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Ten Years of Dapsone in Lepromatous Leprosy: Clinical, Bacteriological and Histological Assessment and the Finding of Viable Leprosy Bacilli*

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Twelve lepromatous patients who had completed 10 to 12.5 years' continuous chemotherapy, principally or entirely with dapsone, were assessed clinically, bacteriologically and histologically. In all 12 the disease showed full clinical response to therapy, although three patients remained smear positive, and two of these still suffered from mild *erythema nodosum leprosum*. However, by mouse footpad inoculation it was shown that seven of the 12 patients still harboured viable *Myco. leprae*. Thus bacterial multiplication was obtained in mice inoculated with 10 of 37 tissue suspensions prepared from extensor skin (4), striated muscle (3), peripheral nerve (2) and smooth muscle (1), although the numbers of positive footpads in each group of mice were small, in keeping with the minute numbers of leprosy bacilli, of variable viability, inoculated. No bacterial enhancement was obtained in thymectomised-irradiated mice, and three of six strains died out on passage; these findings recalling the difficulties encountered by McCune *et al.* in culturing *Mycobacterium tuberculosis in vitro* from tuberculous mice subjected to effective chemotherapy. Three of these strains of *Mycobacterium leprae* (two from skin and one from nerve) from separate patients, were shown to be fully sensitive to dapsone. The importance of these findings is discussed, especially with regard to clinical relapse of leprosy after premature stopping of treatment and to the total duration of dapsone therapy required in lepromatous leprosy.

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Introduction

There are two major paradoxes in the chemotherapy of lepromatous leprosy. First, whereas the initial response to sulphone treatment is encouraging, long-term clinical results may be very unsatisfactory (Jacobson and Trautman, 1971). Second, although the Morphological Index (MI) rapidly falls after commencing dapsone (DDS) therapy, to reach baseline after about 4.5 months (Waters, Rees and Sutherland, 1967), and although after approximately three months' treatment, leprosy bacilli obtained from skin biopsies may no longer infect mouse footpads (Shepard, Levy and Fasal, 1968) yet bacterial reactivation with clinical relapse may occur after many years of treatment.

In the experience gained over the last 10 years at Sungei Buloh Leprosarium (Pearson and Waters, unpublished data), patients who relapse (clinically and histologically) while still receiving dapsone have developed sulphone resistance, whereas those who relapse through failure to continue on therapy in general are still harbouring sulphone-sensitive strains of *Mycobacterium leprae*. It may be argued that some relapses in the latter group are due to re-infection with *Myco. leprae*. But in the majority of cases the weight of probability, both on clinical and on general bacteriological grounds must be that small numbers of viable leprosy bacilli persist in the patient for long periods after the start of successful chemotherapy (see Committee of Experimental Chemotherapy, 1973). We have suggested (Waters and Rees, 1962; Waters, 1967; Ellard, 1974) that these viable bacilli may be present in special sites, such as Schwann cells and smooth and striated muscle fibres.

To investigate this hypothesis, we have examined fully, both clinically and experimentally, 12 fully documented lepromatous patients who had completed 10 to 12.5 years of continuous anti-leprosy treatment. Here we present a preliminary report of our findings.

Materials and Methods

The 12 patients were unselected volunteers from among those still available from a group of some 80 previously untreated lepromatous subjects investigated by one of us (MFRW) during the years 1959-1962 for admission to controlled clinical trials. Full clinical, bacteriological, histological and treatment records were available. Ten were Chinese, two were Southern Indian, all were males. Their ages on first admission ranged from 16 to 66 (average 38) years. Both clinically and histologically, all had been classified LL or LI on the modified Ridley-Jopling spectrum (Ridley and Jopling, 1966; Ridley and Waters, 1969), and all had suffered from moderately or markedly severe leprosy ("L2" or "L3", as defined by Quagliato, Bechelli and Marques, 1970). Their admission smears had given average Bacterial Index (BI) of 4.5 with a range of 3.7 to 5.2 on Ridley's logarithmic scale (Ridley, 1958); the pretreatment MI was known in 11 patients and averaged 44 (range 20 to 74). Their lepromin (Dharmendra) skin test had been negative.

All had received initially one year of dapsone by intramuscular injection, 200 mg twice weekly for the first six weeks and then 300 mg twice weekly. Thereafter the dosage was more variable, between 50 and 400 mg twice weekly, and some patients had been changed to oral dapsone. During their second and fourth year of treatment respectively, two patients with severe *erythema*

nodosum leprosum (ENL) (nos. 4 and 2) had been switched to thiambutosine in the dosage 1 g b.d. for 17 months, before recommencing dapsone. Two other patients (nos. 10 and 11) had received clofazimine (B663) for 12 and 42 months respectively, the former for persisting neuritis and the latter for severe ENL; dapsone was given simultaneously with the clofazimine for six months (no. 10) and for 28 months (no. 11). A fifth (no. 3) had additionally received streptomycin for two years for pulmonary tuberculosis. Nine patients had suffered from ENL, but had been kept throughout on full and regular treatment. Only one patient (no. 1) was not still taking regular treatment at the time of the re-examination—he had stopped against advice eight months earlier, after more than 12 years' continuous treatment. Eight were still living in, or close to the leprosarium, while four outpatients, although living further away, attended regularly for their supplies of dapsone.

The "ten year" assessment was carried out, ten years after commencing treatment in nine patients, eleven years in two and 13 years in one (average 10.5 years, range 9 years 11 months to 13 years 1 month). The patients were fully examined clinically by the same observer as a decade earlier. Photographs were taken and compared with the pretreatment photographs. Smears were taken from both ear lobes and from at least four representative skin sites, the pretreatment sites being chosen unless alternative sites were indicated. Urine specimens were collected for routine examination and for estimation of dapsone concentration. Lepromin (Wade-Mitsuda) and tuberculin (1 TU of RT23) skin tests were performed in eight patients. Biopsies were obtained of extensor skin, triceps muscle, superficial radial nerve, and in five cases also of scrotum for dartos (smooth muscle). Aliquots of skin were fixed in Ridley's fixative, and of all sites in Richardson's solution, for histological examination. Other aliquots were flown on wet ice to the National Institute for Medical Research, London, where they were extracted for acid-fast bacilli (AFB), and the resulting suspensions were inoculated into the footpads of groups of six normal and/or thymectomised irradiated (T900R) mice, whether or not AFB could be detected (Rees, 1971). The mice were harvested individually after 12 months, counted, and suspensions found positive for AFB were passaged.

Additional extensor skin, nerve (superficial radial or sural) and dartos biopsies were taken from nine of the 12 patients from one year to three years eight months after the "10-year" examination (i.e. from 11 years 1 month to 14 years 6 months, average 12 years 9 months after first commencing treatment). During this time all 12 patients were still on regular dapsone treatment, patient no. 1 having been restarted from the date of the principal examination.

Results

CLINICAL

The general appearance of all 12 patients was good, compatible with quiescent, fully treated lepromatous leprosy (Fig. 1). Nine were smear negative, three (patients nos. 2, 7 and 11) were still positive at one or two only of the six sites, where individual BIs were 1+ to 3+; all bacilli seen were fragmented (MIs were 0). Two of the three smear-positive patients had evidence of mild ENL (nos. 7 and 11).

Over the decade, anaesthesia had increased slightly in six patients, although

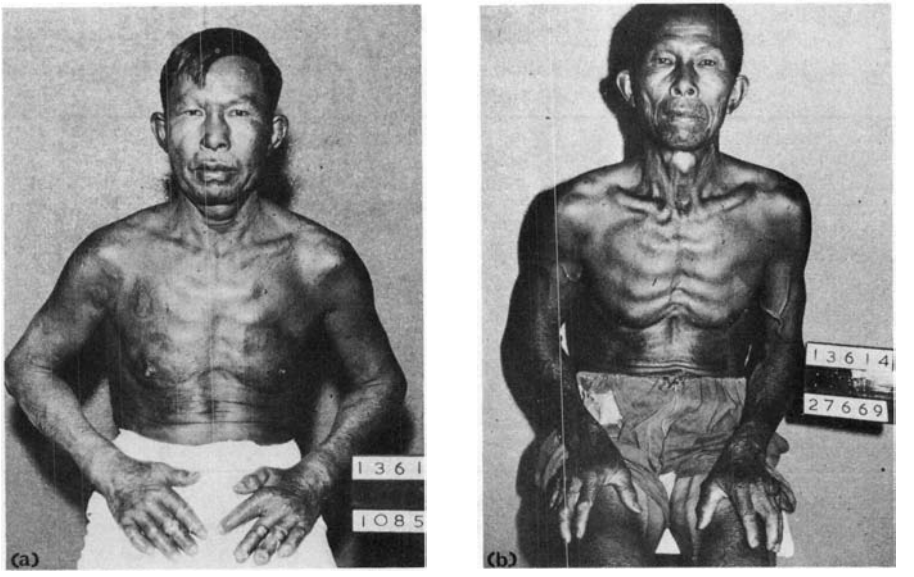

Fig. 1 Representative picture of lepromatous patient (No. 4) before start of treatment (a) and after 10 years of continuous treatment (b).

nerves in general had become smaller. However, two LI patients showed further unilateral enlargement of ulnar nerves originally related to initial borderline lesions on the forearm; both patients (nos. 6 and 10) had suffered from neuritis and had eventually received "nerve slits" after increased muscle wasting had developed. Eight others showed slight increase in muscle weakness and/or wasting (most commonly increased weakness of toe abduction), compared with pretreatment; two showed no significant change. Two patients (nos. 4 and 10) had superficial anaesthetic plantar ulcers at the time of review and a third (no. 3) had developed slight atrophy of some anaesthetic digits over the decade.

None of the 12 patients had undergone a reversal reaction, and the eight who were retested remained lepromin negative.

HISTOPATHOLOGY

Skin. In respect of infiltrating cells, all 12 specimens were abnormal. They showed streaks or clumps of old foamy lepromatous infiltration mainly around dermal appendages, especially sweat glands and arrector pili muscle, but also following neuro-vascular pathways. The cell type was predominantly foamy histiocyte, with occasional lymphocytes and a few plasma cells. The dermal nerves were present in all but two instances and showed changes varying from slight fibrosis to total collagenisation of the endoneurium with multilayering of the perineurium. Granular AFB, or bacterial debris were found in four patients.

Striated muscle (triceps). This was available from 11 patients, and only two showed any abnormality, consisting of a slight histiocytic and lymphocytic

TABLE 1

Details of Myco. leprae *isolates obtained in footpads of mice inoculated with various tissue suspensions from the 12 patients*

Myco. leprae isolates	Origin of tissue suspensions				
	Skin	Muscle	Nerve	Dartos	Total
No. of isolates attempted	13[a]	12	7	5	37
No. of isolates successful	4	3	2	1	10
No. of passages attempted	3	2	1	0	6
No. of passages successful[b]	2	0	1	0	3

[a] Included two skin biopsies from patient no. five.
[b] Skin biopsies from patients nos. 3 and 5; nerve biopsy from patient no. 11.

TABLE 2

Data showing dapsone sensitivity in mice of the three passaged strains of Myco. leprae

Source of *Myco. leprae*	Proportion of mouse footpads showing multiplication			
Patient no. and tissue	Normal mice		T/900R mice[a]	
	UTC[b]	DDS[c]	UTC	DDS
3, skin	–	–	7/12[d]	0/12
5, skin	8/12	0/12	8/8	–
11, nerve	–	–	2/10	0/10

[a] T/900R = mice subjected to adolescent thymectomy followed by whole body irradiation.
[b] UTC = untreated control mice.
[c] DDS = mice fed with 0.0001% dapsone in their diet.
[d] Yields of bacilli were not enhanced in any of the groups of T/900R mice.

infiltrate between fibres. In both patients a single AFB was found in this infiltrate after intensive searching.

Nerve. The superficial radial was available from all 12 patients, and the sural was examined from four. One biopsy was completely normal, and one showed only slight perineurial thickening, barely beyond the limits of normality, but all others were abnormal, often markedly so. Changes varied from slight proliferation of perineurial cells through varying degrees of cellular infiltration to gross disorganization of epi-, peri-, and endoneurial elements. In over half the specimens, foamy histiocytes, destroyed Schwann cells and collagen formation

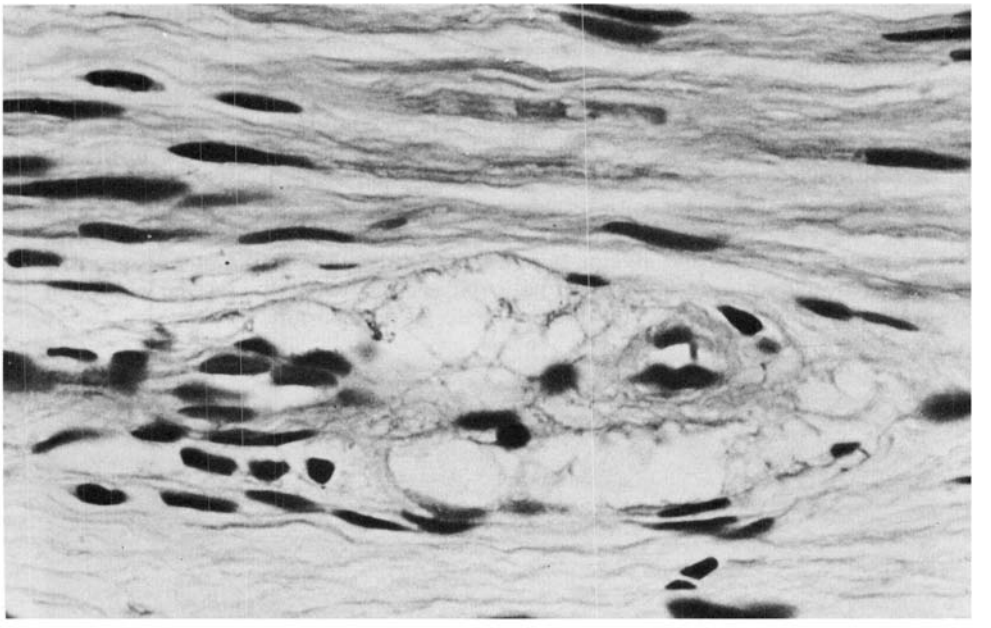

Fig.2.In the substance of endoneurium, foamy histiocytes and occasional lymphocytes have completely replaced Schwann cell, axonal and myelin elements. TRIFF stain. X 3750.

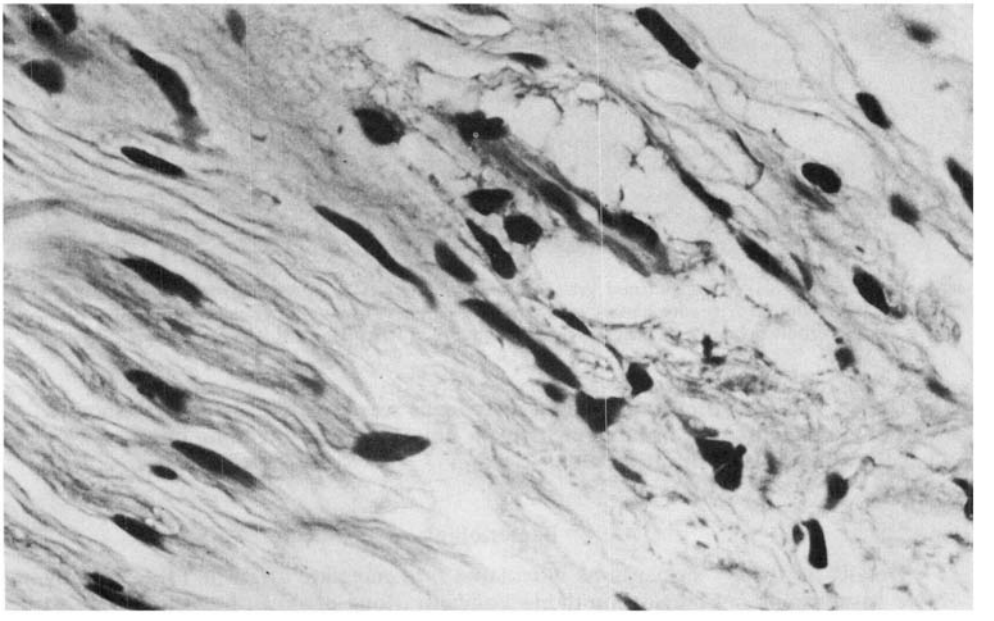

Fig.3.Close to normal neural elements in the endoneurial area (left), the perineurium (right) shows extensive destruction and replacement by foamy histiocytic infiltration. TRIFF stain. X 3750.

were seen in association with axonal and myelin abnormalities (Figs 2 and 3). Infiltrating cells were mainly foamy histiocytes but in several cases lymphocytes, and less commonly plasma cells, were present in considerable numbers (Fig. 4). Epineurial blood vessels were often hypertrophied, especially in the media. In several biopsies it appeared that the entire nerve substance had been replaced by fibrous tissue, consonant with total loss of functioning axons. In one patient with continuous and formerly severe ENL, marked endoneurial changes were seen with destruction of axons and myelin, lymphocytic infiltration, and numerous necrotic cells with pyknotic nuclei. In four patients, acid-fast bacillary material was found in the cytoplasm of histiocytes in either endo- or perineurium.

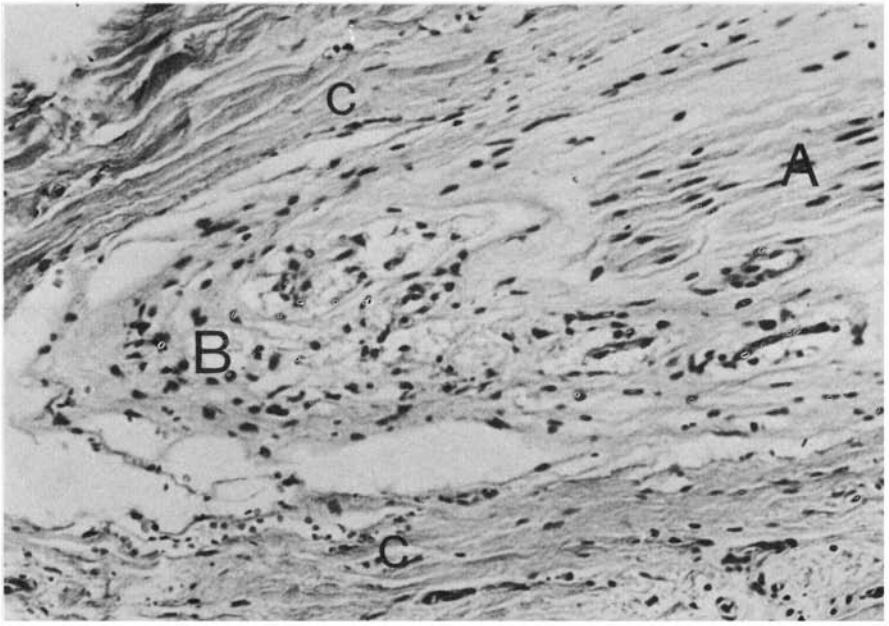

Fig.4. Adjacent to surviving normal axons and Schwann cells at A, the endoneurium (B) shows infiltration with foamy histiocytes, lymphocytes and a few plasma cells. The perineurium at C is multilayered and extensively collagenised. TRIFF stain. X 936.

Dartos (with scrotal skin). Eight specimens were examined. Several showed atrophy and reduction in number of plain muscle fibres, but in no instance were bacilli found or an infiltration seen.

Bacteriology

Details of the 37 suspensions inoculated into mice are given in Table 1. Only five suspensions contained countable concentrations of AFB, but these were so low that mouse inocula consisted of only 1 to 8×10^2 bacilli. For the remaining 32 suspensions in which no AFB could be detected, the numbers of bacilli inoculated were presumably even smaller.

Only two of the five "positive" suspensions multiplied in mice, including one skin specimen from patient no. 5, where seven of 12 footpads showed multiplication. But the other "positive" and the eight "negative" suspensions which also multiplied in footpads, gave very few takes (1 to 3) in each group of animals. Yields also tended to be low, from 5×10^4 to 1.2×10^6 bacilli, and no enhancement was obtained in T900R mice. Passage was attempted of 6 strains yielding positive footpads; three were successful (skin suspensions of patients nos. 3 and 4 and nerve of no. 11) in normal and/or T900R mice, and the bacilli proved fully sensitive to dapsone (0.0001% dapsone in the mouse diet)—see Table 2. Two strains, however, have failed to passage and the third died out on second passage.

Discussion

Even today, 33 years after the introduction of sulphone therapy, there is no accurate data to guide the physician on the duration of dapsone treatment required to cure lepromatous leprosy. Reactivation of the disease after premature stopping of treatment remains a major hazard, especially in view of the high percentage of patients "lost to control" or "out of control" in many national leprosy control schemes (Bechelli, 1971). Some earlier accounts of relapse may well have included borderline-lepromatous (BL) as well as lepromatous (LI and LL) disease, thereby presenting an unduly optimistic picture. The majority of more recent publications have confined themselves to recording the reappearance of leprosy bacilli in the smears of negative patients, without reference to the BI or the MI, nor to histological or clinical signs of relapse, and have failed to allow for sampling error associated with the patchy distribution of fragmented (dead) leprosy bacilli in the skin of much-treated patients. A notable exception is Price (1959), who reported clinical and histological as well as bacteriological relapse in six patients (an incidence of 25%) who had ceased chemotherapy on achieving smear negativity 30 to 66 months (average 44 months) after commencing treatment; on average, clinical relapse developed 27 months after stopping chemotherapy. Noordeen (1971) has recently listed the data required to evaluate the precise significance of relapses occurring in any series of patients. He concluded that (bacteriological) relapse is a common feature of lepromatous leprosy, especially among smear negative patients who discontinue treatment.

There is clearly a great need for more information on the incidence of relapses after various durations of anti-leprosy treatment, and even more urgently for scientific data on their possible mechanisms. This is particularly so, because of the current confusion and misconception concerning the effect of chemotherapy on *Myco. leprae*. We have repeatedly shown that leprosy bacilli respond rapidly to standard anti-leprosy treatment so that the MI reaches baseline in approximately 4.5 months, or with rifampicin after only four to six weeks. Shepard, Levy and Fasal (1968, 1972) and ourselves (Rees, Pearson and Waters, 1970) have likewise shown that it is difficult to infect mice with *Myco. leprae* obtained from the skin of patients treated for three months or more with standard dapsone therapy, or a few days or more with rifampicin. But partly because of the frequent occurrence of relapses, Bechelli and Guinto (1970) and Dharmendra (1973) have cast doubts on the value of the MI and of the mouse footpad infection. In fact, apart from the development of dapsone resistance, there are two simple explanations for relapses. As effective immunity is never normally developed in lepromatous leprosy, re-infection is always theoretically possible once chemotherapy ceases. Alterna-

tively, small numbers of drug-sensitive bacilli may "persist" despite chemotherapy in full dosage, and start to multiply and spread once dapsone treatment is prematurely stopped. This situation is similar to that which may occur, for example, in typhoid fever, subacute bacterial endocarditis, tuberculosis and brucellosis. The phenomenon of microbial persistence has been particularly well described and discussed by McDermott (1958, 1959). Although reinfection cannot be ruled out in every case, in the great majority indirect evidence favours the recrudescence of the original infection; the time interval after stopping therapy is relatively short, the clinical manifestations of such relapses usually resemble those seen in patients who have developed drug resistance and not those of previously untreated leprosy, and drug-sensitive relapses may occur in lepromatous patients who have emigrated to leprosy free areas.

In the work which we now report, we have investigated the persistence of viable leprosy bacilli in a group of 12 lepromatous patients (LI and LL) who had all received a minimum of 10 years chemotherapy principally or entirely with dapsone. To rule out the possibility of reinfection off treatment, all save one were still receiving dapsone regularly, in a dosage currently varying between 200 mg twice weekly by mouth to 400 mg twice weekly by injection; two patients (nos. 7 and 8) had received dapsone in full dosage twice weekly by injection throughout the ten years. Urine tests confirmed the presence of sulphone.

Our choice of sites for investigation was determined in part by reports of solid bacilli persisting in dermal nerves (Dharmendra, 1960) and smooth muscle (Neves, 1961; Ridley, personal communication, 1962; see also Harman, 1968; Leiker, 1972) and our own experience, clinically with striated muscle (Pearson, Rees and Weddell, 1970, 1973), and experimentally with the mouse footpad and hamster ear infections.

Clinically, all 12 patients appeared to have made a good response to continuous chemotherapy given for 10 to 12.5 years. There was no clinical or histological evidence of mycobacterial activity, although three nerve biopsies, from patients nos. 5, 7 and 11, were suggestive of some continuing tissue damage, probably related to ENL. Patients nos. 7 and 11 still suffered from mild ENL of the skin. This quiescent state of the disease compares favourably with the results reported from Carville (Jacobson and Trautman, 1971), although it must be remembered that in our series the duration of active lepromatous leprosy before the start of treatment was shorter, as was also the period of follow-up; more especially, treatment had been continued in full dosage despite ENL in nine and neuritis in a tenth.

Although the leprosy was quiescent, occasional multiplication has been obtained with 10 of 37 tissue suspensions, from seven of the 12 patients, inoculated into mouse footpads. All four tissue sites have been implicated. Three successful passage experiments have revealed that two strains of *Myco. leprae* (no. 3, skin; no. 5, skin) were without doubt fully sensitive to dapsone, and a third (no. 11, nerve) almost certainly so (although the small number of "takes" in the untreated control mice in the latter experiment prevent absolute certainty). Therefore, here is proof positive that viable drug-sensitive bacilli can persist for a full ten years in treated lepromatous patients. In this connection it is of interest that we have also isolated a dapsone-resistant strain of *Myco. leprae* from the striated muscle of a proven sulphone-resistant patient who had been treated continuously for five years with clofazimine and was still on such treatment.

The few takes obtained per group of mice are compatible with the minute

numbers of *Myco. leprae,* of uncertain viability, which were inoculated. Moreover, we failed to obtain enhancement in T900R mice, and during first and second passage three of six strains died out, a situation quite unlike our experience of strains of leprosy bacilli obtained from patients with active leprosy. McDermott and his colleagues have reported that "persisting" strains of *Mycobacterium tuberculosis* in treated experimental tuberculous infections in mice were considerably more difficult to isolate *in vitro* than ordinary populations of the tubercle bacillus. Indeed, immediately after three months' chemotherapy of experimental tuberculosis using certain drug combinations, it was found impossible to isolate any colonies of *Myco. tuberculosis in vitro*; but if similarly infected and treated mice were left for variable periods after the course of chemotherapy had been completed, then it became increasingly easy to isolate such "persisting" bacilli (McCune *et al.,* 1966; McCune, Feldmann and McDermott, 1966). Probably similar factors apply to strains of *Myco. leprae* persisting in well treated lepromatous patients, and we could well have been fortunate to have obtained any multiplication at all in mouse footpads of bacilli obtained from patients currently receiving such long term therapy. We presume, by analogy with the results of McCune *et al.,* that after stopping chemotherapy prematurely, the leprosy bacilli left alive would slowly regain their capacity to grow, multiply and spread. Certainly, three of the same group of some 80 lepromatous patients from which our volunteers were obtained, who absconded from treatment after seven, eight and nine years respectively, were all found to have relapsed (three histologically and two clinically) when they later returned to Sungei Buloh Leprosarium. Furthermore, also by analogy with McCune *et al.,* negative findings in mice, say after 15 or 20 years of dapsone therapy, would not necessarily indicate that all viable "persisters" had died out.

These preliminary results confirm that small numbers of leprosy bacilli may persist for many years in treated lepromatous patients, and suggest that several tissues are involved. Unfortunately, the histology examined so far does not pin-point precisely in which cell-types these bacilli remain alive, although there is little doubt that nerve is involved. We plan to continue and expand this study, and the bacteriological results of the additional biopsies are still awaited. Nevertheless, a number of important conclusions may already be drawn. For control workers, the seriousness of losing lepromatous patients from control is even more evident, as is also the importance of discovering and diagnosing leprosy at the indeterminate and borderline stages before a proportion of such patients are able to develop lepromatous leprosy—thereby avoiding very long term chemotherapy with all its problems and demands. More especially, the achievement of negative smears in lepromatous leprosy is no guide whatsoever as to the safety or otherwise of stopping antileprosy treatment; dapsone therapy must be continued for many more years than ten, at least in all but very early cases ("L2" and "L3") and with our present knowledge, we prefer to continue giving it for life.

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