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Persisting *Mycobacterium leprae* among THELEP trial patients in Bamako and Chingleput

Subcommittee on Clinical Trials of the Chemotherapy of Leprosy (THELEP) Scientific Working Group of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases

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Summary The availability of approximately 75% of the anticipated results with respect to persisting *Mycobacterium leprae* from the THELEP controlled clinical trials in Bamako and Chingleput has made possible an interim analysis. Persisting *M. leprae* were detected in 43 skin-biopsy specimens obtained from 39 patients, among a total of 468 specimens obtained at intervals of 3, 12 and 24 months from 199 patients during treatment with five combined drug regimens. The proportion of specimens in which persisting organisms were discovered appeared not to vary with regimen or duration of treatment. The regimen consisting of a single large initial dose of rifampicin plus daily dapsone was not shown to be less effective, in terms of the proportion of specimens in which persisters were detected, than regimens consisting of rifampicin, dapsone and clofazimine or prothionamide, each drug administered daily. The average number of persisting *M. leprae* per patient was calculated to lie in the range 50,000–250,000 at each of the intervals. The results of these trials lend strong support to the multidrug regimen recommended for treatment of multibacillary leprosy by the World Health Organization Study Group on Chemotherapy of Leprosy for Control Programmes.

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Introduction

In April 1976, at the time of the meeting of the THELEP Planning Committee,[1] it was clear that efforts to control leprosy by treatment of the infectious patients (primarily those patients with previously untreated multibacillary (LL, LI, and BL*) leprosy, and those multibacillary patients who have relapsed as the result of premature cessation of treatment or emergence of drug-resistance[2]) in the community were failing, both because of the inability to maintain patients under treatment for the long duration of dapsone monotherapy required, and because of the increasing frequency of relapse associated with the emergence of dapsone-resistant *Mycobacterium leprae*. It appeared obvious that treatment by regimens composed of two or more drugs, each acting by a different antimicrobial mechanism, would prevent relapse with dapsone-resistant *M. leprae*; however, unless the multidrug regimens led not only to rapid loss of infectivity, but also to early 'cure', it was believed that employment of the regimens was unlikely to add to the effectiveness of programmes of leprosy based on case-finding and treatment. Only regimens that are effective if administered for a limited time, the termination of which will not be followed by a high frequency of relapse, may be expected to lead to improved case-holding. Persistence of viable, drug-susceptible *M. leprae* ('persisters', or 'persisting' *M. leprae*[3]) had already been demonstrated after many years of monotherapy with dapsone or rifampicin,[4,5] and after combined chemotherapy with rifampicin and dapsone,[6] and it was feared that, after the therapy had been withdrawn, persisting *M. leprae* would cause relapse of a large proportion of patients.

Thus, at the time of the THELEP Planning Committee meeting, a central issue in attempting to improve chemotherapy of leprosy was whether administration of combinations of drugs could reduce the proportion of patients harbouring persisting organisms, or reduce the numbers of persisting *M. leprae* in individual patients, so that the risk of relapse following cessation of treatment was diminished. At that time, because it appeared likely that relapse-rates would be unacceptably high, as a consequence of the ubiquity of persisters, it was considered unethical to conduct clinical trials in which chemotherapy of patients with lepromatous leprosy was deliberately stopped and relapse-rates subsequently measured. Therefore, the Planning Committee could not undertake to measure the risk to patients presented by the persisting *M. leprae*. On the other hand, controlled clinical trials could be undertaken among patients with lepromatous leprosy to examine

* Deceased.

† The Ridley–Jopling system of classification of leprosy, a system based on clinical, histopathological and other criteria, employs the following terminology: BL, borderline-lepromatous; LI (or LL_s), sub-polar lepromatous leprosy; LL (or LL_p), polar lepromatous leprosy.[7]

the efficacy of various combined drug regimens in reducing the proportions of patients harbouring persisters, or the numbers of persisting *M. leprae* harboured by patients. At its first meeting, in April 1977, the THELEP Scientific Working Group decided to mount controlled clinical trials among previously untreated patients with lepromatous leprosy at Bamako and Chingleput,[8] in order to compare the proportions of patients treated by each regimen in whose skin biopsy-specimens viable *M. leprae* could be detected at intervals after beginning treatment. Six regimens were selected for study*: two 'maximal' regimens, consisting of rifampicin, dapsone, and prothionamide or clofazimine administered daily for two years; two 'intermediate' regimens, consisting of an initial course of weekly rifampicin and daily prothionamide together with daily dapsone for two years in Bamako, and, in Chingleput, a single initial large dose of rifampicin and daily clofazimine for the first three months, together with daily dapsone for two years; and the same 'minimal' regimen in each centre—an initial large dose of rifampicin and daily dapsone administered for two years. (As explained in the earlier publication,[8] these regimens were to be employed in a study of the ability of combined drug-regimens to eliminate persisters; that the regimens might or might not be suitable for routine use in programmes of leprosy control was not a factor in their selection.)

Materials and methods

The patients recruited into the two trials and the methods employed in the trials, are those already described.[8] In brief, patients with LL, LI or BL leprosy were recruited who denied prior treatment, and in whose urine no dapsone could be detected. At intervals of 3, 12 and 24 months after beginning treatment by one of the three regimens under study at each treatment centre, biopsy-specimens were obtained from the same skin lesions and shipped fresh on wet ice to the National Institute for Medical Research (NIMR), London, where the largest possible number of organisms, to a maximum of 10^5 per footpad, was inoculated into each hind footpad of thymectomized and irradiated (TR)[9] mice, usually eight mice per specimen. After about one year, a period of time theoretically sufficient to permit the multiplication of even a single viable *M. leprae* to a large number, harvests were performed from the hind footpads of all surviving mice. On numerous occasions, the harvested *M. leprae* were diluted, and 10^4 organisms were sub-inoculated into the hind footpads of normal CBA mice. Persisting *M. leprae* were defined as those multiplying to at least 10^6 organisms per footpad from an inoculum of 10^5, or those yielding at least 10-fold multiplication to at least 10^5 organisms per footpad from an inoculum smaller than 10^5, or those multiplying in passage.

Data were analysed by means of the χ^2 and Fisher exact probability techniques for comparison of frequencies and proportions among two or more categories.[10] The most probable number of viable *M. leprae* (MPN) was calculated by means of the Halvorson–Ziegler equation.[11]

Results

FREQUENCY OF PERSISTING *M. LEPRAE*

By 31 December 1984, the results of study of 468 biopsy-specimens—about 75% of results expected—had been reported. These results were derived from the study of skin-biopsy specimens

* Patients were allocated among six treatment-groups, three in each centre; however, one of the five regimens (regimen C) was employed in both centres. The regimens were, in Bamako,

A_2: rifampicin, prothionamide and dapsone, each in a daily dose of 600, 500, and 100 mg, respectively, for two years; C: rifampicin, in a single initial dose of 1500 mg, and dapsone, 100 mg daily for two years; E_2: rifampicin, 900 mg once weekly, and prothionamide, 500 mg daily for the first three months, together with dapsone, 100 mg daily for two years;

and in Chingleput,

A_1: rifampicin, clofazimine and dapsone, each in a daily dose of 600, 100, and 100 mg, respectively for two years; C: as for Bamako; D_1: rifampicin, in a single initial dose of 1500 mg, clofazimine, in a daily dose of 100 mg for the first three months, and dapsone, 100 mg daily for two years.[8]

from 199 patients, of whom 107 were represented by three specimens, and an additional 55 by two specimens. As shown in Table 1, the numbers of specimens were greatest for the 3-month interval, and smallest for the 24-month interval. This was to be expected, because recruitment of patients continued until the latter part of 1983, whereas, by 31 December 1984, results of 24-month specimens were available only for those patients who had been recruited prior to 1982.

Table 1. Proportions of specimens in which persisting *M. leprae* were detected

Duration of treatment (months)	Number 'positive' specimens/total number: Bamako regimen A_2*	C	E_2	Chingleput regimen A_1	C	D_1	Total	%
3	0/11	4/42[0]	4/34	2/37[+]	4/38[x]	4/35[**]	18/197	9·1
12	3/9	2/32	3/27	4/32[+]	2/30	3/32[**]	17/162	10·5
24	1/7	2/20[0]	1/17	0/23	3/20[x]	1/22	8/109	7·3
Total (3+12+24)	4/27 (14·8%)	8/94 (8·5%)	8/78 (10·3%)	6/92 (6·5%)	9/88 (10·2%)	8/89 (9·0%)	43/468	9·2[oo]
Total (12+24)	4/16 (25·0%)	4/52 (7·7%)	4/44 (9·1%)	4/55 (7·3%)	5/50 (10·0%)	4/54 (7·4%)	25/271	9·2[++]

A_2*: rifampicin, prothionamide and dapsone, each in a daily dose of 600, 500, and 100 mg, respectively, for two years; C: rifampicin, in a single initial dose of 1,500 mg, and dapsone, 100 mg daily for two years; E_2: rifampicin, 900 mg once weekly, and prothionamide, 500 mg daily for the first three months, together with dapsone, 100 mg daily for two years; A_1: rifampicin, clofazimine and dapsone, each in a daily dose of 600, 100, and 100 mg, respectively, for two years; D_1: rifampicin, in a single initial dose of 1,500 mg, clofazimine, in a daily dose of 100 mg for the first three months, and dapsone, 100 mg daily for two years.

[0, +, x, **] One patient is represented at both intervals; only 4 of 162 patients represented by > 1 specimen were found to harbour persisters at > 1 interval.

[oo] 95% confidence interval (6·6–11·8).

[++] 95% confidence interval (5·8–12·9).

The data of Table 1 show that persisting *M. leprae* were detected in about 9% of all specimens; the proportions of specimens in which persisting organisms were detected did not differ significantly between centres, among regimens, or as a function of duration of treatment. Also striking is that persisters were detected in two biopsy-specimens from only 4 of the 39 patients; assuming persisting *M. leprae* to be uniformly distributed among the biopsy-specimens, the number of such patients expected by chance is 3, a result not significantly different from 4 ($P > 0{\cdot}90$).

INOCULUM SIZE

The likelihood with which small proportions of viable *M. leprae* may be detected depends, at least in part, on the number of organisms inoculated. But because patients' populations of *M. leprae* diminish substantially during treatment, the proportions of specimens obtained at the later intervals in which persisters were detected may underestimate the true frequency of this phenomenon. Therefore, it is important to examine the numbers of organisms actually inoculated from the biopsy-specimens obtained at the three intervals.

That efforts to detect persisting *M. leprae* were not limited by the number of organisms available for inoculation is shown by the data of Table 2. As shown in the table, 90% (421/468) of the

Table 2. Distribution of inoculum sizes with respect to detection of persisting *M. leprae*

Number of *M. leprae* inoculated	Duration of treatment (months)			
	3	*12*	*24*	*Total*
Number of specimens in which persisters were detected/Total number of specimens (% of total)				
10^5	16/171 (9·4)	11/119 (9·2)	3/52 (5·8)	30/342 (8·8)
$3 \times 10^4 - < 10^5$	2/13 (15·4)	2/15 (13·3)	2/16 (12·5)	6/44 (13·6)
$10^4 - < 3 \times 10^4$	0/5 (<20·0)	1/10 (10·0)	0/20 (<5·0)	1/35 (2·9)
$< 10^4$	0/8 (<12·5)	3/18 (16·7)	3/21 (14·3)	6/47 (12·8)
All $< 10^5$	2/26 (7·7)	6/43 (14·0)	5/57 (8·8)	13/126 (10·3)
All specimens	18/197 (9·1)	17/162 (10·5)	8/109 (7·3)	43/468 (9·2)

There was no significant association between inoculum size and detection of persisting *M. leprae*, *i.e.* the inocula prepared from specimens in which persisters were detected did not differ from those prepared from the remaining specimens, with respect to the numbers of organisms inoculated.

specimens provided inocula of at least 10^4 *M. leprae* for TR mice; and 73% (342/468) of the specimens provided inocula of 10^5 organisms per footpad. Consistent with the expected decrease of size of populations of *M. leprae* in the course of effective treatment, the proportion of specimens yielding inocula of 10^5 decreased with increasing duration of treatment. Thus, at 3 months, 96% (189/197) of the specimens provided an inoculum of at least 10^4 *M. leprae*, and 87% (171/197) provided inocula of 10^5, whereas, after 24 months, these percentages had fallen to 81 (88/109) and 48 (52/109), respectively. However, only two specimens contained so few organisms that none could be counted. In fact, the distribution, with respect to the numbers of organisms inoculated, of specimens in which persisters were detected did not differ significantly from that of all specimens.

PERSISTERS AND DAPSONE-RESISTANCE*

One of the characteristics that might be associated with persistence of *M. leprae* is primary resistance to dapsone, which had been identified in approximately 37% of these patients.[12] As shown in the upper panel of Table 3, however, persisting organisms were detected in the biopsy-specimens of 10% of the patients harbouring dapsone-resistant strains of *M. leprae*, a frequency not

* The detection of persisting *M. leprae* in specimens obtained from patients representing instances of primary resistance to dapsone may appear to contradict the definition of persisting *M. leprae*, which were defined as drug-susceptible[3]; however, this is not the case. In the sense of this definition of persisters, 'drug-susceptibility' refers to the drugs employed in treatment at the time that viable organisms are detected. This is, in fact, the logical basis of combined chemotherapy; organisms that are already resistant to one of the components of the combination at the time that therapy is begun are prevented from multiplication and even killed by the other components of the combination.

Table 3. Relationship between persistence of *M. leprae* and dapsone resistance, according to regimen

Duration of treatment (months)	Bamako regimen			Chingleput regimen			Total	%
	A_2*	C	E_2	A_1	C	D_1		
	Number of specimens demonstrating persisters/ Number of resistant strains							
3	0/3	0/12	1/9	1/7	2/9	2/5	6/45	13·3
12	1/2	0/7	1/7	1/6	1/7	0/5	4/34	11·8
24	0/0	0/3	0/4	0/5	0/4	0/3	0/19	< 5·3
Total	1/5	0/22	2/20	2/18	3/20	2/13	10/98	10·2[o]
	Number of resistant strains demonstrating persisters/ Number of strains, susceptible and resistant, demonstrating persisters							
3	0/0	0/1	1/3	1/1	2/2	2/2	6/9	66·6
12	1/3	0/0	1/2	1/3	1/1	0/2	4/11	36·4
24	0/1	0/1	0/1	0/0	0/1	0/1	0/5	< 20·0
Total	1/4	0/2	2/6	2/4	3/4	2/5	10/25+	40·0[x]

No significant association was encountered between persistence and resistance at any time interval for any regimen, either centre or both centres combined.

* Regimens as defined in Table 1.

[o] Not significantly different from 9·2%, the proportion of all specimens demonstrating persisting *M. leprae* (see Table 1).

+ Although persisting *M. leprae* were detected in 43 specimens, persisters were detected in only 25 specimens obtained from patients whose organisms could be determined to be either susceptible or resistant to dapsone. The remaining 18 specimens were obtained from patients, the susceptibility of whose organisms to dapsone could not be determined.[12]

[x] Not significantly different from 37·4%, the proportion of dapsone resistant among all strains of *M. leprae*, the susceptibility of which to dapsone could be determined.[12]

significantly different from that with which persisting *M. leprae* had been detected in the entire patient-population (approximately 9%). And, as shown in the lower panel of Table 3, the proportion of patients with primary dapsone-resistant strains of *M. leprae* among those in whose biopsy-specimens persisters were subsequently detected, 40%, does not differ significantly from the proportion of all patients harbouring dapsone-resistant strains. Finally, considering those 131 patients, of whose pretreatment isolates the susceptibility or resistance to dapsone could be determined,[12] the distribution with respect to susceptibility or resistance to dapsone of the organisms isolated from the 24 patients, in whose specimens persisting *M. leprae* were detected, did not differ significantly from that of the organisms of the 107 patients, in whose specimens no persisters were detected (see Table 4).

PERSISTERS AND OTHER PATIENT-CHARACTERISTICS

The distributions among those patients in whose specimens persisting *M. leprae* were detected of a number of characteristics, other than susceptibility or resistance to dapsone, observed at the time of admission into the clinical trials in Bamako and Chingleput, are compared in Table 4 with the

Table 4. Relationship between the persisters and pretreatment patient-characteristics

Pretreatment characteristic	Bamako regimens		Chingleput regimens	
	Persisters	No persisters	Persisters	No persisters
Mean age (years)	27·1	28·2	28·0	30·6
Mean LIB	5·4	4·9	5·2	5·3
Mean BI	4·8	4·4	4·3	4·3
Mean LAFBPG	8·5	8·3	8·4	8·4
No. patients with*:				
DDS-susceptible organisms	9	28	6	39
DDS-resistant organisms of:				
Low degree	2	19	4	14
Intermediate degree	1	5	2	2
CLINCLAS° LL	2	12	7	20
CLINCLAS LI	16	58	10	70
CLINCLAS BL	1	10	3	6
HISTCLAS+ LL	0	2	0	1
HISTCLAS LI	16	63	19	92
HISTCLAS BL	3	14	1	3

* The 24 patients with organisms susceptible or resistant to dapsone, in whose specimens persisters were detected, were represented by 25 specimens (see Table 3).

°Clinical classification, according to the classification of Ridley and Jopling.[7]

+ Histopathological classification, according to the classification of Ridley and Jopling.[7]

distributions of these characteristics among those in whose specimens no persisters were detected. Significant associations could not be demonstrated between the presence of persisting organisms and: age, pretreatment values for bacteriological index (BI),[13] logarithmic biopsy index (LIB),[14] and logarithm$_{10}$ of the number of *M. leprae* recovered from the patients' pretreatment biopsy-specimens (LABPG);[15] and initial clinical and histopathological classifications.

NUMBERS OF PERSISTING *M. LEPRAE*

The detection of persisting *M. leprae* in approximately 9% of all specimens, without relation to dapsone-susceptibility or to duration of treatment between 3 and 24 months, with a frequency no greater than that predicted by chance in a second specimen, and the lack of association of the detection of persisters with any other recorded characteristic of the patients indicate that persisting *M. leprae* may be distributed rather uniformly among the patients, and that their detection may be a chance event. If this is the case, one may estimate the absolute numbers of viable organisms persisting at each interval.

Presented in Table 5 are the data describing the 43 specimens in which persisting *M. leprae* were detected. In each case, the number of organisms inoculated and the proportion of inoculated mouse-feet demonstrating multiplication of *M. leprae* have been employed to calculate the MPN, expressed here as the MPN per 10^6 *M. leprae*. Taking as an example the first entry in Table 5, and assuming that multiplication would have occurred in none of 10 footpads inoculated with 10^4 organisms, and in all of 10 footpads inoculated with 10^6 organisms, the MPN of viable *M. leprae* in the inoculum may be calculated by the equation of Halvorson & Ziegler[11] to be approximately 5 per 10^6 total organisms. Assuming that the total (viable plus dead) bacterial population of a patient with BI = 4·0 is 10^{11} (i.e., 10^{BI+7})[16,17] this patient may be calculated to harbour MPN/$10^6 \times 10^{BI+7}$ = approximately 5×10^5 viable *M. leprae*.

Table 5. Calculation of the total numbers of persisting *M. leprae*

Patient number	Inoculum ($\times 10^5$)	Proportion of feet showing multiplication	MPN per 10^6	BI	Total number of persisters* ($\times 10^5$)
3 months					
01024	1·0	4/10	5	4·00	5
01043	1·0	1/10	3	4·33	6
01049	0·30	1/8	10	4·00	10
01050	1·0	1/12	3	4·33	6
01051	1·0	1/12	3	4·33	6
01059	1·0	1/12	3	3·50	1
01073	1·0	1/8	3	4·33	6
01078	0·31	1/8	9	2·33	0·2
01093	1·0	3/14	3	3·83	2
01116	1·0	1/8	3	4·33	6
02033	1·0	2/8	4	4·33	8
02038	1·0	1/2	6	4·33	13
02044	1·0	2/16	3	4·33	6
02045	1·0	1/10	3	4·33	6
02048	1·0	1/8	3	4·00	3
02051	1·0	3/14	3	4·83	23
02067	1·0	1/10	3	4·67	13
02083	1·0	1/10	3	4·67	13
12 months					
01037	0·07	1/14	37	3·17	6
01042	1·0	1/8	3	4·33	6
01043	0·18	1/6	17	4·33	37
01052	0·79	1/8	3	4·17	5
01069	1·0	1/6	3	4·17	5
01074	1·0	1/10	3	3·50	1
01079	1·0	2/12	3	3·87	2
01093	1·0	1/10	3	3·50	1
01103	1·0	6/14	5	3·67	2
02001	1·0	1/14	3	4·17	4
02011	1·0	1/16	3	4·00	3
02018	1·0	1/10	3	4·00	3
02019	1·0	1/8	3	4·17	4
02072	0·55	1/14	5	4·00	5
02073	1·0	4/14	4	3·91	3
02074	0·006	1/8	490	3·74	270
02080	0·002	1/8	140	3·50	46
24 months					
01022	1·0	1/8	3	3·67	1
01047	0·70	1/10	4	4·00	4
01048	1·0	1/12	3	3·17	0·4
01051	1·0	1/8	3	3·17	0·4
02003	0·35	1/12	8	4·00	8
02015	0·05	1/12	57	3·24	10
02044	0·06	1/8	49	2·17	1
02053	0·03	1/12	92	3·50	29

* Total number of persisters = $\text{MPN}/10^6 \times 10^{\text{BI}+7}$.

After treatment for 3 months, the mean BI calculated from the values observed for the 197 patients examined, was 4·42 (unpublished data). Thus, the total population of *M. leprae* of these patients was $197 \times 10^{11 \cdot 42} = 5 \times 10^{13}$. Considering the 18 patients found to harbour persisting *M. leprae* at this interval, one may calculate from the data of Table 5 that they harboured a total of approximately $(\text{MPN}/10^6 \times 10^{\text{BI}+7}) = 10^7$ viable *M. leprae*. Assuming that these were all of the viable *M. leprae* in the total population of 5×10^{13}, the proportion of persisters at the interval is roughly 2 per 10^7 organisms, and the average number of persisters per patient approximately 7×10^4.

At 12 months, the 17 patients in whose specimens persisting *M. leprae* were detected may be seen to have harboured an approximate total of 4×10^7 viable organisms. Assuming these to be the only viable organisms among the total bacterial population of 162 patients, whose mean BI was 3·98, the proportion of viable *M. leprae* is roughly $(4 \times 10^7)/(162 \times 10^{10 \cdot 98}) = 3$ per 10^6 organisms; the average patient harboured approximately 3×10^5 viable organisms.

At 24 months, the 8 patients in whose specimens persisters were detected harboured a total of about 5×10^6 viable *M. leprae*. The 109 patients biopsied at this interval had a mean BI of 3·33, and harboured a total of approximately $109 \times 10^{10 \cdot 33}$ organisms. Thus, the proportion of persisting *M. leprae* at this interval was approximately 2 per 10^6 organisms, and the average patient harboured approximately 5×10^4 viable organisms.

Discussion

The primary objective of the THELEP controlled clinical trials in Bamako and Chingleput was to compare several combined drug regimens among previously untreated patients with lepromatous leprosy, in terms of the frequency with which persisting *M. leprae* could be detected at intervals during treatment. Although results are available for only about 75% of the biopsy-specimens to be obtained, they appeared sufficiently important to justify interim analysis and publication.

These results of the THELEP trials demonstrate that persisting *M. leprae* were detected in approximately 9% of all patients, without relation to regimen, duration of treatment, primary resistance to dapsone, number of *M. leprae* inoculated into mice, or any pretreatment characteristic of the patients—age, BI, LIB, LAFBPG, and clinical and histopathological classifications. Most noteworthy is the lack of association between regimen and frequency with which persisting organisms were detected; persisters were detected no less frequently among patients treated with regimens involving the daily administration of three drugs including rifampicin than among those treated only with a single initial (albeit large) dose of rifampicin in addition to continuous daily dapsone.

That persisting *M. leprae* were detected by the methods employed (single biopsies at intervals of 3, 12 and 24 months, and inoculation of TR mice with no more than 10^5 *M. leprae*) with a frequency that appears to be independent of regimen, duration of treatment, and all of the patient-characteristics studied suggests that detection of persisters was a random event, and that the frequency of detection depended upon the intensity of the search. This contention is supported by the low frequency with which persisters were detected in more than one specimen from the same patient. In no patient were persisting organisms detected in all three biopsy-specimens; assuming that detection of persisters in a given specimen is independent of their detection in any other specimen, the expected frequency of such an occurrence among the 107 patients for whom the results of three specimens were available is $< 0{\cdot}08\%$. On the other hand, had twice as many biopsies been performed, it appears likely that persisting *M. leprae* would have been detected in almost twice as many patients. In fact, a sufficiently intensive search might have revealed the presence of persisters in virtually all patients. In other words, the frequency with which persisters were detected may well have reflected not the proportion of the patients harbouring them, but rather the small proportion of persisters in the patients' bacterial populations.

The foregoing estimates of the numbers of persisting *M. leprae* are based on assumptions, the

validity of which is difficult to test. Other calculations of the mean number of persisters at each interval could have been employed. Alternatives to that used, which actually involved the arithmetic means, are calculations involving the geometric means and the median values. In fact, these alternatives provide smaller estimates, respectively 5×10^4 and 6×10^4 viable organisms per patient after 3 months, rather than the forgoing estimate of 7×10^4 persisters. Another alternative is that of calculating the proportions of viable organisms directly, rather than by means of the MPN calculation; this calculation yields an estimate of 4×10^4 persisting *M. leprae* per patient after 3 months. None of these alternatives appears more readily justified than that employed in the calculations presented. In particular, the direct calculation appears inappropriate, as it assumes non-random behaviour of the organisms, and denies the possibility that some mice might be inoculated with more than the average number of viable *M. leprae*, and other mice with fewer than the average. In fact, although the distribution of *M. leprae* in the inoculum may not be perfectly random,[18] as assumed by the Halvorson–Ziegler equation,[11] there is no basis whatever for assuming them to be uniformly distributed. Finally, the most conservative alternative—that which produces the largest estimates of the numbers of persisting *M. leprae*—appears wisest.

Our confidence in these estimates of the numbers of surviving *M. leprae* would be strengthened, if similar estimates could be derived from other studies, in which the proportions of viable *M. leprae* had been measured by inoculation of comparatively large numbers of organisms into immune-deficient rodents. A few studies are available that permit rough estimates of numbers of persisters. Prior to the THELEP trials, only a very few attempts, involving only small numbers of patients, had been made to detect persisting *M. leprae*. In the first report[4] of persisting organisms in treated patients with lepromatous leprosy, 12 patients who had been treated with well supervised dapsone monotherapy for a minimum of 10 years are described. Thirty-seven biopsy-specimens were obtained from 12 patients, and mice were inoculated by much the same techniques employed in the present study; *M. leprae* were reported to have multiplied in mice inoculated with organisms from 10 of the specimens. Assuming from the data presented in the report an inoculum no greater than 10^3 *M. leprae* per mouse, that each inoculum was administered to 12 mice, that multiplication, in those instances in which it was detected, occurred in no more than 3 of the 12 mice, and that *M. leprae* would have multiplied in all mice inoculated with 10^4 organisms, and in none of the mice inoculated with 10^2 organisms, one may estimate the MPN of the inoculum yielding multiplication as approximately 4 viable per 10^4 total organisms; but because persisters were detected in only 10 of 37 specimens, then the average proportion of viable organisms was 1 per 10^4, a value considerably larger than that of approximately 2 per 10^6 found at the three intervals in this present study. (The value of 1 per 10^4 is, in fact, a minimal figure, because most inocula contained fewer than 10^3 *M. leprae*; therefore, the proportion of organisms capable of multiplying must have been greater.)

Another study represented an attempt to detect persisting *M. leprae* among patients with secondary dapsone resistance who had been administered daily rifampicin as monotherapy for varying periods of time[5]. Unfortunately, the first data susceptible to analysis are those for patients studied after treatment for 5 years. Biopsies were taken from several tissues of 12 patients, and a total of 316 mice were inoculated, in 45 (approximately 1/7) of which multiplication of *M. leprae* was recognized. Assuming an average inoculum no greater than 10^4 organisms per footpad (after effective treatment for 5 years, very few specimens would be expected to yield enough organisms to permit an inoculum of 10^5, and the yield from many specimens would be below the threshold of detectability), the MPN may be calculated to be approximately 3 viable *M. leprae* per 10^5 organisms.

A third study of persisting *M. leprae* represented a comparison, among 11 previously untreated patients with lepromatous leprosy, of daily dapsone as monotherapy with the combination of daily dapsone *plus* daily rifampicin.[6] Employing the data for the proportion of inoculated footpads showing multiplication, analysis of the results for the two regimens demonstrates proportions of 4 and 3 viables per 10^6 organisms, after treatment with dapsone for 3 and 6 months, respectively, and 30 and 3 viables per 10^7 organisms after treatment with the combination dapsone *plus* rifampicin for 3 and 6 months, respectively.

It is reported in a recent study[19] that previously untreated patients with lepromatous leprosy were treated with a single initial dose of 1500 mg rifampicin, together with 100 mg dapsone daily, or weekly 900 mg doses of rifampicin, together with 100 mg dapsone daily. During the first month of treatment, biopsies were performed at four intervals, *M. leprae* were recovered from the biopsy-specimens, and neonatally thymectomized rats were inoculated in both hind footpads with large numbers of organisms. Persisting organisms were detected in 33 of 66 specimens, a frequency much greater than that described in this present report. This greater frequency appears to reflect the much larger numbers of *M. leprae* inoculated into the rats—a mean of approximately 4×10^7 per specimen (Gelber *et al.*, unpublished data), compared to a mean of fewer than 2×10^6 per specimen (8 mice × 2 hind feet × $< 10^5$ *M. leprae* per footpad) in the present study in Bamako and Chingleput. The small proportion of viable *M. leprae* observed—a mean of 7×10^{-8}—is striking. Assuming the average San Francisco patient to have had a BI = 4, the average population of persisters may be calculated to be approximately $6 \times 10^{-8} \times 10^{7+4} = 6 \times 10^3$.

To facilitate comparison with the results of the THELEP trials reported here, the foregoing estimates of the proportions of viable *M. leprae* are shown in Table 6. The assembled estimates suggest that the proportion of persisters is greater for longer durations of treatment; in the course of long-continued effective treatment, the patient's bacterial population may decrease primarily at the expense of dead *M. leprae*. Certainly, during continued effective chemotherapy, the total number of persisting *M. leprae* could not have increased, *i.e.* the organisms could not have multiplied. In fact, although the results of the THELEP trials demonstrate no significant difference of the proportion of persisting *M. leprae* among the three intervals, they do not exclude the possibilities that the frequency of persisters actually increased or decreased; because of the small numbers of specimens in which persisters were detected, the confidence bands around the estimates of the frequency with which persisters were detected are broad.

Whether or not the proportion of persisting *M. leprae* or the frequency with which persisting organisms may be detected changes in the course of continued chemotherapy, the results of the THELEP trials lead to the conclusion that the absolute number of viable *M. leprae* comprising the population of persisting organisms is very small, suggesting that current concepts of the

Table 6. Proportions of persisting *M. leprae* after various chemotherapies

Treatment	Duration (months)	Proportion of patients demonstrating persisters	Proportion of viable *M. leprae* (per 10^6)	Estimated mean number of persisters ($\times 10^4$)	Reference
Dapsone	3	5/6	4	40	6
Dapsone	6	3/6	3	30	6
Dapsone	120	7/12	100	0·1	4
Rifampicin	60	7/12	30	0·3	5
Dapsone + rifampicin	1	13/18	0·07	0·7	19
Dapsone + rifampicin	3	3/5	3	30	6
Dapsone + rifampicin	6	1/5	0·3	3	6
THELEP trial regimens	3	18/197	0·2	7	
THELEP trial regimens	12	17/162	3	30	
THELEP trial regimens	24	8/109	2	5	

chemotherapy of lepromatous leprosy—particularly for chemotherapy directed at the control of leprosy—may be reconsidered.

The results of the THELEP trials in Bamako and Chingleput demonstrate both the strengths and the potential weaknesses of the WHO Study Group regimen for multibacillary leprosy. Prior to beginning chemotherapy, a lepromatous patient's population of viable *M. leprae* may be thought of as being composed of three subpopulations: (i) rifampicin-susceptible non-persisting organisms, representing by far the largest of the sub-populations; (ii) rifampicin-resistant non-persisting organisms, which, by analogy with *M. tuberculosis*,[20] comprise $1:10^7$ of the entire population; and (iii) persisting *M. leprae*. The results of the THELEP trials suggest that treatment with a combined drug-regimen including rifampicin brings about reduction of the number of viable *M. leprae* to no more than 50,000–250,000 organisms during the first 3–24 months of treatment. That no difference was discerned among regimens suggests that a single dose of rifampicin together with daily dapsone for 3 months is sufficient to eradicate sub-population (i). From the results of earlier trials,[21] it appears likely that rifampicin alone is responsible for the rapid killing of this sub-population. If the first dose of rifampicin is insufficient to produce this effect, the effect should certainly have been obtained by the second or third dose; whether the drug is administered daily or intermittently is probably of little importance.

Because, during treatment by the Study Group regimen, the first supervised doses of rifampicin eliminate sub-population (i), the task of dapsone and clofazimine is to eradicate sub-population (ii). This process certainly proceeds more slowly than does killing of the rifampicin-susceptible non-persisters by rifampicin, and may be incomplete in poorly compliant patients.

Finally, one is forced to the disappointing conclusion that none of the three drugs, acting singly or together, is likely to affect the size of sub-population (iii), even after administration for 2 years. On the other hand, recent evidence suggests that the presence of persisting *M. leprae* does not carry a high risk of relapse following termination of treatment.[22,23] Therefore, the critical sub-population in terms of the requirements of chemotherapy for leprosy-control is sub-population (ii), that composed of rifampicin-resistant non-persisters. No presently available combination of drugs will kill these organisms as rapidly as rifampicin-susceptible organisms are killed by rifampicin; and despite their small relative numbers, no other sub-population represents as grave a threat to the success of current leprosy-control efforts. Thus, the results of the THELEP trials suggest that control of leprosy may be achieved with smaller quantities or shorter courses of rifampicin than have been recommended[2]. On the other hand, because of the increasing prevalence of primary resistance to dapsone, prevention of selection of rifampicin-resistant mutant *M. leprae* appears, at least for the present, to depend upon the meticulous use of clofazimine or one of the thioamides.

Another aspect of the foregoing estimates of the numbers of persisting *M. leprae* must also be considered. The estimates are based, of course, on the average BI, and on an assumption relating the BI to the total population of *M. leprae*. Before exploiting these estimates of the number of surviving organisms to modify the currently recommended programme of chemotherapy of multibacillary leprosy for purposes of control, one must take into account the expected variation of individual patients around the average, and the possibility of error in the assumed relationship between BI and total bacterial population. In fact, the BI was found to range from 2·3 to 5·3 after 3 months of treatment, and from 0 to 4·7 after 24 months of treatment (unpublished data). The patients with larger-than-average populations of *M. leprae* may be at greater risk of failure of treatment. Therefore, recommendations for chemotherapy to be employed in controlling leprosy must be formulated with these outlying patients in mind.

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NEWS AND NOTES

Robert Cochrane Fund for Leprosy

The fund, in memory of the contribution of the great leprologist Robert Cochrane, is administered by the Royal Society of Tropical Medicine and Hygiene. It is to be used to finance up to 3 travel fellowships each year to a maximum value of £1200 each.

The intention is to enable leprosy workers to travel for practical training in field work, or in research, or to enable experienced leprologists to travel in order to provide practical clinical training in a developing country. There is no restriction on the country of origin or destination providing the above requirements are fulfilled.

Application forms are available from the Society and must be received by the Society at least 6 months ahead of the proposed trip. All applications must be sponsored by a suitable representative of the applicant's employer or study centre, and agreed by the host organization. A 2 page report on the travel/study should be submitted to the Society within 1 month of the recipient's return. Apply: The Administrator, Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London W1N 4EY.

British Association for the Advancement of Science—150th Annual Meeting, Oxford 1988

This Association was established in 1831 and its aims are: 1, to enhance public understanding and awareness of science and technology and their impact on society; to help people to recognize science as part of our culture and heritage and to share in a scientific approach to solving problems; and 2, to increase public support for science, and to defend the public interests of science.

The Association's work is multidisciplinary, covering all the natural, social and applied sciences. It provides a forum for debate and a focus for educational activities for people of all ages and experience. An independent body, its membership is open to all. The Annual Meeting is the largest general scientific meeting held in Britain. Open to all, its programmes of several hundred talks, exhibitions and demonstrations form a festival of science that attracts several thousand participants each year.

The Scientific Programme covers: physics; chemistry; geology; biological sciences; geography; economics, engineering; anthropology and archaeology; medical sciences; psychology; agriculture and forestry; education; sociology; general; and mathematics. Core topics will include: evolution; molecular electronics; education and science; the challenge for British science; and biotechnology. Lectures will range over such subjects as: The death of the dinosaurs; physics in the home; geology and society; protein engineering; recreation and leisure; the prospects for full employment; robotics and automation; local archaeology; medical imaging; brain localization; the agricultural policy of the EEC; are teachers professionals? and class and public policy.

The Association also has a Youth Section and almost two-thirds of its work involves young people. There will be a great deal to interest younger scientists in the main programme as well as in a special day of events for BAYS (British Association Youth Section). Many delegates will wish to attend the Mason Conferences, during which specialists will describe the latest advances in their various fields. These will be held, among others, by the Institute of Scientific Information, the Institute of Biology, the Royal Society of Chemistry Food Chemistry Group and the Royal Astronomical Society.

For further information contact: Dr D. Morley, British Association for the Advancement of Science, Fortress House, 23 Savile Row, London W1X 1AB.

WHO New Books Catalogue, 1987

This catalogue lists new books and periodicals produced by WHO during the first half of 1987. The main sections include: Communicable Diseases; Education, Training; Epidemiology; Statistics; National Health Management; Pharmaceuticals, and Biologicals. The final pages contain useful information on publications of particular importance, together with an index of titles. Apply to: World Health Organization, Distribution and Sales, 1211 Geneva 27, Switzerland.

Kenya; *Manual of the National Leprosy Tuberculosis Programme*

This is a strongly bound paperback of no fewer than 150 pages, written for 'the clinical officer tuberculosis and leprosy control', published by the National Leprosy and Tuberculosis Control Programme, Ministry of Health of Kenya, Box 20781, Nairobi, Kenya, Africa. Part A covers general information on the programme, Part B deals with leprosy and Part C with tuberculosis. First published 1987, this manual is extraordinarily comprehensive and detailed; it is difficult to find any aspect of control, for either disease, which has not been considered and clarified. It includes numerous charts, diagrams, tables and forms—all of practical importance. In previous issues of this Journal we have drawn attention to excellent manuals from Ethiopia and Nepal. This one from Kenya ranks very high and should surely serve as a model for other countries, perhaps particularly in Africa, for the development of a manual with a truly national basis, for the control of these diseases.