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Glucose Transport in *Acholeplasma laidlawii* B: Dependence on the Fluidity and Physical State of Membrane Lipids

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The uptake of D-glucose by *Acholeplasma laidlawii* B occurs via a mediated transport process, as shown by the following observations: (i) glucose permeates *A. laidlawii* B cells at a rate at least 100 times greater than would be expected if its entry occurred only by simple passive diffusion; (ii) the apparent activation energy for glucose uptake in *A. laidlawii* is significantly lower than that expected and observed for the passive permeation of this sugar; (iii) glucose uptake appears to be a saturable process; (iv) glucose uptake can be completely inhibited by low concentrations of phloretin and phlorizin; and (v) glucose uptake is markedly inhibited at temperatures above 45 C, whereas the passive entry of erythritol continues to increase logarithmically until at least 60 C. The metabolism of D-glucose by this organism is rapid and, at low glucose concentrations, the intracellular radioactivity derived from D-[^{14}C]glucose is at any given time a reflection of the net effect of glucose transport, glucose metabolism, and loss from the cell of radioactive metabolic products. Care must thus be taken when attempting to determine the rate of glucose transport by measuring the accumulation by the cells of the total radioactivity derived from D-[^{14}C]glucose. The rate of uptake of D-glucose by *A. laidlawii* B cells is markedly dependent on the fatty acid composition and cholesterol content of the plasma membrane and exhibits a direct dependence on the fluidity of the membrane lipids as measured by their reversible, thermotropic gel to liquid-crystalline phase transition temperatures. In contrast to the transport rates, the apparent activation energy for glucose uptake above the phase transition temperature is not dependent on membrane lipid composition. At the temperature range within the membrane lipid phase transition region, the apparent activation energy of glucose uptake is different from the activation energy observed at temperatures above the phase transition. This may reflect the superimposed operation within the phase transition region of more than one temperature-dependent process.

Acholeplasma laidlawii B is an organism which is uniquely suited to the study of membrane structure and function. This relatively simple procaryotic microorganism has no cell wall or internal membrane systems. The limiting or plasma membrane, which contains essentially all of the cellular lipid, can thus be easily isolated in relatively pure form. In addition, the fatty acid composition of the membrane lipids can be significantly and reproducibly altered by the simple addition of appropriate exogenous fatty acids to a lipid-poor growth medium (8). When grown in the absence of exogenous cholesterol, the *A. laidlawii* B membrane contains no cholesterol or other steroids, but this organism will incorporate moderate amounts of cholesterol and other sterols if these compounds are added to the growth medium (9, 10). Since alterations in the fatty acid composition or cholesterol content do not significantly affect the qualitative or quantitative distribution of the membrane polar lipids or membrane proteins, this organism is an ideal system in which to study the effect of variations in the nature of the hydrophobic core of the plasma membrane on various membrane properties and functions (for reviews, see references 9, 15; S. Razin, *In* J. F. Danielli, M. D. Rosenberg, and D. A. Cadenhead (ed.), *Progress in Surface and Membrane Science*, in press).

We have previously shown that the rates at which several nonelectrolytes passively permeate intact *A. laidlawii* B cells are markedly dependent on the chemical structure and chain length of the membrane lipid fatty acids (3, 10, 16). The incorporation of branched-chain or unsaturated fatty acids, or fatty acids of re-

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duced chain length, increases nonelectrolyte permeability to a similar extent in both cells and liposomes generated from the total membrane lipid. At temperatures above the membrane lipid phase transition temperatures, the incorporation of cholesterol decreases the nonelectrolyte permeability of both the plasma and liposomal membranes. The mean apparent activation energies calculated for the permeation of several nonelectrolytes into intact cells and into liposomes are the same, within experimental error. The activation energies for permeation are similar to the hydration energies of the permeant molecules, suggesting that nonelectrolytes passively permeate both the biological and artificial membrane systems as single, fully dehydrated molecules. In contrast to permeation rates, which are dependent on both permeant structure and membrane lipid composition, activation energy values for the overall permeation process are dependent only on permeant structure and are not significantly influenced by variations in fatty acid composition or cholesterol content. Finally, the permeability of *A. laidlawii* cells increases as a direct function of the increasing fluidity of the membrane lipids, as measured by their reversible, thermotropic gel to liquid-crystalline phase transition temperatures (3, 11, 16).

The objective of the work described here was to determine whether or not a mediated transport system in *A. laidlawii* B would exhibit a dependence on the fluidity and physical state of the membrane lipids similar to that previously observed for the passive diffusion of nonelectrolytes into this organism.

Tarshis and co-workers have reported that glucose, fructose, and maltose are actively transported by *A. laidlawii* B (17–19). In these studies glucose transport was monitored by simply measuring the intracellular accumulation of sugar-derived radioactivity at times of the order of 5 min. These investigators assumed that the transport of sugars across the plasma membrane was the rate-limiting step in the accumulation of intracellular radioactivity, an assumption supported by their calculation that glucose metabolism (phosphorylation) by cell-free extracts occurs with K_m similar to that observed for glucose accumulation but with an 11-fold higher V_{max} (17). However glucose phosphorylation in cell-free extracts was assayed after the addition of 10 mM adenosine 5′-triphosphate to the reaction mixture. Since the intracellular concentration of adenosine 5′-triphosphate in intact *A. laidlawii* B cells is not known, the objection can be raised that the rate of metabolism in intact cells may be very different from that measured in the cell-free extracts. To clarify this point, we studied the metabolism of radiolabeled glucose at various glucose concentrations and times to assess the effect of glucose metabolism and possible loss of radioactive metabolic products on the apparent rate of glucose accumulation by intact cells. We also undertook a partial characterization of the glucose transport system in this organism to confirm that this process is indeed protein mediated, and to determine whether glucose uptake occurs by an active transport or by a metabolically driven facilitated diffusion system.

MATERIALS AND METHODS

Organism and growth conditions. *A. laidlawii*, strain B (formerly *Mycoplasma laidlawii*), was originally obtained from G.ff. Edward, Wellcome Research Laboratories, Beckenham, Kent, England. Cells were grown at 35 C in a fatty acid-supplemented, lipid-poor growth medium as described by McElhaney and Tourtellotte (11). Exogenous fatty acids were added to the growth medium as sterile ethanolic solutions to yield a final concentration of 0.13 mM. Palmitic acid plus an unsaturated fatty acid were present at a concentration ratio which resulted in the incorporation of each to the level of about 40 to 45 mol% of the total fatty acids of the membrane lipids. Cholesterol (final concentration 25 mg/liter) was added to the growth medium after thorough mixing with ethanolic solution of the appropriate exogenous fatty acids, to insure that cholesterol remained in solution in the growth medium. The cells were harvested in late log phase by centrifugation at 7,000 × *g* for 20 min and washed several times in 200 mM sucrose or in isotonic buffered saline solution before being used for transport measurements. The total cellular protein present in cell suspensions was determined by the sensitive Biuret procedure of Koch and Putnam (6).

Measurement of fluxes. Swelling of cells induced by the uptake of permeant was followed by monitoring the absorbance at 450 nm of a cell suspension (3). A suspension of cells (50 μl) in 200 mM sucrose, to which the cell membrane is impermeable, was added, while stirring, to 4.5 ml of permeant solution at the same concentration in a thermally regulated spectrophotometer cell. As permeant entered the cells they swelled, resulting in a decrease in the light absorbance at 450 nm of the suspension. These cells have been shown to behave as ideal osmometers and to exhibit a linear proportionality between cell volume (proportional to 1/osmolarity) and reciprocal absorbance when suspended in sucrose or other impermeable nonelectrolytes (10). Also, the size and shape of cells grown in the presence of different fatty acids did not show any systematic or significant variation when suspended in 200 mM sucrose solutions; suspension in 200 mM sucrose, which is hypotonic with respect to the growth medium, causes all cells to become spheri-

cal (10). Therefore, the initial rate of increase of (1/A) when cells are added to the permeant solution is a valid indicator of the relative nonelectrolyte permeabilities of the cells.

For measuring radioisotope tracer fluxes, cells were suspended in 100 mM NaCl containing 10 mM $MgCl_2$ and 10 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.4. Cell suspension and radioactively labeled permeant solution in buffered saline were mixed at zero time in a water-jacketed tube and stirred. Portions (200 μl) of the mixture were removed at the desired intervals, using a Gilson Pipetman, and added to 3 ml of ice-cold quench solution containing 0.15 mM phloretin in isotonic buffered saline. After filtration of the quenched cell suspensions through membrane (47-mm diameter, 0.45 μm; Millipore Corp.) filters at 15 lb/in^2 negative pressure, the cells were washed on the filters four times with ice-cold quench solution and the filters were dried and counted for radioactivity in toluene-Omnifluor solution. Zero time samples were obtained by adding cell suspension and labeled permeant solution directly to the quench solution without prior mixing.

Analysis of metabolic products. After incubation with radioactive glucose, cells were added to ice-cold quench solution, then filtered and washed. The membrane (Millipore) filters were immediately frozen in liquid nitrogen, then soluble radioactive material was extracted by placing the frozen filters in 20 ml of boiling distilled water and boiling for 5 min. After removal of the water by lyophilization, the residue was taken up in 0.2 ml of 25% (vol/vol) aqueous acetic acid. These suspensions were centrifuged at top speed for 5 min in the clinical centrifuge, then 50 μl was spotted onto Whatman no. 1 filter paper. Chromatography was carried out for 18 h at room temperature in the descending mode, using Solvent 28 (1 M aqueous ammonium acetate, pH 5.0:95% ethanol, 3:7 [vol/vol]). Reducing sugars were stained with alkaline silver reagent and 2-deoxyglucose with 1% boric acid plus 1% HCl in methanol (1). The patterns of radioactivity on the chromatograms were determined by cutting a 4-cm-wide strip of paper into squares (2 by 2 cm) and counting the radioactivity of each square in toluene-Omnifluor.

Other techniques. Lipid extraction and purification, liposome formation, and differential thermal analysis were carried out exactly as described by McElhaney et al. (10). The fatty acid composition of the membrane lipids was analyzed by gas-liquid chromatography of the fatty acid methyl esters, formed by acid-catalyzed transesterification. Lipid was heated at 70 C for 2 h with 10 ml of anhydrous methanol containing five drops of concentrated H_2SO_4 and then cooled, 20 ml of water was added, and the reaction mixture was extracted twice with 10-ml portions of hexane to obtain the methyl esters.

RESULTS

Metabolism of D-glucose by A. laidlawii. Figure 1 shows the chromatographic pattern obtained from acid-soluble extracts of *A. laidlawii* cells incubated for 1 min at 37 C in the presence of 200 mM D-[^{14}C]glucose. The fact that essentially all the radioactivity occurred where standard D-glucose was found indicates that very little of the intracellular glucose taken up was metabolized under the conditions employed for the measurement of swelling rates. Therefore, use of the swelling rate assay of glucose uptake circumvented the difficulties posed by the rapid metabolism of glucose and subsequent loss of metabolic products observed under other assay conditions.

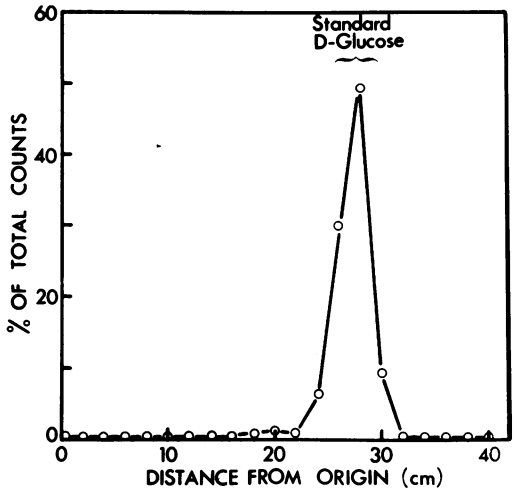

FIG. 1. *Metabolism of 200 mM D-glucose by A. laidlawii. The labeling pattern shown was obtained after chromatography of an acid-soluble extract of cells incubated for 1 min with 200 mM D-[^{14}C]glucose. The position of standard D-glucose is indicated by the bracket. The abscissa represents the percentage of recovered radioactivity in each fraction.*

A much more significant portion of the radioactive glucose taken up was metabolically altered at lower glucose concentrations, indicating that the metabolism of glucose was more readily saturated than its uptake. Figure 2 shows the chromatographic pattern obtained when cells were incubated at 37 C with 0.02 mM D-[^{14}C]glucose for various periods of time. The conditions were similar to those used by Tarshis et al. to measure glucose uptake (18–19). After 1 min, a large proportion of the intracellular radioactivity was present on the chromatogram at a position having the same R_f as glucose phosphate. The fact that the relative proportions of radioactivity at the various positions were only slowly altered from 1 to 30 min suggests that a relatively steady-state labeling pattern of metabolic intermediates was seen after 1 min under these conditions. The effects

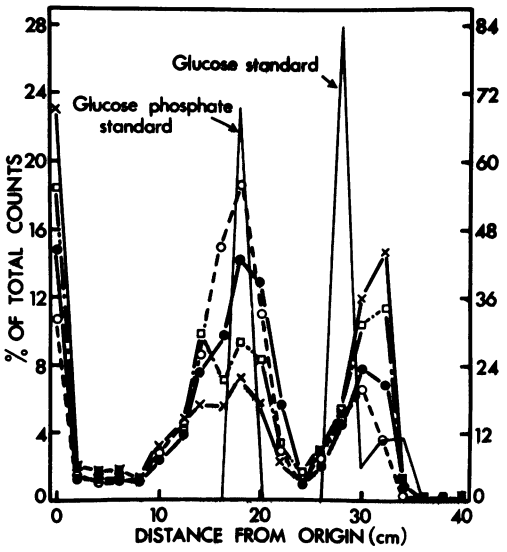

FIG. 2. *Metabolism of 0.02 mM* D-*glucose by A. laidlawii. The labeling patterns shown were obtained after chromatography of acid-soluble extracts of cells incubated at 37 C with 0.02 mM* D-[^{14}C]*glucose. The shaded peak is standard* D-[^{14}C]*glucose. Symbols:* ○, *1-min incubation;* ●, *10 min;* □, *20 min;* ×, *30 min. The abscissa represents the percentage of recovered radioactivity in each fraction.*

of 0.5 mM unlabeled D-glucose and of 10 mM iodoacetic acid on the uptake of 0.2 mM D-[^{14}C]glucose at 37 C (Fig. 3) support this contention. In the absence of iodoacetic acid, a rapid uptake of radioactivity was seen in the first 5 min, followed by a loss of counts after that, presumably resulting from saturation of the metabolic pools, then loss of permeable metabolic products. The acceleration, rather than inhibition, of uptake of radioactivity by the presence of extra unlabeled glucose can be explained by a stimulation of phosphorylation and subsequent metabolism of [^{14}C]glucose due to the adenosine 5′-triphosphate made available by the metabolism of this unlabeled glucose. Iodoacetic acid (10 mM), which has been shown by chromatographic analysis to inhibit glucose metabolism in these cells (data not presented), prevented the initial burst of uptake of label and the subsequent loss of intracellular radioactivity. To minimize the effect on the apparent glucose uptake of the loss from the cells of metabolic products, we sampled at 15-s intervals when measuring the uptake of low concentrations of labeled glucose, rather than after 5 min as was done by Tarshis et al. (18, 19).

Evidence for mediated glucose permeation. A comparison of swelling rates of cells and of liposomes in glucose and in erythritol shows that the swelling of cells in glucose was faster than would be predicted for its uptake by passive diffusion through the lipid bilayer. The swelling rate of cells in glucose at 25 C was about two-thirds of the rate in erythritol (Fig. 4). The apparent swelling rates of liposomes prepared from the total membrane lipids were lower than for the cells themselves because of differences in optical properties. Nevertheless, the swelling rate of liposomes in glucose, which was undetectable at 25 C, was obviously less than two-thirds the rate seen in erythritol. This low glucose permeability of liposomes, compared to cells, was even more apparent at 45 C. There, the apparent swelling rate of liposomes in erythritol was about equivalent to that of cells at 25 C, but the swelling of liposomes in glucose was still undetectable. Since liposomes contain only lipid, these structures can serve as a model for passive diffusion of nonelectrolytes through the lipid bilayer in the *A. laidlawii* B plasma membranes. These results therefore demonstrate that the observed rates of glucose permeation of *A. laidlawii* B cells are much too fast to be accounted for by simple passive diffusion alone and suggest that a mediated

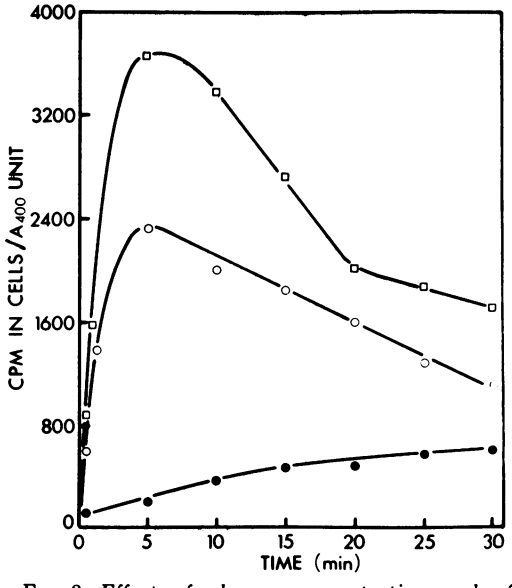

FIG. 3. *Effect of glucose concentration and of iodoacetic acid on* [^{14}C]*glucose uptake by A. laidlawii. The amount of intracellular radioactivity at various times is shown for cells incubated at 37 C with 0.2 mM* D-[^{14}C]*glucose in the presence or absence of added 0.5 mM unlabeled* D-*glucose or 10 mM iodoacetic acid. Symbols:* ○, *control;* □, *0.5 mM unlabeled glucose;* ●, *10 mM iodoacetic acid.*

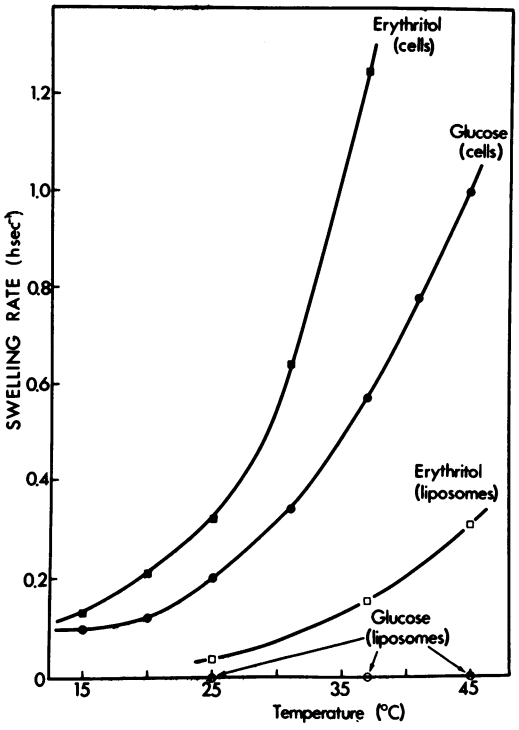

FIG. 4. *Swelling rates of A. laidlawii and derived liposomes in glucose and erythritol. Swelling rates here and in Fig. 5 are expressed in reciprocal hectoseconds (1 hsec = 100 s).*

transport system must be operative in this organism.

The apparent activation energy of glucose uptake by cells grown in the presence of palmitate plus oleate (16:0/18:1_c) or linoleate (16:0/18:2) is about 16 Cal/mol (Table 1). This is further evidence for mediated uptake of glucose, as activation energy values of 19 to 22 and 26 to 29 Cal/mol have been reported for the passive efflux of glucose from sonically treated, unilamellar phosphatidylethanolamine bilayer vesicles and multilamellar phosphatidylcholine bilayer vesicles, respectively (2, 12). The different apparent heats of activation seen with cells supplemented with palmitate plus elaidate (16:0/18:1_t) may result from the fact that the lipid phase transition in these cells fell within the temperature range where the swelling rates are measured. This is discussed later.

A plot of swelling rates versus glucose concentration (Fig. 5) did not pass through the origin. This indicates that an acceleration of glucose uptake occurred at lower glucose concentrations via some saturable process. At high glucose concentrations, glucose appeared to enter the cells by a combination of passive diffusion and mediated transport, since net uptake continued to increase as the glucose concentration increased up to at least 200 mM.

Table 2 gives the effects of the inhibitors phlorizin and phloretin, present in the permeant solution, on swelling rates. Erythritol permeation, which occurs by passive diffusion (10), was not significantly affected by the levels of these compounds used, but glucose and 2-deoxyglucose permeation was strongly inhibited, supporting the suggestion of a mediated pathway for the permeation of these sugars.

The effects of high temperature on glucose and on erythritol permeation are shown in Fig. 6, an Arrhenius plot of swelling rates. The inhibition of swelling in glucose at temperatures above 45 C can be interpreted by assuming a

TABLE 1. *Effect of lipid composition on heats of activation*

Additions to growth medium	Heat of activation (Cal/mol)	
	Erythritol	Glucose
16:0/18:1_t[a]	19.62	14.35
16:0/18:1_t plus cholesterol	26.75	20.91
16:0/18:1_c	18.44	16.64
16:0/18:1_c plus cholesterol	19.16	16.43
16:0/18:2	17.76	16.22
16:0/18:2 plus cholesterol	19.48	16.64

[a] In the fatty acid nomenclature system used, the number before the colon indicates the number of carbon atoms in the fatty acid molecule, and that after the colon is the number of double bonds. The subscript c designates the *cis* configuration for the double bond, and the subscript t, the *trans* configuration.

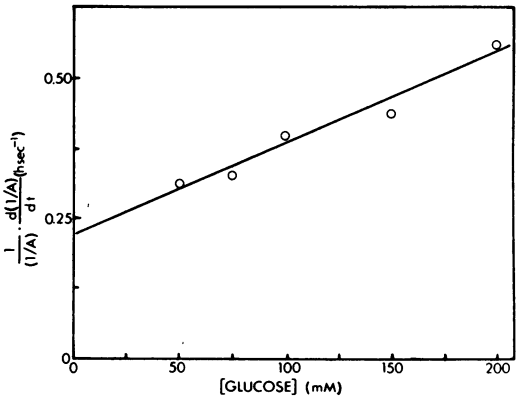

FIG. 5. *Swelling rate of A. laidlawii versus glucose concentration. The total osmolarity of the permeant solution was kept constant by the addition of sucrose.*

TABLE 2. *Effects of inhibitors on swelling rates*[a]

Permeant and assay conditions	Inhibitor used		
	0.2 mM Phlorizin (%)	2.0 mM Phlorizin (%)	0.2 mM Phloretin (%)
D-Glucose, 25 C, 16:0/18:1_t	43	100	100
Erythritol, 25 C, 16:0/18:1_t	18	13	13
2-Deoxyglucose, 25 C, 16:0/18:2	39	85	85

[a] The left hand column gives the permeant used (present at 200 mM), the temperature at which swelling was measured and the fatty acids that were added to the growth medium. The body of the table is percent inhibition.

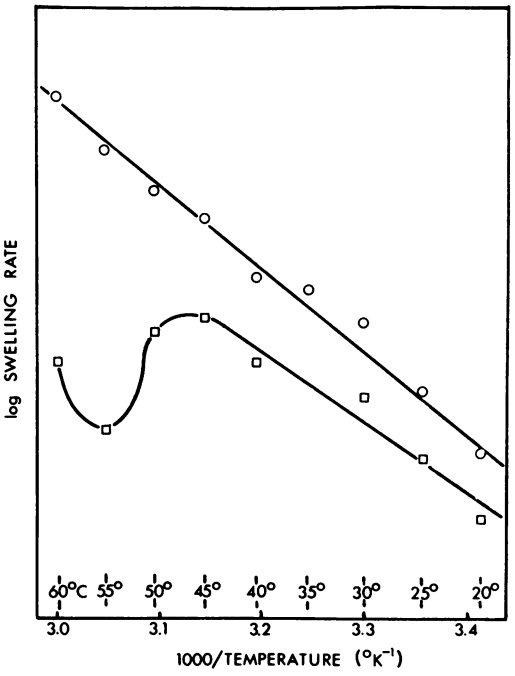

FIG. 6. *Inactivation of glucose transport by high temperature. The logarithm of swelling rate is plotted versus reciprocal temperature (Arrhenius plot). Symbols: O, erythritol; □, glucose.*

thermal inactivation of one or more transport proteins. The increase in swelling rate observed above 55 C may be due to an increase in the passive flux, which would be expected to exhibit a larger activation energy than is seen for mediated transport and thus become relatively more significant in the overall flux observed as the temperature is raised.

Effect of membrane lipid fluidity on glucose flux. The fatty acid compositions of cells grown in the presence of various supplemented fatty acids are given in Table 3. In each case the exogenous unsaturated fatty acid represented nearly one-half of the total fatty acid in the membrane lipid, with exogenous palmitate comprising most of the remaining fatty acid. The presence of cholesterol in the growth medium did not alter the fatty acid incorporation patterns obtained.

Figure 7 is a temperature-based thermogram from the differential thermal analysis of the plasma membranes from cells grown in the presence of various fatty acid supplements. The troughs in these heating curves define the positions of endothermic phase transitions. The large transition centered at 0 C is due to melting of ice in the sample, and the broad troughs represent the crystalline to liquid-crystalline phase transitions of the membrane lipids. For cells grown with palmitate plus oleate, the lipid phase transition occurred near 0 C and was obscured by the ice melting endotherm. As determined from the positions of the lipid phase transitions, the membrane lipid fluidity of these cells decreased in the order of 16:0/18_t < 16:0/18:1_c < 16:0/18:2, as would be predicted from the capillary melting points of the unsaturated fatty acids (18:2 = −5 C; 18:1_c = 10.5 C; 18:1_t = 45.0 C [5]).

Figure 8 gives the swelling rates in erythritol and in glucose of cells grown in the presence of different fatty acid supplements. For both per-

TABLE 3. *Fatty acid compositions of A. laidlawii membrane lipids*

Identity	Fatty acid added as supplement		
	16:0/18:1_t[a]	16:0/18:1_c[b]	16:0/18:2[c]
12:0	6.0	4.3	2.2
13:0	0.9	0.5	0.1
14:0	8.0	6.8	4.4
15:0	0.6	0.8	0.6
16:0	40.1	38.7	32.5
17:0	0.1	0.1	0.2
18:0	—	0.7	1.4
18:1_t	44.2	?[d]	—
18:1_c	?[d]	46.0	3.5
18:2	0.1	1.2	44.4
20:1	—	1.0	—
20:2	—	—	10.5

[a] 0.06 mmol of 16:0 plus 0.07 mmol of 18:1_t per liter of medium.

[b] 0.055 mmol of 16:0 plus 0.075 mmol of 18:1_c per liter of medium.

[c] 0.02 mmol of 16:0 plus 0.11 mmol of 18:2 per liter of medium.

[d] 18:1_c and 18:1_t are not resolved by conventional gas-liquid chromatography analysis so it is not possible to determine whether all the material in the peaks is the isomer designated.

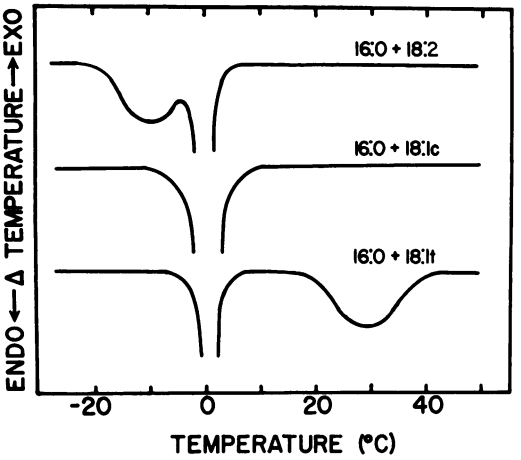

FIG. 7. *Differential thermal analyses of A. laidlawii membrane lipids. The difference in temperature between the reference material and the lipid sample is shown versus the temperature of the heating block. The fatty acid supplements added to the growth medium are indicated on the figure for each trace.*

meants, the swelling rates increased in the order 16:0/18:1_t < 16:0/18/1_c < 16:0/18:2, the order of increasing lipid fluidity. Also, the presence of cholesterol reduced the swelling rate in every case, which is in line with its effect in lowering the mobility and increasing the viscosity of unsaturated lipid bilayers (12). For cells grown in the presence of palmitate plus elaidate, without cholesterol, the swelling rates at 20 C or lower were apparent only. As was previously noted (10), the swelling curves of these cells at these temperatures were biphasic, consisting of an initial anomalously rapid decline in absorbance, followed by a slower decline. In the present work, the swelling rates were calculated from the second, slower portion of the trace. It is postulated that the initial rapid change may be due to lysis resulting from the crystallization of a large portion on the membrane lipid. Indirect evidence that this is indeed the case has already been published (10). In this case, the slower portion would represent swelling of a partially lysed population of cells, and so would not be directly comparable to swelling rates obtained at higher temperatures.

This same effect of membrane lipid fluidity on glucose permeability was seen when the uptake of millimolar concentrations of D-[^{14}C]glucose was followed at 25 C, sampling at 15-s intervals. Under these conditions only the initial burst portion of the uptake of radioactivity was monitored and the efflux of glucose metabolites from the cells should be minimal. The uptake rates of 0.1 or 1.0 mM glucose increased in the order of increasing lipid fluidity (16:0/18:1_t < 16:0/18:1_c < 16:0/18:2) (Fig. 9 and 10).

Table 1 gives the heats of activation for erythritol and D-glucose uptake, obtained from Arrhenius plots of the swelling rates over the temperature range 15 to 60 C. For cells grown in medium supplemented with palmitic plus oleic or palmitic plus linoleic acids, which are above their lipid phase transition over the entire temperature range used for the swelling experiments, the heats of activation do not show a significant systematic change with changing fatty acid composition. This observation would be compatible with the idea that the activation enthalpy is determined by the binding of glucose to a carrier protein at the membrane surface. Significantly higher heats of activation were seen for the permeation of both substances in cells grown in the presence of palmitic plus elaidic acids plus cholesterol, and a lower heat of activation for glucose permeation of cells grown with palmitic plus elaidic acids. Since the membrane lipid phase transition in cells

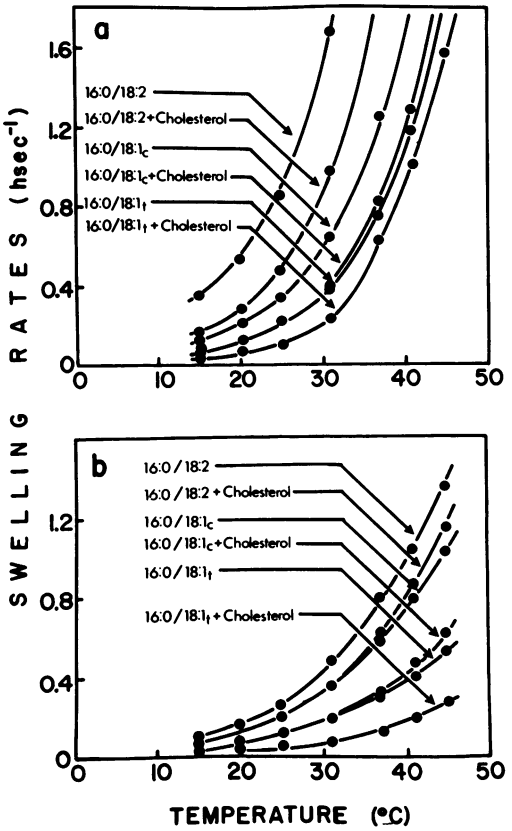

FIG. 8. *Swelling rates of A. laidlawii in erythritol and glucose. Swelling rates are shown as a function of temperature for cells grown in the presence of the lipid supplements indicated. (a) Erythritol; (b) glucose.*

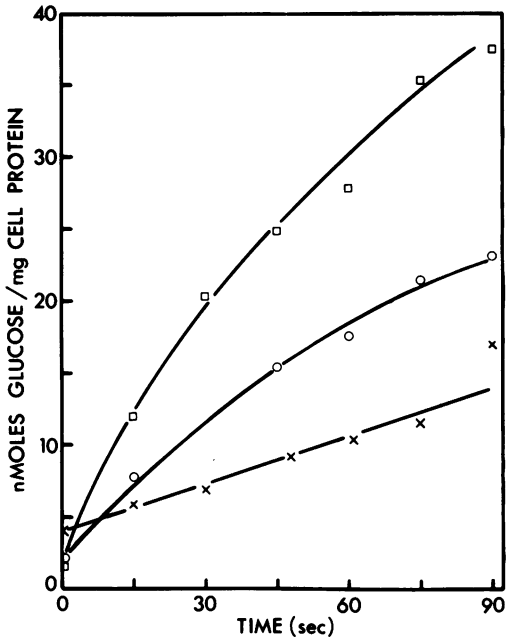

FIG. 9. *Effect of fatty acid composition on uptake of 0.1 mM* D-[^{14}C]*glucose by A. laidlawii. The intracellular radioactivity is shown as a function of time of incubation at 25 C with 0.1 mM* D-[^{14}C]*glucose. Symbols:* □, *16:0/18:2;* ○, *16:0/18:1$_c$;* ×, *16:0/18:1$_t$.*

grown with these fatty acids occurred within the temperature range used for swelling rate measurements (see Fig. 7), the difference in behavior of these cells may reflect a contribution to the apparent rate by some structural rearrangement of membrane lipid within the phase transition region, which alters either the fragility or the permeability of the cells.

It is interesting to note that although the rate of mediated glucose uptake was dependent on the fatty acid composition and cholesterol content of the cell membrane, the apparent enthalpy of activation was not dependent on membrane lipid composition at temperatures above the membrane lipid phase transition temperatures. After the treatment of De Gier et al. (3), it then follows that the changes in uptake rates observed with variations in membrane lipid composition must arise from changes in the entropy of activation. One possible explanation of this result is that insertion into or movement through the plasma membrane by the glucose-carrier complex may be dependent on the packing and fluidity of the hydrocarbon chains of the membrane lipids.

DISCUSSION

The uptake of D-glucose by *A. laidlawii* was more rapid and exhibited a lower heat of activation than would be predicted for its permeation by passive diffusion. It showed saturability under certain experimental conditions and was inhibited by phlorizin or phloretin and by temperatures above 45 C. All these observations support the contention that glucose permeation in this organism occurs via a mediated process. Under the conditions used in this study and by Tarshis et al. (18, 19) to measure the uptake of labeled D-glucose, metabolism of the glucose and loss of radioactivity as metabolic products seemed to play a significant role in determining the amount of intracellular radioactivity observed. Because of this, it was not possible for us to determine whether or not accumulation of glucose against a concentration gradient occurred, or to determine the actual values for the K_m or V_{max} of the transport system itself.

It was established, however, by using a swelling rate assay of permeation and also by measuring the uptake of radioactive glucose at very early times, that the rate of glucose permeation showed a direct dependence on the fluidity of the membrane lipids, similar to that previously observed for the passive diffusion of nonelectrolytes into this organism. The rate of glucose uptake increased as the gel to liquid-crystalline phase transition temperatures of the membrane lipids decreased. At temperatures above the lipid phase transition temperature the incorporation of cholesterol decreases the

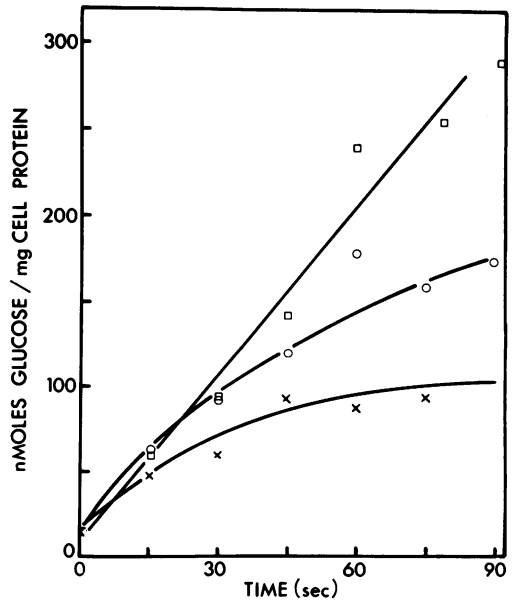

FIG. 10. *Effect of fatty acid composition on uptake of 1.0 mM* D-[^{14}C]*glucose by A. laidlawii. The intracellular radioactivity is shown as a function of time of incubation at 25 C with 1.0 mM* D-[^{14}C]*glucose. Symbols:* □, *16:0/18:2;* ○, *16:0/18:1$_c$;* ×, *16:0/18:1$_t$.*

rate of glucose transport, as would be expected from the known ability of cholesterol to reduce the fluidity of various artificial and natural membranes (9, 12; R. Razin, *In* Danielli et al. (ed.), *Progress in Surface and Membrane Science*, in press). On the other hand, the activation energies for permeation were not dependent on membrane fatty acid compositions if the lipids were above their phase transition temperature, but these activation energies showed significant deviation when permeation was measured within the phase transition region. This dependence of transport on the fatty acid composition of the membrane lipids extends the observations made by Overath et al. (13) and by Linden and Fox (7) of the influence of physical state of the membrane lipids on the β-galactoside and β-glucoside transport systems of *Escherichia coli*. Using unsaturated fatty acid auxotrophs of *E. coli*, up to three discontinuities were seen in the slopes of Arrhenius plots of transport of these sugars. The temperatures at which the discontinuities occurred were dependent on the fatty acid compositions of the membrane lipids, altered by varying the unsaturated fatty acids which were exogenously supplied. Two of these changes in slope could be correlated with the onset and completion of the thermotropic, gel to liquid-crystalline phase transition of the membrane lipids (7).

The dependence of transport on the physical properties of membrane lipids shown with *E. coli* and, in the present work, with *A. laidlawii* B places a constraint on the types of spatial and functional relationships between the membrane lipids and transport proteins which may be postulated. These observations appear inconsistent with models for the transport site featuring a fixed pore of rigid dimensions, or a transport system existing at specific sites which are isolated from the membrane lipid bilayer. Our observations are compatible with mobile carrier models or with other models which predict a dependence of transport rates on the fluidity and physical state of the membrane lipids.

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