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Editorial

CAN ARTHROPODS TRANSMIT LEPROSY?

The mode of transmission and spread of leprosy has still not been defined. Clearly it is of the greatest importance for the control of leprosy to discover the answer to this question. The huge load of *Myco. leprae* in the tissues of patients with borderline and lepromatous leprosy provides an ample source of bacilli for directly infecting contacts, or for infecting the environment and thereby indirectly infecting contacts. It is on this basis that the spread of leprosy has dogmatically been said to arise from skin-to-skin contact during prolonged and intimate contact. However, histological studies on the skin of bacilliferous patients show how rarely organisms are shed from the epidermis. On the other hand large numbers of bacilli are shed from the nasal mucosa and upper respiratory tract, a source of infection that has been particularly stressed by Dr Pedley in his series of excellent papers in *Leprosy Review* (1970, **41**, 31) during the last few years. *Myco. leprae* from this source could enter contacts *via* the upper respiratory tract and lungs in a manner comparable with the transmission of tuberculosis, but as yet there is no direct proof. Because natural infections with *Myco. leprae* have been found in no other animal species than man we can safely assume that the source of infection is man. On this basis it is theoretically possible that *Myco. leprae* could be carried from man to man *via* a temporary host in which the bacilli survive for a limited period, and any insect which bites man could provide such a host. Hence the hypothesis that arthropods—mosquitoes, bedbugs, lice or scabies mites—may play a rôle in the transmission of leprosy. This is not a new hypothesis, but hitherto it has not been tackled scientifically, and only within the last ten years have laboratory methods been available to test the hypothesis. In the following pages of this issue of *Leprosy Review* Dr Balasubrahmanyan and his colleagues in Pondicherry, with the collaboration of Dr Kirchheimer from the U.S.A. Public Health Services Laboratory at Carville, present their preliminary results of studies undertaken in India since 1969 on the possible rôle of arthropods in the transmission of leprosy.

Since the skin of patients with active and untreated borderline and lepromatous leprosy is heavily infected with viable *Myco. leprae,* arthropods at the time of feeding on such patients could pick up bacilli from the dermal tissues, or take up bacilli in the blood feed. Therefore the investigators first confirmed that untreated patients with borderline or lepromatous leprosy had acid-fast bacilli in their peripheral blood. They showed that all such patients had a significant bacteraemia of between 5000 and 500,000 acid-fast bacilli per ml of blood, quantitatively comparable with the bacteraemia observed by Drutz *et al.* [*New Engl. J. Med.*, (1972) **287**, 159] in patients with lepromatous leprosy. These figures are sufficient for the blood feed of an arthropod to contain several hundred *Myco. leprae.* Still more significantly the Pondicherry group applied the mouse-footpad technique to confirm that the acid-fast bacilli present in the blood

from these patients were viable and their pattern of multiplication resembled that of *Myco. leprae.*

The next sequence in their systematic studies was to see whether mosquitoes (*Culex fatigans*) and bedbugs (*Cimex hemipterus*), allowed to feed on patients with untreated lepromatous leprosy, contained acid-fast bacilli. For these experiments, laboratory-bred strains of mosquitoes and bedbugs, which they showed were free of acid-fast bacilli, were allowed to feed freely on lepromatous patients and were then collected, homogenized, and the homogenates stained for acid-fast bacilli. They showed that, after feeding mosquitoes and bedbugs contained small numbers of acid-fast bacilli and these bacilli failed to grow on medium suitable for the cultivation of mycobacteria. They have also inoculated a series of these homogenates from mosquitoes and bedbugs into the footpads of mice and to date have obtained from two groups of mosquitoes, growth patterns of mycobacteria consistent with those characteristic of *Myco. leprae.* In one group of mosquitoes the bacilli were isolated immediately after feeding, but in the other group not until 48 h after feeding, thereby demonstrating that *Myco. leprae* can survive in mosquitoes for the period of time which could elapse before an infected mosquito again fed on man.

Their final series of studies was undertaken on collections of the same species of arthropods obtained from two sources—"patient collections", that is, arthropods from houses in which there was an open case of lepromatous leprosy, and "random collections", i.e., from houses in which there were no patients with leprosy. Groups of arthropods of each species from these collections were homogenized, stained for acid-fast bacilli, inoculated on to medium for the isolation of mycobacteria, and a proportion inoculated into the footpads of mice. From these studies less consistent results have been obtained, since acid-fast bacilli were seen at least as frequently in homogenates of arthropods from random collections as from patient collections. Moreover, from both collections a very small proportion of the homogenates revealed colonies of mycobacteria on culture media. At the time of writing the authors' studies in mice had not been maintained long enough to identify any of the isolates as *Myco. leprae.* Their findings here are eagerly awaited.

The importance of these studies in Pondicherry are that they have established beyond doubt the ability of arthropods to take up acid-fast bacilli at the time of feeding on skin-positive patients with leprosy. Moreover, the investigators have applied the mouse footpad technique to identify the acid-fast bacilli as *Myco. leprae* on the basis of their growth pattern in this experimental model. This is a very considerable achievement, although they still need to demonstrate that the isolates of acid-fast bacilli do invade the dermal and peripheral nerves of the inoculated mice. These same extended and strict criteria must also be applied to the acid-fast bacilli which they have obtained from the arthropod collections from houses with known patients as compared with random samples from non-patient houses. Allowing for these essential further confirmations their studies will show that arthropods can be one mode by which leprosy can be transmitted. However, the very significant observations on the nose in leprosy by Dr Pedley, referred to above, would seem to be a still more likely route of transmission and therefore must be investigated with the same expertise that is currently being applied by the Pondicherry investigators to the rôle of arthropods.

R. J. W. REES

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News and Notes

MORE NEWS FROM LAMBARENE

Following the news item entitled "Lambarene–a new look", which appeared in a recent issue of *Leprosy Review* (1972, **43**, 8), the Executive Committee of the International Association for the Albert Schweitzer Hospital at Lambarene met for the first time in Libreville, capital of the Republic of Gabon, in August of this year. The Committee decided to establish a Foundation with headquarters in Gabon, and with the full participation of Gabonese interests.

A delegation of the Executive Committee was received by His Excellency President Albert Bongo, who expressed his deep appreciation of, and wholehearted support for, the Project as presented to him for the creation of a Rehabilitation Centre at Lambarene. Madame Rhéna Schweitzer-Miller (daughter of the late Dr Schweitzer) introduced to the President the members of the delegation–Mr Larry Gussman (the President of the International Association), Dr Walter Munz and Dr S. G. Browne.

After Dr Munz explained to President Bongo and his advisers the details of the proposed buildings, Dr Browne was asked to give an exposé of the need for such a Centre in the Gabonese Republic, and its functions. Dr Browne said that Gabon could not at present, without help from abroad, look forward to the creation of a Rehabilitation Centre in view of the prior claims of widespread endemic disease and malnutrition. However, the problem of deformity would become increasingly important in the future. The paralyses and mutilations of old and untreated leprosy provided an obvious example of the backlog of preventable conditions that had not been prevented, but tuberculosis, poliomyelitis, congenital deformities and accidents of all kinds (road, mines, lumbering and domestic) accounted for a heavy load of handicapping conditions.

The Rehabilitation Centre would attempt to deal with this problem by providing the specialist or referral services necessary–reconstructive (orthopaedic and plastic) surgery, physiotherapy, occupational therapy, and shoe and splint prosthesis making. The whole would be geared to the needs of a people primarily agricultural, and the object would be not only to restore to human dignity and economic usefulness those who are handicapped in some way, but also to prevent deformity by teaching and training at all levels. The influence of the Centre would thus extend throughout Gabon and to the neighbouring French-speaking countries.

Dr Browne assured the President that, in the spirit of Dr Schweitzer, no patient needing urgent surgical or medical care would ever be turned away, but he did foresee a diminution of the general patient load–a diminution indeed already noticeable in out-patient attendances, major surgery work, and maternity work. In accordance with Government plans for upgrading district hospitals, the hospital at Lambarene *poste* would assume an increasing proportion of the surgical work, and if plans for the creation of a network of rural dispensaries came to fruition,

then the majority of the villagers needing primary medical care for ordinary conditions would be able to obtain it without crowding out the new Centre and the old hospital with patients who could obtain adequate help elsewhere.

Dr Browne then referred to the necessary links to be forged with the *Service contre les Grandes Endémies* and other Government medical services, so that the Centre could function to full capacity. He could forsee a gradual "Gabonization" of the staff, and the eventual complete integration of the Centre into the developing health programme of the Government. The Committee's idea was to create such a hospital complex that this proposed integration would meet with no major difficulty. In conclusion, Dr Browne assured the President of the sense of gratitude and pleasure that the medical staff and Executive Committee experienced as they provided a new Lambarene that would fulfil a real need in Gabon.

The President replied at length, stressing his appreciation of what Lambarene had contributed to the welfare of the country in the past, and welcoming wholeheartedly the present proposals. He hoped that money would be forthcoming, and promised the support of his Government in such matters as the provision of a letter publicly supporting this initiative and emphasizing that it could be part of the Gabonese medical services; approaching the embassies established in Libreville with the object of acquainting them with the attitude and support of the Government; and finally the exemption of import duties on all material to be used in the construction and equipment of the hospital.

NEWS FROM ARGENTINA

Dr L. M. Baliña is President of the Argentinian Society for the Scientific Investigation of Leprosy. The aims of this Society are to publicize the modern outlook on leprosy, and to promote research into the medical, sociological, and psychological aspects of the disease.

Recently, the Society organized a seminar in Buenos Aires, attended by leprologists and social workers, and invited the participants to submit for adjudication a novel having as its central theme the new approach to leprosy.

The Society suggests that special efforts be made in Argentina and other countries to make World Leprosy Week (February, 1973) the occasion for a publicity campaign stressing the scientific advances achieved during the past 100 years in leprosy research, and the challenges remaining for the future.

PUBLIC RELATIONS IN REHABILITATION

Leprosy was adequately represented at the Second International Symposium on Public Relations in Rehabilitation, which was held in Athens from 4 to 8 September, 1972. Dr S. D. Gokhale of Bombay (Assistant Secretary General of the International Council on Social Welfare), Dr A. J. Selvapandian (Professor of Orthopaedic Surgery, Vellore), Dr Ernest P. Fritschi (Chief of Rehabilitation and Surgery, ALERT, Addis Ababa), and Dr S. G. Browne (Member of the International Committee on Public Relations in Rehabilitation) presented papers and took an active part in the discussions.

The Symposium, under the patronage of His Excellency the Minister of Social Services, and held in the magnificent new buildings housing the (Greek) National Foundation for Rehabilitation of the Disabled, brought together some 60

participants from 20 countries. While their common interest was rehabilitation, speakers and listeners represented a wide range of professional activities—from orthopaedic surgeons and audiologists, to organizers of community social services and voluntary agencies. A similarly wide geographical representation was apparent, ranging from North America to India. Poland, Bulgaria, and Jugoslavia were represented, as well as Scandinavia and other European countries.

The papers on leprosy were concerned with "Stigma as an impediment to rehabilitation" (Browne), "Rehabilitation of those afflicted by leprosy" (Selvapandian), "The handicapped, and social stigma in the context of rehabilitation" (Gokhale), and "Community responsibility in rehabilitation of the discharged leprosy patient" (Fritschi).

In the matter of "selling" the idea of rehabilitation, the problems posed by leprosy were frequently cited as providing examples applicable to other handicapping conditions and diseases. The necessity to "educate the educators" was stressed in the context of undergraduate teaching and teacher training colleges. Although the wide diversity of medical and financial resources available in the countries represented might at first sight appear to preclude the emergence of anything like a common mind on the problems of rehabilitation and public relations, the participants showed by their enthusiastic concern for the handicapped and "disadvantaged" that ignorance, indifference, and inertia could be tackled successfully whatever the context.

XII WORLD CONGRESS ON REHABILITATION OF THE DISABLED

From 27 August to 1 September, 1972, over 2000 delegates and associates gathered in Sydney, Australia, for the 12th World Congress on Rehabilitation of the Disabled—the "Golden Jubilee Congress". Converging on Sydney from many countries and representing many branches of medical science concerned with deformities of all kinds and from all causes, the participants were offered plenary sessions and sectional meetings to suit their varied interests.

A "special interest" meeting for leprosy was organized by Dr Grace Warren, of The Leprosy Mission, Hay Ling Chau, Hong Kong. A panel of leprosy workers from Africa, India, Australia, New Guinea, USA, and Hong Kong discussed techniques aimed at minimizing the deformities caused by leprosy. Special emphasis was placed on the necessity for education of the leprosy sufferer, so that he could prevent damage to his anaesthetic tissues. It was pointed out that, because of the diminution of pain perception, many patients became psychologically detached from an anaesthetic part of their body and consequently misused, or even abused, it. This problem, which leprosy shares with other peripheral neuropathies, should be tackled in the light of the conviction that an anaesthetic limb becomes deformed only as the result of neglect.

Another session of particular interest to leprosy workers was on Orthotics and Prosthetics, at which Dr Paul Brand presented a résumé of his recent research activities. He stressed the importance of the summation of repetitive stress in the production of so-called "traumatic" lesions of the anaesthetic extremities, and referred to his work on the use of microcapsules containing dye which ruptured when known pressures were applied. The use of these capsules indicates sites of under or damaging pressure occurring in shoes and prostheses, as well as those sustained during the stresses of walking or standing. Dr Brand also referred to recent work on thermistors, which shows that high temperatures occur in limbs

after use. These "hot spots" give warning of sites of incipient or impending damage, and call for rest and for removal of the causative traumatizing factor. The surgeon's percipient finger-pulp should be able to detect such "hot spots" without the help of sophisticated apparatus, and thus to forestall damage and eventual disability.

XIV INTERNATIONAL CONGRESS OF DERMATOLOGY

Leprosy provided some interesting contributions to the 14th International Congress of Dermatology, which was held in Padua and Venice from 22 to 27 May, 1972.

Dr R. D. Azulay (Brazil) was the co-ordinator of the leprosy symposium which had as its title: "Progress in Leprology". He gave a paper on "Clinical, bacteriological and histopathological results in the treatment of lepromatous leprosy with G 20.320" [B663, Lamprene (Geigy) or clofazimine]. Other speakers were Drs Paul Fasal ("The rôle of laboratory methods in drug trials in lepromatous leprosy"), C. Bhakta Viziam ("Erythema nodosum leprosum—changing aspects"), A. Saul ("Therapy of leprosy"), and L. M. Bechelli ("Controlled field trials on the effect of BCG in the prevention of leprosy").

By general consent the leprosy sessions were among the liveliest and best attended, and once again the interest and importance of leprosy in the world were brought to the attention of many doctors unaware of the progress being recorded in the bacteriology and therapy of leprosy.

TUBERCULOSIS AND LEPROSY—AND MORE ABOUT THE ARMADILLO

The programme of the 25th Congress of the German Society for Tuberculosis and Chest Diseases, held in Hamburg from 19 to 23 September, 1972, reflected not only the great progress registered in the Western World in the control of tuberculosis, but also the growing importance of pulmonary diseases other than tuberculosis and the interest to phthisiologists of non-tuberculous mycobacterial diseases.

About 600 participants gathered from all over Germany, with guests from Great Britain, Switzerland, Holland, USA, and Uganda. Under the dynamic presidency of Professor E. Freerksen of the Borstel Institute for Experimental Biology, and at his suggestion, leprosy was included in the programme, with papers by Dr S. G. Browne ("The epidemiology of leprosy") and a joint contribution by Professor Freerksen and Drs M. Rosenfeld, W. Blenska and E. Kalakowska, M. Chambers, D. L. Leiker, and R. Rhode on their recent experiences with rifampicin, either alone or in combination with other drugs. Dr A. B. Verhagen added a paper on his experiences with a small series of patients treated with rifampicin and other drugs.

Other contributions of interest to those working in the field of leprosy were made by Prof. S. R. Pattyn on "The bacteriology and pathology of mycobacterioses (other than tuberculosis and leprosy)", by Drs R. J. W. Rees and D. N. Mitchell on "The aetiology of sarcoidosis—a reappraisal", and Dr J. L. Stanford on "Burulin—a skin test antigen for the investigation of *M. ulcerans* infection". Some matters touched on in other papers brought to leprologists reminders that erythema nodosum occurs in various fungal infections, both cutaneous and

systemic, and that opportunist mycotic infections might develop in patients under prolonged treatment with antibiotics and corticosteroids.

This interchange of experiences and insights between leprologists and those working in related fields can do nothing but good. Each side can learn from the other as they discuss common problems. To adapt a phrase of George Bernard Shaw's referring to the American and English people as "separated by a common language", we must confess that leprologists have been separated from phthisiologists by a common interest in a different but related mycobacteriosis. Leprosy has certainly been indebted—in microbiology, pathology, therapy, and immunology—to tuberculosis, and now may begin to repay the debt by providing vast opportunities for research, to the lasting benefit of those suffering from either disease.

MORE ABOUT THE ARMADILLO

Dr Eleanor E. Storrs gave a report to the Congress on her research in Louisiana on the production of leprosy in the armadillo. This animal is proving of especial value: it has a low body temperature and a long life-span, and it produces litters of 4 identical young. The number of animals inoculated with *Myco. leprae* in the soft skin of the abdomen and ears has now reached 58, of which 23 show signs of leprosy, 6 of them with disseminated disease. One animal, inoculated with material obtained from the mouse footpad, is showing generalized lepromatous disease, and experiments are in progress for transferring bacilliferous material from one armadillo to another.

Dr Storrs projected some very convincing histopathological slides showing highly bacilliferous tissue and giant globi filled with acid-fast organisms. In certain respects, notwithstanding these resemblances to human disease, the pathological picture showed some interesting divergences. For instance, cellular infiltration in the nerve tissue was less dense, and the concentration of bacilli less intense, than in human disease. In the liver, on the other hand, bacilli were present in great numbers, and massive destruction of liver cells was obvious; the meninges were heavily infected; and in the lungs, consolidation of the tissues with a pneumonia-like exudate was a feature.

Perhaps the most important immediate dividend of this research has been the availability of some 243 g of highly bacilliferous lepromatous material (containing 10×10^{10} organisms per g) from two animals. This vast quantity will allow biochemical and other analyses of *Myco. leprae*—a procedure hitherto impossible.

The essential factors most probably determining the suitability of the armadillo are its low body temperature and some, as yet undetermined, immune mechanism. Dr Storrs pointed out that a disseminated infection resembling human lepromatous leprosy has been noted within a period as short as 10 months after inoculation.

Since some 40% of animals inoculated have so far responded by developing a disseminated mycobacteriosis, and since lepromatous leprosy in the armadillo is a fatal disease, it is interesting to speculate that leprosy might have proved to be the world's most serious disease had the human immunological climate been more propitious to *Myco. leprae* than it actually is.

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Letter to the Editor

was most interested in Professor Vella's letter (*Lepr. Rev.* 1972, **42**, 252) in which he states his opinion about the stigma of the word "leprosy" and his preference for the substitute "Hansenosis". Coming from England, where that stigma is not a cause for personal, social, or preventive problems—so serious in Brazil and other endemic countries of the Romance languages—our hopes of international co-operation are thus strengthened.

In Portuguese, the substitute "hanseniase" (as in "elephantiase", "psoriase") is more euphonic and was preferred by a large majority of fellow-workers and patients consulted before our first terminological changes; translated as "hanseniasis", it was also accepted by most foreign specialists who honoured us with their responses to our questionnaire (*Derm. Int.*, 1969, **8**, 40) and seems to be no more difficult to pronounce than "schistosomiasis", etc. Admittedly, it has the lexicographic defects so clearly pointed out by Professor Vella, but these are apparently less important in Brazil, where both forms "leishmaniase" and "leishmaniose", "esquistossomiase" and "esquistossomose", etc. are used indifferently, and where our hearing is accustomed to the admitted forms "meningococcia", "estreptococcia", "estafilococcia", etc.

However, quite a few correspondents here and abroad have also manifested their preference for "hansenose" ("hansenosis") and this was the form suggested to the Ministry of Health of Peru by a national seminar held there in 1971.

I can speak only for myself, but I believe that most of the 33 Brazilian medical schools and the 4 State public secretariats, as well as most of the authors who have already changed to "hanseniase", would welcome an international agreement around "hansenosis", "Hansen's disease" or any non-eponymic appellation. Our main common objective is to free medical terminology from a stigmatizing pejorative term which has been definitely blocking any and all attempts at public enlightenment and causing immense personal and social suffering—much more than the disease itself—to millions in our area, i.e. our patients and their most unfortunate families.

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The Effect of Antibacterial Drugs on the Ultrastructure of *Mycobacterium leprae* in Human Skin

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Biopsies of skin from patients with lepromatous leprosy were taken before treatment, after 1 year's treatment with DDS, and after 3 and 6 weeks' treatment with DDS, rifampicin or streptomycin, and sectioned and examined in the electron microscope. Longitudinally sectioned *Myco. leprae* were classified into grades of morphological intactness. Analysis of the results showed a significant shift towards less-intact bacteria after 1 year of DDS treatment and after 6 weeks of rifampicin treatment. The assessments agreed qualitatively with conventional morphological indices measured by light microscopy. Some details of the process of degeneration were noted.

Introduction

The first experiments to distinguish between morphologically intact (presumed viable) and degenerate (presumed dead) *Mycobacterium leprae* were made with whole bacteria observed in the electron microscope (Rees, Valentine and Wong, 1960). It was subsequently shown that there was a good agreement between the appearance of each bacterium in the electron microscope and the identical organism stained and observed under the light microscope (Rees and Valentine, 1962). The relation between morphological intactness (solidity) and infectivity has been confirmed (Shepard and McRae, 1965), and the disappearance of solid organisms from tissues has been used to follow the progress of effective antileprosy therapy (Water and Rees, 1962). We have attempted to study the details of the process of degeneration in ultra-thin sections of skin from leprosy patients, both before and after periods of chemotherapy.

Methods

Two series of skin biopsies, A and B, each series from 3 patients with previously untreated lepromatous leprosy, were kindly supplied by Dr J. M. H. Pearson of the (British) Medical Research Council Leprosy Research Unit, Sungei Buloh, Malaysia. Series A consisted of biopsies taken before and after 1 year of chemotherapy with 4,4′-diamino-diphenyl-sulphone (DDS, 600 mg per week); Series B came from patients before, and then 3 and 6 weeks after starting treatment with DDS (600 mg per week), rifampicin (600 mg daily), or streptomycin (1 g daily). The biopsies were divided into halves; one half was used to prepare a suspension of bacteria (Rees, 1964) and the other was fixed for electron microscopy. Biopsies in Series A were fixed in 4% neutral formaldehyde (Richardson, 1960), while material in Series B was fixed in 2.5% glutaraldehyde in 0.085 M-cacodylate buffer, pH 7.4 (Glauert and Thornley, 1966), washed in 2.5% (w/v) sucrose in cacodylate buffer, pH 7.4, containing 0.1 M-calcium chloride, and post-fixed in 1% OsO_4 in veronal buffer (Kellenberger, Ryter and Séchaud, 1958). After fixation both series of biopsies were embedded in Araldite, sectioned, and stained with saturated aqueous uranyl acetate and lead citrate. Sections were examined at 80 kV in a Siemens Elmiskop 101.

Prints of electronmicrographs were examined for longitudinally sectioned bacteria, which were classified visually into 9 grades of morphological intactness, from "apparently intact" to "cell wall and minimal cytoplasm remaining". Examples of organisms from various grades are shown in Fig. 1. Two assessments were made of each biopsy, separated by a period of 21 months, as a test of the objectivity of the classification. In all, 50 sections were classified in each assessment of each biopsy. Comparisons between the assessments before and after treatment were made in two ways. In the first, the grades were scored 1 for Grade 1, 2 for Grade 2, and so on, and mean values and standard deviations calculated. A "standardized normal deviate" was calculated, this being the difference between 2 means divided by the standard error of the difference. This statistic was used to test whether there was a true difference between the 2 means. A value of 1.96 or more, which has only a 5% probability of occurring if there is in fact no difference between the true means, was taken to indicate a real difference.

The second method of comparing findings before and after treatment involved dividing the graded sections into "Grade 1" and "the rest" (corresponding roughly to the "solid"–"degenerate" classification used in light microscopy) and using either χ^2 or Fisher's exact test as appropriate, to determine whether the difference between the numbers of solid bacteria in the 2 groups before and after treatment could be ascribed to chance. A number of transverse sections of bacteria were examined to ascertain if there was any relation between (minimum) diameter, treatment, and apparent morphological intactness.

All the biopsies in Series B were examined "blind", the nature of the treatment, if any, being not known until afterwards.

Fig. 1. Examples of longitudinal sections of *Myco. leprae* in skin biopsies, showing some of the grades of degeneration used in quantitative investigation. (a) Intact bacteria (Grade 1) (EM x 70,000). (b) Early stage of degeneration; cytoplasmic membrane damaged, granular cytoplasm (Grade 2) (EM x 80,000). (c) Considerable degeneration of longer organism (Grade 5) (EM x 80,000). (d) Cell wall and minimal contents only remaining (Grade 9). Note electron-transparent part of wall revealed by contiguous organisms. (EM x 80,000)

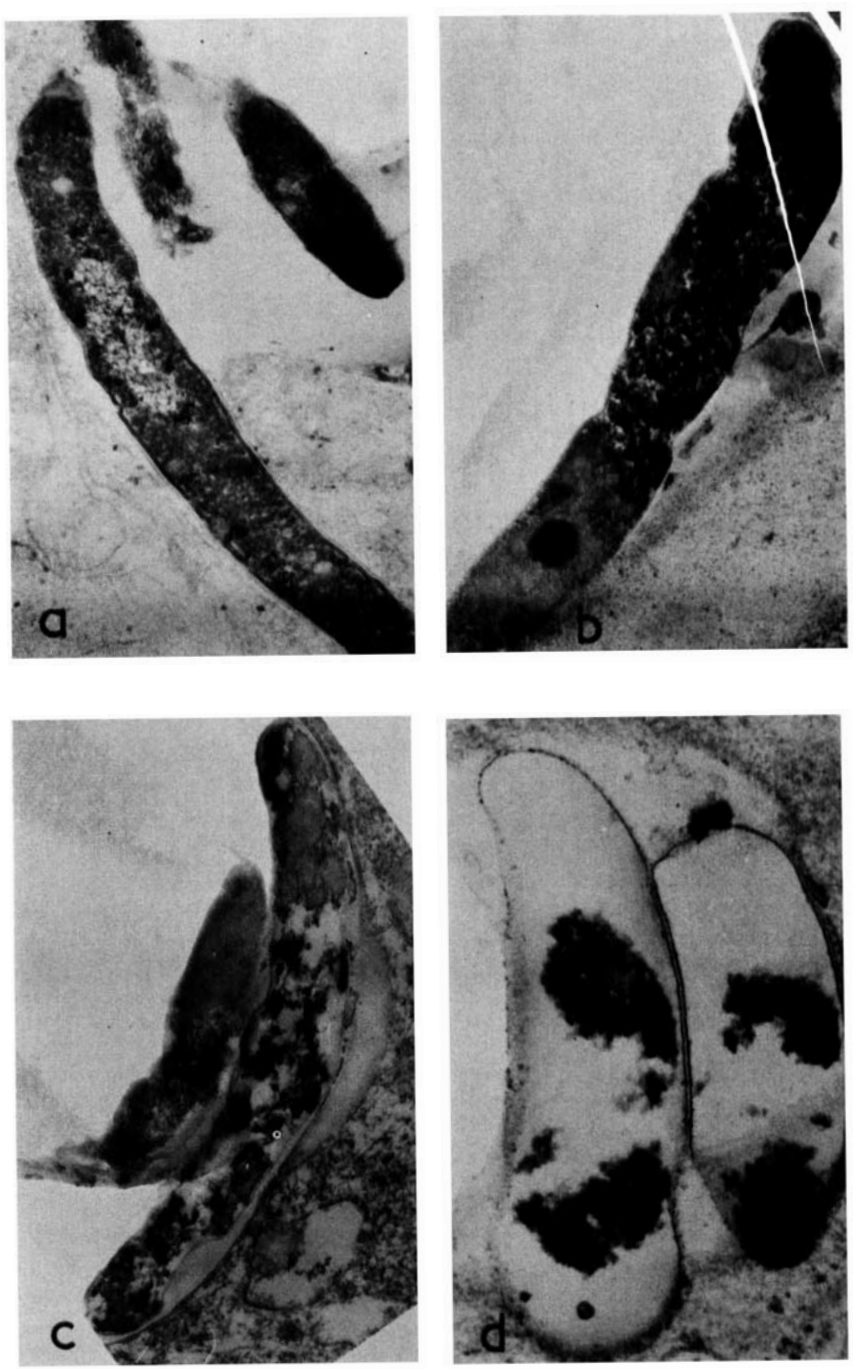

Fig. 1

Morphological indices of the bacterial suspensions (Waters, Rees and Sutherland, 1967) were kindly provided by Dr R. J. W. Rees.

Results

Data about longitudinal sections of bacteria in the three pairs of biopsies in Series A are shown in Tables 1 and 2. Agreement between the two assessments was very good, and both methods of statistical analysis showed that there was a significant shift towards more-degenerate categories after 1 year's treatment with DDS.

TABLE 1

Use of standardized normal deviate (d) to compare morphology of sectioned bacteria in biopsies taken before and after 1 year's treatment with DDS

		First assessment					Second assessment				
Patient no.	Biopsy	No. of sections	Mean[a] grade	± S.D.	d	*P*[b]	No. of sections	Mean grade	± S.D.	d	*p*
16101	Before	50	3.16	2.64	12.29	<0.001	50	3.18	2.62	10.6	<0.001
	After	50	8.16	1.15			50	8.14	1.14		
16052	Before	50	2.96	2.34	9.9	<0.001	50	2.38	1.96	13.1	<0.001
	After	49	6.82	1.49			51	6.88	1.45		
16136	Before	50	5.36	2.41	6.17	<0.001	50	5.34	2.33	6.4	<0.001
	After	50	7.76	1.33			50	7.76	1.32		

[a] Grades were scored from 1 for "intact sections" to 9 for "cell wall and minimal cytoplasm remaining".
[b] *P* is the probability that the observed difference in mean grade would occur by chance if there were no true difference.

TABLE 2

Use of χ^2 or Fisher's exact test to compare proportions of "intact" and "degenerate" sectioned bacteria in biopsies taken before and after 1 year's treatment with DDS

		First assessment				Second assessment			
Patient No.	Biopsy	Grade 1	Grade 2-9	χ^2	*P*[a]	Grade 1	Grade 2–9	χ^2	*P*
16101	Before	22	28	25.7	<0.001	17	33	18.1	<0.001
	After	0	50			0	50		
16052	Before	24	26	28.5	<0.001	28	22	36.2	<0.001
	After	0	49			0	50		
16136	Before	9	41	–[b]	0.001	8	42		0.006
	After	0	50			0	50		

[a] *P* is the probability that the observed difference between proportions of Grade 1 would occur by chance if there were no true difference.
[b] Where no χ^2 is given, Fisher's exact test was used to calculate *P*.

The results of 3 and 6 weeks' treatment with DDS, rifampicin, or streptomycin are shown in Tables 3 and 4. Agreement between the two assessments was good except for the first biopsy from patient 16316 (treated with streptomycin). Also, for the biopsies from this patient the two statistical methods used for comparing

TABLE 3

Use of "standardized normal deviate" (d) to test effect of short periods of treatment on morphology of sectioned bacteria

Patient No. Drug	Week after treatment started	First assessment: No. of sections	Mean grade ± S.D.	d	P[a]	Second assessment: No. of sections	Mean grade ± S.D.	d	P
	0	50	4.72 ± 2.73			50	4.8 ± 2.64		
16308	3	50	4.66 ± 2.25			50	5.00 ± 2.09		
	6	50	6.24 ± 2.37			50	6.2 ± 2.34		
Rifampicin	0 *v* 3			0.12	high			0.42	high
	0 *v* 6			2.98	<0.01			2.82	<0.01
	0	50	6.06 ± 2.2			50	6.26 ± 2.07		
16305	3	50	6.64 ± 1.41			50	6.82 ± 1.23		
	6	50	6.26 ± 1.7			50	6.32 ± 1.69		
DDS	0 *v* 3			1.57	high			1.86	high
	0 *v* 6			0.51	high			0.08	high
	0	50	3.42 ± 1.54			50	2.86 ± 1.68		
16316	3	52	4.37 ± 2.81			50	4.44 ± 2.73		
	6	50	5.06 ± 1.88			50	5.1 ± 1.8		
Streptomycin	0 *v* 3			2.13	<0.05			3.51	<0.001
	0 *v* 6			4.78	<0.001			6.4	<0.001

[a] *P* is the probability of the observed difference between mean grades occurring by chance in the absence of a true difference.

TABLE 4

Use of Fisher's exact test to compare proportions of bacteria in biopsies

Patient No. Drug	Weeks after treatment started	First assessment Grade 1	First assessment Grade 2–9	First assessment P[a]	Second assessment Grade 1	Second assessment Grade 2–9	Second assessment P
	0	7	43		9	41	
16308	3	3	47		2	48	
	6	0	50		0	50	
Rifampicin	0 v 3			0.205			0.029
	0 v 6			0.012			0.001
	0	5	45		4	46	
16305	3	0	50		0	50	
	6	0	50		0	50	
DDS	0 v 3			0.056			0.117
	0 v 6			0.056			0.117
	0	1	49		7	43	
16316	3	8	44		7	43	
	6	1	49		0	50	
Streptomycin	0 v 3			0.031[b]			—[c]
	0 v 6			—[c]			0.012

[a] *P* is the probability that the observed difference between proportions of Grade 1 would occur by chance if there were no true difference.

[b] Increased intactness.

[c] No change in intactness.

pre- and post-treatment findings gave discrepant results. There was no significant shift in the bacterial population after 3 or 6 weeks' treatment with DDS (patient 16305); nor after 3 weeks of rifampicin treatment (patient 16308), but a significant change, by both methods of statistical analysis, after 6 weeks' treatment with this drug.

The morphological indices of the bacteria from biopsies of Series A and B, measured by light microscopy, are shown in Table 5. The results agree qualitatively with those from electron microscopy. No clear-cut pattern was seen in the measurements of transverse sections.

The process of degeneration appeared to follow a definite pattern in the sections. First the cytoplasmic membrane of the bacteria broke down; at the same time the mesosome appeared to disintegrate and small pieces could be seen mingling with the cytoplasmic and nuclear material as it too disintegrated. Finally, the bacterial contents disappeared except for small fragments of cytoplasmic material. The cell wall remained intact throughout; both the inner electron-dense and the outer electron-transparent parts could be observed. If bacteria in the final stage of degeneration were contiguous, the electron-transparent part of the walls could be seen separating them.

Discussion

The use of the morphological index as an estimate of bacterial viability is important clinically as indicating, more rapidly and sensitively than do total

TABLE 5

Morphological indices, by light-microscopy, of Myco. leprae *in biopsies from patients in series B (Data kindly supplied by Dr R. J. W. Rees)*

Patient No. Drug	Weeks after treatment started	Degenerate bacteria (%)
16308	0	87
	3	98
Rifampicin	6	98
16305	0	86
	3	87
DDS	6	87
16316	0	86
	3	95
Streptomycin	6	91

counts, the progress of therapy and the infectiousness of the patient. It has been criticized (Chang and Andersen, 1969) because, at least in some other species of mycobacteria, the appearance of stained organisms can vary with different growth conditions and staining techniques. It is therefore encouraging to find that an ultrastructural degeneration of *Myco. leprae* occurs and corresponds to the changes indicated by light microscopy (and originally observed in unsectioned bacteria in the electron microscope), during antileprosy treatment. The results from the patient treated with streptomycin are equivocal, though they appear to indicate removal of bacteria; streptomycin is, however, regarded as a poorly effective drug against leprosy.

Two sources of error prevent quantitative comparison of light- and electron-microscopical results. Ultra-thin longitudinal sections include only about one-fifth of the whole organism, so that the first stages of the degenerative process may be missed. Further, the sections of necessity include only a very tiny part of the whole biopsy specimen, which in turn represents only a small sample of the patient's parasites. On the other hand, the bacteria are examined *in situ* in their tissue habitat, so that possible artefacts produced by homogenizing do not occur in the electronmicrographs.

Our study did not provide the hoped-for details of the early stages of the degenerative process. It did however draw attention to the persistence of bacterial walls more-or-less empty of cytoplasm; these would not be stained by carbol fuchsin. Although these walls can play no part in the spread of infection, their presence may cause a continuing pathological process in the patient freed from living bacteria by chemotherapy. Such a process cannot be controlled by existing antileprosy drugs.

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Demonstration of *Mycobacterium leprae* and its Viability in the Peripheral Blood of Leprosy Patients*

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Two methods for demonstrating *Mycobacterium leprae* in the blood of leprosy patients are described. On screening the blood of untreated lepromatous patients it was observed that all had bacteraemia. Bacteraemia was also observed in some patients with borderline and in some with treated lepromatous leprosy. Bacteraemia was not found in any one of the 15 patients with tuberculoid leprosy. High bacterial loads and the morphological integrity of the organisms in the skin were correlated with the degree of bacteraemia. The *Myco. leprae* in the blood were found to be viable. The evidence presented allows the conclusion that blood-sucking arthropods can take up viable *Myco. leprae* during a blood meal.

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Introduction

Mycobacterium leprae has been demonstrated not only in the Schwann cells of the nerves and histiocytes of the skin, but also in lymph nodes (Desikan and Job, 1966; Sharma and Srivatsava, 1958), spleen (Powell and Swan, 1955), liver and kidney (Mathur, Gupta and Singaravi, 1961), bone marrow (Gass and Rishi, 1934; Karat, 1966; Low and Dharmendra, 1937), muscle (Convit, Arvello and Mendoza, 1960), nasal mucosa and endothelial cells of capillaries (Fite, 1941). This implies that the bacilli must have disseminated through the blood stream into various organs and tissues. The final objective of our study is to throw light on the rôle of arthropods in the transmission of leprosy. In this connection an effort was made to determine the extent of bacteraemia in patients with untreated lepromatous leprosy and in those under drug treatment for varying periods.

Materials and Methods

The patients studied were selected at random from untreated and treated lepromatous, borderline, and tuberculoid cases. They were attending either

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leprosy clinics around Pondicherry or the out-patient department of the Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry. They were classified clinically according to the type of leprosy from which they suffered. Skin-slit smears were taken from various parts of the body. In collecting the blood samples the syringe was moistened with heparin solution (1000 units per ml) and 4 to 7 ml of blood then drawn from the cubital vein. None of the patients was in a reactional phase or in a febrile state at the time of the collection of blood.

Demonstration of Acid-Fast Bacilli

Two methods were used to demonstrate acid-fast bacilli in the macrophages of the blood.

1. THE BUFFY COAT METHOD

In this method heparinized blood is transferred into a test tube which is kept upright in a refrigerator for 5 h. The plasma is then pipetted off and the leucocytes from the buffy coat are transferred on to a clean glass slide with a capillary pipette. A leucocyte smear is prepared by the usual haematological technique, and air dried. The smear is fixed in formaldehyde vapour and stained by the Ziehl-Neelsen method.

2. THE LEUCOCYTE ADHERENCE METHOD

For this method approximately 4 ml of heparinized blood is kept in the refrigerator. The red blood cells settle down within an hour, leaving most of the leucocytes still in suspension. A drop of leucocyte-rich plasma is then transferred to a clean dry slide and the slide placed in a Petri dish with moist filter paper at the bottom to prevent drying out of the plasma drop. The leucocytes settle down and stick on the slide in about 30 min. The plasma is then gently washed off, using 0.5% phenol saline with the slide held at a slant. Finally the slide is air dried, fixed in formaldehyde vapour, and stained.

Samples of the plasma containing the leucocytes were inoculated in Löwenstein-Jensen medium and into the footpads of mice.

It was observed that in buffy coat smears the plasma with the remaining red cells formed, on drying, a wrinkled film which when stained interfered with the clear visualization of the acid-fast bacteria. Preparation of the smear, staining, and examination took a relatively long time (about 5 to 6 h). To overcome these shortcomings the leucocyte adherence method was employed. With this method the smears were uniform and clear, and the acid-fast bacilli when present could be demonstrated with ease. The total time taken for the examination of blood was also much less (2 to 3 h).

Results

Heparinized blood from 186 leprosy patients was examined. They comprised 151 cases of lepromatous, 15 cases of tuberculoid and 20 cases of borderline leprosy. The total incidence of bacillaemia was 41.4% (77 cases). All 38 untreated lepromatous patients had acid-fast bacilli (AFB) in the peripheral blood (Table 1).

TABLE 1

Incidence and degree of bacteraemia in patients with different types of leprosy

Type of case	Total no. of cases	Total no. of bacteraemic cases	No. of AFB in 500 fields of leucocyte smear 5–20	21–40	41-80	>81
Lepromatous						
(a) Untreated	38	38 (100%)	14	13	4	7
(b) Treated for 6 months	31	14 (45.16%)	6	5	3	
(c) Treated for one year	12	4 (33.3%)	3	1		
(d) Treated for more than one year	70	16 (22.86%)	9	4	2	1
Borderline	20	5 (25%)	5			
Tuberculoid	15	nil				
Total	186	77 (41.4%)	37	23	9	8

Of the 43 lepromatous patients treated for one year or less, 18 (41.86%) had bacteraemia. In 70 lepromatous patients who were treated for more than one year, only in 16 (22.86%) were bacteria seen in the blood. None of the 15 patients with tuberculoid leprosy had AFB in the peripheral blood, while only 5 (25%) of the 20 patients in the borderline group had visible evidence of bacteraemia.

The AFB were seen predominantly in macrophages (Fig. 1). In several instances, particularly among untreated lepromatous cases, the bacilli could be demonstrated also in the polymorphonuclear leucocytes (Figs 2 & 3). Extracellular AFB were present in a few instances. In most cases the bacilli were solidly stained and could be counted with ease, even when inside the macrophages. In

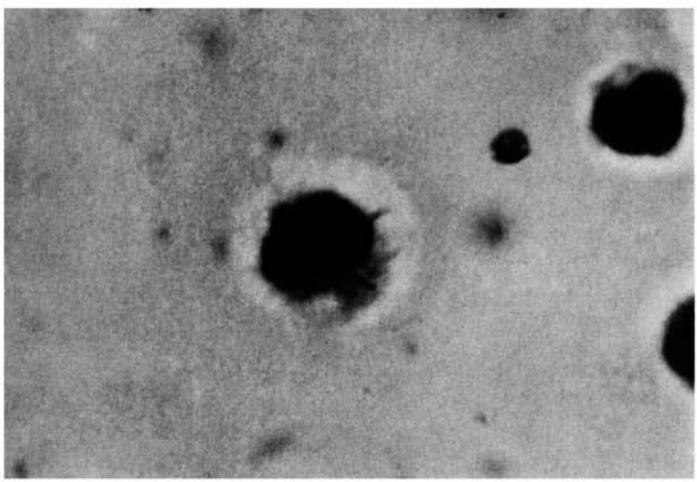

Fig. 1. Solidly stained *Myco. leprae* in a macrophage.

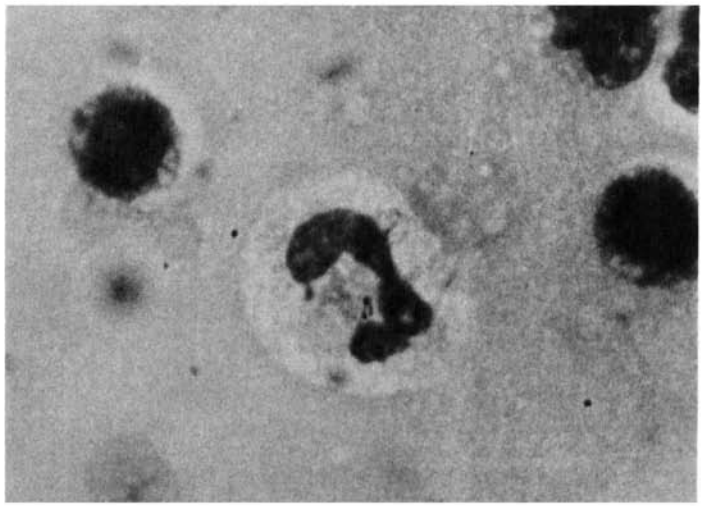

Fig. 2. Granular *Myco. leprae* in a polymorphonuclear leucocyte.

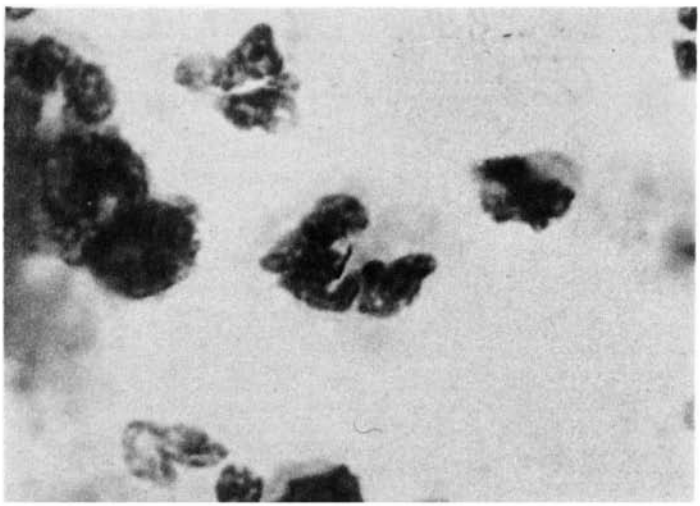

Fig. 3. Solidly stained *Myco. leprae* in a polymorphonuclear leucocyte.

some of the advanced lepromatous cases the macrophages were so packed with bacilli that it was difficult to count them (Fig. 4), particularly in those cases where the macrophages of the blood contained 30 or more bacilli per cell.

The number of bacilli varied from 5 to 120 per 500 fields of leucocyte smears, or expressed as bacteria per macrophage, it ranged from 2 bacilli per 100 cells to 2 bacilli per cell. From these data it was calculated that there were from 5×10^3 to 5×10^5 bacilli per ml of blood.

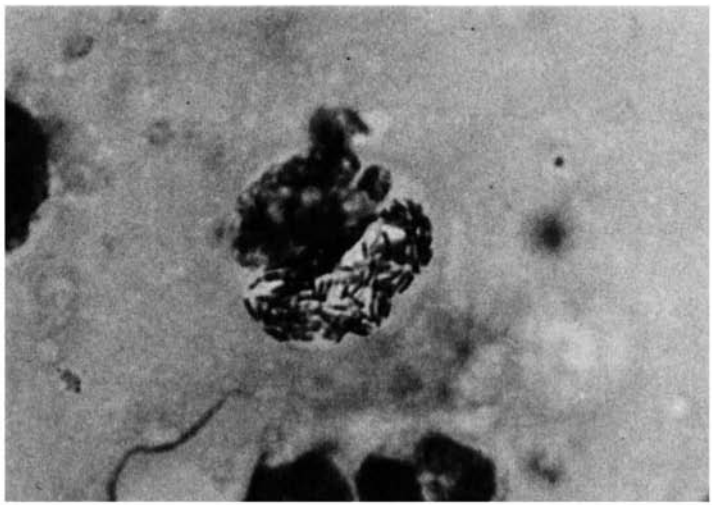

Fig. 4. Macrophage loaded with solidly stained *Myco. leprae.*

Skin slit smears from these patients were routinely examined and the bacterial index on the Ridley scale was found to be between 1 and 4. The morphological index varied from less than 2 to 40%. Aliquots of plasma containing leucocytes, cultured on Löwenstein-Jensen medium did not yield any growth of mycobacteria. Out of 77 samples of blood which were positive for AFB 15 were inoculated into the footpads of mice, and so far multiplication of AFB in the footpads has been obtained in 7 of them. The results for the remaining samples will become available later.

Discussion

Montel (1949) demonstrated leprosy bacilli in the blood of lepromatous patients, especially during febrile states. More recently Drutz and Levey (1970) have reported the continuous nature of bacillaemia in lepromatous leprosy. Our present investigation, besides confirming bacillaemia in lepromatous leprosy, also reports the occurrence of bacillaemia in borderline leprosy and its absence in tuberculoid leprosy. There is evidence of a continuous bacillaemia in all untreated lepromatous patients. The degree of bacillaemia is proportional to the bacterial and morphological indices of organisms in the skin. Moreover, bacilli from the blood of 7 patients were shown to be viable (infectious) in the mouse, but it is not known whether *Myco. leprae* can retain viability in blood containing high concentrations of DDS (dapsone).

According to Beiguelman (1967) macrophages of lepromatous patients do not digest *Myco. leprae,* whereas those of tuberculoid patients do. This may be one of the reasons for the absence of bacteraemia in tuberculoid patients. Although patients in the group with borderline leprosy had bacteraemia less frequently on the whole than those in the group with lepromatous disease, no histopathological classification of the borderline group was made.

There are two possible explanations for the persistence of the bacteraemia in

TABLE 2

Correlation between bacteraemia and bacterial and morphological indices of Myco. leprae *in leprosy*

Type of cases	No. of bacteraemic cases	Bacterial index				Morphological index (%)					No. of AFB in 500 fields			
		1	2	3	4	<2	2–10	11–20	21–30	>31	5–20	21–40	41–80	>81
Lepromatous														
(a) Untreated	38		2	19	17		4	20	9	5	14	13	4	7
(b) Treated six months	14		1	8	5			9	3	2	6	5	3	
(c) Treated for one year	4	1	1	1	1	1	2	1			3	1		
(d) Treated for more than one year	16		1	8	7	3	2	5	3	3	9	4	2	1
Borderline														
Untreated	3			2	1		1	1	1		3			
Treated	2		1	1		1		1			2			

lepromatous patients treated for more than one year. On the one hand the patients might not have taken their drugs regularly, or on the other hand the persistent bacteraemia might signify the emergence of drug-resistant bacilli.

The present investigation has helped in the selection of patients for feeding experiments with laboratory-reared insects. The constancy of bacteraemia in lepromatous patients together with the high prevalence of blood-sucking insects in this leprosy endemic area make it quite possible that arthropods could take up bacilli from patients during a blood meal.

Finally, bacteraemia should be taken into consideration by those in charge of blood banks where there may be the possibility of taking blood from persons with *undiagnosed* leprosy.

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Arthropod Feeding Experiments in Lepromatous Leprosy*

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Laboratory-reared *Culex fatigans* and *Cimex hemipterus* were fed on untreated lepromatous leprosy patients. The presence of acid-fast bacteria in a high proportion of these insects after feeding showed that they can take up the bacilli from the patients' blood. The dependence of infestation of fed insects on the degree of bacteraemia in patients and the detection in *Cimex* of bacteria-laden leucocytes suggest that the insects took up the bacilli along with the blood rather than from the skin. Results of mouse footpad harvests showed multiplication of *Myco. leprae*, and therefore one must conclude that the leprosy bacilli in the insects were viable.

Introduction

The occurrence of mycobacteria in human parasitic arthropods in the field and the possibility of at least some of these being *Myco. leprae* has been shown in our laboratory (Narayanan *et al.*, 1972). The epidemiological significance of this observation is at the present time not clear. To be an effective vector an arthropod must be able to pick up the required number of bacilli from a patient and should contribute to the inoculation of the bacilli into another susceptible person. In addition, the bacilli must be able to survive inside the arthropod for a sufficiently long time, this depending on the feeding habits of the arthropod. This paper deals with feeding experiments conducted with mosquitoes and bed-bugs to investigate the ability of these arthropods to take up the bacilli from untreated lepromatous leprosy patients.

Materials and Methods

For the feeding experiments volunteer untreated lepromatous leprosy patients were selected from both roadside clinics and out-patients of the Department of Skin and Venereal Diseases of the Jawaharlal Institute at Pondicherry and admitted to the infectious diseases ward of the hospital. The patients' blood was investigated before commencing the experiment for the presence of acid-fast

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bacilli. The methods used for this study have been reported separately (Shankara Manja *et al.*, 1972).

Colonies of *C. fatigans* and *C. hemipterus* were established in the laboratory for feeding experiments. Field collected females of *C. fatigans* were allowed to oviposit in the laboratory to start the colonies; the larvae were reared in pans, using yeast powder as food. Laboratory-reared adult females were fed on mice for further propagation. The method adopted by Wattal and Kalra (1961), using wooden traps, was followed for the maintenance of *C. hemipterus* colonies.

Laboratory-reared female mosquitoes about 5 days old were used for the experiments. Female mosquitoes randomly drawn from laboratory colonies were examined microscopically and culturally to ascertain that they were free of acid-fast bacilli. For the feeding experiments with bed-bugs, nymph-stage *Cimex* or fresh adults from laboratory colonies starved for 14 days or more were used. Nymphs and adults drawn at random from laboratory breeding cages were examined microscopically and culturally for contaminating acid-fast bacilli.

The feeding experiments with mosquitoes were done at night in insect-proof rooms of the infectious diseases unit. The volunteers were given 65 mg of Gardinal (phenobarbitone) to ensure undisturbed sleep. A mosquito net was lowered over the bed and secured at the lower sides after the subjects were asleep; known numbers of mosquitoes were then released into the net. Two workers kept watch over the patient throughout the night to render him any assistance if he wanted to come out of the net and to ensure that the mosquitoes did not escape during the process. All mosquitoes fed, unfed, or dead were recovered and accounted for in the morning, and those which had fed were separated for further examination.

Feeding experiments with bed-bugs were conducted during the day. For this purpose a screw cap of a McCartney bottle with perforations on top was used. Four pieces of black paper with slits cut along the sides were snugly fitted inside the cap with spaces between the layers. Known numbers of bugs were released into the cap, using a fine artist's brush. The bugs readily entered the cap through the slits in the black paper and rested inside. The mouth of the cap was covered with fine voile cloth, fastened with rubber bands, and the cap then placed on the body of the volunteer, with the voile side in contact with the skin and secured with bandage cloth. After about 2 h the bugs were taken out for examination.

Fed mosquitoes and bed-bugs were killed by chilling. The wings and legs of the mosquitoes were removed before making a homogenous suspension. Then the mosquitoes and bugs were ground separately in a cold mortar, using 1 ml of 0.1% bovine albumin (fraction V) in Hanks balanced salt solution. This suspension was examined microscopically for acid-fast bacilli (AFB). As the numbers of bacilli in the suspension were too low to be counted, 0.03 ml of the suspension was directly inoculated into the footpads of Swiss albino mice (Rockefeller strain).

Results

The results of the feeding experiments with *Culex* showed that, out of 38 successful feeding experiments, mosquitoes in 27 experiments had taken up AFB, as seen by microscopy (Table 1, Fig. 1). Of the 35 experiments conducted with *Cimex* 18 were microscopically positive for AFB (Table 2, Fig. 2, 3).

The blood samples from volunteers 1 to 5 were not investigated for bacteraemia, but it is reasonable to assume that they were positive because all

TABLE 1

Results of feeding experiments with mosquitoes

Patient No.	Degree of bacteraemia (AFB per 500 fields)	No. of feeding experiments	Nature of feeding	AFB in mosquito homogenate Positive	Negative
1	Not recorded	1	Nil		1
2	Not recorded	1	Nil		1
3	Not recorded	4	1 Nil 3 Fair	3	1
4	Not recorded	1	Poor		1
5	Not recorded	2	Poor	1	1
6	73	3	Poor	3	
7	0	1	Poor		1
8	23	3	Fair	2	1
9	18	1	Fair		1
10	28	3	Good	3	
11	21	3	Fair		3
12	24	3	Fair	2	1
13	16	1	Nil		1
14	48	3	Fair	2	1
16	26	3	Fair	2	1
17	100	3	Fair	3	
19	83	3	Fair	2 + (1)	
20	120	3	Fair	3	

Figures in brackets indicate impression smears.

TABLE 2

Results of feeding experiments with bed-bugs

Patients No.	Degree of bacteraemia (AFB per 500 fields)	No. of feeding experiments	Nature of feeding	AFB in bed-bug homogenate Positive	Negative
10	28	2	Good	0	2
11	21	1	Good	0	1
12	24	2	Good	1	1
12	Not significant[a]	2	Good	0	2
13	16	1	Good	0	1
14	48	4	Good	3	1
15	18	2	Good	0	2
16	26	3	Good	2	1
17	100	3	Good	3	0
18	35	4	Good	1	3
19	83	3	Good	1 + (2)	0
20	120	3	Good	2 + (1)	0
21	63	2	Good	1	1
22	11	3	2 Good 1 Poor	1	2

[a] After about 4 months of treatment with DDS.

Figures in brackets indicate impression smears.

untreated lepromatous leprosy patients have been shown to be bacteraemic (Shankara Manja *et al.*, *loc. cit*). The results of the feeding experiments showed that the microscopical detection of acid-fast bacteria in the insects depends on the degree of bacteraemia of the patient.

In one of the 38 successful feeding experiments with *Culex* mosquitoes, a homogenate of mosquitoes was prepared 48 h after feeding and 0.03 ml of the suspension was inoculated into mouse footpads. The footpad harvest 6 months after inoculation yielded less than 3×10^4 AFB per mouse; after about 9 months the total yield went up to $5 \pm 0.5 \times 10^5$ AFB per mouse. Finally, in about 14 months, it reached a maximum of $1.05 \pm 0.1 \times 10^6$ AFB per mouse. This shows that there was a steady increase in the number of bacilli in the footpads of mice. On re-inoculation almost the same results were obtained in the new group of mice showing a 100 to 300-fold increase of bacilli in between 6 and 9 months. In another experiment with a different volunteer, *Culex* mosquitoes yielded $1.2 \pm 0.2 \times 10^5$ AFB per mouse in about 14 months after inoculation. In this experiment the suspension was prepared immediately after feeding. These bacteria have now been re-passaged into a new batch of mice and the results are awaited. None of the AFB obtained in these experiments grew on Löwenstein-Jensen (L-J) medium. A typical growth pattern in the footpads of mice, together with the inability to grow on L-J medium, are highly suggestive of *Myco. leprae*.

Examples of acid-fast bacilli in homogenates of mosquitoes and bed-bugs are shown in Figs 1 and 2 and the presence of bacilli in a leucocyte in a smear prepared from a bed-bug is shown in Fig. 3.

Discussion

It is a well-known fact that the incidence of viable *Myco. leprae* in patches on the skin of untreated lepromatous leprosy patients is much higher than in the skin

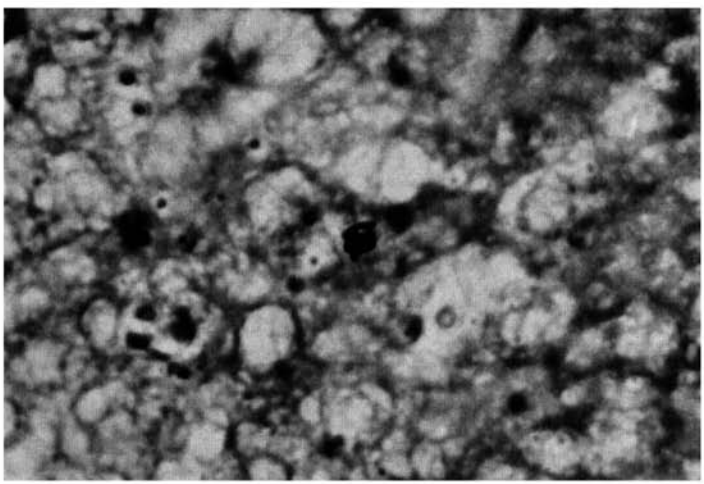

Fig. 1. A clump of solidly stained *Myco. leprae* in ground suspension of mosquitoes fed on a leprosy patient.

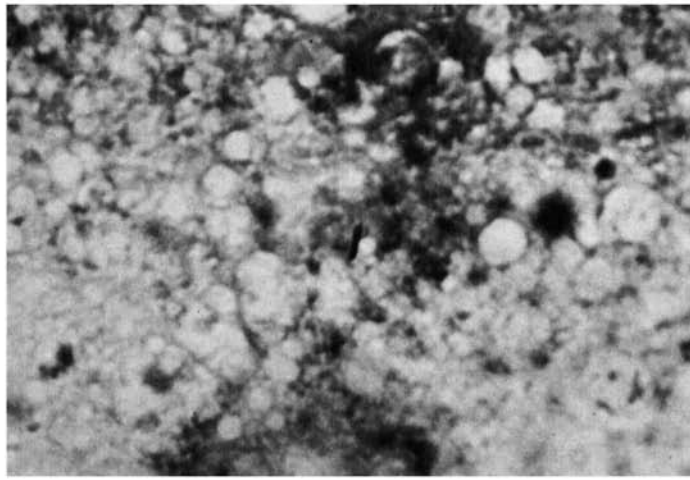

Fig. 2. A single solidly stained *Myco. leprae* in a ground suspension of bed-bugs fed on a leprosy patient.

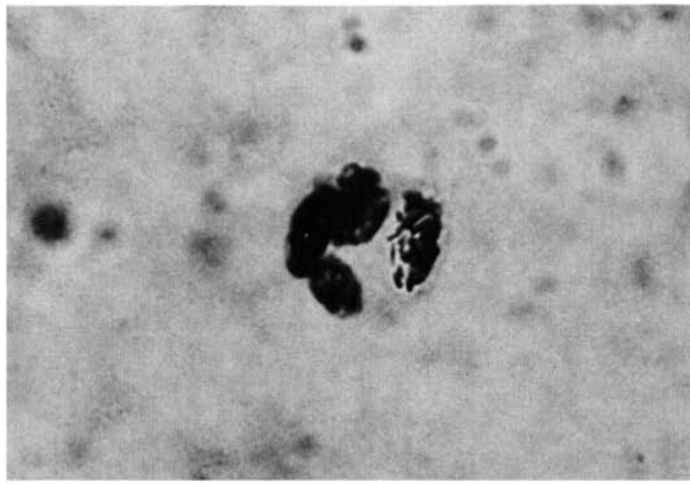

Fig. 3. A partially digested leucocyte loaded with solidly stained *Myco. leprae* in a smear made from a bed-bug after feeding on a leprosy patient.

elsewhere. It has been shown in our laboratory that the degree of bacteraemia increases in relation to the bacterial and morphological indices of the bacteria in the skin-slit smear. It also has been shown in our laboratory that blood-borne *Myco. leprae* are viable. An arthropod feeding on a leprosy patient can ingest *Myco. leprae* either while its mouth parts are penetrating the skin containing the bacteria or along with the blood it draws. While the method of mosquito-feeding experiments used in these studies permits the mosquitoes to suck blood from any

convenient point in the body, the bed-bugs were mostly confined to patches on the body for their feeding. There was, however, no indication that the bugs had ingested more bacilli than the mosquitoes, and this may be because the bacilli are taken up along with the blood by both these insects and not from the skin at the time of penetration. The finding of intact leucocytes containing bacilli in bugs examined after feeding supports this view. Suspensions prepared from fed *Culex* mosquitoes immediately after feeding and again after 48 h were inoculated into mouse footpads and yielded $1.2 \pm 0.2 \times 10^5$ and $1.05 \pm 0.1 \times 10^6$ bacteria respectively in 14 months, showing that the insects had indeed taken up viable organisms.

Twenty pools of laboratory-reared *Culex* mosquitoes and 10 pools of laboratory-reared bed-bugs were quite free of AFB. This makes it very likely that the AFB found in the insects which had fed on patients were taken up during the feeding process. McFadzean and Macdonald (1961) allowed mosquitoes and bed-bugs to feed on lepromatous leprosy patients with highly positive skin smears and then re-fed the insects on patients with tuberculoid leprosy. On the basis that no microlepromin reaction was produced in the tuberculoid patients, they doubted the ability of these insects to pick up the bacilli from the skin. The results of the present study however show that *C. fatigans* and *C. hemipterus* can take up the bacilli along with the blood meal.

Acknowledgements

The authors are grateful to the authorities of Polambakkam Leprosy Centre, the Government Leprosy Treatment and Study Centre, Tirukoilur, the Hemerijckx Rural Welfare Centre, Rawattankuppam and to Mr G. Kasturi, Social Worker, for help in obtaining volunteers for experiments. The authors are also grateful to Messrs J. Venkateswarlu, V. Samuel and M. Leo for their technical assistance.

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Lepr. Rev. (1972) 43, 194-198

Occurrence of *Mycobacterium leprae* in Arthropods*

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Mosquitoes, bed-bugs, head lice and scabies mites were collected from the dwellings of persons suffering from lepromatous leprosy (patient collections) and from those where no known case of leprosy existed (random collections). Suspensions made from pools of these arthropods were used for making smears for acid-fast staining, culture on Löwenstein-Jensen medium and for mouse footpad inoculation. In the patient collections acid-fast bacteria were detected microscopically in 4.1% of *Anopheles*, 3.6% of *Culex*, 22.2% of *Anopheles* and *Culex* (mixed), 4.8% of *Cimex*, 7.4% of *Pediculus* and in a single pool of *Sarcoptes*. In the random collections acid-fast bacteria were found in 7.7% of *Anopheles*, 6.8% of *Culex*, 9.2% of *Cimex*, in none of *Pediculus*, and in 2 out of 3 *Sarcoptes* pools. Footpad multiplication was obtained from 2 *Culex* pools, one collected at random and the other from patients. The findings strongly support the conclusion that the acid-fast bacteria obtained from the two pools of *Culex* were indeed *Myco. leprae*.

Introduction

It is generally believed that the portal of entry of *Myco. leprae* into the human body is through the skin, and since the bacteria by themselves cannot penetrate the skin the view has been put forward that biting arthropods may act as vectors. In support of this assumption several workers have demonstrated acid-fast bacteria (AFB) in arthropods associated with man. Dungal (1960) has discussed the possible rôle played by several arthropods such as lice, scabies mites, fleas, and mosquitoes as vectors. Spickett (1961) incriminated follicular mites also. Until recently it was impossible to establish the identity of these AFB as *Myco. leprae* in such arthropods owing to the lack of a reliable technique, but this handicap has been at least partially removed by the mouse footpad inoculation technique of Shepard (1960). The present study was undertaken to investigate whether mosquitoes, bed-bugs, lice, and scabies mites in the leprosy endemic area of Pondicherry and surrounding places harboured AFB in field conditions. Shepard's mouse footpad technique was used to culture and identify *Myco. leprae*.

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Materials and Methods

The arthropod collections were made from the leprosy endemic area around Pondicherry. Suspensions of the arthropods were prepared for microscopic examination for AFB, for inoculation into Löwenstein-Jensen (L-J) medium for isolating cultivable mycobacteria, and for inoculation into mouse footpads for multiplication. The arthropods were collected early in the morning, the mosquitoes from inside dwellings, bed-bugs from furniture and crevices in the walls and floors of dwellings, and lice and mites from persons. Separate collections were made from leprosy patients and their houses (patient collections), and from houses having no known case of leprosy (random collections). Only female mosquitoes were used for preparation of the suspensions and for convenience arthropods were pooled according to genus and locality, except in some instances when *Anopheles* and *Culex* mosquitoes were pooled together.

For microscopic examination field-collected arthropods were killed by chilling and the wings and legs of the freshly killed arthropods detached, leaving the proboscis intact. All procedures were carried out in a cold sterile Petri dish surrounded by ice. The arthropods were then ground in a sterile mortar or a cooled tissue grinder and suspended in 1 ml of 0.1% bovine albumin in Hanks' balanced salt solution (BSS). Smears were prepared from the homogenate and stained by the Ziehl-Neelsen method. As there were very few AFB in the homogenate it was not possible to concentrate the bacteria for counting. Therefore, 0.03 ml of the material was inoculated into the hind footpads of Swiss albino mice (Rockefeller strain). The remainder of the material was treated with a drop of 4% sodium hydroxide for 30 min at room temperature and then neutralized with 8% hydrochloric acid. Aliquots of this material were cultured on the L-J medium. The culture tubes were incubated at 37° and 25° C for 8 weeks and examined at weekly intervals for growth of mycobacteria.

Footpad harvests were planned to be carried out after 6 months, 1 year, and 1 to 2 years by the method described by Shepard (*loc. cit.*). In some initial cases footpads were harvested after 3 months. The footpad tissue was ground in a tissue grinder, using 0.1% bovine albumin in BSS. The AFB obtained from the footpad were counted by the method of Hanks, Chatterjee and Lechat (1964). Following counting, the footpad homogenates were inoculated into the footpads of new mice. Aliquots of the material were also inoculated on L-J medium.

Results

The results of the microscopical examination and footpad harvests pertaining to arthropod collections from patients' houses and random collections are shown in Tables 1 and 2. Of the 218 *Anopheles* pools from patients' houses 9 were positive microscopically and 71 were inoculated into mouse footpads, but harvests at 3 months, 6 months, and 1 year did not show any acid-fast bacilli. Of 111 *Culex* pools, 4 were positive microscopically, while of 15 inoculated into mouse footpads clear indication of footpad multiplication was obtained in only one instance at 6 months harvest; this inoculum had been microscopically negative. Of 36 mixed pools (*Anopheles* and *Culex*) 8 were positive microscopically and 7 were inoculated into mouse footpads. Acid-fast bacilli were seen in one 3-month harvest, but not in countable numbers; further harvests were negative.

TABLE 1

Incidence of non-culturable acid-fast bacteria (AFB) in arthropod collections from leprosy patients' houses and results of footpad harvests

Arthropods	No. of pools examined	Smears positive for AFB	No. of pools inoculated into mouse footpads	Footpad harvests positive for AFB at: 3 months	6 months	1 year
Anopheles	218	9	71	0	0	0
Culex	111	4	15	0	1 (−) (5.6×10^4)	0
Anopheles and *Culex* mixed	36	8	7	1 (+)[a]	0	0
Cimex	62	3	18	0	0	0
Pediculus	54	4	3	0	–	–
Sarcoptes	1	1	1	1 (+)[a]		

(−), From smear negative pool; (+), From smear positive pool; [a], Not countable.

TABLE 2

Incidence of non-culturable acid-fast bacteria in random arthropod collections and results of footpad harvests

Arthropods	No. of pools examined	Smears positive for AFB	No. of pools inoculated into mouse footpads	Footpad harvests positive for AFB at: 3 months	6 months	1 year
Anopheles	246	19	41	1 (+)[a]	1 (+)[a]	0
Culex	292	20	32	0		1 (−) (1.6×10^6)
Cimex	98	9	18	2 (+) 1 (−)[a]	0	0
Pediculus	44	0	3	–	–	–
Sarcoptes	3	2	2	1 +)[a]		

(−), From smear-negative pool; (+), From smear-positive pool; [a], Not countable.

Thus, out of 62 *Cimex* pools 3 were positive microscopically and 18 were inoculated into mouse footpads, but no indication of multiplication of AFB was seen in footpads up to 1 year. Out of 54 *Pediculus* pools 4 were positive microscopically and 3 of these were inoculated into mouse footpads, but there was no indication of footpad multiplication of AFB. The single pool of *Sarcoptes* was positive microscopically and AFB were detected in the footpad at 3 months' harvest, but not at subsequent harvests.

Microscopically, acid-fast bacilli were more common in random collections. Of the 246 *Anopheles* pools, 19 were positive and 41 were inoculated into mouse footpads. AFB were seen in smears made from footpad material in two instances (at 3 months and 6 months), but there were not enough bacteria to make a count. Of the 292 *Culex* pools, 20 were positive microscopically and 32 were inoculated into mouse footpads. Some indication of multiplication in the mouse footpad was seen in one 1 year harvest; the original inoculum of this was negative microscopically. Of the 98 *Cimex* pools, 9 were positive microscopically and 18 were inoculated into mouse footpads. In 3 cases AFB were seen in 3-month footpad harvests, but not subsequently. All 44 *Pediculus* pools were negative

microscopically, and 3 of these when inoculated into footpads gave negative results. Of the 3 *Sarcoptes* pools 2 were positive microscopically, 2 were inoculated into mouse footpads and AFB were seen in one 3-month harvest, but not subsequently.

The incidence of culturable mycobacteria was low in field collected arthropods—as can be seen in Tables 3 and 4.

TABLE 3

Results of culture on L-J of arthropod material from patients' houses

Arthropods	No. of pools cultured	Smears positive for AFB	No. of pools positive on culture
Anopheles	197	8	3
Culex	85	1	0
Anopheles and *Culex* mixed	35	8	0
Cimex	51	3	2
Pediculus	15	2	1
Sarcoptes	1	1	0
Total			

TABLE 4

Results of L-J culture of arthropod material from random collections

Arthropods	No. of pools cultured	Smears positive for AFB	No. of pools positive on culture
Anopheles	215	19	1
Culex	265	21	3
Cimex	84	9	1
Pediculus	18	0	0
Sarcoptes	2	1	0
Total			

As mentioned above, footpad multiplication was obtained from 2 pools of *Culex* mosquitoes, one in a patient collection and the other in a random collection. The patients' pool suspension inoculated into footpads yielded 5.6×10^4 acid-fast bacilli at 6 months. The material from the footpads was re-inoculated into a second batch of mice in order to study the pattern of multiplication. In about 7 months a 200-fold increase was observed. The random *Culex* pool inoculated into footpads yielded 1.6×10^6 acid-fast bacilli in 1 year.

Discussion

That *Culex* mosquitoes and bed-bugs can take up *Myco. leprae* from leprosy patients, and that the number of bacilli taken up depends on the degree of bacteraemia of the patient, has been demonstrated in our laboratory and is being reported separately (Narayanan *et al.* 1972). The microscopical detection of

non-culturable AFB in field-collected arthropods will therefore depend on whether the arthropods have actually fed on patients with a sufficiently high level of bacteraemia. In order to produce multiplication in mouse footpads, the arthropods should have ingested a sufficient number of viable *Myco. leprae.* In observations made in this laboratory *Myco. leprae* has been found to retain its viability in *Culex* mosquitoes for at least 48 h.

This evidence, together with the isolation of non-culturable mycobacteria from *Culex* mosquitoes in the footpads of mice, strongly suggests that at least a few *Culex* mosquitoes in this endemic area carry viable *Myco. leprae.* The 200-fold increase of acid-fast bacilli in footpads in one case and the ceiling number of over one million AFB obtained in another is typical of footpad multiplication of *Myco. leprae.* It is likely that the randomly collected *Culex* might have taken up *Myco. leprae* from leprosy patients and come to rest in a house having no leprosy patient.

With regard to *Anopheles* mosquitoes, bed-bugs, head lice and scabies mites, in the absence of footpad multiplication it is not possible to draw any conclusions at present. Microscopical detection of acid-fast bacteria by itself is inconclusive because they might not be leprosy bacilli, or even if they were, they might be non-viable. On the other hand, samples found to be negative microscopically might nevertheless contain viable *Myco. leprae,* as shown in two instances where mouse footpad multiplication took place.

When suspensions of arthropod material were inoculated into mouse footpads, inflammatory reactions were observed. This problem is being overcome with the use of anti-inflammatory drugs and the results are expected to show whether the inflammatory reaction can interfere with the proper multiplication of *Myco. leprae* in mouse footpads.

Acknowledgements

The authors are grateful to the authorities of Polambakkam Leprosy Centre, the Government Leprosy Treatment and Study Centre, Tirukoilur, to the Hemerijckx Rural Welfare Centre, Rawattankuppam, and to Mr G. Kasturi, Social Worker, for locating leprosy patients. They are also grateful to Messrs J. Venkateswarlu, V. Samuel and M. Leo for technical assistance.

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Some Further Aids for the Handicapped Leprosy Patient*

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On the basis of his practical experience in the field the author describes four ingenious and, in respect of three of them, inexpensive appliances which have been devised primarily for the use of handicapped leprosy patients—but which could be equally useful to persons disabled from other causes—to enable them to carry on a useful and gainful occupation.

Introduction

Some of the common deformities of leprosy patients are also seen in other conditions such as the ulnar-nerve damage of industrial injury, or the unshapely and stiff hands of sufferers from rheumatoid arthritis.

I consider that "experienced" social workers are often unnecessarily concerned about the "stigma" of special tools and appliances, which they would otherwise recommend. A handle may be "too bulky" and a tool so "unconventional" that the user will "at once be recognized" as suffering from leprosy the moment he uses it outside the institution. I would assert that anything that makes work easier is acceptable, and is usually accepted. Aids for those suffering from deformities caused by leprosy could also be used to help people disabled by other causes, and furthermore, if any of them can be of help to normal people to work more easily or rapidly, then their acceptance is more assured.

The Action-Research Project at the Dr Bandorawalla Leprosy Hospital, Kondhwa, Poona has, as one of its objectives, the experimental social rehabilitation of ex-leprosy patients after vocational training. Carpentry, tailoring, cane-work, sheep breeding, and poultry farming are some of the vocations patients learn under this project before their discharge. A high proportion of these patients have irremediable handicaps. Working for this project, I have had the opportunity of devising, and experimenting with, aids for such patients.

New Aids

Four new aids specially devised for handicapped leprosy patients are described below.

1. THE "KONDHWA" CLOTH-CUTTER (Fig. 1)

This is constructed by mounting a pair of cloth-cutting scissors on a wooden table. One of its blades is rigidly fixed to the table while the other

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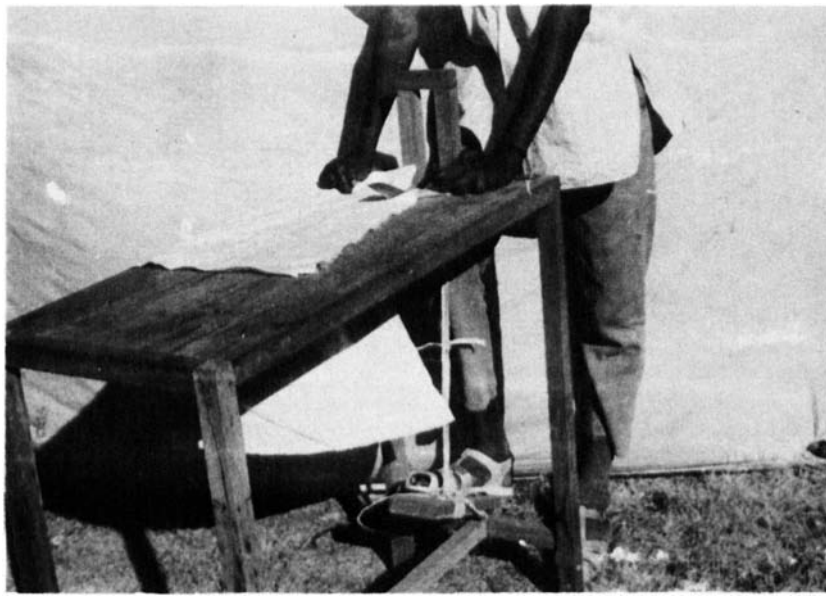

Fig. 1. Unable to use his badly damaged hands for cutting cloth, a patient makes use of the "Kondhwa" cloth-cutter.

is mobile and operated by a foot-pedal and spring. It is useful for patients who lack digits or range of the thumb web necessary for the efficient use of scissors in cutting cloth. Including labour cost, this could be made for less than 70 rupees by using materials of moderate quality. Our disabled patients, when leaving the hospital after a course in tailoring, are given a cloth-cutter if they need one as part of the equipment for setting up in business in their home.

2. SEWING-MACHINE PEDAL SPRING (Fig. 2)

This device helps amputees and those who can use only one foot for operating a sewing-machine. The spring lifts the pedal up when the downward foot pressure is released, thus compensating for the action of the other foot. Any type of spring may be used provided it is coiled, between 8 and 12 in (20 to 30 cm) long, and not too powerful.

3. KNIFE AND BLOCK COMBINATION (Figs. 3-5)

A discarded surgical blade attached to a smoothened wooden handle enables deformed hands to slit hems conveniently when the cloth is held over a wooden block attached to the side of the sewing-machine board. This also protects anaesthetic hands. (Tailors in India slit hems usually by folding the cloth tightly over the index finger of one hand while using a bare razor blade held by the other hand.)

4. THE "KONDHWA" CANE HOLDER (Fig. 6)

Cane-craft is a very profitable trade, but is usually not recommended for leprosy patients because of the high risk of damage to the fingers. The holder

Fig. 2. An amputee operates a sewing-machine by help of the pedal spring.

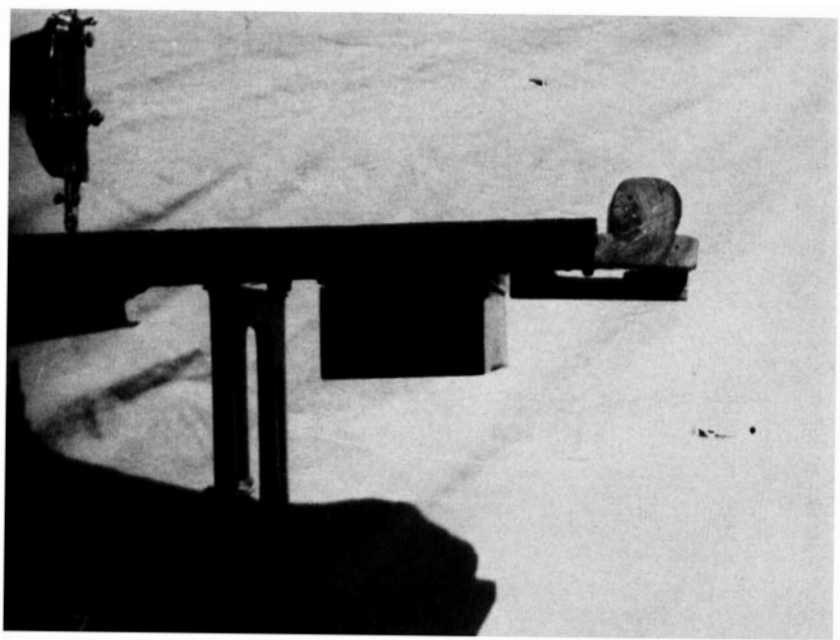

Fig. 3. Sewing-machine with wooden block attached at the side to afford convenience and safety in slitting hems.

Fig. 4. Devoid of fingers except for two stumps, this hand holds the knife in readiness for use with the block.

Fig. 5. Using the knife and block, the almost digitless hand slits a hem.

enables gripping and tightening of the cane to be carried out safely, and can even be used by mobile claw-hands. Cane work is quite safe as long as the cane is not held by the bare hands during work. The holder is made of two blocks of wood, the moulded surface of one block fitting into the grooved surface of the other; both blocks are hinged at one end, from which an arm protrudes from each block (Fig. 6). The grooved and moulded surfaces afford a firm hold of the cane and the protruding arms enable easy opening of the jaws of the holder (Fig. 7).

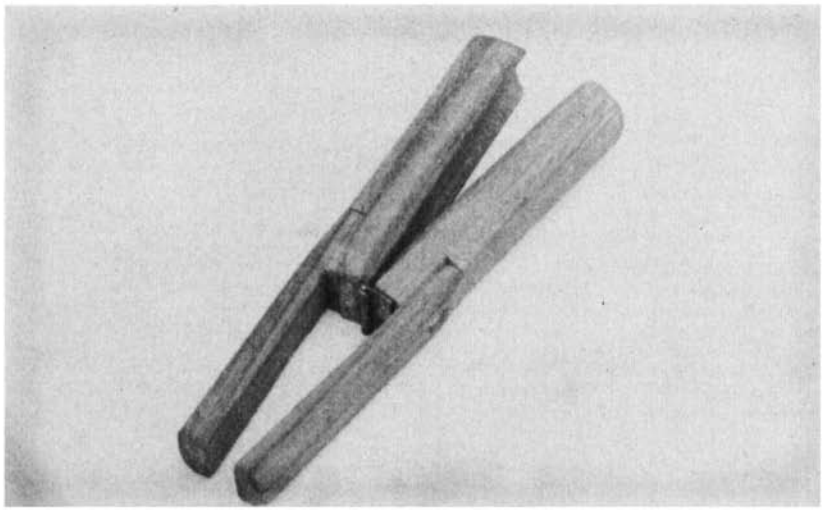

Fig. 6. The "Kondhwa" cane holder.

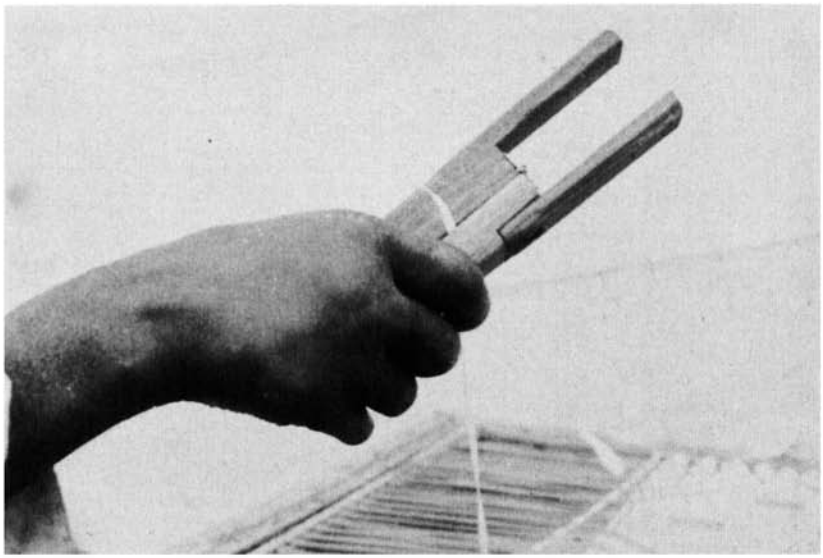

Fig. 7. An anaesthetic hand using the cane holder while working on a cane chair.

Discussion

In poor countries where leprosy is prevalent, and where thousands need physical rehabilitation, the main criterion is practicability. Any aids devised therefore should be cheap to make and convenient to use, no matter how crude their appearance.

Excepting the cloth-cutter, all the aids mentioned in this paper could be made for, at most, 5 rupees. The cloth-cutter costs 70 rupees. These aids could be useful also to many persons disabled from other causes than leprosy, and the cane holder and wooden block and knife might appeal even to normal people.

Acknowledgements

My thanks are due to Dr J. M. Mehta, President, Poona District Leprosy Committee, who is also chief administrator and honorary surgeon to the hospital, and to Mr S. W. Gokhale, Principal Investigating Officer, SRS Action-Research Project, Poona District Leprosy Committee, for allowing me project materials for constructing these devices, for all their encouragement, and for their permission to publish this paper.

Book Review

Handbuch der Haut- und Geschlechtskrankheiten by H. Röckl (Ed.). Vol. 4, Pt. 1 B. Infektionskrankheiten der Haut. II. XI + 568 pp. illustrated, 1970. Springer: Berlin, Heidelberg, & New York, DM 248.

This book is probably meant as a complement to Victor Klingmüller's famous German handbook *"Die Lepra"*. It can certainly not serve as a handbook on leprosy, since it scarcely deals with the practical side of the disease. One looks in vain for such subjects as reconstructive surgery, rehabilitation, and the social and psychological aspects of the disease.

The most important chapter is the first, by G. Klingmüller, on the pathology and clinical picture of leprosy. It is written from the point of view of a dermatological histopathologist, and provides a broad coverage of the literature up to fairly recently. The chapter is well illustrated.

There are two chapters by L. M. Bechelli. The first, dealing with premunition in leprosy, is based mainly on the South American literature before 1964. The second is on treatment; it contains much obsolete material, and fails to mention many new developments. M. I. Quiroga's chapter about prevention and control is almost exclusively based on the South American situation. It is very out of date. It is astonishing to read, in a work dated 1971, a recommendation for "selective compulsory isolation". H. Schmidt writes about serology, but the exciting newer developments in this field are not mentioned.

Though it contains much of interest, the book cannot be recommended to the leprosy worker in the field. It may have a place in the library of large institutions–only they can afford to buy it!

A. H. van Soest

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Abstracts

The following abstracts are reprinted, with permission, from *Trop. Dis. Bull.* 1972, Vol. **69**.

1. **The treatment of leprosy with the sulfones. 1. Faget's original 22 patients. A thirty-year follow-up on sulfone therapy for leprosy**, by R. R. JACOBSON and J. R. TRAUTMAN. *Int. J. Lepr.* 1971, **39**, 726-737.

This interesting and informative report from Carville, U.S.A., gives the case histories of the first 22 patients to have received sulphone therapy for leprosy (Faget *et al.*, *Trop. Dis. Bull.* 1944, v. **41**, 494) and describes their subsequent progress. Disappointingly, it shows that 30 years after commencing treatment, or at the time of death, 13 patients had active lepromatous leprosy (but 2 of these patients can be excluded as they died prematurely). The three most important facts emerging from this report are: (1) all (excepting the 2 who died prematurely) had their disease arrested after taking treatment regularly for varying numbers of years; (2) sulphone resistance tended to develop in those who suffered reactivation of their disease due to stopping treatment or to becoming irregular with treatment; (3) sulphone resistance did not develop in those who continued treatment regularly.

The authors rightly conclude that, in lepromatous leprosy, regular therapy for life is the wisest course to follow.

W. H. Jopling

2. **Controlled drug trial of B663 compared with DDS. Preliminary (48-week) report, by J. G.** TOLENTINO, J. N. RODRIGUEZ, and R. M. ABALOS. *Int. J. Lepr.* 1971, **39**, 738-741.

In a controlled drug trial in the Philippines, lasting 48 weeks, the progress of 16 patients taking clofazimine (Lamprene; B663) was compared with that of an equal number taking dapsone (DDS). Dosage of clofazimine was 200 mg daily six times a week, and of dapsone was 50 mg given twice a week increasing to 600 mg weekly. Clinical and bacteriological progress was equally good in both groups, but erythema nodosum leprosum reaction and acute neuritis occurred less frequently and with less severity in the patients taking clofazimine.

[The controlling effect of clofazimine on lepra reaction in this trial was due to the fact that the drug was given in the high dosage usually reserved for the treatment of lepra reaction; it is highly doubtful if this effect would have occurred with the much smaller, but therapeutically effective, dosage of 100 mg twice a week.]

W. H. Jopling

3. **Traitement de la lèpre lépromateuse par l'ethionamide. (Treatment of lepromatous leprosy with ethionamide)**, by R. ROLLIER and M. ROLLIER. *Maroc méd.* 1972, **52**, 148-166.

This well-documented account of a trial of ethionamide in leprosy attempts an objective reappraisal of the efficacy of this neglected drug, and thus complements the experimental work of Shepard (*Trop. Dis. Bull.* 1971, v. **68**, abstr. 1013). The trial concerned 102 patients and extended over 10 years. Laboratory investigations were inadequate, including examinations of skin biopsy specimens and material obtained from the skin (by the slit-smear technique) and from the "nasal mucus".

All the patients, except one, had lepromatous leprosy. The drug was first given at a dose of 1 g daily; because of side-effects (mainly digestive) the dose was reduced to 0.50 g daily for an adult, and 0.25 g for an adolescent. Bacilli disappeared from the "nasal mucus" and from the skin at a regular rate, and at the end of 4 years of treatment, in the majority of patients, the Bacillary Index had fallen to zero. [The morphology is not indicated.]

Clinical amelioration was also impressive. Early signs of improvement could be seen after 6 months of treatment, and after 2 years 75% of the patients could be classified clinically as "disease arrested". Special attention was paid to lesions of the upper respiratory tract (nasal mucosa, palatal mucosa and the tongue), and to the occurrence of erythema nodosum leprosum. Some evidence is given that the latter might on several occasions have been precipitated by the drug when given in the higher initial dose of 1 g daily.

The authors conclude that ethionamide is active in leprosy and that its activity resembles that of dapsone. Given at the dose recommended, it is relatively free from side-effects. Its price militates against it ever being more than a second-line drug.

S. G. Browne

4. A comparison of the growth curves of the NQ bacillus (*Mycobacterium* sp.) derived by photometric turbidity, microscopic counting, and viability in a tube-dilution-series, by C. V. REICH. *Int. J. Lepr.*, 1971, **39**, 25-33.

This study "was designed to investigate the existence of the unstained, microscopically invisible state in a cultivable species of mycobacteria and to determine whether the state can be associated with biologic activity." The NQ bacillus is a mycobacterium isolated from the ear and testis of hamsters inoculated with material from patients with leprosy. The growth medium was Dubos medium at pH 6.8, but without Tween 80 and with 20% bovine serum. 15 tubes of culture were read daily for turbidity, and the tube which was nearest the daily mean was selected to provide the sample aliquots for viability and microscopic counts. These latter were made by the procedure of Shepard and McRae (*Int. J. Lepr.* 1968, v. **36**,78). The cultures were sampled for 46 days, and viability counts were made using ten 10-fold dilutions. The number of acid-fast bacilli increased rapidly for 10 days reaching a level of 10^8/ml. They remained at this level for a further 15 days and then rose to nearly 7×10^8/ml by the thirtieth day. However, the number of viable units increased for 20 days up to 10^{10}/ml. The "turbidity" increased more slowly to give an estimated 10^8 bacteria/ml after 40 days. The author discusses reasons for the difference between the count of acid-fast bacilli and the viability count, but dismisses clumping as a possible cause. It is suggested that mycobacteria which did not stain by the Ziehl-Neelsen method appeared in the medium after 10 days. The extremely rapid initial rate of growth of almost two logs that occurred in the first 3 days is explained by the author as "inter-conversion from nonstaining to acid-fast [forms]". He concludes that "in the active reproductive phase of a mycobacterial culture, a significant proportion of the population remained unstained by the Ziehl-Neelsen procedure". The author states that his study "is directed ultimately to *Mycobacterium leprae*". [This study poses significant questions, but whether the bacterial population in a leprosy patient after many years of the disease is in "an active reproductive phase" seems doubtful.]

C. S. Goodwin

5. Attempts to establish the armadillo (*Dasypus novemcinctus Linn.*) as a model for the study of leprosy. 1. Report of lepromatoid leprosy in an experimentally infected armadillo, by W. F. KIRCHHEIMER and E. E. STORRS. *Int. J. Lepr.* 1971, **39**, 693-702.

It is reported that an armadillo (*Dasypus novemcinctus*) has developed lepromatoid infection with *Myco. leprae* approximately 14 months after inoculation of leprosy bacilli, from an untreated case of lepromatous leprosy, into the skin of its abdomen and ear lobes. The diagnosis of lepromatoid leprosy is supported bacteriologically by over 1000-fold increase in the inoculation sites of acid-fast bacteria, which do not grow on mycobacterial culture media and which oxidize D-dopa. In addition, these acid-fast bacteria have been found in great numbers at a skin site remote from the inoculated area. The remote skin site was of normal appearance. The inoculated skin sites were converted into massive nodular lesions. The acid-fast bacteria were intracellular, and typical lepra cells made up much of the lepromas. Bacilli were also seen in cutaneous nerves. It is too early yet to evaluate the results of the mouse footpad inoculations of the bacilli. So far, however, sections of the footpads show what one would expect of *Myco. leprae* after one month.

The reasons for attempting transfer of leprosy to the armadillo and the possible future significance of the armadillo in leprosy research have been discussed.

6. **Etude clinique et épidémiologique de 56 cas de lèpre suivis à la Clinique Dermatologique de Bordeaux depuis 1947. (A clinical and epidemiological study of 56 cases of leprosy seen at the Bordeaux Skin Clinic since 1947),** by L. TEXIER. *Med. Afr. Noire,* 1972, **19,** 193-196.

In the 23 years from 1947 to 1969 inclusive, the author made the diagnosis of leprosy on 56 occasions in patients attending his skin clinic. All except 7 had contracted the disease outside Europe; 4 were from Spain, and 3 had caught leprosy in France. Among the 31 patients born in metropolitan France, 10 had been in Government service, 7 in commerce, 5 in the armed services and 5 were missionaries. The 25 immigrant patients were mainly students (8), or workmen (8), or wives and children (6).

The presenting signs that brought patients for diagnosis were confined to the skin in 44, in both skin and peripheral nerves in 8, and in peripheral nerves only in 4. 33 were classified as having tuberculoid leprosy, 20 as lepromatous, and 3 as indeterminate. It is noteworthy that of the 20 patients with lepromatous leprosy, 15 were from metropolitan France. The average minimum "silent" period before signs of leprosy appeared would be under 2 years.

All patients were admitted initially to hospital, and those with lepromatous leprosy were kept in hospital until the nasal mucus no longer harboured *Mycobacterium leprae* (the morphology not being indicated). Patients with tuberculoid leprosy are discharged from hospital after stabilization of treatment has been achieved, and continue taking dapsone at home under medical supervision, reporting to the clinic each month. The standard treatment is dapsone, with a starting dose of 25 mg daily, increasing to a maximum maintenance dose of 200 mg daily. No untoward complications attributable to this dose of dapsone are noted.

Most patients are correctly diagnosed within 2 years of the appearance of the first signs, but 1 patient had to wait 12 years before the diagnosis of leprosy was entertained.

Details are given of the 3 patients who contracted leprosy in France itself. One was a boy in boarding school, 2 of whose classmates (both immigrants) had leprosy. Another was a woman who had looked after a civil servant known to have had lepromatous leprosy. In the third instance, a hotel maid, there is no suggestion of any contact with anyone suffering from leprosy, and the source of her infection remains quite unknown.

S. G. Browne

7. **Immunological phenomena in leprosy and related diseases,** by J. L. TURK and A. D. M. BRYCESON. *Adv. Immunol.,* 1971, **13,** 209-266.

The following is an extract from the authors' introduction:

"Failure of host resistance frequently results from a defect in cell-mediated immunity (CMI). However, hypersensitivity reactions resulting in tissue damage can occur as readily from CMI as from the deposition of immune complexes involving humoral antibody. Such an interaction between immune procedures and a given microorganism can display a wide spectrum of pathological processes, which, in turn, leads to markedly different clinical manifestations.

Such a spectrum is particularly well demonstrated by the recent elaboration of the varied clinical patterns in leprosy. Postulations from time to time related these differences to variations in the host's resistance, yet experimental and clinical evidence has been available only during the last few years. With elucidation of the immunological basis for the disease spectrum in leprosy, a parallel has been sought and found in other infectious diseases. These include especially certain protozoal diseases such as leishmaniasis and others caused by yeasts and fungi such as candidiasis and the systemic mycoses. This review is, therefore, concerned with immunological concepts in leprosy leading to a discussion of analogous states now recognized in other infectious diseases."

[There are 214 references.]

8. **WHO co-ordinated short-term double-blind trial with thalidomide in the treatment of acute lepra reactions in male lepromatous patients,** by C. G. S *et al. Bull. Wld Hlth Org.* 1971, **45,** 719-732.

This is a report of a WHO short-term double-blind trial on male patients suffering from lepromatous leprosy carried out to compare the effect of thalidomide with that of acetylsalicyclic acid in the control of lepra reaction. 214 lepra reactions were treated during a period of 9 months, 116 with thalidomide and 98 with acetylsalicylic acid, and treatment was given for 7 days. Dosage of thalidomide was one tablet (100 mg) 4 times daily for patients over

50 kg in weight, with a reduced dosage for lighter patients, and those on acetylsalicylic acid received an equal number of tablets of 400 mg strength.

Although acetylsalicylic acid had a beneficial effect on some of the symptoms, thalidomide was more effective; its effect on skin lesions and body temperature was greater than on acute nerve and eye manifestations. In the very short period of the trial, side-effects of a serious nature were not encountered in either group, but thalidomide had a tendency to induce leucopenia.

W. H. Jopling

9. **Infection of murine striated muscle with *Mycobacterium leprae*: a study by light and electron microscopy**, by M. M. ESIRI, A. G. M. WEDDELL and R. J. W. REES. *J. Path.* 1972, **106**, 73-80

It has already been shown that in murine infections with *Mycobacterium leprae* the striated muscle fibres are colonized at a very early stage, whether the mice are intact or thymectomized (Rees and Weddell, *Ann. N.Y. Acad. Sci.* 1968, v. **154**, 214). The present paper examines the distribution of *Myco. leprae* in striated muscle over a 2-year period of the infection, and the pathological changes that result.

Bacilli in footpads increased steadily in number from the sixth to the tenth months, the number being about 100 times greater in the thymectomized animals. Microcolonies were almost always intracellular in muscle fibres (in disorganized sarcoplasm), or macrophages. In the footpad they were nearly all in muscle but in the nose they were nearly all macrophages. This might be associated with the fact that mitochondria are more numerous in the muscle fibres of the nose, which have the characteristics of red muscle. It is possible that *Myco. leprae* preferentially colonizes white muscle fibres.

In intact mice the infection produced after several months of low-grade inflammation with only slight patchy damage to muscle fibres. In thymectomized-irradiated mice the greater number of bacilli was associated with more widespread inflammation and damage, which was attributed directly to the bacilli and not secondarily to nerve damage. The nature of the damage to muscle fibres is described and illustrated with electronmicrographs.

D. S. Ridley

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