Editorials

LEPROSY-LIKE DISEASE OCCURRING NATURALLY IN ARMADILLOS

The most important advances in our knowledge of leprosy in the past 15 years have stemmed from studies on experimental infections with *Mycobacterium leprae* in animals. First by Shepard (1960) based on limited infection in the mouse following foot pad inoculation and later by Kirchheimer and Storrs (1971) based on disseminated infection in the 9-banded armadillo (*Dasypus novemcinctus*) following inoculation with *M. leprae*. While both animal models continue to be exploited, and the readily available and laboratory bred mouse is being used throughout the world, the armadillo is mainly used where the species is indigenous and entirely on animals caught from the wild because they fail to breed in captivity. The special importance of the armadillo model is as the sole source of large quantities of *M. leprae* since the organism has not been grown *in vitro*. It is against this background therefore that the recent discovery by Walsh *et al.* (1975) of a leprosy-like disease occurring naturally in armadillos trapped in South West Louisiana has caused so much concern and interest. Their research leading up to this discovery was carried out by Gulf South Research Institute (GSRI), Louisiana, which was where in collaboration with the Public Health Service Hospital, also in Louisiana, the original studies on the armadillo model were undertaken.

The discovery by Walsh and his colleagues was made during studies on naturally occurring diseases among armadillos captured from the wild in South West Louisiana. In their 1975 publication they reported 7 animals with large numbers of acid-fast bacilli in skin lesions, nerves, lymph nodes, spleens and livers at the time of necropsy and when the animals had been housed from 1 day to 15 weeks in the outer holding area at GSRI. The histology of the infected tissues and the distribution of bacilli therein was typical of lepromatous leprosy in man and identical with the picture seen in armadillos with diffuse acid-fast bacillary infection following experimental inoculation with *M. leprae* from man. Bacilli from 4 of the animals so far cultured for 3 months, failed to grow on conventional media for the cultivation of mycobacteria incubated at 32° or 37°C. While the authors drew attention to the resemblance of the AFB isolates to *M. leprae* they referred to other important criteria which were being investigated and their continuing study of the prevalence and distribution of natural leprosy-like disease in armadillos in Louisiana. Already some of these results have recently been published as well as the experience of others equally concerned with and experienced in studies on armadillos from Louisiana for leprosy research. GSRI have now identified 14 naturally infected armadillos trapped 17 to 39 miles from their Institute, representing approximately 10% of uninoculated animals, of which only 2 had been in captivity for more than 5 months before examination (reported in Morbidity and Mortality Weekly Report, 1976).

Furthermore, from the patterns of Mitsuda reactivity in patients with leprosy and loss of acid-fast staining following pyridine extraction of bacilli isolated from armadillos infected in the wild strongly suggest that they are *M. leprae* (Walsh *et al.*, 1976; Morbidity and Mortality Weekly Report, 1976), although their final identification cannot be considered definite. On the other hand, Kirchheimer (1976) at the Public Health Service Hospital, Carville, in South East Louisiana has found no evidence whatsoever of mycobacteriosis in 133 wild armadillos captured within this area of Louisiana or from 87 and 13 similarly captured armadillos from Florida and Texas, respectively.

This new and unexpected data from GSRI has obviously generated deep concern and interest since it indicates for the first time that there could be a non-human source of *M. leprae* (i.e. the armadillo) and therefore an important public health risk for those regions in the world where this animal species is indigenous, but at the same time challenge and possibly jeopardise future advances in leprosy research dependent upon the armadillo model.

Assuming armadillos in the wild in Louisiana are infected with *M. leprae*, is there any evidence that they are a source for infecting man? Epidemiological data does not support such evidence, since leprosy has been endemic in the resident population of Louisiana for 150 years, whereas the 9-banded armadillo has been there only since 1926 (entering then from Texas and since spreading eastwards as far as Georgia and Florida), and in the past 50 years the incidence of leprosy has been decreasing in Louisiana. Moreover, a recently conducted case control study undertaken by the Centre for Disease Control, Atlanta, on leprosy patients reported from Louisiana since 1966 who had no family history of leprosy, showed no greater contact with armadillos than did matched controls (Morbidity and Mortality Weekly Report, 1976).

Thus with no evidence to implicate the armadillo as a source of infecting man with leprosy in Louisiana, what are the implications of the GSRI findings on the use of the armadillo for leprosy research? They could jeopardise the whole future of this important programme. However, because of the importance of the armadillo in providing vast quantities of *M. leprae*, it is to be hoped that no hasty decisions will be made. The data must be used as a timely reminder of the extra precautions which have to be taken for studies entirely dependent on animals caught from the wild. On the basis of GSRI data that 10% of wild armadillos have a spontaneous *M. leprae*-like infection, there are sceptics who challenge whether any infections with *M. leprae* have been transmitted experimentally to armadillos. This extreme view can be discounted, since the incidence in experimentally inoculated armadillos is at least 50%. Also 16 armadillos caught from the wild, with no evidence of gross myobacteriosis, were shipped to us in the UK by GSRI. They were inoculated intravenously here with *M. leprae* from man, and by the end of a year 9 had gross evidence of multiple skin lesions, 8 of which had a skin nodule at the site of the intravenous inoculation.

Nevertheless, in future, detailed procedures for detecting every type of mycobacterium must be used for screening wild armadillos. They must then be held in quarantine for at least 3 months before being finally inoculated with *M. leprae*. Furthermore, at present stocks of experimentally infected armadillos must be inoculated with *M. leprae* from man and not with serially passaged organisms. Although no cultivable species of mycobacteria have so far been isolated from armadillos in Louisiana, Muñoz Rivas (1973) has isolated cultivable strains of *M. avium* and *M. intracellulare* from some 9-banded armadillos caught

from the wild in Colombia, South America. The world-wide distribution of *M. lepraemurium* among wild rats might well, under the environmental conditions in which armadillos live, result in such an infection. However, apparently 9-banded armadillos failed to develop infection inoculated with *M. lepraemurium* (Dr J. Convit, personal communication).

There still remains to be clarified the finding by GSRI of *M. leprae*-like infections in wild armadillos. If further studies on these isolates confirm their identity as *M. leprae,* what is their source? Since within Louisiana, GSRI and Carville are the only centres inoculating large numbers of armadillos with *M. leprae,* and GSRI but not Carville are reporting *M. leprae*-like infections in uninoculated animals caught from the wild, a possible source could be the spread of *M. leprae* from experimentally infected armadillos at GSRI. However, of the 14 armadillos with *M. leprae*-like infection, only 2 had been more than 5 months in captivity at GSRI before manifesting overt disease. Furthermore, all the armadillos had been trapped from locations 17 to 39 miles from GSRI and at locations 18 to 44 miles between each other, whereas the home range of the adult armadillo is believed to be only 8 to 10 acres. While Issar (1976) reported a massive *M. leprae*-like infection in an uninoculated armadillo in 1973, that had been maintained for 2 years in GSRI's outer animal compound, GSRI claim that their discovery was only made since 1974. Because of the importance of these claims, with their varying discrepancies, the only way to clarify them is by a full investigation. This can be achieved by an independent and detailed survey of mycobacteriosis among randomly captured armadillos in Louisiana. Fortunately the Center for Disease Control, Atlanta, are currently undertaking such a survey covering 600 armadillos, and the results of their investigations are eagerly awaited.

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HYPERSENSITIVITY AND IMMUNITY REACTIONS AND CLASSIFICATION

The relationship between delayed hypersensitivity and immunity is a problem with a long and vexed history. Suffice it for the moment to say that delayed hypersensitivity and cell mediated immunity are closely interconnected, the two being operated by the same or related mechanisms. As regards leprosy, new light has been shed on this relationship, and also on the significance of the lymphocyte transformation test (LTT), by recent work at the Armauer Hansen Research Institute and the Medical Research Council Unit at Addis Ababa.

The TT-LL classification scale was originally intended to reflect resistance, immunity or whatever it was that determined the pattern of the disease; but whereas the middle and the lepromatous end were soundly based, mainly on the rate of lysis or removal of *Mycobacterium leprae* in patients on chemotherapy, now known to be a function of cell mediated immunity, there had been no equivalent numerical index that could be applied to the tuberculoid end. It was hoped that the LTT using *M. leprae* antigen might fill this gap, and it was satisfactory therefore to find that the classification scale as originally defined (Ridley and Jopling, 1966) correlated tolerably well with the LTT (Myrvang *et al.*, 1973*a*). The correlation, however, was with groups rather than individuals. Furthermore, it was found that the LTT was virtually negative in patients in the clinically indeterminate stage although many such cases are thought to be self-healing (Myrvang *et al.*, 1973*b*). And when the test was applied to borderline patients in reaction some surprising results were obtained that could not be reconciled with shifts in immunity (Godal *et al*, 1973). A more detailed retrospective analysis of the histology of individual patients in the Myrvang *et al.*, (1973*a*) series also produced surprising results (Ridley, 1974); because although the LTT confirmed most of the histological criteria it failed to correlate with the number of lymphocytes in the lesions which had been one of the pillars of the original classification. Allowing for the special relationship of *M. leprae* to nerve and also epidermis (Ridley, 1973), the features with which the LTT correlated were those of hypersensitivity (Epstein, 1973), All these findings were confirmed in a new prospective study at Addis Ababa in which different, more sophisticated techniques were employed for the LTT amd for the preparation of *M. leprae* antigens (Bjune *et al.*, 1976). This study also confirmed that the LTT was raised during reactions in borderline patients, and demonstrated that this elevation far outweighed the differences in value from one part of the spectrum to another. At the termination of the reaction the LTT fell to its initial level, or lower, whether or not there had been any shift in immunity as judged by classification. The authors concluded (1) that the LTT reflected the destructive aspect of delayed hypersensitivity rather than the protective aspects of immunity, and (2) that although it had served to pin-point precisely the histological features of hypersensitivity in leprosy, the LTT was not a useful tool for the evaluation of individual patients. Not only are the results weighted unduly by the response to reactions, but the individual differences in non-reacting patients in a group are marked; many non-reacting BT patients give almost negative results for reasons that are not yet understood. The immunologists in the Addis Ababa team believe that hypersensitivity and immunity in leprosy may represent responses to separate bacterial antigens. This also in a different context is the view of Youmans (1975) who concludes a review: "delayed hypersensitivity and immunity to tuberculosis

are entirely separate processes that are initiated by animals in response to various components of the tubercle bacillus". The basis for this conclusion was the finding that macrophage migration inhibition factor, which is correlated with tuberculin hypersensitivity, and mycobacterial inhibition factor which inhibits the multiplication of tubercle bacilli, are two separate lymphokines. It had already been found that tuberculin protein exhibited strong tuberculin sensitizing activity, whereas immunity appeared to be enhanced only by a polysacchoride fraction, not protein (Takahashi, 1969). The situation is probably not so simple as this, because the results were influenced not only by the presence of otherwise of adjuvant but also by the route of administration of the antigen; and Warren (1972) reports somewhat conflicting results. Nevertheless there is some foundation for a dual antigen hypothesis which, if confirmed, would indicate the possibility of producing a vaccine that selectively enhanced immunity alone. The dual antigen theory also gives food for thought about the nature of reactions and the basis of classification in leprosy.

Apart from the occasional ENL-like reaction that may be seen in the BL region of the spectrum (Waters and Ridley, 1964; Karat *et al.*, 1967), the great majority of reactions in borderline leprosy patients would appear to belong to Coombs and Gell type IV, i.e. delayed hypersensitivity reactions (DHR). They are always associated with a strong rise in the LTT, and more often than not they are followed by an increase in the histological features of delayed hypersensitivity, the elimination of bacilli and perhaps resolution of the lesion. There is some support here for the idea of two sorts of antigen in that *M. leprae* antigen appears to present differently according to whether it is in Schwann cell or macrophage, with corresponding differences in the LTT response during reversal reactions (Barnetson *et al.*, 1975). In the case of the reversal reactions in BT patients that are being considered by these authors the onset could be explained by a sudden "exposure" of a few bacilli or by a release of a particular sort of antigen from them. But in the case of BL patients in whom there may have been millions of bacilli in the lesions before the reaction commenced it is hardly credible that the onset of DHR could be due to this mechanism; large amounts of antigen must have been exposed for some time beforehand. In such a situation a DHR can most readily be explained by assuming an increase of delayed hypersensitivity in response to the reduction of the bacterial load to the point at which pre-existing antigen becomes "noticed". Conversely in untreated active infections there is normally a progressive fall in delayed hypersensitivity in parallel with the increase in the bacterial load so that DHR is not precipitated. But a delay in this desensitization process would again result in a temporary imbalance between hypersensitivity and bacterial load. This is perhaps the most likely explanation of the occasional reaction that is associated with downgrading. Thus viewing DHR as a whole it is difficult to escape the impression of a quantitative relationship between the level of hypersensitivity on the one hand and the amount of immunologically detected antigen on the other. Such a quantitative relationship was demonstrated in tuberculosis by Romer (1908) and Hamburger (1909), who found that the intensity of the hypersensitivity response was dependent on the amount of antigen administered. Thus the quantitative relationship would appear clinically to be as important as the different antigens hypothesis, though not necessarily incompatible with it.

Turning to classification, the foregoing results with the LTT raise a number of questions. Does the Ridley-Jopling scale reflect any sort of useful immunity or

only a destructive hypersensitivity? Can the two in practice be separated in leprosy? And if so does the disease really present two spectra instead of one?

Taking the last question first, there is some histological evidence of a dual spectrum based on the presence of few or many lymphocytes in lesions. Thus BT tends to link with BL and BB with subpolar leproma. The main difference between these spectra seems to be that, other things being equal, the patient with many lymphocytes will be rather more resistant to downgrading without treatment and more likely to upgrade with treatment (Ridley, 1974). Perhaps because he is more likely to upgrade he is also more likely to react. But it is not possible to equate either of these situations with immunity as opposed to hypersensitivity. The slight modifications proposed for the histological definition of the tuberculoid end of the spectrum, being based on the LTT, further emphasize the hypersensitivity element in classification. But these proposals were only made because a search over many years had failed to disclose any group of histologically or clinically identifiable tuberculoid patients in whom there was a predisposition to resolution of an established infection without destructive DHR. In this respect leprosy appears to differ from cutaneous leishmaniasis, a disease with which it has many other points in common, but in which there is an apparent dissociation of delayed hypersensitivity and immunity. Thus the recidiva form in which hypersensitivity is maximal is non-self healing, though other forms with less hypersensitivity are self-healing (Turk *et al.*, 1970; Bryceson *et al.*, 1972). In leprosy uncomplicated self-healing may in fact come about in the early stage of the infection in a manner indicative of an immune process. Clinically such cases may be identifiable as tuberculoid though it is not possible to predict which tuberculoid or indeterminate patients will be self-healing. There is a need for a simple specific test of immunity. Histologically at this stage there is still as a rule no granuloma (Myrang *et al.*, 1973*b*). Once a granuloma is manifest hypersensitivity tends to be more conspicuous than immunity. No doubt the one is associated with the other, but if there are cases in which immunity is high in relation to hypersensitivity there are no means of detecting them. Indeed, since *M. leprae* in the absence of hypersensitivity appears to be almost avirulent, and since it is neither a physical or chemical irritant, one might suppose that the granuloma which is the cause of tuberculoid symptomatology is almost wholly a reflection of hypersensitivity. Thus one cannot object too strongly if it turns out that classification, at least in the tuberculoid region, is likewise a reflection primarily of hypersensitivity. As things stand the patient with the best prognosis is the one with a TT histology and clinically the minimum extension of the disease. Here again a quantitative relationship between hypersensitivity and antigen is in evidence. The main reason why the antigenic load in leprosy so readily gets out of hand is presumably that bacilli remain hidden for too long in nerves. But another factor could be that *M. leprae*, being of low virulence, is only weakly immunogenic since these two characteristics appear to go together (Brehmer *et al.*, 1969).

If the dual antigen concept points the way to a better vaccine and better skin test antigens, the association of hypersensitivity with antigenic load emphasizes the desirability of early treatment. It is sometimes said with some justification that in view of the medical and social problems a diagnosis of leprosy should not be made until it can be proved. But there are, of course, two reasons why an early lesion may remain dormant for long periods: either because it is self-healing, or because the bacilli remain immunologically undetected while they multiply and

establish themselves. The price of delay in starting treatment is sometimes heavy.

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Qualitative Histology and Quantitative Bacteriology in Various Tissues of 50 Leprosy Patients*

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Fifty patients, 45 males and 5 females, from different parts of the leprosy spectrum and at various stages of the disease and its treatment, were examined both by multiple skin smears, nasal scrapings and also by qualitative histology and quantitative bacteriology of skin, dartos, lymph node, nasal mucosa, muscle and nerve. A total of 797 tissues were studied by histology as well as homogenization.

Our study revealed that the qualitative involvement and quantitative bacillary load in the nerves was highest of all the tissues examined. A high incidence of *M. leprae* in the nerves of tuberculoid patients (40%) as opposed to other tissues–skin (7%), dartos (8%), nasal mucosa (7%), lymph node (7%), voluntary muscle (0%) was also observed. The nerve was also found to be a major and the most important reservoir of *M. leprae*. Scrotal skin biopsy was shown to be a suitable and practical site for diagnosis of leprosy. A smear obtained from the homogenate of the scrotal skin can be a useful investigation when histological facilities are not available. The findings of histology and homogenization correlate fairly well except in the skin where homogenization (24%) was better than histology (18%) for detection of bacilli. Nasal mucosa had a similar bacillary load while the lymph node showed a higher load. The importance of voluntary or involuntary muscle (dartos) as a reservoir of *M. leprae* was not borne out in our study.

Introduction

Mycobacterium leprae was first demonstrated by Hansen in 1873. Since then the presence of *M. leprae* has been associated with leprosy infection in man even though the organism has not yet been cultured *in vitro*. Shepard (1960) demonstrated a limited growth of *M. leprae* in the footpad of normal mice and

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This work was undertaken in the Research Laboratory of the TATA Department of Plastic Surgery, Bombay-8.

Received for publication 22nd January, 1976.

Rees and Weddell (1970) have described lesions in the tissues of mouse resembling those of man.

Though lesions in nerves and other tissues have been known since the earliest writings on this disease (Danielssen and Boeck, 1848), it is the external visible cutaneous lesions which have held the interest of leprologists. The present method of declaring a patient infectious or non-infectious is based on smears from the skin and the nasal mucosa. Histological examination when performed for diagnostic purposes is almost invariably of the skin. Khanolkar (1951, 1955), Dastur (1955), Lumsden (1964) and Antia *et al.* (1970) have described the presence of *M. leprae* in Schwann cells of peripheral nerves. Pearson *et al.* (1970) have demonstrated the organism in the striated muscle of mice and man and state that the Morphological Index is higher in muscle than in skin. Job *et al.* (1969), as well as Harman (1968), demonstrated *M. leprae* in the non-striated muscle of the scrotum. The organisms have been described in the lymph nodes (Turk and Walters, 1968), testes, liver, spleen, bone marrow and even in the brain and cerebrospinal fluid (Vaidya *et al.*, 1970). Almost all tissues are invaded in lepromatous leprosy.

Most of these findings are of a qualitative nature though Pearson has described quantitative findings in human muscle and skin.

This study was undertaken to establish quantitatively the load of *M. leprae* in various tissues. Unlike many others, this was not a post-mortem study, nor was it restricted to the lepromatous side of the spectrum. It involved patients from different parts of the spectrum and at various stages of the disease and its treatment. It was hoped that the study may help to a better understanding of tissue invasion in leprosy and identify tissues which are a repository of *M. leprae*.

Materials and Methods

The nature of this investigation was explained to patients undergoing reconstructive surgery in the Tata Department of Plastic Surgery. Forty-five male and 5 female subjects volunteered to offer tissue biopsies. A detailed history and physical signs were recorded in each case including the duration of the disease and regularity or otherwise of treatment. The Lepromin Test was performed using Mitsuda antigen and read at 21 days. Skin smears by the slit and scrape method were taken from the ear, forehead, cheek, buttock, a skin patch and also from the nasal mucosa, and examined for acid fast bacilli after Ziehl Neelsen staining.

BIOPSIES

The following biopsies were obtained under general anaesthesia.

1. *Skin:* an elliptical biopsy from the margin of one or more of the most recent and active lesions penetrating to deep fascia, in all 50 cases. 80 skin biopsies were obtained.

2. *Nasal mucosa:* a small mucosal biopsy of an average weight of 40 mg was obtained from one surface of the septum in 47 cases. The underlying cartilage was included in some cases.

3. *Dartos:* scrotal skin and underlying dartos muscle biopsy was performed in all 45 male patients.

4. *Muscle:* 84 biopsies were taken from paralysed as well as unparalysed muscles including flexor digitorum sublimis, tibialis posterior, tibialis anterior, flexor carpi ulnaris, first dorsal interosseous, triceps, platysma, etc.

5. *Lymph node:* of the total 56 lymph nodes biopsied, there were 34 axillary, 15 inguina, 6 supratrochlear and 1 cervical region. Multiple biopsies were obtained in 5 cases.

6. *Nerve:* this varied from a bundle biopsy of as little as 16 mg in cases where the nerve was not paralysed to total excision biopsy of a segment of the nerve, weighing 465 mg in a totally paralysed nerve. The total 107 biopsies included: the ulnar nerve in the arm 28, ulnar nerve in the forearm 16, median nerve 4, radial cutaneous nerve 18, index branch of radial cutaneous nerve 21, lateral popliteal 10, posterior tibial 1, dorsal branch of ulnar 4, sural 1, digital 1, greater auricular 2, anterior cutaneous of the leg 1. Multiple nerve biopsies were obtained in 35 cases, the ulnar nerve being biopsied simultaneously above the elbow and in the lower forearm in 18 cases.

7. *Bone Marrow:* this was obtained by sternal puncture after incising the skin in 40 cases.

Method: the instruments were cleaned with spirit between biopsies. Each biopsy was divided approximately into two halves for light microscopy and homogenization. (The weights quoted are only for the tissues which were weighed for homogenization.) The average weight of the tissue for homogenization varied generally between 100 to 400 mg.Formol Zenker was used as a fixative and 5 μm thick sections were stained with TRIFF and Fite Faraco stains. In cases where the biopsied tissue was minute as in the index branch of radial cutaneous nerve or bundle biopsy of a nerve, it was subjected only to light microscopy. All nerve tissues were also stained by the Holmes Silver Technique of 15 μm sections. Suitable biopsies of nerves were also fixed for electronmicroscopy.

A manual glass homogenizer was employed. The tissue was weighed on a Mettler balance accurate to 4 decimals. 0.1% bovine albumin in distilled water was used as diluent in a rough proportion of 1 ml diluent for 200 mg of tissue.

Bacillary count was done as per the method of Rees (1964). A standardized platinum loop with a volume of 0.004 ml was used for producing 4 spots on a glass slide (each of 8 mm diameter). The slides were fixed by inverting them over a beaker of formalin for 5 min and then over a steam bath for 3 min. They were stained by the "cold method" using 2% carbol fuchsin for 20 min. The bacilli were counted under a flat field objective of ×100 magnification. Thirty-two microscopic fields were counted per slide, but if they showed no bacilli, 100 fields were counted to exclude the presence of any bacilli. The Morphological Index of bacilli for each tissue was also noted. In tissues showing the presence of acid fast bacilli, the homogenate was streaked on Lowenstein Jansen medium and 0.03 ml of it was injected into each hind footpad of 10 Swiss white mice without dilution. The histological sections were scanned for the presence of *M. leprae* and for the nature of the cells harbouring them. The disease was also classified histologically. The TRIFF stain and histology were done independently in Bombay and by Dr D. J. Harman at the Leprosy StudyCentre in London.

Results

Fifty cases were studied comprising 45 males and 5 females; age varied from 11 to 51 years. The duration of the disease varied from 11 months to 31 years and the treatment varied from 0 to 30 years. The classification as per the Ridley-Jopling Scale using both clinical and histological criteria and their relation

TABLE I

Type of leprosy and duration of chemotherapy

Type (after Ridley-Jopling)	Period of chemotherapy			
	Untreated	0-4 years	5 years and over	Total
Tuberculoid TT-BT	3	16	4	23
Borderline BB-BL	1	10	3	14
Lepromatous LL	2	2	9	13
Total	6	28	16	50

to treatment is given in Table 1. The duration and regularity of treatment is plotted in Fig. 1.

Table 2 summarises the findings of routine smears and the bacteriological positivity of various tissues per patient and per number of specimens, and includes actual counts /g of tissue.

The diagnostic reliability of homogenization vs. histology was evaluated for each tissue by finding out how often one method gave a positive result when the other had failed.

Applying McNemar's Test to these data shows homogenization to be superior to histology ($P < 0.05$) at least in so far as skin was concerned (Table 3).

The bacteriological positivity of tissues correlated to the classification is shown in Fig. 2. The tuberculoid nerves showed a high percentage of positive results (6 of 15 or 40%), as opposed to other tissues, which gave a very low percentage of positive results (0 to 8%). The difference between nerves and any of the other tissues except the muscle, was not significant ($P > 0.05$) when examined by Fischer's exact test.

Analysis of variance showed that the difference among the tissue averages was

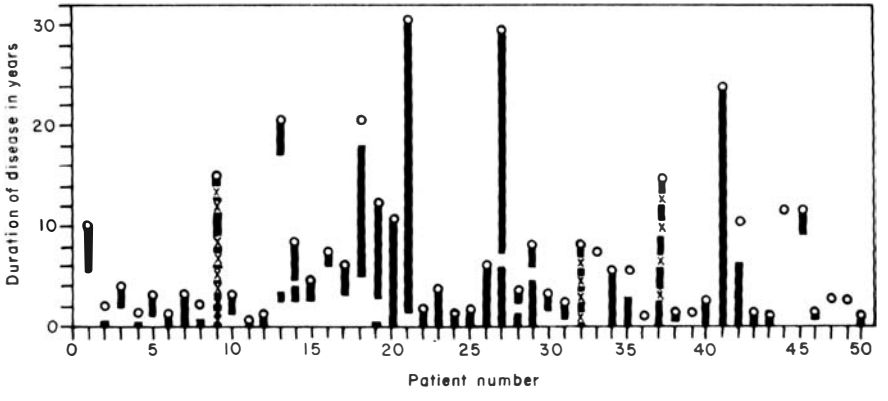


Fig. 1. Duration and regularity of treatment. (o) Earliest symptom; (–) regualr treatment; (– x –) irregular treatment.

TABLE 2

Bacteriological positivity of tissues per patient and per number of specimens

Tissue	No. of patients	Smears % + ve	Histology % + ve	No. of biopsies	Histology % + ve	No. of homogenates	% + ve	Bacterial count ($\times 10^6$)
Nerve	49		60	107	43	79	41	34 ± 10.6
Dartos	40		34	45	34	40	33	5.0 ± 10.7
Lymph node	48		22	56	25	54	25	10.7 ± 9.2
Nose (biopsy)	41		22	47	22	41	20	0.65 ± 10.6
Nose (smear)	48	14						
Skin (biopsy)	50		18	80	15	80	21	1.4 ± 7.6
1 or 2 smears	48	12						
multiple smears	48	18						
Muscle	50		6	84	3.5	84	6	0.17 ± 7.4
Bone marrow	40	0						
				Total 419		Total 378		

Total tissues studied = 797.

TABLE 3

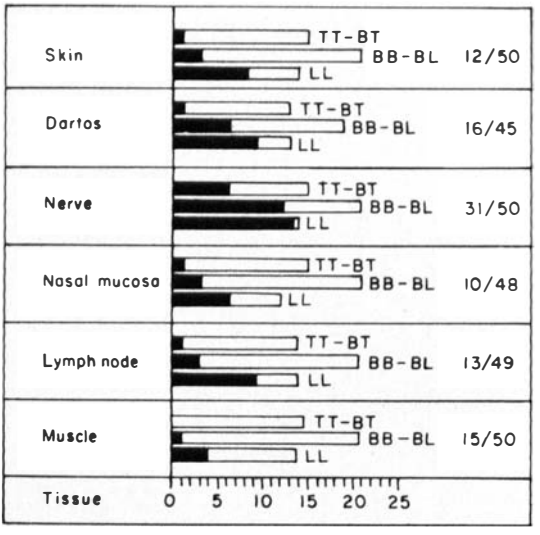
Diagnostic reliability of homogenization and histology by tissues

Tissue	No. + ve by homo only	No. + ve by histo only	x^2 *	Significant difference†
Skin	5	0	5	S
Dartos	1	3	1	NS
Nerve	2	8	3.6	NS
Nasal mucosa	0	0	0	NS
Lymph node	1	2	0.33	NS
Muscle	2	1	0.33	NS

* Calculated by McNemar's Test (without Yates' correction‡).

† Level of significance $P \leqslant 0.05$.

‡ Feinstein, A. R. (1973). *Clinical Pharmacology and Therapeutics* 14, 898.



significant ($F_5,372 = 2.8$; $P < 0.05$). Subsequently, since the number of observations in each group was not the same, a "standard error of difference" was calculated separately for each of the 15 individual differences, and their significance tested by employing the Student's *t*-test. The average load of *M. leprae* in nerve was found to be significantly higher ($P < 0.05$) than that in skin, dartos, nasal mucosa and muscle, but not in lymph node.

HISTOLOGY

The salient findings will be described tissuewise.

Skin: this showed the classical changes as described by most authors.

Dartos: there were marked histological changes in the region of the neurovascular bundles, i.e. Schwannian proliferation, macrophage and lympho-

cytic infiltration. The bacilli seen were almost always restricted to these areas. The smooth muscle of the dartos was rarely infiltrated and showed the presence of bacilli in only 4 cases.

Lymph node: the increase in germinal centres and replacement of the gland by macrophage infiltration in advanced lepromatous leprosy was noted. The bacilli were chiefly in the macrophages, rarely in the dendritic cells of the germinal follicles.

Nasal mucosa: there was loss of cilia and stratification of epithelium in the majority of cases, even in the early ones. This was also observed on naked eye examination of the septum as a process of "creeping epithelialization".

Voluntary muscle: infiltration and presence of bacilli was chiefly in the intramuscular nerves and in the interstitial tissue between nuscle fibres. Actual infiltration of muscle (leprous myositis) was seen only in 3 cases.

Nerves: these showed by far the most advanced histological evidence of disease out of all the tissues examined in any individual patient. Early cases showed Schwann cell proliferation. Bacilli were seen both in the Schwann cells and in macrophages. They were generally better preserved in the Schwann cells while those in macrophages were usually granular.

Conclusions

The following conclusions can be drawn from the findings of this study.

In the cases studied, the routine slit and scrape method of diagnosis of leprosy by a single patch and/or ear lobule as is used in the field, provided only 12% positive results, while nerve biopsies revealed 60% positivity. Multiple skin smears increased the positivity only to 18% while nasal scrapings yielded a 14% positive result. Nasal biopsy increased this to 20%.

As expected, the bacillary load of tissue was lowest in the tuberculoid part of the spectrum and greatest on the lepromatous side.

The findings of histology and homogenization correlate fairly well except in the skin where homogenization (24%) was better than histology (18%) for detection of bacilli. Since this difference was significant (Table 3), which tallies with our clinical impression, homogenization of the skin patch may be used where facilities for histology are not available.

Multiple skin patch biopsies should be carried out whenever possible as they have a better chance of providing a positive result.

The importance of voluntary muscle as a reservoir of *M. leprae*, as described by other authors, has not been borne out in this study. In fact, this study revealed that muscle was the least affected tissue in man. Actual myositis was even rarer than interstitial tissue involvement.

Scrotal skin biopsy has been shown to be a most suitable and practical site for the diagnosis of leprosy both by histological and bacteriological findings. This involvement is chiefly restricted the the nerves. In fact, clinically uninvolved scrotal biopsy is often a better indicator of leprosy than even a clinically involved skin patch in the borderline and lepromatous types. (Pandya and Antia, 1974). Wherever histology facilities are not available, the presence of acid fast bacilli can be ascertained by the examination of a smear obtained from the homogenate. This would prove to be a superior method of bacteriological diagnosis to multiple skin smears or nasal scrapings or even homogenization of skin patch especially in

lepromatous leprosy and could be particularly useful in field studies in the developing countries.

Lymph nodes showed a high rate of bacillary presence. This may indicate that a large number of bacilli are trapped in the lymph nodes for eventual destruction. Analysis showed that it did not seem to matter which lymph nodes were examined in any given case.

Nasal mucosa had a smaller bacillary load. This small load may be due to increased destruction in the earlier phases of treatment.

The same reasons may account for the absence of acid fast bacilli from all the bone marrow smears examined. There was no untreated lepromatous case in this series.

The qualitative involvement and quantitative bacillary load in the nerves was found to be highest of all the tissues examined. Nerve biopsy has been shown to be a feasible procedure and provides the best histological and bacteriological evidence for the diagnosis of leprosy. Selection of one of the clinically most involved nerves gives as good a result as multiple nerve biopsies. It may not at present be a practical method of diagnosis except in special circumstances when diagnosis is in doubt. The index branch of the radial cutaneous nerve or a bundle biopsy of an involved nerve is helpful for diagnosis in early leprosy.

The high incidence of *M. leprae* in the nerves of patients declared negative by the routine skin and nasal scrapings method is disturbing and may emphasize the need for prolonged treatment. It may also account for the high relapse rate even after bacteriological negativity has been demonstrated.

The failure to reach statistical significance in spite of showing a high percentage of positive results in the tuberculoid nerves is very probably due to the small size of the samples. However, the size of the difference suggests that it is clinically important and cannot be ignored.

The high incidence of *M. leprae* in the nerves of tuberculoid patients (40%) as opposed to other tissues (skin (7%), dartos (8%), nasal mucosa (7%), lumph node (7%), voluntary muscle (0%)) may indicate the reason for the high incidence of the nerve involvement so typical of this type of disease.

Our study showed the nerve as a major and most important reservoir of *M. leprae* in man both on qualitative histology and quantitative bacteriology. The bacilli in the nerves were more solid staining when compared with the same in other tissues of the same patient. The bacterial load was highest in the nerves when compared with other tissues in the same patient. Inoculation in the mouse footpad also showed consistently higher growth curves from the nerves when compared with other tissues in the same patient (Bhatt and Antia, 1974). The examination of the index branch of the radial cutaneous nerve has shown that this nerve is the earliest tissue involved even prior to any clinical manifestations (Antia *et al.*, 1974). This leads us to believe that leprosy results in a diffuse though sub-clinical peripheral neuropathy even at the earliest stages of the disease.

ACKNOWLEDGEMENTS

This study was undertaken with a grant from The Wellcome Trust, London.

We wish to thank Dr D. J. Harman of the Leprosy Study Centre, London, for reviewing the histology, Dr A. S. Nanivadekar. M.D., Medical Director, Pfizer Ltd. for help in statistical analysis of results and Dr (Mrs) S. Bhasin and Miss P. V. Bhatt for technical assistance.

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Lepr. Rev. (1976) 47, 185-191

Enumeration of *Mycobacterium leprae* Stained with and without Prior Periodate Oxidation

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Slides prepared with suspensions of *Mycobacterium leprae* recovered from skin biopsy specimens obtained from treated lepromatous leprosy patients and oxidized with periodate contained on the average 36% more acid-fast bacteria (AFB) than did duplicate slides stained by the standard acid-fast stain. By contrast, slides prepared with suspensions of *M. leprae* recovered during logarithmic multiplication in mouse foot pads and treated with periodate contained on the average 16 or 33% fewer AFB than did duplicate slides stained by the standard technique. These results are inconsistent with a non-acid-fast stage in the growth of *M. leprae*.

Introduction

The claim has been made that oxidation of *Mycobacterium tuberculosis* and *M. leprae* renders more of these organisms stainable by an acid-fast staining technique than when acid-fast staining is carried out without prior oxidation (Nyka, 1963, 1967; Reich *et al.*, 1972; Harada, 1973; Mohysen and Alemayehu, 1973). This claim has been reexamined for *M. leprae*.

Materials and Methods

In this laboratory, which has been engaged in the inoculation of mice with *M. leprae* for the past 9 years, it is standard practice to make duplicate preparations of bacterial suspensions on Reich counting slides* for acid-fast staining and enumeration of acid-fast bacilli (AFB). Both slides are routinely fixed in formalin fumes; one slide is stained with a carefully standardized acid-fast stain at room temperature, and the AFB are enumerated (Shepard and McRae, 1968); both the stained and unstained slides are filed in a light-tight box. The exposure of the stained slide to light is minimized between the time it is stained and the time it is finally filed.

For the purpose of this study, 50 pairs of slides were selected from the files. These included 20 pairs prepared between September, 1971 and April, 1973 from

* Bellco Glass, Inc., Vineland, New Jersey, U.S.A.

Received for publication 30th March, 1976.

the biopsy specimens of patients who had been treated with acedapsone or rifampicin for 3 or 6 months (Group 1—"human"), 20 pairs prepared with logarithmic-phase *M. leprae* from mouse foot pad homogenates during the first six months of 1974 (Group 2—"new mouse"), and 10 pairs prepared from mouse foot pad homogenates between January and May, 1971 (Group 3—"old mouse"). Only those pairs were selected that had yielded a minimum of 100 AFB per 60 oil immersion fields when originally counted.

The 50 unstained slides were immersed in a freshly-prepared 10% (w/v) aqueous solution of periodic acid for 4 h, after which they were rinsed with water and stained by the standard acid-fast stain (oxidation for 24 h was found to yield the same results as periodate treatment for 4 h).

C.H. examined all 100 slides from Groups 1, 2 and 3. In addition, 20 slides from Groups 1, 2 and 3 were recoded and reexamined by C.H.—10 stained by the standard technique and 10 after periodate oxidation. L.P.M. examined 20 of the slides from Groups 1, 2 and 3—10 stained by the conventional acid-fast stain and 10 stained after treatment with periodate. In addition to these 100 slides, 10 "human" and 10 "new mouse" acid-fast stained slides previously found by L.P.M. to yield at least 100 AFB per 60 fields were selected for recounting by the same examiner. All 120 slides were coded with a three-digit random number.

The results have been analyzed by the linear regression technique (Goldstein, 1964).

Results

REPRODUCIBILITY OF COUNTS OF AFB

The results of the duplicate counts are plotted in Fig. 1. All 40 points representing duplicate counts made by the same observer were plotted with the first count as the "observer no. 1" value and the second count as the value for "observer no. 2". The duplicate counts by L.P.M. are represented by the open circles. The slope of line a, the regression line calculated for the 20 comparisons, is 0.74, a value significantly smaller than 1. The slope of the regression line for duplicate counts by C.H. (closed circles, line b) is 0.82, a value not significantly different from 1.

It may be more appropriate to consider the absolute differences (the differences without regard to sign) between duplicate counts as the measure of observer error. The mean absolute difference between duplicate counts, expressed as the percentage of the mean of the two counts, is 25% for L.P.M. and 24% for C.H. One may also calculate the regression of the smaller counts on the larger counts in each pair; the slopes of these regression lines are 0.62 for L.P.M. and 0.84 for C.H.; both values are significantly smaller than 1 but not different from each other.

Twenty of the points plotted in Fig. 1 (Δ's, line c) represent counts made by L.P.M. (observer no. 1) and C.H. (observer no. 2). The slope of the regression of the counts by C.H. on those of L.P.M. was 0.76, a value significantly smaller than 1 but not different from 0.74 or 0.82.

Because L.P.M. reexamined 10 slides prepared from human material that had been first examined from 1 to 3 years earlier, it was possible to assess the effect of storage on the number of AFB stained by the conventional AFB stain. The regression of the recent counts on those made originally was 0.78, which is not significantly smaller than 1.

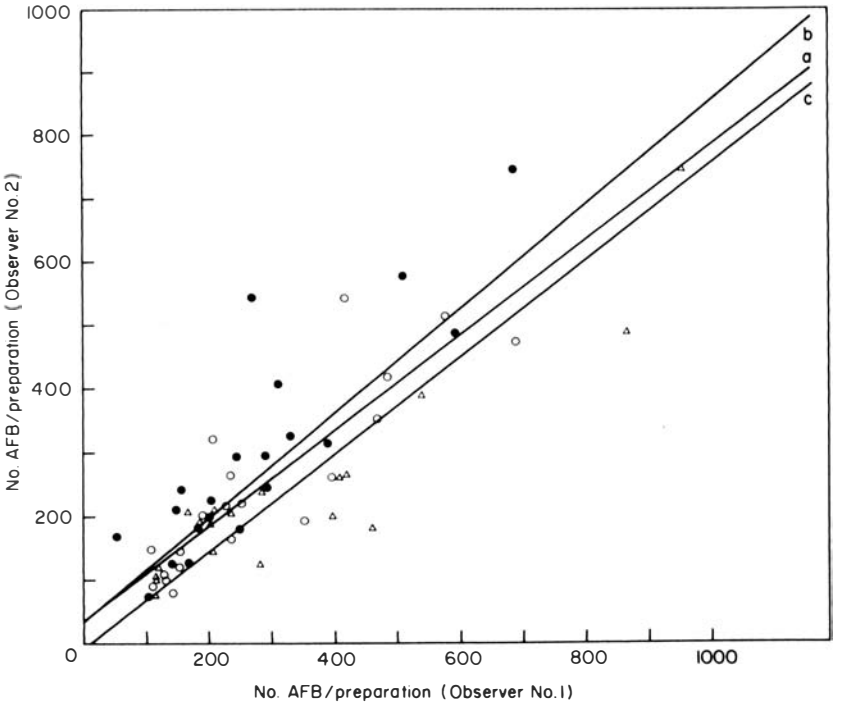

Fig. 1. The number of AFB counted by observer no. 2 as a function of the number counted by observer no. 1 in the same preparation. The lines represent the regression of the number counted by observer no. 2 on that counted by observer no. 1.

L.P.M. vs L.P.M. - (○), line a;
C.H. vs C.H. - (●), line b;
C.H. vs L.P.M. - △'s, line c.

Equations of the regression lines and their correlation coefficients (r) are as follows:

line a - Count no. 1 = 246.4 + (0.744 ± 0.208) (Count no. 2 - 282.3); $r = 0.871$;
line b - Count no. 1 = 275.6 + (0.820 ± 0.224) (Count no. 2 - 298.0); $r = 0.876$;
line c - Count no. 1 = 271.6 + (0.763 ± 0.152) (Count no. 2 - 367.4); $r = 0.926$.

EFFECT OF PERIODATE TREATMENT

In Fig. 2, the numbers of AFB in the acid-fast stained preparations are plotted as a function of the numbers of organisms counted in the periodate-treated preparations. The slope of the regression of the counts in the acid-fast stained preparations on those in the periodate-treated slides prepared from skin biopsy specimens from patients (open circles, line a) was 0.38, a value significantly smaller than 0.6. The slope of the regression of the numbers of organisms in acid-fast stained preparations on those in corresponding periodate-treated slides prepared with *M. leprae* harvested from infected mouse foot pads (closed circles, line b), is 1.07; this value is not significantly different from 1, but is different from 0.38.

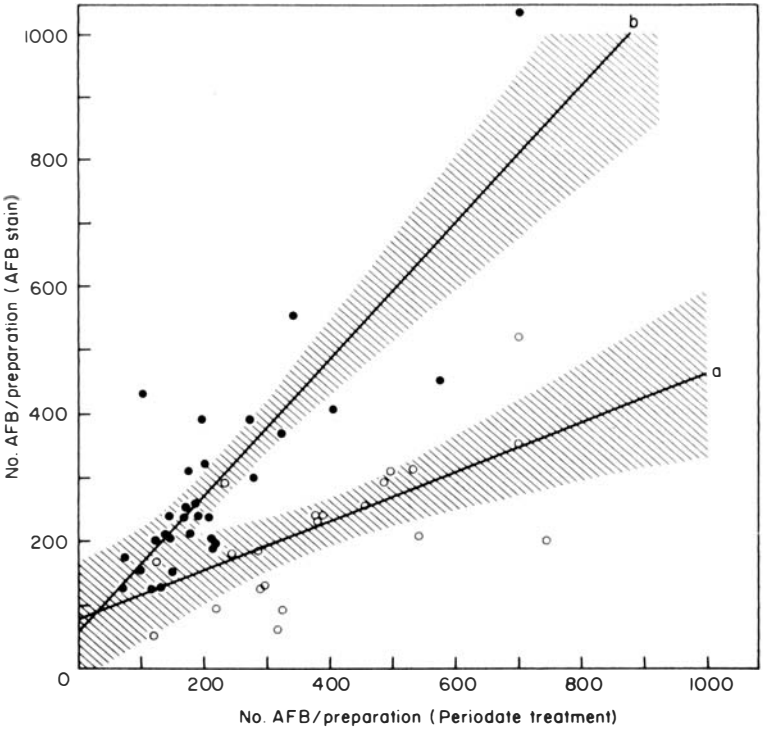

Fig. 2. The number of AFB stained by the standard acid-fast stain as a function on the number stained after periodate oxidation. The lines represent the regression of the number stained by the acid-fast stain on that stained after periodate treatment. Shaded areas represent the 95% confidence bands.

"Human" slides–(○), line a;
"Old mouse" and "new mouse" slides – (●), line b.

Equations of the regression lines and their correlation coefficients are as follows:

line a - Count no. 1 = 227.1 + (0.381 ± 0.214) (Count no. 2 - 393.8); r = 0.662;
line b - Count no. 1 = 289.7 + (1.067 ± 0.259) (Count no. 2 - 219.2); r = 0.847.

Discussion

The purpose of this study was to reexamine claims that periodate treatment rendered stainable *M. leprae* that could not otherwise be stained by the acid-fast technique. The results demonstrate that this is indeed the case for *M. leprae* recovered from patients after treatment sufficient to render the organisms non-infective for mice (Shepard *et al.*, 1972*a,b*). The number of these organisms stained after periodate treatment was, on the average, 36% larger than that directly stained by the acid-fast technique. These data appear consistent with those reported by others.

That we are not measuring the effects of storage has already been shown. The question of an artefact imposed by a systematic difference between observers is

TABLE 1

Comparison of counting error with effects of periodate treatment

Observer		Source	Stain	n	$\bar{D}$* (%)
No. 1	No. 2				
L.P.M.	L.P.M.	New mouse	AFB	10	6.93
L.P.M.	L.P.M.	Human†	AFB	10	20.3
C.H.	C.H.	Human	AFB	10	14.4
C.H.	C.H.	Human	Periodate	10	1.54
L.P.M.	C.H.	Old mouse	AFB	10	16.1
L.P.M.	C.H.	Human	Periodate	10	40.5‡
C.H.	C.H.	Human	Periodate vs AFB§	20	36.4‡
C.H.	C.H.	New mouse	Periodate vs AFB§	20	−32.9
C.H.	C.H.	Old mouse	Periodate vs AFB§	10	−16.4‡

* $\bar{D} = \Sigma$ |200 (Observation no. 1 - Observation no. 2)/(Observation no. 1 + Observation no. 2)|/n, where n = the number of pairs of observations.

† Observation no. 1 - those counts made in the period 1971 to 1973; observation no. 2 - recent counts.

‡ None of the values of $\bar{D}$ was significantly different from 0; the slopes of the regression lines calculated for these sets of data were significantly different from 1, however.

§ Observation no. 1 - those counts made on periodate-treated slides; observation no. 2 - counts made on slides stained by the standard acid-fast stain.

best answered by the data listed in Table 1. Here, the mean difference between duplicate counts or counts on duplicate preparations is expressed as the percent of the mean of the 2 counts. The absolute difference between duplicate counts on the same preparations is the same on the average for the 2 observers. C.H. appears consistently to count fewer organisms than does L.P.M.; this is particularly true for the periodate-treated "human" slides. It may be, then, that C.H. underestimated the effect of periodate treatment, perhaps by regularly dismissing as staining artefacts objects that the more experienced L.P.M. considered *M. leprae*. Thus, the effect of periodate treatment of *M. leprae* recovered from treated patients appears to be real, and may in fact be greater than that we have measured.

The situation with respect to *M. leprae* harvested from mouse foot pad tissues during logarithmic multiplication, however, appears different. In this case, observer C.H. found consistently more AFB stained by the conventional technique than after periodate treatment. And even if C.H. underestimated the number of these organisms stained after oxidation, there is no evidence of an excess of AFB stained after periodate treatment, as was the case with those recovered from treated patients. Thus, the phenomenon is not the result of fading of the acid-fast stained organisms on storage, nor is it the result of observer error.

Reich has reported (1971) that the number of viable organisms in a logarithmic phase culture of a cultivable mycobacterial strain—the "NQ bacillus"—was as much as 100-fold greater than the number enumerated as AFB. He suggested that the failure to be stained by the acid-fast stain was a characteristic associated with "physiologic youth" of this mycobacterial strain. In a later presentation, published only in abstract (Reich *et al.*, 1972), he reported that in approximately half of a group of smears of skin scrapings and bacterial suspensions recovered from the biopsy specimens of skin lesions of patients, periodate treatment

increased the number of AFB by at least 30% above the number counted in the corresponding conventionally-stained preparations. He concluded that, as was the case with cultures of the NQ bacillus, an important fraction of *M. leprae* goes unstained by the standard acid-fast stain, and that, because we have no independent measure of the number of *M. leprae*, "mycobacterial counting that included only the red stained bacilli would provide data that could be invalid for experimental conclusions that were based on the assumption that either the entire population or constant proportions of the population were being investigated." The data presented in this report suggest, on the other hand, that the failure of staining by the standard acid-fast technique is a characteristic of dead or decadent *M. leprae* that are unable to infect the mouse foot pad, whereas "youthful" organisms—those recovered from the mouse foot pad during logarithmic multiplication—stain at least as well by the standard technique as after periodate treatment.

The data presented here suggest, further, that the excess of "human" *M. leprae* stained after periodate oxidation is inconsequential. In an earlier, as yet unpublished study of the counting technique, we found that the *M. leprae* were often randomly distributed in the "circles" of the counting slide; that is, the numbers of organisms encountered in field-by-field counts were consistent with the Poisson distribution (Goldstein, 1964). Moreover, the numbers of organisms counted during the examination of 20 fields along the equator of each of the three circles on the slide fell in the range −50% to +100% of the mean for the 3 circles 90% of the time. Thus, some variation of the numbers of organisms counted in duplicate preparations is to be expected, even when both have been stained simultaneously by the same technique and were examined by the same observer. In order to demonstrate an effect of periodate treatment, the difference between numbers of AFB counted on duplicate slides must be shown to be greater than that attributable to counting error. Our data suggest that the increase of the number of stainable AFB from human material produced by periodate treatment is only a little larger than the apparent increase that might be encountered by chance when the same slide is reexamined by the same or another examiner, and may be no greater than the apparent increase encountered when duplicate but not identical preparations are examined. Certainly, there is no evidence to suggest that staining by the standard acid-fast technique results in a gross underestimate of the number of *M. leprae* in a preparation.

Acknowledgement

This research was partially supported by the U.S. Leprosy Panel of the U.S.-Japan Cooperative Medical Science Program administered by the Geographic Medicine Branch, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland 20014, U.S.A. (Grant R22 AI 07801). The assistance of W. Mark Krushat, Community Medicine Program, Public Health Service Hospital, San Francisco, is gratefully acknowledged.

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Lepr. Rev. (1976) **47**, 193-199

DNCB—Reactivity in Patients with Leprosy In Kenya

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Sensitization followed by graded challenges of dinitrochlorobenzene (DNCB) were performed in 105 leprosy patients (Bantu) in Kenya (22 tuberculoid, 53 borderline and 30 lepromatous leprosy). The results were compared with those obtained in a group of 38 relatives (index cases 5 lepromatous leprosy patients) and in a group of healthy controls (no known household contact with leprosy). All patients showed a diminshed DNCB reactivity as compared to healthy controls. In the group of relatives of lepromatous leprosy patients no decrease of DNCB reactivity (as compared to local controls) was observed.

The percentage of DNCB reactors in healthy controls in Africa proved to be significantly lower than the percentage of DNCB reactors in healthy controls of Caucasion and Negro ancestry in Holland. The factors possibly influencing these results are discussed.

Introduction

In several studies evidence is given that cell-mediated immune reactivity is depressed in patients with lepromatous leprosy. Impaired responses were noted to intracutaneous test antigens (Waldorf *et al.*, 1966; Bullock, 1968), and to contact sensitization with 2,4-dinitrochlorobenzene (DNCB) or picrychloride (Waldorf *et al.*, 1966; Bullock, 1968, Turk and Waters, 1969). Besides prolonged skin allograft survival has been reported in lepromatous patients (Job *et al.*, 1967) and in both tuberculoid and lepromatous patients (Han *et al.*, 1971). Reports on *in vitro* tests with leucocytes of leprosy patients have been contradictory (Dierks and Shepard, 1968; Sheagren *et al.*, 1969; Nelson *et al.*, 1971; Wong *et al.*, 1971) and consequently there is some doubt whether in lepromatous leprosy a generalized depression of CMI reactivity actually occurs.

Moreover, some findings point to an impairment of CMI reactivity in patients with tuberculoid leprosy (Bullock, 1968). Recently Mendes *et al.* (1974) showed significantly impaired responses to DNCB in tuberculoid leprosy patients with normal responses to intracutaneous test antigens (tuberculin, oidiomycin, trichophytin and lepromin).

Because of this controversy of opinion we decided to start a systematic study on the immune reactivity of leprosy patients. In this paper we report on the results of DNCB sensitization testing in leprosy patients in Kenya. Patients representing the whole spectrum of leprosy from polar tuberculoid to polar lepromatous were included in these tests. A comparison was made with similar

Received for publication 11 February, 1976.

tests carried out in relatives of lepromatous patients while results were matched with results obtained in two groups of healthy controls with residence in Kenya and in the Netherlands, respectively.

Methods

SENSITIZATION PROCEDURE

Sensitization tests with D CBNwere performed according to the methods described by Bleumink *et al.* (1974) with minor modifications.

1. The solutions used for sensitization and challenge were prepared at the Biochemical Laboratory (Head: E. Bleumink, Ph.D.), Department of Dermatology, Groningen, The Netherlands, and issued in polythene vials which were sealed after the removal of acetone by evaporation. The vials were stored at 4° C and prior to use in the field were reconstituted with a standard volume of acetone.
2. The sensitization dose of 2 mg DNCB in acetone as a rule was applied to the volar aspect of the forearm. Moslems, however, because of ritually prescribed daily ablutions, were sensitized at the volar aspect of their *upperarm.*
3. Challenges were performed 14 days late; by patchtesting with 3 and 10 ug of DNCB, omitting tests with 30 ug in order to prevent erroneous readings (Hartman, 1976).
4. The reactions were examined 48 h later and graded as 2, 3 or 4 plus reactions omitting 1 plus reactions (erythema only) because of their inconspicuousness on African skin. The sum of the plus reactions is designated as DNCB-score. A score of 2 or more is taken as positive evidence for cell-mediated immune reactivity to this particular component.

PATIENTS AND CONTROLS STUDIED

A. In Kenya three groups of persons were studied:

1. 105 leprosy patients; 75 persons from Western Kenya admitted to the Leprosy Hospital at Alupe and 30 in-patients of the Tumbe Leprosy Centre (Coast Province). Of these 47 were in a reactional state or had recently suffered from a reactional state (reversal reaction or ENL). All of them had received treatment with diaminodiphenylsulphone (DDS) or Lamprene. Some patients were treated with corticosteroids.
2. 38 relatives (first and second degree) of 5 patients with (polar) lepromatous leprosy living in the Coast Province. Physical examination revealed that 3 of them had untreated tuberculoid leprosy.
3. 27 healthy villagers near Tumbe without a family history of leprosy.

B. In the Netherlands 2 groups of controls were studied:

1. A group of 105 Dutch out-patients of the Department of Dermatology at Groningen, presenting with minor skin disease and obviously free of leprosy.
2. A group of 7 healthy negroes of S. American ancestry born in Holland.

The classification of leprosy patients was based on clinical history, physical examination, determination of skin smears and skin biopsies. All patients were (re)classified according to the Ridley-Jopling scale (1966).

Details like age, sex, tribe, reactional state, reason of admission, period of treatment, BCG vaccination and other factors of possible influence on the DNCB reactivity were recorded.

Some biopsies were taken for histological evaluation of our test results.

Finally all volunteers were clinically examined and the clinical history and diseases were noted.

GENERAL HEALTH SCREENING

Physical examination revealed that a great many people in the 3 groups investigated had a palpable spleen. Anaemia was commonly encountered (18%). All of them had a history of repeated attacks of malaria and a number of them (58%) had a history of schistosomiasis. Kwashiorkor and marasmus are well known diseases in the areas under survey.

Results

Leprosy patients both in Alupe as well as in Tumbe Leprosy Centre, were classified by clinical examination in five groups (Table 1). Since DNCB reactivity partly depends upon age, data are given for 4 separate age groups (Table 2). Only 4 out of 105 Patients (4%) with leprosy were found reactive to DNCB as compared to 11 out of 38 in the group of relatives (29%) and 7 out of 27 (26%) healthy controls in Kenya. The difference between the leprosy patients and the latter two groups is statistically significant ($P > 0.01$). The differences are even

TABLE 1

Classification of leprosy patients, reactional state and DNCB reactivity

Type	Admitted at Tumbe	Reactional state	DNCB-test	Admitted at Alupe	Reactional state	DNCB-test
1. TT	3	0	0	–	–	–
2. TB	8	5	0	32	12	1
3. BB	7	3	0	7	3	1
4. BL	6	3	0	28	13	1
5. LL	6	2	0	8	6	1
	30	13	0	75	34	4

TABLE 2

Results of patch tests with DNCB in leprosy patients, relatives of LL patients and controls in Kenya (1) and in Holland (2)

Age years	Leprosy patients		Relatives of LL* patients		Controls in Kenya		Controls in Holland (Dutch)		Controls in Holland (negroes)	
	No	*Pos*	*No*	*Pos*	*No*	*Pos*	*No*	*Pos*	*No*	*Pos*
0-30	37	2	22	7	14	3	48	48	6	6
31-50	54	1	13	3	10	3	35	35	1	1
51-70	12	1	2	1	12	1	10	8	0	0
70	2	1	1	0	1	0	10	2	0	0
	105	4	38	11	27	7	103	92	7	7

No = Number.
Pos = Positive.

* Lepromatous leprosy (polar form).

TABLE 3

Summary of DNCB-tests in leprosy patients and controls in Kenya

		Number Challenged (read)	Positive	%
Group A	Leprosy patients			
	Alupe (Western Kenya)	75	4	5
	Tumbe (Coast Province)	30	0	0
	Total	105	4	5
Group B	Family members of 5 lepromatous patients (1st and 2nd degree)			
	Coast Province	38	11	29
Group C	Control Group	27		
	Coast Province		7	26

Differences between A and B, A and C statistically significant ($P < 0.01$)

more striking (Table 3) if only those persons living in the Coast Province are taken into account. None out of 30 leprosy patients and 7 out of 27 healthy controls could be sensitized to the contact allergen. Also 3 children with high resistant tuberculoid leprosy (TT) found among the relatives of patients were negative to DNCB.

TABLE 4

Data of persons in Kenya with positive reactions to DNCB

Age	Sex	Leprosy class	BCG vaccinated persons	DNCB-score	Palpable spleen*
Group A		Leprosy patients (4)			
40	f	BL	+	8	++
35	f	TB	–	2	–
31	m	BB	–	2	+
33	m	LL	–	2	–
Group B		Family members (11)			
16	f	–	+	5	++
9	f	–	+	2	–
32	f	–	–	4	+
4	f	–	+	8	+
20	f	–	–	2	+++
45	f	–	–	2	–
40	m	–	–	5	–
70	f	–	–	2	++
8	m	–	+	2	–
17	f	–	–	3	–
22	f	–	–	4	–
Group C		Controls (7)	–		
14	f	–	+	5	–
15	m	–	+	5	++
15	f	–	+	2	+
21					
19	f	–	–	4	+++
24	m	–	+	3	–
20	m	–	–	3	+

* Spleen size measured in fingers: + = one finger below the costal arch,
2+ = two fingers great and so on.

The 4 positive reactors among leprosy patients from Western Kenya were classified as tuberculoid-borderline (1), borderline (1), lepromatous-borderline (1) and polar lepromatous (1), (Table 4). In the DNCB positive reactors we found 2 female and 2 male patients. Three of them were in a reactional state either with neuritis or erythema nodosum leprosum. There was no relation to duration of treatment or type of treatment (DDS or Lamprene). All patients receiving corticosteroids showed negative responses. Even very recent polar tuberculoid cases proved to be completely negative to DNCB, even by histological examination of skin biopsies taken from challenge sites in 4 patients.

Prior BCG vaccination did not influence DNCB reactivity.

In persons with a palpable spleen in the 3 groups investigated, no correlation was found between the spleen size and DNCB reactivity. In healthy controls in Holland all individuals under 50 years of age were responding to DNCB sensitization; older individuals were less sensitive (Table 2). All negroes born in Holland could be sensitized. These results sharply contrast with those found in healthy controls in Kenya, of whom only 26% showed reactivity to DNCB upon sensitization. These differences were found highly significant ($P < 0.001$).

Discussion

In leprosy patients of all types a low percentage of reactions to DNCB was found. The fact that only 1 out of 11 tuberculoid patients responded to DNCB challenge does not corroborate the assumption of Turk and Bryceson (1971) that cellular immune reactivity is intact in tuberculoid leprosy. The data presented tend to confirm the observation of Lim *et al.* (1972) stating that difference in immune reactivity in the various types of leprosy might be more a difference of degree than of kind.

The observation of marked impairment of immune reactivity to DNCB both in leprosy patients and also in healthy controls in Kenya contrasts with other investigations, first of all with our own normal results obtained by examining DNCB reactivity in a control group consisting of Negroes and Caucasians born in Holland. Similarly in Africa (Uganda) Ziegler *et al.* (1969, 1970) observed a high rate of DNCB reactivity in small groups of patients with malignant melanoma and Burkitt's lymphoma.

Different explanations can be given for the low percentage of skin reactors to DNCB in leprosy patients and healthy controls in Kenya. Weigand and Gaylor (1974) testing different concentrations of DNCB upon stripped and normal skin of Negroes and Caucasians in USA concluded that the stratum corneum of Negroes provided a more effective barrier to chemical substances like DNCB. This observation is at variance with the finding of Verhagen (1974) that contact dermatitis is not rarely encountered in Kenya, though the number of sophisticated allergens obviously is smaller than in industrialized countries. The fact that Hartman (1976) in Kenya found a lower percentage of contact dermatitis as compared to figures reported from Sweden (Hellgren, 1967) may largely be explained by the above mentioned relative lack of potential allergens in Africa.

Walker *et al.* (1967) in investigating genetic factors operative in contact dermatitis found normal skin reactivity to DNCB, even at low challenge dosage, in Caucasians as well as in Negroes and Asians. The well known fact of the declining rate of positive skin reactors in older groups (e.g. Waldorf *et al,* 1968) cannot explain the low percentage of DNCB reactors neither in our study with few

patients and controls in age groups beyond 50 years nor in Africa as a whole due to the relative preponderance of young age groups in tropical countries.

Impaired immune reactivity to DNCB in bacilliferous infections may be explained by massive infiltration of paracortical zones of lymph nodes by histiocytes containing ingested *M. leprae*, but in paucibacilliferous types of leprosy this explanation cannot be put forward. The common depression of cellular immune reactivity due to malnutrition and to various endemic infections of viral, bacterial or protozoal origin is well known.

Both in India (Chandra, 1972) and in Thailand (Edelman *et al.*, 1973) a significantly lower frequency of sensitization to DNCB (resp. DNFB) has been reported in malnourished children. Similarly in South Africa Smythe *et al.* (1971) demonstrated profound depletion of the thymolymphatic system and severe depression of cellular immune responses in patients with malnutrition as compared to healthy controls.

The synergistic interaction of malnutrition and infection is well recognised on the basis of clinical observations and epidemiological data (Chandra, 1972).

A recent report of a WHO scientific group (1973) sums up a number of viral and bacterial infections, many of them present in tropical countries, primarily causing non-specific loss of cell mediated immunity in man and test animals.

In the Coast province of Kenya schistosomiasis, helminthic infections, tuberculosis, leprosy and filariasis are endemic (Hartman, 1976). The area is known for the hyperendemicity of malaria (Kortman, 1972). Malnutrition in younger age groups is frequently encountered. Therefore the explanation most likely to apply to the reported low percentage of skin reactors to DNCB both in leprosy patients and in healthy controls in Kenya is to be found in environmental factors in which an interplay between malnutrition and parasitic endemic infections is operative.

Future immunological studies of leprosy patients preferably should include investigations to be carried out in an environment in which concurrent endemic disease cannot possibly interfere with the collection of data needed to elucidate the patho-immunological mechanisms involved in leprosy patients.

Acknowledgements

Permission and help was given by the Kenyan Government both in Alupe leprosy hospital, Western Province (Head: A. de Rijk M.D.B.S.) and Tumbe leprosy centre, Coast Province (Head: K. Waaldijk M.D.B.S.). Thanks are due to the Dutch Leprosy Foundation at Amsterdam, for financially supporting this investigation and to the East African Leprosy Research Centre, Alupe (Head: Dr Y. Otsyula) for assistance and transportation facilities.

Furthermore, I thank the Staff of the Department of Dermatology for technical support and helpful discussion. Finally I am most grateful for the cooperation of all volunteers both in Kenya as well as in Holland.

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Lepr. Rev. (1976) **47**, 201-209

Weekly Self-Medication of Leprosy Patients Monitored by DDS/Creatinine Ratios in Urines

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The self-administration of once-weekly doses of 300 mg Dapsone (DDS) by leprosy patients in the Mwanza region of Tanzania was monitored using the urine-test method described by Ellard *et al.* (1974*a*). DDS/creatinine ratios were determined on urine samples voided by 65 supervised leprosy patients on each of 7 successive days following the ingestion of 300 mg DDS. The method was then applied to urine samples collected by means of surprise visits to the homes of 158 out-patients 2 days after the day on which a 300 mg dose of DDS should have been taken. The extent of DDS self-administration by the out-patients was estimated by comparing the results with those obtained from controls given supervised DDS doses and from subjects not taking DDS. Significant amounts of DDS were not detected in the urine samples collected from 30% of the out-patients. Furthermore the average DDS/creatinine ratios of the urine samples of the other out-patients were significantly lower than those from the supervised controls. The implications of these findings to the treatment of leprosy in the Mwanza region and their relevance to other leprosy control schemes is discussed.

Introduction

The successful mass treatment of leprosy is largely dependent on the regularity with which out-patients take the DDS tablets they are given. Furthermore irregularity of DDS treatment is probably one of the main factors that promote the emergence of DDS-resistant strains of *Mycobacterium leprae* (Rees, 1967; Jacobson, 1973). The potential unreliability of daily self-medication of DDS has been demonstrated by Ellard and his co-workers who showed that only about a half of the prescribed DDS doses had been taken by patients in Malawi in the days immediately preceeding their attendance at the clinic (Ellard *et al.*, 1974*b*). Similar irregularity in daily self-medication was found in Ethiopia by Low and Pearson (1974). The effectiveness of costly programmes for the mass treatment of leprosy may therefore be substantially impaired if these findings are typical of the situation in the world's major endemic areas.

The quantitative determination of the ratio of DDS and its acid-labile metabolites to creatinine in the urine provides a simple and relatively sensitive method for monitoring DDS self-administration (Ellard *et al.*, 1974*a*). However,

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Received for publication 4 March, 1976.

in contrast to the patients studied in Malawi and Ethiopia, most leprosy patients in Tanzania are prescribed weekly doses of DDS for self-administration.

This paper describes an investigation of the applicability of Ellard's method to monitor the ingestion of weekly doses of DDS, and an examination of the actual regularity of dapsone self-administration in the Mwanza region of Tanzania.

Methods

COLLECTION OF URINE SAMPLES FROM CONTROLS AND OUT-PATIENTS

Controls. DDS urine samples were collected from 18 adult in-patients (10 male, 8 female) from the leprosy ward of the hospital at Sengerema (Geita district, Mwanza region) and from 49 adult leprosy patients (23 male, 26 female) living in or around the leprosy control centre at Shirati (Tarime district, Mara region). The Sengerema patients were under treatment with weekly doses of 300 mg DDS. The Shirati patients were treated with 200 mg DDS weekly prior to this study. In the last 2 weeks before urine was collected all of these patients were treated with 300 mg DDS once a week, ingested between 09.00 and 10.00 hrs under supervision. Urine samples were collected between 08.30 and 11.00 hrs on the 7 days following the day on which the second supervised dose of 300 mg was taken. The patients were asked to empty their bladders early in the morning prior to passing urine for the required samples. The next dose of DDS was given only after the last urine sample was collected. Blank urine samples were also collected from 67 staff, patients not under treatment with DDS and visitors to the Sengerema and Shirati hospitals.

Out-patients. Urine samples ("field samples") were collected from 158 adult out-patients (80 male, 78 female) living in separate houses in the Mwanza and Kwimba districts of the Mwanza region. Patients were selected who had attended their monthly clinic regularly according to the definition of the WHO Expert Committee on Leprosy (1970). The patients were also selected so as to provide a representative sample from both the small and large clinics throughout each of the 2 districts. Urine samples were collected from these patients by means of surprise visits to their homes between 10.00 and 16.00 hrs 2 days after the day on which the fourth weekly self-administered dose of 300 mg DDS should have been taken. All urine samples mentioned were collected by the authors themselves.

ANALYSES OF URINE SAMPLES.

Urine samples were collected by filling up a small container containing an amount of 2N HCl equivalent to a third of its capacity. The hydrochloric acid served both as a preservative against bacterial growth and as an agent to hydrolyse the acid-labile metabolites of DDS. Previously it had been found that storing such acidified urine samples at room temperature for 1 week did not affect the DDS/creatinine ratios significantly Ellard *et al.*, 1974*a*). During the present study however it was shown that storing urine samples at ambient temperatures for several months influenced the estimations. Comparing aliquots of 31 urine samples stored for 90 days at $-20°C$ with aliquots of the same samples stored at room temperature ($+25°C$), the latter aliquots showed an average decrease in measurable DDS/creatinine ratios of 20%. In this study urine samples were therfore estimated as soon as possible after collection (average delay: 5 days), and only in exceptional cases were stored for a few weeks at room temperature prior to analysis. DDS/creatinine ratios were determined using the method described by Ellard *et al.* (1974*a*). The results from a few samples had to be discarded because

in the determination of acid-labile DDS, protein precipitation occurred after the addition of trichloroacetic acid (about 1% of the samples) or because the presence of sulphonamides was revealed (another 1% of the samples) (Ellard *et al.*, 1974*b*).

Results

DDS/CREATININE RATIOS IN URINES FROM 65 SUPERVISED PATIENTS, COMPARED WITH BLANK VALUES FOUND IN 62 CONTROL URINES

The values found in urines from supervised patients and controls are summarized in Table 1. A distinction was made between the ratios found in urine samples from men and those found in urine samples from women. This is based on the well established fact that the daily excretion of creatinine by women is significantly less than that in men (Documenta Geigy, 1970). The results presented in Table 1 fully justify this distinction and indicate:

1. DDS/creatinine ratios in urines collected on successive days after the ingestion of 300 mg DDS fell relatively slowly. When the ratios were corrected for the mean blank values, the DDS/creatinine ratios fell exponentially with an average half-life of 38 h in both men and women.
2. Higher ratios in urine samples from female patients than in those from male patients.
3. Significant differences between the mean blank values and the mean values of DDS/creatinine ratios in urines from patients taking 300 mg DDS, even on the seventh day after ingestion of the dose.
4. Wide ranges of individual values within each group, so that as early as the third day after ingestion of 300 mg DDS one result overlapped with those obtained from the blank urines.

The ranges, mean values and 95% confidence limits of the means are illustrated in Fig. 1.

DDS/CREATININE RATIOS IN URINES FROM 158 OUT-PATIENTS COMPARED WITH BLANK AND CONTROL VALUES

On the basis of the data summarized in Table 1 urine samples from the out-patients were classified as "positive" or "negative" according to whether the

TABLE 1

DDS/creatinine ratios in urine samples from 65 supervised patients and 62 controls

Days after 300 mg DDS	Number of urine samples		DDS/creatinine ratios (μg/mg)			
			men		women	
	men	women	range	mean ± S_m	range	mean ± S_m
1	32	32	20.0-109.3	73.1 ± 3.0	61.0-138.7	90.1 ± 3.7
2	32	33	14.8- 68.8	50.1 ± 2.0	38.9-107.6	64.9 ± 3.0
3	31	33	8.4- 51.7	33.3 ± 1.8	25.5- 67.2	42.9 ± 2.0
4	32	33	6.4- 40.8	23.1 ± 1.4	15.0- 60.3	29.5 ± 1.6
5	31	33	5.7- 33.8	17.3 ± 1.2	11.5- 57.9	24.2 ± 1.8
6	32	33	3.9- 31.4	12.7 ± 1.2	10.3- 39.4	18.1 ± 1.3
7	31	33	5.3- 19.0	10.1 ± 0.8	4.8- 27.9	12.0 ± 0.9
Blanks	32	30	1.4- 9.8	5.3 ± 0.4	0.0- 12.5	6.0 ± 0.5

Indicated are ranges of values found on each day and mean values with the standard errors of the means (S_m) for every day.

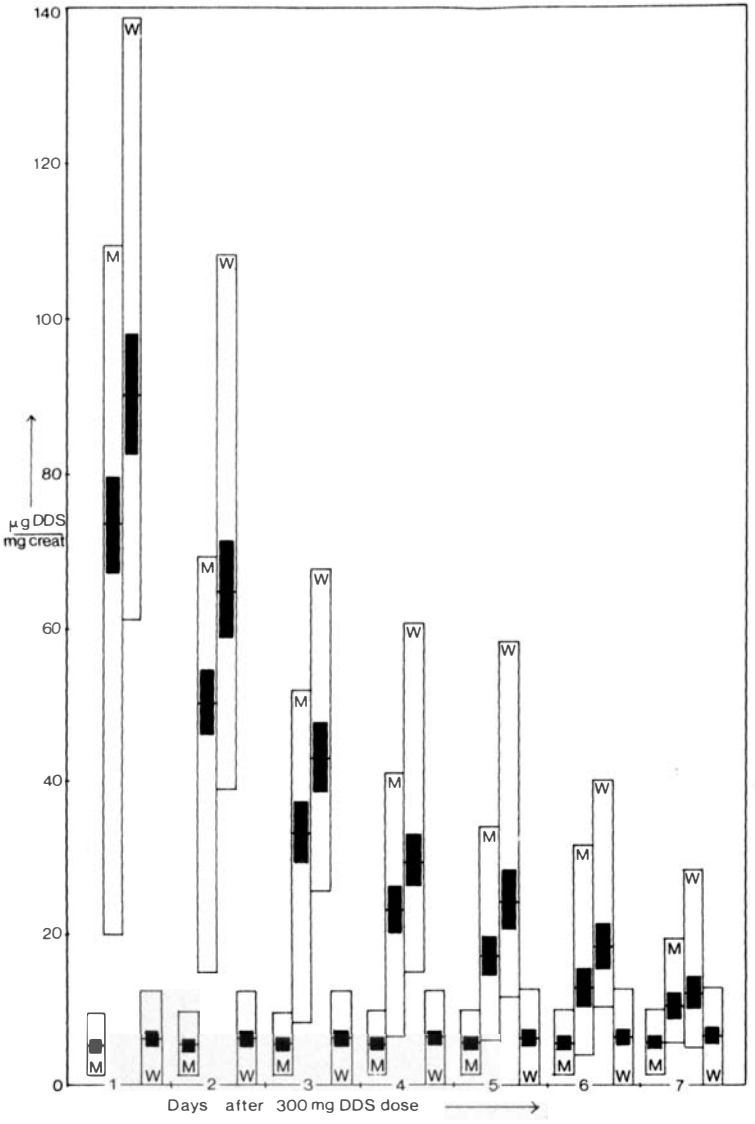

Fig. 1. DDS/creatinine ratios in urines after a 300 mg DDS dose. (□) Range of individual values, M = men. W = women. (■) Mean with its 95% confidence-area.

The ranges and means for each day repeated at the bottom of the figure concern the blank values for men and women.

TABLE 2

DDS/creatinine ratios in urine samples from 158 out-patients in comparison with blank values and second day's standard ratios

Type of urine samples	Number	%	DDS/creatinine ratios (μg/mg) Range	Mean ± S_m
From men				
Blanks	32	–	1.4- 9.8	5.3 ± 0.5
2nd day superv.	32	–	14.8- 68.8	50.1 ± 2.0
Field samples < 10 ("negative")	21	26	2.6- 9.9	6.4 ± 0.5
Field samples > 10 ("positive")	59	74	10.4- 88.3	38.8 ± 2.5
All field samples	80	100	2.6- 88.3	30.3 ± 2.4
From women				
Blanks	30		0.0- 12.5	6.0 ± 0.5
2nd day superv.	33		38.9-107.6	64.9 ± 0.3
Field samples < 12 ("negative")	27	35	0.0- 11.6	6.9 ± 0.6
Field samples > 12 ("positive")	51	65	12.5-105.8	43.4 ± 2.9
All field samples	78	100	0.0-105.8	30.8 ± 2.9

Indicated are ranges of values found in each group and mean values with the standard error of the means (S_m) for each group.

DDS/creatinine ratios (μg/mg) were greater or less than 10.0 (men) or 12.0 (women), respectively. The results obtained when samples were classified in this way are compared in Table 2 with the blank and control values. They indicate that:

1. About 30% of the urine samples were negative and among these samples the mean DDS/creatinine ratios were very similar to those of the blank urines.
2. The mean DDS/creatinine ratios of the positive urine samples were significantly lower than those of the control samples taken 2 days after dosage with 300 mg DDS ($P < 0.0005$).

The frequency distributions of the DDS/creatinine ratios are illustrated in Fig. 2.

Discussion

The organization of the field study was based on the results obtained from the supervised patients and controls. The easiest method of obtaining urine samples from the out-patients would have been to have collected them when the patients attended at the monthly clinic to collect their supply of DDS tablets for the following 4 weeks. However, because of the large overlap between the DDS/creatinine ratios determined among supervised controls 7 days after dosage with 300 mg DDS and the values for untreated subjects (Table 1, Fig. 1), for a high proportion of subjects it would have been impossible to judge with certainty from such urines whether or not the previous dose had been taken. Furthermore it was found that a number of the patients often did not come to the clinics themselves but sent a relative or neighbour to collect their DDS tablets for them. As a

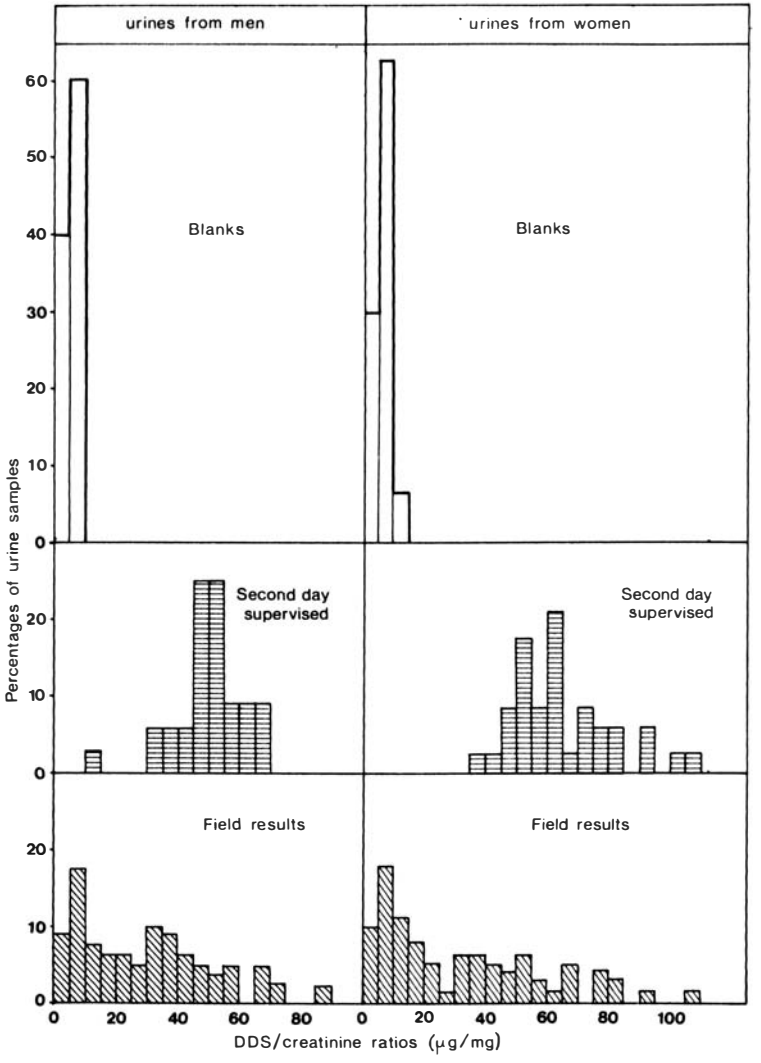

Fig. 2. Frequency distributions of DDS/creatinine ratios.

consequence the more laborious approach of collecting urine samples by means of home visits had to be followed.

End-points for classifying urine samples as positive and negative had to be chosen so that they would misclassify the least numbers of genuinely positive and negative urine samples. The end-points chosen for men and women were 10 and 12 μg DDS/mg creatinine, respectively. If such criteria had been applied to the urine samples collected for up to 4 days from the controls given supervised 300 mg doses of DDS. only 2 of the 258 samples would have been considered as

negative, and only 1 of the 62 blank urine samples would have been classified as positive.

In this study it was decided to collect urine samples 2 days after the day on which a dose of 300 mg DDS should have been taken. Thus positive results would also have been anticipated from patients who had taken only 100 or 200 mg DDS instead of the full 300 mg dose, or who had taken the correct dose a few days too early or one day late. However, it can be reasonably argued that adequate DDS blood levels were probably being achieved in most of those patients from whom urine samples were classified as being positive. Thus Ellard *et al.* (1971) have shown that daily dosage with 1 mg DDS results in the maintenance of DDS blood levels continously in excess of about 0.01 μg/ml and prevents the multiplication of drug sensitive leprosy bacilli in the body. The level of 0.01 μg/ml is considered to be the minimal inhibitory concentration. A dose of 300 mg DDS gives a mean blood level of more than 3 μg/ml (Powell *et al.*, 1967; Glazko *et al.*, 1968). In the present study the mean half-life of DDS determined among the 65 supervised patients was 38 ± 1.2 h for both men and women. This value is significantly higher than those of 21 h (Glazko *et al.*, 1968), 28 h (Peters *et al.*, 1974, 1975), 29 h (Peters *et al.*, 1972) and 31 h (Ellard *et al.*, 1974*b*) reported from determinations of the rates of fall of DDS plasma concentrations. Although the higher DDS half-life determined in the present study was based on renal excretion, DDS blood levels probably fall with the same half-life (Glazko *et al.*, 1968).

It is therefore probable that in the average patient in the Mwanza region of Tanzania DDS blood levels would take about 13 days to fall below the minimal inhibitory concentration against *M. leprae* after giving a 300 mg dose of the drug. Since the DDS/creatinine end-points chosen to discriminate between positive and negative urines were equivalent to the mean ratios in urines from supervised patients 7 days after the ingestion of 300 mg DDS (Table 1, Fig. 1), a positive result indicated that inhibitory DDS blood levels would probably be maintained for at least another 6 days, that is until the next scheduled intake of DDS. Although these calculations are based on mean values, they provide a context from which the possible therapeutic relevance of these results may be discussed.

Similar considerations raise the possibility that a significant proportion of patients providing urine samples that were classified as negative might still have been taking therapeutically significant doses of DDS. However, the similarity between the mean DDS/creatinine ratios of the field urine samples classified as negative and the blank urine samples from the controls (Table 2) indicates that the majority of the negative urine samples were correctly graded. Although no attempt was made in the present study to determine the consistency with which positive or negative urine test results would be given by individual patients, it is probable that the results obtained truly represent the overall picture of DDS self-administration among regularly attending out-patients in the Mwanza region of Tanzania.

If then one keeps the necessary restrictions in mind, it may be said that also *weekly* self-medication of leprosy patients can be monitored by DDS/creatinine ratios in urines. Thus it may be concluded from Table 2 that at any one time about 30% of the out-patients prescribed 300 mg DDS once-weekly are not taking their prescribed weekly doses of 300 mg DDS. The difference between men and women in this regard is not significant. It was also apparent that the mean values of the positive samples were significantly lower than the mean values of the ratios

for control urines taken 2 days after a 300 mg DDS dose. These data may be compared with the results of the study of Ellard *et al.* (1974*b*) in Malawi among patients on a self-medication schedule of 25 mg DDS daily. In the Malawi study 30% of the patients were found to take DDS grossly irregularly. The definitions given in the text are such that this group may be compared with the "negative" groups (30%) in the present study. If one compares our "positive" groups with the rest of the Malawian results, again the results of the 2 studies agree remarkably. The evaluation of the Malawian results in terms of the estimated percentage of doses taken by all out-patients (52-53%) may also be compared with a same evaluation of the present results in which about 50% of the doses were taken. This last figure was confirmed by determining the DDS/creatinine of pools of aliquots prepared from all the urine samples. An exact comparison of our results with those found in Ethiopia (Low and Pearson, 1974) is somewhat more difficult, due to the different way in which the Ethiopian results were presented. The trend of irregularity however is evidently the same.

Carrying out analyses on representative pools of urine samples can reduce the cost of this type of study. On the other hand it also reduces the amount of information gathered. Another way of reducing expenditure is to use the services of local medical helpers to collect the urine samples. This was tried in two other districts of the Mwanza region where 154 urine samples were collected by Health Home Visitors. However, many difficulties were encountered, especially as regards communication and although the overall results did not differ significantly from those presented in Table 2, the authors fear that too much uncertainty was associated with these collections. Nevertheless in some situations local helpers may be able to undertake a great part of the work.

Finally it should be emphasized that the actual situation regarding the regularity of DDS self-administration in the Mwanza region is probably more unfavourable than is suggested by our results. Of the estimated 13,700 leprosy patients in the region only 4312 are registered and of the latter only about 50% attend regularly for treatment. The sample in the present study was taken from this minority who attend regularly for treatment. Those patients who either do not attend at all or who do not attend regularly are obviously not receiving effective treatment.

On the other hand the proportions of these groups of patients still requiring treatment is not known. It is evident however that irregularity is one of the main factors that promote the emergence of DDS-resistant strains of *M. leprae* (Rees, 1967; Jacobson, 1973). Although there are great differences between control schemes, the similarity between the results obtained in the present study and those of studies conducted in Malawi (Ellard *et al.*, 1974*b*) and Ethiopia (Low and Pearson, 1974) suggests that these findings are probably applicable to many other similar schemes. This should be a cause for concern and reflection. Methods of improving the regularity of both clinic attendance and self-medication are clearly required. If such methods cannot be found, the objectives of many current leprosy control schemes may be incapable of realisation.

Acknowledgements

For this study financial support was received from the Netherlands Leprosy Relief Association, from the Working Party for Study Visits to Developing Countries and from the Mwanza Program of the Ministry of Health.

The assistance and cooperation of Dr V. M. Eyakuze, Dr G. R. Brubaker, of their staffs, and of the Mwanza Regional leprosy workers was sincerely appreciated. We are indebted to Dr G. A. Ellard for much valuable advice. Last but not least we want to thank the leprosy patients for their cooperation.

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Temperature Assessment and Plantar Inflammation

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The rehabilitation of the healed plantar ulcer is difficult to monitor by routine methods. Monitoring of skin temperature has been found to detect the early inflammatory response of the soft tissues to the insult imposed during walking. Methods of doing this are discussed and case histories described. The human hand, though quantitatively inaccurate, can effectively detect temperature contrasts. If routine management follows the recognition of early inflammatory responses, deformity can be prevented.

Introduction

The prevention of the plantar ulcer, a common problem in Hansen's disease, is a serious and seemingly unsurmountable task. Patient education, safety procedures and adequate footwear all have been beneficial in reducing the incidence of ulceration of the insensitive foot. Ulceration continues to be a problem regardless of the standard procedures implemented. Modification of the shoe to reduce the pressures on the plantar surface to an acceptable level may be satisfactory for 500 steps, but would the soft tissue tolerate 5000 steps at the same level? It may tolerate 5000 steps, but not 5000 steps every day. Brand (1966) demonstrated that moderate levels of stress, which are acceptable for a few repetitions, may be harmful when continued for long periods of time. As the tissue becomes injured, an inflammatory response with resultant hyperemia develops and, if the irritation continues, the tissue is damaged with subsequent ulceration. Once an opening in the skin exists, infection may occur with the often seen complications.

The hyperaemia due to the inflammatory response of tissues to repeated bouts of stress can be determined by monitoring skin temperature using one of several methods: thermographic unit, liquid crystals, radiometer, thermistor and thermocouple, and the human hand. The advantages and disadvantages of the various methods are listed in Table 1. Although the human hand is not quantitatively accurate, it can be very effective in recognizing increased heat. Health workers and patients can be taught to use their hands or temperature sensitive skin effectively by palpating for "hot areas."

At the U.S.P.H.S. Hospital, Carville, Louisiana, all methods of temperature recording are available and used. This manuscript will deal primarily with thermography as a method of temperature recording, but the information derived from this method may be utilized with the other methods as well.

Received for publication 8th April, 1976.

TABLE 1

Temperature monitoring equipment

	Advantages	Disadvantages
Thermographic units	Rapid screening Accurate (0.2°C) Non-contact	Cost – $30,000(?)
Liquid crystals	Screening capability Inexpensive	Contact necessary Messy Narrow temperature ranges
Radiometer	Rapid spot recording Accurate (0.5°C) Non-contact	Cost – $800.00(?) Poor screening capability
Thermistor or Thermocouple	Inexpensive (less $200) Accurate (0.1°C) Durable Small	Slow response (10-20 s) Contact necessary Poor screening capabilities
Hand or temperature sensitive skin	Free Screening capability Availability anywhere	Inaccurate (< 2°C)

A thermographic unit measures the thermal (infrared) emission of energy from an object and displays it in a manner similar to a television image on a video screen. The range of temperatures is represented with a continuous grey scale with warm areas of the skin showing up as shades of white and cooler areas as darker shades. A single temperature may be electronically highlighted as a bright line. The use of two such "isotherms" allows the operator to measure the absolute temperature and the difference (ΔT) between the highlighted areas. A more detailed discussion of the application of thermography is given by Gershon-Cohen and Barnes (1964).

The surface temperature of skin varies, depending on the location. Generally, skin temperatures become lower as the distance increases from the trunk with certain exceptions. Each individual has a characteristic thermal pattern on the feet and lower legs which is symmetrical if no pathology is present. Differences of greater than 1°C are considered to be significant and should be evaluated. With peripheral nerve involvement, abnormal surface temperature patterns may become evident. They show uniform patterns, however, in individuals unless pathological changes occur.

Levan *et al.* (1969) used thermography to determine the temperature of lepromatous skin lesions and found that large hypopigmented macules were 0.3 to 2°C warmer than the surrounding tissue. They used blood flow studies to confirm that the elevated temperature they found reflected an increase in blood flow. They concluded after testing the patients' local sensitivity to norepinephrine that the hyperaemia was due to autonomic degeneration rather than to an inflammatory response.

Enna and Bergtholdt (1973) reported thermography showed that nerve paralysis found in leprosy patients' hands created unique but consistent thermal patterns.

Harris and Brand (1966) found local warmth to be one of the earliest signs of tarsal disintegration that occurs in the anaesthetic foot, and they used it as a guide to identify active disintegration in anaesthetic feet. Brand *et al.* (1970) studied the temperature patterns of leprosy patients after minor injuries and trauma, and found that anaesthetic limbs often had localized warm areas at the point of stress. They felt it worthwhile to pursue this area of investigation.

After several years of experimentation and observation in thermograms taken of normal feet under stress and of leprosy patients' feet, including feet with complete anaesthesia and often gross deformity and paralysis, it has become obvious that warmth can be an important sign to monitor. Several case studies are presented to demonstrate the findings which have proved to be useful.

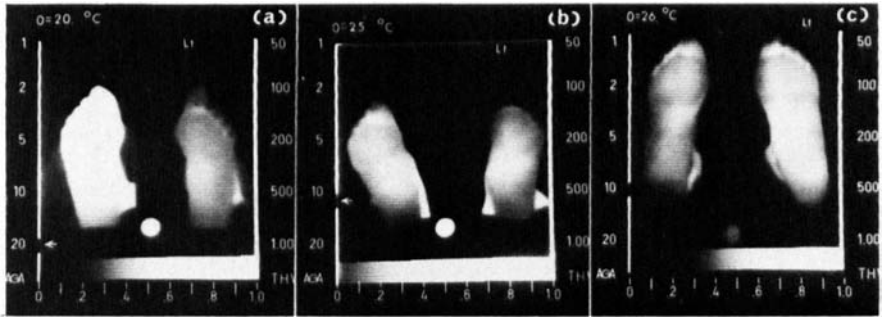

Fig. 1. Thermograms of plantar surface of insensitive feet following blistering of right matatarsal heads. (a) Thermogram 7 days following injury. (b) Thermogram after 6 days of immobilization. (c) Thermogram after 7 days of walking.

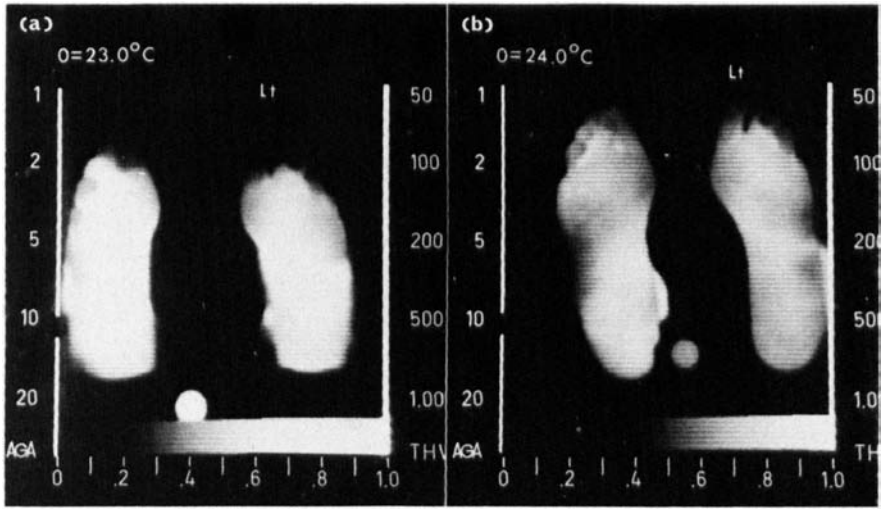

Fig. 2. Thermograms of plantar surface of insensitive feet. (a) Ten days after treatment started. (b) Forty-nine days after treatment started, after one month of routine walking.

Case Studies

METHOD

The feet were exposed in the environmentally controlled room at a temperature of 21°-22° C for 10 to 15 min to allow the normal skin temperature to equilibrate with the room air. This produced a surface temperature difference between the normal and inflamed tissue. The AGA 680 Thermovision* was used throughout the study. The thermograms were recorded on Polaroid film in black and white, and 35 mm slides were made from the color monitor.

Case Study 1. A 42-year-old patient with a diagnosis of inactive dimorphous leprosy for the last year had total anaesthesia of the feet. She had been walking a great deal in small high heel shoes and suffered blisters over the right metatarsal heads. Minimal drainage occurred, but no bacteria were found on culture. Seven days later, no improvement was noted. At this time a thermogram (Fig. 1) showed the entire plantar surface of the right foot was warm and 12° C warmer than the contralateral site. The warmest region (37° C) was over the second, third and fourth metatarsal heads. The leg was placed in a plaster total contact cast for a period of 6 days. The increased warmth present had decreased with a contralateral temperature difference of less than 1° C.

The patient's shoes were modified and instructions were given concerning the necessity of proper shoe wear. One week later the thermogram shows the involved region had a near normal thermal pattern (Fig. 1).

Case Study 2. A 54-year-old patient with inactive lepromatous leprosy and

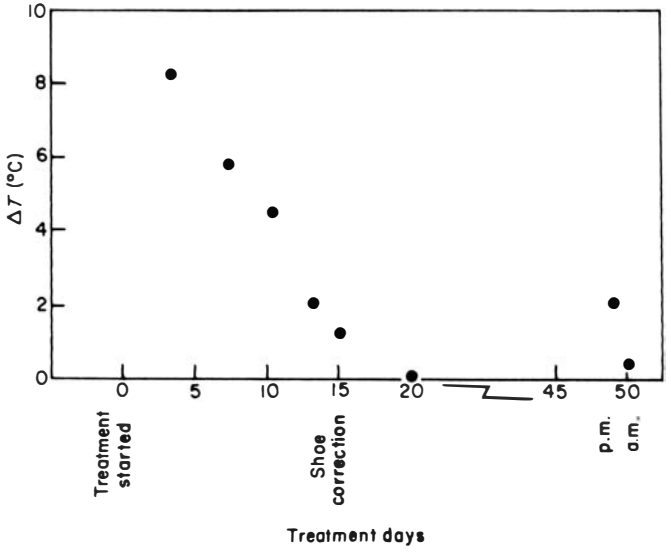

Fig. 3. Table showing the temperature difference between right third toe and left third toe (ΔT) during course of treatment.

* AGA Corporation, 500 County Avenue, Secaucus, New Jersey 07094. Company names are mentioned in this paper for the purpose of identification, and does not imply endorsement by the U.S. Public Health Service.

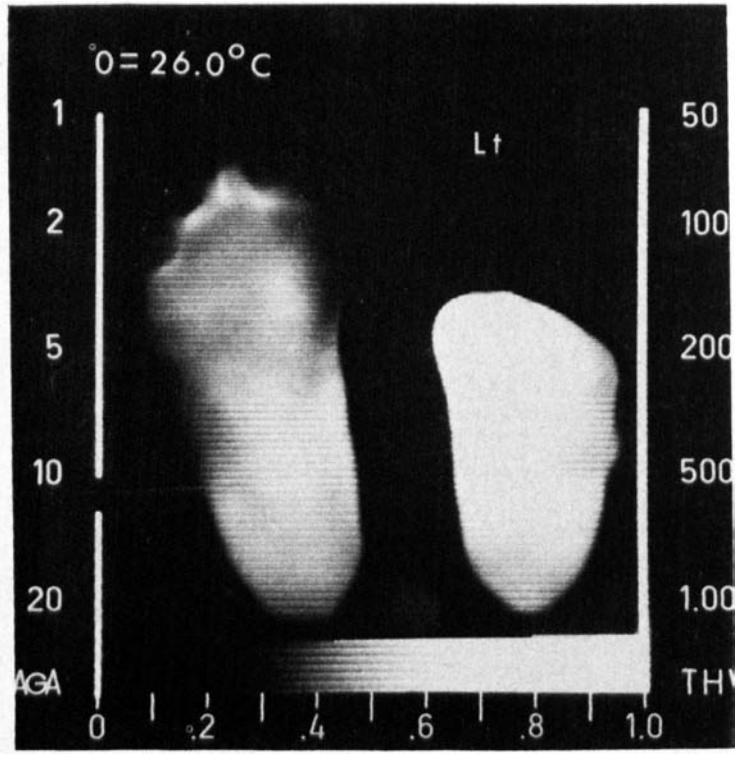

Fig. 4. Thermogram of plantar surface of insensitive feet (left transmetatarsal amputation).

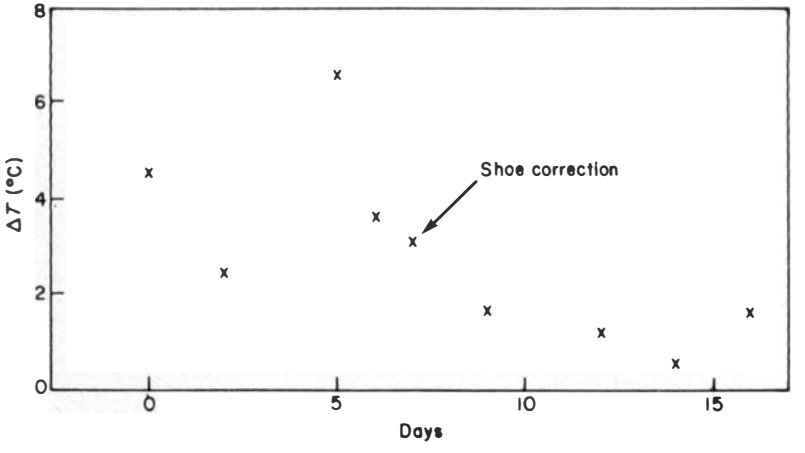

Fig. 5. Table showing the ΔT for end of left first metatarsal vs. contralateral site following observation of hematoma.

total anaesthesia of the feet noticed the right third toe tip had a callus present with a small amount of clear drainage. The treatment prescribed included soaking with an antiseptic soap, elevation and an appropriate antibiotic. Periodic thermograms were taken of the feet starting 3 days later. The involved toe was found to be highly inflamed, being 8.2°C warmer than the contralateral toe. The entire forefoot on the plantar surface was warm with the warmth present over the entire dorsal surface as well. During the course of treatment, as the inflammation was subsiding, the warmth became localized and the temperature difference between the right third toe and the contralateral site decreased (Figs 2 and 3). Fifteen days later, the toe appeared healed and its temperature was the same as the contralateral toe. The patient was sent to the shoe shop for shoe assessment and necessary shoe modifications. The patient began a progressive ambulation routine for 5 days, and was then released to return to work. One month later, a thermogram was taken of the patient's feet following a day of work (Fig. 2). Only a minimal degree of warmth was noted, which returned to a normal thermal pattern the following morning after a night's rest.

Case Study 3. A 68-year-old patient with inactive lepromatous leprosy had a transmetatarsal amputation of the left foot 10 years ago. The patient reported to

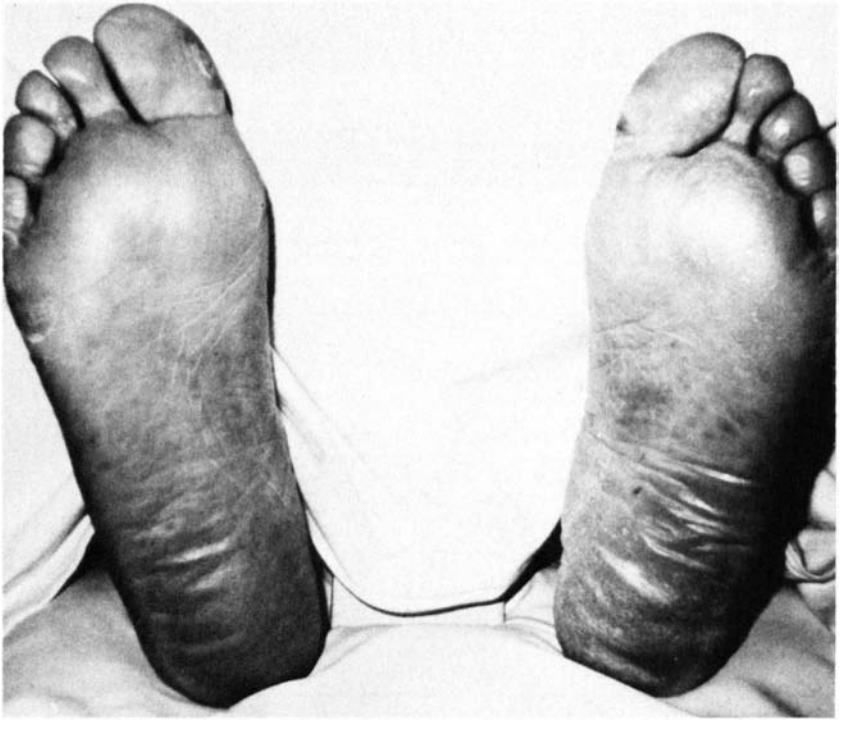

Fig. 6. Photograph of insensitive feet with callosities on right great toe, first, second, fifth metatarsal heads; and left great toe, third and fifth metatarsal heads.

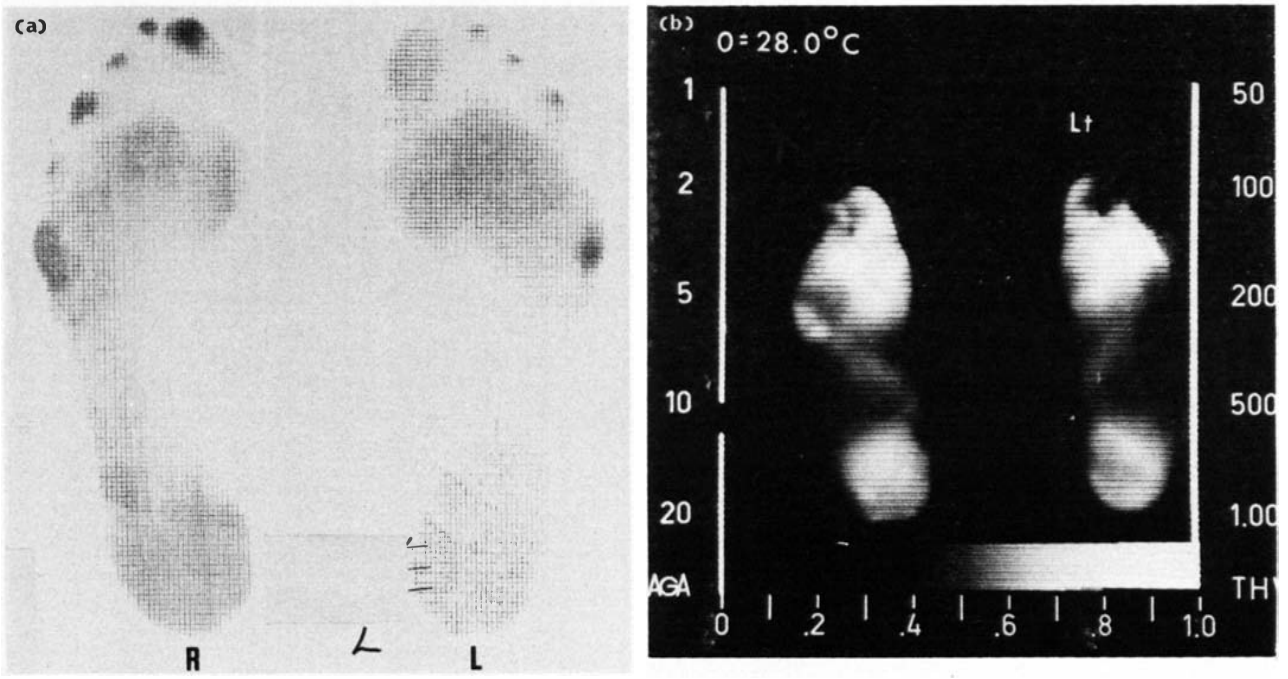

Fig. 7. Harris Footprint Mat showing areas of high pressure during barefoot walk.

Fig. 8. Thermogram of plantar surface of the feet showing "hot spots" over right great toe, first, second and fifth metatarsal heads; and left great toe, third metatarsal head.

the shoe shop with a large callus and a blood blister under the distal aspect of the left first metatarsal. A thermogram (Fig. 4) showed the entire left foot was warm, with the warmest aspect at the end of the first metatarsal, which was 4.4°C warmer than the contralateral site. Soaking, trimming of the callus, and shoe evaluation was prescribed. The condition of the injury become worse. No opening or drainage was noted however, and the ΔT increased to 6.5°C (Fig. 5) by the fifth day of observation. Further shoe pressure evaluations showed the injured area was rubbing the distal-medial edge of the moulded insole. A further shoe modification apparently stopped the excessive repetitive stress to the area, as can be seen by the improvement of the ΔT (Fig. 5), and then by the clinical improvement. By the 14th and 16th days, the foot had cooled to a near normal level, and the wound appeared to be healed.

Case Study 4. A 43-year-old patient with active lepromatous leprosy had practically complete anaesthesia over the entire body. The patient had multiple trimmed callosities over the plantar surface of the feet (Fig. 6). Pressure assessment, using the Harris Foot Mat, demonstrated the areas with heavy callus were the areas under greatest pressure during standing and walking (Fig. 7). A thermogram taken of the plantar surface of the feet showed the areas under greatest pressure were the warmest areas of the feet (Fig. 8). These hot areas suggested that the weight bearing surfaces of the feet were inflamed from walking, and if the stress due to walking increased, breakdown and ulceration would follow.

Discussion

Rehabilitation of the healed ulcer is difficult to monitor by routine methods. Low levels of repetitive walking need to be increased as the healed area toughens, but too much walking is harmful. Current methods of evaluation do not indicate the response of the foot to the toughening process. Monitoring of skin temperature has been found to be a practical method as a guide for a progressive rehabilitation programme by evaluating the tissue's response to the gradual increase in the walking programme.

The insensitive and paralytic foot is best managed before scar and deformity from ulceration reduce the stress threshold of the weight bearing surfaces of the foot. Each plantar ulcer will reduce the patient's foot tolerance to the repetitive stress imposed during walking. Prevention of the ulcer, therefore, should be paramount in the management of insensitive feet. Temperature monitoring has been found to detect the early inflammatory response of the soft tissue to the insult imposed during walking. If routine management is provided upon recognizing the early inflammatory responses, deformity can be averted.

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Lepr. Rev. (1976) **47**, 221-233

Leprosy and the Community

EVALUATION OF THE CAMPAIGN AGAINST LEPROSY IN THE WEST LAKE REGION, TANZANIA

KNUD BALSLEV

Introduction

In accordance with an agreement between the Government of Tanzania and the Swedish Norwegian Save the Children Organizations (RB), RB in 1962 undertook a leprosy eradication programme in the West Lake Region of Tanzania.

Before 1962 the Government and voluntary agencies were treating about 450 leprosy patients in different localities in the region, but no comprehensive programme was established. The 450 patients were taken over by RB and the organization established a programme encompassing all 4 districts of the region.

In January 1973 the programme was taken over by the Government, and RB sponsored one expatriate doctor who has worked in the capacity of Regional Leprosy Officer (RLO).

The RLO has undertaken an evaluation of the project, the results of which are presented in this report

Leprosy Work in Tanzania

Undertaken by the Government and a number of voluntary agencies, leprosy control work is co-ordinated through the National Leprosy Advisory and Co-ordinating Committee (NLACC). The country is divided into 19 regions, in each of these there is a Regional Leprosy Officer working under the Regional Medical Officer (RMO) if the RMO is not the RLO himself. National reports are available from NLACC since 1972.

THE WEST LAKE REGION

This is situated in the north-western corner of the country bordering Rwanda, Burundi to the west, Uganda to the north and Lake Victoria to the east.

Northern border along 1° southern latitude, southern border 3° southern latitude. Elevation 1100 to 2000 m above sea level. It covers an area of 28,750 km^2. Population 1974: 764,000 to 807,000 (estimated). Density varying between 9 and 55/km^2. People mainly Bantu. Rainfall in the different districts varies between 870 and 2280 mm per year. Mean temperature 20°-26°C. There is only one township in the region, Bukoba, with a population of 14,000. Industries: sugar, coffee, tea. Main products: bananas, maize, coffee and in

Received for publication 9 December, 1975

Biharamulo, cotton. Main income per capita per year (gross domestic product): T. Shs. 300 = US $60.

ADMINISTRATIVE STRUCTURES

The smallest unit is the 10-house cell, each having its 10-house chairman (XHC). This system, which includes each and every person in the area, greatly facilitates tracing of patients. Without this well-functioning structure the work of evaluation would have been much more difficult. The region is divided into 4 districts, two of which are divided into 2 sub-districts.

HEALTH SITUATION AND HEALTH SERVICES

The main problems include different forms of malnutrition in children, endemic malaria and foci of sleeping sickness. There are 9 hospitals (Govt and VA), 5 health centres (Govt), and about 100 dispensaries (mostly Govt). With no general practitioners, different forms of indigenous treatment are widely used.

THE CAMPAIGN AGAINST LEPROSY

The methods used have been *intensive case finding* and domicilary treatment. Two small hospitals (Kagemu, 52 beds and Katoke, 32 beds) have been maintained for the treatment of acute, contagious and complicated cases. Because of this policy the problem of rehabilitation has been small compared with many other projects. Most patients maintain themselves or are taken care of by their families. Only about 10 patients have to be taken care of by the authorities (old, blind or otherwise handicapped and without relatives).

Patients are treated in the local dispensaries or other suitable treatment centres where they are seen by a Medical Officer or other supervisor. During the years of the campaign all patients were seen by a Medical Officer each month. During later years the scheme has gradually been changed into a scheme for continued control, where patients are treated by the dispensaries and these are visited by a supervisor (Medical Officer, Medical Assistant or Rural Medical Aid) at bi- to 4-monthly intervals. Standard treatment has been dapsone 200 mg twice weekly for adults.

Case sheets have been kept for all patients treated and comprehensive yearly reports on the activities have been published. This material has been used for the evaluation, supplemented by surveys for the assessment of present status, together

TABLE 1

District	1948	1957	1962	1967	1974	Population density 1974 /km^2
Bukoba	254	308	343	383	444	55
Biharamulo	50	41	61	82	97 (140)	9 (14)
Karagwe	48	63	80	97	125	18
Ngara	105	102	98	94	98	35
The region	457	514	585	656	764	26

The figures for 1948, 1957 and 1967 are taken from the official census figures from those years. The figures for 1962 are interpolations. The figures for 1974 are the estimates made by the Central Statistical Bureau.

with estimation of the number of patients remaining undiagnosed in the region.

Statistics in the report which follows need to be read against the background of population development in the different districts. This is given in Table 1.

The figures are uncertain in the case of Biharamulo, where there has been considerable immigration in recent years. The figure in brackets (140) is a cautious estimate by the Regional Planning Team.

PLAN OF THE INVESTIGATION

In order to assess to what extent control of the spread of leprosy has been attained in the West Lake Region evidence is presented in answer to the following questions:

1. How many potentially contagious cases have been diagnosed in the region during the period in question, and what has been their fate?
2. How many potentially contagious cases are under control, and how efficient is this control?
3. How many contagious or potentially contagious cases are left undiagnosed in the region?
4. How many patients are under treatment out of the total estimated number of patients requiring treatment (diagnosed and undiagnosed), and how many are under regular treatment?
5. Incidence and prevalence rates during the years of the campaign.
6. Relapses of leprosy.
7. Proportion of cases released from control.

1. *How many potentially contagious cases have been diagnosed in the region during the period in question, and what has been their fate?*

In order to illustrate this *all case sheets in the archives* from 1962 to 1974 have been counted. Table 2 gives the results.

TABLE 2

	I	T	BT	BL	L	uncl.	Total
Died	16	380	9	10	118	21	554
Discharged cured	86	2265	35	2	30	24	2442
Disappeared	118	2211	100	39	152	14	2766
On treatment end 1974	1	430	186	132	285	–	1034
Total	221	5286	330	183	585	191	6796

Disappeared stands for: lost sight of, out of control, struck from the register for other reasons than cure or death.

In a number of case sheets classification has been given as *Borderline or Dimorphous* only. These have been listed as BT when skin smears were negative and BL when skin smears were positive.

Uncl. are those case sheets where no classification is given (old case sheets from the beginning of the campaign, mostly taken over from other agencies).

Patients known to have died after discharge or "disappearance" have been entered as died.

The potentially contagious cases are those within the frame. What is known about their fate? How many of them do still represent a danger of spread of the disease in the region?

In order to ascertain this all case sheets have been scrutinized, and where information in the case sheets has been insufficient the patients have been traced by leprosy scouts. The results are given in Table 3 below.

TABLE 3

Fate of BT, BL and L patients 1962-1974

	BT	BL	L	Total
Disappeared before 1962	1	2	36	39
Died	9	10	118	137
Discharged after 4-13 years treatment	33	—	6	39
Old, inactive cases, struck	1	—	6	7
Left district	57	30	93	180
Sought, not found	4	1	13	18
Refused treatment	19	4	9	32
Insufficient address in case sheet	3	2	3	8
Misdiagnosed or doubtful diagnosis	7	1	15	23
Returned to treatment 1975	1	1	1	3
Defaulted end 1974 not yet found	9	—	—	9
On register end 1974	186	132	285	603
Total	330	183	585	1098

Comments. Disappeared before 1962: the case sheets were taken over from other agencies in 1962 but the patients did not reappear. No attempt has been made to trace them.

Discharged: only 6 discharged lepromatous patients remain in the region, they are controlled occasionally.

Left district: most of these are known to have left the region, others have left for an unknown destination.

Sought, not found: these were unknown in the villages where they used to get their treatment. They have probably left the district.

Refused treatment: these either have refused to take medicine or they have failed to come for control after having been called, many of them several times.

Doubtful diagnosis: no signs of leprosy and no evidence in the case sheet to support the diagnosis.

There is a discrepancy between the figures for discharged patients in Tables 2 and 3. The reason is that some case sheets are marked "discharged" but should have been marked "left district", "mis-diagnosed", "refused treatment" or "insufficient information". They have been entered as such in Table 3.

In Table 4 are summarized *all BL and L cases* diagnosed since 1962 and known still to be *present in the region* plus estimated undiagnosed cases calculated as 20% of the estimated total number of undiagnosed cases–see Table 10 and page 228.

Conclusion: Only few of the cases classified as BT, BL and L might still represent a danger of spread of the disease in the region.

Most of the BL and L cases discharged, those struck off as old, inactive and those who have refused treatment are controlled occasionally. None of these have positive skin smears.

TABLE 4

	Under treatment	Discharged	Old, inactive struck	Refused treatment	Estimated undiagnosed
Bukoba	214	4	5	7	30-60
Biharamulo	150	2	1	2	85-123
Karagwe	31	—	—	2	7
Ngara	22	—	—	2	—
Total	417	6	6	13	122-190

2. *How many potentially contagious cases are under control and how efficient is this control?*

Numbers of patients concerned are recorded in the last line of Table 3 as follows

BT	BL	L	Total
186	132	285	603

Regularity of attendance is the commonly used measure for the efficiency of the control.

As regular are counted those patients who have received their medicine (dapsone) for at least 75% of the number of weeks during which they have been under treatment.

Table 5 gives the figures for 1974 for the different districts and sub-districts; per cent regular out of the total number of cases in each group.

TABLE 5

	BT cases	BL cases	L cases	All cases
Bukoba north	48% of 29	71% of 24	65% of 48	61% of 165
Bukoba south	71% of 41	65% of 37	75% of 105	69% of 267
Biharamulo	71% of 86	86% of 52	80% of 98	75% of 429
Karagwe	36% of 11	82% of 11	75% of 20	66% of 102
Ngara Bushubi	79% of 14	86% of 7	75% of 12	72% of 56
Ngara Bugufi	0% of 5	100% of 1	0% of 2	13% of 15
The region	67% of 186	77% of 132	75% of 285	69% of 1034

BT + BL + L: 440 = 73% regular out of 603.

The figures are fairly satisfactory.

It has not been possible to check to what extent the patients actually take their medicine as prescribed. This would require elaborate laboratory examinations (sulphonuria test) for which we do not have the facilities.

The ratio of BL and L cases having negative skin smears to the total number of BL and L cases is another measure of the efficiency of control.

Table 6 shows the distribution of cases by the end of 1974.

TABLE 6

	No recent smear	Smears negative	Smears positive	Total
All BL and L cases	20	270 = 68%	127 = 32%	417

Under regular treatment with dapsone it takes months or years before the positive skin smears become negative, some never do become negative.

Table 7 shows the ratio of positive skin smears to negative skin smears related to length of treatment in years.

TABLE 7

Length of treatment in years	0-2	3-4	5-6	7-8	9-10	11-12	12+	Total
Smear negative	15	28	18	26	39	48	96	270
Smear positive	33	13	14	19	13	13	22	127
Total cases	48	41	32	45	52	61	118	397
Cases with negative smears	31%	69%	56%	58%	75%	79%	81%	
Cases with positive smears	69%	31%	44%	42%	25%	21%	19%	

The figures are as good as can be expected under dapsone treatment. It is possible that they could be improved by the use of alternative drugs (Lamprene) if the persistence of positivity of skin smears is due to resistance to dapsone.

Do the patients come for treatment in the early stages of the disease? When leprosy patients start treatment sufficiently early in the course of their disease, the risk of spread of the infection is lessened and the risk of mutilating disabilities is greatly reduced.

In recent years it has not been possible to undertake active case finding campaigns in the West Lake Region, most patients report themselves or are referred from dispensaries or hospitals.

Table 8 shows the *duration of disease in years before reporting for treatment* for all new patients diagnosed in 1974.

TABLE 8

Years	0-½	½-1	1-2	2-3	3-5	5+	?	Total
No. patients	36	38	23	16	4	13	–	130

Table 9 shows the *disabilities in all new patients* diagnosed in 1974:

TABLE 9

Disability grade (WHO)	I	T	BT	BL	L	Total
0	–	53	17	12	3	85
1	–	24	9	3	3	39
2	–	2	3	–	–	5
3	–	–	1	–	–	1
No. patients	–	79	30	15	6	130

The figures are fairly satisfactory and show that the majority of patients come early for treatment.

The figures could be improved by continued health education of the public and further training of medical personnel.

Conclusion. The control of potentially contagious patients under treatment is satisfactory. A number of new patients still come too late to receive the full benefits of treatment.

3. *How many cases of leprosy are left undiagnosed in the region?*

This has been studied by examination of random samples of the population. As a check on these figures examination of school children has been undertaken.

Random sample surveys have been done in Bukoba, Biharamulo and Ngara districts. For Karagwe district Due Madsens figures from 1970 have been used.

Overall results are given in Table 10.

TABLE 10

	Population 1974	New cases found	Size of sample	Estimated no. undiagnosed	Estimated undiagnosed per 10,000
Southern Bukoba, 4 wards	57,500	5	2724	104	18.0
Northern Bukoba, 36 wards	386,000	1	7886	(48)	(1.2)
Bukoba district, total	443,500	6	10,610	293	6.5
Biharamulo	97,300	18	4086	428	44.0
Same, other estimate	140,000	18	4086	616	44.0
Karagwe, 1970	101,000	22	41,042	33	3.3
Same, 1974	124,800			33	2.6
Ngara	98,200	—	3037	—	
				613-914	

Figures for Northern Bukoba are too small for calculation and are therefore put in brackets.
Figures for Karagwe 1974: if there is the same number undiagnosed cases as there was 1970.

Size of samples:

Bukoba	10,610 = 2.4% of the population
Biharamulo	4086 = 4.2% (2.9%)
Ngara	3037 = 3.1%
Karagwe, 1970	41,042 = 41.0%

School surveys. Overall results of sample school surveys are given in Table 11.

TABLE 11

	No. of schools in area	No. of schools surveyed	No. of pupils examined	No. of new cases discovered
Southern Bukoba	13	13	2501	0
Northern Bukoba	175	29	5228	0
Biharamulo	37	35	6831	7
Karagwe	56	14	2565	0
Ngara	57	42	7788	0

The figures confirm the impression obtained from the sample surveys that control has not been attained in Biharamulo.

4. *How many patients are under treatment out of the total estimated number of patients requiring treatment (diagnosed and undiagnosed), and how many of these are under regular treatment?*

The estimated number of undiagnosed cases (all types) is given in Table 10.

Estimation of number of undiagnosed L cases: total number of new cases 1965-1974: 3124 (Table 14). Of these L cases 210 = 6.7% of 3124. It would therefore appear reasonable to estimate the number of undiagnosed L cases at 7% of the total number of undiagnosed cases.

Estimation of number of undiagnosed BL + L cases: The BL + L rate for 1973-1974 is estimated from Table 14 at 20% of all new cases. It would therefore appear reasonable to estimate the number of undiagnosed BL + L cases at 20% of the total number of undiagnosed cases.

Figures are given in Tables 12 and 13 for:

1. Estimated total number of cases (all cases),
2. Estimated number of L cases,
3. Estimated number of BL + L cases.

(For Biharamulo estimates are based on a population figure of 97,300.)

Conclusion. The goal of regular treatment of 75% of all existing cases requiring treatment has not yet been reached. It has almost been achieved for the BL and L cases on the register (see Table 5).

Active case finding is required in all 4 districts.

Patients under treatment out of estimated total number:

TABLE 12

All cases	No. under treatment	Estimated no. undiagnosed	Estimated total no.	% under treatment
Bukoba	432	293	725	60
Biharamulo	429	427	856	50
Karagwe	102	33	135	76
Ngara	71	–	71	100
The region	1034	753	1787	58
L cases				
Bukoba	153	20	173	88
Biharamulo	98	30	128	77
Karagwe	20	2	22	91
Ngara	14	–	14	100
The region	285	52	337	85
BL + L cases				
Bukoba	214	58	272	79
Biharamulo	150	85	235	64
Karagwe	31	7	38	82
Ngara	22	–	22	100
The region	417	150	567	74

Patients under *regular* treatment out of estimated total number:

TABLE 13

All cases	No. under regular treatment	Estimated total no.	% under regular treatment
Bukoba	285	725	39
Biharamulo	323	856	38
Karagwe	67	135	50
Ngara	42	71	60
The region	717	1787	40
L cases			
Bukoba	110	173	64
Biharamulo	79	128	62
Karagwe	15	22	68
Ngara	9	14	64
The region	213	337	63
BL + L cases			
Bukoba	151	272	55
Biharamulo	124	235	53
Karagwe	24	38	63
Ngara	16	22	73
The region	315	567	56

5. *Incidence and prevalence rates during the years of the campaign.*

Annual incidence rates. There is no practical way of determining this.

The annual rate of newly registered cases gives an approximation. One difficulty is that the international nomenclature has changed during the period under review with different definitions of Indeterminate, Borderline, Dimorphous

TABLE 14

Year	I	T	B	L	Total	% L
1962	311	1734	–	245	2290	10.7
1963	58	530	–	42	630	6.7
1964	2	701	–	17	720	2.3
		T	I + B	L		
1965		501	7	34	542	6.2
1966		262	33	27	322	8.4
1967		374	57	30	461	6.5
	I	T	B	L		
1968	8	312	7	24	351	6.8
1969	4	292	28	13	337	3.8
1970	7	248	55	21	331	6.3
1971	3	167	51	21	242	8.7
1972	9	101	69	11	190	5.8
	I	T	BT BL	L		
1973		102	51 23	14	190	7.4
1974	–	81	44 18	15	158	9.5
Total	402	5405	443	514	6764	7.6

and Intermediate cases. Some cases earlier diagnosed as Tuberculoid now would be called Borderline Tuberculoid.

Lepromatous has been the most uniformly defined type during the period.

Table 14 shows the annual numbers of newly registered cases–quoted from annual reports–and the lepromatous rate in %.

In order to get comparable figures the numbers for 1973 and 1974 include patients transferred from other regions or countries as such patients have been included in the figures for earlier years.

The BL + L rate for 1973 + 1974 is 70 = 20% out of 348. The rate for former years cannot be calculated.

Annual rates of newly diagnosed cases in selected groups. Figures for school children (from annual reports):

TABLE 15

Year	No. examined	No. new cases	New cases per 1000
1962	3732	5	1.3
1963		No surveys	
1964	8604	72	8.3
1965	5557	13	2.3
1966	3330	10	3.0
1967	32,525	12	0.4
1968	10,700	12	1.1
1969	21,692	10	0.5
1970	19,434	12	0.6
1971	5941	19	3.2
1972		No surveys	
1973	6831	7	1.0
1974	8474	0	0.0
1975	9608	0	0.0

1975: figures for surveys done January to April 1975.

Up to 1970 no distinction was made between the districts. All of the 26 new cases diagnosed between 1971 and 1975 were found in Biharamulo district.

Prevalence rates. Unfortunately no survey was undertaken immediately before the project was started in 1962. For political reasons it was found not feasible at that time.

Prevalence in 1951. In that year Ross Innes did a survey of 5 localities in Northern Bukoba (Kyaka, Kabale, Maruku, Kalema, Kamachumu) partly in the same places as our sample surveys, and one locality in Southern Bukoba (Rubungu in Muleba Ward).

His findings were:

Northern Bukoba	4617 persons examined,
	40 cases found = 8.7 per 1000
Southern Bukoba	1181 persons examined,
	23 cases found = 19.4 per 1000
Average for the district	5798 persons examined
	63 cases found = 10.8 per 1000

Prevalence in 1967. In 1967 a general census was made giving population figures for each district.

The total number of patients diagnosed during the 5 years period before 1967 and the 5 years period after 1967 are known from the yearly reports:

TABLE 16

	Population 1967	New patients diagnosed 1962-72	New patients per 1000 population
Bukoba	382,708	3510	9
Biharamulo	81,854	1768	22
Karagwe	97,221	414	4
Ngara	94,312	724	8
The region	658,095	6416	10

The figures for new patients diagnosed include approximately 450 patients taken over from other agencies in 1962. It is not known how many from each district.

The figures in the last column give a good approximation to the prevalence rates for leprosy in 1967.

Prevalence rates 1974. The number of patients on treatment is known from the yearly report for 1974. The number of undiagnosed cases is estimated in Table 10.

TABLE 17

	Population	On treatment	Undiagnosed	Total patients	Prevalence per 1000
Southern Bukoba 4 wards	57,500	92	104	196	3.4
Northern Bakoba 36 wards	386,000	340	(48)	(388)	(1.0)
Bukoba district	443,500	432	293	725	1.6
Biharamulo	97,300	429	428	857	8.8
Same, other estimate	140,000	429	616	1045	7.5
Karagwe 1970	101,000	196	33	229	2.3
Same, 1974	124,800	102	33	135	1.1
Ngara	98,200	71	–	71	0.7

Northern Bukoba. The figures are too small for calculation—only one patient was found among 7886 persons examined.

If the figures for the whole of Bukoba district (including the four wards in Southern Bukoba) are used, the figure for prevalence becomes 1.6.

The prevalence for Northern Bukoba therefore is calculated as 1.0-1.6.

Biharamulo. There are different estimates for the size of the population.

Karagwe. There are no figures for estimated numbers of undiagnosed cases 1974. If the same figure as that for 1970 is used, the prevalence in 1974 becomes 1.1.

Ngara. In order properly to estimate the number of undiagnosed cases a much larger number of people would have to be examined.

TABLE 18

Summary of prevalence rates (per 1000)

	1951	1967	1970	1974
Southern Bukoba, 4 wards	19.4	} 9		3.4
Northern Bukoba, 36 wards	8.7			1.0-1.6
Biharamulo		22		7.5-8.8
Karagwe		4	2.3	1.1
Ngara		8		0.7

It is noted that the figures for different years are calculated in different ways.

6. *Relapses of leprosy*

Table 19 shows the number of relapses in 1973 and 1974 analysed by classification. For comparison is given the total number of patients under treatment at the end of each year and the number of BL + L cases out of these.

TABLE 19

	I	T	BT	BL	L	Relapses total	All cases	BL + L cases
1973								
Bukoba	–	–	1	5	5	11	483	220
Biharamulo	–	–	–	2	5	7	433	130
Karagwe	–	–	1	1	–	2	106	30
Ngara	–	–	–	–	1	1	109	28
The region	–	–	2	8	11	21	1131	408
1974								
Bukoba	–	2	3	4	11	20	432	214
Biharamulo	–	–	5	4	11	20	429	150
Karagwe	–	–	–	1	1	2	102	31
Ngara	–	–	–	1	–	1	71	22
The region	–	2	8	10	23	43	1034	417

Patients counted as relapses are those who, after a period of apparent cure, again get signs of active disease–new patches or nodules, negative skin smears again becoming positive.

For BL and L cases the number of relapses correspond to 4.6 and 7.9% respectively of the number of cases of these types under treatment.

7. *Proportion of cases released from control*

Related to (a) patients under treatment, and (b) patients under treatment and out of control cases.

Table 20 gives the figures for 1974 for the two categories.

TABLE 20

		I	T	BT	BL	L	Total
(a)	No. of patients	1	433	186	132	285	1034
	Patients released	1	129	10	0	0	140
	% Released	100	30	5	0	0	13
(b)	No. of patients	1	488	207	137	289	1122
	Patients released	1	129	10	0	0	140
	% Released	100	26	5	0	0	12

SUMMARY OF RESULTS

Of all contagious and potentially contagious (BL and L) cases diagnosed since 1962 very few of those remaining in the region are out of control.

The control of those BL and L cases who are on the register is satisfactory.

There are few undiagnosed BL and L cases left in Ngara, Karagwe and Bukoba districts with the exception of the southern-most part of Bukoba. In Biharamulo the number of undiagnosed BL and L cases is high.

Southern Bukoba (Ngote, Muleba, Mubunda, Rushwa wards). Population about 60,000 = 14% of the population in Bukoba district. BL and L cases under treatment: 40. Estimated number of BL and L cases left undiagnosed: 20.

Biharamulo district. Population 97,300-140,000. BL and L cases under treatment: 150. Estimated number of BL and L cases left undiagnosed: 100 (86-123).

The disease is under control in the region with the exception of the two areas mentioned.

Both areas are characterized by a higher prevalence than the rest of the region. The high prevalence is (at least partly) due to immigration from more heavily infected areas to the south of them. The campaign has not hitherto been correspondingly intensified in these regions.

MEASURES WHICH SHOULD BE TAKEN

1. *Control measures* should be maintained in all 4 districts at least at the present level; if possible, improved. All patients failing to report for treatment should be traced immediately by dispensary personnel or—if they fail—by Health Home Visitors.
2. *Sample surveys* for further clarification of the situation in Bukoba district in:
   (a) Lubale, Kabilizi ward which is situated between Southern Bukoba and Northern Bukoba as defined in this report.
   (b) Kishanje ward, Bugabo and Kyaka ward, Missenye—in both Ross Innes did surveys in 1951.
3. *Case finding campaigns* in all districts starting in Southern Bukoba and Biharamulo. This should be done by:
   (a) contact survey of all known cases of leprosy.

(b) tracing through co-operation with Ward Executive Officers and Ten-house Chairmen.

4. *Cases with persistently positive skin smears* should be treated with alternative drugs or a combination of drugs.
5. *Records* should be kept in order to continue yearly operational assessment.
6. Each year definite *Targets* should be set for the activities.

Lepr. Rev. (1976) 47, 235-244

Field Workers' Forum

IMMUNOLOGY OF LEPROSY

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This article is taken from the second Edition of "Leprosy for Students of Medicine" by A. D. M. Bryceson and R. E. Pfaltzgraff to be published by Churchill Livingstone in 1977, who have kindly consented to its publication in Leprosy Review.

Introduction

The response of the body to invading micro-organisms involves both non-specific and specific mechanisms of defence. Non-specific mechanisms include the barriers of the skin and mucosae, antisepsis of sebum and mucosal secretions and simple phagocytosis by polymorphonuclear leucocytes and macrophages. Specific mechanisms are those elaborated by the immune response. They contribute to healing and immunity, hypersensitivity and pathology.

Basic Immunology Relevant to Leprosy

INDUCTION OF THE IMMUNE RESPONSE

A micro-organism consists of and produces many different chemical substances which are antigenic. These antigens reach the lymphoid organs (lymph nodes or spleen) where each is potentially capable of inducing an immune response. The type of response induced is determined by a number of factors.

Types of response

No response. This is the rule to 'self' substances, as in autografting of skin for burns, or rarely to foreign antigens.

Immune response. This is the commonest response to most microorganisms and has two components, cellular and humoral. Cell-mediated immunity is mediated by lymphocytes that are processed in foetal life by the *thymus,* and are often referred to as T-lymphocytes. Humoral immunity is mediated by B-lymphocytes and their progeny, plasma cells. In birds B-lymphocytes are processed by the *bursa* of Fabricius, an organ associated with the hind gut and having no anatomical equivalent in mammals.

Tolerance. The antigen kills the receptor cell in the lymphoid tissue or paralyses it in such a way that it fails to initiate an immune response to this and to any subsequent challenge with the same antigen. Both the humoral and cellular

responses are paralysed and the host 'tolerates' the foreign antigen. Occasionally only T-lymphocytes are paralysed.

Immune deviation. Antibody 'deviates' the antigen away from T-lymphocytes (see below) in such a way that cell-mediated immunity is not induced. The humoral response is normal. The situation in lepromatous leprosy (LL) resembles this, in that the individual seems able to produce antibodies but no cell-mediated response to *M. leprae*.

Factors affecting the type of response

Antigen determined

Chemical nature. Some antigens especially polysaccharides and simple chemicals (haptens) with a poor affinity to host proteins are only weakly antigenic.

Molecular size. Substances, including some proteins, of low molecular weight (below about 10,000) are often poorly antigenic, and are liable, under certain circumstances, to induce tolerance. Polymers of these substances and molecules of high molecular weight are more immunogenic.

Concentration. A very high concentration of antigen may induce tolerance rather than immunity. In an already immunized host such a concentration may suppress the expression of cellular immunity (desensitization), producing a situation similar to immune deviation. This mechanism may underlie the process of downgrading in borderline leprosy. A very low concentration of antigen may also be tolergenic.

Solubility. A highly soluble antigen is more likely to achieve a high circulating concentration and be less immunogenic than a poorly soluble antigen.

Route of presentation to lymphoid tissue. Antigens may reach the lymphoid tissue through the blood stream or along the lymphatics. They may be free or taken up by macrophages. Poorly soluble antigens, inoculated intradermally, phagocytosed by macrophages and taken by the lymphatics to the regional lymph node are highly immunogenic, especially with regard to cellular immunity. This is usually the case with *M. leprae.* Highly soluble antigens circulating free in the blood stream are much less efficient in inducing immunity, especially cellular immunity, and may be liable to induce tolerance. In lepromatous leprosy soluble antigens of *M. leprae* are found in the circulation. Their chemical nature and size and effect on the immune response are unknown.

Adjuvants. Certain substances, notably Wax D, a component of walls of *Mycobacteria,* have the property of augmenting both the humoral and cellular immune response to a wide range of antigens inoculated in emulsion with the adjuvants. Cell walls of *M. leprae* probably act as efficient adjuvants.

Host determined

Genetic. Congenital immunological deficiencies of either the cellular or humoral components of the immune response are rare and usually lead to an early death from infection. Genetically determined inability to respond normally to one particular antigen may be a factor in determining the clinical picture and outcome of a particular infection in that individual. Such a mechanism could contribute to the pathogenesis of lepromatous leprosy. Males are most susceptible to some infections than are females. This susceptibility may be a factor influencing the sex pattern of leprosy.

Integrity of lymphoid tissue. For the induction of a normal response the draining lymphoid organ must be intact and not grossly damaged by fibrosis or distorted by infiltration with abnormal substances such as amyloid, abnormal cells as in Hodgkin's disease, or normal cells as with the excessive ingress of macrophages from a large persisting depot of antigen in advanced lepromatous leprosy. If lymphoid tissue is preoccupied by the presence of one antigen it may respond poorly to the presence of another, a condition known as an antigenic competition. These mechanisms could contribute to the partial suppression of cellular immunity to unrelated antigens which is found in some cases of lepromatous leprosy.

Acquired immunosuppression. Some diseases may impair the induction of an immune response to certain antigens (malaria to tetanus toxoid, malnutrition to contact sensitizing agents); others may impair the expression of an immune response (measles to tuberculin, leukaemia to a variety of infections). Some drugs, the commonest of which are the corticosteroids, are immunosuppressive. This knowledge is made use of in the treatment of reactions. The partial, generalised immunosuppression acquired in lepromatous leprosy may render some patients more susceptible to intercurrent infection.

Enhancing antibodies. In situations where cellular immunity provides the effector arm of the immune response, circulating antibody may coat antigen molecules on the surface of cells without damaging that cell, and in this way block the recognition of that antigen by cytotoxic lymphocytes. This phenomenon, known as enhancement, contributes to the establishment and growth of certain tumours.

T-lymphocyte modulation of the immune response. T-lymphocytes help regulate the immune response in several ways. Two important ways are:

1. B-lymphocytes require the cooperation of specifically sensitized T-lymphocytes for the production of antibody to a wide range of antigens (so called thymus dependant antigens).
2. Some T-lymphocytes, generated during the induction of the immune response, regulate the production of effector T-lymphocytes. This is probably a normal physiological feed-back process. On occasion these regulatory cells, which are sometimes called suppressor T-cells, totally inhibit the production of effector cells and so create a situation which resembles immune deviation or selective T-lymphocyte tolerance.

Enhancing antibodies and suppressor T-cells have been little studied in infectious diseases. Their presence, if that could be demonstrated, might provide mechanisms whereby specific cell-mediated immunity was suppressed in lepromatous leprosy.

Changes in lymphoid tissue

During the course of an immune response certain changes take place in the lymph node which receives the antigen, or the spleen in the case of circulating antigens. Antigen from an intradermal site is presented by macrophages which enter through the marginal sinus and pass between the germinal centres into the paracortex. After this the parts of the node which develop depend upon whether humoral or cellular immunity is induced.

Humoral immunity. The cortex expands and germinal centres increase greatly in number and size, often bulging into and distorting the rest of the node. Plasma

cells appear in the medullary cords which become thickened. In a pure antibody response the paracortex becomes thinned. Histological staining with pyronin, which stains ribonucleic acid red, shows the intense activity of germinal centres and medullary cords. These changes are seen in lepromatous leprosy.

Cellular immunity. The paracortical area of the node, whose development is dependent upon the integrity of the thymus in foetal life, expands. It becomes populated by small lymphocytes, which enter through the postcapillary venules, and start to divide. These large pale dividing cells are also pyroninophylic on staining. Small non-pyroninophylic lymphocytes are produced and leave the node through the intermediary sinuses, which become greatly distended. In a pure cellular response the cortex and medulla are thinned. These changes are seen in tuberculoid leprosy (TT).

Most micro-organisms contain antigens which between them elicit both types of response.

Cellular events leading to antibody production and cell-mediated immunity

These events are complex and still imperfectly understood. A simplified concept involves the following stages:

1. Antigen brought to lymphoid tissue sensitizes T- and B-lymphocytes of the specific clone of cells that have the appropriate receptor site on their surface. The sensitized cells divide.
2. T-lymphocytes, on division, give rise to:
   (a) a population of effector cells (cytotoxic or "killer" lymphocytes which are able directly to attack cells coated with the specific antigen. The cells leave the lymphoid tissue *via* the intermediary sinuses, enter the blood stream and recirculate continuously until they reach their "target", where further division may take place.
   (b) a population of memory cells which take part in the secondary immune response.
   (c) helper and suppressor cells which, respectively modulate B- and T-lymphocyte function. It is possible that helper and suppressor effects are simply different physiological functions of effector cells, and do not represent distinct cell populations.
   (d) the secretion of a number of soluble factors (lymphokines) which increase the digestive power of macrophages and mediate the phenomena of delayed hypersensitivity.
3. B-lymphocytes divide several times and give rise to plasma cells which secrete specific antibody. Most plasma cells remain in the lymphoid organs. B-memory cells are produced.

EXPRESSION OF IMMUNE RESPONSE

Humoral immunity (antibody mediated)

There are several ways by which antibody can help the body to get rid of micro-organisms. At the same time 'unwanted' responses to many of the antigens induce a state of hypersensitivity which contributes to the pathology of the disease.

The most important of these mechanisms are:

1. Antibody combines with and neutralizes toxins, as in diphtheria or typhoid, but probably not in leprosy.

2. Circulating antibody (opsonizing or complement fixing) reacts with antigen on the wall of the organism. In the first case rapid and efficient phagocytosis ensues. In the second complement is fixed and the organism is lysed. Neither of these mechanisms seems to be important in defence against leprosy.

3. Antibody passively coats host cells and is available to react with antigen. This antibody is one of two kinds. It may be cytophilic for macrophages, in which case that cell is better enabled to take up micro-organisms; this mechanism may be important in cell medicated immunity to certain facultative intracellular organisms, but it does not increase the digestive power of that macrophage, and may not be relevant in leprosy. Alternatively the antibody is reaginic of the class IgE and is fixed to mast cells. Circulating antigen reacts with the fixed antibody giving rise to the phenomena of local or systemic anaphylaxis. This does not seem to happen in leprosy.

4. Precipitating antibody combines with antigen, which is present in moderate excess, and forms complexes to which complement is fixed. This may take place either in the circulation or in the tissues. Complexes formed in the circulation are deposited, depending on their size, in the endothelial spaces of vessels, notably those in the glomerulus, the skin and synovial membranes. A depot of antigen, in the tissues, such as a lepromatous nodule, can be the origin of a gradient of antigen concentration. Antibody diffuses from the circulation and at the appropriate relative concentrations complexes are formed and deposited. Complement is fixed and the release of its third component attracts polymorphonuclear leucocytes which accumulate, phagocytose the complexes and release enzymes of which the most potent are proteases and which cause tissue damage. Activation of vascular endothelium further encourages adhesion of leucocytes and platlets, thrombosis and haemorrhage. This process is important in the genesis of type 2 reactions in leprosy (erythema nodosum leprosum).

Cell-mediated immunity (lymphocyte and macrophage mediated)

Antibodies do not normally penetrate host cells. Certain organisms have become adapted to intracellular life, and those which are adapted to intramacrophage life are especially well protected. These organisms include *Leishmania, M. leprae, M. tuberculosis,* certain fungi and, to a lesser extent, the more facultative intracellular organisms, *Salmonella* and *Brucella.* Cellular immunity is of especial importance in dealing with infections caused by these organisms. It is also of importance in some viral infections, in graft rejection and in tumour immunity.

There are two main ways in which cells may produce and maintain a state of immunity. They are macrophage activation and lymphocyte cytotoxicity. There are also several mechanisms involved in the production of a state of delayed hypersensitivity which contribute to pathology.

Specifically sensitized T-lymphocytes, produced in the paracortical areas of lymphoid tissues, enter the circulation and 'home' onto the site or sites containing antigen. In the case of leprosy this is predominantly the infected macrophages of the skin and nerves. The phenomena of cell-mediated immunity and delayed hypersensitivity follow. These are:

1. Focalization and division of macrophages.

2. Accumulation of lymphocytes around the macrophages.

3. Alteration of macrophages into epithelioid cells, often with the formation of giant cells. Steps 1, 2 and 3 produce a tubercle, the characteristic histological picture of cell-mediated immunity.

4. Increased enzymatic activity of macrophages and increased ability to digest organisms. This increased activity is not specific to the organism inducing the immune response.

5. Central necrosis, or ulceration of overlying skin.

These phenomena are probably mediated by lymphokines released by lymphocytes as they divide during contact with antigen, which is usually on the surface of macrophages. Lymphokines are not immunoglobulins and have a very short range of action. Their action is therefore only local, in contrast to that of circulating antibody. Lymphokines are assayed by their biological activities.

Ths most important of the lymphokine factors are:

skin reactive factor: this increases capillary permeability and may facilitate the ingress of cells into the lesion,

chemotactic factor: this attracts macrophages,

macrophage migration inhibition factor: this inhibits the natural migratory properties of macrophages and may play a part in the localization of infection. It also increases the digestive powers of macrophages, a property which is probably important in leprosy.

Cytotoxic factors (lymphotoxins): there are several of these, antigen specific and non-specific, acting in different ways. They may be responsible for caseation in tuberculosis, ulceration in cutaneous leishmaniasis and for rejection of homografts. They are probably not so important in defence against leprosy, though they might contribute to type I reactions.

Mitogenic (blastogenic) factor: this causes lymphocytes, both specifically sensitized and non-sensitized, to divide and in doing so to secrete more lymphokines and so increase the reaction and, so long as antigen remains, prolong the reaction.

When the end result of these processes is elimination of invading organisms and protection against reinfection a state of cell-mediated immunity is said to exist; when inflammation alone, cellular or delayed hypersensitivity exists.

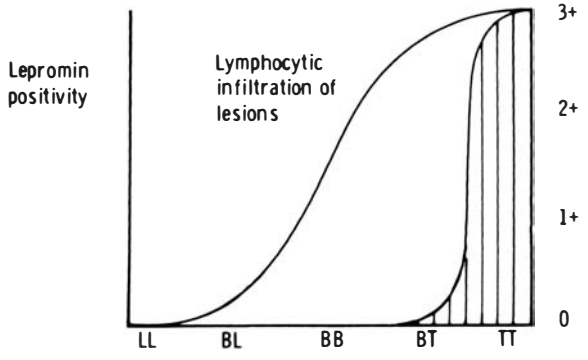

Fig. 1. Cell-mediated immune response, as shown by lymphocytic infiltration of lesions and by lepromin positivity.

Immunological Findings in Leprosy

The pathological and clinical characteristics of leprosy form a spectrum from LL to TT. The factor which mainly determines the place of an individual patient's disease on this spectrum is the extent to which cell-mediated immunity is expressed. In tuberculoid leprosy cellular immunity and hypersensitivity are well developed. In lepromatous leprosy they are absent; in LL induction and/or expression may be impaired. In borderline disease expression rather than induction is impaired. In addition the antibody responses at the poles differ and although this difference does not directly contribute to the patient's position on the spectrum it does contribute to certain other aspects of the disease.

CHARACTERISTICS OF THE IMMUNE RESPONSE AT THE POLES

Tuberculoid leprosy

1. Cellular response

The presence of cellular immunity is shown by certain characteristics of disease at this pole and by some experimental data.

(a) The essential histological characteristic is the tubercle: specialized macrophages (epithelioid cells) focalized by lymphocytes.
(b) Delayed cutaneous hypersensitivity is present and is shown by a positive lepromin test.
(c) Lymph nodes show well developed paracortical areas containing pyroninophilic blast cells. Intermediary sinuses contain numerous non-pyroninophlic lymphocytes. Germinal centres are poorly developed and medullary cords contain few plasma cells.
(d) Lymphocytes from patients with tuberculoid leprosy, cultured *in vitro* in the presence of leprosy bacilli, transform into blast cells, activate macrophages and inhibit their migration.
(e) Macrophages from patients with tuberculoid leprosy can be stimulated, *in vitro*, to digest *M. leprae* if lymphocytes from a tuberculoid patient (but not from a lepromatous patient) are added to the culture.
(f) The disease tends to heal spontaneously.

2. Humoral response

(a) Antibodies to antigens of *M. leprae* and other mycobacteria can be detected in sera of under 10% of patients with TT leprosy by precipitation techniques.
(b) Auto-antibodies are not produced.

Lepromatous leprosy

1. Cellular response

The absence of cellular immunity is shown by:–
(a) There is no tubercle formation. The histological picture is that of the leproma: undifferentiated macrophages, often damaged by oedema and full of bacilli, with no surrounding lymphocytes.

(b) The lepromin test is negative.

(c) Lymph nodes show well developed germinal centres and medullary cords full of plasma cells. Paracortical areas are poorly developed and are replaced by undifferentiated and often bacilliferous macrophages. Intermediary sinuses do not contain lymphocytes.

(d) Lymphocytes from patients with lepromatous leprosy do not transform *in vitro* into blast cells in the presence of *M. leprae*, nor do they activate macrophages or inhibit their migration. The proportion of circulating lymphocytes, capable of binding *M. leprae* to their surface, is much lower in lepromatous than in tuberculoid patients.

(e) Macrophages from lepromatous patients can be stimulated *in vitro* to digest *M. leprae* if lymphocytes from a tuberculoid patient (but not from a lepromatous) are added to the culture.

(f) The disease does not heal spontaneously.

2. *Humoral response* (Fig. 2)

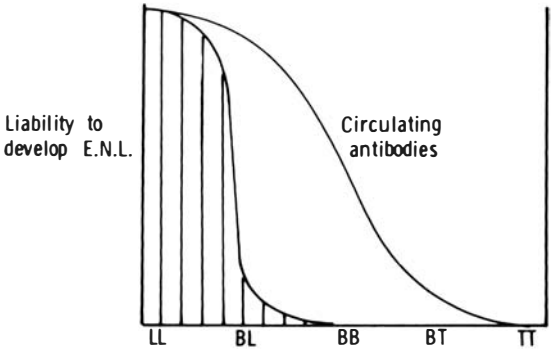

Fig. 2. Humoral response, as shown by presence of circulating antibodies and liability to develop erythema nodosum leprosum.

(a) Antibodies to antigens of *M. leprae* and other mycobacteria can be detected in high titre by complement fixation, precipitation and indirect haemagglutination. Precipitins to up to 5 antigens are present in 80% of lepromatous sera. Serum levels of IgG are raised.

(b) Sera from lepromatous patients do not inhibit, *in vitro*, the blastogenic response to *M. leprae* of lymphocytes from tuberculoid patients. This observation implies the absence of enhancing antibodies in those sera.

(c) Many auto-bodies are produced. They include rheumatoid factor, anti-thyroglobulin antibody, cryoglobulins, C-reactive protein a and false positive biological tests for syphilis such as the Wasserman reaction.

Antibodies in leprosy do not seem to have any protective or useful role. They are unable to get at the intracellular organism. They play a role in type 2 reactions, but in doing so are of no benefit to the patient. It is not known whether autoimmune antibodies cause tissue damage, result from tissue damage or are a manifestation of the adjuvant activity of *M. leprae* in lymph nodes and not directly related to the clinical or pathological pattern of leprosy.

NATURE OF IMMUNE DEFICIENCY IN LEPROMATOUS LEPROSY

The immunological findings in leprosy indicate that there is a deficiency of cellular immunity to *M. leprae* in patients with lepromatous leprosy. The clone of lymphocytes which should respond is absent or unresponsive. As a result of this macrophages are not activated and are unable to digest *M. leprae*. Other features of cellular hypersensitivity are also absent. In LL this deficiency would appear to be absolute, lifelong and not reversible after treatment, which has led people to suggest that a genetic defect may be involved and there is some epidemiological evidence to support this view. On the other hand there is only a relative defect in BL and BB leprosy, and patients may move their position on the spectrum, towards the lepromatous pole if untreated or toward the tuberculoid pole if treated. These facts indicate that the presence of the bacillus itself specifically depresses cellular immunity.

Antibody response is unimpaired in LL. High titres are present to mycobacterial antigens and the response to immunization with typhoid vaccine (TAB) is normal. Enhancing antibody has not been demonstrated.

Thus, in lepromatous leprosy, there exists a state that resembles selective T-lymphocyte tolerance (mediated by antigen or suppressor T-cells) or immune deviation (mediated by antibody). In borderline disease these defects are not absolute and some additional modulating process, such as cellular desensitization, may be operating.

The severe deficiency of cellular immunity in LL is specific for *M. leprae*. There is, however, a partial generalized depression of cellular immunity in some patients with severe LL. They have a reduced number of circulating T-lymphocytes, and *in vitro* tests of lymphocyte function to other antigens may be slightly impaired. Their response to sensitization with some but not all chemical agents (e.g. dinitrochlorobenzene) is impaired, their response to several skin test antigens is depressed, they reject grafts of homologous skin more slowly and they may be more susceptible to incurrent disease. This depression, which tends to recover after treatment, is possibly due to disorganization of lymph node architecture and to antigenic competition.

Several attempts have been made to correct the specific defect of cell mediated immunity in lepromatous leprosy by giving patients lymphocytes or an extract of lymphocytes—transfer factor—from normal or immune individuals. So far there have been no convincing reports that these attempts have altered the course of lepromatous disease. There have as yet been no reports of the use of transfer factor to stop troublesome chronic erythema nodosum leprosum in well treated lepromatous patients, a situation which might be amenable to this sort of immunostimulating therapy.

Further Reading

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News and Notes

IN VITRO CULTIVATION OF LEPROSY BACILLI, A NEW CLAIM

The much heralded claim by Professor Skinsnes and his colleagues for the *in vitro* cultivation of leprosy bacilli which was announced in the world press in late 1975, is now published in the *International Journal of Leprosy* (1975, **43**, 193) under the heading "*In vitro* cultivation of leprosy bacilli on hyaluronic acid based medium. 1. Preliminary report". In this publication the authors state—"The purpose of this presentation is to describe prolific growth of *M. leprae* (and also *M. lepraemurium*) in this medium and to demonstrate the reasonable probability that the claimed *M. leprae* culture is indeed this organism". The essential growth factor is hyaluronic acid incorporated as the sodium salt at a concentration of 0.1% in an otherwise simple medium prepared as a liquid or a solid, by the incorporation of 2% agar. The rationale for incorporating hyaluronic acid into the medium for attempting to cultivate *M. leprae* was based on the earlier observations of Skinsnes and his colleagues that *M. leprae* was particularly concentrated in tissue sites associated with the presence of acid mucopolysaccharides from the host. They were further encouraged to try hyaluronic acid for *in vitro* cultivation from their findings that *M. leprae* multiplied more extensively in mice when bacillary inocula included hyaluronic acid and were followed by repeated injections with hyaluronic acid. Their claim to have cultured *M. leprae in vitro* is based on organisms recovered from these mice and also strains of *M. leprae* obtained directly from patients.

Although it is universally agreed every aspect of our understanding of leprosy would be enhanced by the successful *in vitro* cultivation of the causative organism, no new claim is currently justified until it has been rigorously checked and independently confirmed. Skinsnes and his colleagues have chosen to publish before having their claim checked at other centres. Moreover, the American Leprosy Mission have prematurely launched a major appeal for funds based on Skinsnes' claim.

Final confirmation of Skinsnes' claim is now awaited from those centres to whom he has recently sent his cultures of *M. leprae*.

LEPRA JOINS ILEP

At the 11th General Assembly of ILEP (the International Federation of Leprosy Associations) held in Paris on 27-28 March, 1976, The British Leprosy Relief Association (LEPRA) was welcomed as a new member. Fame Pereo (of Canada) was also admitted. The total membership now stands at 19.

The Annual Report of the Secretary-General, presented to the Assembly, recorded that the Member-Organizations of ILEP raised in the year 1974, the sum of 13.6 million US $, of which about 12 million US $ went to the support of 531 centres or projects, and 0.8 million to research. The number of leprosy patients

under treatment from these centres in 1975 was 1,230,299, of whom 95% were being treated as out-patients. Of the estimated (1972) total number of registered patients under treatment in the world, that is 2.8 millions, those who receive treatment through ILEP-sponsored or ILEP-supported programmes account for 42.6%, or 11.4% of the estimated number of persons needing treatment for leprosy. Over half the centres are responsible for domiciliary treatment schemes, and 147 of them are engaged in mass treatment activities, either as part of national programmes or as independent (usually Mission-sponsored) projects.

Rehabilitation plays an important—but subsidiary—role; a quarter of the centres make protective footwear, two-fifths have some kind of physiotherapeutic activity, and reconstructive surgery of various degrees of sophistication is available at one-fifth of the centres.

The Medical Commission, now under the able chairmanship of Professor Michel Lechat, is continuing to mould the attitudes and activities of the Member-Organizations of ILEP, and is especially concerned with the research projects, to which in 1975 the respectable sum of nearly 1,206,000 US $ was devoted. ILEP helps to fund the IMMLEP Project of the World Health Organization, and is at present exploring the possibility of sponsoring joint leprosy-tuberculosis projects in several countries. The Commission has already alerted Member-Organizations to the grave threat posed to programmes of leprosy treatment and control by the increasing incidence of dapsone-resistance. The advisability and practicability of the integration of leprosy into the general health services continue to be the subject of study by the Commission.

ALERT IS TEN YEARS OLD

The All Africa Leprosy Rehabilitation and Training Centre in Addis Ababa was founded on 11 December, 1965, and at the Annual General Meeting held on 19 March, 1976 the achievements of the first decade were reviewed. The limited initial conception of rehabilitation for those suffering from deformities due to leprosy, and the training of workers from African countries in all aspects of rehabilitation, was a reflection of the origin of the idea for the creation of the Centre, which was the International Society for Rehabilitation of the Disabled, the moving spirit being Professor Paul Brand. A report by Dr Stanley Browne reviewing the possibilities and disadvantages of locations in predominantly English-speaking countries in Africa narrowed the choice, and Ethiopia was finally selected.

From rehabilitation to treatment and control; from an urban institutional centre to a rural demonstration area; from a small group representing the Leprosy Missions and the University to a very broad-based and internationally representative body of sponsors—such has been the progress of ALERT over the past 10 years.

Training courses have been provided for varying periods for physicians, orthopaedic surgeons, rural area leprosy supervisors, rehabilitation technicians, physiotherapists, laboratory technicians and others. Students come from many African countries, and from other continents.

The service reports for the year 1975 and the past decade make impressive reading, regarding both the hospital itself and the numbers of leprosy patients under treatment in Addis Ababa and in a neighbouring control area.

In addition to a most fruitful collaboration with the (British) Medical Research Council, the Armauer Hansen Research Institute, which is structurally an annexe to the hospital but administratively distinct, has made several important contributions to knowledge of the immunological aspects of leprosy.

The Ethiopian Government continues its generous financial support of the service functions of ALERT.

The last annual Kellersberger Lecture, sponsored by the American Leprosy Missions Inc, was given by Prof. Olaf K. Skinsnes; the next will be given by Dr Graham Weddell.

Dr Felton Ross will shortly (June 1976) be relinquishing his post as Director of Training, in which he has done conspicuous work during the difficult first decade of ALERT.

More African countries should be acquainted with the range of facilities provided by ALERT and send key members of their staff to profit from one or other of the courses given. (Address: The Director of Training, ALERT, PO Box 165, Addis Ababa, Ethiopia.)

YEAR OF THE CHILD, 1979

The United Nations has designated 1979 as "The Year of the Child", and has expressed the wish that all governmental and voluntary agencies should emphasize during that year the special needs of children throughout the world.

At the General Assembly of ILEP held in Paris in March 1976, an appeal was made that the problems associated with leprosy in children should provide the springboard for education and fund-raising activities, and that determined efforts should be made in all countries to intensify the search for early leprosy in children so that they may be spared progressive deformity and social ostracism. World Leprosy Day 1979 would be a most appropriate occasion for launching such a campaign.

SECOND REGIONAL CONFERENCE OF DERMATOLOGY

The International Society of Tropical Dermatology is encouraging the holding of regional conferences at which those who practise, teach or conduct research into skin diseases may meet to exchange information on recent research in their respective fields. The second such Conference will be held in Bangkok from 17 to 20 January, 1977. It is being organized mainly by the Dermatological Society of Thailand, with the active support of the Ministry of Public Health and the SEAMEO-Tropical Medicine and Public Health Project.

A full programme for the 4 days is being prepared. Intending participants are invited to make contact with Dr Tongnan Vibhatavanija, the Executive Secretary General, at the Institute of Dermatology, 420/7 Rajvithi Road, Bangkok 4, Thailand.

In addition to papers of general dermatological interest, with special emphasis given to dermatoses common in the countries of South-East Asia, a whole session will be devoted to leprosy, and an exhibition covering many aspects of leprosy and leprosy control in Thailand is being organized by Dr Teera Ramasoota, of the Phrapradaeng Leprosy Institute.

LEPROSY CONGRESS IN CAIRO

The First Leprosy Congress organized in the United Arab Republic was held in the spacious hall of the Kasr el Aini Faculty of Medicine, Cairo University, on 14 and 15 March, 1976. Nearly 500 participants registered, from 13 Arabic-speaking countries of the Near East, with two invited guests from England and France. Since the prime instigator and joint-organizer was Professor M El Zawahry, the internationally known dermatologist, the emphasis was on the skin aspects of leprosy. Almost the entire staffs of the Skin Departments of Cairo and Alexandria Medical Schools were there, in addition to representatives from Tanta, Assiut and Ain Shams.

The platform at the Inaugural Ceremony included the Minister of Health and the Minister for Higher Education, as well as the Dean of the Faculty of Medicine, their presence indicating the interest aroused in official and academic quarters.

Among the two score good papers presented, it would be invidious to cite those of outstanding merit, but mention should be made of excellent work being done in various aspects of immunology, staining techniques, and the nicotonic acid test. The papers will be published (in English) as Proceedings of the Congress.

The revival of interest in leprosy in Egypt serves as a reminder both of the very successful Congress on the International Leprosy Association held in Cairo in 1938, and of the continuing smouldering endemic in that country. There are at present 28,197 leprosy patients registered, of whom about 18,000 are under regular treatment; 1689 are in the two main residential leprosoria (Cairo and Alexandria have 570 beds), and in provincial leprosy hospitals. The relapse rate is said to be in the neighbourhood of 12%; 4128 are classified as lepromatous and 3226 are under 14 years of age. The prevalence rate varies from 0.8 to 2.4 per thousand.

The Congress certainly increased the awareness of the problem of leprosy among dermatologists, and should encourage a more systematic attack on the problem.

Dr Stanley Browne, joint organizer of the Congress with Professor El Zawahry, gave the opening paper on "The Diagnosis of Early Leprosy" and the closing paper in which he summarized recent advances in the various branches of leprosy. The Egyptian dermatologists hope to plan a similar Congress next year.

LEPRA ESSAY COMPETITION FOR MEDICAL STUDENTS IN THE UNIVERSITIES OF OXFORD, EDINBURGH AND BIRMINGHAM: 1976

Following prize essay competitions for medical students in previous years in Oxford and Edinburgh, LEPRA have this year extended the offer to Birmingham, and the subject for all 3 universities is *The transmission of leprosy and tuberculosis in man.*

Entries should be of not more than 10,000 words, but it is emphasized that much shorter essays have won awards in previous years; length is not important. References should be included as in scientific publications. Existing knowledge on the transmission of these diseases should be summarized, but no credit will be given for the mere reproduction of material already published in books or journals. In the case of leprosy, particular attention should be given to constructive criticism of present trends in research on the transmission of this disease and to ideas for future work which might benefit the individual patient and also be of value in world leprosy control. The essay requires neither clinical

experience nor original work and entries from junior students will be most welcome.

Further details are available from the appropriate medical school offices. The closing date is 31 December, 1976 and the judges reserve the right to award the sum of £100 to one candidate, or to divide it amongst several candidates, or to withhold any award if entries are not of sufficient merit.

PERSONAL

Professor Dr Jacob Sheskin, the well-known dermatologist working in Jerusalem, has been awarded the Gold Medal for 1975 of the ancient French scientific society, *Société d'Encouragement au Progrès,* in collaboration with the American Division of the *World Academy of Art and Science.* We offer Professor Sheskin our hearty congratulations.

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Letter to the Editor

"Leprosy" in Standard Text Books

As a Field Supervisor, with only 3 years experience behind me, I was, nonetheless astounded to read the following extract on Lepromatous leprosy in *Diseases of the Skin* by H. O. Mackey, revised edition by John P. Mackey 1968.

Page 288.

Nodular Leprosy
(Lepromatous Leprosy)

"The nodules called lepromata, appear mostly on the forehead, face, ears and hands. The face becomes deformed and assumes the characteristic 'leonine' appearance.

This is the anergic type with many *M. leprae* and gives a negative reaction to lepromin. It is communicable, and has an unfavourable prognosis. The histopathology shows a granuloma made up mainly of histiocytes which contain clumps of *M. leprae*.

Symptoms. The first signs are a husky voice and a nasal discharge which indicate involvement of the mucous membranes. *A malarial type of fever with wasting and diarrhoea is common. The disease is steadily progressive and death occurs on an average in about 8 years. Some cases live on into old age."*

The italicized sentences are the cause of my astonishment.

The statement that death occurs on average in about 8 years is absolutely opposed to all the teaching I have received both here in Sierra Leone and in Albert, Ethiopia. The generalization regarding fever, wasting and diarrhoea I cannot say much about. I can only refer to the 57 lepromatous cases under my care and say that I have never seen wasting in any one of them and as for their fever and diarrhoea this has always responded to the appropriate drugs. Only two cases were referred to hospital and this for ENL.

It would appear we stand very little chance of educating the general public about the nature of leprosy, when would be doctors are given this type of information.

P. J. WITHERS

Leprosy Control Office,
Ministry of Health,
Kenema,
Sierra Leone

Abstracts

1. MICROBIOLOGY

1. MORBID. MORTAL., 1976, v. 25, No. 3, 18, 23. **Leprosy-like disease in wild-caught armadillos—Louisiana.**

Natural infections with bacilli similar to *Mycobacterium leprae* have been found in 14 armadillos trapped in southern Louisiana in 1974 and 1975. Four of the animals had ulcers or subcutaneous nodules suggestive of leprosy. A nasal smear from 1 showed acid-fast bacilli. In 7 of the animals post-mortem examination has revealed invasion of dermal nerves, typical of leprosy. Examination of the other animals has not been completed. Attempts to culture the bacilli have been unsuccessful. Positive Mitsuda reactions at 28 days have been obtained in patients with leprosy tested with lepromin prepared from the tissues of these animals. The 14 infected armadillos represent about 10% of uninoculated animals examined from the trapping areas; most were examined within a month of capture. "The pathologic picture and the Mitsuda reactions at 28 days strongly suggest that the mycobacteria found in these wild armadillos are *M. leprae,* but until other pending confirmatory tests are available, the identification cannot be considered definite." The original report on this finding is by WALSH *et al., J. Reticuloendothelial Soc.,* 1975, v. 18, 347.

F. I. C. Apted

2. OLITZKI, A. L. **Effect of microbial substances of different origins on the growth of *Mycobacterium leprae*. *Israel J. Med. Sci.,*** 1975, v. 11, No. 9, 896-905..

"*Mycobacterium leprae* multiplied in media enriched with substances originating from other mycobacteria, from non-acid-fast *Actinomycetales* or from gram-positive or gram-negative *Eubacteriales.* Most of the *M. leprae* strains did not grow on a synthetic medium containing the amino acids present in *M. smegmatis,* but the growth-promoting effect of sonic extracts of this organism indicated that substances of bacterial origin, other than amino acids, do act with *M. leprae* was verified by their ability to oxidize D-3,4,-dihydroxyphenylalanine (D-dopa test). *M. leprae* did not multiply on Nakamura medium unless at least 40% of medium NM7 enriched by bacterial substances was present. Adequate aeration was essential for multiplication of *M. leprae* in enriched NM7 and No. 3 media."

3. NAKAMURA, M. **[Multiplication of *Mycobacterium lepraemurium* in cell-free liquid medium. 7. Pathogenicity of *M. lepraemurium* cultivated in NC-5 medium.]** *Lepro,* 1975, v. 44, No. 1, 7-12. **[8. Growth of *Myco. lepraemurium* in culture media which were preserved before inoculation.]** *Ibid.,* 13-18. **[9. Effect of transfer of the smeared slide on the growth of *M. lepraemurium.*]** *Ibid.,* 19-23. [In Japanese.]

The English summaries appended to the papers are as follows:—

i. "It was demonstrated that *M. lepraemurium* which were cultivated for 223 days at 30° C by bacillary suspended method and for 172 days by slide culture method, had the ability to infect susceptible mice. In both cases, the procedure of refreshing culture medium resulted in stimulation of the growth of bacilli. In the case of slide culture method, it could be noted that

the infectious abilities of the cultivated bacilli depended upon the number of living organisms, because the growth of bacilli was stimulated by a slide transfer procedure. On the other hand, no pathogenicity was demonstrated when the bacilli were cultivated for 64 days as the control in the EKP medium under the same condition.

"However, the quantitative observation about a relationship between the growth rate of bacilli and the grade of pathogenicity illustrated that multiplied bacilli had the reduced pathogenicities comparing to that of starting material."

ii. "Stabilities of the NC-5 and NC-7 medium for the growth of *M. lepraemurium* were studied. For this purpose, these media were kept at 37°C and 4°C for 1 and 2 months. Using slide culture method, *M. lepraemurium* were cultivated in a freshly prepared medium and the preserved media. In order to see the stabilities of the preserved media, the multiplication of *M. lepraemurium* in the preserved medium was compared to that in a freshly prepared one. The results obtained show that the bacilli multiplied equally in both freshly prepared and preserved media in the case of NC-5 medium, and that, in the NC-7 medium the dominant growth was observed in the medium which was kept for 1 month at 37°C, and the inferior growth was seen in the medium preserved for 2 months at 37°C. Therefore, it could be concluded that the NC-5 medium would be very stable for use."

iii. "The experiments of the factors influencing the growth of *M. lepraemurium* smeared on slides and cultivated in NC-5 medium were carried out as following:

"1. Slide-transfer group: the group in which a smeared slide was transferred to a freshly prepared medium at a definite interval.

"2. Air-exposure group: the group in which a slide was just taken out and put again in the same medium at a definite interval.

"3. Stopper-opening group: the group in which a rubber stopper was just taken out and sealed again at a definite interval.

"4. Control: no treatment group.

"The growths of bacilli treated with the four procedures were compared. The most remarkable growth was observed in the case of the air-exposure group, and the inferior growth was obtained in control group. From the results, it could be presumed that periodical exchange of air in sealed culture medium might be necessary for the growth of bacilli.

"In the experiments of bacillary cultivation, the suspensions of bacilli were inoculated and cultivated in 50 ml of NC-5 medium, which were distributed to a 50 ml-flask 100 ml-flask, and 200 ml-flask, respectively. After two months' cultivation at 30°C, bacterial cells were collected by centrifugation and the wet weights of sediments were measured. The best yield of bacterial cells was obtained when the bacilli were cultivated in a 50 ml-flask, and the poor yield was observed in the case of a 200 ml-flask. Therefore, it would be presumed that *M. lepraemurium* might multiply under a slightly anaerobic condition, rather than aerobic one.

"From the results of two experiments mentioned above, it could be emphasized that the growth of *M. lepraemurium* would unexpectedly depend upon the influence of air."

[See *Trop. Dis. Bull.*, 1975, v. 72, abstr. 1126.]

4. PETERS, J. H., GORDON, G. R., MURRAY, J. F., Jr, FIELDSTEEL, A. H. & LEVY, L. **Minimal inhibitory concentration of dapsone for *Mycobacterium leprae* in rats.** *Antimicrob. Agents Chemother.*, 1975, v. 8, No. 5, 551-557.

"To define the minimal inhibitory concentration (MIC) of dapsone (DDS) for *Mycobacterium leprae* in rats, we determined the relationship between dietary and plasma levels of DDS in uninfected male and female Lewis rats. This knowledge was applied to the design of experiments using rats inoculated in the footpads with *M. leprae*. The MIC for DDS in male and female rats, respectively, was 1.5 to 4.0 ng and 1.8 to 3.0 ng of DDS/ml of plasma, even though the sexes exhibited markedly different concentrations of DDS when receiving the same dietary level of DDS. These values for the MIC of DDS for *M. leprae* in rats are nearly identical to the previously determined MIC of DDS for *M. leprae* in mice."

5. YOSHII, Z. & NAKAMURA, M. **Growth features of *Mycobacterium lepraemurium* cultivated in NC-5 medium.** *Kurume Med. J.*, 1975, v. 22, No. 1, 35-56.

"Morphological features of *Mycobacterium lepraemurium* cultivated on a slide glass in a cell-free liquid medium, NC-5, were precisely observed by a scanning electron microscope. As previously demonstrated, elongation of bacterial cell was noted in the second weeks of cultivation, and thereafter septum formation, division, budding and branching followed. It was obvious that the growth grade of bacilli depended upon the number of bacilli presented on the slide glass; when a small number of bacilli was inoculated on the slide glass, a micro-colony was formed in 10 weeks' cultivation, but further development did not occur even if cultivation was continued."

6. KATO, L. & ISHAQUE, M. **Separation of *Mycobacterium lepraemurium* from the subcutaneous tissues of the rat.** *Int. J. Lepr.*, 1975, v. 43, No. 1, 16-20.

A technically simple method is described for the separation of *Mycobacterium lepraemurium* or *M. leprae* with a degree of purity sufficient for use in spectrophotometric studies. The recovery rate for bacilli is high. The original paper should be consulted for a description of the method.

D. S. Ridley

7. KIRCHHEIMER, W. F., PRABHAKARAN, K., HARRIS, E. B., SANCHEZ, R. M. & SHANNON, E. J. **Preparation of protein from *Mycobacterium leprae* and skin-test responses of vaccinated armadillos.** *Lepr. India*, 1975, v. 47, No. 3, 142-150.

This paper describes details of the method by which 1.6 mg of purified *Mycobacterium leprae* protein was extracted from 62 g liver of a heavily infected armadillo. This protein was used as a skin test antigen in groups of 5 armadillos either not vaccinated or vaccinated 8 months previously with heat-killed armadillo tissue-derived leprosy bacilli in Freund's incomplete adjuvant. [The last paragraph on p. 145 is muddled with regard to controls—perhaps a sentence has been omitted?] Previously vaccinated armadillos developed erythematous areas 4-8 mm in diameter 24 hours after skin testing. These were fading by 48 hours. Skin test reactions elicited 12 months after vaccination persisted up to 72 hours. These reactions were taken to indicate delayed hypersensitivity to *M. leprae* protein. [The presence or absence of immediate (10 minutes) and Arthus (4 hour) sensitivity is not commented upon, nor the histology of the skin test response. The specificity of the response is not described although alluded to in the discussion.]

A. D. M. Bryceson

2. IMMUNOLOGY AND PATHOLOGY

8. RIDLEY, D. S. **Histological classification and the immunological spectrum of leprosy.** *Bull. Wld Hlth Org.*, 1974, v. 51, No. 5, 451-465.

This well illustrated account of the histological classification of leprosy is a record of the system used by the author which has been found to be applicable to all ethnic groups studied to date, as well as to experimental leprosy in mice, and includes a number of new concepts resulting from a re-evaluation of immunological criteria.

[This valuable paper does not lend itself to abstraction and should be studied in the original.]

W. H. Jopling

9. DATE, A. & JOHNY, K. V. **Glomerular subepithelial deposits in lepromatous leprosy.** *Am. J. Trop. Med. Hyg.,* 1975, v. 24, No. 5, 853-856.

Sub-epithelial humps in the glomeruli were seen on electron microscopy in a patient with lepromatous leprosy and erythema nodosum leprosum (ENL). The humps were of the sort known to occur in immune complex nephritis, and, since there was no evidence of recent streptococcal infection, the assumption is that the renal condition was due to ENL. The patient had microscopic haematuria and only trace proteinuria. The total complement and C3 levels were normal, possibly due to low complement consumption.

D. S. Ridley

10. SRIVASTAVA, L. M., AGARWAL, D. P., BENKMANN, H. G. & GOEDDE, H. W. **Biochemical, immunological and genetic studies in leprosy. III. Genetic polymorphism of C3 and immunoglobulin profile in leprosy patients, healthy family members and controls.** *Tropenmed. Parasit.,* 1975, v. 26, No. 4, 426-430.

"One hundred and fourty-eight members from 30 families (64 children) from Ethiopia, where one or more persons were affected with leprosy, were investigated for genetic polymorphism of C3, serum concentration of β1C/β1A-globulin and immunoglobulins A, G and M using high voltage agarose electrophoresis, immunoelectroassay and single radial immunodiffusion techniques respectively. The results are compared with related healthy controls. No association between C3 phenotypes and leprosy could be established through family studies. C3 concentration was, however, lower in leprosy patients. Difficulties and drawbacks of such studies with small families are discussed."

[See *Trop. Dis. Bull.,* (1975, v. 72, abstrs 2618, 2619.]

11. KREISLER, J. M., ARNAIZ, A., PEREZ, B. & BOOTELLO, A. **Lymphocytotoxins in leprosy.** *Int. J. Lepr.,* 1975, v. 43, No. 2, 91-94.

"Occurrence of cold lymphocytotoxins has been observed in 59 leprosy sera. In 46% of the patients, cold lymphocytotoxins were present whereas in healthy controls only 13% showed such antibodies. The highest incidence of alloantibodies was detected in lepromatous leprosy. Levels of autoantibodies, immunoglobulins and C3 were tested in parallel without finding any significant correlations."

12. FLIES, E. L., BACHMANN, A. E., SASIAIN, M. DEL C. & ARES, B. R. Exploración inmunologica en pacientes con lepra indeterminada. **[Immunological studies in patients with indeterminate leprosy.]** *Revta Asoc. Argent. Microbiol.,* 1975, v. 7, No. 3, 81-85.

The English summary appended to the paper is as follows:–

"The immunological competence of 11 patients with indeterminate leprosy was compared with that of 10 normal volunteers of the same age and sex distribution: these controls have not had previous contact with leprosy.

"The following parameters were studies in peripheral blood cells: 1) percentage of lymphocyte bearing surface immunoglobulins, as revealed by immunoflourescence; 2) percentage of lymphocyte bearing complement receptors, as studied by antibody and complement coated erithrocyte rosetting; 3) percentage of T cells, as revealed by spontaneous sheep erithrocyte rosettes; 4) blastogenic and mytogenic response of cultured lymphocytes to PHA, and 5) cell migration inhibition test using lepromin (80×10^6 bacilli/ml) as antigen. Skin reactions to lepromin were also assayed. In the 6 lepromin-positive patients with indeterminate leprosy, no major immunological alterations could be detected. On the contrary, the 5 lepromin-negative patients showed important alterations which could well be considered as precursors of lepromatous leprosy."

13. KAUR, S., MINOCHA, Y. C., SENGUPTA, U. & NAIK, S. **A comparative evaluation of bacteriologic and morphologic indices of *Mycobacterium leprae* in skin, lymph node, bone marrow, nerve and muscle.** *Int J. Lepr.*, 1975, v. 43, No. 1, 55-57.

A comparative evaluation of bacteriological and morphological indices (BI and MI) for leprosy bacilli was made in biopsies of skin, lymph node, sural nerve and quadriceps muscle and in marrow aspirates. There were 15 patients with untreated bacillary positive leprosy, including 5 with erythema nodosum leprosum (ENL) and 2 with dimorphous reactions. Although there were individual variations, neither BI nor MI was generally lower in skin than in any of the other tissues, with the important exception of the patients in reaction. In these patients the MI was often much higher in lymph nodes than in skin, and in a few patients this was also true of the BI. The BI of nerve and muscle was generally lower than in skin, except in one case of ENL and another of dimorphous reaction. The MI of muscle was zero in all biopsies.

[This paper brings out well the point that one reason for the finding of viable bacilli at sites other than skin might be that bacilli in skin (and nerve) are more subject to reactions.]

D. S. Ridley

14. McLEOD, J. G., HARGRAVE, J. C., WALSH, J. C., BOOTH, G. C., GYE, R. S. & BARRON, A. **Nerve conduction studies in leprosy.** *Int. J. Lepr.*, 1975, v. 43, No. 1, 21-31.

Motor and sensory nerve conduction studies were performed on 93 Aborigines from the Northern Territory of Australia; 30 were control subjects, 36 were leprosy patients, and 27 had no abnormalities apart from one or more clinically enlarged nerves of unknown aetiology. In the leprosy group impairment of conduction was demonstrated in the vast majority of clinically abnormal nerves and also in many nerves which were considered normal on clinical examination. Furthermore, it was found possible to locate the segments of nerves in which damage was maximal. In the third group of subjects, abnormal conduction was demonstrated in nearly 50% of the clinically enlarged nerves, and later it was established that leprosy was the cause of the conduction defect in the majority of cases.

The authors conclude that observations on nerve conduction are of considerable value in the diagnosis and management of leprosy.

W. H. Jopling

15. JOB, C. K. & VERGHESE, R. **Schwann cell changes in lepromatous leprosy—an electronmicroscope study.** *Indian J. Med. Res.*, 1975, v. 63, No. 7, 897-901.

"Biopsies from radial cutaneous nerves of 4 untreated lepromatous patients were studied using the electronmicroscope. It was found that *M. leprae* engulfed by Schwann cells grew, and multiplied, building up protective responses against the destructive action of the cell by losing their phagosomal membrane and by producing an inert electron transparent substance around them. However, once the organisms were dead they were digested inside phagolysosomes. The electron transparent substance produced by the cell-bacilli interaction might remain inside the cell for a long time giving it a foamy appearance."

16. LIM, S. D., KISZKISS, D. F., JACOBSON, R. R., CHOI, Y. S. & GOOD, R. A. **Thymus-dependent lymphocytes of peripheral blood in leprosy patients.** *Infection & Immunity*, 1974, v. 9, No. 2, 394-399.

"Study of the number of thymus-derived lymphocytes by the rosette assay (T-RFC) in patients with leprosy reveals that lower than normal numbers of T-RFC are regularly seen in those patients with the active lepromatous form of this disease. Essentially normal numbers of T-RFC were found in inactive lepromatous, borderline, and indeterminate types of leprosy. The lowest percentages and lowest absolute numbers of T-RFC 'were encountered in patients with

lepromatous leprosy resistant to chemotherapy. Patients with lepromatous leprosy complicated by erythema nodosum leprosum show numbers of T-RFC that are more nearly normal than the numbers of T-RFC in patients with uncomplicated lepromatous leprosy. These findings are discussed with respect to the pathogenesis of lepromatous leprosy and the T-RFC deficiency demonstrated in this disease. The possibility that transient defects in T-RFC numbers or function may predispose to lepromatous leprosy is proposed."

17. JOHN, T. J., VIJAYARATHNAM, P., VERGHESE, R. & KRISHNAMURTY, S. **Lymphoblast transformation in leprosy**. *Indian J. Med. Res.*, 1974, v. 62, No. 5, 696-698.

"Phytohaemagglutinin-M induced lymphoblast transformation of peripheral lymphocytes in cell cultures was studied in 21 lepromatous and 7 tuberculoid leprosy patients and in 9 control subjects. There was no appreciable difference in the range or mean of the proportion of blasts in the cultures among the 3 groups. This indicates that thymus dependent lymphocytes are basically normal in number and function in lepromatous leprosy in our locality."

18. CONVIT, J., PINARDI, M. E., RODRÍGUEZ OCHOA, G., ULRICH, M., AVILA, J. L. & GOLDMAN, M. **Elimination of *Mycobacterium leprae* subsequent to local *in vivo* activation of macrophages in lepromatous leprosy by other mycobacteria.** *Clin. Exp. Immunol.*, 1974, v. 17, No. 2, 261-265.

"This investigation studied the possibility of activating lepromatous macrophages by a local *'in vivo'* test.

"Lepromatous macrophages have an evident incapacity for clearing *M. leprae*. This is demonstrated by injecting lepromatous patients with an antigen containing *M. leprae* from human tissue at a concentration of 640×10^6 bacteria per ml. This produces a nodule which, at 30-day biopsy, shows a macrophagic granuloma with numerous bacteria inside the macrophages, proving that these cells are unable to remove *M. leprae*. This incapacity is specific for *M. leprae*, and all other mycobacteria produce a different reaction.

"Local *'in vivo'* stimulation of the lepromatous macrophage was obtained by injecting *M. leprae* in the same concentration as above but mixed with other mycobacteria (*M. lepraemurium* or BCG).

"The mixed antigens produced a tuberculoid granuloma with abundant lymphoid cells. Fite–Faraco stains showed almost no acid-fast bacteria. Therefore, our mixture of antigens had activated the macrophages locally and made them competent for clearing *M. leprae*."

19. BARNETSON, R. S. C., BJUNE, G., PEARSON, J. M. H. & KRONVAAL, G. **Antigenic heterogeneity in patients with reactions in borderline leprosy**. *Br. Med. J.*, 1975, Nov. 22, 435-437.

"Fifteen patients with borderline leprosy who developed 'reversal' reactions were studied from the inception of treatment. Thirteen showed an appreciable increase in lymphocyte transformation (LT) when preparations of *Mycobacterium leprae* were used as antigen. The LT responses to either 'whole' or 'sonicated' preparations of the bacillus in these 15 patients and in 9 others also in reaction correlated with the clinical presentation. Those with skin disease predominating in the reaction showed an appreciable increase in LT when whole *M. leprae* was used as antigen. Those with nerve disease predominating showed an increase with sonicated *M. leprae*. In those with both skin and nerve disease there was an increase with both antigen preparations. The ratios of the LT test results (whole to sonicated *M. leprae*) showed highly significant differences between the three groups."

20. NELSON, D. S., PENROSE, J. M., WATERS, M. F. R., PEARSON, J. M. H. & NELSON, M. **Depressive effect of serum from patients with leprosy on mixed lymphocyte reactions.** *Clin. Exp. Immunol.,* 1975, v. 22, No. 3, 385-392.

"Mixed leucocyte cultures, from 2 normal donors, were set up in media containing human serum from one of the following sources: (a) a pool of normal group AB donors; (b) Chinese, Malay or Indian patients with untreated leprosy; (c) the same patients after effective anti-leprosy treatment; (d) control Chinese, Malay or Indian subjects. Transformation was estimated by measuring the incorporation of tritiated thymidine in the last 24 h of a 7-day culture period. Transformation was impaired in sera from untreated lepromatous patients, but was less impaired or not impaired at all in sera from treated lepromatous patients. The loss of depressive activity after treatment was more marked in Chinese and Indian than in Malay patients. Transformation was also impaired, though to a lesser extent, in sera from patients with untreated tuberculoid leprosy; it was still impaired in sera from treated tuberculoid patients. There was no evidence of specificity in impairment of mixed lymphocyte reactivity and lymphocytotoxic antibodies appeared to play no role. The incidence of hepatitis B antigen and antibody and of anti-nuclear factor were not notably high."

21. YOUNGCHAIYUD, U., PANPATANA, P., JATIKAVANIJ, V. THONGCHAROEN, P. & VIBHATAVANIJA, T. N. **Serum immunoglobulin determinations in leprosy patients.** *J. Med. Ass. Thailand,* 1975, v. 58, No. 6, 304-307.

"The serum immunoglobulins were determined by the single radial diffusion method in 147 cases of leprosy, 49 cases with lepromatous and 98 cases with tuberculoid type. The cases were divided into 3 groups corresponding to the duration of disease. No significant alteration of serum immunoglobulin G, A, M level was demonstrated in all of the tuberculoid patients and in lepromatous cases with the disease than 5 years, but a slight rise in IgA level was found in this group. There was a significant increase in all of the 3 classes of immunoglobulin in lepromatous patients who had had the disease more than 5 years. This finding suggested that the duration of the disease may be one of the factors affecting the variation in serum immunoglobulin level in lepromatous patients."

3. CLINICAL ASPECTS

22. MALIK, R., AHUJA, P. & CHANDRA, K. **Leprosy of the larynx.** *Int. J. Lepr.,* 1975, v. 43, No. 2, 114-115.

"Five instances of lepromatous leprosy involving lesions of the larynx were encountered among a series of 280 laryngeal lesions. These are briefly described as involving the epiglottis in all cases, vocal cords in 2, and extension into the pyriform fossa in 1 instance."

23. ANTIA, N. H., METHA, L., SHETTY, V. & IRANI, P. F. **Clinical, electrophysiological, quantitative, histologic and ultrastructural studies of the index branch of the radial cutaneous nerve in leprosy. 1. Preliminary report.** *Int. J. Lepr.,* 1975, v. 43, No. 2, 106-113.

"The index branch of the radial cutaneous nerve has been demonstrated as a constant nerve which can be readily biopsied under local anesthesia and yields a nerve which is of suitable size for quantitative and qualitative studies both by light and electron microscopy. It supplies a limited but constant area where the sensory loss does not disturb the patient.

"Definite ultrastructural changes have been demonstrated in the clinically normal nerves of leprosy patients. These nerves have also revealed a loss in the small myelinated and unmyelinated fibers, which correlated with the common clinical findings of the absence of

sweating and dissociated sensory loss in this disease. Gross damage has been encountered in nerves which showed only early signs of clinical damage even with refined methods of sensory testing. These nerves would have passed as normal on routine testing by No. 5 nylon. Regeneration of small fibers was noted following loss of large size fibers.

"Nerve conduction velocity may be a useful tool in early diagnosis."

4. THERAPY

24. RUSSELL, D. A., WORTH, R. M., JANO, B., FASAL, P. & SHEPARD, C. C. **Prevention of leprosy by acedapsone.** [Correspondence.] *Lancet,* 1975, Oct. 18, 771.

The acedapsone prophylaxis-treatment programme in the Ponape District of Micronesia continues to provide useful data. The fourth annual post-treatment survey here reported indicates that the earlier intensive campaign has resulted in a declining incidence of new cases of leprosy. However, lapses from regularity of treatment and the emergence of sulphone resistance have complicated the issue. A few new infections have occurred from these cases.

However, the main thesis of the exercise has been confirmed, that is, that susceptible individuals exposed to infection from patients suffering from multibacillary forms of leprosy may be protected by 15 injections of acedapsone given over a period of 3 years.

[See also *Trop. Dis. Bull.,* 1974, v. 71, abstr. 1036.]

S. G. Browne

5. EPIDEMIOLOGY, PREVENTION AND CONTROL

25. KURIAN, P. V., VICTOR, V. & DEVANBU, D. **School survey as an effective method for leprosy control in rural areas.** *Lepr. India,* 1975, v. 47, No. 2, 75-78.

"During the school year June 1973 to April 1974, 85% of the pupils attending 380 schools in Gudiyatham Taluk of Tamil Nadu were examined for evidence of leprosy. Among the 76891 children examined, 217 new cases of leprosy were detected. There was no case of lepromatous leprosy in this group. All except two borderline cases were either indeterminate or tuberculoid. In endemic areas repeated annual examination of the school going population will help considerably in the control of the disease and in educating the people. The results of school surveys carried out in some Indian cities are also included for comparison."

26. AMBLARD, P., AMBROISE-THOMAS, P., DESIRE, C., GOUT, M., MONROSE, M. & SCHNEIDER, R. Aspects actuels de la lèpre à la Martinique. [**Some aspects of leprosy in Martinique today.**] *Bull. Soc. Path. Exot.,* 1975, v. 68, No. 2, 164-171. English summary (7 lines).

Probably introduced into the island by African slaves, leprosy today is prevalent in all districts of Martinique, affecting 5.1 males per 10,000 and 2.45 females.

The authors analyse [rather superficially] the main findings in the records of 844 patients. They report that in 60% the disease showed itself before the age of 30 years, that multiple cases (251) occurred in 109 families, and that no instance of conjugal leprosy was discovered. They do not pretend that their figures are complete, a large proportion of patients admitting that they had had the disease for over 5 years before seeking treatment. Forty-seven per cent of patients are said to have lepromatous leprosy, but leprous rhinitis is quite rare. The standard treatment for three-quarters of the patients is dapsone; the remainder are given a sulphonamide.

S. G. Browne

27. NOORDEEN, S. K. **Evolution of tuberculoid leprosy in a community.** *Lepr. India,* 1975, v. 47, No. 2, 85-93.

"The paper is based on a longitudinal study of a geographically limited rural community highly endemic for leprosy. The entire population in the community numbering about 8000 was ollowed up every year for over 6 years, including cases of leprosy that occurred. Mean incidence of tuberculoid leprosy was found to be 10.6 per 1000 per year. Intensive follow-up of newly detected cases revealed that regression of disease among tuberculoid leprosy cases was a very common feature, the mean inactivation rate being 10.9% per year. Inactivation rates were not influenced by either age or sex. Inactivation rates were comparatively low when more than one part of the body was affected, or when the patient had more than two patches, or when he had involvement or nerve trunks. Inactivation rates were not influenced by treatment, the bulk of inactivation being spontaneous. The epidemiological significance of the findings with regard to leprosy control is reviewed.

28. BROWNE, S. G. **The training and deployment of medical auxiliaries in the leprosy campaign.** *Ann. Trop. Med. Parasit.,* 1975, v. 69, No. 4, 413-416.

Afrer describing the different types of medical auxiliary carrying out essential work in the anti-leprosy campaigns in the tropics and subtropics, the author draws on his experience in the former Belgian Congo in the training and deployment of the most valuable of them all—the polycompetent auxiliary—and gives a detailed account of the curriculum and of the type of service such a qualified auxiliary can render.

W. H. Jopling

6. REHABILITATION AND SOCIAL ASPECTS

29. RAMU, G., DWIVEDI, M. P. & IYER, C. G. S. **Social reaction to leprosy in a rural population in Chingleput District (Tamil Nadu).** *Lepr. India,* 1975, v. 47, No. 3, 156-169.

The authors examine the social reactions to leprosy elicited in a group of 40 patients from a rural area in Tamil Nadu, India, and attempt to draw general conclusions from their data.

In spite of the low degree of physical disability in the group, social rejection, marriage difficulties and economic handicap were quite marked. The prevailing attitudes to the disease itself and to its victims provide a useful background to the study of the actual experiences of sufferers themselves. The stigma of leprosy and social rejection are more evident in the educated and the more affluent classes of society. Physicians as well as social agencies could do much to break down the walls of prejudice and ignorance that still surround leprosy in the rural areas of a country like India.

S. G. Browne

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Printed in Great Britain by The Whitefriars Press Ltd., London and Tonbridge