Fatty Acid Composition, Distribution, and Requirements of Two Nonsterol-Requiring Mycoplasmas from Complex but Defatted Growth Media*

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ABSTRACT: The fatty acid composition, distribution, and requirements of two nonsterol-requiring mycoplasmas, *Mycoplasma* sp. KHS and *Mycoplasma laidlawii* B, grown in complex but exhaustively defatted growth media have been determined by high-resolving capillary gas chromatography. The residual fatty acid content of the two defatted growth media employed were 0.007 and 0.003%, respectively, with the unsaturated fatty acid content of each being less than 40% of the total (<0.0028 and 0.0014%, respectively). All cells were harvested during their late logarithmic phases of growth. Neither the qualitative nor quantitative fatty acid composition of these organisms or their anatomical components mimicked the trace residual fatty acid content of the growth medium that had survived solvent or solvent plus charcoal–lipid extraction. The need for preformed unsaturated fatty acids for growth of these organisms was judged to be minimal or nonexistent when grown under the conditions described. The monoenoic fatty acid synthetic capabilities of these organisms was also shown to be, at best, meager. Addition of various preformed long-chain monoenoic acids of the *cis* configuration to defatted growth media failed to either alter the growth rate, increase the cellular yields, or affect the microscopic morphology of the organisms examined. All cells and anatomical components showed an exceedingly high-saturated fatty acid content with the total unsaturated fatty acids present being almost negligible. The origin of these unsaturated fatty acids, in large part, was attributed to absorption from the medium. While palmitic acid is regarded as the predominating saturated fatty acid of microorganisms, myristic acid predominated in the lipids of these two mycoplasmas. Some evidence was obtained for the presence of exceedingly small quantities of branched methyl fatty acids in both organisms and anatomical components. The fatty acid composition of membranes and cytoplasm was found to mimic that of whole cells. Data were obtained illustrating a great variability in fragility between the membranes of *Mycoplasma* sp. KHS and *M. laidlawii* B, two sterol-nonrequiring mycoplasmas, when obtained under comparable growth conditions and, otherwise, treated in identical fashion. The seemingly anomalous findings concerning an absolute requirement for an unsaturated fatty acid for growth of *M. laidlawii* documented by others and the findings presented herein are discussed.

Some information is available concerning fatty acid requirements and composition of sterol-nonrequiring mycoplasmas. For example, Razin and Rottem (1963), employing a partially defined medium containing bovine plasma albumin fraction V as the only undefined ingredient, documented a need for an unsaturated fatty acid for growth of the fermentative saprophyte *Mycoplasma laidlawii* when this fraction was lipid preextracted prior to use. The fatty acid content and composition of these cells, however, was not determined during these nutritional studies. Similarly, utilizing ill-defined but solvent-extracted media, Smith *et al.* (1965) and Razin *et al.* (1966b) showed the predominating presence of saturated over unsaturated acids in *M. laidlawii* phospholipids. However, as mentioned by Smith *et al.* (1965) the significance of the fatty acid types found could not be assessed since the preextraction procedures employed did not remove all of the free fatty acids present in the culture medium. It has been shown that mycoplasmas, like bacteria, may mimic the fatty acid composition of the growth medium. To our knowledge, no concentrated effort has been made to specifically detail the composition and anatomical distribution of the fatty acids within a sterol-nonrequiring mycoplasma or to determine its absolute requirements for unsaturated fatty acids when grown in a complex medium essentially devoid of all fatty acids. Results of studies undertaken to fill these voids are the subject of this report. This report also documents the initial use of the relatively new and high-resolving technique of capillary (Golay) column gas chromatography in the study of mycoplasma lipids. A similar study has appeared with *Streptococcus pyogenes* and derived stable L form (Panos *et al.*, 1966). Part of these results have been reported in preliminary form (Henrikson and Panos, 1968).

Experimental Section

Microorganisms and Growth Media. Two sterol-nonrequiring mycoplasmas, *Mycoplasma* sp. KHS and *M.*

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laidlawii B, were kindly provided by Dr. P. F. Smith (University of South Dakota, Vermillion, S. D.). The organisms were routinely grown in a liquid medium (37°) consisting of 2% tryptose (Difco Laboratories, Inc., Detroit, Mich.) which had been exhaustively lipid preextracted in the dry state, 0.5% sodium acetate, 0.5% sodium chloride, 0.5% glucose added separately after sterilization, and thallium acetate (1:2500, w/v). For certain studies, the medium employed contained a tryptose "mixture" consisting of one part of solvent-extracted tryptose plus four parts of solvent-extracted tryptose treated with charcoal. Charcoal treatment was performed according to the methods of Chen (1967). Both media were adjusted to pH 8.0 and sterilized by autoclave.

For growth of *Mycoplasma* sp. KHS in tryptose "mixture" medium, cells were adapted and maximum growth achieved after 10–20 subtransfers. Regardless of the organisms or media used, cellular yields (dry weight) per liter of medium ranged from 15 to 20 mg at harvest. Where necessary, the fatty acids added to either growth medium were dissolved in 70% ethanol. The absolute alcoholic content of the media never exceeded 1.4% and did not significantly effect growth of either organism. The final concentration of all fatty acids tested ranged from 0.5 to 2.0 μg per ml of medium.

Tryptose Lipid Extraction. To tryptose (600 g) in the dry state was added 2 l. of chloroform–methanol (2:1, v/v) and the mixture was stirred continuously for 2 hr at room temperature. Following removal of chloroform–methanol, the tryptose was transferred to a Büchner funnel and percolated with an additional liter of this solvent mixture. Following removal of all solvent *in vacuo*, dried tryptose was next extracted three times with 2.5 volumes each of ethyl ether at 4°. All ether extractions were performed with constant and vigorous agitation with two extractions performed for 2 hr each, and the final extraction was allowed to continue overnight. The procedure by which constituted complex media could be rendered lipid free by acidification, lyophilization, and subsequent solvent extraction, prior to reconstitution and inoculation, was also attempted. However, no greater decrease in the residual fatty acids remaining by more than that observed with the multiple solvent extraction procedure above was observed.

Harvest. Cells were obtained during their late logarithmic phases of growth (usually after 30 hr) by Sharples centrifugation. Samples were removed for dry weight determinations after being washed twice (37,000*g* for 10 min) with 0.2 M phosphate-buffered saline (pH 7.5). Three additional washes were performed in the presence of magnesium chloride (0.01 M) plus DNase to facilitate cellular dispersion and prevent medium components from being occluded prior to lyophilization (Panos *et al.*, 1966). Growth curves were determined turbidimetrically with the aid of a Zeiss spectrophotometer at 420 mμ and correlated well with those determined earlier by dry weight and viable counts.

Membrane and Cytoplasm Preparations. Cellular fractionation was achieved by repeatedly passing a suspension of organisms through the orifice of a Biotech "X-Press." Breakage (>95%) was followed by dark-phase microscopy. The homogenates so obtained were subjected to differential centrifugation (37,000*g* for 20 min) for recovery of membrane and cytoplasm anatomical components. These components were prepared and stored as previously detailed (Panos *et al.*, 1966). Cytoplasmic fatty acids refer to those present in the nonsedimentable fraction after differential centrifugation.

Fatty Acid Extractions, Standards, and Chromatographic Methods. Fatty acids were extracted by the alkaline hydrolysis techniques of Hofmann *et al.* (1957). For accuracy, the appropriate correction was made after methylation of the extracted fatty acids and the total cellular content expressed as the free acids. All standards were obtained from Applied Sciences Laboratories, University Park, Pa., or the Hormel Institute, Austin, Minn. Esterified fatty acid mixtures were resolved by capillary gas chromatography as has been described (Panos, 1965; Panos *et al.*, 1966). A 150-ft capillary column coated with Carbowax K-20M + V-93 (99:1) (Perkin-Elmer Corp., Norwalk, Conn.) replaced that used previously for certain of these studies (Panos *et al.*, 1966). The designated abbreviations ($C_{13}T$?, $C_{14}T$?) of the tentatively identified branched methyl fatty acids are based upon their total carbon atom content. Controls included hydrolysis of large quantities of dried and extracted tryptose or tryptose "mixture" for residual fatty acid content and composition analyses.

Chemical Methods. Methylation and hydrogenation procedures, infrared analyses, and protein determinations of cytoplasm and membrane preparations were performed as has been described (Panos *et al.*, 1966; Weinbaum and Panos, 1966).

Results

The fatty acid and nonsaponifiable lipid content of the sterol-nonrequiring mycoplasmas and of their respective membranes is tabulated in Table I. Although close agreement was noted in the nonsaponifiable lipid content of each preparation, their fatty acid content differed markedly. Whereas whole cells of *Mycoplasma* sp. KHS contained 25% more fatty acid than did *M. laidlawii* B cells, the latter's membranes contained 42% more fatty acid than did those from the former. Similarly, *M. laidlawii* B cytoplasm exhibited a greater total fatty acid content (by 45%) than did *Mycoplasma* sp. KHS cytoplasm (100.09 and 54.71 μg per mg of protein, respectively). *M. laidlawii* B membranes are more fragile than those from *Mycoplasma* sp. KHS (P. F. Smith, personal communication). This was confirmed by the finding that, under identical membrane isolation and preparation procedures, the membrane content of whole cells of *Mycoplasma* sp. KHS and *M. laidlawii* B were 30.3 and 12.9%, respectively. Razin *et al.* (1963) have reported that the membrane comprises approximately 35% of the dry weight of certain mycoplasmas. Thus, the increased fatty acid content observed in these *M. laidlawii* B membrane preparations is probably due to their continued fragmentation during the purification procedure. The equally higher fatty acid content of its cytoplasm would seem to substantiate fragmentation of this isolated component. Apparently, such fragmen-

TABLE I: Whole Cell and Membrane[a] Fatty Acid Content of Two Sterol-Nonrequiring Mycoplasmas.

Cellular Material	% Dry Weight: Non-saponifiable Lipid	% Dry Weight: Total Fatty Acid
Mycoplasma sp. KHS	0.42	4.30
Mycoplasma sp. KHS membranes	1.94	8.82
M. laidlawii B	0.56	3.43
M. laidlawii B membranes	2.01	15.19

[a] From late logarithmically grown cells in lipid-preextracted growth medium (chloroform–methanol, 2:1, and ethyl ether).

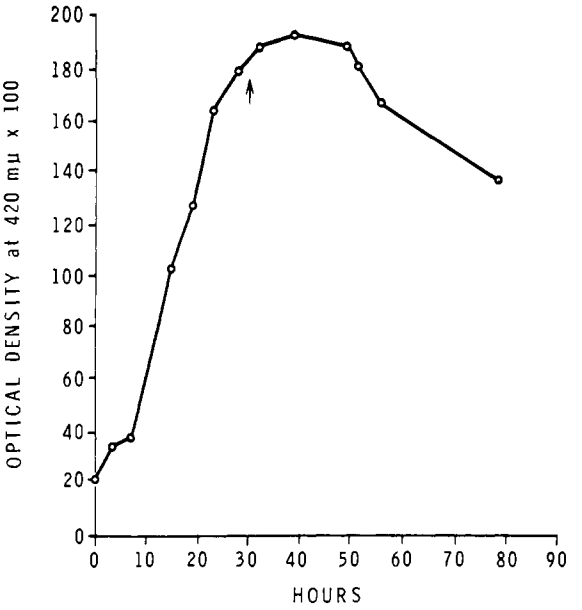

FIGURE 1: Growth curve of *Mycoplasma* sp. KHS growing in solvent-extracted tryptose medium. Arrow indicates point of harvest.

TABLE II: Total Fatty Acid Content of *Mycoplasma* sp. KHS from Lipid-Preextracted Tryptose Growth Medium before and after Additional Treatment with Charcoal.

	Per Cent[a]: Tryptose[b]	Per Cent[a]: Tryptose (1:5)[c]
Whole cells		
Fatty acid	4.30	2.40
Cellular S/U fatty acid ratio[d]	9.05	23.37
Nonsaponifiable lipid	0.42	0.42
Growth medium (uninoculated control)		
Fatty acid	0.007	0.003
Medium S/U fatty acid ratio	1.66	1.26
Nonsaponifiable lipid	0.0162	0.0009

[a] Per cent of total dry weight of cells or lyophilized growth medium. [b] Extracted with chloroform–methanol (2:1) and ethyl ether. [c] Mixture of one part extracted tryptose plus four parts extracted and charcoal-treated tryptose. [d] S/U = saturated/unsaturated fatty acid ratio. Determined by capillary gas chromatography.

tation was not encountered during isolation of membranes from the *M. laidlawii* B used by Razin *et al.* (1963) and may reflect structural or enzymatic differences within species. They may, however, also be the result of the methods used for these membrane preparations ("X-Press" *vs.* osmotic lysis). As is apparent, the data verified the expected high fatty acid content of isolated membranes and the relatively low nonsaponifiable lipid content of these intact sterol-nonrequiring mycoplasmas. Infrared analyses of all free and methylated fatty acid mixtures failed to reveal the presence of hydroxylated or cyclopropane-containing fatty acids and of the presence of acids with the *trans* configuration.

Tryptose, the only undefined ingredient for growth of these mycoplasmas, was rendered almost totally fatty acid free (0.007% remaining, Table II) by a multiple solvent extraction procedure (see Experimental Section). Further treatment of this lipid-extracted tryptose with charcoal, while reducing the residual fatty acids remaining still further almost completely destroyed its growth-promoting abilities. Spectrophotometric analyses indicated that considerable protein and nucleotide materials as well as lipids had been removed by this charcoal treatment. Thus, for certain studies with *Mycoplasma* sp. KHS in a medium containing an even lower residual fatty acid content (0.003%), the tryptose "mixture" described earlier (see Experimental Section) was used. The total unsaturated fatty acid content of both of these defatted media was less than 40% of the residual acids surviving lipid extraction. After adaptation, growth medium prepared with tryptose "mixture" supported growth of this organism to the same extent as media prepared with solvent-extracted tryptose alone. A typical growth curve for *Mycoplasma* sp. KHS, but characteristic of both mycoplasmas, appears in Figure 1. The growth rate (generation time 6.5 hr) and microscopic morphology of *Mycoplasma* sp. KHS in these two media remained unaltered and was almost identical with that experienced with nonlipid-extracted medium. Table II presents data obtained with *Mycoplasma* sp. KHS when grown in lipid-preextracted media with and without the addition of charcoal-treated tryptose (tryptose "mixture"). It should be noted that (a) the fatty acid content of the medium routinely employed in these

studies (medium control), although practically nil after solvent extraction alone, could be lowered still further when employing the tryptose "mixture"; (b) although the nonsaponifiable lipid content of such a "mixture" medium was almost nonexistent, whole cells from this medium contained as much nonsaponifiable lipid as did those from media with solvent-extracted tryptose, indicating net synthesis rather than medium absorption of nonsaponifiable lipids; and (c) while the fatty acid content decreased by 44% when cells were grown with the tryptose "mixture," their saturated–unsaturated fatty acid ratio increased markedly, as compared with cells grown with solvent-extracted tryptose alone.

Figure 2 illustrates a typical chromatographic pattern obtained by capillary gas chromatography of methylated fatty acids from the membranes of *Mycoplasma* sp. KHS. Certain facets require emphasis: (a) the meager amount of C_{16}- and C_{18}-unsaturated fatty acids present in such mixtures as emphasized by the low attenuation ($\times 1$, $\times 2$) necessary for their detection, (b) the preponderance of C_{12}- and C_{14}-saturated acids as indicated by the high attenuations ($\times 50$) necessary for their containment on the chromatogram, and (c) the capable resolution of C_{16} and C_{18} positional isomers found in such membrane and whole cell fatty acid mixtures by the capillary column. The insert illustrates an even greater resolution of the extremely small quantities of the positional isomeric monoenoic C_{18} acids from such membranes as recently achieved with an improved column. The relative merits of these two type columns is discussed elsewhere (Panos and Henrikson, 1969).

Capillary gas chromatographic analyses of the residual fatty acids (0.007%) remaining in solvent-extracted tryptose revealed the presence of finite quantities of unsaturated acids identified as linoleic, oleic, and *cis*-vaccenic acids by comparison of their relative retention times with standards and by their quantitative conversion into stearic acid upon hydrogenation. Thus, the origin of these quantitatively meager acids in these cells is uncertain and is most probably due, in large part, to their absorption from the growth medium.

Table III tabulates the fatty acid composition of *Mycoplasma* sp. KHS and *M. laidlawii* B whole cells and anatomical components in the solvent-extracted medium and of the changes in unsaturated fatty acid content of *Mycoplasma* sp. KHS when grown in a medium whose fatty acid content had been reduced still further (tryptose "mixture"). As is apparent, unusual C_{13}- and C_{14}-saturated acids were detected in all preparations. These acids were not disturbed by hydrogenation and possessed relative retention times indicative of branched methyl fatty acids. These and a C_{14} and two

TABLE III: Total Fatty Acid (Per Cent) Composition and Distribution of Two Sterol-Nonrequiring Mycoplasmas.[a]

	Mycoplasma sp. KHS				*M. laidlawii* B		
Fatty Acid	Whole Cells	Membrane	Cytoplasm	Whole Cells (1:5 Medium)	Whole Cells	Membrane	Cytoplasm
Capric	0.42	0.85	0.58	0.06	0.20	0.15	0.04
Lauric	18.78	29.65	21.31	7.95	8.31	11.93	11.34
C_{13} saturated (C_{13}T ?)[b]	0.38	0.34	0.38	0.12	1.20	0.40	0.18
Tridecanoic	0.73	0.43	0.58	0.81	0.97	0.75	0.38
C_{14} saturated (C_{14}T ?)[b]	TR	TR	TR	TR	0.82	0.29	0.12
Myristic	40.61	44.94	47.94	45.20	39.27	57.28	51.70
C_{14} $\Delta^{9,10}$	TR	TR	TR	TR	TR	TR	TR
C_{14} unsaturated XK[b]	1.65	1.61	1.44	0.48	1.60	0.85	0.43
Pentadecanoic	0.76	0.29	0.37	1.04	1.29	0.84	0.48
Palmitic	27.11	14.85	19.39	38.65	37.61	22.37	32.80
C_{16} $\Delta^{7,8}$	TR	TR	TR	TR	TR	TR	TR
C_{16} $\Delta^{9,10}$	0.45	0.60	0.70	0.07	TR	TR	TR
C_{16} unsaturated XK[b]	TR	0.14	TR	TR	TR	0.08	0.03
C_{16} unsaturated XK[b]	0.54	0.24	0.32	0.32	0.83	0.43	0.19
Margaric	TR	TR	TR	0.16	TR	0.11	0.06
Stearic	1.27	0.53	0.80	1.81	1.48	0.86	0.47
C_{18} $\Delta^{9,10}$	3.75	3.05	3.36	1.86	3.13	2.03	0.95
C_{18} $\Delta^{11,12}$	1.38	0.84	1.05	0.78	1.53	0.81	0.40
Linoleic	2.18	1.64	1.78	0.59	1.76	0.83	0.41
Saturated/unsaturated ratio	9.05			23.37	10.30		

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

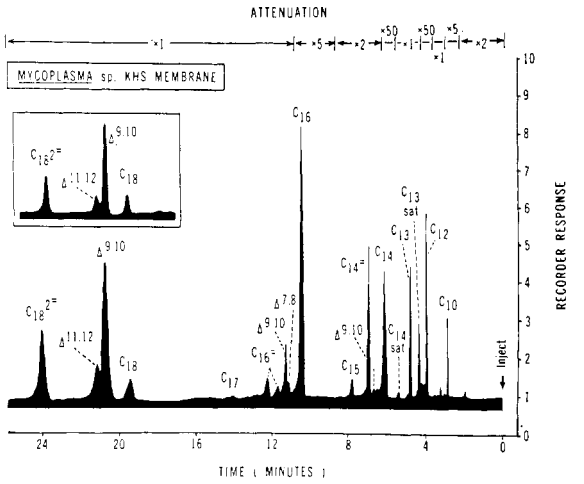

FIGURE 2: Typical capillary gas chromatographic pattern of total membrane fatty acid methyl esters from late logarithmically grown cells in lipid-preextracted tryptose medium. Column: capillary, Carbowax 1540, 150 ft at 185°; total amount injected = 0.019 μl; no split. Insert illustrates higher resolution of C_{18} positional isomeric mixture achieved with subsequent use of Carbowax K-20M + V − 93 (99:1) as liquid phase.

C_{16} monoenoic acids whose unsaturations were seemingly close to the ω end of their molecules, as deduced from positional isomeric log relative retention time plots, were not present in the residual fatty acid content of these lipid-preextracted media. Verification of chain length of these positional isomers was achieved by noting their disappearance, with a concomitant increase in their corresponding homologous saturated acids, upon hydrogenation. The capable resolution of C_{16} positional isomers by capillary column chromatography has been documented (Panos, 1965) and verified (Ackman and Castell, 1967). Growth of *Mycoplasma* sp. KHS in these two media showed that while the saturated/unsaturated acid ratio could be increased markedly, the predominating saturated component remained myristic acid. This shift in the cellular fatty acid ratio upon addition of charcoal-treated tryptose to the growth medium was due, in the main, to a decrease in the unsaturated fatty acid content. While the saturated acid content increased by only 6% from that in cells from solvent-extracted medium, the unsaturated fatty acids decreased by 59% when grown in a medium whose residual fatty acid content was 0.003%. As expected, both organisms failed to show a preferential distribution of their fatty acids for membrane or cytoplasm components. The fatty acid composition of each anatomical component mimicked that of the intact organism.

Discussion

This study has presented the first detailed analyses of the fatty acid composition, distribution, and requirements of two sterol-nonrequiring mycoplasmas grown in complex but defatted media by high resolving capillary gas chromatography. As opposed to bacteria in which the major saturated acid, by far, is known to be palmitic acid, myristic acid predominated in the whole cells, and anatomical components of *Mycoplasma* sp. KHS and *M. laidlawii* B. It was observed that as the chain length increased a progressive decrease in the concentration of the higher saturated acids occurred (*i.e.*, myristic, palmitic, and stearic acids). Credance in these results providing insights into the biosynthetic capabilities of these organisms were (a) in uninoculated medium controls the trace total fatty acids remaining possessed an opposite ratio of three times more palmitate than myristate, (b) the qualitative results of others (Pollack and Tourtellotte, 1967; Rottem and Razin, 1967) of the synthesis of only long-chain saturated fatty acids *via* labeled acetate in certain related mycoplasmas, and (c) the finding that neither the qualitative or quantitative fatty acid composition of these organisms mimicked that of the trace residual fatty acid content of the defatted growth media employed. This documents the first report of myristic acid as the predominating saturated fatty acid in the total fatty acids from these mycoplasmas and their anatomical components.

Although an absolute need for preformed long-chain monoenoic acids for growth of *M. laidlawii* has been stated when grown in a minimal and defatted medium (Razin and Rottem, 1963), the data presented herein indicate that this fact cannot be generalized and probably depends upon the organism and nature of the growth medium employed. Thus, when grown in a complex but defatted medium, these data suggest that *M. laidlawii* B and *Mycoplasma* sp. KHS do not appear to require an unsaturated fatty acid for growth and that the presence of small quantities of such acids in whole cells and anatomical components is probably due to absorption from the growth medium. This is substantiated by the finding of a dienoic acid from the uninoculated medium, linoleic acid, in all of the cellular lipid extracts. An unequivocal conclusion concerning the absolute need for monoenoic acids by these mycoplasmas is not yet possible. This is due to (a) the lack of a suitable chemically defined growth medium, (b) the presence of trace but perceptible unsaturated acids in the crude but defatted media used in these studies, and (c) an indication of *some* degree of unsaturated acid biosynthesis prevailing in these organisms (see below). Nevertheless, support for a lack of need for such acids in *Mycoplasma* sp. KHS and *M. laidlawii* B is lent by certain facts. The total C_{18} monoenoic acid (4.66–5.13%) and C_{16} monoenoic acid precursor (trace, 0.45%) content of these two mycoplasmas was extremely low. This is in sharp contrast to the large C_{16} and C_{18} monoenoic acid content of gram positive and negative bacteria from lipid-free media (from 35 to 63% of total fatty acids) illustrating a most necessary need for monounsaturated acids by the *Eubacteriales*. The total unsaturated fatty acid content of these two sterol-nonrequiring mycoplasmas never exceeded 10% and was usually less than 5% of all the acids obtained when grown under the conditions described. These differences in the monoenoic acid content between these two microbial orders become more meaningful when it is realized that the total fatty acid content of these mycoplasmas (2–4%) and of bacteria in general (3–5%) do not vary appreciably.

The significant differences in whole cell saturated/unsaturated fatty acid ratios, as compared with those of uninoculated medium controls, together with the total meager unsaturated fatty acid content of isolated membranes also emphasizes the overwhelming preference for saturated acids for structural purposes in these two organisms. The saturated/unsaturated fatty acid ratio of bacteria may range from less than 1 to 2 (O'Leary, 1967). This ratio, however, could be markedly varied for these mycoplasmas depending upon the defatted medium used. Thus, and as exemplified with *Mycoplasma* sp. KHS, an already saturated acid "favored" ratio of 9.05 was increased to 23.37 without any apparent alteration whatsoever being observed in either its growth rate, dry weight yield, or cellular morphology. Finally, the addition of either oleic, *cis*-vaccenic, palmitoleic, or *cis*-5,6-tetradecenoic acids to defatted media failed to (a) stimulate growth beyond that observed in their absence, (b) increase cellular yields, or (c) alter the morphology (spheres as deduced by phase microscopy) of these mycoplasmas. When supplied to the growth medium, however, such acids were absorbed to an appreciable extent and certain of these were elongated by *Mycoplasma* sp. KHS (Panos and Henrikson, 1968). It should be noted that in a tryptose medium containing albumin and employing much larger concentrations of unsaturated fatty acids (*i.e.*, 50 μg/ml of medium), Razin *et al.* (1966a) have been able to alter the morphology of *M. laidlawii* B to the filamentous form.

As already discussed, the presence of extremely small quantities of monoenoic acids in the lipids of these two mycoplasmas makes it seem highly unlikely that they are absolute requirements for growth or structure. This does not, however, negate the possible need for monoenoic acids by these organisms when grown in a minimal medium. Such a relationship would be reminiscent of the earlier nutritional studies concerning the biotin-unsaturated fatty acid interrelationship of bacteria. This might explain these seemingly anomalous findings with those of Razin and Rottem (1963) for the absolute requirement of an unsaturated fatty acid for growth of *M. laidlawii* B in a minimal and defatted medium, a requirement apparently obviated when this organism is grown in a complex but defatted medium. Aside from medium differences between these respective laboratories, these results may also reflect changes in the cultural characteristics of the *M. laidlawii* B employed due to long years of laboratory propagation. An apparent difference has already been noted in the resulting stability of isolated membranes from these two cultures of *M. laidlawii* B (see Results).

The data obtained, however, have unequivocally demonstrated that in complex but defatted media the *de novo* synthesis of long-chain monoenoic acids in *Mycoplasma* sp. KHS and *M. laidlawii* B is, at best, only meager. As already mentioned, the existence of some degree of unsaturated acid biosynthesis cannot be denied. Thus, while the presence of a C_{14} and two C_{16} positional monoenoic acid isomers was detected in these organism, but not in medium controls, the combined content of these acids never exceeded 2% of the total acids extracted.

Finally, while membrane and cytoplasm results mimicked those of whole cells, a great difference in the total membrane fatty acid content of *Mycoplasma* sp. KHS and *M. laidawii* B was noted. This, coupled with the continued fragmentation of *M. laidlawii* B membranes during these purification procedures, attests to a degree of variability of membrane fragility that may be more common than hitherto supposed from nutritionally related mycoplasmas (*i.e.*, sterol nonrequiring) when obtained under comparable growth conditions and, otherwise, treated in identical fashion.

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