Comparison by Gas-Liquid Chromatography of the Fatty Acids of *Mycobacterium avium* and Some Other Nonphotochromogenic Mycobacteria

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Fifty-eight strains of nonphotochromogenic mycobacteria representing nine different serotypes were studied, including 38 strains of *Mycobacterium avium* and 20 strains of Battey bacilli. The lipids were extracted from whole cells, saponified with potassium hydroxide, and esterified with diazomethane. The fatty-acid profiles determined by gas-liquid chromatography included saturated fatty acids ranging from C₁₀ to C₂₀, plus some unsaturated analogues and a branched-chain acid. No consistent differences in the fatty-acid profiles were observed between strains of *M. avium* and Battey bacilli. Quantitative differences in the means (significant at *P* < 0.01) were observed in the relative amounts of 14:0, 16:1, 18:0, 18:1, and a branched-chain acid among strains of the same serotype. We were unable to differentiate the fatty-acid profiles of recently isolated strains or those maintained in culture for more than 2 years.

The difficulty of identifying certain slowly growing nonphotochromogenic mycobacteria (group III) has led to designating them as a group by such terms as “Battey-avium complex” (9), “avian-Battey group” (12), “avian-like strains” (3), and “mycobacteria of the avian group” (14). These microorganisms have been discussed together as *Mycobacterium intracellulare-M. avium*, which are “not usually distinguished from one another” (8). For convenience, we use the term “Battey-avium group” to include the Battey bacilli and *M. avium* of all known serotypes.

Our interest in the fatty acids of the Battey-avian group was prompted by a study with gas-liquid chromatography of the lipids of *M. kansasii*, in which it was found that all of 35 strains had an assortment of fatty acids different from those of *M. avium, M. marinum, M. tuberculosis*, and certain skotochromogens (15). Also, other workers had reported that the fatty-acid profiles of five strains each of *M. avium* and Battey bacilli are similar (4, 6). The purpose of this study was to reveal any similarities or differences between *M. avium* and Battey bacilli isolated from man and animals based on analysis of cellular fatty acids.

**MATERIALS AND METHODS**

**Microorganisms.** The 38 strains were identified serologically by the method of Schaefer (10), who kindly furnished the antisera. The serotypes and the sources are listed in Table 1.

One loopful of a 10-day-old culture on Lowenstein-Jensen medium was transferred to 300 ml of Proskaer-Beck liquid medium (Difco) in each of two 1,000-ml Erlenmeyer flasks. Duplicate or triplicate cultures were made for each organism to insure reliability of the analysis. All cultures were incubated at 37 °C for 28 days in air containing 5.0% carbon dioxide; with each batch of specimens, a flask of uninoculated medium was included for control tests. At the end of 28 days, subcultures on Lowenstein-Jensen medium were made from each flask; smears were made from growth in each flask and stained for acid-fast bacilli and with Gram stain to detect contamination.

**Preparation of bacterial fatty acids for gas-liquid chromatography.** The flasks of culture and the uninoculated media were autoclaved at a pressure of 15 lb for 5 min (121 °C) and cooled to room temperature. Preliminary studies revealed that autoclaving had no effect on the fatty acid profiles of mycobacteria. Also, no fatty acids were detected in the uninoculated media. The cells were harvested by centrifugation in 50-ml tubes at 3,000 rev/min for 20 min at a radius of 20.0 cm.

The packed cells of each specimen (amounts varied from 5 to 15 ml) were suspended in a mixture containing 3.0 ml of 33.0% potassium hydroxide plus 15.0 ml of methyl alcohol. The mixture was heated for 1 hr on a steam table and cooled at room temperature. The nonsaponifiable material was extracted with hexane and discarded. After the residue was acidified with 15% sulfuric acid to pH 2.0, the fatty acids were extracted with distilled ligroin (boiling point, 60 to 80 °C) and esterified with diazomethane (13).

**Gas-liquid chromatography.** The methyl esters of
The bacterial fatty acids were analyzed by gas chromatography; a hydrogen flame ionization detector (F-M model 500-1609) was used. The specimens were injected directly onto the chromatographic column. The columns were 6 ft (1.83 m) long and were packed with diethylene glycol succinate (15%) on siliconized Chromosorb G-AW (60 to 80 mesh) and with methyl silicone (SE-30) on Chromosorb W (60 to 80 mesh). The columns were operated isothermally at temperatures of 200 C and 250 C, respectively, with a detector temperature of 275 C. Fatty acids in the bacterial specimens were identified by comparison with retention times of known standards containing saturated and unsaturated fatty acids (Fig. 1 and Table 2). The identification of unknown cellular fatty acids for which no standards were available was based on plots of the log of retention time versus the number of carbon atoms in each acid (Fig. 1).

RESULTS

A chromatogram of the fatty-acid methyl esters present in a representative strain of Battey bacillus serotype Boone is shown in Fig. 2. This chromatogram was prepared with a silicone column. The fatty-acid profile of this strain of serotype Boone and the profiles of the other 57 strains of Battey-avian mycobacteria were similar and included the

### Table 1. Serotype and source of cultures

| Serotype | Source and no. of cultures |
|----------|---------------------------|
|          | Human | Chicken | Swine |
| M. avium I | 6     | 2      | 4     |
| M. avium II | 3    | 5      | 18    |
| Arnold    | 2     | 1      | 0     |
| Boone     | 2     | 0      | 0     |
| Wilson    | 3     | 0      | 0     |
| Yandle    | 3     | 0      | 0     |
| Davis     | 1     | 0      | 1     |
| Watson    | 1     | 0      | 2     |
| Howell    | 2     | 2      | 0     |

### Table 2. Kinds and relative amounts of fatty acids in Battey bacillus serotype Boone

| Carbon no. and no. of C-C double bonds | Retention time (min) of fatty acids | Fatty acid of serotype Boone (%) |
|----------------------------------------|--------------------------------------|----------------------------------|
|                                        | In DEGS<sup>a</sup> | In SE-30<sup>b</sup> | Mean<sup>c</sup> | SD<sup>d</sup> |
| 8:0                                    | 1.7                        | 0.8                        | T                |               |
| 10:0                                   | 2.9                        | 2.2                        | T                |               |
| 11:0                                   | 3.7                        | 3.0                        | T                |               |
| 12:0                                   | 5.0                        | 3.8                        | T                |               |
| 14:0                                   | 8.4                        | 6.7                        | 5.3              | 0.4           |
| 15:0                                   | 10.7                       | 8.8                        | T                |               |
| 16:0                                   | 14.5                       | 11.0                       | 35.1             | 2.8           |
| 16:1                                   | 17.2                       |                            | 2.0              | 0.1           |
| 17:0                                   | 19.1                       | 14.8                       | 2.7              | 0.2           |
| 18:0                                   | 24.8                       | 19.4                       | 9.0              | 1.2           |
| 18:1                                   | 28.8                       | 18.5                       | 7.4              | 0.6           |
| 19:0                                   | 32.4                       | 25.2                       | 8.4              | 0.9           |
| 19:1                                   | 21.6                       |                            | 19.1             | 1.8           |
| 20:0                                   | 42.4                       | 32.7                       | 2.1              | 0.1           |
| 20:1                                   | 49.6                       | 31.0                       | T                |               |
| 21:0                                   | 56.0                       | 42.4                       | T                |               |
| 22:0                                   | 72.0                       | 54.4                       | 1.9              | 0.1           |
| 22:1                                   | 84.4                       | 51.4                       | 1.6              | 0.2           |
| 23:0                                   | 96.4                       | 70.2                       | T                |               |
| 24:0                                   | 126.0                      | 90.0                       | 2.4              | 0.3           |
| 24:1                                   | 144.4                      | 84.6                       | 1.4              | 0.1           |

<sup>a</sup> Diethylene glycol succinate coated on siliconized Chromosorb G-AW.

<sup>b</sup> Methyl silicone rubber gum coated on Chromosorb W.

<sup>c</sup> Mean value of separate analyses on three specimens. T = trace.

<sup>d</sup> Standard deviation.

<sup>*</sup> B denotes a branched carbon skeleton.

![Fig. 1. Graph of log of retention time of fatty acids plotted against the carbon number of saturated and monoenoic fatty acids for both polyester and silicone columns.](image1)

![Fig. 2. Gas-liquid chromatographic profile on silicone column of methyl esters of fatty acids extracted from cells of a representative strain of Battey bacillus serotype Boone.](image2)
same fatty-acid components. The relative amounts of fatty acid (mean value for triplicate analyses) of serotype Boone shown in Fig. 2 are given in Table 2 with corresponding standard deviations.

The values represent relative mass units of fatty acids per sample. These units were determined by integration of strip-chart tracings obtained from chromatographic analysis of the fatty acids from bacterial cells. Relative mass units represent the amount of a specific fatty acid divided by the amount of the fatty acid (16:0) present in the mixture. Statistical analysis of the data, employing analysis of variance, shows that differences significant at the 0.01 level existed in the amount of the acid 14:0 (mean value) among the nine serotypes (Table 3). In addition, differences significant at the 0.01 level were found in various strains within the same serotype. These differences between isolates of the same serotype were larger than the differences due to laboratory error (Table 3).

No differences significant at the 0.01 level were detected among the nine different serotypes in the amount of the fatty acid 16:1 (Table 4). However, differences were present at the 0.01 level for different strains within serotypes (Table 4).

Statistical analyses were made at the 0.01 level to determine differences among relative amounts of 18:0 (Table 5), 18:1 (Table 6), and 19B (Table 7). The evidence is not sufficient to conclude that the means of the nine serogroups are not equal with regard to the relative amounts of 18:0, 18:1, and 19B fatty acids. However, the evidence is sufficient to conclude that the relative amounts of each of these three acids are not equal for strains within serotypes.

**DISCUSSION**

The cultural and biochemical similarities between *M. avium* and other members of the Battey-avian group have been noted by many workers (2, 3, 5, 7, 9, 16). Definite serological
differences exist between microorganisms of this group (11, 12); however, the serological differences between *M. avium* and Battey bacilli do not relate necessarily to pathogenicity for chickens (1, 11, 17).

Our study included recently isolated strains and some strains which had been cultured on artificial media for at least 2 years. No consistent differences were detectable in the kinds of fatty acids extracted from the cells. Strains that were pathogenic for chickens, Japanese quail, and rabbits had fatty-acid profiles similar to those of the nonpathogenic strains.

Our analyses of cellular fatty acids suggest that *M. avium* and Battey bacilli are closely related organisms. Chromatograms of one strain of *M. avium* serotype II which was found to be pathogenic for quail and rabbits and one strain of serotype Watson which was not pathogenic for quail and rabbits were identical; both of these two serotypes were isolated from swine.

Differences in fatty-acid synthesis may be more sensitive indicators of gene heterology than differences in antigenic properties of cells. Further characterization of chemical profiles of cells could provide a firm basis for resolving the taxonomic relationship of these bacteria.

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LITERATURE CITED

1. Anz, W., D. Lauterbach, G. Meissner, and I. Willers. 1970. Vergleich von Sensitin-Testen an Meerschweinchen mit Serotyp und Hühnervirulenz bei M. avium- und M. Intra-cellulare-Stämmen. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. I Orig. 215:536–549.

2. Bojalil, L. F., and J. Cebóns. 1960. A comparative study of nonphotochromogenic mycobacteria and Mycobacterium avium. Amer. Rev. Resp. Dis. 81:382–386.

3. Bönicker, R. 1967. Die Differenzierung „atypischer“ Mycobakterierien. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. I Orig. 205:260–268.

4. Cattaneo, C., G. C. De Ritis, M. Lucchesi, P. Rossi, and S. Ferrari. 1965. Analisi gas chromatografica degli acidi grassi (C11-C20) presenti nei microbatteri. Ann. Ist. “Carlo Forlanini” 25:349–388.

5. Köppler, W. 1968. Zur Taxonomie der Gattung Mycobacterium. II. Klassifizierung langsam wachsender Mycobakterien. Z. Tuberk. Erkrankungen Thoraxorgane 129:321–328.

6. Lucchesi, M., C. Cattaneo, and G. C. De Ritis. 1967. The chromatographic separation of fatty acids (C11-C20) from mycobacteria. Bull. Int. Union Tuberc. 39:65–70.

7. Pattyn, S. R. 1967. A study of group III non chromogenic mycobacteria: correlation of chicken virulence with other in vitro characters among 20 strains. Z. Tuberk. Erkrankungen Thoraxorgane 127:41–46.

8. Runyon, E. H. 1967. Mycobacterium intracellulare. Amer. Rev. Resp. Dis. 95:861–865.

9. Runyon, E. H., G. P. Kubica, W. C. Morse, C. R. Smith, and L. G. Wayne. 1970. Mycobacterium, p. 112–136. In J. E. Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.

10. Schaefer, W. B. 1965. Serologic identification and classification of the atypical mycobacteria by their agglutination. Amer. Rev. Resp. Dis. 92 (Suppl): 85–93.

11. Schaefer, W. B. 1968. Incidence of the serotypes of *Mycobacterium avium* and atypical mycobacteria in human and animal diseases. Amer. Rev. Resp. Dis. 97:18–23.

12. Schaefer, W. B., K. J. Birn, P. A. Jenkins, and J. Marks. 1969. Infection with the avian-Battey group of mycobacteria in England and Wales. Brit. Med. J. 2:412–415.

13. Schlenk, H., and J. L. Gellerman. 1960. Esterification of fatty acids with diazomethane on a small scale. Anal. Chem. 32:1412–1417.

14. Tacquet, A., B. Devulder, F. Tison, and B. Polspoel. 1966. Le rôle pathogène pour l’homme des mycobactéries du groupe avium. Rev. Pathol. Comp. 64:495–475.

15. Thoen, C. O., A. G. Karlson, and R. D. Ellefson. 1971. Fatty acids on *Mycobacterium kansasi*. Appl. Microbiol. 21:628–632.

16. Wayne, L. G. 1966. Classification and identification of mycobacteria. III. Species within group III. Amer. Rev. Resp. Dis. 92:919–928.

17. Yoder, W. D., and W. B. Schaefer. 1971. Comparison of the seroagglutination test with the pathogenicity test in the chicken for the identification of Mycobacterium avium and Mycobacterium intracellulare. Amer. Rev. Resp. Dis. 103:173–178.