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Growth of Group IV Mycobacteria on Medium Containing Various Saturated and Unsaturated Fatty Acids

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Seventy-one strains of 15 species of rapidly growing mycobacteria were studied for their susceptibilities to fatty acids with 2 to 20 carbons by the agar dilution method at pH 7.0. Most mycobacteria other than potential pathogens (*Mycobacterium fortuitum* and *Mycobacterium chelonei*) were resistant to saturated fatty acids, except for lauric acid ($C_{12:0}$) (MIC, 6.25 to 25 μg/ml) and capric acid ($C_{10:0}$) (MIC, 50 to 100 μg/ml). *M. fortuitum* and *M. chelonei* were substantially insusceptible to these fatty acids. Unsaturated fatty acids with 16 to 20 carbons, except for $C_{20:5}$, were highly toxic to group IV mycobacteria other than *M. fortuitum*, *M. chelonei*, *Mycobacterium smegmatis*, and *Mycobacterium phlei*, these being highly resistant to all the unsaturated acids, except for $C_{16:1}$, $C_{18:3}$, and $C_{20:5}$. Introduction of double bonds to C_{16} to C_{20} fatty acids caused a marked increase in their activities that depended on the increase in the number of double bonds, at least up to three or four. *M. fortuitum* and *M. chelonei* were more resistant to the unsaturated fatty acids (particularly to $C_{20:3}$ and $C_{20:4}$) than the other group IV mycobacteria.

Certain types of long-chain fatty acids, such as lauric and myristic acids and most unsaturated fatty acids, are highly toxic to various gram-positive cocci (3, 4, 10, 13), gram-positive rods (7, 13, 21, 27), and gram-negative cocci (21, 36). Mycobacteria are also highly susceptible to the long-chain fatty acids, as reviewed by Kanai and Kondo (13). They tested the susceptibilities of some slowly and rapidly growing mycobacteria to various saturated (C_8 to C_{18}) and unsaturated ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$, and $C_{20:4}$) fatty acids and found that most mycobacteria were highly susceptible to lauric ($C_{12:0}$), myristic ($C_{14:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), and arachidonic ($C_{20:4}$) acids but resistant to other fatty acids tested (15, 16). The inhibitory pattern of fatty acids against mycobacteria is consistent with that of fatty acids against *Bacillus megaterium* (7) and *Neisseria gonorrhoeae* (21), suggesting a common mechanism in the killing or growth inhibition of bacteria, including that of mycobacteria by long-chain fatty acids. We now report the detailed susceptibility patterns of 71 strains in 15 species of rapidly growing mycobacteria to various fatty acids (C_2 to C_{20}).

MATERIALS AND METHODS

Organisms. Five strains each of *Mycobacterium fortuitum* (5), *Mycobacterium chelonei* subsp. *abscessus* (18), *M. chelonei* subsp. *chelonei* (18), *Mycobacterium smegmatis* (9), *Mycobacterium phlei* (9), *Mycobacterium neoaurum* (31), *Mycobacterium thermoresistibile* (29), *Mycobacterium vaccae* (2), *Mycobacterium diernhoferi* (35), and *Mycobacterium chitae*, four strains each of *Mycobacterium rhodesiae* (32), *Mycobacterium parafortuitum* (33), *Mycobacterium duvalii* (28), and *Mycobacterium flavescens* (1), three strains of *Mycobacterium aurum* (34), and two strains of *M. gilvum* (28) were studied. The sources of the strains are as follows: *M. fortuitum* ATCC 6841 (American Type Culture Collection [ATCC], Rockville, Md.), ATCC 23010 (H. C. Engbaek, Statens Seruminstitut, Copenhagen, Denmark), and ATCC 23012, ATCC 23029, and ATCC 23048 (L. F. Bojalil, Facultad de Medicina, Mexico); *M. chelonei* subsp. *abscessus* ATCC 14472 (ATCC), ATCC 23003 (L. G. Wayne, Veterans Administration Hospital, San Fernando, Calif.), 335R (E. H. Runyon, Veterans Administration Hospital, Salt Lake City, Utah), YAMAMOTO (K. Urabe, Hiroshima University, Hiroshima, Japan), and MATSUMURA (clinical isolate by H. Saito); *M. chelonei* subsp. *chelonei* ATCC 19537 (I. Tarnok, Forschungsinstitut Borstel, Borstel, Federal Republic of Germany), ATCC 19540 (R. Bönicke, Forschungsinstitut, Federal Republic of Germany), ATCC 23013 (ATCC), 860 (H. W. B. Engel, Rijks Instituut Voor de Volksgezondheid, Utrecht, The Netherlands), and 882 (J. L. Stanford, Middlesex Hospital Medical School, London, England); *M. smegmatis* ATCC 14468, ATCC 19979, ATCC 23011, ATCC 23028, and ATCC 23037 (ATCC); *M. phlei* ATCC 19249 and ATCC 23042 (ATCC), Wa-40 and Wa-289 (L. G. Wayne), and TRUDEAU (Centers for Disease Control, Atlanta, Ga.); *M. rhodesiae* 5295, 5296, 5297, and 5302 (M. Tsukamura, National Sanatorium Chubu Chest Hospital, Aichi, Japan); *M. parafortuitum* ATCC 19686, ATCC 25807, and ATCC 25809 (ATCC) and ATCC 19687 (M. Tsukamura); *M. neoaurum* ATCC 25790, ATCC 25791, ATCC 25794, ATCC 25795, and ATCC 25796 (ATCC); *M. aurum* ATCC 23366, ATCC 25792, and ATCC 25793 (ATCC); *M. duvalii* NCTC 358, NCTC 509, NCTC 514, and NCTC 8645 (W. J. Gunthorpe, The Middlesex Hospital Medical School); *M. thermoresistibile* ATCC 19527, ATCC 19529, ATCC 25813, and ATCC 25816 (ATCC) and 1029 (M. Tsukamura); *M. gilvum* 35 and 391 (W. J. Gunthorpe); *M. flavescens* ATCC 14474 (M. Hori, Osaka University, Osaka, Japan), ATCC 23009 and ATCC 23033 (ATCC), and 3362 (M. Tsukamura); *M. vaccae* ATCC 25950, ATCC 25951, ATCC 25953, and ATCC 25954 (ATCC) and 2742-6 (M. Tsukamura); *M. diernhoferi* ATCC 19340 and ATCC 25958 (ATCC) and SN1412, SN1413, and SN1414 (R. Bönicke); and *M. chitae* ATCC 19627, ATCC 19628, ATCC 19629, ATCC 25805, and ATCC 25806 (ATCC).

Fatty acids. Acetic acid ($C_{2:0}$), butyric acid ($C_{4:0}$), caprylic acid ($C_{8:0}$), capric acid ($C_{10:0}$), lauric acid ($C_{12:0}$), myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), linolenic acid ($C_{18:3}$), arachidic acid ($C_{20:0}$), and arachidonic acid ($C_{20:4}$) were purchased

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from Wako Pure Chemical, Osaka, Japan. Caproic acid ($C_{6:0}$) was obtained from P-L Biochemicals, Inc., Milwaukee, Wis., and eicosapentaenoic acid ($C_{20:5}$) was purchased from Sigma Chemical Co., St. Louis, Mo. Palmitooleic acid ($C_{16:1}$), eicosenoic acid ($C_{20:1}$), eicosadienoic acid ($C_{20:2}$), and eicosatrienoic acid ($C_{20:3}$) were obtained from Alltech Associates, Inc., Applied Science Div., Gardena, Calif.

Antimycobacterial activity of fatty acids. The MICs of fatty acids against test organisms were determined by using the agar dilution method with heart infusion agar containing 4% glycerol. The organisms grown on 1% Ogawa egg medium (22) were scraped off into a sterile test tube containing glass beads, dispersed into 1 or 2 drops of distilled water containing 1% Tween 80 by a vigorous shaking within 10 s, and suspended in ca. 5 ml of distilled water. This preparation was kept at room temperature for 5 min to precipitate bacterial clumps. The upper part of the suspension was withdrawn and adjusted to an optical density of 0.1 at 540 nm by using a Spectronic 20 colorimeter (Shimadzu, Kyoto, Japan). The resulting bacillary suspension contained ca. 10^7 organisms per ml. Fatty acids were dissolved or emulsified into 50 mM sodium bicarbonate buffer (pH 8.3) containing 5% dimethyl sulfoxide and 0.1% Tween 80 by vigorous mixing at 100°C or, if necessary, by a 1-min sonication with the Handy Sonic sonicator (model UR-20P; Tomy Seiko Co., Tokyo, Japan), serially diluted with distilled water, and then mixed with heart infusion agar supplemented with 4% glycerol. One loopful (1 mm in diameter) of the bacterial suspensions was streaked onto agar plates 2 cm in length. Growth of bacteria was observed after incubation at 37°C (32°C for *M. chelonei* subsp. *chelonei* and *M. rhodesiae*) for 7 days.

RESULTS

Antimycobacterial activity of saturated fatty acids. Table 1 shows the susceptibilities of various group IV mycobacteria to the saturated fatty acids with the carbon numbers of C_{10} to C_{14}. Lauric acid ($C_{12:0}$) inhibited the growth of *M. rhodesiae*, *M. parafortuitum*, *M. neoaurum*, *M. aurum*, *M. duvalii*, *M. thermoresistibile*, *M. gilvum*, *M. flavescens*, *M. vaccae*, *M. diernhoferi*, and *M. chitae* at concentrations of 6.25 to 25 μg/ml but was essentially nontoxic to *M. fortuitum*, *M. chelonei*, *M. smegmatis*, and *M. phlei*. Capric acid ($C_{10:0}$) showed antibacterial activity to some degree (MIC, 50 to 200 μg/ml), except with *M. chelonei* and *M. vaccae*. It should be noted that *M. fortuitum*, *M. smegmatis*, and *M. phlei* were somewhat susceptible only to capric acid (MIC, 100 μg/ml). The saturated fatty acids other than lauric and capric acids were essentially nontoxic to all the rapidly growing mycobacteria, with some exceptions (see Table 1, footnote *a*).

Antimycobacterial activity of unsaturated fatty acids. Tables 2 and 3 show the susceptibilities of rapidly growing mycobacteria to the unsaturated derivatives of C_{16}, C_{18}, and C_{20} fatty acids. Palmitooleic acid ($C_{16:1}$) was highly toxic to most of the test organisms (MIC, 3.2 to 6.25 μg/ml), except for *M. fortuitum* and *M. chelonei* (MIC, 12.5 μg/ml). Oleic acid ($C_{18:1}$) showed a similar or somewhat higher toxicity against *M. neoaurum*, *M. aurum*, *M. duvalii*, *M. thermoresistibile*, *M. gilvum*, *M. flavescens*, *M. vaccae*, *M. diernhoferi*, *M. chitae* (MIC, 1.6 to 6.25 μg/ml), and *M. parafortuitum* (MIC, 12.5 μg/ml), as compared with palmitooleic acid. Oleic acid was not toxic to *M. fortuitum*, *M. chelonei*, *M. smegmatis*, and *M. phlei* (MIC, ≥400 μg/ml). Linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids showed an antimicrobial activity similar to that seen with oleic acid against the organisms which were highly susceptible to oleic acid, whereas the activity of these C_{18} fatty acids against the oleic acid-resistant species such as *M. fortuitum*, *M. chelonei*, *M. smegmatis*, *M. phlei*, and *M. rhodesiae* increased, depending on the increase in the number of double bonds (Fig. 1; Tables 2 and 3).

Eicosenoic acid ($C_{20:1}$), a fatty acid with one double bond like palmitooleic and oleic acids, showed essentially no antibacterial activity against all the group IV mycobacteria so far tested (MIC, ≥400 μg/ml). However, antimycobacterial activity of C_{20} fatty acids against organisms other than *M. fortuitum* and *M. chelonei* increased in the case of

TABLE 1. Susceptibility of various group IV mycobacteria to capric, lauric, and myristic acids[a]

Species	No. of strains	MIC (μg/ml): At which 40% of the strains were inhibited			At which 80% of the strains were inhibited		
		$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$
M. fortuitum	5	100	>400	>400	100	>400	>400
M. chelonei subsp. *abscessus*	5	>400	>400	>400	>400	>400	>400
M. chelonei subsp. *chelonei*	5	>400	100	>400	>400	200	>400
M. smegmatis	5	100	>400	>400	100	>400	>400
M. phlei	5	100	>400	>400	100	>400	>400
M. rhodesiae	4	100[b]	6.25[b]	400[b]	100[c]	12.5[c]	400[c]
M. parafortuitum	4	100[b]	25[b]	400[b]	200[c]	400[c]	>400[c]
M. neoaurum	5	100	25	400	200	25	400
M. aurum	3	50[d]	6.25[d]	25[d]	100[e]	12.5[e]	100[e]
M. duvalii	4	100[b]	25[b]	400[b]	100[c]	25[c]	400[c]
M. thermoresistibile	5	100	25	400	100	25	>400
M. gilvum	2	50[b]	1.6[b]	100[b]	100[e]	12.5[e]	400[e]
M. flavescens	4	100[b]	12.5[b]	400[b]	100[c]	12.5[c]	400[c]
M. vaccae	5	>400	25	400	>400	25	400
M. diernhoferi	5	50	25	200	50	25	400
M. chitae	5	100	12.5	400	100	25	400

[a] The MICs of the fatty acids for each mycobacterial strain were determined by three or more separate experiments. All the mycobacterial species were highly resistant (MICs were ≥400 μg/ml) to the saturated fatty acids other than those indicated in this table, except for the following cases: $C_{16:0}$ against *M. rhodesiae* and *M. aurum*; $C_{18:0}$ against *M. rhodesiae*, *M. aurum*, and *M. gilvum*; and $C_{20:0}$ against *M. aurum* (in these cases, the MIC at which 30 or 50% of the strains were inhibited was 200 μg/ml).

[b] The MICs at which 50% of the strains were inhibited.

[c] The MICs at which 75% of the strains were inhibited.

[d] The MICs at which one of the three strains was inhibited.

[e] The MICs at which all of the test strains were inhibited.

TABLE 2. Susceptibility of various group IV mycobacteria to unsaturated fatty acids[a]

Species	No. of strains	MIC at which 40% of the strains were inhibited (μg/ml)								
		$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:1}$	$C_{20:2}$	$C_{20:3}$	$C_{20:4}$	$C_{20:5}$
M. fortuitum	5	12.5	>400	200	25	>400	50	400	400	50
M. chelonei subsp. *abscessus*	5	12.5	>400	100	25	>400	100	400	>400	50
M. chelonei subsp. *chelonei*	5	12.5	>400	100	25	>400	400	400	200	50
M. smegmatis	5	6.25	400	50	6.25	>400	50	12.5	6.25	25
M. phlei	5	3.2	400	6.25	6.25	>400	12.5	3.2	3.2	12.5
M. rhodesiae[b]	4	3.2	100	6.25	3.2	400	12.5	6.25	6.25	25
M. parafortuitum[b]	4	3.2	12.5	6.25	6.25	400	25	3.2	6.25	12.5
M. neoaurum	5	3.2	1.6	3.2	3.2	400	6.25	3.2	3.2	12.5
M. aurum[c]	3	3.2	1.6	3.2	3.2	400	3.2	1.6	3.2	12.5
M. duvalii[b]	4	3.2	3.2	3.2	3.2	400	6.25	3.2	6.25	12.5
M. thermoresistibile	5	3.2	1.6	3.2	3.2	>400	3.2	1.6	1.6	12.5
M. gilvum[b]	2	3.2	1.6	1.6	3.2	200	3.2	1.6	1.6	12.5
M. flavescens[b]	4	3.2	1.6	1.6	3.2	400	3.2	1.6	1.6	12.5
M. vaccae	5	6.25	3.2	3.2	6.25	400	12.5	3.2	6.25	12.5
M. diernhoferi	5	6.25	1.6	3.2	3.2	200	6.25	3.2	3.2	12.5
M. chitae	5	3.2	1.6	3.2	3.2	400	3.2	1.6	3.2	12.5

[a] See Table 1, footnote *a*.
[b] The MICs at which 50% of the strains were inhibited are indicated.
[c] The MICs at which one of the three strains was inhibited are indicated.

eicosadienoic ($C_{20:2}$) and eicosatrienoic ($C_{20:3}$) acids, depending on the increase in the number of double bonds. The activity of arachidonic acid ($C_{20:4}$) was nearly the same as that of eicosatrienoic acid, but the activity tended to decrease in the case of eicosapentaenoic acid ($C_{20:5}$) (Fig. 2). *M. fortuitum* and *M. chelonei* were highly resistant to the unsaturated C_{20} fatty acids, except for eicosadienoic ($C_{20:2}$) and eicosapentaenoic ($C_{20:5}$) acids.

DISCUSSION

In the present study, the detailed susceptibility patterns of rapidly growing mycobacteria against various saturated and unsaturated fatty acids were investigated. We found that organisms other than the potential pathogens (*M. fortuitum* and *M. chelonei*) were most susceptible to lauric acid ($C_{12:0}$), among the saturated fatty acids, and resistant to the short-chain (C_2 to C_8) fatty acids, as well as to fatty acids with more than 14 carbons (Table 1). These are much the same findings as noted for *Bacillus subtilis* and lipopolysaccharide layer-deficient *Salmonella typhimurium*, although these two bacteria were most susceptible to myristic acid ($C_{14:0}$), as reported by Miller et al. (21). Kondo and Kanai (15) obtained similar results on the antimycobacterial activity of saturated fatty acids, in which myristic acid was most active against slowly and rapidly growing acid-fast bacilli such as *Mycobacterium tuberculosis*, *Mycobacterium intracellulare*, and *M. fortuitum*. This discrepancy between their findings and ours may be due to the differences in the experimental methods; Miller et al. (21) determined the concentration of fatty acid causing 50% inhibition of bacterial growth by turbidity measurement in a shaking culture system by using liquid medium (containing proteose peptone, soluble starch, IsoVitaleX enrichment, and so on [pH 7.2]), and Kondo and Kanai (15) measured bactericidal activity in acetate buffer (pH 5.6). We determined the MICs of fatty acids against test organisms on heart infusion agar containing 4% glycerol (pH 7.5). The present data confirm the findings that the balance of lipophilic and hydrophilic moieties is important for the exhibition of antimicrobial activity of fatty acids against rapidly growing mycobacteria (16). However, this is not

TABLE 3. Susceptibility of various group IV mycobacteria to unsaturated fatty acids[a]

Species	No. of strains	MIC at which 80% of the strains were inhibited (μg/ml)								
		$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:1}$	$C_{20:2}$	$C_{20:3}$	$C_{20:4}$	$C_{20:5}$
M. fortuitum	5	12.5	>400	200	25	>400	100	400	400	100
M. chelonei subsp. *abscessus*	5	12.5	>400	200	25	>400	100	400	>400	50
M. chelonei subsp. *chelonei*	5	12.5	>400	200	25	>400	400	400	400	50
M. smegmatis	5	6.25	400	100	12.5	>400	50	12.5	100	50
M. phlei	5	6.25	400	6.25	6.25	>400	50	12.5	6.25	25
M. rhodesiae[b]	4	3.2	100	6.25	3.2	400	12.5	6.25	6.25	25
M. parafortuitum[b]	4	3.2	12.5	6.25	6.25	>400	50	6.25	6.25	25
M. neoaurum	5	6.25	6.25	6.25	6.25	>400	12.5	6.25	6.25	25
M. aurum[c]	3	6.25	6.25	3.2	3.2	400	6.25	1.6	6.25	12.5
M. duvalii[b]	4	3.2	3.2	3.2	3.2	400	12.5	3.2	6.25	12.5
M. thermoresistibile	5	3.2	3.2	3.2	3.2	>400	3.2	3.2	1.6	12.5
M. gilvum[c]	2	3.2	1.6	3.2	3.2	400	12.5	12.5	1.6	12.5
M. flavescens[b]	4	3.2	1.6	3.2	3.2	400	3.2	1.6	1.6	12.5
M. vaccae	5	6.25	3.2	3.2	6.25	400	12.5	6.25	6.25	25
M. diernhoferi	5	6.25	3.2	3.2	3.2	400	12.5	3.2	6.25	25
M. chitae	5	6.25	1.6	3.2	3.2	400	6.25	3.2	3.2	12.5

[a] See Table 1, footnote *a*.
[b] The MICs at which 75% of the strains were inhibited are indicated.
[c] The MICs at which all of the test strains were inhibited are indicated.

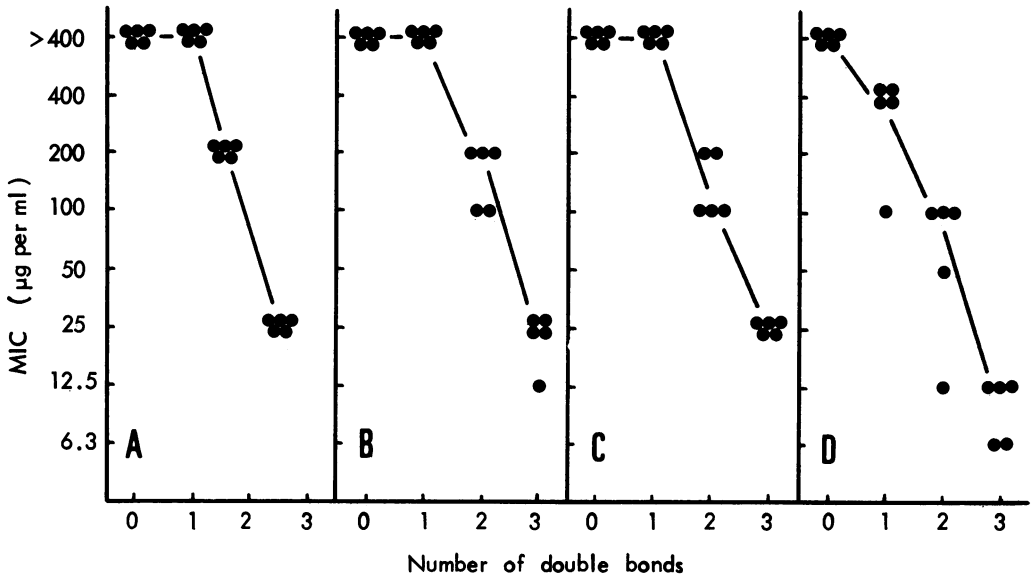

FIG. 1. Dependency upon the number of double bonds of the antibacterial activity of C_{18} fatty acids against *M. fortuitum* (A), *M. chelonei* subsp. *abscessus* (B), *M. chelonei* subsp. *chelonei* (C), and *M. smegmatis* (D). The MICs of each fatty acid against five strains per species are plotted.

always essential, as *N. gonorrhoeae* is highly susceptible to the methyl ester of palmitic acid (21), and treating *Escherichia coli* with NaN_3 results in an uncoupling of its oxidative phosphorylation and makes the methyl ester of lauric acid lethal (6).

With some exceptions, unsaturated fatty acids exhibited a potent antimycobacterial activity against rapidly growing mycobacteria (Tables 2 and 3). It is noteworthy that the antimycobacterial activity of C_{18} and C_{20} fatty acids was potentiated by the introduction of double bonds, depending

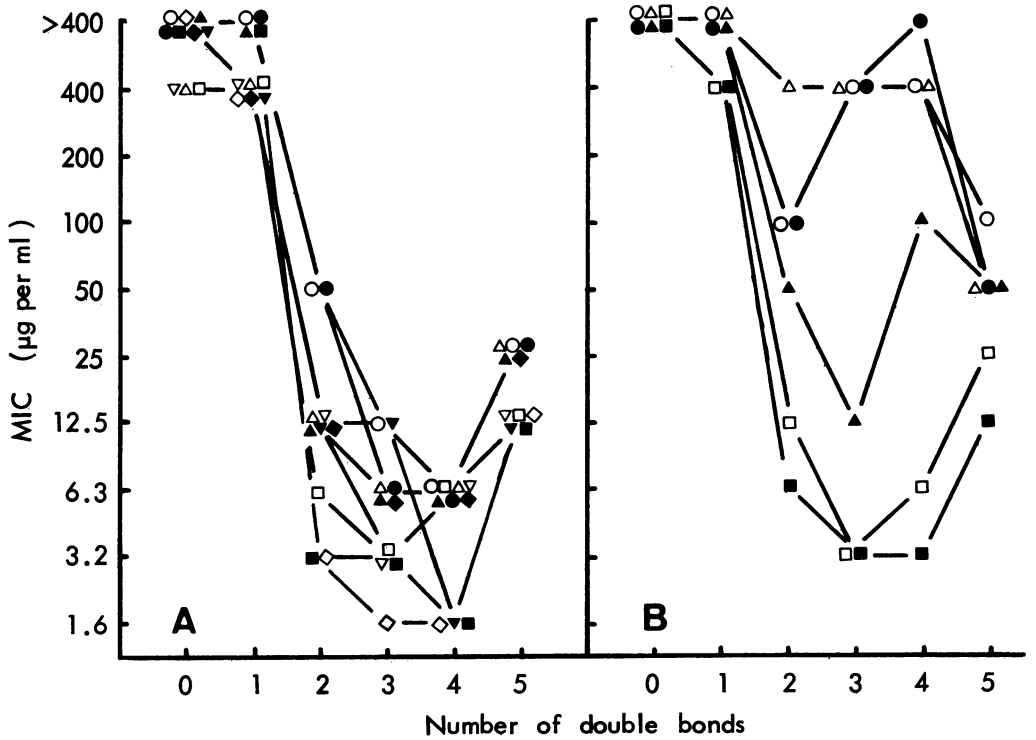

FIG. 2. Dependency upon the number of double bonds of the antibacterial activity of C_{20} fatty acids against group IV mycobacteria. (A) Scotochromogenic and photochromogenic mycobacteria. Symbols: ○, *M. phlei*; △, *M. rhodesiae*; ●, *M. parafortuitum*; ▲, *M. neoaurum*; □, *M.aurum*; ▽, *M. duvalii*; ■, *M. thermoresistibile*; ▼, *M. gilvum*; ◇, *M. flavescens*; ◆, *M. vaccae*. (B) Nonphotochromogenic mycobacteria. Symbols: ○, *M. fortuitum*; ●, *M. chelonei* subsp. *abscessus*; △, *M. chelonei* subsp. *chelonei*; ▲, *M. smegmatis*; □, *M. diernhoferi*; ■, *M. chitae*. Each plot represents the MICs at which 75 or 80% of the strains were inhibited, except for *M. aurum* and *M. gilvum*, for which the MIC at which 100% of the strains were inhibited was indicated.

on their number. The order of the activity was $C_{18:0} < C_{18:1} \leq C_{18:2} \leq C_{18:3}$ and $C_{20:0} \leq C_{20:1} < C_{20:2} < C_{20:3} \simeq C_{20:4} > C_{20:5}$. These findings are consistent with the observations of other investigators regarding the inhibitory actions of fatty acids against *Staphylococcus aureus* (3), *Streptococcus faecalis* (4), *B. megaterium* (7), *M. tuberculosis* (16), and *Mycobacterium bovis* (16). The present results indicate the importance of solubility, limiting area, and the ability to behave as a liquid in a solution of a given fatty acid, as reported by other investigators (7, 13, 21). It has been postulated that fatty acids exhibit their antibacterial action by inserting their nonpolar moieties into the phospholipid layer of bacterial cell membrane (13, 16) and that this causes a change in membrane permeability (13), alteration of the functions of some membrane-bound proteins (15, 27), and uncoupling of the oxidative phosphorylation system (13, 27). In the case of rapidly growing mycobacteria, the limiting area of a given fatty acid does not seem to be so decisive as proposed by Galbraith et al. (7), because the antimycobacterial activity of eicosapentaenoic acid ($C_{20:5}$) was lower than the activities of $C_{20:3}$ and $C_{20:4}$ fatty acids (Fig. 2).

Potential pathogens among group IV mycobacteria (*M. fortuitum* and *M. chelonei*) were highly resistant to all the fatty acids, except for palmitooleic and linolenic acids, which showed moderate antimycobacterial action. Long-chain fatty acids such as palmitooleic, oleic, and linoleic acids, which are major fatty acids with antimicrobial activity in the lysosomal lipid fraction, are important for the mycobactericidal activity of macrophages (11, 13, 14, 17). Activated macrophages secrete a large amount of palmitooleic, oleic, and linoleic acids (11), and phospholipids in phagocytic cell membrane can be cleaved by exogenous or endogenous phospholipase A (14, 17), generating antimycobacterial fatty acids than were other, nonpathogenic mycobacteria, are pholipase A is enhanced by interaction of the organisms with the lysosomal membrane derived from phagocytic cells (17). The present results, which show that *M. fortuitum* and *M. chelonei*, potential pathogens among group IV mycobacteria, were much more resistant to C_{18} and C_{20} unsaturated fatty acids than to other nonpathogenic mycobacteria, are consistent with these observations. Although unsaturated C_{18} fatty acids are important for the antimycobacterial action of phagocytic cells (14), the role of highly unsaturated C_{20} fatty acids cannot be excluded for the following reasons: *M. smegmatis*, a representative nonpathogenic mycobacterium, is more susceptible to $C_{20:3}$ and $C_{20:4}$ fatty acids than are *M. fortuitum* and *M. chelonei*, whereas *M. smegmatis* has a resistance to $C_{18:1}$ and $C_{18:2}$ fatty acids similar to that of *M. fortuitum* and *M. chelonei*. This may indicate a certain role for highly unsaturated C_{20} fatty acids in the killing mechanisms for rapidly growing mycobacteria other than *M. fortuitum* and *M. chelonei* by phagocytes. Our findings that *M. fortuitum* and *M. chelonei* were highly resistant to most of the fatty acids and our previous observations (25) that *M. chelonei* was effectively killed by activated macrophages indicate that fatty acids play only a minor role in the killing of these two mycobacteria by activated macrophages and that the antimycobacterial ability of the phagocytic cells can be attributed to their function in the generation of active oxygen (12, 20, 25) and to bactericidal proteins (19, 23, 24). Here, we examined the growth-inhibitory activity of fatty acids at pH 7.5, because phagosomal pH in phagocytic cells is known to increase temporarily to 7.5 and this is associated with enhanced production of active oxygen (8, 26). Studies on the synergism between fatty acids and active oxygen in in vitro mycobactericidal system are under way.

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LITERATURE CITED

1. **Bojalil, L. F., J. Cerbón, and A. Trujillo.** 1962. Adansonian classification of mycobacteria. J. Gen. Microbiol. **28**:333–346.
2. **Bönicke, R., and S. E. Juhasz.** 1964. Beschreibung der neuen Species *Mycobacterium vaccae* n. sp. Zentralbl. Bakteriol. Paraseitenkd. Infektionskr. Hyg. Abt. I Orig. **192**:133–135.
3. **Butcher, G. W., G. King, and K. G. H. Dyke.** 1976. Sensitivity of *Staphylococcus aureus* to unsaturated fatty acids. J. Gen. Microbiol. **94**:290–296.
4. **Carson, D. D., and L. Daneo-Moore.** 1980. Effects of fatty acids on lysis of *Streptococcus faecalis*. J. Bacteriol. **141**:1122–1126.
5. **da Costa Cruz, J.** 1938. *Mycobacterium fortuitum* um novo bacilo acido-resistente patogenico para o homen. Acta Med. (Rio de Janeiro) **1**:298–301.
6. **Fay, J. P., and R. N. Farias.** 1977. Inhibitory action of a non-metabolizable fatty acid on the growth of *Escherichia coli*: role of metabolism and outer membrane integrity. J. Bacteriol. **132**:790–795.
7. **Galbraith, H., T. B. Miller, A. M. Paton, and J. K. Thompson.** 1971. Antibacterial activity of long chain fatty acids and reversal with calcium, magnesium, ergocalciferol and cholesterol. J. Appl. Bacteriol. **34**:803–813.
8. **Geisow, M. J., P. D. Hart, and M. R. Young.** 1981. Temporal changes of lysosomal and phagosomal pH during phagolysosome formation in macrophages: studies by fluoresence spectroscopy. J. Cell. Biol. **89**:645–652.
9. **Gordon, R. E., and M. M. Smith.** 1953. Rapidly growing, acid fast bacteria. I. Species descriptions of *Mycobacterium phlei* and *Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann. J. Bacteriol. **66**:41–48.
10. **Greenway, D. L. A., and K. G. H. Dyke.** 1979. Mechanism of the inhibitory action of linoleic acid on the growth of *Staphylococcus aureus*. J. Gen. Microbiol. **115**:233–245.
11. **Hemsworth, G. R., and I. Kochan.** 1978. Secretion of antimycobacterial fatty acids by normal and activated macrophages. Infect. Immun. **19**:170–177.
12. **Johnston, R. B., Jr.** 1978. Oxygen metabolism and the microbicidal activity of macrophages. Fed. Proc. **37**:2759–2764.
13. **Kanai, K., and E. Kondo.** 1979. Antibacterial and cytotoxic aspects of long-chain fatty acids as cell surface events: selected topics. Jpn. J. Med. Sci. Biol. **32**:135–174.
14. **Kanai, K., and E. Kondo.** 1980. Phospholipase A_2-induced antimycobacterial activity in the membrane fraction obtained from peritoneal exudate cells of guinea pigs. Jpn. J. Med. Sci. Biol. **33**:87–101.
15. **Kondo, E., and K. Kanai.** 1971. The lethal effect of long-chain fatty acids on mycobacteria. Jpn. J. Med. Sci. Biol. **25**:1–13.
16. **Kondo, E., and K. Kanai.** 1977. The relationship between the chemical structure of fatty acids and their mycobactericidal activity. Jpn. J. Med. Sci. Biol. **30**:171–178.
17. **Kondo, E., and K. Kanai.** 1982. Lipolytic enzyme activity of phagocytic cells as affected by the presence of tubercle bacilli. Kekkaku **57**:1–7.
18. **Kubica, G. P., I. Baess, R. E. Gordon, P. A. Jenkins, J. B. G. Kwapinski, C. McDurmont, S. R. Pattyn, H. Saito, V. Silcox, J. L. Stanford, K. Takeya, and M. Tsukamura.** 1972. A cooperative numerical analysis of rapidly growing mycobacteria. J. Gen. Microbiol. **73**:55–70.
19. **Lehrer, R. I., M. E. Selsted, D. Szklarek, and J. Fleischmann.** 1983. Antibacterial activity of microbicidal cationic proteins 1 and 2, natural peptide antibiotics of rabbit lung macrophages. Infect. Immun. **42**:10–14.
20. **Lowrie, D. B.** 1983. How macrophages kill tubercle bacilli. J. Med. Microbiol. **16**:1–12.
21. **Miller, R. D., K. E. Brown, and S. A. Morse.** 1977. Inhibitory action of fatty acids on the growth of *Neisseria gonorrhoeae*. Infect. Immun. **17**:303–312.
22. **Ogawa, T., and K. Saba.** 1949. The quantitative culture method for tubercle bacilli: on the case of cultivation of bacterial

suspension. Kekkaku **24**:13–18.

23. **Patterson-Delafield, J., R. J. Martinez, and R. I. Lehrer.** 1980. Microbicidal cationic proteins in rabbit alveolar macrophages: a potential host defense mechanism. Infect. Immun. **30**:180–192.
24. **Pruul, H., B. L. Wetherall, and P. J. MacDonald.** 1983. Bactericidal activity of a granule extract from human polymorphonuclear leukocytes against *Bacteroides* species. Infect. Immun. **41**:1373–1375.
25. **Saito, H., H. Tomioka, and T. Watanabe.** 1981. H_2O_2-releasing function of macrophages activated with various mycobacteria based on wheat germ agglutinin and phorbol myristate acetate triggering. J. Reticuloendothel. Soc. **29**:193–204.
26. **Segal, A. W., M. Geisow, R. Garcia, A. Harper, and R. Miller.** 1981. The respiratory burst of phagocytic cells is associated with a rise in vacuolar pH. Nature (London) **290**:406–409.
27. **Shew, C. W., and E. Freese.** 1972. Effects of fatty acids on growth and envelope proteins of *Bacillus subtilis*. J. Bacteriol. **111**:516–524.
28. **Stanford, J. L., and W. J. Gunthorpe.** 1971. A study of some fast-growing scotochromogenic mycobacteria including species descriptions of *Mycobacterium gilvum* (new species) and *Mycobacterium duvalii* (new species). Br. J. Exp. Pathol. **52**:627–637.
29. **Tsukamura, M.** 1966. *Mycobacterium thermoresistibile*: a new species (preliminary report). Med. Biol. (Tokyo) **72**:187–190.
30. **Tsukamura, M.** 1967. *Mycobacterium chitae*: a new species. Jpn. J. Microbiol. **11**:43–47.
31. **Tsukamura, M.** 1972. A new species of rapidly growing, scotochromogenic mycobacteria, *Mycobacterium neoaurum* Tsukamura n. sp. Med. Biol. (Tokyo) **85**:229–233.
32. **Tsukamura, M., S. Mizuno, N. F. F. Gane, A. Mills, and L. King.** 1971. *Mycobacterium rhodesiae* sp. nov. A new species of rapid-growing scotochromogenic mycobacteria. Jpn. J. Microbiol. **15**:407–416.
33. **Tsukamura, M., H. Toyama, and S. Mizuno.** 1965. *Mycobacterium parafortuitum*, a new species. Med. Biol. (Tokyo) **70**:232–235.
34. **Tsukamura, M., and S. Tsukamura.** 1966. *Mycobacterium aurum*, a new species. Med. Biol. (Tokyo) **72**:270–273.
35. **Tsukamura, M., H. J. van der Meulen, and W. O. K. Grabow.** 1983. Numerical taxonomy of rapidly growing, scotochromogenic mycobacteria of the *Mycobacterium parafortuitum* complex: *Mycobacterium austroafricanum* sp. nov. and *Mycobacterium diernhoferi* sp. nov., nom. rev. Int. J. Syst. Bacteriol. **33**:460–469.
36. **Walstad, D. L., R. C. Reitz, and P. F. Sparling.** 1974. Growth inhibition among strains of *Neisseria gonorrhoeae* due to production of inhibitory free fatty acids and lysophosphatidylethanolamine: absence of bacteriocin. Infect. Immun. **10**:481–488.