monotherapy for the entire duration of observation—5 to 9 years. When one considers that a single high-grade DDS-resistant *M. leprae* bacillus dividing once in 12 days should yield 10^{12} *M. leprae* in only 1½ years, the observed response to DDS monotherapy seems spectacular. Previous assumptions that mouse test drug resistance was, in the long run, equivalent to clinical drug resistance in patients seem contrary to accumulating evidence.

Our criteria for growth in mouse experiments require a sixfold or greater increase in the number of *M. leprae* remaining in the foot pad 24 hr after inoculation ([3]). In fact, we observed a 12-fold or greater increase in every experiment. This is unlikely to be due to chance.

We are glad to know that the correspondents will publish findings similar to ours on the response to DDS monotherapy compared between the 1960s and 1970s. They agree that the efficacy of DDS monotherapy has not diminished over the years. We are content with the corroboration afforded by their observations. Our own inferences have been fully spelled out in the paper, and will be judged by the readers. We obviously do not oppose the use of DDS suggested by the correspondents.

The interesting claim that "mortality is higher among lepromatous cases who do not respond to treatment and worsen clinically," is not supported by any evidence in the letter or by a reference.

We hope that previous papers by other workers on DDS resistance will receive a similar critical evaluation by the correspondents.

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REFERENCES

1. Almeida, J. G., Joseph, P. S., Sarangapani, G. and Chacko, C. J. G. "Drug-resistant proportion test" for *M. leprae* to quantify the proportion of drug-resistant *M. leprae* in a sample using the mouse foot pad. Int. J. Lepr. **52** (1984) 468–470.
2. Almeida, J. G., Joseph, P. S., Sarangapani, G. and Chacko, C. J. G. The mouse foot-pad test—sensitive to small proportions of drug-resistant bacilli in a sample of *M. leprae*. Indian J. Lepr. **56** (1984) 10–14.
3. Evans, M. J., Newton, H. E. and Levy, L. Early response of the mouse footpad to *Mycobacterium leprae*. Infect. Immun. **7** (1973) 76–85.
4. Warndorff-van Diepen, T., Aredath, S. P. and Mengistu, G. Dapsone-resistant leprosy in Addis Ababa: A progress report. Lepr. Rev. **55** (1984) 149–157.

Drug Sensitivity Testing of *M. leprae*

To the Editor:

We have been surprised by the content of the discussion and the conclusions reached by the authors in the Almeida, *et al.* paper that appeared in the 1983 September issue of IJL (1983 **51** 366–379); namely, a) that patients may respond to dapsone (DDS) monotherapy despite a high degree of dapsone resistance, and consequently b) that results of mouse foot pad sensitivity tests do not indicate whether patients will respond to DDS monotherapy.

Concerning the first point, the conclusion of the authors is not fully supported by the data they present. Actually, their whole reasoning is based upon the results of bacterial smears under routine DDS monotherapy. When the BI decreases, patients are considered as having DDS-sensitive infection, and when the BI is reported to increase, patients are considered as having DDS-resistant infection. When the authors biopsied 128 patients treated with DDS for at least three years with increasing BI and inoculated the specimens into the foot pads of mice for sensitivity testing, they observed 26 failures to grow *Mycobacterium leprae* (20%). Among the 102 *M. leprae* strains that grew, 90 were DDS resistant (77 with high-degree DDS resistance). When the authors biop-

sied 14 patients treated with DDS for at least three years with decreasing BI and inoculated the specimens for sensitivity testing, they observed 8 failures to grow (57%); among the 6 strains that grew, 1 was DDS sensitive and 5 resistant to DDS (high degree). It is well known that all steps in the preparation, staining, and reading of skin smears are difficult to standardize. Thus we would conclude that, in the published data, there is a good correlation between the assessment of clinical deterioration by skin smears and the mouse foot pad assessment. The five observed discrepancies would form the few exceptions that confirm the rule. Therefore, we would certainly not support the conclusion of the authors that the mouse test cannot discriminate between patients deteriorating and patients improving, especially when there is no evaluation of the accuracy and adequacy of their method used to diagnose deterioration or improvement.

Moreover, one would not support the authors' implicit conclusion that the mouse food pad sensitivity tests are unreliable. It is true that it is by analogy with *M. tuberculosis* that wild strains of *M. leprae* are assumed to contain about one drug-resistant mutant in 10^6 sensitive organisms. Such a proportion has practical implications in the performance of drug sensitivity testing in tuberculosis. If the inoculum used for the sensitivity test contains as many as 10^9 viable units, a situation which is easily realized, a fully sensitive wild strain of *M. tuberculosis* will give confluent growth of colonies on drug containing medium, and thus may be considered as drug resistant. To prevent false conclusions due to the use of heavy inocula, Canetti, *et al.* ([1]) strongly recommended the use of defined and low inocula (about 10^2 and 10^4 viable units) for sensitivity testing, a recommendation now widely understood and accepted by those who work in the field of tuberculosis chemotherapy.

Let us now consider the conditions under which drug sensitivity tests are done in leprosy. First of all, the inoculum used for *M. leprae* drug sensitivity testing has always been low, about 5×10^3 AFB (of which perhaps 10–20% are usually viable). Given the assumed proportion of 10^{-6} drug-resistant mutant in a wild strain of *M. leprae,* the probability for a drug-resistant mutant to have been inoculated is very low, as thus is the probability for a fully sensitive strain to be considered as resistant. Secondly, the sensitivity to DDS is judged by the growth of AFB in the foot pads of mice that have been fed with three different concentrations of DDS in the diet. The highest concentration, 0.01% in the diet, is selected to give blood levels in the mouse as high as those obtained in patients treated with a full daily dose of DDS (100 mg or 1.6 mg/kg). When *M. leprae* is able to grow in the foot pads of mice fed with 0.01% DDS in the diet, it is also able to grow in man despite treatment with a full dose of DDS. Twenty years' experience has shown this in a number of studies. Therefore, correlation between mouse foot pad data and clinical data under chemotherapy should be excellent. When exceptions are now found, the accuracy of the newly collected data should be considered.

For mouse foot pad sensitivity testing, accuracy means not only a low inoculum but also adequate concentrations of the drug in the mouse diet and an assessment of *M. leprae* growth in the drug-treated mice as soon as the control mice are positive. The first condition has already been mentioned. The second condition is self-evident. The third condition is important because a strain which would have been considered as partially resistant might well be interpreted as fully resistant . It is because every specialist is aware of such risks that mouse sensitivity testing is done everywhere with great care and in a strictly standardized manner.

Concerning the accurate assessment of whether a patient is deteriorating or improving under chemotherapy, we would like to point out two essential ideas.

1. For routine assessment of multibacillary patients under chemotherapy, the use of a standardized BI technique is certainly commendable. However, there is ample evidence of the limitations of this technique.

2. In view of these limitations, when the purpose is to demonstrate a possible need to reconsider the whole concept of drug sensitivity testing of *M. leprae* then comprehensive data, including clinical, bacteriological, and histopathological findings, are needed, as well as the accurate assessment

of the drug intake. What has been necessary to establish the present concept itself should be used to challenge it.

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REFERENCE

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.

Response to Dr. Grosset, *et al.*

To the Editor:

We thank the correspondents for their interest in our papers, and are happy to note that they do not dispute those of our findings of most practical importance. Our population-based study in an established leprosy control program directly observed dapsone resistance in a leprosy-endemic area. Previous estimates had relied on clinic- or hospital-based studies.

One thousand out of 1224 lepromatous and borderline lepromatous patients on dapsone monotherapy in the 1320 km^2 area of Gudiyatham Taluk, India, were found to have been smear negative for three years or more. Smear negativity was found to indicate a markedly reduced risk of dapsone (DDS)-resistant infection. Seventy-six patients, a very small group, remained continuously smear positive despite treatment, and only this group had a high prevalence of DDS-resistant infection.

This small "high-risk" group that emerges during dapsone monotherapy deserves the fullest possible concentration of efforts and resources. Theoretical predictions that dapsone-resistant infections would threaten every "multibacillary" patient are not supported by evidence from leprosy control programs in endemic areas. On the contrary, data showing the continuing efficacy of dapsone monotherapy, after two decades, were presented by independent investigators from Polambakkam, Chingleput, and Salur (all in South India) at the biennial conference of the Indian Association of Leprologists in November 1983.

The correspondents seem to feel that mouse test drug resistance is equivalent to clinical drug resistance in patients. In our view this is not supported by the evidence which, in fact, comes from several sources. Pearson, *et al.* ([4]) found patients who responded for over 53 months (4½ years) to DDS monotherapy, after the mouse foot pad test had grown high-grade DDS-resistant *Mycobacterium leprae.* Jacobson ([3]) observed that patients diagnosed by the mouse foot pad test to harbor primary dapsone-resistant *M. leprae,* and treated initially with DDS monotherapy, showed a response that was "completely normal as measured by all the usual criteria." Warndorff-van Diepen ([6]) showed that after even "high-grade" dapsone-resistant *M. leprae* grew in mice, patients yielding such organisms attained smear negativity and clinical inactivity despite continuing on dapsone monotherapy.

It seems to us that the mouse foot pad test for drug resistance has suffered from the omission of a control group of patients. While patients deteriorating on DDS monotherapy invariably yielded dapsone-resistant *M. leprae* in mice, it was assumed that patients responding favorably to DDS monotherapy would not do so. No "control" group of responding patients was ever tested. Such a control group has now become available from our study, and 5 out of 6 responding patients yielded *M. leprae* resistant to high-dosage dapsone (0.01% w/w) in the mouse diet.

The mouse foot pad test for drug resistance, as described by Pettit and Rees ([5]), seems exquisitely sensitive to the presence of a few drug-resistant *M. leprae* in predominantly drug-sensitive strains. We have subsequently demonstrated that strains of *M.*