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THE EFFECT OF ALTERATIONS IN FATTY ACID COMPOSITION AND CHOLESTEROL CONTENT ON THE NONELECTROLYTE PERMEABILITY OF *ACHOLEPLASMA LAIDLAWII* B CELLS AND DERIVED LIPOSOMES

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SUMMARY

1. The fatty acid composition and cholesterol content of the membrane lipids of *Acholeplasma laidlawii* B were systematically altered and the rates at which glycerol and erythritol passively diffuse into intact cells and into liposomes prepared from the total membrane lipid were measured at a variety of temperatures.

2. The permeability of intact cells and derived liposomes is markedly dependent on the chemical structure and chain length of the fatty acids incorporated into the membrane lipids. Incorporation of branched-chain or unsaturated fatty acids, or fatty acids of reduced chain length, increases nonelectrolyte permeability to a similar extent in both cells and liposomes.

3. The nonelectrolyte permeability of both the plasma and liposomal membrane is reduced by the incorporation of cholesterol.

4. The mean activation energy values calculated for the permeation of glycerol and erythritol into intact cells are 18.2 and 21.3 kcal/mole, respectively. These values are the same, within experimental error, as those calculated for the liposomal system and suggest that glycerol and erythritol permeate both the biological and artificial membrane systems as single, fully dehydrated molecules.

5. 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 affected by variations in fatty acid composition or cholesterol content.

6. The permeability of intact cells and derived liposomes is a function of the fluidity of the membrane lipids as measured by their reversible, thermotropic gel to liquid–crystalline phase transition temperatures. Cells or liposomes placed in isotonic permeant solutions undergo spontaneous lysis when the temperature is reduced to the point where most of the membrane lipids exist in the gel state.

INTRODUCTION

Several studies concerned with the relationship between the fatty acid composition, cholesterol content and the nonelectrolyte permeability of artificial lipid bilayer membrane systems have appeared[1,2]. These studies demonstrate that the

permeability of lipid bilayers toward glycerol and glucose is markedly dependent on the nature and chain length of the fatty acid constituents of the lipid molecules and on the presence or absence of cholesterol in these membrane systems. Phospholipids containing long-chain saturated fatty acids form tightly packed bilayers whose permeability is relatively low. Phospholipids containing branched-chain or unsaturated fatty acids, or short-chain saturated fatty acids, produce more permeable bilayer membranes due to the reduced attractive forces between adjacent lipid molecules. The presence of cholesterol decreases molecular motion in the hydrocarbon chains of unsaturated phospholipids in bilayer membranes resulting in a closer packing[3] and a decreased nonelectrolyte permeability[1,2].

If biological membranes do indeed contain a lipid bilayer core which functions as a permeability barrier, one would expect that the nonelectrolyte permeability of these membranes would be affected by alterations in fatty acid composition and cholesterol content, in a manner at least qualitatively similar to that observed in artificial lipid bilayer systems. Apparently because of the inability to produce large, controllable changes in the fatty acid composition and sterol content of most organisms, little work has been done toward elucidating the relationship between lipid composition and permeability in biological systems. The fatty acid composition and cholesterol content of the membrane lipids of *Acholeplasma laidlawii* B (formerly *Mycoplasma laidlawii* B), however, can be dramatically and systematically altered by the addition of exogenous fatty acids or sterols to a lipid-poor growth medium[4]. In this paper we report on the effect of such variations in the hydrophobic portion of the membrane lipids on the nonelectrolyte permeability of the plasma membrane of *A. laidlawii* and we compare the permeabilities of intact cells of this organism with liposomes prepared from the total membrane lipids. Some preliminary results from this study have been presented previously[5].

EXPERIMENTAL

A. laidlawii B cells were grown in lipid-poor growth medium and harvested in late log phase by centrifugation as described previously[6]. Suitable quantities of exogenous fatty acids were added to the growth medium as sterile ethanolic solutions (10 mg/ml) before inoculation. In the cholesterol experiment it was necessary to add the cholesterol (25 mg/l) to the growth medium after thorough mixing with ethanolic solutions of exogenous fatty acids to insure that cholesterol remained in solution. Collected cells were washed and equilibrated in 200 mM sucrose and the permeability of these cells was then determined by optical measurement of the initial swelling rates in 200 mM solutions of glycerol or erythritol as described by Bangham *et al.*[7] and De Gier *et al.*[2]. A Zeiss PMQII spectrophotometer equipped with a high-speed magnetic stirring bar, special water-jacketed cuvette, and recorder, was used to measure absorbance changes with time. The total membrane lipid was quantitatively extracted from the remainder of the harvested cells by the method of Bligh and Dyer[8] and freed from traces of non-lipid contaminants by silicic acid column chromatography. Liposomes were prepared from this purified lipid as described previously[2], except that the lipid was dispersed in 50 mM KCl–50 mM $MgSO_4$ solution. The presence of Mg^{2+} was found to reduce the ζ potential of the liposomes from -65 to about -18 mV and to increase their osmotic response as monitored optically.

The glycerol and erythritol permeabilities of these liposomes were then measured by exactly the same technique as utilized for intact cells. Because of differences in the size, surface area and ionic environment between whole cells and derived liposomes, a quantitative comparison of the relative permeabilities of the two membrane systems was not attempted.

The differential thermal analysis was carried out on a DuPont 900 Thermal Analyzer using glass macro tubes with glass beads as a reference material. Analyses were done at the highest instrumental sensitivity at heating rates of 3–10 °C per min. Cooling rates of up to 10 °C per min could be obtained by circulation of N_2 gas, chilled by passage through a copper coil immersed in liquid N_2, through the differential thermal analysis cell. Membranes were prepared by osmotic lysis of cells in distilled water and collected by centrifugation in a Beckman L2-65B ultracentrifuge at $30\,800 \times g$ for 60 min. The yellow membrane pellet was then resuspended in distilled water and centrifuged again at $110\,000 \times g$ for 60 min. After careful removal of the supernatant, the tightly-packed membrane pellet thus obtained was transferred by means of a Pasteur pipet into the differential thermal analysis sample tube. The sample tube itself was placed in an International Clinical Centrifuge and spun at top speed for 3 min to insure that all air bubbles were removed and that the membranes were tightly packed. Aqueous dispersions of the total membrane lipid were prepared by adding a 4-fold excess (w/w) of distilled water to a thin film of lipid, previously dried overnight under vacuum in the presence of P_2O_5, followed by vigorous mechanical agitation in the presence of several glass beads.

RESULTS

Cellular volume and optical extinction

It has been shown that volume changes occurring during the osmotic swelling of liposomes prepared from natural phospholipids can be easily followed by optical means, since a linear relationship between volume and reciprocal absorbance is observed experimentally[7,9]. Since these liposomes behave as nearly ideal osmometers, the permeability of these spherical lipid bilayer membrane systems can be determined by optical measurement of initial swelling rates (initial rates of volume change) in isotonic solutions of nonelectrolytes[7]. Synthetic lecithins containing a variety of different fatty acids form liposomes which are equivalent with respect to optically monitored changes in volume[2]. We have observed that liposomes prepared from the total membrane lipid of *A. laidlawii* (data not presented) also behave as nearly equivalent, ideal osmometers despite alterations in fatty acid composition or cholesterol content.

The situation with intact cells is more complex. Alterations in fatty acid compositions have a marked effect on cell morphology. Cells grown in saturated or isobranched fatty acids exist in the growth medium primarily as single spheres or short swollen filaments, while cells grown in unsaturated fatty acids exist primarily as long, fine filaments[4]. These marked differences in cell size and shape precluded a simple and straightforward application of the optical swelling technique to measure cellular permeability. We found, however, that by equilibrating the cells in 200 mM sucrose (hypotonic to growth medium) filamentous cells were converted nearly quantitatively to spheres, apparently by a swelling and pinching-off of filamentous

segments. After this treatment photomicrography revealed predominantly spheres with no obvious differences in the size and shape of cells grown in different fatty acids. Also, as shown in Fig. 1, these sucrose-equilibrated cells behave as equivalent, ideal osmometers at sucrose concentrations above about 50 mM, exhibiting a similar, linear proportionality between cell volume ($1/A$) and osmolarity. Therefore the optical swelling rate assay as utilized here is a valid indicator of the relative nonelectrolyte permeabilities of both intact cells and derived liposomes.

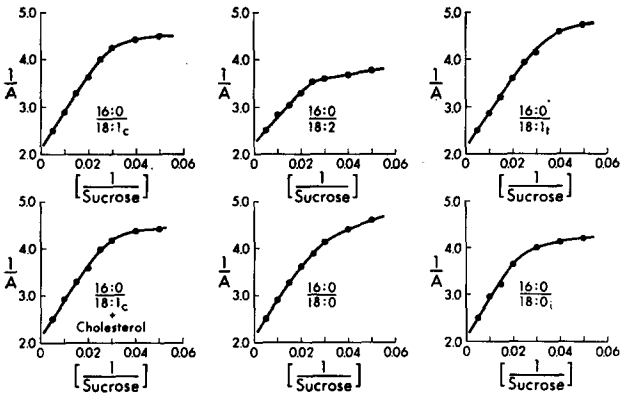

Fig. 1. Relationship between the reciprocal of the absorbance at 450 nm (cell volume) of *A. laidlawii* B cells, grown in various combinations of exogenous fatty acids, and the reciprocal of the sucrose concentration in which the cells were swollen until equilibrium. Cells were originally washed and equilibrated in 200 mM sucrose. The fatty acids are designated by the number of carbon atoms, followed by the number of double bonds; *c* and *t* indicate the *cis* and *trans* configurations of the double bonds and *i* indicates a methyl group attached to the penultimate carbon atom.

Fatty acid composition

The fatty acid compositions of the total membrane lipids extracted from *A. laidlawii* cells grown in various combinations of exogenous fatty acids are shown in Tables I and II. In Table I it can be seen that when appropriate quantities of palmitate *plus* either isostearic, elaidic, oleic, or linoleic acid are added to the growth medium, the branched-chain or unsaturated fatty acid accounts for about half of the total fatty acid while palmitic acid accounts for the bulk of the remainder; relatively small amounts of lauric and myristic acid are present as a biosynthetic background[10]. However, when palmitate and stearate are added, there is a relatively poor incorporation of stearic acid coupled with an increased biosynthesis of shorter-chain saturated fatty acids and an increased uptake of residual exogenous oleic and linoleic acids from the growth medium. Thus *A. laidlawii* appears to respond to the incorporation into the membrane lipids of the relatively higher melting stearate residues by a compensatory uptake of over 30 mole % of various lower melting fatty acids. We shall return to this point shortly.

The fatty acid compositions of the total membrane lipids from cells grown in oleic acid plus either myristic, palmitic, or stearic acid, with and without cholesterol, are shown in Table II. Oleic acid accounts for approximately half of the total

TABLE I

THE FATTY ACID COMPOSITION OF THE TOTAL MEMBRANE LIPIDS FROM *A. LAIDLAWII* B CELLS GROWN IN VARIOUS EXOGENOUS FATTY ACIDS

The total membrane lipid was extracted and purified as described in the text. Methyl esters of fatty acids were prepared by heating with acidified methanol and extracted with hexane. The fatty acid methyl esters were then analyzed on a Barber-Colman series 5000 gas–liquid chromatograph equipped with a hydrogen-flame ion detector and glass column packed with 15% diethyleneglycol succinate on Anakrom ABS at 170 °C. Data represent mole % fatty acid found.

Fatty acids found	*Fatty acids added to the growth medium:*				
	16:0 (30 mM) / *18:2 (100 mM)*	*16:0 (60 mM)* / *18:1$_{cis}$ (70 mM)*	*16:0 (70 mM)* / *18:1$_{trans}$ (60 mM)$_{iso}$*	*16:0 (30 mM)* / *18:0 (100 mM)*	*16:0 (30 mM)* / *18:0 (100 mM)*
12:0	3.8	1.2	1.8	6.4	7.3
14:0	7.3	4.5	2.8	12.8	12.2
16:0	40.4	45.9	44.7	32.1	39.1
18:0	Trace	0.2	Trace	Trace	28.2
18:0$_{iso}$	—	—	—	48.5	—
18:1	Trace	48.0	50.6	Trace	8.1
18:2	48.4	0.2	Trace	Trace	4.9

TABLE II

THE FATTY ACID COMPOSITION OF THE TOTAL MEMBRANE LIPIDS FROM *A. LAIDLAWII* B CELLS GROWN IN VARIOUS EXOGENOUS FATTY ACIDS WITH AND WITHOUT CHOLESTEROL

Data represent mole % fatty acids found.

Fatty acids found	*Fatty acids added to the growth medium:*				
	14:0 (80 mM) / *18:1$_{cis}$ (50 mM)*	*16:0 (60 mM)* / *18:1$_{cis}$ (70 mM)*	*18:0 (80 mM)* / *18:1$_{cis}$ (50 mM)*	*16:0 (60 mM)* / *18:1$_{cis}$ (70 mM)*	*+ cholesterol (25 mg/l)*
12:0	1.4	1.2	1.9	1.3	
14:0	27.5	4.5	6.8	4.3	
16:0	22.2	45.9	12.4	45.1	
18:0	1.6	0.2	27.8	0.2	
18:1$_{cis}$	47.2	48.0	50.1	48.7	
18:2	Trace	0.2	0.5	0.3	

fatty acid in each case with the exogeneous saturated fatty acid accounting for most of the remainder. Thus in these samples the ratio of total saturated to unsaturated fatty acid is nearly equal but the average chain lengths of the saturated fatty acyl groups are appreciably different. Note also that the addition of cholesterol to the growth medium along with exogeneous palmitic and oleic acid does not alter the fatty acid compostition of the cellular lipids with respect to the addition of these exogenous fatty acids alone.

Mechanism of permeation

We have three major lines of evidence which indicate that glycerol and erythritol enter *A. laidlawii* cells by a passive diffusion process. Firstly, as illustrated in Fig. 2, glycerol and erythritol permeation rates are linearly related to the concentration of the permeant in the isotonic suspension medium up to a permeant concentration of at least 200 mM, as expected for a simple diffusion process. Facilitated, protein-mediated transport systems normally show a saturation effect at permeant concentrations well below 50 mM[11,12]. Secondly, the initial swelling rates in glycerol and erythritol continue to increase exponentially up to a temperature of at least 60 °C, whereas mediated transport systems in mesophiles generally show a decline and eventual loss of activity below about 50 °C[11,12]. Finally, the entry of glycerol and erythritol into intact cells is not inhibited by a variety of compounds including phloretin, *p*-chloromercuribenzoate and *N*-ethylmaleimide which are potent inhibitors of many other transport systems. Also, as will be discussed shortly, the high activation energies characteristic of the glycerol and erythritol permeation process argue for a passive diffusion mechanism[11,12].

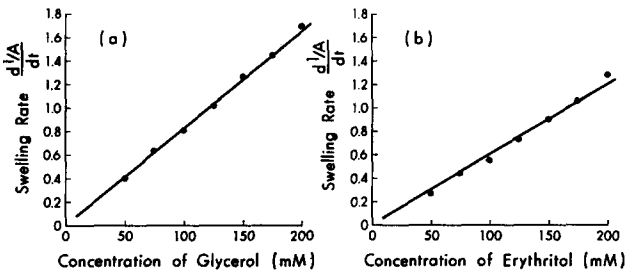

Fig. 2. (a) The relationship between the initial swelling rates of *A. laidlawii* B cells and the glycerol concentration in the isotonic solution in which the cells were suspended. Initial swelling rates were measured at 17 °C as described in the text. (b) The relationship between the initial swelling rates of *A. laidlawii* B cells and the erythritol concentration in the isotonic solution in which the cells were suspended. Initial swelling rates were measured at 37 °C. In both experiments cells were grown in the presence of palmitic and oleic acids and osmotically equilibrated in 200 mM sucrose. The glycerol or erythritol concentrations were varied from 50 to 200 mM and the total nonelectrolyte concentration of the suspending medium was maintained at 200 mM by the addition of appropriate amounts of sucrose.

Permeation rates

The initial swelling rates in isotonic glycerol solutions of intact cells and derived liposomes of various fatty acid compositions are presented as a function of temperature in Fig. 3. All preparations show a similar strong dependence of glycerol permeation rate on temperature. In addition, the nature and length of the lipid hydrocarbon chains are seen to be important in determining glycerol permeability. As illustrated in Figs 2a and 2b, glycerol permeability decreases in the following order for both cells and liposomes: 16:0/18:2> 16:1/18:1_{cis}> 16:0/18:0> 16:0/18:1_{trans}> 16:0/18:0_{iso}. An inspection of Figs 2c and 2d also reveals that glycerol permeability increases as the average chain length of the saturated fatty acyl groups decrease and that the incorporation of cholesterol significantly decreases

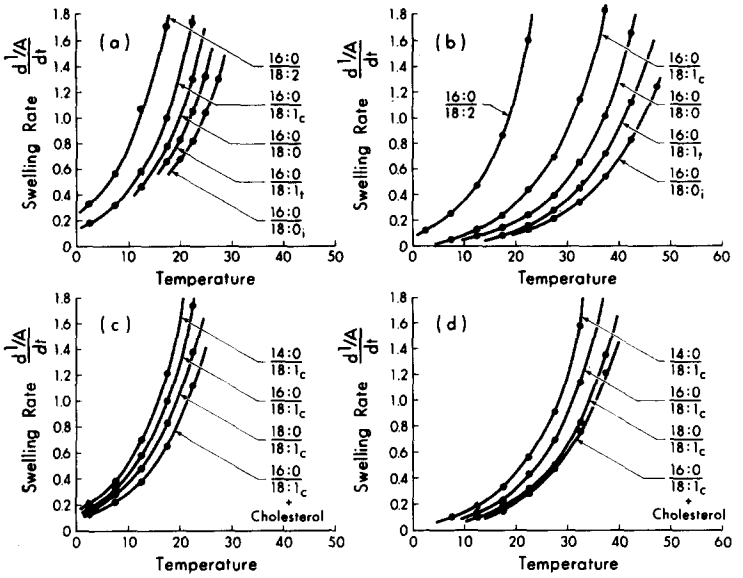

Fig. 3. (a) and (c). Initial swelling rates in isotonic glycerol of intact cells of *A. laidlawii* B grown in the presence of different combinations of fatty acids, with or without cholesterol, as a function of temperature. (b) and (d). Initial swelling rates in isotonic glycerol of liposomes, prepared from the total membrane lipids of *A. laidlawii* B, as a function of temperature.

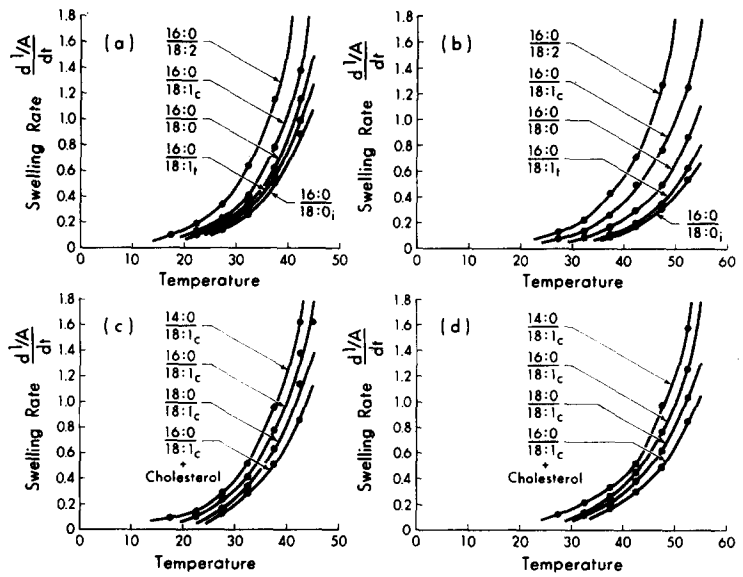

Fig. 4. (a) and (c) Initial swelling rates in isotonic erythritol of intact cells of *A. laidlawii* B, grown in the presence of different combinations of fatty acids, with and without cholesterol, as a function of temperature. (b) and (d) Initial swelling rates in isotonic erythritol of liposomes, prepared from the total membrane lipid of *A. laidlawii* B, as a function of temperature.

permeation rates by a similar factor in both the biological and artificial lipid bilayer membrane systems.

The initial swelling rates of intact cells and derived liposomes in isotonic erythritol solutions are presented as a function of temperature in Fig. 4. As expected, cells and liposomes are less permeable to the larger erythritol molecules at any particular temperature. However, a similar marked dependence of erythritol permeation rates on temperature, fatty acid composition and cholesterol content as noted with glycerol is evident in both the biological and artificial lipid bilayer membrane systems.

Activation energies of permeation

Arrhenius plots of the data reported in Figs 3 and 4 revealed linear relationships between the logarithm of the initial swelling rates and the reciprocal of the absolute temperature and permitted a calculation of an activation energy for the overall permeation process. The activation energies obtained are presented in Table III,

TABLE III

ACTIVATION ENERGIES CALCULATED FOR THE PERMEATION OF GLYCEROL AND ERYTHRITOL INTO *A. LAIDLAWII* B CELLS AND LIPOSOMES

Fatty acids added	*Activation energy (kcal/mole)*			
	Glycerol		*Erythritol*	
	Cells	*Liposomes*	*Cells*	*Liposomes*
16:0+18:2	18.2	20.2	20.5	20.7
16:0+18:1$_{cis}$	18.5	18.9	21.8	21.0
16:0+18:1$_{trans}$	19.4	17.3	21.5	23.8
16:0+18:0$_{iso}$	19.0	16.7	20.3	21.9
16:0+18:0	16.8	17.7	21.1	21.6
18:1$_{cis}$+14:0	18.6	18.6	21.7	21.2
18:1$_{cis}$+16:0	18.5	18.9	21.8	21.0
18:1$_{cis}$+18:0	17.3	17.6	22.6	20.3
18:1$_{cis}$+16:0+ cholesterol	17.8	17.6	21.2	21.3
Mean ± S.D.	18.2 ± 0.9	18.1 ± 1.1	21.3 ± 0.7	21.5 ± 1.1

which can be summarized as follows: (i) the activation energies for the permeation of glycerol or erythritol across the plasma membrane of *A. laidlawii* are the same, within the limits of experimental error, as for the permeation of these substances across an artificial lipid bilayer membrane of the same lipid and fatty acid composition; (ii) the energy of activation for the permeation of glycerol is in all cases lower than that obtained with erythritol, the mean values being 18.2 kcal/mole and 21.4 kcal/mole, respectively; (iii) alterations in the fatty acid composition or cholesterol content of either intact cells or liposomes affect activation energy values only slightly, if at all.

Permeability and membrane lipid phase transitions

Careful inspection of Fig. 3a will reveal that cells grown in palmitic *plus* either oleic or linoleic acids exhibit "normal" swelling curves when placed in isotonic glycerol solutions at temperatures as low as 2 °C. That is, swelling rates decrease regularly and exponentially with decreasing temperature as the temperature is lowered from above 40 down to 2 °C. On the other hand, cells grown in palmitic acid *plus* either isostearic, elaidic, or stearic acids show anomalous optical behavior below about 25, 20 and 15 °C, respectively. This anomalous behavior is illustrated in Fig. 5 for cells grown in palmitic and elaidic acids. Swelling rates decrease regularly and exponentially with decreasing temperature until approximately 20 °C. Below this temperature, instead of the expected relatively slow decrease in absorbance normally accompanying cell swelling, a rapid drop in absorbance occurs followed by a period of slow decline. This phenomenon is noted also in isotonic erythritol or glucose solutions in the same temperature range and with liposomes prepared from the membrane lipid of cells grown in palmitic and elaidic acids. The anomalous optical swelling is thus characteristic of particular lipid fatty acid compostions, irrespective of whether these lipids are arranged in a biological or an artificial lipid bilayer membrane, and is not greatly influenced by the nature of the permeant molecules present.

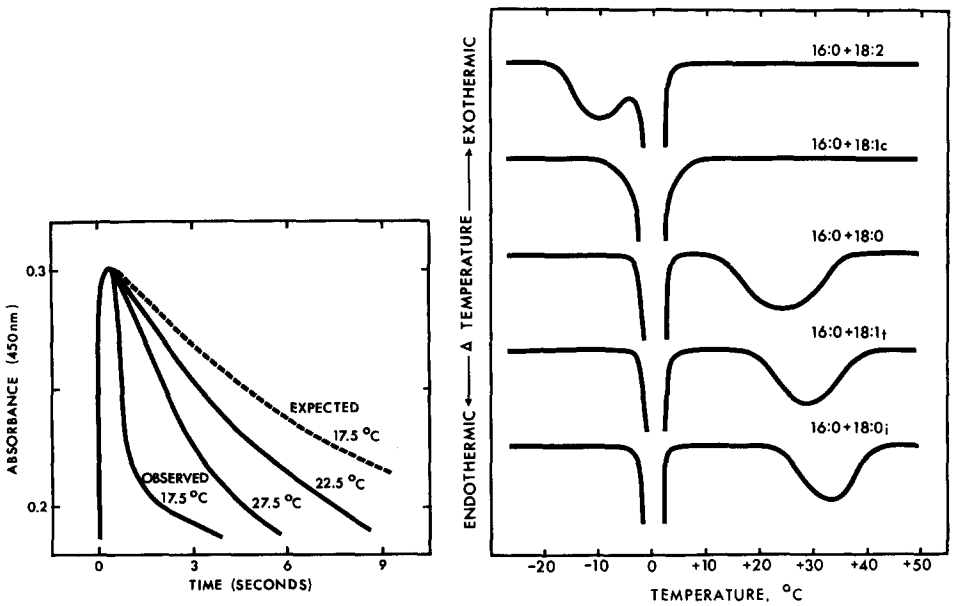

Fig. 5. The relationship between the reciprocal of the absorbance at 450 nm of intact *A. laidlawii* B cells and time as a function of temperature. Cells were grown in the presence of palmitic and elaidic acids and the swelling curves were monitored as described in the text.

Fig. 6. The temperature-base thermograms of isolated *A. laidlawii* plasma membranes enriched in various combinations of fatty acids. The temperature differentials between the samples and inert reference are plotted as a function of the temperature of the reference. The thermograms were obtained as described in the text using a heating rate of 7 °C per min. The large endotherm centered around 0 °C is due to melting of the ice from the excess water associated with the membrane preparations. The broad endotherm due to the lipid phase transition in the membrane preparation enriched in palmitic and oleic acids is partly obscured by the ice endotherm.

Previous studies[13,14] led us to expect that reversible, thermotropic gel to liquid–crystalline lipid phase transitions might occur in the temperature range in which this swelling anomaly was noted and somehow be connected with this behavior. We therefore analyzed isolated plasma membranes from cells grown in various combinations of exogenous fatty acids by differential thermal analysis in order to detect these possible phase transitions. The thermograms are presented in Fig. 6 for membranes from cells grown in palmitic *plus* either linoleic, oleic, elaidic, isostearic or stearic acids. As can be seen, broad phase transitions, occurring over a range of 20–25 °C, are detected in all cases. These same reversible, thermotropic gel to liquid–crystalline phase changes were also noted for isolated membrane lipids dispersed in excess distilled water and they occurred over the same temperature range, within experimental error, as noted for plasma membranes of similar fatty acid composition. Membranes enriched in oleic or linoleic acids, as expected, exhibit no phase changes within the temperature range of the permeability determinations and cells grown in these fatty acids exhibit normal swelling behavior. On the other hand, membranes enriched in elaidic, isostearic or stearic acids did exhibit phase transitions within the temperature range of the permeability assay and cells enriched in these acids do show anomalous optical swelling behavior. In Table IV the transition temperature range, transition midpoint temperature, and the temperature below which anomalous swelling is first noted are presented. We can see that a definite correlation exists between the thermal transitions and the swelling anomaly. When most of the membrane lipids become converted to the gel state, the anomalous swelling behavior first becomes evident. Cells supplemented with oleic or linoleic acids do not show permeability anomalies because the lipids do not become appreciably crystalline at temperatures above 2 °C.

It should also be noted that the permeability of intact cells is closely correlated to the fluidity of the membrane lipids as measured by their phase transition temperatures. The lower the transition temperature, the greater the nonelectrolyte permeability of the membrane system at a given temperature.

TABLE IV

THE LIPID PHASE TRANSITION AND OPTICAL SWELLING ANOMALY TEMPERATURES OF *A. LAIDLAWII* B CELLS GROWN IN VARIOUS COMBINATIONS OF FATTY ACIDS

Fatty acids added to growth medium	*Phase transition temperature range* (°C)	*Phase transition midpoint temperature* (°C)	*Temperature below which optical anomaly occurs* (°C)
16:0+18:2	−12 to 0*	−10	<2
16:0+18:1$_{cis}$	−11 to −8	− 2*	<2
16:0+18:0	10 to 37	25	15±2
16:0+18:1$_{trans}$	16 to 41	29	20±2
16:0+18:0$_{iso}$	21 to 43	33	25±2

* These temperatures are estimates because the lipid phase transition endotherms were partly obscured by the melting of the ice from the excess water associated with the membrane preparations.

DISCUSSION

The rate at which nonelectrolytes enter intact *A. laidlawii* B cells and derived liposomes depends on both the nature of the permeating molecule and on the lipid composition of the membrane system. Thus in both cells and liposomes glycerol is appreciably more permeable than erythritol. This finding is expected since the permeation rate for biological membranes in general is inversely related to the cube of the molecular weight of the permeant[16,20]. Thus the erythritol molecule would be predicted to permeate more slowly than the smaller glycerol molecule.

The permeation rates of glycerol and erythritol are also markedly dependent on the nature and length of the lipid hydrocarbon chains in both the biological and artificial membrane systems studied here. Previous studies[1,2] utilizing liposomes prepared from synthetic phospholipids demonstrate that the initial swelling rates in glycerol or glucose decrease in the following order: 16:0/18:2> 16:0/18:1_{cis}> 16:0/18:1_{trans}> 16:0/18:0_{iso}> 16:0/18:0 and 14:0/18:1_{cis}> 16:0/18:1_{cis}> 18:0/18:1_{cis}. The orders of decreasing initial swelling rates observed in this study for both cells and liposomes were as follows; 16:0/18:2>16:0/18:1_{cis}>16:0/18:0> 16:0/18:1_{trans}>16:0/18;0_{iso} and 14:0/18:1_{cis}>16:0/18:1_{cis}>18:0/18:1_{cis}. Thus the relative permeabilities are identical for all systems with one exception. The anomalously high permeability of cells grown in palmitic and stearic acids is doubtless due to the incorporation of relatively large amounts of lower-melting shorter chain saturated and unsaturated fatty acids as discussed earlier: the liposomes generated from the synthetic lecithin of course contain only stearate and palmitate. That the melting point of membrane lipids from cells grown in palmitate and stearate does indeed fall between those of cells grown in palmitate and oleate and palmitate and elaidate is confirmed by the differential thermal analysis results. Thus, increasing the fluidity of the lipid bilayer, either by introducing methyl branches or double bonds which interefere with hydrocarbon chain packing or by decreasing the chain length, leads in all cases to a similar increase in permeability in both intact cells and liposomes.

The presence of cholesterol in the membrane of intact cells and derived liposomes results in a definite decrease in glycerol or erythritol permeability. These results are in line with several recent investigations which demonstrated that cholesterol-depleted erythrocytes showed an increase in glycerol permeability[15] and that increasing quantities of cholesterol reduce the nonelectrolyte permeability of artificial lipid bilayer membranes[12]. Cholesterol has been shown to lower the hydrocarbon chain mobility of various artificial and natural membranes[3,16,17].

In contrast to permeation rates, the activation energy for the permeation of glycerol or erythritol through the cellular or liposomal membrane system is independent of fatty acid composition or cholesterol content and dependent only on the structure of the permeant molecule. This result suggests that the energy-limiting step in nonelectrolyte permeation may be the movement of permeant molecules from the aqueous interface into the hydrophobic phase. The high activation energies observed in this study are reasonably close to the expected enthalpy of dehydration values for glycerol and erythritol and support the idea that the two nonelectrolytes diffuse through both the biological and liposomal membrane as single, fully dehydrated molecules. For a detailed thermodynamic analysis of the permeation of a number of nonelectrolytes across the *A. laidlawii* membrane and also across several other

biological and liposomal membrane systems, the reader is referred to an earlier publication[18].

The existence of thermotropic gel to liquid–crystalline phase changes is known to have a profound effect on the stability and permeability of artificial membrane systems. Closed phospholipid vesicles (liposomes), for example, cannot be formed at temperatures below the phase transition temperature of the phospholipid constituents[2,19]. If liposomes are formed from phospholipids above their phase transition temperature and the permeability of these structures is then monitored as the temperature is decreased, a dramatic change in permeability is noted near the phase transition temperature[2,19]. The dramatic permeability increase which occurs upon the crystallization of the hydrocarbon chains may result from the formation of "holes" or faults in the phospholipid vesicles, since these structures may not be able to retain a closed configuration and accomodate the now tightly-packed and linearly-arranged lipid bilayer lamallae. The unexpectedly rapid decline in absorbance noted with both intact cells and derived liposomes in this study at temperatures within the phase transition range suggests that lysis may be occurring as the membrane lipids approach a fully crystalline state. The observation of broken cells and cell "ghosts" by phase microscopy supports this suggestion. We therefore postulate that the conversion of the pliable liquid–crystalline lipid bilayer to a rigid, planar crystalline state may result in a fracture of the cell membrane due to the inability to accommodate linear arrays of solidified bilayer into small, spherical cells. The resultant cell lysis is probably the cause of the optical swelling anomalies noted earlier.

The close similarities noted in this study between the plasma membrane of *A. laidlawii* and the artificial lipid bilayer membrane derived from it support our earlier suggestion, derived from thermal analytical data[13,14], that at least the major portion of the lipid in the membrane of this organism is arranged in a bilayer structure. Further support for this suggestion has recently come from X-ray diffraction[20,21] and electron spin resonance studies[22]. The hydrophobic core of this lipid bilayer apparently forms the major permeability barrier in intact cells.

ACKNOWLEDGEMENTS

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