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Elongation *in vitro* of *Mycobacterium lepraemurium* as a Distinction from *Mycobacterium leprae* [1]

P. D'Arcy Hart and R. J. W. Rees[2]

A liquid cell-free medium was described by Hart and Valentine ([3,4]) in which considerable elongation of *Mycobacterium lepraemurium* (Douglas strain) was evident, though without multiplication. Negative results with a few strains of *Mycobacterium leprae* from experimentally infected mice led us to consider whether elongation in this medium might be a distinctive characteristic of *M. lepraemurium*. Accordingly, we have examined for this property all other authentic strains of *M. lepraemurium* available to us—numbering six and including the well-known Odessa and Hawaii strains—as well as a large number of strains of *M. leprae*, and, in addition, a group of noncultivable strains resembling *M. lepraemurium*.

METHODS

M. lepraemurium. The six strains were Odessa, Hawaii, Kumamoto, Keishiko, Kurume and Fukuoka. All were provided as freeze-dried preparations derived from mouse lepromata by Prof. S. Nishimura and Dr. T. Mori.

M. leprae. Twelve of the 30 strains were obtained from the foot pads of mice infected experimentally from human leprosy tissue. The remaining 18 strains were obtained directly from human leprosy tissue.

Other noncultivable strains of mycobacteria. Osaka 1, an *M. lepraemurium*-like strain isolated from a healthy mouse ([6]), was provided by Professor Nishimura in a saline suspension. Five strains were provided by Dr. K. R. Chatterjee, who had isolated them from livers of mice with systemic infection after inoculation of human leprosy tissue ([1]). A strain of cat leprosy was given to us by Miss M. Barnett and Dr. S. R. M. Bushby. Three mycobacterial strains were encountered in these laboratories, becoming the dominant organism after the third and fourth passages, respectively, from mouse foot pad infections originating from inoculated human leprosy tissue; the intense local swelling, high bacterial counts, histologic appearances of the lesions, infectivity in fresh mice, and drug susceptibilities, all indicated *M. lepraemurium* rather than *M. leprae*.

Assessment of elongation. The liquid medium used (pH 6.2-6.5) was that of Hart and Valentine ([4]), to which penicillin in the amount of 200-400 units/ml. was added in some tests; inoculation of bacilli and assessment of elongation after two to four weeks' incubation, were carried out as previously described.

The strains of *M. lepraemurium* were tested with the use of the freeze-dried preparations, and also, later, of infected organs (usually liver) of mice that had been inoculated intravenously with the same preparations (6 mice per strain) 8 to 14 months previously. The freeze-dried preparations were reconstituted in small volumes of 0.1 per cent albumin in 0.9 per cent NaCl. They were then diluted with the albumin-saline so as to contain about 2×10^9 organisms/ml., and 0.2 ml. of this suspension was added to 8 ml. of medium. The infected organs were homogenized and the bacilli finally suspended ([4]) in 0.1 or 1.0 per cent albumin-saline to give 2×10^9/ml., and similarly inoculated into the medium.

The strains of *M. leprae* also were

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[2]P. D'Arcy Hart, M.D. and R. J. W. Rees, F.C.Path., National Institute for Medical Research, Mill Hill, London N.W.7, England. *Requests for reprints* should be addressed to Dr. Rees.

tested with the use of suspensions containing 2×10^9 bacilli/ml., or as near this figure as obtainable, derived from the homogenates of the mouse foot pad tissue or of human biopsy material ([7]).

The test of Osaka 1 was made directly from the saline suspension. Dr. Chatterjee's strains were tested with the use of homogenates either of infected mouse livers or of foot pads of mice infected therefrom. The strain of cat leprosy was assessed with the use of homogenate of liver from an experimentally infected mouse. The apparent contaminants of human leprosy-infected mouse foot pads were tested with the use of homogenates of the latter.

RESULTS

The results (Table 1) from the freeze-dried preparations of *M. lepraemurium* were varied, only two of the six strains giving unequivocal elongation. This variation could be explained either by elongation as an inconsistent property of this species, or by the low viability of the organisms in the cases of failure to elongate (the lyophilization having been too lethal).

The degree of success of the attempts to infect the organs of mice from the same freeze-dried preparations (column 3) appears to have a correlation with the degree of bacillary elongation originally obtained (column 2). Such correlation of infectivity with elongation would be expected if defective elongation from certain of the freeze-dried preparations was an indicator of low viability.

On the other hand, the finding (column 4) of marked elongation in every case where organ involvement, and consequently a liberal supply of fresh healthy bacilli for testing *in vitro*, was obtained, indicates that there is no inherent inability to elongate among these strains. Indeed, in this way good elongation was demonstrated in four of these six strains of *M. lepraemurium*, no fresh bacilli from viscera being available for the remaining two strains.

The results (Table 2) for *M. leprae* were in strong contrast. Whether the microorganisms were derived from mouse foot pads or directly from human tissue, elongation was uniformly absent in all 30 strains. To exclude the foot pad source as a factor, *M. lepraemurium* (Douglas strain) was inoculated into mouse foot pads; in due course tests were made with the homogenized tissue and the usual elongation was observed.

TABLE 1. *Elongation* in vitro *of* Mycobacterium lepraemurium (*i*) *from freeze-dried preparations, and its relation to the latter's infectivity in mice, and* (*ii*) *from fresh mouse preparations.*

	Freeze-dried preparations		Fresh mouse preparations
Strain	Bacterial elongation	Infectivity in mice: gross disease in organs[a]	Bacterial elongation
Odessa	Partial[b]	1 nodule	++
Hawaii	Partial	+	++
Kumamoto	+	+	++
Keishiko	Doubtful	0	Not available
Kurume	+	+	++
Fukuoka	0[c]	0	Not available

[a] Killed at 8–14 months (presence of lesions was associated with profuse acid-fast bacilli in impression smear; absence was associated with sparse, degenerate bacilli); mice killed at 5–8 months all showed no lesions.
[b] A second ampoule gave no elongation.
[c] A second ampoule also gave no elongation.

TABLE 2. *Elongation* in vitro *of strains of* Mycobacterium leprae.

Number of strains from mouse foot pads	Number of strains from human leprosy tissue	Total	Bacterial elongation
12	18	30	0

In every one of the other 10 noncultivable strains examined, elongation was observed. In addition, comparison of the drug susceptibility of elongation in the Chatterjee strains with that of *M. lepraemurium* (Douglas strain) revealed that, like the latter, elongation was inhibited by isoniazid ([2]), but not by dapsone or streptomycin.

DISCUSSION

All the authentic strains of *M. lepraemurium* that could be tested satisfactorily gave definite and striking elongation in the medium employed, whereas none of the strains of *M. leprae* did. This contrast suggests that elongation *in vitro* under these conditions is a characteristic distinction, and might have practical use as such, for example, in easily identifying *M. lepraemurium* as a tissue contaminant in experimental studies of *M. leprae in vitro.*

The risk of such contamination is evident from the observation that healthy mice can carry *M. lepraemurium*-like strains ([6]); our finding of elongation in one of these (Osaka 1) would be consistent with this identification.

The possibility of a carrier condition must be taken into account in considering the Chatterjee strains examined by us. In their elongation, and the pattern of the drug susceptibility of this elongation, these strains resembled *M. lepraemurium* rather than *M. leprae*; histologic appearances and some other properties also showed similarities to the former species.

Our three strains that have overgrown experimental human leprosy infections, and were suspected on other grounds of being contaminating *M. lepraemurium,* also gave typical elongation.

The occurrence of elongation in the cat leprosy strain is consistent with the report ([5]) that the organism responsible has the properties of *M. lepraemurium.*

Thus in this varied group of noncultivable mycobacteria, all of which resembled or were suspected of being *M. lepraemurium,* the elongation test would appear to support this interpretation.

The present study is confined to *M. lepraemurium* or *M. lepraemurium*-like organisms, and to *M. leprae.* We have not tested any other noncultivable mycobacteria. Cultivable mycobacteria (e.g., *M. tuberculosis* and *M. johnei*) in an adverse environment, like many bacterial species, show some elongation, but this is much less striking than that of *M. lepraemurium* and is not confined to an acid pH range; in the present medium elongation is slight and temporary and is followed by multiplication.

If ability to elongate *in vitro* under the conditions described is an inherent feature of *M. lepraemurium,* a corollary is that an observation of defective or absent elongation with a suspension known to contain bacilli of this species in adequate quantity, as in the case of certain of the present freeze-dried preparations, can be regarded as indicative of low viability.

SUMMARY

A total of seven authentic strains of *Mycobacterium lepraemurium,* 30 strains of *M. leprae,* and 10 other noncultivable strains of mycobacteria resembling or suspected of being *M. lepraemurium,* were tested in "elongation medium." In two of the strains of *M. lepraemurium* the tests had to be confined to freeze-dried preparations, in which viability appeared to be low; with each of the other five, for which fresh tissue preparations were also obtainable, elongation was observed. Elongation was observed also with each of the 10 *M. lepraemurium*-like strains. In contrast, none of the 30 strains of *M. leprae* (all tested with fresh tissue homogenates) showed elongation.

It is suggested that elongation *in vitro* may prove useful in easily distinguishing *M. lepraemurium* from *M. leprae.*

RESUMEN

Un total de siete cepas auténticas de *Mycobacterium lepraemurium,* 30 cepas de *M. leprae,* y 10 otras cepas de mycobacteria no cultivable parecidos o sospechosos de ser *M. lepraemurium,* se ensayaron en "elongation medium." En dos de las cepas de *M. lepraemurium* las pruebas tuvieron que reducirse a preparaciones congeladas y secas, en las cuales la viabilidad pareció ser baja; con cada una de las otras cinco, para las cuales preparación de tejidos frescos fueron también conseguidas, se observó elongación. También se observó elongación con cada una de las 10 cepas parecidas a *M. lepraemurium.* En contraste, ninguna de las 30 cepas de *M. leprae* (todas probadas con homogenizados de tejidos frescos) mostraron elongación.

Se sugiere que la elongación *in vitro* puede ser útil para la facil distinción entre *M. lepraemurium* y *M. leprae.*

RÉSUMÉ

Dans un "milieu d'élongation" (elongation medium), on a étudié un total de sept souches authentiques de *Mycobacterium lepraemurium,* de trente souches de *M. leprae,* et de dix autres souches non cultivables de mycobactéries ressemblant à *M. lepraemurium* ou soupçonnées d'être *M. lepraemurium.* Pour deux des souches de *M. lepraemurium,* les épreuves ont été limitées à des préparations congelées-desseches, dans lesquelles la viabilité s'est révélée être faible. Avec chacune des cinq autres souches, pour lesquelles des préparations tissulaires fraiches avaient également été obtenues, on a observé une élongation. L'élongation a également été notée avec chacune des 10 souches ressemblant à *M. lepraemurium.* Par contre, aucune des trente souches de *M. leprae,* qui toutes avaient été étudiées avec des homogénéisats de tissue frais, n'ont montré de l'élongation.

On suggère que l'élongation *in vitro* peut se révéler utile pour distinguer facilement *M. lepraemurium* de *M. leprae.*

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REFERENCES

1. Chatterjee, K. R. Experimental transmission of human leprosy infection to a selected laboratory-bred hybrid black mouse. Internat. J. Leprosy **26** (1958) 195-204.
2. Hart, P. D'Arcy, Rees, R. J. W. and Valentine, R. C. Isoniazid resistant and dependent strains of *Mycobacterium lepraemurium* studied *in vivo* and *in vitro.* J. Path. & Bact. **84** (1962) 105-111.
3. Hart, P. D'Arcy and Valentine, R. C. Elongation of a leprosy bacillus (*Mycobacterium lepraemurium*) in a cell-free medium. Nature (London) **185** (1960) 58-60.
4. Hart, P. D'Arcy and Valentine, R. C. Growth (without multiplication) of *Mycobacterium lepraemurium* in a cell-free medium. J. Gen. Microbiol. **32** (1963) 43-53.
5. Lawrence, W. E. and Wickham, N. Cat leprosy: infection by a bacillus resembling *Mycobacterium lepraemurium.* Australian Vet. J. **39** (1963) 390-393.
6. Nishimura, S., Kawaguchi, Y., Kohsaka, K. and Mori, T. Contamination of healthy mice with murine leprosy-like acid-fast bacillus. La Lepro **33** (1964) 245-256.
7. Rees, R. J. W. Limited multiplication of acid-fast bacilli in footpads of mice inoculated with *Mycobacterium leprae.* British J. Exper. Path. **45** (1964) 207-218.