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SPECIAL ARTICLE

Drug resistance in leprosy—a review

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Although the introduction of dapsone made possible the effective chemotherapy of leprosy, we have required more than 30 years to learn how to use the drug properly. From the beginning, dapsone was used as monotherapy in a variety of dosages, although, by the time the drug was in use worldwide, it had already been established that cavitary pulmonary tuberculosis could not be cured by monotherapy with any antituberculosis drug. As a result, drug resistance in leprosy has become a serious problem, and demands immediate action.

Dapsone resistance was first proved[1,2] soon after multiplication of *Mycobacterium leprae* in the mouse foot-pad had been described.[3,4] It was estimated that, during the period 1964–66, the prevalence of dapsone resistance was only 2 per 1000.[5] However, the situation had changed by 1976 by which time it was apparent that dapsone resistance was already a significant problem.[6] Since the establishment in 1977 of the Scientific Working Group on Chemotherapy of Leprosy (THELEP) of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases,
in a number of leprosy-endemic areas to assess the true magnitude of the threat to leprosy-control activities.[7] Similar studies have also been carried out outside THELEP. As a result of these studies, not only is secondary dapsone resistance understood to be distributed worldwide, with rapidly increasing prevalence rates and alarming annual incidence rates in some areas;[8] also, primary dapsone resistance has been detected with unexpectedly high prevalence rates.[9,10] In addition, secondary resistance to bactericidal drugs other than dapsone, i.e. rifampicin (RMP)[11,13,50], ethionamide (ETH)[14–16], and clofazimine (CLO)[17],
to two bactericidal drugs, i.e. doubly resistant strains, have also been detected.[11,13,17,50]

Basic knowledge and speculation regarding drug resistance in leprosy

DEFINITION OF DRUG RESISTANCE

Epidemiologically, there are two types of drug resistance. Secondary or acquired resistance, a result of inadequate chemotherapy, is usually accompanied by a classic history, i.e. initial improvement, followed by deterioration despite continued treatment.[18] The other type is primary resistance, which occurs in patients who have not received chemotherapy, and results probably from infection with drug-resistant organisms that originated from another patient who had relapsed with secondary resistance.

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ETIOLOGY OF DRUG RESISTANCE

M. leprae still canot be cultivated *in vitro*, and *M. leprae*-infection of the foot-pad of the immunologically normal mouse does not develop bacterial populations large enough to contain drug-resistant mutants. Thus, our knowledge of how drug resistant *M. leprae* develop is only fragmentary. However, drug resistance of *M. tuberculosis* has been extensively studied. The following observations on *M. tuberculosis* might be useful in understanding the etiology of drug resistance in leprosy.

(i) Resistant bacilli are present in wild strains, i.e. bacterial populations that have never been exposed to an antituberculosis drug. This phenomenon was first demonstrated after the discovery of streptomycin, and was later found to be characteristic of other antituberculosis drugs as well. These resistant bacilli occur spontaneously as the result of mutational events. The frequency and degree of resistance of these mutants in a wild strain depend on many factors, such as the origin of the strain, the drug, its concentration, and the size of the bacterial population. The mutation frequency, which is closely related to the degree of resistance, is 10^{-6} to 10^{-7} for low-, and 10^{-8} to 10^{-9} for high-degree resistance. Therefore, among 10^{9} *M. tuberculosis*, one may expect to find 100–1000 naturally occurring low-resistant mutants, 10–100 moderately resistant mutants, and 1–10 highly resistant mutants against any drug to which resistance occurs in step-wise rather than single-step fashion.[19, 23]
(ii) The development of drug resistance during treatment is probably the result of a selective process. Whereas a majority of susceptible organisms are killed by the drug, the resistant mutants survive and multiply. Finally, they replace the susceptible organisms in the population. Drug resistance is not the result of a progressive adaptation of susceptible organisms to the drug to which they are exposed; no homogenously resistant strain has ever reverted to susceptibility.[19, 21]

If these observations can be applied by analogy to *M. leprae*, drug resistance during monotherapy is more likely to occur in multibacillary (LL, BL and BB) leprosy. Only 10^{8} *M. tuberculosis* are commonly found in pulmonary cavities before chemotherapy.[20] By conservative estimate, an untreated multibacillary patient has a total of 10^{11} organisms, 10% of which are viable.[24] Assuming the frequency of the spontaneously resistant mutants of *M. leprae* to be of the same order as for *M. tuberculosis*, one would expect the following outcomes:

(i) when the treatment is regular and in full dosage, only the highly resistant mutants are likely to survive after a period of favourable response;
(ii) when the treatment is only in a low dosage of the drug, even the low-resistant mutants will survive;
(iii) if the compliance with treatment is poor, unfortunately a frequent occurrence,[25] the resistant mutants will survive, because drug concentrations in blood or tissues cannot always be maintained above certain critical levels. It is possible that, initially, there may exist only mutants with low-degree resistance, and that the mutants with higher degree resistance arise only as the result of further mutations.[23, 26]

Once the surviving resistant mutants multiply to a certain level, clinical signs of relapse occur. Should the patient receive regular treatment in full dosage, relapse occurs quite late and mainly with high-degree resistance. On the other hand, should the patient receive treatment in low dosage or irregularly, relapse occurs earlier and mainly with low- or intermediate-degree resistance, as shown by the studies in Malaysia and Ethiopia.[5, 6, 8, 18, 27, 28]

If these assumptions are true, treatment failure caused by drug resistance must be expected in every multibacillary patient under monotherapy. Thus far, however, no data have demonstrated a prevalence rate of secondary dapsone resistance greater than 30% in any area in which dapsone has been used as monotherapy for more than 30 years (see Table 1). This means that at least 50% of multibacillary patients do not develop drug resistance. It may be argued that, because of the exquisite susceptibility of *M. leprae* to dapsone, and also because of the very long generation time of

Table 1. Results of surveys of secondary dapsone resistance

Country	Number at risk*	Minimal prevalence (% per year)	Incidence (% per year)	Degree of resistance	Reference
Burundi	925	37	—	majority high	(29)
Costa Rica	200	68	1·0	majority high	(30)
China				majority high	(31)
(Jiangsu)	236	51	—	or intermediate	
(Shanghai)	777	86	—	majority high	(32)
Ethiopia	1500	100†	3·0	mostly intermediate or low	(5, 18)
India					
(Karigiri)	1580	95	—	majority high	(33–35)
(Chingleput)	660	29	—	majority high	(unpublished data)
Israel	100	37	—	intermediate	(36)
Malaysia					
(1964–66)	5000	2	0·1	high	(2, 5)
(1973)	5000	25	0·3	majority high	(37)
Mali	105	57	3·0	intermediate or high	(38, 39)
Upper Volta	355	70	—	majority high	(40)

* All multibacillary patients who began dapsone monotherapy at least 5 years ago and were still living during the survey.[7]

† About one-third of the resistance confirmed by mouse foot-pad test.

M. leprae, clinical signs of resistance will take many years to appear. However, it can hardly be imagined that the majority of them will ever relapse. The explanation for this is not evident. On the other hand, there is no indication that monotherapy can prevent the development of drug resistance; therefore, that not all patients treated with monotherapy relapse with drug resistance does not justify treatment of patients with monotherapy.

Proof of drug resistance

Drug resistance should be suspected in a multibacillary patient who has relapsed, either under treatment or after he has stopped treatment, or whose clinical response is less favourable than expected, or in a new patient who may have had an intimate contact with a secondary resistant case.

Two methods have been used to prove drug resistance.

CLINICAL TRIAL

This method, which can be easily carried out anywhere,[23] has been used mainly for patients with *prima facie* evidence of secondary drug resistance.[18, 23, 27, 28] After the patient has been fully assessed clinically, histopathologically, and bacteriologically, he is given the tested drug in full dosage. The

full dosage of dapsone is 300–400 mg twice weekly by injection or 100 mg daily by mouth, either under supervision or while monitoring the urine to confirm the presence of sulphone. Clinical assessment, skin smears and skin biopsy are repeated regularly during the trial. If his strain of *M. leprae* is fully susceptible to the drug, the patient will respond to treatment at the same rate as other previously untreated and sensitive patients. In drug-resistant cases, several different patterns of response may be seen. Patients who have previously been on regular, full dosage treatment will show no, or only partial, response for a brief period, and then deteriorate. In other patients, especially those previously on low or irregular dosage, the initial response to the treatment will resemble that of drug-sensitive patients. However, after a time new, histopathologically active lesions appear with elevated MI, and old lesions become reactivated. Proof of resistance by clinical trial may require from 3 months to more than 5 years.[27]

Clinical trial is suitable only for those patients who relapse with active, new lesions with high BI and MI. However, in field surveys, employing the susceptibility test in the mouse foot-pad, resistant strains have been isolated even from patients without clinical relapse. The clinical trial pays more attention to the change of clinical signs and MI. The former indicator seems to be too subjective, and the latter too difficult to standardize. More importantly, patients with low-degree resistance may require as long as 5 years for confirmation of resistance,[23, 28] thus delaying combined therapy. For these reasons, clinical trial has generally been giv
resistance.[8]

DRUG SUSCEPTIBILITY TEST IN THE MOUSE FOOT-PAD

Because mouse inoculation is not possible unless sufficient organisms are recovered from skin biopsy specimens, such tests can be carried out only among multibacillary patients with a BI ≥ 3 in at least one skin lesion. The patient can be started on combined therapy immediately after the biopsy specimen, without awaiting the results of the study. Moreover, susceptibility of the *M. leprae* to a series of drugs can be tested simultaneously by this method.

Immunologically normal mice are inoculated with $5–10^3$ or 10^4 *M. leprae* into one or both hind foot-pads, and divided into several groups. One group is kept as untreated controls and fed normal diet; the rest are divided into drug-treated groups and fed special diets, into which have been incorporated different concentrations of drug. For example, 3 treated groups are fed diets containing 0·0001%, 0·001% or 0·01% dapsone. Usually 6–12 months after inoculation, mice are sacrificed and the acid-fast bacilli are harvested from the foot-pad tissue. If no multiplication of *M. leprae* is observed in the control group, the test is considered to have failed, possibly because the proportion of viable bacilli in the inoculum was too low. If multiplication of *M. leprae* is observed only in the control group, and not in the drug-treated groups, the strain is considered susceptible to the drug tested. If multiplication of *M. leprae* is observed in some or all drug-treated mice, the strain is resistant, and the degree of resistance is reflected by the highest concentration of drug in the diet that permits multiplication of *M. leprae*.

Drug susceptibility testing by the mouse foot-pad technique is quite reliable. However, the test is available in only a limited number of laboratories, and the majority of leprosy-control programmes and leprosaria do not have access to mouse foot-pad facilities. Therefore, setting up regional or national reference laboratories may be desirable.

Regarding drug-susceptibility testing by this technique, the following aspects need to be discussed, and some of them require further study.

The critical concentration of drug, and the critical proportion of resistant organisms as criteria of drug resistance

The only satisfactory definition of resistance is based on the behaviour of wild strains.[41] In

tuberculosis, a critical concentration of drug and a critical proportion of resistant organisms had been set up as criteria of drug resistance.[19, 21]

The critical concentrations of drugs for the diagnosis of resistance in the mouse foot-pad test are the concentrations of drug in the mouse diet, these being 0·0001% dapsone, 0·0001%–0·001% CLO, 0·003% RMP, and 0·01% ETH or prothionamide (PTH).

The more important problems appear to be in relation to bactericidal drugs other than dapsone. The numbers of wild strains fully titrated against these drugs have been small, and the criteria for the critical concentrations of these drugs are not reliable. Therefore, many more wild strains of *M. leprae* should be titrated for susceptibility to RMP, CLO, and ETH/PTH. These drugs have not yet been used in several endemic areas, so there remain good opportunities to carry out these crucial studies.

To determine whether a population of *M. tuberculosis* is drug resistant or not, it is not enough to establish the presence of resistant bacilli; all large bacillary populations contain some. Therefore, it is necessary to determine whether the proportion of resistant bacilli is abnormally large.[19, 21] So far, there are no data regarding the proportion of resistant *M. leprae* in a bacillary population. It may be assumed that when the population of a wild strain of *M. leprae* is no larger than 10^6, it will behave as a homogeneous population. As soon as the population becomes large, e.g. 10^{10} or more, it is no longer homogeneous for any drug, and has become a mixed population, because of the spontaneous occurrence of resistant mutants. However, the inoculum for the mouse foot-pad is usually of the order of 10^4 organisms, the majority of which are dead. As the frequency of the spontaneous drug-resistant mutants is very low, probably no larger than 10^{-6}, there is no possibility of detecting these resistant mutants before treatment. After several months' treatment with dapsone or several days' treatment with RMP, the majority of the viable drug-susceptible bacilli have been killed. But because the total number of bacilli does not change significantly, the proportion of viable bacilli decreases to such a level that their capacity to infect mice is lost.[42]

The resistant mutants cannot be isolated unless they have multiplied to a certain level. By this time, the viable bacterial population has again become virtually homogeneous, because the number of susceptible bacilli has decreased to a low level as 'persisters'. Therefore, at no time does it appear possible to isolate in the mouse a mixed (drug-susceptible and drug-resistant) population of *M. leprae*. The isolation of drug-resistant bacilli indicates that the resistant mutants have multiplied, and that the patient is a resistant case.

Nevertheless, in some drug-susceptibility tests in mice, only a small proportion, e.g. 1/10 or even 1/20, of animals treated with the lowest concentration (0·0001%) of dapsone demonstrate multiplication of *M. leprae*, whereas the majority of animals in the control group show multiplication. One explanation of such results is that the population of *M. leprae* is mixed, with the majority susceptible to 0·0001% dapsone, and a minority resistant. There are alternative explanations.

To test the possibility that the patient's population of *M. leprae* is mixed, and, more importantly, to evaluate the relationship between a resistant strain and a resistant patient, the following experiment is proposed. From those tests in which only a small proportion of animals fed 0·0001% dapsone demonstrate multiplication of *M. leprae*, bacilli should be recovered from drug-treated mice, and designated inoculum A. Bacilli should also be recovered from control animals and designated inoculum B. The bacilli should be passaged to groups of control mice and to animals administered 0·0001% dapsone in diet. If, at harvest, a majority of the treated animals inoculated with inoculum A and a small proportion of the treated animals inoculated with inoculum B demonstrate multiplication of *M. leprae*, then there is a mixed population. If, on the other hand, a majority of animals from both treated groups show multiplication, the population is homogeneous, and probably there was some technical error in the original experiment. Finally, if both inocula behave similarly in both control and treated mice, reproducing the results noted in the original test, one should consider the possibility of phenotypic variation of susceptibility to dapsone among members of a single clone of *M. leprae*.

Standardization of the drug-susceptibility test in the mouse foot-pad

Although the mouse foot-pad technique has been basically standardized through workshops and some documents,[43] the following aspects of the susceptibility test also require standardizing.

Criteria of multiplication of M. leprae. The smallest number of *M. leprae* detected by present counting methods is rather large. It can hardly be concluded that multiplication of *M. leprae* has occurred if 2×10^4 AFB per foot-pad are harvested after inoculation of 5×10^3 or 10^4 AFB; a count of 2×10^4 AFB per foot-pad indicates that only 2 or 3 AFB were observed during counting, and these could be merely the inoculated bacilli. It would be better to define 10^5 AFB per foot-pad as the criterion of multiplication of *M. leprae*.

Mouse diet. The drug-containing mouse diets should be analysed regularly by standard methods, to ensure that the proper concentrations of drugs are in the diets. Some drugs, such as RMP and other rifamycin derivatives, are unstable, especially after they have been incorporated into the mouse diet. Also, the procedures for preparing and preserving the diets should be standardized.

Minimal number of animals per group. In some biopsy specimens, the proportion of viable bacilli is quite low. The more animals inoculated and harvested, the greater the possibility of detecting multiplication of *M. leprae*. After multiplication of *M. leprae* has been confirmed in the control group, each treated group should have at least 4 mice. When manpower is available, harvesting of mice should be done individually. If multiplication of *M. leprae* has occurred in only a small proportion of mice, it may be masked by pooling the foot-pad tissues from several mice.

Main observations on drug resistance in leprosy

RESISTANCE TO DAPSONE

With respect to drug resistance, dapsone is the most important of the antileprosy drugs, apparently because it has been widely used as monotherapy for more than 30 years. It is still the only drug available for most leprosy patients in developing countries.

Dapsone is extremely effective in inhibiting the multiplication of *M. leprae*. This has been confirmed both in experimental studies in the mouse[44-46] and in small scale clinical trials.[47] Dapsone-resistance is identified when bacilli obtained from patients multiply in mice receiving 0·0001% or more dapsone in the diet. The degree of resistance is defined as low, intermediate or high, depending upon the strain's ability to multiply in mice administered 0·0001%, 0·001%, or 0·01% dapsone.[8] Because the degree of resistance varies remarkably among strains, and because the risk of emergence of dapsone-resistant infection differs between patients treated initially with low dose sulphone and those treated initially with dapsone in full dose,[37] the mutation that produces dapsone resistance has been identified as 'step-wise' rather than 'single-step'.

Secondary dapsone resistance

At the moment, secondary dapsone resistance has been detected in more than 25 countries.[8] Pearson reviewed the available data on secondary resistance to dapsone in 1981.[5] Since then, sporadic instances of secondary dapsone resistance proved in the mouse foot-pad have also been reported from Guadeloupe, [13] Martinique,[13] Indonesia,[48] Nepal[49] and New Caledonia.[50]

The results of surveys for secondary dapsone resistance are summarized in Table 1. Most of the recent surveys have followed the THELEP Protocol.[7] Four studies followed-up the same population for varying durations and, therefore, were able to estimate the incidence rates. Currently, 2 more formal surveys are being carried out in Burma and in India. Resistant cases have been detected in all these areas, but the data are incomplete. Based on clinical examinations, the

prevalence and incidence rates in Burma appear to be the highest—of the order of 200 per 1000 and 30 per 1000 per year[51] respectively.

Based on the available data, it is clear that secondary dapsone resistance is now a worldwide phenomenon. A majority of the resistant strains of *M. leprae* are of intermediate- or high-degree resistance. Generally speaking, some study areas listed in Table 1 have quite effective leprosy control programmes. For example, in Malaysia, many patients have been treated for many years as in-patients. Their treatment has been reasonably regular and well supervised. In most cases, dapsone had been used in full dosage. Therefore, the Malaysian figures for prevalence and incidence of dapsone-resistant leprosy can be taken as the best results that can be achieved by dapsone monotherapy.[5] The poorer the quality of the control programme, the more likely is the occurrence of dapsone resistance and its rapid increase. Recently, the alarming figure of a 3% annual incidence rate has been reported from both Ethiopia [18] and Mali,[39] and this is probably also true in Burma.[51] This is a clear indication of the seriousness of the situation.

Among the dapsone-resistant patients, the duration of treatment before relapse is much longer than in drug resistance in tuberculosis, perhaps because of the very long generation time of *M. leprae*. This duration varies remarkably, ranging from 2[18] to 24 years,[27] and appears to correlate with the dosage of dapsone and regularity of treatment. If resistance develops among the patients receiving low dose dapsone or irregular treatment, it usually develops within fewer than 10 years of treatment, and frequently with low or intermediate levels of resistance.[23, 27]

Primary dapsone resistance

Five primary dapsone-resistant patients, all proved in the mouse foot-pad, were reported in 1977.[9] Subsequently, several surveys were carried out. The available data are summarized in Table 2. In

Table 2. Results of surveys of primary dapsone resistance

Country	Number of patients		Prevalence (per 1000)	Degree of resistance	Reference
	Total	Resistant			
China	20	10	500	low or intermediate	(Ji *et al.* unpublished data)
Ethiopia	29	16	550	majority low	(9)
India					
(Chingleput)	56	21	375	majority low	(10)
(Karigiri)	12	5	420	low or high	(52)
Korea	18	4	222	majority low	(56)
Mali	40	14	350	majority low	(10)
Nepal	15	13	870	mostly high	(53)
Philippines	55	2	36	low or intermediate	(57)
Malaysia	22	6	270	majority high	(5)
Martinique & Guadeloupe	17	12	700	low or intermediate	(54)
USA					
(Carville)	93	18	190	majority low	(16)
(San Francisco)	54	1	18	low	(55)

addition, sporadic cases have also been reported from Burundi,[29] India[69] and Indonesia.[48] Although the available data are still limited, primary dapsone resistance appears to have become ubiquitous. Until now, it has gone unrecognized in many places, perhaps because it has not been sought.

In tuberculosis, it is not easy to differentiate between primary resistance and undiscovered secondary resistance. The term 'initial drug resistance' has been used for resistance among newly discovered patients, when it is impossible to obtain a reliable history.[22] One may question whether or not the so-called primary dapsone-resistant patients had in fact received prior dapsone treatment. Of course, this possibility must be kept constantly in mind. In certain areas with poor registration systems, some undiscovered secondary dapsone-resistant cases might be included as primary resistant patients; however, such a possibility should be much less likely in leprosy than in tuberculosis. In most developing countries, leprosy patients can receive treatment only from certain clinics, and usually their records are kept in these clinics. In addition, relapsed cases usually have some old lesions, and might be distinguished clinically from the new patients. The data on primary resistance from India and Mali, shown in Table 2, were obtained from THELEP controlled clinical trials.[10] Considerable efforts, including urine analysis for dapsone, were undertaken to ascertain that the patients admitted to these trials had not been previously treated. The prevalence rates of primary resistance in India and Mali are not significantly lower than the figures obtained elsewhere.

If one compares the data in Tables 1 and 2, one may see that, with the exception of the Philippines, the prevalence rate of primary dapsone resistance is much higher than that for secondary resistance. Such a result, although unexpected, may be explained. The calculations for prevalance rates of secondary and primary dapsone resistance are entirely different. The denominator for secondary resistance is the total number of patients at risk, regardless of their skin-smear status. But most of these patients have been negative. The denominator for primary resistance is the total number of tested patients, all of whom are untreated multibacillary patients having lesions with BI ≥ 3. In Shanghai, for instance, [32] the number of patients at risk of secondary resistance was 777; of these, 92 (11·8% of the total) were skin-smear positive, and most likely the main sources of infection in the community. Sixty-seven of these 92 patients, representing 73% of the skin-smear positive group, had at least one lesion with BI ≥ 3, and the proportion found to have resistance by mouse foot-pad was no smaller than 62%. Even ignoring the infectivity of patients with BI < 3, the proportion of patients with resistance among the main sources of infection, *i.e.* all skin-smear positive multibacillary patients, should be greater than 45% (62% $\times$ 73%). This could explain the high rate of prevalence of primary dapsone resistance.

By definition, primary dapsone-resistant patients are those infected with organisms from resistant patients. Therefore, it must be assumed that primary resistance, unlike the secondary resistance, occurs in at least as high a proportion of paucibacillary as that of multibacillary patients. One cannot demonstrate that such patients have resistance by inoculation of mice, because too few organisms can be recovered from the skin-biopsy specimens. If the primary dapsone-resistant paucibacillary patients are treated with dapsone monotherapy, some of them may undergo serious deterioration, and even downgrade towards the lepromatous end of the spectrum.

Because drug resistance is an inherited trait, the mutants isolated from the primary resistant patients should have the same degree of resistance as the source of infection, i.e. secondary resistant patients. In tuberculosis, reversion of resistant strains of *M. tuberculosis* to susceptible has never been observed.[19-21] Very few data regarding the stability of resistance of *M. leprae* can be found in the literature, but there are a few resistant strains which remained dapsone-resistant for several years after the patients had changed their drugs.[23] The reason that most strains of *M. leprae* isolated from primary dapsone-resistant patients were of low-degree resistance, whereas the majority of strains isolated from secondary dapsone resistant patients have shown intermediate- or high-degree resistance, is still unknown. Perhaps the explanation lies in the long incubation period of multibacillary leprosy. Most patients recognized with primary resistance today were infected 10 years ago or more, at which time the characteristics of strains from secondary resistant patients may have been different from those of today. Because of the low degree of resistance, the majority of

primary dapsone-resistant patients may be expected to respond to treatment with dapsone in full dosage. However, dapsone must be combined with other drugs; otherwise the patients are very likely to relapse with high-degree resistance in the course of time.

RESISTANCE TO OTHER BACTERICIDAL DRUGS

Not much data on resistance to other bactericidal drugs can be found in the literature because these drugs have not been used as widely as dapsone and because resistance to them has not been systematically sought. However, during the past 10 years, a number of patients have been treated with these other drugs, after they have relapsed under dapsone monotherapy. Unfortunately, many patients had either been kept on monotherapy, or were given in addition such bacteriostatic drugs as thiambutosine (DPT). Because of high cost (of RMP) and side-effects (pigmentation in the case of CLO, poor tolerance in the case of ETH/PTH), the regularity of treatment with these other bactericidal drugs may have been even poorer than that with dapsone. One may anticipate that leprosy patients resistant to these drugs will appear in the near future, unless they are changed to combined therapy.

RMP

RMP kills *M. leprae* with exceptional speed, as proved in both experimental studies and clinical trials.[58 60] Within a few days after a single dose of 600 or 1500 mg RMP, 99·9% of *M. leprae* from an untreated patient have been shown to be killed.[61] Such a rapid bactericidal effect has deeply impressed both the clinicians and the patients. Therefore, since the mid 1970s, RMP has been increasingly used despite its high cost. One might have expected that, after the experience of dapsone monotherapy, medical personnel would have rejected the use of RMP as monotherapy. Unfortunately, this has not been the case. As early as 1976, two RMP-resistant patients had been reported.[11] So far, 4 of 16 patients, who had been treated with RMP monotherapy in a dosage of either 300 mg or 600 mg daily, have developed resistance within 3–5 years.[16] This indicates that, despite its rapid bactericidal effect, the development of secondary resistance to RMP, used as monotherapy, occurs earlier than in the case of dapsone monotherapy. Recently, 9 more resistant strains have been reported.[13, 50] However, this regrettable situation does not deny the effectiveness of RMP. It is still the most powerful weapon we have. Even without any new powerful drugs, one might still expect that leprosy can be completely treated by regimens containing RMP, provided RMP is always combined with other antileprosy drugs capable of preventing the development of resistance to it.

CLO

The bactericidal effect of CLO to *M. leprae* has been confirmed in the mouse[62] as well as in clinical trial.[63] Because of its anti-inflammatory effect, it can also be used to prevent or control lepra reactions. The drug was introduced into the treatment of leprosy in the 1960s, but it was not until 1982 that the first CLO-resistant leprosy patient was reported [17] [this strain has since been found susceptible to 0·001% CLO, and resistant to 0·0001% CLO in another laboratory (L Levy, personal communication)], despite many patients having been treated with CLO monotherapy during the intervening years. The repository character of the drug appears to have delayed the appearance of resistance, as had been observed before with regular, full dosage dapsone monotherapy. 'One way' cross-resistance between CLO and RMP had been demonstrated in *Mycobacterium* sp. 607 by Morrison, i.e. the RMP-resistant strain was fully susceptible to CLO, but the CLO-resistant strain was also resistant to RMP.[64] The presence of this phenomenon should be tested in *M. leprae* as soon as possible.

ETH and PTH

Prima facie evidence of ETH-resistance was reported[14] in 7 of 102 patients treated with 500 mg ETH as monotherapy; 4 of these were later confirmed as resistant by mouse foot-pad test.[15] In addition, one of the three patients treated with ETH 500 mg daily as monotherapy in Carville developed ETH-resistance.[16]

In leprosy, the potential of cross-resistance between thioamides and thiacetazone (TBI) as well as DPT is a serious concern. All of these compounds have in common the group —CH—NH_2. Cross-resistance between TBI and DPT in *M. leprae* has been demonstrated, although not in every strain[65, 66] (Ji *et al.*, unpublished data). Cross-resistance between TBI and ETH in *M. tuberculosis* has also been reported.[21] Cross-resistance between ETH and PTH is to be expected, because the parts of the molecules responsible for antibacterial activity are identical; this has been confirmed in *M. leprae*.[66] The problem is that both TBI and DPT were quite widely used during the 1960s, and TBI is still being used as a component of combined therapy. Therefore, some leprosy patients must be harbouring mutants resistant to these 2 drugs. If cross-resistance between the thioamides and TBI as well as DPT exists in *M. leprae*, not only will this decrease the effectiveness of the thioamides, but it will also seriously threaten the effectiveness of multidrug regimens which contain thioamides. Pattyn and Colston reported that 2 ETH-resistant strains were also resistant to TBI and DPT, but that 3 DPT-resistant strains were susceptible to PTH.[66] Although this result is reassuring, the number of strains studied for cross-resistance is insufficient, and no TBI-resistant strain was included in the reported study. Therefore, there is urgent need for further evaluation of cross-resistance among TBI, DPT and ETH or PTH. Until the situation has been clarified, it would be preferable not to treat with multidrug regimens containing PTH patients who previously had been treated with either TBI or DPT for more than 2 years.

In conclusion, it seems unnecessary to conduct additional surveys for secondary dapsone-resistant leprosy, but it is necessary to collect more data with respect to resistance to RMP, CLO and PTH, in an attempt to estimate the magnitude of the threat to the prospects of combined therapy.

Prevention and treatment of drug resistance

Prevention of resistance is more important than treatment from the epidemiological point of view, and is also less expensive than treatment of drug-resistant patients. As mentioned previously, many patients will relapse with drug resistance if they are treated with bactericidal antileprosy drugs as monotherapy.

By analogy with the treatment of tuberculosis, to prevent the development of drug resistance, every multibacillary leprosy patient should be given combined therapy from the very first day of treatment. The spontaneous mutants resistant to any one drug should be fully susceptible to the other drugs. When the proportions of mutants resistant to any 2 of the drugs are 10^{-m} and 10^{-n} respectively, the proportion of doubly resistant mutants in a previously untreated bacterial population will be $10^{-(m+n)}$.[21] Assuming that the total number of organisms in an untreated multibacillary leprosy patient is 10^{11}, and that 10% of them are viable, the population must consist of 3 fractions: the largest fraction, more or less 10^{10}, is composed of the organisms susceptible to both dapsone and a second bactericidal drug, e.g. RMP; a second fraction includes about 10^4 mutants resistant to dapsone; and the third fraction includes the same number of mutants resistant to RMP. Because the proportion of mutants resistant to either dapsone or RMP is 10^{-6}, the frequency of a doubly resistant mutant is 10^{-12}, and the possibility of finding an individual organism resistant to both dapsone and RMP is practically nil. If the patient is treated with dapsone plus RMP, RMP will kill the mutants resistant to dapsone, and, reciprocally, dapsone will kill the mutants resistant to RMP. Thus, drug resistance is prevented.

However, it should be emphasized that this description is valid only if the bacterial population

is normally susceptible to these 2 drugs, and treatment with the 2 drugs is started simultaneously. If the patient is treated with dapsone monotherapy for a certain period, and then RMP is added, the situation is no longer the same as that just described. Because of the period of dapsone monotherapy, the proportion of dapsone-resistant mutants might have increased by selection. Once the dapsone-resistant mutants have multiplied to a certain extent, e.g. to $\geqslant 10^6$, a certain number of RMP-resistant, i.e. doubly resistant-mutants will have appeared among the dapsone-resistant mutants, leading thereby to failure of combined therapy. Moreover, as mentioned earlier (p. 272), primary dapsone resistance is now quite common among untreated patients; therefore, the *M. leprae* of an untreated patient may no longer be fully susceptible to dapsone. Because the proportion of dapsone-resistant mutants is large even before treatment, and because the absolute number of the dapsone-resistant mutants is large enough to include a few RMP-resistant mutants, combined chemotherapy with dapsone plus RMP, even if started simultaneously, may fail, as RMP cannot kill the doubly resistant mutants. In order to prevent multiplication of the doubly resistant mutants, a third bactericidal drug must be included in the multidrug regimen. This is the reason for recommending, at this stage, that all multibacillary patients be treated with a combined regimen including 3 bactericidal drugs.[8]

Until now, most secondary-dapsone resistant patients have been treated with CLO or RMP,[67, 68] usually as monotherapy or combined with a bacteriostatic drug. Although the presence of dapsone resistance has not altered the rate of initial response to the other drug, it is still possible for resistance to the second drug to develop, as if that drug had been given as monotherapy for a long period. Thus, the organisms become doubly resistant. In tuberculosis, double resistance is frequently observed after successive monotherapy.[21] At least 12 doubly resistant strains of *M. leprae* have been reported—11 strains resistant to dapsone and RMP,[11, 13, 50] and 1 strain to dapsone and CLO.[17] Because only 4 bactericidal antileprosy drugs with different mechanisms are currently available, the patient cannot receive proper treatment once a doubly resistant strain develops; moreover, he will also become a most dangerous source of infection. Therefore, prevention of such mutants is extremely important.

There have been a certain number of dapsone-resistant patients who had earlier been skin-smear negative for varying periods under dapsone monotherapy and had subsequently relapsed.[18, 23, 27, 28] In order to prevent relapse caused by drug resistance, smear-negative multibacillary patients never previously treated with a multidrug regimen should also be retreated with multidrug regimens for fixed periods.

In short, all categories of multibacillary leprosy patients should receive combined therapy with bactericidal drugs. The regimen recommended for treatment of multibacillary leprosy by the WHO Study Group on Chemotherapy of Leprosy for Control Programmes[8] has been designed to be effective in such patients.

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Viability of *Mycobacterium leprae* within the gut of *Aedes aegypti* after they feed on multibacillary lepromatous patients: a study by fluorescent and electron microscopes

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Summary This paper describes the viability of *Mycobacterium leprae* within the gut of mosquitoes after they have bitten bacilliferous lepromatous patients. In the test experiments prestarved female *Aedes aegypti* were allowed to feed on bacilliferous leprosy patients, while in the control experiments mosquitoes were fed on a glucose-water-lepromin mixture containing dead *M. leprae*. The insects were sacrified for 7 consecutive days, their guts were dissected out and excreta were collected. These preparations were mounted and examined for acid-fast bacilli (AFB) by: 1, fluorescent staining technique; 2, by a light microscope using acid-fast staining technique; and 3, also by scanning as well as transmission electron microscopes. AFB were found in the gut and also in excreta but more abundantly in the earlier days after blood meal.

The fluorescent staining technique showed that AFB within the gut of mosquitoes became non-viable (red stain) after 4 days of blood meals. It also demonstrated multiplication of the viable bacilli (green stain) during early days. It was further observed that most of the solid bacilli quickly became granular and non-viable (red stain). Ultrastructural studies confirmed these findings and demonstrated membrane bound dividing bacteria within the gut of the insects mostly within 72 h after a blood meal. No such cell-division was found in the gut of mosquitoes artificially fed on the glucose-water-lepromin mixture.

These data together with light microscopic findings lend support to the transient multiplication of viable *M. leprae* within the gut of the mosquitoes after the bacilli were taken up from the circulation of lepromatous patients at least during the early period following a blood meal. However the possibility of transmission of the illness into humans by mosquito bites seemed to be remote,

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because of the short viable time, quick fragmentation and elimination of the ingested bacteria from the gut of the insects.

Introduction

Acid-fast bacillaemia has been demonstrated in untreated borderline or lepromatous leprosy patients.[1] Laboratory bred mosquitoes (*Culex fatigens*), previously allowed to feed on lepromatous leprosy patients, often contain leprosy bacilli,[2] which was transmitted into mouse foot-pad[3] and some remained viable up to 48 h after the blood meal.[4] Thus in some parts of the world these arthropods might play a significant role in at least a mechanical transport of *M. leprae*. However, there is a paucity of data regarding the alterations in the morphology that might take place in *M. leprae* within their gut. Also their exact survival period within the insect's intestine remains to be determined. The present work is an attempt to find out: 1, whether there is any multiplication of the ingested bacteria; 2, to determine their survival time within the gut of the mosquitoes; and 3, also to document the ultrastructural changes occurring in *M. leprae* within their gut till they are excreted. These results have been compared with control experiments in which the mosquitoes were allowed to feed on a mixture of glucose, water and lepromin (containing non-viable *M. leprae*) in a proper proportion.

Materials and methods

A BREEDING OF MOSQUITOES IN STERILE CONDITION*

Adult female *Aedes aegypti* mosquitoes were obtained from a laboratory bred mosquito colony, kept at the Malaria Research Centre, Indian Council of Medical Research, Delhi.

B HUMAN MATERIAL

Ten (9 males and 1 female) leprosy patients including 9 LL and 1 maculo-anaesthetic case volunteered for the feeding experiments. Their age varied from 20 to 42 years. The duration of their illnesses ranged from 1 to 7 years. Diagnosis was based on clinical examinations and histological classification.[5] All cases except one 22-year-old patient with maculo-anaesthetic (MA) type of leprosy

* Filter paper containing desiccated mosquito eggs was kept in alcohol for a few minutes, and thereafter floated in 0·5 l of sterile distilled water, covered with a sterile net and kept at 28°C in a sterile chamber. The eggs were hatched next day; 50 units of neomycin sulphate and 50 units of polymyxin B sulphate were added. Every alternate day autoclaved dog biscuit and yeast were given as food. pH was maintained at 6·5 by changing water every third day. Fresh antibiotics were added when the water was changed. Pupa and adult mosquitoes were formed.

were on antileprosy treatment at the time of the study. Bacillary index was estimated, which varied from +2 to +4. One of the authors (KS) volunteered for the feeding experiment. He was taken as a control.

C FEEDING EXPERIMENTS PERFORMED ON BACILLIFEROUS LEPROMATOUS PATIENTS OR HEALTHY VOLUNTEERS

(1) A cage (8″ × 8″ × 8″) made of iron wires was covered on all sides by mosquito net cloth. In a typical experiment 40–50 prestarved female mosquitoes were taken. There was an opening through which the volunteers introduced their hands. The mosquitoes were starved for 12–24 h and then allowed to bite on the hands for 5 to 10 min during the day-time.

(2) The biting experiment was also performed with the apparatus described in Figure 1. The advantage of this apparatus is that the mosquitoes could be allowed to feed on a particular skin lesion.

After the blood meal mosquitoes were kept in the cage with proper humidity. A sugar source (raisins soaked in water or cotton wool soaked in glucose-water) was offered to the insects.

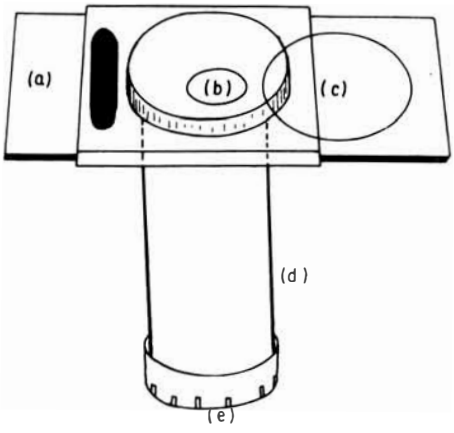

Figure 1. A plastic cylinder (d) had wire netting at one end (e) and a sliding stage with one opening at the other end (a). The mosquitoes were introduced through the opening (b) and thereafter it is closed. Before the biting experiments, the cylinder was put on the exposed skin of the volunteer, with the net (e) touching the skin. The mosquitoes bite the volunteers through the net (e). After biting the mosquitoes are taken out through (c) for dissection.

D FEEDING OF MOSQUITOES ON A GLUCOSE-WATER-LEPROMIN MIXTURE

Two volumes of 5% glucose solution were mixed with 1 volume of armadillo derived lepromin (World Health Organization) containing 4×10^7 bacilli/ml. This mixture was soaked in cotton wool and was kept on the mosquito cage net for 1–2 h and thereafter replaced by a plain pad containing glucose water. Sterile precautions were taken in the preparation of the mixture as far as possible.

E COLLECTION OF EXCRETA FROM MOSQUITOES

The excreta was collected from the glucose-water pad, mounted in a drop of phosphate buffer and stained for AFB.

F DISSECTION OF THE GUT OF THE MOSQUITO AND PREPARATION OF SAMPLES FOR THE DEMONSTRATION OF ACID-FAST BACILLI

Dissection of midguts was done according to the method described by Vanderberg & Gwadz[6] and they were examined for normal bacterial flora.*

Two to four fed mosquitoes were etherized at 24-h intervals and thereafter up to 7 days. The proboscii was cut and the guts were dissected out under an ordinary dissecting microscope by using 2 needles, in a drop of normal saline on a microscopic slide. The samples thus obtained were processed for light and electron microscopy. Excreta were collected from the water pad and were smeared either on microscopic slides or on grids for electron microscopy.

G STUDY OF THE VIABILITY OF MYCOBACTERIAL CELLS BY FLUORESCENT STAINING PROCEDURE

The method described by Kvach & Veras[7] was followed. In brief, 5 mg fluorescein diacetate (FDA) (Sigma) per ml acetone was prepared. 2 mg ethidium bromide (EB) (Sigma, USA) was dissolved in 1 ml Hanks' balanced salt solution (HBSS), pH 7·4, containing 0·05% Tween-80. A fresh working solution of FDA/EB was prepared by diluting the above FDA solution tenfold in acetone. A 0·02 ml volume of the diluted FDA was added to 5 ml HBSS containing 0·05% Tween-80. A 0·01 ml of EB stock solution was added to 5 ml HBSS containing FDA. The fixed smears of mosquito guts were stained with FDA/EB, incubated at room temperature for 10 min, wet mounted, sealed and seen under a Leitz microscope with filer BP-390 at the excitation and filter 2 at the barrier. Green cells were considered to be viable and the red stained bacteria were considered to be dead.

H LIGHT MICROSCOPY

The gut and excreta smears were stained by Ziehl–Neelsen stain, examined under an oil immersion objective of a Leitz light microscope and documented photographically.

* Mosquitoes were etherized, guts were dissected with sterile needles under a dissecting microscope in an inoculation chamber. These tissues were teased out in a drop of sterile saline, smeared on glass slides and stained with Gram stain. Part of the tissue suspension was cultured on blood agar and McConkey plates. No Gram positive or Gram negative bacilli were seen or grown.

ELECTRON MICROSCOPY

(1) *Scanning electron microscopy*. The intact guts from control-unfed, control-fed, patient-fed mosquitoes were sonicated in alcohol at 30 kHz frequency for 30–45 s. The sonication time was standardized by sonicating the gut tissues for different time durations (15, 30, 45 and 75 s). Sonication for a longer time completely smashed the gut as well as the bacteria.

The intact sonicated as well as teased guts of mosquitoes fed on patients and a control were mounted on polished aluminium discs with a very fine layer of quickfix, examined under a JSM 35 scanning electron microscope with a resolution power of 60 A and the results were documented.

(2) *Transmission electron microscopy*. Blood was taken from the gut of the mosquito, at 24 h intervals for 96 h, diluted 1:4 in phosphate buffer (pH 7·0) on microscopic slides. Formvar-coated grid (300 mesh) was floated upside down on blood-buffer mixture and the grid was placed on a moist filter paper and a drop of freshly prepared solution of 1% uranyl acetate was placed on it to stain the bacteria negatively. The method has been described earlier.[1]

Similar grids were also prepared from the blood in the gut of mosquitoes fed on control volunteers.

Transmission electron microscopy was also performed with mosquitoes fed artificially on a glucose-water-lepromin mixture. Guts and excreta were mounted on coated grids at regular intervals after feeding and stained with 1% uranyl acetate.

Results

A FLUORESCENT MICROSCOPY: A FEEDING EXPERIMENT ON LEPROMATOUS PATIENTS

Table 1 describes the viability of the ingested *M. leprae* by *A. aegypti* after biting bacilliferous lepromatous patients. The percentage of green solid bacteria on the 1st day was 36%; it increased slightly (48%) on the 3rd day and thereafter it decreased to 26% on the 4th day. After the 4th day no green coloured solid bacteria was seen. The percentage of red coloured solid bacteria increased steadily from the 3rd day onwards. The slight increase of green coloured solid bacteria on the 3rd day might be due to transient bacterial multiplication, which had been confirmed by transmission electron microscopy (Figure 4(d) and (e)). Table 1 further showed that the ingested bacilli quickly became fragmented. Most interestingly the beaded bacilli took red coloured stain.

B LIGHT MICROSCOPY

(1) *Feeding experiment on lepromatous patients*. A total number of 81 prestarved female *A. aegypti* were taken and divided into 2 groups for 2

Table 1. Studies on viability and morphology of *M. leprae* within the gut of the *A. aegypti* after they bit on bacilliferous lepromatous patients

Days of bite on which mosquitoes were sacrificed	Total number of solid green and red coloured bacilli counted	Total number and (%) of green coloured bacilli (viable)	Total number and (%) of red coloured bacilli (non-viable)	Total number of beaded‡ bacilli (red coloured) counted	No. beaded bacilli × 100 / No. solid bacilli
1st	80*	27 (35·75)	53 (64·25)	1	1·25
3rd	46*	22 (47·82)§	24 (52·18)	5	10·87
4th	69*	18 (26·08)	51 (73·91)	27	39·13
5th	8†	0	8‡ (100)	16†	200
6th	36*	0	36‡ (100)	125‡	347·22

* Randomly counted in 3 experiments consisting of 3 mosquitoes.

† Randomly counted in only 1 experiment, consisting of 1 mosquito. The low bacterial count might be due to the fact that this mosquito did not suck blood well.

‡ All ingested *M. leprae* were dead on the 5th day of bite.

§ This rise in the percentage of green coloured bacilli might be due to the bacterial multiplication within 48 h of bite (Figure 4(d) and (e)). Alternatively this initial increase of the percentage of viable bacteria might be also due to a declumping effect as a result of surface changes in the dying mycobacteria.[11]

experiments. In the 1st experiment, 51 mosquitoes were allowed to bite 2 LL patients and, in the 2nd experiment, 30 mosquitoes were fed on 3 LL volunteers.

The blood was found in the gut up to 48 h and thereafter it was absorbed. The incidence of AFB in the gut of mosquitoes was studied 2 h after the bite and thereafter followed daily up to 7 days. Four mosquitoes were sacrified every 24 h, and gut from each insect was dissected separately, smeared and stained for acid-fast bacilli. Thus from the 2 experiments 8 slides were prepared every day. The incidence of positivity was very high initially. Sixty-two per cent of the total number of slides screened showed AFB up to 48 h after feeding (Figure 2(a)). Thereafter not only the incidence of AFB positivity per slide became less but also the bacilli were less in number. However, solidly stained AFB were seen in the gut of mosquitoes up to 120 h following blood meals and thereafter mostly beaded AFB were seen.

(2) *Artificial feeding experiments on glucose-water-lepromin mixture.* In a control experiment, 60 prestarved female adult *A. aegypti* mosquitoes were allowed to feed on the glucose-water-lepromin mixture. Their guts were dissected every 24 h for 5 days and processed for demonstration of AFB by light as well as by electron microscopes.

The number of AFB found in the gut of mosquitoes thus fed artificially were much larger than observed in the gut of mosquitoes fed on leprosy patients. Large globi of AFB, solidly stained, were seen 24 h after feeding. This was probably due

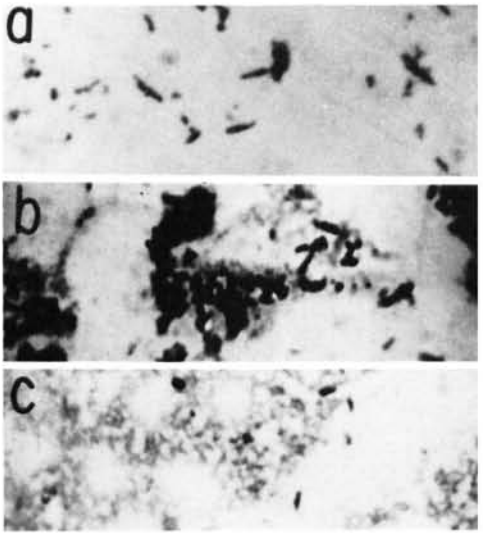

Figure 2. (a) Shows mostly solid acid-fast bacilli and few fragmented *M. leprae* in the gut of one female adult *A. aegypti*, 48 h after biting a lepromatous patient. This is the 2nd feeding experiment in which 30 mosquitoes were allowed to bite 3 lepromatous patients.

(b) Shows large numbers of solidly stained *M. leprae*. These are obtained from the gut of a female *Aedes aegypti* fed on glucose-water-lepromin mixture. The sample was taken 24 h after artificial feeding on lepromin.

(c) Shows fragmented acid-fast bacilli in the excreta of female *Aedes aegypti* 24 h after feeding on glucose-water-lepromin mixture.

to the high concentration of solid bacteria in the lepromin suspension. Unlike the AFB seen in the gut of mosquitoes fed on leprosy patients, practically no fragmented bacilli were seen in this experiment. Even when these mosquitoes were sacrificed 72 h after artificial feeding, the contents of the gut showed globi of solidly stained *M. leprae* and some fragmented bacilli (Figure 2(b)).

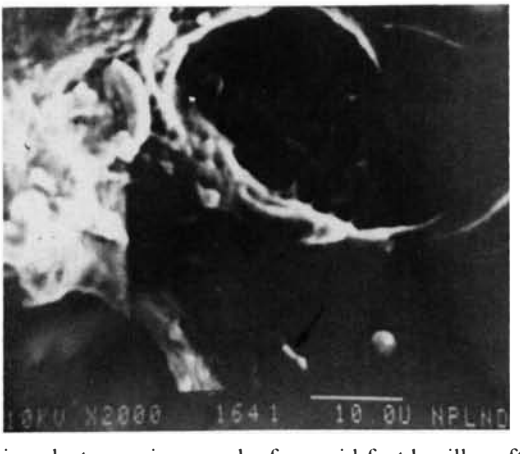

Figure 3. Shows a scanning electron micrograph of an acid-fast bacillus after 3 guts from 3 female *Aedes aegypti* were sonicated. These mosquitoes were allowed to feed on a maculo-anaesthetic leprosy patient who had a bacillary index of +2. Mosquitoes were sacrificed 48 h after biting. The left upper part of the figure shows the collapsed gut membrane. One AFB is seen in dividing stage. (× 2000).

The excreta collected in this experiment, 24 and 96 h after feeding, however, demonstrated mostly beaded acid-fast bacilli, but the number of bacilli gradually decreased (Figure 2(c)). This experiment was terminated 4 days after artificial feeding.

(3) *Control experiments.* (a) *Control unfed mosquito group.* Twenty control unfed female *A. aegypti* were included in this experiment. None showed any acid-fast bacilli either in the gut or excreta. (b) *Control-fed mosquito group.* Twenty-five control female *A. aegypti* fed on a normal volunteer were followed every 24 h

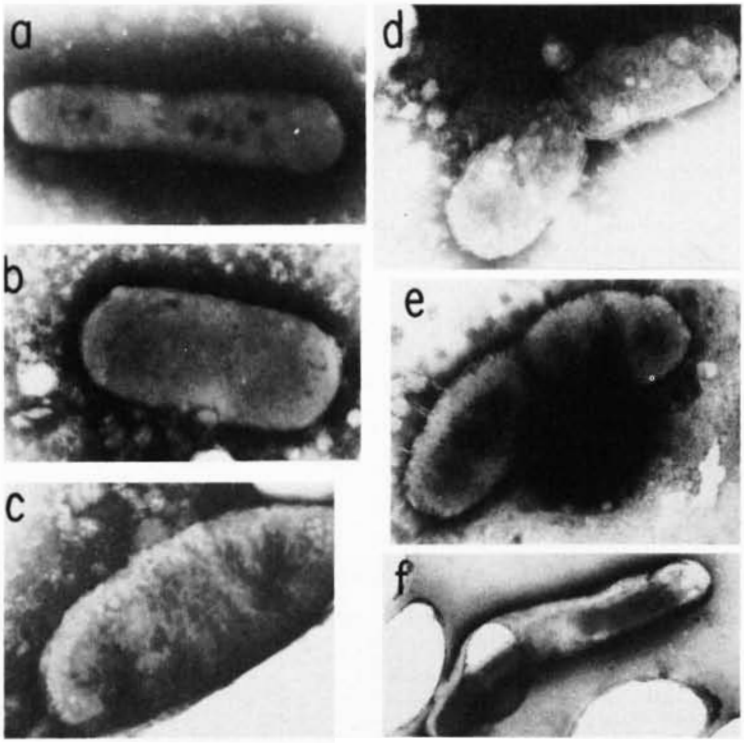

Figure 4. (a) Shows a characteristic ultrastructure of *M. leprae* within the gut of a female *Aedes aegypti* after 24 h of feeding on a maculo-anaesthetic patient. Electron dense areas are visible. (× 55,000).

(b) Shows the ultrastructures of *M. leprae* within the gut of a mosquito 24 h after feeding on a LL patient. Outer membrane of bacteria is not intact. (× 33,000).

(c) Shows the ultrastructures of *M. leprae* in the gut of a mosquito 72 h after biting a LL patient. The particle shows mesosomal structures at the poles. Distinct band structures are visible, which show division of bacteria. (× 42,000).

(d) Shows a dividing mycobacterium within the gut of a mosquito 72 h after feeding on a LL patient. Outer double layered cell membrane is distinctly seen. One of the daughter cells shows budding. (× 42,000).

(e) Shows dividing *M. leprae* in the gut of a mosquito 48–72 h after biting a LL patient. Pilli like structures are visible on both the daughter cells. (× 27,000).

(f) Shows an electronmicrograph of *M. leprae* within the gut of a mosquito artificially fed on lepromin.

for 7 days. No acid-fast bacilli could be demonstrated in the gut or excreta of these mosquitoes.

C ELECTRON MICROSCOPY

Demonstration of the ultrastructures of acid-fast bacilli in the gut of mosquitoes fed on leprosy patients. (i) *Scanning electron microscopy*: Seventy female *A. aegypti* were allowed to bite a maculoanaesthetic patient. Two to three mosquitoes were sacrified every 24 h. Figure 3 illustrates a typical scanning electron micrograph of a dividing acid-fast bacillus within the gut of the mosquito 48 h after feeding. (ii) *Transmission electron microscopy*: Figure 4 (a) illustrates an electron micrograph of an acid-fast bacillus within the gut of a female mosquito fed on a maculo-anaesthetic patient. The picture was taken 24 h after biting. Figure 4(b) and 4(c) show the ultrastructure of *M. leprae* in the gut of the mosquito 24 and 72

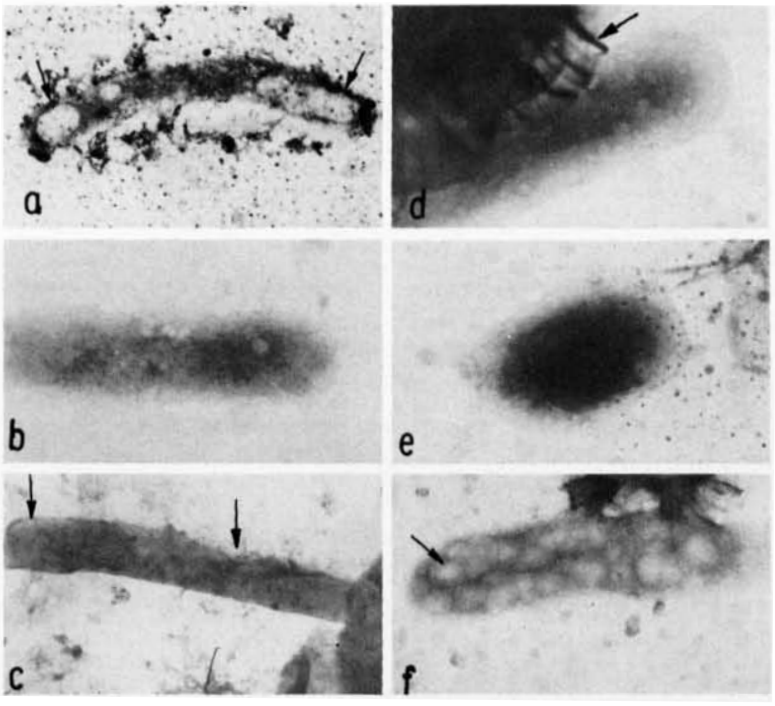

Figure 5. Control electron micrographs of *M. leprae* in lepromin and alterations in the ultrastructures of acid-fast bacilli within the gut of mosquitoes fed on a glucose-water-lepromin mixture.

(a) and (b) Electron micrograph of a pure suspension of *M. leprae* in armadillo-derived lepromin. Mesosomal structures are visible in (a) (× 20,000).

(c) Alteration of the ultrastructures of *M. leprae* within the gut of a mosquito 72 h after feeding on a mixture of glucose-water-lepromin. Double membranes are partially visible. Middle portion of the bacteria is partially digested (× 42,000).

(d), (e) and (f) Alteration of the ultrastructures of *M. leprae* within the gut of a mosquito 96 h after feeding on a mixture of glucose-water-lepromin. Vaculations within the bacteria are seen in (d) and (f). Musculature of the gut of mosquito is also visible in (d) and (f) (× 27,000).

h after biting the LL patient. Figure 4(d) and (e) clearly depict dividing acid-fast bacilli in the gut of mosquitoes after 72 h of biting a LL patient. Figure 4(d) demonstrates a symmetrical membrane structure.

Results of electron microscopy of the guts obtained from mosquitoes fed artificially on the glucose-water-lepromin mixture were similar to that observed in insects fed on leprosy patients (Figure 5). The number of AFB within the gut gradually decreased after artificial feeding. Many AFB were observed up to 96 h after feeding; thereafter occasional AFB were seen. After 4 days of feeding, lysis of AFB started (Figure 5(f)) and by 168 h lysis was almost complete. An electronmicrograph of *M. leprae* in the excreta of mosquitoes fed on the glucose-water-lepromin mixture after 72 h of artificial feeding showed fragmented and disrupted bacteria.

When the guts of mosquitoes fed on healthy volunteers were examined under a transmission electron microscope, digestion of erythrocytes and monocytes was seen after 24 h of biting. Musculature of the mosquito gut as well as virus-like particles was also visible inside the gut.

Discussion

The work of Narayanan *et al.*[9] aroused the interest of leprologists in the possibility of transmission of leprosy by arthropods. They suggested that for successful transmission, 3 essential requirements are necessary: 1, acquisition of sufficient number of living bacilli; 2, sustenance of the acquired bacilli in viable form until they are transmitted in new hosts; and 3, the ability of parasites to multiply in the vector.

To demonstrate the viability of *M. leprae* in the intestines of mosquitoes we have used the fluorescent staining procedure.[7] The staining method incorporated FDA and EB mixture. FDA entered the live cells, enzymatically hydrolysed by acetylesterase, rapidly accumulated in the cytoplasm and appeared green under ultraviolet light indicating viability. EB entered dead cells, combined with DNA and appeared red. Thus viability of the ingested green coloured bacteria was correlated with the presence of native acetylesterase within *M. leprae*. After the 4th day of bite no green coloured bacteria were seen within the mosquito gut indicating thereby the destruction of the enzyme of *M. leprae*.

In order to demonstrate the ability of *M. leprae* to multiply in mosquitoes, arthropods were allowed to bite bacilliferous leprosy patients. In a control experiment, they were also allowed to suck a mixture of glucose-water and armadillo-derived lepromin containing dead *M. leprae*.

The earlier investigators studied the transmission of *M. leprae* by mosquitoes by light microscopy, which had not allowed them to study any alteration in the ultrastructures of *M. leprae* in the adverse environment of the gut of the insects. We therefore decided to study this aspect of transmission of leprosy by mosquito.

As observed by previous workers, we also found that the number of *M. leprae*

in the gut of mosquitoes was larger initially and became scanty in the later part of the week after blood meals as well as after artificial feeding of lepromin. Electron-dense materials (negative staining) have been visible (Figure 4a). Similar electron-dense substances have recently been described by Hirata in longitudinal serial sections of *M. leprae*.[10] Mesosomal structures and nuclear apparatus have also been seen in a dividing bacterium (Figure 4(c)). Most interestingly both transmission (Figure 4(d) and (e)) as well as scanning electron microscopy (Figure 3) and also fluorescent technique could demonstrate bacterial cell division in the gut of mosquitoes within 72 h after biting. Another interesting finding is that solid AFB found in the excreta of the mosquitoes were not only small in number but they were also disrupted and fragmented. No *M. leprae* were found in dividing stage in the gut of mosquitoes which sucked lepromin (Figure 5). The most striking finding is a symmetrical membrane profile (Figure 4(d)). This is characteristic of *M. leprae* and is not shared by other cultivable species of mycobacteria.[8] However it cannot be taken as a definitive identification of *M. leprae*, since during fixation with 1% uranyl acetate, we had not used divalent cations like calcium or magnesium,[8] which might have affected the ultrastructure of *M. leprae*.

We are thus tempted to postulate from the similarity of electron micrographs of the bacteria within the gut of mosquitoes fed on patients (Figure 4) and lepromin-glucose-water mixture (Figure 5) that binary division of *M. leprae* within the gut of *A. aegypti* was possible at least in the early days after blood meals. This view is further substantiated by the observed increase of the percentage of green stained bacilli from 36% to 48% on the 3rd day of bite (Table 1). Since the number of green stained *M. leprae* rapidly decreased in the gut of the mosquitoes after 96 h and since red stained beaded bacilli increased rapidly in number after the 4th day of bite (Table 1) and further since the excreta showed fragmented and disrupted AFB, it is possible that digestive enzymes of the gut of the vectors might have affected the ultrastructures of *M. leprae* and probably have killed the parasites. However, detailed enzyme studies are necessary to substantiate our hypothesis.

Our findings of binary division of *M. leprae* in the gut of mosquito within 72 h after biting leprosy patients (Figure 4(d) and (e)) is contrary to the existing notion that propagation time of *M. leprae* is sufficiently long and the lifespan of the arthropods is relatively short. Thus substantial multiplication of *M. leprae*, if at all, could have not been possible within the gut of the mosquitoes. Therefore the observed transient multiplication of *M. leprae* within the gut of mosquito under an electron microscope might have 2 explanations:

(1) it is possible that under the gut environmental conditions, such as the contaminated water offered to the insects, saprophytic mycobacteria might have multiplied in the gut. But had it been so, the percentage of the green stained AFB should have been progressively increased during all the days of our experiment following blood meals. One might also argue that the staining technique would not differentiate between AFB and any bacterial flora in the gut of mosquito, such

as *E. coli*, so often present in it. We had demonstrated that the intestines of mosquitoes bred under sterile conditions were free from Gram positive and Gram negative bacteria. Furthermore the guts of control unfed mosquitoes and those of mosquitoes fed on normal volunteers showed no AFB like bacilli by EM. Even if the guts of our mosquitoes contained bacteria other than *M. leprae*, then the ratio of the numbers of green to red bacilli should be equal during all periods instead of the observed progressive decrease of green and rapid rise of red bacteria (Table 1). Furthermore the symmetric geometry of the outer and inner membranes of the mycobacteria as observed in the electron micrograph in our study (Figure 4(d)) as well as the similarity of the electron micrographs of *M. leprae* in the gut of lepromin fed insects (Figures 4 and 5) rule out this possibility.[8]

(2) Alternatively binary fission of the *M. leprae* (Figure 4(d)) and 4(e)) which might have started in the circulation of the patient at the time of blood meal was seen within 72 h of the feeding experiment. However, under the adverse environment of the gut of the arthropod, *M. leprae* became quickly non-viable (red stained) and eliminated. Further experiments are necessary to substantiate this posulate.

Acknowledgment

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Suppression of *Mycobacterium leprae*-induced leucocyte migration inhibition following lepromin injection in healthy contacts of leprosy. Preliminary observations

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Summary Lymphokine production to PHA and *Mycobacterium leprae* was measured using the leucocyte migration inhibition test before and after lepromin skin testing in 7 healthy contacts of leprosy patients. There was suppression of responses to *M. leprae* following lepromin injection, but the responses to PHA were unaffected: this may indicate the presence of protective immunity to leprosy in these subjects.

Introduction

Leprosy is a very chronic disease. Infectious cases are likely to excrete *M. leprae* for months or years prior to diagnosis, and for weeks or months after the commencement of treatment, thus exposing their household contacts to prolonged bombardment with antigenic material. The normal response of exposed subjects is the development of protective immunity; few acquire progressive disease. However, the prolonged exposure suggests a special need for a mechanism to avoid the development of an overactive immune response which could be harmful to the subject.

A possibly suitable control mechanism has been demonstrated *in vitro*. Regulation of the immune response is a function of suppressor cells, and mycobacterial antigens have been shown to induce suppressor cells which exerted antigen-specific suppression in lymphocyte cultures. The subjects were healthy

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individuals who were probably immunized against leprosy by prolonged exposure[11] or against tuberculosis by BCG vaccination.[4] Similarly another study[9] demonstrates *M. leprae* induced suppression of Con A responses of mononuclear cells from armadillos which appeared to be resistant to infection with *M. leprae*. This rather consistent pattern of results contrasts with the highly varied results of similar studies in which cell cultures from leprosy patients were used.[1,3,6] It has been suggested[11] that this antigen specific suppression regulates (and thus indicates the presence of) protective immunity in leprosy.

Hitherto studies of *M. leprae* induced immune suppression in man have been '2 stage' experiments. Suppressor cells have been generated by exposure of cell cultures to antigens of *M. leprae*: their effect has been measured by incorporating the cells into a second culture. In the present study we re-exposed healthy leprosy contacts to *M. leprae* in standard dosage by Mitsuda lepromin testing them, and assessed the resulting immune suppression by using the leucocyte migration inhibition test (LMIT) before and after lepromin testing to measure cell mediated responses to *M. leprae* antigens.

Materials and methods

Seven healthy members of the scientific and technical staff of Dhoolpet Leprosy Research Centre, Hyderabad, India, who had been working in close contact with leprosy patients for more than 3 years, were skin tested with Mitsuda lepromin (armadillo-derived, containing 4×10^7 bacilli per ml), and the late reaction (21 day) was recorded. Blood was drawn from these subjects twice to measure their LMIT responses, once before performing the skin test and again when the 21 day reaction was read.

The LMIT was performed exactly as described earlier.[8] This method is a modification of the original method described by Soborg & Bendixen.[10] Briefly, 7 ml of the anti-coagulated blood was added to 3 ml of 3% gelatin (Sigma Chemicals, USA) in saline in a culture tube. After thorough mixing it was kept at 37°C in an incubator for 45 min. The leucocyte-rich plasma was then aspirated to pellet the cells by centrifugation and subsequently for washing thrice. The cell pellet was resuspended in Minimum Essential Medium (MEM) (Bios, Bombay, India). Leucocyte concentration was adjusted to 3×10^7 cells/ml and the cell viability was checked with 0·25% Trypan Blue. The capillaries (Arthur Thomas Co., USA) were loaded with the leucocyte suspension and centrifuged at 1000 rpm for 5 min in a swing-out rotor centrifuge. Then, the capillaries were cut at the cell-medium interface and kept in polystyrene chambers which were filled with MEM containing 20% foetal calf serum (Microlab, Bombay, India) with or without antigen or mitogen and were sealed with cover-slips. Each test was run in triplicate. After 18 h of incubation at 37°C, the areas of migration were measured with planimetry.

The Migratory Index (MI) was calculated as follows:

$$MI = \frac{\text{Average area of migration with antigen}}{\text{Average area of migration without antigen}}$$

The stimulants used were the mitogen, Phytohaemagglutinin-P (PHA-P) obtained from Difco, USA which was used at 10 μg/ml. (In the dose-response study, this concentration gave optimal responses without agglutination of leucocytes. At lower concentrations (i.e. 1, 2 and 5 μg/ml) the LMI responses were weak while at higher concentrations (i.e. 20 μg and 25 μg/ml) agglutination of leucocytes was observed in the migration chambers.) *M. leprae* antigens, whole bacilli (MLW) and sonicated preparation (MLS) of the same batch (Batch No. AB 51) were kindly supplied by Dr R J W Rees, NIMR, London. They were used at $2{\cdot}5 \times 10^7$ bacilli/ml concentration (or equivalent concentration in the case of MLS) which were previously shown to be optimal for this system.[8]

Students 't' test was used for statistical analysis.

Results

The responses of each subject, before and after lepromin testing, to PHA, MLS and MLW are shown in Table 1. The responses to PHA were remarkably stable, and the means before and after lepromin testing were almost identical. The MLS responses were variable (4 showed little change, 3 suppression); they suggested

Table 1. Individual migratory indices of 7 healthy contacts before and after lepromin skin testing and their lepromin reaction

Subject number	PHA		MLS		MLW		Lepromin reaction (mm)
	Before	After	Before	After	Before	After	
1	0·70	0·79	0·88	0·94	0·67	0·82	7
2	0·79	0·80	1·08	1·05	0·63	1·01	10
3	0·72	0·68	0·84	0·75	0·81	0·83	7
4	0·71	0·58	0·66	0·84	0·60	1·02	6
5	0·56	0·76	0·57	1·09	0·79	0·95	6
6	0·86	0·79	0·66	0·98	0·74	1·02	10
7	1·22	1·19	0·91	0·95	0·90	1·09	3
Mean =	0·79	0·81	0·80	0·94	0·73*	*0·96	7
±	±	±	±	±	±	±	±
SE =	0·09	0·06	0·06	0·04	0·04	0·04	0·9

* Indicates significant ($P < 0{\cdot}01$) difference in mean values.

post-lepromin suppression, but the difference was not significant. However, the MLW responses were suppressed (i.e. the Migratory Indices were clearly elevated) in all subjects except one after lepromin testing, the mean figures showing a significant difference ($P < 0{\cdot}01$). When MLS and MLW results were pooled there was still suppression which was significant at $P < 0{\cdot}01$ level.

There was no correlation between the amount of suppression detected and the size of lepromin reaction.

Discussion

This preliminary study differs in 2 major ways from others which have demonstrated antigen specific immune suppression in man. Firstly, suppression was induced *in vivo*, suggesting that the previous studies were not simply detecting an *in vitro* artefact. Secondly, a different detection system, the LMIT, was used. This technically simple test measures lymphokine production, but can be much influenced by other effects. However, measurements of lymphokine production may be potentially more specific indicators of immune responses than are tests involving lymphoproliferation such as the LTT. It has been reported[5] that, compared with the LTT, the LMIT showed less cross-reactivity between leprosy and tuberculosis infections. In the present study the more marked MLW responses suggest that our subjects responded preferentially to surface antigens of the *M. leprae* preparations they received; but direct lymphokine assays will be needed to prove such discrimination between different antigens of *M. leprae*. The stability of the PHA responses indicates that the altered responses to *M. leprae* were specific and induced by the lepromin injection.

This study has demonstrated antigen specific immune suppression in subjects probably immunized against *M. leprae* by exposure, supporting the view of Stoner *et al.*[11] that such a phenomenon might be part of a normal, native protective response against infection. The possibility that this phenomenon could be used to indicate the presence of protective immunity deserves further exploration, particularly as it might be applied as part of the short term evaluation of potential anti-leprosy vaccines. In view of the demonstrated defect in antigen-specific lymphokine production by lepromatous leprosy patients,[2,7] tests that measure lymphokine production may be more suitable than the standard LTT for this purpose.

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Effect of thalidomide on induction of antibody synthesis in mice to the T-independent antigen, DNP-Ficoll

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Summary Thalidomide enhances *de novo* IgM antibody synthesis in mice to DNP-Ficoll. The immunocompetent cells responsive to thymic independent antigens like DNP-Ficoll are macrophages and the B lymphocytes. Enhancement of immunoresponsiveness to DNP-Ficoll seems to be due to augmentation of macrophage function by thalidomide.

Introduction

Since 1965, thalidomide has been used in alleviating the signs and symptoms of a major medical complication of lepromatous leprosy, erythema nodosum leprosum (ENL).[1] Histologically, the hallmarks of ENL lesions are vasculitis or vascular necrosis, oedema and inflammation with infiltrates of neutrophils affecting the entire dermis and subcutaneous fat.[2] The factor or factors that trigger the influx of neutrophils is unknown. For thalidomide to be effective in ENL, it must interfere with one or more of the essential steps in the pathogenesis of this syndrome.

Since it has been suggested that ENL is an immune complex mediated disease,[3, 4] we have investigated the effect of thalidomide on humoral immunological responses. Thalidomide significantly inhibits an IgM but not an IgG response in mice to the T-cell-dependent antigen, sheep erythrocytes; it also selectively decreases serum IgM concentrations among leprosy patients being treated for their ENL.[5] These observations have prompted us to speculate that a clinically relevant site of action for thalidomide in ENL is on the synthesis of IgM antibody. If thalidomide acts on immunocompetent cells, the cell target of the drug must be among the macrophage, IgM antibody forming B cell and helper or suppressor lymphocytes.

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To exclude T cell interaction and to determine the effect of thalidomide on cell interactions of the macrophage and B cell type, we investigated the effect of thalidomide on IgM responsiveness of mice to DNP-Ficoll. B cell responsiveness to this nitrophenol conjugated polysaccharide is not dependent on T cell help but has been shown to require a phagocytic accessory and B cell interaction.[6] The accessory cells are adherent, phagocytic and must be viable.

Materials and methods

Animals. Locally bred female Swiss–Webster mice 8–12 weeks of age were used. Thalidomide (Grunenthal GMBH, 5190 Stolberg/RhLd., Federal Republic of Germany) was incorporated in a 0·03% w/w basis in powdered Rodent Lab Chow (Ralston Purina Co., St Louis, MO).

DNP^{40}-AECM-Ficoll (Biosearch, San Rafael, CA) was resuspended in sterile saline and injected intravenously into mice. Picrylsulphonic acid, 2,4,6-trinitrobenzinesulphonic acid (TNBS) was purchased from Sigma Chemical Co., St Louis, MO, and conjugated to sheep erythrocytes (SRBC) (Microbiological Associates, Bethesda, MD) to produce trinitrophenol (TNP) substituted cells as described in an earlier study.[7]

Enumeration of plaque-forming cells (PFC). TNP substituted sheep erythrocytes were plated with mouse spleen cells as described previously, for enumeration of SRBC-plaque-forming cells (PFC).[5] Plaques were corrected for background PFC to SRBC and the contribution of myeloperoxidase positive cells (monocytes-macrophages). Results are expressed as PFC/million peroxidase negative cells. Statistical analysis was done using a Student's *t*-test.

Results

A preliminary experiment was to determine the dose of DNP^{40}-Ficoll in mice which would result in 50% of the maximum PFC response to TNP substituted SRBC. Classically at the median effective dose (ED_{50}), a dose response curve is steepest, and this is therefore the area at which inhibition or enhancing phenomena resulting from a drug treatment would be most readily demonstrable. The ED_{50} for DNP^{40}-Ficoll induction of a 4 day direct PFC response was interpolated to be $10^{3·5}$ ng (Figure 1). To assess the effect of thalidomide on the responsiveness of mice to DNP^{40}-Ficoll, mice were fed thalidomide at 0·03% w/w in powdered rodent chow for 7 days. Control mice received no thalidomide. (Thalidomide fed at this concentration for 3 days achieves a mean blood level of intact drug in mice of 0·84 μg/ml[5], and this is essentially equivalent to that of 0·9 μg/ml achieved in humans following 100 mg oral doses.)[8] After 7 days, both groups of mice were injected with an ED_{50} dose of $10^{3·5}$ ng of DNP^{40}-Ficoll. Four

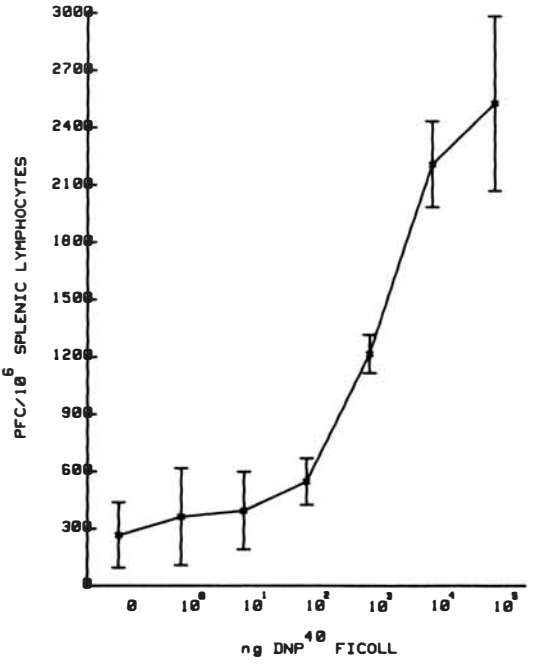

Figure 1. Four day direct PFC (mean ± SD) of 4 mice per group to DNP^{40}-Ficoll.

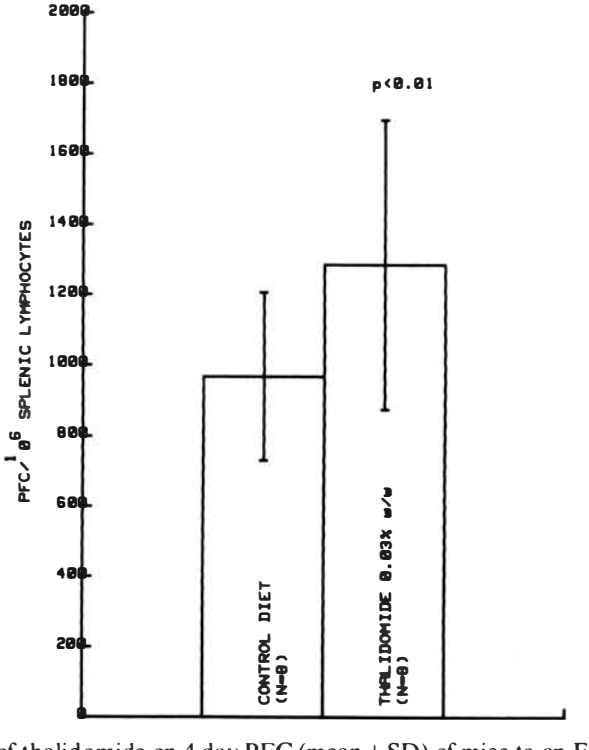

Figure 2. The effect of thalidomide on 4 day PFC (mean ± SD) of mice to an ED_{50} dose ($10^{3.5}$ ng) of DNP^{40}-Ficoll.

days later, spleens from both groups of mice were enumerated for their PFC to TNP substituted SRBC. As may be seen in Figure 2, thalidomide enhanced the PFC responsiveness of mice to DNP^{40}-Ficoll.

Discussion

The pathogenesis of ENL may be viewed as an immunological puzzle. If the mechanism of action of thalidomide in inhibiting immunological and inflammatory events were known, it would facilitate an understanding of the immunopathology of ENL. We,[5] and others,[9] have shown thalidomide to be effective in suppressing a primary humoral response to sheep erythrocytes. The development of optimal antibody responses to T-cell-dependent antigens like SRBC requires the participation of and interactions among at least 3 distinct types of cells in the immune response: macrophages; thymus-derived cells (T cells); and precursors of antibody-producing cells (B cells).

Macrophages have the critical function of presenting antigen in a highly immunogenic form to T and B cells to initiate the immune response.[10] As tested by reticuloendothelial system clearance of colloidal carbon in mice, thalidomide does not decrease macrophage phagocytic capability.[5] On the contrary, a significantly enhanced phagocytic index over controls was observed in animals fed 0·03% w/w thalidomide for 7 days. In this study, mice were fed thalidomide under similar conditions of time and dose. The immunoreactive cells in focus using the T-independent antigen DNP^{40}-Ficoll, are the macrophage and the B cell. Since immunoresponsiveness was enhanced, the function of either the macrophage or B cell (or both) was enhanced by thalidomide. In view of thalidomide's increasing RES clearance of colloidal carbon in mice, and its reported enhancement of monocyte function in a lepromatous patient,[11] an augmentation of macrophage function by thalidomide could be an explanation for the enhanced PFC response to DNP^{40}-Ficoll and the reduced PFC responses to SRBC. Thalidomide has been shown to stabilize the membranes of human and rat liver lysosomes.[12] By stabilizing membranes, the drug could retard the release of antigens from phagolysosomes. Retention of antigen within phagolysosomes could result in enhanced enzymatic degradation. Antigens, especially proteins on SRBC, could be degraded to less immunogenic moieties. Polysaccharide carrier conjugates like DNP^{40}-Ficoll which are more difficult to degrade could be more efficiently processed and presented to B cells.

The enhanced IgM antibody responsiveness to DNP^{40}-Ficoll would seem to exclude the B lymphocytes as a target site for thalidomide's effect on the humoral immune responses. The target site for thalidomide among the macrophages and T lymphocytes remains to be elucidated. Thalidomide has been shown to decrease T helper cells in the peripheral blood of healthy males.[13] Thus thalidomide could inhibit IgM antibody responses to T helper cell dependent antigens like SRBC or possibly *M. leprae* by inhibiting T helper cell activity.

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Comparative studies in human and armadillo derived Mitsuda lepromin

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Summary This study is on 2 groups of leprosy patients using the Mitsuda lepromin test. The first group of 37 patients received 0·1 ml of both human (Lepromin-H) and Armadillo lepromin (Lepromin-A) in concentrations of 40 million bacilli per ml. The average readings for Lepromin-A and -H were 4·7 mm and 2·1 mm respectively. Using Nested analysis of variance, the Lepromin-A readings were found statistically significantly (2·2 times) higher than the Lepromin-H readings. The second group of 60 patients received 40 M bacilli per ml of Lepromin-A and 100 M bacilli per ml of Lepromin-H. The average readings for Lepromin-A and -H were 6·9 mm and 6·3 mm respectively. This study suggests that with the paucity in supply of Lepromin-H, Lepromin-A can be used as a good substitute in concentrations of 40 M bacilli per ml in comparison with the Lepromin-H using 100 M bacilli per ml.

Introduction

The Mitsuda lepromin reaction is an important indicator of the ability of the host to mount a cell mediated immune response to *Mycobacterium leprae*. The lepromin test was described by Hayashi[1] and Mitsuda.[2] The standard Mitsuda lepromin reaction is read after 3–4 weeks. A reading of more than 5 mm is taken as positive. The preparation of lepromin has been standardized by WHO.[3] Studies[4,5] have suggested that the lepromin test was one of the most useful indicators to determine the risk of relapse in patients treated with Dapsone monotherapy.

It is becoming increasingly difficult to obtain human lepromatous leprosy tissue to prepare Mitsuda lepromin. However, lepromin prepared from armadillo tissue is now becoming more easily available.[6] If lepromin is to be used as a criterion to determine the treatment and release from control of patients, then armadillo lepromin will have to be used. This study compares the Mitsuda lepromin reactions using armadillo (Lepromin-A, 40 M bacilli per ml) and human lepromin (Lepromin-H, 40 and 100 M bacilli per ml).

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Materials and methods

This present study is on 2 groups of leprosy patients of all classifications. The first group of 37 patients received both human and armadillo lepromin in concentrations of 40 M bacilli per ml. The second group of 60 patients were given 40 M bacilli per ml of Lepromin-A and 100 M bacilli per ml of Lepromin-H.

Three of the larger clinic sub-centres of the Schieffelin Leprosy Research and Training Centre in Karigiri, Gudiyatham Taluk were chosen for the study. This area has been previously described.[7] Patients of all classifications were randomly chosen for the study.

Human lepromin (Lepromin-H) injections of 0·1 ml and armadillo lepromin (Lepromin-A) were given intradermally. The injections were randomized to one or other arm by use of calculator generated random numbers. Each patient received both human and armadillo lepromin, either in the left or right arm. The lepromin readings were taken 4 weeks later by a minimum of 2, up to a maximum of 6 observers according to availability. The observers were all paramedical staff or medical officers belonging to the leprosy control unit who had had previous experience and training in the reading of the lepromin test.

Neither the patients nor the observers knew which arm had been given Lepromin-A or Lepromin-H. One arm was read at a time and the readings noted by the chief investigator or the senior paramedical worker who assisted in the study. The observers were not allowed to compare the reading of both arms before recording their results.

Lepromin-H containing 40 M bacilli per ml was obtained from the National Institute of Medical Research Laboratories, Mill Hill, London, UK. Lepromin-A of the same strength, 40 M bacilli per ml, was obtained from WHO Headquarters, Geneva. Lepromin-H of concentration 100 M bacilli per ml, was prepared at the Laboratories of the Schieffelin Leprosy Research and Training Centre, according to standard protocol suggested by the World Health Organization.[3]

Results

STUDY 1

The age, sex and type of leprosy in the 37 patients who received 0·1 ml of human and armadillo lepromin of the concentration 40 M bacilli per ml is given in Table 1. Their average lepromin readings according to age and type are given in Table 2. The average Mitsuda lepromin readings using the armadillo lepromin (Lepromin-A) was consistently higher than the human lepromin (Lepromin-H) readings (Table 2).

The results were analysed by the technique of Nested 2-way analysis of variance,[8] using the individual readings. There was no significant difference in

Table 1.

	Study 1*					Study 2†				
	0–14 years	15–24 years	25 years and above	Total		0–14 years	15–24 years	25 years and above	Total	
IND M/F	—	—	1/1	1/1	2	—	1/0	0/1	0/2	2
TT	2/3	1/1	4/3	7/7	14	3/3	4/5	5/7	12/15	27
BT	—	—	4/8	4/8	12	1/0	2/2	9/4	12/6	18
BL and LL	—	0/1	5/3	5/4	9	0/1	3/0	7/2	10/3	13
Total	2/3	1/2	14/15	17/20	37	4/4	10/7	21/14	24/26	60

* Distribution of the 37 patients in Study 1.

† Distribution of the 60 patients in Study 2.

Study 1: Lepromin-A and -H, 40 M bacilli per ml; Study 2: Lepromin-A 40 M and Lepromin-H 100 M bacilli per ml.

IND, Indeterminate; TT, Tuberculoid; BT, Borderline Tuberculoid, BL; LL, Borderline Lepromatous and Lepromatous; M/F, Number of males/females.

reading between the sexes or ages. The Lepromin-A readings however were statistically significantly higher ($P < 0{\cdot}001$) than the Lepromin-H readings. The overall average lepromin reading using Lepromin-A was 4·7 mm and Lepromin-H 2·1 mm.

STUDY 2

A similar method of analysis as for Study 1 was used. Table 1 gives the age, sex and type of leprosy in the 60 patients who received 0·1 ml of Lepromin-H of concentration 100 M bacilli per ml and 0·1 ml of Lepromin-A, concentration of 40 M bacilli per ml. The average lepromin readings of the 60 patients, by age and type are given in Table 2. This table shows that the lepromin readings using Lepromin-A and -H were very similar. There was no significant difference in the readings when the ages and sexes were compared. When the lepromin readings using Lepromin-A and -H were analysed as in Study 1, there was no significant difference in the readings. The overall average Mitsuda lepromin reading using Lepromin-A was 6·9 mm and the average lepromin reading using Lepromin-H was 6·3 mm.

Discussion

One study[9] using similar concentrations of Lepromin-A (175 M bacilli per ml) and Lepromin-H (160 M bacilli per ml), reported an exaggerated response in

Table 2. Average lepromin reading by age and type (mm)

Study	Lepromin	Type	0–14 years			15–24 years			25 years and above			Total		
			No. of patients	*A	*H	No. of patients	*A	*H	No. of patients	*A	*H	No. of patients	*A	*H
1	A and H 40 M bacilli per ml.	IND	—	—	—	—	—	—	2	6·8	3·5	2	6·8	3·5
		TT	5	5·7	3·1	2	7·5	3·9	7	7·6	2·9	14	6·9	3·1
		BT	—	—	—	—	—	—	11	4·9	2·1	11	4·9	2·1
		BL+LL	—	—	—	1	0·0	0·0	9	1·0	0·4	10	0·9	0·3
	Total No.		5			3			29			37	4·7	2·1†
2	A 40 M bacilli per ml. H 100 M bacilli per ml.	IND	—	—	—	1	5·8	5·8	1	4·7	7·3	2	5·3	6·6
		TT	6	8·6	7·3	9	10·8	10·7	12	9·7	8·4	27	9·8	8·9
		BT	1	8·4	5·6	4	7·3	6·5	13	6·4	5·7	18	6·3	5·8
		BL+LL	1	0·4	0·0	3	0·0	0·7	9	2·5	1·9	13	1·8	1·5
	Total No.		8			17			35			60	6·9	6·3†

IND, Indeterminate; TT, Tuberculoid; BT, Borderline Tuberculoid; BL+LL, Borderline Lepromatous and Lepromatous.
* Average lepromin readings (mm); † Overall average of readings.

patients receiving Lepromin-A. Using the same concentration of Lepromin-A and -H (160 M bacilli per ml) another study[10] also obtained an exaggerated result in individuals receiving Lepromin-A.

Three double blind trials[11] were carried out to determine the late reactivity of 103 leprosy patients and unaffected persons to different concentrations of human and armadillo lepromin. They suggested that a concentration of armadillo lepromin of 1 M bacilli per ml would be best for routine work in comparison with the human lepromin. A study[6] reviewing the work of comparative studies of human and armadillo lepromin, states that several workers have shown concordance of Mitsuda reaction evoked by Lepromin-A and Lepromin-H.

A study[12] of 41 leprosy patients using Lepromin-H in concentration of 80 M bacilli per ml and Lepromin-A 40 M bacilli per ml, found that the Mitsuda lepromin reading was significantly higher using Lepromin-H.

In the present study the Mitsuda lepromin readings using 40 M bacilli per ml of Lepromin-A and -H, were significantly different. The average reading of Lepromin-H and -A were 4·7 and 2·1, respectively. However, the lepromin readings using Lepromin-A, 40 M bacilli ml and Lepromin-H 100 M bacilli per ml were similar (6·90 versus 6·3 mm), and not statistically different.

This study suggests that for obtaining comparative readings in human and armadillo lepromin, a concentration of 40 M bacilli per ml, of Lepromin-A compares favourably with 100 M bacilli per ml of Lepromin-H. Thus, for routine use of the lepromin test, the above strengths are recommended.

Previous studies at Karigiri[4] indicated that lepromin reaction is one of the most important criteria for determining the risk of relapse in paucibacillary leprosy. It was also suggested that lepromin positivity could be used as a criterion to determine chemotherapeutic regimens. The important constraint however was the unavailability of Lepromin-H. This study provides rationale for the possible substitution of Lepromin-A in the concentration of 40 M bacilli per ml, instead of Lepromin-H concentration 100 M bacilli per ml. Field studies are planned to evaluate this possibility.

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Diagnostic efficiency of paramedical workers in leprosy

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Summary The diagnostic efficiency of 9 paramedical workers trained in leprosy was assessed with regard to the misdiagnoses and wrong diagnoses made by them during their involvement in a recent leprosy case detection (survey) programme. The workers missed (misdiagnosed) 10·5 leprosy cases per thousand persons examined by them during the survey. Of the 316 new cases detected by workers, 55 (17·4%) were wrongly diagnosed as leprosy, mostly non-lepromatous (N) type. Amongst the correctly diagnosed cases, 98% N-type cases were correctly classified by them; 3 out of 9 (33%) borderline-tuberculoid (BT) cases were over-diagnosed as borderline lepromatous (BL) type. The clinical activity status of 39 (16·3%) out of 240 leprosy cases, all N-type, was either over-assessed as active (11·7%) or under-assessed as inactive (4·6%).

The implications, and suggestions to improve the technical skills of workers to achieve optimal efficiency in their work, are discussed.

Introduction

Since leprosy case detection depends on the diagnostic efficiency of paramedical workers (PMWs) employed in the programme, their training and technical skills should be kept up-to-date for effective leprosy control. One study[1] has assessed the diagnostic efficiency of trained PMWs engaged in the 7th total population survey (Jan 1981–June 1982) in a part of Chingleput District, of Tamil Nadu, India, and extended suggestions to improve their diagnostic efficiency. This operational research project was carried over to the next population survey, ie the 8th total (July 1982–July 1983), of the same area, to study: 1, the quantum of leprosy patients being missed (misdiagnosis) by PMWs during the survey; and 2, their efficiency in diagnosis and classification of leprosy cases newly detected by them during the survey.

Materials and methods

Nine trained and experienced (5–20 years) PMWs examined about 94% of 85,000

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persons during the total population survey (July 1982–July 1983) of 52 villages in Chingleput District. Most of these villages are thickly populated and well connected with roads. During the last 7 surveys of this area by these workers, a very good rapport has been established with the community.

A total of 630 new cases were recorded during the 8th survey period of that area. Of these 630 new cases, only 316 untreated patients could be re-examined and confirmed by an experienced medical officer within 3–4 months of their detection by workers. The remaining 314 cases were not included in the present study on account of 1, their non-availability for confirmation (69); 2, diagnosis by another medical officer in a mobile treatment unit on their voluntary reporting (30); and 3, inclusion of cases in a chemotherapeutic trial after confirmation by other doctors (215). Presuming the diagnosis of the Medical Officer to be correct, the diagnostic efficiency of PMWs was thus assessed on those 316 cases newly detected by them and confirmed by the same Medical Officer.

For estimating the number of leprosy cases being missed (misdiagnosed) by workers during the survey, all the persons reported free from leprosy (healthy) by 9 PMWs during 13 days' (randomly selected) survey of their respective areas on different dates were re-examined within 2–3 days by an experienced medical officer (MO). Of the total 879 persons examined by PWMs (67 persons/PMW/day), 809 were reported to be free from leprosy (healthy). Out of these 809 healthy persons, only 667 could be re-examined by the medical officer as others were not available. The missing case rate (MCR) was thus estimated on these 667 persons with the help of the following formula:

$$\text{MCR} = \frac{\text{No. of leprosy cases detected by medical officer}}{\text{No. of persons reported free from leprosy by PMWs and re-examined by medical officer}} \times 100$$

Results

MISDIAGNOSIS OF LEPROSY BY PMWS

On re-examination of 667 persons reported free from leprosy by PMWs, the Medical Officer on clinical evidence diagnosed (detected) 7 active cases of leprosy (Table 1) thereby indicating a MCR of 1·05%, i.e. 10·5 cases missed per thousand persons examined by workers. It could be said that these 7 cases were overlooked by workers during the survey.

PMWS' EFFICIENCY IN DIAGNOSIS AND CLASSIFICATION OF LEPROSY

Of the total of 316 new cases detected by PMWs, 55 (17·4%) were wrongly diagnosed as leprosy, mostly non-lepromatous (N) type (Table 2). Wrong diagnosis was most common in children. All these 55 wrongly diagnosed cases,

Table 1. Leprosy cases misdiagnosed by PMWs

Sl. No.	Age (yr) and sex	Type of leprosy	Site of lesion	Remarks
1	37 Male	PN	LP nerve (left)	(i) All cases of pure neuritic leprosy had mild to moderate thickened and tender LP nerves with below knee area of anaesthesia, for duration of 01–03 years.
2	20 Male	PN	LP nerve (left) and left post. and ant. tibial nerves	
3	18 Male	PN	LP nerve (right)	
4	39 Male	I	Scapular region (left)	(ii) All cases of indeterminate leprosy showed impairment of fine touch and pinprick pain sensations on their single patch (1–1·5 cm diameter) of 4–5 months duration.
5	32 Female	I	Right lumbar region (back)	
6	24 Female	I	Right cheek	
7	3 Male	I	Left lat. abdomen	

PN, pure neuritic; I, early indeterminate; LP, lateral popliteal nerve

Table 2. PMWs' efficiency in leprosy diagnosis

Diagnosis by PMWs	N	N?L	Total
Correct diagnosis	246 (80·39%)	9 (90·00%)	255 (80·70%)
Wrong diagnosis as leprosy	54 (17·65%)	1 (10·00%)	55 (17·40%)
Suspected by MO as having leprosy	6 (1·96%)	—	6 (1·90%)
Total	306	10	316

Note. All the above N?L cases were borderline-tuberculoid (BT) type, and there was no case of lepromatous (L) type.

mostly children (> 60%), had fungal, malnutritional and skin lesions other than leprosy.

Of the 246 non-lepromatous cases correctly diagnosed by workers, 240 (97·6%) were also correctly classified; however, 3 out of 9 (33·3%) N?L (BT) cases were over-diagnosed as borderline-lepromatous (BL) type (Table 3(a)). The bacteriological examination by slit skin smear technique was carried out on all the N?L cases on the day of their detection by the workers themselves. All these cases showed bacterial negativity, which was not made known to the Medical Officer who re-examined these cases.

Although the activity status of all N?L type lesions was assessed correctly by

Table 3(a). PMWs' efficiency in classification (types) of leprosy lesions

Classification by PMWs		N	N?L	Total
	Correct	240 (97·56%)	6 (66·67%)	246 (96·47%)
Wrong*	Over-diagnosis	—	3 (33·33%)	3 (1·18%)
	Under-diagnosis	6 (2·44%)	—	6 (2·35%)
	Total	246	9	255

* Over-diagnosis, mild type being classified as severe type. Under-diagnosis, severe type being classified as mild type.

Table 3(b). PMWs' efficiency in classification (activity status) of leprosy lesions

Activity status by PMWs		N	N?L	Total
	Correct	197 (83·47%)	04 (100%)	201 (83·75%)
Wrong*	Over-assessment	28 (11·86%)	—	28 (11·67%)
	Under-assessment	11 (4·66%)	—	11 (04·58%)
Total		236	04	240

Note. Since no information on activity status was provided by PMWs on 10 N and 5 N?L (total 15 cases), they were excluded.

* Over-assessment, inactive lesions assessed as active, and under-assessment, active lesions assessed as inactive.

PMWs, 39 out of 236 (16·52%) N-type lesions were either over-assessed as active (11·86%) or under-assessed as inactive (4·66%) (Table 3(b)).

The above cross-sectional observations are almost similar to our experiences reported earlier.[1]

Discussion

The misdiagnosis of leprosy was most common in young adult males, perhaps due to hurry and lack of interest and motivation amongst both the PMWs and persons (patients) resulting in improper clinical examination by the worker(s) ignoring standard methods and criteria. On the other hand, the wrong diagnosis was more frequent in children, possibly due to the workers' insufficient knowledge, training and skills to differentiate other common skin lesions from early leprosy. The extra-leprosy-consciousness while working in a high endemic area could also lead to wrong/over diagnosis by workers, as experienced earlier,[1] that out of the total of 215 persons suspected of having leprosy by workers, only 75 (35%) were found to be suffering from leprosy and the remainder had no evidence of disease when re-examined by a medical officer. Moreover, the inadequate qualitative supervision and assessment of PMWs' work could adversely influence their efficiency. It is needless to point out that poor diagnostic efficiency of workers has considerable implications on patients for their treatment, the community for transmission of disease, and on the leprosy programme as such.

As emphasized in another study[1] the diagnostic efficiency of workers can be appreciably improved by constant good quality of training, supervision, and evaluation of their work by motivated and experienced medical officers. It is felt necessary that the workers should be equipped with a suitably designed *manual* highlighting the policy and operational methodology guidelines to carry out their work uniformly, efficiently and effectively. In order to quantify the misdiagnosis and wrong diagnosis of leprosy by workers, about 5–10% of the surveyed population should be regularly counter-checked by the non-medical supervisors concerned; and also by the medical officer(s) during their supervisory visits to the area. All the cases detected by the workers should be concurrently confirmed by experienced medical officers in their respective units, then only a list of the known cases should be prepared and reported to higher levels for meaningful planning and evaluation of the leprosy situation and control programme. The delayed case confirmation may slightly inflate the figure of wrong diagnosis by PMWs as a proportion of early leprosy lesions may self-heal in the course of time.

Another important relevant point which needs to be mentioned is that although the PMWs form the pillars of our National Leprosy Programme(s), their involvement in planning and decision making has been quite passive and opportunities for their better prospects while in jobs have been limited. In

addition to various administrative and social aspects, the above factors are to a large extent responsible for their reducing interest, motivation and efficiency in leprosy work. To look into various problems of workers and to gather and utilize their valuable field experiences for planning, as well as to allow them to benefit from each other's knowledge and experience; the active involvement of peripheral workers in local/state/national level workshops should be of paramount importance to improve the overall efficiency and effectiveness of the leprosy programme. Likewise to sustain their interest in leprosy work, their job prospects could be enhanced by opening at least 3 promotional avenues, i.e. Non-medical Supervisor, Assistant and Deputy Leprosy Control Unit Officers, with appropriate training before each promotion to the deserving candidates. Now is the time to consider these aspects when we are aiming to eradicate leprosy by the end of this century.

Acknowledgments

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Hypersensitivity reaction to dapsone. Four case reports

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Summary Hypersensitivity reaction to the most commonly used antileprosy drug, dapsone, is becoming an increasingly important problem in the field of leprosy treatment. This undesired reaction to dapsone is more common than was thought previously. In this article 4 cases seen at the Nonsombun Leprosy Hospital during the years 1982–83 are reported. The 4 leprosy patients (2 cases of BL, 1 case of BT and 1 case of TT) presented with cutaneous and systemic manifestations of hypersensitivity to dapsone. The diagnosis was confirmed by trial dose in 3 cases.

Introduction

Dapsone is still the drug of choice in treating leprosy in most countries. It is also being used in many other skin diseases. We have the impression that we are seeing hypersensitivity and other side effects to this drug more commonly than before.

Hypersensitivity is confined to the first 6 weeks of treatment. The skin manifestations of hypersensitivity reaction to dapsone are exanthematous eruptions, exfoliative dermatitis, toxic epidermal necrolysis and Stevens–Johnson syndrome (erythema multiforme bullosum). The systemic manifestations include fever, eosinophilia, mononucleosis, lymphadenopathy, hepatitis, etc. A fatal hypersensitivity reaction known as 'DDS Syndrome' was described by leprologists in the early years of its use.

Case Reports

No. 1. A 50-year-old female patient (weight 35–40 kg), with active BL leprosy, was registered in the OPD clinic in 1982 and was started on antileprosy drugs including dapsone (50 mg daily) clofazimine and rifampicin (according to the national regime). She came back in the fourth week with reddish itchy papular rashes which were more marked on the sun exposed areas, namely, the face, neck,

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upper chest and forearms. There were rashes on the trunk too. The patient complained of fever and weakness and there was associated redness of eyes and generalized lymphadenopathy. A few blisters and small ulcers were seen on the lower lip. The original leprosy lesions were obscured due to the rash.

She was admitted and all drugs were stopped temporarily. Only antihistamines and topical lotions were given. After 2 weeks when the skin was clean she was given a trial dose of dapsone 50 mg and kept under close clinical observation. Within 4 hours she complained of feeling 'hot' and developed itching and burning associated with erythematous papular rashes mainly on the face and upper extremities. The conjunctivae were congested too. All symptoms subsided following antihistamines. Now she is on alternative treatment for leprosy.

No. 2. A 24-year-old female patient (weight 52 kg) with active BL leprosy was started on treatment with all 3 drugs in the OPD clinic in 1983. After about a month she was brought back with jaundice and skin rashes. On examination the patient was quite weak, febrile and moderately jaundiced. There was generalized lymphadenopathy and conjunctivitis. The liver was enlarged and tender. The skin rashes were miliariform and mainly over the face, trunk and upper extremities, and associated with itching and burning. The lips were blistered. The leprosy lesions were not visible except the few papules and nodules.

The patient was admitted, all antileprosy drugs were stopped and she was treated with prednisolone and topical lotions only. After about 2 months she was fit for discharge and was sent out on clofazimine. She was not challenged with dapsone since it was thought very risky, considering the severity of her initial illness.

During the course in the hospital and while still on small doses of prednisolone, she had developed repeated rashes with itching mainly on her face and forearms whenever she went out in the sun.

No. 3. A 35-year-old male patient (weight −60 kg) presented at the OPD clinic in 1983 with generalized erythroderma and exfoliative dermatitis. On further questioning he showed a packet of dapsone 100 mg tablets which he had received from a local health centre and had been taking for over one month. He had also brought supplies of antihistamines as well as prednisolone tablets which had been given by 3 different private doctors over the past 3 weeks. But none of them had instructed him to stop dapsone—probably he did not volunteer to tell them that he was taking dapsone. It was stopped on admission here.

The patient complained of itching and burning all over the body, as well as fever and weakness. On examination he had generalized lymphadenopathy. The leprosy patches were not clearly visible but there were fairly large islands of normal looking skin on both legs. The patient claimed that those were the sites of hypopigmented patches earlier, for which he was given dapsone by the health centre. The islands were dry and anaesthetic, not itchy and there was no redness or exfoliation on them. One lateral poplitial nerve was thickened. He was clinically classified as BT leprosy.

The dermatitis cleared in 6 weeks on treatment with prednisolone and topical applications. He too developed mild rash on face and forearms when exposed to the sun after prednisolone was stopped, but required only antihistamines. Later when all lesions were cleared he was given a trial dose of dapsone 50 mg. After about 2 hours he complained of feeling feverish and burning over the face. This was soon followed by erythema, itching and papular rashes over face, trunk and upper extremities. The face was slightly puffy and the eyes were congested. All symptoms subsided with antihistamines. He was discharged after another week with no specific treatment, but with the instruction to return after 2 months for re-examination for activity of leprosy and alternative treatment if needed. But he never returned.

No. 4. A 45-year-old female patient (weight 59 kg) was brought to the OPD in 1983 with severe exfoliative dermatitis following 3 weeks of dapsone 100 mg daily, given at the local health centre. The face was oedematous and the lips were blistered and ulcerated. There were ulcers even on the oral mucosa. The patient was febrile and had generalized lymphadenopathy and conjunctivitis.

The patient was admitted, dapsone was stopped and the dermatitis was treated with prednisolone and topical applications. After about 6 weeks when all lesions had cleared and when she was off prednisolone she was given a trial dose of dapsone 50 mg under close clinical observation. Within 3–4 hours she developed symptoms such as fever, itching and burning over the face with exanthematous rashes on the face, trunk and upper extremities. Symptoms subsided with antihistamines. Later she was examined for leprosy. There was only a small area of anaesthesia on the right elbow, with no nerve thickening. The patient said that

Table 1. Summary of the main manifestations in the 4 patients

Cases	Cutaneous manifestations	Systemic manifestations
No. 1 F 50 yr BL	Itching, burning, exanthematous rash	Fever, weakness, lymphadenopathy, congestion of eyes, ulcers and blisters on lip
No. 2 F 24 yr BL	Itching, burning, exanthematous rash, recurrence of rash on exposure to sun	Fever, weakness, conjunctivitis, hepatitis, lymphadenopathy
No. 3 M 35 yr BT	Itching, burning, erythroderma, exfoliation, recurrence of rash on exposure to sun, leprosy lesions free of involvement	Fever, weakness, lymphadenopathy
No. 4 F 45 yr TT	Itching, burning, exfoliative dermatitis	Fever, weakness, swelling of face, lymphadenopathy, conjunctivitis, ulcers of mouth

she had a patch over the same site. So she was classified as a case of TT leprosy, the activity of which was to be decided later. She was not given any treatment on discharge, but was instructed to come back after 2 months for re-assessment. She did not return in 1983, but presented again in July, 1984 with severe exfoliative dermatitis and a packet of dapsone 50 mg tablets! The tablets were forced on her by the local health worker when he found out that she was not taking dapsone. She admitted taking just a single tablet, after which she developed the dermatitis and reported here.

In all these 4 patients dapsone hypersensitivity was clinically diagnosed and this was supported by the fact that symptoms subsided when dapsone was stopped and all 4 were treated with prednisolone/antihistamines. In the third patient, though he was treated with prednisolone and antihistamines along with dapsone, his symptoms and signs did not improve until the offending drug, dapsone, was stopped. In all but one patient the diagnosis was confirmed by reproducing the signs and symptoms when a single trial dose was given. No significant mental disturbance occurred in any of the 4 patients. Summary of the main manifestations in the 4 patients is shown in Table 1.

Discussion

The cutaneous and systemic manifestations presented by all 4 patients were compatible with those of dapsone hypersensitivity[10] as well as the DADPS Syndrome described earlier.[2] All patients fulfilled most of the criteria for hypersensitivity reaction, namely the symptoms occurred within 6 weeks of starting dapsone, and the manifestations included most of the following: fever, lymphadenopathy, hepatitis and exanthematous skin rash.[4] Two patients had severe skin manifestation in the form of exfoliative dermatitis. Due to lack of laboratory facilities a complete blood count was not done to look for eosinophilia or mononucleosis. The sequence of syndrome usually is dermatitis, hepatitis, lymphadenopathy and mononucleosis of which dermatitis is always present though the others may or may not be present.[2] Such cases may even prove fatal if not diagnosed and treated in time and/or manifest in the form of more severe dermatological emergencies such as toxic epidermal necrolysis[5] and Stevens–Johnson syndrome (erythema multiforme bullosum).[1]

Such cutaneous and systemic complications of dapsone in the field of leprosy had been encountered by leprologists even earlier.[7, 8] The author had come across similar cases while a medical officer at the Schieffelin Leprosy Research and Training Centre, Karigiri, India.[9] But in some of those cases the manifestations were more of an allergic nature (symptoms appearing even after a single dose) rather than hypersensitivity.

Hypersensitivity is not dose related[10] as in the case of toxicity and therefore giving the drug in gradually increasing small doses may not be of help.[3] In a

previously sensitized person even a small dose may be dangerous. With the availability of alternative and effective antileprosy drugs, it is not necessary to desensitize these patients presently. Moreover, this practice may predispose to dapsone resistance. But it should be remembered that such patients with proved hypersensitivity should be given alternative treatment as severe hypersensitivity can be fatal.

One of the purposes of this paper is to point out that the BT leprosy lesions were not involved in the hypersensitivity manifestation. The lesions were free of symptoms or signs. A similar feature was noticed by the author in the patients in India, but to the author's knowledge such a feature has not been documented in earlier reports. The possible explanation is that in BT (and TT) lesions due to the complete damage of the sympathetic nerve fibres, there is absence of axon reflex which is responsible for the histamine response to the offending allergen, leading to the cutaneous manifestations seen. This fact is supported by the finding of no such feature in BL lesions where there is no complete damage to the nerve fibres in early stages.

The reason for 2 of the patients developing fresh skin rashes on exposure to sun when no fresh dose of the drug was taken could be that in highly sensitized subjects even small doses of the offending drug, when combined with ultraviolet light are sufficient to trigger the hypersensitive reaction. It is reasonable to assume that in previously involved and damaged skin there may be prolonged retention of small amounts of the photoallergic bacteriostatic agent.

It is important to remember that hypersensitivity reactions may not be as uncommon as was previously thought.[6] Dapsone is usually given under field conditions by paramedical workers and patients who receive dapsone alone do not get such close supervision as those on rifampicin or lamprene. Such patients, when they develop dermatitis, hepatitis, etc, are usually referred to the general hospitals and thus may not even come to the notice of leprologists. So the actual number of such cases could be even more than those so far documented.

Acknowledgments

I am grateful to all the above patients who have been very co-operative. Thanks are due to the director of the leprosarium for granting me permission to publish. Thanks are also due to the authorities of the doctors' library at Khon Kaen University Medical School for permitting me to use their materials for reference.

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Migration and proliferation of Schwann cells in adult human leprous nerve cultures

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Summary The migratory and proliferative activities of Schwann cells affected with leprosy were studied in explant cultures of leprous nerves maintained for 4 weeks *in vitro*. In these cultures, it was observed that Schwann cells harbouring *Mycobacterium leprae* failed to migrate from the explant, attach to the culture surface and proliferate. These cells, therefore, were either sloughed off or still localized to the explant region at the end of the culture period. Hence, no outgrowths of Schwann cells were obtained from highly bacilliferous lepromatous nerve cultures. This was a direct inhibitory effect of the intracellular organism on the host. There was no evidence of the effect being mediated through the release of any soluble product. Unparasitized Schwann cells, however, exhibited normal migration, attachment to culture surface and proliferation. Therefore, a good outgrowth of Schwann cells comparable to that from normal nerve was obtained from tuberculoid nerve cultures. Fewer Schwann cells migrated from the bacteriologically negative lepromatous nerve explant, which displayed a normal proliferative activity. From the borderline tuberculoid nerves, there was migration and proliferation only of unparasitized cells.

This study, thus, demonstrates that *M. leprae* inhibits migratory and proliferative activity of the host Schwann cells.

Introduction

Leprosy is primarily a disease of the peripheral nerve and its Schwann cells.[1,2] In the lepromatous form of the disease, the Schwann cells harbour *Mycobacterium leprae*.[3] While in the tuberculoid form, which shows infiltration by the immunocompetent cells, it does not harbour the causative organism.[1,4] This study was undertaken to observe the alteration, if any, in the functional status of the Schwann cells in these 2 forms of the disease.

Utilizing the *in vitro* nerve culture technique, the migratory and proliferative capacity of the leprosy affected Schwann cells were assessed, as these activities

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precede the interaction and association of Schwann cells with the axons during the process of regeneration and remyelination of the nerve.[5, 6]

Materials and methods

NERVE BIOPSIES

Leprosy patients undergoing routine nerve biopsy for diagnostic purposes were used in this study. A part of the biopsy was collected under sterile conditions in minimal essential medium containing crystalline penicillin (100 IU). Under the dissection microscope, the nerve was cleaned, the perineurium freely incised and the interfunicular connective tissue removed. Each funicle was then cut into 1 to 2 mm squares. Twenty explant cultures were set from each biopsy.

CULTURE TECHNIQUE

The funicular pieces were briefly trypsinized (0·25% in phosphate buffered saline) and explanted on glass coverslips coated with collagen by the method of Bornstein.[7] The coverslips were then transferred to sterile plastic Petri dishes (Falcon, USA), and fed with a growth medium consisting of 75% Dulbecco's modified essential medium, 20% foetal calf serum (Gibco) and 5% chick embryo extract (50% EE), 600 mg% glucose and antibiotics made up of 100 iu of crystalline penicillin, 200 μg streptomycin and 50 iu of mycostatin. Cultures were incubated at 36°C in 100% humidity. On the third day, cytosine arabinoside at a concentration of 10^{-5} M was added to these cultures for 48 h to inhibit the excessive growth of fibroblasts,[8] after which the growth medium was renewed twice a week.

MIGRATION AND ATTACHMENT

Cultures were viewed regularly under the phase contrast optics of an inverted microscope (IM 35 Carl Zeiss). Observations were made on the migration from the explant and the attachment to the culture surface of the outgrowing cells. The drained growth medium was collected and checked for the sloughing of the cells by centrifuging the medium at 800 rev/min. In the event of a cell pellet, smears were prepared on the microscope slides, fixed in 3% formaldehyde for 15 min and stained using Ziehl–Neelsen's method.

PROLIFERATION

The proliferative capacity of the cultured cells was assessed by their ability to synthesize DNA. For this 1 μc/ml of ^{3}H-thymidine (specific activity 15,200 MC/

mmol) was added to the culture for 24 h. Then the coverslips were washed with balanced salt solution, fixed in acid–alcohol and coated with Ilford K5 emulsion diluted 1:1 in distilled water. The emulsion was air dried and the coverslips were stored at 4°C in light-proof boxes. After 7 days, the coverslips were developed, fixed and stained using Ziehl–Neelsen's method.

LIGHT MICROSCOPY

Cultures were fixed for Sudan black staining and light microscopic analysis in 3% glutaraldehyde in 0·1 M sodium phosphate buffer, pH 7·2, overnight at 4°C. They were rinsed, post-fixed in 2% osmium tetroxide in 0·1 M buffer pH 7·2 for 1 h at room temperature, rinsed several times, dehydrated to 70% ethanol and stained with 0·5% Sudan Black in 70% ethanol. Then, the cultures were rehydrated and mounted in glycerine jelly. Replicate cultures were stained for acid-fast organisms by the Ziehl–Neelsen method and for non-specific esterase according to the method described by Bancroft.[9] All the explant cultures were scanned under the light microscope for the type of the cells in the explant mass and in the culture outgrowth.

Results

Nerve biopsies were obtained from 12 patients from the radial cutaneous, sural or ulnar nerves. The patients were classified clinically, bacteriologically and histologically as tuberculoid, borderline and lepromatous according to the Ridley–Jopling classification.[10] Two normal nerve biopsies were taken from volunteers. Relevant clinical data of the biopsied nerves is presented in Table 1.

In both the normal and the leprous nerve explant cultures, 2 types of cells were identifiable, the Schwann cells and fibroblasts. The Schwann cells were identified under phase-contrast optics by their long spindle shape, denser cytoplasm and narrow elongated nucleus. These cells were positively stained with Sudan Black and non-specific esterase and exhibited slow proliferation. Fibroblasts were recognized by their broad flattened morphology, weak staining with Sudan Black and non-specific esterase and formation of underlying carpet layer to Schwann cells (Figure 1). The proportion of these 2 cell types varied depending upon the type of leprous nerve explant culture. The proportion of Schwann cells in the culture outgrowth of different types of leprous nerve explant cultures is depicted in Table 2 and their proliferative activity is compared in Table 3.

TUBERCULOID NERVE

Within 3–4 days of culture, the migration of cells from the explant became evident. The light microscopic picture of the outgrowth from a tuberculoid nerve

Table 1. Clinical details of patients from whom nerves were biopsied

Patient No.	Age	Sex	Diagnosis	Duration of leprosy (years)	Treatment (years)	Nerve
FMR 36/82	17	F	Normal	—	—	Digital nerve
FMR 15/83	13	M	Normal	—	—	Sural
FMR 11/83	18	F	Lepromatous +ve*	2	Untreated	Index branch radial cutaneous
FMR 2/83	25	F	Lepromatous +ve	12	2 months	Index branch radial cutaneous
FMR 20/83	35	F	Lepromatous +ve	7 months	Nil	Ulnar (dorsal) branch
FMR 12/81	35	M	Lepromatous −ve*	12	12	Ulnar
FMR 44/82	—	M	Lepromatous −ve	32	32	Index branch radial cutaneous
FMR 25/82	14	M	Borderline tuberculoid	1	1	Ulnar
FMR 30/82	19	M	Borderline tuberculoid	3	2	Index branch radial
FMR 4/83	14	M	Tuberculoid	1	6 months	Cutaneous branch ulnar
FMR 21/82	34	M	Tuberculoid	5	4	Radial cutaneous
FMR 16/83	21	M	Tuberculoid	1	1	Ulnar

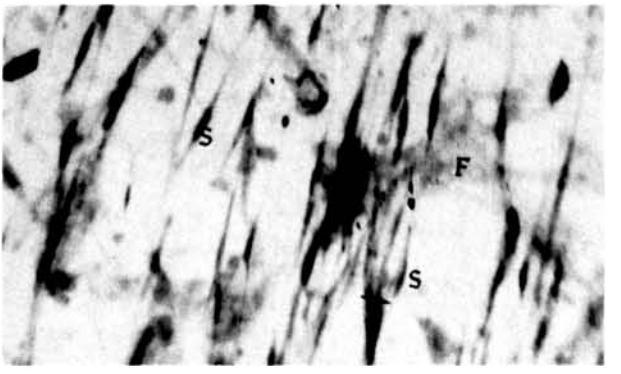
* Bacteriological status of the skin smear

Table 2. Outgrowth characteristics of leprous nerve explant cultures. Comparisons made between 20-day-old cultures

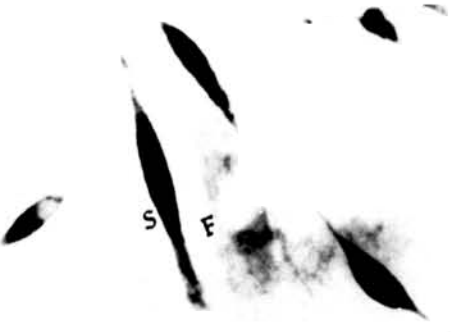
Nerve	Explant		Proportion of cell types in the outgrowth*	
	Number of cultures	Cellular outgrowth (%)	Schwann cells (%)	Fibroblasts (%)
Normal	40	85·0	89·3 ± 6·4	10·7 ± 2·1
Lepromatous −ve	40	75·0	21·3 ± 4·8	78·5 ± 10·2
Lepromatous +ve	160	0·0	—	—
Borderline tuberculoid†	60	80·0	53·1 ± 8·2	46·9 ± 9·4
Tuberculoid	120	87·5	82·0 ± 10·2	18·0 ± 3·2

* Results represent mean ± standard deviation. Cells were counted from several fields of each culture under ×63 oil immersion objective of standard WL microscope (Carl Zeiss).

† Outgrowth only of cells not harbouring bacilli.



(a)



(b)

Figure 1. Relationship of Schwann cells and fibroblasts in tuberculoid nerve explant culture. Schwann cells (S) lying over the fibroblasts (F): (a) Ziehl–Neelsen stain (× 1025); (b) Non-specific esterase (× 1200).

Table 3. Proportion of Schwann cells cultured from leprous nerves incorporated with ^{3}H-thymidine

Nerve	Schwann cells labelled (%)
Normal	49·2 ± 5·3
Lepromatous −ve	33·1 ± 4·2
Lepromatous +ve	—
Borderline tuberculoid	40·0 ± 3·2
Tuberculoid	48·2 ± 6·4

Results represent mean ± standard deviation of 10 cultures. Cells were counted from several fields of each culture under × 63 oil immersion objective.

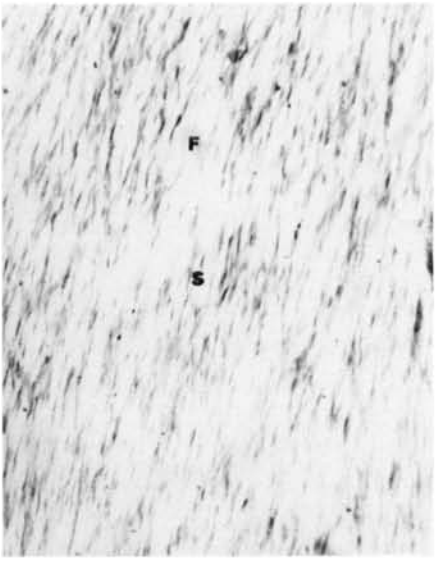


Figure 2. A representative light microscopic picture of a portion of tuberculoid nerve explant culture. Note large number of Schwann cells and a few fibroblasts. Sudan Black B stain (× 350).

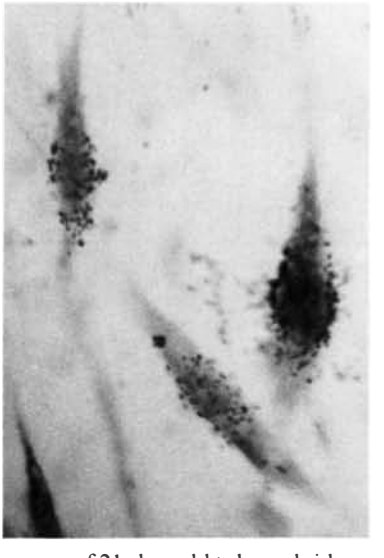


Figure 3. A portion of autoradiogram of 21-day-old tuberculoid nerve explant culture. Schwann cell nuclei are heavily labelled with ^{3}H-thymidine (× 1200).

explant is shown in Figure 2. The culture outgrowth is dense and is made primarily of Schwann cells. On quantitation, the Schwann cells formed 82%, while fibroblasts formed 18% of the outgrowth (Table 2). The rich outgrowth of Schwann cells in these cultures was due both to the good migration from explant as well as active proliferation of the Schwann cells *in vitro*. At the end of 3 weeks in culture, 48·2% of the Schwann cells were synthesizing DNA (Figure 3 and Table

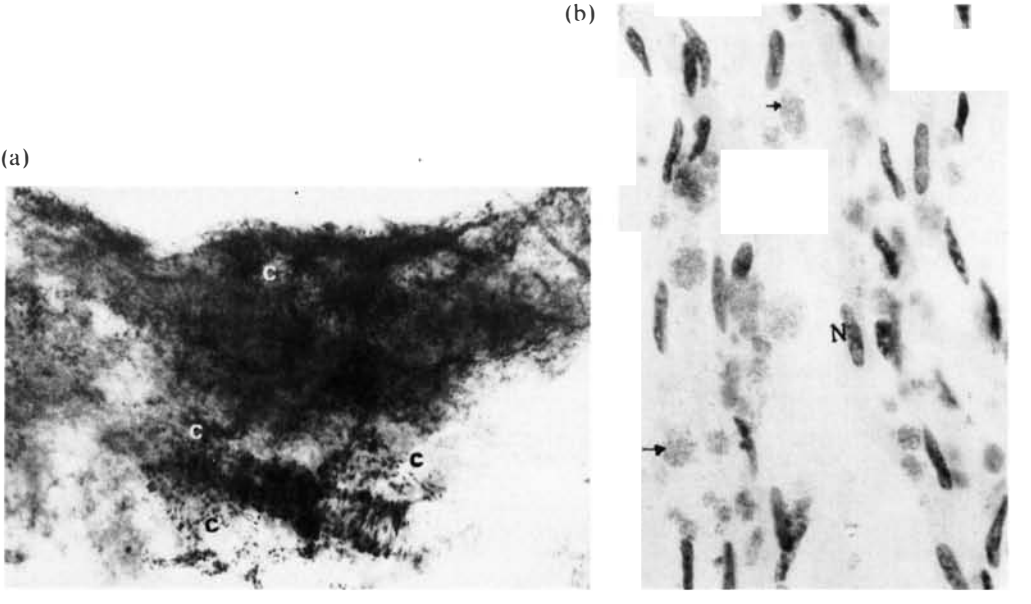


Figure 4(a). Bacteriologically positive lepromatous nerve explant containing cells (C). No evidence of cellular outgrowth from explant even after 3 weeks of incubation *in vitro* (× 52).

Figure 4(b). Autoradiogram of teased nerve fibres of bacteriologically positive lepromatous nerve maintained for 7 days *in vitro*. Note the intracellular bacilli (arrow) and non-incorporation of ^{3}H-thymidine by the cells. N = Nucleus (× 850).

3). The cellular outgrowth from the tuberculoid nerve explant was comparable to that from the normal human nerve explants with respect to density and proportion of cell types (Table 2).

BORDERLINE TUBERCULOID NERVE

Outgrowth from the borderline tuberculoid explants contained predominantly Schwann cells not harbouring *M. leprae* even though the explant region contained a mixed population of cells, both *M. leprae* harbouring and non-harbouring. Schwann cells formed 57% of the cellular outgrowth. These cells showed proliferative activity (Table 3).

BACTERIOLOGICALLY POSITIVE LEPROMATOUS NERVE

There was no migration of cells from the explant of highly bacilliferous nerve and hence no outgrowth of cells from these nerves, although the explant region contained viable looking cells loaded with *M. leprae* (Figure 4(a) and (b)). Some of these nerves were teased and maintained *in vitro*. The Schwann cells contained bacilli in clumps and globi, and did not incorporate ^{3}H-thymidine. Therefore in

these cultures there was no activity of migration, attachment and proliferation of the cells throughout the culture period. From time to time there was sloughing of the cells, which when smeared and stained using Ziehl–Neelsen's method, revealed cells to be loaded with acid-fast organisms. At times, there was evidence of attempted extension or migration of cells from the edge of the explant, which when followed, were found to be floating in the medium suggesting their inability to attach to the culture surface.

The lack of migratory and proliferative activity of *M. leprae*-harbouring Schwann cells was a direct effect of the organism on the host cells as the supernatant from the bacteriologically positive lepromatous nerve cultures had no effect on the outgrowth of cells from normal and bacteriologically negative lepromatous explant cultures, thus ruling out the possibility of the effect being mediated through any soluble product.

BACTERIOLOGICALLY NEGATIVE LEPROMATOUS NERVE

In these cultures, there was predominant outgrowth of fibroblasts, Schwann cells forming only 21·5% of the cellular outgrowth, a value much lower compared to that of normal or tuberculoid explant cultures. The low proportion of Schwann cells in these cultures was due to migration of fewer cells from the explant. A good proportion of migrated cells synthesized DNA (Table 3).

Discussion

The nerves of leprosy patients were cultured *in vitro* in order to study the migratory and proliferative function of Schwann cells in this disease. The results demonstrate that intracellular *M. leprae* inhibit the migratory and proliferative functions of the host Schwann cells without affecting the neighbouring unparasitized Schwann cells. Cells not harbouring bacilli exhibited normal migration, attachment to the culture surface and proliferation. In these cultures Schwann cells were identified by their characteristic bipolar spindle shape, a criterion also utilized by others.[11–13] The light microscopic picture of these leprous Schwann cells cultured in this manner were identical to that described in another study[11] in cultures of normal nerves. These observations demonstrate that the light microscopic morphology of unparasitized Schwann cells is not altered in this disease. These cells also stain with Sudan Black B and non-specific esterase more intensely than the fibroblast. The specific staining effect of Sudan Black B and non-specific esterase has also been reported.[12]

The proportion of Schwann cells in the outgrowth of the leprous nerve cultures was determined by the proportion of *M. leprae* not harbouring Schwann cells in the explant which in turn was determined by the types of leprosy the nerve was affected by. Therefore, maximum outgrowth of Schwann cells was observed

in the tuberculoid nerve explant culture. However, these results do not suggest that the Schwann cells are not affected in the tuberculoid form of the disease, but demonstrate that a large proportion of the Schwann cells present in the nerve in this form of the disease have normal proliferative and migratory activity.

The inhibitory effect of *M. leprae* on the proliferative and migratory activity of Schwann cells observed in this study supports our earlier observations on organized nerve culture in which we reported that the intracellular *M. leprae* inhibited the proliferative activity, as well as the alignment and association with the axons of host Schwann cells.[14, 15] The mechanism by which *M. leprae* alters the functional status of the parasitized host cell is not yet understood and is under study. These organisms are not toxic and the protein synthesis of the host is unaffected.[15] But physical occupation of the host cytoplasm by these organisms may alter the organization as well as the content of the cytoskeletal element, which determines the proliferative as well as the migratory activity of the cells in general.[16, 17]

Since there is extensive collagenization of the nerve in chronic lepromatous leprosy,[18] it could be postulated that the cells harbouring *M. leprae* could not migrate out of the explant because of entrapment by the collagen matrix. That this is not so was shown by the ability of the cells from the equally highly collagenized bacteriologically negative nerves[19] to migrate out of the explant. Further, in borderline tuberculoid leprosy where there was a fairly even distribution of cells containing *M. leprae* and those not containing these organisms, the ability of only the cells not containing *M. leprae* to migrate also confirms that this is a defect induced in the host cells by *M. leprae*.

The properties of migration, attachment and proliferation of Schwann cells in the peripheral nerve are important for the association and interaction with axons as well as for the organization of the nerve during development.[20, 21] In the adult this process operates during the regeneration of the nerve.[22, 23] Our observations indicate that the cells harbouring *M. leprae* may not be able to participate in such regenerative processes of the nerve. However, with prolonged chemotherapy, if the intracellular bacilli are cleared, these properties may be restored as observed in the bacteriologically negative lepromatous nerve explant cultures. Poor regeneration seen in the tuberculoid spectrum of the disease may be due to the degeneration of the axons as well, possibly induced by the infiltrating granuloma,[24, 25] since these Schwann cells *in vitro* display normal proliferation and migration.

This study also demonstrates that it is possible to cultivate Schwann cells *in vitro* from the leprous nerve, thus providing possibilities for the study of the cell at the membrane and molecular level in this disease.

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Plasma cells in caseous necrosis of nerves in leprosy

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Summary A large number of plasma cells were identified by direct immunoperoxidase staining in caseous nerve abscesses of all 14 borderline, borderline tuberculoid and polar tuberculoid leprosy patients. The specificity of the secreted antibody appeared to be directed against mycobacterial antigenic determinants. This hitherto unreported observation stresses the role of local humoral immune mechanisms in the pathogenesis of the tuberculoid form of leprosy.

Introduction

Caseous nerve abscesses in earlier reports[1] were mainly associated with the resistant tuberculoid form of leprosy, or primary neuritis; both groups being classically associated with active epithelioid cell granulomas surrounded by a cuff of lymphocytes and a marked absence of acid-fast bacilli. In such a state, humoral immunity was understood to play an insignificant role in the killing or expression of DTH reactions to *Mycobacterium leprae*, a belief having many adherents even today.

Since 1980, caseous nerve abscesses were observed by us in 14 leprosy patients exhibiting polar tuberculoid, borderline tuberculoid, or borderline form of the disease but with no clinical signs of lepra reactions. We report here on the general morphology and inflammatory cell pattern in caseated nerve abscesses, stressing particularly our consistent observation of a large number of plasma cells in all of the 14 patients exhibiting caseous nerve abscesses.

Methods

The treatment status of the 14 patients varied from untreated to a duration of 6 years. The nerves from which the biopsies were obtained included 6 ulnar nerves,

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2 index branches of the radial cutaneous nerves, 2 greater auricular and median nerves and 1 each of sural and intercostal nerves.

The biopsies were obtained during surgery for treatment and evacuation of abscess(es). A fine wedge-shaped biopsy was taken from the wall of the abscess before evacuation, fixed in Formol–Zenker and processed routinely for light microscopy. The sections were stained by the routine Trichrome Fite–Farraco (TRIFF) and Fite methods, the latter being imperative for visualization of acid-fast bacilli.

The presence of plasma cells was demonstrated by direct application of anti-human IgG or IgM (1:20) conjugated to peroxidase (DAKOPATTS). The reaction was developed using 3,3′diaminobenzidene tetrahydrochloride (SIGMA), with Harris' haematoxylin as a counter stain.

Attempts to demonstrate mycobacterial sub-cellular antigen fractions were made using anti-BCG (1:50, DAKOPATTS) in the peroxidase-anti peroxidase (PAP) system.[2]

A portion of the biopsy from 3 BT patients was teased and chopped. Both tissues as well as extracted cells were cultured in MEM for a period of 48 hr. The supernatants were collected and used for running against standard human immunoglobulin preparation (IgG + IgM + IgA, WHO) in a standard continuous buffer SDS–PAGE (concentration of separating gel 10%) to assure the presence of secreted antibody.

The supernatants were also applied in the troughs in a standard immunoelectrophoretic assay and run against homogenized nerve extracts of two normal Swiss white mouse sciatic nerves or *M. leprae* sonicates placed in the wells.

Observations

The abscesses were observed chiefly in the major nerve trunks but they also occurred in cutaneous nerves, lying within the nerve itself or breaking through the epineurium to form a collar-stud extension. The abscesses were visualized as a series of one or more nodular swellings in the nerve which on longitudinal epineurotomy were seen generally to lie in a linear fashion along one or two nerve fascicles.

A typical histological picture of the abscess wall is demonstrated in Figures 1 and 2. The lesions were generally restricted to one or two funicles that were grossly enlarged and showed dense deposits of collagen in the interfunicular region, in the perineurium and in the sub-perineurial endoneurial area. Except for the thickened perineurium, there was total destruction of the normal structure of the funicle.

The centre of the amorphous caseous mass consisted largely of degenerating pyknotic nuclei without a defined cell wall. Just immediate to the caseous material was a layer of large mononuclear cells of the epitheliod variety often fusing to

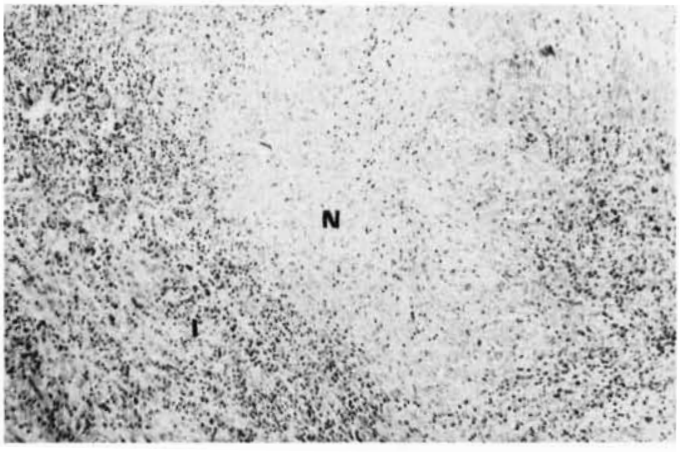

Figure 1. T.S. of greater auricular nerve of BT patient showing central necrotic mass (N) surrounded by a dense collar of inflammatory cells (I) (× 372).

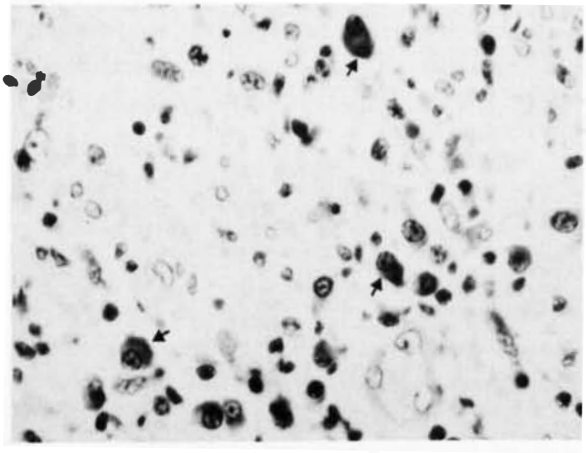

Figure 2. T.S. of greater auricular nerve of the same BT patient. A magnified view of inflammatory cell collar demonstrating abundance of plasma cells (→ .)These were identified by localization of peroxidase conjugated anti-human IgG/M. Counter stain Harris haematoxylin (× 948).

form giant cells. These cells seemed positive for collagenase activity. The inner wall of this intermediate layer appeared synonymous with the outer wall of the caseous mass and showed the transition of cells from the normal to the degenerated state.

Towards the wall of the caseous funicle, these cells were joined by a large number of plasma cells, mainly in clusters, generally restricted to the cellular layer but sometimes within the necrotic mass. The plasma cells extended as far as the perineurial cell layers in association with epitheliod cells and moderate numbers of lymphocytes. This sub-perineurial zone also displayed enhanced vascularity

and it was possible to observe migration of plasma cells and very occasionally polymorphonuclear cells through these blood vessels. Very few PMNs if any, were noted in the cellular layers. The perineurium was thickened and showed the presence of active perineurial cells. It was interrupted at places by epitheliod cells, plasma cells and moderate numbers of lymphocytes. No acid-fast bacilli could be demonstrated within or around the caseous mass.

The repeated presence of plasma cells was confirmed by their typical morphology during routine staining and by direct application of HRP conjugated anti-human IgG or IgM (Figure 2). Dense Ig staining was obtained intracellularly in and around the caseous mass. The plasma cells around the caseous mass were seen to secrete predominantly IgG and IgM. However, the caseous mass itself stained only for IgM, a feature consistent with caseous lymph nodes in tuberculosis (Ridley, personal communication).

Precipiting lines were consistently observed when these supernatants were run against sonicated *M. leprae* in an immunoelectrophoretic system. No reaction was noted against homogenized mouse sciatic nerve. This feature correlated with the finding of granular antigen deposit within epitheliod-like cells in the subperineurial zone in these nerves as detected by anti-BCG antibody via the PAP system. Therefore despite the striking absence of detectable integral acid-fast bacilli in these biopsies, the locally synthesized antibody appeared to be directed against bacterial antigens.

Discussion

The singular feature hitherto unreported is the repeated presence of a large number of Ig secreting plasma cells in the caseous necrotic mass. Such a lesion is not, as previously thought, restricted to polar tuberculoid leprosy, and can occur, as shown here in the borderline groups of patients also with no clinical symptoms of lepra reactions. The inflammatory cell pattern is not of the classical CMI granuloma, is more compatible with a borderline type of response and unlike the liquefied abscesses of ENL lesions is devoid of PMN's as well as acid fast bacilli.[3] The formation of such a lesion probably has its basis in a critical proportion of antigen, antibody and CMI.[4]

The specificity of these antibodies secreted in the caseous mass, like those synthesized in *M. leprae* infected skins,[5] are directed against bacterial determinants. Whether these antigens have been liberated because of an initial cell-mediated immune reaction remains speculative but there is some evidence to state that plasma cells could predispose to caseous necrosis, since in two patients, these cells were noted in large numbers in the precaseous stage.

We postulate on the basis of observations made in cutaneous leishmaniasis, that caseous necrosis may be associated with the killing (or clearance) of *M. leprae* at localized sites. Leishmania amastigotes were seen to be most rapidly

eliminated by focal necrosis as opposed to compact granulomatous formations that paradoxically enhanced bacterial growth.[6]

In summation this report underlines the strong association between local humoral immune mechanisms and the paucibacillary form of leprosy.

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A patient with nerve abscesses due to leprosy

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Summary A patient with borderline-tuberculoid leprosy who developed nerve abscesses along the line of cutaneous nerves is described. The treatment of choice is surgical removal of the abscess together with combination chemotherapy.

Introduction

A nerve abscess is a relatively rare complication of leprosy. Because it is not frequently encountered and *Mycobacterium leprae* does not grow on artificial culture media, the diagnosis may be missed unless the clinical picture is recognized. A patient with borderline tuberculoid leprosy, who developed such nerve abscesses, is described.

Case report

A 25-year-old Surinam–Javanese male noticed a hypopigmented hypaesthetic area above the right knee and subsequently a diagnosis of leprosy was made in Surinam. He received diamino-diphenyl sulphone (DDS)-monotherapy for $1\frac{1}{2}$ years. He left Surinam and migrated to the Netherlands but did not have any therapy in the $2\frac{1}{2}$ years before presenting at the Department of Dermatology, University Hospital, Leiden. His right leg showed a markedly depigmented, hypaesthetic macule just above the right knee. In addition there was a longitudinal swelling of rubbery consistency, starting from the depigmented area and running upwards for a distance of 10 cm (Figure 1).

A skin biopsy from the hypaesthetic macule showed a marked, partly granulomatous infiltrate with epithelioid cells, macrophages, and lymphocytes,

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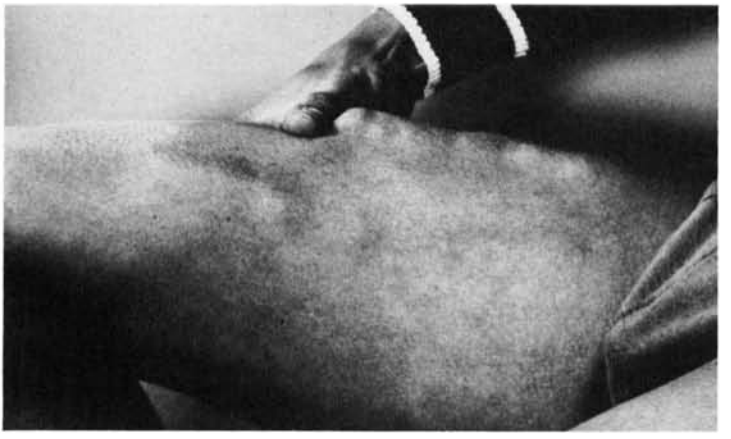

Figure 1. Abscesses due to leprosy along anterior cutaneous branches of the femoral nerve in the upper leg.

localized in the upper dermis, surrounding sweat glands and nerves. The Ziehl–Neelsen stain did not show acid-fast bacilli. Earlobe and nose scrapings revealed no acid-fast bacilli. The lepromin reaction after 4 weeks was 8 mm in diameter; the PPD was negative. Cultures for *M. tuberculosis*, atypical mycobacteria, aerobic and anaerobic bacteria and fungi remained negative, as well as a mouse foot-pad test for *M. leprae*. Physical and laboratory examination and roentgenograms of chest, thoraco-lumbar spine and upper legs did not show abnormal findings. There was no evidence of calcification in the affected leg. As the diagnosis of borderline tuberculoid leprosy was confirmed, the patient was treated with the combination of 100 mg DDS and 600 mg rifampicin daily. In spite of treatment the rubbery swelling gradually progressed and a second, similar, swelling developed medially to the first one. After several months the swelling became fluctuating and pus was aspirated. Microscopical examination showed scanty acid-fast bacilli with granular forms.

As both abscesses were located in the course of the anterior cutaneous branches of the femoral nerve, the most likely diagnosis was an abscess of these nerves. The affected nerves were surgically removed *in toto*. There were no complications. The hypaesthetic area did not enlarge. Histological examination of the surgical specimen showed necrosis surrounded by epithelioid cells. Some remnants of nerve endings were found. There were no acid-fast bacilli. The combination therapy of dapsone and rifampicin was continued and in the follow-up period of over 1 year, no further abscesses were observed and the skin lesions did not show signs of activity.

Discussion

This patient had borderline tuberculoid leprosy. Nerve abscesses are uncommon

and they are mainly found in patients with non-lepromatous leprosy.[1 4] A few cases have been described in patients with lepromatous leprosy.[5 8]

Nerve abscesses due to leprosy have been reported from India and less commonly from Africa and America.[1,2,4]

The reaction to *M. leprae* that is responsible for the clinical form of the disease is said to be HLA-linked,[9] but environmental factors or concomitant infections with other mycobacteria may also influence the clinical picture of leprosy.[10] The pathogenesis of nerve abscesses is still speculative. An increase in cellular immunity against dead *M. leprae* may be responsible for the abscess formation. Depending on the presence of viable bacilli or their antigenic remnants in nerves and in skin, the nerves may become reactive, while the skin lesions may remain unaltered as happened in the patient described in this article.

The slow development of abscesses in the absence of constitutional symptoms, in association with an unchanging hypopigmented macule, excluded erythema nodosum leprosum.

Although nerve abscesses are an uncommon complication of leprosy, the typical localization along the course of cutaneous nerves, as in this case, should lead to a correct diagnosis. Other causes of cutaneous abscesses, such as atypical mycobacteria, should be ruled out. The treatment of choice is surgical removal of the abscess, together with combination chemotherapy.

Acknowledgment

We are grateful for the advice of Professor Dr E Scheffer, Laboratory of Pathology, State University, Leiden, The Netherlands.

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Histiocytosis X in a patient with leprosy. A case report

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Summary A patient known to have lepromatous leprosy developed multicentric histiocytosis X from which he died. Initial misinterpretation of the histological findings resulted in delay in diagnosis and institution of treatment. The clinical implications are discussed.

Introduction

Foamy histiocytes (Virchow cells) are characteristic of the granulomatous lesions of multibacillary leprosy. Histiocytosis X is another condition in which large numbers of histiocytes are a typical finding on microscopy. The similarity between the histopathological features in the two conditions presents a possible pitfall, and was responsible for delay in diagnosis in the case described.

Case report

A 41-year-old Melanesian male presented to Port Moresby General Hospital in July 1983 with a ten-month history of swelling of the left knee, and swelling of the right elbow for 3 months. Leprosy had been diagnosed in 1969 and his subsequent course had been uncomplicated. At the time of admission he was taking 100 mg per day each of dapsone and clofazimine.

On examination he was febrile and the left knee and right elbow were warm,

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firm, tender and swollen. There was local bony tenderness adjacent to both joints. The range of movement of the elbow was from 20 to 90 degrees, and of the knee from 0 to 90 degrees. The left quadriceps was wasted but there was no effusion in the knee. His leprosy was clinically quiescent, and there was no hepatosplenomegaly. Haemoglobin was 12·3 g/dL, white cell count 6000/cu mm (neutrophils 79%, lymphocytes 10%, eosinophils 10% and monocytes 1%), and the ESR 29 mm/hr. Rheumatoid factor was negative. Radiographically there was patchy rarefaction of the bone ends around both affected joints; a chest radiograph was normal. The principal working diagnosis was of a chronic infective process.

Arthrotomy of the left knee revealed a striking profusion of whitish cheese-like tissue encroaching onto the articular surfaces, and apparently infiltrating the synovial membrane and periosteum. The bone of the distal femur was very friable. Biopsies of the abnormal tissue (Figure 1) contained collections of histiocytic cells and were thought to be consistent with lepromatous leprosy. Biopsies from the femur revealed only necrotic lamellar bone and plentiful leucocytes. Bacterial cultures of both tissues were negative.

Ten days postoperatively thalidomide (100 mg/day) was commenced for a type II leprosy reaction, and rifampicin (600 mg monthly) was added for its mycobactericidal effect, in view of a possible diagnosis—despite the sites—of leprous osteitis.

Both joints remained stiff and painful, and over the next 10 weeks the swelling and tenderness in the left knee progressively increased. A firm, tender left inguinal lymph node appeared and 2 centimetres of hepatomegaly developed. The serum calcium was normal. Aspiration of the knee produced turbid fluid, and a second

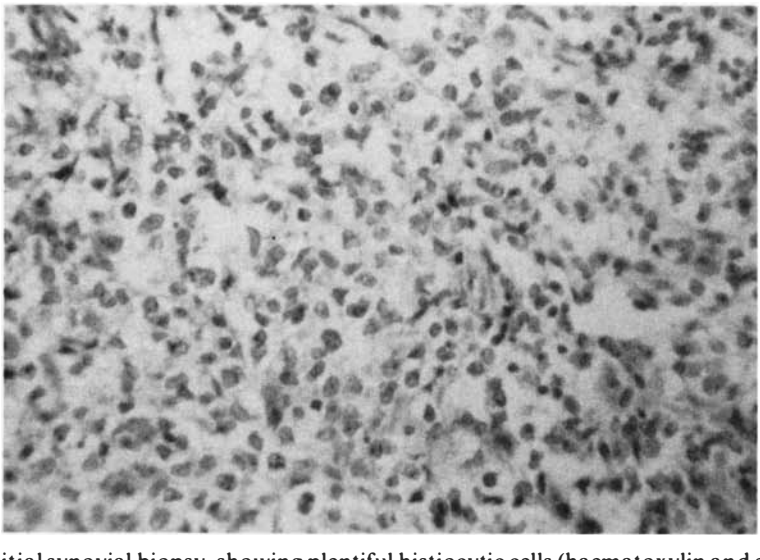

Figure 1. Initial synovial biopsy, showing plentiful histiocytic cells (haematoxylin and eosin, ×40).

arthrotomy three-and-a-half months after admission revealed that the synovium was now almost completely replaced by large amounts of soft yellowish-white material which bled freely when incised. Biopsies were taken from this tissue and from the distal femur, and also from a mass of large friable left inguinal nodes, some apparently replaced by the same abnormal tissue as found in the knee.

Histopathology of the specimens from the knee showed infiltration of the bone (Figure 2), synovium and subcutaneous connective tissue by masses of ill-defined cells with pale eosinophilic cytoplasm and irregular nuclei with a distinct nuclear membrane and a nucleolus. Some mitoses were present, and no acid-fast bacilli were seen. The lymph-nodes (Figure 3) showed replacement by a mixture of plasma cells and the abnormal cells seen distally. Four pathologists, 1 in Port Moresby, 2 in Brisbane, and 1 in Oxford, independently agreed on a diagnosis of histiocytosis, probably histiocytosis X.

Postoperatively the patient developed a deep wound infection with *Streptococcus pyogenes*, with associated hepatomegaly, jaundice and hypoalbuminaemia. His condition settled on intravenous penicillin therapy. Four months after admission his haemoglobin had fallen to 6·4 g/dL, with a white cell count of 3900/cu mm (neutrophils 67%, lymphocytes 13%, eosinophils 19%, monocytes 1%). Bone marrow examination showed generalized hyperplasia with erythroblastic predominance, but no evidence of malignancy.

Over the next 2 weeks the left knee began to enlarge further rapidly, with the development of satellite nodules in the proximal thigh. A smooth, circumscribed ballottable mass was palpable in the right paraumbilical region, separate from the liver, and not moving with respiration. Intravenous pyelography gave no further

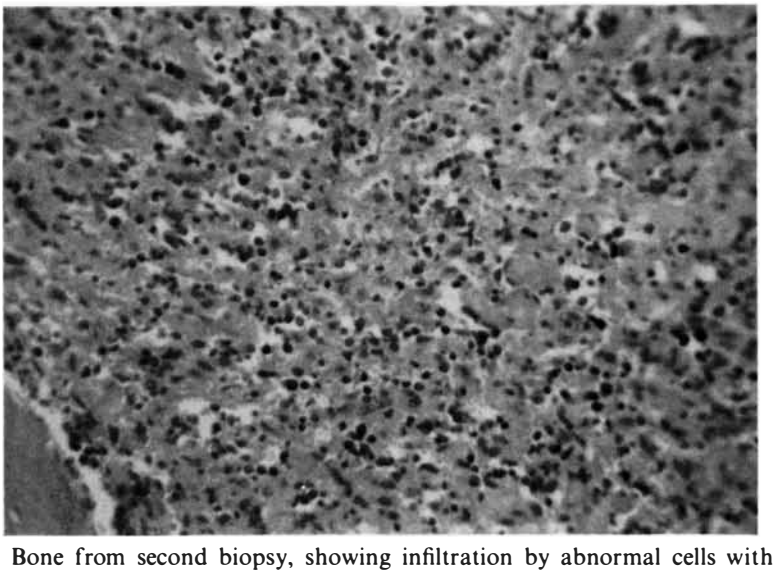

Figure 2. Bone from second biopsy, showing infiltration by abnormal cells with eosinophilic cytoplasm and irregular nuclei (haematoxylin and eosin, × 20).

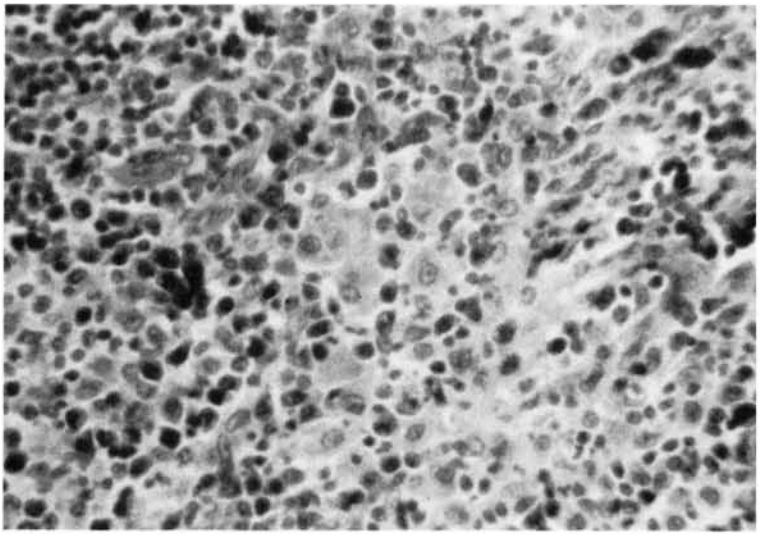

Figure 3. Lymph node containing plasma cells and infiltration of abnormal cells as in Figure 2 (haematoxylin and eosin, ×40).

helpful information. Chemotherapy was commenced with intermittent intravenous vinblastine, with continuous allopurinol cover and prednisolone 40 mg daily. Within 2 weeks there was an obvious reduction in the size of both the knee and the abdominal mass, but the arthrotomy wound then broke down completely and bled freely, necessitating transfusion, and despite further doses of vinblastine the patient's general condition deteriorated and he died 7 months after admission. Permission for autopsy was withheld.

Discussion

When a resident of a tropical country presents with subacute symptoms referable to multiple bones or joints a chronic infective process such as tuberculosis is among the leading differential diagnoses. In the case described, after exclusion of the commoner infective causes, we were left with a histological picture apparently consistent with leprous osteitis, albeit in an unusual site. It was only when the patient's condition continued to deteriorate despite mycobactericidal chemotherapy that the findings were reviewed and further investigations were undertaken leading to the definitive diagnosis.

Many patients with leprosy ultimately develop secondary bony changes, but true invasion of the skeleton by *Mycobacterium leprae* is responsible for less than 10% of such cases, occurring in 2–3% of all leprosy patients. The great majority of radiologically apparent bone abnormalities in leprosy are due to 'aseptic necrosis' in anaesthetic limbs, with or without superadded pyogenic infection introduced

through ulcers or burns. One study[1] postulates that episodes of leprous osteitis or the development of leprous osteomata may be provoked by lepra reactions causing activation of macrophages already containing leprosy bacilli. In their experience it is usually the small bones of the hands and feet which are affected, and they describe almost complete replacement of the bone marrow by large histiocytes, some with 'pink granular cytoplasm', together with some lymphocytes and plasma cells.

Exactly the same cells are present in the lesions of the histiocytosis X complex (eosinophilic granuloma, Hand–Schuller–Christian disease, and Letterer-Siwe disease), where again the histiocytes have been described as containing 'abundant eosinophilic cytoplasm'.[2] Indeed, one study[3] contends that this disease probably represents a reaction of macrophages to an as-yet unidentified infective or toxic agent.

Another study[4] points out that histoid leprosy, a rare form of lepromatous leprosy occurring in long-standing patients treated with sulphones, closely resembles fibrous histiocytoma, both grossly and microscopically. It has been suggested that the lesions occur as a result of the emergence of sulphone-resistant bacilli, which can be demonstrated intracellularly with special stains.

Histiocytosis X is a rare condition, usually affecting the flat bones of children[2] with an estimated annual incidence in the United Kingdom of 0·5 per million.[5] In Papua New Guinea, with a population of 3 million, a total of 4 cases were reported to the Tumour Registry over the 5 years to 1983 (unpublished data). The natural history of the disease is unpredictable, though the course is commonly self-limiting. Affected joints are permanently damaged. Recent reviews[2,3,6] have pointed out that study of the clinical syndromes characterized by the development of eosinophilic granulomatous lesions is made very difficult by their relative rarity, by multiple systems of classification based on questionable criteria, and by the inability to predict the outcome from the histopathological findings. The patient reported here was certainly older than the average, and his disease followed an uncharacteristically aggressive course, despite treatment with vinblastine which is generally held to be the most effective single agent for the management of such cases.

The estimated prevalence for leprosy in Papua New Guinea is 280 per 100,000 (Department of Health, unpublished data). Although 'common things occur commonly' is an axiom which should guide much clinical practice in a small developing country where diagnostic and treatment facilities of all kinds are in short supply, this is not always the case.

Acknowledgments

We are grateful to Drs K Misch and Rosemary Ashby of Port Moresby General Hospital for performing the histopathological examinations; to Dr J White of

Port Moresby General Hospital for the bone marrow examination; and to Dr R Axelsen of the Royal Brisbane Hospital and Dr C G Woods of the Nuffield Orthopaedic Centre, Oxford, who also provided opinions on the material from the second biopsy.

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SPECIAL ARTICLE

Leprosy in the Yemen Arab Republic

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Summary The present situation with regard to leprosy control in the Yemen Arab Republic (North Yemen) is briefly reviewed. It is believed that there are currently about 1800 registered cases, of whom 1200 have active disease. The authors estimate that there are between 6000 and 8000 cases in the country. There is a 130-bed hospital in Taiz, called the City of Light, which acts as a central leprosarium. Facilities for diagnosis and treatment are also available in other parts of North Yemen, but the overall situation with regard to the competence of health staff, laboratory facilities, chemotherapy, reactions, eye complications, deformities and rehabilitation is far from satisfactory. A plea is made for the appointment of a leprosy specialist and the urgent development of a national leprosy control programme, including the appropriate training of personnel.

Introduction

The Yemen Arab Republic (North Yemen) is located on the south-west edge of the Arabian Peninsula and has a population of about 8·6 million. It is bordered on the north by Saudi Arabia, on the west by the Red Sea, on the south and south-east by People's Democratic Republic of Yemen, (South Yemen), and the east by the Great Arabian desert. It encompasses about 200,000 km.^{2}

Geographically, North Yemen can be conveniently divided into 4 divisions, each having its own characteristic altitude, climate, and vegetation. These divisions are: (1) the *coastal lowlands of Tihama* which stretch along the Red Sea from Saudi Arabia in the north to Bab-el Mandab in the south. Hodiedah, with a population of 120,000, is the capital of this region; (2) the *foothills and middle heights*, at an altitude of 200–1500 m and situated between the Tihama and the central highlands; (3) the *central highlands* which exceed 1500 m in elevation and include Nabi Shu'ayb (3760 m), the highest mountain in North Yemen, Sana'a (population about 280,000), the capital of North Yemen, Taiz (population about 120,000), the second largest city in the country, and Ibb (population about 34,000), are all situated in the central highlands; (4) the *eastern semi-desert*

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plateau slopes gently eastward and drops to an elevation of 1000 m and borders the Empty Quartar, El-Rub El-Khali.

About 1·4 million of the population live and work outside the country. Much of what they earn is sent to their families in North Yemen and serves as a major source of income for the population there. Unlike Saudi Arabia to the north, there are no petroleum reserves in the Yemen Arab Republic. In 1980, the average annual per capita income was 2039 Rial (4·8 Rials less than $1·00 US).

The individual income of North Yemen is negatively affected by the widespread habit of chewing khat (*Catha edulis*), a green leaf plant cultivated on the mountain slopes. The social activity of chewing khat, the exhaustion following its use, and the cost of purchasing it, all serve to reduce the useful income of the population, especially as the coffee plant is no longer cultivated for export.

Magnitude of the problem of leprosy in North Yemen

Although progress in the care of patients with leprosy in North Yemen was at first slow, it has started to accelerate. Recently, the problem was evaluated by one of us (SKN) who visited North Yemen in the Spring of 1983 on behalf of the World Health Organization (WHO).

In the years from 1940 to 1960, patients were isolated and offered little medical care. In 1964, a sanatorium was built for leprosy patients, who were either hospitalized or given medication in the home. In the 1970s the sanatorium was improved, new wards were added, and it was named the City of Light. This facility is located in Taiz and is run by the Missionaries of Charity, a Catholic organization founded by Mother M. Teresa of Calcutta, India. There is a 130-bed hospital and homes for patients and their families who have been rejected by their community. Patients who are judged non-infectious are encouraged to return to their homes and occupations, but if they are rejected by their communities, they are allowed to live in the sanatorium where they are engaged in various occupations, including sewing for women, and farming, carpentry, stone cutting, building, and taxi-driving for men. There is a small dispensary which was used by one of us (HBO) for out-patient ophthalmic surgery, but general surgery or intra-ocular surgery is provided for the patients at the general hospital. Medical care is given by one of us (YA-Q), who attends the sanatorium twice weekly.

Although WHO recorded a figure of 850 registered cases in 1975,[1] information obtained from this visit suggested that a figure of 1800 is more accurate, with about 1200 suffering from active disease. It has recently been suggested[2] that there may be as many as 18,000 cases of leprosy in the Yemen, but by reasonable estimates, admittedly based on limited data, our belief is that a total of 6000–8000 is more likely. Unfortunately, there is marked social stigma against the disease. About one-half of the relatively high endemic governorates include Damar and Sana'a located in the central highlands, and Hodeidah, located in the coastal lowland of Tihama. The disease is relatively uncommon among children.

Facilities for treatment are available in Al-Hudahdah and in Jeblah (in the central highlands) and in Damar. The treatment offered in these areas, however, is not well organized and only minimal facilities for skin-smear examination and treatment of complications are available. This is also true for the sanatorium. Furthermore, there are no mobile teams for the early detection and treatment of the disease. There are no qualified physiotherapists, nor is there physiotherapy equipment for the treatment of the handicapped patients, although minimal international collaboration for patient care and research work is now available.

Conclusions and recommendations

There is an important need for an individual interested in leprosy to be appointed at the Ministry of Health to ensure full teamwork in the problem. Under this person's direction, a national plan for the

control of leprosy should be prepared. The plan should include means of early identification and early treatment of patients, follow-up on treatment, prevention of deformities, and the rehabilitation of patients with deformities. Mobile teams for case detection which would work first in the highly endemic areas, and later in the other areas, should be mobilized. Members of the community should be educated so that they can help in the early detection and assist in preventing social rejection. Personnel should be trained in physiotherapy for prevention of deformities and the rehabilitation of patients with deformities. Laboratory personnel should be trained in the preparation and examination of smears for the identification of acid-fast organisms. The sanatorium at Taiz (the City of Light) should be upgraded to serve as the national hospital training centre for the medical and paramedical personnel. (Initially, the training of personnel who would work at the City of Light could be arranged at the All Africa Leprosy and Rehabilitation Training Centre (ALERT) in Addis Ababa.) Leprosy should be part of the curriculum of the new medical school being built at Sana'a and in the Health Manpower School at Sana'a. International collaboration and consultation should be sought to help in training personnel, research, and for financial support of these programmes.

To accomplish these goals, an international consultant(s) should be sought to help ensure sound policies and to help implement the programmes. Financial support for equipment, personnel, and patient care should be obtained from international organizations. The mobile teams which are formed should be attached to regional hospitals where adequate facilities for diagnosis, laboratory examination, and the treatment of complications can be handled. Combined chemotherapy, as recommended by the WHO study group on Chemotherapy of Leprosy for Control Programmes,[3] should be implemented as soon as reasonably possible. In order that this can be accomplished safely and effectively, a high priority must now be given to leprosy work generally, both in the training of health personnel and laboratory technicians, in upgrading the City of Light and in the development of physiotherapy for the prevention of deformity and the care of the deformed.

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SPECIAL ARTICLE

Communicating with photographs in a leprosy hospital in Nepal

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Introduction

Many of the devastating health problems of the developing world could be prevented by adequate health education about sanitation, diet, living conditions and seeking early medical advice. The people most in need of this education present a major communication problem as the majority of them are illiterate. Health educators are increasingly using pictures to give information to these illiterate village people. Recent cross-cultural research[1,2] suggests, however, that the type of information that can be delivered through pictures needs careful examination. There is a great danger in assuming that pictures and symbols which convey certain information in the developed world will convey a similar message in a Third World setting. Communicating with pictures is not independent of the people they are aimed for and ethnocentricity must be avoided at all costs.

Photographic teaching aids for health education about leprosy were made for use in Anandaban Leprosy Mission Hospital, 12 miles south of Kathmandu, the capital of Nepal. The effective treatment of leprosy requires considerable education of medical staff, the general public and patients. The major educational needs are the recognition of the early lesions by medical staff allowing early treatment and the education of the patient with the disease. By these means the mutilating deformities can be avoided. In Nepal 1% of the population suffer from leprosy, frequently with appalling physical, social and economic consequences. The fact that patients suffer severe deformity represents in part a failure of communication by medical personnel.

There are considerable barriers to the communication of health information in Nepal. The 15 million population are scattered among the formidable Himalayan mountains in a country that has never been colonized and until the middle of this century was cut off from outside influences. The vast majority of the population are subsistence level farmers, with an average annual income per capita of less than $100/year and only 10–20% of the population are literate. Until effective vaccination against *Mycobacterium leprae* is developed educators will have to continue to battle against the terrain, widespread illiteracy, under-development and poverty of Nepal.

The projects involved producing aids for leprosy health education using photographs. This made us aware of the great difficulty in producing appropriate teaching materials in the developing world. Photography offers an accurate representation of a subject which can either be used for recognition or imparting information.

The function function of recognition was used in taking clinical photographs to help educate

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medical staff to recognize leprosy in its early stages. Aratacus in AD 94 said of leprosy that 'The physician, from inattention or ignorance of the patient's ailment, does not apply his art to the commencement when the disease is very feeble.' Today early diagnosis is more important because advances in drug therapy mean the disease is rapidly controllable.

To give information using photographs is more difficult. The aim of the second project was to produce a set of teaching aids to help inform a patient on how to look after his anaesthetic feet, which is a major cause of deformity in leprosy. The series of teaching aids was assessed by staff at the hospital and by experienced health educators attending a conference at the hospital. They made many valid constructive criticisms of the teaching aids and considered that photographic teaching aids were more suitable for medical staff than patients. This illustrates the difficulty in trying to create communication aids for a different cultural group than your own and the importance of a pre-test with an educational aid.

Project 1: Colour clinical photographs

AIM

Anandaban Hospital is involved in teaching all levels of medical personnel from senior doctors to trainee basic level health workers. The best way of learning about leprosy is to see patients and be actively involved in their management. There is, however, a role for photographs to reinforce and supplement this practical experience especially when time is limited. One problem in Nepal is the display of photographs: bright sunlight and limited electricity make it difficult to use slide projectors. Our aim was to take colour transparencies that would then be used to produce 20″ × 30″ prints that would be mounted in plastic coats to improve their durability.

METHOD

Consenting in-patients and out-patients at clinics with suitable lesions were photographed using a Pentax ME Super 35 mm camera. A standard 50 mm lens was used for most of the photographs, with a 2 × close-up lens stopped at f8–f11 for close-up shots. To ensure sufficient detail in the final prints Kodachrome 25 transparency film was used. It was found that frequently the shade offered the best light for photographs avoiding the very intense shadow of direct sunlight. A neutral background was achieved by stretching a large light blue sheet over a 6 ft × 6 ft screen. It was intended to produce a clear, sharply focussed, picture of the particular lesion in front of the blue screen which was itself out of focus.

RESULTS

About 150 photographs were taken of which about 80 were technically good. Of these, 30 were made into postcard-sized prints and sent to Anandaban Hospital so they could choose which, if any, they wanted copies of. The superintendent requested copies of all 30 and thought they would be of use in the health education of staff. These were produced using money provided by Lepra.

Project 2: Producing teaching aids to help with health education and the care of anaesthetic feet in leprosy patients

AIM

Many of the horrific deformities of the hands and feet of leprosy patients could be prevented if adequate precautions were taken by the patient. Teaching patients to look after their anaesthetic feet and hands is one of the greatest challenges of health education in leprosy.

The danger of anaesthesia is quite a difficult topic to understand. The recently diagnosed leprosy patient who has normal-looking but insensitive feet, causing him no problem, may see no reason for time-consuming foot care. Even patients with severe ulceration of their painless feet may only seek medical help because of the smell of rotting flesh!

Anandaban Hospital is a referral centre for ulcer care and so has a large number of patients with foot ulcers and is very actively involved with health education about foot care. Prototype teaching aids were produced using photographs. The aim of these was to help communicate to patients the important points of anaesthetic foot care, supporting the talks and practical demonstrations with a visual input. It was hoped that accurate representation by photographs would help people to understand difficult concepts such as how neglect of foot care would lead to deformity.

Teaching aids are a fashionable area of health education. There is a tendency to produce them without evidence that they are helpful or needed, often assuming that high quality glossy pictures must be advantageous. For this reason it was decided to produce prototypes and assess them before making the final version.

METHOD

Time was spent helping treat patients on the ulcer wards, seeing at first hand the problems of anaesthetic feet. Staff and patients were asked what they considered to be the most important problems before deciding what information to communicate with the teaching aids.

Each teaching aid was 18″ × 16″ and consisted of between 2 and 6 mounted black and white photographs. Black and white had the advantage that we were able to develop the prints ourselves. This reduced the cost and also allowed considerable manipulation of the size and composition of the final prints.

To get feedback on the teaching aids 9 were displayed and comments were requested from members of staff of the hospital. Special attention was given to the answers of paramedical workers who would be likely to use them. Further comments were obtained from questionnaires completed by people attending a conference held at the hospital entitled 'The training of middle level health workers in Nepal'. Many attending this conference had considerable experience of health education in Nepal.

The photographs were only accompanied by one- or two-word titles. It was hoped that avoiding detailed captions allowed the best assessment of how effective the pictures were in conveying the message. It was intended, however, that the teaching aids would always be accompanied with an oral explanation by staff.

The teaching aids were modified according to the comments made on them and mounted in a large album, as this offered both protection and portability. The final assessment of their value will come from long term use by patients and staff.

RESULTS

The teaching aids were enthusiastically received at the hospital, 20 out of 25 questionnaires completed and many valid and constructive points made:

(i) *Value of teaching aids.* Eighteen people replied that they considered the teaching aids to be useful and said they would use them themselves if they were teaching about anaesthetic feet.

(ii) *Useful for patients or medical staff?* People were asked whom they considered the teaching aids were suitable for. The results are shown below:

		Middle	Basic	
Doctors	Nurses	Health worker	Health worker	Patients
10	12	10	11	7

(iii) *Comments on the teaching aids.* There was an initial reluctance to discuss or write down the problems of the teaching aids. When it was realized that criticism was genuinely wanted many people produced many constructive comments on the photographs, the teaching aids and the use of photographic teaching aids in general.

(a) *Photographs.* There were many favourable comments about the photographs. However, some were misinterpreted: many people thought the picture of sweating looked like blisters rather than sweat. Photographs showing only part of a person or part of a foot were considered likely to be misunderstood by patients.

(b) *Teaching aids.* The teaching aids with only 2 pictures and a minimum of symbols were preferred. As expected the teaching aids were not considered adequate on their own; many respondents requested written or oral explanation.

(c) *Concept of teaching aids.* It was questioned whether photographs were suitable for use with village people who were not used to photographic representation. Alternative approaches of education were suggested including puppets, clay models and drama.

Discussion

The 2 projects were both photographic but had very different aims in what and to whom they communicated. They met with varying degrees of success which reflected the value of photographs and teaching aids in the different situations.

The clinical photographs were rapidly accepted by the staff of Anandaban Hospital. Photographs can be an excellent way to accurately convey the appearance of a subject if the viewer is accustomed to pictorial and photographic representation. The aim of such 'identification' photography is simply to provide a clear picture of the subject against a featureless background. With modern photographic equipment and attention to detail this is a simple task.

The teaching aids were more ambitious, aiming to convey information to patients who were not used to pictures and photographs. When producing teaching aids it is vital to pre-test them. Hospital staff and other people involved in health education in Nepal were used for this. This was not a good sample as they represented the educated minority of the Nepalese population rather than the illiterate majority. Despite this, their considerable experience of giving health education to Nepali villagers did mean that they were able to point out many problems with the teaching aids. This enabled alterations to be made before a further trial with patients was done.

The teaching aids were considered to be more useful for staff than patients. The unsuitability of the aids for use with patients reflects the fact that they are based on western ideas of picture interpretation. Studies in Nepal and elsewhere[1, 2] have shown that the rural villager in Third World countries interprets pictures in a very different way. This difference is principally attributable to reduced exposure to pictures. Educated members of Third World countries, such as the staff of the hospital, are exposed to more pictures and are more likely to interpret them in a western fashion.

To understand fully what a picture rèpresents requires interpretation of certain visual clues. Many villagers in Third World countries have difficulties with depth perception in pictures. Hudson's classical work on Black South Africans[3] showed that pictorial depth clues of object size, object superimposition and perspective were often not correctly interpreted. Questions on relative distances between objects were answered in terms of two-dimensional proximity only. The inability to interpret a picture three-dimensionally greatly alters a person's perception of what a picture represents. Example of this are: Nepalese villagers on seeing a line drawing of a cube showing 3 faces considered there to be 3 cubes[1] and over one-third of them when showed a picture of a man from behind stepping up on to a box considered that the raised leg was abnormally short.

Failure to see a picture as symbolic representation results in very literal interpretations. This can prove a great problem to health educators. A health worker in rural India used a 2 ft × 3 ft picture of a fly to illustrate a talk on the need for hygiene and the covering of food because of insect

transmitted contamination. When obtaining feedback about his talk the villagers said it was an interesting story but fortunately they did not have flies that big! Similarly Hudson[4] found that posters not showing the whole person were misinterpreted as showing people with missing limbs. Many of the leprosy teaching aids produced showed only part of a person and so were likely to be misunderstood.

Teaching aids not only require an understanding of what the picture represents but also comprehension of the meaning of the picture. In a western society we are used to deriving meaning from a picture but this is not the case for the Nepali villager who rarely sees pictures. Even with posters in which villagers accurately recognized the individual components of a picture they did not link them together. Linking pictures together by arrows to suggest a causal relationship is unsuccessful as an arrow has an arbitrary symbolic meaning which is not understood.[2]

Analysing the substantial difficulties in communicating with pictures to villagers in Nepal, Fussel & Haaland concluded that pictures could not be used to convey information on their own but did have a role in supporting other means of communication.

What role, if any, do the teaching aids have in communicating education about living with anaesthetic feet? The response to the questionnaires and subsequent review of the literature shows that there were many shortcomings of the original teaching aids (e.g. multiple photographs, showing parts of people or limbs, linked by arrows, etc.). Considerable modifications were made and they are now being tested in the education of staff and patients. It is likely that they will be helpful for staff who are used to interpreting pictures and photographs. For patients, their role is far less certain. They do not have a role on their own, but they may form a useful addition to the educators' armoury of talks and demonstrations. Only long term use will decide this.

Whatever others learn from the clinical photographs and teaching aids, a great deal was learnt producing and testing them. A photograph is an accurate two-dimensional representation of a subject, but this does not mean it will necessarily be recognized and communicate information to the viewer. The vast problems of communicating to rural villagers in Third World countries are not simply overcome by western technology but will need continued assessment and modification of the teaching materials used.

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Obituaries

JAMES CECIL PEDLEY, MRCS, LRCP 1905–1985

Cecil Pedley died on 11 February 1985. Beyond the inevitable sadness and sense of loss, we thank God for a long life of devoted and constructive service. Cecil Pedley was a committed Christian who spent most of his adult life as a medical missionary, much of it in demanding and pioneering situations.

Born in 1905, he qualified as a MRCS, LRCP, at the Middlesex Hospital, London, in 1931, and from 1935 to 1944 served in Lanchow, NW China, where he first met and treated leprosy sufferers. From 1949 to 1955 he worked in Kashmir, Simla, and the Kulu Valley, setting up 2 rural hospitals, including 1 near the Tibetan border. He also organized basic health care delivery in areas which involved isolation and strenuous trekking.

In 1956 he joined The Leprosy Mission (then The Mission to Lepers), and served in India before secondment to Nepal. During his 16 years there he was associated with the development of the Anandaban Leprosy Hospital, and with the United Mission to Nepal. It was during this time that he completed his important study of several years on the major role of nasal secretion in the transmission of leprosy.

He also undertook leprosy surveys in Afghanistan and, at the age of 69, when most people are well into retirement, he accepted an invitation from The Leprosy Mission to share in establishing a new integrated general medical and leprosy hospital in Bhutan, and an associated leprosy control programme. At the end of 1974 he finally returned to England, where he continued in general practice until his death.

Behind the recitation of dates and places, we see the dedicated and courageous spirit of a great man. Like all the truly great, he showed a deep and true humility, never seeking publicity, content to work hard and quietly, and a willingness constantly to accept new challenges. His contribution in the field of leprosy was a deeply professional and compassionate care for his patients, and his ability to look beyond the received wisdom and to undertake studies, at grass roots level, which have contributed significantly to our understanding of the transmission of leprosy. Throughout his life he was sustained by his faith, and by his wife, Betty. Our loving sympathy and prayers are offered to her and the family.

EDDIE ASKEW

Long before I met Cecil Pedley, I had the opportunity to examine slides from biopsies he sent from Nepal to the Leprosy Study Centre in Wimpole Street, London, and to read the voluminous and fascinating letters and reports which passed between him and Douglas Harman over a period of years. I soon realized that the material submitted, whether of skin, peripheral nerve or other tissues, was of extremely high quality as were his notes on clinical findings and treatment. Cecil Pedley recognized the importance of the nose and nasal mucus in the transmission of leprosy long before the subject was 'revived' by workers in this country and elsewhere and I also have a letter from him—which I greatly treasure—describing counts which he made in Tansen on the numbers of leprosy

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bacilli in one drop of breast milk from a patient with untreated lepromatous leprosy. Although he was modest about his own observations and impressions, he derived great pleasure from the publication of a series of papers in the leprosy journals on aspects of leprosy which were always of great practical importance and I was privileged to assist in some of those concerned with neuropathology.

We have lost a colleague whose contribution to the study of leprosy has in some ways still to be fully recognized but its quality will surely stand the test of time. Shortly after his final retirement to this country, I met him in a blinding gale above Hartland Quay and we soon came to share a common love of the North Devon coast.

A COLIN McDOUGALL

CHARLES C SHEPARD, MD 1914–1985

Dr Charles C Shepard, Chief of the Leprosy Section at the Centers for Disease Control in Atlanta, Georgia, USA for over 30 years and internationally renowned as the first person to obtain reproducible multiplication of *Mycobaterium leprae* in an animal model, the foot-pads of mice, died unexpectedly of heart failure on 18 February 1985 at the age of 70. His death occurred at a time in his long career when, as he would have undoubtedly wished it, he remained at his peak in intellectual productivity.

His mouse foot-pad inoculation procedure revolutionized research in leprosy, creating the whole field of study of experimental infections in the disease and moving leprosy research into the mainstream of laboratory investigation. Rarely has any field benefitted so greatly from a single discovery.

While best known to leprologists for his work in this disease, he made significant early contributions to the diagnosis, natural history, and epidemiology of Rocky Mountain spotted fever, Q-fever, and typhus. Despite full commitments to leprosy, he was the co-discoverer of the Legionnaires' disease bacterium (*Legionella pneumophilia*) following the well-known outbreak of pneumonia due to this organism in Philadelphia in 1976.

Charles Carter Shepard was born in Ord, Nebraska, on 18 December 1914. After attending Stanford University from 1932 to 1935, he transferred to Northwestern University in Chicago where he received his bachelor's, master's, and medical degrees. In 1941 he became a commissioned officer in the US Public Health Service and served in that capacity until reaching mandatory retirement age in 1978, when he transferred to the US Civil Service. He worked at the National Institutes of Health (NIH) from 1942 to 1948 where he developed techniques which allowed the serologic differentiation of epidemic and murine typhus. In 1948–49, he spent a year studying in the laboratory of Arne Tiselius in Uppsala, Sweden, learning the then newly discovered electrophoresis and physical separation techniques which would revolutionize protein chemistry and, in turn, the fields of immunology and biochemistry. After another year at the NIH, Dr Shepard joined the Rocky Mountain Laboratory in Hamilton, Montana in 1950, where he remained until 1953 studying the ecology and epidemiology of Rocky Mountain spotted fever and Q-fever. He then joined the Centers for Disease Control in Atlanta, where he continued his outstanding work with rickettsiae and began his incredibly productive career with the leprosy bacillus.

Dr Shepard received many awards in recognition of his outstanding contributions to microbiology in general as well as leprosy, including the Gorgas Medal (1962), the Kimble Methodology Award (1962), the Philip R Edwards Award (1946), the World Leprosy Day Award (1970), the Centers for Disease Control Medal of Excellence (1977), the US Public Health Service Meritorious Service Medal (1964) and Distinguished Service Medal (1978), the Raoul Follereau

Award (1978), and the Richard and Hilda Rosenthal Award (1979). He belonged to numerous scientific and professional societies and served on the WHO Steering Committees on the Immunology of Leprosy (IMMLEP) and Therapy of Leprosy (THELEP), the WHO Advisory Panel on Leprosy, The Heiser Program for Research in Leprosy, was chairman of the National Hansen's Disease Center Research Advisory Committee, and for many years served as the chairman of the US Leprosy Panel of the US–Japan Cooperative Medical Science Program of the National Institute of Allergy and Infectious Diseases. He was a devoted member and supporter of the International Leprosy Association, serving as a Councillor for the Americas and as a member of the Board of Directors of the *International Journal of Leprosy* at the time of his death.

Although he had authored 179 publications and a number of others were in press or in preparation at the time of his death, 'Shep''s productivity and contributions to science in general, and to leprosy in particular, cannot be measured. His love of music, art, nature, and of life itself set him apart. His quiet logic, gentle guidance, and calm wisdom as well as an immense store of scientific knowledge pervaded any meeting with him. His influence extended far beyond his published works—to the people who learned from him about science, about leprosy, and about life. The world of leprosy is a better place because of Charles C Shepard. He leaves a rich legacy to us and a deep responsibility to continue the work.

ROBERT C. HASTINGS

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Domiciliary and Field Work

Pedabarograph to measure the pattern of foot pressure

The following is extracted from *Hospital Doctor*, 25 April 1985: 'Dr Janet Hughes of The Department of Rehabilitation, Northwick Park Hospital, Harrow, Middlesex, UK, has used the pedabarograph (first described by Elftmann in 1934) to study foot pressure. The apparatus measures the pattern of foot pressure on the ground using a thick sheet of glass which has a light source on either side and a layer of thin metal on top.

When the patient steps on its surface, light is reflected in proportion to the pressure applied.

High pressure 'hot spots' can be looked at objectively and instantly, and records of pre- and post-operative patterns kept using a polaroid camera.

This means that comparisons can be made between different surgical procedures applied to each problem.

Various insole materials can also be compared and the most appropriate can be supplied to the individual.

However, the latest advances have been made with the 'dynamic' recording of patients walking across the pedobarograph.

A computer-based image processing system, devised at the Royal Hallamshire Hospital, Sheffield, and now supplied to Northwick Park, allows the information recorded on videotape to be analysed.

The data can be presented as pressure versus time plots for any given area of the foot, or as 'isopressure contour plots'—rather like the weather map.

Using the technique, surgeons can decide on the most suitable operation.

Teaching and Learning Material; Leprosy Documentation Service, Amsterdam, 1985

The Leprosy Documentation service (INFOLEP) in Amsterdam has produced a compendium of teaching and learning material in leprosy in a ring binder, which includes detailed information on what has been produced from many different parts of the world. The introductory pages include an account of the ILA Workshop on teaching and training in New Delhi, 1984. The language headings include—Amharic, Arabic, Chinese, Dutch, English, French, German, Hausa, Hindi, Ilocano, Italian, Japanese, Korean, Marathi, Nepali, Portuguese, Russian, Samoan, Sesotho, Spanish, Swahili, Tagalog, Tamil, Telegu, Thai. Enquiries to Leprosy Documentation service (INFOLEP), *at their new address*: Wibautstraat 135, 1097 DN Amsterdam, the Netherlands.

Manual for Multiple Drug Therapy from Ethiopia; ALERT, 1985

We are most grateful to Dr Marijke Becx-Bleumink, Director of ALERT Leprosy Control, PO Box 165, Addis Ababa, Ethiopia, for sending the latest edition of this excellent manual. The Foreword explains that this developed from the original version prepared in late 1983, in association with senior staff of the National Leprosy Control Programme and a short-term WHO consultant. From the experience gained in the 112 leprosy treatment centres during the period November 1983–November 1984, certain amendments were made and these are now included in the edition dated January 1985. The chapter headings include information and instruction on categories of patients; release from treatment before MDT is introduced; criteria for release from treatment of patients who have already been treated for many years; diagnosis and classification of new patients; registration of new and old patients for MDT; the multiple drug regimens; procedures during treatment; slit-skin smears; side-effects of the drugs and complications; duration of treatment; follow-up after release from treatment; re-treatment; reporting and evaluation of the completion of MDT. The whole work is extremely practical and has been very well thought out. This must be one of the best manuals for the implementation of MDT so far prepared and other control programmes could learn a great deal from it.

OXFAM: Guidelines for Tuberculosis Control Programmes in Developing Countries

This is an OXFAM Memorandum in their Practical Guide series, produced by the OXFAM Health Unit and written by Dr Paul Shears. It is a strongly bound paperback of 59 pages. The cost is £1.50 and it is obtainable from OXFAM, 274 Banbury Road, Oxford OX2 7DZ. Fifteen chapters cover all aspects of tuberculosis control and there are 7 appendices dealing with tuberculin surveys, sputum microscopy, drug dosages, drug adverse effects, drug resistance, measuring drug compliance. There is also a list of useful addresses and literature. This is an up-to-date and extremely valuable booklet with a great deal of practical information based on actual experience. It deserves a wide circulation.

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Expanding cards for clinical features and multiple drug therapy in leprosy (India)
We are most grateful for 2 'expanding cards,' both from India and of very similar format, which will undoubtedly be of great value for diagnosis, classification and treatment of leprosy. The first on 'Clinical Features in Leprosy' is a diagnostic card, published by Hind Kusht Nivaran Sangh, 1, Red Cross Road, New Delhi, India 110001, which gives a written description of those forms of leprosy grouped by WHO as paucibacillary and multibacillary, together with colour prints to illustrate each type of leprosy, from Indeterminate, through the spectrum to Lepromatous. The second on 'Multi-Drug Therapy for Leprosy' is published by the Indian Association of Leprologists, Central JALMA Institute for Leprosy, Agra, UP, India, and has similar expanding cards showing the skin lesions and the chemotherapy for both pauci- and multibacillary leprosy. Pure neuritic leprosy is grouped with paucibacillary for this purpose. This card on MDT emphasises the 2 important differences with regard to MDT in India, as recommended by this Association, (1) in contrast to the strict WHO recommendations (*Chemotherapy of leprosy for control programmes*, Technical Report Series 675, WHO, Geneva, 1982)—namely that for multibacillary cases, the 3 drugs, dapsone, clofazimine and rifampicin are given at the outset as intensive therapy for 21 days, under supervision, and (2) that in the case of paucibacillary leprosy, the dual therapy with dapsone and rifampicin is continued for 6 months, or 'until clinical inactivity'.

These 2 excellent folding cards are almost completely complementary and could well be in the hands of all staff who have responsibility for MDT in India. They will be useful not only for health personnel but also for health education of patients and they both deserve a very wide distribution.

Orientation in Leprosy for Doctors: HKNS, India
This excellent booklet of 28 pp has been written by Dr and Mrs Thangaraj and Dr K C Das in India and is published by Hind Kusht Nivaran Sangh, 1, Red Cross Road, New Delhi 110001, India. Its aim is '. . . to give short orientation in leprosy to all medical doctors and to ensure their involvement in the National Leprosy Eradication Programme.' Following an introduction, there are sections on nerves affected by leprosy; clinical features of pauci- and multibacillary leprosy; differential diagnosis; reactions; neuritis; care of the feet and hands. The more intensive involvement of doctors and medical students in leprosy in India might well have an enormously beneficial effect in current attempts to find and treat the estimated 4 million cases in that country and we wish this booklet every possible success.

Leprosy can be cured; a challenge for CIBA–GEIGY
This company have kindly sent this booklet of 32 pp produced in Basle, May 1985 and available from CIBA–GEIGY Ltd, Pharma Division, PH9, CH-4002, Basle, Switzerland. It describes their past, present and intended future contribution to leprosy, including the production of dapsone, rifampicin and clofazimine and plans which are currently being made for the presentation of these 3 drugs in a 'bubble' or 'calendar' pack for the treatment of multibacillary forms of leprosy. There is a life-size illustration of the prototype of this pack on page 26, clearly showing the tablets and capsules to be administered under supervision monthly, and at home on a daily basis. The back cover carries an important message from Dr S K Noordeen, Chief Medical Officer, Leprosy, WHO:

'*Through multidrug therapy (MDT) as recommended by WHO it is now possible to effectively deal with not only the ever increasing problem of drug resistance to dapsone but also to reduce the period of chemotherapy, thus encouraging patient compliance to treatment. There is every reason to hope that with the wider application of MDT the control of leprosy in the world could be greatly accelerated.*'

Teaching and Learning Materials from the Leprosy Mission in India
Particularly for readers working in India, we draw attention to the availability of teaching and learning materials in leprosy from The Leprosy Mission (Southern Asia), CNI Bhavan, 3rd Floor, 16 Pandit Pant Marg, New Delhi 110001, India (Dr/Mrs E S Thangaraj). The list is very similar to that which has been in use by TLMI in London for several years, but it should be noted that charges (in rupees) are made for every item. The many valuable items available are numbered on the front page indicating their suitability for health programme planners, health educators, shoe workshop managers, junior health workers, supervisors, multi-purpose workers, laboratory technicians, physiotherapy technicians, medical students, surgeons. Apply to TLM in India at the address above.

Queensland Department of Health, Visiting Medical Specialist—Leprosy
A medical practitioner registered in Queensland or eligible for such registration with specialist training in leprosy (Hansen's Disease) is required for two consecutive months annually to provide consultative services to the Health Department, visit other parts of Queensland in connection with the treatment of Hansen's Disease, conduct a weekly clinic at The Princess Alexandra Hospital, and provide training for medical staff in Hansen's Disease. Alternative arrangements for sessional work can be negotiated. $81.35—$117.90 per 3 hour session (depending on year of registration).

Please forward application to: The Director, Division of Specialized Health Services, Health and Welfare Building, 63–79 George Street, Brisbane, Australia, or telephone (07) 224 5665 and make an appointment to see Dr A.M. Patel.

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Reports, News and Notes

Submission of material to 'Reports, News and Notes' or to 'Domiciliary and Field Work' in *Leprosy Review*; an appeal from the Editor

During the past 10 years or so we have printed many hundreds of items of information under these, or similar headings, and although we may be changing the titles of these sections again slightly in 1986, the type of material included will remain essentially the same. We are always looking for information, news, notices or comments in these pages of the Journal, which are of *practical* value to those working in leprosy control. High on the list of priorities comes anything to do with teaching, learning or training. In this context, we wish to record our gratitude to those many individuals and centres in different parts of the world who have supplied information about materials and training courses through the years. It is however now becoming clear that we cannot by any means keep up with the full range of activities and productions, worldwide, even through the excellent channels established by ILEP. We therefore appeal to our readers to submit material for these sections of the Journal and to keep us informed, repeatedly, about training courses and the development of new teaching or learning material. We greatly prefer brief communications, with specific details of names and addresses to contact for further information. Suitable material of this kind will be acknowledged from this Office—and published—without delay. *Editor.*

Mycobacterium leprae* monoclonal antibodies and recombinant DNA from *M. leprae

IMMLEP have established a Bank of such monoclonal antibodies and expect soon to set up a Bank of recombinant DNA clones. These will be available to qualified investigators. Enquiries/requests, with brief outline of project, should be sent to Dr S K Noordeen, Secretary, IMMLEP Steering Committee, World Health Organization, 1211 Geneva 27, Switzerland.

Heiser Program for Research in Leprosy, 1986

The brochure for 1986 introduces the Program as follows: Dr Heiser set up his fund in The New York Community Trust and stipulated that income generated be used not to treat patients but to try to find a cure or preventative for leprosy. The New York Community Trust, a public foundation designed to carry out the charitable purposes of donors, met with medical experts and scientists to determine the best approach. It was decided that the 3 most important objectives should be: to attract the brightest, most highly motivated young biomedical scientists to train in research fields related to leprosy; to support the training efforts of laboratories and senior investigators who are experienced in leprosy research; and to promote collaborative research studies of leprosy and encourage international sharing of scientific information.

In considering research on leprosy, the fields selected as among the most relevant for the study of this disease are:

Cultivation of Mycobacterium leprae (Hansen's bacilli)
Immunology of mycobacterial infection
Experimental transmission of leprosy
Pharmacology of anti-leprosy drugs

The following awards have been established and are available—Postdoctoral Research Fellowships; Research Grants; Visiting Research Awards. Apply to the Heiser Program for Research in Leprosy, 450 East 63rd Street, New York, New York 10021, USA.

New leprosy vaccine trial

From the *Lancet*, 20 July 1985:

Leprosy carries with it a unique stigma, though only a small proportion of those infected eventually suffer the gross disfigurement and deformity commonly associated with the disease. Dapsone has remained the best

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treatment, but it must be taken for a long time, it may not be well tolerated, it may not be active against bacteria in the resting stage, and it may provoke resistance to its earlier benefits. It has proved impossible to cultivate *Mycobacterium leprae* in vitro, so development of a vaccine has been slow. It had, however, been observed that BCG conferred some protection against leprosy with varying degrees of efficacy around the world. When killed *M. leprae* derived from the infected nine-banded armadillo became available, it was decided to combine organisms from this source with BCG in an attempt to induce cellular immunity. A randomized controlled trial of about 90,000 people is to be carried out in Karonga, Malawi, to compare the protective efficacy of this combination with a single standard dose of BCG and to compare single BCG vaccination with repeated BCG or with the combination. The trial is to be funded by members of ILEP (International Federation of Anti-Leprosy Associations), including the British Leprosy Relief Association (LEPRA). This trial is the second such trial (succeeding another large trial of over 60,000 people in Venezuela) and could begin, with the collaboration of the London School of Hygiene and Tropical Medicine, LEPRA, the Malawian health authorities, the World Health Organization, the World Bank, and the United Nations Development Project, in a few months. It will take about 10 years for results to emerge.

Armadillos for leprosy research from Mississippi, USA

We have received a letter from Dr B J Gormus, Research Scientist at Tulane University, Delta Regional Primate Research Center, Covington, Lousiana 70433, USA, informing us that armadillos are available for shipment for leprosy research from the State of Mississippi. Further details are available from Mr Sal Cefalu, 1000 Manson Avenue, Metairie, Louisiana 70001, USA.

Technical Guide for Smear Examination for Leprosy by Direct Microscopy

This 34-page guide, produced by the Leprosy Documentation service (INFOLEP) in Amsterdam, was first issued in 1983 (English only). Five thousand copies were printed and they have been distributed mainly to members of the International Federation of Anti-Leprosy Associations (ILEP); to the Health Unit of OXFAM in Oxford for inclusion in the OXFAM–LEPRA pack of teaching materials on leprosy, and to The Leprosy Mission International in London for distribution as part of their Teaching and Learning Materials service. A second edition, with minor corrections, is under discussion. A Spanish edition is already available. Dr Corroller of the Association Française des Foundations Raoul Follereau has very kindly translated the guide into French and this is now being prepared for the press. WHO has arranged for translation into Arabic, and offers have been received for Thai, Indonesian, Chinese and Portuguese. It is hoped that it will in the near future be possible to send several thousand copies to the Voluntary Health Association of India, for distribution from Delhi. Plans are also being made for a much wider and more positive distribution of this guide to all leprosy-endemic areas from 1985 onwards.

Epidemiology of leprosy in relation to control; WHO, 1985

This report of a WHO Study Group is published in their Technical Report Series as Number 716, from WHO, Geneva, Switzerland, 1985. It deals with: current knowledge of the epidemiology of leprosy; the measurement of leprosy and its control; proposals for future research. There are 4 annexes on terminology, epidemiological and operational indicators in leprosy control, OMSLEP individual patients form and WHO disability grading scale. Particularly for those who are responsible for Multiple Drug Therapy (MDT) and field work, the definitions on page 53, including those of 'adequate treatment' and 'regular treatment' and the indicators in Annex 2 are of considerable interest.

Handbook of the Association of Medical Research Charities, UK, 1984–85

This handbook of 41 pages is available from the Association of Medical Research Charities, The Development Trust for the Young Disabled, Royal Hospital and Home for Incurables, West Hill, Putney, London, SW15 3SW. The Introduction opens: 'The Association of Medical Research Charities was founded in 1972 and provides a forum for the chief executives of major medical research charities to meet on a regular basis. The meetings give members an opportunity to discuss strategy and organization and to hear from visiting speakers about trends and developments of significance for medical research. The Association has played a key role in improving personal contact between members and has thus provided a basis for collaboration on major projects and has prevented unnecessary duplication of effort. The object of this booklet is to assist applicants for funds by providing essential information on each of the member charities.' The list of members includes LEPRA, together with many organizations in the UK dealing with cancer, leukaemia, heart disease, multiple sclerosis, asthma, arthritis, mental health and muscular dystrophy. The annual income of the 35 member charities in 1983 amounted to UK £128 million, for medical care and research of which no less than £77 million was spent purely on research.

ALERT, Addis Ababa; Nineteenth Annual General Meeting, March 1985

The All Africa Leprosy and Rehabilitation Training Centre (ALERT), founded on 11 December 1965 held its Annual General Meeting in Addis Ababa, 22 March 1985, and was attended by delegates from sponsoring organizations, as well as representatives of a number of embassies in Addis Ababa. The meeting was presided over by the President of ALERT, Dr Gizaw Tsehai, the Minister of Health of Ethiopia. The Directors of the various departments of ALERT and the Executive Director gave brief reports on the activities during 1984. Of special interest and importance was the continuation and extension of Multidrug Therapy (MDT) which was started in ALERT's control area in January 1983. The MDT programme was extended to the capital, Addis Ababa, and surrounding districts. Total number of patients under MDT at the end of the year was 2868, 448 paucibacillary and 2420 multibacillary patients. In the year under review 4367 patients were released from treatment, 3032 after dapsone monotherapy and 1335 after MDT. All patients released after MDT were paucibacillary patients, as the multibacillary patients have not yet finished their treatment period of 2 years. The Director of ALERT's Leprosy Control, Dr M Becx-Bleumink informed the assembly that the MDT programme will be extended to new districts in 1985.

The Director of Training, Dr J Warndorff gave an outline of the training activities in 1984. ALERT had 73 participants on the International courses, and 220 participants on the National courses. A total of 55 students had in-service training during the year.

Dr Warndorff also gave the encouraging information that out of a total of 17 participants at the WHO Workshop on Reorientation of Leprosy Control for English Speaking Countries, which took place in Banjul, Gambia in July 1984, 13 had received their training at ALERT. This figure is evidence of ALERT's dominant role in the training of leprosy staff in anglophone Africa. The Director of Hospital Services, Dr Taye Tadesse informed the assembly that the Hospital Services had been further improved in 1984, in spite of the fact that the Hospital was short of medical staff during most of the year. ALERT is operating a hospital of 207 beds, shared amongst medicine, surgery and ophthalmology with 104, 90 and 13 beds respectively. ALERT is also rendering extensive services to out-patients, especially in the field of dermatology.

Dr J Warndorff, who is the Director of Training since 1980 will terminate his service in 1985. Mr B Johannessen, the Executive Director since 1979 will also leave ALERT in 1985.

The assembly recorded its appreciation to the outgoing directors for their excellent and hard work during their appointment periods, and for their contributions to the development of ALERT.

Leprosy; a medical film from Science Service, Berlin

In co-operation with the German Leprosy Relief Association, Science Service, Berlin has produced a training film on leprosy, which has now been up-dated and extended in order to include latest results of scientific research and information on multiple drug therapy. The film is 16 mm, Eastman Color Kodak, lasts 26 minutes and costs DM 2100 for optical sound track, or DM 2200 for magnetic sound. Enquiries to Science Service Berlin, Audiovision GmbH, Thamasisstrasse 11-1000, Berlin.

TALMILEP; ILEP Steering Committee on Teaching and Learning Materials

At the recent meeting of the International Federation of Anti-Leprosy Associations; ILEP (234 Blythe Road, London W14 0HJ) in Luxembourg, further meetings and discussions took place to define the status and functions of TALMILEP which was formed as a sub-group of Ad Hoc Working Group No. 5 (Training) in June 1983.

TALMILEP has undertaken to stimulate, facilitate and co-ordinate efforts to provide and distribute teaching and learning materials in leprosy, worldwide, and to devise the most appropriate mechanisms for achieving this within ILEP. Its main aims are as follows:

To co-ordinate efforts for providing all categories of leprosy workers, worldwide, with teaching and learning materials; to promote the development of high quality materials by ILEP member associations, adequately covering all relevant subject aspects, and avoiding unnecessary duplication of work; to set up active distribution networks and thus ensure worldwide availability of materials; to make information about teaching and learning materials generally available.

Eventually, it should be possible for TALMILEP to assist ILEP member associations in answering such questions as: how many copies of a particular booklet are needed for a particular category of leprosy workers, in which languages, and in which countries?; which resource persons can be called upon to translate, adapt, or illustrate material, for example from English into Vietnamese?; where can material be printed at low cost?; how can a new publication be field tested?; how can material be actively and effectively distributed in a particular region?; which materials are best suited of the needs of, for example, leprosy field workers in Ghana?; what material is currently available in, for example, Portuguese?

Six ILEP member organizations are currently involved in TALMILEP, the participants are:

DAHW: Ms K Rossler, acts as secretary and co-ordinates printing and publication.
TLMI: Ms J Neville, co-ordinates distribution in the English language.

ALM: Dr W F Ross, co-ordinates assessment activities.
NSL: Dr D L Leiker, acts as chairman.
Ms I Kalf, co-ordinates survey activities.
FFF: Dr le Coroller, co-ordinates distribution of material in the French language.
DF: Mr L de Meersman, co-ordinates the preparation and production of material.
ILEP C/B: Ms S Lacey, assists with survey activities within ILEP.
WHO: Dr S K Noordeen, maintains contacts with the Global Health Learning Materials Programme of WHO.

Most of the work of TALMILEP is done by correspondence. In addition, the group meets 4 times a year: working meetings are held in April and September (in Europe). Meetings are also held during ILEP Working Sessions in June and December and these are open to all delegates.

The agenda centres around a list of 'items in progress'. In relation to each individual item, survey, assessment, production and distribution aspects are discussed.

For further information please contact: The Secretary TALMILEP, Ms K Rössler, German Leprosy Relief Association, PO Box 348, D-8700 Wurzburg, West Germany.

Recent ideas and progress in the treatment of leprosy; Erwin Stindl Memorial Oration, 1985, India

We are grateful to the Project Director of the Greater Calcutta Leprosy Treatment and Health Education Scheme, 35/1/A, Old Ballygunge 1st Lane, Calcutta-700-019, India, for sending this 40-page booklet which records the oration given by Dr B K Girdhar (Central Jalma Institute for Leprosy, Agra) in memory of Mr Erwin Stindl. Dr Girdhar covers the recent history of the development of chemotherapy for leprosy, notably with multiple drugs, as we know it today and goes on to consider the properties of dapsone, rifampicin, clofazimine, the thioamides, thiacetazone and other drugs in considerable detail. There are no fewer than 161 references to this masterly review of the chemotherapy of leprosy. The booklet is available from the above address at a cost of Rs 15. Considerable emphasis is given to the Jalma finding that 6 months of dual therapy of paucibacillary cases with dapsone and rifampicin is '. . . too short a time for patients to show objective and even subjective improvement . . .'. They recommend continuation of treatment in paucibacillary cases up to the point of disease inactivity. The concluding paragraph of this Oration calls for quotation in full:

'To sum up, I will say we have the availability and knowledge about more potent antileprosy drugs but the outcome is going to be determined by the sincerity and zeal with which we do the work in the field, in programmes like case detection, case holding, defaulter retrieval and drug delivery. It is not the the initiation of the chemotherapeutic programme which is important but the continued maintenance of the initial vigour which is more important for success of chemotherapy in control and eradication of this disease in the years to come.'

***Chinese Journal of Dermatology*, Number 18, 1985**

We have a continuing exchange with various journals in the People's Republic of China, which includes *The Chinese Journal of Dermatology*, c/o Institute of Dermatology, Chinese Academy of Medical Sciences, 100, Jiangwangmiao Taipingmen, Nanjing, Jiangsu, People's Republic of China. It is perhaps not realized that this dermatology journal, and some other journals from China, frequently carry articles of considerable importance on leprosy. For instance, the one referred to above has an important contribution by Dr Ji Baohong (Secretary, Steering Committee of the Scientific Working Group on the Chemotherapy of Leprosy, WHO) entitled 'Observation on bactericidal activity of several drugs against *M. leprae* by proportional bactericidal test.' Abstracts are in English; text in Chinese.

Thalidomide; Chemie Grunenthal stops production and distribution

Among many important matters discussed at the 44th Meeting of the Medical Commission at the recent ILEP meeting in Luxembourg, was the decision by Chemie Grunenthal in Germany to stop production and sale of thalidomide. A few months prior to this meeting it was understood that they might be handing over all remaining stocks to WHO in Geneva and at one time it was proposed that WHO might take responsibility for distribution. However at the above Medical Commission meeting, Dr S K Noordeen reported that a more recent decision by WHO had decided against this. A discussion then took place on possible mechanisms for maintaining supplies of thalidomide for responsible leprosy workers (for the treatment of type 2 reactions in lepromatous leprosy). Meanwhile it was reported that the only company known to produce and export thalidomide is Interbras, Petrobras, Comercio International SA, Rua de Rosario 90, Rio de Janeiro RJ, CEP 20041, Brazil. [From correspondence with Dr D V A Opromolla, we have in fact also received the name and address of another source of thalidomide in Brazil; Fundacao Ezequiel Dias (FUNED), Rua Conde Pereira Carneiro No 90, Gameleira, CEP 30,000 Belo Horizonte, Minas Gerais, Brazil. Further information can be obtained from Dr Aguinaldo Goncales, Director da Divisao Nacional de Dermatologia Sanitaria, Ministerio de Saude, sala 809 80 andar, Esplanada dos Ministerios, Bloco 11, 70,000, Brasilia, Brazil. In recording this information, we emphasize that it is the personal responsibility of any who contact these agencies to clarify formulation, chemical content and dosage. *Editor*.]

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Letters to the Editor

HEPATIC, RENAL AND OTHER SYSTEM ABNORMALITIES IN PATIENTS WITH LEPROMATOUS LEPROSY IN NIGERIA

Sir,

We recently investigated 68 patients with lepromatous (LL) leprosy in the Leprosy Teaching and Training Centre and the Ahmadu Bello University Teaching Hospital in Zaria, mainly in order to assess their hepatic and renal function. Forty-six male and 22 female patients were included; their ages ranged from 16 to 60 years and the duration of their leprosy from 1 to 40 years. Forty out of the total of 68 had suffered from leprosy for more than 10 years. All had received varying periods of antileprosy treatment, mainly with dapsone monotherapy in a dose of 50–100 mg daily.

All 68 showed clear clinical evidence of active or inactive lepromatous leprosy and virtually all patients had bilateral inguinal lymphadenopathy (which may also have been due to lepromatous leprosy). Our specific findings in the hepatic and renal systems are as yet incomplete and will be the subject of further study, but the main point of this letter is to draw attention to a wide range of conditions other than leprosy which we recorded in this group. Fifteen had mild to moderate oedema of the feet, 3 hypertension, 1 jaundice and 3 had a haemoglobin of less than 10 g/100 ml. Sixteen out of 40 patients whose stools were examined for hookworm were positive. From 40 patients whose urine was examined, 32 had abnormalities in the form of albinuria, with or without red or white cells, 4–10 per high power field. One patient had cervical tuberculous lymphadenopathy, proven on biopsy. A variety of liver function abnormalities were discovered in 60 out of a total of 68 patients, and of 2 biopsied, 1 showed mixed nodular cirrhosis and schistosomal parasites and the other, a granulomatous reaction.

From this limited study, it is clear that some findings, such as the granuloma in one of the liver biopsies and the bilateral inguinal lymphadenopathy in many cases, may well have been due to leprosy. It is, however, our impression that conditions *other* than leprosy accounted for most of the abnormalities noted. Viral, parasitic, nutritional and other factors, including alcoholism, have to be considered.

If confirmed, this seems to us to be an important observation, which has not always been acknowledged in similar studies in the literature, notably from India. At least in this part of the world, it has to be accepted that many clinical and laboratory abnormalities in leprosy patients may be due to a wide range of other conditions.

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FAILURE OF PASSIVELY TRANSFERRED LEPROSY LYMPHOCYTES TO DEMYELINATE PERIPHERAL NERVE

Sir,

Recent studies on the interaction between *Mycobacterium leprae* and cells of the immune system have raised the question whether peripheral nerve damage in leprosy is (a) incidental to the cell-mediated immune response to the bacilli in a neural milieu,[1,2] (b) the result of specific humoral and cellular mechanisms directed against neural components,[3, 4] or (c) a combination of the 2 factors. We passively transferred human leprosy lymphocytes intraneurally to peripheral nerves of guinea-pigs and looked for cell-associated demyelination.

The donors of circulating lymphocytes were 8 patients with BT leprosy (2 of them undergoing Type I reaction) and 4 normal individuals. Fifty microlitres of the lymphocyte cell suspension in RPMI 1640 (containing $3{\cdot}75–9 \times 10^6$ cells), or RPMI 1640 alone were injected into the sciatic nerves of guinea-pigs. (Cell separation, counting and viability testing were kindly performed for us by Dr Sita Naik and colleagues at the K.E.M. Hospital Bombay.) The animals were sacrificed on day 4 and the nerves collected for light microscopy. (In an analogous study in Guillain-Barre polyneuropathy, Feasby *et al.*[5] found no evidence of host rejection of intraneurally injected heterologous (human) lymphocytes up to 4 days post-injection.) The histopathologic features were rated semi-quantitatively.

Normal endoneurial contents with a few or moderate numbers of lymphocytes in or outside the perineurium . . . 1

Localized demyelination without cells (attributed to injection damage) . . . 2

Normal endoneurial contents in the presence of lymphocytes . . . 3

Demyelination in the presence of lymphocytes . . . 4

Statistical comparison between the ratings awarded to nerves injected with leprosy lymphocytes and normal lymphocytes and RPMI 1640 alone showed no evidence that demyelination was significantly correlated with the presence of leprosy cells. These observations are compatible with the reported *in vitro* absence in leprosy of circulating T lymphocytes specifically responsive to peripheral nerve myelin. Neither has there been evidence of anti-myelin antibodies in leprosy.[3,4] Specific anti-axonal antibody has been detected in the sera of some lepromatous and tuberculoid leprosy patients but there was no correlation with the degree of nerve damage, and hence no pathogenetic significance.[4] The probability that the 'by-stander' effect causes major nerve fibre damage in non-lepromatous leprosy appears strong.

S S PANDYA & S S NAIK

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AN APPRAISAL OF MALIGNANT PLANTAR ULCER

Sir,

There are few reported cases of malignant change in plantar ulcer in leprosy. The number dwindles if lesions not commencing on the sole are excluded. It is suggested that the remarkably low incidence of malignant change in these ulcers is due to the fact that the plantar surface of the foot is minimally exposed to sunlight. Published case reports support this notion.

Reports in English of malignant change in plantar ulcer in leprosy are remarkably few. This may be because the condition is truly rare, because it is unrecognized, or because it appears to have no particular features warranting publication, or indeed for all 3 reasons.

In Papua New Guinea squamous carcinoma arising in old tropical ulcer scars is very common, and the principal function of the Artifical Limb Factory is to provide prostheses for patients whose amputations were for 'S.C.C. leg'. Against this background the regular appearance of malignant change in plantar ulcers in my own practice has not seemed remarkable, and a score or more of cases seen since 1965 remain inadequately documented. We have even had 2 in the ward at the same time.

However, when every surgical unit on our coast has cases of 'S.C.C. leg' in the ward nearly all the time, so that students pass them by in search of something more interesting, 1 or 2 malignant plantar ulcers a year seem a very small yield given the large number of chronic ulcers presumably at risk.

The scars from tropical ulcer, and from burns, which become malignant, are those which have healed by granulation so that the eventual scar is an area of intense local albinism. Examination of the malignancies arising in such scars almost always clearly reveals their origin in the most depigmented area.

For this reason it was long ago suggested that sunlight was an important aetiological factor.[1] This should not be surprising, given the photobiological axiom that keratoses and epitheliomata may be regarded as normal processes that occur in white skin if exposure to sunlight is continued for long enough.[2] It seems unlikely that malignancy arising in chronic ulcers or scars on the front of the leg and those a few centimetres lower, on the sole, should have differing aetiology.

An examination of published case reports gives some support to this suggestion. Job & Riedel[3] reported 4 cases. Their case 1 seems not to have been a plantar ulcer in the anatomical sense, having occurred on the lateral side of a varus foot, in an area obviously exposed to sunlight.

Their case 2, of which they publish a photograph, shows an ulcer on the lateral side of the heel and lower leg, once again exposed to sunlight. Their other 2 cases were true plantar ulcers, but are not illustrated.

Riedel[4] subsequently published details of another case in which the ulcer was on the plantar aspect of the heel and was connected to the lateral malleolus by a sinus. In the absence of more information it is not possible to speculate on the extent to which the depigmented scar was exposed to the sun in this case.

Srinivasan & Desikan[5] described 13 cases of cauliflower growths, only 3 of which were frankly malignant, all low grade. The other 10 were reported to show pseudo-epitheliomatous hyperplasia, and their good response to local excision confirms this diagnosis.

This condition is occasionally seen in the mouths of betel-nut chewers, and sometimes at the site of an old tropical ulcer, but has not been identified on the sole in my patients.

The fact that Srinivasan[5] was able to collect only 3 malignant plantar ulcers in many years of very busy practice in leprosy suggests that malignant change is fairly rare in plantar ulcer in India.

Andersen[6] recently reported 2 cases of cutaneous squamous carcinoma in leprosy, only one of which was on the sole. He provides a photograph of the case, showing wide depigmentation around the lesion. The similar scar on the instep suggests that the foot was burnt at some time.

Discussion

The base of a chronic ulcer, certainly of plantar ulcer in leprosy, is made of granulation tissue, with no epithelium. Carcinoma is by definition malignant change in epithelium. Granulation tissue alone therefore cannot give rise to squamous carcinoma. It must arise in the surrounding squamous epithelium, or in the scar of a healed ulcer.

The report by Srinivasan and Desikan[5] does not provide detail about depigmentation, but 2 of the other 3 papers describe and/or illustrate malignant change in scarred depigmented areas. The only published photograph of a truly malignant, truly plantar, ulcer in which the immediately surrounding skin is visible is Andersen's, and in this case depigmentation is very obvious.

Intense depigmentation such as follows tropical ulcer or full-thickness burns is far from the rule in plantar ulcer. Ulcers of many years duration frequently have virtually normally pigmented skin right up to the edges.

Therefore it is suggested that malignant plantar ulcer is rare because the lesion is comparatively protected from direct sunlight and because in general the surrounding epithelium is insufficiently depigmented to be at serious risk. Chronic sepsis, *per se*, is not the cause of malignant change in plantar ulcer.

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A Note from the Editor to Contributors of *Leprosy Review*

During the past year we have occasionally had problems with the receipt, acknowledgement, posting and preparation of manuscripts, and we take this opportunity to draw attention to a few points which may ease the editorial 'process'.

1 Envelopes and packaging. Quite a number of manuscripts have been received with the envelope frayed, or even open along the edges. A strong envelope is essential and the use of plastic 'grips' or spines to hold pages together should be avoided, since they cut through the paper.

2 Originals and copies. We need a clear (black) original and an equally clear copy. We usually have to make at least two additional copies and this is impossible from a faint or poor quality original. Good quality photostat copies are preferable to carbon copies. Artwork, especially lettering, should be sufficiently clear to stand a reduction of about 60%.

3 Return of manuscripts to authors. The current costs of correspondence and air mail postage incurred by this Journal are already considerable. We regret that it is not possible, except under exceptional circumstances, to return manuscripts, photographs or artwork to authors. If a paper has not been found suitable for publication, we retain it here for reference for a period of 1 year, after which it is discarded.

4 Addressing. All matters to do with *manuscripts*, *publishing*, *printing* and the *editorial 'process'* should be addressed to the Editor or Editorial Assistant at the Slade Hospital, Headington, Oxford OX3 7JH, England. All matters to do with *subscriptions to the Journal*, *postage* and *distribution*, should be referred to LEPRA, Fairfax House, Causton Road, Colchester CO1 1PU, England. On several occasions, authors have changed address without letting us know. Please indicate your address for reply or any likely change of address in the near future.

5 Titles. Both for the purposes of our own indexing of this Journal, but even more importantly for general indexing and abstracting systems, it is important in most instances to get 'leprosy' (or some related word) into the title as a general guide to the subject matter.

6 Summaries. Authors, especially those working in pure or basic science, are asked to keep in mind that this Journal has a wide-ranging readership. Many subscribers do not appreciate the significance of scientific data *per se* and it would be of great help if authors could include in their summaries a brief note explaining *why* the study was undertaken and *what the results mean*, in terms which are likely to be comprehensible to the reader whose background is not scientific.

Thank you,
EDITOR

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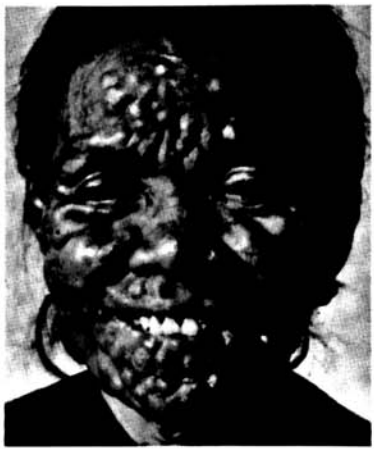

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