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THE EFFECT OF DIFFERENT FATTY ACID AND STEROL COMPOSITION ON THE ERYTHRITOL FLUX THROUGH THE CELL MEMBRANE OF *ACHOLEPLASMA LAIDLAWII*

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SUMMARY

1. A technique is described whereby the [^{14}C]erythritol equilibrium flux through the *Acheloplasma laidlawii* cell membrane is measured. Cells are separated from the surrounding medium by filtration through membrane filters. It is essential that the *A. laidlawii* cells are suspended in isotonic NaCl. Cells suspended in sucrose media are fragile and easily damaged by applying a mechanical force such as ultrafiltration under negative pressure.

2. The [^{14}C]erythritol equilibrium flux for cells grown on different fatty acids is in the order of cells grown on linoleic acid > oleic acid > elaidic acid > stearic acid.

3. It is indicated that the permeation of glycerol through the *A. laidlawii* cell membrane at temperatures in the transition of the membrane lipids predominantly occurs *via* those parts of the membrane where the fatty acid chains of the membrane lipids are still in the liquid crystalline state.

4. The *A. laidlawii* cells remain intact upon cooling below the temperature of the phase transition of the membrane lipids. However, at temperatures below the phase transition of the membrane lipids *A. laidlawii* cells are damaged when mechanical forces such as ultrafiltration or osmotic swelling are applied.

5. The incorporation of cholesterol into the *A. laidlawii* cell membrane decreases the [^{14}C]erythritol flux for cells grown on linoleic, oleic, elaidic and stearic acid.

6. Cholesterol, epicholesterol, cholestanol, epicholestanol, ergosterol, stigmasterol and coprostanol, when present in the growth medium, are all incorporated in the *A. laidlawii* cell membrane to about the same extent.

7. The 3α-hydroxy isomers epicholesterol and epicholestanol and the sterol with a *cis*-structured steroid nucleus, coprostanol, do not significantly alter the [^{14}C]-erythritol flux through the membrane.

8. The 3β-hydroxy sterols with a flat steroid nucleus cholesterol, cholestanol and ergosterol all decrease the [^{14}C]erythritol flux. Stigmasterol does not influence the [^{14}C]erythritol flux to a marked extend.

9. For the reduction in permeability of the *A. laidlawii* cell membrane a 3β-hydroxyl group and a planar configuration of the sterol molecule are essential requirements. This is in good agreement with similar studies using liposomes and monolayers.

10. It is demonstrated that the liquefying effect of cholesterol on the membrane lipids of *A. laidlawii* can be very important in maintaining proper membrane functioning in growing cells. A 3β-hydroxyl group on the sterol molecule is an absolute requirement for this liquefying effect.

INTRODUCTION

Acholeplasma laidlawii is a unique organism for the study of membrane properties because it lacks a cell wall and its cytoplasmic membrane can be easily isolated. Moreover, the fatty acid composition of the membrane lipids can be controlled to a certain extent by supplying different fatty acids in the growth medium[1]. The acholeplasmas in contrast to the mycoplasmas do not require sterols for growth. However, when cholesterol is supplemented in the growth medium this sterol is incorporated unaltered in the membrane[2,3]. This property makes *A. laidlawii* very suitable for studying the role of sterols in a biological membrane.

Sterols, especially cholesterol, are important constituents of many biological membranes. The exact function of cholesterol in the membrane is not yet known. In studies using a variety of model membrane systems it was shown that cholesterol can affect the packing of the fatty acid chains in the model membrane, thereby producing changes in the permeability to several electrolytes and non-electrolytes[4–6]. When part of the membrane-associated cholesterol of erythrocytes was removed by incubating the erythrocytes with egg lecithin liposomes the permeability of the membrane to glycerol was increased[7]. Also with some fungi the effect of cholesterol upon the permeation of several compounds through the cell membrane was demonstrated[8,9]. In our laboratory it was found that the incorporation of cholesterol in the *A. laidlawii* cell membrane decreased the initial swelling rate of the cells in isotonic glycerol and erythritol[3,10]. It was concluded that cholesterol reduced the permeability of the *A. laidlawii* cell membrane to these solutes as compared to the sterol-free cells[3,10]. In this paper the effect of cholesterol upon the erythritol permeability of *A. laidlawii* cells is described using a technique where transport of radioactively labelled erythritol through the membrane is measured. The same technique was explored to measure the effect of fatty acid composition of the membrane lipids upon the erythritol permeability. McElhaney *et al.*[10] demonstrated that the initial swelling rate of *A. laidlawii* cells in isotonic glycerol was dependent upon the fatty acid composition of the membrane lipids.

Demel *et al.*[11] showed that only sterols having a 3β-OH group, a planar ring system and a hydrophobic side-chain at C-17 were capable of reducing the permeability of bilayers of egg lecithin liposomes to erythritol, glycerol, glucose and Rb^+. The same requirements for the sterol molecule were found in monolayer studies where the interaction between sterols and 18:0/18:1-lecithin leading to a reduction in the area per molecule was measured[12] as well as for the reduction of spin label mobility in egg lecithin bilayers effected by cholesterol[13]. In order to determine the structural requirements of the sterol molecule for its specific interaction in a biological membrane we incorporated in the present study various sterols in the cell membrane of *A. laidlawii*. The effect of these sterols upon the permeability of the *A. laidlawii* membrane to glycerol and erythritol was measured using both the initial swelling rate

method[3,10] and the method in which transport of radioactively labelled erythritol through the membrane is determined.

MATERIALS AND METHODS

Organism and growth conditions

A. laidlawii strain B cells were grown in 0.1–1-liter quantities of lipid-poor tryptose medium as described before[3]. Fatty acids (60–120 μM) and sterols (25 mg/l of culture) were added to the growth medium at 37 °C as sterile ethanolic solutions. Fatty acids and sterols were added together to the growth medium to prevent precipitation of the sterols. By this method all fatty acids and sterols tested could be homogeneously dissolved in the growth medium.

Cells were harvested in late log phase of growth by centrifugation for 15 min at $14500 \times g$ in the GSA rotor of a Sörvall centrifuge. To prevent possible damage of the cells by passing the transition temperatures of the membrane lipids, oleic acid- and linoleic acid-grown cells were centrifuged at 4 °C, whereas stearic acid- and elaidic acid-grown cells were isolated at room temperature (21 ± 1 °C). The cells were washed once with the same solution in which the permeability experiment was performed. The final cell pellet was gently homogenized in a volume of fresh solution about 30% that of the cell pellet. This thick cell suspension was used for the permeability experiments.

In some experiments membrane lipids were labelled by growing the cells in the presence of 5 μCi of [1-^{14}C]palmitic acid per l of culture. *A. laidlawii* cell membranes were prepared as described before[14].

Permeability assays

[^{14}C]Erythritol equilibrium flux determination. About 0.5 ml of a thick cell suspension was transferred to a small flask containing 3–6 μCi [^{14}C]erythritol. The thick cell suspension was either prepared in 200–400 mM sucrose + 10 mM erythritol or 150 mM NaCl + 10 mM erythritol. In some experiments also 10 mM $MgCl_2$ or 10 mM Tris–HCl, pH 7.5, were present. The cells were incubated for 1 h at room temperature. Periodically the cell suspension was shaken carefully to ensure complete homogenisation of the system. The flux experiment was initiated by transferring 50 μl of the cell suspension to 25 ml of the same solution as used for preparing the thick cell suspension. In experiments where the net [^{14}C]erythritol flux was measured, the 10 mM erythritol was omitted in the solution to which the 50 μl of thick cell suspension were added. The solution was stirred with a magnetic bar at a moderate speed. We observed that cells were easily damaged at high stirring rates. At time intervals samples of 1 ml containing approximately 100 μg of cell protein were withdrawn from the solution and transferred onto a 25-mm diameter Sartorius or Millipore ultramembrane filter with a pore size of 0.45–0.80 μm. Cells were separated from the medium in less than 5 s by applying a negative pressure of about 6 cmHg. The cells were washed once on the filter with 5 ml of the same solution at the same temperature used for the flux experiment. The filters were dried at room temperature and transferred to a scintillation vial containing 15 ml of a solution containing 5 g PPO and 0.3 g POPOP per l toluene. The amount of ^{14}C radioactivity was determined with a Packard-Tricarb scintillation counter. Corrections for quenching were made,

if necessary, using the channel ratio method. If necessary, enough Biosol V BBS 3 (Beckman) was added to make the counting solutions completely clear.

Erythritol and glycerol permeability as measured by the initial swelling rates of cells in isotonic glycerol or erythritol. This method has been described in detail before[3,10,18]. In all cases the osmotic behaviour of the cells in solutions having different sucrose concentrations was tested[3,10,18].

Cytocryt determination

The cytocryt of the thick cell suspensions used for the permeability experiments was measured by determining the volumes of the cell suspension that could be penetrated by the permeant molecule erythritol and by the non-permeant molecule sucrose. A thick cell suspension of about 3 ml was divided in two parts. One part was incubated with 0.25 μCi [^{14}C]erythritol (spec. act. 4 Ci/mole) per ml of cell suspension and the other part with 0.25 μCi [^{14}C]sucrose (spec. act. 5 Ci/mole) per ml of cell suspension. The incubation was carried out at room temperature for 1 h. After the incubation the cells were centrifuged at room temperature for 15 min at 10000 rev./min in the SS_{34} rotor of a Sörvall centrifuge. The amount of ^{14}C radioactivity in 50 μl of cell-free supernatant and in 50 μl cell suspension before centrifugation was determined. The cytocryt, the percentage of the volume of the cell suspension that was penetrated by [^{14}C]erythritol but not by [^{14}C]sucrose, was calculated using the following equation:

$$\text{Cytocryt} = \left\{\left(1 - \frac{\text{cpm [}^{14}\text{C]sucrose in 50 } \mu\text{l cell suspension}}{\text{cpm [}^{14}\text{C]sucrose in 50 } \mu\text{l supernatant}}\right) - \left(1 - \frac{\text{cpm [}^{14}\text{C]erythritol in 50 } \mu\text{l cell suspension}}{\text{cpm [}^{14}\text{C]erythritol in 50 } \mu\text{l supernatant}}\right)\right\} \times 100\,\%$$

The first term represents the volume that is not permeable to sucrose, *i.e.* membranes and cytoplasma; the last term represents the volume that is not permeable to [^{14}C]-erythritol, *i.e.* membranes. This volume was also determined in another, independent way. An aliquot of the cell suspension was freeze-dried and another transferred to distilled water. The membranes of the latter were removed by centrifugation and the supernatant was freeze-dried. After correcting the weight of the freeze-dried cells for the weight of the solutes and cytoplasmic components present, we calculated the volume of the membranes by using a value of 1.17 g/cm^3 for the density of *A. laidlawii* membranes[15]. Both methods yielded comparable data for the volume of membranes in the thick cell suspension.

Lipid extraction

Lipids were extracted from the *A. laidlawii* cells according to the procedure of Bligh and Dyer[16]. Residual protein was removed by column chromatography over silica gel, mesh 70–230 (Merck). Total lipids were eluted with excess chloroform–methanol (1:9, v/v). After evaporation of the solvent, the lipids were dissolved in diethyl ether. Silica gel was removed by centrifugation of the ether solution. The ether was evaporated and the amount of total lipids determined by weight.

Analytical methods

Protein was quantitated according to the method of Lowry *et al.*[17] using bovine serum albumin as standard. The fatty acid pattern of the membrane lipids was determined as described before[3]. The amount of cholesterol, epicholesterol, coprostanol, stigmasterol and ergosterol incorporated in the *A. laidlawii* membrane was determined using the modified Liebermann–Burchard method[18]. Each of these sterols developed a characteristic absorbance spectrum between 250 and 650 nm with the Lieberman–Burchard reagent. The spectra were recorded with a Perkin–Elmer 356 double beam spectrophotometer. The spectra of the different sterols before and after incorporation into the *A. laidlawii* membrane were identical. Cholestanol and epicholestanol could not be determined by this method.

Materials

[U-^{14}C]Erythritol (spec. act. 4 mCi/mmole) and [U-^{14}C]sucrose (spec. act. 5 Ci/mole) were purchased by the Radio Chemical Centre (Amersham, England). Cholest-5-en-3β-ol (cholesterol), 5α-cholestan-3β-ol (cholestanol) and cholest-5,7,22-trien-24-methyl-3β-ol (ergosterol) were obtained from Fluka AG (Buchs, Switzerland). Cholest-5-en-3α-ol (epicholesterol) and 5α-cholestan-3α-ol (epicholestanol) were manufactured by Mann Research Laboratories (New York, U.S.A.). Cholest-5,22-dien-24-ethyl-3β-ol (stigmasterol) and cholest-5-en-3-one were obtained from Koch-Light Laboratories (Colnbrook, Buchs, England), 5β-cholestan-3β-ol (coprastanol) from K and K Laboratories (Hollywood, Calif., U.S.A.), cholest-4,6-dien-3-one from British Drug Houses (Poole, England), androstan-3β-ol and androstan-3α-ol from Ikapharm (Ramat-Gan, Israel). The purity of the sterols was checked by thin-layer chromatography as described before[12]. The sterols requiring purification were recrystallized several times from ethanol. All other chemicals were analytical grade.

RESULTS

Determination of the [^{14}C]erythritol flux of A. laidlawii cells

After loading *A. laidlawii* cells with [^{14}C]erythritol we measured the rate at which [^{14}C]erythritol was lost from the cells after diluting the cell suspension with unlabelled solution. A linear relationship between the logarithm of the amount of [^{14}C]erythritol in the cells and time was obtained (see, for instance, Fig. 4). Two important parameters can be obtained from such data: first, the amount of [^{14}C]-erythritol present in the cells on the filter at zero time can be calculated and, second, the half-time of [^{14}C]erythritol flux can be measured. According to Mawe and Hempling[19] we can calculate an equilibrium flux for the permeant molecule from the equation:

$$\ln \frac{R_1}{R_0} = \frac{f}{S} t \tag{1}$$

R_1, amount of [^{14}C]erythritol in the cells at time t; R_0, amount of [^{14}C]erythritol in the cells at zero time; t, time in min; S, erythritol concentration (moles/l); f, equilibrium flux in moles$\cdot$s$^{-1}\cdot$l cells^{-1}.

The relation between the half-time of [^{14}C]erythritol flux ($R_1 = 1/2\ R_0$) and the flux can be calculated to be

$$f = -\frac{S \cdot \ln 2}{t_{\frac{1}{2}}} \tag{2}$$

We attempted to study the erythritol permeability in a sucrose medium because we wanted to compare the [^{14}C]erythritol flux directly with the erythritol permeability as measured by the initial swelling rate of *A. laidlawii* cells when they are transferred from a sucrose solution to isotonic erythritol[3,10]. The results of [^{14}C]erythritol flux measurements in sucrose solutions are given in Table I. The amount of radioactivity in an aliquot of the cell suspension which was applied to the filter and the amount of radioactivity on the filter at zero time were determined. From these data the percentage of the radioactivity present in the cells on the filter at zero time (t_0 %) could be calculated. By dividing the percentage of the radioactivity present in the cells on the filter at zero time (t_0 %) by the cell cytocryt one obtains the fraction of the *A. laidlawii* cells which remain intact on the membrane filter. It is obvious that in all cases a considerable rupture of cells on the membrane filter occurs (Table I). Different sucrose concentrations, addition of 10 mM $MgCl_2$ or 10 mM Tris–HCl, pH 7.5, or working at different temperatures between 0 and 30 °C had no marked effect on the survival of the cells on the filter. By labelling the *A. laidlawii* membranes by growing the cells on [^{14}C]palmitic acid we determined that the membrane filters with pore sizes of 0.45, 0.60 and 0.80 μm retained all the [^{14}C]radioactivity. Whole cells and membranes can not pass these filters so that the low percentages of erythritol remaining on the filter (Table I) cannot be explained by a loss of [^{14}C]erythritol passing through the filter together with intact cells. By doing protein determinations on the filtrate and the solution which was applied to the filter and by determining the amount of cytoplasmic and membranous protein present in *A. laidlawii* cells we could calculate that in isotonic sucrose more than 50% of the cells lost their cytoplasmic proteins on the filter. Apparently *A. laidlawii* cells in sucrose solutions become

TABLE I

EFFECT OF DIFFERENT SUCROSE SOLUTIONS AND DIFFERENT PORE SIZES ON THE FRAGILITY OF *A. LAIDLAWII* CELLS ON ULTRAMEMBRANE FILTERS

Filtration solutions used: (1) 200 mM sucrose+10 mM erythritol+10 mM $MgCl_2$; (2) 400 mM sucrose+10 mM erythritol+10 mM $MgCl_2$; (3) 200 mM sucrose+10 mM erythritol. Cells were grown on 0.03 mM palmitic acid and 0.03 mM elaidic acid. Filtrations were carried out at 20 °C using cell suspensions with a cytocryt of 9.5%.

Filtration solution used	*Pore diameter used* (μm)	*% of the radioactivity in the cells on the filter at zero time*	*% of the cells survived on the filter*
1	0.60	2.3	24
2	0.60	2.0	21
3	0.60	1.1	12
1	0.80	4.2	44
2	0.80	3.3	35
3	0.80	1.9	20

TABLE II

EFFECT OF REPLACEMENT OF SUCROSE BY NaCl UPON THE FRAGILITY OF *A. LAIDLAWII* CELLS ON ULTRAMEMBRANE FILTERS

Cells were grown on 0.03 mM palmitic and 0.03 mM elaidic acid. Filtrations were carried out at 20 °C using membrane filters with pore size 0.60 μm.

Filtration solution	*% radio-activity on the filter at zero time*	*Cytocryt of the thick cell suspension*	*% cells survived on the filter*
150 mM NaCl +10 mM erythritol+10 mM $MgCl_2$	7.3	8.0	91.4
200 mM sucrose+10 mM erythritol+10 mM $MgCl_2$	2.8	11.5	24.2

rather fragile. This is also indicated in experiments were K^+ efflux was measured using a K^+-specific electrode. Cells in sucrose lost their K^+ rather fast. This resulted in cell shrinking as visualised by electron microscopy and cytocryt determinations (unpublished results). Also prolonged dialysis of *A. laidlawii* cells in isotonic sucrose solutions lysed the cells completely (van der Neut-Kok, E., personal communication). The increased fragility of the cells in sucrose solutions is probably caused by the removal of ions from the cytoplasmic membrane which causes loss of the intracellular ions. As demonstrated for cells grown on elaidic acid almost all of the cells remained intact on the filter at 20 °C when isotonic NaCl was used in the filtration solution instead of sucrose (Table II). In the sucrose solution 24.2% of the cells retained their [^{14}C]erythritol on the filter whereas in isotonic NaCl 91.4% of the cells remained intact. In all other experiments described hereafter, the filtration solution was 150 mM NaCl containing 10 mM erythritol and the filters used had a pore diameter of 0.60 μm. The presence of 10 mM $MgCl_2$ or 10 mM Tris–HCl, pH 7.5, was not essential for this effect. The results obtained with filter pore sizes of 0.45, 0.60 and 0.80 μm were identical, the filtration rate being somewhat decreased by the 0.45-μm filter. No significant binding of [^{14}C]erythritol to *A. laidlawii* membranes occurred in both the sucrose and the NaCl media.

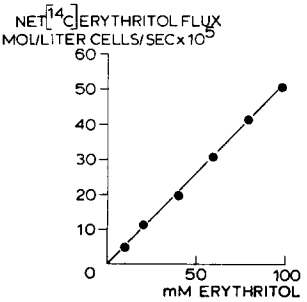


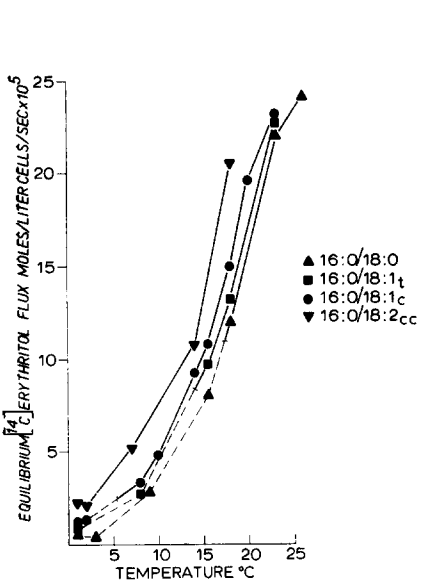
Fig. 1. [^{14}C]Erythritol net flux through the *A. laidlawii* cell membrane as a function of the erythritol concentration. Cells were grown on 0.03 mM palmitic acid and 0.03 mM elaidic acid. The flux experiment was performed at 17 °C.

In order to check whether active transport was involved in the erythritol permeation in NaCl we studied the concentration dependence of the net [^{14}C]erythritol flux (Fig. 1). The linear relationship between the net erythritol flux and the erythritol concentration is consistent with the idea that erythritol passes the *A. laidlawii* cell membrane by simple diffusion.

The effect of different fatty acid and cholesterol incorporation on the [^{14}C]erythritol flux through the A. laidlawii cell membrane

The [^{14}C]erythritol equilibrium flux of *A. laidlawii* cells grown on different fatty acids in the presence and absence of cholesterol is shown in Figs 2 and 3. Identical results were obtained for three different sets of cell suspensions. The fatty acid composition of the total membrane lipids and the amount of incorporated cholesterol are given in Table III. The fatty acids supplemented to the growth medium were incorporated to a different extent in the *A. laidlawii* membrane lipids. The most saturated stearic and the less saturated linoleic acid were incorporated to a lesser extent than oleic and elaidic acid. This is in agreement with previous observations[20].

The amount of cholesterol incorporated in the cell membrane was independent of the fatty acid composition of the membrane lipids. The fatty acid patterns of cells grown with or without cholesterol were identical. It is obvious that the [^{14}C]erythritol equilibrium flux is dependent on the fatty acid composition and the cholesterol con-



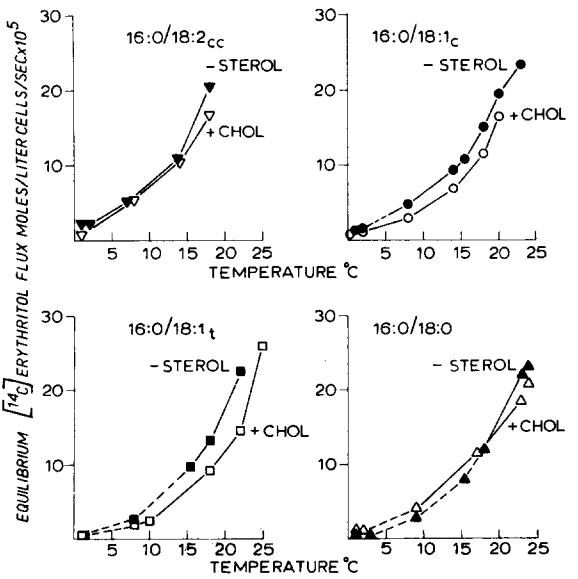


Fig. 2. Effect of the fatty acid composition of *A. laidlawii* membrane lipids on the [^{14}C]erythritol equilibrium flux through the cell membrane. Cells were grown on 0.03 mM palmitic acid and 0.03 mM of the fatty acid indicated in the figure. See text for explanation of the dashed lines.

Fig. 3. Effect of cholesterol incorporated in the *A. laidlawii* membrane on the [^{14}C]erythritol equilibrium flux through the cell membrane. Cells were grown on 0.03 mM palmitic acid and 0.03 mM of the fatty acid indicated, in the presence or absence of 25 mg cholesterol per l of growth medium. See text for explanation of the dashed lines.

TABLE III

FATTY ACID COMPOSITION AND CHOLESTEROL CONTENT OF *A. LAIDLAWII* CELL MEMBRANES

Fatty acids supplemented in the medium. 0.03 mM of each fatty acid	*Amount of cholesterol present in the medium (mg/l culture)*	*Fatty acid composition (mole %)*									*Amount of cholesterol incorporated (weight % of total lipids)*
		12:0	*14:0*	*16:0*	*18:0*	*18:1c*	*18:1t*	*18:2*	*18:3*	*unknown*	
16:0/18:2cc	—	3.5	7.9	45.7	1.9	3.6	—	33.2	—	4.2	0.0
16:0/18:2cc	25	4.8	8.2	44.0	1.3	3.1	—	32.6	—	6.0	10.0
16:0/18:1c	—	3.4	9.9	48.4	2.7	35.8	—	2.2	—	1.3	0.0
16:0/18:1c	25	3.6	8.2	46.1	2.3	37.5	—	2.3	—	1.6	8.0
16:0/18:1t	—	3.2	8.0	42.9	2.0	—	44.1	1.2	—	0.7	0.0
16:0/18:1t	25	3.0	5.9	39.0	2.2	—	48.4	1.1	—	0.4	10.0
16:0/18:0	—	9.5	19.0	46.1	8.2	3.6	—	6.2	0.3	7.1	0.0
16:0/18:0	25	12.5	18.5	41.2	8.5	3.5	—	6.8	0.4	8.6	9.4

tent of the membrane. The erythritol permeability of the cells grown on different fatty acids was in the order linoleic acid > oleic acid > elaidic acid > stearic acid. The differences in the [^{14}C]erythritol flux of the cells grown on different fatty acids are smaller than could be expected if equal amounts of each fatty acid were incorporated in the cell membrane lipids. This indicates that *A. laidlawii* is equipped with a mechanism to control the fatty acid composition of its membrane lipids such that the permeability of the membranes to non-electrolytes of cells grown on different fatty acids is not dramatically different. This is in agreement with the findings of Romijn *et al.*[20]. The activation energies for the [^{14}C]erythritol equilibrium flux ranged from 20–25 kcal. McElhaney *et al.*[10] found that the initial swelling rate of *A. laidlawii* cells in isotonic glycerol was also in the order linoleic acid-grown cells > oleic acid-grown cells > elaidic acid-grown cells > stearic acid-grown cells. This already indicates that the correlation between the two techniques for measuring non-electrolyte permeability of the *A. laidlawii* cell membrane is good. Cholesterol incorporation into the *A. laidlawii* cell membrane reduced the [^{14}C]erythritol equilibrium flux of cells grown on linoleic, oleic, elaidic and stearic acid (Fig. 3). This reduction in permeability was most pronounced when cells were grown on oleic or elaidic acid. This is in agreement with our previous work where we demonstrated that the initial swelling rate of *A. laidlawii* cells in isotonic erythritol and glycerol was decreased when cholesterol was incorporated in the cell membrane[3,10]. At lower temperatures, indicated by dotted lines in Figs 2 and 3, we observed a marked decrease in the percentage of radioactivity in the cells at zero time (t_0 %). The temperature at which this decrease

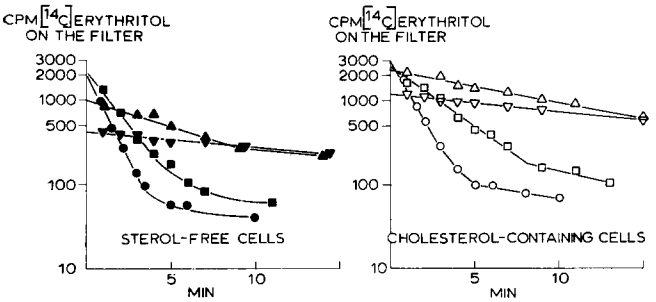


Fig. 4. Effect of temperature on the loss of [^{14}C]erythritol from *A. laidlawii* cells grown on elaidic acid in the absence and presence of cholesterol. Cells were grown on 0.03 mM palmitic acid and 0.03 mM elaidic acid in the presence and absence of 25 mg cholesterol per l of growth medium. Flux experiments were performed at 23.1 °C (●, ○); 18.0 °C (■, □); 8.0 °C (▲, △) and 0.2 °C ▼, ▽). The corresponding half-times of [^{14}C]erythritol equilibrium flux in min were:

Temp. (°C)	*Sterol-free cells*	*Cholesterol-containing cells*
23.1	0.65	0.80
18.0	1.0	2.0
8.0	4.4	6.0
0.2	17.0	15.0

in t_0% occurred was dependent on the fatty acid composition and cholesterol content of the membrane.

These temperatures were 17.5, 14 and 5 °C when cells were grown without cholesterol on stearic, elaidic and oleic acid, respectively. Cells grown on linoleic acid did not show this decrease in t_0% at lower temperatures. Cholesterol incorporated into the *A. laidlawii* cell membrane was capable of reducing these temperatures by about 7 °C. This effect is demonstrated in Fig. 4 for cells grown on elaidic acid with or without cholesterol. Sterol-free cells showed a decreased t_0% value between 18 and 8 °C, whereas cholesterol-containing cells showed a decreased t_0% value between 8 and 0.2 °C. That the phase transition in the membrane lipids of *A. laidlawii* is involved in this decrease of the t_0% value at lower temperatures is indicated by several experimental facts: (1) the temperatures below which a decrease in t_0% occurs for elaidic acid-grown cells coincides with the temperature at which almost all of the fatty acid chains in the membrane lipids become crystalline as measured with differential scanning calorimetry[3]. (2) The temperatures below which a decrease in t_0% occurs are lowered when cholesterol is incorporated into the *A. laidlawii* cell membrane. This decrease in transition temperature is consistent with the liquefying effect of cholesterol on the membrane lipids in the intact *A. laidlawii* membrane[3]. (3) The temperatures for cells grown on stearic, elaidic and oleic acid with or without cholesterol below which a decrease in t_0% occurs are identical to the temperatures below which *A. laidlawii* cells apparently swell very quickly in isotonic glycerol or erythritol[3]. This increased apparent swelling rate was attributed to bursting of the cells caused by the inability of a cell with its membrane lipids in a crystalline form to swell[3]. The decrease in the percentage of the radioactivity in the cells at zero time can be the result of two possible processes. (1) At a temperature below the phase transition of the membrane lipids the *A. laidlawii* cell is very fragile and breaking of the membrane occurs without the application of any physical force. This would permit erythritol and cytoplasmic components to leave the cell. (2) The *A. laidlawii* cell at a temperature below the phase transition of the membrane lipids is stable enough in solution to retain its cytoplasmic solutes. However, by applying a physical force such as filtration under negative pressure the membrane breaks and the erythritol, together with the cytoplasmic components, will be lost from the cells.

In order to check the first possibility we did the following experiments: *A. laidlawii* cells were grown on 0.03 mM elaidic acid and 0.03 mM [^{14}C]palmitic acid without cholesterol and isolated at 22 °C. The cells were washed once with 150 mM NaCl containing 10 mM erythritol at 22 °C. 50-μl-thick cell suspension was added to 25 ml 150 mM NaCl containing 10 mM erythritol at 22 °C and another 50 μl to 25 ml of the same solution at 0 °C. After 30 min incubation the cells were spun down at 22 °C and 0 °C, respectively, and the protein content and amount of [^{14}C]-radioactivity in the supernatant were determined. Another 50 μl of thick cell suspension was added to 25 ml distilled water at room temperature, causing lysis of the cells. After 30 min incubation the membranes were spun down and the amount of protein and radioactivity in the supernatant determined. In all cases less than 1% of the radioactivity was present in the supernatant. The results of the protein determination are given in Table IV. After correcting for the amount of the already extracellular protein present in the supernatant of the cells in 150 mM NaCl+10 mM erythritol 22 °C one can calculate that less than 1% of the cytoplasmic proteins are

TABLE IV

EFFECT OF TEMPERATURE ON THE RELEASE OF PROTEIN FROM A SUSPENSION OF *A. LAIDLAWII* CELLS

See text for details.

Medium in which the cells were present	*Temperature (°C)*	*Amount of protein in the supernatant (μg/ml)*
150 mM NaCl+10 mM erythritol	22	9.9 ± 0.2
150 mM NaCl+10 mM erythritol	0	10.1 ± 0.1
Distilled water	22	4.2 ± 0.4

released from the cells when they are brought below the transition temperature of the membrane lipids. This experiment excludes the first possibility mentioned before. In order to test the second possibility *A. laidlawii* cells were grown on 0.03 mM [^{14}C]palmitic and 0.03 mM elaidic acid. With these cells a normal erythritol flux experiment was performed except that the [^{14}C]erythritol was omitted. The amount of ^{14}C radioactivity and protein applied to the membrane filter and the amount of ^{14}C radioactivity and protein were determined. No radioactivity could be detected in the filtrate at all temperatures tested. The results of the protein determination are given in Table V. The amount of protein in the filtrate could be corrected for the amount of protein present which was already extracellular by measuring the protein content of 1 ml of supernatant when the cells were spun down as described before. The difference in the amount of protein in the supernatant of the cells at 23 °C in Tables IV and V is caused by differences in the cytocryt of the two cell suspensions. It is obvious that a marked increase in the amount of cytoplasmic protein in the filtrate caused by breaking of the cells on the filter occurs below the temperature of the phase transition of the membrane lipids. From these data we conclude that *A. laidlawii* cells at temperatures below the phase transition of the

TABLE V

EFFECT OF TEMPERATURE ON THE LOSS OF CYTOPLASMIC PROTEINS FROM *A. LAIDLAWII* CELLS ON A MEMBRANE FILTER

Cells were grown on 0.03 mM [^{14}C]palmitic acid and 0.03 mM elaidic acid.

Temperature (°C)	*Amount of protein applied to the filter (μg/ml)*	*Amount of protein in the filtrate (μg/ml)*	*Amount of protein in the filtrate produced by breaking of the cells on the filter (μg/ml)*
23.0	126 ± 6	17.6 ± 0.8	0.1
16.0	124 ± 7	24.0 ± 1.6	6.4
9.0	132 ± 8	42.5 ± 2.5	34.9
1.0	138 ± 8	66.5 ± 4.0	48.9

membrane lipids are easily damaged by applying a physical force such as filtration under negative pressure or osmotic swelling.

The [^{14}C]erythritol equilibrium flux below the temperature of the phase transition of the membrane lipids (Figs 2–4) represents only the permeability of that part of the cells which remained intact on the filter. For this reason a comparison between the erythritol permeabilities of *A. laidlawii* cells below and above the transition temperature of the membrane lipids cannot be made.

The effect of the incorporation of different sterols upon the [^{14}C]erythritol flux through the A. laidlawii cell membrane

In order to investigate the structural requirement of the sterol molecule for its specific interaction with other lipid components in the *A. laidlawii* cell membrane resulting in a decreased glycerol and erythritol permeability, *A. laidlawii* cells were grown in media supplemented with 0.03 mM palmitic acid, 0.03 mM oleic acid and different sterols. *A. laidlawii* cells could not grow when the medium was supplemented with the 3-ketosteroids cholest-5-en-3-one and cholest-4,6-diene-3-one. These sterols also inhibited cholesterol-stimulated growth of *Mycoplasma mycoides*[21]. No growth of *A. laidlawii* cells was observed in a growth medium supplemented with androstan-3β-ol and the cells showed very little growth when androstan-3α-ol was present in the growth medium. For this reason no permeability assays where performed on these cells. When the growth medium was supplemented with cholesterol, epicholesterol, cholestanol, epicholestanol, coprostanol, ergosterol or stigmasterol good growth was observed.

Only in the case of epicholesterol and epicholestanol the lag period of growth was increased with about 5 h. In our growth experiments we noticed an interesting phenomenon: A culture of *A. laidlawii* cells grown on stearic acid reached a lower

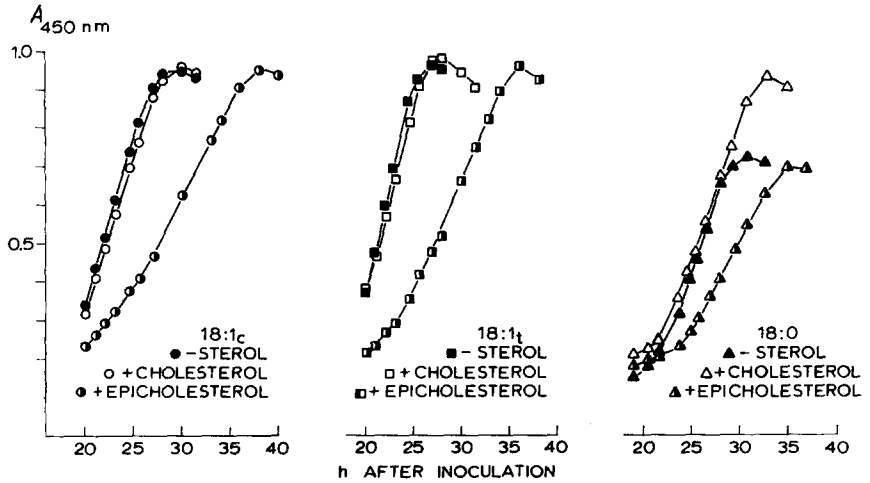


Fig. 5. Effect of cholesterol and epicholesterol on the growth of *A. laidlawii* cells in media containing different fatty acids. Cells were grown at 37 °C on 0.06 mM oleic (18:1c), elaidic (18:1t) or stearic acid (18:0) in the presence or absence of 25 mg cholesterol or epicholesterol per l of culture. Cultures were inoculated with 1% of log-phase *A. laidlawii* cells. Growth was monitored as the absorbance of the culture measured at 450 nm.

absorbance than cells grown on oleic or elaidic acid (Fig. 5). A possible explanation for this effect is that due to the increased incorporation of stearic acid the fatty acid chains in the membrane lipids become almost crystalline at growth temperature[22]. Stearic acid-grown cells in late log phase have a swollen appearance and lyse quickly[22,26]. The presence of cholesterol together with stearic acid in the medium had a marked influence on the growth of the cells. The velocity of growth in log phase was unaltered but the point where the absorbance reaches its maximum is increased to the values obtained for oleic acid- or elaidic acid-grown cells (Fig. 5). This can be explained by a liquefying effect of cholesterol upon the membrane lipids. The fatty acid chains of the membrane lipids remained more liquid and the membrane can still fulfill its peculiar properties. In this experiment we have an example of the importance of the liquefying effect of cholesterol in maintaining proper membrane functioning. As indicated in Fig. 5, cholesterol has no influence on the growth of cells in a medium supplemented with oleic or elaidic acid. With epicholesterol, the 3α-OH isomer of cholesterol, the liquefying effect of cholesterol on the paraffin chains in 18:0/18:1c-lecithin could not be demonstrated[3]. The inability of epicholesterol to liquefy the crystalline fatty acid chains in a membrane is also demonstrated in Fig. 5. Epicholesterol incorporation in the *A. laidlawii* membrane together with stearic acid was unable to increase the absorbance of the culture over the value obtained for the sterol-free cells.

The amount of stigmasterol, ergosterol, and coprostanol incorporated in the *A. laidlawii* cell membrane was 9, 14 and 12 weight% of total lipids, respectively. Comparable data were obtained for cholesterol and epicholesterol[3]. The characteristic absorbance spectrum which the sterols developed in the Liebermann–Burchard reaction (see Materials and Methods) was identical before and after incorporation of the sterols in the *A. laidlawii* cell, indicating that the sterols were incorporated as such in the cell membrane. Cholestanol and epicholestanol could not be quantitated by this method. That these sterols are incorporated to the same extent as the sterols mentioned before is indicated by the study of Smith[2] who demonstrated that the related *Mycoplasma* strain 07 incorporated equal amounts of cholesterol, epicholesterol, cholestanol and epicholestanol. Moreover, we observed that the characteristic shift in the ultraviolet spectrum of the polyene antibiotic filipin, indicating an interaction between the antibiotic and the sterol, was identical for cells grown on cholesterol and cholestanol, indicating that both sterols were incorporated to an equal extent (to be published). The fatty acid composition of the membrane lipids was not altered by the incorporation of the different sterols in the *A. laidlawii* cell membrane. The [^{14}C]erythritol equilibrium flux and the initial swelling rates of *A. laidlawii* in isotonic glycerol and glycerol were measured; the results are given in Tables VI, VII and VIII. All permeability data are related to the permeability of the sterol-free cells at 20 °C.

Cholesterol and cholestanol incorporation into the *A. laidlawii* cell membrane reduced the initial swelling rate of the cells in isotonic glycerol and erythritol (Tables VII, VIII) and the [^{14}C]erythritol equilibrium flux through the cell membrane (Table VI) as compared with the sterol-free control cells. The reduction in permeability produced by cholestanol is somewhat less as that produced by cholesterol. Apparently the $\Delta 5$ double bond of cholesterol is not required for the reduction in glycerol and erythritol permeability caused by cholesterol in this biological mem-

brane. This is in agreement with the studies of Demel *et al.*[11] who showed that both sterols decreased the permeability of liposomal egg lecithin bilayers to glucose, erythritol, glycerol and Rb^+. Of the plant sterols ergosterol and stigmasterol that are incorporated into the *A. laidlawii* cell membrane, only ergosterol had a significant

TABLE VI

EFFECT OF DIFFERENT STEROLS INCORPORATED IN THE *A. LAIDLAWII* CELL MEMBRANE ON THE [^{14}C]ERYTHRITOL EQUILIBRIUM FLUX THROUGH THE MEMBRANE

Cells were grown on 0.03 mM palmitic acid and 0.03 mM oleic acid.

Sterol present in the growth medium	*Relative [^{14}C]erythritol equilibrium flux*		
	10 °C	*15 °C*	*20 °C*
None	37	60	100
Cholesterol	28	43	66
Cholestanol	33	51	71
Ergosterol	30	47	75
Stigmasterol	37	53	89
Epicholesterol	34	52	—
Epicholestanol	41	60	87
Coprostanol	41	59	90

TABLE VII

EFFECT OF DIFFERENT STEROLS INCORPORATED IN THE *A. LAIDLAWII* CELL MEMBRANE ON THE INITIAL SWELLING RATE OF THE CELLS IN ISOTONIC GLYCEROL

Cells were grown on 0.03 mM palmitic acid and 0.03 mM oleic acid.

Sterol present in the growth medium	*Relative initial swelling rate in isotonic glycerol*		
	10 °C	*20 °C*	*30 °C*
None	53	100	206
Cholesterol	29	60	—
Cholestanol	43	74	172
Ergosterol	47	80	142
Stigmasterol	49	85	157
Epicholesterol	50	95	—
Epicholestanol	50	93	203
Coprostanol	41	105	201

TABLE VIII

EFFECT OF DIFFERENT STEROLS INCORPORATED IN THE *A. LAIDLAWII* CELL MEMBRANE ON THE INITIAL SWELLING RATE OF THE CELLS IN ISOTONIC ERYTHRITOL

Cells were grown on 0.03 mM palmitic acid and 0.03 mM oleic acid.

Sterol present in the growth medium	*Relative initial swelling rate in isotonic erythritol*		
	20 °C	*30 °C*	*40 °C*
None	100	195	434
Cholesterol	53	133	324
Cholestanol	63	133	340
Ergosterol	79	165	328
Stigmasterol	88	176	351
Epicholesterol	89	200	450
Epicholestanol	93	183	431
Coprostanol	80	280	384

effect on the [^{14}C]erythritol equilibrium flux and the initial swelling rates of the cells in isotonic glycerol and erythritol (Tables VI, VII and VIII). This indicates that a 24-methyl group in the hydrophobic chain of the sterol molecule and double bonds at C-7 and C-22 do not interfere very much with the interaction occurring between the sterols and other membrane compounds leading to a decreased permeability. Ergosterol incorporation into egg lecithin liposomes is also capable of reducing the permeability of several non-electrolytes[11]. Stigmasterol, although incorporated to about the same extent as the other sterols, only had a moderate effect on the permeability of the *A. laidlawii* cell membrane to glycerol and erythritol (Tables VI, VII and VIII). Stigmasterol can reduce the glycerol and erythritol permeability of liposomes prepared from egg lecithin[11]. This might indicate that the extra ethyl group at C-24 of the sterol molecule, which produces a more bulky hydrophobic side chain, interferes with the interaction between the sterol and other membrane lipids. The 3α-OH isomers epicholesterol and epicholestanol present in the *A. laidlawii* cell membrane show much less effect on the glycerol and erythritol permeability of the membrane than the corresponding 3β-OH isomers cholesterol and epicholesterol (Tables VI, VII and VIII). This is in agreement with our previous findings that epicholesterol only had a small effect on the initial swelling rate of *A. laidlawii* cells in isotonic glycerol and erythritol[3]. A 3β-OH group is an absolute requirement for the sterol molecule for the reduction in the glycerol and erythritol permeability of the *A. laidlawii* cell membrane. The same has been stated for the liposomal bilayer system[11]. Coprostanol differs from cholestanol in the structure of the A–B ring. In cholestanol the A–B ring is *trans*-oriented, producing a flat steroid nucleus; in coprostanol the *cis*-oriented A–B ring gives a steroid nucleus which is bent. The permeability to erythritol and glycerol of egg lecithin liposomes is only slightly affected by copro-

stanol[11]. Coprostanol incorporation into the *A. laidlawii* cytoplasmic membrane only had a small effect on the glycerol and erythritol permeability of the membrane (Tables VI, VII and VIII). A flat steroid nucleus seems to be a prerequisite for the permeability-lowering effect of a sterol both in the model membrane and the biological membrane of *A. laidlawii*. Mycoplasma, strain 07, which has an absolute sterol growth requirement failed to grow on coprostanol[2]. Tables VI and VIII show that there is a good correlation between the effect of different sterols incorporated into the *A. laidlawii* cell membrane on the erythritol permeability as measured by the initial swelling rate of the cells in isotonic erythritol and the [^{14}C]erythritol equilibrium flux measured in NaCl medium.

DISCUSSION

In the present study we have described a technique by which the transport of [^{14}C]erythritol through the membrane of *A. laidlawii* is measured. By this technique the loss of radioactivity from cells loaded with [^{14}C]erythritol is determined by separating cells from the surrounding medium by ultramembrane filtration under negative pressure. From the rate of loss of radioactivity from the cells a erythritol equilibrium flux can be calculated. The *A. laidlawii* cells suspended in sucrose media become rather fragile and most of these cells are damaged on the membrane filter. This effect is not observed when sucrose is replaced by NaCl in the filtration solution. Almost all the cells remain intact on the membrane filter when the cells are suspended in isotonic NaCl. Apparently monovalent ions play an important role in maintaining a stable membrane, which is in agreement with the findings of Cho *et al.*[23] who demonstrated that, although *A. laidlawii* has no absolute growth requirement for Na^+, a certain amount of Na^+ in the growth medium is essential for optimal growth.

The linear relationship that exists between the erythritol net flux through the *A. laidlawii* cell membrane and the erythritol concentration in the NaCl solution indicates that erythritol passes the membrane by simple diffusion. The permeability of several non-electrolytes through the bilayers of lecithin liposomes is strongly dependent on the nature of the fatty acid acylated to the glycerol moiety of the lecithin molecule[4]. McElhaney *et al.*[10] demonstrated that the initial swelling rate of *A. laidlawii* cells and derived liposomes in isotonic glycerol was dependent on the fatty acid composition of the membrane lipids. In this study we have shown that the [^{14}C]-erythritol equilibrium flux through the *A. laidlawii* membrane is also dependent on the fatty acid composition. The [^{14}C]erythritol equilibrium flux through the *A. laidlawii* membrane is in the order linoleic acid > oleic acid > elaidic acid > stearic acid-grown cells. The same order has been found using the initial swelling rate method[10].

The phase transition occurring in elaidic acid-grown cells is broad and ranges from about 16 °C up to 43 °C (ref. 3). If one assumes that this broad transition is caused by a gradual melting of hydrocarbon chains in the interior of the bilayer in such a way that at each temperature during the transition both crystalline and liquid crystalline fatty acid chains are present, one can calculate from Fig. 8 of ref. 3 the temperature dependence of the amount of both phases during the transition (see Fig. 6). In this calculation it is assumed that there is a fairly constant cal/g value over the whole transition. It is obvious that at the growth temperature (37 °C) about 20% of the lipids are in the crystalline state. From Fig. 9 of ref. 3 and Fig. 2 of this

paper the permeability to glycerol and erythritol expressed as the initial swelling rate in isotonic solutions or as the [^{14}C]erythritol equilibrium flux can be plotted as a function of the percentage of the membrane lipids which are in the liquid crystalline state (Fig. 7). A linear relationship between the glycerol and erythritol permeability and the percentage of the membrane lipids in the crystalline state is obtained (Fig. 7). This linear relationship indicates that the temperature dependence of the diffusion of non-electrolytes through the *A. laidlawii* cell membrane with its membrane lipids

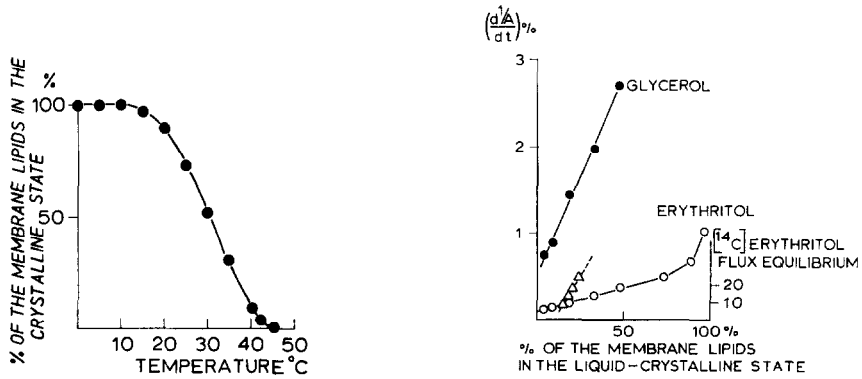


Fig. 6. Percentage of the membrane lipids of *A. laidlawii* in the crystalline form as a function of temperature. Data are taken from ref. 3, Fig. 8. Cells were grown on 0.06 mM elaidic acid.

Fig. 7. Glycerol and erythritol permeability of *A. laidlawii* cell membranes at temperatures in the transition of the membrane lipids. Initial swelling rate of cells in isotonic glycerol (●) or erythritol (○) and the [^{14}C]erythritol equilibrium flux (△) at temperatures in the transition of the membrane lipids is given as a function of the percentage of the membrane lipids in the liquid crystalline state. Cells were grown on 0.06 mM elaidic acid.

in the transition can be described as a preferential diffusion through liquid crystalline parts of the lipid bilayer in the membrane of which the surface changes as a function of the temperature. This can only be true if the average thermal motion of the hydrocarbon chains in the liquid–crystalline parts of the lipid bilayer in the membrane is not dependent on the temperature in the transition. Qualitatively, this fits with the reasoning that, by lowering the temperature in the transition of the membrane lipids, those lipids will crystallize first which have the highest transition temperature and the lowest thermal motion.

At lower temperatures we observed (Figs 2 and 4) that considerable breakage of the *A. laidlawii* cells occurred on the membrane filter. These temperatures were 17.5, 14 and 5 °C for cells grown on stearic, elaidic and oleic acid, respectively. Cells grown on linoleic acid remained intact on the filter at all temperatures tested. These temperatures coincide with the temperatures at which the swelling of *A. laidlawii* cells in isotonic glycerol or erythritol apparently becomes very rapid (ref. 3 and unpublished observations). This increased apparent swelling rate as detected by a rapid decrease of the absorbance of the cell suspension was attributed to bursting of part of the cells caused by the inability of a cell with its membrane lipids in a crystalline form to swell. In the case of the elaidic acid-grown cells all fatty acid chains of the membrane lipids of *A. laidlawii* become "crystalline" at 16 °C as measured by differ-

ential scanning calorimetry[3]. From these data we conclude that *A. laidlawii* cells with all their membrane lipids in a crystalline form become very fragile, which leads to breakage of the cells on the membrane filter. A similar phenomenon has been observed with an unsaturated fatty acid-requiring mutant of *E. coli* K1060[24]. The most striking differences in this behaviour between the two organisms are (1) the breakage of the *A. laidlawii* cells on the membrane filter below the transition temperature of the membrane lipids is accompanied by a release of cytoplasmic proteins; this was not observed with the *Escherichia coli* cells which might indicate that the cell wall can retain these proteins[24]. (2) When *A. laidlawii* cells are suspended in NaCl media below the transition temperature of the membrane lipids no loss of cytoplasmic proteins is observed when no big mechanical stress is applied to the cells (Table IV). This indicates that the *A. laidlawii* cells with all its lipids in a crystalline form is itself stable in solution, and that no damage of the membrane occurs by passing the transition temperature of the membrane lipids. This is also supported by the finding that no loss of intracellular K^+ is observed when cells are brought below the transition temperature of the membrane lipids of *A. laidlawii* (van der Neut-Kok, E., unpublished). In the case of the *E. coli* mutant, however, a dramatic loss of K^+ is observed when the cells are suddenly cooled below the transition temperature of the membrane lipids[24]. This indicates that there is a considerable difference between the integrity of the *A. laidlawii* and *E. coli* cell membrane when the temperature is passed at which the membrane lipids become crystalline.

Cholesterol incorporated in the *A. laidlawii* membrane reduced the [^{14}C]-erythritol equilibrium flux above the temperature at which all membrane lipids become crystalline through the membranes of cells grown on linoleic, oleic, elaidic and stearic acid. The initial swelling rate in isotonic glycerol and erythritol of *A. laidlawii* cells grown on linoleic, oleic, elaidic and stearic acid is also decreased when cholesterol is incorporated into the membrane[3,10]. We observed that the temperatures below which rupture of the cells on the membrane filters occurs are decreased by about 7 °C in the presence of cholesterol. This can be explained by a liquefying effect of cholesterol on the membrane lipids of *A. laidlawii*[3].

Cholesterol lowers the erythritol and glycerol permeability of the elaidic acid-grown cells up to the lower end of the phase transition (Fig. 5 and ref. 3). This can be explained as discussed above by presuming that the permeation to glycerol and erythritol only occurs *via* these part of the membrane which remain liquid in the transition. Cholesterol will lower the glycerol and erythritol permeability of these liquid crystalline domains in the membrane. The liquefying effect of cholesterol on a variety of lipid bilayers including the *A. laidlawii* cell membrane is well documented[3,25]. In this study we have presented evidence that this liquefying effect of cholesterol can be very important for maintaining proper membrane functioning (Fig. 5). *A. laidlawii* failed to grow in a medium supplemented with cholest-5-en-3-one or cholest-4,6-dien-3-one, which is in agreement with the observations of Butler and Knight[27]. The growth of the cholesterol-requiring *M. mycoides* was inhibited by 3-ketosteroids[21].

Part of the cholesterol of erythrocytes is replaced by 3-ketosteroids after incubating the erythrocytes with 3-ketosteroid-rich liposomes. This leads to an increased glycerol permeability and osmotic fragility of the erythrocytes[7]. Similar results were obtained in permeability studies using egg lecithin liposomes containing 3-keto-

steroids[11]. Apparently 3-ketosteroids induce alterations in the membrane leading to an increased permeability or fragility.

No or poor growth of *A. laidlawii* cells was observed when the medium was supplemented with sterols missing the hydrophobic side chain at C-17. The sterols cholesterol, epicholesterol, cholestanol, ergosterol, stigmasterol and coprostanol were incorporated into the *A. laidlawii* membrane when present in the growth medium.

The sterols having a 3α-OH group, epicholesterol and epicholestanol, and the sterol with a bent *cis*-structured steroid nucleus, coprostanol, did not change the glycerol and erythritol permeability of the membrane very much. Of the sterols having a 3β-OH group and a flat steroid nucleus only stigmasterol showed almost no effect on the glycerol and erythritol permeability. The other sterols tested caused a considerable reduction in the glycerol and erythritol permeability of the *A. laidlawii* membrane. In model membrane systems it was found that for the specific interaction occurring between sterols and lecithin, leading to a decreased permeability for several solutes[11], a reduction in the area per molecule in a monolayer of lecithin[12] and a reduction in the mobility of the fatty acid chains in egg lecithin[13], the sterol molecule was required to have a 3β-OH group, a planar, intact ring system and a hydrophobic side-chain at C-17. In the biological membrane of *A. laidlawii* the first two requirements also seem to be a prerequisite for the permeability-lowering effect of the incorporated sterol. In contrast to the model membrane systems, an ethyl group in the side-chain of the sterol molecule (stigmasterol) seems to interfere with the interaction between the sterol and other membrane components in the *A. laidlawii* membrane.

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