Fatty Acid Interconversions in *Mycoplasma* sp. KHS*

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ABSTRACT: It has been shown earlier that the unsaturated fatty acid biosynthetic capability of *Mycoplasma* sp. KHS is practically nil. This study has utilized this organism as a representative member of the sterol-nonrequiring mycoplasmas and has demonstrated its inherent ability to form long-chain monoenoic fatty acids when supplied with appropriate precursors. The use of palmitoleic ($C_{16}\Delta^{9,10}$) and *cis*-5,6-tetradecenoic acids has revealed that the position of unsaturation within these precursors influences to a considerable degree the subsequent abilities of this mycoplasma to form a particular positional isomeric octadecenoic acid from them. Under the conditions studied, the formation as well as the absorption of *cis*-vaccenic acid ($C_{18}\Delta^{11,12}$) by whole cells was favored over that of its isomer, oleic acid ($C_{18}\Delta^{9,10}$). Analogies are drawn and the results discussed in terms of recent bacterial lipid findings. For example, the data obtained tend to implicate a possible common branching point in fatty acid biosynthesis by seemingly reflecting a sensitive point in saturated fatty acid biosynthesis amenable to varying degrees of disturbance depending upon the monoenoic acid supplied to the growth medium. It was found that although unsaturated fatty acid elongation does occur upon addition of suitable precursors, a concomitant but increasing disturbance on saturated acid biosynthesis also occurs as the chain length of the unsaturated acid precursor added is decreased. Major saturated fatty acid biosynthesis terminated at a chain length of 16 carbon atoms. No increase in either the saturated or unsaturated fatty acid content of this organism was noted when the precursor added was β-hydroxydecanoic acid.

The results obtained with a similar but opposite geometrici somer, palmitelaidic acid, have provided insights into the specificity of fatty acid utilization by *Mycoplasma* sp. KHS. These findings have pointed to at least two "sets" of enzymes being involved in the utilization of long-chain monoenoic fatty acid by whole cells of this nonsterol-requiring mycoplasma: (a) those unconcerned with geometrical or positional isomerization but necessary for long-chain fatty acid incorporation into complex structural lipids and (b) those involved in the elongation of only those acids with the *cis* configuration. Similarities and differences in fatty acid interconversions between bacteria and this mycoplasma are discussed.

Smith (1964), in his review of the comparative physiology of pleuropneumoniae-like (mycoplasma) and L-form organisms, pointed out our then meager knowledge of lipid biosynthesis in these microorganisms. Since then some information has accumulated concerning the synthesis of long-chain fatty acids in the *Mycoplasmataceae*. For example, Pollack and Tourtellotte (1967) reported on the synthesis of long-chain-saturated fatty acids from sodium acetate-1-^{14}C by *Mycoplasma laidlawii* strains A and B and *Mycoplasma* sp. A60549. Rottem and Razin (1957) confirmed this finding in the sterol-nonrequiring *M. laidlawii* by showing inhibition of saturated fatty acid biosynthesis upon addition of palmitic and stearic acids, presumably by end-product inhibition. Both groups concluded that these organisms were unable to synthesize unsaturated fatty acids from acetate when grown in a complex growth medium. Similarly, Rodwell (1968) demonstrated growth of a sterol-requiring mycoplasma, *M.* strain Y, in a partially defined medium when elaidate was the only fatty acid added. This organism could neither (a) synthesize saturated or unsaturated fatty acids, (b) desaturate saturated acids, or (c) alter the chain length of either.

As most recently documented from these laboratories, *Mycoplasma* sp. KHS is almost devoid of long-chain monoenoic acids when grown in a complex but defatted growth medium (Henrikson and Panos, 1969). However, an indication of a meager unsaturated fatty acid biosynthetic capacity prevailing in this sterol-nonrequiring mycoplasma was apparent. Since lipids form an integral part of the membrane of the mycoplasmas, an understanding of the extent of inhibition of unsaturated fatty acid biosynthesis in these organisms becomes necessary to augment our knowledge of differences between mycoplasmas (Smith, 1966) and of bacteria and their L forms (Panos, 1966) if meaningful biochemical comparisons are to be made. This investigation, therefore, utilized *Mycoplasma* sp. KHS as a representative member of the sterol-nonrequiring mycoplasmas and was concerned with answering the following questions: (1) Given appropriate precursors, can this organism form C_{16} and C_{18} monoenoic acids? (2) If so, to what extent does the position of the double bond within the precursor influence its metabolism; *i.e.*, is there a preference or specificity for isomeric precursors favoring the oleic

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or *cis*-vaccenic acid "series." Finally (3), does the geometric configuration of the precursor supplied influence the degree and extent of chain elongation. Part of these results have been presented in preliminary form (Panos and Henrikson, 1968a).

Experimental Section

Microorganism, Growth Medium, and Cultivation. Mycoplasma sp. KHS was kindly supplied by Dr. P. F. Smith (University of South Dakota, Vermillion, S. D.). The solvent-extracted (defatted) tryptose growth medium was prepared as recently detailed (Henrikson and Panos, 1969). Similarly, the methods for determining the late logarithmic phase of growth for this organism as well as the harvesting, washing, and storage procedures employed were identical with those used earlier (Henrikson and Panos, 1969). The growth inhibitory concentration of all fatty acids was determined prior to their use in large-scale cultivation (8–50 l.) experiments. All fatty acids were added to the growth medium as ethanol (70%) solutions and the concentrations used were such as to avoid any degree of growth inhibition. The final concentration of each fatty acid used (palmitoleic, palmitelaidic, and 2-hydroxydodecanoic acids) was 1 μg/ml of medium with the following exceptions: *cis*-5,6-tetradecenoic acid (2 μg/ml), mixture of oleic and *cis*-vaccenic acids (0.70 μg/ml), and β-hydroxydecanoic acid (0.25 μg/ml). For the oleic–*cis*-vaccenic acid mixture, the amount of each isomer added included that already known to be present as residual trace components of the extracted medium (Henrikson and Panos, 1969). The final concentration of each of these octadecenoic acid isomers present in the medium, therefore, was made equal (0.35 μg/ml).

Fatty Acid and Complex Lipid Extractions, Standards, and Chromatographic Methods. Fatty acids were extracted from lyophilized whole cells, resolved by capillary gas chromatography, and identified as has been described (Panos *et al.*, 1966; Panos, 1965). Monoglycosyldiglyceride and phospholipids were extracted from lyophilized cells and isolated by silicic acid column chromatography (Cohen and Panos, 1966). Positional isomeric *cis*-octadecenoic acids ($\Delta^{10,11}$ and $\Delta^{12,13}$) were gifts from Dr. F. D. Gunstone (The University, St. Andrews, Scotland). Most standards and precursors were obtained from Applied Sciences Laboratories, University Park, Pa., or the Hormel Institute, Austin, Minn. Optically inactive 2-hydroxydodecanoic acid was obtained from the Aldrich Chemical Co., Milwaukee, Wis., and D-(−)-β-hydroxydecanoic acid (Birge *et al.*, 1967) was isolated from *Pseudomonas aeruginosa* rhamnolipid generously supplied by Dr. J. A. Hayashi (Presbyterian-St. Lukes Hospital, Chicago, Ill.). Following hydrolysis, extraction (Jarvis and Johnson, 1949; Hofmann *et al*, 1957), and methylation of β-hydroxydecanoic acid, purification of both hydroxylated acids was achieved by preparative thin-layer chromatography (silica gel G, 250 mμ) employing petroleum ether (bp 38–52°)–ethyl ether (9:1, v/v) as the solvent system. Visualization was by water spray and ultimate purity (>93%) determined by capillary gas chromatography. Separation and purification of *Mycoplasma*-saturated and *cis*- and *trans*-monoenoic acid fractions in experiments utilizing palmitelaidic acid as the precursor were accomplished by preparative silver nitrate (15%) impregnated thin-layer chromatography (silica gel G, 250 mμ). The solvent system and visualization techniques were the same as above. Methods employed for the purification and deacylation of isolated glyco- and phospholipids have been described (Cohen and Panos, 1966). In addition, the purity of monoglycosyldiglyceride was confirmed by thin-layer chromatography essentially as described by Smith and Koostra (1967). Monoglycosyldiglyceride from *Mycoplasma* sp. KHS migrated as did standard monoglucosyldiglyceride supplied by Dr. P. F. Smith after isolation from *M. laidlawii*, strain B.

Chemical Methods Methylation, hydrogenation, and infrared analyses were performed as had been detailed elsewhere (Panos *et al.*, 1966; Weinbaum and Panos, 1966).

Results

A typical chromatogram of the fatty acid methyl esters of membranes isolated from *Mycoplasma* sp. KHS grown in a complex but defatted growth medium has been presented (Hendrikson and Panos, 1969). Also, all membrane lipid results were found to mimic those of whole cells. Without exception, the chromatographic patterns obtained were conspicuous by the presence of only meager quantities of C_{14} and C_{16} monoenoic acids and of a minimal oleic ($C_{18}\Delta^{9,10}$) and *cis*-vaccenic ($C_{18}\Delta^{11,12}$) acid content. The source of these monoenoic acids was attributed, in the main, to the trace lipids in the growth medium remaining after lipid preextraction. Surprisingly, and in addition to finding a predominately saturated fatty acid content in this organism (saturated/unsaturated fatty acid ratio = 9.05), myristic acid and not palmitic acid emerged as the major acid in this organism and its anatomical components (cytoplasm and membrane) (Henrikson and Panos, 1969).

Figure 1 compares the fatty acid profiles of *Mycoplasma* sp. KHS whole cells cultivated in the presence of added *cis*-5,6-tetradecenoic or palmitoleic ($C_{16}\Delta^{9,10}$) acids, known precursors of octadecenoic acid positional isomers in bacteria. As is apparent, both of these acids were absorbed and elongated. In the case of the former precursor, an appreciable quantity of the $C_{16}\Delta^{7,8}$ isomer was formed from it. However, only a small quantity of this isomer was, in turn, elongated to form oleic acid. In contrast to these findings, a significant amount of *cis*-vaccenic acid resulted when the precursor was palmitoleic acid. It must be noted that in the preparation of capillary gas chromatograms, such as Figure 1, for publication measurable retention time differences between adjacent positional isomers, particularly those with a chain length of 16 carbon atoms or less, may become obscure during the tracing and photographic reduction process. For this reason accurate measurements can only be made from original chromatograms.

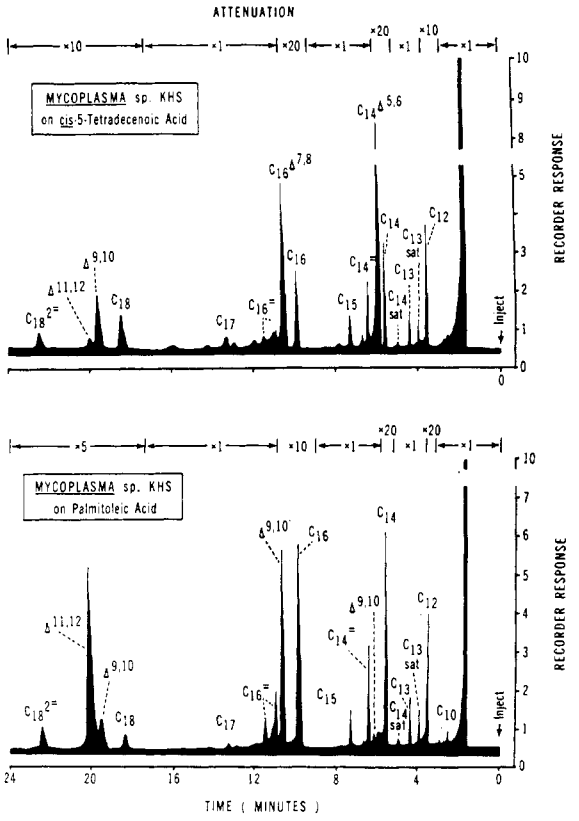

FIGURE 1: Capillary gas chromatographic patterns of total fatty acid methyl esters of *Mycoplasma* sp. KHS grown in the presence of *cis*-5,6-tetradecenoic and palmitoleic acids, respectively. Late logarithmically grown cells from complex defatted growth medium. Column: capillary, Carbowax K-20M + V-93 (99:1), 150 ft at 185°.

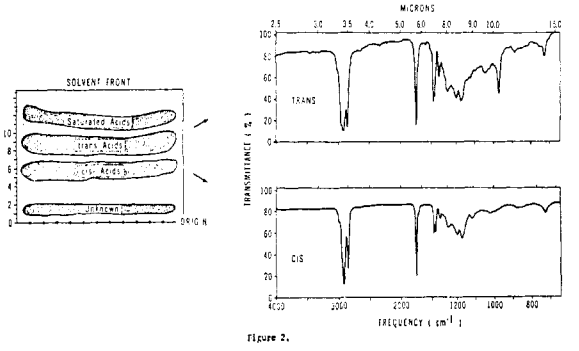

FIGURE 2: Fractionation of the total fatty acids of *Mycoplasma* sp. KHS grown with palmitelaidic acid by thin-layer chromatography and the resulting infrared spectra of the isolated *cis* and *trans* fractions. Tracing from thin-layer chromatography plate (silica gel G + 15% $AgNO_3$; 250 mμ thick). Total amount methylated fatty acids per plate = 2.9 mg. Solvent system: petroleum ether and ethyl ether (9:1 v/v). Infrared spectra as films between KBr crystals.

It had been shown previously that streptococcal C_{16}-$\Delta^{7,8}$ and $\Delta^{9,10}$ isomers are separable using a capillary column with a coating material (Carbowax 1540) similar to that utilized in these studies; the separation factor (Ackman and Castell, 1967) for these isomers was 1.029 (Panos, 1965). Similarly, utilizing a capillary column coated with butanediol succinate, Ackman and Castell (1967) obtained a separation factor of 1.026. Under the conditions indicated in Figure 1, the separation factor for these two isomers with a different polyester liquid phase (Carbowax K-20M) was 1.022. The relative merits of Carbowax 1540 and K-20M for bacterial fatty acid analyses by capillary column gas chromatography is discussed elsewhere (Panos and Henrikson, 1968b). Since relative retention times (palmitate = 1) are not identical for hexadecenoic acid isomers on different polyester liquid phases, the position of the double bond within the C_{16} and C_{18} monoenoic acids formed from the precursors added was obtained by (a) relative resolution comparisons after cochromatography with microbial lipid mixtures known to contain a particular C_{16} positional isomer, (b) from corrected relative retention time plots constructed in these laboratories from appropriate positional isomeric standards, and (c) cochromatography with authentic oleic or *cis*-vaccenic acids for identification of the resulting elongated isomeric octadecenoic acid end product. Certain methylated mixtures of positional isomeric C_{18} monoenoic acid standards ($\Delta^{9,10}$, $\Delta^{11,12}$, and $\Delta^{12,13}$) are capable of being adequately resolved by a relatively recently coated capillary column of the type used in these studies (C. Panos, unpublished results). However, although octadecenoic acid isomeric mixtures whose double bonds are in the $\Delta^{9,10}$ and $\Delta^{10,11}$ position are not resolved by this column, each has a distinctly different retention time when chromatographed separately. Mixtures of $\Delta^{10,11}$–$\Delta^{11,12}$ (*cis*-vaccenic) isomers, on the other hand, are resolved without difficulty. The identification of oleic acid, therefore, was confirmed by determining the differences in resolution between $\Delta^{10,11}$–$\Delta^{11,12}$ and $\Delta^{9,10}$–$\Delta^{11,12}$ standard mixtures and comparing these results with similar data obtained from mycoplasma extracts containing added *cis*-vaccenic acid. *cis*-Vaccenic acid was easily resolved from any one or a mixture of all of the esterified C_{18} positional isomeric standards employed ($\Delta^{9,10}$, $\Delta^{10,11}$, and $\Delta^{12,13}$). No identification of positional isomers was attempted by mere referral to previous or published relative retention time data.

Table I tabulates the quantitative results obtained from the chromatographic patterns of *Mycoplasma* sp. KHS cultivated in the presence and absence of the acids studied. Certain points require emphasis: (a) Growth of this organism with added palmitoleic acid resulted in an increase in the total fatty acid content as well as a tremendous increase in the amount of *cis*-vaccenic acid (20.45%) as compared with that found in control cells grown in the absence of added precursor (0.65%). (b) In contrast to these findings and although a dramatic increase in the $C_{16}\Delta^{7,8}$ isomer was evident when cells were grown with a shorter monoenoic acid, *cis*-5,6-tetradecenoic acid, the quantity of oleic acid resulting from this $C_{16}\Delta^{7,8}$ intermediate formed was less dramatic; only 7.90% as compared with the control (3.31%). This was appreciably less than that of its isomer, *cis*-vaccenic acid, as formed from palmitoleic acid. (c) Cells grown in the presence of an equal mixture of oleic and *cis*-vaccenic acids, although apparently absorbing more *cis*-vaccenic

TABLE I: Fatty Acid Composition of *Mycoplasma* sp. KHS[a] Cultivated in the Presence of Various Monoenoic Acids.

Fatty Acid	Composition of Total Fatty Acids (%)			
	Control	Palmitoleic	*cis*-5,6-Tetradecenoic	Oleic–*cis*-Vaccenic Mixture
Capric	0.21	TR	TR	0.43
Lauric	19.56	7.03	1.84	13.10
C_{13} saturated (C_{13}T?)[b]	0.21	0.12	0.06	0.17
Tridecanoic	0.46	0.20	0.10	0.36
C_{14} saturated (C_{14}T?)[b]	TR[b]	TR	TR	TR
Myristic	40.44	19.99	8.65	34.03
$C_{14}\Delta^{5,6}$			26.35	
$C_{14}\Delta^{9,10}$	TR	TR	TR	TR
C_{14} unsaturated XK[b]	1.51	0.53	0.24	2.18
Pentadecanoic	0.45	0.25	0.17	0.34
Palmitic	29.91	21.39	12.88	20.94
$C_{16}\Delta^{7,8}$	TR		33.99	TR
$C_{16}\Delta^{9,10}$	0.32	20.96		0.48
C_{16} unsaturated XK[b]	TR	0.19	TR	TR
C_{16} unsaturated XK[b]	0.44	0.28	TR	0.52
Margaric	TR	TR	TR	TR
Stearic	0.97	1.51	4.55	1.50
$C_{16}\Delta^{9,10}$	3.31	4.83	7.90	10.52
$C_{18}\Delta^{11,12}$	0.65	20.45	1.14	12.71
Linoleic	1.57	2.26	2.11	2.71
Saturated/unsaturated ratio[b]	11.82	1.02	0.39	2.43
% total fatty acids	4.30	5.82	3.89	3.26

[a] Late logarithmically grown cells from complex-defatted growth medium. Analyses by capillary gas chromatography. [b] T = tentative identification of branched methyl acids; numeral indicates total carbon atoms. XK = unknown, TR = trace.

than oleic acids, never markedly exceeded a total octadecenoic acid content than cells grown with palmitoleic acid; *i.e.*, 23.23 and 20.45%, respectively. (d) Growth in the presence of palmitoleic, *cis*-5,6-tetradecenoic, or oleic–*cis*-vaccenic acids resulted in a significant depression in saturated fatty acid biosynthesis. For example, cultivation with *cis*-5,6-tetradecenoic acid resulted in an appreciable decrease in the absolute cellular content of lauric, myristic, and palmitic acids as compared with the control. The greater total unsaturated fatty acid content in this organism when grown in the presence of this C_{14} monoenoic acid precursor, as compared with the other lipids tested, is apparent in the saturated/unsaturated fatty acid ratios tabulated (Table I). Comments concerning the unknown C_{14} and C_{16} unsaturated fatty acids detected and of the tentatively identified C_{13} and C_{14} methyl branched acids have been presented (Henrikson and Panos, 1969).

Although not tabulated, it was found that cells grown in the presence of β-hydroxydecanoic acid isolated from *Ps. aeruginosa* rhamnolipid or 2-hydroxydodecanoic acid failed to show either an increase in the unsaturated acid content or a decrease in the saturated acids synthesized. However, growth of *Mycopolasma* sp. KHS in the presence of 2-hydroxydodecanoic acid resulted in an increase of palmitic (39.97%) by 10% as compared with cells grown in the absence of these additions (Table I). The fatty acid content of these cells when grown with these hydroxylated acids was 4.65 and 6.20%, respectively.

Figure 2 illustrates a typical tracing depicting the resolution achieved by thin-layer chromatography, for the separation of geometrical isomers, of the fatty acid mixture from *Mycoplasma* sp. KHS grown with palmitelaidic acid, the *trans* isomer of palmitoleic acid. The infrared patterns of these resolved fatty acid fractions after their elution are also presented. The initial uptake of palmitelaidic acid by whole cells was confirmed in two ways: (a) chromatographically, by the appearance of a fraction with an R_F identical with that of fatty acid standards possessing the *trans* configuration, and (b) by infrared analyses revealing the characteristic *trans* configurational band at 10.3 μ in this fraction. As is apparent, this spectral band was absent in the infrared pattern from the thin-layer chromatography fraction containing acids with the *cis* configuration.

The capillary gas chromatographic results of these *cis* and *trans* fractions isolated by thin-layer chromatography are presented in Figure 3. The resolution of the entire fatty acid content of this sterol-nonrequiring

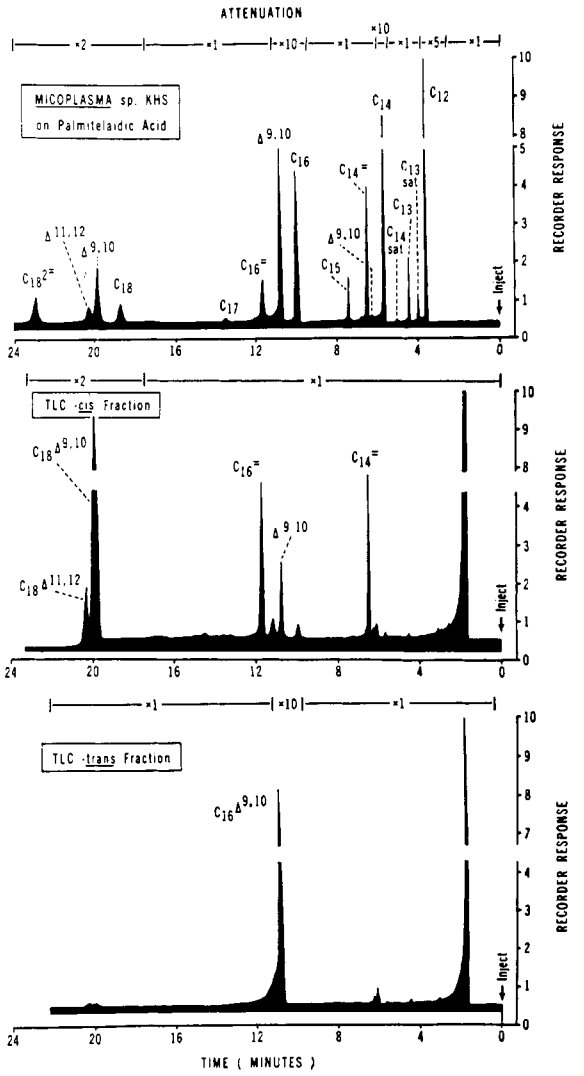

FIGURE 3: Capillary gas chromatographic comparison of the total fatty acid methyl esters of *Mycoplasma* sp. KHS grown with palmitelaidic acid and of the fatty acid content of its *cis* and *trans* isomeric fractions isolated by thin-layer chromatography. Column: capillary, Carbowax K-20M + V-93 (99:1), 150 ft at 185°.

organism prior to thin-layer chromatography is presented for comparison. These data unequivocally demonstrate that although palmitelaidic acid was absorbed from the medium, it was neither elongated nor degraded as evidenced by the absence of unsaturated acids in the C_{18} monoenoic acid region or the appearance of shorter acids. The *cis* isomeric fatty acid content is obvious when a comparison is made of the acids present in the thin-layer chromatographic *cis* fraction with that of the total mixture.

Table II tabulates the fatty acid composition and distribution of this mycoplasma when cultivated in the presence of palmitelaidic acid. The data illustrate that following absorption, palmitelaidic acid is incorporated to an appreciable extent into the total phospholipids and a monoglycosyldiglyceride of this organism. The monoglycosyldiglyceride, however, revealed a greater lauric, myristic, and palmitic acid and a lower palmitelaidic acid content than that present in the total phospholipids extracted. The inability of this organism to elongate this precursor to the homologous *trans*-vaccenic acid is quantitatively illustrated (see also control cells, Table I). This is in sharp contrast to the formation of *cis*-vaccenic acid to the extent of 20.45% of the total lipids when this organism was grown in the presence of the identical positional but opposite geometrical isomer, palmitoleic acid. Nevertheless, the total fatty acid yield obtained was identical and the saturated/unsaturated fatty acid ratio similar to cells grown in the presence of palmitoleic acid (Table I)

Discussion

Although *Mycoplasma* sp. KHS is almost devoid of long-chain monoenoic acids when grown in a complex but defatted growth medium, these studies have demonstrated an inherent ability to form such acids when supplied with appropriate precursors. The data presented have illustrated a two-carbon chain-elongation procedure for unsaturated fatty acid biosynthesis seemingly analogous to that in most bacteria. Others have already shown that saturated but not unsaturated fatty acids in a

TABLE II: Fatty Acid Composition and Distribution of *Mycoplasma* sp. KHS[a] Grown in the Presence of Palmitelaidic Acid.

Fatty Acid	Composition of Total Fatty Acids (%)		
	Whole Cells	Total Phospho-lipids	Mono-glycosyl-diglycer-ide
Capric	0.39	TR	TR
Lauric	10.65	3.23	5.43
C_{13} saturated (C_{13}T?)	0.25	TR	0.13
Tridecanoic	0.49	0.26	0.35
C_{14} saturated (C_{14}T?)	TR	TR	TR
Myristic	22.47	20.12	27.98
$C_{14}\Delta^{9,10}$	TR	TR	TR
C_{14} unsaturated	1.13	0.79	0.80
Pentadecanoic	0.53	0.48	0.53
Palmitic	22.36	25.90	31.72
$C_{16}\Delta^{9,10}$	31.12	39.38	25.73
C_{16} unsaturated XK			
C_{16} unsaturated XK	0.40	0.45	0.43
Margaric	TR	0.11	TR
Stearic	1.82	1.59	1.56
$C_{18}\Delta^{9,10}$	4.34	3.94	2.96
$C_{18}\Delta^{11,12}$	1.69	1.19	0.96
Linoleic	2.37	2.49	1.41
Saturated/unsaturated Ratio	1.44		
% total fatty acids	5.81		

[a] See Table I for footnotes.

closely related mycoplasma, *M. laidlawii*, arise entirely or in part from acetate (Pollack and Tourtellotte, 1967; Rottem and Razin, 1967). In these studies, *Mycoplasma* sp. KHS, with a generation time of 6.5 hr, readily absorbed and elongated the precursors added without a prior need for adaptation Although the saturated/unsaturated fatty acid ratio became markedly reduced to that generally found in bacteria (O'Leary, 1967) during growth with precursors, no change in either the growth rate or microscopic morphology of this organism was ever apparent.

The use of palmitoleic ($C_{16}\Delta^{9,10}$) and *cis*-5,6-tetradecenoic acids, suitable precursors in the biosynthesis of long-chain fatty acids in bacteria, has revealed that the position of unsaturation within the precursor influences to a considerable degree the subsequent ability of this mycoplasma to form a particular isomeric octadecenoic acid. This is most apparent in the amount of *cis*-vaccenic ($C_{18}\Delta^{11,12}$) and oleic ($C_{18}\Delta^{9,10}$) acids formed from the respective precursors utilized (Table I). In this respect, it is of interest to point out that while oleic acid is regarded as the predominating octadecenoic acid of higher forms of life, its positional isomer, *cis*-vaccenic acid, prevails in bacteria. Thus, while the mycoplasma have been ascribed by some as being akin to higher forms of life, this preference for *cis*-vaccenic acid formation seemingly represents an attribute more common to bacterial metabolism. Similarly, it is of interest to note that in the presence of an equal mixture of these two terminating isomeric octadecenoic acids, *Mycoplasma* sp. KHS absorbed more *cis*-vaccenic acid than oleic acid.

The differences in the amount of oleic and *cis*-vaccenic acids formed by *Mycoplasma* sp. KHS when supplied with either isomeric acid precursor has illustrated that the enzymatic deficiency (specificity?) for oleic acid biosynthesis lies in the elongation of its immediate hexadecenoic acid precursor and not in the formation of this *cis*-7,8-hexadecenoic acid. The elongation of palmitoleic (*cis*-9,10-hexadecenoic) acid to *cis*-vaccenic acid, on the other hand, proceeded to approximately the same extent as did the formation of *cis*-7,8-hexadecenoic acid from *cis*-5,6-tetradecenoic acid.

Under the growth conditions employed and drawing analogies from recent bacterial findings (Kass and Bloch, 1967), the inability of *Mycoplasma* sp. KHS to synthesize appreciable quantities of the homologous *cis*-vaccenic and/or oleic acid series in the absence of added precursors could reflect a metabolic alteration or inhibition of a possible common branching point for the synthesis of saturated and unsaturated acids. Such an intermediate common to both saturated and unsaturated fatty acid biosynthesis has been found in *Escherichia coli* and identified as β-hydroxydecanoate. The data obtained here add impetus to the possible presence of a branching point in *Mycoplasma* sp. KHS by seemingly reflecting a sensitive point in saturated fatty acid biosynthesis amenable to varying degrees of disturbance depending upon the monoenoic acid supplied. For example, cultivation of *Mycoplasma* sp. KHS with *cis*-5,6-tetradecenoic acid resulted in a significant depression of the saturated fatty acid content (lauric, myristic, and palmitic acids). These results are not due merely to an uptake of precursor as may be seen by calculation of the absolute content of each of these saturated acids and may, indeed, be indicative of an effect upon a circumvented or altered branching point for possible synthesis of unsaturated fatty acids in this sterol-nonrequiring mycoplasma.

In further support of this assumption was the finding of a similar but now less dramatic effect on saturated fatty acid biosynthesis when cells were grown with palmitoleic acid. Since palmitoleic acid may represent a precursor further removed from a possible common branching site, its effect might be expected to be less pronounced than one in closer proximity to it; as, for example, the results obtained with added *cis*-5,6-tetradecenoic acid. We had shown earlier that myristic acid and not palmitic acid is the predominating saturated fatty acid in *Mycoplasma* sp. KHS (Henrikson and Panos, 1967). Growth of this organism with palmitoleic acid resulted in a decrease of both lauric and myristic acids. However, although these decreases were not as pronounced as those when *cis*-5,6-tetradecenoic acid was supplied, they were sufficient to alter the absolute concentrations of these saturated acids. Thus, palmitic acid equalled that of myristic acid or emerged as the predominating saturated acid in this mycoplasma when the precursor was either palmitoleic or *cis*-5,6-tetradecenoic acids.

The addition of a mixture of both terminating octadecenoic acids, isomers furthest removed from a possible common branching point, continued to result in a notable but still lesser decrease in the major saturated fatty acids synthesized. Here, however, myristic acid remained the predominating saturated fatty acid. Thus, although unsaturated fatty acid elongation does occur upon addition of suitable precursors, the data obtained also reflect a concomitant but increasing disturbance upon the biosynthesis of saturated fatty acids as the chain length of the unsaturated acid supplied is decreased.

During these studies stearic acid remained as only a minor component of the total fatty acid content of this mycoplasma. Seemingly, therefore, major saturated fatty acid biosynthesis terminates at a chain length of 16 carbon atoms in this sterol-nonrequiring mycoplasma when grown in a complex but defatted medium.

If a branching point is possible within *Mycoplasma* sp. KHS it would probably be altered or inhibited at or below a common intermediate similar to that in *E. coli; i.e.*, β-hydroxydecanoic acid. Some credance in this hypothesis is indicated by the inability to alter either the saturated or unsaturated fatty acid content of this mycoplasma when grown in the presence of β-hydroxydecanoic acid isolated from *Ps. aeruginosa* rhamnolipid. Thus, one of several possibilities, for example, may be the lack of a specific dehydrase for the formation of the intermediate C_{10} monoenoic acid necessary for its elongation to *cis*-vaccenic acid. This would account for the absence of significant quantities of monoenoic acids in this mycoplasma and, also, for the labeling of only saturated acids *via* labeled acetate in related myco-

plasmas (Pollack and Tourtellotte, 1967; Rottem and Razin, 1967).

The inability to metabolize β-hydroxydecanoic acid may also be due to its being the inappropriate stereoisomer. In this case, the point of inhibition would not necessarily have to be a dehydrase step but could, instead, be in the formation of such a hypothesized hydroxylated intermediate. However, Birge *et al.* (1967) have employed β-hydroxydecanoic acid from *Ps. aeruginosa* rhamnolipid for the study of saturated fatty acid biosynthesis in *E. coli via* β-hydroxydecanoyl-ACP.[1] A means for metabolizing 2-hydroxydodecanoic acid nevertheless was apparent since growth in the presence of this acid resulted in an increase of only palmitic acid in these late logarithmically grown cells.

The results obtained with a similar but opposite geometric isomer, palmitelaidic acid, have provided some insights into the specificity of fatty acid utilization by this *Mycoplasma*. A degree of nonspecificity was clearly evident by the similar uptake and incorporation of the hexadecenoic acid precursor added regardless of whether it was of the *cis* (palmitoleic) or *trans* (palmitelaidic) configuration and by the identical total fatty acid content obtained when this organism was grown in the presence of either geometrical isomer (Tables I and II). Since only palmitoleic acid was elongated to *cis*-vaccenic acid, it would seem that at least two "sets" of enzymes are involved: (a) those unconcerned with geometrical or positional isomerization but necessary for long-chain fatty acid incorporation into complex cellular lipids and (b) those involved in the elongation of only those acids with the *cis* configuration. With the exception of elaidic acid, *trans* isomers have been found less active than the corresponding *cis* form in the nutrition of various bacteria (Cheng *et al.*, 1951; Hofmann and Panos, 1954).

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[1] ACP = acyl carrier protein.