Structure and composition of the outer connective tissue sheaths of peripheral nerve

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ABSTRACT

A regular pattern of parallel reflections with a periodicity of ~ 39 μm has been detected on peripheral nerve fascicles. The reflections have been found to originate from a layer of wavy epineurial collagen fibrils arranged in register around the entire circumference of the fascicle. The waves were observed to be disposed in parallel with the plane of the fascicular sheath and along its axis. The pattern was observed in cut or relaxed fascicles in situ as well as in isolated and split layers of the epineurium. The pattern was not observed on nerve fascicles under tension. An additional structural feature consisting of longitudinally disposed elastic fibres was also detected among the epineurial collagen fibrils. The perineurial collagen associated with sheets of cells was found to be disposed in flattened waves as in the inner layer of the epineurium. The period of the waves, however, was much shorter, in the range of 6–9 μm. From the nature of the wavy structure it can be surmised that on stretching or contraction the sheath length may change, thus accommodating displacement and movement of nerve fibres.

Key words: Tamarin monkey; rat; human epineurium; perineurium; collagen fibrils; elastic fibres.

INTRODUCTION

The outer epineurial connective tissue layer which surrounds peripheral nerve fascicles has been investigated rather infrequently (Thomas, 1963; Sunderland, 1965; Ushiki & Ide, 1990). In contrast, the perineurial layer which forms an effective diffusion barrier around the endoneurial space has received considerably more attention (see, e.g., Low, 1976; Oldfors, 1980; Thomas et al. 1993).

The connective tissue layers surrounding the nerve fibres were correctly described by Ranvier (1878) who identified 'tissu conjonctif périfasciculaire' (perifascicular connective tissue) and the 'gaine lamelleuse' (lamellar sheath) corresponding respectively to the epineurium and perineurium, which currently adopted terms were originally proposed by Key & Retzius in 1873.

Up to the present it has been generally assumed that the epineurium consists of oblique and longitudinally arranged collagen fibrils and elastic fibres (Thomas, 1963; Gamble & Eames, 1964) with additional areolar connective tissue and vascular components (Sunder-

land, 1965). Thin collagen layers separating the perineurial cell laminae were assumed to be arranged in a double spiral system enveloping the entire nerve fascicle (Glees, 1943), or to be mainly longitudinal (Thomas, 1963), in all directions (Gamble & Eames, 1964) or in a circular and oblique manner (Sunderland, 1965). More recently Ushiki & Ide (1990) investigated the arrangement of the collagen fibrils in the entire peripheral nerve fascicle using thin section transmission and scanning electron microscopy. Following the digestion of all cellular components, dispersed wavy bundles of collagen, arranged obliquely to the axis of the fascicle, were observed in the epineurial regions. Perineurial collagen was presented as a sheet of fibrils forming an undulating lacework structure.

The appearances of the peripheral nerve sheaths presented here confirm previous reports (Sunderland, 1965) that there are 2 distinct layers within the epineurium: an outer one consisting of areolar connective tissue with vascular components, and an inner one consisting of collagen fibrils and elastic fibres.
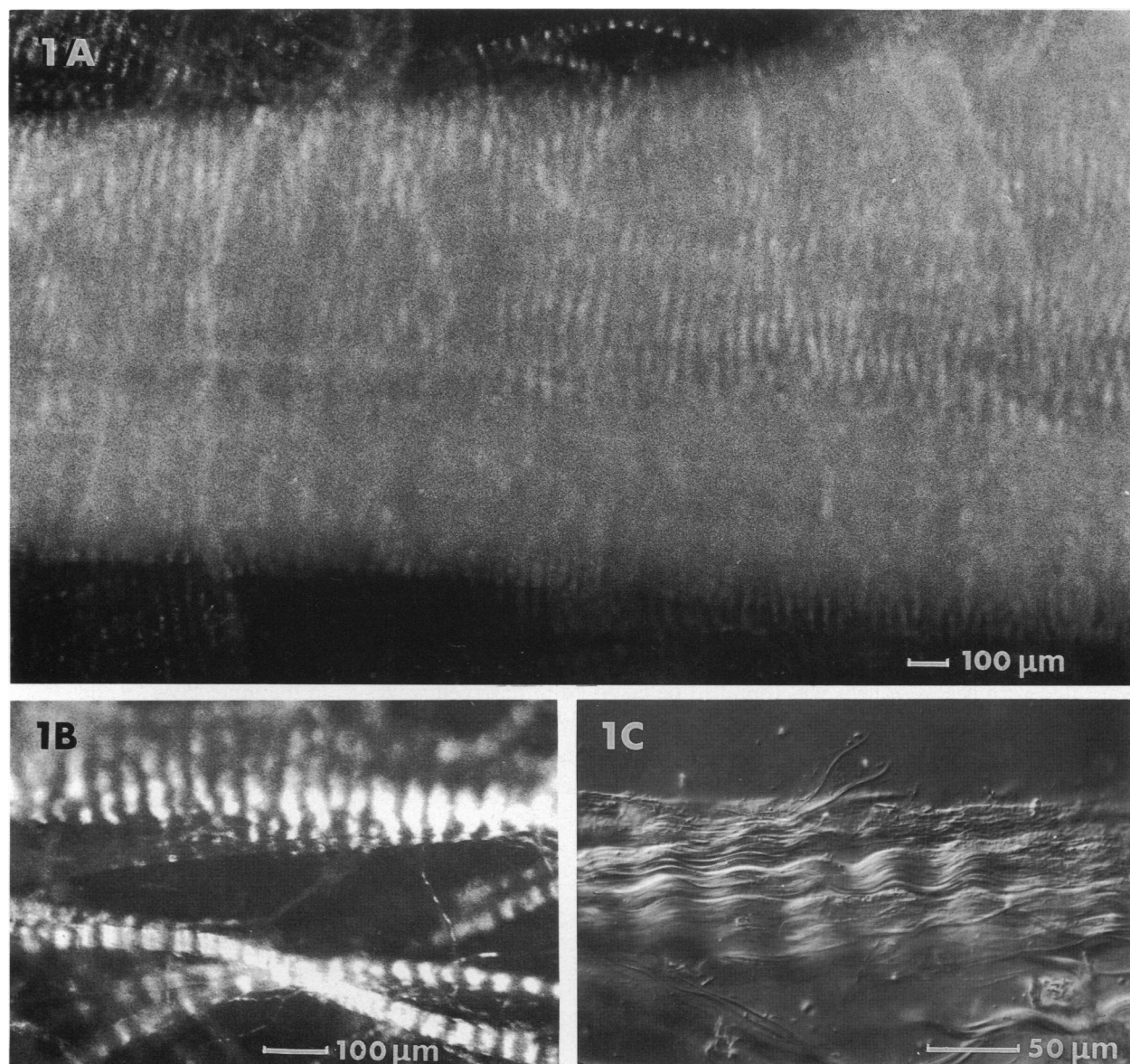
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Fig. 1. (*A*) Fixed and flattened outer sheath of human sciatic nerve suspended in saline and illuminated along the fascicular axis. Parallel reflections, spaced at 39 μm and disposed at 90° to the nerve axis can be observed. × 58. (*B*) Enlarged fragment of the sheath showing strong regular reflections. × 93. (*C*) Wavy aspect of the sheath revealed by Nomarski optics. × 280.

The present study proposes that the dense collagenous layer forming the inner part of the epineurium, when not under tension, is arranged in a regular wavy pattern which surrounds the entire fascicle. It is also proposed that the collagenous waves which are disposed in register around the fascicle are arranged along and in parallel with the plane of the sheath. The 3-dimensional disposition of the perineurial collagen fibrils is similarly arranged in planar wavy layers, which however have a much shorter period when compared with that of the inner part of the epineurial sheath.

These results have partly appeared in abstract form (Stolinski, 1994).

MATERIALS AND METHODS

Human sciatic nerves were dissected from embalmed cadavers (previous consent was obtained in each case). Brachial nerves were similarly removed from an anaesthetised (Saffan 1.5 ml/kg) and subsequently killed Tamarin monkey (*Saguinus labiatus*). Sciatic nerves were also removed from freshly killed or perfused adult Wistar rats. Tissues were fixed in formol calcium for light microscopy and half strength Karnovsky's fluid for scanning electron microscopy. Unfixed rat sciatic nerve and minor branches were also photographed in situ.

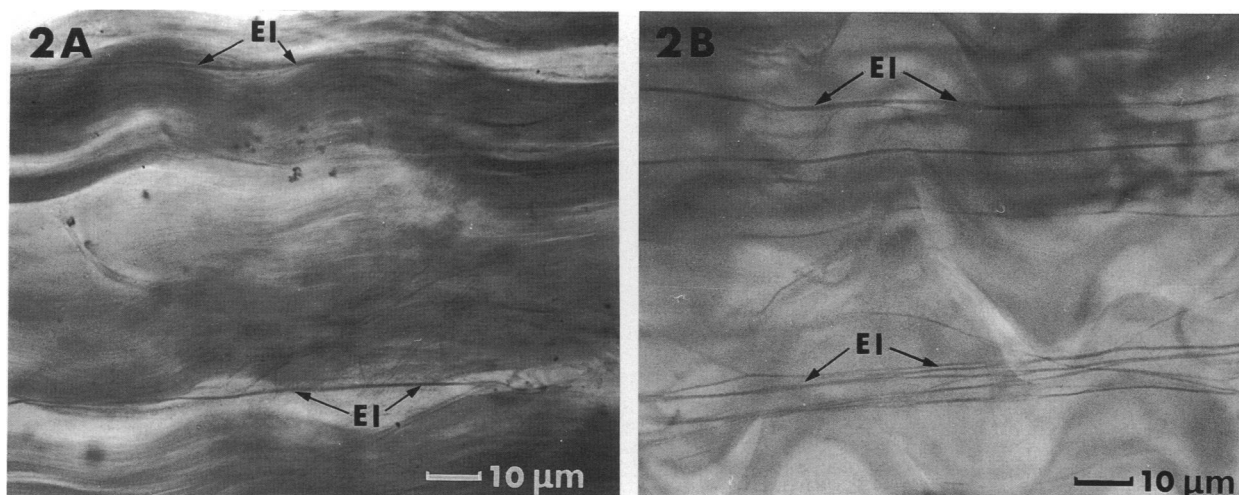The fixed outer sheath of the nerve fascicle was

Fig. 2. Human and rat elastic fibres (El) viewed against the wavy background of collagen fibrils. Miller's stain. × 720.
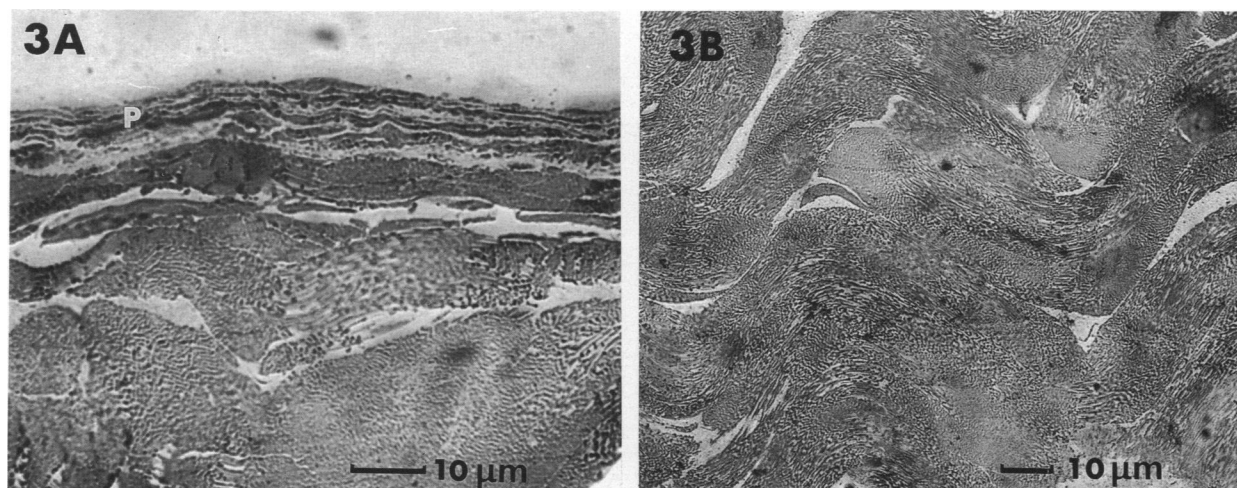

Fig. 3. Isolated Tamarin monkey nerve sheath, sectioned transversely and tangentially to the fascicular axis. (*A*) Shows the flattened bundles of collagen fibrils. P, perineurium. × 590. (*B*) Shows the wavy arrangement of the collagen bundles. × 1000.

dissected under the binocular microscope. After cutting the fascicle longitudinally into two halves the nerve fibres were removed leaving the isolated outer sheath, comprising both epineurium and perineurium. This was placed on a glass slide in a drop of phosphate buffered saline and was flattened with a coverslip. In some cases, the outermost sheet of areolar tissue was removed. The flattened sheath was illuminated at approximately 30° to the plane of the sample and the reflected image was viewed against a black background using a × 4 objective. The isolated sheath was also stained with Miller's solution, dehydrated and mounted in DPX in order to detect elastic fibres.

The periodicity of the reflections was measured in 6 regions originating from 4 fascicles. Measurements were made on fixed intact and isolated sheaths of fascicles from the human sciatic nerve.

For conventional light microscopy, flattened isolated layers of the outer sheath were embedded in acrylic resin. Sections (1 µm) were cut parallel to and across the sheath, both along the axis of the fascicle, and were subsequently stained with silver methenamine.

For scanning electron microscopy, fixed tissues were dehydrated in graded concentrations of acetone up to pure acetone and critical point dried. The superficial layer in some samples was removed from dried specimens using double-sided adhesive tape. Specimens were subsequently sputter coated with gold.

RESULTS

A regular parallel wavy pattern of circumferentially arranged reflections originating from the outer sheath of the nerve fascicles was observed in a reflecting mode. The pattern was observed when an inclined
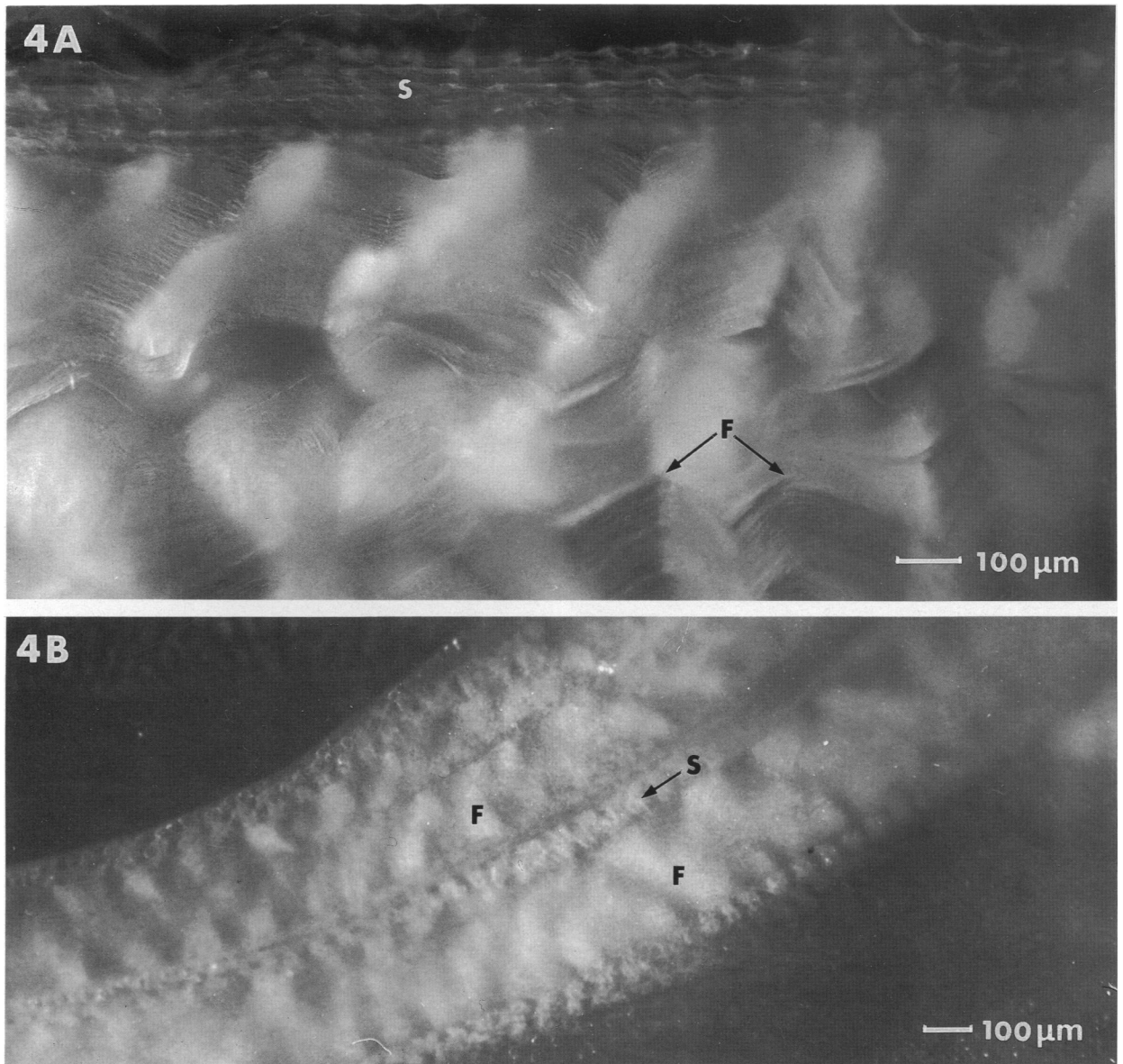
Fig. 4. (*A*) Fixed Tamarin monkey brachial nerve cut longitudinally along the fascicular axis and viewed in reflected light. Nerve fibres show the characteristic zigzag bands of Fontana (F). The cross cut outer sheath (S) shows a laminar arrangement with periodic reflections. × 94. (*B*) Unfixed rat nerves in situ. Two minor branches of the sciatic nerve are observed with reflected light. Zigzag bands of Fontana (F) and reflections from the epineurium (S) can be observed. × 70.

parallel beam of light was directed along the axis of the fascicle (Fig. 1*A*). Figure 1*B* shows the same pattern at higher magnification as viewed on an isolated strip of the sample. Figure 1*C* shows a similar strip as viewed using Nomarski optics. The regular periodicity of the wavy pattern of the fixed human tissue was estimated to be in the range of 37–41 μm.

Elastic fibres in the inner layer of the human and rat epineurium were visualised in a spread preparation by staining the tissue with Miller's method. The fibres (Fig. 2) were observed to be arranged longitudinally along the axis of the fascicle with occasional 'Y' links between the strands.

Silver methenamine-stained sections prepared from the isolated and flattened inner layer were cut tangentially and across both along the axis of the fascicle. Parallel layers of fibrillar bundles were observed external to the perineurium in a section cut at right angles to the plane of the sheath (Fig. 3*A*). The same tissue cut tangentially to the plane of the sheath revealed collagen fibrils arranged in sinusoidally shaped waves (Fig. 3*B*).

Bands of Fontana as reflected from a zigzag configuration were observed when a segment of the fascicle was cut longitudinally (Fig. 4*A*). The interior of the fascicle revealed the extent of the zigzags which
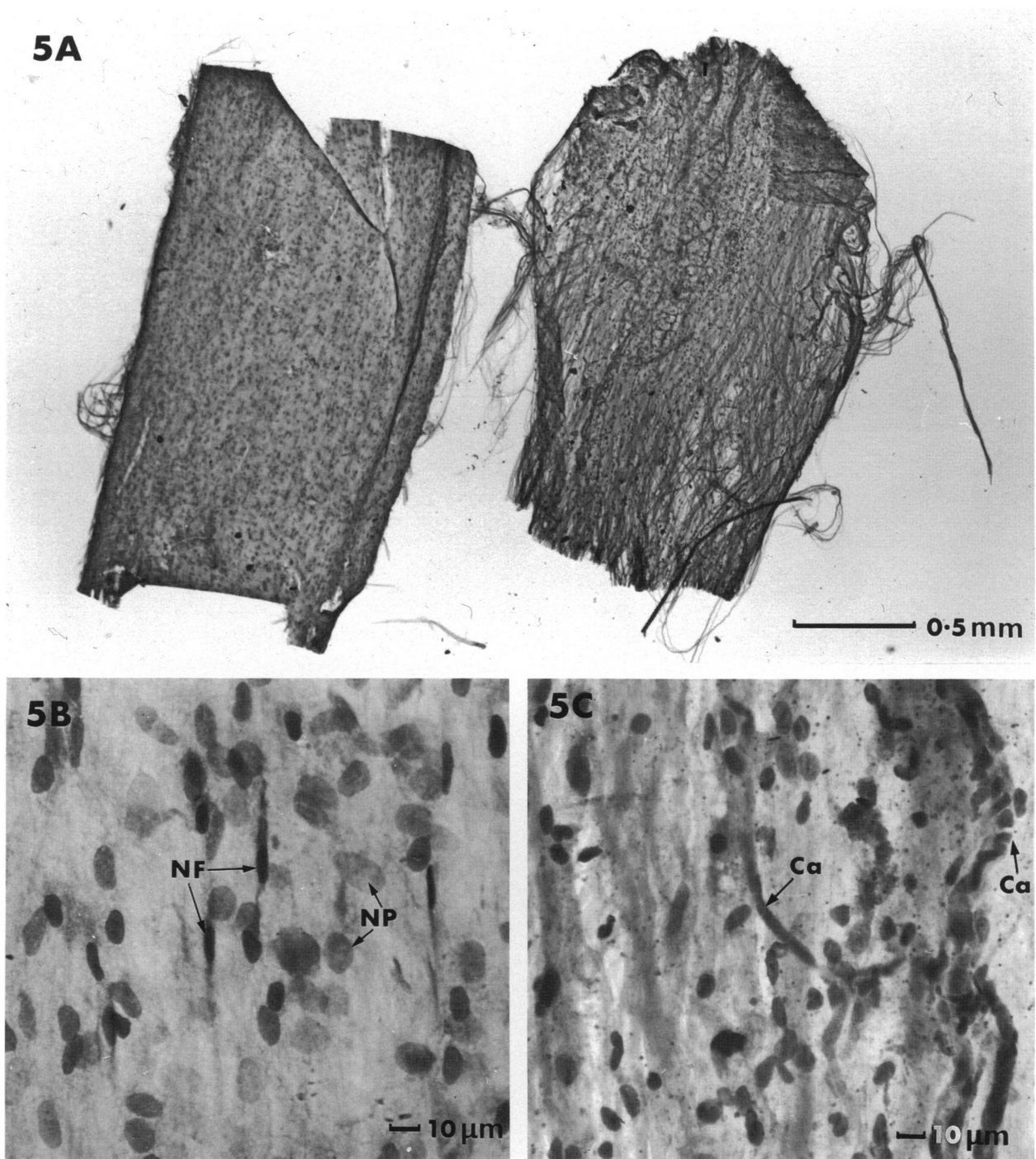
Fig. 5. (*A*) Spread preparation of isolated and split outer nerve sheath from Tamarin monkey brachial nerve showing the inner (left) and outer (right) fascicular layers. × 39. (*B*) Enlarged fragment of the inner layer showing large oval nuclei of perineurial origin (NP) and elongated nuclei of fibroblasts (NF) arranged along the fascicular axis. × 450. (*C*) Enlarged fragment of outer layer showing typical constituents of areolar connective tissue. Ca, capillaries. × 450. Spread preparation stained with Azure II and mounted with DPX.

on average had a 170 μm periodicity. Compared with the rectilinear length of the nerve fibres it was estimated that they were approximately 17% longer in the zigzag mode.

Unfixed rat sciatic nerve was photographed in situ (Fig. 4*B*). Bands of Fontana were readily observed in the reflected mode. Reflections originating from the epineurium were also observed.

Figure 5*A* shows an isolated epineurial sheath of the monofascicular Tamarin monkey brachial nerve which was split into its two constituent layers. The inner layer (left of Fig. 5*A*, *B*) shows large oval nuclei
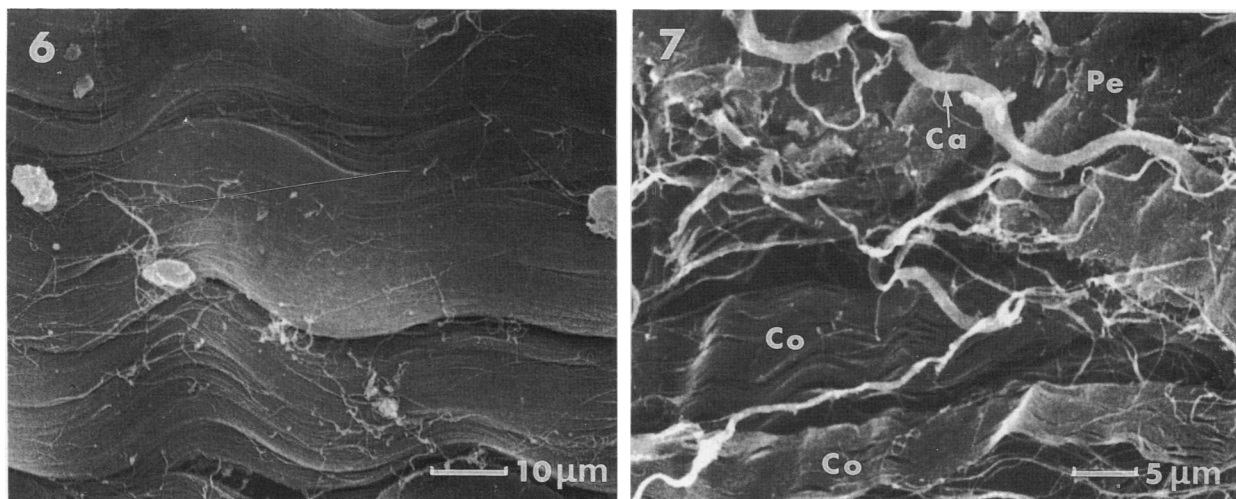
Fig. 6. Scanning electron micrograph of human sciatic nerve sheath showing wavy collagen fibrils with a 35 μm period originating from the inner epineurial layer. × 1020.

Fig. 7. Scanning electron micrograph of rat sciatic nerve sheath showing a layer of perineurial cells (Pe) and the underlying wavy collagen layer (Co) with a period of approximately 6 μm. Ca, capillary. × 1800.

and elongated nuclei arranged along the axis of the fascicle. The outer layer (right of Fig. 5A, C) shows the characteristic features of areolar tissue with a well developed vascular network. This spread preparation was stained with Azure II and mounted with DPX, hence the collagen is not visualised on the micrograph. The different structural features of the two layers at higher magnification are shown in Figure 5A, C.

A regular wavy disposition of collagen fibrils of 60–80 nm diameter arranged in register is shown in Figure 6. This scanning electron micrograph of the human inner epineurial layer shows a period of 36 μm and an amplitude of approximately 4 μm. Figure 7 portrays the perineurial cell layer with an associated capillary. The thin sheet of collagen associated with this layer shows a regular wavy pattern of 60 nm diameter fibrils with a period of approximately 6 μm.

DISCUSSION

The present study confirms the previous report of Sunderland (1965) who described two distinct layers within the epineurial sheath. In peripheral nerves of the monofascicular type, the outer epifascicular epineurium consists of the local vascular network within areolar connective tissue components, disposed among randomly and partly longitudinally arranged bundles of collagen fibrils (Fig. 5A, C). Underlying this tissue, loosely attached, is a dense collagenous

layer which in reflected light shows a regular pattern, indicating the presence of wavy collagen fibrils (Figs 1, 6). The arrangement of the collagen fibrils in this layer was deduced from tangential and transverse sections of the sheath which were cut along the axis of the fascicle (Fig. 3). The tangentially cut sheath displayed a regular wavy pattern while the cross-sections shows flat layers of collagen cut across and disposed in parallel to the plane of the sheath. These two images indicate that the disposition of waves is arranged in parallel to the plane and along the axis of the fascicle. The regular pattern of reflections (Fig. 1) also indicates that the collagenous waves are disposed in register around the entire surface and within the fascicle. This last observation contradicts the model of epineurial collagen arrangement as suggested by Ushiki & Ide (1990) where bundles of fibrils are shown to be generally disorganised.

Interspaced between the dense wavy layers of collagen, straight longitudinally arranged elastic fibres of 0.25–0.5 μm diameter were observed (Thomas, 1965; Ushiki & Ide, 1990) (Fig. 2). Their probable functions are to retain the sheath under tension and to maintain its overall elasticity. The epineurial layer was found to be closely associated with the underlying perineurial layer from which it could not be separated mechanically. Collagen associated with perineurium shows a similar wavy appearance as in the inner epineurial layer. However, the period of the waves was found to be only 6 μm as compared with 39 μm for the epineurial layer. This could be a function of
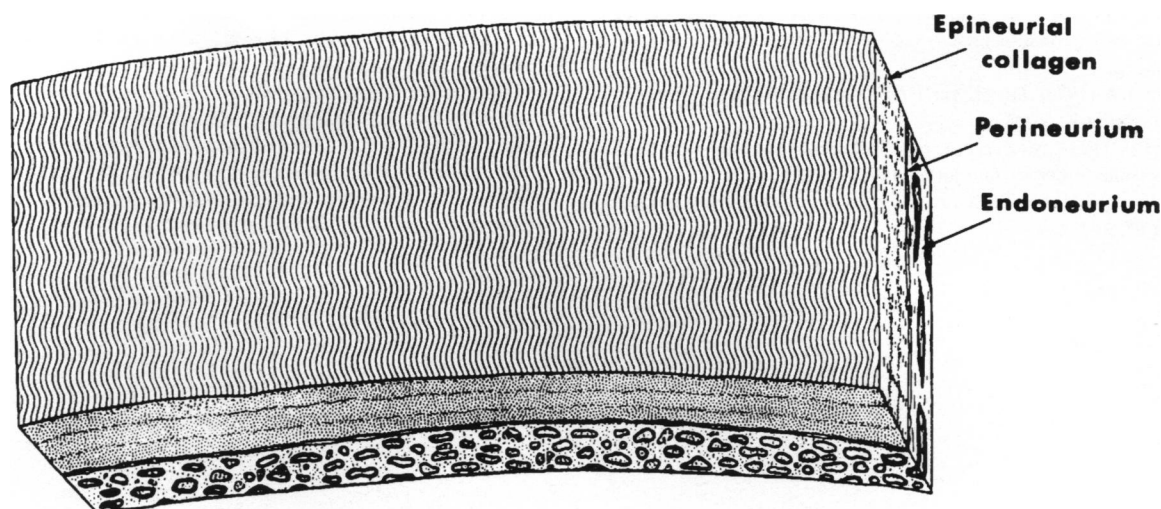
Fig. 8. Schematic representation of the outer peripheral nerve showing the wavy pattern of the epineurial collagen. The outer areolar epineurial layer is not illustrated.

fibril diameter which according to Gamble & Eames (1964) is 20 nm smaller than the epineurial fibrils which have a diameter of 80 nm.

Substantial changes of the overall length of both mono- and multifascicular peripheral nerve have to be accommodated in the vicinity of the large synovial joints of the limbs. Stretching the nerve fibres, which are normally arranged in a zigzag pattern (Clarke & Bearn, 1972), theoretically allows for an increase in length of about 20%. This amount of elongation, according to the experiments conducted by Haftek (1970), should not result in overstretching and consequent damage to the fibres. It is unlikely that such a spare capacity to accommodate stretching would not be matched by a similar elongation of the fascicular sheath. The epineurium, due to its wavy collagen arrangement, should also accommodate stretching on elongation without tensioning the collagen fibrils. It is significant that stretching of the sheath could not be attained if the collagen fibrils, which are regarded as not capable of extension, were arranged simply in straight parallel bundles along the axis of the fascicle. The outer epineurial connective tissue layer which closely resembles areolar tissue can also accommodate stretching and can easily deform as is commonly observed in the vicinity of other tissues or organs.

The isolated sheath and cut nerves which are not under tension exhibit very strong striated reflections which were shown in Figure 1A and B. In situ, the striation can also be observed but only on some fascicles (Fig. 4B) while others show a smooth appearance. This suggests that some fascicles are under tension, while those that are not exhibit the

characteristic striations. The possibility also has to be considered that due to postmortem effects, longer nerves can undergo stretching and remain under considerable tension, resulting in absence of the pattern.

Finally, the general conclusion can be made that the wavy organisation of the epineurial sheath (Fig. 8) forms a very efficient dynamic structure which effectively protects the nerve fibres on stretching and bending.

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