VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 64, No. 4, pp. 253–268

ISSN 0015–5659
www.fm.viamedica.pl

Schwann units in the human foetal phrenic nerve

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[Received 27 August 2005; Accepted 27 October 2005]

In three human foetuses aged 15, 17, and 23 weeks the number of axons surrounded by single Schwann cells was counted. These Schwann cell/axon complexes form the Schwann units. The largest Schwann units in the foetus aged 15 weeks contained 232 axons, in the foetus of 17 weeks the number was 140 and in the foetus of 23 weeks the largest units contained 65 axons.

Key words: human neuroembryology, myelination, Schwann units, phrenic nerve

INTRODUCTION

In the mammalian peripheral nervous system the neural crest is a multipotent precursor population that gives rise to a wide variety of cell types including neurons and Schwann cells [5]. Schwann cells are the PNS counterpart of oligodendrocytes. They surround all peripheral axons, whether myelinated or not, while oligodendrocytic processes do not surround unmyelinated axons in the CNS.

The process of a single Schwann cell may surround many unmyelinated axons. Finally, Schwann cells are covered by a basal lamina, whereas oligodendrocytes are not. The co-ordinated differentiation of axons and myelin-forming cells and the selective elimination of axons, dendrites, and synaptic connections without the death of parent neurons require reciprocal interactions between neurons and glial cells [18, 21]. In higher mammals glial cells vastly outnumber neurons and reciprocal signalling between neurons and glial cells forms the functional units during the development of the nervous system [16].

Schwann cells are the principal glial cells in the peripheral nervous system. Schwann cell precursors give rise to two mature Schwann cell forms, namely myelin-forming and non-myelin-forming Schwann cells [12, 14]. In the adult each myelinating Schwann cell surrounds a single axon and forms a myelin sheath. Each non-myelin-forming Schwann cell surrounds many axons in troughs along its surface. Another type of non-myelin-forming cell is found at the neuromuscular junction and this cell is known as a terminal or perisynaptic Schwann cell [8]. As development proceeds, axon bundles are progressively subdivided and segregated by premyelinating Schwann cell processes, and a one-to-one Schwann relationship of cell to axion is eventually established prior to myelination [28]. Premyelinating Schwann cells exit the cell cycle, synthesise a basement membrane and begin to myelinate associated axons [28]. This occurs in human foetuses around the 13th week [26, 27]. Bundles of axons surrounded by one Schwann cell can be referred to as a Schwann unit.

Several investigations have described a consecutive decrease of axons in Schwann units with the advancement of development [2, 3, 6, 7, 19, 20, 23, 24, 25]. The aim of the present paper is to count the axons in the largest Schwann units in the foetal phrenic nerve.

MATERIAL AND METHODS

The upper thoracic parts of the phrenic nerves were removed in three human foetuses of 135, 149

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and 220 mm C-R length. The ages of the foetuses investigated were 15, 17, and 23 postovulatory weeks. Immediately after operation the nerves were fixed by immersion in cooled 1.2% glutaraldehyde for one hour. The material was then placed for two hours in 2% glutaraldehyde buffered to pH 7.4 with cacodylate. The nerve pieces were rinsed in cacodylate buffer for 24 hours and postfixed in 1% osmium tetroxide. Thin and semi-thin sections were stained with uranyl acetate and lead citrate. The thin sections were examined and photographed in an electron microscope and montages were then constructed. All axons in the montage were counted and the axon diameters measured. The measurements were made using a Leica imaging system.

RESULTS

Foetus of 135 mm C-R length (15 weeks)

The processes of single Schwann cells surrounded many axons, forming Schwann units. The Schwann cells and their processes were covered by a basal lamina. The largest Schwann units contained up to 232 axons (Fig. 1). The diameter of the axons in a particular unit varied from 0.1 to 2.0 μm (Fig. 2). Nerve fascicles were surrounded by distinct perineurium, which at this stage of development is not a regular laminar structure (Fig. 3). The endoneurium contains fascicles of collagenous fibres, glycogen granules, and isolated fibroblasts (Fig. 4, 5). The processes of Schwann cells invaded the nerve fascicles, dividing them into smaller units, some of which contained two or three axons (Figs. 3–5). In the foetus investigated single axons surrounded by Schwann cell processes were observed (Fig. 3–6). The first turns of these processes were thick and irregular. The diameter of the myelinating axons ranged from 0.5 to 2.0 μm.

Foetus of 149 mm C-R length (17 weeks)

In this foetus the following important developmental events were observed:

— a decrease in the number of axons in a Schwann unit;
— the formation of compact myelin;
— an increase in the number of myelinating fibres.

The largest Schwann units contained 140 axons. Most units contained 30 and 18 axons (Fig. 7). Many axons were at the onset of myelination (Fig. 8). Well myelinated fibres were present, containing major dense lines and intraperiod lines with compact myelin (Fig. 9). Schmidt-Lanterman clefts were also present in these fibres (Fig. 9). The number of myelin lamellae may reach 11. The diameters of axons in a Schwann unit varied from 0.2 to 1.5 μm. The diameters of the myelinating axons ranged from 0.6 to 2 μm. The perineurium showed a regular laminated structure.

Foetus of 220 mm C- R length (23 weeks).

In this period of development the number of myelinated fibres in the phrenic nerve increased. The largest Schwann units had 65 axons (Fig. 10). The nerve fibres were in various phases of myelinogenesis (Fig. 11, 12). The diameter of unmyelinated fibres varied from 0.3 to 2 μm. Myelinated fibres had diameters ranging from 0.8 to 3 μm. The perineurium presented a laminar structure with blood vessels and collagenous fibres (Fig. 13). Collagenous fibres and glycogen granules were present in the endoneurium.

DISCUSSION

During development Schwann cells migrate along bundles of axons, proliferate, and synthesise a basal lamina consisting of laminins, fibronectin, type IV collagen and other components [13]. The β1 integrins present on undifferentiated Schwann cells mediate the Schwann cell/basal lamina interactions which are essential for myelinogenesis [4]. Merosin (laminin -2), which is a substrate adhesion molecule produced by Schwann cells [15], is implicated in peripheral myelinogenesis. It is present in the endoneurial basement membrane of developing and mature peripheral nerves [11] and promotes Schwann cell migration [1]. By 11 weeks Schwann cells in human foetal nerves express β4 integrins and are surrounded by continuous basal laminae [11]. This coincides with our earlier studies on the myelination of human peripheral nerves, in which the onset of myelin formation was found at the end of the 13th week [26].

The major integral membrane protein of peripheral nerve myelin is protein zero (P_0), a member of a very large immunoglobulin superfamily [17]. This protein makes 40–80% of the protein complement of peripheral nerve myelin. The interactions between the extracellular domains of P_0 molecules expressed on one layer of the myelin sheath and those of the apposing layer yield a characteristic regular periodicity that can be seen as the intraperiod line representing the extracellular apposition of the myelin bilayer as it wraps around itself [10]. In peripheral nerves, although other molecules are present in small quantities in compact myelin (for example, MAG

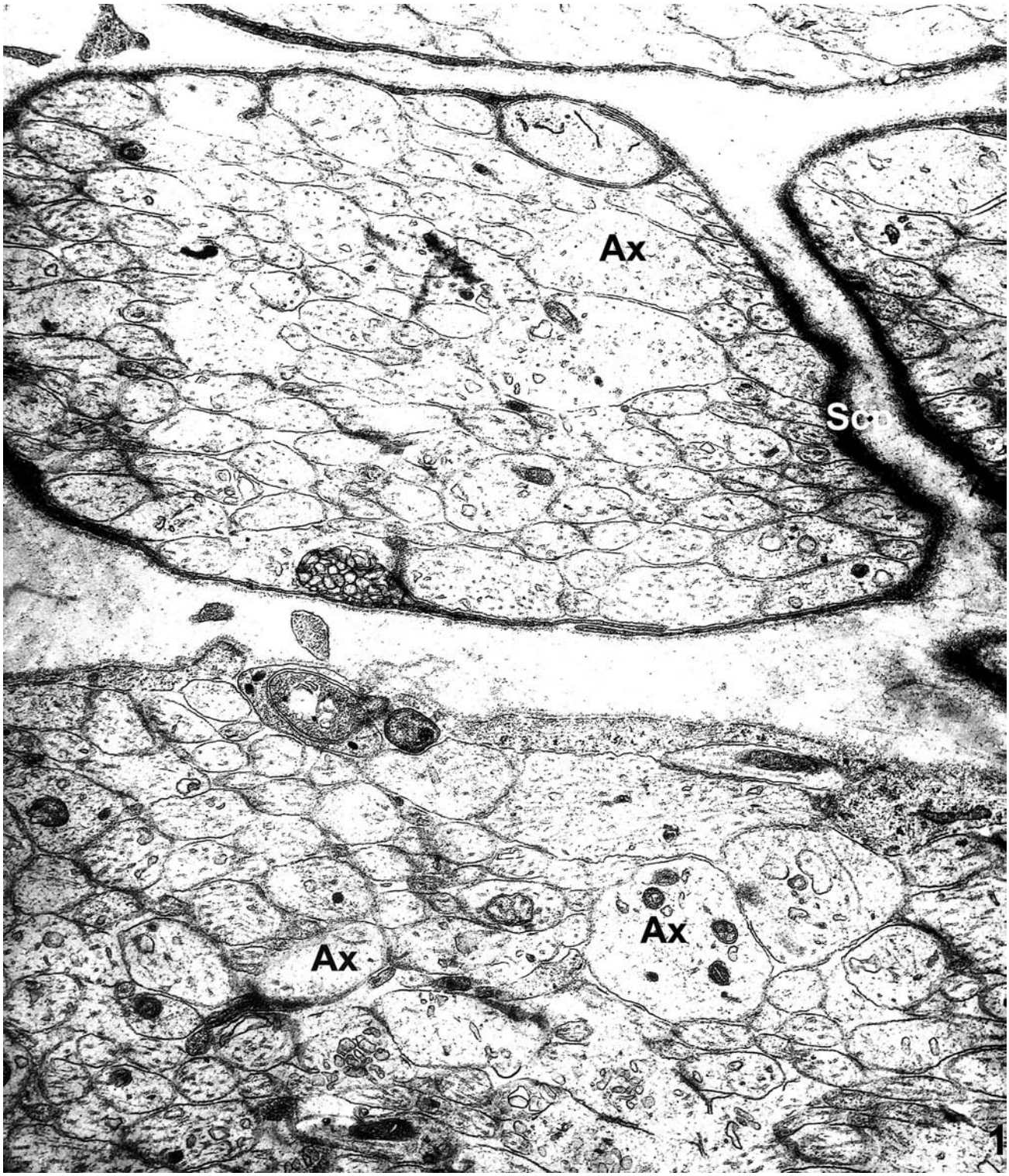

Figure 1. The left phrenic nerve in a human foetus of the 15th week. × 17500. Ax — axon, Scp — Schwann cell process.

PMPZZ) and may have important functions, compaction is accomplished solely by P_0–P_0 interactions at both extracellular and intracellular surfaces [9, 22].

It is known that the onset of myelin formation is preceded by axon isolation and a relationship of one axon to one Schwann cell [3, 6, 7]. Compact myelin formation is observed in human foetuses by the 17th week. This was found in our earlier studies on foetal nerves in which foetal age was precisely established [2, 23–27].

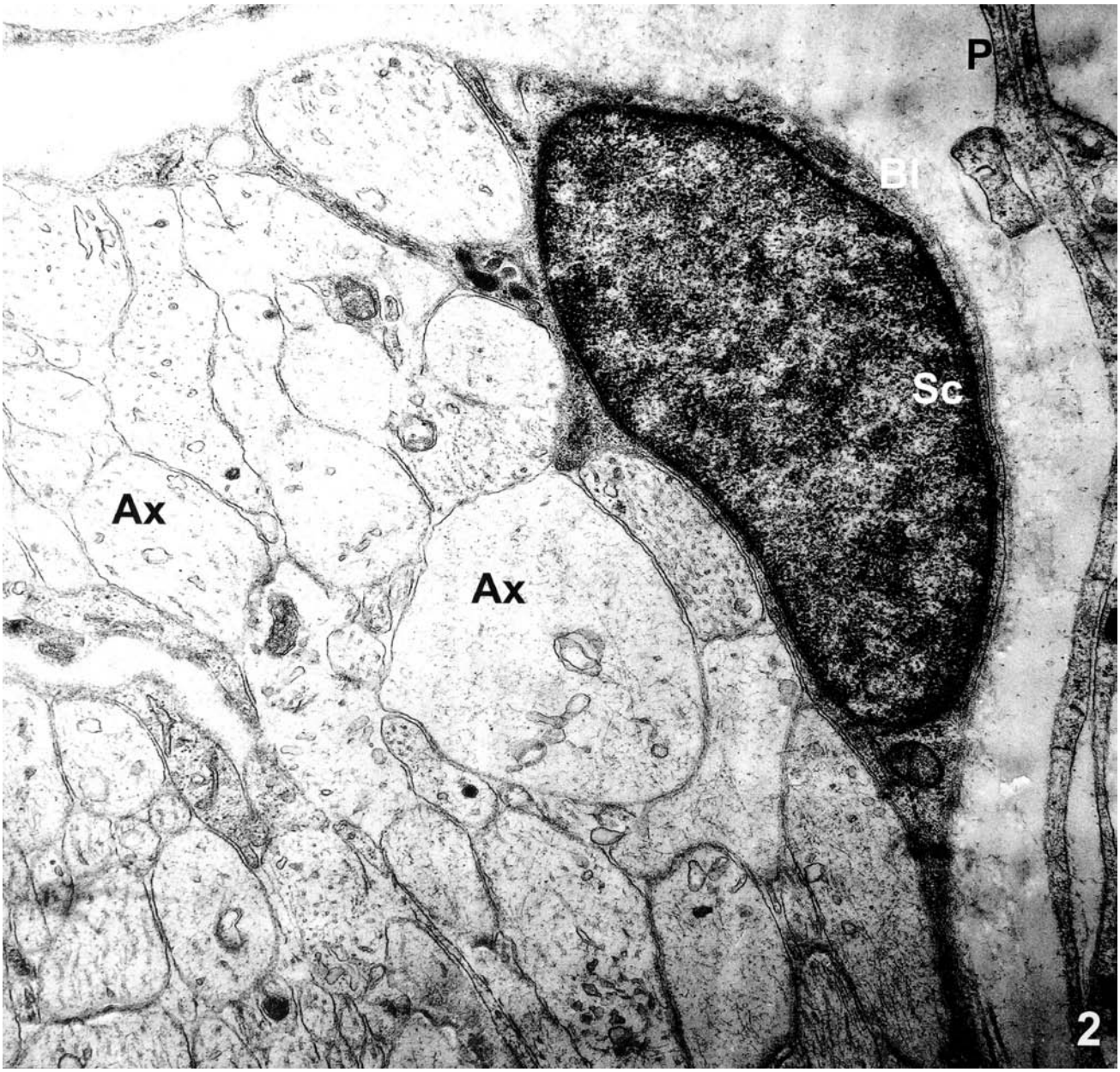

Figure 2. The left phrenic nerve from a human foetus of the 15th week. × 24500. Ax — axon, Sc — Schwann cell, Bl — basal lamina, P — perineurium.

The number of nerve processes surrounded by one Schwann cell is reduced with the advancement of development. This was confirmed in the present study. It was also observed that in the investigated foetuses aged between 17 and 23 weeks myelination progresses rapidly.

Ochoa [19] observed in the sural nerve of a human foetus of 9 weeks 800 axons bounded by one Schwann cell, and Gamble [6] found that in a foetus at 11 weeks of menstrual age the largest bundles contained more than 450 unmyelinated axons enveloped in one Schwann cell.

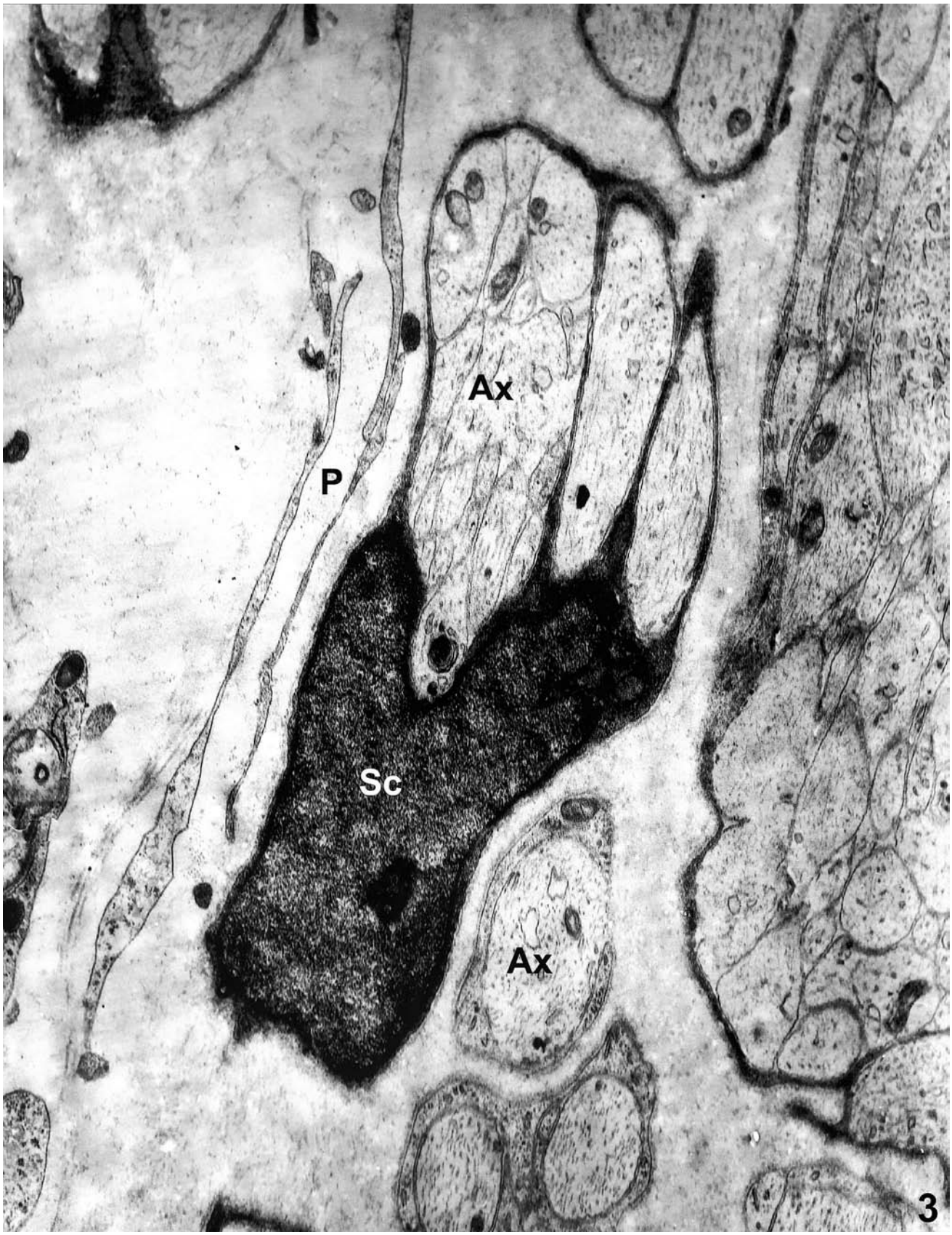

Figure 3. Axons (Ax) at the onset of myelination in the left phrenic nerve in a human foetus of the 15th week. × 17500. P — perineurium, Sc — Schwann cell.

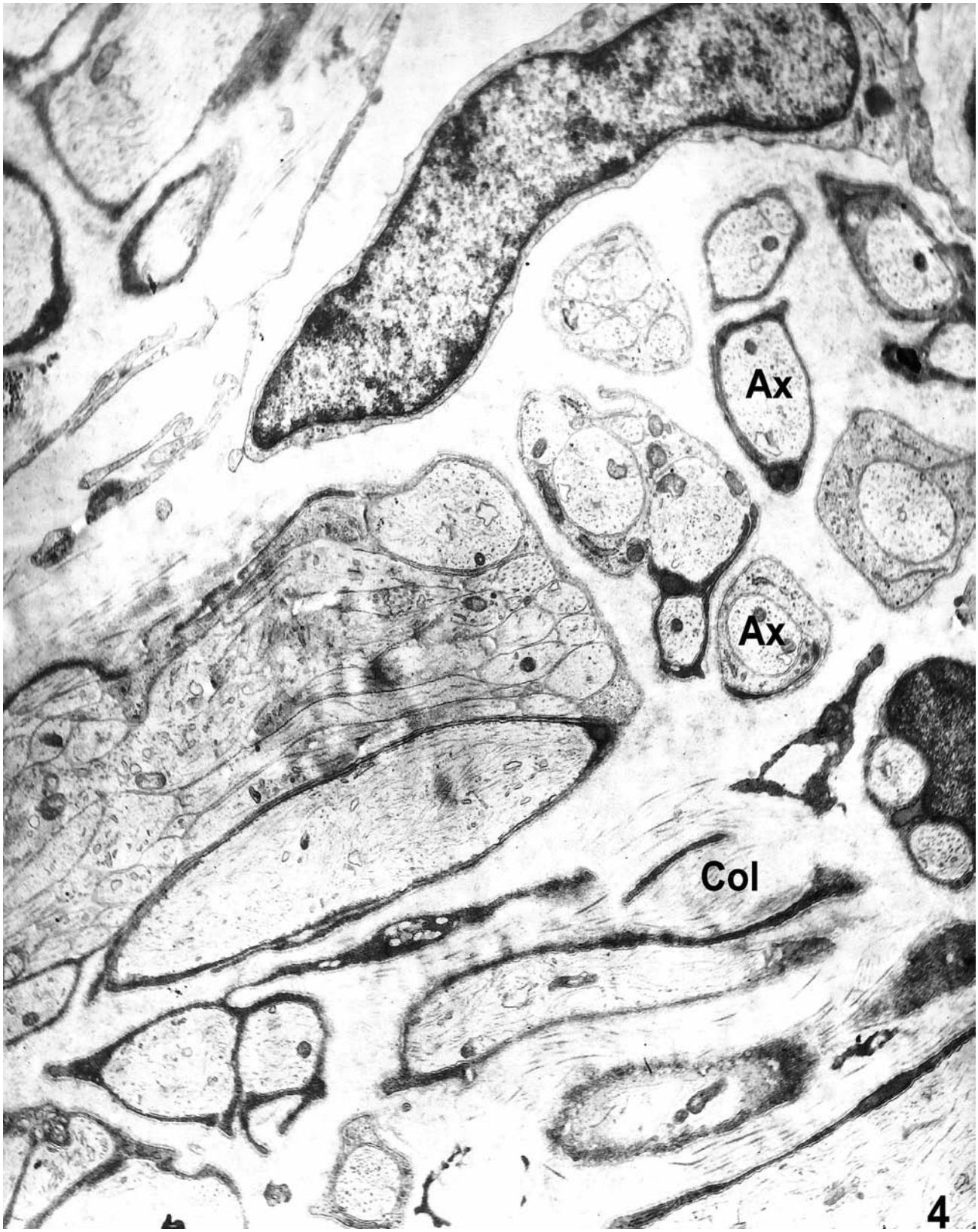

Figure 4. The left phrenic nerve in a human foetus of the 15th week. × 10000. Ax — axon, Col — collagen.

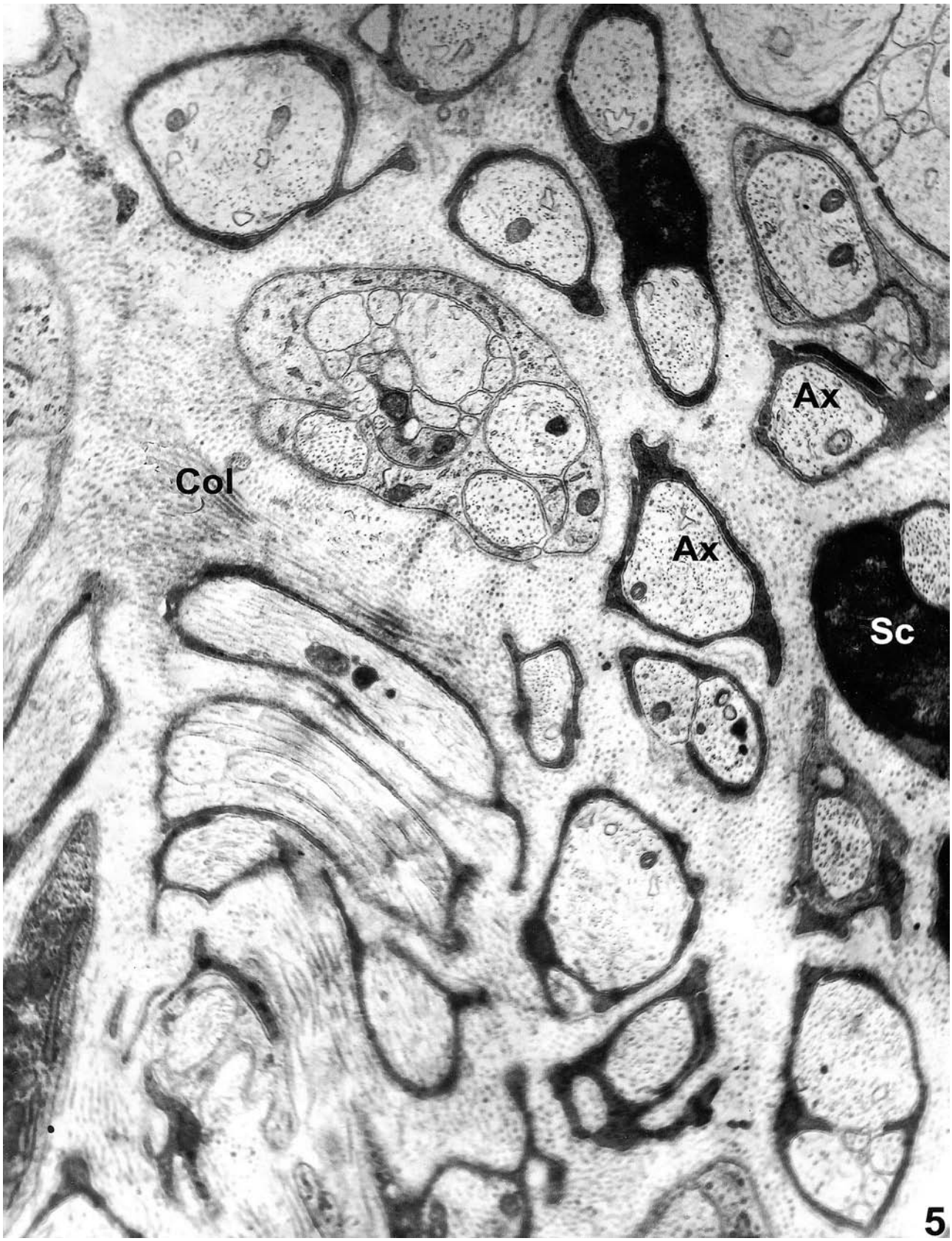

Figure 5. The left phrenic nerve in a human foetus of the 15th week. × 18000. Ax — axon, Col — collagen, Sc — Schwann cell.

Figure 6. Axon (Ax) at the early stage of myelination in the left phrenic nerve in a human foetus of the 15th week. × 79000. Col — collagen.

Figure 7. The phrenic nerve in a human foetus of the17th week. × 35000. Ax — axon, Scp — Schwann cell process.

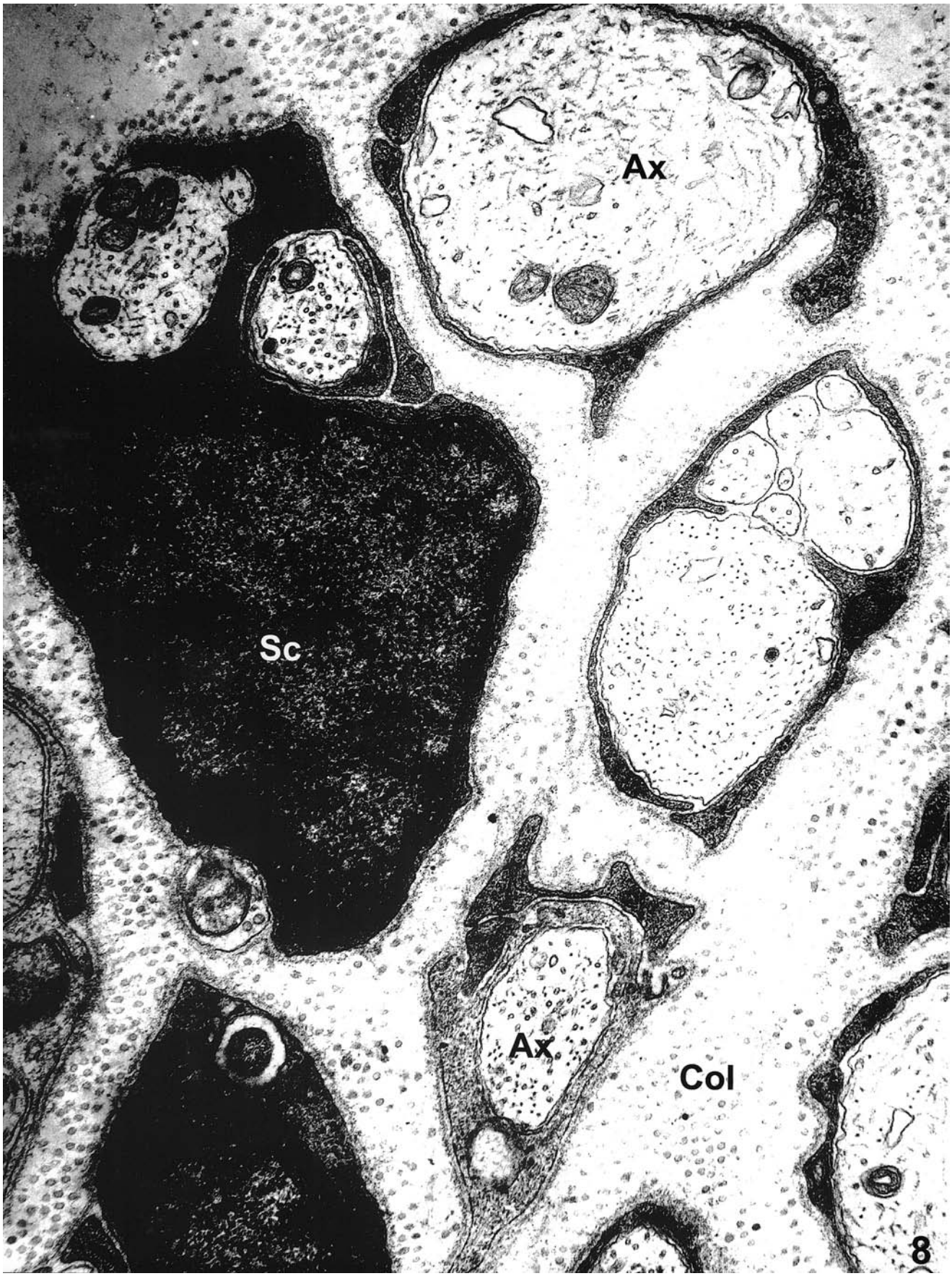

Figure 8. The right phrenic nerve in a human foetus of the 17th week. × 42000. Ax — axon, Col — collagen, Sc — Schwann cell.

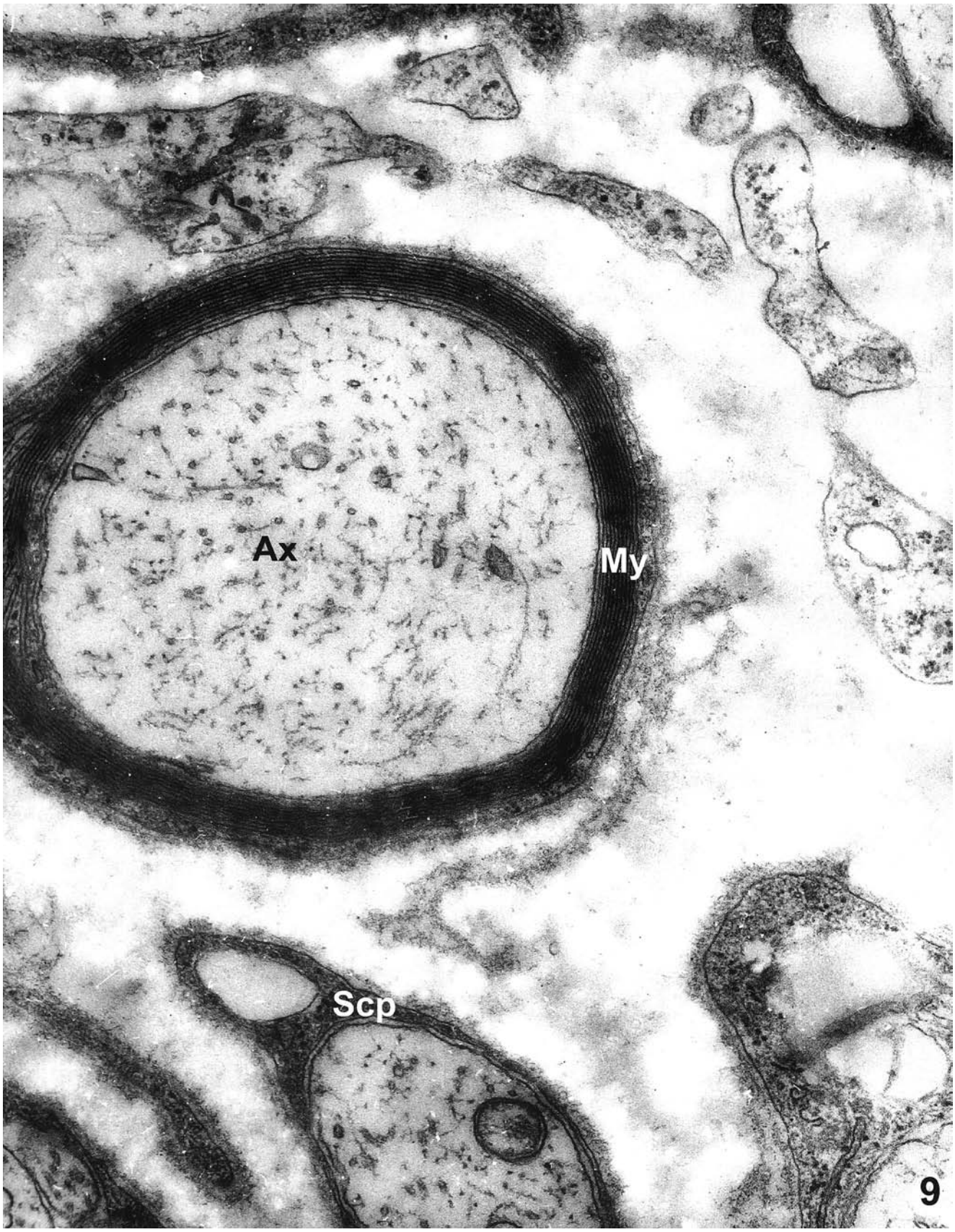

Figure 9. The phrenic nerve in a human foetus of the 17th week. × 56000. Ax — axon, My — myelin, Scp — Schwann cell process.

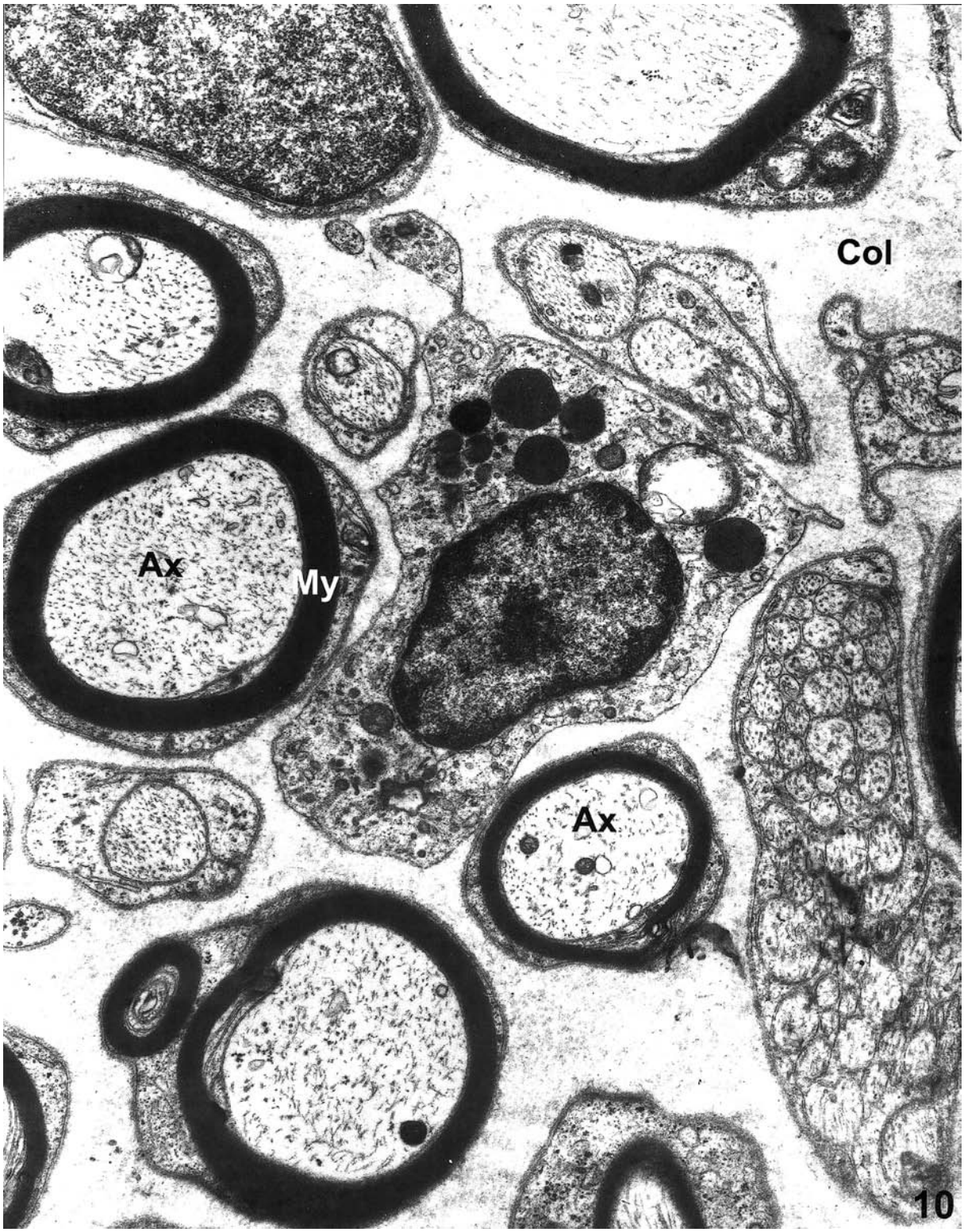

Figure 10. Myelinated fibres of the left phrenic nerve in a human foetus of the 23rd week. × 10000. Ax — axon, Col — collagen, My — myelin.

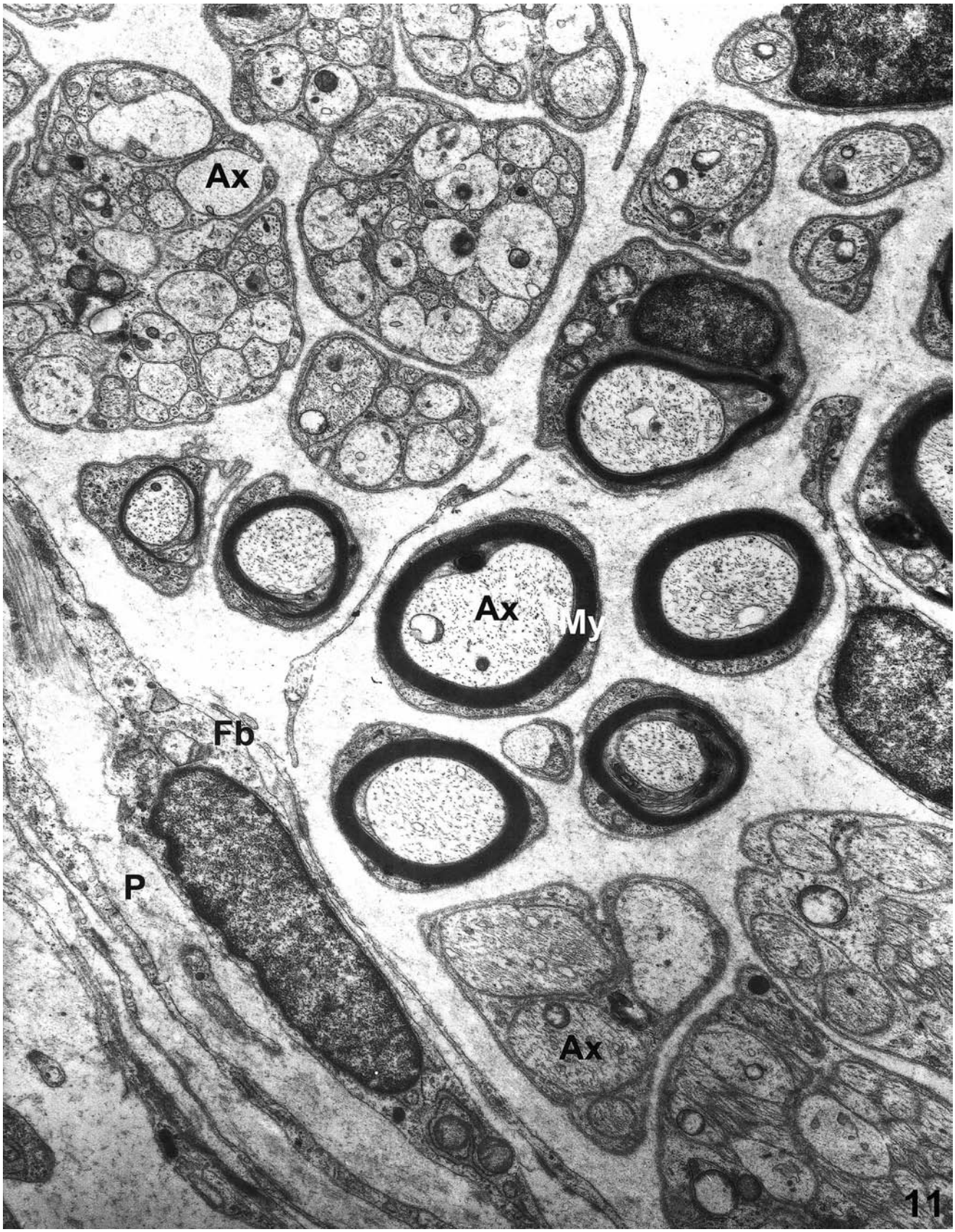

Figure 11. The left phrenic nerve in a human foetus of the 23rd week. × 8400. Ax — axon, Fb — fibroblast, My — myelin, P — perineurium.

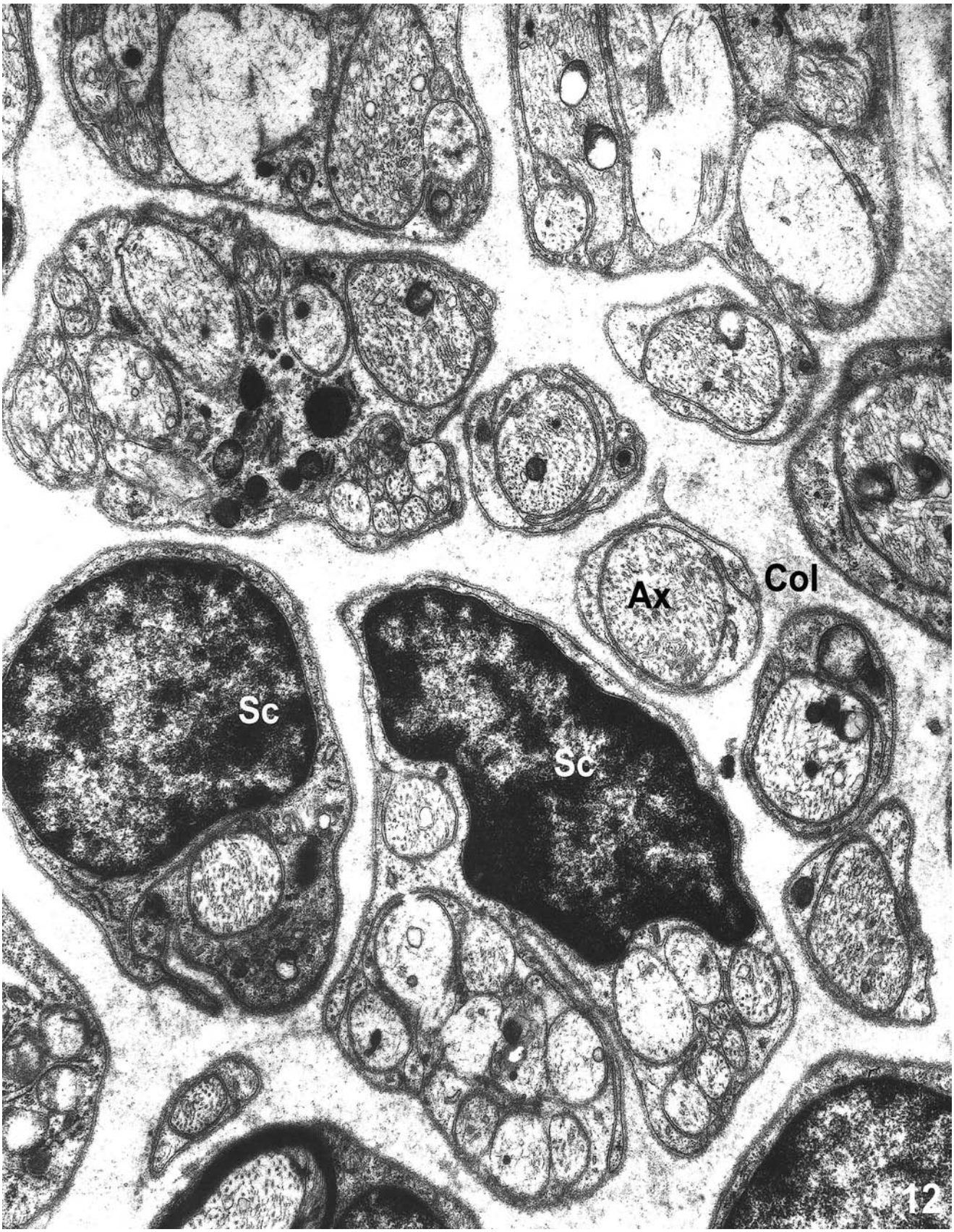

Figure 12. Myelinated fibres of the left phrenic nerve in a human foetus of the 23rd week. × 15500. Ax — axon, Col — collagen, Sc — Schwann cell.

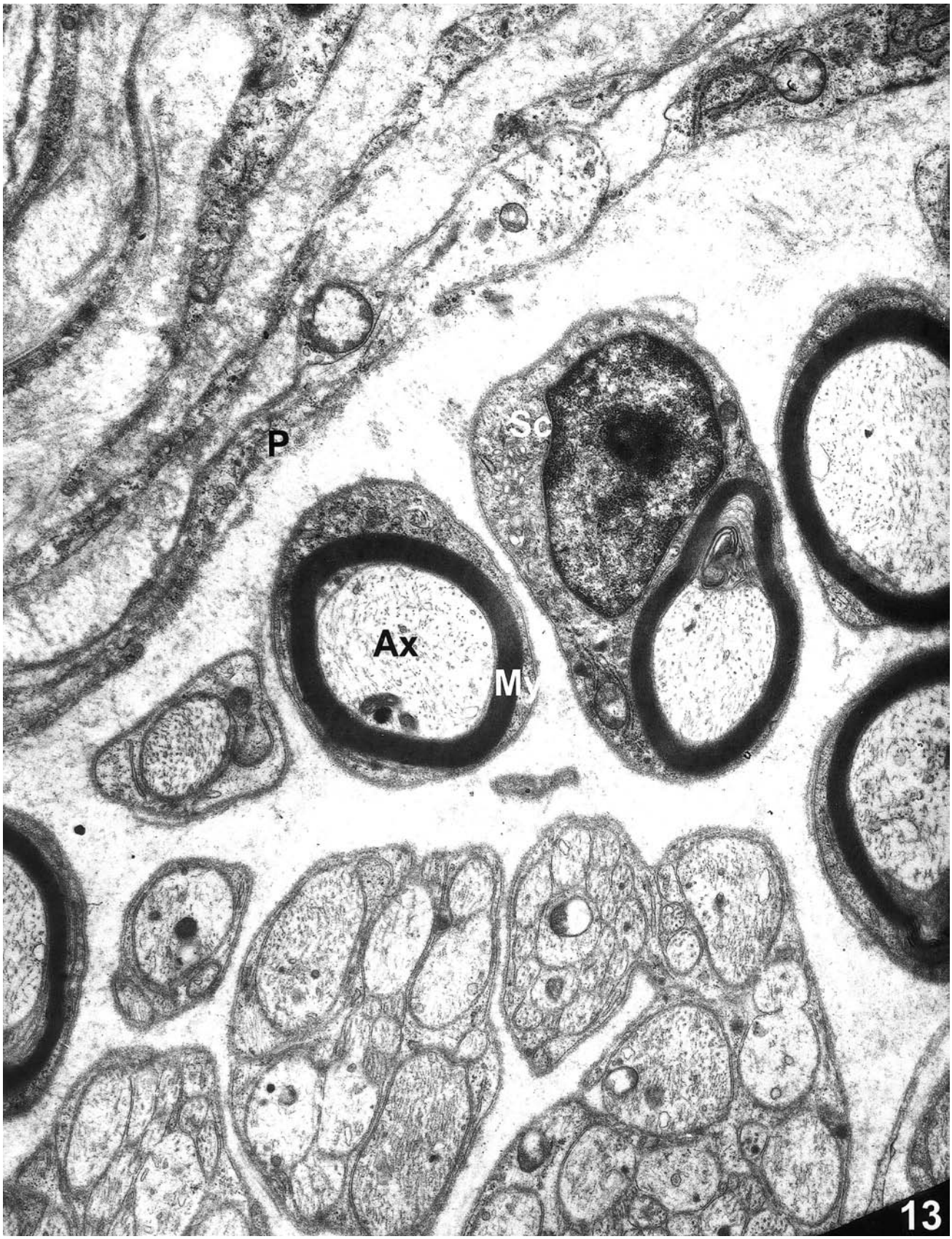

Figure 13. The left phremic nerve in human foetus of 23rd week. × 12500. Ax — axon, My — myelin, P — perineurium, Sc — Schwann cell.

REFERENCES

1. Anton ES, Sandrock AW, Matthew WD (1994) Merosin promotes neurite growth and Schwann cell migration in vitro and nerve regeneration in vivo: evidence using an antibody to merosin, ARM-1. Dev Biol, 164: 133–-146.
2. Bruska M, Piotrowski A (2004) Development of the myelin sheath of the hypogastric nerves in a human foetus aged 23 weeks. Folia Morphol, 63: 289–301.
3. Fraher JP (1978) Quantitative studies on the maturation of central and peripheral parts of individual ventral motoneuron axons. I. Myelin sheath and axon calibre. J Comp Neurol, 144: 233–252.
4. Fernandezvalle C, Gwynn L, Wood PM, Carbonetto S, Bunge MB (1994) Anti-β1 integrin antibody inhibits Schwann cell meylination. J Neurobiol, 25: 1207–1226.
5. Gaiano N, Fishell G (2002) The role of Notch in promoting glial and neural stem cell fates. Ann Rev Neurosci, 25: 471–490.
6. Gamble HJ (1966) Further electron microscope studies of human foetal peripheral nerves. J Anat, 100: 487–502.
7. Gamble HJ, Breathnach AS (1966) An electron microscope study of human foetal peripheral nerves. J Anat, 99: 573–584.
8. Georgiou J, Charlton MP (1999) Non-myelin-forming Perisynaptic Schwann cells express protein zero and myelin-associated glycoprotein. Glia, 27: 101–109.
9. Giese KP, Martini L, Lemke G, Soriano P, Schachner M (1992) Mouse P_0 gene disruption leads to hypomyelination, abnormal expression of recognition molecules, and degeneration of myelin and axons. Cell, 71: 565––576.
10. Hof PR, Trapp BD, de Vellis J, Claudio L, Colman DR (1999) The cellular components of nervous tissue. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR (eds.). Fundamental neuroscience. Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto, 41–70.
11. Jaakkala S, Savunen O, Halme T, Uitto J, Peltonen J (1993) Basement membranes during development of human nerve: Schwann cells and perineurial cells display marked changes in their expression profiles for laminin subunits and β1 and β4 integrins. J Neurocytol, 22: 215–230.
12. Jessen K R, Mirsky R (1998) Origin and early development of Schwann cells. Microsc Res Tech, 41: 393–402.
13. Kamiguchi H, Hlavin ML, Yamasaki M, Lemmon V (1998) Adhesion molecules and inherited disease of the human nervous system. Ann Rev Neurosci, 21: 97–125.
14. Le Douarin N, Dulac C, Dupin E, Cameron-Curry P (1991) Glial cell lineages in the neural crest. Glia, 4: 175–184.
15. Leivo I, Engvall E (1988) Merosin, a protein specific for basement membranes of Schwann cells, striated muscle, and trophoblast, is expressed late in nerve and muscle development. Proc Natl Acad Sci USA, 85: 1544–1548.
16. Lemke G (2001) Glial control of neuronal development. Ann Rev Neurosci, 24: 87–105.
17. Lemke G, Axel R (1985) Isolation and sequence of the gene encoding the major structural protein of peripheral myelin. Cell, 40: 501–513.
18. Luo L, O'Leary DDM (2005) Axon retraction and degeneration in development and disease. Ann Rev Neurosci, 28: 127–156.
19. Ochoa J (1971) The sural nerve of the human foetus: electron microscope observations and counts of axons. J Anat, 108: 231–245.
20. Ochoa J, Mair WGP (1969) The normal sural nerve in man. I. Ultrastructure and numbers of fibers and cells. Acta Neuropathol, 13: 197–216.
21. Rougon G, Hobert O (2003) New insights into the diversity and function of neuronal immunoglobulin superfamily molecules. Ann Rev Neurosci, 26: 207–238.
22. Shapiro L, Fannon AM, Kwong PD, Thompson A, Lehmann MS, Grübel G, Legrand JF, Als-Nielson J, Colman DR, Hendrickon WA (1995) Structural basis of cell-cell adhesion by cadherins. Nature, 374: 327–337.
23. Woźniak W (1982) Compact myelin formation in the human nerves during intra-uterine development. Folia Morphol, 41: 157–162.
24. Woźniak W (1983) Axon diameter and myelin formation in the developing human nerves. Folia Morphol, 42: 63–66.
25. Woźniak W, Bruska M (1986) Fine structure and myelination of the greater splanchnic nerves in human fetuses. Folia Morphol, 45: 192–205.
26. Woźniak W, O'Rahilly R (1981) Fine structure and myelination of the developing human vagus nerve. Acta Anat, 109: 218–230.
27. Woźniak W, O'Rahilly R, Bruska M (1982) Myelination of the human fetal phrenic nerve. Acta Anat, 112: 281–296.
28. Zorick TS, Lemke G (1996) Schwann cell differentiation. Curr Opin Cell Biol, 8: 870–876.