ULTRASTRUCTURE OF DEVELOPING HUMAN INTRAMUSCULAR NERVES

GUILBERTO MINGUETTI, PhD *
W. G. P. MAIR, MD, FRCPath **

The developing intramuscular nerves were studied by electron microscopy, the material being obtained from human foetuses of nine weeks to nine months development. Already at nine weeks there occur amongst the muscle cells bundles of naked axons surrounded by a few Schwann cells. As development advances the Schwann cells proliferate by mitosis and each Schwann cell begins to surround smaller groups of axons and even single axons: the latter are destined to become myelinated fibres. Obvious myelinated axons are first observed in foetuses of eighteen weeks. At this stage well developed nodes of Ranvier and Schmidt-Lantermann clefts are also present in the myelin around the nerve cells. At twelve weeks development the nerves show several layers of concentric cells around the axons and these are cells of the perineurium which is very well developed at sixteen weeks: at eighteen weeks small blood vessels are seen between the perineurial cells.

MATERIAL AND METHODS

The material used in this investigation is derived from twenty seven human foetuses ranging from nine weeks to nine months development. They have been studied in detail in longitudinal and transverse sections by electron microscopy; some 300 grids were examined. Foetuses of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 24, 28, 32, 36 and 40 weeks were studied.

The age of the minute foetuses was established by the obstetricians who supplied the specimens and who supplied the specimens and was calculated according to the crown-rump length. The ages of foetuses of 5, 7, 8 and 9 months development were based on the menstrual age. They were newborn prematures who died within 24 hours

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Trabalho realizado no Instituto de Neurologia da Universidade de Londres (Queen Square): * Assistant Professor, Department of Internal Medicine (Neurology), Federal University of Paraná, Curitiba, PR, Brasil; ** Consultant Neuropathologist, Institute of Neurology, Queen Square, London.

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after birth. Specimens were removed soon after death in these cases to avoid the occurrence of post mortem changes in the muscle.

The muscle was usually taken from the thigh, even that from the minute foetuses, and laid immediately on a piece of card and kept slightly stretched by means of pins applied to either end of the specimen. The specimens were then immersed in cold 3% glutaraldehyde in Sörensen's phosphate buffer at pH 7.4. After 2 hours of fixation the muscle held by the pins was released and cut in small pieces of about 1-2mm thick. These were washed in two changes of Sörensen's buffer at pH 7.4 for 15 minutes each.

Post fixation was carried out for 2 hours at room temperature in cold 1% osmium tetroxide in Michaelis' Veronal- acetate buffer at pH 7.4. After being washed in distilled water and dehydrated in ascending grades of alcohols, they were placed successively in propylene oxide and in a mixture of equal parts of propylene oxide and Epon 812.

Finally they were embedded in fresh Epon mixture. Sections, 1-2μ thick were cut on an LKB Ultratome and stained with toluidine blue by the method of Trump, Smuckler and Benndítt (1961) and examined by light microscopy. Thin sections of appropriate regions were collected on copper grids, stained by uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Siemens Elmiskop I.

RESULTS

Nerves can be identified within the muscle of the foetus at nine weeks: the nerves consist of groups of many closely packed axons of varying diameter (Fig. 1 and 2). The axons are surrounded by the cytoplasm of one or more Schwann cells. With maturation the number of Schwann cells steadily increases: they multiply by mitosis (Fig. 3 and 5) and in the developing nerves centrioles are frequently found near the nucleus of the Schwann cells (Fig. 6). As development advances fewer axons are enveloped by each Schwann cell: there is still however marked variation in the diameter of the axons (Fig. 6 and 7). As early as ten weeks development there occur some Schwann cells which contain only one axon and these Schwann cells may be isolated from other structures (Fig. 4). Single axons in a Schwann cell are those which are destined to become myelinated. Each Schwann cell is covered by a layer of basement membrane and next to the basement membrane occur collagen fibrils. By twelve weeks development gaps begin to appear between the bundles of axons surrounded by Schwann cell cytoplasm and many collagen fibrils surround the Schwann cell cytoplasm lodging these axons (Fig. 6). Fibroblasts can also be identified at this stage between the Schwann cells. Schwann cells containing only one axon with a long and undulating mesaxon may also be seen at twelve weeks development (Fig. 6).

Already at twelve weeks concentrically arranged cells surround the bundles of axons which form the nerve. These cells are the perineurial cells (Fig. 6 and 8). They have an elongated nucleus and long cytoplasmic processes which surround the various nerves. At sixteen weeks the perineurial cells although well differentiated

(Fig. 8), do not like the perineurial cell in the adult exhibit any basement membrane surrounding their plasma membranes. However, a considerable number of collagen fibrils occur between the perineurial cell. The cytoplasm of the perineurial cell contains mitochondria and a considerable amount of rough endoplasmic reticulum, many ribosomes and some vesicles particularly near the nucleus (Fig. 8). Blood capillaries occur between the perineurial cell in the foetus of eighteen weeks (Fig. 14).

Up to sixteen weeks development none of the axons are myelinated but at eighteen weeks many Schwann cells contain only one axon which is surrounded by multiple layers of myelin (Fig. 9, 10, 11 and 12), but some axons of the same diameter as the myelinated axons are however unmyelinated (Fig. 13). At eighteen weeks both the Schmidt- Lantermann clefts (Fig. 10) and the nodes of Ranvier (Fig. 12) of the myelinated nerves are well differentiated. The layer of Schwann cell cytoplasm around the myelin sheath and between the axolemma and the myelin sheath (Fig. 11 and 12) is very scanty. At twenty eight weeks the nerves found amongst the muscle fibers present the same structural appearance as those seen in the adult (Fig. 15). The structures within the axons from nine weeks are well defined and consist of filaments, tubules, mitochondria and also occasional vacuoles are readily identified.

COMMENTS

The earliest stages of the developing muscle examined already showed evidence of the formation of nerves. This was at nine weeks. The nerves consist of large collections of axons within the cytoplasm of one or two Schwann cells which are covered by basement membrane: this in turn is surrounded by bundles of collagen fibrils.

As maturation advances the Schwann cells multiply and each Schwann cell contains fewer axons than at earlier stages in development. In addition processes of Schwann cells contain small groups of axons. Collagen fibrils surround the basement membrane of the Schwann cells and their processes. Some Schwann cells at twelve weeks development contain only one axon. Often these axons have a very long mesaxon a structure first defined by Robertson (1955), but as yet there is no evidence of the formation of myelin.

How the spiralled lamellae of the myelin sheath are formed has been the subject of continuous discussion. No definite solution has yet been found but the most appropriate explanation is that they are formed by rotation of the Schwann cell around the axon. This is supported by observations made by several authors including Geren (1954), Peterson and Murray (1955), Murray (1959) and Pomarat et al. (1967). Unfortunately, it was not possible in the present work to detect how the myelin sheath is formed, but by eighteen weeks the single axons in many of the Schwann cells are already surrounded by multiple layers of myelin. The myelin sheath is at this time formed of multiple layers and in longitudinal sections of nerves the Schmidt-Lantermann clefts, whose structure was first elucidated by electron microscopy in 1958 by J. D. Robertson, are well differentiated. At this stage the nodes of Ranvier are also

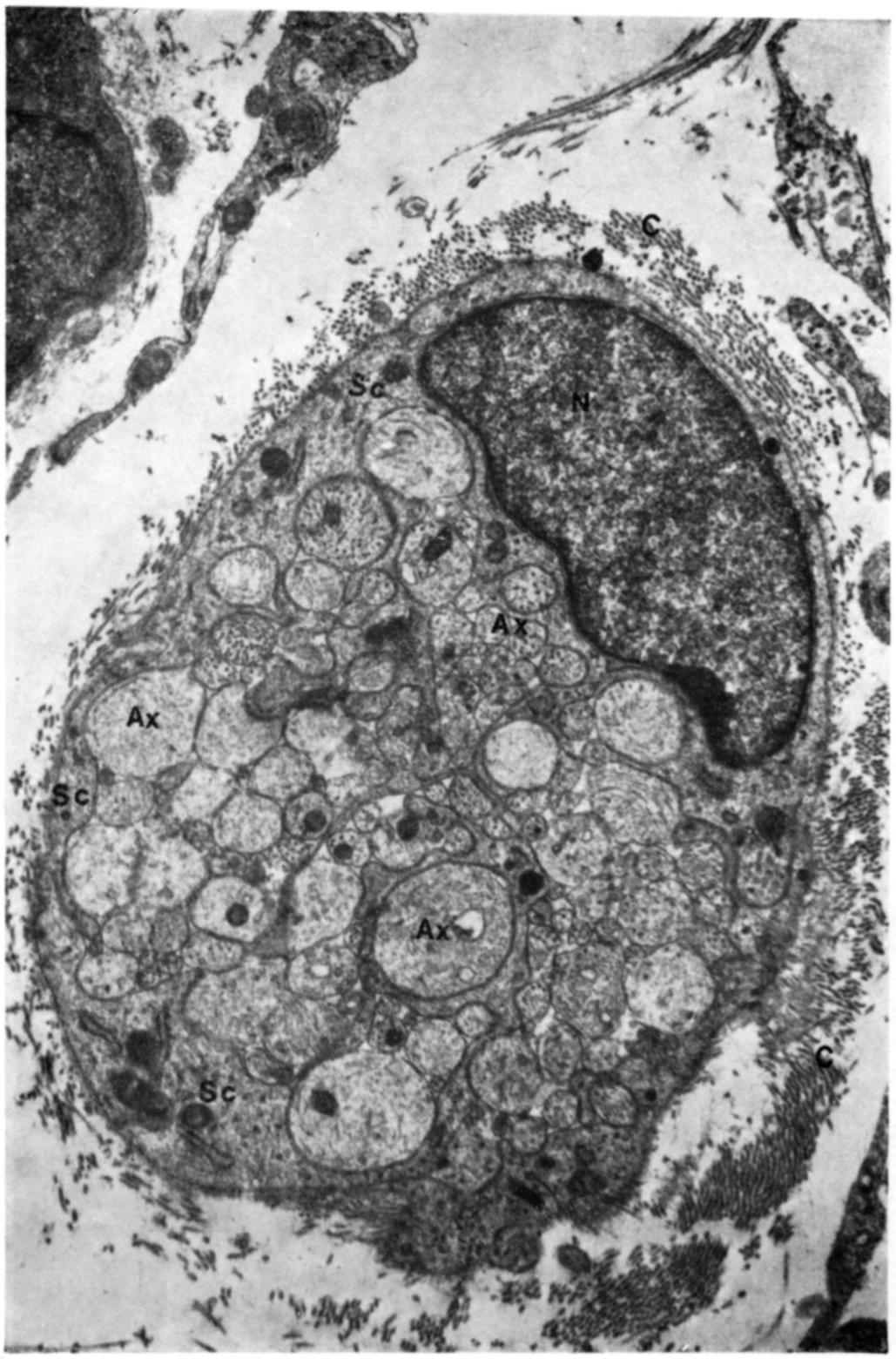

Fig. 1 — One Schwann cell with a prominent nucleus extends its cytoplasm around a group of closely applied axons of a developing nerve. The plasma membrane of each axon is clearly defined and in the axon occur neurofilaments, neurotubules and occasional mitochondria. Groups of collagen fibrils are seen surrounding the nerve 9 weeks, X 12,000. Ax = axons, C = collagen, N = nucleus, Sc = Schwann cell.

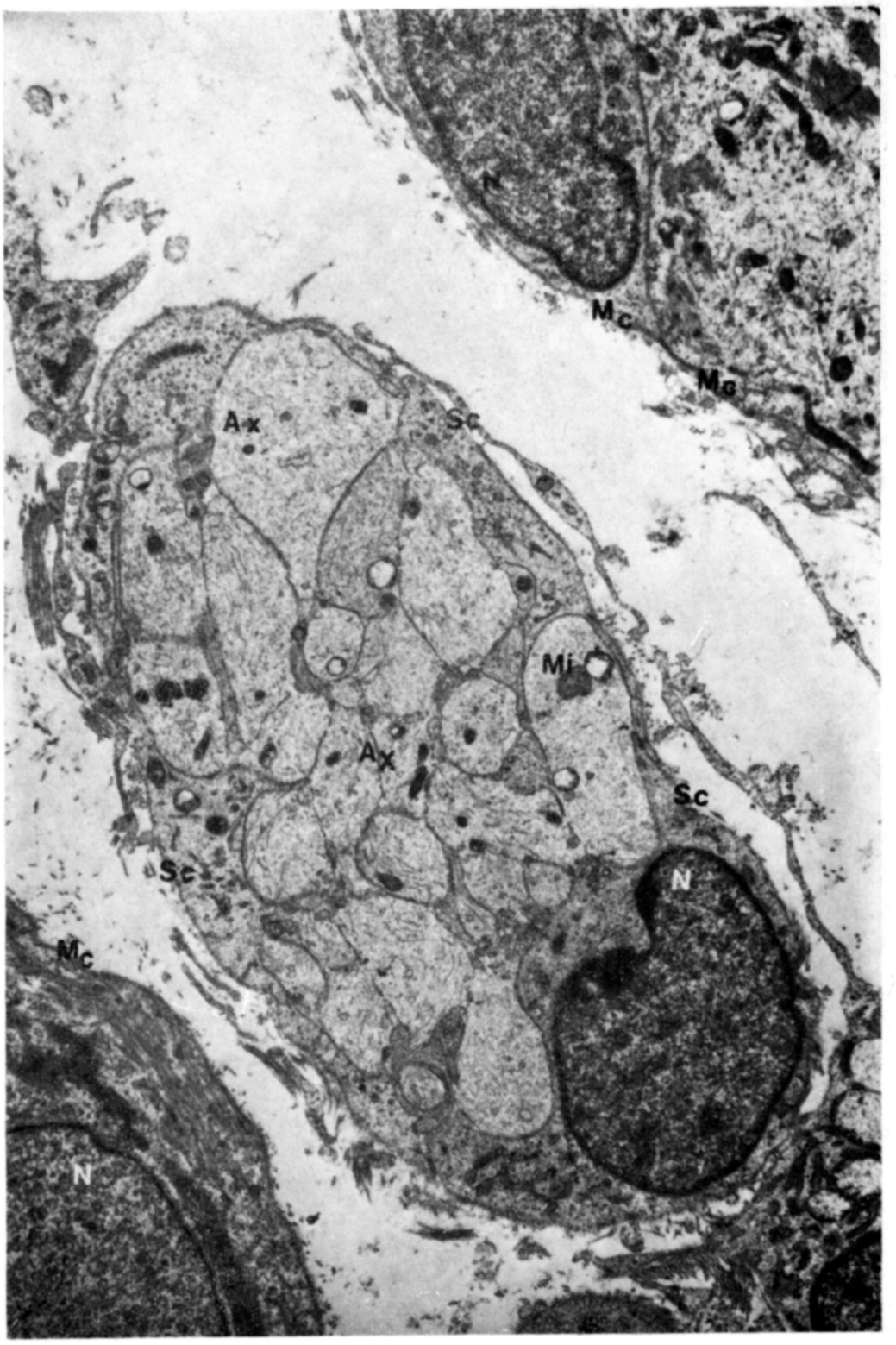

Fig. 2 — A developing nerve lies between muscle cells and consists of a group of axons, each axon with a well defined axolemma, surrounded by a Schwann cell. 10 weeks, X 5,000. Ax = axons, Mc = muscle cells, Mi = mitochondria, N = nucleus, Sc = Schwann cell.

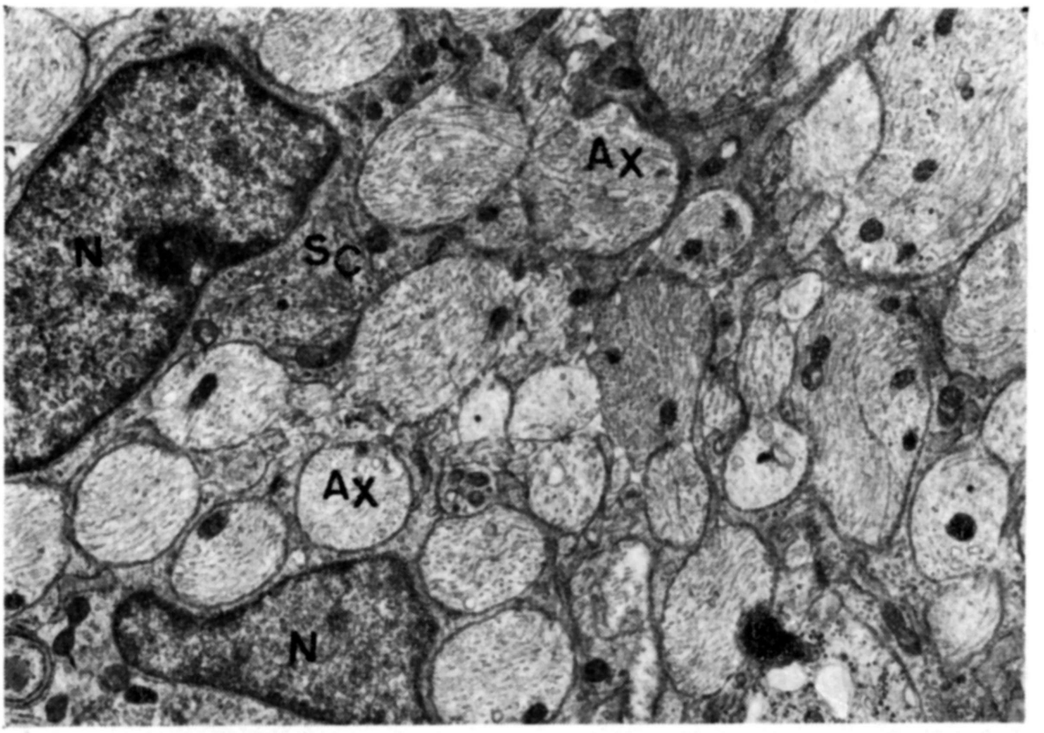

Fig. 3 — As development advances more Schwann cells appear in the nerve and their cytoplasm extends between individual axons. 10 weeks, X 12,000. Ax = axon, N = nucleus, Sc = Schwann cell.

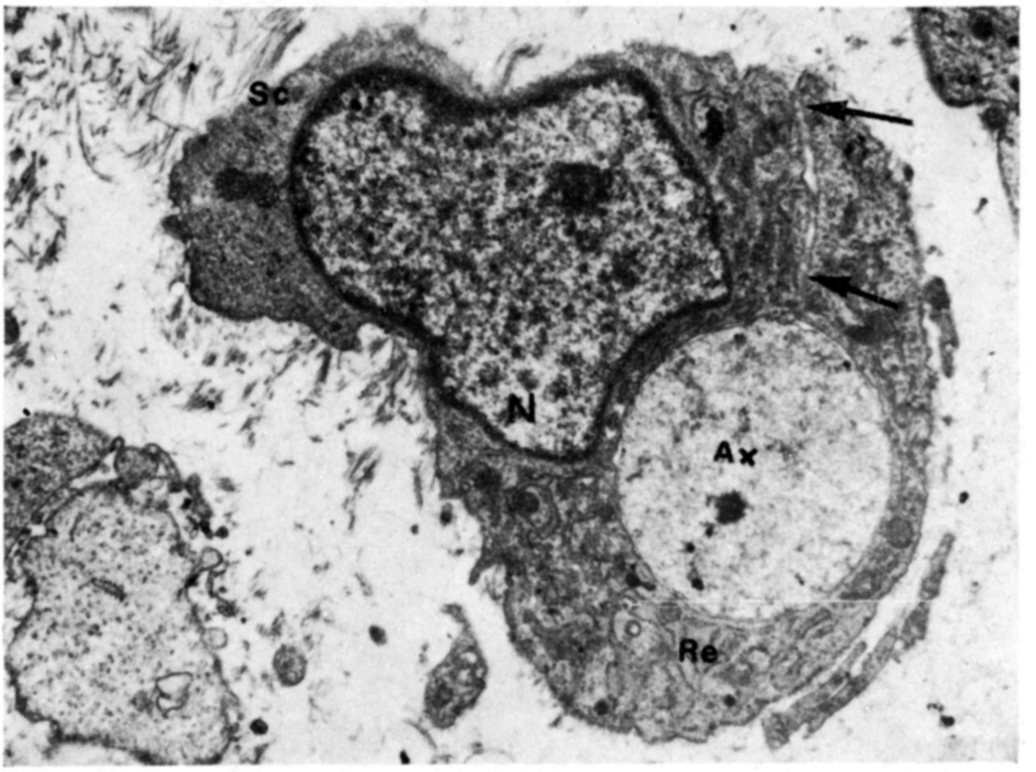

Fig. 4 — Eventually axons which are destined to become myelinated lie solitary in the cytoplasm of the Schwann cell. The mesaxon (arrowed) of this nerve fibre does not yet show any evidence of encircling the axon and neurofilaments and neurotubules are not seen. The Schwann cell cytoplasm is very rich in ribosomes and rough endoplasmic reticulum. 10 weeks, X 6,000. Ax = axon, N = nucleus, Re = rough endoplasmic reticulum, Sc = Schwann cell.

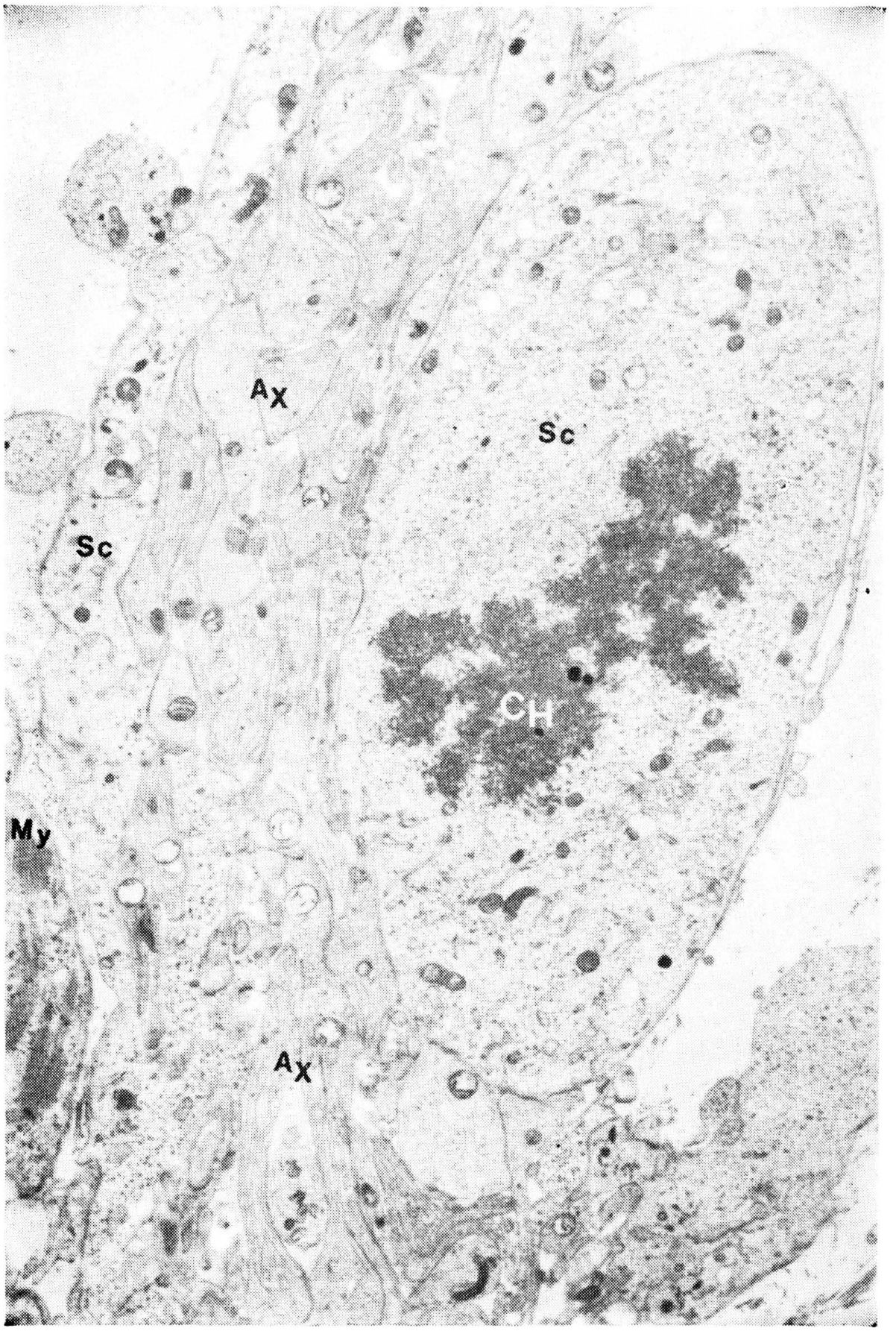

Fig. 5 — An oblique section of a nerve shows a Schwann cell in mitosis and processes of Schwann cell cytoplasm at the periphery of collagen fibrils. 14 weeks, X 20,000. Ax = axons, C = collagen, My = Myotube, Sc = Schwann cell.

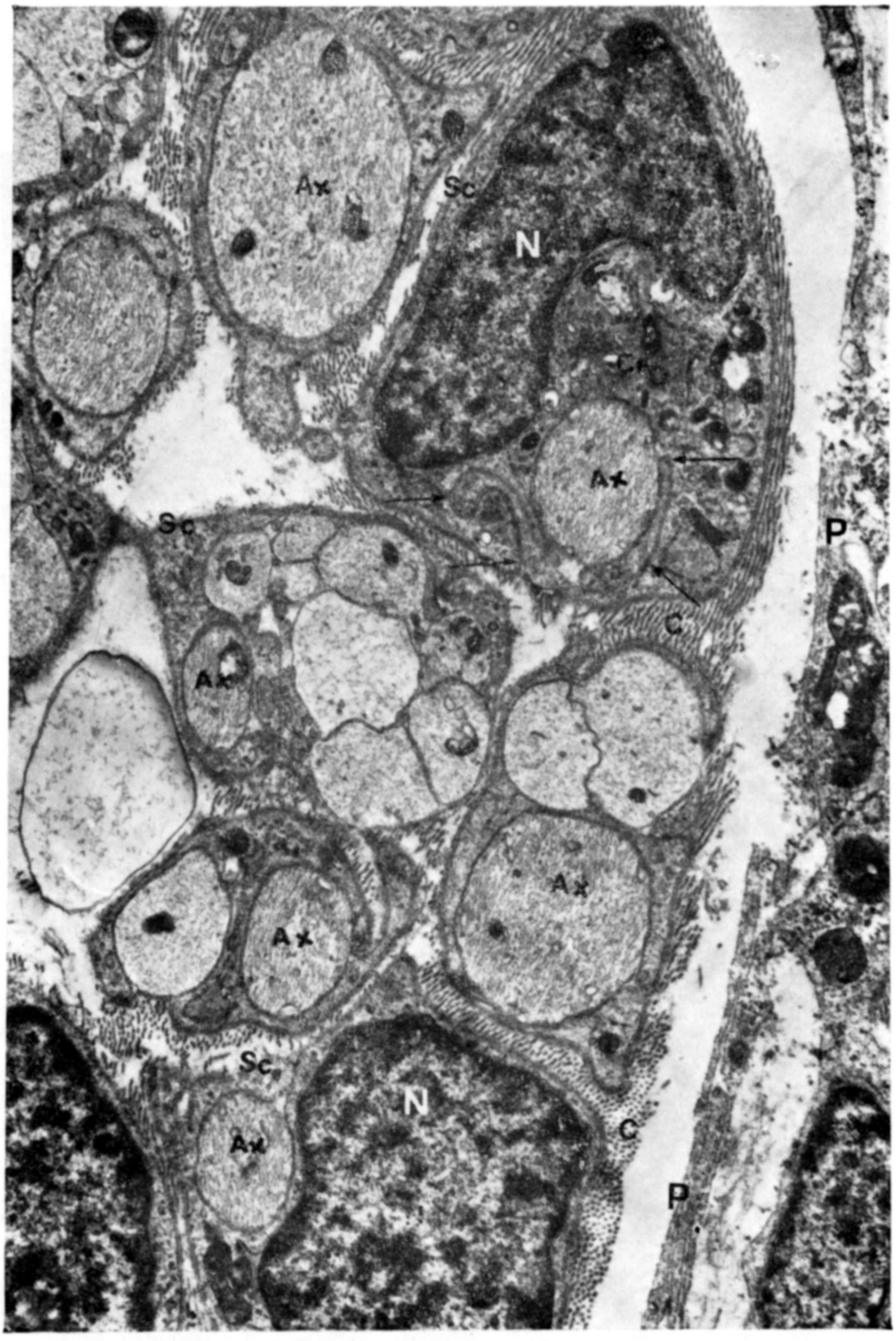

Fig. 6 — A transversse section of a developing nerve shows a single axon in each of the two Schwann cells lying top and bottom right. The mesaxon is arrowed. In the top cell it is prolonged and undulating suggesting that myelination is about to occur. In the Schwann cell at the top two centrioles lie between the nucleus and the axon. Groups of axons are enclosed by processes of Schwann cell cytoplasm. The plasma membranes of the Schwann cells and their processes are covered by basement membrane and in turn by numerous collagen fibrils. To the right are closely applied cell processes of developing perineurial cells. Neurotubules and neurofilaments are evident in the axoplasm of all axons. 12 weeks, X 13,000. Ax = axons, C = collagen, Ce = centrioles, N = nucleus, P = perineurial cells, Sc = Schwann cells.

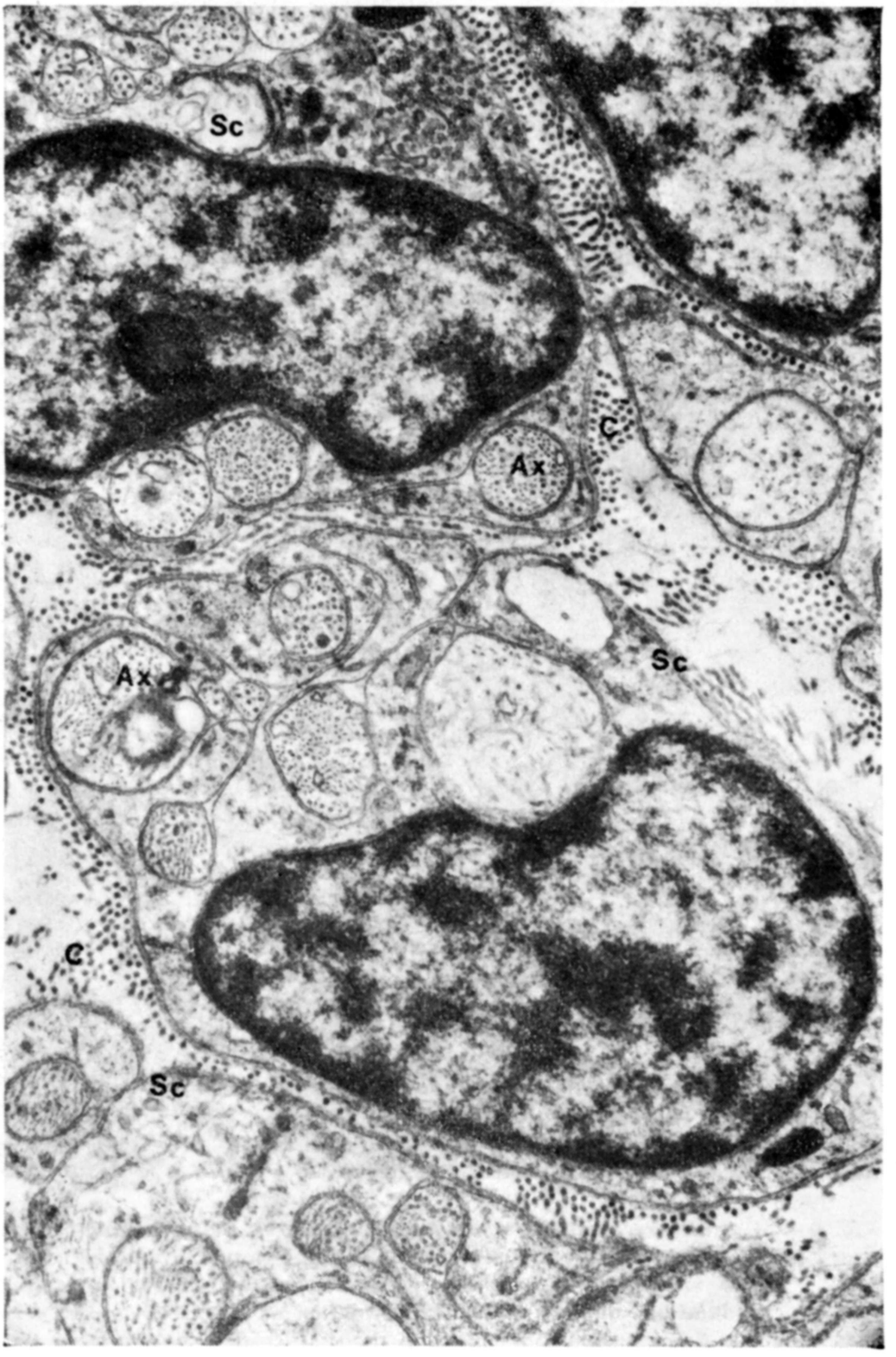

Fig. 7 — Neurotubules and neurofilaments are clearly seen in all axons. The Schwann cells are covered by basement membrane and collagen fibrils. 14 weeks, X 20.000. Ax = axons, C = collagen, Sc = Schwann cell.

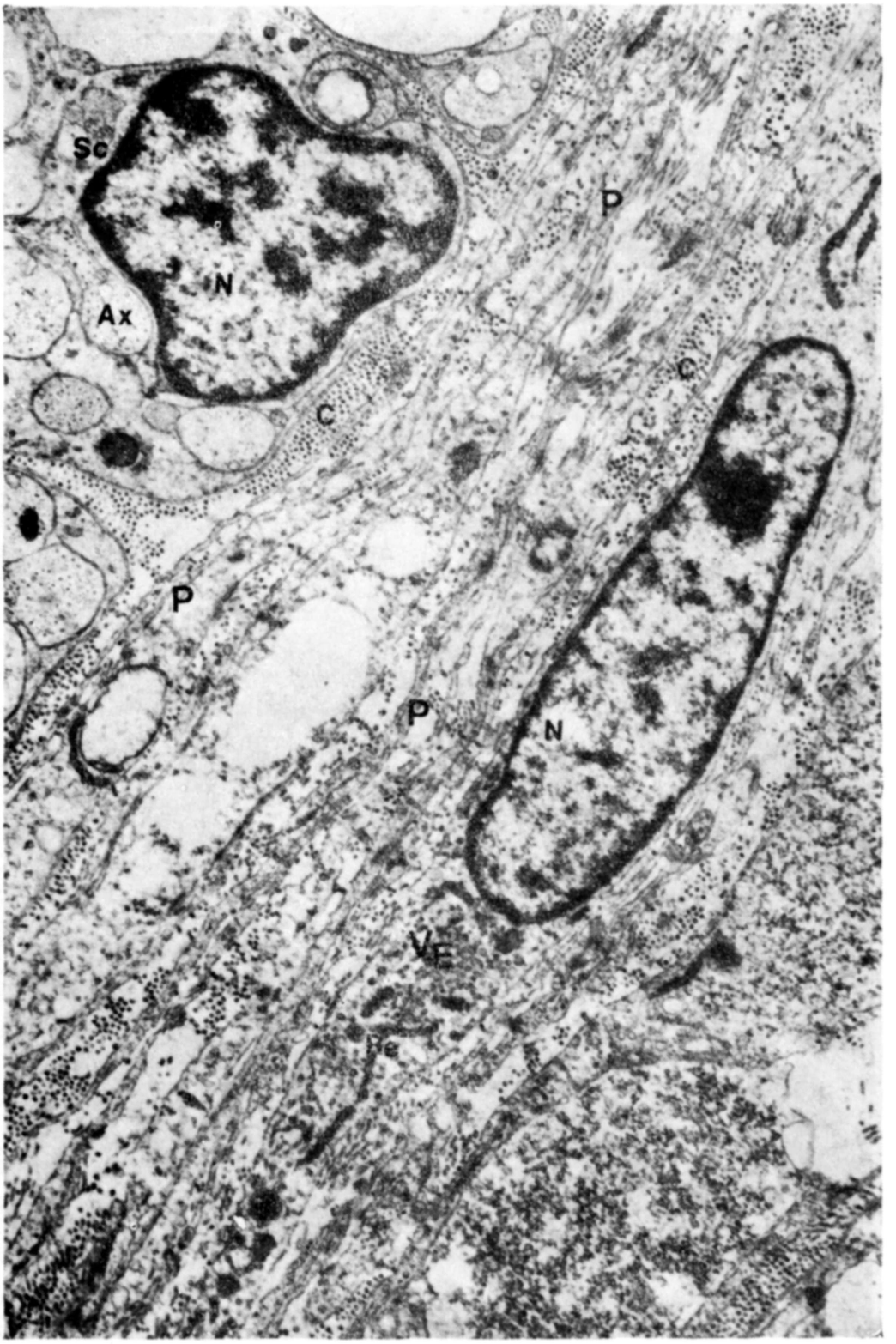

Fig. 8 — Top left lie a Schwann cell and processes of a Schwann cell containning axons. Collagen lies between them and also between the obliquely running perineurial cells. One of the perineurial cells contains a nucleus with a well marked nucleolus and its cytoplasm is rich in vesicles and rough endoplasmic reticulum. No basement membrane is apparent on the surface of these cells. 16 weeks, X 10,000. Ax = axons, C = collagen, N = nucleus, P = perineurium, Sc = Schwann cell, Ve = vesicles.

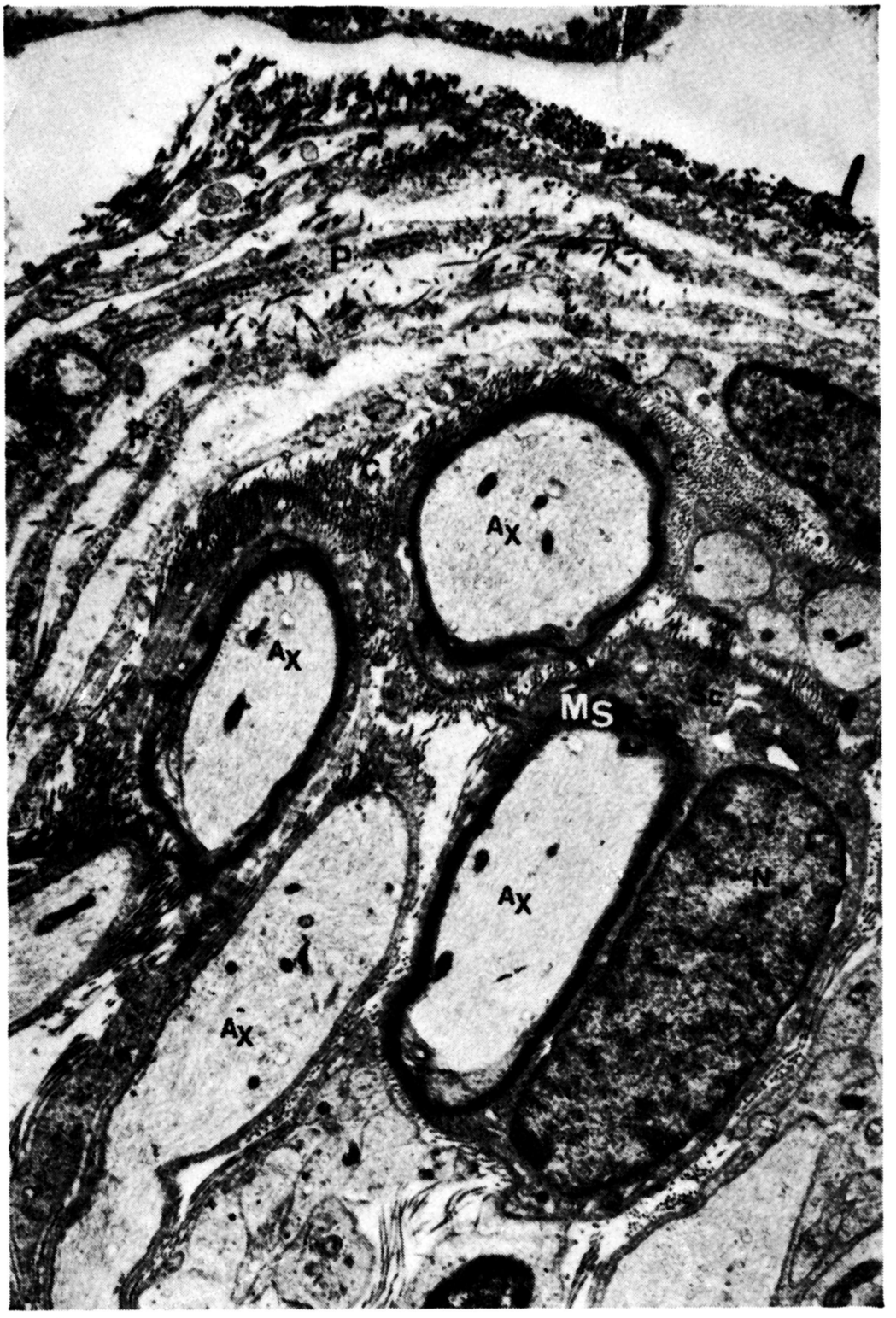

Fig. 9 — An oblique section of a nerve shows that some axons now possess a dense myelin sheath. A single Schwann cell surrounds each myelinated axon and also some of the isolated axons which are not yet myelinated. Basement membrane invests the Schwann cells and collagen fibrils lie between them. Concentric layers of perineurial cells surround the nerve. 18 weeks, X 20,000. Ax = axons, C = collagen, My = myelin sheath, N = nucleus, P = perineurial cell, Sc = Schwann cell.

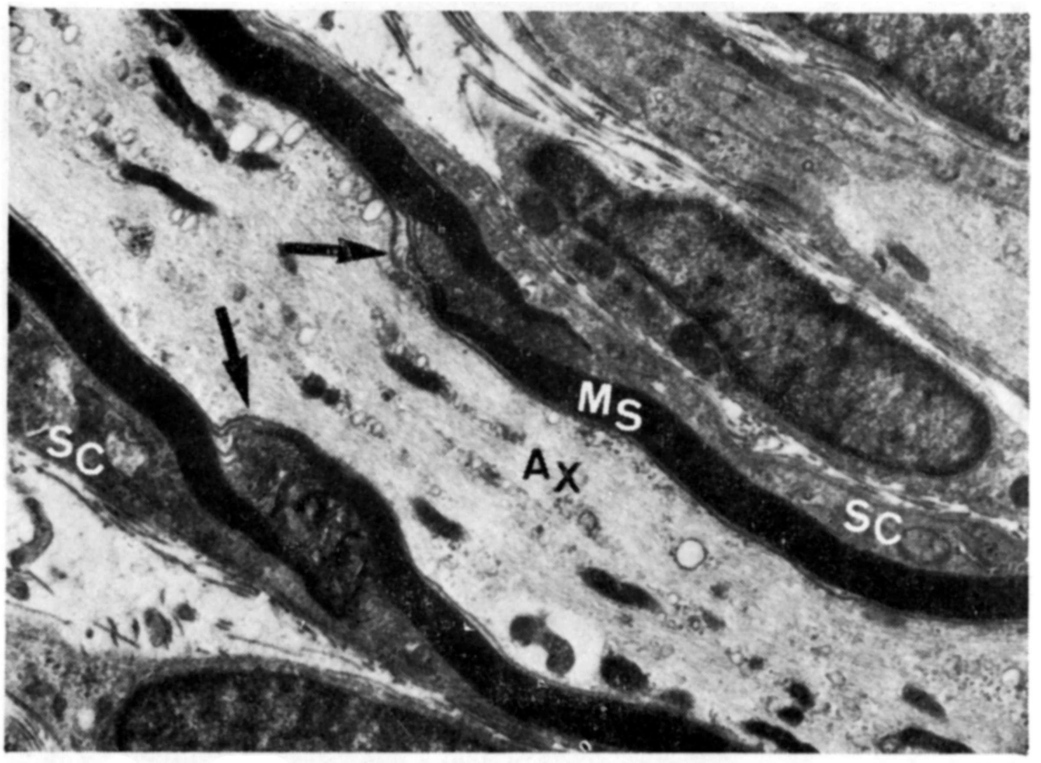

Fig. 10 — In the myelin sheath of the nerve cells occur Schmidt-Lantermann clefts (arrowed). Neurotubules, neurofilaments and mitochondria are evident in the axons. A definite layer of Schwann cell cytoplasm is seen around the myelin sheath. 18 weeks, X 12,000 Ax = axon, Ms = myelin sheath, Sc = Schwann cell.

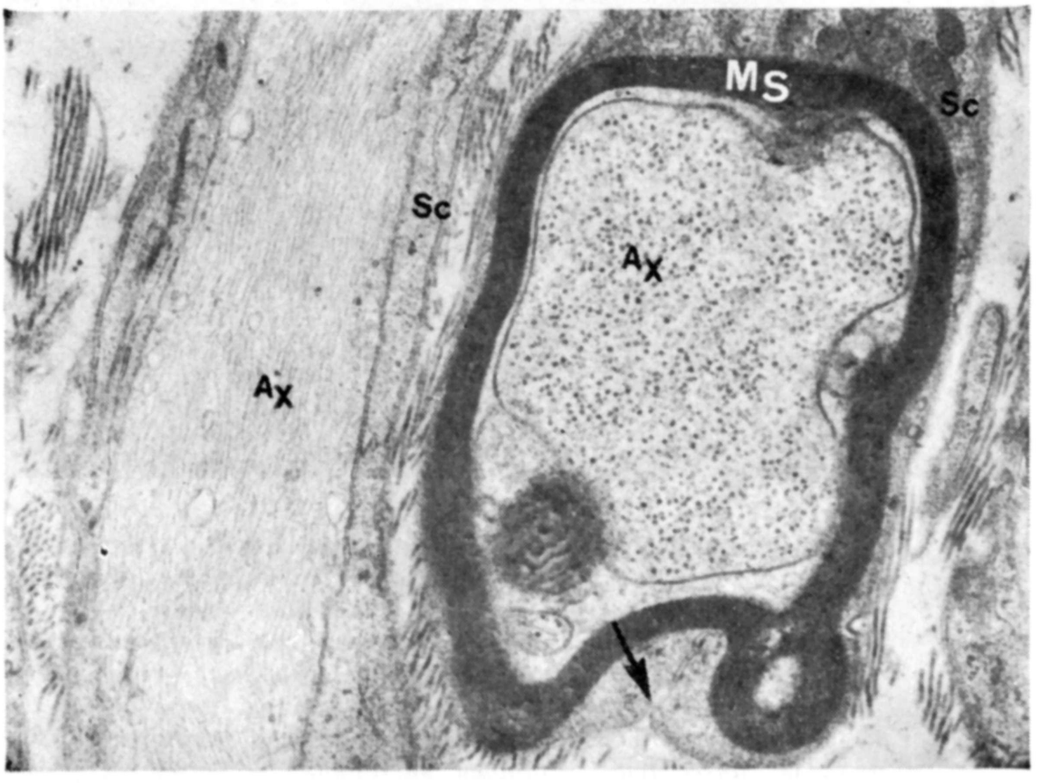

Fig. 11 — Two axons are shown. The axon on the left (longitudinal section) is not myelinated and that on the right (cross section) shows a well formed myelin sheath. Both have a large amount of neurofilaments, neurotubules and a well defined axolemma. The external mesaxom is arrowed in the axon already myelinated. 18 weeks, X 15,000. Ax = axon, Ms = Myelin sheath, Sc = Schwann cell.

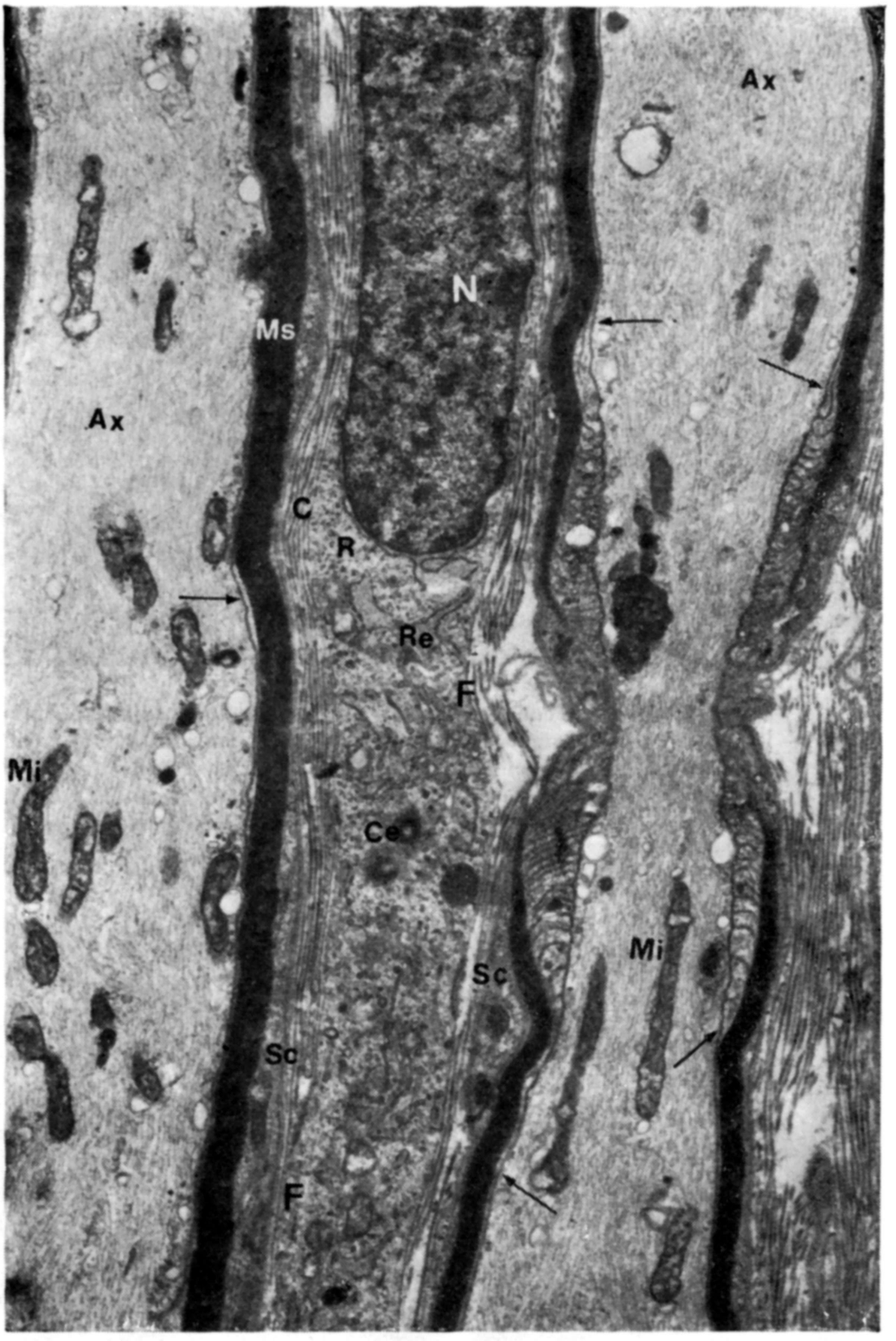

Fig. 12 — Two well myelinated axons are cut longitudinally. The myelin is bounded by a distinct membrane, the axolemma (arrowed) and a narrow space lie between it and the myelin which is surrounded by a thin sheet of Schwann cell cytoplasm. The axons contain many neurofilaments and some mitochondria. The nerve on the right shows the distinct terminal loops of myelin which form the node of Ranvier where the diameter of the axon is constricted. Collagen surrounds the basement membrane which invests the Schwann cell. A fibroblast with a dense nucleus and cytoplasm rich in ribosomes and rough endoplasmic reticulum lies between the two nerves. In the cytoplasm near the pole of the nucleus lie two centrioles. 18 weeks, X 13,000. Ax = axons, C = collagen, Ce = centrioles, F = fibroblast, Mi = mitochondria, Ms = myelin sheath, N = nucleus, R = ribosomes, Re = rough endoplasmic reticulum, Sc = Schwann cell.

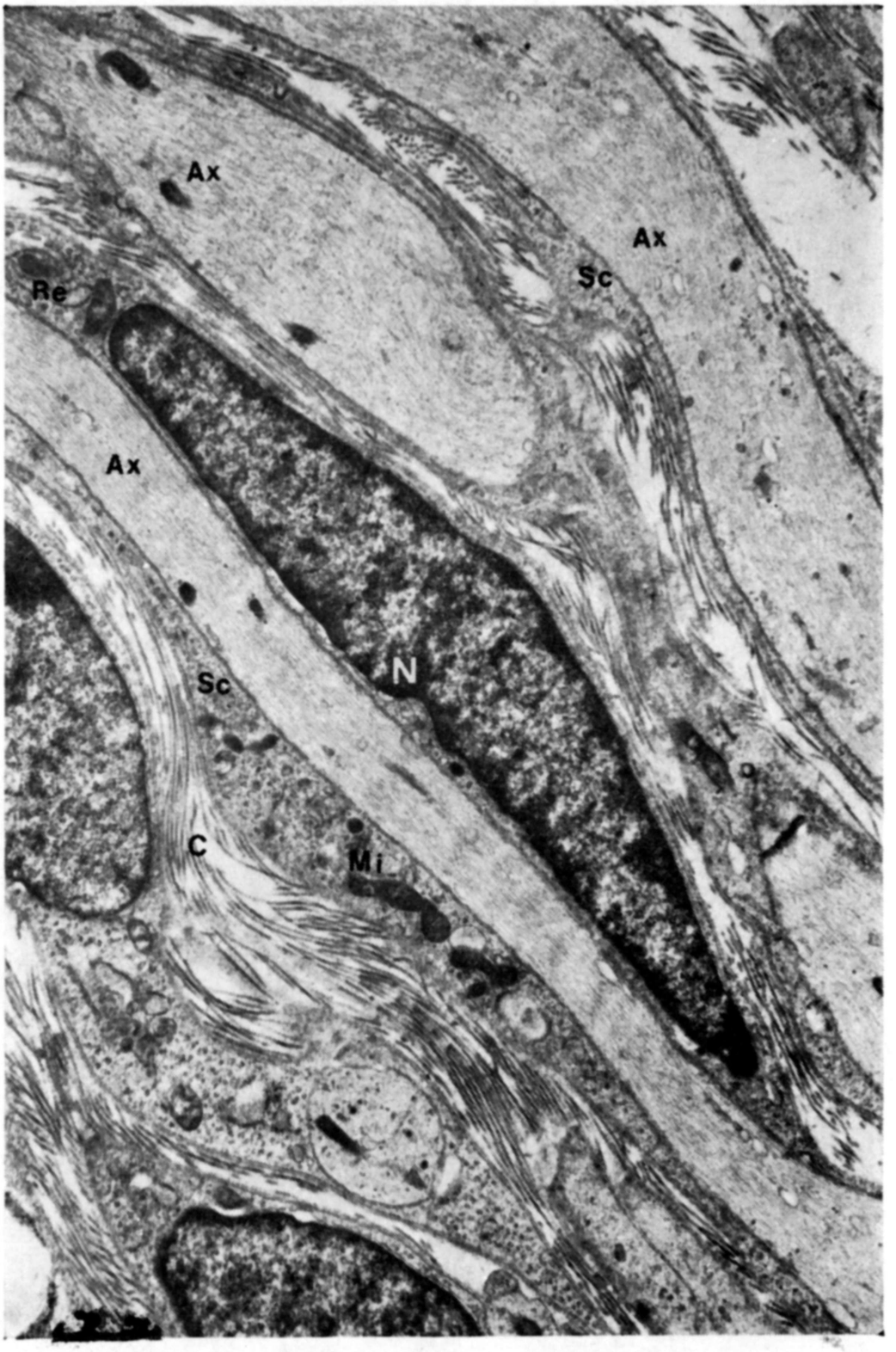

Fig. 13 — A Schwann cell in the centre of the illustration surrounds a single unmyelinated axon cut longitudinally. The Schwann cell nucleus is elongated and has a fairly dense chromatin. 18 weeks, X 7,500. Ax. = axon, C = collagen, Mi = mitochondria, N = nucleus, Sc = Schwann cell.

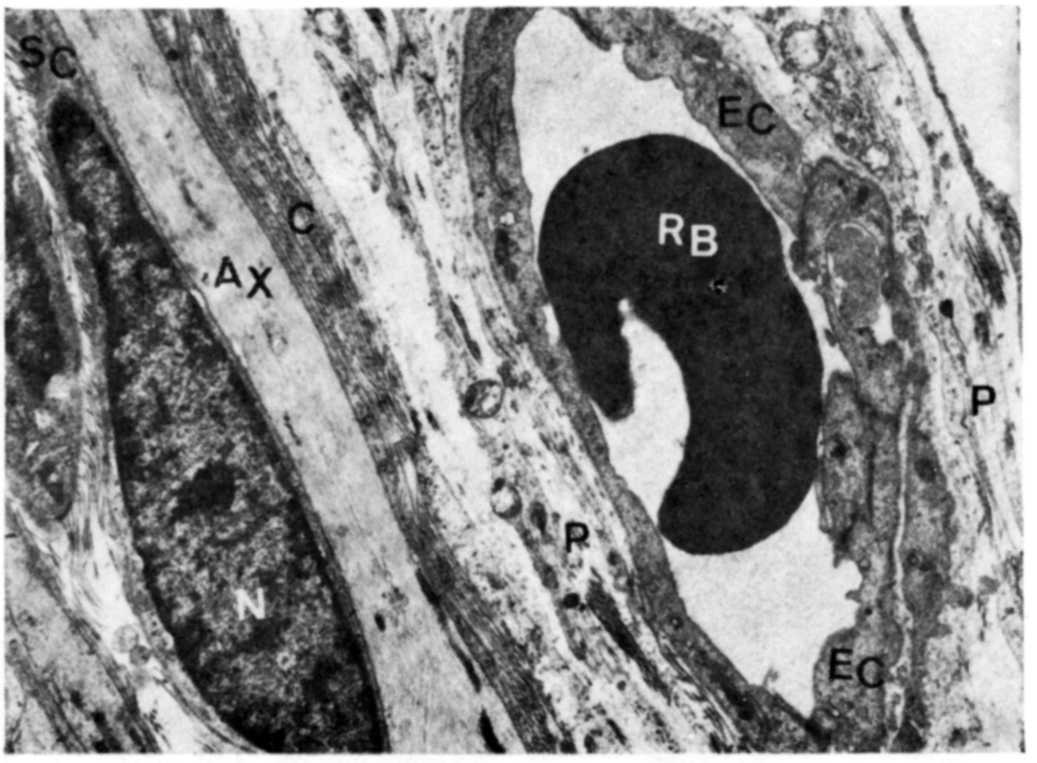

Fig. 14 — Blood vessels lined by closely applied endothelial cells occur within the capsule of perineurial cells surrounding the developing nerve. 18 weeks, X 7,000. Ax = axon, C = collagen, Ec = endothelial cell, N = nucleus, P = perineurial cell, Rb = red blood cell, Sc = Schwann cell.

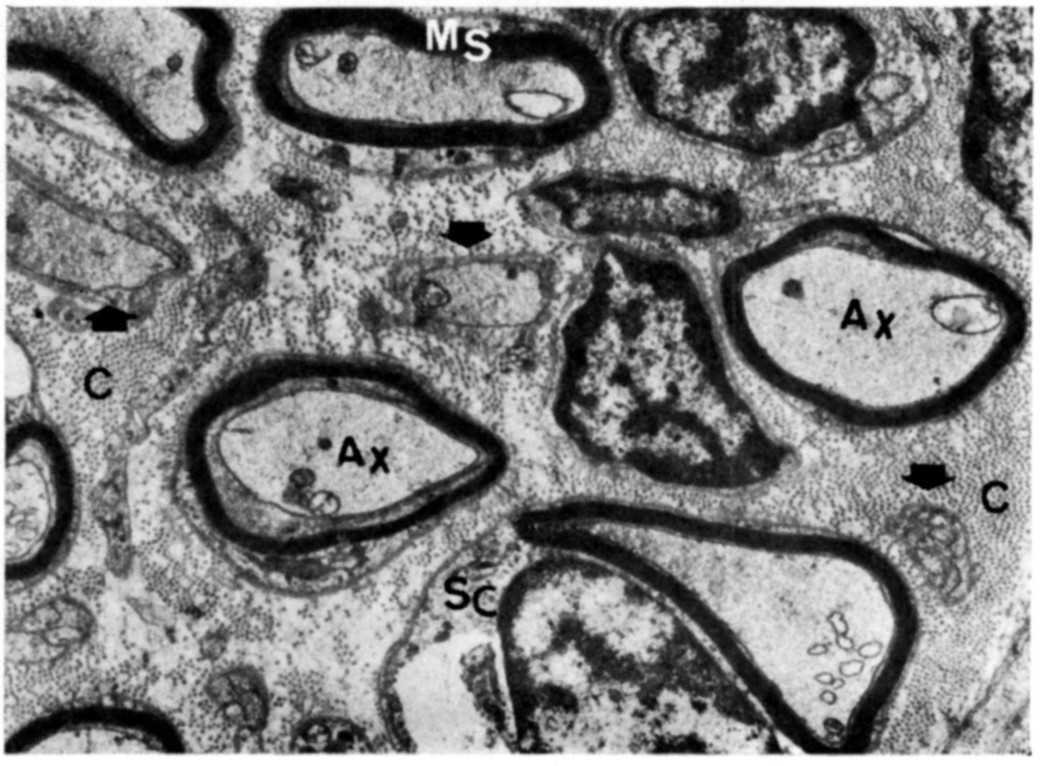

Fig. 15 — At 23 weeks development many nerve fibres are already well myelinated and there is no marked variation of their diameter. The layer of Schwann cell cytoplasm around the myelinated fibres is relatively scanty. Some unmyelinated axons (arrowed) can be seen amongst the myelinated nerve fibres. A large amount of collagen surrounds the nerve fibres. 28 weeks, X 6,000. Ax = axon, C = collagen, My = myelin sheath, Sc = Schw cell.

well formed and at the nodes the axons are somewhat constricted. On either side of the node the terminal loops of the myelin sheath has the characteristic pattern of that seen in the adult. Curiously, Ochoa (1971) found that the onset of myelination in sural foetal nerves also starts at the age of eigthteen weeks. Individual axons which are of the same diameter as the myelinated fibres also lie singly within Schwann cells but as yet no myelin formation can be seen around them. From the earliest stages of the axons observed neurofilaments, neurotubules, vesicles and mitochondria can be identified within them. These structures run parallel to the long axis of the axons. Microtubules were first described in the neuron by Palay (1956, 1958). It is of interest that in immature animals (Bodian, 1966; Peters and Vaughn, 1967) nerve fibers contain many microtubules but very few neurofilaments. Peters and Vaughn (1967) state that in the optic nerve of rats neurofilaments are common only at about five days after birth and they postulate that the neurofilaments are formed by the break down of the walls of the microtubules. In the present investigation, it is interesting to note that neurofilaments and neurotubules are seen abundantly in the axons of the intramuscular nerves of foetuses at all stages of development and that the number of such structures steadily increases with maturation.

Fibroblasts also occur amongst the Schwann cells containing the axons but they are relatively few compared to the number of Schwann cells. Undoubtedly the fibroblasts contribute to the formation of collagen within the nerves but most authorities now accept that the Schwann cells themselves contribute to the formation of the collagen around them.

The first evidence of the formation of perineurium around the nerves is seen at twelve weeks and at this stage the perineurium is not complete but by sixteen weeks the perineurium is well differentiated and consists of several layers of slender elongated cells surrounding the nerve. In the foetus of eighteen weeks blood vessels-the "vasa nervorun"-occur between the perineurial cells. From the earliest evidence of the formation of the perineurium, collagen fibrils occur between the perineurial cells but not even at the later stages of the nerves observed is there any evidence of the formation of basement membrane around them.

RESUMO

Ultraestrutura de nervos intramusculares humanos em desenvolvimento.

Terminações nervosas intramusculares são observadas já na nona semana de vida intra-uterina e consistem de grupos de axônios não mielinizados, de diâmetro variável, envolvidos por poucas células de Schwann e sem nenhum espaço entre os axolemas. Nesta e nas fases imediatamente seguintes as células de Schwann são comumente vistas em mitose. Na décima segunda semana, pequenos espaços preenchidos por fibras colágenas aparecem entre os axônios que são agora envolvidos em pequenos grupos ou individualmente pelas células

de Schwann as quais , por sua vez, aparecem em maior número. Naqueles axônios únicos a célula de Schwann envolvente apresenta mesaxônios, o que constitue nos primeiros sinais de mielinização. Ainda nesta fase não observadas células perineurais pouco diferenciadas. Por volta da décima sexta semana os axônios e o perineuro atingem um grau avançado de diferenciação, embora ainda não se possa visualizar bainhas de mielina. Somente na décima oitava semana de desenvolvimento é que são visualizadas as bainhas de mielina com suas típicas incisuras de Schmidt-Lantermann e nodos de Ranvier perfeitamente formados. Ainda na décima oitava semana aparecem perfeitamente formados os "vasa nervorum" que se dispoem no sentido longitudinal das fibras nervosas, envoltos pelo tecido conjuntivo das mesmas. Da vigésima oitava semana em diante o aspecto encontrado é semelhante ao das terminações nervosas do adulto.

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Current address of Dr. Guilberto Minguettei: Rua Brigadeiro Franco 122 — 80000 Curitiba, PR — Brasil.