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Incorporation and Elongation of Fatty Acid Isomers by *Mycoplasma* laidlawii A*

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ABSTRACT: These studies have dealt with the fatty acid metabolism of *Mycoplasma laidlawii* A, a nonsterol-requiring organism having an absolute requirement for an octadecenoic or cyclopropane ring containing fatty acid for growth. This organism exhibited an inherent capacity for long-chain monoenoic acid formation and incorporation when supplied with positional dodecenoic or tetradecenoic acid precursors. It was noted that the efficiency of elongation of *cis*-tetradecenoic isomeric precursors depended upon the position of the double bond. This organism also incorporated and elongated an unnatural isomer, *trans*-5-tetradecenoic acid, to *trans*-7-hexadecenoic acid although the efficiency of elongation was considerably less than that observed with the *cis* homolog.

The inability to continue the elongation of hexadecenoic acids to terminating octadecenoic acids emphasized the absolute need for at least an 18-carbon-containing fatty acid for growth and presents the first report of the inability of unsaturated short-chain fatty acids to replace this requirement. Although lactobacillic acid, a cyclopropane ring containing fatty acid, replaced the need of an octadecenoic acid for growth, a marked preference for *cis*-vaccenic, its precursor in bacterial cells, was noted when both were added to the growth medium. Studies over the growth cycle of this mycoplasma illustrated that myristic acid was the predominating saturated fatty acid in the membrane polar lipids of young cells while palmitic acid predominated as the cells grew older.

Recent studies from this laboratory have shown that two nonsterol-requiring mycoplasmas, *Mycoplasma* sp. KHS and *M. laidlawii* B, are capable of growth in the absence of added octadecenoic acids. When analyzed by highly sensitive capillary gas chromatography, the fatty acid content of each organism was almost completely devoid of monoenoic acids (Henrikson and Panos, 1969). However, *Mycoplasma* sp. KHS had the ability to form octadecenoic acids when supplied with appropriate monoenoic acid precursors (Panos and Henrikson, 1969). Although some information is available on the ability of positional and stereoisomeric monoenoic acids to serve in the nutrition of certain bacteria (Hofmann and Panos, 1954), practically nothing is known concerning the fate of the unnatural *trans* isomer once added to the medium. In the mycoplasma, Rodwell (1968) had shown that *trans*-9-octadecenoic (elaidic) acid was capable of replacing the growth requirement for both saturated and unsaturated fatty acids of a sterol-requiring mycoplasma, *Mycoplasma* sp. Y. This *trans* isomer was incorporated unchanged into its membrane lipids. Likewise, *Mycoplasma* sp. KHS incorporated significant quantities of palmitelaidic acid into its complex lipids without subsequent isomerization or elongation (Panos and Henrikson, 1969). The incorporation of elaidic acid into membrane lipids of *M. laidlawii* A and B has also been demonstrated (McElhaney and Tourtellotte, 1969; Rottem and Panos, 1969a). Most recent isotopic evidence for the lack of isomerization of a *trans* acid in *M. laidlawii* A is presented elsewhere (Rottem and Panos, 1969a).

Some indirect information is available suggesting the presence of minute amounts of cyclopropane ring containing fatty acids in the mycoplasma (O'Leary, 1962; Pollack and Tourtellotte, 1967). These fatty acids are formed by the insertion of a methyl group into an unsaturated chain and are capable of replacing biotin in the nutrition of certain bacteria (Hofmann, 1963). Most recently, these ring-containing acids have also been shown to serve in lieu of an octadecenoic acid for growth of a mycoplasma when grown in a defatted medium (Rottem and Panos, 1969a).

The following studies have provided information currently lacking in the area of fatty acid metabolism by the nonsterol-requiring mycoplasma. They have utilized a mycoplasma known to require an octadecenoic acid for growth to detail the metabolism of preformed monoenoic positional and stereoisomers and of a cyclopropane ring containing fatty acid in *Mycoplasma laidlawii* A. Also, the effect of these acids upon saturated fatty acid biosynthesis and the relationship of membrane fatty acid content and composition to changes in the growth phase has been established. Part of these results have been presented in preliminary form (Rottem and Panos, 1969b).

Experimental Section

Organism and Growth Conditions. M. laidlawii (oral strain) was obtained from S. Razin (The Hebrew University, Hadassah Medical School, Jerusalem, Israel) and is related to *M. laidlawii* A (Rottem and Razin, 1967). Cells were grown in 4–6 l. of medium consisting of 2% tryptose (Difco Laboratories Inc., Detroit, Mich.) which had been lipid pre-

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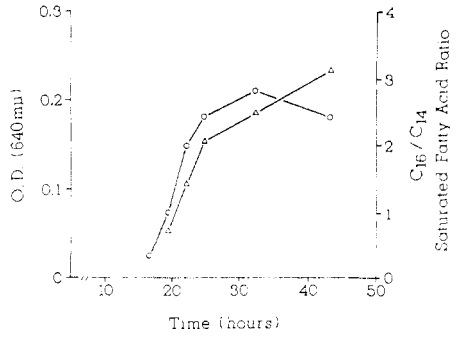

FIGURE 1: Changes in the relative saturated fatty acid composition over the growth cycle of *M. laidlawii* A. Cells grown in lipid-preextracted medium containing an octadecenoic acid (2 μg/ml of medium); optical density (○), saturated fatty acid ratio (Δ).

extracted as before (Henrikson and Panos, 1969), 0.5% glucose, 0.5% sodium acetate, 0.25% charcoal-treated bovine serum albumin (kindly provided by Armour Pharmaceutical Co., Kankakee, Ill.), 2–4 μg/ml of oleic or *cis*-vaccenic acid, and 500 units/ml of penicillin G. Final pH was 8.0. For elongation experiments, octadecenoic acid precursors were added (2 μg/ml) in addition to an octadecenoic acid (2 μg/ml) necessary for growth. All fatty acid additions were as alcoholic solutions with the final concentration of absolute ethanol not exceeding 0.1–0.2% of the growth medium. The residual fatty acid content of the tryptose–albumin ingredients of the growth medium was 0.0059% (w/w) (Rottem and Panos, 1969a). All cells were harvested after 24–36-hr incubation (37°) by centrifugation (17,000*g* for 20 min) and washed once with 0.25 M NaCl.

Mycoplasmal membranes were obtained by osmotic lysis. Washed sedimented cells were resuspended in 100 ml of deionized water and incubated at 37° for 15 min. Membranes were collected by centrifugation (34,000*g* for 45 min), washed three times with deionized water, and lyophilized.

Lipid Extractions and Chromatographic Methods. Total lipids were extracted from lyophilized membranes with chloroform–methanol (2:1, v/v) according to Folch *et al.* (1957). Lipid extracts were dried under a stream of nitrogen and redissolved in 0.5 ml of chloroform and pojar lipids were separated from neutral lipids by silicic acid chromatography. Lipid extracts in chloroform were applied to columns (6 × 100 mm) of activated (Unisil) silicic acid (100–200 mesh, Clarkson Chemical Co., Inc., Williamsport, Pa.) prewashed with chloroform. Neutral and polar lipids were eluted in succession with 20 ml of chloroform and 20 ml of methanol, respectively (Ansell and Hawthorne, 1966). Polar lipid fractions, which account for over 90% of the total membrane lipids, were dried under nitrogen and the fatty acids obtained after alkaline hydrolysis (Hofmann *et al.*, 1957). Esterified fatty acid mixtures were resolved by capillary column gas chromatography (Panos *et al.*, 1966) using a polar column (150 ft × 0.01 in.) coated with Carbowax K-20M + V-93 (99:1). Geometrical isomers were resolved on a nonpolar capillary column coated with Apiezon-L + Co-880 (90:10). Both coating materials are the products of Perkin-Elmer Corp. (Norwalk, Conn.).

Chemical Methods. Methylation, hydrogenation, and infrared analyses were performed as described elsewhere (Panos *et al.*, 1966; Weinbaum and Panos, 1966). *trans*-5-Tetradecenoic acid was prepared by the isomerization of *cis*-5-tetradecenoic acid according to Sax (1953). The resulting *trans* isomer was separated from contaminating quantities of the *cis* isomer by thin-layer chromatography, after methylation, on silica gel G plates containing 15% (w/v) $AgNO_3$ and 0.01% (w/v) Rhodamine 6G. Plates were developed with petroleum ether (bp 38–46°)–diethyl ether (9:1, v/v) and the *cis* and *trans* areas visualized under ultraviolet light. The *trans* area was scraped off plates, extracted three times with 15 ml of petroleum ether, and hydrolyzed to the free acid. Purity of the methyl ester was greater than 95% as judged by capillary column gas chromatography. Lactobacillic (*cis*-11,12-methyleneoctadecanoic) acid was obtained from the methylated fatty acids of *E. coli* E-26 by fractional distillation in a spinning-band type, semimicro Piros Glover fractionating column (Hofmann *et al.*, 1952). The growth, fatty acid extraction, methylation, etc., of this gram-negative bacterium has been detailed (Weinbaum and Panos, 1966). Methyl lactobacillate obtained by vacuum distillation was found to be 85% pure as judged by capillary gas chromatography. The free acid was recrystallized (twice) at −20° from petroleum ether (bp 36–42°) before use.

Fatty Acids. Myristoleic, *cis*-5-tetradecenoic, palmitoleic, *cis*-vaccenic, and oleic acids were purchased from the Hormel Institute (Austin, Minn.). Lauroleic acid was the product of Applied Science Laboratories (State College, Pa.). All fatty acids were greater than 99% pure. 1-[^{14}C]Oleic acid (57.2 mCi/mmole) was the product of Applied Science Laboratories (State College, Pa.).

Results

Aside from a high *cis*-vaccenic acid content because of incorporation from the medium, typical chromatograms of the fatty acid methyl esters of membrane polar lipids from *M. laidlawii* A illustrated myristic and palmitic acids as the major saturated components. When fatty acid analyses were performed over the entire growth cycle of this organism (oleic acid grown cells), a significant change was observed in the relative amounts of myristic and palmitic acids of the membrane polar lipids (Figure 1). While early logarithmic phase cells contained a greater amount of myristic acid, cells at the late stationary phase of growth possessed more palmitic acid (three times) than myristic acid in their membrane polar lipids. All membrane preparations, regardless of the growth phase from which they originated, contained approximately the same total fatty acid content (17.6–18.1%) and showed only a slight change in their saturated:unsaturated fatty acid ratios; from 0.51 in the early logarithmic phase to 0.56 in the late stationary phase of growth. Likewise, no marked change was noted in the amount of preformed octadecenoic acids (oleic or *cis*-vaccenic) incorporated into these membrane polar lipids from the medium during growth as determined by quantitative capillary column gas chromatography and by the constant ratio of radioactivity per mg of fatty acid mixture from membrane polar lipids of cells grown with 1-[^{14}C]oleic acid (1 μCi/l.).

The requirement for an octadecenoic acid for growth of *M. laidlawii* A could not be fulfilled by *cis*-5-tetradecenoic or palmitoleic (*cis*-9-hexadecenoic) acids known to be precursors of oleic or *cis*-vaccenic acids, respectively, in bacteria

TABLE I: Fatty Acid Composition of Membrane Polar Lipids of *M. laidlawii* A in the Presence of Various Positional and Geometrical Tetradecenoic Acid Isomers.[a]

Fatty Acid	Composition of Total Fatty Acids (%)			
	cis-Δ^{11}-C_{18} (Control)	*cis*-Δ^{11}-C_{18} and *cis*-Δ^{5}-C_{14}	*cis*-Δ^{11}-C_{18} and *trans*-Δ^{5}-C_{14}	*cis*-Δ^{11}-C_{18} and *cis*-Δ^{9}-C_{14}
Capric	0.12	0.15	0.08	0.08
Lauric	7.21	6.21	4.66	3.02
C_{13} satd (C13T?)[b]	0.52	0.41	0.42	0.15
Tridecanoic	1.40	0.75	0.61	0.26
C_{14} satd (C14T?)[b]	0.25	0.14	0.14	0.07
Myristic	33.90	17.99	12.96	7.49
cis-Δ^{5}-C_{14}		18.82		
trans-Δ^{5}-C_{14}			36.34	
cis-Δ^{9}-C_{14} (myristoleic)				8.90
Pentadecanoic	1.62	0.68	0.70	0.40
Palmitic	18.67	9.12	9.25	5.30
cis-Δ^{7}-C_{16}		11.16		
trans-Δ^{7}-C_{16}			4.80	
cis-Δ^{11}-C_{16}				35.77
Margaric	0.21	0.18	0.16	0.08
Stearic	1.27	0.94	0.85	0.90
cis-Δ^{9}-C_{18} (oleic)	2.26	3.61	2.15	2.10
cis-Δ^{11}-C_{18} (*cis*-vaccenic)	31.55	28.94	26.02	32.57
cis-Δ^{13}-C_{18}				1.31
Linoleic	1.06	0.92	0.86	1.10
Unsatd:satd ratio[b]	0.53	1.59	2.32	4.81
% Total fatty acids	18.2	17.6	18.8	21.6

[a] Early logarithmically grown cells from complex-defatted growth medium containing 2 μg/ml of each acid. Analyses by capillary column (polar) gas chromatography. [b] T = tentative identification as branched methyl acids. Unsatd = unsaturated, satd = saturated.

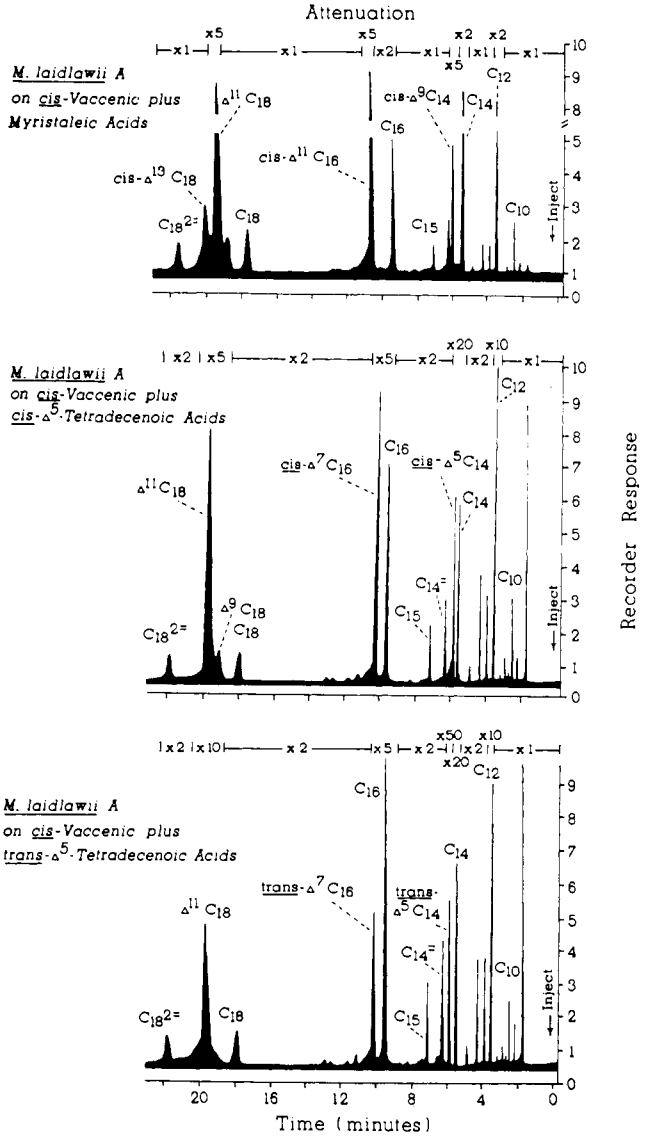

FIGURE 2: Capillary chromatograms of methylated fatty acids from membrane polar lipids of *M. laidlawii* A grown with *cis*-vaccenic acid plus tetradecenoic acid positional or geometrical isomers. Column: Carbowax K-20M, 150 ft at 185°.

(O'Leary, 1967). However, when various tetradecenoic acid isomers were added to the growth medium in the presence of an octadecenoic acid, this mycoplasma incorporated these octadecenoic acid precursors into its polar lipids and even elongated them. Figure 2 illustrates representative fatty acid chromatograms of *M. laidlawii* A polar lipids from cells grown in the presence of various tetradecenoic acid isomers. Determination of the position of an unsaturation or cyclopropane ring within esterified long-chain fatty acids by capillary gas chromatography has been detailed (Panos, 1965; Panos and Henrikson, 1968). The limitations of such a method for structural characterization and the validity of accurate relative retention time data being possible from only original chromatograms prior to their reduction in size for publication have been detailed (Panos and Henrikson, 1969). The quantitative data from such chromatograms are tabulated in Table I. As is apparent, this mycoplasma is capable of elongating these tetradecenoic acid isomers with the formation of appreciable quantities of corresponding hexadecenoic acids. However, only minute quantities of these latter acids were, in turn, further elongated to their homologous octadecenoic acid isomers. Because of the residual trace lipids of the growth medium (0.0059%) and its minute but perceptible oleic acid content (Rottem and Panos, 1969a), any further elongation of *cis*-7-hexadecenoic acid to *cis*-9-octadecenoic (oleic) acid was difficult to establish. However, that some elongation to an octadecenoic acid did occur was evident by the minute amount of *cis*-13-octadecenoic acid present in the membrane polar lipids when this organism was grown in a medium supplemented with *cis*-9-tetradecenoic (myristoleic) acid. This unusual octadecenoic acid isomer was not present in the residual fatty acids of the uninoculated medium control.

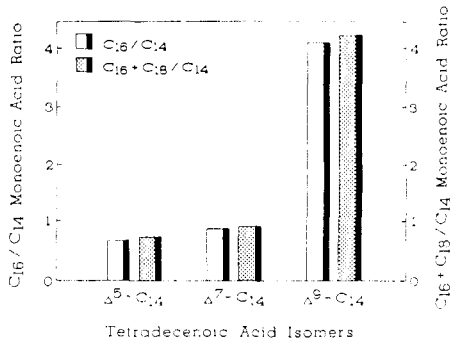

FIGURE 3: Elongation efficiency of various tetradecenoic acid isomers by *M. laidlawii* A. Cells grown in lipid-preextracted medium containing *cis*-vaccenic and *cis*-5-, or 9-tetradecenoic or *cis*-5-dodecenoic acids. *cis*-7-Tetradecenoic acid after elongation of *cis*-5-dodecenoic acid. Isomeric analyses by capillary column gas chromatography. $C_{16} + C_{18}$:C_{14} monoenoic acid ratio includes only newly synthesized octadecenoic acid isomers.

Although extensive incorporation and subsequent elongation (30–44% of total fatty acid content) of preformed tetradecenoic acid isomers resulted when added to the growth medium, no marked changes were noticed in either the amount of *cis*-vaccenic acid taken up from the growth medium or in the total percentage of membrane polar lipids. However, an increase in the unsaturated:saturated fatty acid ratio of the polar lipids from 0.53 (control cells grown with *cis*-vaccenic acid only) to 4.81 when cells were grown with *cis*-vaccenic and *cis*-9-tetradecenoic (myristoleic) acid did occur.

The relative amounts of the various tetradecenoic acids incorporated and elongated to their corresponding hexadecenoic acid isomers by this mycoplasma was dependent upon the position of the double bond. Figure 3 illustrates that with these tetradecenoic acid isomers, efficiency of elongation increased as the distance between the double bond and carboxyl group was increased. Of the total content of *cis*-5-tetradecenoic acid and its elongated product, *cis*-7-hexadecenoic acid, from cells grown with this tetradecenoic acid isomer, 63% was incorporated as the isomer and only 37% further elongated. When cells were grown with *cis*-9-tetradecenoic acid, only 20% of the combined isomer and product remained as the tetradecenoic acid isomer. The fact that the resulting C_{16}:C_{14} monoenoic acid ratio in membrane polar lipids is not markedly different from that of the $C_{16} + C_{18}$:C_{14} monoenoic acid ratio (Figure 3), when preformed C_{14} monoenoic acid isomers were supplied indicates again the inability of this mycoplasma to synthesize an octadecenoic acid. Additional evidence for the inability of this mycoplasma to elongate a hexadecenoic acid is the fact that its polar lipids, when grown in the presence of *cis*-9-hexadecenoic (palmitoleic) acid, contained 18% of this isomer yet no significant elongation was observed. As with the tetradecenoic acid isomers, *cis*-5-dodecenoic (lauroleic) acid was also incorporated from the growth medium and elongated by this mycoplasma. By comparison, however, the total amount incorporated and elongated was very low. Although the *cis*-5-dodecenoic acid content of membrane polar lipids was less than 0.5%, its *cis*-7-tetradecenoic acid (6.8%) and *cis*-9-hexadecenoic acid (5.6%) elongation products were detectable. No indication of an octadecenoic acid product was evident.

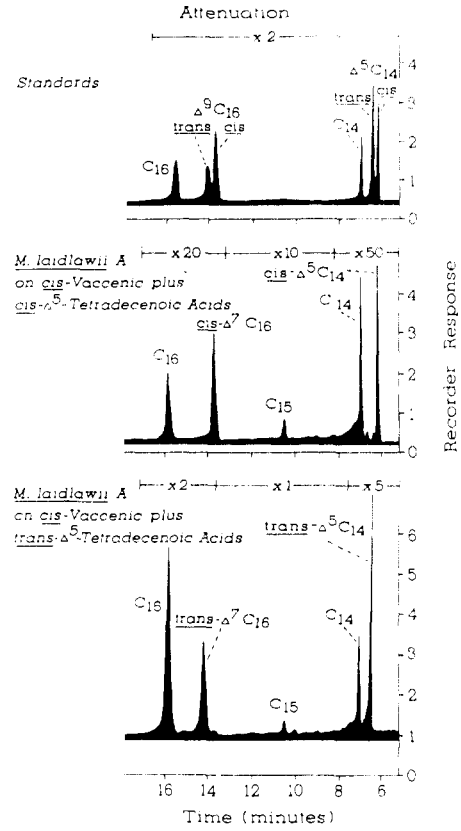

FIGURE 4: Partial capillary column gas chromatograms of methylated fatty acids from membrane polar lipids of *M. laidlawii* A grown with *cis*-vaccenic acid and *cis*-5-tetradecenoic or *trans*-5-tetradecenoic acids. Column: Apiezon L, 150 ft at 190°.

trans-5-Tetradecenoic acid was also incorporated into membrane polar lipids. The incorporation of this *trans* acid was confirmed by infrared analyses revealing the characteristic *trans* configurational band at 10.3 μ. As was expected, this band was absent in infrared patterns of fatty acid polar lipids from cells grown with the corresponding *cis* isomer. Of the total *trans* acid incorporated, 88.7% was found as *trans*-5-tetradecenoic acid while only 11.7% was elongated to 7-hexadecenoic acid. This elongation product accounted for only 5% of the total membrane fatty acids. The question of whether these cells were capable of elongating a *trans* acid or if isomerization had preceded elongation was solved by the use of a nonpolar capillary column (Apiezon-L) capable of resolving geometrical as well as positional isomers. Figure 4 illustrates the resolution of geometrical isomers by this column. Analyses of fatty acids from membrane polar lipids of cells grown with *trans*-5-tetradecenoic acid indicated that the 7-hexadecenoic acid formed was of the *trans* configuration, illustrating an ability by *M. laidlawii* A to elongate a *trans*-monoenoic acid.

The ability of a long-chain cyclopropane ring containing fatty acid to substitute for *cis*-vaccenic or oleic acids as the octadecenoic acid growth requirement for *Mycoplasma laidlawii* A has been detailed (Rottem and Panos, 1969a). Table II illustrates that lactobacillic (*cis*-11,12-methyleneoctadecanoic) acid could replace the octadecenoic acid requirement of this mycoplasma and was incorporated into

membrane polar lipids. Although *M. laidlawii* A incorporated both lacotobacillic and *cis*-vaccenic acids, the latter was preferred when both were added as mixtures or when added individually to the growth medium. It should, perhaps, be pointed out that while cyclopropane ring containing fatty acids possess certain chemical properties characteristic of monoenoic acids, they are saturated acids (Hofmann, 1963). For these ratio determinations (Table II), lactobacillic acid has been included in the total saturated acid content. There was no significant differences noted in the incorporation of *cis*-vaccenic or lactobacillic acids in these studies (Table II) when early logarithmic or stationary phase cells were used.

TABLE II: Fatty Acid Composition of Membrane Polar Lipids of *M. laidlawii* A Grown with *cis*-Vaccenic and Lactobacillic Acids.[a]

Fatty Acid	Composition of Total Fatty Acids (%)		
	cis-Vaccenic Acid	Lactobacillic Acid	*cis*-Vaccenic and Lactobacillic Acids
Capric	0.08	0.16	0.04
Lauric	0.59	0.77	0.36
C_{13} satd (C13T?)	0.08	0.15	0.10
Tridecanoic	0.04	0.09	0.05
C_{14} satd (C14T?)	0.09	0.08	0.01
Myristic	6.30	11.65	6.20
Pentadecanoic	0.10	0.20	0.48
Palmitic	14.80	22.30	12.20
Margaric	1.16	1.98	1.06
Stearic	3.50	6.95	3.29
Oleic	7.06	12.84	6.40
cis-Vaccenic	63.05	3.21	51.51
Linoleic	2.40	3.65	1.85
Lactobacillic		35.99	15.36
Unsatd:satd ratio	2.85	0.39	1.50
% Total fatty acids	18.6	17.4	18.2

[a] Medium contained 4 μg/ml of each acid or total mixture, otherwise footnotes are the same as for Table I. Cells harvested at the stationary phase of growth.

Discussion

These studies have dealt with the fatty acid metabolism of *M. laidlawii* A, a nonsterol-requiring organism having an absolute requirement for an octadecenoic or cyclopropane ring containing fatty acid for growth (Rottem and Panos, 1969a). The emphasis of these studies has been on the membrane polar lipids. Aside from illustrating the degree of fatty acid alteration possible within this cellular component, these investigations have illustrated the inherent capacity of this organism for some degree of long-chain monoenoic acid formation when supplied with appropriate precursors. These findings have augmented earlier but recent results obtained with another mycoplasma devoid of any requirement for an octadecenoic acid (Panos and Henrikson, 1969). However, the data differ in that while *Mycoplasma* sp. KHS was able to elongate isomeric precursors to the octadecenoic acid level, *M. laidlawii* A was not. Although *M. laidlawii* A was capable of some octadecenoic acid formation, as exemplified by the elongation of myristoleic acid, no significant chain elongation occurred above a chain length of 16 carbon atoms. Thus, the ability to elongate short-chain monoenoic acids but the inability to form sufficient octadecenoic acids for growth from them has illustrated a continued elongation capacity by this mycoplasma even though its performed octadecenoic acid requirement was being fulfilled. The inability to continue the elongation of hexadecenoic acids to terminating octadecenoic acids emphasizes the absolute requirement for at least an 18-carbon-containing fatty acid for growth (Rottem and Panos, 1969a,b, 1970) and documents the first report of the inability of unsaturated short-chain fatty acids to replace this need. The explanation for this requirement lies in the lack of enzymes (specific dehydrase?) necessary for the continued elongation of hexadecenoic acids in addition to an inability for their *de novo* synthesis. The ability of a cell-free system from this mycoplasma for *de novo* synthesis of saturated and unsaturated long chain fatty acids has been detailed elsewhere (Rottem and Panos, 1970).

The preference of *M. laidlawii* A for the elongation of a particular tetradecenoic acid positional isomer depended upon the position of the double bond and points to a selectivity previously alluded to with the mycoplasma. Earlier, a preference for the elongation of palmitoleic over that of an isomer, *cis*-7-hexadecenoic acid, to *cis*-vaccenic and oleic acids, respectively, had been demonstrated by *Mycoplasma* sp. KHS (Panos and Henrikson, 1969). The marked preference for the elongation of myristoleic acid over that of other positional tetradecenoic acid isomers by *M. laidlawii* A, permitted an extended study of this ability and indicates that a correlation exists between position of unsaturation and the degree of elongation of monoenoic fatty acids by the nonsterol-requiring mycoplasma. Although the resulting unsaturated:saturated fatty acid ratio of the membrane polar lipids was markedly altered, no significant effect was ever noticed on either the growth rate, morphology or per cent membrane fatty acid content of *M. laidlawii* A after addition of such fatty acid precursors. As noted earlier (Panos and Henrikson, 1969), a marked reduction in myristic and palmitic acids also occurred with this mycoplasma when dodecenoic, tetradecenoic, or hexadecenoic acids were added to the growth medium. However, it should be pointed out that while increasing the growth response of *M. laidlawii* A (Rottem and Panos, 1969a), a decrease in its saturated fatty acids could also be demonstrated upon increasing (from 2 to 4 μg per ml) the monoenoic or cyclopropane ring containing fatty acid content of the growth medium.

As already stated, cyclopropane ring containing fatty acids are widespread in bacteria (O'Leary, 1967), are formed by the methylation of a monoenoic acid across the double bond (Hofmann, 1963), and their presence in the mycoplasma is only speculative. Evidence for the ability of a cyclopropane-ring containing (lactobacillic) acid to substitute for the octadecenoic acid growth requirement of *M. laidlawii* A has been presented (Rottem and Panos, 1969a). Herein, a marked preference for *cis*-vaccenic acid was exhibited by *M. laidlawii*

A when lactobacillic and *cis*-vaccenic acids were added simultaneously to the growth medium. This greater preference for an octadecenoic acid may explain the lower growth response of this mycoplasma to cyclopropane ring containing fatty acids noted elsewhere (Rottem and Panos, 1969a).

The Eubacteriales contain major proportions of both monoenoic and saturated fatty acids and some information is available concerning the effect of age on their fatty acid content and composition (Kates, 1964; O'Leary, 1967). However, nothing is known concerning the relationship of fatty acid composition to the growth phase in the Mycoplasmatales. This study has demonstrated the importance of the growth phase upon the saturated fatty acid content of the membrane polar lipids of *M. laidlawii* A. Although myristic acid predominated in young cells, palmitic acid predominated once cells began to reach midlogarithmic growth. These findings confirmed the earlier results obtained from membranes of young cells of two sterol-nonrequiring mycoplasmas that had been grown in a medium almost devoid of fatty acids (Panos and Henrikson, 1969). As with the monoenoic acid elongation results already discussed, major saturated fatty acid synthesis for *M. laidlawii* A also terminated at 16 carbon atoms (palmitic acid). Therefore, the growth phase of the Mycoplasmatales, like bacteria, must be determined before lipid studies can be meaningfully interpreted and valid comparisons made.

Although comparatively little information is at hand, certain mycoplasma are known to utilize the *trans* isomers of various fatty acids. While the *trans* isomer is considered the unnatural isomer in bacterial metabolism, both a sterol-requiring and -nonrequiring mycoplasma have been found to incorporate *trans* isomers. Rodwell (1968) had shown that elaidic acid could replace both the saturated and unsaturated fatty acid growth requirements of *Mycoplasma* sp. Y. Panos and Henrikson (1969) illustrated the incorporation of major proportions of palmitelaidic acid into the total phospholipid and monoglycosyldiglyceride content of *Mycoplasma* sp. KHS grown in a "lipid-free" medium. However, subsequent elongation or isomerization of these *trans* isomers was not observed in these studies. *Mycoplasma laidlawii* A is the first organism, thus far, found capable of incorporating a *trans* isomer (*trans*-5-tetradecenoic acid) and elongating it.

Acknowledgment

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