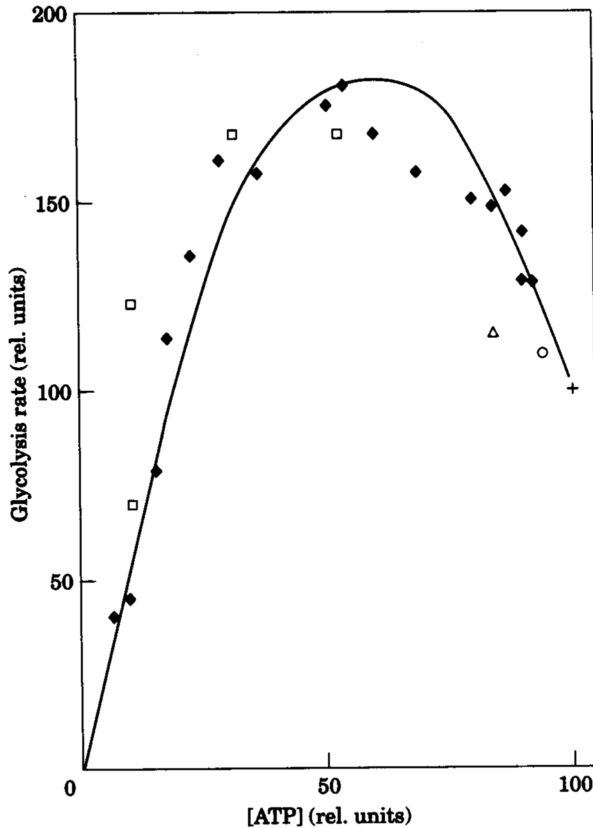

FIG. 1. The normalized steady-state rate of glycolysis as a function of the intracellular concentration of ATP in the human erythrocyte. The curve was computed using equations (18–22) (Ataullakhanov *et al.*, 1980). The point corresponding to the normal physiological values of ATP concentration and glycolysis rate is indicated by cross. Different symbols correspond to experimental points from the following studies: (Ataullakhanov *et al.*, 1981*a*)—◆; (Halperin *et al.*, 1987)—□; (Segel *et al.*, 1975)—△; (Blum *et al.*, 1969)—○. In each experiment, the normal physiological values of the glycolysis rate and the ATP concentration were taken as 100 relative units.

dence of the experimental results of the studies of regulatory characteristics of glycolysis in human erythrocyte and the model predictions were obtained.

Energy metabolism of the erythrocyte comprises glycolysis and the ATP-consuming processes. Adenylate and energy metabolisms are closely linked. Adenylate metabolism in the erythrocyte serves to synthetize AMP from adenine or adenosine and to irreversibly degrade AMP (Dean & Perrett, 1976; Meyskens & Williams, 1971; Paglia *et al.*, 1986; Rapoport *et al.*, 1979; Van den Berge & Bontemps, 1990). Adenylate kinase reaction converts AMP to the other components (ADP and ATP) of the adenylate pool. As previously (Ataullakhanov *et al.*, 1996), adenylate metabolism enter the model as the irreversible reactions of one-step synthesis and one-step degradation of AMP.

TABLE 1
Stationary values of variables obtained in the model at normal physiological values of parameters

Variable	Value	Units
ATP	1	mM
ADP	0.1	mM
AMP	0.01	mM
G6P	0.19	mM
Glycolysis rate (v_{HK})	$1.11\cdot10^{-4}$	mM s^{-1}
Na_i^+	10	mM
K_i^+	130	mM
Volume	76	μ^3

The dynamics of adenylate nucleotide concentrations may be given by the following sets of equations, describing the interaction between adenylate and energy metabolisms:

$$\frac{d}{dt}\left([G6P]\frac{V}{V_0}\right) = \frac{v_{HK} - v_{PFK}}{1 + 1/K_{PGI}} \tag{23}$$

$$\frac{d}{dt}\left([ATP]\frac{V}{V_0}\right) = (2C-1)v_{PFK} - v_{ATPase} - v_{HK} - 2v_s - v_{AK} \tag{24}$$

$$\frac{d}{dt}\left([ADP]\frac{V}{V_0}\right) = v_{ATPase} + v_{HK} + 2v_s + 2v_{AK} - (2C-1)v_{PFK} \tag{25}$$

$$\frac{d}{dt}\left([AMP]\frac{V}{V_0}\right) = v_s - v_d - v_{AK} \tag{26}$$

$$\frac{[ADP]^2}{[AMP][ATP]} = 1 \tag{27}$$

where v_{HK} denotes the rate of hexokinase reaction that was given by expression (21); v_{PFK} is the rate of phosphofructokinase reaction that was given by expression (22); v_{ATPase} is the rate of ATP hydrolysis by transport Na^+/K^+ ATPase; v_s denotes the rate of *de novo* synthesis of AMP [in eqns (24) and (25) this term reflects the averaged overall consumption of ATP for AMP synthesis]; v_{AK} is the rate of adenylate kinase reaction; v_d denotes the rate of AMP degradation; C is the coefficient reflecting the stoichiometry of ATP production in glycolysis. Under normal conditions, $1 < C < 2$.

The rate of AMP synthesis is assumed to be constant ($v_s = \text{const} = 5.6\cdot10^{-6}$ mM s^{-1}) and corresponding to about 3% of the glycolytic flux in human

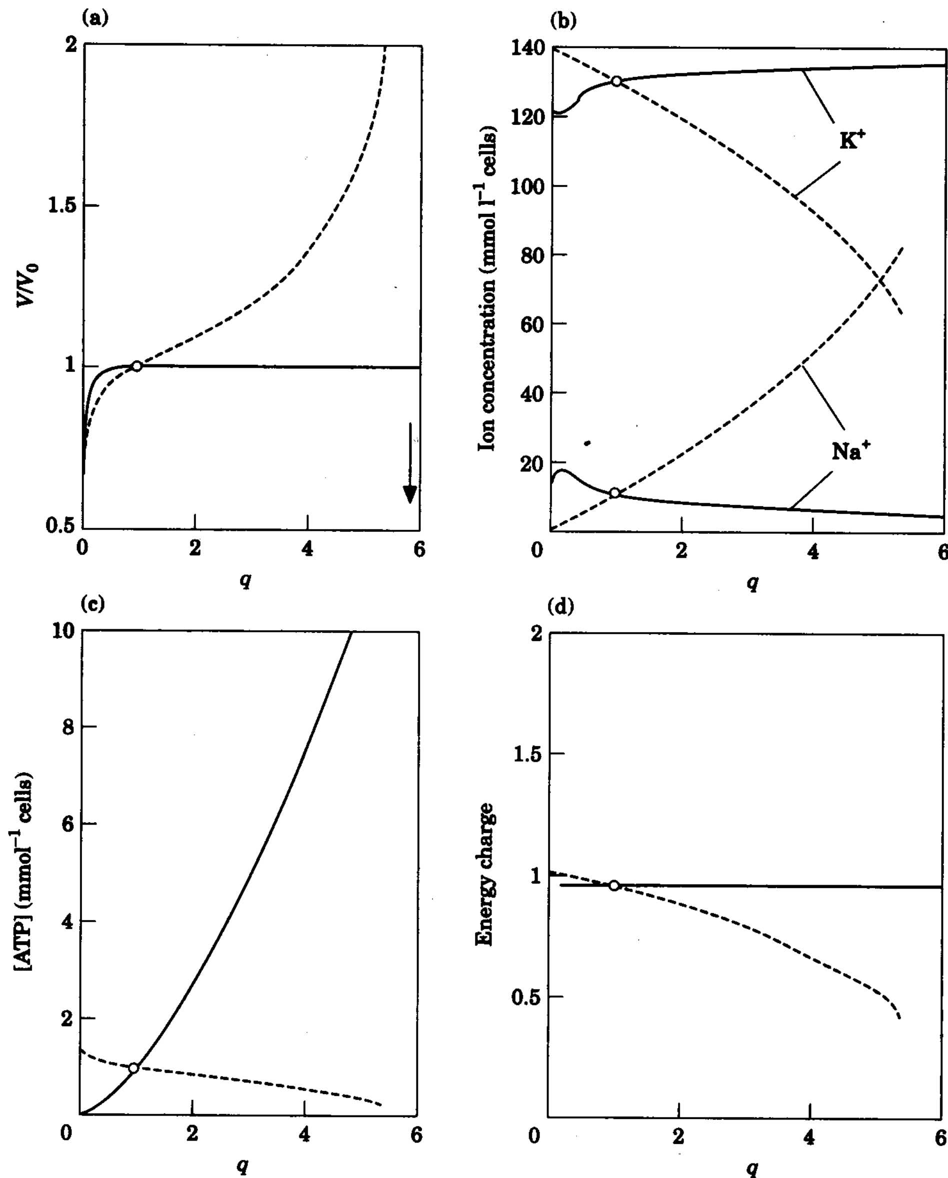

FIG. 2. Steady-state values of (a) the erythrocyte volume, (b) intracellular concentrations of K^+ and Na^+, (c) ATP concentration, and (d) the energy charge as a function of the cell membrane permeability to cations. The circle corresponds to the normal physiological state of the erythrocyte. The value of the instant change in membrane permeability that corresponds to the transient two-fold increase in the cell volume is indicated by arrow. Dashed lines show the results obtained when terms describing metabolism of adenylates were excluded from the model and adenylate pool was kept at a constant level [1.11 mmoles/(l cells) for $V = V_0$].

erythrocyte. This low value of the rate of adenylate metabolism is optimal to keep cell metabolism in the stable state and to provide effective control of the adenylate pool value (Ataullakhanov *et al.*, 1996). The rate of AMP degradation is taken as:

$$v_d = \alpha_d \frac{[\text{ATP}]}{[\text{AMP}]} \qquad (28)$$

where $\alpha_d = 5.6 \cdot 10^{-8}\ \text{mM s}^{-1}$. Such dependence provides most effective stabilization of the intracellular concentrations of ions under varying permeability of the cell membrane (Ataullakhanov *et al.*, 1996).

The equilibrium in the adenylate kinase reaction allows us to exclude the rate of adenylate kinase from (23–27). The final set of the model equations contains eqns (1–5) and (23–27).

3. Results

Analysis of the model showed that it has a single steady-state solution in the region of parameters that

is of special interest. The solution is a stable node. The kinetic behavior of the model is characterized by three time constants. The relaxation times of the energy metabolism are on the order of tens of minutes. They reflect the rates of changes in the energy charge and concentration of glucose-6-phosphate. The time constant for the changes in the intracellular concentrations of Na^+ and K^+ is several hours. The slowest changes are those in the erythrocyte volume and adenylate pool. The relaxation times of these variables are nearly 100 hr.

Normal physiological values of the ion concentrations, metabolites, glycolysis rate and erythrocyte volume, obtained using the model, are presented in Table 1. The model results, presented in Fig. 2, show how the permeability to cations of the cell membrane affects the steady-state volume, energy charge, and concentrations of ATP, Na^+ and K^+ in the erythrocyte. For comparison, the results obtained when terms describing metabolism of adenylates were excluded from the model and adenylate pool was kept at a constant level [1.11 mmoles/(l cells) for $V = V_0$] are also presented (dashed lines). If the adenylate pool were unchangeable, an approximate five-fold increase in the membrane permeability would double the steady-state value of the cell volume. Such volume values are ultimately high for the erythrocyte because of the transformation to the spherical form. If the adenylate pool were governed by some control mechanism, stabilization of the erythrocyte volume might be considerably better: it could tolerate appreciably greater variations in the cell membrane permeability. The model shows that even a 15-fold increase in the membrane permeability only slightly affects the steady-state value of the cell volume.

Figure 3 demonstrates the model kinetics of transition processes in the erythrocyte after a jump of the membrane permeability in the case when adenylate pool is regulated. The instant changes in the membrane permeability may well be associated with the transient cell volumes far exceeding the steady-state values. Note that only steady-state values of the cell volume are under stabilization control. The model also predicts volume stabilization of the erythrocyte when the activities of hexokinase and phosphofructokinase or stoichiometry (parameter C) of ATP production in glycolysis vary (Fig. 4).

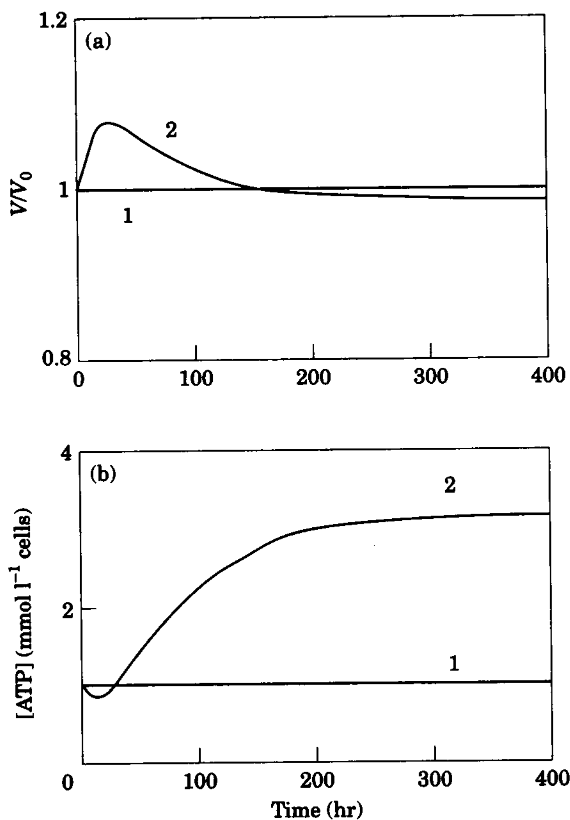

FIG. 3. Dynamics of the changes in (a) the erythrocyte volume and (b) intracellular ATP concentration after a jump in the membrane permeability to cations computed using the model. Line 1 corresponds to the normal physiological state of the erythrocyte ($q = 1$). Line 2 was computed in assumption of the two-fold instant increase in the membrane permeability ($q = 2$).

4. Discussion

Analysis of this model, in which ion homeostasis and energy metabolism of the erythrocyte are described in more detail, confirmed the conclusions of the earlier simple model about the potential regulatory role of adenylate metabolism (Ataullakhanov *et al.*, 1996). As we expected, the more complete model of the erythrocyte metabolism is also able to provide the ideal volume stabilization. To this end, the feedbacks that control the adenylate pool were introduced into the metabolism of adenine nucleotides. Specifically, we assumed that the rate of AMP degradation is activated by ATP and inhibited by AMP. As a result, adenylate pool (and ATP concentration) could alter in a way that allowed transport ATPase to operate more effectively, stabilizing the concentrations of cations and, as a consequence, the cell volume.

In this model, the erythrocyte retains its volume even when permeability of its membrane increases by 15-fold. A further increase in the membrane permeability leads to the loss of the non-zero stable steady state because of limitations of the glycolysis flux: glycolysis fails to provide the necessary increase in the rate of ATP production. The actual permissible changes in the membrane permeability have to be

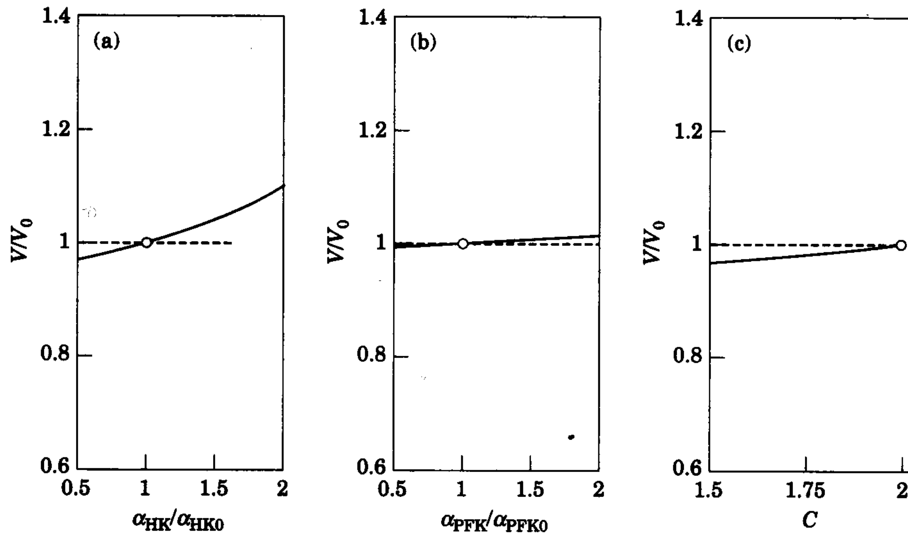

FIG. 4. The steady-state value of the erythrocyte volume as a function of the activity of (a) hexokinase, (b) phosphofructokinase, as well as of (c) the parameter C, specifying the stoichiometry of ATP production in glycolysis. Dashed lines show the results obtained when terms describing metabolism of adenylates were excluded from the model and adenylate pool was kept at a constant level [1.11 mmoles/(l cells) for $V = V_0$].

lower. The erythrocyte volume may significantly deviate from the steady-state value during the transient processes after a rapid increase in the membrane permeability (Fig. 3). Note that only the steady-state volume is stabilized. The transient values may exceed the permissible two-fold increase in the erythrocyte volume when the membrane permeability instantly increases by some rather high value [shown by the arrow in Fig. 2(a)]. These results suggest that adenylate metabolism better stabilizes the erythrocyte volume in the case of slow long-term changes in cell parameters.

Analysis of the model revealed satisfactory stability of the cell volume over large changes in parameters of glycolysis. Figure 4 demonstrates that a somewhat better volume stabilization can be achieved when adenylate pool does not change. However, the activity of hexokinase may increase only by 1.6-fold. A further increase in the hexokinase activity is associated with the loss of stability of the steady state. On the other hand, two-fold or greater variations in the activities of glycolytic enzymes (including hexokinase) are known for erythrocytes (Mohrenweiser *et al.*, 1981; Lestas *et al.*, 1982; Brewer, 1967). When the control able to adjust the adenylate pool is assumed, the range of permissible variations in the activity of hexokinase may even exceed the observed extremes of its variation.

Our model description of the adenylate metabolism in terms of its feedback control is largely abstractive, but it suggests a convenient way to study the regulatory properties of this metabolic system, both analytically and numerically (Ataullakhanov *et al.*, 1996). The use of more natural and generally accepted expressions for the rates of enzyme reaction will hardly change the behavior of the model (and the results obtained) provided that all basic regulatory properties of the metabolic system are retained. Figure 5 shows the erythrocyte volume as a function of the membrane permeability that was calculated using the following more realistic expression for the rate of AMP degradation [instead of expression (28)]:

$$v_d = \alpha_d\left(\frac{[\mathrm{AMP}]}{[\mathrm{AMP}] + K_1}\right)\frac{1 + [\mathrm{ATP}]/K_2}{1 + [\mathrm{AMP}]/K_3} \qquad (29)$$

where $\alpha_d = 6.1 \cdot 10^{-7}$ mM s^{-1}, $K_1 = 10^{-5}$ mM, $K_2 = 10^{-2}$ mM, $K_3 = 10^{-3}$ mM.

Both expressions describe the activation of AMP degradation by ATP and its inhibition by AMP. However, the form of expression (29) is more conventional: a majority of the actual enzymatic processes are described by similar expressions. Comparison of the results presented in Figs 2(a) and 5 demonstrates that the use of expression (29) in the model [instead of expression (28)] does not change the model behavior with respect to the volume stabilization: the erythrocyte volume remains stable over large changes in the membrane permeability to cations.

Our description of adenylate metabolism basically differs from that proposed by Shauer *et al.* (1981) and Joshi & Palsson (1989, 1990). They suggest that the rate of AMP synthesis is proportional to the

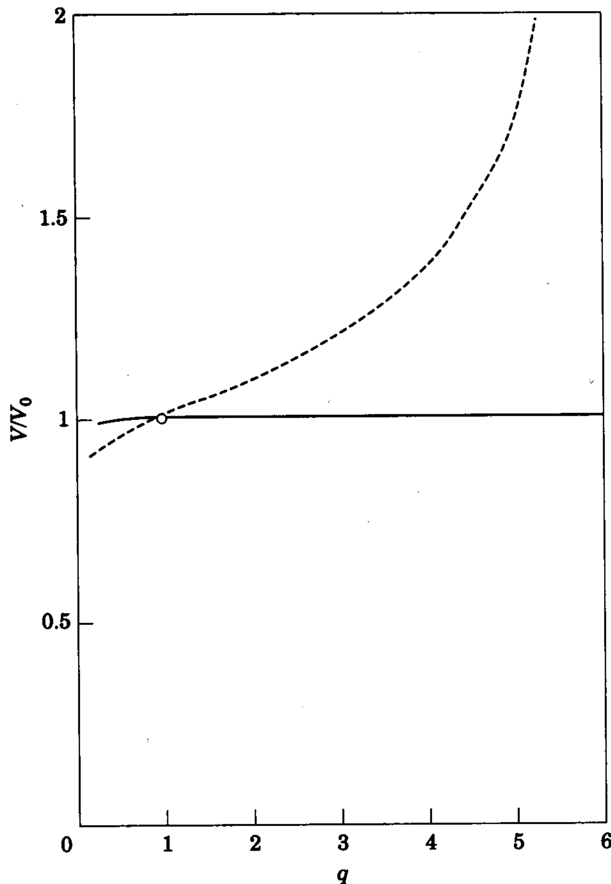

FIG. 5. The steady-state volume of the erythrocyte as a function of cell membrane permeability to cations computed using expression (29) for AMP degradation instead of expression (28). The circle corresponds to the normal physiological state of the erythrocyte. Dashed line shows the results obtained when terms describing metabolism of adenylates were excluded from the model and adenylate pool was kept at a constant level [1.11 mmoles/(l cells) for $V = V_0$].

concentration of ATP, and the rate of AMP degradation is proportional to the concentration of AMP. With these assumptions, our simple mathematical model gives poor volume stabilization over changes in the membrane permeability (Ataullakhanov *et al.*, 1996). Our estimations obtained for the physiologically normal values of the parameters using the model Joshi & Palsson (1989, 1990), also showed that the erythrocyte volume is strongly affected by changes in the membrane permeability. These results are similar to those obtained using our model in assumption of the steadiness of adenylate pool.

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APPENDIX

List of Special Characters Used in the Text

α—activities of ferments
υ—rates of reactions
$\Delta\varphi$—membrane potential