

Dapsone-Resistant Leprosy—The THELEP Approach¹

At the time of the first meeting, in April 1977, of the Scientific Working Group (SWG) on Chemotherapy of Leprosy (THELEP) of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, the problem of dapsone-resistant leprosy appeared to be one of considerable urgency. As related in the accompanying review of the subject by Pearson (⁴), a limited number of reports of surveys of dapsone resistance were available. Only two of these surveys were country-wide (^{3,6}), whereas others may have yielded biased results, having been conducted without particular attention to the representativeness of the patient sample studied. For whatever reason, the results of the several surveys varied widely. In addition, the existence of clinically-evident dapsone resistance had not been recognized in many leprosy-endemic countries. The wide variation among estimates of the prevalence of secondary dapsone resistance (the situation in which at the start of chemotherapy the great majority of the patient's *Mycobacterium leprae* are susceptible to dapsone; after initially responding to treatment with dapsone, the patient's disease relapses, and the majority of his organisms are then found to be dapsone-resistant) led to two conflicting interpretations. The problem of dapsone resistance had been exaggerated, and schemes based on dapsone as monotherapy remained the most cost-effective means of controlling leprosy. Alternatively, the problem of dapsone resistance had already assumed alarming proportions, and there loomed the prospect of being required to control leprosy without the assistance of one of the most effective drugs known.

The THELEP SWG decided upon a two-pronged approach to this problem. First, formal surveys of the prevalence of dap-

sone resistance were to be undertaken in a number of leprosy-endemic countries to assess the true magnitude of the problem. Simultaneously, the efficacy of various combined-drug regimens, designed both to prevent drug-resistant relapse and to minimize the numbers of persisting *M. leprae*, was to be determined by controlled clinical trial. Because formal surveys of the prevalence of dapsone resistance had never previously been undertaken under field conditions, a standard protocol was designed that could be applied to any leprosy control program in which some initial documentation of the patients was available. The protocol, which appears in the appendix to this paper, must be adapted to the local situation in which it is to be employed.

In brief, the protocol specifies that the survey is to include all patients with disease initially classified as lepromatous according to the Madrid classification who began treatment with dapsone at least 5 years earlier. Patients with a BI in one smear-site ≥ 3 are suspected of dapsone resistance; depending upon the number of such suspects, all or a randomly chosen portion of these patients are to be biopsied for inoculation of mice and dapsone-susceptibility testing of their *M. leprae*. The diagnosis of dapsone resistance is made only in mice. Purposely excluded as criteria for selection for biopsy and mouse inoculation were clinical appearance of relapse and evidence of irregular treatment. The BI criterion was chosen not as a criterion of relapse, but rather because it ensured the recovery of sufficient organisms to permit inoculation of mice. Thus, this simple and direct protocol does not require standardization of clinical descriptions, nor definition of regularity of treatment; on the other hand, it implies susceptibility-testing in mice of the *M. leprae* of all patients at risk of dapsone resistance from whom sufficient organisms can be recovered.

The protocol also envisions ongoing surveillance for primary resistance. It requires biopsy and mouse inoculation and drug-susceptibility testing of recovered *M. leprae* from all "new" patients with a BI in one lesion ≥ 3 . At least in retrospect, the

¹ Received for publication on 30 June 1981. This article is based on a presentation made at the October 1980 meeting of the Scientific Working Group of THELEP in Geneva. It is our understanding that similar publications will be printed in the December issue of LEPROSY REVIEW.—RCH

proportion of previously untreated patients who can be shown to harbor larger-than-normal proportions of drug-resistant *M. leprae* may be taken as a measure of the size of the pool of drug-resistant, infectious patients in the community.

Subsequent to the development of the protocol, THELEP-sponsored surveys of the prevalence of secondary dapsone-resistant leprosy were undertaken in Burma, Upper Volta, and two districts in South India, and surveys of the prevalence of primary resistance were undertaken in Ethiopia and the Philippines. With respect to primary resistance, dapsone-resistant organisms were recovered from 16 of 24 consecutive patients studied in Addis Ababa⁽⁵⁾ and two of 55 consecutive patients biopsied in Cebu⁽²⁾. The first estimate of the prevalence of dapsone resistance in Gudiyatham Taluk, South India, is 2.3 per 100⁽¹⁾; repeated annual surveys are planned in this survey area, and an estimate of incidence of dapsone-resistant relapse will also be obtained.

In order to encourage wider use of the mouse foot pad technique for detecting drug resistance, THELEP, together with the South-East Asia Regional Office of WHO, sponsored the "Mouse Foot-Pad Technique Standardization and Application Workshop," which was held at the Central Leprosy Teaching and Research Institute, Chingleput, South India, 19 November–14 December 1979. Among the 20 participants were scientists from Bangladesh, Bolivia, Burma, China, Cuba, India and Indonesia. Subsequent to the workshop, dapsone resistance has already been proven in Jakarta, Rangoon, and Shanghai.

During the period 1977–1980, in addition to the information on prevalence of dapsone resistance generated by THELEP-sponsored activities, individual cases have been recognized by mouse foot pad tests in a number of leprosy-endemic countries. Perhaps more importantly, however, a change in the climate of opinion appears to have occurred. Initial combined-drug therapy of patients with lepromatous leprosy is now widely advocated, and it appears very likely that such a policy will become almost universally implemented during the next decade.

With the rational use of multiple drug

therapy, the proportion of new cases who develop dapsone-resistant leprosy will certainly fall sharply. In addition, if it proves possible to give a period of supplementary chemotherapy to patients with lepromatous leprosy who had previously received only dapsone monotherapy, it may well be possible to arrest the present epidemic of secondary dapsone-resistant leprosy.

There is thus widespread acceptance that dapsone-resistant leprosy, although a serious problem, can be effectively dealt with; and there is some measure of agreement on the means to do so. The prevention of dapsone-resistant leprosy need no longer await proof of its widespread presence in an area. Therefore, it was agreed at the third meeting of the THELEP SWG in October 1980, that further formal surveys of secondary dapsone-resistant leprosy should not be undertaken by THELEP. Instead, THELEP will undertake to promote the development of national and regional laboratories capable of carrying out the mouse foot pad technique and of measuring the susceptibility to drugs of *M. leprae*. These new laboratories will be best able to serve local needs by demonstrating the occurrence in their communities of drug-resistant relapse, by undertaking ongoing programs of surveillance for primary resistance, and by propagating the mouse foot pad technique.

REFERENCES

1. BALRAJ, V., JESUDASAN, K., CHACKO, C. J. G., CHRISTIAN, M., TAYLOR, P. M., FRITSCHI, E. P. and JOB, C. K. Prevalence of secondary dapsone resistance in Gudiyattam Taluk, the leprosy control area of the Schieffelin Leprosy Research and Training Centre, Karigiri. 1. Preliminary report. *Int. J. Lepr.* **48** (1980) 397–401.
2. GUINTO, R. S., CELLONA, R. V., FAJARDO, T. T. and DE LA CRUZ, E. C. Primary dapsone-resistant leprosy in Cebu, Philippines. *Int. J. Lepr.* **49** (1981) 427–430.
3. LEVY, L., RUBIN, G. S. and SHESKIN, J. The prevalence of dapsone-resistant leprosy in Israel. *Lepr. Rev.* **48** (1977) 107–112.
4. PEARSON, J. M. H. The problem of dapsone-resistant leprosy. *Int. J. Lepr.* **49** (1981) 417–420.
5. PEARSON, J. M. H., HAILE, G. S., BARNETSON, R. ST. C. and REES, R. J. W. Dapsone-resistant leprosy in Ethiopia. *Lepr. Rev.* **50** (1979) 183–199.
6. PETERS, J. H., SHEPARD, C. C., GORDON, G. R., ROJAS, A. V. and ELIZONDO, D. S. The incidence of DDS resistance in lepromatous patients in Costa Rica: their metabolic disposition of DDS. *Int. J. Lepr.* **44** (1976) 143–151.

Appendix

PROTOCOL FOR SURVEYS OF DAPSONE RESISTANCE¹

1. Background. Since the first report by Pettit & Rees (1964) of relapse of lepromatous leprosy accompanied by the emergence of dapsone-resistant *Mycobacterium leprae* proven in the mouse foot pad, patients who have relapsed with dapsone-resistant *M. leprae* have been recognized in widely-scattered treatment centers. Prevalence surveys have recorded 2.5 cases of dapsone resistance per 100 patients with lepromatous leprosy at risk in Malaysia (Meade, *et al.*, 1973); 6.8 cases per 100 patients at risk in Costa Rica (Peters, *et al.*, 1976); and 3.7 cases per 100 patients at risk in Israel (Levy, *et al.*, 1977). In a survey of incidence, Pearson, *et al.* reported (1976) about 3 cases of dapsone resistance per year per 100 patients at risk in Ethiopia. The definition of patients at risk varied from survey to survey. Moreover, the Costa Rican and Israeli surveys were countrywide, whereas the Malaysian and Ethiopian surveys were conducted within leprosy treatment centers. Therefore, it is difficult to compare these figures. Nevertheless, they stand in sharp contrast to the failure to recognize relapse of lepromatous leprosy associated with dapsone-resistant *M. leprae* in many treatment centers.

Many experts believe that therapy of lepromatous leprosy with dapsone alone is accompanied by an unacceptably high risk of dapsone resistance, and that monotherapy should be abandoned in favor of some combined-drug regimens. Other experts are not convinced of the danger of dapsone monotherapy, and justify its continued use in terms of cost-effectiveness. It is important to resolve this lack of consensus. If the prevalence surveys were biased, producing inflated estimates of the risk of dapsone resistance, one could not justify heavy investment of limited resources in combined chemotherapy. If, on the other hand, dapsone resistance has become ubiquitous, but has been unrecognized simply because it has not been sought, the more expensive combined treatment may prove more cost-effective in the long term.

This protocol, designed for surveys of the prevalence of lepromatous leprosy patients whose course has been complicated by the emergence of dapsone-resistant *M. leprae* during dapsone monotherapy, has resulted from the desire of the THELEP Scientific Working Group to conduct surveys of the prevalence of dapsone-resistance in a number of leprosy endemic countries, in an attempt to measure the magnitude of

the threat to leprosy control activities presented by resistance to dapsone.

2. Study designs. In simplest terms, the therapist wishes to know the *incidence* of dapsone resistance and to identify *risk factors*. This knowledge would enable him to design more effective chemotherapeutic regimens. Although these matters are also of interest to the leprosy control physician, he will be more interested in the *prevalence* of dapsone resistance. It is the leprosy patients who currently serve as source cases for transmission of dapsone-resistant infection that present the gravest threat to his leprosy control program.

Measurement of the incidence of dapsone resistance is probably not possible in the real world. Such measurements, whether retrospective or prospective, could be made only in those leprosy control areas possessing both excellent records and excellent follow-up that had not introduced systematic treatment with secondary drugs. These requirements appear mutually exclusive; a leprosy control program that has maintained excellent records and patient follow-up over a number of years is very likely also a program in which treatment with secondary drugs had been introduced early and continuously. On the other hand, it appears possible to find leprosy control programs in which treatment with secondary drugs has not been introduced on a large scale, and in which records adequate for a *point-prevalence* survey have been maintained. Although one could not measure the incidence of dapsone resistance in such an area, one might nevertheless be able to define risk factors by the *case-control* method.

There appears, finally, to be a sound theoretical basis to the belief that the proportion of patients with newly-diagnosed BL and LL leprosy who are found to have "primary" resistance to dapsone is a measure of the prevalence of "secondary" resistance in that community. A program of surveillance for primary resistance to dapsone can be enlarged to detect primary resistance to other drugs, with only minimal additional effort.

The term "resistance," as used heretofore and henceforth, is defined as that *demonstrated in the mouse foot pad system*. With this definition in mind, it is the purpose of this protocol to describe surveys of the point-prevalence of secondary dapsone resistance and of primary resistance to dapsone (and other drugs, should this be desired). These surveys are to be sponsored by THELEP in collaboration with local leprosy control authorities. By *secondary resistance* is meant the situation of the patient who begins therapy with the great majority of his *M. leprae* susceptible to the drugs employed. After an initial response to treatment, which may endure for 20 years, his disease relapses, and the majority of his *M. leprae* are found to be drug resistant. By *primary resistance* is meant the situation of the patient, the majority of whose organisms are drug-resistant before any treatment has been instituted.

¹ Prepared by the Subcommittee on Dapsone Resistance Surveys of the Steering Committee of the Chemotherapy of Leprosy Programme (THELEP), Special Programme for Research and Training in Tropical Diseases, World Health Organization (Dr. M. F. Lechat, Dr. D. L. Leiker, Dr. L. Levy, Dr. S. K. Noordeen and Dr. J. M. H. Pearson).

Because mouse inoculation is not possible unless sufficient bacilli are recovered from a skin biopsy specimen, THELEP-sponsored surveys of secondary dapsone resistance will be based on inoculation of immunologically normal mice with *M. leprae* recovered from skin biopsy specimens obtained from patients with lepromatous leprosy who began sulfone treatment at least five years earlier, who have continued in treatment, and who demonstrate a BI ≥ 3 in at least one skin lesion. One group of inoculated mice will be held as untreated controls; dapsone will be administered, incorporated in the mouse chow in one of three concentrations, to other groups of mice inoculated with *M. leprae* recovered from the same biopsy specimen. Patients will be subjected also to a formal, standardized clinical assessment, and the MI (or "solid ratio") will be measured, so that one may measure in retrospect the reliability with which these simpler criteria may be used to detect patients whose organisms would be proven resistant to dapsone by mouse inoculation.

A brief description of the statistical basis of the survey design may assist one to understand the design of the survey protocol that follows. First, one assumes that a leprosy control area encompassing a population of about one million has a leprosy prevalence of 1 per 100 population, and that 10 per 100 leprosy patients have the lepromatous form of the disease. If the prevalence of dapsone resistance is 5 to 10 per 100 lepromatous patients then one should detect 50 to 100 patients with dapsone resistance. Second, one assumes that it is not necessary to measure the prevalence of dapsone resistance with great precision. That the prevalence is actually 8 per 100 rather than 7 per 100 lepromatous patients would be of little importance in designing a control strategy. On the other hand, if the prevalence were 20 per 100, one would plan a control program greatly different from that appropriate to a prevalence of 2 per 100. The Subcommittee believed that it would not be necessary to measure the prevalence of dapsone resistance with a precision greater than ± 30 –50%.

Our statistical consultant² has calculated that 25 mouse foot pad tests would permit one to estimate prevalence with 26% uncertainty if only 70% of the 25 patients so tested proved to have resistant *M. leprae*, assuming that the organisms from all 25 patients were infective for mice (that is, included at least 1 viable per 1000 organisms). Testing biopsy specimens that contained viable *M. leprae* from 50 patients, one could estimate prevalence with 28% uncertainty if only half of the patients were subsequently proven to have dapsone-resistant organisms. If one tested specimens from 100 patients, half of whom were shown to have resistant organisms, one could estimate the prevalence of dapsone resistance with an uncertainty of 20%.

The measurement of susceptibility to dapsone of *M. leprae* recovered from 50 to 100 specimens appears feasible, either in a new laboratory located near the survey site, or in an existing laboratory located at some distance. One would still have a reasonably precise estimate of the prevalence of dapsone resistance, even if as many as half of the mouse tests were "wasted"—that is, the biopsy specimens contained enough organisms to provide an inoculum of 1000–10,000 organisms per foot pad, but the proportion of viable organisms was smaller than 1 per 1000, the threshold for detection in the mouse, and *M. leprae* did not multiply even in untreated mice.

This survey design assumes that all of the patients with dapsone resistance will be included among the patients treated with sulfones for at least five years who are found to have a BI ≥ 3 . Undoubtedly, also included in this number will be patients whose *M. leprae* are largely susceptible to dapsone, but whose BI has remained high because of irregular treatment; some of these may have a proportion of viable organisms large enough to infect mice (1 viable per 1000 total), whereas others may not; it is the patients of this last group who will represent the "wastage." Finally, one must admit the possibility that patients with dapsone resistance whose BI has not yet increased to the level of ≥ 3 will not be included among the patients whose organisms are inoculated into mice. To the extent that these patients are not recognized, the measured prevalence of dapsone resistance will represent an underestimate of the true prevalence. On the other hand, these patients will be discovered on subsequent surveys. And it could be argued that, with so low a BI, they do not yet represent a threat to the public's health.

3. Requirements of participating centers. There must be, at the very least, a register of leprosy patients admitted for treatment. This register must include both the initial classification of the disease and the date on which treatment was initiated. There must be registered a minimum of 1000 living patients with lepromatous (according to the Madrid classification) leprosy resident within a geographically defined area.

Dapsone therapy must have been introduced at least 15 years before the date of the survey. There must have been no systematic use of clofazimine or rifampin.

4. Conduct of the survey.

4.1. Preliminary steps.

4.1.1. Site visit. A site visit will be made by a member of the THELEP Steering Committee to assess the feasibility of a dapsone resistance survey. Staff members will be interviewed; laboratory and other equipment will be inspected; and patient records will be examined. Assistance will be given in the development of a detailed, specific protocol and budget request. Finally, the Steering Committee will hear a report of the site visit and review the protocol and budget.

4.1.2. Training. Before the survey can begin, key personnel must be trained. The physician responsible

² Mr. J. Duppenhaler, Health Statistics Methodology, World Health Organization.

for the survey must be trained in standard smear-taking and biopsy techniques. A laboratory technician must be trained in the measurement of the BI according to Ridley's scale. A nurse or clerk must be trained in the management of survey records. If mouse inoculation is to be carried out near the survey site, personnel must be trained in the required laboratory and mouse husbandry techniques.

4.1.3. *BI*. Measurement of the BI as practiced at the center will be verified by an exchange of slides with an established center. All necessary modifications of smear-taking, staining and microscopy techniques will be made before the survey begins.

4.1.4. *Mouse inoculation*. One of the purposes of the site visit described in 3.1.1 is to assess the feasibility of establishing at the center a laboratory for the inoculation of immunologically normal mice, or alternatively, to consider the ease with which skin biopsy specimens could be transported to an already-established laboratory. If the establishment of a new laboratory at the center is at all possible, this is the preferred alternative. The laboratory must be established before the survey is begun.

5. The survey.

5.1. *Selection of patients for study of point-prevalence of secondary dapsone resistance*. Available patient records will be examined, and those of patients with a recorded pretreatment diagnosis of lepromatous leprosy according to the Madrid classification will be abstracted. Those patients not known to have died who began treatment with a sulfone drug at least five years earlier and who are still under treatment, regardless of the degree of regularity of attendance, will be listed according to the last-known place of residence, and strategies will be mapped for approaching the patients in order to perform clinical assessment, skin smears and skin biopsies. Based on the estimate of the number of living patients with lepromatous leprosy treated with dapsone monotherapy for a minimum of five years whose last BI was ≥ 3 , the proportion of these patients to be biopsied for mouse inoculation will be determined.

5.2. *Procedure to be followed with each patient*. This procedure must be designed at the survey site in the course of developing the detailed specific protocol. The optimal procedure is certain to differ from center to center. It will be necessary to see all patients with lepromatous leprosy who began treatment with sulfones at least five years earlier and who have remained in treatment, whatever the degree of regularity, to the

time of the survey. The patient must be interviewed; formal clinical assessment must be carried out; a urine specimen must be obtained for analysis for dapsone; smears for measurement of the BI must be made, fixed, stained, and examined; of the patients with BI in one site ≥ 3 , all or a sample will be subjected, with their consent, to skin biopsy and inoculation of normal mice. The most efficient means of doing this may well differ importantly from center to center; for example, it may prove possible in one center to bring patients to the center; in a second center, although it may not be possible to bring patients to the center, it may prove possible to make repeated visits to the patients' homes; in a third center, it may be necessary to take smears, stain and examine them, and take skin biopsy specimens all at the time of a single visit. The various procedures are fully described in several appendices to the Standard (THELEP) Protocol for Chemotherapy Trials in Lepromatous Leprosy.

5.3. *Primary dapsone resistance*. As a matter of routine, all newly diagnosed leprosy patients with a BI in one lesion ≥ 3 will be subjected, with their consent, to skin biopsy; the organisms recovered from the skin biopsy specimens will be inoculated into mice and the susceptibility to dapsone (and other drugs) tested.

6. *Ethical considerations*. The survey should pose no ethical problems. The procedures of skin scraping for measurement of the BI, and of skin biopsy for inoculation of mice are accepted clinical procedures. Although they will be performed, in this instance, in the course of a research activity, it is clearly in the patients' interest that these procedures be carried out. In any case, none of these procedures will be carried out without the patients' consent.

REFERENCES

1. MEADE, T. W., PEARSON, J. M. H., REES, R. J. W. and NORTH, W. R. S. The epidemiology of sulphone-resistant leprosy. *Int. J. Lepr.* **41** (1973) 684.
2. PEARSON, J. M. H., ROSS, W. F. and REES, R. J. W. DDS resistance in Ethiopia—a progress report. *Int. J. Lepr.* **44** (1976) 140.
3. PETERS, J. H., SHEPARD, C. C., GORDON, G. R., ROJAS, A. V. and ELIZONDO, D. S. The incidence of DDS resistance in lepromatous patients in Costa Rica: their metabolic disposition of DDS. *Int. J. Lepr.* **44** (1976) 143–151.
4. PETTIT, J. H. S. and REES, R. J. W. Sulphone resistance in leprosy. An experimental and clinical study. *Lancet* **2** (1964) 673.

QUESTIONNAIRE

1. Name of institution _____
2. Sponsorship of institution _____
3. Name of respondent _____
4. Estimated population included in leprosy control scheme _____
5. Estimated prevalence of leprosy per 1000 population _____
6. Estimated proportion of lepromatous (Madrid classification) patients _____
7. Number of square miles/kilometres included in leprosy control scheme _____
8. Estimated proportion of registered patients who reside within boundaries of scheme _____
9. Number of patients registered _____
10. In what year was sulfone therapy introduced to area? _____
11. What additional antimicrobial drugs have been used?

	When introduced?	How many patients treated?
Clofazimine	_____	_____
Rifampin	_____	_____
Thiacetazone	_____	_____
Ethionamide	_____	_____
Prothionamide	_____	_____
Other	_____	_____
12. What proportion of your registered patients is seen at a leprosarium or hospital? _____
13. What proportion of your registered patients is seen at an outlying clinic? _____
14. What proportion of your registered patients is seen with some regularity? _____
15. How do you define "regularity" as used in question No. 14? _____
16. Describe below your clinical laboratory, histopathological, and animal facilities.