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An electron microscope study of the connective tissues of human peripheral nerve

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INTRODUCTION

It is well known that the endoneurial connective tissues play an important although imprecisely understood part in the processes of nerve regeneration and repair (Guth, 1956). It is also clear that the perineurium is important as a diffusion barrier between the nervous elements and the tissue fluids which surround the nerve trunk (Huxley & Stampfli, 1951; Krnjević, 1954). Recent electron microscopic studies of peripheral nerves in the rabbit (Thomas, 1963) and rat (Gamble, 1964) have confirmed the classical findings of Key & Retzius (1876) and of Ranvier (1878) although they are at variance with the findings of Causey and Barton (1959) regarding the number and probable function of the endoneurial fibroblasts.

Human peripheral nerves have not been studied extensively by electron microscopy and we are consequently grateful to Mr G. L. W. Bonney and Mr P. Chesterman, Orthopaedic Surgeons at St Mary's Hospital, for providing biopsy specimens. Such material is obviously of limited extent and may not always be normal, nor so well preserved as that obtainable from experimental animals. The results have been encouraging and are of interest.

MATERIALS AND METHODS

The material consisted of:

(1) A small branch of the medial cutaneous nerve of the forearm severed during transposition of the ulnar nerve at the elbow. There was no reason to believe that this specimen was in any way abnormal. The patient's age was 53 years.

(2) A small piece of the middle trunk of a child's brachial plexus encountered during exploration of the plexus at which the decision was made to amputate the limb. This child, aged 6 years, had birth injuries involving upper motor neurones and ascending tracts of the cord; one upper limb was anaesthetic and paralysed although electrical stimulation showed that nerve conduction was possible in both sensory and motor fibres of the peripheral nerves.

(3) A piece of the median nerve of a 68-year-old man whose arm was amputated after an earlier operation had failed to prevent the recurrence of a sarcoma; this earlier operation, performed 5 months previously, had involved the removal of some of the muscles of the forearm ordinarily supplied by the median nerve.

The pieces of tissue were fixed for 3 hr. in buffered 1% osmium tetroxide and stained with 1% phosphotungstic acid for 3 hr. during the dehydration which preceded imbedding in Araldite. Sections were cut with Porter-Blum and Huxley microtomes, mounted upon uncoated copper grids and examined with a Siemens Elmiskop I electron microscope.

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RESULTS

The medial cutaneous nerve of the forearm was the most extensively studied of the three specimens and its connective tissues are described below. The other specimens were similar in their structure and are described only in so far as they differ from the medial cutaneous nerve of the forearm.

Endoneurium

Collagen fibrils, often disposed in densely packed blocks, are abundant and form a conspicuous feature of the endoneurium. Almost all the fibrils are directed longitudinally and range from 300–650 Å. in diameter. There is little to suggest that the collagen forms two separate and concentric layers about the nervous elements so that it is not possible to recognize the sheaths of Plenk-Laidlow and of Key & Retzius which have been described in electron microscope studies of rabbit nerves (Thomas, 1963) and of rat nerves (Gamble, 1964). Microfibrils are occasionally seen among the collagen fibrils but elastin has not been recognized.

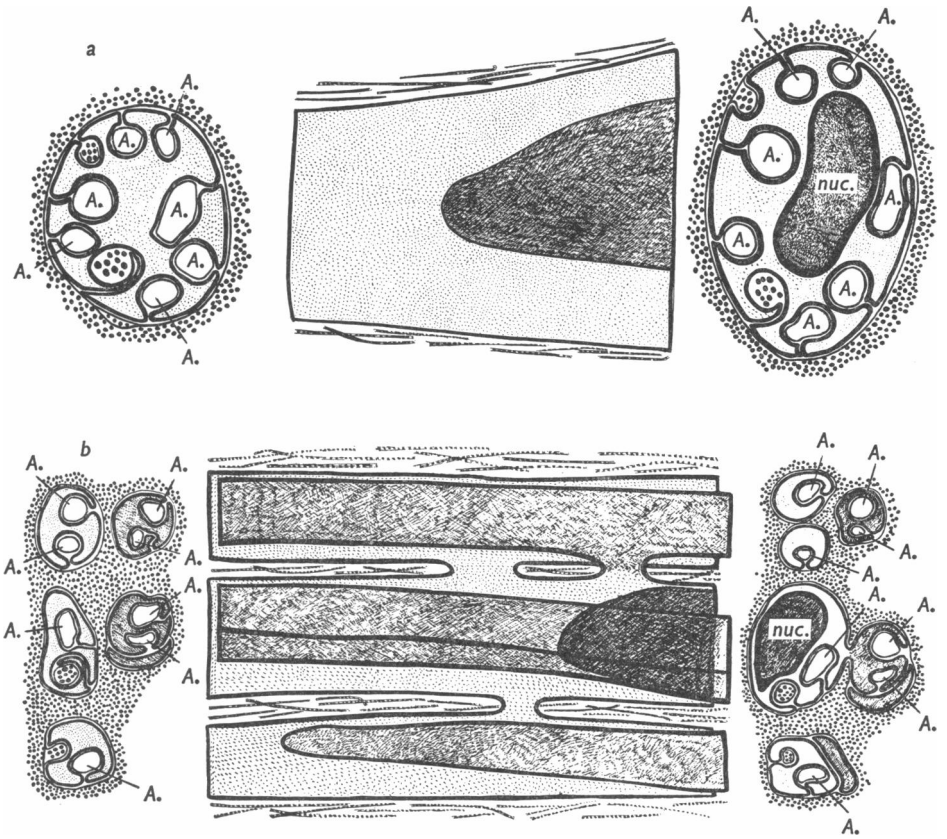
Collagen pockets of the kind described in the rat's sural nerve (Gamble, 1964) are numerous in, and form a conspicuous feature of, this human nerve. Processes of Schwann cells bearing unmyelinated axons may be indented by a bundle of collagen fibrils, or may be so deeply invaginated that a bundle of collagen fibrils, together with an encapsulating layer of basement membrane, becomes suspended from the cell surface by a double layer of its plasma membrane in a manner similar to that by which an axon is suspended by its mesaxon (Pl. 1, fig. 1). The invaginated collagen fibrils may be of any size within the range found in endoneurial collagen. Occasionally (Pl. 1, fig. 2) a tiny and unidentified cytoplasmic process shares the invagination with the collagen pocket, lying between the plasma membrane and the basement membrane which encapsulates the collagen.

Many collagen pockets are invaginated into cell processes which resemble those of Schwann cells but lack the invaginated axons by which they can be identified with certainty. These pockets, however, differ from those found in Schwann cells in that the invaginated collagen bundle is not encapsulated by basement membrane and so lies in direct contact with the plasma membrane of the invaginated cell (Pl. 1, figs. 3 and 4). Such pockets also occasionally contain tiny cytoplasmic processes as well as collagen fibrils but the nature of these processes is not known. Another variety of collagen pocket is seen (Pl. 2, fig. 5) where three small and unidentified cytoplasmic processes are enclosed by a common and unusually thick basement membrane and themselves invest a small bundle of collagen fibrils. Whatever the arrangement of these collagen pockets, the fibrils lie in an intercellular space and none has been seen lying intracellularly.

Careful inspection of bundles of unmyelinated nerve fibres shows that the larger bundles are almost always of several Schwann cell processes enclosed by a common basement membrane, each process being invaginated by one, or occasionally, two axons (Pl. 2, fig. 6). More commonly small Schwann cell processes (invaginated by single axons) are separated from their fellows by investing basement membrane and by a variable number of collagen fibrils. Examination of cross-sections through or near the nuclear regions of Schwann cells of unmyelinated axons (Pl. 2, fig. 6)

suggests that long cytoplasmic processes arise from this part of the cell and form a parallel array in the long axis of the nerve: each process is invaginated by one or two axons, and often by a pocket of collagen too, and is surrounded by a matrix of endoneurial collagen from which it is separated by its basement membrane (Text-fig. 1). The Schwann cell/unmyelinated axon complex is thus permeated by longitudinally directed collagen fibrils which must add to its tensile strength and which are, perhaps, an expression of the general abundance of endoneurial collagen in this nerve.

The other cellular elements of the endoneurium are those usually described although mast cells have not yet been identified by electron microscopy. Capillary



Text-fig. 1. Diagrams to indicate relationships between Schwann cell and unmyelinated axons (*A* represents an axon). (*a*) as found in deeply placed human nerves and in the nerves of various experimental animals (references to these are given in discussion on p. 659). Seven axons and two collagen pockets are shown to maintain essentially similar relations to a Schwann cell at two cross-sectional levels, one through the nuclear region of the cell, the other through its single, large cytoplasmic process. (*b*) as found in the medial cutaneous nerve of the forearm of man. Long cytoplasmic processes extend from the nuclear region of the Schwann cell and may themselves divide. Most of these processes are invaginated by one or two axons and sometimes by a pocket of collagen. Interstitial collagen invests many of the processes. One small process bears neither axon nor collagen pocket and ends short of its fellows.

endothelial cells and pericytes are present and are easily distinguished from fibroblasts by their investment by basement membrane. Fibroblasts characteristically lack basement membrane over all but tiny parts of their surfaces, and possess an electron dense and relatively complex cytoplasm while their processes often extend for considerable distances and may wrap around bundles of nerve fibres and their associated endoneurial collagen (Pl. 3, fig. 8). Occasionally the cytoplasm of fibroblasts is invaginated by bundles of collagen (Pl. 2, fig. 7) which lie in direct contact with the plasma membrane.

Perineurium

The perineurium exhibits a characteristic lamellar structure of flattened cell processes alternating with layers of collagen (Pl. 4, fig. 10). Most of the collagen fibrils lie in the long axis of the nerve but occasional bundles pursue oblique or circumferential courses. The individual fibrils are 400–800 Å in diameter and scattered microfibrils are present among them although no elastin has been seen.

The nuclei of the perineurial cells are very flattened and the slightly granular cytoplasm contains small, scattered mitochondria and rough walled vesicles, of rather watery appearance, up to 0·5 μ in their long axes. The plasma membranes bear scattered indentations suggestive of pinocytosis and the presence of tiny (up to *ca.* 0·1 μ diameter), smooth walled vesicles in the cytoplasm tends to support this suggestion (Pl. 3, fig. 9).

From seven to nine cellular layers are present in the perineurium, the thickness of the layers ranging from *ca.* 1 μ in the nuclear regions to 0·1 μ, or less, in the more attenuated parts. The overall thickness of the perineurium is approximately 5 μ and in some regions a surprisingly large part of this is contributed by extremely thick basement membranes which cover the cellular layers. These may be as much as 0·5 μ thick (as compared with the 250–500 Å thickness of basement membranes of endoneurial cells) and perineurial collagen may be embedded in them.

Epineurium

The epineurium consists largely of longitudinally directed collagen fibrils many of which are larger (600–1100 Å in diameter) than those found in endoneurium and perineurium. Fibroblasts are embedded in the collagen and are most frequently seen as small attenuated cytoplasmic processes. Microfibrils are occasionally seen and scattered elastin fibres often occur in close proximity to the processes of fibroblasts.

The features described above have all been seen in the medial cutaneous nerve of the forearm. In the other two specimens endoneurial collagen is much less abundant, tends to be of smaller diameter (up to *ca.* 500 Å) and is only rarely invaginated as 'pockets' in the cytoplasm of Schwann cells. Furthermore, the complexes of small Schwann cell processes singly (occasionally doubly) invaginated by unmyelinated axons, and permeated by collagen, which characterize the medial cutaneous nerve of the forearm, are rarely seen in the other specimens: here most of the unmyelinated axons occur as multiple invaginations of large Schwann cell processes.

DISCUSSION

The invagination of bundles of endoneurial collagen to form 'pockets' associated with the Schwann cells of unmyelinated nerve fibres has been described in the sural nerve of the rat. Here similarly invaginated 'pockets' are also found in other basement membrane-covered cells which lack only the axons that would have allowed their identification as Schwann cells (Gamble, 1964). The collagen fibrils in these 'pockets' have a variable relationship to the cytoplasmic membrane of the associated cell; sometimes a basement membrane capsule intervenes and wholly surrounds the fibrils but sometimes the basement membrane forms an incomplete capsule or is wholly lacking, so that the fibrils are in direct apposition with the plasma membrane.

The demonstration of numerous 'pockets' of collagen of three distinct types in the endoneurial cells of the medial cutaneous nerve of the forearm of man is an extension of the findings in the rat sural nerve. In the human nerve, 'pockets' occur in relation to three types of cell: the Schwann cells of unmyelinated nerve fibres, similar cells which possess basement membrane but which lack axons, and fibroblasts. This last relationship is seen only occasionally but is apparently identical with that described by Seong Soo Han (1961) and by Clark (1962) in the lymph nodes of rats and mice respectively where cytoplasmic processes of reticular cells (fibroblasts) often enfold bundles of collagen fibrils. Where collagen 'pockets' are invaginated into human Schwann cells, a complete capsule of basement membrane invariably intervenes between the fibrils and the plasma membrane. Where similar 'pockets' are invaginated into the other (unidentified) basement membrane-covered cells then the collagen fibrils consistently lack a capsule of basement membrane and, consequently, lie in direct contact with the plasma membrane. Incomplete capsules of basement membrane around the invaginated fibrils have never been seen in any type of collagen pocket in human nervous tissue.

The presence of collagen pockets in collagen secreting cells (fibroblasts) is not surprising and their presence in Schwann cells might be suggestive of a collagen secreting role for these cells also. This possibility has already been discussed (Gamble, 1964) in relation to the peripheral nerves of the rat where collagen pockets are considered to represent a secondary relationship between cell and fibrils comparable with and similar to that between Schwann cell and axon. It might express a tendency for Schwann cells to wrap themselves around any suitably oriented, elongated structure in their immediate neighbourhood. This view is reinforced by the observation, in human nerve, that whenever a 'pocket' of collagen invaginates an undoubted Schwann cell the fibrils are encapsulated by basement membrane which was, presumably, invaginated with them.

The present findings provide little other evidence which bears upon the suggestion that endoneurial collagen is secreted by Schwann cells. This proposition has been based mainly upon an assumed scarcity of endoneurial fibroblasts but it seems likely that Causey & Barton (1959) underestimated the fibroblast content of peripheral nerves (Thomas, 1963; Gamble, 1964) and that Nathaniel & Pease (1963*a*) overestimated the collagen content of nerve roots: their figures 1 and 2 do not support their claim that a 'good deal' of endoneurial collagen is present and it

has been shown by Gamble (1964) that nerve root collagen is very scanty. It may be that Schwann cell basement membranes are permeable to tropocollagen as Nathaniel & Pease (1963*b*) claim, at least under their experimental conditions. In normal peripheral nervous tissue, however, all endoneurial collagen lies outside the Schwann cell basement membrane and if it is assumed that the collagen precoursers have all permeated through these basement membranes it becomes difficult to assign any useful role to the fibroblasts which share the intercellular space.

The secretion of collagen into 'pockets' by enveloping cytoplasmic processes is easier to envisage where these 'pockets' occur in the other (unidentified) type of basement membrane-covered cell and in fibroblasts. In these situations the fibrils are not separated from the plasma membrane by an encapsulating layer of basement membrane. It is unfortunate that more precise identification of the basement membrane-covered cells is not possible. They are 'Schwann-like' in being covered by basement membrane (a characteristic which is equally typical of pericytes) but by the direct contact of their plasma membrane with invaginated collagen fibrils they resemble fibroblasts.

It is possible only to speculate about the function of these collagen pockets. In fibroblasts and in the Schwann-like cells they may represent the secretion of the invaginated cell. In Schwann cells they may be mere 'accidents', invaginated in place of axons which they resemble in being elongated in the axis of the nerve. Possibly they have a role in strengthening the Schwann cell/unmyelinated axon complexes with which they are associated. Their greater frequency in superficial nerves (as in the medial cutaneous nerve of the forearm of man and the sural nerve of the rat) than in more deeply placed and more protected nervous structures (such as the median nerve of man and sacral nerve roots of the rat) lends some support to this suggestion.

Additional strengthening of the Schwann cell/unmyelinated axon complexes in the human medial cutaneous nerve of the forearm might result from their permeation by collagen fibrils which has been described above. In other nerves a single Schwann cell or Schwann cell process is often invaginated by a quite large number of unmyelinated axons; up to sixteen axons in the sympathetic trunk of the cat (Elfvin, 1961; figs. 3, 5) and up to ten axons in a cutaneous nerve of the rhesus monkey (Pease & Pallie, 1959; figs. 2, 8) and in the sural nerve of the rat (Gamble, 1964; figs. 3, 5). In the human medial cutaneous nerve of the forearm single invagination of axons into Schwann cell processes is the rule and invagination by two or more axons is rare. Since the nuclei of Schwann cells bearing unmyelinated axons are not especially numerous in this nerve the condition observed must be explained in terms of a multiple branching of the Schwann cell to form an extensive, parallel array of cytoplasmic processes elongated in the axis of the nerve. Many of these processes are separately enveloped by basement membrane and surrounded by collagen fibrils so that the Schwann cell/unmyelinated axon complex is traversed from end to end by longitudinally oriented collagen fibrils. Moreover, many of the individual Schwann cell processes are invaginated by one or more pockets of collagen, and by these two methods the Schwann cell/axon complex is much reinforced with collagen.

So far as is known the medial cutaneous nerve of the forearm was 'normal' and the relative abundance of its endoneurial collagen not attributable to any known pathological condition. It may be that the endoneurium of cutaneous nerves normally contain more collagen than more deeply situated and better protected nerve trunks. Weddell (1962) has pointed out that the small cutaneous nerve bundles of middle-aged adults normally contain a proportion (1 in 100–500) of axons which exhibit the classical signs of degeneration and regeneration. Some of these, it may be supposed, have reacted or are reacting to minor local traumata (such as local bruising) and the effects upon the axons are, presumably, transient. If the axonal degeneration which occurs under these circumstances is accompanied by local collagen proliferation then that collagen might be expected to persist long after all sign of axonal damage and repair has disappeared. 'Normal' in the present context can only mean 'not known to have been, nor to be, grossly affected by lesions', and its validity can be tested only by comparison with similar specimens which become available.

In other respects the endoneurial connective tissues were as usually described (e.g. Thomas, 1963). The presence of grossly thickened patches of basement membrane upon perineurial cells is at present an isolated observation which cannot be discussed usefully until additional material becomes available for study.

SUMMARY

1. The connective tissues of human peripheral nerves have been shown to be similar to those of rabbit and rat.

2. The collagen 'pockets' recently described in the sural nerve of the rat are present in human nerves and are numerous in the medial cutaneous nerve of the forearm.

3. The Schwann cells associated with unmyelinated axons in the medial cutaneous nerve of the forearm are frequently composed of multiple cytoplasmic processes extending in parallel array from the nuclear region, each process invaginated by a single axon (occasionally by two axons) and often separated from its fellows by its own basement membrane and by collagen.

4. It is suggested that the formation of collagen 'pockets' invaginated into Schwann cells may be an expression of a tendency for these cells to engulf any suitably orientated, elongated structure in their immediate neighbourhood. This condition, like the permeation of endoneurial collagen between the parallel array of Schwann cell processes, may be a means of strengthening these structures in superficial nerves.

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We wish to thank Mr R. J. Fant for the photography.

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EXPLANATION OF PLATES

All figures are from the medial cutaneous nerve of the forearm.

PLATE 1

Fig. 1. A Schwann cell process forms a mesaxon which bifurcates (arrow) to suspend an axon (*a.*) by one limb and a collagen pocket (encapsulated by basement membrane) by the other.

Fig. 2. Several small Schwann cell processes are enclosed by a common basement membrane. Single axons (*a.*) are invaginated into two of these processes, one of which is also invaginated by a small collagen pocket (its fibrils encapsulated by basement membrane) and a small and unidentified cytoplasmic process (arrow).

Fig. 3. Several unidentified but basement membrane-covered cytoplasmic processes are invaginated by collagen pockets. One of these (*p.*) is enlarged as fig. 4.

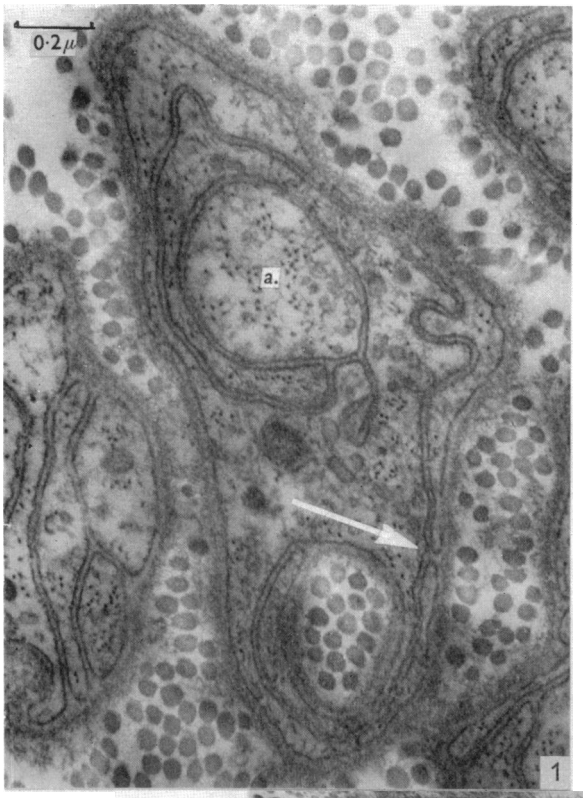
Fig. 4. One of the basement membrane-covered cytoplasmic processes shown in fig. 3 is here enlarged to show collagen fibrils of a 'pocket' in direct contact with the plasma membrane of the invaginated process. The 'pocket' also contains two tiny and unidentified cytoplasmic processes.

PLATE 2

Fig. 5. A 'pocket' of collagen fibrils lies between three cytoplasmic processes (*p.*) which are enclosed by a common basement membrane. Other collagen fibrils lie enmeshed in folds of basement membrane which lie free in the interstitial space.

Fig. 6. Four Schwann cells processes (*s.*) are each invaginated by a single axon (*a.*), and are enclosed by a single continuous basement membrane. Finger-like processes (*p.*) of Schwann cell cytoplasm project from the surface of the cell, one of them forming a shallow pocket containing collagen fibrils.

Fig. 7. The surface of a fibroblast is indented to form shallow and deep (arrow) collagen pockets.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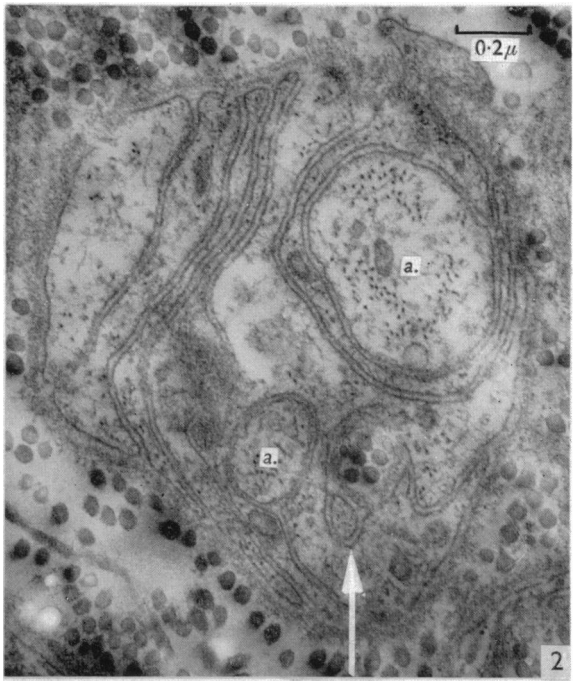
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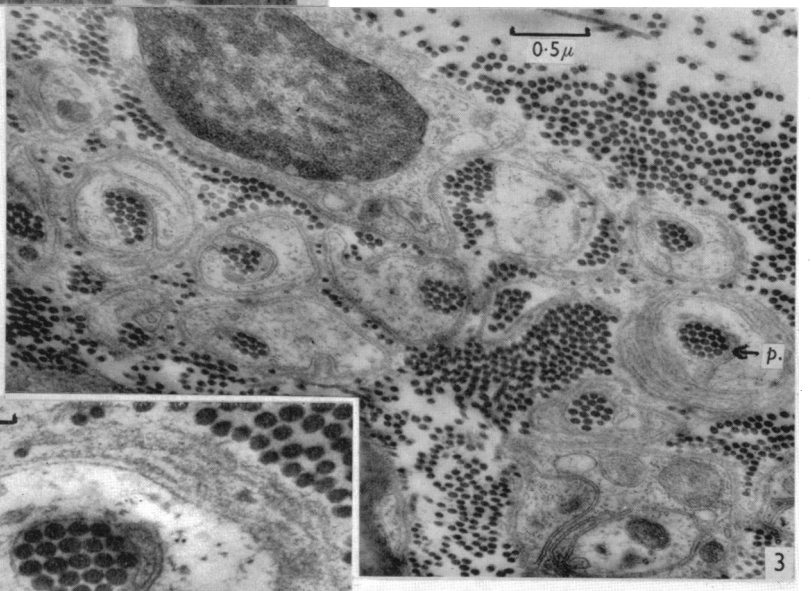
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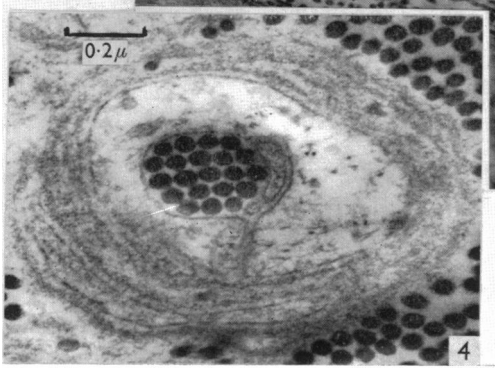
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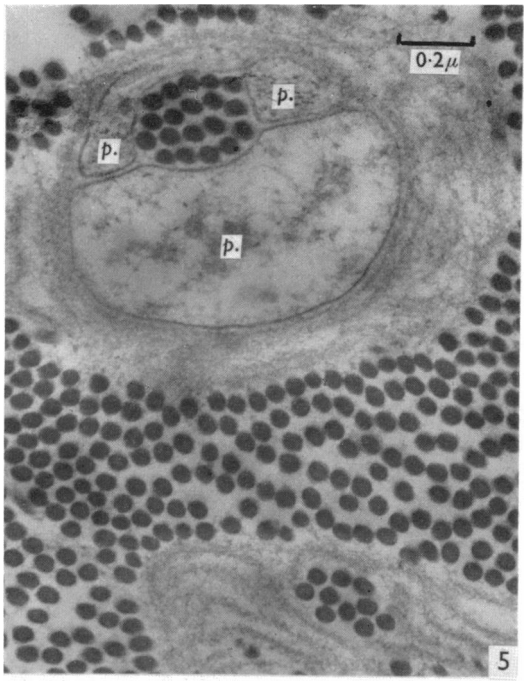
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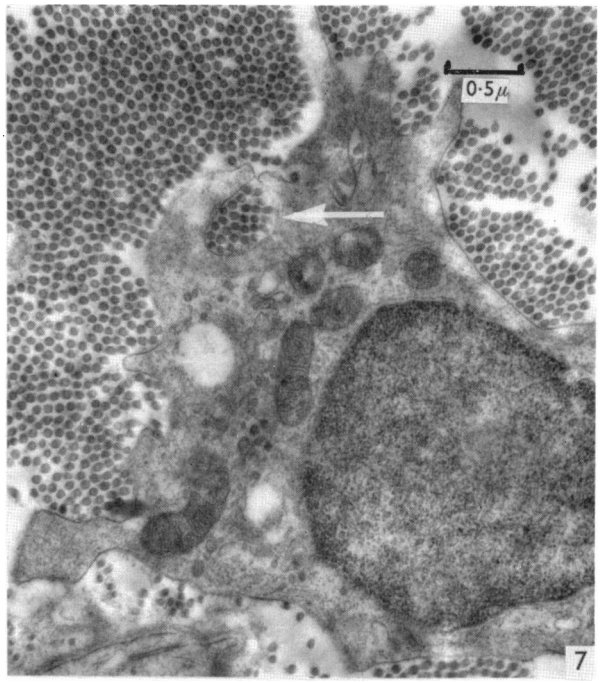
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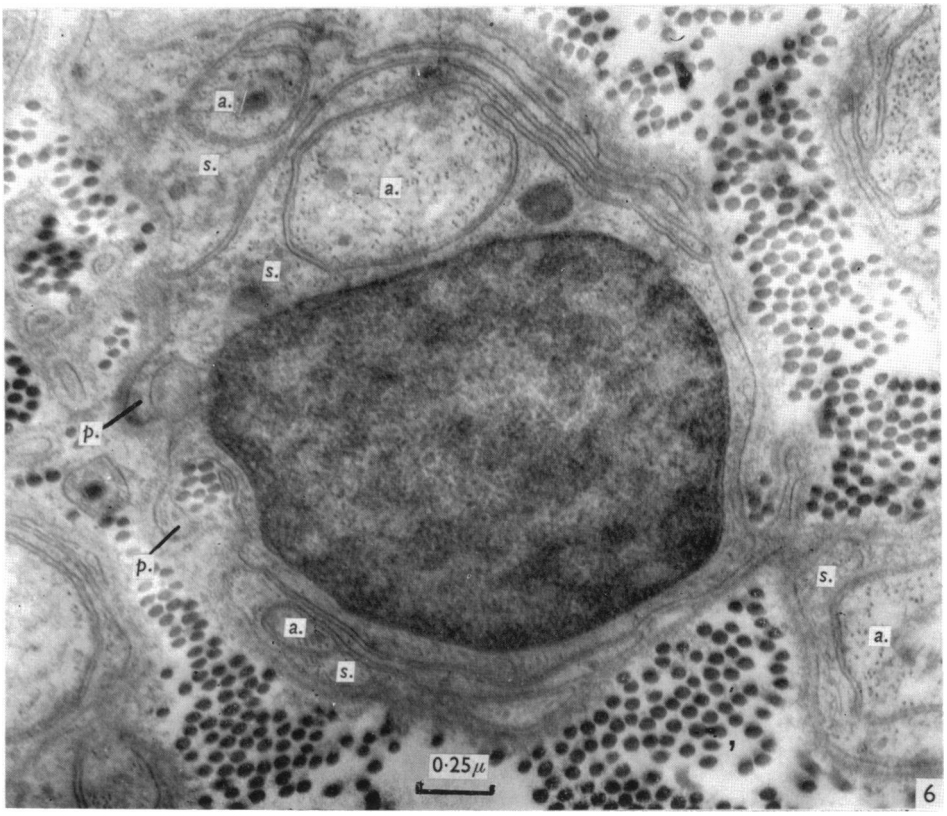
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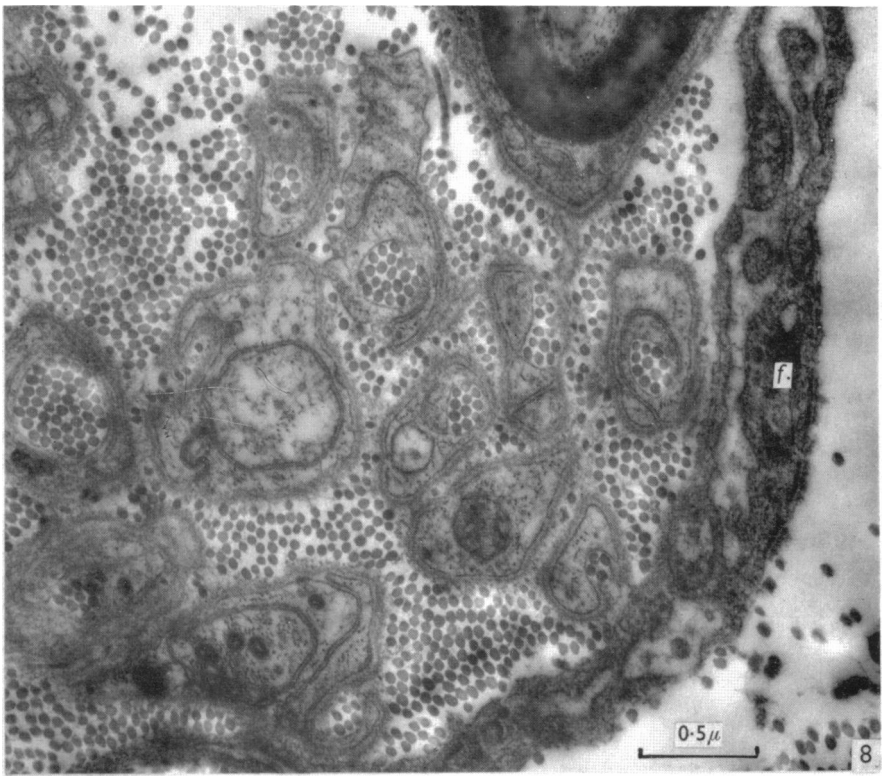
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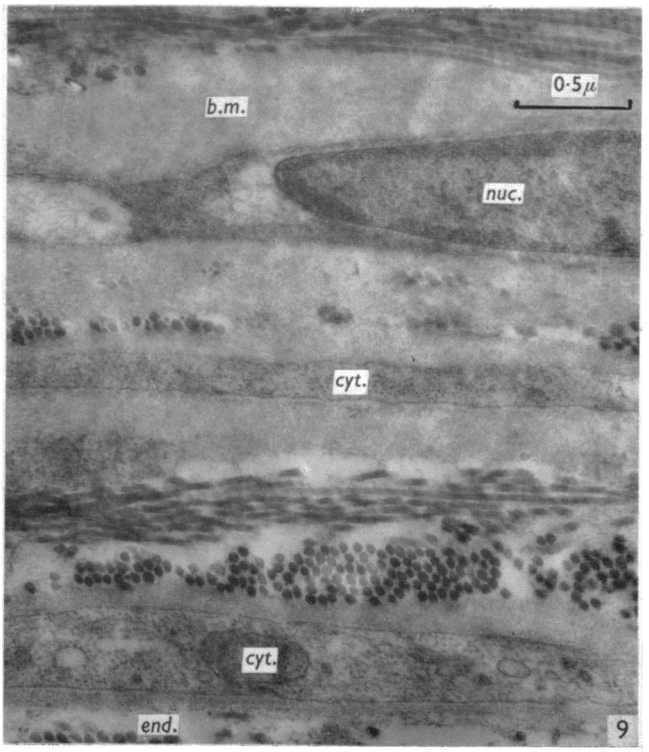
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PLATE 3

Fig. 8. An elongated process of a fibroblast (*f.*) is wrapped around endoneurial collagen, unmyelinated axons in Schwann cell processes and other cytoplasmic processes invaginated by collagen 'pockets'.

Fig. 9. The nucleus (*nuc.*) and cytoplasmic extensions (*cyt.*) of perineurial cells are shown, covered by unusually thick basement membrane (*b.m.*). The basement membrane abutting upon the endoneurium (*end.*) is of normal thickness.

PLATE 4

Fig. 10. This section passes from epineurium (*epi.*) through the whole thickness of the perineurium to the endoneurium (*end.*). Elastin (*el.*) is seen set in the thick collagen fibrils of the epineurium. Endoneurial and perineurial collagen (*col. per.*) is of rather smaller calibre. The perineurium here contains eight cytoplasmic layers (one of which is nucleated at *nuc.per.*) but a large part of its bulk is made up of unusually thick basement membranes (*b.m.*).