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# SOME STUDIES ON THE FATTY ACID COMPOSITION OF TOTAL LIPIDS AND PHOSPHATIDYLGLYCEROL FROM *ACHOLEPLASMA LAIDLAWII* B AND THEIR RELATION TO THE PREMEABILITY OF INTACT CELLS OF THIS ORGANISM

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SUMMARY

1. *Acholeplasma laidlawii* B (previously denoted as *Mycoplasma laidlawii* B) was grown in the presence of equimolar mixtures of palmitic or stearic acid and the following unsaturated fatty acids: elaidic, oleic, linoleic, or arachidonic acid.

2. Significant differences were observed in the fatty acid composition of total lipids and phosphatidylglycerol after growth in the presence of the various mixtures of fatty acids. Decreasing amounts of unsaturated fatty acids were incorporated in the following order: elaidic > oleic > linoleic > linolenic > arachidonic acid. This decrease was found to be counterbalanced by an increased uptake of saturated fatty acids.

3. The permeability of intact cells of *Acholeplasma*, after growth on the indicated mixtures of fatty acids was found to be maintained within certain limits. These observations indicate that *A. laidlawii* B is equipped with a mechanism to control the fluidity of its membrane lipids and, hence, the permeability of its membrane.

4. The positional distribution of saturated and unsaturated fatty acyl chains at the C-1 and C-2 positions of phosphatidylglycerol of *A. laidlawii* B appeared to be introduced during the first step of its biosynthesis, namely the formation of phosphatidic acid.

5. *A. laidlawii* B was found to elongate palmitoleic acid.

## INTRODUCTION

*Acholeplasma* cells are surrounded by a single lipoprotein membrane, which comprises the majority of the cellular lipids[1]. Several investigators have shown that the fatty acid composition of the lipids of *Acholeplasmas* and *Mycoplasmas* can be altered substantially by the addition of fatty acids to a lipid-poor growth medium[2,3]. Because the relative proportions of the various phospholipids and glycolipids remain

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unaffected under these conditions[4], the *Acholeplasmas* are extremely suitable organisms for investigating the effect of variations in the fatty acyl moieties of the lipids on the permeability properties of their membrane. McElhaney *et al.*[4] studied the permeability of glycerol for intact cells of *A. laidlawii* strain B, grown on mixtures of palmitic acid and either elaidic, oleic or linoleic acid. The fatty acids were added to the growth medium in such a ratio that the membrane lipids contained approximately equimolar amounts of palmitate and one of the unsaturated fatty acids. Both for intact cells or *Acholeplasma* and for liposomes prepared from the *Acholeplasma* membrane lipids, the glycerol permeation strongly increased in the order elaidic < oleic < linoleic acid. These findings were consistent with previous studies by De Gier *et al.*[5] and Demel *et al.*[6] on the permeability of liposomes prepared from various synthetic phospholipids.

In the present study a somewhat different approach was chosen for studying the permeability properties of *Acholeplasma* membranes. The cells were grown in the presence of equimolar amounts of a saturated fatty acid, *viz.* palmitic or stearic acid, and an unsaturated fatty acid, *viz.* elaidic, oleic, linoleic or arachidonic acid, and the permeability for both glycerol and erythritol was determined. If the *Acholeplasma* cell would possess the capacity to keep the permeability of its membrane constant within certain limits, despite the variety of the fatty acyl groups of its membrane lipids, one would expect that the incorporation of unsaturated fatty acids would strongly decrease in the range elaidic > oleic > linoleic > linolenic > arachidonic acid. If, on the other hand, equal amounts of the various unsaturated fatty acids would be incorporated into the Acholeplasma membrane lipids, one would expect a most significant increase in permeability of cells grown on the unsaturated fatty acids in the order mentioned above.

It has been shown that under certain conditions, *e.g.* in anaerobically grown yeast[7], short-chain saturated fatty acids replace long-chain monoenoic fatty acids at the 2-position of phospholipids. Demel[8] showed that synthetic phospholipids having either a short-chain saturated or a long-chain monoenoic acid at the 2-position exhibited a very similar behaviour in monomolecular films. It was, therefore, thought of interest to include also short-chain saturated fatty acids in the present study on the permeability of *Acholeplasma* membranes.

McElhaney and Tourtellotte[9] studied the positional distribution of the fatty acyl chains of phosphatidylglycerol of *A. laidlawii* strain B, grown in the presence of an extensive series of fatty acids. These authors found that the affinity for the 2-position increased in the following order: saturated < *trans*-monoenoic < *cis*-monoenoic < cyclopropane < *cis*-dienoic fatty acids. In order to extend the observations of McElhaney and Tourtellotte[9], the affinity of linolenic and arachidonic acid for the 2-position of phosphatidylglycerol was also determined in the present study. Moreover, it was investigated, by using various radioactively labelled fatty acids, whether the asymmetric positional distribution observed in phosphatidylglycerol is already accomplished in phosphatidic acid, the first phospholipid presumably formed during the biosynthesis of phosphatidylglycerol.

## EXPERIMENTAL

### *Organism and conditions of growth*

The organism used throughout these studies was *A. laidlawii* strain B.

*A. laidlawii* cells were grown in a lipid-poor tryptose medium, containing glucose, penicillin and bovine serum albumin, as described in detail by McElhaney and Tourtellotte[9]. Equimolar mixtures of two fatty acids, one of which always being either palmitic or stearic acid, were added as sterile ethanolic solutions up to a total concentration of 0.12 mM. Growth was carried out in 100 or 200 ml of culture medium by static incubation at 37 °C. Cells were harvested in the late logarithmic phase of growth as estimated by measurement of the absorbance at 450 nm, by centrifugation at 13000 × *g* for 15 min in a cooled (3 °C) Sorvall RC-28 centrifuge. Traces of growth medium were removed by resuspending the cells in 0.1 M Tris–0.125 M KCl (pH 7.4) buffer followed by sedimentation at 37000 × *g* for 30 min in Sorvall centrigufe.

*Extraction and separation of Acholeplasma lipids*

Total lipids were extracted according to the method of Folch *et al.*[10]. Neutral and polar lipids were separated by means of column chromatography on silicic acid (70–325 mesh, Merck, Germany). Neutral and polar lipids could be eluted from the silica with chloroform and chloroform–methanol (4:1, v/v), respectively. The polar lipids from *Acholeplasma* were fractioanted through thin-layer chromatography on silicagel G (Merck, Germany) using chloroform–methanol–water (65:35:4, v/v/v) as a developer. The separated components were visualised by slight exposure to iodine vapour or, when polyunsaturated fatty acids were present, under violet light after spraying with a 0.005% (w/v) solution of Rhodamine 6G in water.

*Fatty acid analysis*

*(A) Total lipids.* An appropriate aliquot of the total lipid extract, representing approximately 2 mg of total lipids, was evaporated to dryness under reduced pressure and the residue subsequently heated, under nitrogen atmosphere, in 18 ml methanol–sulphuric acid (95:5, w/v) for 2 h at 70 °C. After cooling 2 ml of 10 M NaOH was added and the heating at 70 °C continued for 1 h. The resulting reaction mixture was extracted three times with 25-ml portions of pentane to remove non-saponifiable materials. The combined pentane fractions were extracted once with 30 ml methanol–water (1:2, v/v) and then discarded. The methanol–water layer was added to the original aqueous layer and, after acidification, the fatty acids could be extracted completely with three 25-ml portions of pentane. The combined pentane layers were evaporated to dryness *in vacuo* and the free fatty acids converted into their corresponding methyl esters by treatment with an ethereal solution of diazomethane[11].

*(B) Phosphatidylglycerol.* The phosphatidylglycerol fraction was scraped from the silica thin-layer plate and transferred directly into a tube containing 18 ml of methanol–sulphuric acid (95:5, w/w). Transesterification was carried out under nitrogen atmosphere for a period of 2 h at 70 °C, followed by extraction of the methylesters with three 25-ml portions of pentane.

Analysis of the methyl esters of the fatty acyl consistuents of both the total lipid mixtures and of phosphatidylglycerol fractions were performed using a Packard gaschromatograph model 878, equipped with a flame ionization detector and a 3% EGSS-X column.

*Positional distribution of the fatty acyl constituents of phosphatidylglycerol*

After separation of the polar lipids on silica thin-layer plates as described above,

the phosphatidylglycerol fraction was exhaustively eluted from the silica with chloroform–methanol (1:1, v/v) and subsequently subjected to hydrolysis with pure pancreatic phospholipase $A_2$ (EC 3.1.1.4) as described previously[12]. The resultant 1-acyl lysophosphatidylglycerols and fatty acids were separated *via* thin-layer silica G plates, developed in chloroform–methanol–water (65:35:4, v/v/v) and their fatty acid composition determined as described above for phosphatidylglycerol.

*Permeability of A. laidlawii cells*

*Acholeplasma* cells were washed in 200 mM sucrose and finally suspended in 2 ml of this medium. The permeability for glycerol or erythritol was determined at various temperatures by measuring the initial swelling rates of intact *Acholeplasma* cells in isotonic solutions of these compounds using a Vitatron UFD photometer as described by de Gier *et al.*[5] for determination of the swelling rate of liposomes and as applied by McElhaney *et al.*[4] and de Kruyff *et al.*[13] to intact *A. laidlawii* cells.

*Experiments with radioactively labelled fatty acids*

In the mid-lag of growth 20 $\mu$Ci of a $^3$H- and 5 $\mu$Ci of a $^{14}$C-labelled fatty acid were added to a 100-ml cell culture which has been growing in the presence of the same non-labelled fatty acids. After the addition of the radioactive acid, growth was allowed to continue for 1 h, after which the cells were harvested and the polar lipids isolated as described above.

*Positional distribution of labelled fatty acids in phosphatidic acid*

Phosphatidic acid was isolated from the polar lipid mixture by means of thin-layer chromatography on silica thin-layer plates impregnated with 0.25 M oxalic acid using chloroform–methanol–conc. HCl (87:13:0.5, v/v/v) as a developer. Carrier phosphatidic acid was added to facilitate identification. The phosphatidic acid fraction was eluted from the silica with chloroform–methanol–0.1 M HCl (2:7:1, v/v/v) and converted into dimethylphosphatidates by the method described by Possmayer *et al.*[14]. The dimethylphosphatidates were purified by means of silica thin-layer chromatography using hexane–diethyl ether–acetic acid (20:80:1, v/v/v) as a developer. After elution from the silica with chloroform–methanol (80:20, v/v) the dimethyl phosphatidates were degraded with pancreatic lipase (EC 3.1.1.3), as described by Possmayer *et al.*[14], to ascertain the positional distribution of the fatty acids incorporated into phosphatidic acid.

*Radioactivity measurement of methyl esters separated by gas chromatography*

In order to investigate possible interconversions of fatty acids during the growth the fatty acyl constituents of the *Acholeplasma* lipids were separated, as methyl esters, by gas–liquid chromatography using an F and M gas chromatograph Model 720 equipped with a 12% polyethylene glycol adipate column, a thermal conductivity detector and a Packard fraction collector Model 830. The methyl esters leaving the gas chromatograph were collected in small glass tubes plugged with glass wool, which were transferred directly into scintillation vials for assay of radioactivity.

*Assay of radioactivity*

Radioactivity was assayed in a Packard Tricarb liquid scintillation spectro-

meter Model 3003. The samples were counted in scintillation vials containing 15 ml of a solution of 0.5% (w/v) PPO and 0.03% (w/v) dimethyl-POPOP in toluene. Quench corrections were made using the external standard method.

*Chemicals*

Lauric, myristic, palmitic and stearic acid were obtained from Fluka A.G. (Switzerland); elaidic, oleic, linoleic and linolenic acid from Koch-Light (England) and arachidonic acid from the Hormel Institute (U.S.A.). Radioactive fatty acids were purchased from New England Nuclear (Boston, Mass., U.S.A.). [$^{3}$H]Arachidonic acid from Applied Science Lab. Inc. (State College); [$^{14}$C]palmitoleic and [$^{14}$C]elaidic acid from the Radiochemical Centre, Amersham (England). Bovine serum albumin was obtained from Calbiochem (Los Angeles, Calif. U.S.A.).

## RESULTS AND DISCUSSION

As already shown by various authors, dramatic differences are observed in the fatty acid patterns of *Acholeplasma* lipids after growth in the presence of different fatty acids[2,9]. In none of these studies was the uptake of unsaturated fatty acids with more than two double bonds, reported. Fig. 1 presents the fatty acid composition of

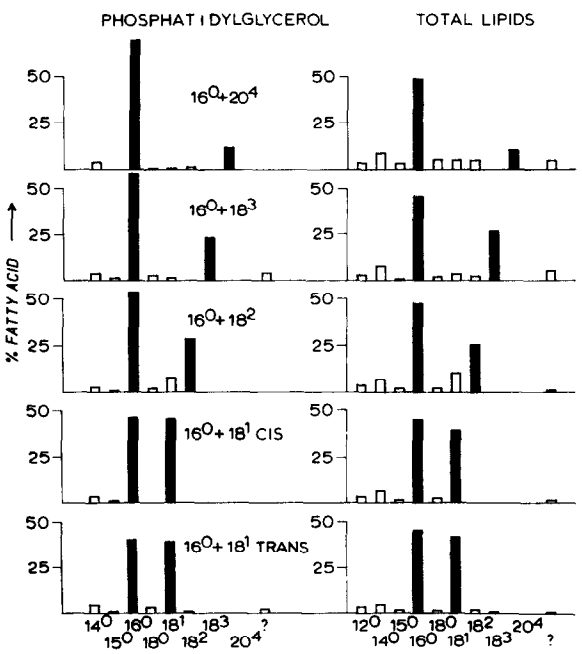


Fig. 1. Fatty acid composition of phosphatidylglycerol and total lipids of *A. laidlawii* B grown in the presence of different combinations of palmitic acid *plus* an unsaturated fatty acid. (The fatty acids added to the medium are indicated by solid bars.)

the total lipids and of phosphatidylglycerol of *A. laidlawii* strain B, grown in the presence of equimolar mixtures of palmitic acid and one of the following unsaturated fatty acids: elaidic, oleic, linoleic, linolenic and arachidonic acid respectively.

Both oleic and its *trans*-isomer elaidic acid are taken up into the total lipids and phosphatidylglycerol in amounts equimolar to that of palmitic acid. However, decreasing amounts of unsaturated fatty acids are incorporated into total lipids and phosphatidylglycerol in the following order: linoleic acid > linolenic acid > arachidonic acid. In phosphatidylglycerol, the diminished amount of unsaturated acids incorporated, appears to be counterbalanced by higher levels of palmitic acid. In the total lipids of *Acholeplasma* this compensation is less clear though there is a definitely increased uptake of palmitic acid when the organism is grown in the presence of polyunsaturated fatty acids, there also seems to be an increase in the level of short-chain fatty acids, especially myristic and pentadecanoic acids. These short-chain fatty acids were not present in the growth medium in significant amounts, but probably result from biosynthesis by *Acholeplasma* cells. The small amount of oleic and linoleic acid found in *Acholeplasma* lipids even when grown in the absence of these fatty acids, must originate from residual amounts of these acids in the lipid-poor medium, since it is known that *A. laidlawii* does not possess the enzymes required for the biosynthesis of unsaturated fatty acids[15].

A similar decrease in the uptake of dienoic and trienoic fatty acids, when compared to monoenoic fatty acids, has been found in an unsaturated fatty acid auxotroph of *Escherichia coli* by Esfahani *et al.*[16]. Probably both *E. coli* and *A. laidlawii* possess a regulatory mechanism which controls the total degree of unsaturation of their membrane lipids.

Fig. 2 presents the fatty acid composition of the total lipids and of phosphatidyl-

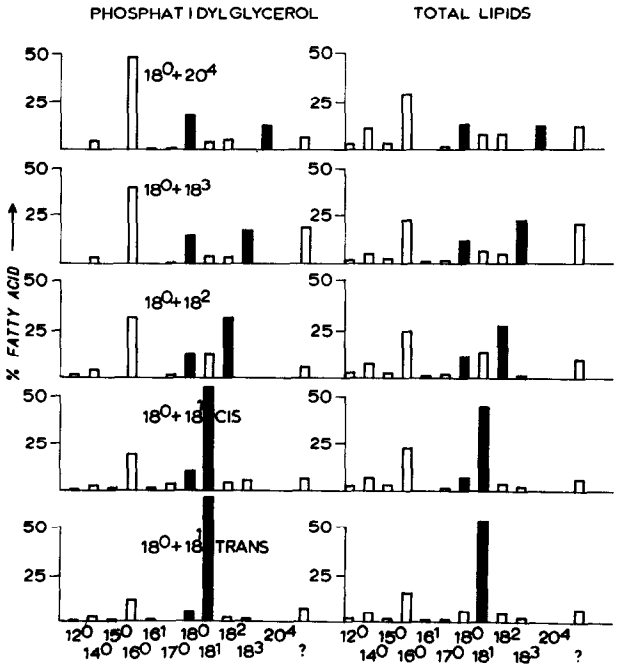


Fig. 2. Fatty acid composition of phosphatidylglycerol and total lipids of *A. laidlawii* B grown in the presence of different combinations of stearic acid *plus* an unsaturated fatty acid. (The fatty acids added to the medium are indicated by solid bars.)

glycerol of *A. laidlawii* grown in the presence of equimolar amounts of stearic acid and the same series of unsaturated fatty acids. Similarly as observed in the various mixtures with palmitic acid, a strong decrease can be noted in the uptake of unsaturated fatty acids in the order elaidic > oleic > linoleic > linolenic > arachidonic acid. The monoenoic acids, particularly elaidic acid, were incorporated into *Acholeplasma* lipids in much higher amounts when provided to the medium in combination with stearic than with palmitic acid. The strong decrease in the uptake of unsaturated fatty acids with increasing degree of unsaturation, is accompanied by enhanced levels of stearic and palmitic acid. The latter acid was not provided to the growth medium, but must have been formed by *Acholeplasma* cells. In the presence of exogeneous fatty acids in the growth medium the biosynthesis of fatty acids by the *Acholeplasma* cell is normally suppressed[15]. However, the presence of exogeneous stearic acid, particularly in combination with polyunsaturated fatty acids, appears to induce the biosynthesis of myristic and palmitic acid, perhaps to avoid accumulation of stearate residues in the membrane lipids. In contrast to the findings with palmitic acid, significant amounts of unidentified fatty acids with a longer retention time than linoleic acid were found, especially in the total lipids fraction, if the *Acholeplasma* cells were grown in the presence of stearic acid.

The preservation of a certain degree of unsaturation of its membrane lipids during growth in the presence of equimolar mixtures of palmitic or stearic acid and a variety of unsaturated fatty acids, as shown in Figs 1 and 2, may be an effort on the part of the *Acholeplasma* cell to control the permeability properties of its membrane. To investigate this the permeability of *Acholeplasma* cells grown in the presence of the various equimolar mixtures of saturated and unsaturated fatty acids was determined by measuring the initial swelling rates of the intact cells in isotonic glycerol and erythritol. Fig. 3. presents the relative initial swelling rates in glycerol at 15 °C and in erythritol at 25 °C and 37 °C. The initial swelling rate of cells grown in the presence of equimolar amounts of palmitic or stearic acid and arachidonic acid is arbitrarily set at 100. A significant increase in permeability can be noted in the order: elaidic <

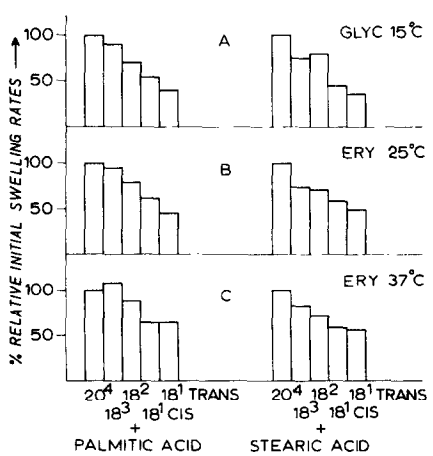


Fig. 3. Relative initial swelling rates in isotonic glycerol or erythritol of intact cells of *A. laidlawii* B. The initial swelling rate of cells grown in the presence of palmitic or stearic acid and arachidonic acid is arbitrarily set at 100.

oleic < linoleic < linolenic < arachidonic acid, both in the presence of palmitic and stearic acid as saturated fatty acids. However the increase in permeability would have been much more pronounced if equimolar amounts of the various unsaturated fatty acids were incorporated. Indeed McElhaney *et al.*[4] reported that the relative swelling rate in glycerol at 15 °C for *Acholeplasma* cells containing equimolar amounts of palmitic and elaidic, palmitic and oleic, and palmitic and linoleic acid respectively, showed a ratio of 0.3:0.6:1.0, whereas the corresponding ratio for cells grown in the presence of equimolar amounts of these fatty acids (Fig. 3) is: 0.6:0.8:1.0. At present no data are available to compare the permeability of *Acholeplasma* cells grown on equimolar amounts of palmitic and linolenic or arachidonic acid with that of cells containing equimolar amounts of these acids. However, the results presented in Figs 1, 2 and 3, already indicate that *A. laidlawii* possesses a mechanism to control the fluidity of its membrane lipids and the permeability of its membrane barrier, at least within certain limits.

Table I shows the fatty acid composition of *Acholeplasma* total lipids and phosphatidylglycerol after growth in the presence of equimolar mixtures of palmitic or stearic acid and lauric, myristic or palmitoleic acid, respectively. *A. laidlawii* B has the capacity to elongate both lauric and myristic acid: if *A. laidlawii* B is grown in the presence of stearic acid and either lauric or myristic acid significant amounts of palmitate are formed. (Table I). If the organism is grown in the presence of stearic and lauric acid, a substantial level of myristic acid is found in the lipids. These observations endorse previous findings that both lauric and myristic acid can be elongated to palmitate[15]. Panos and Henrikson[17] have demonstrated that *Mycoplasma* sp. KHS is able to elongate palmitoleic acid into *cis*-vaccenic acid. However *Mycoplasma* strain Y (Rodwell[18]) and *Acholeplasma laidlawii* strain B (Panos and Rottem[19]) were shown to be devoid of the enzymes required to elongate palmitoleic acid.

TABLE I

FATTY ACID COMPOSITION OF TOTAL LIPIDS AND PHOSPHATIDYLGLYCEROL OF *A. laidlawii* B. GROWN IN THE PRESENCE OF DIFFERENT COMBINATIONS OF FATTY ACIDS

| *Fatty acid added* | % *Fatty acid* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *12:0* | *13:0* | *14:0* | *15:0* | *16:0* | *16:1* | *17:0* | *18:0* | *18:1* | *18:2* | *Unidentified* |
| | *Total phospholipids* | | | | | | | | | | |
| 16:0+16:1 | 3.1 | 0.3 | 7.2 | 1.0 | *48.3* | *10.5* | — | 1.4 | 24.6 | — | 3.5 |
| 16:0+14:0 | 2.5 | 0.5 | *28.1* | 1.3 | *51.5* | + | + | 2.9 | 3.9 | 3.0 | 6.3 |
| 16:0+12:0 | 5.7 | 0.5 | 24.8 | 1.2 | *58.9* | + | + | 2.7 | 3.9 | 2.3 | + |
| 18:0+16:1 | 3.0 | 0.5 | 6.3 | 1.7 | 18.2 | *10.5* | 2.0 | *7.5* | 37.2 | 3.1 | 10.0 |
| 18:0+14:0 | 2.7 | 0.9 | *33.9* | 2.6 | 37.8 | + | 0.6 | *9.8* | 4.8 | 4.3 | 2.6 |
| 18:0+12:0 | *6.8* | 0.5 | 24.2 | 1.7 | 33.4 | 1.0 | 1.2 | *13.5* | 4.1 | 5.9 | 7.7 |
| | *Phosphatidylglycerol* | | | | | | | | | | |
| 16:0+16:1 | + | + | 2.8 | 1.2 | *41.0* | 6.4 | — | 1.8 | 46.8 | + | — |
| 16:0+14:0 | 1.3 | + | *38.1* | + | *54.8* | — | — | 4.1 | 1.7 | + | — |
| 16:0+12:0 | *5.2* | + | 24.8 | + | *61.3* | — | — | 4.0 | 2.2 | 2.5 | — |
| 18:0+16:1 | 0.9 | 1.0 | 1.9 | 0.9 | 14.0 | *8.9* | 0.7 | *8.9* | 59.8 | 3.0 | — |
| 18:0+14:0 | 1.2 | 0.6 | *25.6* | 1.3 | 28.6 | + | 2.6 | *32.6* | 2.0 | 3.3 | 2.2 |
| 18:0+12:0 | *3.4* | 0.4 | 24.0 | 0.9 | 24.0 | 0.5 | + | *41.0* | 4.3 | 1.5 | — |

The results mentioned in Table I show that in contrast to *Acholeplasma* A, *Acholeplasma* B has the capacity to elongate palmitoleic acid into octadecenoic acid.

This observation was confirmed by adding [1-$^{14}$C]palmitoleic acid to the growth medium. The majority of the radioactivity incorporated into the *Acholeplasma* lipids was recovered as octadecenoic acid.

Remarkably high is the incorporation of stearic acid when added to the growth medium in combination with lauric or myristic acid, whereas only low levels of stearate are taken up into the *Acholeplasma* lipids when this acid is present in the growth medium in combination with unsaturated fatty acids (Fig. 2).

The relative initial swelling rates of *Acholeplasma* cells grown on equimolar mixtures of palmitic acid and hexadecenoic, myristic or lauric acid, respectively, indicate that the permeability of cells grown on short-chain saturated fatty acids does not deviate significantly from that of cells grown on long-chain monoenoic acids (Table II). This observation is in agreement with the finding of Demel[8] that phospho-

TABLE II

RELATIVE INITIAL SWELLING RATES IN ISOTONIC GLYCEROL AND ERYTHRITOL OF INTACT CELLS OF *A. laidlawii* B GROWN IN THE PRESENCE OF DIFFERENT COMBINATIONS OF FATTY ACIDS

The initial swelling rate of cells grown in the presence of palmitic or stearic acid and palmitoleic acid is arbitrarily set at 100.

| *Fatty acid added* | *Relative initial swelling rate* | | |
|---|---|---|---|
| | *In glycerol* 15° C | *In erythritol* | |
| | | 25 °C | 37 °C |
| 16:0+16:1 | 100 | 100 | 100 |
| 16:0+14:0 | 79 | 62 | 76 |
| 16:0+12:0 | 56 | 42 | 57 |
| 18:0+16:1 | 100 | 100 | 100 |
| 18:0+14:0 | 43 | 37 | 29 |
| 18:0+12:0 | 21 | 25 | 28 |

lipids having either a short-chain saturated or a long-chain monoenoic acyl group, behave rather similarly at the air–water interphase of a monolayer system.

*Acholeplasma* cells grown on equimolar mixtures of stearic acid and lauric or myristic acid are much less permeable than cells grown on stearic acid and palmitoleic acid. This has probably to be attributed to rather high levels of stearate incorporated into the *Acholeplasma* lipids, when this acid is present in combination with lauric or myristic acid.

Many naturally occurring phospholipids are characterized by an asymmetric positional distribution of their fatty acyl constituents: saturated fatty acids are located predominantly at the 1-position, whereas polyunsaturated acyl groups are encountered mainly at the 2-position. The distribution of monounsaturated fatty acids is somewhat less pronounced, though they are generally located at the 2-position. (See reviews by van Deenen[20], Hill and Lands[21], van den Bosch *et al.*[22].) The positional distribution of fatty acids among the 1- and 2-position of *Acholeplasma* phospholipids does not represent an exception to this general rule of positional specificity. McElhaney and Tourtellotte[9] studied the positional distribution of phosphatidylglycerol of *A. laidlawii* strain B after growth in the presence of an extensive series of different fatty acids. They found an increasing affinity for the 2-position of phosphatidylglycerol in the following order: saturated < branched chain < *trans*-monoenoic < *cis*-monoenoic < cyclopropane < *cis*-dienoic fatty acids. It has been demonstrated by *in vitro*

and *in vivo* studies that the asymmetric distribution of acyl chains in phospholipids of rat liver is introduced during the first step of their *de novo* synthesis, namely the formation of phosphatidic acid (for a review see ref. 22). It was thought of interest to investigate whether the asymmetric distribution of the fatty acyl constituents of phosphatidylglycerol of *Acholeplasma* is also introduced in the first step of its biosynthesis. For this purpose *Acholeplasma* cells were grown in the presence of equimolar amounts of palmitic acid and one of the following unsaturated acids: elaidic, oleic, linoleic, linolenic and arachidonic acid. In the mid-logarithmic phase of growth trace amounts of radioactive palmitic and one unsaturated fatty acid were added, and growth was allowed to continue for 1 h. The positional distribution of the various radioactive fatty acids among the 1- and 2-position of phosphatidic acid is shown in Fig. 4. The positional distribution of unlabelled fatty acids in phosphatidylglycerol is

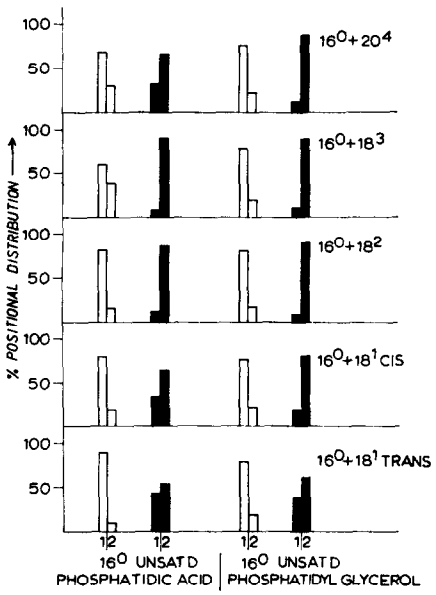


Fig. 4. Positional distribution of palmitic acid and unsaturated fatty acid in phosphatidic acid and in phosphatidylglycerol from *A. laidlawii* B. (The unsaturated fatty acids are indicated by solid bars.)

also presented in Fig. 4. The results show, in agreement with the findings of McElhaney and Tourtellotte[9] that the polyunsaturated fatty acids are directed preferentially to the 2-position of phosphatidylglycerol. The preference for the 2-position is, as could be expected, somewhat less for oleic acid and, particularly, for elaidic acid. The affinity of linoleic and linolenic acid for the 2-position is almost equal, whereas the preference of arachidonic acid for the 2-position is, surprisingly, somewhat less pronounced than that of the less unsaturated linoleic and linolenic acids.

As shown in Fig. 4, the asymmetrical positional distribution of the acyl groups of phosphatidylglycerol is present in phosphatidic acid as well. Actually, the affinity of the various unsaturated fatty acids for the 2-position of phosphatidylglycerol and phosphatidic acid, respectively, is strikingly similar.

Probably, the enzyme(s) of *A. laidlawii* involved in the transfer of fatty acid constituents to the 1- and 2-position of phosphatidic acid show a similar selectivity towards the structural characteristics of the fatty acyl moiety as observed in other cells.

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