J. Anat. (1987), **152**, pp. 189–203
With 7 figures
Printed in Great Britain

The CNS–PNS transitional zone of rat cervical dorsal roots during development and at maturity. A morphological and morphometric study

JOHN P. FRAHER AND MARGARET M. SHEEHAN
Department of Anatomy, University College, Cork, Ireland

(*Accepted* 12 *August* 1986)

INTRODUCTION

Each dorsal and ventral spinal nerve root is attached to the cord surface by a number of rootlets. An outgrowth of central nervous tissue, termed the *central tissue projection*, extends distally into the proximal part of those sensory rootlets which have been studied to date (Tarlov, 1937; Berthold & Carlstedt, 1977; Moll & Meier, 1983). This projection tapers in a proximodistal direction within the rootlet and is surrounded by peripheral tissue. That segment of rootlet which contains both central and peripheral tissue is termed the *transitional zone*.

Within a given species the form of the central tissue projection, and therefore of the interface between the central and peripheral parts of the nervous system, varies widely between different sensory (Fraher, 1985, unpublished observations; Fraher & Delanty, 1986, in preparation) and motor nerves (Fraher, 1978; Fraher & Kaar, 1982; Moll & Meier, 1983; Fraher & Kaar, 1986). Such variations are the end results of developmental interactions between the same central and peripheral tissue classes. Accordingly, a study of the development of transitional regions having a variety of adult forms will help to elucidate the underlying morphogenetic mechanisms. This in turn will provide the necessary background for the interpretation of pathological changes involving the transitional zone and of central–peripheral tissue interactions in nervous system transplantation experiments.

This investigation consists of a morphological and morphometric study of the rat cervical dorsal rootlet transitional zone during development and at maturity. Preliminary studies (Sheehan, Fraher & O'Sullivan, 1984) have shown that its overall appearance differs substantially from that of dorsal rootlets in the cat sacral region, described by Berthold & Carlstedt (1977) and Carlstedt (1981). General ultrastructural features of the developing and mature transitional zone have been described for cat sacral dorsal rootlets (Berthold & Carlstedt, 1977; Carlstedt, 1981), rat cervical ventral rootlets (Fraher, 1978; Fraher & Kaar, 1982), rat and mouse cervical ventral rootlets (Moll & Meier, 1983) and rat lumbar ventral rootlets (Kaar, 1984; Fraher & Kaar, 1984, 1985, 1986; Kaar & Fraher, 1985). Accordingly, ultrastructural features will be considered in this study only insofar as they differ from the general pattern as previously described.

MATERIALS AND METHODS

Wistar albino rats were used in this investigation. Three animals were studied at 2, 3, 4 and 5 days postnatum, 8 animals at 6, 12, 20 and 70 days and 3 at 300 days postnatum. Animals were taken from litters delivered 21·5 days $\pm$ 2 hours following mating. Their ages were known to within 2 hours.

Animals were anaesthetised with anaesthetic ether and then perfused through the left ventricle with a solution containing 2·5% paraformaldehyde and 2·0% glutaraldehyde in orthophosphate buffer at pH 6·6–6·8, preheated to 38 °C. Perfusion was continued for 15–20 minutes. To aid fixation of tissue at 20 days and older, perfusate was also introduced into the sacral part of the subarachnoid space and allowed to escape through a cranial burr-hole. The spinal roots were irrigated in this way for one additional hour. Following bilateral laminectomy, the seventh cervical segment of the spinal cord and the attached spinal nerves were removed and bathed in primary fixative for a further hour. After treatment with buffer and osmium tetroxide, they were washed in distilled water and stained *en bloc* with 2% aqueous uranyl acetate (Hayat, 1975). After staining, they were dehydrated in ethanols, placed in epoxypropane and embedded in Araldite.

All sections were cut using a Reichert OMU4 ultramicrotome. Specimens cut in a plane transverse to the cord produced longitudinal sections of rootlets. Those cut in parasagittal planes produced transverse sections of rootlets. In each of the four youngest age groups and at 300 days, the rootlets of two animals were sectioned transversely and those of the third longitudinally. In each of the remaining age groups, the rootlets of six animals were sectioned transversely and those of two longitudinally. Rootlets were sectioned transversely by means of serial semithin (0·45 μm) sections, with three series of thin (80–110 nm) sections taken at equidistant intervals along the transitional zone. Sections were taken proceeding in a proximodistal direction, beginning medial to the rootlet–cord junction and ending beyond the distal limit of the central tissue projection. Most series of sections at 12 days and older extended distally beyond the level at which the rootlets joined together and therefore into the root itself. The number and sequence of all semithin and thin sections were known. Section thickness was accurately known. That of all thin sections was estimated on the basis of their interference colour spectra (Peachey, 1958), while that of the semithin sections was determined by accurately calibrating the ultramicrotome. Thin sections were post-stained with lead citrate (Reynolds, 1963). Semithin sections were stained with toluidine blue. These were examined in a Reichert Polyvar photomicroscope and photographed at negative magnifications of from $\times 20$ to $\times 400$. Thin sections were examined in Corinth 500 and JEOL JEM 1200 EX electron microscopes. Most electron micrographs were taken at negative magnifications of from $\times 1000$ to $\times 5000$. The precise magnification of each electron micrograph was determined by photographing a standard calibration grid under the same conditions of magnification as the thin sections during each photographic session.

At each age, transverse and longitudinal series of sections were examined to determine the form, appearance and position within the rootlet of the central tissue projection. Central and peripheral tissues could be distinguished from one another at the light microscopical level by their differential uptake of toluidine blue and by the identification of the pale astrocytic cytoplasmic barrier bounding the central tissue projection (Figs. 3, 4). Differentiation was confirmed by comparison with the serial electron micrographs closest to them. Each transversely sectioned transitional zone was followed throughout its entire length on serial sections. Since all section thicknesses were known, transverse sections were used to determine central tissue projection length. The distribution of projection length was determined at each age (Fig. 6). Rootlet cross sectional area was measured midway along the transitional zone, using a MOP-Videoplan image analysis system (Table 1). The relationship of central tissue projection length to rootlet cross sectional area was examined statistically at each age, using a Tectronix 4051 Graphics Display Unit and Tectronix 4662 Flat Bed

plotter (Fig. 7). Age changes in the above features were studied to determine morphologically and morphometrically the form of the mature transitional zone and the patterns of its growth and differentiation.

OBSERVATIONS

General rootlet form and arrangement

Each root was attached to the cord by means of four to eight short rootlets (Fig. 1). These approached the cord at a shallow angle and were attached to it along a strip (the *attachment zone*) on its dorsolateral surface. The transition between the peripheral and the central nervous systems lay at and distal to this level. Medial to this the fibre bundle comprising each rootlet projected above the surrounding cord as a ridge which became progressively less prominent as the bundle gradually sank into the cord. Up to 6 days the rootlets tended to be oval on cross section and were sometimes separated by gaps. At 12 days and after, they were more closely apposed to one another and were approximately rectangular on cross section.

Ultrastructure

The early development of the transitional zone in the cervical dorsal root resembled that in cervical (Fraher, 1978; Fraher & Kaar, 1982) and lumbar (Fraher & Kaar, 1986) ventral rootlets of the rat and in dorsal rootlets of the mouse (Moll & Meier, 1983) and cat (Carlstedt, 1981). At the end of the first week postnatum and subsequently, a central tissue projection extended distally as far as the definitive root (Figs. 2*a*, 4). Its surface was made up of astrocyte processes which varied widely in form and size (Fig. 2*b*, *c*). This formed the central–peripheral interface. The processes were derived from astrocyte perikarya which lay within, and sometimes at the surface of, the central tissue projection (Figs. 2*a*, *d*, respectively). At younger ages some of the latter were apposed to one another in an epithelioid fashion (Fig. 3*d*). As in other transitional regions, the central ends of the most proximal peripheral internodes lay in grooves or invaginations of the surface of the central tissue projection (Fig. 2*e*). The central–peripheral transitional nodes lay at the central ends of these. In their development and mature form these nodes closely resembled those of the lumbar region (Fraher & Kaar, 1984). The central tissue projection resembled central nervous tissue generally in its internal structure (Fig. 2*a*). Astrocyte processes extended distally from its surface into the endoneurial space between the peripheral internodes (Fig. 2*b*). These were considerably more sparse and the glial fringe which they formed was correspondingly more open than that described in the cat by Berthold & Carlstedt (1977) and Carlstedt (1981).

Morphology of the central tissue projection

Up to *3 days* dorsal rootlets contained no central tissue. At that stage the central–peripheral interface was a smooth relatively flat plane which was in line with the curvature of the spinal cord perimeter (Figs. 3*a*, 5*a*). It therefore faced dorsolaterally. After this, the central tissue projection gradually grew distally into the rootlet, displacing peripheral tissue as it did so (Fig. 3*b*). It lay closer to the anterior than to the posterior rootlet surface at all stages. At *5 days* the projection had a highly irregular surface and gave rise to spikes of central tissue which extended distally for 20 μm or more beyond the main projection, to interdigitate with peripheral nervous tissue. Up to and including this stage, peripheral tissue formed the surface of the entire rootlet. After this the central tissue projection became increasingly complex in form.

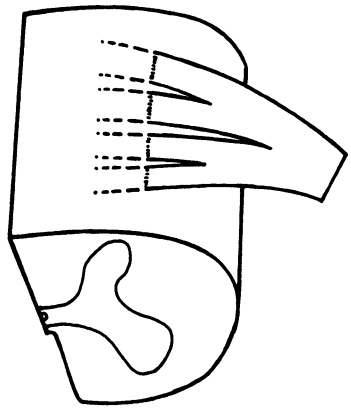
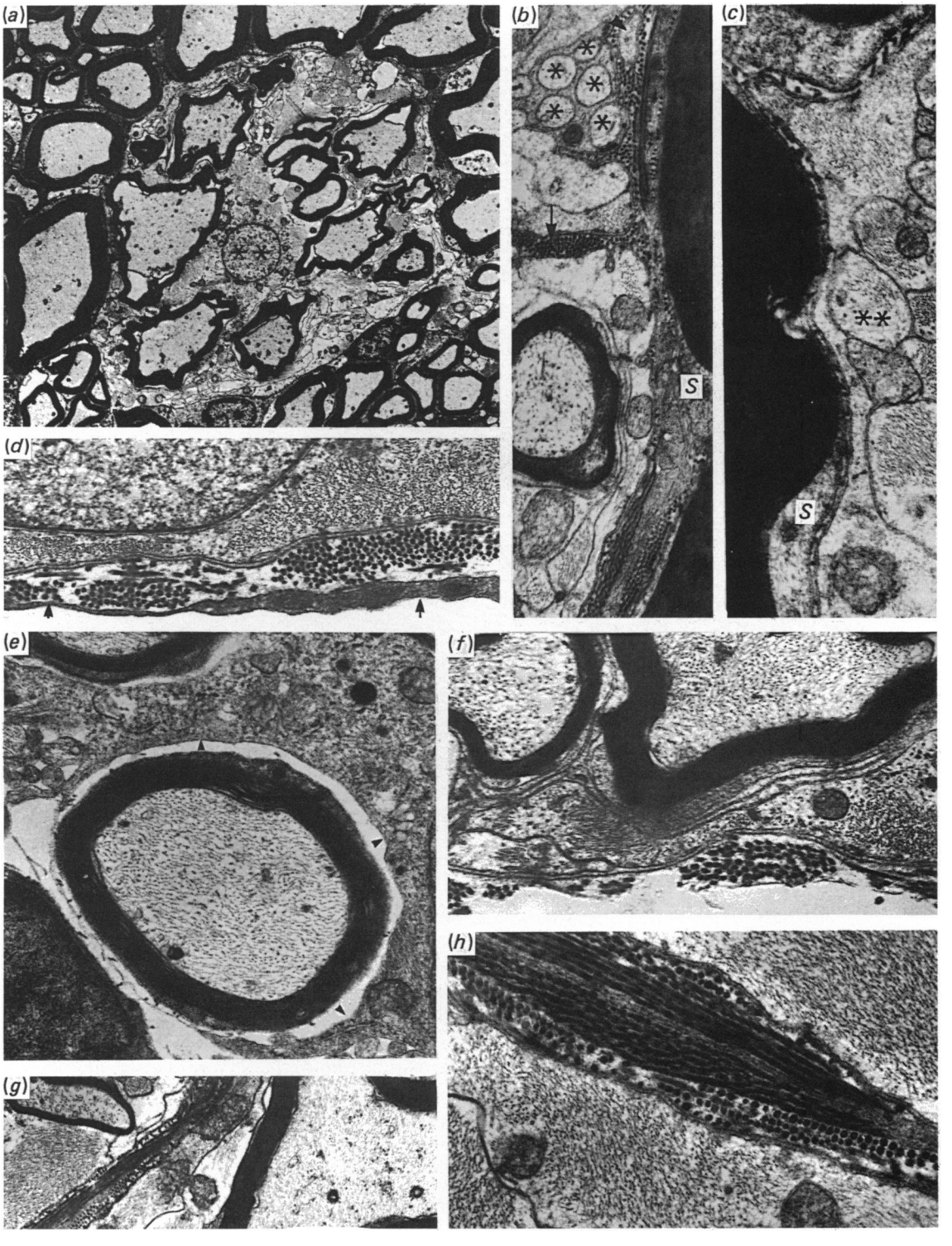


Fig. 1. Diagrammatic view of the dorsal aspect of the spinal cord, showing the manner in which the dorsal spinal nerve root is formed from its constituent rootlets. Medial to the level at which it becomes attached to the cord, each rootlet is continued centrally as a ridge on the cord surface (dotted outlines).

At *6 days* (Fig. 3*d*) it had an irregular and sometimes jagged outline. Because of this, transitional nodes of adjacent fibres were commonly separated by longitudinal distances of up to 30 μm. Over much of its length it formed the anterior surface of the rootlet (Figs. 2*d*, *f*, 3*e*, 4). By *12 days* the projection had increased in length, so that in most cases it extended through the entire length of the rootlet and its distal portion lay in the root. Its surface had become generally smoother than previously. Over most of its extent it was shaped like a long segment of a dorsoventrally flattened, distally tapering wedge with a rounded apex (Fig. 3*e*, 5*c*). Its distal extremity sometimes gave rise to two shallow dome-shaped projections. It continued to form a substantial part of the anterior surface of the rootlet over the transitional zone. The rootlet was markedly asymmetrical insofar as the projection was placed ventrally in it.

Accordingly, peripheral nervous tissue extended further proximally on the posterior than on the anterior rootlet surface. Furthermore, a thin strip of peripheral tissue commonly extended centrally for 200 μm or more beyond the level at which the great majority of fibres had entered the central nervous system (Figs. 4*l*, 5*d*), thereby further increasing rootlet asymmetry.

Fig. 2(*a–h*). Ultrastructure of central tissue projection. (*a*) General appearance of transversely sectioned rootlet at 70 days. Peripheral nervous tissue surrounds the central tissue projection. This consists of pale astrocytic processes surrounding centrally myelinated axons and includes an astrocyte perikaryon with its nucleus (**). × 2020. (*b*, *c*) Transverse sections through 70 day central tissue projections. The surface of the mature central tissue projection consists of multiple layers of astrocyte processes of widely varying form. On section, many are flattened and a proportion are elliptical or polygonal (**). Some envelop individual axons (*) of an unmyelinated bundle. Collagen fibres clothe the projection surface and lie in the spaces separating it from the nearby Schwann cells (*S*). Some lie in deep clefts projecting into it (arrow). Their numbers and density vary from one site to another (compare (*b*) with (*c*)). An astrocyte process (arrowhead) passing distally from the surface of the projection lies among the collagen fibres. (*b*) × 17420; (*c*) × 22770. (*d*) Part of the surface of the 70 day central tissue projection is made up of an astrocyte perikaryon. Here the projection also forms the ventral surface of the rootlet. Arrows, rootlet sheath. × 18500. (*e*) To show the central end of a peripheral internode lying in a groove (arrowheads) on the surface of a 12 day central tissue projection. × 14300. (*f*) To show multiple layers of astrocyte processes forming the surface of a 70 day central tissue projection where the latter comprises the anterior surface of the rootlet. × 21000. (*g*, *h*) Overall view (*g*) and detail (*h*) of connective tissue septum between astrocyte processes forming the surfaces of central tissue projections of adjacent rootlets. (*g*) × 11400; (*h*) × 27700.


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
S
S

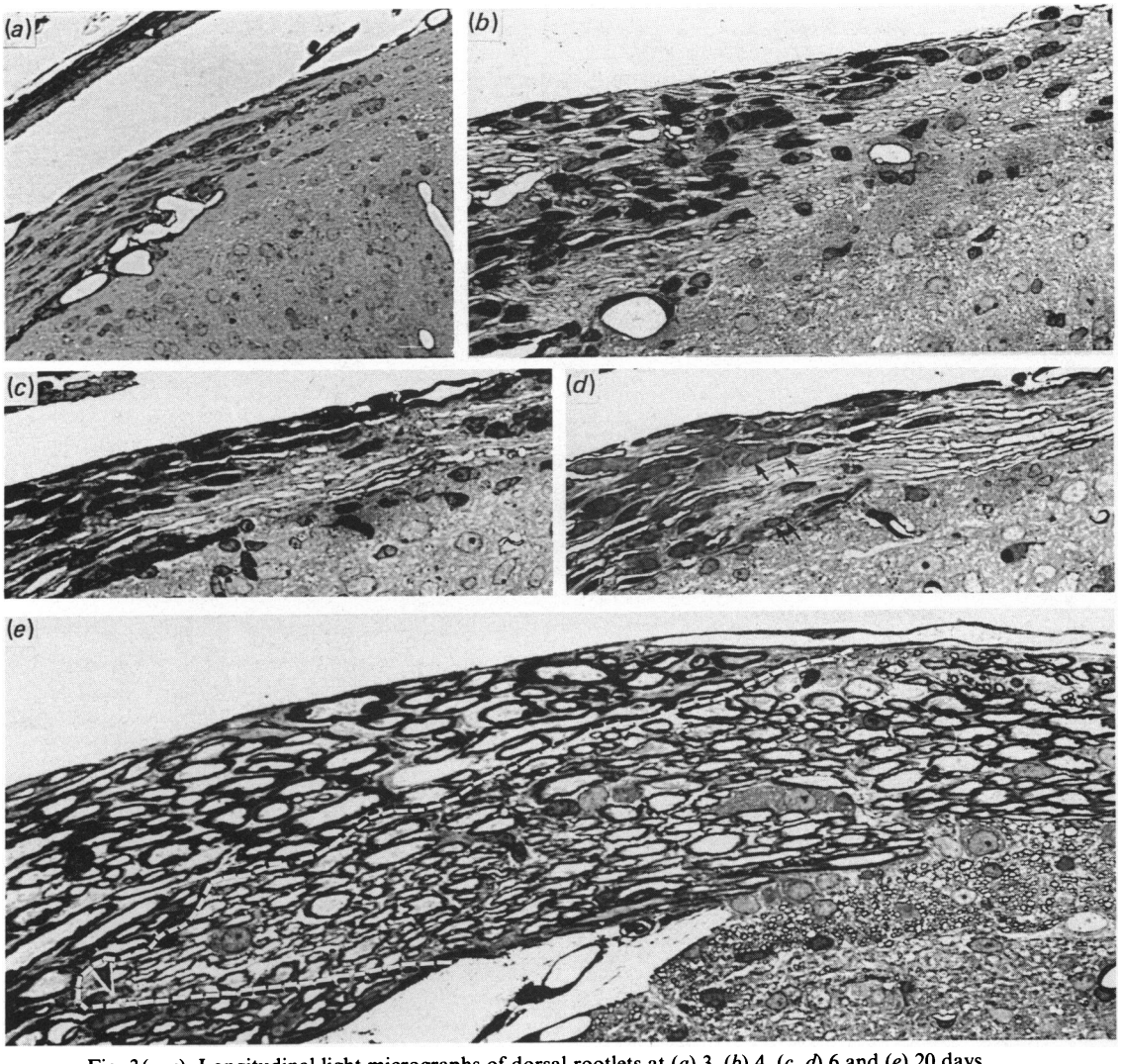
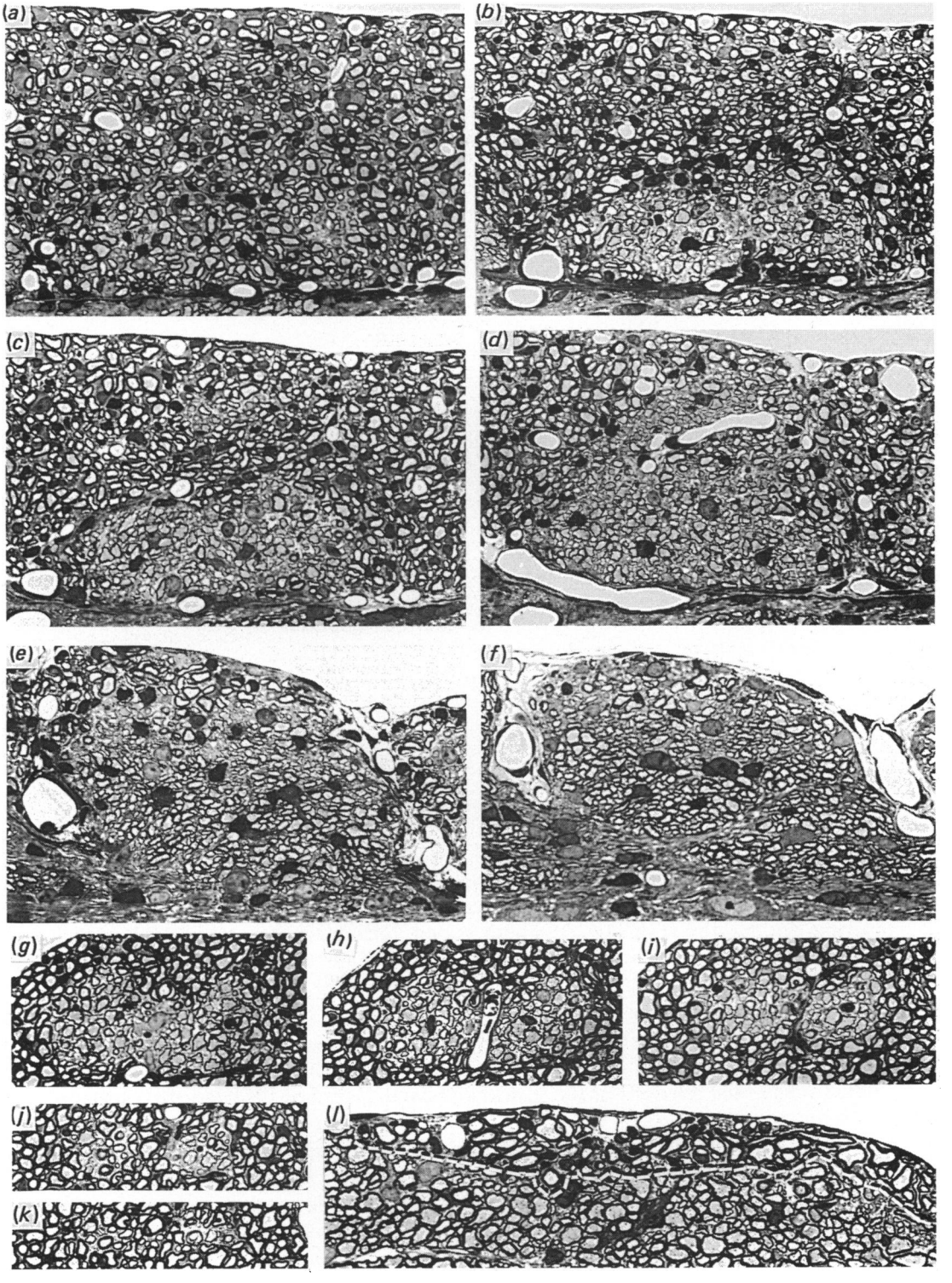


Fig. 3(*a–e*). Longitudinal light micrographs of dorsal rootlets at (*a*) 3, (*b*) 4, (*c*, *d*) 6 and (*e*) 20 days postnatum. At 6 days, note (i) the epithelioid arrangement (arrows) of astrocyte perikarya at the dorsal surface of the central tissue projection, (ii) astrocyte perikarya forming the surface of the central tissue projection also comprising the anterior surface of the rootlet (double arrow). Note the transitional node of Ranvier (arrowhead) at the apex of the 20 day projection (outlined). (*a*) × 375; (*b*) × 364; (*c*) × 390; (*d*) × 390; (*e*) × 350.

By *20 days* the central tissue projection had increased further in complexity and had developed the morphological features typical of maturity. It generally consisted of three continuous segments (Fig. 5*d*). The proximal one of these was shaped like a segment of a wedge and corresponded to most of the 12 day projection. From this the second segment arose and consisted of a dorsoventrally flattened, conical projection

Fig. 4(*a–l*). (*a–f*) Serial transverse sections through the transitional zone of a 12 day rootlet proceeding in a distoproximal direction. Level (*f*) is central to the transitional zone. The central tissue projection (pale) lies closer to the anterior than to the posterior surface of the rootlet. × 280. (*g–k*) Serial transverse sections through a 12 day rootlet, proceeding in a proximodistal direction, showing the central tissue projection bifurcating. × 260. (*l*) Transverse section through a 20 day dorsal rootlet showing a narrow strip of peripheral tissue (outlined) dorsal to the central tissue projection, 200 μm central to the level at which most fibres have passed into the CNS. × 260.


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)

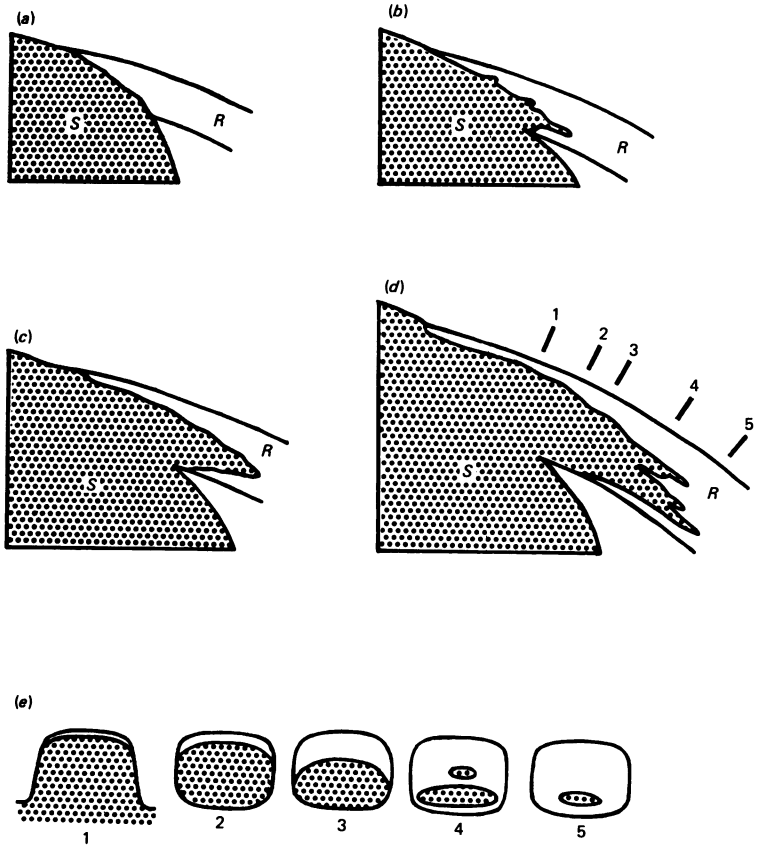


Fig. 5(*a–e*). Diagrammatic longitudinal sections through a dorsal rootlet (*R*) showing the form of the central tissue projection at (*a*) 3, (*b*) 6, (*c*) 12 and (*d*) 20 days postnatum. (*e*) shows the profiles of transverse sections through the rootlet at the levels indicated in (*d*). Central tissue, dots; peripheral tissue, white; *S*, spinal cord.

which tapered distally and was approximately oval on cross section. From the distal end of this a third segment generally arose, consisting of a number of distinct slender tapering outgrowths (Fig. 4*g–k*). These varied considerably in length. They each contained a small bundle of myelinated axons. At *70* and *300 days* the central tissue projection resembled that at 20 days (Fig. 5*d*). However, the overall dimensions of each segment tended to increase progressively with age. Figure 5*e* shows diagrammatically the appearance of the mature central tissue projection and its position within the rootlet at various levels along the transitional zone. This shows that the central tissue formed substantial portions of the rostral and caudal (termed the *collateral*) surfaces of the rootlet.

When traced centrally, each central tissue projection came to occupy a progressively increasing proportion of the cross sectional area of its rootlet. Distal to the levels at which adjacent rootlets became apposed and joined with one another, their projections commonly fused together, or were separated only by layers of collagen fibres (Fig. 2*g*, *h*). Those collagen fibres closest to the rootlet surfaces ran parallel to the long axis of the rootlet. Between these layers, collagen bundles ran circumferentially around the rootlets.

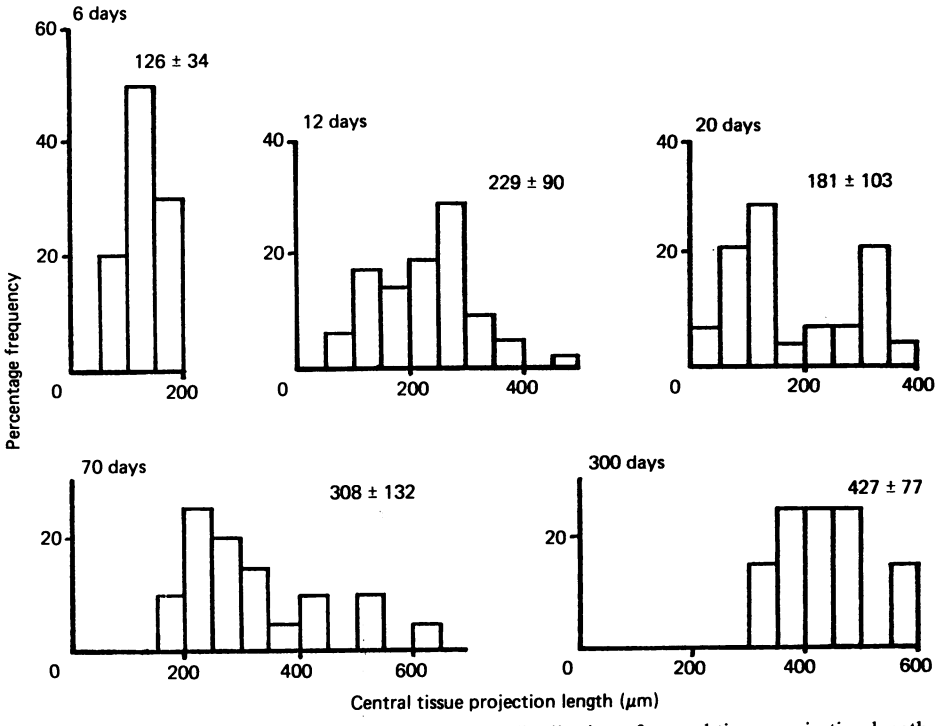


Fig. 6. Percentage frequency histograms showing the distribution of central tissue projection length at the ages indicated. Mean projection length and its standard deviation are also shown.

Morphometry

Central tissue projection length varied widely between rootlets (Table 1; Fig. 6). The length distributions for each succeeding pair of ages overlapped extensively with one another. All were unimodal except for that at 20 days, which was bimodal. The mean length generally tended to increase with age except between 12 and 20 days when it decreased substantially. The greatest rate of increase took place over the first interval (Table 1). The mean central tissue projection length and its standard deviation were also calculated for each animal. Mean projection length varied somewhat between the animals at each age, but the values were almost never statistically different from one another. Only two exceptions were found: One 12 day animal had a mean projection length significantly smaller than half of the remaining animals at that age. At the 20 day stage, mean projection length in one animal was significantly greater than in all the remainder. In that animal all projections were around 300 μm long.

The relative lengths of the various segments which made up the central tissue projection at 20 days and later varied widely and without a constant pattern. For example, the relative length of the second and third segments varied by a factor of up to 10. Furthermore, in some cases the former was the longer of the two, in others the latter. These variations were independent of the total length of the projection.

Rootlet cross sectional area (Table 1) varied markedly (commonly by an order of magnitude) between the component rootlets of each root studied. The larger rootlets tended to lie towards the middle of the attachment zone of each root. Mean area increased over all the age intervals studied, including that between 12 and 20 days, when projection length decreased. However, the increase over that interval was less

Table 1. *Growth rates of rootlet cross sectional area (CSA) and central tissue projection (CTP) length*

	CSA ($\mu m^2/100$)					CTP length (μm)				
Age (Days)	$\bar{x}$ (±S.D.)	$d\bar{x}$	dt	$d\bar{x}/dt$	$d\bar{x}/\bar{x}.dt$ (%)	$\bar{y}$ (±S.D.)	$d\bar{y}$	dt	$d\bar{y}/dt$	$d\bar{y}/\bar{y}.dt$ (%)
6	93 (±34)					126 (±34)				
		43	6	7·2	8		103	6	17	14
12	136 (±81)					229 (±90)				
		18	8	2·3	2		−48	8	−6	−3
20	154 (±133)					181 (±103)				
		213	50	4·3	3		127	50	2·5	5
70	367 (±173)					308 (±132)				
		77	230	0·3	0·1		119	230	0·5	0·2
300	444 (±97)					427 (±77)				

$\bar{x}$, mean rootlet CSA for all animals at the specified age; $d\bar{x}$, increase in area over interval; dt, length of interval (days); $d\bar{x}/dt$, daily increase in CSA over interval; $d\bar{x}/\bar{x}.dt$, daily increase in CSA over interval, expressed as percentage of area at beginning of interval; $\bar{y}$, mean CTP length for all animals at the specified age; $d\bar{y}$, increase in length over interval; dt, length of interval (days); $d\bar{y}/dt$, daily increase in length over interval; $d\bar{y}/\bar{y}.dt$, daily increase in length over interval, expressed as percentage of length at beginning of interval.

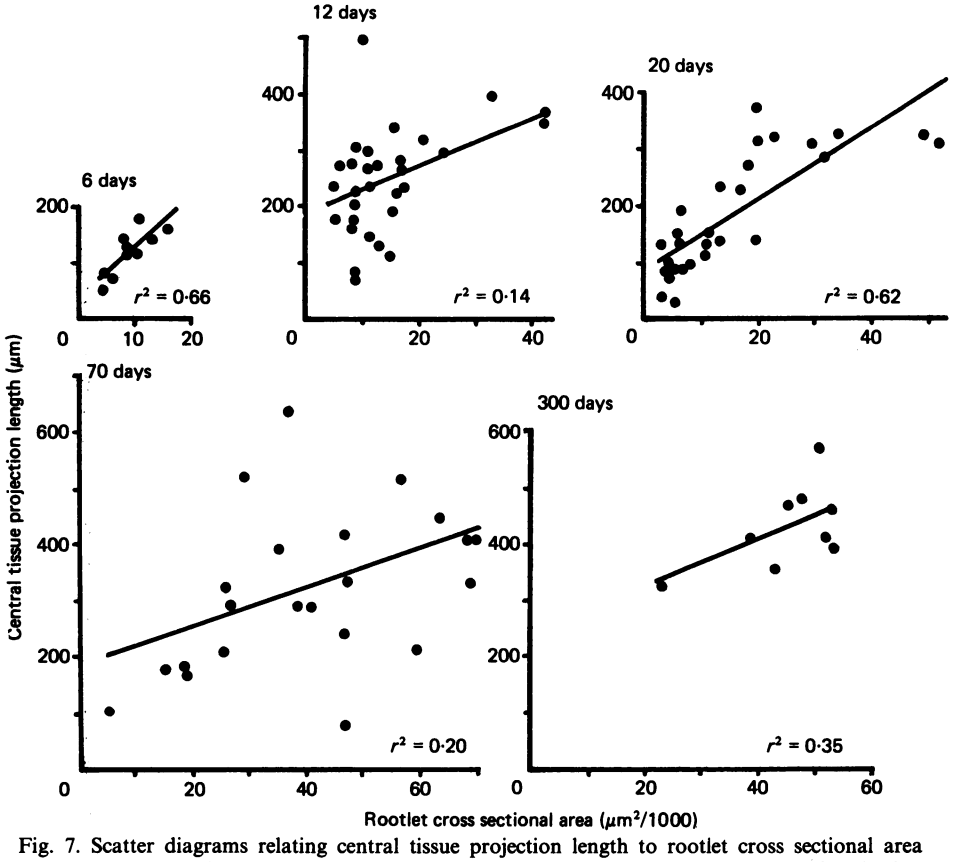


Fig. 7. Scatter diagrams relating central tissue projection length to rootlet cross sectional area (expressed in units of 1000 μm^2), at the ages indicated. The proportion of the variance of projection length attributable to a linear regression between it and rootlet cross sectional area (r^2) is given in each case.

Table 2. *Proportion of ventral and collateral rootlet surfaces composed of central nervous tissue*

Age (days)	Ventral (V) mean ± S.D.	Collateral (C) mean ± S.D.	Ratio of means V/C
6	0·82 ± 0·08	0·69 ± 0·11	1·19
12	0·81 ± 0·09	0·59 ± 0·16	1·37
20	0·74 ± 0·11	0·47 ± 0·11	1·57
70	0·84 ± 0·10	0·54 ± 0·15	1·56
300	0·65 ± 0·16	0·37 ± 0·12	1·76

than over those immediately preceding or following it. The daily rate of increase between 70 and 300 days was substantially less than that at earlier stages.

Although there was a general tendency for larger rootlets to have longer central tissue projections, scatter diagrams (Fig. 7) relating projection length to rootlet cross sectional area showed this relationship to be variable. At the mature stages it was weak. The best fitting curve was linear at 12 and 300 days and logarithmic at the other ages. However, the extent to which the two types of curve fitted the data differed little at each stage. The relationship between central tissue projection length and rootlet *diameter* was also examined. In each case the diameter was calculated as that of the circle having the same area as the rootlet. At each age the relationship was very similar in all respects to that between projection length and rootlet cross sectional area.

Asymmetry of the central tissue projection

Soon after the central tissue projection first appeared, its astrocytic limiting membrane came to form the proximal part of the surface of the rootlet (Fig. 2*d*, *f*). At all stages the projection was asymmetrical within the rootlet, being displaced away from the central axis of the rootlet towards its anterior surface. Accordingly, astrocytic processes formed a larger part of its anterior surface than of its collateral surfaces (Fig. 5*e*). Peripheral tissue formed the remainder of the rootlet surface and made up most or all of its posterior surface. Though astrocyctic tissue made an increasing contribution to the anterior rootlet surface as age advanced, the proportion which this constituted showed no clear tendency to vary with age (Table 2). By contrast, the proportion of the collateral surface made up by central tissue tended to decrease with age. This reflected the increasing asymmetry of the rootlet, as the central tissue both within the rootlet and at its surface gradually shifted anteriorly. This asymmetry was quantified as the ratio between the two proportions mentioned above (Table 1). The ratio decreased with age by a factor of 1·5.

Mitotic and degenerating cells

Mitotic Schwann cells, oligodendrocytes and astrocytes were most common at 3 days and less so at 6 days while only a few were noted at 20 days. They had a similar frequency in peripheral and central tissue at each age at which they were present. At 3 days they were usually within the rootlet tissue. However at 6 days the great majority were located along the rootlet periphery. Degenerating cells were observed at 3 and 6 days, again with a relatively similar frequency in central and peripheral tissues. Their numbers were also small.

DISCUSSION

Age changes in central tissue projection morphology and dimensions

The shape and complexity of the central tissue projection change considerably with age. The central–peripheral interface is at first approximately planar. The projection first appears at 4 days postnatum and from then until about the end of the second week it elongates rapidly. This process is disorganised and as a result the central–peripheral interface is jagged. During the third postnatal week, reorganisation occurs at the expense of elongation. As the projection comes to be shaped like a dorsoventrally flattened wedge it decreases in length. Its surface becomes smoother and remains so subsequently. After this the projection again elongates. Between 6 days and maturity its length increases approximately threefold. Over the same period its volume increases approximately eight times (Fraher, 1985, unpublished observations). Its shape becomes more complex. It develops one or more long, distally tapering processes which in some cases branch. It may therefore possess up to three orders of branching. Branching bears no clear relationship to projection length. Among the longest examples, some are unbranched while others possess second or third order branches. Central tissue projections may also possess irregularities in cat sacral dorsal rootlets (Carlstedt, 1981) and in rat ventral lumbar spinal nerve rootlets (Fraher & Kaar, 1986), but they are neither as marked nor as frequent, nor are they as constant in form as the present examples.

From the beginning the projection lies asymmetrically in the rootlet, being closer to its anterior than to its posterior surface. This asymmetry increases with maturation as the projection comes to lie progressively further anteriorly in the rootlet. In accordance with this, central tissue forms substantial proportions of the anterior and collateral rootlet surfaces. These changes imply either that peripheral nervous tissue withdraws distally, or that central tissue actively migrates distally and becomes organised to build up the proximal part of the rootlet. These events, as well as considerable elongation of the central tissue projection, take place in the absence of substantial numbers of mitoses. It is therefore likely that cell migration, increase in cell size and elaboration of cellular processes play an important part in the development of the central tissue projection. Furthermore, it is likely that astrocyte process organisation is plastic, since the projection successively expands distally, retracts and expands again between the second and fourth weeks postnatum. The tissue dynamics involved in the growth of the dorsal rootlet and the proximal part of the root are therefore complex.

Projection lengths vary widely at each age, so much so that the lengths of some zones at 20 days postnatum already lie within the adult range. Rootlet cross sectional area also varies substantially at each age. Furthermore, the two parameters bear no close statistical relationship to one another. The proportion of the variance of projection length which can be accounted for in terms of its linear regression on rootlet cross sectional area varies from 18 to 69%. This suggests that any tendency for the two parameters to be closely related is disturbed by other, more obscure factors. For example, the proximal and distal limits of neighbouring central tissue projections are commonly at closely similar levels, even though their rootlets have widely differing cross sectional areas. It is therefore possible that factors unrelated to rootlet size, such as rootlet proximity, may tend to maintain these limits level with one another during development. A correlation between transitional zone length and rootlet size in rat lumbar ventral rootlets was suggested by Kaar (1984) and in feline S1 dorsal rootlets by Carlstedt (1981). However, neither author determined the nature of the relationship, its strength, or if it varied with time.

Comparison with transitional zone development in other areas

The growth phases of the central tissue projection in the cervical dorsal rootlet resemble those in some other areas. The period of most rapid growth in length occurs at the onset of its development, between 3 and 6 days. This is also the most rapid growth period of the projection into the L5 ventral rootlet (Fraher & Kaar, 1986). Between 12 and 20 days the cervical dorsal rootlet projection decreases in length. The bimodality of its distribution (Fig. 6) suggests that the extent of the reduction varies substantially between rootlets. A decrease also ocurs in the L5 projection, but at an earlier phase, viz. between 6 and 12 days (Fraher & Kaar, 1986). In both locations this may represent a distal overgrowth of central tissue which engulfs previously peripheral fibre segments. After this, projection length increases at a progressively decreasing rate in both locations.

By contrast, the development of the transitional zone in the cervical dorsal rootlet differs fundamentally from that in the cervical ventral rootlet, as described by Fraher (1978) and by Fraher & Kaar (1982), where during the period immediately after birth, a short central tissue projection grows into the ventral rootlet. This reaches a maximum length of only 14 μm at 3 days postnatum. Its appearance coincides with the initial outgrowth of central tissue into the dorsal rootlet. However, the ventral rootlet projection soon disappears. Perhaps because of distal outgrowth of central tissue (Fraher, 1978; Fraher & Kaar, 1982) Schwann cells become invaginated progressively deeper below the cord surface and the transitional zone sinks into the cord, coming to lie with its distal limit at the level of the cord surface. In this respect transitional zone development in the cervical ventral rootlet differs from that in all other locations studied to date, in which the central tissue projection tends to undergo a progressive and gradual increase in length, though with occasional transient decreases, as discussed above. This qualitative difference suggests that special conditions operate in the ventral cervical region which favour Schwann cell invagination deep to the cord surface, thereby providing for mechanical stability (Fraher & Kaar, 1986).

In general, zone length is considered to be greater in dorsal than in ventral nerve rootlets (Skinner, 1931; Tarlov, 1937; Kaar, 1984). Gamble (1976) states that the length of the glial segment of the transitional zone may be as much as two or three millimetres long in the roots of the cauda equina of large animals. This suggests that transitional zone length may be proportional to animal size. However, the length of the dorsal rootlet central tissue projection in the mouse (Moll & Meier, 1983) and in the cat (Berthold & Carlstedt, 1977) overlap extensively with the ranges of values found in the present study for the rat, despite the differences in dorsal root length and in overall body size between the three species. It is therefore likely that any correlation between body dimensions and central tissue projection length is relatively imprecise.

The mature form of the central tissue projection in rat cervical dorsal rootlets is considerably more complex and variable in its definitive form than that in the cat sacral dorsal rootlet as described by Berthold & Carlstedt (1977). The latter is regularly conical and circular in cross section. Its long axis coincides with the central axis of the rootlet. It is considerably less variable in form, generally lacking second order branches. When present, these are shorter than in the rat. Third order branches are not described. The mature central tissue projection in the rat lumbar ventral rootlet (Fraher & Kaar, 1986) is considerably smaller and more variable in form than that of the cervical dorsal rootlet. Each possesses irregularities in form, but those of the former have a much less constant pattern. Both are asymmetrically placed in the rootlet. That in the former lies posterior to the central axis of the rootlet. Thus both types lie towards the surface of the rootlet closest to the spinal cord as it runs in the subarachnoid space.

SUMMARY

Each seventh cervical dorsal nerve root is attached to the spinal cord surface by four to eight rootlets. A tapering outgrowth of central nervous tissue, the *central tissue projection*, extends distally into the proximal part of each rootlet in the immediate postnatal period. The central ends of the most proximal peripheral internodes surround this projection. Thus a length of rootlet contains both CNS and PNS tissue. This is termed the *transitional zone.*

Material was processed by standard preparative techniques for electron microscopy. Serial semithin and ultrathin sections were made over the entire extent of several transitional zones at ages ranging from 2 to 300 days postnatum. Central tissue projections were reconstructed in three dimensions and analysed morphometrically.

The morphology of the central tissue projection varies during development. At first, it forms an irregular projection into the anterior portion of the rootlet. It than elongates and takes the form of a dorsoventrally flattened, distally tapering wedge. By 20 days postnatum it has attained its definitive form. This consists of three segments: a proximal wedge-shaped portion, similar to that described above; continuous with this is a distally tapering, dorsoventrally flattened, cone-shaped segment which generally branches into two or more slender projections of central tissue. The latter comprise the third segment. The projection comes to form a substantial proportion of the anterior, proximal and distal surfaces of the dorsal rootlet from an early stage.

The mean length of the central tissue projection increases progressively over all intervals studied, except that between 12 and 30 days postnatum, when a reduction in length is associated with reorganisation of the morphology of the projection. Projection length varies considerably between rootlets and is relatively weakly correlated with rootlet cross sectional area. There is a great deal of overlap between the distributions of projection lengths at all stages between 20 and 300 days.

The authors wish to thank Mr D. O'Leary and Ms B. Rea for their technical assistance and Ms V. Paul for typing the manuscript. The JEOL JEM 1200 EX electron microscope was purchased with the aid of a grant from the Wellcome Trust.

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 S1 dorsal rootlets. *Acta physiologica scandinavica, Suppl.* **446**, 23–42.

CARLSTEDT, T. (1981). An electron-microscopical study of the developing transitional region in feline S1 dorsal rootlets. *Journal of the Neurological Sciences* **50**, 357–372.

FRAHER, J.P. (1978). The maturation of the ventral root–spinal cord transitional zone. An ultrastructural study. *Journal of the Neurological Sciences* **36**, 427–449.

FRAHER, J. P. & KAAR, G. F. (1982). The maturation of the ventral root–spinal cord transitional zone. Part 2. A quantitative ultrastructural study of the dynamics of its early development. *Journal of the Neurological Sciences* **53**, 63–75.

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.

HAYAT, M. A. (1975). *Positive Staining for Electron Microscopy*. New York: Van Nostrand Reinhold Co.

KAAR, G. F. (1984). Development of the transitional region between central and peripheral nervous systems. A morphological and quantitative study of the L5 ventral root. 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.

MOLL, C. & MEIER, C. (1983). The central-peripheral transition zone of cervical spinal nerve roots in jimpy mutant and normal mice. Light and electron-microscopic study. *Acta neuropathologica* **60**, 241–251.

PEACHEY, L. D. (1958). Thin sections. I. A study of section thickness and physical distortion produced during microtomy. *Journal of Biophysical and Biochemical Cytology* **4**, 233.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208.

SHEEHAN, M. M., FRAHER, J. P. & O'SULLIVAN, V. R. (1984). The ultrastructural appearance of oligodendrocytes in the developing CNS-PNS transitional zone of the dorsal rootlet. *Proceedings of the Royal Microscopical Society* **19**, Suppl. 12–13.

SKINNER, H. A. (1931). Some histological features of the cranial nerves. *Archives of Neurology and Psychiatry* **25**, 356–372.

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