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The Prevalence of Dapsone-resistant Leprosy in Israel*

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The prevalence of dapsone-resistance among patients with lepromatous leprosy treated in Israel for a minimum of 8 years was 3.7 per 100.

Introduction

For many years after the introduction of sulphone drugs in the treatment of leprosy in the 1940's, the belief was widely held that the risk of emergence of sulphone-resistant mutants of *Mycobacterium leprae* was negligible. However, beginning with the report of Pettit and Rees (1964) of the first patients from whom *M. leprae* resistant to dapsone (4,4′-diaminodiphenylsulphone, DDS) were isolated, it has become clear that relapse of lepromatous leprosy during sulphone monotherapy because of the emergence of sulphone-resistant organisms is by no means a rare occurrence. Efforts have been made recently to assess the risk in quantitative terms (Meade *et al.*, 1973; Peters *et al.*, 1976). In this paper, we report the results of such an effort among patients with lepromatous leprosy in Israel.

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Methods and Materials

After diagnosis, practically all leprosy patients in Israel are treated and observed as outpatients, under the supervision of the 14 subdistrict offices of the Ministry of Health. All of the inpatient records and abstracts of the outpatient records are filed at the Government Hospital for Hansen's Disease in Jerusalem, where approximately 25 patients are currently hospitalized. The outpatients who live in and near Jerusalem are seen regularly in the clinic located at the Hospital by one of us (J.S.), who also sees all of the other patients periodically in their home communities.

For the purpose of this study, the records of all 114 patients with leprosy classified as "lepromatous" who began treatment prior to 1966 were examined and abstracted. Of the 114 patients, 20 had been lost prior to the beginning of this study in 1974; 17 patients had died, 2 had emigrated, and one had simply been lost to follow-up. There was no evidence that any of these patients had died or emigrated because of their leprosy. Therefore, the value of the denominator to be used in calculating the prevalence of dapsone-resistant leprosy is 94.

Twenty patients who had kept their clinic appointments faithfully and who were believed to have taken their treatment regularly were observed to have suffered a relapse of their disease process, or to have failed to improve, on the basis of their clinical appearance and the continued appearance of acid-fast bacteria (AFB) in smears of skin scrapings, despite a minimum of 8 years of treatment. Skin biopsy specimens were obtained from active-appearing lesions of these patients by means of a 6 mm scalp punch, sealed in sterile tubes, placed together with wet ice in a vacuum flask, and shipped by air to San Francisco. The specimens were received in San Francisco within 72 h of biopsy, at which time ice remained in the flask. In San Francisco, the specimens were processed for mouse inoculation by published methods (Shepard, 1960; Shepard and McRae, 1968).

Twenty mice were inoculated with the organisms recovered from each specimen. Beginning 3 or 4 months after inoculation, one mouse from each group was killed for measurement of the "incubation period" (IP), the number of months elapsed between inoculation of the mice and the demonstration of AFB within 30-40 cells in histological sections of the inoculated foot-pad tissues. After evidence of multiplication of *M. leprae* was noted in a monthly section, a harvest was performed from the pooled tissues of 4 foot-pads. If no multiplication was apparent by the 12-month section, a harvest of *M. leprae* was performed from a pool of the inoculated foot-pad tissues of all surviving mice. From the number of AFB harvested and the number of days elapsed between inoculation of the mice and harvest, the "generation time" (G) was calculated as if all of the inoculated bacilli had multiplied at a constant rate between inoculation and harvest. Values for the IP $\leqslant 12$ months and for $G < 100$ days indicate that *M. leprae* had multiplied, and, therefore, that the inoculum had contained organisms infective for the mouse and presumably viable.

When *M. leprae* were found to have multiplied in mice, they were recovered by harvest and subsequently passaged to groups of 60 mice. Beginning on the day of passage, dapsone incorporated into the mouse diet in a concentration of 10^{-4}, 10^{-3}, or 10^{-2} g% was administered to 3 subgroups of 15 mice each, whereas the remaining subgroup received drug-free diet. Dapsone administration was continued until a harvest from the foot-pads of untreated control mice revealed that the *M. leprae* had multiplied to a level near 10^6 AFB per foot-pad. At this time,

M. leprae were harvested from pools of the tissues of 4 foot-pads of the treated mice of all 3 subgroups. Susceptible organisms were defined as those that failed to multiply in mice administered dapsone.

Results

Several characteristics of the entire group of patients, and of the 20 patients selected for further study, are summarized in Table 1. A little more than one-quarter of the patients had been born in Israel. About one-third of the patients were female. The 20 patients suspected of harbouring dapsone-resistant *M. leprae* did not differ from the larger group of 114 patients in terms of birthplace, sex, or year of birth.

TABLE 1

Characteristics of patient population

	Place of birth		Sex		Year of birth	
	Israel	Abroad	Female	Male	Before 1921	1921 or later
Total number	30*	84	37	77	56	58
Number lost	6	14	5	15	N.A.†	N.A.
Number at risk	24	70	32	62	N.A.	N.A.
Number studied	4	16	4	16	9	11

* All of these patients were born before 1948, the year the State of Israel was established.
† Not available.

The results of the study of the 20 skin biopsy specimens submitted for mouse inoculation are summarized in Table 2. No AFB were recovered from the specimens of 8 patients—nos 15, 46, 125, 131, 153, 199, 203 and 218; therefore, no mice were inoculated with *M. leprae* from these patients. In the case of 3 additional specimens—those from patients nos 171, 184 and 191, only one AFB was seen in the 60 oil-immersion fields examined on each counting slide. The numbers of AFB recovered were therefore very small, resulting in very small inocula. No evidence of multiplication of *M. leprae* was encountered in the mice inoculated with organisms recovered from any of these specimens. Nine specimens contained enough AFB to permit mice to be inoculated with 5000 organisms per foot-pad. The organisms from 3 of these specimens—those from patients nos 109, 193 and 202—did not prove infective for mice, and the organisms from a fourth specimen—that of patient no. 85—were only marginally infective, multiplying in the foot-pad of the mouse killed for histopathological examination after 10 months, but in none of the 8 mice sacrificed for harvest of *M. leprae* 398 days after inoculation. Five skin biopsy specimens—those of patients nos 42, 50, 58, 135 and 287—contained *M. leprae* infective for mice.

The results of testing these 5 strains of *M. leprae* for susceptibility to dapsone are presented in Table 3. Two of the strains—those isolated from the specimens of patients no. 42 and 287—were fully susceptible to dapsone, in that the organisms failed to multiply in mice fed any of the dapsone-containing diets. *M. leprae* of the 3 remaining patient-strains were partially resistant to dapsone, multiplying in mice fed 10^{-4} and 10^{-3} g% dapsone, although at a slower rate than in control

TABLE 2

Results of mouse inoculation

Patient no.	Inoculum		Harvest	
	No. AFB per specimen ($\times 10^5$)	No. AFB per foot-pad ($\times 10^3$)	Incubation period (months)	Generation time (days)
15	< 0.20	N.I.*		
42	409	5.0	8	43.0
46	< 0.20	N.I.		
50	29.8	7.08	9	39.3
58	1580	5.0	4	30.6
85	129	5.0	10	> 100
109	57.7	5.0	> 12	> 100
125	< 0.20	N.I.		
131	< 0.20	N.I.		
135	1430	5.0	8	40.8
153	< 0.20	N.I.		
171	0.32	0.12	> 12	> 100
184	0.30	0.33	> 12	> 100
191	0.20	0.20	> 12	> 100
193	536	5.0	> 12	> 100
199	< 0.20	N.I.		
202	697	5.0	> 12	N.H.†
203	< 0.20	N.I.		
218	< 0.20	N.I.		
287	199	5.0	8	39.4

* N.I., not inoculated.
† N.H., not harvested.

TABLE 3

Results of dapsone-susceptibility studies

Patient no.	Dapsone concentration (g%)			
	0	10^{-4}	10^{-3}	10^{-2}
	Generation time (days)			
42	23.7	> 100	> 100	> 100
50	14.9	33.6	32.1	> 100
58	22.3	31.2	52.3	> 100
135	22.8	32.7	41.8	> 100
287	25.4	> 100	> 100	> 100

mice, and failing to multiply in mice administered dapsone in the largest concentration.

In addition to these 20 patients whose *M. leprae* were suspected to be resistant to dapsone, examination of the medical records revealed 5 additional patients whose smears of skin scrapings contained AFB in 1973 or 1974. One of these patients died before a biopsy could be performed and the specimen shipped to San Francisco. No specimens were obtained from the 4 remaining patients, who

were thought to be demonstrating satisfactory progress without a change of treatment on clinical grounds.

Thus, there were 25 patients suspected of harbouring dapsone-resistant *M. leprae*; skin biopsy specimens were obtained from 20. Of these 20, 11 did not contain enough AFB to permit study of their susceptibility to dapsone. Of the remaining 9 specimens, the organisms recovered from 4 were not infective or only marginally infective for mice. The organisms recovered from the specimens of 2 patients were infective for mice but fully susceptible to dapsone, whereas the *M. leprae* of 3 patients were resistant to low concentrations but susceptible to a high concentration of dapsone in the mouse diet. If specimens had been obtained from the 5 patients not studied, it appears likely that 3 specimens would have contained insufficient organisms for inoculation of mice, one would have contained organisms not infective for mice, and one would have contained *M. leprae* capable of multiplying in mice, with a 50% likelihood of being resistant to dapsone. Thus, the numerator for the calculation of the prevalence of patients harbouring dapsone-resistant *M. leprae* is 3.5, and the prevalence is 3.5 per 94 patients, or 3.7 per 100.

Discussion

The purpose of this study was to estimate the frequency with which dapsone-resistant *M. leprae* emerge after years of treatment of lepromatous leprosy patients in Israel with sulphone monotherapy. Studies of this kind are ordinarily difficult to carry out. The patients who have relapsed with the emergence of resistant organisms, who form the numerator, are usually easily recognized, and are therefore well-known to leprosy treatment centres. The difficulty lies in calculating the denominator, the number of patients at risk; only in a few jurisdictions have good records been maintained and virtually complete patient follow-up practised.

Two such studies have already been reported. Meade and his coworkers (1973) reported a frequency of 2.5 per 1000 among patients beginning treatment in Malaysia with dapsone in full dosage, and 7.8 per 100 among patients who began treatment with solapsone. Peters *et al.* (1976) reported a frequency of 6.8 per 100 among Costa Rican patients treated for a minimum of 7 years. During the first years of sulphone therapy in Costa Rica, patients were treated with sulphoxone. The frequency of dapsone resistance in Israel—3.7 per 100, appears to represent an intermediate value. There is nothing to suggest a disproportionate number of relapses among the 20 patients lost to follow-up.

It has been pointed out (Pearson *et al.*, 1975, 1976) that consistent treatment with dapsone in full dosage results in the emergence of mutant strains of *M. leprae* that multiply in mice administered dapsone in a dosage of 10^{-2} g%, the largest dosage usually employed in measuring the susceptibility of strains of *M. leprae* to dapsone. On the other hand, treatment with dapsone in low dosage or with dapsone derivatives produces mutant *M. leprae* that multiply in mice administered dapsone in lower dosages (10^{-4} and 10^{-3} g%) but not in those mice administered the largest dosage. Such "low resistance" mutants were not encountered in Malaysia, where those patients beginning treatment with solapsone were all subsequently transferred to treatment with dapsone in full dosage (Pearson *et al.*, 1975). In the Costa Rican study, half of the 12 dapsone-resistant patient-strains of *M. leprae* isolated were found to be low-resistance mutants; this suggests that

the use of dapsone in full dosage, said to have been started in 1960, may not have been universal (Peters *et al.*, 1976).

In Israel, a variety of treatment regimens has been used, so that it is difficult to characterize them in a few words. Prior to 1950, patients were treated with thiacetazone or sulphoxone. Dapsone, in daily doses of 25-100 mg, has been generally used since 1950. Until the introduction of thalidomide in 1964, however, the dosage of dapsone was frequently interrupted, or sulphoxone or solapsone was substituted when patients experienced severe lepra reactions. Also, solapsone was administered weekly by injection as a supplement to dapsone when patients were thought to be irregular in their self-administration of dapsone. Finally, solapsone was sometimes substituted for dapsone as a convenience to those patients required to work away from their homes. That the 3 dapsone-resistant mutants isolated from Israeli patients were of the low resistance variety appears consistent with these facts.

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