The Effect of Straight-chain Saturated, Monoenoic and Branched-chain Fatty Acids on Growth and Fatty Acid Composition of Mycoplasma Strain γ

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SUMMARY

The fatty acid growth requirements of a sterol-requiring Mycoplasma (strain γ), unable to synthesize or alter the chain length of either saturated or unsaturated fatty acids, were investigated. In some cases adaptation was required for growth when mycoplasmas were transferred to media of differing fatty acid composition. No straight-chain saturated acid supported growth when tested alone, but good growth was obtained with three branched-chain acids (isopalmitate, isostearate and anteisoheptadecanoate) tested singly and with some straight-chain saturated acids when tested in pairs (e.g. when equal proportions of C_{12} and C_{22} acids were supplied together).

Of the unsaturated fatty acids tested, *cis*-12-octadecenoate and two *trans*-octadecenoates (elaidate and *trans*-vacenate) supported good growth alone. Little or no growth was obtained with any *cis*-monoenoic acid of the oleic acid series unless a straight-chain acid was also supplied and both the chain length of the monoenoic acid and the position of the double bond had a marked effect on the range of saturated acids with which it could be successfully paired. With myristoleate, long-chain saturated acids of chain length C_{20} , C_{22} gave the best growth; with oleate the range extended from C_{12} to C_{20} (optimal at C_{17}) and with erucate from C_{10} to C_{18} (optimal at C_{15}). When the double bond was near the carboxyl group, as in *cis*-6-octadecenoate, strain γ grew only when paired with C_{14} to C_{16} saturated acids; as it became further removed from the carboxyl group as in *cis*-vacenate, addition of a single saturated acid from an extended range (C_{10} to C_{24}) could support growth.

Only those fatty acids supplied in the growth medium were present in the lipids of the organism, and in cases where strain γ grew well when a single fatty acid was supplied, this was the sole fatty acid found. Marked differences in morphology were observed in mycoplasmas of differing fatty acid composition.

INTRODUCTION

The effects on the fatty acid composition of the membrane lipids of varying the unsaturated fatty acid supplied for growth have been studied with auxotrophs of *Escherichia coli* (Silbert, Ruch & Vagelos, 1967; Esfahani, Barnes & Wakil, 1969), in *Acholeplasma laidlawii* strain A (Rottem & Panos, 1969) and strain B (McElhaney & Tourtellotte, 1969, 1970). All of these organisms could synthesize saturated fatty acids and none required or could synthesize a sterol. The experiments with *E. coli* auxotrophs suggest the operation of regulatory mechanisms controlling the chain length of the saturated acids synthesized, and the relative amounts of saturated and unsaturated acids incorporated into the lipids in order to maintain their physical properties within narrow limits. Strain γ is a sterol-requiring mycoplasma

incapable of synthesizing saturated or unsaturated fatty acids, of desaturating or isomerizing unsaturated fatty acids or of altering the chain length of either (Rodwell, 1968, 1971). It was of interest to determine the fatty acid growth requirements of an organism totally devoid of fatty acid synthetic ability.

The fatty acid growth requirements of strain γ in a partly defined medium (medium C), containing defatted bovine serum albumin (BSA) to bind fatty acids were reported previously (Rodwell, 1967). Growth in medium C was, in some cases, limited by the availability of cholesterol. Better growth was obtained in a modified medium (medium C 2) containing in addition a pronase-treated defatted serum protein fraction with cholesterol-dispersing activity (Rodwell, 1969). While it was possible to alter and control the fatty acid composition of the membrane lipids to a large extent by altering the fatty acids supplied for growth in medium C 2, the lipids always contained fatty acids other than those added, in amounts varying from 4 to 8% of the total. With unsuitable fatty acid additions the proportions of these 'extraneous' fatty acids were even greater and, as was later realized, could have pronounced effects on growth.

The present paper reports the effects of a range of fatty acids on growth and fatty acid composition of strain γ , growing in a medium (FAP medium) with a low degree of fatty acid contamination.

METHODS

Mycoplasma strains γ , 801 and strain γ 5 of *Mycoplasma mycoides* have been described in previous publications. They were maintained by monthly subculture in BVFOS medium (Turner, Campbell & Dick, 1935). Strain γ was purified by three single-colony transfers in PPLO horse serum agar. The growth conditions and the preparation of inocula were as described previously (Rodwell, 1971). The size of the inoculum used represented a dilution of mycoplasmas by a factor of about 10,000.

Fatty acid poor medium (FAP medium). This medium had the same composition as medium C 2 (Rodwell, 1969) except that the concentrations of the purines and pyrimidines was doubled, since it was found that growth in medium C 2 was limited by uracil and guanine, and charcoal-treated BSA was replaced by BSA treated by the mild alkaline methanolysis procedure described below. The concentration of BSA was 1.6 g./l.; fatty acids were added to a total concentration of 0.1 mM (0.05 mM in each where two were added), i.e. the initial molar ratio of fatty acids to BSA was in all cases 3.7. The concentration of pronase-treated fraction C was 0.4 g./l.

Measurement of growth. Growth was measured at intervals during incubation by turbidity at 660 m μ . The maximum extinction of cultures in FAP medium with suitable fatty acid additions was 0.7 to 0.8. An extinction of 0.7 corresponded to a mycoplasmal dry weight of approximately 0.5 mg./ml., and the mycoplasmal protein of 0.3 mg./ml. These relationships were determined on mycoplasmas grown with elaidate, elaidate+palmitate, and oleate+palmitate. There were marked differences in morphology in mycoplasmas grown with these fatty acids. Despite these differences, turbidity was closely related to cell mass and protein (Table 1).

Mild alkaline methanolysis of BSA. Fatty acids were removed from BSA by mild alkaline methanolysis. Ten g. of fraction V (Pentex Incorporated, Tankakee, Illinois, U.S.A.) 'fatty acid poor', previously dried *in vacuo* over P₂O₅, was suspended in 100 ml. methanol at 0° and 100 ml. of 2 M-sodium methoxide at 0° added to the stirred suspension. Solution was rapid and complete. After 1 h. at 0° under N₂, the solution was chilled to -8° and the protein precipitated by the slow addition of 60 ml. 0.4 M-aqueous acetic acid, the temperature being

kept below 0°. The mixture was cooled to -8° and 1 l. ethanol at -8° added. The precipitate was collected by centrifugation, washed with ethanol, and ethanol + ether (1 + 1 by vol.), and finally with ether on a Büchner funnel in the cold, care being taken not to allow the cake to crack. The product, after removal of ether *in vacuo*, was fully soluble in water. Solutions (4 %, w/v) were pH 7.0 to 7.2 without adjustment and were sterilized by filtration through a Seitz filter pad which had been extracted successively with chloroform + methanol, ethanol + acetic acid, ethanol, water.

Table 1. Relationship between extinction (*E*), cell protein and dry weight in cultures of *Mycoplasma* strain Y grown with different fatty acids

Cell dry weight and protein were determined on mycoplasmas washed in 0.02 M-phosphate buffer containing 0.01 M-MgSO₄. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard.

Fatty acids* supplied	<i>E</i>	Dry weight (mg./ml. culture)	<i>E</i> /dry wt	Protein (mg./ml. culture)	<i>E</i> /protein
18:1 (9t)	0.56	0.42	1.35	0.25	2.22
18:1 (9t) + 16:0	0.74	0.54	1.38	0.33	2.25
18:1 (9c) + 16:0	0.54	0.43	1.26	0.25	2.13

* Fatty acid abbreviations in this and other tables: the number before the colon is the number of carbon atoms; the number after the colon is the number of double bonds. The position and configuration of the double bond is shown in parentheses, e.g. 18:1 (9t), *trans*-9-octadecenoic acid.

Table 2. Removal of fatty acids from bovine serum albumin (fraction V) by mild alkaline methanolysis

Material and treatment	Fatty acid analysis					
	μ moles/g.	Composition (moles %)				
		BSA	16:0	18:0	18:1	18:2
Alk. meth. washings*	3.4	19	39	19	16	8
Alk. meth. washings-BF ₃ treated†	7.7	19	42	17	15	7
C/M/HCl extract-BF ₃ treated‡	4.0	22	45	14	14	6
Alk. meth. washings of charcoal-treated BSA-BF ₃ treated§	5.2	20	42	18	14	6

* The washings after alkaline methanolysis were combined, and Me 15:0 added as an internal standard. The solvents were removed *in vacuo* and the fatty acid methyl esters extracted from the residue into hexane and analysed by g.l.c.

† As for * except that the residue after removal of solvents was treated with 10 % BF₃ in methanol for 1 h. at 64° under N₂ to methylate free fatty acids.

‡ One vol. aqueous solution fraction V (from the same batch) was extracted with 19 vol. chloroform + methanol + conc. HCl (200 + 100 + 1, by vol.). The extract was washed with 0.2 vol. water, the lower phase dried and the residue after removal of solvent treated with BF₃ in methanol at 64° for 1 h. under N₂.

§ Fraction V solution was charcoal-treated (Chen, 1967), the solution lyophilized, treated by alkaline methanolysis and analysed as in †.

Fatty acid methyl esters were analysed in the alkaline methanolysis extracts before and after treatment with BF₃ in methanol to methylate free fatty acids. Considerable amounts of fatty acids were removed by the methanolysis procedure (Table 2) even from a sample of fraction V which had been treated with charcoal (Chen, 1967). Since a total of 3.4 μmoles fatty acid methyl esters/g. BSA was found in the methanolysis extracts before BF₃ + methanol treatment a large proportion of the fatty acids in the BSA was esterified.

Sources and purification of fatty acids. Methyl *cis*-vacenate and methyl *cis*-12-octadecenoate were obtained from the Hormel Institute (Austin, Minnesota, U.S.A.), petroselinic acid from Sigma Chemical Company (St. Louis, Missouri, U.S.A.) and *trans*-vacenic acid from E. Light and Co. (Colnbrook, Buckinghamshire). The other fatty acids were obtained from Applied Science Inc. (Pennsylvania, U.S.A.). The straight-chain saturated fatty acids had a purity greater than 99 % as judged by gas-liquid chromatography (g.l.c.) of their methyl esters, with the exception of myristic acid which contained a total of 5.7 moles % of saturated acid impurities. These were removed by reversed-phase preparative thin-layer chromatography (t.l.c.) of the methyl esters on silanized silica gel G plates developed in 10 % (v/v) methanol in water (Ord & Bamford, 1967). The purified methyl myristate was recovered from the plates and saponified as described below for the monoenes. Most of the *cis*-monoenoic acids contained substantial amounts of *trans*-monoene impurities, and some of them (as well as the *trans*-monoenoic acids) contained easily detectable amounts of saturated acid impurities when the methyl esters were examined by silver ion t.l.c. (Ag-t.l.c.; Morris, 1964), and the plates were sprayed with the relatively insensitive 2',7'-dichlorofluorescein reagent and viewed under u.v. irradiation. All of the monoenoic fatty acids, with the exception of myristoleic acid in which impurities were not detected, were purified by preparative Ag-t.l.c.

The bands containing the purified methyl esters were scraped from the plates and the methyl esters eluted with chloroform + methanol (100 + 1, v/v). After removal of the solvent, the residues were dissolved in methanol + water (95 + 5, v/v) and passed through small columns of Dowex 50 (H⁺) – washed and packed in the same solvent – to remove traces of Ag⁺ and dye. The elutes were standardized by g.l.c.

The branched-chain fatty acids had purities from 65 to 90 % as received, the impurities consisting of branched- and straight-chain saturated acids. They were purified by preparative g.l.c. of their methyl esters.

For addition to growth media, the methyl esters were saponified. The methyl esters (20 μ moles) were warmed, with gently shaking under N₂, with 0.5 ml. ethanol + aqueous M-KOH (90 + 10, v/v) at 50° for 1 h. and the solutions diluted with water to a final mM concentration. The saponification conditions were critical; more severe conditions (higher temperatures or stronger alkali) caused a substantial amount of alkali isomerization of the *cis*-monoenes. Solutions of the sodium salts of free fatty acids were prepared by warming them with a two- to threefold excess of dilute alkali.

Fatty acid analysis. Fatty acid analyses were made as described previously (Rodwell, 1971) except when lipids containing caprate were to be analysed. In this case, methanolysis was done in sealed tubes under the conditions described by de Man (1967), and the methanolysis mixtures analysed by g.l.c. without concentration or further treatment.

Microscopy. Suspensions of freely floating organisms in the growth medium were examined by dark-ground light microscopy. The need to immobilize the organisms, by pressure or other possibly damaging methods, was avoided by using electronic flash illumination for photography.

For electron microscopy, cultures were centrifuged and the mycoplasmas suspended in 0.25 M-NaCl containing 0.01 M-MgSO₄ and 0.02 M-phosphate (pH 7.0) at 0°. Four volumes of the same solution containing in addition 5 % (w/v) glutaraldehyde were added, and after 2–16 h. at 0° the mycoplasmas were sedimented and suspended in water. To ensure an even and representative distribution, organisms were transferred to the electron microscope grids by a pseudoreplication technique. Drops of the fixed suspensions were applied to the surface of agar plates (2 %, w/v, Noble agar in water), and, after the suspending medium had been

absorbed, the organisms were stripped from the agar surface on a collodion film which was then transferred to the specimen grid. The specimens were examined without shadowing or staining.

RESULTS

Adaptation to growth in FAP medium with different fatty acids

In contrast to the results obtained with medium C 2 (Rodwell, 1971) there was no detectable growth of strain γ in FAP medium in the absence of added fatty acids, even after prolonged incubation, and only a trace of growth in FAP medium plus various purified *cis*-monoenoic acids (e.g. palmitoleate, oleate) which in medium C 2 gave growth followed by cellular lysis.

Table 3. *Effect of fatty acids in inoculum cultures on growth rates (doubling time in h.) of Mycoplasma strain γ in FAP medium with elaidate or trans-vaccenate plus palmitate or stearate*

Fatty acids supplied	Inoculum grown with		
	18:1 (9t)	18:1 (9t) + 16:0	18:1 (9t) + 18:0
18:1 (9t)	2.4	3.5	3.6
18:1 (9t) + 16:0	2.7	2.4	2.6
18:1 (9t) + 18:0	2.5	2.8	2.8
	18:1 (11t)	18:1 (11t) + 16:0	18:1 (11t) + 18:0
18:1 (11t)	2.3	4.0	3.3
18:1 (11t) + 16:0	2.6	2.3	2.5
18:1 (11t) + 18:0	2.5	2.4	2.5

When FAP medium was supplemented with various fatty acids which gave good growth in medium C 2, and inoculated with strain γ grown in BVFOS medium, there was slow non-exponential growth. The growth curves with elaidate as an example are shown in Fig. 1. The doubling time (t) was about 6.5 h. Growth in subculture in the same medium was normal ($t = 2.3$ h.), and the growth curve was very similar to that in medium C 2 + elaidate inoculated with BVFOS-grown organisms ($t = 2.4$ h., Fig. 1). The addition of the saponified alkaline methanolysis extract from fraction V, or of a mixture of fatty acids approximating this in composition, improved growth in FAP medium + elaidate inoculated with BVFOS-grown organisms, but had no effect on growth of organisms previously adapted to growth in FAP medium + elaidate.

Adaptation was not required for growth of BVFOS-grown organisms in FAP medium with some pairs of fatty acids. For example, with *trans*-vaccenate + palmitate or with erucate + myristate, growth of strain γ was almost the same whether the inoculum had been grown in BVFOS medium or in FAP medium plus the respective fatty acid pairs.

There did not appear to be a high degree of specificity towards particular fatty acids in the adaptation process. For example, strain γ when adapted to growth with elaidate or *trans*-vaccenate alone also grew well with oleate + palmitate and vice versa. However, when strain γ was adapted to growth with elaidate or *trans*-vaccenate + palmitate or stearate it grew poorly with the *trans*-monoenes alone (Table 3).

The phenomenon of adaptation to growth with different fatty acids was observed also with *Mycoplasma* strain 801 and with strain v5 of *Mycoplasma mycoides*; but with these strains, adaptation was irregular and occurred only occasionally. In one trial, strain v5 grown in BVFOS medium grew well after a lag period in FAP medium with erucate + myristate, and also after a longer lag period with oleate and heptadecanoate. Organisms

adapted to growth in FAP medium with oleate+heptadecanoate grew well in subculture with these fatty acids ($t = 2.3$ h.) and also with erucate+myristate ($t = 2.5$ h.), whereas organisms grown with erucate+myristate grew well with erucate+myristate ($t = 2.4$ h.) but poorly with oleate+heptadecanoate.

Fig. 1.

Fig. 2.

Fig. 1. Adaptation of *Mycoplasma* strain Y to growth in FAP medium+elaidate. ○, Medium C2 + elaidate inoculated with organisms grown in BVFOS medium; ●, FAP medium+elaidate inoculated with organisms grown in BVFOS medium; △, FAP medium+elaidate inoculated with organisms grown in same medium.

Fig. 2. Growth of *Mycoplasma* strain Y in FAP medium with pairs of straight-chain saturated fatty acids. *Mycoplasmas* for caprate series were grown with caprate+behenate; for laurate series with laurate+behenate and for myristate series with myristate+behenate. ●, Caprate+behenate; ○, laurate+behenate; ▽, laurate+arachidate; △, myristate+behenate.

Growth of strain Y with straight-chain saturated fatty acids

There was no growth of strain Y in FAP medium with any of the straight-chain saturated acids from C_{10} to C_{24} , when added one at a time. When pairs were added and the media inoculated with BVFOS-grown organisms there was variable growth with caprate+behenate, laurate+arachidate, laurate+behenate and myristate+behenate, and no growth with palmitate, stearate or lignocerate plus shorter or longer chain acids. Growth was tested in subculture with caprate, laurate or myristate plus each of the longer chain acids from C_{16} to C_{24} , the inocula for each series being grown with the respective shorter chain acid+behenate. Only the same pairs gave growth in subculture, best growth being obtained with laurate+behenate (Fig. 2).

Effect of chain length of cis-monoenoic fatty acids (oleic acid series) on growth with straight-chain saturated fatty acids

Growth of strain Y was tested in FAP medium with each monoene alone (0.1 mM) or together with each member of a series of even numbered straight-chain saturated acids (0.05 mM in each) varying in chain length from 10 to 24 carbon atoms, and in most cases including also the odd-numbered C_{15} and C_{17} acids. The same suspension was used to

inoculate each series, and was grown in FAP medium with the monoene plus the saturated acid of optimum chain length (as determined in previous tests in medium C 2). Thus the inoculum culture for the myristoleate series was grown with myristoleate+behenate; for the palmitoleate series with palmitoleate+stearate; for the oleate series with oleate+heptadecanoate and for the erucate series with erucate+myristate.

There was only a slight amount of growth, accompanied by extensive lysis, with any of these *cis*-monoenoic acids when tested alone, but good growth when oleic acid or homologues of 14 to 22 carbon atoms were paired with a straight-chain saturated acid of suitable chain length. The range of straight-chain saturated acid with which each of the *cis*-monoenes could be successfully paired, and the growth rate with each pair are summarized in Table 4. Two trends are evident: (i) the chain length of the saturated acid for optimum growth decreased with increasing chain length of the *cis*-monoene, and (ii) the range of saturated acids which gave good growth increased with increasing chain length of the *cis*-monoene. Thus with myristoleate there was good growth when paired with behenate only, while with erucate there was good growth with saturated acids of 10 to 20 carbon atoms optimum at C₁₄ to C₁₆.

There was no growth in FAP medium and a purified sample of nervonate (24:1 (15c)) when paired with any of the saturated acids. Strain Y grew in FAP medium with an impure sample of nervonate (containing small amounts of saturated and *trans*-monoene impurities) +myristate, but there was no growth when these organisms were inoculated into FAP medium with the purified acid+myristate. In experiments reported previously (Rodwell, 1967) the optimum chain length of saturated fatty acids from growth in medium C with nervonate (unpurified) was from 12 to 14 carbon atoms.

Effect of positional isomerism in cis-octadecenoic acids. Growth of strain Y was tested with the 6, 11 and 12 *cis*-octadecenoates, each being supplied alone or paired with a straight-chain saturated acid. The inocula for these series were grown in FAP medium containing the respective monoenes+palmitate. The range of saturated acids with which each could be successfully paired may be compared with the results obtained with oleate (Table 4). The position of the double bond had a marked effect. When it was near the carboxyl end of the molecule, as in petroselinate (*cis*-6-octadecenoate) there was good growth – but at a slow rate – only within the restricted range of C₁₄ to C₁₆ saturated acids (optimum C₁₅), and no growth in the absence of a saturated acid. When the double bond was further removed from the carboxyl end as in *cis*-vacenate (*cis*-11-octadecenoate) or *cis*-12-octadecenoate, the range of saturated acids giving good growth extended from C₁₀ to C₂₂ (optimum C₁₆ to C₁₈) although short- and long-chain acids decreased growth rates. With these isomers there was an increasing amount of growth in the absence of a saturated acid, *cis*-12-octadecenoate giving good growth (Fig. 3).

Effect of configurational isomerism in octadecenoic acids. Strain Y grew well in FAP medium with elaidate and *trans*-vacenate alone and when paired with a straight-chain saturated acid of 10 to 24 carbon atoms, but, as already described, the source of the inoculum had a marked effect. When the organisms for inoculum were grown with the *trans*-monoenes alone, the saturated acids of intermediate chain length (C₁₆ to C₂₀) resulted in growth after a long lag period; when they were grown with the *trans*-monoenes+palmitate best growth was obtained with elaidate+palmitate or stearate and with *trans*-vacenate+myristate or palmitate (see Table 3).

Fig. 3.

Fig. 3. Effect of chain length of straight-chain saturated fatty acids on growth of strain γ in FAP medium + *cis*-12-octadecenoate; mycoplasmas for inocula were grown with *cis*-12-octadecenoate + palmitate. ●, *cis*-12-Octadecenoate alone; △, *cis*-12-octadecenoate + palmitate; ▽, *cis*-12-octadecenoate + stearate; □, *cis*-12-octadecenoate + myristate; ▲, *cis*-12-octadecenoate + arachidate; ▼, *cis*-12-octadecenoate + laurate; ○, *cis*-12-octadecenoate + caprate.

Fig. 4.

Fig. 4. Growth of Mycoplasma strain γ in FAP medium with isopalmitate and straight-chain saturated fatty acids. Mycoplasmas for inocula were grown with isopalmitate alone. ●, Isopalmitate alone; ○, isopalmitate + laurate; △, isopalmitate + myristate; ▽, isopalmitate + stearate; ▲, isopalmitate + arachidate; ▼, isopalmitate + behenate.

Table 4. Growth rates of strain γ in FAP medium with *cis*-monoenoic or branched-chain fatty acids alone, and paired with a straight-chain saturated fatty acid

The figures in the first column are the growth rates (doubling time in h.) with the *cis*-monoenoic or branched-chain acids alone; those in the succeeding columns are the growth rates when paired with a straight-chain saturated acid.

<i>cis</i> -Monoenoic or branched-chain acid	Straight-chain saturated acid										
	None	10:0	12:0	14:0	15:0	16:0	17:0	18:0	20:0	22:0	24:0
Myristoleate	—	—	—	—	NT	(4.2)	NT	—	—	2.8	—
Palmitoleate	—	—	—	(5.5)	4.9	3.5	2.8	2.9	4.8	—	—
Oleate	—	—	4.2	3.1	3.2	3.0	2.9	3.0	4.1	—	—
Erucate	—	2.6	2.6	2.3	2.2	2.3	NT	2.5	—	—	—
Petroselinat	—	—	—	6.6	4.9	6.8	—	—	—	—	—
<i>cis</i> -Vaccenate	—	4.5	3.6	3.3	2.8	2.4	2.1	2.2	3.3	3.8	NT
<i>cis</i> -12-Octadecenoate	5.1	(6.1)	4.9	3.9	2.7	2.5	2.3	2.4	4.0	(4.4)	NT
Isopalmitate	2.4	2.5	2.7	4.4	NT	—	NT	—	2.9	2.4	2.5
Isostearate	3.3	2.5	4.7	—	NT	—	NT	—	—	L	2.8
Anteisoheptadecanoate	2.5	2.7	2.6	3.1	NT	3.5	NT	3.0	2.6	2.5	2.7

—, No growth, or a trace of growth; NT, not tested; L, good growth after long lag period — rate not determined; ( ), poor or non-exponential growth — rate approximate.

Growth with branched- and straight-chain saturated fatty acids. Strain Y grew well with isopalmitate, isostearate and anteisoheptadecanoate alone, but with isostearate the growth rate was slower (Table 4). As with the *trans*-octadecanoates, the addition of straight-chain saturated acids had marked effects of growth when the organisms for inoculum were grown with the branched-chain acids alone.

Mycoplasmas grown with each of these branched-chain acids alone were inoculated into FAP medium containing the branched-chain acids alone, or paired with a straight-chain acid of 10 to 24 carbon atoms. With isopalmitate (Fig. 4, Table 4) short, straight-chain acids (caprate, laurate) decreased the amount of growth, but had little effect on growth rate. Myristate markedly decreased the growth rate, and there was no growth with palmitate. With longer chain acids (stearate, arachidate) growth rates increased as compared with myristate, and with behenate or lignocerate the growth curves were very similar to that with isopalmitate alone. With isostearate growth was inhibited by a wider range of straight-chain acids, laurate decreased the growth rate and there was no growth with myristate,

Table 5. *Fatty acid composition of the lipids of Mycoplasma strain Y after growth in FAP medium with different fatty acids*

Fatty acids supplied		Culture extinction	Total incorporated (μ moles/100 ml. culture)	Fatty acid composition (moles %)		
(1)	(2)			(1)	(2)	Others
14:1 (5c) + 22:0		0.51	4.0	75	22	3
18:1 (9c) + 14:0		0.68	7.1	24	73	3
18:1 (9c) + 17:0		0.78	7.0	54	43	2
22:1 (13c) + 16:0		0.78	5.0	47	53	< 1
18:1 (12c)		0.54	6.9	99		1
18:1 (12c) + 17:0		0.72	9.8	49	49	2
18:1 (9t)		0.55	6.0	99		1
14 Me 15:0*		0.59	5.3	97		3
14 Me 15:0 + 22:0		0.58	6.7	36	61	3
14 Me 16:0†		0.57	4.8	96		4

* Isopalmitate.

† Anteisoheptadecanoate.

palmitate, stearate or arachidate. The addition of a very short chain normal acid (caprate) increased the growth rate. With anteisoheptadecanoate there was good growth with all of the straight-chain acids, but acids of intermediate chain length (C_{14} to C_{18}) decreased growth rates. Whether strain Y could be adapted to growth with branched-chain acids plus straight-chain saturated acids of intermediate chain length was not determined.

Fatty acid composition of lipids of mycoplasmas grown with different fatty acids

The fatty acid composition of the lipids of strain Y grown with different fatty acids was determined in the stationary phase only. The results of some of these analyses selected to illustrate the following points are listed in Table 5. (i) The lipids contained smaller amounts (a maximum of 4 moles %) of 'extraneous' fatty acids, i.e. fatty acids other than those added to the growth medium, than was found previously in the lipids of mycoplasmas grown in medium C 2. (ii) In all cases where good growth was obtained with a single fatty acid, this was virtually the only fatty acid present in the lipids. (iii) Where pairs of fatty acids were supplied, the proportions incorporated in stationary-phase mycoplasmas varied from the extreme values of 75:22 for the pair myristoleate+behenate and 24:73 for the pair oleate+myristate, to equimolar proportions, e.g. for the pair *cis*-12-octadecenoate+heptadecanoate. (iv) The relationship between culture extinction, and total fatty acid

incorporation varied quite widely. (v) In the case of the pair *cis*-12-octadecenoate + heptadecanoate the amount incorporated approached closely the amount supplied (100 μ moles/100 ml. medium). (vi) In all cases where pairs of fatty acids were supplied, substantial amounts of both became incorporated into the lipids of stationary-phase organisms, even with those acids which gave good growth alone.

Effect of fatty acid composition on morphology

Growth with different fatty acids had marked effects on morphology. Cultures were examined during exponential growth, and in the stationary phase by dark field and by electron microscopy. The morphology as seen in electron micrographs was similar to that of the live organisms viewed by dark-ground microscopy. Representative electron micrographs of strain γ after growth with selected fatty acids are illustrated (Figs. 5, 6). When grown with laurate + behenate, exponential phase cultures consisted mostly of plump particles of irregular shape and size (Fig. 5*a*); they did not differ markedly in stationary-phase cultures apart from an increase in particle size (Fig. 5*b*). Exponential phase cultures grown with oleate + palmitate (Fig. 5*c*) contained numerous beaded filaments, many of them branched, and these cultures differed from exponential phase cultures grown with elaidate (Fig. 6*a*) or elaidate + palmitate (Fig. 6*c*). Stationary-phase cultures grown with these fatty acids (Figs. 5*d*, 6*b*, *d*) contained numerous elongated forms with swellings of variable size. When unfixed preparations from these cultures were examined by dark-ground microscopy, these latter forms appeared as rigid objects with distensions, in contrast to the more flexible beaded filaments illustrated in Fig. 5*c*.

DISCUSSION

The objective of the work described in the present paper was to determine the extent to which the fatty acid composition of the membrane lipids of strain γ could be varied. Unlike the fatty acid auxotrophs of *Escherichia coli* which have been examined, or *Acholeplasma laidlawii*, strain γ is unable to compensate for differences in physical properties in the fatty acids supplied by synthesizing saturated fatty acids of varying chain length or by altering the chain length of unsaturated fatty acids. The results showed that the fatty acid composition could be varied widely, but in many cases adaptation was required for growth when mycoplasmas were transferred to media of differing fatty acid composition.

What this involved was not determined. Adaptation to growth with different fatty acids was not observed previously in medium C 2, with the exception of growth with elaidate + stearate. If selection was involved, the contaminating fatty acids in medium C 2 might have provided an assortment of fatty acids which allowed a limited amount of growth, thus providing a larger population from which to select organisms capable of growth with the new fatty acids. Other possibilities are that the newly synthesized lipids of different composition and properties are not incorporated readily within the membrane structure of the organisms inoculated until the existing lipid species have been replaced, or that some other change in membrane composition or structure must occur before the new lipid species can be inserted. It is curious that adaptation is apparently not required for growth of BVFOS-grown organisms of strain γ with some pairs of fatty acids (e.g. *trans*-vaccenate + palmitate or erucate + myristate) but is required with oleate + palmitate, although oleate and palmitate are the most abundant fatty acids in the lipids of BVFOS-grown organisms. It is possible that when strain γ is grown with pairs of fatty acids such as *trans*-vaccenate + palmitate, these are incorporated in just the right proportions so that the physical properties

Morphology of *Mycoplasma* strain γ after growth in FAP medium with different fatty acids. Electron micrographs: bar marker = $1\ \mu\text{m}$. Dark-ground photographs (inset), bar marker = $5\ \mu\text{m}$.

Fig. 5. (a) Exponential phase organisms grown with laurate+behenate. (b) Stationary phase organisms grown with laurate+behenate. (c) Exponential phase organisms grown with oleate+palmitate. (d) Stationary phase organisms grown with oleate+palmitate.

Fig. 6. (a) Exponential phase organisms grown with elaidate. (b) Stationary phase organisms grown with elaidate. (c) Exponential phase organisms grown with elaidate+palmitate. (d) Stationary phase organisms grown with elaidate+palmitate.

of the lipids approximate closely to those of BVFOS-grown organisms – hence no adaptation is needed. Yet another possibility is that adaptation involves the selection of organisms with acylating enzymes of altered specificity.

The results of altering the chain length, the position and the configuration of the double bond in monoenoic acids on the requirement for saturated fatty acids are in general agreement with the results reported previously for growth in medium C (Rodwell, 1967). The findings, such as the inverse relationship between the chain length of *cis*-monoenoic and saturated fatty acids for optimum growth, are consistent with the idea (van Deenen, 1965; Chapman, Owens & Walker, 1966) that the physical properties of the polar lipids in the membrane should lie within narrow limits, but the wide range of fatty acids which gives good growth is difficult to reconcile with this concept unless some other change in structure or composition compensates for differences in properties of the component fatty acids.

There are several ways in which compensation might be effected. In the cases where two fatty acids are supplied, the proportions of each incorporated might vary. In the present work, organisms were analysed in the stationary phase only, and the proportions may alter markedly during growth (Rodwell, 1971). However, strain γ grew with elaidate, *trans*-vacenate, *cis*-12-octadecenoate, and each of three branched-chain fatty acids, and in each case the lipids contained virtually only one fatty acid. The melting-points of the free fatty acids indicate that there may be considerable differences in physical properties, such as the endothermic phase transition temperatures in lipids containing these fatty acids. The melting-point of *cis*-12-octadecenoic acid (27 to 28°) is close to that of petroselinic acid (28 to 29°) but the requirement for saturated acids for growth with these two fatty acids is quite different. The *trans*-9- and 11-monoenoic acids have melting-points in the range 45 to 47°. It would be of interest to compare growth with the *cis*-4- and 14-octadecenoic acids, which have similar melting-points (42 and 46.5° respectively, Gunstone & Ismail, 1947). Isopalmitic and isostearic acids melt at considerably higher temperatures (62.4 and 69.5° respectively) while anteisoheptadecanoic acid melts at 36.8° (Weitkamp, 1945).

Variations in the amount of cholesterol incorporated with different fatty acids may modify the fluidity of the hydrophobic region. The proportions of the different lipid classes might vary in mycoplasmas grown with different fatty acids: the nature of the polar groups has been shown to influence the packing of the acyl chains in phospholipid monolayers (Chapman *et al.* 1966). However, no differences in the proportions of the major glycerolipid classes were detected in mycoplasmas grown with elaidate or elaidate + behenate (Plackett & Rodwell, 1970), or in *Acholeplasma laidlawii* strain B of varying fatty acid composition (McElhaney & Tourtellotte, 1970). Hydrophobic interactions between the acyl chains of the fatty acids and the hydrophobic regions of membrane proteins may also modify the hydrocarbon chain fluidity.

At all events, it is possible with these strains of *Mycoplasma* to alter the fatty acid composition of the membrane lipids within wide limits, and to control it very precisely. These alterations may be expected to have pronounced effects on membrane properties, such as the passive permeability to various solutes. Further work will be directed towards studying the effects of variations in the fatty acid composition of membrane lipids on membrane properties.

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