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Hypothesis: Do phases of immunosuppression during a *Mycobacterium leprae* infection determine the leprosy spectrum?

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Summary The failure of the cell-mediated immune response to *Mycobacterium leprae* in leprosy is amply documented, but the immunoregulatory mechanisms involved are unknown. It is suggested that suppressor mechanisms could explain the spectrum of immunity observed in leprosy, and a primary specific immunodeficiency need not be invoked. Three major phases of suppression can be identified. The primary suppression phase may be a consequence of the neural predilection of *M. leprae* which insures preferential exposure of bacillary antigens to suppressor cells in the spleen, rather than to effector cells in the draining lymph node. This view of the leprosy spectrum can accommodate recent findings of an HLA association of tuberculoid leprosy. It also has implications for the interpretation of leprosy vaccine trial data.

Despite many years of intensive research since the pioneering studies of Mitsuda in 1919, the reasons for the failure of the immune system in leprosy remain obscure and controversial. Successful chemotherapy of leprosy and clinical management of its complications are possible with our present state of knowledge, but the development of effective methods for immunotherapy and immunoprophylaxis of leprosy will be greatly facilitated by a clearer understanding of the immunoregulatory derangements which underlie the leprosy spectrum.

Deficiencies of the host

The spectrum of leprosy is the result of an infinitely varied interaction of the intracellular bacterial parasite, *Mycobacterium leprae*, and its human host.

* Adapted from a contribution to the booklet commemorating the Tenth Anniversary of the Armauer Hansen Research Institute.

Efforts to understand this complex host–parasite relationship may focus either on the deficiencies in the defences of the host or on the escape mechanisms employed by the parasite. In the decade just ended, a major effort has been directed toward elucidating the immunologic deficiencies of the human host. A variety of specific and non-specific immunodeficiencies have been documented, both *in vivo* and *in vitro*.¹ However, the question of which of these alterations are causes of the infection and which are its effects remains unresolved.

Particular attention has been given to the possible immunogenetic basis of a specific immunodeficiency in leprosy. In numerous studies, the first of which was reported from AHRI,² the frequency of HLA types in groups of leprosy patients and in controls has been analysed. These studies, and more recent ones which have examined the influence of HLA-D identity with a lepromatous patient on the lymphocyte transformation response to *M. leprae* of normal siblings of the patient,³ have generally failed to support the concept of an HLA-linked specific immunodeficiency in leprosy. However, studies of shared HLA haplotypes in families of leprosy patients in Surinam⁴ and India^{5,6} have shown evidence of an HLA association of susceptibility to tuberculoid leprosy, but not to lepromatous leprosy. A study of HLA-DR antigens in Mexican leprosy patients⁷ is in accordance with the family data. Of course, a gene controlling a specific immunodeficiency (immune response gene), or one controlling susceptibility to lepromatous leprosy by another mechanism, could be linked to a locus other than HLA. Additional family studies in several populations are needed to clarify the influence of HLA-linked or other genetic factors in susceptibility to lepromatous leprosy.

Strategies of the parasite

It is likely that the decade ahead will also see additional research efforts to delineate the escape mechanisms employed by the parasite in its fight for survival. Escape mechanisms of *M. leprae* which have already been identified include its ability to penetrate and grow in non-defensive cells (e.g. Schwann cells,⁸ smooth muscle fibres⁹ and endothelial cells¹⁰ which cannot be activated by immunological mechanisms to destroy the invader. Even within mononuclear phagocytes which do have the capability to be activated by sensitized T cells to destroy intracellular parasites, *M. leprae* may possess an activity which blocks phagosome–lysosome fusion and thereby forestalls its own destruction.¹¹

Another important escape mechanism is likely to be the ability of the parasite to generate suppressor cells in its host.^{12–16} Why these suppressor cells subvert the immune response in one individual and not in another becomes a central question. One possibility is that the induction of suppression in leprosy and the concomitant dissemination of the infection may require the

establishment of a focus of infection in a peripheral nerve.¹⁷ The peripheral nerve endoneurium, which is where the bacilli are localized, has long been known to lack lymphatic vessels.⁸ Consequently, bacilli in the nerve have access to the circulation from bacillated endothelial cells,¹⁸ but they do not have access to lymphatic drainage. Evidence from experimental animal models suggests that immunological suppression develops following preferential stimulation of the central lymphon compartment (spleen) via the circulation.¹⁹ It is thus possible that leakage of bacilli into the circulation at an early stage of the infection promotes the development of suppressor cells in the spleen. At the same time, peripheral lymphon stimulation, i.e. activation of effector cells in the draining lymph node, which is essential for the establishment of cell-mediated immunity (CMI), is by-passed. In other words, the leprosy bacillus, by its fastidious choice of cellular habitat, may accomplish an 'inversion' of the immune response, i.e. excessive central lymphon stimulation prior to effective peripheral lymphon stimulation. The result could be a specific suppression of the immune response to *M. leprae* antigens which is mediated by suppressor cells formed in the spleen.

The postulated importance of the lack of lymphatics in the site of predilection for the development of suppression in leprosy is analogous to that shown for privileged sites for allografts. Sites such as the hamster cheek pouch and the anterior chamber of the rat eye lack lymphatics, thus giving alloantigens preferential access to the spleen via the circulation which causes suppression of allograft rejection.²⁰

Already there is experimental evidence that lack of access to lymphatic vessels may also be crucial in allowing an intracellular parasite to evade the host's defence mechanisms. In experimental cutaneous leishmaniasis, the blocking of the access of the inoculum to lymphatic vessels transforms a self-healing local infection into a disseminated and progressive disease.²¹

The generation of suppressor cells in the mouse is controlled by genes within the I region of H-2, i.e. within the major histocompatibility complex (MHC).²² Since the human analogue of the murine I region is thought to be the HLA-D locus, it follows that some of the HLA-D-associated factors⁵ which govern susceptibility to *M. leprae* infection could operate by controlling suppressor cell generation. If so, we have in the process of suppressor cell induction the convergence of the parasite's escape mechanisms and the host's genetically controlled immunoregulatory mechanisms which influence the outcome of a leprosy infection.

The leprosy spectrum: a spectrum of suppression?

The spectrum of leprosy could arise from the varied interaction of the slowly enlarging bacillary population with the host's immune system. The nature of

this interaction may determine the relative amounts of suppression and immunity which are generated. The leprosy spectrum as described by Ridley, Jopling, and Waters^{23,24} can be derived from this interplay of suppression and immunity as follows:

POLAR TUBERCULOID LEPROSY (TT)

TT leprosy, in its purest form, may represent a localization of the infection to the primary intraneural focus. Neither bacillary dissemination, nor the suppression which accompanies it, has occurred, and the immune response to *M. leprae* in polar tuberculoid leprosy is, therefore, unimpaired.

BORDERLINE LEPROSY (BT, BB, BL)

If, on the other hand, a suppression phase accompanies the dissemination of the infection from its primary focus, the borderline types of leprosy may result. It is the interruption of this state of suppression with the subsequent appearance of delayed type hypersensitivity (DTH) to *M. leprae* antigens which precipitates active clinical leprosy. Either decreased suppressor function or a stronger antigenic stimulus for production of effector cells, or a combination of the two, could tip the balance in favour of immunity and cause the eruption of the multiple infiltrated skin lesions characteristic of borderline leprosy. Borderline leprosy ranges from the paucibacillary borderline tuberculoid (BT) to multibacillary borderline lepromatous (BL) depending on the length of the preceding suppression phase and the extent of bacterial multiplication and dissemination.

A second phase of suppression may follow the active phase of borderline leprosy if continued bacterial multiplication occurs despite the established DTH. This is the suppression phase which accompanies downgrading into subpolar lepromatous leprosy. Alternatively, the second phase of suppression may be interrupted by a 'reversal' reaction. Reversal reactions frequently, but not invariably, occur following the initiation of chemotherapy.²⁵ The mechanisms which promote the resurgence of DTH leading to reversal reaction are not known. However, reversal of a block in phagosome–lysosome fusion¹¹ could occur in treated patients if the block is maintained only by metabolically-active bacilli. The 'unblocking' of fusion in macrophages might then provide an important stimulus to *M. leprae*-specific DTH, as the macrophage has an essential role in the induction and expression of sensitized T cells.²⁶

LEPROMATOUS LEPROSY (LLs AND LLp)

Leprosy of both the subpolar and polar types represents a fully disseminated infection which encompasses much of the reticuloendothelial system (RES),

including the bone marrow, spleen, liver, and lymph nodes. Subpolar lepromatous leprosy (LLs) includes those lepromatous patients who have downgraded following an earlier borderline stage.²⁴ Polar lepromatous leprosy (LLp) is distinguished by a merging of the first and final phases of suppression which precludes the emergence of the immunity (or hypersensitivity) which characterizes borderline leprosy.

PHASES OF SUPPRESSION

Three major phases of suppression can thus be postulated (Fig. 1). Phase I, or primary suppression, is the phase of suppression which precedes signs of clinical leprosy. When primary suppression is overcome by rising DTH, the typical lesions of borderline leprosy occur. Phase II, or secondary suppression, is the phase of suppression which follows onset of clinical borderline leprosy and permits downgrading toward lepromatous leprosy (subpolar). Following the

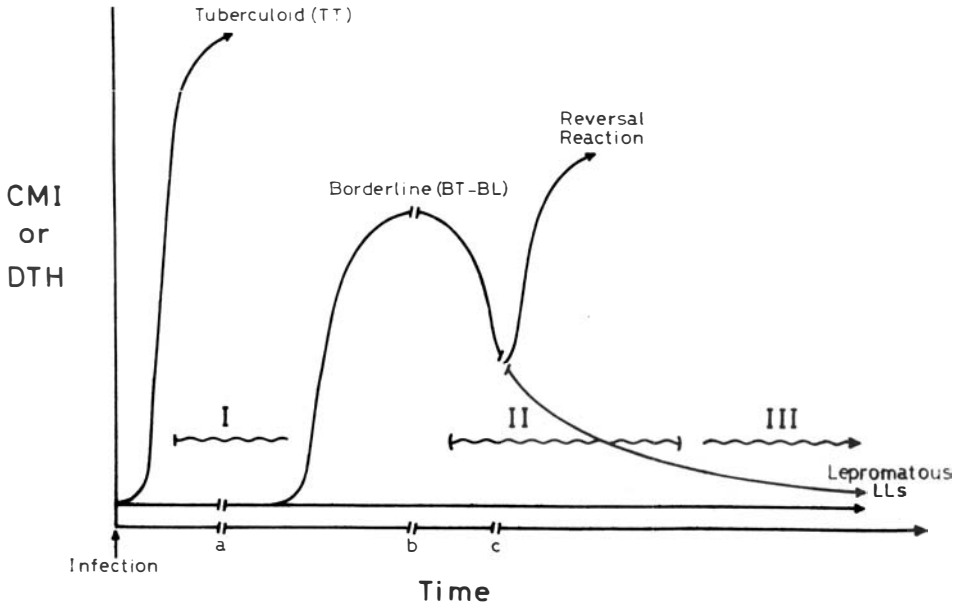


Figure 1. Phases of suppression which determine the spectrum of leprosy. Three phases of suppression (indicated by wavy lines) could explain the observed spectrum of clinical leprosy. For definition of Phases I, II, and III see text. This diagram is similar to that of Godal *et al.*^{27,28} who assumed that the late cell-mediated immune response to *M. leprae* seen in borderline and lepromatous patients was due to a variable lag period prior to attainment of threshold levels of bacillary antigen, rather than to an active suppression as is assumed here. The time scale is not defined because it varies widely in individual patients. The incubation period of borderline leprosy has been estimated to be from 2 to 5 years.²⁸ The breaks in the time scale serve to emphasize (a) the variable length of Phase I suppression which presumably determines in part the nature of the borderline disease (BT, BB or BL) and which in turn influences both (b) the duration of the active phase of a borderline infection and (c) the time of onset of reversal reaction following the initiation of chemotherapy.²⁵

decline in bacillary viability resulting from chemotherapy, secondary suppression may be dramatically reversed with the onset of a reversal reaction. Phase III, or tertiary suppression, is the irreversible suppression of the lepromatous state, both polar and subpolar.

Elucidation of the cellular mechanisms responsible for the observed phases of suppression is hampered by the lack of experimental *M. leprae* infections which parallel the spectrum of human leprosy. However, other mycobacterial infections of mice will continue to provide useful insights into the immunoregulatory events which may underlie the leprosy spectrum.²⁹⁻³⁴

COROLLARIES

There are several corollaries to the postulated phases of suppression:

(a) There is no 'innate' level of immunity which is characteristic of each type of leprosy. The widely variable lymphocyte transformation test (LTT) responses and levels of lymphocytic infiltration of the lesions of borderline leprosy^{35,36} are not readily accommodated by a static picture of the leprosy spectrum which assumes a steadily declining CMI capacity across the spectrum. These wide variations can rather be attributed to differences between patients who are still in the active phase of a borderline leprosy infection and those in whom secondary suppression has already set in. It seems likely that the active phase is shorter in borderline lepromatous patients than it is in borderline tuberculoid patients because of the much heavier bacterial burden in the former group. Therefore, most borderline lepromatous patients have passed through the active phase of the disease by the time they self-report to the clinic, and, on the average, their lymphocyte transformation responses to *M. leprae* are much lower than those in the borderline tuberculoid group, as are the number of lymphocytes in the lesions.^{35, 36}

(b) Polar tuberculoid leprosy results when bacilli in the primary focus of infection fail to induce adequate suppression. Some failures of suppression resulting in TT leprosy would likely occur simply because bacilli in the primary focus gain early access to the peripheral lymphon compartment via lymphatic vessels. The relationship of local spread to systemic dissemination would be influenced by the location of the primary focus. Alternatively, some of the individuals who develop tuberculoid leprosy may do so because they have an HLA-linked impairment of their ability to generate suppressor cells in response to *M. leprae*. This could explain the apparent HLA association of susceptibility to tuberculoid leprosy noted above.

(c) The mechanisms underlying the primary suppression are identical in individuals destined to become either borderline or lepromatous patients. However, in borderline leprosy the suppression is interrupted early. In polar lepromatous leprosy it is not interrupted before persistent (tertiary) suppression sets in following complete dissemination throughout the RES. It is important to note that the irreversible suppression follows complete dissemi-

nation of the infection, but does not precede it. In other words, although the lepromatous patient has passed into the irreversible Phase III, in its earlier stages his disease passed through a phase of primary suppression (phase I) which under different circumstances might have been interrupted to precipitate one or the other type of borderline leprosy.

Implications for vaccine trials

Various vaccination strategies have been proposed which derive from their proponents' views on the pathogenesis of leprosy.¹⁷ No matter which of the proposed vaccines is eventually tested, a deduction from this last corollary has implications for the interpretation of the trial data. If it is true that the early stages of suppression are qualitatively identical in individuals incubating borderline or lepromatous leprosy, then it seems possible that an effective anti-leprosy vaccine (ALV) would be able to precipitate subclinical lepromatous leprosy as one of the borderline types. The factors which control the natural evolution of the disease are incompletely understood. However, the balance of suppression *vs.* immunity is influenced by immunological perturbations caused by pregnancy³⁷ and possibly by such factors as intercurrent infections, malnutrition, injury, and stress in general.

Another important factor could be the distribution of bacilli in the body. In particular, the relative numbers of bacilli reaching the central and peripheral lymphon compartments may be important. It seems likely, therefore, that the intradermal inoculation of an ALV could provide a potent stimulus to the peripheral lymphon compartment and thereby tip the balance in favour of immunity. The evidence obtained by Convit *et al.*³⁸ for a conversion of Mitsuda-negative indeterminate leprosy to determinate (borderline) leprosy by intradermal inoculation of *M. leprae* combined with BCG supports this possibility.

Thus at certain stages in the evolution of the disease vaccination might precipitate borderline leprosy in individuals who otherwise would have continued to progress silently towards polar lepromatous leprosy.³⁹ This would complicate the interpretation of vaccine trials because some of these subclinical lepromatous cases in the unvaccinated control group could get safely through the trial period undetected due to the fact that the incubation period of lepromatous leprosy may range up to ten years or more. From a public health point of view, precipitation by vaccination as 'closed' cases those early infections which might otherwise have gone on to become infectious 'open' cases is highly desirable. However, this factor, in itself, will tend to increase the incidence of leprosy in the vaccinated group compared to the control group, and, depending on the incidence of lepromatous leprosy in the trial region, will make the overall protection rate appear less than it actually is. Selection of lepromin-negative individuals for inclusion in the vaccination trial, as would likely be done, would tend to accentuate the problem because individuals in

early subclinical stages of a lepromatous leprosy infection are thought to be found largely in the lepromin-negative group. Unfortunately, subclinical lepromatous leprosy cannot be identified easily by any of the diagnostic methods currently available.

The design of an effective vaccine and a successful field trial of it depends on an understanding of the forces which shape the leprosy spectrum. The concept that three major phases of suppression can account for the complexities of the leprosy spectrum accommodates much of the available evidence. However, further experiments will be required to establish its validity as a basis for design and testing of a leprosy vaccine.

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Editorial note

1. Special issue of Leprosy Review: 'Leprosy and Primary Health Care'

In one of the later numbers of *Leprosy Review*, **52**, 1981, we are intending to devote the entire number to the urgent and important topic of Leprosy and Primary Health Care. Invitations have already been issued to experts in the international field who may be able to contribute, and it is also hoped to review existing WHO and other documents which may be relevant. Meanwhile we welcome spontaneous contributions, letters or comments from readers who feel they have something to say about a matter which is clearly of enormous importance to the methodology of leprosy control between now and the year 2000.

2. News and Notes; Leprosy and the Community; Reviews and Abstracts.

We apologize for the very short entries under several of these headings in recent numbers of the journal. This is due to pressure on space, mainly from original articles, but it is hoped to catch up with a backlog of interesting material during 1981.

3. Vancouver style.

In the transition period during 1980, we have been far from strict, and not always consistent, in this style of printing, which is now in extensive use by a number of well-known journals. From now on, however, we shall attempt to use it more accurately, and it is therefore essential that contributors submit their manuscripts in this style.

EDITOR

ELISA inhibition technique for the demonstration of sulphones in body fluids

II. A new method to monitor leprosy patient compliance under field conditions*

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Summary An enzyme-linked immunosorbent assay (ELISA) for sulphones in urine is described. This method for monitoring dapsone (DDS) self-administration is compared with the quantitative determination of DDS/creatinine (D/C) ratios, currently used. Urine samples collected from 10 volunteers on 14 consecutive days after taking single doses of 100 mg DDS are studied, together with pretreatment samples and urine containing standard amounts of DDS. The lowest quantity of DDS still visually detectable by ELISA inhibition is 0.01 $\mu\text{g/ml}$ urine. All samples obtained up to 4–10 days after the 100 mg DDS doses are positive by this method, whereas the first negative D/C results occur on the third day. The method is especially apt to monitor substantial failure in DDS self-administration without using sophisticated instruments.

Introduction

A current method to monitor the self-administration of dapsone (DDS) by leprosy patients is based on colorimetric estimation of the DDS/creatinine (D/C) ratio in urine. The method discriminates between blank control urine samples and urine samples of patients on DDS doses as low as 25 mg per day. Also urine samples collected within 2 or 3 days of a single 200 mg DDS dose were classified positive by this technique. The method was especially advocated by Ellard and colleagues¹ for quantitative estimation of percentages of self-administered doses that are actually being taken by groups of out-patients. With this aim it has been applied successfully to leprosy patients in Africa and Asia.^{2–13}

*This investigation received support from the Chemotherapy of Leprosy (THELEP) component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, from the Netherlands Leprosy Relief Association (Nederlandse Stichting voor Leprabestrijding) and from the Italian Leprosy Relief Association (Amici dei Lebbrosi).

However, the method has several limitations:

- (1) Variations in D/C ratios between controls on the same supervised DDS dose are too large for conclusions to be drawn as to the number of missed doses of DDS that might have preceded an individual low D/C ratio.¹
- (2) The estimation of low D/C ratios is limited by other diazotizable substances causing apparent D/C values in blank control urines. These values vary within a population, and also between different populations.^{1,2,4}
- (3) Compliance estimation may be hindered by the presence in urine of sulphonamides that cross-react with DDS in the colorimetric estimation. Measurement at a second wavelength is needed to avoid misleading results.²
- (4) The method requires a spectrophotometer, a costly and vulnerable instrument, not readily available in rural leprosy endemic areas.

In view of these limitations it is understandable that the WHO Expert Committee on Leprosy in its fifth report¹⁴ asks for *simpler methods for analysing urine for dapsona content*. The increasing incidence of resistance of *Mycobacterium leprae* to DDS makes a convenient method for checking leprosy patient compliance urgently needed in order to distinguish between relapse due to non-compliance and that due to the emergence of sulphore-resistant *M. leprae*. Such a method is also of importance in attempts to improve DDS self-administration, since poor compliance is probably a factor promoting the emergence of sulphone resistance.

We have therefore developed an enzyme-linked immunosorbent assay (ELISA) for DDS in urine. It is an inhibition technique essentially corresponding to an idea of Engvall and Perlmann,¹⁵ first applied by Holmgren and Svennerholm in 1973¹⁶ for the detection of *Vibrio cholerae* antigen in sera. Preliminary experiments with urine samples from 44 Kenyan leprosy patients have shown the high sensitivity of the basic technique, using whole rabbit anti-DDS serum and enzyme conjugated anti-rabbit IgG serum.¹⁷ The main tool for the test in its present form is the sulphones specific antibody-enzyme conjugate described in our previous paper.¹⁸ This paper describes the method for detecting sulphones in urine, and compares its qualities with those of the D/C method.

Materials and methods

URINE SAMPLES

Each of 10 healthy volunteers took a single oral dose of 100 mg DDS. Urine samples were collected immediately before the DDS doses were taken and on 14 consecutive days thereafter, from each volunteer one 10 ml sample per day. Early morning urine was avoided. Half of each sample was preserved by the

addition of a few grains of thymol. To each of the other 5 ml portions 2.5 ml 2 N HCl was added.

REAGENTS FOR ELISA

The following reagents were prepared according to the prescriptions laid down in the previous paper:¹⁸ Bovine Serum Albumin-DDS conjugate (BSA-DDS), lyophilized in carbonate buffer of pH 9.6 in vials containing 20 μ g BSA-DDS each, stored at room temperature; sulphones specific enzyme-immunoglobulin conjugate (the E-Ig dialyzate of the previous paper), mixed in portions of 125 μ l dialyzate with 500 μ l normal horse serum and 50 μ l Tween 20, lyophilized and stored at +4°C; purified 5-aminosalicylic acid (5AS), lyophilized and stored at room temperature in portions of 10 mg per vial.

Other reagents used were thymol, Tween 20, 5% aqueous phenol red solution, diluted ammonia solution, HCl, NaOH, H₂O₂, and phosphate buffered saline (PBS), containing 1.72 g Na₂ HPO₄ · 12H₂O, 0.254 g KH₂ PO₄ and 8.5 g NaCl per l of water.

DEMONSTRATION OF SULPHONES BY ELISA

Before analysis each urine sample was roughly adjusted to pH 7 by the addition of a few drops of diluted ammonia solution or diluted HCl, using phenol red as indicator. The presence of DDS and its conjugates in urine samples was demonstrated by an ELISA inhibition technique. A vial with lyophilized buffered BSA-DDS was filled with 10 ml tap water, dissolving the conjugate. To each of the 96 wells of a microtiter tray 100 μ l of this solution was added. The tray was covered (e.g. with a second one) and left to incubate for 15 min at 56°C. Then the tray was emptied and washed 4 times with PBS containing 0.05% Tween 20 (PBS/Tween), each time leaving the fluid in the wells for 1 min. Before the next incubation 25 μ l PBS/ Tween was added to each well, followed by 25 μ l of a urine sample. A vial with lyophilized E-Ig (one portion as described above) was filled with 5 ml tap water, dissolving the conjugate. To each well of the tray 50 μ l of this solution was added. The tray was again covered and left to incubate for 15 min at 56°C. It was then emptied and washed 4 times with PBS/Tween as described above. Finally to each well 100 μ l substrate was added, prepared by dissolving a 10 mg lyophilized 5AS portion in 9 ml distilled water, and then mixing it with 1 ml of a 0.05% solution of H₂O₂ in distilled water. After leaving the tray for 2 h at room temperature, the colour intensities resulting from the test samples were compared with those resulting from the pretreatment samples, and from a mixture of the latter to which standard amounts of DDS were added. Comparison was made with the naked eye.

ESTIMATION OF D/C RATIOS BY SPECTROPHOTOMETRY

DDS/creatinine ratios of the acidified urine specimens were estimated as described by Ellard and colleagues.¹

Results

Figure 1 is a photograph of ELISA results using the urine samples from 5 of the 10 volunteers. The first 2 rows show the results after using standard amounts of DDS in urine. The lowest quantity of DDS still detectable in these standard samples by naked eye is 0.01 $\mu\text{g/ml}$ urine, i.e. 0.25 ng/well (wells E 1–2). Likewise, in urine specimens collected up to 8 days after the 100 mg dose of DDS the naked eye could easily detect sulphones by ELISA. In the case of one volunteer even the urine collected on the tenth day was unmistakably positive (well C8). Similar results were obtained with the other urine samples, except in those of one volunteer, when the limit of detection was reached already on the fourth day after the DDS dose was taken. DDS/creatinine determinations proved that this particular volunteer was an extremely rapid DDS eliminator with an estimated $T_{\frac{1}{2}}$ (half-life) for DDS of 11 h, compared to the average $T_{\frac{1}{2}}$ among this group of volunteers of 22 h (range 11–34 h).

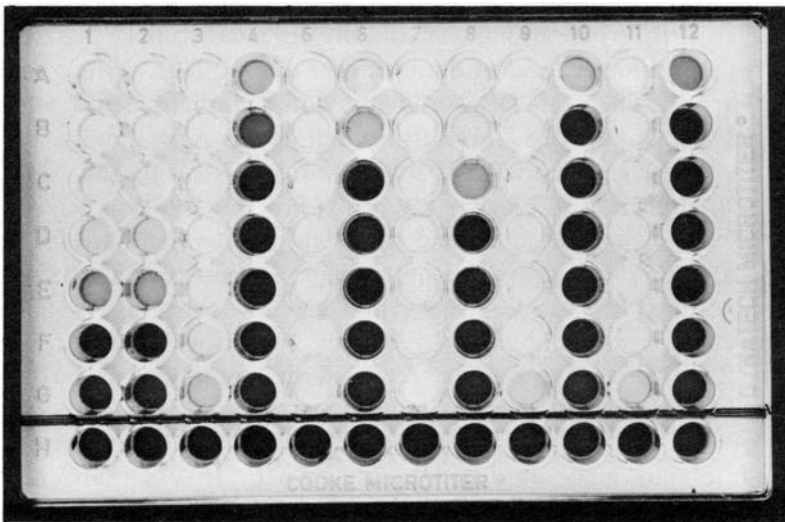


Figure 1. ELISA on urine samples to detect sulphones. Rows 1 and 2: duplicate mixed urine samples containing standard amounts of DDS, respectively 100 (A), 10 (B), 1 (C), 0.1 (D), 0.01 (E), 0.001 (F), 0.0001 (G) and 0 (H) $\mu\text{g/ml}$. Rows 3, 5, 7, 9 and 11: urine samples from 5 different volunteers, collected respectively before treatment (H), or on day 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), 6 (F) and 7 (G) after taking 100 mg DDS. Rows 4, 6, 8, 10 and 12: urine samples from the same 5 volunteers, collected respectively before treatment (H, duplicate), or on day 8 (A), 9 (B), 10 (C), 11 (D), 12 (E), 13 (F) and 14 (G) after taking 100 mg DDS.

Table 1 compares the numbers of urine samples positive by D/C ratios with the numbers positive by ELISA. To obtain an end-point for the positive classification by D/C ratios either 2 or 3 standard deviations (0.7) were added to the mean pretreatment value (2.8), resulting in an end-point of 4.2 and 4.9 respectively. Using an end-point of 4.2, all samples collected up to 3 days after the 100 mg dose of DDS are positive by D/C ratio, but among those samples collected during the last days of the experiment some false positives are likely to have occurred. With an end-point of 4.9, only 7 out of 10 samples are positive on the third day, but false positives are excluded. The much greater sensitivity of the ELISA is apparent from the table. When $T_{\frac{1}{2}}$ values of DDS are normal, urines may be expected to be positive for at least a week after the taking of a single dose of 100 mg DDS.

The ELISA results were identical, whether incubations were done for 15 min at 56°C, for 30 min at 37°C, or for 90 min at 20°C.

Table 1. D/C and ELISA analysis of urine samples after single oral doses of 100 mg DDS (10 subjects)

Time (Days)	D/C ratios ($\mu\text{g/ml}$)*	Number D/C > 4.9 [†]	Number D/C > 4.2 [‡]	Number pos. by ELISA
1	22.5 (15.2–27.8)	10	10	10
2	10.4 (6.5–14.3)	10	10	10
3	6.8 (4.3–10.3)	7	10	10
4	4.5 (2.7– 7.4)	3	5	10
5	4.1 (2.5– 6.4)	3	4	9
6	3.2 (2.1– 5.2)	1	2	9
7	3.0 (1.7– 4.3)	0	1	9
8	2.9 (2.1– 4.7)	0	1	9
9	2.7 (1.5– 3.8)	0	0	3
10	2.5 (1.5– 3.6)	0	0	1
11	2.4 (0.9– 3.2)	0	0	0
12	2.5 (0.4– 4.6)	0	1	0
13	2.7 (2.0– 4.4)	0	1	0
14	2.5 (0.8– 4.7)	0	1	0

* means and ranges

[†] mean of pretreatment values (2.8) plus three standard deviations (0.7)

[‡] mean of pretreatment values plus two standard deviations

Discussion

An important advantage of the ELISA compared to the D/C method is its higher sensitivity, even though it is read by naked eye. Reliable, positive results are obtained for up to 4–10 days after giving a single dose of 100 mg DDS, equivalent in effect to the situation at the end of a period of daily dosage with 50 mg DDS. Thus, finding negative results from urine samples of patients who

were meant to be self-administering such daily doses would indicate substantial failure in self-medication. This is clinically far more important than the occasional missed dose, and cannot be revealed with precision by the less sensitive D/C method.

Another advantage of the ELISA is its relative simplicity. No sophisticated instruments are required. A single microtiter tray will suffice for duplicate samples of 36 test specimens, 6 positive and 6 blank controls. For positive controls one might either use urine specimens containing a range of known DDS concentrations, as in this study, or serial dilutions of a mixture of urine samples from a small number of patients, whose DDS administration is strictly supervised. The latter will be easier to prepare in the field. It will also allow a more adequate comparison between control and test samples since both will contain a natural mixture of conjugated and unconjugated sulphones, each with its own affinity for the E-Ig conjugate.¹⁸

The turning-point of colour change should be included in the series of dilutions of the control urine. If that is done, calculations based on a $T_{\frac{1}{2}}$ of 1 day will give an indication of what the average negative urine result implies. It should be stressed, however, that the broad variations in diuresis and in $T_{\frac{1}{2}}$ for DDS do not allow any conclusions to be drawn as regards individual patients in anything more than general terms such as *substantial non-compliance*. Even quantitative tests, that include the creatinine determination as a correction factor for the diuresis, can avoid this limitation only by the laborious way of comparing individual results with those obtained after the same patient had taken supervised doses of DDS.^{1,2} Those who are interested in the omission of only 2 or 3 daily DDS doses should make different dilutions of the test samples, to find out at which dilutions a positive sample turns negative. Conclusions must again be based on average $T_{\frac{1}{2}}$ values, with the unavoidable restriction that a slow eliminator remains longer positive than a rapid eliminator, all other factors being equal.

For some leprosy workers such semi-quantitative interpretations of the ELISA may appear unnecessary or too troublesome. They want a simple *yes* or *no* answer to the compliance question. In this case, it may only be necessary to include 6 positive and 6 negative control urine samples, all undiluted. Any negative test result points to substantial non-compliance without further interpretation. Strict standardization of the reagents, giving reproducible tests under changing circumstances, does permit this simplification, thus making the test more attractive for the man in the field.

Although in the laboratory it was convenient to carry out our incubations at 56°C, clearly in the field incubations would be best done for 90 minutes at ambient temperature.

The shelf-life of two of the three lyophilized reagents is very long. They remained stable even when being kept at 56°C for one month. The third reagent, the specific E-Ig conjugate, remains in optimal condition kept at 4°C for one year, and at 37°C for 25 days.

The total production costs of the three lyophilized reagents per microtiter tray are about US \$15 (E-Ig, \$13; BSA-DDS, \$1; 5AS, \$1). These figures are an indication of the present situation. Other costs are the tray (\$0.50), the washing fluid and some chemicals (\$0.50 per tray). The trays may be recycled by intensive washing with 1% sodium dodecyl sulphate (SDS) and water, or with 1 N NaOH and carbonate buffer. If the investment of US \$100 for an adjustable pipette is prohibitive, simple plastic pipettes (\$0.25 each), calibrated for drops of 25 or 50 μ l of water and operated with a small balloon may be used.

In conclusion, this ELISA inhibition technique may be of some help to workers supervising leprosy treatment in endemic areas, especially where a growing resistance of *M. leprae* to sulphones occurs.

Acknowledgements

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Changes in neutrophil motility accompanying dapsone and rifampicin therapy

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Summary Dapsone has been shown to stimulate the motility of normal neutrophils *in vitro* and the neutrophils of patients with lepromatous and tuberculoid leprosy *in vivo*. It is suggested that dapsone possesses immunostimulatory activity.

Introduction

We have previously reported that dapsone therapy in patients with lepromatous leprosy was associated with progressive improvement of neutrophil migration and mitogen-induced lymphocyte proliferation (Anderson, Gatner, Imkamp & Kok, 1980).¹ These observations could have been due to a primary immunopharmacological action of the drug or alternatively to a secondary phenomenon due to the antimicrobial activity of the drug (as a result of decreased levels of bacteria-derived serum inhibitors of leucocyte functions). We have now investigated the *in vitro* effects of dapsone on the motility of normal neutrophils and the *in vivo* effects in patients with tuberculoid leprosy receiving dapsone only and rifampicin or dapsone only in a group of patients with lepromatous leprosy.

Patients and methods

PATIENTS

Twenty-four new untreated admissions (6LL, 6BL, 8BT and 4BB) were classified according to the clinical and histopathological criteria of Ridley and

Jopling (1966).² The 6LL and 6BL patients comprised the lepromatous leprosy group and the 8BT and 4BB patients formed the tuberculoid leprosy group.

EXPERIMENTAL PROTOCOL

Patients in the lepromatous leprosy group received dapsone only (DDS, 100 mg daily) for the first week of therapy after which therapy with rifampicin only for 6 weeks (600 mg daily) was commenced. Testing of neutrophil motility was performed prior to dapsone therapy, 2 h after the ingestion of a single 100 mg oral dose (to investigate possible immunopharmacological effects) and one week after the ingestion of 100 mg dapsone daily; testing was also performed after the 6-week period of rifampicin therapy. In patients with tuberculoid leprosy neutrophil motility was tested prior to dapsone therapy and 4 weeks after therapy with 100 mg dapsone daily.

STUDIES OF NEUTROPHIL MOTILITY

Neutrophils were obtained from heparinized venous blood (5 units heparin/ml) processed as previously described (Anderson *et al*, 1980)¹ and resuspended to a final concentration of 5×10^6 /ml. One leucoattractant was used, viz. endotoxin-activated autologous serum (EAS) prepared by activation of fresh serum with 100 μ g/ml of bacterial endotoxin (*E. coli*: 0127: B8 Difco, Detroit, Michigan, USA). Neutrophil motility was assessed as previously described (Anderson and van Rensburg, 1979).³ Chambers were incubated and the results expressed as the average number of cells reaching the lower surface of the 5 μ -pore size Millipore filter after 3 h incubation and expressed as an average for triplicate filters.

Dapsone powder for *in vitro* experiments was obtained from Lennon Laboratories (Pty) Ltd, Port Elizabeth, South Africa, and dissolved in HBSS. The concentration range investigated was 10^{-6} M– 10^{-2} M. In these experiments to assess the effects of dapsone on the motility of neutrophils from normal adult volunteers to autologous EAS an incubation period of 2 h was used. Dapsone was present with the neutrophils in the upper compartment of the chemotaxis chamber throughout the incubation period.

Results

In vitro effects of dapsone

Results on the migration of normal neutrophils are shown in Table 1. Dapsone at concentrations of $> 10^{-4}$ M caused stimulation of motility which was statistically significant at concentrations of 10^{-3} M, 2.5×10^{-3} M and 5×10^{-3} M.

Table 1. The effects of dapsone *in vitro* on the migration of normal neutrophils to autologous EAS

Dapsone concentration	Neutrophil migration to EAS	P value*
Control	169 ± 18 [†]	
1 × 10 ⁻³ Molar	243 ± 20	< 0.05
2.5 × 10 ⁻³ Molar	266 ± 21	< 0.05
5 × 10 ⁻³ Molar	259 ± 22	< 0.05

*Statistical analysis was performed by the Student's *t* test for paired means.

[†]Results as the mean value in cells/microscope high power field for six different individuals

In vivo effects of dapsone and rifampicin

Results in patients with lepromatous leprosy are shown in Table 2. Dapsone therapy was associated with significantly improved neutrophil motility to autologous EAS. Rifampicin therapy was associated with a slight decrease in neutrophil motility (Table 2).

Table 2. Effects of dapsone only and rifampicin only on neutrophil migration to autologous EAS in patients with lepromatous leprosy

Pre-therapy	Neutrophil migration to EAS		
	2 h after ingestion of 100 mg dapsone	After 1 week 100 mg dapsone daily	After 6 weeks' therapy with rifampicin only (600 mg daily)
28.5 ± 12.6*	57.4 ± 28.4 (< 0.05) [‡]	75.3 ± 20.4 [†] (< 0.05)	44.8 ± 15.9

*Results as the mean value with standard error as cells/high power field.

[†]Dapsone therapy stopped at this stage prior to commencement of rifampicine therapy.

[‡]P value calculated by the Wilcoxon Signed-Rank test.

In vivo effects of dapsone therapy

Results in patients with tuberculoid leprosy are shown in Table 3. Ingestion of dapsone caused a significant increase of neutrophil motility.

Table 3. Effects of Dapsone only on neutrophil migration to EAS in patients with tuberculoid leprosy

Neutrophil migration to EAS	
Prior to dapsone therapy	4 weeks after ingestion of 100 mg dapsone daily
109.2 ± 23.4*	200.0 ± 38.1 (< 0.05) [†]

*Results as mean cells/microscope high power field with standard error.

[†]P value calculated by the Wilcoxon Signed-Rank test.

Discussion

In this study dapsone at concentrations of $>10^{-4}$ M has been shown to stimulate neutrophil motility *in vitro*. These findings suggest that *in vivo* stimulation of leucocyte migration would require serum levels of dapsone of $> 25 \mu\text{g/ml}$ which indicates that dapsone stimulation would not be achieved following ingestion of 100 mg of dapsone. However we have previously reported for levamisole that concentrations of this agent required to stimulate neutrophil motility *in vivo* are considerably less than those required *in vitro* (Anderson, Oosthuizen, Theron & van Rensburg, 1979).⁴

In order to investigate the immunopharmacological activity of dapsone the effects of the drug on neutrophil motility were studied prior to and 2 h after the ingestion of a single 100 mg oral dose. Significant stimulation of neutrophil migration was observed which justifies our previous suggestion (Anderson *et al.* 1980)¹ that dapsone may possess immunostimulatory activity. It was not established if the dapsone-mediated changes in motility were cell or serum-related. Rifampicin therapy was associated with a slight decrease in neutrophil motility *in vivo*. Stendahl, Molin and Dahlgren (1970)⁵ have reported that dapsone *in vitro* had no effects on PMN migration to zymosan-activated serum which is not in agreement with this report. These differences are probably related to the low concentrations of dapsone investigated by Stendahl *et al.*⁵

These findings show that dapsone in addition to antimicrobial and suspected anti-inflammatory activity (Thomson and Souhami, 1975)⁶ possesses immunostimulatory properties. There is the suggestion that dapsone therapy may be involved in the pathogenesis of erythema nodosum leprosum (ENL) since dapsone causes increased migration of neutrophils and may therefore also increase the locomotion of mononuclear cells and macrophages. Further studies are in progress to investigate the mechanism of dapsone-mediated stimulation of neutrophil motility.

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Activity of four clofazimine analogues against *Mycobacterium leprae*

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Summary Four clofazimine analogues were found active against *Mycobacterium leprae* in the mouse footpad system, but none was as active as clofazimine itself. The results suggest the importance of the two *p*-chlorosubstituents that are a structural feature of clofazimine.

Introduction

Except for one compound [2-anilino-5-phenyl-8-chloro-3,5-dihydro-3-cyclohexyliminophenazine, B1912] studied by Shepard *et al*,¹ no analogue of clofazimine [2-(6-chloroanilino)-5-(6-chlorophenyl)-3, 5-dihydro-3-isopropyliminophenazine, B663] had been screened for activity against *Mycobacterium leprae*. Such a study became possible when the late Dr VC Barry, Medical Research Council of Ireland, Dublin, agreed to furnish four analogues; the number of analogues to be screened initially was limited by the need to resynthesize each in 10-g quantities. Therefore, the activity against *M. leprae* of clofazimine and the four analogues was investigated in the mouse footpad system by Shepard's kinetic method.^{2,3} In addition, the half-time of disappearance of each analogue from the mouse carcass was determined, to permit more precise comparisons of the antimicrobial activity of the compounds.

Materials and methods

Locally-bred weanling BALB/c mice were inoculated in the hind footpad, each footpad receiving 5,000 *M. leprae* of the same strain. Compounds were administered, either incorporated into the mouse chow in several

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concentrations, or in weekly intraperitoneal doses, for periods of about 90 days, beginning 60 or 75 days after inoculation. Clofazimine was tested at a dietary concentration of 0.1 mg per 100 g chow, the minimal effective concentration for this strain of *M. leprae*,⁴ and in an intraperitoneal dose of 25 μ g (approximately 1 mg/kg body weight); the analogues were tested in dietary concentrations equimolar to 0.1, 1.0 and 10 mg clofazimine per 100 g mouse chow, and in intraperitoneal doses equimolar to that of clofazimine. Groups of untreated mice served as controls. Harvests of *M. leprae*, usually from a pool of four footpads, were performed at intervals by published methods,^{5,6} and bacterial growth curves were constructed from the results of the harvests. Activity of the compounds is expressed in terms of the 'delay' of bacterial multiplication in treated mice compared to that in control mice;^{2,3} a delay of at least 30 days may be considered significant. In separate experiments, mice were administered a single intraperitoneal dose of each of the analogues, dissolved in propylene glycol, equimolar to 4 mg clofazimine/kg body weight; pairs of mice were sacrificed at intervals during the following 21 days, the carcasses were homogenized, and the clofazimine analogues were extracted from the trichloroacetic acid-precipitates of the homogenates, as previously described for clofazimine.⁴ The concentrations of the analogues were determined spectrophotometrically by their native absorbance in 20% H₂SO₄, and the half-time of disappearance of each analogue from the mouse carcass was calculated.

Results

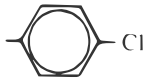
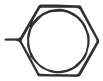
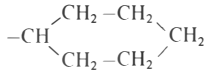

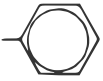

The results are summarized in Table 1. All four analogues of clofazimine demonstrated activity against *M. leprae*, but none was as active as clofazimine itself. Three analogues – B669, B749 and B775 – demonstrated only one-tenth the activity of clofazimine, whereas B1056 appeared only about 1/100 as active as clofazimine.

The half-time of disappearance of clofazimine from the mouse carcass had been determined previously to be 7 days.⁴ Two of the analogues – B749 and B1056 – were found to disappear at about the same rate as did clofazimine, whereas the two remaining compounds disappeared more rapidly.

Discussion

None of the four analogues of clofazimine was as active against *M. leprae* as was clofazimine itself. In the cases of B669 and B775, the lesser degree of antimicrobial activity may simply reflect the more rapid disappearance of the two analogues. On the other hand, that B749 demonstrated roughly 10-fold the

Table 1. Studies of clofazimine analogues

Compound	R ₁	R ₂	Dosage	Delay* (days)	T ^{1 2 †} (days)
Clofazimine‡		-CH(CH ₃) ₂	25 µg [§] 0.0001 g/g	160 182 112	7
B 669 [¶]			23 µg 0.000094 g/g 0.00094 g/g 0.0094 g/g	0 19 182 150	2
B 749 [¶]		-(CH ₂) ₂ -N-(CH ₂ -CH ₃) ₂	27 µg 0.00011 g/g 0.0011 g/g 0.011 g/g	6 11 200 455	7.5
B 775 [¶]		-CH ₂ -CH-(CH ₃) ₂	21 µg 0.000085 g/g 0.00085 g/g 0.0085 g/g	0 13 133 162	0.4
B 1056 ^{¶**}		-(CH ₂) ₂ -N-(CH ₂ -CH ₃) ₂	25 µg 0.0001 0.001 0.01	0 23 40 258	8

*Delay in multiplication of *M. leprae* in treated mice compared to that in untreated mice, corrected for period of drug administration.

†Determined from analyses of mouse carcasses after intraperitoneal administration.

‡Generously furnished by Dr W Vischer, CIBA-GEIGY, Basle, Switzerland.

§ Dosages shown as quantities of compounds were administered up weekly between day 60 and day 142.

|| Dosages shown as concentrations of compounds were administered, incorporated in the mouse chow, between day 75 and day 165.

¶ Generously synthesized and furnished by Dr Barry.

**Furnished as the hydrochloride salt.

activity of B1056 suggests the importance of the *p*-chloro-substituent on the two benzene rings; it may be that the *p*-chlorophenyl-analogues of B669 and B775 would have been more active than the latter compounds. It is interesting that, against murine infection with *M. tuberculosis*, all four analogues were much less active than was clofazimine; B775 was the most active of the four analogues, and B1056 the least active.⁷ And B673 and B776, the *p*-chlorophenyl-analogues of B669 and B775, respectively, were each much more active in murine tuberculosis than the corresponding compound without the *p*-chloro-substituent.⁷

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Choice of sites for study of slit skin smears

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Summary In view of recent publications drawing attention to the importance of fingers and toes as sites for slit skin smears in leprosy, a study has been carried out on patients in India to compare the bacterial load and morphology of bacilli at various sites in both treated and untreated lepromatous patients. Although the ear lobes gave maximum bacteriological and morphological indices in most instances, a few cases gave higher values at elbows, fingers and toes. In three patients, bacilli could be detected only from sites other than ear lobes. The importance of taking slit skin smears from peripheral sites, including fingers and toes, is stressed.

Introduction

The bacteriological status of leprosy patients is usually assessed by the slit skin smear method of Wade (1963)¹ as described by Cochrane (1964).² The bacterial index (BI) is used as a semiquantitative index for the assessment of bacterial load. Morphological index (MI) is still assumed to be a sensitive index for assessment of chemotherapeutic efficacy and detection of bacterial resistance. Skin slit smears from various sites have been used by different workers for diagnosis, assessment of drug therapy and early detection of drug resistance. With the increasing importance of drug resistance, to obtain a meaningful result there is a real need for standardization of methods of assessment of drug effectiveness including the simplest available method of slit skin smears. Although serial biopsies have been recommended for the assessment of bacteriological changes (Leiker, 1971)³ for patients on therapy, the slit skin smear method has been shown to be sensitive enough for bacteriological and morphological changes (Izumi, 1971).⁴ Various sites have been recommended by different workers and the number suggested varies from one to eleven sites. In spite of all its limitations, MI still remains the only method for routine estimation of viability of *Mycobacterium leprae* in skin slit smears.

Ridley *et al* (1976)⁵ have reported highest BI and MI from smears taken from fingers. Fingers were also stressed to be the important site for picking up solid staining bacilli in long-standing treated lepromatous cases when other sites may be bacteriologically negative (Jopling *et al*, 1979).⁶ The present study was undertaken to compare the bacterial load (BI) and their morphology (MI) at various sites in treated and untreated lepromatous patients and to find out the site, which in addition to conventional sites, yielded more bacilli and for a longer time under treatment. Besides the fingers, toes were also included to see if this could provide the same or more information than that obtained from fingers alone.

Material and methods

Forty patients with lepromatous leprosy, irrespective of age and sex, attending the leprosy clinic of the Postgraduate Institute of Medical Education and Research, Chandigarh, India, were taken up for the study. There were twenty each in the treated and untreated groups. All untreated patients were taken for the study irrespective of the duration of the disease. All treated patients had taken treatment with only dapsone for a duration of 5–9 years. Almost all the patients either wore 'chappals' (footwear with almost whole of dorsum of foot and toes exposed) or nothing at all.

Skin slit smears were taken from both ear lobules, both elbows and dorsum of middle phalanges of the middle finger/toe of both hands/feet (terminal phalanges were not taken because many patients had almost total resorption/ulceration especially of the toes) and if the middle finger/toe was missing adjoining finger/toe was taken. The slides were stained with Ziehl–Neelsen method. Bacteriological index (BI) was estimated according to the method of Ridley (1964)⁷ and morphological index (MI) according to the method of Waters and Rees (1962).⁸ The slides were studied by at least two observers without being aware of the previous bacteriological status and the site. Any gross discrepancy was rechecked by taking fresh smears and in the case of minor discrepancies (MI only), the mean of two figures was taken.

Results

The results are given in Table 1.

Bacteriological index (BI) of untreated patients:

- (1) Ear lobes gave uniformly higher values as compared to those from elbows (higher in 13).
- (2) Ear lobes gave values which were higher as compared to those from fingers (higher in 16).

Table 1. BI and MI at various sites in treated and untreated lepromatous patients

Sr No	Name	Age & Sex	Rt EL		Lt EL		Rt Elb		Lt Elb		Rt Fing		Lt Fing		Rt toe		Lt toe	
			BI	MI	BI	MI	BI	MI	BI	MI	BI	MI	BI	MI	BI	MI	BI	MI
1	HS	49 M	2+	26%	1+	NP	3+	13%	--	--	1+	NP	--	--	--	--	--	--
2	JK	45 F	5+	3%	6+	3%	4+	2%	4+	3%	4+	5%	4+	6%	4+	5%	4+	4%
3	RC	36 M	5+	4%	5+	4%	4+	3%	4+	5%	3+	8%	2+	7%	4+	10%	4+	8%
4	AS	45 M	4+	10%	4+	8%	4+	4%	3+	3%	1+	NP	1+	NP	1+	NP	1+	NP
5	GD	45 F	4+	12%	4+	11%	5+	14%	3+	16%	1+	NP	3+	10%	2+	8%	1+	NP
6	RD	30 F	2+	25%	1+	NP	3+	26%	1+	NP	2+	20%	1+	NP	--	--	--	--
7	L	25 F	4+	27%	4+	25%	4+	47%	4+	38%	3+	32%	4+	27%	4+	26%	4+	34%
8	PD	32 M	4+	2%	4+	3%	2+	1%	3+	1%	1+	NP	1+	NP	1+	NP	3+	1%
9	BS	70 M	2+	2%	1+	NP	2+	3%	1+	NP	1+	NP	1+	NP	--	--	--	--
10	MR	38 M	5+	2%	5+	2%	3+	5%	5+	5%	3+	6%	3+	8%	3+	3%	3+	4%
11	SS	50 M	2+	10%	2+	7%	2+	12%	1+	NP	1+	NP	1+	NP	--	--	--	--
12	RR	46 M	4+	16%	4+	21%	3+	18%	3+	15%	4+	24%	4+	26%	2+	14%	2+	25%
13	MC	25 M	2+	8%	1+	NP	1+	NP	1+	NP	1+	NP	1+	NP	1+	NP	1+	NP
14	K	18 F	3+	8%	3+	6%	1+	NP	2+	5%	1+	NP	1+	NP	1+	NP	1+	NP
15	P	37 M	3+	52%	3+	50%	4+	20%	4+	18%	4+	18%	4+	16%	4+	20%	4+	16%
16	MP	40 M	3+	1%	2+	1%	2+	3%	1+	NP	1+	NP	1+	NP	1+	NP	1+	NP
17	H	28 M	6+	26%	5+	28%	4+	29%	3+	34%	3+	30%	3+	31%	4+	28%	4+	29%
18	UK	29 M	4+	0%	4+	0%	3+	1%	3+	1%	2+	4%	2+	5%	4+	0%	4+	0%
19	PC	34 M	3+	5%	3+	5%	4+	10%	1+	NP	1+	NP	1+	NP	1+	NP	1+	NP
20	HS	49 M	2+	8%	1+	NP	3+	7%	1+	NP	--	--	--	--	3+	3%	1+	NP
21	JK	50 F	--	--	--	--	1+	NP	--	--	--	--	--	--	--	--	--	--
22	GP	38 M	1+	NP	1+	NP	1+	NP	2+	6%	1+	NP	1+	NP	1+	NP	1+	NP
23	PNJ	68 M	--	--	--	--	--	--	1+	NP	--	--	--	--	1+	NP	--	--
24	S	42 M	1+	NP	1+	NP	1+	NP	1+	NP	1+	NP	1+	NP	1+	NP	1+	NP
25	SR	25 M	1+	NP	2+	6%	1+	NP	1+	NP	1+	NP	1+	NP	--	--	--	--
26	G	35 M	1+	NP	1+	NP	--	--	--	--	1+	NP	1+	NP	--	--	--	--

Choice of sites for study of slit skin smears

Table 1. BI and MI at various sites in treated and untreated lepromatous patients (*continued*)

Sr No	Name	Age & Sex	Rt EL		Lt EL		Rt Elb		Lt Elb		Rt Fing		Lt Fing		Rt toe		Lt toe	
			BI	MI	BI	MI	BI	MI	BI	MI	BI	MI	BI	MI	BI	MI	BI	MI
27	RS	50 M	1+	NP	1+	NP	—	—	—	—	—	—	—	—	—	—	—	—
28	K	30 F	—	—	—	—	—	—	—	—	1+	NP	—	—	—	—	—	—
29	SD	36 F	1+	NP	3+	12%	—	—	—	—	—	—	—	—	—	—	—	—

Patients No 30 to 40: No bacilli could be seen at any of the above-mentioned sites, hence not charted.

Patients No 1–20: Untreated; 21–40: Treated.

EL – Ear lobule; Elb – Elbow; Fing – Finger; NP – MI not possible.

- (3) Ear lobule values were consistently higher than those obtained from toes (higher in 16).

Morphological index (MI) of untreated patients:

- (1) Ear lobules showed higher number of solid staining bacilli than those seen in smears from elbows (higher in 10, lower in 8).
- (2) Ear lobules gave higher MI values than those from fingers (higher in 13 and lower in 7).
- (3) Ear lobules gave values for MI which were higher than those seen in smears from toes (higher in 13).

Bacteriological index (BI) of treated patients:

- (1) No bacilli could be detected at any of the sites mentioned above in eleven patients.
- (2) Out of the other nine, maximum number (6) showed bacilli in the ear lobules followed by fingers (5), elbows (4) and toes (3).
- (3) In three patients bacilli could be detected from sites other than ear lobules (elbows—2, fingers—1, and toes—1; one patient gave positive results from elbows and toes).

Morphological index (MI) of treated patients.

- (1) Except in three situations (Elbow—1, Ear lobules—2) the bacilli were too scanty to enable counting.
- (2) Solid and fragmented bacilli were seen almost equally from all the sites wherever the smears were positive.

Discussion

It is evident from the results that BI and MI values were generally higher in the ear lobules in untreated as well as treated patients. There were, however, occasions where the BI and MI were higher at elbows, fingers and toes, especially in untreated lepromatous patients. At least in treated patients, where the MI was zero from ear lobules, solid staining bacilli could be detected from elbows and fingers.

The ear lobules uniformly showed bacilli more frequently than the other sites, but the differences were more significant in three (16.6%) long-treated patients, in whom no bacilli could be detected in the ear lobules, but were detectable from other sites like elbows, fingers and toes. The percentage though small is significant in relation to patients on long-term follow-up for the assessment of therapeutic response and discovery of resistant strains.

There is no uniformity of opinion regarding the selection and number of sites for demonstration of bacilli. One to eleven sites have been recommended by various workers (Davison, 1961;⁹ Dharmendra, 1967;¹⁰ Browne, 1959;¹¹

1966;¹² 1967;¹³ Leiker and Carling, 1969;¹⁴ Garrod and Ellard, 1968;¹⁵ Cochrane, 1964;² Bryceson and Pfaltzgraff, 1973;¹⁶ Levy, 1969;¹⁷ Jopling, 1971).¹⁸ Levy (1969),¹⁷ however, observed wide variation both in BI and MI from different sites.

Ears were found to have the higher BI followed by nearly similar results for buttocks in males and thighs in females. Arm, chest and back have been shown to have the lowest BI (Gideon and Job, 1965;¹⁹ Padma, 1965).²⁰ Recently Ahmed *et al* (1979)²¹ found higher BI from ear lobes, buttocks, face, fingers and toes whereas legs, arms and back were found to have the lowest BI. MI was found to be correspondingly high where BI was more. In a similar study, Kaur *et al* (to be published)²² found higher BI and MI in ear lobes and fingers as compared to that from knees and elbows. Hiramalini *et al* (1978)²³ found fingers and uncovered toes to be the most productive sites, even better than ear lobules. The present study for untreated and treated lepromatous patients confirms the findings of Gideon and Job (1965),¹⁹ Padma (1965)²⁰ and Ahmed *et al* (1979),²¹ in showing that ear lobules have the maximum BI and MI as compared to the other sites, except in isolated cases where elbows, fingers and toes gave marginally higher values. In eleven out of the twenty long-treated patients, no bacilli could be detected at any site. In the remaining nine, in whom bacilli were demonstrated, ear lobules were positive in six followed by elbows, fingers and toes. In three patients, bacilli could be detected only from sites other than the ear lobes.

On the basis of the above observations it is recommended that in addition to the standard ear lobe smears it is rational and essential to study multiple sites including the peripheral sites like fingers and toes. This has been previously recommended (Hiramalini *et al*, 1978;²³ Ahmed *et al*, 1979;²¹ Jopling *et al*, 1979;⁶ Ridley *et al*, 1976).⁵

Why do bacilli remain viable in the peripheral areas? We consider that there is more to it than the coolness of the parts only. A lower drug concentration consequent to various peripheral vascular abnormalities (Kaur *et al*, 1976)²⁴ may be the reason for persistence of bacilli. The inclusion of superficial cutaneous twigs in the slit on the dorsal aspects of fingers and toes may theoretically improve the chances of finding the bacilli at these sites. All this, however, should not detract from the importance of studying multiple sites, which are essential to reveal any persistent bacilli in the skin.

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The mononuclear cell series in leprosy: an ultrastructural report

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Summary Ultrastructural observations have been carried out on the mononuclear phagocytic series, the host cells of *Mycobacterium leprae*, throughout the spectrum of leprosy. The changes seen in these cells at various points in the immunological spectrum indicated differences ranging from the state of non-specific stimulation which occurs on the entry of *M. leprae* into the macrophage to the more specific activation which takes place in the presence of immune mechanisms. The most highly differentiated of the cell types was the epithelioid cell which appeared when hypersensitivity was maximal, in tuberculoid lesions and in positive Mitsuda skin tests. Cells described in the literature as 'A' type epithelioid thought to be an immature epithelioid cell had much rough endoplasmic reticulum while 'B' type epithelioid cells thought to be an end cell had numerous smooth lined vesicles. Lepromatous leprosy was characterized by a small undifferentiated immature bacteria-laden macrophage. An intermediate cell stage was seen in BB leprosy. The cells of BB leprosy were activated macrophages with high phagocytic potential. All gradations in the proportion of endoplasmic reticulum to vesicles of macrophages, from the activated cell seen in BB to 'A' type epithelioid cells seen in low antigen, high resistance BT to mainly 'B' type cells in downgrading BT were encountered. This supported the continuous spectrum of macrophage development seen throughout the spectrum of leprosy.

Introduction

Ultrastructural studies on the spectrum of leprosy have been carried out with special reference to degradation and absorption of bacilli,^{1,2} and to 'bacillated epithelioid cells' in borderline leprosy³ and to certain aspects of tuberculoid leprosy.⁴ Nishiura,⁵ described the electron microscopical appearances of cells in the spectrum of leprosy as it existed at that time (this was before the present immunological spectrum was described). Most of these studies were carried out on material fixed and processed in ways which today would not be regarded as optimal for ultrastructural preservation and presentation. The present work,

therefore, is concerned with a re-examination of the host cell, the macrophage, and the changes seen in it at various points in the immunological spectrum of leprosy. A preliminary account of this work has already been presented.⁶ The present report describes more particularly the epithelioid cell and its relation to other cells of the spectrum.

Material

PATIENTS

Investigations were carried out on biopsies taken from eight patients with BT (borderline-tuberculoid) leprosy. One patient was undergoing an upgrading BT reaction (BTR). Two BB (mid-borderline) and three BL (borderline-lepromatous) cases were included as well as three patients with LL (polar lepromatous) leprosy. One of these patients was in relapse following resistance to treatment. Two Mitsuda skin tests performed on two BT patients were examined 30 days after intradermal injection of antigen (heat-killed *M. leprae* extracted from human tissue). One histoid biopsy from a patient not previously treated for LL leprosy was also examined. This biopsy has been reported elsewhere. Material was obtained from the Sungei Buloh Leprosarium, Malaysia; The Armauer Hansen Research Institute, Addis Ababa, Ethiopia; The State Hospital, Gongola, Nigeria and the Hospital for Tropical Diseases, London.

Methods

Biopsies were divided into two, half being fixed, examined and classified according to Ridley.⁷ Routine stains were haematoxylin eosin, PAS, Mallory's trichrome and reticulin by Gomori. The other half was placed in ice cold 3% glutaraldehyde in cacodylate buffer pH 7.4 and quickly cut into small pieces measuring 1 mm. Fixation was carried out for 24 h before transferring to a washing buffer solution. The material was post fixed in osmium tetroxide and embedded in an araldite epon mixture. For orientation and comparative study semi-thin sections from multiple sites of each sample were cut and stained by methylene blue-azur-basic fuchsin. Ultra thin sections were cut and stained with uranyl acetate and lead citrate and examined in a AEI 801 microscope.

Results

LEPROMATOUS LEPROSY (ACTIVE RELAPSE) (LL)

Descriptive terminology of cell types is given in Table 1.

Table 1. (By permission of Dr D O Adams.)

Histology	Size	Shape	Nucleus	Nucleolus	Chromatin	Cytoplasm	Cell border	
Mononuclear cell	Small	Round	Central, round	None	Dark	Scanty, basophilic	Distinct	
Immature macrophage	Medium	Round	Central, round	Small	Slightly vesiculated	Slightly increased; Slightly foamy	Well-defined	LL
Mature macrophage	Medium – large	Polygonal	Fairly, large	Large, eosinophilic	Open, vesiculated; slightly marginated	Less foamy	Cell touch lightly granuloma	BL
Immature epithelioid cell	Large	Oval	Large, eccentric, kidney-shaped	Less prominent	Completely vesiculated; slightly marginated	Hyaline, eosinophilic	Cells touch closely	BT/ BB
Epithelioid cell	Large	Elongated	Large, eccentric, kidney-shaped	Large, eosinophilic	Marginated	Abundant, pale	Cells touch closely Tubercles	BTR

Light microscopy

The granuloma was composed of macrophages laden with *M. leprae*. The cells appeared eosinophilic and there was no foamy change.

Electron microscopy

Macrophages were closely interdigitating cells with distinct margins. Few bacilli were seen in them. Mitochondria were sparse and Golgi apparatus was well developed, showing dilated vesicles and lamellar cisternae characteristic of cells in active synthesis.

LEPROMATOUS LEPROSY (QUIESCENT) (LL) (Fig. 1)

Light microscopy

Macrophages filled with *M. leprae* in various stages of degeneration were seen in sheets. Foam cells were predominant. Lymphocytes were few and inconspicuous.

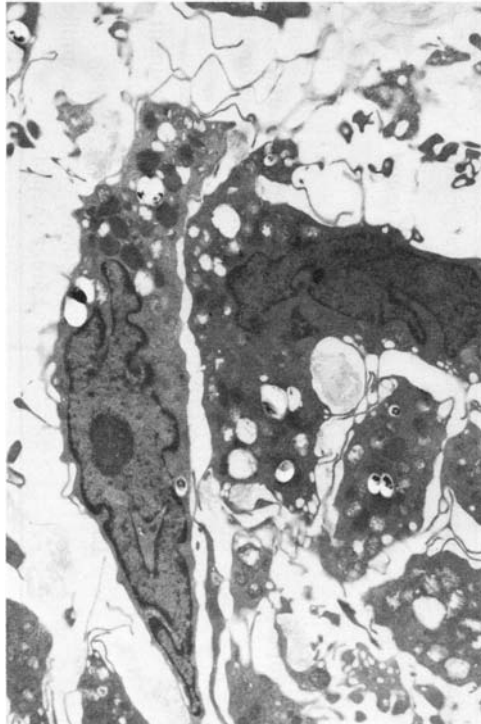


Figure 1. LL leprosy. Macrophages have cytoplasm filled with degrading bacilli. Many pseudopodia and membraneous marginal processes are characteristic. $\times 8000$.

Electron microscopy

The macrophages were characterized by a large, irregular-shaped nucleus with a marginal band of chromatin. The nuclear membrane was clearly visible and nuclear pores could be seen. In the cytoplasm there was scanty endoplasmic reticulum of a granular type, a Golgi apparatus with small structural elements and moderate numbers of mitochondria of small size and typical structure. Dense bodies which would not clearly be demonstrated as lysosomes were present. Residual bodies and myelin bodies were seen. The cytoplasm was thrown into a number of pseudopodia some of which were long and villous like and these gave the appearance of being closely bound together. Numerous interdigitating endoplasmic processes extended from the membrane of each cell and it was these long processes devoid of any cytoplasmic organelles which bound the cells closely together in a compact mass. Varying amounts of cytoplasm occupied the cell dependent on the bacterial load. Bacteria were in various stages of degradation. Electron transparent zones were present and caused the cytoplasm of the cell to be pushed to the boundary giving a foamy appearance by light microscopy. The cytoplasm contained a few mitochondria and no other recognizable organelles. Other cell types seen were fibroblasts and the intracellular space was occupied by remnants of bacteria-containing cells. No extracellular bacilli were seen.

BORDERLINE LEPROMATOUS (BL) (Fig. 2)

Light microscopy

Macrophages were similar to those of LL active relapse. No foam cells were seen. Bacilli were less numerous than in LL. Lymphocytes and plasma cells were numerous. Some areas of the section showed epithelioid cell foci.

Electron microscopy

Macrophages were similar to those of lepromatous leprosy but with fewer bacilli. They had long cytoplasmic processes which made intimate contact with neighbouring cells, often lymphocytes. In other areas larger cells with few bacilli had more cytoplasm relative to the nucleus and large numbers of lysosomes. No phagosome-lysosome fusion was noted. The membrane of these cells was thrown into folds. Mitochondria were numerous. This area corresponded to 'epithelioid foci' seen in thick section. Other cell types included large numbers of secretory cells of plasmacytoid appearance with much rough endoplasmic reticulum arranged in concentric lamellae. These cells had one or two endocytosed *M. leprae*. The nucleus was large, oval and stellate because of electron dense material interspersed between electron transparent zones. The cells were of the size of small macrophages and lymphocytes were always in communication with them by short villous projections.

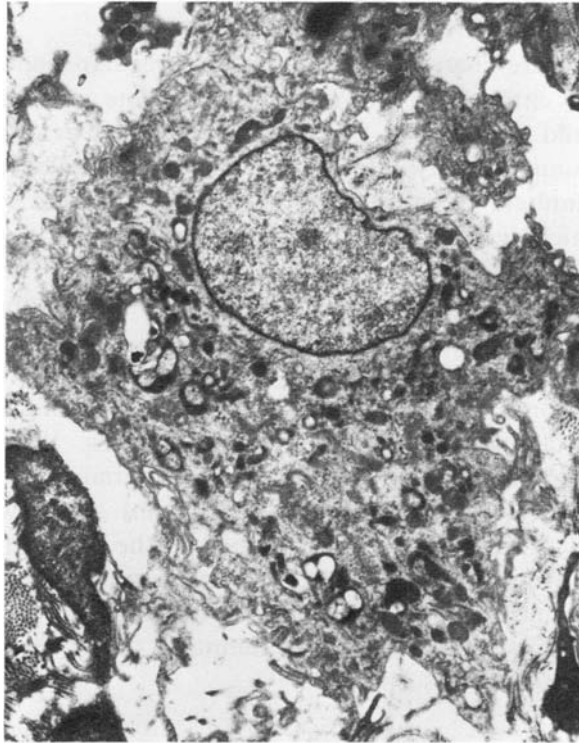


Figure 2. BL leprosy. Macrophages have more cytoplasm and fewer bacilli than LL. Lysosomes are present. $\times 8000$.

MID BORDERLINE (BB) (Fig. 3)

Light microscopy

Sheets of epithelioid cells were seen. There were a few bacilli in some cells.

Electron microscopy

The predominating cells were larger than those of BL and smaller than epithelioid cells of BT leprosy. The cytoplasm was filled with many mitochondria and Golgi apparatus was well developed with typical structural elements. Endoplasmic reticulum was scanty and in short strands. Some vesicles were seen. One or two bacilli were endocytosed. These did not cause any disorganization of organelles. The cells were arranged close together separated by granular oedema. The margins did not interdigitate. The nucleus was large and oval with dense granular chromatin. The cell had an activated appearance and was intermediate between those of BL and mature epithelioid cells of BT. This was evident in the proportion of endoplasmic reticulum to cytoplasmic organelles.

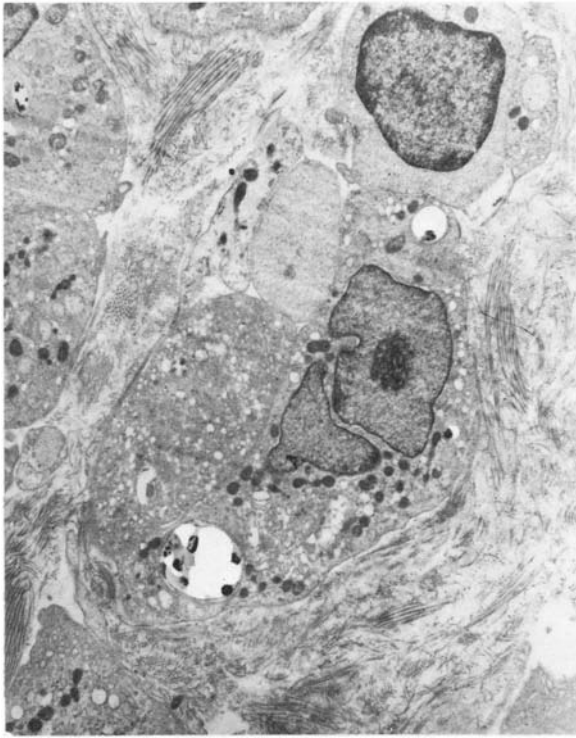


Figure 3. BB leprosy. Activated macrophages have many cytoplasmic organelles and one or two bacilli. $\times 8000$.

BORDERLINE TUBERCULOID (BT) (Fig. 4)

Light microscopy

Epithelioid cell granulomas were found in three types: (1) as a sheet of cells with a peripheral ring of lymphocytes, (2) as a small central focus cuffed by several layers of lymphocytes and (3) as a 'tubercle', each cell being bounded by lymphocytes which were also found at the periphery. Langhan's giant cells were often seen.

Electron microscopy

Epithelioid cells were distinguished by their large size, abundant cytoplasm and large pale oval nucleus. Two types of epithelioid cell could be distinguished. One, the 'A' type cell had much endoplasmic reticulum, a well-developed Golgi apparatus, numerous small mitochondria and smooth interdigitating cell margins. The second type of epithelioid cell was vesiculated. This was consistent with 'B' type of epithelioid cell. The margins of the vesicles were smooth.

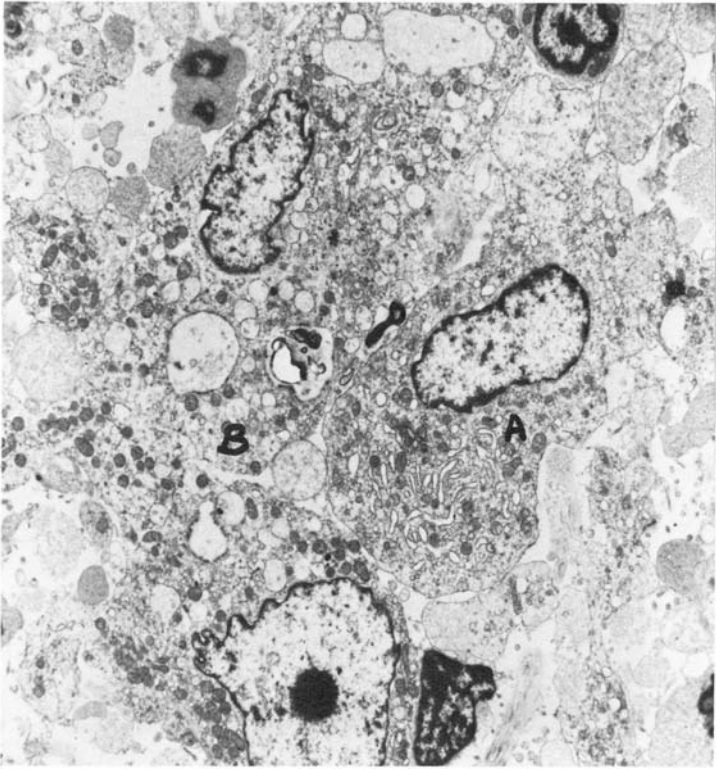


Figure 4. BT leprosy. 'A' and 'B' type epithelioid cells 'A' type has much endoplasmic reticulum. 'B' type is characteristically vacuolated. $\times 8000$.

No endoplasmic reticulum was present. Mitochondria were small and few in number in this type of cell. Cell margins were often indistinct, although sometimes they were smooth and interdigitating. The three types of epithelioid cell granulomas distinguished by light microscopy were composed of 'A' and 'B' type cells in varying proportions. In (1) 'A' and 'B' type cells could be distinguished side by side; in (2) 'B' type cells predominated, and in (3) 'A' type cells predominated. When bacilli were found they were present either sequestered in macrophages (Fig. 5), or they were found in a macrophage surrounded by epithelioid cells all of which were orientated in the direction of the bacillus-containing cell. All stages of cells intermediate between 'A' and 'B' type epithelioid cells were seen. Some cells had both vesicles and small amounts of endoplasmic reticulum (Fig. 6). Giant cells of the Langhan's pattern were sometimes present. Like the epithelioid cells these were of two varieties: those which contained many small mitochondria, vesicles and endoplasmic reticulum (Fig. 7) and those which were mainly vesiculated with no endoplasmic reticulum (Fig. 8). Large numbers of vesicles containing granular material were seen at the periphery of the cells in the intercellular space of these lesions of BT leprosy.

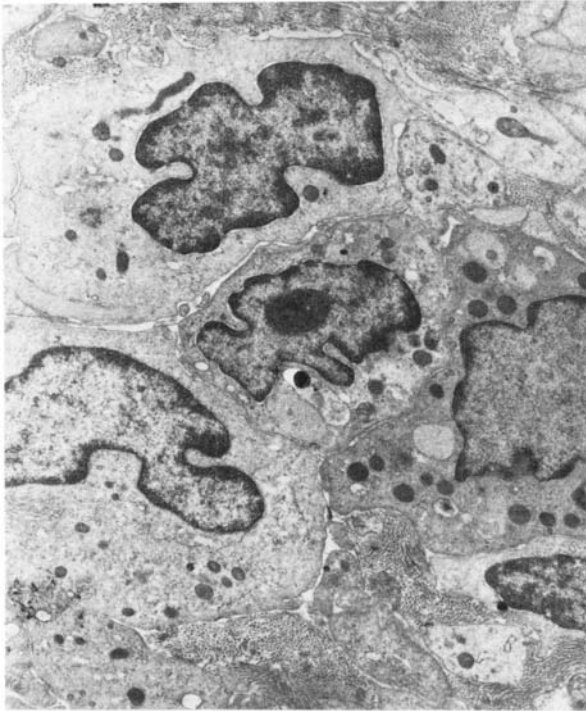


Figure 5. BT leprosy. *M. leprae* sequestered in macrophages. $\times 8000$.

BORDERLINE TUBERCULOID REACTION (BTR) (Fig. 9)

Light microscopy

Scattered foci of 'tubercles' were seen. Epithelioid cells were arranged spirally, separated by much oedema. Lymphocytes were present among the epithelioid cells. No bacilli were found.

Electron microscopy

Typical 'A' type epithelioid cells were seen sometimes separated by oedema and sometimes closely interdigitating. These cells had abundant endoplasmic reticulum, well-developed Golgi apparatus and few if any vesicles. Mitochondria were numerous. In addition to epithelioid cells there were large pale cells with much cytoplasm relative to the nucleus. The cytoplasm of these cells was composed of many free polyribosomes and in some cells organization of the polyribosomes into rough endoplasmic reticulum could be distinguished. The nucleus of these cells was large, oval and pale with much chromatin at the rim. The appearance was of a blast cell. Death of epithelioid cells was seen as degenerate mitochondria, no Golgi apparatus and degenerating cell margins (Fig. 10).

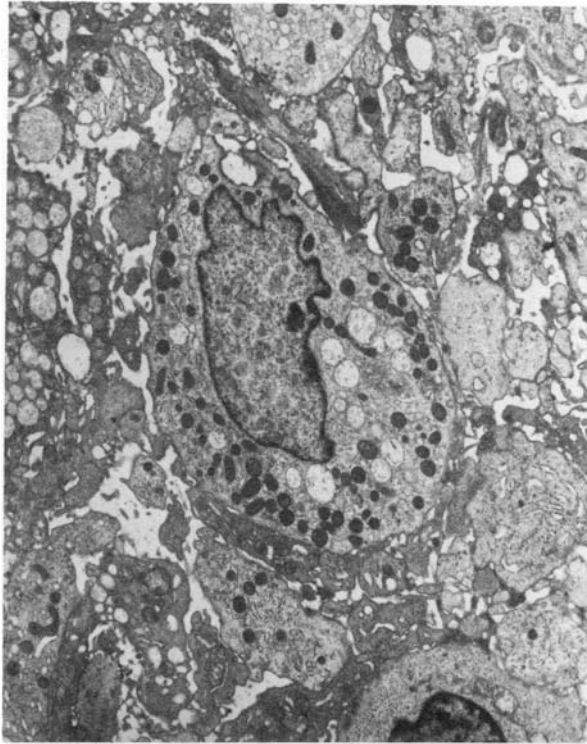


Figure 6. BT leprosy. Epithelioid cell intermediate between 'A' and 'B' type cell. $\times 8000$.

MITSUDA SKIN TEST

Light microscopy

The lesion was extremely vascular and one area of polymorph necrosis was seen. Epithelioid cells and Langhan's giant cells were also present among other cells of the mononuclear phagocyte series. Lymphocytes and plasma cells were also seen.

Electron microscopy

Epithelioid 'A' type cells were separated by oedema. Sometimes these cells appeared as plasmacytoid cells with much rough endoplasmic reticulum. Plasmacytoid cells were large and ovoid with an eccentric nucleus and well-developed Golgi apparatus with enlarged cisternae and lamellae. Occasionally one or two dead (heat killed) *M. leprae* were endocytosed and occupied variable positions in the cell. Typical 'A' type epithelioid cells had no bacilli in them (Fig. 11). The necrotic area was composed of polymorphs and degenerate cells. In one area there was a coagulum of cell constituents and debris. This was a very homogeneous mass.

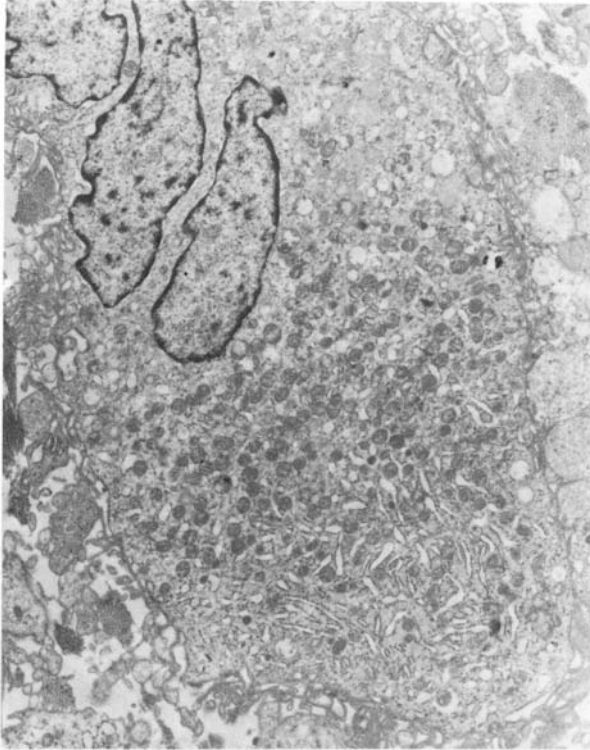


Figure 7. BT leprosy. Langhans giant cell with much endoplasmic reticulum similar to ‘A’ type epithelioid cell. $\times 8000$.

Discussion

The differentiation of cells of the mononuclear phagocyte series (MPS) through the spectrum of leprosy supported the concept of a continuous spectrum of these cells as reported by Adams.⁸ The intermediate cell stage occurred in BB and the highest differentiation was seen in borderline tuberculoid leprosy (Polar tuberculoid leprosy was not examined). This was the epithelioid cell.

Lepromatous leprosy is divided into the active phase and quiescent phase. The macrophage in each group was quite distinct although in both cases, an undifferentiated cell. In active disease the cell had smooth margins and very large nucleus as is characteristic of monocytes or dividing macrophages. There was much cell death in such lesions. In the absence of thymidine labelling and kinetics these features might indicate high cell turnover. Alternatively in quiescent leprosy macrophages were filled with fat foam and degenerate bacilli. Very long filamentous projections extended from the cytoplasmic margin binding neighbouring cells. These features are found in long-lived macrophages.

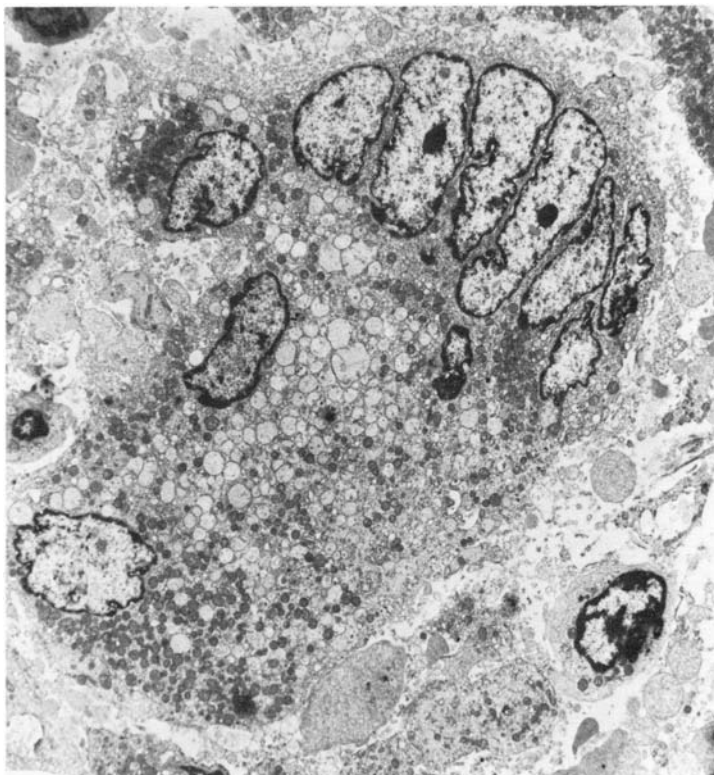


Figure 8. BT leprosy. Langhans giant cell with many vesicles similar to 'B' type epithelioid cell. $\times 8000$.

Electron microscopical observations of the various phases of LL lesions thus supported the histological appearance which led to the view that in active especially histoid lepromas there is a high cell turnover, in regressing lesions with foamy effete macrophages a low turnover rate.^{9, 10}

Epithelioid cells have been described as 'A' and 'B' type cells.^{11, 12}

The cells of BB leprosy represented an intermediate cell stage between macrophages and highly activated cells which could not with certainty be distinguished from immature epithelioid 'A' type cells. No epithelioid 'B' type cells were ever encountered in BB, and mature epithelioid cells may not occur in this group except when the lesion inclines to the BT pole, bacterial density is low and the lesion is contained. BB leprosy is an unstable group and rarely remains long as BB.

Both 'A' and 'B' cell types were present in the BT samples examined here. Epithelioid cells lose their Fc receptors and express only the C₃ receptor.^{13, 14} This might indicate altered cell function such as the removal of immune complexes¹⁵ when the Fc receptor is no longer necessary. Well-developed endoplasmic reticulum may be an indication of a secretory role where the

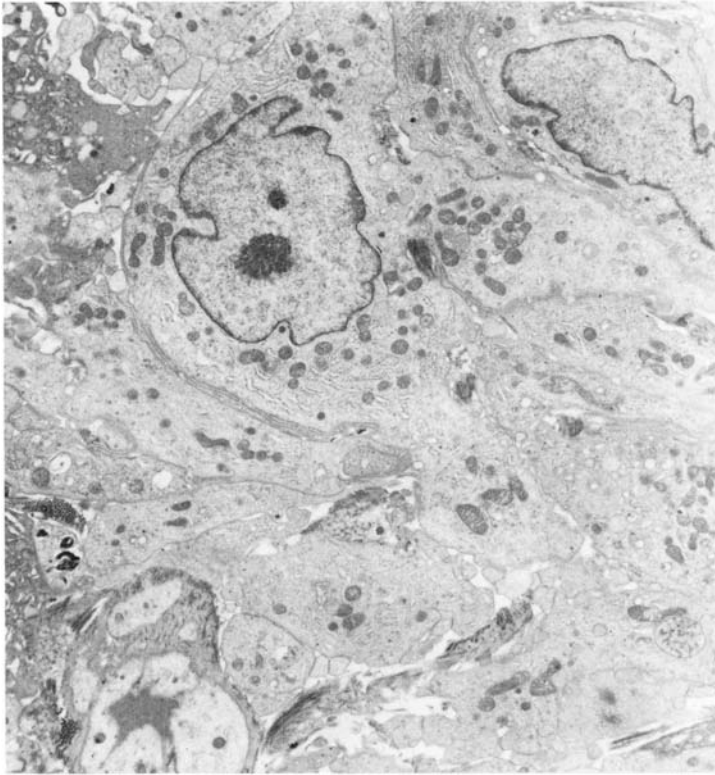


Figure 9. BT in upgrating reaction. 'A' type epithelioid cell. $\times 8000$.

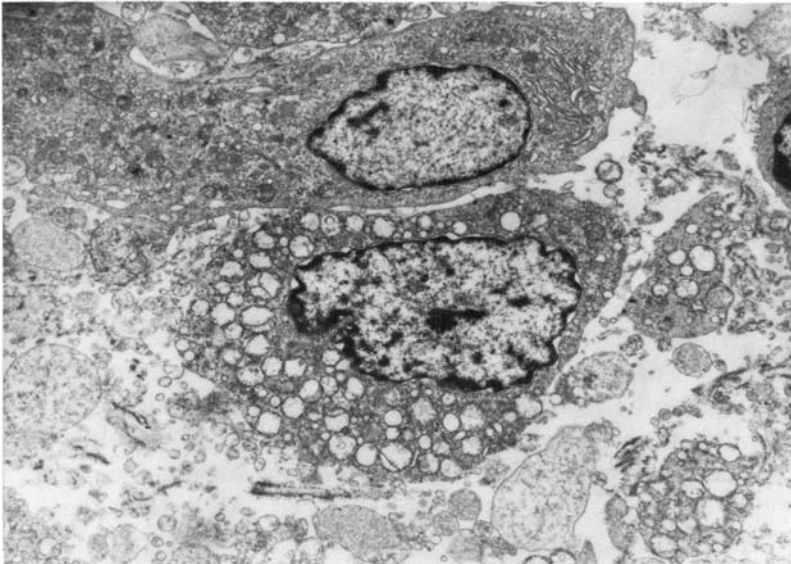


Figure 10. Death of epithelioid cell. One cell shows intact mitochondria, Golgi apparatus and endoplasmic reticulum. The other cell shows swollen degenerate mitochondria, no Golgi and indistinct cell margins. $\times 8000$.

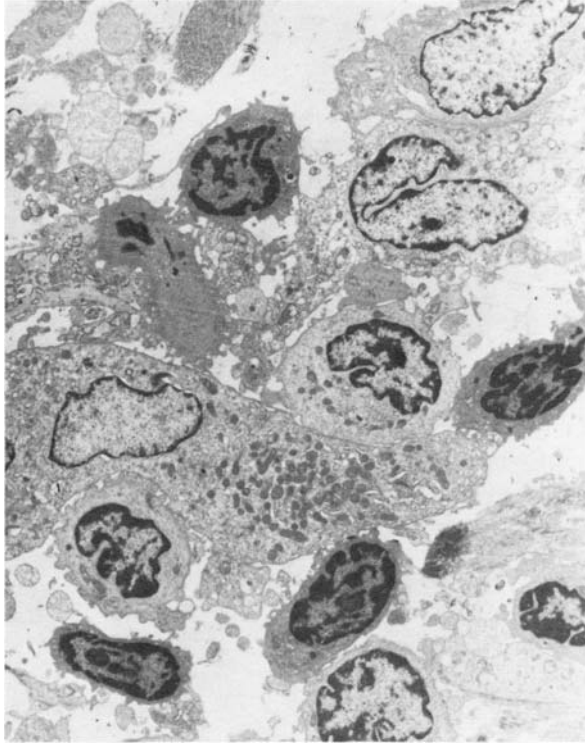


Figure 11. Mitsuda skin test – Epithelioid cell surrounded by lymphocytes. $\times 4000$.

product of secretion, usually a protein, is used by the cell itself resulting in a metabolically activated cell, or for export. Experimental evidence (personal observation) indicates that some immune complexes stimulate the macrophage to develop much rough endoplasmic reticulum for its own activation. The stimulus which governs the differentiation of cells of the mononuclear phagocyte series in the direction of epithelioid cells in leprosy could not be elucidated. Low bacterial density and high host resistance play a part. When small numbers of *M. leprae* were found they were seen sequestered in macrophages. Epithelioid cells in such areas were seen not in 'tubercles' but as a sheet of cells composed of both 'A' and 'B' type cells. The random arrangement of these cells suggests the instability of the lesion and its predisposition to downgrade. Lymphocytes were rarely seen in such lesions. The nature of the cells both of 'A' and 'B' type occurring together would suggest the degeneration of the 'A' type cell into the vesiculated 'B' cell. All intermediate stages of degeneration of the 'A' type cell, beginning with mitochondrial degeneration and proceeding to increase and coalescence of small vesicles, and the eventual typical 'B' cell with much vesiculation and devoid of rough endoplasmic reticulum were seen. The positive PAS reaction seen by light microscopy in the

vesicles of 'B' epithelioid cells indicates a glycoprotein which may be precipitated as a coagulum when these cells disintegrate. This process would be similar to that seen when some epithelial cells degenerate.¹⁶ The process is not exactly understood. The vast amounts of intercellular material seen in BT lesions in this study would support this hypothesis. No such material was seen among the macrophages of LL leprosy. The way in which epithelioid cells degenerated indicated that the degeneration is brought about by functional exhaustion, not by physiological means, physiological degeneration being variable as in lepromatous leprosy. Degeneration in macrophages of lepromatous leprosy was seen as cloudy swelling, fat and foam. The finding of epithelioid cells only in hypersensitivity states suggests that this cell occurs only when the immune response is maximal and that it is therefore a marker for immunological granulomas. Epithelioid cells were not associated in this study with fibroblasts as they are in sarcoidosis.

Acknowledgements

I am very grateful to my husband Dr D S Ridley for his comments, to Dr R J W Rees for arranging the supply of material and to Drs A B G Laing, M F R Waters and Anthony Bryceson for biopsy material and clinical data. The study was supported by LEPROA.

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Dapsone compliance in North-east India

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Summary The dapsone/creatinine ratios of urine samples collected from out-patients attending a mission hospital clinic in North-east India indicated that only about a half of the patients had ingested their prescribed dapsone within the previous 24 h.

Introduction

Previous investigations undertaken in various parts of Africa and Asia have shown that leprosy patients often self-administer their prescribed dapsone treatment irregularly.¹⁻⁵ This report describes a study of dapsone compliance among patients being treated by the Leprosy Mission Hospital, Muzaffarpur, in North-east India.

Methods

Urine samples were collected from male staff members of the hospital who were not taking dapsone, from male in-patients 24-h after their previous daily supervised dose of 100 mg dapsone and from male out-patients at the time of their visit to collect a further stock of 100 mg dapsone tablets for daily self-administration. A small crystal of thymol was then added to each urine sample and the samples stored without refrigeration at ambient temperatures of 75° to 100°F for 4 months until analysis in London. Urinary concentrations of diazotisable compounds (as dapsone equivalents) were determined by a modification of the Bratton and Marshall procedure, creatinine concentrations were determined using the alkaline picrate method, and 'dapsone/creatinine' ratios then calculated.⁶

Results and discussion

In about a fifth of the samples (14 out of 72) abnormally low concentrations of creatinine (less than 0.1 mg/ml) were encountered despite seemingly normal levels of diazotisable compounds. It was surmized that in these samples creatinine must have been bacterially degraded, although the reason why the thymol had apparently failed to act as a preservative is not understood. The creatinine concentrations of the remaining 58 samples appeared to be normal (range 0.16 to 1.84 mg/ml, mean 0.53 mg/ml). Although the numbers of control samples were small, the results obtained were in accord with those obtained in previous studies from Addis Ababa in Ethiopia¹ and from Dichpalli in India.⁷ Thus the mean dapsone/creatinine ratios ($\mu\text{g}/\text{mg}$) averaged 9 for the 5 controls not on dapsone as compared with 7 from Addis Ababa and Dichpalli, while those from the supervised controls being treated with 100 mg dapsone a day averaged 92 as compared with 80 from Addis Ababa and 102 from Dichpalli.

The results obtained from the 44 satisfactory urine samples collected from the out-patients were interpreted in the light of those from the treated and untreated controls in Muzaffarpur, Dichpalli and Addis Ababa. Dapsone/creatinine ratios of greater than 50, indicating that a dose of 100 mg dapsone had been taken within the 24 h prior to the clinic, were found in only about a half of the urine samples. Furthermore 11 of the urine samples had dapsone/creatinine ratios of less than 16.8, the highest value for the untreated controls, indicating that about a quarter of the patients had probably not taken their prescribed treatment for at least the previous 3–4 days. The results of this small study therefore support the findings of previous investigations indicating that poor dapsone compliance is probably very widespread. It was also apparent that some patients failed to appreciate that their running out of dapsone tablets was often a consequence of missed out-patient clinic appointments. Hence the importance of educating patients about the dangers of irregular treatment.

Acknowledgements

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An assessment of dapsone self-administration in Gudiyatham Taluk. How should urinary dapsone/creatinine ratios be used?

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Summary The self-administration of daily doses of 50 mg and 100 mg dapsone by leprosy patients in Gudiyatham Taluk, South India, was monitored by measuring urinary dapsone/creatinine ratios in out-patients compared with a group of in-patient controls on supervised doses of dapsone. A new method is described to analyse the results of such a survey; if used widely this would allow comparison between the results of surveys in different areas. Overall 37% of the 189 out-patients tested were judged to be taking dapsone regularly. The results were the same whether patients were tested at clinics or at surprise home visits. The implications of these results are discussed with reference to leprosy control schemes and to further studies of this kind.

Introduction

Dapsone is still the most important drug used in the treatment of leprosy, it is cheap and effectively controls the disease if patients take the tablets regularly. In South India, patients with leprosy are prescribed dapsone on a daily basis, a 25 mg tablet for children, a 50 mg tablet for adults unless they have Lepromatous leprosy, when a 100 mg tablet is prescribed.

In Gudiyatham Taluk in South India, which has a population of 420,000, there are over 8,000 patients who suffer from leprosy: they live at home in villages or small towns, and attend monthly clinics located within 5 km of their homes. Initially patients attend a clinic each month to receive their supply of dapsone tablets, but later they come four times a year and receive 3 months' supply of dapsone. In order to encourage regular attendance, Paramedical Workers visit patients on the day before they should come to a clinic, and on average 75% of patients then attend as requested.

I set out to find out how many of these patients, who came to clinics and collected their dapsone tablets, actually ingested the tablets when they went home. The patients are expected to take daily tablets for at least five years and it would not be surprising if compliance was low. Studies in other parts of the

world using urinary dapsone/creatinine ratios to assess compliance, have indicated that only about a half of patients take their tablets regularly,¹⁻³ but a study in 1976 from Karigiri Hospital in Gudiyatham Taluk indicated that about three-quarters of patients were taking their dapsone regularly.⁴

Sampling methods

The out-patient clinics at Gudiyatham Taluk are divided into three areas: Area I is within 20 km of the hospital, Area II within 80 km and Area III within 150 km. Ten clinics were randomly chosen for the study, four from Area I, two from Area II and four from Area III. Urine samples were obtained from a total of 73 patients at their visit to their out-patient clinic. Urine samples were also collected from 116 patients from the same clinics by means of surprise visits to their homes. These were carried out early in the morning by the author and a Paramedical Worker, and were at least a week before the next clinic visit was due. All patients were randomly selected on the morning of the visit to rule out any special attention by Paramedical Workers to the patients involved in the study.

Information was collected for each patient about age, disabilities, date of registration, mode of detection, attendance at clinics in the previous year, type of leprosy and the distance they had to come to the clinic.

Urine tests to estimate compliance

The urinary ratios of the concentration of dapsone plus its diazotisable metabolites to creatinine (D/C ratios) were measured by the method described by Ellard *et al*,⁵ using a Unicam SP 600 spectrophotometer provided by Karigiri Hospital and standards provided by Dr Ellard. 'Blank' D/C ratios were determined by analysing urine samples from 22 healthy members of the hospital staff at Karigiri who were not taking dapsone. Significant blank ratios are due to the presence of natural diazotisable compounds in the urine.

In order to assess the D/C ratio in fully supervised patients urine samples were collected from 36 in-patients at Karigiri Hospital. Twentyfour patients received 50 mg dapsone daily and 12 patients received 100 mg dapsone daily given in the morning by the nurses. All of these patients provided urine samples 24 h after taking a dose of dapsone, and some provided a further sample 6 h after taking the next dose.

Results

CONTROLS

The mean 'blank' D/C ratio for the 22 staff members not taking dapsone was 7.5 $\mu\text{g}/\text{mg}$, with individual results ranging from 1.1 to 16.0 $\mu\text{g}/\text{mg}$. In the fully

supervised patients taking 50 mg dapsone daily the mean D/C ratio at 24 h was 48.9 $\mu\text{g}/\text{mg}$ (standard deviation 11.2 $\mu\text{g}/\text{mg}$), and in those taking 100 mg dapsone daily it was 83.8 $\mu\text{g}/\text{mg}$ (SD 28.2 $\mu\text{g}/\text{mg}$). The distribution of individual control and out-patient D/C ratios is shown in Fig. 1.

Development of criteria for assessing compliance

The D/C ratios of the urine samples collected from the controls were analysed in such a way as to enable criteria to be developed to judge whether or not it was likely that out-patients had ingested their last dose of dapsone within the previous 24 h prior to the collection of the urine sample. For this purpose I assumed that dapsone is fully absorbed within 6 h and that the urinary rate of elimination of dapsone and its diazotisable metabolites thereafter falls exponentially.⁶ The half-life for the elimination of dapsone and its metabolites could therefore be calculated from the mean 6 h and 24 h D/C ratios of the supervised controls after subtracting the mean blank D/C ratio (7.5 $\mu\text{g}/\text{mg}$) since this does not change with time. This yielded a mean half-life of 25 h which is similar to that of 24 h found by Ellard.⁶ This half-life was then employed to estimate the scatter of D/C ratios that might have been expected at 48 h. The observed 24 h D/C ratios of the controls after supervised daily doses of 50 and 100 mg dapsone together with the calculated 48 h ratios are illustrated in Fig. 2. The 24 h and 48 h distributions overlapped and the horizontal lines shown in Fig. 2 represent the D/C ratios that would imply that there was an equal chance that such a patient had ingested their last dapsone dose either 24 h or 48 h previously I therefore classified out-patients as 'regular' in self-administering dapsone if their D/C ratios exceeded these cut-off points (37.5 and 60 $\mu\text{g}/\text{mg}$ respectively for dosage with 50 or 100 mg dapsone daily), and 'irregular' if they fell below such values yet exceeded the highest D/C ratio for the untreated controls (16 $\mu\text{g}/\text{mg}$). Out-patients with D/C ratios of less than 16 $\mu\text{g}/\text{mg}$ were classified as 'grossly irregular'. The distributions of D/C ratios illustrated in Figs. 1 and 2 indicate that out-patients classified as 'regular' had probably taken a dose of dapsone with the previous 24 h, that those judged to be 'irregular' had probably omitted to take a dose of dapsone the previous day but had in all probability self-administered some treatment within the 48 h preceding collection of the urine sample. By contrast 'grossly irregular' patients had probably taken no drug in the previous 3 or 4 days.

OUT-PATIENTS

Tables 1 and 2 summarize this classification of the out-patients and include estimates of the average time since each group of patients last ingested a dapsone tablet. These times were estimated by dividing the mean D/C ratio of the out-patients by that of the controls after correcting for the blank and

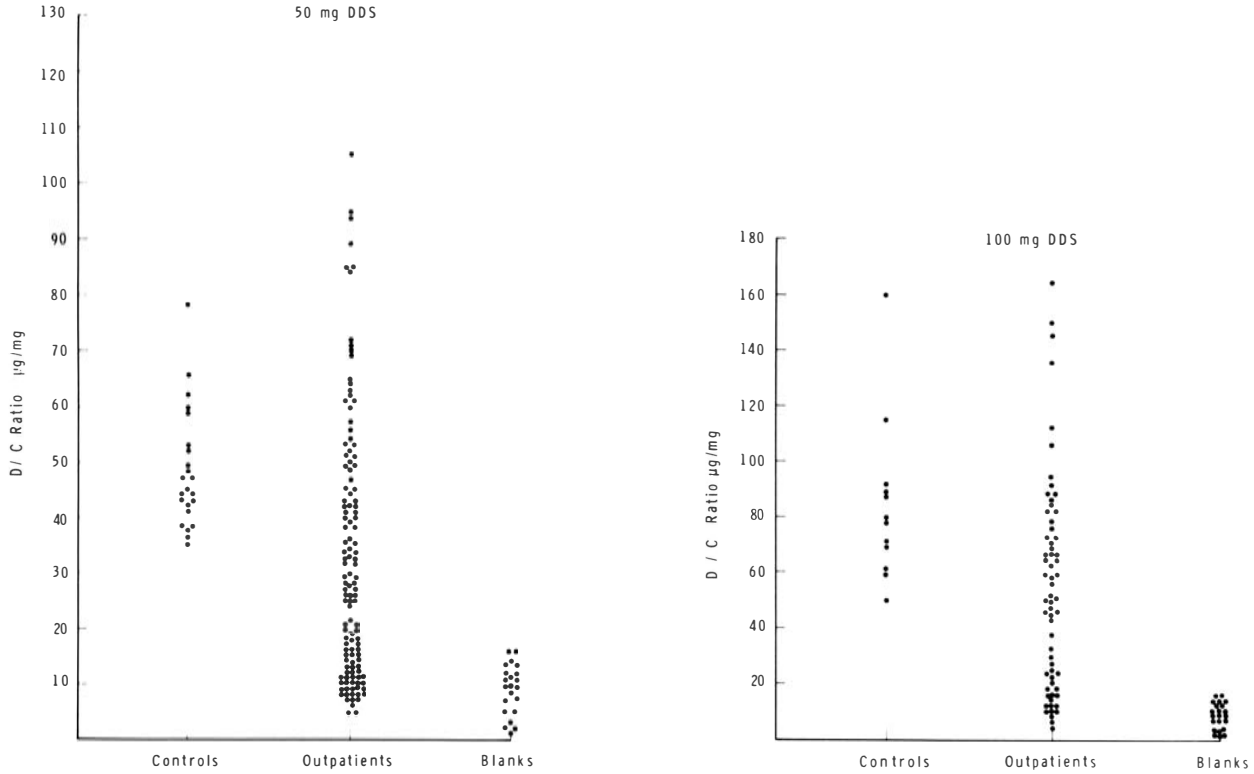


Figure 1. Urinary D/C ratios in controls and out-patients taking 50 mg and 100 mg dapsone daily.

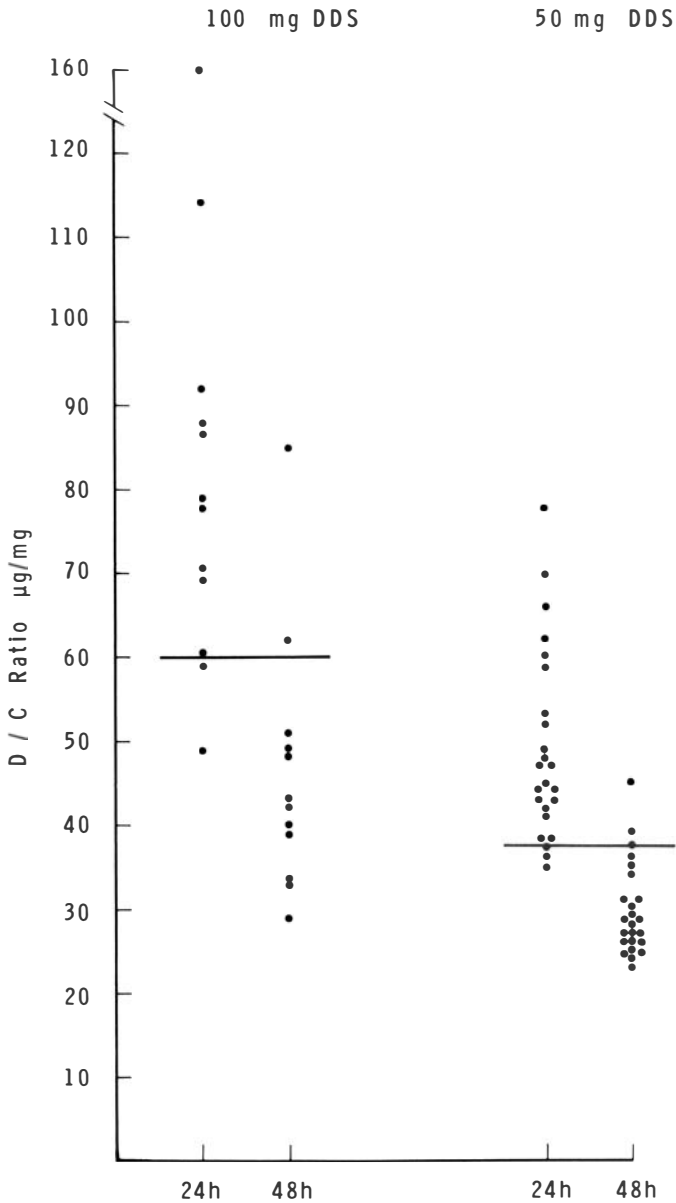


Figure 2. Urinary D/C ratios at 24 h and 48 h in controls on 50 mg and 100 mg dapsone.

utilizing the calculated half-life of 25 h for the assumed exponential fall in the rate of elimination of dapsone plus its diazotisable metabolites.

Of 189 out-patients tested at clinics and at home, 71 were classified as 'regular', 58 as 'irregular' and 60 as 'grossly irregular'. The overall compliance was similar whether measured at clinics or at home-visits between clinics. Of 15

Table 1. Comparison of D/C ratios of urine samples from supervised controls and out-patients on 50 mg DDS daily

	Number	Mean D/C ratio $\mu\text{g}/\text{mg}$	Range of D/C ratios $\mu\text{g}/\text{mg}$	Out-patient mean* control mean	Hours since* last DDS dose
Controls	24	48.9	35.3–78.8	–	24
‘Regular’ out-patients	45 (36%)	59.3	38.2–124	1.25	16
‘Irregular’ out-patients	34 (27%)	27.6	16.4–37.3	0.49	50
‘Grossly irregular’ out-patients	46 (37%)	9.8	3.2–16.0	0.06	128
Total out-patients	125	32.5	3.2–124	0.6	42

*See text.

Table 2. Comparison of D/C ratios of urine samples from supervised controls and out-patients on 100 mg DDS daily

	Number	Mean D/C ratio $\mu\text{g}/\text{mg}$	Range of D/C ratios $\mu\text{g}/\text{mg}$	Out-patient mean* control mean	Hours since* least DDS dose
Controls	12	83.8	49–159	—	24
'Regular' out-patients	26 (41%)	88.8	60–160	1.07	22
'Irregular' out-patients	24 (37%)	37.0	16.1–58	0.39	58
'Grossly irregular' out-patients	14 (22%)	10.9	5.2–15.5	0.04	136
Total out-patients	64	64	5.2–160	0.59	43

* See text.

patients tested on both occasions, the compliance of 7 was the same both times, in 4 it was worse at home and in 4 it was better. Thus compliance was not significantly influenced by the day on which the sample was collected.

Considerable variation was found in the compliance of patients attending different clinics, from the worst where only 1 patient in 11 was regular to the best where 10 out of the 16 patients were regular. These sample sizes were however too small for the differences to be statistically significant. Compliance was not related to the distance of the clinic from the hospital, nor was it related to the attendance rate at the clinic. As far as individual patients were concerned, missing clinics in the previous year did not mean that they ingested fewer tablets when they did collect them from the clinic. Fiftyone per cent of the patients whose homes were between 2 and 5 km from the site of the clinic were classified as 'regular' in contrast to only 30% of those living under 2 km from the clinic ($p = 0.01$ using chi-square test). Eleven per cent of patients who were found to have leprosy during general surveys of the population were classified as 'regular' compared with 39% of those who presented voluntarily at clinics and 53% of those found as contacts of known patients ($p = 0.02$). Twentyeight per cent of tuberculoid patients were 'regular' compared with 42% 'regular' in other groups ($p = 0.05$).

The disability of the patients did not affect their compliance, and patients who had only been registered for one year or less were no better at taking their tablets than those who had been registered for longer periods.

Discussion

While measuring the dapsone concentration in acidified urine samples, I noted that on addition of the nitrite reagent a pink colouration developed in about a fifth of the samples. Hence if one measured the OD immediately before the addition of *N*-1-naphthyl-ethylene-diamine-dihydrochloride, rather than before the addition of nitrite as suggested in the original method of Ellard, the blank D/C ratio could be reduced in such samples by up to 75%.

An attempt was made to assess the compliance of children prescribed 25 mg dapsone daily, but it was impossible to interpret the results because of the wide range of D/C ratios in the controls (12–90 $\mu\text{g}/\text{mg}$). Although a wide range in creatinine excretion might be expected due to the difference in the weight of the children, the individual D/C ratios did not correlate with the size of the children. As there were few children in the hospital, out-patient children were used as controls and the variability of results may have been due to inadequate supervision.

The importance of good control results cannot be overemphasized, and in future studies of this kind it would be of value to collect urine samples 24 h and 48 h after a supervised course of dapsone in at least 20 patients. The

distribution of the 24 h and 48 h ratios can then be used to assess the best criteria for discriminating between 'regular' and 'irregular' patients.

This study has shown that urine samples taken at clinics are representative of the level of compliance of the population; as long as the tests are unexpected at clinics, it is not necessary to carry out surprise home visits when assessing the compliance of the population. However this should be reassessed at a future date, since encouragement by paramedical workers might improve tablet-taking just before the clinics without altering compliance in the period between clinics.

The most worrying group in the study were the 12 lepromatous patients who appeared to be 'grossly irregular' in self-administering their prescribed dapsone treatment. These patients could become infectious again if they do not take dapsone, and also have a greater danger of developing dapsone-resistant disease. About 20 dapsone-resistant cases have already been detected at Karigiri. These 'grossly irregular' lepromatous patients need special encouragement to improve their compliance. Thus they might be hospitalized for a short period (3–4 days) so that a series of consecutive daily doses of dapsone could be given under supervision. A urine sample would then be obtained prior to the final supervised dose to provide a control D/C ratio for comparing results from subsequent urine samples obtained during routine out-patient treatment.

The results of this study emphasize the important role that paramedical workers should play to inspire patients not only to attend clinics regularly but also to take their dapsone each day.

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The histology of erythema nodosum leprosum. Variant forms in New Guineans and other ethnic groups

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Summary The histology of erythema nodosum leprosum (ENL) shows some distinctive variations, which were considered in relation to ethnic groups in Papua New Guinea, Malaysia, Mexico and a miscellaneous group of ENL patients. In highland patients of Papua New Guinea the reaction involved the connective tissues of the dermis more than the lepromatous granuloma, and the chief features were oedema and fibrinoid necrosis followed by very heavy fibrosis. Infiltration of neutrophils was a subsidiary finding. In Malaysia, some of the features of the necrotizing form of ENL, previously described, were detectable fairly regularly even in less severe non-necrotizing lesions. In Mexican patients who developed ENL rather than the Lucio form of reaction, the ENL was of the classic type and did not differ from the ENL seen in the miscellaneous group.

ENL appears to be a complex of reactions whose form may perhaps be modified by ethnic factors as well as by the immune status within the spectrum.

Introduction

Clinically erythema nodosum leprosum (ENL) varies considerably in its severity and in the extent to which it causes systemic complications, but otherwise its skin lesions are recognizably similar.¹ Histologically the classic form of ENL, essentially an acute inflammation superimposed on a lepromatous granuloma, is a well recognized entity,^{2,3,4} though among the Bantu the reaction was found to be more definitely subcutaneous² than in the other two studies from India³ and the Philippines.⁴ There are some significant variants of this pattern. An escharotic or necrotizing form of reaction found in south Vietnam⁵ and Malaysia⁶ was considered to be a form of ENL rather than the Lucio form of

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reaction which, despite some claims, has not been confirmed in patients other than those of Mexican or Central American origin. Another histologically divergent form of ENL is one that consists mainly in a disturbance of the dermal connective tissue rather than the granuloma.⁷ It is relatively uncommon, is often correlated clinically with a quite mild but otherwise typical form of ENL, or it may even be clinically non-reactive. It has, therefore, received little attention.

In the present paper we report an apparently unique group of cases of ENL of the connective tissue type in patients from the highlands of Papua New Guinea, in some of whom the reaction was clinically severe. This group has already been the subject of previous reports^{8,9} though so far its histology has not been described. We have taken the opportunity to make a brief comparative study of the variant forms of ENL and their relationship to certain ethnic groups.

Patients

Papua New Guinea group

The patients were seen at Togoba Leprosy Hospital, near Mt Hagan, which serves an ethnically compact population of New Guineans in the eastern and western highlands. The study was a prospective investigation of secondary amyloidosis and ENL, and the patients were to some extent selected as being potential candidates for ENL. Although one of the objects of the study concerned the effect of colchicine, none of the patients was on this drug at the time of biopsy. Only a few were on clofazimine, and steroids were reserved for the severest reactions.

Clinically the reaction pattern of these patients differed from those in Sepik River Area (seen at Aitape). Among those at Togoba were some with small discrete ENL lesions of the classical type which lasted 2–4 days. A larger number produced large tender coalescent areas of deep panniculitis which were hot to touch and had the appearance of *peau d'orange*. They involved the extensor surfaces of arms and thighs, and also the buttocks, and they lasted many days or weeks. In spite of their severity the skin lesions remained intact, and suppuration was not observed. Unlike the patients with ENL at Aitape, these highland patients rarely developed iritis, arthritis or orchitis; but they had high fever and felt unwell.

Sixty-three biopsies were received from 55 patients with LL or BL leprosy. Of these 41 were initial biopsies taken in the clinically non-reacting state, 22 were taken during a subsequent reaction. The biopsies were processed and examined in London.

Mexican group

Eighteen biopsies of Mexican patients with ENL were available for study and also, for comparison, 13 biopsies of Lucio's reaction. They were obtained and processed at the Los Angeles County/University of Southern California Medical Center. The patients had not received anti-inflammatory drugs.

Malaysian group

Biopsies were available from 25 patients with LL leprosy and ENL seen during a 7-year period at the Medical Research Council Unit at Sungei Buloh, Malaysia. Four were included in a previous report.⁶ Of the 23 whose racial origin was known 18 were Chinese, 2 Malay, 3 Indian. Owing to the chronic nature of the reaction some of the more severe cases had been partially immuno-suppressed with steroids. The biopsies were processed and examined in London.

Miscellaneous group

For comparison with these three ethnically fairly distinct groups, 12 miscellaneous biopsies of ENL lesions were examined from patients from various other parts of the world who were seen in London. They were not receiving anti-inflammatory drugs.

The biopsies in these four groups were all unselected except for the exclusion of histologically trivial cases. All the biopsy specimens extended down to the subcutis. They were processed routinely and stained with haematoxylin-eosin and a modified Fite stain. Special stains were used in selected cases.

Results

ENL in Papua New Guinea

Of the 22 biopsies of clinical reaction sites, the reaction histologically was of the ENL type in 20. Of these 20, 3 were the classic non-necrotizing form of ENL associated with neutrophil infiltration in the subcutis or in and around a lepromatous granuloma, described below. The cases identified clinically as 'classical' were among these. The other 17 reactions in this group consisted of ENL of the type that involves primarily the dermal connective tissue. In addition, surprisingly, clear histological signs of ENL were found in 17 of the 41 clinically non-reacting biopsies, 1 classical, 16 of the dermal type.

Thus there were in all 33 cases of ENL of the dermal type, though only 17 of these were clinically reactive. Of the 33 cases 7 were histologically acute inflammatory, 5 were transitional and 21 were in a chronic, fibrosing, stage. All the acute cases were clinically reactive, but 3 transitional cases and 13 of the 21

chronic cases were clinically non-reacting, despite quite dramatic histological abnormalities in some of them. All these dermal ENL reactions were in patients of the LL type, and they presented no unusual features apart from the reaction, either histologically or bacteriologically.

The acute stage was characterized by severe extra-cellular oedema of the connective tissues of the dermis, associated with degenerative changes in the collagen and elastosis (Fig. 1). In some areas degeneration had progressed to

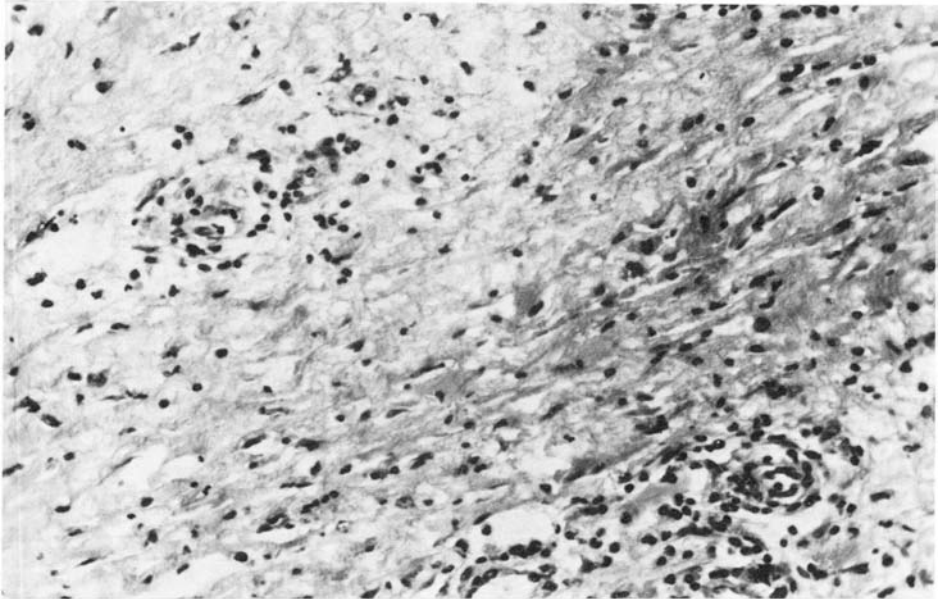


Figure 1. Acute ENL of the 'dermal' or connective tissue type. Severe degeneration of collagen with some oedema. Note the absence of polymorphs and granuloma. H&E. $\times 200$.

fibrinoid necrosis (Fig. 2). The most acutely affected areas were infiltrated by primitive young fibroblasts with large pale nuclei which were not far removed morphologically from mesenchymal cells (Fig. 3), and in these areas eosinophils were sometimes present. These changes were most marked in the deep and mid-zone of the dermis. In the superficial zone there was only a generalized proliferation of swollen young fibroblasts in the interstices of the collagen bundles, without disturbance of the collagen. In parts of the lepromatous granuloma and in the subcutaneous fat generally there was oedema and an infiltrate of lymphocytes, mononuclear cells, plasma cells and a few polymorphs. A similar inflammatory reaction affected the capillaries and arterioles, whose walls were greatly swollen some cases. There was little fat necrosis. In only two cases were there aggregates of polymorphs as in classical ENL, one in the subcutis, the other in the mid-dermis.

The transitional or subacute lesions were marked by the presence of small foci of fibrinoid change and infiltration of fleshy young fibroblasts, and in

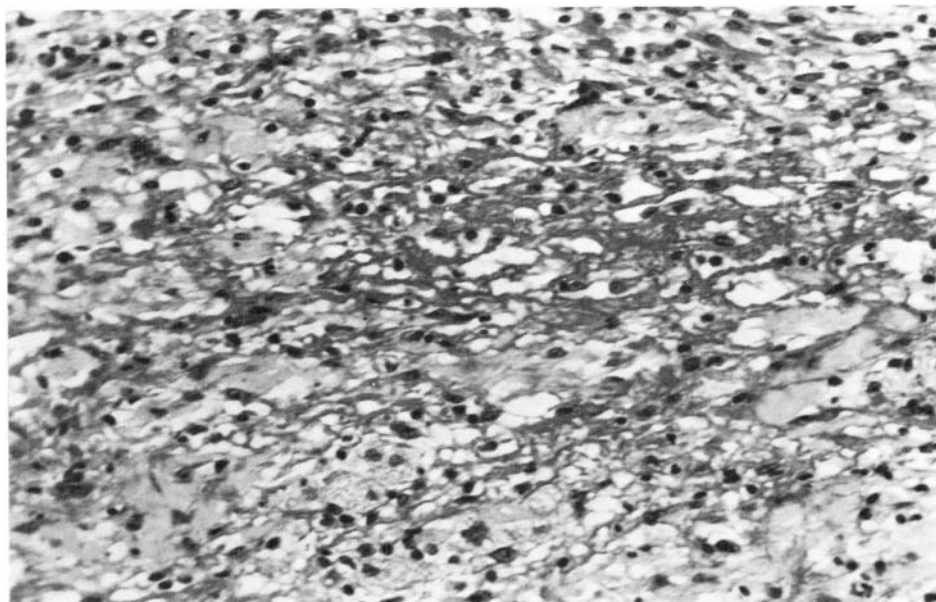


Figure 2. Acute dermal ENL. Fibrinoid necrosis. H&E \times 400.

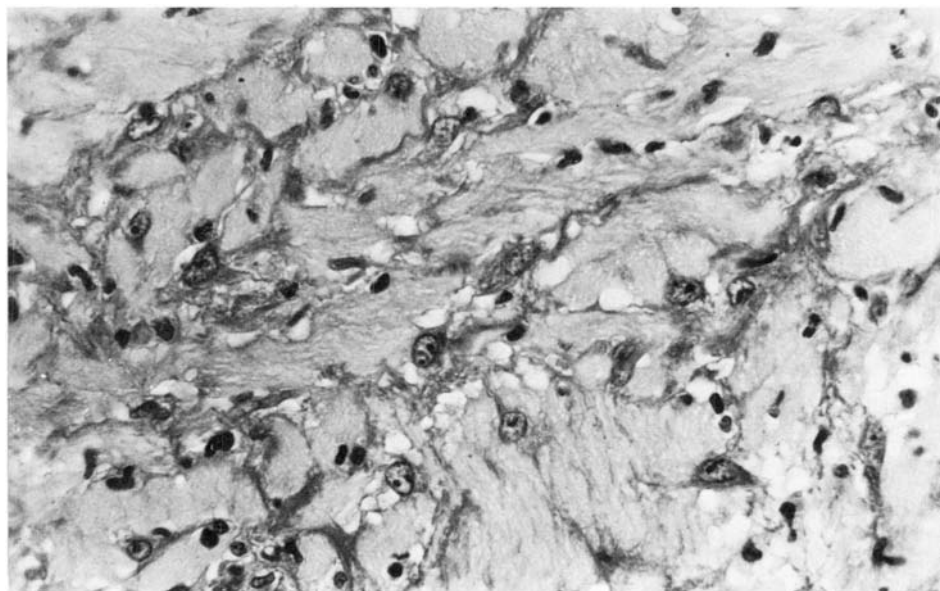


Figure 3. Acute dermal ENL. Influx of primitive young fibroblasts and some oedema. H&E. \times 625.

other areas by a heavy proliferation of more mature fibroblasts, sometimes associated with new capillary formation, which tended to invade the

lepromatous granuloma (Fig. 4). Elsewhere, especially on the periphery or in the subcutis, there might be coarse bands of collagen and fibrocytes which resembled a keloid (Fig. 5), though this development was more typical of the chronic fibrosing stage. There was no clear-cut demarcation of the two stages.

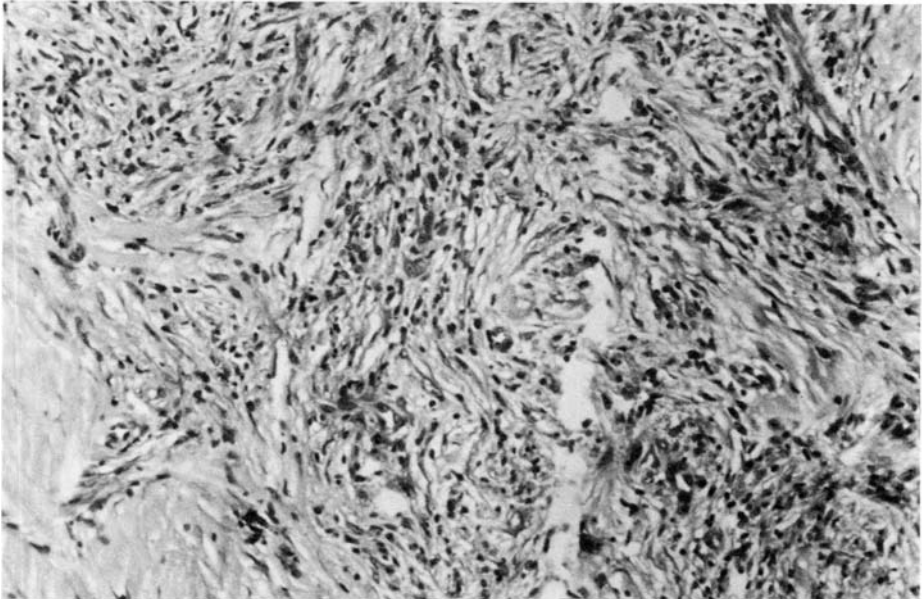


Figure 4. Subacute dermal ENL. Proliferation of fibroblasts and some new capillary formation. H&E. $\times 250$.

In the early chronic as in the subacute stage, chronic vasculitis of greatly swollen arterioles was a conspicuous feature in a few cases (Fig. 6). In the dermis generally oedema had not completely subsided, and plasma cells and mast cells were always present, in variable numbers (Fig. 7). Plasma cells, often quite numerous, were found in dermis as well as the granulomatous areas. The latter were often infiltrated also with lymphocytes, and in two cases numbers of polymorphs were present associated with necrosis in the centre of the granulomatous mass. In the later lesions the lymphocytes and polymorphs had disappeared though some plasma cells and mast cells persisted.

ENL in the Malaysian, Mexican and Miscellaneous groups

All the patients were classified as LL. Despite the points of difference the reactions in the three groups presented a sufficiently homogeneous histological picture to be considered together, and they shared in common most of the

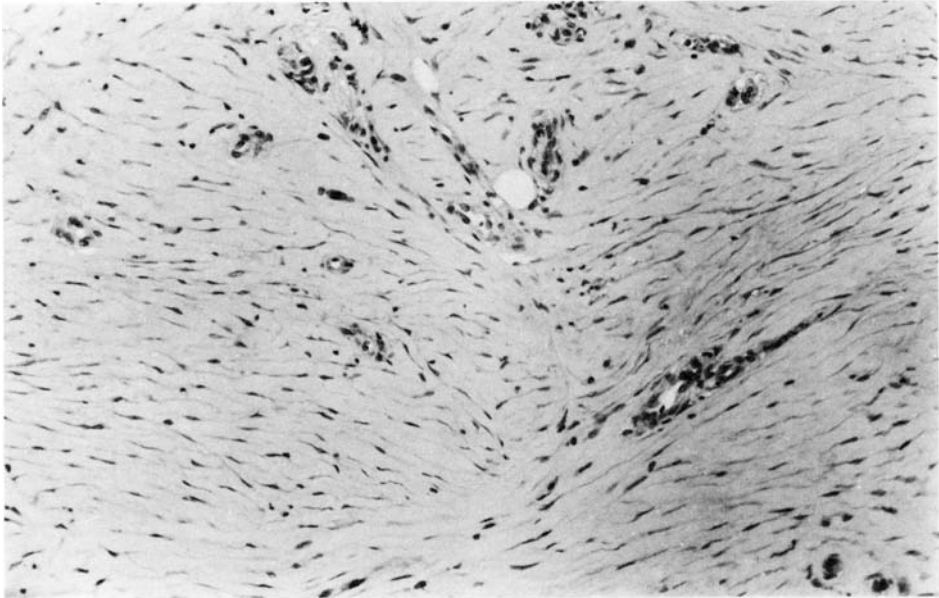


Figure 5. Chronic dermal ENL (or periphery of subacute stage lesion). Coarse fibrosis resembling a keloid and extending into the subcutis. H&E. $\times 120$.

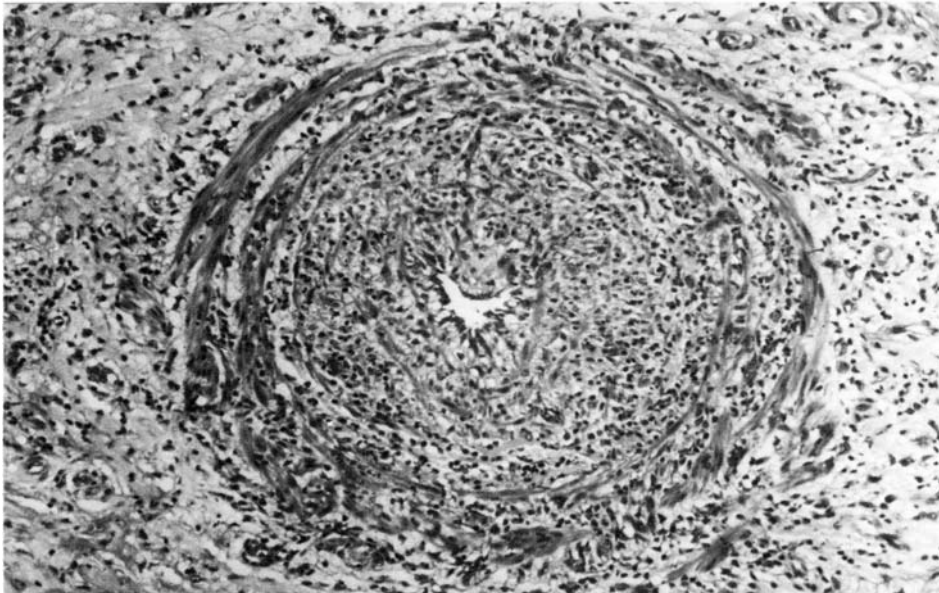


Figure 6. Chronic or subacute dermal ENL. Severe chronic vasculitis in a greatly swollen blood vessel. H&E. $\times 150$.

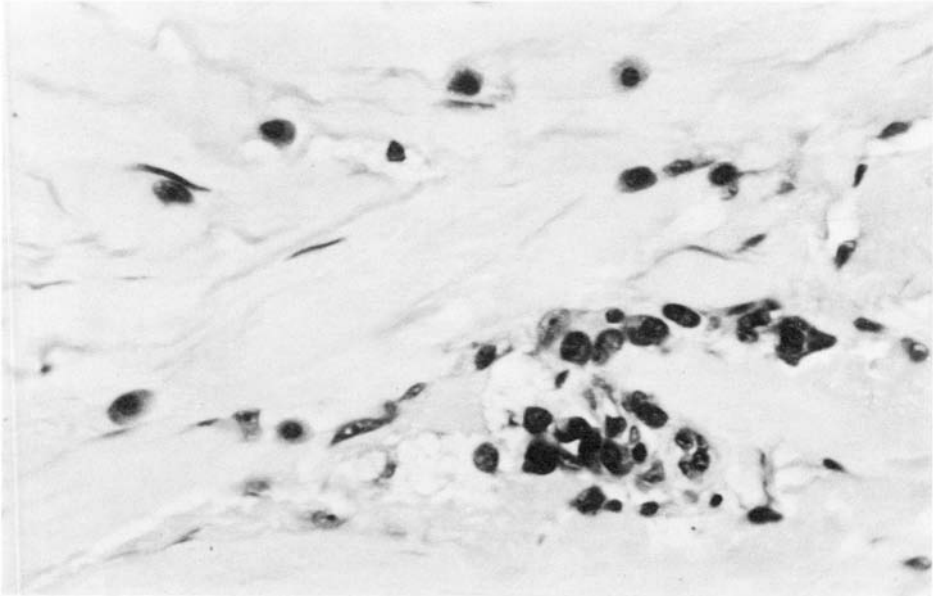


Figure 7. Chronic or subacute dermal ENL. Oedema of dermis with plasma cell infiltrate and increase of mast cells. H&E. $\times 500$.

features commonly associated with ENL. The groups were found to be comparable in regard to the character of the neutrophilic and lymphocytic infiltrate, the irregular incidence of vasculitis and the range of the indices which reflected the numbers and morphology of bacilli. However, marked differences were noted as to the anatomical site of the most intense inflammatory reaction and the amount of oedema in the superficial dermis.

The distribution of the main sites of the inflammatory reaction is summarized in Table 1. In both Mexicans and the miscellaneous group it was

Table 1. Primary site of inflammation in three groups of patients with ENL

	Mexican (18)	Miscellaneous (12)	Malaysian (25)
Subcutis and deep dermis	15	9	2
Deep and superficial dermis	3	3	15
Superficial dermis only	0	0	8

located predominantly in the subcutis and deep dermis. Occasionally it was equally severe in the superficial dermis, but the superficial dermis never had an inflammatory reaction more intense than that seen in the deep dermis or subcutis. In contrast, among Malaysian patients the reaction was characteristically more superficial. The deep dermis and subcutis was the site of the most severe inflammatory reaction in only 2 of 25 patients (Figs. 8 and 9). Oedema of the



Figure 8. ENL (Malaysia). The reaction involves the granuloma, not the dermis; even in a non-necrotizing case it is predominantly in the superficial zone. H&E. $\times 20$.

superficial dermis was well developed only among Malaysian patients and at times it could be severe (Fig. 10).

Complete necrosis of a portion of granuloma was seen in one Malaysian case, and small areas of fibrinoid necrosis in a few. There was no ulceration or vesiculation. Three of the lesions appeared to involve granuloma in a wedge-shaped area radiating from a point in the subcutis to an arc of epidermis. Heavy lepromatous involvement of small arteries in the subcutis was noted in some cases, but vasculitis in these vessels was not marked though it was often seen in the smaller vessels of the mid-zone.

Lucio's reaction and necrotizing ENL

Lucio's reaction was distinguished from the potentially necrotizing ENL of Malaysians by a number of features. Ischemic necrosis of the epidermis and superficial dermis (Fig. 11) was found in 11 of 13 patients with Lucio's

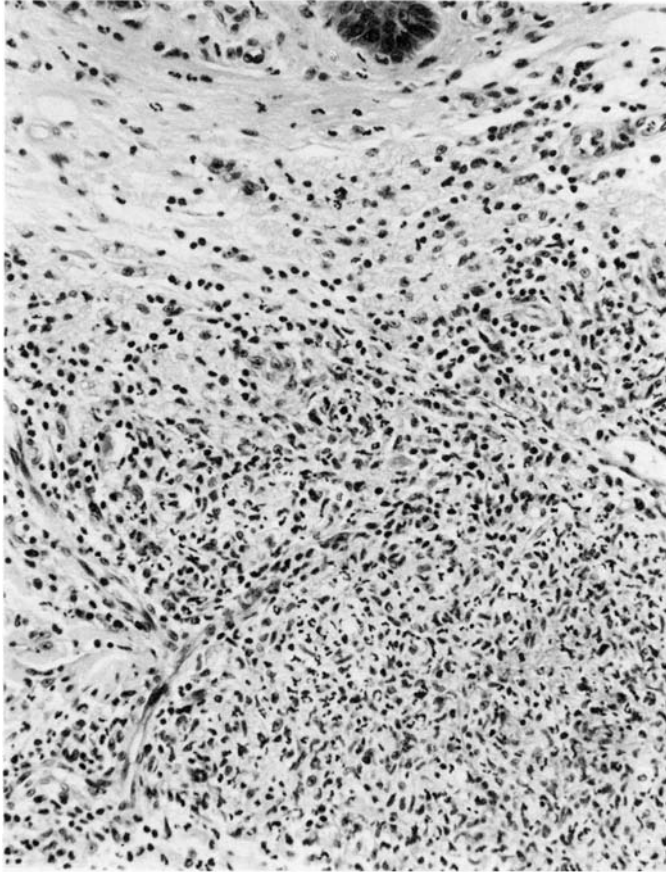


Figure 9. ENL (Malaysia). Detail of Fig. 8. The reaction is of the classical type, with a polymorph infiltrate. H&E. $\times 150$.

reaction but in none of the 25 Malaysians with ENL, even in the presence of severe oedema. Oedema of the superficial dermis was not a prominent feature in any case of Lucio's reaction, unlike ENL in Malaysia (compare Figs. 11 and 10). Lucio's reaction was characterized by a sparse inflammatory response (Fig. 11). Some neutrophilic reaction might occur, particularly on the border between the viable and infarcted tissue, as in a myocardial infarction. In none of the 13 patients with Lucio's reaction was the neutrophilic reaction ever as well developed as what was regularly found in ENL of Malaysians (compare Figs. 11 and 8).

Other markers of Lucio's reaction not found in Malaysian ENL included comparatively poor development of granulomas in the superficial dermis, prominent endothelial proliferation and abundant AFB in endothelial cells. In areas of endarteritis ENL lesions also showed abundant AFB but these areas were more localized.

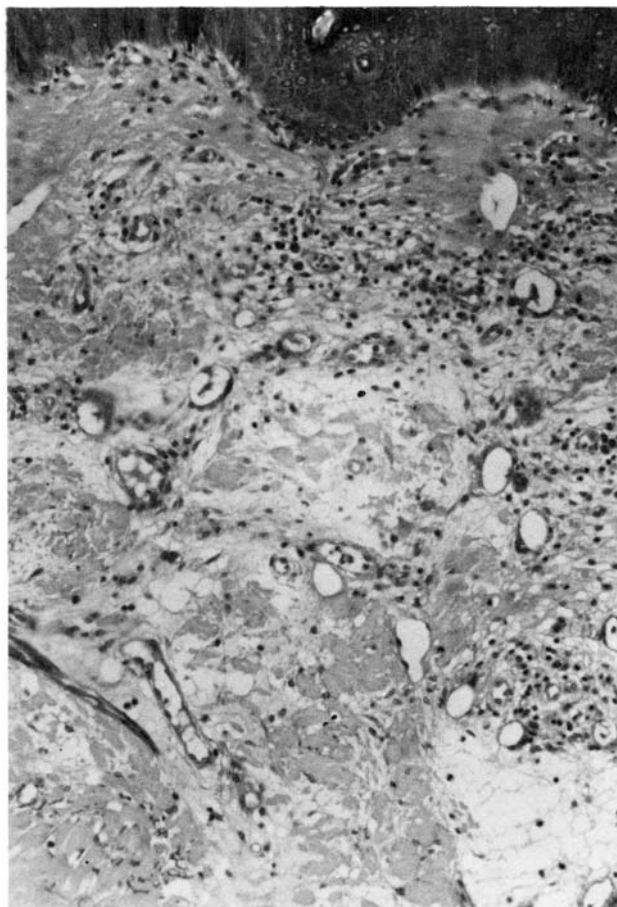


Figure 10. ENL necroticans (Malaysia). Severe oedema is the main feature of this reaction, which is predominantly superficial. H&E. $\times 120$.

Discussion

The reaction encountered in the highlanders of Papua New Guinea appears to be almost unique, despite the occasional finding of the same sort of reaction in a milder form in patients elsewhere.⁷ The involvement of the connective tissues ranged so far beyond the confines of the granulomatous masses that it was impossible to decide to what extent, if at all in some cases, it was centred on the granuloma which constitutes the lesion of lepromatous leprosy. To a less extent the connective tissues of the dermis are involved in reversal reactions.⁷ Multiple autoantibodies have been observed in patients with leprosy and particularly in those at the lepromatous pole. In the New Guinea leprosy population, antibodies to collagen were detected with the highest prevalence in



Figure 11. Lucio reaction. There is ischaemic necrosis of the epidermis and subepidermal zone without much oedema or cellular infiltration. H&E. $\times 20$.

LL patients.¹⁰ It is unclear whether this was due to the disturbance of collagen, or whether these antibodies were actually augmenting the ENL reaction. In the presence of large amounts of mycobacterial antigens, acting as a local adjuvant, it may be that these antibodies are involved with the pathogenesis of the severe dermal reaction.

The escharotic, haemorrhagic or vesicular type of ENL found in parts of south-east Asia is histologically recognizable as ENL despite atypical features. Selected cases have already been described.^{5,6} The contribution of the present study has been to show that in an unselected series of cases from Malaysia the atypical features (the primary involvement of the superficial zone of the dermis and the severe oedema) present a fairly constant pattern. The superficial distribution was a feature of even mild reactions. Why this should be so is not clear, though the superficial distribution helps to explain the tendency of this form of reaction to become necrotic. Harter and Kim⁵ refer to a deep, underlying panvasculitis in the subcutis. We could not confirm this, possibly because

our biopsies did not go deep enough into the subcutis, or because of the partial immunosuppression in some cases. Nevertheless, this observation would be in keeping with the occasional finding of wedge-shaped involvement of the skin with ENL in the Malaysian patients, which was presumably related to a large deep vessel. Furthermore, it may be noted that the finding of immunoglobulin in the vessel walls in ENL has been reported in Malaysian patients,¹¹ but not in those from Mexico,¹² Columbia¹³ or immigrants to New York City.¹⁴

The Malaysian type of ENL was found to differ distinctively from Lucio's reaction. This is a confirmation of previous reports,^{5,6} and it is also the only direct comparison of these two types of reaction. It was also found that when ENL, as opposed to Lucio's reaction, occurred in Mexican patients it was of the classical deep node type.

From the foregoing conclusions it would appear that ENL is by no means a uniform reaction, and that its form may be influenced by ethnic factors. Some of the confusion that formerly surrounded the classification of leprosy was at one time attributed to ethnically different disease patterns. It is now accepted that these differences are due mainly to variations in the position of ethnic groups within the immunological spectrum. In a previous study we suggested that the predisposition of Mexican patients to develop the Lucio type of reaction might be due to an ultra-low immune level.¹⁵ By contrast, Malaysian patients who develop ENL are mainly of the sub-polar type of leproma, a form which was first described from Malaysia.¹⁶ Another factor that might influence the form of a reaction would be the size of blood vessel involved in the vasculitis and immune process.¹⁵ But neither of these factors appears to be relevant to the ENL reported here from New Guinea. If some of the predisposition to different patterns of ENL is directly due to ethnic factors, they would presumably be mediated by the HLA system. There is an association between B27 and immune complex diseases, especially those of which iritis is a feature. Iritis is a point of distinction between the classical and New Guinea (dermal) form of ENL. Although ENL is attributed to immune complexes,¹¹ these are more likely to be extravascular,¹⁷ not circulating as originally proposed.

ENL and Lucio would appear to represent a complex of reactions in which Lucio is perhaps the most distinctive clinically and the most specific ethnically. Fundamentally the dermal form of ENL may prove to be more disparate. The inter-relationships of the complex are as yet no more clear than the mechanisms at work.

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Selective loss of *Mycobacterium leprae* responsiveness of circulating lymphocytes in primary neuritic leprosy

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Summary Sixteen patients with Primary neuritic leprosy characterized by neural involvement in the absence of dermal lesions were studied. Eight healthy contacts from non-endemic areas formed the control group. T and B cell numbers as well as lymphocyte transformation responses to Concanavalin A (Con A), Phytohaemagglutinin (PHA) and PPD were not found to be significantly decreased in the majority of the neuritic patients. However, there was a selective uniform lack of *M. leprae* induced lymphocyte transformation in this group (Median stimulation index – 1.05). In 3 of the patients, the antigen specific unresponsiveness was present even after 5 years of chemotherapy. The clinical extent of neural involvement and serum factors did not appear to contribute to the antigen specific deficit observed in this form of leprosy.

Introduction

The clinical features of leprosy are predominantly associated with skin and nerve involvement, the causative organism, *Mycobacterium leprae*, having a particular affinity for skin and neural tissue. Being an obligate intracellular organism it mainly resides in the tissue macrophages and Schwann cells. Dermal nerve involvement is observed in the tuberculoid, borderline and lepromatous forms of leprosy, (Ridley & Jopling, 1966).¹ The larger peripheral nerves appear to be affected early in tuberculoid leprosy and only in the later phases of lepromatous leprosy (Cochrane, 1964;² Dharmendra, 1978).³ In borderline leprosy, neuritis during reactions, forms a serious complication which may lead to permanent damage to the nerve (Barnetson *et al*, 1978).⁴

Nerves may also be affected primarily in leprosy without an apparent involvement of skin or dermal nerves. Controversy exists as to the exact position of this form of leprosy in the general spectrum of the disease. Neural anaesthetic lesions were recognized as a separate entity in the Cairo Congress Classification (1938)⁵ and were grouped into lepromatous, tuberculoid and indeterminate forms in the Madrid Congress Classification (1953).⁶ The latter classification was mainly based on the number of nerves involved in the absence of skin lesions. Pure neuritic patients are frequently seen in India, though the occurrence of this form of leprosy has regional variations (Noordeen, 1972).⁷ Some workers classify such lesions in a manner similar to dermal lesions. However, according to the Indian system of classification, primary or pure neuritic leprosy is grouped as a separate clinical entity (Dharmendra & Chatterji, 1953).⁸ Neuritis in nerves draining active or healed lesions has been designated as secondary neuritic leprosy. The primary neuritic group is further subdivided into mononeuritic (single nerve involvement) or polyneuritic (two or more nerves affected) leprosy (Dharmendra, 1978).³ The patients included in the current study have been graded according to the above criteria.

Strong evidence exists to indicate that host-determined immunological responses are responsible for the leprosy spectrum (Ridley & Jopling, 1966;¹ Myrvang *et al*, 1973).⁹ Yet the mechanisms leading to nerve damage in leprosy remain unknown. Recent reports indicate that patients undergoing reactions in borderline leprosy develop neuritis which is associated with enhanced lymphocyte transformation to *M. leprae* antigens (Barnetson, 1978).⁴ As far as we are aware, immunological data in patients with primary neuritic leprosy is scant. The present study was therefore undertaken on 16 patients with primary polyneuritic and mononeuritic forms of leprosy. Enumeration of (a) circulating T and B cells and (b) lymphocyte transformation to T cell mitogens, specific *M. leprae* antigen and PPD were undertaken to assess the immunological status of these patients.

Materials and methods

SUBJECTS

Sixteen patients between the age groups of 23 to 70 years attending the leprosy clinics of All India Institute of Medical Sciences and Lok Nayak Jaya Prakash Narayan Hospital, Delhi, were included in this study. Fourteen were male and 2 were females. None of the patients had active or healed dermal lesions. Four patients had mononeuritic leprosy, 3 had ulnar nerve involvement and one had affection of the lateral popliteal nerve; 12 others had thickening of 2 or more nerves and were placed in the polyneuritic group (Dharmendra, 1978).³ One patient with mononeuritic and 2 with polyneuritic leprosy had been treated with DDS (300 mg/week) for 5 years. Detailed neurological examination for

sensory and motor functions were performed on all the patients (Table 1). Lepromin skin tests using whole autoclaved bacilli were available in 8 patients. The other patients were not obtainable for reading of the skin test at the appropriate time. Eight healthy individuals from the laboratory and clinical staff between the age groups of 25 to 50 years, who were not residents of endemic areas but had been constantly exposed to leprosy patients and *M. leprae* antigens for 3–5 years were included as a control group of healthy contacts.

Table 1. Clinical status of 16 patients with primary neuritic leprosy

	Number of patients	
	Polyneuritic leprosy	Mononeuritic leprosy
Number of involved nerves		
(a) One	Nil	4
(b) Two to four	12	Nil
Nerves involved		
(a) Ulnar	12	3
(b) Median	10	Nil
(c) Radial	2	Nil
(d) Lateral popliteal	5	1
(e) Posterior tibial	3	Nil
Muscle changes		
(a) Flattening only	2	Nil
(b) Wasting	12	4
(c) Deformity	5	1
Sensory loss		
(a) Thermal	12	4
(b) Touch	12	4
Trophic changes		
(a) Dry glossy skin	10	2
(b) Blisters	Nil	Nil
Motor nerve conduction velocity		
(a) Normal	4	Nil
(b) Delayed	8	4

NERVE CONDUCTION STUDIES

A medelec 2 channel EMG machine (UK) was used to study the affected nerves in the patients. Motor nerve conduction velocities below 40 m/sec were considered abnormal (The normal Indian subjects showed a range of 40–60 m/sec).

NERVE BIOPSIES

Biopsies were only performed on the radial cutaneous nerves in 4 patients with multiple nerve involvement and sensory loss in the area supplied by the nerve. Biopsies were not attempted in patients with single nerve or only motor nerve involvement for ethical reasons. Histopathological specimens were stained with Haematoxylin and Eosin, and Ziehl Neelson Laxol. Fast blue followed by Periodic acid Schiff reagent was used for delineation of myelin.

SEPARATION OF LYMPHOCYTES

Lymphocytes were isolated from sterile heparinized venous blood (10 IU of preservative free Heparin per ml) by Ficoll-Isopaque density gradient centrifugation according to Boyum (1968).¹⁰ The lymphocytes collected from the interface were contaminated with 5 to 15% monocytes and 1–2% neutrophils. They were washed twice with Eagles' Minimum Essential Medium and resuspended in RPMI 1640 (Gibco Biocult) to a concentration of 1×10^6 per ml. 10% pooled AB serum was added to the aliquots of cells used for lymphocyte transformation tests.

ENUMERATION OF T AND B CELLS

(i) T-cells were identified by spontaneous rosette formation with sheep red blood cells treated with AET (2 aminoethylisothiuronium bromide hydrobromide, Sigma Chemical Co) by a modified method of Kaplan and Clark (1974).¹¹

(ii) B cells were identified by surface markers for immunoglobulin and activated complement as described earlier (Nath *et al*, 1977).¹²

(a) Immunoglobulin marker was identified by direct immunofluorescence using FITC conjugated antihuman immunoglobulin (Capell Labs). In brief, 3×10^6 lymphocytes in 100 μ l of MEM were mixed with 50 μ l of an optimum dilution of the fluorescein-labelled antiserum, incubated at 4°C for 45 min, washed thrice with MEM and finally suspended to 100 μ l. One drop of the cell suspension was put on a slide covered with a coverslip and sealed with nail varnish. Fluorescence was visualized using a Carl Zeiss universal microscope with an HBO 200 mercury lamp and KP 500 excitation filter.

(b) Rosette formation with sheep erythrocytes coated with anti-sheep erythrocyte antibody and mouse complement was used to identify B cells with receptors for activated complement (Bianco Patrick & Nussensweig, 1970).¹³ 300 lymphocytes were counted for each individual test.

LYMPHOCYTE TRANSFORMATION TEST

Microcultures were performed in round bottom microtitre plastic plates (Nunc Intermed, Denmark). Each well contained 2×10^5 lymphocytes in 200 μ l of

RPMI 1640 buffered with HEPES (Gibco Biocult) and supplemented with 10% pooled AB serum, penicillin (100 IU/ml) and streptomycin (100 µg/ml). The cultures were incubated at 37°C in a humidified chamber containing 5% CO₂ in air. 25 µl of stimulants were added to test cultures and consisted of (i) Concanavalin A (Pharmacia Uppsala 600 µg/ml) and phytohaemagglutinin (Difco Labs 800 µg/ml); (ii) antigens: (a) PPD (Ministry of Agriculture and Fisheries, UK, 50 µg/ml). (b) Whole *M. leprae* were derived from skin nodules of untreated lepromatous leprosy patients by homogenization of skin and two washings with PBS. The bacilli were autoclaved prior to use. The concentrations used were 5×10^6 and 10^7 bacilli per ml: these were chosen after performing dose response curves on lymphocytes from normal subjects and tuberculoid patients. Mitogen and antigen stimulated cultures were terminated after 3 and 6 days respectively. Fourteen–sixteen hours prior to harvesting 25 µl of ³H-thymidine containing 1 µCi (Bhabha Atomic Research, Trombay, Sp activity 6.4 mCi per mmol) was added to each culture. The cells were collected on glass fibre paper using automatic harvester. ³H-thymidine incorporation was determined and the results given as counts per minute (CPM) in a liquid scintillation counter. All cultures were performed in quadruplicate.

Due to the long period involved in the study, lymphocytes from each set of patients were tested in parallel with control subjects. As technical variations inherent in such protracted studies are inevitable, the results were expressed as stimulation indices rather than counts per minute. A stimulation index above 2 was considered to indicate stimulation with particular reference to antigen stimulated cultures.

STATISTICAL ANALYSIS

The results were analysed by nonparametric methods using Mann Whitney U Test (Siegel, 1956).¹⁴

Results

CLINICAL FEATURES

The 16 patients studied were in the adult age group and predominantly male. Their general condition and nutritional status was good. Skin tests using Mitsuda type lepromin were negative in all eight of the patients studied. The details of the neurological features of the primary neuritic patients in this study are given in Table 1. It was noted that absence of dermal lesions, presence of nerve thickening with tenderness only on deep pressure and sensory loss in the area of nerve supply were universal features. Motor nerve conduction velocities were delayed in the majority of the patients studied. Mononeuritic and polyneuritic groups could not be distinguished by any objective criteria (except on

the basis of the number of nerves involved). Therefore, all neuritic patients were grouped together for the other parameters.

HISTOPATHOLOGY OF RADIAL CUTANEOUS NERVES

For ethical reasons biopsies of nerve trunks were not done. Four patients with polyneuritic leprosy who had sensory loss in the skin areas supplied by the radial cutaneous nerve were biopsied. Histological examination indicated thickening with multilayered appearance of the perineurium in all the nerves. Mild to moderate infiltration with mononuclear cells and lymphocytes was observed. Acid fast bacilli were seen in the perineurium and in many of the Schwann cells. Patchy demyelination as well as moderate nerve fibre damage was noted.

CIRCULATING T AND B CELL NUMBERS

Table 2 shows the percentage of T and B cells in the peripheral blood of neuritic patients and healthy contacts. It may be observed that the percentages of B cells are within the normal range. The T cell numbers on the other hand appear marginally reduced in the patients as compared to the normal subjects. This was not found to be statistically significant.

Table 2. Circulating T and B Cell numbers in patients with primary neuritic leprosy and healthy contacts

	Mononuclear cells		
	T Cells	Ig(+) Cells	EAC Rosettes
Healthy contacts (8)			
Range	58 – 77	10 – 16.5	8.6 – 14.4
Median	71.2	11.8	13.0
Mean ± SD	68.8 ± 9.1	12.7 ± 2.7	12.4 ± 2.4
Neuritic patients (16)			
Range	54 – 73	8 – 10.5	8.1 – 14
Median	67.8	10.5	12.0
Mean ± SD	65.9 ± 5.8	11.2 ± 2.26	10.4 ± 1.9

Figures in parentheses indicate number of individuals studied.
SD = Standard deviation.

LYMPHOCYTE TRANSFORMATION

(i) *T Cell Mitogens*: Peripheral blood lymphocytes from patients and healthy contacts were stimulated in parallel experiments with optimal doses of PHA and Con A. No difference in responsiveness to mitogens was noted between the mononeuritic and polyneuritic groups. Eleven of the primary neuritic patients showed lower stimulation indices to Con A and 7 showed decreased levels to

PHA as compared to the control subjects (Fig. 1). The median values for Con A and PHA in the patient group were 29.0 and 43.9 respectively whereas the control group showed medians of 66.7 and 73.4 for Con A and PHA respectively. Statistical analysis by non-parametric methods (Mann Whitney U Test) did not reveal any significant differences between the neuritic and control groups. This may be due to the wide range of responsiveness to T cell mitogens seen in the patients.

(ii) *Antigens*: PPD and autoclaved integral *M. leprae* were used for *in vitro* antigen stimulation of lymphocytes. Fig. 1 shows the stimulation indices of individual patients and healthy contacts. It may be noted that responsiveness to PPD also showed a wide scatter and did not indicate any statistical difference between the two groups. The Median stimulation index in the neuritic and control groups was 4.7 and 7.4 respectively.

The important feature of this study was the specific lack of responsiveness to *M. leprae* in the neuritic group. Seven of the eight healthy contacts on stimulation showed *M. leprae* stimulation indices ranging from 2.0 to 3.8 with a Median value of 2.5. In contrast all the neuritic patients studied showed a stimulation index which ranged from 0.63 to 1.4 (Median 1.05). The statistical difference between the two groups was found to be highly significant ($p < 0.002$).

Discussion

Neural involvement is a predominant feature of leprosy in the presence and the absence of dermal lesions. *M. leprae* has been shown to reside mainly in the Schwann cells (Job, 1971;¹⁵ Pearson & Ross; 1975)¹⁶ and infrequently within the axons of peripheral nerves (Boddingius, 1974).¹⁷ Most of the studies to date focus attention on the status of dermal and peripheral nerves in the general leprosy spectrum. Clinical (Cochrane, 1964;² Dharmendra, 1952;¹⁸ 1978;³ Pearson & Ross, 1975),¹⁶ electrophysiological (Antia *et al*, 1975;¹⁹ Mehta *et al*, 1975)²⁰ and extensive morphological evidence (Iyer, 1965;²¹ Job & Desikan 1968;²² Job, 1971;¹⁵ Dastur, Rammohan & Shah, 1972;²³ 1973;²⁴ Dastur, 1978;²⁵ Pearson & Weddell, 1975)²⁶ regarding the involvement of nerves is available in the literature. However, the pure or primary neuritic form of leprosy has not been investigated fully and the consensus does not exist as to its exact status in the leprosy spectrum.

Immunological studies pertaining to nerve involvement in leprosy have been few. In recent years, Bjune *et al* (1976)²⁷ and Barnetson (1978)⁴ drew attention to enhanced antigen induced lymphocyte responses in borderline leprosy patients who developed neuritis during reactions. Primary neuritic leprosy as seen in Indian patients is a unique example of the preferential predilection of *M. leprae* for the peripheral nerves rather than the dermal nerves of

skin (Dharmendra, 1978).³ This feature is even more striking when it is realized that in the peripheral nerve as well as in the dermal nerves, the host cell for *M. leprae* is the Schwann cell. Though strong evidence exists to indicate that host determined immunological factors are responsible for the varied spectrum of leprosy, no data is available on the immunological status of this neuritic group of patients.

In the present study, except for the number of nerves affected, differences regarding the clinical extent of sensory loss, nerve damage, motor nerve conduction velocities, lepromin skin tests and immunological parameters were not distinguishable in the mononeuritic and polyneuritic groups. Therefore, all the 16 patients were included in the broad neuritic group. It was also considered more pertinent to take healthy contacts from non-endemic areas as a control group, in order to minimize non-quantifiable environmental effects of mycobacterial load of the endemic regions.

In general, normal ratios of T and B cells were found in the peripheral blood of the neuritic group. The mean and median values of T cells were marginally decreased but this reduction was not statistically significant. The functional ability of the peripheral lymphocytes to respond to general T cell mitogens showed a wide range in the patients. Though median stimulation indices appeared lower than those found in the control group, statistical significance could not be demonstrated. Clinical reassessment of the patients did not show any differences between the 'high' and 'low' responders to T cell mitogens. Poor nutritional status was not responsible for the decreased T cell functions. The 3 patients who had been treated for 5 years with dapsone showed low mitogenic stimulation (Con A 17 to 25; PHA 4.2 to 25.7). This may be related to the suppressive effect of dapsone on lymphocyte transformation as reported by Sengupta *et al* (1978).²⁸

The predominant feature noted in the neuritic patients was the selective lack of *M. leprae* stimulated *in vitro* responses of peripheral lymphocytes. The degree of unresponsiveness corresponded to the values obtained in lepromatous leprosy patients of the same ethnic group (Nath *et al*, 1977);¹² and was significantly lower than the values obtained in the healthy contacts who were studied in parallel ($p < 0.002$). Repeated testing in some patients continued to show low responsiveness to *M. leprae*. It is evident that the lymphocytes from these patients are capable of responding to a cross-reacting 'recall' antigen such as PPD (Fig. 1).

The antigen specific unresponsiveness in neuritic leprosy may be due to (a) *The type of antigen used*: It had been shown in patients with reactions that sonicated bacilli gave enhanced responses in lymphocyte transformation test due to the possible release of 'cytoplasmic' antigens (Bjune *et al*, 1976).²⁷ In our study autoclaved bacilli were also expected to release multiple antigens. The type of antigen used may not be the cause of the observed unresponsiveness as the same batch of antigen was proved to be a good stimulant in contacts

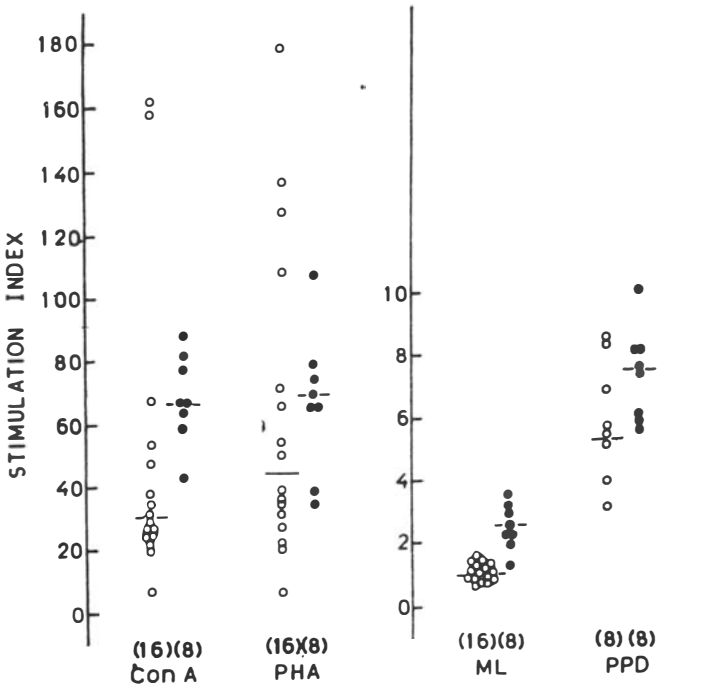


Figure 1. Lymphocyte transformation to Concanavalin A (Con A), Phytohaemagglutinin (PHA), *M. leprae* and purified protein derivative (PPD) in 16 primary neuritic patients (○) and 8 healthy contacts (●).

Median Stimulation Index for each group are shown (—); Mann Whitney U Test analysis showed $p < 0.002$ only for *M. leprae* stimulation.

and tuberculoid patients who were tested in parallel. (b) *Serum Factors*: It was considered possible that suppressor factors may be present in the patients' serum. Lymphocytes from the neuritic group were washed repeatedly and cultured in pooled AB serum which had been tested for its ability to support lymphocyte transformation: (c) *Extent of Nerve Involvement*: No correlation was found between the extent of neural damage and antigen responsiveness. Both *in vivo* and *in vitro* antigen responses were lacking in our patients.

The reasons for the specific loss of antigen stimulation in this form of leprosy are not clear from the present study and need further elucidation. *M. leprae* resides within the Schwann cells of the nerves and this may form a 'protected' site which precludes contact with circulating lymphocytes. No information is available regarding the presence or absence of *M. leprae* antigen on the surface of infected Schwann cells to assess the mode of antigen presentation to specific lymphocytes. Active suppressor mechanisms involving a subclass of T cells or macrophages may be responsible for the observed inhibition of *M. leprae* responses (Gershon, 1975).²⁹

Depletion of antigen reactive lymphocytes from the peripheral blood of the neuritic patients seems the most likely explanation. It may be inferred from

earlier reports of variable lepromin reactivity (Dharmendra, 1978;³ Job, Victor & Chacko, 1977)³⁰ and the presence of lymphocytes in the nerve biopsies of our patients and those of others (Job *et al*, 1977)³⁰ that antigen specific lymphocytes are generated in neuritic leprosy. It is possible that such cells are selectively diverted to antigenic sites in the nerves thus leading to a depletion in the peripheral blood.

The patients studied by us appeared to have a stable form of the disease and did not show reactions at the time of examination. It is thus not possible to know whether the immunological enhancement noted in the neuritis due to reactions in borderline leprosy (Barnetson, 1978)⁴ is also applicable in Primary Neuritic Leprosy. The selective loss of *in vitro* responsiveness to *M. leprae* seems a long-lasting phenomenon as observed in 3 patients who had been with dapsone for 5 years.

Acknowledgements

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Obituary

JOSE N RODRIGUEZ

MD; CPH (Hopkins); MPH (Hopkins)

(1897–1980)

Dr Jose N Rodriguez, internationally renowned and dedicated leprologist was born in San Marcelino, Zambales, Philippines, on 1 December 1897. He completed his Medical Course at the University of the Philippines in 1922, a Certificate of Public Health in 1932 and a Master in Public Health in 1948 from the Post Graduate School of Public Health, Johns Hopkins University. He attended post-graduate courses in Tropical Medicine and Leprology at the Calcutta School of Medicine, and dermatology courses from Western Reserve University, Columbia University, USA, and Hospital St Louis, Paris, France.

His long, dedicated, and brilliant career in the field of leprosy control started soon after completing his medical course in 1922. He served the Philippines government in various capacities at the Culion Leper Colony and the Eversley Childs Sanitarium. He was the first Chief of the Central Luzon Sanitarium and the Division of Sanitaria (1951–56) and the first Director of the Bureau of Disease Control in 1957. He remained in this position up to his retirement on 1 December 1961.

Dr Rodriguez established a Leprosy Control Project in Cebu, Philippines, which eventually served as the framework of the National Leprosy Control Program in the Philippines. This leprosy control scheme became the model for other Asian countries where he had several times served as WHO Short-term Consultant to the governments of Taiwan, Korea, Singapore, Malaya and the British Solomon Island.

The competence and dynamic leadership of Dr Rodriguez can be attested by his numerous memberships in local and international scientific and medical societies, his official representation as Philippine delegate to international health and leprosy conferences, and various citations that were awarded to him during his career. Leprologists all over the world have recognized and respected his more than one hundred scientific articles published in various journals, which are considered classic in the field of leprosy and its control. In 1954, the government of Spain conferred on him the 'Orden Civil de Sanidad Con

Categoria de Encomienda Con Place'. He was also conferred Knight, Order of Hospitalier of the Holy St Sepulcre in Jerusalem. In 1974, he was the recipient of the much coveted Damien-Dutton Award in recognition of his outstanding contributions and life-long dedication to leprosy control.

Dr Jose N Rodriguez, clinician, epidemiologist, researcher, educator, and administrator died on 27 February 1980 at the age of 83 years. His death certainly leaves a void in the field of leprosy that will be very difficult to fill, particularly in the Philippines. However, his contributions in the field of leprosy and its control will long be remembered.

FERNANDO A JOSE, JR

Leprosy and the community

We are extremely grateful to Dr H W Wheate, Secretary to the Medical Commission of ILEP, for the following report.

THE NATIONAL LEPROSY TRAINING CENTRE, WAU, SOUTHERN SUDAN

In the Southern Sudan, after the conclusion of the Civil War in 1971, the Government was faced with the tremendous task of reconstruction. Like much else, health services had been severely disrupted, hospitals and dispensaries destroyed and personnel dispersed or lost. Although there were no exact figures, it was known that leprosy prevalence was higher in the South than in other parts of the country. It was also recognized that the return and resettlement of refugees, as well as the movement of large numbers of the population associated with the work of reconstruction would be likely to increase the risk of transmission of the disease, and that this, in its turn, would lead to increased disability and invalidity and so to a reduction in the net benefits of this reconstruction to the community at large. From the beginning, therefore, the Government sought the assistance of WHO so that leprosy control would be an integral part of its health care delivery planning.

It was clear from the outset that considerable financial aid from international and non-governmental agencies would be required to re-establish even a modest basic health service and that one of the priority needs would be for the training of the personnel required at dispensary and health unit level. The conception of a rural leprosy control programme, based on a referral hospital and training centre which, being a practical example of the basic principles of community health could provide appropriate training in leprosy for the health personnel of the whole country, was readily accepted both by the Government and the German Leprosy Relief Association, which undertook to finance the construction and to run the Centre for an initial period of two years.

The practical difficulties were immense. There was no local source of building materials and the only reliable means of communication with the capital, Khartoum, was by air. Accordingly, it was decided to bring in the necessary supplies from Kenya and to carry out the work with local labour, supervised by expatriate volunteer technicians. Inevitably, there were delays and frustrations, but slowly all the interdependent parts of this comprehensive, self-sufficient unit, were completed and in November 1979 the Centre was officially opened – though, of course, work both in the hospital and in the rural leprosy control area had already started before this.

The Centre is about 20 km from the town of Wau and occupies an area of 6 sq km, part of which has been developed as farmland to provide food crops, fruit and vegetables for the hospital. There are well-constructed houses for the staff, including six for senior staff families. The hospital complex comprises administrative offices, an out-patient department, wards with 50 beds, and ancillary services (X-ray, operating theatre, physiotherapy department, laboratory and pharmacy). There is a separate building for the hospital kitchen and laundry and extensive workshops to ensure regular maintenance of both buildings and transport, and one for orthopaedic appliances and footwear.

The training unit is adjacent to the hospital and comprises 2 lecture rooms, library, canteen and accommodation for 30 students.

The Leprosy Control Programme is being developed in coordination with the Primary Health Care Programme in the Bahr el Ghazal province and provides an important learning experience for the village health workers now in training. Of the other health problems in the area, onchocerciasis is one of the most important. A special effort is being made to develop an area around Wau as a teaching and demonstration area which will not only provide examples of all types of leprosy and of its complications but will also provide practical lessons in the integration of leprosy control into a Primary Health Care programme.

The first training course was held in April/May 1980 and was a four weeks' orientation in leprosy for tutors at nursing schools and senior medical assistants responsible for refresher and in-service training. This was followed by a week's Seminar for Assistant Commissioners of Health, in which representatives from the Headquarters, Ministry of Health, Khartoum, and from the University of Khartoum also participated and at which the policy and the training programme were fully discussed. The plans agreed included participation in the post-graduate DPH course, brief orientation courses for medical students, courses for tutors at nursing training schools and refresher courses for serving personnel of the existing leprosy programmes. Some of these courses will be in Arabic. Visiting consultant lecturers will be invited to assist at some courses as necessary.

The construction of the National Leprosy Training Centre at Wau has been a very expensive and very time-consuming project, but the cooperation established between all parties concerned argues well for its future and for its potential contribution to the basic health services of the Sudan.

Field Workers Forum

84 SLIDES ON LEPROSY; MEDDIA, THE ROYAL TROPICAL INSTITUTE, AMSTERDAM

This series of 84 slides on leprosy, *Leprosy in the Dark Skin*, prepared by Dr D L Leiker, in collaboration with Professor W Peters of the London School of Tropical Medicine and Hygiene and WHO, is available on microfiche, or in the form of unmounted slides. There is a written text in English (soon to be available in French and Spanish), with the following main sections – Bacteriology, Epidemiology, Diagnosis, Classification, Leprosy reactions, Chemotherapy, Complications, Differential Diagnosis and Control.

The photomicrographs are of high quality and provide an immense amount of valuable information in compact form. We congratulate Dr Leiker and his colleagues on this initiative and wish the set every possible success and a wide distribution.

Apply to MEDDIA, Royal Tropical Institute, 63 Mauritskade, Amsterdam Oost, The Netherlands.

ALERT, PO BOX 165, ADDIS ABABA, ETHIOPIA (THE ALL AFRICA LEPROSY AND REHABILITATION AND TRAINING CENTRE). COURSES OF INSTRUCTION DURING 1981

Monday 12 January – Saturday 22 February:

Senior Rural Area Supervisors Course on Clinical Leprosy and Leprosy Control, (2 visiting lecturers invited.)

Monday 30 March – Saturday 11 April:

Course on Surgery in *Leprosy* for Surgeons and Medical Officers with experience in surgery.

Monday 11 May – Saturday 6 June:

Course for Medical Officers – I Emphasis on Clinical Leprosy and on Teaching of Leprosy.

Monday 21 September – Saturday 31 October:

Course for Physiotherapists who are working in Leprosy Projects or are going to do so.

Monday 5 October – Saturday 31 October:

Course for Medical Officers – II Emphasis on Leprosy Control.

Monday 16 November – Saturday 28 November:

Surgical Workshop (For Members of Working Group only).

SCHIEFFELIN LEPROSY RESEARCH & TRAINING CENTRE, KARIGIRI, SOUTH INDIA. SCHEDULE OF TRAINING COURSE FOR THE YEAR 1981.

Course	Qualification	Duration	Commencing date	No. of seats	Fees in Rs.
For Doctors					
(a) Condensed course in Leprosy	Doctors & Senior Medical personnel	1 week	19 January 6 April 7 September	20	25
(b) Medical students course	Undergraduates	1 week	Pooja Holidays	20	—
(c) Medical Officers course	Medical personnel engaged in leprosy work	6 weeks	2 February 13 July	16	75
(d) Ophthalmic aspects in Leprosy	Qualified medical personnel (included in 6 weeks' course)	3 days	9-11 February 20-22 July	12	10
For Non-Medical personnel					
(a) Non-medical Supervisors course	Fully qualified para-medical workers with a minimum of 3 years' experience	4 months	8 June	12	200
(b) Orientation course in Leprosy	For paramedical personnel (Nurses, Physios, OT and administrators) 1 week condensed course + 3 weeks in-service training	1 month	19 January 6 April 7 September	6	—
(c) Para-medical workers Course	SSLC passed, graduates preferred	6 months	3 August	20	200
(d) PMW Refresher course	Qualified PMWs	3 weeks	8 June	20	50
(e) Leprosy for general health workers	Persons now working or trained as general health workers	1 week	31 March 17 November	20	30
(f) Physiotherapy technicians' Course	SSLC passed, graduates preferred	9 months	15 June	8	200
(g) Laboratory technicians' Course	SSLC passed, PUC preferred	12 months	1 July	4	150
In-Service Training					
(a) In-service training in medicine, Surgery, Pathology, Control & Laboratory Technology	For qualified medical personnel	9 months	By arrangement	—	—

SCHEDULE OF TRAINING COURSE FOR THE YEAR 1981 (Continued)

Course	Qualification	Duration	Commencing date	No. of seats	Fees in Rs.
In-Service Training					
(b) Prosthetic Technicians	SSLC passed, PUC preferred	18 months	January & July	3	—
(c) Shoe-makers' Course	V standard with knowledge of English preferred	6 months	by arrangement		
(d) Smear Technicians	SSLC passed Qualified Lab. Technicians	3 months 1 month	by arrangement		50
(e) Medical Record-keepers	SSLC with proficiency in typing and good English	1 month 2 months	by arrangement		50

Instructions to candidates:

These courses are recognised by the Government of Tamil Nadu and the Government of India. Candidates will be awarded Government recognized certificates.

In-service Training for Doctors: In the case of in-service training, Medical personnel are expected to carry out routine regular duties in the concerned departments like any other members of staff in that particular department.

All correspondence to the Training Officer, SLR and T Centre, SLRS PO, via KATPADI 632 106, NA Dt S. India.

ILEP: MEMBER ORGANISATIONS & MEDICAL COMMISSION

We are most grateful to the General Secretary of ILEP for permission to publish the following list of addresses of the Member Organizations and members of the Medical Commission of ILEP (The International Federation of Anti-Leprosy Associations, 234, Blythe Road, London W14 0HJ)

Member-associations/Association-members

Aide Aux Lepreux Emmaus-Suisse (ALES)
9 Spitalgasse
CH 3011 Berne
Switzerland/Suisse
Tel. 31-22.77.97

American Leprosy Missions (ALM)
1262 Broad Street
Bloomfield, NJ 07003
USA
Tel. 201-338 9197

Amici Dei Lebbrosi Ocsi (AL)
4 Via Borselli
I 40135 Bologna
Italy/Italie
Tel. 51-42.38.09

Amigos De Los Enfermos De Lepra (AEL)
Marques de Santa Ana
20 Bajo derecha
E – Madrid 10, Spain/Espagne
Te. 1-221.80.11

Amis Du Pere Damien, Les (APD)
16 rue Stévin
B 1040 Bruxelles, Belgium/Belgique
Tel. 2-230.66.05

Association Francaise Des Fondations Raoul Follereau (FF)
33, rue de Dantzig
F 75015 Paris, France
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ILEP (Continued)

Member-associations/Association-membres

Association Luxembourgeoise Des
Fondations Raoul Follereau (FL)
26, rue Maréchal Foch (rez-de-chaussée)
L – Luxembourg
Tel. 352/44.66.06

British Leprosy Relief Association (LEPRA)
Fairfax House
Causton Road
GB – Colchester CO1 1PU
Great Britain/Grande Bretagne
Tel. 206-62286/7

Catholic Relief Services (CRS)
11, rue de Cornavin
CH 1201 Geneva
Switzerland/Suisse
Tel. 22-31.46.54 Telex: 23866 CRS CH

Deutsches Aussaetzigen-Hilfswerk (DAHW)
Postfach 348
D 8700 Wuerzburg
West Germany/Allemagne Federale
Tel. 931-50.784 Telex: 68583 dahw d

Evangelische Leprahilfswerk (ELH)
Deutsches Institut für Ärztliche Mission
24 Paul Lechler Strasse
D 7400 Tuebingen
West Germany/Allemagne Federale
Tel. 7071-46.85

Fame Pereo (FP)
2065 Ouest, rue Sherbrooke
Montreal H3H 1G6
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Canada
Tel. 514-931.3867

Fondation Pere Damien (FOPERDA)
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Reppersbergstrasse 66
D – 6600 Saarbrücken 1
West Germany/Allemagne Federale
Tel. 681/53260

Leprosy Mission, The (TLM)
50 Portland Place
GB – London W1N 3DG
Great Britain/Grande Bretagne
Tel. 637 2611

Leprosy Trust Board
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NZ – Christchurch
New Zealand/Nouvelle Zelande
Tel. 3 63-685

Nederlandse Stichting Voor Leprabestrijding
Koninklijk Instituut voor de Tropen
63, Mauritskade
NL 1092 AD Amsterdam
Netherlands/Pays-Bas
Tel. 20-93.89.73 Telex: 15080 KIT NL

Ordre de Malte (C.I.A.L. de) (OM)
3, Place Claparède
CH 1200 Geneva
Switzerland/Suisse
Tel. 22-46.86.87

Order of Charity, The (OC)
61, Berners Street
GB – Ipswich IP1 3LN
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Tel. 473-51.469

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Red Barnet (RD)
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DK – 2820 Gentofte
Denmark/Danemark
Tel. 1-68.08.88

Redd Barna (RN)
Lilletorget 1
N – Oslo 1
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Tel. 2-41.33.78

ILEP (Continued)

Member-associations/Association-membres

Sanatorio San Francisco de Broja
Fontilles (SF)
E – Alicante
Spain/Espagne
Tel. 965-78.13.58

Sasakawa Memorial Health Foundation (SJ)
Sabokaikan Bldg
2-7-5 Hirakawa-Cho, Chiyoda-Ku
J – Tokyo 102
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E – Alicante
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News and Notes

INDIA; ANNUAL REPORTS FROM THREE CENTRES

We are extremely grateful to have received reports from our colleagues in India as follows:

(1) Hind Kusht Nivaran Sangh. The Indian Leprosy Association

Annual Report for 1979, including detailed accounts of activities in the various state branches.

(2) Central Jalma Institute for Leprosy, Tajganj, Agra (Indian Council of Medical Research)

Annual Report for 1979, under the headings – Treatment and Service Facilities, Clinical and Therapeutic Studies, Immunology, Microbiology, Morphological Studies, Biochemistry, Animal Experimentation, Field Study Unit, Training and Publications.

(3) Bombay Leprosy Project. 6/27 Amar Bhuvan, Sion East, Bombay 400 022

Annual Report for 1979, describing Methodology, Staff, Activities, Prevalence Rates in Slums, Treatment, Lectures, Health, Education, Public Relation Activities, Rehabilitation and Research.

These excellent accounts of the continuing fight against formidable odds in India should be studied in the originals, which are available on request from the appropriate directors.

ARMAUER HANSEN RESEARCH INSTITUTE, PO BOX 1005, ADDIS ABABA, ETHIOPIA. CONFERENCE ON IMMUNOLOGICAL ASPECTS OF LEPROSY, TUBERCULOSIS AND LEISHMANIASIS, 27–30 OCTOBER 1980, ADDIS ABABA HILTON HOTEL

This conference, which was attended by a highly representative number of world experts, included the following papers on leprosy:

- (1) JK Seydel, West Germany. Drug Development for Therapy of Leprosy Studies in Cell-Free and Whole Cell System of Mycobacteria.
- (2) E Freersken, West Germany. Eradication of Leprosy and Tuberculosis Using Inoprodian and Rifampicin (Background and Findings).
- (3) ABG Laing, Malaysia. Thalidomide in the Treatment of Erythema Nodosum Leprosum in Malaysia.
- (4) WF Kirchheimer, USA. Advances in Leprosy Research.
- (5) BR Chatterjee, India. Leprosy Endemicity in India – A Changing Profile.
- (6) E Van Praag, Tanzania. A Screening Survey on Leprosy and the Possible Role of Village Ten Cell Leaders in Case Detection in Shuheza District, Tanzania.
- (7) JK Nsimbambi, Ethiopia. All Africa Leprosy and Rehabilitation Training Centre (ALERT), Goals and Activities.

- (8) F Portaels, Belgium, Parameters influencing the *In Vitro* Cultivation of *Mycobacterium lepraemurium*.
- (9) R Sher, South Africa. Trace Elements and Vitamin A Metabolism in the Various Leprosy Sub-Types.
- (10) M Nuti, Italy. Lymphocytotoxic Antibodies in Leprosy. Preliminary Data..

ILEP; 35th MEETING OF THE MEDICAL COMMISSION, HOTEL DEI PRINCIPI, ROME, 10 DECEMBER 1980

In a following number, if space permits, it is intended to give an account of some of the interesting matters discussed at this meeting. Meanwhile we are grateful to Dr H Wheate for the following information:

The ILEP Medical Commission met recently in Rome and issued two important recommendations concerning the use of Rifampicin and Clofazimine:

Rifampicin

Should be prescribed only by a medical practitioner, should never be given as monotherapy, and should never be given unsupervised for self-treatment at home.

Clofazimine

Because of the numerous reports of toxic phenomena following the prolonged administration of Clofazimine (Lamprene/CIBA-GEIGY) in doses exceeding 300 mg daily, as treatment for ENL, the ILEP Medical Commission is of the opinion that clinicians using this valuable drug should exercise considerable caution in giving it in doses of 300 mg or more for periods exceeding 3 months.

LEPROSY SYMPOSIUM

A Symposium in leprosy with emphasis on leprosy in Europe will be held from 1 to 3 May 1981 in Santa Margherita Ligure, near Genoa, Italy. Further details may be obtained from the Italian leprosy relief organization: Amici di Raoul Follereau, Via Borselli 4, 40135 Bologna, Italy.

Letter to the Editor

Does the suppression of the immune response in pre-clinical lepromatous leprosy affect both cell-mediated and humoral responses to *Mycobacterium leprae* antigens?

Sir,

My article on 'Importance of the neural predilection of *Mycobacterium leprae* in leprosy' was summarized superbly by Dr Jopling in your September 1980 number (p. 269). I only wish to emphasize here, as I did not do originally, that little, if any, information is available on the question of the detailed cellular mechanisms of suppression in leprosy. It is, therefore, not yet possible to predict whether antibody to *M. leprae* antigens is consistently produced during the postulated suppression phase which accompanies very early (pre-clinical) lepromatous leprosy, i.e. before skin smears become positive for AFB.

One possibility, as was suggested, is that the suppression of specific cell-mediated immunity (CMI) to *M. leprae* is accompanied by a humoral immune response to antigens of *M. leprae*. However, the alternative possibility cannot be excluded, i.e. that the suppression at that early stage is mediated by suppressor cells which suppress both cell-mediated and antibody responses to *M. leprae*.

This question is important because the possibility of very early serodiagnosis of lepromatous leprosy would seem to depend on the answer. A serological test would not only be of value in leprosy control programmes, but it might also find application in the exclusion of infected individuals from vaccine trials and/or in the early identification of lepromatous leprosy in the trial group.

If suppression of CMI to *M. leprae* in very early lepromatous leprosy is accompanied by formation of significant amounts of antibody, then efforts at very early serodiagnosis are well founded. If, on the other hand, the suppression extends over the antibody response as well, then early detection of lepromatous leprosy by serological methods may be difficult. In that case the level of various antibody specificities in these individuals may not differ from those of healthy individuals in the community who were subclinically infected. The antibody levels would presumably follow the bacterial load and might become 'positive' no earlier than skin smears stained for AFB.

In a study of the immune response to *M. leprae* in lepromin-negative

indeterminate leprosy¹ there was evidence for a failure of both CMI and the antibody response. Sera were assayed for precipitins to mycobacterial antigens. This would suggest that early suppression may involve both CMI and the antibody response, and bodes ill for early serodiagnosis of lepromatous leprosy. However, the question of antibody responses in lepromin-negative indeterminate leprosy must be re-examined with the more sensitive and/or specific assays recently developed in several laboratories.^{2,3,4,5,6} With the highly sensitive fluorescent antibody test, in which 36 out of 44 tuberculoid (TT) sera (82%) were positive, 2 out of 4 indeterminate sera were positive.² With the ELISA technique, 3 out of 7 indeterminate sera were positive.⁶

GERALD L STONER

References

- ¹ Myrvang B, Godal T, Feek CM, Ridley DS, Samuel DR. Immune response to *Mycobacterium leprae* in indeterminate leprosy patients. *Acta Path Microbiol Scand*, sect B, 1973, 81, 615–20.
- ² Abe M, Izumi S, Saito T, Mathur SK. Early diagnosis of leprosy by indirect immunofluorescence. *Lepr India*, 1976, 48, 272–76.
- ³ Harboe M, Closs O, Bjune G, Kronvall G, Axelsen NH. *Mycobacterium leprae* specific antibodies detected by radioimmunoassay. *Scand J Immunol*, 1978, 7, 111–20.
- ⁴ Yoder L, Naafs B, Harboe M, Bjune G. Antibody activity against *Mycobacterium leprae* antigen 7 in leprosy: Studies on variation in antibody content throughout the spectrum and on the effect of DDS treatment and relapse in BT leprosy. *Lepr Rev*, 1979, 50, 113–21.
- ⁵ Abe M, Minagawa F, Yoshino Y, Ozawa T, Saikawa K. Fluorescent leprosy antibody absorption (FLA-ABS) test for detecting subclinical infection with *Mycobacterium leprae*. *Int J Leprosy*, 1980, 48 (in press).
- ⁶ Ulrich M. Serological studies in leprosy using the ELISA technique. Paper presented to the 3rd IMMLEP Scientific Working Group Meeting, Geneva, 24–26 June 1980.

Book Reviews

Diuciphon: Experimental and Clinical Data by N M Goloschapov, Moscow.

This booklet of 60 pages describes a new anti-leprosy drug named Diuciphon, synthesized and investigated in the USSR. Its chemical name is: para-para-bis (2,4-dioxo-6-methyl-pyrimidinyl-5-sulphono-amino)-diphenyl sulphone, and its empirical formula is: $C_{22}H_{20}N_6O_{10}S_3$. Clinical trials began in March 1971, and the drug was officially approved by the USSR Ministry of Health in March 1973. It is a white powder, stable, soluble in water, and can be administered orally or by intramuscular injection as a 10% solution prepared immediately before use. The highest concentration in blood is reached in 3–4 h. After oral administration it is no longer detected in the blood at 24 h, but after injection a measurable amount is detected at 48 h, hence the drug cannot be given orally less frequently than twice a day, and by injection less frequently than once in 48 h. It is excreted via the urine.

The trial

The trial reported in this booklet was carried out in 3 clinical centres in the USSR after exhaustive investigations in mice and rats. One hundred and twenty-one leprosy patients were divided into 3 groups, and before giving a summary of the trial I must make the reservation that I encountered some difficulty in gaining the information I considered important; first, because in groups 1 and 2 non-lepromatous patients are included and their progress is not assessed separately from that of the

lepromatous patients, and, secondly, because of some discrepancies between text and tables.

Group 1 consisted of 20 patients who had not received previous treatment. Eleven were lepromatous and 9 non-lepromatous, and all 20 had active skin lesions. *Clinical results:* all skin lesions healed at 6 months in 5 patients, at 12 months in 4 more, at 24 months in 6 more, at 36 months in 4 more, and at 48 months in the last patient.

Bacteriological results: 10 patients had positive nasal scrapes at the beginning of treatment (it is odd that they were negative in one of the lepromatous patients), and nasal scrapings became negative in all 10 by 12–24 months. Skin smears were positive in 14 of the 20 patients, with solid-staining bacilli in 11. By 6 months solid bacilli were found in 10, by 12 months in 5, by 24 months in 2, and by 36 months no solids were found. *Histological results:* 16 patients had solid-staining bacilli in biopsies of skin lesions (10 in great numbers). These disappeared in 5 patients by 14–24 months, and in the remainder by 36 months. Four lepromatous patients developed histological evidence of upgrading.

Group 2 consisted of 76 patients, all previously treated. Sixty-nine were lepromatous and 7 were non-lepromatous. Only 60 are discussed in the text (those detained as in-patients), the remaining 16 being difficult to follow up as out-patients, but we are not told if all 60 were lepromatous. Of the 60 patients 39 had active skin lesions, 8 had positive nasal scrapings, 33 had positive skin smears (17 with solid-staining AFB), and 57 had AFB in skin sections

(solid in 25). *Clinical results:* improvement was slow. After 12 months skin lesions had healed in 15 (i.e. of 39 originally with skin lesions), and after 24 months skin lesions had healed in another 16; thus 8 patients still had skin changes at 24 months. *Bacteriological results:* at 12 months nasal scrapings became negative in all 8 patients who were positive originally. As regards the 17 who had solid-staining bacilli in skin smears, by 12 months only 6 had solids, by 24 months there were only 2, and by 36 months no solid-staining AFB were seen. *Histological results:* of the 25 patients who had solid bacilli in biopsies when the trial started, none remained at 24 months. In 4 patients there was histological upgrading from lepromatous to 'a tuberculoid type' – actually, borderline.

Group 3 consisted of 25 lepromatous patients resistant to other anti-leprosy drugs (including Dapsone). Twenty had active skin lesions, 8 had positive nasal scrapings, and all had positive skin smears (solid and granular bacilli). *Clinical results:* at 12 months skin lesions had healed in 13, at 24 months in one more, and at 36 months in 5 more (i.e. in 19 out of 20). *Bacteriological results:* all solid-staining bacilli had disappeared by 25–36 months. *Histological results:* solid bacilli disappeared more slowly than in skin smears, and after 4–5 years of treatment 2 patients had a few solids among the granular forms, 11 had large numbers of granular AFB only, 7 had occasional single acid-fast granules, and 5 had no AFB. Two patients showed histological evidence of upgrading.

Full case histories are given of one patient in group 1, one in group 2, and of two patients in group 3, together with black-and-white photographs.

Conclusions

Diuciphon is effective in all types of leprosy, including Dapsone-resistant leprosy. No toxic effects or reactions were observed throughout the trial. The tendency for Diuciphon to stimulate histological upgrading was demonstrated in 4 patients in

group 1, 4 in group 2, and 2 in group 3 (one with clinical upgrading in addition).

In the final pages of the booklet notes are given, together with tables, of the progress of 87 patients in a control group who were treated with various antileprosy drugs other than Diuciphon, and less satisfactory results were recorded.

Dr Goloschapov has written to tell me that Diuciphon has been patented in the UK, USA, France and Japan, and will be marketed in 1981. He is hopeful that the drug will prove of value in the treatment of systemic sclerosis, lupus erythematosus, and rheumatoid arthritis, and investigations are in progress. I understand that copies of the booklet are obtainable in English, French, Spanish and German, and anyone requiring a copy should apply to: V/O 'Medexport', 31, bldg 2, Kakhovka Street, Moscow 113461, USSR. A leprologist requiring a small supply of Diuciphon for treating a patient suffering from drug-resistant leprosy should write to Dr Edward Arminakovich Babayan, Head of the Department of New Medical Treatments, USSR Ministry of Health, Moscow.

W H JOPLING

Standard Management of Tuberculosis and Leprosy in Papua New Guinea, by Myra Kennedy. Boroko, Hebamo Press, Papua New Guinea, Undated.

This practical handbook of 128 pages describes the national policy for the control of tuberculosis and leprosy throughout Papua New Guinea based on case findings, treatment, immunization, health education, and contact tracing. Principal clinical manifestations of the commonly-encountered forms of tuberculosis and of the various types of leprosy are described, and there are 9 good black-and-white photographs of leprosy lesions. The keeping of records is given detailed coverage, together with the making of sputum smears and skin smears. Treatment of tuberculosis is based on 3 drugs – Streptomycin, Isoniazid (INAH), and Thiacetazone (TB1), and dosage schedules are given for patients over 40 kg in

weight, for those under 40 kg, and for children, and side effects are discussed. The majority of patients will need hospital admission for the first 8 weeks (during the time of Streptomycin injections) and then can continue oral therapy as out-patients for 16 months, but admission must not be compulsory. Only a small minority of leprosy patients will need admission, and forced admission is condemned; those who are 'skin smear positive' are given daily treatment with Dapsone (DDS) 100 mg and Clofazimine (Lamprene) 100 mg for 12 weeks, after which they continue on Dapsone alone, while 'skin smear negative' patients are given Dapsone alone in daily dosage of 100 mg. Length of treatment ranges from 2 to 3 years for indeterminate leprosy to life for those on the lepromatous side of the spectrum. The importance of regular treatment and the taking of skin smears at regular intervals is emphasized, with notes on the side effects of these two anti-leprosy drugs and on Dapsone resistance. There is a chapter on Lepra reaction and nerve damage, and one on the prevention and treatment of plantar ulcer. BCG in the prevention of tuberculosis is fully described, to be given three times to children (at or soon after birth, at 7 years, and at 13 years) and once to adults who have never been immunized. The place of BCG in the prevention of leprosy is not mentioned. The tuberculin (Mantoux) test is described, and sound advice is given regarding contact tracing and health education for patients and their families.

The information within these pages will prove of great value to medical auxiliaries, not only in Papua New Guinea but throughout the tropics, but the reviewer would like to see some minor alterations and additions in the next edition: (1) the inclusion of finger smears in the diagnosis and follow-up of lepromatous patients (Jopling, 1979);¹ (2) dosage of Clofazimine in the treatment of leprosy is unnecessarily high; one capsule of 50 mg daily for the first 12 weeks would be adequate for an adult, especially if a single oral dose of 1,200 mg

Rifampicin is given on the first day of treatment to render a lepromatous patient non-infectious and rapidly to reduce the number of living AFB. It should be noted that Ciba-Geigy are now manufacturing 50 mg capsules. The statement on page 105 that 'children with positive skin smears can safely be given Clofazimine 100 mg daily' requires correction; 50 mg on alternate days would be quite adequate; (3) in the treatment of pulmonary tuberculosis in developing countries a strong case can be made for reducing the course of chemotherapy to 6 months (Scott, 1978);² for the first 2 months the patient receives daily treatment with 4 drugs (Streptomycin 1 g, Isoniazid 300 mg, Rifampicin 450–600 mg, and Pyrazinamide 1.5–2.0 g) and during the remaining 4 months he receives the standard combination of Thiacetazone 150 mg plus Isoniazid 300 mg daily.

W H JOPLING

References

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- ² Stott H. The treatment of pulmonary tuberculosis in the developing countries. *Trans Roy Soc Trop Med Hyg*, 1978, 72, 564–9.

Dermatology in General Medicine. Textbook and Atlas (2nd ed.), edited by T B Fitzpatrick, A Z Eisen, K Wolff, I M Freedberg and K F Austen. McGraw-Hill Book Company, New York. Price £63.

This is the second edition of a book which has already been highly praised in dermatological and other circles. Including the index, it runs to 1,884 pages; it is 8 cm thick and weighs over 7 kg. There are seven main parts with the following titles: 'Introduction to dermatology in general medicine', 'Biology and pathophysiology of skin', 'Disorders primarily arising in the skin and mucous membrane', 'Dermatology and internal medicine', 'Disorders due to microbial agents', 'Therapy of dermatological disorders' and 'Dermatologic atlas'.

The pages dealing with 'bacterial diseases with cutaneous involvement' are exceptionally well written and the section on leprosy,

by S G Browne, is a delight to read. The UK price seems to have jumped from around £49 to £63 in a matter of months and this will rule out possession of this remarkable book for most individuals – which is a pity, for it is currently claimed by experienced dermatologists and clinicians to be the best of its type available.

Dermatology Update. Reviews for Physicians (1979 edition). Elsevier, New York–Oxford. Price not stated, probably around £13.00.

Those who are not already familiar with other titles in this series (paediatrics, cardiology, psychiatric medicine, drug therapeutics and clinical immunology) may wonder how it is possible to justify yet another ‘update’ or review book of this size on the subject of dermatology. Yet half an hour is more than enough to demonstrate its value. This is a beautifully produced and printed book of 456 pages, designed, as the preface says, ‘... to update dermatologists, internists, paediatricians, general practitioners, pathologists, and physicians-in-training in dermatologic progress. A variety of the subjects are reviewed in depth and accompanied by current and complete references. Significant progress is reported in genetics, such as relationship of HLA antigens to dermatologic disease; in an understanding of the heritable disorders of connective tissue diseases, and in the approach and in the diagnoses of heritable skin diseases. Some of the advances in immunology have resulted in a better understanding of the blister diseases and the collagen–vascular diseases, such as cutaneous necrotizing venulitis and mixed connective tissue disease; these too are thoroughly reviewed. The intriguing variants of morphea (localized scleroderma), particularly eosinophilic fasciitis, as well as the newer knowledge of systemic sclerosis, are described.’ There are excellent sections on infectious diseases, including mycobacterial infections of the skin; the section on leprosy is difficult to fault. This is by no means a book for the novice, either in dermatology or leprosy, but those with

knowledge of these subjects will find it fascinating – some might even say essential reading.

Functional Histology: a Text and Colour Atlas, by P R Wheater, H G Burkitt and V G Daniels. Illustrated by P J Deakin; foreword by R Warwick. Churchill Livingstone, Edinburgh, London and New York, 1979. Price £19.50.

This is a robust, hardback book, measuring 19 × 25 cm, running to 278 pages, including an excellent index, and is an outstandingly well written and illustrated account of mammalian cells and tissues (largely from human sources). The idea for this book ‘... arose from the teaching experience of the authors with Open University students studying histology for the first time. Their ideas were consolidated and developed whilst the authors studied medicine as mature students.’ The illustrator, Philip Deakin, was originally trained in graphics, then graduated in physiology before proceeding to medicine, which he is currently completing. The authors begin their preface with the words ‘Histology has bored generations of students’ and go on to say that they believe this is almost certainly because it has been regarded as a study of structure, isolated from function. This remarkable book goes a very long way to correcting this approach; it should also be of great value to pathologists.

Tuberculosis Case-finding and Chemotherapy: Questions and Answers, by K Toman. WHO, 1978. Price 32 Swiss francs.

This is a cloth-bound book of 239 pages, 16 × 24 cm and 2 cm thick. It poses over 50 questions on the most important and practical aspects of tuberculosis under the main headings of case-finding and chemotherapy, and then answers them in detail. There is a preface by the Director General of WHO, Dr H Mahler. Professor John Crofton of the Department of Tuberculosis in Edinburgh has already reviewed this valuable book in *Tubercle* 60 (1979),

page 195, and his tribute ends: 'Everyone interested in tuberculosis must be grateful to Dr Toman for his industry, his skill in construction and his capacity to crystallize out the practical implications of research. For this is essentially a practical book for the man in the field.'

This book makes interesting reading, if only because of the comparatively confident way in which various regimes of chemotherapy in tuberculosis can be discussed. But it is also a highly informative and educational book, without parallel (regrettably) in the leprosy field.

(Earlier this year, IUAT and ILEP members were circulated on the matter of a reduced price and enquiries could still be sent to these agencies.)

Leprosy. Proceedings of the XIth International Leprosy Congress, Mexico City, November 13–18, 1978, Excerpta Medica, Amsterdam–Oxford–Princeton, 1980, edited by F Latapi, A Saul, O Rodriguez, M Malacara and S G Browne. Price 170 Dutch florins.

This is the definitive publication for the XIth International Leprosy Congress, produced from camera-ready typed scripts submitted by participants prior to the event. The main headings are: epidemiology and control, experimental leprosy, clinical aspects, microbiology, immunology, social aspects, experimental chemotherapy, clinicopathological aspects, nerve damage, therapy, rehabilitation and workshop summaries.

The standard of production and clarity are high, but the price (about £36 sterling; \$75, US) will put it far beyond most individuals. This is regrettable, for, until the next Congress in 1983, it carries in considerable detail the views of many

world experts in the field of leprosy, and as such it should be freely available to the widest possible readership.

A C McDOUGALL

Mister Leprosy, by Phyllis Thompson. Published jointly by Hodder and Stoughton and the Leprosy Mission England and Wales. Paper, 220 pp. £1.50.

A life-time of service to those who suffer from leprosy was recognized today by the publication of a biography on the life of Dr Stanley Browne, GMG, OBE. Published jointly by Hodder and Stoughton and the Leprosy Mission England and Wales, the book has been written by Phyllis Thompson, and is a story of dedicated Christian service by a man born in the dreary streets of New Cross, in South-East London, who has become one of the most respected figures in the treatment and control of leprosy throughout the world.

Tracing his life from the early family days in London, as a boy in a secondary school, as a student at King's College, London, as a missionary doctor in Africa, and as a consultant reaching the top of his profession, Phyllis Thompson unfolds the fascinating story of a brilliant and dedicated doctor who firmly believes that God has called him into each situation, and has given him a definite task to perform. The book reveals the hardships which faced Stanley Browne throughout his life, and the chapters on missionary activity in the Belgian Congo (now Zaire), provide a good account of the development of medical work in that country. Those who know Phyllis Thompson's other books will appreciate her readable style as she reveals a life of prayer and hard work which has been honoured internationally.

Abstracts

1. GIDOH M, TSUTSUMI S, NARITA M, FUKUSHI K. **Influences of several anti-leprosy drugs on immunological state of experimental animals.** (1979) *Jap J Lepr*, 1979, 48, 4–169.

In a previous paper (Gidoh *et al*, *Jap J Leprosy* 48, 1979, 7–8) these authors provided evidence that DDS can suppress acute inflammation and cellular exudation in several experimental systems, while exacerbating the chronic inflammation involved in adjuvant-induced arthritis. In contrast, B663 was able to suppress this type of chronic inflammation. They speculated that the effect of B663 might be due to the activation of suppressor T-cells, leading to the normalization of cell-mediated immunity.

In the present paper the authors have investigated the effects of these and other drugs, including levamisole, on the antibody and delayed skin-test responses to sheep erythrocytes of mice and guinea-pigs. They also looked at the percentage of guinea-pig peripheral blood lymphocytes which formed rosettes with rabbit erythrocytes. Such cells are assumed to be mainly T-lymphocytes. Dapsone was given daily for 10 days at between 10 and 100 mg/kilo, whereas B663 was used daily at 30 mg/kilo. The use of such large doses must cast doubt on the relevance of the results to man. Nevertheless the findings were interesting.

There was no convincing effect of either drug on antibody or delayed skin-test responsiveness of normal animals unless the dose was so large that the animals were ill. On the other hand dapsone significantly decreased the percentage of guinea-pig lymphocytes which formed rosettes with rabbit erythrocytes, whereas B663 did not,

or sometimes, like levamisole, caused a slight increase. These effects were enhanced in thymectomized animals. The authors suggest therefore, that thymectomized animals may provide a sensitive assay for any immunomodulatory effects of these drugs.

Comment

The efficacy of dapsone in the treatment of several conditions not known to be caused by an infectious agent has prompted the suggestion from many authors that in the treatment of leprosy it may have effects on the immune response of the host, as well as effects on the organism. Indeed the rather low serum levels which can be achieved with dapsone have led some authors to doubt the existence of a direct antibacterial effect. The demonstration of apparently dapsone-resistant *Mycobacterium leprae* by the mouse foot-pad technique can never completely refute this argument, since it does not exclude the possibility that such organisms are resistant to a dapsone-induced change in the immune response, rather than to the drug itself. However the need for such a tortuous hypothesis has now diminished, because mycobacterial species have been found (Portaels, *personal communication*) which are sensitive to very low dapsone levels comparable to those achieved in the patient's serum.

Nevertheless it remains theoretically possible that direct effects of dapsone on the host are also important and therefore, that failure of dapsone therapy could sometimes be due to host factors.

G A W Rook

2. TEN DAM HG, HITZE KL. **Determining the prevalence of tuberculosis infection in populations with non-specific tuberculin sensitivity.** *Bulletin of the WHO* 1980 58, 475-83.

A simple and interesting concept is presented in this paper based on tuberculin testing before and after BCG vaccination. Basically the authors say that whereas positive responses due to infection with tubercle bacilli or previous vaccination are not enhanced after BCG, tuberculin reactions due to sensitization by non-tubercle bacilli are increased in size after BCG vaccination. Their explanation for this is that only *Mycobacterium tuberculosis* and its variants including BCG can sensitize to the species specific antigens of this species. Thus a reaction to shared mycobacterial antigens will be increased in size by the addition of sensitization to the species specific antigens.

This simple hypothesis enables the authors to estimate the prevalence of tuberculosis infection by measuring the percentage of persons in a given age group who

do not have previous BCG scars, but do have positive responses to tuberculin that are not increased in size by subsequent BCG. Applying their method to Dahomey and Mauritania the authors arrive at the figures of 10.7% and 17.5% as the prevalences of tuberculosis in the two countries respectively. In each case this is less than half of the prevalence calculable on the basis of a single tuberculin test result. The same was true when the authors applied their principles to the published work of others on Burundi.

An omission from this work was detail on how soon after BCG the second tuberculin test was performed, although this would not undermine the hypothesis. More serious today was their lack of consideration of the probability that not all positive responses to tuberculin are by the same immunological mechanism. If this is finally proven to be the case then perhaps the assessment of disease prevalence are erroneous or only fortuitously correct and the authors' system might prove better as a measure of susceptibility.

J L Stanford

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1. Browne, S. G.: Lepr. Rev. 37, 141 (1966)

2. Azulay et al.: Lepr. Rev. 46 (Suppl.) 99 (1975)

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