*Br. J. exp. Path.* (1971) **52**, 627.

# A STUDY OF SOME FAST-GROWING SCOTOCHROMOGENIC MYCOBACTERIA INCLUDING SPECIES DESCRIPTIONS OF *MYCOBACTERIUM GILVUM* (NEW SPECIES) AND *MYCOBACTERIUM DUVALII* (NEW SPECIES)

J. L. STANFORD AND W. J. GUNTHORPE

*From The Bland-Sutton Institute, Middlesex Hospital Medical School, London*

Received for publication April 26, 1971

**SUMMARY.—Twenty strains of fast-growing scotochromogenic mycobacteria with similar saccharolytic activities, including type strains of *Mycobacterium acapulcense* and *Mycobacterium flavescens* were compared in cultural, biochemical and immunodiffusion tests with established strains of 15 other mycobacterial species. The 20 strains could be divided into 3 separate groups. One of these included the type strains of *M. acapulcense* and *M. flavescens* which are considered synonymous and for which the latter name is recommended. The other 2 groups differed from all named species with which they were compared, and are considered as new species for which the names *Mycobacterium gilvum* and *Mycobacterium duvalii* are proposed. Species descriptions are given for the 2 new species and for *M. flavescens*.**

THE scotochromogenic mycobacteria are a large group of commonly encountered organisms which have proved particularly difficult to classify. The named slow-growing species are *Mycobacterium xenopi* (originally *xenopei*) Schwabacher and *Mycobacterium marianum* Penso (*scrofulaceum* Prissick and Masson). The fast-growing species include *Mycobacterium phlei* Lehmann and Neumann, *M. acapulcense* (originally *acapulcensis*) Bojalil, Cerbon and Trujillo, *M. flavescens* Bojalil *et al.*, *Mycobacterium gordonae* Bojalil *et al.*, and *Mycobacterium aurum* Tsukamura. The name *Mycobacterium aquae* Galli-Valerio is ill-defined and probably includes several species. Scotochromogenic strains of the usually non-pigmented *Mycobacterium avium* and of the photochromogen *Mycobacterium kansasii* occur, and at least 1 of the scotochromogenic species, *M. xenopi*, may have non-pigmented variants.

The study described here is limited to a defined group of fast-growing scotochromogens.

## MATERIALS AND METHODS

### *Selection of strains*

Sixty strains of scotochromogenic mycobacteria, including type strains or other well-established strains of each of the named species, were tested for their rate of growth on Löwenstein–Jensen medium at 25°. Thirty-two of the strains produced good growth after 7 days incubation and these were selected for examination of their action on sugars according to the method of Gordon and Smith (1953). It was found that they fell into 3 groups dependent on the number of sugars that they fermented. The group with moderate saccharolytic activity, consisting of 20 strains, was selected for special study, and will be referred to henceforth as the " study group " of organisms.

In the tests to be described these organisms were compared with representative strains of each of the other named species of fast- and slow-growing scotochromogens with strains of *M. avium*, *M. kansasii*, *Mycobacterium marinum* and with strains of 6 of the fast-growing usually non-pigmented species.

*The strains*

1. The " study group ":
   1. 35—isolated from sputum (Dr. A. Beck)
   2. 40—N.C.T.C. 10270 *M. acapulcense*
   3. 51—N.C.T.C. 8645 Jose I
   4. 70—N.C.T.C. 358 Duval's lepra bacillus
   5. 74—N.C.T.C. 10271 *M. flavescens*
   6. 132—isolated from pleural fluid (Dr. A. Beck)
   7. 204—*M. aquae* type 3 A2 from Professor Tacquet
   8. 205—*M. aquae* type 3 B from Professor Tacquet
   9. 213—isolated from sputum (Dr. A. Beck)
   10. 301—isolated from urine (Dr. A. Beck)
   11. 332—isolated from gastric washings (Dr. N. Simmons)
   12. 391—isolated from pleural fluid (Dr. A. Beck)
   13. 392—isolated from sputum (Dr. A. Beck)
   14. 452—ATCC 14474 *M. flavescens*
   15. 570— } isolated as contaminants
   16. 571— } from other cultures
   17. 572— }
   18. 578—N.C.T.C. 521 " Currie" } No longer maintained by N.C.T.C.
   19. 579—N.C.T.C. 509 " Kedrowsky " }
   20. 580—N.C.T.C. 514 " Duval's Chrome " }
2. Other scotochromogenic strains:
   1. 10—*M. xenopi*—isolated from sputum (Dr. A. Beck)
   2. 60—N.C.T.C. 10042 *M. xenopi*
   3. 15—*M. marianum* (*scrofulaceum*) (Dr. A. Beck)
   4. 22—*M. marianum* (*scrofulaceum*) (Dr. J. Marks)
   5. 76—N.C.T.C. 10267 *M. gordonae*
   6. 266—*M. gordonae*—isolated from a rain water tank (W.J.G.)
   7. 277—N.C.T.C. 10438 *M. aurum* (type I)
   8. 278—N.C.T.C. 10439 *M. aurum* (type II)
   9. 3—N.C.T.C. 8156 *M. phlei*
   10. 86—N.C.T.C. 8157 *M. phlei*
   11. 203—*M. aquae* type 3A1 from Professor Tacquet
   12. 209—*M. aquae* type 2 from Professor Tacquet
   13. 211—*M. aquae* type 1 from Professor Tacquet
   14. 212—*M. aquae* type 1 from Professor Tacquet
3. Other mycobacteria:
   1. 42—N.C.T.C. 8551 *M. avium*
   2. 8—*M. kansasii* isolated from sputum (Dr. A. Beck)
   3. 50—N.C.T.C. 2275 *M. marinum*
   4. 124—*Mycobacterium chelonei* (strain I.W. see Inman, Beck, Brown and Stanford, 1969)
   5. 81—N.C.T.C. 2891 *Mycobacterium ranae* (*fortuitum*)
   6. 333—A.T.C.C. 4445 *Mycobacterium thamnopheos*
   7. 1—N.C.T.C. 333 *Mycobacterium smegmatis*
   8. 295—*Mycobacterium diernhoferi* } (Originally from Dr. R. Bönicke,
   9. 296—*Mycobacterium vaccae* } supplied by Dr. R. Muser)

*Bacteriological tests*

The colonial appearance of the organisms growing on Löwenstein–Jensen medium was noted and the cell morphology was studied on Ziehl–Neelsen stained films. The strains were tested for their ability to grow at 25, 37, and 45°.

Ability to utilise sugars was investigated for the fast-growing strains by the method of Gordon and Smith (1953). Utilisation of citrate was tested using Simmons' medium, and results were read after 7 days incubation. The amidase spectrum of each strain was obtained using the method of Bönicke (1962). Production of catalase (Middlebrook, 1954), nitrate reductase (Virtanen, 1960), arylsulphatase (Whitehead, Wildy and Engbaek, 1953) read after 3 days and hydrolysis of Tween 80 (Wayne, 1962) within 10 days were investigated for each strain.

The majority of strains in the study group and representative strains of each of the other species were tested for sensitivity to streptomycin, sodium aminosalicylate, isoniazid, ethionamide, cycloserine, ethambutol, and rifampicin. *Mycobacterium tuberculosis* H37Rv was included in each of the batches of tests and results were recorded as resistance ratios. A ratio of 2 or less was taken as an indication of sensitivity to the drug.

*Serological tests*

Immunodiffusion studies were performed as previously described (Stanford and Beck, 1968) with certain modifications. Antigens were released from organisms by treatment of thick suspensions with an M.S.E. 100 Watt ultrasonic disintegrator for 15 min. with the peak distance set between 8–10 $\mu$m. The immunising injections used for production of the rabbit antisera were prepared by emulsifying a volume of the crude antigen prepared in the ultrasonic disintegrator with an equal volume of a mixture of 8·5 parts Bayol F and 1·5 parts Arlacel A. Antigens were prepared from each of the strains and antisera were prepared in pairs of rabbits to 5 of the strains in the study group (35, 40, 51, 70, and 74). Antisera to strains of the other species considered here were available from previous or concurrent studies. Each of the antigen extracts was tested with each of the antisera in the immunodiffusion studies.

## RESULTS

*Bacteriological examinations*

With the exception of microscopic appearances the cultural data obtained on the scotochromogenic organisms is shown in Table I. Microscopically all the study group of strains were almost indistinguishable from each other. They were acid-fast pleomorphic rods of moderate length. Among the other scotochromogens, *M. aurum* was coccobacillary, *M. marianum* consisted of short rods, *M. gordonae* of long rods, and the other strains were rather more pleomorphic.

The results of the carbohydrate utilisation tests and the enzymic tests for the scotochromogenic and the non-scotochromogenic strains are shown in Tables II and III. The results of the drug sensitivity tests are shown in Table IV.

*Serological examinations*

*The "study strains".*—When tested with the antisera to *M. acapulcense* strain 40, and *M. flavescens* strain 74, the study strains could be divided into 2 groups. One group contained strains 40, 74, 204, 213, 301, 332, 452, 570, 571, 572, and 578, each of which possessed the same 10 antigens demonstrable with both antisera. Members of the 2nd group possessed only 6 antigens demonstrable with these antisera (Fig. 1).

Similarly with the antiserum to strain 35, 2 groups could be distinguished. One group containing strains 35, 132, 205, 391, and 392, possessed 11 demonstrable antigens, and the other group possessed only 6 of these.

The antisera to strains 51 and 70 also distinguished 2 groups. Twelve antigens were demonstrable with both these antisera in strains 51, 70, 579 and 580 (Fig. 2), and only 6 antigens were demonstrable with all the other strains (Fig. 3)

*Scotochromogenic organisms outside the study group.*—The results were the same

TABLE I.—*Cultural Characteristics of Scotochromogenic Mycobacteria*

| | No. | Growth at 24 | Growth at 37 | Growth at 45 | Speed of growth in days 7− | Speed of growth in days 7+ | Colony type | Pigmentation |
|---|---|---|---|---|---|---|---|---|
| | 35 | + | + | − | + | − | smooth | pale yellow |
| *M. acapulcense* | 40 | + | + | − | + | − | rough | bright yellow |
| | 51 | + | + | − | + | − | smooth | bright yellow |
| | 70 | + | + | − | + | − | rough | bright yellow |
| *M. flavescens* | 74 | + | + | − | + | − | rough | bright yellow |
| | 132 | + | + | − | + | − | smooth | pale yellow |
| *M. aquae* 3A2 | 204 | + | + | − | + | − | rough | bright yellow |
| *M. aquae* 3B | 205 | + | + | − | + | − | smooth | pale yellow |
| | 213 | + | + | − | + | − | rough | bright yellow |
| | 301 | + | + | − | + | − | rough | bright yellow |
| | 332 | + | + | − | + | − | rough | bright yellow |
| | 391 | + | + | − | + | − | smooth | pale yellow |
| | 392 | + | + | − | + | − | smooth | pale yellow |
| *M. flavescens* | 452 | + | + | − | + | − | rough | bright yellow |
| | 570 | + | + | − | + | − | rough | bright yellow |
| | 571 | + | + | − | + | − | rough | bright yellow |
| | 572 | + | + | − | + | − | rough | bright yellow |
| | 578 | + | + | − | + | − | rough | bright yellow |
| | 579 | + | + | − | + | − | rough | bright yellow |
| | 580 | + | + | − | + | − | rough | bright yellow |
| *M. xenopi* | 10 | − | + | + | − | + | rough | pale yellow |
| *M. xenopi* | 60 | − | + | + | − | + | rough | pale yellow |
| *M. marianum* | 15 | + | + | − | − | + | smooth | bright yellow |
| *M. marianum* | 22 | + | + | − | − | + | smooth | bright yellow |
| *M. gordonae* | 76 | + | + | − | + | − | smooth | bright yellow |
| *M. gordonae* | 266 | + | + | − | + | − | smooth | bright yellow |
| *M. aurum* | 277 | + | + | − | + | − | smooth | bright yellow |
| *M. aurum* | 278 | + | + | − | + | − | smooth | bright yellow |
| *M. phlei* | 3 | + | + | + | + | − | rough | buff to yellow |
| *M. phlei* | 86 | + | + | + | + | − | rough | buff to yellow |
| *M. aquae* 3A1 | 203 | + | + | − | + | − | smooth | pale yellow |
| *M. aquae* 2 | 209 | + | + | − | + | − | smooth | bright yellow |
| *M. aquae* 1 | 211 | + | + | − | + | − | smooth | pale yellow |
| *M. aquae* 1 | 212 | + | + | − | + | − | smooth | bright yellow |

The specific names shown on the left of the table are those under which the strains were received. Strains not bearing names were previously unidentified.

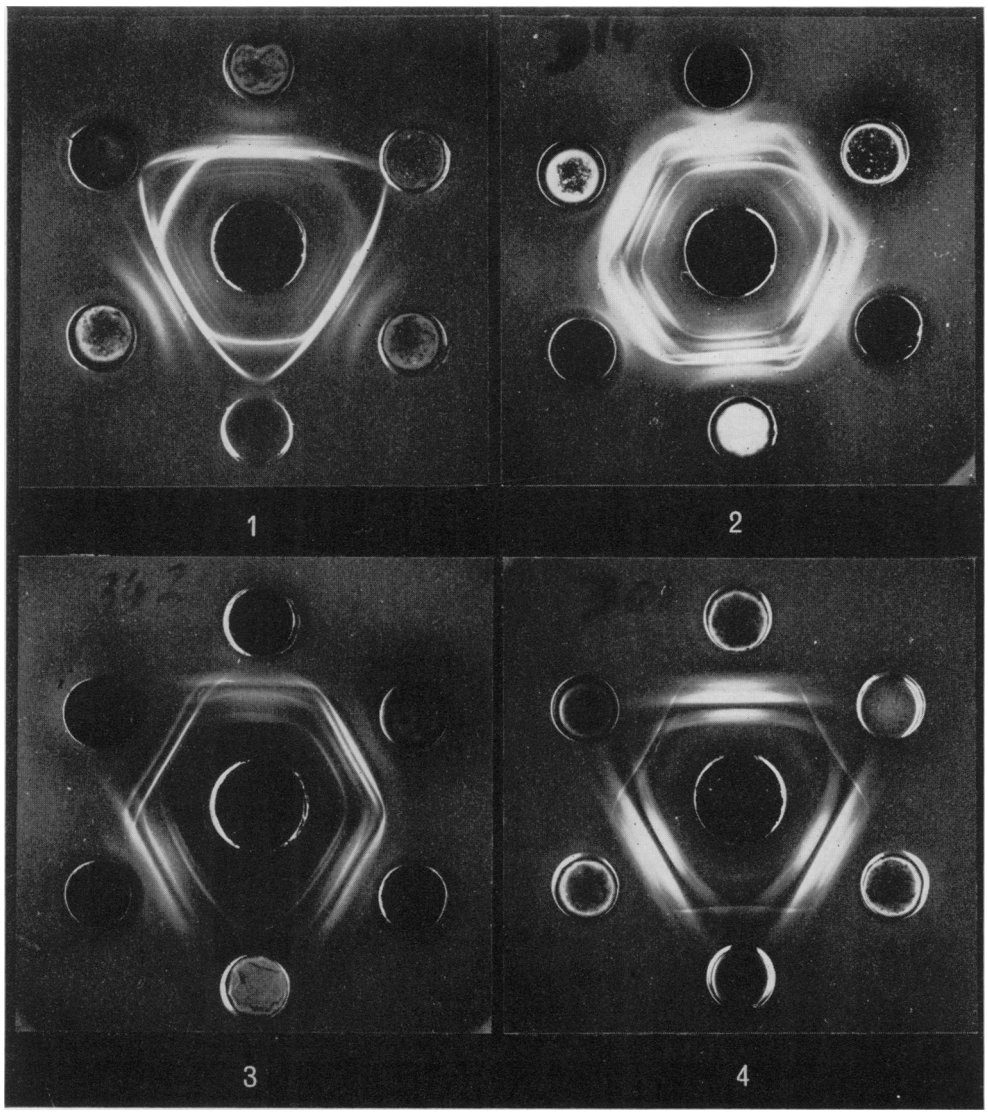
## EXPLANATION OF PLATE

FIG. 1.—Immunodiffusion test showing reaction between an antiserum to *M. flavescens* (central well) and antigens prepared from the type strain of *M. flavescens*. NCTC 10271 (top, lower left and lower right wells), *M. gilvum*, proposed type strain (upper right well), *M. duvalii*, proposed type strain (upper left well), and *M. vaccae* (bottom well).

FIG. 2.—Immunodiffusion test showing reaction between an antiserum to *M. duvalli* (central well) and antigens prepared from the proposed type strain of *M. duvalli* (top, lower left and lower right wells), and 3 other strains of this species.

FIG. 3.—Immunodiffusion test showing reaction between an antiserum to *M. duvalii* (central well) and antigens prepared from the proposed type strain of *M. duvalii* (top, lower left, and lower right wells), a second strain of this species (upper right well), the type strain of *M. flavescens* (upper left well), and the proposed type strain of *M. gilvum* (bottom well).

FIG. 4.—Immunodiffusion test showing reaction between an antiserum to *M. gilvum* (central well) and antigens prepared from the proposed type strain of *M. gilvum* (top, lower left and lower right wells), *M. aquae*, 3A1, strain 203 (upper right well), *M. aquae* 1, strain 211 (bottom well) and *M. gordonae*, strain 266 (upper left well).




Stanford and Gunthorpe.

TABLE II.—*Saccharolytic Activity of Mycobacteria*

| | | Arabinose | Dulcitol | Erythritol | Galactose | Glucose | Inositol | Lactose | Mannitol | Mannose | Raffinose | Rhamnose | Sorbitol | Trehalose | Xylose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 35 | − | − | − | − | + | + | − | + | + | − | − | + | + | − |
| *M. acapulcense* | 40 | − | − | − | − | + | − | − | + | + | − | − | + | + | − |
| | 51 | − | − | − | − | + | − | − | + | + | − | − | + | + | − |
| | 70 | − | − | − | − | + | − | − | + | + | − | − | + | + | − |
| *M. flavescens* | 74 | − | − | − | − | + | − | − | + | + | − | − | + | + | − |
| | 132 | − | − | − | − | + | + | − | + | + | − | − | + | + | − |
| *M. aquae* 3A2 | 204 | − | − | − | − | + | − | − | − | + | − | − | − | + | − |
| *M. aquae* 3B | 205 | − | − | − | − | + | + | − | + | + | − | − | + | + | − |
| | 213 | − | − | − | − | + | − | − | − | + | − | − | − | + | − |
| | 301 | − | − | − | − | + | − | − | − | + | − | − | − | + | − |
| | 332 | − | − | − | − | + | − | − | − | + | − | − | − | + | − |
| | 391 | − | − | − | − | + | + | − | + | + | − | − | + | + | − |
| | 392 | − | − | − | − | + | + | − | + | + | − | − | + | + | − |
| *M. flavescens* | 452 | − | − | − | − | + | − | − | + | + | − | − | − | + | − |
| | 570 | − | − | − | − | + | − | − | − | + | − | − | − | + | − |
| | 571 | − | − | − | − | + | − | − | − | + | − | − | − | + | − |
| | 572 | − | − | − | − | + | − | − | − | + | − | − | − | + | − |
| | 578 | − | − | − | − | + | − | − | + | − | − | − | + | + | − |
| | 579 | − | − | − | − | + | − | − | + | + | − | − | + | + | − |
| | 580 | − | − | − | − | + | − | − | + | + | − | − | + | + | − |
| *M. marianum* | 15 | − | − | − | − | + | − | − | − | − | − | − | − | − | − |
| *M. marianum* | 22 | − | − | − | − | + | − | − | − | − | − | − | − | − | − |
| *M. gordonae* | 76 | − | − | − | − | + | − | − | − | + | − | − | − | − | − |
| *M. gordonae* | 266 | − | − | − | − | + | − | − | − | + | − | − | − | − | − |
| *M. aurum* | 277 | + | − | + | + | + | + | − | + | + | − | + | + | + | + |
| *M. aurum* | 278 | + | ± | + | + | + | + | − | + | + | + | + | + | + | + |
| *M. phlei* | 3 | + | − | − | + | + | − | − | + | + | − | − | + | + | − |
| *M. phlei* | 86 | + | − | − | + | + | − | − | + | + | − | − | + | + | + |
| *M. aquae* 3A1 | 203 | − | − | − | − | + | − | − | − | ± | − | − | − | − | − |
| *M. aquae* 2 | 209 | − | − | − | − | + | − | − | − | − | − | − | − | − | − |
| *M. aquae* 1 | 211 | − | − | − | − | + | − | − | − | − | − | − | − | − | − |
| *M. aquae* 1 | 212 | − | − | − | − | + | − | − | − | − | − | − | − | − | − |
| *M. marinum* | 50 | − | − | − | − | + | − | − | − | − | − | − | − | − | − |
| *M. chelonei* | 124 | − | − | − | − | + | − | − | − | + | − | − | + | + | − |
| *M. ranae I* | 81 | − | − | − | − | + | − | − | − | + | − | − | − | + | − |
| *M. thamnopheos* | 333 | − | − | − | + | + | − | − | + | + | − | − | + | + | − |
| *M. smegmatis* | 1 | + | + | + | − | + | + | − | + | + | − | + | + | + | + |
| *M .diernhoferi* | 295 | + | − | − | + | + | + | ± | + | + | − | − | + | + | + |
| *M. vaccae* | 296 | + | − | + | + | + | + | − | + | + | − | + | + | + | + |

Strains of *M. avium, M. kansasii,* and *M. xenopi* have been omitted because the method used to test saccharolytic activity is not suitable for such slow-growing species.

TABLE III.—*Enzymic Activity of Mycobacteria*

| | | Acetamide | Benzamide | Urea | Isonicotinamide | Nicotinamide | Pyrazinamide | Salicylamide | Allantoin | Succinamide | Malonamide | Nitratase | Catalase | Arylsulphatase | Tween 80 | Citrate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 35 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | + |
| *M. acapulcense* | 40 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| | 51 | − | − | + | − | + | + | − | − | − | − | + | + | − | + | − |
| | 70 | − | − | + | − | + | + | − | − | − | − | + | + | − | + | − |
| *M. flavescens* | 74 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| | 132 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| *M. aquae* 3A2 | 204 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| *M. aquae* 3B | 205 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| | 213 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| | 301 | − | − | − | − | + | + | − | − | − | − | + | + | + | + | − |
| | 332 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| | 391 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | + |
| | 392 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | + |
| *M. flavescens* | 452 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| | 570 | − | − | − | − | + | + | − | − | − | − | + | + | + | + | − |
| | 571 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| | 572 | − | − | − | − | + | + | − | − | − | − | + | + | + | + | − |
| | 579 | − | − | + | − | + | + | − | − | − | − | + | + | − | + | − |
| | 578 | − | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| | 580 | − | − | + | − | + | + | − | − | − | − | + | + | − | + | − |
| *M. xenopi* | 10 | − | − | − | − | + | + | − | − | − | − | − | − | + | − | − |
| *M. xenopi* | 60 | − | − | − | − | + | + | − | − | − | − | − | − | + | − | − |
| *M. marianum* | 15 | − | − | + | − | − | − | − | + | − | − | − | + | − | − | − |
| *M. marianum* | 22 | − | − | + | − | + | + | − | − | − | − | − | + | − | − | − |
| *M. gordonae* | 76 | − | − | + | − | + | + | − | + | − | − | − | + | − | + | − |
| *M. gordonae* | 266 | + | + | − | − | + | − | − | + | − | − | − | + | − | + | − |
| *M. aurum* | 277 | ± | + | + | − | + | + | − | + | − | − | + | + | − | + | − |
| *M. aurum* | 278 | + | + | + | − | + | + | − | + | − | − | − | + | + | + | − |
| *M. phlei* | 3 | + | − | + | − | + | + | − | − | − | − | + | + | − | + | − |
| *M. phlei* | 86 | + | − | + | − | + | + | − | − | − | − | + | + | + | + | − |
| *M. aquae* 3A1 | 203 | − | − | + | − | + | + | − | − | − | − | − | + | − | − | − |
| *M. aquae* 2 | 209 | − | − | − | − | − | + | − | − | − | − | − | + | − | + | − |
| *M. aquae* 1 | 211 | − | − | + | − | + | + | − | − | − | − | − | − | − | − | − |
| *M. aquae* 1 | 212 | − | − | + | − | − | + | − | − | − | − | − | + | − | − | − |
| *M. avium* A | 42 | − | − | − | − | + | + | − | − | − | − | − | + | − | − | − |
| *M. kansasii* | 8 | − | − | + | − | + | − | − | − | − | − | + | + | + | + | − |
| *M. marinum* | 50 | − | − | + | − | + | + | − | − | − | − | − | + | + | + | − |
| *M. chelonei* | 124 | + | − | + | − | + | + | − | + | − | − | − | + | + | − | + |
| *M. ranae I* | 81 | + | − | + | − | − | − | − | + | − | − | + | + | + | − | + |
| *M. thamnopheos* | 333 | ± | − | + | − | ± | + | − | + | − | − | − | + | − | + | − |
| *M. smegmatis* | 1 | + | + | + | + | + | + | + | − | + | − | + | + | − | + | + |
| *M. diernhoferi* | 295 | + | + | + | + | − | + | + | + | − | − | + | + | + | ± | + |
| *M. vaccae* | 296 | + | + | + | + | + | + | − | + | + | − | − | + | + | + | |

Among the amidase results, + means production of a blue colour, ± a deep green colour, and − a yellow or pale green colour. Nitratase activity refers to the reaction after 4 hr incubation; arylsulphatase after 3 days incubation, and hydrolysis of Tween 80 within 10 days incubation. The ± result in this latter test means that the inoculum became red but the medium remained yellow in colour.

TABLE IV.—*Sensitivity to Antituberculous Drugs*

| | | Streptomycin | P.A.S. | Isoniazid | Ethionamide | Cycloserine | Ethambutol | Rifampicin |
|---|---|---|---|---|---|---|---|---|
| | 35 | R | R | R | R | S | S | R |
| *M. acapulcense* | 40 | S | R | R | R | R | S | R |
| | 51 | R | R | R | R | R | S | R |
| | 70 | S | R | R | S | S | S | R |
| *M. flavescens* | 74 | S | R | R | S | S | S | R |
| | 132 | R | R | R | R | S | S | R |
| *M. aquae* 3A2 | 204 | R | R | R | S | R | R | R |
| *M. aquae* 3B | 205 | R | R | R | R | R | S | R |
| | 213 | R | R | R | R | R | S | R |
| | 301 | R | R | R | R | R | R | R |
| *M. xenopi* | 10 | S | S | S | S | S | S | S |
| *M. xenopi* | 60 | R | R | R | S | S | S | S |
| *M. marianum* | 15 | R | R | R | S | S | S | R |
| *M. marianum* | 22 | R | R | R | S | S | R | R |
| *M. gordonae* | 76 | R | R | R | S | S | S | R |
| *M. gordonae* | 266 | R | R | R | R | R | S | R |
| *M. aurum* | 277 | S | R | R | R | R | S | R |
| *M. aurum* | 278 | R | R | R | R | R | R | R |
| *M. avium* | 42 | R | R | R | R | R | R | R |
| *M. kansasii* | 8 | R | R | R | S | S | S | S |
| *M. chelonei* | 124 | R | R | R | R | R | R | R |
| *M. ranae I* | 81 | R | R | R | R | R | R | R |
| *M. thamnopheos* | 333 | S | R | R | R | R | R | — |
| *M. smegmatis* | 1 | S | R | R | R | R | S | R |

In this table S indicates a resistance ratio of 2 or less and R indicates a ratio of 4 or more.

whichever of the antisera to the study strains were used. *M. xenopi*, *M. marianum*, *M. gordonae* and the strains of *M. aquae* 203, 209, 211, and 212 possessed 4 demonstrable antigens (Fig. 4). *M. phlei* and *M. aurum* possessed 6 demonstrable antigens (Fig. 1).

Similarly antisera raised to strains of *M. xenopi*, *M. marianum* and *M. phlei* could demonstrate 4, 4 and 6 antigens respectively in extracts of representative strains of the study group.

*Non-scotochromogenic organisms.*—Again the results were the same whichever of the antisera to the study strains were used. *M. avium*, *M. kansasii* and *M. marinum* possessed 4 demonstrable antigens, and *M. chelonei*, *M. ranae*, *M. thamnopheos* and *M. smegmatis* possessed 5. The strains of *M. diernhoferi* and *M. vaccae* alone possessed all 6 antigens shared by *M. phlei*, *M. aurum* and all the strains of the study group.

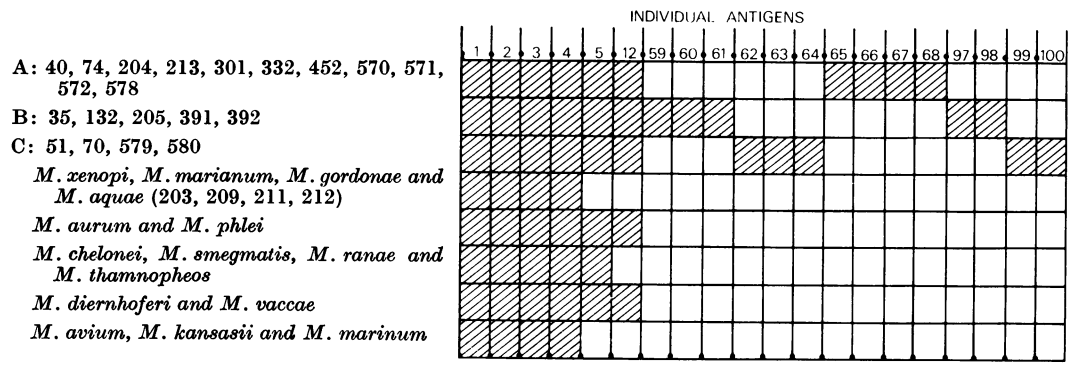



FIG. 5.—Histogram showing the demonstrated antigenic inter-relationships between organisms of the study group and those of other mycobacterial species. A, B and C refer to the same groups so labelled in Table V.

With antisera raised to strains of *M. kansasii*, *M. ranae*, *M. smegmatis* and *M. diernhoferi* 4, 5, 5 and 6 antigens respectively could be demonstrated in representative strains of the study group.

All the serological results are expressed in the histogram (Fig. 5).

## DISCUSSION

Examination of the tables of cultural and biochemical results shows that the study strains are remarkably uniform in their properties, and that they can be readily differentiated from strains of all the other species examined. Omitting colonial appearances, the study group can be divided into 5 subgroups on a basis of the results for 6 tests. These are oxidation of inositol, mannitol and sorbitol, utilisation of citrate, urease and arylsulphatase activity. This division into subgroups is shown in Table V.

The sensitivity test results were of little value in differentiation within the study group and neither did they separate them for other fast-growing organisms.

The serological results clearly separate the study strains from all other species tested, and divided them into 3 quite distinct groups (marked A, B and C in Fig. 5) which coincide with the biochemical groups (Table V).

The question remains whether the biochemically and serologically defined clusters differ at the specific or subspecific level. This can most easily be answered by analogy with the results obtained for other well-defined mycobacterial species. There is plenty of evidence for biochemical variation between strains of a single species and the demonstrated biochemical differences between members of the study group could easily be put down to this. However, when analogies are drawn between the serological results for the study group and those previously published for other species (Stanford and Beck, 1968, 1969; Beck and Stanford, 1968; Stanford and Muser, 1969; Stanford and Gunthorpe, 1969) the evidence is strongly in favour of the 3 serological groups being three separate species. They share no antigens between themselves that they do not also share with other mycobacteria, and each of them possesses antigens specific to itself alone.

TABLE V.—*Biochemical and Serological Differences Between Members of the Test Group of Strains*

| Strains | Inositol | Mannitol | Sorbitol | Urease | Aryl-sulphatase | Citrate | Bio-chemical sub-group | Serological groups |
|---|---|---|---|---|---|---|---|---|
| 301 | − | − | − | − | + | − | 1 | A |
| 570 | − | − | − | − | + | − | | |
| 572 | − | − | − | − | + | − | | |
| 332 | − | − | − | + | + | − | 2 | |
| 204 | − | − | − | + | + | − | | |
| 213 | − | − | − | + | + | − | | |
| 571 | − | − | − | + | + | − | | |
| 40 | − | + | + | + | + | − | 3 | |
| 74 | − | + | + | + | + | − | | |
| 452 | − | + | − | + | + | − | | |
| 578 | − | + | + | + | + | − | | |
| 35 | + | + | + | + | + | + | 4 | B |
| 132 | + | + | + | + | + | + | | |
| 205 | + | + | + | + | + | + | | |
| 391 | + | + | + | + | + | + | | |
| 392 | + | + | + | + | + | + | | |
| 51 | − | + | + | + | − | − | 5 | C |
| 70 | − | + | + | + | − | − | | |
| 579 | − | + | + | + | − | − | | |
| 580 | − | + | + | + | − | − | | |

The figures 1–5 for biochemical subgroups and the letters A, B and C for serological groups have no significance beyond convenience.

The inclusion of the type strains of both *M. flavescens* and *M. acapulcense* in one of the species is in agreement with the work of others (Pattyn, Herman-Boveroulle and van Ermengem, 1968). Since both names were published simultaneously in the same paper by the same authors (Bojalil, Cerbon and Trujillo, 1962) neither has chronological priority over the other. The original descriptions of *M. acapulcense* and *M. flavescens* are very similar, and since there is very little to choose between the names on this basis, the more descriptive and commonly used *M. flavescens* is recommended.

The name *M. aquae* Galli-Valerio, of which there is no type strain, and little information, was recommended by Bönicke (1962) for the fast-growing scotochromogens, 2 varieties of which were described on the basis of their ability to split urea. Tacquet and his colleagues (Tacquet, Tison, Plancot, Devulder and Roos, 1967), who supplied our strains of this name, used *M. aquae* as a " temporary taxonomic entity " in which they distinguished 6 groups. Recently, Wayne

(1930) considered *M. aquae* to be invalid, and recommended that strains of that name should be considered as *M. gordonae*.

Thus the 2 strains called *M. aquae* in our study group, which differ considerably from the type strain of *M. gordonae*, are misnamed and do not create a taxonomic problem.

Three of the strains isolated from cases of leprosy were originally thought to be *M. leprae*, but they are obviously not so and this designation need be considered no further.

Thus the 3 species of the study group are *M. flavescens* and 2 un-named species for which the names *Mycobacterium gilvum* and *Mycobacterium duvalii* are proposed. Two of the strains designated *M. gilvum* were isolated from sputum, and 2 others were from pleural fluid. In no case was the organism thought to be acting as a pathogen, and in no case was it recovered twice from the same patient.

All 4 strains designated *M. duvalii* were isolated long ago from cases of leprosy.

There is insufficient evidence at this stage to state whether either of the 2 newly described species is potentially pathogenic for man or animals.

## SPECIES DESCRIPTIONS

*Mycobacterium flavescens* Bojalil, Cerbon and Trujillo (synonymous with *M. acapulcense*).

Pleomorphic acid-fast bacillus producing deep-yellow pigmented rough colonies on Löwenstein–Jensen medium at 25° and 37°, but not at 45° within 7 days. Able to oxidise glucose, mannose and trehalose, some strains also split mannitol and sorbitol. None of them oxidises arabinose, dulcitol, erythritol, galactose, inositol, lactose, raffinose, rhamnose, or xylose. Most strains have an amidase spectrum of 3, 5, 6 (urease, nicotinamidase and pyrazinamidase). All strains reduce nitrates to nitrites, produce catalase and arylsulphatase, and hydrolyse Tween 80 within 5 days. They are resistant to isoniazid, sodium aminosalicylate and rifampicin. The species is characterised by the possession of 4 species-specific antigens demonstrable in immunodiffusion tests.

*Mycobacterium gilvum* (new species)

Pleomorphic, acid-fast bacillus producing pale yellow, pigmented, smooth colonies on Löwenstein–Jensen medium at 25° and 37° but not at 45° within 7 days. Able to oxidise glucose, inositol, mannitol, mannose, sorbitol and trehalose, but not arabinose, dulcitol, erythritol, galactose, lactose, raffinose, rhamnose, or xylose. Strains utilise citrate, have an amidase spectrum of 3, 5, 6 (urease, nicotinamidase and pyrazinamidase), reduce nitrates to nitrites, produce catalase and arylsulphatase, and hydrolyse Tween 80 within 48 hr. They are resistant to isoniazid, sodium aminosalicylate and rifampicin. The species is characterised by the possession of 5 species-specific antigens demonstrable in immunodiffusion tests.

The specific epithet *gilvum* is derived from the Latin *gilvus*: pale yellow. This refers to the colour of colonies growing on Löwenstein–Jensen medium. The proposed type strain (No. 35 in this paper) which comes from sputum, has been deposited with the National Collection of Type Cultures.

*Mycobacterium duvalii* (new species)

Pleomorphic acid-fast bacillus producing bright yellow pigmented rough or smooth colonies on Löwenstein–Jensen medium at 25° and 37° but not at 45° within 7 days. Able to oxidise glucose, mannitol, mannose, sorbitol and trehalose, but not arabinose, dulcitol, erythritol, galactose, inositol, lactose, raffinose, rhamnose and xylose. Strains have an amidase spectrum of 3, 5, 6 (urease, nicotinamidase and pyrazinamidase), reduce nitrates to nitrites, produce catalase, but not arylsulphatase, and hydrolyse Tween 80 within 5 days. They are resistant to isoniazid, sodium aminosalicylate and rifampicin. The species is characterised by the possession of 6 species-specific antigens demonstrable in immunodiffusion tests.

The specific epithet *duvalii* refers to the late Professor C. W. Duval who isolated 2 of the strains, believing them to be *M. leprae*. The proposed type strain (No. 70 in this paper) already exists in the National Collection of Type Cultures under the name Duval's Lepra Bacillus NCTC 358.

We would like to thank Dr. A. Beck of St. Mary's Hospital for providing many of the strains and for his help in the preparation of this paper. Professor Tacquet of the Pasteur Institute, Lille, Professor S. R. Pattyn of the Prince Leopold Institute of Tropical Medicine, Antwerp, Dr. R. Muser of the Animal Hygiene Institute, Heidelberg, Dr. J. Marks of the Tuberculosis Reference Laboratory, Cardiff, and Dr. N. Simmons of Chase Farm Hospital, Enfield, Middlesex, provided some of the other strains, and to them we are most grateful.

## REFERENCES

Beck, A. and Stanford, J. L.—(1968) *Tubercle*, **49**, 226.
Bojalil, L. F., Cerbon, J. and Trujillo, A.—(1962) *J. gen. Microbiol.*, **28**, 333.
Bonicke, R.—(1962) *Bull. Un. int. Tuberc.*, **33**, 13.
Duval, C. W. and Wellman, C.—(1912) *J. Infect. Dis.*, **2**, 116.
Gordon, R. E. and Smith, M. M.—(1953) *J. Bact.*, **66**, 41.
Middlebrook, G.—(1954) *Am. Rev. Tuberculosis*, **69**, 471.
Pattyn, S. R., Hermans-Boveroulle, M. T. and van Ermengem, J.—(1968) *Zentbl. Bakt-ParasitKde.*, **207**, 509.
Stanford, J. L. and Beck, A.—(1968) *J. Path. Bact.*, **195**, 131.—(1969) *J. gen. Microbiol.*, **58**, 99.
Stanford, J. L. and Gunthorpe, W. J.—(1969) *J. Bact.*, **98**, 375.
Stanford, J. L. and Muser, R.—(1969) *Tubercle* **50**, Supplement.
Tacquet, A., Tison, F., Plancot, T., Devulder, B. and Roos, P.—(1967) *Bull. Un. int. Tuberc.*, **39**, 36.
Virtanen, S.—(1960) *Acta tuberc scand.*, **48**, Supplement.
Wayne, L.—(1962) *Am. Rev. resp. Dis.*, **86**, 579.—(1970) *Internat. J. Systematic Bact.*, **28**, 149.
Whitehead, J. E. M., Wildy, P. and Engbaek, H. C.—(1953) *J. Path. Bact.*, **65**, 451.