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Editorial

AFTER MULTIDRUG THERAPY (MDT): WHO IS RESPONSIBLE FOR CONTINUING CARE?

Before considering who is responsible for providing continuing care, it is necessary to look at the present situation regarding multidrug therapy (MDT).

Most leprosy endemic countries in the world have introduced, or are in the process of introducing the WHO recommended MDT regimens for leprosy control.[1] However, data available in 1986[2] showed that only 8·8% of the total number of registered cases in the world were receiving MDT. This was four years after the WHO Study Group recommended that, as a matter of urgency, combined chemotherapy regimens should be introduced in all leprosy control programmes.

This slow progress in the introduction of MDT is understandable to planners and field staff alike, and is a cause for concern. It is partly due to inadequate financial and personnel resources, and very often due to transport problems. It could well be argued that with limited resources, those that are available must first be used for implementing MDT, and that it is unrealistic to consider continuing care until all leprosy patients have been treated with MDT. However, the importance of patient care was underlined at a recent meeting of WHO and nongovernmental organizations.[3] Some programme planners are already aware of this, and recognize that continuing patient care not only helps the individual but also reinforces the credibility of the MDT programme.

Surveillance and continuing care

Many leprosy control programmes plan for the surveillance of both paucibacillary (PB) and multibacillary (MB) patients after the completion of chemotherapy in order to monitor and treat relapse or reaction. The mechanism for doing this varies from country to country. For instance, PB patients are checked annually for 4 years in western Nepal; at 3, 6 and 8 months after release from treatment in Ethiopia, and annually for 2 years in India. Multibacillary (MB) patients in India and Ethiopia are asked to report annually for 5 years for clinical and bacteriological examinations; in Nepal, the annual surveillance period is 8 years, and in Bhutan 10 years. In these countries patients are not followed up if they do not report voluntarily for examination. Indeed, some patients in Nepal requested not to be followed up either by a home visit or by letter.

But continuing care is more than surveillance, and is usually designed to meet the felt needs of patients who have been discharged from chemotherapy, but who are still faced with a variety of problems. Some will need help with readjustment problems or job training: the elderly and disabled may need residential care; those with insensitive feet will need protective footwear. Others may develop deformity after the completion of chemotherapy, and require teaching and care. There will need to be a flexibility of approach to continuing care, just as there is in the surveillance of patients after release from treatment.

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Already some leprosy control programmes have made provision for continuing care, and this varies from country to country, according to the number of leprosy patients and the resources available. In Lesotho, some disabled patients are provided with a pension, and are able to purchase subsidized protective footwear. The National Leprosy/Tuberculosis programme in Kenya[4] is proposing to have a care register to list those patients who will require long-term care for insensitive hands, feet or eyes, the provision of footwear or reconstructive surgery. In India,[5] an ambitious 3-year scheme 'Care after Cure' is in operation, and is designed to follow up all patients who have been released from control since the early 1970s. They number about 9700, of which 30% or 3000 had visible deformity on discharge. Perhaps 2000 still need care; the project aims to contact them and review them medically and offer help in the form of social service or employment opportunity.

Timing

During the preparation phase for the introduction of MDT, and during the first year of implementation, there is an increased workload for the staff.[6] Obviously if continuing care is proposed, it is not feasible to attempt it early on in the programme. However, after the discharge of many PB patients from treatment, the caseload will be reduced and staff will, in theory, have more time for patient care—both for those still receiving treatment, and for others needing continuing care.[7]

But in some projects, it may already be too late to reassign staff time to continuing care, as staff in the leprosy control programme have been given new tasks, or have been diverted to other work. All patients on treatment are usually screened before the introduction of MDT, and many inactive PB cases released from treatment. If a disability register is kept at this point for those who need continuing care and protective footwear, then this will assist in later planning and budgeting.

Resources

Having identified those patients who need continuing care, the next question will be 'who will provide it?' In some projects it will be appropriate to select leprosy control or health centre staff who have shown an aptitude for communicating with patients and the community in which they live. It may also be useful to look wider and enlist the help of others outside the health centre team. Perhaps a physiotherapist at the district hospital will be able to supervise the production of footwear,[8] or the local village carpenter help with the production and repair of simple artificial limbs.

Another possibility in the future will be to cooperate with the Community Based Rehabilitation Worker (CBRW)[9] in continuing care for leprosy patients. This is a new approach to the care of the disabled in the community and is closely related to primary health care. Until now rehabilitation services have been town based, but CBRWs will make these services available to people with physical, mental and sensory disabilities within their own community. To make this concept a reality, a one-year course is now available to train the teachers and supervisors of CBRWs.[10] It is encouraging to see that sessions on the prevention of disability in leprosy are included in the course.

Many governments find it difficult to finance the implementation of MDT and look to nongovernmental organizations (NGOs) for assistance. Would it not be appropriate that these agencies, because of the flexibility of their approach take some of the responsibility for continuing care by providing funds or experienced staff?

One purpose of this article has been to stimulate thought and discussion about the need for continuing care for selected leprosy patients after the completion of chemotherapy, and to suggest possible approaches. Perhaps the key activity will be the selection of a suitable person to plan and

co-ordinate continuing care, with the ability to enlist the help of a wide variety of people in order to provide this care.

Finally, continuing care needs to be planned in consultation with the patients themselves. The main responsibility for continuing care will rest with the patients and their families and it is therefore logical to invite them to contribute their ideas to the ongoing discussion.

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Note. As this issue goes to press our attention has been drawn to more recent data on MDT coverage published in the *WHO Statistics Annual*, December 1987, pp 23–4.

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TEACHING MATERIALS AND SERVICES

Essential Drugs Monitor; WHO

The *Essential Drugs Monitor* is a newsletter produced and distributed by the WHO Action Programme on Essential Drugs and Vaccines and by the Division of Public Information and Education for Health. Since the Action Programme was launched in 1981, more than 100 countries have either drawn up essential drugs lists or started projects in support of primary health care, providing reliable essential drugs and vaccines which: meet people's common health needs; have significant therapeutic value; are acceptably safe; and offer satisfactory value for money.

All correspondence should be addressed to the Editor, *Essential Drugs Monitor*, World Health Organization, CH-1211 Geneva 27, Switzerland.

Number 5, 1987 includes a note on the front page of a generous grant from the Dutch: 'The Netherlands Special Development Cooperation Programme has made a grant of more than US $10 million to the Action Programme. Out of this amount, about 95% will be used over the next 3 years to reinforce national essential drug policies in Gambia, Kenya, Malawi, Sudan and Yemen Arab Republic. The rest will be used for parts of WHO's Revised Drug Strategy (see *Monitor 3–1987)*.'

Page 2 describes the Uganda Essential Drugs Manual: 'First Edition, Ministry of Health, Republic of Uganda, 1986. For health workers dispensing from the Ministry's Essential Drugs Kit (23 drugs for PHC). Introduces the ED concept, the kit and programme management. Other chapters cover receiving and storing drugs, healing without drugs, problem-solving, and treatment and prevention. In English. Copiously illustrated. Available from: Ministry of Health, Kampala, Uganda.'

Also on page 2 the following is described: *Price Indicator on International Low-Price Sources for Essential Drugs*, Third Edition, Medico International, 1987. Pocket guide subtitled 'Rational Drug Therapy in Facts and Comparisons', giving dollar prices for 9 different sources for essential drugs on WHO's Model List. This edition includes an increased range of generic suppliers. Lists of drugs given in English only, but introduction and notes in French and Spanish also. Available from: Medico International, Hanauer Landstrasse 147-149, D-6000 Frankfurt am Main, Federal Republic of Germany, or from the Action Programme.

On page 6 there is an important note for those in leprosy and tuberculosis who may have doubts about the quality and stated quantity of rifampicin in capsules from 'low-price' sources: 'Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce, WHO, 1985. In English, French and Spanish. Contains the WHO recommendations on ensuring the quality of drugs in international commerce.'

Finally, there is encouraging news from Thailand: Thailand is on target, or nearly so, in 5 major areas of its National Drug Policy. A WHO essential drugs review team, visiting in 1986, was impressed by what had been achieved in 5 years through coordinated efforts by government departments, especially the Food and Drug Administration.

Availability of drugs. By 1986, half of all villages (24,000 out of 50,000) in Thailand had Drug and Medical Products Funds (drug cooperatives) providing primary health care drugs.

Essential Drugs Monitor is probably essential reading for those responsible for leprosy (and tuberculosis) control. Are we paying enough attention to the absolutely regular supply of all the drugs needed for the treatment of leprosy in adult and child dosage? *Editor.*

Journal of Compliance Health Care, USA

The Journal provides a central source of knowledge for confronting the pervasive problems caused by noncompliance, as it affects patients' quality of life, the cost and effectiveness of health care, and the morale and the malpractice concerns of service providers.

Contributions are encouraged from the fields of medicine, nursing, psychology, public health, dentistry, rehabilitation, nutrition, pharmacology, social work, medical sociology and anthropology, law, and management and administration.

Topics to be covered include: disease and regimen factors, identification of patient and provider variables, and facilitating and blocking variables in attaining compliance; the clinical roles of health care providers in preventing, monitoring, and reducing patient noncompliance, health planning, fiscal analyses, and other administrative and policy decisions as these affect compliance responses. The Journal will also feature book reviews, information on forthcoming workshops and conventions, position openings, job and training situations wanted, letters to the editor, and resources and requests for materials, information, and research.

We have just received the inaugural issue: Volume 1, Number 1, Spring 1986, and take this opportunity to wish this new journal every success. The contents list includes an article on an intervention to improve compliance to year-long isoniazid (INAH) therapy for tuberculosis and we look forward to the possibility that future issues of this journal will address the matter of compliance to dapsone, clofazimine and rifampicin in leprosy. Editorial office: Dr Raymond A Ulmer, Editor in Chief, The Journal of Compliance and Health Care, The Noncompliance Institute of Los Angeles, 1888 Century Park East, Suite 800, Los Angeles, CA 900067, USA.

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Characteristics of the multiplication of dapsone-resistant strains of *Mycobacterium leprae* in mice*

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

Accepted for publication 9 September 1987

Summary Twenty-seven per cent of the 49 strains of *Mycobacterium leprae* isolated in the course of the THELEP controlled clinical trials of combined chemotherapy of lepromatous leprosy in Bamako and Chingleput, and found to be resistant to dapsone multiplied in significantly fewer mice administered dapsone than in mice administered the dapsone-free diet.

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Also participating in this study were Drs R D McDermott and G F R Hilson, Department of Medical Microbiology, St George's Hospital Medical School, London, UK. Mr J L Duppenthaler,

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World Health Organization, Geneva, Switzerland, provided statistical consultation throughout the trials, and Mrs M Anker, World Health Organization, performed the statistical analyses.

Introduction

During the years 1978–1983, trials of combined chemotherapy were carried out among 215 patients with previously untreated multibacillary (LL, LI and BL) leprosy at the Institute Marchoux, Bamako, Mali, and the Central Leprosy Teaching and Research Institute, Chingleput, South India.[1]. Of the 131 patients, the susceptibility of whose strains of *Mycobacterium leprae* to dapsone could be determined, 49 (37·4%) were found to harbour strains resistant to the drug; all but 10 of these strains were resistant only to 0·0001 g dapsone per 100 g mouse diet, the smallest concentration of the drug tested, and no strain resistant to the largest concentration (0·01 g per 100 g mouse diet) was encountered.[2]

In the course of the study, it was observed that a proportion of the strains of dapsone-resistant *M. leprae* did not multiply in as many of the mice administered dapsone as in the mice administered drug-free diet. Because there was no ready explanation for this phenomenon, the relevant data have been analysed.

Materials and methods

The methods employed to test the susceptibility to dapsone of the pretreatment isolates of *M. leprae*, are those already described.[2] In brief, before beginning treatment by means of one of the trial regimens, a biopsy-specimen was obtained from a skin lesion of each patient and air-shipped on wet ice to London. In the Department of Medical Microbiology, St George's Hospital Medical School, the specimens were homogenized, and the *M. leprae* were recovered, counted, diluted to provide an inoculum of 10^4 organisms per footpad, and inoculated into the right-hind footpads of CD-1 mice. Groups of 8 mice were administered a drug-free diet, and groups of 5–7 mice were fed diets into which had been incorporated dapsone in concentrations of 0·0001, 0·001 or 0·01 g per 100 g diet. Approximately six months after inoculation, several control mice (those administered the drug-free diet) were killed, and *M. leprae* were harvested from the inoculated footpads. If *M. leprae* were found not to have multiplied after 6 months, additional harvests were performed from control mice 3 months later. Similarly, if *M. leprae* were found not to have multiplied after 9 months, harvests were performed from additional control mice after 12 months. When the organisms were noted to have multiplied in control mice, harvests were performed from all surviving treated mice. *M. leprae* were determined to be resistant to dapsone in a given concentration if they multiplied to $\geq 10^5$ per footpad in at least one mouse administered dapsone in that concentration. The data were analysed by means of the χ^2 and Fisher exact probability techniques for comparison of frequencies.[3]

Results

As demonstrated by the examples presented in Table 1, three patterns of multiplication were observed. The results of testing the pretreatment isolate from patient 1061 for resistance to dapsone reveal that multiplication had occurred in almost all of the mice administered the drug-free diet and the diet containing dapsone in the concentration of 0·0001 g per 100 g, and in none of the mice administered dapsone in the concentration of 0·001 g per 100 g diet. Thus, this strain, which is resistant to the smallest concentration of dapsone, and susceptible to the intermediate concentration, multiplied virtually as readily in mice administered the largest concentration of dapsone permitting multiplication as in those administered the drug-free diet.

Table 1. Results of dapsone-resistance measurement on pretreatment isolates of three patients

Concentration of dapsone (g%)	Results of mouse-by-mouse harvests ($\times 10^4$)	Proportion of mice showing multiplication	P*
Patient 1061†			
0	150, 98, 61, 39, 26, 14, 11, 8·4	7/8	0·28
0·0001	32, 25, 21, 13, 12, 8·8, 6·4, < 1·0	5/8	
0·001	5·4, 3·6, 3·6, 1·2, < 1·0, < 1·0, < 1·0	0/7	
Patient 1117			
0	37, 30, 15, 7·1, 4·9, 3·1	3/6	0·59
0·0001	120, 40, 16, 12, 3·6 2·7, 1·3	4/7	0·21
0·001	22, 16, 12, 7·1	3/4	
0·01	6·7, 4·0, < 1·0, < 1·0	0/4	
Patient 1001			
0	140, 130, 120, 81, 19, 10, 6·0, 4·4	6/8	0·05
0·0001	11, 5·2, 4·4, 4·0, 3·2, 2·4	1/6	
0·001	< 1·0, < 1·0, < 1·0, < 1·0, < 1·0, < 1·0	0/6	

* The probability, calculated by Fisher's exact test,[3] that the adjacent pairs of results have been drawn from the same population.

† The patient number consists of the number of the centre (1, Chingleput; 2, Bamako) and the three-digit number representing the order in which the patient was recruited.

A second pattern is exemplified by the results of testing the susceptibility to dapsone of the *M. leprae* isolated from the pretreatment biopsy-specimen of patient 1117. In this case, the organisms may be seen to have multiplied in only a fraction of the mice, but in much the same fraction of mice administered drug-free diet and those administered diets into which dapsone had been incorporated in concentrations of 0·0001 or 0·001 g per 100 g diet. This strain, which manifests an intermediate degree of resistance to dapsone, also multiplied as readily in the mice administered the largest concentration of dapsone permitting multiplication as in control mice, but, probably because the proportion of viable organisms in the inocula was small, multiplication occurred in an average of only 60% of the mice.

The third pattern is exemplified by the results obtained from the study of the pretreatment specimen of patient 1001. In this case, the *M. leprae* may be noted to have multiplied in 6 of 8 mice administered the drug-free diet, but in only 1 of 6 mice administered 0·0001 g dapsone per 100 g diet, a significantly smaller proportion ($P = 0{\cdot}05$).

The results of inoculating mice with *M. leprae* of the 49 dapsone-resistant strains are summarized in Table 2, in which are listed, for each strain, both the numbers of mice in which no evidence of multiplication was found, and the numbers of mice in which the organisms were found to have multiplied. With respect to those 39 strains exhibiting a low degree of resistance, 9 (23%) were found to have multiplied in significantly fewer dapsone-treated than control mice. Among the 10 strains that demonstrated an intermediate degree of resistance, 3 multiplied more readily in control mice than in mice administered dapsone in the smallest concentration; one of these strains and one additional strain multiplied more readily in control animals than in mice administered dapsone in the intermediate concentration. Thus, 13 of the 49 (27%) dapsone-resistant strains manifested the third pattern of multiplication, multiplying in significantly fewer treated that control mice.

In Table 3 are shown the distributions of specimens demonstrating different patterns of multiplication. The *M. leprae* of the majority [28 of 48 (58%)] of the strains demonstrating

Table 2. Strains of *M. leprae* manifesting resistance to dapsone

Patient No.	Number of mice yielding: Dapsone concentration (g/100 g diet) 0		0·0001		0·001	
	$<10^5$	$\geq 10^5$	$<10^5$	$\geq 10^5$	$<10^5$	$\geq 10^5$
Low-grade resistance						
1001*	2	6	5	1†	6	0
1008	0	6	5	2†	7	0
1025	1	7	5	2†	7	0
1028	3	2	2	3	7	0
1050	0	4	3	3	5	0
1053	5	3	7	1	4	0
1055	6	2	7	1	ND‡	
1060	4	4	4	4	5	0
1061	1	7	3	5	7	0
1062	2	6	2	4	7	0
1064	2	6	4	1	5	0
1067	6	2	6	1	5	0
1078	3	3	5	1	4	0
1093	2	5	6	1†	5	0
1097	3	3	4	3	5	0
1104	3	4	4	2	5	0
1108	0	4	3	2	5	0
1116	2	5	5	1	5	0
2008	0	3	8	1†	10	0
2018	6	3	6	1	7	0
2028	1	7	3	3	7	0
2030	0	6	5	2†	7	0
2032	6	3	7	1	6	0
2037	6	1	6	1	ND	
2043	0	3	2	3	5	0
2051	2	3	4	2	4	0
2054	1	5	5	1†	6	0
2057	1	4	5	1	4	0
2059	3	0	5	1	5	0
2060	3	3	5	1	4	0
2064	0	6	4	1†	5	0
2069	ND		5	1	10	0
2071	3	4	3	4	6	0
2075	3	4	6	1	5	0
2087	0	7	2	5	5	0
2089	1	6	6	1†	5	0
2090	3	4	5	1	5	0
2095	3	4	6	1	5	0
2098	2	8	2	6	10	0
Intermediate resistance						
1059	3	5	8	0†	4	1
1080	0	6	1	5	2	2
1037	0	5	1	4	2	5
1117	3	3	3	4	1	3
2025	1	6	6	2†	6	1†
2026	0	7	2	6	5	1†
2034	2	5	0	7	2	4
2072	3	3	6	1	4	1
2088	1	5	5	1†	4	1
2096	2	5	4	2	4	1

* The patient is shown in the first column. In the remaining columns are entered the numbers of mice yielding on harvest the number of organisms shown at the head of each column. In no case did *M. leprae* multiply in mice administered dapsone in the concentration of 0·01 g per 100 g diet.

† $P \leq 0{\cdot}05$ by Fisher's exact test,[3] when this distribution of mice demonstrating multiplication and those not showing multiplication is compared with that in the untreated control mice.

‡ Not done.

Table 3. Proportion of mice showing multiplication as a function of degree of resistance and concentration of dapsone

Degree of resistance	Dapsone concentration (g%)	Proportion of mice showing multiplication				
		<0·20	0·20–0·39	0·40–0·59	0·60–0·79	>0·80
		(Numbers of specimens)				
Low	0	2	5	11	5	15*
	0·0001	19	7	6	6	0*
Intermediate	0	0	0	2	3	5
	0·0001	3	2	1	1	3
	0·001	2	4	1	3	0

* For one strain, no harvests were reported from untreated mice.

resistance to the smallest or intermediate concentrations of dapsone were found to have multiplied in more than 60% of the mice administered dapsone-free diet, whereas, in the mice administered dapsone in the concentration of 0·0001 g per 100 g diet, this pattern of multiplication was found in the case of only 6 of 38 (16%) strains demonstrating resistance to dapsone at this level, and in the mice administered 0·001 g dapsone per 100 g diet, in the case of only 3 of 10 strains resistant at this level.

Discussion

That the *M. leprae* of 49 of the 131 strains multiplied in mice administered dapsone in some concentration provided an opportunity to analyse the patterns of multiplication of dapsone-resistant organisms in dapsone-treated mice. More than 25% of the strains multiplied in significantly fewer of the mice administered dapsone than in mice administered dapsone-free diet. Hastings has described[4] a similar study of 75 strains of *M. leprae* resistant to dapsone, in which harvests (presumably from pooled mouse footpad tissues) from mice administered the largest concentration of dapsone permitting some multiplication were reported consistently to yield fewer organisms than were obtained from simultaneous harvests from mice administered smaller concentrations of dapsone or control diet.

The explanation for this phenomenon is obscure. Some authors[4–9] have suggested the coexistence in the biopsy-specimen of more than a single strain of *M. leprae*, the strains exhibiting different degrees of resistance to dapsone. In the case of primary resistance, however, such an hypothesis does not appear tenable. Because infection with *M. leprae* probably involves only one or, at most, a very small number of viable organisms, it appears unlikely indeed that the patients were infected *ab initio* by *M. leprae* representing a mixture of drug-resistant and drug-susceptible strains. One might consider as an alternative the possibility of superinfection by a second strain differing in susceptibility to dapsone from the strain causing the first infection. Although superinfection has been proposed as the cause of some instances of relapse,[10] however, no evidence has been produced to show that superinfection occurs under any circumstances.

A second alternative explanation, that of phenotypic variation of susceptibility among the members of a genetically homogeneous population, has been proposed by Ji.[11] It should be possible to exclude infection by a mixture of susceptible and resistant strains of *M. leprae* by experiments in mice. Ji[11] suggested as one experimental approach, simply subinoculating organisms harvested from both control and dapsone-treated mice into new groups of mice, and repeating the test of

susceptibility on both isolates. If the pattern of multiplication in both control and untreated mice of the organisms isolated from treated mice was the same as that in the original test, i.e. greater multiplication in control than in dapsone-treated mice, one could exclude the possibility of infection by a mixture of strains.

It appears more likely that, in mice administered dapsone in or near the minimal effective dosage, *M. leprae* multiply more slowly than in control mice or mice administered dapsone in a less than maximally effective dosage. Such a phenomenon has been described by Seydel[12] in the case of a laboratory-derived, dapsone-resistant strain of '*M. lufu*'.

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Lepr Rev (1988) **59,** 11–15

Superdelayed parturition in armadillos: a new mammalian survival strategy

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Summary. Armadillos are generally believed to have a gestation period of 8–9 months that includes a 3- to 4-month period of embryonic diapause. Nevertheless, 21 females bore litters 13–24 months after capture and subsequent isolation from males. Two of these animals were pregnant in successive years. Evidence is presented that this is a facultative survival mechanism, induced by stress, that has not been previously reported among mammals.

Introduction

Armadillos (*Dasypus novemcinctus*) are placental mammals (Order Xenarthra) that are usually monestrous and monovular.[1] In North America, parous females ovulate during July and August and nulliparous animals in November. After summer ovulation and mating, embryos develop to the blastocyst stage and then enter a diapause period of 3–4 months. Implantation of a single blastocyst usually takes place in late November or early December,[1] after which the cell mass buds twice to yield 4 embryos.[2,3] Monozygous quadruplets are born in the spring about 5 months after implantation.[1] Thus gestation is believed to last for 8–9 months including embryonic diapause. However, we have found that some females bear young 1–2 years after the generally accepted time for parturition, a phenomenon not previously reported among mammals.

Materials and methods

This finding resulted from the use of wild-caught armadillos in leprosy research[4] so that large numbers of them are housed in laboratories throughout the world. To minimize injuries, all animals in our colonies in Florida and England were housed separately in plastic cages for 1–3 years before they were killed for harvest of leprosy bacilli. Cage design and the protocols used precluded copulation after capture. These procedures are described in detail elsewhere.[5]

Animals were collected in groups of 10–40 in Central Florida from 1979 to June 1986. The dates of capture were governed by the requirements of the leprosy programme; therefore, sampling of the wild population was not uniform from month to month.

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Results

Of 633 females that were housed in Florida, 179 (28%) bore litters during their first parturition season in captivity.

Of 336 females that survived a second parturition season, 15 produced litters 13–20 months after capture (Table 1). Two of these animals were also pregnant during their first year in captivity. A sixteenth animal gave birth to a litter of 4 females during her third season in captivity, 24 months after capture and 32 months after she last mated, assuming that this took place in the wild during July and August.

The birth rate among females delivering young during their second year in captivity was only 18% of that found during the first year, but under some circumstances can be much higher. Eight females captured in Florida during November 1984 were sent by air freight to England in January 1985. They were shipped in separate containers and were caged individually thereafter. One of these was killed in August 1985. During the spring of 1986, five of the seven surviving animals produced litters 16 months after capture (Table 1).

The finding that 70% of the females shipped to England experienced delayed parturition indicated that this phenomenon might be induced by stress, and thus could serve as a facultative

Table 1. Litters produced by armadillos during their second & third parturition seasons in captivity

Year	Date of birth	Months post-capture	Composition of litter
1981	23 Mar	16·2	3 M
	31 Mar	17·7	3 F
	10 Apr	17·8	3 U
	14 Apr	16·9	4 F
1982	7 Jan‡	12·7	4 M
1983	21 Mar	15·6	U U
	31 Mar	19·9	4 M
	4 May	15·8	4 M
1984	26 Mar	12·7	4 M
	25 Apr	15·2	U U
1985	1 Feb‡	12·6	4 M
	11 Feb	24·3	4 F
	7 Mar†	13·7	3 F
	11 Mar	14·0	U U
	22 Apr	15·2	4 F
1986	15 Feb*	15·4	4 M
	1 Mar*	15·8	U U
	4 Mar*	15·9	4 F
	11 Mar*	16·2	4 M
	18 Mar*	16·3	4 M
	30 Mar	16·7	2 M

* Born in England.
† In third parturition season.
‡ Found in necropsy.
M, males; F, females; U, unknown due to cannibalism by dams.

survival mechanism. Major stresses to which these animals are subjected are capture and, where applicable, shipment to other laboratories. On relating month of capture to incidence of delayed births it was found that 20 delayed births occurred among 204 animals procured from November to February (Table 2). Of 132 animals captured from March to October, only one exhibited delayed parturition.

Insight into this phenomenon can be gained by relating the month of capture to birth rates during the first parturition season in captivity. A bimodal distribution with a minimum in November is obtained (Figure 1). Only 5% of animals captured during ovulation months (July and August) bore young the following spring, whereas 17% of animals captured during embryonic diapause (September and October) had litters. Productivity fell to 7% for November captures, the month during which blastocyst implantation most often takes place, but increased to 32% for December captures. The birth rate peaked at 52% for animals captured in February, but declined thereafter, since many bore young in the wild before capture.

November is a critical month as shown by the finding that only 7% of females captured then had litters during their first year in captivity, compared to 18% in the second year. Of 17 females captured in November of 1984, none had young the following spring. However, of 12 animals that survived a second season in captivity, 6 produced litters. For all other months, first-year births predominated overwhelmingly. This suggests that an additional year of diapause is induced by capturing animals when blastocyst implantation is at its peak, possibly because stress-induced changes of hormonal levels in newly captured animals inhibit implantation[1,6] or delay postimplantation development.

The average date on which parturition occurred differed for first and second season births. The average date of first season births was May 4 ± 20 days based on 112 litters. The litters that were born to dams captured during parturition months (March–June) were excluded to avoid making the result biased. This date did not vary significantly with the year or month of capture, and thus is characteristic for armadillos in their first year in captivity.

The average date of delayed births was March 24 ± 23 days. The difference between the two dates is $41 \pm 5{\cdot}6$ days, or more than 7 standard deviations. Thus animals maintained for one year or more under standard conditions of temperature, day-length and nutrition gave birth one month earlier in the year of parturition than those newly brought in from the wild.

Reproduction delay in armadillos is of longer duration than for all other mammals. The longest period known previously was for a marsupial, the tammar wallaby (*Macropus eugenii*) that has an embryonic diapause of 11 months and a development period of 28 days.[7] Assuming that armadillos ovulate on 1 August and undergo a 5-month period of embryonic development, reproduction delay for animals giving birth during their second season in captivity was $15 \pm 0{\cdot}8$ months. The animal

Table 2. Relation between the month of capture and the percent of females producing young during their first and second parturition seasons in captivity

	First season		Second season	
Month captured	No. of females	With young (%)	No. of females	With young (%)
Nov	71	7	57	18
Dec	41	32	30	7
Jan	137	42	87	7
Feb	89	52	30	7
Others	295	19	132	<1
Total	633	28	336	6

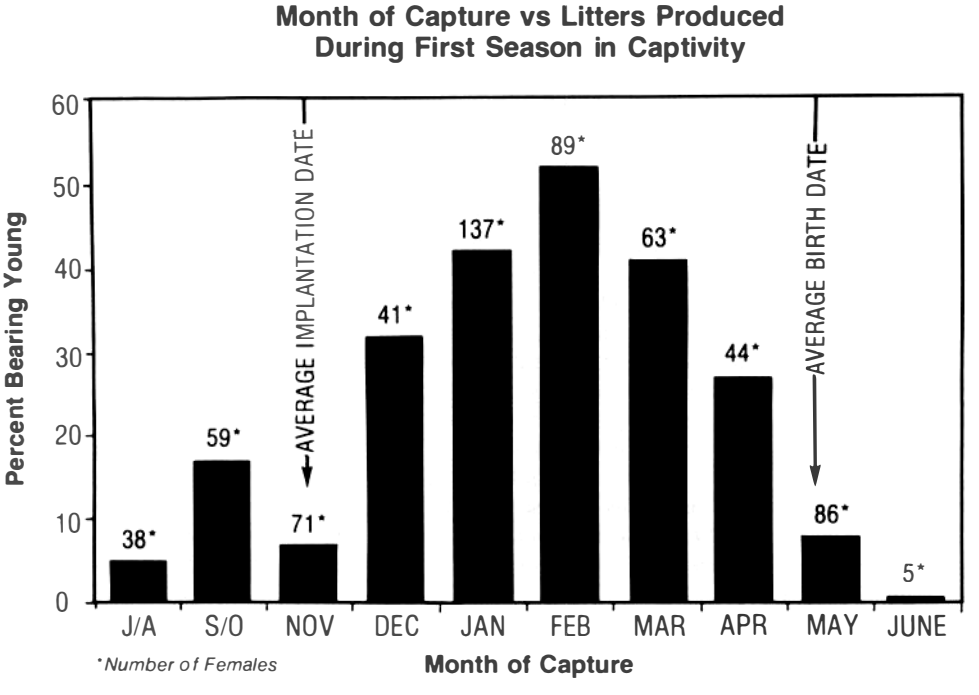

Figure 1.

that produced young during her third season had a delay of 23–24 months. Thus delay in armadillos can extend into successive parturition seasons, while the reproduction cycle of the wallaby is completed within one year. Also, wallaby blastocysts resume growth in the current season if the young in the pouch dies or is otherwise removed, while delay in armadillos persists until the following implantation season.

Discussion

The most probable explanation of delayed parturition in armadillos is that stresses experienced during the peri-implantation period prolong the generally accepted diapause of 3–4 months for an additional year, and in one case 2 years. However, this does not explain why 2 females were pregnant in successive years. Diovulation in armadillos is rare;[1,3] nevertheless, it is possible that two fertilized ova were produced by each female during the same year, and one was stored for 15 months while the other developed. Alternately, ova could have been produced in successive years, and the later ones fertilized by sperm that remained viable in the reproductive tracts of the females. Thus superdelayed embryo activation combined with diovulation or delayed fertilization provide working hypotheses that can be used to design studies on *in vivo* storage of embryos and gametes.

Discovery of superdelayed parturition helps to clarify anomalies in the reproduction strategy of armadillos. Basically, they lean heavily toward 'K' selection. This conclusion is based on body-weight (3–5 kg), time required to reach sexual maturity (29 months), longevity (10–12 years), monovularity, implantation of only one blastocyst each year, long gestation period and birth of precocial young. However, armadillos also have characteristics that link them with 'r' selected

species. Thus they have the lowest encephalization quotient among Xenarthrans (0·371) and one of the lowest among mammals.[8]

This combination of relatively small brain size with low birth rate would be expected to limit their range and reduce prospects of species survival. Instead, *D. novemcinctus* is by far the most successful Xenarthran, and in the last century and a half has expanded its range from a small bridgehead in South Texas to all of the Gulf Coast and many contiguous states of the United States.[9]

Capacity to implant only one embryo each year could presage extinction in a species in which maternal care is minimal. However, evolutionary development of polyembryony, unique to Dasypodinae, increased birth rate 4-fold and at the same time insured preservation of individual genomes by natural cloning. Now it has been shown that female armadillos are able to bear young for one or more seasons after their last contact with males. This phenomenon could play an important role in their territorial expansion and survival of the species.

Acknowledgments

The authors appreciate the faithful cooperation of Ms Jan Stoddard who diligently supervised the Florida animal colonies and Mr A Mullard who supervised the colonies in England throughout the programme. We are grateful for the advice and encouragement of the late Professor Ralph W Wetzel and dedicate this paper to his memory. The colonies of armadillos in the UK and USA were supported by the Immunology of Leprosy (IMMLEP) component of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases. Additional support for the USA colonies was provided by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health.

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LEPROSY CONTROL AND FIELD WORK

Charts for multiple drug therapy (MDT) in paucibacillary and multibacillary leprosy

In the December issue of *Lepr Rev*, 1987; **58:** 438–9 diagrams were printed which explain the drugs, dosages and periods of treatment for paucibacillary and multibacillary leprosy, according to WHO recommendations. They are normally produced on card (A4 size) rather than paper, the information being printed on both sides, with laminating and sealing of the edges in plastic. A recent estimate in Oxford, UK, for the printing of 1000 of these charts came to about £100.00, with an additional £480.00 for laminating. They are intended for desk or clinic use by all those responsible for the implementation of MDT; they are also of value in small group teaching. Further enquiries to this *Editorial Office*.

WHO: Disability Prevention and Rehabilitation in Leprosy, 1987

This WHO Report (WHO/CDS/LEP 87.3) is of a consultation on disability prevention and rehabilitation in leprosy which took place in Geneva, March 1987. The two subjects were considered under the following main headings: introduction, background information and state-of-the-art, preventing and limiting disabilities, rehabilitation, research and recommendations. The list of participants was as follows:

Dr (Mrs) Brand, National Hansens's Disease Centre, Carville, LA 70721, USA; Dr S D Gokhale, International Leprosy Union, A-2, Rasadhara Co-operative Housing Society Ltd, 385 S.V.P. Road, Girgaum, Bombay-400 004, India; Dr W Felton Ross, American Leprosy Mission, One Broadway, Elmood Park, New Jersey 07407, USA; Dr Maria Leide de Oliviera, Ministry of Health, Esplanada dos Ministérios, Bloc 11, Sala 809, 70058 Brasilia, Brazil; Dr E Pupulin, Amici de Raoul Follereau, Via Borselli, 4, 40135 Bologna, Italy; Mrs J Santos Valdez, Volunteers for the Rehabilitation of the Handicapped and the Disabled Inc., 99 North Drive, Baclod City, Philippines; Dr H Srinivasan, Director, Central Jalma Institute for Leprosy, Taj Ganj, Agra-282001, India; Ms J Watson, The Leprosy Mission International, 50 Portland Place, London, W1N 4DG, UK.

Leprosy Control in the People's Republic of China

Dr Ma Haide, Chairman of the China Leprosy Association/Foundation, Hou Hai Bei He Yan 24, Beijing, People's Republic of China, recently wrote with greetings and his message included the information that during 1986 they treated a further 20,000 patients, whose treatment continued for another year before release. In 1988 they anticipate that they still have about 80,000 patients and PRC is appealing for international support towards basic eradication by the year 2000. Contributions may be sent to the above address. Bank transfer: Bank of China, Beijing, PRC Acct. No 71405516.

Drug Distribution for MDT; Stocks at Various Levels of the Health Service

During discussions recently with Dr Cesar Viardo (Leprosy Control Service, Manila) and Dr Yo Yuasa (Sasakawa Memorial Health Foundation, Tokyo, Japan), it was noted that stocks of antileprosy drugs in the Philippines are held as follows: 24 months' supply at Department of Health level; 12 months' at Regional; 6 months' at Provincial; 3 months' at District; 2 months' at Rural Health Centre and 1 month at Barangay Health Service, i.e. the peripheral, level. These arrangements clearly relate to a particular administrative and health service structure, but they give a useful indication of periods which have been found workable in practice. If the number of cases to be treated is large and the pace of MDT implementation fast, consideration might be given to ensuring that purchase and stocking at Department of Health level, i.e. central, national, is in the order of 3, rather than 2 years.

The Use of Essential Drugs; Technical Report Series, WHO

Technical Report Series 722, Second Report of the WHO Expert Committee on The Use of Essential Drugs is published by the World Health Organization (1985), and is obtainable from the Office of Publications, WHO, Geneva, Switzerland, or from any established medical bookshop. The contents are as follows: 1, Introduction; 2, Guidelines for establishing a national programme for essential drugs; 3, Criteria for the selection of essential drugs; 4, Guidelines for the selection of pharmaceutical dosage forms; 5, Quality assurance; 6, Drug utilization surveys; 7, Research and development; 8, Specialized applications of the essential-drugs concept; 9, Updating of lists of essential drugs; 10, Model list of essential drugs (Fourth revision)—10.1 Alphabetical list of essential drugs; 11, Changes made in revising the model list; 12, Essential drugs and primary health care—12.1 Criteria for the selection of drugs for primary health care, 12.2 A model list of drugs for primary health care; 13, Drug information and education activities—13.1 National responsibilities, 13.2 The role of WHO; and 14, Glossary of terms used in the report.

Dapsone and clofazimine are included under antileprosy drugs and rifampicin, ethionamide and prothionamide under antibacterial drugs (pages 18 and 19).

Lepr Rev (1988) **59,** 17–24

Ultrastructure of human foetal Schwann cells in tissue culture infected with *Mycobacterium leprae*

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Accepted for publication 1 June 1987

Summary Human Schwann cells from foetal peripheral nerves were grown in tissue culture and infected with *Mycobacterium leprae.* After fixation for electron microscopy the ultrastructural features of infected Schwann cells were studied. The findings reproduce previous ultrastructural findings of adult human Schwann cells in tissue culture and clearly demonstrate that *M. leprae* infects cultured Schwann cells. Most of the *M. leprae* remained electron dense, suggesting retained viability, and did not appear to induce any toxic change in the Schwann cells.

Introduction

Infection of the Schwann cell by *Mycobacterium leprae* is considered to be an important factor in the pathogenesis of human leprosy. Ultrastructural observations have confirmed the presence of *M. leprae* in Schwann cells both in the human disease[1,2] and in armadillos infected with the bacterium.[3,4]

In the last decade there has been considerable progress in tissue culture of various constitutents of peripheral nerves and it is now possible to selectively culture Schwann cells[5,6] and ultrastructural observations have been made on such cells.[7]

Recently, cultured human foetal Schwann cells from peripheral nerves have been successfully infected with *M. leprae.*[8] We now describe the ultrastructural features of these infected cells.

Materials and methods

TISSUE SAMPLES

Presumed healthy human foetuses of 8 to 20 weeks gestational age were obtained from the tissue bank at the Royal Marsden Hospital. The study of the foetal material was approved by the Ethical Committee of the Brompton Hospital.

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CELL CULTURES

Primary cultures

The peripheral nerves were dissected out. The connective tissue surrounding the nerve was removed under the dissecting microscope. The nerves were placed in calcium and magnesium free Dulbecco's modified Eagles medium (DMEM) which was obtained from the Zoology Department of University College, London. The peripheral nerves were placed in 35 mm diameter plastic Petri dishes (Nunc, Denmark) with 200 μl of 0·4% collagenase and 200 μl of 0·25% trypsin. The nerves were cut into small pieces and were then incubated in 10% CO_2 at 36°C for 30 min. After this period the fluid was pipetted off under the dissecting microscope and replaced with 200 μl of collagenase and 200 μl of trypsin. The Petri dish was returned to the CO_2 incubator for another 30 min. This process was repeated a third time so that the total enzyme digestion was 1·5 hr. An equal volume of Eagles minimum essential medium plus 0·02M HEPES (MEM-H; pH 7·4) and 15% heat inactivated foetal calf serum (FCS) (Northumbria Biologicals Ltd) was added. The nerves were dissociated by two cycles of trituration through number 21 and 23 hypodermic needles (Becton Dickinson) and the suspension was transferred to a centrifuge tube and centrifuged at 180 g for 10 min at room temperature. The supernatant was removed and the pellet resuspended in DMEM plus 15% FCS and a count was made using haemocytometer. One to two ml of cellular suspension was placed in 50 ml flasks (Falcon) to be used for subcultures. After 24 hr of incubation cytosine arabinoside 10^{-5}M (Sigma) was added for 48 hr to kill the dividing fibroblasts. After the removal of cytosine arabinoside by changing the medium the cells were maintained in DMEM + 15% FCS. The medium was changed twice weekly. The Schwann cells were identified by immunofluorescent staining with the specific neuroectodermal antigen S-100.[8]

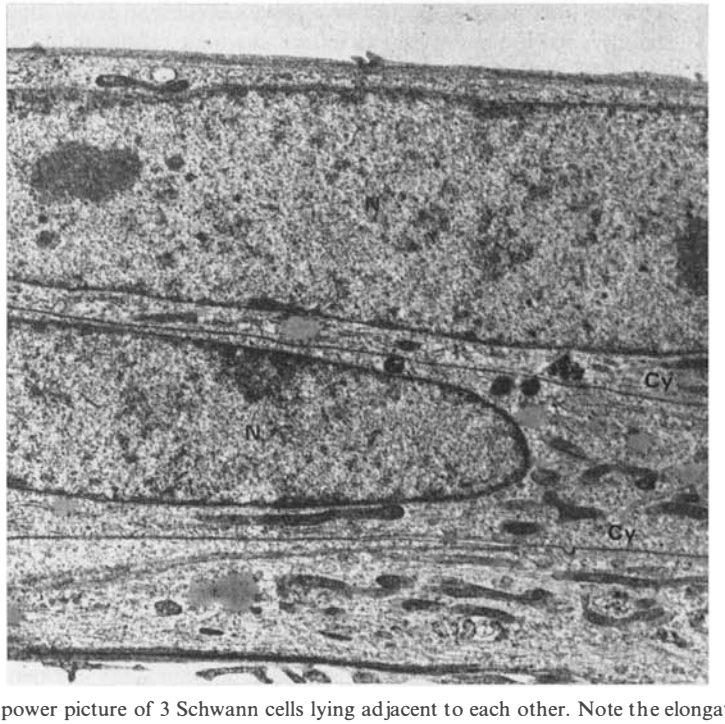

Figure 1. Low power picture of 3 Schwann cells lying adjacent to each other. Note the elongated nuclei (N), prominent nucleoli and narrow cytoplasm (Cy). (Original, × 12,000).

Secondary cultures

Several days later the flasks were rinsed and then 0·25% trypsin plus 0·002% EDTA in calcium and magnesium free DMEM was added. The flasks were transferred to the incubator and left at 37°C for 30 min. Cells were then harvested by centrifugation for 10 min at 260 g, resuspended in the feeding medium and counted on a haemocytometer. The cell suspension was then transferred onto Thermanex (EMScope) cover slips which had previously been coated by immersion in D-poly-l-lysine, 10 μg/ml (Sigma). Approximately 15–20,000 cells were seeded on each cover slip.

The cover slips were placed in 24 well tissue culture plates containing DMEM plus 15% FCS (Libro) and incubated in 10% CO_2 at 36°C.

After 7 days growth, half the cultures were infected with fresh suspensions of armadillo-derived *M. leprae* containing 5×10^6 6×10^6 organisms. After a further 3 days the cover slips were immersed in 2% glutaraldehyde in 0·1M cacodylate buffer pH 7·4 for 1·5 hr at room temperature, then for half an hour at 4°C. They were then rinsed in 0·1M cacodyulate buffer pH 7·4 and immersed in 1% buffered osmium tetroxide for 45 min. After another rinse in the buffer they were dehydrated in alcohols, immersed in propylene oxide and embedded in Araldite. After hardening, the cover slips were removed with forceps and thick, 1μ, sections were cut from selected areas and stained with toluidine blue, and Schwann cells were identified. Thin sections were then cut with a Cambridge microtome, stained with uranyl acetate and lead citrate and viewed in an AE1 801 electron microscope.

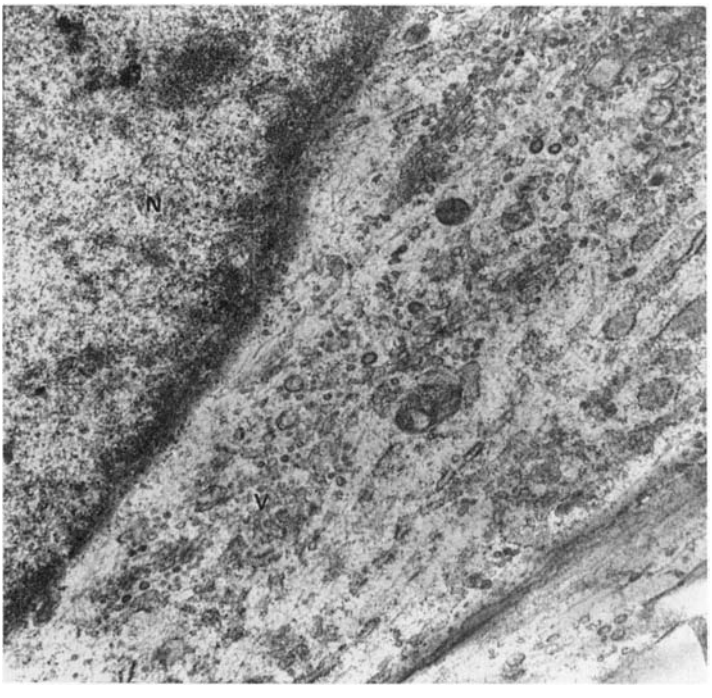

Figure 2. Perinuclear region of Schwann cell nucleus (N). Note extensive dense cored vesicles (V). Only occasional microtubules are present. (Original, ×23,500).

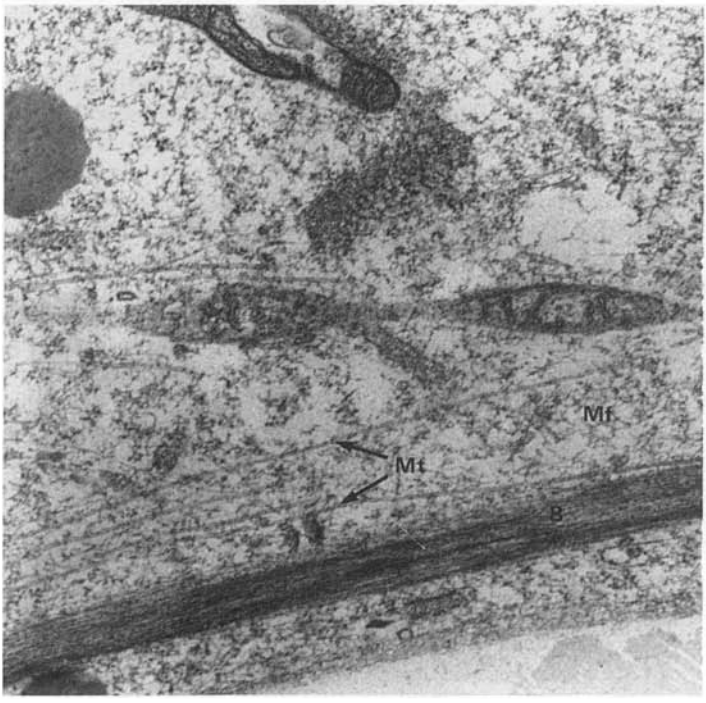

Figure 3. Polar cytoplasm of Schwann cell. Note the prominent microtubules (Mt) and how they are banded together to form a bundle (B). Microfilaments (Mf) are interspersed between the microtubules. (Original, ×42,000).

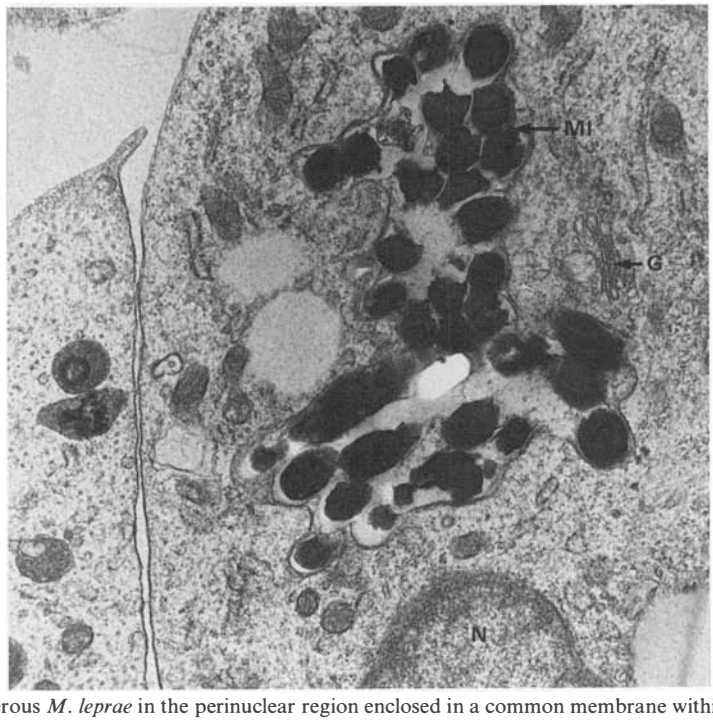

Figure 4. Numerous *M. leprae* in the perinuclear region enclosed in a common membrane within the Schwann cell cytoplasm. Nucleus (N), *M. leprae* (Ml), Golgi apparatus (G). (Original, ×33,600).

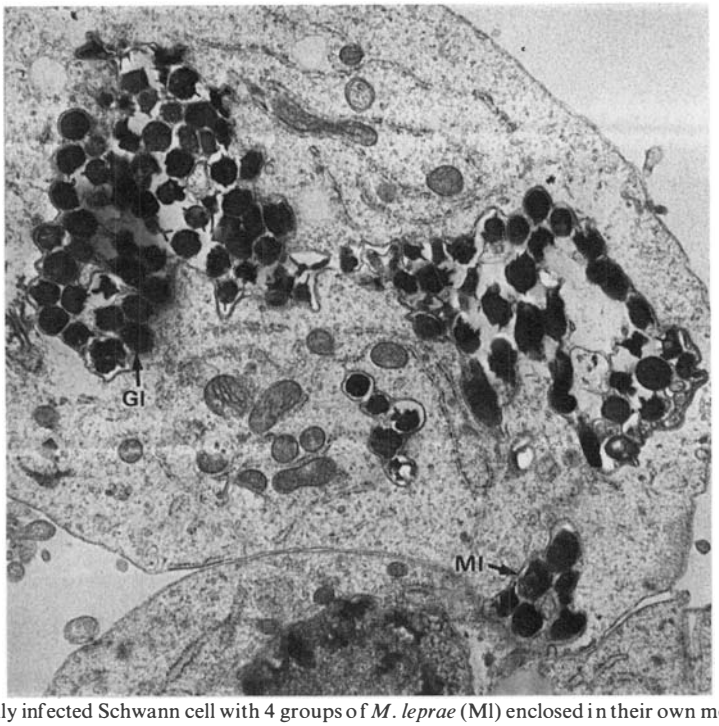

Figure 5. Heavily infected Schwann cell with 4 groups of *M. leprae* (Ml) enclosed in their own membrane. Globi (Gl). (Original, × 20,000).

Results

Schwann cells in cultures not infected with *M. leprae* had the following ultrastructural characteristics:

1 The cells were bipolar with an elongated nucleus and prominent nucleoli (Figure 1).
2 Golgi apparatus was present in the perinuclear region together with many free ribosomes and dense core vesicles (Figure 2).
3 Microtubules and microfilaments were sparse in the perinuclear region but were especially prominent in the processes (Figure 3).
4 Basement membranes were not present around the Schwann cells.
5 Fibroblasts were seen occasionally and were distinguished by their smaller size, the presence of extensive rough endoplasmic retinculum and the paucity of microtubules and microfilaments.

In cultures infected with *M. leprae*, the bacteria were found in abundance in Schwann cell cytoplasm (Figure 4), mostly in the perinuclear region. Some cells were heavily infected and globi were observed (Figure 5). Usually three or more bacteria were surrounded by an electron-dense common membrane (Figure 6) and none were seen lying free within the cytoplasm. The majority of *M. leprae* showed an even staining but occasional bacteria appeared fragmented (Figure 7). No fusion of phagosomes and lysosomes were observed.

In spite of the infection by *M. leprae* the appearance of the Schwann cells was similar to that in the control culture. That is, the cells were bipolar and the processes contained the characteristic microtubules and microfilaments while dense core vesicles, ribosomes and golgi apparatus were present in the perinuclear cytoplasm.

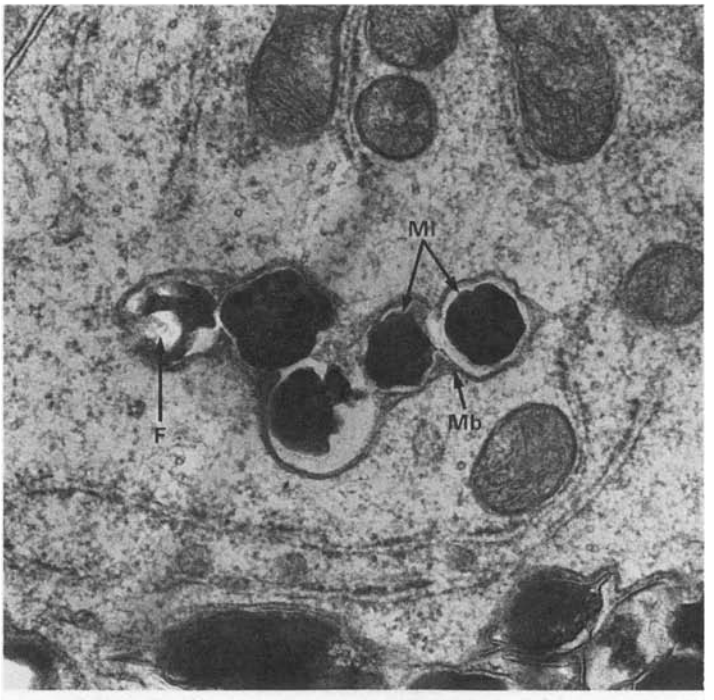

Figure 6. Five *M. leprae* (Ml) enclosed in a common membrane within the Schwann cell cytoplasm. Membrane (Mb), Mitochondria (M). One bacterium is fragmented (F). (Original, × 72,000).

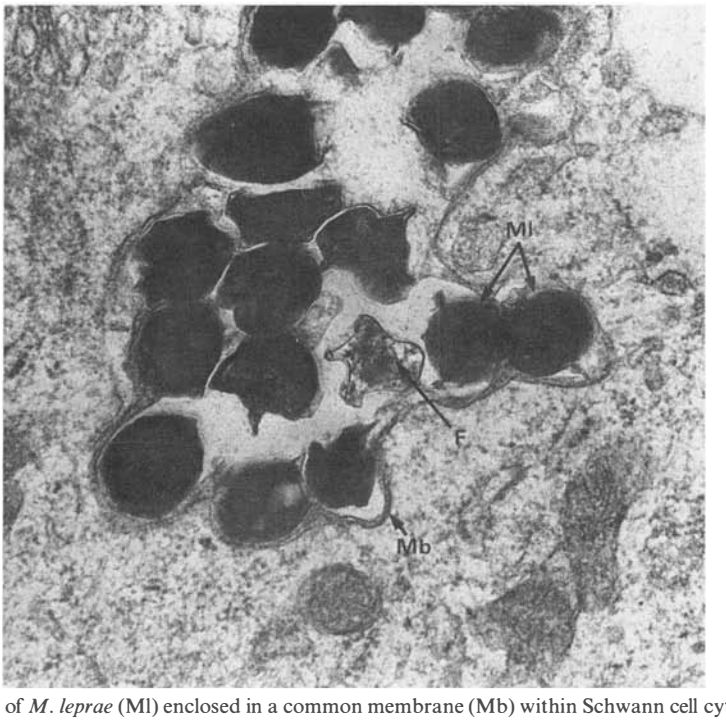

Figure 7. Group of *M. leprae* (Ml) enclosed in a common membrane (Mb) within Schwann cell cytoplasm (Cy). Most *M. leprae* shown are evenly stained but one bacterium is unevenly stained (F) and is probably not viable. (Original, × 72,000).

Discussion

The ultrastructural features of the Schwann cells observed here were very similar to those described by Askanas *et al.*[7] who grew the cells from nerve biopsies as explant cultures. Thus cultured Schwann cells of foetal peripheral nerves appear to possess the characteristic ultrastructure of their adult counterparts.

Schwann cells in tissue culture are not surrounded by a basement membrane so unfortunately this criterion cannot be used to distinguish them from fibroblasts. We have, however, already shown in a parallel study on the same cultures that cells of this morphology are S-100 positive,[8] thus confirming that they are Schwann cells. Also, the ultrastructural features already described, although not absolute, do permit a distinction as Askanas *et al.* have noted.[7] The lack of basement membrane in cultured Schwann cells is thought to be due to the absence of neurons. Thus, when neurons and Schwann cells are cultured together, a basement membrane is formed.[9]

This study provided confirmation of the light microscopic findings that *M. leprae* can infect human Schwann cells in tissue culture.[8] In a parallel study[8] on the same culture, 71% of the Schwann cells contained one or more acid-fast bacilli 48 hr after infection. The ultrastructural findings closely resemble those found in the Schwann cells of nerves of leprosy patients and in those of armadillos infected with *M. leprae*. We did not, however, observe any bacteria lying free in Schwann cell cytoplasm as described by Job.[1] Indeed, in our experiments the membranes surrounding the bacilli appeared unusually prominent and may possibly be a special feature of Schwann cells in tissue culture infected with *M. leprae*; an observation requiring further investigation.

Most of the *M. leprae* observed here were evenly stained, suggesting that they were still viable. Thus human Schwann cells in tissue culture do not appear to destroy viable *M. leprae* although other workers have claimed that such bacilli are destroyed within rat Schwann cells.[10] The few fragmented bacilli may well have been dead before they were added to the cultures. Also, the ultrastructural appearance of the Schwann cells was not altered by the presence of *M. leprae* within them, suggesting that the bacteria were not unduly toxic. Moreover, the Schwann cell is responsible for making myelin in the peripheral nerve, yet ultrastructural studies have shown that the presence of *M. leprae* does not cause gross structural abnormalities in the myelin sheath.[1]

The experiments described here show that it is possible to reproduce the findings which occur in Schwann cells in nerves of leprosy patients. These techniques could therefore be used to study various aspects of the interaction between *M. leprae* and the Schwann cells.

Acknowledgments

We are most grateful to Dr J Colston, National Institute for Medical Research, London, for the gift of the armadillo-derived leprosy bacilli and Dr Wong, tissue bank, Royal Marsden Hospital, for supplying foetal tissues. We also thank Mr J Bowles for technical assistance.

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NEWS AND NOTES

Meralgia paraesthetica (Bernhardt's syndrome)

Dr Terence Ryan, Medical Adviser to St Francis Leprosy Guild, London, has drawn our attention to an interesting communication in a recent issue of the German dermatological journal *Der Hautarzt*. It is entitled 'Alopecia in Meralgia paraesthetica'; Krause, K-H *et al.*, from the Neurologische Universitatsklinik, Heidelberg, West Germany; *Der Hautarzt*, 1987; **38:** 474–6. Two patients are described with characteristic loss or impairment of sensation over a quite extensive area on the antero-lateral aspect of the thigh (on one side). The interesting (and apparently new) observation in this article is that both cases showed loss of hair growth in this area. This syndrome is due to nipping or damage to the lateral cutaneous nerve of the thigh and such cases have, on several occasions, been misdiagnosed as having leprosy. Clinicians may be interested to consult this paper in the original.

Fifty-three interesting things to do in your lectures

This amusing and stimulating book carries a more serious message. It is a highly readable paperback of 157 pages, written by Graham Gibbs, Sue and Trevor Habeshaw, published by Technical and Educational Services Ltd, 37 Ravenswood Road, Bristol BS6 6BW. Price £6.00. The main chapter headings are: structuring the process; improving student notes; using handouts; structuring and summarizing content; linking lectures; holding attention; active learning during lectures and checking on learning. This little book will do us all a lot of good and is well worth the money.

WHO. Report on Educational Material for Patients

This full title is 'Report of an informal working group on educational material for patients' DAP/85.10. This meeting held in New Delhi, October 1985 was convened by the WHO Action Programme on Essential Drugs. Essentially it deals with educating patients on the correct use of drugs, including those used in leprosy. It is important reading for all concerned with health education and patient motivation. Apply: Action Programme on Essential Drugs and Vaccines, WHO, 1211 Geneva 27, Switzerland.

Lepr Rev (1988) **59,** 25–30

Further observations on the breeding and rearing of BALB/C nude (nu-nu) mice under normal laboratory conditions

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Accepted for publication 16 September 1987

Summary An account is given of the breeding and rearing of BALB/C (nu-nu) mice under normal laboratory conditions, in a research institute in Thailand. Starting with twenty pairs of mice in 1980 over 4,000 have now been successfully bred and reared in this unit: on reaching adult age (30 days), the mortality rate is nil.

A detailed description is given of the housing unit, cages, bedding, diet, animal husbandry and neonatal management. The approach used is demanding in terms of professional time and the need for constant attention to detail—but it is successful.

Our experience is in striking contrast to published evidence on the absolute need for specific pathogen-free conditions for this animal model.

Introduction

In previous publications we described our preliminary findings on the rearing of BALB/C nude (nu-nu) mice without recourse to specific pathogen-free conditions[1] and our experience in the inoculation of the hind footpads with *Mycobacterium leprae* of human origin.[2] Since that time, we have continued to breed and rear nude mice in this institute under similar, normal laboratory conditions with considerable success. The colony of healthy nude mice now exceeds 4,000 and this paper gives a detailed account of the principles and techniques developed here since 1980.

Materials and Methods

In 1979, Professor K Kohsaka of Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, sent 20 pairs of BALB/C mice to this Institute, of which 20 were nu-nu males and 20 nu/+ females. From these we developed the present colony which now numbers over 4,000.

GENETIC CONSTITUTION

The animals are in every way identical to those used by Kohsaka for his previous studies in Japan,[3–6]

0305-7518/88/059025+06 $01.00

and by other research workers from different parts of the world.[7–12] We have not encountered congenital deformities, unexpected hair or eye colour, or obvious evidence of tumours in any of the animals produced here since 1980. Although detailed genetic typing has not been done, it appears that the original stock and progeny are identical with BALB/C nude (nu-nu) mice used in other parts of the world.

CAGES

The mice are kept in standard plastic boxes 17 × 25 × 12 cm, with metal grid covering. The boxes are lined with coarse wood shavings obtained from a local saw-mill; the composition is variable, but teak and plywood, among others, are most common. Throughout the period of study, bedding has been changed twice-weekly.

FOOD

We use a standard laboratory mouse 'pellet' supplied by an agency in Bangkok, which contains crude protein (fish origin) 24%; crude fat 3%; fibre 4%; ash 8·5%; calcium 1·6%; phosphorus 1·0%; nitrogen-free extract 4·8% and water 12·0%. Detailed studies of intake have not been carried out, but we estimate that each adult consumes approximately 4 g daily; perhaps more during lactation and certainly less with progressive ageing.

ROOM SIZE

Cages are kept on wooden shelves in rooms measuring 7 × 5 × 3 m (for breeding) and 5 × 5 × 3 m (for rearing) both of which have narrow windows placed high up in the walls so that there is never any possibility that nude mice will be exposed to direct sunlight (which produces phototoxic dermatitis in this model).

NUMBER OF MICE PER CUBIC METRE OF ROOM SPACE

The number of mice accommodated in a room of the dimensions given above has increased steadily through the years, but in the last 12 months it has averaged 17. Figures above this produce a foul atmosphere from decomposition of urine and the production of ammonia; the influence of overcrowding on mice inoculated with *M. leprae* of human origin is clearly adverse.

AIR-CONDITIONING, TEMPERATURE, HUMIDITY

Although not absolutely essential, we have installed a single air-conditioning machine (capacity 18,000 BTU, Climatrol, window type) in 1980. This was due mainly in order to deal with foul-smelling air due to putrefaction of urine and ammonia formation, which often reached unacceptable levels for laboratory staff. It was, however, soon discerned that its introduction increased sexual activity in the mice and the number of progeny per litter: see Results and Discussion. The environmental temperature in this area ranges 21°C–39°C throughout the year with a mean of 30°C. The humidity ranged 55%–75%, with a mean of 65%. Air-conditioning reduces these figures in the mouse room to 23–28°C and 45%–60% respectively.

SELECTION OF PAIRS FOR BREEDING

We select strong, healthy-looking mice only, aged 8 weeks for breeding. The male is BALB/C nu-nu (nude) and the female BALB/C nu/+ (white, hairy). The female nu-nu have only rudimentary,

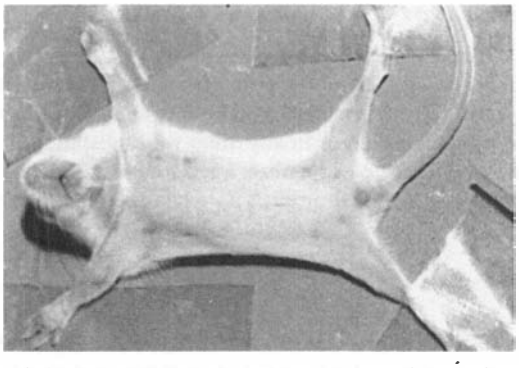

Figure 1. Spots of nipples are clearly observed at 12 days of age in female white mouse.

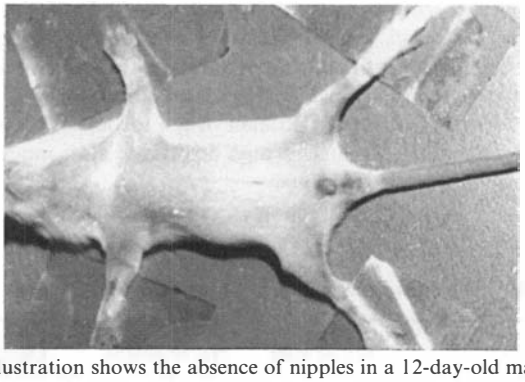

Figure 2. This illustration shows the absence of nipples in a 12-day-old male white mouse.

almost invisible nipples (and do not lactate); they breed normally but are thus unable to suckle the babies. The pairs are housed in separate cages, with two changes of wood shavings each week.

NEONATAL MANAGEMENT

From the litters, which range from 3 to 13 in number, with an average of 8, we redistribute nude (nu-nu) mice at 7 days in groups of five only to one (nu/+) mother, taking care that those from the real mother and the 'surrogate' mother were all born on the same day. This is done because white mice are stronger than nude mice; they displace the nude mice from the nipple and gain weight at their expense. At the same time (7 days) we group 5–10 white mice with one mother. When they are 11–12 days old it is possible to decide on the sex of the white mice by inspecting the abdomen for nipples (Figure 1); they are by this time obvious in the female and absent in the male (Figure 2). We then take out from the colony all white male mice, since they are of no breeding value to the further maintenance of the stock.

ADULTHOOD AND LIFE-SPAN

By 30 days the mice are fully developed adults and we separate them from the parents. They live on average for 70–75 weeks, but after about 50 weeks some of them lose weight, become slow in their movements and develop the well recognized 'hump-back' spinal curvature.

GENERAL ASPECTS OF HUSBANDRY AND STOCK MAINTENANCE

Attention to the technical details described above is essential on a daily basis. We record the number and condition of all mice born every day of the year. It is essential to start each breeding batch with healthy robust parents and it is our practice to take out and kill baby mice which seem weak, abnormally small in the neonatal period or in any other way substandard. Careful attention to the milk supply from lactation is vital; the white mice, aged 7 days in groups of 5–10 with one mother take less milk in the first few days, but soon pick up when the males are removed at 11–12 days.

Results

Using the methodology and techniques described above, we usually lay down 180 pairs of adult mice for breeding in 180 separate cages at any one time. From there, in due time, we obtain about 100 nude mice per month. With the continuing use of adults (30 days and over) for experimental purposes in this unit, together with the natural death rate at 70–75 weeks, we are able to keep the total number of mice in the room under control. We have no evidence that overcrowding is hazardous for young nude mice, or for young adults, though it should obviously be avoided if only to avoid air pollution by foul-smelling urine and ammonia. In the case of more mature adults inoculated in the footpad with *M. leprae* of human origin for leprosy studies, we have, however, strong evidence that overcrowding produces a high death rate after inoculation.

Table 1. Nude mice monthly death rate (room = 5 × 5 × 3 m).

Month	Year	No. of mice per month	No. of deaths per month	Deaths per month (%)
1	1983	1468	151	10·28
2	1983	1454	147	10·11
3	1983	1476	154	10·50
4	1983	1468	162	11·30
5	1983	1484	158	10·64
6	1983	1492	154	10·32
7	1983	1496	167	11·16
8	1983	1532	177	11·55
9	1983	1502	161	10·71
10	1983	1543	172	11·14
11	1983	1569	195	12·42
12	1983	1608	198	11·75
1	1984	1632	204	12·50
2	1984	1462	164	11·21
3	1984	1402	145	10·34
4	1984	1367	137	10·02
5	1984	1304	112	8·58
6	1984	1322	104	7·86
7	1984	1311	97	7·39
8	1984	1253	95	7·58
9	1984	1216	87	7·15
10	1984	1208	81	6·70
11	1984	1137	61	5·36
12	1984	1125	48	4·26

Table 2. Nude mice death rate after inoculating and rearing together (room of 5 × 5 × 3 m).

Group	No. of mice per group	Total No. of mice in the room	12 months		15 months		18 months	
			death	%	death	%	death	%
1	80	1454–1608	19	23·75	24	30·0	33	41·25
2	120	1454–1608	31	25·83	43	35·83	56	46·66
3	40	1402–1632	9	22·5	13	32·5	17	42·2
4	80	1304–1632	21	26·25	27	33·75	34	42·5
5	100	1253–1632	25	25·0	34	34·0	48	48·0
6	90	1208–1632	21	23·33	27	30·0	38	42·22
7	100	1137–1632	13	13·00	18	18·0	27	27·0
8	40	1024–1311	3	7·50	5	12·5	6	15·0
9	80	984–1024	6	7·50	6	7·5	8	10·0
10	80	973–984	4	5·0	4	5·0	5	6·25

As noted above, the parents are selected for health and vigour. We also take out and kill baby mice (at any stage before 30 days) if they are obviously underweight, weak, damaged by trauma, or in any other way substandard. Those attaining adulthood at 30 days are thus well fed and robust. In the 7 years of this study, we have had very few deaths between the ages of 35 days and 70 weeks.

Discussion

The high success rate using the approach described above is in marked contrast to published work which emphasizes the absolute need to maintain nude mice under specific pathogen-free conditions at all times. In Nepal, Samuel[13] reported excellent results in breeding and rearing in 1984. To our knowledge, this is the only reported instance of success in normal laboratory conditions other than our own. Our ability to breed and rear nu-nu mice under the normal conditions described above is difficult to explain. It is possible that the original genetic stock was in some or other particularly robust and that the local temperature, humidity and diet are all favourable. It is however our belief that these factors are of relatively small significance compared with the vitally important matter of meticulous day-to-day husbandry of the colony, including the careful selection of healthy mice for breeding purposes.

Acknowledgments

I would like to express my gratitude for much assistance in these studies from the Sasakawa Memorial Health Foundation, Japan; the Raj-Pracha-Samasai Institute, Leprosy Division and the Department of Communicable Disease Control, Ministry of Health, Bangkok, Thailand.

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NEWS AND NOTES

WHO Symposium on Ocular Leprosy: London, September 1987

A symposium on ocular leprosy took place at the International Centre for Eye Health, Institute of Ophthalmology, London, 21–23 September 1987. It was organized by Professor Gordon Johnson, Director of the Department of Preventive Ophthalmology, and Dr Paul Courtright of the Proctor Foundation, San Francisco and was funded by the WHO through its Programmes for Leprosy and the Prevention of Blindness with contributions from LEPRA and Dutch and German Leprosy Missions.

The meeting, which took the form of a workshop, was attended by 15 ophthalmologists with a special interest in ocular leprosy—Dr J Anderson (London), Dr M Brand (USA), Dr F Brandt (Germany), Dr A Cherinet (Ethiopia), Mr T ffytche (London), Dr M Hogeweg (Holland), Professor G Johnson (London), Mr M Kerr-Muir (London), Professor P Lamba (India), Dr G Lim (Philippines), Dr B Ostler (USA), Dr R Pokhrel (Nepal), Dr N Suryawanshi (India), Dr K Waddel (Uganda) and Dr G Warren (Thailand). Dr M Jacob (India), pathologist, and Dr P Courtright, epidemiologist, both specializing in ocular leprosy, were also present.

The WHO was represented by Dr K Nordeen, Chief, Leprosy, WHO, Dr R Pararajasegaram, Regional Advisor to WHO (SEARO) and Dr B Thylefors, Programme Manager of Prevention of Blindness, WHO.

The meeting covered a wide range of topics relating to ocular leprosy including its global distribution, the present state of knowledge of its clinical and pathological manifestations and its prevention and management in the field. The training needs for all levels of personal working in leprosy were discussed and a policy for integration of ocular leprosy management in National Leprosy and Blindness Prevention programmes was formulated.

A report of the meeting will be published by the WHO and it is intended that its recommendations will be presented to the 13th International Leprosy Congress at the Hague in 1988.

(Contributed by Mr T J ffytche, Consultant Ophthalmic Surgeon, St Thomas' Hospital, London SE1 7EH.)

Lepr Rev (1988) **59,** 31–35

Inhibition of mononuclear leukocyte transformation *in vitro* by dihydrophenazines in comparison to clofazimine

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Accepted for publication 7 May 1987

Summary To identify the molecular structures of the antileprosy drug clofazimine which mediate its immunosuppressive activity the effects of ten phenazine derivatives on phytohaemagglutinin-stimulated mononuclear leukocyte (MNL) transformations were investigated. It was found that modifications in the substituent at position 2 of the dihydrophenazine moiety decreased the antiproliferative activity. The nature of the chemical group in position 2 also influenced the immunomodulatory effect of halogenation in the paraposition of the phenyl-rings and anilino-rings.

Introduction

Clofazimine (B663)[1–4] has become an established part of the standard antimycobacterial chemotherapy of leprosy.[5,6] Due to its efficacy in the treatment of erythema nodosum leprosum reactions[5] and various nonmicrobial skin disorders,[7] immunosuppressive effects have been attributed to this agent in addition to its antimycobacterial properties. A variety of techniques have been employed to investigate this immunosuppressive activity *in vitro* and *in vivo*,[8,9] including phytohaemagglutinin (PHA)-stimulated lymphocyte transformations and leukocyte migration assays, which were inhibited by clofazimine at therapeutic concentrations.[10,11]

In an attempt to identify the molecular structure(s) responsible for this clofazimine-mediated immunosuppressive activity we have investigated the effect of ten dihydrophenazine-derivatives on mononuclear leukocyte (MNL) transformation *in vitro* in comparison to clofazimine. The results were correlated with the previously described antimycobacterial properties of the compounds.[3]

Methods

AGENTS

1 *Selection*

All agents investigated in this study were synthesized by Dr J F O'Sullivan, Medical Research

0305-7518/88/059031+06 $01.00

Council of Ireland Laboratories, Dublin. A cross-section of ten dihydrophenzine-derivatives was chosen in order to examine the importance of various substitutions for the inhibition of MNL transformation to PHA in comparison to the antimycobacterial effects. Since the insertion of chlorine in the paraposition of the phenyl-rings and anilino-rings has been found to strongly augment the activity of the agents against murine tuberculosis (TB),[3] all compounds were investigated in their chlorinated and unchlorinated forms. The significance of this halogen was further examined by including the fluorinated form of B663, namely B980, in the study. Since aposafranone derivatives, in which the nitrogen substituent at position 2 of the phenazine core has been replaced by oxygen, were inactive *in vitro* and against murine TB,[3] we also included four representatives of this group of agents in the present study, namely B3722, B433, B685 and B432. The significance of the anilino-group of clofazimine in position 3 of the phenazine molecule was investigated using compounds in which this group had been substituted by a hydroxyl group (B3722, B433). Similarly the importance of the isopropyl-imino-group in position 2 of clofazimine was examined by replacing it with an imino-group resulting in B628 and B283. The chemical precursors of B663 and its unchlorinated analogue B670, which are the imidazophenazines B654 and B621, were also included in this study, since they were virtually without antimycobacterial activity.[3] The chemical structures of the agents are shown in Table 1.

2 *Solubilization*

One milligramme of each compound was solubilized in 0·7 ml dimethyl sulphoxide at 56°C, 0·3 ml hot distilled water was added immediately prior to dilution of the agents in Hepes- (N-2-hydroxyethyl piperazine-N′-2-ethane sulphonic acid, Sigma Chemical Co., St Louis, Mo., USA) buffered tissue culture medium 199 (M199, Grand Island Biological Co., NY, USA) to the concentrations required. All compounds were compared to the appropriate solvent control.

Cell preparation. MNL were obtained from heparinized venous blood of healthy volunteers as previously described.[10] The cells were suspended to a concentration of 4×10^6 MNL/ml in M199.

Effect of test agents on MNL transformation to phytohaemagglutinin. This assay was performed according to the method previously described.[10] For dose-response studies the test agents and the solvent control were added to the wells of the micro tissue culture plates at final concentrations of 0·3–10 μg/ml.

Expression and analysis of results. Results are expressed as mean values of four different experiments. Statistical analyses were performed by the Student's *t*-test (paired *t*-statistic).

Results

Effect of test agents on MNL transformation to phytohaemagglutinin. Apart from the compounds B670, B628, B685 and B432 all agents investigated caused statistically significant inhibition of MNL transformation at concentrations $\geqslant 0{\cdot}6$ μg/ml with *P*-values between $< 0{\cdot}05$ and $< 0{\cdot}005$. At a concentration of 0·3 μg/ml only the compounds B663, B980, B3722 and B654 significantly inhibited PHA-stimulated blastogenesis (*P*-values between $< 0{\cdot}05$ and $< 0{\cdot}025$). Results are shown in Figure 1.

Discussion

The dihydrophenazine-derivative clofazimine (B663) is one of the standard drugs in the treatment of leprosy.[5,6] Serum concentrations of approximately 1 μg/ml are achieved with this agent,[6] but since it is concentrated in macrophages[12–14] and in fat, tissue levels are significantly higher.[15] Apart from its antimycobacterial properties clofazimine also possesses anti-inflammatory activity.[8] *In vitro* it

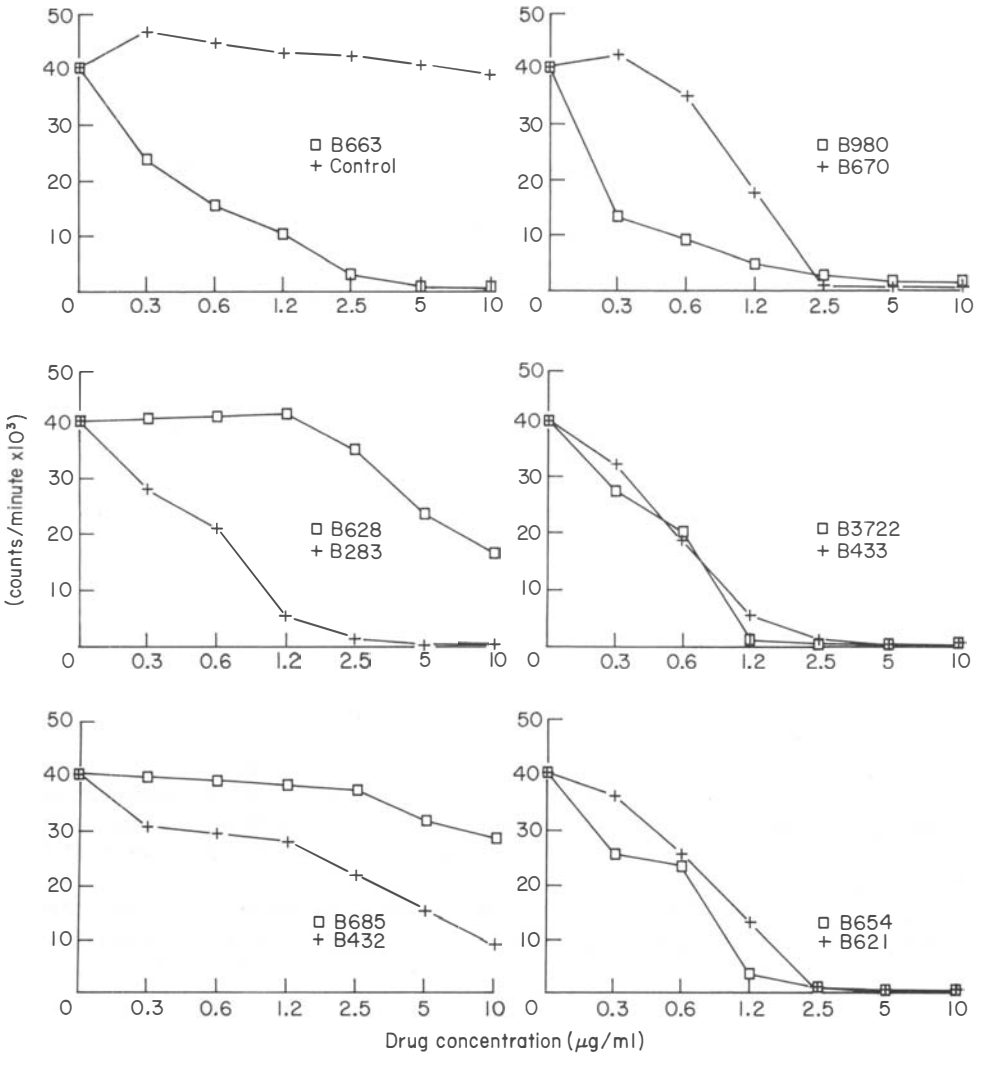
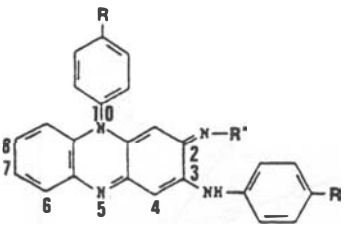


Figure 1. The effect of ten phenazine-derivatives on MNL transformation to PHA relative to clofazimine (B663) and a drug-free solvent control. Results are shown as man counts/minute $\times 10^3$ of four different experiments.

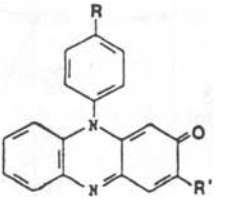
significantly inhibits PHA-stimulated MNL transformation and leukocyte migration at therapeutic concentrations[10,11] and similar effects have been observed after oral administration of the drug.[10]

In the present study we have used 10 dihydrophenazine-derivatives to correlate the chemical substitutions with the inhibitory activity of clofazimine on MNL transformation. Our findings indicate that this effect was dependent on the chemical groups in position 2 of phenazine. When the isopropyl-imino-group in position 2 of clofazimine was replaced by an imino-group (B628) or an oxygen (B685/B432), the antiproliferative potency of the compounds was decreased. The nature of the chemical group in position 2 of these agents also influenced the immunomodulatory effect of halogenation in the paraposition of the phenyl-rings and anilino-rings: the halogen-free analogues

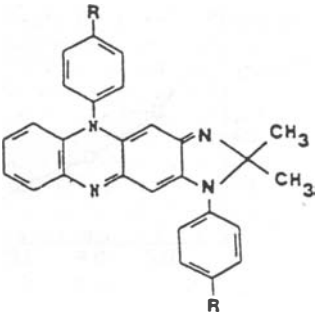
Table 1. Chemical structures of ten phenazine derivatives in comparison to clofazimine (B663)



B663: R = Cl, R′ = $CH(CH_3)_2$



B3722: R = Cl, R′ = OH



B654: R = Cl

B670: R = H, R′ = $CH(CH_3)_2$

B433: R = H, R′ = OH

B621: R = H

B980: R = F, R′ = $CH(CH_3)_2$

B685: R = Cl, R′ = NHC_6H_4Cl

B628: R = Cl, R′ = H

B432: R = H, R′ = NHC_6H_5

B283: R = H, R′ = H

of B628 and B685—B283 and B432—were more potent inhibitors of MNL transformation than their chlorinated counterparts, whereas the antiproliferative potency of the other compounds tested was increased by the presence of chlorine. Interestingly B685 and B283 as well as B670, B654 and B621 were all virtually inactive against murine TB, whereas B628 increased the median survival time of *Mycobacterium tuberculosis*-infected mice, though not to the same extent as clofazimine.[3] Therefore most of the agents investigated apparently possessed little or no antimycobacterial activity in murine TB, but had nevertheless retained antiproliferative effects equivalent to clofazimine.

It is concluded that the suppressive activity of dihydrophenazine-derivatives on MNL transformation to PHA depends on the nature of the chemical group in position 2 of the compounds tested. Furthermore the antiproliferative effects of the agents are influenced by interrelations of the group in position 2 with halogen-substitution of the paraposition of the phenyl-rings and anilino-rings. Our results also indicate that the antimycobacterial and immunosuppressive properties of clofazimine are probably unrelated.

Acknowledgment

The authors wish to thank Dr M K Felten for the production of the figures.

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NEWS AND NOTES

Robert Cochrane Fund for Leprosy

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

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

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

Posters and other Visual Material for Health Education in Developing Countries

Mr Bob Linney, Holly Tree Farm, Walpole Lane, Walpole, Halesworth, Suffolk, UK, continues to develop his interest, previously reported in this Journal, on the production of posters and other visual material for health education in Third World countries. From his commercial poster studio in London, Mr Linney has branched out into the production of coloured posters on health issues in developing countries, giving emphasis to the involvement of local artists and technicians and to the importance of getting to grips with local customs and culture. He has already organized workshops in India and Nepal. Pretesting and further testing of any poster developed are considered essential, in view of the evidence he has documented for widespread misinterpretation of many, perhaps the majority of posters produced in the health field. He is currently working towards the foundation of a charity called 'Health Images' which will concentrate on Third World needs, using local ideas and expertise whenever possible. Bob Linney (address above) would like to hear from anyone who would be interested in cooperation or who would like to learn more about his work.

NEWS AND NOTES

Ciba-Geigy Leprosy Fund

In 1986 the Pharmaceutical Division of Ciba-Geigy Ltd established the Ciba-Geigy Leprosy Fund with a budget of Sfr. 3 million (US $1·7 m) for an initial period of 3 years.

Purpose of the Fund

The Ciba-Geigy Leprosy Fund has been established to support leprosy control programmes which implement or intend to implement multidrug therapy (MDT) as recommended by the World Health Organization (WHO).

The primary emphasis of the Fund is to help create the preconditions for the correct implementation of MDT and thus to increase the total number of patients on MDT.

Conditions for funding

The proposed field activities should be undertaken in a programme which currently uses or wishes to introduce the WHO recommended MDT regimens to treat leprosy patients.

Only one project proposal may be submitted per year by each institution or agency. The Ciba-Geigy Leprosy Fund reserves the right to visit project sites. The use of the Fund's resources may be audited. Research dimensions can be added to projects (e.g. costs involved in MDT implementation, acceptance of MDT, effectiveness of measures taken to prevent disabilities). All applicants must agree to supply a bi-annual progress report on the specific activities supported by the Fund. Field projects must use the Ciba-Geigy form to give the project specific details.

Application procedure

Proposals must be submitted to the: Ciba-Geigy Leprosy Fund, PO Box, K-24.2.09, CH-4002 Basle, Switzerland before 15 February or 15 August.

All proposals must be accompanied by the following documentation: the objective of the proposed activities; detailed plan of action which includes methods, targets, timetable, staff; detailed budgetary request; details of other support reveived and requested.

In the case of field projects the following additional information is required: a brief description of the project area (e.g. size, population, patients by type, attendance rates) and the leprosy control efforts in the country; current extent of MDT implementation; a copy of the most recent annual report.

Selection criteria

Given a limited budget and the fact that most projects will require financing during the 3 year life span of the Fund, priorities must be set for the types of projects that would be favoured.

Only project proposals submitted by institutions (e.g. governments, non-governmental organizations and voluntary organizations) will be considered.

Any application should receive the approval of the government of the country where the project will be executed.

Field projects will be given top priority. Eighty per cent of the budget will be allocated to such projects.

Preference will be given to an intensification of ongoing work but projects which start from scratch are not excluded.

The contribution of the Ciba-Geigy Leprosy Fund, either in a professional or a financial sense, should be a significant one in the context of the total project.

The number of patients treated in the course of the project is of great importance.

The existence of the preconditions for the correct use of MDT (e.g. trained personnel, laboratory facilities, basic infrastructure, health education, or their creation as part of the project is essential.

Specific activities within the context of a project (e.g. health education, training) will be financed rather than contributing to the total project costs.

Ciba-Geigy Leprosy Fund, PO Box, K-24.2.09, CH-4002 Basle. *Telephone* (061) 36 26 55; *Telex* 962 355; *Telefax* (061) 36 22 39.

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Evaluation of inexpensive blocking agents for ELISA in the detection of antibody in leprosy

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Summary In leprosy research, ELISA is currently being used to quantitate antibody concentrations in leprosy patients and their contacts. The advent of *Mycobacterium leprae* specific synthetic antigens has tremendously increased the sensitivity and specificity of the detection system being used. It allows researchers to monitor the effectiveness of chemotherapy and also permits early detection of lepromatous patients likely to spread the disease and those contacts who have contracted it. The use of this detection system has now gained popularity amongst researchers in various countries throughout the world. However, its use in some countries is still being hampered by availability and high costs of reagents, particularly, blocking agents. We compared 5 blocking agents commonly used and found 10% skimmed milk or nonfat dry milk to be the most suitable. It is as effective a blocking agent as those popularly used. It did not adversely affect the pattern of the ELISA response expected of high and moderate reacting sera. It is relatively inexpensive compared to bovine serum albumin (BSA) or normal goat serum (NGS), readily available (it can be purchased at local grocery stores), stable at room temperature and very simple to prepare.

Introduction

An important problem in the epidemiologic study of leprosy is the lack of reliable technology for detecting subclinical infections in patients infected with *Mycobacterium leprae* and detecting the disease in early stages. It is well known that the FLA–ABS test first established by Abe[1] was demonstrated as a tool for these purposes.[2] Recently, however, a new, simple, rapid, sensitive and quantitative technology, enzyme-linked immunosorbent assay (ELISA), has been developed and is now widely used in the quantitation of humoral antibodies for both clinical and research applications. The ELISA is now being applied to leprosy serology and in addition to increased sensitivity, it avoids the subjective analysis associated with fluorescent antibody tests.

In some countries where both technology and resources are limited, the transfer of this

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technology has met some difficulties. One problem encountered in the beginning was the quality of water used in sensitive assays. It was found that glass distilled water was a requirement.[3] Another problem is the availability and cost-effectiveness of blocking agents. At present, some countries that are using the ELISA technology need to purchase their blocking agents from foreign suppliers because known, effective, pre-tested blocking agents are not available locally. Such long distance transactions are not only cumbersome but also time consuming. It may take as long as 6 months before a shipment is received, making it difficult to anticipate when the next order should be placed.

Another major obstacle is the cost of these blocking agents. Some, for example BSA (bovine serum albumin) can cost as much as US$797·00 per kg, plus shipping and handling. This can be a major problem for laboratories operating on a very limited budget. In this article, we compare the efficacy and cost of different blocking agents and discuss their advantages and disadvantages for use in countries where availability of reagent grade chemicals is limited.

Materials and methods

SERA

Leprosy patients were clinically and histologically classified according to the Ridley–Jopling scale.[4] The sera were obtained from LWM laboratory in Cebu, Philippines and stored in aliquots at −70°C until used. The lepromatous (high and moderate reactors) and normal sera were diluted 1:1000 in appropriate blocking agents.

ANTIGEN

ND-O-BSA (synthetic disaccharide containing neo-glycoconjugate), was kindly provided by Dr Brennan (Colorado State University, Fort Collins) under NIH contract.[6] This antigen was diluted to a concentration of 0·1 μg/ml with a volatile buffer, 0·01M ammonium acetate-carbonate, pH 8·2.[7]

CONJUGATE

Peroxidase conjugated goat antihuman IgM (mu-chain specific, Cappel Laboratories) was diluted 1:6000 in appropriate blocking agents.

SUBSTRATE

O-phenylenediamine 0·04% in citrate buffer (pH 5·0) 0·003% hydrogen peroxide.

BLOCKING AGENTS

5% and 10% bovine serum albumin (BSA, w/v), 10% crude egg albumin (EAC, w/v) filtered, 10% skimmed milk or nonfat dry milk (SM, w/v), 10% normal goat serum (NGS, v/v) 10% and 20% egg white albumin (EA, v/v) filtered, all diluted in PBSTW20 (phosphate buffered saline with 0·1% Tween 20) pH 7·4. All are prepared fresh daily and discarded after use to prevent bacterial contamination.

TEST

The procedure for ELISA was conducted in the following manner:

1 Coating plates. Add 50 μl of prepared ND-O-BSA into each well. Incubate plates 14–18 h at 37°C.

2 Blocking. Prior to blocking, add 100 μl of PBSTW20 to each well and soak for 15 min, then wash plates 3 times to remove unbound antigen. Add 100 μl of appropriate blocking agent into each well (skip first column for blank). Incubate plates for 1 h at 37°C.
3 First antibody reaction. Aspirate off the blocking agent from each plate and add 50 μl of diluted sera in duplicate wells. Save the last row for controls and conjugate background. Incubate at 37°C water bath for 1 h, then wash plates 3 times.
4 Second antibody reaction. Add 50 μl of diluted conjugate into each well except blank column. Incubate at 37°C for 45 min. After incubation, aspirate off conjugate, soak plates for 3 min, then wash 3 times.
5 Colour development. Add 50 μl of OPD solution to each well and incubate plates at 37°C water bath for 20 min. Stop reaction by adding 50 μl of 2·5N sulphuric acid. Mix well. Read absorbance at 492 nm.

Results

COMPARATIVE RESULTS OF VARIOUS BLOCKING AGENTS

Sera were routinely diluted 1:1000 to permit evaluation of high reacting lepromatous sera. The background levels of normal sera did not vary significantly at dilutions ranging from 1:500 to 1:1000.

It can be seen in Table 1 that both 10% BSA and 20% EAC, blocked the least. Normal sera were reading above 1·0 (A). Even with the moderate reactors, there were still readings above 2·0 (A). The high concentration of these two blocking agents prevented them from blocking efficiently. The same two blocking agents at a lower concentration blocked better. It was observed that the high concentration of albumin (protein) resulted in the production of large bubbles in this viscous solution. These bubbles obstruct the critical contact necessary between the blocking agent and the

Table 1. Comparative blocking efficiency of blocking agents

Sera No.	Blocking agents*							
	5% BSA	10% BSA	10% NGS	10% EA	20% EA	10% EAC	5% EAC	10% SM
High reactors								
1	1·63†	1·61	1·73	2·10	1·69	1·47	1·39	1·12
22	2·46	2·20	2·40	2·65	2·14	1·56	1·66	1·95
95	2·65	2·19	2·44	2·77	2·41	0·79	2·05	1·91
180	1·81	1·73	1·64	1·95	1·55	1·40	0·81	0·87
Moderate reactors								
10	0·37	0·39	0·36	0·37	0·26	0·25	0·23	0·16
23	0·67	2·20	0·59	0·87	2·17	0·56	0·31	0·36
32	0·31	0·26	0·24	0·31	1·91	0·12	0·29	0·11
35	0·52	1·71	0·42	0·55	1·51	0·40	0·32	0·26
Normal sera								
2	0·10	1·38	0·07	0·11	1·19	0·04	0·17	0·04
3	0·15	1·79	0·11	0·14	1·58	0·07	0·28	0·06
19	0·13	1·67	0·09	0·13	1·53	0·04	0·32	0·08
33	0·15	0·10	0·10	0·14	0·08	0·05	0·13	0·05

* BSA, bovine serum albumin; NGS, normal goat serum; EA, egg albumin, fresh; EAC, egg albumin, crude; SM, skimmed milk.
† Absorbance at 492 nm.

Table 2. Comparative absorbance range of the best blocking agents

Sera	10% NGS*	10% EAC	10% SM
High reactors ($n=12$)	2·35±0·60†	2·16±0·60	2·13±0·60
Moderate reactors ($n=12$)	0·32±0·20	0·30±0·20	0·27±0·20
Normal sera ($n=12$)	0·08±0·05	0·08±0·04	0·05±0·02

* NGS, normal goat serum; EAC, egg albumin crude; SM, skimmed milk.

† Mean ± range of ELISA absorbance at 492 nm.

Table 3. Comparative expense of blocking agents*

Normal goat serum, mycoplasma tested, Gibco	$6·22/100 ml of 10% NGS
Bovine serum albumin 98–99% purity Sigma	$7·97/100 ml of 10% BSA
Egg albumin, crude dried egg white, Grade II, Sigma	$0·20/100 ml of 10%EAC
Egg albumin, fresh egg white	$0·03/100 ml of 10%EA
Skimmed milk, instant non-fat dry milk	$0·05/100 ml of 10%SM

* Cost based on prices (US$) in Honolulu, Hawaii (1986).

binding sites of the plates. The best blocking reagents were: 10% NGS, 10% EAC, 10% SM. These resulted in low normal sera values without inhibiting the reactivity of the high and moderate reactors. Although the maximum range of reactivity decreased with the effectiveness of the blocking agents, the distinction between strong, moderate and normal sera became clearer. The 5%BSA and 10% EA were not as efficient in blocking but did permit a clear distinction between high reactors, moderate reactors and normal sera.

COMPARATIVE RANGE OF VALUES FOR THE BEST BLOCKING AGENTS

The three most efficient blocking agents were selected based on the values observed in Table 1: 10% NGS, 10% EAC, 10% SM. These reagents were compared with 36 sera as summarized in Table 2. Twelve samples were chosen for each group of high reacting, moderate reacting and normal sera. To avoid any plate variation, each group was loaded on one plate diluted in the three different blocking agents: column 1, blank; columns 2, 3, 4, 10% NGS; columns 5, 6, 7, 10% EAC; columns 8, 9, 10, 10% SM; columns 11, 12, conjugate. The same procedure was followed for the moderate and normal sera. As can be seen in Table 2, the lowest range for normal sera was found with 10% SM without adversely decreasing the response of the moderate and high reactors.

COMPARATIVE COST OF THE DIFFERENT BLOCKING AGENTS

Table 3 compares the relative cost of the different blocking agents per 100 ml of a 10% solution. Bovine serum albumin and normal goat serum are the two most expensive blocking agents costing US$7·97/100 ml and US$6·22/100 ml respectively. Skimmed milk and both fresh and crude egg albumin were found to be the least expensive: US$0·03 to US$0·20 per 100 ml.

Table 4. Comparison of mean OD values obtained using 10% skimmed milk and 10% NGS

Type of Sera	# Samples	10% SM*	10% NGS†	Deviation‡
Multibacillary§	31	0·52 ± 0·57	0·54 ± 0·57	0·03
Paucibacillary	32	0·05 ± 0·04	0·03 ± 0·04	0·02
Contacts¶	35	0·07 ± 0·09	0·07 ± 0·10	0·02
Normals	39	0·04 ± 0·04	0·04 ± 0·04	0·03

* Mean OD ± standard deviation for values using 10% skimmed milk as the blocking agent.

† Mean OD ± standard deviation for values using 10% normal goat serum as the blocking agent.

‡ Mean deviation between OD values obtained using 10% skimmed milk and 10% normal goat serum.

§ Sera from multibacillary patients.

¶ Sera from contacts of multibacillary patients.

COMPARATIVE ABSORBANCE READINGS OBTAINED USING 10% SM AND 10% NGS

A field evaluation using 10% SM as a blocking agent was conducted in Cebu, Philippines. Four different samples representing three brands of powdered skimmed milk were purchased and used during the course of this study. A total of 548 serum samples were analysed for IgM antibody against the natural disaccharide, ND-O-BSA. The group consisted of: 39 normals, 137 paucibacillary cases, 156 new contacts, 95 old contacts, 78 new multibacillary cases and 48 old multibacillary cases. No difference in value of positive and negative controls could be found with the use of skimmed milk from different brands. One hundred and thirty-seven samples were chosen at random, and their absorbance readings compared with those obtained in Hawaii using 10% NGS as the blocking agent. Results of the evaluation are compared in Table 4. The mean deviation in absorbance values obtained using 10% NGS and 10% SM are as follows: multibacillary, 0·03; paucibacillary, 0·02; contacts (new and old), 0·02; normals, 0·03.

Discussion

In the ELISA procedure, one of the first requirements is the blocking of plates to prevent nonspecific binding of serum antibodies and enzyme reagents. Failure to sufficiently block available binding sites leads to increased absorbance (A) readings and therefore, false positive reactions of supposedly negative sera. An effective blocking agent is thus of utmost importance. For some countries, the efficacy of a blocking agent is not the only criterion needed to successfully apply the ELISA technology. Several laboratories that use this technology in their research, are still being hampered by costly reagents, primarily blocking agents that also require special attention with shipping and handling. To accelerate the process of transferring this valuable technology to these regions therefore, requires blocking agents that are not only very effective but also affordable and easily available.

Judging from the values of both Tables 1 and 2, we find 10% SM to be the most effective blocking agent, giving a lower range for absorbance readings. The other blocking agents, 10% NGS (normal goat serum) and 10% EAC (egg albumin, crude) could also be considered as effective blocking agents since their overall absorbance range are not too far off from those of 10% SM. However, they both have their drawbacks: both require cold temperatures for storage (10% NGS at −20°C, 10% EAC requires 0–4°C). This cold temperature requirement becomes a problem when they need to be shipped to some countries. Some areas do not have efficient delivery services

available to them that will guarantee frozen or refrigerated conditions upon arrival of the reagents. Skimmed milk on the other hand is available in 3·2 oz packets that can be stored at room temperature for prolonged periods of time alleviating the problem of inadequate freezer and refrigerator storage space and eliminating cold temperature shipping. It can also be purchased at local grocery stores avoiding the troublesome process and delays associated with ordering from foreign suppliers.

Another favourable aspect of skimmed milk is its simple preparation. All that is required is weighing of the blocking agent and then dissolving in PBSTW20. It dissolves quite readily, unlike 10% EAC which requires 30 min of constant stirring with a magnetic stir bar and filtering to remove any undissolved sticky aggregates.

Economically, skimmed milk is comparatively very inexepensive, with a cost of 5 cents per 100 ml for a 10% preparation. One litre of a 10% solution will block four plates, dilute 152 samples plus 4 controls at 1 : 500 (10 μl of sample and 4990 μl diluent), and dilute 20 ml of conjugate at 1 : 6000 for a total cost of 50 cents.

Fresh egg albumin, a cheaper and easily available blocking agent has been used by one of us at the National Institute of Dermatology in Nanjing, China. It blocks well compared to others and could possibly be used as an alternative, however, we find its preparation to be quite tedious, requiring careful aspiration of the white portion of the egg and a subsequent filtration to remove coagulated aggregates.

In some countries, skimmed milk or nonfat dried milk (BLOTTO) is already used by researchers in technologies other than microtiter ELISA, for example: recombinant DNA and Western blot techniques.[6] Although bovine products such as skimmed milk and bovine albumin as well as normal goat serum may contain cross-reactive immunoglobulins, these reagents did not adversely interfere with the ELISA system we tested.

An exhaustive evaluation using skimmed milk as the blocking agent was conducted at LWM Cebu, Philippines. We compared the values obtained in Cebu with those obtained using 10% NGS here in Hawaii. The OD values were very much comparable with a mean deviation of 0·02 for paucibacillary and contact samples, and 0·03 for multibacillary and normal samples. As can be seen in Table 4 there is no significant variation in the mean and standard deviation between the OD values obtained using the two blocking agents. No significant deviation was observed in the OD values when we compared results obtained with the skimmed milk used in Cebu and skimmed milk used in Hawaii.

We recommend the use of skimmed milk as an alternative blocking agent for leprosy ELISA because it blocks very effectively, is simple to prepare, does not require cold temperatures for shipping and storage, is more easily available than other conventional blocking agents and is quite inexpensive to use. It is relatively pure since it is a pasteurized product and is screened by the dairy industry for bacterial contamination.

Since initial presentation of this work at the group meeting sponsored by the World Health Organization, Western Pacific Region held in Manila, June 1986, 10% SM/PBS Tween 20 blocking system has been used successfully in Manila, Philippines and Bangkok, Thailand.[7,8]

Acknowledgments

This research was supported by the Leonard Wood Memorial American Leprosy Foundation, Rockville; National Institute of Allergy and Infectious Diseases Grant #R22 A1 24154; Pacific Health Research Institute, Honolulu; and the University of Hawaii Office of Research Administration. We thank the Philippine Government Ministry of Health for their cooperation in the collection of sera used in this study. We also thank Ms Manuela Luisa Parrilla for her excellent technical assistance at Leonard Wood Memorial Research Laboratory, Cebu, Philippines.

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TEACHING MATERIALS AND SERVICES

English Language Book Society (ELBS); catalogue

The ELBS Student Editions are low-priced editions of British publishers' books, chosen by an advisory committee for their value to students in developing countries. They are priced at between one third and a half of the price of the cheapest publishers' editions and are made available to students by a subsidy from the British Government in approximately 80 countries:

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West Indies and Atlantic Antigua, Bahamas, Barbados, Belize, British Virgin Islands, Cayman Islands, Dominica, Guyana, Jamaica, Monserrat, St Kitts-Nevis, Anguilla, St Lucia, St Vincent, Surinam, Trinidad and Tobago, Turks and Caicos Islands, St Helena.

Middle-East Egypt, Jordan, Sudan.

The main subjects covered are: social sciences, pure sciences and applied sciences. Under medical sciences (p 25) and medical microbiology (pp 27/28) alone, there are numerous entries of considerable interest. Many important books, including several on laboratory work in developing countries, etc., are either available or under development or revision. In countries where there is a British Council Library, an exhibition set of these ELBS books is usually kept for consultation. Books cannot be supplied free: they should be purchased direct from local booksellers. Source: ELBS, The British Council, 11 Portland Place, London W1N 4EJ.

NEWS AND NOTES

XIIIth International Leprosy Congress, 11–17 September, 1988, The Hague, The Netherlands

Full details of the sessions have already been printed in this journal; see Number 1, **58,** 1986.

The Congress Location and Hotel Accommodation: The 13th ILA Congress will be held in The Netherlands Congress Centre, The Hague, The Netherlands, from 11–17 September 1988. Hotel accommodation will be provided in several price categories ranging from ca. Dfl. 50,-to Dfl. 250,- and more. *Congress Bureau*: For all information concerning the congress, please contact the Congress Bureau: QLT Convention Services, Keizersgracht 792, 1017 EC Amsterdam, The Netherlands. Tel. +31 (0)20-26 1372, Tlx. 31578 inter nl att qlt. This Meeting is co-sponsored by the World Health Organization.

XIIth International Congress for Tropical Medicine and Malaria, September 1988

This congress will be held in the International Congress Center RAI in Amsterdam from 18–24 September 1988, immediately after the International Leprosy Congress (above).

Information can be obtained at: Organisatie Bureau, Amsterdam, Europaplein 12, 1078 GZ Amsterdam, The Netherlands. Tel. +31 (0)20-440807, Tlx. 13499.

Meeting of the International Society of Dermatology, Oxford, September 1988

A joint meeting of this Society with the International Society of Dermatopathology will take place in Oxford, UK, 4–8 September, 1988. There will be at least two sessions on leprosy, including histopathology, together with exhibits and demonstrations, one of which will come from the Wellcome Institute of Tropical Medicine in London. Further details; Mrs Christine Cherry, Department of Dermatology, the Slade Hospital, Headington, Oxford, OX3 7JH, England.

Mini-leprosy Guide, 1988, National Leprosy Organization, India

Mr Tare of the Gandhi Memorial Leprosy Foundation, PO Hindinagar, Wardha 442 103, India, has kindly sent a copy of this excellent guide or diary which, as in previous years, contains a great deal of basic information about leprosy. The opening pages (in red print) deal with diagnosis, classification, treatment, etc, and the final pages with sources of further information, a list of journals on leprosy, together with details of teaching–learning material available from GMLF and other agencies. The inclusion of so much useful information in a diary, which is likely to be in almost daily use by many people, is clearly an excellent idea—and one which might well be copied in French, Spanish, Portugese and some local languages.

Innovations in Medical Education; University of New South Wales

The latest publication from the School of Medical Education, University of New South Wales, PO Box 1, Kensington NSW 2033, Australia, is entitled 'Implementation and Innovations in Medical Education' (1987). It records the proceedings of an intercountry workshop sponsored by the World Health Organization and held at the WHO Western Pacific Regional Teacher Training Centre for Health Personnel in the above university. The main contents included: the medical curriculum and primary health care; trends in the medical curriculum; case studies in educational innovation; organizational arrangements for curriculum development; planning and managing change; evaluation of educational innovation. One of the annexes gives a list of the participants and their affiliations. This publication is a valuable reference source for anyone involved with changes in curricula in medical education (and should in fact also be in the hands of medical school staff even if they are not considering changes). It may also help those who are currently attempting to draft outline curricula or modules for the teaching of leprosy in medical schools.

The Global AIDS Epidemic in 1987–88; WHO

Press Release WHO/34 of 16 December 1987 gives an excellent account of the present world situation in general terms, including reference to the dramatic increase in the number of countries reporting AIDS during 1987. Another document, WHO Features, Number 114, December 1987, deals with the subject in much greater detail: 'The AIDS pandemic poses serious questions for public health worldwide. What is the state of AIDS in the world today? How does the pandemic affect specific regions? What are the prospects for a vaccine or treatment? What role does WHO play? In an interview with Dr Jonathan Mann, Director of the World Health Organization's Special Programme on AIDS, these and other questions are addressed. It includes also a comprehensive review of activities over the past year as well as recent developments in the global AIDS epidemic.'

Lepr Rev (1988) **59,** 45–52

Association of IgG and IgM antibodies to phenolic glycolipid-1 antigen of *Mycobacterium leprae* with disease parameters in multibacillary leprosy patients

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Summary IgG and IgM antibodies to the phenolic glycolipid-1 (PGL-1) antigen of *Mycobacterium leprae* were assessed using an enzyme-linked immunosorbent assay (ELISA) in 77 multibacillary leprosy patients. No correlations were found when their absorbance values were compared to: disease type and duration, bacillary load, reactional status, or concurrent secondary infection. A statistical difference was seen between patients with and without neurological deficiency.

Introduction

Cellular immune response to *Mycobacterium leprae* is under intensive investigation but following purification of antigens and the development of more sensitive techniques interest in humoral response has also risen. The first studies used the cross-reacting antigen 7 in a sophisticated radioimmunoassay.[1,2] A more recent study[3] demonstrated a unique phenolic glycolipid antigen of *M. leprae* which differs from the glycolipids found in other mycobacteria.

This specificity of PGL-1 of *M. leprae* has led to the development of the use of ELISA to detect circulating antibody in leprosy patients.[4–6] Elevation of PGL-1 IgG and IgM globulin levels occurs in multibacillary leprosy but this is less marked, or absent, in paucibacillary disease.

Considerable variation of globulin levels appears to occur even between patients with the same classification of multibacillary disease. This investigation compares some of the factors which may account for this variation including: disease type and duration, bacillary load, reactional status, concurrent secondary infection and neurological deficiency.

Materials and methods

Seventy-seven multibacillary leprosy patients were selected for the study, all of whom were either outpatients or short-term inpatients of the Tropical Diseases Unit at Harare Central Hospital, Zimbabwe.

0305-7518/88/059045+08 $01.00

Disease classification in all cases was based on the Ridley–Jopling[7] scheme using clinical and histopathological criteria. The duration of disease ranged from 3 months to 40 years and some patients had previously received monodapsone therapy. All were currently receiving a 2-year regimen of rifampicin 600 mg and clofazimine 300 mg once monthly and clofazimine 50 mg and dapsone 100 mg daily.

Reactional status was assessed clinically as either reversal reaction (RR) or erythema nodosum leprosum (ENL). Neurological deficit was detected using sensory and voluntary muscle testing. Anaesthesia of hands and feet was assessed with pressure graduated single nylon filaments and by two-point discrimination tests. Cotton wool wisps or a light-pressure nylon filament was used to detect corneal anaesthesia. Motor deficit of the hand was determined by flexion and extension of the wrist and metacarpophalangeal joints and by adduction and abduction of the digits. Plantar flexion and dorsiflexion, tarsal inversion and eversion and flexion and extension of metatarsal-phalangeal joints were used to determine similar motor deficit of the feet. Facial nerve involvement was assessed by weakness of facial musculature including blink and eye closure.

Serum was separated from whole blood and stored, without preservative, at $-20°C$ until use. Specimens were thawed once only for the purpose of this investigation.

The ELISA used was basically that of an earlier study[8] as modified for use with PGL-1.[5] Specifically PGL-1 (kindly provided by Dr P Brennan) was mixed with 0·05M carbonate buffer pH 9·2 and suspended by sonication with a probe at 45 Watts for 90 s (Ultrasonics, Inc., Plainville, NY, USA).

The suspended antigen was diluted in the same buffer to a concentration of 2 μg in 50 μl, the volume which was added to 48 wells of a 96-well polyvinyl flatbottom microtitre plate (Cook Labs., Alexandria, Va, USA). The remaining 48 wells received 50 μl of the carbonate buffer without antigen.

The plates were covered with a plastic lid and incubated overnight at 4°C. After incubation, the wells were washed 3 times for 7 min each with 200 μl of wash buffer consisting of 0·01 M phosphate buffered saline (PBS) pH 7·2 containing 1% bovine serum albumin (Fraction V, Sigma, St Louis, Mo, USA). After washing, the wells were blocked by the addition of 100 μl of 0·01M PBS – pH 7·4 containing 5% BSA and incubated at 37°C for 1 h.

The wells were then washed as before and 50 μl of each serum sample, diluted 1:300 in wash buffer, was added to quadruplicate wells of the microtitre plate: 2 with antigen and 2 not containing antigen. Each plate also contained negative controls (pooled serum samples from two normal individuals) (Mean $\pm$ SD Delta Abs. for IgG = 0·134 $\pm$ 0·043; IgM = 0·012 $\pm$ 0·014, N = 72); positive multibacillary controls (pooled serum samples from one polar lepromatous and one subpolar lepromatous patient), (Mean $\pm$ SD Delta Abs. for IgG = 0·314 $\pm$ 0·065; IgM = 0·077 $\pm$ 0·032, N = 72); and positive paucibacillary controls (serum samples from two borderline tuberculoid patients) (Mean $\pm$ SD Delta Abs. for IgG = 0·159 $\pm$ 0·072; IgM = 0·045 $\pm$ 0·020, N = 72). The plates were incubated at 37°C for 45 min, removed and washed as before.

Horseradish peroxidase conjugated IgG fraction of goat antihuman IgG (γ-chain specific) and horseradish peroxidase conjugated IgG fraction of goat antihuman IgM (μ-chain specific) (Cappel Labs., Cochranville, Pa, USA) were diluted in wash buffer. Fifty microlitres of the peroxidase-linked goat antiserum to human IgG and IgM heavy chain each containing 35 ng of the precipitating antibody was added to quadruplicate wells of the microtitre plate: 2 with antigen and having been incubated with serum and 2 without antigen and having been incubated with serum.

The plates were incubated for 45 min at 37°C when they were removed and washed as before. After washing, 50 μl of a solution containing 0·4 mg/ml orthophenylene-diamine (Sigma, St Louis, Mo, USA) with 0·002% H_2O_2 in 0·02M sodium acetate buffer pH 5·5 was added to each well. The plates were incubated at room temperature for 10 min when the reactions were stopped by adding 5 N HCl – 50 μl per well.

Absorbances were read at 492 nm with a spectrophotometer (Titertek Multiscan, Flow Labs., Richmond, Va, USA). Mean absorbance values and coefficient of variation for each set (pair) of like

wells were calculated. If the coefficient of variation for either set exceeded 20%, values were not taken and tests were repeated. The antibody reactivity to PGL-1 for each serum sample was calculated by subtracting the mean absorbance of duplicate samples in the carbonate buffer coated wells from the mean of duplicates of the PGL-1 coated wells.

Results

Absorbance values for IgG and IgM globulins to PGL-1 are presented in Figures 1–6. The duration of disease (Figure 3(a) and 3(b)) is calculated from the year of onset indicated by the patients and are not necessarily the same as the onset of treatment.

Patients with neural deficit resulting from known episodes of RR are excluded from Figure 4. At the time when blood specimens were taken 14 patients were experiencing RR and 22 had ENL (Figure 5). Eighteen patients had secondary infection at the time the specimen was taken or within 2 months previously (Figure 6).

Discussion

The presence of PGL-1 antibody in sera of leprosy patients is a specific marker of the disease. The ELISA provides a convenient method of assessing antibodies to PGL-1 and with careful internal controls is suitable for handling large numbers of specimens. The method is relatively inexpensive and can be incorporated easily into leprosy control programmes. Although the methodology can be

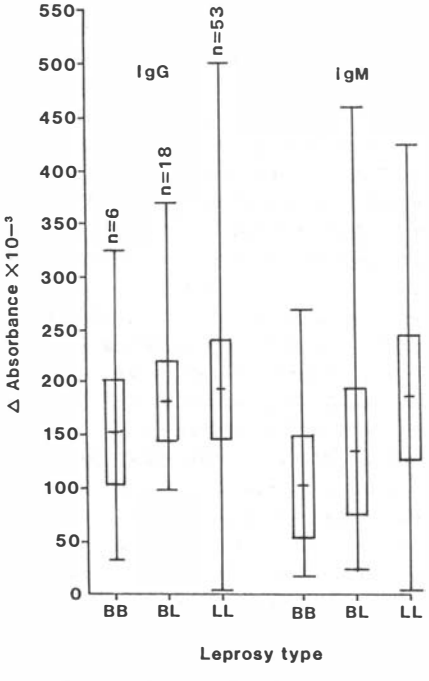

Figure 1. IgG and IgM absorbance values and leprosy classification. (The range of results is indicated by the vertical line, the standard deviation by the vertical column and the mean by the horizontal bar.)

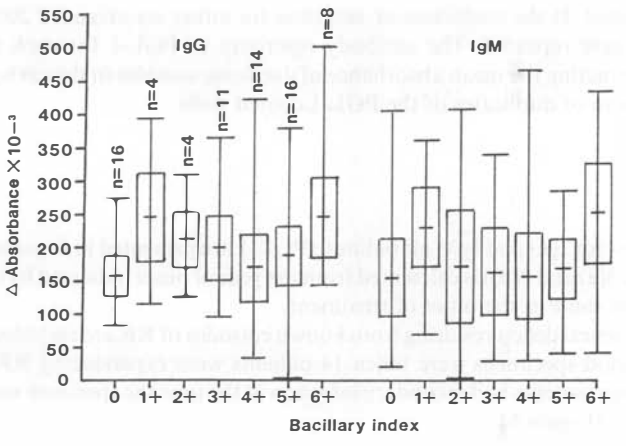

Figure 2. IgG and IgM absorbance values and bacillary index (assessed by slit skin ear smears). The BI of 4 cases was not recorded at the time blood was collected.

standardized, in our experience there is a wide variation in the IgG and IgM absorbance values to PGL-1 from patients within the lepromatous spectrum. This observation is in agreement with previously reported findings among LL patients.[4]

Good correlations with parameters including clinical classification and bacterial indices (BI) with IgM absorbance values to the deacylated PGL-1[6] or the native PGL-1[9,10] have been reported. The results presented here are in contrast to these observations. While showing an upward trend in IgG and IgM absorbance values from mid-borderline to lepromatous, the wide scatter and overlap indicated a marked lack of correlation. A similar wide deviation and lack of correlation was seen in absorbance values of IgG and IgM antibodies to PGL-1 and BI. This observation was in agreement with those of a previous study.[11] They found no significant correlation between IgG, IgM and IgA absorbance values to PGL-1 and BI among lepromatous patients.

Clearly there are distinct differences among laboratories, especially when patients at the lepromatous end of the spectrum have been assayed for their antibody responses to PGL-1. Such opposing findings could be explained by variations in methodology, reagents and experimental design.

This ELISA methodology required the use of secondary antibodies. We attempted to standardize our methodology by using equal ng of precipitating peroxidase conjugated anti-μ and anti-γ reagents. However, the equalization of the number of peroxidase molecules conjugated to the antiglobulins, their capacity and affinity to react with the two heavy chains of IgG and the 10 heavy chains of IgM cannot be standardized. Furthermore it is not clear whether discontinuous solid phase assays like the ELISA which require frequent washing steps measure antibody concentration of affinity.[12] The enormous range of responses to this *M. leprae*-specific antigen is not easy to explain. It seems that the humoral immune response to *M. leprae* antigens varies between patients but individually is remarkably stable.[13] Individual response may be mediated by hereditary factors and contact with environmental mycobacterial antigens.[14] A strong response to other mycobacterial antigens may interfere with the stimulating effect of glycolipids on the humoral immune system.

In addition the presentation of the PGL-1 antigen could be masked. *In vitro* the lipid content of the whole native antigen might block binding sites on the ELISA, although this may not be the case *in vivo* where recognition and humoral response may depend on exposed saccharide precursors. If this were the case it could explain the lack of a significant rise in antibody level from 1+ to 6+

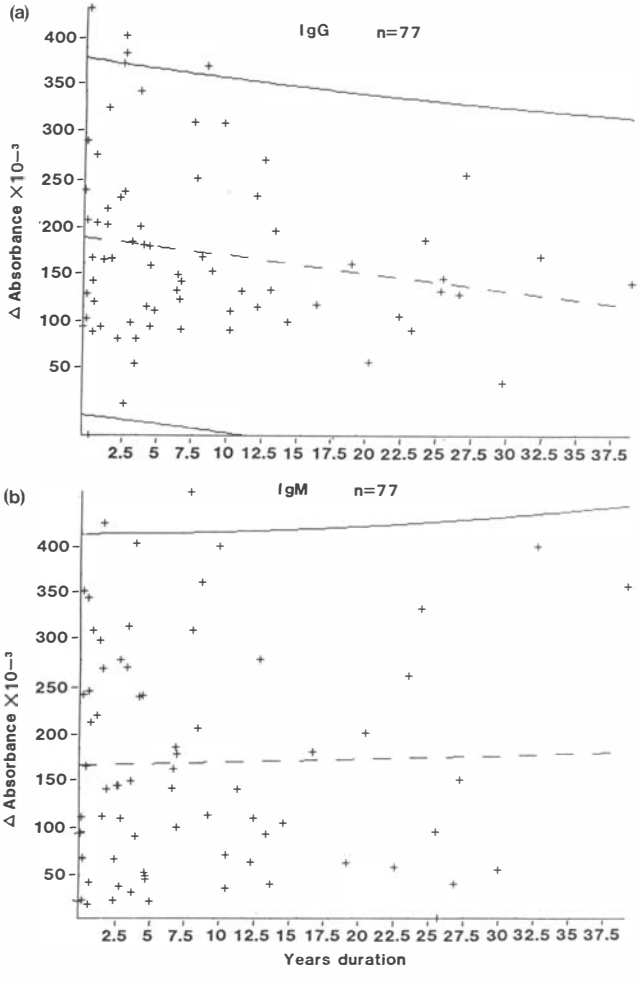

Figure 3(a). IgG absorbance values and disease duration. The central dotted line indicates the regression line interval (solid bounded by the 95% confidence line). (b) IgM levels and disease duration.

bacteriological index as a significant level of lipid is found in the skin of multibacillary leprosy patients.[15]

Duration of disease also appears to have little effect on absorbance values for IgG and IgM anti-PGL-1 immunoglobulins although there is a suggestion in the results presented here that IgG levels fall faster than IgM. This is in agreement with observations made by Melson for whole *M. leprae* antigen.[16] Chronicity of these levels might be explained by the enormous load of antigen present in multibacillary cases which must take many years to clear.

If neurological deficity can be taken as evidence of progression through the higher resistance paucibacillary forms of the disease then absence of such trauma might indicate onset nearer the lepromatous pole. Comparison of IgG and IgM absorbance values to PGL-1 showed that patients presenting neural deficit had significantly lower IgM absorbance values than patients presenting

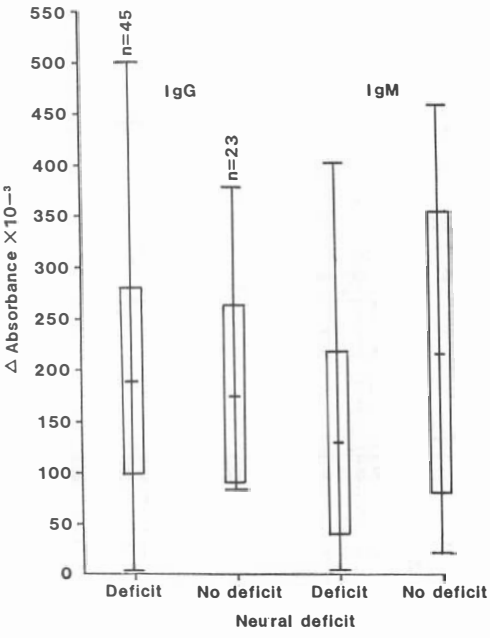

Figure 4. IgG and IgM absorbance values of patients with and without neural deficit. (Nine patients with neural deficit known to be due to RR were omitted.)

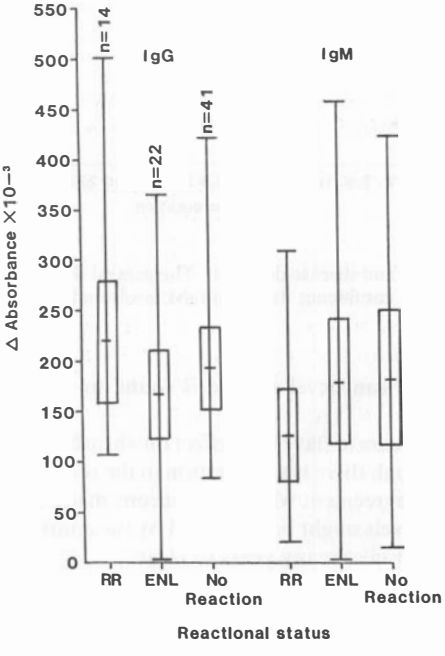

Figure 5. IgG and IgM absorbance values in RR, ENL and non-reactional patients.

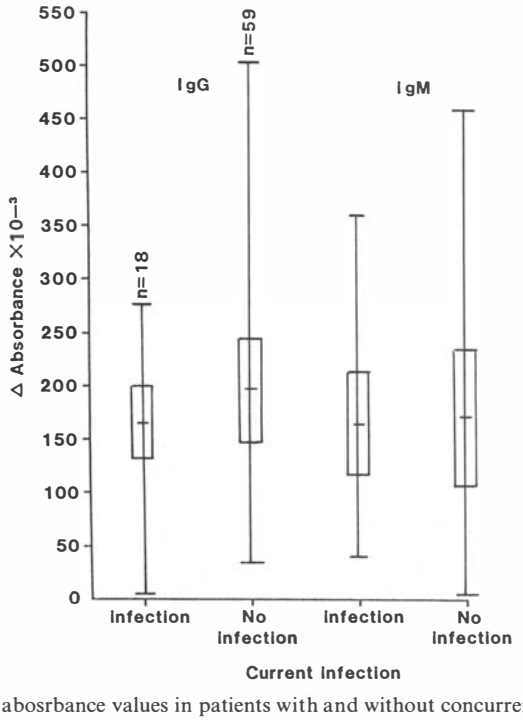

Figure 6. IgG and IgM abosrbance values in patients with and without concurrent secondary infections.

with no neural deficit (Students *t*-test, $P < 0{\cdot}02$). There was no significant difference with IgG anti-PGL-1 absorbance values and neural deficit.

Reactional status appears not to bias IgG and IgM immune response to PGL-1 although the mean IgG absorbance values were higher than IgM in RR. This was not seen in patients presenting with ENL.

Melson *et al.*[16] and Cruickshank & Ellis[17] found an increase in immunoglobulin during ENL. These investigators however, followed individual patients during the course of the reactional episodes and were not assessing a single serum specimen as in our series.

Many leprosy patients exhibit a secondary infection but no evidence of nonspecific polyclonally-induced antibodies produced as the result of such infection was seen in this investigation.

From the results obtained it appears that detection of immunoglobulins against native PGL-1 antigen is of limited value in differentiating between the multibacillary forms of leprosy. With the exception of neurological deficit other factors including bacillary load, duration of disease, reactional status and secondary infections seem not to influence the IgG and IgM absorbance values to PGL-1.

Acknowledgments

We would like to thank Mrs J Els for her technical assistance, Mr M Mabena for preparing the figures and Mrs M Masterman for typing the manuscript.

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Are hypersensitivity reactions to dapsone becoming more frequent?

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Summary Hypersensitivity reactions to dapsone, which were common in the late 1940s and early 1950s and then virtually disappeared, have now reappeared in the last 5–6 years. Review of the literature and a postal survey of centres using dapsone on a mass scale confirms that the reaction has reappeared. The explanation for this is unclear but may be related to the use of dapsone combined with other drugs. These reactions are rare and some centres treating large numbers of patients with dapsone have not experienced any cases. Dapsone must still be regarded as a safe preparation.

Introduction

Diaminodiphenylsulphone (dapsone) has proved a safe and effective drug, since its introduction in the 1940s, in the management of a variety of conditions from leprosy and malarial prophylaxis to dermatitis herpetiformis. The toxic effects and adverse reactions to dapsone have been well documented. In the early years after the introduction of dapsone to clinical use hypersensitivity reactions to dapsone occurred frequently but then rapidly declined. Few cases were reported between the mid 1950s and the late 1970s. However, over the last 5 or 6 years case reports of hypersensitivity reactions to dapsone have reappeared in the literature. This paper reviews the literature on hypersensitivity reactions to dapsone and presents the findings of a postal survey carried out in 1986.

Literature review

A number of adverse reactions associated with dapsone have been reported sporadically including agranulocytosis,[1] peripheral neuropathies,[2] toxic epidermal necrolysis,[3] hypoalbuminaemia,[4] phototoxicity,[5] nephrotic syndrome,[6] and haemolysis[7]. The toxic effects associated with dapsone in high dosage have also been well documented and these include renal impairment,[8] haemolytic jaundice,[9] hepatitis,[10] methaemoglobinaemia,[11] psychosis,[12] and optic atrophy.[13] The frequency of adverse reactions appears to be associated with dosage, and is higher when the dose is in excess of 100 mg daily. There have been a number of review articles[14,15] on the side-effects of dapsone but the concensus remains that dapsone is an extremely safe drug.

0305-7518/88/059053+06 $01.00

Hypersensitivity reactions to sulphones were reported[16] as early as 1944 when used to treat tuberculosis and later[17] in the treatment of leprosy. Many adverse reactions were encountered in the early use of the sulphones and attempts were made to find a less toxic derivative.[18] The earliest reports of a hypersensitivity reaction to dapsone was published in 1949 based on work in Nigeria.[19] In this early description of the reaction it was thought that dapsone precipitated glandular fever. The 'illness' arose early in the treatment and was characterized by fever, lymphadenitis, splenomegaly, jaundice, abnormal liver function tests, mononucleosis, and dermatitis including generalized exfoliation. Positive Paul Bunnell tests and mononucleosis led to the diagnosis of glandular fever. The authors suggested a gradual build up in dose to 300 mg daily was advisable to avoid this complication.

Lowe reviewed the situation in 1950[20] when reporting 3 further cases of hypersensitivity reaction and advised early withdrawal of dapsone, the use of antihistamines followed by desensitization. Another publication reported liver biopsy evidence of hepatic damage in 3 cases of the reaction.[21] In 1951 the reported frequency of hypersensitivity to dapsone was 2%,[22] fatal outcomes associated with exfoliation were reported and the relation between the reaction and doses in excess of 100 mg daily noted. In 1957 Barnes coined the name dapsone syndrome[23] and advised lowering the dose of dapsone.[24] Other workers reported an incidence of hypersensitivity to dapsone when used on a mass scale.[25,26] Case reports appeared from Malaya[27] and New Guinea.[28]

From 1956 until 1980 there were only 2 reports of the reaction in the literature. A number of

Table 1. Case reports of hypersensitivity reaction to dapsone 1980–86

Year	Country (reference)	Patient age	Patient sex	Daily dose of dapsone (mg)	Other drugs used
1980	India[33]	40	F	100	—
1980	India[34]	30	F	50	—
1980	India[34]	30	M	50	—
1981	*USA[35]	17	M	100	—
1981	USA[36]	16	F	50	—
1981	India[38]	?	?	?	?
1982	Australia[39]	49	F	150	—
1982	Denmark[40]	33	F	100	—
1984	Malaysia[41]	17	F	100	Rifampicin
1984	Malaysia[41]	18	F	100	—
1984	Malaysia[41]	61	M	200	—
1984	India[42]	?	?	50–100	—
1984	India[42]	?	?	50–100	—
1985	Thailand[43]	50	F	50	R+C
1985	Thailand[43]	24	F	?	R+C
1985	Thailand[43]	35	M	100	—
1985	Thailand[43]	45	F	100	—
1985	Guyana[44]	—	—	?	R+C
1985	Guyana[44]	—	F	?	—
1985	India[45]	35	M	100	R+C
1985	India[46]	7	F	25	—
1985	India[46]	25	M	100	Rifampicin
1985	India[46]	26	F	100	R+C
1986	*Papua New Guinea[47]	20	M	100	Clofazimine
1986	Papua New Guinea[47]	20	M	100	Clofazimine
1986	*India[48]	40	M	100	—
1986	*USA[49]	49	F	50	—

R+C refer to use of rifampicin and clofazimine.
* Refers to fatal cases.

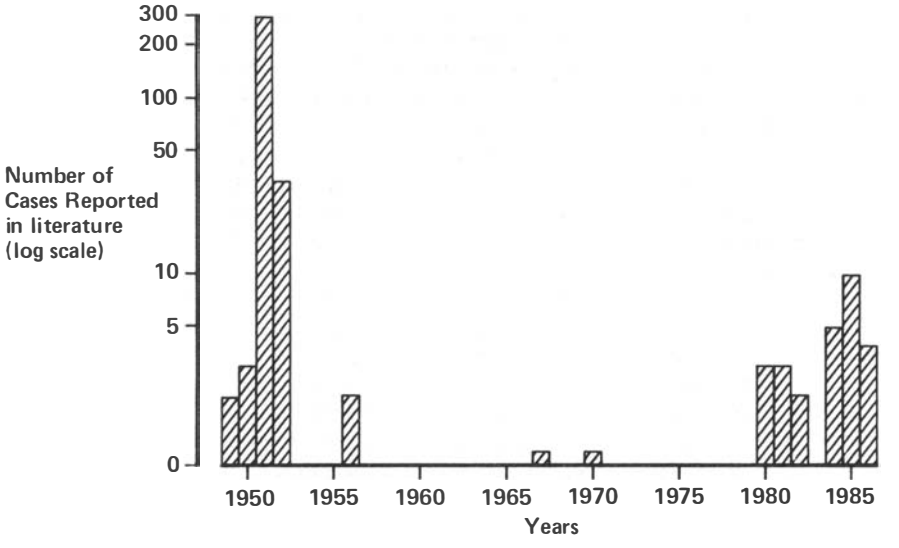

Figure 1. Number of cases of hypersensitivity reaction to dapsone reported in the literature (1949–86).

papers were published on the management of the reaction; the use of cortisone in sensitivity reactions[29] and methods of desensitization;[30] but the only case reports of hypersensitivity reactions to dapsone were in 1967[31] and in 1970.[32] There are other reports in the literature, during this 24-year period, of jaundice associated with dapsone but they do not have the other diagnostic features of hypersensitivity and are mostly haemolytic. Then from 1980 to the present there were 27 case reports published (Table 1). The published case reports of hypersensitivity reactions to dapsone between 1949 and the present have been plotted (Figure 1).

The characteristics of the most recent 27 cases of hypersensitivity reaction were that 9 were in men and 14 in women (4 the sex was not reported) in the age range 7–50 years. Twenty-two were in leprosy patients, 2 in the treatment of dermatitis herpetiformis and one each in the treatment of acne vulgaris, psoriasis and vasculitis. In 23 out of the 27 the dose of dapsone was specified; 16 were on dapsone alone and the remainder were taking dapsone as part of the multidrug regimen (Table 1).

Postal survey

In order to estimate whether these reported cases represented all cases occurring and to estimate the frequency of occurrence of hypersensitivity a postal survey was undertaken. Leprosy centres were chosen as they were likely to be treating large numbers of new cases with dapsone. Since the syndrome occurs within 6 weeks of commencement of dapsone therapy only new cases were of interest. A total of 73 centres were included covering Asia, South and Central America and Africa and replies were received from one-third. Many centres did not treat many new cases and were thus unlikely to encounter the reaction.

Five of the 26 centres replying had recently encountered cases of hypersensitivity and provided case histories of at least 10 episodes (Table 2). These centres were in Nigeria, Korea, India, Zambia and Thailand. Four of the cases were receiving multidrug therapy with clofazimine and rifampicin along with dapsone. Two were from previous years and associated with dapsone alone. One centre

Table 2. Case reports of hypersensitivity reaction to dapsone from a postal survey in 1986.

Year	Country (reference)	Patient age	Patient sex	Daily dose of dapsone (mg)	Other drugs used
1982	*Zambia	16	M	100	—
1982	*Zambia	10	F	50	—
1984	*Zambia	46	M	100	Rifampicin
1985	Zambia	38	M	100	R+C
1986	Thailand	32	M	100	R+C
1986	Thailand	52	M	100	R+C
1986	Nigeria	?	F	100	R+C
1986	Nigeria	?	M	100	—
1986	Nigeria	?	?	100	—
1986	Korea	74	M	100	R+C
1986	India	more than one but no details			

R+C refers to use of rifampicin and clofazimine.
* Refers to fatal cases.

although admitting to experiencing cases did not provide clinical details. It was not possible to give an overall estimate of the frequency of the dapsone syndrome but it varied from 0 to 2% of new cases treated.

Discussion

Review of the literature on case reports of hypersensitivity reactions to dapsone shows the reaction to be common (2–12%) in the early years after the introduction of dapsone to clinical use, and then to virtually disappear from the literature between 1956 and 1980. The reasons for this seem unclear and no serious attempt has been made to explain the apparent disappearance. Three distinct explanations seem possible: firstly, the reaction has continued to occur but has not been recognized; secondly, the reaction has continued to occur, has been recognized but has not been reported, and finally, the reaction in fact virtually disappeared over this period. The first explanation seems unlikely due to its severity, while the second is possible having discussed the problem with many mass users of dapsone, although the sudden reappearance of case reports from 1980 onwards describe the hypersensitivity reaction as an unusual occurrence.

One conclusion from these arguments is that hypersensitivity reactions to dapsone did virtually disappear between 1956 and 1980. A proposed explanation is that the incidence of the reaction is related to dose;[35] in the 1950s the recommended dose of dapsone was reduced from 300 mg to 100 mg daily. However, it has been argued that hypersensitivity reactions to dapsone[36] or to any other drug[50] are not dose dependent. Explanations on the genetic basis of hypersensitivity reactions cannot explain sudden changes; and changes in manufacture of dapsone have not been investigated.

The sudden reappearance of reports of the hypersensitivity reaction to dapsone since 1980 also needs to be considered. Publication bias may be a possibility but from the results of the postal survey support the fact that the syndrome is occurring widely. There has been no major increase in the use of dapsone recently but since 1982[51] multidrug regimens, including dapsone, have been recommended in the treatment of leprosy. It is possible that these more complex multidrug regimens lead to wrong administration of dapsone, there is also the potential for drug interactions. About half of the recent cases of hypersensitivity since 1980 have been associated with dapsone

monotherapy which does not support a drug interaction therapy as a complete explanation. There have been well-documented cases of hypersensitivity reactions with similar characteristics associated with clofazimine[52] and rifampicin,[53] this may well appear to exaggerate the frequency of hypersensitivity to dapsone. One other consideration is that dapsone is prepared in combination with pyrimethamine for malarial prophylaxis and may be dispensed in this form instead of dapsone alone,[54] adverse reactions in these circumstances may therefore not necessarily be to dapsone.

Acknowledgments

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NEWS AND NOTES

Back numbers of *Leprosy Review* required

We continue to receive a number of requests for complete sets of *Leprosy Review* and for individual missing numbers. The stocks of back numbers stored by LEPRA are limited. We therefore appeal to anyone who may have an unwanted collection, or odd numbers of the journal, either for donation to a *bone fide* applicant, or for sale. Within reasonable distance of Oxford or London we would be more than happy to arrange for collection or to negotiate payment for transport by train. *Editor.*

Wellcome Tropical Institute Museum, London

Professor Eldryd Parry, Director of the Wellcome Tropical Institute, has recently written as follows:

'I am writing to inform you about our plans to redevelop the Wellcome Tropical Institute Museum (formerly the Wellcome Museum of Medical Science). You will have used the museum in the past and we hope your interest will continue after the changes have been completed.

Plans to redesign the museum and its displays are now being effected. A museum with new material is being constructed on the ground floor of the Wellcome Tropical Institute, 200 Euston Road. The present museum in the Wellcome Building, 183 Euston Road, will close on 27 November 1987.

I will write again when the new museum is ready to open, which will be in 1988. In the meantime our Malaria Exhibition will reopen in the Institute on 14 December 1987.

For further information about the museum, the exhibition (or indeed about the Institute) please contact Dr Alan Knell on 01 387 4477, ext 3365, or write to: The Wellcome Tropical Institute Museum, 183 Euston Road, London NW1 2BP.'

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Care of plantar ulcerations: comparing applications, materials and non-casting

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Summary We have reviewed our experience with plantar ulcer care in our population of 525 patients treated between 1982 and 1987. Patients were treated with standard plaster of Paris casts, alternative methods of casting, and without casting. Of the 24 patients who received casts, all healed, while 23 of the 30 patients healed without casting. The average healing time for those who were casted was $5{\cdot}6 \pm 2{\cdot}6$ weeks and for those uncasted, $8{\cdot}1 \pm 6{\cdot}6$ weeks ($P = 0{\cdot}1$). It was concluded that ulcers can heal without casting and that alternative casting procedures offer certain significant advantages. Such approaches are especially applicable to the current leprosy population being treated in the community and remaining ambulatory.

Introduction

Plantar ulceration, a direct consequence of insensitivity, is a frequent and serious complication in leprosy. Nonhealing neuropathic ulcers often become infected and, if they remain untreated, result in osteomyelitis and bony absorption, thus producing a deformed foot. A deformed foot is even more prone to ulceration. If this cycle of ulceration is repeated often enough, amputation may be necessary. Amputation can be avoided by prompt treatment to close the ulcer, medication to cure the infection, proper footwear for protection of the anaesthetic foot and education of the patient.

The traditional method of applying a plaster of Paris—total contact case (POP–TCC) has been described by others.[1–7] Since 1982 we have had many patients at our clinic who have refused application of a TCC for various reasons, such as inability to use crutches while the cast dries, or living conditions necessitating climbing long flights of steps. The majority of today's leprosy patients are not being hospitalized but instead are working or going to school in either rural or urban areas. For them the appearance of a cast and the wearing of it are often an embarrassment and a hindrance. Thus an alternative to the traditional POP–TCC which would be lighter in weight and permit immediate ambulation without crutches would offer advantages that would enable us to convince some patients to accept casting. For this purpose we have examined several alternatives of both materials and methods of application. In this paper we will review our experience in the care of plantar ulcers in leprosy patients utilizing the standard POP cast, alternative casting procedures, and results obtained without casting.

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Table 1. Casting—theme and variations.

1 Traditional below knee plaster of Paris (POP) total contact cast (TCC).

Materials: Stockinette, orthopaedic felt, POP, board and walking heel or stirrup.

Application: To assist in cast removal orthopaedic felt is applied in a strip down the leg dorsum from top of cast to base of toes and a piece over each malleolus. Then a thin 'egg-shell' layer of POP is applied and moulded well into the leg, followed by supporting layers of POP and application of a board and walking heal or stirrup.

Advantages	Disadvantages
a Materials are generally available.	a Drying time is 24 h or longer.
b Cost is low.	b Some patients cannot use crutches during drying time.
c POP moulds well.	c Walking heel puts cast close to ground thus getting it wt/dirty.
d Ulcerations usually heal without complications	d Cast is thick and heavy.
	e Cast is difficult to remove because it is not bivalved.

2 Total contact cast reinforced with Scotch Cast reinforcing strips.*

Materials: Stockinette, orthopaedic felt, POP, Scotch Cast reinforcing strips, walking device (board and heel or stirrups).

Application: Apply as for technique 1 substituting Scotch Cast reinforcing strip instead of POP immediately after 'egg-shell' layer, then complete cast with POP and walking device.

Advantages	Disadvantages
a Drying time is slightly less.	a Crutches are still needed until the cast dries.
b Cast is lighter and stronger.	b Removal of cast is more difficult due to the hardness of the reinforcing material.
c Materials are generally available.	
d POP and Scotch Cast adhere well to each other.	
e This variation has all the advantages of the traditional below knee TCC.	

3 TCC 'egg-shell' of POP with surrounding cast padding and Scotch Cast to complete.

Materials: Stockinette, cast padding, POP, Scotch Cast, walking device.

Application: Over stockinette apply cast padding only to cover malleoli and any bony prominences. Apply 'egg-shell' layer of POP and mould into leg. Wrap thick layer of padding around 'egg-shell' layer and apply Scotch Cast using as many layers as needed for durability. Apply either heel or stirrup or supply patient with cast boot.

Advantages	Disadvantages
a Patient can ambulate $\frac{1}{2}$ h after application of cast.	a It appears likely that ulcers do not heal as quickly.
b Crutches are not needed.	

Table 1. Casting—theme and variations.

c Cast is light weight and strong.
d Walking device is not needed.
e Removal is simplified by bivalving the cast and cutting the 'egg-shell' layer with plaster-cutting scissors.

4 Orthopaedic padded cast using Scotch Cast only.

Materials: Stockinette, cast padding, Scotch cast, and cast boot.

Application: Apply several layers of cast padding over stockinette, then apply layers of Scotch cast to desired thickness for durability.

Advantages	Disadvantages
a This has all the advantages of technique 3.	a Further breakdown at ulcer site was noted, possibly because padding holds drainage from the ulcer against the skin causing surrounding area to macerate.

5 Shoe cast.

Materials: Stockinette, cast padding, orthopaedic felt, POP, board and heel.

Application: Apply padding over stockinette at ankle if cast is to cover malleoli. Apply orthopaedic felt down front of foot for cast removal, then apply POP as a shoe with board and heel.

Advantages	Disadvantages
a Application is not difficult.	a Cast can cause rubbed areas around the ankle.
b Cast is much higher than a below knee cast.	b Even slight looseness can make ambulation unsafe.
c Removal of cast is easy.	

6 Scotch cast as a total contact cast.

Materials: Stockinette, orthopaedic felt, Scotch Cast.

Application: Apply double layer of stockinette, then apply a thin strip of $\frac{1}{8}$ in thick orthopaedic felt down each side of the leg to cover malleoli and from below malleoli to base of the toes, as well as around leg at top of cast. Apply Scotch Cast in the same manner as POP total contact cast using palm pressure to mould. Walking device can be used or a cast boot.

Advantages	Disadvantages
a Cast is quick drying ($\frac{1}{2}$ h) and no crutches are needed.	a Materials are more expensive.
b Cast is light weight and strong.	b Scotch cast is not available in all countries.
c Application is not difficult.	
d Removal is simplified by bivalving.	

Materials and methods

This report describes our experience with plantar ulcerations in our leprosy population of 525 patients treated from October 1982 to April 1987. In those years 56 casts were applied to 24 patients (16 male, 8 female), ages 14–65 with an average age of 45·9 years. The casting techniques utilized are described in detail in Table 1. Technique 1, the traditional POP–TCC with walking heel or Böhler-type stirrup iron, was applied a total of 24 times to 16 patients. An unpadded POP–TCC has been an excellent means to hasten healing of plantar ulceration while permitting reasonable amounts of ambulation. The traditional method of applying a TCC employs POP as the casting material, with minimal padding used only for pressure areas and as an aid to cast removal. A walking device, board and heel or walking stirrup (Böhler-type iron) is applied and after complete drying of POP the patient may ambulate while healing of the ulcer progresses. Technique 2, POP cast with Scotch Cast (Orthopedic Products, St Paul, Minn, USA) reinforcing strip, was applied a total of 19 times to 9 patients; technique 3, POP 'eggshell' layer finished with Scotch Cast, was applied a total of 2 times to 1 patient; technique 4, Scotch Cast padded cast, was applied a total of 3 times to 3 patients; technique 5, a shoe cast of POP, was applied 1 time to 1 patient; and, technique 6, Scotch Cast as a TCC, was applied a total of 8 times to 3 patients (see Table 2).

All of the patients received, as part of the treatment plan, moulded shoes and insoles with instructions concerning shoe care and wearing time, gait and foot care. The patients' feet were measured and a POP mould for shoes was prepared before the first cast was applied, and, where possible, the shoes were adjusted before final fitting between cast changes, usually at the third week of casting.

Additionally in this report we describe our experience with 40 patients whose ulcers were treated without TCC. The reasons for not casting were: 1, the patient was elderly and unsteady in ambulating; 2, the patient refused casting; 3, the patient could not use the crutches necessary until the cast dried (due to clawed hands); 4, the patient was already hospitalized either for the ulcer or other cause and was, at the time, non-weight bearing on bed rest; 5, the patient had immediate amputation of the ulcerated foot; 6, the ulcer was infected; and 7, the patient left the area immediately after ulcer evaluation.

When the patient was not casted, the ulcer was usually debrided, cleaned well, and dressed with a topical ointment and dry sterile dressing. The patients were then given dressings and instructions for

Table 2. Results of six techniques used for healing plantar ulcers.

Technique used	Patients in sample	Average time cast on (weeks)	Healed	Approx. cost of materials (US $)
1 POP–TCC	16	5½	16	20·00
2 POP, SC Reinforcing	9	5½	9	30·00
3 POP, Padding & SC	1	4	0	20·00
4 Ortho. Padded Cast	3	5	1	35·00
5 Shoe Cast of POP	1	3	0	10·00
6 SC–TCC	3	7½	3	40·00

Those who did not heal using Techniques 3, 4 and 5, were casted with another technique to achieve full healing.

home care as well as being provided with moulded shoes and inserts. They were instructed to limit ambulation and, if possible and necessary, were given crutches and shown non-weight bearing gait. Antibiotics were prescribed if indicated. Surgical procedures such as bony debridement, grafting or amputation were done when conservative treatment failed.

One patient had bilateral ulcerations of long-standing without treatment and had to be casted for extended periods. A variety of techniques were used in applying casts to this patient. However, as the ulcers usually recurred within days of healing, this patient's problem was finally solved by a flap graft. Due to the length of time this patient was casted and recasted, he has been omitted from the statistics. However, because his case is illustrative of many of the most difficult problems confronting ulcer patients and resulted in a salutary outcome, we have decided to present this case in depth (see Results). Also presented is a short summary of a case which required amputation.

Results

We have treated 64 patients (40 male, 24 female, mean age 45·9 years) who had plantar ulcers. The average size of the ulcer at its widest part was 1·9 cm with a range of 0·7–4·5 cm. Of those 64, 24 had casts applied at least once; all of those casted healed completely. The initial casting in four patients did not result in complete healing. However, these patients were then casted with a different technique and achieved full healing. Of those 24 healed ulcers, casts were left on the patient for an average of 2·7 weeks, and reapplied an average of 1·8 times, resulting in an average time of healing of $5{\cdot}6 \pm 2{\cdot}6$ weeks.

Forty patients were treated without casting from October 1982 to April 1987. This group included 23 male and 17 female patients with an age range of 23 to 75 years (mean age 48 years). The size of the ulcers ranged from 1 to 5 cm with an average size of 1·6 cm. Twenty-three ulcers were healed, 7 remain unhealed, and 10 were lost to follow-up (usually having moved out of the area). Thus of those who could be followed (30 patients) 22% did not heal. In those that eventually healed, healing time of 1 week to 28 weeks was required, an average healing time of $8{\cdot}1 \pm 6{\cdot}6$ weeks. Healing also occurred in those who had amputations (4). Two Symes amputations were required in patients with Charcot joints, one patient having a severely infected foot. Because of repeated infection and osteomyelitis two patients required toe amputations, one of the great toe and the other the fifth toe.

Of those casted, all ulcers were eventually healed, whereas of those not casted, 7 did not heal. Of the 7 who did not heal, 6 of those patients, when seen initially at our clinic, presented with the ulcer, claiming it had repeatedly healed and reopened over many years. Some of these patients have had repeated infections at the ulcer site. The time to healing in those casted compared to those not casted was not significantly different ($P = 0{\cdot}1$). The occurrence of breakdown of the ulcer after healing was not significantly different between those who were casted (10 out of 24) and those who were not casted (10 out of 30). For those who accepted TCC, the average time between healing and reoccurrence of ulceration at the same site was $9\frac{1}{2}$ months, SD $\pm 2{\cdot}6$ months (range: 1–24 months), whereas for those who were not casted the average time was $10\frac{1}{2}$ months, SD $\pm 6{\cdot}6$ months (range: $1\frac{1}{4}$–27 months). The time to breakdown in the casted versus those not casted was not different ($P = 0{\cdot}2$).

Using techniques 1 and 2, healing time was similar, or about $5\frac{1}{2}$ weeks. Few complications developed during wearing time. In the single patient treated by two applications of technique 3 treatment was discontinued after a total of 4 weeks as the ulcer appeared to be increasing in size and macerating, possibly due to the longer drying time of the POP layer with padding applied around it. Using technique 4, one patient had complete healing in 3 weeks but 2 other patients had only minimal healing in 6 weeks. With technique 5, the cast became loose and the patient removed it at home. At his request, it was discontinued. On one patient not included in the statistics, using technique 5 there was some healing of the ulcer after 3 weeks but as the cast caused rub wounds around the ankle, it was discontinued. Technique 6 was tried for the first time on a patient who was

able to return often to the clinic for monitoring. The first application was removed after 6 days to evaluate skin condition and ulcer size. As the patient's skin was not reddened on any area and as the ulcer had some healing evident, the cast was reapplied in the same manner as the first one. When the second application was removed 16 days later, the ulcer was pin-hole size and the skin was in excellent condition. The second patient was noncompliant, insisting on walking on a still-wet POP–TCC. Although there was some healing on removal of the cast, there was a rub ulcer on the medial malleolus. A SC–TCC was applied and removed 7 days later. The skin of his leg and foot was in excellent condition and the ulcers healed to where another cast was not necessary. (Full healing occurred within 1 week while the patient ambulated with moulded shoes.) The third patient had a very deep, large (approximately 3 cm) ulcer. The first and second SC–TCC applications were each removed after 1 week. After the second week, the skin around the ulcer was macerated; therefore, the cast was not immediately reapplied. Instead, the patient used a nonweight bearing crutch gait while ambulating for 3 days and changing the dressing daily to keep the ulcer and surrounding area dry. After 3 days the skin returned to normal and the ulcer was clean. Petrolatum was rubbed on the surrounding skin but not on the ulcer and a third SC–TCC was applied for 2 weeks. Presently, the ulcer is pin-hole size after $7\frac{1}{2}$ weeks of casting and there is no need for further casting. The patient will have drop-foot surgery shortly to decrease the chances of reulceration.

Included here are summaries of two patients, one requiring amputation and one whose ulcer was healed without recurrence by means of a flap graft.

AMPUTATION

In July 1984 a 44-year-old male who had been lost to follow-up for many years attended the clinic with infected right foot plantar ulceration. Skin lesions clinically and histologically demonstrated relapsed lepromatous leprosy. The ulcer was approximately 5×12 cm in length on the plantar surface and extended 0·5–2·5 cm deep. The entire foot was inflamed, oedematous and malodorous. The patient was septic and had a fever (temperature 39·3°C) and chill. He stated the ulcer, which was nonhealing for 4 to 5 years, had been infected for 6 months but that he had only felt sick for the last 2 weeks. He was immediately hospitalized and after several days of treatment he was well enough to commence nonweight-bearing crutch gait training in preparation for the amputation of his foot. In August he had a partial amputation at the level of the forefoot, and the wound was left open to drain for 2 weeks. A second revision of the Symes amputation was then completed and the patient was hospitalized for $5\frac{1}{2}$ weeks following surgery. During that time a prosthesis was supplied and ambulation progressed well. His stump to date remains in excellent condition and he has returned to his employment as a truck driver and labourer.

FLAP GRAFT

In September 1983, a 63-year-old male presented with bilateral plantar ulcerations (the right foot had 2 ulcers of approximately 3 cm each and the left foot had 1 ulcer approximately $1\frac{1}{2}$ cm) which he stated were recurrent for 20 years. In the remaining months of 1983 his right foot was casted 8 times (frequency of cast changes was due to heavy exudate from the ulcers). In January 1984, the cast was applied 4 times resulting in healing to pin-point size. At that time a decision was made to attempt to heal the left foot while the patient wore a moulded shoe on the right. Between February and May 1984 the left leg was casted 7 times, resulting in complete healing. To date (June 1987) the ulcer has not recurred on the left.

As the right foot ulcer continued to increase in size, a cast was once again applied and between July 1984 and March 1985 his right foot was casted 9 times with intervals when the patient refused casting for personal reasons and, instead, used crutches. In early March 1985, he slipped while climbing a ladder and deeply cut his fifth toe. The cast was removed and the toe was surgically debrided, then splinted and bandaged. The patient was then nonweight-bearing on crutches for

1 month. All ulcers and the toe appeared healed at that time. Breakdown occurred within 2 weeks of full ambulation and between April and May 1985 a cast was applied 6 times without effecting healing.

A decision was thus made to have a surgical flap procedure carried out. This was done in June 1985. However, the flap became infected and a second procedure was done in August 1985. In December 1985 the patient returned from a trip with an ulcerated blister over a deep bursa, above the grafted area. This was permitted to commence healing using non weight-bearing crutch gait. Then 3 stitches were taken to fully close the skin. From March 1986 to present (April 1987) the right foot has remained healed, and the patient ambulates well using moulded shoes and insert.

Discussion

Peripheral neuropathy of the lower extremity which involves only sensory loss without motor impairment can still be a debilitating problem. Preventing plantar ulceration involves a lifetime of caution in matters which the sensory-privileged person cannot imagine. Often a break in the skin is not noticed until it is large and possibly smells from infection or until blood is noticed on the socks or insoles of the shoe. Unless the patient has become aware of the need to look at the bottom of his feet daily, it is unlikely that the beginning of a plantar ulcer will be noticed until it is quite large.

From information gathered on the 40 noncasted patients, it would seem that nonweight-bearing (either by casting or other means) is not an absolute necessity for healing plantar ulcers. In our experience, if a patient is willing to do ulcer care at home, to wear moulded shoes at all times, and to limit walking, it is possible for plantar ulcers to heal. However, the average healing time with a cast is 6 weeks as compared to an average healing time of over 2 months for the noncasted foot. Additionally, noncasted ulcers are more commonly subject to infection.

To meet the needs of the patient who is not hospitalized and who must work or go to school, thereby necessitating more than minimal ambulation and less time for self-care of the ulcer, it is beneficial to have available other means for healing than the traditional application of POP–TCC. Results of several variations used in this clinic are recorded in this paper. As the Scotch Cast–TCC was evaluated on only 3 patients, it is not certain yet to what extent it can replace POP. However, the patients stated that they definitely preferred the lightness of the cast as well as its eliminating the need to use crutches during drying time. The cast is certainly easier to remove than the POP cast and healing time appears about the same. In light of these findings, it would be useful to continue evaluating this type of cast for use in healing plantar ulcerations. Further evaluation of alternative materials and new means of applying them would benefit the current population of leprosy patients who are largely treated in the community and wish to remain ambulatory.

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NEWS AND NOTES

Robert White Fellowships in Immunology

The British Society for Immunology will consider awarding fellowships, in the memory of Professor Robert White, to individuals from developing countries in order to aid or further their education or scientific experience in Immunology. The Society interprets these aims in the broadest possible terms and consideration will be given (for example) to support travel to study in a University or to gain experimental or technical and scientific expertise or for the purchase of books, journals or equipment.

All members of the Society, and particularly those resident overseas, are urged to bring the Robert White Fellowships to the attention of their colleagues. For further details of the scheme, and any enquiries about it, please contact The British Society for Immunology, 11 Hobart Place, London SW1N 0HL.

VI World Congress of Dermatology, 1989

This congress is to be held at the Convention Center of Hotel Nacional-Rio, Rio de Janeiro, Brazil on 29 April to 3 May 1989. It is sponsored by International Society of Dermatology: Tropical, Geographic and Ecologic, whose executive president is Professor Rubem David Azulay. The following events are being scheduled: 18 courses; 6 special lectures; 7 symposia; 6 forum; 4 special symposia; 72 short communications; 240 mini-case presentations.

For further information: Secretariat Congregare, Marketing de Conferências, Av. Rio Branco, 185, Suite 912. Tel. (021) 533.0105–20040 Rio de Janeiro, RJ, Brazil.

Corrections

1 Immunological effects of lepromin testing in Sri Lankan population groups I. Effect of repeated lepromin testing. Pinto, MRM, Eriyagama, NB, Pemajayantha, V & Fish, DG. *Lepr Rev*, 1987; **58:** 119–28.

p. 125, line 2: for 'increase' read 'decrease' and for 'dimunition' read 'increase'.

2 A study of leprosy and other skin diseases in school children in the state of Amazonas, Brazil. *Lepr Rev*, 1987; **58:** 233–7.

Reference 9 in the above paper has been wrongly cited. It should read

'*Int J. Lepr*, 1977; **45:** 360–3.'

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Cataract extraction in leprosy patients

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Summary Forty-one eyes of 36 leprosy patients were operated on for cataracts. The ocular findings contributing to blindness among the 41 operated eyes were corneal opacity (26·8%), old uveitis (36·6%) and glaucoma (7·3%). Shallow anterior chamber in the early postoperative period was observed in 26·8% of cases. The use of systemic corticosteroids definitely reduced the incidence of postoperative uveitis (2·4%). Thirty-seven (90·24%) eyes showed improvement in their visual acuity of two Snellen's lines or more after surgery. We conclude that cataract surgery may help in rehabilitation of already disabled and handicapped leprosy patients.

Introduction

No other disease is surrounded by so much prejudice in the eyes of the general public as leprosy. The total number of cases throughout the world is now placed at over 12 million.[1] It is estimated that about 25% cases of both lepromatous and nonlepromatous leprosy patients ultimately show ocular involvement[2] and if given enough time all patients with lepromatous leprosy will develop ocular complications.[3] With the introduction of sulphones and other chemotherapeutic drugs in the last two decades, leprosy rarely causes death in the absence of secondary infections and certain other complications.[2] Ocular lesions are seen more frequently with increasing age and duration of the disease.[4] Chronic iritis produces iris atrophy with small nonreacting pupil which exaggerates the visual impairment caused by lens changes and corneal opacities.[5]

Blindness in leprosy superimposes an intolerable burden and seriously threatens a patient's quality of life. Cataract extraction is perfectly feasible in leprosy patients provided the disease is quiescent.[2,6,7] In the present study, the visual outcome along with operative and postoperative complications of cataract extraction in 41 eyes of 36 leprosy patients have been evaluated.

Materials and methods

Thirty-six leprosy patients were admitted in the Department of Ophthalmology, JIPMER, Pondicherry from March 1983 to August 1986 for cataract surgery. Leprosy was diagnosed by clinical signs and laboratory investigations. The patients with positive skin test were not admitted

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for surgery. All patients had received some form of antileprosy therapy earlier. Complete ocular examination was done including visual acuity, tonometry, slit-lamp examination and funduscopy.

All the patients were given dapsone 100 mg/day from the day of surgery. Systemic steroid (prednisolone 40 mg/day) was started in patients with old uveitis and tapered within 2 to 3 weeks after surgery. Lens extraction was achieved with cryo-probe and 0·25 ml each of Dexamethasone and Gentamicin was injected subconjunctivally after surgery. Complete iridectomy was accomplished opposite clear cornea in patients with central corneal opacity and also in miosed, nonreacting pupils. In patients with synechiae, synechiotomy was performed before extracting the lens. All the patients were followed up for a maximum period of 6 months.

Observations

Forty-one eyes of 36 leprosy patients (lepromatous 29, borderline 2 and tuberculoid 5) were operated for cataract. There were 26 (72·3%) males and 10 (27·7%) females with mean age of 55·8 and 47·5 years respectively. Twenty-four patients had severe involvement of the face and/or limbs. Visual acuity for distant vision of 6/60 or more in the better eye with best correction was present in 12 (33·3%) patients only. Out of 72 eyes, 5 (6·9%) had no perception of light (anterior staphyloma 3, phthisis bulbi 1, glaucoma with ciliary staphyloma 1). The preoperative and postoperative visual acuity in 41 eyes is shown in Figure 1. Thirty-seven (90·24%) eyes showed improvement in their visual acuity of two Snellen's lines or more after surgery. Four (9·75%) patients showed no improvement (panophthalmitis 1, total glaucomatous optic atrophy 2, central leucomatous corneal opacity 1). An analysis of ocular findings contributing to blindness in 41 operated eyes is shown in Table 1. The operative and postoperative complications are shown in Table 2.

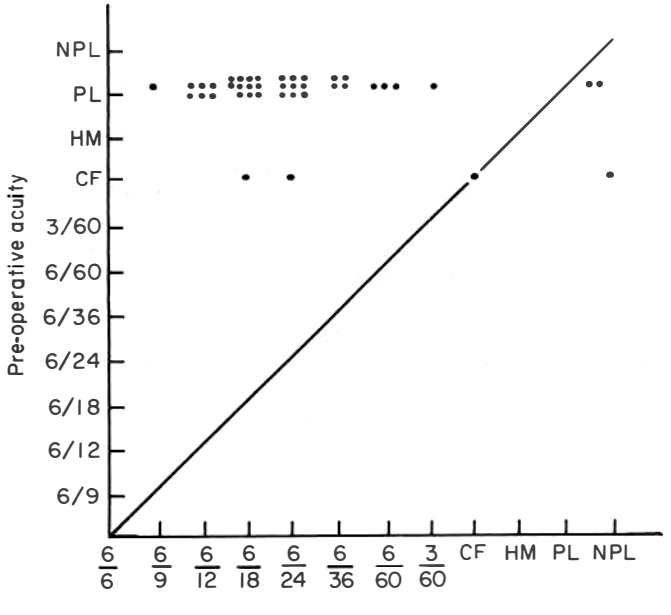

Figure 1. Postoperative visual acuity in 41 eyes.

Table 1. Ocular findings contributing to blindness among 41 operated eyes.

Corneal opacity	11 (26·8%)
Old uveitis	15 (36·6%)
Glaucoma (Chronic simple glaucoma 1) and secondary glaucoma 2)	3 (7·3%)

Table 2. Operative and postoperative complications in 41 lens extractions.

Unplanned extracapsular extraction (In two capsule was removed)	5
Vitreous loss	1
Postoperative uveitis	1
Postoperative hyphaema	5
Postoperative shallow anterior chamber	11
Panophthalmitis	1
After cataract	3

Discussion

The reported incidence of ocular involvment in leprosy varies from 5·87% to 80% in India and 47% to 74·2% abroad.[7] The eyes are involved in leprosy in 4 ways: i, abnormal exposure of the eyes secondary to involvement of the 5th and 7th cranial nerves; ii, inflammation of the eyes secondary to the infiltration by the leprosy bacillus; iii, infiltration of the eyes and/or eyelids by the leprosy bacillus; and iv, complication secondary to involvement of neighbouring structures—eyelids, lacrimal glands and nasolacrimal drainage system.[8] The visual loss in leprosy is due to the combined effect of corneal and lens opacities associated with chronic uveitis.[5] The crystalline lens is never directly invaded by bacilli in a leprotic lesion and the lens changes are those seen in a normal ageing population.[9,10] A complicated cataract may develop after chronic uveitis and acute iritis associated with hypersensitivity.[9,11,12] Toxic cataract may develop with the use of corticosteroids in lepra reaction.[2,13] ffytche[6] found that the average age of the patients 61·5 years for men and 62·7 for females represented a lower average age than that of non-leprosy patients undergoing cataract surgery. In the present series, the average age of the patients 55·8 years for men and 47·5 for females, suggests that there are other factors apart from senile changes in lens which definitely accelerate the development of cataract.

Functional blindness is defined as visual acuity for distant vision of 6/60 (20/200) or less in the better eye with correction. Blindness is a grave problem among leprosy patients. Courtright *et al.*[14] reported 11% of leprosy patients with functional blindness in South Korea, and in 4·3% of eyes, there was no perception of light. Advanced cataract was observed in approximately 5% of eyes. Prasad *et al.*[7] reported 76% success of cataract surgery in leprosy patients and in most of the cases (73·2%), the improvement of vision was up to 3 lines of Snellen's chart. In another series, ffytche[6] reported visual improvement in 90% of lepromatous leprosy patients and in 60% this improvement was 2 Snellen's lines or more. In the present series, 90·2% eyes showed visual improvement of two or more Snellen's lines.

ffytche[6] reported higher incidence of operative complications in 81 eyes which included 14 vitreous loss and 14 unplanned extracapsular extraction as compared to present series of 5

unplanned extracapsular extraction and one vitreous loss in 41 eyes. This may be due to different techniques of lens extractions—forceps combined with expression in the former and cryo-extraction in the latter. Prasad *et al.*[7] reported a higher incidence of postoperative uveitis (16%) as compared to 2·4% in the present series. This might be due to use of systemic steroids which prevented the reactivation of old uveitis. Surprisingly 26·8% eyes showed shallow anterior chamber in the immediate postoperative period. All resolved within 3 to 5 days with conservative management. This might be due to various factors like the use of systemic steroids and corneal anaesthesia, due to involvement of ophthalmic division of the trigeminal nerve which results in delayed wound healing.

Blindness is the most expensive of all causes of serious disablement. It not only makes an individual handicapped and miserable but also puts unnecessary burden on the community. This is more so in leprosy patients who might be disabled due to deformities of hands and feet. Cataract surgery provides a reasonably safe surgical answer to restore the vision in the leprosy patients who are blind due to opacification of lens.

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A borderline leprosy lesion on the palate: a case report

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Summary A patient with borderline tuberculoid leprosy was found to have involvement of hard palate which was histologically compatible with borderline leprosy (BB) in reaction. The possible modes of involvement of palate are discussed.

Introduction

Infiltration of the tissues like palate, gums and nasal mucosa has often been reported in lepromatous cases. Oral involvement was found in 19% of the cases.[1] Another study[2] observed that 60% of the lepromatous cases had oral involvement, and others[3] observed that 57% of the 40 lepromatous cases had oral affection. In nonlepromatous cases, the disease does not involve the mucous membrane and it seems that lesions in these patients, usually do not cross the mucocutaneous junction.

Recently we came across a patient of borderline leprosy having leprosy lesion on mucosa of hard palate. Because of the rarity of mucosal lesion in nonlepromatous patients the case is being reported.

Case report

A 45-year-old Hindu female attended the OPD with complaints of gradually appearing multiple analgesic erythematous patches all over the body during the previous five years. She had taken 100 mg of DDS and 10 mg of prednisolone daily for 4 weeks. She had also received a single dose of 600 mg of rifampicin at the initiation of the therapy.

On examination, she was found to have multiple, widespread, asymmetrical, raised, erythematous, oedematous, dry scaly lesions with well-defined margins. All the lesions were analgesic. Lesions were present on the face, trunk and extremities. None of the nerve trunks were thickened and there were no associated deformities. The skin smear report was as follows: on right ear negative, lesion on forehead negative, lesion on left leg positive (I+). Mitsuda reaction to lepromin was strongly positive (+++). The patient was diagnosed as having borderline tuberculoid leprosy in reaction.

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On detailed examination a raised pinkish patch of about 2×3 cm with 3 small ulcers, was noticed on the mucosa of hard palate on the left side of midline (Figure 1).

Histopathology

A biopsy specimen from the lesion on the left forearm shows skin with normal keratinization. The epidermis shows flattened rete pegs. The dermis is almost totally filled by a granuloma, composed of numerous macrophages. In some areas there is focalization and collection of epithelioid cells and poorly formed giant cells. Numerous lymphocytes and an almost equal number of plasma cells are seen throughout the granuloma, either scattered diffusely or forming cuffs around the focal collections. The nerves are replaced by epithelioid cell collections. Fite stain shows acid-fast bacilli in small groups within the macrophages (BI-3+). The picture is consistent with BT/BB leprosy.

A section through the palate shows dense granuloma covered by stratified squamous epithelium. The granuloma consisted mostly of macrophages, with a dense infiltration by lymphocytes. Several plasma cells are also seen. In areas there are focal collections of histiocytes and immature epithelioid cells with abundant eosinophillic cytoplasm. In this area infiltrating cells are mainly lymphocytes and hardly any plasma cells observed there. Fite stain shows diffusely scattered acid-fast bacilli (BI-3+). Bacilli were very rare in the focalized area. In view of a very large number of capillaries and some oedema the lesion could be in reaction. The histological picture is consistent with BB type of leprosy in reaction.

Treatment and progress

She was put on monthly pulsed rifampicin 600 mg, dapsone 100 mg and prednisolone 5 mg daily. At the end of 4 months the lesions on the skin and also the palate lesion showed evidence of regression. The ulcers on the palate lesion healed, but the pinkish raised lesion was still evident. Unfortunately the patient disappeared subsequently and further follow up has not been possible.

Discussion

Presence of borderline lesion on palate in a case of BT/BB leprosy is an odd presentation and to the best of our knowledge, has not been reported so far.

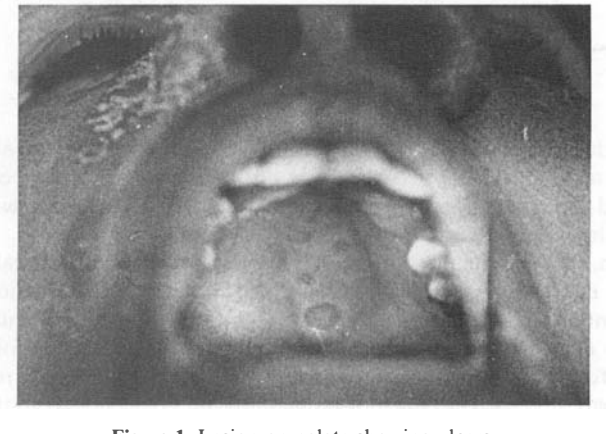
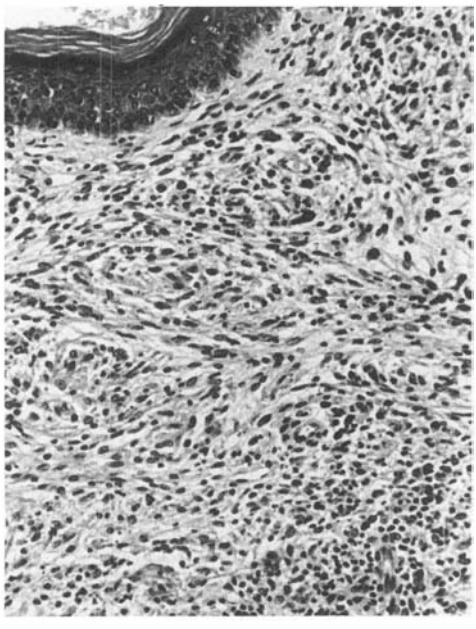


Figure 1. Lesion on palate showing ulcers.



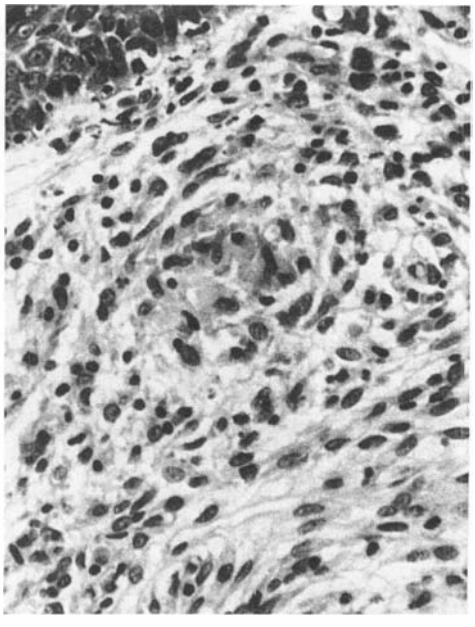


Figure 2. Section through skin lesion showing extensive granuloma with epithelioid cells and a large number of lymphocytes (H & E original × 200).

Figure 3. High power view of granuloma of skin showing epithelioid cell focus (H & E original × 400).

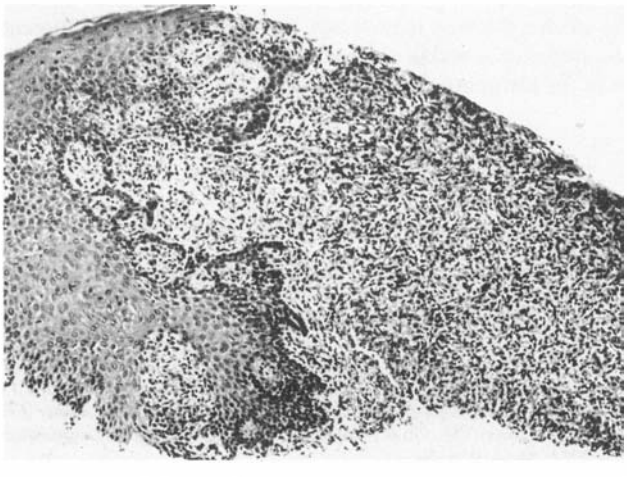


Figure 4. Photomicrograph of palate lesion showing extensive granuloma in the subepithelial tissue (H & E original × 100).

In the past various theories have been put forward to account for the site of selection and localization of nonlepromatous lesions. The possibility of a lesion appearing at the site of initial inoculation was suggested earlier.[4] Case reports regarding lesion at the site of initial inoculation support this view.[5–7] In the present report, though the lesion was noticed at the time of the first examination, because of its asymptomatic and insidious nature, it was difficult to ascertain whether it was the first lesion or appeared after the skin lesions.

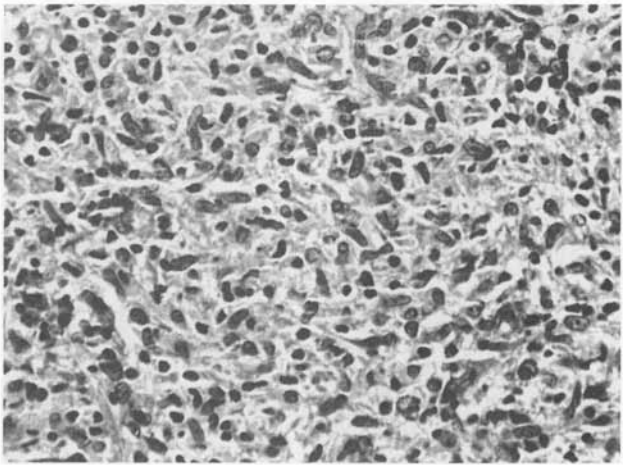


Figure 5. High power view of palate lesion, showing a granuloma composed of histiocytes, plasma cells and lymphocytes (H & E original ×400).

Lower temperature on both the surfaces of the palate, might have contributed to the localization of the lesion at this site, as it has been suggested that *M. leprae* has predilection for cooler areas of the body.[8,9] It is possible that in the present case the palate lesion occurred by haematogenous spread of the bacteria. Bacteraemia has been shown in 8 out of the 11 investigated TT/BT cases.[10] As such haematogenous spread of the infection in TT/BT cases cannot be ruled out.

It is interesting to note that the palate lesion showed a histological picture at a lower level of immunological scale. Such a diversity in histology has been reported in different skin lesions of the same patient.[11] Also a dichotomy in skin and nerve lesions has been observed.[12] One could therefore expect a divergence in the histopathology of lesion on the mucosa compared to one on the skin.

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Lepr Rev (1988) **59,** 75–82

SPECIAL ARTICLE

Immunopathology of leprosy granulomas—current status: a review

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Introduction

The term granuloma was first introduced by Virchow as a tumor like mass or nodule of granulation tissue. Granulomas have been defined in different ways. One of the common definitions could be 'A granuloma is a localized collection of cells of the mononuclear phagocyte system with or without the admixture of other inflammatory cell types.[1] The 'Koch phenomenon' was probably the first experimentally induced granuloma. It is an accelerated enhanced dermal reaction evoked by live or dead tubercule bacilli in guinea-pigs presensitized with tubercle bacilli.[2] This is probably due to the release of lymphokines following, the interaction between the tubercle bacilli and specifically sensitized T cells in the blood or tissues. The infiltrates of this reaction contain lymphocytes and epithelioid cells. The relationship between epithelioid cells and macrophages was recognized by Metchnikoff as early as 1888.[3] In this context, leprosy is an interesting disease for investigation for two main reasons: a, it affects a large population in the world; and b, the histopathological features of lesions and cells in the granulomas bear a positive correlation with the delayed hypersensitivity reaction seen in these patients. Thus it could serve as a useful model for studying the immunopathological aspects.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* where granulomatous inflammation affecting the skin and nerves is a prominent manifestation. This disease exhibits a spectrum with tuberculoid leprosy, a high immune, paucibacillary form at one end and lepromatous leprosy, a low immune, multibacillary form at the other end. The lesions in tuberculoid leprosy are characterized by an epithelioid cell granuloma with abundant lymphocytes forming dense collections around the epithelioid cells. On the other hand, lepromatous leprosy is characterized by a granuloma composed of sheets of macrophages loaded with *M. leprae* along with plasma cells and a few lymphocytes diffusely distributed into the granuloma. Most of the studies to understand the immunological mechanisms in leprosy have been mainly carried out using *in vitro* tests on peripheral blood derived lymphocytes and monocytes.[4] However, the major site of immunological reaction in leprosy, i.e. the skin has been largely uninvestigated due to methodological limitations. This has been overcome to a considerable extent with the use of monoclonal antibodies directed against cell surface antigens. They have proved to be an important tool to analyse the nature of lymphocytes and other cell types in humans.[5] The present review is limited to the progress made in the last five years in understanding the immunopathology of leprosy granulomas. Most of the

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studies described in this review have used the Ridley–Jopling scale for the classification of leprosy patients.

Characteristics of lymphocytes in leprosy granulomas

NONREACTIONAL STATES

The characterization of lymphocytes in the leprosy granulomas was done using rosetting techniques. The majority of lymphocytes formed rosettes with sheep erythrocytes indicating the presence of T cells. They were mostly seen to be associated with epithelioid cell granulomas and showed maximal density in tuberculoid leprosy. A graded reduction was observed in borderline leprosy with a severe reduction in lepromatous leprosy.[6] This was further evident by lymphocytes not forming EA and EAC rosettes, in tuberculoid lesions. However, the lymphocytes in the BL lesions rosetted with EA and EAC suggesting that they were mainly B cells.[7] The lymphocytes also exhibited nonspecific esterase activity (marker of T cells) in the leprosy granulomas.[8]

The above observations has been further supported by the immunohistological analysis of leprosy granulomas carried out, using monoclonal antibodies defining T cell subsets and Ia like antigens by four groups of workers.[9–12] Most lymphocytes in leprosy lesions were positive for OKT3 and Ia like antigens indicating thereby the presence of activated T cells. Maximal numbers of these cells were seen in tuberculoid granulomas in close association with the epithelioid cells. A decline in their numbers were observed over the leprosy spectrum. In disseminated multibacillary lepromatous leprosy, only a few positive cells were visualized. The ratio of OKT4+/OKT8+cells was higher in tuberculoid lesions in comparison to lepromatous lesions. In treated lepromatous patients, a high proportion of T cells was noticed. An important difference, was observed in the microanatomical distribution of the functional subsets of lymphocytes. In the tuberculoid lesions, the lymphocytes expressing phenotype of helper T cells (T4+) were diffusely scattered being present both amongst the epithelioid cells as well as in the lymphocytic cuff surrounding these cells. In contrast, lymphocytes expressing phenotype of suppressor T cells (T8+) were arranged in 'ring like' manner mainly in peripheral lymphocytic cuff. However, no such demarcation of lymphocyte subsets was observed in lepromatous lesions. That the microanatomical pattern was more important than the numerical value of lymphocytes was further indicated by studies on reactional leprosy lesions. During both ENL and type I reactions of BL which occur in patients with lepromatous type of leprosy, T cells of helper phenotype entered the lesions in large numbers, sometimes reaching levels observed in BT lesions, yet the microanatomical pattern showed scattering of both T8+(suppressor) and T4+cells and nonformation of organized granuloma.[9,10,12] However, such a distribution was not noticed in the studies of Van Voorhis *et al.*[11] This could be because a, fewer numbers of patients in each group, or b, sampling error. It would appear therefore that not only the presence of helper or suppressor T cells but their distribution may indicate the development or nondevelopment of effective immunity in the lesions. Such a type of concept is brought out by various workers in other conditions where epithelioid cell granulomas are prevalent, e.g. sarcoidosis,[10,13] Kveim reaction,[14] by our studies on Mitsuda skin tests in leprosy patients[15,42] and in American cutaneous leishmaniasis.[39] Similar findings of the presence of T cells and their distribution has been observed in the lymph nodes of leprosy patients.[58] The lymphocytes in both the tuberculoid and lepromatous granulomas expressed *M. leprae* specific antigens (shown using monoclonal antibodies to soluble antigens of *M. leprae*[16]) and also expressed fibronectin.[17] This leads to the question as to what is responsible for the organization and maintenance of effective immunity in tuberculoid granulomas. Modlin *et al.*[18] and Longley *et al.*[47] have made an attempt to answer it using monoclonal antibodies defining interleukin 2 (IL2) and T cell subsets. Their study reveals that disorganized lepromatous granuloma contains significantly fewer densely staining IL2+cells than highly organized tuberculoid granuloma, thus leading to diminished production of

IL2 in lepromatous leprosy. Further, IL2+cells also stained with antileu 4, antileu 3a antibodies but not with antileu 2a antibodies. This finding suggests that IL2 bearing cells were helper/inducer T cells. Working independently, Nilsen *et al.*[59] have made similar observations in the immunohistological analysis of nerve granulomas in leprosy.

REACTIONAL STATES

Histologically it has been shown that a large number of lymphocytes enter the lesion during type I reaction of BL and ENL reactions. The nature of lymphocytes has been examined with monoclonal antibodies. The lymphocytes entering the reactional leprosy lesions were predominantly activated T lymphocytes with a preponderance of T4+cells. The ratio of T4+/T8+cells was increased in reactional BL and ENL lesions in comparison to nonreactional states. The distribution pattern of T8+cells was similar to T4+cells both being diffusely scattered among bacilli laden macrophages in these lesions. Reactional BT lesions showed a mild increase in pan T cells and the microanatomical distribution of T4+ and T8+cells was identical to that seen in nonreactional tuberculoid lesions. Though ENL and reversal reactions of BL were thought to have different mechanisms of initiation, yet they showed similar T cell types and pattern in the lesions.[12,19,20] There was an increase of IL2 positive cells in ENL lesions in comparison to nonreactional lepromatous lesions.[45] This clearly suggests that T cells (particularly helper/inducer T cells) are involved in the pathogenesis of these reactions, in leprosy.

These results in turn strongly support one of the earliest findings of the pathogenesis of Type I leprosy reactions in mice. It was shown that T cells were responsible for the elicitation of these reactions. The inflammatory infiltrates contained predominantly small lymphocytes and activated macrophages.[52] So, the presence of increased numbers of T4+cells in these lesions may cause the activation of macrophages due to delayed hypersensitivity reaction and thus the fragmentation of the bacilli. This is particularly seen in ENL lesions. These macrophages may also release increased amount of pyrogenic factors. Further, the T4+cells could help in the production of antibodies which ultimately results in the formation of immune complexes. All these phenomenon are evident in patients of leprosy undergoing reaction.[53,54,56]

In vivo* skin reaction to killed *Mycobacterium leprae

Lepromin reaction is one of the parameters which may be used; a, in the assessment of the immune status of a leprosy patient or a contact; b, to test the efficacy of an immunoprophylactic agent; and c, to study the immunological mechanisms involved in the formation of hypersensitivity mediated epithelioid cell granulomas. The type of lepromin reaction depends upon the method of preparation of the antigen. For example, whenever the organisms are sonicated and soluble components are injected, it gives only an early reaction. However, if the killed organisms are inoculated intact, it gives a late reaction.[21] Our group has carried out studies to understand the mechanism of elicitation of lepromin reaction by characterizing the cells in the infiltrates with monoclonal antibodies. Standard Dharmendra lepromin has been used which elicits both the early and late reaction. Both these reactions were positive in tuberculoid patients and negative in lepromatous patients. The infiltrates of early reaction comprised of lymphocytes and polymorphonuclear leucocytes. A high proportion of cells in these infiltrates were activated T cells expressing OKT11, Leu 3a, OKT8 and Ia-like antigens. Ia-like antigens were not discernible on polymorphonuclear leucocytes.[15] Similar types of observations were made in other skin reactions, e.g. delayed hypersensitivity reaction to PPD in humans,[22,23] described earlier and our studies using armadillo derived leprosin,[15] purified mycobacterial antigen (MY1) from *M. leprae*.[24] The granulomas of Mitsuda reaction was characterized by the presence of lymphocytes and epithelioid cells. The immunological characteristics of cells in the Mitsuda reaction was similar to that seen in tuberculoid leprosy lesions.[15,43] This

has been further confirmed using armadillo derived leprosin coupled to liposomes as antigen in leprosy patients.[42] Further the immunohistology of skin responses have been recently used: a, to distinguish direct reactions from cross-reactions in delayed hypersensitivity reactions in humans elicited by various antigens;[44] b, to understand the entry of T lymphocytes in the lepromatous granulomas;[48] and c, to study the kinetics of the elicitation of lepromin reaction in leprosy patients.[60] Thus these experiments are of potential importance to the vaccination studies undertaken in leprosy.[57]

IMMUNOGLOBULINS AND COMPLEMENT COMPONENTS

Ridley *et al.*[25] have evaluated the immunoglobulins, complement components, plasminogen, lysozyme, C-reactive protein and α-1-antitrypsin, in leprosy granulomas using immunoperoxidase staining. These factors were produced in higher amounts in TT and LL with a dip in the BT-BB region (except C-reactive protein and α-1-antitrypsin). The immunoglobulins were present mainly in plasma cells and lymphocytes.

Accessory cells in leprosy granulomas

MACROPHAGES

Macrophages are involved in antigen presentation and elicitation of immune response. They carry receptors for C3 component of complement, FC component of IgG, exhibit esterase activity and express Ia-like antigens. Two groups of workers have assessed the macrophage membrane characteristics using EA and EAC rosetting in leprosy lesions. Ridley *et al.*[7] have observed maximal adherence of EAC to cells of the mononuclear phagocyte series (MPS) at the tuberculoid end of the spectrum with less adherence towards LL. No EA adherence to MPS cells was seen in tuberculoid patients but increased adherence in the leromatous granulomas. This suggests that epithelioid cells possess only C3 receptors and no receptor for FC component of IgG. However, Gupta *et al.*[6] have reported that both epithelioid cells to tuberculoid leprosy and foam macrophages containing AFB showed adherence to EA and EAC. Further, the presence of nonspecific esterase was uniformly observed in the lesions across the leprosy spectrum. The difference observed in the two studies may be due to type of red cells and immunoglobulin used for preparing EA.

A generalized marker (using monoclonal antibodies to Ia like antigens) have been used to characterize the macrophages in the granulomas.[9 12] Most of the macrophages from the granulomas of both tuberculoid and lepromatous leprosy expressed Ia-like antigens. This was a feature even in reactional states.[19,20] However, in certain large granulomas of tuberculoid leprosy, the central epithelioid cells lacked the expression of Ia-like antigens.[9,26] This expression of Ia-like antigens in leprosy lesions was further quantitated. A significant difference in the expression was noticed.[40] The lack of Ia antigen expression was also observed in the granulomas of Mitsuda reaction[15] and in epithelioid cells of experimental mycobacterial granulomas in guinea-pigs.[27] In contrast, Ridley & Ridley[28] claim that Ia antigens are expressed only by cells in the granulomas of tuberculoid leprosy but not in lepromatous leprosy. This discrepancy in the results was due to the latter having used formalin fixed tissues (which could destroy some of the antigens) and a different type of Ia antibody which was not monoclonal. Further, macrophages from both the granulomas expressed fibronectin[17] and *M. leprae* specific soluble antigens.[16,56] The presence of fibronectin in these granulomas, may suggest that, this molecule when released within lesion may play some role in the regulation of granuloma formation and in the resolution of the lesion. It has been demonstrated that the majority of mononuclear cells in the granulomas of BL leprosy express the phenotype of macrophages. Two other populations expressing phenotypic characteristics of interdigitating cells and Langerhans cells were also present.[41] More important is that the monoclonal antibodies have

been used recently to differentiate between epithelioid cells and macrophages in the granulomas of leprosy and sarcoidosis.[51]

LANGERHAN'S CELLS (LC)

Accessory cells other than macrophages have been shown to play an important role in the presentation of antigen to T cells.[37] Langerhan's cells present in the skin have been shown to participate in experimental allergic contact dermatitis and delayed hypersensitivity reactions.[37,38] These cells bear receptors for Fc component of IgG, and C3 component of complement, express Ia like antigens and contain high concentrations of ATP-ase enzyme.[29] LC can be defined by a specific OKT6 monoclonal antibody.[30]

Four groups of workers have reported on the status of LC in leprosy. Mathur *et al.*[31] using ATP-ase staining showed that the number of LC were reduced in LL in comparison to TT. Jihe *et al.*[32] have reported reduced numbers of ATP-ase positive LC in skin lesions of tuberculoid and borderline patients in comparison to normally appearing skin from the same patients. Narayanan *et al.*[26] have used OKT6 antibody for defining LC. Normal numbers of LC was observed across the leprosy spectrum. However, the dermal granulomas of tuberculoid leprosy showed a high proportion of nondendritic T6+cells scattered in the mononuclear infiltrates surrounding the epithelioid cells. These cells were not detectable in the lepromatous lesions. Morphologically the T6+cells in the dermis lacked dendritic appearance. A similar observation was made by Modlin *et al.*,[18] and Kaplan & Cohn.[48] The discrepancy in results could be where ATP-ase has been used as a marker of LC, it is possible that the differences in the content of this enzyme, may explain the appearant reduction in LL. No difference in the numbers of OKT6+epidermal LC have been noticed in reactional states of leprosy[19] and among various types of skin reactions.[15,24,50] However, a small proportion of T6+cells was noticed in the infiltrates of these reactions. So, these results particularly using monoclonal antibody (T6) may suggest that the pathogenesis of the tuberculoid lesion may be different from that of lepromatous lesion.

In vitro studies on leprosy granulomas

Three groups have been working along these lines. Lai Fat *et al.*[33] using *in vitro* techniques have demonstrated the synthesis of immunoglobulins and complement in skin lesions across the spectrum. IgG synthesis occurred in small amounts in tuberculoid leprosy, a distinct amount in borderline leprosy and a large amount occurred in lepromatous leprosy. Synthesis of C3 was found only in some cultures of these granulomas. The same group[34] have shown the synthesis of anti *M. leprae* antibodies *in vitro* from the skin lesions of leprosy patients.

Our group has recently reported that, it is possible to prepare a single cell suspension from the leprosy granulomas by collagenase treatment and study some of the properties of cells *in vitro*. The first part[35] involves the characterization of cells using rosetting and histochemical techniques. The granulomas were found to contain lymphocytes and macrophages. The number of lymphocytes were significantly higher in the suspensions of tuberculoid granuloma in comparison to the suspensions from the lepromatous granuloma. A high percentage of lymphocytes from the tuberculoid granuloma formed rosettes with sheep erythrocytes and also showed the presence of esterase as dots in the cytoplasm. However, the lymphocytes did not form rosettes with EAC. This indicates that these lymphocytes appear to be T cells. Most of the macrophages from both the granulomas were esterase positive, exhibited peroxidase activity and did not carry receptors for C3 component of complement. The macrophages from tuberculoid granuloma were nonadherent to plastic surface, while those from the lepromatous granuloma were adherent. In the second part[36] these cells have been further characterized using monoclonal antibodies. The results showed a good correlation with *in situ* characteristics as described earlier. Modlin *et al.*[45] have also made single cell

suspension from the leprosy granulomas and separated T4 and T8 positive cells. These cells were exposed to IL2 and the T4, T8 cell lines were established. The T8 cell lines from lepromatous granulomas showed significant suppressor activity in comparison to the T8 cell lines derived from tuberculoid granulomas.[46,49] Further, a comparison has been made of the characteristics of dermal granulomas of tuberculoid and lepromatous leprosy by culturing them *in vitro*. It was observed that lepromatous granulomas release soluble factors which significantly reduced the viability and division of lymphocytes derived from peripheral blood of normal individuals in comparison with tuberculoid granulomas.[55]

Conclusions

The nature and characteristics of infiltrating cells in leprosy granulomas has been elucidated. It has been possible to understand some of the features of antigen presenting cells like macrophages and LC in these lesions. In particular, the lack of expression of Ia-like antigens by epithelioid cells *in situ* and in experimental mycobacterial granulomas could suggest that these cells may not be involved in antigen presentation. More interestingly, macrophages from lepromatous granuloma express abundant Ia-like antigens and therefore may possess the ability to present antigen. Incidentally, a large proportion of these macrophages were adherent to plastic surface. It is known that adherent cells are involved in antigen presentation. Nonreactional lepromatous lesions contain only occasional positive T lymphocytes while during reactional phase, there was an influx of large numbers of T lymphocytes. Recent attempts to isolate the cells from the dermal granulomas have proved to be successful and has given way for studying functional characteristics of lesional cells. With these available facts, it is hoped that an investigation along the following lines will be possible in the future: a, Mechanism leading to lymphocyte deficit in lepromatous granulomas; b, To clone the T4+ and T8+cells from lesions and to assess their characteristics; c, To study the characteristics of lesional macrophages and their products; and d, Role of Langerhans cells and T6+cells in the development of leprosy lesions.

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Obituary

CARINE WETERMAN

Medical Mission Sister

1929–1987

Carine entered the Society of Catholic Medical Mission Sisters in 1950, in Holland. From 1954 to 1957 she trained as a laboratory technician in Utrecht and in 1960 came to Malaŵi to set up the laboratory of the newly established Holy Family Mission Hospital in Phalombe (Southern Malaŵi). In 1970 she worked for one year in Ethiopia to help set up the laboratory in a Hospital of the Medical Mission Sisters.

It was Dr Molesworth, the Medical Director, who appreciated the importance of a first class laboratory service for the Leprosy Control Project which he initiated in 1966 in Malaŵi, and it was John Eldon, the Manager, who spotted the talents of Carine when he visited the Sisters of Holy Family Hospital in Phalombe and he soon invited her to come and work for LEPRA. Over the years, first in Blantyre and later in Lilongwe, Sister Carine was instrumental in improving the quality of all aspects of leprosy-laboratory work throughout Malaŵi. She developed, and maintained for many years, a system of regular quality control and the reading of leprosy smears. Through her we, and later others, realized that a consistently high standard of laboratory work in leprosy is feasible, given the basic equipment and staff pertaining in Malaŵi. Her practical sound sense and her gift as a teacher found recognition in the fact that she was invited to conduct courses outside Malaŵi, to give her comments on drafts of publications. Neighbouring countries sent their laboratory technicians to her for specific training in leprosy-laboratory work and its supervision. It is to her personal credit that LEPRA's laboratory work in Malaŵi has become outstanding in the eyes of our peers, and that it enabled LEPRA to introduce WHO/MDT, without delay or much difficulty, in Malaŵi in 1983.

Living alone in Blantyre, and later in Lilongwe, Carine kept close contact with her fellow nuns, spending her weekends with the Sisters in Kasina. Through the Mother House of the Society of Medical Mission Sisters Carine regularly obtained financial support for LEPRA's work in Malaŵi; in particular she acquired a double-head for a microsope which facilitated enormously the teaching of reading slit-skin smears.

During the last two years before her retirement at the end of 1984 she trained a successor and felt that he was sufficiently prepared to take over from her. Thus she had rounded off her work with us in a logical manner, and could be satisifed with what she had accomplished in her 13 years with LEPRA. Nevertheless we were sorry to see her leave us and to miss her stable and charitable influence. Early in 1985 she left Malaŵi for Holland.

Carine died on 27 August 1987 in Holland, where she stayed with the Medical Mission Sisters in Mook.

G BOERRIGTER

Lepr Rev (1988) **59,** 84–89

Letters to the Editor

BLISTER CALENDAR PACKS FOR THE TREATMENT OF PATIENTS IN LEPROSY CONTROL PROGRAMMES WITH MULTIPLE DRUG THERAPY (MDT)

Sir,

With reference to the article published by Georgiev and Kielstrup on Blister Calendar packs (*Lepr Rev* 1987; **58:** 249–55), we would like to report that 4 districts in India, where MDT implementation is supported by DANIDA, have started using these blister packs. Up to now, about 7000 MB and 10,000 PB patients have been given blister packs. Our observations over the past 3 months indicate that blister packs have become extremely popular among patients. They take capsules and tablets as instructed and regularly come to service delivery points with empty packets. Similarly, the staff find the blister packs handy and time-saving when preparing for the clinic, delivering drugs to patients and counting tablets taken at the end of a month.

DANIDA has initiated a field trial in 4 districts to evaluate the advantages of blister packs over bulk drugs. A 10% sample is taken for trial, with a control group, and various parameters monitored. The results of this multicentre trial will be communicated later.

C R REVANKAR & BIRTE H SØRENSEN

DANIDA Assisted National Leprosy
Eradication Programme (MDT Programme)
7 Golf Links Area
New Delhi 110 003
India

SPILLAGE OF MYCOBACTERIA IN THE LABORATORY: DECONTAMINATING PROCEDURES

Sir,

I would be most grateful for advice on the best procedure to follow in the event of accidental spillage of mycobacteria in the laboratory. We are in the process of setting up various projects here, which include the cultivation of quite large quantities of mycobacteria. It is possible, despite every precaution, that we may have spillage. I would at the same time appreciate advice on what to do in the case of noncultivable but pathogenic mycobacteria, such as *Mycobacterium leprae.*

S K KAR

Regional Medical Research Centre
Indian Council of Medical Research
Nandankanan Road
Bhubaneswar-751005 Orissa, India

REPLY

We referred this request to Dr Colston's laboratory at the National Institute for Medical Research, Mill Hill, London, NW7 1AA and he kindly supplied a copy of the 'Code of Practice for a Category II Laboratory'. The main subheadings read: general information; personal precautions; centrifuging; negative pressure hoods; decontamination and cleaning; servicing; accidents and emergencies. Under decontamination, paragraph 5 reads:

> 'Always swab insides of hoods after use, and also swab benches if they have been contaminated with Hycolin when finishing work in the laboratory. Hycolin is diluted and used as a 1% solution in water. Diluted Hycolin must be discarded whenever it shows signs of deterioration (turning brown, scum or sediment formed) and in any case must be replaced weekly. Keep it in a stoppered bottle.'

And paragraph 6 reads:

> 'Spills should be covered with a Hycolin-soaked cloth and left for 10 minutes, then mopped up using swabs and if necessary a dustpan. All material, including the dustpan must be treated as infected and autoclaved. Inform the safety officer of any major spill.'

Mr David Day, Safety Supervisor, Bacteriology Department, John Radcliffe Hospital, Headington, Oxford OX3 9DU, has written with the following additional information:

> 'Hycolin is one of the clear phenolic disinfectants which are recommended for general bacteriological use. They do not attack metals and, not being greatly inactivated by organic matter, are suitable for treating tuberculous materials. It should be noted that they are not active against viruses. Other clear phenolics include 'Clearsol', 'Printol', 'Stericol' and 'Sudol'.
>
> Whilst 'Chloros' (page 4 of your Code of Practice for Category II Laboratory) is not recommended for disinfection of tuberculous materials due to its failure to penetrate sputum, etc., hypochlorite will in fact kill the organisms. It may, therefore, be effective against a split culture.'

We would be interested to hear about standard laboratory practices with regard to spillage of mycobacteria from workers in other countries.

EDITOR

ECHO FOR LEPROSY

Sir,

Irene Brightmer, in *Lepr Rev*, 1987; **58:** 69–78, describing a spatial study of leprosy in Cross River State, Nigeria, concludes that a fresh approach is needed, involving education, a steady reliable flow of all necessary drugs and involvement of people at the village level. With such conclusions governments worldwide would agree. I would specially emphasize her second concluding point in which she states:

> 'a steady and reliable flow of all necessary drugs must be assured. A means must also be found of making the new multiple drug therapy more widely available and this will require the development of techniques to make it simple to administer.'

ECHO (the supply of Equipment to Charity Hospitals Overseas) is a charitable agency whose purpose is to supply the best quality pharmaceuticals and medical equipment at the lowest prices to mission and voluntary hospitals all over the world. The biggest growth in ECHO's pharmaceutical programme has been in the supply of leprosy drugs and by 1980 some 260 million dapsone tablets were being manufactured annually for ECHO for world leprosy needs.

The awareness in the 1970s of the worldwide problem of drug resistance in leprosy and of the need to implement multiple drug therapy has produced both a new urgency and a new financial constraint. The urgency is to treat patients with a full course of multiple drug therapy with the highest patient compliance and the lowest default rate possible in order to avoid drug resistance. The financial constraint is that of the actual cost of multiple drug therapy. In the light of these facts it is at once more important and more difficult to assure a steady and reliable flow of all necessary leprosy drugs.

ECHO supplies 1900 mission and voluntary hospitals in 120 countries and, by virtue of its bulk purchase of leprosy drugs, is able to obtain very favourable discounts and to pass these on to leprosy programmes worldwide.

During a three-month period this year ECHO has despatched very large quantities of dapsone, clofazimine and rifampicin overseas in response to orders received. Countries to which these leprosy drugs have been sent are Bhutan, Burma, India, Korea, Laos, Nepal, Niger, Nigeria, Thailand and Uganda. It is ECHO's privilege and task to supply leprosy workers with the drugs required for the enormous but finite task of treatment and control of leprosy worldwide.

In addition to the supply of leprosy drugs ECHO supplies medical and surgical equipment which is of low cost, robust, of good quality and appropriate technology. Orthopaedic and reconstructive surgical equipment can be ordered through ECHO, and one of the most popular tools for laboratory work (as in the examination of leprosy smears) is the ECHO Alpha microscope, made to ECHO's specifications and field tested and proved in remote rural situations.

In recent years one of the most urgent and frequently recurring requests to ECHO has been for vehicles. This need has occurred especially in rural health programmes. Such transport needs are specially relevant to leprosy survey and control work with the typical pattern of a wide network of clinics in relation to a central clinic or small hospital. Such has been the interest and demand that ECHO have, this year, established a Land Rover department. Diesel and petrol Land Rovers of right- or left-hand drive are built up in ECHO's workshop to an 'as new' specification. The recipient receives a vehicle as good as new—and better in the sense that it is purpose built. At the outset when an order for one of the Land Rovers is received by ECHO the person making the order indicates his or her requirements. The order form has space to indicate preference for left- or right-hand drive, for stretcher fittings, for tropicalization of the roof, for additional winch, air-conditioning of the driver's compartment, colour of bodywork, and signs, markings or logos to be painted on the vehicle. These vehicles sell for between £6,400 and £6,700.

Whether leprosy drugs, medical and surgical equipment or Land Rovers are purchased, ECHO has its own freight department with expertise in sea and airfreight, sea and air mail and documentation. We would encourage those interested who are visiting the UK to spend a few hours with us at Coulsdon viewing the warehouse, stocks, facilities and meeting our staff.

It is ECHO's aim to equip leprosy staff and other health workers with essential tools for the job. Further details of ECHO's services are available on request.

J TOWNSEND

ECHO
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Coulsdon,
Surrey CR3 2HR

A POSSIBLE MODE OF ENTRY TO THE BODY OF *MYCOBACTERIUM LEPRAE*

Sir,

It is remarkable that after more than a century of research on leprosy the mode of transmission of *Mycobacterium leprae* is still unknown. The major mode of exit was quickly elucidated[1] although the work was ignored for some years before enjoying a recent revival.[2] But the mode of entry of the bacillus still remains unclear.[3]

Whilst writing a university dissertation recently on the mode of transmission of *M. leprae*, I considered the importance of the distribution of initial skin lesions in assessing the skin as a potential mode of entry of the bacillus. Several good quantitative surveys have been performed this century[4–9] and an attempt at assimilating this data was made. The conjecture was that if the initial lesion is formed at the initial point of entry of the infection then the distribution of such lesions should be biased towards those regions of the body which are frequently exposed. If such a biased distribution were to be found, then this would lend support to the skin as a portal of entry of the bacillus. If, however, the distribution were random, then this would suggest a systemic distribution of the bacilli from some other portal of entry, e.g. the upper respiratory tract.

The data from 6 surveys, covering 1288 patients, were simply accumulated and 4 zones of skin area assessed: 1, the head; 2, arms and hands; 3, trunk/buttock/thighs; and 4, legs and feet. Also two broad age groups, children and adults, were delineated (574 adults, 714 children). The accumulated

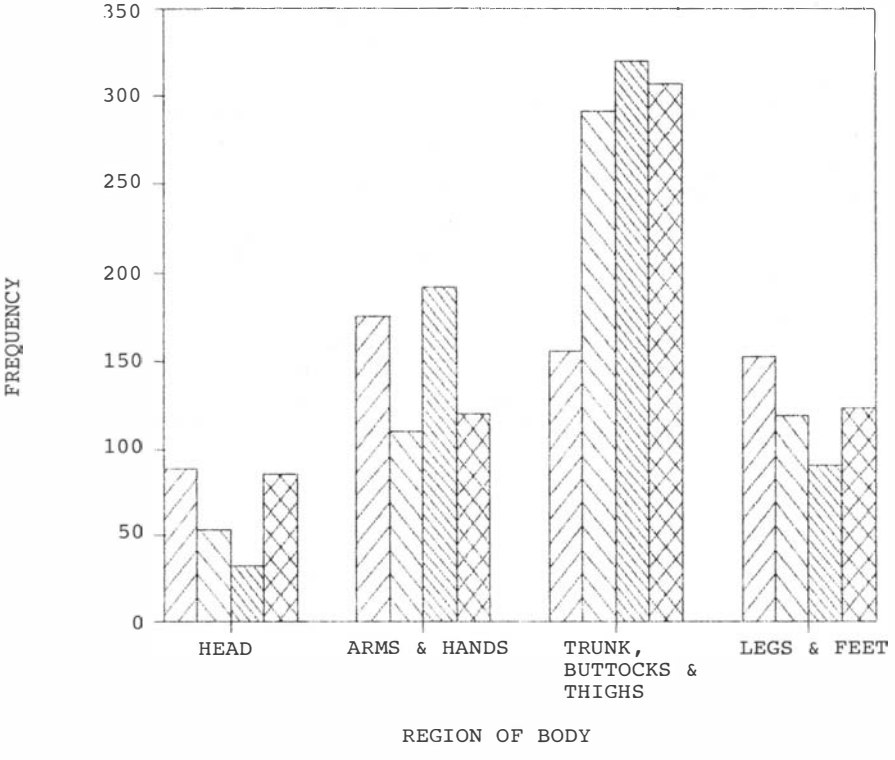

Figure 1. The site of the initial skin lesion in leprosy, the accumulated data of six studies. ▨, the expected frequency for adults, and ▨, for children, if predicting a random distribution based on the skin area of each body region. ▨, the observed adults' and ▨, the observed children's distributions.

Table 1. Initial lesion location—accumulated data

Site of first lesion	Adults			Children		
	Observed	Expected	χ^2	Observed	Expected	χ^2
Head	89	53	4·26007	47·8	96·363	3·42865
Arms and hands	176	110·4	6·79089	196·6	135·2651	3·89630
Trunk, buttocks and thighs	156	291·4	10·9606	359·4	344·7654	0·08702
Legs and feet	153	119·2	1·66972	110	138·4772	0·82042
			23·6813			8·23241

or 'observed' values were compared with values predicting a random distribution of lesions, calculated on the basis of the surface area of the skin in each of the regions,[10]—the 'expected' values (Figure 1, Table 1).

The deviations of the observed values away from the values predicting the random distribution were calculated and plotted (Figure 2). The significance of these deviations was assessed with the chi-squared test, and the adults' distribution ($P = < 10–8$) was found to be more significant than the childrens' ($P = 0·05$).

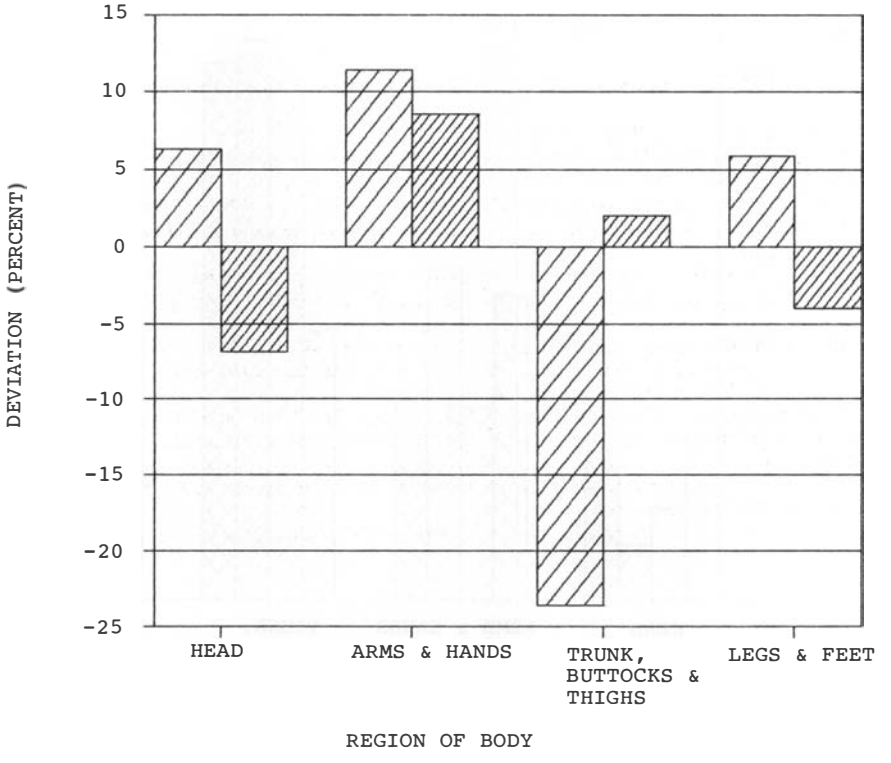

Figure 2. The deviation of the initial lesion distributions in adults and children away from a calculated random distribution (0%). ▨, the adult distribution, and ▨, the child.

The adult distribution, as see in Figure 2, is very significantly depressed in the trunk/buttock/thighs region, whereas the childrens' distribution is not. In the adult, these regions of the body are the most frequently covered, whereas children in these parts of the world at the times of these studies were generally unclothed or scantily clad. Thus the results do indeed, suggest that the initial lesion distribution is influenced by the degree to which body regions are exposed, and this supports the skin as a mode of entry of the bacillus.

There are however several problems associated with the collection and interpretation of this data. There is a lack of consideration of the nasal mucosa as a site for initial lesions and there is very little detail as to the type of clothing worn. Furthermore, it has been suggested that the site of the initial lesion may not be linked in any way to the initial site of entry of the bacillus, but may be controlled by a number of other predilecting factors, e.g. peripheral nerve turnover rates[11] temperature,[12] and blood supply. There is also the problem of the lack of any convincing experimental or histopathological evidence, despite persistent studies,[13,14] for the entry of *M. leprae* through the skin. Despite these problems, the distribution data presented here strongly suggests that the skin may be an important mode of entry of *M. leprae*.

M MACHIN

Oriel College
Oxford

(These observations are developed from a Dissertation Project on 'The Mode of Transmission of Leprosy' for the Final Honour Schools of Physiological Sciences, University of Oxford, 1987.)

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Teaching Materials and Services

Dermatology in Basic Health Services

The following were the conclusion and recommendations of the International Workshop 'Dermatology in Basic Health Services' on behalf of the Scientific Secretariat of the 17th World Congress on Dermatology (CMD) and the German Foundation for International Development (DSE), 20–23 May 1987, Berlin (West):

The epidemiology of skin diseases in Africa, Asia and Latin America indicates that in general about 30% of patients seeking medical help are suffering from skin affections. It appears that a majority of the skin diseases are associated with poor socioeconomic conditions, lack of environmental hygiene, inappropriate housing and environmental conditions favouring parasitic, bacterial and fungal infections. Since not sufficient dermatologists are available—some countries do not have any—the strengthening of dermatology within primary health care is clearly needed.

Existing data extracted from hospital records and population surveys should be reviewed epidemiologically and additional well-planned studies are needed to define priorities for the prevention and care of skin diseases, leprosy and STD to identify target populations.

There is need for development of a methodology for rural and peri-urban dermatological care by using reproducible and realistic models. Control of skin diseases, leprosy and sexually transmitted diseases requires the development of inexpensive, appropriate health education materials designed for health personnel of different levels.

The national governments and international and bilateral agencies of technical assistance ought to recognize the overall importance of skin diseases and in so doing support and finance a better planning and integration of dermatology into primary health care.

A global strategy is needed for international collaboration amongst developing countries and with the developed parts of the world. WHO should give greater support and priority to dermatological work in developing countries.

There is a need to develop basic national formularies meeting the requirements of primary dermatological care. Basic medicaments including traditional medicines should preferably be produced locally and at low cost. To facilitate the availability of formulations regarded as essential for the treatment of common skin diseases at primary health care level, it is recommended to include them in WHO's Model List of Essential Drugs.

The creation of regional centres for basic applied and clinical research and training in dermatology should be supported.

Enquiries: German Foundation for International Development Reicherwerder, D-1000 Berlin (West) Germany.

University of Dundee: Centre for Medical Education

Although scheduled for March 1987 and thus too late as information in this issue, we nevertheless draw attention to three courses offered by the Centre for Medical Education, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland: 1, curriculum planning; 2, assessment in medical education; and 3, design and production of learning resources. These are all designed for medical and paramedical teachers. Apply to the above address for details of numerous other courses.

This Centre now has very considerable experience in medical education, including curriculum planning. Countries with continuing defects in their approach to teaching and learning in leprosy might seriously consider a formal approach to the Director of this Centre, for advice. *Editor.*

Leprosy Relief Organization Munich e.V. (AHM)

The Leprosy Relief Organization Munich e.V. was founded in 1972 by a group of Munich citizens with the following target: Relief activities within the leprosy complex.

These worldwide relief activities carried out by AHM include the following fields of equal importance: 1, *Medical Field*: Financial support of leprosy-projects, hospitals, leprosy wards. Medical mass examination, medical therapies; 2, *Social Field*: Financial support of rehabilitation centres and measures, preventative measures, leprosy health education.

The complex relief activities of AHM are based on the following ideas resulting out of experience. The main point is to recognize leprosy, which is today a curable disease, at the earliest possible stage. This also means the enlightenment of those not suffering from leprosy in developing countries affected. In other words, the relief activities launched by AHM are not only understood and practised as a medical process of healing, but also preventatively as a process of learning especially for the youth of the developing countries. Not only will medical relief measures be carried out by trained staff, but the attitude towards leprosy by the population in the developing countries will be changed positively. The stigma of leprosy will be removed and thus psychological preconditions will be achieved to heal people with modern means at the earliest stage.

As a result of the 12th International Leprosy Congress in New Delhi 1984, an active and permanent working relationship between the AHM and Gandhi Memorial Leprosy Foundation, Wardha came into being. The GMLF received significant input from the AHM. The AHM also cofounded the Centre of Social Science for Leprosy in Wardha, through its sponsorship of the Centre's Director, Professor Mutatkar. Research of this kind is absolutely necessary to introduce cost saving measures and to break the stigma of leprosy within the population.

On 1 April 1987 AHM opened an office in Pune, India to continue and put into practice the work which was started at the Social Science Research Centre in Wardha. Professor Mutatkar, founder of the Wardha Centre, has agreed to act as an advisor on the advisory committee. Dr Mutatkar, a leprosy specialist and an experienced leprosy officer, is the regional director of AHM-India Office in Pune.

Further enquiries: AHM, Zenettistrasse 45, D-8000, Munich 2, West Germany.

Teaching and Learning Materials on Leprosy for India, TLMI

Dr R H Thangaraj has now retired as the Leprosy Mission's Director for Southern Asia. However, Dr (Mrs) E S Thangaraj will continue her work as Medical Coordinator and will still supervise the distribution of literature. All correspondence and orders should be addressed to Dr (Mrs) E S Thangaraj, Medical Coordinator, The Leprosy Mission, Salur 532 591, Vizianagram District, Andhra Pradesh, India.

OXFAM–LEPRA, Oxford, UK. A mini-pack of teaching materials on leprosy

Following the development and distribution of a larger pack of teaching–training materials on leprosy during the past 2 or 3 years, OXFAM in cooperation with LEPRA have assembled 100 packs containing only 8 items, as follows:

1 *Chemotherapy of Leprosy for Control Programmes* (1983). Technical Report Series 675, 1211 Geneva 27, Switzerland.
2 *OXFAM Memorandum on the Implementation of Multiple Drug Therapy (MDT) for Leprosy* (1984). The Health Unit, OXFAM, 274 Banbury Road, Oxford OX2 7DZ, UK.
3 *Leprosy* (1979) by Bryceson and Pfaltzgraff. Published by Churchill Livingstone, Edinburgh, UK.
4 *The Diagnosis and Management of Early Leprosy* (1983) by Browne. Published by the Leprosy Mission International, London, UK.
5 *Better Care in Leprosy* (1978). Published by the Voluntary Health Association of India, New Delhi, India.
6 *Insensitive Feet* (1981) by Paul Brand. Published by the Leprosy Mission International, London, UK.
7 *Technical Guide for Smear Examination for Leprosy by Direct Microscopy* (1983) by Leiker and McDougall. Published by the Leprosy Documentation Service (INFOLEP), Amsterdam, the Netherlands.
8 *Atlas of Leprosy* (1983). Published by the Sasakawa Memorial Health Foundation, Tokyo, Japan.

Intended mainly for: Medical students, medical officers (with or without experience of leprosy), leprosy control officers, nurses, tutors and other potential teachers.

In view of the high cost of postage by air or surface mail, OXFAM strongly recommends 'personal' delivery. Copies may be obtained by calling at OXFAM in Oxford during normal working hours or by writing to The Health Unit, OXFAM, 274 Banbury Road, Oxford OX2 7DZ, UK. Delivery, especially for bulk orders, may also be possible through embassies and consulates in London and by liaison with ILEP, the International Federation of Anti-Leprosy Associations, 234 Blythe Road, London W14 (Tel. 01–602 6925) which holds twice-yearly meetings, often abroad, cost UK £10.

International Disability Education and Awareness

Based at William House, 101 Eden Vale Road, Westbury, Wiltshire, BA13 3QF, England, this organization runs courses periodically on disability and rehabilitation. The broad description introducing a recent course ran as follows: 'We welcome anyone who is working or is about to work overseas with people with disabilities and those concerned with work in this field. Practical and theoretical sessions provide a forum for participants and tutors to share their experiences, skills and ideas and to look at some of the fundamental issues behind Disability and Development.'

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News and Notes

Retirement of Lepra's Director G Francis Harris, MC

Francis Harris joined Lepra in 1962 as Deputy to the then General Secretary Bill Crisham.

Concerned that the Association he had joined was simply one which funded other people's work and aware that the fundraisers of that time would appreciate a treatment scheme that was run by Belra (as the Association was then known) the two men explored the possibility of working in Africa.

Three countries were approached and asked if they would consider having a control project and the most constructive reply came from Malaŵi, where the President for Life, Hastings Banda, was a doctor.

The setting up of this project in 1965 and its progress is now part of history. Suffice to say that our control work in Malaŵi is something of which all of us connected with Lepra are most proud, and the 50,000 discharged patients most grateful.

After a long search for a suitable project director the Lepra Evaluation Project began in 1979. Simply; the project aims to find out who is at risk from the disease so that when a satisfactory vaccine is found, there will be no doubt as to whom it should be given.

It has to be said that the particular project has had to be steered through sometimes stormy waters, but again all of us connected with Lepra are grateful for the way that our Director had encouraged the 200 people who work on this project, to carry on their endeavours.

As readers of *Leprosy Review* know, in January 1986 we began the first trial of a new antileprosy vaccine to be held on the African continent. All of us working with Lepra await the first results with eagerness for the vaccine is the work of the former Chairman of our Medical Advisory Board, Dr Dick Rees.

In 1973 Francis Harris, with the assistance of Dick Rees reorganized Lepra's Medical Advisory Team and from that time onwards we have had a Medical Advisory Board to which both Directors have felt able to turn for support. This work, which is in addition to that for which they are paid and which in itself is at times extremely demanding, is very much appreciated.

As Director, Francis Harris has brought many changes to the home front. He has moved the office three times, saying each time it would be the last and in 1975 the Association began its work in Colchester with a second and smaller Head Office in London. Lepra now has approximately fifty staff working throughout the United Kingdom in eighteen different locations. Not an easy task to manage but to date we are flourishing. Each part of the Lepra Kingdom from Dundee in the north to Exeter in the south-west is working hard and in close co-operation with the research centres in Scotland, through London to Oxford and other compass points too many to mention. All of this takes a great deal of work and much travel, all of which Francis Harris has done willingly and well.

On the fundraising side he has seen the Association's income go from £166,681 to over £2,000,000 (and the management of any organization with an income of this size and expenditure to match, is hard work). Not only in encouraging and improving grants which go forward to Lepra's Executive Committee but the day to day handling of Lepra's monies. Their entry into Lepra's coffers is erratic, two thousand being banked one day and over twenty another if a legacy or unexpected gift has been received. Whether to forward it to our brokers or to keep the money on deposit against a need we expect to arise shortly is always a problem, and takes up time if we are to get it right.

One fundraising task of which he is most proud is Lepra's participation in Help Cards, an organization which Lepra joined in its earliest years and of which he was Chairman for ten years.

Another is his presidency of ILEP (the Federation of Anti-Leprosy Associations) a post which he held from 1984 to 1986.

He recognized the importance of these Associations working for the control of leprosy to be doing so in partnership with one another. Here again he has had to steer this particular ship through sometimes turbulent waters and has done so with strength and sensitivity to the needs of his fellow members.

Francis has a saying with which many of those who read these words are familiar: 'How is this action or that going to help the leprosy patient?' Of one thing he need have no fear. He has, through his work as Director of Lepra, helped the leprosy patient personally and, equally importantly, seen the leprosy patient helped forward in so many ways, not the least of which is the adoption of multidrug treatment and, he hopes soon, of a vaccine which should lead to the eventual eradication of this shameful disease.

JOY MAITLAND, Director
of Fundraising and Publicity

New Director for LEPRA, Neil Winship

Lepra, the British Leprosy Relief Association, has appointed a new Director. Mr Neil Winship, a Natural Sciences graduate, served 22 years in the Royal Tank Regiment before leaving to help with famine relief efforts in the Sudan.

Mr Winship, who first came face to face with leprosy in the course of his widely travelled army career, commented: '. . . Having witnessed the effects of leprosy at first-hand in Southern Sudan and elsewhere, I wholeheartedly support efforts towards its eradication.'

When he left the army in 1985, critical conditions in the Sahel prompted him to volunteer to help Band Aid with their Sudan Trucking. Six months later he became Logistics Manager for the International relief charity, World Vision, working in Central Sudan. He soon became involved however, in the task of getting emergency food supplies through to the Churches' Relief Committee in the Southern Sudanese two of Wau, which at that time was surrounded by rebel troops.

He returned to England in March 1987 and worked alongside Mr Francis Harris, Lepra's Director since 1962, before formally taking up his post on 1 January 1988.

'Clearly it is vital that I gain a sound, if layman's understanding of leprosy; its enigmatic nature, the treatment, and hopes for prevention' said Mr Winship. 'While a passing but rusty knowledge of organic chemistry, and a familiarity with microscopy should help a bit, far more significantly by scientific background has helped me appreciate the scope and complexity of the leprosy clinical and research fields.'

Mr Winship travelled to Malaŵi in October to observe the large scale epidemiological survey and vaccine trial, as well as the treatment programmes that Lepra is carrying out there. 'This is our flagship project' he said, 'and my visit will be an essential, as well as, stimulating part of my induction.

However, what has already become clear to me during my short time with Lepra is the challenge in extending our activities in co-operation with host Governments to make the multidrug therapy available to a greater number of sufferers worldwide.'

Editorial Note: Leprosy Control and Field Work; Teaching Materials and Services; News and Notes

Due to the extraordinary number of articles awaiting publication, we have taken the decision to use various blank spaces and pages in the main body of the journal for items normally printed separately, under the above headings. We apologize for any adverse effect this may have on reprints and for any inconvenience to our readers in locating information on the items concerned. *Editor.*

Material for 'Leprosy Control and Field Work' 'Teaching Materials and Services', 'News and Notes'

In recent years it has become increasingly apparent that many readers of this journal, particularly those for whom the scientific articles are of limited significance, greatly appreciate the 'general' pages devoted to the above subjects. These sections are intended to carry information of *practical* value in leprosy control and the clinical management of patients. Teaching–learning materials, courses of instruction, sources of funds for travel and further study, are amongst the most helpful items. We have no shortage of material for these pages from various parts of the world and would be happy to continue selecting and publishing what seems most helpful. However, it would be preferable (and possibly less biased . . .) if suitable items of information could be supplied *by readers of the journal.* The presentation should be brief and should include addresses for further contact, details of costs, postal charges, etc, if relevant. We have a circulation of about 1500 to over 100 different countries, with considerable international indexing, 4 times yearly. Please submit suitable material, so that these pages can be used to maximum advantage. *Editor.*

Zimbabwe Leprosy Association

Dr J A Warndorff, leprosy specialist in Zimbabwe, has kindly written with the following information, which is extracted from the Report of the Chairman of the ZLA, 1987:

'The main focus of activity during the year—indeed of the past four years—has been on the leprosy settlement Mutemwa where the building programme has replaced most old huts. A policy of reintegration of leprosy victims into communities is being actively pursued and a significant step forward was taken when fourteen leprosy victims, together with some eighty 'dependants' who had joined them at Mutemwa over the years were resettled, with the help of social welfare grants, on small plots in the rural areas. With the completion

of the major part of the building programme at Mutemwa, seventy leprosy victims now live there in comfort. There remain some 7000 identified leprosy sufferers in Zimbabwe, most living in the rural areas, and the dermatalogical programme will undoubtedly identify more. Serious cases are hospitalized at the Tropical Diseases Unit in Harare. The Association is therefore turning its attention increasingly to support for the national leprosy programme and has provided equipment for it, as well as for the Tropical Diseases Unit. Communication between Government, the Leprosy Mission and the Association has improved but there is need for better coordination of their activities to ensure maximum impact on the fight against leprosy and to avoid duplication of effort. There is a Leprosy Coordinating Committee on which I represent the Association and I hope that, following today's election of office bearers, I shall be able to give more time to this aspect of work in the field of leprosy. There is some confusion in the minds not only of the general public but also of our members about the aims and objects of the Association. While it is self-evident that, by our very nature and name, we are engaged in the fight against leprosy, our main and best-known activity has been, for twenty years, the administration and maintenance of Mutemwa. It is felt, in some quarters, that we have devoted a disproportionate amount of activity and funding to this area, at the expense of the greater need of the identified leprosy sufferers. I have requested a meeting with the Ministers of Health and of Social Welfare, at which our responsibilities for the village may be confirmed and defined. Once this has been done, it is the intention of the Executive Committee to revise and update the Association's Constitution and then to conduct a public relations exercise with a view to recruiting new members and raising further funds for the fight against leprosy, which will extend beyond the confines of Mutemwa.'

Strong envelopes for posting from India

We are grateful to Dr V V Dongre of Hind Kusht Nivaran Sangh for drawing our attention to the trade name of a strong envelope which is available in stationery shops in India; 'Kaynox Covers'. These are strongly made and reinforced inside with gauze-like cloth. These are ideal for booklets, heavy manuscripts, etc. Please use them! Many of our original articles, mailed in standard envelopes, arrive only by the skin of their teeth. *Editor.*

AIDS; WHO guidelines for the safety of leprosy workers

In a previous number of *Lepr Rev*, 1987; **58:** 207 we printed the full text of the WHO document WHO/CDS/LEP/87.1: 'Guidelines for personnel involved in collection of skin smears in leprosy control programmes for the prevention and control of possible infection with HIV', and once again we draw the attention of all programme managers and leprosy workers to these important recommendations.

Leprosy in South America

The following is from the *Bulletin of the Pan-American Health Organisation*, 1986; **20:** No. 2.

Aside from mainland Chile, leprosy is endemic everywhere in the Americas. Although the recording system is deficient and outdated, 318,001 cases were registered in 1984, 68% of them in Brazil. Overall, around 20,000 new cases are reported each year. The largest numbers of cases appear to occur in Argentina, Brazil, Colombia, Mexico, Paraguay, and some Caribbean countries.

Despite important progress in the understanding of leprosy immunity, much of the health staff in many countries is unacquainted with leprosy epidemiology or with leprosy control methods and lacks the means for diagnosing, treating, and monitoring cases. Moreover, the disease is still an object of social stigma in most communities, primarily because appropriate up-to-date information is lacking, and this circumstance hampers leprosy prevention, outpatient treatment, and rehabilitation.

Close to half of the cases diagnosed as contagious forms (lepromatous and dimorphous cases). In Central America the incidence is generally low, ranging from about 0·04 cases per 1,000 inhabitants per year in Guatemala to 0·22 per 1,000 in Costa Rica. Some Caribbean countries (Guyana, Guadeloupe, and Martinique) have relatively high rates—between 2 and 10 cases per 1,000 inhabitants—but the proportion of contagious cases (lepromatous and dimorphous) is lower than in most other countries. In the Amazon area and some parts of the Andes there are foci of high endemicity where the prevalence can reach 30 cases or more per 1,000. The proportions of unspecified and tuberculoid cases vary widely from one country to another, but account for about 20% and 23%, respectively, of cases in the hemisphere as a whole. In an estimated 30% of the reported cases the disability involved is of grades II and III.

The current strategy for leprosy control is based on reduction of the sources of infection in the community through early detection of cases and the supervised administration of multidrug treatment. It is also necessary to provide for better implementation of control programmes by making extensive use of the health services network.

The Journal of Tropical Medicine and Hygiene

Published for the London School of Hygiene & Tropical Medicine

Edited by **Professor D.J. Bradley** *Department of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London WC1E 7HT, UK*

The Journal of Tropical Medicine and Hygiene is an international journal which has been well established for many years in the field of tropical medicine throughout the world, and covers all aspects of modern diagnosis and treatment. Drawing on studies in the disciplines of immunology, haematology, parasitology, epidemiology, etc., the journal presents a multi-disciplinary approach to the subject. The emphasis of its editorial content is on material of the highest quality concerning community health, epidemiology and preventive medicine.

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Edited by **Dr R. Hay** *St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ, UK*

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++ A CIBA-GEIGY CONTRIBUTION + TO THE FIGHT AGAINST LEPROSY ++

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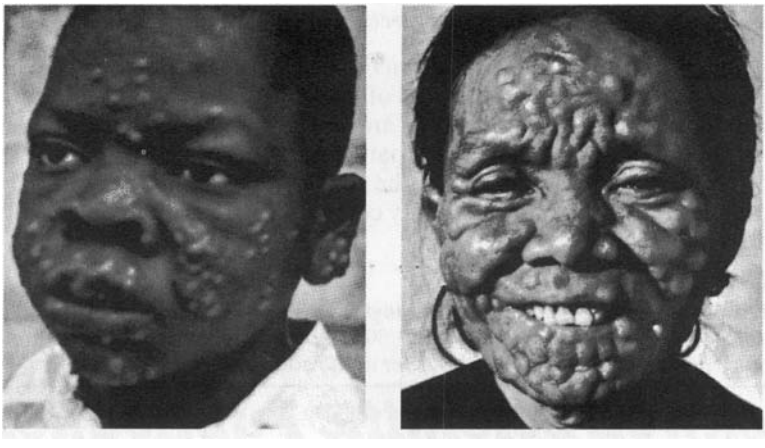
(= clofazimine)
and
®Rimactane Ciba
(= rifampicin)



Two highly effective drugs for use in the treatment of leprosy

Lamprene
Capsules of 50 mg and 100 mg

Composition: Clofazimine. *Capsules* of 50 mg and 100 mg. Indications: Lamprene, employed in combination with dapsone and rifampicin (®Rimactane), serves as treatment for multibacillary forms of leprosy, such as lepromatous (LL), borderline lepromatous (BL), and mid-borderline (BB) leprosy, as well as erythema nodosum leprosum (ENL). Combined chemotherapy is necessary in order to prevent the emergence of resistant strains of *M. leprae*. Dosage: *Adults* (of approx. 60 kg body weight): for the treatment of multibacillary leprosy (LL, BL, BB) the WHO (World Health Organisation) recommends the following dosage schedule: Lamprene: 300 mg once a month under surveillance + 50 mg once a day as self-medication. Rifampicin: 600 mg once a month under surveillance. Dapsone: 100 mg once a day as self-medication. This threefold combination should be administered for at least 2 years and, whenever possible, until such time as the skin smears become negative. If the patient develops ENL, the treatment with dapsone and rifampicin should be continued as before, whereas the dosage of Lamprene should be raised to at the most 300 mg per day. These high daily doses must not be given for longer than 3 months. *Children:* Children should receive lower doses adapted to their body weight. Administration: The capsules should be taken at mealtimes or together with milk. Contra-indication: Known hypersensitivity to clofazimine. Precautions: Leprosy patients suffering repeatedly from abdominal pains and diarrhoea, as well as those with liver or kidney damage, should if possible not be treated with Lamprene. Treatment with daily doses of Lamprene exceeding 100 mg should not be continued for longer than 3 months, and during this time the patient should be kept under medical supervision. If gastro-intestinal symptoms develop during the treatment, the dosage should be reduced or the interval between doses prolonged. In the event of persistent diarrhoea or vomiting, the patient should be hospitalised. Pregnancy and lactation: As in the case of any form of drug therapy, Lamprene should be employed with caution during pregnancy, especially in the first 3 months. Clofazimine crosses the placental barrier and causes temporary discoloration of newborn infants. The active substance also passes into the breast milk. Unwanted effects: The following side effects have been observed: Reddish to dark-brown discoloration of the skin and of the leprous lesions, particularly in pale-skinned patients at sites exposed to light. Discoloration of the hair, conjunctiva, cornea, and lacrimal fluid, as well as of sweat, sputum, urine, and faeces. This discoloration is reversible, although in the case of the skin it often does not disappear completely until some months after the cessation of treatment. Dryness of the skin, ichthyosis, pruritus, photosensitivity, acneform eruptions, and non-specific skin rashes. Nausea, vomiting, abdominal pains, diarrhoea, anorexia, loss of weight, and eosinophilic enteropathy. Storage: Protect from heat and moisture. Packages: 100 capsules of 50 mg or 100 mg. Further information is available on request.

Rimactane
Capsules of 150 mg and 300 mg

Composition: Rifampicin. *Capsules* of 150 mg and 300 mg. Indications: *Leprosy:* in combination with other antileprosy drugs as treatment for lepromatous and dimorphous (borderline) forms of leprosy, as well as in patients with other forms of leprosy, in whom intolerance of, or resistance to, other antileprosy drugs is encountered. Administration: At least ½ hour before a meal on an empty stomach according to WHO recommendations. Contra-indications: Hypersensitivity to rifamycins. Jaundice associated with reduced bilirubin excretion. Note: Daily treatment with Rimactane is generally better tolerated than intermittent therapy. Resumption of treatment with Rimactane after termination of a course of long-term therapy with the drug involves risks and should therefore, if possible, be avoided. In patients with *liver diseases,* as well as in *severely undernourished patients,* treatment with Rimactane entails a higher risk and its therapeutic benefits should therefore be weighed against the possibility of its causing further damage. If such treatment is necessary, the dosage must be correspondingly reduced. During pregnancy the use of Rimactane should, if possible, be avoided. Rimactane passes into the breast milk. Mothers in whom its use proves unavoidable should refrain from breast-feeding their infants. Unwanted effects: Gastro-intestinal disturbances; disorders of hepatic function, e.g. mild transient elevation of the transaminase values, may occur–chiefly at the start of treatment–but do not generally necessitate discontinuation of the medication; isolated occurrences of jaundice, leucopenia, and eosinophilia; particularly in patients taking Rimactane intermittently or in patients in whom daily treatment is resumed after a temporary interruption, side effects–possibly of immunopathological origin–may take the form of influenza-like symptoms ('flu syndrome) and, in rare instances, of cutaneous manifestations, thrombocytopenia, purpura, and fever, as well as of acute renal failure, dyspnoea, or haemolytic anaemia. If serious complications occur, such as thrombocytopenia, purpura, renal failure, or haemolytic anaemia, treatment with Rimactane should be stopped at once and not reinstituted at a later date. Packages: 8, 16, and 80 capsules of 150 mg; 8 and 40 capsules of 300 mg. Further information is available on request.

1. Chemotherapy of leprosy for control programmes, Report of a WHO Study Group, WHO Technical Report Series 675, WHO, Geneva 1982.
2. S. J. Yawalkar, J. Languillon, S. K. Hajra, A. C. McDougall, S. Gosh, D. V. A. Opromolla, C. J. S. Tonello. Once-monthly rifampicin plus daily dapsone in initial treatment of lepromatous leprosy. Lancet 1199, 29 May 1982.