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ANAEROBIC STIMULATION OF SUGAR TRANSPORT IN AVIAN ERYTHROCYTES

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Summary

The kinetic parameters of the sugar transport in avian erythrocytes were evaluated under aerobic and anaerobic conditions. In anaerobic cells, transport measurements with 3-*O*-[^{14}C]methylglucose resulted in a set of similar dissociation-like constants. Thus the Michaelis constants of 3-*O*-[^{14}C]methylglucose entry and exit, K_{so} and K_{si}, were 8 and 7 mM, respectively. The equilibrium exchange constant, B_s, and the counterflow constant, R_s, were 9 and 11 mM, respectively. The activity constant for 3-*O*-methylglucose transport, F_s, defined as V/K_m, was 4 ml/h per g. This set of kinetic constants was compatible with a symmetrical mobile-carrier model. In contrast, the Michaelis constant for glucose entry, K_{go}, was 2 mM and less than the counterflow constant, R_g (8 mM). This result could be accounted for by slower movement of the glucose-carrier complex than the free carrier. The activity constant for glucose transport, F_g, was 5 ml/h per g.

Under aerobic conditions, two of the dissociation-like constants (K_{si} and B_s) for 3-*O*-methylglucose transport were significantly larger than those obtained in anaerobic cells, but the remaining two (K_{so} and R_s) remained unchanged. The values for K_{so}, K_{si}, B_s and R_s were 8, 26, 20 and 8 mM, respectively. The activity constant, F_s, decreased to 2 ml/h per g. These changes in kinetic constants were consistent with the hypothesis that anoxia accelerated sugar transport by releasing free carrier that was previously sequestered on the inside of the cell membrane.

Introduction

Sugar transport in avian erythrocytes was shown to be regulated by hormones and metabolic factors [1—3]. Although the acceleration of sugar trans-

port by anoxia or catecholamines correlated with a fall of intracellular high-energy phosphates, the exact mechanism of these effects remains unknown. In addition, it is not clear whether the sugar transport system in avian erythrocytes could be adequately described by the classical carrier theory [4,5]. Controversy still exists on the applicability of the carrier model to the sugar transport system in human erythrocytes [6].

In the present study, the kinetic behavior of the sugar transport system in avian erythrocytes was characterized under both aerobic and anoxic conditions. The adequacy of the carrier model, without simplifying assumptions [5], was tested using established rejection criteria [7,8]. Anoxia decreased some dissociation-like constants, but increased the activity constant. A regulatory mechanism compatible with these changes is proposed.

Methods

Blood was collected from the neck veins of geese into polycarbonate bottles containing heparin (2 mg/100 ml blood) and washed three times by centrifugation and resuspension in ice-cold Krebs-imidazole glycylglycine buffer, pH 7.4 [2]. Leucocytes were thoroughly removed during the washing procedure. Erythrocytes were stored overnight at 4°C and washed once immediately before use.

Cells were suspended in Krebs-imidazole-glycylglycine buffer to which was added 5 mM glucose, 5 mM fumarate, 10 mM KH_2PO_4, 2.7 mM adenine and 5 mM inosine, with or without 3-*O*-methylglucose, and incubated at 37°C under aerobic conditions for 3 h as described previously [2]. Cells were then washed three times with ice-cold substrate-free buffer, and resuspended in substrate-free buffer with or without 3-*O*-methylglucose. The cell suspensions were incubated at 37°C for a further 90 min, under aerobic or anaerobic conditions, before measurements of sugar entry or exit were made. Gravimetric determinations of total water content and hematocrits of the cell suspensions were performed routinely.

The appropriate experimental procedures for determinations of individual transport constants are given in the subscripts of the tables and figures.

Results

Kinetics of glucose transport

Earlier studies [1] on effects of energy-deprivation on the kinetics of glucose transport in avian erythrocytes were carried out by following glucose disappearance from the incubation medium. A potentially more sensitive technique to determine rates of glucose uptake was to estimate ^3H_2O appearance in the medium during incubation with 2-[^{3}H]glucose [9]. The validity of this method depended on four assumptions: (i) the Embden-Meyerhof pathway accounted for virtually all glucose metabolism, (ii) all the ^{3}H in the C-2 position was released as ^3H_2O by either phosphoglucoisomerase, triosephosphate isomerase or enolase, (iii) ^3H_2O equilibrated rapidly between extracellular and intracellular compartments, and (iv) transport of 2-[^{3}H]glucose was rate-limiting for production of ^3H_2O. As seen in Table I, rates of ^3H_2O production did not match

TABLE I

COMPARISON OF GLUCOSE UPTAKE AND ^3H_2O PRODUCTION BY AVIAN ERYTHROCYTES USING 2-[^{3}H]GLUCOSE

Red cell suspensions (50% hematocrit) were incubated as described in Methods in buffer containing either 0, 10, 30, 60 or 100 mM 3-*O*-methylglucose to achieve a range of intracellular 3-*O*-methylglucose concentrations. After washing, the cells were incubated for an additional 90 min with 0, 4, 11, 23 or 38 mM 3-*O*-methylglucose under aerobic or anaerobic conditions. Following this period of incubation, 2-[^{3}H]-glucose (0.01 mM) and 3-*O*-methylglucose (0, 4, 10 or 20 mM) were added as indicated in the Table, and samples were taken at 0, 5, 15, 30 and 45 min. Streptomycin (1 mg/ml) and penicillin G (1000 units/ml) were present in both incubation periods. Samples were transferred rapidly to test tubes sitting in an ice-bath. After centrifugation, supernatants were collected and analyzed for ^3H_2O by passage of 0.5 ml of supernatant through a 0.8 × 1.5 cm column of Dowex-1-borate followed by elution with 1.5 ml of water [10]. Radioactivity in the total eluant was determined in a liquid scintillation spectrometer. Supernatant glucose was assayed fluorimetrically using the hexokinase and glucose-6-phosphate dehydrogenase reactions [11]. Rates of glucose uptake were obtained from slopes of the plot of log extracellular glucose concentration versus time. Rates of ^3H_2O production were obtained from slopes of the plot of ^3H_2O accumulation versus time. Both rates are normalized to a standard extracellular glucose concentration (0.01 mM). The number in parenthesis indicates the fraction of glucose uptake appearing as ^3H_2O. The experiment was performed twice with similar results.

Conditions	Initial 3-*O*-methylglucose concentration (mM)		Glucose uptake μmol/h per g	^3H_2O production μmol/h per g
	Extracellular	Intracellular		
Aerobic	0	0	0.0142	0.0058 (41%)
	4	0	0.0114	0.0027 (24%)
	10	0	0.0087	0.0015 (17%)
	20	0	0.0039	0.0009 (24%)
	4	4	0.0162	0.0065 (40%)
	11	11	0.0165	0.0067 (40%)
	23	23	0.0156	0.0030 (19%)
	38	38	0.0087	0.0025 (29%)
Anoxic	0	0	0.0446	0.0106 (24%)
	4	0	0.0330	0.0054 (16%)
	10	0	0.0208	0.0025 (12%)
	20	0	0.0155	0.0015 (10%)
	4	4	0.0265	0.0081 (30%)
	11	11	0.0178	0.0050 (28%)
	23	23	0.0170	0.0027 (16%)
	38	38	0.0089	0.0016 (18%)

the corresponding rates of glucose disappearance, indicating that assumptions (i) and/or (ii) were not justified in the avian erythrocyte. Further, the fraction of glucose uptake appearing as ^3H_2O was affected by incubation under anaerobic conditions or by addition of 3-*O*-methylglucose. Thus, measurement of ^3H_2O production from 2-[^{3}H]glucose could not be taken as an index of glucose uptake in avian erythrocytes.

In order to equate kinetics of glucose utilization with those of glucose entry, intracellular phosphorylation of the sugar must be sufficiently rapid to maintain low levels of intracellular glucose. Under these conditions, glucose utilization was limited principally by glucose penetration across the membrane. Previous measurements [1] of glucose and sorbitol spaces indicated that glucose was restricted to the extracellular compartment since the sugar was distributed

TABLE II

MEASUREMENT OF INTRACELLULAR GLUCOSE

Cell suspensions (50% hematocrit) were incubated aerobically or anaerobically for 90 min in Krebs-imidazole glycylglycine buffer, pH 7.4 [2]. 20 min following addition of varying concentrations of 2-[^{3}H]-glucose, [G_o], samples (0.5 ml) were taken for estimation of intracellular glucose. Samples were rapidly ejected into 6 ml of buffer layered on top of 7 ml of silicone oil (General Electric, SF1154) in a conical tube sitting in an ice-bath. Samples were spun, the aqueous layer was aspirated and the sides of the test-tube repeatedly rinsed by addition and aspiration of isotonic saline. The same procedure was applied to the silicone oil layer. The remaining cell pellet was hemolyzed with 1 ml 10% perchloric acid. The cell extract was collected and neutralized (pH 7) with KOH. An aliquot of the cell extract was added to 0.3 ml of Tris buffer (100 mM, pH 8) containing 13 mM $MgCl_2$ and 0.6 mM ATP. Hexokinase and phosphoglucoisomerase were added and the mixture was allowed to react at room temperature for 1 h with occasional mixing. The reaction mixture was then analyzed for ^3H_2O as described in Table I. Incubation under these conditions resulted in conversion of 85% of the radioactivity in 2-[^{3}H]glucose standards (Amersham) to ^3H_2O. The remaining radioactivity could not be converted to ^3H_2O by prolonging the incubation time and was presumed to represent impurities in the 2-[^{3}H]glucose. An equal aliquot was treated in a similar manner except hexokinase was omitted. Intracellular glucose, [G_i], was calculated as follows: [G_i], mM = [^3H_2O (hexokinase + phosphoglucoisomerase), dpm − ^3H_2O (phosphoglucoisomerase), dpm]/[Specific activity of extracellular glucose, dpm/μmole · 0.85 · volume of intracellular water, ml]. In parallel experiments with [^{14}C]sorbitol as extracellular marker, it was found that no more than 0.3% of the extracellular fluid was trapped in the cell pellet. Data was presented as mean ± S.E.M. from 3 to 5 separate experiments.

Conditions	Extracellular glucose (mM)	Intracellular glucose (mM)	$[G_i]/[G_o]$
Aerobic	0.0091 ± 0.0003	0.0008 ± 0.0004	0.09
	0.099 ± 0.013	0.0022 ± 0.0009	0.02
	0.21 ± 0.008	0.0037 ± 0.0019	0.02
	1.08 ± 0.12	0.011 ± 0.0049	0.01
	3.08 ± 0.42	0.03 ± 0.01	0.01
	10.67 ± 1.04	0.14 ± 0.07	0.01
	29.40	0.26	0.01
	45.49 ± 0.25	0.22 ± 0.07	0.005
	66.26	0.31	0.005
Anoxic	0.0087 ± 0.0009	0.0005 ± 0.0002	0.05
	0.086 ± 0.016	0.0034 ± 0.0010	0.04
	0.20 ± 0.015	0.0046 ± 0.0023	0.02
	1.20 ± 0.19	0.027 ± 0.0054	0.02
	3.17 ± 0.41	0.056 ± 0.02	0.02
	12.76 ± 1.84	0.11 ± 0.04	0.01
	33.67 ± 3.76	0.26 ± 0.10	0.01
	55.96 ± 3.55	0.27 ± 0.02	0.005

in a volume slightly less than that of sorbitol, an extracellular marker. An alternative method to measure intracellular glucose is described in Table II. This technique is more sensitive since (i) the cold buffer on top of the silicone oil cooled the cells in a sufficiently short time to stop cell metabolism and transport, and diluted the medium glucose concentration approximately 25-fold to reduce further glucose entry and (ii) in vitro conversion of 2-[^{3}H]-glucose to ^3H_2O was a more precise measure of extremely low quantities of glucose than fluorimetry or spectrophotometry. As seen in Table II, intracellular glucose concentrations were not significantly different from zero at low external glucose levels (0.01—0.1 mM), under both aerobic and anaerobic conditions. At high external glucose concentrations, intracellular glucose levels were

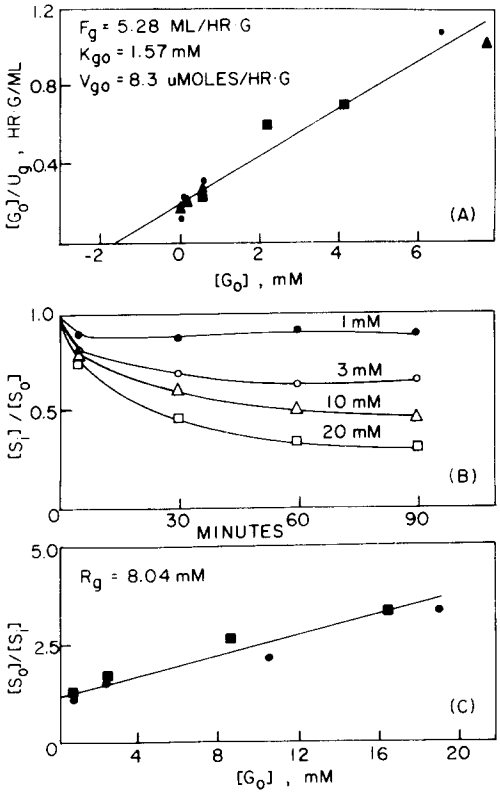

Fig. 1. Measurement of K_{go}, the Michaelis constant of glucose entry, and R_g, the counterflow constant in anaerobic cells. For measurement of K_{go}, cells were suspended at 50% hematocrit and incubated as described in Methods. Antibiotics were added as described in Table I. After the anaerobic incubation, 0.1, 0.3, 1 or 10 mM glucose were added to the cell suspensions. Samples were taken at 5, 15, 30, 45, 60 and 90 min. Glucose in the supernatants was assayed enzymatically [11]. The rate law governing glucose uptake was

$$U_g = (F_g([G_o] - [G_i]))\Big/\left(1 + \frac{[G_o]}{K_{go}} + \frac{[G_i]}{K_{gi}} + \frac{[G_o]}{R_g}\frac{[G_i]}{B_g}\right),$$

where U_g was glucose uptake rate; $[G_o]$, $[G_i]$, extra- and intracellular glucose concentrations; K_{go}, K_{gi}, Michaelis constants of glucose entry and exit; R_g, B_g, the counterflow and equilibrium exchange constants for glucose; and F_g, the activity constant for glucose transport [7]. As shown in Table II, $[G_i]$ was negligible and the equation could be simplified to $[G_o]/U_g = (1/F_g) + ([G_o]/F_gK_{go})$. The least square line is fitted to data from 3 experiments, indicated by the solid circles, squares and triangles in panel A. For measurement of R_g, red cell suspensions (10% hematocrit) were incubated as above, except that 3-*O*-[^{14}C]methylglucose (8 μM) was present in both the preliminary and subsequent anaerobic incubation periods. At the end of the anaerobic incubation, the intracellular concentration of 3-*O*-[^{14}C]methylglucose was equal to that in the incubation medium. Immediately after the second incubation, 1, 3, 10, or 20 mM glucose was added to the medium. Samples were taken at 5, 30, 60 and 90 min and analyzed for supernatant glucose and intracellular 3-*O*-[^{14}C]methylglucose as described previously [2]. These measurements are shown in panel B. In the presence of glucose, the equation governing the final equilibrium distribution of 3-*O*-methylglucose as a function of extracellular and intracellular glucose concentrations was $[S_o]/[S_i] = (1 + [G_o]/R_g)/(1 + [G_i]/R_g)$, where $[S_o]$, $[S_i]$ were the extracellular and intracellular 3-*O*-methylglucose concentrations, and $[G_o]$, $[G_i]$, and R_g were as defined above [5]. Since $[G_i]$ was negligible over the full range of $[G_o]$ employed (Table II), the equation could be simplified to: $[S_o]/[S_i] = 1 + [G_o]/R_g$. The least square line in panel C is fitted to data from two experiments.

only 1—2% of those in the external medium. Thus, over the range of glucose concentrations (0.1—10 mM) used to estimate glucose transport constants (Fig. 1), rates of glucose utilization approximated rates of glucose entry.

In anaerobic cells, glucose entry followed Michaelis-Menten kinetics (Fig. 1a). The V of glucose entry, V_{go}, was 8 μmol/h · g and the Michaelis constant of glucose entry, K_{go}, was 1.57 mM. The latter figure agreed reasonably well with that of Wood and Morgan [1] who reported a value of 0.8 mM. The maximal rate of glucose entry was greater than previously reported (3.36 μmol/h per g) [1]. This difference could be accounted for by a greater extent of energy-deprivation in the present experiments, due to longer periods of incubation. Glucose transport constants in aerobic cells could not be evaluated with precision in the present periods of incubation since the rate of glucose uptake was very slow. Earlier work [1], however, indicated that energy-deprivation resulted primarily in a 3-fold-increase of V_{go} with little or no effect on K_{go}.

To test the hypothesis that movements of glucose-carrier complex and free-carrier are rate-limiting and have identical rate-constants [4], R_g, the counter-flow constant for glucose, was measured in anerobic cells by following counter-flow of 3-*O*-methylglucose by glucose. As seen in Fig. 1b, 3-*O*-methylglucose left the cells against its concentration gradient in response to the addition of glucose and reached a new equilibrium distribution in less than 90 min. Fig. 1c reveals that R_g was 8 mM, significantly different from K_{go} as measured in the same batch of cells. The value of R_g was consistent with that reported previously (5 mM) [2]. These earlier experiments also indicated that R_g was approximately 5 mM in aerobic cells [2].

Kinetics of 3-O-methylglucose transport

A more detailed analysis of the effects of energy-deprivation on sugar transport could be achieved by studying the movements of a non-metabolized sugar such as 3-*O*-methylglucose. For determination of K_{so}, the Michaelis constant of 3-*O*-methylglucose entry, and B_s, the equilibrium exchange constant for 3-*O*-methylglucose, three types of experiments were performed: (1) inhibition of glucose entry by 3-*O*-methylglucose, (2) entry of 3-*O*-[^{14}C]methylglucose into either sugar-free cells (K_{so}) or cells containing unlabeled 3-*O*-methylglucose at concentrations equal to those in the external medium (B_s), or (3) exit of 3-*O*-[^{14}C]methylglucose into medium containing unlabeled 3-*O*-methylglucose at the same concentration as present within the cell (B_s).

Measurement of B_s, the equilibrium exchange constant, required that the extracellular and intracellular 3-*O*-methylglucose concentrations be equal. This condition was achieved by pre-loading the cells with 3-*O*-methylglucose, washing the cells free of external sugar, and resuspending them in medium containing concentrations of 3-*O*-methylglucose equal to those in the cells. It was found that after 3 h of incubation in 10, 30, 60 and 100 mM of extracellular 3-*O*-methylglucose, the intracellular sugar concentrations were 4.10 ± 0.23, 10.73 ± 0.47, 22.65 ± 1.12, and 37.88 ± 2.96 mM, respectively (3 observations). Thus, the 3-*O*-methylglucose concentrations in the resuspending media were routinely chosen to be 4, 11, 23 and 38 mM. However, prolonged incubation in substrate-free buffer decreased the intracellular ATP levels from an initial value of 5.67 ± 0.26 mM to 2.41 ± 0.09 mM after 3 h. Acceleration of

transport was found to accompany the fall in ATP levels [2]. In this case, the half-time of entry of 3-*O*-[^{14}C] methylglucose (12 μM) decreased from 118 min, with no incubation, to 42 min after 3 h. In order to prevent acceleration of sugar transport during the initial 3-*O*-methylglucose loading period, substrates listed in Methods were added to the Krebs-imidazole glycylglycine buffer. Incubating the cells in buffer containing these substrates for 3 h increased intracellular ATP levels somewhat (5.67 ± 0.26 mM to 7.61 ± 0.43 mM). The half-time of tracer 3-*O*-[^{14}C] methylglucose entry was 122 min, not significantly different from the control value. The concentrations of 3-*O*-methylglucose attained inside the cells after 3 h were not affected by the addition of substrates.

In the competition experiments, rates of glucose uptake at low glucose concentrations (0.01 mM) were monitored since at this concentration no intra-

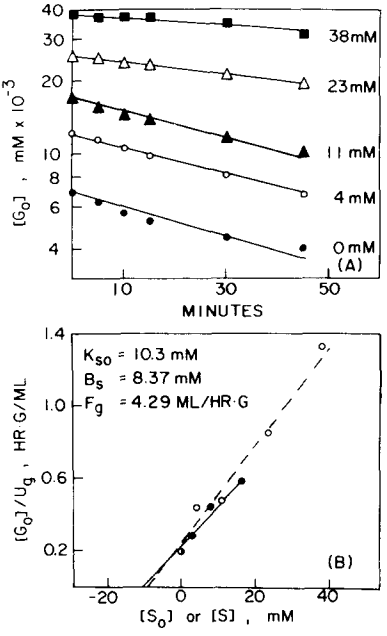

Fig. 2. Determination of K_{SO}, the Michaelis constant for 3-*O*-methylglucose entry, and B_S, the equilibrium exchange constant, by competitive inhibition of glucose uptake in anaerobic cells. A single red cell suspension was incubated under conditions described in Table I. Rates of glucose uptake by cells equilibrated with a range of 3-*O*-methylglucose concentrations were obtained from rates of glucose disappearance from the media (panel A). The external glucose concentrations increased in direct proportion to the 3-*O*-methylglucose concentration since the 3-*O*-methylglucose (Calbiochem, A grade) contained trace amounts (0.1%) of glucose. When 3-*O*-methylglucose was present at equal concentrations inside and outside the cell, the rate law governing glucose uptake was: $U_g = F_g[G_O]/(1 + [G_O]/K_{gO} + [S]/B_S)$, where U_g, $[G_O]$ and K_{gO} were defined as in Fig. 1; [S] was the 3-*O*-methylglucose concentration at equilibrium across the membrane; and B_S, the equilibrium exchange constant of 3-*O*-methylglucose [5]. Under the experimental conditions, $[G_O]/K_{gO} << 1$ (Fig. 1a), the rate law could be simplified to $[G_O]/U_g = 1/F_g + [S]/F_gB_S$. The plot $[G_O]/U_g$ vs. [S] is drawn as open circles and dotted line in panel B. When only external 3-*O*-methyglucose was present, the initial rate for glucose entry was $U_g = F_g[G_O]/(1 + [G_O]/K_{gO} + [S_O]/K_{SO})$, where K_{SO} was the Michaelis constant for 3-*O*-methylglucose entry; the other parameters have been defined in Fig. 1 [5]. As discussed above, the equation could be simplified to $[G_O]/U_g = (1/F_g) + ([S_O]/F_gK_{SO})$. The plot $[G_O]/U_g$ versus $[S_O]$ is drawn in panel B as solid circles and solid line. These experiments were performed twice with similar results.

cellular glucose was detected (Table II) and an insignificant fraction of free carrier was occupied ($[G_o] << K_{go}$, Fig. 1a). As seen in Fig. 2a, rates of glucose uptake by anaerobic cells decreased progressively as 3-*O*-methylglucose concentration at equilibrium across the membrane was increased, indicating competition between two sugars for exchange. The value of B_s was 8 mM (Fig. 2b). Glucose uptake also was inhibited in cells exposed to only external 3-*O*-

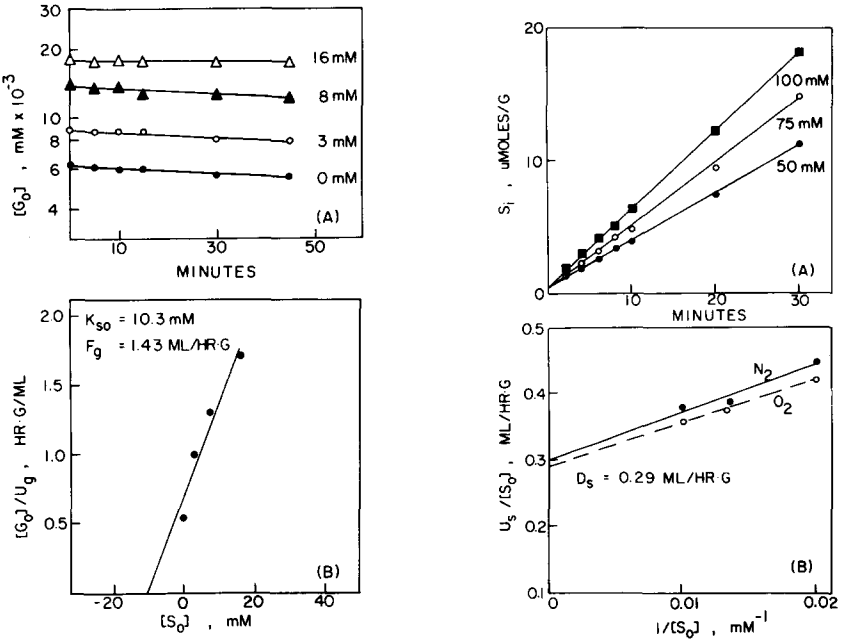

Fig. 3. Measurement of K_{SO}, the Michaelis constant for 3-*O*-methylglucose entry, by competitive inhibition of glucose uptake in aerobic cells. Red cell suspensions were incubated under conditions described in Table I. Rates of glucose uptake were obtained from panel A and K_{SO} was determined (B) as described in Fig. 2. This experiment was performed twice with similar results.

Fig. 4. Measurements of D_s the diffusion constant for 3-*O*-methylglucose. Red cell suspensions (10% hematocrit) were incubated as described in Methods without the addition of 3-*O*-methylglucose. Immediately after the second incubation period, 3-*O*-[^{14}C]methylglucose (50, 75 or 100 mM) was introduced into the medium. Samples were taken at the times indicated (panel A) and analyzed for intracellular 3-*O*-[^{14}C]methylglucose as described previously [2]. Rates of sugar entry were obtained from the slopes of the linear plots. The rate law governing 3-*O*-[^{14}C]methylglucose entry was

$$U_s = \left(F_s([S_o] - [S_i])/\left(1 + \frac{[S_o]}{K_{so}} + \frac{[S_i]}{K_{si}} + \frac{[S_o]}{R_s}\frac{[S_i]}{B_s}\right)\right) + D_s([S_o] - [S_i]),$$

where U_s represented 3-*O*-[^{14}C]methylglucose uptake rate; $[S_o]$, $[S_i]$, extracellular and intracellular 3-*O*-[^{14}C]methylglucose concentrations; K_{so}, K_{si}, the Michaelis constants of 3-*O*-methylglucose entry and exit; B_s, R_s, the equilibrium exchange constant and counterflow constant for 3-*O*-methylglucose; and F_s and D_s, the activity constant and diffusion constant of 3-*O*-methylglucose. The first term on the right side of the equation represented the contribution of carrier-mediated transport to 3-*O*-[^{14}C]methylglucose uptake while the second term represented the diffusion component. Under initial conditions of sugar entry, the rate law reduced to: $U_s = (F_s[S_o]/(1 + [S_o]/K_{so})) + D_s[S_o]$. When $[S_o] >> K_{so}$ (Figs. 2 and 3) the equation could be further simplified to: $U_s/[S_o] = ((F_sK_{so})/[S_o]) + D_s$. A plot of $U_s/[S_o]$ vs. $1/[S_o]$ gives a straight line with a y-intercept equal to D_s (panel B). This experiment was performed twice with aerobic cells and 6 times with anaerobic cells with similar results.

methylglucose (data not shown). The value of K_{so} was not significantly different from B_s. Rates of glucose uptake in aerobic cells in either the presence or absence of external 3-*O*-methylglucose were very low (Fig. 3a) and could not be determined as precisely as in anaerobic cells. The K_{so} in aerobic cells also was 10 mM (Fig. 3b), but this value is the least reliable of the transport constants that were measured. The activity constant for glucose transport, F_g, was 1/3 of that found in anaerobic cells.

Measurement of the entry and exit of 3-*O*-[^{14}C]methylglucose was complicated by the fact that the sugar permeates avian erythrocytes both by carrier-mediated transport and simple diffusion [1]. It was necessary, therefore, to determine the diffusion constant, D_s, of 3-*O*-methylglucose in order to segregate the observed flux into two components (Fig. 4). At high concentrations of 3-*O*-methylglucose, sugar entry was mainly by diffusion, the rate law being $U_s \cong D_s([S_o] - [S_i])$. When $[S_i]$ was small compared to $[S_o]$, $U_s \cong D_s[S_o]$ and the increase in $[S_i]$ was linear (Fig. 4a). No significant differences were found in the values of D_s measured under aerobic or anaerobic conditions (Fig. 4b). The diffusion constant ranged from 0.12 to 0.30 ml/h per g in different batches of cells. For calculation of the diffusion components of sugar entry, D_s was determined in various batches of cells in which K_{so} and B_s were measured.

Kinetics of 3-*O*-[^{14}C]methylglucose entry into sugar-free cells and cells equilibrated with 3-*O*-methylglucose under anaerobic conditions are shown in Fig. 5. The value of B_s was in excellent agreement with that obtained from competition experiments (Fig. 2). The discrepancy in K_{so}, as estimated in competition experiments and by 3-*O*-[^{14}C]methylglucose entry was attributed to backflux of the labeled sugar. Using time-course simulation and assuming $K_{so} = K_{si} = R_s = B_s$ (see below), the K_{so} obtained by least-square fitting was 6 mM, a value more compatible with that obtained from competition experiments (10 mM). Backflux was minimal in equilibrium exchange experiments since labeled sugar was diluted by unlabeled 3-*O*-methylglucose already present in the cell. In aerobic cells, transport constants could not be evaluated in this way, since carrier activity was low and most of the 3-*O*-methylglucose entry was by diffusion [1].

Although F_s, the activity constant, could be determined theoretically from reciprocal of the y-intercept in a $[S]/(U_s - D_s[S])$ vs. [S] plot (Fig. 5c), it also could be measured independently by following 3-*O*-[^{14}C]methylglucose entry at very low sugar concentrations (Fig. 6). In these experiments, F_s ranged from 1.5 to 2.3 ml/h per g in aerobic cells and 2.9 to 4.5 ml/h per g in anaerobic cells, indicating an increase in this constant in anaerobic cells.

Phloretin is an inhibitor of sugar transport in human red cells [12] and avian erythrocytes [3]. Sugar entry in the presence of phloretin was due mainly to diffusion. Phloretin inhibited 3-*O*-[^{14}C]methylglucose entry in aerobic and anaerobic cells to the same entry rate (Fig. 6), indicating that D_s was the same in both types of cells. The value of D_s was similar to that previously reported (0.42 ml/h per g) [3], but was somewhat higher than that determined by 3-*O*-[^{14}C]methylglucose entry (Fig. 4). This difference is attributed to the fact that a small fraction of sugar entry in the presence of phloretin was due to carrier-mediated transport.

Measurement of the kinetic parameters of 3-*O*-methylglucose efflux allowed for evaluation of the effects of anoxia on K_{si}, the Michaelis constant of sugar exit, R_s, the counterflow constant, B_s, and F_s. Calculation of the constants was dependent upon accurate measurements of the initial rates of exit (Fig. 7).

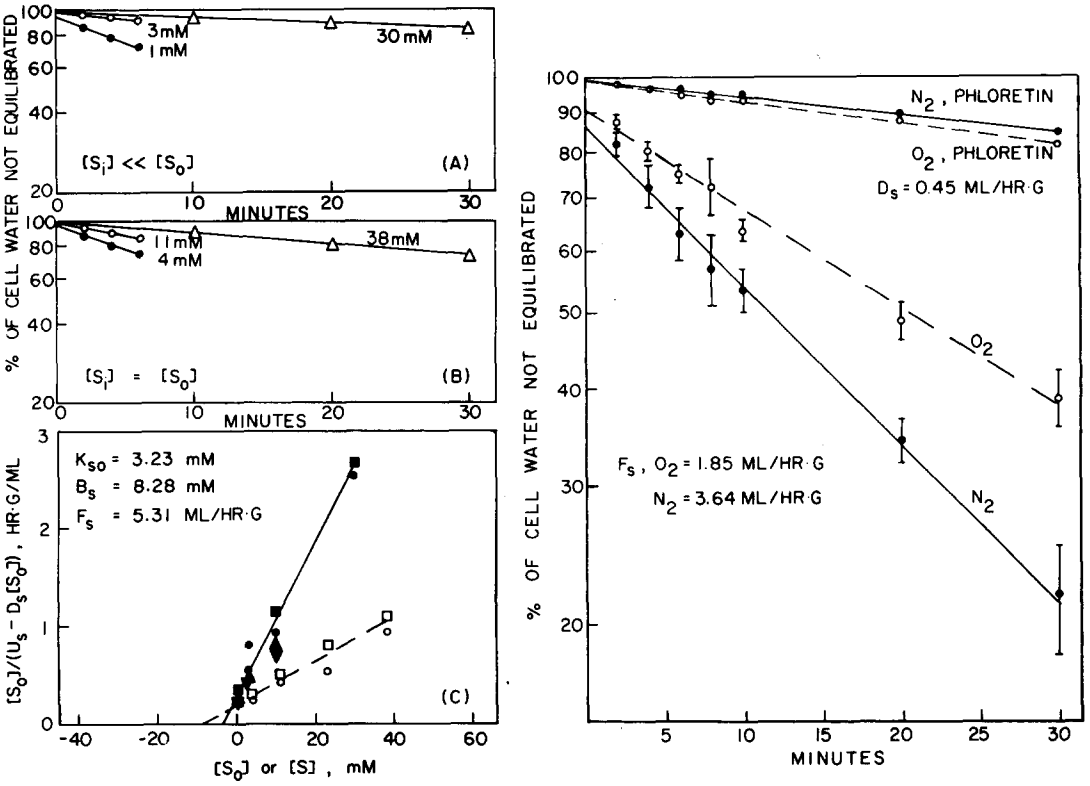

Fig. 5. Determination of K_{so} and B_s by 3-*O*-[^{14}C]methylglucose entry. Red cell suspensions (10% hematocrit) were incubated under conditions described in Table I. After the anaerobic incubation, 3-*O*-[^{14}C]-methylglucose (0.008, 1, 3, 10 or 30 mM) was added. Samples were taken at the times indicated and analyzed for 3-*O*-[^{14}C]methylglucose. The time course of 3-*O*-[^{14}C]methylglucose equilibration in sugar-free cells and cells equilibrated with 3-*O*-methylglucose are shown in panels A and B, respectively. Half-times of equilibration, $t_{1/2}$, were calculated for each sugar concentration and were used to compute the ratio $U_s/[S]$ by the following formula: $U_s/[S] = 0.693/(1/W_o + 1/W_i)$ (dry cell mass) $t_{1/2}$ where W_o and W_i were the extracellular and intracellular volumes, respectively [5]. When initial rates of 3-*O*-[^{14}C]-methylglucose entry into sugar-free cells were measured, the rate law (Fig. 4) could be simplified to: $[S_o]/(U_s - D_s[S_o]) = (1/F_s) + ([S_o]/F_sK_{so})$, where all the parameters were defined as in Fig. 4. Data for estimation of K_{so} are indicated by solid symbols and solid line in panel C. Under equilibrium exchange conditions, the rate law was: $[S]/U_s - D_s[S] = (1/F_s) + ([S]/F_sB_s)$, where [S] was the concentration of sugar equilibrated across the membrane and the other parameters were defined as in Fig. 4. Data for estimation of B_s are indicated by open symbols and dotted line in panel C. Experiments were performed 4 times for estimation of K_{so} (circles, squares, triangles, inverted triangles) and twice for determination of B_s (circles, squares).

Fig. 6. Measurement of F_s by 3-*O*-[^{14}C]methylglucose entry and of D_s by phoretin inhibition. Red cell suspensions (10% hematocrit) were incubated as described in Methods. After the second incubation period, 3-*O*-[^{14}C]methylglucose (8 μM) and phloretin (1 mM), as indicated, were added. Samples were taken at the times indicated and analyzed for 3-*O*-[^{14}C]methylglucose. The rate law for entry of 3-*O*-methylglucose at very low concentration was: $U_s = (F_s + D_s)[S_o]$, where the parameters were defined as in Fig. 4. As indicated in Fig. 5, the ratio, $U_s/[S_o]$, was calculated from the $t_{1/2}$. The experiment to determine F_s was performed 5 times but to estimate D_s only once.

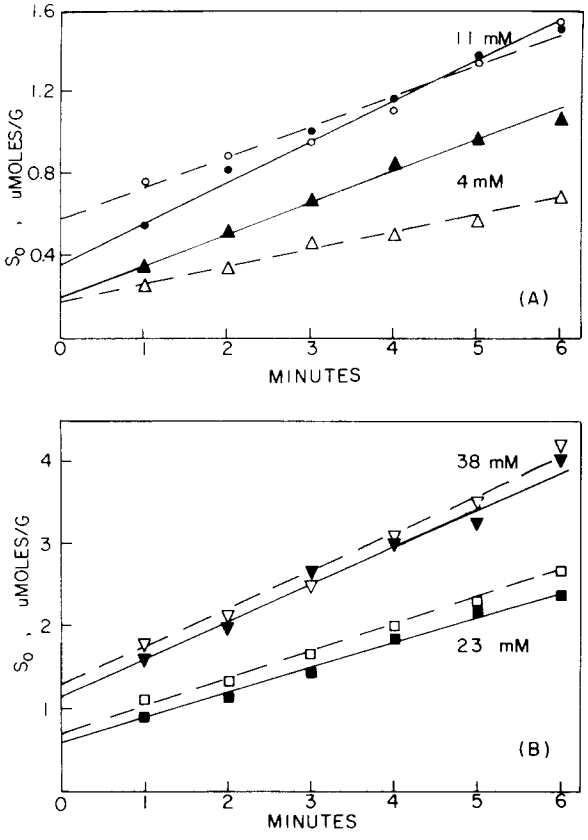

Fig. 7. Exit of 3-*O*-[^{14}C]methylglucose from aerobic and anaerobic cells. Red cell suspensions (10% hematocrit) were incubated in buffer containing 3-*O*-[^{14}C]methylglucose, as described in Methods and Table I. After the second incubation, the cells were washed 3 times and rapidly added to sugar-free buffer. Samples were taken at the times indicated and radioactivity in the supernatants was determined. Rates of 3-*O*-[^{14}C]methylglucose exit under aerobic (open symbols, dotted lines) and anaerobic (solid symbols, solid lines) conditions were calculated from the slopes of the lines.

Since the intracellular sugar concentration did not fall more than 15% during the first 6 min of sugar efflux, the rate of appearance of sugar in the medium was linear. Initial exit rates were higher in anaerobic cells at low 3-*O*-[^{14}C]-methylglucose concentrations (panel a), but were similar in both types of cells at high sugar concentrations (panel b). These results suggest that the maximal rate of 3-*O*-methylglucose exit, V_{si}, was not appreciably affected by anoxia. Appearance of 3-*O*-[^{14}C]methylglucose also was linear when the medium contained unlabeled 3-*O*-methylglucose at concentrations equal to those in the cells or 100 mM 3-*O*-methylglucose (data not shown). These results indicated that the specific activity of intracellular 3-*O*-[^{14}C]methylglucose did not decrease substantially in the first 6 min.

Transport constants from 3-*O*-[^{14}C]methylglucose exit experiments were fitted by a least-squares method (Table III). Limits of D_s, K, and F_s were derived from independent measurements of F_s (Figs. 5 and 6), D_s (Figs. 4 and 6), K_{so} and B_s (Figs. 2, 3 and 5). The values of V_s, F_s, and D_s derived from

TABLE III — NUMERICAL ITERATION OF TRANSPORT CONSTANTS FROM 3-*O*-[^{14}C]METHYLGLUCOSE EXIT EXPERIMENTS

Cell suspensions were incubated as described in Fig. 7. The rate law describing 3-*O*-[^{14}C]methylglucose exit: (a) into sugar-free buffer (K_{si} experiment) was: $U_S = (F_S[S_i]/(1 + [S_i]/K_{si})) + D_S[S_i]$. (b) under equilibrium exchange conditions (B_S experiment) was: $U_S = (F_S[S_i]/(1 + [S_i]/B_S)) + D_S[S_i]$ (c) under infinite-trans conditions (K_{it} experiment) was:

$$U_S = \left(F_S K_{S0}[S_i]/R_S/\left(1 + \frac{[S_i]\,K_{SO}}{R_S B_S}\right)\right) + D_S[S_i]$$ where all the parameters have been defined in Figure 4. The infinite-trans constant, K_{it}, was $R_S B_S/K_{SO}$.

Each dissociation-like constant, K, was calculated from a set of 3 or more paired values of $[S_i]$ and U_S. This was achieved by a FORTRAN program in which paired $[S_i]$ and U_S were the input values and F_S, K and D_S were the output values such that

$$\sum_{J=1}^{N}\left(U_S(J) - \frac{F_S[S_i(J)]}{1 + [S_i(J)]/K} - D_S[S_i(J)]\right)^2$$ $N = 3$ or 4, was minimal. Allowed range of transport constants: F_S, 0.1 to 10 ml/per g, increments of 0.1; K, 0.5 to 30 mM, increments of 0.5; D_S 0.1 to 0.45 ml/h per g, increments of 0.01. Each transport constant was determined in 2 to 3 separate experiments with similar results.

Conditions	Dissociation-like constant measured	$[S_o]$ (mM)	$[S_i]$ (mM)	Rate of exit, (μmol/h per g)		% Difference	Constants		
				Observed	Calculated		F_S, (ml/h per g)	K, (mM)	D_S, (ml/h per g)
Aerobic	K_{si}	0	11	10.75	11.29	− 5.02			
		0	23	18.67	17.86	4.34	1.4	21.5	0.10
		0	38	22.53	23.02	− 2.17			
Aerobic	B_S	4	4	5.33	5.06	5.07			
		11	11	11.87	11.70	1.43	1.0	17.5	0.45
		23	23	20.15	20.29	− 0.69			
		38	38	29.26	29.08	0.62			
Aerobic	K_{it}	100	4	4.39	5.27	−20.05			
		100	11	13.73	11.93	13.11	1.1	15.0	0.45
		100	23	18.64	20.34	− 9.12			
		100	38	29.72	28.93	2.66			
Anoxic	K_{si}	0	4	7.13	7.13	0.03			
		0	11	13.16	13.16	0.00	2.4	8.5	0.15
		0	38	22.28	22.37	0.40			
Anoxic	B_S	4	4	10.67	8.87	16.87			
		11	11	15.95	17.42	− 9.22	2.6	8.5	0.45
		23	23	26.09	26.49	− 1.53			
		38	38	36.05	35.16	2.47			
Anoxic	K_{it}	100	4	9.47	9.79	− 3.38			
		100	11	18.89	18.70	1.01	3.2	11.0	0.10
		100	23	26.43	26.11	1.21			
		100	38	30.90	31.10	− 0.65			

TABLE IV

SUMMARY OF TRANSPORT CONSTANTS

Data are reported either as individual observations or as the mean ± S.E.M. The figure in parenthesis indicates the number of observations. Units for the constants are: K_{go}, R_g, K_{so}, K_{si}, R_s, B_s, and K_{it} in mM; F_g, F_s, and D_s in ml/h per g; and V_{go}, V_{gr}, V_{so}, V_{si}, V_{sr}, and V_{sb} in μmol/h per g.

Transport constant	Experiment	Aerobic	Anoxic
K_{go}	Glucose uptake	0.35 *	1.35, 1.72, 0.80 *
R_g	3-*O*-Methylglucose counterflow	5 **	7.10, 8.69, 5 **
F_g	Glucose uptake	1.98 ± 0.36 (3)	4.98 ± 0.30 (5)
V_{go}	Theoretical derivation, F_gK_{go}	1.04 **	7.64, 3.36 *
V_{gr}	Theoretical derivation, F_gR_g	9.9 **	39.32, 24.90 **
K_{so}	Competitive inhibition	10.29, 5.89	10.31, 10.79
K_{so}	3-*O*-Methylglucose entry	--	6.0, 5.5
K_{si}	3-*O*-Methylglucose exit	21.50, 30.00	7.17 ± 1.33 (3)
B_s	Competitive inhibition	--	8.37, 11.83
B_s	3-*O*-Methylglucose entry exchange	—	7.46, 9.42
B_s	3-*O*-Methylglucose exit exchange	20.17 ± 2.19 (3)	7.67 ± 1.92 (3)
K_{it}	3-*O*-Methylglucose exit	20.50 ± 2.75 (3)	11.00, 12.00
R_s	Theoretical derivation, $K_{it}K_{so}/B_s$	8.22	10.92
D_s	3-*O*-Methylglucose entry	0.23, 0.29	0.25 ± 0.03 (6)
D_s	Phloretin inhibition	0.46	0.43
D_s	3-*O*-Methylglucose exit, computer-fit	0.30 ± 0.06 (8)	0.30 ± 0.06 (8)
F_s	Tracer 3-*O*-methylglucose entry	1.85 ± 0.22 (5)	3.64 ± 0.51 (5)
F_s	3-*O*-Methylglucose exit, computer-fit	1.55 ± 0.39 (6)	2.65 ± 0.40 (8)
V_{so}	Theoretical derivation, F_sK_{so}	13.75	25.63
V_{si}	Theoretical derivation, F_sK_{si}	43.78	22.55
V_{sb}	Theoretical derivation, F_sB_s	34.29	27.00
V_{sr}	Theoretical derivation, F_sR_s	13.97	34.34

* Wood and Morgan [1]

** Whitfield and Morgan [2]

measurements of sugar exit from anaerobic cells were in excellent agreement with those obtained by other experimental approaches. It appeared that in either aerobic or anaerobic cells, K_{si}, B_s and K_{it}, (the infinite-trans constant, Table III), were similar. Anoxia decreased the dissociation-like constants, K_{si}, B_s and K_{it}, and increased F_s. As a result, maximal rates of sugar exit were not affected.

The constants of glucose and 3-*O*-methylglucose transport measured in present and previous experiments [1,2] are summarized in Table IV.

Comparison of rates of heterologous and homologous sugar exchange

Since K_{go} was smaller than R_g in anaerobic cells (Fig. 1), it was of interest to determine whether external glucose had an effect on 3-*O*-[^{14}C]methylglucose efflux. As shown in Fig. 8, efflux of 3-*O*-[^{14}C]methylglucose was slower in the presence of external glucose than external 3-*O*-methylglucose. This effect was apparent at 4 and 38 mM intracellular 3-*O*-[^{14}C]methylglucose and in aerobic and anoxic cells. This is in contrast to the anomalous hetero-trans-stimulation observed in human red cells [13,14].

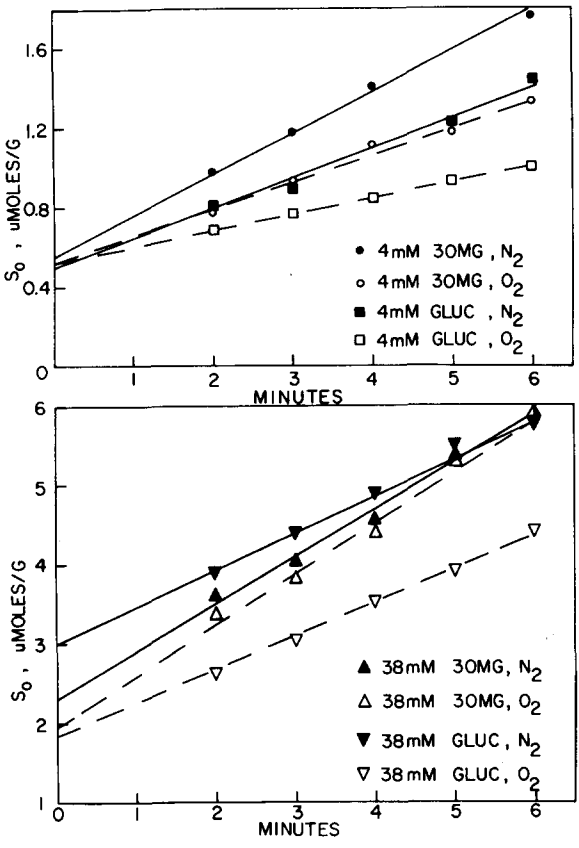

Fig. 8. Homologous and heterologous exchange of 3-*O*-[^{14}C]methylglucose from aerobic and anaerobic cells. Red cell suspensions (10% hematocrit) were incubated as described in Fig. 7. After the second incubation, washed packed cells were rapidly added to buffer containing either glucose or 3-*O*-methylglucose at either 4 or 38 mM. Data was presented as in Fig. 7. In aerobic cells (open symbols, dotted lines), rates of homologous sugar exchange were 7.69 μmol/h per g at 4 mM and 42.06 μmol/h per g at 38 mM; rates of heterologous sugar exchange were 4.76 μmol/h per g at 4 mM and 26.17 μmol/h per g at 38 mM. In anaerobic cells (solid symbols, solid lines), rates of homologous sugar exchange were 11.75 μmol/h per g at 4 mM and 36.72 μmol/h per g at 38 mM ; rates of 3-*O*-[^{14}C]methylglucose/glucose exchange were 9.39 μmol/h per g at 4 mM and 29.29 μmol/h per g at 38 mM. This experiment was performed twice with similar results.

Discussion

Carrier versus non-carrier models

A number of observations on glucose transport in human erythrocytes has been difficult to reconcile by the classical carrier theory [6,13—17]. Three new non-carrier models were postulated to supplant the carrier hypothesis [18—20]. In addition, theoretical considerations have provided rejection criteria for the carrier hypothesis. The first criterion was developed by Lieb and Stein [8] for the conventional symmetrical carrier [4] and required K_m^{Zt}, the zero-trans constant, to be less than K_m^{ic}, the infinite-cis or Sen-Widdas constant [21]. In our notation, K_m^{Zt} should be K_{si} and K_m^{ic} should be R_sB_s/K_{si}. Under anaerobic conditions, K_m^{Zt} was 7 mM and K_m^{ic} was 13 mM, an observation consistent with

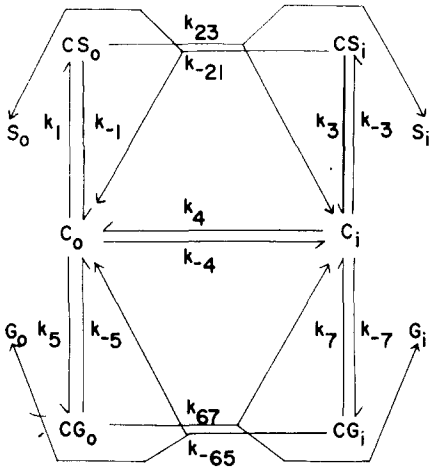

Fig. 9. The simple carrier model: C_o and C_i are the free-carriers; CS_o, CS_i, the 3-*O*-methylglucose-carrier complexes; CG_o and CG_i, the glucose-carrier complexes; i and o denote inside and outside; the rate constants for the individual steps in the transport cycle are denoted by k_1, k_{23}, etc. This model is essentially the same as that of Regen and Tarpley [7] except that (a) no unstirred layers are present, and (b) assignment of the numerical subscripts to the reaction constants is reversed. Thus reaction constants associated with glucose-carrier complex bear subscripts with digits 5, 6 and/or 7 while those associated with 3-*O*-methylglucose-carrier complex bear subscripts with digits 1, 2 and/or 3.

carrier symmetry. In aerobic cells, K_m^{Zt} was 26 mM, but K_m^{ic} was only 6 mM. This observation would suggest that symmetrical carrier model must be rejected as a description of sugar transport in aerobic cells. Another rejection criterion was developed by Regen and Tarpley [7] for the carrier model with no simplifying assumptions [5]. For this carrier hypothesis to be internally consistent, the four dissociation-like constants must obey the relationship $1/B_s + 1/R_s = 1/K_{so} + 1/K_{si}$. In the anoxic experiments, $1/B_s + 1/R_s$ was 0.21 mM^{-1} and $1/K_{so} + 1/K_{si}$ was 0.26 mM^{-1}. In aerobic cells, $1/B_s + 1/R_s$ was 0.17 mM^{-1} and $1/K_{so} + 1/K_{si}$ was 0.16 mM^{-1}. The experimental findings were in excellent agreement with theoretical predictions of the carrier hypothesis.

The observation that $K_{so} \cong K_{si} \cong B_s \cong R_s$ in anaerobic cells indicated that the carrier was symmetrical for 3-*O*-methylglucose transport, i.e., movements of free carrier and sugar-carrier complex were equal and rate-limiting. Under these conditions, the dissociation-like constants (K_{so}, K_{si}, R_s, B_s) were synonymous with the dissociation constant of the 3-*O*-methylglucose-carrier complex which was identical at both interfaces. This result also eliminated the requirement for the so-called "unstirred layer effect" that was necessary to accomodate sugar transport data in human erythrocytes [7,18].

The carrier model with no simplifying assumptions [5] also could accomodate glucose transport data in anaerobic cells (Fig. 9). The maximal rates of glucose transport in entry and counterflow experiments were given by the following expressions [7]:

$$V_{go} = F_g K_{go} = C_t/(1/k_{67} + 1/k_4) = 7.64\ \mu mol/h\ per\ g$$

$$V_{gr} = F_g R_g = C_t/(1/k_4 + 1/k_{-4}) = 39.3\ \mu mol/h\ per\ g$$

where

$$F_g = C_t \frac{k_4}{k_4 + k_{-4}} \cdot \frac{k_5 k_{67}}{k_{-5} + k_{67}}$$

$$K_{go} = \frac{k_{-5} + k_{67}}{k_5} \cdot \frac{k_4 + k_{-4}}{k_4 + k_{67}}$$

$$R_g = k_{-4} \frac{(k_{-5} + k_{67})}{k_5 k_{67}}$$

$$[C_t] = [C_o] + [C_i] + [CG_o] + [CG_i] + [CS_o] + [CS_i]$$

Comparison of the values for V_{go} and V_{gr} indicated that k_{67} was less than k_{-4}. Therefore, movement of the glucose-carrier complex was slower than that of free carrier. This prediction was given strong support by the independent observation that the efflux of intracellular 3-*O*-[^{14}C]methylglucose into buffer containing glucose was slower than that into buffer containing 3-*O*-methylglucose. This observation indicated that the glucose-carrier complex was translocated across the membrane at a slower rate than 3-*O*-methylglucose-carrier complex. It follows that in anaerobic cells in which movements of free carrier and 3-*O*-methylglucose-carrier complex were approximately equal, the glucose-carrier complex had a slower mobility than free carrier. An opposite observation was reported for the human erythrocyte [17].

Modulation of sugar transport in avian erythrocytes

Stimulation of sugar transport by anoxia was unlikely to be due to de novo carrier synthesis because (a) inhibition of oxidative metabolism would be expected to inhibit protein synthesis and (b) agents that mimic the anoxic effect, e.g. cyanide, had a rapid effect [1]. The effects of anoxia, therefore, must be ascribed to increased catalytic efficiency of pre-existing carriers due to (a) enhanced carrier mobility, (b) release of carriers from an immobilizer, or (c) increased affinity of carrier for sugar. Kinetic measurements could distinguish among several hypothetical modes of regulation, but not among others. To relate different mechanisms of regulation to observed kinetics, transport constants must be defined according to the model in Fig. 9:

$$F_s = C_t \frac{k_4}{(k_4 + k_{-4})} \cdot \frac{k_1 k_{23}}{(k_{-1} + k_{23})} = C_t \frac{k_{-4}}{(k_4 + k_{-4})} \cdot \frac{k_{-3} k_{-21}}{(k_3 + k_{-21})}$$

$$K_{so} = \frac{(k_{-1} + k_{23})(k_4 + k_{-4})}{k_1 (k_4 + k_{23})}$$

$$K_{si} = \frac{(k_3 + k_{-21})(k_4 + k_{-4})}{k_{-3}(k_{-4} + k_{-21})}$$

$$R_s = \frac{(k_3 + k_{-21}) k_4}{k_{-3} k_{-21}} = \frac{(k_{-1} + k_{23}) k_{-4}}{k_1 k_{23}}$$

$$B_s = \left(\frac{k_1 k_4}{(k_{-1} + k_{23})(k_4 + k_{-4})} + \frac{k_{-3} k_{-4}}{(k_3 + k_{-21})(k_4 + k_{-4})} \right)^{-1}$$

a) Enhancement of carrier mobility. If translocations of free carrier and sugar-carrier complex are accelerated under anaerobic conditions (equal increase in k_{23}, k_{-21}, k_4 and k_{-4}), F_s will increase, but K_{so}, K_{si}, B_s and R_s will remain unchanged. If translocation of only sugar-carrier complex is accelerated (equal increase in k_{23} and k_{-21}), R_s, K_{so} and K_{si} will decrease, F_s will be higher, and B_s will not be affected. If only translocation of free carrier is accelerated (equal increase in k_4 and k_{-4}), R_s, K_{so} and K_{si} will be elevated, F_s and B_s will remain unchanged. These mechanisms are not compatible with the experimental observations in that F_s increased in anaerobic cells while K_{si} and B_s decreased and K_{so} and R_s remained unchanged.

b) Carrier sequestration. The catalytic efficiency of the carrier might be masked in aerobic cells by transfer to an intracellular location remote from the membrane, by covalent modification to an immobile form or by non-covalent interaction with an immobile component of the membrane or cytosol. These mechanisms are kinetically equivalent and might be called sequestration, since the carrier would stay on the side where the inhibiting event occurred. Sequestration could be considered a dead-end inhibition. Sequestration which acts on loaded and empty carriers to the same degree will not distinguish itself kinetically; but if it acts specifically on loaded or empty carrier, then it will affect the empirical constants (F_s, K_{so}, K_{si}, etc). in characteristic ways, which will also reveal the side of the membrane on which the sequestration occurs. If these characteristics disappear upon stimulation, sequestration is further implicated as the regulatory mechanism. Sequestration specific for a given form (e.g., $C_i + X \rightleftharpoons CX_i$) will appear kinetically as a stabilization of that form, i.e., a reduction of coefficients (in this example, k_{-3} and k_4) leading away from that form. This is because the coefficient k_4 is defined as the rate of outward translocation divided by $[C_i] + [CX_i]$. For k_{-3}, a bimolecular event, the coefficient is defined as the rate of formation of sugar-carrier complex divided by $([C_i] + [CX_i]) \cdot [S_i]$. Looking at the definitions, we see that proportional reduction in k_{-3} and k_4 results in lower F_s, higher K_{si}, higher B_s, no change in R_s, and little or no change in K_{so}. The high K_{si} and B_s relative to R_s and K_{so} observed in aerobic cells is then compatible with sequestration of C_i. It is not indicative of sequestration of C_o (lowering of k_1 and k_{-4}), which would result in high K_{so} and B_s relative to R_s and K_{si}. Moreover, anoxic stimulation resulted in higher F_s and a fall in K_{si} and B_s to values near K_{so} and R_s, while K_{so} and R_s were little affected by anoxia. This is the constellation of changes expected with reduced sequestration of C_i in anaerobic cells. Sequestration of CS_o and/or CS_i would have very different effects. For example, sequestration of CS_i (lowering k_{-21} and k_3) would not affect F_s, R_s, or K_{so}, but would lower K_{si} and B_s. Against this possibility are the observations that K_{si} and B_s were high (not low) in aerobic cells and anoxia was accompanied by a rise in F_s and a fall (not a rise) in K_{si} and B_s. Likewise, sequestration of CS_o (lowering of k_{23} and k_{-1}) would not affect F_s, R_s, or K_{si}, but would lower K_{so} and B_s. Against this possibility are the observations that B_s was high (not low) in aerobic cells, and anoxia was accompanied by a rise in F_s, a fall in K_{si}, and a fall (not a rise) in B_s. It appears, then, that sequestration of C_i is a possible regulatory mechanism, but sequestration of other forms is not indicated. This mechanism also accounts for the asymmetry of sugar transport in aerobic cells

($K_{so} = R_s < K_{si} = B_s$). Sequestration of C_i has been implicated in studies of glucose transport in human erythrocytes [7].

c) Increased affinity of carrier for sugar. If anoxia exerts its effect by either covalent modification of the carrier or by non-covalent binding of regulatory substances to the carrier such that its affinity for sugar increases (equal reduction in k_{-1} and k_3), F_s will be higher, but K_{so}, K_{si}, R_s and B_s will be lower. This mechanism would be compatible with our experimentsl findings only if K_{so} decreases in anaerobic cells. This possibility is unlikely but cannot be denied categorically since the measurement of K_{so} in aerobic cells was technically difficult.

Glucose versus 3-O-methylglucose transport kinetics

Previous studies [1,2] indicated that maximal rates of glucose transport increased in energy-deprived cells. In energy-poor cells, F_g increased while K_{go} and R_g were essentially unchanged. Measurements of K_{gi} and B_g were not possible due to intracellular metabolism of glucose. This incomplete set of transport constants for glucose is compatible with sequestration of C_i in aerobic cells. As discussed above, glucose-carrier complex appeared to have a lower mobility than free carrier. This behavior was not altered in anaerobic cells suggesting that it was not involved in transport regulation.

If the correct model of regulation of 3-*O*-methylglucose transport involved a decrease affinity for the sugar rather than sequestration of C_i in aerobic cells, the mechanism would be incompatible with the constants observed for glucose. This conclusion would imply that 3-*O*-methylglucose was a poor analog for studies of the regulation of glucose transport. As discussed above, the present study does not favor this possibility.

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