

## A. INTRODUCTORY REVIEW OF LEPROSY RESEARCH

# The Impact of Experimental Human Leprosy in the Mouse on Leprosy Research

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In no field of medicine has greater progress been made than with the infectious diseases—all within the last hundred years. This has applied to all diseases caused by bacteria and, more recently, many of those caused by viruses. These advances in knowledge, whether they have been on the microbiologic, pathologic, preventive or therapeutic side, have evolved, at every stage, either from studies related to *in vitro* cultivation of the causative organism and/or from infections in experimental animals. Unfortunately the causative organism of human leprosy, *Mycobacterium leprae*, is the one exception, since it has still not been cultured *in vitro* and only since 1960 (22) has it been shown to infect experimental animals. The appreciation of these facts not only accounts for the limitations in knowledge and progress of leprosy but also the persistence of mysticism and dogma in this field. On the more creditable side it explains the indirect approaches and the nonhuman leprosy models used by those who earlier bravely worked on leprosy research. It equally explains why so few research workers could afford to study leprosy in competition with the exciting progress then being made in all the other infectious disease fields. Finally, it was obvious that as soon as *M. leprae* could be grown there would be unleashed almost a century of outstanding experimental work! This breakthrough came in 1960 when Shepard showed that *M. leprae* could be transmitted to mice by footpad inoculation.

The theme of my lecture, therefore, will be a review of progress and assessment of the impact of experimental leprosy in the mouse on the field of leprosy research with-

in the last ten years, bearing in mind that all the studies had by necessity to be adapted to *in vivo* conditions.

### BACTERIOLOGIC CHARACTERISTICS OF *M. leprae* BASED ON INFECTION IN THE MOUSE

The initial (8, 22) and subsequent main studies were undertaken in mice, although subsidiary studies indicate that other rodents, including rat (4), hamster (30) and *mystromys* (1) behave similarly.

Under standard conditions of inoculation of *M. leprae* in the footpad or ear of mice (8, 22) the pattern of multiplication is reproduced by all strains of *M. leprae* obtained from patients with active disease, irrespective of the type of leprosy, race or region of the world from which they come. In the logarithmic phase of multiplication the generation time is 12-13 days (27), longer than that of any other species of mycobacterium. The bacteriologic pattern of response is reproducible on serial passage through mice, apparently indefinitely, without a change in virulence or pathogenicity. Moreover, the "Mitsuda reactivity" of bacilli recovered from mice is identical to that elicited by Mitsuda lepromin in patients with leprosy (2, 25). From a review of the literature and my personal knowledge of centers actively engaged in studies on experimental leprosy in the mouse I would estimate that these general characteristics are currently based on some 400 strains of *M. leprae* from studies undertaken by laboratories in Belgium, Holland, India, Japan, Malaysia, the Philippines, United Kingdom and USA. Although in more precise studies small differences have been reported in the susceptibility of individual strains of mice to infections with *M. leprae* (26), multiplication of *M. leprae* has been obtained in all out-

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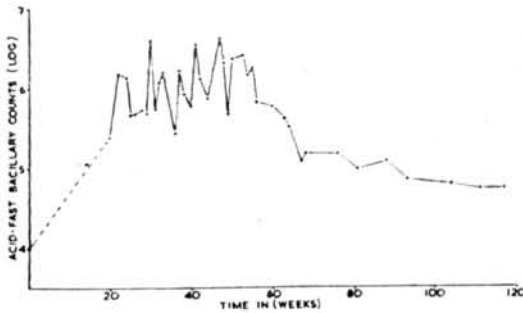


FIG. 1. Growth curve of *M. leprae* in the ears and footpads of mice following local inoculation of  $10^4$  bacilli.

bred and pure-line strains of mice studied so far.

In normal mice the logarithmic phase of multiplication of *M. leprae* is maintained for 6-8 months, followed by a plateau and then a regression phase (Fig. 1). Multipli-

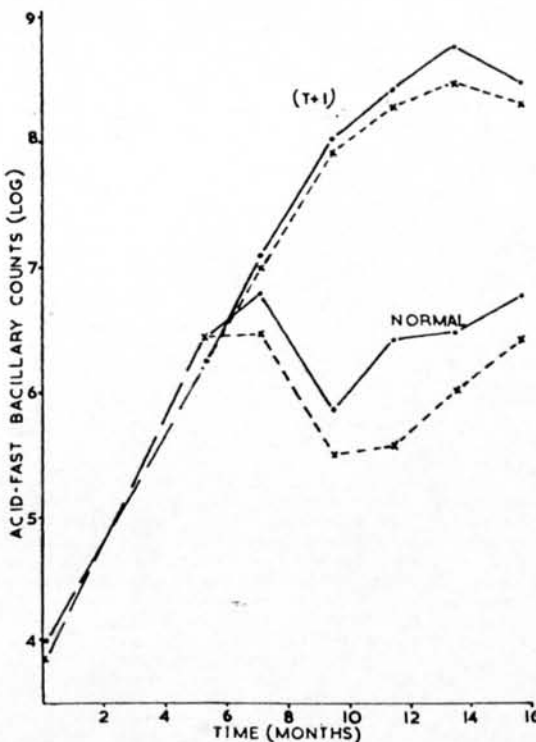


FIG. 2. Growth curves of *M. leprae* in the footpads of normal and thymectomized-irradiated (T + I) mice inoculated locally with  $10^4$  bacilli. — = Total count; x-----x = "Viable" count.

cation, however, occurs only with smaller inocula (routinely  $5 \times 10^3 - 10^4$  acid-fast bacilli) since inocula of  $10^6$  or more fail to multiply. More recently it has been shown that this limited phase of logarithmic multiplication can be extended by reducing the immunologic capacity of the mice by thymectomy followed by total body irradiation (900 r) before infection, necessitating a small, life-saving injection of syngeneic bone-marrow cells (<sup>9, 17</sup>). In such immunologically deficient mice—predominantly a deficiency in cell-mediated immunity—the period of multiplication is extended, but the generation time is unchanged (Fig. 2). This immunologic depressive procedure for obtaining enhanced infections with *M. leprae* was originally described by Rees in England (<sup>9</sup>), subsequently confirmed by Shepard and Congdon in America (<sup>24</sup>) and more recently by C. K. Job and D. L. Leiker (personal communications) in India and Holland, respectively.

#### CLINICAL AND HISTOLOGIC MANIFESTATIONS OF DISEASE IN MICE INFECTED WITH *M. leprae*

These manifestations have been based on systematic observations made on mice inoculated locally in the footpads or ears or intravenously or intraperitoneally with *M. leprae*, in both normal and immunologically deficient animals, followed throughout their life-span, and are summarized in Table 1.

Clinical manifestations were of two types, skin lesions and deformities. Skin lesions were confined to the immunologically-deficient mice, presenting as nodular swellings in the feet or ears, and affecting no more than five per cent of the animals at risk. Such skin nodules were seen in the hind footpads of locally inoculated mice (Fig. 3) or in the hind or forefoot pads or ears of intravenously inoculated animals. A proportion of the nodules in the hind footpads eventually ulcerated (Fig. 4), possibly due to secondary infection, but in the light of more recent observations on the associated damage to peripheral nerves in these animals they are more likely to have arisen from loss of sensation. Direct smears prepared from the surface of these ulcers or

TABLE 1. Clinical and histologic manifestations of disease in mice infected with *M. leprae*.

Clinical		Histologic		
Skin	Nerves	Tissue sites	Cells	Type of leprosy <sup>a</sup>
Nodules/ulcers	Muscle weakness Deformities Clonus Paralysis "Foot-drop"	Skin: (foot, ear and tail) Nose	Macrophages Muscle Capillary endothelial Perichondrial	LL-BT
		Nerves: Dermal and peripheral trunks	Perineurial Schwann	
		Testis	Interstitial	

<sup>a</sup> Classification is according to Ridley and Jopling (20) and Ridley and Waters (21).

after superficial scarification, always contained acid-fast bacilli, exemplifying the ease in which *M. leprae* are shed from skin surfaces.

While skin nodules appeared only in a proportion of the immunologically deficient mice, and within a period of 6-8 months, deformities of the hind legs were observed

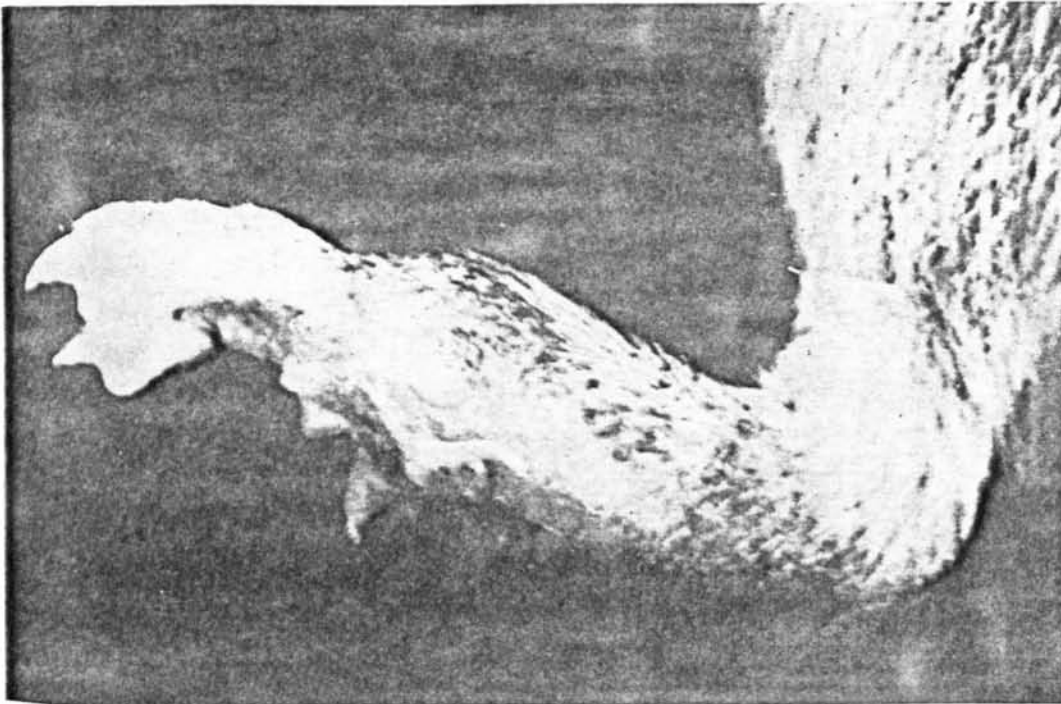


FIG. 3. Nodular swelling of hind footpad in a thymectomized-irradiated mouse injected locally with  $10^7$  *M. leprae* 11 months earlier.

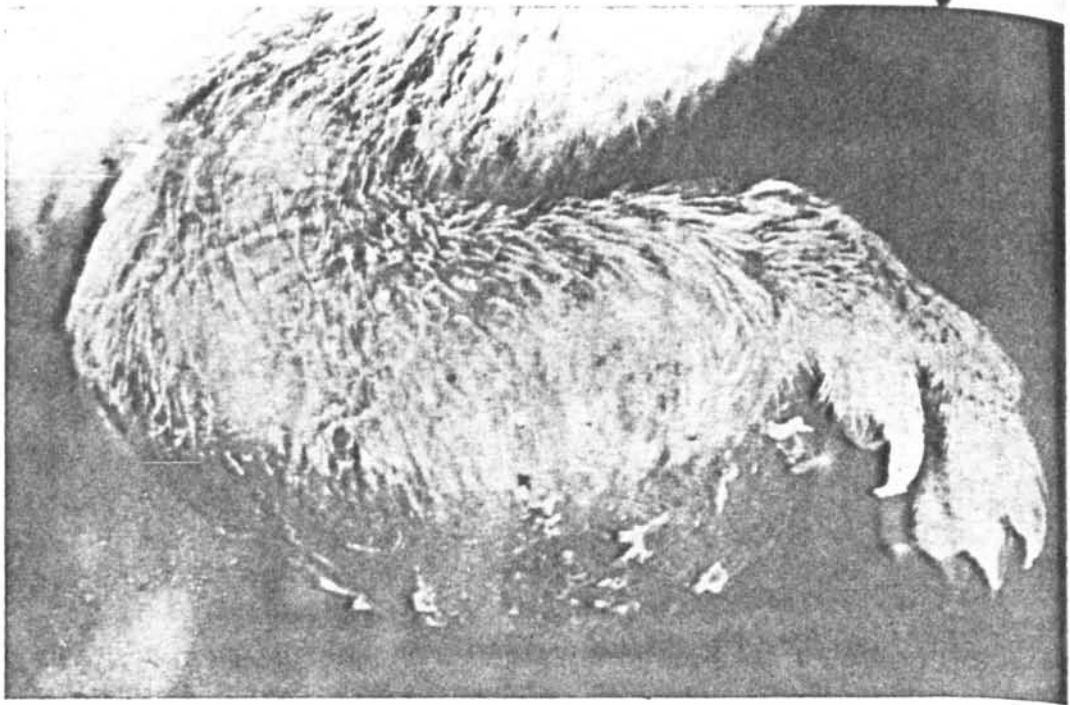


FIG. 4. Nodular swelling and plantar ulcer of hind footpad of a thymectomized-irradiated mouse inoculated locally with  $10^7$  *M. leprae* 15 months previously.

in a high proportion of both normal and immunologically deficient mice, but not before 20 or more months after inoculation. These deformities presented wide variations from minor to major muscle weakness of the hind limb and from minor to major manifestations of hind-leg paralysis (15).

In the most severe and more rare cases the changes were sufficient to affect the gait of the animals, associated with "dragging" of the hind legs. These deformities were most readily manifest by holding the mice vertically by gripping them at the nape of the neck and the tail. In this position normal

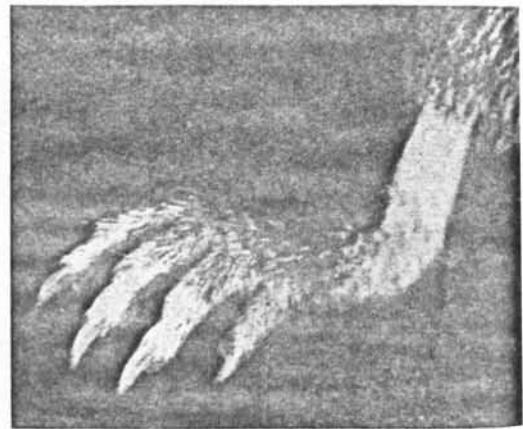
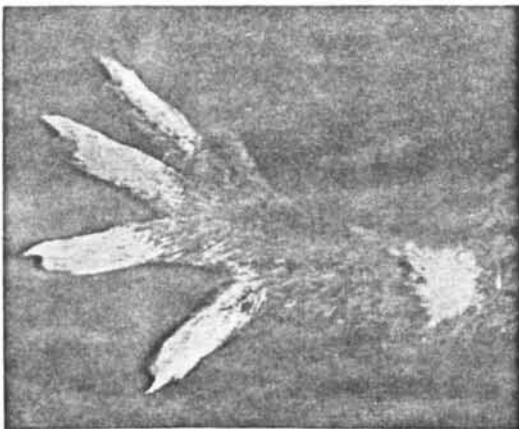


FIG. 5. Foot deformities in mice with leprosy. (A) Normal resting position of foot in uninfected mouse. (B) Abnormal resting position of foot—"foot-drop"—from a mouse inoculated locally with  $10^4$  *M. leprae* 27 months previously.

and uninfected mice at all ages retain their hind feet in flexion with their toes spread, whereas the infected mice are unable to retain this position and therefore the hind-leg remains in extension with the toes remaining together (Fig. 5). In the latter, more severe condition, mice retained in this position rapidly developed a fine tremor or gross clonus of their hind legs, manifestations never seen in normal mice.

Systematic studies on the isolation of acid-fast bacilli in homogenates of tissues from various sites following local footpad or ear inoculation of *M. leprae* in immunologically deficient mice showed that systemic spread was eventually a universal feature. However, spread was confined to selected sites, namely the skin of the paws and ears (when these sites were not locally inoculated) and tail, nose, main nerve trunks (studies mainly confined to sciatic and brachial nerves) and testes. In immunologically deficient mice inoculated intravenously with *M. leprae* there was also systemic spread, but this again was confined to the same specific sites. It was on the basis of these observations in immunologically deficient mice that we were led to a re-investigation of the earlier claim that spread of infection failed to occur following local inoculation of *M. leprae* in normal mice. The new studies have shown that spread of infection also occurs in normal mice, including those inoculated intravenously, and is confined to the same sites of predilection. Spread of infection in normal mice differs only from that in immunologically deficient mice by taking longer to reach a detectable number of acid-fast bacilli.

In order to correlate the histologic features of infections with *M. leprae* in mice with the yield or distribution of bacilli, paired organs were taken, one for histologic examination and the other for bacterial count of individual tissues divided equally, for the two respective assessments. Moreover, these dual assessments were made at regular intervals throughout the life-span of the animals and have provided information on the type of cells infected with *M. leprae* and the cellular responses related to the bacterial population and the

age of infection. These studies have provided a very clear pattern of the type of cells in which bacilli are present and, under various conditions, the type of cells in which the bacilli multiply most freely. Thus initially and throughout the period of bacillary multiplication in both normal and immunologically deficient mice striated muscle fibers are the cells in which the bacilli multiply most readily (Fig. 6). The special and unexpected predilection of striated muscle fibers for *M. leprae* was investigated in greater detail at regular intervals within the first 48 hours of inoculation. These studies showed that following footpad inoculation of *M. leprae* a few bacilli entered local muscle fibers and that after a few days the only other organisms retained in the footpad were present within macrophages or endothelial lining cells of capillaries<sup>(31)</sup>. At the local site of inoculation in normal mice this cellular pattern of distribution was retained for the next 6 month period. However, in this period there was a significant increase in the num-

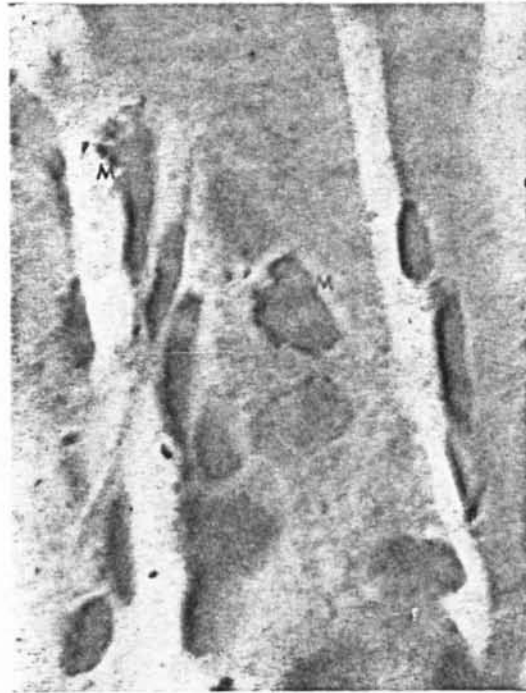


FIG. 6. "Micro-colonies (M) of acid-fast bacilli within a striated muscle fibre of the footpad of a mouse inoculated locally with  $10^4$  *M. leprae* 9 months previously.

ber of bacilli within striated muscles and at this site the majority of the organisms remained intact, staining uniformly with carbol fuchsin. Similarly intact bacilli were present in the endothelial lining cells of capillaries, whereas organisms in macrophages stained irregularly, indicating degenerative changes<sup>(13)</sup>. After six months there was a decrease in the number of bacilli within striated muscle fibers. However, a few usually well stained bacilli could always be found in the endothelial lining cells of capillaries, particularly intramuscular capillaries and those associated with nerve fibers. A new picture began to appear 12 and more months after inoculation, with the appearance of well stained bacilli within perineurial and Schwann cells of cutaneous nerve fibers in the skin of the footpad (or of the ear, if this site had been inoculated). From 20 months onward well stained bacilli also appeared within perineurial and Schwann cells of large nerve trunks, including the sciatic and brachial nerves. Exactly the same cellular pattern of distribution of bacilli was seen in locally

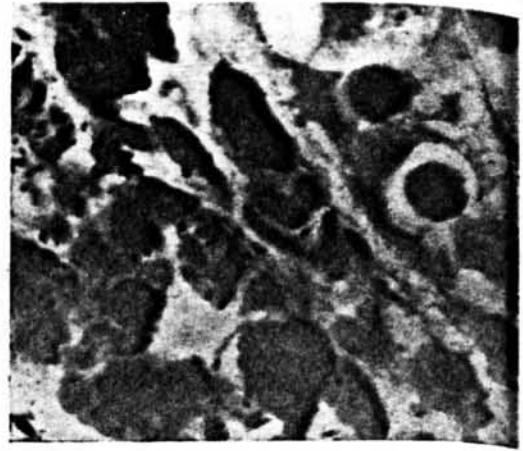
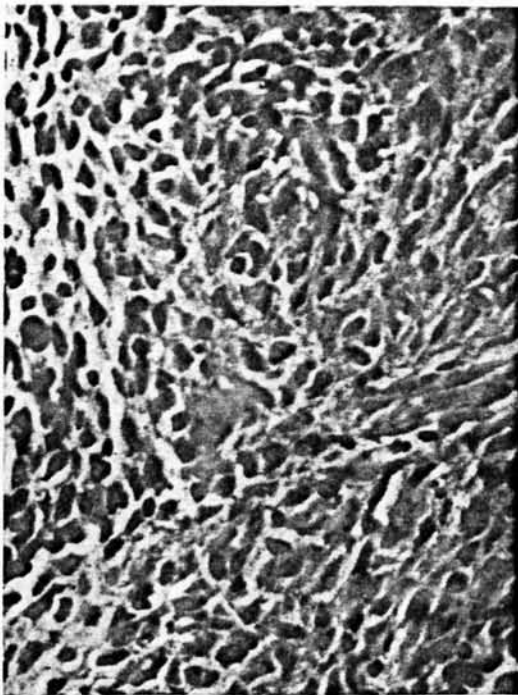
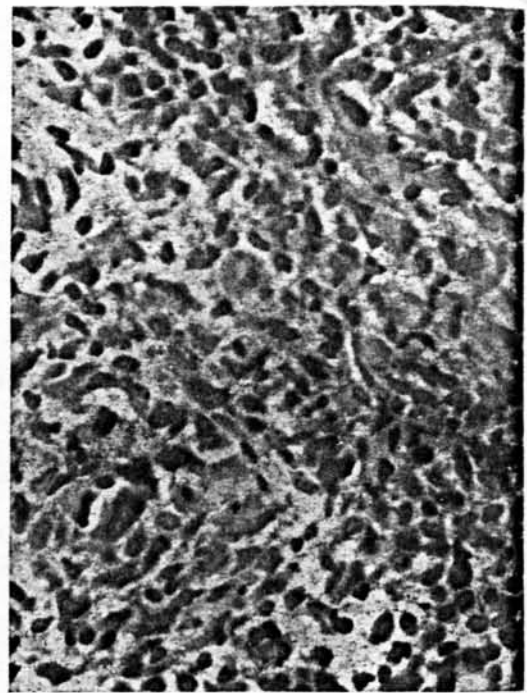


FIG. 7. Testis showing large number of acid-fast bacilli within interstitial cells and macrophages. From a thymectomized-irradiated mouse inoculated intravenously with  $7 \times 10^7$  *M. leprae* 16 months previously.

inoculated and immunologically deficient mice. However, in such mice after initial multiplication of organisms in striated muscle fibers an increasing number of bacilli accumulated within macrophages and in



A



B

FIG. 8. Borderline leprosy. Epithelioid granuloma showing epithelioid cells surrounded by lymphocytes. (A) Mouse: deep dermis of footpad 2 years after local inoculation of  $10^4$  *M. leprae*. (B) Man: deep dermis from the shoulder.

these cells the organisms remained intact. There were two other cell types in which *M. leprae* were found, namely perichondrial cells and, in male mice, the interstitial cells of the testis (Fig. 7).

In addition to the distribution of bacilli within cells, the histologic studies also provided a picture of the cellular responses to the infections in normal and immunologically deficient mice. In normal mice inoculated with *M. leprae* there was a surprising absence of cellular infiltration within the local site of inoculation other than a few macrophages and lymphocytes. The cellular response was minimal and not characteristic of any of the forms of leprosy seen in man. However, by 20 months and later there developed at local or distal sites of predilection well formed epithelioid granulomata (18) with features resembling those seen in patients with near-tuberculoid type leprosy (20). The appearance of these granulomata in mice compared with near-tuberculoid type (borderline) leprosy in man is shown in Fig. 8.

In immunologically deficient mice the cellular response at the local site of inoculation for the first six months was minimal and as uncharacteristic of any of the forms of leprosy seen in man as the response in normal mice. However, by eight months at the local site of inoculation and at variable times later at distal sites of predilection in these mice or mice inoculated intravenously, histiocytic "granulomata" developed with features resembling those seen in patients with lepromatous type leprosy (20, 21). The appearance of these lesions in mice compared with lepromatous-type leprosy in man, is shown in Fig. 9. The cellular response was predominantly histiocytic, infiltrating the dermis but leaving a cell-free area immediately deep to the epidermis, resembling the clear zone seen in patients with lepromatous leprosy. The histiocytes were enlarged, their cytoplasm had a foamy appearance and the cells were stuffed with acid-fast bacilli—features characterizing the Virchow cell seen in patients with lepromatous leprosy. Although this

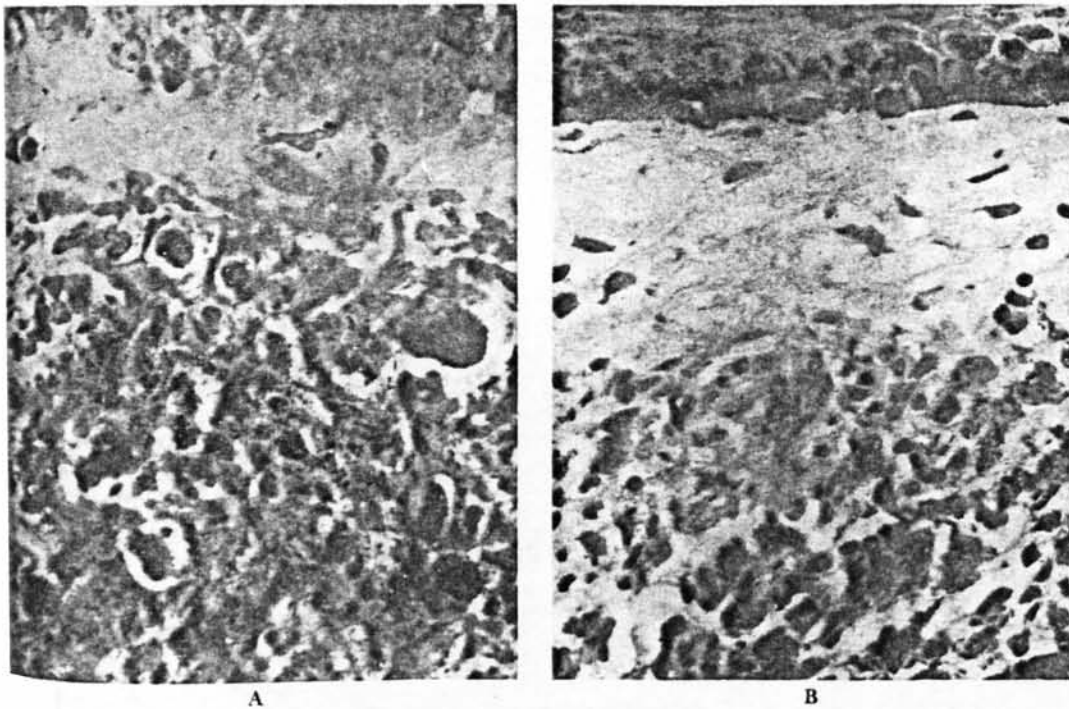


FIG. 9. Lepromatous leprosy. Globi loaded with bacilli and foam (Virchow's) cells in all stages of development are seen in the dermis that is separated by a clear zone from the epidermis. (A) Mouse: skin from footpad of a thymectomized-irradiated animal 9 months after inoculation of  $10^6$  *M. leprae*. (B) Man: skin from the upper thigh.

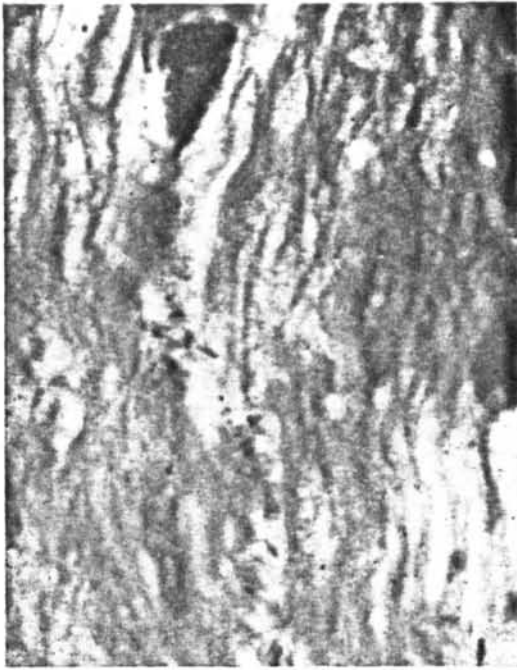


FIG. 10. Medial plantar nerve showing acid-fast bacilli in Schwann cells. From a thymectomized-irradiated mouse inoculated in the footpad with  $10^4$  *M. leprae* one year previously.

"lepromatous" picture was present in all the immunologically deficient mice and persisted throughout the life-span of some, the majority of these histiocytic lesions were invaded later by a variable number of lymphocytes. Lymphocytic invasion was associated with an increase in the proportion of degenerate bacilli within the histiocytes and, in a proportion of the animals, with the appearance of epithelioid cells. This spectrum of cellular changes replicated the histologic pictures classified in man as borderline type leprosy, covering the range defined by Ridley and Jopling (<sup>20</sup>) BL-BT.

Twelve months or later following local or intravenous inoculation of *M. leprae* in normal or immunologically deficient mice, bacilli were found in dermal and peripheral nerves. The bacilli were found within Schwann and perineurial cells. In the earlier infections these infected cells were not associated with damage to nerve fibers (Fig. 10). By 15 months, and always by the time there was gross evidence of hind-leg

deformities, there was histologic evidence of destruction of nerve fibers (Fig. 11).

#### EFFECT OF LYMPHOID CELL REPLACEMENT ON INFECTIONS WITH *M. leprae* IN IMMUNOLOGICALLY DEFICIENT MICE

Mice made immunologically deficient by thymectomy and irradiation, when donated lymphoid cells from the spleen and lymph nodes of normal syngeneic mice prior to inoculation with *M. leprae*, showed little or no enhancement of their infection (Fig. 12). Further studies were undertaken using thymectomized-irradiated mice with an established infection resulting from the local inoculation of *M. leprae* into the ears and footpads of the animals 10 months previously. A similar donation of syngeneic lymphoid cells from normal mice into these



FIG. 11. Lower segment of sciatic nerve from a thymectomized-irradiated mouse with severe "foot drop", inoculated intravenously with  $3 \times 10^8$  *M. leprae* 25 months previously. Electron micrograph showing endoneurium with a scarcity of nerve fibers and severely damaged myelinated fibers. A = destroyed axon with almost complete loss of myelin; M = various stages in destruction of myelin.



animals resulted, within ten days, in a gross inflammatory reaction of the infected skin of the footpads and ears. Over the next 40 days both footpads continued to swell slowly and remained inflamed, but the skin covering them never ulcerated. At about day 40 it was also noticed, for the first time, that both the ears were showing signs of change. Their margins looked somewhat red and were slightly swollen, but at no point was the skin ulcerated. During the next five months the ears gradually shrank in size and, in particular, lost their smooth graceful outlines and took on an irregular "nibbled" appearance, but, although the overlying skin became cyanosed, it never ulcerated. During this same five months period the footpads gradually became less swollen, were no longer red and inflamed, and, indeed, the overlying skin became wrinkled. Lymphoid-cell-donated and non-donated animals were killed at intervals up to five months and proportions of the tissues were taken for bacteriologic assessments and histologic examination (12, 14). The bacteriologic assessments showed that multiplication of *M. leprae* ceased abruptly and the residual organisms were destroyed (Table 2). Histological examination showed invasion of the lesions by lymphoid cells with edema, followed later by deposition of collagen and subsequent fibrosis and the formation of epithelioid granulomata with large numbers of degenerate organisms. The acute inflammatory changes observed in the footpads and ears of thymec-

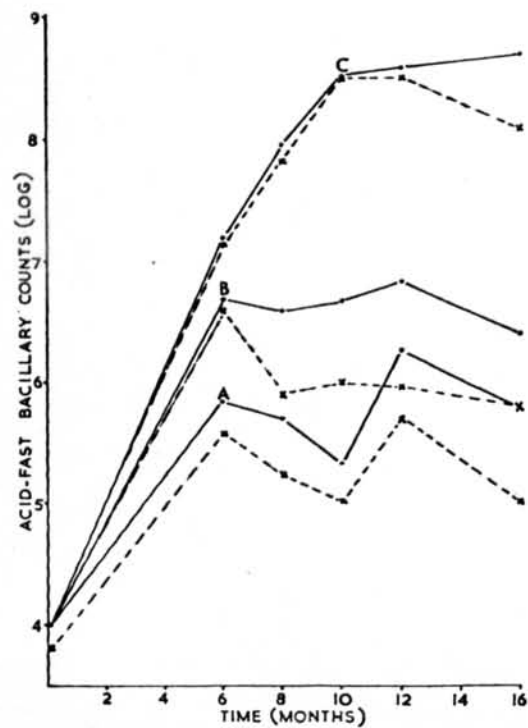


FIG. 12. Growth curves of *M. leprae* in skin sites of normal (A) and thymectomized-irradiated (B) mice and the effect of lymphoid tissue replacement (C). Inoculum was  $10^4$  bacilli. Each point represents the mean count from both ears and both hind footpads. Total count: ●—●—●; 'viable' count: x—x—x.

tomized-irradiated mice with an established leprosy infection following replacement by lymphoid cells from normal mice, closely resembled the macro- and microscopical pictures referred to as "reversal-type

TABLE 2. Effect of lymphoid cells on established (10 months) thymectomized-irradiated infection.

Treatment	Total yield and proportion of degenerate bacilli at 15 months					
	Ears		Footpads		Nose	
	Yield	Degen- erate (%)	Yield	Degen- erate (%)	Yield	Degen- erate (%)
Lymphoid cells	$6.7 \times 10^8$	100	$3.8 \times 10^8$	98	$2.4 \times 10^8$	90
No lymphoid cells	$7.3 \times 10^9$	46	$5.2 \times 10^9$	78	$3.1 \times 10^9$	61

reactions" in man (12, 19). It is of particular interest that reversal reactions in man are followed by an increase in the patient's resistance leading to a shift in the type of leprosy from the lepromatous toward the tuberculoid form—a shift exactly replicated in the mice-donated lymphoid cells. Elsewhere in this symposium Gaugas *et al.* describe similar reversal reaction brought about by transplants of syngeneic neonatal thymus grafts in thymectomized-irradiated mice with established infections with *M. leprae*. All these experimental procedures involving lymphoid cells and the thymus underline the role of cell-mediated immunity in determining the host response to infections with *M. leprae*.

From accumulated data from large numbers of thymectomized-irradiated mice inoculated with *M. leprae* there is no doubt that the infection is enhanced during the first period of eight to ten months with an associated histologic picture resembling the lepromatous form of disease seen in man. However, from then onward a very wide spectrum of response is seen in individual animals. In some, bacterial multiplication continues with a persisting lepromatous picture, whereas in others bacterial multiplication continues at a diminished rate or ceases abruptly, and these bacteriologic variables are histologically associated with invasion of the lesions by lymphocytes, with superimposed tendencies for epithelioid-type granulomatous formation. Because these spontaneous, but variable, changes observed in thymectomized-irradiated mice inoculated with *M. leprae* resemble the gross changes brought about by inoculation of lymphoid cells or grafts of thymus glands from normal animals, it would seem reasonable to speculate that the spontaneous changes are brought about by the gradual return of immunologically competent cells in a proportion of the thymectomized-irradiated animals.

#### EXPERIMENTAL CHEMOTHERAPEUTIC STUDIES IN MICE INFECTED WITH *M. leprae*

Once infections with *M. leprae* were obtained in the mouse footpad, the model

provided the first opportunity for experimental chemotherapy. A review of these studies and their impact on our knowledge of the chemotherapy of human leprosy are presented elsewhere in this symposium by Shepard. However, experimental leprosy infections in the mouse also provided the first opportunity for directly identifying the emergence of drug-resistant mutants of *M. leprae* in patients showing relapse, despite continued therapy. By combined studies on the multiplication of *M. leprae* and the serum concentration of dapsone in treated mice, it has been shown that the human leprosy bacillus from previously untreated patients is exquisitely sensitive to dapsone in animals, being inhibited by serum concentrations of 0.02  $\mu\text{g}$  dapsone/ml. serum (11, 28). These serum levels in the mouse contrast significantly with the serum levels of approximately 2.0  $\mu\text{g}$  dapsone/ml. of serum obtained in man given standard doses of 100 mgm dapsone/daily. Current systematic studies on the dapsone sensitivity in mice of strains of *M. leprae* isolated from patients who have relapsed during treatment with dapsone, have shown that a proportion of these strains multiply freely in the footpads of mice given doses of dapsone resulting in serum levels of 2.0  $\mu\text{g}/\text{ml}$ . These results provide conclusive evidence that a proportion of relapses occurring in patients treated with dapsone are due to the emergence of drug-resistant mutants (10, 11). Their bacilli behave differently, being no longer inhibited by serum concentrations of dapsone equivalent to those obtained by standard 100 mgm. dapsone daily (7). Similar studies in mice have established the fact of emergence of thiambutosine-resistant strains of *M. leprae* in a high proportion of patients receiving this drug for two or more years (10). The speed and frequency of the emergence of resistance to thiambutosine is in contrast with that of dapsone where, from our experience, resistance is infrequent and is observed only after seven years of treatment.

#### SUMMARY OF THE CHARACTERISTICS OF *M. leprae* FROM STUDIES IN THE MOUSE

The main part of my lecture has been

allocated to a description of the bacteriologic characteristics and the histopathologic manifestations elicited in mice inoculated with *M. leprae*. The main characteristics are summarized as follows:

(1) *M. leprae* has a uniquely long generation time (13 days).

(2) The rate and pattern of multiplication of *M. leprae* is unrelated to the source of the organism from patients by race, country of origin or type of leprosy.

(3) The bacilli can be serially passaged, apparently indefinitely, without a change in virulence or pathogenicity.

(4) Multiplication of *M. leprae* is inhibited by dapsone and other antileprosy drugs that are efficacious in man.

(5) Mouse-grown bacilli have the same Mitsuda antigenicity as Mitsuda-type lepromin prepared from organisms from patients when tested in patients with all types of leprosy.

(6) *M. leprae* multiplies preferentially in normal and immunologically-deficient mice at specific sites, viz., skin, muscle, nose, nerves and testes.

(7) Within these sites *M. leprae* has a specificity for striated muscle fibers, and perineurial, Schwann, capillary endothelial and Sertoli cells and macrophages.

(8) These sites and cells of predilection for the multiplication of *M. leprae* are unrelated to the source of the organisms from patients by race, country of origin or type of leprosy.

(9) Although local inoculation of *M. leprae* in normal and immunologically-deficient mice results first in a local lesion, with the passage of time systemic spread occurs, for which there is overwhelming evidence that it is hematogenous in origin.

(10) Whatever the site of inoculation and whether in normal or immunologically-deficient mice, there is eventually infection of dermal and peripheral-nerve trunks, resulting in gross and microscopic destruction of both myelinated and nonmyelinated nerve fibers.

(11) In normal mice the cellular response to infections with *M. leprae* results eventually in epithelioid granulomata, repeating the histologic picture of tuberculoid-type leprosy seen in man. In immuno-

logically-deficient mice the cellular responses replicate those seen in patients with lepromatous-type leprosy. In a proportion of both normal and immunologically-deficient mice there may develop, still later, cellular responses replicating intermediate (borderline) cellular responses with features of both tuberculoid and lepromatous leprosy, again replicating the borderline types of leprosy seen in man.

(12) Finally, the cellular response and the multiplication or survival of *M. leprae* in immunologically-deficient mice with established infections can be influenced by the inoculation of the animals with syngeneic and immunologically competent lymphoid cells from normal animals, or by grafting them with thymus tissue from neonatal mice.

Thus the mouse infection has provided a means of studying the bacteriologic properties of *M. leprae*, which for other bacteria are normally studied *in vitro*. Moreover, the most important feature of the histologic manifestations of infections with *M. leprae* in the mouse is their remarkable resemblance to the manifestations of the disease processes seen in patients with leprosy.

#### EXPERIMENTAL LEPROSY IN THE MOUSE AND ITS CLINICAL IMPLICATIONS

While it was anticipated that our knowledge of leprosy would be advanced once experimental models were available for studying the causative organism in the laboratory, the actual impact has transcended the most optimistic prophecies. This pleasing situation has arisen from the fact that the mouse has provided a susceptible host for the multiplication of *M. leprae*, but once the infection has been established, disease processes have developed that replicate all the specific features of leprosy in man. In this final section are summarized some of the most important clinical applications that have been developed from studies of experimental human leprosy in the mouse.

(1) Combined bacteriologic and pharmacologic methods have been applied for determining the antileprosy activity of dap-

sone and other drugs known to be efficacious in the treatment of human leprosy, and for determining the antileprosy activity of new drugs and the minimal inhibitory concentrations of all these drugs against *M. leprae*. These studies have revealed the exquisite sensitivity of *M. leprae* to dapsone, and, on this basis, to the new repository derivative—4,4'-diacetyldiaminodiphenyl sulfone (DADDS)—which releases daily small doses of dapsone in the order of the minimal inhibitory concentration of the drug against *M. leprae* in the mouse (23). From these studies in the mouse preliminary trials of DADDS in man have shown that the drug is active and releases concentrations of dapsone comparable to those released in mice (20). Still more recently, the new semisynthetic antibiotic rifampicin was shown first to be active against *M. leprae* in the mouse and then tested in man, where it was confirmed as efficacious and, moreover, able to kill *M. leprae* more rapidly than dapsone (16). The killing power of rifampicin compared with that of dapsone was confirmed by demonstrating, in the mouse, that bacilli failed to multiply in the mouse much sooner when recovered from patients receiving rifampicin than from those receiving dapsone.

(2) The mouse footpad infection has established for the first time the emergence of strains of *M. leprae* resistant to dapsone and to thiambutosine and the application of this method can now be used to assess the incidence of drug resistance.

(3) The characteristic of leprosy in man is the very wide range of clinical manifestations—from patients in whom the bacilli multiply freely, indicating little or no host resistance, to those in whom there are few bacilli, indicating a high degree of host resistance. In the mouse, bacilli from all these various types of leprosy, show a similar growth curve. These results suggest that the disease pattern in man is a reflection on variations in the host response and not on variations in the virulence of the organism. This conclusion is particularly reinforced by studies of infections with *M. leprae* in normal and in immunologically deficient mice, where the more resistant and less resistant forms of the disease, respectively,

can be reproduced. Furthermore, the more progressive form of the infection in immunologically-deficient mice can be reversed by transfusion of immunologically-competent lymphocytes from syngeneic animals. Such reconstituted mice manifest clinical and histologic evidence of acute inflammation at all the sites infected with *M. leprae*. These acute inflammatory changes resemble those seen in patients undergoing so-called "reversal reactions." These episodes in the mice, as in man, are followed by a shift in the leprosy pattern, changing from the lepromatous toward the tuberculoid type. Thus all these experimental models indicate that the disease pattern in response to infections with *M. leprae* has an immunologic basis. These experimental results in mice have stimulated investigations into the immunologic capacity of patients manifesting different patterns of leprosy. Although these studies are still in their early stages, they are already revealing overwhelming evidence of the part played by the host's defense mechanisms in determining the type of overt disease.

(4) In the mouse model, infections with *M. leprae* involve peripheral nerves, corresponding to the predilection for nerves by this bacillus in man, a property shared by no other species of mycobacterium. Therefore, the mouse has provided an experimental model for attempting to study the pathogenesis of nerve damage, which is the most important complication of leprosy in man. In the mouse, systematic studies on early phases of nerve involvement are beginning to provide evidence that the perineurium is the primary site of damage. Thus, in all peripheral nerve lesions in mice infected with *M. leprae*, where there is destruction of nerve fibers, a part or whole of the perineurium is destroyed and replaced by collagen fibers (Fig. 13). Certainly these more gross findings are consistent with the histology of nerves from patients with leprosy, where there is gross thickening of the perineurium and evidence of deposition of collagen and fibrosis. Systematic studies on the early changes in peripheral nerves of mice infected with *M. leprae*, before there is destruction of nerve fibers, frequently show intact perineurial

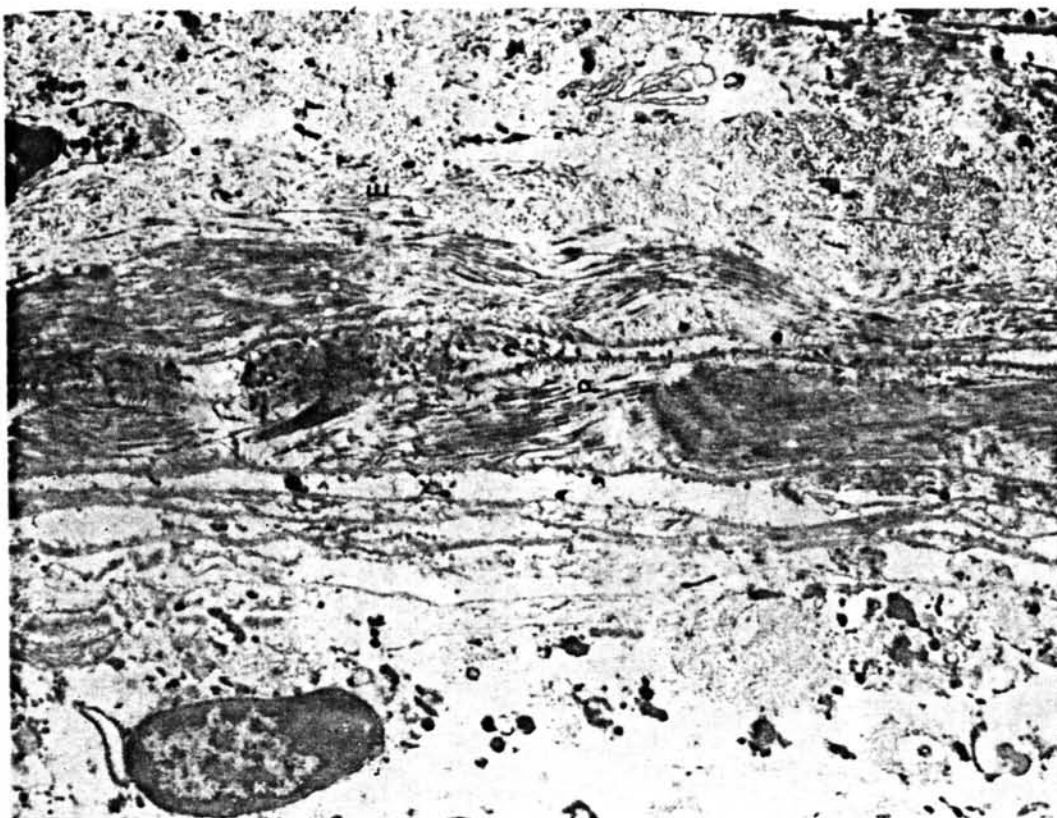


FIG. 13. Upper segment of sciatic nerve from the same mouse as in Fig 11. Electron micrograph showing almost complete destruction of perineurium with replacement by collagen. P = Perineurium; E = Epineurium.

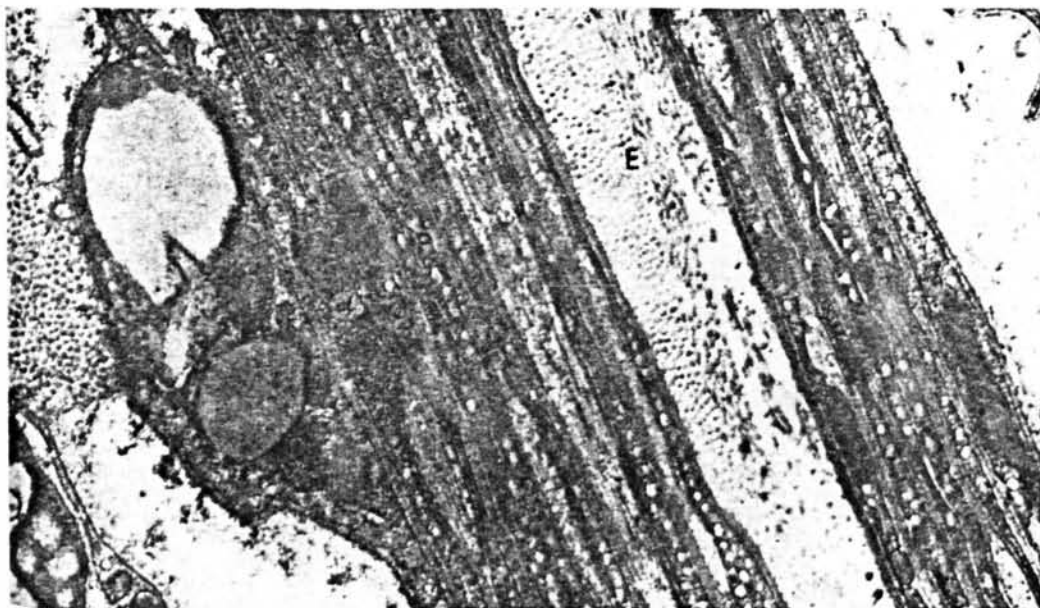


FIG. 14. Upper segment of sciatic nerve from a thymectomized-irradiated mouse with mild "foot drop", inoculated in the footpad with  $10^6$  *M. leprae* 21 months previously. Electron micrograph showing the perineurium (P) of two nerves with intervening epineurium (E). Note the thickened basement membranes of all the perineurial cells.

cells but with gross thickening of their basement membranes (Fig. 14). Taken together, these findings point to the perineurium as the "target site" in the attack on nerves in leprosy, and show that the subsequent damage to nerve fibers may result from a breakdown in the peripheral nerve-blood barrier normally maintained by an intact perineurium. Such a situation is seen in congenital hypertrophic neuropathy, where the primary lesion is a deficiency in the formation of the perineurium.

(5) In all infections with *M. leprae* in the mouse, striated muscle fibers appeared to be the cells of predilection for the bacilli and for their successful multiplication in the early phases of infection. Hitherto, this was the only feature of infections with the leprosy bacillus in the mouse which had not been described in man. On the basis of these observations in the mouse, a series of muscle biopsies were examined from patients with all types of leprosy, and the results indicated a similar situation in man<sup>(6)</sup>. A summary of these findings is given in Table 3. Similar observations have also been obtained by Job *et al.*, in studies on biopsies of striated muscle fibres in patients with leprosy<sup>(5)</sup>. These observations on the

importance of striated muscle fibers as a site in which *M. leprae* enter and multiply preferentially in the mouse, have stimulated further studies on the hitherto well documented information in man, that *M. leprae* were frequently found in arrectores pilorum muscles, i.e., nonstriated muscle of the skin. Thus Harman<sup>(3)</sup> in particular has found *M. leprae* in nonstriated muscle fibers of the breast and scrotum. The evolution of infections with *M. leprae* in the mouse, therefore has revealed the apparent importance of muscle fibers as a site in which the bacilli are preferentially protected against the normal defense mechanism. These observations suggest that muscle fibers may also be the cells in which the bacilli enter in the earliest phases of the infection in man. In order to test this hypothesis studies are planned to see whether acid-fast bacilli are present in muscle fibers obtained from otherwise healthy contacts of patients with active leprosy.

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TABLE 3. Presence of acid-fast bacilli (AFB) in muscle biopsies from leprosy patients.

Type of disease*	Biopsies		
	Number	AFB present	AFB in muscle fibers
Lepromatous (LL-BL)	20	16	13
Borderline (BB)	4	3	2
Tuberculoid (BT-TT)	9	5	5
Total	33	24	20

\* Classification is according to Ridley and Jopling<sup>(20)</sup> and Ridley and Waters<sup>(21)</sup>.

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