A Comparison of Four Species of Mycobacteria

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SUMMARY: Expanded descriptions of *Mycobacterium phlei* Lehmann & Neumann, *M. smegmatis* (Trevisan) Lehmann & Neumann, *M. fortuitum* Cruz, and the species tentatively designated as *M. rhodochrous* (Overbeck) Gordon & Mihm are presented. These characterizations are based on 40, 95, 80, and 79 strains respectively, upon which 39 different tests and observations were made.

In 1951, a taxonomic study was begun of the nocardias, streptomycetes, and rapidly growing strains of mycobacteria. Subsequently, a goodly number of more slowly growing strains of mycobacteria was received, mainly from diagnostic laboratories. After varying periods of cultivation, some of these slowly growing strains grew as abundantly at 28° as those of *Mycobacterium phlei*, for example, and it became evident that separation of mycobacteria by their growth rate was both arbitrary and unfounded. In 1956, therefore, we included in this taxonomic investigation the so-called atypical, chromogenic, and other variously designated strains, the cause of increasing concern in the diagnostic laboratories. These strains were compared with those of the four species of mycobacteria described earlier, and stable, correlating criteria were sought for their specific identification. Thus far, this search has perversely yielded only additional means of recognizing strains of the species already characterized. As these additional criteria are especially useful for identifying strains of *M. fortuitum*, a species represented by more than 20 % of our strains of mycobacteria obtained from laboratories associated with human and veterinary medicine, the expanded descriptions are presented here.

METHODS

The strains of *Mycobacterium phlei*, *M. smegmatis*, *M. fortuitum*, and *M. rhodochrous* included those listed previously (Gordon & Smith, 1953, 1955; Gordon & Mihm, 1957) and those given in Tables 1-4.

Microscopic examination. The cultures were grown on glycerol agar (peptone, 5 g.; beef extract, 3 g.; glycerol, 70 ml.; agar, 15 g.; soil extract, 250 ml.; tap water, 750 ml.; pH 7.0) or on blood base agar (Difco) plus 7 % (v/v) glycerol for 5 days at 28° and stained by the Ziehl-Neelsen method.

As the strains of mycobacteria were compared with those of *Nocardia* spp. and *Streptomyces* spp., observations of colonial morphology were made on Bennett's agar (yeast extract, 1 g.; beef extract, 1 g.; N-Z Amine A (Sheffield Farms Inc., New York, N.Y.), 2 g.; glucose, 10 g.; agar, 15 g.; distilled water, 1000 ml.; pH 7.3) and on soil-extract agar (peptone, 5 g.; beef extract, 3 g.;

agar; 15 g.; soil extract, 250 ml.; tap water, 750 ml.; pH 7.0). One loopful of a 7- to 14-day culture in glucose broth was inoculated into tubes of Bennett's agar and soil-extract agar that had been melted and cooled to 45°. After careful mixing of the inoculum, approximately 1 ml. of each agar was pipetted

Table 1. *Additional strains identified as Mycobacterium phlei*
Lehmann & Neumann

Laboratory no.	Name when received, source, and strain name or number
N 525	<i>Mycobacterium phlei</i> Lehmann & Neumann; NCTC (525)
A 11728	<i>M. phlei</i> ; ATCC (11728); J. M. Desranleau
A 11758 a	<i>M. phlei</i> ; ATCC (11758 a); G. Penso
589	<i>M. phlei</i> ; J. Glover, Univ. of Liverpool (Crottin); A. Lutz
594	<i>M. phlei</i> ; J. W. Hornibrook, Nat. Inst. of Health, Bethesda, Md. (581)
811, 812	<i>M. phlei</i> ; L. S. McClung, Indiana Univ. (X 39, IUMC)
854, 855	<i>M. phlei</i> ; E. Lederer, Inst. de Biologie Physico-Chimique, Paris (Fleole Pasteur, ICI)
N 10,008	<i>Mycobacterium</i> sp.; NCTC (10,008, Kedrowsky's bacillus)
682-684	<i>Mycobacterium</i> spp.; E. R. Brown, USAF Hospital, Austin, Texas (2, syphilitic lesion; 3, sputum; 1, gastric washings)

Table 2. *Additional strains identified as Mycobacterium smegmatis*
(Trevisan) Lehmann & Neumann

Laboratory no.	Name when received, source, and strain name or number
818, 814	<i>Mycobacterium avium</i> Chester; L. S. McClung, Indiana Univ. (X 85, A)
A 11314	<i>M. butyricum</i> Bergey <i>et al.</i> ; ATCC (11314); F. Stimpert (04909)
582	<i>M. chelonae</i> Bergey <i>et al.</i> ; J. W. Hornibrook, Nat. Inst. of Health, Bethesda, Md. (174)
A 11759	<i>M. lacticola</i> Lehmann & Neumann; ATCC (11759); G. Penso (Milch)
580	<i>M. ranae</i> (Küster) Bergey <i>et al.</i> ; J. Q. Heplar, Long Island Biological Assn., Cold Spring Harbor, N.Y. (Steenken)
675	<i>M. ranae</i> ; E. N. Azarowicz, Univ. of Calif. (lymph node)
A 11727	<i>M. smegmatis</i> (Trevisan) Lehmann & Neumann; ATCC (11727); J. M. Desranleau
672	<i>M. smegmatis</i> ; A. Abrams, Walter Reed Army Medical Center (52-L-1)
853	<i>M. smegmatis</i> ; E. Lederer, Inst. de Biologie Physico-Chimique, Paris
595	<i>M. stercoris</i> Bergey <i>et al.</i> ; A. Abrams (52-Q-1)
519	<i>Mycobacterium</i> sp.; G. Pacheco, Inst. Oswaldo Cruz, Brazil (190, sputum)
592, 593	<i>Mycobacterium</i> spp.; J. W. Hornibrook (370, 430, associated with human leprosy)
661	<i>Mycobacterium</i> sp.; J. W. Hornibrook (366, associated with rat leprosy)
815	<i>Mycobacterium</i> sp.; L. S. McClung (X 40)
831	Unidentified isolation; C. C. Campbell, Walter Reed Army Medical Center (573)

on to a sterile microscope slide inside a sterile, moist chamber and incubated at 28°. The slides were examined (magnification, $\times 430$) at 3, 7 and 14 days for the types of colony present. An alternative method was the pipetting of the inoculated agar into the depression of a sterile, hanging-drop slide inside a sterile, moist chamber, filling the depression $\frac{2}{3}$ to $\frac{3}{4}$ full. Before observation (magnification, $\times 100$), the culture was covered with a cover slip, held in place with 'Vaseline'.

Decomposition of casein. Skim milk or skim-milk powder suspended in the required amount of water and an equal volume of 2% (w/v) water agar were sterilized separately by autoclaving, cooled to 47°, mixed, and poured into sterile plates (5 plates from 100 ml.). Each culture was streaked on a plate and inspected for clearing of the casein after incubation at 28° for 7 and 14 days (Hastings, 1908).

Table 3. *Additional strains identified as Mycobacterium fortuitum Cruz*

Laboratory no.	Name when received, source, and strain name or number
N 2006	<i>Mycobacterium fortuitum</i> Cruz; NCTC (2006)
662-666	<i>M. fortuitum</i> ; S. McMillen, Hektoen Inst. for Medical Research (Tb-11, Tb-166, Tb-167, 266, 2338)
922	<i>M. fortuitum</i> ; F. B. Seibert, Henry Phipps Inst. (P.H.S. 12); S. McMillen (12, sputum)
961-964	<i>M. fortuitum</i> ; L. G. Wayne, Veterans Administration Hospital, San Fernando, Calif. (76, 212, 213, 214)
691-696	<i>M. minetti</i> Penso <i>et al.</i> ; D. W. Will, Olive View Sanatorium, Olive View, Calif. (Goring, C18, C19, enteriditis, C70, C75); G. Penso
N 2891	<i>M. ranae</i> (Küster) Bergey <i>et al.</i> ; NCTC (2891); ATCC (110)
N 3631	<i>M. tuberculosis</i> (Schroeter) Lehmann & Neumann; NCTC (3631, avian type from mesenteric glands of a cow)
N 1542*	<i>Mycobacterium</i> sp.; NCTC (1542); Král Collection (fish)
12, 13	<i>Mycobacterium</i> spp.; R. E. Gordon, Dept. of Agric., Washington, D.C. (soil)
478	<i>Mycobacterium</i> sp.; P. Stuart, Ministry of Agric. and Fisheries, Weybridge, Surrey (Ms. 229.52)
518, 520-522	<i>Mycobacterium</i> spp.; G. Pacheco, Inst. Oswaldo Cruz (189, sputum; 191, gastric juice; 192, sputum; 193, urine)
640, 667, 723, 725	<i>Mycobacterium</i> spp.; D. Yegian, State Tuberculosis Hospital, Ray Brook, N.Y. (58112, 58111, 58108, 58109); M. E. Clark (sewage)
644-646, 726	<i>Mycobacterium</i> spp.; D. Yegian (58104, 58102, 58106, 58113); M. E. Clark (sputum)
681	<i>Mycobacterium</i> sp.; E. N. Azarowicz, Univ. of Calif. (M18)
720	<i>Mycobacterium</i> sp.; G. Altmann, Tel Hashomer Hospital, Israel (TH/2886, gastric juice)
786-788	<i>Mycobacterium</i> spp.; J. Ross, Univ. of Washington (Oak Ridge, Columbia River, Carson; fish)
789 A, 789 B	<i>Mycobacterium</i> spp.; J. Ross (variants of Chambers Creek; fish)
936, 937, 945	<i>Mycobacterium</i> spp.; U.S. Public Health Service, Muscogee County Health Dept., Ga. (P.H.S. 10, P.H.S. 11, P.H.S. 81; sputum)
965-967	<i>Mycobacterium</i> spp.; L. G. Wayne (80, 211, 313)
970	<i>Mycobacterium</i> sp.; D. H. Howard, Univ. of Calif. (134, skin ulcer)
981	<i>Mycobacterium</i> sp.; L. F. Affronti, Henry Phipps Inst. (P.H.S. 383); E. H. Runyon
734	Unidentified isolation; C. C. Campbell, Walter Reed Army Medical Center (931)
820	Unidentified isolation; O. Kanner, Veterans Administration Hospital, Oteen, N. C. (Hagans, sputum)

* Same as ATCC strain 9820, erroneously listed by Gordon & Smith (1955) as NCTC strain 2014.

Decomposition of tyrosine. Each culture was streaked once across a plate containing approximately 20 ml. of the following medium: peptone, 5 g.; beef extract, 3 g.; agar, 15 g.; tyrosine, 5 g.; distilled water, 1000 ml.; pH 7.0. An even distribution of the crystals of tyrosine was insured by placing 0.5 g.

of tyrosine in a 250 ml. flask, adding 100 ml. of the remaining components, autoclaving, cooling to 47°, and carefully mixing the agar before and during pouring. After 14 and 21 days of incubation at 28°, the plates were observed for the disappearance of the crystals underneath and bordering the growth.

Hydrolysis of starch. Duplicate plates of the following medium were streaked and incubated at 28°: peptone, 5 g.; beef extract, 3 g.; agar, 15 g.; potato

Table 4. *Additional strains identified as Mycobacterium rhodochrous (Overbeck) Gordon & Mihm*

Laboratory no.	Name when received, source, and strain name or number
804	<i>Erythrobacillus havamiensis</i> ; L. S. McClung, Indiana Univ. (RH 181); R. S. Breed (4001)
802	<i>Mycobacterium agreste</i> Gray & Thornton; L. S. McClung (RH 176); R. S. Breed (2565)
801	<i>M. rubropertinctum</i> (Hefferan) Ford; L. S. McClung (RH 175); R. S. Breed (185)
816, 819	<i>Mycobacterium</i> spp.; L. S. McClung (B 161B, 41 B)
568-570	<i>Nocardia</i> spp.; N. M. McClung, Univ. of Kansas (14, 15, 16; soil)
729	<i>Nocardia</i> sp.; L. Ajello, Communicable Disease Center, Chamblee, Ga. (M 172-6); W. M. Bowman.
764	<i>Nocardia</i> sp.; D. M. Webley, Macaulay Inst., Aberdeen (P ₂); B. Treccani
822	<i>Nocardia</i> sp.; M. P. Lechevalier, Rutgers Univ. (24E, soil)
836, 838, 839	<i>Nocardia</i> spp.; J. B. Davis, Magnolia Petroleum Co., Dallas, Texas (18-68, 18-2, 107-308; utilize ethane, Davis, Chase & Raymond, 1956)
A 12483	<i>Nocardia</i> sp.; ATCC (12483); Amer. Cyanamid Co.
766	<i>Proactinomyces caprae</i> ; D. M. Webley (T ₃)
765A, 765B	<i>P. opacus</i> (den Dooren de Jong) Jensen; D. M. Webley (variants of T ₁)
759, 760	<i>P. ruber</i> Krassilnikov; D. M. Webley
805, 809	Unidentified isolations; L. S. McClung (RH 185, RH 210); R. S. Breed (B17, GLF)
1022	Unidentified isolation; C. C. Campbell, Walter Reed Army Medical Center (188, resected lung tissue)

starch, 10 g.; distilled water, 1000 ml.; pH 7.0. The starch was suspended in 50 ml. of cold water prior to its addition to the melted agar. Before the plates were poured, the agar was thoroughly mixed to distribute the starch evenly. One plate was flooded with 95% ethanol at 5 days, and the other at 10 days. After 20-30 min., the unchanged starch became white and opaque, while a clear zone underneath and around the growth measured the hydrolysis of the starch (Kellerman & McBeth, 1912).

Temperatures of growth. Cultures were made on slants of glycerol agar, heated or cooled to the desired temperature in a water bath, then transferred to a water bath at the same temperature inside a constant temperature incubator. The water level and temperature of the water bath were carefully watched. The cultures were examined for growth after 5-7 days at temperatures of 35° or above, and after 3 weeks at 10°.

Survival at 60°. Slants of glycerol agar were inoculated, quickly heated to 60° in a water bath, then placed in another water bath at 60° inside a constant

temperature incubator. After 4 hr., the cultures were rapidly cooled, incubated at 28° for 14 days, and inspected for growth.

Acid production from carbohydrates. One-half ml. of a 10 % (w/v) aqueous solution of each carbohydrate, sterilized by autoclaving, was pipetted aseptically to 5 ml. of sterile, inorganic nitrogen agar base: $(\text{NH}_4)_2\text{HPO}_4$, 1 g.; KCl, 0.2 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g.; agar, 15 g.; distilled water, 1000 ml. (Ayers, Rupp & Johnson, 1919). The pH value of this medium was adjusted to 7.0 before the addition of 15 ml. of a 0.04 % (w/v) solution of bromocresol purple. Cultures on each carbohydrate agar were observed for the acid colour of the indicator after 7 and 28 days of incubation at 28°.

Utilization of organic acids as carbon sources. Modifications of Koser's (1924) citrate agar were prepared by adding 2 g. of Na benzoate, Na citrate, Na lactate, Ca malate, mucic acid, Na oxalate, or Na succinate to NaCl, 1 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g.; $(\text{NH}_4)_2\text{HPO}_4$, 1 g.; KH_2PO_4 , 0.5 g.; agar, 15 g.; distilled water, 1000 ml.; 0.04 % (w/v) solution of phenol red, 20 ml. To insure a slightly acid colour of the indicator in the sterilized agar, the pH value was adjusted before autoclaving as follows: benzoate, 7.0; citrate, 6.8; lactate, 6.8; malate, 7.2; mucate, 6.8; oxalate, 6.7; and succinate, 6.8. Use of the inorganic acid was indicated by the alkaline colour of the phenol red after 4 weeks of incubation at 28°.

Production of urease. Ten ml. of a 15 % (w/v) solution of urea, sterilized by filtration, was combined with 75 ml. of the following autoclaved broth: KH_2PO_4 , 9.1 g.; Na_2HPO_4 , 9.5 g.; yeast extract, 0.1 g.; phenol red, 0.01 g.; distilled water, 1000 ml.; pH 6.8 (Rustigian & Stuart, 1941). The mixture was pipetted aseptically to sterile plugged test tubes in 1.5 ml. amounts and heavily inoculated with actively growing, 2- or 3-day-old cultures. Urease was demonstrated by the alkaline reaction of the phenol red after 5, 7, 14, 21, and 28 days at 28°.

Sensitivity to dyes. Malachite green oxalate, methyl violet, and pyronin B agars were made by adding 0.01 % (w/v) of each dye to glycerol agar. Slants of each of the three agars and one of glycerol agar as a control were inoculated from a 7- to 14-day-old culture in glucose broth with a loop, 2.5 mm. in outside diameter. After incubation at 28° for 28 days, growth on the agars was recorded.

MacConkey agar. 7- to 14-day-old cultures in glucose broth were streaked on a slant of MacConkey agar (Difco) and on a control slant of glycerol agar with a loop, 2.5 mm. in outside diameter. Growth and colour change of the neutral red indicator were noted after 7 and 28 days at 28°.

RESULTS

Mycobacterium phlei Lehmann & Neumann

The 13 strains of this species added to the previously listed 27 (Gordon & Smith, 1953, 1955) were initially labelled *Mycobacterium phlei* or *Mycobacterium* spp. As misnamed strains or strains with specific names in synonymy were not received, annoying problems of nomenclature were eliminated.

When stained by the Ziehl-Neelsen method, cultures of the 13 strains showed coccobacilli; small, short rods; or small, pleomorphic rods. Forty to 95 % of the rods retained the carbol fuchsin. Each of the 13 strains formed three or more of the following types of colonies: dense colonies with smooth edges; dense colonies with a halo or outcroppings of filaments; densely or loosely filamentous colonies; and thin, flat colonies of cells, sometimes resembling a medusa-head. Cultures growing in the smooth stage showed dense colonies with smooth edges, dense colonies with a fringe of short filaments, and tiny, filamentous colonies. Cultures growing in the rough stage had few, if any, dense colonies with smooth edges, and their filaments were longer and more extensive. During the 14 days of observation, the filaments of 10 of the 13 cultures fragmented into shorter forms.

The macroscopic appearance and physiological reactions of the 13 new strains did not differ from those of the 27 previously studied, and the results of the tests applied to the 40 strains of this species are presented in Table 5. The most important reactions for separating strains of *Mycobacterium phlei* from those of *M. smegmatis*, *M. fortuitum*, and *M. rhodochrous* were: growth at 52°; lack of growth at 10°; survival at 60° for 4 hr.; acid production from arabinose and sorbitol; failure to form acid from dulcitol, inositol, or rhamnose; inability to utilize benzoate, mucate, or oxalate; inhibition of growth by a 0.01 % (w/v) concentration of malachite green, methyl violet, or pyronin; and lack of growth on MacConkey agar. Although the following properties had no value in distinguishing strains of *M. phlei* from those of the other three species, they were considered part of the pattern of reactions of this species: failure to decompose casein or tyrosine; hydrolysis of starch; acid formation from mannitol and mannose; inability to produce acid from lactose, raffinose, or α -methyl-D-glucoside; use of citrate, lactate, malate, and succinate as sources of carbon; and development of urease. Variation by a strain in one or two respects from the pattern of these 28 more dependable reactions did not prevent its identification as *M. phlei*.

Mycobacterium smegmatis (Trevisan) Lehmann & Neumann

Mycobacterium chelonae Bergey *et al.* (1923) was the only specific name among those of the 17 strains listed in Table 2 that had not been represented by one or more of the 78 strains previously identified as strains of *M. smegmatis*. The original description of *M. chelonae*, Friedmann's (1903) turtle bacillus, was based mainly on variable characteristics, but did not definitely challenge the authenticity of strain no. 582. The name *M. chelonae* was, therefore, accepted in the synonymy of *M. smegmatis*.

Two of the 17 strains were initially labelled *Mycobacterium ranæ* (Küster) (Bergey *et al.* 1923). The original account of this species (Küster, 1905) described the first strain as unable to grow at 37°. As growth at 45° was a very stable characteristic of strains of *M. smegmatis*, Küster's strain was not believed to have been *M. smegmatis*. These two strains and seven others previously listed, received as *M. ranæ* from laboratories in the U.S., were regarded as misnamed and not representative of *M. ranæ*.

Eight cultures of the 17 were composed of pleomorphic rods, often unevenly stained by the Ziehl-Neelsen technique. The rest of the cultures were slender rods of short or medium length. In the 17 cultures, 25-100% of the rods retained the carbol fuchsin. Each culture showed two or more of the following

Table 5. *Comparison of physiological characteristics*

Property	40 strains of <i>M. phlei</i> (% positive strains)	95 strains of <i>M. smegmatis</i> (% positive strains)	80 strains of <i>M. fortuitum</i> (% positive strains)	79 strains of <i>M. rhodochrous</i> (% positive strains)
Decomposition of				
Casein	0	0	0	0
Tyrosine	0	0	0	76
Hydrolysis of starch	100	100	100	97
Growth at				
52°	100	0	0	3
45°	100	100	15	54
40°	100	100	70	76
35°	100	100	95	90
28°	100	100	100	100
10°	0	44	46	100
Survival at 60°, 4 hr.	90	0	0	22
Acid from				
Arabinose	98	100	3	0
Dulcitol	0	98	0	0
Erythritol	52	100	18	4
Galactose	73	99	10	5
Glucose	91	100	100	97
Inositol	0	100	9	16
Lactose	0	0	0	0
Mannitol	100	98	26	100
Mannose	94	100	100	100
α -methyl-D- glucoside	0	0	0	0
Raffinose	0	0	1	0
Rhamnose	0	100	0	8
Sorbitol	100	100	3	100
Trehalose	73	99	100	96
Xylose	64	99	5	17
Utilization of				
Benzoate	0	95	3	80
Citrate	94	99	95	87
Lactate	100	100	100	99
Malate	100	100	100	99
Mucate	0	100	0	0
Oxalate	0	99	0	0
Succinate	97	100	99	100
Production of urease	98	100	96	75
Growth on dyes				
Malachite green	3	100	100	1
Methyl violet	0	99	100	0
Pyronin	5	96	100	0
Colour change of MacConkey agar	0	43	96	0

colonial types: dense colonies with smooth edges; dense colonies with a fringe or outcroppings of filaments; dense colonies with a halo of cells; loosely filamentous colonies; and very thin colonies of rods, often resembling a medusa-head. During incubation, the filaments of 11 cultures fragmented into short rods.

The growth and physiological characteristics of these 17 strains were similar to those of the 78 previously assigned to *Mycobacterium smegmatis*. The physiological reactions of the 95 strains of this species are given in Table 5. Distinctive properties were: growth at 45° but none at 52°; failure to survive at 60° for 4 hr.; production of acid from arabinose, dulcitol, erythritol, inositol, rhamnose, sorbitol, and xylose; utilization of benzoate, mucate, and oxalate; and growth on agar with 0.01 % (w/v) malachite green, methyl violet, or pyronin. Other criteria comprising the pattern of reactions of this species were: failure to decompose casein or tyrosine; hydrolysis of starch; acid production from galactose, glucose, mannitol, mannose, and trehalose; failure to form acid from lactose, α -methyl-D-glucoside, or raffinose; use of citrate, lactate, malate, and succinate; and the presence of urease.

Among all the mycobacteria in this collection (those in Table 5 plus 205 specifically unassigned strains) utilization of mucate and oxalate was peculiar to strains of *Mycobacterium smegmatis*.

Mycobacterium fortuitum Cruz

The nomenclature of this species is still unsettled. In 1955, Gordon & Smith found it difficult to believe that a strain of such a widely distributed species (soil, water, lesions in man, cattle, and cold-blooded animals) had not been described and named long before *Mycobacterium fortuitum* Cruz (1938). Had Cowan's (1956) proposal of the use of a common name for a species, whose scientific name was not established, been available, it would have been seriously considered.

In 1958, NCTC strain no. 2891 of *Mycobacterium ranae* was added to this collection. This strain was received by the NCTC many years ago from the ATCC as strain no. 110. Unlike the present ATCC strain no. 110 and eight other misnamed ones initially labelled *M. ranae* from laboratories in the U.S., NCTC no. 2891 was a strain of *M. fortuitum* and agreed more closely with Küster's first description of *M. ranae* (1905). Küster stated that his strain did not grow at 37°; NCTC strain no. 2891 grew at 40°, but not at 45°. The maximum temperatures of growth of the 80 strains representing *M. fortuitum* in this collection, however, varied considerably. There were four, isolated from fish, that did not grow at 35°; 19 others that did not grow at 40°; and 12 that grew slightly at 45°. After a few more years of cultivation *in vitro*, the 23 strains that did not grow at 40° will be tested again. If a substantial number of them, including the four from fish, grow at 40°, variation of the maximum temperature of growth of NCTC strain no. 2891 will be accepted, and the name *M. ranae* will be considered in the nomenclature of this species.

Mycobacterium minetti Penso *et al.*, typified by strains nos. 691 to 696, was previously assigned to the synonymy of *M. fortuitum*. According to our

records, NCTC strain no. 3631, initially labelled *M. tuberculosis* avian type, was one of the four NCTC strains identified as *M. minetti* by Penso *et al.* (1952).

Thirty-five of the 52 strains of *Mycobacterium fortuitum* acquired since 1955 exhibited thin, very short to long rods, and the remaining 17 were composed of pleomorphic rods. The rods of 27 strains showed 90% or more retention of the carbol fuchsin; the rods of 10 strains, 50–85%; and those of 15 strains, 5–45%. The colonial morphology of the 52 strains was similar to that described for *M. smegmatis*. A strain growing in the smooth stage produced a preponderance of dense colonies as illustrated in Pl. 1, fig. 1. Thin colonies of rods (Pl. 1, fig. 2) were also formed. Most of the colonies of a strain growing in the rough stage, on the other hand, were loosely filamentous, and their filaments were fairly extensive (Pl. 1, figs. 3, 4). The filaments of all but 12 strains fragmented into shorter forms.

The growth of cultures of *Mycobacterium fortuitum* on glycerol agar was off-white or cream-coloured, soft and butyrous, or waxy and nodular. Occasionally an old culture on glycerol agar formed a black soluble pigment, as dark as the pigment of cultures of *Bacillus subtilis* var. *niger* on tyrosine agar. When first observed, these pigmented cultures were replated, but contaminants were not found. The physiological characters of the 80 strains assigned to this species are listed in Table 5. The most useful reactions for recognizing strains of *M. fortuitum* were: scant growth, if any, at 45°; lack of growth after 4 hr. at 60°; inability to form acid from arabinose, dulcitol, rhamnose, sorbitol, or xylose; failure to utilize benzoate, mucate, or oxalate; growth on malachite green, methyl violet, and pyronin agars; and growth on MacConkey agar changing the agar from deep fuchsia to yellowish pink or yellow. Other characteristics of this species were: inability to attack casein or tyrosine; hydrolysis of starch; demonstration of acid from glucose, mannose, and trehalose; failure to form acid from α -methyl-D-glucoside, lactose, or raffinose; use of citrate, lactate, malate, and succinate; and production of urease.

The 205 specifically unassigned strains of mycobacteria in this collection were also inoculated on malachite green, methyl violet, and pyronin agars. As some strains grew on one, two, or three of these agars, resistance to the three dyes was not limited to strains of *Mycobacterium fortuitum* and *M. smegmatis*. When streaked on MacConkey agar, the 205 strains grew slightly, if at all, and did not change the colour of the indicator during 6 weeks of incubation. A colour change of MacConkey agar was, therefore, peculiar to the strains of *M. fortuitum* and 43% of those of *M. smegmatis*.

Mycobacterium rhodochrous (Overbeck) Gordon & Mihm

This taxon has often been described in this laboratory as a good species in search of a genus. Its strains in this collection have been placed by different investigators in the following genera: *Bacillus*, *Bacterium*, *Erythrobacillus*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Proactinomyces*, *Rhodococcus*, and *Serratia*. Although strongly tempted to assign this species to the genus *Corynebacterium*, Gordon & Mihm (1957) would not do so without a comparative

study of the corynebacteria. It was tentatively allotted, therefore, to the genus *Mycobacterium* because the colonies of 54 of the 56 strains of this species were like those of mycobacteria. Colonies of strains of *M. rhodochrous* growing in the smooth state (Pl. 2, figs. 5, 6) resembled those of smooth strains of mycobacteria (Pl. 1, figs. 1, 2), rather than colonies of nocardias as typified by *Nocardia asteroides* (*N. farcinica*). Acid-fastness was also considered. Many strains of this species, of which only nos. 381, 382, and 386 remain, were isolated during a study of the acid-fast bacteria in soil (Gordon & Hagan, 1937). When first examined, they were as strongly acid-fast as strains of *M. phlei* or *M. smegmatis*. After a few transfers, however, most of them no longer retained the carbol fuchsin. Eight of the 56 strains assigned to *M. rhodochrous* in 1957 showed traces of acid-fastness. Retention of the carbol fuchsin was, therefore, accepted as a variable characteristic, providing little aid in the generic location of the species.

After more study, discussions with other investigators, and second thoughts, it is conceded that this species cannot be satisfactorily allocated to a genus until a comparative examination of the corynebacteria has also been made. Such a study will undoubtedly reveal other scientific names deserving consideration in the nomenclature of this species. In the meantime, and in the absence of a generally accepted common name, *Mycobacterium rhodochrous* is used with the understanding that it may be only a temporary designation, both generically and specifically.

There were only four strains, among the 23 of this species acquired since 1957, which initially bore generic and specific combinations of names not previously encountered. The authority for the name *Erythrobacillus havaniensis*, under which strain no. 804 was received, was not found. The specific epithet was assumed to be that of *Bacillus havaniensis* Sternberg (1892), a species previously listed in the synonymy of *Mycobacterium rhodochrous*. Jensen (1932) assigned *M. opacum* den Dooren de Jong (1927) to the genus *Proactinomyces*. As strains nos. 765 A and 765 B conformed to the original description of *M. opacum*, *Proactinomyces opaca* was accepted in the synonymy of *M. rhodochrous*. Authority for the name *Proactinomyces caprae* was not found, and this specific epithet may or may not be that of *Streptothrix caprae* Silberschmidt (1899), a species in the synonymy of *Nocardia asteroides* (Gordon & Mihm, 1957).

The cellular morphology of the 23 more recently examined strains of this species varied from coccoid forms to short branching filaments and easily accounted for several different generic designations given this species. Some cultures on glycerol agar exhibited only coccoid forms; others, pleomorphic cells; others, rods of various lengths; and others, pleomorphic rods and branching, swollen filaments. Four of the 23 strains retained traces of carbol fuchsin. The colonies of each of 20 of the 23 strains belonged to two or more of the following types: dense colonies with smooth edges or a thin halo of cells; dense colonies with a fringe or outcroppings of filaments; loosely filamentous colonies; and thin, filmy colonies of cells, sometimes resembling a medusa-head. The filaments of these 20 strains fragmented into shorter forms. Plate 2,

figs. 5, 6, illustrate the colonies predominantly formed by a smooth strain of *Mycobacterium rhodochrous* and Pl. 2, figs. 7, 8, show the colonies of the same strain growing in the rough stage. The colonies of the three remaining strains and those of two of the 56 strains previously studied, were more like those of some strains of *Nocardia* spp. Although these five strains had all the other properties of *M. rhodochrous*, they could not be assigned to the genus *Mycobacterium* or to the genus *Nocardia* on the basis of their colonial morphology.

The results of the physiological tests applied to the 79 strains assigned to *Mycobacterium rhodochrous* are given in Table 5. The distinguishing characters of the species, in addition to scant, if any, retention of the carbol fuchsin, were: growth at 10°; formation of acid from sorbitol; failure to produce acid from arabinose, dulcitol, or erythritol; inability to use mucate or oxalate; and lack of growth on malachite green, methyl violet, pyronin, or MacConkey agar. Although all strains did not dissolve the crystals of tyrosine or utilize benzoate, these two reactions, when positive, aided greatly in the recognition of strains of *M. rhodochrous*. Other characteristics in the specific pattern were: failure to decompose casein; hydrolysis of starch; acid from glucose, mannitol, mannose, and trehalose; failure to form acid from lactose, α -methyl-D-glucoside, or raffinose; and use of lactate, malate, and succinate. Deviation by a strain in one or two respects from the pattern of these twenty-five more reliable characteristics did not prohibit its identification as a strain of this species.

DISCUSSION

The search for correlating characteristics common to old type strains, variants of the same strain, and new isolates of a species is never-ending in this taxonomic laboratory. The reliability of each character for the identification of a species is determined by application of the test or observation to as many strains of the particular species as possible. If 99 of 100 strains of *Mycobacterium smegmatis*, for example, use oxalate as a source of carbon, while only 40 grow on MacConkey agar, much more dependence is placed on utilization of oxalate than on growth on MacConkey agar in the recognition of strains of *M. smegmatis*. To accommodate for microbial variation, use of oxalate is supported by other similarly tested criteria such as acid-fastness; growth at 45°; lack of growth at 52°; acid formation from arabinose, dulcitol, inositol, and rhamnose; production of urease; and growth on pyronin agar. With a distinctive group of 20 to 25 reliable properties for the description of a species, variation by a strain from the pattern of reactions in one or two respects is expected, but does not interfere with the identification of the strain.

So many observations for the recognition of a strain are not feasible, of course, in a diagnostic laboratory. The use of MacConkey and oxalate or mucate agars may, however, be valuable as a presumptive test for the identification of *Mycobacterium fortuitum*. Strains of mycobacteria growing on MacConkey agar and changing the colour of the indicator, but not utilizing oxalate or mucate, can be expected, on the basis of this study, to belong to *M. fortuitum*.

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EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Colonies of strain 448 of *Mycobacterium fortuitum* on Bennett's agar at 6 days ($\times 680$).
Fig. 2. Colony of strain 1025 of *M. fortuitum* on soil-extract agar at 10 days ($\times 780$).
Fig. 3. Colony of strain 964 of *M. fortuitum* on soil-extract agar at 12 days ($\times 780$).
Fig. 4. Portion of colony of strain 1026 of *M. fortuitum* on soil-extract agar at 10 days ($\times 780$).

PLATE 2

- Figs. 5, 6. Colonies of R. S. Breed's strain KBMC (W 3406) of *Mycobacterium rhodochrous* on Bennett's agar at 6 days ($\times 560$ and $\times 690$ respectively).
Fig. 7. Colony of R. S. Breed's strain KBMC (370) of *M. rhodochrous* on Bennett's agar at 5 days ($\times 680$).
Fig. 8. Portion of colony of R. S. Breed's strain KBMC (370) of *M. rhodochrous* on Bennett's agar at 11 days ($\times 680$).

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R. E. GORDON & J. M. MIHM—COMPARISON OF *MYCOBACTERIUM* SPP. PLATE 1
(Facing p. 748)