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An electron-microscope study of human foetal peripheral nerves

BY H. J. GAMBLE AND A. S. BREATHNACH

Departments of Anatomy, St Thomas's Hospital Medical School, S.E. 1 and St Mary's Hospital Medical School, W. 2

INTRODUCTION

The ultrastructure of adult peripheral nerve is well documented, with recent studies of the connective tissues (Thomas, 1963; Gamble, 1964; Gamble & Eames, 1964) amplifying numerous accounts of Schwann cell/axon relationships (e.g. Geren, 1954, 1956; Robertson, 1958; Causey, 1960, 1962). Geren's (1954) demonstration of the mode of myelin formation was, of course, based upon the study of chick embryo material, while the study of rat foetal nerves by Peters & Muir (1959) supports Harrison's (1924) classical concept of Schwann-cell migration from neural crest. There are, however, other features of the adult ultrastructure which might be illuminated by further study of foetal material, and an opportunity to do so, while extending the observations to human tissues, arose recently when a pregnancy was terminated by hysterectomy at the fourteenth week. Apart from its intrinsic interest, and the opportunity it provides of confirming some of the results of Peters & Muir, this material allows: (*a*) some consideration of the claim of Harvey & Burr (1926) that the perineurium, like the Schwann cells, is derived by migration from neural crest; and (*b*) investigation of the ability of Schwann cells to invaginate collagen 'pockets', which has recently been demonstrated in adult peripheral nerve fibres (Gamble, 1964; Gamble & Eames, 1964). In the work reported here comparisons are made of the ulnar nerve at the elbow with cutaneous nerves in arm, lower forearm and face.

MATERIALS AND METHODS

The foetus, aged 14 weeks (menstrual) was obtained at hysterectomy. Small blocks of tissue from the ulnar nerve (at the elbow) and from the skin of the arm, forearm, and face were fixed for 3 hr. in chilled, buffered, 1% osmium tetroxide, dehydrated in graded alcohols and embedded in Araldite. Some blocks were stained in bulk with 1% P.T.A. in 90% alcohol before embedding. Thin sections were cut on a Huxley ultra-microtome and examined with a Siemens Elmiskop I electron microscope. Sections of blocks not stained in bulk were stained with lead hydroxide on the grid. Similarly processed material obtained by biopsy of adult human forearm skin was also examined.

RESULTS

Ulnar nerve

Schwann cells or their cytoplasmic processes are readily identifiable by their investment by basement membrane and by their relationships with unmyelinated nerve fibres. Their cytoplasm is less homogeneous than in adult tissue, endoplasmic

reticulum, Golgi apparatus and vesicular structures often being conspicuous (Fig. 1). The Schwann cell/axon complexes seen in transverse section include the Schwann-cell nucleus almost as often as not, so that it may be inferred that the overall length of the cell is short in relation to the nucleus. In consequence of this, Schwann-cell nuclei are far more frequently seen than in adult nerve (Figs. 1–3).

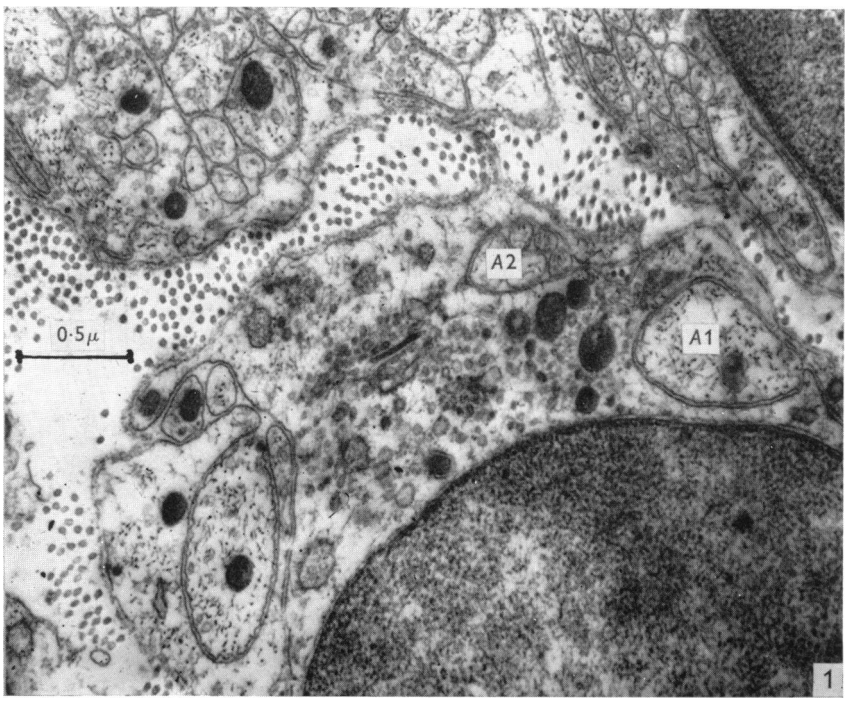

Fig. 1. Foetal ulnar nerve, showing nuclear region of Schwann cell with many cytoplasmic organelles. Axons are invaginated into the cell, singly at *A* 1 and in a group of five at *A* 2. Basement membranes cover the surfaces of this and adjacent Schwann cells. (× 30,000; 'stained' with P.T.A.)

The axons in the ulnar nerve are all alike in being unmyelinated but they vary considerably in size, ranging from *c.* 0·1 μ to a little over 1·0 μ in diameter (Fig. 2). They also vary considerably in their detailed relationship with the Schwann cells, where singly invaginated axons are seldom seen, the great majority occurring in bundles of variable number. In Fig. 2 a Schwann cell is shown invaginated at one point by a bundle of three axons and at another point by a bundle of *c.* thirty axons; many other axons are also related to this cell but the details of their relationships cannot clearly be made out.

Collagen pockets invaginated into Schwann cells have been searched for with care but none has been seen truly invaginated into the cell, although fibrils do frequently occupy shallow indentations upon the cell surface (Fig. 2). The endoneurial collagen present in the interstitial spaces is probably less abundant and certainly of smaller calibre than is found in the adult (250–450 Å diameter com-

pared with 300–650 Å diameter, Gamble & Eames, 1964). Almost all the fibrils are orientated longitudinally, and although periodicity may be seen in a few obliquely cut fibrils it has not been possible to measure it. The collagen is not organized to form recognizable sheaths of Plenk–Laidlaw and of Key and Retzius, and neither microfibrils nor elastin has been recognized in the endoneurium.

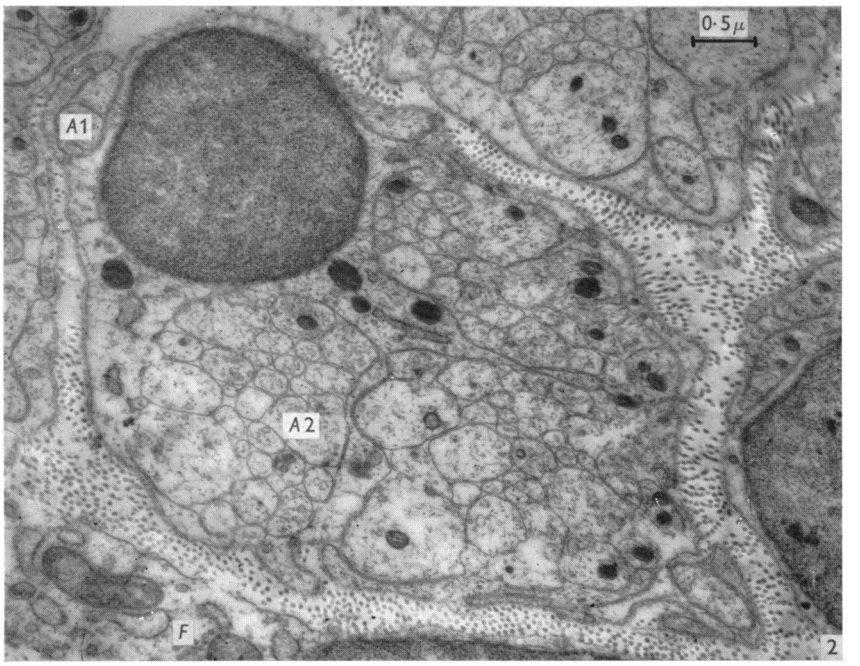

Fig. 2. Foetal ulnar nerve showing Schwann cell invaginated by three axons at *A* 1 and by *c.* 30 axons in the bundle *A* 2. Some fifty to sixty additional axons are related to this cell but their precise relationships cannot be made out. A fibroblast (*F*) lies in the endoneurium at the bottom of the picture, its cytoplasm being rich in inclusions and bounded by a cytoplasmic membrane in which defects are apparent in places; it lacks basement membrane. (× 16,000; 'stained' with P.T.A.)

Cells identified as fibroblasts are quite numerous in the endoneurium. Their identification is based upon the following: (1) they lack investing basement membrane; (2) they have no intimate relationship with axons; (3) they often contain large cisternae (as well as extensive endoplasmic reticulum); and (4) their plasma membranes may be defective over short distances (Figs. 2, 3). In these last two characteristics they resemble the migrating and synthesizing fibroblasts illustrated by Chapman (1962).

Endoneurial mast cells have not been seen and blood vessels are of normal structure except that endoplasmic reticulum is unusually conspicuous in both endothelial cells and pericytes. The ulnar nerve is covered by a thick membrane (*c.* 15 μ) formed by cells alternating with collagen. By its position at least, this membrane corresponds with the perineurium of adult nerve, but it differs from the adult in both the nature and the arrangement of its constituent cells.

Foetal perineurial cells lack investing basement membrane, contain an extensive endoplasmic reticulum together with other cytoplasmic organelles and exhibit frequent breaks in their plasma membranes. They thus resemble the fibroblasts of the endoneurium (Fig. 4). They further resemble fibroblasts in being wholly separate from their fellows; they no not appear to make contact even at their edges but form layers by overlapping rather than by joining to form sheets. Besides the larger, usually nucleated parts of the cell many attenuated cytoplasmic processes traverse the interstitial spaces so that an accurate count of the cellular layers of the perineurium is difficult; there seem to be, however, parts of at least seven cells in the thickness of perineurium at any point.

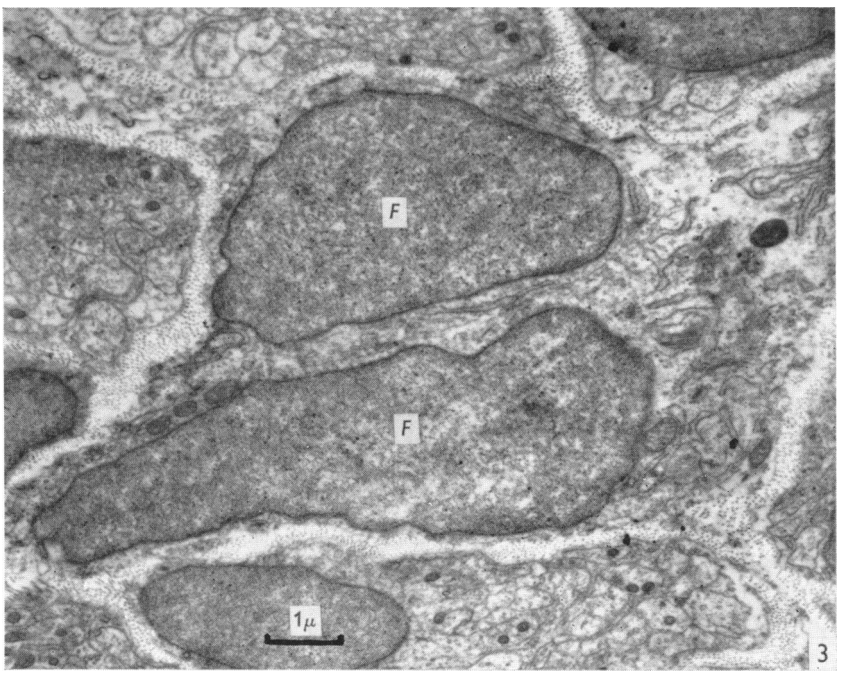

Fig. 3. Foetal ulnar nerve at low magnification showing two fibroblasts (*F*) lying between Schwann cells in three of which nuclei are in the plane of section. (×10,000; 'stained' with P.T.A.)

Perineurial collagen is of similar size (250–450 Å diameter) to that of the endoneurium and, like that collagen, is almost wholly of longitudinal orientation. Fine filamentous material (possibly microfibrils) occurs occasionally in the interstitial spaces of the perineurium, most commonly in association with breaks in the plasma membrane of the perineurial cells. Perineurial elastin fibres have not been seen. Nothing corresponding with the epineurium of adult peripheral nerve has been identified. The outermost cellular elements of the perineurium are covered by a thin layer of collagen but this does not differ in calibre, orientation or other character from the perineurial collagen.

Cutaneous nerve bundles

The Schwann cell/axon complexes of the small cutaneous nerve bundles are usually of very similar appearance to those seen in the ulnar nerve but sometimes (as in Fig. 5) the complex is formed by two undoubtedly separate Schwann cells which, from opposite sides, enwrap the associated axons. The axons of such a complex may number as many as 150, ranging in size from 0·15 to 2·0 μ diameter, and identing (not invaginating) the Schwann cells.

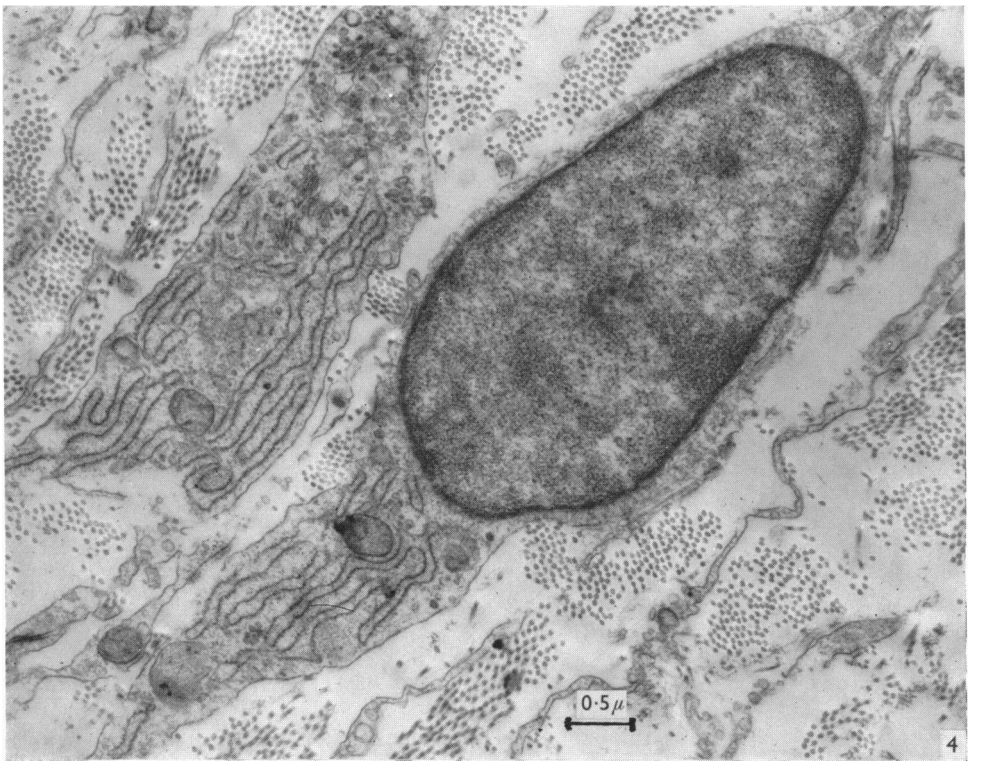

Fig. 4. Foetal ulnar nerve showing fibroblast-like cells and their attenuated processes which, with interstitial collagen, form the perineurium. The cytoplasm of these cells is rich in endoplasmic reticulum and other inclusions and is bounded by plasma membrane in which defects occur in places. The cells are not invested by basement membrane. ($\times$ 18,000; 'stained' with P.T.A.)

Neither the basement membrane of the Schwann cells nor the endoneurial collagen has been very clearly seen in small cutaneous nerve bundles, although they are present. Fibroblasts, blood vessels and other endoneurial components have not been seen in these tiny cutaneous nerves, which usually consist of a single Schwann cell with its associated axons wrapped around by a single layer (even a single cell) of perineurium (Fig. 6). The perineurial cells, like those of the ulnar nerve, resemble fibroblasts. Sometimes a Schwann cell/axon complex lies apparently free

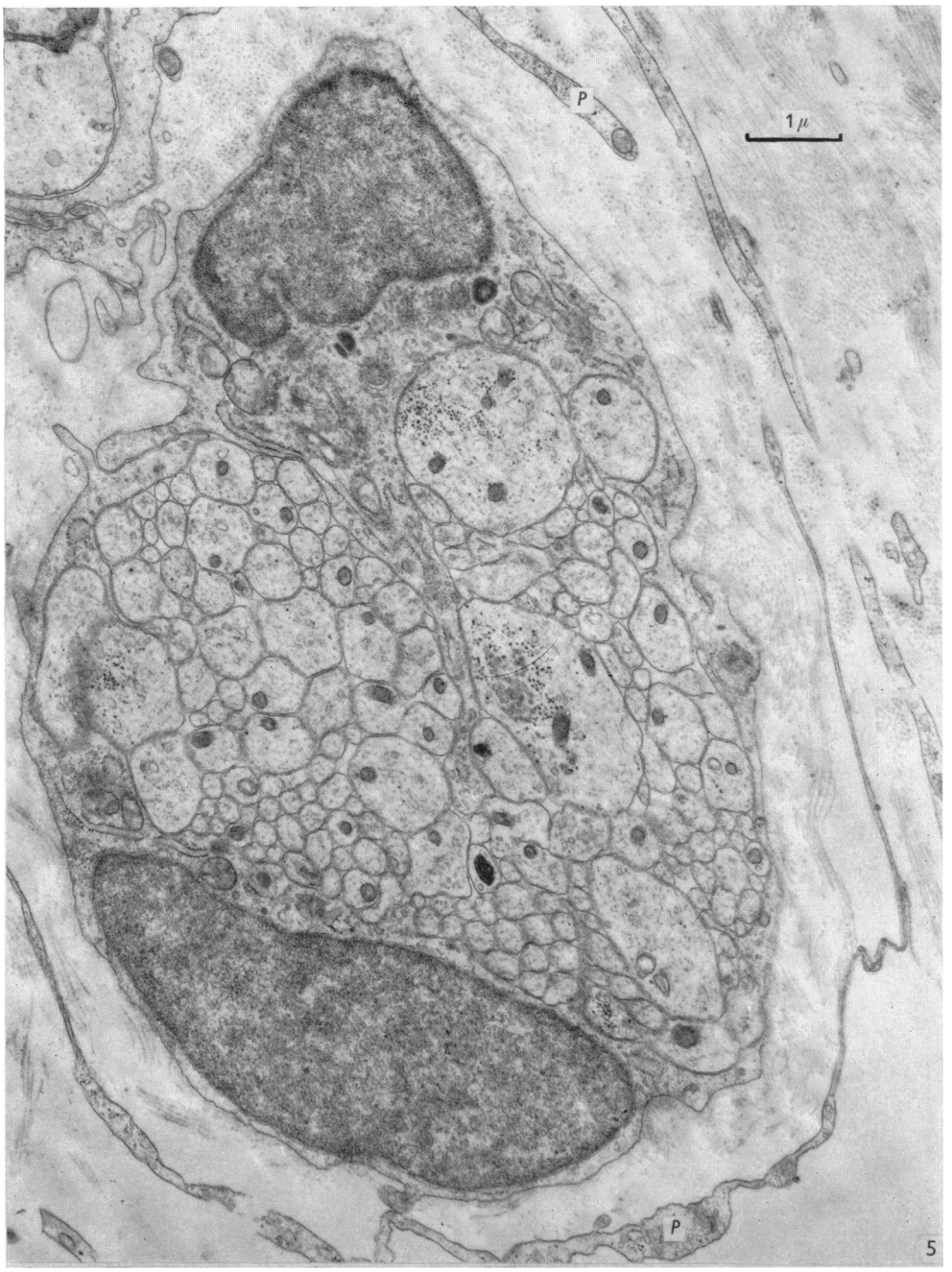

Fig. 5. Foetal cutaneous nerve, showing two Schwann cells which are together associated with *c.* 150–180 axons. Schwann-cell cytoplasm is rich in endoplasmic reticulum and other inclusions and the outer aspect of the cells is covered by an ill-defined basement membrane. Where processes of the two cells meet, the contact is simple without apparent specialization of the plasma membrane. Attenuated cytoplasmic processes (*P*), uncovered by basement membrane, loosely surround the Schwann cell/axon complex, being separated from it by a little endoneurial collagen. (× 13,000; 'stained' with lead hydroxide.)

in the dermal collagen, close to but never in contact with the basement membrane of the epidermis: more frequently such complexes lie in close association with small cutaneous blood vessels (Fig. 7) and are, presumably, concerned with their innervation.

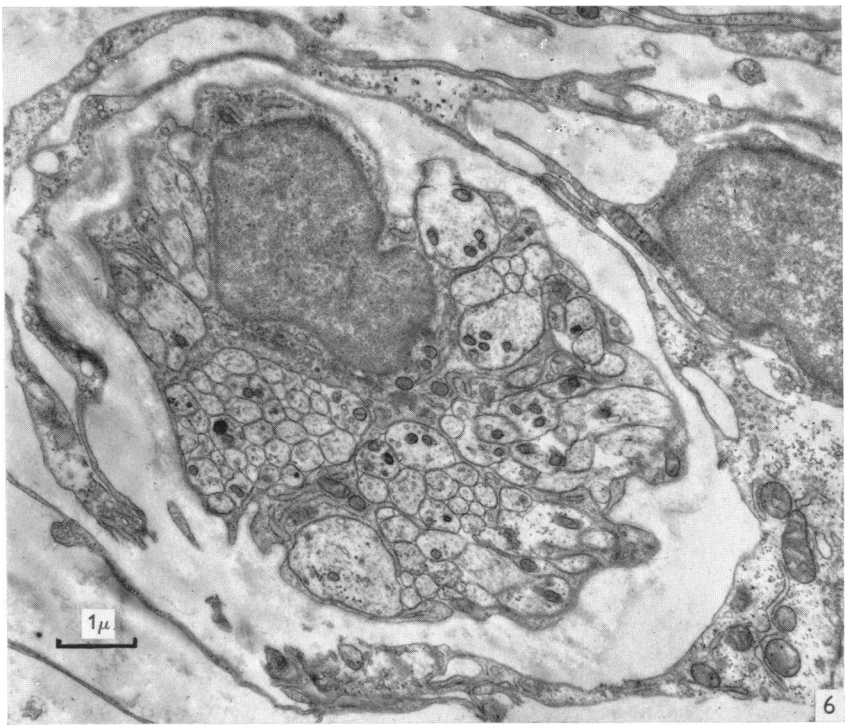

Fig. 6. Foetal cutaneous nerve invested by perineurial cell of very 'active' appearance but lacking basement membrane. (×10,000; 'stained' with lead hydroxide.)

Adult cutaneous nerves, by contrast, contain very few axons in their near-terminal regions (Figs. 8, 9). Like foetal cutaneous nerves they may possess or lack perineurial investment. They have never been seen making contact with the basement membrane of the epidermis. Where adult cutaneous nerves possess an investment of perineurium (Fig. 9) then the perineurial cells are themselves invested by basement membrane as in other adult nerves (Thomas, 1963; Gamble, 1964).

DISCUSSION

The stage of Schwann cell/axon development reached in the 14-week human foetal ulnar nerve appears to correspond closely with that described by Peters & Muir (1959) in 19½-day and 20½-day rat foetal digital nerves. Schwann cells are rather uniformly distributed through the substance of the nerve where their processes wrap around large and small bundles of axons but are only occasionally invaginated by single axons. It is possible that the small cutaneous nerves from this human

foetus are less developed than those of the ulnar nerve in that no truly and singly invaginated axon has been seen; but it cannot be said that the time taken for Schwann cells to migrate peripherally from the neural crest has led to any obvious difference in the maturation of the more peripheral nerve fibres.

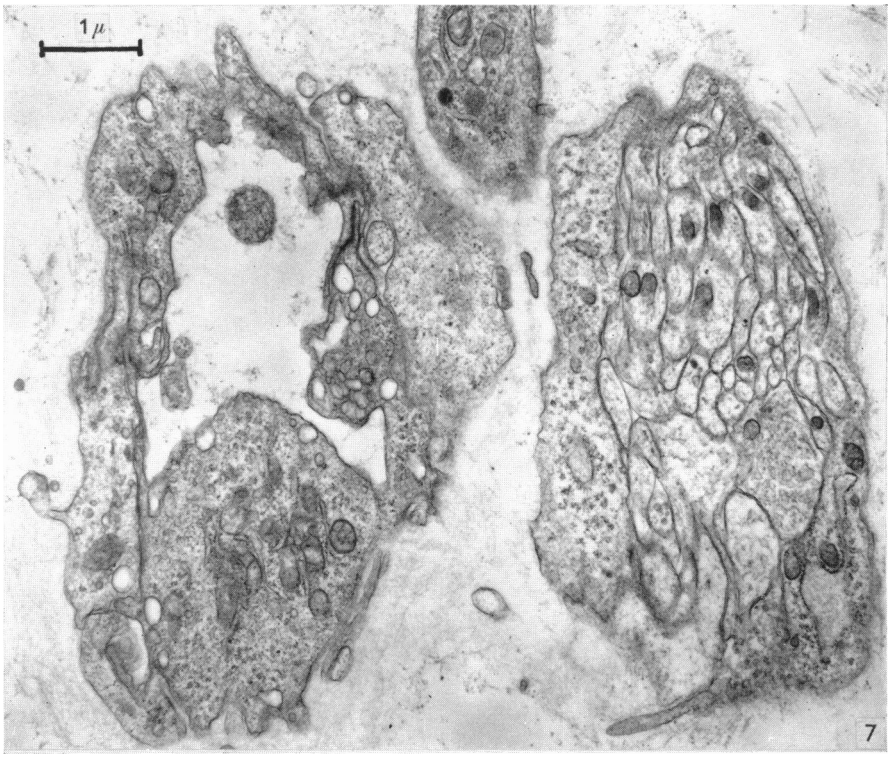

Fig. 7. Foetal cutaneous nerve bundle, lacking perineurial investment, lying beside cutaneous blood vessel. (×13,000; 'stained' with lead hydroxide.)

Endoneurial fibroblasts occur in the human foetal nerve but were not seen in rat nerves until early post-natal stages (Peters & Muir, 1959). In foetal rat nerves 'fibroblasts' were seen only in the capsule and were there described as forming the 'perineurial sheath' or 'epineurium'. There seems to be little doubt that similar cells form the covering of both rat and human foetal nerves and that these cells closely resemble 'active' fibroblasts not only in the structure of their cytoplasm and in their lack of basement membrane but also in the mode of their association one with another; cells overlap one another and no signs of adhesion between adjacent cells may be seen. The perineurial cells of adult nerve are very different in internal structure, are covered by basement membranes and are joined edge to edge by 'closed contacts' to form continuous cellular sheets (Thomas, 1963; Gamble, 1964). It seems probable that the ability of the adult perineurium to act as a 'diffusion barrier' to certain ions (Huxley & Stampfli, 1951; Causey & Palmer, 1953), drugs

(Crescitelli, 1951; Crescitelli & Geissmann, 1951), silver salts (Lehmann, 1953), toxins (Fedinec, 1958) and certain infective agents (Shanthareevappa & Bourne, 1963) must differ from the foetal perineurium of such different structure. The stage of development at which the perineurial cells take on their adult structure is not known. The illustrations of rat nerves (Peters & Muir, 1959) throw no light on the problem of the appearance of the basement membrane, but do show that the cells are still of very 'active' fibroblast-like structure in the 4-day post-natal rat.

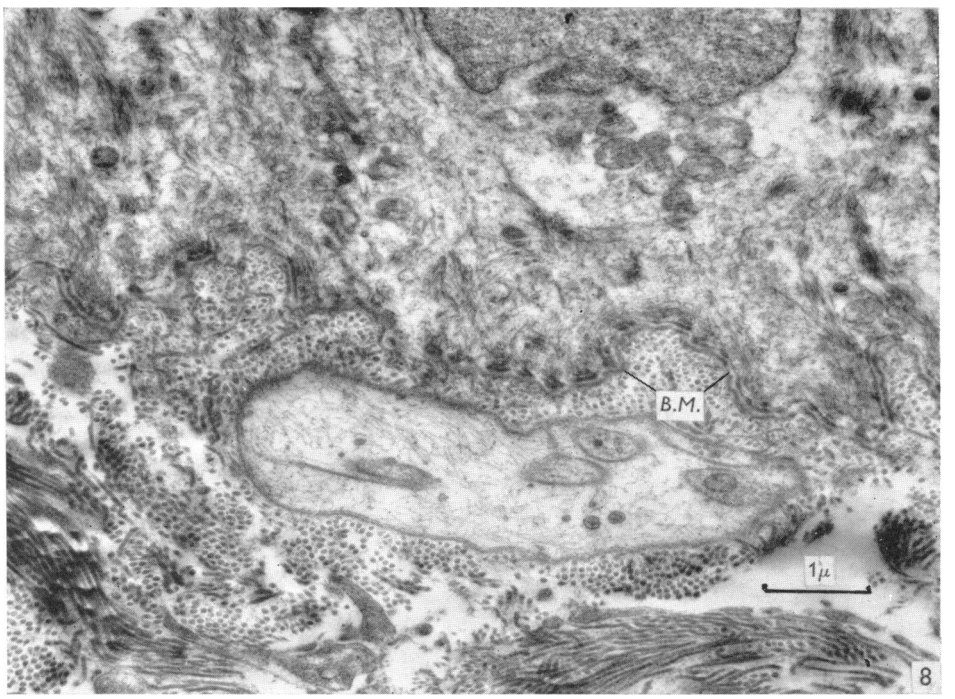

Fig. 8. Adult cutaneous nerve consisting of four axons invaginated into a Schwann-cell process which lies within 0·5μ of the basement membrane (*B.M.*) of the epidermis. No perineurium surrounds this Schwann cell/axon complex. ($\times$ 14,000; 'stained' with P.T.A.)

It is generally accepted that Schwann cells are derived from neural-crest ectoderm (Harrison, 1924) and some evidence points to a similar origin of the perineurial cells (Harvey & Burr, 1926; Harvey, Burr & van Camperhout, 1933). It is, then, surprising that the two cell types derived from a common origin in the neural crest should develop one of their adult characteristics, namely, basement membrane, at different stages of development, for Schwann cells possess and perineurial cells lack investing basement membrane in the 14-week human foetal nerve. It is possible that the Schwann cells, as they migrated peripherally, inherited a pre-existing basement membrane which had invested the associated axons before their own arrival. Only the investigation of earlier stages in the development of peripheral nerves could show whether such 'naked' axons were invested by basement membrane; examination of the figures published by Smith & Dempsey (1957) suggests

that the 'naked' axons of the adult organ of Corti lack a basement membrane. No 'naked' axons have been identified in human foetal skin but since the identification of axons there depends upon their having a characteristic relationship with Schwann cells, 'naked' axons and terminals could exist unrecognized in both dermis and epidermis despite careful searching.

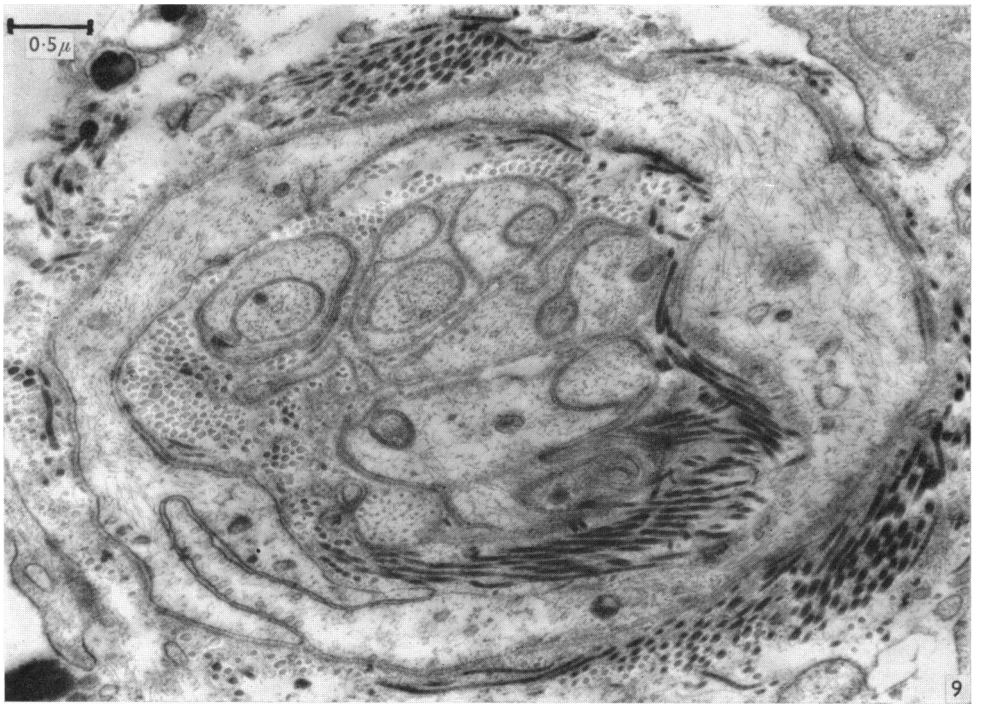

Fig. 9. Adult cutaneous nerve invested by perineurial cell with 'watery' cytoplasm but with well-defined basement membrane. (×22,000; 'stained' with P.T.A.)

It is worth commenting upon the very large number of axons which may be associated with a single Schwann cell in the foetal ulnar nerve (e.g. *c.* ninety axons in Fig. 2) and comparing this with the far smaller numbers seen in adult nerves (for references, see Gamble & Eames, 1964). Presumably the adult condition is achieved by mitotic division of the Schwann cells (which need occur only three or four times to reduce the Schwann cell/axon ratio from 1:100 to 1:12 or 1:6) but some of the reduction may also occur through the disappearance of axons, as seems to happen in the early stages of axon regeneration. It has been pointed out that a large proportion of the Schwann cells seen in cross-sections of the nerve are sectioned through their nuclear regions so that the cells cannot be very much longer than their own nuclei. Possibly mitosis can occur only when the cells are of this shape (it is difficult to envisage the mitotic division of a Schwann cell elongated to the 200–300 μ length found in the adult), and before their relationship with axons has gone beyond simple indentation of the cytoplasm.

SUMMARY

1. The ulnar nerve and cutaneous nerve bundles from a 14-week human foetus have been examined by electron microscopy.

2. Unmyelinated axons occur mainly in large bundles (up to 100 axons) indenting the surface of a Schwann cell. Occasionally a small bundle of axons is indented more deeply, but singly invaginated axons are only very rarely seen.

3. The axons, which occur in bundles, range widely in size, from 0·1 to 2·0 μ, and the rare cases of single invagination do not occur preferentially in relation to the larger axons.

4. The cellular elements of the endoneurium differ from the adult in the richness of cytoplasmic inclusions; Schwann cells, endothelial cells and fibroblasts alike are rich in endoplasmic reticulum and Golgi aparatus is often prominent. The cell types are identifiable by their other characteristics, which resemble those of the adult.

5. The perineurium of the nerve trunk and of the fine cutaneous nerves is composed of collagen and flattened fibroblast-like cells which lack basement membrane and overlap their neighbours. In adult nerves basement membranes are prominent and edge-to-edge contacts occur between cells to form continuous sheets.

6. In the fine cutaneous nerves the enclosure of a bundle of axons may be shared between two Schwann cells which lie side by side in a shared tube of basement membrane, resembling an early stage of development in rat foetal nerves.

7. The findings have been discussed.

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