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# Isolation of a strain of *Mycobacterium lepraemurium* from normal laboratory mice

BY

S. R. PATTYN and G. VERDOOLAEGE-VAN LOO

*Summary* — Isolation of a strain of *Mycobacterium lepraemurium* from « normal » laboratory white mice is related.
It's implication on foot pad passage work with *M. leprae* is discussed.

Nishimura *et al.* (1964) and Nishimura (1966) stressed the possibility that normal laboratory mice might harbour *Mycobacterium lepraemurium*.

Hart and Rees (1968) mention 3 instances where *M. leprae* after 3 or 4 passages in mouse foot pads became replaced by *M. lepraemurium*.

From 1966 on, we made some efforts to detect *M. lepraemurium* in the NMRI strain of white mice reared in our Institute.

## Passage history of the strain

In February 1966, both hind foot pads of 12, 10 months old, female breeders were harvested in the same way as is done for *M. leprae* harvests (Shepard, 1960).

Ziehl Neelsen stains of these suspensions showed in 10 out of 12 animals, acid fast bacilli (AFB) ranging from 1 to 15 bacilli. All these suspensions were inoculated into Loewenstein-Jensen medium (LJ) and incubated for 3 months at 33 °C. The rest of each suspension was added to 5 ml liquid Sula medium containing 5 percent calf serum. These were incubated at 30 °C.

Some of these cultures gave rise to contaminant non-acid fast organisms or molds after some time. No acid fast organisms could be cultured.

One mouse foot pad harvest was also inoculated into both hind foot pads of 12, four week old, mice (see accompanying scheme).

One year later, 5 mice of this group were still alive. Their foot pads were harvested with following results :

Animal No 1 : both hind feed : 5 AFB.
Animal No 2 : both hind feed : 9 AFB.
Animal No 3 : both hind feed : 30 AFB.
Animal No 4 : left foot : 2 AFB.
right foot : 3 AFB.
Animal No 5 : left foot : 4 AFB.
right foot : 3 AFB.

Inoculations on L-J remained negative for mycobacterial growth. The suspension from which a preparation showed 30 AFB was again inoculated into the foot pads of 12 mice.

Examination of 3 mice, 10 and 11 months later, showed :

Mouse No 1 : $4.4\ 10^5$ AFB/foot pad.
Mouse No 2 : 3 AFB/foot pad.
Mouse No 3 : $1.8\ 10^6$ AFB/foot pad.

The $4.4\ 10^5$ harvest was now inoculated into foot pads of 12 mice and intravenously into 6 mice.

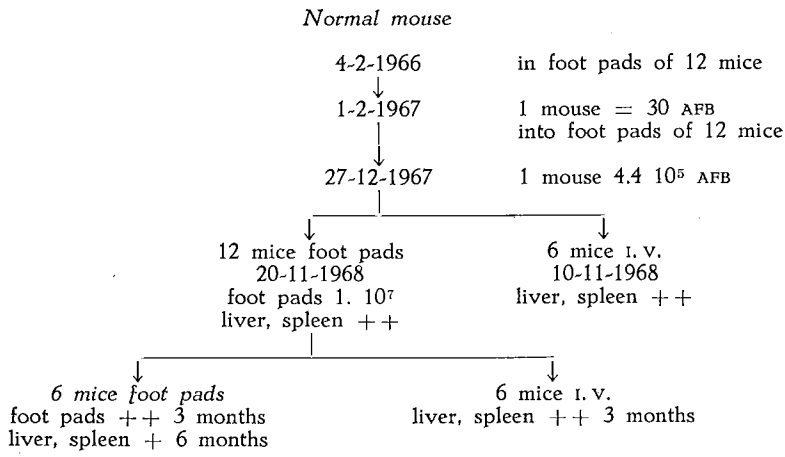
Ten months later the animals inoculated in the foot pads had more than $10^7$ AFB locally and their livers and spleens were heavily positive for AFB. So were livers and spleens of the I. V. inoculated mice.

Foot pad passage of this material showed more than $10^5$ AFB in the feet within 3 months and spread to liver and spleen.

Histologically spleens showed masses of macrophages studded with AFB, so were reticulo-endothelial cells in the liver. In the foot pads, bacilli were also located in masses intracellularly within histiocytes of the sub- and intermuscular connective tissue. In contrast with leprosy bacilli where many bacilli are non solid, degenerated forms, when numbers are high; these bacilli were almost all « solids » and some specimens showed a tendency to branching. Some bacilli were apparently also located within muscle cells or muscle associated cells. Many bacilli were longer than *M. leprae* in mouse foot pads.

Incubation of these bacilli into the medium described by Hart and Valentine (1963) gave rise to elongation after 2 to 3 weeks. Controls were a known strain of *M. lepraemurium* (also showing elongation) and *M. leprae* harvests showing no elongation.

**Passage history of the strain**




## Discussion

The uncultivable AFB isolated from « normal » mice and giving rise to liver and spleen involvment after intravenous and foot pad inoculation of mice behave as *M. lepraemurium* (Pattyn, 1965).

The elongation obtained in the medium of Hart-Valentine (1963) confirms this identification (Hart and Rees, 1968).

It is difficult to state precisely at which passagelevel the strain was actually isolated : from the original mice started with in 1966 or at the first or second passage made respectively in February and December 1967.

This finding, as those of Nishimura *et al.* (1964, 1966) and Hart and Rees (1968) shows how carefully each passage level of *M. leprae* in mouse foot pads as originally described by Shepard (1960) has to be controled.

These controls in our laboratory consist of histological examination of foot pads, examination of Ziehl stains of spleen suspension of harvested mice and inoculation of harvests on L-J medium.

If any doubt subsists I. V. inoculation into mice should be performed and elongation in the medium of Hart and Valentine should be looked for.

In one instance of a passage of an *M. leprae* strain we found some mice whose food pads showed histological lesions incompatible with *M. leprae* lesions, which may have been due to *M. lepraemurium*. This material was not studied further.

That such contaminations of *M. leprae* strains do not occur more frequently, is probably due to the fact that at each passage level of *M. leprae*, dilutions of harvests are made.

The danger of contamination or/and replacement of *M. leprae* strains by *M. lepraemurium* varies perhaps from one laboratory to another and with the mouse strains used. This danger should however always be kept in mind.

*Résumé* — **Isolement d'une souche de** *Mycobacterium lepraemurium* **à partir de souris blanches normales.**

Une souche de *Mycobacterium lepraemurium* fut isolée à partir de souris blanches « normales ».

Les implications sur l'entretien des souches de *M. leprae* chez la souris sont discutées.

*Samenvatting* — **Isolering van een** *Mycobacterium lepraemurium* **stam uit normale witte muizen.**

Een *Mycobacterium lepraemurium* stam werd uit « normale » witte laboratorium muizen geïsoleerd.

De weerslag hiervan op het onderhoud van *M. leprae* stammen bij de muis wordt besproken.

Departement van Bakteriologie, Prins Leopold Instituut voor Tropische Geneeskunde, Nationalestraat 155, Antwerpen, Belgium.

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