Eur. J. Biochem. *115*, 359–365 (1981)

The Regulation of Glycolysis in Human Erythrocytes

The Dependence of the Glycolytic Flux on the ATP Concentration

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(Received March 24/October 27, 1980)

1. According to the mathematical model, the dependence of the rate of glycolysis on the ATP concentration can be represented by the bell-shaped curve with the descending part at the concentration of ATP close to physiological values. The existence of the descending part is a result of the strong inhibition of phosphofructokinase by ATP and hexokinase by glucose 6-phosphate and provides stabilisation of the intracellular concentration of ATP.

2. Using arsenate as an uncoupler of the oxidation of glucose and ATP synthesis, the dependence of the rate of glycolysis on the ATP concentration in erythrocytes was measured. This dependence (glycolysis characteristic) is represented by a bell-shaped curve. The normalized glycolysis characteristics are the same for all the donors investigated.

3. The increase of permeability of the erythrocyte membrane to monovalent cations by the polyene antibiotic levorin leads to an increase of glycolytic rate in erythrocytes (40–70%) and to a decrease in the ATP concentration (15%) and glucose 6-phosphate (25–60%). The data obtained with levorin, and also other results on Na, K-ATPase activation are in a good agreement with the characteristic obtained using arsenate.

4. In all cases, inhibition of Na^+, K^+-ATPase of erythrocytes by ouabain leads to the decrease of the rate of lactate production (10–20%) and increase of the concentration of glucose 6-phosphate (14–40%). The ATP concentrations remains almost unchanged or is only slightly increased. The rate of glucose utilisation in erythrocytes from different donors show two types of behaviour. In the first type of erythrocyte it drops proportionally to the decrease in the lactate production rate; in the second type of erythrocyte it remains unchanged.

5. The experimental results presented are in qualitative agreement with the predictions of the mathematical model.

The problem of the regulation of energy metabolism in erythrocytes have been extensively studied. Many important features of the control mechanisms have been elucidated [1]. It has become apparent that the control of energy metabolism in erythrocytes must provide stabilisation of the intracellular concentration of ATP to ensure the concerted functioning of the numerous reactions in which ATP involved. This stabilisation is shown experimentally using the change of the ATPase activity in the cell [2–5]. The general principles of energy metabolism control in the cell have been reviewed [6, 7]. In particular, it has been shown that the ATP stabilisation during the change of ATPase activity can be achieved at the expense of the descending part of the curve describing the dependence of the steady-state ATP production on the ATP concentration (we will call this dependence the glycolysis characteristic throughout this paper). The form of this characteristic determines the stabilisation capacity of glycolysis. A number of hypotheses on the glycolysis control mechanism and ATP stabilisation in erythrocytes and some mathematical models of this metabolic system have appeared in the literature [4, 8–11]. After critical examination of the earlier mechanisms suggested we have proposed a mathematical model of the Embden-Meyerhof pathway for human erythrocytes and calculated the glycolysis characteristic [12].

Abbreviations. Glucose-6-*P*, glucose 6-phosphate; fructose-6-*P*, fructose 6-phosphate.

Enzyme. ATPase or ATP phosphohydrolase (EC 3.6.1.3).

In this paper we wish to present the results on the quantitative measurements of the glycolysis characteristic using inhibition of Na^+,K^+-ATPase by ouabain, its activation by levorin and uncoupling by arsenate as tools for gradual variation of steady-state concentration of ATP in erythrocytes.

MATERIALS AND METHODS

Donor blood was collected in vessels containing 12 I.U. of heparin/ml of blood. The samples were centrifuged 10 min at $1000 \times g$, then the plasma and the buffy coat were removed with a pipette. The packed cells were resuspended in an equal volume of medium (see below), the suspension was centrifuged 10 min at $1000 \times g$ and the supernatant was removed with a pipette. The washing procedure was repeated twice. A salt solution containing 125 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.2 mM sodium phosphate, 2–10 mM glucose, 20–50 mM Tris/HCl pH 7.3–7.5 was used for washing and incubation of erythrocytes. The washed erythrocytes were diluted in the incubation solution (hematocrit $\approx$ 40%) and incubated in thermostated glass or teflon tubes at 37 °C with constant stirring. The time from the beginning of blood collection to the beginning of incubation was 1–2 h. The samples of erythrocytes from only one donor were used in each separate experiment. Ouabain was added as a dry substance, and levorin was added as a freshly prepared aqueous solution of sodium salt in a volume not exceeding 1 µl/ml of suspension. The required concentration of arsenate and

orthophosphate were obtained by addition of 1 M solutions of disodium orthophosphate or arsenate. After addition of the effectors the suspension was incubated for six hours. To determine the concentration of metabolites the small samples were withdrawn, the proteins were precipitated by 0.5 M perchloric acid (2 ml of acid/ml of the suspension); the sediment was removed by centrifugation of the samples for 5 min at $1000 \times g$, the supernatant was removed and neutralized with a solution of K_2CO_3 to pH 7.0.

The concentration of glucose, lactate, ATP, ADP, AMP, and glucose-6-*P* were measured by enzymatic methods according to [13]. Ouabain was obtained from Calbiochem, levorin (30000 I.U./mg) from Penza Factory of Medical Preparations (USSR).

The Mathematical Model and Principles of the Measurement of the Glycolysis Characteristic

The mathematical description is given by the following set of equations:

$$[\text{glucose}] = \text{constant}$$

$$\frac{\mathrm{d}\,[\text{glucose-6-}P]}{\mathrm{d}\,t} = v_{\text{HK}} - v_{\text{PGI}}$$

$$\frac{\mathrm{d}\,[\text{fructose-6-}P]}{\mathrm{d}\,t} = v_{\text{PGI}} - v_{\text{PFK}}$$

$$\frac{([\text{ADP}])^2}{[\text{ATP}] \cdot [\text{AMP}]} = K_{\text{eq}}^{\text{AK}} = 2.0 \qquad [14,15]$$

$$[\text{ATP}] + [\text{ADP}] + [\text{AMP}] = \alpha = 1\ \text{mM}\,. \qquad (1)$$

Under natural conditions and in our experiments the hexokinase is saturated by glucose and the solution of Eqn (1) does not depend on the glucose concentration. The rates of hexokinase (v_{HK}), phosphoglucoisomerase (v_{PGI}) and phosphofructokinase (v_{PFK}) reactions are described as follows:

$$v_{\text{HK}} = \alpha_{\text{HK}} \frac{\dfrac{[\text{ATP}]}{K_{\text{m,ATP}}^{\text{HK}}}}{R_1 + \dfrac{[\text{ATP}]}{K_{\text{m,ATP}}^{\text{HK}}} + \dfrac{[\text{glucose-6-}P]}{K_{\text{i}}^{\text{HK}}}} \qquad (2)$$

$R_1 = 4$; $K_{\text{m,ATP}}^{\text{HK}} = 1\,\text{mM}$; $K_{\text{i}}^{\text{HK}} = 0.007\,\text{mM}$; $\alpha_{\text{HK}} = 11.5\,\text{mM/h}$ [16–19].

$$v_{\text{PGI}} = \alpha_{\text{PGI}} \frac{\dfrac{[\text{glucose-6-}P]}{K_{\text{m,glucose-6-}P}^{\text{PGI}}} - K_{\text{eq}}^{\text{PGI}} \dfrac{[\text{fructose-6-}P]}{K_{\text{m,glucose-6-}P}^{\text{PGI}}}}{1 + \dfrac{[\text{glucose-6-}P]}{K_{\text{m,glucose-6-}P}^{\text{PGI}}} + \dfrac{[\text{fructose-6-}P]}{K_{\text{m,fructose-6-}P}^{\text{PGI}}}} \qquad (3)$$

$K_{\text{m,glucose-6-}P}^{\text{PGI}} = 0.3$ mM; $K_{\text{m,fructose-6-}P}^{\text{PGI}} = 0.2$ mM; $K_{\text{eq}}^{\text{PGI}} = 3$; $\alpha_{\text{PGI}} = 360$ mM/h [20–22].

$$v_{\text{PFK}} = \alpha_{\text{PFK}} V \frac{\dfrac{[\text{ATP}]}{K_{\text{m,ATP}}^{\text{PFK}}} \left\{1 + 2\left(\dfrac{[\text{AMP}]}{K_{\text{a,AMP}}^{\text{PFK}}} + R_2\right)\right\}}{\left(1 + \dfrac{[\text{ATP}]}{K_{\text{m,ATP}}^{\text{PFK}}}\right)\left(1 + R_2 + \dfrac{[\text{AMP}]}{K_{\text{a,AMP}}^{\text{PFK}}}\right) + \left(\dfrac{[\text{ATP}]}{K_{\text{i,ATP}}^{\text{PFK}}}\right)^7} \qquad (4)$$

$$V = \frac{[\text{fructose-6-}P]}{[\text{fructose-6-}P] + 1.1};$$

$$K_{\text{m,ATP}}^{\text{PFK}} = \frac{0.08\,[\text{fructose-6-}P]}{[\text{fructose-6-}P] + 0.14}\ (\text{mM});$$

$$K_{\text{i,ATP}}^{\text{PFK}} = \frac{0.75\,([\text{fructose-6-}P] + \beta)}{[\text{fructose-6-}P] + 0.6}\ (\text{mM});$$

$R_2 = 10$; $K_{\text{a,AMP}}^{\text{PFK}} = 0.01$ mM; $\beta = 0.17$ mM; $\alpha_{\text{PFK}} = 360$ mM/h [23,24].

The steady-state ATP production rate v^+ is determined as

$$v^+ = 2\,v_{\text{GLU}} = 2\,v_{\text{HK}}\,. \qquad (5)$$

The solution of Eqn (1) for the steady-state conditions makes it possible to build up the dependence of the glucose utilisation rate (v_{GLU}) or, because of stoichiometry given in Eqn (5), the rate of ATP production on ATP concentration. We will call this dependence of v_{GLU} on ATP as 'glucose characteristic'. In our model the glucose characteristic is bell-shaped and the point typical for physiological conditions is situated on the descending part of the curve (Fig. 1). The existence and slope of the descending part of the curve (Fig. 1) is strongly dependent on the inhibition constant of hexokinase by glucose-6-*P* (Eqn 2). The value of this constant published by different authors varies greatly [15–19]; thus the real characteristic of erythrocyte glycolysis cannot be calculated from the data in literature.

It follows from the steady-state solution of Eqn (1) that the variation of ATP concentration leads to a strong change of [glucose-6-*P*]. In fact in this model [glucose-6-*P*] is the stabilising variable and [ATP] is the stabilised one.

We have undertaken the experimental measurement of the glucose characteristic of glycolysis.

This characteristic could be obtained by changing the total ATPase activity of erythrocytes (see Fig. 1) and measuring the stationary rates and concentration of ATP. However, as far as we know, no methods exist to produce a sufficiently large change of ATPase activity in native erythrocytes without interference of side-effects.

The independent method of obtaining the characteristic is using the uncoupling of oxidation and ATP production in glycolysis. When the uncoupler is added, the rate of ATP production falls. This should lead to a decrease of ATP concentration. Thus, by changing the concentration of the uncoupler and obtaining different stationary values of [ATP] and corresponding rates of glucose consumption it is possible to obtain the glucose characteristic. For this purpose we used arsenate. In glycolysis arsenate can enter the glyceraldehyde-3-phosphate dehydrogenase reaction [25–29]. The 1-arseno-3-phosphoglycerate which forms, breaks down rapidly to arsenate and 3-phosphoglycerate, resulting in prevention of ATP formation in phosphoglyceratekinase reaction. All the following stages remain unchanged [30].

The part of the characteristic which can be obtained using alternatively arsenate or ATPase activation, may serve for mutual controls. Moreover, the decrease of ATPase activity makes it possible to obtain the part of characteristic at the ATP level higher than physiologically normal value (Fig. 1). Some results on the effect of change in the activity of Na^+, K^+-ATPase on glycolysis in erythrocytes had been obtained earlier [2–5, 31, 32].

RESULTS

The Effect of Arsenate

The addition of arsenate to the suspension of erythrocytes leads to changes in all the metabolites measured. The new value of the glycolysis rate and the concentrations of the metabolites are reached 3 h after the addition of arsenate and remained constant during further incubation (Fig. 2). The transition period may be due to the rather slow penetration of arsenate into erythrocyte [33]. Measurements of the

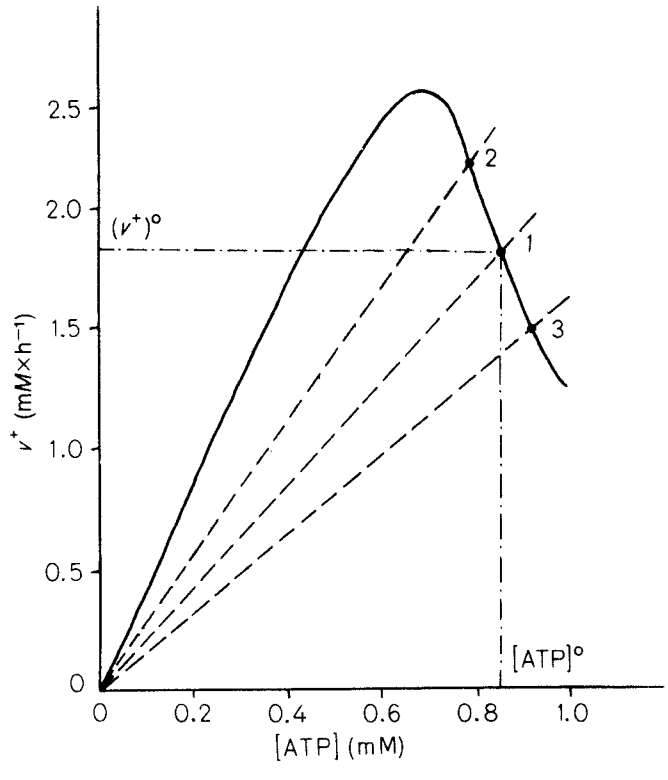

Fig. 1. *Theoretical glycolysis characteristic.* Computed dependence of ATP production $v^+ = 2\,v_{GLU}$ on the ATP concentration in the erythrocyte (solid line). Broken lines — hypothetical dependence of the rate of total ATPase of erythrocytes on [ATP]; normal (1), activated (2) and inhibited (3) ATPase. The intercept of the glycolysis characteristic with the line representing total ATPase determines the stationary ATP concentration and the rate of its production. Normal ATPase (1) determines the physiological values of the ATP concentration, $[ATP]^\circ$ and the rate of glycolysis, $(v^+)^\circ$

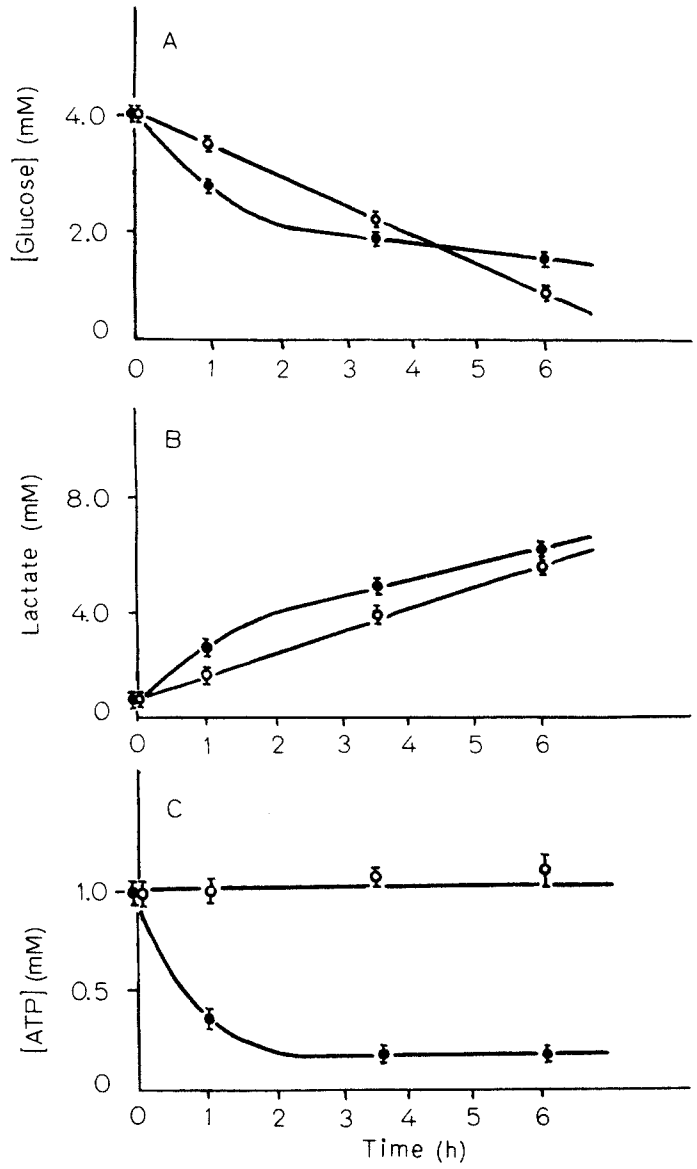

Fig. 2. *Effect of arsenate on the erythrocyte glycolysis.* Changes in concentration of glucose (a), lactate (b) and [ATP] (c) during incubation of erythrocytes (hematocrit 47%) with arsenate. (O——O) Control; (●——●) arsenate (7.5 mmol/l suspension)

steady-state values of the concentrations were thus made 3 h after the addition of arsenate. The results are given in Table 1. It can be seen that with the increase of arsenate concentration the concentration of ATP and glucose-6-*P* decrease monotonously, while the glucose utilisation rate first increases and then falls down.

The addition of orthophosphate leads to a considerable increase of the rate of glucose utilisation and to a slight increase in [ATP]. The concentration of glucose-6-*P* remains practically unaltered. If the sum of arsenate and orthophosphate was maintained constant, the effect of arsenate was found to be the same. The measurement of the ADP and AMP concentration in one experiment showed that the total concentration of ATP, ADP and AMP are constant throughout the incubation period and does not depend on the arsenate concentration.

The Effect of Levorin

In the preliminary experiments we found that levorin in concentrations not exceeding 0.1 μg/ml of erythrocyte suspension does not change glycolysis. On the other hand, levorin in a concentration higher than 0.3 μg/ml leads to hemolysis of erythrocytes during incubation. Hence the concentration of levorin used was 0.3 μg/ml. At this concentration the changes in the erythrocytes volume during incubation did not exceed 10%. It can be seen from Table 2 that the addition of levorin causes considerable enhancement of the glucose utilisation rate and lactate formation.

The Effect of Ouabain

In parallel experiments with levorin (blood from the same donor) the effect of ouabain (Strophantin G) was studied. The results of these experiments (Table 2) are in a good agreement with the data obtained earlier on the influence of strophantin K on erythrocytes [5]. As seen from Table 2, ouabain reduces the rate of lactate formation, slightly enhances the concentration of ATP (not more than 5%) and the concentration of glucose-6-*P* increases considerably. In most cases the rate of glucose utilisation decreases and the lactate/glucose ratio remains constant. In the samples from some donors, however, the glucose utilisation rate remains unchanged and the lactate/glucose ratio decreases (see donor 3, Table 2). In experiments with levorin and ouabain the sum: [ATP] + [ADP] + [AMP] remains unaltered.

Normalized Glucose Characteristic

Using the data presented in Table 1 it is possible to make a plots of the interdependence between the glucose utilisation rate and the ATP concentration in the erythrocytes for each donor. In all cases, the general character of the curve corresponds to the theoretical predictions. However, these curves are difficult to compare because of the large differences in the values of v_{GLU}, [ATP] and [glucose-6-*P*] in the samples from different donors. To compare the results, it was found more suitable to use the relative units, i.e. the values obtained in the control (in the absence of effectors) are taken as 100%. The results of all the experiments expressed in such relative units are given in Fig. 3. As it is seen, the dependence of the rate of glucose utilisation on the ATP concentration can be represented as a single curve, despite the great differences in the glucose utilisation rate and the ATP concentration in the samples from different donors in the control experi-

Table 1. *The effects of arsenate and orthophosphate on glycolysis in human erythrocytes*
The stationary values of rates and concentrations determined after 3 h of preincubation of erythrocytes are given. Accuracy: v_{GLU}: 5%. v_{LAC}: 5%, [ATP]: 10%, [glucose-6-*P*]: 20%

Donor	Arsenate	Orthophosphate	Hematocrit	v_{GLU}	$\frac{v_{LAC}}{v_{GLU}}$	[ATP]	[Glucose-6-*P*]
	mmol/l suspension		%	mmol × l cells^{-1} × h^{-1}		µmol/l cells	
1	0	1.2	47	0.98	2.0	1020	–
	0.5	1.2	48	1.77	1.9	560	–
	5.0	1.2	46	0.77	2.4	140	–
2	0	1.2	50	0.90	1.7	1340	–
	0.5	1.2	47	1.36	2.0	1080	–
	2.5	1.2	47	1.22	2.7	310	–
	5.0	1.2	50	0.92	2.8	–	–
3	0	1.2	47	1.28	1.7	900	–
	1.0	1.2	47	1.98	1.8	410	–
	2.5	1.2	47	1.87	1.9	–	–
	7.5	1.2	47	0.46	2.2	140	–
4	0	1.2	50	0.63	2.4	390	–
	0	4.0	43	0.90	2.7	430	–
	2.0	2.0	53	1.70	1.9	200	–
	4.0	1.2	51	0.72	3.6	70	–
5	0	1.2	35	0.67	2.7	580	–
	0	15.0	32	1.50	1.5	780	–
	5.0	10.0	32	2.60	1.2	340	–
	7.5	7.5	33	1.70	1.7	140	–
	15.0	1.2	33	0.31	2.3	60	–
6	0	1.2	50	0.60	2.2	760	60
	0	13.0	42	1.13	2.0	1000	70
	1.0	12.0	43	1.96	2.2	610	15
	3.0	11.0	43	1.96	2.2	420	5
	3.0	10.0	43	1.88	2.2	360	–
	5.0	8.0	42	1.25	–	230	–
	7.0	7.0	42	1.25	1.7	150	–
	13.0	1.2	42	0.24	–	50	–
7	0	1.2	32	1.14	1.8	530	47
	0.4	1.2	32	1.74	1.5	460	33
	5.5	1.2	31	1.80	1.5	190	10
8	0	1.2	36	0.86	1.7	830	110
	0.17	1.2	36	1.11	2.0	750	65
	0.30	1.2	36	1.22	1.8	750	51
	0.60	1.2	36	1.36	2.2	580	35
	1.20	1.2	37	1.51	2.0	430	22
	4.0	1.2	35	1.39	2.5	240	11
9	0	1.2	42	0.87	–	1350	101
	0.36	1.2	43	1.12	–	1240	76
	1.10	1.2	42	1.30	–	1130	47
	3.75	1.2	43	1.46	–	810	17
	0	20.0	40	1.58	–	1660	94
	0.34	20.0	41	1.76	–	1580	76
	1.00	20.0	41	1.76	–	1500	–
	3.40	20.0	40	2.27	–	1390	34

ments. The increase in the concentration of the sum of arsenate and orthophosphate (donors 4, 5, 6, 9, Table 1) leads to a proportional change in the dependence of v_{GLU} on [ATP]. This can be seen if the values of v_{GLU} and [ATP] without arsenate are taken as 100%. Again, the results of the experiments can be represented as a single curve (Fig. 4).

The question arises to what extent the characteristic obtained in presence of arsenate corresponds to the characteristic of unmodified glycolysis. The main sources of possible artifacts are the side effects of arsenate. The pentavalent arsenic compounds can be reduced to trivalent state (arsenite) capable of inactivating the enzymes through the reaction with –SH groups [25, 26, 34, 35]. In the special experiments it was shown that during incubation the appearance of arsenite compounds is very slow and to the end of incubation does not exceed 4–6% of the added arsenate [33]. The existence of the steady state of glycolysis during the incubation suggests that the inactivation by arsenite is negligible. The possible effector action of the arsenate on the enzymes (similar to orthophosphate) may also be a source of artifacts. The part of the characteristic obtained by changing ATPase activity makes it possible to evaluate the magnitude of the errors due to the effector action of arsenate. The data, obtained with levorin, and also the results previously described [3, 4] are in good agreement with the characteristic obtained using arsenate (see Fig. 5). The changes of glucose-6-*P* concentration in

Table 2. *The effect of levorin and ouabain on glycolysis in human erythrocytes*
Accuracy: v_{GLU}: 5%; v_{LAC}: 5%; [ATP]: 10%; [glucose-6-*P*]: 20%

Donor	Levorin	Ouabain	v_{GLU}	v_{LAC}	[ATP]	[Glucose-6-*P*]
	μg/ml suspension		mmol × l cells^{-1} × h^{-1}		μmol/l cells	
1	0	0	1.06	–	1160	49
	0	100	0.84	–	1230	66
	0.3	0	1.57	–	990	19.5
2	0	0	0.87	2.18	1300	60
	0	100	0.77	1.90	1300	80
	0.3	0	1.30	3.22	1100	34
3	0	0	0.80	1.86	1100	40
	0	100	0.80	1.54	1150	56
	0.3	0	1.15	2.57	950	30
4	0	0	0.94	1.90	1400	70
	0	100	0.79	1.70	1400	80
	0.3	0	1.60	3.30	1200	33

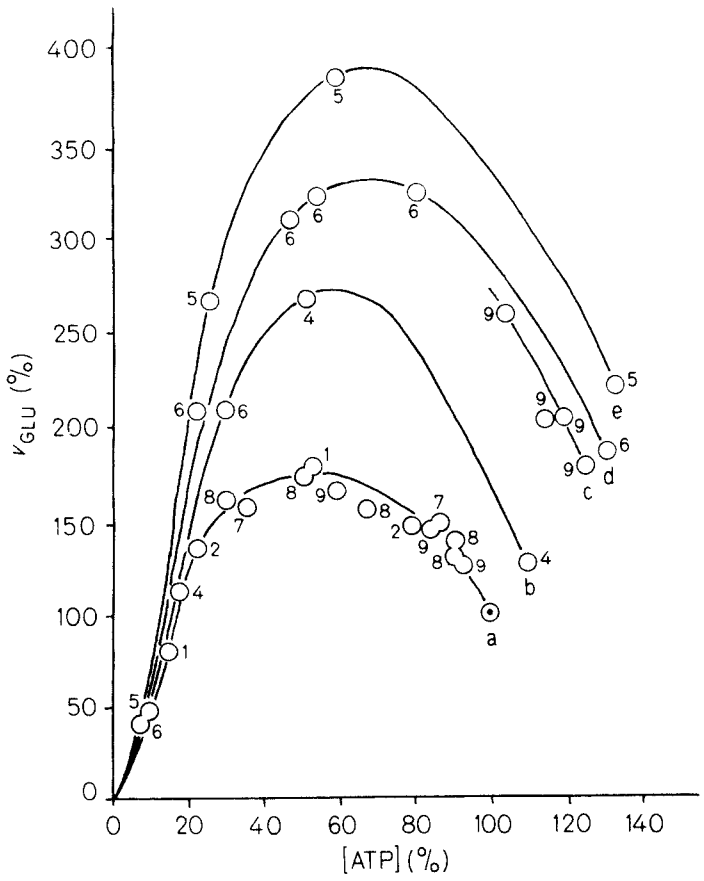

Fig. 3. *Dependence of the rate of glucose utilisation on the concentration of ATP.* The numbers in the circles correspond to the numbers of the experiments in Table 1. In each experiment the values of the glucose utilisation rate and the ATP concentrations obtained in the control were taken as 100%. (a) The orthophosphate concentration equals 1.2 mM; (b, d, e) the sum of orthophosphate and arsenate concentrations equal 4, 15 and 13 mM respectively; (c) the orthophosphate concentration equals 20 mM

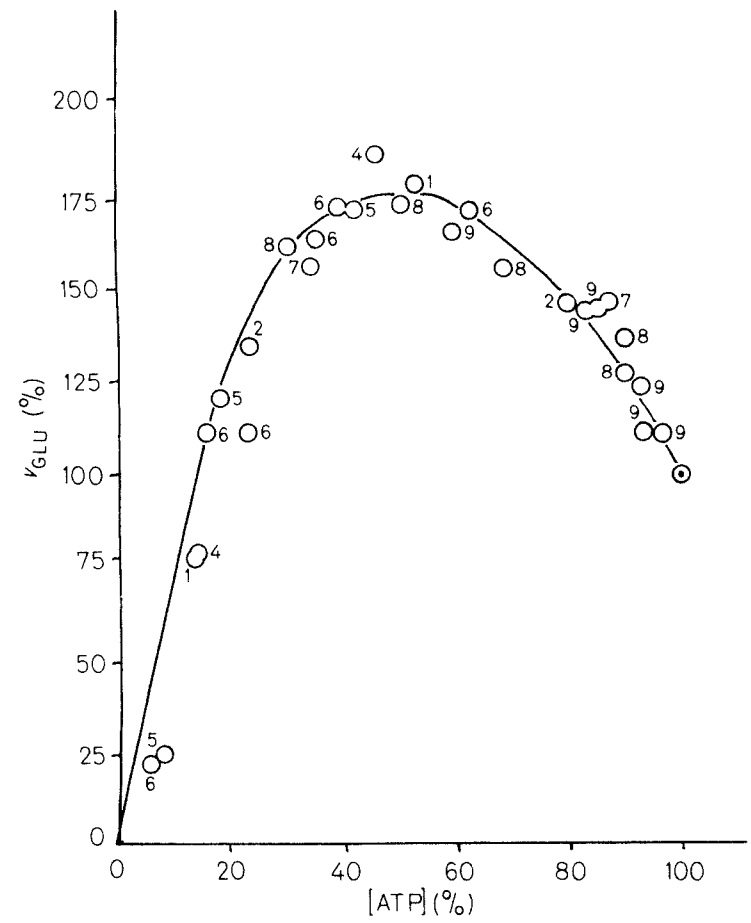

Fig. 4. *The normalized glucose characteristic of human erythrocytes.* The numbers in the circles correspond to the numbers of the experiments in Table 1. Solid line – theoretical curve. In each experiment the values of the glucose utilisation rate and the ATP concentrations obtained without arsenate were taken as 100%. It is seen that normalized characteristics coincide in all cases in spite of the great variability of absolute values

presence of levorin or arsenate are the same. Besides, the fact that the increase of orthophosphate concentration (when the sum of arsenate and orthophosphate was kept constant) modifies the curve proportionally, also indicates that the characteristic obtained with arsenate was only slightly different from the real one (Fig. 4).

DISCUSSION

The data obtained are in accord with the above-mentioned conceptions of the control of glycolysis in erythrocytes. The existence of the descending part of the glycolysis characteristic at high ATP concentration and the significant strong slope of the curve should provide a strong stabilization of the intracellular concentration of ATP over a wide range of the ATPase activity. The behaviour of the stabilising variable (the glucose-6-*P* concentration) is in good agreement with the considered mechanism of a control. The individual variations of the physiological values of the glycolysis rate and the concentrations of the metabolites can be explained by the differences in activity of certain enzymes and the pool of adenylates in erythrocytes of different donors. With certain changes of the coefficients it is possible to fit the calculations from the Eqns (1–5) to the experimental data, given in Table 1 for any individual donor. For example, for donor 8 a good agree-

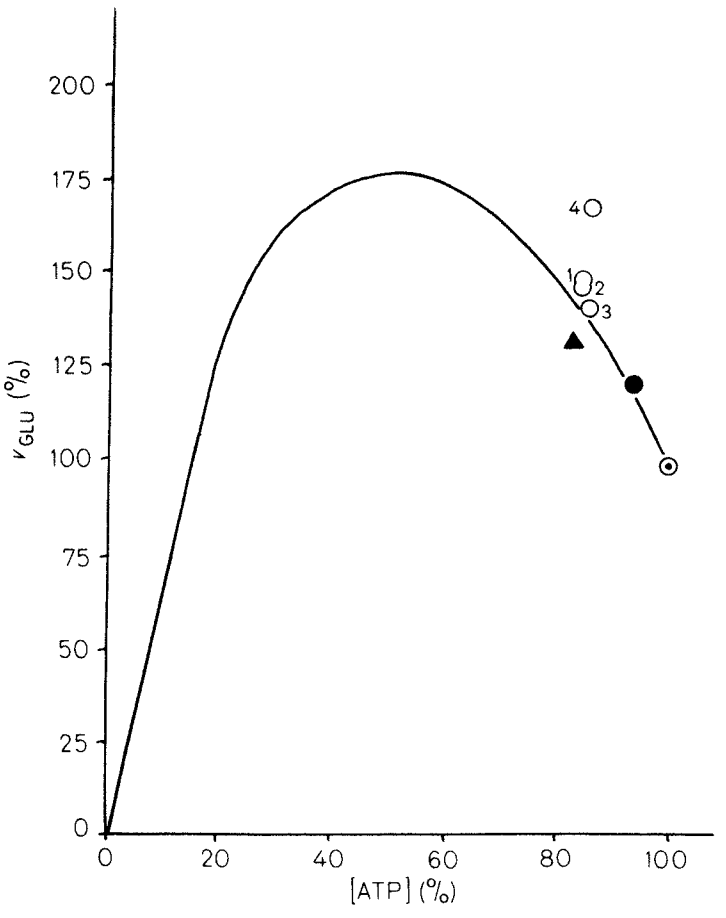

Fig. 5. *Comparison of effects of arsenate and of Na^+,K^+-ATPase activation.* The solid line is taken from Fig. 4; open circles, activation of Na^+,K^+-ATPase by levorin. The numbers in the circles correspond to the numbers of the experiments in Table 2. Closed circle and triangle: activation by amphotericin B (the values are taken from [3] and [4] respectively). In each case the values of rates and [ATP] in controls are taken as 100%

ment is obtained with the following values of the coefficients in the model:

$$K^{PFK}_{i,ATP} = \frac{0.31\,([\text{fructose-6-}P] + \beta)}{[\text{fructose-6-}P] + 0.114}\,[\text{mM}]\,.$$

$R_1 = 1$; $R_2 = 0$; $K^{HK}_{i,\text{glucose-6-}P} = 0.0115$ mM; $\alpha_{HK} = 12$ mM/h; $\beta = 0.94 \cdot 10^{-3}$ mM; $K^{AK}_{eq} = 0.5$; $\alpha = 0.965$ mM; $\alpha_{PFK} = 302.4$ mM/h. The other parameters are the same as before. The calculated curve constructed with the relative values is shown in Fig. 4 (solid line). Eqns (1–5), however do not predict the coincidence of normalized characteristics for different donors. Moreover, without changing the functional types of the formulae for the rates of hexokinase and phosphofructokinase reactions it is impossible to explain the invariability of a normalized characteristic by changes of the enzymes activities and adenylate pool only. Thus, the problem of the invariance of the normalized characteristic of glycolysis remaines unresolved.

The effect of ouabain on the energy metabolism of erythrocytes revealed the presence of two types of glycolysis control. In the erythrocytes from donors of the first type the ATP production drops when ATP utilization is decreased and this is due to decrease of glucose consumption. This type of glycolysis behaviour is well described by the model suggested. In the erythrocytes of donor of the second type ouabain does not diminish the glucose utilisation, but decreases the lactate-glucose ratio, which is indicative of the enhanced flow of the metabolites through hexose-monophosphate shunt.

Inhibition of Na^+,K^+-ATPase by ouabain causes a considerable increase of glucose-6-*P* concentration in erythrocytes. The constant value of v_{GLU} under the conditions where [glucose-6-*P*] increases contradicts the model. It may be proposed that the dependence of v_{GLU} on [glucose-6-*P*] in the erythrocytes of donor of the second type differs from the one accepted above. A great variation of the values of $K^{HK}_{i,\text{glucose-6-}P}$ (2.5–70 μm) reported by different authors for isolated hexokinase [16–19] indicate the possibility of the presence of different hexokinases in erythrocytes. The results of Rijksen et al. [36] show the possibility of the presence in erythrocytes of two isoenzymes of hexokinase with a different sensitivity to glucose-6-*P*. However, it is possible that the alteration of the dependence of v_{HK} on [glucose-6-*P*] in the erythrocytes with the second type of control is a result of ouabain side effect and not a consequence of the natural hexokinase properties.

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