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Observations on the development of the connective tissues of developing human nerve*

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The origins of the endoneurial collagen of peripheral nerves and their roots have not yet been determined. Ochoa (1976) has recently commented upon the presence of collagen in endoneurial clefts some weeks before the earliest appearance of endoneurial fibroblasts and consequently attributed collagen production to the immediately adjacent Schwann cells. The occurrence of collagen in 'pockets' invaginated into the Schwann cells of unmyelinated fibres (Gamble, 1964) was interpreted as showing a tendency in such cells to enwrap any suitably sized and orientated structure, but Thomas (1973) thought the phenomenon more probably indicative of a capacity of Schwann cells to replace degenerated axons with newly formed collagen. It was remarked also (Ochoa, 1971) that although collagen pockets may be quite numerous in young adult human nerves they had not appeared in the sural nerve of a human fetus of 18 weeks of intrauterine life, i.e. at a stage of development when Schwann cells are extremely active in the establishment of complex interrelationships with unmyelinated axons.

In the course of work directed to the study of the development of the human trochlear nerve some observations have been made which are pertinent to the problem of the origin of the endoneurial collagen. They are reported and discussed below.

MATERIALS AND METHODS

Parts of the trochlear nerve were dissected from human fetuses of 9·2 and 10 cm crown–rump length which were obtained at hysterotomy and believed to be normal in development. Their ages, from the histories, were estimated to be 10 and 13 weeks respectively.

The cerebrum and the tentorium cerebelli were removed and the brain stem bathed with chilled 5% glutaraldehyde in phosphate buffer at pH 7·3 while the intraorbital part of the trochlear nerve was exposed. (For the larger specimen, the roof of the orbit was then removed and its contents similarly bathed.) Short lengths of the trochlear nerve, from just proximal to the point where it pierced the dura mater (and in the larger specimen from just proximal to its entry into the superior oblique muscle) were then removed for further fixation (*c.* 4 hours) in buffered glutaraldehyde. After several rinses in chilled 10% solution of glucose, similarly buffered, the tissues were further fixed in a buffered 1% solution of osmium tetroxide for 1 hour. After dehydration, and embedding in Araldite, thick sections (*c.* 1 μm)

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were cut and stained with a mixture of Azur II and methylene blue (Richardson, Jarrett & Fink, 1960) and examined by light microscopy. Thin sections were mounted on uncoated grids, stained with uranyl acetate, and examined in an A.E.I. EM6B electron microscope.

Serial sections were often obtained, although the exact sequence was not always certain on the grid. Preservation of tissue was generally good.

Through the kindness of Professor A. S. Breathnach of the Department of Anatomy, St Mary's Hospital Medical School, London, we also had available for study some sections taken through the finger of a 9·5 cm crown–rump length human fetus in which digital nerves were well preserved.

OBSERVATIONS

(1) 9·2 *cm crown–rump length specimen* (*estimated age* 10 *weeks*) – *intracranial part of nerve, equivalent to a nerve root*

The whole cross section of the nerve within its cellular sheath measured some 54×25 μm. In a typical cross section, *c.* 4000 unmyelinated axons were arranged in 18 bundles separated by narrow fissures of endoneurial space in which collagen fibrils were present, but were nowhere numerous. A very small number of fibroblasts, or their cytoplasmic processes, was also seen. No blood vessel was seen. The main concentration of endoneurial collagen lay immediately within the cellular sheath of the nerve.

The perineurium, or root sheath, consisted of rather flattened cells devoid of basal lamina and ill-provided with cytoplasmic organelles, arranged in two or three layers between which scattered groups of collagen fibrils were trapped. In much of its extent small blood vessels were present; fused to it a tiny bundle of nerve fibres (wholly separate from the main trunk) probably represented a meningeal nerve, the nervi tentorii.

As mentioned above, some 4000 axons with their associated Schwann cells were divided by clefts of endoneurial space into 18 bundles. In such a bundle, some hundreds of axons (of $< 0{\cdot}1$ up to $1{\cdot}4$ μm in diameter) were invested and infiltrated by branching sheets of Schwann cell cytoplasm stemming from one of several such cells present in the bundle at any point along its length. Many apparently separate Schwann cell processes were present in every section examined, but it was not possible to determine the number of separate cells from which they originated. The whole outer aspect of a nerve bundle was formed by Schwann cell cytoplasm, mostly smooth, but occasionally giving off processes to enwrap collagen fibrils over short distances, and everywhere covered by basal lamina. Sheets of Schwann cell cytoplasm investing and infiltrating among the axons were in places so thin (< 20 nm) as to be incapable of containing cytoplasmic organelles. At points of bifurcation, or at their origins from the perinuclear region, they were often thicker and then contained one or two small mitochondria and/or a small vesicle of granular endoplasmic reticulum in addition to ribosomes. The perinuclear cytoplasm itself almost always lay alongside an endoneurial cleft. It was never extensive, but often contained Golgi apparatus, several small mitochondria, and several profiles of granular endoplasmic reticulum, as well as coated vesicles. Basal bodies and centrioles were occasionally seen, but no sign of mitotic activity was ever recognized.

Beside the major clefts of the endoneurial space, which could easily be traced laterally into communication one with another, were minor endoneurial spaces

containing collagen fibrils apparently embedded within the nerve bundles. They seemed to make no communication with other spaces, but sometimes, traced through near-serial sections, they did in fact communicate with the major clefts. The fate of others was not determinable. In general, such spaces were bounded by basal lamina covered processes of Schwann cell cytoplasm: occasionally, however, the basal lamina was deficient. Similar spaces were present also in one of the other specimens and are illustrated from it. Endoneurial collagen fibrils were all of small diameter, ranging from *c.* 17 to *c.* 50 nm.

(2*a*) 10 *cm crown–rump length specimen* (*estimated age* 13 *weeks*) – *intracranial part of nerve, equivalent to a nerve root*

In this part of the nerve Schwann cell complexes were small (1–60 axons) and were separated by an extensive network of endoneurial spaces. The collagen fibrils in these spaces were of diameters ranging from 20 to 53 nm. A complete count of a typical cross section showed there were 475 separate Schwann cell/axon complexes among which were found 150 Schwann cell nuclei. Basal lamina was present on the outer aspects of all Schwann cells and also on the endothelial cells of the single capillary vessel lying in the endoneurial space. The endoneurial spaces also contained processes of fibroblasts, of which 20 contained nuclei.

The perineurium, or root sheath, consisted of several lamellae (up to 10 in places), each of which was extremely thin except where it was thickened to contain the nucleus and, sometimes, quite extensive granular endoplasmic reticulum lying alongside the nucleus. All the lamellae were wholly devoid of basal lamina. Longitudinally running collagen fibrils were present between the cytoplasmic lamellae and were occasionally found in local concentrations. There was a marked concentration of collagen fibrils immediately within the innermost perineurial lamella.

The nerve contained *c.* 6000 axons of diameter ranging from $< 0{\cdot}1$ up to $2{\cdot}1$ μm. The Schwann cell elements of a complex consisted of one (or several, apparently separate) cytoplasmic process (or processes), sometimes of very complex form investing and infiltrating among the axons so that the great majority of these were in actual contact with Schwann cell cytoplasm. Except immediately alongside the nucleus, where a few mitochondria and a little granular endoplasmic reticulum were often seen, Schwann cell cytoplasm contained remarkably few organelles. The greater part of its extent was in extremely flattened sheets, too thin in most cases to contain anything much larger than a ribosome. No sign of mitotic activity was seen in any Schwann cell.

(2*b*) *Intraorbital part of trochlear nerve, i.e. true peripheral nerve*

This part of the nerve consisted of a very thin perineurium surrounding a mass of Schwann cell/axon complexes which was divided into eight very large nerve bundles by narrow endoneurial spaces which extended centrally from the endoneurial space lying immediately within the perineurium. Basal lamina was present upon those Schwann cell processes which confronted the endoneurial spaces. The spaces contained scattered fibrils but no blood vessels nor fibroblasts. In a typical cross section there were *c.* 35 Schwann cell nuclei and 5300 axons of $< 0{\cdot}1$ up to $3{\cdot}1$ μm diameter.

The perineurium consisted of no more than five cytoplasmic lamellae, extremely thin in the greater parts of their extent and not more than 3 μm thick in sections or alongside the nucleus, where a little granular endoplasmic reticulum was sometimes

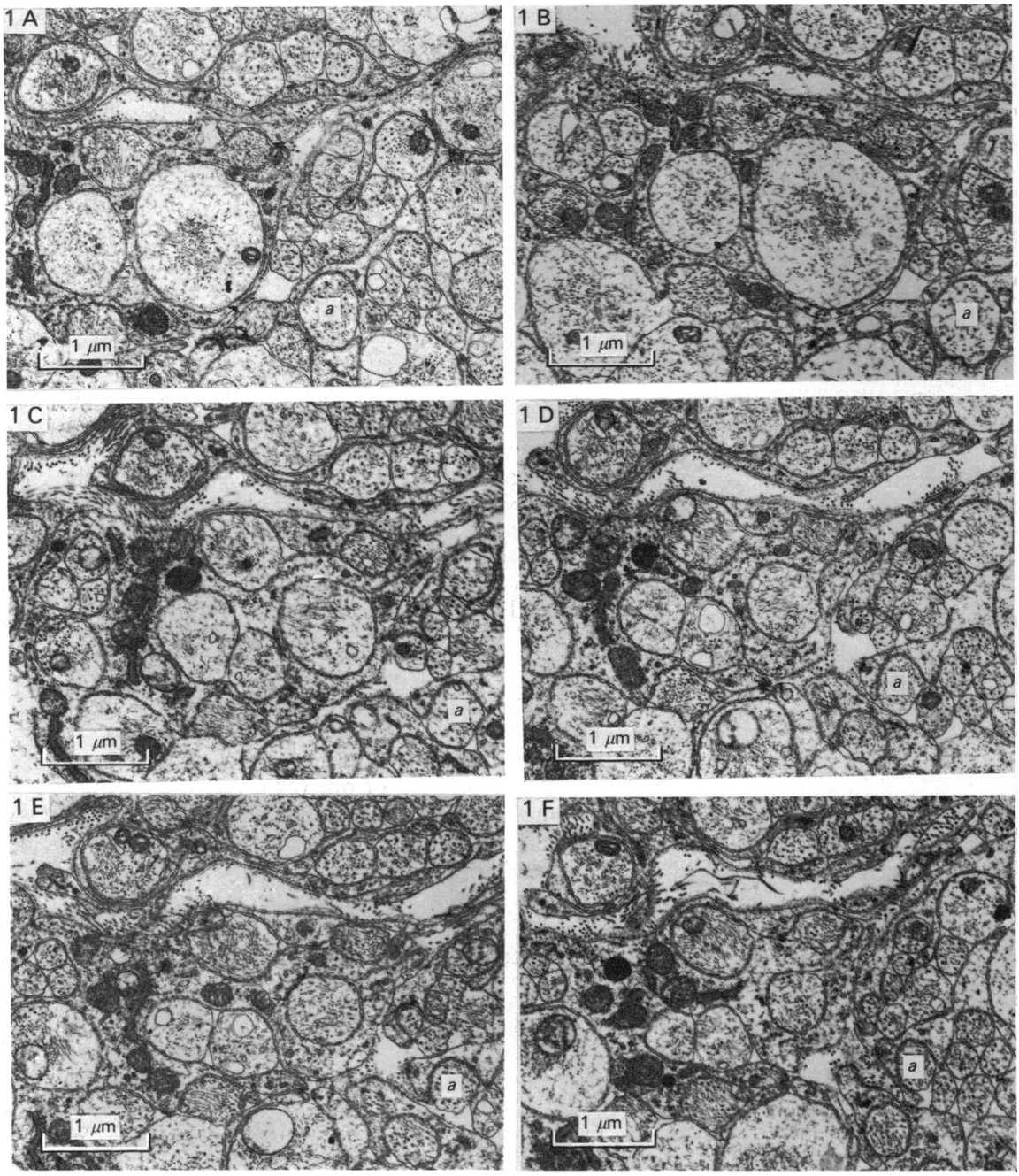


Fig. 1. Near-serial electron micrographs (× 15000) made from the intraorbital part of the trochlear nerve of a 10 cm crown–rump length specimen (age estimated to be 13 weeks). Immediately to the left of the axon (*a*) in each section is a Schwann cell cytoplasmic process confronting a small cleft of endoneurial space. The process contains a vesicle, empty but coated in B, containing collagen fibrils in C, D and E, and apparently absent (at both ends of the sequence) from A and F.

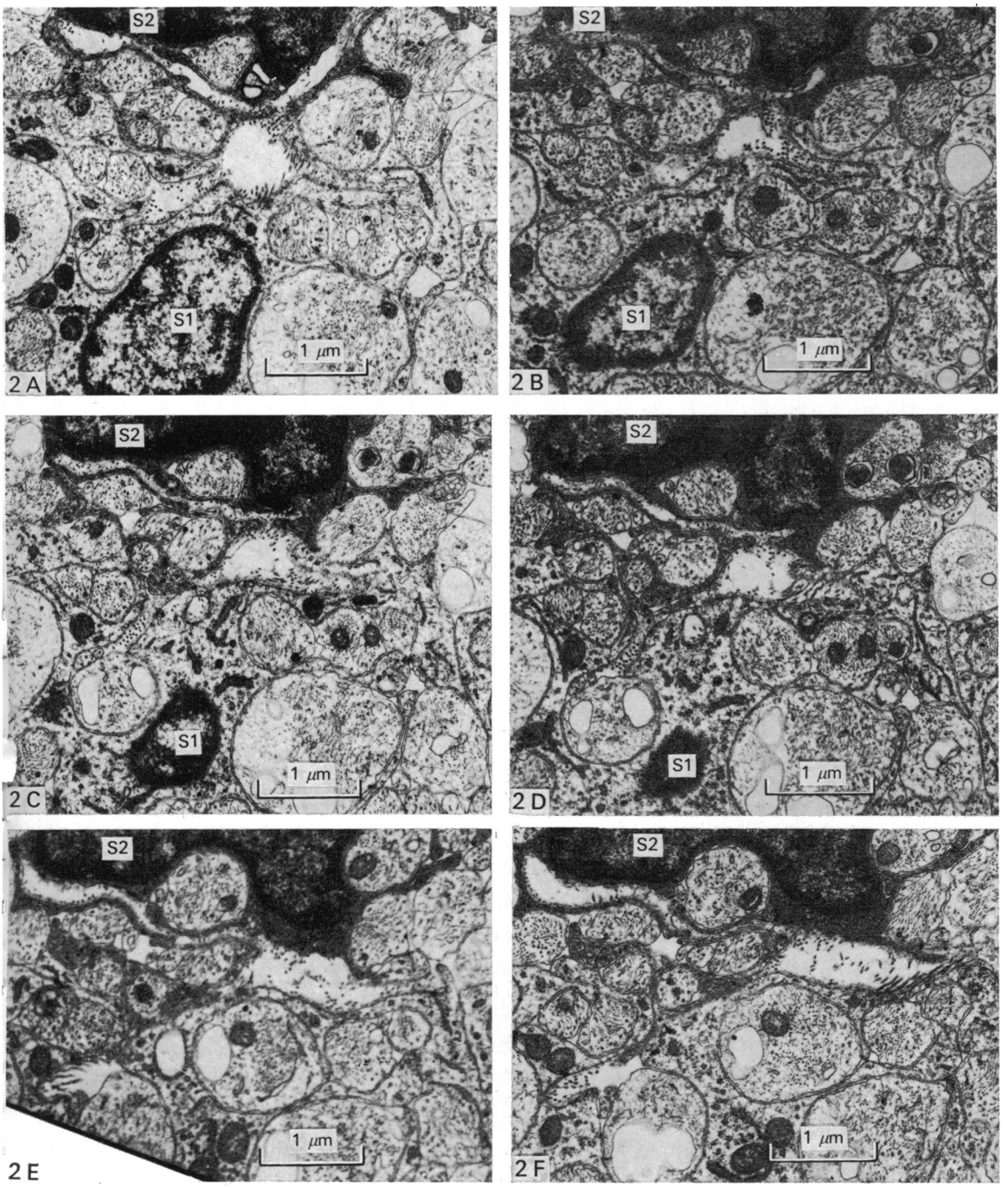


Fig. 2. Near-serial micrographs of (×15000) made from the intraorbital part of the trochlear nerve of a 10 cm crown–rump length specimen (age estimated to be 13 weeks). In A, cytoplasm close to the nucleus of a Schwann cell (S1) confronts a cleft of the endoneurial space. As the sections pass closer to and beyond the pole of this nucleus (B–E) a vesicle appears in the cytoplasm, linked by 'meso' to the endoneurial space in C, wholly independent in D and E, and absent from F. In E the vesicle is coated but empty; in D and C it contains six collagen fibrils, which seem to correspond with the six fibrils now outside the cell in the intermediate part of the endoneurial space shown in B. An extensive cleft of endoneurial space alongside the Schwann cell nucleus S2 is bounded on its other side, and across a blind end, by a slender cytoplasmic process from the same cell.

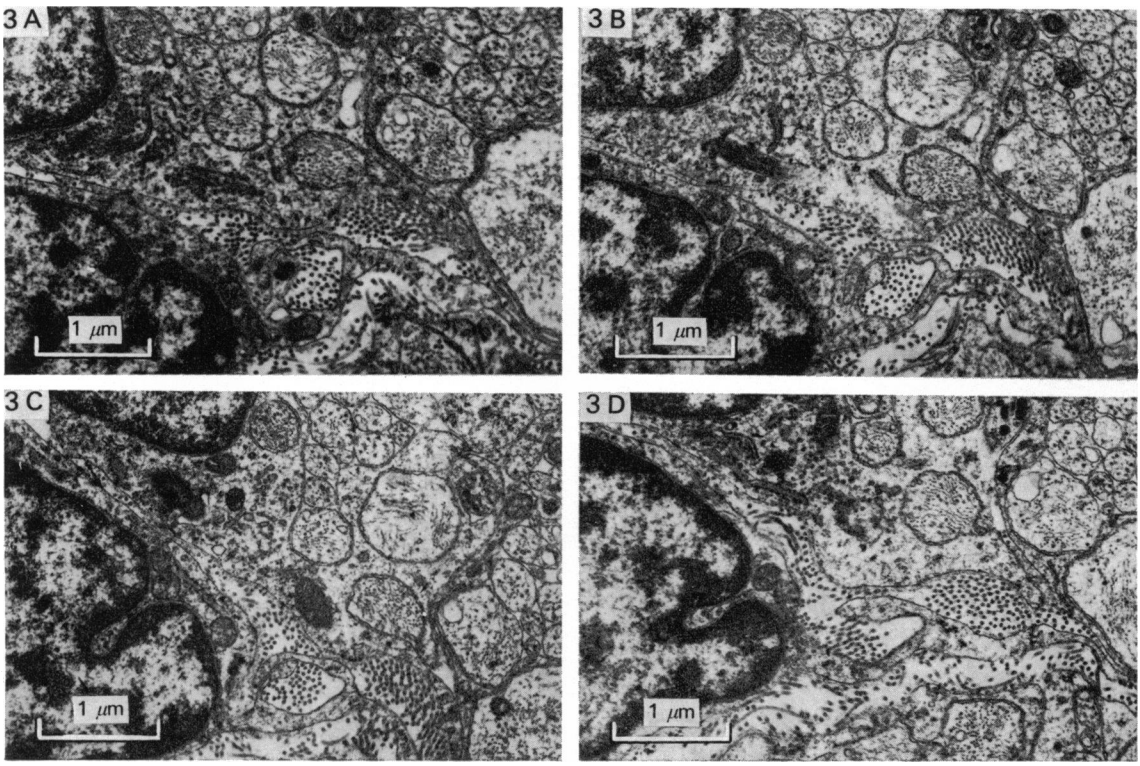


Fig. 3. Near-serial electron micrographs (×15000) made from the intraorbital part of the trochlear nerve of 10 cm crown–rump length specimens (age estimated to be 13 weeks). The Schwann cell at lower left (with deeply indented nucleus) projects flaps of cytoplasm to overlap each other and so enwrap a bundle of collage fibrils (A–C). In D, the processes are shorter and the 'pocket' widely open to the more general endoneurial space. Cytoplasm alongside the other Schwann cell nucleus contains Golgi apparatus and coated vesicles in A, a cilium in B and C and granular endoplasmic reticulum in B and D.

seen. No basal lamina was seen in relation to these cells, and inter-lamellar collagen was extremely scanty at best and in most of the sheath wholly absent. Blood capillaries were present in and on the outer aspect of the sheath, but none was seen to penetrate through it into the endoneurial space.

In addition to the sub-perineurial endoneurial space, in which collagen was conspicuous, although fibrils were of small diameter (12–42 nm), narrow clefts extended centrally into the body of the nerve; many of these ended blindly by the apposition of their walls but others linked up to divide the great mass of Schwann cell/axon complexes into eight major fascicles. Many additional spaces containing collagen (sometimes only four or five fibrils) and bounded by processes of Schwann cells were separate from the major subdividing clefts: when traced through many serial sections some ended blindly by direct apposition of the bounding Schwann cells.

Occasionally, collagen fibrils lay within a vesicle in Schwann cell cytoplasm, and in two cases the vesicle, traced through serial sections, ended blindly at both ends (Fig. 1A–F). In other cases such a vesicle was shown to end blindly at one end but to open into an endoneurial space at the other, where its own complement of collagen fibrils added to those already present (Fig. 2A–F). Close to the point of exit from

vesicle into endoneurial space (Fig. 2C) the collagen pocket resembled those described by Gamble (1964) in adult rat and by Gamble & Eames (1964), Ochoa & Vial (1967) and Eames & Gamble (1970) in adult human nerves. The vesicles associated with this collagen were often coated over parts of their extent (Figs. 1B,2E,3D).

Flaps of Schwann cell cytoplasm were frequently seen projecting into the major endoneurial spaces, either to end there freely or to be bent over to contact the main part of the cytoplasm and so trap some collagen fibrils (Fig. 3A–D) which, usually, in serial sections, could be seen to escape from the trap after a short distance. As might be expected, the extremities of Schwann cell cytoplasmic flaps sometimes tapered off to appear as free-lying cytoplasmic processes in the endoneurial space, but when serial sections allowed their tracing these were found, without exception, to be continuous with one of the Schwann cells confronting the space. No fibroblast process was ever identified in the endoneurial spaces.

Schwann cell cytoplasm formed almost the whole of the surfaces bounding endoneurial clefts except, very occasionally, when an axon (covered by basal lamina) did so. In general, Schwann cells exhibited extraordinary complexity of form, as shown, for example, in Figures 4A and B. In this cell a few mitochondria and scanty endoplasmic reticulum lay alongside the nucleus but the greater part of the cytoplasm extended into wide spreading processes (long flutings in the length of the cell) too slender, for the most part, to contain an organelle of any size much larger than a ribosome: in places they were slightly thickened and contained a mitochondrion or short lengths of granular endoplasmic reticulum. By direct measurement, the cell in the section shown in Figure 4B presented *c.* 152 μm of its plasma membrane to various structures: *c.* 18 μm to basal lamina confronting one large and three small clefts of the endoneurial space (the bulk of this was perinuclear cytoplasm), *c.* 12 μm to processes of cytoplasm belonging to other Schwann cells, 5 μm to other of its own processes and the remainder (*c.* 117 μm) to unmyelinated axons, investing these singly, multiply, and (in some cases) incompletely. The examination of 15 near-serial sections through this Schwann cell, proximally and distally along its length, showed that its basic pattern of branching cytoplasmic processes persisted, although changing in detail, so that:

(1) Some processes of cytoplasm became detached while others, originally separate, joined the main body of the cell.

(2) The extent of the Schwann cell surface confronting endoneurial spaces changed as neighbouring cytoplasmic processes increased or decreased their areas of contact and of overlapping.

It was clear that the eight very large fascicles of Schwann cells and axons were partially subdivided by the endoneurial clefts which occurred within them, and it seems likely that extension of these spaces would soon, with further development, provide boundaries to more numerous but smaller nerve fibre bundles. It was often quite impossible to predict where such boundaries would occur but, where different Schwann cell processes were immediately contiguous and were traceable to regions where they did confront endoneurial space, it was easy to envisage the later formation of an extension of this space between them.

Further sections of this part of the nerve were examined after a few thick (1 μm) sections had been cut. The branching pattern of the endoneurial spaces was still recognizable, but some of the spaces once in continuity were now independent and others, once independent, had linked up with those communicating with the subperineurial space.

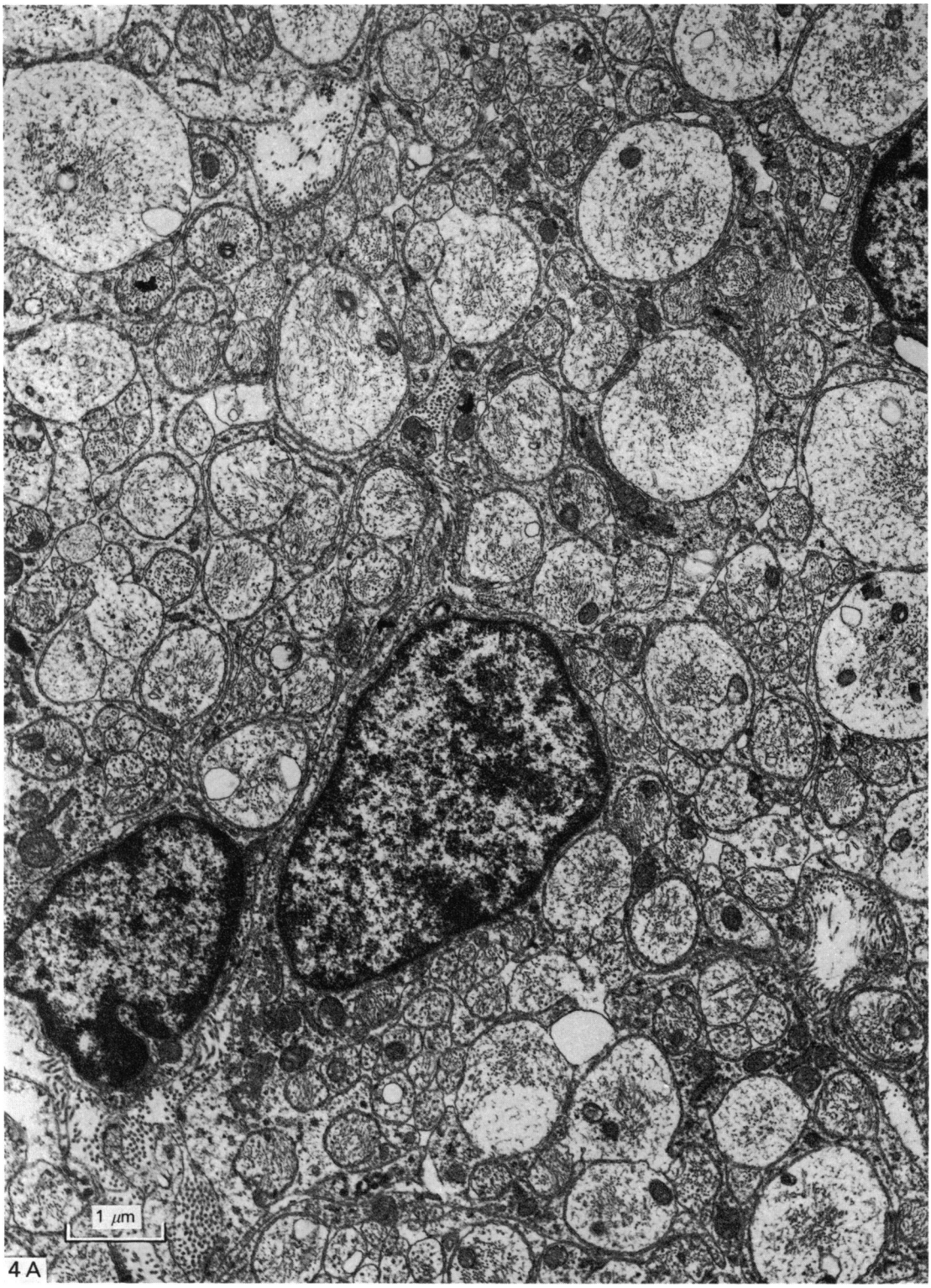


Fig. 4A. Electron micrograph (× 15000) made from the intraorbital part of the trochlear nerve of a 10 cm crown–rump length specimen. To show Schwann cell processes (including the nuclei of three cells) and axons in a part of one of the eight huge fascicles which make up this part of the nerve.

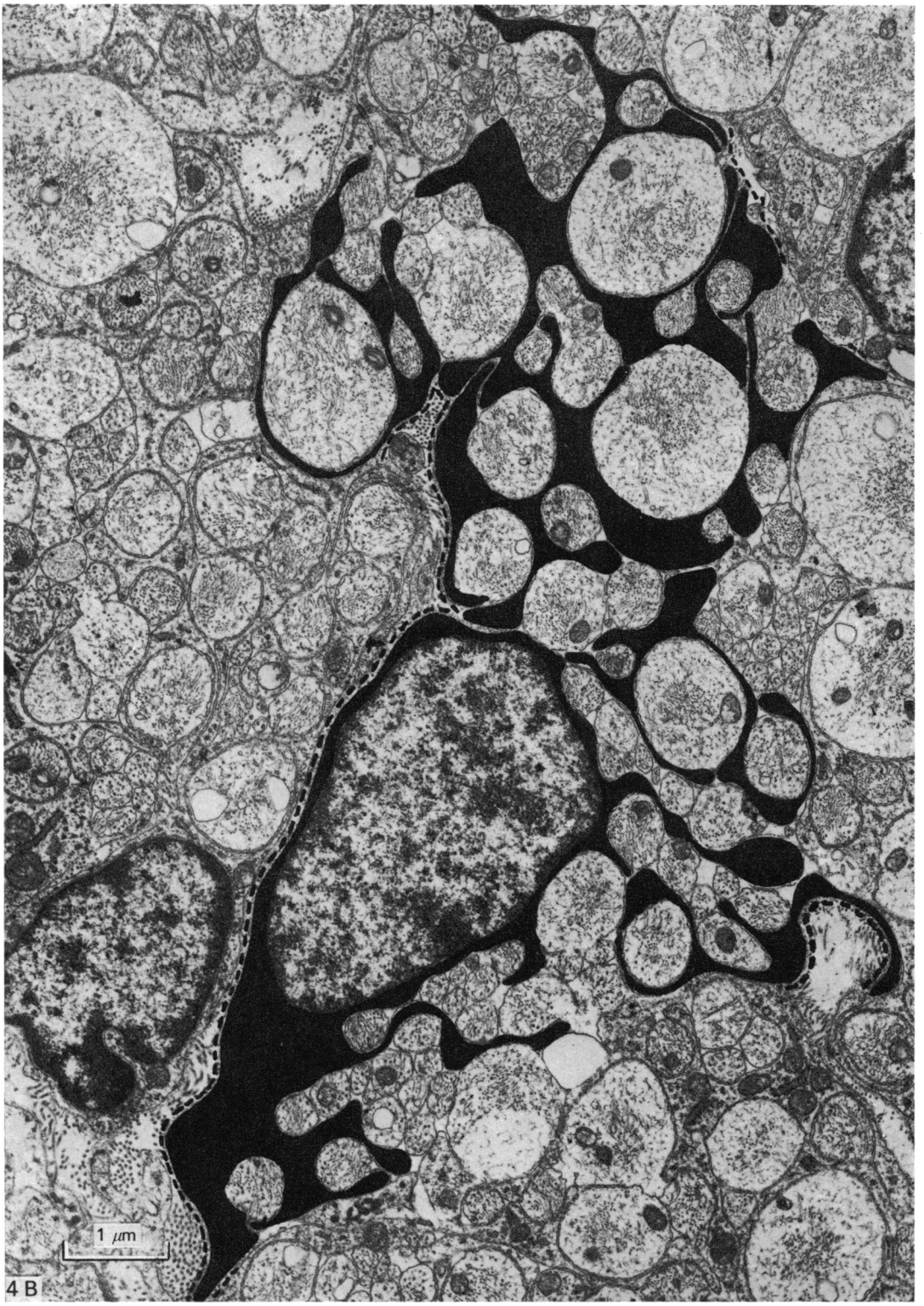
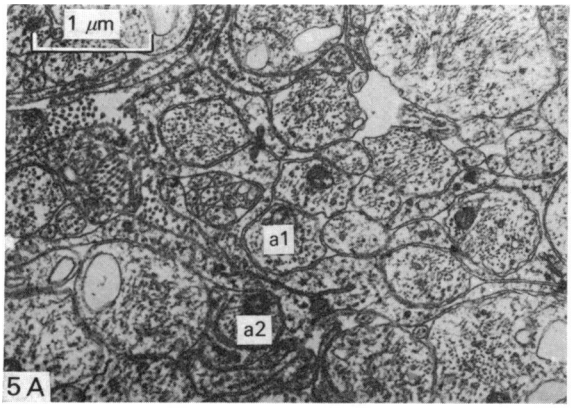
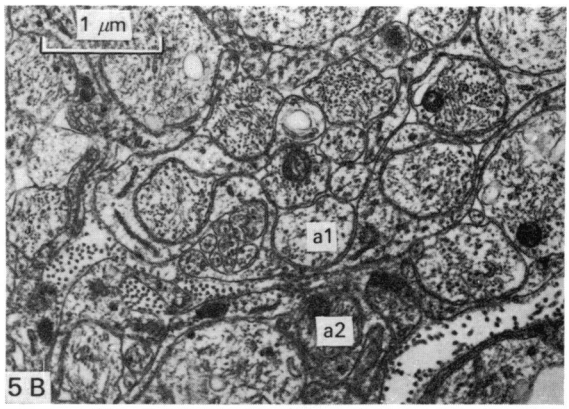


Fig. 4B. As in 4A but inked in as 'aide memoire': solid black to show extent of one Schwann cell's cytoplasmic processes; in dashed line to show extent of the same cell's confrontation with endoneurial space.


1 μm
a1
a2
5 A


1 μm
a1
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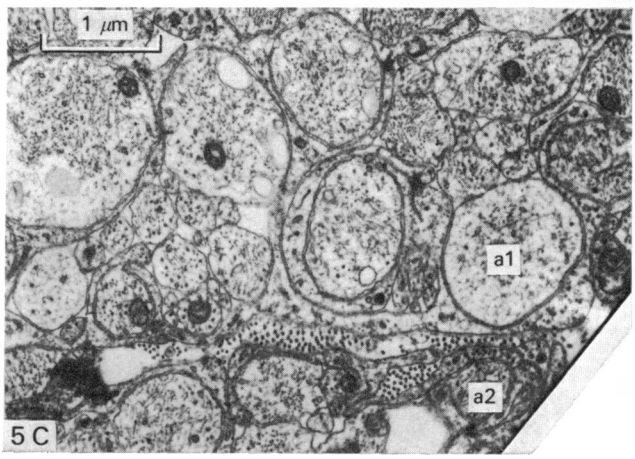
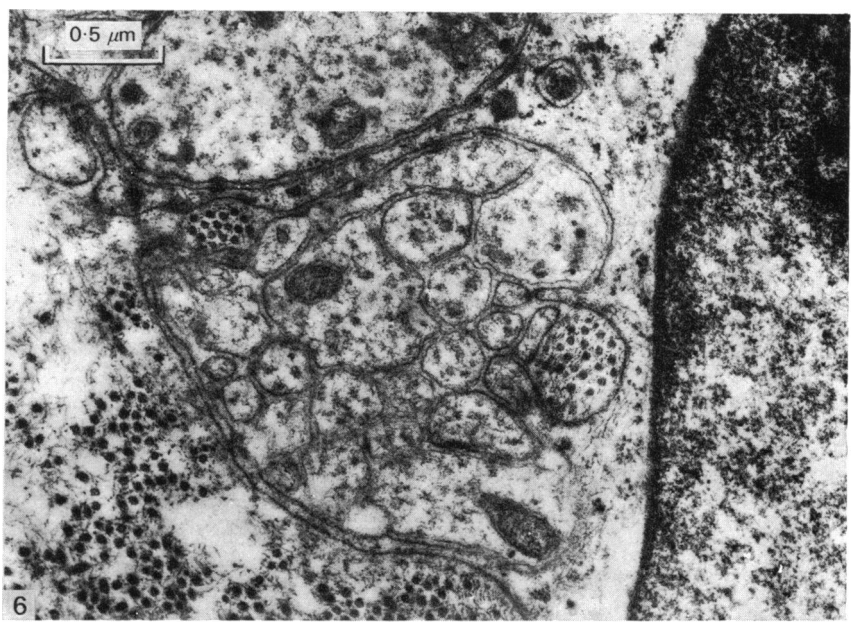

1 μm
a1
a2
5 C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6

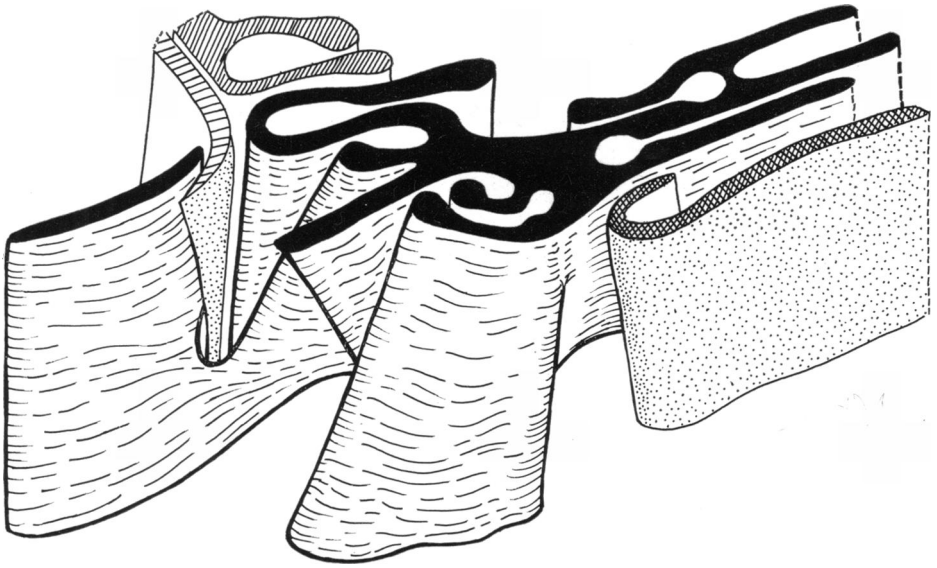

Fig. 7. Line drawing of reconstruction of Schwann cell processes shown in Figs. 5A–C and of 12 other near-serial cross sections.

Mention has been made of separate processes of Schwann cell cytoplasm which, by examination of serial sections, were shown to link up with others. An example is illustrated in Figure 5A–C: a relatively thick process of cytoplasm contained a flattened vesicle and lay between two separated parts of the endoneurial space (Fig. 5A); in near-serial sections the two spaces were joined (Fig. 5B, C), apparently by division of the cytoplasmic process through the axis of the flattened vesicle. A reconstruction of this has been made in Figure 7.

Not infrequently clefts of the endoneurial space were bounded along sides and across a blind end by cytoplasm of the same Schwann call, i.e. the cleft was not between adjacent Schwann cells but was a deep incision into a single cell. An example of this is seen in Figure 4A–F where endoneurial space extends across the top of the

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Fig. 5. Near-serial electron micrographs (×15000) made from the intraorbital part of the trochlear nerve of a 10 cm crown–rump length specimen (age estimated to be 13 weeks). (A). A process of Schwann cell runs laterally across the figure and contains a wide, flattened vesicle which points toward collagen-containing endoneurial space at its left extremity. Axons a1 and a2 lie on either side of the process. (B). This process is divided into two by extension of the endoneurial space to the right, apparently along a line corresponding with the vesicle seen in A. Axon a1 is related to one process, axon a2 to the other. (C). The endoneurial space has extended further to the right to link up with the apparently separate space seen in A and B.

Fig. 6. Electron micrograph made from digital nerve of a human fetus of 9·5 cm crown–rump length (×30000). Two slender cytoplasmic processes extend from the nucleus of a Schwann cell to enwrap *c.* 20 axons, the wrapping being completed by a group of collagen fibrils plugging a narrow interval between their extremities. Another bundle of collagen fibrils is deeply set within the nerve fibre bundle, largely bounded by two additional, but tiny, processes of Schwann cell cytoplasm.

image between the nuclear region of the cell and an extensive flap of cytoplasm which runs almost parallel with it.

(3) *Digital nerve from finger of* 9·5 *cm crown–rump length human fetus*

The sections of fetal digital nerve kindly supplied by Professor Breathnach included the Schwann cell/axon complex of which a part is shown in Figure 6. Two slender processes of cytoplasm extended from the nuclear region to invaginate communally some 20 small axons, a small bundle of some 17 collagen fibrils (set in an amorphous matrix and surrounded by basal lamina) being set in the narrow interval between the tips of the processes to complete the axonal investment. Deep within the axonal bundle, and largely invested by two more, much shorter, cytoplasmic processes, was another bundle of collagen fibrils. This part of the endoneurial space was separated from the general endoneurial space around the whole Schwann cell/axon complex by several layers of small axons which were separated from each other by no more than the usual gaps of *c.* 20 nm width, i.e. there was no free communication between the two collagen-containing spaces.

DISCUSSION

Many of the fine structural differences between the intracranial parts of the trochlear nerve of the 9·2 cm and 10 cm crown–rump length specimens, and between the intracranial and intraorbital parts of the nerve from the latter specimen, are indicative of very marked differences in maturation, the one attributable to a 2–3 weeks difference in age (rather poorly reflected by a difference in size), the other attributable to a proximodistal gradient of maturation along the length of the nerve.

Immaturity of the (intracranial) nerve from the 9·2 cm crown–rump length (10 weeks estimated age) specimen is shown by the paucity and small calibre of the endoneurial collagen, by the very infrequent occurrence of fibroblasts, by the small extent of the endoneurial spaces and in the consequent large size and small number (18) of the nerve bundles. The ratio of Schwann cells to axons is not possible to determine; many Schwann cell cytoplasmic processes appear detached from their nuclear regions. The number of Schwann cell nuclei showing in a typical cross section was 20, and these were associated (with an unknown additional number of Schwann cells) with *c.* 4000 axons to give 1:200 for this particular ratio.

The equivalent part of the 10 cm crown–rump length (13 weeks estimated age) specimen was very advanced by comparison. Collagen was present in greater quantity and as slightly larger fibrils and spread through a very much more complex system of endoneurial spaces so distributed as to divide the nerve into 475 bundles. This specimen contained several fibroblasts in the endoneurial spaces. The number of Schwann cell nuclei showing in a typical cross section was 150 and these were associated with *c.* 6000 axons to give 1:40 for this particular ratio. The distal (intraorbital) part of the same nerve was very immature by comparison; scanty and very slender (12–42 mm) collagen fibrils occupied slender endoneurial spaces so disposed as to divide the nervous tissues into some 8–10 extremely large bundles. Examination of the nerve at intervals of a few microns along its length showed that the pattern of this subdivision was not fixed and that an apparently separate fascicle at one point might have divided to join other now apparently separate fascicles. Despite this interchange, the number of fascicles remained small and the fascicles themselves large. The number of Schwann cell nuclei showing in a typical cross section was

c. 35 which, associated with *c.* 5300 axons, gave *c.* 1:150 for this particular ratio. (In this respect, perhaps, the intraorbital nerve of the older specimen was more mature than the intracranial part of the nerve of the younger.)

In terms of the development of endoneurial spaces, and of collagen fibrils within them, the degree of maturity in the younger (10 weeks) and in the intraorbital part of the older (13 weeks) trochlear nerves described have seemed to correspond closely with that described by Ochoa (1971) in the human fetal sural nerve at 9 weeks. In marked contrast, Cravioto (1965) demonstrated collagen and spaces only immediately within the perineurial sheath of a 12 weeks human fetal sciatic nerve; elsewhere in this specimen Schwann cells were closely packed together without even basal lamina separating them. It is not surprising that maturation of a cranial nerve should be in advance of one so caudally situated as the sciatic, but it is surprising that maturation of the sciatic should lag behind that of one of its own distal branches. If this precocious maturation in the sural nerve were limited to the extent and disposition of its endoneurial spaces and fibrils it might be explicable as a response to its relatively exposed situation close to the surface of the limb; but, in fact it appears to extend to the Schwann cell/axon complexes also; there are no Schwann cells still unassociated with axons, as there are in the sciatic nerve. In this respect, however, the sural nerve at 9 weeks appears less mature than the two specimens of trochlear nerve which its endoneurial tissues resemble; its Schwann cells enclose large bundles of axons but scarcely penetrate between them. In the two trochlear nerves the Schwann cells are of extremely complex form so that only a minority of axons fail to contact some part of the Schwann cell cytoplasm.

Ochoa (1971) commented that "the presence of endoneurial collagen before the appearance of fibroblasts favours the view that Schwann cells participate in the formation of collagen (Nathaniel & Pease, 1963; Thomas, 1964)" and the present results would tend to support this view. It is true that, in Ochoa's 9 weeks sural nerve, and in our specimens, endoneurial spaces extend to the sub-perineurial region where collagen is conspicuous and which is bounded by cells which, though extremely flattened, still are equipped with quite extensive granular endoplasmic reticulum and have something of the appearance of active fibroblasts. At the same time, collagen is present in quantity only on their endoneurial, or inner, aspect and in only minute and scattered groups of fibrils on their outer aspect. It might appear strange that such cells, if capable of synthesizing collagen at all, should be so polarized; no corresponding polarization of their cytoplasmic organelles has been observed. If it should be assumed, nevertheless, that the inner perineurial cells were involved in the production of the sub-perineurial collagen, there remains that collagen which, scanty though it may be, still is found at relatively considerable distances from the perineurium but immediately adjacent to Schwann cells.

It has been remarked that a great part of the Schwann cells present in the fetal trochlear nerves occurs as sheets no more than 20 nm thick, except, commonly, where they split. Here, the cytoplasm contains some granular endoplasmic reticulum and mitochondria and, close to the nucleus, contains, additionally, Golgi apparatus, dense bodies and coated vesicles. It would be true to say, however, that the Schwann cells do not present an especially 'active' appearance and yet it is quite certain that they are extremely active, at least in the production of enormous quantities of plasma membrane (in one section, a Schwann cell exhibited a cytoplasmic circumference of *c.* 152 μm). It is also true that the great bulk of the Schwann cell perinuclear cytoplasm lies alongside endoneurial space, i.e. most of the rather scanty organelles also do so.

The presence of 'collagen pockets' in Schwann cells (Gamble, 1964) has sometimes been taken as evidence of collagen synthesis by Schwann cells (Thomas, 1973), most probably after some local injury had caused degeneration of an unmyelinated axon, the collagen forming within the space that had once held that axon. Collagen pockets have not commonly been seen in human fetal nerve (Ochoa, 1976) but there is evidence of their, at least, occasional presence (Figs. 2C, 3, 6) in a form similar to that first described, and some evidence also of the occurrence of collagen more completely within the cytoplasm of Schwann cells in fetal trochlear nerve (Fig. 1C, E). In both situations, basal lamina may separate collagen from Schwann cell cytoplasm or basal lamina may be absent. When absent, the Schwann cell may contain a coated vesicle opening to the collagen-containing space. The evidence favouring the idea that endoneurial collagen is produced by Schwann cells remains incomplete (and is perhaps likely to continue so until its production by a pure culture of Schwann cells has been demonstrated) but is reinforced by the additional circumstantial evidence presented here. The apparent absence of fibroblasts, or of even their slender cytoplasmic extensions, from an immature specimen (13 weeks intraorbital) conforms to Ochoa's (1971) finding and constitutes additional, but negative, circumstantial evidence. Reference has been made to the maturation of our various specimens, mainly in terms of the amount and disposition of endoneurial space and the numbers and sizes of collagen fibrils occupying it. In general, the size ranges of the axons in the various specimens conform but are, perhaps, less reliable criteria of maturation for the following reasons:

(*a*) Some of the largest axons in all specimens are of a pale and watery appearance, sometimes containing swollen but empty vesicles. We suggest that these may be undergoing 'physiological' degeneration and are swollen in consequence. (See, for example, Figs. 1A, B, 2A–F).

(*b*) Many axons vary considerably in calibre along their length without, meanwhile, exhibiting any evidence of abnormality (see many examples in Figs. 1, 2, 5).

We are aware of disparities in axonal counts, as between the younger and older specimens, of the intracranial part of the nerve, and as between intracranial and intraorbital parts of the same (older) nerve. While not attempting to explain these completely, we suggest that the following factors are relevant:

(1) New axons, or their collaterals, are being produced while some of their predecessors are undergoing physiological degeneration. The combined effect of these changes is likely to vary at different ages, and at different points along the length of a given nerve.

(2) In traversing the length of the cavernous sinus the trochlear nerve may lose afferent fibres present in its intraorbital part and passing centrally thereafter in the ophthalmic division of the trigeminal nerve. However, the number of such fibres, and the timing of their outgrowth to enter this part of the nerve are not known so that it is impossible to judge of their importance. Even the smallest of cranial nerves is more complex than might be wished!

SUMMARY

Trochlear nerves from two human fetuses, and digital nerves from a third, have been examined by electron microscopy.

Very marked differences in maturation were found between trochlear nerves of fetuses of ages differing only by 2–3 weeks, and between proximal and distal parts of the same trochlear nerve.

Immaturity was reflected in paucity of endoneurial space and collagen and in the rarity, or virtual absence, of endoneurial fibroblasts. Circumstantial evidence of collagen formation by Schwann cells has been presented and discussed.

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REFERENCES

CRAVIOTO, H. (1965). The role of the Schwann cell in the development of human peripheral nerves. *Journal of Ultrastructure Research* **12**, 634–651.

EAMES, ROSEMARY A. & GAMBLE, H. J. (1970). Schwann cell relationships in normal human cutaneous nerves. *Journal of Anatomy* **106**, 417–435.

GAMBLE, H. J. (1964). Comparative electron microscopic observations on the connective tissues of a peripheral nerve and a spinal nerve root in the rat. *Journal of Anatomy* **98**, 17–25.

GAMBLE, H. J. & EAMES, ROSEMARY, A. (1964). An electron microscope study of the connective tissues of human peripheral nerve. *Journal of Anatomy* **98**, 655–663.

NATHANIEL, E. J. H. & PEASE, D. C. (1963). Collagen and basement membrane formation by Schwann cells during nerve regeneration. *Journal of Ultrastructure Research* **9**, 550–560.

OCHOA, J. (1971). The sural nerve of the human foetus: electron microscope observations and counts of axons. *Journal of Anatomy* **108**, 231–245.

OCHOA, J. (1976). The unmyelinated nerve fibre. In *The Peripheral Nerve* (ed. D. N. Landon). London: Chapman and Hall.

OCHOA, J. & VIAL, D. (1967). Behaviour of peripheral nerve structures in chronic neuropathies with special reference to the Schwann cell. *Journal of Anatomy* **102**, 95–111.

RICHARDSON, K. C., JARRETT, L. & FINK, E. H. (1960). Embedding in epoxy resin for ultrathin sectioning in electron microscopy. *Stain Technology* **35**, 313–323.

THOMAS, P. K. (1964). The deposition of collagen in relation to Schwann cell basement membrane during peripheral nerve regeneration. *Journal of Cell Biology* **23**, 375–382.

THOMAS, P. K. (1973). The ultrastructural pathology of unmyelinated nerve fibres. In *New Developments in Electromyography and Clinical Neurophysiology*, vol. 2 (ed. J. E. Desmedt), pp. 227–239. Basle: Karger.