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Editorial

SACRED COWS AND HELPFUL MICE or THE TRANSMISSION AND SPREAD OF LEPROSY

We may smile at some of the ideas and beliefs of the pre-scientific era—at the miasmas and the phlogiston, the humours and the clysters. We rather look down on the old historic battles between Danielssen and Hansen, representing respectively the champions of the traditional view that leprosy was hereditarily transmitted, and the advocates of the new-fangled theory that this disease-cum-myth was actually due to a specific micro-organism. With all our newly acquired knowledge, however, we may still be in danger of repeating some of the unverified assumptions that have been taken over uncritically from the past. Despite the continuing helpfulness of the co-operative mouse, with its footpads and its immunological apparatus—intact, or experimentally rendered deficient—"sacred cows" still exist. Some of them wax fat, get sleek and respectable, even reproduce their kind. They are referred to with fitting awe; no-one doubts their existence, or their essential rightness. They receive deferential acclaim in text-books, learned articles, lectures . . . until some bold spirit has the temerity, the effrontery, to ask a question or cast tentative doubts on their sacrosanct inviolability.

"Leprosy is spread by skin-to-skin contact", it is said. We have all said it at some time or other. When we add "prolonged and intimate contact", we conveniently fail to define the kind of contact. Some alternative explanation had to be sought when the hereditary theory of transmission was shown to be untenable. There were the family infections, persisting for generations, the household and community foci, and the demonstration of *Myco. leprae* in discharges from patients with multibacillary types of leprosy.

The histopathologists, however, were always insisting on the extreme rarity of organisms in the cells of the epidermis, and also in the subepidermal clear zone. Extremely few organisms seemed to manage to scale this double barrier, and those that did so, appeared to be no longer viable. Rather more bacilli were to be found in the secretory cells and the acini of the sweat glands, and in the cells lining the hair follicles of the skin. And some could be demonstrated in the galactophorous ducts of the lactating mammary gland, and in the renal glomeruli. But the uncounted millions seemed to be safely imprisoned behind an impervious and impassable tissue barrier.

On another page (167), Pedley pursues, with the minimum of apparatus, his critical questionings of the bacterial basis for the old assumptions underlying the "skin-to-skin" theory of the transmission of *Myco. leprae*. According to his findings, acid-fast organisms are extremely rare on the surface of the intact skin, whatever may be happening in the underlying dermis. They are demonstrably numerous, of course, in the discharge from lepromatous ulcers (as distinct from neuropathic ulcerations of the extremities), and also in that from the nasal mucosa. Other observers have been similarly struck by the paucity of acid-fast organisms on the skin, and have emphasized the importance of the bacillary load in the dermis and adjacent lymphatic nodes. Concentration methods of skin scrapings have given equivocal results, of doubtful significance when the organisms have not been positively identified. In the light of present suggestive findings, then, "close contact" need no longer mean "skin-to-skin contact", but rather proximity or propinquity, a physical nearness sufficient for air-borne infective material from the nasal mucosa or from contaminated fomites to be brought into relation with an epithelial surface of a susceptible human being. It will embrace intrafamilial proximity as well as any contact in a community where the prevalence of leprosy reaches 1%.

The importance of these observations is far-reaching. Epidemiological investigations of the kind reported recently in the pages of *Leprosy Review* should be pursued with added vigour and precision. A simple and practical method for roughly determining the infectivity of the individual patient, namely by bacteriological examination of the nasal discharge, is now placed in the hands of the field worker reluctant to make use of an instrument for obtaining material from the skin or nasal mucosa. While histological examination of skin sections is still the indispensable arbiter of the presence of *Myco. leprae*, their morphology, and the tissue response they evoke, the administrative categorization of patients into "open" and "closed" may conveniently be decided on bacteriological examination of typical examples of nasal mucus. Contaminating acid-fast organisms obtained from the vestibule are never found agglomerated in globi. This examination, or a series of such examinations, will thus be of help in establishing or confirming the diagnosis and classification, and be useful in the ascertainment of contagiousness, cure, and relapse. It may provide as good evidence as the usual scraping of the septal mucosa, and in a manner more acceptable to the patient.

For some time, nasal washings from patients with untreated lepromatous or borderline leprosy have furnished viable bacilli for mycobacteriological investigation in the experimental animal.

Just as mycotic pathogens have been isolated from the surface of the skin, fomites, and dust, and thereafter cultured and identified, so it is not too much to hope that the day may not be far distant when *Myco. leprae* may be prised from the places where it lurks—in or on human tissues, in people known to have leprosy, or perhaps even in those not under suspicion or with inapparent infections, in contacts, in carriers, and also possibly in extra-human reservoirs—and positively identified.

The role of fomites contaminated by discharge from the nasal mucosa or from open bacilliferous ulcers needs to be reassessed, and the length of time that *Myco. leprae* remains viable after release from the body should be determined by the footpad inoculation technique.

The source of viable organisms needs also to be investigated anew, for discordant findings abound; for instance, the unduly high frequency with which patients with tuberculoid leprosy apparently act as index cases for infections among contacts; the route of exit of viable organisms during the *poussées bacillifères* of reactional tuberculoid leprosy; the possibility that non-stainable or filterable forms of *Myco. leprae*, or L-forms, exist at certain stages in the life-cycle of an organism that has diverse taxonomic affinities; the apparently sudden burgeoning of mycobacterial activity when indeterminate macules become patently lepromatous, or when bacilliferous papules arise in normal skin.

There are thus many unsolved problems surrounding the emergence of *Myco. leprae* through the intact epithelial surfaces. The upper respiratory mucosa seems to hold pride of place; the gastro-intestinal mucosa plays a negligible role; the intact skin, including mucocutaneous junctions, is of debatable importance. Once again, the clinician, the epidemiologist, and the experimental microbiologist are called to collaborate in investigating these intriguing problems, questioning the "sacred cows" and enlisting the co-operation of the humble mouse.

News Items

PHYSICAL THERAPY CONGRESS AT AMSTERDAM

The Sixth International Congress of the World Confederation for Physical Therapy was held in Amsterdam from 27 April to 2 May, 1970, under the patronage of Her Royal Highness Princess Margriet of the Netherlands; some 1600 participants gathered from more than 40 countries.

The International Leprosy Association was officially represented by Dr. S. G. Browne, its Secretary-Treasurer.

At the opening ceremony, Miss Glen Park, President of the World Confederation, spoke in appreciative terms of the increasing contacts and links between physiotherapists and other medical workers, and gave a special welcome to representatives from the various international organizations present.

Although leprosy did not figure as prominently on the programme as its world importance would seem to merit, several papers given were of interest to physical therapists treating leprosy patients, and Professor A. J. Selvapandian's film, "Physiotherapy in Leprosy", was shown.

AWARD TO DR. S. G. BROWNE

The medal of the Royal African Society for "dedicated service to Africa" has been awarded to Dr. S. G. Browne, O.B.E., Director of the Leprosy Study Centre and Chairman of the Editorial Board of *Leprosy Review*.

The citation refers to Dr. Browne's 23 years' service in the former Belgian Congo as a Baptist medical missionary, followed by 7 years in Nigeria, where he was Director of the Leprosy Research Unit at Uzuakoli, and goes on to say, "his unrivalled knowledge of leprosy research, treatment and control has enabled him to travel widely throughout the world on survey and demonstration work, and his prolific writings and programmes on the subject have done much to change the attitudes of both medical and lay people towards the disease".

BIRTHDAY GREETINGS TO DR. ERNEST MUIR

Leprosy Review extends its sincere greetings to Dr. Ernest Muir, C.I.E., C.M.G., K.I.H. gold medal and bar, on the occasion of his 90th birthday, on 17 June, 1970. Dr. Muir is the doyen of British leprologists, a man honoured and respected throughout the world.

To celebrate the occasion, The Leprosy Mission gave a small tea-party at its new London Headquarters (50 Portland Place, W.1) by kind invitation of the Chairman of Council, Sir Harry Greenfield, C.S.I., C.I.E. Lady Templer, the wife of the Lord Lieutenant of London, received the guests. Greetings were voiced by many who had known Dr. Muir over the years, including Dr. R. G. Cochrane, C.M.G.

BELGIUM PAYS ITS TRIBUTE TO THE LATE DR. FR. HEMERIJCKX

The memory of Dr. Hemerijckx, whose death was recently announced in the columns of *Leprosy Review* (1970, **41**, 65), will be kept alive in Belgium, and indeed throughout the world, in at least two ways: a memorial statue in Grimbergen, and a fund to be devoted principally to the training of leprosy workers. Sunday, 7 June, was set aside for the unveiling of the statue and the public announcement of the creation of the "*Fonds Docteur Hemerijckx*".

The proceedings began with a crowded service in the Abbey Church at Grimbergen, where Dr. Hemerijckx and his family worshipped. A fitting tribute was paid, in the course of the sermon, to the life and activities of the doctor, and especially to his work in the Congo from 1929 onwards, and then from 1955 in India. After the service, the congregation proceeded in brilliant sunshine to a plot of greensward, tastefully planted with spring flowers and rhododendrons in bloom, bordering the main road in the centre of the Flemish town of Grimbergen, some 8 miles from Brussels.

After Grimbergen's Mayor (Monsieur A. de Winter, Member of the Belgian Senate and an ex-Minister) had spoken in eulogistic terms of Dr. Hemerijckx and his work, he unveiled the statue, which consists of a bronze relief of the well-known bearded, smiling face, surrounded by symbolic figures suggesting "the clinics under the trees" in Congo's Kasai Province and India's Polambakkam. Monsier M. Huybrechts, Vice-President of the Damien Foundation, also spoke, and Professor Jan Hemerijckx, worthy son of his father, thanked those present on behalf of the family. Floral tributes presented by many organizations and personalities were laid at the foot of the statue.

After a reception at the Town Hall given by the Mayor of Grimbergen, and a lunch offered by the *Amis du Père Damien* for foreign guests and Committee members, the company drove to Brussels to take part in a public meeting organized as a memorial to Dr. Hemerijckx and held in the spacious hall of the Bibliothèque Royale.

Under the gracious chairmanship of the renowned cancer specialist, Professor J. Maisin, now President of the *Amis du Père Damien*, the Belgian people and nation paid their tribute of respect to a good man who for 40 years had devoted himself to the well-being of leprosy sufferers. Many references were made to his pioneering insistence on the then novel concepts of domiciliary treatment, rehabilitation, and comprehensive care of those afflicted. Dr. Hemerijckx was able to carry these ideals from the Congo to India, where they were to meet with rather more resistance from traditional attitudes and prejudices.

On behalf of the Medical Commission of ELEP, Dr. L. P. Aujoulat, its Chairman, spoke in warm terms, underlining the personal qualities of the man, which were expressed in his work. The honorary rector of Ghent University, Professor J. J. Bouckaert, spoke of Dr. Hemerijckx's influence in the world of practical help to leprosy sufferers and training of auxiliary workers.

After the showing of a film made in East Africa by the Dutch Leprosy Foundation, the "Dr. Hemerijckx Memorial Fund" was publicly inaugurated. Already the townsfolk of Grimbergen and of Ninore (where the doctor was born, in 1902) have made considerable contributions to the Fund, and it is confidently hoped that public bodies, commercial undertakings, and many people of goodwill will wish to associate themselves with the Fund. The interest on invested moneys will be devoted to training leprosy workers, so that the ideals towards which Dr. Hemerijckx worked so ardently may be furthered and practised.

Monsieur Raoul Follereau, Honorary President of ELEP, made a characteristically eloquent and impassioned plea for the continuation of the work of Belgium's "second Father Damien", and a representative of the Minister of Health brought the good wishes of the Belgian Government to the assembly and warmly commended its object.

The immediate past-Chairman of ELEP, Monsieur A. Récipon, was also present; and Dr. S. G. Browne, Secretary-Treasurer of the International Leprosy Association and a colleague for many years of Dr. Hemerijckx in the Congo, represented the Association at the events of the day, and conveyed to the organizers the greetings of the President and Council.

CO-ORDINATION OF LEPROSY CONTROL IN EAST AFRICA

The three countries comprising the East African Community—Kenya, Tanzania, and Uganda—have, since attaining independence, maintained many medical links. Among these links is the Council that fosters and co-ordinates medical research.

Since January, 1969, and thanks to the stimulus provided by the Conference on Mycobacterial Diseases held in Dar es Salaam in that month (reported in *Leprosy Review* (1969) **40**, 139), an increasing interest in leprosy and leprosy control has been evident. In Tanzania, a National Leprosy Advisory and Co-ordinating Committee has been formed, and is now functioning (*Leprosy Review* (1969) **40**, 142). In April, 1970, a working meeting attended by 30 doctors and senior auxiliary staff engaged in leprosy, was held at Makerere University, Kampala, Uganda. The main task of the meeting was to draw up principles of leprosy control that would be generally applicable throughout the three countries represented. Detailed draft proposals concerned with such matters as records, treatment, and training were prepared by panels and submitted to the full conference. After long and at times lively discussions, these proposals were accepted with slight amendments. (Copies of the recommendations in their final form may be obtained on application to Dr. Lomholt, P.O. Box 7051, Kampala, Uganda).

The conference was followed by the inaugural

meeting of the East African Leprosy Workers' Association plans for which had been laid at the Dar es Salaam Conference. The Secretary-General of the East African Community had graciously accepted the office of President of the Assocation. The *East African Leprosy Bulletin*, whose appearance was noted in these pages (*Leprosy Review* (1970) **41**, 8) publishes reports on the progress of leprosy work in the area.

Many projects for leprosy control in East Africa are still in the preliminary planning stages, and their implementation over the course of the next few years will be followed with interest. However, the fact that 2 such schemes, in Kenya, are already being implemented, is to be attributed in part to the stimulus afforded by the Dar es Salaam working meeting, together with the East African Leprosy Workers' Association and the *Bulletin*. Dr. A. R. B. H. Verhagen (to whom we are indebted for the gist of this report) is the energetic Editor of the *Bulletin*.

A FILM ON LEPROSY

The Gandhi Memorial Leprosy Foundation (P.O. Hindinager, Wardha, Maharashtra, India) has produced a black-and-white documentary film entitled "Protection Against Leprosy". Its running time is 19 minutes, and it is available in either 16-mm or 35-mm. At present, only the English version is on sale, but it is hoped shortly to prepare dubbed versions in all 16 languages listed in the Constitution of India. The fact that Dr. R. V. Wardekar, until recently Director of the Foundation, was responsible for the script is a sufficient recommendation. It is hoped to show the film in all cinema theatres in India.

Prints of the film (with English commentary) are now available on application to the Films Division, Ministry of Information and Broadcasting, Government of India, 32 Apollo Street, Fort, Bombay 1. For educational institutions and Government departments the price per print (payable on order) is Rs. 630 (plus Rs. 38.08 excise duty) for the 35-mm version, and Rs. 378 (plus Rs. 10.15 excise duty) for the 16-mm version; and Rs. 684 (plus Rs. 38.08), and Rs. 396 (plus Rs. 10.15), respectively, for private organizations and individuals. Those interested in purchasing a print dubbed in a language other than English are invited to enquire of the Films Division regarding the date when the version they desire will be available.

THE MALAWI LEPROSY CONTROL PROJECT

The Interim Report of the Leprosy Control Project in Malawi, conducted by the British Leprosy Relief Association (LEPRA), has recently been published. It deals with the first 4 years of activity since the Project was inaugurated in 1966. Details of the progress achieved were given in the paper by Dr. B. David Molesworth that appeared last year in *Leprosy Review* (1969) **40**, 237).

This Interim Report provides additional information concerning finance and administration and brings the story up to date. It emphasizes the essentially pragmatic nature of the project and the practicability in the African setting of the principles of leprosy control that have been widely advocated in theory, yet all too rarely put into practice. Already, more than 10,000 leprosy patients have been admitted to treatment; over 46,000 children under 15 years of age have received BCG vaccination; whole population surveys in selected villages and in selected groups have been undertaken; and regular mobile circuits (by motor vehicle, or bicycle) have brought diagnosis, treatment, and education in the use of anaesthetic extremities within the reach of even the most isolated villages in the Project area. A commendable apportionment of time and energy, as between the scattered patients under domiciliary treatment and the claims of the 36-bed central hospital block in Blantyre, is evident in the Report. The crucial importance of training of medical personnel, and also of public relations (through the press and radio, and lectures and demonstrations) has not been forgotten.

We append below verbatim sections of the Report likely to be of interest and value to readers of *Leprosy Review* facing similar problems and opportunities in leprosy control.

Research aspects of the Project

The research potentials in a Project of this magnitude are considerable but they have to be balanced against the overall priority of initiating and establishing the Project as a demonstration model for the control of leprosy. In retrospect, the wisdom of adhering to this priority is made clear from the foregoing interim report, since within a period of only 4 years, 10,000 leprosy patients have been brought under treatment. Moreover, it is equally clear that this has been achieved only by a series of modifications in the original plans based on observations and experience gained in the field. The acceptance of such modifications in the sciences is defined as "research" and in the control of disease as "operational research". Therefore, the application of operational research methods already has played a major role in the successful initiation of the Project and will continue to take priority throughout. However, as soon as the Project was firmly established, other research projects were introduced and are being added as the opportunities arise.

1. The Malawi Project has joined an International Study of trials on the slow-release derivative of dapsone—DADDS—which is given by injection once every 75 days. Preliminary results are encouraging.

2. Another study has as its objective the evaluation of Lamprene and thalidomide as alternatives to corticosteroids for the treatment of serious reactional episodes in patients with lepromatous leprosy.

3. A study has just been initiated to determine whether patients with leprosy who deteriorate despite treatment with dapsone have become resistant to the drug. This study will be undertaken on a collaborative basis between the Department of Chemistry at Blantyre and the National Institute for Medical Research in London. Detailed studies will be made on blood levels of dapsone in the patients and on *Myco. leprae* in their skin, with the view to detecting drug-resistant strains (if present): the mouse footpad technique will be used (in London) to this end.

4. Recent studies in London and Oxford have shown unexpectedly that leprosy bacilli are found in the muscles of patients in both the early stage of the infection and in treated patients whose skin smears are negative. These important observations will now be further investigated on selected patients in the Project in collaboration with workers in London and Oxford.

Interim conclusions

While the time is not yet ripe for making definitive observations on the Project, the following interim conclusions may be drawn in an attempt to evaluate the progress already achieved and the lessons learned.

1. The objectives of the Project, and the presuppositions on which these objectives were formulated, are inherently sound. It is in practice proving possible, with the methods employed, to register the great majority of leprosy patients requiring treatment within a delimited area. Good public relations and persistent and widespread health education are proving of even greater importance than was at first realized.

2. Careful planning of mobile circuits, and the supplementing of motor transport by bicycles, can bring leprosy treatment to within 3 miles of every person needing it. The insistence on domiciliary treatment is already proving beneficial in many ways: (a) it ensures patient co-operation and goodwill, and a high regularity rate of attendance; (b) it prevents disruption of family life, of the farming calendar, and (in the case of children) of schooling; (c) in the absence of whole-population surveys, it encourages patients with early and suspicious lesions to present themselves for examination; and (d) it plays a significant role in preventing progressive damage to insensitive extremities.

3. The Project provides data concerning such matters as: the distribution of leprosy, the type of leprosy, the patterns of transmission, the occurrence of deformities, the prevention of nerve damage by early treatment—data that should prove useful for future research into the epidemiology of leprosy in the African environ-

ment. The registering on punch-cards of basic information in respect of every patient will provide a permanent record of the findings on admission to treatment and of progress toward arrest of the disease.

4. The possible value of BCG vaccination in affording protection to children within the Project area will be followed with great interest. Where the prevalence of leprosy is of the order found in Malawi, in a population distributed in the existing density per square mile, no distinction need be made between household contacts and general contacts. A decided reduction in the leprosy incidence among the vaccinated child population should shortly become evident.

5. Treatment for tuberculosis could, with administrative advantage, be combined with a leprosy control project similar to that under consideration. The theoretical objection that patients suffering from lepromatous leprosy (being probably more susceptible to tuberculosis) should not be unnecessarily exposed to infection by being brought into contact with patients with "open" pulmonary tuberculosis, should not be forgotten when the feasibility of such combined schemes is being discussed.

6. In spite of all efforts, case-finding activities have not yet attained full coverage. It has been abundantly demonstrated that self-reporting and good public relations will bring to light most of those suffering from leprosy and in need of treatment, but some patients will remain unidentified. For this and other reasons, isolated sporadic cases may from time to time be found within the Project area, and even small foci of leprosy. This eventually would not detract from the overall validity of the objectives, but would serve to emphasize one aspect of the problem of leprosy control.

As Dr. Molesworth and his colleagues enter the phase of consolidation and complete coverage of the assigned area, they may be assured of the good wishes of all.

Lepr. Rev. (1970) **41**, 136-154

New Prospects for the Study of Leprosy in the Laboratory*

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Although *Mycobacterium leprae* was identified earlier than *Myco. tuberculosis*, it has still not been cultured *in vitro* and only in 1960 was an infection obtained in laboratory animals. However, important advances have been made in the field of experimental leprosy in the last decade due to the development of new techniques and models for studying *Myco. leprae in vivo*, thus overcoming the limitations imposed by a non-cultivable mycobacterium. Quantitative techniques using *Myco. lepraemurium* provided the first model for developing an indirect method for distinguishing dead (non-infectious) from living (infectious) bacilli, based on morphological differences in organisms stained by the Ziehl-Neelsen method. However, the most important advances resulted from the limited and localized growth of *Myco. leprae* when inoculated into the footpads of mice and, later, the more substantial and generalized multiplication of *Myco. leprae* in immunologically deficient mice (thymectomized and irradiated with a dose of 900 r). Moreover, in the immunologically deficient animals, the infection eventually resulted in a disease replicating that of lepromatous type leprosy in man, including the involvement of peripheral nerves. The results from these studies and the future prospects for the study of leprosy in the laboratory are reviewed in this article.

Of the mycobacterial diseases affecting man, leprosy is second only to tuberculosis in presenting a world health problem affecting more than 10 million people, most of whom live in newly developing tropical and subtropical countries. Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an obligatory intracellular parasite often giving rise to virtually no symptoms. Clinically, the disease manifests itself most commonly in the skin, nose, upper respiratory tract and peripheral nerves and its form is related to the capacity of the individual to destroy the organism. The effect on nerves is responsible for the serious disabilities and deformities of the feet, hands and face, and a Scientific Meeting on Rehabilitation in Leprosy (1961) estimated that in at least 25% of all cases there is some degree of deformity.

It is of interest that *Myco. leprae* is the only species of mycobacterium which affects nerves in either man or animals. This unique property deserves special study, for it is likely to increase our knowledge both of the organism and of peripheral nerve fibres.

Patients with leprosy present a wide range of symptoms and signs, ranging between one or other of the 2 polar forms of the disease; namely, tuberculoid leprosy, in which there are few bacilli, and lepromatous leprosy, in which the number of bacilli is extremely high. Thus, a study of the pathogenesis of leprosy requires knowledge drawn from a wider range of scientific disciplines than for any of the other mycobacterial diseases. Hitherto, progress has been severely restricted because *Myco. leprae* cannot, as yet, be grown *in vitro* and has only been grown *in vivo* to a limited extent since 1960 (Shepard, 1960*a*, *b*). This is unfortunate because the bacterium was one of the first to be linked specifically to a human disease.

It is obvious that, if the causative organism cannot be cultivated or the disease transmitted to experimental animals, neither the bacteriology nor the pathology of the infection can be studied in the laboratory. The lack of success

*(Reprinted from *Bull. Wld Hlth Org.*, 1969, **40**, 785-800, by kind permission of Chief, Technical Publications, WHO.)

with these techniques has seriously limited the scope of fundamental and applied research in the leprosy field since the isolation and identification of causative organisms, and their subsequent cultivation *in vitro* and *in vivo* has resulted in the control of most of the bacterial diseases of man. Failure to cultivate and transmit leprosy in the laboratory has not been due to lack of effort. The first attempts were made by Hansen and since then by many bacteriologists and pathologists. From time to time, claims to success have been made but, until recently, none has been upheld although, between 1874 and 1930, all the other important human pathogens were successfully cultured by one means or another. Since 1960, however, techniques have been developed for the transmission of leprosy to animals, and the situation has been transformed.

This review is confined to a consideration of the experimental models developed during the last 10 years which have now reached a stage that permits *Myco. leprae* to be studied on a sound experimental basis. Although it is still impossible to grow the bacilli *in vitro*, these models have already contributed significantly to the study of leprosy in man and are likely to contribute even more during the next 10 years. Although the most important advances arose from the development in 1960 of techniques for transmitting leprosy to animals, it was a prerequisite that experimental models using other species of mycobacteria were developed before the laboratory study of human leprosy was started. The study of models, particularly those using *Myco. lepraemurium*, established the method for determining, indirectly, the infectivity of *Myco. leprae* and the techniques of tissue culture adapted to the cultivation of human leprosy bacilli.

MODELS BASED ON COMPARATIVE STUDIES WITH *MYCO. LEPRAEMURIUM*

An indirect method for determining the viability of leprosy bacilli

Most species of bacteria are completely destroyed when they die within an infected host, but mycobacteria are an exception since they retain both their bacillary form and the property of staining with carbol fuchsin when they are no longer alive. However, in a *Myco. leprae* infection it was not possible to determine directly whether bacilli in the patient were alive or dead because they could not be cultured. Therefore, indirect methods for measuring their viability had to be used. For these studies the closely related *Myco. lepraemurium* proved to be an ideal model since, although the rat leprosy bacillus also failed to grow *in vitro*, its viability could be assessed in terms of infectivity by determining its ability to produce disease following its reinoculation into animals. Early studies using electron microscopy showed that viable *Myco. lepraemurium*, that is, bacilli that infected animals, could readily be distinguished from non-viable bacilli, that is, bacilli that failed to infect animals (McFadzean and Valentine, 1959; Rees, Valentine and Wong, 1960). The essential differences were that the cell wall of the non-infectious (i.e. dead) bacillus, though still intact, was no longer uniformly filled but contained only electron-dense aggregates of disorganized protoplasmic material, whereas the infectious form was uniformly electron-dense. Evidence that this was a general phenomenon was provided by using an entirely different species of bacterium, *Escherichia coli*, which had the added advantage that viability could quickly be tested by means of conventional viable colony counts on solid media. The results of such studies showed that there was a close correlation between the decrease in the number of colonies and the increase in the proportion of degenerate forms of *E. coli* seen under the electron microscope. Furthermore, the degenerate changes which took place in *E. coli* resembled those seen in *Myco. lepraemurium* (Rees, Valentine and Wong, 1960).

Once it had been established that living and dead forms of bacilli could be distinguished, a new set of comparisons was undertaken to see how far the morphological changes seen under the electron microscope were correlated with those shown by the light microscope when *Myco. lepraemurium* was stained with carbol

fuchsin using the routine Ziehl-Neelsen method. Rees and Valentine (1962) developed a new technique that allowed individually identified and stained bacilli to be examined first under the light microscope and then under the electron microscope. Close agreement was found between the proportion of degenerate forms of *Myco. lepraemurium* seen under the electron microscope and the proportion of bacilli showing irregular staining with carbol fuchsin under the light microscope. From these studies it appeared that the electron-dense material within the cell wall of the organism corresponded exactly to the part staining with carbol fuchsin. It was therefore concluded that all forms of *Myco. lepraemurium* showing irregular staining were dead and only those showing uniform or "solid" staining were likely to be viable. It was reasonable to expect that this assumption could be extended to the human leprosy bacillus since the same morphological changes were likely to be shared by all species of mycobacteria. This expectation was fully confirmed when, using the same technique, suspensions of *Myco. leprae* were examined by both light and electron microscopy (Rees and Valentine, 1962). More recently, it has been possible to confirm this assumption by demonstrating experimentally that only the uniformly staining forms of *Myco. leprae* are capable of multiplying in the mouse footpad (Rees, 1965*b*; Shepard and McRae, 1965).

Rate of multiplication of leprosy bacilli in vivo

One explanation for the chronicity of human leprosy might be that *Myco. leprae* divides more slowly than other bacteria or even other mycobacteria; this hypothesis could be tested in murine leprosy since the natural and experimental infections are known to be chronic. By applying quantitative techniques it has been possible to follow the total number of stained acid-fast bacilli in the tissues of animals during the infection and therefore to determine the rate of multiplication or the generation time of *Myco. lepraemurium*. Several different groups of workers (Hilson and Elek, 1957; Hobby *et al.*, 1954; Rees, 1957) have found that even in susceptible animals, the bacilli divide only every 10 to 14 days. This very long generation time is unique for micro-organisms and even for other species of mycobacteria. It is most unlikely that *Myco. leprae* would have a shorter generation time than that of *Myco. lepraemurium*. The long generation time must be considered when claims of successful cultivation or transmission of *Myco. leprae* are being assessed, particularly if the claim suggests that the organism is multiplying rapidly. It is even more important to accept a long generation time when methods for attempting to cultivate or transmit *Myco. leprae* are being planned. The model has been shown to be applicable by Shepard and McRae (1965), who demonstrated that *Myco. leprae* has a generation time of 13 to 25 days in the mouse footpad (see p. 144).

Time taken to kill leprosy bacilli and the fate of dead organisms following chemotherapy

It is known that in patients receiving successful chemotherapy, in particular after treatment with diaminodiphenylsulfone, a very long time elapses before bacilli disappear from the lesions. Whether this was due to the slow rate at which the drug killed the bacilli or to the inability of the host to dispose of the dead organisms could only be a matter of conjecture in the absence of direct or indirect evidence. However, when murine leprosy was used as a model to study the effect of chemotherapy on the rate of kill (as determined by the proportion of irregularly stained, "dead", bacilli) and the ability of the host to dispose of the bacilli killed by chemotherapy *in vivo* (Rees and Waters, 1963), indirect evidence on this point became available. Mice heavily infected with *Myco. lepraemurium* were treated with isoniazid and the total number of bacilli and the total numbers of viable (solidly staining bacilli) were determined in the treated and untreated groups at regular intervals for more than a year. The results showed that isoniazid was very effective since the untreated animals all died of gross infection within 100 days, whereas the treated mice lived for more than a year. Furthermore, 73% of the bacilli in the treated mice showed irregular staining after only 28 days and the proportion

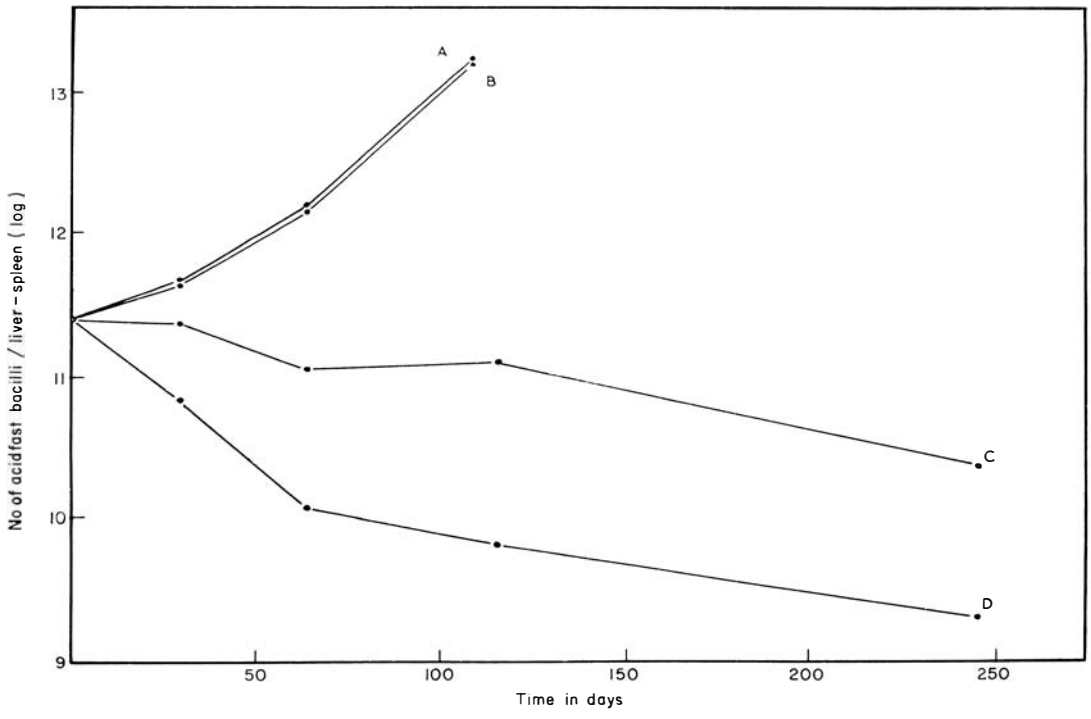

FIG. 1

"Viable" and total populations of *Myco. lepraemurium* in the liver and spleen of untreated and isoniazid-treated mice*. (Reproduced, by permission, from Rees and Waters, 1963.) A = Untreated, total count; B = Untreated, viable count; C = Isoniazid-treated, total count; D = Isoniazid-treated, viable count.
*All untreated mice died with gross infection by day 100.

had reached 93% by the 63rd day of treatment. There was, therefore, a 90% kill within 50 days of treatment. On the other hand, the fall in the total number of stained bacilli in the tissues of the treated mice was much slower; it took 250 days for a 90% fall in numbers to occur (Fig. 1). Thus, although isoniazid was effectively bactericidal within a relatively short time, dead *Myco. lepraemurium* were not nearly so quickly removed from the infected tissues. After continuous treatment with isoniazid for 1 year the proportion of solidly staining acid-fast bacilli started to rise again; in other words, viable organisms were reappearing. The total number of such organisms steadily increased and within 3 months the animals died of *Myco. lepraemurium* infections. Bacilli recovered from animals that had relapsed under treatment with isoniazid were inoculated into healthy mice which were also treated with isoniazid. These animals showed no response to the drug and the infections took the same course as in untreated controls (Hart, Rees and Valentine, 1962). This indicated, without doubt, that the recurrence of active disease in the mice used in the first experiment was due to the appearance of isoniazid-resistant organisms and that the relapse was heralded by the reappearance of bacilli which were morphologically viable.

Use of tissue culture systems for the cultivation of Myco. lepraemurium in vitro

Myco. lepraemurium, like *Myco. leprae*, is predominantly an intracellular parasite and so far neither organism has been cultured in bacteriological media. The dependence on an intracellular environment strongly suggested that tissue-culture methods might offer a means

of growing *Myco. lepraemurium in vitro*. The first successful claims of limited multiplication of *Myco. lepraemurium* in tissue culture systems were made by Wallace, Elek and Hanks (1958) and by Rees and Wong (1958). Since then, techniques have been developed using an established strain of rat fibroblast cells (Rees and Garbutt, 1962) or cultures of mouse macrophages (Chang, 1960) in which indefinite and continuous multiplication of *Myco. lepraemurium* has been obtained. The success of these tissue-culture methods for *Myco. lepraemurium* provides a wealth of knowledge which can be applied directly to the problems concerned with the very similar, slow-growing organism *Myco. leprae*. In particular, the studies have stressed the importance of using quantitative methods for determining the total number of acid-fast bacilli present at the beginning and at the end of each culture period, and therefore basing multiplication on absolute increases in the bacillary population. The value of examining the morphology of the bacilli as a sensitive means of determining their survival in tissue culture has also been made clear. It is significant that the characteristics of *Myco. lepraemurium* maintained in continuous cultivation in tissue-culture systems for more than 3 years have not changed (Rees and Garbutt, 1962). *Myco. lepraemurium* grown in tissue culture do not multiply in bacteriological media, still have a generation time of 10 to 12 days and retain their pathogenicity for mice and rats. These observations are of considerable importance since they confirm the generally accepted view of the stability of bacterial populations, which is in sharp contrast to so many of the acid-fast bacillary strains, claimed to be *Myco. leprae*, which were isolated from leprosy patients. These strains had a wide and variable range of characteristics which were, unjustifiably, explained on the basis of adaptation or mutation.

Application of the results of studies on murine leprosy to human leprosy

Myco. lepraemurium has been extensively used as a model in leprosy research, although more recently its value has been criticized. Such criticism is justifiable only when murine leprosy has been used uncritically as a model for the human infection—namely, for screening potential antileprosy drugs and in comparative pathological and histological studies. Where murine leprosy has been used to answer specific questions or to test hypotheses directly related to *Myco. leprae*, these studies have provided valuable information. It is clear from the results of these studies that a new and valuable technique was available that could now be used in man to determine the viability of *Myco. leprae* in patients and thus to assess precisely their probable response to chemotherapy, the infectivity of the various types of leprosy, and, by histological studies, the viability of *Myco. leprae* in different tissues. Thus the morphological index (MI), that is, the percentage of solidly staining bacilli seen in smears or sections from leprosy patients, could now be used as the measure of viability.

The routine bacteriological examination of patients is based on smears prepared from diseased skin or a nasal scraping and, after staining the smear by the carbol fuchsin method, the density of bacilli (this is known as the bacteriological index (BI) is scored, irrespective of the morphological appearance of the bacilli. The progress of the patient under treatment is then judged by the rate of disappearance of bacilli from these smears, that is, by the fall in the BI. It has long been considered that even the most active antileprosy drugs, including diaminodiphenylsulfone, leave much to be desired since in patients with the more severe lepromatous type of leprosy many years elapse before negative smears give evidence of a "cure". Hitherto, it has been assumed that more active drugs are required to kill *Myco. leprae* more rapidly than diaminodiphenylsulfone, an assumption that was based on the fact that the BI falls so slowly. Now that it has been demonstrated conclusively that the viability of *Myco. leprae* can be assessed on the basis of their morphology rather than on the total number of the bacilli, it has been possible to show, in carefully controlled studies, that a very high proportion of bacilli are killed in 3 months in

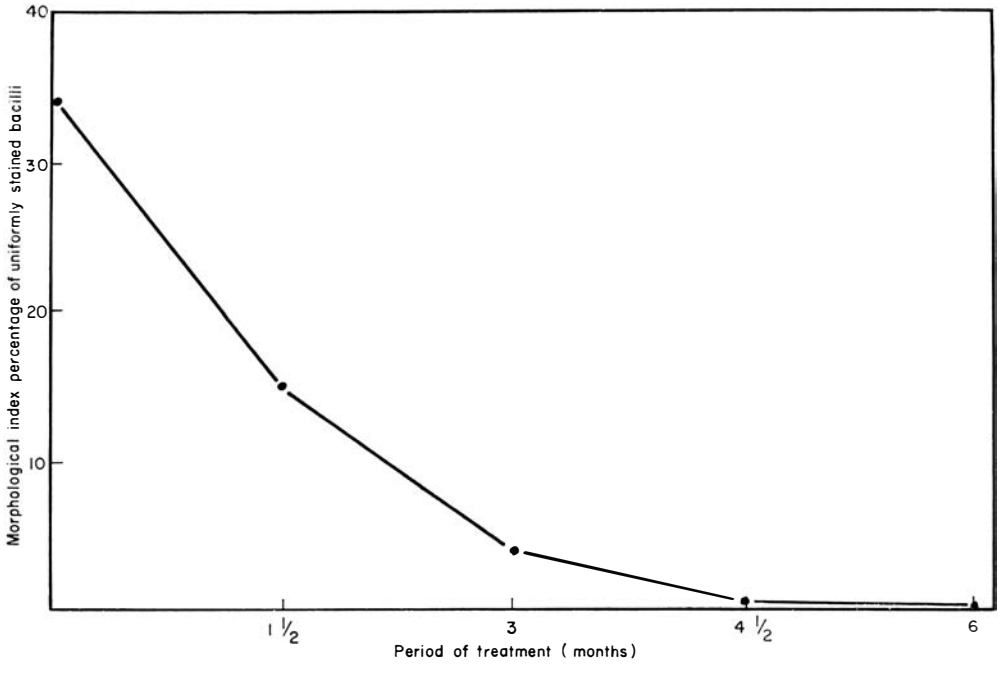

FIG. 2

Effect of treatment with diaminodiphenylsulfone (100 mg daily) for 6 months on the Morphological Index (MI) in 6, previously untreated, lepromatous patients. (100 mg daily).

patients receiving standard treatment with diaminodiphenylsulfone (Waters and Rees, 1962). This observation suggests that persisting lesions and many of the manifestations of leprosy, including reactions of the erythema nodosum leprosum type, that follow the initial phase of chemotherapy must be due, in part, to the presence of dead bacilli. It implies that a more rapid cure will be achieved only if other drugs or methods are found that could be used, after the initial killing of leprosy bacilli with standard antileprosy drugs, to enhance the host's ability to dispose of dead, but still intact, leprosy bacilli.

Thus the introduction of the MI as an index of viability has not only provided a rapid method for determining, within a period of only 3 to 6 months (Fig. 2), the activity of potential antileprosy drugs (Waters, Rees and Sutherland, 1967) but has led to an entirely new approach to the problem of leprosy chemotherapy. Furthermore, an increase in the MI during treatment provides a sensitive measure of the patient's deterioration, whether this is due to failure to take the drug or to the emergence of drug resistance (Pettit and Rees, 1964; Pettit, Rees and Ridley, 1966) (see p. 146).

EXPERIMENTAL LEPROSY IN ANIMALS

Before describing advances that have been made in the field of experimental transmission of leprosy to animals since 1960, it is pertinent to review briefly the general problems and methods used in animal transmission experiments and the criteria for assessing successful claims. These were defined in detail by the Technical Committee on Pathology and Experimental Transmission (1963) at the Eighth International Congress of Leprology in Rio de Janeiro, Brazil. Bacilli for inoculating laboratory animals should be obtained from patients with untreated leprosy and with a high MI and, because of the possible contamination of skin by other cultivable mycobacteria, biopsy specimens should not be taken

from ulcerated lesions. Moreover, all suspensions prepared for purposes of inoculation should be cultured on a variety of media suitable for isolating mycobacteria. In addition to each group of animals inoculated with fresh suspensions of bacilli, there should be a group inoculated with heat-killed organisms and an uninoculated group of animals. Quantitative bacteriological methods should be used in order to determine the number of organisms inoculated and subsequently to determine the number of organisms present in the animals. These rigorous methods and checks were introduced in order to exclude as far as possible the inoculation and subsequent development of infections with contaminating strains of mycobacteria, and also the possibility that the animals themselves might be carriers of mycobacteria. This latter possibility has been demonstrated very clearly by the elegant work of Nishimura and his colleagues working at Osaka in Japan, showing that a proportion of apparently healthy mice and other rodents can be carriers of a murine leprosy-like infection (Nishimura *et al.*, 1964). The criteria for assessing successful claims for transmission of leprosy were also rigorously defined. In addition to counting the number of bacilli isolated from the animal tissues, the organisms should be cultured, using again media suitable for growing mycobacteria. Successful transmission of an infection from one patient should be reproduced from others, using the same experimental conditions. A standard type lepromin should be prepared from the bacilli harvested from the animals and compared with a similarly prepared human lepromin from patients with tuberculoid and lepromatous leprosy; these tests should be carried out and read blindly. Finally, a histopathological examination should be made of the infected tissues including, in particular, a careful examination of the nerves, using the requisite staining methods.

Experimental leprosy in normal animals

Undoubtedly the most important direct contribution to the study of leprosy since the identification of *Myco. leprae* by Hansen has been the transmission of experimental leprosy to animals. This was achieved first by Shepard (1960*a*, 1960*b*), at the Communicable Disease Center, Atlanta, Ga., USA, who showed that a reproducible and limited infection could be produced when the footpads of mice were inoculated with *Myco. leprae*. Moreover, an identical type of infection has now been produced in other centres throughout the world.

General features

The infection obtained in the mouse footpad is a local one and is dependent upon the number of bacilli inoculated. Thus inocula of 5×10^3-10^4 *Myco. leprae* multiply 100-fold in 6 to 8 months, whereas larger inocula fail to give a higher yield, and inocula of 10^6 bacilli fail to show any multiplication. Moreover, having multiplied as far as they are able, the bacilli gradually die (Rees, 1964). A similar type of infection has been obtained subsequently in the ears of mice and in the ears and footpads of hamsters (Waters and Niven; 1965, 1966) and in the footpad of the rat (Hilson, 1965). Although these experimental infections give only limited multiplication of *Myco. leprae*, they are reproducible and can be maintained indefinitely in the laboratory by passage and are adaptable to quantitative analysis. It is probable that more than 200 strains of *Myco. leprae* derived from individual patients with active disease originating from nearly every part of the world have produced an identical type of infection when inoculated into footpads of mice. For example, in our own series we obtained successful transmission with all 89 strains of bacilli isolated from individual patients with active leprosy from different parts of the world and with different types of the disease (79 patients with lepromatous leprosy and 10 with borderline tuberculoid leprosy). The sources of the 89 strains is shown in the following tabulation.

Origin	*No. of strains*
Africa	
Central	1
East	1
West	3

Burma	6
India	8
Malaysia	66
Malta	1
Pakistan	1
Samoa	1
West Indies	1

These results are important since they indicate that the virulence of strains of *Myco. leprae* for the mouse is similar, irrespective of whether the strains were derived from patients with the more tuberculoid or more lepromatous form of the disease, thus suggesting that the pattern of disease in man is determined by the host and not by the parasite. However, these findings do not exclude the possibility of strain difference in *Myco. leprae* of the type, for example, that has been shown with *Myco. tuberculosis* in animals where both *Myco. tuberculosis hominis* and *Myco. tuberculosis bovis* are virulent in the guinea-pig but only the latter is virulent in the rabbit.

The evidence that the infection produced in the footpads or ears of various rodents, including the mouse, rat and hamster, is overwhelmingly in favour of the infection being due to the human leprosy bacillus. Although this is now universally accepted, it is important to recapitulate the criteria which were used to establish this evidence and the care that was taken by those working in the field to satisfy all the criteria that are outlined in the following section. The criterion of bacterial multiplication was based on precise quantitative methods so that the number of bacilli inoculated could be compared with the number finally harvested in the footpads of mice. The pattern and rate of multiplication were uniformly reproducible with all strains of *Myco. leprae* derived from untreated patients. All the inocula and harvests were cultured on media suitable for isolating mycobacteria, in order to exclude the possibility that the infection was caused by a cultivable organism. Lepromin prepared from bacilli harvested from the mouse footpad infections was compared with standard Mitsuda-type lepromin prepared from man in a series of patients with different types of leprosy and the pattern of response was identical (Shepard and Guinto, 1963). The histology of the footpad infection in mice was also studied and the pattern of response was shown to be similar for all strains of *Myco. leprae* and, although the cellular changes were indeterminate and not characteristic of the polar types of leprosy as seen in man, they were not characteristic of those produced by any other known species of mycobacteria (Rees and Weddell, 1968). Moreover, in a proportion of the infected animals acid-fast bacilli were found, occasionally late on in the infection, within nerves of the footpad or in the sciatic nerve (Wiersema *et al.*, 1965; Rees and Weddell, 1968) and therefore showed a selectivity for peripheral nerves shared by no other species of mycobacteria.

Although the infection produced by the local inoculation of *Myco. leprae* into the ears or footpads of rodents was a limited one and multiplication only occurred when the number of bacilli inoculated was less than 10^6, bacilli could be harvested from these infections and reinoculated into animals, where again they multiplied and reproduced the same bacteriological and histological patterns of response. Therefore, because the characteristics of *Myco. leprae* were not changed by serial passage, this method could be used for maintaining indefinitely experimental infections with *Myco. leprae* for study in the laboratory (Rees, 1965*b*; Shepard, 1965*b*). Moreover, a similar pattern of infection has been obtained in the many different strains of mice used although more detailed comparisons suggest that the CBA and BALB/C strains of mice are the most susceptible (Shepard and Habas, 1967).

RECENT APPLICATIONS OF INFECTIONS IN THE FOOTPADS OF MICE FOR THE STUDY OF *MYCO. LEPRAE*

Once it was established that *Myco. leprae* could be transmitted to animals it was hoped that this experimental infection would provide the first opportunity for studying *Myco. leprae* in the laboratory. These hopes have been fully justified;

within less than 10 years, despite the limited nature of experimental human leprosy in normal animals, this *in vivo* technique has provided new information of great importance concerning the properties of *Myco. leprae*. For practical reasons the mouse footpad infection with *Myco. leprae* has been chosen by most laboratories working in this field.

Generation time of Myco. leprae

During the logarithmic phase of multiplication in the mouse footpad a generation time of 13 to 25 days has been observed (Shepard and McRae, 1965). This very long generation time is consistent with the chronicity of the disease and the long incubation period observed in man and, although comparable to the generation time of *Myco. lepraemurium* it is otherwise unique, even for the slowly multiplying Mycobacteriaceae.

Drug sensitivity testing

The footpad infection, in spite of its limited nature, has been used successfully for testing drugs for antileprosy activity. Thus it has been shown that when *Myco. leprae* is injected into the footpads of mice treated with known antileprosy drugs the organisms fail to multiply. Therefore, the mouse footpad infection provides, for the first time, a specific test for screening new antileprosy drugs and the search is no longer restricted to drugs that are known to be active against *Myco. tuberculosis* (Table 1). Hitherto, chemotherapy in leprosy had necessarily evolved from an entirely empirical basis and even the regimen of treatment with diaminodiphenylsulfone, which has been the standard used for leprosy since 1943, was established by trial and error. This was due to the fact that although diaminodiphenylsulfone has slight *in vitro* and

TABLE 1

Tests of activity of drugs against *Myco. leprae* using the mouse footpad technique

Drug	*Dose (% in diet)*	*No. of strains tested*	*Activity**	*Reference*
Diaminodiphenylsulfone	0.1–0.0001		+	Rees (1965*b*), Shepard (1964), Shepard and Chang (1962, 1964)
Sulfadimethoxine	0.1	4	+	Rees (1965*b*)
Sulformethoxine	0.04	2	+	Rees (1965*b*)
Sulformethoxine	0.1 (3 times weekly)	1	+	Rees (1965*b*)
Diphenylthioureas				
Thiambutosine	0.1	2	0	Shepard and Chang (1964)
Thiambutosine	0.1	6	+	Rees (1965*b*)
Ba-22′330†	0.1	1	+	Rees (1967*a*)
Ba-36′223‡	0.1	1	+	Rees (1967*a*)
SU-2079[s]	0.1	1	+	Rees (1967*a*)
Thiacetazone	0.1		P	Shepard and Chang (1964)
Thiacetazone	0.2	6	+	Rees (1965*b*)
Clofazimine	0.01	1	+	Shepard and Chang (1964)
Clofazimine	0.006	1	+	Rees (1965*b*)
Streptomycin	2 mg/day[n]		+	Shepard and Change (1964)
Isoniazid	0.01		+	Shepard and Chang (1962, 1964)
p-Aminosalicylic acid	0.6		+	Shepard and Chang (1962, 1964)
Cycloserine	0.5		P	Shepard and Chang (1962, 1964)
Ethambutol	0.25		0	Shepard and Chang (1964)
Ditophal	0.5		0	Shepard and Chang (1964)
Pyrazinamide	0.5		0	Shepard and Chang (1964)
Capreomycin	10 mg/day	1	+	Shepard (1964)

* + = Full activity; P = partial activity; 0 = inactive.

† Ba-22′330 = 4-(3-carboxypropoxy)-4′-dimethylamino-diphenylthiourea
‡ Ba-36′223 = 4-dimethylamino-4′-(4-hydroxybutoxy)-dephenylthiourea
} thiambutosine metabolites.

[s] SU-2079 = 4-butoxy-4′-diethylaminoethoxy-diphenylthiourea

[n] Once-daily injections.

in vivo activity against *Myco. tuberculosis* in guinea-pigs, it is ineffective against tuberculosis in man. Thus on an empirical basis it has been accepted that the standard dose of diaminodiphenylsulfone should be 100 mg daily; however, there are important practical and clinical advantages to be gained by modifying this regimen. For example, intermittent treatment with diaminodiphenylsulfone could more easily be supervised than daily treatment. Furthermore, leprologists are now tending to advocate smaller doses of diaminodiphenylsulfone because these appear to reduce the frequency and severity of acute reactions (exacerbation) with their attendant nerve and eye complications but are equally effective in controlling the disease. On account of these trends, the mouse footpad technique is used not only for screening drugs but has also been specifically applied to determine, for the first time, the minimal inhibitory concentration (MIC) of diaminodiphenylsulfone *in vivo* against strains of *Myco. leprae* from previously untreated patients (Shepard, 1967*b*; Rees, 1967*a*, 1967*b*). The sensitivities of 5 "wild" strains of *Myco. leprae* from patients in Malaysia were tested in this way using concentrations of 0.0001% and 0.00001% of diaminodiphenylsulfone in the diets of the mice. The results are shown in the following tabulation:

Strain	0.0001%	0.00001%
1	Sensitive	Sensitive
2	Sensitive	Resistant
3	Sensitive	Resistant
4	Sensitive	Resistant
5	Sensitive	Resistant

The MIC was determined by feeding groups of mice with diminishing concentrations of diaminodiphenylsulfone in their diet and determining the concentrations of diaminodiphenylsulfone in the sera of each group. The results of these studies are shown in Table 2, from which it is clear that the MIC for diaminodiphenylsulfone against wild strains of *Myco. leprae* is approximately 0.015 μg/ml. Thus, *Myco. leprae* in the mouse is exquisitely sensitive to diaminodiphenylsulfone; an unexpected finding, because all other species of mycobacteria are relatively insensitive to sulfones and even the diaminodiphenylsulfone-sensitive micro-organisms such as group A streptococci (Francis and Spinks, 1950) and *Plasmodium berghei* (Thompson, Oleszewski and Waitz, 1965) are 3 to 100 times less sensitive than *Myco. leprae* to diaminodiphenylsulfone. The standard treatment of 100 mg of diaminodiphenylsulfone per day for man gives serum concentrations of approximately 1.5 μg/ml. Assuming that it is permissible to extrapolate these findings from mouse to man, they suggest that a daily dose of 1 mg of diaminodiphenylsulfone would be effective in man. This means that there is strong support from the animal studies for encouraging trials, using lower doses of diaminodiphenylsulfone in man, to reduce the incidence of reactions without the fear of diminishing the therapeutic efficacy of the drug.

TABLE 2

Concentration of diaminodiphenylsulfone in the sera of mice fed different levels of drug in the diet

Dose of diaminodiphenylsulfone *Percentage in diet**	*Mg/kg of body-weight*	*Concentration of diaminodiphenylsulfone in serum or plasma*
0.1	200.0	12.5
0.025	50.0	3.3
0.01	20.0	1.0†
(Man: 100 mg/day)	(2.0)	(1.5)
0.001	2.0	0.015†
0.0001	0.2	0.015†
(Man: 1 mg/day)	(0.02)	(0.018)†
0.00001	0.002	(0.0015)‡

*g/100 g.
†Estimated by Glazko's method.
‡Calculated concentration. Serum concentration varies linearly with doses between 0.1% and 0.0001%. Actual value is below level of detectability.

Drug-resistant strains of Myco. leprae

The result which has emerged from the systematic investigation of footpad infections has been direct proof of the existence of drug-resistant strains of *Myco. leprae*. Carefully controlled investigations on specially selected patients who showed active disease despite treatment with diaminodiphenylsulfone for at

TABLE 3

Sensitivity of strains of *Myco. leprae* from relapsed diaminodiphenylsulfone-treated patients* to diaminodiphenylsulfone†

Strain	*Country of origin*	*Sensitivity to diaminodiphenylsulfone: percentage of drug in the diet*					
		0.1	0.025	0.01‡	0.001	0.000	0.00001
1		R	R				
2		R					
3	Malaysia	R					
4		S	R				
5		S	R				
6	India	R	R				
7	Malaysia		R	R			
8	India		S	R			
9	West Africa		R	R	R		
10	India		S	R			
11			S	R	R	R	R
12			S	R	R	R	R
13			S	R	R	R	R
14			S	R	R	R	R
15	Malaysia		S	R	R	R	R
16			R	R	R	R	R
17			R	R	R	R	R
18			R	R	R	R	R
19		S		R	R	R	R

*All these patients failed to respond bacteriologically, histologically or clinically to a rigorously controlled period of not less than 6 months on a supervised dose of 600 mg of diaminodiphenylsulfone per week.

†A dosage of 0.01% of diaminodiphenylsulfone in the diet of mice gives a serum concentration of 1 μg/ml and is therefore of the same order as the concentration in man on a regimen of 100 mg/day. Thus the level of resistance in the 19 strains of *Myco. leprae* determined in mice is consistent with the therapeutic failure of diaminodiphenylsulfone in the patients.

‡S=sensitive; R=resistant.

least 10 years, combined with studies on the diaminodiphenylsulfone sensitivity of bacilli from these patients using the mouse footpad technique, have shown that a proportion of such patients are infected with diaminodiphenylsulfone-resistant strains of bacilli (Pettit and Rees, 1964; Pettit, Rees and Ridley, 1966; Adams and Waters, 1966; Rees, 1967*b*). To date, 19 diaminodiphenylsulfone-resistant strains from individual patients in different parts of the world have been detected using the mouse footpad test (Table 3). From the data presented in Table 2 on the serum levels obtained in patients on regimens of 100 mg of diaminodiphenylsulfone daily (1.5 μg/ml), and from the MIC of diaminodiphenylsulfone for wild strains of *Myco. leprae* calculated from the footpad test (0.015 μg/ml), relapses under such doses of diaminodiphenylsulfone in man due to the emergence of drug-resistant strains, gives a resistance ratio of 100. The results from Table 3 show clearly that the degree of resistance developed by all 19 strains had a resistance ratio of not less than 100. The relevant data on resistance studies which show that the correlation between studies in man and mouse are satisfactory, are summarized opposite.

Although the mouse footpad test has provided the first direct evidence of the emergence of resistance to diaminodiphenylsulfone, the phenomenon appears to be rare, and may have resulted from the use of excessively high doses of diaminodiphenylsulfone. It has to be admitted that the use of smaller doses of diaminodiphenylsulfone, resulting in concentration of diaminodiphenylsulfone in the serum and tissues nearer to the MIC of diaminodiphenylsulfone for *Myco. leprae*, could lead to the emergence of a greater number of resistant strains. This possibility must be weighed against the advantages to be gained by a significant diminution in the incidence of serious reactions that are

Man	100 mg of diaminodiphenylsulfone/day; serum level=1.5 μg/ml
Mouse	MIC of diaminodiphenylsulfone; serum level=(0.015 μg/ml)
Man	1 mg of diaminodiphenylsulfone/day; serum level=(0.018 μg/ml)
Man/mouse	(100 mg of diaminodiphenylsulfone/day; serum level in man) / (MIC in mouse)="therapeutic ratio"=1.5/0.015=100
Man/mouse	Similarly, strains of *Myco. leprae* resistant to treatment with 100 mg of diaminodiphenylsulfone/day in man would be expected to have a resistance ratio of not less than 100
Mouse/man	This expectation was confirmed: diaminodiphenylsulfone-resistant strains of *Myco. leprae* from patients receving 100 mg of diaminodiphenylsulfone/day multiplied in mice fed doses of diaminodiphenylsulfone resulting in serum levels from 1–12.5 μg of diaminodiphenylsulfone/ml, giving a resistance ratio of 100–1250

believed to be directly related to high doses of diaminodiphenylsulfone.

Similar but less extensive studies have demonstrated the emergence of thiambutosine-resistant strains of *Myco. leprae* (Rees, 1967*a*, 1967*b*): such strains show cross-resistance to thiacetazone, a feature shared by thiambutosine-resistant strains of *Myco. tuberculosis* (Konopka *et al.*, 1955).

Effect of BCG vaccination against Myco. leprae

The footpad infection technique provides an experimental model for investigating the prophylactic effects of various vaccines and has already demonstrated that vaccination by BCG significantly inhibits the multiplication of *Myco. leprae* (Shepard, 1965*a*, 1966). This finding is important although it is not unique since it has already been shown experimentally that vaccination by BCG can produce protective immunity against species of mycobacteria other than *Myco. tuberculosis* (Fenner, 1957). Still more recently, Shepard and Ribi (1968) have shown that vaccination with the cell-wall fraction of BCG incorporated in an oily base is as protective as living BCG, weight for weight, against infections with *Myco. leprae* in the footpads of mice. Thus, mouse footpad infections with *Myco. leprae* provide an important model for investigating the value of prophylactic vaccinations against human leprosy which, if they could eventually be applied to man, would be expected to play a major role in the eradication of the disease. The current importance of these experimental observations that BCG prevents the multiplication of *Myco. leprae* in the mouse footpad is that they support the preliminary results in man that BCG vaccination significantly reduces the incidence of early type leprosy in child contacts in Uganda (Brown and Stone, 1966; Brown, Stone and Sutherland, 1968).

EXPERIMENTAL LEPROSY IN MICE WITH REDUCED IMMUNOLOGICAL CAPACITY

The successful transmission of human leprosy to animals in 1960 provided the first and only means of studying *Myco. leprae* in the laboratory. Although this experimental model has, in less than a decade, provided more information on the human leprosy bacillus than was available previously, progress in the field of leprosy research was still restricted by the limited nature of the infection. Clearly, the next step was to determine the factor or factors preventing *Myco. leprae* from multiplying freely in mice or in other rodents. On the assumption that the infection was limited by the development of immunity, various methods for reducing the immunological capacity of mice were investigated in an attempt to enhance the infection. The assumption was confirmed when it was demonstrated that enhanced infections with *Myco. leprae* could be obtained in mice following thymectomy and wholebody irradiation (with 900 r) as a means of reducing their immunological capacity.

General features of the infection in thymectomized irradiated mice inoculated with Myco. leprae

It has now been established (Rees, 1965*a*, 1965*b*; Rees and Weddell, 1968; Rees *et al.*, 1967) and confirmed (Gaugas, 1967; Shepard and Congdon, 1968) that when the immunological capacity of mice is reduced by thymectomy plus irradiation (with 900 r), *Myco. leprae* inoculated locally into the footpads or ears multiply more freely and yield 100 to 1000 times more bacilli per site than in normal animals (Fig. 3), and that in due course the infection spreads to other sites. Moreover, similarly treated mice become heavily infected in specific sites when inoculated intravenously with *Myco. leprae* (Rees and Weddell, 1968; Rees *et al.*, 1967). Although, in these animals, the generation time is not reduced, the bacilli continue to multiply for a longer period. The spread of infection, in locally inoculated animals, is also highly selective. The

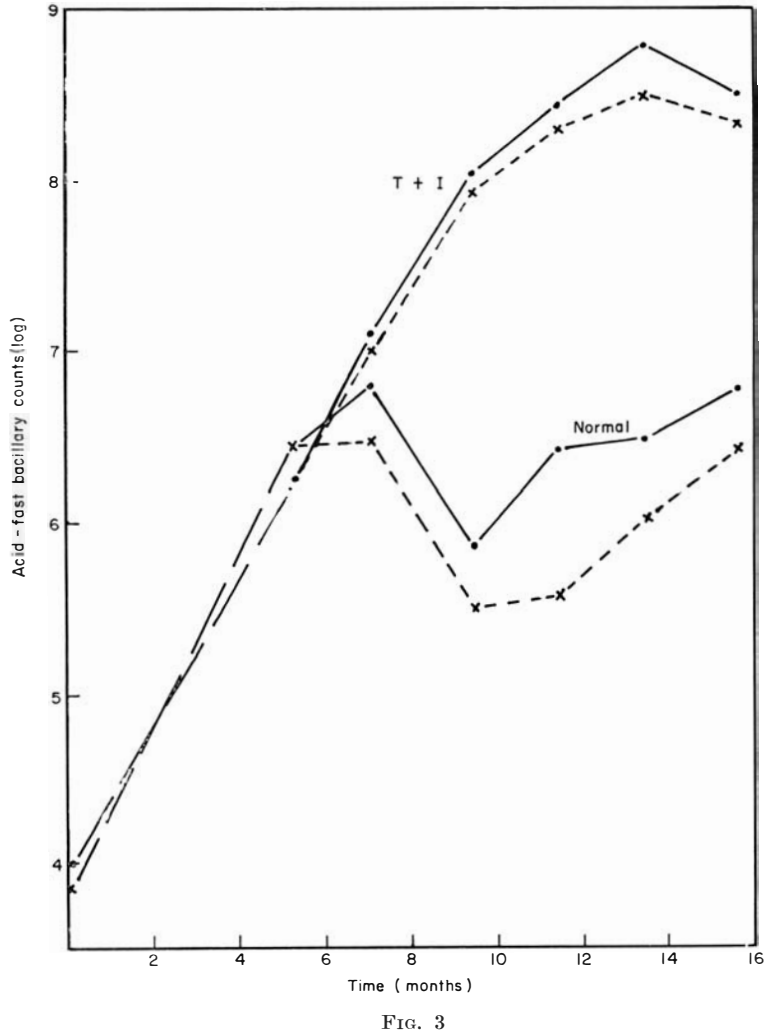

FIG. 3

Growth curves of *Myco. leprae* in the footpads of normal and thymectomized, irradiated (T+I; dose 900 r) mice inoculated with 10^4 bacilli (data taken, by permission, from Rees *et al.*, 1967). Continuous lines, total number of *Myco. leprae*; broken lines, number of viable *Myco. leprae*.

sites of predilection are in the skin of the ears, hind and fore paws, the tail and also the nose (Table 4).

TABLE 4

Localization and yield of *Myco. leprae* in a thymectomized, irradiated* mouse 19 months after the intravenous injection of 3×10^7 bacilli

Organ or tissue	*Estimated yield of bacilli* *Per site* ($\times 10^6$)	*Percentage of total yield*		*Degenerate bacilli* (%)
Footpads:				
Hind	1740	18		56
Fore	960	10		60
Total	2700	28	95	59
Ears	4800	49		50
Nose	1800	18		61
Muscle of leg	76	0.8		30
Muscle of body	160	1.6		31
Skin of tail	21	0.2		89
Skin of body	5	0.05		55
Liver	130	1.3		82
Spleen	53	5.0		87
Lung	10	0.1		70
Total	9755			

*Dosage: 900 r.

The same rigorously controlled criteria have been used to identify *Myco. leprae* in the enhanced infections that were used in normal animals and have included testing the sensitivity of the organisms to diaminodiphenylsulfone (Rees and Weddell, 1968; Rees *et al.*, 1967) and the production of lepromin (Draper, Rees and Waters, 1968).

In addition to the higher yields of bacilli from such animals it has been shown that later in the infection there is frequently nodular swelling of the footpads (Fig. 4) and the histology of the lesions replicates that seen in patients with lepromatous or borderline-type leprosy (Fig. 5) (Rees and Weddell, 1968; Rees *et al.*, 1967). Thus there is heavy infection of the peripheral nerves in the skin sites referred to (Fig. 6). Positive smears can be obtained from nasal swabs and the histological picture shows typical foam cells and degenerative changes in some of the infected peripheral nerves (Rees and Weddell, 1968). There is increasing evidence that these changes occur with the slow and partial recovery

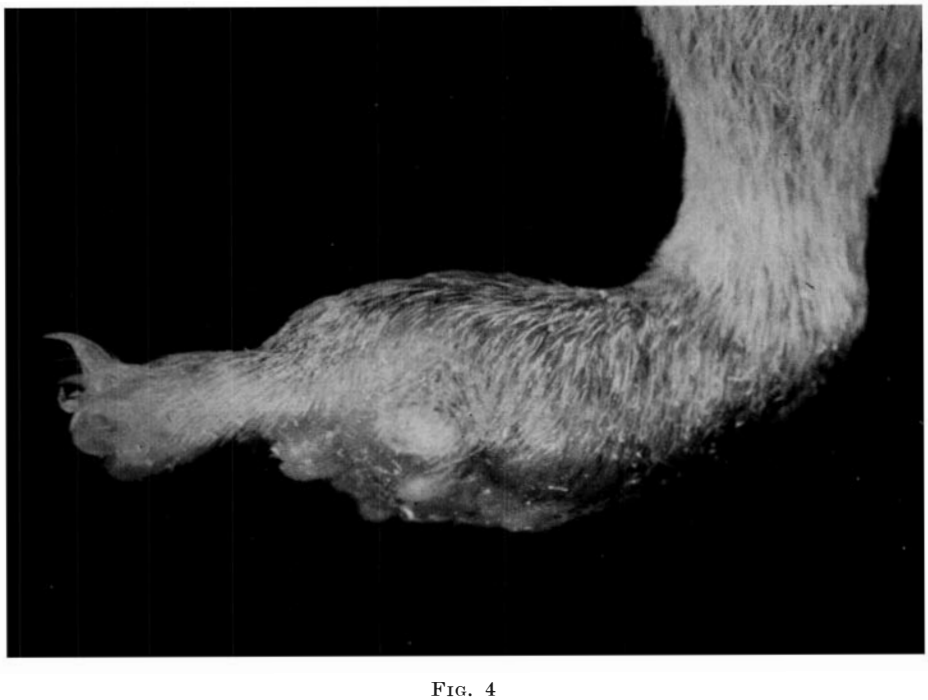

FIG. 4

Nodular swelling of hind footpad of a thymectomized, irradiated mouse inoculated locally with 10^4 *Myco. leprae* 9 months previously.

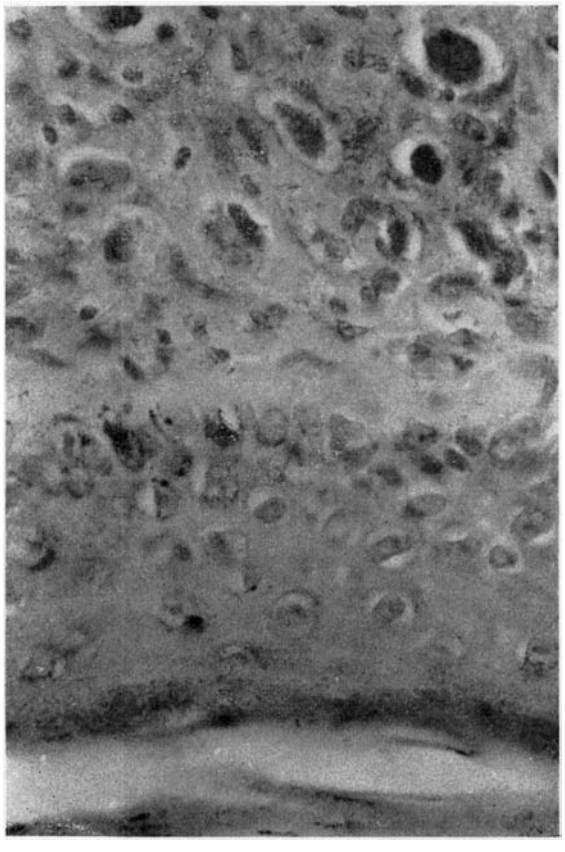

FIG. 5

Skin from the nodular, swollen footpad of a thymectomized, irradiated mouse inoculated 8.5 months previously with 10^8 *Myco. leprae*. Globi loaded with bacilli and foam (Virchow's cells) are seen in the dermis that is separated by a clear zone from the epidermis. These features replicate those seen in lepromatous leprosy in man. Stained: haematoxylin and cold carbol fuchsin. × 2160.

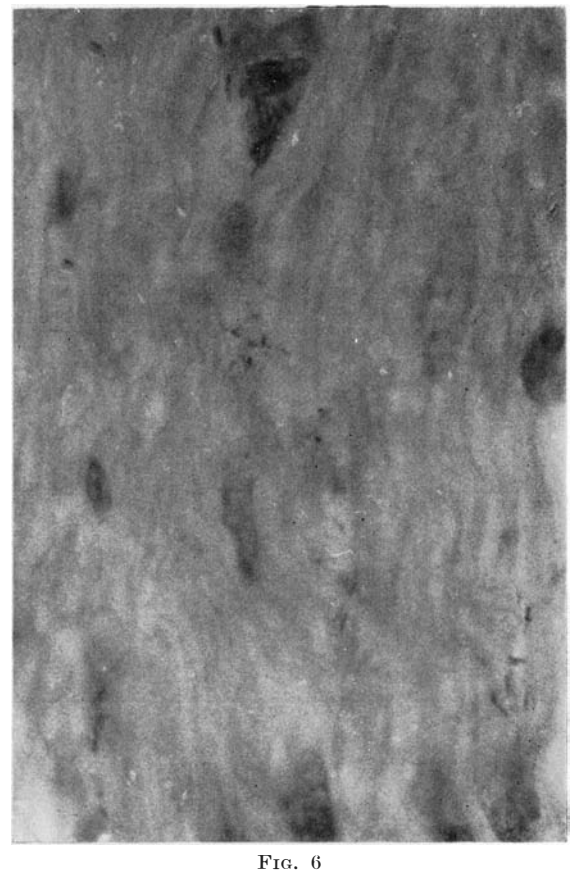

FIG. 6

Medial plantar nerve from the same mouse as in Fig. 5 to show Schwann cells infected with *Myco. leprae*. Stained: haematoxylin and cold carbol fuchsin. × 4500.

of the immunological capacity of the animals and can be enhanced in animals with established infections by the donation of immunologically competent syngeneic lymphoid cells from normal mice (Fig. 7) (Rees and Weddell, 1968).

FUTURE PROSPECTS

The successful transmission of human leprosy to animals (with the prospect of maintaining the infection indefinitely by serial passage) and the reproduction of some of the major characteristics of the human disease in laboratory animals by reducing their immunological capacity, provide, for the first time, a means of studying *Myco. leprae* in the laboratory.

Chemotherapeutic studies

The enhanced infection provides a more rapid method for screening new drugs against *Myco. leprae* and for applying the more quantitative and kinetic methods already developed in normal mice for studying the action of anti-leprosy drugs (Shepard, 1967*a*). In particular, the larger infections obtained in thymectomized, irradiated mice will provide a more sensitive method for determining and comparing the bacteriostatic, as well as the bactericidal,

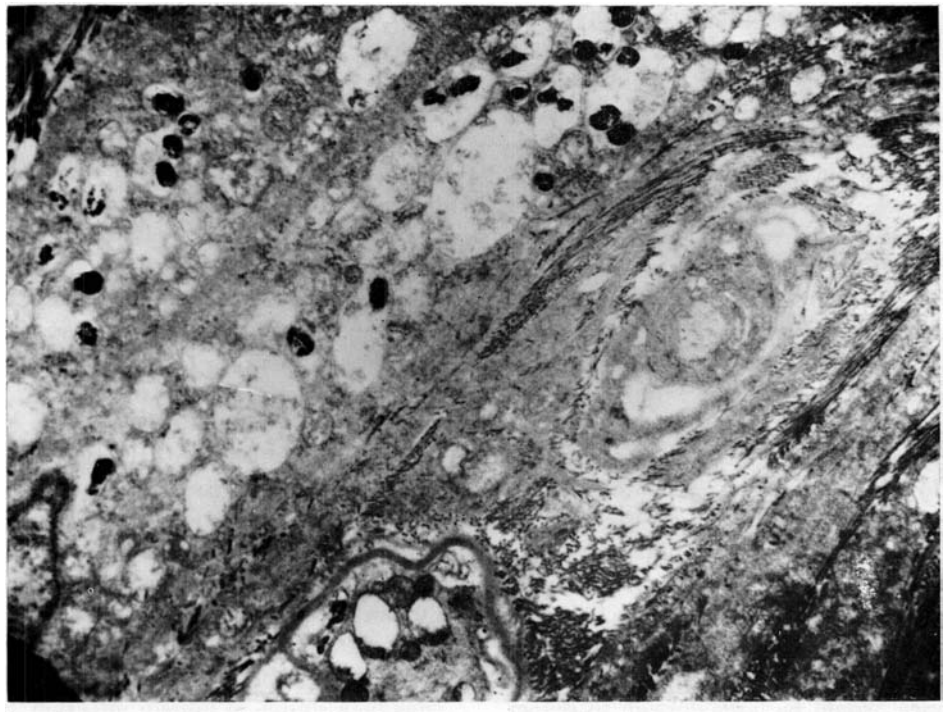

FIG. 7

Electron photomicrograph of dermis from ear of thymectomized, irradiated mouse inoculated with 10^7 *Myco. leprae* locally in both ears and footpads 15 months previously and given syngeneic lymphoid cells from a normal mouse 5 months before this preparation was made. (The degeneration of nerve axons, myelin rings, and Schwann cells in 2 myelinated nerve fibres can be seen.) × 31500

effects of drugs on *Myco. leprae*. Although the application of the MI to chemotherapeutic studies in man indicates that a very high proportion of bacilli in the skin are killed, by diaminodiphenylsulfone, for example, within a period of 3 to 6 months, there is strong clinical evidence that patients relapse unless diaminodiphenylsulfone treatment is maintained for several years (Quagliato *et al.*, 1961). One possible explanation is that there are specific sites in the body in which diaminodiphenylsulfone and other antileprosy drugs are ineffective, or less effective, and that such sites provide a source of viable organisms. There are suggestions from histological studies on human tissues that arrectores pili muscles and Schwann cells may still harbour healthy bacilli when those in surrounding tissues are very degenerate. The heavy and generalized infection which can be obtained in intravenously inoculated thymectomized, irradiated mice provides an ideal model for investigating these possibilities since the cellular pattern of infection exactly mimics that used in man, including parasitization of both muscle and Schwann cells. By treating such animals with antileprosy drugs, including drugs labelled with radiactive isotopes, it should be possible to determine both the distribution of the drugs and of degenerate and normal bacilli, at an intracellular level.

Emergence of drug-resistant strains of Myco. leprae

The footpad infection technique in normal and thymectomized, irradiated mice is the only method available at present for detecting the

emergence of drug-resistant strains of *Myco. leprae* and the method could now be used to survey the importance of drug resistance on a world-wide basis. Because of the increasing use of low-dose regimens of diaminodiphenylsulfone, there is obviously a danger of diaminodiphenylsulfone resistance emerging. Under field conditions, however, if such resistance emerged it might remain undetected by the routine clinical and bacteriological methods until it had reached serious proportions. Because diaminodiphenylsulfone is the standard form of treatment throughout the world, such an occurrence would be disastrous to the control of leprosy. Every effort should therefore be made now to devise suitable experimental models which might be used to predict the rate of emergence of diaminodiphenylsulfone-resistant strains of *Myco. leprae* in animals receiving decreasing doses of the drug. The bacterial populations in an established infection in intravenously inoculated, thymectomized, irradiated mice, would be large enough to detect resistance since such mice have a bacterial population of 10^{10} and drug-resistant mutants could be expected in a proportion of $1 : 10^7$.

Routes of infection

The routes of infection for leprosy in man are still unknown and the much more susceptible, thymectomized, irradiated mice provide an experimental model for investigating this important problem. The nose, upper respiratory tract, alimentary tract and skin are all routes of infection that should be studied. Moreover, because it is known that thymectomized, irradiated mice heavily infected with *Myco. leprae* excrete bacilli from their nasal mucosa (Rees and Weddell, 1968), they could be used as "open cases" to determine their infectiousness for highly susceptible but non-infected thymectomized, irradiated mice, housed in the same cages.

Sources of infection other than man

There has been much discussion on whether vectors, either insect or mammalian, could be involved in the transmission of leprosy from man to man and whether any domestic or wild animals can be infected with *Myco. leprae* and therefore be a source of bacilli to infect man. The successful transmission of human leprosy to normal or thymectomized, irradiated mice might be used in 2 ways to study these possibilities. The mouse footpad technique could provide, for the first time, a reliable method of identifying as *Myco. leprae* any non-cultivable, acid-fast bacilli isolated from potential vectors. On the other hand, and perhaps even more important, there is the observation that the intravenous inoculation of *Myco. leprae* into normal mice can produce, towards the end of their life, an infection of the nose and the paw skin. Because the inoculation of *Myco. leprae* into the footpads of rats results in a pattern of infection similar to that in mice, it is likely that both species are equally susceptible to the human leprosy bacillus. It is possible, therefore, that wild mice and rats in leprosy endemic areas could be infected with *Myco. leprae* and thus be a source of human infection. Since both rats and mice are present in large numbers in all epidemic and endemic leprosy areas throughout the world, it is suggested that this possibility should be investigated by sample surveying.

Application of enhanced infection for studying the pathogenesis of human leprosy

Leprosy in man presents a wide clinical spectrum ranging from the tuberculoid type, where there are few bacilli and the patient has a high degree of immunity, to the lepromatous form, where there are many bacilli and the patient has little or no resistance. Superimposed on these very variable clinical forms is the common feature that peripheral nerves are infected. *Myco. leprae* is the only species of mycobacterium known to infect nerves in either man or animals and the extent of damage to the infected nerves appears to depend on the immunological capacity of the host. Thus, in tuberculoid-type leprosy, infection of nerves results in the destruction of axons, whereas in lepromatous leprosy, the nerves can be heavily infected with bacilli without damage to the axons. The "target cell" for parasitization by

Myco. leprae within nerves is the Schwann cell. In addition to the variable clinical picture "reactional" episodes may occur and on these occasions the existing lesions, or new ones, present as sites of acute inflammation. These episodes are likely to have an immunological basis; certainly, an increase in the immunological capacity of the patient must play a major role in one type of reaction since it is followed by a shift in the clinical picture from the lepromatous towards the tuberculoid form of the disease. The term "reversal reaction" has been applied to such a shift.

The importance of nerve involvement in all forms of leprosy, together with the very wide range of clinical, bacteriological and histological forms, and the way in which each seems to be dependent upon fine differences in the immunological capacity of the patient, have been stressed in order to illustrate the complexity of leprosy in man. On account of the chronicity and complex nature of the disease in man, it is probable that the final elucidation of the pathogenesis of human leprosy will be achieved only if contributions are made from the experimental studies and such contributions can only be made if the human disease can be duplicated in experimental animals. This prerequisite seems likely to be achieved because already the inoculation of *Myco. leprae* into mice subjected to thymectomy and total body irradiation has reproduced completely the lepromatous type of disease seen in man (Rees and Weddell, 1968).

These 2 areas of immunological research in leprosy are, of course, importantly related. Mice have been shown to develop lepromatous-type infection when they are subjected to procedures (thymectomy and irradiation) that produce a profound and long-lasting immunological depression, and the immunological depression in lepromatous patients has been made clear by well-tried immunological procedures (*Int. J. Leprosy*, 1968). Fortunately, it is possible to increase the immunological capacity of treated mice at will (by the intraperitoneal inoculation of syngeneic lymphoid cells from normal mice) and already preliminary results of such manipulations show that the progressive form of lepromatous leprosy can be halted or that reversal reactions can be produced which result in a shift from the lepromatous form of the disease to the tuberculoid form seen in man (Rees and Weddell, 1968). Therefore, there is every reason to believe that these models can and should be developed in order to provide means to study the pathogenesis of the different forms of human leprosy, to study the etiology of nerve involvement and damage and to study the role of immunology in these processes.

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Mycobacterium leprae in the Striated Muscle of Patients with Leprosy*

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Biopsies of striated muscle from leprosy patients showed the presence of *Myco. leprae* in 16 out of 20 biopsies from lepromatous cases, 3 out of 4 borderline, and 5 out of 9 tuberculoid cases; in most cases the bacilli lay chiefly within muscle fibres. Bacillary counts performed on homogenates of 22 skin and muscle biopsies (13 lepromatous, 3 borderline and 6 tuberculoid) showed that the concentration of bacilli in lepromatous cases was from 100 to 1000 times greater in skin than in muscle, but that in this group of treated patients a higher proportion of muscle bacilli were solid staining. In tuberculoid leprosy the total number of bacilli may be greater in muscle than in skin. Muscle involvement in human leprosy may precede the appearance of skin lesions and could also play a significant part in the development of relapse and drug resistance.

INTRODUCTION

When mouse footpads are inoculated with *Mycobacterium leprae* (Shepard, 1960; Rees, 1964), the bacilli are placed in the hypodermis close to the intrinsic muscles; they are not injected into the muscles or into the dermis of the skin. Nevertheless, they enter muscle fibres remarkably quickly, being found within them after as short a time as one hour, but they are not found in the dermal nerves until 12 to 14 months later (Weddell *et al.*, 1970).

During the early months of active bacterial multiplication which follow such inoculations, there is local spread of *Myco. leprae* from muscle to muscle in the footpad. This probably takes place *via* blood vessels, for bacilli are also found in the endothelial cells of intramuscular blood capillaries. However, at this stage by far the greatest number of bacilli is found within muscle fibres, and there is little cellular response to their presence (Palmer *et al.*, 1965). Other mycobacteria, including *Myco. "Charbotier"*, *Myco. "Chatterjee"*, *Myco. lepraemurium*, *Myco. marianum*, *Myco. tuberculosis*, *Myco. ulcerans* and BCG, do not behave in this way when tested under the same experimental conditions (Rees and Weddell, 1968).

By contrast, striated muscle involvement in patients with leprosy has been recorded only on very few occasions (Ishihara, 1959; Convit *et al.*, 1960), and then as lepromatous infiltration between muscle fibres rather than bacilli within muscle cells. However, the observations in mice summarized in the foregoing paragraph have stimulated the investigation of both smooth and striated muscle as possible sites of election for bacillary multiplication in man. It is well known that the arrectores pilorum muscles often contain bacilli when the latter are scanty or absent elsewhere in hairy skins, and Harman (1968) has shown that bacilli are also present in other skin sites containing abundant smooth muscle, e.g. the foreskin, the dartos muscle, and the nipple. Moreover, he noted that in many cases a higher proportion of bacilli were solid-

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†This Laboratory is designated a WHO Regional Reference Centre for *Myco. leprae*.

staining within these muscle fibres than in the surrounding tissues, which suggested that smooth muscle was, to some extent, at least, a protected zone where bacilli could either survive or multiply more readily than elsewhere.

Job *et al.* (1969), also stimulated by the findings in mice inoculated with *Myco. leprae*, examined a series of biopsies of human striated muscle from 6 patients with lepromatous leprosy and reported the presence of bacilli within the muscle fibres of all of them.

The consistency with which striated muscle is found to be a site of predilection and multiplication for *Myco. leprae* in both normal and immunologically handicapped mice (Rees and Weddell, 1970), together with the observations of Harman (1968) and Job *et al.* (1969) in man, led us to predict that *Myco. leprae* might well be safe from cell-mediated immunological attacks provided they remained confined within muscle fibres and did not multiply sufficiently to destroy their host cells. If this hypothesis is correct, then we might expect to find bacilli in striated muscle not only in patients with lepromatous leprosy, but also in patients with other forms of the disease. To test this we undertook a comparative histological and bacteriological study of biopsies from striated muscle in treated and untreated patients with all types of leprosy, from lepromatous through borderline to tuberculoid. The results of this investigation are reported below.

MATERIALS AND METHODS

Biopsies were taken from Malay, Chinese, Indian and Gurkha patients. They were processed for examination as follows.

(1) Biopsies from 22 patients were fixed and processed for histological examination only. (2) Biopsies from 11 patients were subdivided. A small segment from each was fixed and processed for histological examination and the remainder was homogenized and used for bacterial counts. (3) Biopsies from 9 patients were homogenized and used for bacterial counts only.

TABLE 1

Type of leprosy, treatment and reactional status of patients studied

*Type of disease**	*Untreated*		*Treated*		*Total*
		ENL	*DDS resistant*	*ENL+ DDS resistant*	
Lepromatous (BL; LI; LL)	8	10	4	3	19
Borderline (BB)	4	–	–	–	1
Tuberculoid (BT; BT/TT)	10	–	–	–	1

*Classification according to Ridley & Jopling (1966); Ridley and Waters (1969).

The form of the disease from which the patients were suffering is set out in Table 1. Briefly, there were 8 untreated cases of lepromatous, 4 of borderline, and 10 of tuberculoid leprosy. Of the treated cases, 19 were lepromatous and of these, 10 had erythema nodosum leprosum (ENL).

The use of proportionately larger numbers of biopsies from patients with treated lepromatous leprosy with and without ENL, including patients who had developed sulphone-resistant infections, was deliberate because we thought that such patients might be particularly liable to harbour bacilli in their muscles despite anti-leprosy treatment.

The biopsies used only for histological examination were taken in the following way. A routine diagnostic skin biopsy was removed under local anaesthesia (2% procaine). Next, using a clean set of instruments, the incision was deepened to reach the underlying muscle and a discoid fragment about 0.5 cm in diameter was cleanly removed with fine scissors. The excised skin and muscle were fixed for immersion in buffered 10% formaldehyde solution (Richardson, 1960).

The material for bacillary counts was taken in the same way, but the amount removed was larger. The biopsies were placed on wet ice in a vacuum flask and despatched by air to the National Institute for Medical Research, London, where they were weighed, homogenized,

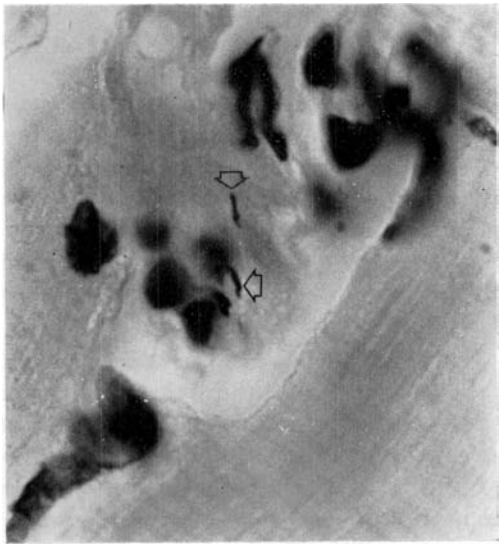

FIG. 1

Acid-fast bacilli in striated muscle (arrows). They lie close to an intramuscular capillary and are solidly stained; muscle fibres containing bacilli tend to be friable and this is no exception.

FIG. 2

Acid-fast bacillus in a muscle fibre in transverse section.

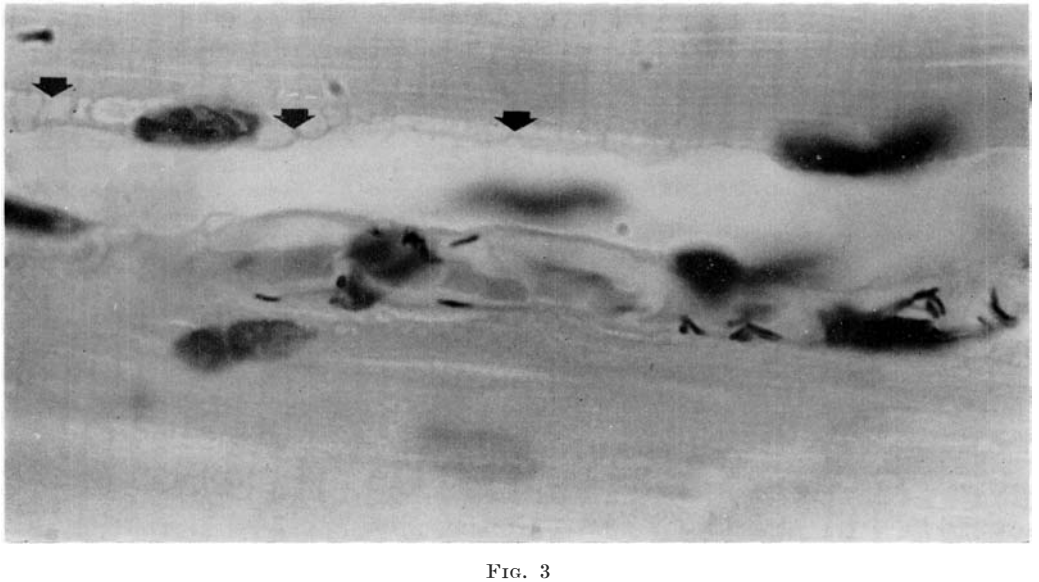

FIG. 3

Bacilli, chiefly in the endothelial cells of an intramuscular capillary. Note lipofuchsin granules at points of arrows.

and bacillary counts performed on the skin and muscle fractions separately by the method used for mouse footpad tissue (Rees, 1964). In order to increase the sensitivity of the technique, however, these tissues were homogenized in minimal volumes of fluid, not more than 2 ml being used for the skin and only 1 ml for the muscle. As a result, counts of as little as 3.4×10^3 bacilli per g tissue homogenate could be detected.

The muscle biopsies came from the triceps brachii or quadriceps femoris muscles in all but 3 cases; in these 3 they came from the deltoid, platysma, and frontalis muscles respectively.

In 3 cases, outside this series, we obtained biopsies from muscles in patients with lepromatous leprosy undergoing surgical operations. Two came from the internal oblique muscle during hernia repairs and one from the peroneus longus muscle during an orthopaedic operation. All these specimens were processed for examination by light microscopy as described above.

RESULTS

Histological observations

1. *Muscle.* A total of 33 muscle biopsies were taken from 32 patients (see Table 2). One patient suffering from ENL was biopsied twice, the biopsies being a year apart. Acid-fast bacilli were found in 11 out of 12 biopsies from patients with lepromatous leprosy, but not in reaction, and in 5 out of the 8 biopsies from patients with ENL. They were also found in 3 untreated cases with borderline leprosy, but not in the treated case, and finally in 5 of the 8 patients with tuberculoid leprosy, but none in the patient who had had treatment. Bacilli were, therefore, found in 24 of the 33 biopsies taken.

Bacilli were found within muscle fibres in 20 of the 24 biopsies in which they were present (Figs 1 and 2). In 4 of these 20 cases bacilli were also found in the endothelial cells lining blood vessels (Fig. 3), in macrophages scattered along the neurovascular bundles between groups of muscle fibres and extracellularly between muscle fibres. In 4 biopsies only were no bacilli found lying within muscle fibres; in one of these they were lying extracellularly in connective tissue, in 2 others in endothelial cells of muscle capillaries (Fig. 4) and in the remaining case large numbers of bacilli were seen in the Schwann and perineurial cells of an intramuscular nerve bundle as well as in macrophages and an endothelial cell of a muscle capillary.

TABLE 2
Summary of histological findings in all muscle biopsies

*Type of disease**	*No. of biopsies*		*Tissues in which acid-fast bacilli were found*				
	Total	*With AFB†*	*Muscle fibres*	*Blood vessel wall*	*Nerves*	*Macrophages in NVB‡*	*Extra cellular*
Lepromatous							
(a) Untreated	6	5	5	1	0	5	1
(b) Treated	4	4	3	2	0	0	0
(c) ENL	7	4	3	1	1	1	0
(d) DDS resistant No ENL	2	2	2	0	0	0	0
(e) DDS resistant ENL	1	1	0	1	0	0	0
Borderline							
Untreated	3	3	2	1	0	0	1
Treated	1	0	0	0	0	0	0
Tuberculoid							
Untreated BT	5	3	3	0	0	0	2
Untreated BT/TT	3	2	2	0	0	0	0
Treated BT		0	0	0	0	0	0

*Classification according to Ridley & Jopling (1966); Ridley and Waters (1969).
†Acid-fast bacilli.
‡Neuro-vascular bundles.

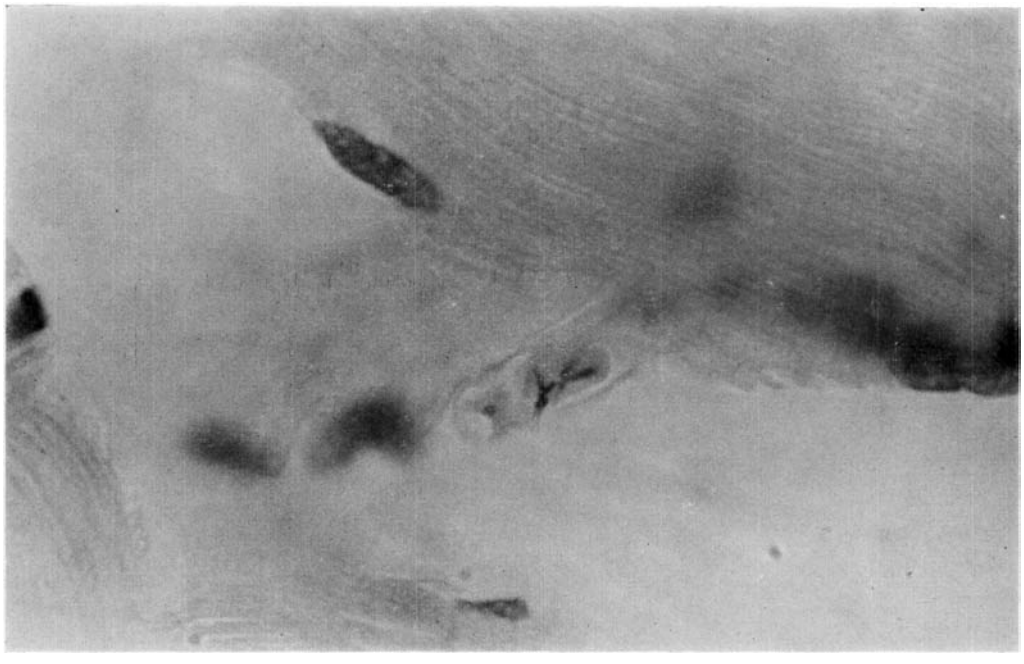

FIG. 4

Solidly stained bacilli in the endothelial lining of a blood capillary. The capillary has been torn from the muscle fibre and the bacilli are lying close to a segment of muscle which has come away with the capillary. The nuclei of the capillary lining cells are more darkly stained than the muscle fragment.

In all patients with either tuberculoid or borderline leprosy the intramuscular bacilli lay singly, and there were no inflammatory cells near the muscle fibres in which they were situated. This was not always so in biopsies from cases of lepromatous leprosy. Indeed, in one patient there were zones in which muscle fibres had been replaced by foam cells (Fig. 5), and in half the specimens there were well-defined areas where the muscle fibres had been so severely damaged that they were difficult to identify without examining the tissue under phase-contrast conditions. Macrophages were sometimes present in these zones, and in 2 cases bacilli were present in these sites in higher concentration than elsewhere in the specimen and were present in micro-colonies as well as singly (Fig. 6). There were never many acid-fast bacilli in these muscle biopsies; in 10 of the 24 positive specimens, for example, only one such bacillus was found after several hours of searching, but in very few of our cases were inflammatory cells present.

In undamaged muscle fibres in 17 out of 20 biopsies from patients with lepromatous leprosy, in one out of 4 biopsies from patients with borderline leprosy, and in 9 out of the 11 biopsies from patients with tuberculoid leprosy there were variable numbers of weakly stained acid-fast granules (lipofuchsin). They sometimes resembled irregularly stained bacilli and some may have originated from them. However, the majority of the granules were paler, more globular and quite unlike fragmented bacilli seen in other tissues. The granules lay just beneath the sarcolemma and mostly close to nuclei, which were often irregular in outline and less basophilic than their neighbours (see Fig. 3). In all such cases there were zones in which muscle fibres contained nuclei which were aggregated into clumps or strung together in chains (Fig. 7). There was no evidence of in-

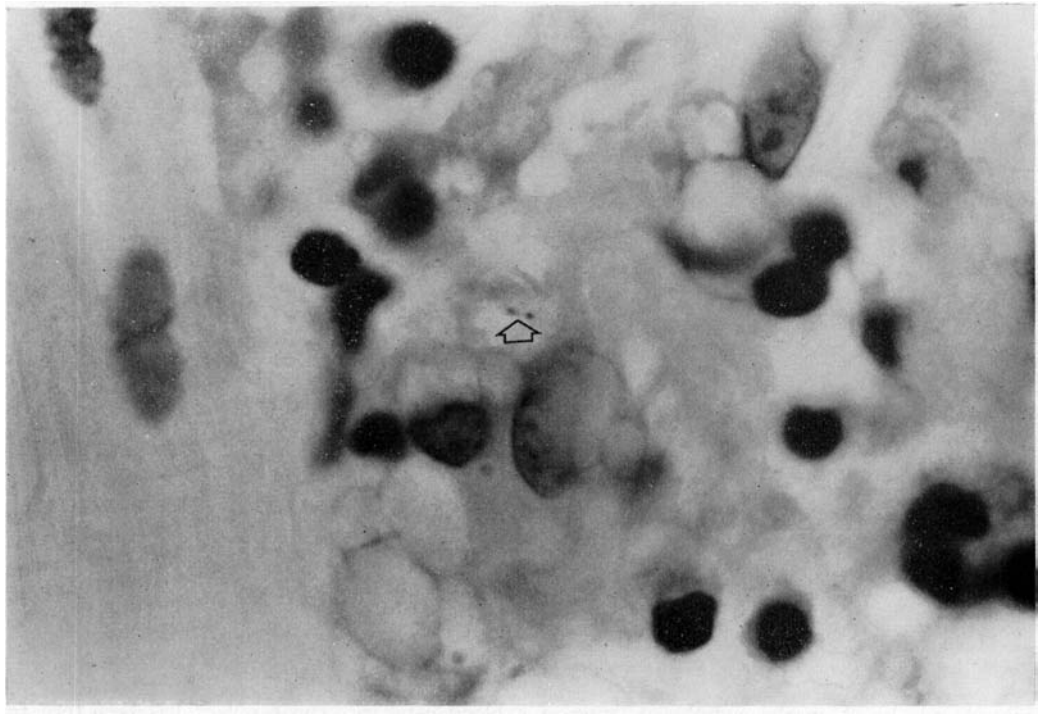

FIG. 5

Foam cells in a neurovascular bundle between muscle fibres. Note the lymphocytes and the characteristic dumb-bell appearance of the irregularly stained bacillus (arrows). Bacilli close to lymphocytes commonly assume this form (Rees and Weddell, 1968).

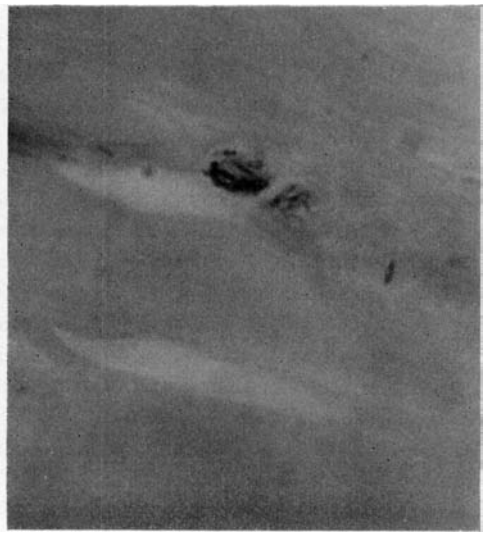

FIG. 6

Microcolony of solidly stained bacilli in a muscle fibre. Note the absence of inflammatory cells.

flammation surrounding any of the muscle biopsies in our series.

2. *Muscle biopsies from patients taken during surgical operations.* These all came from patients with lepromatous leprosy receiving antileprosy treatment, none of whom had any clinical signs of muscle involvement. In the 2 biopsies which were taken from internal oblique muscles of the abdomen the findings were comparable with those in our series from the quadriceps femoris and triceps brachii muscles. There were single isolated solid-staining bacilli in muscle fibres and in one of the cases there were micro-colonies of bacilli. However, in neither case was there any inflammatory cells present among the muscle fibres. The biopsy from the peroneus longus muscle of the leg, however, was full of bacilli. In focal zones around blood vessels there was a great deal of muscle destruction (Fig. 8). Fibres had been completely or partially des-

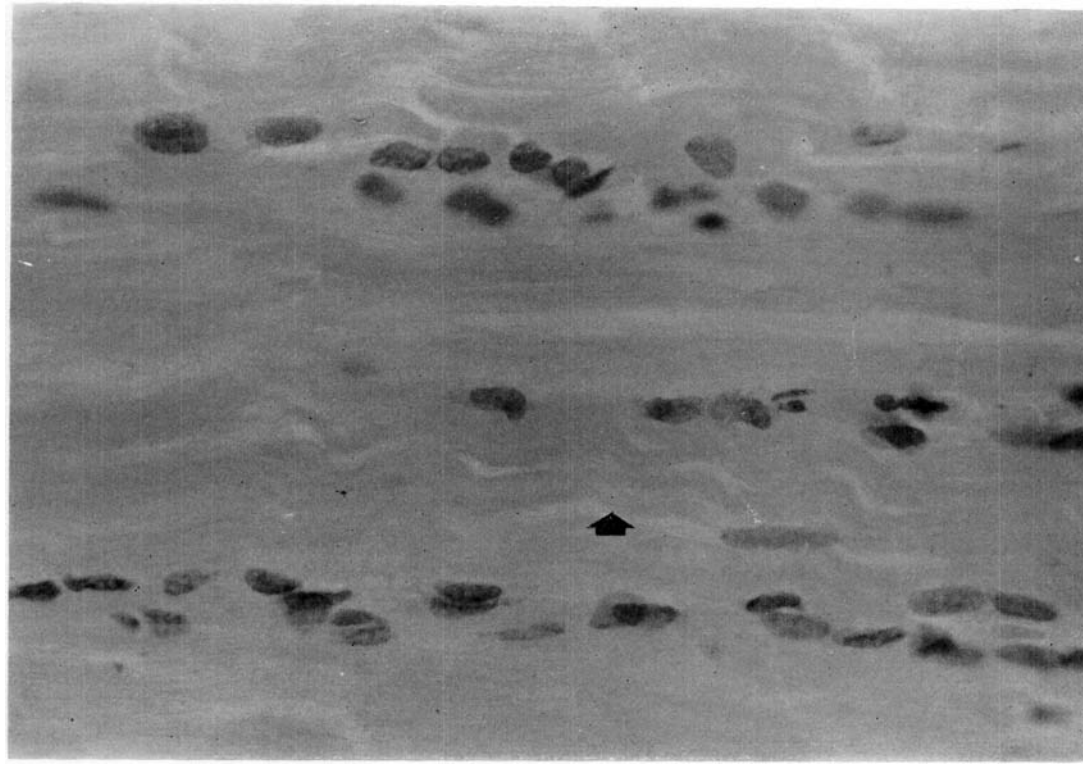

FIG. 7
Bunched nuclei in muscle fibres lying between bundles of collagen fibres which have a characteristic wavy appearance (arrow). They have undoubtedly replaced destroyed muscle fibres.

TABLE 3
Presence of acid-fast bacilli; comparison of skin and muscle histology

Type of leprosy	*Presence of acid-fast bacilli*			
	Muscle		*Skin*	
	Positive	*Negative*	*Positive*	*Negative*
Lepromatous				
(BL; LI; LL)	10	3	10	3
ENL	4	2	4	2
No ENL	6	1	6	1
Borderline				
(BB)	3	0	2	1
Tuberculoid				
(BT; BT/TT)	5	0	2	3

*Classification according to Ridley and Jopling (1966) and Ridley and Waters (1969).

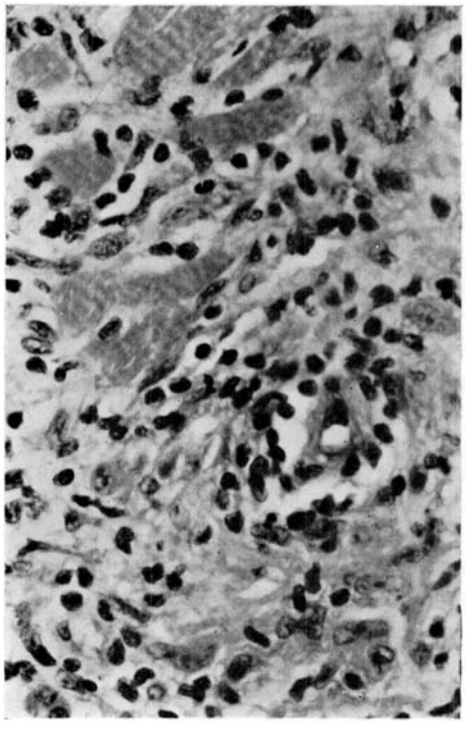

FIG. 8
Muscle fibre destruction around a blood vessel "cuffed" with lymphocytes

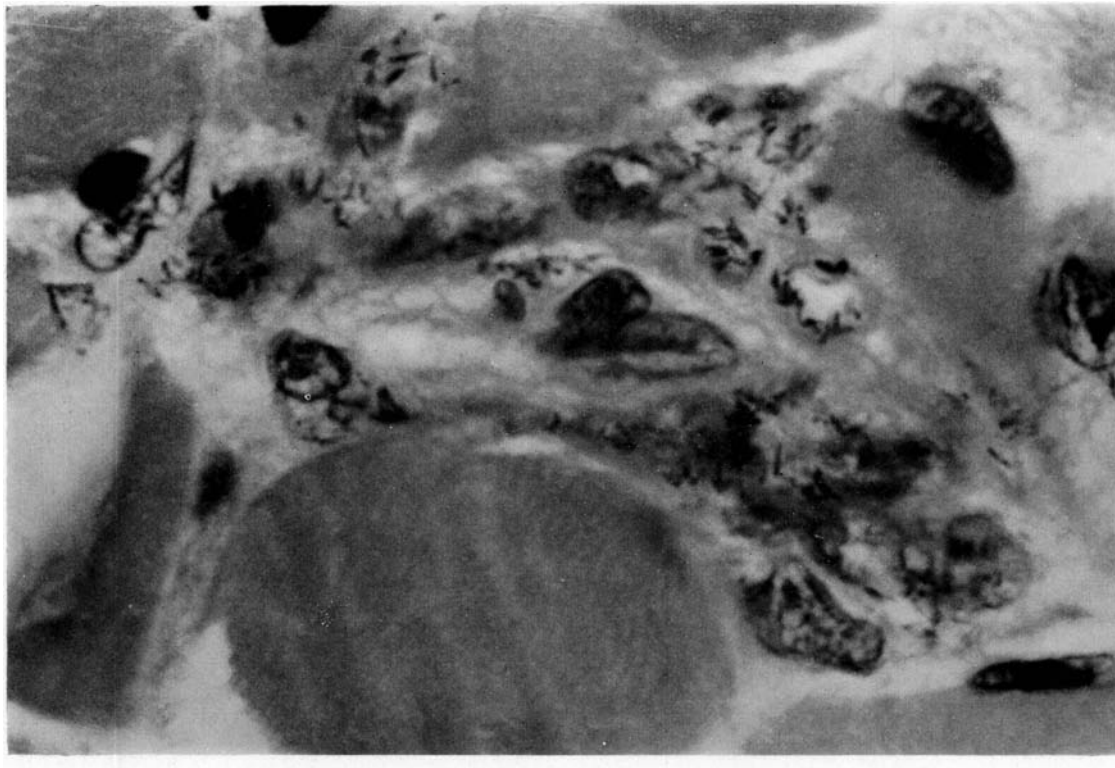

FIG. 9

Muscle fibres undergoing exhaustion and destruction due to the number of bacilli they are nourishing. Note the absence of inflammatory cells. The nuclei seen have all the characteristics of muscle cell nuclei and the bacilli are all solidly stained. A neighbouring muscle cell is quite unaffected.

troyed, a number of macrophages containing irregularly stained bacilli and lymphocytes were present, but most of the bacilli in the muscle remnants were solidly stained. In other regions of the section there were muscle bundles entirely free from infection and it was noteworthy that some muscle fibres in the badly affected zones were also free from infection and looked entirely normal (Fig. 9).

3. *Skin.* When taking muscle biopsies through skin incisions care was taken to see whether, macroscopically, there had been any evidence of spread of the infection from the hypodermis into the deep fascia covering the muscle. In no case from which we took biopsies was there any evidence of such spread.

The skin biopsies were examined histologically and in all cases confirmed the clinical diagnosis. They were next compared with the muscle biopsies, section by section, particularly in respect of the presence or absence of bacilli; the results are set out in Table 3. In some of our cases only muscle biopsies were taken for histological examination, so that this comparison is available for only a limited number of cases. However, as far as they go it is clear that among the cases with lepromatous leprosy, there is a close parallel between skin and muscle with respect to the presence of bacilli. In borderline leprosy, bacilli were found in muscle in 3 cases, but in one of these there was none in the skin. However, the most striking observation is the presence of solid-staining bacilli in muscle in so many cases of tuberculoid leprosy and their relative rarity in the skin of these same patients.

Clearly, histology is not the method of choice

to settle a quantitative issue of this kind. Because of this it was decided to make direct bacterial counts from tissue homogenates and in particular to try to express the results in terms of morphological indices.

4. *Bacilli in skin and muscle homogenates.* Twenty-two combined skin and muscle biopsies were taken from 18 patients for homogenization. Bacilli were found in 15 of the muscle biopsies and in 19 of the skin biopsies. Moreover, as can be seen in Table 4, they were found in muscle

TABLE 4

Presence of acid-fast bacilli in homogenates of skin and muscle biopsies from 18 patients

*Type of leprosy**	*No. of patients*	*No. of biopsies*	*No. of biopsy-homogenates with acid-fast bacilli*	
			Muscle	*Skin*
Lepromatous (LL; LI; BL)	10	13	11	13
Borderline (BB)	3	3	2	3
Tuberculoid (BT; BT/TT)	5	6	2	3

*Classification is according to Ridley and Jopling (1966) and Ridley and Waters (1969).

from patients with borderline and tuberculoid as well as lepromatous leprosy. In Tables 5 and 6 we have set out our observations in greater detail. This was necessary to guard against the possibility that the bacilli found in the muscle homogenates were merely contaminants from the skin. We can be certain that the majority were not, firstly because of our histological findings in the cases from which small segments were available for this purpose; and secondly, though the evidence in the other cases is indirect it is persuasive. It rests on the fact that the morphological indices in muscle and in skin are in sharp contrast and this would be most unlikely if the muscle bacilli had been contaminants from the skin.

Figures for the bacillary counts in the patients with lepromatous leprosy are shown in Table 5. There were 100 to 1000 times more bacilli in skin than in muscle, but there was a higher proportion of solid-staining organisms in the muscle, particularly in patients who were suffering from ENL. The figures for patients with borderline and tuberculoid leprosy are shown in Table 6. The counts were considerably lower, but the same trend is evident.

DISCUSSION

The mouse has been shown to provide a very accurate model for studying the pathogenesis of human leprosy (Rees and Weddell, 1968; Rees and Weddell, 1970). There was one noticeable difference, however—in mice the bacillation and subsequent infection of striated muscle fibres was a prominent feature, whereas this is not thought to be the case in man. The present comparative studies were undertaken because of the overwhelming evidence that striated muscle was an important site of multiplication of *Myco. leprae* in experimental leprosy. Thus, when muscle biopsies from patients with lepromatous leprosy were found to contain acid-fast bacilli, a series of patients with the full spectrum of leprosy was investigated. It is now clear that, in this respect also, the mouse provides a model for the disease in man, and that muscle infection plays a part in the pathogenesis of human leprosy.

All the muscle biopsies were taken through a skin lesion, but the dermal granuloma never extended through the deep fascia. Moreover, the different venous drainage of skin and of muscle makes it impossible for bacilli to pass directly from one to the other. Thus the presence of acid-fast bacilli in both skin and muscle is clear indication of systemic spread. Similarly, although the muscle biopsies were taken from zones of muscle immediately beneath skin lesions this does not mean that these sites are the only ones likely to harbour bacilli.

Some interesting and important points emerge from these studies:

(1) In most lepromatous cases, the concentration of bacilli in muscle was 100- to 1000-fold less than in skin; but the proportion of solid-staining organisms was often 10 or more times greater. This trend was particularly striking in

TABLE 5

Number of acid-fast bacilli (AFB) in homogenates of muscle and skin from 10 previously treated patients with lepromatous leprosy

Case No.	*Treatment*		*Clinical status*	*Muscle*		*Skin*		*Site of bacilli histologically in muscle*
	Drug	*Duration (year)*		*AFB/g tissue*	*Solid staining AFB (%)*	*AFB/g tissue*	*Solid staining AFB (%)*	
(A) (*Histology incl.*)								
9055	DDS	21	DDS resistant; ENL	1.6×10^{6}	18	$1.0\%10^{8}$	2	Nerves; blood vessel walls.
16004	DDS	1.5	ENL	1.8×10^{6}	28	1.3×10^{9}	2	Muscle, neurovascular bundles.
16004 2nd biopsy	DDS	2.5	ENL	Nil	—	2.4×10^{6}	2	None seen.
16030	DDS	2.5	ENL	6.6×10^{4}	2/5 (40)	1.8×10^{6}	1	Muscle.
16107	DDS	0.75	No ENL	1.6×10^{5}	1/9 (11)	5.2×10^{7}	0	Muscle.
16147	DDS	16	DDS resistant	1.3×10^{6}	32	1.3×10^{9}	13	Muscle, blood vessel walls.
(B) (*No Histology*)								
9302	—	Nil for 7 yrs	No ENL	1.2×10^{5}	2/4 (50)	8.1×10^{7}	25	
10607	DDS	18	ENL; DDS resistant	Nil	—	1.8×10^{8}	1	
10835	DDS	17	DDS resistant	5.4×10^{4}	2/8 (25)	9.5×10^{8}	4	
16011	DDS	1.5	ENL	2.1×10^{4}	0/2 (0)	1.7×10^{7}	1	
16217	DDS	10	DDS resistant	4.6×10^{5}	5/17 (30)	1.0×10^{8}	12	
9055 2nd biopsy	DDS	22	DDS resistant	7.1×10^{5}	19	5.4×10^{7}	5	
16147 2nd biopsy	B 663	16.5	DDS resistant	1.1×10^{5}	2/5 (40)	2.1×10^{10}	0	

TABLE 6

Number of acid-fast bacilli (AFB) in homogenates of muscle and skin from 8 previously untreated patients with borderline and tuberculoid leprosy

Case No.	*Type of leprosy**	*Muscle*		*Skin*		*Site of bacilli histologically in muscle*
		AFB/g tissue	*Solid staining AFB (%)*	*AFB/g tissue*	*Solid staining AFB (%)*	
(A) (*Histology incl.*)						
16159†	BT/TT	2×10^{5}	3/4 (75)	0 ($<3.6\times10^{5}$)	—	None seen.
16162	BB	0 ($<7.7\times10^{3}$)	—	4.0×10^{5}	1/17 (6)	Muscle; loose connective tissue.
16164	BT/TT	0 ($<10^{4}$)	—	0 ($<10^{4}$)	—	Muscle.
16173	BT	0 ($<1.7\times10^{5}$)	—	2.1×10^{5}	1/2 (50)	Muscle.
16175	BB	8.9×10^{4}	1/3 (33)	3.0×10^{7}	0	Blood vessel wall.
16253	BT/TT	8.4×10^{3}	2/4 (50)	0 ($<1.4\times10^{4}$)	—	Loose connective tissue.
(B) (*No Histology*)						
16155	BT	0 ($<10^{4}$)	—	1.2×10^{6}	0/13 (0)	
16163	BB	1.5×10^{4}	0/3 (0)	9.9×10^{7}	6	
16173‡ 2nd biopsy	BT	0 ($<1.7\times10^{5}$)	—	2.1×10^{5}	1/2 (50)	

*Classification is according to Ridley and Jopling (1966) and Ridley and Waters (1969).

†This biopsy was made after 3 weeks' treatment with DDS.

‡Repeat biopsies of muscle after 3 and 4 months' treatment with DDS both gave negative counts.

patients who had received previous treatment, and makes it clear that the muscle bacilli were not contaminants from the skin. This ob-observation suggests that bacilli can survive and multiply in striated muscle more readily than in the skin, and that in this site they are less liable to attack, either by the normal defence mechanisms of the body or by drugs. Certainly there was a striking absence of inflammatory cells in relation to the muscle bacilli in almost all our biopsies from human muscle. This finding closely matches the absence of inflammatory response in the early and late stages of the muscle infection in the mouse footpad. However, in some cases of treated lepromatous leprosy there is an inflammatory response in human peripheral limb muscles. The process is symptomless and closely matches the process seen at particular periods in the footpad infection in mice (Weddell *et al.*, 1970). It is interesting that, as in the mouse, a few individual muscle fibres in a bundle are spared and some whole bundles escape. This suggests that *Myco. leprae* multiply more freely in some muscle fibres than others, a point already emphasized by Esiri (1969).

It is also of interest that as long as the muscle fibres are intact the bacilli within them are solidly stained, but once they get into macrophages and in association with lymphocytes they degenerate. A few degenerate bacilli are also found in completely exhausted muscle fibres in the absence of lymphocytes. It is perhaps of more than passing interest that the most heavily infected muscle from man which we have yet seen came from the leg rather than the thigh, arm, or face. It suggests that the more peripheral the infection is, the more the bacilli are able to multiply. This is certainly the case in mice where the footpad musculature is more heavily infected than the rest of the limb musculature, after infection by the intravenous route.

Our observation, then, strongly suggests that muscle offers a protected site where bacilli can lodge and multiply in the early stages of infection, where they could persist after an apparent cure, and from which they might spread to cause a relapse when treatment is stopped. It is also possible that such bacillary reservoirs are related to the development of drug resistance, and they may even play some part in the aetiology of ENL.

Just how *Myco. leprae* enter target cells, i.e. muscle fibres and the perineurial and Schwann cells of nerve fibres, remains a mystery. However, one of the more interesting observations in this series of patients was the presence of so many solidly stained bacilli in the endothelial lining cells of intramuscular capillaries, and this in the complete absence of inflammatory cells. Moreover, bacilli were found in the capillary endothelium of muscle biopsies from patients with tuberculoid as well as from patients with lepromatous leprosy.

It has been found both in mice and in patients, that intramuscular capillaries with bacilli in their wall tend to be more than usually adherent to the muscle fibres in which they are virtually embedded. For man this is illustrated in Figs 3 and 4. It appears, therefore, that capillaries in target organs probably have endothelial lining cells to which circulating *Myco. leprae* readily adhere and subsequently enter.

It is highly significant that bacilli were seen histologically in 5 of 7 biopsies from patients with tuberculoid (BT or TT) leprosy. They were few in number, and it often required 2 or more hours' searching of serial sections before a single bacillus was found, but it was striking that in most cases they were solid-staining, and lay within muscle fibres and capillary walls, neither of which had any inflammatory cells near them. The number of bacilli found in homogenates of skin and muscle from such cases suggests that they are present in comparable concentrations in skin lesions and in striated muscle, but as the bulk of the body musculature is in the order of 100 times greater than that of the skin lesions in a case of tuberculoid leprosy, a greater number of bacilli may well lie within striated muscle.

The significance of these findings is heightened by the small samples of muscle which were examined. They were taken from random sites in

patients who had no symptoms or signs of a muscle disorder, and the weight of each homogenized muscle biopsy was in the order of 0.25 g. The specimens examined histologically were smaller still and only a fraction of a total biopsy could be searched for bacilli in a few hours. Nevertheless, at least one bacillus was seen histologically in 5 out of 8 muscle biopsies from patients with untreated tuberculoid leprosy. In other words, bacilli were commonly found on examining a few milligrams of randomly selected striated muscle taken from patients with tuberculoid leprosy. In one case, bacilli were even found on 2 occasions by the less sensitive but quantitative technique of homogenization. The distribution of bacilliin muscle is uneven; this has been noted in single biopsies and also demonstrated most strikingly in the first biopsy from case 16004, which had the highest count of all in the homogenized muscle, but in which only a single bacillus was found histologically. Nevertheless, muscle bacillation may well play a significant part in the pathogenesis of human leprosy.

These studies were initiated by the finding that when mice were infected with *Myco. leprae*, striated muscle was the first tissue to be affected. The same may be true of human leprosy and our findings are compatible with this hypothesis. That is to say, it is possible that when leprosy becomes clinically apparent and skin lesions are visible, bacilli have previously been multiplying within striated muscle. Further studies, however, including biopsy of muscle from leprosy contacts and footpad inoculation to confirm that these acid-fast bacilli are indeed *Myco. leprae*, will be required to elucidate this aspect of the pathogenesis of human leprosy.

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We are most grateful to our colleague, Dr. M. F. R. Waters, of the Leprosy Research Unit, Sungei Buloh Leprosarium, Malaysia, who provided many biopsies, and to Dr. D. S. Ridley of the Hospital for Tropical Diseases, London, for histological classification of the patients. The Leprosy Research Unit, Sungei Buloh, is jointly administered by the British Medical Research Council and the Malaysian Ministry of Health.

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Summary of the Results of a Search of the Skin Surface for *Mycobacterium leprae*[*]

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The surface of the skin of 24 patients with active lepromatous leprosy was searched for the presence of *Myco. leprae* by the method of taking composite skin contact smears (C.S.C.S.) as described in the author's previous paper in *Leprosy Review* (1970) **41**, 31; the patients included 11 of those previously examined by this method. The total area searched was 813 sq. cm (a page of *Leprosy Review* is approximately 450 sq. cm in area). The results are shown in Table 1.

TABLE 1

Region	*Area*	*No. of AFB*
Face	505 sq. cm	25
Breasts, thighs, arms and back	308 sq. cm	3
Total	813 sq. cm	28

COMMENTS

In order to search the total area of 813 sq. cm 86 C.S.C.S. were used. This took about 50 hours of microscope work. It will be noted that, of this area, 505 sq. cm were face skin, in which there are probably more sweat and sebaceous glands than anywhere else in the body. Because *Myco. leprae* are sometimes seen in these glands, it has been supposed that they emerge in great numbers on to the surface of the skin—many of them being in "live" or active form. However, only 25 acid-fast bacilli (AFB) in this area of face skin were found. These were all present in 4 of the patients, all of whom had infected nasal mucus secretion. In addition, one of them had a small discharging sore on the border of one ear; a smear from the discharge showed that it was heavily loaded with *Myco. leprae*, mostly in solid-rod form. A C.S.C.S. compiled from both sides and the edge of the enlarged lobe of this ear showed 5 solid staining bacilli. When I took the smear from this ear the sore was not discharging, but was sealed off by a hard serous crust which had to be removed to make the smear (of the discharge). Thus it would not be unreasonable to suppose that these 25 bacilli had found their way on to the skin surface from the infected nasal secretion present in each of these 4 cases, and from the discharging sore. These 4 patients accounted for 85 of the 505 sq. cm of face skin examined. Thus there remained 420 sq. cm of face skin from which *no* bacilli were picked up by the C.S.C.S. method. The 3 remaining rather doubtful acid-fast organisms are referred to in Case 8 of the author's previous paper (*Lepr. Rev.* (1970) **41**, 31).

DISCUSSION

The belief that *Myco. leprae* are discharged on to the surface of the skin from sweat and sebaceous glands of lepromatous skin appears to be incompatible with these findings, especially when it is remembered that the skin of the face probably contains many more sweat and sebaceous glands than anywhere else on the surface of the body. If the finding of these organisms in sweat and sebaceous glands or in the cells of the epidermis of lepromatous skin has given rise to the belief that "innumerable" *Myco. leprae*—many of them assumed to be "live"—are emerging on to the skin surface, the following question arises: Why does the C.S.C.S. method fail to pick them up?; although this method does *not* fail to pick up bacilli:

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(1) shed on to the skin from nearby discharging sores; or (2) which have found their way on to the skin of the face of patients whose nasal mucus secretion is infected with *Myco. leprae*; or (3) organisms which were placed on the skin (for control testing of the C.S.C.S. method) by smearing on to the skin: (a) infected nasal mucus secretion, or (b) infected tissue "juice" from skin slit scrapings?

CONCLUSIONS

From this extended search the following conclusions can be made:

(1) *Myco. leprae* do not emerge from intact lepromatous skin.

(2) Skin to skin transmission of the organism is therefore unlikely to occur. Thus, by the process of elimination, the most likely mode of transmission is by ingestion or inhalation. The former has been proved by finding *Myco. leprae* in the mother's milk, and in the lining cells and lumina of the milk ducts of lactating women with active and untreated lepromatous leprosy. (See references in the author's previous paper, *Lepr. Rev.* (1970) **41**, 31.)

PREVENTION

In the prevention of the transmission of leprosy special attention should be paid to the following points:

(1) The nasal mucus secretion of a patient with active lepromatous leprosy (especially the untreated case) should *always* be examined for the presence of *Myco. leprae* as this is the index of the patient's infectivity. In this connection one must be specially watchful for patients with the non-apparent lepromatous type of the disease, who could otherwise pass unnoticed and yet have highly infectious nasal mucus secretion. (See Case 9 in the author's previous paper.)

(2) Instruction should *always* be given to a patient with infected nasal mucus secretion on how to dispose of his nose-blowings, and also his sputum, in a hygienic manner.

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Alterations in Sweat Response in Skin Lesions of Leprosy

A Dermometric Study*

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Sweat response and tactile sensibility has been studied by the use of dermometry and strength of tactile stimulus in the skin lesions of 45 leprosy patients. Tuberculoid lesions showed severe impairment to sweating, while the majority of lepromatous macules showed a fairly normal sweat response. The sweat response in borderline leprosy appeared variable. There was a close correlation between alteration in sweat response and impairment of tactile sensibility in these lesions.

Anhidrosis as a clinical manifestation of leprosy has been well recognized and adequately documented (Duhring, 1877; Jeanselme and Giraudeau, 1931; Degotte, 1942). It is characterized clinically by the absence of visible sweating on the affected skin in the presence of an appropriate stimulus and environment. A study of anhidrosis therefore immediately leads to the problems of (1) stimulating the sweat glands, and (2) estimating the degree of sweat response. The sweat glands may be stimulated by cholenergic drugs. They may also be stimulated to produce thermoregulatory sweating by elevation of body temperature. The sweat response may be judged by direct visualization (Kahn and Rothman, 1942), by various colorimetric procedures (Minor, 1927; Gutmann, 1940; Oden and Holstein, 1954; Karat *et al.*, 1969) and by measurement of electrical skin resistance (Richter and Katz, 1943).

Muir (1938) applied the pilocarpine test and Arnold (1944) the mecholyl test to study the sweat response in leprosy. Wade (1954) in an editorial entitled "Neglected Electrical Testing" records some observations made by Suskind who, using a neurodermometer on 9 patients, found a neat correlation between loss of sweat response and a clear-cut measurable rise in electrical skin resistance. These observations of changes in sweat response have often been used to record sensory changes in denervated skin (Moberg, 1958).

The purpose of this paper is to present the technique of dermometry as used to study the sweat response in skin lesions in various types of leprosy. It also attempts to find a correlation, if any, between sweat response and tactile sensibility in these areas of skin.

NEUROHISTOLOGY OF SKIN LESIONS OF LEPROSY

The neurohistology of skin lesions of leprosy has been studied and reported by Decoud (1948), Gass and Balasubrahmanyan (1954) and more recently by Dastur (1955) and Weddell *et al.*, (1964). Dastur considers the density of innervation greatest in "normal" skin, rather less dense in skin from "lepromatous" lesions and least dense in skin from "neural" lesions. He states that "in anaesthetic patches, no nerves were seen and it was significant that in such specimens the cells of sweat and sebaceous glands as well as smooth muscle fibres could no longer be identified".

Weddell maintains that in established untreated tuberculoid lesions the tissues of the dermis are grossly disorganized but that the only structures which appear to be completely destroyed are the neural elements. He remarks that in established untreated lepromatous lesions, there is, as far as can be judged, a full

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complement of axons. He describes the neurohistological picture in these lesions as that of ischaemia with a minimal amount of nerve destruction. In borderline leprosy, he suggests, the pictures seen form a series between those in tuberculoid lesions and those in lepromatous lesions; those in the borderline to tuberculoid spectrum show a greater tendency towards destruction of neural elements, while those in the borderline to lepromatous spectrum show less damage to axis-cylinders and more of the changes which are seen following ischaemia.

MATERIAL

In this study, in which 45 leprosy patients took part, the series included 10 cases of tuberculoid leprosy, 10 of lepromatous leprosy, 20 of borderline leprosy (of which 10 were borderline to tuberculoid (BT) and 10 were borderline to leproma (BL)), and 5 cases of indeterminate leprosy. Since interpretation of the results requires experience with normal individuals under similar experimental conditions, 15 normal subjects were also included to study "area differences", individual variations in sweat response, and tactile sensibility.

METHODS

Heating

Thermoregulatory sweating was produced by local application of heat, using radiant heat lamps, or by placing the patient in direct sunlight to raise the skin temperature to about 38°C. Visible sweating occurred within 7 to 10 min. under these conditions; the patient was then removed to a closed room to minimize evaporation of sweat and the skin lesions were tested.

Dermometry

It has been known for some time that denervated dry skin and normally moist skin show distinct differences in electrical resistance. The study of this phenomenon, called dermometry, was popularized by Richter and Katz in 1943, and the dermometer used in our experiments was similar to that used by those workers for their study. The fixed electrode consists of a zinc plate about 6 cm square which is fastened to a sweating area, usually the axilla. The movable electrode consists of a copper rod about 5 mm in diameter with an insulated handle. The electrodes are connected to a box which contains a microammeter reading 0 to 50, 2 rheostats, a switch, and a 4.5 V dry battery.

The movable electrode is pressed lightly against the skin to be tested. By means of the variable resistance in the instrument the current flowing through the completed circuit is adjusted so that only a minimal flow is allowed. The movable electrode is then moved over the area of the skin lesion and the deflections of the microammeter, if any, carefully noted. When a sudden change in the flow of current was found, the area was further explored until the line of demarcation between areas of high and low resistance was located. The point was then marked with a skin marking pencil. When a second line of demarcation was determined, a line was drawn between the 2 points. The process was repeated until finally the entire areas of high and low resistance were defined. Often the change from high to low resistance occurred within a few millimetres of each other. Over non-sweating, dry skin no reading was obtained. The movable electrode was then passed over a comparable area of apparently normal skin on the contralateral side and the needle deflections again carefully recorded. These areas were then tested for tactile sensibility.

Tactile sensibility

In all cases the areas to be tested were shaved before the application of stimuli. Threshold stimulus in skin lesions and mirror areas on the corresponding sides of the body was determined by using the bending pressure of 1-in. long nylon filaments graded from 1 to 5 according to increase in diameter of the filament. The subjects, who remained seated in a relaxed position, were asked to keep their eyes closed during the application of stimuli and to point to the spot when a touch was felt. Stimulation was commenced with the filament of smallest diameter and was progressed to the larger diameter filaments in each case.

RESULTS

Table 1 shows the results of sweat tests in microamperes in 15 normal individuals from corresponding areas of skin on either side of the body.

TABLE 1
Normal subjects

Area tested	*Right (mean) microamps*	*Left (mean) microamps*
Face (cheek)	50	50
Axilla	50	50
Chest	48	47
Back	47	46
Buttock	46	42
Thigh (anterior)	30	30
Abdomen	48	48
Forearm (anterior)	27	26
Knee	10	8
Elbow (posterior)	5	5

Table 2 shows the results of sweat tests conducted on skin lesions and normal areas of skin in 10 patients with tuberculoid leprosy. In all cases tactile sensibility was tested in the corresponding areas.

TABLE 2
Tuberculoid leprosy

Over skin lesions		*Over control areas*	
Sweat response (microamps)	*Loss of tactile sensibility 1–5 nylon*	*Sweat response (microamps)*	*Loss of tactile sensibility 1–5 nylon*
0	1–5	30	Nil
0	1–5	50	Nil
0	1–5	50	Nil
0	1–5	50	Nil
0	1–5	50	Nil
0	1–5	50	Nil
0	1–5	50	Nil
0	1–5	25	Nil
0	1–5	30	Nil
0	1–5	40	Nil

Table 3 shows the results of sweat tests in 10 cases of borderline leprosy in the tuberculoid spectrum (BT), with corresponding tests for tactile sensation in skin lesions and apparently normal areas.

TABLE 3
Borderline-Tuberculoid leprosy

Over skin lesions		*Over control areas*	
Sweat response (microamps)	*Loss of tactile sensibility 1–5 nylon*	*Sweat response (microamps)*	*Loss of tactile sensibility 1–5 nylon*
5	1–3	50	Nil
30	1	50	Nil
20	Nil	30	Nil
30	1	50	Nil
*0/10	1–5/1–3	50	Nil
30	1	50	Nil
*0/20	1–5/1–4	30	Nil
*0/20	1–5/1–3	25	Nil
*10/20	1–3/1–2	25	Nil
0	1–5	25	Nil

Correlation coefficient $(r)=0.911$; $P<0.001$.
*These skin lesions showed a variation in sweat reponse within the area tested and 2 readings were obtained in each case. A corresponding variation in tactile sensibility was also recorded as shown in the tabulation.

Table 4 records the results of sweat tests in 10 cases of borderline leprosy in the lepromatous spectrum with results of tests for tactile sensibility in skin lesions and apparently normal areas.

TABLE 4
Borderline-Lepromatous leprosy

Over skin lesions		*Over control areas*	
Sweat response (microamps)	*Loss of tactile sensibility 1–5 nylon*	*Sweat response (microamps)*	*Loss of tactile sensibility 1–5 nylon*
50	Nil	50	Nil
30	Nil	30	Nil
10	1–5	50	Nil
20	Nil	25	Nil
15	Nil	20	Nil
10	1–2	20	Nil
5	1–3	20	Nil
10	1–2	50	Nil
5	1–2	30	Nil
50	Nil	50	Nil

Correlation coefficient $(r)=0.739$; $0.01<P<0.02$.

In Table 5 the results of sweat tests and tactile sensibility tests conducted in lepromatous macules are recorded and compared with those in apparently normal areas on the contralateral side.

TABLE 5
Lepromatous leprosy

Over skin lesions		*Over control areas*	
Sweat response (microamps)	*Loss of tactile sensibility 1–5 nylon*	*Sweat response (microamps)*	*Loss of tactile sensibility 1–5 nylon*
15	1–3	30	Nil
20	Nil	20	Nil
0	1–5	20	Nil
25	Nil	30	Nil
20	Nil	25	Nil
20	Nil	25	Nil
20	Nil	20	Nil
0	1–5	15	Nil
30	Nil	30	Nil
25	Nil	30	Nil

Correlation coefficient (r)=0.946; $P<0.001$.

Table 6 records the results of sweat tests and tactile sensibility tests in skin lesions and apparently normal areas in 5 cases of indeterminate leprosy.

TABLE 6
Indeterminate leprosy

Over skin lesions		*Over control areas*	
Sweat response (microamps)	*Loss of tactile sensibility 1–5 nylon*	*Sweat response (microamps)*	*Loss of tactile sensibility 1–5 nylon*
0	1–5	20	Nil
15	Nil	15	Nil
20	Nil	20	Nil
20	Nil	20	Nil
20	Nil	20	Nil

Correlation coefficient (r)=0.968; $0.001<P<0.01$.

COMMENTS

Sweat glands are governed by the sympathetic nervous system, through post-ganglionic fibres from the thoraco-lumbar sympathetic plexus. Under ordinary circumstances generalized sweating occurs in response to a rise in the environmental temperature and this is regulated by the anterior hypothalamus. Emotional sweating is caused by pain, anxiety, and such other emotional changes and is known to occur in the palm of the hand and sole of the foot. The final stimulus is mediated by the release of acetylcholine at nerve endings whereby the sweat gland is stimulated to activity.

In the 15 normal healthy control subjects studied it was found that there was a variation in sweat response in different parts of the body. Thus, the response appeared to be high in certain areas such as the axilla, chest, back, abdomen and face. It was particularly low at the point of the elbow and in front of the knee. It was also observed that sweating varied in "mirror areas" in the same individual. These observations were similar to those previously reported (Shelley *et al.*, 1950).

In the 10 cases of tuberculoid leprosy (Table 2) there was complete absence of sweat response in the skin lesions, with a clear line of demarcation between the lesion and the surrounding skin as indicated by deflection of the microammeter needle. In all cases there was complete loss of touch to all grades of nylon filament (1 to 5). This picture of loss of touch concurrently with loss of sweat response suggests destruction of nerve elements in the affected area. The loss of sweating, however, could be in part due to destruction of sweat glands by tuberculoid granulomatous infiltration (Sato, 1938; Arnold, 1948).

In the 10 cases of borderline leprosy in the tuberculoid spectrum (Table 3), the sweat response in the skin lesions was less than that in the apparently normal areas in all cases. It was also noted that there was some degree of loss of tactile sensibility in every case. It was interesting to record in 4 patients with this type of skin lesion a variation of sweat response in the same lesion with a corresponding loss of tactile sensibility to various grades of nylon filaments. The pattern of sensory changes generally followed the pattern of sweat response in these lesions. The decreased sweat response associated with impairment of tactile sensibility suggests that there is partial destruction of nerve elements to a variable extent within the lesion. In this group of patients sweat response and tactile sensibility were found to be closely correlated (r=0.911).

Among the 10 borderline cases in the lepromatous spectrum (Table 4), a diminished sweat response was found in 5 skin lesions. Loss of

tactile sensibility to various grades of nylon filaments was also noted within these lesions. The other 5 skin lesions showed a normal sweat response with no loss of tactile sensibility. In this group there is probably less direct involvement of nerve endings in the skin lesion until late in the course of the disease, with the result that the lesions still retain a fair amount of tactile sensibility and the ability to sweat. These skin lesions showed a fair degree of correlation between sweat response and tactile sensibility ($r=0.739$).

In macular leproma, the sweat response and tactile sensibility were similar in the lesion and in apparently normal skin in 7 out of a total of 10 cases (Table 5). Since in lepromatous leprosy the disease is widely disseminated and large areas of skin are involved, the sweat response was further compared with that of healthy controls and found to be similar. Three skin lesions showed a marked reduction in sweat response and tactile sensibility. These lesions could have deteriorated from the borderline or indeterminate zone to the lepromatous zone. Loss of tactile sensibility and sweat response were found to be highly correlated in this group of lesions ($r=0.946$).

In 5 cases of indeterminate leprosy, loss of both sweat response and tactile sensibility was recorded in only one instance. The remaining 4 patients showed a normal sweat response and tactile sensibility within the skin lesions. This suggests that local destruction of nerve endings in skin lesions of the indeterminate type is relatively uncommon and probably a late manifestation. In this group also, sweat response and tactile sensibility were found to be highly correlated ($r=0.968$).

This study suggests a fairly good correlation between loss of tactile sensibility in a skin lesion in leprosy and loss of sweating in the same lesion when tested by dermometry. The study also suggests the existence of a gradation in loss of tactile sensibility which corresponds to a gradation in the degree of sweat loss. One may therefore infer that the major cause of interference with sweat response in specific skin lesions of leprosy is probably the loss of autonomic nerve supply rather than specific involvement of sweat glands in the granulomatous inflammatory process.

SUMMARY

Sweat response and tactile sensibility have been studied by the use of dermometry and strength of tactile stimulus as represented by 5 grades of nylon filaments in the skin lesions of 45 patients with tuberculoid, borderline, lepromatous and indeterminate leprosy.

Practically all tuberculoid lesions showed severe impairment of sweating whereas the majority of lepromatous macules showed a fairly normal sweat response. A normal sweat response was also noted in the majority of indeterminate lesions.

The sweat response in borderline leprosy appeared to be variable. Diminished sweat response was more frequently recorded in borderline-tuberculoid lesions than in borderline-leproma lesions.

There was a close correlation between alteration in sweat response and impairment of tactile sensibility in these lesions, suggesting that the alteration in sweat response in skin lesions of leprosy is probably mostly due to loss of autonomic supply rather than to destruction of sweat glands.

The simplicity and accuracy of the technique makes it a useful objective method of testing sweat response and tactile sensibility in the skin lesions of leprosy.

It is suggested that dermometry be employed to determine tactile sensibility in those patients, such as children and the aged, whose co-operation is generally difficult to obtain.

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Peripheral Nerve Abscess in Leprosy

Report of Three Cases Encountered in Dimorphous and Lepromatous Leprosy*

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A review of the literature pertaining to peripheral nerve abscess in leprosy is presented. Relevant features indicate that it is rare, occurring most commonly in males and almost exclusively in patients with tuberculoid leprosy; also that it may involve either the peripheral trunk or cutaneous branches, which involvement is correlated with definite clinical patterns. Three cases recently encountered at Carville are reported. They are of interest because they occurred in lepromatous and dimorphous cases of leprosy which were complicated by acute ENL reaction. Also, each abscess originated within the trunk of the ulnar nerve and had different features of interest. The principles of surgical management of caseation and abscess are discussed. In view of the infrequent occurrence of this condition fuller reporting of such cases is advocated, for this would aid in providing a better understanding of the entity.

There are relatively few articles on peripheral nerve abscess in the medical literature, and these are limited to cases due to leprosy. Certain relevant features are emphasized: the incidence is considered to be rare, and it is noted to occur almost exclusively among males who have tuberculoid leprosy. Either the peripheral nerve trunk or the cutaneous branches may be affected. The pathology is variable, with the diagnosis of an abscess established on the basis of microscopic findings, or gross evidence of caseation, and definite cavitation with liquefied purulent exudate. Reports have emanated from India, Africa, Japan, and South America. Wade (1955) stated that peripheral nerve abscess has not been observed in the Philippines. Also, no cases have been reported from Hawaii or the National Leprosarium at Carville.

Notable of earlier reported case-histories is that they are brief, contain inadequate information, and there is a lack of uniformity of data pertinent to the subject. Though the greatest number of cases are encountered in men, one case is reported by Priestman (1967) in an 11-year-old girl with reacting tuberculoid leprosy, and Saikawa (1950) described one case of multiple abscesses involving a tuberculoid macular case in an old woman. Only one case is reported in a patient with dimorphous leprosy (Sehgal *et al.*, 1967).

Also, the report of nerve abscess in lepromatous leprosy is only occasional. Thus, Job and Bhaktaviziam (1967) report the case of an abscess involving a subcutaneous nerve, the diagnosis of which was established histologically, and Sato (1956) reports 3 cases of abscesses in lepromatous leprosy, one of which involved the ulnar nerve trunk. Differentiation is made between the clinical manifestations resulting from involvement of the nerve trunk and the cutaneous branches. It was pointed out by Lowe (1934) that trunk involvement was common in Dichpali in contrast to involvement of cutaneous nerves in patients in Calcutta, and that there was a different pattern of clinical manifestations corresponding to each.

The nerve trunks reported involved are: (a) the ulnar nerve at the elbow, this being the most frequently cited, (b) the common peroneal, (c) the posterior tibial, (d) the external popliteal, and (e) one case involving the sciatic nerve (Mukherjee and Ghosh, 1956). Cutaneous nerves reported having abscesses include (a) the great

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auricular, (b) the cutaneous nerves of the arm, (c) the cutaneous nerve of the forearm, (d) the cutaneous nerve of the median in the arm, (e) the cutaneous branch of the ulnar on the dorsum of the hand, (f) the radial nerve branch to the dorsum of the hand, (g) the posterior cutaneous nerve of the leg, (h) the superficial peroneal, (i) the saphenous, and (j) the sural nerve. Although abscesses have been encountered in cutaneous branches of the median (Gupta, 1962) and radial (Wheate, 1964) nerves, it could not be determined from the literature that the trunk of either nerve has been involved.

Detailed descriptions of both the gross and the microscopic pathology are generally wanting. However, the findings reported in the various papers cited are comparable. The diagnosis of an abscess is established from both its histopathological and gross pathological features. Gross findings vary from caseous tissue to liquefied or inspissated, yellow, purulent exudate. The disintegrated tissue may appear as a dry grumous or cheesy material. Caseous nodularity of nerves in tuberculoid leprosy is also described (de Souza Campos, 1936); however, they are not always identified as abscesses. In other instances, the disintegrated tissues are autolysed, and there is a purulent exudate. These findings are reported in both the tuberculoid and lepromatous types of leprosy. The *Mycobacterium leprae* bacilli are demonstrable in only approximately 50% of cases (Lowe, 1934), which have been reported to be dominantly of the tuberculoid type. The size of the abscess varies. The majority are observed clinically as a localized swelling. On the other hand, the actual abscess associated with a swollen nerve may be so small that the diagnosis is established only incidentally during surgery or by the pathologist when examining the surgical specimen (Casile *et al.*, 1954). Localized swelling does not always indicate a true abscess, as we have observed repeatedly upon surgical exploration. Fusiform neural enlargement is often due to leprous inflammation. There may be localized cellular necrosis within the swelling, but the neural framework is intact, and the affected focus remains adherent. Minute abscesses are encountered incidentally. On the other hand, giant-sized abscesses are occasionally seen (Gupta, 1962; Mukherjee and Ghosh, 1956) which suggest, and require differentiation from, a tumour.

Reports of nerve abscess in lepromatous leprosy are few, and in only one instance is the nerve trunk reported to have been involved as compared to cutaneous nerve involvement (Sato, 1956). Interest in the subject of nerve abscess and pure neuritic lesions in lepromatous leprosy has been indicated by Wade (1955). Whereas the question whether nerve abscess occurs in lepromatous leprosy has now been answered (Sato, 1956; Job and Bhaktaviziam, 1967), a related question submitted was "Do they occur without coincident skin lesions?" This question is raised because nerve abscesses have developed in patients with lepromatous leprosy who are in acute reaction. It appears pertinent to this inquiry that the site of the abscess should be indicated in relation to the lesions of erythema nodosum leprosum (ENL), whether it be cutaneous or truncal in origin.

In the past few years, nerve abscesses have been encountered in 3 cases of lepromatous and dimorphous leprosy at Carville. In view of the rarity of reports on peripheral nerve abscesses observed in types of leprosy other than the tuberculoid, and because each case presents different features of interest, it is felt that the report of these 3 cases is warranted.

CASE REPORTS

No. 2549, male, date of birth 16-12-25; Filipino, with dimorphous leprosy (BL). Trouble intermittently with ENL reactions and was receiving steroid therapy. Clinically, there was persistent pain and tenderness accompanying a localized swelling of the ulnar nerve at a site proximal to the medial aspect of the elbow. On 11-7-63 a neurolysis and transposition of the ulnar nerve was done. Three small areas of caseation necrosis were associated with a fusiform enlarged nerve trunk. The abscesses were situated beneath the epineurium, which had to be incised to evacuate them. The material

was grossly caseous with numerous acid-fast bacilli. The pathologist's report of tissue submitted was as follows: "The nerve branches and bundles are greatly scarred with distorted architecture. However, all showed a few bacilli which stain well". Diagnosis was leprous neuritis, active (report signed G. L. Fite).

Comment

This case of dimorphous leprosy (BL) represents an incidence in which multiple small areas of caseation containing acid-fast bacilli were associated with a fusiform enlarged ulnar nerve trunk showing scarring and a distorted architecture.

No. 2543, female, date of birth 11-8-39, Filipino, with lepromatous leprosy (LL). There had been a recent ENL reaction which responded to prednisone but had recurred with ulnar nerve pain. "Hydeltrasol" was injected around and into the nerve without relief of pain. Two previous left ulnar neurolyses had been performed, on 8-5-63 and 31-10-63 respectively, by different surgeons. Because of persistent localized pain and swelling which did not respond to non-surgical measures, a third neurolysis was done on 24-4-64. An ulnar nerve abscess was suspected pre-operatively. At operation a greenish-coloured neural bundle was noted. It was incised and a soft exudate extruded. Tissue was submitted to pathology. The report stated: "Skin fragments possessed a few scattered lepromatous foci of small to moderate numbers of bacilli. In an area of the subcutaneous tissue there is a foreign body granulomatous type lesion showing some partly acid-fast; it suggests the residue of inoculated material. The perineural scar tissue shows much more of the foreign-body reaction, many giant cells containing weakly acid-fast ovoid bodies suggesting colloidal particles. Another area shows many brilliant orange granules, also with some foreign-body change. The impression of previous infection or surgical intervention with residue is very strong. The nerve bundle with abscess is more of a foreign-body change, including all and more of those described. Diagnosis: Foreign-body reaction about ulnar nerve, left. Probably residual from prior injection of material in oily suspension".

Comment

This case of lepromatous leprosy (LL) represents a foreign-body abscess in an ulnar nerve possessing leprous disease and complicated with scarring from 2 previous procedures. It cannot be determined if it is superimposed upon a pre-existing leprous abscess, even though acid-fast bacilli were present. It is presumed, therefore, in view of the dominant foreign-body reaction, that the abscess was probably iatrogenic in origin.

No. 2757, male, date of birth 12-3-21, Mexican, with lepromatous leprosy. This patient developed an ENL reaction which responded satisfactorily to prednisone except for the persistence of a painful and tender localized swelling involving the right ulnar nerve, proximal to the elbow. On 6-6-69 an abscess of the ulnar nerve trunk was evacuated surgically and the nerve was lysed and transposed. Postoperatively there was an exacerbation of the ENL reaction, although the symptoms and signs referable to the ulnar nerve had completely subsided. Because of the exacerbation of ENL, in spite of further prednisone, therapy was supplemented with thalidomide, following which the reaction subsided. Smears obtained from the viscid exudate of the abscess revealed numerous acid-fast bacilli (AFB). The pathology report was as follows: "(a) Subcutaneous tissue, right arm, revealed fibro-fatty tissue with moderate size vessels and prominent small intact nerves. Stains for AFB reveal rare fragments within certain small nerves. Bacterial index (BI)=0–1+, morphological index (MI)=0. (b) Epineurium right ulnar nerve reveals dense fibrous tissue with scattered chronic inflammatory cells, predominantly lymphocytes. In the larger piece of tissue, old foamy histiocytes are present. Stains for AFB reveal a focus of rare, beaded bacilli within the larger focus and on occasion in smaller pieces of the fibrous tissue. BI=0–2+, MI=0. (c) Fascicules, right

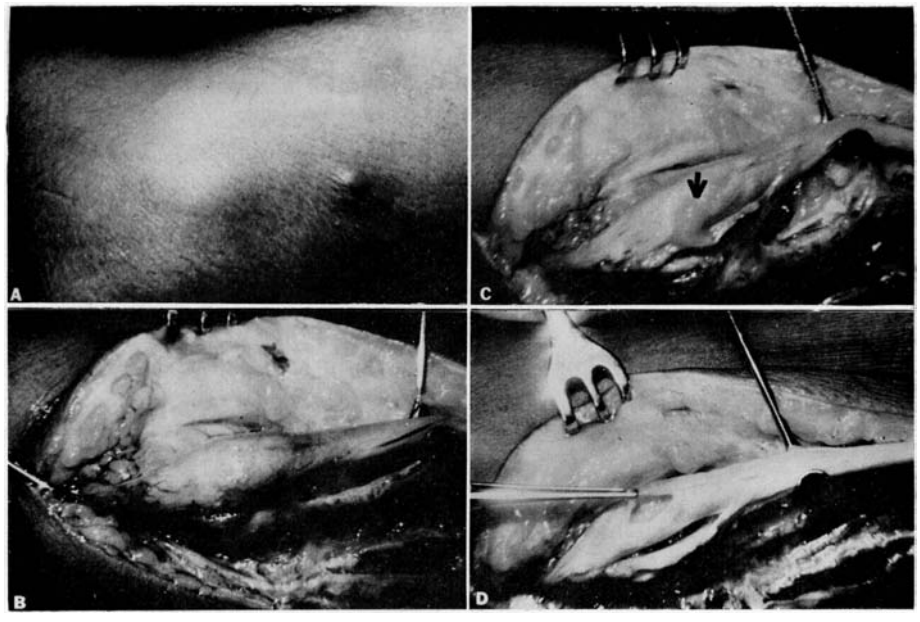

FIG. 1

Leprous abscess of ulnar nerve proximal to epicondylian groove. A, Localized swelling medial aspect forearm near the elbow; B, Fusiform swelling of the nerve; C, Arrow pointing to a purulent exudate escaping from cavity; D, Cavity following evacuation.

ulnar nerve, reveals cross-section of a very small nerve showing a definite lepromatous infiltrate with focal fibrosis. A moderate amount of oedema separates remaining nerve axons. Stains for AFB reveal generally moderate numbers of beaded bacilli with a rare solid noted only in one section. (d) Medial intramuscular septum, right arm, reveals fibro-fatty tissue; in addition, these reveal small nerves, possessing lepromatous infiltrate and slight prominence of the perineurium. Stains for AFB reveal moderate number of beaded bacilli within the nerves. A focus of what appears to be made on H & E section contains also moderate numbers of beaded bacilli. (c) and (d) BI=4+, MI=0. Diagnosis: Tissue right arm, lepromatous leprosy, active, with moderately severe involvement of nerves''.

Comments

This is a case of lepromatous leprosy in which a peripheral nerve abscess is encountered within the ulnar nerve trunk at a site proximal to the elbow (Fig. 1). Although its development is associated with an episode of ENL reaction, it was centrally located within the nerve, and there was no apparent contiguous involvement to skin lesions. Thus, this peripheral nerve abscess encountered in a case of lepromatous leprosy has its origin within the nerve trunk and is not related to coincident ENL skin lesions.

DISCUSSION

Peripheral nerve abscess appears to be unique to leprosy. Although the majority of reported cases have been in patients with tuberculoid leprosy, it is also encountered in dimorphous and lepromatous cases. It occurs in both sexes, but with greater frequency in the male. An abscess may involve either the peripheral nerve trunk or the cutaneous branches, and the lesion may be independent of, or contiguous with, skin lesions of acute ENL reaction.

A review of published reports reveals a lack of uniformity of basic information on the

FIG. 2
A saccular leprous abscess communicating with the tibial nerve within the popliteal space.

subject matter in many instances. A better understanding of the subject could be attained if the adequate biomedical data were provided in each case. It should include sex, age, type of leprosy, the basis for establishing the diagnosis of abscess, the site of neural involvement, that is whether trunk or cutaneous, the presence or absence of acute reaction, and the relation of the abscess to ENL lesions of the skin if such exists. It would also be of value to know the functional status of these nerves during the period that the abscess was present and also following surgical treatment.

The criteria for establishing the diagnosis of peripheral nerve abscess may be controversial. The terms "caseation" and "cold abscess" are common to both tuberculosis and leprosy. In tuberculosis, the classic sequence of focal necrosis, followed by caseation, and subsequently a cold abscess is not unfamiliar. A similar progression of events is seen in peripheral neuritis of leprosy. Caseation literally means "of the nature of cheese". Histologically it consists of a necrosis of tissue with complete loss of structure. A finding which resembles but is not true caseation is the presence of an area of cellular necrosis within which there is a continuity of collagen framework. The necrotic substance is adherent and cannot be removed by scraping with a blunt instrument, as caseous material can be. In this instance the fasciculus undergoes cellular necrosis without caseation. The process may be focal or continuous and may be located between fasciculi which are intact and functional. It may progress to such complete destruction of all tissue elements that the material becomes caseous, whereupon it can be easily removed by simply "spooning it out" with a dull instrument. Following caseation, a true abscess may occur. A granulomatous

exudate producing a cold abscess develops slowly, whereas an abscess complicated by secondary infection is manifested more acutely. When an abscess forms within the nerve trunk, it may either burst through the perineurium but remain contained within the epineurium to produce a localized fusiform swelling, or it may migrate, extending by a narrow tract that leads to a saccular swelling within adjacent soft tissues (Fig. 2). In our practice we confine the surgical diagnosis of abscess to gross collections of caseous material, and semi-fluid or fluid purulent exudate. When an abscess co-exists with adjacent caseous material, we advise evacuation of the abscess together with the caseous nerve bundle that has given rise to it. This permits of primary closure and healing of the wound. If only the purulent exudate is evacuated, then either the abscess may recur or a sinus tract communicating with the focus of caseous residue will develop. On the other hand, caseation alone does not necessarily require surgical intervention except when it results in enlargement of the nerve that increases the intraneural tension whereby it produces pain or progressive paralysis.

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Chemotherapeutic Trial of Combined Capreomycin and Diasone*

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INTRODUCTION

In Hawaii, as in all areas where the lepromatous type of leprosy is common, there is a proportion of patients that do not respond well to sulphone therapy. From Hale Mohalu Hospital, Hansen's Disease Faculty for the State of Hawaii, 5 of these patients with lepromatous leprosy who had been receiving sulphone treatment for many years—4 of them since 1946 and 1 since 1947—and had had both high- and low-dosage treatment were selected for the trial. In all of these cases sulphone resistance was suspected. It was therefore decided to try them on combined therapy with Capreomycin and Diasone, giving Capreomycin, 1 g intramuscularly 6 times a week and Diasone 0.15 g 3 times a week. Capreomycin was chosen (Black *et al.*, 1962; Colestos and Oriot, 1964; Gunella *et al.*, 1964; Stark *et al.*, 1962) because it had been investigated by Shepard (1964) using the mouse footpad method and appeared to have a reasonable chance of success. Diasone (sulfoxone sodium) was chosen because these patients appeared to tolerate the sulphone drug in small doses better than other sulphones that they had taken. It was decided to use combined therapy because of better patient acceptance, as well as the chance for better results. No attempt has been made in this study to compare this combination with other methods of treatment.

In all 5 cases the leprosy was of the lepromatous (LL) type. The original diagnosis in each case had been made by experienced leprologists by clinical examination, biopsy, and the scraped-incision technique. All had had several subsequent biopsy and numerous skin-smear examinations. All had been under the treatment of experienced leprologists for years; all were lepromin negative. During the course of this study, also, all the patients were seen once a month by an experienced independent leprologist (Dr. Claude Caver) (Pettit and Rees, 1967; Waters *et al.*, 1967).

LABORATORY DATA

Complete blood counts, consisting of determination of hematocrit, white blood cell count, haemoglobin and differential values were done on all cases before beginning the study, then once a month for 6 months, and later at 2-monthly intervals; these showed no deviations from the normal range and no changes of significance. The urine was analysed before the study and once a month for 6 months, and thereafter at 2-monthly intervals; no marked deviation from normal was found and no significant changes occurred.

Blood urea nitrogen levels and thymol turbidity tests, carried out before the beginning of the study and also every 3 months, all gave results within normal limits.

Sulphone blood levels, determined monthly, showed a variation of between 0.03 and 1.3 mg per 100 ml, these levels being considered satisfactory. Chest X-ray examinations made before the study started and again 6 months later all showed normal results. An audiometer test was made every 3 months; in all cases the results stayed within normal limits.

Biopsy indices were not done. Skin smears were taken by the standard scraped-incision

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TABLE 1

		Age	*Weight*	*Sex*	*Race*
1	Case 3712 W.M.H.	37	117	F	Portuguese
2	Case 3731 B.K.	36	188	M	Hawaiian
3	Case 8957 K.N.B.	35	210	F	Hawn/Caucasian
4	Case 3878 B.H.R.	55	176	F	Portuguese
5	Case 3894 E.M.M.	32	169	M	Hawaiian

	Case 3712		Case 3731		Case 8957		Case 3878		Case 3894	
Date	*BI*	*MI*	*BI*	*MI*	*BI*	*MI*	*BI*	*MI*	*BI*	*MI*
22-11-67	6	27	6	25	5	20	5	30	6	32
10-1-68	6	25	4	12	5	20	5	31	6	21
8-2-68	6	27	5	18	5	22	5	25	6	20
6-3-68	5	24	4	19	4	25	4	20	5	21
9-4-68	6	20	4	16	5	18	4	18	6	18
15-5-68	6	14	4	19	5	9	3	9	6	12
12-6-68	5	5	5	6	5	5	3	3	6	12
11-9-68	5	5	3	6	5	6	3	7	6	10
30-10-68	5	3	3	1	5	2	4	2	6	6

technique before beginning the study, and then every month for 6 months and subsequently every 2 months; staining was by the Ziehl-Neelsen technique, and all slides were examined by the same experienced technician. Since all cases showed generalized heavy infiltration, the ear was chosen as the site for taking the skin smears. The Bacterial Index (BI) was used as follows:

- 6+ Many clumps of bacilli in an average microscopic field (over 1000 bacilli).
- 5+ 100-1000 bacilli in an average microscopic field.
- 4+ 10-100 bacilli in an average microscopic field.
- 3+ 1-10 bacilli in an average microscopic field.
- 2+ 1-10 bacilli on average in 10 microscopic fields.
- 1+ 1-10 bacilli on average in 100 microscopic fields.

The Morphological Index (MI) is the percentage of solid staining forms. Table 1 shows the results obtained.

It will be noted that the BI in each patient changed very little but in every case the MI fell by the greatest amount between the fourth and sixth months.

In 4 out of the 5 patients, clinical improvement paralleled the above laboratory data to some extent, showing considerable improvement at 6 months and more at the end of a year. The fifth patient, 3894, began to show small leprous nodules about the sixth month, but these gradually regressed.

One patient, 3731, developed a dermatitis after 9 months. This cleared within one month during which Capreomycin was discontinued and Benadryl given. At the end of a month, Capreomycin was resumed and Benadryl continued without recurrence of the dermatitis. At the end of a year another patient asked that the Capreomycin be discontinued temporarily because of soreness at the site of injection. No other ill-effects were noted.

CONCLUSIONS AND SUMMARY

1. Because Capreomycin needs to be given almost daily by intramuscular injection, its use would not seem to be practicable except in cases that do not respond to sulphones.
2. Capreomycin and Diasone in combination appear to be worthy of further, more extensive trial in cases that have not responded to sulphones.
3. Any ill-effects from this drug combination appear to be minimal.

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Follow-up of Application of Plaster-of-Paris Casts for Non-infected Plantar Ulcers in Field Conditions*

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This investigation of the frequency of recurrence of plantar ulcer after the removal of a plaster cast showed that while the overall recurrence rate was 40%, over half (55%) recurred within the first 3 months after removal and 72% did so within 6 months. The recurrence rate was higher (45%) in deformed feet than in "normal" feet (36%). The importance of preventing development of a first ulcer is emphasized and the prophylactic effect of wearing microcellular rubber shoes is discussed.

INTRODUCTION

In the Danish Leprosy Control Project at Pogiri, Andhra Pradesh, the routine treatment of non-infected plantar ulcers consists of the application of below-knee walking plaster-casts, which are applied in the field as well as in hospital. These patients are followed up at regular intervals as far as is feasible in field conditions, and preliminary results of 549 cases were presented at the Ninth International Leprosy Congress, London, 1968, and at the Eleventh All India Leprosy Workers' Conference, New Delhi (see Cap *et al.*, 1968).

Since then, information about 313 additional cases has become available. The total number of ulcers found to be cured at removal of the plaster-cast, and followed up after at least one year, amounts at present to 862. The results are presented in this paper. The terminology and classification used are in accordance with the recommendations of the WHO Expert Committee.

MATERIAL AND METHODS

When this information was collected, some 27,300 patients were registered for treatment in the project, and 8016 had already been released from control. Out of a total of 35,316 patients under treatment or under observation, 9391 (26.5%) have anaesthetic feet, and 2196 are suffering from plantar ulcers, i.e. 7.2% of the total number of patients or 23.4% of the patients with anaesthetic feet.

One of the objectives of the physiotherapy department is to prevent the development of plantar ulcers in patients with anaesthetic feet, through health education, and by providing them with simple nail-less microcellular rubber (MCR) shoes.

A plaster-cast is applied for non-infected plantar ulcers, but patients with infected ulcers are admitted to hospital for a few days and a P.O.P. (plaster-of-Paris) cast applied as soon as the infection has subsided. It is not known how many of the existing plantar ulcers in the project are non-infected and suitable for treatment with a plaster-cast.

The healing rate lies between 80 and 85%. An ulcer is considered healed only when it is completely closed. When the ulcer is persistent, the failure is mainly due to the fact that patients themselves remove the cast shortly after application. Sometimes it happens that the plaster-cast is damaged in such a way that it becomes useless.

In the 862 cases which could be followed-up, and which form the subject of this study, the ulcer was found to be cured on removal of the

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plaster. It was intended to re-examine all those patients after a 12-month period, but this was not possible in field conditions, because it would take several months before all clinics could be visited by the mobile physiotherapy team. It sometimes happened that the patient did not attend the programme although he was requested to do so, or he may have been absent from his village at the time. For these reasons the great majority of the patients were seen between 18 and 24 months, the average period being 19 months. In a very few cases, the period between removal of P.O.P. and follow-up was more than 3 years.

There was no difference in the recurrence rate in relation to the duration of the period of observation (see Table 1).

TABLE 1

Recurrence of plantar ulcers in relation to the period of observation after removal of the P.O.P.

Period of observation in months	*Recurrence*		*Total*	
	Number	%	*Number*	%
12-18 months	175	42.4	413	47.9
18-24 months	116	40.0	290	33.6
24-32 months	42	36.8	114	13.2
32-36 months	5	31.2	16	1.9
37-42 months	4	33.3	12	1.4
42 months and over	6	35.3	17	2.0
Total	348	40.4	862	100.0

RESULTS

General

As in the previous report (Cap *et al.*, 1968) the recurrence rate is 40%, 348 plantar ulcers having recurred out of the total group of 862. The additional group of 313 patients with ulcers did not change the recurrence rate in any way.

As can be seen in Tables 2 and 3, there is no difference in the recurrence rate between the sexes, nor in relation to the form of leprosy; no patients with the indeterminate type of leprosy were treated for plantar ulcers with a plaster-cast, and the group under review therefore contains only those with lepromatous or tuberculoid leprosy.

TABLE 2

Recurrence of plantar ulcer after P.O.P. in relation to sex

Sex	*Recurrence*		*Total*	
	Number	%	*Number*	%
Male	229	39.7	577	66.9
Female	119	41.8	285	33.1
Total	348	40.4	862	100.0

TABLE 3

Recurrence of plantar ulcers in relation to the classification

Classification	*Recurrence*		*Total*	
	Number	%	*Number*	%
Lepromatous	96	42.3	227	26.3
Tuberculoid	252	39.7	635	73.7
Indeterminate	–	–	–	–
Total	348	40.4	862	100.0

Recurrence in relation to the period of time after removal

Information about the period of time which elapsed between removal of the plaster-cast and recurrence of the ulcer was obtained by questioning the patient and also from the notes on the treatment cards which were kept by the clinic-workers.

Table 4 shows that out of 862 healed ulcers, 193 or 22.3% recurred within 3 months and 29.2% within 6 months after removal of the P.O.P. From then onwards, the recurrence rate increases more slowly.

TABLE 4

Period of time between removal of P.O.P. and recurrence of plantar ulcer (862 cases)

Period after removal in months	*Recurrence*	
	Number	%
0-3 months	193	22.3
3-6 months	252	29.2
6-9 months	281	32.6
9-12 months	322	37.4
12 months and over	348	40.4

TABLE 5

Period of time between removal of P.O.P. and recurrence of plantar ulcer

Period after removal in months	*Recurrence*	
	Number	%
0-3 months	193	55.4
3-6 months	59	17.0
6-9 months	29	8.3
9-12 months	41	11.8
12 months and over	26	7.5
Total	348	100.0

Considering only the recurred ulcers, it can be seen from Table 5 that of 348 recurred ulcers more than 55.4% did so within the first 3 months after removal of the plaster. Nearly 72.4% had recurred before 6 months had elapsed. Only in 26 cases (7.5%) did the ulcer recur after having been closed for more than 12 months.

The first few months immediately following the removal of the plaster-cast thus seem to be decisive in the possible recurrence of plantar ulcers.

Duration of the ulcers

In our previous study the impression was gained that the recurrence rate was higher in long-standing ulcers than in ulcers of short duration; out of 549 ulcers, 123 were reported to have been present less than one year before treatment. Of the latter, 39 or 31.7% recurred, while in the 426 long-standing ulcers 180 or 42.3% recurred.

In the present study the difference has flattened out; thus, 78 or 35.0% of the 223 ulcers with a duration of less than one year recurred, while of the 633 long-standing ulcers, 267 or 42.2% recurred. The difference is statistically not significant. (See Table 6.)

Results in normal feet and in deformed feet

In our previous study it was observed that of 295 patients with normal feet the ulcer recurred in 105 or 35.6%, and that of the 254 patients with deformed feet, 114 or 44.9% suffered a recurrence. The opinion was then

TABLE 6

Recurrence of plantar ulcer in relation to its duration previous to the application of P.O.P.

Duration of plantar ulcer	*Recurrence*		*Total*
	Number	%	
0-3 months	30	36.1	83
3-6 months	25	32.1	78
6-12 months	23	37.1	62
Less than 1 year	78	35.0	223
1 year	104	39.0	267
2-6 years	120	42.1	285
6-10 years	35	52.2	67
10 years and over	8	57.1	14
More than 1 year	267	42.2	633
Total	345	40.3	856*

*Information about duration of the plantar ulcer is not available for 6 patients: the ulcer recurred in 3 and did not recur in the 3 remaining.

expressed that the difference in the recurrence rate would increase still more if only those deformities which change the weight-bearing area were taken into consideration.

An attempt was therefore made to clarify this point and the present group was split into 3 sub-groups (see Table 7). This table shows that in 354 patients with otherwise normal feet, 128 or 36.2% had a recurrence of the ulcer.

The group of 189 patients with absorption of the feet is not very homogeneous and is therefore kept separately. It included patients with slight absorption of the toes, as well as patients with gross absorption and subsequent changes in the

TABLE 7

Recurrence of plantar ulcers after P.O.P. application in patients with deformed feet, and in patients with otherwise normal feet

	Recurrence		*Total*	
	Number	%	*Number*	%
Normal feet	128	36.2	354	41.2
Feet with absorption	75	39.7	189	21.9
Deformed feet	145	45.7	317	36.9
Total	348	40.5	860*	100.0

*Information about status of foot missing in 2 cases.

weight-bearing area; here 75 or 39.7% of them had recurrent ulcer. By "deformed feet" is mainly meant changes in the weight-bearing area due to previous bone involvement, and also drop-foot and combined deformities. Out of this group of 317 patients the ulcer recurred in 145 or 45.7%.

It is obvious that with a more detailed and more scientifically reliable classification of the deformities, the percentage of recurrences in feet with a deformed weight-bearing area would still increase.

Recurrence and site of the ulcers

The recurrence rate is highest in feet with multiple ulcers (see Table 8). The great majority of these feet are deformed. Out of 139 patients with multiple ulcers, 73 or 52.5% recurred. In the group of 718 patients with single ulcers, the recurrence rate was 38.1%.

Ulcers under the calcaneus recurred more frequently (45.5%), followed closely by ulcers under the 1st metatarsal head (42.1%). Then come the ulcers under the other metatarsal heads and the great toe (36%), and the lowest recurrence rate was observed in ulcers under the base of the 5th metatarsal bone (24.3%). (See Table 8.)

TABLE 8

Recurrence of plantar ulcers in relation to the site

Site	*Recurrence*		*Total*	
	Number	%	*Number*	%
Single ulcers				
Calcaneus	40	45.5	88	10.3
1st metatarsal head	64	42.1	152	17.7
5th metatarsal head	24	36.4	66	7.7
2nd, 3rd and 4th metatarsal head	88	36.2	243	28.4
Great toe	44	36.1	122	14.2
Base of 5th metatarsal	9	24.3	37	4.3
Other	5	50.0	10	1.2
Single ulcers	274	38.1	718	83.8
Multiple ulcers	73	52.5	139	16.2
Total	347	40.5	857*	100.0

*No information available about the site of the plantar ulcer in 5 patients: in 1 the ulcer recurred and in 4 it did not recur.

"Slippery slope"

Information was obtained from 837 patients about the existence of previous ulcers. The plaster-cast was applied for the first ulcer in 243 patients. It recurred in only 46 or 18.9% of them, as compared with a recurrence rate of 40.1% for the whole group. It is notable that where there had been a previous ulcer which left scar tissue after healing, the recurrence rate becomes more than twice as high. In a group of 217 patients who had had one ulcer previously, it recurred in 98 or 45.2%. From then onwards the recurrence rate increases slowly from 48.5%, when there were 2 previous ulcers, to 52.6% when there were more than 2 previous ulcers (see Table 9). These findings clearly show that the first ulcer is the most dangerous and should be avoided at all costs.

An attempt was then made to find out whether the ulcers which recurred almost immediately after removal of the plaster-cast (see p. 185) did so in patients with multiple

TABLE 9

Recurrence of plantar ulcers in relation to the previous ulcers (slippery slope)

	Recurrence		*Total*	
	Number	%	*Number*	%
I	46	18.9	243	29.0
II	98	45.2	217	25.9
III	82	48.5	169	20.2
IV-VIII	110	52.6	209	24.9
Total	336	40.1	837*	100.0

*Information about scars of previous ulcers is not available for 24 patients, 12 without recurrence of the ulcer and 12 with recurred ulcer.

I : The P.O.P. was applied for the first ulcer to occur, there having been no ulcer previously.

II : The P.O.P. was applied for the second ulcer, the first being cured with or without plaster-cast.

III : The P.O.P. was applied for the third ulcer, the first and second being cured with or without plaster-cast.

IV-VIII : Previous to the application of the plaster-cast, there had been 2 to 7 ulcers, which had been cured with or without application of P.O.P.

TABLE 10
Distribution on "slippery slope" of patients whose ulcer recurred within 3 months after removal of the plaster-cast

Number of ulcers	*Number of patients*	%
I	58	31.2
II	50	26.9
III	38	20.4
IV-VIII	40	21.5
Total	186*	100.0

*Information about scars of previous ulcers is not available for 7 cases.

previous ulcers and extensive scar tissue. However, a comparison between Tables 9 and 10 shows that the proportion of patients for whom a plaster-cast was applied for 1, 2 or 3, or more previous ulcers, is the same in the total group as in the group in which the ulcer recurred within 3 months after removal.

FOOTWEAR

One of the points of the health education programme of the physiotherapy department is to convince patients with anaesthetic feet to wear MCR shoes.

The shoes are provided by the Project and cost about 10 rupees, but the patients can buy them from us for 3 rupees, which is about the same price as they pay for cheap, locally made shoes.

As a rule MCR shoes are not given free, so as to make sure that there is some personal motivation on the part of the patients.

TABLE 11
Number of patients with anaesthetic feet and having MCR shoes

	Number of patients with anaesthetic feet	*Number with shoes* 1968 *Number*	1969 *Number*	%
Without ulcers	7,195	365	866	12.0
With ulcers	2,196	376	684	31.1
Total	9,391	741	1,450	15.4

At present 1450 patients are wearing MCR shoes, or 15.4% of patients with anaesthesia of the feet, with or without ulcers (see Table 11). It is much easier to convince patients who are suffering from plantar ulcers to wear these shoes, and 31.1% of them have been provided with protective footwear.

Of the 7195 patients with anaesthesia of the feet without plantar ulcers, only 866 (or 12%) have MCR shoes. It is, however, very encouraging to see that the number of patients in this category who are wearing MCR shoes has risen from 365 to 866, an increase of 135%. In the group of patients who had already had ulcers previously, the numbers rose from 376 to 684, or by 82%. The relative higher increase of shoe-wearing in the former group gives an indication of the influence of the health-education programme.

It is not possible in field conditions to have a measurement of the efficacy of footwear in the prevention of plantar ulcers, but data have been collected about the recurrence of plantar ulcers after P.O.P. in relation to the use of shoes.

It is rather surprising to find, as Table 12 shows, that there is little difference in the recurrence rate between patients who have MCR shoes and those who have not. Several explanations can be put forward. In this part of India footwear is uncommon among the agricultural population and lower social strata. The great majority of our patients belong to these categories, and although they are taught that they have to wear their shoes "24 hours a day", it is not at all certain that they in fact do so.

TABLE 12
Recurrence of plantar ulcer after P.O.P. application, in patients with and without MCR shoes

Number of patients	*Recurrence* *Number*	%	*Total* *Number*	%
Patients with shoes	175	43.3	404	46.8
Patients without shoes	173	37.7	458	53.2
Total	348	40.4	862	100.0

In other words, there may be a difference between "having shoes" and "wearing shoes".

The standard MCR shoes, when worn regularly, last for 4 to 6 months, but it sometimes happens that the patient neglects to have them repaired, or fails to replace them when they are beyond repair. Another reason is that patients who have had several ulcers previous to the P.O.P. application, and also those with deformed feet, are more willing to wear shoes. Out of 243 patients for whom a plaster-cast was applied for the first ulcer, 103 or 42.4% are wearing shoes, whereas 123 or 58.8% of the 209 patients who have had more than 3 ulcers previously have MCR shoes (see Table 13). Again, 149 patients (41.8%) with normal feet wear shoes, against 180 (56.8%) of the 370 patients with deformed feet (see Table 14). Both groups represent the great majority of the patients for whom a plaster-cast has been applied, and their plantar ulcers are prone to recur whatever kind of footwear they are provided with; the footwear supplied by the Centre is unavoidably of a simple and standardized type, which may not be entirely suitable for such feet.

TABLE 13
Number of patients having MCR shoes in relation to the number of previous ulcers

	Number of patients	*Patients wearing MCR shoes*			
		Recurrence		*Total*	
		Number	%	*Number*	%
I	243	21	20.4	103	42.4
II	217	45	45.0	100	46.0
III	169	40	51.2	78	46.1
IV-VIII	209	69	56.2	123	58.8
Total	837	175	43.3	404	48.2

TABLE 14
Number of patients with normal and deformed feet, having MCR shoes

	Number of patients	*Number of patients having MCR shoes*	
		Number	%
Normal feet	354	149	42.1
Feet with absorption	189	75	40.2
Deformed feet	317	180	56.8
Total	860*	404	47.0

*Information missing about 2 patients.

SUMMARY AND CONCLUSIONS

Over an average period of 19 months after removal of a plaster-cast for non-infected plantar ulcer, it was found that the ulcer had recurred in 348 or 40.4% of 862 patients whose ulcer had been healed at the time of removal of the plaster.

The first 6 months after removal of the plaster is the most dangerous period for recurrence of plantar ulcers: 72.4% of all recurrences took place during this period, and 55.4% occurred during the first 3 months after removal. No relationship was observed between the recurrence rate and sex, or classification and duration of the plantar ulcer previous to treatment.

The recurrence rate was higher (45.7%) in 317 patients with deformed feet than in 354 patients with "normal" feet (36.2%). The recurrence rate is also related to the site of the ulcer. The most important factor seems to be the presence of scar tissue due to previous

TABLE 15
Recurrence rate in patients with normal and deformed feet in relation to shoes

	With shoes			*Without shoes*			*Total number of patients*		
	No.	*Recurred*	%	*No.*	*Recurred*	%	*No.*	*Recurred*	%
Normal feet	149	59	39.6	205	69	33.6	354	128	36.2
Feet with absorption	75	31	41.4	114	44	38.6	189	75	39.7
Deformed feet	180	85	47.2	137	60	43.8	317	145	45.7
Total	404	175	43.3	456	173	37.9	860	348	40.4

TABLE 16
Patients with P.O.P. application for ulcer under 5th metatarsal head in relation to drop-foot

	Recurred	*Not recurred*	*Total*
Patients with drop-foot	6	14	20
Patients without drop-foot	18	28	46
Total	24	42	66

TABLE 17
Patients with P.O.P. application for ulcer under the base of 5th metatarsal bone in relation to drop-foot

	Recurred	*Not recurred*	*Total*
Patients with drop-foot	3	2	5
Patients without drop-foot	6	26	32
Total	9	28	37

ulcers. Of a group of 243 patients for whom the plaster-cast was applied for the first ulcer, the ulcer recurred in only 45 (18.9%). This clearly shows the absolute necessity of directing the greater part of our activities towards the prevention of the first ulcer, through health education, teaching patients with anaesthetic feet how to take care of them, and to persuade them to wear nail-less microcellular rubber shoes, of which simple standardized models are provided by the Project.

At present 1450 patients are wearing MCR shoes, or only 15.4% of those who ought to use protective footwear. It is encouraging to observe that the number of shoe-wearing patients who have anaesthetic feet but no plantar ulcer has increased in one year from 365 to 866. The number of patients in this group who are wearing shoes has increased proportionally more than in the group of patients with plantar ulcers. It represents at present 12% of all patients (7195) with anaesthetic feet without plantar ulcer. It looks as if the health-education programme is slowly catching up in the prevention and treatment of plantar ulcers, and that gradually more and more patients are reached at the appropriate period, that is, *before* the ulcer develops.

In the Project no data are available concerning the value of protective footwear in the prevention of plantar ulcers.

The data presented with regard to the recurrence of plantar ulcers and the wearing of footwear are not very encouraging: the recurrence rate is the same in patients with shoes as in patients without shoes. But these figures may be misleading, since proportionally more patients with deformed feet and multiple previous and present plantar ulcers are wearing shoes; in these cases recurrence of the ulcer is very frequent and it is questionable whether the standardized type of shoes they are provided with do give full protection.

It is also not known how regularly the shoes are worn, not only because most people in the area are accustomed to walk bare-footed, but also because several social, economic, and even religious factors are not in favour of constant wearing of shoes. The health-education programme is being directed along these lines.

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Lepr. Rev. (1970) **91,** 191-192

Obituary Notice

DR. H. JOCELYN SMYLY, 1882-1970

With the passing of Dr. Smyly at the age of 87, one of the few remaining medical links with "old" China has been severed, and the cause of leprosy has lost an enthusiastic advocate.

Henry Jocelyn Smyly was born on 7 October, 1882, in Dublin, the eldest son of Sir William Josiah Smyly, one-time Master of the famous Rotunda (Maternity Hospital) in Dublin. His paternal grandmother was the founder of the Smyly Homes, which (it was thought) inspired Dr. Barnardo to begin a similar work in England.

Although his schooldays were interrupted by 4 years of illness, necessitating prolonged immobilization for tuberculosis of the spine, Jocelyn Smyly on entering Trinity College, Dublin, soon gave promise of outstanding intellectual ability, becoming senior moderator and winning the "big gold medal" as the best student in 5 years. He obtained the degree of M.A., and graduated in medicine (M.B., CH.B., B.A.O.) in 1911. The following year, he gained both the M.D. and the Fellowship of the Royal College of Surgeons of Ireland.

Within a year, he was off to China as a missionary under the London Missionary Society, assuming an appointment as Associate in Medicine at the Peking Union Medical College. After the First World War, he came back to England, but after furlough returned to Peking under the auspices of the Society for the Propagation of the Gospel. Here he worked until 1928, in which year he was appointed to the Chair of Clinical Medicine at the Shantung Christian University at Cheloo. Successive generations of students and nurses whom he taught there had cause to be grateful to the Professor who lectured so competently and taught them basic medicine so thoroughly.

He was repatriated to England in 1941, and spent the next 6 years in enforced absence from his beloved China. However, undaunted, he took up geriatric research at Guy's and Tooting Bec Hospitals, and did "locums" for general practitioners in war-time England. After the war, back in Cheloo and faced with the disruption of the hospital work caused by the Japanese occupation, Dr. Smyly threw himself into the task of rebuilding, only to be met by another threat—this time from the Communists. He stayed at his post as long as he could, but finally retired from Cheloo in 1951.

It was at Cheloo that he became interested in leprosy, having taken clinical charge of a small hospital for leprosy patients just outside the campus and city. He resolved to restrict his general medical interests and reading and to concentrate on the study of leprosy. This he did with such good effect that he became extremely knowledgeable about many aspects of this disease. On his way back to England, he visited several leprosy centres in India, not (as he put it) as a distinguished Professor of Medicine, but rather as an eager student, sitting at the feet of younger men who were experienced in the various aspects of leprosy.

In 1954, he was asked temporarily to relieve Dr. Neil Fraser, who was building up the work of The Leprosy Mission at the Island of Happy Healing (Hay Ling Chau) near Hong Kong. This furlough relief was extended into the following year. Dr. Smyly entered enthusiastically into every side of the work, revelling especially in the pathological investigations and the teaching of medical students. He was in his element again. It was at this time that he made the acquaintance of Dr. Robert Cochrane, son of the Dr. Thomas Cochrane who was Founder of the Peking Union Medical College, with whom he had first worked in China.

Back in England again, he could not remain idle. He visited his doctor son in Northern Rhodesia in 1958, found out that the post of Government Leprologist was falling vacant, and was appointed to it at the age of 76. He was there for 2 years, before finally "retiring" to England. He soon joined Dr. Robert Cochrane in London at the Leprosy Study Centre, examining and reporting on histological sections of leprosy lesions and helping in the work of the

Centre. It was then that he wrote his contribution to the second edition of *Leprosy in Theory and Practice* (1964), edited by Cochrane and Davey.

Full of helpfulness and good works, Dr. Smyly retained a lively interest in all aspects of leprosy. He wrote to *The Daily Telegraph* in May, 1970, protesting at the use of the word "leper" in its columns. Within a few days of his death, he was discussing some of the latest work in leprosy and recently published papers on cell-mediated immunity. On the morning of the day he died, he was cycling in Kingston, Surrey, near his home.

Dr. "Jock" Smyly will be missed in China, Africa, and Britain. He was a good man, a real Christian who lived out his faith, and was genuinely helpful to all in need. He had a well-furnished mind, and professionally was extremely competent. Physically very agile, and mentally very alert right up to the end, he has left his mark for good in many places. A small and sprightly man, always neatly turned out and rather precise of utterance, Dr. Smyly leaves a wide circle of those who are proud to have been his friends.

Our sympathy goes out to the gracious lady who was his helpmeet for 49 years to the day, and to his two sons.

S.G.B.

Abstracts

The following 3 abstracts are reprinted, with permission, from *Trop. Dis. Bull.*, 1970, **67**, 4:

1. **La lutte contre la lèpre en Afrique centrale** (Leprosy control in central Africa), by R. Labusquière. *Acta Leprol.*, 1969, No. 36, 5-18.

The thesis developed by the author in this paper is that, in the ex-French colonies of central and west Africa, experience during the past 15 years demonstrates conclusively that leprosy can be controlled and that the numbers of new infections show a progressive reduction. He supports this contention with sober statistics drawn from the 5 countries comprising the French-orientated Union, in which high prevalence rates (above 10% in some districts, and 45% in some villages) and dispersal of the population presented a challenge to the public health administrator and the mobile leprosy teams.

The total population covered in the report is about 10 millions; 165,576 patients were under treatment for leprosy at the end of 1968, 41,335 having been discharged "disease arrested" since the beginning of the campaign. For the last 2 years, the number of new infections registered is about a third of those discharged. Attention is drawn to the 2 countries (Cameroun and Gabon) whose progress lags far behind that of the other 3, and the valid explanation is offered that these 2 have failed to adopt modern methods of mass control.

[This paper is both salutary and reassuring. It provides evidence to support the thesis that leprosy can be controlled in the environment of central and west Africa, where, despite inherent difficulties of communications and scattered populations, mass treatment measures can be applied persistently by supervised teams of medical auxiliaries. It is salutary in the sense that these results can be paralleled by extremely few leprosy control projects in those populous countries where the prevalence of leprosy is high, where the lepromatous/tuberculoid ratio is also high, and where prejudice against leprosy is greater. The local success registered in populations totalling 10 millions does not unfortunately invalidate the far from optimistic conclusions based on much larger figures from countries where only 1 in 5 of those with leprosy are at present able to get treatment.]

S. G. Browne.

2. **Investigations de sept gynécomasties chez le lépreux africain** (A study of 7 cases of gynaecomastia in African patients with leprosy, by A. Carayon, L. Maydat, P. Bobin and F. Blin. *Bull. Soc. Méd. Afr. Noire Lang. Fr.*, 1969, **14** (3), 498-506.

By means of the lymphographic technique that they have developed, the authors visualized the testiculo-funicular lymphatic vessels in 7 patients who had gynaecomastia and long-standing lepromatous leprosy. They were able to demonstrate some degree of lymphatic stasis in these vessels in all the patients. The associated non-inflammatory oedema of the Leydig cells, accompanied by the specific leprosy lesions in the testis itself, is held to provide an adequate pathological explanation of the hormonal imbalance that results in gynaecomastia in leprosy.

S. G. Browne.

3. **An open trial of indomethacin therapy in exacerbated phases of lepromatous leprosy,** by G. Thomas, A. B. A. Karat, S. Karat and P. S. S. Rao. *Indian J. Med. Sci.*, 1969, **23**, 68.

Indomethacin, a non-steroidal drug with anti-inflammatory and antipyretic properties, was given to 19 patients suffering from lepra reaction and to 1 patient who had previously had a reaction. Eighteen patients received 50 mg thrice daily with food, and 2 received half this dose. The all-round effect was only slightly better than could have been expected from parenteral antimony. Side-effects included abdominal pain, headache, vertigo, nausea and vomiting. The drug was discontinued in 8 patients, either because of side-effects or ineffectiveness.

W. H. Jopling.

The following 8 abstracts are reprinted, with permission, from *Trop. Dis. Bull.*, 1970, **67**, 5:

4. **Caractères épidémiologiques et cliniques de la lèpre dans la région de Manga, en Haute-Volta. Confrontation avec d'autres observations dans ce pays** (Epidemiological and clinical characteristics of leprosy in the Manga region, Upper Volta, compared with other observations in the same country, by H. Sansarricq, E. Steen and M. Sauvaget *Méd. Trop.*, 1969, **29** (2), 208-28.

This article presents detailed findings in a selected district in which the leprosy prevalence was studied in depth. The total population comprised 25,443 inhabitants in 28 villages scattered over a circumscribed savannah-type plateau. For 20 years regular annual medical examinations of the people had been carried out for leprosy and other endemic diseases, and treatment for leprosy had been available throughout that period.

The prevalence rate of leprosy at the last survey was almost 25 per 1000 inhabitants, and the highest rates were found in both men and women aged between 35 and 45 years. Since the prevalence thereafter showed a sharp decline, it is suggested that perhaps leprosy shortened the life-span. [This suggestion is

highly debatable, in view of the further facts adduced concerning the slight severity of the disease.]

Among the 636 patients with leprosy in the area, only 8 had lepromatous leprosy, giving a lepromatous rate of 3 per 10,000 of the population, or 1.25% of the cases of leprosy diagnosed. No patient was diagnosed as having borderline leprosy [a rather strange observation].

Diagnosis was made on clinical grounds, supported by bacteriological findings from skin smears. The annual incidence of new infections fell from 1.66 per 1000 in 1964 to 0.51 per 1000 in 1967. The initial lesions appeared on parts of the body usually covered by clothing in about 57% of the patients.

Physical disability attributable to leprosy was found in 9.4%. [This is probably an underestimate, since anaesthesia of the extremities was not included.]

The treatment was oral dapsone. Patients with lepromatous leprosy were advised to take treatment for life, and those with tuberculoid or indeterminate leprosy had 3 years of continuous treatment before being placed on observation. After 3 years' observation, and in the absence of clinical signs of relapse, the disease was considered to be arrested.

Comparisons are made between these findings and those (especially leprosy prevalence rates) in neighbouring areas.

S. G. Browne.

5. **Leprosy in Singapore: a survey of this disease between the years 1962-1967,** by K. K. Yew. *Singapore Med. J.*, 1969, **10** (3), 194.

This paper is based on figures derived from statistics of patients with leprosy who have been notified and registered at the Government Skin Clinic. In the 6 years 1962-67, 1358 patients were registered, making a running total since 1951 of 6087 in a population of nearly 2 million, a prevalence rate of 3.08 per 1000. Of the 1358 patients, 510 were classified as positive (lepromatous 287, borderline dimorphous 77, and reactional tuberculoid 146), and 848 as negative (tuberculoid 460, neural 192, and indeterminate 196). According to the histories given by the patients, signs of leprosy had been noted for under a year by 493 patients, and from 1 to 5 years by 309. The majority of patients were aged between 16 and 35 years. Analysis of the prevalence rates according to racial origin indicated that among the Indian and Pakistani community the rate was 1 : 4600, among the Chinese 1 : 8100, while among the Malays it was 1 : 23,300.

[These figures are interesting, but admittedly incomplete. The criteria for separating "positive" from "negative" are not indicated. The meaning of "neural" leprosy is uncertain. Where leprosy prevalence rates depend largely on lay suspicion, and where contact examinations are restricted, the real prevalence of leprosy may be much higher than the figures suggest. Similarly, the higher prevalence of leprosy among the Indian and Pakistani community may reflect many other factors besides a racial "proneness" to infection.]

S. G. Browne.

6. **Erythema nodosum leprosum: a clinical manifestation of the Arthus phenomenon,** by S. N. C. Wemambu, J. L. Turk, M. F. R. Waters and R. J. W. Rees. *Lancet*, 1969, 1 Nov., 933.

Histological features of skin lesions of erythema nodosum leprosum (ENL) include perivascular infiltration with polymorph leucocytes and fibrinoid necrosis of blood vessels. A similar histological picture is seen in laboratory animals in lesions due to the Arthus phenomenon, which is due to the deposition of immune complexes (antigen, antibody and complement) in and around blood vessel walls. Immune complexes are found in the circulation of patients with chronic serum-sickness, some symptoms of which are similar to systemic symptoms of ENL. Frozen sections of ENL skin lesions in 17 patients with lepromatous leprosy, and of lepromatous skin lesions in 6 patients without ENL were examined by fluorescence microscopy for the components of immune complexes. [For details of technical methods the original paper should be consulted.] Immunoglobulin (antibody) and complement "were demonstrated . . . in the areas of perivascular polymorph infiltration in the dermis of ENL lesions in 10 out of 17 patients with this condition", but in none of the 6 patients without ENL. Although *Mycobacterium leprae* failed to stain with fluorescein conjugate prepared with Freund's adjuvant (containing *Myco. tuberculosis*), staining with this conjugate in 7 ENL lesions was interpreted as indicating the presence of a soluble mycobacterial antigen from dead *Myco. leprae* in the immune complexes, because no staining occurred with conjugated sera prepared without *Myco. tuberculosis*. All of the patients with ENL had normal or high serum levels of the C'_3 per ml. High immunoglobulin levels were found both in patients with ENL and those without ENL. [Whether serum levels of the different components of complement are usually increased or decreased in patients with lepromatous leprosy with and without ENL would seem to require further study.]

C. S. Goodwin.

7. **The histoid leproma. Its characteristics and significance,** by J. N. Rodriguez. *Int. J. Lepr.*, 1969, **37** (1), 1-21.

This paper gives an account of histoid lesions in leprosy and describes investigations on 35 patients with histoid lesions at the Eversley Childs Sanatorium in the Philippines. Twenty-eight were relapsed patients, and the remaining 7 were new and untreated patients. Most were classified as lepromatous but a few were borderline-lepromatous, and the typical histoid nodules were erythematous, round or oval, regular in outline, and shiny. Subcutaneous nodules were rarer and were confined to relapsed patients. Bacilli in the histoid lesions were usually longer than the bacilli in the non-histoid lesions of the same patient, and globi were absent or scanty. Histoid nodules of non-relapsing patients tended to heal on treatment with dapsone (DDS), but the reverse held good in relapsed patients; in fact, in some of these patients new histoid nodules

appeared while under treatment. The view is put forward that the sulphone-resistant bacilli in histoid lesions of relapsed patients are mutant organisms that have merged from a predominantly sulphone-susceptible bacterial population following prolonged treatment with dapsone. Animal footpad studies are to be carried out to verify this assumption.

The paper is illustrated by 12 photographs.

W. H. Jopling.

8. **Chemotherapeutic trials in leprosy. 7. Trial of 50 mgm DDS twice weekly in the treatment of lepromatous leprosy,** by J. M. H. Pearson and J. H. S. Pettit. *Int. J. Lepr.*, 1969, **37** (1), 40-45.

Fifteen patients with lepromatous leprosy in the leprosarium, Sungei Buloh, Selangor, Malaysia, were treated for 12 months with 50 mg dapsone (DDS) twice weekly by mouth. Progress was as satisfactory as would have been expected on a dosage of 300 mg twice weekly, and there was no reduction in the incidence and severity of reactional states. [The results of this trial are not unexpected, and the abstracter would like to see them compared with those obtained from a dosage of 5 mg twice weekly.]

[For Parts 1-6, see *Trop. Dis. Bull.*, 1964, **61**, 161; 1966, **63**, 656; 1967, **64**, 1211; 1968, **65**, abstrs. 584 and 925; 1970, **67**, abstr. 600.]

W. H. Jopling.

9. **A rapid qualitative spot test for the detection of dapsone in urine,** by J. H. Peters, S. C. Lin and L. Levy. *Int. J. Lepr.*, 1969, **37** (1). 46-51.

In California, urine specimens from patients with leprosy receiving oral dapsone and intramuscular DADDS, from healthy volunteers receiving no drugs, and from volunteers taking aspirin, were treated with ammonium sulphate, sodium hydroxide and ethylene dichloride and shaken for 15 minutes. An aliquot of the extract was evaporated to dryness under a stream of nitrogen, the residue dissolved in ethanol and 5 µl (equivalent to 0.5 ml urine) applied to filter paper. After 5 minutes the spot was sprayed sequentially with hydrochloric acid and ethanol, aqueous sodium nitrate, ammonium sulphamate and N-1-naphthylethylenediamine in ethanol. The intensity of the violet colour of the spot was related to the amount of dapsone in the sample. Assay by thin-layer chromatography and spectrophotometry confirmed the accuracy of the spot test, its limit of sensitivity being 0.1 µg dapsone per ml of urine. Urines obtained up to 24 hours after the ingestion of 10 mg dapsone, or up to 6 days after 50 mg dapsone, were routinely positive by the spot test. [The technical skill and equipment needed for this "rapid spot test" place it beyond the reach of most leprosaria, and the effort involved might be equally well spent determining the specific concentration of dapsone in the urine (see Goodwin and Sparell, *Trop. Dis. Bull.* 1970, **67**, abstr. 599).]

C. S. Goodwin.

10. **Human leprosy in normal mice,** by R. J. W. Rees, A. G. M. Weddell, E. Palmer and J. M. H. Pearson. *Br. med. J.*, 1969, 26 July, 216.

Because the number of leprosy bacilli present in the footpads of mice, following injection of lepromatous tissue by the method described by Shepard (*Trop. Dis. Bull.*, 1961, **58**, 214), decreases after 8 to 10 months, it has been generally assumed that the infection is self-limiting, but in the present paper the authors report that multiplication does in fact continue and that the infection spreads to other sites.

In the experiments reported, the number of bacilli present in the inoculation site in the footpad were found to decrease as usual, but histological examination showed that the infection had persisted with small numbers of bacilli present in dermal neurovascular bundles, lying in epineurial histiocytes and sometimes in perineurial and Schwann cells, 2 years after infection. At this time the most striking feature of infection was the presence in the hypodermis and among muscle fibres of an epithelioid-cell granuloma which contained few bacilli and was surrounded in places by lymphocytes; when fully developed, these lesions resembled those of the borderline human disease. At 2 years or later, bacilli were often found in the nose and uninoculated footpads.

In order that the significance of these observations should be appreciated a brief outline of the present knowledge of leprosy in man is given and it is concluded that the normal mouse provides an accurate model for studying the early stages in pathogenesis of the human disease.

S. R. M. Bushby.

11. **Minimal effective dosages in mice of clofazimine (B 663) and of ethionamide against** *Mycobacterium leprae*, by C. C. Shepard. *Proc. Soc. Exp. Biol. Med.*, 1969, **132** (1), 120.

This article presents important data to justify treating leprosy patients with "spaced ingestion" (e.g. monthly doses) of clofazimine (B 663, Lamprene), or with much smaller doses than are currently used, which may avoid the distressing skin pigmentation sometimes accompanying clofazimine therapy in light-skinned people.

Mice were inoculated in the footpad with 5000 *Mycobacterium leprae* and then given diets containing varying concentrations of clofazimine from 0.01% to 0.000001%, or ethionamide from 0.1% to 0.00001%. Some animals received the drug from the day of inoculation for 183 days, while in the other animals the bacilli were allowed to multiply for 76 days and then the drug was given until 167 days after inoculation. Groups of 4 mice from an inoculated, untreated, control group, and from each of the dosage-schedule groups were sacrificed at 50-day intervals up to 400 days after inoculation, and "the counts of *Myco. leprae* were carried out on pools of the footpad tissues". The minimal effective dose of ethionamide was 0.01% (about 10 mg/kg/day) and its action ceased when the drug was stopped, suggesting a bacteriostatic action alone.

The minimal effective dose of clofazimine was 0.0001%, which is equivalent to 0.1 mg/kg/day, or 7 mg/day in man. After this dosage schedule was completed multiplication of *Myco. leprae* was delayed for at least 80 days. In mice receiving 0.01% clofazimine the characteristic pigment could still be found in the footpad tissues 219 days after the drug was discontinued. [This slow elimination of clofazimine has been detailed by Vischer *et al.*, *Beitr. Klin. Tuberk.*, 1958, **119**, 59.]

[The abstracter has given monthly doses of clofazimine after a loading dose to untreated patients with lepromatous leprosy, with excellent results.]

C. S. Goodwin.

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12. **Manifestaciones iniciales de la lepra en la adolescencia y pubertad** (Initial manifestations of leprosy in adolescence and puberty), by D. F. Contreras. *Actas DermoSifiliog.*, 1968, **19**, 459-466.

It is generally recognized that the diagnosis of leprosy in an adult is a late diagnosis. The manifestations in children have been described frequently, but there is a paucity of records on leprosy in adolescence and puberty. The author has seen some 300 initial manifestations in patients less than 16 years old but only 14 among those between 16 and 20 years. The latter were children of patients who had spent their infancy in a leprogenous environment and displayed mild macular skin lesions manifested by hypochromia. For some years the author has emphasized differences between the indeterminate leprosy of infants and adolescents, consisting principally of a greater tendency to hypochromia in children than adults, and anhidrosis and alopecia, which are more clearly evident in adults and adolescents than children. There is reason to believe that lesions first recognized in adolescence had their origin during puberty. All observations point to the importance of search for initial lesions during this period. The disease is generally of indeterminate character at that time. Localization varies greatly; the anterior surface of the thighs is a common site.

E. R. Long.

13. **Sweating under cellulose tape. A test of autonomic function,** by A. B. A. Karat, S. Karat and C. A. Pallis *Lancet,* 1969, **1**, 651-652.

The ability to sweat in a given part of the body is a useful guide to the integrity of its autonomic nerve supply. Leprosy may interfere with sweating in a variety of ways. In areas where the disease is endemic there is widespread need for a simple and reliable test of sudomotor function. In the test suggested by the authors sweat evaporation is prevented by covering the quinizarin indicator, previously lightly sprinkled on the area to be investigated, by a broad layer of adhesive cellulose tape. Exposure to the sun was sufficient, making it possible to dispense with heat cradles. Although the sweating response must have been diffuse, areas of skin under the cellotape invariably showed early evidence of sweating, while adjacent areas of skin, similarly sprinkled with the indicator, failed to change colour (presumably because of simultaneous evaporation of any sweat produced). Three photographs illustrate the results of this procedure.

N. D. Fraser.

14. **The sensitivity to dapsone (DDS) of *Mycobacterium leprae* from patients with and without previous treatment,** by C. C. Shepard, L. Levy and P. Fasal. *Amer. J. Trop. Med. and Hyg.*, 1969, **18**, 258-263.

Thirty-two isolates of *Myco. leprae* from 27 patients with leprosy were tested in mice for sensitivity to 0.0001% DDS in the diet. All 11 isolates from previously untreated patients and 6 from patients with some treatment were sensitive to 0.0001% DDS. This dosage in mice is estimated to produce blood and tissue concentrations of about 0.02 μgm/ml, or 1/100 the concentrations produced in man by standard dosages of DDS. Since 0.00001% DDS was usually not effective, the usual minimum inhibitory concentration of DDS for *Myco. leprae* in untreated patients appears to lie between 0.02 and 0.002% μgm/ml. Fifteen isolates from 10 other patients with previous treatment were found resistant to 0.0001% or more DDS in the diet of mice. These patients had begun treatment 11 to 20 years previously. Seven had begun with glucosulfone and 2 with sulfoxone. It seems possible that the irregularity of the DDS supplied by these drugs contributed to the appearance of DDS-resistant *Myco. leprae.*

Authors' summary.

15. **Las formas sub-microscopicas del bacilo de Hansen en la lepra humana. Nota previa** (Submicroscopic forms of Hansen's bacillus in human leprosy. Preliminary note), by J. Gay Prieto and G. G. Gonzalez. *Med. Cutanea,* 1968, **2**, 599-605.

In 1 case of early lepromatous leprosy in the deep layer of the dermis, close to the bacillary remains and in the wax capsule of the bacilli it was possible to see a small body with a dark centre and clear perpheral area wrapped in a thin membrane. In 2 plates degenerated bacilli and large L cells could be seen, inside of which several elementary bodies were observed. In another case of tuberculoid leprosy it was also possible to see, beside the degenerated bacilli, the big L cells. These findings permit the assumption that *Myco. leprae* has a cycle like that of many other germs. In certain circumstances the bacilli lose the acid-fast resistance, becoming granular and adopting forms similar to those described by Convit in the hamster. Afterward, around the bacillary remains, elementary bodies appear which are wrapped in the beginning in the bacillary membrane, like big L cells. Finally, the membrane breaks and the elementary bodies are released.

From authors' summary.

16. **Estudio histológico de la reacción de Mitsuda, en pacientes de lepra lepromatosa y su valor pronóstico en los casos bacteriológicamente negativos** (Histologic study of Mitsuda reaction in lepromatous leprosy patients and its prognostic value in bacteriologically negative cases), by O. Reyes. *Med. Cutanea*, 1968, **3**, 135-139.

The literature reviewed by the author offers conflicting views on the meaning of a positive Mitsuda test in patients with lepromatous leprosy who have received specific treatment for many years and are negative to bacteriologic examinations at the time of the study. In this study, more than 176 patients in such conditions were tested with integral lepromin antigen, and 78 clinically negative responses and 98 positive, with more than 3 mm diameter, were found. Biopsies of the positive reactions gave the following results: 76 showed positivity of varying intensity; 9 were negative; 3 showed an isopathic reaction; 1 gave a picture similar to a fibrohistiocytoma; 1 showed the structure of a rheumatic nodule; and 1 was a reaction of the giant cell type. Eight patients with a histologically positive Mitsuda reaction in whom treatment had been stopped, had relapses with bacteriologically positive lesions. It is concluded that in lepromatous patients without bacilli after many years of treatment, a positive Mitsuda reaction does not permit evaluation of the degree of of resistance of the patient, and has no prognostic value.

Author's abstract.

17. **Enhancing effect of antilymphocytic globulin on human leprosy infection in thymectomized mice,** by J. M. Gaugas. *Nature, Lond.*, 1968, **220**, 1246-1248.

Administration of antilymphocytic serum (ALS) to thymectomized mice enhances the course of infection by *Myco. leprae.* From heterologous rabbit ALS, globulins (ALG) were precipitated by ammonium sulfate, and administered at weekly intervals to thymectomized and normal mice killed 9.5 months after footpad inoculation of *Myco. leprae* from a leproma from an untreated leprosy patient. In normal mice bacillary multiplication was spontaneously arrested when the bacilli reached a little more than a million. Thymectomy plus administration of ALG, however, increased susceptibility to the point of 30-fold multiplication of this figure. The generation time of 14 to 26 days appeared unchanged. Heavily parasitized macrophages were prominent, but the enhanced lesions were almost devoid of lymphocytes. Careful search failed to show spread of infection to adjacent regions of the body.

E. R. Long.

Corrigenda

1 Dr. Schulz has asked us to point out that in her paper with H. H. L. Pentz on "Leprosy Control in South Africa" (*Lepr. Rev.* 1970, **41**, 15-19) the following corrections should be made:

(i) In Table 1 the heading to Col. 2 should read: "No. of population *in thousands*".

(ii) In Fig. 1 the caption should read: "x—x—x, no. of new cases per 100,000 population" (and NOT per million as inadvertently stated).

2. We regret that in the Letter to the Editor from Dr. W. H. Jopling (*Lepr. Rev.* 1970, **41**, 62) the Table was wrongly and misleadingly set out. It should have been as follows:

TABLE 1

Classification of lepra reaction (reaction in leprosy)

Name of reaction	*Type of leprosy involved*	*Main clinical features*	*Main histological features (in dermis)*	*Main haematological findings*
Type 1 reaction	Tuberculoid, borderline, and lepromatous	Erythema and swelling of some or all of the leprosy skin lesions; nerve swelling and pain; oedema of extremities	In "reversal reaction" there is oedema, diminution in number of acid-fast bacilli, and increase in defensive cells such as lymphocytes, epithelioid cells, and giant cells In "downgrading reaction" there is oedema, increase in acid-fast bacilli, and diminution in the number of defensive cells	Nil
Type 2 reaction	Lepromatous; some cases of borderline-lepromatous	Any of the following, singly or in combination: erythema nodosum leprosum, nerve pain, bone pain, joint pain, fever, malaise, lymphadenitis, rhinitis, epistaxis, iridocyclitis, epididymo-orchitis, proteinuria. In severe cases, erythema nodosum leprosum lesions may become vesicular or bullous and break down	Oedema. Polymorphonuclear infiltration of dermis. Swelling of capillary endothelium. In necrotizing reactions there is capillary necrosis with fibrinoid patches in and around affected vessels	Polymorphonuclear leucocytosis. Raised erythrocyte sedimentation rate. Increased serum gamma globulin. Anaemia sometimes

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