Effect of Temperature on Kinetics and Symmetries of the Hexose Transporter of Novikoff Rat Hepatoma Cells[†]

Peter G. W. Plagemann,* Robert M. Wohlhueter, John Erbe, and Patricia Wilkie

ABSTRACT: We have used rapid kinetic techniques to measure the accumulation of radioactively labeled 3-*O*-methyl-D-glucose to transmembrane equilibrium in Novikoff cell suspensions as a function of temperature. Arrhenius plots of the maximum velocities of isotopic exchange and zero-trans entry were continuous between 6 and 39 °C (E_a = 15–19 kcal/mol). Equilibrium exchange and zero-trans entry of 3-*O*-methylglucose at six concentrations (1–30 mM) at 16, 27, and 35 °C conformed to appropriate integrated rate equations derived for a simple transporter. The hexose transporter exhibited directional symmetry, but the loaded carrier moved about 2 times faster than the empty carrier at all three temperatures investigated. Thus, the differential mobilities of loaded and empty carrier are not affected by temperature in this range. The Michaelis–Menten constant for equilibrium exchange increased about 2-fold with increase in temperature between 16 and 35 °C.

The effect of temperature on hexose transport[1] or uptake[1] has been investigated in a number of cell systems, but the results have been conflicting, even within the same cell system (Table I). In some studies, Arrhenius plots exhibited distinct discontinuities with higher Arrhenius activation energies below the transition temperature than above it. But in other studies, no discontinuities were detected, and, in general, the activation energies reported have been widely variant.

Temperature transitions in transport systems, if they exist, may have important implications with respect to the structural organization and function of the transporter. For example, they could reflect a phase transition of the lipids embedding the transporter and would suggest great homogeneity of such lipids. However, the interpretation of discontinuities in Arrhenius plots of the rates of uptake of a metabolizable hexose, such as D-glucose or 2-deoxyglucose, is problematic.

Uptake of a metabolizable hexose is, reduced to its simplest form, a two-step process, so that the apparent activation energy or discontinuity in it does not necessarily reflect the behavior of the transporter per se. For example, the rate of uptake of 2-deoxyglucose in cultured cells attains a steady state within about 30 s (Graff et al., 1978; Plagemann & Estensen, 1980). The linear phase of uptake that follows and lasts for at least several minutes corresponds to the intracellular accumulation of phosphorylated products of 2-deoxyglucose. Its slope depends on the capacities of the cell to both transport and phosphorylate the hexose, which, of course, may vary independently of each other with respect to both physiology and temperature.

We have, therefore, reanalyzed the temperature dependence of hexose transport in Novikoff cells with a nonmetabolizable hexose, 3-*O*-methylglucose, as substrate. The development of rapid sampling techniques has made it feasible to assess initial rates of 3-*O*-methylglucose transport from time courses of transmembrane equilibration in cultured animal cells for which the half-time of transmembrane equilibration in the first-order substrate range is 10–40 s (Wohlhueter et al., 1976; Graff et al., 1978, 1981).

Using these techniques, we have recently investigated the kinetics of zero-trans[2] entry and exit and equilibrium exchange[2] of 3-*O*-methylglucose in various mammalian cell lines at 25 °C. The results indicated that the mobilities of the hexose transporters of these cells are indifferent with respect to direction (directional symmetry) but that the loaded carrier moves 2–14 times faster than the empty carrier (Graff et al., 1981; Plagemann et al., 1981). Thus, in the present study, we have also determined the effect of temperature on the kinetic parameters of the hexose transporter and the loaded/empty carrier mobility ratio.

Experimental Procedures

All procedures were as described in detail previously (Wohlhueter et al., 1978, 1979; Graff et al., 1981). Novikoff cells were harvested from exponential-phase cultures and suspended to (2–4) × 10^7 cells/mL in glucose-free basal medium. Time courses of accumulation of 3-*O*-[^{3}H]methyl-D-glucose (ICN) to transmembrane equilibrium in the zero-trans or equilibrium exchange mode were determined by a rapid sampling technique which consists of mixing cell suspension and substrate in short intervals with a dual-syringe apparatus. The mixtures are delivered into tubes containing silicone oil and mounted in an Eppendorf microcentrifuge, and uptake is terminated by centrifuging the cells through the oil. Radioactivity per cell pellet was corrected for that attributable to extracellular space measured with [^{14}C]inulin and converted to picomoles per microliter of intracellular water as measured with ^3H_2O.

Zero-trans entry and inward equilibrium exchange were measured in the same cell population, and each time course consisted of 15 time points. Complete kinetic analyses of the

---

[†] From the Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455. *Received November 7, 1980.* This work was supported by U.S. Public Health Service Research Grant AM 23001.

[1] We make an operational distinction between "transport" and "uptake" of a radiolabeled substrate. "Transport" denotes solely the transfer of unmodified substrate across the cell membrane as mediated by a saturable, selective carrier. "Uptake" denotes the transfer of radioactivity from exogenous labeled substrate to intracellular space or components regardless of metabolic conversions.

[2] We generally follow the nomenclature and definitions of kinetic constants employed by Eilam & Stein (1974). "Zero trans" (zt) designates the transport of a substrate from one side of the membrane (the cis side) to the other side (the trans side), where its concentration initially is zero. "Equilibrium exchange" (ee) designates unidirectional flux of radiolabeled substrate from one side to the other side of the membrane, where the substrate is held at equal concentration. Arbitrarily, we designate the outside and inside faces of the membrane as 1 and 2, respectively.

0006-2960/81/0420-3366$01.25/0

Table I: Temperature Responses of Hexose Uptake in Various Cell Systems

cell type	substrate	exptl protocol[a]	transition temp (°C)	E_a (kcal/mol)[b] above	E_a (kcal/mol)[b] below	ref
human erythrocytes	D-glucose	zt_{21}	not definitive	7–10	20	Sen & Widdas (1962)
	D-glucose	ee	20	10–13	19–22	Lacko et al. (1973)
	D-glucose	zt_{21}, ee	none	16–18		Hankin & Stein (1972)
	D-galactose	zt_{12}	15	17	53	Ginsburg & Yeroushalmy (1978)
		zt_{21}	5	25	40	
		ee	5	23	66	
human fibroblasts	D-glucose	zt_{12}	none	5–9		Werdan et al. (1980)
rat liver cells	D-glucose	zt_{12}	18	7	22	Zala & Perdue (1980)
chick embryo vesicles	D-glucose	zt_{12}	11	18	48	Baur & Heldt (1977)
untransformed and RSV-transformed[c] chick embryo cells	deoxyglucose	zt_{12}	none	15–18		Kletzien & Perdue (1974a,b), Weber et al. (1976)
Novikoff rat hepatoma	deoxyglucose	zt_{12}	30	5–9	17–22	Plagemann & Erbe (1975)
mouse BALB/3T3	deoxyglucose	zt_{12}	none	3.6		Bose & Zlotnick (1973)
MSV-transformed[c] BALB/3T3	deoxyglucose	zt_{12}	none	7.4		
murine neuroblastoma	deoxyglucose	zt_{12}	none	5–9		Walum & Edström (1976)
rat thymocytes	3-*O*-methylglucose	zt_{12}	20	11	83	Yasmeen et al. (1977)
stimulated rat thymocytes	3-*O*-methylglucose	zt_{12}	15		23	

[a] See footnote 2. [b] E_a, Arrhenius activation energy above and below transition temperature. [c] RSV, Rous sarcoma virus; MSV, mouse sarcoma virus.

hexose transporter were conducted as described previously (Graff et al., 1981). In brief, the approach was as follows: The following integrated rate equation (eq 1) (Wohlhueter

$$N_{2,t} = N_{2,\infty}\left[1 - \exp\left(-\frac{V^{ee}t}{K^{ee} + S}\right)\right] \qquad (1)$$

et al., 1978, 1979) was fitted by the method of least squares to equilibrium exchange data pooled for six 3-*O*-methylglucose concentrations (1–32 mM), where $N_{2,t}$ = intracellular concentration of radioactivity at time t, $N_{2,\infty}$ = intracellular concentration of radioactivity at $t = \infty$ and is equal to N_1, the concentration of radioactivity per equivalent volume of medium, and S = substrate concentration which is equal on both sides of the membrane. The fit yields estimates of K^{ee} and V^{ee} ($1/R^{ee}$), the apparent Michaelis–Menten constant and maximum velocity for equilibrium exchange, respectively.

Then the integrated zero-trans entry equation (Wohlhueter et al., 1978, 1979)

$$S_{2,t} = S_1\left[1 - \exp\left(-\frac{t + (R_{21} + R_{ee}S_1/K)S_{2,t}}{KR_{oo} + R_{12}S_1 + R_{21}S_1 + S_1^2R_{ee}/K}\right)\right] \qquad (2)$$

was fitted to zero-trans entry data pooled for the same six 3-*O*-methylglucose concentrations, whereby R_{ee} was fixed at the value estimated from the equilibrium exchange data and R_{12} was held equal to R_{21}. In eq 2, $S_{2,t}$ = concentration of intracellular substrate at time t ($S_{2,0} = 0$), S_1 = extracellular concentration of substrate (and is taken as a constant), K is a limit Michaelis–Menten constant, and the R terms are resistance factors, proportional to the time of round trip of the carrier in one of four modes: (1) loaded on the inbound trip and empty on the outbound trip (R_{12}); (2) empty on the inbound and loaded on the outbound trip (R_{21}); (3) empty in both directions (R_{oo}); (4) loaded in both directions (R_{ee}). The R parameters are reciprocals of the corresponding maximum velocities; for example, $R_{ee} = 1/V_{ee}$ and $R_{12} = 1/V_{12}^{zt}$ (maximum velocity of zero-trans entry).

The fit of eq 2 to zero-trans entry data with the constraints in parameter independence indicated above yields best fitting values of $R_{12} = R_{21}$ and K, from which the following parameters were calculated:[2] $K_{12}^{zt} = K_{21}^{zt}$ (Michaelis–Menten constant for zero-trans entry and exit, respectively) $= KR_{oo}/R_{12}$; $V_{12}^{zt} = V_{21}^{zt} = 1/R_{12}$; $K^{ee} = KR_{oo}/R_{ee}$; $R_{oo} = 2R_{12} - R_{ee}$ and R_{oo}/R_{ee}; the R_{oo}/R_{ee} ratio indicates how much more rapidly the loaded carrier moves than the empty carrier. Initial zero-trans velocities (v_{12}^{zt}) were calculated for a given substrate concentration as the slope of the computed theoretical curves for $t = 0$: $v_{12}^{zt} = S_1/(KR_{oo} + R_{12}S_1)$.

For an analysis of time courses of exchange at a single 3-*O*-methylglucose concentration, eq 1 was fitted with K^{ee} fixed at 15 mM for an estimate of V^{ee}. Initial velocities of equilibrium exchange (v^{ee}) were calculated for a given substrate concentration as the slope of the computed theoretical curve for $t = 0$: $v^{ee} = V^{ee}S/(K^{ee} + S)$. In cases of zero-trans entry time courses at a single substrate concentration, we fitted eq 2 to the data with K fixed at 5 mM and all R parameters held equal to obtain an estimate of R and calculated v_{12}^{zt} as the slope of the computed curve at $t = 0$: $v_{12}^{zt} = S_1/(KR + S_1R)$. Monte Carlo experiments have shown that neglecting the differential mobilities of loaded and empty carriers in the computer fit has only a minor effect on the estimate of v_{12}^{zt} from a progress curve (Graff et al., 1981). v_{12}^{zt} is also relatively insensitive to variations in K.

Results

Figure 1 illustrates Arrhenius plots for initial velocities of zero-trans entry and inward equilibrium exchange of 3-*O*-methylglucose at concentrations of 20 and 60 mM, respectively, that is, at over 80% of saturation of the carrier (see below). No discontinuities were detected for either zero-trans influx or equilibrium exchange flux, and the activation energies were about the same for both (15–19 kcal/mol, corresponding to a Q_{10} of about 2.6 between 25 and 35 °C). Similar results were obtained in two other zero-trans entry experiments with 20 and 0.8 mM 3-*O*-methylglucose (apparent activation energies of 15 and 20 kcal/mol, respectively).

In other experiments, we investigated the effect of temperature on the kinetic parameters and symmetries of the hexose transporter. Zero-trans entry and equilibrium exchange were measured at six concentrations of 3-*O*-methylglucose (1–32 mM) in the same population of cells at 16, 27, and 35 °C. Equation 1 was fitted to the equilibrium exchange data for each temperature to estimate the Michaelis–Menten constant and maximum velocity of exchange, K^{ee} and $V^{ee} = 1/R_{ee}$, respectively (for details, see Experimental Procedures).

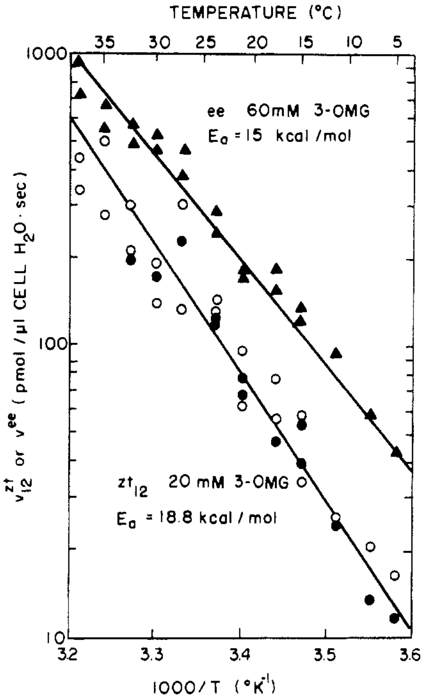

FIGURE 1: Temperature dependence of zero-trans influx and equilibrium exchange flux of 3-*O*-methylglucose in Novikoff cells. One suspension of about 2 × 10^7 cells/mL in glucose-free basal medium was assayed for zero-trans influx of 20 mM 3-*O*-[^{3}H]methylglucose (418 cpm/pmol) and another suspension for 3-*O*-[^{3}H]methylglucose exchange (273 cpm/µL) at 60 mM 3-*O*-methylglucose as described under Experimental Procedures. Samples of the cell suspensions, the substrate solution, and the apparatus were thermally equilibrated before assay at the temperatures indicated on the upper abscissa. All time courses of intracellular accumulation of radiolabeled 3-*O*-methylglucose consisted of 12 time points encompassing 120–600 s, depending on concentration. Equation 1 was fitted to each isotope exchange time course with K^{ee} fixed at values interpolated from those given in Table II to obtain an estimate of V^{ee} for calculation of initial exchange velocities, $v^{ee} = V^{ee}S/(K^{ee} + S)$. Equation 2 was fitted to each zero-trans entry time course with K fixed at 5 mM and all R parameters held equal to obtain an estimate of R for calculation of the initial zero-trans velocity, $v_{12}^{zt} = S_1/(KR + S_1R)$. The curves are regression lines on v_{12}^{zt} and v^{ee} and correspond to the indicated Arrhenius activation energies (E_a).

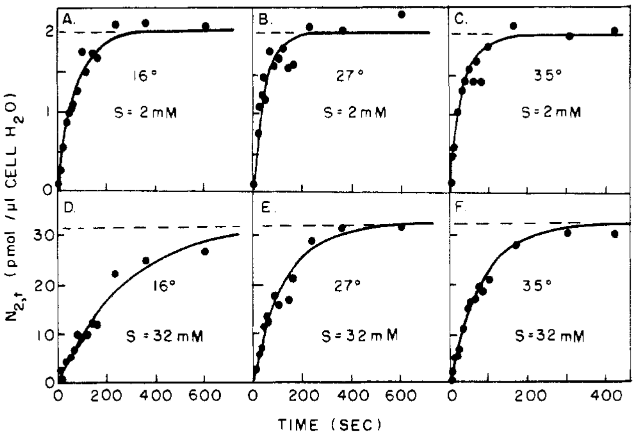

FIGURE 2: Representative time courses of inward equilibrium exchange of 2 (A–C) and 32 mM (D–F) 3-*O*-methylglucose at 16, 27, and 35 °C (taken from experiments for which kinetic parameters are summarized in Table II). The curves drawn are theoretical ones obtained by fitting eq 1 to exchange data pooled for six 3-*O*-methylglucose concentrations (1–32 mM) at each temperature.

Table II: Kinetic Parameters for Zero-Trans Entry (zt_{12}) and Equilibrium Exchange (ee) of 3-*O*-Methylglucose at Various Temperatures[a]

exptl protocol	kinetic parameters	temp (°C) 16	27	35
ee	K^{ee} (mM)	9.7 ± 1.0	13.2 ± 1.8	19.9 ± 2.4
	V^{ee} [pmol/(µL of cell H_2O·s)]	152 ± 7	342 ± 22	643 ± 42
	$r_{y,\hat{y}}$	0.9886	0.9888	0.9926
	V^{ee}/K^{ee} (10^3/s)	15.7	26.0	32.4
	R_{ee} (s/mM)	6.6	2.93	1.56
zt_{12}	K (mM)	5.0 ± 0.5	8.5 ± 0.5	7.1 ± 1.2
	$R_{12} = R_{21}$ (s/mM)	11.0 ± 0.4	3.67 ± 0.67	2.68 ± 0.16
	$r_{y,\hat{y}}$	0.9600	0.9920	0.9514
	$K_{12}^{zt} = K_{21}^{zt}$ (mM)	7.0	10.0	10.1
	$V_{12}^{zt} = V_{21}^{zt}$ [pmol/(µL of cell H_2O·s)]	91	272	373
	V^{zt}/K^{zt} (10^3/s)	13.0	27.2	37.0
	K^{ee} (mM)	11.8	12.9	17.4
	R_{oo} (s/mM)	15.5	4.41	3.31
	R_{oo}/R_{ee}	2.35	1.52	2.44

[a] The details of the experiment are described under Experimental Procedures. The radioactivity concentration was 325 cpm/µL, irrespective of 3-*O*-methylglucose concentration; intracellular water space was 9.3 µL/cell pellet, and extracellular space available to substrate was 2.1 µL/cell pellet. Fit parameters are stated ±standard error of estimate. $r_{y,\hat{y}}$ = correlation coefficient.

Representative time courses of exchange of 2 and 32 mM 3-*O*-methylglucose at the three temperatures are illustrated in Figure 2. Then the integrated zero-trans equation (eq 2) was fitted to the zero-trans data for each temperature with R_{ee} fixed at the measured value corresponding to that temperature and with R_{12} held equal to R_{21} (as justified by the observed directional symmetry of the carrier). The regression yields estimates of K and $R_{12} = R_{21}$ from which the Michaelis–Menten constants and maximum velocities of zero-trans entry and exit, $K_{12}^{zt} = K_{21}^{zt}$ and $V_{12}^{zt} = V_{21}^{zt}$, respectively, and R_{oo} were calculated. The computed kinetic parameters are summarized in Table II and show the following:

(1) The data for all three temperatures were consistent with the simple carrier model. Equations 1 and 2 adequately described the equilibrium exchange and zero-trans entry data as indicated by the high correlation coefficients. Furthermore, in each case, the first-order rate constants for equilibrium exchange (V^{ee}/K^{ee}) and zero-trans entry (V^{zt}/K^{zt}) were about the same, as is implicit in the simple carrier model. This finding attests to the internal consistency of the data and adequacies of the computational technique. Internal consistency is also indicated by the fact that values for K^{ee} were similar whether obtained directly from the equilibrium protocol or calculated from the zero-trans data ($K^{ee} = KR_{oo}/R_{ee}$). The same was true for the value of K computed from the zero-trans data (Table I) and that calculated from the experimentally determined equilibrium exchange parameters ($K = K^{ee}R_{ee}/R_{oo}$ = 4.2, 8.6, and 8.2 mM for 16, 27, and 35 °C, respectively).

(2) K^{ee} was significantly higher than K_{12}^{zt} as reported previously for 25 °C (Graff et al., 1981). A plot of ln K^{ee} vs. $1/T$ yielded an estimate of the apparent enthalpy of carrier/substrate dissociation ($-\Delta H°'$) equal to about 6.8 kcal/mol in analogy to the van't Hoff relationship: $d(\ln K_{eq})/[d(1/T)] = -\Delta H°'/R$.

(3) The differential mobility of loaded and empty carriers, as quantitated by R_{oo}/R_{ee}, was similar (about 2-fold) at all three temperatures. Higher mobility[3] of loaded than empty

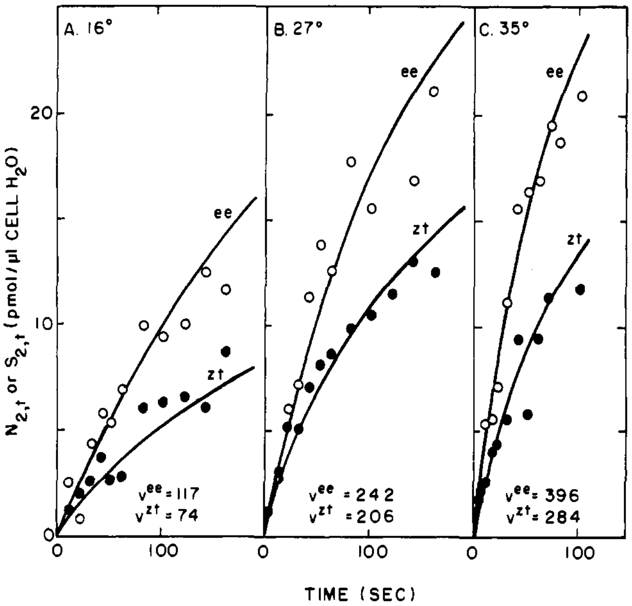

FIGURE 3: Comparison of initial time courses of zero-trans entry (zt) and equilibrium exchange (ee) of 32 mM 3-*O*-methylglucose at 16, 27, and 35 °C (taken from experiment for which kinetic parameters are summarized in Table II). The initial velocities of entry (v_{12}^{zt}) and exchange (v^{ee}) are those calculated from the best fitting parameters for S = 32 mM: $v_{12}^{zt} = S_1/(KR_{oo} + R_{12}S_1)$ and $v^{ee} = V^{ee}S/(K^{ee} + S)$; the curves drawn are the corresponding theoretical ones.

carrier is manifest in trans acceleration of transport. The trans effect is clearly demonstrated by a comparison of the initial time courses of inward equilibrium exchange and zero-trans entry of 32 mM 3-*O*-methylglucose at the three temperatures shown in Figure 3.

We have also examined the effect of prolonged incubation at 25, 37, and 42 °C on the capacity of Novikoff cells to transport 3-*O*-methylglucose. The data in Table III show that zero-trans entry was little, if at all, affected by a 60-min incubation of the cells at these temperatures. Even a 5-min heating at 47 °C had only a minor effect. The results were confirmed in two other experiments.

Discussion

The activation energies observed here for zero-trans influx and equilibrium exchange flux of 3-*O*-methylglucose in Novikoff cells are similar to those reported for zero-trans efflux and equilibrium exchange flux of D-glucose in human erythrocytes (Sen & Widdas, 1962; Hankin & Stein, 1972; Lacko et al., 1973). They are similar also to the activation energies reported for the uptake of 2-deoxyglucose by Novikoff cells (<31 °C; Plagemann & Erbe, 1975) and by chick embryo fibroblasts (Kletzien & Perdue, 1974a,b; Weber et al., 1976), and of D-glucose by isolated rat liver cells (<18 °C; Baur & Heldt, 1977). The activation energy for another facilitated diffusion system in Novikoff cells, that for nucleoside transport, is also of similar magnitude, namely, 18.3 kcal/mol (Wohlhueter et al., 1979).

The temperature dependences of the Michaelis–Menten constants for the transport of hexoses (cf. Table II) and nucleosides (Wohlhueter et al., 1979) in Novikoff cells are also similar and reflect a 2–3-fold increase as temperature increases from 15 to 35 °C. A similar change in the Michaelis–Menten constant with temperature has been reported for zero-trans exit and equilibrium exchange of D-glucose in human red cells (Sen & Widdas, 1962; Lacko et al., 1973), although those for D-galactose zero-trans entry and exit and equilibrium exchange in these cells were little affected by temperature (Ginsburg & Yeroushalmy, 1978).

Table III: Effect of Incubation at Various Temperatures on the Capacity of Novikoff Cells to Transport 3-*O*-Methylglucose[a]

temp (°C)	time (min)	ν_{12}^{zt} [pmol/(μL of cell $H_2O \cdot s$)]	correlation coefficient, $r_{y,\hat{y}}$
25	0	183 ± 41	0.9131
	60	162 ± 16	0.9739
37	0	185 ± 28	0.9295
	30	193 ± 17	0.9803
	60	164 ± 14	0.9859
42	0	159 ± 12	0.9821
	30	144 ± 25	0.9420
	60	145 ± 11	0.9868
47	5	128 ± 20	0.9220

[a] Samples of a suspension of 1.6 × 10^7 cells/mL in glucose-free basal medium were incubated at the indicated temperatures. After the stated time periods of incubation, aliquots of each suspension were equilibrated at 25 °C and assayed for the zero-trans influx of 10 mM 3-*O*-[^{3}H]methylglucose (44 cpm/nmol) as described under Experimental Procedures. Sampling times were 2, 7, 12, 17, 22, 30, 40, 50, 60, 80, 100, 120, 180, 300, and 480 s. Equation 2 was fit to each progress curve with K fixed at 5 mM and all R parameters held equal for an estimate of R from which the initial velocity ν_{12}^{zt} was calculated as the slope of the theoretical curve at $t = 0$; $\nu_{12}^{zt} = S_1/(KR + S_1R)$.

The Arrhenius plots for 3-*O*-methylglucose transport in Novikoff cells showed no significant discontinuities. The same is true for nucleoside transport in these cells (Wohlhueter et al., 1979). The reason for the temperature transition in 2-deoxyglucose uptake by Novikoff cells at about 31 °C (Plagemann & Erbe, 1975; see Table I) is not known but may reflect a shift from transport as the main, rate-determining step in uptake below the transition temperature to phosphorylation as the main, rate-determining step above the transition temperature. This explanation may also apply to biphasic Arrhenius curves obtained for the uptake of metabolizable hexoses in other cell systems (see Table I). In general, there is the question of whether the Arrhenius activation energies reported for the uptake of hexoses that are rapidly metabolized by the cells reflect those of the hexose carrier. Less readily apparent is an explanation for the discrepancy between our results and those reported for 3-*O*-methylglucose uptake in rat thymocytes (see Table I; Yasmeen et al., 1977). Kinetic analyses of 3-*O*-methylglucose transport in rat thymocytes, however, are complicated by the simultaneous operation of two transport systems with 10–20-fold differences in velocities in populations of these cells (Yasmeen et al., 1977; Reeves, 1977a,b; Whitesell et al., 1977) which might be differently affected by temperature (Yasmeen et al., 1977). Rat thymocytes also differ from the types of cultured cell lines we have investigated in that only about 50% of their intracellular H_2O space is accessible to 3-*O*-methylglucose (Whitesell et al., 1977), and 3-*O*-methylglucose uptake is stimulated 3–4-fold by ATP depletion (Reeves, 1977a). Why Arrhenius curves for D-glucose transport in human red cells were observed to be concave upward in one study (Sen & Widdas, 1962), linear between 5 and 45 °C in another study (Hankin & Stein, 1972), or to show a distinct discontinuity at 20 °C in a third study (Lacko et al., 1973) is also still unresolved.

The fact that V^{ee} and V_{12}^{zt} for 3-*O*-methylglucose transport exhibit equal temperature dependence indicates that the temperature dependences of the mobilities of loaded and empty

---

[3] Greater mobility of the loaded carrier is manifest as "trans stimulation" or "accelerated countertransport" [see Eilam & Stein (1974) and Widdas (1980)]. "Mobility" connotes some macromolecular movement, if only a conformational shift, and not necessarily carrier translocation as suggested by the term "mobile carrier".

carriers are equivalent, in spite of the absolute difference in these mobilities. The same observation applies to the nucleoside carrier of Novikoff cells (Wohlhueter et al., 1979), but there it was presumed to be a natural consequence of equal mobilities of loaded and empty carriers. The reasons for the difference in mobility of loaded and empty hexose transporter are not known. In the case of D-glucose transport in human erythrocytes, Lacko et al. (1972) have suggested that movement of the empty carrier in the membrane is impeded by hydrophilic groups on the carrier which become neutralized as a result of binding of D-glucose. If such a mechanism also applies to the hexose carrier of cultured cells, the nucleoside transporter must differ in this respect since the mobilities of nucleoside-loaded and empty carrier are the same. It is also apparent that hexose transport in human red cells differs greatly from that in cultured animal cells. It exhibits directional asymmetry, in addition to a higher mobility of loaded than empty carrier [see Widdas (1980)], and some recent work indicates the operation of two simultaneous antiparallel carriers with high and low affinity binding sites on opposite sides of the membrane (Eilam, 1975; Ginsburg, 1978).

Our results also indicate that the hexose transporter of Novikoff cells is relatively heat stable. Heating of the cells at 47 °C for 5 min has no significant effect on their capacity to transport 3-*O*-methylglucose. This finding implies an inhibition of phosphorylation rather than of transport as the reason for the marked reduction in long-term uptake of 2-deoxyglucose caused by such heat treatment in Novikoff cells (Renner et al., 1972). We also have not observed the 2-fold stimulation of 3-*O*-methylglucose transport caused by heating at elevated temperatures reported for rat thymocytes (Reeves, 1977c).

References

Baur, H., & Heldt, H. W. (1977) *Eur. J. Biochem. 74*, 397.

Bose, S. K., & Zlotnick, B. J. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2374.

Eilam, Y. (1975) *Biochim. Biophys. Acta 401*, 364.

Eilam, Y., & Stein, W. D. (1974) *Methods Membr. Biol. 2*, 283.

Ginsburg, H. (1978) *Biochim. Biophys. Acta 506*, 119.

Ginsburg, H., & Yeroushalmy, S. (1978) *J. Physiol. (London) 282*, 399.

Graff, J. C., Wohlhueter, R. M., & Plagemann, P. G. W. (1978) *J. Cell. Physiol. 96*, 171.

Graff, J. C., Wohlhueter, R. M., & Plagemann, P. G. W. (1981) *Biochim. Biophys. Acta 681*, 320.

Hankin, B. L., & Stein, W. D. (1972) *Biochim. Biophys. Acta 288*, 127.

Kletzien, R. F., & Perdue, J. F. (1974a) *J. Biol. Chem. 249*, 3366.

Kletzien, R. F., & Perdue, J. F. (1974b) *J. Biol. Chem. 249*, 3375.

Lacko, L., Wittke, B., & Kromphardt, H. (1972) *Eur. J. Biochem. 25*, 447.

Lacko, L., Wittke, B., & Geck, P. (1973) *J. Cell. Physiol. 82*, 213.

Plagemann, P. G. W., & Erbe, J. (1975) *J. Membr. Biol. 25*, 381.

Plagemann, P. G. W., & Estensen, R. D. (1980) *J. Cell. Physiol. 104*, 105.

Plagemann, P. G. W., Wohlhueter, R. M., Graff, J., Erbe, J., & Wilkie, P. (1981) *J. Biol. Chem. 256*, 2835.

Reeves, J. P. (1977a) *J. Biol. Chem. 252*, 4876.

Reeves, J. P. (1977b) *J. Cell. Physiol. 92*, 309.

Reeves, J. P. (1977c) *Arch. Biochem. Biophys. 183*, 298.

Renner, E. D., Plagemann, P. G. W., & Bernlohr, R. W. (1972) *J. Biol. Chem. 247*, 5765.

Sen, A. K., & Widdas, W. F. (1962) *J. Physiol. (London) 160*, 392.

Walum, E., & Edström, A. (1976) *Exp. Cell Res. 97*, 15.

Weber, M. J., Hale, A. H., Yau, T. M., Buckman, T., Johnson, M., Brady, T. M., & Larossa, D. D. (1976) *J. Cell. Physiol. 89*, 711.

Werdan, K., Lehner, K., Cremer, T., Stevenson, A. F. G., & Messerschmidt, O. (1980) *Hoppe-Seyler's Z. Physiol. Chem. 361*, 91.

Whitesell, R., Hoffman, L. H., & Regen, D. M. (1977) *J. Biol. Chem. 252*, 3533.

Widdas, W. F. (1980) *Curr. Top. Membr. Transp. 14*, 166.

Wohlhueter, R. M., Marz, R., Graff, J. C., & Plagemann, P. G. W. (1976) *J. Cell. Physiol. 89*, 605.

Wohlhueter, R. M., Marz, R., Graff, J. C., & Plagemann, P. G. W. (1978) *Methods Cell Biol. 20*, 211.

Wohlhueter, R. M., Marz, R., & Plagemann, P. G. W. (1979) *Biochim. Biophys. Acta 553*, 262.

Yasmeen, D., Laird, A. J., Hume, D. A., & Weidemann, M. J. (1977) *Biochim. Biophys. Acta 500*, 89.

Zala, C. A., & Perdue, J. F. (1980) *Biochim. Biophys. Acta 600*, 157.