The Effect of Long Chain Fatty Acid Isomers on Growth, Fatty Acid Composition and Osmotic Fragility of *Mycoplasma laidlawii* A

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SUMMARY

Growth of *Mycoplasma laidlawii* A in a tryptose medium, with lipid pre-extracted, containing charcoal-treated bovine plasma albumin, occurred only after addition of long-chain fatty acids. Of the long-chain fatty acids tested, positional as well as geometrical isomers of octadecenoic acids were effective in growth promotion; *trans* isomers were more effective in promoting growth and in lowering osmotic fragility than their corresponding *cis* isomers. Tetradecenoic or hexadecenoic acids did not replace the growth requirement for an octadecenoic acid. Although fatty acids containing cyclopropane-rings promoted growth, a greater toxicity and lower growth response was observed. No fatty acid isomerization by this mycoplasma was detected and the octadecenoic acids added to the growth medium were recovered from membrane polar lipids unchanged. Fatty acid analyses revealed that myristic and palmitic acids were the major saturated fatty acid components of the membrane polar lipids. No differences were found in the fatty acid content or composition of membrane polar lipids from mycoplasmas grown at 25° and 37°. Major differences in the fatty acid composition between glycolipids and phospholipids were not apparent.

INTRODUCTION

The mycoplasma cell is bound by a lipoprotein membrane containing virtually all of the cellular lipids (Razin, 1967). While mycoplasmas not requiring sterols are capable of synthesizing saturated fatty acids from acetate (Rottem & Razin, 1967*a*; Pollack & Tourtellotte, 1967) some require an exogenous octadecenoic acid for growth (Razin & Rottem, 1963; Razin, Tourtellotte, McElhaney & Pollack, 1966) and others do not (Henrikson & Panos, 1969; Panos & Henrikson, 1969). Thus far, all have been found capable of incorporating both saturated and unsaturated fatty acids into their membrane polar lipids (Razin *et al.* 1966; Rottem & Razin, 1967*a*).

Practically nothing is known about the possibility of replacing the unsaturated fatty acid requirement for growth of a non-sterol-requiring mycoplasma with monoenoic positional or geometrical isomers or by fatty acids containing a cyclopropane ring. The ability to alter the fatty acid composition of mycoplasmal membranes by changing the lipid composition of the growth medium and the resulting effect on osmotic fragility of intact cells has been reported (Razin *et al.* 1966). An incorporation of elaidic acid into membrane lipids of the sterol-requiring *Mycoplasma* sp. Y, was reported by Rodwell (1968). This acid replaced both the saturated and un-

saturated fatty acid growth requirements and was incorporated, unchanged, into the membrane lipids of this organism. The incorporation of *trans* monoenoic acid isomers as well as cyclopropane ring containing and methyl-branched fatty acids into membrane lipids has also been reported for certain non-sterol-requiring mycoplasmas (Panos & Henrikson, 1969; McElhaney & Tourtellotte, 1969).

The present work examines the effect of various positional and geometrical monoenoic acids and of the cyclopropane-ring containing acids on growth and osmotic fragility of *Mycoplasma laidlawii* A, an octadecenoic acid-requiring organism. The fatty acid composition of the membrane polar lipids of this organism when grown in the presence of these exogenously supplied fatty acids is also discussed.

METHODS

Organism and growth conditions. *Mycoplasma laidlawii* (oral strain) was kindly provided by Dr S. Razin (The Hebrew University—Hadassah Medical School, Jerusalem, Israel). This strain is related to *M. laidlawii* A (Rottem & Razin, 1967*b*) and requires an octadecenoic acid for growth. For growth experiments, mycoplasmas were grown in 5 ml. medium consisting of 2% (w/v) tryptose (Difco Laboratories Inc., Detroit, Michigan, U.S.A.) from which lipid had been extracted (Henrikson & Panos, 1969), 0.5% (w/v) glucose, 0.5% (w/v) sodium acetate, 500 units penicillin G/ml. and 0.25% (w/v) bovine plasma albumin fraction V (Armour Pharmaceutical Co., Kankekee, Illinois, U.S.A.) which had been treated with charcoal to remove fatty acids according to Chen (1967). Each tube received 0.05 ml. of an ethanolic solution (70%, v/v in water) containing various amounts of the fatty acid to be tested and 0.05 ml. of inoculum grown in the medium described above with 2 μ g. oleic acid/ml. The initial inoculum contained about 10^7 colony-forming units (c.f.u.)/ml. medium as determined by the colony-count technique of Butler & Knight (1960). Inoculated tubes were incubated statically at 37°. Growth was estimated after 24 to 36 hr by viable count (Butler & Knight, 1960) and/or extinction at 400 nm. For the analyses of fatty acids from membrane polar lipids, organisms were grown in 4 l. volumes of medium as described above, supplemented with 2 to 4 μ g./ml. of a particular monoenoic fatty acid. For isotope experiments, [$1-^{14}\text{C}$]oleic acid (57.2 mC/m-mole) or [$1-^{14}\text{C}$]elaidic acid (52 mC/m-mole) was added to the growth medium (1 μ C/l.). For osmotic fragility experiments, 100 ml. of medium supplemented with 10 to 20 μ g./ml. of a monoenoic or cyclopropane-ring containing fatty acid was used. Organisms were routinely harvested after 24 to 36 hr at 37° (17,000 *g* for 20 min.), washed once with 0.25 M-NaCl and the amount of cell protein determined according to Lowry, Rosebrough, Farr & Randall (1951).

Assessment of osmotic fragility. A portion of washed mycoplasmas was suspended in a small volume of 0.25 M-NaCl and 0.05 ml. of suspension added to test-tubes containing 2 ml. 0.25 M-NaCl or 2 ml. 0.05 M-NaCl. The change in turbidity (from 0.500 extinction) was measured at 500 nm. with a Zeiss PMQ II spectrophotometer after incubation at 37° for 15 min. and the results expressed as % lysis in 0.05 M-NaCl.

Lipid extraction and chromatography. Lyophilized membranes isolated by osmotic lysis of washed organisms as described by Razin *et al.* (1966), were extracted with 20 volumes of chloroform + methanol (2 + 1, v/v) according to Folch, Lees & Sloane-Stanley (1957). The lipid extract was dried under a stream of nitrogen, dissolved in

0.5 ml. chloroform and applied to a 1.6×10 cm. column of activated silicic acid (Unisil, 100 to 200 mesh, Clarkson Chemical Co. Inc., Williamsport, Penna., U.S.A.) that had been washed with chloroform. Neutral lipids were eluted with 200 ml. chloroform followed by 200 ml. methanol which eluted the polar lipids (Ansell & Hawthorne, 1964). After removal of neutral lipids, separation of phospholipids from glycolipids was achieved by successive elution with 200 ml. acetone and 150 ml. chloroform + methanol (1 + 1, v/v) (Shaw, Smith & Kostra, 1968). Total phosphorus in lipid fractions was determined according to Fiske & SubbaRow (1925) after digestion of samples with 70 % perchloric acid at 120° for 24 hr.

Analyses of fatty acids. The alkaline hydrolysis-fatty acid extraction methods of Hofmann, Henis & Panos (1957), modified by the omission of bicarbonate washings, were used throughout. Methylation of the free fatty acids, hydrogenation and infrared analyses were performed as described elsewhere (Panos, Cohen & Fagan, 1966; Weinbaum & Panos, 1966). Separation of methylated fatty acid geometrical isomers was achieved by thin-layer chromatography on silica gel G plates containing 15 % (w/v) AgNO_3 and 0.01 % (w/v) Rhodamine 6G. Methylated fatty acid mixtures were dissolved in benzene and applied on plates as bands with each plate containing approximately 200 μg . of material. The developing solvent was a mixture of light petroleum (b.p. 38° to 46°) + diethyl ether (9 + 1, v/v). Fatty acid zones were located under ultraviolet radiation. For recovery of labelled acids in radioactive experiments, bands were scraped off plates, transferred to tubes and the silica gel extracted (3 times) with multiple portions (5 ml.) of light petroleum. After transfer to scintillation vials and subsequent removal of solvent under a stream of nitrogen, 10 ml. of scintillation fluid (3 g. 2,5-diphenyloxazol and 0.1 g. 1,4-bis-(5 phenyl:oxazolyl-2)-benzene in 1 l. toluene) was added and samples counted in a Packard Tricarb Scintillation counter. Esterified positional monoenoic acid isomers, together with the other components of such mixtures, were resolved by capillary column gas chromatography using a 150 foot (40.6 m.) $\times$ 0.01 inch (0.25 mm.) column coated with Carbowax K-20 M + V-93 (99 + 1) (Perkin-Elmer Corp., Norwalk, Conn., U.S.A.) as described previously (Panos *et al.* 1966; Panos & Henrikson, 1968). The designated abbreviations ($\text{C}_{13}\text{T?}$, $\text{C}_{14}\text{T?}$) of the tentatively identified branched methyl fatty acids are based upon their total carbon atom content.

Chemicals. Oleic, *cis*-vaccenic, elaidic and *trans*-vaccenic acids were purchased from the Hormel Institute (Austin, Minn., U.S.A.) and were greater than 99 % pure. [$1\text{-}^{14}\text{C}$]Oleic acid (57.2 mc/m-mole) and [$1\text{-}^{14}\text{C}$]elaidic acid (52 mc/m-mole) were the products of Applied Science Laboratories (State College, Penna., U.S.A.) and were checked for purity before use. Dihydrosterculic (*cis*-9,10-methyleneoctadecanoic) acid had been prepared by the hydrogenation of *Sterculia foetida* kernel oil according to the method of Tausig (1955) following low-temperature crystallization of the free acid and purification of the methyl ester by thin-layer chromatography as described above. Lactobacillic (*cis*-11,12-methyleneoctadecanoic) acid was obtained by vacuum fractional distillation of methylated fatty acids derived from *Escherichia coli* E-26 grown in a defined medium (Hofmann, Lucas & Sax, 1952; Weinbaum & Panos, 1966).

RESULTS

The fatty acid composition of the tryptose+albumin ingredients of the growth medium is given in Table 1. The intensive extraction of dried tryptose and bovine serum albumin resulted in an almost insignificant combined fatty acid content remaining, giving approx. 0.5 μ g. unsaturated fatty acid/ml. medium when reconstituted. Charcoal treatment of bovine serum albumin decreased its fatty acid content from 0.147 % to 0.008 % without changing the relative amount of each of its residual acids. The dominant fatty acids in charcoal-treated albumin preparations were oleic (29 %), palmitic (25 %) and stearic acids (22 %). Almost no growth of *Mycoplasma laidlawii* A occurred in this extracted medium without the addition of an octadecenoic acid.

Table 1. *Residual fatty acid composition and content of the lipid-extracted tryptose+albumin ingredients of the growth medium**

Fatty acids	Composition (%)
Lauric	0.33
C ₁₈ sat'd	0.04
Tridecanoic	0.04
C ₁₄ sat'd	0.04
Myristic	2.87
C ₁₄ unsat'd	0.10
Pentadecanoic	0.31
Palmitic	33.22
Margaric	0.30
Stearic	1.80
Oleic	38.27
Linoleic	0.67
Total fatty acids = 0.0059%	

* Tryptose extracted with chloroform+methanol and ethyl ether, albumin by charcoal treatment

The absolute requirement of *Mycoplasma laidlawii* A for an unsaturated fatty acid has been described (Razin *et al.* 1966). This requirement can be fulfilled by oleic acid and to a lesser degree by linoleic or linolenic acids (Razin & Rottem, 1963). Figure 1 shows that this mycoplasma can be grown in a tryptose pre-extracted medium containing 0.25 % (w/v) charcoal-treated albumin with other fatty acids as well. Either positional or geometrical isomers of octadecenoic acids served as effective growth promoters. Furthermore, a *trans* isomer, elaidic (*trans*-9-octadecenoic) acid, was more active in promoting growth than its corresponding *cis* isomer (oleic acid). Likewise, two cyclopropane-ring containing acids, lactobacillic (*cis*-11,12-methyleneoctadecanoic) and dihydrosterculic (*cis*-9,10-methyleneoctadecanoic) acids were able to replace this need for an octadecenoic acid for growth. Although shorter-chain monoenoic fatty acids such as lauroleic, *cis*-5-tetradecenoic, myristoleic or palmitoleic were devoid of growth-promoting abilities, they were none the less incorporated into complex lipids and some elongated. This aspect will be dealt with in a subsequent publication. The optimal concentration of the various octadecenoic fatty acids for growth was 2.5 to 5.0 μ g./ml. medium, higher concentrations began to exert a toxic effect. The lowest growth response was found to occur with the cyclopropane ring-

containing fatty acids (Fig. 1), with about 87% inhibition of growth at 15 $\mu\text{g./ml.}$ medium (Table 2). A similar toxic effect was not observed with any of the other positional or geometrical octadecenoic acid isomers tested. Also, marked lysis occurred when mycoplasmas were grown with 4 $\mu\text{g./ml.}$ of either of these ring-containing fatty acids upon reaching the stationary phase of growth.

Fig. 1. The effect of long-chain fatty acid isomers on growth of *Mycoplasma laidlawii* A in a lipid pre-extracted tryptose medium. Δ , Oleic (*cis*-9-octadecenoic) acid; $\circ$, *cis*-vaccenic (*cis*-11-octadecenoic) acid; $\square$, elaidic (*trans*-9-octadecenoic) acid; $\bullet$, dihydrosterculic (*cis*-9,10-methyleneoctadecanoic) acid; $\blacktriangle$, lactobacillic (*cis*-11,12-methyleneoctadecanoic) acid. Growth estimated after 36 hr.

Table 2. Growth inhibition of *Mycoplasma laidlawii* A by long-chain fatty acids

Organisms grown in a lipid pre-extracted medium containing 0.25% charcoal-treated albumin. Fatty acids added as ethanolic solutions.

Fatty acid	Concentration ($\mu\text{g./ml.}$)	Colony-forming units/ml.	Inhibition* (%)
Elaidic	5	8.3×10^9	
	15	6.1×10^9	26.5
Oleic	5	6.1×10^9	
	15	2.4×10^9	60.7
Dihydrosterculic	5	2.4×10^9	
	15	3.1×10^8	87.1

* As compared to mycoplasmas grown with fatty acid, 5 $\mu\text{g./ml.}$

Quantitative results from capillary column gas chromatograms are given in Table 3 and illustrate the intensive incorporation of *cis*-vaccenic acid into membrane polar lipids as well as their high myristic acid content as compared with that of the growth medium (Table 1). The total fatty acid content of these membranes was 18.6%. No changes in fatty acid content or in the relative amount of the various fatty acids of the membrane polar lipids was noticed when *Mycoplasma laidlawii* A was grown at 25°.

A comparison of the fatty acid content of membrane phospho- and glyco-lipids is shown in Table 3, which also shows a small but consistently greater *cis*-vaccenic acid content of the phospholipids always apparent during these studies. Glycolipid fractions were eluted from silicic acid columns with acetone and contained approximately 40 % of the membrane polar lipids. Membrane phospholipids were similarly eluted with chloroform + methanol (1 + 1, v/v). These fractions contained over 43 % of the total polar lipids and all of the organic phosphorus of the membrane lipids.

Table 3. *Fatty acid composition (%) and distribution of Mycoplasma laidlawii A membrane polar lipids**

Organisms grown in a lipid pre-extracted medium containing 0.25% charcoal-treated albumin. *cis*-Vaccenic acid added as ethanolic solution.

Fatty acid†	Polar lipids	Phospholipids	Glycolipids
Capric	0.01	0.01	0.01
Lauric	7.04	6.72	7.96
C ₁₈ sat'd (C ₁₃ T?)	0.49	0.40	0.39
Tridecanoic	1.02	0.92	0.98
C ₁₄ sat'd (C ₁₄ T?)	0.02	0.01	0.02
Myristic	30.12	28.62	31.40
C ₁₄ unsat'd	0.40	0.38	0.25
C ₁₄ unsat'd	0.93	0.91	0.93
Pentadecanoic	1.48	1.47	1.43
Palmitic	16.61	15.01	17.12
Margaric	0.01	0.01	0.01
Stearic	1.27	1.32	1.06
<i>cis</i> -Vaccenic	39.09	41.98	38.01
Linoleic	1.30	1.15	1.20
Unsat'd/sat'd ratio	0.72	0.80	0.70

* Mycoplasmas grown with *cis*-vaccenic acid, 2 µg./ml., average of three experiments.

† Unsat'd = unsaturated, sat'd = saturated.

When *Mycoplasma laidlawii A* was grown with elaidic acid the fatty acid profile and composition of its membrane polar lipids was the same as that for organisms grown with oleic acid. However, the characteristic configurational *trans* band (10.3 µ) appeared in the infrared spectra of those methylated fatty acid mixtures from membrane polar lipids of mycoplasmas grown with elaidic acid (Fig. 2). The possibility that part of the elaidic acid was isomerized to the corresponding *cis* isomer was excluded by the use of labelled fatty acids in the growth medium and the subsequent separation of geometrical octadecenoic acid isomers by thin-layer chromatography. Table 4 shows that this mycoplasma was incapable of isomerizing an octadecenoic acid either from or to the *trans* configuration. All of the [1-¹⁴C]elaidic or [1-¹⁴C]oleic acid added to the growth medium was recovered from membrane polar lipids without redistribution of radioactivity. The low amount of radioactivity appearing in the *cis* and *trans* areas of the respective *trans* and *cis* thin-layer chromatographic fractions (Table 4) was due to impurities in the isotopic reagents as supplied by the vendor.

An increase in the concentration of oleic or elaidic acid in the growth medium up to 12 µg./ml. resulted in an increase in the total amount of each acid incorporated into the mycoplasmas (Fig. 3). However, further increases in the external fatty acid concentration did not increase incorporation. The maximal amount of octadecenoic

acid/mg. cell protein under these conditions was approx. 150 μ g. Although no differences were noted in the amount of oleic or elaidic acid incorporated into *Mycoplasma laidlawii* A, a marked change in its osmotic fragility was observed. This difference was most pronounced when the concentration of such acids in the growth medium increased above 5 μ g./ml. The differences in cell yields, viable counts and osmotic fragility of *M. laidlawii* A grown with relatively high concentrations of various fatty acids are summarized in Table 5. The *trans* isomer, elaidic and *trans*-vaccenic acids improved growth and increased the resistance of the organisms to osmotic lysis more than did their corresponding *cis* isomers, oleic and *cis*-vaccenic acids or their homologous *cis*-cyclopropane-ring containing fatty acids, dihydrosterculic and lactobacillic acids.

Fig. 2. Infrared spectra of methylated fatty acids from membrane polar lipids of *Mycoplasma laidlawii* A. I, Grown with oleic acid, 2 μ g./ml. II, Grown with elaidic acid, 2 μ g./ml. Arrow indicates *trans* band.

Table 4. Incorporation of geometrical octadecenoic acid isomers into *Mycoplasma laidlawii* A membrane polar lipids

Organisms grown in a lipid pre-extracted medium containing 0.25 % charcoal-treated albumin and labelled fatty acid indicated. Fatty acids added as ethanolic solutions. Methylated fatty acids separated into fractions by thin layer (AgNO_3) chromatography

Fractions	Radioactivity (counts/min.)		
	R_F	[1- ^{14}C]Oleic acid	[1- ^{14}C]Elaidic acid
Saturated acids	0.85	42	33
<i>Trans</i> acids	0.66	120	6218
<i>Cis</i> acids	0.45	4843	270

DISCUSSION

A growth requirement for long-chain fatty acids has been established for certain mycoplasmas: *Mycoplasma* var. *mycoides mycoides* and *Mycoplasma* sp. Y require both an unsaturated and a saturated fatty acid for growth (Rodwell & Abbot, 1961;

Table 5. *Effect of octadecenoic and cyclopropane ring containing fatty acid isomers on growth and osmotic fragility of Mycoplasma laidlawii A*

Organisms grown in lipid pre-extracted tryptose medium containing 0.25 % charcoal-treated bovine serum albumin with fatty acids indicated.

Fatty acid added to growth medium	Concentration ($\mu\text{g./ml.}$)	Cellular yields (mg. protein/100 ml.)	No. of c.f.u.* (c.f.u./ml.)	Osmotic fragility†
Oleic	20	1.72	6.20×10^8	44.5
	10	2.94	2.60×10^9	47.0
Elaidic	20	3.86	1.65×10^{10}	26.4
Dihydrosterculic	10	2.00	7.00×10^8	58.6
<i>cis</i> -Vaccenic	20	1.61	4.35×10^8	53.6
	10	3.10	2.30×10^9	55.0
<i>trans</i> -Vaccenic	20	3.45	4.65×10^9	32.1
Lactobacillic	10	1.90	8.25×10^8	52.6

* C.f.u. = colony-forming units.

† Percentage lysis in 0.05 M-NaCl (0.25 M-NaCl as control).

Fig. 3. Incorporation of octadecenoic acid isomers into *Mycoplasma laidlawii A* and their effect on osmotic fragility. Incorporation: oleic acid (○) and elaidic acid (●). Osmotic fragility: organisms grown with oleic (□) or elaidic acids (■). Fragility determined after 24 hr.

Rodwell, 1967). However, while some non-sterol-requiring strains require an unsaturated fatty acid for growth (Razin & Rottem, 1963; Razin *et al.* 1966), others do not (Henrikson & Panos, 1969; Panos & Henrikson, 1969). In the present studies, the requirement for an unsaturated fatty acid by *M. laidlawii A* was fulfilled by either positional or geometrical octadecenoic acid isomers, but not by shorter-chain mono-

enoic fatty acids. The inability of shorter-chain fatty acids to be elongated beyond their respective hexadecenoic acid isomers by this mycoplasma has been discussed by Panos & Rottem (1969). Growth of *M. laidlawii* A was greatest when elaidic acid replaced oleic acid as the octadecenoic acid requirement. Elaidic acid provided a greater growth response and was less toxic at higher concentrations than its corresponding *cis* isomer under otherwise identical growth conditions. Rodwell (1968) reported that both the saturated and unsaturated fatty acid needs of *Mycoplasma* sp. Y could be fulfilled by elaidic acid. This greater response of *M. laidlawii* A with elaidic acid may be due to a better packing of its membrane polar lipids necessary for greater growth and cellular activity. Surprisingly, cyclopropane-ring-containing fatty acids (lactobacillic and dihydrosterculic acids) were able to substitute for oleic acid in the growth medium. While these acids are known to replace the octadecenoic acid requirement for certain bacteria (Hofmann & Panos, 1954), their presence in the mycoplasmas has been only speculative (O'Leary, 1962; Pollack & Tourtellotte, 1967). Although nothing is known about the physical effects of ring-containing fatty acids on, for example, lipoidal membrane packing sequences, it is known that 'the cyclopropane-ring appears to restrict the number of "economical" modes of packing the long chains into a crystal lattice' (Hofmann, 1963). Thus the greater toxicity and lowest growth response of mycoplasmas grown with lactobacillic or dihydrosterculic acids and the lysis of *M. laidlawii* A, once the stationary phase of growth was reached, may conceivably be related to an indiscriminate incorporation resulting in an alteration of the spatial requirements for membrane stability, with eventual lysis.

The fatty acid composition of mycoplasma lipids has been a subject of previous publications (Razin *et al.* 1966; Rodwell, 1968; Panos & Henrikson, 1969). A comparison of the fatty acid profile of mycoplasma lipids with that of the growth medium has also been reported (Smith, Kostra & Henrikson 1965; Razin *et al.* 1966). Using high-resolving capillary gas chromatography, Henrikson & Panos (1969) showed that when *Mycoplasma* sp. KHS or *M. laidlawii* B were grown in a medium almost devoid of fatty acids, myristic acid comprised almost 50% of their total fatty acids. The presence of significantly greater amounts of myristic acid than palmitic acid in the membrane polar lipids from *M. laidlawii*, together with the reverse observed in uninoculated growth-medium controls, reflected a net synthesis of myristic acid and confirmed the earlier results of others concerning the *de novo* synthesis of saturated fatty acids from acetate (Rottem & Razin, 1967a; Pollack & Tourtellotte, 1967). Contrary to these whole-cell growth experiments, stearic acid was the major saturated fatty acid synthesized by a cell-free system from this organism (Rottem & Panos, 1969), indicating the possibility of an altered regulatory mechanism once cellular integrity had been destroyed. The fatty acid content of the complex lipids from *M. laidlawii* A revealed minute but consistent differences in the saturated fatty acid content of the glycolipid and phospholipid fractions. However, their over-all similarity in fatty acid composition indicated the use of a possible common precursor (2,3-diglycerides?) for the syntheses of gly co- and phospho-lipids (Kates, 1966).

Elaidic acid was incorporated into the membrane polar lipids to the same extent as its corresponding *cis* isomer, indicating that the enzymes catalysing the esterification of the fatty acids do not discriminate between *cis* and *trans* isomers in *Mycoplasma laidlawii* A. An equal incorporation of *cis* and *trans* isomers into the 2 position of phosphatidylcholine was described by Lands (1965) for mammalian cells. The fact

that most of a *trans* isomer added to the growth medium could be recovered unchanged from *Mycoplasma* sp. Y and *Mycoplasma* sp. KHS had been mentioned previously (Rodwell, 1968; Panos & Henrikson, 1969). Our findings that no isomerization of a *trans* octadecenoic acid occurred in *M. laidlawii* A confirms these earlier results. The use of radioactive geometrical octadecenoic acid isomers made the investigation of the lack of isomerization possible by a method with greater sensitivity than that of capillary gas chromatography.

Mycoplasmas are relatively resistant to osmotic lysis as compared to bacterial protoplasts (Razin & Argaman, 1963). Since almost all of the polar lipids reside in the mycoplasmal membrane (Smith, 1967), it is reasonable to assume that changes in membrane lipid composition may result in changes in the elasticity of this cellular component. The stability of myelin has been assumed to be due to its high saturated fatty acid content, allowing for a closer packing of its phospholipids (O'Brien, 1965). Likewise, Chapman, Owens & Walker (1966), using an artificial system, showed that phospholipids containing a *trans* acid formed a more condensed monolayer than those containing the corresponding *cis* derivative. Among the mycoplasmas it has been found that unsaturated long-chain fatty acids increase the resistance of *Mycoplasma laidlawii* B to osmotic lysis (Razin *et al.* 1966), presumably because of increased membrane elasticity. These authors inferred that unsaturated fatty acids may allow for a looser packing of the mycoplasmal membrane lipids resulting in greater elasticity. However, our studies showed that although osmotic fragility decreased with increasing concentrations of oleic acid in the growth medium, elaidic acid lowered fragility still further. The need for an optimal ratio of saturated:unsaturated fatty acids for both growth and osmotic stability of the mycoplasmal membrane has been suggested (Razin *et al.* 1966). The ability of elaidic acid to serve both the unsaturated and saturated fatty acid requirement for growth of a sterol-requiring mycoplasma (Rodwell, 1968) seemingly supports this assumption. Although no change in the saturated or unsaturated fatty acid content of the membrane polar lipids was noted when *M. laidlawii* A was grown with elaidic acid, as compared with oleate grown cells, the possibility still remains that this *trans* acid may be more suitable in maintaining an optimal lipoidal membrane orientation for better growth and decreased osmotic fragility.

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