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A numerical study of cervical and thoracic ventral nerve roots

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INTRODUCTION

Numerous workers have studied the total numbers of ventral root axons capable of being counted with the light microscope (Hatai, 1903; Dunn, 1912; Duncan, 1934*a*; Moyer & Kaliszewski, 1958).

Branching of nerve fibres is known to occur in developing, adult and regenerating nerves, and it has been observed in the living by Speidel (1933, 1964). The literature up to 1950 dealing with fibre branching has been reviewed by Sunderland & Lavarack (1953). Evidence in favour of such branching has been found in frog sciatic and femoral nerves (Dunn, 1902), in rat peroneal nerves (Greenman, 1913), in branches of nerves to cat leg muscles (Eccles & Sherrington, 1930) and in human (Bors, 1925; Swensson, 1949) and dog cranial nerves (McLean, 1927). These studies, as well as those for total numbers of ventral root axons mentioned above, have all been undertaken at the light microscope level and are therefore limited by the inability of the light microscope to demonstrate the finer axons.

The present study was carried out on rat ventral nerve roots. The electron microscope was used to examine the changes taking place during the perinatal period in the total number of axons per root as well as in the numbers belonging to various classes of axon within the root. Evidence suggestive of axon branching was also considered.

In the course of another study dealing with myelination (Fraher, 1973), each of a number of rat lower cervical ventral roots had been sectioned transversely at several levels, the first and last levels being at some distance from one another. The opportunity was therefore taken of carrying out a limited study of the manner in which the numbers of myelinated, and of various classes of unmyelinated, axons varied over distances of the order of several hundreds of microns along the roots. The pattern thus found for these roots, which contain predominantly somatic efferent fibres, was compared with that for roots which also contain visceral efferent fibres, by studying in the same manner mid-thoracic ventral roots over a similar age range.

For the purposes of the present investigation the following terms are defined:

(i) *Compact myelin* is said to be present only when (at least) two parts of the same spiral mesaxon separated by one turn have coalesced so that both period and intraperiod lines have appeared. The latter line is frequently less clearly demonstrable in newly formed compact myelin. Any fibre whose sheath contains compact myelin thus defined will be referred to as being myelinated.

(ii) *Noncompact myelin* is said to be present when either the mesaxon describes

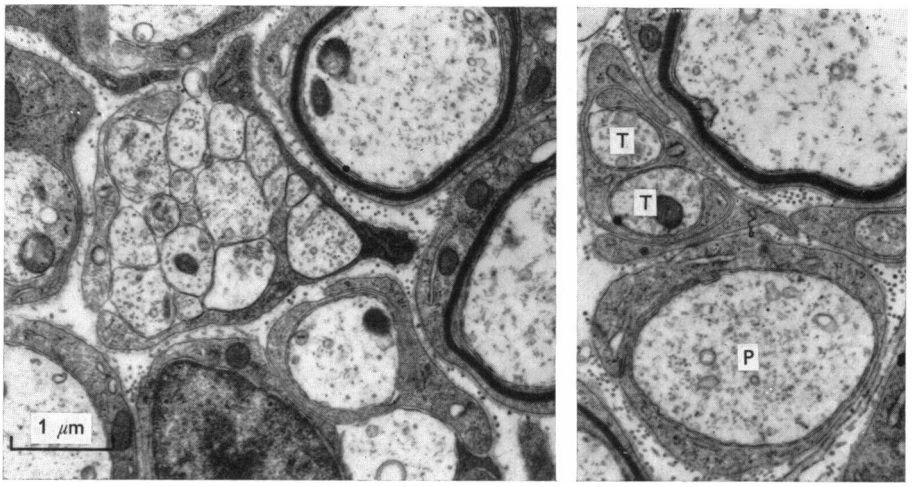

Fig. 1. Electron micrographs showing (*a*) a packet of fetal axons, (*b*) transitional axons (T), and (*c*) a promyelin axon (P).

less than one complete turn about the axon, or the mesaxon has completed one or more spiral turns about its axon but has nowhere condensed to give rise to period and intraperiod lines. Such axons are regarded as unmyelinated.

(iii) *Fetal axon.* Detailed accounts of the morphological changes occurring in peripheral nerves during their maturation have been given by Peters & Muir (1959), Cravioto (1965), Gamble & Breathnach (1965), Gamble (1966), Allt (1969), Ochoa (1971), Webster, Martin & O'Connell (1973) and Martin & Webster (1973). Particularly during the early stages of development it is frequently the case that axons are arranged in groups or packets of two or more, each packet being in a distinct compartment bounded by one or more Schwann cells. The individual axons are separated from one another by shallow gaps, there being no visible structure between them (Fig. 1). Such axons are *fetal axons* (Friede & Samorajski, 1968). The packets will be referred to as fetal packets. One Schwann cell may envelop more than one fetal packet.

(iv) *Transitional axon.* As a packet of axons enveloped by a single Schwann cell matures, the individual axons are progressively separated from one another, so that the original packet is divided into a number of smaller packets, each of which is further subdivided, thus progressively reducing the number of axons in each packet. Even with axon/Schwann cell ratios which are greater than unity, therefore, a single axon may become the only occupant of a particular groove on the Schwann cell surface. Such an axon will be referred to as a *transitional axon* (Fig. 1) and corresponds to the *segregated axon* of Webster, Martin & O'Connell (1973).

(v) *Promyelin axon.* In all cases examined in the present study, it is only when the segregation process has produced (in T.S.) the appearance of a single axon in a single Schwann cell that compact myelin can be formed. An unmyelinated axon enclosed in one Schwann cell at a particular level will be referred to as a *promyelin axon* (Fig. 1). It, together with its enveloping Schwann cell will be referred to as a

promyelin fibre (Friede & Samorajski, 1968), because it is thought to have the potential of myelination. Webster *et al.* (1973) have shown that a given axon may be assigned to more than one of the above classes at different levels of one and the same Schwann cell. Very few axons were observed which were not in contact with a Schwann cell. Such axons, if solitary, were included in the transitional class; if in a group of two or more they were classed as fetal. Thus, every axon observed in each root studied could be assigned to one of the above four classes, viz. myelinated, fetal, transitional and promyelin.

MATERIALS AND METHODS

The present study was carried out on fetal rats at a *post-coitum* age (accurate to within one hour) of 20 days as well as on newborn and postnatal animals aged 1, 2, 6, 12, 17 and 21 days. The method of fixation and embedding of tissue has been previously described (Fraher, 1972). Following ether anaesthetization the animal was perfused through the left ventricle with a solution at 4 °C of 4% paraformaldehyde and 0·5% glutaraldehyde in phosphate buffer at a pH of 7·2. Bilateral laminectomy was then carried out, and the 12 to 14 most rostral spinal medullary segments, together with the attached pairs of roots and proximal parts of the spinal nerve trunks, were carefully exposed under a dissecting microscope. These were removed *en bloc*, osmicated, and the spinal medulla divided by transverse sections into segments. The segments were embedded in Araldite, note having been taken of the curvature, shape and orientation of each ventral root and its constituent rootlets.

The roots chosen for investigation were from two levels: lower cervical (C_6 and C_7) and mid-thoracic (T_5 and T_6). In the cervical region two roots were studied from 20 day fetuses (each from a different litter mate), two roots from 1 day postnatal animals and one each from newborn, 2, 6, 12 and 21 day postnatal animals. In the thoracic region two roots (each from a different litter mate) were studied in the newborn and the 2 day old, but only one root from a 20 day fetus and from animals 1, 6 and 17 days old. At two days the cervical and thoracic roots were taken from the same animal.

The change in number of axons was studied over a length of root which varied between specimens from 50 to 250 μm. Distances between proximal and distal levels of section were as follows:

(i) *Cervical*: 20 day fetal, root 1: 100 μm; 20 day fetal, root 2: 192 μm; newborn: 180 μm; 1 day, root 1: 200 μm; 1 day, root 2: 100 μm; 2 days: 200 μm; 6 days: 185 μm; 12 days: 184 μm; 21 days: 150 μm.

(ii) *Thoracic*: 20 day fetal: 50 μm; newborn, root 1: 50 μm; newborn, root 2: 131 μm; 1 day: 200 μm; 2 days, root 1: 140 μm; 2 days, root 2: 110 μm; 6 days: 220 μm; 17 days: 125 μm.

Ultrathin transverse sections were made at either end of the length studied. These were stained with lead citrate (0·2%) and uranyl acetate (saturated solution in 50% ethanol) and examined in an AEI (Metropolitan-Vickers) EM6 electron microscope. For the purposes of the present investigation it was essential that all ultrathin sections used should be sufficiently close to the transverse to enable the outlines of all unmyelinated axons to be clearly distinguished, particularly those which were members of fetal axon packets. Only those sections fulfilling this criterion were studied because

it was frequently difficult to distinguish adjacent fetal axons from one another if the plane of section departed considerably from the transverse.

Serial thick (0·5–1·0 μm) transverse sections were made of the intervening segment of the root, using a Porter-Blum MT2 automatic ultramicrotome. These, stained with a mixture of 0·8 % toluidine blue and 0·2 % pyronin B, were examined at intervals along the root to ensure that the plane of section remained approximately transverse throughout, and that no nerve fibres joined or left the root or its constituent rootlets over the length covered by the study. Both of these conditions were fulfilled in every case.

An electron micrographic montage was made of the whole cross section of all the roots under investigation, or of all their constituent rootlets, at the proximal and distal ends of the lengths studied. The electron optic magnification differed from one root to another, being $\times 2500$, $\times 4000$ or $\times 6000$. In order to check the magnification, a calibration grid was photographed, under the same conditions of magnification as the root, at each photographic session.

At both proximal and distal levels the numbers of myelinated, promyelin, transitional and fetal axons were counted and the percentage of the total made up by axons of each class was calculated. In order to decrease observer error, counts were made twice in each root. The difference in the number belonging to each class over the length of the root was also noted and was expressed as a percentage of the number at the proximal level per 100 μm distance along the root. In addition, the number of axons in each fetal packet was noted at both levels for each root. The difficulty of distinguishing between fetal axons and Schwann cell processes has been noted by Robertson (1960) and by Dyck & Hopkins (1972). Only those profiles which were unequivocal sections through unmyelinated axons were counted. However, in only a very small proportion of cases was there any doubt as to the nature of the profile sectioned.

OBSERVATIONS

Figure 2 shows the total numbers of axons in cervical and thoracic ventral roots at the ages indicated. The numbers present in the more mature cervical roots were considerably less than those of the earlier ones. The thoracic totals did not show such a dramatic fall during the period under review.

The segregation of axons from one another proceeds more rapidly in the cervical than in the thoracic roots. The proportion of fibres having an axon/Schwann cell ratio of unity had become large in the cervical roots shortly after birth (Fig. 3). In the thoracic region such fibres still comprised less than one half of the total at 17 days (Fig. 3). These trends are followed fairly closely by the changing proportion of the total made up by the myelinated axons in each case.

Figures 4 and 5 show the numbers and percentages made up by the various classes of axon at both proximal and distal root levels over a similar range of ages in the cervical and thoracic regions respectively. It is clear that the spectra of axon numbers and percentages in the cervical roots differ considerably from those in the thoracic over the period encompassed by the present study; the percentage made up by myelinated axons is much larger, and by fetal axons much smaller, in the cervical roots when similar stages are compared.

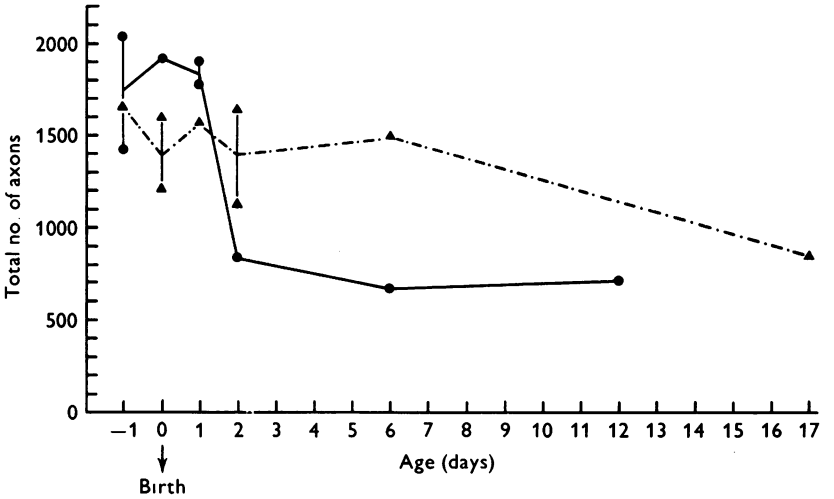

Fig. 2. Graphs showing total numbers of axons contained in cervical (circles) and thoracic (triangles) ventral roots at various ages. In all cases the numbers given refer to those at the most proximal level of section of the root.

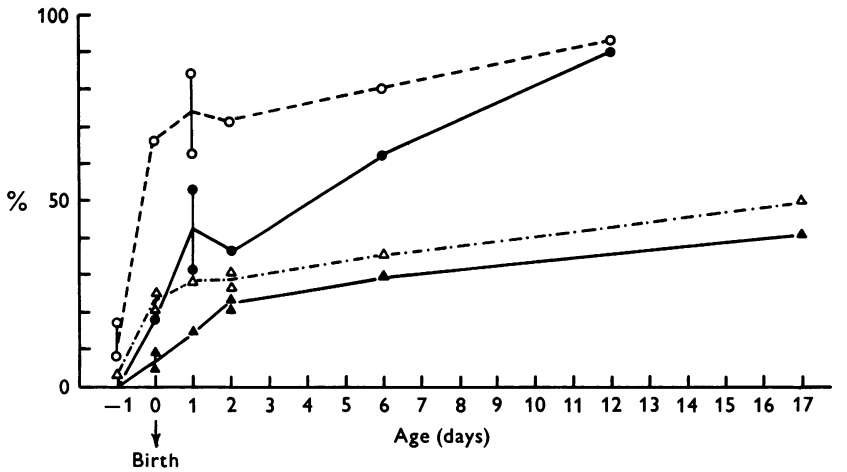

Fig. 3. Graphs showing the percentages of the total number of axons in each root examined which were made up (i) by myelinated and (ii) by fully segregated (myelinated together with promyelin) axons. Cervical roots: myelinated axons – closed circles; fully segregated axons – open circles. Thoracic roots: myelinated axons – closed triangles; fully segregated axons – open triangles.

The degree of segregation of individual axons may be taken as an indicator of root maturity. The histograms relating to the 20 day fetal cervical roots show that the degree of maturity may vary considerably between corresponding roots in different animals whose ages were closely similar (Fig. 4). Marked variation was also noted in the maturity of the component rootlets of individual roots.

In one 20 day fetal and in the 1 day postnatal animal, all rootlets making up

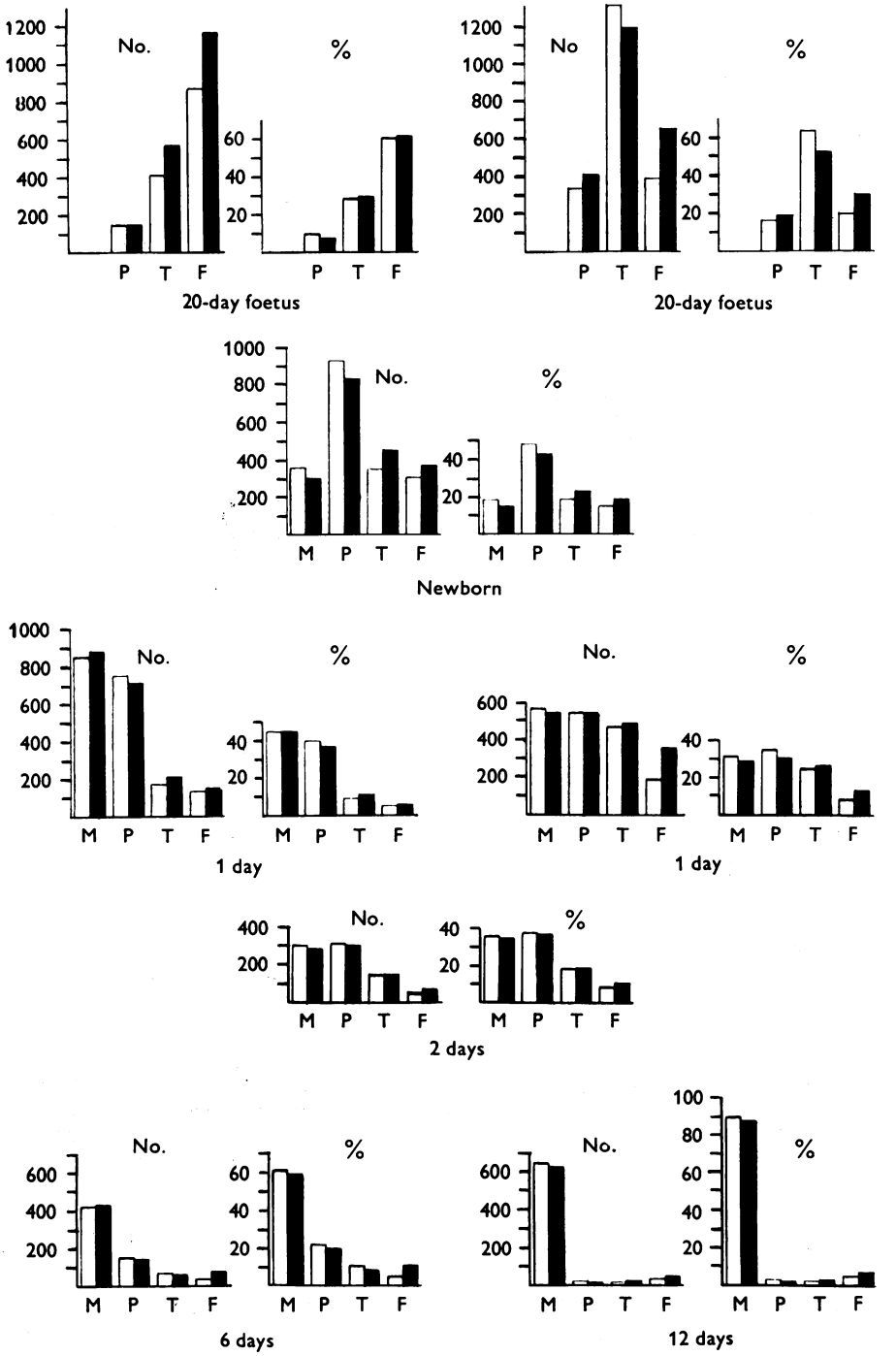

Fig. 4. Diagrams showing (i) the numbers and (ii) the percentages of the total made up by myelinated (M), promyelin (P), transitional (T) and fetal (F) axons at proximal and distal levels of cervical ventral roots at the ages indicated. Proximal levels – unshaded bars. Distal levels – shaded bars.

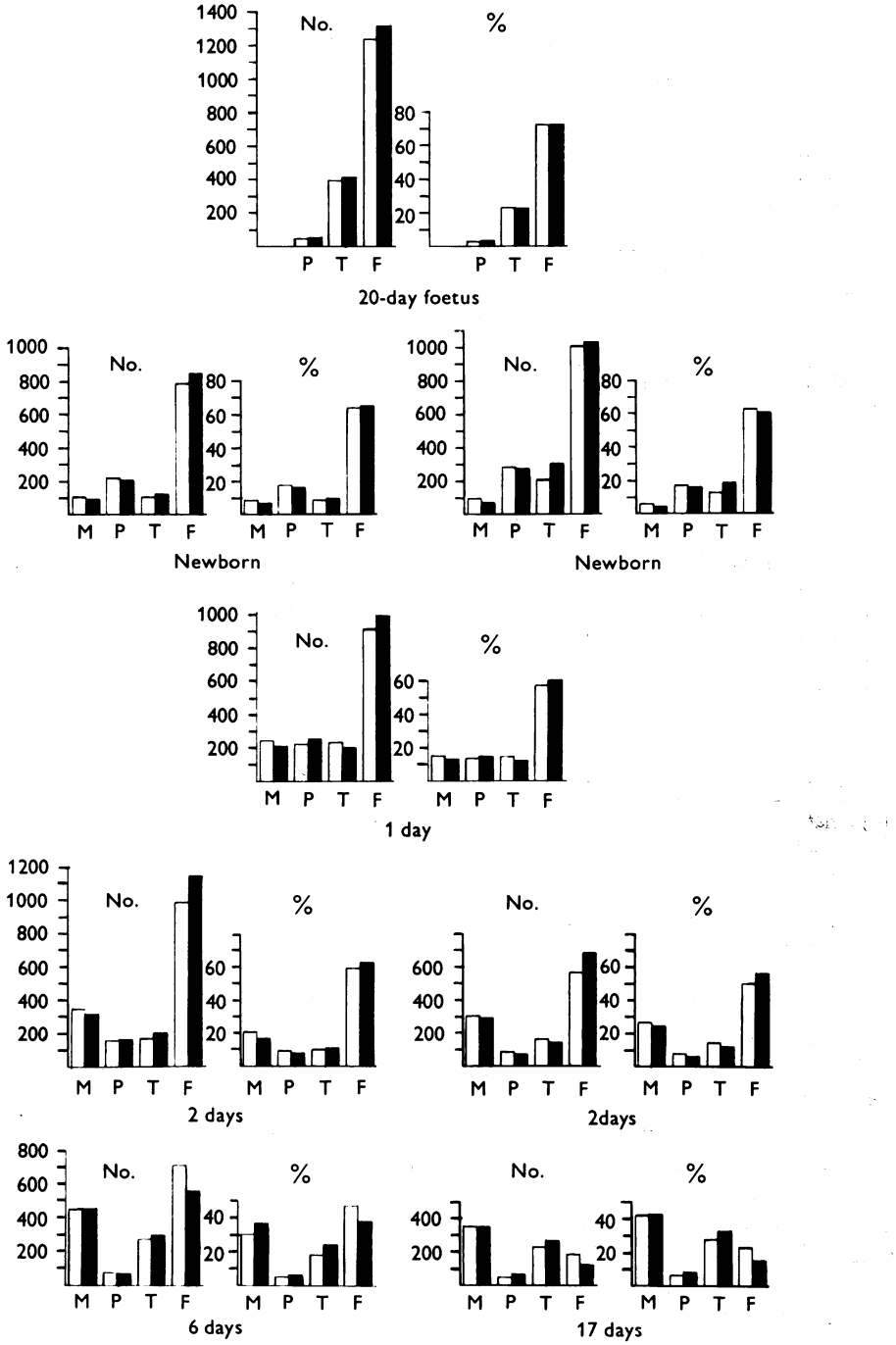

Fig. 5. Diagrams showing (i) the numbers and (ii) the percentages of the total made up by myelinated (M), promyelin (P), transitional (T) and fetal (F) axons at proximal and distal levels of thoracic ventral roots at the ages indicated. Proximal levels – unshaded bars. Distal levels – shaded bars.

Table 1. *Cervical roots*

This table shows the *change with distance* along each cervical root studied both of the numbers of axons in each class, and of the total number of axons per root. In order to facilitate comparison between roots, the change is expressed as a percentage increase or decrease over a distance of 100 μm along the root in each case. The difference between the numbers at the proximal and distal levels is expressed as a percentage of that at the proximal.

	Age							
Axon class	20 day fetus	20 day fetus	New-born	1 day	1 day	2 days	6 days	12 days
Myelinated	—	—	−6·6	+1·8	−3·2	−2·7	+0·5	−0·3
Promyelin	+1·4	+11·6	−5·4	−2·3	−0·2	−1·4	−2·5	−5·4
Transitional	+35·6	−4·6	+15·4	+12·4	+3·8	+0·4	−5·2	+21·6
Fetal	+33·4	+32·9	+11·9	+4·8	+87·5	+13·3	+55·4	+19·0
Total	+30·8	+5·3	+1·1	+1·3	+9·6	−0·4	+2·4	+0·2

Table 2. *Thoracic roots*

This table shows the *change with distance* along each thoracic root studied both of the numbers of axons in each class, and of the total number of axons per root. In order to facilitate comparison between roots the change is expressed as a percentage increase or decrease over a distance of 100 μm along the root in each case. The difference between the numbers at the proximal and distal levels is expressed as a percentage of that at the proximal.

	Age							
Axon class	20 day fetus	New-born	New-born	1 day	2 days	2 days	6 days	17 days
Myelinated	—	−20·0	−18·1	−6·4	−6·3	−1·2	−0·2	0
Promyelin	0	−4·5	−0·8	+9·5	+3·6	−11·1	−1·2	+26·5
Transitional	+8·0	+24·6	+33·5	−6·3	+14·2	−7·7	+3·4	+12·9
Fetal	+13·3	+5·5	+1·7	+4·5	+11·1	+17·8	−9·9	−27·2
Total	+11·4	+4·8	+4·2	+1·9	+8·7	+7·4	−4·2	−1·1

the cervical root were studied separately at three different levels. Considerable differences were evident not only between the axon spectra but also between the manner in which the numbers of contained axons changed per unit length in each from one level to another.

Tables 1 and 2 relate to the change in numbers of axons in each class between the different levels of the various roots. To facilitate comparison the change in each case is expressed as a percentage increase or decrease over a distance of 100 μm. In all but four of the roots the number of myelinated axons decreased proximodistally; in two cases (1 and 6 day old cervical) it underwent a slight increase; in the remaining case (17 day old thoracic) it remained unchanged. The fixation of the unmyelinated axons in the 21 day old cervical root was poor, allowing counts to be made of the myelinated axons only: 715 of these were counted at both levels, which were separated by 150 μm.

Both promyelin and transitional axon numbers behaved variably, though the

Table 3. *Cervical roots*

For each root studied data are given for the following classes: (i) all axons in the root; (ii) all fully segregated axons (myelinated together with promyelin); (iii) all partially segregated axons (transitional together with fetal).

In the case of each of the above classes the total number of axons is given at the proximal (p) and distal (d) levels of section, as well as the difference between these values ($p-d$). Since distances between proximal and distal levels of section varied from one root to another, in order to facilitate comparison between roots, an increase or decrease in number of axons in each class over a standard distance of 100 μm is also given: $(p-d)/100\,\mu$m.

	(i) Total				(ii) Fully segregated				(iii) Partially segregated			
Age (days)	Prox (p)	Dist (d)	$p-d$	$p-d$/ 100 μm	Prox (p)	Dist (d)	$p-d$	$p-d$/ 100 μm	Prox (p)	Dist (d)	$p-d$	$p-d$/ 100 μm
20 day fetus	2041	2256	+215	+112	337	412	+75	+39	1704	1844	+140	+73
20 day fetus	1439	1882	+443	+443	146	148	+2	+2	1293	1734	+441	+441
Newborn	1931	1953	+22	+12	1275	1134	−141	−78	656	819	+163	+90
1	1907	1957	+50	+26	1601	1596	−5	−2	306	361	+55	+28
1	1779	1946	+167	+167	1111	1092	−19	−19	668	854	+186	+186
2	841	835	−6	−3	621	596	−25	−13	220	239	+19	+10
6	682	712	+30	+17	571	568	−3	−2	111	144	+33	+18
12	716	730	+14	+8	664	659	−5	−3	52	71	+19	+10

Table 4. *Thoracic roots*

For each root studied data are given for the following classes: (i) all axons in the root; (ii) all fully segregated axons (myelinated together with promyelin); (iii) all partially segregated axons (transitional together with fetal).

In the case of each of the above classes the total number of axons is given at the proximal (p) and distal (d) levels of section, as well as the difference between these values ($p-d$). Since distances between proximal and distal levels of section varied from one root to another, in order to facilitate comparison between roots, an increase or decrease in number of axons in each class over a standard distance of 100 μm is also given: $(p-d)/100\,\mu$m.

	(i) Total				(ii) Fully segregated				(iii) Partially segregated			
Age (days)	Prox (p)	Dist (d)	$p-d$	$p-d$/ 100 μm	Prox (p)	Dist (d)	$p-d$	$p-d$/ 100 μm	Prox (p)	Dist (d)	$p-d$	$p-d$/ 100 μm
20 day fetus	1670	1779	+109	+273	49	56	+7	+18	1621	1723	+102	+255
Newborn	1234	1293	+59	+118	331	315	−16	−32	903	978	+75	+150
Newborn	1602	1691	+89	+68	380	354	−26	−20	1222	1337	+115	+88
1	1591	1652	+61	+31	454	463	+9	+5	1137	1189	+52	+26
2	1668	1832	+164	+117	510	486	−24	−17	1158	1346	+188	+134
2	1129	1212	+83	+75	391	376	−15	−14	738	836	+98	+89
6	1495	1358	−137	−62	521	517	−4	−2	974	841	−133	−60
17	832	821	−11	−8	403	420	+17	+14	429	401	−28	−22

former tended more often to show a decrease, and the latter an increase, proximo-distally.

In all cases examined for fetal axons, apart from the 6 and 17 day thoracic, their numbers increased proximo-distally (Figs. 4, 5); the relative magnitudes of these changes are shown in Tables 1 and 2. The fetal axons made up a greater percentage

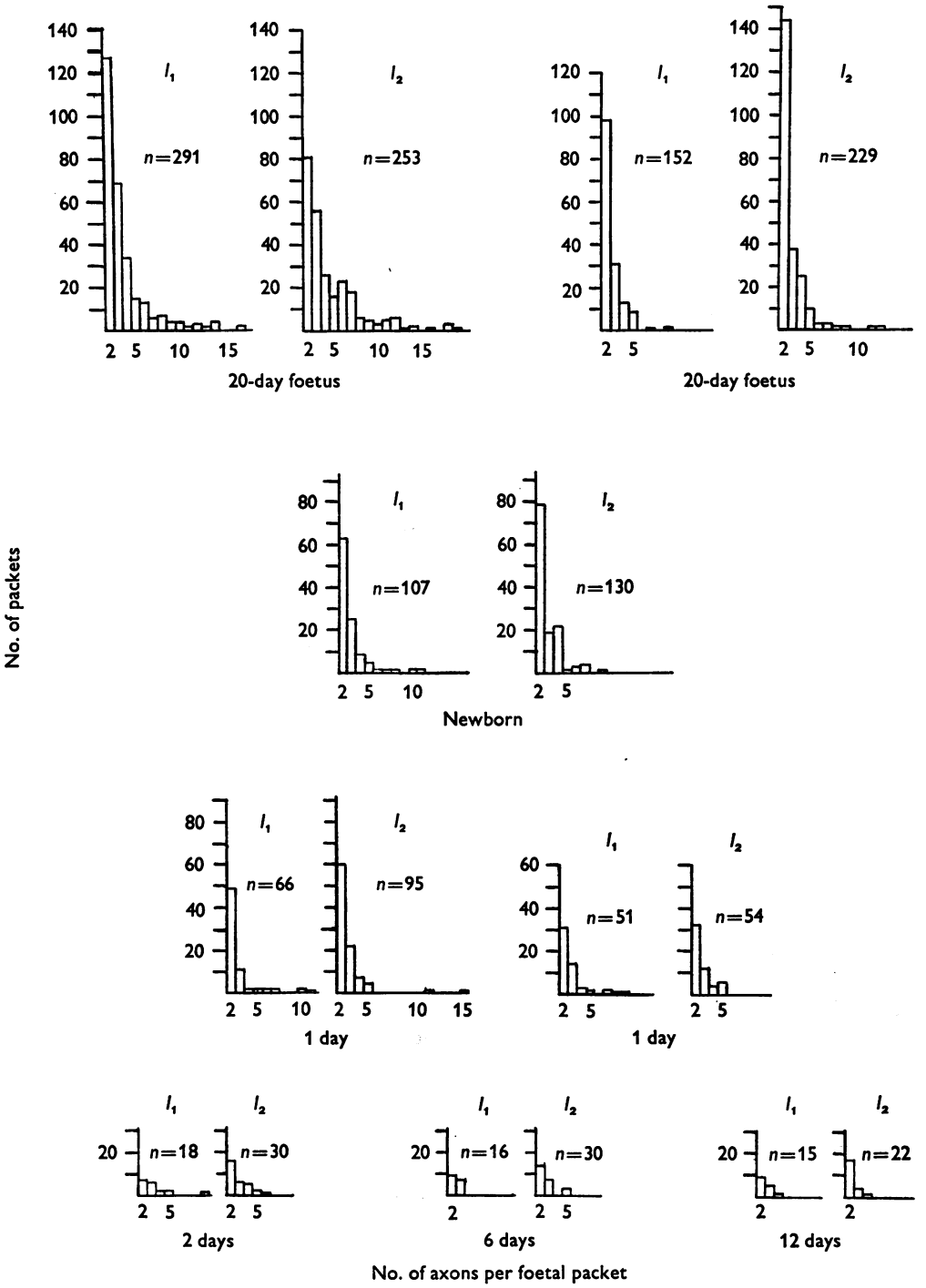

Fig. 6. Frequency histograms showing the total numbers of fetal packets containing the indicated numbers of axons observed over the whole transverse section of cervical ventral roots at different levels at the ages shown. Ordinates: numbers of fetal packets. Abscissae: fetal packet size. n = total number of fetal packets in root; l_1 = transverse level 1 (proximal); l_2 = transverse level 2 (distal).

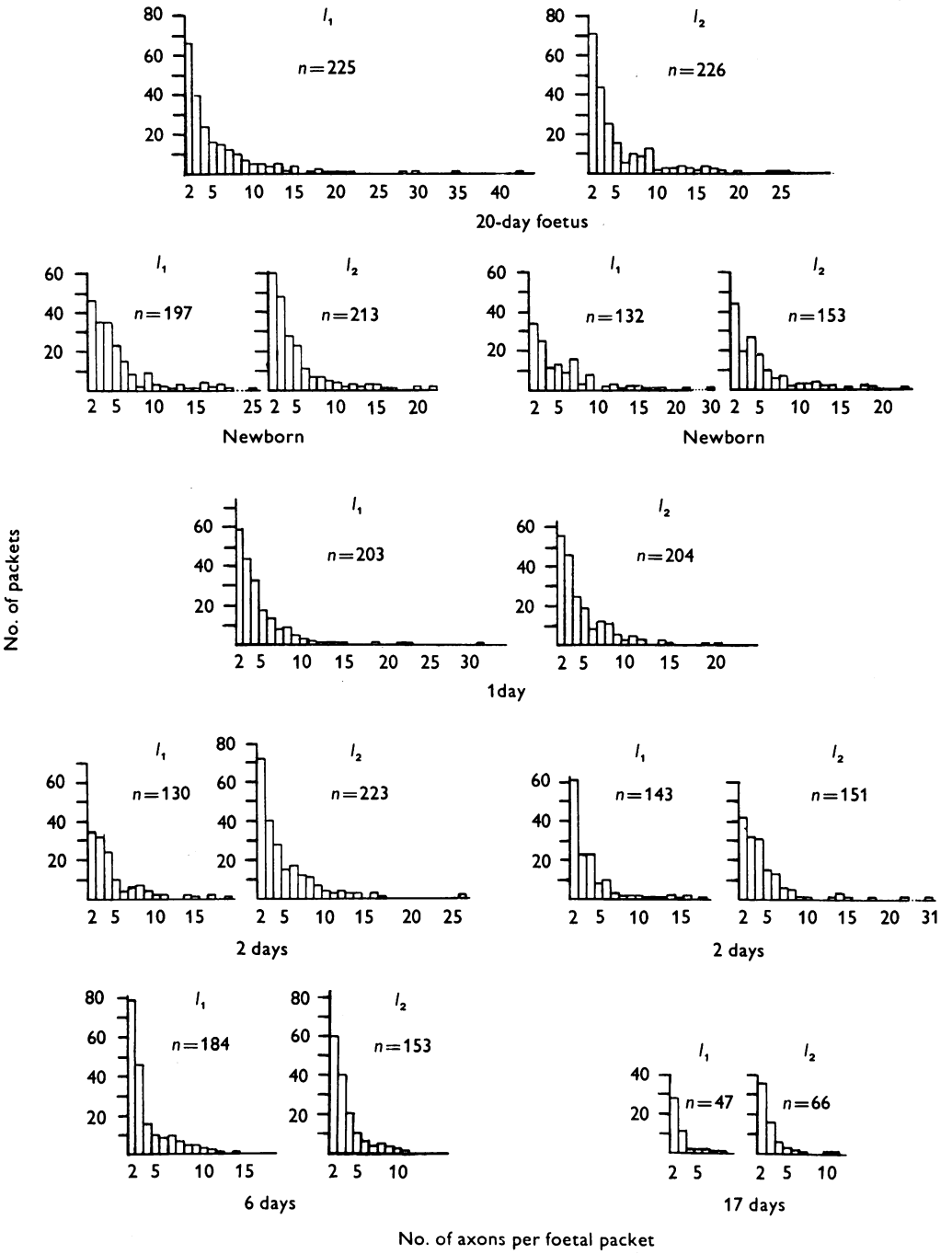

Fig. 7. Frequency histograms showing the total numbers of fetal packets containing the indicated numbers of axons observed over the whole transverse section of thoracic ventral roots at different levels at the ages shown. Ordinates: numbers of fetal packets. Abscissae: fetal packet size. n = total number of fetal packets in root; l_1 = transverse level 1 (proximal); l_2 = transverse level 2 (distal).

of the total distally than proximally in all cervical roots, but only in the thoracic roots of one newborn, the 1 day and both 2 day old animals (Figs. 4, 5).

Tables 3 and 4 show, at proximal and distal levels of section, the total numbers of axons present in each root, as well as the total numbers of fully segregated (myelinated together with promyelin) and of partially segregated (transitional together with fetal) axons. Distances between the levels varied from one root to another. Because of this, differences in axon numbers are expressed (i) as the *actual* increase, or decrease and (ii) in order to facilitate comparison between the different roots, as a *calculated* increase or decrease over a standardized distance of 100 μm. It is clear that the total number of axons generally tends to show a proximo-distal increase, though this is less marked in the older roots. Much the greater proportion of this increase is due to a rise in the number of partially segregated axons. The last-mentioned set shows an increase in all cases but two (the 6 and 17 day thoracic), whereas the numbers of fully segregated axons generally show only minor variations from one level to another.

From Figures 6 and 7 it is evident that the distribution of the numbers of fetal packets containing given numbers of axons tends to show an exponential decrease with increasing packet size. These figures clearly show the much greater degree of segregation from one another of cervical than of thoracic axons at similar ages. Also, the total number of fetal packets was greater distally than proximally in all roots examined except for the least mature of the 20 day cervical fetal roots and the six day postnatal thoracic.

It is of interest to note that only a single structure resembling an axonal growth cone was observed (in one of the 20 day cervical fetal roots). Also, features suggestive of unmyelinated axon degeneration were uncommon.

DISCUSSION

It has generally been found, using the light microscope, that a progressive increase takes place in the total number of axons per ventral root over a considerable period after birth (Hatai, 1903; Dunn, 1912; Duncan, 1934*a*; Moyer & Kaliszewski, 1958). Duncan (1934*a*, *b*) concluded that the full complement of axons may have been present in the roots at an earlier stage than is suggested by the light microscope findings, and that myelination of existing axons, rather than the outgrowth of new ones, explains the increases observed. Clearly, all of these studies were severely limited by the inability of the light microscope to demonstrate the finer axons.

Duncan (1934*a*) found that the number of myelin sheaths steadily increased in the 8th thoracic ventral root of the rat for at least 300 days after birth but that the adult number of myelin sheaths was present in the largest lumbar ventral root by the 18th day after birth. The present findings of a relatively slow rate of increase with age in the proportion of the total made up by myelinated axons in the 5th or 6th thoracic ventral root are compatible with the above observations of Duncan. The similarity may be due to the slow rate of maturation of preganglionic autonomic fibres in both cases. That the rate of maturation of cervical ventral roots in the present study (as judged by the proportion of axons which were myelinated) is compatible with that found by Duncan (1934*a*) for lumbar roots (as summarized above)

is probably related to the absence of an autonomic component from both sets of roots.

Though corresponding spinal nerves were examined in the present study, the total number of axons per root varied considerably between roots of similar age. Similar variation was noted in the light microscope studies of Duncan (1934*b*) and of Moyer & Kaliszewski (1958), even in adult roots. The animals used in the present study commonly showed pre- or post-fixation of the cervical enlargement of the spinal cord, and the variation noted was likely to have been influenced by this factor.

The present findings seem to indicate the presence in the cervical roots at and before birth of more than the adult complement of axons (Fig. 2). This is followed by a decrease in number which begins soon after birth and seems to reach a fairly constant value by the age of about one week in the cervical roots. Taking into account the total number of axons per root, as well as the percentage made up by the various classes in the earlier stages, it seems likely that the decrease may result from a loss not only of fetal and transitional, but also of promyelin axons. Aguayo, Terry & Bray (1973) found that a decrease takes place over approximately the same period of time in rat cervical sympathetic unmyelinated axons. However, the fact that few roots were examined in the present study in the two to six day postnatal period precludes a more precise estimate of the timing and rate of the fall in numbers.

Surprisingly few degenerating unmyelinated axons were seen, perhaps due to the rapidity of the process. A loss of axons from ventral roots during development has also been found for the Anuran tadpole (Prestige & Wilson, 1972). This correlates with the finding of degeneration of Anuran ventral horn motoneuron cell bodies during development by Hughes (1961). Among mammals, Romanes (1946) found that the number of ventral horn cells in mouse lumbosacral enlargement normally decreased during the first week after birth. Also, Prestige (1965, 1967) has observed degeneration of Anuran dorsal root ganglion cells during maturation.

Comparison of proximal with distal axon counts indicated a tendency for myelinated axons to be less numerous distally in the younger roots. This may reflect a central-peripheral gradient in nerve maturation. It may be due to the presence of axons which are only beginning to myelinate in those parts closest to the central nervous system.

That promyelin axons were generally fewer in number distally could result from their division or from their sharing of Schwann cells with other axons at more peripheral levels. Alternatively both of these factors might be operative. Such behaviour would also conform to the central-peripheral gradient in fibre maturation. Recent observations (Webster, 1971; Webster *et al.* 1973) indicate that a given axon may be assigned to different classes at different levels of one and the same Schwann cell; the degree of segregation of an axon from others enveloped by the same Schwann cell tended to be greater in the juxtanuclear region than at more proximal or distal levels of the cell. This situation would tend to obscure the central-peripheral gradient, particularly if the proximal section by chance showed more of one type of axon than the distal one. However, the examination of such large numbers of axons as were studied would help to overcome the above tendency by diminishing the influence of the level along a Schwann cell at which any given axon was sectioned.

It follows from the findings of Webster *et al.* (1973) that, on tracing axons along

a nerve, a repeating systematic change in numbers belonging to each class of axon would be found if, at a given age, all Schwann cells were of equal length and were in phase with one another along the nerve. This would obscure any tendency for axons to become less segregated from one another distally. However, even if all Schwann cells were of equal length, they would be most unlikely to be in phase with one another. This is because the axons comprising a single given root emerge from the spinal cord from all parts of an area on its antero-lateral surface whose width is about 20% of the total transverse diameter of the spinal cord (Fraher, 1973, unpublished observations).

The increase in total numbers of axons in the younger animals over very short distances is most marked in the thoracic roots (Tables 1 and 2). It is largely attributable to the increase in the combined total of the incompletely segregated (transitional and fetal) axons (Tables 3 and 4). By the nature of the method it was impossible to identify the class of axons at the proximal level which contributed most to the increase in the number of fetal axons at the distal level, assuming that branching was responsible.

If permanent branching were responsible for the distal increase in axon number, then eventually, as the root matured, the number of myelinated axons should undergo considerable proximo-distal increase per unit length. This increase should, however, be less than that for fetal axons, because of the growth in length of the roots. However, the myelinated axons showed a slight increase in two cases only. This could be due to a greater proportion of myelinated axons having by chance been sectioned through an unmyelinated segment, or a node, proximally than was the case distally. This, together with the fact that the rate of change of fetal axon numbers was found to vary over short distances is suggestive of the impermanence of these branches. There was no evidence of injury to the animals. In any case, the pattern of number change showed a continuous trend involving fetal as well as postnatal roots. The former were unlikely to have been traumatized *in utero*.

The peripheral nerves of the 14 week menstrual age human fetus studied by Gamble & Breathnach (1965) were somewhat less mature than the 20 day fetal rat ventral roots examined in the present study; they corresponded more closely to 18 day roots. These authors found a very marked decrease in the total numbers of axons contained in small cutaneous nerves when fetal material was compared with adult. They proposed that Schwann cell division with consequent diminution of the axon/Schwann cell ratio as well as disappearance of axons may account for the fall in numbers of the latter.

Another possible explanation for the large numbers of axons counted in the younger roots in the present study could lie in the looping of dorsal root axons into the ventral root as they grow distally, before proceeding on their way into the spinal nerve, in a manner similar to that found in relation to both the optic nerve and tract in the chiasmal region of man (see Crosby, Humphrey & Lauer, 1962). However, no evidence was found of any reversal of direction on the part of myelinated axons followed by means of serial sections through distances of 250 μm along roots (Fraher, 1973).

Thus it seems likely that numbers of unmyelinated axons are lost during maturation of the ventral roots. Also, though the number of roots studied was small, there is

some evidence that a proportion of the incompletely segregated axons branch freely during earlier stages of development, and that many of these branches are transient, arising at least in part in the root itself.

SUMMARY

The present study was carried out on a limited number of rat ventral nerve roots. Evidence is presented suggesting the occurrence of a decrease, shortly after birth, in the total number of axons in lower cervical roots.

The observations are compatible with the hypothesis that the decrease noted was at the expense of the unmyelinated rather than the myelinated axons.

The thoracic roots, however, did not show such a fall in totals during the period when the numbers were falling in cervical roots.

Evidence is also presented suggesting that unmyelinated ventral root axons may branch relatively freely during early development, but the branches are transient.

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