

## “Drug-resistant Proportion Test” for *M. leprae* to Quantify the Proportion of Drug-resistant *M. leprae* in a Sample Using the Mouse Foot Pad<sup>1</sup>

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The mouse foot pad test for drug-resistant *Mycobacterium leprae* has so far been used as a qualitative rather than a quantitative test (<sup>3,4</sup>). No differentiation has been made between strains of *M. leprae* containing only a small proportion of drug-resistant *M. leprae* and strains consisting mostly of drug-resistant *M. leprae*. The objective of this study was to find out whether such differentiation is possible using the mouse foot pad test.

The experiments were carried out at the Radda Barmen Research Laboratories of the Schieffelin Leprosy Research and Training Center, Karigiri, India, where a large mouse foot pad laboratory has been in operation since 1970.

### METHODS

The techniques of foot pad inoculation, harvest, and counting of *M. leprae* were identical to those routinely used in the mouse foot pad test (<sup>3,4</sup>). *M. leprae* used in the experiments were obtained from the *M. leprae* “bank” of the Radda Barmen Research Laboratories. Bacilli of the strain “R” were taken from the foot pads of mice continuously fed dapsone (DDS) in the concentration of 0.01% w/w in their diet. Bacilli of the strain “S” were obtained from mice fed a diet without DDS which had already been

shown to be inhibited completely by 0.01% DDS in the diet. Mouse diets were routinely tested for DDS concentrations.

Two different inocula were prepared: Inoculum 1 = only “R” bacilli; Inoculum 2 = 10% “R” bacilli plus 90% “S” bacilli. Each inoculum was injected into the hind foot pads of 14 CBA mice, delivering 10<sup>4</sup> *M. leprae* per foot pad. From the day of inoculation, eight mice (controls) were fed a normal mouse diet, while six (treated mice) were fed the same diet mixed with 0.01% DDS.

From one month after inoculation, monthly foot-by-foot harvests were performed on one control mouse until *M. leprae* reappeared in the foot pads (<sup>2</sup>). Six weeks later, harvests of two controls and two treated mice were performed. Each mouse was randomly assigned a number, either 1 or 2. Counts were compared between the four corresponding foot pads from controls and treated mice, respectively.

Counts of *M. leprae*, for the present purpose, are most meaningfully expressed as logarithms to the base 2, since the number of viable *M. leprae* doubles during a cycle of multiplication. The randomization test (<sup>6</sup>) was used to test the difference between the means of the two samples for statistical significance. The test yields the exact probability of finding the observed arrangement of values if, in fact, the means of the two samples do not differ.

### RESULTS

Harvest results were obtained on four foot pads each from the controls and the treated mice for each of the inocula. The Table shows bacterial counts with the difference (number of doublings) in counts between the controls and the treated mice. Inoculum 1 yielded significantly smaller differences between the controls and the treated mice

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THE TABLE. *Mouse foot pad harvest results.*

Mouse no.	Foot pad	Harvested <i>M. leprae</i> (log <sub>2</sub> no. of AFB) from mice with					
		Inoculum 1			Inoculum 2		
		Control	Treated	Difference	Control	Treated	Difference
1	Left	5.67	4.46	1.21	8.73	1.58	7.15
	Right	5.98	4.75	1.23	8.17	1.58	6.59
2	Left	7.01	4.75	2.26	8.40	1.00	7.40
	Right	6.81	4.58	2.23	8.06	2.00	6.06

than did inoculum 2 ( $p = 0.0143$ ). Also, for each inoculum the four counts from the control mice were significantly higher than those from the treated mice (in each case  $p = 0.0143$ ).

#### DISCUSSION

Methods to measure the proportion of resistant bacilli in a sample have long been used in tuberculosis (1). However, such methods are not reported to have been tried in leprosy. The results presented indicate that the "drug-resistant proportion test," a simple modification of the mouse foot pad test, is capable of differentiating between samples of *M. leprae* containing differing proportions of resistant bacilli.

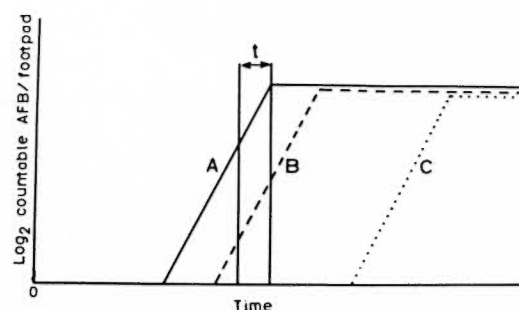
"R" bacilli in the present experiment were taken from mice continuously fed 0.01% DDS in their diet, and may therefore be regarded as predominantly DDS resistant. "S" bacilli, on the other hand, had been shown to be completely inhibited by 0.01% DDS in the mouse diet and may be regarded as predominantly DDS sensitive. The difference in harvest counts between the controls and the treated mice for each inoculum appears to be inversely related to the proportion of resistant *M. leprae* in the inoculum.

The explanation for this observation is likely to be as follows. Shepard (5) found that the incubation period until the appearance of a microscopic granuloma in the mouse foot pad was inversely related to the number of *M. leprae* inoculated. Therefore, in mice treated with a drug, the inocula containing predominantly drug-resistant *M. leprae* should have a shorter incubation period than the inocula containing small proportions of drug-resistant *M. leprae*.

The Figure depicts this concept schemat-

ically. A represents the growth of *M. leprae* in the control mice. If every bacillus in the sample tested is drug resistant, then the growth of *M. leprae* in the treated mice should not be delayed compared to the growth in the control mice. If, on the other hand, every bacillus in the sample tested is drug sensitive, then the growth in the treated mice should be infinitely delayed. Between these two extremes, differing proportions of drug-resistant *M. leprae* should give correspondingly different delays of growth in the treated mice as compared to the controls. Thus, B and C in The Figure represent growth of *M. leprae* in treated mice for two separate strains of *M. leprae*. C is found to be more delayed than B, indicating that the strain giving line C has a smaller proportion of resistant *M. leprae* than the strain giving line B. It would be interesting to speculate why harvest counts in the present experiment were lower in the treated mice than in the control mice, even for inoculum 1. This deserves further study.

Harvest during the phase marked  $t$  in The Figure allows the best estimate of the delay



THE FIGURE. Schematic representation of events during the "drug-resistant proportion test" for *M. leprae*.

in growth between the controls and the treated mice. The end of phase t is indicated by the attainment of the plateau level of growth in the control mice. In practice, the t phase is unlikely to be missed if comparisons between the controls and the treated mice are made six weeks after the first appearance of countable bacilli in harvests from the control mice.

Thus, merely by performing harvests earlier than usual the mouse foot pad test can be used quantitatively rather than only qualitatively. Even two mice (four foot pads) each in the control and the treated groups are sufficient to discriminate between inocula with tenfold differences in the proportion of resistant bacilli. Inclusion of one or two more mice per group should enhance the discriminative ability of the test. Further experiments to calibrate the test are in progress. The "drug-resistant proportion test" should be applicable to all drugs active against *M. leprae*.

#### SUMMARY

The mouse foot pad test has not previously been used quantitatively to discriminate between samples of *Mycobacterium leprae* with differing proportions of drug-resistant *M. leprae*. The "drug-resistant proportion test" is a simple modification of the routine mouse foot pad test. It is demonstrated to distinguish between samples of *M. leprae* with a tenfold difference in the proportion of dapsone-resistant *M. leprae*.

#### RESUMEN

La prueba en el cojinete plantar del ratón no se ha utilizado para discriminar cuantitativamente entre muestras de *Mycobacterium leprae* conteniendo diferentes proporciones de *M. leprae* resistentes a drogas. La prueba de "la proporción resistente a la droga" es

una modificación simple de la prueba del cojinete plantar rutinaria. La prueba puede distinguir entre muestras que difieren 10 veces en su proporción de *M. leprae* resistente a la dapsona.

#### RÉSUMÉ

L'épreuve sur coussinet plantaire de la souris n'a pas été utilisée jusqu'à présent de manière quantitative pour distinguer les échantillons de *Mycobacterium leprae* selon la proportion de bacilles résistant aux médicaments qu'ils contiennent. L'épreuve portant sur "la proportion de bacilles résistant aux médicaments" n'est qu'une simple modification de l'épreuve de routine pratiquée sur le coussinet plantaire de la souris. On montre ici que cette épreuve permet de distinguer des échantillons de *M. leprae* qui présentent une différence de 1 à 10 dans la proportion des bacilles résistant à la dapsona.

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#### REFERENCES

1. CANETTI, G., FOX W., KHOMENKO, A., MAHLER, H. T., MENON, N. K., MITCHISON, D. A., RIST, N. and SMELEV, N. A. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. *Bull. WHO* **41** (1969) 21-43.
2. EVANS, M. J., NEWTON, H. E. and LEVY, L. Early response of the mouse footpad to *Mycobacterium leprae*. *Infect. Immun.* **7** (1973) 76-85.
3. REES, R. J. W. Limited multiplication of acid fast bacilli in the footpads of mice inoculated with *M. leprae*. *Br. J. Exp. Pathol.* **45** (1964) 207-218.
4. REES, R. J. W. Drug resistance of *Mycobacterium leprae*, particularly to DDS. *Int. J. Lepr.* **35** (1967) 625-636.
5. SHEPARD, C. C. The experimental disease that follows the injection of human leprosy bacilli into footpads of mice. *J. Exp. Med.* **112** (1960) 445-454.
6. SIEGEL, S. The randomization test for two independent samples. In: *Nonparametric Statistics for the Behavioural Sciences*. International student ed. Tokyo: Kogakusha Co., Ltd., 1956, pp. 152-156.