Additional Serotypes of *Mycobacterium scrofulaceum*, *Mycobacterium gordonae*, *Mycobacterium marinum*, and *Mycobacterium xenopi* Determined by Agglutination

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Agglutination serotypes were investigated by testing mycobacterial cultures from various sources with a large battery of antisera raised in rabbits. Absorption of agglutinins was performed when necessary to clarify serological relationships. Types in addition to those already described were recognized as follows: *Mycobacterium scrofulaceum*, serotype Cole; *Mycobacterium gordonae*, seven serotypes (34 strains isolated from various water sources were serotype Marshall); *Mycobacterium marinum* types 1 and 2; and *Mycobacterium xenopi*. It is proposed that these types be numbered, respectively, as follows: *Mycobacterium avium* complex, serotype 44; *M. gordonae*, serotypes 1 to 7 (Kowal, Marshall, Szent, Puntal, Moore, Lanton, and Brown, respectively); *M. marinum*, serotypes 1 and 2; and *M. xenopi*. The strains of *M. xenopi* included in this study could not be separated into different types by the techniques utilized.

Three serotypes of *Mycobacterium scrofulaceum* are recognized and have been designated as numbers 41, 42, and 43 (4, 7). Preliminary reports on three or four types of *Mycobacterium gordonae* were presented by Ryneerson and Wolinsky in 1967 (Am. Rev. Resp. Dis. 96:155) and in 1970 (Correlation of serological biochemical and clinical aspects of slowly growing mycobacteria; Round Table on "Immunological Approaches to Mycobacterial Taxonomy;" 70th Annu. Meet. Am. Soc. Microbiol., Boston, 1970). Twenty-four strains of *Mycobacterium marinum* were found to be antigenically homogeneous by agglutination (6), and nothing has been published to our knowledge concerning the antigenic varieties of *Mycobacterium xenopi*. The purpose of this report is to document the serological relationships which allow us to propose additional serotypes for the mycobacteria of these species.

MATERIALS AND METHODS

Antiserum production. The mycobacteria used for production of antisera are shown in Table 1. The strains were grown on Middlebrook 7H10 agar slants, washed off with phosphate-buffered saline (pH 7.0 to 7.2), and pipetted into sterile tubes. Large clumps were removed by settling, after which the suspensions were streaked out on 7H10 agar plates and blood agar plates to assure purity. After heating in a 70 C water bath for 70 min, the suspensions were centrifuged at 2,500 rpm and washed three times with sterile buffer. The cell density was adjusted by a Hopkins tube so that 1 ml of suspension contained 0.01 ml of packed cells, and the suspen-

sions were stored in vaccine bottles. Each culture suspension was injected intravenously into two or three 8- to 10-lb. (approximately 3.6- to 4.5-kg) albino rabbits. The animals received a series of six injections at 3- or 4-day intervals, starting with 0.1 ml and gradually increasing to 1.0 ml. A few days after the last injection, the agglutination titer for the homologous culture was determined on a sample of venous blood. One or two further injections were given if the titer was less than 1:640. The rabbits were bled finally from the heart, and the sera were tubed with 1:10,000 merthiolate as preservative and frozen for later use.

Agglutinations. Agglutinations and absorptions were done by the methods of Schaefer (4,5). In addition, twofold dilutions were made in buffered saline to titer the sera with both homologous and heterologous strains. Agglutination was judged after 3 to 4 h at 37 C and checked after overnight refrigeration. The titer was defined as the highest dilution of serum which produced a definite, although not necessarily complete, agglutination.

Strains were identified at the specific level by the criteria described by Kubica (2). The cultures examined for agglutination either were isolated in this laboratory from patient material, soil, and water, or were stock strains acquired from various sources, as indicated in the tables.

RESULTS

One new *M. scrofulaceum* serotype was recognized. The results for the four strains of the proposed serotype Cole are shown in Table 2. These strains had the characteristic biochemical and tinctorial reactions of *M. scrofulaceum*. No cross-reactions with any other *M. scrofula-*

TABLE 1. *Strains used for antiserum production*

Strain no.	Source ^a	Serotype	Mycobacterial species
356	P-5, received from Runyon (White, sput, silicosis)	Cole	<i>M. scrofulaceum</i>
357	P-6, received from Runyon (Cole, sput, lung disease)	Cole	<i>M. scrofulaceum</i>
154	Kowalczyk, sput, cas, CMGH	Kowal	<i>M. gordonae</i>
144	Tap water, CMGH	Kowal	<i>M. gordonae</i>
430	Soil, New Jersey	Puntal	<i>M. gordonae</i>
848	Legeza, sput, cas, CMGH	Puntal	<i>M. gordonae</i>
187	Szentendrei, tonsil, CMGH	Szent	<i>M. gordonae</i>
186	Pentek, tonsil, CMGH	Szent	<i>M. gordonae</i>
46	Marshall, lung resection, UHC	Marshall	<i>M. gordonae</i>
48	O'Neil, sput, CMGH	Marshall	<i>M. gordonae</i>
64	Wohl, lung resection, UHC	Marshall	<i>M. gordonae</i>
778	Jolly, gastric, cas, CMGH	Marshall	<i>M. gordonae</i>
949	Lanton, gastric, cas, CMGH	Lanton	<i>M. gordonae</i>
617	Soil, Cleveland, Ohio	Lanton	<i>M. gordonae</i>
1083	Brown, sput, cas, CMGH	Brown	<i>M. gordonae</i>
209	Soil, Shaker Lakes, Cleveland, Ohio	Brown	<i>M. gordonae</i>
429	Soil, yard of Moore, Cleveland, Ohio	Moore	<i>M. gordonae</i>
595	Soil, Cleveland, Ohio	Moore	<i>M. gordonae</i>
610	Soil, Cleveland, Ohio	Moore	<i>M. gordonae</i>
613	Soil, Cleveland, Ohio	Moore	<i>M. gordonae</i>
338	Received from Runyon as #1328; originally from Schaefer, USPHS #474	1	<i>M. marinum</i>
347	Received from Runyon as #2733, ATCC #11565	1	<i>M. marinum</i>
962	Fox, olecranon bursa fluid, UHC	2	<i>M. marinum</i>
1010	Mattingley, skin lesion, Memorial Hospital, Easton, Md.	2	<i>M. marinum</i>
1181	Serum and culture received from Schaefer: #931, Hernandez ^b	1	<i>M. marinum</i>
1182	Serum and culture received from Schaefer: #661 and 662, Ellender ^b	1	<i>M. marinum</i>
1111	Serum and culture received from Schaefer: #712, Francingues ^b	2	<i>M. marinum</i>
1183	Serum and culture received from Schaefer: #799, Geraci ^b	2	<i>M. marinum</i>
336	Received from Runyon as #1162; originally from Schaefer	?	<i>M. marinum</i>
344	Received from Runyon as #2337; knee lesion strain recovered from mouse	?	<i>M. marinum</i>
871	Ebenger, hand abscess, CMGH	?	<i>M. marinum</i>
351	NCTC #10042 (type strain)		<i>M. xenopi</i>
668	Soil, Montana, received from Runyon as #3244		<i>M. xenopi</i>
1174	Serum and culture received from Schaefer: Cardiff, #25592 ^b		<i>M. xenopi</i>
1173	Serum and culture received from Schaefer; originally from J. Hawkins, #72-1097		<i>M. xenopi</i>

^a Abbreviations: sput, sputum; cas, casual isolate, defined as a strain isolated from human material once or twice, but not significant in causing disease; CMGH, Cleveland Metropolitan General Hospital; UHC, University Hospitals of Cleveland; USPHS, U.S. Public Health Service.

^b These materials were provided by the U.S.-Japan Cooperative Medical Science Program, NIAID, through W. B. Schaefer.

TABLE 2. *Agglutination titers with proposed Cole serotype of M. scrofulaceum*

Strain no.	Source	Agglutination titer to antiserum:	
		356	357
357	Cole, sputum, Runyon P-6	1:320	≥1:1,280
28	Cole, sputum ^a	1:320	1:640
356	White, sputum, Runyon P-5	1:640	≥1:1,280
ATCC 23406	IWGMT study ^b	1:640	1:2,560
161-4	Fish tank, Cleveland, Ohio, Aquarium	1:320	1:320

^a Our stock culture from same source as culture P-6 (patient at Trudeau Sanatorium).

^b International Working Group on Mycobacterial Taxonomy; received as part of a cooperative study.

TABLE 3. *Agglutination titers with proposed Kowal serotype of M. gordonae*

Strain no.	Source ^a	Agglutination titer to antiserum:	
		144	154
154	Kowalczyk, sputum, CMGH	>1:1,280	≥1:2,560
144	Tap water, CMGH	>1:2,560	>1:2,560
360	ATCC 14470 (type culture), Runyon P 15	≥1:2,560	1:1,280
ATCC 23398	IWGMT ^b study, tap water	1:1,280	1:1,280
ATCC 23399	IWGMT study	1:2,560	1:2,560
997	Jones, sputum, CMGH	>1:1,280	1:640

^a For abbreviations, see footnote a, Table 1.

^b See footnote b of Table 2.

TABLE 4. *Agglutination titers with proposed Marshall serotype of M. gordonae*

Strain no.	Source ^a	Agglutination titer to antiserum:			
		46	64	48	778
46	Marshall, lung resection, UHC	1:640	1:320	≥1:2,560	1:1,280
64	Wohl, lung resection, UHC	1:320	1:160	1:1,280	1:320
48	O'Neil, sputum, CMGH	1:320	1:160	≥1:1,280	1:1,280
778	Jolly, gastric, CMGH	1:160	<1:80	≥1:2,560	>1:2,560
4	Crawley, sputum, CMGH	1:1,280	1:640	≥1:2,560	1:2,560
1060	Media contaminant, Bacty Lab, CMGH	1:1,280	1:1,280	1:1,280	1:1,280
1064	Ruttencutter, sputum, CMGH	1:160	1:320	1:1,280	1:320
34	Strains recovered from various water sources: taps, drinking fountains, zoo, and aquarium ^b			1:1,280	1:1,280

^a For abbreviations, see footnote *a*, Table 1.^b Goslee and Wolinsky, *Am. Rev. Resp. Dis.*, in press. Once the four type strains were established as one serotype, only the #48 and #778 sera were used to agglutinate the water strains.

ceum serotypes were observed, nor were there any significant cross-reactions with other species of mycobacteria or their antisera.

Seven *M. gordonae* serotypes were recognized, as depicted in Tables 3 to 9. Their agglutinations were all clear-cut, and no significant cross-reactions among these serotypes were observed. A cross-reaction between serotype Marshall and *Mycobacterium avium* complex serotype 4 was seen, but absorption of the type 4

TABLE 5. *Agglutination titers with proposed Szent serotype of M. gordonae*

Strain no.	Source ^a	Agglutination titer to antiserum:	
		186	187
187	Szentendrei, tonsil, CMGH	>1:640	>1:1,280
138	Grady, sputum, CMGH	>1:1,280	>1:1,280
186	Pentek, tonsil, CMGH	>1:1,280	>1:1,280
281	Soil, Cleveland, Ohio	≥1:1,280	>1:640
567	Soil, Cleveland, Ohio	>1:640	>1:640
569	Soil, Cleveland, Ohio	>1:640	>1:640
679	Croff, lymph node, CMGH	1:2,560	>1:2,560
781	Payne, gastric, CMGH	1:1,280	1:1,280
956	Oster, sputum, CMGH	>1:1,280	>1:1,280
ATCC 23285	IWGMT ^b study	>1:2,560	>1:2,560
ATCC 23415	IWGMT study	>1:1,280	>1:1,280
25-2	Water, Niagara River	1:640	1:1,280
73-1	Water, kitchen tap, Oxford, Ohio	1:640	≥1:2,560

^a For abbreviations, see footnote *a*, Table 1.^b See footnote *b*, Table 2.TABLE 6. *Agglutination titers with proposed Puntal serotype of M. gordonae*

Strain no.	Source ^a	Agglutination titer to antiserum:	
		430	848
50	Puntal, gastric, CMGH	1:640-1,280	1:1,280
40	Galbreath, sputum, CMGH	1:2,560	1:2,560
74	Swanson, lung, UHC	1:2,560	1:1,280
430	Soil, New Jersey	1:2,560	1:640
565	Soil	1:1,280	1:2,560
848	Legeza, sputum, CMGH	1:1,280	1:5,120
999	Leckwatch, sputum ^b		
	5/15/72	1:1,280	1:1,280
	8/2/72	1:1,280	1:640
	11/3/72	1:1,280	1:320-640
1077	Leckwatch, sputum ^b		
	4/18/73	1:2,560	1:1,280
	7/15/73	1:2,560	1:1,280
	9/17/73	1:1,280	1:640

^a For abbreviations, see footnote *a*, Table 1.^b Repeated isolations, probably representing tracheobronchial colonization rather than disease association, from a patient at CMGH and Cuyahoga County Clinic.

serum with Marshall strains produced a negligible reduction in the type 4 homologous titer. It is noteworthy that the four Brown serotype cultures had an optimum growth temperature of 30 to 34 C.

The *M. marinum* strains could be divided into two agglutination groups. The results for type 1 are given in Table 10. It may be seen that a uniform titer of agglutination was not achieved by the five typing sera, including two made in this laboratory and three supplied by Schaefer. Indeed, even repeat tests with the same strain and serum sometimes gave discrep-

TABLE 7. *Agglutination titers with proposed Moore serotype of M. gordonae*

Strain no.	Source	Agglutination titer to antiserum:			
		429	610	595	613
429 ^a	Soil, Moore, Cleveland, Ohio	>1:2,560			
610 ^a	Soil, Cleveland, Ohio	1:1,280	>1:1,280		
595	Soil, Cleveland, Ohio	1:2,560	1:1,280	1:1,280	≥1:5,120
613	Soil, Cleveland, Ohio	1:2,560	1:640	1:640	1:1,280
616	Soil, Cleveland, Ohio	≥1:5,120	1:2,560	1:1,280	≥1:5,120
634	Soil, Cleveland, Ohio	1:1,280	1:1,280	1:5,120	1:5,120

^a These cultures were lost before other antisera were made.

TABLE 8. *Agglutination titers with proposed Lanton serotype of M. gordonae*

Strain no.	Source ^a	Agglutination titer to antiserum:	
		617	949
949	Lanton, gastric, CMGH	≥1:640	1:640
617	Soil, Cleveland, Ohio	1:640	1:320
998	Lanton, sputum, Cuyahoga County Clinic	1:1,280	1:1,280
87-1	Water, drinking fountain, Cleveland, Ohio (liter sample)	≥1:2,560	≥1:2,560
87-2 ^b	Water, drinking fountain, Cleveland, Ohio (liter sample)	≥1:1,280	≥1:2,560
87-3 ^b	Water, drinking fountain, Cleveland, Ohio (liter sample)	1:640	1:2,560
88-1	Water, drinking fountain, Cleveland, Ohio (swab sample)	1:2,560	1:2,560
88-2 ^b	Water, drinking fountain, Cleveland, Ohio (swab sample)	≥1:2,560	≥1:2,560
88-3 ^b	Water, drinking fountain, Cleveland, Ohio (swab sample)	≥1:2,560	≥1:2,560
88-4 ^b	Water, drinking fountain, Cleveland, Ohio (swab sample)	≥1:2,560	≥1:2,560

^a For abbreviations, see footnote a, Table 1.

^b Different colonies from original isolation media.

TABLE 9. *Agglutination titers with proposed Brown serotype^a of M. gordonae*

Strain no.	Source ^b	Agglutination titer to antiserum:	
		209	1083
1083	Brown, sputum, CMGH (5/26/73)	≥1:1,280	≥1:1,280
1084	Brown, sputum, CMGH (5/24/75)	1:1,280	1:1,280
209	Soil, Shaker Lakes, Cleveland, Ohio	1:1,280	1:1,280
1095	Leindecker, gastric, Euclid General Hospital, Cleveland, Ohio	≥1:1,280	≥1:1,280

^a All strains in this serotype had an optimum growth temperature of 30 to 34 C.

^b For abbreviations, see footnote a, Table 1.

glutinate upon repeated subculture, and no titer could be obtained with our type 2 serum pair. Cross-absorptions between type 1 and 2 cultures and sera were performed. The type 2 strains rarely reacted with type 1 sera, and when this occurred the cross-reacting antibody was easily absorbed out with only a small reduction in homologous titer. Similarly, absorption of our type 2 serum 962 with our type 1 strain 338 resulted in no reduction of the homologous titer.

Table 12 shows the serological reactions of the 10 *M. xenopi* strains in our collection which could be suspended smoothly without spontaneous agglutination. For the most part, these strains reacted equally with all four sera (our pair and Schaefer's pair). Absorption of agglutinins was performed with an additional seven rough strains which were not suitable for agglutination, and the results of a representative set of absorptions are shown in Table 13. The two strains from which our serum pair was derived (351, 668) removed the *M. xenopi* agglutinins from their own sera and did not agglutinate significantly with Schaefer's homologously absorbed sera. A similar relationship was found between Schaefer's two strains and our serum

ant results. Similar inconsistencies were noted with sera made from three other strains, numbers 336, 344, and 871.

The reactions of *M. marinum* type 2 are presented in Table 11. Only one of the strains reacted strongly with all four type 2 sera, but 1010 and 1111 were not agglutinated by Schaefer's serum 799. Culture 1183 tended to autoag-

TABLE 10. *Agglutination titers with proposed serotype 1 of M. marinum*

Strain no.	Source ^a	Agglutination titer to antiserum:							
		338	347	931 ^b	661 ^b	662 ^b	336	344	871
338	Our serotype strain, Runyon 1328	≥1:1,280	1:80	≥1:640	1:320	1:320	1:80	1:320	1:40
347	Our serotype strain, Runyon 2733	≥1:2,560	1:320	≥1:640	≥1:640	≥1:640	1:640	1:1,280	1:320
336	Runyon 1162	≥1:1,280	Nil ^c	≥1:640	1:320	Nil	1:160 ≥1:1,280 ^d	1:80	Nil
344	Runyon 2337	≥1:1,280	Nil 1:640	≥1:640	≥1:640	≥1:640	1:160	≥1:1,280 1:320	1:160
871	Ebenger, hand abscess, CMGH	≥1:2,560	≥1:2,560	1:320	1:160	Nil	1:640	1:1,280	≥1:1,280
341	Runyon 2312, originally from W. F. Shinn (Magnusson 172)	1:320 1:1,280	1:80 1:80 1:160	1:640	1:640	Nil	1:320	1:320	Nil
928	Ellender, Schaefer's 661 and 662 ^b	≥1:1,280	≥1:640	≥1:640	≥1:640	≥1:640	≥1:1,280	≥1:1,280	≥1:1,280
1052	Russo, scratch from aquarium, University Hospital, Cleveland, Ohio	1:640	≥1:1,280 Nil	≥1:640	≥1:640	1:320	1:320	1:160	≥1:1,280
1181	Hernandez, Schaefer's 931 ^b	≥1:1,280	1:80	≥1:640	1:160	Nil	1:640	1:320	1:320

^a For abbreviations, see footnote *a*, Table 1.^b Provided by the U.S.-Japan Cooperative Medical Science Program, NIAID, through W. B. Schaefer.^c Designates a titer of less than 1:40.^d Two figures indicate different titers obtained on repeat tests.TABLE 11. *Agglutination titers with proposed serotype 2 of M. marinum*

Strain no.	Source ^a	Agglutination titer to:			
		Our sera		Schaefer's sera ^b	
		962	1010	712	799
962	Fox, bursa, UHC	≥1:1,280	≥1:1,280	≥1:640	≥1:640
1010	Mattingley, skin, Maryland	≥1:1,280	1:320	≥1:640	Nil ^c
1111	Francinques, Schaefer's 712 ^b	≥1:1,280	1:640	≥1:640	Nil
1183	Geraci, Schaefer's 799 ^b	— ^d	— ^d	1:640	1:1,280

^a For abbreviations, see footnote *a*, Table 1.^b Provided by the U.S.-Japan Cooperative Medical Science Program, NIAID.^c Designates a titer of less than 1:40.^d Culture autoagglutinated.

pair. The "unknown" strain 1155 removed all agglutinins from Schaefer's sera, but it absorbed relatively little of the agglutinins from our 351 serum (usually a twofold drop in homologous titer on repeated testing) and only slightly more (fourfold drop) from our 668 serum. The other six rough strains reacted in a similar fashion.

DISCUSSION

A fourth serotype of *M. scrofulaceum*, serotype Cole, has been proposed. Of the five strains in this group, two originated from the

same patient, although they arrived in the collection by different routes. These strains and the White strain (356) were considered to be human pathogens. ATCC 23406 was received as part of an international cooperative study, and its association with human disease is uncertain. One strain was recovered from aquarium water.

M. gordonae is an environmental saprophyte which is not usually associated with disease in man. Gunthorpe and Stanford (1) found five species-specific antigens by immunodiffusion, and Pattyn et al. (3) studied over 30 strains,

TABLE 12. Agglutination titers with proposed *M. xenopi* serotype

Strain no.	Source ^a	Agglutination titer to:			
		Our sera		Schaefer's sera ^b	
		351	668	927	907
351	NCTC 10042 (type strain)	1:640	1:1,280	1:1,280	1:640
668	Runyon: Montana 3244	1:320	1:1,280	1:320	1:160
669	Runyon: Arkansas 3254	1:320	1:320	1:320	1:160
967	Keyerleber, sputum, CMGH	1:640	1:320	1:320	1:320
1127	Hawkins: water, 74-0202, West Haven, Conn.	1:640	1:640	1:1,280	1:1,280
1130	Hawkins: Faucher, sputum, 74-0704	1:320	1:640	1:1,280	1:1,280
1131	Hawkins: Faucher, sputum, 74-0781	1:320	1:640	1:640	1:640
1132	Hawkins: MacNevin, sputum, 74-0782	1:320	1:640	1:1,280	1:1,280
1173	Schaefer's 927: originally from Hawkins 72-1097	1:640	1:640	1:640	1:320
1174	Schaefer's 907: Cardiff 25592	1:640	1:640	1:80	1:160

^a For abbreviations, see footnote a, Table 1.^b Provided by the U.S.-Japan Cooperative Medical Science Program, NIAID.TABLE 13. *M. xenopi*: results of absorptions with homologous strain and with strain 1155^a of our serum pair (351, 668) and Schaefer's serum pair (907, 927)^b

Strain no.	Source	Agglutination titer to antiserum:										
		351 ^c	351	668	668	668	927	927	927	907	907	907
		351	1155	668	668	1155	927	1173	1155	907	1174	1155
351	NCTC 10042	Nil ^d	1:320	1:80	Nil	— ^e	1:80	Nil	—	1:320	Nil	—
668	Runyon: Montana 3244	Nil	—	1:320	Nil	1:80	1:320	Nil	—	1:160	Nil	—
1173	Schaefer's 927 ^b	Nil	—	1:640	Nil	—	1:640	Nil	Nil	1:320	Nil	—
1174	Schaefer's 907 ^b	Nil	—	1:640	Nil	—	1:80	Nil	—	1:160	Nil	Nil

^a Received from Hawkins, West Haven, Conn.^b Provided by the U.S.-Japan Cooperative Medical Science Program, NIAID.^c Indicates serum 351 absorbed with strain 351.^d Designates a titer of less than 1:40.^e Agglutination not done.

mostly recovered as laboratory contaminants, by agglutination with four antisera, but neither group of investigators separated the species into serotypes. The results presented herein indicate that at least seven agglutination types may be recognized. No single serotype or group of serotypes contained a larger proportion than the others of the human-derived strains. Most of the *M. gordonae* strains in our collection were not typable with the seven serum pairs (unpublished observations), but the majority of the typable strains were of the Marshall serotype. In particular, of 80 *M. gordonae* strains recovered from water, 34 were serotype Marshall, 34 were not typable with the sera used, and the remaining 12 were distributed among the other six types (Showalter and Wolinsky, submitted for publication). Despite its evident antigenic heterogeneity, it may be possible to delineate additional serotypes of *M. gordonae* by further investigation.

Two types of *M. marinum*, 1 and 2, were separated clearly by absorptions, but several

M. marinum strains were agglutinated by only one of the pair of our as well as of Schaefer's type sera. Three of these strains were selected as possible representatives of a third type. After making antisera to these strains, it was found that the questionable strains and their antisera were closely related, but probably not identical, to type 1. Further investigation will be necessary to clarify these relationships.

The agglutinations of *M. xenopi* gave somewhat conflicting results. Absorptions indicated that our type strains and Schaefer's type strains were antigenically homogeneous. Similarly, the "unknown" strains absorbed most of the agglutinins from Schaefer's sera. With our sera, however, these strains absorbed relatively little agglutinating activity, causing a drop in homologous titer no greater than four-fold. Thus, it was not possible by the techniques used to clearly recognize more than one type among the *M. xenopi* strains included in this study.

The results with *M. marinum* and *M. xenopi*

emphasize the importance of using at least a pair of antisera to establish the serotype of an unknown strain, as recommended by Schaefer (4). It may be desirable to use a trio of typing sera for certain more broadly reactive groups.

The proposed new serotypes could be included in the suggested numbering scheme (7) as follows: *M. avium* complex, serotype 44 (*M. scrofulaceum*, Cole); *M. gordonae*, serotypes 1 to 7 (Kowal, Marshall, Szent, Puntal, Moore, Lanton, and Brown, respectively); *M. marinum*, serotypes 1 and 2; and *M. xenopi*.

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REPRINT REQUESTS

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