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The Collagen of Permanently Damaged Nerves in Human Leprosy[1]

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Studies of collagen have shown that at least four different types of this protein exist in human tissues. Collagen type I is present in the dermis, bone, tendon, and dentin; collagen type II is found in hyaline and elastic cartilages, and collagen type III is present associated mainly with collagen type I in arteries and in the uterus. Collagen type IV has been described as a constituent of basement membranes (9). Recent biochemical observations have shown the presence of collagen types I and III in the human femoral nerve (11).

Results from this laboratory have shown that the increase in birefringency observed in tissue sections, stained with Picrosirius and studied with polarization microscopy, is specific for collagen (5). Furthermore, this method permits the distinction of collagen types I and III (6). Under these conditions, collagen type I appears as thick, bright (strongly birefringent), red or yellow fibers. Collagen type III is characterized by thin, pale (weakly birefringent), greenish fibers. When applied to nerve sections, this method localized collagen type III in the endoneurium and collagen type I in the epineurium (7).

Additional observations with electron microscopy showed that collagen fibrils in the endo- and perineurium have distinctly smaller diameters than those of the collagen fibrils in the epineurium. Nerves therefore have two distinct collagen populations. These populations can be characterized by means of light and electron microscopy, and they are localized in different structural compartments of the nerve (8).

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Since leprous lesions are known to induce proliferation of collagen in nerves (1, 2, 4, 12), it was of interest to apply both the Picrosirius-polarization method and electron microscopic observation to peripheral nerves from leprosy patients. Our observations strongly suggest that, in these lesions, collagen proliferation occurs in two separate compartments with increases of collagen type III in the peri- and endoneurium and of collagen type I in the epineurium.

MATERIALS AND METHODS

Clinical material. Segments of peripheral nerves from ten chronically treated leprosy patients, whose clinical diagnosis had been confirmed by histopathological studies, were used. These nerve segments were obtained in the course of clinically-indicated surgical procedures involving affected portions of the nerves. We are dealing therefore with selected samples of leprous neuritis in all of which the pathologic changes in the nerve lesions were characterized by a proliferation of collagen (fibrosis) in the three nerve compartments (endo-, epi-, and perineurium).

Normal control nerves were obtained from autopsies.

The Picrosirius-polarization method. One-half of the excised segment of each nerve was fixed in Bouin's fluid for 24 hr, dehydrated, embedded in paraffin, and sectioned at 5 μm. These sections were stained for one hr in 0.1% Sirius Supra Red F3BA as described (5) and counterstained with hematoxylin. The slides were studied with an optical microscope fitted with polaroid filters. To observe the thin pale fibers of collagen type III, very strong illumination was necessary. This was made possible by overloading the voltage of the illumination lamp.

Electron microscopy. The other half of each biopsy was fixed in 2% glutaraldehyde

in 0.1 M phosphate or cacodylate buffer for two hr, followed by a second fixation in 1% osmic acid for one hr. Block staining was performed in 0.5% uranyl acetate overnight. The material was embedded in araldite, sectioned in a LKB ultratome, and double stained in uranyl acetate and lead citrate. Micrographs were obtained with a Zeiss EM 9 S electron microscope.

The diameters of collagen fibrils were measured in suitably enlarged micrographs with a Bausch & Lomb measuring magnifier. The magnification of the microscope was calibrated with a diffraction grating. At least 200 measurements were made in four electron micrographs in each case.

RESULTS

Light microscopy. The study of the nerve biopsies with the Picrosirius-polarization method showed a remarkable increase of birefringent material in the endo-, peri-, and epineurium when compared to normal control nerves (Fig. 1). Since birefringency is, in this method, specific for collagen, the increase of birefringent material denotes an increase in the amount of collagen present in the biopsies. Further observation of this material disclosed the presence of several thin parallel layers of collagen (birefringent material) concentrically disposed in transverse sections of the nerve, subdividing it into an inner and an outer region (Figs. 1b and 3).

Polarization microscopy showed that two different types of collagen could be clearly distinguished in these nerves: one type was formed by thin, pale (weakly birefringent), greenish fibers typical of collagen type III ([6]). In normal nerves, these collagen fibers formed a delicate network surrounding the nerve fibers. In the biopsies from leprosy patients these collagen type III fibers appear in two sites. The first site corresponds to the endoneurium although its appearance is different from that of normal nerves. In this location the fibers appear as irregular agglomerates of thin fibers that occupy part, if not all, of the place originally occupied by the nerve fibers (Fig. 2). The second site corresponds to the perineurium, which appears in most biopsies as the inner portion of the parallel concentric layers described above. It is interesting to note that the perineurium, which is not easily observed in normal nerves due to its thinness, is clearly evident in most of the leprosy biopsies studied as the result of collagen proliferation in between the layers of perineural cells (Fig. 3).

The other type of collagen observed in nerves is made up of thick, bright (strongly birefringent), yellow or red fibers. These fibers correspond to collagen type I, which is present in the epineurium of normal nerves. The biopsies from leprosy patients show a thickening of the epineurium due to an increase in the amount of this type of fibers (Fig. 1). In most of the biopsies from leprosy patients, this nerve sheath can be divided into a compact outer region and a laminated inner region circumscribing the perineurium.

Electron microscopy. The electron microscopic study confirmed the observations made with the Picrosirius-polarization method. The nerve biopsies from the leprosy patients showed compartments when compared to normal control nerves (Fig. 4).

The study of our leprosy material showed that as in normal nerves ([8]), two different populations of collagen can be easily distinguished by the diameter of their fibrils (Fig. 5). A population of thinner fibrils with a smaller average diameter was found in the endo- and perineurium while a population of thicker fibrils was characteristic of the epineurium (Table 1). The diameters of the

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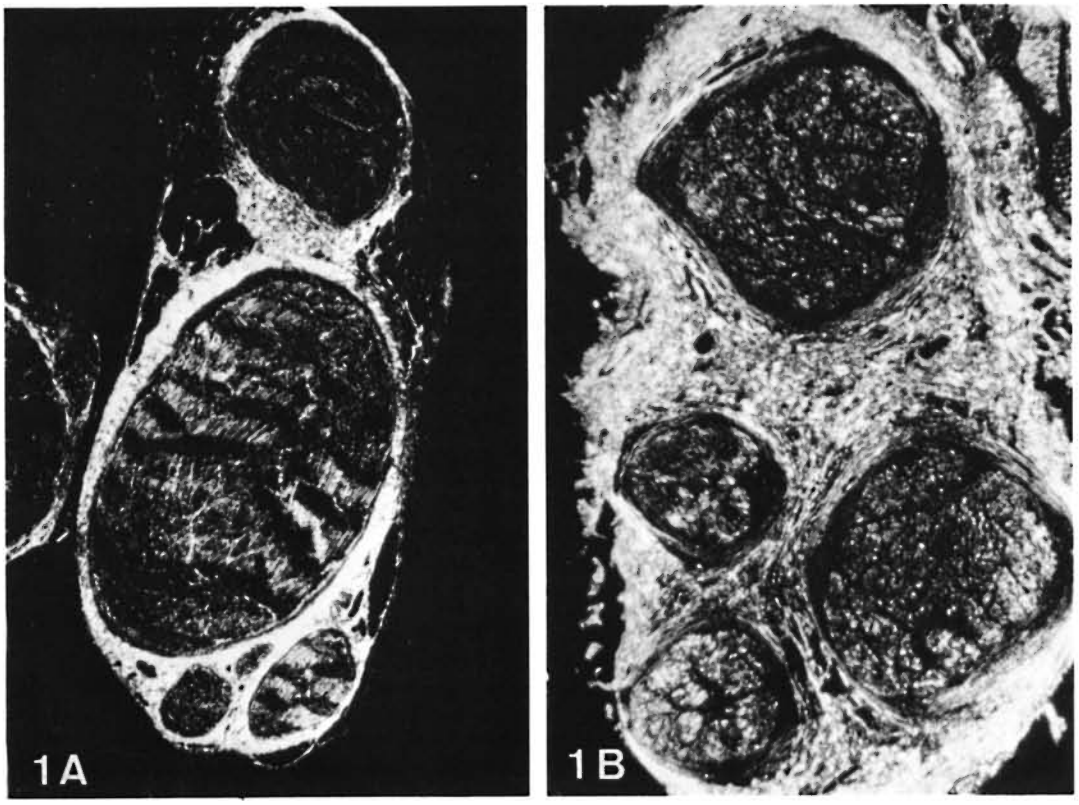
FIG. 1. Photomicrographs of cross sections of normal (A) and leprous (B) nerves, stained with Picrosirius and observed with polarization microscopy. All birefringent structures are collagen. Observe the remarkable increase of collagen induced by the leprous infection. (×60)

FIG. 2. Photomicrographs of a leprous nerve stained by Picrosirius and observed with normal (A) and polarization (B) microscopy. The epineurium (E) is formed by thick, bright collagen fibers showing a yellow or red color when observed with polarization while the endoneurium (EN), is formed by pale, thin fibers that present a greenish color. The perineural cell layers (P) are separated due to retraction during the histological procedure. (×265)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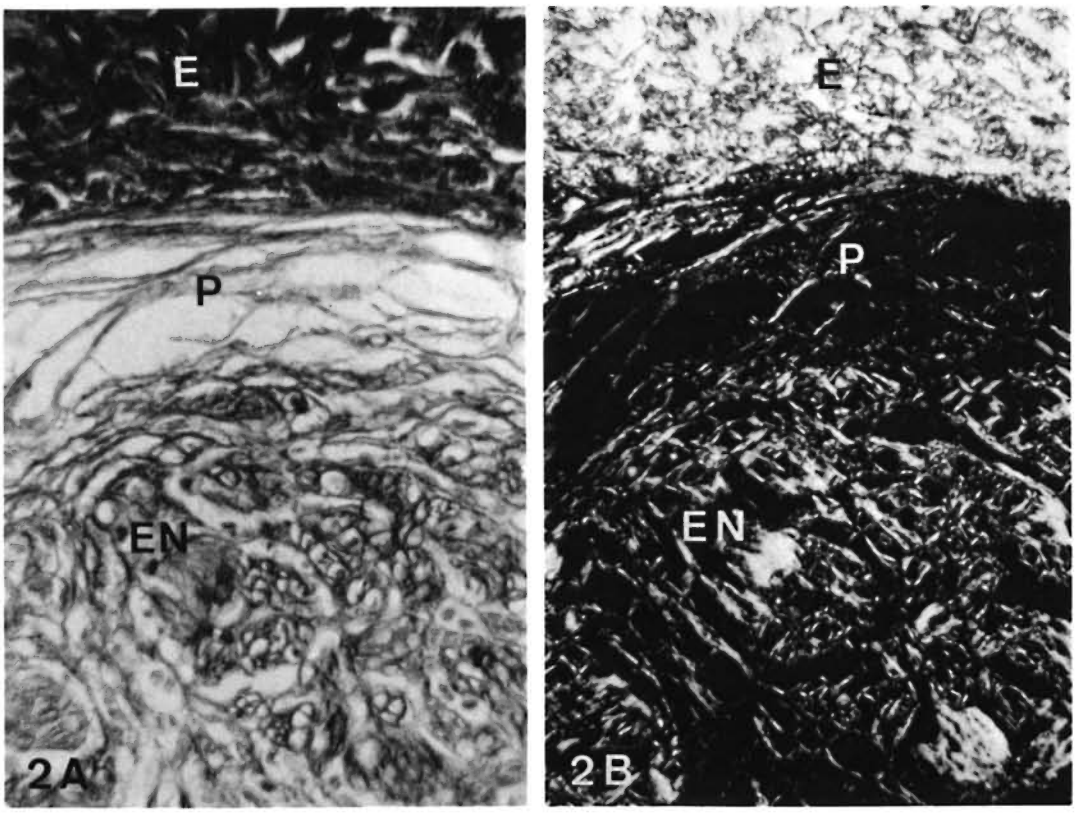
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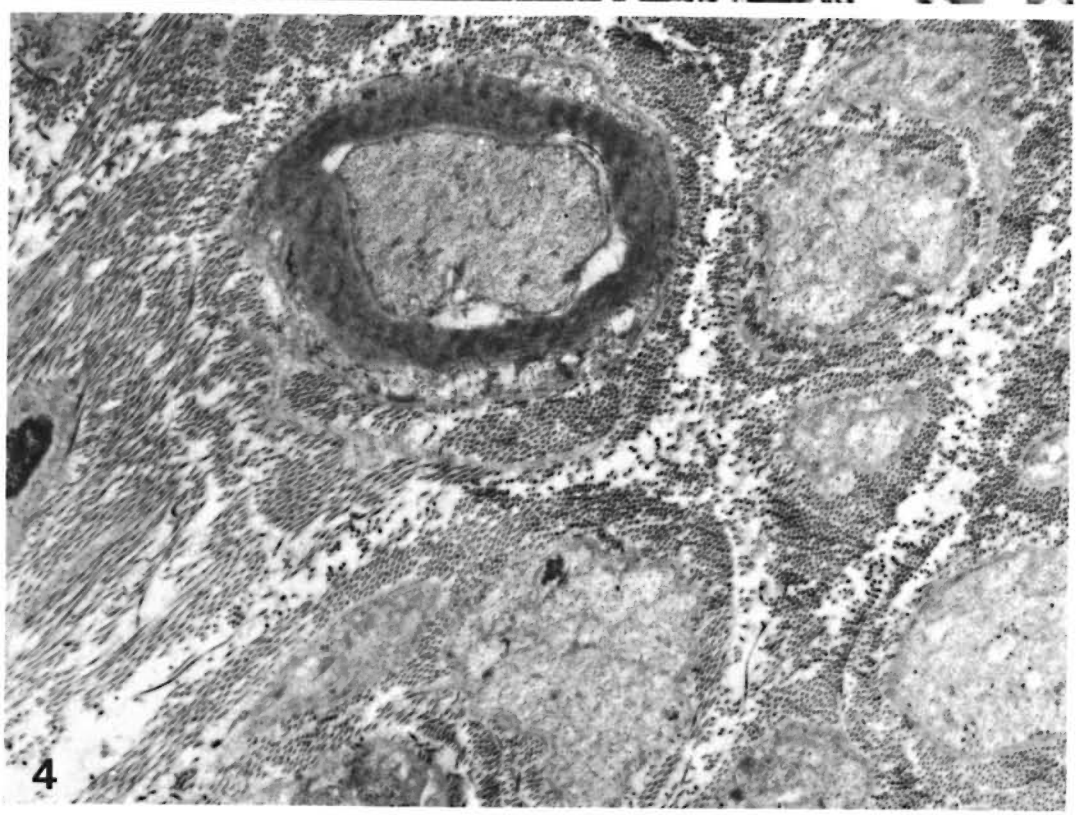
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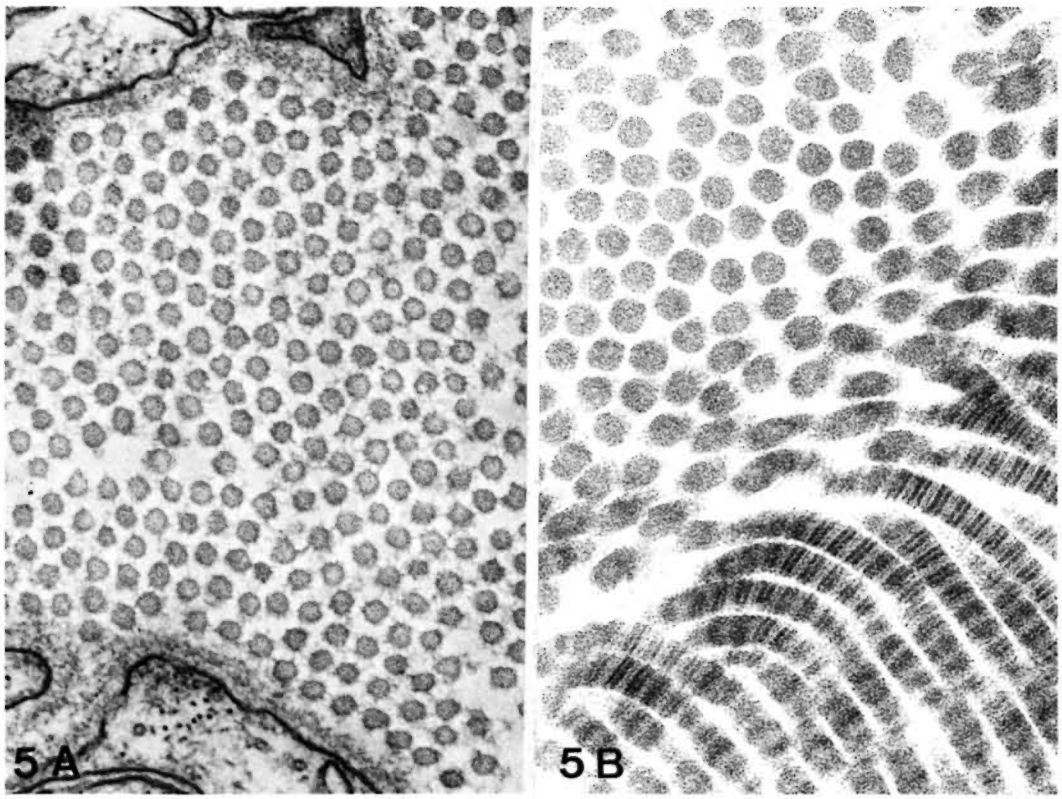

FIG. 5. Electronmicrographs of the thin collagen fibrils of the endoneurium (A) and thick collagen fibrils from the epineurium (B) at the same magnification, illustrating the presence of 2 different collagen populations in leprous nerves. (×60,000)

TABLE 1. *Average diameters (mean ± S.D.) of collagen fibrils present in the endo- and epineurium of nerves from human leprosy patients.*

Case No.	Nerve studied	Epineurium (nm)	Endoneurium (nm)	Epi-/endo-ratio
1	radial cutaneous branch	88.54 ± 11.26	47.78 ± 7.08	1.85
2	musculo-cutaneous	78.30 ± 9.83	58.62 ± 7.96	1.34
3	great auricular	68.38 ± 6.83	38.25 ± 5.29	1.79
4	great auricular	89.98 ± 10.89	57.23 ± 7.77	1.57
5	ulnar	65.80 ± 5.84	32.67 ± 4.60	2.01
6	medial plantar	66.20 ± 5.89	36.48 ± 4.34	1.81
7	radial cutaneous branch	89.08 ± 6.60	46.73 ± 4.95	1.91
8	palmar cutaneous branch	69.78 ± 6.84	36.31 ± 6.67	1.92
9	ulnar	72.52 ± 6.47	39.51 ± 5.32	1.84
10	radial cutaneous branch	89.24 ± 9.47	53.28 ± 8.83	1.67

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FIG. 3. Leprous nerve stained by Picrosirius, and observed with polarization microscopy, showing the thickened perineurium which presents several concentric layers of collagen in between the perineural cells. This is responsible for the marked increase in the width of the perineurium when compared to a normal nerve. The epineurium (right) shows characteristic, thick, bright fibers which contrast with the thin, pale fibers of the endoneurium (left). (×435)

FIG. 4. Electronmicrograph of a leprous nerve showing a marked increase in the endoneural collagen. (×7800)

TABLE 2. *Comparison between the average diameters (mean ± S.D.) of collagen fibrils from the endo- and epineurium of normal and leprous nerves.*

	Number of cases	Epineurium (nm)	Endoneurium (nm)	Epi-/endo-ratio
Normal nerves[a]	5	75.43 ± 7.83	50.01 ± 4.24	1.51
Leprous nerves[b]	10	77.78 ± 7.99	44.68 ± 6.28	1.74

[a] Average of two ulnar, two femoral, and one musculo-cutaneous nerve. Data from Junqueira, *et al.* (8)
[b] Average of Table 1.

thin and thick fibrils present in the biopsies from leprosy patients were similar to the values obtained from normal nerves (Table 2).

DISCUSSION

Our results confirm those in the literature (1,2,4,12), showing that leprosy lesions in nerves promote an increase in their collagen content. They show that despite the changes induced by this disease, the distribution of collagen types I and III in the nerve compartments remains the same as observed in normal controls.

Several lines of evidence suggest that Schwann cells are the site of collagen production in the endoneurium (7) and that the collagen produced is type III (8). This agrees with Job and Verghese (3), who suggested that Schwann cells play an important role in the production of collagen in leprous nerves. The fact that Schwann cells are the preferential site of invasion by *Mycobacterium leprae* (2,10), promoting leprous neuritis characterized by the proliferation of collagen, shown in the present paper to be type III, supports the above-mentioned contention.

The results of our study on the collagen of leprous nerves show that despite the remarkable increase in the amount of this protein, its other characteristics such as fibril diameters, localization, and types of collagen present show no difference when compared to normal nerves.

SUMMARY

The collagen of nerve biopsies from ten leprosy patients was studied by the Picrosirius-polarization method and electron microscopy. It was observed that leprosy promotes a marked increase in nerve collagen content. Despite the changes induced by this disease, the localization of collagen types I and III in the endo-, peri-, and epineurium remains the same as in normal nerves.

RESUMEN

Se estudió el colágeno de los nervios obtenidos de diez pacientes leprosos utilizando el método de Picrosirius-polarización, y la microscopía electrónica. Pudo comprobarse que la lepra produce un notable incremento del contenido de colágeno en el nervio. A pesar de los cambios provocados por esta enfermedad, la localización del colágeno de los tipos I y III en el endo-, peri-, y epineuro, se mantiene igual a la de los nervios normales.

RÉSUMÉ

Le collagène a été etudié dans des biopsies nerveuses de dix cas de lèpre par la méthode du Picrosirius-polarisation et la microscopie électronique. On a observé que la lèpre produit une augmentation notable de la quantité de collagène dans le nerf. Malgré les changements produits par cette maladie, la localisation du collagène des types I et III dans l'endo-, péri-, et epinèvre reste semblable à celle des nerfs normaux.

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