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Temperature-Dependent Changes in Activation Energies of the Transport Systems for Nucleosides, Choline and Deoxyglucose of Cultured Novikoff Rat Hepatoma Cells and Effects of Cytochalasin B and Lipid Solvents

Peter G.W. Plagemann and John Erbe

Department of Microbiology, Medical School,
University of Minnesota, Minneapolis, Minnesota 55455

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Summary. The initial rates of transport of uridine, thymidine, purines, choline and 2-deoxy-D-glucose by cultured Novikoff rat hepatoma cells were determined as a function of temperature between 5 and 41 °C. Arrhenius plots of all transport systems exhibited sharp breaks in slope; between 17 and 23° for uridine, thymidine and hypoxanthine-guanine transport and between 29 and 32° for choline and 2-deoxy-D-glucose transport. The activation energies for the transport systems changed from 15–26 kcal/mole below the transition temperatures to 4–9 kcal/mole above the transition temperatures. Propagation of the cells in the presence of *cis*-6-octadecenoic acid which results in marked changes in the lipid composition of cell membrane, had little effect on the temperature characteristics of the various transport systems. Similarly, propagation of the cells for 24 hr in media containing Tween 40 or nystatin had no effect on the capacity of the cells to transport the various substrates or on the temperature dependence of the transport systems. The presence of ethanol, phenethyl alcohol or Persantin at concentrations that inhibited thymidine and 2-deoxy-D-glucose transport between 40 and 70% also did not alter the transition temperatures or activation energies for the transport of these substrates. Cytochalasin B, on the other hand, shifted the transition temperature for 2-deoxy-D-glucose transport to higher temperatures in a concentration-dependent manner, whereas it had no effect on the temperature dependence of thymidine transport.

Although proteins or glycoproteins most likely are the active components of membrane transport systems, recent evidence indicates that various membrane functions may be influenced by the composition of the membrane lipids. It was first demonstrated for *Escherichia coli* that the Arrhenius plots for β-glucoside and β-galactoside transport are biphasic and that the transition temperatures (the temperature at which a transition in slope occurs) can vary as much as 23° depending on the essential fatty acid used for the propagation of the organism (Wilson & Fox, 1971). The latter finding suggests that the transition temperature for

the transport processes reflects phase separations in the lipid environment of transport sites in the membrane, presumably from a more highly to a less highly ordered (or more fluid) state with increase in temperature (Wilson & Fox, 1971). Furthermore, since the activation energy is higher below than above the transition temperature, the more ordered structure seems to impede the transport process. The activity of the $(Na^+ + K^+)$-ATPase (EC 3.6.1.3) in mammalian cells exhibits a similar transition in activation energy near 20° whether measured in crude or purified preparations of native enzyme (Gruener & Avi-dor, 1966; Charnock, Cook & Casey, 1971; Kimelberg & Papahadjopoulos, 1972; Grisham & Barnett, 1973). Upon replacement of the lipids in the native complex with dipalmitophosphat idylglycerol the transition occurs at 32° rather than at 20° (Kimelberg & Papahadjopoulos, 1972). Spin-labeling studies have shown that gel-liquid transitions of the membrane lipids of the ATPase occur at the same temperature as the change in activation energy of the enzyme, and that the transition temperature of the lipids is unaltered by removal from the enzyme protein (Grisham & Barnett, 1973). Facilitated diffusion systems of animal cells are similarly influenced by the lipid environment in the membrane. The activation energy of the adenosine transport system of rabbit alveolar macrophages is about 27 kcal/mole below and 15.7 kcal/mole above 25 °C (Berlin, 1973). The transport system for α-aminoisobutyrate of cultured mouse cells exhibits a major transition temperature at about 20°, but several minor discontinuities in the Arrhenius plot were also observed (Wisnieski, Parkes, Huang & Fox, 1974). The Hyp-Gua[1] transport system of cultured Novikoff cells also exhibits a transition temperature at about 20° (Zylka & Plagemann, 1975) and the present study shows that the transport systems for nucleosides, choline and 2-deoxy-D-glucose of these cells all exhibit three- to fourfold changes in activation energy, but that the major transition temperatures differ for some of the transport systems. We have also investigated the question of whether lipid substitutions in the membrane or various inhibitors of the transport systems affect the transition temperatures and apparent activation energies of these transport systems.

Materials and Methods

Cell Culture

Novikoff rat hepatoma cells (subline N1S1-67) were propagated in suspension culture in Swim's medium 67 and enumerated with a Coulter counter as described previously

1 *Abbreviations:* Hyp, hypoxanthine; Gua, guanine; BM42B, basal medium 42; Urd, uridine; dThd, deoxythymidine.

(Plagemann & Swim, 1966; Ward & Plagemann, 1969). In all experiments, cells were harvested from cultures in the mid-exponential phase of growth (between 1.1 and 2.0×10^6 cells/ml) by centrifugation at about $400 \times g$ for 1 min.

Transport Measurements

Cells were suspended in the following basal media: (a) BM42B (Plagemann & Erbe, 1974*a*) for measuring Urd and dThd transport; (b) choline-free BM42 (Plagemann, 1971) for measuring choline transport; and (c) D-glucose-free BM42 (BM42A, Renner, Plagemann & Bernlohr, 1972) for measuring 2-deoxy-D-glucose transport. The suspensions were supplemented with ^{3}H-labeled substrate and incubated at various temperatures ($\pm 0.1°$) as indicated in the appropriate experiments. Duplicate samples of cell suspension were analyzed for radioactivity in total cell material (acid-insoluble plus acid-soluble) as described previously (Plagemann, 1971; Renner *et al.*, 1972; Plagemann & Erbe, 1974*a*). Values for duplicate samples generally did not vary more than 10%. The acid-soluble pools were extracted from labeled cells with perchloric acid and the labeled components were separated chromatographically as described in the same publications.

Materials

Unlabeled nucleosides, [methyl-^{3}H]dThd, [5-^{3}H]Urd, [8-^{3}H]Gua, and [methyl-^{3}H] choline were purchased from Schwarz/Mann, [G-^{3}H] 2-deoxy-D-glucose from New England Nuclear, unlabeled 2-deoxy-D-glucose and choline chloride from Sigma Chemical Company, and cytochalasin B from Aldrich Chemical Co., Milwaukee, Wisconsin. 2,6-bis (diethanolamino)-4,8-dipiperidinopyrimido (5,4-*d*) pyrimidine (Persantin) was a gift from Geigy Pharmaceuticals, Yonkers, N.Y.

Results and Discussion

The incorporation of Urd, dThd, choline and 2-deoxy-D-glucose into total cell material between 15 and 41° was approximately linear with time for at least 3 or 5 min and chromatographic analysis of the acid-soluble pools showed that most of the radioactivity in the acid-soluble pool was associated with phosphorylated intermediates (not shown). These results were similar to those reported for the incorporation of these substrates at 37° (Plagemann & Roth, 1969; Plagemann & Erbe, 1972; Renner *et al.*, 1972) and support the view that uptake was the rate-limiting step in the incorporation of these substrates into total cell material and that the initial rate of incorporation into total cell material, therefore, was an approximate estimate of the initial rate of total substrate uptake. With decrease in temperature below 15°, the incorporation of the various substrates deviated progressively more from linearity, particularly at concentrations in excess of the transport K_m's for the substrates (about 0.5 μM for dThd, 6 μM for choline, 15 μM for Urd and 1.5 mM

for 2-deoxy-D-glucose). These decreases in incorporation rates with time of incubation at lower temperatures were probably due to failure of the cells to phosphorylate the substrates rapidly enough for efficient trapping of the transported substrates. Since substrate phosphorylation of the transported substrate at low temperatures seemed more efficient at low substrate concentrations, we generally used substrate concentrations in the range of the transport K_m or below in the detailed studies on the effect of temperatures on transport rates (*see below*).

Previous evidence indicated that, in addition to being taken up by the specific transport systems, monosaccharides (Renner *et al.*, 1972), nucleosides (Plagemann, 1970*c*) and choline (Plagemann, 1971) enter Novikoff rat hepatoma cells by a process which shows nonsaturation at least up to concentrations 10–20 times above the transport K_m, a relatively low Q_{10} (*see* Fig. 1) and thus may represent simple diffusion through the membrane (Plagemann & Richey, 1974). Initial rates of total substrate uptake, therefore, represent the sums of the initial rates of uptake by the specific transport systems and by the nonsaturatable process. At 37° and low substrate concentrations only a small proportion of the total rate of uptake is due to the nonsaturatable process, but with a decrease in temperature it makes a progressively larger contribution to the rate of total substrate uptake (*see* Fig. 1; Plagemann & Richey, 1974). In the present study all transport rates (Fig. 2) were estimated by subtracting estimated rates of uptake by the nonsaturatable process from the rate of total substrate uptake (*see* Neame & Richards, 1972; Plagemann & Richey, 1974). The rates of substrate uptake by the nonsaturatable process at the various temperatures were estimated by drawing lines through the origin parallel to the linear portions of the dose-response curves illustrated in Fig. 1 (for clarity these lines are not shown in Fig. 1). Uptake rates by the nonsaturatable process were similar for the various substrates (Fig. 1); for instance, between 1 and 2 pmoles/10^6 cells/min at 37° and a substrate concentration of 5 μM in the medium.

The Arrhenius plots for the transport of Urd, dThd, choline and 2-deoxy-D-glucose all exhibited major changes in slope with activation energies between 15 and 23 kcal/mole below the transition temperature and of 4–9 kcal/mole above the transition temperature (Fig. 2 and Table 1). The Q_{10} values changed from between 3–4 to 1.3–1.5, respectively. The transition temperatures were largely independent of the substrate concentration used in the transport rate measurements (Fig. 2) and the Arrhenius plots of the V_{max} values for Urd and dThd transport calculated from the data in Fig. 2*A* and 2*B*, respectively, exhibited changes in

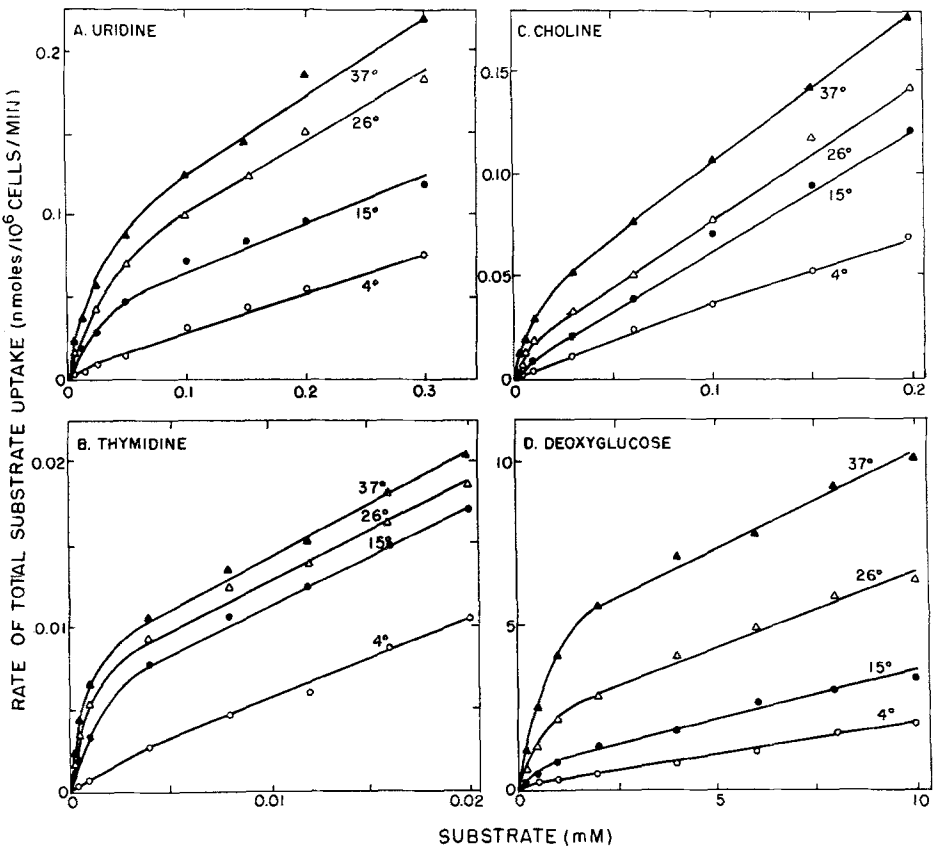

Fig. 1. Initial rates of incorporation of Urd (*A*), dThd (*B*), choline (*C*) or 2-deoxy-D-glucose (*D*) into total cell material (acid-soluble plus acid-insoluble) as a function of substrate concentration and temperature. N1S1-67 cells from an exponential phase culture were suspended to 4×10^6 cells/ml in (*A* and *B*) BM42B, (*C*) choline-free BM42 or (*D*) BM42A which had been equilibrated at the appropriate temperatures. After another 1–2 min of equilibration at the appropriate temperatures, samples of these suspensions were supplemented as follows: (*A*) with 5, 10, 20 or 50 μM [^{3}H] Urd (28 cpm/pmole) or 50 μM [^{3}H] Urd plus unlabeled Urd to 100, 150, 200 or 300 μM; (*B*) with 0.2, 0.5, 1, or 4 μM [^{3}H] dThd (290 cpm/pmole) or 4 μM [^{3}H] dThd plus unlabeled dThd to 8, 12, 16, or 20 μM; (*C*) with 2, 5, 10, 30 or 60 μM [^{3}H] choline (25 cpm/pmole) or 60 μM [^{3}H] choline plus unlabeled choline to 100, 150, or 200 μM; (*D*) with 0.2, 0.5, 1 or 2 mM [^{3}H] 2-deoxy-D-glucose (0.55 cpm/pmole) or 2 mM [^{3}H] 2-deoxy-D-glucose plus unlabeled 2-deoxy-D-glucose to 4, 6, 8, or 10 mM. All samples were incubated in a gyrotory shaker water bath at 200 rpm at the appropriate temperatures and after 3 and 6 min duplicate 1-ml samples of each suspension were analyzed for radioactivity in total cell material (acid-soluble plus acid-insoluble). All points represent averages of the duplicate 3-min values

slope similar to those illustrated for the individual concentrations (not shown). The K_m values for the transport of nucleosides, choline and 2-deoxy-D-glucose were about the same at various temperatures between 20 and 40° (*see* Fig. 5 and Plagemann, 1970*c*; Plagemann, 1971; Plage-

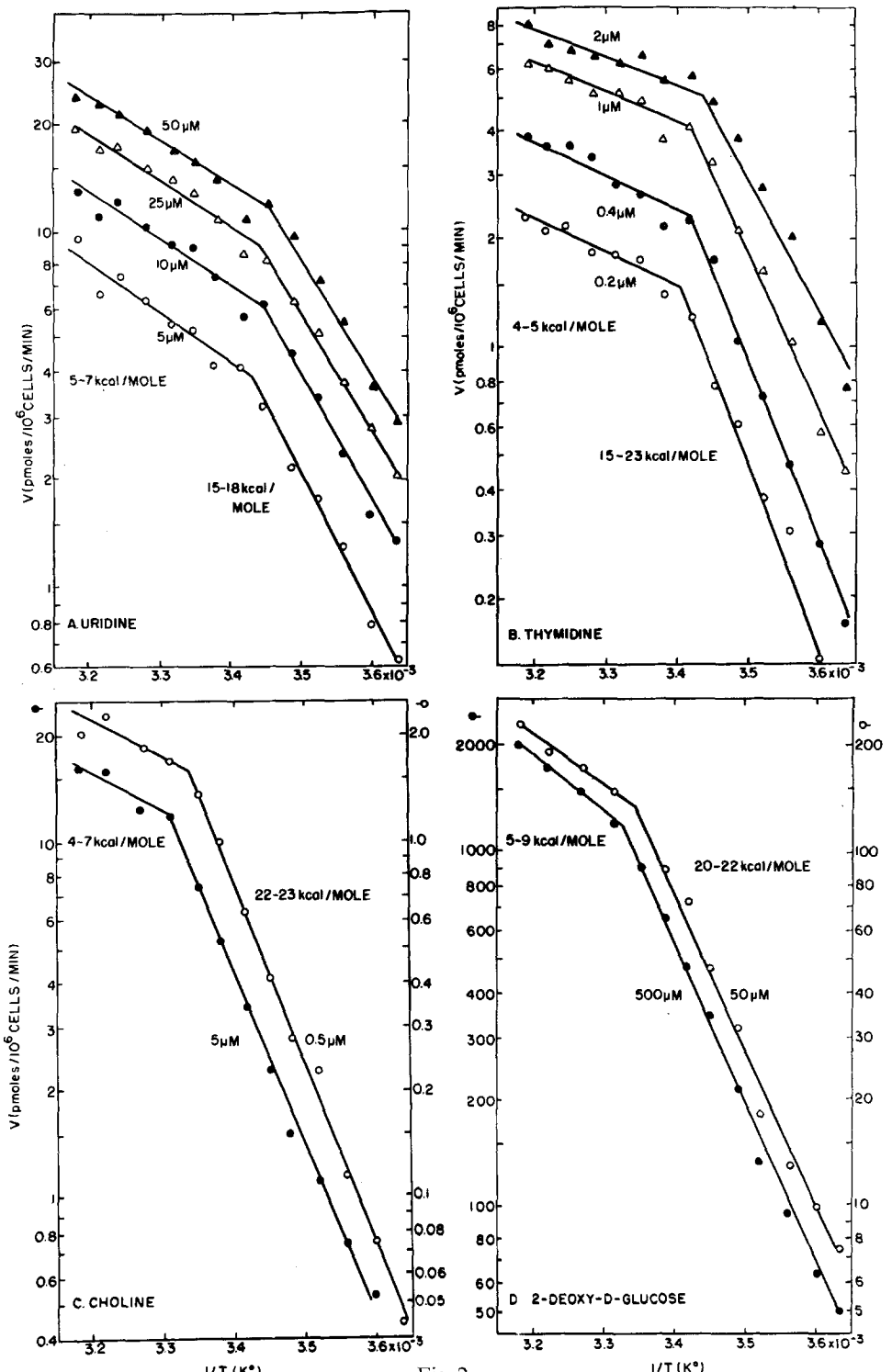
50μM
25μM
10μM
5μM
5-7 kcal/MOLE
15-18 kcal/MOLE
A. URIDINE
V(pmoles/10^6 CELLS/MIN)
2μM
1μM
0.4μM
0.2μM
4-5 kcal/MOLE
15-23 kcal/MOLE
B. THYMIDINE
4-7 kcal/MOLE
22-23 kcal/MOLE
5μM
0.5μM
C. CHOLINE
1/T (K°)
5-9 kcal/MOLE
20-22 kcal/MOLE
500μM
50μM
D 2-DEOXY-D-GLUCOSE
3.2
3.3
3.4
3.5
3.6x10^-3

Fig. 2

Table 1. Activation energies of various transport systems of Novikoff hepatoma cells

Substrate	Transition temperature[a] (°C)	kcal/mole		Previously published data		
		Below transition	Above temperature	Temperature range (°C)	kcal/mole	Ref.
Uridine	17–23	15–18	5–7	6–37	8.7	Plagemann, 1970*c*
Thymidine	17–23	15–23	4–7	22–37	11	Plagemann & Erbe, 1972
Choline	29–32	17–23	4–7	24–37	16	Plagemann, 1971
2-Deoxy-D-glucose	29–32	17–22	5–9	22–37	15.6	Renner *et al.*, 1972
Guanine, Hypoxanthine[b]	17–23	22–26	5–6			

[a] Range of values observed in the experiment illustrated in Fig. 2 and at least three additional experiments of the same type.
[b] From Zylka and Plagemann, 1975.

mann & Erbe, 1972; Renner *et al.*, 1972). In contrast, the K_m for adenosine transport by alveolar macrophages was found to increase approximately threefold with an increase in temperature from 15 to 40° (Berlin, 1973).

The major shift in activation energy of the four transport systems of Novikoff cells, however, occurred at different temperatures. In the experiment illustrated in Fig. 2 the transition temperatures for Urd and dThd transport fell between 17 and 23°, whereas those for choline and 2-deoxy-D-glucose transport fell between 29 and 32°. Very similar results were observed in at least four experiments of this type for each substrate (*see* Figs. 3 and 4) and all values for the estimated major transition

Fig. 2. Temperature dependence of the transport of Urd (*A*), dThd (*B*), choline (*C*) and 2-deoxy-D-glucose (*D*). Duplicate 1 or 1.5 ml samples of an exponential phase culture were centrifuged and the cell pellets were equilibrated at the indicated temperatures (from 2 to 41°) for 0.5 min. The cells were then suspended in 1 ml of the appropriate basal media (*see* Materials and Methods) which had been equilibrated at the same temperature and contained the following concentrations of labeled substrates: (*A*) 5, 7.5, 10, 25 or 50 μM [^{3}H] Urd (32 cpm/pmole); (*B*) 0.2, 0.3, 0.4, 0.6, 1 or 2 μM [^{3}H] dThd (500 cpm/pmole); (*C*) 0.5 μM (700 cpm/pmole) or 5 μM (70 cpm/pmole) [^{3}H] choline; or (*D*) 50 μM (7 cpm/pmole) or 500 μM (0.7 cpm/pmole) [^{3}H] 2-deoxy-D-glucose. All samples were incubated stationary in a water bath at the appropriate temperatures for 5 min and then analyzed for radioactivity in total cell material. All values were corrected for uptake by the nonsaturable process (*see text*). All points are averages of duplicate samples. For clarity the curves for some of the Urd and dThd concentrations have been omitted

temperatures and activation energies fell within relatively narrow ranges which are summarized in Table 1. The Arrhenius plots for Hyp-Gua transport by Novikoff cells are similar to that for dThd and Urd and typical values are included in Table 1. The transport rate measurements, however, were not accurate enough to determine whether minor discontinuities occurred in the Arrhenius plots as reported for the transport of α-aminoisobutyrate (Wisnieski *et al.*, 1974). There was also some uncertainty about the accuracy of transport rate estimates at low temperatures because the rates were relatively low and relatively large corrections (up to 50–60%) for uptake by the nonsaturatable process had to be made. Therefore values below 3–5% of the maximum rate at 41° have been excluded.

In previous studies (*see* Table 1) the existence of transition temperatures of the various transport systems of Novikoff cells were not detected partly because Arrhenius plots were based on an insufficient number of experimental points (3 or 4 V_{max} values) and partly because very limited temperature ranges were employed in order to avoid complications due to nonsaturatable uptake. Thus, the previously published activation energies for the various transport systems represent combinations between those above and those below the transition temperatures (compare values in Table 1).

The transition temperatures for Urd, dThd and Hyp-Gua transport in N1S1-67 cells are lower than that (25°) reported for adenosine transport by alveolar macrophages (Berlin, 1973). A change in activation energy at about 20° has also been reported for D-glucose exchange transport in human erythrocytes (Lacko, Wittke & Geck, 1973), although a similar study failed to detect a transition temperature for this system (Hankin & Stein, 1972). The transition temperature for 2-deoxy-D-glucose transport in N1S1-67 cells is significantly higher than that reported for the D-glucose transport system of erythrocytes. The finding that the transition temperature for the choline and 2-deoxy-D-glucose transport systems are 10–15° higher than those for the nucleoside and Hyp-Gua transport systems suggests that the lipid environments of these two groups of transport systems differ. This finding is in agreement with the view that localized areas of different lipid composition exist within the membrane and that these localized areas may undergo phase separations independent of each other. Hence, at certain temperatures the membrane may exist as a mosaic of areas of both solid and fluid lipids in the membrane. The two groups of transport systems also differ with respect to their metabolic stability and sensitivity to inactivation by *p*-chloromercuric

benzoate. The nucleoside and Hyp-Gua transport systems are metabolically unstable, whereas the choline and 2-deoxy-D-glucose transport systems are relatively stable (Plagemann, Richey, Zylka & Erbe, 1975; Zylka & Plagemann, 1975). The nucleoside and Hyp-Gua transport systems are also more sensitive to inactivation by sulfhydryl reagents than the latter two (*see* Plagemann & Richey, 1974). It remains to be determined, however, whether these different properties of the two groups of transport processes are interrelated.

Steele and Jenkin (1972) have reported that the presence of *cis*-6-octadecenoic acid (melting point = 29°) in the culture medium markedly reduces the growth rate of N1S1-67 cells in suspension culture (*see* Fig. 3*B*) and results in the reduction of the quantity of alkyl diacyl glycerols associated with the cells and in a marked substitution of 18:1 for 16:0 and 18:0 fatty acids in the alkyl diacyl glyceride and triglyceride fractions of the total cell lipids. Furthermore, the concentration of dihexosyl ceramides was slightly increased, whereas the monohexosyl ceramide concentration was correspondingly reduced. Recent results indicate that the chemical changes in the plasma membrane lipids of N1S1-67 cells after growth in medium containing *cis*-6-octodecenoic acid are similar to those previously reported for whole cell lipids (Wennerstrom & Jenkin, *personal communication*). We have compared the temperature dependencies of the various transport systems in N1S1-67 cells that had been propagated in the presence of *cis*-6-octadecenoic acid for 27 hr with those of normal N1S1-67 cells. The transport capacities for Urd, dThd, choline and 2-deoxy-D-glucose were about the same for both untreated and treated cells and no significant differences in transition temperatures and activation energies were observed (not shown), except for a slightly lower activation energy for 2-deoxy-D-glucose transport above the transition temperature in treated than in untreated cells (Fig. 3*A*). The latter difference seems significant since the same difference was apparent in companion transport measurements with 500 μM 2-deoxy-D-glucose (not shown) and because such differences were not observed with replicate samples of an untreated population of cells when analyzed in a single experiment. Nevertheless, the overall results indicate that the lipid changes induced by *cis*-6-octadecenoic acid in N1S1-67 cells had little effect on the temperature characteristics of the various transport processes of these cells.

In other experiments we have propagated N1S1-67 cells for 48 hr in culture medium containing 40 μg nystatin per ml, or 0.01% (v/v) Tween 40. At these concentrations, both substances were only slightly

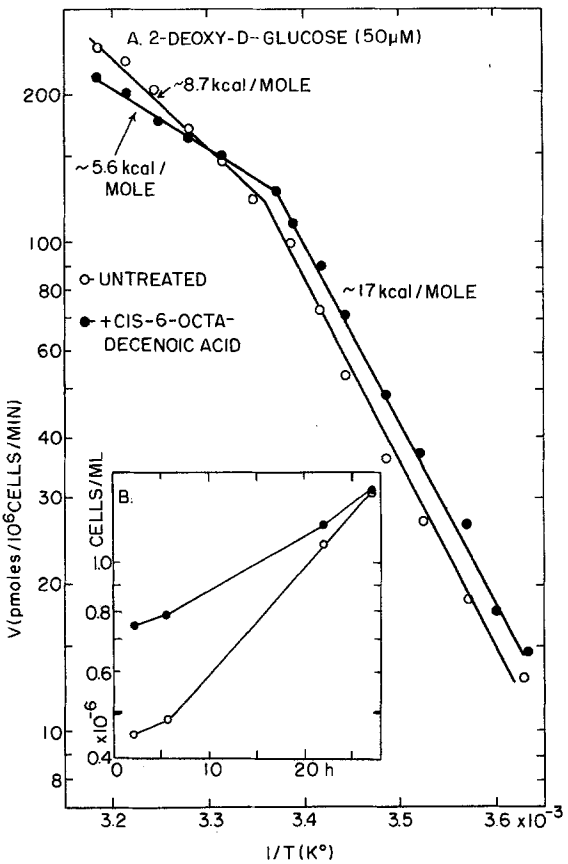

Fig. 3. Temperature dependence of 2-deoxy-D-glucose transport in cells propagated in absence and presence of *cis*-6-octadecenoic acid. Suspensions of cells were propagated for 27 hr in Swim's medium 67 with and without 125 μg of *cis*-6-octadecenoic acid per ml premixed with fatty acid-free serum albumin (*see* Steele & Jenkins, 1974) and monitored for cell density (*B*). Then duplicate samples of each suspension were analyzed for their initial rates of 2-deoxy-D-glucose transport as a function of temperature (*A*) as described in the legend to Fig. 2

toxic reducing growth by not more than 10–20% over the 48-hr incubation period. The polyene antibiotic nystatin has been shown to inhibit the active transport of monosaccharides by certain eucaryotic cells, presumably by interacting with cholesterol in the membrane thereby altering membrane fluidity (Komor, Komor & Tanner, 1974). Tweens are nonionic detergents and have been shown to enhance the uptake of various drugs by increasing the permeability of the plasma membrane (*see* Riehm & Biedler, 1972). N1S1-67 cells propagated in the presence of nystatin or Tween 40, however, exhibited an unaltered capacity to

transport dThd and 2-deoxy-D-glucose and the Arrhenius plots for the transport systems of these cells were identical to those of untreated control cells (data not shown). We are presently attempting to incorporate other fatty acids with varying melting points into plasma membrane lipids of Novikoff cells in order to further assess the effect of membrane fluidity on the temperature characteristics of the various transport processes. The propagation of mouse L cells with various Tween supplements or fatty acid-bovine serum albumin complexes has been shown to cause changes in fatty acid composition of total cell lipids (Wisnieski, Williams & Fox, 1973; Williams, Wisnieski, Rittenhouse & Fox, 1974; Ferguson, Glaser, Bayer & Vagelos, 1975), but the effect of these lipid changes on membrane transport in L cells has not been assessed as yet.

It has been observed that lipid solvents such as ethanol (Plagemann & Erbe, 1974*b*; Scholtissek, 1974), phenethyl alcohol (Plagemann & Roth, 1969; Plagemann, 1970*a*) and dimethylsulfoxide (Collins & Roberts, 1971; Scholtissek, 1974) markedly inhibit the transport of nucleosides, purines, monosaccharides and choline. Although the inhibitions are of an apparent simple competitive type, the effect is most likely mediated through a reversible interaction of the solvents with the plasma membrane (*see* Plagemann & Richey, 1974). This view is supported by the fact that all transport systems investigated are inhibited to a similar extent and that the effect is observed at solvent concentrations just below those that cause lysis of the cells. As long as the cells are not rendered stainable by trypan blue by this treatment, however, the inhibitions are readily reversed by removal of the solvents. The inhibitions, therefore, probably, involve a reversible structural alteration of the membrane most likely by affecting the properties of the plasma membrane lipids. In view of these findings we have determined the effect of ethanol and phenethyl alcohol on the temperature response of dThd and 2-deoxy-D-glucose transport by N1S1-67 cells. In addition, the effect of two other competitive inhibitors of nucleoside, purine and monosaccharide transport, cytochalasin B and Persantin, were investigated. Cytochalasin B is a very potent inhibitor of 2-deoxy-D-glucose transport ($Ki = 0.5$–1 μM; Estensen & Plagemann, 1972), but also, though less effectively, inhibits dThd transport ($Ki = 6$ μM, Plagemann & Estensen, 1972). At concentrations of 10 μM, cytochalasin B also inhibits various other membrane-related processes such as cell movement, cytokinesis, and phagocytosis (*see* Plagemann & Richey, 1974). These effects, however, seem to be unrelated to the inhibition of monosaccharide transport (Estensen & Plagemann, 1972). Persantin inhibits the transport of the same sub-

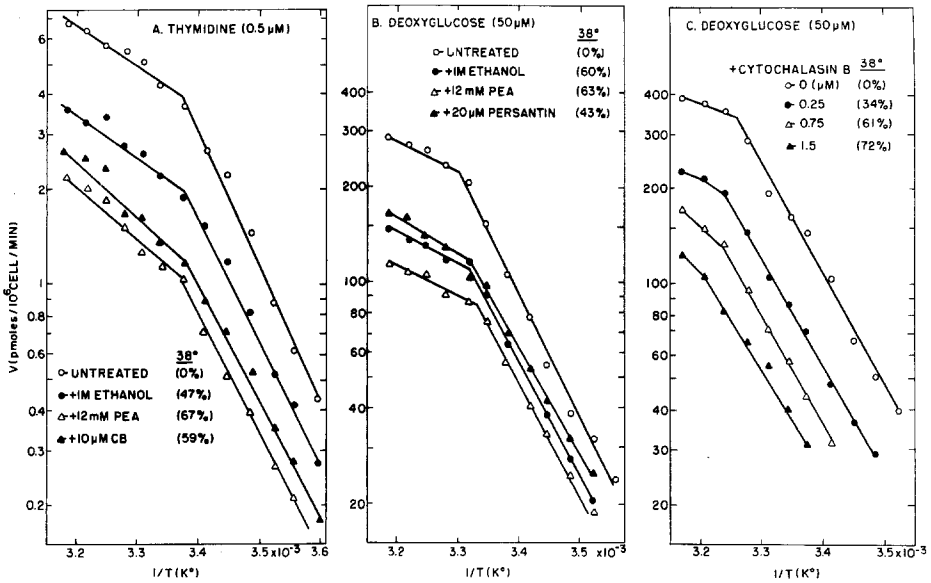

Fig. 4. Effect of ethanol, phenethyl alcohol, Persantin and cytochalasin B on the temperature dependence of dThd and 2-deoxy-D-glucose transport. The experiment was conducted as described in the legend to Fig. 2, except that the samples were supplemented where indicated with 1 M ethanol, 12 mM phenethyl alcohol, 20 μM Persantin or the indicated concentrations of cytochalasin B and that the incubation period was 3 rather than 5 min. In (*A*) the medium was BM42B containing 0.5 μM [^{3}H] dThd (700 cpm/pmole) and in (*B* and *C*) BM42A containing 50 μM [^{3}H] 2-deoxy-D-glucose (0.7 cpm/pmole). All points are averages of duplicate samples. The values in brackets in each frame indicate the per cent inhibition of the initial rate of transport at 38°

strates as cytochalasin B, but has no effect on additional membrane processes (*see* Plagemann & Richey, 1974).

Fig. 4 illustrates the results from these experiments. All inhibitors were employed at concentrations that inhibited dThd and 2-deoxy-D-glucose transport between 40 and 70% at 38° (*see* inserts, Fig. 4). The results show that the presence of 1 M ethanol or 12 mM phenethyl alcohol had no significant effect on the transition temperature of either the dThd or 2-deoxy-D-glucose transport system or on the activation energies below and above the transition temperature (Fig. 4*A* and *B*). The experimental variations in these tests, however, are such that alterations in transition temperature of less than 3° cannot be detected. Nevertheless, spin-labeling studies using TEMPO (Shimsheck & McConnell, 1973) have shown that 1 M ethanol has also no effect on the transition temperature of the lipids in kidney medulla membranes (L. Sillerud and R.E. Barnett, *in preparation*).

The presence of 10 μM cytochalasin B also had no effect on the temperature response of dThd transport (Fig. 4*A*) and 20 μM Persantin had no effect on the temperature response of 2-deoxy-D-glucose transport (Fig. 4*B*). These results also indicate that the relative inhibition of the two transport systems by the various inhibitors was independent of the temperature of incubation. This finding coupled with the fact that the phosphorylation of these substrates is not inhibited by phenethyl alcohol, ethanol, Persantin or cytochalasin B at the concentrations employed in the present study (*see* Plagemann & Richey, 1974) support our conclusion that the shift in activation energy at the transition temperature reflects a property of the transport systems and is not simply due to a shift from transport to phosphorylation as the rate-limiting step.

In contrast to the results in Fig. 4*A* and *B*, cytochalasin B treatment caused an increase in the transition temperature for 2-deoxy-D-glucose transport, although it had no effect on the activation energy below the transition temperature (Fig. 4*C*). The transition temperature increased progressively with an increase in cytochalasin B concentration in the medium. Furthermore, the effect did not seem to be due to a temperature-dependent alteration in the affinity of cytochalasin B for the glucose transport system. The results in Fig. 5 show that the inhibition of 2-deoxy-D-transport by cytochalasin B was of an apparent simple competitive type at both 20° and 40° and that the *Ki* of inhibition was also about the same at both temperatures (about 0.5 μM). Colchicine treatment of macrophages causes a change in the transition temperature of adenosine transport similar to that of 2-deoxy-D-glucose transport of N1S1-67 cells induced by cytochalasin B (Berlin, 1973). Colchicine and colcemid competitively inhibit the transport of various nucleosides, purines, choline, but not of 2-deoxy-D-glucose by mammalian cells (Mizel & Wilson, 1972; Berlin, 1973; Plagemann & Erbe, 1974*b*). The inhibition is not mediated by an interaction with microtubules since vinblastine, which interacts with microtubules as effectively as colchicine, has no effect on nucleoside transport (Plagemann, 1970*b*; Berlin, 1973), whereas hemicolchicine, which fails to react with microtubules, is an effective transport inhibitor (Mizel & Wilson, 1972; Berlin, 1973). One interpretation of these results suggests that the interaction of cytochalasin B or colchicine with the membrane or certain transport systems alters the fluidity of the lipids associated with these transport systems. Various transport systems, however, do not seem to be affected equally since cytochalasin B had no effect on the temperature transition of dThd transport. This difference could be related to the fact that much higher concentrations of cytochala-

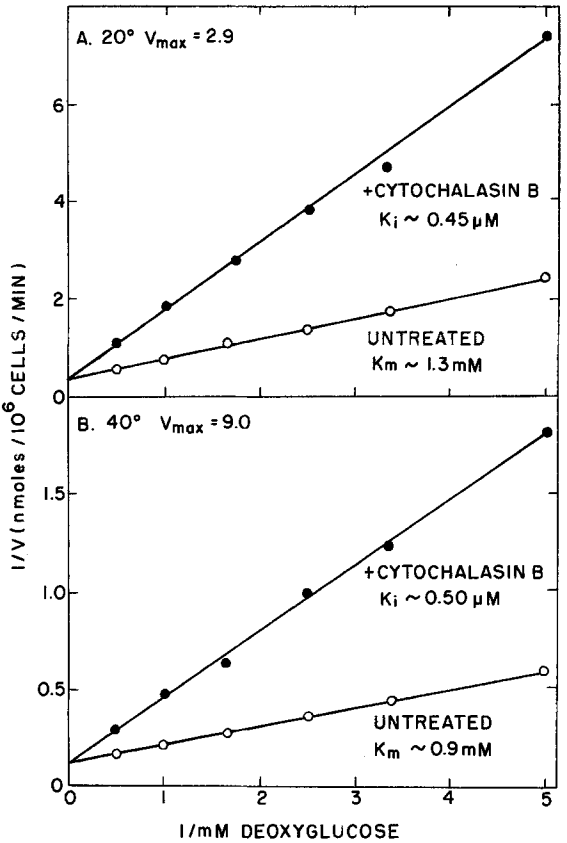

Fig. 5. Effect of cytochalasin B on 2-deoxy-D-glucose transport as a function of substrate concentration at 20° and 40°. Samples of a suspension of 2×10^6 cells/ml of BM42A were equilibrated at 20° (*A*) or 40° (*B*) for about 2 min. Duplicate 1-ml samples of each suspension were then supplemented where indicated with 1 μM cytochalasin B and 0.2, 0.3, 0.4, 0.6, 1 or 2 mM [^{3}H] 2-deoxy-D-glucose (0.7 cpm/pmole). The samples were incubated for 3 min at the appropriate test temperature and then analyzed for radioactivity in total cell material. All points are averages of the duplicate samples

sin B are required to inhibit dThd transport than D-glucose transport. The effect of cytochalasin B on membrane structure or fluidity, for instance, might vary with its concentration and the type or location of the responsible lipids within the membrane. Cytochalasin B at a concentration of 10 μM, also had no effect on choline transport regardless of the temperature of incubation (15–40°; data not shown). The membrane changes induced by the lipid solvents also failed to be reflected in alterations in the temperature response of the two transport systems. Further studies are, therefore, clearly required to elucidate the factors determining the temperature response of the various transport systems

and the underlying mechanism of the temperature transition, particularly to what extent the membrane lipids are involved.

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