Editorial

DO FLIES TRANSMIT LEPROSY?

Exactly 3 years ago an editorial in *Leprosy Review* (1972, **43**, 165) posed the question "can arthropods transmit leprosy?", in reviewing the compelling experimental evidence from studies on arthropods (mosquito, bed bug and scabies mite) presented by Narayanan and his colleagues (*Leprosy Review,* 1972, **43**, 188 and 194). They showed that these blood sucking insects fed on active lepromatous patients contained in their stomachs acid-fast bacilli (AFB) which failed to grow on conventional bacteriological media but multiplied when injected into mice, and their growth characteristics resembled those of *Mycobacterium leprae.* Therefore these arthropods potentially could be an intermediate host for the transmission of *Myco. leprae.* In the Review, however, it was pointed out that the number of *Myco. leprae* carried by an arthropod in a blood meal from a lepromatous patient would be very small compared to the vast numbers of bacilli shed in nasal secretions from the same patient. Therefore, potentially indirect transmission from an environment contaminated by such secretions would be a more likely and hazardous source. In the meantime, the studies by Davey and Rees (*Leprosy Review,* 1974, **45**, 121) fully substantiate the potential hazard from these nasal secretions and that *Myco. leprae* in shed secretions in a dessicated state for 2-7 days, are still infectious in mice.

Now, in this number of *Leprosy Review,* J. G. Geater, working in Bhutan presents on the basis of simple but well conceived studies, the potential role of several common genera of flies in the transmission of leprosy. In this rapidly expanding field of studies on the modes of spread of *Myco. leprae* from infectious lepromatous patients, Geater's findings are of particular importance because they strongly implicate the fly as an intermediate insect carrier of *Myco. leprae* from shed and heavily infected nasal secretions direct to human contacts or by indirectly contaminating man's environment. With the limited facilities available to Geater in Bhutan his studies were based on three universally common genera of flies–*Musca* (housefly), *Calliphora* (blue-bottle) and *Stomoxys* (biting stable fly). He first showed that none of these representative genera caught in the wild state well away from the Leprosarium contained AFB in whole fly homogenates. However, when flies from these three genera were caged with a fresh specimen of heavily *Myco. leprae* infected nasal secretion from an untreated lepromatous patient, they were attracted to it and fed gluttonously upon it. Immediately following feeding the flies were separated and killed 1 h, 1, 2 and 3 days later. Each fly was dissected and pools of legs, mouth-piece, abdominal wall and stomach contents of each were homogenized, smears from homogenates prepared and stained with carbol fuchsin for AFB, after exposing to both acid and alcohol. The results showed that the flies in feeding on nasal secretions became

heavily infected with AFB on their legs, mouth-pieces and abdominal walls as well as containing large numbers of AFB within their stomachs. The morphology of the AFB resembled *Myco. leprae* and in particular, at all these sites, globus formation was common. This was the more or less universal finding 1 h after feeding, but a small proportion of the flies retained diminishing numbers of AFB at these various sites up to 3 days later; in particular, persisting AFB on their abdominal walls and within their stomach contents. Still more importantly, Geater showed that when the flies were caged with infected nasal secretion at one end of the cage and at the other end glass slides coated with albumin and with drops of sugar, while they preferred to feed on the nasal secretions they wandered from time to time and fed on both sites. After 2 h of such exposure the slides were removed and stained for AFB. Of 10 such slides examined only one was negative, the remaining 9 contained many AFB and in 6 slides globi were present. In a subsidiary experiment it was shown that flies allowed to feed upon ulcerated skin lesions of a highly positive untreated lepromatous patient also became similarly contaminated with AFB in all sites examined. Thus Geater has established that flies have, as is well known, a predilection for feeding upon human secretions and that, as in other situations, by so feeding the outer surfaces of the flies become contaminated with the material and *ipso facto* with any contaminating micro-organisms. Likewise their stomachs are similarly contaminated and can be a source of spreading the contaminating microorganisms to wherever they next feed, by direct contact or by regurgitation from the digestive tract which occurs at the time of each feed. With the limitations available to Geater he was unable to use mouse inoculation to establish the AFB from the flies as *Myco. leprae* or their viability and it is of paramount importance that such studies now be undertaken. However, the studies of Davey and Rees would strongly suggest that *Myco. leprae* is robust enough to survive on flies for at least 2 days.

Thus, common species of flies must now be seriously considered as vehicles by which *Myco. leprae* could be readily carried from infected nasal or dermal secretions, either directly to another person, or indirectly to the environment at large. These studies on flies, as do those on nasal secretions increase significantly the ways by which leprosy could be spread. However, the eventual importance of all these studies will depend upon the actual mode of transmission of leprosy to man, which has still not been defined, although it is increasingly unlikely to be solely by close and prolonged skin-to-skin contact. Transmission of leprosy to man via the respiratory and gastrointestinal tracts must be reconsidered as they could be continuously exposed to *Myco. leprae* infeoted dust, water, food or feeding utensils. These possible routes of transmission do not lessen or exclude the importance of skin as a site of invasion by *Myco. leprae.* Moreover, since the mode of transmission of leprosy has still not been defined, it is important that all possible routes should be considered by those concerned with control and prevention of leprosy in the field and leprosy research. Until the mode of transmission of leprosy is defined, every effort should be made to minimize the risk of spread of *Myco. leprae* and Geater's findings indicate the importance of controlling fly populations in and around leprosy units.

R. J. W. Rees

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Lepromatous Leprosy in the Nose After One Year of Dapsone Treatment: Clinical and Bacteriological Findings

R. P. E. BARTON

E.N.T. Department, St. Mary's Hospital, London, W.2

and

L. M. HOGERZEIL

Victoria Hospital, Dichpalli, Andhra Pradesh, India

In 1972/73 a series of patients with early untreated lepromatous leprosy in South India were studied and the clinical, bacteriological and histopathological findings in the nose have been reported. The involvement of nasal tissues and the enormous numbers of viable bacilli escaping from the nose into the environment were of such clinical and epidemiological importance that it was clearly essential to repeat the studies after a period of standard out-patient chemotherapy with dapsone. This paper describes the clinical and bacteriological findings in 16 patients out of the original group of 34, who could be contacted after one year. Attention is drawn to the fact that although almost all patients showed marked improvement on clinical and nasal examination, in a significant percentage solid-staining *Mycobacterium leprae* could be demonstrated particularly in scrapings and biopsies of the nasal mucosa, and also in mucus of nose-blow specimens. It is considered that the findings in patients who failed to attend would almost certainly be worse and the problem of ensuring a high follow-up rate is discussed. Four patients in this series who had bacteriological evidence of persisting infectivity after a year of dapsone showed no definite abnormality on simple clinical examination of the nose, and while this is an extremely important part of the initial assessment and diagnosis in suspected lepromatous leprosy, its value is less in the follow-up of patients on treatment as it is often uninformative and may even be misleading. Careful bacteriological examination of the nasal mucus and nasal smears is of as great importance as the information obtained from routine slit-skin smears.

Introduction

During a 3 month period in the winter of 1972/73 a series of patients with early lepromatous leprosy was studied intensively at Victoria Hospital, Dichpalli in South India. The initial results of these studies were presented at the Tenth International Leprosy Congress at Bergen (Barton *et al.*, 1973) and subsequently, detailed accounts of the clinical findings (Barton, 1974), bacteriology (Davey and

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Barton, 1973; Davey and Rees, 1974) and histology (McDougall *et al.*, 1975) have been published. From these studies the nose has been confirmed both as a site of predilection for *Mycobacterium leprae* and also as the most potent source of exit of viable bacilli from the body, emphasizing the importance of patients with untreated lepromatous leprosy in the transmission of this disease within the community. Early cases are particularly dangerous in this respect as the nose may be heavily involved before the general clinical signs become obvious.

It was therefore considered essential to obtain follow-up data in order to assess the detailed response of individual patients to treatment and additionally to evaluate the continuing risk, if any, posed by these patients to the community. Therefore, although this paper is concerned primarily with the results of chemotherapy in lepromatous leprosy, the problem of persuading patients to accept adequate treatment will also be discussed.

Material

In the original series 34 patients with early lepromatous leprosy had the following investigations: clinical and nasal examination, lepromin test, routine multiple slit-skin smears, multiple nasal smears, bacteriological examination of the nasal discharge and biopsy of the nasal mucosa at several sites and of the skin. Intranasal and clinical photographs were taken in most cases although in some patients it was not possible to carry out every investigation. When the work-up on each patient was completed treatment was commenced with dapsone, rising by monthly increments from an initial dosage of 15 mg a week in divided doses to reach 300 mg in the fifth and subsequent months. Patients were normally admitted at the start of treatment and the mean length of inpatient stay was 3.3 months, ranging from nil to 12 months, as some patients refused admission while others were reluctant to leave the hospital.

One patient had died and one was a schoolboy who had stayed in the hospital's school hostel. The remaining 32 were therefore out-patients at the time of this follow-up study which took place over a period of 4 weeks in early 1974. These patients were sent letters in their own languages asking them to attend the hospital between certain dates, but when it became clear that the initial response was poor, members of staff were sent to the last known address of the non-attenders to attempt to trace them and to invite further attendance.

Sixteen patients attended for follow-up and a further 3 were known to be taking dapsone regularly but had adequate and acceptable reasons for not attending specifically for this study. Thus only 56% of the original group appeared to be in contact with the hospital between 12 and 15 months after their initial attendance.

The majority of those who attended for follow-up were prepared to stay for only 24-48 h and thus investigations had to be somewhat limited compared with those at the initial attendance. In particular, it was thought important to obtain biopsies of the nasal mucosa in preference to nasal smears. However, nasal and skin biopsies were obtained from 14 patients, nasal smears from 6, the nasal discharge was analysed in 15, and all 16 patients had routine slit-skin smears with full clinical and nasal examination.

Results

REGULARITY OF TREATMENT

Thirteen of the 16 patients (81%) appeared to have taken dapsone regularly in the prescribed dosage over the course of the year. Since discharge the other 3 had probably taken 6 months supply of dapsone in 9, 10 and 12 months respectively. Thus no patients who attended for follow-up had received less than a total of 6 months treatment subsequent to their first attendance.

SYMPTOMS

All the patients noted improvement in both general health and in nasal symptoms. General symptoms amounted to no more than occasional tingling sensations in the extremities together with some dryness of the hands and feet.

Eleven patients (69%) still had some symptoms from the nose, the most common of which was crust formation. As this study was conducted in the "dry" season this finding was not altogether surprising. However, in all patients who reported persistent nasal symptoms, they were slight compared with the situation that existed prior to treatment, when in all 16 patients they had been severe and often distressing.

Table 1 summarizes the incidence of nasal symptoms.

TABLE 1

Summary of the nasal symptoms in 16 patients with lepromatous leprosy, initially and after one year of dapsone treatment

	Crust formation	Bleeding	Obstruction	No symptoms
Before treatment	11	10	10	0
After one year of dapsone	9	5	3	5

CLINICAL AND NASAL EXAMINATION

As might have been predicted, the patients who did return for follow-up purposes were those who had taken dapsone on a regular, or reasonably effective, basis and were likely to be doing well. This was indeed so, and all 16 patients were generally fit and healthy. Fourteen out of the 15 adults were at work and there was no medical reason preventing the one unemployed man from pursuing his trade. The 16th patient was a bright and active member of his school.

General examination showed a regression of physical signs, and no new problems were noted although one patient suffered transient ENL. Typical clinical improvement is shown in Figs 1 and 2. As the patients were all selected as having early lepromatous leprosy, the improvement in systemic signs, which were often only slight originally, was overshadowed by an impressive improvement in the nasal state. Originally the nose was involved, often heavily, in all 16 of these patients: a typical finding is illustrated in Fig. 3. After a period of 12-15 months treatment the nasal state had improved in all patients and indeed in 12 (75%) the nose was passed as normal on clinical examination. The criteria to be fulfulled before a nose could be accepted as normal, in this instance, were that there should

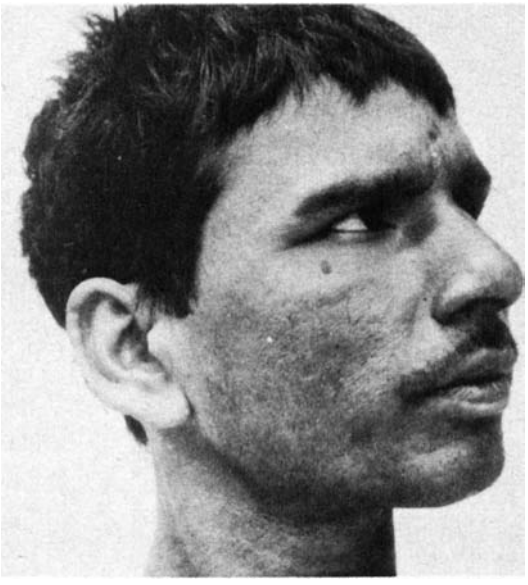

Fig. 1. Typical clinical finding prior to treatment.

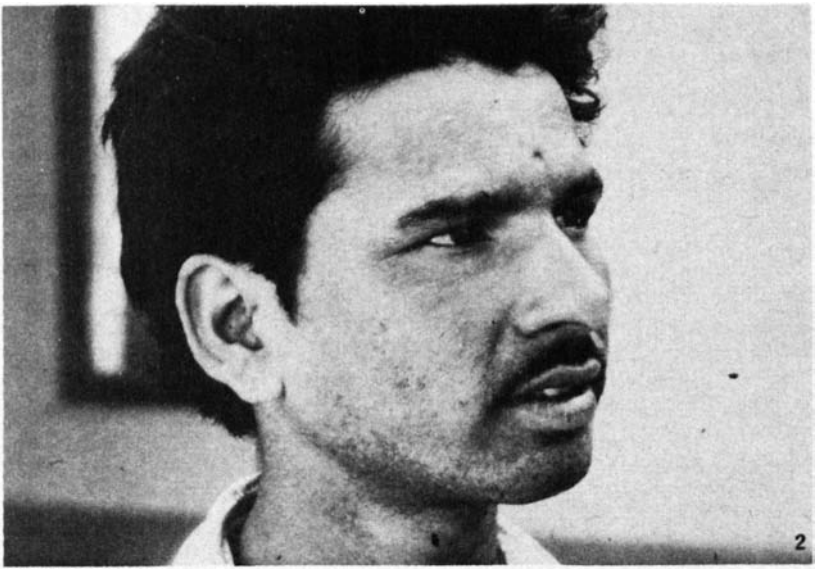

Fig. 2. Typical clinical finding after one year of dapsone.

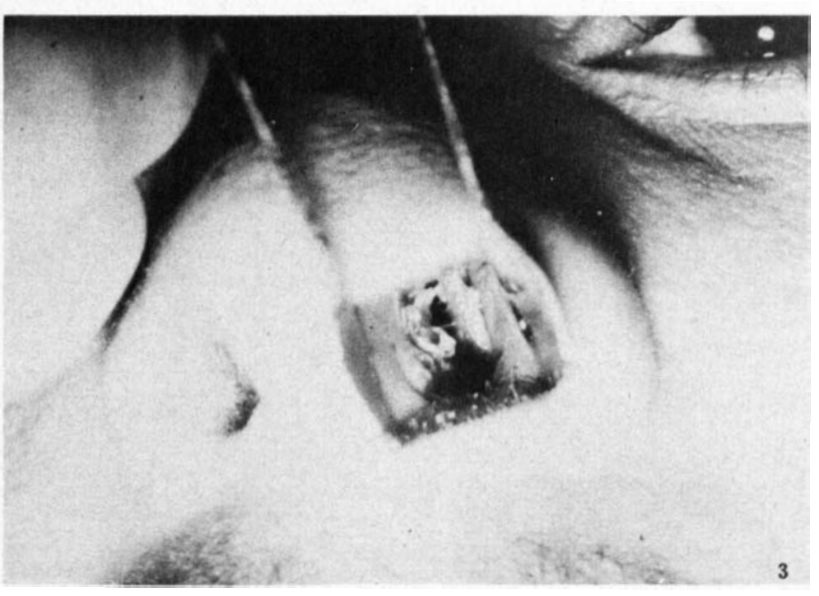

Fig. 3. Typical intranasal finding prior to treatment.

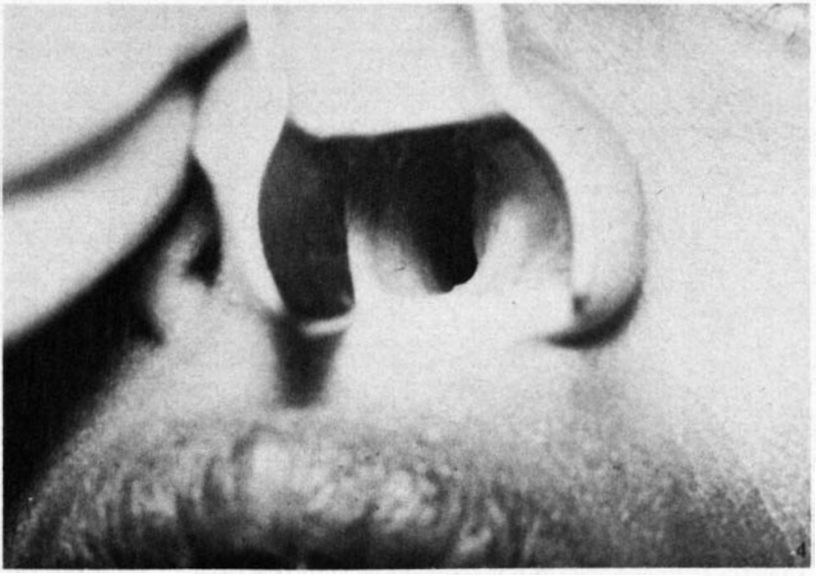

Fig. 4. Typical intranasal finding after one year of dapsone.

be no mucosal lesion detectable on anterior or posterior rhinoscopy, that the airways should be patent and that there should be no destruction of bone or cartilage. Slight reduction in the size of the anterior part of the inferior turbinate, provided that the mucosa was otherwise normal, was allowed, despite the fact that this physical sign is typical, though not pathognomonic, of lepromatous infiltration which has undergone resolution.

Figure 4 illustrates the effect on the nasal mucosa of one year of chemotherapy in a typical case. Four of the 16 patients, although improved, could not be passed as having a normal nose, although in only one was there thought to be definite activity clinically. These patients were:

Case B4. Possible remaining infiltration of the anterior end of the left inferior turbinate. Multiple nasal smears gave an average Bacteriological Index (BI) of 1.2 with a Morphological Index (MI) of zero. The nasal biopsies showed no evidence of solid-staining organisms.

Case B7. There appeared to be definite evidence of remaining lepromatous infiltration, and biopsies confirmed the presence of solid staining organisms in the nasal mucosa, although the nasal discharge had a zero MI (BI 2).

Case B9. An old, and previously noted, septal perforation was present, thus precluding a "normal" classification. However, the nasal mucosa was otherwise healthy and there was no bacteriological or histological evidence of active infection.

Case B21. A moderate degree of atrophic rhinitis, not otherwise encountered in this follow-up series, was present. Biopsies showed no solid-staining organisms.

BACTERIOLOGY

(a) *The nasal discharge.* The mucus and secretions of the nose were collected as an early morning "nose-blow" specimen and examined for acid-fast bacilli in the routine manner. One patient, who could spend only 8 h at the hospital, produced no specimen. Of the remaining 15, 10 had negative specimens. Three patients each had a BI of 2 and an MI of zero, while in 2 (patients B13 and B22) the MI was positive and the bacteriology of these patients is shown in detail in Table 2. Nasal biopsies confirmed the presence of solid-staining organisms in patient B13 but not in patient B22.

(b) *Multiple nasal smears.* The technique for this investigation is described in detail elsewhere (Davey and Barton, 1973). Briefly, the nasal mucosa is gently

TABLE 2

The bacteriology of patients B13 and B22

Patient	Specimen	Before treatment		After one year of dapsone	
		BI	MI	BI	MI
B13	Skin	4.8	2.8	4.5	0.6
	Nasal discharge	5	4	2	0-2
	Nasal smear	4.1	6.5	2	0.3
B22	Skin	5.3	2.8	4.1	0.8
	Nasal discharge	*		3.3	0.2
	Nasal smear	4.8	8	1.2	0.1

* No proper specimen obtained.

scraped with the sharpened, flattened end of a bicycle wheel spoke (Browne, 1965) and thus the specimen produced includes the superficial cells of the surface epithelium. Several sites in the nose should be examined and it is the anterior end of the inferior turbinate, and not the septum, which is most likely to yield a positive result. In addition to the 2 patients with potentially viable bacilli in the nasal discharge a further 2 (B.1 and B34), showed a positive MI on nasal smears. Their figures were BI 0.8/MI 0.1 and 1.5/0.1 respectively. Unfortunately these were the only patients in the follow-up series who did not have nasal biopsies taken, and thus their histological status is unknown. However, it is interesting to note that the nasal discharge in both these patients had a zero MI. The significance of this observation will be discussed below. Prior to treatment the nasal smears for the group had an average BI of 4.5 and MI of 5.9.

(c) *Skin smears.* The average figure for the group prior to treatment was BI 4.4, MI 2.4 and on follow-up these figures had fallen to 3.8 and 0.4. In 5 patients, after treatment, the MI was zero and in the remaining 11 it was 1 or less.

Discussion

Only 19 (56%) of the original 34 patients were known to be in contact with the hospital and receiving dapsone regularly between 12 and 15 months after their initial attendance. One patient had died, thus giving a total default rate of 14 out of 34 (41%). This is in line with those from several series reviewed recently by Davey (1974). A high default rate is not, incidentally, a problem peculiar to leprosy, for Booth (1972) noted default rates of 50% and 55% in 2 follow-up surveys of patients in London who had undergone elective ear surgery. However, it is a matter of great concern that so many patients with highly infectious lepromatous leprosy who have attended a hospital, well known for its sympathetic care and consideration, should fail to return for continuing treatment. Although the problem of travelling considerable distances in rural India is involved, the implication is that these patients, after initial improvement as evidenced by those who did return for follow-up, lack the understanding and motivation to return for further supplies of dapsone. Inevitably after cessation of a 3-6 month course of treatment, relapse and the capacity to infect others will recur. Further efforts to ensure the highest possible follow-up rate are thus obviously called for.*

We have reported elsewhere (Barton *et al.*, 1973) that intranasal pathology early in the disease is frequently quite out of proportion to what might be expected from a general examination of the patient. It is gratifying now to be able to report the considerable clinical regression of the nasal component of the infection after this period of treatment and also improvement in the general state of the patients. Although the nose appeared normal in 12 out of 16 patients (75%), 11 (69%) still had some symptoms, albeit mild, from the nose and the fact that 5 (31%) still had occasional bleeding suggests a potential, though possibly transient, breach in the integrity of the nasal mucosa. Under these circumstances and in the presence of persistent viable bacilli in the submucosa it is possible that a patient may retain the ability to infect others despite an apparently normal nose with satisfactory skin smears and even an MI of zero for the "nose blow", as in patients B1 and B34. Davey and Rees (1974) have demonstrated "the extraordinary sensitivity of *Myco. leprae* in the nose to even small doses of dapsone .... within a few weeks". However, 4 patients (25%) in this series still

had solid staining bacilli in either the nasal mucus or in the superficial layers of the nasal mucosa after a minimum of 12 months regular dapsone. It would clearly be wrong to label these patients "non-infectious" despite the enormous clinical improvement. It is important to note that in none of these 4 patients was the clinical appearance of the nose pathognomonic of leprosy, nor even suggestive of persistent activity following earlier lepromatous infection. It is of interest that the one patient (B7) in this series in whom the nasal mucosa did show definite remaining infiltration clinically and who had solid-staining organisms in the nasal biopsies, had no solid-staining bacilli in the nasal mucus at the time of examination.

Three patients who had received regular dapsone for periods of 2, 5 and 8 years were investigated similarly to the main follow-up group and, though the numbers are too small to be statistically significant, it is interesting to note that no clinical, bacteriological or histological activity was detected in the noses of these patients.

From these observations it would appear that the majority of patients with early lepromatous leprosy will be clinically well, with marked regression of nasal and skin infiltration, when treated with adequate amounts of dapsone for 12-15 months. Although it has generally been thought that the majority of such cases will be no longer capable of transmitting the disease provided that treatment is continued, the present study indicates that a percentage of such patients may remain potentially infectious; this amounted to 25% in those patients who actually attended for follow-up and would surely have been greater amongst the non-attenders. Therefore, the bacteriology of the nasal smears and discharge is clearly as important as the routine skin bacteriology and should become a standard diagnostic and follow-up investigation in lepromatous leprosy. Furthermore, these tests should ideally be repeated each time that the patient is seen, as it is quite probable that the nasal discharge of a patient such as B7, mentioned above, may have been positive on another occasion. The case for routine nasal examination as a diagnostic procedure has been argued elsewhere (Barton, 1974), but as a follow-up investigation it assumes less importance, for it may well be uninformative and even be misleading; the 4 patients in this series who had bacteriological evidence of persisting infectivity showed no definite intranasal abnormality on simple clinical examination.

* The one year follow-up rate for patients outside this special research group at Victoria Hospital has been around 70%. One of us (LMH) feels that a possible reason for the low follow-up rate in the group reported here is fear of undergoing again the original extensive investigations.

Acknowledgement

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Lepromatous Leprosy in the Nose After One Year of Dapsone Treatment: Histopathological Findings

A. C. McDOUGALL

and

A. G. M. WEDDELL

Department of Human Anatomy, Oxford University, Oxford OX1 3QX

and

R. J. W. REES*

National Institute for Medical Research, Mill Hill, London NW7 1AA

Following one year of supervised dapsone treatment, the histopathology of 32 biopsies of nasal septum and turbinate from 14 lepromatous patients is described. In one patient who may originally have had a borderline element, bacilli had completely disappeared, but they were present in all others, together with cellular changes typical of resolving lepromatous leprosy. In virtually every case, the integrity of overlying respiratory and vestibular epithelium had been restored, thus containing the infiltrate in the submucosa. Although 99% of all bacilli were fragmented or granular, occasional solid-staining forms were found in macrophages in 4 patients and in 2 of these, solid-staining bacilli were also seen in the cytoplasm of endothelial lining cells of capillaries. A year of dapsone produced marked general improvement in the histopathological appearances, but the finding of solid-staining (and therefore presumably viable) bacilli in several biopsies is clearly disconcerting. Furthermore, some patients were found by a completely independent assessor to have positive noseblows or smears, sometimes associated with epistaxis.

These nasal tissues were compared with skin in each patient, and the study serves as a reminder that the nasal mucous membrane is vascular, delicate, of considerable surface area and "open" to the exterior. Further studies are indicated on the nose in lepromatous leprosy as an important area for bacillary lodgement, multiplication and dissemination and as one in which *Myco. leprae* might persist despite conventional drug treatment.

Introduction

Following detailed, almost prophetic observations over 75 years ago (Sticker, 1897; Schäffer, 1898), on the importance of the nose in leprosy, this organ did not attract systematic investigation until the publication of Shepard (1960, 1962,

*Requests for reprints should be addressed to R. J. W. Rees.

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1965) and more recently those of Job *et al.* (1968) and Pedley (1970, 1973) who re-emphasized the highly bacilliferous nature of the nasal mucosa and nasal mucus. These led to the first comprehensive clinico-histopathological study of the nose, involving 34 patients in India with early untreated lepromatous leprosy, from whom an average of 4 biopsies were taken from particular sites of the septum and turbinates under local anaesthesia; the clinical, bacteriological and histopathological findings in these patients have already been published (Barton, 1974; Davey and Rees, 1974; Davey and Barton, 1973; McDougall *et al.*, 1975). On examination of over 120 biopsies by light microscopy, the histopathology was highly revealing for the tissue localization of *Mycobacterium leprae*, the high density and morphological index (MI) of bacilli in many situations, and the varied methods of "escape" of bacilli from the tissues into the nasal discharges, and thus to the exterior.

Immediately after examination, these patients were started on dapsone treatment. In view of the extremely high density and MI of bacilli in most of the pre-treatment tissues, and because of the obvious public health importance of the nasal mucus in lepromatous leprosy, it was considered essential to repeat the nasal biopsies after a period of supervised drug therapy. From the original series, 14 patients satisfying the necessary criteria were available for biopsy on the ENT surgeon's second visit to India, and the present paper describes the histopathology in 32 biopsies taken from their septum and turbinates after one year of dapsone treatment.

Patients and Methods

From an original group of 34 patients with early, untreated active lepromatous leprosy, 14 who had attended regularly for one year were available for nasal biopsies. These were taken under direct vision, using local anaesthesia, and either dissected out with a small-bladed scalpel or punched. Fixation was in buffered formaldehyde; tissues were embedded in paraffin wax, cut at 4-5 μm, and stained with "TRIFF" (Wheeler *et al.*, 1965) and the Fite Faraco modification of Ziehl-Neelsen. From each patient, an average of 2.3 biopsies were taken, 32 in all, made up of 20 turbinate and 12 septal biopsies. The procedure was entirely painless throughout, and there were no complications. From an area of likely activity, a skin biopsy was taken at the same time and processed in exactly the same way.

Dapsone (DDS) was given by mouth, beginning in most cases with small doses, but rising to a standard 300 mg weekly. There was no evidence of toxicity or intolerance and ENL occurred transiently in only one patient during the period of study.

Results

INTEGRITY OF THE EPITHELIUM

In every instance, whether from vestibular (squamous epithelium) or respiratory area (pseudo-stratified epithelium) the covering was intact and apparently healthy. All biopsies thus looked "cleaner", more compact and orderly, in marked contrast to many in the original untreated series, where both types of epithelium were often secondarily infected, necrotic, denuded or weakened by direct invasion of inflammatory capillaries accompanied by a highly

bacillated infiltrate (Fig. 1). In this follow-up series bacilli were not found in prickle cells of squamous epithelium or in the substance of columnar epithelium, nor were they seen escaping in polymorphs or macrophages across the epithelial layer. Even when bacillated and infiltrated—as was frequently the case—the infected submucosa was thus never in open contact with the interior of the nose. The mucous blanket was clean and did not contain bacilli.

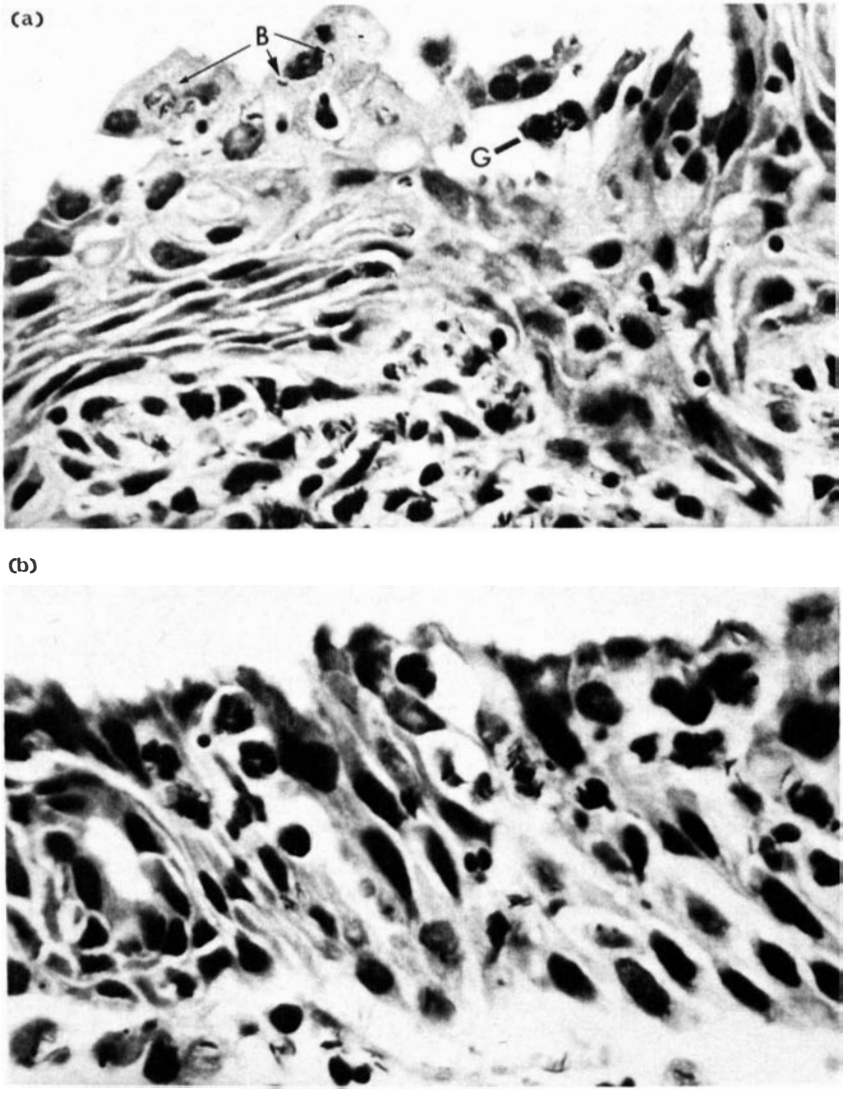

Fig. 1. Pre-treatment biopsies. (a) Squamous epithelium from the vestibular area; a densely bacillated infiltrate rises into the basal layers and solid-staining bacilli (B) and globi (G) are seen escaping from the necrotic epithelial surface into the nasal cavity. (b) Pseudo-stratified columnar epithelium from the respiratory area; solid staining bacilli and globi are seen in macrophages and polymorphs in the substance of the mucous membrane (TRIFF x 1250).

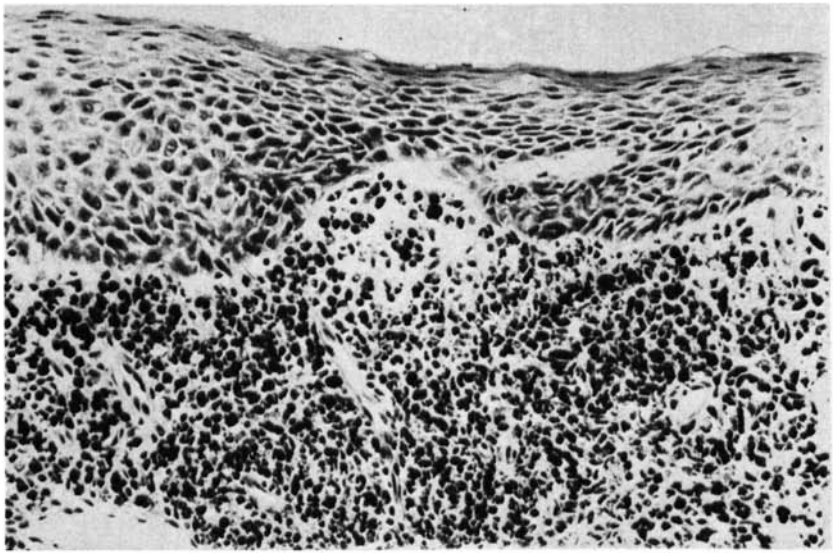

Fig. 2. Pre-treatment biopsy; anterior part of nasal septum. The main constituent of the infiltrate is globi, which rise into direct contact with basal layers of squamous epithelium (Fite-Faraco x 312).

SUBEPITHELIAL CLEAR ZONE

Whereas the untreated tissues had frequently shown an intensely bacillated infiltrate rising into direct contact with the basal layers of epithelium (Fig. 2), this was not found after one year of dapsone, and in several patients a clear zone was clearly visible in vestibular and respiratory areas (Fig. 3).

NUMBERS AND MORPHOLOGY OF BACILLI

From the 14 patients biopsied, one had no bacilli in any tissue examined, and it is thought this may have been the result of effective therapy in a patient with an original borderline element. In all others, bacilli were present in at least one, and usually in two or three of the tissues submitted; from the total of 32, only 5 (16%) were negative on prolonged searching. Using the Bacteriological Index (BI) of Ridley (1964) for the assessment of bacillary density in sections, the BI was zero in 5 (16%), approximately 1-3 in 7 (21.5%) and 4-5 in the remaining 20 (62.5%). These figures were significantly reduced compared with the original untreated series, but a much greater contrast was obvious in the matter of morphology. Bacilli were universally either fragmented or granular in 26 biopsies (81%). However, solid-staining bacilli with both Triff and Fite-Faraco techniques, were present in 4 biopsies. These lay mainly in macrophages of the submucosa of septum, inferior or middle turbinates (Fig. 4) but were also seen in 2 cases in the cytoplasm of endothelial lining cells of capillaries (Fig. 6). No bacilli, either solid or non-solid staining were found free in blood of lymph.

HOST CELLS FOR BACILLI

In two cases, fragmented or granular bacilli were found in mucous secreting glands and in tiny filaments of the trigeminal nerves in the submucosa.

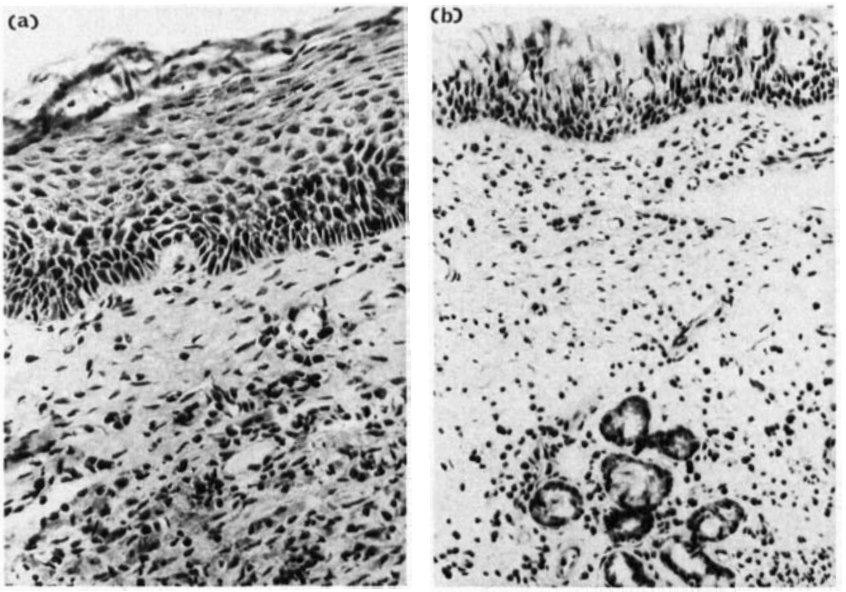

Fig. 3. After a year of dapsone; epithelial integrity has been markedly restored in squamous epithelium of vestibule at (a) and pseudo-stratified columnar at (b) and in both a clear zone is apparent between residual infiltrate and basal epithelium. (a) TRIFF x 500. (b) TRIFF x 312.

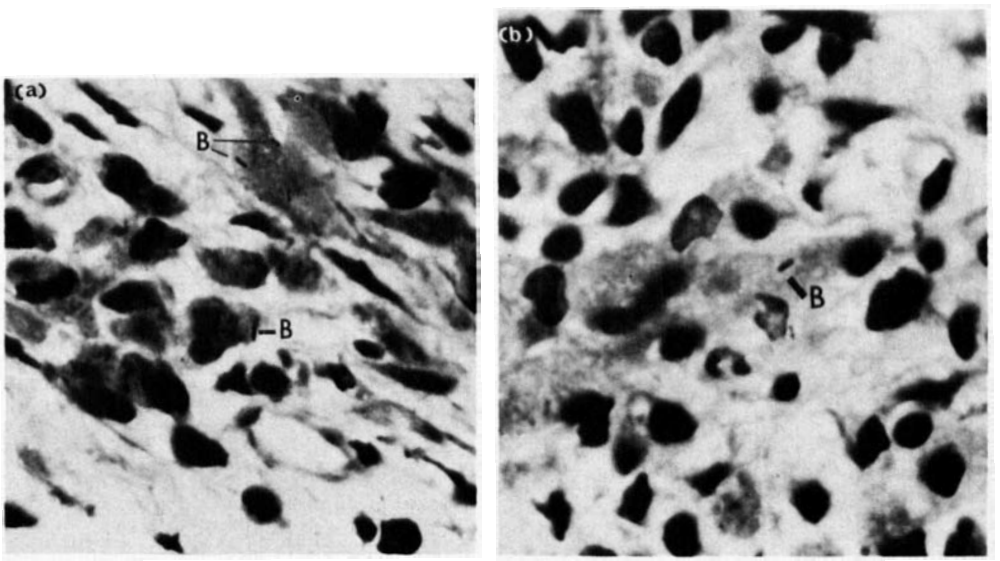

Fig. 4. After a year of dapsone; solid-staining bacilli (B) are seen in the cytoplasm of macrophages in the submucosa of anterior septum (a) and left middle turbinate (b) (TRIFF x 1250).

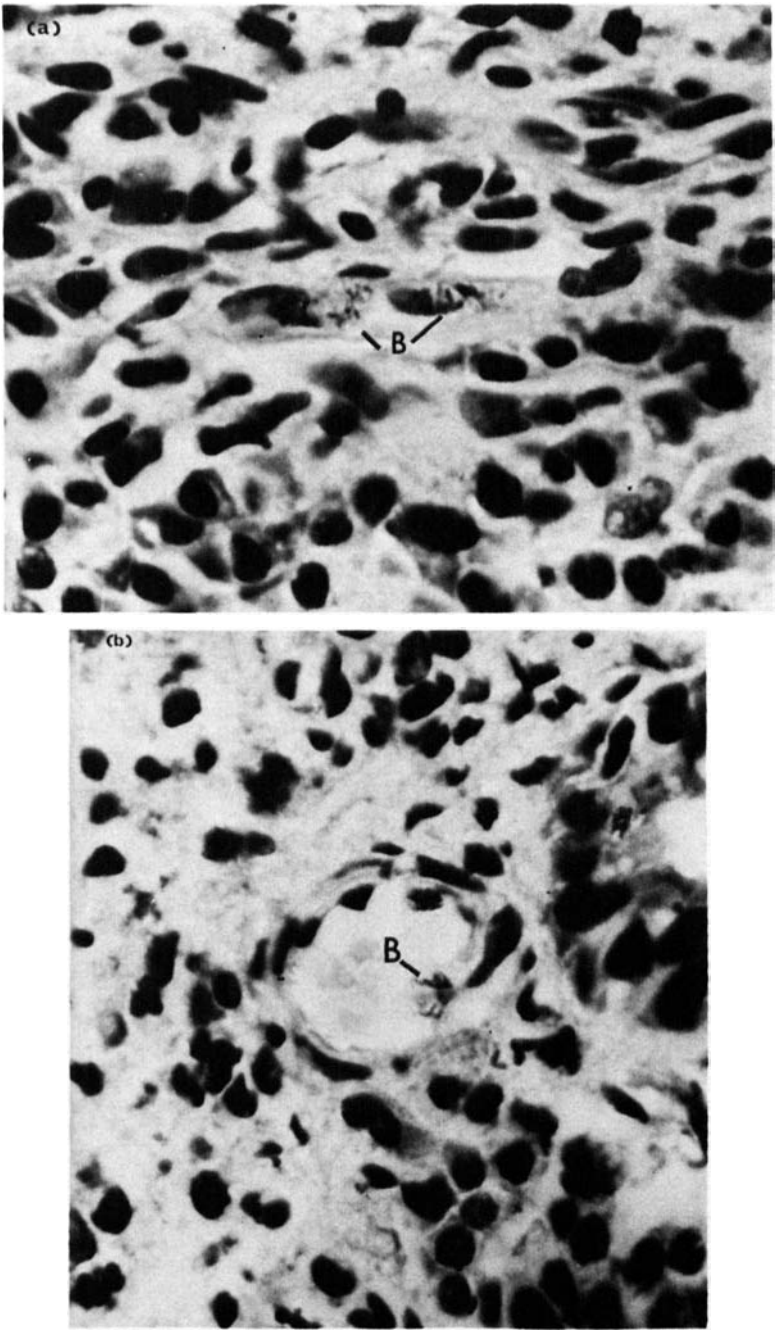

Fig. 5. After a year of dapsone. (a) Left septum, anterior; (b) left middle turbinate. Non-solid staining bacilli are present (B) in the cytoplasm of endothelial lining cells of capillaries in the submucosa (TRIFF x 1250).

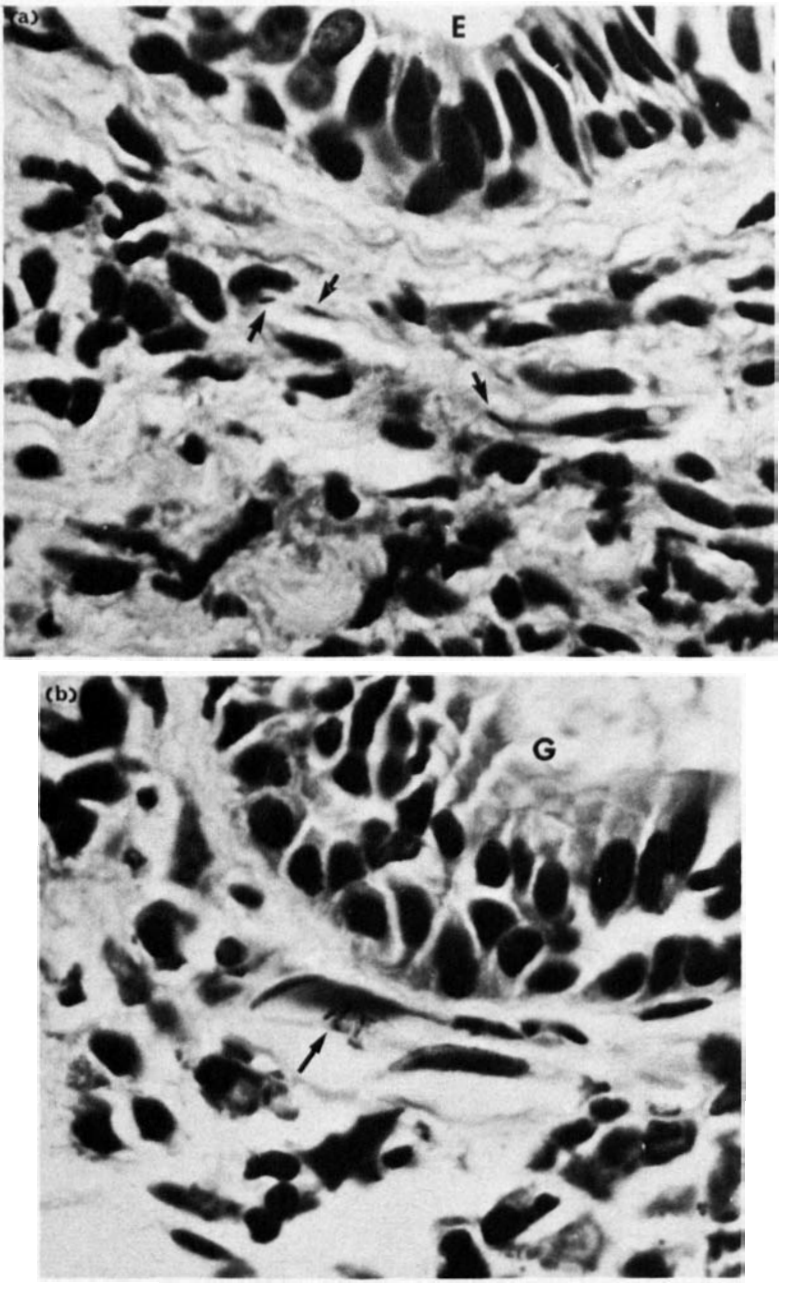

Fig. 6. After a year of dapsone. Solid-staining bacilli (arrowed) are seen in the cytoplasm of capillary endothelial cells; left septum anterior; (a)–above–subjacent to columnar epithelium (E) and (b)–below– adjacent to a mucous secreting gland (G) (Fite-Faraco x 1250).

Polymorphs were not frequent, and in contrast to those of the pre-treatment series, never contained bacilli. Whilst over 90% of all bacilli were located in the cytoplasm of macrophages, they were not infrequently seen, in fragmented or granular form, in some of the endothelial lining cells of capillaries (Fig. 5), arterioles, venules and venous sinuses.

THE CELLULAR INFILTRATE

Well over three-quarters of all tissues showed a definite, and often quite widespread infiltrate in the submucosa, immediately apparent at low magnifications. Although this was mainly histiocytic, many fields showed considerable numbers of plasma cells, mast cells, lymphocytes and eosinophils. As noted above, polymorphs were infrequent, compared to the original series, and areas of frank suppuration or necrosis were not found.

HISTOPATHOLOGY OF SKIN BIOPSIES TAKEN AT THE SAME TIME

All biopsies revealed an infiltrate and bacillary distribution typical of lepromatous leprosy after one year of effective treatment. Foamy vacuolation was frequent in macrophages; bacilli here and elsewhere were all fragmented or granular. Nerves, when shown, were bacillated and often collagenized in both endo- and perineurial areas, with thickening of the latter. Granular bacilli were found in arrector pili muscles in several instances. Particular attention was paid to blood vessel endothelium, and granular bacilli were indeed found in one patient, though in small numbers. The numerous factors which limit any sensible comparison between skin and nasal tissues in lepromatous leprosy will be discussed below, but at this point it may be relevant to add that after one year of treatment, there is a tendency for the nasal pathology, especially under squamous epithelium, to resemble that found in the dermis even to the establishment of a clear sub-epithelial zone.

Discussion

One year after supervised dapsone treatment, the main points of interest in the histopathology are as follows: (1) a marked reduction in the percentage of solid-staining organisms, (2) the presence of a fairly dense and extensive infiltrate in the submucosa, sometimes pleomorphic but mainly histiocytic and broadly resembling that seen in the dermis of skin, (3) the restoration of intact, "clean" epithelium in both vestibular and respiratory areas, (4) the presence of solid-staining bacilli in endothelial lining cells in 2 patients and of non-solid staining bacilli in the same situation in several others, and (5) of solid-staining bacilli in macrophages in 4 patients.

The importance of the nose in leprosy and of the nasal discharge as a source of infection was emphasized by a group of writers at the end of the last century (Jeanselme and Laurens, 1897; Sticker, 1897; Schäffer, 1898; Goldschmidt, 1891) and later by Muir (1921, 1929) and Rogers and Muir (1946). Important clinical observations were recorded on lesions of the upper respiratory tract and nasal perforation by Pinkerton (1938), Wise (1954) and later by Job, Karat and Karat (1966, 1968) and Goodwin (1967). Reports on the value of nasal smears have almost certainly been confused by the different techniques employed in obtaining them, but in an important series on nasal smears taken in Africa, Browne (1959, 1966*a*, *b*) has drawn attention to the fact that they tend to show a

high BI and MI, with many globi, and that they may persist in the nose after disappearance from other "routine" sites. In a detailed histopathological study of 120 biopsies from early untreated lepromatous patients (McDougall *et al.*, 1975), the tissue origin of the enormous numbers of bacilli excreted from the nose was clearly revealed. In the present biopsies after one year of treatment there is a very striking reduction in the numbers of bacilli and in their MI, together with a marked improvement in the overlying epithelium. In the pre-treatment tissues, vast accumulations of solid-staining bacilli lay loosely underneath an epithelial area which was necrotic, purulent, denuded (Fig. 1), and thus indeed to be described as an open ulcer (Shepard, 1962). Reports on the rapid disappearance of bacilli from the nose under chemotherapy have been summarized (Goodwin, 1967) and these are in accord with the clinical appearances and most of the nose-blows and smears in this series, and with the recorded reduction of counts of bacilli in nasal washings after treatment (Shepard, 1962). However, the fact that 4 patients still had some solid-staining and thus presumably viable bacilli in sections, and that several in the series had positive nose-blows or smears or epistaxis, underlines the potential hazard of a cavity which is at least 200 cm^2 in surface area (Davis and Hertzman, 1957), highly vascular, and probably of special importance for the lodgement and multiplication of *Myco. leprae*.

Following restoration of epithelial integrity, our material shows that the situation comes to resemble that seen in skin, where the epidermis—despite a relatively rapid turnover time (Cameron and Thrasher, 1971)—normally forms a barrier to the outward movement and escape of bacilli. Apart from radiation injury, the sloughing of a large number of cells from an epithelial surface will usually stimulate the renewal of those which lie below (Evans, 1973, personal communication), but in the case of untreated lepromatous leprosy such large amounts of nasal epithelium and basement membrane may be lost that this mechanism cannot operate. Indeed, the simple structural and histological differences between nasal epithelium and epidermis, particularly as regards strength and blood supply, limit the value of any comparison. It is for instance not generally appreciated that bacilli in skin are to a large extent physically confined, where their morphology represents a mixture of viable and non-viable forms, the latter analogous to the "acid-fast skeletons" which have been described for tuberculosis (Medlar, Bernstein and Steward, 1952). By contrast many tissues from the untreated lepromatous nose have revealed large areas where the MI was very high, but where the bacilli are not physically confined, giving the opportunity for organisms to be continuously discharged from the open, ulcerated mucous surface. This mechanical factor may be of prime importance in accounting for the higher MI found in the nose as compared to skin, although factors such as microvessel architecture (Kanan and Ryan, 1975), temperature, pH, oxygen and carbon dioxide tension may all contribute to the nasal tissues as being particularly favourable for the multiplication of *Myco. leprae*. Following treatment, the mucosa improves very considerably, but as the histopathology and the accompanying clinical results show, bacilli may still emerge after a year of treatment, no doubt from mucous glands or capillaries and as a result of incidental surface damage from nose-picking or intercurrent infection.

Although the present nasal tissues were much less vascular than those taken before treatment, the comparatively small numbers of bacilli in endothelial lining cells contrast with their profusion, often in solid-staining form, in pre-treatment biopsies. Furthermore, in the latter we have observed bacilli in both solid- and

non-solid-staining form free in the blood of small nasal vessels, whereas they were not found in this tissue in the present study. Our data do not shed light on the possible method of disposal of such bacilli from nasal vessels, but it would appear likely that a year of dapsone has greatly reduced any contribution which the nose might make to the continuous bacteraemia of lepromatous leprosy (Drutz, Chen and Lu, 1972; Shankara Manja *et al.*, 1972).

It is important to note that our observations are based on biopsies from only 14 patients (41%) out of an original series of 34. Whilst 5 more were known to be taking treatment regularly, this still leaves 15 (44%) lepromatous patients, who despite all efforts by the hospital staff, were out of control at the time of this follow-up study and not biopsied. It is of course likely that their disease is once again progressive. The fact that bacteriological findings in the present group who did in fact attend for a year of dapsone were not entirely reassuring, emphasizes the need for further study of the nose in lepromatous leprosy as a site of lodgement, multiplication and dissemination of *Myco. leprae.*

Acknowledgements

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The Fly as Potential Vector in the Transmission of Leprosy

JOHN G. GEATER*

In controlled experiments specimens of 3 genera of flies *Musca, Calliphora* and *Stomoxys* proved capable of taking up relatively large numbers of solid staining AFB and globi from infected nasal mucus from untreated lepromatous patients and also from the surface of ulcerating highly infiltrated skin and nodules. Both *Musca* and *Calliphora* are capable of depositing these on distant surfaces. Bacilli can contaminate the mouthparts of *Stomoxys* after feeding on nasal mucus and ulcers, and this provides a possible means for direct inoculation of *Myco. leprae.* Flies may thus have an important role in the transmission of leprosy, the "infectious" patients from this angle being the one with positive nasal mucus or highly infiltrated and ulcerating skin. Every effort should be made to control flies in and around leprosy units.

Introduction

In considering the possible role of arthropods in the transmission of leprosy, attention has focussed on various types of biting arthropods (Dungal, 1960; Narayana *et al.*, 1972). Before *Mycobacterium leprae* can be injected into a recipient they must first be ingested by the insect, and the source of such bacilli has been assumed to be either the few organisms which might adhere to the mouthparts during puncturing of the skin, or else bacilli circulating in the peripheral blood of lepromatous patients as demonstrated by Shankara Manja *et al.* (1972) and taken up with the blood feed. It would seem probable, however, that large numbers of bacilli would need to be taken up by any potential vector in order that it might by natural means transmit an infecting dose.

There is one group of insects, well known as potent spreaders of many infections, which is invariably and all too abundantly present wherever there is leprosy. This group comprises the several genera of flies, amongst which are the housefly (*Musca*), the bluebottle (*Calliphora*) and the biting stable fly (*Stomoxys*), all of which are known to spread a variety of infections owing to their habit of feeding on human and other excretia and mechanically transporting bacteria to food or onto wounds (even very minor breaks in the skin surface). Infection is transmitted mechanically by flies on their feet or by the excretion of ingested organisms in their faeces or in the drop of liquid that is regurgitated to moisten their food while feeding (Davey and Wilson, 1965). In addition, the biting *Stomoxys* could introduce bacteria into the skin whilst

* Previously: The Leprosy Mission, Mongar Hospital, East Bhutan.
Address for reprints: 78, Westwood Rd, B73 6UN, England.
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feeding, and it has been observed at this centre that infected fly-bites form a major cause of skin sepsis presenting in out-patients during that time of year when these flies abound.

Pedley (1970, 1973, *a, b*) showed that very considerable numbers of *Myco. leprae* were discharged from the noses of untreated lepromatous patients, and Davey and Rees (1974) showed these to be viable outside the body for 1.75 days, and even in one case for 7 days. Such nasal secretions could readily be ingested by flies or adhere to their feet, and would form a far more concentrated reservoir than bacteraemic blood or the relatively few bacilli which would be encountered during skin puncture. If it could be shown that *Myco. leprae* in sufficient numbers are carried by flies having fed on such sources, then the possibility must exist of organisms being implanted into broken skin surface by the insects or even injected into the skin if they are present on the mouthparts of *Stomoxys*.

Another major source of bacilli is the exudate from ulcerating nodules of some highly positive lepromatous patients.

The purpose of this work was to examine the possible uptake of *Myco. leprae* from these two sources by 3 representative flies–*Musca, Stomoxys*, and *Calliphora*–and also to examine whether *Stomoxys* can pick up bacilli when biting on intact skin. The question of whether flies actually transport bacilli from these sites to others was also examined.

Method

Flies of genera *Musca, Stomoxys* and *Calliphora* were captured away from the hospital. Numbers of each species were killed and the whole insect crushed and homogenized. The homogenate was stained for mycobacteria (decolourizing with acid alone) and a careful microscopical search carried out. This involved in each case a search of approximately 2000 fields before assessing them as negative.

Nasal mucus was obtained from untreated lepromatous patients. Specimens were copious, purulent and fluid, and exhibited large numbers of *Myco. leprae*. Within a month of starting treatment the nasal discharge became significantly less copious, had a lower bacterial load and was less readily ingested by flies.

Living flies were placed in separate large containers together with heavily bacillated nasal mucus smeared on a square of gauze. Granules of sugar were also placed in the container as an alternative food source for the flies. The flies were observed to feed voraciously upon the nasal mucus, generally prefering it to the cleaner food source. Often they placed their feet on the mucus whilst feeding.

After feeding on the mucus the flies were transferred to other containers. Some were killed and examined immediately. Others were kept alive for variable periods, being fed on syrup, before being killed and examined. The mouthparts and the legs were dissected from the flies and crushed into a small drop of saline to free any adherent bacilli. The gut contents were extruded and a smear made. Mouthparts, legs and gut contents were examined separately after staining for acid-fast bacilli (AFB). Although no AFB were found in any of the control flies, it is possible that a few saprophytic mycobacteria could be present on occasional flies, and to exclude this possibility decolourization was carried out subsequently with acid-alcohol.

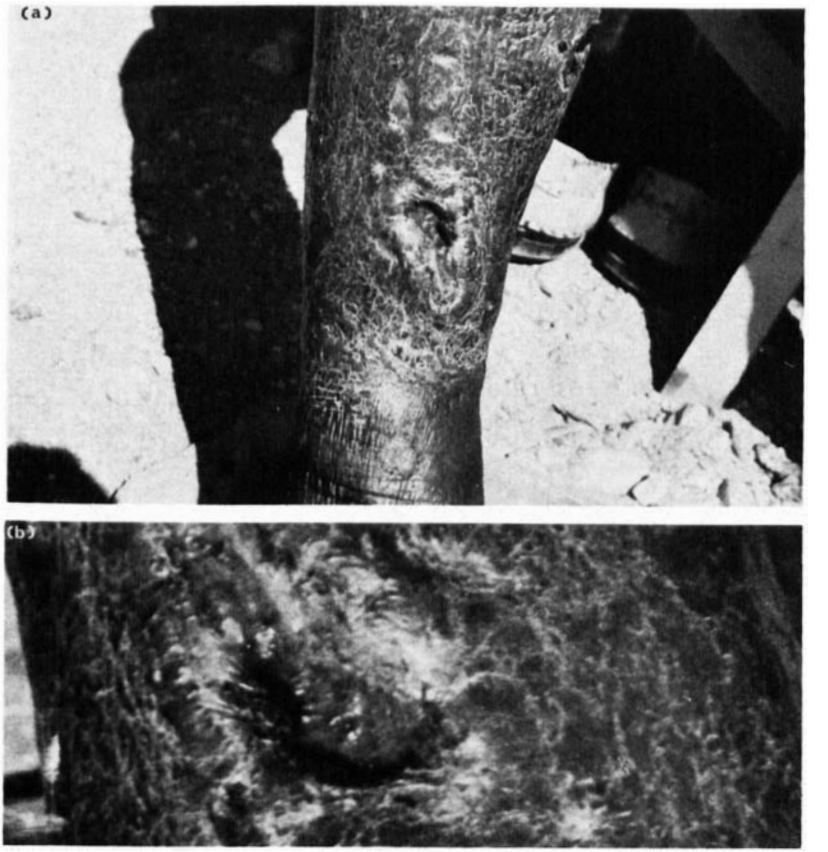

Fig. 1. Fly feeding on lepromatous ulcer. This fly was killed after 10 min and found to have solid staining AFB on legs, in the mouthparts and in the gut, 15 globi being counted in the gut and 3 in the mouthparts along with 20 individual bacilli.

In order to investigate the uptake of bacilli from ulcers and skin, flies were placed in containers over areas of skin of lepromatous patients including ulcerating nodules, and in the case of *Stomoxys* over areas of intact skin. They were left in position until the flies had been observed feeding, which in general they did readily. To reduce the artificial element in this work, certain of the flies which spontaneously alighted and fed on the ulcers were killed and examined.

Results

No AFB were found in any of the control flies of the three representative genera chosen—*Musca, Stomoxys* or *Calliphora.*

(a) UPTAKE FROM MUCUS FEEDING

Results are tabulated in Table 1. All flies of the three genera examined within one hour of feeding were found to have taken up bacilli from the nasal

TABLE 1

Uptake of acid/alcohol-fast mycobacteria by flies fed on nasal mucus from untreated lepromatous patients

Fly Genera	Time of examination after feed	Total number of flies	Number of positive flies: presence of globi											
			Legs				Mouth				Abdomen			
			+	S	S+	Globi	+	S	S+	Globi	+	S	S+	Globi
Musca	1 h	13	10	6	4	50	9	8	1	1	12	6	6	27
Musca	1 day	8	1	1	–		3	3	–		8	3	5	62
Musca	2 days	2	0	–	–		1	1	–		1	1	–	
Musca	3 days	3	0	–	–		0	–	–		3	2	1	8
Stomoxys	1 h	6	3	3	–		4	3	1	1	5	3	2	3
Stomoxys	1 day	2	0	–	–		0	–	–		2	1	1	2
Calliphora	1 h	3	2	–	2	7	2	1	1	7	2	–	2	25
Calliphora	1 day	2	0	–	–		0	–	–		1	1	–	
Calliphora	3 days	2	0	–	–		0	–	–		2	2	–	2 (small)
Total no. of flies examined		41												

\+ No. of flies with AFB present.
S No. of flies with less than 20 solid staining bacilli present.
S+ No. of flies with more than 20 solid staining bacilli present.
Globi Total no. of globi counted in positive groups.

mucus onto some part of their bodies. The majority of bacilli were to be found in the contents of the gut, sometimes in very large numbers. For instance, in one *Musca* acid/alcohol-fast bacilli were present in almost all the 500 microscope fields examined of smears of intestinal contents, as well as 31 globi.

Musca. Twelve of the 13 *Musca* killed within one hour of feeding had solid staining bacilli in their intestinal contents, 6 of them having globi present. Bacilli were also found on the mouthparts of 9 of the 13 flies and on the legs of 10, 4 of these having globi present and one having as many as 41 globi, although some of these were small.

Solid staining bacilli persisted in the gut for 3 days. At 24 hours they were present, often in large numbers, in the guts of all 8 flies examined. Thereafter there was a rapid diminution in. numbers, one of the two flies killed after 48 h had a few bacilli, and whilst all 3 flies examined at 72 h did have bacilli in the gut, only 3 solid staining bacilli were detected in one and 6 in the other. The third, however, still contained 8 globi.

Bacilli were still present on the legs of one and in the mouthparts of 3 of the 8 flies examined at 24 h. They were also present in the mouthparts of one fly after 48 h.

Stomoxys. Being a small fly, as might be expected, fewer bacilli were to be found after feeding. Even so, all but one of the 8 examined were found to have taken up bacilli from the nasal mucus, and 3 of them contained globi, one after 24 h. If these flies are potentially to inject *Myco. leprae* into the skin, the numbers of bacilli found in mouthparts are important. Solid staining bacilli were found in the mouthparts of 4 out of 6 *Stomoxys* flies tested immediately after a meal of nasal mucus. One globus was found.

Calliphora. Results obtained with bluebottles closely approximated to those obtained with the large numbers of *Musca.* Two of the 3 examined shortly after feeding contained very large numbers of bacilli, especially in their intestinal contents. Two had bacilli on their mouthparts one with 7 globi. Globi were also found on the feet of 2 of the 3. More globi and solid bacilli were found on the feet and mouthparts of these 2 bluebottles than on the equivalent sites of *Musca* or *Stomoxys.* Excretion of bacilli from the gut appeared to proceed rapidly, although small globi were still detectable in the guts of the two *Calliphora* examined after 3 days.

(b) UPTAKE FROM THE SKIN

No bacilli were found in any *Stomoxys* feeding on the intact skin of mild lepromatous volunteers (BI between 2 and 3). In the case of 6 *Stomoxys* placed on the earlobes of a highly lepromatous patient (BI 5.2), solid staining bacilli were found in the intestines of 4 flies after biting. Numbers of solid staining bacilli were low, a total of 20 in the 4 positive flies, with a maximum of 9 in any one fly. No bacilli were found in the mouthparts of the flies tested.

The uptake of bacilli by *Stomoxys* feeding on ulcerated skin was greater, and solid staining bacilli were detected on the mouthparts of 3 out of 5 flies. There were 25 solid staining mycobacteria present on the mouthparts of one fly. All 5 of each *Musca* and *Calliphora* which were killed after feeding on ulcerating skin of the highly bacilliferous volunteer were found to contain bacilli, and these were present on all 3 parts examined—legs, mouthparts and abdomen. The

number of globi taken up seemed to be lower than the uptake from nasal mucus, but one *Musca* was found with 15 globi in its intestinal contents and 3 on its mouthparts. This fly happened to be one which had spontaneously alighted to feed on the ulcer and was killed at the conclusion of the meal.

Do Flies Transmit *Mycobacterium leprae*?

Having shown that flies of the 3 genera studied can take up AFB, the question remains that if they are to be considered vectors they must be shown to carry the bacteria from the source to a receptor surface, and there deposit the bacteria. In order to demonstrate this, microscope slides coated with albumin were placed at one end of a large container and a small quantity of nasal mucus was placed at the other end. A few grains of sugar were placed on the slides, and pairs of *Musca* or *Calliphora* admitted to the containers.

The flies fed readily on the nasal mucus and frequently also on the sugar grains, alternating between the two over the course of 1 or 2 h, at the end of which time the slides were removed and stained. Of the 10 slides examined, only one showed no AFB. Globi were present on 6 of the 10 slides, and 2 of the slides, both of which were in a container with 2 *Calliphora* for a 2 h period had solid staining bacilli, and often globi, in virtually every part of the slide examined.

Discussion

The acid-fast bacilli in the nasal mucus of untreated lepromatous patients have been shown to be *Myco. leprae* capable of inducing infection in the mouse foot-pad (Davey and Rees, 1974). Whilst some 300 different species of acid-fast bacilli are known, the number which retain stain when decolourized with acid-alcohol is small and includes *Myco. leprae*. This fact, the absence of any mycobacteria in the controls, and the demonstration of globi in a large number of the flies examined after feeding on nasal mucus or ulcer exudate from lepromatous patients suggests that at least a large number of the bacilli demonstrated were *Myco. leprae*. Of these a great number were solid staining, suggesting viability, but confirmation of this must await the results of mouse foot-pad inoculations.

In view of the known methods of spread of bacteria by flies—on the feet, in the faeces and in the "vomit drop" secreted during the course of feeding, and in the case of *Stomoxys* by contamination of the mouthparts—the finding of solid staining *Myco. leprae* in relatively large numbers on feet, mouthparts and in the gut of flies would suggest that all these mechanisms could play a role in the transfer of viable organisms from nasal mucus or the ulcerating skin of a highly lepromatous patient to a suitable receptor area.

It is one thing to demonstrate that an arthropod fortuitously takes bacilli into its body when feeding and something quite different to claim it as a vector. A vector must also be shown to release the bacilli in a viable form to receptor tissues. Whilst we have not thus far fully complied with this condition, the finding of solid staining bacilli and globi on the albumin coated slides on which *Musca* and *Calliphora* had been feeding, shows that mechanical transfer can occur, and it is possible that the same mechanism could operate when a fly feeds on a defect in the skin of a hitherto uninfected person. Nor would this

be a purely passive or random chance as the flies are positively attracted to any defect of skin surface, which would thus seem to provide a potential site for the lodgement and possible later multiplication of bacilli.

Whilst the intact epidermis would not seem to provide a portal of entry for *Myco. leprae,* implantation of bacilli into breaks of the skin surface could allow entry of them into the dermis or even provide for haematogenous spread. The demonstration of contamination of the mouthparts of *Stomoxys* after feeding on nasal mucus or ulcer suggests that direct inoculation by this means is possible.

Uptake of bacilli was undoubtedly greatest in all 3 species tested after feeding on infected nasal mucus. Feeding on ulcerating highly infiltrated or nodular skin also provided many bacilli. It is likely that other genera of flies of similar feeding habits (e.g. *Fannia, Lucilia*) would give similar results. Of less importance would seem to be the uptake of bacilli in the blood meal of *Stomoxys* biting intact skin of even highly positive patients with bacteraemia, because of the small number of *Myco. leprae* in blood compared with the large number on nasal mucus or ulcer exudate. The patient with a lower BI would not seem to pose a significant risk.

Conclusions

Whilst full confirmation of these findings awaits the results of mouse foot-pad experiments, it has been shown that:

1. Flies of several genera are capable of taking up relatively large numbers of solid staining *Myco. leprae* from infected nasal mucus and the surface of ulcerating highly infiltrated skin or nodules.
2. Both *Musca* and to a greater extent *Calliphora* are capable of depositing these on distant surfaces.
3. Bacilli can contaminate the mouthparts of *Stomoxys* after feeding on mucus or ulcers, and this provides a possible means for direct inoculation of *Myco. leprae.*
4. In view of the above, the "infectious patient" with regard to possible transmission by flies, is the one with positive nasal mucus or highly infiltrated and ulcerating skin.
5. Every effort should be made to control flies in and around leprosy units in order to reduce the possible risk of transportation of leprosy bacilli to the surrounding population, as well as for obvious reasons of general hygiene.

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Multicentre Controlled Comparative Trial of Clofazimine and Dapsone in Low Dosages

T. F. AHRENS

Biological Research Dept., Pharmaceuticals Division, CIBA-GEIGY Ltd, Basle, Switzerland

and

J. H. S. PETTIT

174, Jalan Tuanku Abdul Rahman, Kuala Lumpur, Malaysia

and

D. S. RIDLEY

Path. Hospital for Tropical Diseases St. Pancras Way, London NW1 0PE, England

and

LUCIA GLAUS

Biometrical Unit, Pharmaceuticals Division, CIBA-GEIGY Ltd, Basle, Switzerland

A multicentric, controlled double-blind trial was undertaken to compare the efficacy of low-dosage therapy with dapsone and clofazimine in lepromatous leprosy. The results, which were evaluated centrally allow the conclusion that lowering of the dosage, either of dapsone or of clofazimine, is not followed by a proportional loss of antibacterial activity. The incidence of lepra reactions, especially ENL, in the clofazimine group was significantly lower and skin discolouration no worse than in the dapsone group. The use of clofazimine in low doses would appear to serve a useful purpose and is recommended for periods of up to one year.

Introduction

The therapeutic activity of clofazimine in human lepromatous leprosy, which was first reported by Browne and Hogerzeil in 1962, has been assessed in a large number of clinical investigations. By the end of 1974, about 250 papers on this subject, dealing altogether with more than 2000 patients, had been published.

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* Requests for reprints should be addressed to Dr Ahrens.
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The administration of clofazimine in daily doses of 300 mg was found to produce discolouration of the skin. This proved less troublesome at a dose of 100 mg twice weekly, and the results obtained in a few patients were otherwise comparable to those achieved in the initial phases of treatment with other anti-leprosy drugs (Waters, 1968). It remained to be seen whether this low dosage level would still exert an anti-inflammatory action, as had been suggested by Browne (1965) and others (Karat *et al.*, 1970; Tolentino *et al.*, 1971).

With dapsone, attempts to lower the dosage were originally prompted by the hope that this would decrease the incidence of erythema nodosum leprosum (E.N.L.). Short-term observations indicated that a dosage of 50 mg of dapsone twice weekly elicited a satisfactory antibacterial effect (Pettit and Rees, 1967; Pearson and Pettit, 1969).

Accordingly, it was decided to compare the results of low-dose therapy with dapsone and clofazimine as regards both antibacterial activity and the incidence of reactions. In view of the well-known difficulties in finding a sufficient number of suitable patients in any single institution, a double-blind trial was planned on a multicentric basis. The protocol for this trial was ready in 1968, but the drawbacks inherent in the multicentre method delayed its completion until the end of 1973.

Methods

The following investigators co-operated in the multicentric double-blind clinical trial:

Dr P. N. Behl and Dr Bhatia, Delhi (India)
Dr D. S. Chaudhury, Elmina (Ghana) and Liteta (Zambia)
Dr T. F. Davey and Dr A. Butt, Dichpalli (India)
Dr Z. A. Fazelbhoy and Dr Daud, Karachi (Pakistan)
Dr M. Flowers and Dr B. L. Whitty, Chandraghona (Bangladesh)
Dr M. Harahap, Medan (Indonesia)
Dr M. Hermes, Mwena-Ndanda (Tanzania)
Dr B. Pérez P., Trillo (Spain)
Dr Rajagopalan, Johore Bahru (Malaysia)
Dr C. Reyes C., Mérida (Mexico)
Dr K. Robson, Mount Hagen (Papua, New Guinea)
Dr E. Rodríguez C., Asunción (Paraguay)
Dr R. B. W. Smith, Honiara (Solomon Islands)
Dr T. Smith, Chiang Mai (Thailand)
Dr R. Thakolkaran, Kozhukully (India)
Dr. A. G. Warren, Hay Ling Chau (Hong Kong)

AIMS OF THE STUDY

The main purpose was to compare the antibacterial effects of low dosages of dapsone (50 mg) and clofazimine (100 mg), both administered twice weekly, for a period of 48 weeks. The incidence and severity of any lepra reactions or skin pigmentation occurring in either of the two treatment groups were to be noted.

CRITERIA FOR ADMISSION AND EVALUATION

The conditions of acceptance were that the patients should be male, over 12 years of age and suffering from active, purely lepromatous leprosy. They were also to be untreated cases or at least they were not supposed to have received any antileprosy treatment for the last 5 years. Patients suffering from other severe diseases in addition to leprosy in particular from tuberculosis or diseases requiring corticosteroid therapy, were excluded.

From the histological point of view, cases classified according to Ridley and Jopling (1962, 1970) as LL, including the sub-group LI (Ridley and Waters, 1969), were considered acceptable. All other classifications were excluded.

The histopathological index (HI), formerly known as LIB (Ridley, 1967; Ridley and Hilson, 1967) was determined, using the granuloma index (GI) as a factor, i.e. the fraction of the dermis accupied by granuloma in tenths. No cases showing a GI of less than 0.15 were considered for evaluation.

The bacteriological diagnosis was made on the basis of the bacterial index (BI) according to Ridley (1968), using a logarithmic scale from 0 to 6, and the morphological index (MI), indicating the percentage of evenly and deeply staining *Myco. leprae* in skin scrapings (Waters and Rees, 1962). No cases with a MI of less than 5, or a BI of less than 1.5 were included in the evaluation.

PROCEDURES

The 16 investigators listed above selected the patients according to a pre-established protocol. It was left to the investigator's discretion to withdraw a particular patient from the trial, if need be.

Clinical and bacteriological assessments were repeated at 6-weekly intervals throughout the period of treatment, and biopsies at 6-monthly intervals. The material for biopsies and smears was always taken from the same lesions. Biopsies were sent to London (D.S.R.), skin smears to Kuala Lumpur (J.H.S.P.) and clinical reports to Basle (T.F.A.). The appropriate examinations and evaluations of results were made at these centres.

The trial substances were supplied to the investigators in numbered bottles containing capsules of either 50 mg dapsone or 10 mg clofazimine, which were identical in appearance. Other material, including the fixative for the biopsies, was completely uniform. The allocation to treatment was randomized. Neither the investigators nor the present authors had knowledge about the key during the trial. There was, however, the possibility to have the code broken by an independent person in case of unwanted side effects. One of us (T.F.A.) took the responsibility for notifying the investigators of any unfavourable bacteriological or histological evolution that might make it necessary to stop the trial in the best interest of a particular patient.

Description of the Treatment Groups

Altogether 138 patients were selected in the 17 above-mentioned institutions for inclusion in the study. Of these, 44 proved primarily unsuitable, so that the population studied comprised in all 94 patients. Twenty-six of these were African, 51 Asian, 6 Melanesian, 4 Latin American and 7 European. The mean body weights ranged from 47.8 kg in Thai patients to 64.7 kg in Europeans. There was no significant difference between the two groups in respect of age.

Histologically, the dapsone-treated patients 48 in total, were classified as LL in 15, LI in 21 and BL in 12 cases. The distribution of the clofazimine-treated group ($n = 46$) was 12 for LL, 23 for LI and 11 for BL.

The nature and distribution of lesions were recorded in detail but, for lack of space, are not presented here. It may be stated that both groups were fully comparable as to their clinical features, with the one exception that diffuse infiltrations were more frequent among the LL/LI patients within the clofazimine group than among the corresponding cases of the dapsone-group. This difference was statistically significant.

As could be foreseen, the number of patients in both treatment groups diminished continuously during the trial. The rate of this process was about equal in both groups: the dapsone group initially comprised 48 patients; after 24 weeks there were 35, and by the end of the trial, after 48 weeks, 27. The respective numbers for the clofazimine-treated group were 46, 34 and 22 patients. Prolongation of the trial beyond the first year took place only in 4 patients, thus not allowing statistical analysis.

Results

In the following description of the bacterial and histopathological results, only the LL and LI patients are considered. BL patients, however, were taken into consideration together with the former for the evaluation of side effects and lepra reactions.

BACTERIAL FINDINGS

The bacteriological findings made at start of trial and during therapy with the two trial drugs are shown in Table 1. In both treatment groups, there was a significant decrease in the MI, which was practically zero by the 18th week. The decrease in the BI—as was to be expected—was slower.

TABLE 1

Bacteriological findings in evaluable LL/LI patients at the beginning of and during treatment

Week	Dapsone				Clofazimine			
	BI		MI		BI		MI	
	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$
0	25	3.648	16	15.225	22	3.714	14	17.493
6	21	3.300	14	6.450	18	3.567	11	3.609
12	22	3.427	14	1.720	22	3.450	12	3.800
18	19	3.447	12	0.208	19	3.363	8	0.338
24	21	3.257	13	0.085	20	3.140	11	0.136
30	5	2.900	4	0.100	8	3.775	6	0.200
36	17	3.412	10	0.015	16	3.244	8	0.063
42	3	4.167	2	0.100	3	3.733	4	0.075
48	14	3.079	8	0.025	16	2.725	8	0.038
Initial ranges	1.7–4.7		5.5–28.0		1.5–4.8		5.3–49.0	
Regression coefficient for week 0-18, b_c =			−0.063				−0.055	
Regression coefficient for week 0-48, b_c =	−0.010				−0.02			

The decrease in the bacteriological indices was expressed by means of the linear regression coefficients, which were compared statistically for the two treatment groups. A method proposed by Bliss (1967) was used to combine the individual regression lines. In the regression analysis of the MI, only the obviously linear part of the semilogarithmic curve was taken into consideration, i.e. the section relating to the first 18 weeks of treatment. The analysis of both the MI and the BI does not show a statistically significant difference between the two treatment groups.

HISTOPATHOLOGICAL FINDINGS

Only those patients were assessed histologically whose lesions showed sufficient activity at the beginning. Subsequently, the activity of the lesions was classified as "regressing", "quiescent" or "healed". No statistical analysis was made of the distribution of cases among these purely qualitative categories, but steadily progressive healing took place in response to both treatments. It is particularly noteworthy that none of the lesions revealed any signs of activity after 24 weeks' treatment, either with dapsone or with clofazimine.

TABLE 2

Histopathological findings in evaluable LL/LI patients at the beginning of and during treatment

Week	Dapsone				Clofazimine			
	GI		HI		GI		HI	
	n	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$
0	18	0.46	18	5.2	20	0.53	20	5.3
24	17	0.24	18	4.5	20	0.33	20	4.8
48	13	0.16	13	3.6	12	0.27	12	4.3
Initial ranges	0.2–0.7		4.6–5.9		0.15–0.9		4.75–5.9	
Regression coefficient, b_c =	−0.012		−0.034		−0.008		−0.020	

Quantitative data on the improvement observed are listed in Table 2, which shows the mean values of the Granuloma Index (GI) and the Histopathological Index (HI), the latter being the product of the individual GI and the BI at a given time. In both treatment groups, the extension of the granuloma within the dermis diminished linearly. Analysis of variance proved that the reduction was statistically significant. The same was the case with the HI.

As is evident from the gradient of the regression lines, determined by the three points of measurement, the thickness of granuloma diminished slightly more rapidly in response to dapsone than in response to clofazimine. This difference is statistically not significant.

CLINICAL FINDINGS

In 69 patients, the investigators came to a general conclusion about the clinical progress. On dapsone 27 patients improved, 6 were unchanged and 3 became worse (i.e. new lesions appeared during therapy). On clofazimine, 31 improved and 2 remained unchanged. As the improvement could not be quantified it was not amenable to statistical analysis.

A comparison of the body-weight before and during treatment of the LL/LI

patients in both treatment groups revealed an average increase of 2.0 kg (from 50.4 to 52.4 kg) after 24 weeks in the dapsone group and 4.0 kg (47.4 to 51.4 kg) after 18 weeks in the clofazimine group. Although the average body-weight of the patients in the clofazimine group was slightly lower than that of the dapsone-treated patients, it was never significantly different.

SIDE EFFECTS

Discolouration of the skin is regarded as a common side effect of clofazimine. Hypermelanosis of lesions has been observed as well as an extensive red discolouration. Although dapsone can also give rise to cutaneous pigmentation, especially of the lesions, there was nevertheless occasion to fear that it might occur more frequently in the patients treated with clofazimine and thus jeopardize the double-blind design of the trial.

TABLE 3

Frequency and localization of skin discolouration

Localization	Dapsone		Clofazimine		Total	
	abs.	%	abs.	%	abs.	%
Lesions only	2	(4.2)	3	(6.5)	5	(5.3)
Generalized	8	(16.6)	7	(15.2)	15	(16.0)
Lesions and generalized	3	(6.3)	2	(4.3)	5	(5.3)
Total	13	(27.1)	12	(26.0)	25	(26.6)
Size of treated group	48	(100.0)	46	(100.0)	94	(100.0)

In the total of 94 patients, altogether 25 instances of discolouration were recorded, the distribution of which is shown in Table 3. The times when this phenomenon was first reported were almost evenly spread over the entire duration of the trial in both treatment groups. Patients of darker hue (African) and of lighter hue (Chinese, European) were also equally represented in both treatment groups.

One may conclude that at least at this dose level, there is no significant difference between the two drugs in this respect. Since there was no clear-cut predominance of generalized discolouration in the group treated with clofazimine it may be inferred that this side effect was less pronounced than would have been expected at a daily dosage of, say, 100 mg.

Other side effects were observed in 10 of the 48 patients on dapsone and in 12 of the 46 on clofazimine. The nature and the distribution of these are shown in Table 4. The side effects were usually mild and not always clearly related to the drug administered. Treatment had to be withdrawn on account of side effects in two cases receiving dapsone (1 rash, 1 anaemia) and two receiving clofazimine (1 rash, 1 gastric complaints).

LEPRA REACTIONS

During the 48-week trial, 47 of the 94 patients, i.e. exactly half of the whole series, suffered at least one reactional episode. There were about twice as many dapsone- as clofazimine-treated patients amongst those showing reaction. Table 5 illustrates these relations. The difference between the two treatment groups is statistically significant at the 95% probability level (χ^2 6.252, d.f. 2). The

TABLE 4

Kind and frequency of other supposed side effects

Nature of side effect	Dapsone	Clofazimine	Total
General (weakness, dizziness, headache)	4	3	7
Gastro-intestinal (abdominal pain, nausea)	3	2	5
Anaemia	1	1	2
Vascular (Oedema, Epistaxis, Phlebitis)	4	3	7
Pain (bone and joint pain, myalgia)	2	2	4
Epidermal (rash, pruritus)	2	6	8
Total of occurrences	16	17	33
Patients concerned	10	12	22

TABLE 5

Patient population according to presence or absence of lepra reaction

Patients showing	Dapsone	Clofazimine	Total
ENL	14	7	21
Other reactions only	16	10	26
No reaction	18	29	47
Total	48	46	94

frequency and severity of ENL and all types of reaction including iritis, orchitis, stasis syndrome, panniculitis, acute exacerbation of lesions, or neural reactions, were higher in the dapsone-treated group than in the clofazimine-treated group. The frequency of reactive phases can vary enormously from one patient to the other and combinations are manifold. Because of this great complexity it is difficult to describe the events adequately. For example, the cumulative frequency of bouts of ENL while on dapsone (24 times in 14 patients) and on clofazimine (8 times in 7 patients) is depicted in Fig. 1.

The nature of the curve in this cumulative presentation, whether rising or horizontal, may be regarded as an indication of the stress imposed upon the treating and nursing personnel.

Seven patients (4 on dapsone, 3 on clofazimine) were withdrawn from the trial on account of lepra reactions. As a rule, however, the concomitant treatment given for the reactions (antimonials, chloroquine and in some cases steroids) sufficed to suppress the inflammatory processes.

Discussion and Conclusions

THE RESULTS OF THE TRIAL

The results presented here were obtained on a multicentric basis. Although the patients treated originated from very different countries and circumstances, they were nevertheless homogeneous in respect of their histological classification. Evaluation of the bacteriological and histopathological progress was strictly limited to LL/LI patients, whereas the assessment of side effects and lepra reactions included also the BL patients.

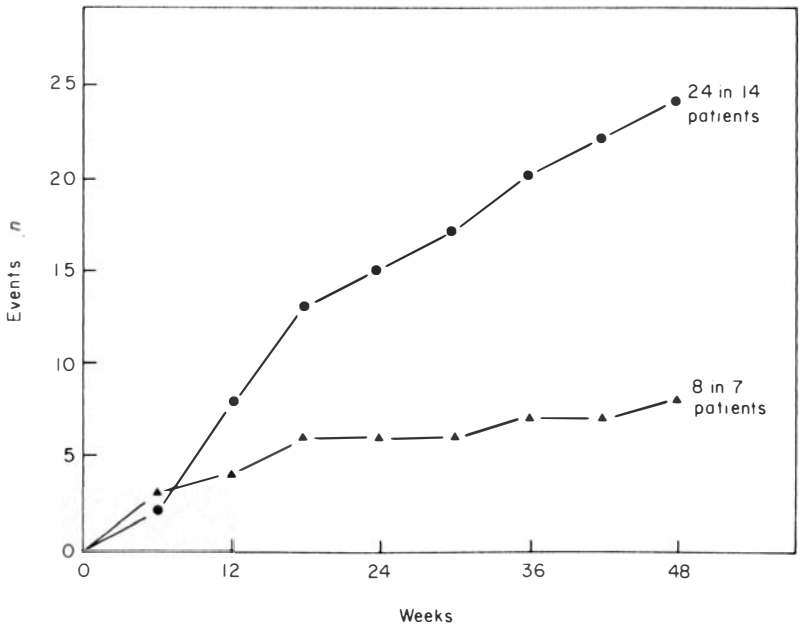

Fig. 1. Cumulative frequency of bouts of ENL while on dapsone (●–●) (14/48 patients) or clofazimine (▲–▲) (7/46 patients).

Comparison of the results obtained in this study demonstrates, in a statistically sound manner, that low-dose therapy with both these agents elicited an almost identical short-term antibacterial effect. This was manifested by a decline in the MI, which approached zero after about 18 weeks' treatment. As was to be expected, there was little change in the BI up to the first year of treatment, and during this time fluctuations were noted in both groups. This factor was less pronounced in the GI and the HI: here the decline was almost linear, and there was no significant difference between the two treatments. From the clinical point of view also, the two groups appear to have made equal progress.

It should be borne in mind that the reason for administering low doses of dapsone was the hope of diminishing the incidence of lepra reactions. However, though Karat *et al.* (1969), found that the severity of ENL was diminished when dapsone was given in doses of only 35 or 10 mg weekly, neither they, nor Leiker and Carling (1969) who administered 20 mg weekly noted any difference in the incidence of reactions. In the present trial the incidence, severity and duration of lepra reactions were not noticeably less than would have been expected on high doses of dapsone. This applies especially to ENL. Compared with the clofazimine-treated patients, about twice as many patients on dapsone displayed ENL and, individually, their bouts of reaction were about twice as frequent. Reversal reactions, resulting histologically in a shift towards the other pole were too rare (3 on dapsone, 2 on clofazimine) to allow statistical analysis.

In the case of clofazimine the object of trying low-dosage treatment was to

avoid, at least partly, the inconvenience of red discolouration. (The hypermelanosis which sometimes occurs with both sulphone and clofazimine therapy seems unavoidable.) The results clearly show that this complication, if present, was not a problem here, thus supporting the view that it is a dose-dependent phenomenon.

The conclusion therefore appears to be that clofazimine is superior to dapsone at the low dosage level, and for a limited period of time. It should be recommended in cases where treatment phases of this kind are indicated. No conclusions can be reached concerning the relative value of the two treatments over a period of more than one year. Although at the time when the present trial was initiated there were a number of reports favouring the use of low dose therapy the current view of many workers is that monotherapy at low dose over a long period invites the risk of bacterial resistance (Jacobson, 1973; Meade *et al.*, 1973; Gelber *et al.*, 1974). Although these reports all refer to sulphones, and although the minimal effective dose of clofazimine may be as low as about 7 mg a day (Shepard, 1969) this objection probably has general application.

The original intention was that any BL patients would be used only for a study of the effect of clofazimine on reversal reactions. In the event there were scarcely any BL patients except those of the "histiocytic" type (Ridley and Jopling, 1966) with few lymphocytes. It has since been observed that the response of these patients is similar to those of the LI group, and it has been proposed that with certain provisos they should now be graded as LI (Ridley, 1975). Consequently, there were few reversal reactions and the BL group could be disregarded for the bacteriological and histological evaluation. It is not recommended that true BL patients should be included in this sort of trial. The dearth of such patients in this series is to the credit of the clinicians in selecting lepromatous patients.

THE PROBLEMS OF THE MULTI-CENTRE METHOD

Difficulties are always likely to arise in clinical trials conducted on a multicentric basis, unless virtually unlimited funds and resources are available to ensure that all organizational requirements are met. This double-blind study proved no exception. Loss of information and the long duration posed major problems of the sort familiar enough in therapeutic trials in any chronic disease.

There was no independent clinical observer, whose task it would have been to assess the clinical progress in a consistent manner. In a trial covering such a vast area as this one did, it is, however, difficult to achieve this ideal, as Waters *et al.* (1967) have already pointed out. In the present study, each investigator was impartial in so far as he had no knowledge of the treatments administered, and his statements were based mainly on the presence or absence of any marked or persistent deterioration in a patient's condition. Regular contact with an independent observer interested in the trial would certainly have afforded more encouragement and incentive to the investigators. Better communications would probably have helped to increase the volume of evaluable data and reduce the number of drop-outs. Under the circumstances, histology is the only approach that affords opportunity for uniform evaluation and assessment. As to the value of the methods used in this trial, there appears to be no doubt that this type of investigation can supply answers to preformulated questions, as long as they relate to measurable parameters or to the presence or absence of particular phenomena.

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Lepr. Rev. (1975) **46**, 297-301

Case Report—Calcification of the Ulnar Nerve in Leprosy

B. P. B. ELLIS

Leprosy Officer, Ministry of Health, Rhodesia

Introduction

Calcification of the peripheral nerves in leprosy is rare (Nalasco, 1936; Trapnell, 1965; Pant and Seghal, 1967; Jopling, 1971) and may be associated with a previous history of nerve abscess, itself an uncommon complication (Browne, 1957; Seghal *et al.*, 1967; Enna and Brand, 1970).

In view of the rarity of this condition the following case is reported.

Case Report

An alert African female juvenile presented at the Harare Central Hospital Neurological clinic with right ulnar and median nerve paralysis of two months duration. She was referred to the leprosy clinic for investigation.

History

She reported that in August 1972 a coppery coloured lesion appeared on the extensor surface of her right upper arm and elbow. Shortly afterwards she experienced pain and paraesthesia along the flexor aspect of the arm from the elbow distally to her ring and little fingers. She then noted progressive clawing of all her digits, impaired opposition of the thumb and weakness of dorsi-flexion of her wrist. Finally the right arm became anaesthetic from the elbow downwards, and the skin lesion on her upper arm disappeared.

She had had no previous illness or injury, nor did her family suffer any chronic disease.

On Examination

No skin lesions were detected after repeated natural light examinations.

The right great auricular, radial, ulnar and median nerves were all enlarged; the radial was tender, as was the ulnar which had an easily palpable fusiform swelling above the medial epicondyle. Anaesthesia extended from the elbow downwards to include the hand.

The right hand had fixed clawing of the middle finger; wasting of the thenar and hypothenar eminences; weakness of the lumbricals and interoseii; impaired

* Received for publication, 7th August 1975

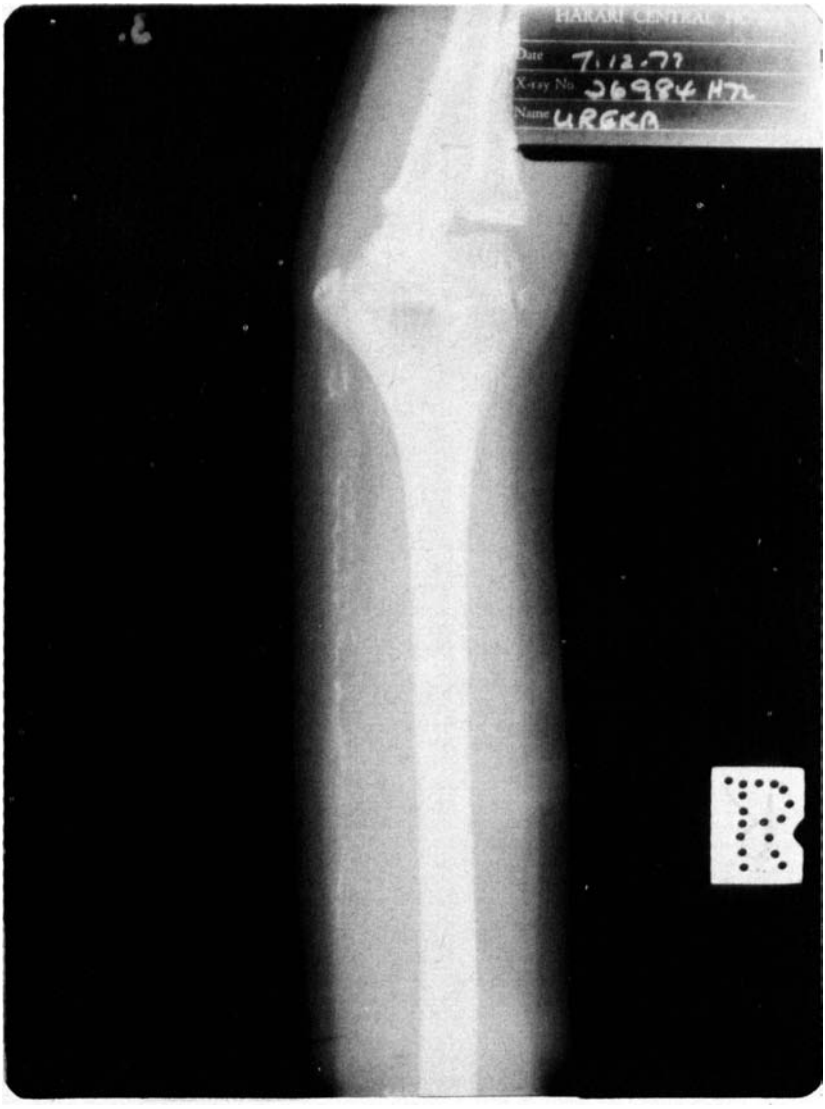

Fig. 1. Calcification of the medial and lower aspects of the ulnar nerve at the level of the right lower humerus.

opposition of the thumb and an unstable pinch. There was partial wrist drop, with hyper-extension of the metacarpophalangeal joints on attempting to straighten the fingers.

The Lepromin test was positive (++) and the Heaf test negative. Except that she had both *Schistoma mansoni* and *haematobium,* all other investigations were either normal or within normal limits.

A skin biopsy from the area where the patient alleged the coppery lesion had

appeared 2 months previously showed no histological abnormality. Unfortunately parental permission for a nerve biopsy was not obtained.

Radiology

Radiology showed "calcification of the medial and lower aspect of the ulnar nerve at the level of the right lower humerus" (Fig. 1).

Follow-Up

In January 1975 the patient presented again for reassessment, having had, since discharge, continuous dapsone therapy as an out-patient at her local clinic.

She still had no skin lesions and her peripheral nerves, with the exception of the right ulnar, were of normal size on palpation. The right ulnar had a large fusiform swelling above the medial epicondyle easily visible as such on extending the arm. On palpation the swelling was firm and tense, but not adherent, and could be tracked above and below until the nerve became apparently of normal size. The area of anaesthesia was now limited to the ulnar distribution of the hand, and the intrinsic motor deficit confined to the ulnar and median nerves.

Radiology showed that "the calcification of the ulnar nerve is markedly extended in comparison with the previous film (1972)" (Fig. 2).

Discussion

The diagnosis of leprosy in this case is based upon the clinical signs of peripheral nerve involvement and the history of a spontaneously healing skin lesion.

Cochrane (1964) stressed that a diagnosis of leprosy is seldom justified unless one of two cardinal signs is present, namely, clinical signs of nerve involvement and the demonstration of *Myco. leprae* in the skin.

Peripheral nerve thickening in the tropics and subtropics should always suggest leprosy according to Jopling and Morgan-Hughes (1965) and they give as differential diagnosis two rare neurological diseases—familial progressive hypertrophic interstitial neuritis (Déjerine-Sottas) and primary amyloidosis affecting peripheral nerves.

The radiological appearance of calcification of nerves has been described by some workers (Campos, 1942; Campos, 1946; Floch and Destombes, 1951; Saikawa, 1951) as blobs consistent with calcification of old nerve abscesses; whilst others (Trapnell, 1965; Ramanujam and Ramu, 1966) as widespread flakes without evidence of abscess formation. Contreras *et al.* (1961) described their case as giving the "impression of a bony tissue and shedding off of particles which seem to be veritable sequestrae".

The literature contains a number of reports of nerve abscess in leprosy, albeit most authors agree that it is an uncommon complication with a predilection for the high resistant form of leprosy in the male.

An analysis of 1500 leprosy patients undergoing treatment at the Harare Central Hospital revealed two abscess cases, both tuberculoid, one an adult male and the other a female child.

Lowe (1934) observed that frequently a single nerve abscess is the only sign of active leprosy and that in many such cases the disease undergoes spontaneous

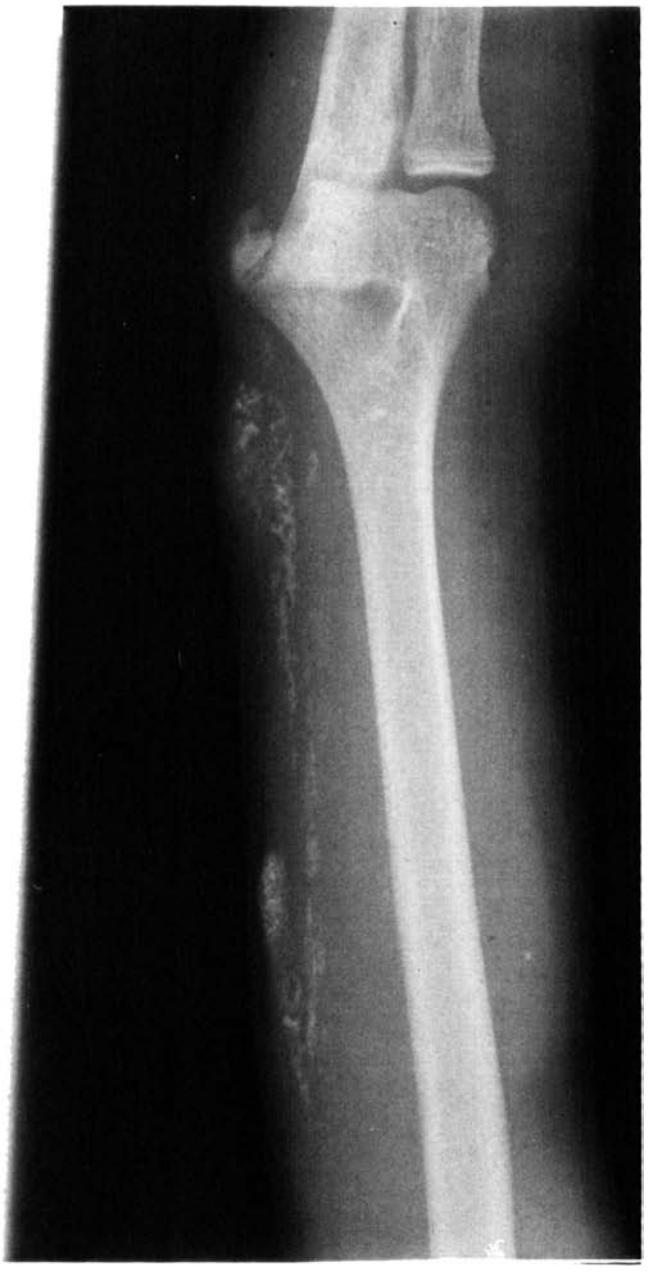

Fig. 2. Follow-up radiograph showing calcification has extended.

arrest. He considered that abscess formation was associated with a substantial immunity, a finding supported by Campos (1936).

Browne (1965) commented on the infrequency of caseation in peripheral nerves and describes the lesions as small areas of caseating autolysis, local and circumscribed accumulation of fluid being rarely sufficiently large to justify the term abscess.

In two cases at Harare Central Hospital, both males with tuberculoid leprosy, where the ulnar nerves were explored surgically, one had multiple areas of caseation both within and surrounding the nerve from the wrist to the axilla; the other had several fusiform swellings above and below the medial epicondyle without caseous material.

Enna and Brand (1970) describing the surgical appearance of affected nerves in leprosy report "the presence of an area of cellular necrosis within which there is a continuity of collagen framework without caseation. This process may be focal or continuous, and may be located between fasciculi which are fundamental and intact. It may progress to the destruction of all tissue elements that the material becomes caseous. Following caseation a true abscess may form.

"When an abscess forms within the nerve trunk, it may either burst through the epinurium to produce a localized fusiform swelling, or it may migrate, extending a narrow tract that leads to a saccular swelling within adjacent soft tissue."

The basis of calcification of the peripheral nerves in leprosy may be therefore caseation, abscess formation and, uncommonly, deposition of calcium within or adjacent to the affected nerves. This process may be halted dependent upon host resistance, and the caseous material retained within the epineurium to become calcified, as in the case reported here.

Acknowledgements

To Dr N. Morgenstern, Specialist Radiologist, Ministry of Health for advice and interpreting the radiographs; Professor J. G. Cruickshank, Dean of the Medical Faculty, University of Rhodesia for his encouragement, and to the Secretary for Health (Dr E. Burnett Smith) for permission to publish.

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News and Notes

WHO AND LEPROSY CONTROL

Leprosy control figured on the Agenda of the 28th World Health Assembly at its recent meeting in Geneva.

The wording of the resolution passed on that occasion was as follows:

Recalling resolutions WHA 5.28 and WHA 27.58, and

Noting that leprosy control measures can reduce substantially the prevalence of leprosy when undertaken with sustained effort for a sufficiently long period,

1. RECOMMENDS that:

(a) intensive case detection be carried out to ensure early diagnosis particularly in children;

(b) infectious cases be identified, and when possible be submitted initially to closely supervised treatment to minimize infectiousness and thus the spread of disease;

2. EMPHASIZES the need for health services to integrate leprosy control as a regular continuing activity; and

3. REQUESTS the Director-General to lay greater stress on the training of multidisciplinary staff to improve levels of competence in leprosy control.

It is to be hoped that Member States will wholeheartedly implement this resolution, and thus take a major step forward in leprosy control.

ELEP BECOMES ILEP

As announced briefly in the last Number of *Leprosy Review*, the Federation of European Leprosy Associations, known hitherto as ELEP, has become intercontinental and has consequently changed its name to ILEP.

For some years, the Canadian organization, the *Secours aux Lépreux*, which is associated with the international body known as the *Fondation Follereau*, has been a member of ELEP. The American Leprosy Missions, Inc., and the Sasakaway Memorial Health Foundation (of Japan) have now been admitted as full members of ELEP. Hence the reason for the change of name: ELEP has become ILEP.

The recently-published statistical summary makes interesting reading. No fewer than 605 centres are being assisted financially with annual grants now totalling about £3,750,000. The number of leprosy patients being treated is 1,106,560—an impressive proportion of the total number being treated by all agencies, government and voluntary, throughout the world. By far the highest expenditure is devoted to leprosy control schemes, mobile services and outpatient clinics, some of which form part of ational leprosy programmes.

This year, about £305,000 will be devoted to leprosy research. At a meeting of ILEP held in Paris (5-8 June, 1975), the IMMLEP Project was again warmly

commended to the Member-Organizations; it is hoped that about £40,000 will be granted annually for the next five years in support of this ambitious co-operative effort in leprosy research.

THE VICTOR HEISER AWARDS FOR RESEARCH IN LEPROSY

These awards are funded by a bequest from Dr Victor Heiser, a well-known doctor with a world-wide experience in public health medicine and a life-long interest in leprosy and leprosy research. The primary purpose of the awards is to foster basic biomedical research in fields related to leprosy and to encourage national and international cooperation and research exchange in the study of this disease.

Two categories of awards are available:

1. *Postdoctoral Research Fellowships*

Candidates should have the Ph.D. or M.D. degree and be at the beginning or early stage of postdoctoral training in a field of basic biomedical science directly related to leprosy. Applications will be accepted either from individuals directly, or from heads of laboratories active in leprosy research, for authorization to appoint a fellow. Up to two years of support will be provided at stipend levels between $10,000 and $14,000 per annum.

2. *Visiting Research Awards*

Applicants should be established investigators in leprosy who wish to carry out a specific project at a distant institution. Per diem and travel support will be provided for up to six months of collaborative research. Preference will be given proposals that plan field/clinical experience with leprosy.

The deadline for receipt of all applications is February 1, 1976

Further information and instructions for making application may be requested from Ms. Caroline R. Stanwood, Director, Heiser Fellowship Program for Research in Leprosy, 1230 York Avenue, New York, New York 10021.

INTERNATIONAL SOCIETY OF PEDIATRIC DERMATOLOGY

The International Society of Pediatric Dermatology—has been formed recently by a group of Dermatologists and Paediatricians.

Those interested to become members of the Society can be either Paediatricians or Dermatologists, or both.

Officers of the Society are: Dr Ramón Ruiz-Maldonado, President; Dr Lawrence Solomon, Secretary; and Dr Coleman Jacobson, Treasurer.

For further information write to: L. Solomon, M.D., P.O. Box 6998, Chicago, Ill. 60680 U.S.A.

INTERNATIONAL LEAGUE OF DERMATOLOGICAL SOCIETIES

Following the meeting of the International Committee of Dermatology, more information is now available regarding the XV International Congress of Dermatology which will take place in 1977 in Mexico City. Seven Sub-

Committees have been appointed to prepare the scientific programmes. Patient presentations, instructional courses, symposia dealing with recent developments in dermatology, guest lectures, topics of current interest, workshops, informal discussion groups, free communications and audiovisual programmes are all envisaged during the Congress. Abstracts of papers to be given during the Congress must be furnished by 1 January, 1977, must not exceed 200 words in length and must be in English. The Address of the Secretary General, Dr Felix Sagher, is, Department of Dermatology, Hadassah University Hospital, P.O. Box 499, 91000 Jerusalem, Israel.

HONOURS FOR LEPROSY WORKER

On the recommendation of its Board of Science, the British Medical Association has awarded the Stewart Prize to Dr Stanley G. Browne. The award is made at about two-yearly intervals in recognition of important work on the spread of epidemic disease. In addition to his "outstanding work" on leprosy, Dr Browne has made important contributions on onchocerciasis, yaws and other tropical conditions.

In recognition of his "continuous services toward sufferers from leprosy in Korea", Dr Browne has been made an Honorary Member of the Korean Society of Leprologists. During his last visit to Korea, he was guest speaker at the Annual Meeting of the Society in Seoul.

At a recent meeting of the Dermatological Society of South Africa held in Durban, Dr Browne was admitted to the Honorary Membership of the Society in recognition of his contribution to the Third South African International Dermatological Congress. He gave papers on "Recent Immunological Concepts and Leprosy", "Skin Manifestations of Onchocerciasis" and "Treponemal Depigmentation". Professor J. Gay-Prieto was also admitted to the Honorary Membership of the Society.

AWARDS TO LEPROSY WORKER

Miss Grace V. Bennett, a qualified nurse who has been in charge of The Leprosy Mission's programme in Korea since the departure of the doctor, has recently been appointed O.B.E. by Her Majesty the Queen. She has also been awarded the Dongbaeg Medal by the Korean Government for her valuable services to leprosy sufferers. The Mission's base at Taegu, attached to the Skin Department of the University, serves as a centre from which mobile teams fan out into the surrounding countryside for diagnosis and treatment of leprosy and education of patients and their families.

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Leprosy and the Community

LEPRA ANNUAL REPORT 1975

The 51st Annual Report of LEPRA condenses into relatively few pages packed with information a notable contribution to the cause of leprosy control during 1974, and reveals the concern of very many people all over Britain. Income during 1974 rose to a record £447,884, and made it possible to increase the amount spent on grants and services to leprosy work by £78,000 above the record figure achieved in 1973.

For over 50 years the declared objective of LEPRA has been the eradication of leprosy. At present its main guidelines towards this objective are: leprosy research and the dissemination of information; prevention and cure of leprosy in children; the training provision and support of indigenous medical staff workers; assistance to governments and other organizations in support of effective leprosy work; and integrated control schemes providing domiciliary treatment. All this is offered on a totally non-sectarian basis.

During 1974 the increasing concern of LEPRA with research made possible the importation of 20 armadillos into Britain and their continuing study as well as the support of a series of other research projects. Assistance in the care and treatment of children was given to 97 centres mostly in Africa and Asia. The comprehensive leprosy control project in Malawi now enters its tenth and final year, with Dr Molesworth, O.B.E., now Adviser to the Malawian Government for Leprosy Control. The original project has led to nation-wide developments and demonstrates successfully how leprosy can be tackled when government and voluntary agencies are able to co-operate fully. The involvement of LEPRA in control schemes in Sierra Leone, Uganda, Zambia, Ethiopia, Guyana and India continues. In India and Indonesia support is given to multipurpose rural health projects. The training of indigenous medical and other staff has been encouraged.

Last, but not least, this Journal has been sustained and its expansion in both size and subsidized distribution made possible. We join with numerous supporters and well-wishers in congratulating LEPRA on a year of outstanding progress. Long may its beneficent work continue.

LEPROSY MISSION CENTENARY ANNUAL REPORT

"A YEAR OF GRACE", the Annual Report of The Leprosy Mission for 1974, its Centenary Year, reveals both the astonishing breadth of the Mission's activities and the depth of concern for leprosy sufferers and the control of leprosy felt by Christian communities in many countries. The Mission, now very much an international organization, enjoyed a record income of £1,486,111 in 1974, an increase of £340,000 over 1973, and this, in spite of inflation, enabled the extensive work of the Mission in Asia and Africa to be sustained and developed.

India was the scene of the pioneer early work of the Mission, and is still the country where its most extensive and concentrated work is undertaken. The wellknown research and training centre at Karigiri continues to develop. It was the historic work of Paul Brand on the cure and prevention of deformities in leprosy which gave this centre its international reputation, and with the appointment of Dr Fritschi as Superintendent, this aspect will certainly be carried forward. The large-scale leprosy control project in Gudiyatham Taluk among a population of 400,000 is now coming to its years of fruitful assessment, and leprosy workers everywhere in Asia await the findings with interest. At many centres elsewhere in India the concern of the Mission for the individual patient is an object lesson to all. Community concern and closer co-ordination with Government are signs of health and progress.

In East and South East Asia, the phasing out of Hay Ling Chau, the "Isle of Happy Healing", with leprosy sufferers integrated into general medical work marks a new stage in leprosy control in Hong Kong, and has enabled Dr Grace Warren to undertake a wider role as Adviser in reconstructive surgery throughout Asia. In Korea, Thailand, Indonesia and New Guinea there are important developments in integrated co-operation with Government Health Service.

In many countries in Africa the Mission has a long tradition of co-operation with a wide variety of national Churches and Missions, and thanks to its support, leprosy control has advanced and large numbers of patients have been encouraged. Further developments are now planned in Zaire.

With its enlightened policies, its international outlook and the breadth of its interest, the Mission never forgets "the one, and the one, and the one," a principle which comes out so clearly in the Report. We offer all concerned our good wishes for 1975.

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Letter to the Editor

Fixation of Skin Biopsies

While we should like to draw attention to the helpfulness of Dr D. J. Harman's useful article on biopsies in leprosy (*Lepr. Rev.* **46**, 125), we should like also to comment on his fixation procedure, which is the one described by Wheeler (1964) and which is the source of some confusion. Although it is often refered to as Lowy's or Ridley's fixative this method does in fact differ from the one in use in our laboratory in three important respects: (1) in mixing solutions A and B at the time of the biopsy, (2) in the use of additional acetic acid (the quantity was inadvertently omitted in the article but Wheeler recommends 5%), and (3) in the much longer period of fixation. The first and last of these modifications were introduced to make the fixation procedure more convenient or reliable for use in the field, while the second is probably an attempt to compensate for the third. If these changes are helpful for field work, which is not necessarily always the case, it is proper that the procedure should be recommended for this purpose. But wherever practicable we would recommend the following simple method, which is a slight modification of the one by Lowy (1956).

Fixative:	Formaldehyde (40%)	10 ml
	Mercuric chloride	2 g
	Acetic acid, glacial	3 ml
	Distilled water to	100 ml

This solution becomes mature after about 24 h when a small amount of white precipitate settles to the bottom. It keeps for a month, perhaps longer. Procedure: Fix a skin biopsy specimen for 1½ to 2 h. The time may vary somewhat according to the size of the specimen but it should never be more than 3 h. Transfer to 70% alcohol without washing. The specimen can be left thus for as long as convenient and despatched to the laboratory.

It is well recognized that the preferences of laboratory workers sometimes differ. Nevertheless, Wheeler's procedure departs from standard histological practice in two respects. Fixatives containing formalin and acetic acid are prepared in one solution; Zenker's and Helly's fluids are prepared in two parts because they contain potassium dichromate in addition to either acetic acid or formalin, and fixation in fluids containing mercuric chloride never exceeds 3 h. We think that the method here recommended gives appreciably better results, though we do not wish to imply that Wheeler's method gives bad results, or that it may not be advantageous on occasion for use in the field. The main object of this letter is to clear up a source of confusion.

The Hospital for Tropical Diseases
St. Pancras Way,
London NW1 0PE

D. S. RIDLEY
M. J. RIDLEY

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Wheeler, E. A. (1964). In *Leprosy in Theory and Practice.* (Eds R. G. Cochrane and T. F. Davey), p. 626. Bristol: John Wright and Sons Ltd.

COMMENT BY DR HARMAN

Dr and Mrs Ridley are quite correct in drawing attention to the differences we have introduced in the fixation method as recommended by them, and I do apologize for not making this clear in my article. May I just state that our aim at this Centre is to provide a simple biopsy method which will give a good histopathological picture not only suitable to the pathologist for diagnosis, classification, assessment and prognosis, but also useful to the worker in the field for study and teaching purposes. The slight changes in the Ridley fixative method were introduced with this end in view, and we have found from experience that they have not affected the final result to any appreciable extent. This is extremely fortunate as we depend upon the formaldehyde–mercuric chloride–acetic acid combination to give suitably fixed tissues for leprosy purposes and also to give us satisfactory results with our staining technique.

D. J. HARMAN

Lepr. Rev. (1975) **46**, 311-319

Abstracts

1. GODAL, T., MYRVANG, B., STANFORD, J.-L. & SAMUEL, DOROTHY R. **Recent Advances in the Immunology of Leprosy with Special Reference to New Approaches in Immunoprophylaxis.** *Bull. Inst. Pasteur,* 1974, 273

This long paper makes exciting reading. The earlier sections describe clearly, concisely and comprehensively the recent work which has placed our understanding of the immunology of leprosy on firm foundations, given meaning to the clinical and pathological spectrum of the disease and its complications, and provided a fresh approach to its epidemiology. For this alone the paper is very valuable, but in substantial concluding sections on Immunotherapy and Immunoprophylaxis important new work is described.

This concentrates particularly on the search for a cross-reactive mycobacterium inducing protection against *Myco. leprae.* Screening of 20 different mycobacterial strains indicated that while BCG had some action, the vole bacillus (*Myco. microti*) is antigenically more closely related to *Myco. leprae* than is BCG, its cross reactivity to *Myco. leprae* being both at a higher level and more persistent. Furthermore, in rabbits this reactivity could be increased three to four fold if *Myco. duvalii* was mixed with BCG or the vole bacillus. A sphere of study of immediate importance in the development of a vaccine for prophylactic use in leprosy is thus opened up. Reference is also made to evidence which suggests that the addition of *Myco. leprae* to BCG in rabbits can provoke an *Myco. leprae* specific LTT response which is not achieved with BCG or *Myco. leprae* alone. Clearly we are on the threshold of an important advance in the immunoprophylaxis of leprosy.

T. F. Davey

2. BROWNE, S. G. **Some Aspects of the History of Leprosy: The Leprosie of Yesterday.** *Proc. R. Soc. Med.,* 1975, v. 68, 485.

This masterly paper, read by invitation before the Royal Society of Medicine, first gives a concise account of early records of leprosy in Asia and Africa, proceeds to trace the spread of the disease by soldiers, sailors and merchants through the Mediterranean Collateral and into Europe, and then concentrates on the history of the disease in England and Scotland. An impressive array of records is sifted with great refinement, and a picture of leprosy emerges which demolishes several myths and is authenticated by the sound scholarship and great experience of the author. The text is enlivened by some interesting quotations. It is impossible briefly to summarize the content of this paper, which itself should be read and treasured by leprologists everywhere.

T. F. Davey

The following Abstracts are reprinted, with permission, from *Tropical Diseases Bulletin,* 1975, May to July.

3. HASSELBLAD, O. W. **Psycho-social aspects of leprosy.** *Bull. Pan. Am. Hlth Org.,* 1974, v. 8, No. 4, 283-8.

" . . . While there are no certain solutions for the psycho-social problems of leprosy, a number of positive steps have proven productive. These include a variety of measures to assist the

patient's development of a sound mental attitude during diagnosis; a public health approach to leprosy management that permits the person being treated to remain at home; treatment of leprosy cases at general medical facilities rather than special facilities; accurate and carefully thought-out programs of public health education; and health education of the patient and his family aimed at prevention and treatment of the adverse psychological effects of his condition."

4. NEBOUT, M. Observation d'un cas de Lèpre tuberculoïde nodulaire consécutive à des scarifications rituelles. **[Report of a case of nodular tuberculoid leprosy following ritual scarification.]** *Méd. Afr. Noire,* 1974, v. 21, No. 11, 867-70. English summary.

In 1945, three years after ritual scarifications were made with an unsterile metal instrument on a male aged 15 years of the Central African Republic, large nodules began to appear in the resulting cheloid scars on the face. Nearly 25 years later, similar nodules developed in a symmetrical pattern on the thoracic wall and afterwards on the limbs. Some loss of sensation was demonstrable in the skin over the lesions. No other signs were present (the state of the peripheral nerves is not recorded) and the chest X-ray was normal. Although no *Mycobacterium leprae* were seen, the histological diagnosis of "typical tuberculoid leprosy" was made. After a year's standard treatment, the nodular lesions had completely disappeared. The author suggests that leprosy bacilli must have been introduced at the time of the ritual scarifications through a breach in the skin.

He summarizes usefully the 9 recorded cases in which percutaneous introduction of leprosy bacilli is presumed, largely on anecdotal history.

[The present report leaves many crucial questions unanswered (and unasked). A very rare form of nodular tuberculoid leprosy, which disappeared spectacularly after a year's treatment, requires better substantiation than the author provides before it can be admitted as another instance of an inoculated leprosy infection.]

S. G. Browne

5. MYRVANG, B., NEGASSI, K., LØFGREN, M. & GODAL, T. **Immune responsiveness to *Mycobacterium leprae* of healthy humans.** *Acta Path. Microbiol. Scand. Sect. C,* 1975, v. 83, No. 1, 43-51.

"Immune responsiveness to *Mycobacterium leprae* was studied in various groups of healthy humans. Contacts of leprosy patients responded significantly more than non-contacts by the methods of leucocyte migration inhibition, lymphocyte transformation and early and late lepromin testing. By classifying responses of strengths found in non-contacts as negative, 71.2% of medical attendants, the main category of contacts, were responders by the leucocyte migration inhibition test, 44.2% by the lymphocyte transformation assay and 50.0% by the early lepromin reaction. On the other hand, no degree of the late lepromin reaction was found solely in *Myco. leprae*-exposed people. While the assays of leucocyte migration inhibition, lymphocyte transformation and early lepromin testing thus may be considered useful for detection of healthy individuals expose to *Myco. leprae,* the late lepromin reaction appears unsuitable as a measure of exposure. Besides the association of negative responses by leucocyte migration inhibition, lymphocyte transformation and early lepromin tests, there was in the group of non-contacts a significant quantitative correlation between early and late lepromin reactions. In the group of medical attendants significant correlations were observed between the results of all tests employed."

6. DASGUPTA, A., MEHRA, N. K., GHEI, S. K. & VAIDYA, M. C. **Histocompatibility antigens (HL-A) in leprosy.** *Tissue Antigens,* 1975, v. 5, No. 2, 85-7.

"HL-A antigens of 70 leprosy patients and 40 normal healthy individuals were determined by the standard microlymphocytotoxicity test. Both lepromatous and non-lepromatous leprosy patients were tested for the presence of 11 HL-A antigens, and the frequency of each specificity

was compared with that in a normal population of the same ethnic group. Although the statistical significance of HL-A8 specificity was found to be marginal in lepromatous leprosy patients, when using ordinary 2 x 2 statistics, there did seem to be a decreased frequency of HL-A9 among the non-lepromatous type. Other antigens tested did not reveal any significant differences between the two groups of subjects."

7 SHARMA, C. S. G. **Effect of broxyquinoline and broxaldine in leprosy**. *Lancet*, 1975, 15 Feb., 405.

A patient with lepromatous leprosy in a reaction stage was found to have intestinal amoebiasis and was advised to take a combination of broxyquinoline 500 mg and broxaldine 100 mg 3 times a day for 1 week, after which treatment for leprosy was to have been started. However, the patient did not return and was not seen again for 6 months, during which period he had continued to take broxyquinoline and broxaldine in the dosage suggested. He had not taken any specific treatment for leprosy, nor had he visited a leprosy centre. The lepromatous lesions showed "remarkable clinical improvement", and it was decided to try this treatment in further cases of lepromatous leprosy. So far 12 patients have been treated, 6 for 9 months and 6 for 6 months. Appreciable chemical and bacteriological improvement was noticed within 3-4 months, the nodules shrivelled and flattened, and there was a fall in both the morphological and bacteriological index [no details]. No serious adverse reaction was seen. The author, from Madras, suggests that the possibility that a combination of broxyquinoline and broxaldine might have some specific effect in leprosy should be investigated.

F. I. C. Apted

8. BERGEL, M. **Actividad cancerigena de la diaminodifenilsulfona (D.D.S.). [Carcinogenic activity of diamino-diphenylsulphone (DDS).]** *Publções Cent. Estud. Leprol.*, 1973, v. 13, Nos 1/2, 30-40. English summary p. 41.

25 young rats were placed on a diet in which the content of diaminodiphenylsulphone was gradually raised to 0.3%. They were kept on this diet for 25 months, some being killed for examination at suitable intervals. In 11 rats examined within 15 months of the start, none showed tumours. Among 5 rats examined between 16 and 22 months, one rat showed highly malignant fibrosarcomas in the peritoneum and mesentery.

Eight rats were examined after 24 or 25 months and 7 of them showed tumours—reticularosarcomas, adenocarcinomas *etc.*, of the intestine, liver, spleen and thyroid. In 15 control rats on a normal diet, killed after 25 months, there were no significant tumours. The author refers to two reports that cancers are unduly common among leprosy patients treated with dapsone.

F. Hawking

9. BECHELLI, L. M. *et al.* **BCG vaccination of children against leprosy: nine-year findings of the controlled WHO trial in Burma.** *Bull. Wld Hlth Org.*, 1974, v. 51, No. 1, 93-9.

This further report on the findings of the WHO Burma trial will be read with great interest by all concerned with the control of leprosy. It should be studied in conjunction with the previously published reports of the trial and with the reports of the Uganda and Karimui trials [*Trop. Dis. Bull.*, 1971, v. 68, abstr. 83; 1974, v. 71, abstrs 739 and 740.]

A consistent protection rate of about 20% was observed during the second, third and fourth follow-up examinations. Previous infection with tuberculosis as indicated by tuberculin reactions of 10 mm or greater would not seem (on these results) to confer any substantial protection against subsequent challenge by leprosy.

The highest protection rate, that is 38%, was seen in children 0-4 years at intake.

No correlation could be found between the size of the tuberculin reaction and the form of leprosy developing in children, in either the group receiving BCG vaccination or in the control group. Most of these cases were of indeterminate or tuberculoid leprosy, and no case of

lepromatous leprosy appeared in either group during the study; the explanation advanced for this latter observation is that all detected persons with leprosy were treated.

The incidence of leprosy among household contacts was found to be 3.2 times that among others, irrespective of BCG vaccination.

The authors conclude that the protective effect of BCG vaccination found in the conditions of the Burma trial is not sufficiently substantial to warrant the widespread use of BCG with the aim of preventing leprosy or of affecting its trend in other areas in the world with similar epidemiological features.

The tables and the comment merit detailed and informed study.

S. G. Browne

10. IVANOVA, N. N. **[The results of parallel investigation of the content of xanthurene and 5-oxiindolylacetic acids in the urine of patients with leprosy.]** *Vest. Derm. Vener.*, 1975, No. 2, 16-19. [In Russian.]

The English summary appended to the paper is as follows:

"Parallel studies were carried out to determine the content of xanthurene acid (XA) according to the method of G. Ya. Vilenkina (1965) and of 5-oxiindolylacetic acid (OIA) by the method of Udenfriend *et al.* (1955) in the daily urine of 17 apparently normal subjects of the control group and of 57 patients with leprosy (42 [41?] with lepromatous, 12 undifferentiated, 3 with tuberculoid and 1 with dimorphic borderline forms). In 28 out of 42 patients with lepromatous leprosy the content of XA in the urine was found to be increased considerably (76.95 ± 7.68 mg/day, $P < 0.001$), as compared to that in the subjects of the control group (21.15 ± 1.96 mg/day). As the disease regressed the XA content showed a trend to normalization (46.16 ± 11). The XA content was increased also in 5 out of 12 patients with undifferentiated leprosy, in some patients with tuberculoid and borderline leprosy. Increased excretion of 5-OIA in the urine was observed in 21 out of 42 patients with lepromatous leprosy (4.27 ± 0.3 mg/day, $P < 0.001$ against 2.38 ± 0.189 mg/day in the controls), in 6 out of 12 patients with undifferentiated leprosy (5.13 ± 0.948 mg/day, $P < 0.011$) and in one patient with dimorphic leprosy (5.8 mg/day).

"The increase in the content of XA and 5-OIA acid in the urine of patients with leprosy attests to disorders of kinurenine and serotonine ways of triptophane metabolism."

11. QUISMORIO, F. P., REA, T. H., LEVAN, N. E. & FRIOU, G. J. **Immunoglobulin deposits in lepromatous leprosy skin. Presence of deposits in apparently uninvolved skin and occurrence of serum antiepithelial antibodies.** *Arch. Derm.*, 1975, v. 111, No. 3, 331-4.

"Immunoglobulin deposits were detected in 10 of 13 biopsy specimens from apparently uninvolved skin of patients with lepromatous leprosy. There were deposits of IgM at the dermoepidermal junction in the skin of 5 patients, and deposits of IgM along the dermal collagen and elastic fibers in the skin of the other 5. The deposits were eluted with acid buffers and high molarity salt solution. Circulating IgC antibodies to intercellular substance of epithelial cells, similar to those present in pemphigus vulgaris, were found in 25% of patients with lepromatous leprosy who were studied. These antibodies appeared to be different from the skin-bound immunoglobulin deposits."

12. DELVILLE, J. Microbiologie de la lèpre. Comportement et affinités tinctoriales due bacille de Hansen dans les lésions lépreuses. **[Microbiology of leprosy. Behaviour and staining properties of Hansen's bacillus in leprosy lesions.]** *Ann. Soc. Belg. Méd. Trop.*, 1974, v. 54, No. 6, 457-62. English summary (6 lines).

This unorthodox and unconventional report will achieve the author's purpose if it causes microbiologists to question accepted standards for the identification, by its staining properties, of the causative organism of leprosy. After a brief summary of selected previous work, that has indicated that *Mycobacterium leprae* may possibly be present in all varieties of leprosy in forms

that are not revealed by standard staining procedures for acid- and alcohol-fast organisms, the author reports that the employment of variations of such techniques as those of Ziehl-Neelsen, Gram and Schiff, may reveal numerous bacilli that are not optically present when the ordinary Ziehl-Neelsen technique is used. Moreover, he has found many such bacilli in histological sections from lesions in which they are usually regarded as extremely scanty.

By Schiff staining, he concludes that these bacilli contain polysaccharides and, by an immunofluorescent technique using sera prepared from diphtheroidlike organisms, isolated by him from leprosy lesions, he deduces affinity, if not identity.

[These provocative suggestions need to be confirmed by other workers and the identity of the organisms established by available procedures (such as, for example, mouse footpad inoculation, the demonstration of specific phenoloxidases, and typical surface structure revealed by scanning electron microscopy) before their *bona fides* can be admitted.]

S. G. Browne

13. REES, R. J. W., McDOUGALL, A. C. & WEDDELL, A. G. M. **The testis in mice infected with *Mycobacterium leprae*.** *J. Path.*, 1975, v. 115, No. 2, 73-9.

"Following inoculation either locally or intravenously with *Myco. leprae* of human origin, the histopathology and bacteriology of the testis in experimental mice is described. Normal mice, and mice rendered immunologically deficient by thymectomy and whole-body irradiation, were studied.

"Attention is drawn to a heavy bacillation of the testis in mice from both groups. Bacilli were found in and beneath albuginea, but mainly in interstitial cells and in macrophages surrounding the tubules. The percentage of solidly staining bacilli was high, and globi were frequent.

"The study showed that the testis in mice is particularly favourable for the lodgment and multiplication of *Myco. leprae* following either local or intravenous inoculation.

"The significance of this in relation to the metabolism of the leprosy bacillus and to the frequent occurrence of testicular damage in the lepromatous male patient is discussed."

14. MILLAN, J. & LE CORROLLER, Y. Le dépistage systématique dans la lutte contre la maladie de Hansen: résultats obtenus en Guadeloupe dans le secteur de Grande-Terre. **[Systematic case-finding in the leprosy campaign. Results obtained in Guadeloupe, in the Grande-Terre sector.]** *Méd. Afr. Noire,* 1974, v. 21, No. 10, 695-703.

The authors summarize the results obtained through an intensive case-finding programme in a sector containing about half of the total leprosy patients in a country of a third of a million inhabitants and a leprosy prevalence rate of 5.7 per 1000.

As regular whole-population surveys are impracticable, the programme depends upon the application of widely-accepted principles to ensure that leprosy is diagnosed as early as possible. General practitioners discover, in their ordinary clinics, about half the total number of leprosy patients diagnosed annually. Co-operation with other doctors who regularly examine selected populations (for example, work-people) is another fruitful source of new cases. The examination of contacts should, it is admitted, be more extensive and better organized than it is. The most hopeful feature of the programme was the introduction of a mobile team, which concentrated on the examination of schoolchildren; 52% of new cases were discovered through this activity, and these included 46% of the new cases of lepromatous leprosy. With the lowering of the average age at diagnosis, the numbers of schoolchildren suffering from self-healing forms of leprosy have increased, but the authors consider that the efforts are justified if some patients with early lepromatous or near-lepromatous leprosy are thereby brought to light.

S. G. Browne

15. KRONVALL, G., HUSBY, G., SAMUEL, D., BJUNE, G. & WHEATE, H. **Amyloid-related serum component (protein ASC) in leprosy patients.** *Infection & Immunity,* 1975, v. 11, No. 5, 969-972.

"The presence of amyloid-related serum component, protein ASC, in serum samples from 63 leprosy patients was investigated. Protein ASC was detected in 38% of the patients. A correlation to the disease spectrum of leprosy was apparent: polar lepromatous cases, 64% positive; borderline lepromatous, 50%; borderline tuberculoid, 36%; subpolar tuberculoid, 17%; and polar tuberculoid, negative. Antibody activity against the a antigen of *Mycobacterium leprae* was also determined, showing a similar correlation to the disease spectrum. Serum samples from 23 apparently healthy Ethiopians serving as controls showed a protein ASC incidence of 22%. This figure is significantly higher than the frequency found by others among healthy Norwegian blood donors. Immunoglobulin M levels among patients were elevated in the borderline lepromatous and polar lepromatous groups. The three tuberculoid groups did not differ in this respect from the control group but were all elevated as compared to a normal Caucasian serum pool. Although raised immunoglobulin M levels seemed to parallel increased frequencies of protein ASC in the patient groups as well as in controls, this correlation might be only secondary to a primary derangement in T-cell function."

16. LIM, S. D., TOURAINE, J. L., STORKAN, M. A., CHOI, Y. S. & GOOD, R. A. **Leprosy XI. Evaluation of thymus-derived lymphocytes by an antihuman T-lymphocyte antiserum.** *Int. J. Lepr.,* 1974, v. 42, No. 3, 260-265.

"T lymphocytes were evaluated in the peripheral blood of patients with various forms of leprosy, using a heterologous antiserum specific for human T cells. A significant decrease in T lymphocyte numbers was observed in cases of active lepromatous leprosy but not in the active lepromatous, borderline or indeterminate forms of the disease.

"Patients with lepromatous leprosy resistant to chemotherapy showed a lower level of T lymphocytes than did drug sensitive patients, while patients with lepromatous leprosy complicated by *erythema nodosum leprosum* showed higher levels than did those with uncomplicated lepromatous leprosy. Evaluation of T lymphocytes by microcytotoxicity test with the anti T-cell serum used in this study proved to be as accurate as the nonimmune or spontaneous formation of rosettes with the sheep red blood cells after incubation at 37° C."

17. KAUR, S., CHAKRAVARTI, R. N. & WAHI, P. L. **Liver pathology in leprosy.** *Lepr. India,* 1974, v. 46, No. 4, 222-225.

"Twenty six patients suffering from leprosy, classified clinically, bacteriologically and histopathologically as lepromatous-14, tuberculoid-9 and dimorphous-3, have been studied for liver involvement.

"Thirteen out of fourteen lepromatous patients showed specific granulomata in the liver. Acid fast bacilli were found in 12. A direct relationship between the intensity of skin lesions and hepatic involvement was found. Five out of the nine tuberculoid cases showed epithelioid granulomata. Acid fast bacilli were not found in any of these. Two of the dimorphous cases showed granulomata and one was positive for acid fast bacilli. Amyloid deposit was not found in any of the liver biopsies."

18. GUPTA, J. C., GUPTA, D. K. & GUPTA, A. K. **Hepatic lesions in leprosy.** *Lepr. India,* 1974, v. 46, No. 4, 226-233.

"Liver biopsies from 50 cases of leprosy, 43 cases of lepromatous leprosy and 7 cases of nonlepromatous leprosy, admitted into the skin, V.D. and Leprosy department of the Medical College Hospital, Jabalpur, during the period from April 1971 to October 1972 have been studied histopathologically in detail employing H & E stain as well as special staining techniques for reticulin, amyloid and A.F.B.

"Granuloma constituted the commonest specific lesion in liver in both the major types of

leprosy in lepromatous and nonlepromatous. Other changes noted included, Kupffer's cell hyperplasia, reticulin condensation in and around granuloma, increased connective tissue and cloudy swelling. No amyloid deposit could be detected. A.F.B. could be demonstrated in granulomas in all the cases of lepromatous leprosy.

"The changes noted have been discussed and compared with those of the previous workers.

"It is postulated that granuloma constitutes the commonest and specific type of lesion in liver in cases of leprosy of the two major types. Other changes noted could be attributed to long duration of illness or simultaneous malnutrition or associated other infections or effects of prolonged administration of drugs."

19. KELKAR, S. S., NIPHADKAR, K. B., KHARE, P. M. & GHARPURAY, M. B. **Environment and carriage of hepatitis B antigen in leprosy**. *Indian J. Med. Res.*, 1974, v. 62, No. 12, 1794-1799.

"Hepatitis B antigen carriage was studied in 152 patients with leprosy attending the out-door of the Sassoon General Hospitals, Poona. This group consisted of 37 cases of lepromatous leprosy and 115 of tuberculoid leprosy. Sera from the patients were studied for presence of hepatitis B antigen by both immunoelectroosmophoresis and agar-gel diffusion. Agar-gel diffusion was much less sensitive than immunoelectroosmophoresis. The carriage rates for hepatitis B antigen were 13.5% in the lepromatous leprosy patients (5 positive in 37) and 4.3% in the tuberculoid leprosy patients (5 positive in 115). The accommodation, sanitation and source of water of each patient was established by careful interrogation. The environment, in terms of these three parameters was best with tuberculoid leprosy patients, less so in the lepromatous leprosy patients not carrying the hepatitis B antigen and worst in the 5 patients with lepromatous leprosy and carrying the hepatitis B antigen. Carriage of hepatitis B antigen appears to be related to poor environmental circumstances which seem to favour transmission of the hepatitis B virus.

20. LEVY, L. **Superinfection in mice previously infected with *Mycobacterium leprae*.** *Infection & Immunity*, 1975, v. 11, No. 5, 1094-1099.

"Previous studies of the protection of mice by prior infection with *Mycobacterium leprae* in one hind footpad against challenge with *Myco. leprae* in the opposite hind footpad had produced conflicting results; therefore, the problem was restudied. In several experiments, BALB/c mice were inoculated first in the right hind footpad with 5000 *Myco. leprae* and then challenged in the left hind footpad with 5000 *Myco. leprae* of the same strain at intervals after primary infection, at the same time that uninfected mice were inoculated. Multiplication of the *Myco. leprae* of the secondary challenge inoculum occurred at the same rate and to the same level as multiplication in uninfected mice when challenges were made soon after primary infection. Multiplication was slowed but proceeded to the same level in previously infected as in uninfected mice when the challenges were administered between 76 and 106 days after primary infection (47 to 17 days before the *Myco. leprae* of the primary inoculum had multiplied to the level of 10^6 organisms per footpad). Finally, the *Myco. leprae* of a secondary challenge administered at the time that the organisms of the primary inoculum had multiplied to 10^6 per footpad or later not only multiplied more slowly in previously infected than in control animals, but multiplication in the previously infected animals reached a lower maximum. These results are similar to those observed when mice previously infected with *Myco. bovis* (BCG), *Myco. marinum, Toxoplasma gondii,* or *Besnoitia jellisoni* were challenged with *Myco. leprae.*"

21. JOB, C. K., CHACKO, C. J. G., VERGHESE, R. & PADAM SINGH, S. **Enhanced growth of *Myco. leprae* in the foot pads of thymectomized irradiated mice.** *Lepr. India,* 1974, v. 46, No. 4, 216-221.

"In 10 separate experiments 10 different strains of 10^4 *Myco. leprae* obtained from untreated lepromatous leprosy patients were injected into the foot pads of thymectomized irradiated (T 900r) Swiss albino mice. Normal mice were also given the same number of organisms into

their foot pads and were used as controls. It was shown that in all the 10 experiments immunological suppression by thymectomy and total body irradiation produced a marked enhancement of the growth of *Myco. leprae* in the foot pads which is 10 to 100 times more than that in normal mice. Controlled environmental temperature of the animal house at 20-22°C and careful screening of the T 900r mice from exposure to other infections were necessary for these animals to survive for long periods. In normal mice the infection with *Myco. leprae* persisted for its life time without producing any ill effects on them."

22. LEVY, L., NG, H., EVANS, M. J. & KRAHENBUHL, J. L. **Susceptibility of thymectomized and irradiated mice to challenge with several organisms and the effect of dapsone on infection with *Mycobacterium leprae*.** *Infection & Immunity,* 1975, v. 11, No. 5, 1122-1132.

The authors conclude that because these thymectomized and irradiated mice "were not greatly immunosuppressed, they would not have provided a model of human lepromatous leprosy suitable for chemotherapeutic studies".

23. HOLMES, I. B. **Minimum inhibitory and bactericidal dosages of rifampicin against *Mycobacterium leprae* in the mouse foot pad: relationship to serum rifampicin concentrations.** *Int. J. Lepr.*, 1974, v. 42, No. 3, 289-296.

"The minimum dietary dosage of rifampicin (RMP) suppressing the growth of eight strains of *Mycobacterium leprae* in the mouse foot pad has been determined. Graded dosages of the drug were administered continuously to mice infected with *Myco. leprae* from the day of inoculation. The growth of six strains was suppressed by 0.001% RMP in the diet: the remaining two strains were suppressed by 0.0003% and 0.003% RMP respectively.

"By use of the kinetic technic of Shepard, the bactericidal effect on three strains of *Myco. leprae* of graded dietary dosage of RMP administered for 56 days has been determined. Considerable bactericidal activity was observed with dosages greater than the minimum inhibitory dosage (MID). The MID (0.001%) was bactericidal against strain SBL 16237 (1.17% survival), bacteriostatic against strain SBL 16325 (100% survival) and weakly bactericidal against strain SBL 16263 (13.9% survival).

"Serum RMP concentrations in mice receiving graded dietary dosages of the drug were estimated by a microbiological assay technic using *Sarcina lutea*. A linear relationship between dosage and resultant serum RMP concentrations was found. The MID of RMP for six *Myco. leprae* strains (0.001%) was equivalent to a serum RMP concentration of 0.2 μg/ml. For the two remaining strains the MID was equivalent to a serum concentration of 0.06-0.09 and 0.9 μg/ml respectively. The minimum bactericidal dosage of RMP (0.003%) gave serum levels of approximately 0.9 μg/ml. Serum RMP concentrations equivalent to the minimum inhibitory and bactericidal dosages for *Myco. leprae* are maintained for long periods in patients receiving a daily RMP dosage of 600 mg which has been used in recent clinical trials of the drug in the treatment of leprosy."

24. LEVY, L. **The activity of chaulmoogra acids against *Mycobacterium leprae*.** *Am. Rev. Resp. Dis.*, 1975, v. 111, No. 5, 703-705.

"The activity of the crude sodium salts of the fatty acids of chaulmoogra oil and of hydnocarpic and chaulmoogric acids against *Mycobacterium leprae* was studied in mouse footpad infection. Multiplication of the organisms was inhibited when the salts were administered intraperitoneally and subcutaneously 3 times per week, and when chaulmoogric acid was administered intraperitoneally 5 times per week in half the equivalent dose. Dihydrochaulmoogric acid was also active, whereas palmitic acid was not. Hydnocarpic acid administered intraperitoneally once per week in a dose equivalent to half that of the sodium salts of the chaulmoogra fatty acids was not effective. The demonstration that chaulmoogra fatty acids possess activity against *Myco. leprae* lends weight to our earlier suggestion that a

study of compounds analogous to these acids may yield effective antimicrobial agents with a unique mechanism of action."

25. LEVY, L. & ULLMANN, N. M. **Inhibition of multiplication of *Mycobacterium leprae* by several antithyroid drugs.** *Am. Rev. Resp. Dis.*, 1975, v. 111, No. 5, 651-655.

"Multiplication of *Mycobacterium leprae* in the mouse footpad was inhibited when mice were fed, mixed in their diet, 0.05% methimazole, 0.066% USP thyroid powder, methimazole plus thyroid powder, 0.15% 5-*n*-heptyl-2-thioxo-4-thiazolidinone, 0.1% propylthiouracil, and 0.1% thiambutosine for 154 days, beginning on the day of inoculation. All of the treatment regimens, except for the 2 containing thyroid powder, decreased the plasma concentrations of thyroxine and protein-bound iodine. It is suggested that the 2 antithyroid drugs, methimazole and propylthiouracil, and the 2 antimicrobial agents, heptylthioxothiazolidinone and thiambutosine, all of which possess structural features in common, may exert the antithyroid and antimicrobial effects through a common mechanism."

26. YOSHIZUMI, M. O., KIRCHHEIMER, W. F. & ASBURY, A. K. **A light and electron microscopic study of peripheral nerves in an armadillo with disseminated leprosy.** *Int. J. Lepr.*, 1974, v. 42, No. 3, 251-259.

"The lesion of peripheral nerve observed in an armadillo which developed lepromatoid leprosy following experimental infection with *Myco. leprae* was found to be similar by light and electron microscope examination to the peripheral nerve lesion of human lepromatous leprosy. Bacilli were found primarily within macrophages, endothelial cells, perineurial cells and Schwann cells of unmyelinated fibers. Destruction of nerve tissue appeared to have a perivascular distribution. The pattern of bacillation with predominant involvement of blood vessels suggests hematogenous dissemination of *Myco. leprae* in the armadillo. These observations taken together constitute evidence that armadillos with the disseminated form of leprosy are suitable models for the study of the neural lesions of human lepromatous leprosy.

27. MATSUO, Y. **Studies of *Mycobacterium lepraemurium* in cell culture. I. Continuous multiplication in cultures of mouse foot pad cells.** *Jap. J. Microbiol.*, 1974, v. 18, No. 4, 307-312.

"A serial increase in the number of *Mycobacterium lepraemurium* with successful subcultures has been obtained in the mouse foot pad (MFP) cell culture. Special attention has been given to maintaining the infected cells for longer periods: (1) the infected cells were incubated at 30°C rather than at 37°C, and (2) the concentration of serum in the culture medium was reduced from 10 to 2% as soon as a monolayer growth of the transferred cells was obtained. There have been cumulative bacterial increases of 1.47×10^{17} and 1.84×10^{15} fold for the Kurume-42 strain during a period of 1255 days, and 2.23×10^{9} and 3.89×10^{5} fold for the Hawaiian strain during periods of 831 and 572 days. The overall generation times were estimated at 22.0, 24.8, 26.8, and 30.8 days, respectively. All attempts to grow the acid-fast bacilli obtained in cell cultures on artificial culture media have failed. The ability of the organisms to produce typical lesions in mice has been well preserved."

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