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The vascularisation of the central–peripheral transitional zone of rat lumbar ventral rootlets: a morphological and morphometric study

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INTRODUCTION

Bundles of lumbar ventral motoneuron axons run obliquely across the ventrolateral white matter of the spinal cord. They emerge as ventral rootlets through a strip of cord surface (the *ventral rootlet exit zone*). Immediately distal to their emergence from the cord adjacent rootlets are separated by the *inter-radicular space* (Kaar & Fraher, 1986). Groups of rootlets converge and eventually join to form the ventral roots. Each rootlet can therefore be subdivided into an *intramedullary* segment within the cord, an *emergent* segment where it passes through the cord surface and a *free* segment distal to the cord surface (Fig. 2*d*) (Fraher & Kaar, 1986). From an early stage of development an irregular tapering projection of central nervous tissue extends distally into both emergent and free parts of each fifth lumbar ventral rootlet in the rat. It is surrounded by the central ends of the proximal Schwann cells of the rootlet. The length of rootlet containing central and peripheral tissue is termed the *transitional zone*. This short length of rootlet contains all the central–peripheral transitional nodes of Ranvier (Fraher & Kaar, 1984, 1985; Kaar & Fraher, 1985).

The blood supply to the transitional zone is of interest for a number of reasons. Firstly, it needs to be sufficient to meet the metabolic requirements of the high concentration of nodes in the zone. Secondly, reports regarding the existence of anastomoses between the vessels of the spinal cord and those of spinal nerve roots are at variance: Berthold & Carlstedt (1977) found that blood vessels at the dorsal rootlet transitional zone lack continuity with those of the underlying spinal cord in the cat. However, other studies on rat cervical (Fraher, 1976, unpublished observations) and lumbar (Kaar, 1983, unpublished observations) ventral rootlets suggest that such anastomoses do exist. Thirdly, previous studies (Tarlov, 1937; Steer, 1971; Haller, Haller & Low, 1972; Gamble, 1976; Berthold & Carlstedt, 1977) have been concerned only with blood vessels at spinal nerve root attachments in the adult, and have not dealt with their development.

The present study consists of a morphological and morphometric examination of the blood vessels supplying the rat fifth lumbar ventral rootlet transitional zone both during development and at maturity. The pronounced developmental changes in the transitional zone blood supply are examined in the light of concurrent maturation of adjacent tissues, in particular the transitional nodes of Ranvier.

Table 1. *Data for capillaries and postcapillary venules related to ventral rootlets and for ventral rootlet cross sectional area. 20f:20 day fetal*

	Capillaries							Postcapillary venules			
Age (days)	Mean rootlet cross sectional area (μm^2)	No. of rootlets examined	Mean number of capillaries/ rootlet	Mean percentage of rootlet length related to capillaries	Mean distance from capillary to rootlet centre (μm)	Diameter (μm) Mean	Diameter (μm) Range	Percentage of rootlets with related venules	Mean number of venules per rootlet	Diameter (μm) Mean	Diameter (μm) Range
20f	51·8	24	1·1	57	8·8	7·4	4·7–13·3	63	0·4	10·9	9·2–11·2
1	100·4	22	1·1	41	11·5	8·1	4·5–10·8	100	0·7	11·6	8·8–17·3
3	150·5	36	0·9	63	16·6	7·5	4·6–13·3	39	0·3	15·7	10·8–22·8
6	334·9	24	1·1	51	13·5	8·4	5·1–18·3	100	0·4	17·6	11·0–25·3
12	542·0	31	1·2	48	17·8	7·4	5·1–10·5	81	0·5	16·9	7·8–31·5
20	618·8	33	1·5	92	23·7	7·6	4·1–12·6	27	0·2	13·4	9·5–17·6
300	2372·6	14	1·4	96	39·5	8·5	6·0–14·6	64	0·5	30·0	17·7–52·3

MATERIALS AND METHODS

Ten Wistar albino rats were studied at each of the following ages: 20 days fetal, 1, 3, 6, 12, 20 and 300 days postnatum. The methods of preparation, embedding, sectioning and staining of tissue, as well as the manner in which magnifications were calculated have been described previously (Fraher & Kaar, 1984). At each age between 14 and 40 ventral rootlets were sectioned by alternate sequential series of thin and semithin transverse and longitudinal sections of the free, emergent and intramedullary parts of each rootlet. Intramedullary segments were sectioned as far centrally as their emergence from the ventral horn grey matter of the spinal cord. Thin sections were taken at regular intervals of 5 μm. Semithin sections were examined using a Reichert Polyvar photomicroscope. Thin sections were examined using Corinth 500 and JEOL 1200 EX electron microscopes.

At each age light micrographic montages were made covering the entire extent of a number of ventral rootlet exit zones, the rootlets of which were sectioned transversely. Electron micrographic montages were made of portions of each zone studied, and were used to determine the nature of each blood vessel examined, according to the criteria of Rhodin (1967, 1968, 1974) and of Simionescu & Simionescu (1977). Each vessel studied was traced on serial sections along the entire extent of the transitional zone to determine its course, connections and branching pattern.

At each age portions of a number of exit zones were selected at random on the light and/or electron micrographic montages. From these the following quantitative data were determined (Table 1). The cross sectional area of each rootlet within the selected area was measured at proximal, middle and distal levels, using Kontron MOP and MOP-Videoplan Image Analysis Systems. The mean of the three measurements was then calculated. From such data the mean transitional zone cross sectional area was found for each age. The diameter of the lumen of each transversely

sectioned blood vessel which had a circular profile was measured. Where the profile was oval the diameter was estimated as the mean of the long and short dimensions of the lumen. The mean diameter was calculated for each type of blood vessel at each age. At each age the vascularity of the transitional zone was estimated in two ways by following, on serial sections, the entire length of the rootlet containing the transitional zone. Firstly, the ratio of the number of each type of blood vessel to the number of rootlets was calculated from the montages. Secondly, the proportion of the length of each transitional zone which was related to each type of blood vessel was determined. A vessel was said to be *related* to a rootlet if no other structure intervened between the vessel and the rootlet sheath.

OBSERVATIONS

Ultrastructure of blood vessels related to the transitional zone

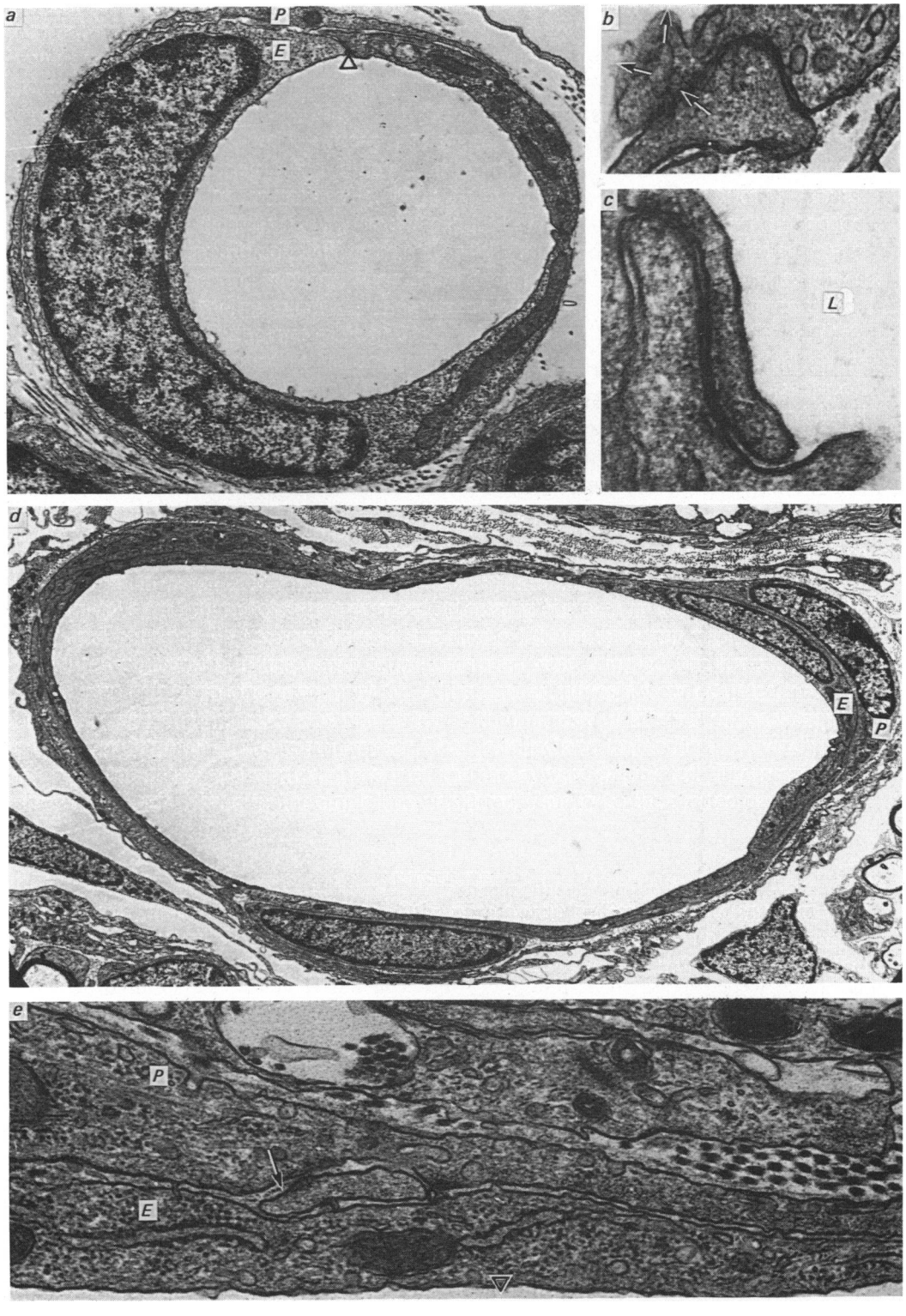
All vessels present were either capillaries or postcapillary venules. Capillaries had walls consisting of an inner single continuous unfenestrated endothelial layer and an outer incomplete layer of pericytes (Fig. 1*a*). Endothelial cells possessed occasional micropinocytotic vesicles on the luminal aspect. The junctions between endothelial cells varied. In some the edges simply abutted together. In others, they were oblique, overlapped (Fig. 1*b*) or 'tongued and grooved' (Fig. 1*c*) along bands 1 μm or more wide. Towards the luminal end of each junction the plasma membranes commonly formed a zonula occludens. Elsewhere they were separated by a 5–10 nm gap. A continuous basal lamina lay outside the endothelial cells. Pericytes (Fig. 1*a*) resembled endothelial cells and contained occasional lysosome-like bodies. The basal lamina covering their inner surface fused with that of the endothelial cells. Postcapillary venules were usually of greater calibre than capillaries, the majority being more than 10 μm in diameter (Fig. 1*d*). The wall resembled that of the capillaries except that the outer layer of pericytes was complete (Fig. 1*e*) and was surrounded by a continuous basal lamina.

Arrangement of blood vessels of the transitional zone

Blood vessels did not occur within rootlets. They formed a closely-knit network adjacent to the emergent rootlets (Fig. 2*a–c*). They lay in the inter-radicular space and were therefore related to the rootlet sheath cells and pial cells bounding this space (Kaar & Fraher, 1986). Each rootlet was related to at least one blood vessel and some were related to as many as four. The blood vessels ran parallel to the rootlets. They were most frequently (40–60 %) positioned medial or lateral, and least frequently (10–30 %) dorsal to the rootlet. At the cord surface they branched to form a closely-knit network. In this location between 10 and 35 % of the vessels or their branches traversed the cord surface (Fig. 2*c*, *d*), where they were continous with similar vessels running parallel and immediately adjacent to the intramedullary rootlets (Fig. 2*d*) as far centrally as the grey matter.

Blood vessel entry into the spinal cord

Where each vessel penetrated the cord it was surrounded by a narrow, funnel-shaped perivascular space bounded by astrocyte processes continuous with the astrocytic limiting layer of the cord surface. The space surrounding smaller blood vessels was around 250 nm wide at its opening but narrowed rapidly to around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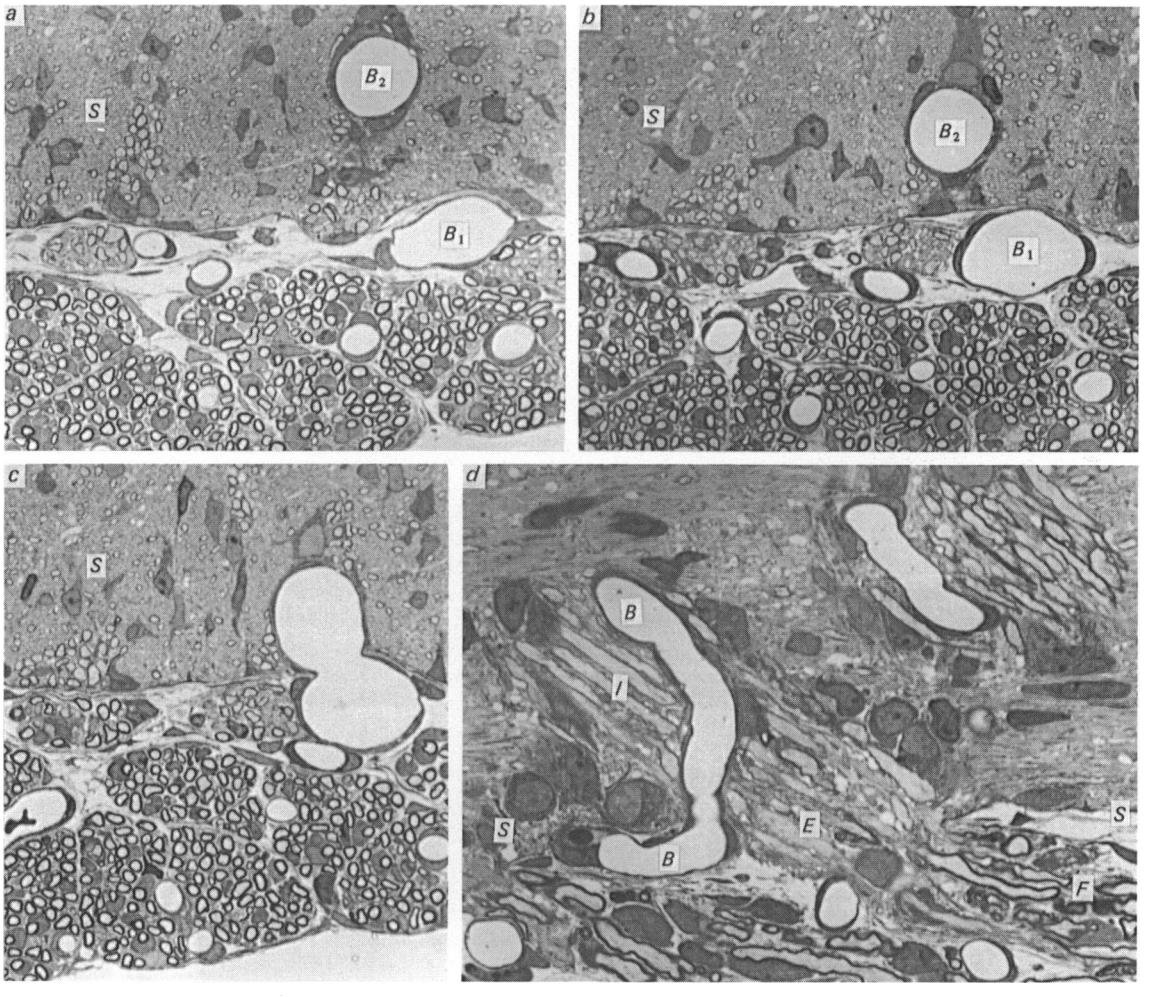

Fig. 2 (*a–d*). (*a–c*) Serial transverse sections through transitional zones of 6 day postnatal rootlets. Blood vessels related to free (B_1) and intramedullary (B_2) rootlets are continuous with one another at the spinal cord surface. *S*, spinal cord. × 590. (*d*) Longitudinal section through intramedullary (*I*), emergent (*E*) and free (*F*) parts of 6 day ventral rootlet, showing a blood vessel (*B*) running from the inter-radicular space into the spinal cord with the rootlet. *S-S*, spinal cord surface. × 700.

Fig. 1 (*a–e*). (*a*) Transverse section through capillary in inter-radicular space. *E*, endothelial cell; *P*, pericyte. Note tight junction (arrowhead) where endothelial layers meet. × 19400. (*b*) Apposed margins of some endothelial cells are overlapped and irregular. Note obliquely sectioned plasma membranes (arrows). × 48000. (*c*) Apposed edges of some endothelial cells are 'tongued and grooved'. *L*, lumen. × 88300. (*d*) Transverse section through a postcapillary venule. Its wall consists of inner endothelial (*E*) and outer pericyte (*P*) layers. × 3800. (*e*) Enlargement of (*d*). Where adjacent endothelial (*E*) and pericyte (*P*) layers are separated by 30 to 50 nm, a basal lamina (arrow) is present. Micropinocytotic vesicles occur in both endothelial cells and pericytes. Arrowhead, lumen. × 36650.

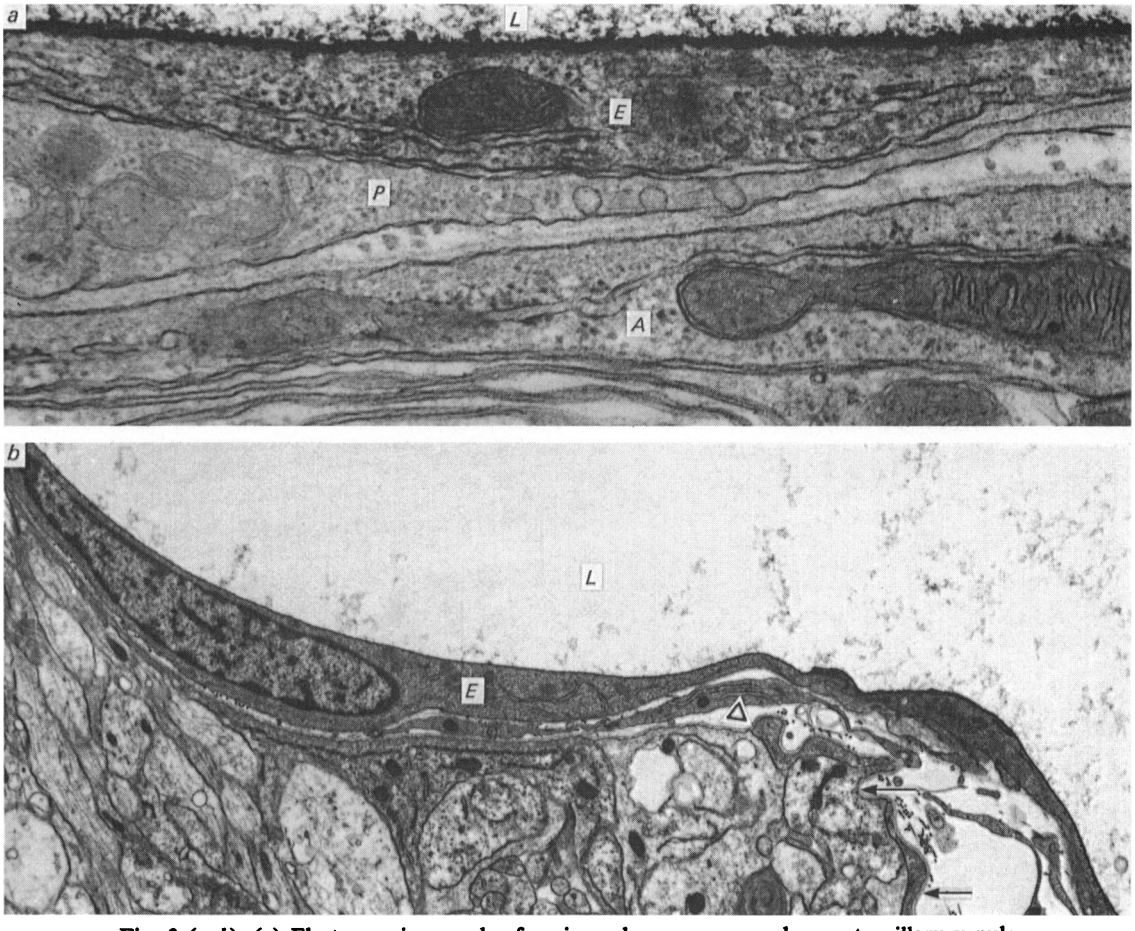

Fig. 3 (*a–b*). (*a*) Electron micrograph of perivascular space around a postcapillary venule. *A*, astrocyte process; *E*, endothelial cell; *P*, pericyte; *L*, lumen. × 50800. (*b*) Electron micrograph of longitudinal section through a large capillary passing through the cord surface (arrows) at 6 days. The superficial part of the perivascular space contains a pial cell process (arrowhead). *E*, endothelium; *L*, lumen. × 7100.

75 nm at a depth of 5–10 μm. It was lined by astrocytic and vascular basal laminae (Fig. 3*a*). It contained sparse collagen fibres, but no pial cell processes extended into it. The space surrounding some larger blood vessels was more substantial (Fig. 3*b*). Between 6 and 300 days its width at the cord surface increased from 0·5–1·0 μm to 2·0–5·0 μm and its length increased from 5 μm to 30 μm. Pial cell laminae extended into its more superficial part (Fig. 3*b*).

Blood vessels lying distal to the transitional zone

Distal to the inter-radicular space, rootlets were aggregated into bundles and were separated by multilayered sheaths (Kaar & Fraher, 1986). The vessels lay among these sheath cell layers which generally completely separated them from the nerve fibres in the rootlets. Occasionally, where the sheath was fenestrated, only extracellular space intervened between the blood vessel and the rootlet fibres.

Quantitative changes in blood vessels

Quantitative data for capillaries which were related (see Materials and Methods) to the rootlets are given in Table 1. At each age at least one capillary was related to each free rootlet. Some rootlets were related to more than one capillary and *vice versa*. From 20 days fetal to 6 days postnatum the numbers of capillaries and rootlets were approximately equal. After this, capillary numbers increased. At 20 days and subsequently they outnumbered rootlets by up to 50 %. The percentage of the length of transitional zone which was directly related to one capillary or more fluctuated between 40 and 60 % up to 12 days postnatum. By 20 days it was over 90 % and was at about the same level at 300 days. The mean distance from the capillary wall to the centre of the related rootlet doubled between 20 days fetal and 12 days postnatum. It had increased by a further one third by 20 days. It almost doubled between 20 and 300 days. Mean capillary diameter remained similar at around 8 μm at each age. At each age over 85 % of all capillaries were less than 10 μm in diameter. Postcapillary venules were considerably greater than this in calibre (Table 1). Their mean diameter tended to increase with age, especially between 20 and 300 days. The proportion of rootlets related to one, or more, postcapillary venule fluctuated considerably between ages and showed no age-related change.

DISCUSSION

The blood vessels of the transitional zone resemble those occurring in the central nervous system (Caley & Maxwell, 1970; Phelps, 1972; Hannah & Nathaniel, 1974; Sturrock, 1981) and within the endoneurium of peripheral nerves (Thomas, 1963; Lundborg & Brånemark, 1968; Olsson, 1975). Zonulae occludentes probably prevent diffusion of protein and other molecules between the vascular lumen and the perivascular space, as has been demonstrated experimentally by Olsson & Reese (1971) for endoneurial and cerebral vessels.

The absence of blood vessels from rootlet endoneurium is in contrast to Gamble's (1976) conclusion that nerve root endoneurium contains capillaries. In this respect roots resemble peripheral nerves in general. In the latter the endoneurium contains capillaries (Thomas, 1963; Burkel, 1967; Gunderson & Low, 1968; Lundborg & Brånemark, 1968; Olsson, 1975). Berthold & Carlstedt (1977) state that blood vessels at the dorsal root transitional zone lie within the endoneurial space. However, those which they illustrate are surrounded by root sheath cells.

The network of vessels in the inter-radicular space is continuous peripherally with the vessels of the root and centrally with those in the ventrolateral white column of the spinal cord. The latter arrangement differs from that at the dorsal rootlet attachment zone in the cat. Here, both during development (Carlstedt, 1981) and at maturity (Berthold & Carlstedt, 1977) the blood vessels running proximally on the rootlet do not enter the cord but deviate to join vessels on the cord surface. As a result, dorsal roots may be more susceptible to ischaemia than ventral roots, since the former lack anastomotic communication with blood vessels in the central nervous system.

There is a substantial increase in the vascularisation of the ventral rootlet transitional zone between 12 and 20 days postnatum, both in terms of an increase in the number of capillaries and in the proportion of the length of the rootlet related to capillaries. This is not related to the onset of myelination in the transitional zone

which occurs around birth (Kaar, 1984). It occurs after the stage of maximal increase in transitional zone cross sectional area as the present study shows and after the onset of myelination in the ventrolateral white column of the spinal cord, which occurs between 6 and 12 days (Kaar, 1984). The increase is, however, associated with functional maturation of gamma fibres, the majority of which are likely to be functionally immature at 12 days. At that stage the transitional node is still immature and presumptive in form (Fraher & Kaar, 1984), with the result that impulse conduction is likely to be incomplete and irregular. By 20 days after birth almost all gamma fibres possess definitive transitional nodes (Kaar & Fraher, 1985) and are therefore likely to be functionally mature. Since gamma motoneuron activity appears to be essential for stretch reflex activity and for smooth and load-responsive muscle contraction (Guyton, 1981), this functional maturity is likely to lead to increased alpha motoneuron activity. As a result, the metabolic requirements in both groups of fibres increase and the increased vascularisation observed may occur in response to this.

The age-related increase in vascularisation is somewhat offset by the progressive increase in the distance from the capillary wall to the centre of the rootlet, resulting from growth in rootlet cross sectional area. This change is most marked between 20 and 300 days. It is not accompanied by any decrease in capillary calibre. The short distance between capillaries and rootlet fibres at 20 days probably ensures a sufficient supply of nutrients to maintain nerve function as well as growth. The nerve fibres in the centre of the rootlet are furthest from their blood supply. The distance involved (approximately 40 μm) is within the range of distances of cells in general from capillaries (25–50 μm) given by Guyton (1981) and is considerably less than the value of 100 μm or more for nerve fibres in feline dorsal rootlets, given by Berthold & Carlstedt (1977). Metabolite diffusion for all fibres in the rat ventral rootlet transitional zone is therefore likely to be highly efficient. Efficiency is likely to be even greater because the present estimate does not include capillaries separated from rootlets by other capillaries, which would further enhance the diffusion process. The rich blood supply to the transitional zone probably reflects its large nutrient requirements, in particular in relation to the high concentration of nodes. In contrast to the progressively increasing vascularisation of rootlets by capillaries over the period studied, the number of postcapillary venules per rootlet shows no age-related trend. The increase in venular calibre and the probable increase in venular length which occur after 20 days probably compensate for this and at least maintain, if not increase, the capacitance of the adult venular network, compared to that during development.

The age-related changes in the vascularisation of the transitional zone differ considerably from those of the adjacent spinal cord. Sturrock (1981, 1982) examined the vascularisation of the developing ventral white matter of mouse and rabbit spinal cord and found that increased vascularity coincides with the onset of myelination and with a period of rapid increase in spinal cord cross sectional area. After that, vascularisation diminishes substantially due to a decrease in the size and/or the number of blood vessels. Neither of these changes takes place in the vascularisation of the lumbar ventral rootlet transitional zone, perhaps reflecting sustained metabolic requirements of the central–peripheral transitional nodes.

SUMMARY

The blood vessels supplying the central–peripheral transitional zone of rat fifth lumbar ventral spinal nerve rootlets were examined during development and at maturity. At all stages all vessels were either capillaries or postcapillary venules. They lay in the spaces between the rootlets, being entirely absent from the endoneurial spaces. A proportion of these vessels communicated with those supplying the adjacent spinal cord. In this respect they differed from those supplying the dorsal rootlet transitional zone, at least in the cat, where no such communication occurs. During the first week after birth, at least one capillary was directly related to each rootlet, generally over about half the length of the transitional zone. Subsequently vascularity increased considerably. At three weeks postnatum, and subsequently, capillaries outnumbered rootlets by up to 50 % and almost the entire length of the transitional zone was related to one capillary or more. This change was related to the maturation of the transitional nodes of gamma axons, which is likely to be related to increased alpha and gamma motoneuron activity. These changes were somewhat offset due to the fact that rootlet diameter increased with age. As a result, the distance between the capillary wall and the centre of the rootlet almost doubled between 20 and 300 days postnatum. The diameter of the capillaries did not change with age but that of the postcapillary venules increased.

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REFERENCES

BERTHOLD, C.-H. & CARLSTEDT, T. (1977). Observations on the morphology at the transition between the peripheral and central nervous system in the cat. II. General organisation of the transitional region in S_1 dorsal rootlets. *Acta physiologica scandinavica, Suppl.* **446**, 23–42.

BURKEL, W. E. (1967). The histological fine structure of perineurium. *Anatomical Record* **158**, 177–190.

CALEY, D. W. & MAXWELL, D. S. (1970). Development of the blood vessels and extracellular spaces during postnatal maturation of rat cerebral cortex. *Journal of Comparative Neurology* **138**, 31–48.

CARLSTEDT, T. (1981). An electron-microscopical study of the developing transitional region in feline S_1 dorsal rootlets. *Journal of the Neurological Sciences* **50**, 357–372.

FRAHER, J. P. & KAAR, G. F. (1984). The transitional node of Ranvier at the junction of the central and peripheral nervous systems. An ultrastructural study of its development and mature form. *Journal of Anatomy* **139**, 215–238.

FRAHER, J. P. & KAAR, G. F. (1985). The development of alpha and gamma motoneuron fibres in the rat. II. A comparative ultrastructural study of their central and peripheral myelination. *Journal of Anatomy* **141**, 89–103.

FRAHER, J. P. & KAAR, G. F. (1986). The lumbar ventral root–spinal cord transitional zone in the rat. A morphological study during development and at maturity. *Journal of Anatomy* **145**, 109–122.

GAMBLE, H. J. (1976). Spinal and cranial nerve roots. In *The Peripheral Nerve* (ed. D. N. Landon). London: Chapman & Hall.

GUNDERSON, L. L. & LOW, F. M. (1968). Perineurium and connective tissues of peripheral nerve. *American Journal of Veterinary Research* **29**, 455–461.

GUYTON, A. C. (1981). *Textbook of Medical Physiology*, 6th ed. Philadelphia: W. B. Saunders Co.

HALLER, F. R., HALLER, A. C. & LOW, F. M. (1972). The fine structure of cellular layers and connective tissue space at spinal nerve root attachments in the rat. *American Journal of Anatomy* **133**, 108–124.

HANNAH, R. S. & NATHANIEL, E. J. H. (1974). The postnatal development of blood vessels in the substantia gelatinosa of rat spinal cord – an ultrastructural study. *Anatomical Record* **178**, 691–710.

KAAR, G. F. (1984). Ph.D. thesis, National University of Ireland.

KAAR, G. F. & FRAHER, J. P. (1985). The development of alpha and gamma motoneuron fibres in the rat. I. A comparative ultrastructural study of their central and peripheral axon growth. *Journal of Anatomy* **141**, 77–88.

KAAR, G. F. & FRAHER, J. P. (1986). The sheaths surrounding the attachments of rat lumbar ventral roots to the spinal cord: a light and electron microscopical study. *Journal of Anatomy* **148**, 137–146.

LUNDBORG, G. & BRÅNEMARK, P.-I. (1968). Microvascular structure and function of peripheral nerves. *Advances in Microcirculation* **1**, 66–68.

OLSSON, Y. (1975). Vascular permeability in the peripheral nervous system. In *Peripheral Neuropathy* (ed. P. J. Dyck, P. K. Thomas & E. H. Lambert). Philadelphia: W. B. Saunders Co.

OLSSON, Y. & REESE, T. S. (1971). Permeability of vasa nervorum in perineurium in mouse sciatic nerve studied by fluorescence and electron microscopy. *Journal of Neuropathology and Experimental Neurology* **30**, 105–119.

PHELPS, C. H. (1972). The development of the glio-vascular relationship in the rat spinal cord. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **128**, 555–563.

RHODIN, J. A. G. (1967). The ultrastructure of mammalian arterioles and precapillary sphincters. *Journal of Ultrastructure Research* **18**, 181–223.

RHODIN, J. A. G. (1968). Ultrastructure of mammalian venous capillaries, venules and small collecting veins. *Journal of Ultrastructure Research* **25**, 452–500.

RHODIN, J. A. G. (1974). Cardiovascular system. In *Histology, a Text and Atlas* (ed. J. A. G. Rhodin). New York: New York Medical College.

SIMIONESCU, N. & SIMIONESCU, M. (1977). The cardiovascular system. In *Histology* (ed. R. O. Greep & L. Weiss). New York: McGraw-Hill.

STEER, J. M. (1971). Some observations on the fine structure of rat dorsal spinal nerve roots. *Journal of Anatomy* **109**, 467–485.

STURROCK R. R. (1981). A quantitative and morphological study of vascularisation of the developing mouse spinal cord. *Journal of Anatomy* **132**, 203–221.

STURROCK R. R. (1982). A quantitative study of vascularisation of the prenatal rabbit spinal cord. *Journal of Anatomy* **135**, 89–96.

TARLOV, I. M. (1937). Structure of the nerve root. I. Nature of the junction between the central and peripheral nervous system. *Archives of Neurology, Neurosurgery and Psychiatry* **37**, 555–583.

THOMAS, P. K. (1963). The connective tissue of peripheral nerve: an electron microscopic study. *Journal of Anatomy* **97**, 35–44.