Quantitative, Histologic and Ultrastructural Studies of the Index Branch of the Radial Cutaneous Nerve in Leprosy and its Correlation with Electrophysiologic Study[1]

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Detection of the early involvement of the peripheral nerves in leprosy would help in arresting the crippling deformities following nerve damage. The reported observations on nerve involvement in leprosy ([3, 4, 6, 7, 8]) are definitely not of early lesions. The basis of this study was to observe what may be the earlier changes in peripheral nerves in leprosy. An earlier paper ([1]) on correlation of electrophysiologic values with the clinical status of the patient, in particular with the sensory changes in the territory of the index branch of the radial cutaneous nerve (IRC), was studied. Here the emphasis is laid more on histologic, quantitative and ultrastructural changes and their correlation with changes in conduction velocity.

MATERIALS AND METHODS

As in our previous communication ([1]) the leprosy cases were divided into two groups on the basis of clinical testing of different sensory modalities. Eight nerves in group I were designated LN where the area supplied by the index branch of the radial cutaneous nerve was clinically normal. Five nerves in group II were designated L where the area supplied by the index branch of the radial cutaneous nerve was clinically slightly affected. Five nerves from normal volunteers served as controls.

Three lengths of each nerve were taken (Fig. 1). The middle segment B, about 1 cm long, was fixed in 2.5% glutaraldehyde and processed by routine method for electron microscopy and embedded in araldite. The proximal, about 1.5 cm length C, was fixed in formol Zenker for light microscopic study, and the distal 1 cm length A was fixed in 2.5% glutaraldehyde and processed by the method of Spencer and Thomas ([10]) for fiber teasing.

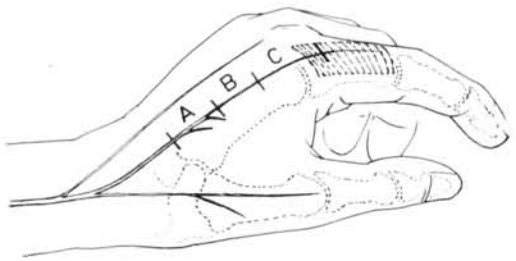

FIG. 1. Diagrammatic representation of the index branch of the radial cutaneous nerve (IRC) and its area of sensory supply (shaded area).

The sites of biopsy: A—for light microscopy, B—for electron microscopy, and C—for fiber teasing.

Semithin sections of one to two microns were cut from the entire fascicle of the nerve on a Porter Blum Ultramicrotome I. The sections were stained with 1% toludine blue in 1% borax. These sections were studied under light microscopy. For the purpose of fiber counting, the complete fascicle of the nerve was photographed at ×40 magnification and the print was enlarged to ×1000. The fiber count and measurement of the diameter of individual fibers were done simultaneously by using a Perspex cursor ([2]). Fiber density was then calculated and the histogram was constructed of fiber diameter against number of fibers. Ultrathin sections showing silver or pale golden colors picked up on a 50 micron copper grid, were dried and stained with uranyl acetate and lead citrate. These grids were examined under a Philips EM 200. The selected areas were photographed and studied by prints.

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RESULTS

Observations of control specimens (NN). The healthy volunteers presented responses to various sensory modalities as follows. Their response to sensation of touch and pressure was 3.61 gm to 3.84 gm, where they could point out the areas that were touched. The minimum distance between two point discrimination which was appreciated, varied from 0.7 cm to 0.9 cm. These volunteers very well appreciated the temperature difference of hot (60°C) and cold (0°C). The sensation of pain by pin prick with a weight of 4 gm was felt. The feeling of movement of hair was appreciated. The electrophysiologic normal average values for nerve conduction were latency to onset 2.2 msec, latency to peak amplitude 20 μv. Duration of potential was worked out to be 19 msec.

Quantitation of fibers. Normal fiber density varied from 6,000 to 12,000 fibers/square mm. These fibers were divided into three different groups:

1) Fibers of 7μ diameter or above were grouped as large fibers.
2) Medium-sized fibers had a diameter between 4μ to 7μ.
3) Small-sized fibers were of a diameter below 4μ.

This histogram of normal nerves gave a mean ratio of the small to medium to large-sized fibers as 2:2:1 (Fig. 2).

Study of semithin sections. Fibers of various diameters were evenly distributed throughout the section (Fig. 3). The perineurium was composed of seven to eight closely packed layers. One or two mast cells were seen, and also 4/5 capillaries were seen.

Ultrastructure. The normal nerve showed a few collagen fibers. The arrangement of unmyelinated bundles was different from that described in mouse sciatic nerve by Ochoa and Mair (9). The mouse sciatic nerve showed compactly packed groups of unmyelinated axons with one Schwann cell encircling each group completely. Our biopsy specimen of normal human nerves in the controls revealed unmyelinated axons which were not as compactly arranged. Schwann cells encircling them had different pattern (Fig. 4). Their processes were reaching out to the neighboring axon. This gave an appearance of each axon having separate Schwann cells without the nucleus being in sight, as described previously (1). Schmidt-Lantermann (SL) clefts were noticed in a few myelinated fibers.

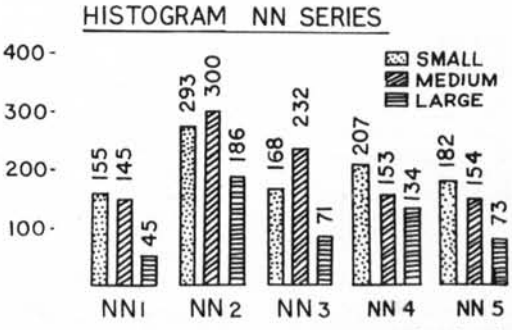

FIG. 2. Histogram showing normal distribution of large (>7μ diameter), medium (4 to 7μ diameter), and small (<4μ diameter) fibers.

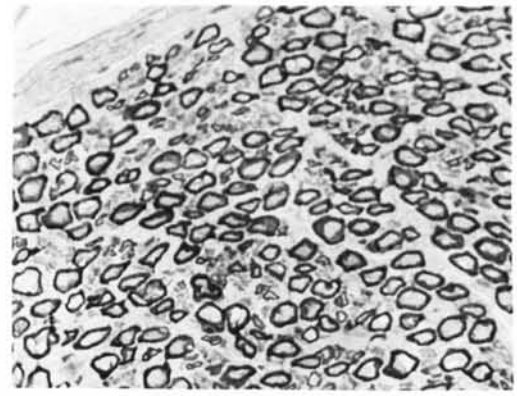

FIG. 3. Transverse section (TS) of part of normal IRC nerve showing different-sized myelinated fibers and part of perineurium. Araldite embedded semithin section. Toluidine blue stain. Original ×360.

FIG. 4. TS of a group of unmyelinated fibers (NM) and a myelinated fiber (M) of a normal IRC. Uranyl acetate, lead citrate. EM, original ×18,200.

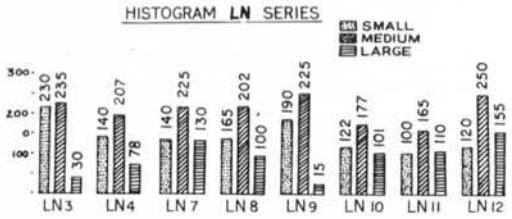

FIG. 5. Histogram showing distribution of large, medium and small fibers in LN series of cases. There is loss of small fibers in cases LN-7, LN-8, LN-10, LN-11, and LN-12.

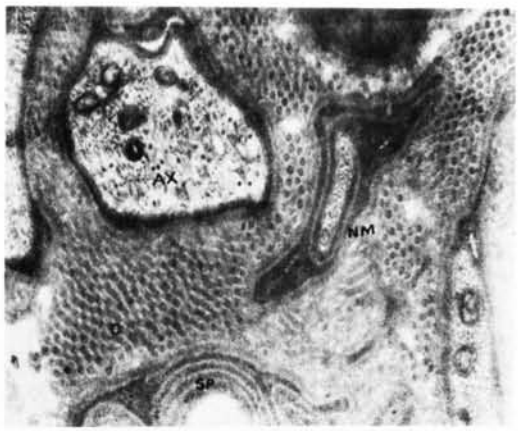

FIG. 6. TS of an axon from IRC belonging to LN series. Note the comparatively large axon (AX) devoid of myelin, proliferation of Schwann cells (SP) surrounding the unmyelinated axons (NM) and increased endoneurial collagen (C) (case LN-11). Uranyl acetate, lead citrate. EM, original ×53,800.

Summary of observations of LN series. This group consisted of leprosy patients with clinically normal IRC nerves, as tested by the methods enumerated previously. Electrophysiologically, the conduction velocity values of these nerves were in the upper range of normal or slightly delayed. The histograms of this group of nerves revealed loss of small-sized fibers in five of eight cases (Fig. 5). There was a slight fall-out of large-sized fibers in two instances.

At the ultrastructural level, however, early pathologic changes were observed. There was thickening of basement membrane of perineurial cells. The perineurial layers had more collagen between the cells. Proliferation of basement membrane of endothelial cells lining the blood vessels was evident. A few large naked axons were seen (Fig. 6). These could be myelinated fibers, showing loss of myelin. The amount of collagen was increased. There was Schwannian proliferation seen around unmyelinated axons (Fig. 6).

Teased fiber preparations of four nerves showed segmental demyelination in small-sized fibers. SL clefts in some large fibers were increased.

Observations on L series. In this group of leprosy patients the sensory modalities of touch and temperature were affected in the area supplied by the IRC nerve. This indicates involvement of the nerve to some degree. In two cases no action potential could be recorded electrically. One of these had one large fiber and 45 medium-sized fibers and the fiber density was low, i.e., 4,036/sq mm (Fig. 7). In the other case there was no

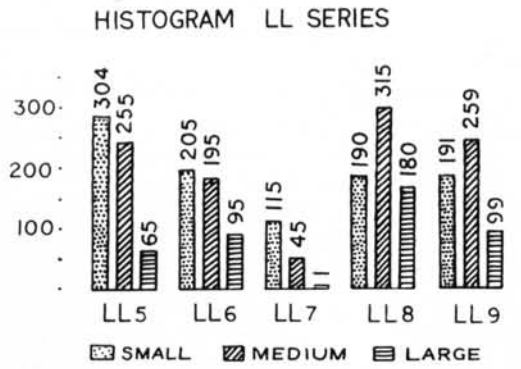

FIG. 7. Histogram showing distribution of large, medium and small fibers in L series of cases. Cases L-5 and L-7 show loss of large fibers.

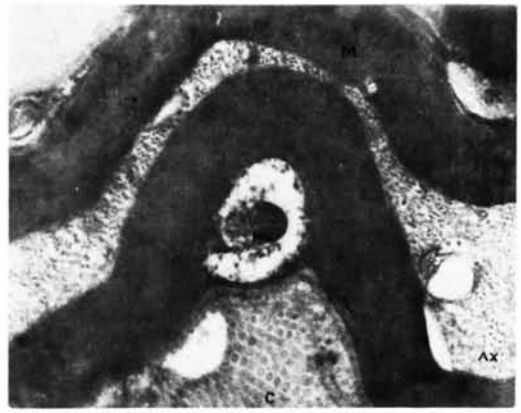

FIG. 8. TS of part of myelinated fiber (M) (case L-5) showing *M. leprae* (ML) in the Schwann cytoplasm. Uranyl acetate, lead citrate. EM, original ×40,000.

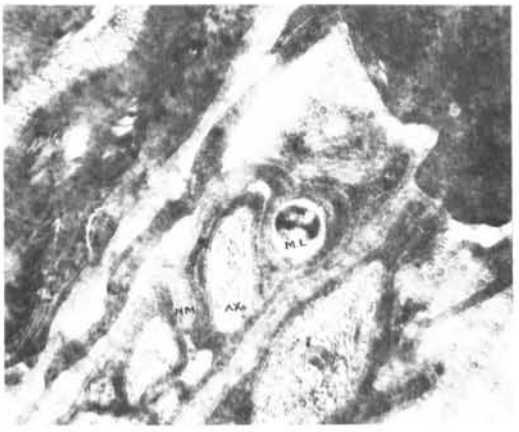

FIG. 9. TS of a group of unmyelinated axons (NM) (case L-6). One showing *M. leprae* (ML) in the Schwann cytoplasm. Uranyl acetate, lead citrate. EM, original ×53,800.

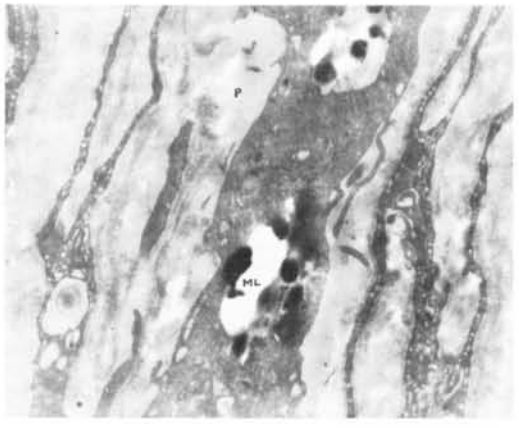

FIG. 10. TS of part of perineurial cells showing *M. leprae* (ML) in the cytoplasm of the perineurial cells (P) (case L-9). Uranyl acetate, lead acetate. EM, original ×15,600.

apparent fall in number of large-sized fibers. The fiber density was within normal range 8,851 fibers/sq mm.

Teased fiber preparations revealed mainly segmental demyelination in small-sized fibers and some early features of Wallerian degeneration in large-sized fibers as seen under the electron microscope. Most of the fibers when seen showed evidence of myelin degeneration. When sensory action potential could not be recorded the patient was submitted to repeated electrophysiological examination to rule out errors in the method. The absent sensory action potential was attributed to the degenerative changes seen in the fibers.

Three cases were of BL type of leprosy. Bacilli were seen in the cytoplasm of Schwann cells of myelinated fibers and unmyelinated fibers (Figs. 8, 9). They were also seen in macrophages, fibroblasts, perineurial cells and endothelial cells (Fig. 10). In this group, the amount of endoneurial collagen was much more than in the LN group. The perineurium was thickened, having an increased number of layers. In addition, the increase in collagen between the layers was much more marked than in the LN series. Numerous Schwann processes were seen encircling collagen pockets (Fig. 11). On the

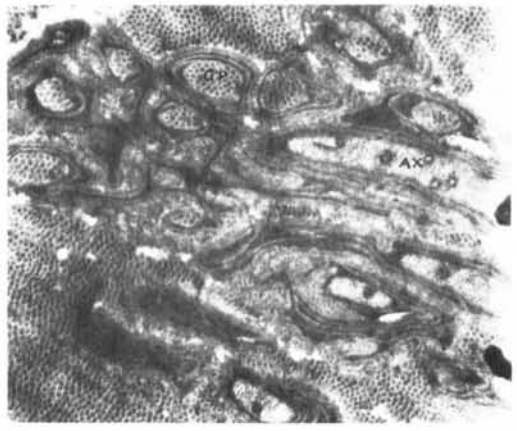

FIG. 11. TS of collagen pockets (CP) (case L-8) enclosed within the Schwann processes. Some unmyelinated axons (AX) are also seen. Uranyl acetate, lead citrate. EM, original ×26,000.

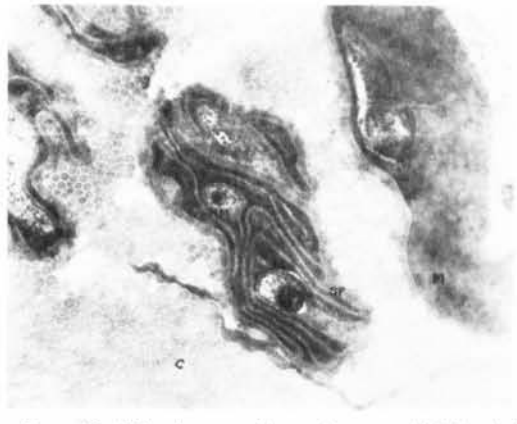

FIG. 12. TS of unmyelinated axons (NM) with proliferated bands of Schwann cytoplasm (SP) which is opening at one end. A part of myelinated fiber (M) is also seen. Uranyl acetate, lead citrate. EM, original ×53,800.

FIG. 13. Teased fiber preparation showing demyelination of segments (case L-5). Glutaraldehyde-fixed nerve, post-fixed with OsO_4. ×108.

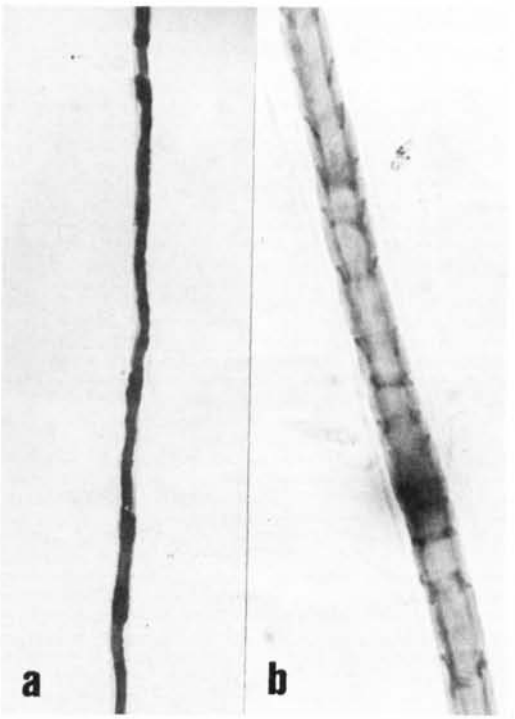

FIG. 14a. Teased fiber preparation showing Wallerian type of degeneration (case L-5). Glutaraldehyde-fixed nerve, post-fixed in OsO_4 ×514.

FIG. 14b. Teased fiber preparation showing increased number of Schmidt-Lantermann (SL) clefts (case L-9). Glutaraldehyde-fixed nerve, post-fixed in OsO_4. ×514.

whole, there was increased Schwann cell activity. Many unmyelinated axons were surrounded by the Schwann process all around except in some sectors, giving an appearance of a defect in the collar of Schwann cell cytoplasm (Fig. 12). This gave an impression of unmyelinated axons extruding out, as described by Taxi ([11]). Many thinly myelinated fibers with comparatively large axons were also noticed. They could be regenerating fibers.

Teased fiber preparations of cases L-5, L-6 and L-9 showed segmental and Wallerian type of degeneration (Figs. 13, 14a). Case L-9 showed a large number of SL clefts (Figs. 14b, 15, 16).

Postoperative follow-up. Postoperative clinical charting done three weeks after biopsy of IRC showed complete loss of all sensory modalities in the area of its distribution. The clinical examination done three months later showed almost complete restoration of sensation in the above area.

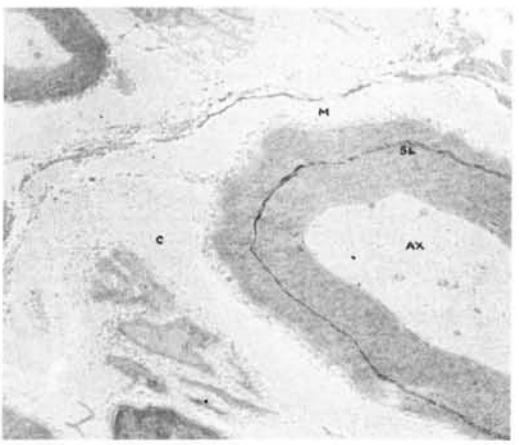

FIG. 15. Part of a large myelinated fiber (M) showing SL clefts (case L-9). EM, original ×11,400.

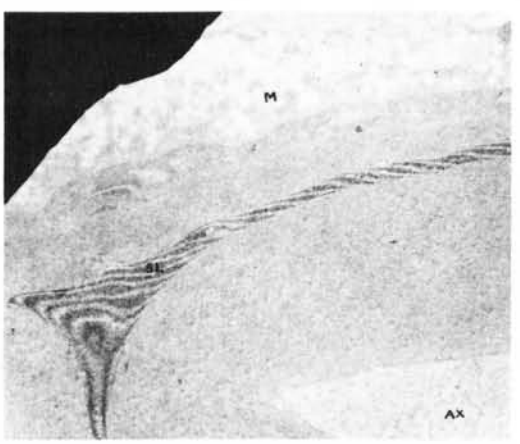

FIG. 16. TS of magnified view of SL cleft which is an oblique cytoplasmic channel between the axon and the Schwann cytoplasm. EM, original ×51,000.

RESULTS

Ultrastructural changes. *Perineurium.* Involvement of perineurial cells in leprous pa-

tients has been reported by other workers ([3, 6]). In our LN series of cases very minimal but significant change was seen in the perineurium. There was thickening of the basement membrane of the perineurial cells. There was no increase in the number of layers of perineurial cells. But in the early stages, the thickening was due to increased amount of collagen in between the perineurial cells. In slightly advanced cases, the thickening was due to an increase in number of layers of perineurial cells as well as an increase in collagen between them. Perineurial cells seem to harbor a large number of *M. leprae*. In our two BL cases (L-5, L-9) many *M. leprae* were seen in the perineurial cells.

Blood vessels. The blood vessels mainly showed proliferation of basement membrane of endothelial cells. In BL type of leprous nerves endothelial cells were seen harboring *M. leprae* as observed by Job ([6]) and Dastur ([3]).

Collagen. Collagenosis of nerves in leprosy and other chronic neuritis has been resolved at the ultrastructural level by many previous workers ([3, 4, 6]). Significant increase of collagen in leprous nerves was one of the striking features observed in the present study.

Myelinated fibers and the Schwann cells. Degenerating myelinated fibers were rarely seen in LN series of cases. SL clefts were noticed in some large myelinated fibers, which according to earlier workers ([3, 13]) is one of the early features of Wallerian degeneration. At some places large axons devoid of myelin were noticed. By the size of the axons they were more likely to be demyelinated small myelinated fiber than unmyelinated axons. This is confirmed by the teased fiber preparations which showed segmental demyelination of small myelinated fibers. Cases L-5, L-6, L-8 and L-9 showed signs of myelin degeneration with ovoid formation. In case L-9 there was marked increase in the number of SL clefts, which were beautifully demonstrated in teased fiber preparations (Fig. 14b) as well as ultrastructurally.

It was observed that Schwann cell activity was much more evident around unmyelinated fibers than around myelinated fibers. In the BL type of case, Schwann cells of myelinated fibers were seen harboring *M. leprae*. No evidence of onion bulb formation, which is a sign of chronic recurring demyelination and remyelination process, was seen in any specimen.

Unmyelinated fibers and Schwann cells. At many places unmyelinated fibers gave an appearance suggesting that the Schwann cell columns were breaking open to allow the axons to extrude (Fig. 5). This type of degeneration of unmyelinated fibers was described by Taxi ([11]) as quoted by Thomas ([12]). Probably in leprous nerves this is one type of degeneration of unmyelinated fibers. Another frequent feature of unmyelinated fibers was the presence of "collagen pockets" in which groups of collagen fibrils were surrounded by Schwann cell processes.

Similar collagen pockets were not found in relation to Schwann cells of myelinated fibers. These collagen pockets most likely indicate the site of degenerated unmyelinated axons. These pockets were seen in larger number in advanced cases. This has been described by Thomas in his study on unmyelinated fibers ([12]).

Schwann cell activity was seen always around unmyelinated fibers.

DISCUSSION

A significant fallout of small-sized fibers observed in five of seven of the LN cases differs from the impression of Dastur *et al* ([3]) who commented on the loss of large-sized fibers. These authors did not do regular quantitative study of the myelinated fibers. Since there is no correlative data in their report it is not possible to know the actual status of the nerve clinically. Two cases of the present study, where a fall-out of large-sized fibers was encountered, did show slightly advanced changes, electrophysiologically as well as ultrastructurally. Thus, it is possible that the small-sized fibers are the ones which are affected initially in leprosy patients.

Quantitative study of unmyelinated fibers has not been done in the present study and is also not reported by other authors. Observations at the ultrastructural level reveal degenerative changes in the axons and Schwann cell activity around unmyelinated axons. It appears that in early cases of neural damage in leprosy unmyelinated and the small myelinated fibers are affected initially. The large-sized fibers are damaged subsequently. This is followed by increase in small-sized fibers occasionally which may be

regenerating fibers. Segmental demyelination was seen in small-sized fibers.

Weller and Nester ([14]) in their study of segmental demyelination in diphtheritic neuritis, suggest that the mechanism of demyelination in nerve fibers of less than 5μ in diameter differs from that in fibers greater than 5μ. Myelin breakdown occurs earlier in the smaller fibers (less than 5μ) and involves the whole internodal segment at the same time. In the large fibers (more than 5μ) demyelination starts at the nodes of Ranvier with gradual widening of the gap between the Schwann cells. Only later is the whole segment involved in degeneration. In the LN series of the present study, in four of eight patients, nerve teasing could be done and all of them showed evidence of segmental demyelination in small myelinated fibers involving one to three segments. There was no trace of Wallerian degeneration, except for the increased number of SL clefts in some of the large myelinated fibers and in some places widening of the nodal gap as noticed by Webster ([13]) and Dastur and Razzak ([5]).

In the L series those which belonged to BL type of leprosy showed segmental and also Wallerian type of degenerative changes in teased nerve preparation. Irrespective of type of leprosy, segmental demyelination seems to predominate in the early stage and Wallerian degeneration sets in as the disease progresses, ultimately resulting in total destruction of nerve fibers.

Correlation of clinical, electrophysiological, quantitative and ultrastructural features. It can be seen from the observations in all the three groups that in the LN series of cases where the clinical findings were normal for all the sensory modalities, nerve conduction studies nevertheless revealed some abnormalities. While the amplitude (19 μv) and duration (1.7 msec) of the sensory action potential were comparable to the normal values, in two thirds of cases the sensory velocity was within the upper limit of normal or slightly delayed. In LL series of cases clinically the nerves were so minimally involved that the usual methods of testing the leprosy patient with one inch No. 5 nylon would have missed the sensory deficit. Finer graded nylon testing showed a slightly higher range of normal (4.56 gm). Electrophysiologically in three cases (L-5, L-6 and L-9) of the LL series where the sensory conduction was in the upper limit of normal or delayed, a fair number of large-sized fibers were preserved. In cases L-8 and L-9 where no sensory action potential could be recorded the nerve showed total absence of large-sized fibers. In cases L-9 and L-8 even though the fiber counts at light microscopic level were normal, at the ultrastructural level most of the fibers showed degenerative changes. It may be because of these degenerative changes that there was no synchronous volley coming at the pickup point and hence the nerve failed to conduct.

It is evident that for clinical impairment to occur the damage to the nerve has to be quite extensive. While early nerve damage involving particularly the small-sized fibers could be detected by studying conduction velocity, use of an "average" which is more sensitive might help to detect the damage to the small-sized fibers even earlier.

SUMMARY

A correlative study of clinical, electrophysiological, quantitative, histopathologic and ultrastructural changes seen in 13 instances of the index branch of the radial cutaneous nerve was undertaken in leprosy patients.

Testing by graded nylon proved to be more reliable than any other conventional clinical tests. The quantitative studies revealed that small-sized fiber loss was encountered in the early stages of nerve involvement in leprosy followed by loss of large-sized fibers with or without increase in small-sized fibers. Segmental demyelination of small-sized fibers was seen in early stages of degeneration in leprosy nerves irrespective of type of leprosy. Wallerian degeneration was encountered in advanced stages with total destruction of the nerves.

At the ultrastructural level clinically normal nerves of leprosy patients (LN series) showed minimal but significant changes, such as thickening of the basement membrane of perineurial cells, as well as an increase in the amount of collagen between the perineurial layers. Marked increases in the amount of endoneurial collagen were noticed. Axons devoid of myelin, probably demyelinated fibers, were occasionally observed. Slight proliferation of the basement membrane of the endothelial cells was also observed. These changes were of a more advanced nature in the clinically involved cases

of leprosy (L series). Two BL types of cases from the L series, showed presence of *M. leprae* in Schwann cell cytoplasm of myelinated and unmyelinated fibers, perineurial cells, in endothelial cells and macrophages.

In cases having impaired sensory modalities (L series), thickening of the perineurium was due to increase in the number of layers of perineurial cells in addition to the increase in collagen.

Probably one of the ways in which unmyelinated fibers degenerate is by splitting the Schwann cell columns and extrusion of the axons. Collagen pockets were seen in the LN series of cases and much more frequently in the L series of cases. These are probably the degenerated nonmyelinated fibers being replaced by collagen fibrils. Postoperative clinical charting was done on the patient whose IRC nerves were removed. It showed complete restoration of all modalities of sensation in the area of its distribution by the end of three months.

RESUMEN

Estudio correlativo de las alteraciones clínicas, electrofisiológicas, cuantitativas, histopatológicas y ultraestructurales en 13 estudios de la rama índice del nervio cutáneo radial en pacientes con lepra.

Las pruebas con nylon graduado demostraron ser más confiables que cualquiera otra prueba clínica convencional. Los estudios cuantitativos revelaron que en las etapas iniciales de la lepra, se encuentra pérdida de las fibras pequeñas, seguida por pérdida de las fibras grandes con o sin aumento de las fibras pequeñas. La desmielinización segmentada de las fibras pequeñas se observó en las etapas tempranas de la degeneración de los nervios con lepra, en cualquiera de los tipos de lepra. La degeneración Walleriana se encontró en las etapas avanzadas, con total destrucción de los nervios.

A nivel ultraestructural, los nervios clínicamente normales de la serie NL mostraron cambios mínimos pero significativos, tales como engrosamiento de la membrana basal de las células perineurales, como también aumento de la cantidad de colágeno entre las capas perineurales. Se observó un marcado aumento de la cantidad de colágeno endoneurial. En forma ocasional se observaron axones desprovistos de mielina. También se observó una ligera proliferación de la membrana basal de las células endoteliales. Estas alteraciones fueron de naturaleza más avanzada en la serie L de casos. Dos casos tipo LD de la serie L, mostraron presencia de *M. leprae* en el citoplasma de las células de Schwan de las fibras mielínicas y no mielínicas, células perineurales y en las células endoteliales y macrófagos.

En la serie de casos LL en engrosamiento del perineuro se debió a aumento del número de capas de células perineurales, además del aumento del colágeno.

Probablemente una de las formas en que las fibras desmielinizadas degeneran, es diviendo las columnas de las células de Schwan y destruyendo los axones. Se observaron depósitos de colágeno en la serie de casos NL y con mucha mayor frecuencia en la serie de casos L. Estos son probablemente las fibras no-mielínicas degeneradas que están siendo reemplazadas por fibrillas de colágeno. Se hizo un estudio clínico post-operatorio de los pacientes a quienes se les habían extirpado los nervios RIC, el cual mostró completa restauración de la sensibilidad en el área de distribución después de tres meses.

RÉSUMÉ

Une étude a été entreprise pour étudier les corrélations existant entre les modifications cliniques, électrophysiologiques, quantitatives, histopathologiques, et ultrastructurelles observées au niveau de la branche principale du nerf cutané radial chez des malades de la lèpre. L'exploration par des fibres de nylon d'épaisseur graduée, s'est révélée plus fiable que toute autre épreuve clinique conventionnelle. Les études quantitatives ont révélé une perte des filets de petite dimension dans les phases précoces de la lèpre, suivie par une perte des filets de plus grande dimension, avec ou sans augmentation du nombre des filets de petite dimension. Une démyélinisation segmentaire des filets de petite dimension a été relevée dans les phases précoces de dégénérescence des nerfs chez les lépreux, et ceci quel que soit le type de lèpre. Une dégénérescence wallérienne a été observée dans les phases avancées, avec destruction totale des nerfs.

Au niveau ultrastructurel, les nerfs cliniquement normaux appartenant à des malades de formes LN, ont témoigné de modifications minimes mais significatives, telles que l'épaississement de la membrane basilaire des cellules périneurales, de même qu'une augmentation de la quantité de collagène entre les couches périneurales. Une augmentation notable de la quantité de collagène endoneural, a été noté. Occasionnellement, on a pu observer des axones dépourvus de myéline, qui étaient probablement des fibres démyélinisées. Une légère prolifération de la membrane basilaire des cellules endothéliales a également été relevée. Ces modifications étaient plus prononcées chez les cas lépromateux. Deux malades atteints de forme BL dans la série L, ont révélé la présence de *M. leprae* dans le cytoplasme des cellules de Schwann des fibres myélinisées et non myélinisées, des cellules périneurales, de même que dans les cellules endothéliales et dans les macrophages.

Dans la série L de cas, l'épaississement du perin èvre était dû, outre à l'augmentation de collagène, à l'accroissement du nombre des couches de cellules périneurales.

Il est vraisemblable que la séparation des colonnes de cellules de Schwann, suivie de l'irruption à l'extérieur des axones, constitue l'une des manières par lesquelles les fibres non myélinisées entrent en dégénérescence. Des poches de collagène ont été observées dans la série LN de cas, et ceci beaucoup plus fréquemment chez les malades atteints de la forme lépromateuse. Cette poche était probablement constituée par des fibres non myélinisées en dégénérescence, qui avaient été remplacées par des fibrilles de collagène. On a procédé à la recherche de la topographie des troubles cliniques postopératoires des malades dont les nerfs radiaux cutanés avaient été excisés. On a pu en conclure à un retour total de la sensibilité, dans tous ses aspects, dans la zone de distribution de ce nerf, après trois mois.

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