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Clinical, Electrophysiological, Quantitative, Histologic and Ultrastructural Studies of the Index Branch of the Radial Cutaneous Nerve in Leprosy I. Preliminary Report [1, 2]

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Sensory loss in the skin is known to every clinician dealing with leprosy. This may be either in the skin patch, a glove or stocking distribution or affect the territory supplied by the major nerve trunk. It is also well known that loss of sweating is one of the earliest signs of leprosy and generally precedes loss of thermal sensation. Touch is the final modality to be lost. Clinical experience thus indicates that there is dissociated loss of cutaneous nerve function in leprosy, the finest unmyelinated fibers controlling autonomous function probably being the earliest to be affected, followed by the small myelinated fibers serving thermal sense, and lastly the largest fibers which are responsible for sensation of touch. Nevertheless, the studies of Dastur *et al* (2) demonstrate loss of large fibers preceding that of the smaller ones.

The majority of the above studies dealt with nerves which had been severely damaged by the disease process. Even the studies on nerves which showed early changes on normal cutaneous testing were very likely to be on nerves in a state of fairly advanced involvement because the normal methods of clinical testing are crude and not capable of testing for graded loss of sensation. It is also known that considerable nerve fiber loss has to take place before clinically detectable sensory loss is manifest, Weddell and Miller (15).

Many of the previous studies have been of an isolated nature. The studies on electrophysiology of Magora *et al* (11), Hackett *et al* (5), Verghese *et al* (14), and Karat *et al* (9) do not correlate their findings with morphological changes, while the studies on histopathology and ultrastructure of nerve by Fite (4), Khanolkar (10), Iyer (6), Job and Desikan (8), Nishiura (12), Job (7), Dastur *et al* (2, 3), do not correlate with the electrophysiological changes in the examined nerve if any.

This study was undertaken to observe what may be the earliest changes in the peripheral nerves in leprosy and to correlate the histopathologic and ultrastructural changes with any detectable changes in conduction velocity and also with the clinical status of the patient, particularly as relating to the sensory changes in the area supplied by the nerve. The senior author's experience in earlier studies has indicated that it would probably be necessary to study clinically normal nerves in patients suffering from leprosy if the earliest preclinical changes are to be observed. It was also decided to use more refined methods for sensory testing. A nerve was sought which could be readily available for biopsy from not only leprosy patients but also from normal volunteers without leaving any serious or permanent sensory deficit.

MATERIALS AND METHODS

Index branch of the radial cutaneous nerve (IRC). Pearson and Weddell (13) described biopsy branches of the radial cutaneous nerve in leprosy as did also Dastur *et al* (3). These were presumably random excisions of one of the many terminal branches of this nerve. Adamson *et al* (1) described the use of the index branch of the radial cutaneous nerve in the transfer of an innervated skin flap from the radial aspect of the index finger which was based on a neurovas-

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cular pedicle and used to supply sensation to an insensitive thumb. This flap had been used by one of us and suggested the possibility of using this anatomically constant nerve for the present study. In order to confirm the constancy of this nerve, dissection of 25 hands of cadavers was undertaken in the anatomy department.

Figure 1 illustrates the constant position of this nerve, which lies lateral to the tendon of the muscle extensor indices. After giving off two fine branches to the web of the thumb at the level of the base of the index finger metacarpal, it remains as a single nerve bundle under the deep fascia until it reaches the neck of the metacarpal after which it divides into its terminal branches. The nerve is not only constant in position and hence readily identifiable at surgery, but is of a diameter suitable for both light and electron microscopy, including total fiber counting.

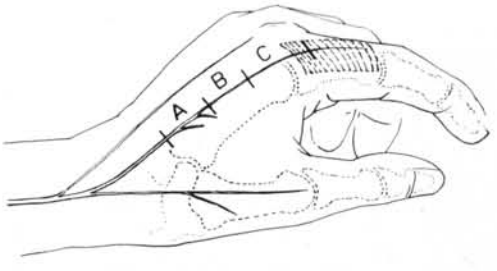

FIG. 1. Diagrammatic representation of the index branch of the radial cutaneous nerve and its area of sensory supply (shaded area). The sites of biopsy were: A—for light microscopy; B—for electron microscopy; C—for fiber teasing.

Normal control material. Thirty-three nerves were studied by sensory testing and for nerve conduction velocity. Five of these nerves were subjected to biopsy. These studies in normal persons not suffering from leprosy or any other neurological disease helped to establish the normals against which the findings of the leprosy patients' nerves could be compared.

Leprosy material. Of the 13 leprosy patients in the study, 10 were borderline tuberculoid (BT) and 3 were borderline lepromatous (BL). These patients were involved in a larger study in which they were subjected to detailed clinical and histopathologic investigations.

On the basis of clinical sensory testing the leprosy cases were further divided into two categories in which eight cases showed no sensory changes in the territory supplied by the IRC (LN) and the remaining five showed some changes, even if only of a minimal nature, on sensory testing as shown in Table 1. A total of 20 leprous nerves were included in the sensory testing and electrophysiologic part of the study. Thirteen of these nerves were biopsied.

Clinical testing. The normal procedure for sensory testing by cotton wool, No. 5 nylon, pinprick and hot and ice cold water was considered inadequate for observing finer changes. Therefore, the following method was adopted to test the modalities of touch, pain and temperature. The whole hand was tested with special attention paid to the area supplied by the IRC nerve. Nylon filaments which bend under graded weights (No. 5 nylon is too coarse as compared with the graded nylon where the normal range is 3.61 gm to 3.84 gm pressure.) were used to test the sensation of touch and pressure (Fig. 2). Pain was tested with a straight sharp cutting surgical needle loaded with a four gram weight. Temperature was tested at the two levels of 50°C to 60°C and 0°C. Copper cylinders tapered at one end were coated with araldite except at the testing point and were dipped in water heated to 60°C and in ice for a prolonged period. These cylinders retained their temperatures at constant levels

TABLE 1. *Summary of case types and procedures.*

	N[a]	LN[b]	L[c]	Total
Sensory testing	33	15	5	53
Electrophysiologic studies	33	15	5	53
Biopsy	5	8	5	18

[a] N = Normal nerves of healthy volunteers, control group.
[b] LN = Clinically normal nerves in leprosy patients.
[c] L = Clinically affected nerves in leprosy patients.

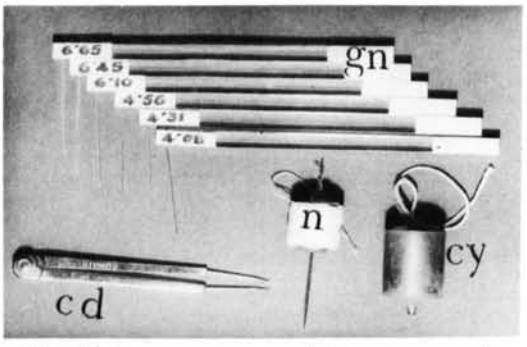

FIG. 2. Instruments used in sensory testing: gn—graded nylons for touch and pressure sensation; cy—copper cylinder completely coated with areldite except at the tip; n—4 gm weighted needle for testing sensation of pain; cd—compass divider to measure two point discrimination.

for 15 minutes. Hair movement was tested by using a needle. Two point discrimination was measured by using a compass divider (Fig. 2).

Nerve conduction velocity. Surface electrodes were used throughout the study. The radial cutaneous nerve was stimulated in the lower forearm and the antidromic impulse was recorded over the index branch under study at a distance of 2.5 cm proximal to the metacarpophalangeal joint. The distance between the points of stimulus and recording was kept constant at 14 cm. A two channel DISA electromyograph with a Tetronix storage oscilloscope was used. The following values were recorded: latency to onset, latency to peak, peak to peak amplitude and the duration of the potential. Normal values were established statistically from the findings in 33 healthy subjects.

Biopsy. The biopsy of the IRC nerve was performed under general anesthesia or under local anesthesia by blocking the nerve trunk in the forearm. A tourniquet using a sphygmomanometer cuff was employed for a maximal period of five to ten minutes. The nerve was exposed and, using a microtechnic, it was dissected from its bed with minimal handling of the part which was to be studied. The middle segment was fixed on a card for electron microscopy, the proximal for light microscopy, and the distal for fiber teasing.

The proximal segment, A (Fig. 1), fixed in formol Zenker solution was sectioned longitudinally and transversely stained with Triff ([16]) and examined under the light microscope. The middle segment, B, fixed in glutaraldehyde and processed for electron microscopy, was sectioned transversely. Semithin, one micron sections, stained by toludine blue were studied for myelinated fiber count while ultrathin sections were studied by electron microscopy. The distal segment, C, fixed in glutaraldehyde and postfixed in osmium was studied by fiber teasing.

RESULTS

Control values. Clinical testing and nerve conduction velocity findings of the five healthy volunteers were as follows.

Sensory testing. Graded nylon sensation was felt by all volunteers at 3.84 gm. Distinct perception of temperature difference was between 50° C to 60° C, and pinprick was felt at 4 gm. Two point discrimination was noticeable between a minimum of 0.7 cm and a maximum of 0.9 cm of distance.

Postoperative sensory testing. Examination three weeks after nerve biopsy revealed a loss of all modalities of sensation over the radial half of the dorsum of the proximal phalanx of the index finger. At three months the majority showed recovery of sensation. No patient complained of this being a handicap.

Electrophysiologic findings. The details of the electrophysiologic study will be published elsewhere. The mean values of normal nerve conduction velocity were: latency to onset—2.2 msec ± 0.4 (SD 2); latency to

TABLE 2. *Average electrophysiologic values.*

Group of patients	Latency to onset (msec)	Latency to peak (msec)	Peak to peak amplitude (μV)	Duration of potential (msec)
N[a]	2.2 + 0.4	2.7 + 0.4	19 + 12	1.6 + 0.4
LN[b]	2.4 + 0.4	2.8 + 0.4	19 + 12	1.7 + 0.4

[a] N = Normal nerves of healthy volunteers, control group.
[b] LN = Clinically normal nerves in leprosy patients.

TABLE 3. *Fiber count values per square millimeter.*

Group of patients	Mean fiber count	Mean large fiber (L) (>7μ)	Mean median fiber (M) (4-7μ)	Mean small fiber (S) (<4μ)	Mean ratio of L:M:S
N[a]	500	102	197	201	1:2:2
LN[b]	452	90	211	151	1:2:1.5
L[c]	502	88	213	201	1:2:2.2

[a] N = Normal nerves of healthy volunteers, control group.
[b] LN = Clinically normal nerves in leprosy patients.
[c] L = Clinically affected nerves in leprosy patients.

peak—2.7 msec + 0.4 (SD 2); peak to peak—19 V + 12 (SD 6); duration of potential—1.6 msec + 0.4 (SD 2). Experimental findings are given in Table 2.

Quantitative histopathology. Details will be published elsewhere but the general findings are noted in Table 3. The diameter of the IRC varied between 0.25 mm to 0.315 mm and the myelinated fiber density between 6,000 and 12,000 fibers per square millimeter.

Fiber teasing. This showed segmental demyelination in the small fibers (below 4μ) in the LN series. The L series showed segmental plus Wallerian degeneration and a marked increase in Schmidt-Lantermann clefts was observed. The latter was also noted on electron microscopy.

Electron microscopic findings. Details will be published elsewhere but the general findings are here noted.

LN series. In six of eight cases the nerve showed a definite increase in endoneurial collagen, thickening of basement membrane of perineurial cells (Fig. 3), presence of demyelinated axons suggestive of segmental demyelination, and considerable proliferation of Schwann cell processes especially those surrounding unmyelinated axons (Fig. 4). Although no quantitative study was done, there seemed to be a definite decrease in nonmyelinated fiber density and collagen deposition in the empty spaces within the Schwann cells following loss of these fibers.

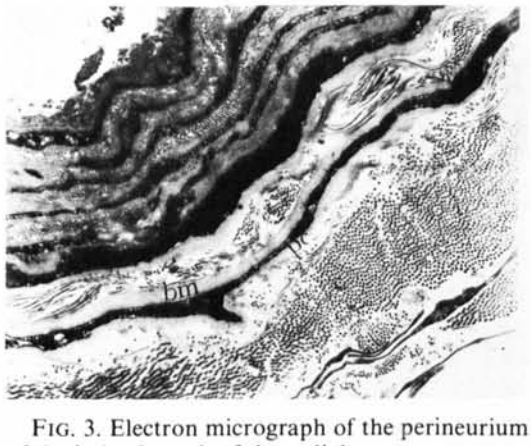

FIG. 3. Electron micrograph of the perineurium of the index branch of the radial cutaneous nerve, showing thickened basement membrane (bm) and increased collagen (c) between the perineurial layers (pc). ×11,000.

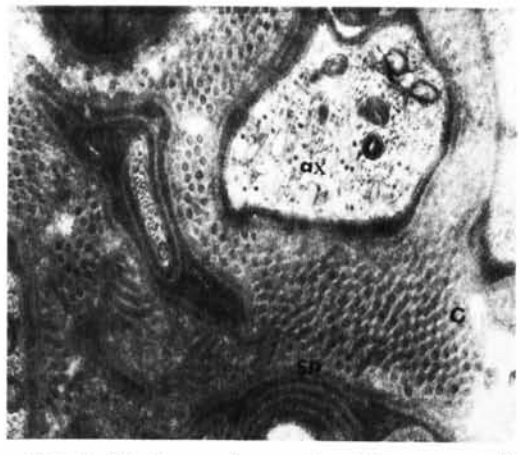

FIG. 4. Electron micrograph of large unmyelinated axon (ax) showing increased proliferation of Schwann cell processes (Sp). There is an increased amount of collagen (c). ×53,800.

L series. All cases showed the above changes in a more advanced stage, including changes suggestive of Wallerian degeneration in two cases (L-5 and L-9).

Analysis of five cases in L series. The L series (Table 4) was too small and differences between individual nerves were so great that it is difficult to generalize in this series. On the other hand, the N and NL series results have been fairly constant in both groups. In the L series in two nerves of

TABLE 4. *Analysis of five cases in L series.*

Laboratory no.	Nerve conduction	Quantitative histology finding	Ultrastructure
L-5	Upper limit of normal	Normal	Marked changes +
L-6	Upper limit of normal	Normal	Marked changes +
L-9	Delayed	Large size fiber loss	Advanced changed ++ 90% show SL
L-7	Absent	Only one large size fiber	Damage +++
L-8	Absent	Normal histogram	Damage +++

the BL patients (L-5 and L-6) there was no apparent fiber loss but they, nevertheless, showed marked damage to the fibers in terms of Wallerian and segmental demyelination with Schwanian hypertrophy, collagenization and delayed nerve conduction. The other two L series cases (L-7 and L-8) showed absence of sensory action potential. One of these showed only one large size fiber while the other showed a normal histogram though electron microscopy revealed marked degenerative changes in almost all large fibers.

DISCUSSION

Our previous studies have revealed gross histopathologic changes in nerves of leprosy patients who had early sensory changes demonstrated by the usual clinical methods. It was therefore evident that if early nerve changes were to be studied in this disease it would be necessary to refine our technics for sensory testing and also to select nerves which were either normal or showed the earliest changes on such testing.

After establishing the norms in healthy volunteers (N) in respect to sensory testing, nerve conduction velocity, quantitative histology and electron microscopy for the index branch of the radial cutaneous nerve, similar studies were undertaken on clinically normal (LN) (Figs. 5 and 6) and slightly affected (L) (Fig. 7) nerves in patients who had leprosy in other parts of the body.

Report of the LN cases showed definite pathologic changes on electron microscopy. It was interesting to note that there was a significant reduction in the small myelinated fibers in five of eight cases without any change in the large myelinated fibers. Electron microscopy in these cases also revealed loss of unmyelinated fibers.

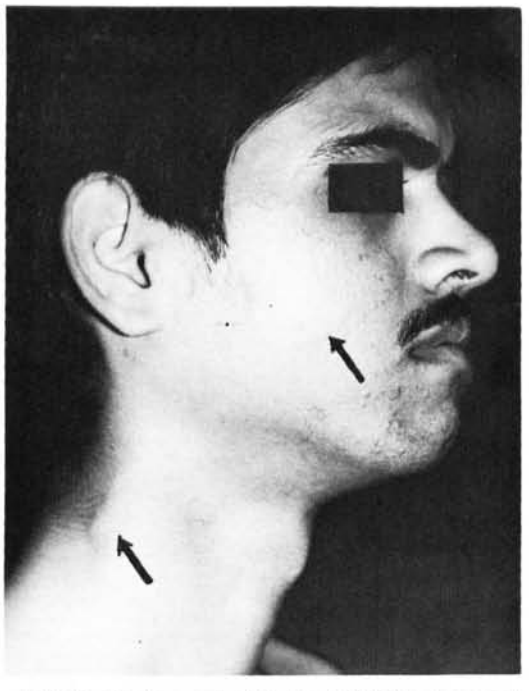

FIG. 5. Photograph of patient (O.R.), man aged 19 years, of LN series. Upper arrow shows a depigmented patch on the cheek while lower arrow shows the thickened greater auricular nerve.

Electrophysiologic studies showed that in two thirds of the cases the sensory velocity was in the upper limit of normal or slightly delayed, while the amplitude and duration of the action potential was within the normal range. This illustrates that the disease results in a diffuse peripheral neuropathy even at a stage where clinically it is restricted to a limited part of the body, i.e., a patch or a single thickened nerve. This cannot be de-

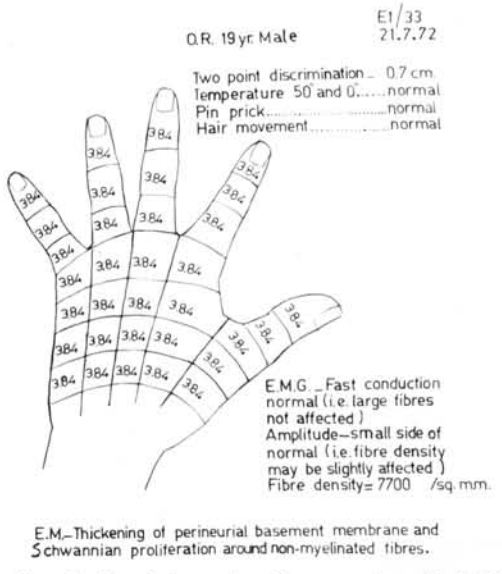

FIG. 6. Hand chart showing mapping of all different normal sensory modalities. Electrophysiological values and electron microscopic observation show definite involvement of the nerve. Figures in divided segments represent pressure in grams measured by graded nylon, which is within the normal range (3.61 gm to 3.84 gm).

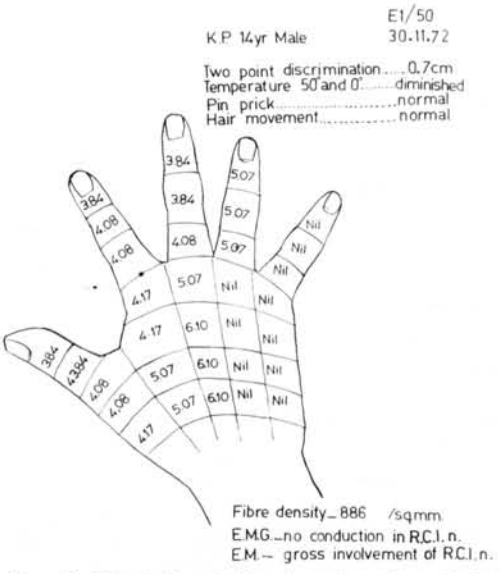

FIG. 7. Whole hand charting of patient (K.P.), male aged 14 years, of L series, showing disturbed sensory modalities on clinical examination. There was gross involvement of the nerve as demonstrated by electrophysiologic and electron microscopic studies.

tected by routine methods of clinical testing or histopathology although it is evident by electron microscopy. Although leprosy clinically presents a classical picture of dissociated fiber loss and suggests that loss of small fibers precedes that of the large ones, previous studies have indicated that loss of large sized fibers predominates ([5, 6]). The LN series, which probably is the study of the earliest nerve changes in this disease, reveals selective loss of small myelinated and unmyelinated fibers. There is also some evidence of delay in conduction velocity in these nerves. The use of an "averager" may help to further elaborate such changes by revealing diminution or loss of the third peak which is formed by the slow and finest fibers.

The L cases reveal the marked discrepancy between clinical testing, electrophysiologic and ultrastructural changes. Using normal clinical methods of testing (i.e., No. 5 nylon) these nerves would not have revealed any sensory changes in their area of innervation since only with the more refined and graded methods of testing used by us were early changes recorded.

Delayed conduction in the three cases (L-5, L-6 and L-9) was probably the result of damage to all the fibers as revealed by electron microscopy. The absence of electrical nerve potential in L-7 is explained by the almost complete loss of large-sized fibers. The total absence of conduction velocity in L-8 in the presence of normal histogram was, at first glance, confusing but the electron microscope revealed degenerative changes in most of the fibers.

In one case of LN (LN-3), where there was a decrease in large size fibers, there was simultaneous marked increase in small size fibers, most of which were probably efforts at regeneration. This was also noted in L-7.

SUMMARY

The index branch of the radial cutaneous nerve has been demonstrated as a constant nerve which can be readily biopsied under local anesthesia and yields a nerve which is of suitable size for quantitative and qualitative studies both by light and electron microscopy. It supplies a limited but constant area where the sensory loss does not disturb the patient.

Definite ultrastructural changes have been demonstrated in the clinically normal

nerves of leprosy patients. These nerves have also revealed a loss in the small myelinated and unmyelinated fibers, which correlated with the common clinical findings of the absence of sweating and dissociated sensory loss in this disease. Gross damage has been encountered in nerves which showed only early signs of clinical damage even with refined methods of sensory testing. These nerves would have passed as normal on routine testing by No. 5 nylon. Regeneration of small fibers was noted following loss of large size fibers.

Nerve conduction velocity may be a useful tool in early diagnosis.

RESUMEN

Se ha demostrado que la rama índice del nervio radial cutáneo es un nervio constante que puede ser fácilmente biopsiado bajo anestesia local, proporcionando un nervio de un tamaño adecuado para estudios cualitativos y cuantitativos, tanto por medio del microscopio de luz como con el microscopio electrónico. Proporciona un área limitada pero constante donde la pérdida de la sensibilidad no molesta al paciente.

En nervios clínicamente normales de pacientes con lepra, se han demostrado cambios ultraestructurales evidentes. Estos nervios también han revelado una pérdida de las fibras pequeñas, mielínicas y no-mielínicas, que se relaciona con los hallazgos clínicos de ausencia de sudoración y pérdida de sensibilidad disociada, que se encuentran corrientemente en esta enfermedad. Se han encontrado lesiones importantes en nervios que mostraban sólo indicios de lesión clínica, aun con métodos refinados de estudio sensorial. Estos habrían pasado como normales en las pruebas de rutina por medio de Nylon No 5. Se observó que después de la pérdida de las fibras grandes se producía una regeneración de las fibras pequeñas.

La velocidad de conducción nerviosa puede ser un método útil para el diagnóstico temprano.

RÉSUMÉ

La branche du nerf cutané radial innervant l'index est un nerf dont la présence est constante et qui, comme on l'a démontré, peut être facilement biopsié sous anesthésie locale. La biopsie permet de disposer d'un nerf d'une dimension satisfaisante pour des études quantitatives menées tant par la microscopie optique que par la microscopie électronique. Ce nerf dessert une zone limitée mais constante où la perte de la sensibilité n'entraîne pas d'inconvénients pour le malade.

Des modifications très nettes dans l'ultrastructure ont été démontrées au niveau de nerfs cliniquement normaux provenant de malades de la lèpre. Ces nerfs ont également révélé une diminution des petites fibres myélinisées et non-myélinisées; cette observation peut être mise en relation avec les observations cliniques habituelles concernant l'absence de sudation et les troubles dissociés de la sensibilité qui sont observés dans cette maladie. Des lésions importantes ont été relevées dans des nerfs qui ne présentaient que des signes précoces de troubles cliniques, mis en évidence sans avoir recours à des méthodes raffinées pour l'exploration de la sensibilité. Ces nerfs auraient été considérés comme normaux lors d'épreuves de routine par des fibres de nylon de calibre 5. On a noté, à la suite d'une disparition des grosses fibres nerveuses, une régénération des petites fibres.

Les études de vitesse de conduction nerveuse peuvent constituer un outil utile pour le diagnostic précoce.

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