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Glucose transport in human red cell membranes. Dependence of age, ATP, and insulin

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Glucose self-exchange flux (J_{ex}) and net efflux (J_{net}) in human red cells and ghosts were studied at 25 °C and pH 7.2 by means of the combined use of the Millipore-Swinnex filtering method and the continuous flow tube method to show the dependence of time of storage after aspiration, ATP and insulin. In fresh cells (RBC), ghosts (G), ghosts with 2 mM ATP (G +), and cells stored at 4 °C > 60 days (OC) both J_{ex} and J_{net} follow simple Michaelis-Menten kinetics where $J = J^{max} \cdot C_i \cdot (K_{1/2} + C_i)^{-1}$. J_{ex}^{max} and J_{net}^{max}(nmol · cm^{-2} · s^{-1}), respectively, was: (RBC) 0.27 and 0.19, (G) 0.24 and 0.27, (G +) 0.23 and 0.24, (OC) 0.23 and 0.20. $K_{1/2,ex}$ and $K_{1/2,net}$ (mM), respectively, was: (RBC) 7.5 and 1.3, (G) 4.8 and 14.2, (G +) 11.6 and 6.8, (OC) 3.8 and 9.0. In ghosts, the ATP-dependent fraction of the permeability shows a hyperbolic dependence on glucose concentrations lower than 80 mM. Insulin up to 1 μM had effect on neither J_{ex} nor J_{net} in RBC. Based on reported values of cytochalasin B binding sites the turnover rate per site in RBC appears to be as high as in maximally insulin-stimulated fat cells. Our results suggest that the number of transport sites remains constant, independent of age, ATP and insulin.

Introduction

In human red cells D-glucose is transported by facilitated diffusion, which means that the passive transport is governed by the presence of a transport system in the membrane. The kinetics of glucose transport have been studied extensively for decades in order to characterize further the transport system. Originally it was assumed that the transport was mediated by small entities in the membrane that 'ferry' the glucose molecules across the membrane after binding at one side of the membrane, and subsequently liberate the glucose molecule at the other side of the membrane. The kinetics of such 'carrier-mediated' glucose transport still apply, though the concept of the structure of the transport system has changed considerably by the introduction of the 'fluid mosaic membrane model' [1].

According to the model, integral membrane proteins that span the membrane lipid bilayer may create hydrophilic pathways which mediate transport of lipid-insoluble solutes. Recently it has been demonstrated that one class of the integral proteins that are named band 4.5 proteins after the location in SDS gel electrophoresis compiles the glucose transport system [2].

Among the many attempts to characterize the kinetics of glucose transport across the red cell membrane, it has been postulated that the transport system undergoes an ageing process which

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also changes the kinetics of the transport [3]. Further, it has been shown that glucose transport appears to be four times larger in the presence of ATP [4], which is surprising in the light of the generally accepted view that ATP is not required for passive transport processes. Finally, it appears generally accepted though not well-documented that insulin does not increase glucose transport [5].

The three questions raised above were further investigated by means of methods that have shown their reliability in studies of transport processes which proceed more rapidly than glucose transport.

Materials, Methods and Calculations

Fresh blood was obtained by venous aspiration into heparin and was processed immediately.

Outdated transfusion blood in CPDA (citrate, phosphate, dextrose, adenine, 63 ml/450 ml blood) was obtained from the blood bank and was kept in the refrigerator until use. The outdated blood was used over a period of 63 to 90 days after it was drawn from the donor.

The electrolyte media were made by use of grade chemicals and had the following compositions (mM): 150 KCl; 0.5 KH_2PO_4; 0–300 D-glucose for the red cell experiments, and 165 KCl; 0.5 KH_2PO_4; 0–80 D-glucose for ghost experiments. The media were all titrated to pH 7.2 with 0.1 mM KOH.

The isotope used was D-[U-^{14}C]glucose with a specific activity of 270 mCi/mmol (Amersham International, Amersham). The insulin used was porcine insulin (mol. wt. 6000 g/mol, NOVO). The albumin used was bovine albumin fraction V, and the ATP was the disodium salt (Sigma).

Preparation of red cell ghosts. Ghosts were made by a modified procedure of that described by Schwoch and Passow [6]. (Schnell, K., personal communication). The heparinised blood sample was washed once and resuspended in 165 mM KCl (50 vol%). The cells were hemolyzed at 0 °C by adding a 10-times larger volume of 3 mM $MgSO_4$ and 3.8 mM acetic acid. pH between 5.9–6.2 was obtained by titrating with 2 M Tris. 5 min later resealing at 0 °C for 10 min was initiated by adding a solution (volume: 1 : 10 of the hemolyzing solution) of 1.99 M KCl, 25 mM Tris. In experiments with ATP, the resealing solution contained 24 mM ATP in order to obtain a final intracellular concentration of ATP of 2 mM. During the resealing, pH was adjusted to 7.2 by adding 2 M Tris. Next, the cell suspension was kept on water bath at 38 °C for 45 min to complete the resealing procedure. After resealing, the red cell ghosts were loaded with D-glucose and the radioactive isotope as were the intact cells.

The red blood cells. The red blood cells were washed once in the appropriately titrated medium and titrated to pH 7.2 with either CO_2 or 1 M KOH, and then washed 3 or 4 times in the glucose medium concerned before labelling with the radioactive D-glucose. The cells (and ghosts) stood for at least 5 min at room temperature to ensure that isotopic equilibrium between the cell water and the extracellular phase was obtained. The cells (and ghosts) were isolated by centrifugation (49000 × g for 15 min) in 0.8 ml nylon tubes or 10 ml tubes as described previously [7]. Except for the ghost preparation, all procedures were carried out at room temperature. The efflux experiments were all carried out at 25 °C shortly after the preparation procedures in order to reduce the fraction of metabolized glucose.

In the insulin-albumin experiments, a slightly different procedure was followed as the red cells after two washes in 150 mM KCl, 0.5 mM KH_2PO_4 were divided into four equal portions. One portion was washed in the control medium, one in the control medium with 0.5 g albumin/100 ml, one in the control medium containing insulin (0.02 or 1 μM) and one portion in the control medium containing both albumin and insulin in the above mentioned concentrations. In a few experiments with Insulin the cells were incubated for 24 h at 4 °C (Hct 50%) before labelling with the radioactive D-glucose isotope, and subsequent centrifugation.

Mean cell volume, radioactivity and cell water. The mean cell volume of ghosts was determined by measuring the Hct and performing a cell count (Coulter Counter, model DN).

The radioactivity of the cell free filtrates, the equilibrium samples, the radioactivity of cells, and incubation medium after precipitation with per-

chloric acid, were determined by liquid scintillation spectrometry [8].

The cell water fraction was determined by drying cell samples for 24 h at 105 °C to constant weight, and calculating the water fraction as (1 − (dry weight/wet weight)). Assuming that 1 g of cell solids equals $3.1 \cdot 10^{10}$ normal erythrocytes and that each erythrocyte has a membrane area of $1.42 \cdot 10^{-6}$ cm^2, i.e. $3.1 \cdot 10^{10}$ cells have a total area of $4.4 \cdot 10^4$ cm^2, the ratio between cell water volume (V, cm^3) and cell membrane area (A, cm^2) can be calculated. V/A appeared to be independent of all glucose concentrations lower than 250 mM (V/A (cm) $= 4.51 \pm 0.20$, $n = 10$).

Both net efflux and self-exchange experiments were carried out by measuring the unidirectional efflux of radioactive D-glucose from cells whose total water volume was much smaller than the volume of the non-radioactive suspension medium. In this experimental situation the more than 100-times larger extracellular compartment ensures that the efflux of labelled glucose can be considered unidirectional, as the probability of labelled glucose molecules being transported back into the cells is negligible.

Two different methods for measuring the unidirectional efflux were used depending on the concentration of glucose and thus the efflux rate of labelled glucose. At low concentrations (< 20 mM glucose) with efflux rates > 0.6 s^{-1} the continuous flow tube method [7] is the superior method for measuring glucose transport. At glucose concentrations > 20 mM glucose, with efflux rates < 0.6 s^{-1}, the Millipore-Swinnex filtering technique [8] was used.

In experiments with outdated red cells the order in which the efflux of glucose at different concentrations was measured was accidentally chosen to exclude any connection between a deviation from a linearity in a plot against concentration and the time of storing of the outdated red blood cells before use.

Rate coefficient, permeability and flux. The rate coefficient of the transport process, k(s^{-1}), was determined by linear regression analysis of $\ln(1 - (a_t/a_\infty))$ vs. time, where a_t and a_∞ are the radioactivity in the sample at time t, and at equilibrium, respectively. k was used to calculate the permeability, P (cm · s^{-1}), and the unidirectional flux, J (mol · cm^{-2} · s^{-1}) from the relation, which defines the unidirectional flux

$$J = P \cdot C_i = k \cdot V \cdot A^{-1} \cdot C_i \quad (1)$$

where C_i is the intracellular concentration of glucose.

Results

Donor variation

Table I shows a series of self-exchange and net efflux experiments with red blood cells from different donors. The permeability in self-exchange experiments with blood from the same donor at most varies from $5.0 \cdot 10^{-6}$ to $6.0 \cdot 10^{-6}$ cm · s^{-1} as varies the permeabilities at self-exchange in red cells from different donors. The range of net efflux permeabilities in red cells from different donors shows a similar size. The table furthermore shows that the self-exchange permeability is larger than the net efflux permeability, the ratio of the mean permeabilities being 1.2.

Outdated vs. freshly drawn blood

Fig. 1 depicts glucose self-exchange flux, J_{ex}, and net efflux, J_{net}, as a function of the cellular glucose concentration at pH 7.2 and 25 °C, in freshly drawn blood (Fig. 1a) and in outdated bank blood stored at 4 °C for more 60 than days (Fig. 1b). Both under conditions of self-exchange and net efflux the concentration dependence of glucose transport could be described by simple Michaelis-Menten kinetics. Outdated bank blood shows a lower maximum flux than does the freshly drawn blood, and there is a grater spread of the results.

An analysis of the results depicted in a Hanes plot (Table II) shows that in fresh and in outdated cells, respectively, J_{ex}^{max} declined by 16% from 0.27 to 0.23 nmol · cm^{-2} · s^{-1}, while values of J_{net}^{max} are similar, 0.19 and 0.20 nmol · cm^{-2} · s^{-1}. The affinity of glucose to the glucose transporter is inversely related to the half-saturated constant, $K_{1/2}$ (mM). $K_{1/2}$ under self-exchange conditions decreased 50% from 7.5 to 3.8 mM in the outdated cells, while $K_{1/2}$ under net efflux conditions increased from 1.3 in fresh cells to 9.0 mM in outdated cells. Table III shows that the differences

TABLE I

GLUCOSE SELF-EXCHANGE AND NET EFFLUX IN RED BLOOD CELLS FROM DIFFERENT DONORS (pH 7.2 and 25 °C)

The glucose concentration in the wash and self-exchange medium was 40 mM. The ratio of the mean values of P_{ex} and P_{net} is 1.2. The subscripts numbering the donor refer to experiments done at different times. The rate coefficient in each experimental series represents the mean ± S.D. of three determinations. The asterisk denotes that the value is the mean of two determinations.

Donor	C_o (mM)	C_i (mM)	k (±S.D.) (s^{-1})	V/A (10^5 cm)	J (nmol·cm^{-2}·s^{-1})	P (10^6 cm·s^{-1})
Self-exchange						
JB_1	40.0	37.5	0.13(±0.003)	4.54	0.22	6.0
JB_2	40.0	37.4	0.12(±0.010)	4.11	0.19	5.0
JOW	40.0	34.3	0.13(±0.005)	4.46	0.20	5.7
SP_1	40.0	35.9	0.10(±0.006)	4.75	0.18	5.0
MR	40.0	39.2	0.13 *	4.41	0.23	5.9
$\bar{x}(n=14)$			0.12(±0.012)	4.45(±0.22)	0.20(±0.021)	5.48(±0.49)
Net efflux						
JB_3	0.0	36.8	0.10(±0.003)	4.31	0.16	4.5
JOW	0.0	34.3	0.11(±0.008)	4.46	0.17	5.0
SP_2	0.0	35.3	0.09(±0.008)	4.44	0.14	3.9
MR	0.0	39.2	0.10(±0.003)	4.41	0.18	4.6
$\bar{x}(n=12)$			0.10(±0.010)	4.49(±0.15)	0.16(±0.018)	4.50(±0.45)

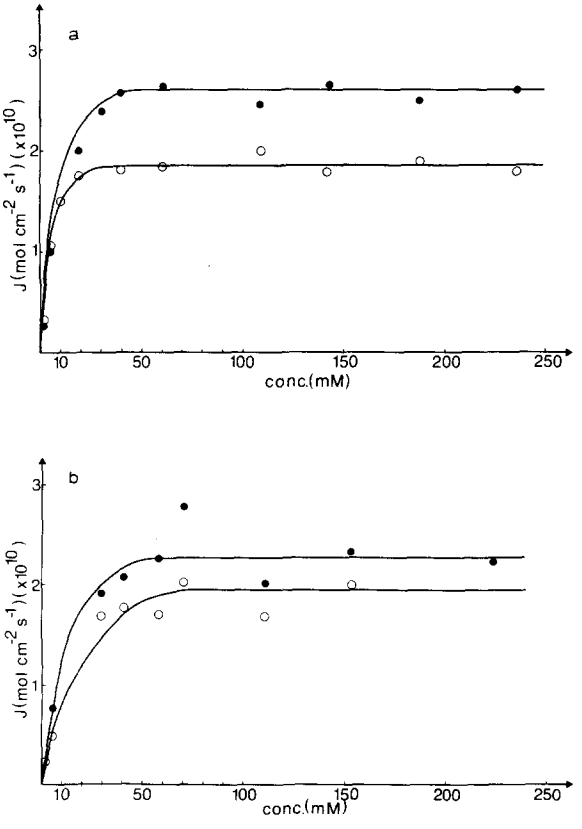

Fig. 1. Glucose self-exchange flux (●) and net efflux (○) in freshly drawn (Fig. 1 a) and outdated (Fig. 1 b) human red blood cells as a function of the intracellular glucose concentration, C_i. The four curves are drawn according to the Michaelis-Menten equation: $J = J^{max} \cdot C_i \cdot (K_{1/2} + C_i)^{-1}$, where J^{max} is the maximum flux, and $K_{1/2}$ is the glucose concentration at which the flux, J, is half the maximum flux. The blood used for the experiments shown in Fig. 1 b was stored more than 60 days. Each point is an average of two or more flux experiments. All experiments were done at 25 °C, pH 7.2.

in flux and permeability in the self-exchange experiments neither are due to changes in the ratio V/A (cm) nor to changes in C_i (mM), but appear to depend on a change of the membrane permeability. Table IV shows that both in self-exchange mode and net efflux mode the permeability coefficient decreased slightly with time in outdated blood cells. Experiments with red blood cells stored cold for more than 100 days failed because of hemolysis caused by the increased fragility of the cells.

Insulin

The experiments were carried out at 25 °C, pH 7.2, and at two glucose concentrations of 5 and 40

TABLE II

GLUCOSE TRANSPORT IN FRESHLY DRAWN AND OUTDATED BANK BLOOD CELLS EXPRESSED BY THE HALF SATURATION CONSTANT, $K_{1/2}$, AND THE MAXIMUM GLUCOSE FLUX, J^{max} (pH 7.2 and 25 °C)

A summary of four linear-regression analysis of the reciprocal permeability (P^{-1}, s·cm^{-1}) vs. the intracellular concentration of glucose (C_i) (Hanes plot), where $K_{1/2}$ is the intersection of the curve with the abscissa, and J^{max} is the reciprocal of the slope of the curve. The correlation coefficient (r) was ≥ 0.99 in all four analyses.

	$K_{1/2}$ (mM)	J^{max} (nmol·cm^{-2}·s^{-1})
Freshly drawn		
exchange	7.5	0.27
net efflux	1.3	0.19
Bank blood		
exchange	3.8	0.23
net efflux	9.0	0.20

TABLE IV

GLUCOSE SELF-EXCHANGE AND NET EFFLUX AT 60 mM GLUCOSE IN RED CELLS AT DIFFERENT TIMES AFTER ASPIRATION (pH 7.2 and 25 °C)

The age of the red blood cells in days refers to the number of days passed after venous aspiration into a CPDA medium. The cells were stored at 4 °C. The data represent mean values ± S.D. of three or four experiments.

Age (days)	C_i (mM)	k (±S.D.) (s^{-1})	V/A (10^5 cm)	J (nmol·cm^{-2}·s^{-1})	P (10^6 cm·s^{-1})
Self-exchange					
0	61.7	0.10(±0.004)	4.28	0.26	4.31
68	56.9	0.09(±0.004)	4.43	0.22	3.95
90	56.3	0.07(±0.010)	4.42	0.18	3.22
Net efflux					
0	61.7	0.07(±0.006)	4.28	0.18	2.98
68	56.9	0.07(±0.005)	4.43	0.17	2.95
90	56.3	0.06(±0.002)	4.42	0.15	2.64

mM. The effect of 0.02 and 1 μM insulin was determined under conditions of glucose self-exchange and net efflux as shown in Table V. The results show a slight increase of glucose transport by the addition of insulin and albumin, which was added to the solution to prevent insulin binding to test tubes and glass materials. However, the table also shows that albumin by itself increased glucose transport somewhat. Our results thus show no insulin-stimulated increase, neither under self-exchange conditions nor under net efflux conditions with 5 mM intracellular glucose under which experimental mode the transport sites facing by the external solution become unloaded.

ATP

We measured the glucose flux and permeability in ghosts prepared without and with 2 mM ATP intracellularly from freshly drawn human red

TABLE III

GLUCOSE SELF-EXCHANGE AND NET EFFLUX AT 1 AND 40 mM GLUCOSE IN FRESH AND OUTDATED RED BLOOD CELLS (pH 7.2 and 25 °C)

The data are average values of three experiments. The asterisk denotes average values of two experiments. The outdated cells were stored at 4 °C for more than 60 days.

	C_o (mM)	C_i (mM)	k (±S.D.) (s^{-1})	V/A (10^5 cm)	J (nmol·cm^{-2}·s^{-1})	P (10^6 cm·s^{-1})
Self exchange						
fresh	1	1.04	0.57(±0.049)	4.80	0.029	27.5
outdated	1	0.96	0.37 *	5.17	0.018	18.9
fresh	40	39.2	0.15(±0.023)	4.41	0.260	6.6
outdated	40	39.4	0.12(±0.003)	4.42	0.210	5.3
Net efflux						
fresh	0	1.04	0.69(±0.046)	4.80	0.034	32.9
outdated	0	0.96	0.45(±0.20)	5.17	0.020	23.4
fresh	0	39.2	0.10(±0.003)	4.41	0.180	4.6
outdated	0	39.4	0.10 *	4.42	0.180	4.5

TABLE V

THE EFFECT OF INSULIN ON GLUCOSE TRANSPORT IN HUMAN RED BLOOD CELLS (pH 7.2 and 25 °C)

Albumin and Insulin were added to the wash and the efflux media. The flux values are average values from three or more experiments.

Type of expt.	Glucose C_o (mM)	Glucose C_i (mM)	Albumin ($g \cdot l^{-1}$)	Insulin (μM)	J ($nmol \cdot cm^{-2} \cdot s^{-1}$)
Self exchange	40	38.2	0	0	0.20(±0.028)
	40	38.2	0	0.02	0.23(±0.006)
	40	38.2	5	0	0.25(±0.014)
	40	38.2	5	0.02	0.25(±0.009)
	5	4.4	0	0	0.16(±0.010) [a]
	5	4.4	0	0.02	0.18(±0.009) [a]
	5	4.4	5	0	0.18(±0.021) [a]
	5	4.4	5	0.02	0.19(±0.006) [a]
Net efflux	0	4.6	0	0	0.10(±0.003) [b]
	–	4.6	5	1	0.10(±0.001) [b]

[a] The cells were incubated for 24 h at 4 °C before the experiments.

[b] A different donor.

blood cells. The standard procedure of preparing ghosts implies that the intracellular content is diluted at least 12 times. Thus no specific procedure was used to reduce further the intracellular ATP concentration which is in the micromolar range in such ghosts. The results are presented in Fig. 2, which shows the permeability in resealed ghosts and resealed ghosts with 2 mM ATP as a function of the cellular glucose concentration (C_i). At low glucose concentrations the permeability of the ATP-enriched ghosts was more than 10-times larger than the permeability in ATP depleted ghosts. The difference in permeability declines to near zero as glucose concentration is raised towards 80 mM.

Table VI shows the effect of addition of 2 mM ATP intracellularly on $K_{1/2}$ and J^{max}, both under conditions of self-exchange and net efflux as determined in Hanes plots of the reciprocal permeability ($1/P$) vs. the cellular glucose concentration (C_i). The half saturation constant, $K_{1/2,ex}$, increased from 4.8 mM in control ghosts to 11.6 mM in ghosts with 2 mM ATP intracellularly while J_{ex}^{max} was almost constant, 0.24 and 0.23 $nmol \cdot cm^{-2} \cdot s^{-1}$.

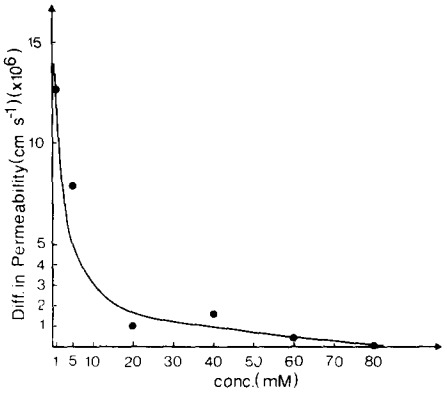

Fig. 2. The difference in glucose self-exchange permeability of ghosts with 2 mM ATP and control ghosts as a function of glucose concentratrion. Each point is the difference between the averaged value of three self-exchange experiments with 2 mM ATP-ghosts and three control experiments (25 °C, pH 7.2).

In the net-efflux experiments $K_{1/2,net}$ changed from 14.2 mM control ghosts to 6.8 mM in ghosts with 2 mM ATP. J_{net}^{max} was 0.27 and 0.24 $nmol \cdot cm^{-2} \cdot s^{-1}$, respectively in control ghosts and ATP-containing ghosts.

TABLE VI

EFFECT OF ATP ON GLUCOSE TRANSPORT IN RESEALED GHOSTS (pH 7.2 and 25 °C)

The values are obtained from an analysis of reciprocal permeability vs. intracellular glucose concentration (Hanes plot) in control ghosts and ghosts with 2 mM ATP intracellularly. ATP level in control ghosts was < 200 μM, because the preparation procedures imply a >12-times dilution of the intracellular contents, including approx. 2 mM ATP in intact, freshly drawn cells.

	$K_{1/2}$ (mM)	J^{max} ($nmol \cdot cm^{-2} \cdot s^{-1}$)	Correlation coefficient
Self-exchange			
control	4.8	0.24	0.988
+2 mM ATP	11.6	0.23	0.983
Net efflux			
control	14.2	0.27	0.975
+2 mM ATP	6.8	0.24	0.999

Discussion

Donor variation

In the present study we measured both self-exchange flux and net efflux in red blood cells from different donors to elucidate whether glucose transport shows a donor variation. The results of Table I show that the permeability both at self-exchange and net efflux varies from donor to donor. The table furthermore shows that a day-to-day variation of self exchange permeability in red cells from the same donor is as large as the interindividual variation.

The results underline that care must be taken when a comparison of results is undertaken. It should be noted that the spread in data cannot be ascribed to an artifact of the method, because the half time of self-exchange in these experiments were of the order of about 6 s, which is considerably slower than the minimum half time of 1–2 s that can be determined with the Millipore-Swinnex filtering method used here.

We see that net efflux permeability is lower than self-exchange permeability. This confirms previous studies (see Ref. 9). Though our data qualitatively agree with the concept that self-exchange flux is larger than net efflux, it must be noted that the ratio P_{ex}/P_{net} of the mean values is only 1.2 as compared to a ratio above 2 in another study using the same technique [10].

Glucose transport in outdated cells

Weiser, Razin and Stein [3] showed that the affinity of the glucose transporter to glucose at self-exchange (equilibrium exchange) was decreased in outdated red blood cells, as the half saturation constant, ($K_{1/2}$), increased from 12.7 mM in freshly drawn cells to 21.2 mM in outdated red cells. Weiser et al. [3] further showed that J_{ex}^{max} decreased from 0.26 nmol $\cdot$ cm^{-2} $\cdot$ s^{-1} in fresh red cells to 0.21 nmol $\cdot$ cm^{-2} $\cdot$ s^{-1} in outdated blood cells. Jacquez [4] who performed influx experiments, reported that influx was reduced in cold stored cells. The analysis of Weiser et al. is based upon determinations of the rate of glucose transport by means of a stopper solution method whose time resolution may be a problem, especially at low glucose concentrations, where the glucose transport proceeds rapidly [11]. Further, Weiser et al. [3] in their analysis used the rate coefficients, that only represent the permeability coefficients if the cell water volume remains constant (cf. Eqn.1).

In the present study we used two rapid filtration techniques [8,12], that have proven their applicability in determination of rapid transport processes. Our results of determination of $K_{1/2,ex}$ do not agree with the results of the study of Weiser et al. [3]. In our self-exchange experiments $K_{1/2,ex}$ was 7.5 mM in fresh cells while it decreased to 3.8 mM in outdated cells (Table II). In both studies J_{ex}^{max} both qualitatively and quantitatively showed a similar reduction in outdated red cells compared to fresh cells. Qualitatively the notion of Jacquez [4] on glucose influx agrees with the self-exchange and net efflux studies.

Our study shows that J_{ex}^{max} changes but very little with time, indicating that the number of transport sites is constant. Furthermore, the experiments show that the affinity of the glucose transport system to glucose varies with time. It is surprising that the change in affinity goes in different directions, depending on the type of experiments: $K_{1/2,ex}$ decreases and $K_{1/2,net}$ increases with time. One would a priori expect that the change of affinity for self-exchange and net efflux were in the same direction.

Since insufficient time resolution of the methods applied in the present study can be excluded, the observed variations of affinity with time may reflect a time-dependent change of the kinetics of the transport system. One possible explanation may be that the asymmetry of the transport system [13] undergoes a change with time that results in the observed, oppositely directed changes of $K_{1/2}$ for self-exchange and net efflux. Further studies which include influx measurements with an improved influx technique are necessary to elucidate the time-dependent changes of glucose transport kinetics in outdated red blood cells.

Insulin

Zipper and Mawe [10] demonstrated that insulin increased glucose maximum net efflux from red cells by almost 50%, whereas there was no significant effect on self-exchange flux. They interpreted the results in terms of a mobile carrier system with which insulin interacts when the sys-

tem is in the glucose-unloaded form facing the external solution. Three reasons made us do glucose self-exchange and net efflux experiments with insulin: (i) The mobile carrier hypothesis has been abandoned in advantage of the concept of solute transport mediated by confirmational changes in integral proteins. (ii) More recent studies of insulin action on glucose transport across cell membranes have revealed that albumin must be present to prevent insulin from binding to the glass and other materials used during the experiment. (iii) Within the last decade insulin preparations have been further purified so that a possible insulin effect per se now can be demonstrated.

Our results (Table V) demonstrate no effect of insulin not even at 1 μM which is an upper concentration used for stimulation of transport of glucose and glucose analogs in e.g. fat cells [14]. The lack of effect both at self-exchange and net efflux was independent of whether the cells were preincubated with or acutely exposed to insulin. Thus we conclude that human red cells in contrast to other cells, such as fat and muscle cells, transport glucose at a rate independent of insulin.

Cytochalasin B is a well-established inhibitor of glucose transport. If one assumes that the number of cytochalasin B binding sites per red cell membrane varies $(0.4–3.5) \cdot 10^5$ [2], a 'one way' turnover number for glucose is $(1.2–13) \cdot 10^3$ molecules/s per site at body temperature [11], where similar calculations for insulin-stimulated fat cells based on the data of Vinten et al. [15] reveal a 'one way' turnover in these cells of $(1–6) \cdot 10^3$ glucose molecules/s per site.

Thus it appears that glucose is transported at a maximum rate across the human erythrocyte membranes that ensures a maximum glucose transport capacity in the blood independent of physiological and pathophysiological variations of the insulin concentration in plasma.

ATP

The fact that glucose transport is a passive facilitated diffusion process implies that the transmembrane movement of glucose is not energy requiring, and thus not linked to the metabolism of the cell and the consumption of ATP. However, Jacquez [4] showed that the capacity of the transport system in human red cell ghosts varied with the intracellular concentration of ATP by comparing glucose influx at 1 mM extracellular glucose in ATP-depleted red cells and freshly drawn red cells. He found a 20–30% decrease of influx in depleted cells, and that the effect was reversible in resealed ghosts containing ATP. Jacquez concluded that the effect of ATP on glucose transport was caused by a change only of J^{max}, while $K_{1/2}$ remained constant. However, Carruthers [16,17] recently demonstrated that the transport asymmetry, both for $K_{1/2}$ and J^{max} of glucose efflux and influx in intact red cells disappeared in resealed ghosts, and was restored by addition of ATP to the interior of ghosts or the exterior of inside-out vesicles. It is not clear, what causes the discrepancy of the effect of ATP on $K_{1/2}$ and J^{max} for influx in Jacquez' study [4] of initial uptake at 1 mM glucose by means of a stopper solution method and Carruthers' studied [16,17] at 60 mM glucose by means of a turbidimetry method. A temperature effect may be a possible cause as the study of Jacquez was done at 5 °C, and those of Carruthers at room temperature.

Our results of glucose net efflux in resealed ghosts in the concentration range 0–80 mM glucose neither agree with the results of efflux experiments of Carruthers [16,17]. He found that $K_{1/2}$ for net efflux in 'freshly outdated' red blood cells was 28 mM, decreased to 10 mM in resealed ghosts, and regained the value of 28 mM in resealed ghosts containing ATP. In contrast, we show here that $K_{1/2}$ increased from 1.3 mM in freshly drawn red cells, or 9 mM in outdated blood cells, to 14 mM in control ghosts having less than 200 μM ATP, and decreased to about 7 mM in resealed ghosts with 2 mM ATP intracellularly (cf. Tables II and VI). Our results show that both in intact red cells and ghosts, $K_{1/2}$ for net efflux increased as the ATP level was reduced. It is not clear to us what causes the oppositely going results in Carruthers' and our study. One possible cause may be that we studied glucose transport in the concentration range 1–80 mM, while Carruthers study was only done at 60 mM.

Our results of $K_{1/2}$ differing from Carruthers' [16,17] cannot simply be ascribed to a methodological error in our study. First, the combination of the Millipore-Swinnex filtering method and the continuous flow tube technique applied here has

shown the ability and reliability to determine transport processes which proceed much faster [7,11,12]. Secondly, our determinations of $K_{1/2}$ under self-exchange do not show a similar variation as observed for net efflux experiments. Under self-exchange conditions, $K_{1/2}$ was 7.5 mM in freshly drawn cells, decreased to 4.8 mM in control ghosts, and increased to 11.6 mM in ghosts with 2 mM ATP. It should be noted that it is a consonant finding that a similar low $K_{1/2}$ (3.8 mM) was also determined for glucose self-exchange in outdated cells, which are assumed to have a low ATP level, as was determined in control ghosts whose ATP level has been diluted by at least 12 times during the preparation procedure.

The present results of $K_{1/2}$ in intact red cells, and resealed ghosts qualitatively correspond fully, and quantitatively agree fairly well. We see that from Tables II and VI by comparing freshly drawn red cells and ghosts containing 2 mM ATP, and by comparing outdated red blood cells and control ghosts having a low ATP concentration. Under self-exchange conditions $K_{1/2}$ is lower (the affinity of the transport system is higher) in cells with low ATP levels. Under net efflux conditions $K_{1/2}$ is higher (the affinity is lower) in cells with low ATP levels. The cause of the oppositely directed effect of ATP under the two different experimental conditions is not known. If the different effects of ATP on the affinity of the transport system are related to the asymmetry of the system, the effects may be absent at body temperature where net efflux and self-exchange have similar values of $K_{1/2}$ and J^{max} [11].

Our results of Table VI further suggest that the number of glucose transporting sites in the red cell membrane is not altered by ATP, as indicated by the constant J^{max}. The present results are consistent with the concept that no facilitated diffusion processes require energy to proceed as might be the interpretation of an ATP-dependent J^{max}.

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