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A Possible Role of Adenylate Metabolism in Human Erythrocytes: Simple Mathematical Model

FAZOIL I. ATAULLAKHANOV, SVETLANA V. KOMAROVA AND VICTOR M. VITVITSKY

National Research Center for Hematology, 125167 *Moscow*, *Russia*

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A simplified mathematical model of cell metabolism describing ion pump, glycolysis and adenylate metabolism was developed and investigated in order to clarify the functional role of the adenylate metabolism system in human erythrocytes. The adenylate metabolism system was shown to be able to function as a specific regulatory system stabilizing intracellular ion concentration and, hence, erythrocyte volume under changes in the permeability of cell membrane. This stabilization is provided via an increase in adenylate pool in association with ATPases rate elevation. Proper regulation of adenylate pool size might be achieved even in the case when AMP synthesis rate remains constant and only AMP degradation rate varies. The best stabilization of intracellular ion concentration in the model is attained when the rate of AMP destruction is directly proportional to ATP concentration and is inversely proportional to AMP concentration. An optimal rate of adenylate metabolism in erythrocytes ranges from several tenths of a percent to several percent of the glycolytic flux. An increase in this rate results in deterioration of cell metabolism stability. Decrease in the rate of adenylate metabolism makes the functioning of this metabolic system inefficient, because the time necessary to achieve stabilization of intracellular ion concentration becomes comparable with erythrocyte life span.

Introduction

The mammalian erythrocyte is one of the most simple and well-studied mammalian cells. Attempts to create a complete and comprehensive mathematical description of the erythrocyte's biochemistry and physiology have a long history. This work was pioneered by Rapoport and his colleagues (Rapoport *et al*., 1974) who published the first mathematical model of glycolysis in human erythrocytes. This group also made an important contribution to the experimental studies of erythrocyte glycolysis by determining kinetics of many important enzymes in red blood cells (Friedman & Rapoport, 1974; Gerber *et al*., 1974; Kuhn *et al*., 1974). During the same period Atkinson (1968) and Sel'kov (1972, 1975) formulated the main principles of regulation of energy metabolism. Specifically, it was suggested that the cell energy charge $\{([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP])\}$ must be stabilized. This principle was further examined in Ataullakhanov *et al*. (1977). It has been shown that the comprehensive description of regulation of glycolysis in red blood cells is confined to a single dependency: the rate of ATP production as a function of ATP concentration. This dependency was found experimentally to be a bell-shaped curve with a steeply descending part at the physiological ATP concentrations (Ataullakhanov *et al*., 1978, 1981a), confirming the original insights about energy charge stabilization. It was also found that the initial models of red cell glycolysis predicted a much lower degree of ATP stabilization than was experimentally determined. After revision of the kinetics of several glycolytic enzymes, a revised mathematical model, capable of an accurate quantitative description of the experimental data, was constructed (Ataullakhanov *et al*., 1980b, 1981a).

In parallel with the study on glycolysis, the models of other metabolic systems in erythrocytes were developed. The model of the glycolytic chain was complemented by a description of the pentose phosphate pathway and redox metabolism (Ataullakhanov *et al*., 1981b). This combined model

was experimentally verified and corrected (Ataullakhanov *et al.*, 1981c). It was concluded that a ratio of the reduced glutathione to the size of the glutathione pool $\{[GSH]/([GSH] + 2[GSSG])\}$ plays a role analogous to the energy charge in energy metabolism in redox metabolism.

The glycolytic model was extended further by including the reactions of synthesis and degradation of adenine nucleotides (Schauer *et al.*, 1981). The functional role of adenylate metabolism in erythrocytes remains poorly understood. Mammalian erythrocytes do not have nucleic acids and their related metabolism, which is the most evident application of adenylate metabolism in nucleated cells. It was suggested in Schauer *et al.* (1981) that adenylate metabolism may serve to improve stabilization of the energy charge, however, this has not been tested experimentally.

Brumen & Heinrich (1984) made a first attempt to combine the models of energy metabolism with the models of volume regulation developed by Jacobsson (1980) and Tosteson & Hoffman (1960). The most complete, up-to-date, quantitative model of human erythrocytes was published by Joshi & Palsson (1989, 1990), who combined glycolysis, the pentose phosphate pathway, the 2,3-DPG bypass, adenylate metabolism, redox metabolism, osmotic regulation and transport processes. In this model, as in earlier works, adenylate metabolism is thought to improve the energy charge stabilization.

We initiated an investigation of a possible functional role of adenylate metabolism of the erythrocytes in view of the apparent failure of previous models to explain the observation that increased membrane permeability correlates with a significant, more than two-fold, increase in the intracellular ATP concentration found for some pathologies (Lichtman & Miller, 1970; Wallas, 1974; Kramer *et al.*, 1976; Mansell *et al.*, 1981; Illner & Shires, 1982). Indeed, according to the traditional models, the increased membrane permeability should activate Na^+,K^+-ATPase, leading to a decrease in the intracellular ATP concentration (for example, see Joshi & Palsson, 1990). We propose that the rise in ATP concentration upon increase in membrane permeability serves to protect the cell from swelling. In response to the increase in membrane permeability the ATP concentration is elevated, so that the rate of ion pumping increases and cell volume remains constant. What is the biochemical mechanism that ensures this regulation? We believe that the changes in the intracellular ATP concentration are realized through the activity of the system of adenylate metabolism. Regulation of the adenylate metabolism is aimed to stabilize cell volume through the adjustment of absolute ATP concentration, rather than to improve the energy charge stabilization.

The aim of the present work is to analyse a hypothesis that the system of adenylate metabolism participates in regulation of ion homeostasis and, as a result, improves the cell volume stabilization. This stabilization is achieved through the hypothetical feedbacks, which lead to increase in the ATP concentration in response to a prolonged increase in the ATP consumption.

We constructed a simple mathematical model to examine the requirements, which the postulated feedback regulation must meet in order to improve the erythrocyte's resistance to lithic disturbances. We also analyse whether this feedback regulation destabilizes the metabolic steady-state of the erythrocyte. The constructed model is used only to address these issues and can not be applied for quantitative descriptions of red cell metabolism. However, the results of the present analysis should help to construct a revised, complete model of the red blood cell.

The analysis of the model leads to the following novel results:

(1) We show that the proper regulation of the adenylate pool size can improve stabilization of the intracellular ion concentration and, hence, cell volume. This regulation requires the postulated feedbacks, which were not included in the existing mathematical models of the red cell metabolism (Schauer *et al.*, 1981; Joshi & Palsson, 1990).

(2) The model predicts existence of a single stationary state. The steady-state level of the ATP concentration grows with the growing permeability of cell membrane, while the energy charge remains almost unchanged. This agrees well with the data on some pathologies (Syllm-Rapoport *et al.*, 1969; Lichtman & Miller, 1970; Wallas, 1974; Kramer *et al.*, 1976; Mansell *et al.*, 1981; Illner & Shires, 1982) and red cell ageing (Dale, 1991).

(3) The model explains why the observed rates of adenylate metabolism are significantly lower than the rate of glycolysis (Bishop, 1961; Lallanne & Willemot, 1980).

Hypothesis

One of the main tasks of erythrocyte metabolism is to maintain an optimal interrelation between cell volume and cell surface. Such optimum must exist because a decrease of surface-to-volume ratio impairs an erythrocyte's ability to pass through narrow capillaries. On the other hand, an increased

surface-to-volume ratio leads to an increase in intracellular viscosity, which also deteriorates erythrocyte's rheology (Koutsouris *et al*., 1985; Clark, 1989). Stabilization of the surface-to-volume ratio can be realised by controlling the cell volume (Tosteson & Hoffman, 1960; Evans & Skalak, 1980).

Here we examine the hypothesis that the adenylate metabolism system in human erythrocytes is a regulatory system changing the size of adenylate pool in order to improve cell volume stabilization. We base our hypothesis on the following postulates:

(1) Stabilization of the erythrocyte volume is provided by stabilization of intracellular ion concentration. This follows from the fact that erythrocyte volume is determined by osmotic pressure (hence, by intracellular ion concentration) (Savitz *et al*., 1964; Freedman & Hoffman, 1979).

(2) Rate of ion pump in the erythrocyte rises proportionally with increase in intracellular Na^+ and ATP concentrations.

Transport Na^+,K^+-ATPase is the main ion pump in erythrocytes. There is no consensus in the literature concerning possible dependence of this ATPase on ATP concentration. In numerous studies performed on the isolated enzyme or reconstructed transport systems, the Michaelis–Menten constant for ATP was found to be quite low, implying a week dependence of transport ATPase on ATP concentration in the cell (Glynn & Karlish, 1976; Robinson & Flashner, 1979). However, there are serious arguments supporting the notion that in intact erythrocytes the rate of operation of the transport Na^+,K^+-ATPase, as well as total ATPase, strongly depends on ATP (Ataullakhanov *et al*., 1980a; Kennedy *et al*., 1986).

(3) The rate of ATP reproduction from ADP in glycolysis (glycolytic flux) as a function of ATP concentration is represented by a bell-shaped curve with a steeply descending part at ATP concentrations close to the size of the adenylate pool.

This dependence was obtained experimentally in humans (Blum *et al*., 1969; Segel *et al*., 1975; Ataullakhanov *et al*., 1978, 1981; Halperin *et al*., 1987) and other mammalian erythrocytes (Ataullakhanov *et al*., 1985) and it is in good agreement with theoretical ideas about functioning of cell energy metabolism (Atkinson, 1968; Reich *et al*., 1976; Ataullakhanov *et al*., 1977, 1980b; Otto *et al*., 1977; Rapoport *et al*., 1977). The bell-shaped dependence ensures, that when energy consuming processes in erythrocytes are activated, leading to a decrease in ATP concentration, glycolytic flux increases replenishing ATP level. Similarly, when ATP consumption decreases, glycolysis is inhibited, and again, ATP concentration remains almost unchanged.

(4) Essential for our hypothesis is the postulate that adenylate metabolism provides an increase in size of the adenylate pool and, therefore, intracellular ATP concentration when ATPases (in particular ion pumps) are activated.

As an illustration of our hypothesis let us consider the sequence of events taking place after the permeability of the erythrocyte membrane has been increased (Fig. 1). There will be an increased influx of sodium ions, followed by a growth in the intracellular sodium concentration. It will cause, in turn, activation of transport Na^+,K^+-ATPase pumping Na^+ out from the cell. If the increase in permeability of the membrane is not too large the intracellular sodium concentration will rise until the sodium outflux, caused by the activated Na^+,K^+-ATPase compensates for the increased sodium influx. Stabilization of the intracellular Na^+ concentration will be achieved at the expense of a decrease in [ATP] consumed by the pumps. The decrease in [ATP] will activate glycolysis and ATP stores will be partially replenished. Eventually, the new steady state will be established, (point b in Fig. 1) characterized by the increased intracellular sodium concentration, activated Na^+,K^+-ATPase, a decreased ATP concentration and activated glycolysis (as compared with the initial undisturbed state shown by point a in Fig. 1). If under these conditions adenylate metabolism was able to actively increase the size of the adenylate pool, then the corresponding increase in ATP concentration would provide an additional activation of Na^+,K^+-ATPase (point c in Fig. 1). As a result, the sodium outflux, which is necessary to compensate for the increased influx would be achieved at an intracellular sodium concentration lower than that when the adenylate pool remains unchanged. Obviously, much better stabilization of intracellular ion concentration and thereby of erythrocyte volume would be achieved. Ideally, the increased adenylate pool might provide sufficient activation of Na^+,K^+-ATPase to keep the initial intracellular ion concentration and the cell volume unchanged (as shown by point c in Fig. 1) leading to the independence of erythrocyte ion concentration and volume from variations in cell membrane permeability.

Description of the Mathematical Model

To test our hypothesis we constructed a mathematical model describing in simplified form a portion of erythrocyte metabolism. The model describes transmembrane ion gradient (e.g. sodium gradient)

with high and constant extracellular concentration and low intracellular concentration; ion pump, which transfers ions out from the cell, glycolysis as an energy source and adenylate metabolism system. The latter changes adenylate pool size and provides an exchange between different components of the adenylate pool via adenylate kinase reaction.

Dynamics of the intracellular ion concentration and concentrations of adenylate pool components in our model are described by the following system of the differential equations:

$$\frac{dI}{dt} = U_1 - 3U_2 \tag{1.1}$$

$$\frac{dT}{dt} = U_3 - U_2 - U_4 + U_5 - U_6 - 2U_7 \tag{1.2}$$

$$\frac{dD}{dt} = U_2 - U_3 - 2(U_5 - U_4) + U_6 + U_7 \tag{1.3}$$

$$\frac{dM}{dt} = U_5 - U_4 + U_6 + 2U_7 - U_8 \tag{1.4}$$

Where I, T, D and M are the intracellular concentrations of ions, ATP, ADP and AMP respectively;

U_1 = rate of passive ion influx in the cell;
U_2 = rate of ATP consumption by the ion pump;
U_3 = rate of ATP reproduction from ADP in glycolysis (glycolytic flux);
U_4 = rate of ATP or AMP consumption and half of ADP production rate in adenylate kinase reaction;
U_5 = rate of reversed adenylate kinase reaction;
U_6 = rate of adenosine kinase reaction;
U_7 = rate of adenine phosphorybosil transferase reaction;
U_8 = total rate of AMP degradation in AMP deaminase and purine nucleotidase reactions.

The first equations of the system (1.1–1.4) describe the dynamics of the intracellular ion concentration. In this equation

$$U_1 = PJ \tag{2}$$

$$U_2 = W_2IT \tag{3}$$

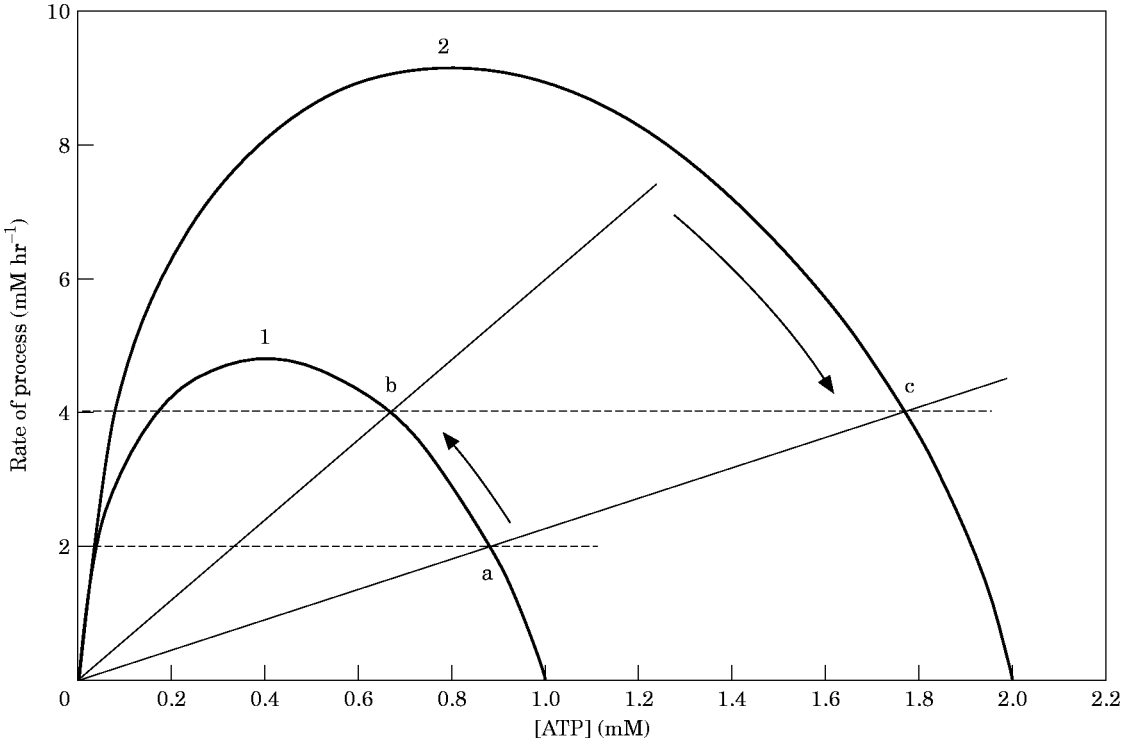

FIG. 1. Illustration of the hypothesis concerning the possible influence of changes in size of adenylate pool on stabilization of ion concentration in an erythrocyte. The dependence of ATP production rate in glycolysis on ATP concentration was calculated using results of Kholodenko *et al*. (1981), and Kholodenko (1983*a*) for adenylate pools of 1 mM (curve 1) and 2 mM (curve 2). The solid straight lines represent rates of ATP consumption by the ion pump as a function of ATP concentration. The points of the intersections of the lines representing ATP producing and ATP consuming processes determine the steady states of energy metabolism and are indicated by letters a, b and c. The dashed lines depict the rates of ion influx for normal and for two-fold increased cell membrane permeability. The arrow pointing up indicates activation of ion pump ATPase caused by a two-fold increase in cell membrane permeability. The arrow pointing down indicates the evolution of the activated ion pump ATPase rate caused by the increasing adenylate pool.

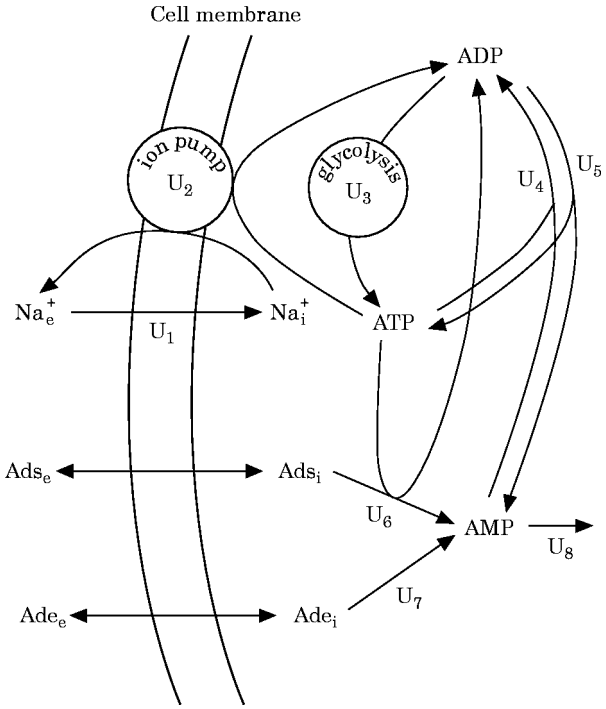

FIG. 2. Scheme of the interaction between ion transport, energy metabolism and adenylate metabolism in a human erythrocyte. Abbreviations: Ade, adenine; ADP, adenosine diphosphate; Ads, adenosine; AMP, adenosine monophosphate; ATP, adenosine triphosphate; Na^+, sodium ions; U_1, rate of passive ion influx in the cell; U_2, rate of ATP consumption by the ion pump; U_3, rate of ATP reproduction from ADP in glycolysis (glycolytic flux); U_4, rate of ATP or AMP consumption and half of ADP production rate in adenylate kinase reaction; U_5, rate of reversed adenylate kinase reaction; U_6, rate of adenosine kinase reaction; U_7, rate of adenine phosphoribosil transferase reaction; U_8, total rate of AMP degradation in AMP deaminase and purine nucleotidase reactions. Indexes e and i denote extra and intracellular constituents respectively.

where P = cell membrane permeability, J = constant extracellular ion concentration, W_2 = the ion pump activity. We include in our model only one ion gradient. Although it was shown that two oppositely directed transmembrane ion gradients provide an improved passive stabilization of erythrocyte volume when cell membrane permeability is disturbed (Moroz *et al*., 1989) a single gradient is sufficient to regulate and stabilize cell volume. We also disregard passive ion outflux. The ion pump in our model consumes one molecule of ATP to transfer three ions out from the cell.

The last three equations of the system (1.1–1.4) describe dynamics of ATP, ADP and AMP concentrations according to the metabolic scheme of Fig. 2. This scheme is based on the data on adenylate metabolism stoichiometry and its connection with energy metabolism in human erythrocytes (Meyskens & Williams, 1971; Dean & Perrett, 1976; Rapoport *et al*., 1979; Paglia *et al*., 1986). The equations take into account ATP consumption during synthesis of phosphoribosylpyrophosphate, which is the second substrate for adenine phosphoribosyl transferase.

In these equations the expression for the rate of glycolysis

$$U_3 = W_3 T^{0.52} M^{0.41} \tag{4}$$

was obtained earlier (Kholodenko *et al*., 1981; Kholodenko, 1983a) as an approximation of the experimental dependence of glycolytic flux on ATP concentration in human erythrocytes. Here W_3 is a parameter reflecting glycolytic activity in the cell.

The rates of adenosine kinase and adenine phosphoribosyl transferase reactions depend on adenosine and adenine concentrations respectively. Due to high permeability of erythrocyte membrane to adenosine and adenine (Moore & Ledford, 1977; Muller *et al*., 1980; Moser *et al*., 1989) the intracellular concentrations of these compounds are equal to their blood plasma levels. Since we cannot take into account variations in the plasma concentrations of these compounds, and the known data on kinetic properties of adenosine kinase, and adenine phosphoribosyl transferase in erythrocytes are scanty, we assume that the rates of these reactions are constant and equal to each other. In this way we can introduce a new parameter:

$$U = U_6 + U_7 = 2U_6 = 2U_7 \tag{5}$$

which describes the rate of AMP synthesis *de novo*.

Equation (1.4) includes the total rate of AMP degradation in purine 5′-nucleotidase and AMP deaminase reactions (U_8). In a generalized form:

$$U_8 = W_8 T^n M^k \tag{6}$$

where W_8 is the activity of the AMP degradation process, and n and k are parameters reflecting the dependence of AMP degradation on [ATP] and [AMP] respectively. The rate of AMP degradation in our model depends on [ATP] as well as on [AMP] because ATP is known as a strong effector of purine 5′-nucleotidase and AMP deaminase (Askari & Rao, 1968; Yung & Suelter, 1978; Itoh, 1981; Bontemps *et al*., 1986; Itoh *et al*., 1986; Van den Berghe *et al*., 1988). Possible dependence of AMP degradation rate on [ADP] is included indirectly in expression (6) because there are only two independent components of the adenylate pool (e.g. AMP and ATP) due to the highly active adenylate kinase in the erythrocytes (Mansell *et al*., 1981; Mohrenweiser *et al*., 1981) and, hence, ADP concentration can be expressed as a function of ATP and AMP concentrations. It is

convenient to express the activity of the AMP degrading process as:

$$W_8 = UW \tag{7}$$

where W is the normalized activity of the AMP degrading process.

Now let us exclude the rate of adenylate kinase reaction using new variables:

Energy pool

$$E = 2T + D \tag{8}$$

and the adenylate pool

$$A = T + D + M \tag{9}$$

and by introducing an equation for adenylate kinase equilibrium:

$$D^2 = TM. \tag{10}$$

Finally, taking into account expressions (2–10) we transform system (1.1–1.4) as follows:

$$\frac{dI}{dt} = U_1 - 3U_2 = PJ - 3W_2IT \tag{11.1}$$

$$\frac{dE}{dt} = U_3 - U_2 - U_6 - 3U_7 = W_3T^{0.52}M^{0.41} - W_2IT - 2U \tag{11.2}$$

$$\frac{dA}{dt} = U_6 + U_7 - U_8 = U - W_8T^nM^k = U(1 - WT^nM^k) \tag{11.3}$$

$$D^2 = TM \tag{11.4}$$

Here:

$$T = (A + 3E - (6AE - 3E^2 + A^2)^{0.5})6^{-1} \tag{12}$$

$$M = (7A - 3E - (6AE - 3E^2 + A^2)^{0.5})6^{-1} \tag{13}$$

It should be noted that the power-law approximations of the reaction rates used here can provide a satisfactory description of any enzymatic process over wide intervals of substrate and effector concentrations. The efficiency of this approach was demonstrated earlier (Savageau, 1969; Kholodenko *et al*., 1981; Kholodenko, 1983a,b; Roels, 1983; Voit & Savageau, 1987; Sorribas & Savageau, 1989; Shiraishi & Savageau, 1993).

The aim of the present study is to investigate, using the mathematical model whether adenylate metabolism can stabilize intracellular ion concentration under perturbations of cell membrane permeability. In particular, the behaviour of the model (11.1–11.4) depending on n, k and U has been studied.

Investigation of the model

The evolution in time of the intracellular ion concentration [Fig. 3(a)] and adenylate pool [Fig. 3(b)] following abrupt alterations of the membrane permeability were computed from system (11.1–11.4). The initial reference steady state in Fig. 3 was obtained for the following values of the parameters:

$$P = P_r = 0.06\ (\mathrm{hr}^{-1}) \tag{14.1}$$

$$J = J_r = 100\ (\mathrm{mM}) \tag{14.2}$$

$$W_2 = W_{2r} = 0.2\ (\mathrm{mM}^{-1}\,\mathrm{hr}^{-1}) \tag{14.3}$$

$$W_3 = W_{3r} = 13.48\ (\mathrm{mM}^{0.07}\,\mathrm{hr}^{-1}) \tag{14.4}$$

$$W = W_r = 0.01\ (\mathrm{mM}^{-(n+k)}) \tag{14.5}$$

$$U = U_r = 0.02\ (\mathrm{mM\ hr}^{-1}) \tag{14.6}$$

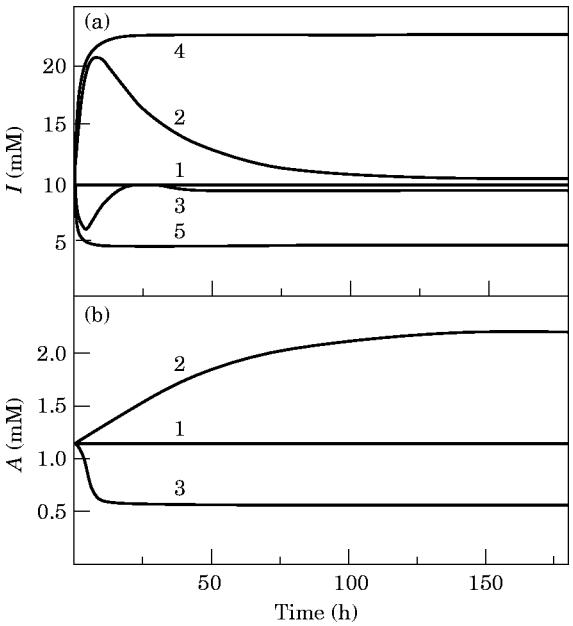

FIG. 3. Kinetics of the changes in intracellular ion concentration (a) and adenylate pool (b) following the abrupt alterations in membrane permeability. The initial steady state values of I and A (lines 1) were obtained for the values of parameters given by (14.1–14.8). Lines 2 and 3 were obtained for parameter P increased up to $0.12\,\mathrm{l\,hr}^{-1}$ and decreased down to $0.03\,\mathrm{l\,hr}^{-1}$ at $t = 0$ respectively. Lines 4 and 5 were computed as 2 and 3, but at constant adenylate pool $A = 1.11$ mM [equation (11.3) was omitted from the system 11.1–11.4].

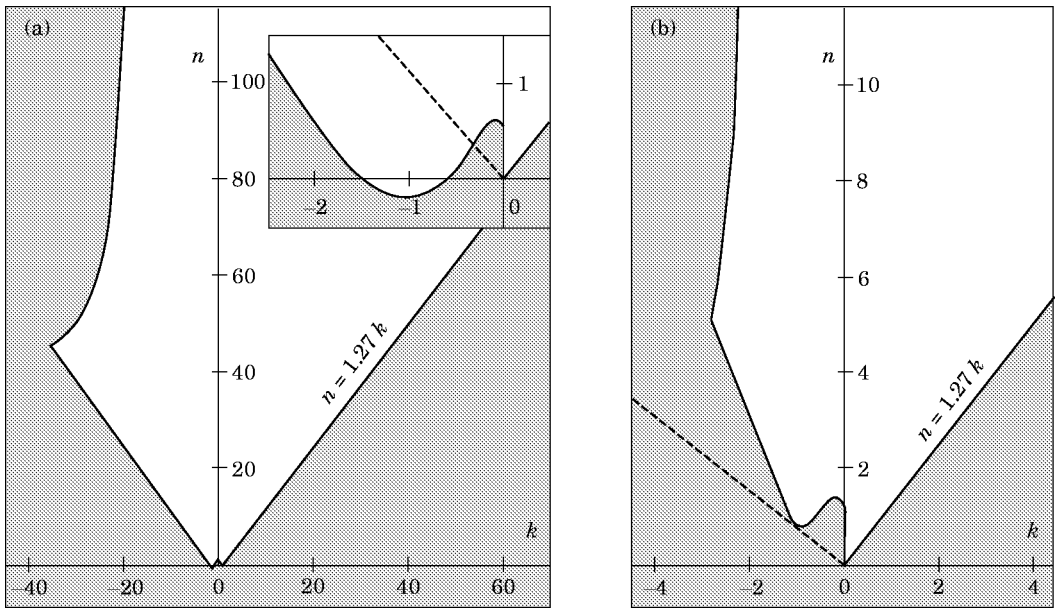

FIG. 4. The region of existence of stable stationary state of system (11.1–11.4) in the plane of parameters n and k (unhatched area). (a) — $U = Ur = 0.02$ mM hr^{-1}, the vicinity of the zero point is shown separately in the box frame. (b) — $U = 0.2$ mM hr^{-1}. The remaining parameters are determined by (14.1–14.5). The dashed lines correspond to the n/k ratios which provide an ideal stabilization of the stationary intracellular ion concentration.

$$k = k_r = -1 \tag{14.7}$$

$$n = n_r = 1.2 \tag{14.8}$$

Here index r indicates the parameter values, which provide the reference steady state in the model. For these values of the parameters, the steady state conforms well to physiological concentrations and fluxes of ions and metabolites in human erythrocytes (Feig *et al*., 1972; Segel *et al*., 1975; Kramer *et al*., 1976; Rapoport I. *et al*., 1977; Lalanne & Willemot, 1980; Ataullakhanov *et al*., 1981; Mansell *et al*., 1981; Ataullakhanov *et al*., 1984):

$$I_s = I_{sr} = 10 \text{ mM} \tag{15.1}$$

$$T_s = T_{sr} = 1 \text{ mM} \tag{15.2}$$

$$D_s = D_{sr} = 0.1 \text{ mM} \tag{15.3}$$

$$M_s = M_{sr} = 0.01 \text{ mM} \tag{15.4}$$

$$U_{2s} = U_{2sr} = U_{3s} = U_{3sr} = 2 \text{ mM hr}^{-1} \tag{15.5}$$

Here index s indicates the steady state values of concentrations and fluxes and index sr indicates those related to the reference steady state.

As one can see from Fig. 3(a), after some initial transitional processes the new stationary levels of the intracellular ion concentration are established. Significant stabilization of the intracellular ion concentration is achieved due to the adjustment in size of the adenylate pool (lines 2 and 3 in Fig. 3). In contrast, alteration of the membrane permeability leads to a prominent change in the stationary intracellular ion concentration if the adenylate pool remains constant, i.e. when eqn (11.3) is excluded from system (11.1–11.4) [lines 4 and 5 in Fig. 3(a)].

In general form the stationary levels of the intracellular ion concentration and adenylate pool components are given by the following steady-state solution of system (11.1–11.4):

$$I_s = PJ(3W_2)^{-1}W^{-0.41x}F^{-kx} \tag{16.1}$$

$$T_s = W^{0.41x}F^{kx} \tag{16.2}$$

$$D_s = W^{-0.47x}F^{0.5(k-n)x} \tag{16.3}$$

$$M_s = W^{-0.52x}F^{-nx} \tag{16.4}$$

$$x = (0.52k - 0.41n)^{-1} \tag{16.5}$$

$$F = (PJ + 6U)(3W_3)^{-1} \tag{16.6}$$

The region of existence of stable steady state of the system (11.1–11.4) in the plane of parameters n and k is represented in Fig. 4. As one can see, this region includes the area of biochemically realistic n and k values, i.e. with absolute values from 0.5 to 2. In this region the steady state is always single.

Now, let us examine whether in our model the stationary ion concentration may be stabilized. A stabilization coefficient is typically employed in order to estimate the degree of stabilization. Here it is more convenient to use a reversed value. We will call it a

sensitivity coefficient. By definition the sensitivity coefficient Q, which describes the sensitivity of stationary intracellular ion concentration (I_s) to the alterations of cell membrane permeability (P) is given by:

$$Q = \frac{\mathrm{d} \ln I_s}{\mathrm{d} \ln P} = \frac{\mathrm{d}I_s}{\mathrm{d}P} \frac{P}{I_s} \tag{17}$$

The better the stabilization, the smaller the sensitivity coefficient. Ideal stabilization means that the stabilized value does not depend on the parameters, therefore the sensitivity coefficient should be equal to zero. In our model the sensitivity coefficient Q is given by the following analytical expression:

$$Q = (0.41nk^{-1} - 0.66U(PJ)^{-1} + 0.48)/((1 + 6U(PJ)^{-1})(0.41nk^{-1} - 0.52)) \tag{18}$$

As one can see, the sensitivity coefficient Q is a hyperbolic function of n/k ratio. The dependence of sensitivity coefficient, Q, on the n/k ratio for other parameter values defined by conditions (14.1; 14.2; 14.6) is shown in Fig. 5. Left and right branches of the graph correspond to the negative and positive values of k respectively. The ideal stabilization ($Q = 0$) is achieved at $n/k = -1.12$. Stabilization of I_s gets worse with deviation of n/k ratio from this value. Negative values of Q in Fig. 5 mean the appearance of over-regulation, which is a decline in I_s with increasing P. As one can see from Fig. 5, a satisfactory stabilization of I_s cannot be achieved in the model for positive k.

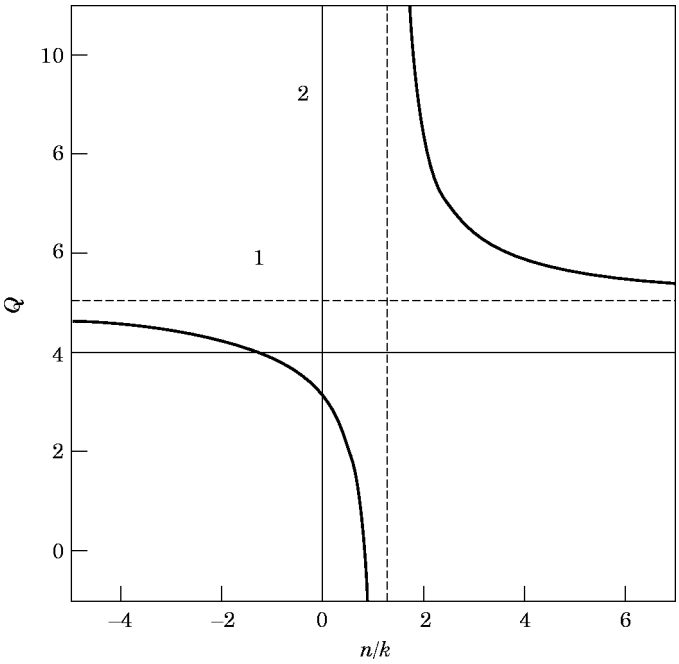

FIG. 5. The coefficient of sensitivity of the stationary intracellular ion concentration to the alterations in cell membrane permeability $Q = \mathrm{d} \ln Is/\mathrm{d} \ln P$ as a function of n/k ratio. The remaining parameters are defined by (14.1–14.6). The dashed lines are the asymptotes: $n/k = 1.27$ and $Q = 1$.

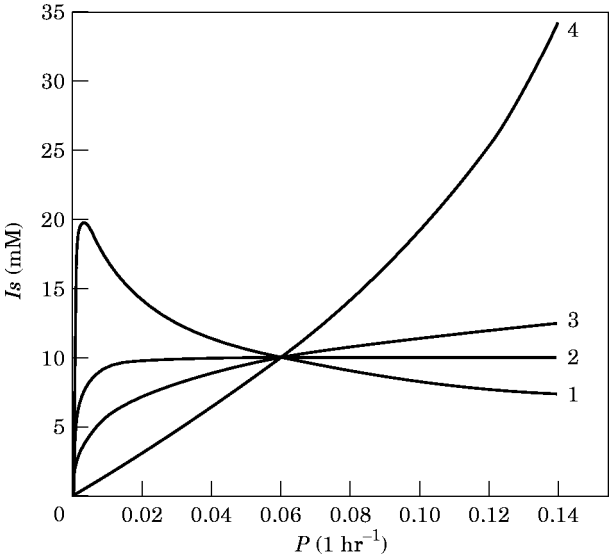

FIG. 6. The influence of n/k ratio on the dependence of stationary intracellular ion concentration Is on the membrane permeability P. All lines were computed for $k = -1$ and varying n: $1 - n/k = -0.5$, $2 - n/k = -1.12$, $3 - n/k = -2$. The remaining parameters were defined by (14.2–14.6). Line 4 was computed for the constant adenylate pool $A = 1.11$ mM.

The dependencies of stationary intracellular ion concentration on cell membrane permeability obtained for different values of n/k ratio are shown in Fig. 6, where curve 2 corresponds to the ideal stabilization of I_s and curve 1 corresponds to over-regulation.

The dependence of the stationary level of adenylate pool (A_s) on cell membrane permeability in our model is also determined by the n/k ratio. When $k < 0$ and $n > 0$ the stationary level of the adenylate pool grows monotonously with increasing cell membrane permeability. The dependence of A_s on P becomes steeper when the n/k ratio grows from $-\infty$ to 0 (not shown). When $n/k = -1.12$ (the ideal stabilization of I_s), the adenylate pool grows proportionally to P [Fig. 7(a), line 1]. In this case adenylate pool growth is mainly determined by the increase in intracellular ATP concentration [Fig. 7(b), line 1], whereas the energy charge remains almost unchanged [Fig. 7(c), line 1]. When n and k are of the same sign, the stationary value of the adenylate pool depends non-monotonously on P. For small P values the adenylate pool declines with increasing P, but then grows (not shown).

In our model parameter U means the value of metabolic flux in adenylate metabolism. In the

reference steady state determined by (14.1–14.8) the value of parameter U constitutes 1% of the stationary glycolytic rate. Increase in U diminishes the region of existence of stable stationary solution of the system (11.1–11.4) in the n, k plane of parameters (Fig. 4). At the same time it leads to an increase in the n/k ratio for which ideal stabilization of I_s is obtained [as it follows from (18)]. If the U value is increased ten-fold, then the ideal stabilization of I_s cannot be achieved within the region of existence of the stable steady state of the system (11.1–11.4) [Fig. 4(b)]. Further increase in U makes it impossible to achieve any significant stabilization of I_s within the region where steady state is stable. If U becomes smaller than U_r then the region of existence of stable stationary solution of the system (11.1–11.4) expands significantly, while the n/k ratio at which the ideal stabilization of I_s is observed decreases slightly. When U tends to zero, the n/k ratio, providing the ideal stabilization of I_s, tends to -1.17. Clearly, however, when parameter U declines significantly (e.g. U is ten times smaller than U_r) regulation of the adenylate pool becomes inefficient, because the time necessary to produce a substantial change in the adenylate pool becomes comparable with the lifespan of an erythrocyte in the bloodstream. Thus, we conclude that the optimal flux in the adenylate metabolism lies within tenths of a percent to several percent of glycolytic flux. Its upper limit is determined by the requirement of the stability of cell metabolism. It is constrained in its lowest value in order to guarantee that the adenylate pool is adjusted within the lifespan of an erythrocyte.

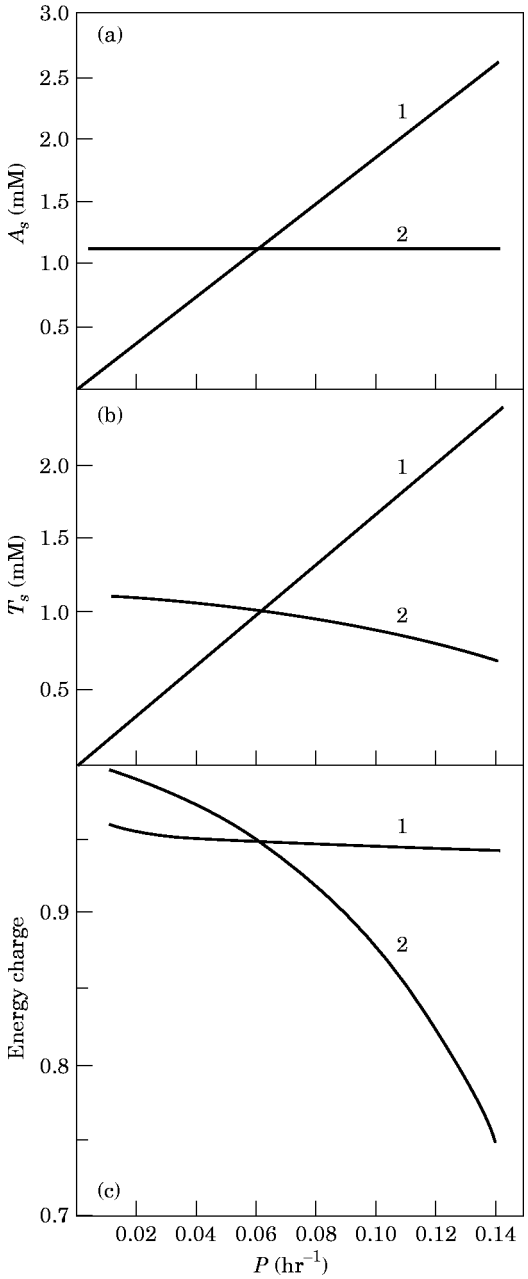

FIG. 7. Stationary values of adenylate pool (a), ATP concentration (b), and energy charge (c), plotted against membrane permeability. Lines 1 were computed from the model (11.1–11.4) for the parameters defined by (14.2–14.8); lines 2 were computed at the same conditions but for the constant adenylate pool $A = 1.11$ mM.

Conclusions

The results obtained demonstrate that effective stabilization of the intracellular ion concentration after alteration of cell membrane permeability might be achieved by the functioning of the adenylate metabolism system. The stabilization is provided by adenylate pool growth with increasing ATP consumption rate. To obtain necessary regulation of adenylate pool size it is sufficient to regulate the rate of AMP destruction while the rate of AMP synthesis may remain unchanged. The required regulation of the rate of AMP destruction, in its turn, may be obtained over a wide range of values of parameters n and k. Importantly, the best stabilization of intracellular ion concentration may be obtained in the model at the realistic values of n and k when rate of AMP destruction is directly proportional to [ATP] and inversely proportional to [AMP] (i.e. when ATP activates and AMP inhibits AMP destruction). ATP is known to be an activator of enzymes destroying AMP (Ascari & Rao, 1968; Yung & Suelter, 1978; Bontemps *et al*., 1986; Van den Berghe *et al*., 1988). The existence of substrate inhibition was not found in kinetic studies of these enzymes *in vitro*, although it may exist *in vivo*. Significant change in the kinetics of the transient processes may be obtained in our model if a possible dependence of AMP production rate on

the adenylate pool components is taken into account. However it is unlikely that steady-state behaviour of the model will be changed.

For the fixed parameters of adenylate metabolism, n and k, the efficiency of stabilization of the intracellular ion concentration decreases with increase in adenylate metabolism flux. At the same time the region in which a stable steady state exists also diminishes. Thus, it seems likely that the low value of the adenylate metabolism flux in erythrocytes (Bishop, 1961; Rapoport *et al*., 1977; Lalanne & Willemot, 1980; Ataullakhanov *et al*., 1984) is imposed by the need to stabilize cellular metabolism. On the other hand, the value of adenylate metabolism flux determines the speed of response of the system. Consequently, the stabilization of erythrocyte ion concentration by regulation of the adenylate pool must occur over the comparatively long time periods (several hours and even days) required to alter the adenylate pool significantly. Most likely, this regulation might serve to compensate slow and constant changes, such as the alterations in membrane permeability, glycolytic activity, ion pump activity arising during cell ageing or some pathological process.

Thus, we conclude that the results of the model considered, support our hypothesis that adenylate metabolism in human erythrocytes may serve as a special regulatory system which provides a significant improvement of stabilization of intracellular ion concentration and, hence, erythrocyte volume.

Interestingly, a high degree of stability of ion concentration is accompanied in the model by a remarkable stabilization of energy charge, while the intracellular ATP concentration and adenylate pool vary significantly.

Consistent with our hypothesis, the increase in intracellular ATP concentration in erythrocytes, which is observed in some pathologies (Syllm-Rapoport *et al*., 1969; Lichtman & Miller, 1970; Wallas, 1974; Kramer *et al*., 1976; Mansell *et al*., 1981; Illner & Shires, 1982) and during cell ageing (Dale, 1991), may be interpreted as a result of adenylate pool growth that compensates for any increase in cell membrane permeability that might have occurred due to these conditions. Individual variability in ATP concentration in erythrocytes of different healthy persons (Brewer, 1967; Lichtman & Miller, 1970; Wallas, 1974; Kramer *et al*., 1976; Mansell *et al*., 1981; Ataullakhanov *et al*., 1984) may also be connected with variations in adenylate pools which compensate for differences in cell membrane permeability arising from some internal (genetic) or external factors.

It would be very interesting to investigate the system (11.1–11.4) using experimentally determined dependencies of AMP production and consumption rates on concentrations of substrates, products and effectors. Unfortunately, relevant descriptions of the kinetics of adenosine kinase, adenine phosphoribosil transferase and purine 5′-nucleotidase reactions in erythrocytes are not available. Investigation of a more detailed model could, for instance, clarify the question of the redundancy of the paths of AMP synthesis and destruction in human erythrocytes. The redundancy may result from different functional roles of the paths, or it may serve to ensure the reliability of adenylate metabolism. The observation that the complete absence of one of the paths of AMP synthesis—adenine phosphoribosyl transferase, or one of the paths of its destruction—AMP deaminase, does not lead to any detectable alterations in the erythrocytes (Dean *et al*., 1978; Valentine & Paglia, 1980; Nobori *et al*., 1986; Ogasavara *et al*., 1986) supports the latter, and points to the significance of adenylate metabolism in red blood cells.

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REFERENCES

Askari, A. & Rao, S. N. (1968). Regulation of AMP deaminase by 2,3-diphosphoglyceric acid: A possible mechanism for the control of adenine nucleotide metabolism in human erythrocytes. *Biochim. Biophys. Acta* **151,** 198–203.

Ataullakhanov, A. I., Ataullakhanov, F. I., Vitvitsky, V. M., Zhabotinsky, A. M. & Pichugin, A. V. (1985). 2,3-diphosphoglycerate shunt and stabilization of ATP level in mammalian erythrocytes. *Biochemistry* (*N.Y.*) **50,** 1005–1011.

Ataullakhanov, F. I., Vitvitsky, V. M., Zhabotinsky, A. M., Kholodenko, B. N. & Ehrlich, L. I. (1977). Quantitative model of glycolysis in the human erythrocyte—I. Dependence of the steady rate of glycolysis on the concentration of ATP. *Biophysics* **22,** 483–488.

Ataullakhanov, F. I., Vitvitsky, V. M., Zhabotinsky, A. M., Pichugin, A. V., Platonova, O. V., Kholodenko, B. N. & Ehrlich, L. I. (1978). Quantitative model of human red blood cell glycolysis. II. Effect of arsenate on glycolysis. Experimental investigation of the relationship between the rate of glycolysis and ATP concentration. *Biophysics* **23,** 1029–1033.

Ataullakhanov, F. I., Buravtsev, V. N., Vitvitsky, V. M., Dibrov, B. F., Zhabotinsky, A. M., Pichugin, A. V., Kholodenko, B. N. & Ehrlich, L. I. (1980a). Connection between rate of ATP-consuming processes and ATP concentration in intact erythrocytes. *Biochemistry* (*N.Y.*) **45,** 1075–1079.

Ataullakhanov, F. I., Buravtsev, V. N., Vitvitsky, V. M., Dibrov, B. F., Zhabotinsky, A. M., Pichugin, A. V., Kholodenko, B. N. & Ehrlich, L. I. (1980*b*). A mathematical model of the energy metabolism of the erythrocytes. Independence of the normalized characteristic of glycolysis from individual peculiarities of the donors. *Biochemistry* (*N.Y.*) **45,** 1267–1273.

Ataullakhanov, F. I., Vitvitsky, V. M., Zhabotinsky, A. M., Pichugin, A. V., Platonova, O. V., Kholodenko, B. N. & Ehrlich, L. I. (1981*a*). The regulation of glycolysis in human erythrocytes. The dependence of the glycolytic flux on the ATP concentration. *Eur. J. Biochem.* **115,** 359–365.

Ataullakhanov, F. I., Buravtsev, V. M., Zhabotinsky, A. M., Norina, S. B., Pichugin, A. V. & Ehrlich, L. I. (1981*b*). Interaction of Embden–Meyerhoff pathway and hexose monophosphate shunt in erythrocytes. *Biochemistry* (*N.Y.*) **46,** 723–731.

Ataullakhanov, F. I., Zhabotinsky, A. M., Pichugin, A. V. & Toloknova, N. F. (1981*c*). Dependence of the rate functioning of the pentose pathway in erythrocytes on the degree of reduction of glutathione. *Biochemistry* (*N.Y.*) **46,** 530–541.

Ataullakhanov, F. I., Vitvitsky, V. M., Zhabotinsky, A. M., Pichugin, A. V., Pomazanov, V. V. & Titkova, N. F. (1984). Effects of glycolysis on adenylate metabolism in human erythrocytes. *Biochemistry* (*N.Y.*) **49,** 104–110.

Atkinson, D. E. (1968). *Cellular Energy Metabolism and its Regulation*. New York: Acad. Press.

Bishop, C. (1961). Purine metabolism in human blood studies *in vivo* by injection of 14C-adenine. *J. Biol. Chem.* **236,** 1778–1779.

Bontemps, F., Van den Berghe, G. & Hers, H. G. (1986). Identification of a purine-5′-nucleotidase in human erythrocytes. *Adv. Exp. Med. Biol.* **195B,** 283–290.

Blum, S. F., Shohet, S. B., Nathan, D. G. & Gardner, F. H. (1969). The effect of amphotericin B on erythrocyte membrane cation permeability: its relation to *in vivo* erythrocyte survival. *J. Lab. Clin. Med.* **73,** 980–987.

Brewer, G. J. (1967). Genetics and population studies of quantitative levels of adenosine triphosphate in human erythrocytes. *Biochem. Genet.* **34,** 25–34.

Brumen, M. & Heinrich, R. (1984). A metabolic osmotic model of human erythrocytes. *Biosystems* **17,** 155–169.

Clark, M. R. (1989). Computation of the average shear-induced deformation of red blood cells as a function of osmolality. *Blood Cells* **15,** 427–439.

Dale, G. L. (1991). Re-examination of factors which influence erythrocyte senescence using a new model of red cell ageing. *Adv. in the Biosciences* **81,** 41–49.

Dean, B. M. & Perrett, D. (1976). Studies on adenine and adenosine metabolism by intact human erythrocytes using high performance liquid chromatography. *Biochim. Biophys. Acta* **437,** 1–15.

Dean, B. M., Perrett, D., Simmonds, A. M., Sahota, A. & Van Acker, K. J. (1978). Adenine and adenosine metabolism in intact erythrocytes deficient in adenosine monophosphate—pyrophosphate phosphoribosyltransferase: A study of two families. *Clin. Sci. Mol. Med.* **55,** 407–412.

Evans, E. A. & Skalak, R. (1980). *Mechanics and Thermodynamics of Biomembranes*, Boca Raton, Florida: CRC Press.

Feig, S. A., Segel, G. B., Shohet, S. B. & Nathan, D. G. (1972). Energy metabolism in human erythrocytes. II. Effect of glucose depletion. *J. Clin. Invest.* **51,** 1547–1554.

Freedman, J. & Hoffman, J. H. (1979). Ionic and osmotic equilibria of human red cells treated with nistatin. *J. Gen. Physiol.* **74,** 157–185.

Friedmann, H. & Papoport, S. (1974). *Enzymes of the Red Cell. A Critical Catalogue. Cellular and Molecular Biology of Erythrocytes*. Tokyo: University Tokyo Press.

Gerber, G., Preissler, H., Heinrich, R. & Rapoport, S. (1974). Hexokinase of human erythrocytes. Purification, kinetic model and its application to the conditions in the cell. *Eur. J. Biochem.* **45,** 39–52.

Glynn, I. M. & Karlish, J. D. (1976). ATP hydrolysis associated with an uncoupled sodium flux through the sodium pump: Evidence for allosteric effects of intracellular ATP and extracellular sodium. *J. Physiol.* **256,** 465–496.

Halperin, J. A., Brugnara, C., Kopin, A. S., Ingwall, J. & Tosteson, D. C. (1987). Properties of the Na^+-K^+ pump in human red cells with increased number of pump sites. *J. Clin. Invest.* **80,** 128.

Illner, H. & Shires, G. T. (1982). Changes in sodium, potassium and adenosine triphosphate contents of red blood cells in sepsis and septic shock. *Circ. Shock.* **9,** 259–267.

Itoh, R. (1981). Regulation of cytosol 5′-nucleotidase by adenylate energy charge. *Biochim. Biophys. Acta* **659,** 31–37.

Itoh, R., Oka, J. & Ozasa, H. (1986). Regulation of the cytosol 5′-nucleotidase of the heart by adenylate energy charge. *Adv. Exp. Med. Biol.* **195B,** 299–303.

Jacobsson, C. J. (1980). A mathematical model of cell volume regulation in human red blood cells. *Amer. J. Physiol.* **238,** C196–203.

Joshi, A. & Palsson, B. O. (1989). Metabolic dynamics in the human red cell. Part I–II. *J. theor. Biol.* **141,** 515–545.

Joshi, A. & Palsson, B. O. (1990). Metabolic dynamics in the human red cell. Part III–IV. *J. theor. Biol.* **142,** 41–85.

Kennedy, B. G., Lunn, G. & Hoffman, J. F. (1986). 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.* **87,** 47–72.

Kholodenko, B. N., Dibrov, B. F. & Zhabotinsky, A. M. (1981). Regulation of erythrocyte energy metabolism. Dependence of glycolysis characteristics on donor individual parameters. *Biophysics* **26,** 501–506.

Kholodenko, B. N. (1983*a*). A model of energy metabolism of erythrocytes. Scale invariance of regulatory characteristics. In: *Mathematical Modelling in Immunology and Medicine*. (Marchuk, G. I. & Belykh, L. N., eds) pp. 311–318. Amsterdam: North Holland Publishing Company.

Kholodenko, B. N. (1983*b*). Power-low approximation and similarity properties of metabolic regulatory characteristics. *Biophysics* **28,** 674–681.

Koutsouris, D. L., Delatour-Hanss, E. & Hanss, M. (1985). Physico-chemical factors of erythrocyte deformability. *Biorheology* **22,** 119–132.

Kramer, H. J., Gospodinov, D. & Kruck, F. (1976). Functional and metabolic studies on red blood cell sodium transport in chronic uraemia. *Nephron* **16,** 344–358.

Kuhn, B., Jacobash, G., Gerth, C. & Rapoport, S. (1974). Kinetic properties of the phosphofructocinase from erythrocytes of rats and rabbits. *Eur. J. Biochem.* **43,** 437–450.

Lalanne, M. & Willemot, J. (1980). Adenine and hypoxanthine salvage in erythrocytes of eight mammalian species. *Comp. Biochem. Physiol.* **66B,** 367–372.

Lichtman, M. A. & Miller, D. R. (1970). Erythrocyte glycolysis, 2,3-diphosphoglycerate and adenosine triphosphate concentration in uremic subject: Relationship to extracellular phosphate concentration. *J. Lab. Clin. Med.* **76,** 267–279.

Mansell, M. A., Allsop, J., North, M. E., Simmonds, R. J., Harkness, R. A. & Watts, R. W. E. (1981). Effect of renal failure on erythrocyte purine nucleotide, nucleoside and base concentrations and some related enzyme activities. *Clin. Sci. Mol. Med.* **61,** 757–764.

Meyskens, F. L. & Williams, H. E. (1971). Adenosine metabolism in human erythrocytes. *Biochim. Biophys. Acta* **240,** 170–179.

Mohrenweiser, H. W., Fielek, S. & Wursinger, K. H. (1981). Characteristics of enzymes of erythrocytes from newborn infants and adults: Activity, thermostability, and electrophoretic profile as a function of cell age. *Amer. J. Hematol.* **11,** 125–136.

Moore, G. L. & Ledford, M. E. (1977). The uptake and egress of adenine from human red blood cells *in vitro*. *Transfusion* **17,** 38–43.

Moroz, I. A., Ataullakhanov, F. I., Kiyatkin, A. B., Pichugin, A. V. & Vitvitsky, V. M. (1989). A mathematical model of erythrocyte volume stabilization. *Biologicheskie membrany* **6,** 409–419.

Moser, G. H., Schrader, J. & Deussen, A. (1989). Turnover of adenosine in plasma of human and dog blood. *Amer. J. Physiol.* **256,** c799–c806.

MULLER, M. M., KUZMITS, R., FRASS, M. & MAMOLI, B. (1980). Purine metabolism of erythrocytes in myotonic distrophy. *J. Neurol*. **223,** 59–66.

NOBORI, T., YAMANAKA, H., KAMATANI, N., NISHIOKA, K. & MIKANAGI, K. (1986). The prevalence of purine metabolic disorders in Japan. *Adv. Exp. Med. Biol*. **195A,** 35–38.

OGASAWARA, N., GOTO, H., YAMADA, Y. & HASEGAWA, I. (1986). Deficiency of erythrocyte type isozyme of AMP deaminase in human. *Adv. Exp. Med. Biol*. **195A,** 123–127.

OTTO, M., JACOBASCH, G. & SVETINA, S. (1977). Properties of the hexokinase-phosphofructokinase system on the basis of an extended PFK-model. *Acta biol. med. germ*. **36,** 581–585.

PAGLLIA, D. E., VALENTINE, W. N., NAKATANI, M. & BROCKWAY, R. A. (1986). Mechanism of adenosine 5′-monophosphate catabolism in human erythrocytes. *Blood* **67,** 988–992.

RAPOPORT, I., BERGER, H., ELSNER, R. & RAPOPORT, S. (1977). pH Dependent changes of 2,3-bisphosphoglycerate in human red cells during transitional and steady states *in vitro*. *Eur. J. Biochem*. **73,** 421–427.

RAPOPORT, I., RAPOPORT, S., MARETZKI, D. & ELSNER, R. (1979). The breakdown of adenine nucleotides in glucose-depleted human red cells. *Acta biol. med. germ*. **38,** 1419–1429.

RAPOPORT, T. A., HEINRICH, R., JACOBASCH, G. & RAPOPORT, S. (1974). A linear steady-state treatment of enzymatic chains. A mathematical model of glycolysis of human erythrocytes. *Eur. J. Biochem*. **42,** 107–120.

RAPOPORT, T. A., OTTO, M. & HEINRICH, R. (1977). An extended model of the glycolysis in erythrocytes. *Acta biol. med. germ*. **36,** 461–468.

REICH, J. G., SEL'KOV, E. E., GEIER, TH. & DRONOVA, D. (1976). Elementary properties of energy-regenerating pathways. *Studia Biophysica* **54,** 57–76.

ROBINSON, J. D. & FLASHNER, M. S. (1979). The (Na^+,K^+)-activated ATPase. Enzymatic and transport properties. *Biochem. Biophys. Acta*. **549,** 145–176.

ROELS, J. A. (1983). In: *Energetics and Kinetics in Biotechnology*. Amsterdam: Elsevier Science Publishers B.V.

SAVAGEAU, M. A. (1969). Biochemical systems analysis. II. The steady-state solutions for an n-pool system using a power-low approximation. *J. theor. Biol*. **25,** 370–379.

SAVITZ, D., SIDEL, V. W. & SOLOMON, A. K. (1964). Osmotic properties of human red cells. *J. Gen. Physiol*. **48,** 79–94.

SCHAUER, M., HEINRICH, R. & RAPOPORT, S. (1981). Mathematische modellierung der Glykolyse und des Adeninnukleotidstoffwechsels menschlicher erythrozyten. *Acta biol. med. germ*. **40,** 1659–1697.

SEGEL, G. B., FEIG, S. A., GLADER, B. E., MULLER, A., DUTCHER, P. & NATHAN, D. G. (1975). Energy metabolism in human erythrocytes: The role of phosphoglycerate kinase in cation transport. *Blood* **46,** 271–278.

SEL'KOV, E. E. (1972). Nonlinear theory of regulation of the key step of glycolysis. *Studia biophysica* **33,** 167–176.

SEL'KOV, E. E. (1975). Stabilization of energy charge, oscillations and multiplicity of stationare states as a result of purely stoichiometric regulation. *Eur. J. Biochem*. **59,** 151–157.

SHIRAISHI, F. & SAVAGEAU, M. A. (1993). The tricarboxylic acid cycle in *Dictyostellium discoideum*. Systemic effects of including protein turnover in the current model. *J. biol. Chem*. **286,** 16917–16928.

SORRIBAS, A. & SAVAGEAU, M. A. (1989). A comparison of variant theories of intact biochemical systems. 1: Enzyme–enzyme interactions and biochemical systems theory. *Math. Biosci*. **94,** 161–193.

SYLLM-RAPOPORT, I., JACOBASCH, G., PREHN, S. & RAPOPORT, S. (1969). On a regulatory system of the adenine level in the plasma connected with red cell maturation and its effect on the adenine nucleotides of the circulating erythrocyte. Lack of relation between ATP-level and life span of the erythrocyte. *Blood* **33,** 617–627.

TOSTESON, D. C. & HOFFMAN, J. F. (1960). Regulation of cell volume by active cation transport in high and low potassium sheep red cell. *J. Gen. Physiol*. **44,** 169–194.

VALENTINE, W. N. & PAGLIA, D. E. (1980). Erythrocyte disorders of purine and pyrimidine metabolism. *Hemoglobin* **4,** 669–681.

VAN DEN BERGHE, G., BONTEMPS, F. & VINCENT, M. F. (1988). Cytosolic purine 5′-nucleotidase of rat liver and human red blood cells: Regulatory properties and role in AMP dephosphorylation. *Adv. Enz. Reg*. **27,** 297–311.

VOIT, E. O. & SAVAGEAU, M. A. (1987). Accuracy of alternative representations for integrated biochemical systems. *Biochemistry* **26,** 6869–6880.

WALLAS, C. H. (1974). Metabolic studies on the erythrocyte from patient with chronic renal disease on haemodialysis. II. ATP metabolism. *Brit. J. Haematol*. **27,** 145–152.

YUNG, S.-L. & SUELTER, C. H. (1978). Human erythrocyte 5′-AMP aminohydrolase. Purification and characterization. *J. biol. Chem*. **253,** 404–408.