INTERNATIONAL JOURNAL OF LEPROSY

Volume 41, Number 1
Printed in the U.S.A.

Ultrastructure of Lepromatous Nerves. Neural Pathogenesis in Leprosy[1]

Darab K. Dastur, Y. Ramamohan and J. S. Shah[2]

That all leprosy is neural leprosy [17] and is so from its very inception [21] is now well-established [8, 24]. Affecting as it does some 15 million patients, with a quarter of them presenting disabilities and deformities, leprosy constitutes the single largest peripheral nerve disorder [7, 9]. Of the two polar types of leprosy, tuberculoid and lepromatous, it is the latter type with its astronomical number of *Mycobacterium leprae* which is suitable for studying host-parasite relations in the presence of deficient immunity in man. In contrast to this, very few bacilli are seen in the tuberculoid type, probably due to host resistance operating through cell-mediated immunity which appears to contain the infection [38].

Earlier histologic work, spread over a hundred years as reviewed elsewhere [6,-24], has clearly established an essential feature of the disease to be involvement of the peripheral nervous system, and the affinity of *M. leprae* for peripheral nerves [22] and Schwann cells in particular [7,-24, 44], and the sparing of the central nervous system. While there is no dearth of histological studies on peripheral nerves in leprosy using the light microscope, the same is not true for ultrastructural studies employing the electron microscope (EM). The latter approach is expected to throw further light on the neural pathogenesis in leprosy.

In the last few years, however, using electron microscopy, Nishiura and his colleagues [25, 26, 27] have reported *M. leprae* in axons, in Schwann cells of the cords of Büngner, in lepra cells in the endoneurium and have also described bacterial microenvironment and regeneration of axons in peripheral nerves in lepromatous cases [25]. Imaeda and Convit [19] reported similar findings in cutaneous nerves in skin lesions. Job [20] showed *M. leprae* in Schwann cells of myelinated and unmyelinated fibers and also in macrophages, endothelial cells and perineurial cells. He confirmed the impressions of Weddell *et al* [44], Lumsden [24] and Dastur [9] that the Schwann cell was the target cell in leprosy. Other studies have elucidated bacterial morphology [4, 18, 28] and the recognition of degenerating and living forms with electron microscopy [33]. Rees and Weddell and their colleagues [34, 35, 43] have recently evolved experimental models to elucidate the pathogenesis of leprosy in mice infected with *M. leprae*, and have sought to relate their findings to human leprosy.

When the present investigation was initiated two years ago, a number of important pathologic questions relating to the peripheral nervous system still appeared unanswered. These pertained to the mechanism of ingress and dissemination of *Mycobacterium leprae* within peripheral nerves; the interactions between the invader and the various neural constituents, parenchymal and supporting, and the inflammatory cells; the type and pattern of degeneration of nerves; the extent and nature of the regenerative effort; the role of connective tissue in nerve damage; the possible axonal participation in harboring and disseminating *M. leprae;* and changes in the perineurium. The basic unclarified problem in the neural pathology of leprosy, therefore concerned the evolution of the disease in nerves.

The present study was undertaken with the object of attempting an answer to these specific and general questions regarding leprous involvement of the peripheral nervous system. It forms part of a larger multipronged investigation employing histologic,

1 Received for publication 18 July 1972.

2 D. K. Dastur, M.D., D.Sc., M.R.C.P.; Y. Ramamohan, M.Sc.; and J. S. Shah, F.R.C.S., Neuropathology Unit, Postgraduate Research Laboratories, Grant Medical College and J. J. Hospitals, Bombay-8; and the Acworth Leprosy Hospital, Bombay, India.

histochemical and ultrastructural methods and using early untreated cases of tuberculoid and lepromatous leprosy. The electron microscopic findings in nerves from tuberculoid cases have been detailed elsewhere ([13]); the histochemical changes and their correlation to light microscopy will be published subsequently. The present investigation reports ultrastructural changes in lepromatous nerves and relates these to neural pathogenesis in leprosy.

MATERIALS AND METHODS

The four cases of lepromatous leprosy being presented here were selected from the outpatients attending the Acworth Leprosy Hospital in Bombay. The clinical diagnosis of these patients was established with the help of a clinical leprologist of the same hospital, where bacteriological examination of skin-snips from the ear and any overt lesion was also carried out. Three of the patients were of the lepromatous (LL) variety and one of the borderline lepromatous (BL) type. The material consisted of one or two branches of the radial cutaneous nerve in three cases, and in the fourth case a branch of the musculo-cutaneous nerve from the foot.

While three of these cases had not received any treatment for leprosy, the fourth had received treatment with DDS for two years but had discontinued treatment for the past three years and therefore is considered to be untreated for all practical purposes. The patients chosen were young adults who were fairly well-nourished, non-alcoholic and without evidence of any other peripheral nerve disorder.

These patients were subjected to careful clinical examination with particular attention to overt skin lesions and to cutaneous

TABLE 1. *Clinical and operative findings.*

Serial no.	Neuropath. no.	Age & sex	Type of leprosy	Clinical	Sensory status	Nerve appearance
1	NP/F/511	M 26	LL	Skin lesions (nodular ears) ±[a] Cutaneous nerves +[b] Large nerves + (tender)	Minimal impairment to PP & CW[c]	Thick yellow & adherent +
2	NP/F/985	M 30	BL	Skin lesions + Cutaneous nerves ++ Large nerves ++ (tender)	Anesthesia	Thick & adherent +
3	NP/G/26	M 33	LL	Skin lesions (nodules) + Cutaneous nerves ++ Large nerves + (tender)	Normal Normal	Thick & adherent ++
4	NP/G/198	M 20	LL	Skin lesions, nodules, all 4 limbs & face ++ Vague hyperpigmentation ± Cutaneous nerves ± Large nerves +	Minimal impairment to PP & CW	Not thickened

[a] ++ = marked change; + = clearly noticeable; ± = slight change; − = no change.
[b] While nerves of this category were thickened in general, the nerve biopsied was not.
[c] PP = pin prick; CW = cotton wool (light touch).

sensations in the area supplied by the nerve as tested by cotton wool and pin prick and occasionally hot and cold tubes. Palpable thickening of the nerve chosen for biopsy and of other peripheral nerves, and areas of sensory loss in their territories were also looked for. The branch of the nerve selected for biopsy was generally not thickened, or only slightly thickened, and there was no appreciable loss of sensation in its territory of supply. The clinical features in each case are summarized in Table 1.

The surgical exploration of the nerve was carried out under local anesthesia by the Hon. Plastic Surgeon of the Acworth Leprosy Hospital (J. S. S.), assisted by the neuropathologist (D. K. D.). The appearance of the nerve branches exposed (generally the two most radial branches of the radial-cutaneous nerve) as they course from the wrist to the heads of metacarpal bones, their feel and the nature of surrounding tissues and any adhesions to them, were carefully observed.

Four to six centimeter lengths of one or two selected branches from each patient were excised. These specimens were divided into roughly four equal parts for the following sets of observations: 1) fixation in 4% glutaraldehyde for ultrastructural study; 2) fixation in neutral buffered formalin for histochemistry; 3) fixation in routine formalin for paraffin embedding; and 4) teased fiber preparations for various histological examinations.

After treatment in Millonig's buffer, the portions destined for electron microscopy were treated with osmic acid, blocked in araldite, cut as semithin sections for light microscopy and cut as ultrathin sections which were finally treated with uranyl- acetate and lead citrate ([36]). The semithin sections of about 2μ thickness were examined and photomicrographs of cross-sections of nerves were taken at identical magnifications for counting the number of myelinated fibers (Table 2). Another set of semithin sections was stained with toluidine blue. The grids bearing ultrathin sections were examined at the Tata Institute of Fundamental Research, using a Philips EM200 model electron microscope, at 80 kv, and the electron micrographs obtained on plates were studied in the usual enlarged prints.

The paraffin sections of the portion meant for histological examination were stained with hematoxylin-eosin, picro-Mallory's method for connective tissues and myelin, Holme's silver impregnation for axons, and Fite-Faraco's method for acid-fast bacilli.

The nerve fiber teasing was carried out according to the method of Cavanagh and Jacobs, but utilizing Sudan black as the myelin stain ([14]).

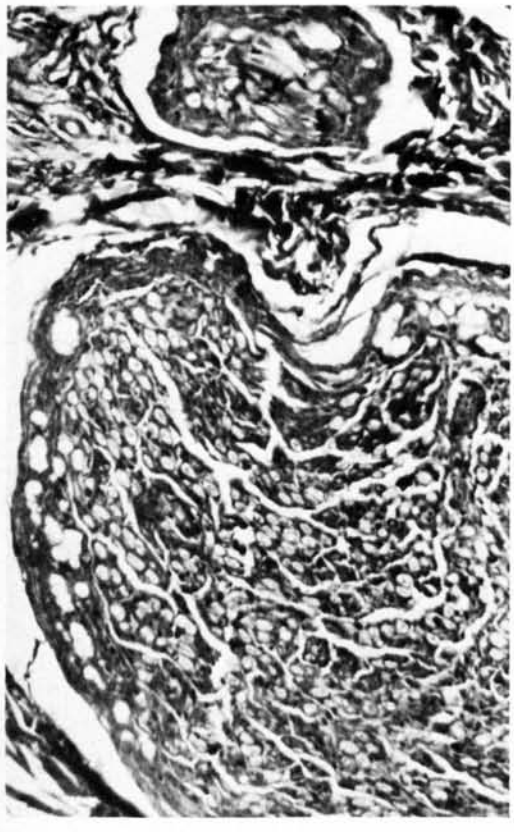
OBSERVATIONS

The clinical and surgical findings in the four patients are summarized in Table 1. Two of the three floridly lepromatous patients showed clear nodules, while one showed only thickening of the ears. The patient with BL type of leprosy had anesthetic patches on a foot and the leg. At nerve exploration three of these four patients, who had shown clinical thickening of the selected nerve, showed thickening, discoloration and adhesions more clearly. The most advanced lepromatous case

TABLE 2. *Relative numbers of large and small myelinated fibers.*

Serial no.	Neuropath. no.	Large and medium sized myelinated fibers	Small myelinated fibers	Tot. no. of myelinated fibers
1[a]	NP/F/972	113	83	196
2[a]	NP/G/166	72	70	142
1	NP/F/511	62	19	81
2	NP/F/985	13 (medium sized only)	47	60
3	NP/G/26	31	68	99
4	NP/G/198	40	45	85

[a] Controls.



A

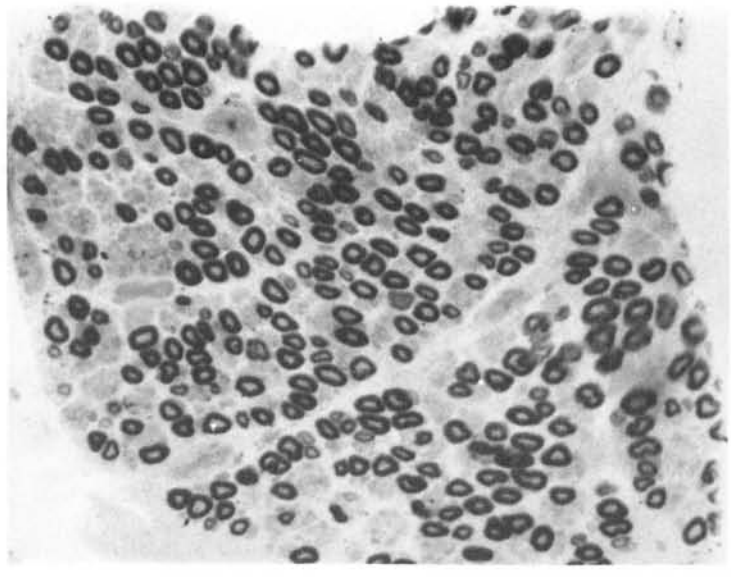
B

FIG. 1. (NP/F/511). A. Paraffin cross-section of nerve showing a number of well-preserved myelinated fibers amidst increased endoneurial collagen; perineurium thickened and vacuolated. Picro-Mallory, X 250. B. Same nerve a short distance away showing bacilli in Schwann cells and bacillary granules in vacuoles of perineurium. Fite-Faraco, X 1,400.

(NP/G/198) showed doubtful thickening of the nerve clinically and no adhesions or gross changes at operation.

Histologic and ultrastructural changes. The three more florid LL cases, with greater bacillation of nerves as compared to the BL case, revealed a wide variety of neural change. The ultrastructural changes in the nerves from these four cases will be illustrated in relation to the more obvious histologic changes, having constant reference to the proportions of large and small myelinated fibers in the nerve from each of the four patients, compared to similar fiber ratios in the normal nerves. This data is summarized in Table 2. There was a depletion of the large myelinated fibers in all four nerves, this being most marked in the BL nerve (NP/F/985).

The least affected lepromatous patient, a young man in very good physical condition who reported only for a slight thickening of ears, presented minimal thickening of the nerve (radial-cutaneous, LL) at operation (Table 1, NP/F/511). On histologic examination this showed moderate diffuse endoneurial fibrosis and the presence of vacuoles in the thickened perineurium of many funiculi of this nerve (Fig. 1-A). The Fite stain showed that these vacuoles contained chiefly granular forms of bacilli and that there were also more intact organisms in the Schwann cells (Fig. 1-B). Consistent with the minimal sensory impairment observed clinically, semithin sections of araldite embedded nerve tissue showed fair preservation of large myelinated fibers (Fig. 2-A). However, comparison with a



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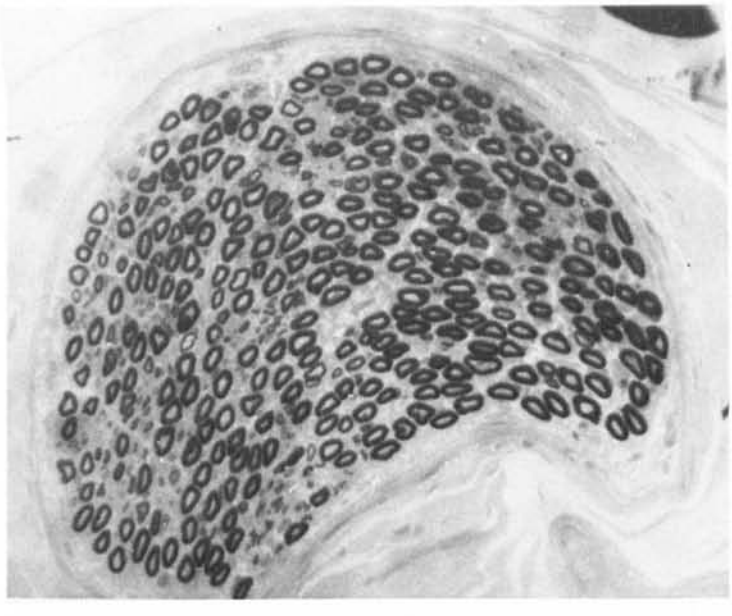


B

FIG. 2. A. Same nerve as in Figure 1; semithin araldite section showing some loss of large myelinated fibers. B. Identical section of normal nerve (NP/F/972). Both OsO_4, X 250.

normal radial-cutaneous nerve (Fig. 2-B) and counting of large and small myelinated fibers in both nerves, revealed an actual reduction in the number of these fibers in the leprosy nerve as compared to the control nerves (19 and 62 fibers respectively) in fields of identical size (Table 2). There was relative prominence in the leprous nerve of areas containing nonmyelinated fibers and Schwann cells (Fig. 2-A).

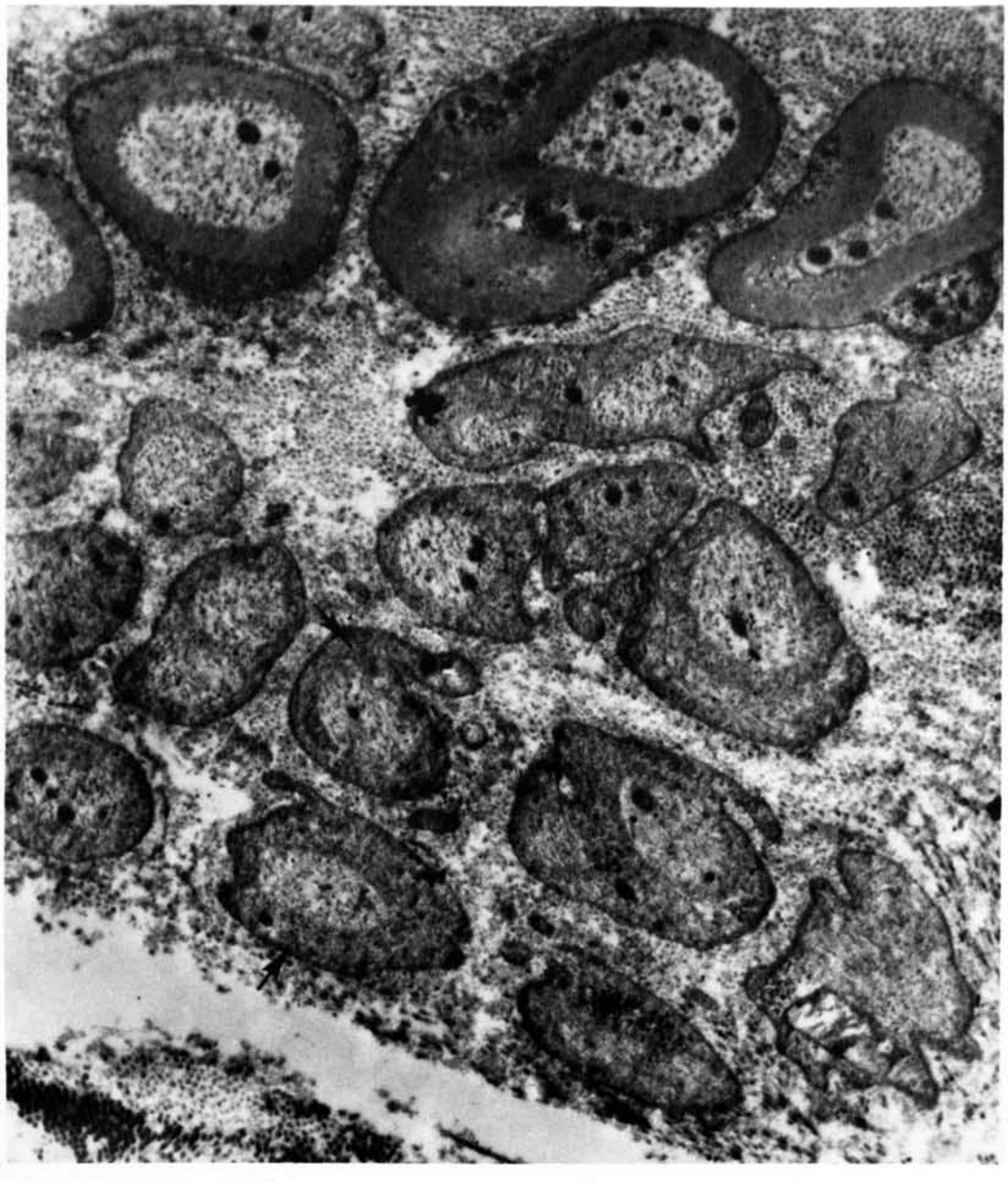


FIG. 3. Same nerve as in Figure 1; showing thickening of Schwann cytoplasm (arrows) around individual nonmyelinated fibers; note four small myelinated fibers in a row at the top. EM, OsO_4, uranyl acetate and lead citrate, X 33,500.

Electron microscopic examination showed that the unmyelinated fibers were quite prominent throughout and with rather bulky Schwann cytoplasm surrounding them (Fig. 3). This was seen at two different levels of the nerve. The Schwann cell processes were quite pronounced in some places, and suggested the formation of bands of Büngner. Along with this there was a tendency towards grouping of small myelinated fibers suggesting a regenerative effort. Redundant basement membranes of empty Schwann cells, containing at best myelin debris or axon remnants (Fig. 4) were frequently encountered in this nerve. At times several such ghost outlines of

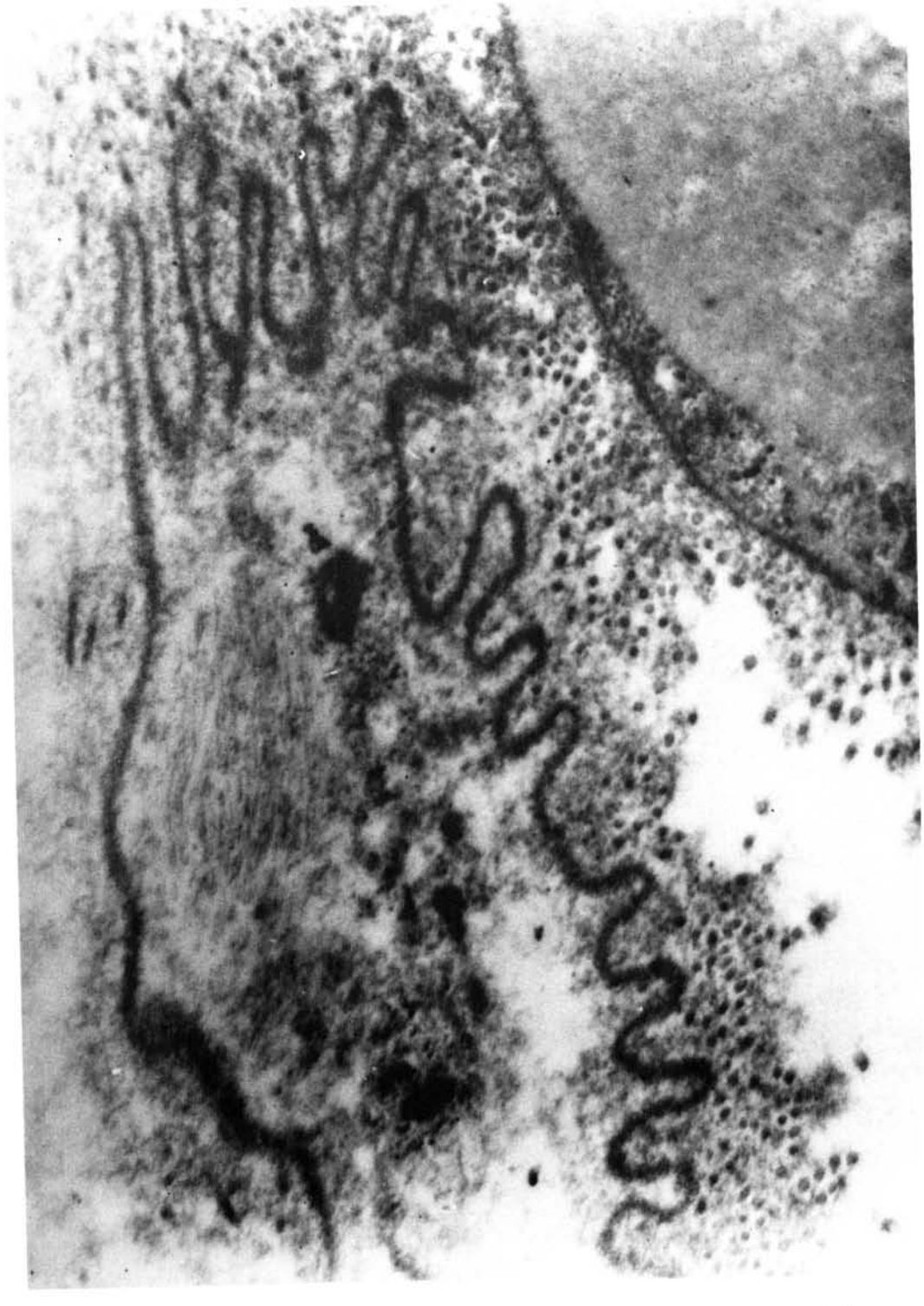


FIG. 4. Same nerve as in Figure 1; accordion-like basement membrane of degenerated Schwann cell and fiber with only a few axon filaments and debris inside; small sector of large well-myelinated fiber in one corner. As in Figure 3, X 45,000.

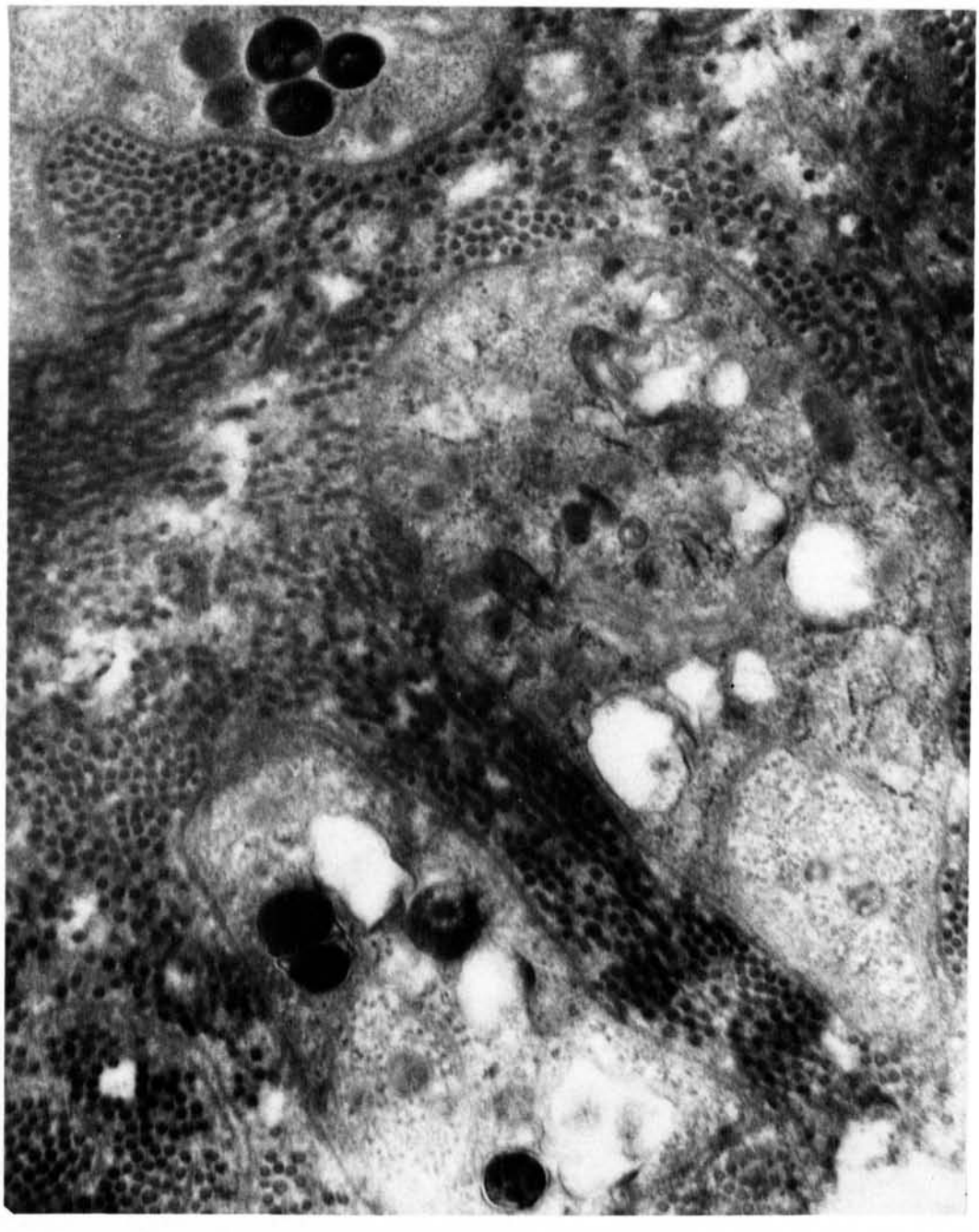
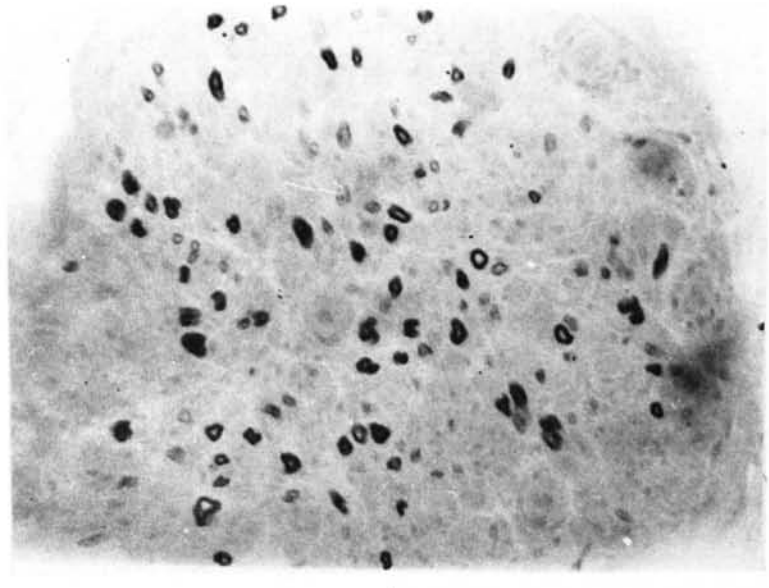


FIG. 5. Some nerve as in Figure 1; three unmyelinated fibers with clear basement membranes showing intact *M. leprae* (with surrounding halos) in two of them, and empty vacuoles in two of them; one clear axon seen in two of the fibers; note increased collagen. Same as in Figure 3, EM, X 41,000.

degenerated fibers were seen together. Another feature of note was the prominence of Schmidt-Lantermann (SL) clefts in many of the large myelinated fibers, with an occasional fiber showing two concentric SL clefts. Mycobacteria were present in Schwann cells of nerve fibers, preferentially of unmyelinated fibers (Fig. 5). The organisms were generally seen as circular, densely osmiophilic bodies representing viable



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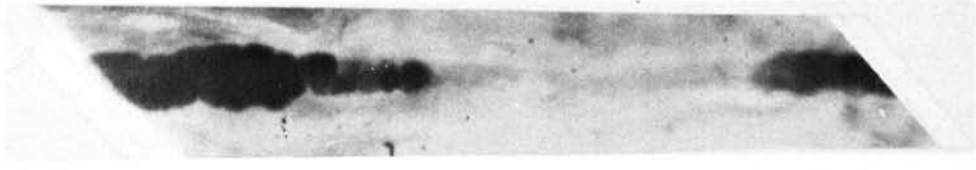


B

FIG. 6. (NP/F/985). A. Semithin araldite section through one nerve bundle showing severe loss of large myelinated fibers and ?-remaining, ?-regenerating small myelinated fibers of varying shape and thickness. OsO_4, X 250. B. Teased fiber from adjacent portion of same nerve showing segmental loss of myelin with irregularly thickened coil of myelin on one side. Sudan black, X 625.

bacilli, with a small clear halo around the cell wall. Within the same and adjacent Schwann cells empty spaces representing bacilli that had disappeared (?-degenerated), and at times lined by a broken or intact thin black line (?-phagocytic membrane), were observed.

Consistent with our light microscopic findings, Schwann cells did not contain many degenerating *M. leprae* and, in this respect, differed from the organisms in the perineurium of the same nerve to be considered later. The bacilli were invariably encountered within Schwann cytoplasm, the remaining axons of such unmyelinated fibers not harboring bacilli (Fig. 5). *M. leprae* were also seen in cell processes which could have belonged to Schwann cells or fibroblasts, and occasionally in elongated cells in the endoneurium which probably were fibroblasts. Rarely, circular or oval, granular or homogenous bodies of lesser osmiophilia than the bacilli were encountered near them; such as the paired bodies in the upper Schwann cell in Figure 5. Their nature will be discussed.

The overall impression gathered on examination of semithin and ultrathin sections was that of axonal type of degeneration, evidenced especially by depletion of myelinated fibers, and this was confirmed in teased fiber preparations which showed various stages of Wallerian degeneration including droplet formation.

The branch of the musculo-cutaneous nerve from the foot of the BL case (Table 1, NP/F/985) showed severe degenerative changes with a loss of almost all large

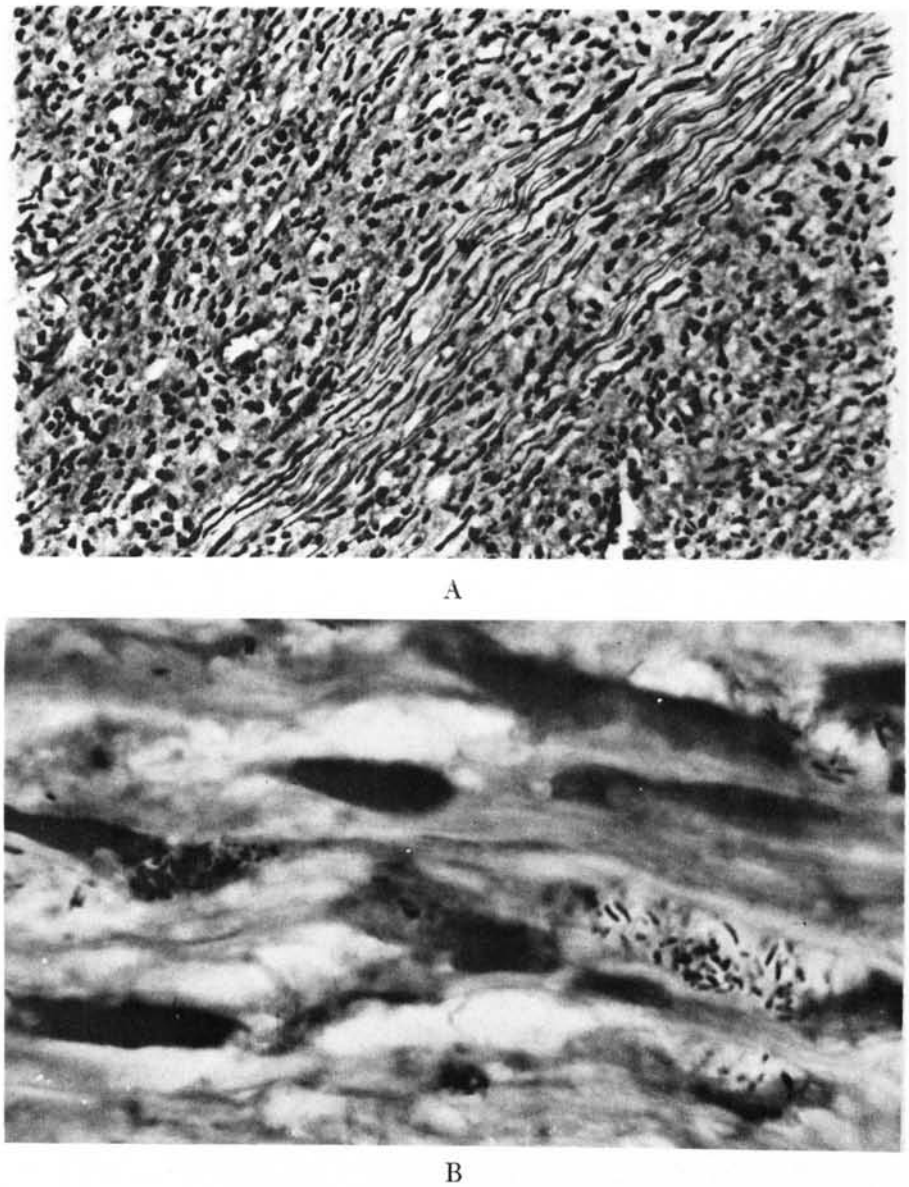
myelinated fibers (Fig. 6-A and Table 2). This was consistent with the anesthesia detected clinically in the territory of this nerve. Ultrathin sections revealed a number of bacilli (which were also seen in paraffin sections) in the Schwann cytoplasm, especially of unmyelinated fibers. Both degenerating and intact forms of *M. leprae* were seen. Very small myelinated fibers, some with fewer than the full complement of myelin lamellae, and at times with prominent nuclei suggesting regeneration, were seen in places and confirmed the impression gathered on semithin sections. At the same time the rest of the nerve matrix was filled with compact cords of nonmyelinated fibers (Fig. 6-B), suggesting regeneration and with fibroblasts. Both of these showed *M. leprae*. Teased fiber preparations confirmed the presence of thin myelinated fibers only, many of them showing elongated nodal gaps which at times extended over sizeable portions of one or two internodes, constituting a form of segmental demyelination. However, there was some associated axonal degeneration.

The third patient was (radial-cutaneous nerve) a more advanced lepromatous case with overt skin lesions as well as neural involvement (Table 1, NP/G/26). Semithin sections showed considerable loss of myelinated fibers (the ratio of large to small myelinated fibers being 31:68, Table 2), with clear increase of intervening Schwann cell processes. Paraffin sections also confirmed this fiber degeneration and in addition showed considerable infiltrations in parts of the nerve by inflammatory cells. Silver impregnated sections evidenced varying degrees of axonal preservation and loss, with occasional clustered sprouts of very fine smooth fibers suggesting regeneration (Fig. 7-A). The enormous bacillation of the nerve was revealed even in semithin sections stained only with toluidine blue (nonspecific bacillary stain), when they were seen filling up Schwann cytoplasm and this was confirmed by Fite-Faraco's method (Fig. 7-B) and by fluorescent technics.

Both teased fiber preparations and semithin sections of longitudinally oriented specimens showed various stages of Wallerian degeneration including the possible early stage (see Discussion) of an irregular increase in the number of SL clefts in many of the internodes (Fig. 8-A). This was also noticeable on electron microscopy of longitudinally oriented nerves when the close proximity of successive pairs of SL clefts was noticeable. At the same time overt axonal degeneration was also seen (Fig. 8-B).

The most striking feature on examination of the nerve through conventional ultrathin cross sections was the presence of very large numbers of *M. leprae* in a number of fibers at two different levels of one nerve and in a second nerve branch examined in this way (Fig. 9). The parasitized fibers almost always showed considerable destruction of both axon and myelin, but the basement membrane remained intact, confirming the light microscopic impression of the bacilli being in Schwann tubes. While the majority of bacillated Schwann cells again appeared to be of nonmyelinated fibers, at times their shape, size and configuration suggested, as in Figure 9, that they were degenerating myelinated fibers. The bacilli were more in the center of Schwann tubes, but never clearly within the axons. Closer examination showed the organisms again to be in both viable and degenerating forms, the latter presenting a moth-eaten appearance or with a break in the bacillary wall (Figs. 9 and 10), as in the first case. Besides the still recognizable bacilli and the bacillary debris, faintly osmiophilic granular bodies, probably lysosomes, were encountered in the Schwann cytoplasm (Fig. 9). The integrity of the myelin, axon and nuclei of nerve fibers not harboring *M. leprae* was in marked contrast to the degenerative changes, often with vacuolation and swelling of the "cell," observed in the parasitized fibers (Fig. 10).

Many of the blood vessels encountered on electron microscopy confirmed the light microscopic observation of bacilli in endothelial cells. In Figure 11-A, the cytoplasm of an endothelial cell of a small blood vessel shows bacilli in different states of preservation, the more degenerating organisms being surrounded by larger clear

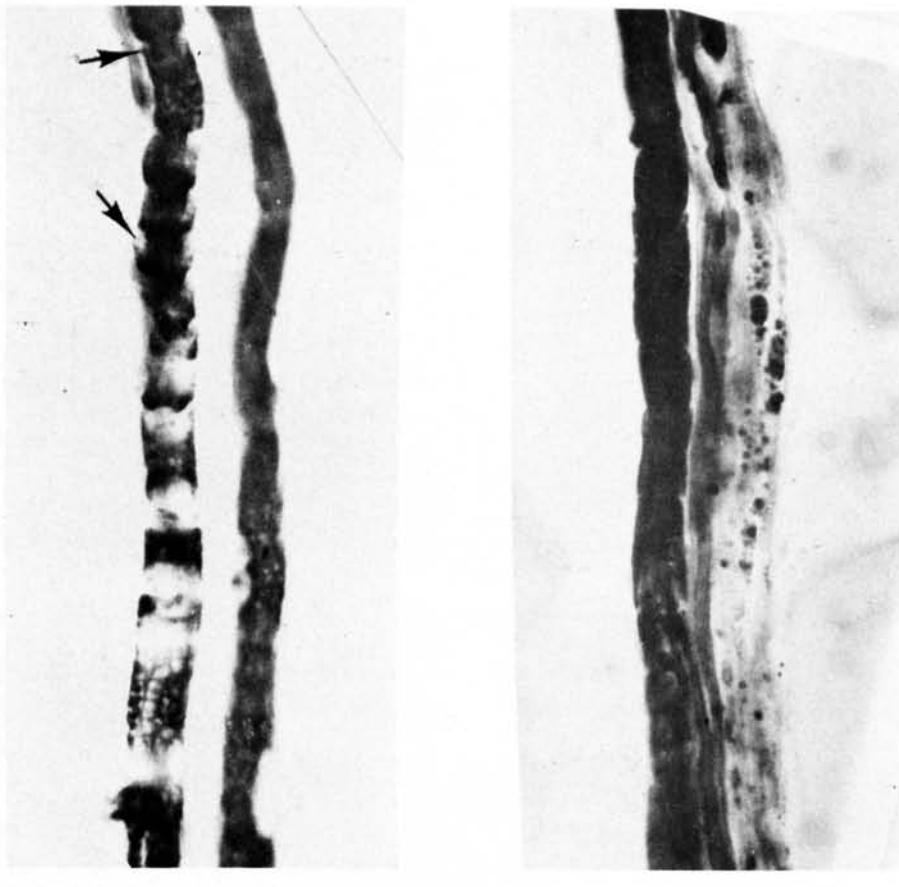


A

B

FIG. 7. (NP/G/26). A. Part of a nerve bundle showing dense infiltration by mononuclear cells, total absence of large axons and a small central group of ?-remaining, ?-regenerating, fine smooth axons. Holmes' silver, X 250. B. Same branch showing clusters of bacilli in swollen Schwann tubes. Fite-Faraco, X 1,400.

spaces. Bacilli were also seen, though infrequently, within pericytes in this nerve and in fact around the vessel illustrated in Figure 11-A. The nerve from the fourth case of the present series, who had more advanced lepromatous leprosy than others, showed more florid parasitization of the endothelial cells by intact bacilli. At times the endothelial cells were ballooned and thinned by organisms, vacuoles and granular osmiophilic material, so that they bulged into the lumen of the vessel (Fig. 11-B). At the point of thinning, they appeared to be rupturing and discharging their contents into the lumen of the vessel, as *M. leprae* were seen floating free in the plasma (Fig. 17-B). This was actually observed in a few instances, the bacilli in such



A B

FIG. 8. Same nerve as in Figure 7; teased fibers. A. One well-myelinated fiber and the other with constrictions at the SL clefts (arrows) which are increased. B. Advanced axonal degeneration with droplet formation in fiber. Both Sudan black, X 625.

cases being generally in the solid viable form. Vessels with endothelial proliferation of this kind showed proliferation and plication of their basement membranes (Fig. 11-B).

The nerve from this fourth case (musculo-cutaneous, foot, LL, Table 1, NP/G/198) also showed on light microscopy, a combination of proliferation, degenerative and inflammatory changes. Even in relatively better preserved areas, especially in frozen sections stained with hematoxylin and eosin, one could notice irregularities and ballooning of myelin (Fig. 12-A). Here, but especially in areas with more sheath cell proliferation and axonal degeneration, enormous and uniform bacillation of Schwann cells was observed with bacillary stains as well as in fluorescent preparations (Fig. 12-B). Teased fiber preparations showed Wallerian degeneration, comparable to that observed in the other two lepromatous cases.

Semithin and ultrathin secions of this nerve revealed moderate diffuse depletion of large and small myelinated fibers (compare Fig. 2-B), prominence of interlying Schwann tissue and thickening of perineurium (Fig. 13-A). Closer examination revealed clusters of *M. leprae* in Schwann cells of unmyelinated and less frequently, of myelinated fibers (Fig. 13-B). Electron microscopy confirmed the great frequency of bacillation of unmyelinated fiber groups,

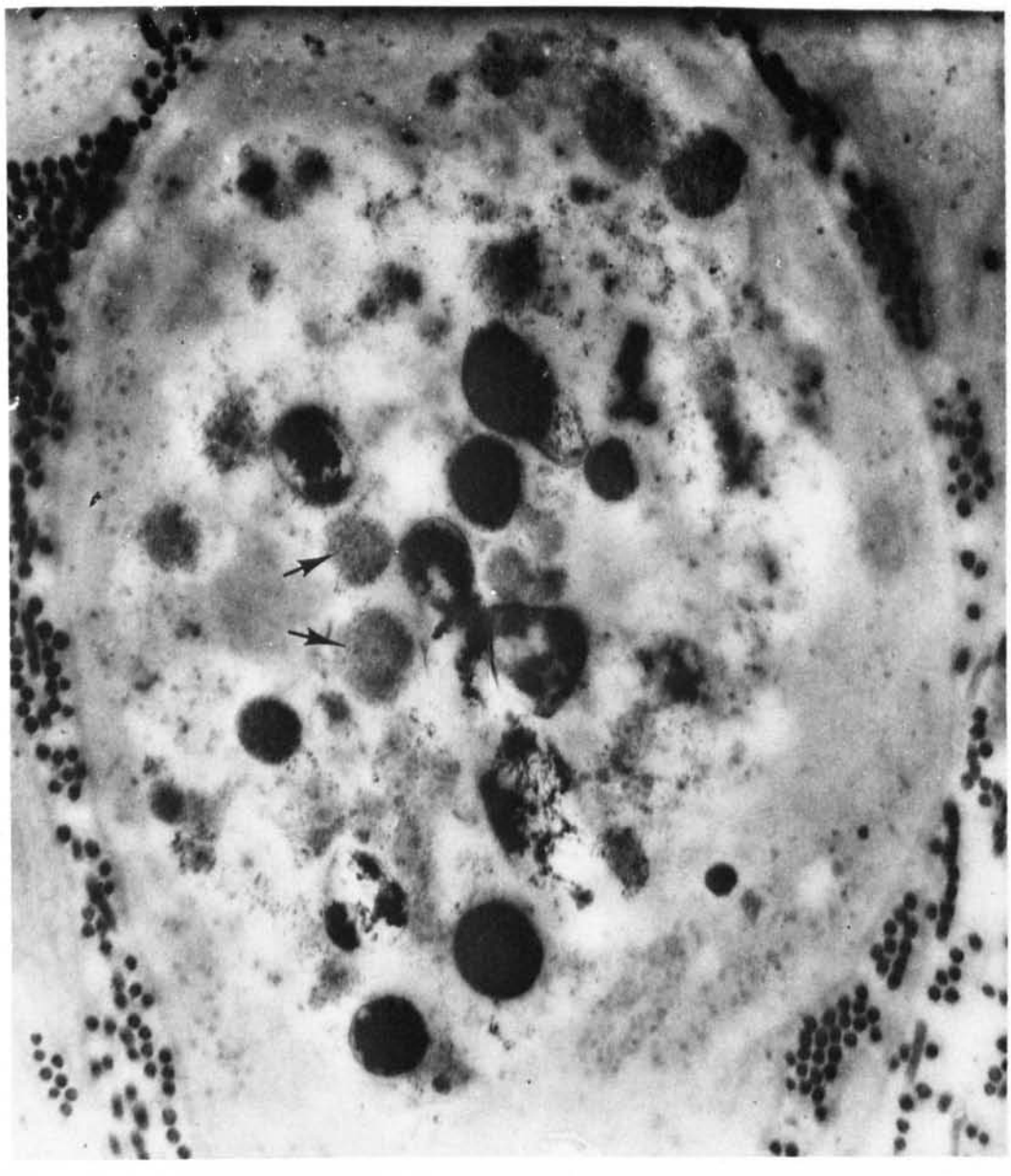


FIG. 9. Same nerve as in Figure 7; severe baccillation of a Schwann cell of ?-originally myelinated fiber. Intact and degenerating forms of *M. leprae*, faintly osmiophilic granular bodies, probably lysosomes (arrows) and intact basement membrane of swollen Schwann cell. As in Figure 3, X 54,000.

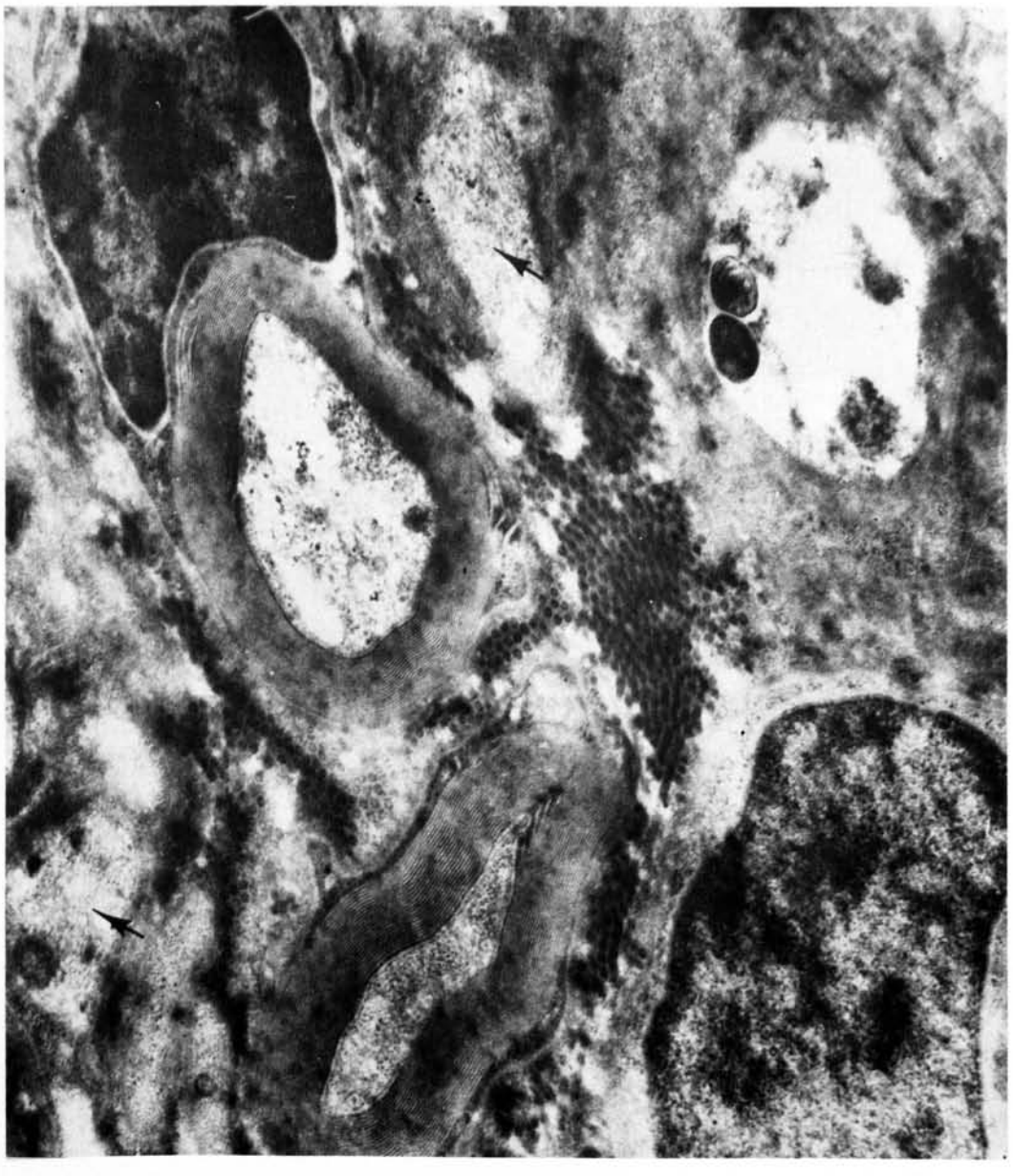


FIG. 10. Same nerve as in Figure 7; two small well-myelinated fibers which are not parasitized; and intact and degenerating bacilli in a large vacuole in ?-Schwann cell of one of the disorganized, unmyelinated fiber groups (arrows). Note prominent nuclei. Same as in Figure 3, X 41,000.

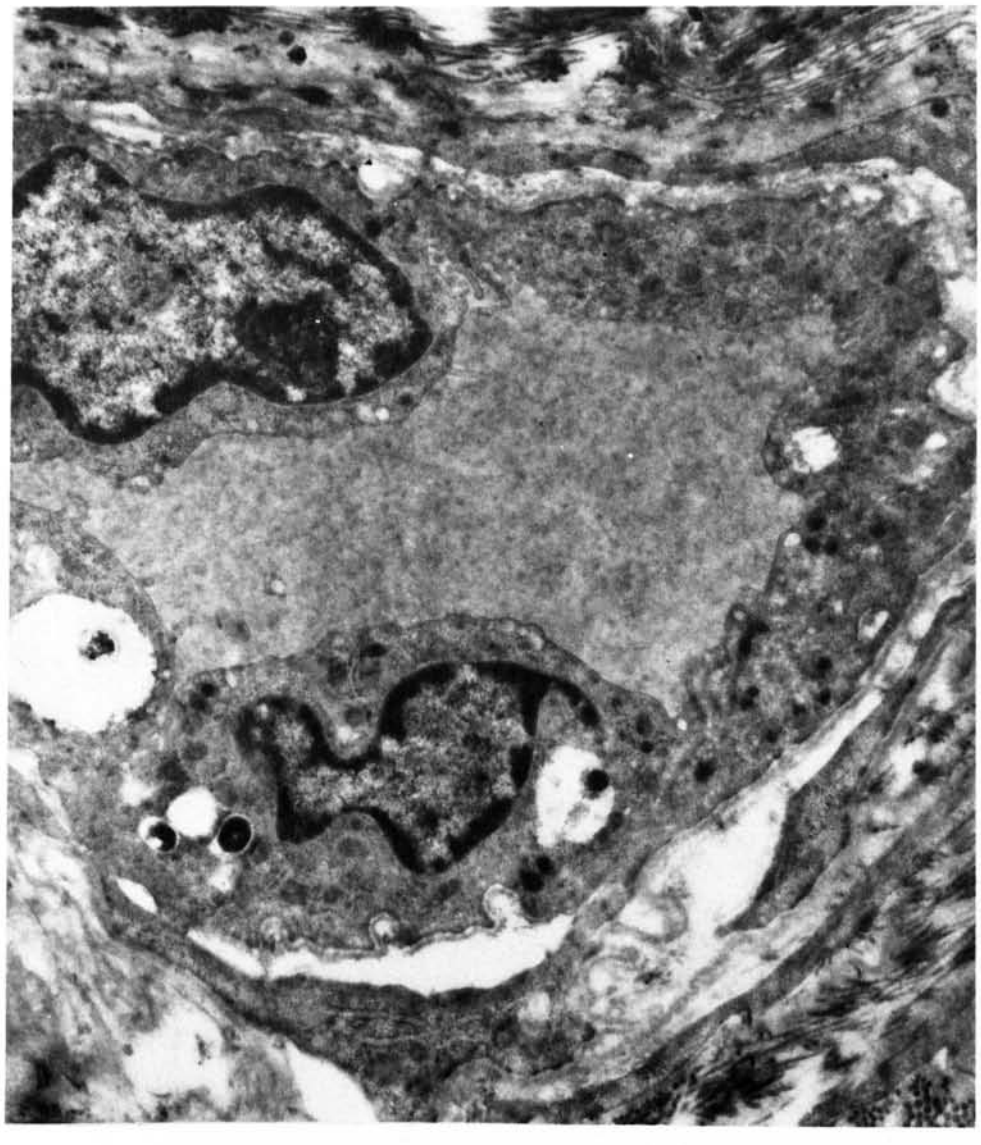


FIG. 11. A. Same nerve as in Figure 7; a small endoneurial blood vessel with prominent endothelial cells containing intact and degenerating forms of *M. leprae,* with or without spaces around them.

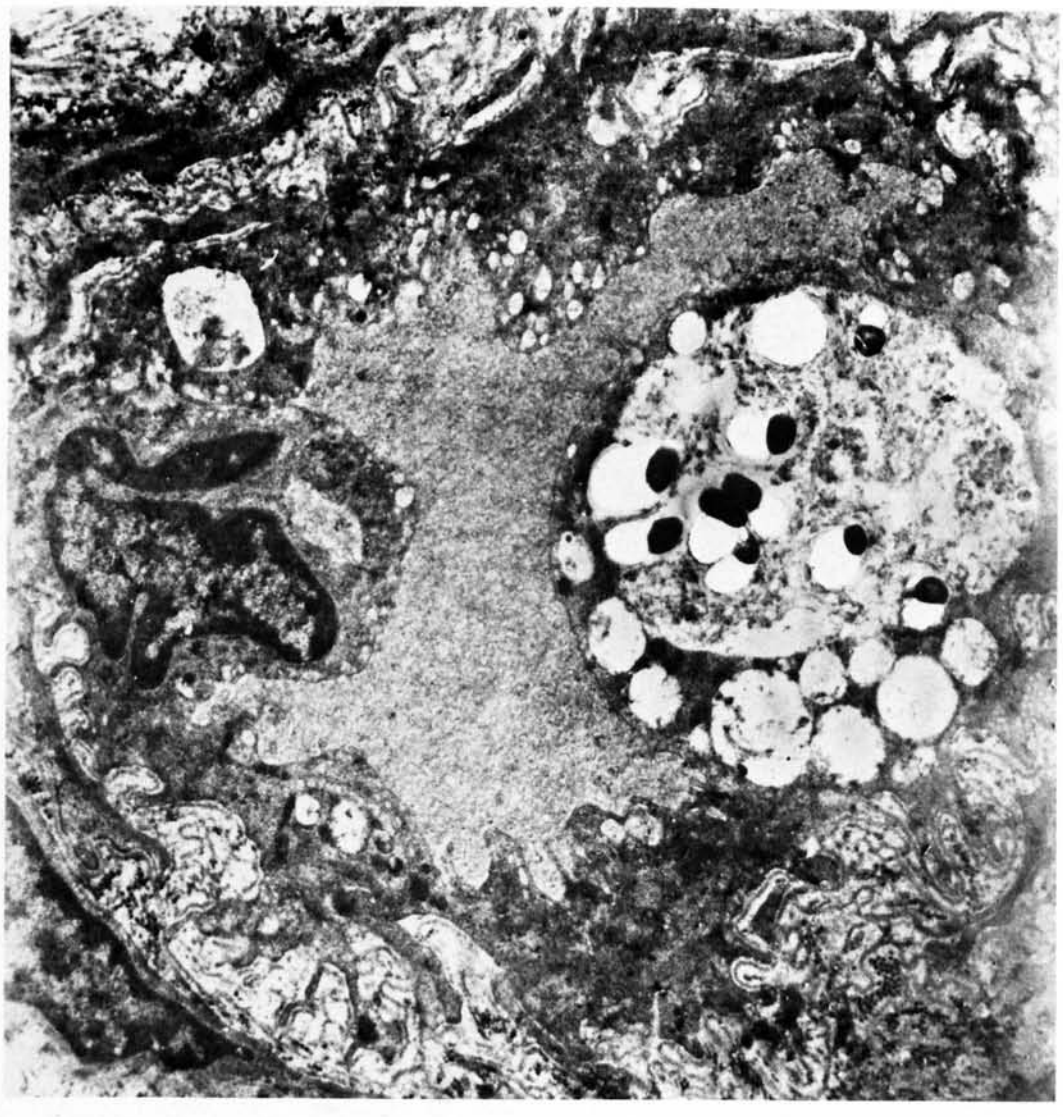
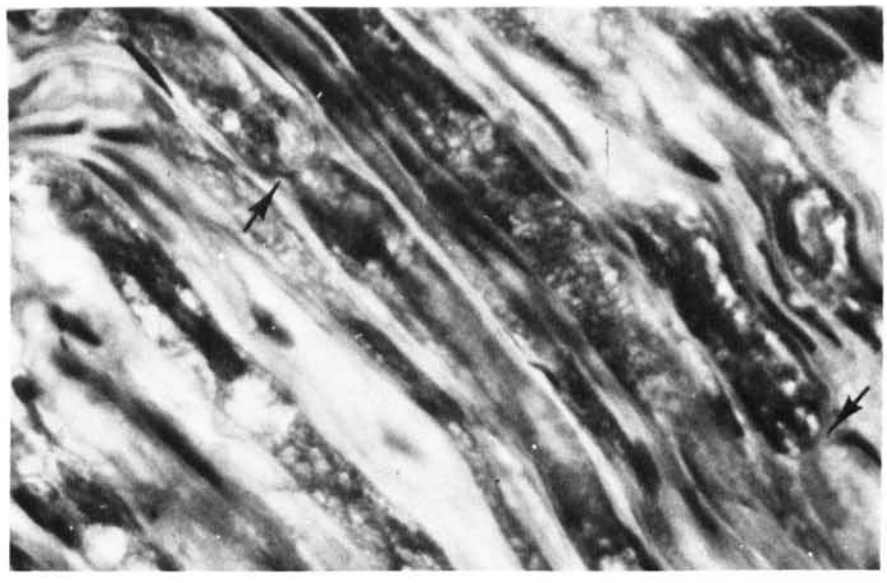


FIG. 11. B. (NP/G/198). Excessively swollen endothelial cell containing intact *M. leprae* together with homogenous and granular material, bulging into the lumen of a small blood vessel; note several small pinocytotic vacuoles along inner side of endothelial cells and proliferated basement membrane outside. Same as in Figure 3; A X 13,500, B X 13,500.



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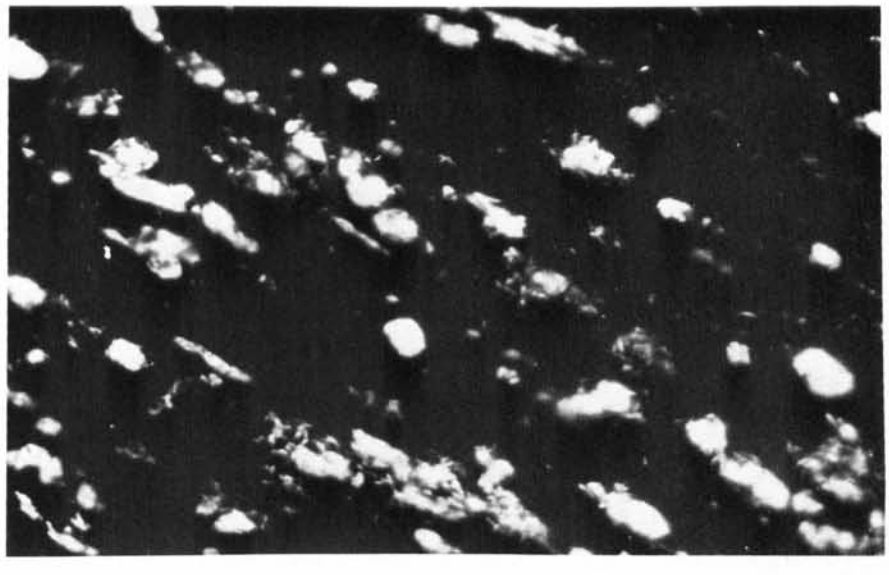
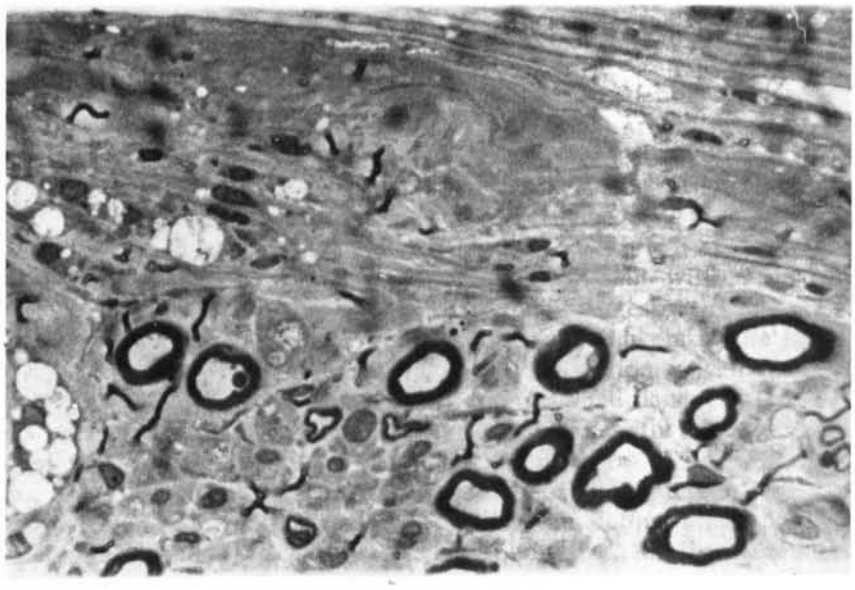


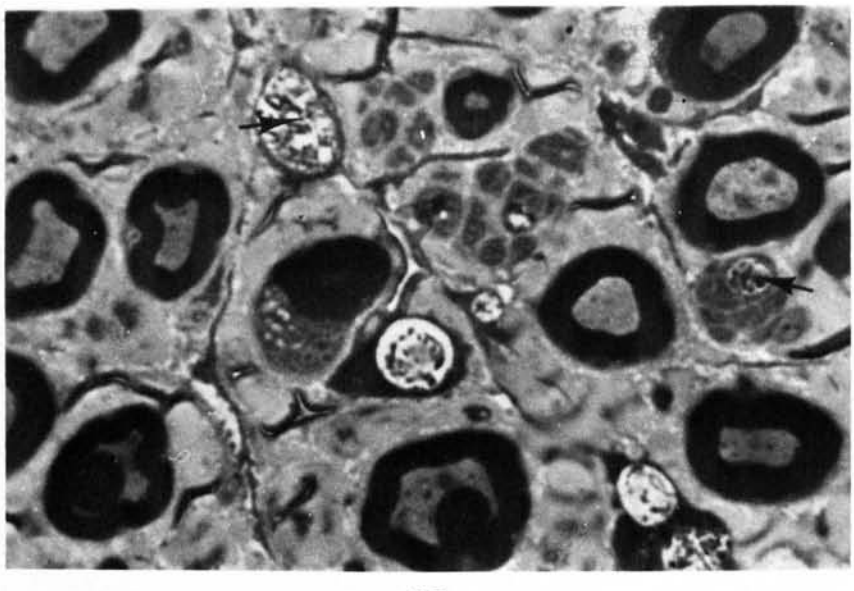
B

FIG. 12. Same nerve as in Figure 11-B. A. Frozen section showing thick and thin myelinated fibers appearing spongy or vacuolated; nodes of Ranvier seen (arrows). Hematoxylin and eosin, X 625. B. Paraffin section under fluorescent light, showing dense clusters of *M. leprae* arranged in rows, in one nerve bundle. Rhodamine-auramine, X 625.

the majority of organisms being of the solid viable type. The Schwann cells of these unmyelinated fibers were ballooned or formed irregular cords bearing less than the normal complement of axons (Fig. 13-C). There was a clear contrast between the original thicker myelinated fibers (Fig. 13-D) and very thinly myelinated, possibly regenerated, fibers (Fig. 13-C). Occasional inflammatory cells or vacuolated histiocytes were seen within the funiculus (Fig. 13-C).

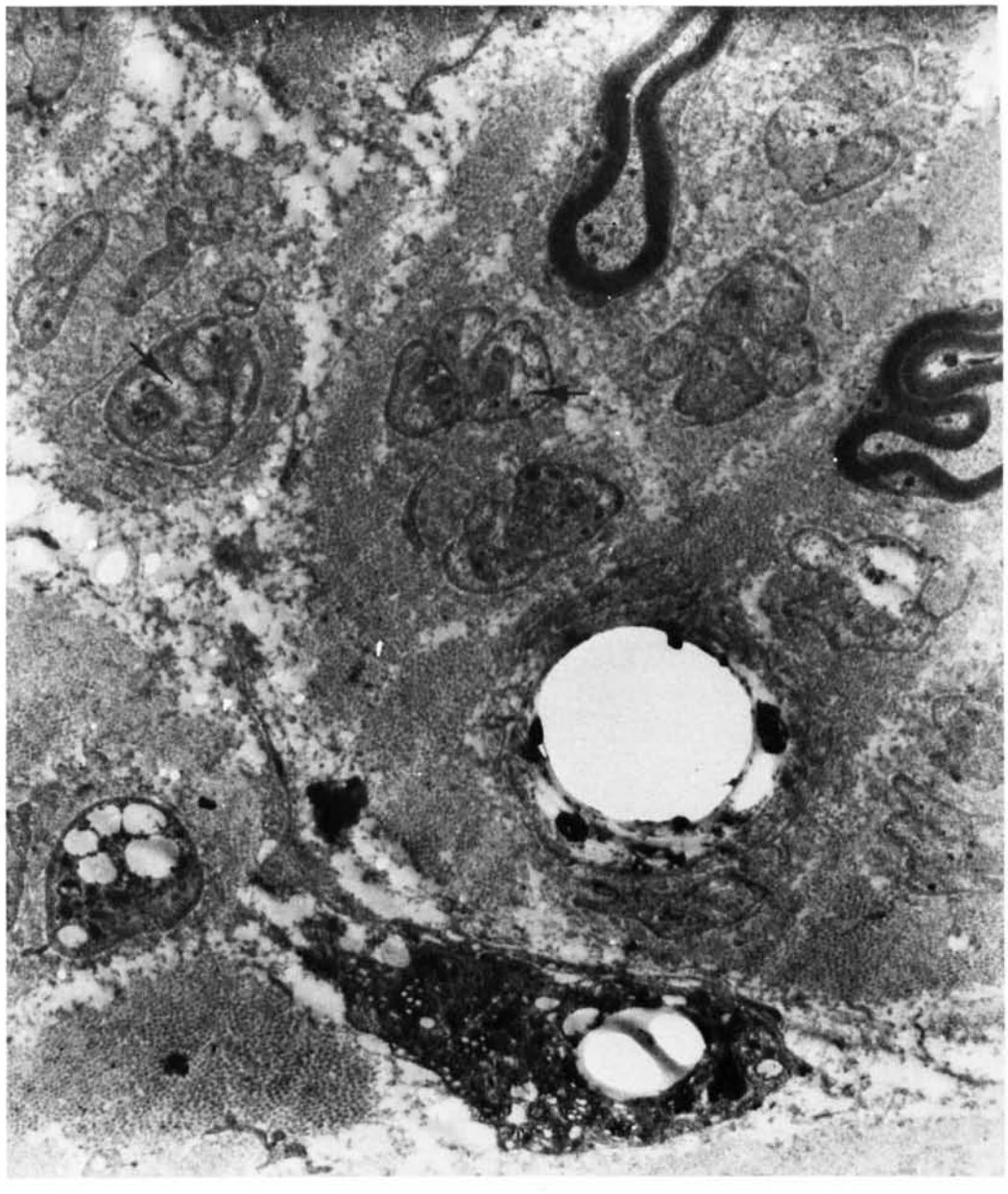


13A

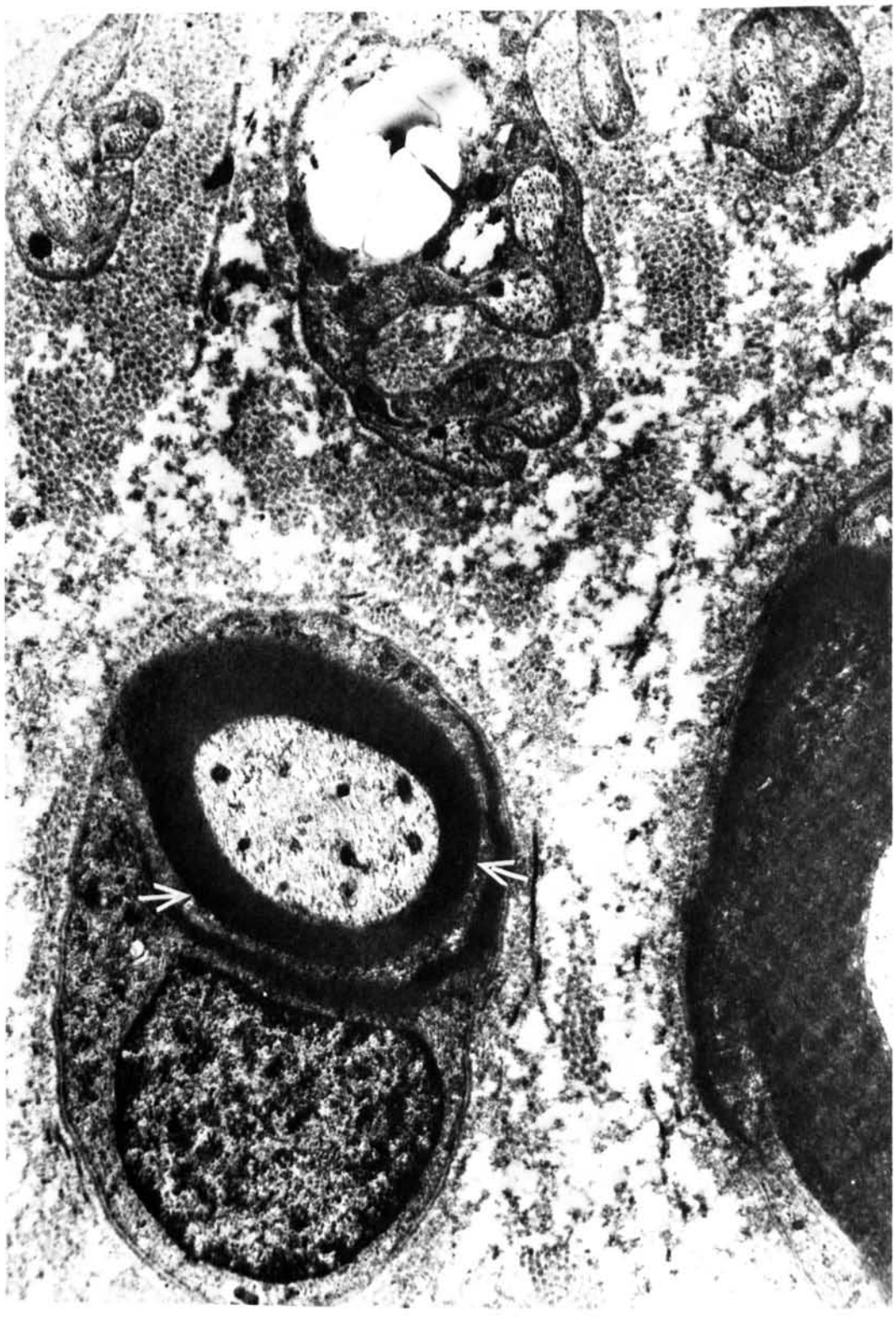


13B

FIG. 13. Same nerve as in Figure 12. A. Periphery of a funiculus showing moderate loss of myelinated fibers, prominent Schwann tissue and thickened perineurium. Vacuoles in Schwannian and perineurial cells contain bacilli. B. Clusters of *M. leprae* in unmyelinated fibers (arrows), rarely in myelinated fibers which are preserved here. C. Similar to above, but with only thinly myelinated, ?-regenerating fibers; prominent cords of Schwann cells, some hearing unmyelinated fibers (arrows); and one histiocyte with vacuoles at site of ?-previous bacillation. D. Closer view of irregular cords of Schwann cells of unmyelinated fibers. Contrast small and large myelinated fibers, the former with an excentric SL cleft (arrows) suggesting proximity to a node of Ranvier. Both C and D show bacilli only in Schwann cells of unmyelinated fibers, and increased collagen. A and B. Semithin sections with toluidine blue only, X 625 and X 1,400 respectively. C and D. Ultrathin sections as in Figure 3, X 5,000 and X 11,000 respectively.



13C



13D

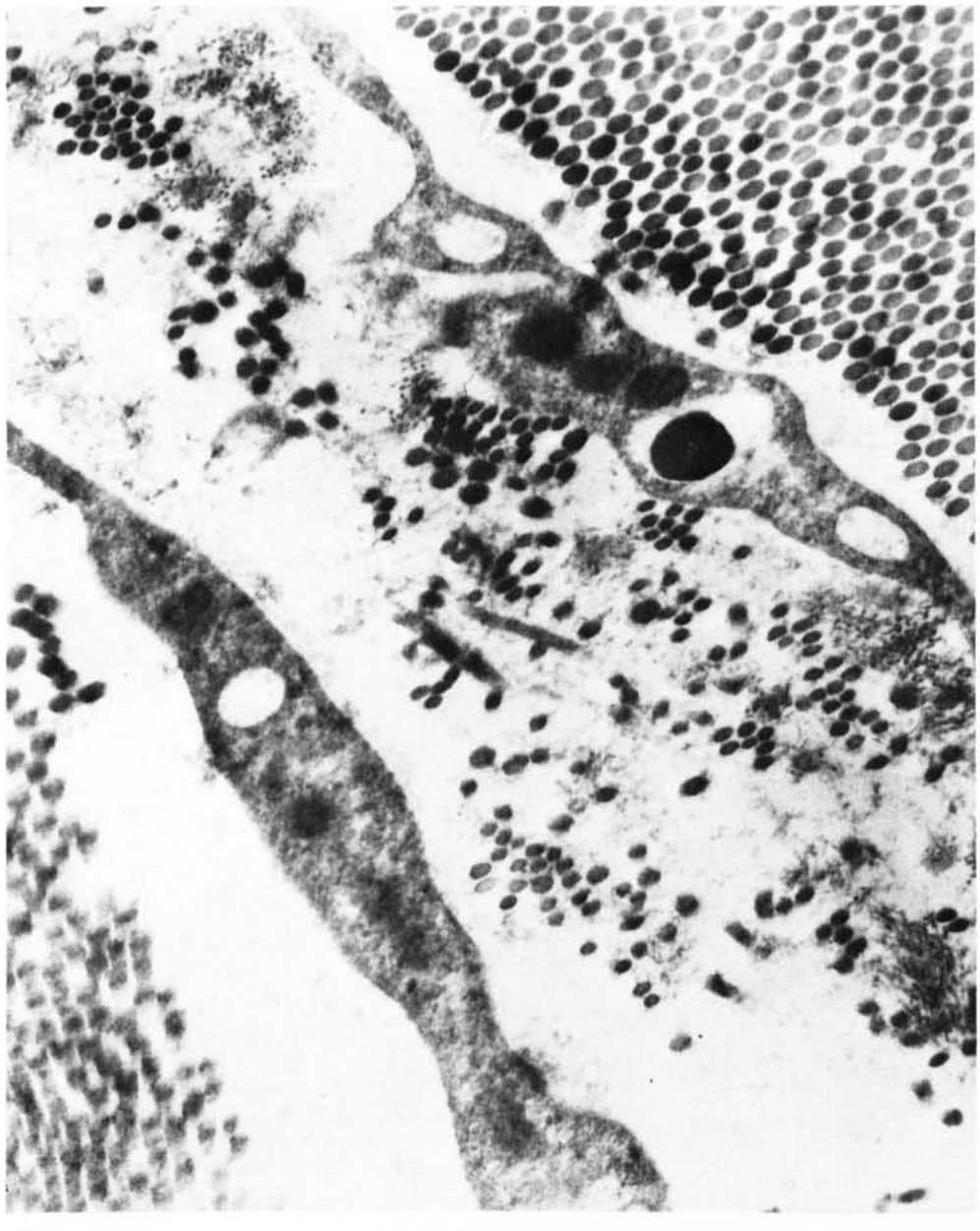
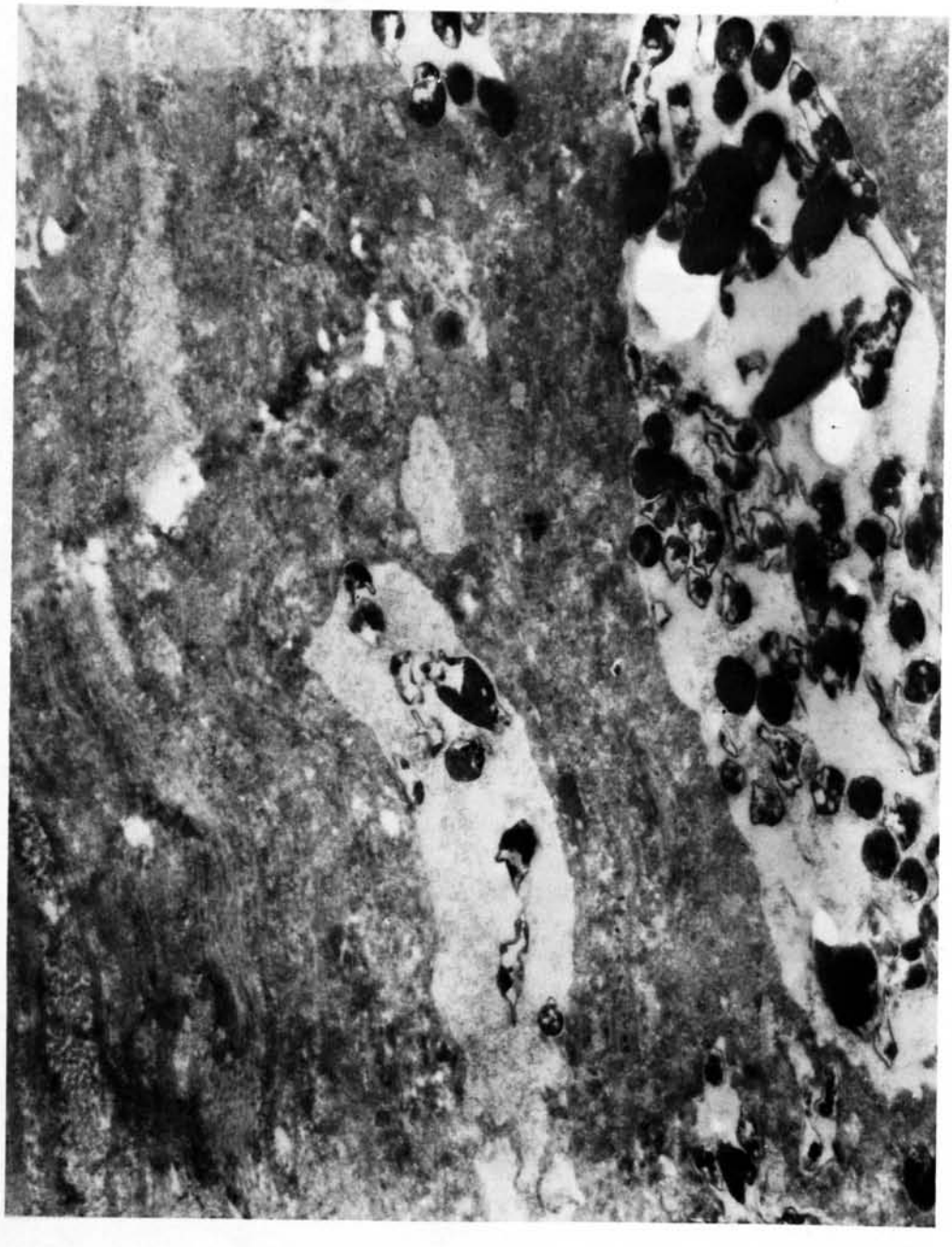


FIG. 14. Same nerve as in Figure 12. Nerve showing bacilli and/or empty vacuoles in fibroblasts in the perineurium. Same as in Figure 3, X 41,000.

Of equal interest was the presence in the endoneural collagen and in interfunicular connective tissue, of elongated cells without a basement membrane and with highly extended processes, probably fibroblasts, containing solid forms of *M. leprae* or empty vacuoles suggesting their earlier presence (Fig. 14).

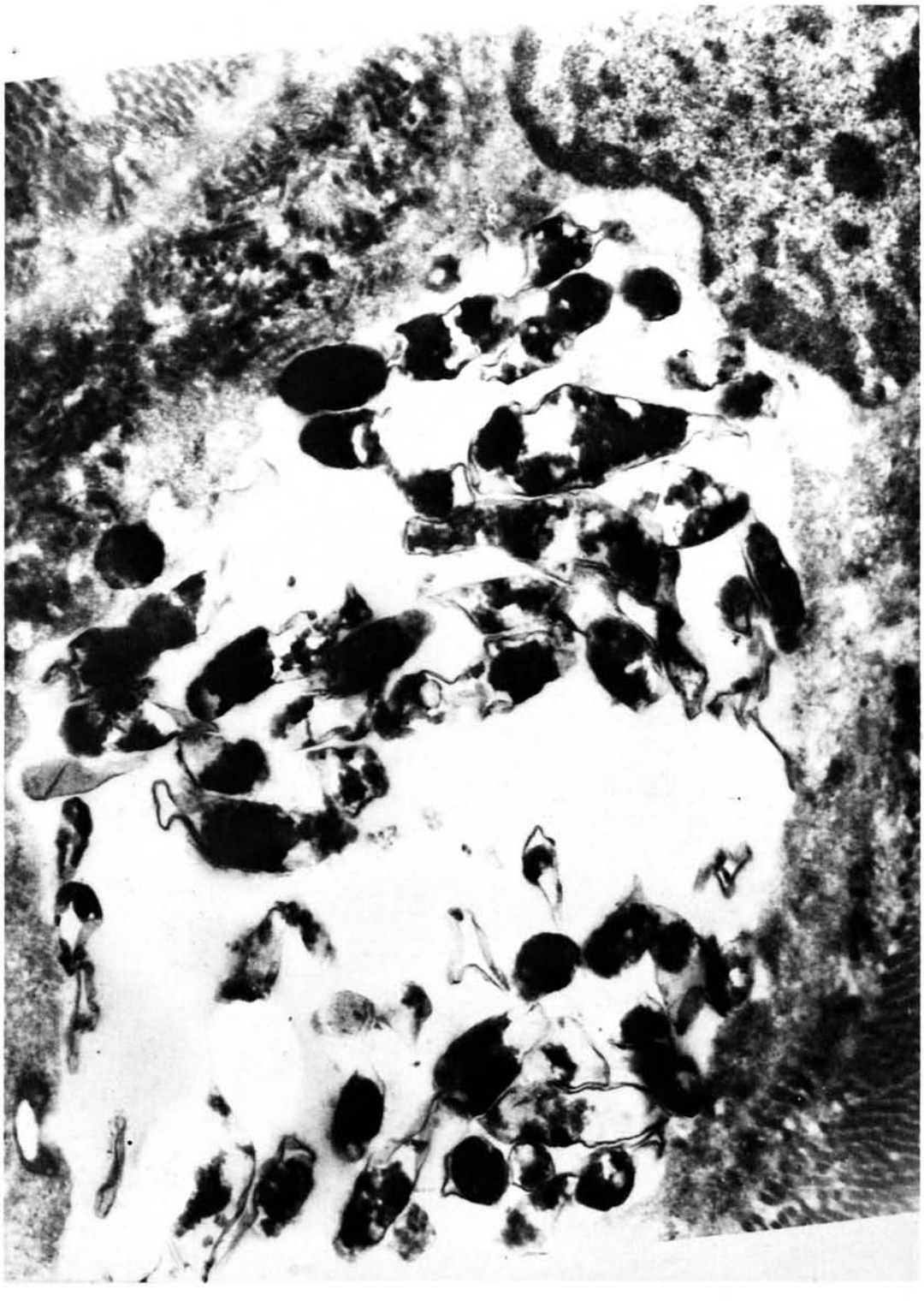
In all of these four lepromatous cases dense collagenization of the endoneurium was a clearly evident electron microscopic feature (Figs. 3, 5, 10, 13-C, 13-D, 14). This collagen enveloped and separated individual myelinated fibers and unmyelinated fibers or cords, and its significance will be discussed later.

The changes in the perineurium were, in all four cases, striking enough to merit a



15A

FIG. 15. A. Same nerve as in Figure 1; portion of thickened perineurium showing formation of lakes filled with intact and degenerating *M. leprae*, amidst homogenous and granular material. B. ? — macrophage, ? — swollen perineurial cell in the same perineurium, showing formation of globus containing mainly degenerating *M. leprae;* granular cytoplasm along periphery of globus and nucleus in top right corner. Both as in Figure 3; A X 18,000, B X 32,000.



15B

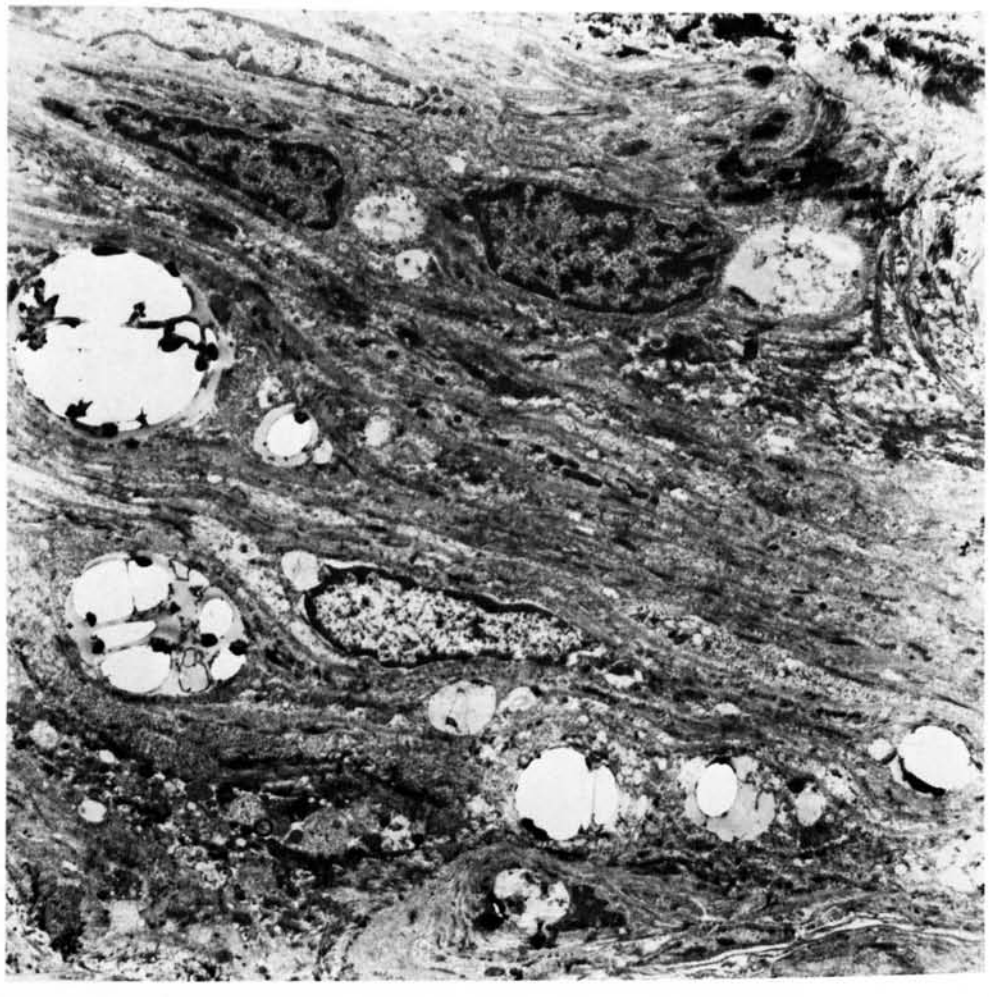


FIG. 16. Same nerve as in Figure 12. Proliferated distended perineurial cells, many with *M. leprae,* compare with Figure 13-A. As in Figure 3, X 3,000.

separate description. In the BL case, the thickening of the perineurium was due, in places, to accumulation of collagen and prominent fibroblasts, and in other places to proliferation of perineurial cells, as has also been observed in tuberculoid nerves ([13]). Two of the four cases showed interesting changes in and amongst the perineurial cells themselves. Thus, in the first case (NP/F/511) large lakes of faintly osmiophilic granular material had formed amongst the perineurial cells, containing dense accumulations of *M. leprae* in various states of degeneration, as well as in compact forms (Fig. 15-A). There was no phagocytic membrane around these lakes and the bacilli could be said to be in direct contact with the cytoplasm of the host cells. Closer examination showed weird shapes of bacillary cell walls with patchy loss of bacillary osmiophilia (Fig. 15-B). The fourth case showed fewer bacilli in the perineurium but their presence was apparent even in semithin sections and clearly ascertained in ultrathin sections (Fig. 16). They were in the cytoplasm of the swollen perineurial cells, with characteristic halos around them. The basement membrane of the perineurial cells also appeared thick.

While electron microscopic details of the cell reaction within both lepromatous and tuberculoid nerves will be considered in a

later paper along with the inflammatory reaction in skin lesions, two or three important cells may be considered here. Within one of the tuberculoid nerves reported elsewhere ([13]), there were a number of large mononuclear cells. The most striking of these were cells with an excess of organelles (like the one illustrated in the upper part of Fig. 17-A). Besides mitochondria, these cells included faintly osmiophilic granular bodies, with or without vacuoles in them and clearly separate from the rest of the cell cytoplasm. They appear to be distended lysosomes and their granularity was comparable to that observed in the lakes of material containing *M. leprae* detected in the perineurium (Fig. 15-A). This cell was possibly an epithelioid cell, the significance of whose augmented lysosomal content will be discussed later in relation to the lepra cell.

The other cells observed in this exudate, and in one of the lepromatous nerves (NP/G/198) were large mononuclears with mitochondria and one or more large or small vacuoles, indicating a greater phagocytic propensity. They contained very little or no lysosomal material compared to epithelioid cells, but showed bacilli in cytoplasmic vacuoles when encountered in lepromatous nerves (Fig. 17-B). Besides these two types of large cells, a number of small mononuclears with denser nuclei and scantier cytoplasm could be recognized in the exudate in this nerve and in others.

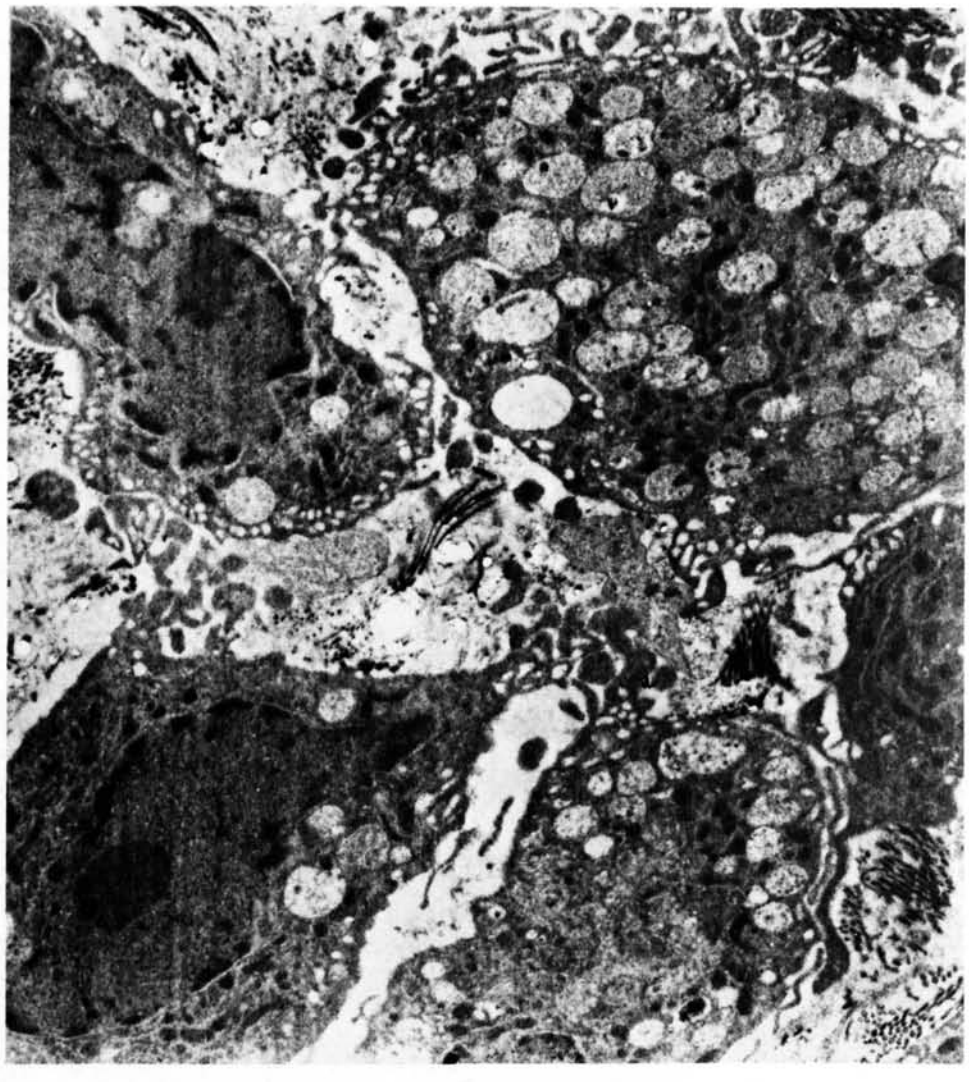
In two of the lepromatous cases of the present series and in two of the eight tuberculoid nerves ([13]), plasma cells were observed electron microscopically in the intraneural exudate (Fig. 17-B). They were identified by the characteristic, abundant rough endoplasmic reticulum and by the nucleus with peripheral clumped heterochromatin. Mast cells were also seen in nerves of both types of leprosy, though more frequently in the lepromatous.

DISCUSSION

Some of the problems raised in the introduction, especially concerning the relation of neural constituents to *M. leprae*, merit discussion.

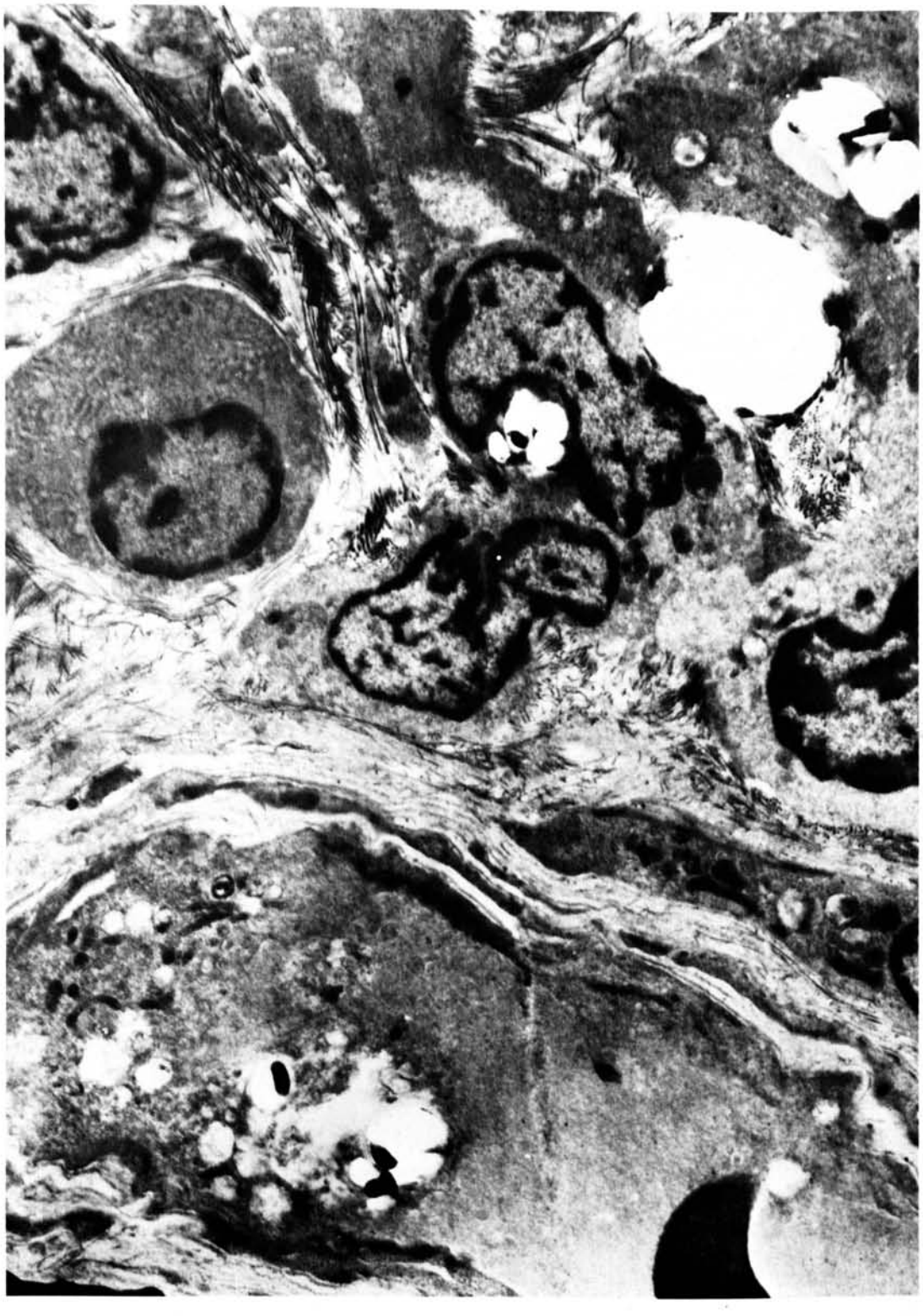
While the nerve does appear to be the "target organ" in leprosy, it now appears that the Schwann cell cannot be considered the sole "target cell" in leprous nerves. Electron microscopy has convincingly confirmed that besides the Schwann cell, the endothelial cell of blood vessels and the specialized perineurial cell also harbor *M. leprae* with equal facility and in comparable populations. We are impressed by the fact that these three cells share the common morphologic property of having a basement membrane. There does not seem to be any clearly discernible common cytoplasmic constituent in these cells which would enable one to suspect a specific congenial chemical substrate. All of them contain a fair amount of mitochondrial substance, Schwann cells the most, and none of them contain as much endoplasmic reticulum as do fibroblasts or epithelioid cells. We have also been impressed by the clear presence, though to a much lesser extent, of *M. leprae* in cells without a basement membrane, such as large mononuclears lepra cells) in the interfunicular exudates and fibroblasts in the endoneurium or perineurium. Weddell *et al* ([43]) also mentioned *M. leprae* in fibroblasts in nerves of mice experimentally inoculated, and Brown and Draper ([5]) have clearly shown "activated" rat fibroblasts in tissue culture behaving as macrophages towards *M. lepraemurium*.

Cells of recognized "immunologic competence" such as the lymphocyte and the plasma cell, were also encountered in both polar types of leprosy. Though the plasma cell with its immunoglobulin synthesizing activity (reflected by the prominent endoplastic reticulum), is more frequent in tuberculoid nerves and skin lesions, it also occurs in untreated lepromatous patients, and probably contributes to what little immunologic response prevails in this type of leprosy. In all our cases the endoplasmic reticulum was abundant and of the rough variety. While the plasma cells did not contain any bacilli, the cisternae of some occurring in lepromatous nerves were dilated and contained granular material or small vacuoles. Larger, possibly pinocytotic, vacuoles were seen in one of our tuberculoid cases. This feature was of interest in view of the recent demonstration of phago-



17A

FIG. 17. A. (NP/F/873). Intrafunicular exudate in a BT type nerve showing parts of four large cells, probably epithelioid, two with nucleolated nuclei, and all with mitochondria, small vacuoles and peripheral cytoplasmic processes. The cell in the top right corner shows a number of faintly osmiophilic membrane-bound bodies, probably lysosomes, containing granular material and vacuoles or denser particles. B. Same nerve as in Figure 12. Inflammatory cells around perineurial blood vessel: *M. leprae* seen in the lumen and disorganized endothelial cell of the vessel, and inside vacuoles in the mononuclear macrophages. Note the plasma cell with dark cytoplasm; suspicious vacuoles in the pericyte; and absence of lysosomes in any cell. Both as in Figure 3, A X 6,500, B X 6,300.



17B

cytic activity of plasma cells towards *Treponema pallidum* in the syphilitic chancre [2] where the organisms were located in membrane-bound spaces in relation to lysosome-like granules.

The other important event in the Schwann cell of lepromatous nerves appears to be the damage done to its inhabitants, viz, the myelin and the axon in myelinated fibers and the axon in nonmyelinated fibers. In cases of very heavy bacillation the persistence of basement membranes of Schwann cells with only a small quantity of Schwann cytoplasm and a large aggregation of *M. leprae*, creates the picture of Schwann tubes filled with bacilli, a process which terminates in the formation of empty redundant folds of effete Schwann membranes. These may be the so-called "Cords of Büngner," reported by Nishiura [25] in lepromatous nerves, and which may also arise from proliferated cords of unmyelinated fibers as observed by us. Such a drastic degenerative change in Schwann cells has also been reported recently by Job [20] in his study of lepromatous nerves. Hence, the earlier concept [9, 44] that there is a symbiotic relationship between Schwann cells and *M. leprae* must be modified. The host cells, and this applies to the endothelial and perineurial cell as well, do not actually thrive in the presence of the parsite.

At the same time the invaders, *M. leprae*, do not seem to remain unscathed, many of them showing evidence of degeneration in the form of a blotchy depletion of the bacillary osmiophilic substance or, less frequently, a break in their cell wall, even in untreated lepromatous patients. These changes are consistent with the descriptions of nonviable *M. leprae* [33]. The point of practical importance here is the capacity of the host cell, either Schwannian, endothelial or perineurial, to present a "milieu interieur" which lyses or otherwise adversely affects the metabolism of *M. leprae* in the absence of any chemotherapeutic support. One possible cytoplasmic constituent of these cells which can act as a potent antibacterial agent is the lysosome. Membrane-bound granular bodies, probably lysosomes, were encountered close to the bacilli within Schwann cells, and large mononuclear cells in the neural exudate, in both lepromatous and tuberculoid nerves. The moderately osmiophilic granularity that we observed in the large lakes among the perineurial cells, also containing *M. leprae*, appeared lysosomal in origin. Thus, in both these situations the bacilli were in direct contact with the organelles of the host-cell cytoplasm, without any intervening phagocytic membrane, a possible mechanism accounting for degeneration of some of them. Such changes are generally seen after prolonged treatment with sulphones [18]. This kind of degeneration may also perhaps result from a failure of nutrition of large numbers of bacilli by the host cell harboring them; this was suggested as a possible contributing factor in Virchow cells by Aquino and Skinsnes [1]. In lepromatous we have found strong acid-phosphatase activity (lysosomal) in interfunicular histiocytes as well as in Schwann cells bearing *M. leprae*, whereas in borderline tuberculoid cases non-bacillated, large mononuclear and proliferating Schwann cells showed such activity. The implications of this will be discussed in a later publication.

In all the lepromatous nerves, Schwann cells of unmyelinated fibers harbored *M. leprae* preferentially compared to those of myelinated fibers. The reason for this greater phagocytic propensity on the part of unmyelinated fiber cells is not clear. Schwann cells in general, and especially of nonmyelinated fibers, are known to phagocytose *M. leprae* and *M. lepraemurium* [43, 44], and also nonbiologic matter such as carbon particles [31], or polystyrene [3]. Similar phagocytic activity is also noticed on the part of perineurial cells which can ingest ferritin [40]. The proliferative capacity of the Schwann cell is nonspecific and almost unlimited. The activity of Schwann cell cytoplasm in the midst of degenerating and regenerating unmyelinated fibers has been clearly demonstrated by Dyck and Hopkins [15] in crushed autonomic nerves in the mouse. Our first case was unusual not only in having a greater concentration of bacilli in the perineurium than in the Schwann cells, but in the

fact that many at the former site were degenerating while most at the latter site were intact. The question therefore arises whether, at least in this nerve, the aggregation and breakdown of *M. leprae* in perineurial lakes indicated that the bacilli had been there longer, and whether they were on their way in or way out of the nerve bundle, or both. The presence of bacilli in the perineurial cells, in greater or lesser numbers, in all our lepromatous cases and in one of our tuberculoid cases ([10]) where mycobacteria were found, would suggest that the perineurium is an important pathway for them to enter into and come out of nerve bundles. This is of pathogenetic significance and probably the perineurium deserves more attention than it has received so far in the problem of neural involvement in human lepromatous leprosy. In any event, in none of our cases could we confirm the suggestions of Khanolkar ([21]) and Nishiura ([25]) that even a few *M. leprae* were clearly within axis cylinders.

In an earlier study of teased nerve fiber preparations we demonstrated that in tuberculoid leprosy Wallerian (or axonal) type degeneration predominates, while segmental demyelination is rare; and even in lepromatous leprosy axonal degeneration is more frequent than segmental demyelination ([14]). The latter was expected with a primary Schwann cell disorder which this type of leprosy appeared to constitute. In the present investigation, electron microscopy demonstrated why Wallerian degeneration preponderated even in lepromatous nerves: there was almost total destruction of both axon and myelin of parts of those fibers which had been heavily bacillated, with consequent distal degeneration of these fibers. This was again confirmed by the teased fiber preparations of the present material, all three LL cases showing axonal degeneration up to the stage of droplet formation along with normal fibers. The association of these two types of nerve degeneration, earlier considered to be distinctive and separate, in a group of fibers or even in the same fiber is now being recognized, e.g., in the neuropathy of chronic alcoholism ([14a]) and in uremic nephropathy ([37]). Here the primary process appears to be axonal degeneration.

Another feature of early Wallerian degeneration which manifests itself more frequently in tuberculoid leprous neuropathy, and which has been described in detail in our paper on tuberculoid nerves ([13]), is the occurrence of SL clefts in large myelinated fibers, more frequently than in normal nerves. To a lesser extent, this feature was evident in the leprous nerves of the present series also. Webster ([41]) first convincingly demonstrated that in nerves experimentally crushed, an irregular increase in the number of SL clefts in certain segments of nerve fibers was a sign of early Wallerian degeneration, but there appear to be hardly any accounts of human neuropathies showing increase in SL clefts as evidence of early axonal degeneration, subsisting along with other fibers manifesting advanced degeneration of this type. At least two of the LL cases of the present series and all tuberculoid cases with some large nerve fibers preserved showed this combination very clearly.

Regeneration of nerve fibers is more difficult to assess. Histologic evidence of such regeneration in the terminal sensory and motor areas, i.e., among the nerve fibers in the skin ([6]) and muscle ([7]) repectively, and in routine paraffin sections of leprous nerves has been published earlier ([8, 9, 12]). In the latter publication on large nerves excised at operation, and in a subsequent study of large nerves removed at necropsy (unpublished observations), the maximal regenerative effort in the form of fine axon sprouts concurrent with degenerating fibers, was observed proximal to sites of selective nerve damage, e.g., in the median nerve in the middle of the forearm. The picture was at times reminiscent of the regenerating funiculets of fibers in irradiated homo-nerve-grafts in the popliteal nerve of the dog ([11]).

Regeneration after axonal degeneration and remyelination following segmental demyelination has been noted in leprous nerves through teased fiber studies ([14]). In the current investigation, araldite embedded semithin sections of both lepromatous and tuberculoid nerves revealed a

clustered prominence of small myelinated fibers at sites of loss of large myelinated fibers, suggesting regeneration. This was confirmed in some cases by electron microscopy which also revealed prominent cords of unmyelinated fibers also suggesting regeneration of such fibers. In the BL case the presence of small myelinated fibers in semithin sections, and of the above described cords of nonmyelinated fibers in ultrathin sections, indicated that the majority of both were possibly regenerating fibers. Such grouping of regenerating myeinated or unmyelinated fibers has also been been observed in other human neuropathies such as in aging (11), or with isoniazid intoxication (29), and after experimental crushing of an autonomic nerve (15). The picture is further complicated when some of the regenerated fibers also show early degenerative or demyelinative changes. This too was possibly occurring in the nerve of the BL patient illustrated here, where in the thinly myelinated fibers in teased preparations fresh segmental demyelination had commenced in some fibers.

In attempting to consider the overall pathogenesis of nerve damage in leprosy, one is inclined to agree with Pearson and Weddell (32) that considerable obscurity still prevails. Nevertheless, on the basis of the light microscopic findings of others and ourselves, and especially of our current electron microscopic findings, a reasonably reliable outline of the pathogenesis may be sketched.

It now appears that the initial onslaught on the peripheral nerves in leprosy, especially of the lepromatous variety, may be through one of two, or possibly both mechanisms, viz, neurogenic and vasogenic. Studies on the neuropathology of early cutaneous lesions of leprosy (6, 21), point to more extensive damage to intradermal nerve twigs than to other dermal adnexa and constituents. In vitally stained wholemounts of skin the finer and superficial nerve filaments tended to degenerate and disappear earlier than the others (6). Such a hypothesis is consistent with the notion of ingress of bacilli first and directly into the skin, either through an intact (21) or through an abraded or punctured epithelium (44), although there is still no consensus on this point (24, 42).

The predilection of *M. leprae* for Schwann cells and endothelial cells would then lead to the second stage in the pathogenesis of this multifaceted disorder. There might be an essentially neural progression of the infection along the Schwann cells, a sort of "Schwannian relay" which we envisaged earlier (9). The more widespread dissemination to other nerves and tissues would naturally be through the blood stream, since endothelial cells are now shown to discharge bacilli into the lumen of the parasitized vessels.

While the phagocytic activity of the Schwann cell is established, an actual transmission of bacilli from one Schwann cell to another still remains to be demonstrated even by electron microscopy. However, since leprous nerves of all types do show Schwann cell processes, and since these processes or cords also contain *M. leprae*, especially in lepromatous nerves, it is quite conceivable that even as the degenerating Schwann cells discharge their load of bacilli, other relatively intact Schwann cells become parasitized. It is not unlikely that the more delicate and probably more vulnerable Schwann cell architecutre of the nonmyelinated fibers (which we often saw distended with bacilli) is more amenable to development of a globus and to rupture. Their microbial content, which has never been seen lying free in the endoneurium, would be taken up by other Schwann cells in lepromatous nerves, or somehow destroyed in tuberculoid nerves. The perineurial cells and the interfunicular macrophages could be responsible for lateral extension of the disease to adjacent nerve bundles, at least in lepromatous leprosy.

In either type of leprosy, since the parenchymal elements of the nerve fiber are destroyed, the subsequent Wallerian degeneration of the distal parts of these fibers constitutes a more readily understandable chapter in the pathogenesis of leprosy. Perhaps of greater interest is the proximal extension of the neurogenic spread of the disease which appears to be restricted at the level of the posterior root ganglia

where, according to Lumsden ([24]), they were detected first by Sudakewitsch in 1887, and clearly demonstrated by Lie ([23]) and Ermakova ([16]). The bacilli are either in or, more likely, on the spinal ganglion cells, i.e., in the capsule cells around them ([10]). These capsule cells are structurally and functionally homologous to the Schwann cells of peripheral nerves.

The deleterious implication of increased endoneurial and perineural collagen to the already compromised nerve fibers, by introducing a further compressive and ischemic factor in the pathogenetic mechanisms, has to be kept in mind. It appears to be an early accompaniment of leprous neuritis and not just a replacement fibrois and has been discussed in our paper on tuberculoid nerves since it is more pronounced in that type of leprosy.

The detection of occasional cases with multiple and totally irregular involvement of nerves, particularly in lepromatous leprosy, can be accounted for today only on the basis of a vascular spread of *M. leprae*. This concept was first stressed by Weddell *et al* ([44]), and such a process could occasionally result in the formation of stray foci of inflammation around AFB in far flung organs such as the spinal cord ([23]), and the brain in experimental leprosy of the mouse ([39]).

SUMMARY

The ultrastructural details of one or two nerve branches each from four untreated cases of lepromatous leprosy (three of the LL type and one of BL type) are presented, along with correlations from light microscopic findings.

There was depletion of large myelinated fibers with or without some increase in myelinated fibers and proliferation of Schwann cells.

M. leprae almost constantly parasitized Schwann cells, endothelial and perineurial cells. Bacilli were not encountered in the myelin or the axon.

There was a much greater predilection of *M. leprae* for Schwann cells of unmyelinated fibers, only occasional myelinated fibers being parasitized at some sites. Myelin and axon or the axon alone of unmyelinated fibers tended to be destroyed in heavily bacillated Schwann cells with consequent Wallerian degeneration of these fibers, often only basement membrane remaining. This was confirmed in teased fiber preparations which showed droplet degeneration in three cases, only one case showing segmental demyelination. Early stages of Wallerian degeneration were evidenced by an increase in the number of Schmidt-Lantermann clefts in the large myelinated fibers, though not to the extent seen in tuberculoid nerves.

Lymphocytes and plasma cells were encountered along with lepra cells in the intraneural inflammatory exudates.

The neural pathogenesis in leprosy is discussed in the light of these and related findings and it is indicated that both a Schwannian passage as well as a vascular dissemination of the bacilli probably occur in all types of leprosy, this being most evident in the lepromatous type. The intraneural extension of the disease might occur through rupturing of swollen Schwann cells and bacillary phagocytosis by other Schwann cells while the dissemination throughout the body, with irregular involvement of nerves, would be the result of hematogenous spread of infection. The heavy bacillation of the perineurium in these cases pointed to its possibly significant role in the movement of *M. leprae* between nerve bundles and, thereby, in the neural pathogenesis of leprosy.

RESUMEN

Se presentan los detalles ultraestructurales de una o dos ramas nerviosas de cada uno de cuatro casos de lepra lepromatosa no tratada (tres de tipo LL y uno de LD), correlacionándolos con los hallazgos obtenidos con el microscopio de luz.

Se observó un vaciamiento de las fibras mielínicas grandes con o sin algún aumento de fibras mielínicas.

El *M. leprae* se encontró casi siempre parasitando las células de Schwann, las células endoteliales y perineurales. No se encontraron bacilos en la mielina ni en el axón.

El *M. leprae* demostró mayor predilección por las células de Schwann de las fibras desmielinizadas, sólo ocasionalmente se observaron fibras con mielina parasitadas en algunos sitios. La mielina y el axón, o el axón solo, cuando

estaban relacionados con células de Schwann, tendían a estar destruídos en asociación con células que contenían bacilos, con una consecuente degeneración Walleriana de estas fibras, a menudo sólo permanecía la membrana basal. Esto se confiró en preparaciones de fibras aisladas, que mostraron degeneración por sectores en tres casos; sólo en un caso se observó desmielinización segmentada. Las primeras etapas de la degeneración Walleriana se evidenciaban por un aumento de las hendiduras de Schmidt-Lanterman en las fibras mielínicas grandes, aunque nunca en la cantidad que se observan en los nervios tuberculoides.

En los exudados inflamatorios intraneurales se encontraron, además de las células de lepra, linfocitos y células plasmáticas.

Se discute la patogénesis neural en la lepra, en relación a estos hallazgos y otros similares, y se indica que probablemente en todos los tipos de lepra se produzca un pasaje por las células de Schwan además de una diseminación vascular de los bacilos, la cual sería más evidente en el tipo lepromatoso. La extensión intraneural de la enfermedad puede producirse por ruptura de células de Schwann repletas de bacilos y fagocitosis de los bacilos por otras células de Schwann, mientras que la diseminación por todo el cuerpo, con compromiso irregular de los nervios, sería el resultado de ladiseminación hematógena. La gran cantidad de bacilos en el perinervio que se observa en estos casos, sugiere que posiblemente tenga un rol preponderante en el movimiento del *M.leprae* entre los haces nerviosos y por lo tanto en la patogénesis neural de la lepra.

RÉSUMÉ

Les détails de l'ultrastructure d'une ou de deux branches nerveuses, chacune provenant de quatre cas de lèpre lépromateuse non traitée, sont présentés. On founit également les corrélations obtenues à partir d'examens à la microscopie optique. Trois des sujets étudiés étaient du type LL, et le dernier du type BL.

On a constaté une déficience en grandes fibres myélinisées, avec ou sans augmentation des fibres myélinisées.

Presque toujours, *M. leprae* parasitait les cellules de Schwann, les cellules endothéliales, et les cellules du périnèvre. On n'a pas rencontré de bacilles dans la myéline ou dans les axones.

On a observé que *M. leprae* présentait une prédilection beaucoup plus prononcée pour les cellules de Schwann des fibres non myélinisées, un parasitisme des fibres myélinisées n'étant relevé qu'occasionnellement à certains endroits. Le myéline et l'axone, ou bien l'axone seul, en rapport avec les cellules de Schwann, tendaient à être détruits, en association avec la présence de cellules hébergeant des bacilles. Il s'ensuivait une dégénérescence Wallérienne de ces fibres, la membrane basale constituant souvent le seul reliquat. Cecil a été confirmé dans des préparations de fibres dissociées, qui montraient une dégénérescence en goutelettes dans trois cas, alors qu'un seul cas présentait une démyélinisation segmentaire. Les stades précoces de la dégénérescence Wallérienne ont été mis en évidence par une augmentation du nombre d'indentations de Schmidt-Lantermann dans les grandes fibres myélinisées, encore que ce phénomène ne se soit pas produit dans la même mesure au niveau des nerfs tuberculoïdes.

Les cellules lymphocytaires et les plasmocytes ont été rencontrées, avec des cellules lépreuses dans les exsudats inflammatoires intraneuraux.

La pathogénèse des troubles nerveux dans la lèpre est discutée à la lumière de ces observations et d'autres observations comparables. Il en ressort que dans tous les types de lèpre, il se produit à la fois un passage Schwannien et une dissémination vasculaire des bacilles, ces phénomènes étant plus prononcés dans le type lépromateux. L'extension intranerveuse de la maladie peut se produire par le truchement d'une rupture des cellules de Schwann élargies, et une phagocytose bacillaire par d'autres cellules de Schwann, alors que la dissémination dans l'organisme, avec atteinte irrégulière des nerfs, serait, elle, le résultat d'une dissémination hématogène de l'infection. La présence de nombreux bacilles dans le périnèvre de ces cas, souligne le rôle significatif éventuel que jouerait la migration de *M. leprae* à travers les faisceaux nerveux, dans la pathogénèse des troubles nerveux de la lèpre.

Acknowledgements. We are grateful to the Lady Tata Memorial Trust for the research grant for this investigation and to the Tata Institute of Fundamental Research for the generous use of their electron microscope. Acknowledgement is also due to the Social & Rehabilitation Service of the US Department of Health, Education and Welfare for the donation of an ultramicrotome for this work.

Special thanks are due to Miss Z. A. Razzak for the teased fiber preparations, V. P. Kate for routine histological preparations, A. S. Dabholkar for histochemistry and N. S. Solanki for the preparation of the electron micrographs.

We are also thankful to Dr. A. C. Parikh, Medical Officer, Acworth Leprosy Hospital, for help in the selection of cases.

Editor's note. Slight differences between the French and Spanish summary translations and the original summary were occasioned by last minute changes by the author after this office had received the work of the translators.

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