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A Co-operative Numerical Analysis of Nonscoto- and Nonphotochromogenic Slowly Growing Mycobacteria

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SUMMARY

A co-operative taxonomic study has been performed on slowly growing non-pigmented mycobacteria (Runyon's group III). Phenetic data on 89 strains, studied in 18 laboratories, were collected and analysed by a numerical taxonomic method. A variety of immunological properties, lipid analyses and measures of pathogenicity were analysed independently to establish correlation with numerical classification. *Mycobacterium gastri*, *M. nonchromogenicum*, *M. terrae*, *M. avium* and *M. xenopi* were recognized by almost all participants as distinct species. *Mycobacterium novum* was considered to be synonymous with *M. terrae*. A clearcut distinction could not be made between *M. avium* and *M. intracellulare*; the majority of participants in the study recommend that *M. intracellulare* be reduced to a synonym of *M. avium*. A minority of authors cannot agree with this proposal.

INTRODUCTION

Because of the variety and complexity of the tests used for classifying and identifying mycobacteria, the International Working Group on Mycobacterial Taxonomy (IWGMT) has undertaken a number of co-operative taxonomic studies. This has permitted pooling of data for numerical analysis and has enhanced our understanding of the status of species of slowly growing scotochromogenic mycobacteria (Wayne *et al.* 1971) and rapidly growing mycobacteria (Kubica *et al.* 1972).

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Slowly growing mycobacteria which produce little or no pigment fall into Runyon's group III (Wayne, Runyon & Kubica, 1969) but the status of certain species within this group is uncertain. Thus Wayne (1966, 1967) and Kubica, Silcox & Hall (1973) have proposed that *Mycobacterium intracellulare* be reduced to a synonym of *M. avium*, whereas Runyon (1967) has argued for the retention of these organisms as separate species. Similarly, there remains some ambiguity about the distinction between *M. terrae* and *M. nonchromogenicum* and between *M. gastri* and nonpigmented strains of *M. kansasii* (Wayne, 1967; Norlin, Lind & Ouchterlony, 1969). In the face of these unresolved problems, the IWGMT undertook a co-operative study on the taxonomy of mycobacteria which meet the criteria for inclusion in Runyon's group III. This is a report on that study, in which investigators from 18 different laboratories (coded A to R) participated. The investigation was carried out under the permissive philosophy and the other ground rules presented in the report of the prior study on scotochromogenic mycobacteria (Wayne *et al.* 1971).

METHODS

The 89 cultures examined in this study were selected from the collection of the two co-ordinators, G. Meissner and K. H. Schröder. Most cultures met the criteria for Runyon's group III, although members of other groups were included for comparison. The type strains of species under study were included whenever possible. The cultures were processed, coded and distributed by the American Type Culture Collection (ATCC) as described earlier (Wayne *et al.* 1971).

Eight laboratories submitted a total of 292 characters per culture: these 292 characters were converted to binary form (i.e. 1 or 0), suitable for numerical analysis. Data on immunology, pathogenicity and lipid patterns were not included in the numerical analysis, but were reserved for tabulation and correlation with the clustering behaviour of the test strains. After 'repetitious' and 'irrelevant' data (Wayne *et al.* 1971) were deleted there remained 118 characters, which were actually used in the numerical analysis of pooled data to yield a conventional $n \times n$ matrix table. A preliminary sequence was established by highest single link and subsequent re-sorting was performed manually to yield clusters of maximum internal density on the 85 % printout. Negative matches were included.

Such matrices were also prepared for each individual laboratory without, however, deletion of any of the repetitious or irrelevant data. Two additional laboratories (I and J) submitted data after the numerical analysis was completed but before the code was broken. These data were treated in the analysis of clustering behaviour observed by individual laboratories, but were not included in the pool. Inspection of the raw data submitted by these investigators suggested that no important additional information was lost by omitting them from the pool.

After completion of the numerical analysis it was possible to identify and tabulate those characters which permitted differentiation of clusters derived from the study.

The ground rules for participation in the study, and the rules of anonymity adopted for the prior IWGMT studies (Wayne *et al.* 1971; Kubica *et al.* 1972) were applied to the present study.

RESULTS

Numerical taxonomy based on 118 pooled characters separated the 89 strains into 8 clusters segregated at the 70 to 85 % matching (m.) level (Figs. 1 to 4). The mean internal matching score (m.i.m.s.) of each cluster and mean matching score (m.m.s.) between clusters

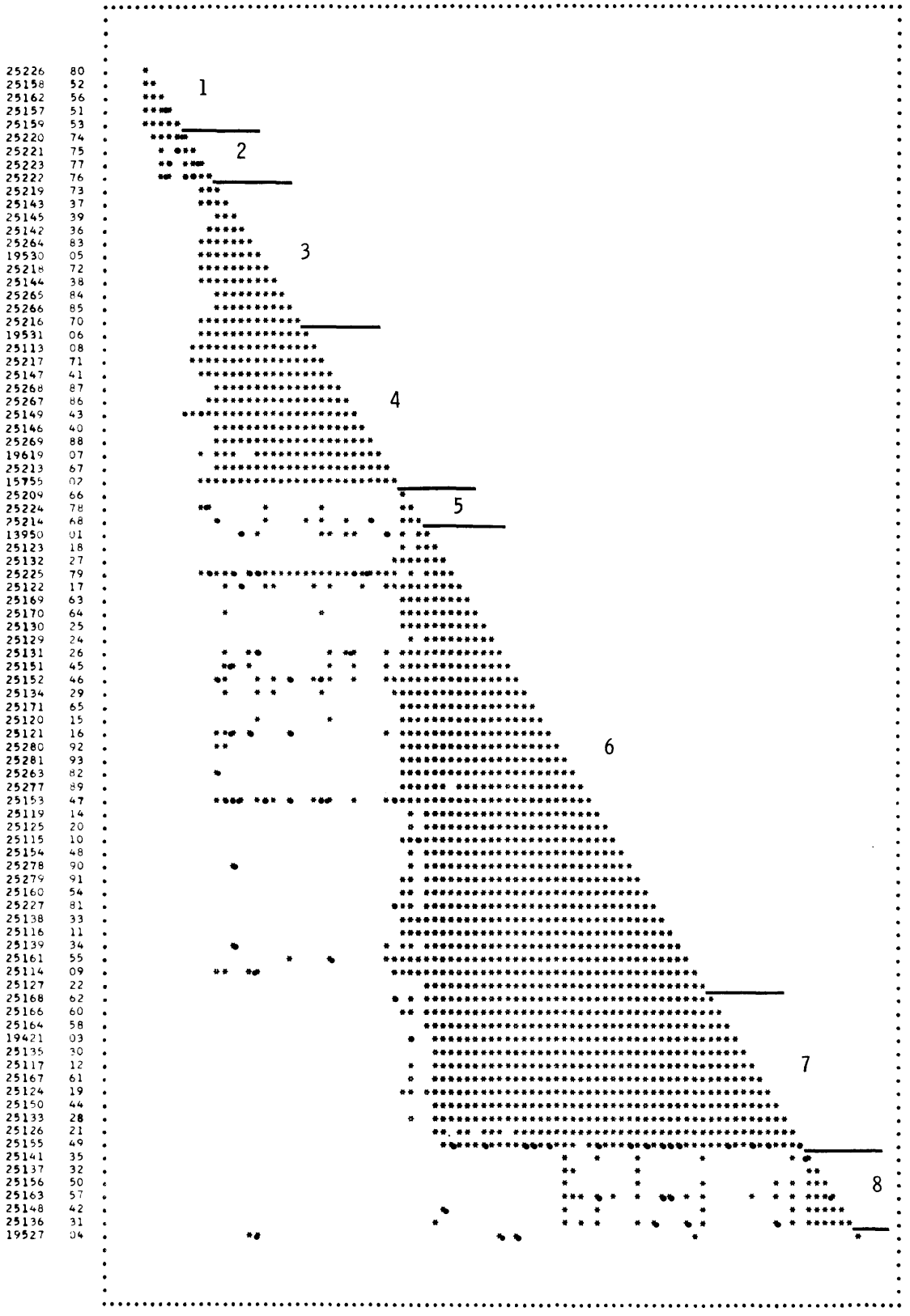

Fig. 1. Evolution of clusters at the 70 % matching score level, by numerical analysis of 118 pooled phenetic characters.

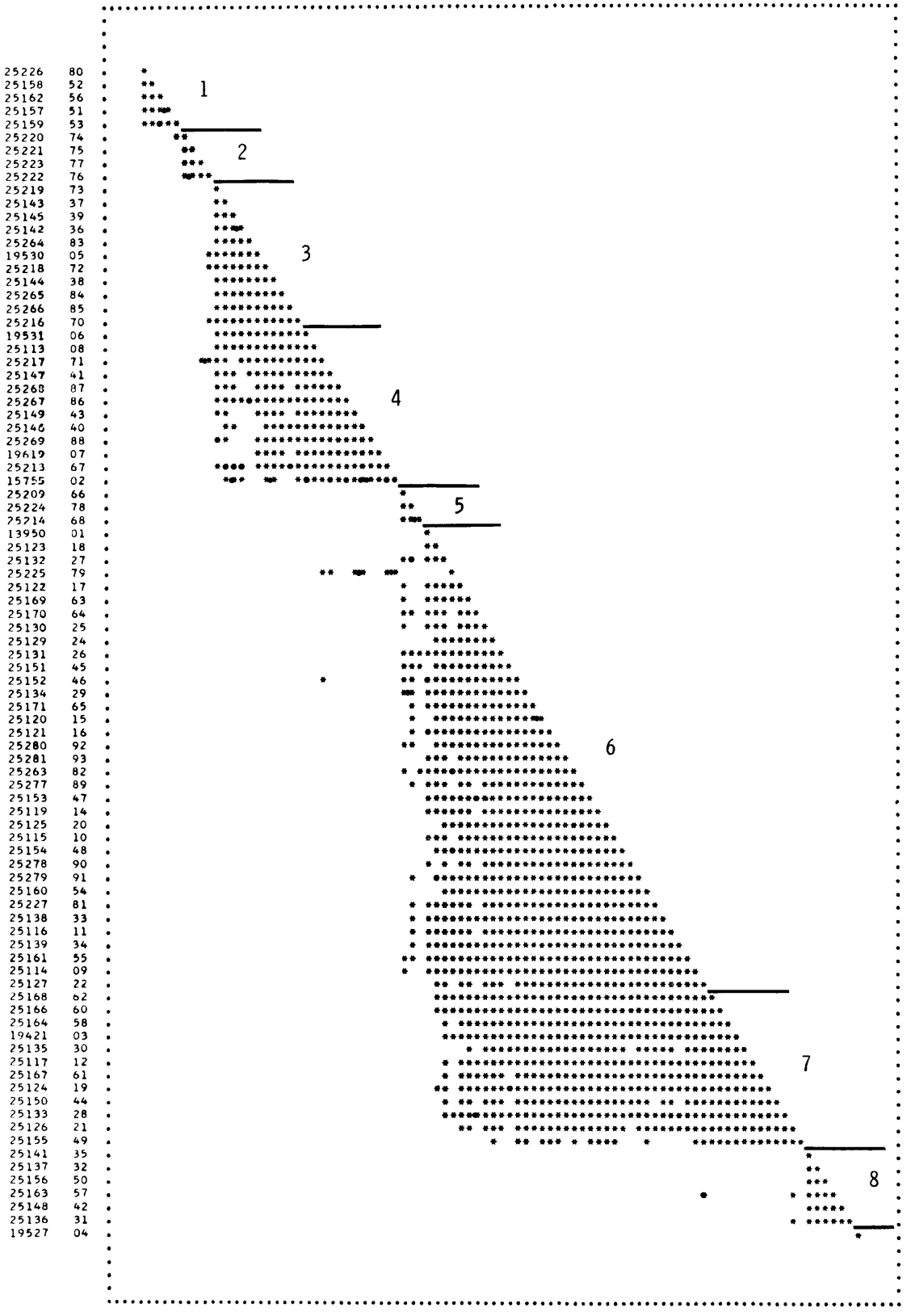

Fig. 2. Evolution of clusters at the 75 % matching score level, by numerical analysis of 118 pooled phenetic characters.

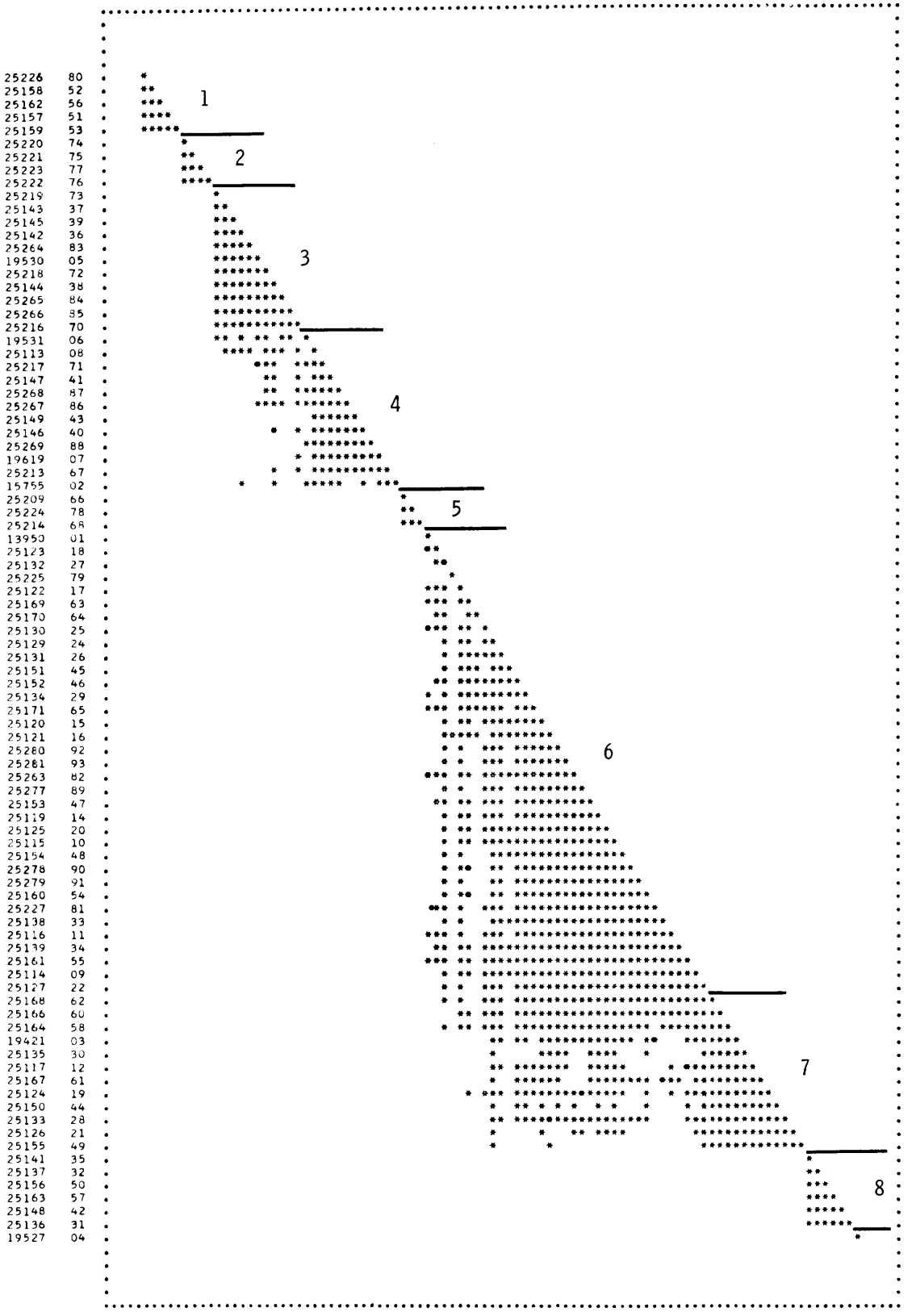

Fig. 3. Evolution of clusters at the 80 % matching score level, by numerical analysis of 118 pooled phenetic characters.

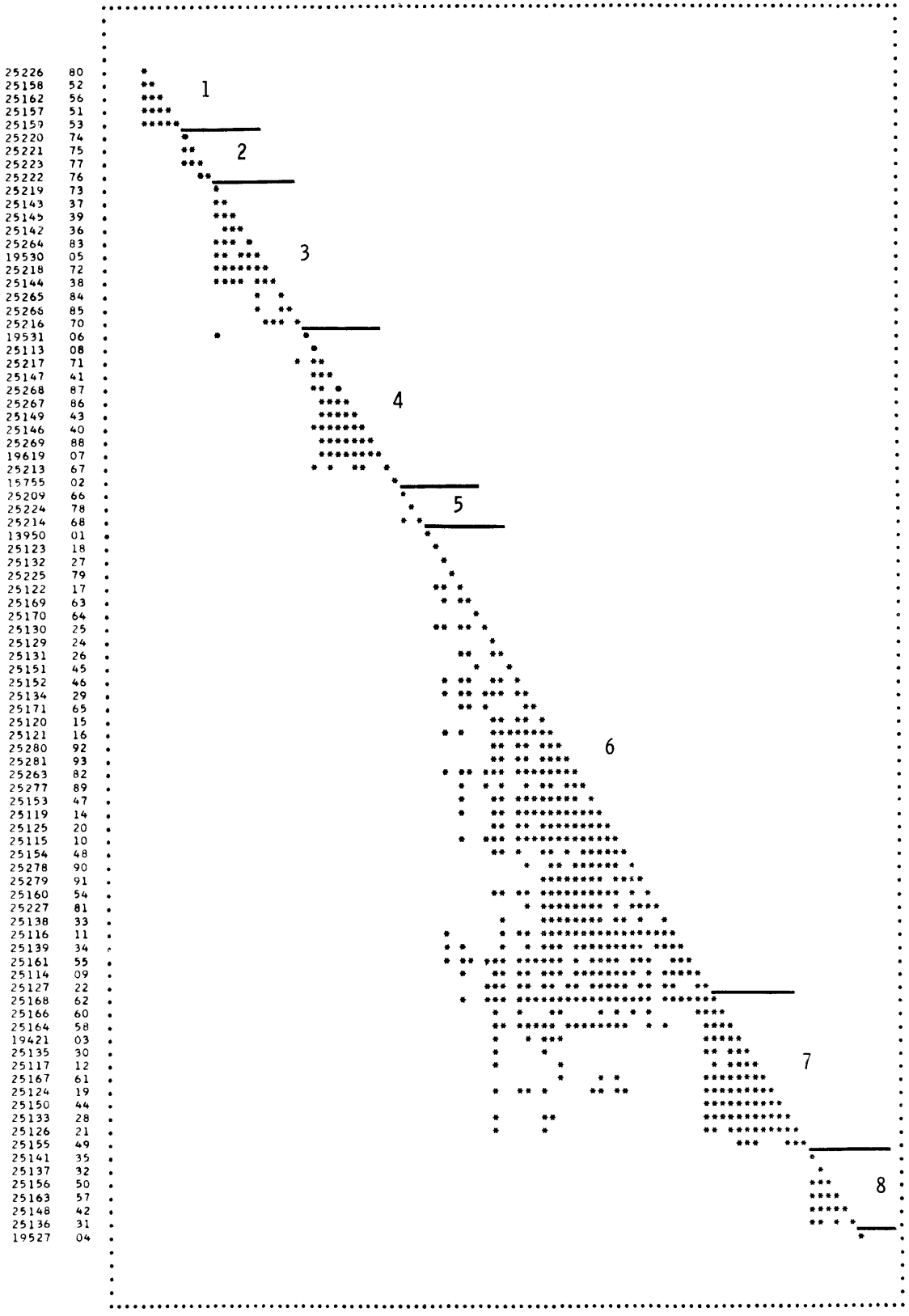

Fig. 4. Evolution of clusters at the 85 % matching score level, by numerical analysis of 118 pooled characters.

Table 1. *Matching scores (% m.) (below and left of diagonal) and actual numbers of characters difference (above and to right of diagonal), expressed as mean intra- and intertaxon values for the eight clusters as identified in the original analysis of pooled data*

The matching scores are rounded to the nearest 1 % and the characters are rounded to the nearest whole numbers.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	9 / 91	33	47	45	46	52	53	45
M. kansasii (non-pigmented)	2	69	14 / 87	37	35	40	43	48	56
M. nonchromogenicum	3	57	67	16 / 86	24	38	36	40	60
M. terrae	4	57	68	77	16 / 85	37	37	40	58
M. scrofulaceum	5	57	64	66	66	19 / 83	29	34	49
M. intracellulare	6	50	60	66	65	73	16 / 84	20	37
M. avium	7	48	55	62	61	67	80	11 / 88	35
M. xenopi	8	57	49	46	46	56	65	66	15 / 86

Pooled data, 118 characters; m.i.m.s. 86 %, m.m.s. 71 %.

for the pooled analysis have been calculated in terms of percentages and of actual numbers of characters (Table 1). Only one culture, *M. thermoresistible* ATCC19527 which is a rapid grower, failed to fit any cluster at a 70 % level. In some cases the clusters corresponded to single named species. In other cases, however, the clustering behaviour failed to support separate species status for strains bearing different names and thus supported contraction of certain species to synonymy.

The identities of the clusters derived from the analysis of pooled data and a brief description of some definitive characteristics are as follows:

Cluster 1 consists of strains of *M. gastri* and had a m.i.m.s. of 91 % and a m.m.s. of 69 % or less to all other clusters.

Cluster 2 consists of strains of nonpigmented *M. kansasii* and had a m.i.m.s. of 87 % and

a m.m.s. of 69 % or less to all other clusters. This cluster is most readily distinguished from *M. gastri* by nitrate reduction, thermostable, high catalase activity, drug susceptibility, and agglutination and lipid patterns.

Cluster 3 includes the type strain of *M. nonchromogenicum* Tsukamura (ATCC19530). It had a m.i.m.s. of 86 %, a m.m.s. to *M. terrae* Wayne of 77 % and a m.m.s. to all other clusters of 67 % or less. Properties which support separation from *M. terrae* are nicotinamidase, pyrazinamidase and, to a lesser extent, aryl sulphatase.

Cluster 4 includes the type strain of *M. terrae* Wayne (ATCC15755) and the type strain of *M. novum* Tsukamura (ATCC19619). It had a m.i.m.s. of 85 % and a m.m.s. to all clusters other than *M. nonchromogenicum* of 68 % or less. *M. novum* appears to be synonymous with *M. terrae* Wayne. Thus there is evidence that the distinction between *M. terrae* Wayne and *M. nonchromogenicum* Tsukamura is sufficient to justify continued separation into two species.

Table 2. *Mean matching scores (% m.) and mean actual numbers of characters difference within and between clusters, as determined from separate analysis of the entire data from each laboratory participating in the study*

Discrepancies between numbers of characters difference and % m. for any comparison are a consequence of missing data, either single test results or complete omission of a given culture. Thus the number of characters recorded for each laboratory represent the *maximum* number of different characters determined per culture in that laboratory.

(*a*) Lab A, 57 characters; m.i.m.s. 87 %, m.m.s. 68 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	1 / 97	14	20	18	17	18	18	13
M. kansasii (non-pigmented)	2	64	8 / 82	16	15	15	12	11	18
M. nonchromogenicum	3	47	58	3 / 90	8	11	8	8	15
M. terrae	4	48	58	76	3 / 90	12	9	9	19
M. scrofulaceum	5	56	63	68	65	8 / 80	9	9	16
M. intracellulare	6	38	57	72	64	71	6 / 83	7	14
M. avium	7	34	55	69	61	69	81	3 / 90	14
M. xenopi	8	66	51	57	44	58	64	63	7 / 86

Table 2 (*cont.*)

(*b*) Lab B, 34 characters; m.i.m.s. 79 %, m.m.s. 70 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	2 / 94	5	7	7	6	10	13	9
M. kansasii (non-pigmented)	2	79	6 / 76	7	8	8	10	12	12
M. nonchromogenicum	3	73	74	4 / 84	5	10	8	10	10
M. terrae	4	72	72	80	6 / 78	9	8	10	10
M. scrofulaceum	5	78	72	63	66	6 / 81	10	14	10
M. intracellulare	6	62	64	68	69	64	8 / 71	9	9
M. avium	7	52	58	61	62	55	69	7 / 76	11
M. xenopi	8	64	55	61	61	63	64	60	8 / 69

(*c*) Lab C, 36 characters; m.i.m.s. 87 %, m.m.s. 78 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	3 / 88	3	7	7	8	9	10	10
M. kansasii (non-pigmented)	2	87	2 / 92	6	6	6	7	7	9
M. nonchromogenicum	3	71	75	4 / 87	4	8	6	7	10
M. terrae	4	72	76	85	5 / 84	8	6	6	10
M. scrofulaceum	5	67	76	69	70	6 / 80	8	7	10
M. intracellulare	6	62	72	76	78	70	4 / 83	4	7
M. avium	7	59	71	74	76	72	83	3 / 89	5
M. xenopi	8	59	63	60	58	61	70	78	1 / 94

Table 2 (*cont.*)

(*d*) Lab D, 31 characters; m.i.m.s. 92 %, m.m.s. 77 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	0 / 100	5	7	10	11	14	14	16
M. kansasii (non-pigmented)	2	85	1 / 97	6	5	10	15	16	19
M. nonchromogenicum	3	78	79	2 / 93	6	10	10	10	15
M. terrae	4	69	82	81	3 / 90	14	13	13	19
M. scrofulaceum	5	65	67	67	53	1 / 97	9	10	11
M. intracellulare	6	55	52	67	57	71	3 / 90	3	6
M. avium	7	55	48	67	56	68	90	2 / 94	6
M. xenopi	8	48	40	51	40	64	80	79	1 / 98

(*e*) Lab E, 41 characters; m.i.m.s. 83 %, m.m.s. 72 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	7 / 79	8	11	11	13	14	15	13
M. kansasii (non-pigmented)	2	74	6 / 83	9	9	12	13	15	17
M. nonchromogenicum	3	63	74	5 / 80	6	10	11	12	15
M. terrae	4	66	73	81	4 / 88	9	13	15	18
M. scrofulaceum	5	56	63	64	69	6 / 80	9	11	13
M. intracellulare	6	49	56	57	57	70	6 / 80	6	9
M. avium	7	46	47	50	48	62	79	3 / 90	8
M. xenopi	8	55	44	42	38	58	71	75	5 / 85

Table 2 (*cont.*)

(*f*) Lab F, 40 characters; m.i.m.s. 90 %, m.m.s. 77 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	2 / 95	9	11	11	13	15	16	10
M. kansasii (non-pigmented)	2	73	3 / 90	10	10	11	12	12	11
M. nonchromogenicum	3	65	69	4 / 88	4	10	11	12	11
M. terrae	4	65	69	88	2 / 92	10	10	10	10
M. scrofulaceum	5	61	66	68	69	5 / 87	7	7	7
M. intracellulare	6	51	60	63	66	80	6 / 81	6	6
M. avium	7	47	59	60	65	78	81	3 / 91	6
M. xenopi	8	61	54	54	58	72	77	76	2 / 93

(*g*) Lab G, 36 characters; m.i.m.s. 86 %, m.m.s. 78 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	5 / 87	4	11	10	9	10	9	11
M. kansasii (non-pigmented)	2	89	2 / 95	9	9	8	8	9	12
M. nonchromogenicum	3	68	73	5 / 87	8	10	9	10	15
M. terrae	4	73	73	76	6 / 83	10	9	8	11
M. scrofulaceum	5	72	75	70	69	4 / 88	5	9	11
M. intracellulare	6	72	75	74	74	84	4 / 84	7	9
M. avium	7	74	73	71	78	74	81	4 / 88	9
M. xenopi	8	67	64	57	69	67	74	74	5 / 84

Table 2 (*cont.*)

(*h*) Lab H, 42 characters; m.i.m.s. 88 %, m.m.s. 77 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	6 / 86	11	16	15	12	15	15	12
M. kansasii (non-pigmented)	2	72	4 / 90	9	8	12	13	15	14
M. nonchromogenicum	3	61	78	5 / 88	5	13	10	10	14
M. terrae	4	63	79	87	5 / 88	12	10	11	15
M. scrofulaceum	5	69	72	68	70	5 / 88	11	11	10
M. intracellulare	6	64	67	74	76	74	5 / 86	7	9
M. avium	7	64	63	75	74	72	82	5 / 88	11
M. xenopi	8	68	64	63	63	73	78	73	4 / 90

(*i*) Lab I, 40 characters; m.i.m.s. 90 %, m.m.s. 75 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	2 / 94	12	12	11	11	14	14	12
M. kansasii (non-pigmented)	2	67	3 / 91	7	9	8	8	8	16
M. nonchromogenicum	3	67	79	1 / 96	5	12	8	8	18
M. terrae	4	70	73	85	5 / 87	12	11	11	19
M. scrofulaceum	5	69	76	65	64	4 / 89	9	9	11
M. intracellulare	6	59	76	75	67	75	3 / 90	3	12
M. avium	7	59	76	77	67	75	91	2 / 94	13
M. xenopi	8	63	55	46	44	68	65	65	6 / 83

Table 2 (*cont.*)

(*j*) Lab J, 50 characters; m.i.m.s. 84 %, m.m.s. 75 %.

Cluster name	No.	1	2	3	4	5	6	7	8
M. gastri	1	4 / 90	10	15	17	14	17	17	18
M. kansasii (non-pigmented)	2	80	5 / 89	11	14	14	13	15	17
M. nonchromogenicum	3	69	78	7 / 84	10	15	13	15	16
M. terrae	4	65	72	78	9 / 81	18	15	15	16
M. scrofulaceum	5	70	70	69	64	8 / 83	12	13	13
M. intracellulare	6	64	72	73	69	74	8 / 83	10	12
M. avium	7	64	68	70	69	73	79	9 / 81	12
M. xenopi	8	62	66	67	66	72	74	75	8 / 83

Cluster 5 consists of three strains of *M. scrofulaceum*. These were included for comparative purposes. The species was described in detail in a previous co-operative study (Wayne *et al.* 1971). This small cluster had a m.i.m.s. of 83 % and a m.m.s. of 73 % or less to all other clusters.

Clusters 6 and 7 had m.i.m.s. values of 84 and 88 %, respectively, and an 80 % m.m.s. between them. Cluster 6 includes the original strain of *M. intracellulare* (ATCC13950) and cluster 7 the reference culture of *M. avium* (ATCC19421). With the exception of their great similarity to one another and a 73 % m.m.s. between clusters 5 and 6, these strains had m.m.s. values of 67 % or less to other clusters. The characters used for this numerical analysis do not support continued separation of these clusters into two species. As will be discussed later in this paper, some distinctions are seen in terms of immunologic and lipid analyses.

Cluster 8 consisting of strains labelled *M. xenopi* (Schwabacher, 1959) had a m.i.m.s. of 86 % and a m.m.s. of 66 % or less to all other clusters. This cluster is most readily distinguished from clusters 6 and 7 by colonial and cellular morphology, drug susceptibility, aryl sulphatase activity, growth temperatures, and immunologic tests.

The summaries of results of numerical analysis performed independently on data from each of ten laboratories are presented in Table 2. The number of characters determined by individual laboratories ranged from 31 to 57. Optimal taxonomic resolution would be

reflected in a maximum difference between percentage homogeneity of a cluster (m.i.m.s.) and mean percentage matching to other clusters (m.m.s.). This difference ranged from 9 to 19 % in different laboratories and does not appear to be dependent on the number of characters determined in each laboratory. However, the difference of means for the pooled data (Table 1) is 15 %, whereas the mean difference of means for the ten laboratories treated independently (Table 2) is only 12%. In general, the clustering seen in the pooled data is reflected in that seen with data from individual laboratories. The numbers for matching scores (% m.) and actual numbers of characters difference in Table 2 do not correspond exactly. Although the total number of characters is indicated for pooled data and for each laboratory, all data were not always available for all strains, and this introduced the discrepancy.

From the total of 118 test characters, 47 were selected as being the most definitive for separation of these taxa on the basis that at least 85 % of the strains in one or more clusters were positive for that character *and* at least 85 % of the strains in one or more of the remaining clusters were negative for that character. The reactions of the members of the seven clusters to these 47 characters (Table 3) showed that most cluster pairs could be differentiated from one another on the basis of characters derived from all types of tests included in the numerical analysis.

As indicated earlier, immunologic data, lipid patterns and pathogenicity data were not included in the numerical analyses, but were reserved for correlation with clustering behaviour after that analysis was completed. Immunologic data were submitted by 13 laboratories, in 8 of which no other tests were performed. Seven laboratories serotyped the strains by the whole cell agglutination method of Schaefer (1965). One of these laboratories, K, used direct agglutination with unabsorbed sera and with absorbed sera (Schaefer, 1967), and performed absorption tests with some of the cultures as well. The other laboratories used only the direct agglutination test with unabsorbed sera. Agglutination serotypes could not be established for clusters 3, 4 and 8 (*M. nonchromogenicum*, *M. terrae* and *M. xenopi*) because of spontaneous agglutination of these strains but were observed in the other five clusters (Table 4). The serotype numbering scheme used in this report is the one proposed by Wolinsky & Schaefer (1973) to replace the older scheme, which included names as well as arabic and roman numerals.

Some strains cross-reacted with more than one type of serum and columns are included in Table 4 to provide for such strains. Strains in clusters 1, 2 and 5 agglutinated as single, homogeneous serotypes. For cluster 6, there is a relatively heterogeneous population of 14 strains at the top portion of cluster 6 (strains 13950 to 25171) (see Figs. 1 to 4) and a more homogeneous population of 21 strains in the lower portion (strains 25210 to 25127); both groups gave different distributions of serotypes. The phenetically heterogeneous group is also serotypically heterogeneous (Table 4). The group with greater phenetic homogeneity shows a concentration of strains around a different set of serotypes; 69 % of the reports indicated *avium* complex serotype 8 (formerly serotype 'Davis') either alone or in a cross-reacting combination. Both of these subclusters yielded a few agglutinations corresponding to *avium* complex serotype 1 or 2/3. Ninety-five per cent of agglutination reaction reported for the 12 strains in cluster 7 corresponded to *avium* complex serotypes 1 or 2/3 or some cross-reacting combination of these. The 'original' strain of *M. intracellulare* in cluster 6 (ATCC-13950) could not be serotyped in laboratory K but was reported as *avium* complex serotype 16 in three other laboratories. The reference culture of *M. avium* in cluster 7 (ATCC19421) was consistently reported as *avium* complex serotype 2/3. Disagreements in serotyping between laboratory K and other laboratories were fairly common for cluster 6 strains

Table 3. *Distribution of 47 characters which show greatest resolving power among 7 clusters. Cluster 5 is not tabulated as only 3 strains of M. scrofulaceum were included for reference purposes*

Character	No.	Positive (%)						
		M. gastri	*M. kansasii* var. *album*	*M. nonchromogenicum*	*M. terrae*	*M. intracellulare*	*M. avium*	*M. xenopi*
		1	2	3	4	6	7	8
Enzymic activity								
Phosphatase	1	100	100	100	100	2	0	0
Tween hydrolysis, 5 day	2	100	100	100	100	0	0	0
α-Esterase	3	0	0	0	25	94	100	100
β-Esterase	4	0	0	36	16	97	100	100
Pyrazinamidase	5	0	0	81	0	97	100	100
Urease	6	100	100	0	16	2	0	0
Nicotinamidase	7	100	100	81	0	100	100	100
β-Galactosidase	8	0	0	100	100	2	0	0
Catalase, 68 °C	9	0	100	100	100	91	25	100
Catalase > 45 mm foam	10	0	100	100	100	14	0	0
Nitrate reduction, high	11	0	100	0	100	0	0	0
Nitrate reduction, very high	12	0	100	0	83	0	0	0
Aryl sulphatase, 3 day	13	0	0	0	0	0	0	100
Aryl sulphatase, 10 day	14	60	100	100	58	97	0	100
Nitrite reduction, 14 day	15	100	100	100	100	89	14	0
Tween opacity	16	0	50	100	100	100	100	100
Tellurite reduction	17	0	75	100	40	75	47	0
Growth								
At 22 °C	18	0	100	100	100	88	41	0
At 40 °C	19	0	75	90	9	77	100	100
At 25 °C	20	100	100	100	100	100	100	0
On glycerol agar	21	0	75	100	100	97	90	0
On glucose (glutamate N)	22	20	100	90	41	94	33	0
At 42 °C	23	0	0	27	0	71	91	100
At 45 °C	24	0	0	0	0	34	8	100
On propanol agar	25	60	25	0	66	94	8	83
Lebek niveau	26	80	0	81	100	84	100	100
Morphology								
Corded	27	100	100	0	0	0	0	0
Rods not coccoid	28	100	100	18	0	11	20	100
Smooth colony type	29	0	100	63	33	96	91	7
Xenopi colony type	30	0	0	0	0	0	0	100
Resistant to:								
Ethambutol, 5 μg	31	0	0	0	0	100	100	100
Oleate	32	0	0	90	100	100	100	100
Isoniazid, 10 μg	33	0	0	100	100	54	100	16
Thiosemicarbazone, 10 μg	34	0	0	100	100	87	100	66
Isoxyl	35	20	0	100	100	100	100	83
$NH_2OH.HCl$, 250 μg	36	20	0	100	100	94	100	66
Ethambutol, 1 μg	37	0	100	0	0	82	100	83
Chloramphenicol, 20 μg	38	0	100	90	100	70	66	50
Rifampin, 20 μg	39	0	100	50	0	79	100	33
Isoniazid, 1 μg	40	0	100	100	100	88	100	0
Streptomycin, 5 μg	41	0	100	90	90	92	100	0
Kanamycin, 20 μg	42	0	100	81	100	77	100	0
Ethionamide, 10 μg	43	0	50	0	100	88	100	0
Viomycin, 30 μg	44	0	75	100	100	74	100	0
p-Aminosalicylate, 10 μg	45	0	25	90	100	92	100	0
p-Nitrobenzoate	46	0	75	54	91	94	100	66
Ethionamide, 40 μg	47	0	50	0	0	46	100	50

Within each type of test character, the order has been arranged such that characters which achieve similar distinctions appear together.

Table 4. *Distribution of strains by phenetic clustering and agglutinating serotype*

Cluster	Species	No. strains in cluster	Lab(s)	No. reports by serotype																														
						Avium complex serotype no.																														
				'gastri'	*'kansasii'*	1	2/3*	1+2/3	4	8	9	10/11*	1+8	1+9	1+8+10/11	2/3+8	2/3+6	4+8	7	12	13	14	16	17	19	20	7+12+13	14+20	41		?
1	*M. gastri*	5	All	15																														1
2	*M. kansasii*	4	All		18																													0
5	*M. scrofulaceum*	3	K																										3					0
			Other																										6					2
6	*M. intracellulare* 13950–25171	14	K																4	1	1	2			3									3
			Other				2												9	5	2	6	4	1	10	3	18							5
	M. intracellulare 25120–25127	21	K			2	1		1	12	1		2	1					1															0
			Other			5	7	1	2	66	2	4			4			2	1	2							1							6
7	*M. avium*	12	K			1	11																											0
			Other			1	39		2							3	1											1						3

* The *avium* complex serotypes 2 & 3, and 10 & 11 were not well differentiated at the time of this study and are combined as 2/3 and 10/11, respectively.

Table 5. *Distribution of strains by phenetic clustering, agglutinating, serotype and lipid patterns*

				Lipid pattern previously associated with serotype (Laboratory M)							
						avium complex					
Cluster	Serotype*	No. strains examined		*'gastri'*	*'kansasii'*	3	7	8	2	Pattern not classified	No specific lipids
1	*'gastri'*	5		5							
2	*'kansasii'*	4			4						
6	*avium* complex 3	1				1					
6	*avium* complex 7	6					4				2
6	*avium* complex 8	7						5			2
6	*avium* complex 1+8	2								2	
7	*avium* complex 2	9							7	2	

* Assignment to *'gastri'* serotype is based on the agglutination report from laboratory A. All other serotype assignments are based on the agglutination report from laboratory K.

Table 6. *Distribution of strains by clustering behaviour, lipid pattern and label on culture as originally received by study co-ordinator*

Cluster	Cluster species	No. of strains with mycobacterial lipid pattern					Original label on cultures as received
		A	*B*	*C*	*D*	*E*	
3	*M. nonchromogenicum*	5	1	0	0	0	*M. nonchromogenicum*
		1	3*	0	0	0	*M. terrae* Tsukamura
4	*M. terrae*	0	5	1*	0	0	*M. terrae* Wayne
		0	0	4	0	1	*M. novum*

* The type strain is included.

(21 disagreements in 157 reports, or 13 %), and less common for cluster 7 strains (3 disagreements in 47 reports, or 6 %).

One laboratory, M, determined lipid patterns of extracts of some of the strains by the thin-layer chromatographic method of Marks & Szulga (1965). There was good correlation between lipid patterns previously associated with agglutination serotype by laboratory M and the actual agglutination serotypes of the strains reported by laboratories A and K (Table 5). Although two strains of *avium* complex 7 serotype are tabulated as having no specific lipids for group III, they were reported to have a lipid pattern similar to that seen with *M. gordonae* which belongs to Runyon's group II.

Nomenclatural confusion continues to surround *M. nonchromogenicum* (cluster 3) and *M. terrae* (cluster 4) (see Wayne, 1967). Some of the cultures originally collected by the study co-ordinators were labelled *M. nonchromogenicum* (Tsukamura, 1965). Others were named

Table 7. *Specificity difference (sp.d.) scores of sensitins and phenetic clustering behaviour*

The differences (mm), observed in laboratory L, are between homologous and heterologous reactions to intradermal tests in guinea pigs immunized with the strains used for preparation of the sensitins. Strains were arranged by manual sorting so that strains giving small differences from one another are clustered together. The lowest sp.d. scores therefore appear along the diagonals.

ATCC no.	Cluster		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
19530	3		0																		
25264	3		4	0																	
25142	3		4	2	0																
19531	4		4	3	4	0															
25144	3		2	6	3	2	0														
25143	3		3	4	2	3	1	0													
25145	3		4	4	3	2	1	2	0												
25218	3		5	5	4	5	2	2	1	0											
25267	4		7	5	7	4	3	1	1	5	0										
25147	4		6	6	9	10	5	4	5	4	2	0									
25149	4		7	5	7	7	7	5	5	4	0	0	0								
25146	4		8	8	7	7	6	6	5	4	0	3	2	0							
19619	4		8	7	9	6	9	7	8	5	3	2	3	3	0						
25269	4		7	7	6	6	4	6	6	8	1	2	2	1	4	0					
25217	4		6	8	7	6	5	6	8	6	3	6	6	7	8	3	0				
15755	4		8	7	8	5	9	8	10	9	5	6	6	5	9	5	6	0			
25268	4		14	14	13	13	13	12	12	9	9	12	11	10	13	11	14	5	0		
25265	3		15	15	17	16	15	16	16	17	7	16	15	15	16	16	17	16	22	0	
25216	3		17	19	18	19	18	15	16	16	16	15	16	15	19	19	17	19	20	25	0

M. terrae Tsukamura (1966), which was proposed as a new name for *M. nonchromogenicum* and not validly published. Still others bore the name *M. terrae* Wayne (1966) and *M. novum* (Tsukamura, 1967). These all appeared in either cluster 3 or 4. Spontaneous agglutination of these strains has prevented their serotyping. Three lipid patterns were found to predominate for strains in these clusters, with considerable overlapping between cluster 3 strains originally labelled *M. terrae* Tsukamura and cluster 4 strains originally received as *M. terrae* Wayne (Table 6).

Three laboratories reported on the specificity of delayed sensitin skin reactions in guinea pigs. Culture filtrates (laboratories L and P) or trichloroacetic acid precipitates of such filtrates (laboratory N) of test strains were injected intradermally into guinea pigs previously immunized with heat-killed organisms suspended in liquid paraffin or with culture filtrates in Freund's incomplete adjuvant. The specificity differences (sp.d.) of the sensitins (Magnusson, 1971) reported by two laboratories (Tables 7 to 9) are measures of the relatedness of immunogenic structures of the corresponding pairs of strains. The smaller the differences are, the more closely related the immunogenic structures under study.

Table 8. *Specificity difference (sp.d.) scores of sensitins, phenetic clustering behaviour, and serotype*

The differences (mm), observed in laboratory L, are between homologous and heterologous reactions to intradermal tests in guinea pigs immunized with the strains used for preparation of the sensitins. Sequence of strains arranged as given in Table 7. The first two strains were included for comparison only.

Sequence	ATCC no.	Cluster	Serotype		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	25266	3	—		0																
2	25213	4	—		4	0															
3	25123	6	19		14	16	0														
4	25129	6	7		18	15	10	0													
5	25171	6	7		12	12	6	2	0												
6	25134	6	19		14	17	7	3	2	0											
7	25153	6	8		11	8	10	5	3	3	0										
8	25119	6	8		10	17	7	4	2	1	0	0									
9	25121	6	8		12	17	10	10	6	7	2	0	0								
10	25115	6	8		10	14	12	6	4	3	0	0	1	0							
11	25133	7	2		14	18	12	7	5	3	1	0	4	1	0						
12	25116	6	8		12	12	10	7	5	2	−1	1	2	0	1	0					
13	25154	6	1		13	18	10	8	6	3	0	−1	2	0	1	1	0				
14	25227	6	9		11	15	10	9	7	5	−1	−3	2	−1	1	0	−2	0			
15	25166	7	2		15	16	13	9	5	6	2	1	5	−1	2	1	−1	2	0		
16	25114	6	1		14	21	14	9	7	4	2	1	4	1	7	0	1	2	2	0	
17	25168	7	2		11	13	11	10	8	4	2	1	7	2	2	3	6	4	2	3	0

The results obtained in laboratory L with sensitins from 19 strains from phenetic clusters 3 and 4 showed that most cluster 3 strains (*M. nonchromogenicum*) present a homogeneous cluster by sensitin analysis (Table 7). Of the first eight cultures in the sequence, seven belong to phenetic cluster 3, and one, ATCC19531, had appeared to be intermediate between phenetic clusters 3 and 4, and had arbitrarily been assigned to cluster 4. This strain showed greatest similarity to members of cluster 3 by sensitin analysis but it also had a low sp.d. to ATCC-15755, the type culture of *M. terrae* (cluster 4). The strains in sequence positions 9 to 17 all belonged to phenetic cluster 4 (*M. terrae*). They showed less homogeneity than did *M. nonchromogenicum* but rather distributed themselves along a diverging continuum by sensitin analysis. The type strain of *M. terrae* (ATCC15755) showed little similarity by sensitin analysis to most other strains of phenetic cluster 4. The last two strains in the sequence, ATCC25265 and ATCC25216, had been grouped in phenetic cluster 3, but showed sp.d. values of 15 mm or greater against all other strains, with one exception.

The results obtained in laboratory L with sensitins from 17 strains, 15 of which were derived from clusters 6 and 7, showed that the majority of strains belonged to phenetic cluster 6 (*M. intracellulare*), especially to serotype 8 of that cluster (Table 8). Members of

Table 9. *Specificity differences of sensitins according to phenetic clustering behaviour and lipid pattern*

The differences (in mm), observed in laboratory P, are between homologous and heterologous reactions to intradermal tests in guinea pigs immunized with the strains or culture filtrates used for preparation of the sensitins. Underlined figures are less precise than other figures, because of weak homologous reactions. The lipid pattern designations for cluster 6 and 7 strains refer to the serotypes which correspond to those lipid patterns. Blank spaces in this Figure indicate that the test was not performed on the corresponding pair.

Sequence	ATCC no.	Cluster no.	Lipid pattern	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	25142	3	*A*	—																								
2	25143	3	*A*	−1	—																							
3	25144	3	*A*	−3	−3	—																						
4	25264	3	*A*	0	0	0	—																					
5	19530	3	*B*	1			3	—																				
6	19531	4						1	—																			
7	25265	3	*B*	6	6	7	5			—																		
8	25266	3	*B*	4	3	5	7	7		1	—																	
9	25216	3	*B*	9	9	11	14			3	3	—																
10	25267	4	*B*	7	10	6	5			10	11	10	—															
11	25268	4	*B*	5	9	4	2			6		12	2	—														
12	25269	4	*B*	7	10	5	4			7			3	0	—													
13	25147	4	*C*							7	8	6	2	4		—												
14	25149	4	*C*	11	13	12	15			7	7	10	3	2		2	—											
15	25146	4	*E*	5	9	7	12			8		10	2	5	4	4	1	—										
16	15755	4	*C*					8	9	11	8		6						—									
17	25217	4	*B*	9	11	9	13			9	10	9	7	7		5	8	6		—								
18	25152	6	7																		—							
19	25132	6	42																		1	—						
20	25117	7	2/3				10				9						11		8				—					
21	25120	6	8				9				13						10		13		8	9	1	—				
22	25121	6	8																		6	4		−1	—			
23	25138	6	8																		7	9	6	2	5	—		
24	25139	6	8																		6	6	4	0	−1	0	—	
25	25119	6	8				10				17						12		15		10	9	5	−3	7	3	1	—

Table 10. *Distribution of strains by comparative sensitin tests, phenetic clustering behaviour and lipid pattern in laboratory N*

Cluster no.	Lipid pattern	Total no. of strains	ATCC no. of strains inducing maximum hypersensitivity to sensitin of the following strains:				
			25145	25219	19530	15755	19619
			*LP *A*	LP *B*	LP *B*	LP *C*	LP ?
3	*A*	6	25145	25142	—	—	—
			25264	25143	—	—	—
			—	25144	—	—	—
			—	25218	—	—	—
3	*B*	5	—	25219	25266	—	—
			—	25265	—	—	—
			—	19530	—	—	—
			—	25216	—	—	—
4	*B*	3	—	—	—	25267	—
			—	—	—	25268	—
			—	—	—	25269	—
4	*C*	3	—	—	—	15755	25113
			—	—	—	—	25213
4	n.d.†	2	—	19531	—	—	19619

LP, lipid pattern of reference strain; ?, LP not established; n.d., not determined.

this serotype tended to cluster together by sensitin analysis, but were not well differentiated from other serotypes of this cluster. Strain 25123 (phenetic cluster 6) showed little sensitin cross-reaction with any of the other strains. Only three strains of cluster 7 (*M. avium*) were examined. They were of the most common serotype 2 and were not distinguishable from cluster 6 strains by sensitin analysis.

Of the results obtained in laboratory P, with sensitins from 25 strains (Table 9), much of the data on strains in phenetic clusters 6 and 7 were considered to be less reliable because homologous reactions less than 10 mm in diameter were seen, so criteria previously used for classification according to sp.d. scores may not be applicable. This laboratory also sought to correlate the sensitin and lipid patterns. Two homogeneous groups (sequences 1 to 6 and 7 to 9) corresponding to cluster 3 (*M. nonchromogenicum*) and one homogeneous group (sequence 10 to 15) corresponding to cluster 4 (*M. terrae*) were recognized, along with two intermediate cluster 4 strains (sequence 15 to 16) (see Table 9). Strain ATCC19531 showed most sensitin cross-reactivity with cluster 3 strains and ATCC15755 showed poor cross-reactivity with other strains. There was, however, overlap in sensitin specificity between clusters 3 and 4. The single cluster 7 (*M. avium*) strain which was included showed marked sensitin cross-reactivity with cluster 6 (*M. intracellulare*) strains with lipid patterns corresponding to serotype 8.

Results of comparative skin tests of five sensitins in guinea pigs immunized with 19 isolates which appeared in phenetic clusters 3 and 4 were reported by laboratory N (Table 10). The sensitin which yielded the largest reaction was coded as positive for purposes of the analysis.

Table 11. *Distribution of strains by cytoplasmic antigen serogroup and phenetic clustering, with subdivision according to agglutination serotype or lipid pattern*

Cytoplasmic antigen data in this Table were submitted by laboratory Q.

Phenetic cluster	Agglutination serotype	Lipid pattern	No. of strains examined	No. strains falling into cytoplasmic antigen serogroup				
				M. kansasii	*M. non-chromo-genicum – M. terrae*	*M. intra-cellulare (B)*	*M. avium (A)*	Not classified
1	*gastri*		4	3		1		
2	*kansasii*		4	3		1		
3		*A*	4		2	1		1
3		*B*	4		2	1		1
4		*B*	2		1	1		
4		*C*	4		1	3		
4		*E*	1					1
6	7		6		1	3		2
6	8		7			6		1
6	1+8		2			2		
6	3		1				1	
7	2		9			1	8	

In general, results of sensitin testing correlated very well with phenetic clustering behaviour but the lipid patterns showed some intercluster overlap.

Immunodiffusion tests were employed in two laboratories (see Kubin, Lind, Matuskova & Norlin, 1971). In laboratory Q, the organisms were cultured in Sauton medium and disrupted by ultrasonic treatment. The resulting material consisted of a suspension of whole living bacteria and cell walls in a solution of protoplasmic cell contents. It was used directly for immunodiffusion test, or emulsified in Freund's incomplete adjuvant for immunization of rabbits. In laboratory O, culture filtrates of the reference strains were used without purification, but admixed with Freund's incomplete adjuvant, for immunization of rabbits or sheep for production of reference antisera. Culture filtrates of the strains to be studied were concentrated and fractionated to yield appropriate test antigens, containing reactive proteins or polysaccharides.

The results submitted by laboratory Q were recorded in terms of an overall precipitin pattern, i.e. those combinations of precipitinogens found in and considered to be representative of the extracts of cultures of the species recognized by the system (Table 11). According to this laboratory, *M. gastri* could not be distinguished from *M. kansasii*. Likewise, *M. terrae* and *M. nonchromogenicum* were not distinguished from one another. Laboratory Q did, however, achieve a rather striking separation of *M. avium* from *M. intracellulare*. (After the study code was broken and data reviewed, it became evident that the *M. intracellulare*

Table 12. *Distribution of strains by cytoplasmic antigens (precipitinogens) tested in laboratory O with M. avium reference system in comparison with clustering behaviour and agglutination serotypes*

Cluster	Agglutination serotype	No. strains tested	No. strains showing 5 or more precipitinogens	No. strains showing precipitinogen			
				b	c	d	e
3		8	0	0	8	0	0
4		8	0	2	8	0	0
6	8	9	5	9	9	4	3
	3, 1, 9	3	3	3	3	3	3
	12, 16 19, 42	5	5	5	5	4	0
7	1	2	2	2	2	2	2
	2	5	4	5	5	4	5
8	*xenopi*	5	1	3	5	1	0

serogroup results seen with one strain each of *M. gastri* and *M. kansasii* and with two strains each of *M. nonchromogenicum* and *M. terrae* were due to contamination. Because it is the policy of the IWGMT that no data be changed after the code is broken, these results remain in Table 11.)

In laboratory O the culture filtrate antigens were tested by the aid of a precipitation reference system derived from *M. avium*. The distribution of strains according to the presence of five or more precipitinogens in common with the reference antigen, and according to demonstration of certain precipitinogens designated b, c, d and e in the reference system, could distinguish the 16 strains of clusters 3 and 4 (*M. nonchromogenicum* and *M. terrae*) from other clusters by the paucity of the total number of shared precipitinogens and by the absence, in almost every strain, of precipitinogens b, d and e (Table 12). Precipitinogen b is common to all strains of clusters 6 and 7 (*M. intracellulare* and *M. avium*) and appears in three of five strains of cluster 8 (*M. xenopi*). Nineteen out of 24 strains from clusters 6 and 7 demonstrate five or more precipitinogens with the *M. avium* reference system. With one exception all seven strains of cluster 7 (*M. avium*) show the presence of the precipitinogens d and e, while 6 out of 17 strains of cluster 6 (*M. intracellulare*) are lacking precipitinogen d and for 11 strains from this cluster precipitinogen e is not revealed.

Three laboratories submitted data on pathogenicity of test strains for chickens, rabbits, mice and guinea pigs (see Tables 13 to 15) (Anz, Lauterbach, Meissner & Willers, 1970; Engbaek, Vergmann & Baess, 1970). Cluster 6 or 7 strains of serotype 2 or 3 were the most virulent, killing most heavily-inoculated chickens or rabbits within two months. The serotype 1 strain which fell into phenetic cluster 7 appeared slightly more pathogenic for these animals than two strains of that serotype which fell into cluster 6. Among other cluster 6 strains, those of serotype 8 or 4+9 were more pathogenic than members of other serotypes. Strains of serotypes 1, 2 and 3 gave rise to lung lesions in mice and guinea pigs in all cases, whereas strains with other serotypes often failed to do so (Table 14). Tuberculosis of the

Table 13. *Pathogenicity in chickens and rabbits compared with serotype and clustering behaviour*

Serotype	Cluster	No. strains	Hen – 1·0 mg intravenously				Hen – 0·1 mg intravenously				Rabbit – 1·0 mg intravenously			
			Died*	Sacrificed†			Died	Sacrificed			Died	Sacrificed		
			++	+to++	±	Neg.	++	+to++	±	Neg.	++	+to++	±	Neg.
2	7	10	17	8	0	0	5	12	0	0	13	5	0	0
3	6	1	3	0	0	0	3	0	0	0	1	0	0	0
1	6	2	1	2	2	1	0	2	1	1	1	2	1	1
1	7	1	3	0	0	0	0	1	0	0	1	0	0	0
8	6	14	2	15	12	6	1	13	6	8	0	10	9	4
4+9	6	3	3	2	1	1	2	1	1	1	1	3	0	2
Misc.	6	12	0	4	13	15	0	1	5	18	0	4	3	20

++, Heavy tuberculosis of liver anord/ spleen; +, moderate tuberculosis of liver and/or spleen; ±, few lesions of liver and/or spleen.

* Died within 2 months of infection.

† Sacrificed 2 months after infection.

Table 14. *Pathogenicity in mice and guinea pigs compared with serotype and clustering behaviour*

Serotype	Cluster	No. strains	Result	Mice with lesions in			Guinea pigs infected		
							Subcutaneous		Intra-cardiac lesions
				Lung	Kidney	Spleen	Localized	Generalized	
2	7	10	+	10	0	7	2	0	n.d.
			0	0	10	3	2	4	n.d.
3	6	1	+	1	0	1	n.d.	n.d.	n.d.
			0	0	1	0	n.d.	n.d.	n.d.
1	6	2	+	2	0	1	2	0	1
			0	0	2	1	1	1	0
1	7	1	+	1	0	1	0	0	1
			0	0	1	0	1	1	0
8	6	14	+	7	0	11	8	0	6
			0	8	15	4	13	21	4
4+9	6	3	+	3	0	1	2	0	n.d.
			0	1	4	3	2	4	n.d.
Misc.	6	12	+	6	0	5	9	0	3
			0	8	12	7	11	5	2

n.d., Not determined.

Table 15. *Correlation between source, time since original isolation, serotype, colony type and virulence for chickens, as determined in laboratory F*

Cluster	Serotype	Origin of strains	Total no. strains	No. chickens inoculated	Time since original isolation (years)																					
					0·5 to 1						1 to 2				Over 2				Total							
					Colony sm.t.			Colony sm.s.			Colony sm.t.		Colony sm.s.		Colony sm.t.		Colony sm.s.		Colony sm.t.			Colony sm.s.				
					No. strains	No. chickens		No. strains	No. chickens		No. strains	No. chickens		No. strains	No. chickens		No. strains	No. chickens		No. strains	No. chickens		No. strains	No. chickens			
						d+	s+		d+	s+		d+	s+		d+	s+		d+	s+		d+	s+		d+	s+		d+	s+
7	2	Man	3	8	1	2	0	0	—	—	1	3	0	0	—	—	0	—	—	1	2	1	2	5	0	1	2	1
		Birds	4	10	0	—	—	0	—	—	0	—	—	0	—	—	1	0	2	3	4	4	1	0	2	3	4	4
		Domestic animals	2	6	1	3	0	0	—	—	0	—	—	0	—	—	0	—	—	1	3	0	1	3	0	1	3	0
6	2	Pigeon	1	3	1	3	0	0	—	—	0	—	—	0	—	—	0	—	—	0	—	—	1	3	0	0	—	—
Subtotal serotype 2			10	27	3	8	0	0	—	—	1	3	0	0	—	—	1	0	2	5	9	5	5	11	2	5	9	5
6+7	1	Man	4	10	1	2	0	1	1	1	1	0	1	0	—	—	0	—	—	1	0	1	2	2	1	2	1	2
6	8	Man	10	26	1	1	1	0	—	—	1	1	1	3	0	2	2	1	2	3	0	2	4	3	4	6	0	4
		Chicken	3	8	0	—	—	0	—	—	0	—	—	0	—	—	1	0	2	2	0	3	1	0	2	2	0	3
		Pig	2	4	1	0	1	0	—	—	0	—	—	0	—	—	0	—	—	1	0	2	1	0	1	1	0	2
Subtotal serotype 8			15	38	2	1	2	0	—	—	1	1	1	3	0	2	3	1	4	6	0	7	6	3	7	9	0	9
6	4 or 9	Man	2	5	1	1	1	0	—	—	1	2	0	0	0	—	—	0	—	—	0	—	2	3	1	0	—	—
6	Other	Man	11	29	0	—	—	1	0	1	0	—	—	0	—	—	0	—	—	10	0	2	0	—	—	11	0	3
Grand total			42	109	7	12	3	2	1	2	4	6	2	3	0	2	4	1	6	22	9	15	15	19	11	27	10	19

sm.t., Colony smooth, transparent; sm.s., colony has a central dome which gradually becomes flat toward the periphery – these colonies often give rise to sm.d. forms. d+, chicken died of infection, with heavy tuberculosis; s+, chicken was sacrificed with moderate to heavy tuberculosis.

spleen occurred more irregularly, but most often with serotype 8 strains. Because of differences in mode and dose of infection, this comparison is difficult to interpret. The same is true of the guinea pig data. More than half of the guinea pigs had no local lesions after subcutaneous infection; generalized tuberculosis never was observed. After intracardiac infection, 8 of 12 animals showed septic organ tuberculosis.

In laboratory F two or three hens were inoculated with each of 42 strains of avian serotype cultures, and the degree of disease produced was correlated with origin of culture, length of time the strain was maintained in the culture collection, serotype, and colonial morphology. The results (Table 15) give the following impressions: (i) *M. avium* serotype 2 (and possibly serotypes 4 and 9) strains are most virulent, regardless of phenetic cluster, serotype 1 and 8 strains are of intermediate chicken virulence, and the other avian serotypes are of a very low order of virulence; (ii) the serotype is a more important determinant of virulence than is the species of animal from which the strain was first isolated; (iii) cultures of sm.t. colony type are more virulent than those of sm.s. colony type; (iv) the longer a culture is kept in a collection after its original isolation, the more likely it is to change from an sm.t. to an sm.s. colony form. (See Anz & Meissner, 1972; Pattyn, 1967; Schaefer, Davis & Cohn, 1970; Moehring & Solotorovsky, 1965.)

DISCUSSION

The data derived from this study provide a rational basis for resolving some of the taxonomic ambiguities among the mycobacteria of Runyon's group III. They also provide further insight into the kinds of tests which are most useful for identifying mycobacteria.

A word of caution is in order, however, regarding the use of data in Table 3 for determinative purposes. Under the permissive philosophy of this study, techniques were not precisely specified and experienced investigators may disagree with certain data presented in that Table. This may be especially true of antibiotic susceptibility data. Some investigators incorporate drugs in agar media and others in inspissated egg, which may make a marked difference to results obtained. The IWGMT is presently conducting co-operative studies for assessing reproducibility of techniques employed in mycobacterial systematics.

Throughout this report, the term phenetic analysis has been used to include data derived from studies of drug resistance, pigmentation, biochemical activities, conditions of growth, etc. Results of immunological and lipid analysis and virulence tests were treated separately for two reasons. First, it is useful to employ a separate, independent set of tests to see how well they correlate with results obtained in the primary phenetic analysis (Jones & Sneath, 1970). Second, inclusion of certain kinds of data in the numerical analysis would dilute their impact disproportionately. This occurs when there are 20 or 30 possible serologic types, and a single strain can only be ascribed to one and occasionally two types. This excessive dilution could be minimized by use of similarity (s.) instead of matching (m.) scores, by exclusion of negative matches. This, however, introduces another serious problem, the question of definition of a positive character state. Thus, resistance to a given drug or accumulation of some metabolic product may represent either the presence or absence of an enzyme or transport system, and in the absence of precise knowledge of the mechanism of the test response it is not possible to be consistent in ascribing a positive or a negative value to the observed response.

The data derived from this study confirm the identity of *M. gastri* as a distinct species. The phenetic numerical analysis of pooled data (Figs. 1 to 4 and Table 1) yields a sharply defined cluster and clearly separates *M. gastri* (cluster 1) from non-pigmented strains of *M. kansasii* (cluster 2). The phenetic analysis from a number of individual laboratories

achieves the same result, although the tests employed in other laboratories do not (Table 2). The uniqueness of *M. gastri* is also supported by agglutination serotype and lipid analysis (Tables 4 and 5), but not by the immunodiffusion studies in laboratory Q (Table 11). (See also Wayne, 1971.)

Mycobacterium nonchromogenicum (cluster 3) and *M. terrae* (cluster 4) are clearly separated from all other species included in Fig. 1 and Table 2, but the distinction between these two species is less sharp. Thus *M. nonchromogenicum* has a mean intracluster matching score of 86 %, *M. terrae* has a mean score of 85 %, and the mean intercluster matching score between the two is 77 %, i.e. a resolution factor of 8 to 9 %. Lipid analyses demonstrate that strains with lipid pattern *A* correlate with cluster 3 and those with lipid patterns *C* and *E* correlate with cluster 4 (Table 6). There is an overlap, in that strains of lipid pattern *B* occur in both clusters. The sensitin results obtained in laboratories L and P (Tables 7 and 9) indicate a homogeneous cluster corresponding to phenetic cluster 3, and a transitional group, of lesser homogeneity, leading into phenetic cluster 4. The sensitin studies performed in laboratory N (Table 10) provide a clearer resolution, with minimal overlap between these two species. Thus, although distinctions are not as great as between other pairs of species, the fact that several independent sets of analyses all provide resolution between cluster 3 and cluster 4 leads to the conclusion that *M. nonchromogenicum* Tsukamura and *M. terrae* Wayne should remained separate species, and *M. novum* Tsukamura should be reduced to synonymy with *M. terrae*.

The resolution between clusters 6 and 7 (Fig. 1 and Table 2) is very small. Thus *M. intracellulare* (cluster 6) has a mean intracluster matching score of only 84 %, and the mean intercluster matching score between clusters 6 and 7 (*M. avium*) is 80 %, i.e. a resolution factor of 4 %. Furthermore, examination of Figs. 1 to 4 indicates that cluster 6 is composed of a fairly heterogeneous subcluster of 14 strains, and a more homogeneous subcluster of 21 strains. The group of greatest phenetic heterogeneity is composed of a variety of agglutination serotypes, whereas the more homogeneous subcluster consists of a disproportionate number of strains of serotype 8 (Table 4). The agglutination data, the immunodiffusion data (Tables 11 and 12), and the animal pathogenicity studies (Tables 13, 14 and 15) all support the conclusion of Kubica & Silcox (1973) that 'They [*M. avium* and *M. intracellulare*] appear to be host-adapted varients of the same species, having a number of distinctive serotypes'. The different degrees of pathogenicity seen in different strains appear to be expressions of serotype rather than species differences. Accordingly it is the recommendation of the majority of the present authors that *M. intracellulare* be reduced to a synonym of *M. avium*, and that mycobacteria resembling those in clusters 6 and 7 of this study be considered strains of *M. avium* of a variety of serotypes.

It was not possible to obtain unanimous agreement among the 22 authors of this report on the recommendation cited above concerning *M. avium* and *M. intracellulare*. Kleeberg, Lauterbach, Magnusson, Meissner, Schaefer and Willers felt that the two names should be retained, possibly as an *M. avium–intracellulare* complex, until further tests provide resolution of the question. Accordingly it was agreed that these six participants would present a statement of their reasoning. That statement is as follows:

> 'A strain can be identified when it resembles (cannot be distinguished from) a type or a reference strain, and when at the same time it can be distinguished from other type or reference strains. As mentioned above, cluster 6 included the type strain of *M. intracellulare* (ATCC13950) and cluster 7 the reference culture of *M. avium* (ATCC19421). It cannot be concluded, however, on this basis that all of the other 34 strains included in cluster 6 were

identified as *M. intracellulare*, or that all of the other 11 strains included in cluster 7 were identified as *M. avium* in this study.

'Among the 34 strains included in cluster 6, 24 have a matching score of 75 % or more when compared with the type strain of *M. intracellulare* and thus, possibly, cannot be distinguished from this strain.

'All of the other 11 strains included in cluster 7 and six strains included in cluster 6 have a matching score of 85 % or more when compared with the reference strain of *M. avium*, and thus, possibly, cannot be distinguished from this strain. It is remarkable, however, that two of these six strains (ATCC25151 and ATCC25134) could not be distinguished from the type strain of *M. intracellulare* either with the criterion used above. Thus in the present study, 23 to 25 of the strains in cluster 6 can be identified as *M. intracellulare*, all of the 12 strains in cluster 7 and four to six of the strains included in cluster 6 can be identified as *M. avium*, and the remaining strains in cluster 6 should be classified in other species, provided the criteria used above for distinction of strains are correct. Unfortunately, this study has not permitted measurement of the variability of the matching scores and thus has not permitted a determination as to whether these criteria are correct.

'Consequently, the present study has not permitted identification of all strains of *M. avium* and *M. intracellulare* included in the study. However, this was not the main object of the study.'

Cluster 8 (*M. xenopi*) appeared so distinctive by phenetic analysis, that the paucity of serological data on these organisms in this study should be no deterrent to recognition of *M. xenopi* as a well defined species.

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