Z. Zellforsch. 124, 103–130 (1972)
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A Study of Degeneration and Regeneration in the Divided Rat Sciatic Nerve Based on Electron Microscopy

II. The Development of the "Regenerating Unit"

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Received April 15, 1971

Summary: In the first six days after division myelinated axons in the proximal stump of rat sciatic nerves produce collateral and terminal sprouts. These are present as circumscribed "groups" which are positively distinguishable from clusters of non-myelinated axons. Two types of "groups" are identifiable, and their distribution in some of the nerve segments is analysed. Their evolution was followed in sequential nerve segments, the initial 'tight' structure becoming looser between 7 and 10 days, and myelinated axons appeared in them during this time. At this stage a complete basal lamina was present surrounding the entire "group". Some of the cells in the "groups" did not have the characteristics of Schwann cells. Between 7 and 10 days after division alveolate vesicles and densely staining material in the cisternae of the rough surfaced endoplasmic reticulum were prominent in Schwann cells in the distal part of the proximal stump. It is thought that both types of "group" are developed from single myelinated axons and the name "regenerating unit" is proposed for both types. Their relationship to "clusters", seen in the distal stump of regenerating peripheral nerves, and "onion bulbs", present in some peripheral neuropathies, is discussed.

Key words: Peripheral nerves — Myelinated axons — Regeneration — Sciatic nerve, rat.

This report is a continuation of a previous paper which describes the traumatic degeneration of myelin (Morris *et al.*, 1972a) in the proximal stump of a divided rat sciatic nerve.

Material and Methods

A full description of the materials and methods used in this study has been given in Morris *et al.* (1972a).

Observations

It is an obvious truism that to detect the changes in the proximal stump following nerve section it is necessary to be conversant with the structure of normal nerve. In the normal mature peripheral nerve Schwann cells containing myelinated axons were not associated with any other axons, myelinated or non-myelinated, and Schwann cells associated with non-myelinated axons had only one or, rarely,

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*** The authors have great pleasure in acknowledging the expert technical assistance of Mrs. Frances Burton. G. W. would also like to thank the British Medical Research Council, the Wellcome Trust and LEPRA (British leprosy relief association) for financial assistance without which this work could not have been completed.

Table 1. *Distribution of non-myelinated axons among Schwann cells in normal nerve*

No. of axons /Schwann cell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	20+
No. of clusters	497	140	61	43	28	23	33	19	30	18	13	9	15	15	7	7	10	14	5	3	3	9
% of total		28	12	9	6	5	7	4	6	4	3	2	3	3	1	1	2	3	1	1	1	2
No. of axons	2988	140	122	129	112	115	198	133	240	162	130	99	180	195	104	105	160	238	90	57	60	219
% of total		5	4	4	4	4	7	4	8	5	4	3	6	7	3	4	5	8	3	2	2	7

two axons in any one invagination of the Schwann cell surface membrane (see Figs. 2, 3 in Morris *et al.*, 1972a). In sciatic nerves of normal rats aged two months (the same age as the experimental animals at the time of nerve section) and sampled at the same anatomical level as the nerve section in the experimental animals, the ratio of myelinated axons to non-myelinated axon clusters was 4:1 (1992 myelinated axons: 497 non-myelinated axon clusters). In the 497 clusters of non-myelinated axons, the mean number of axons associated with each Schwann cell was 6.01 (2987 axons in 497 clusters). The distribution of the non-myelinated axons in these Schwann cells in normal nerve is illustrated in Table 1. The maximum number of axons associated with a single Schwann cell was 36, and Schwann cells associated with more than 20 non-myelinated axons accounted for only 2% (9 out of 497) of the total number of non-myelinated clusters. The maximum diameter attained by a non-myelinated axon was 1.5 μm. All the Schwann cells associated with both myelinated and non-myelinated axons possessed a single layer of basal lamina which was closely applied to the surface of the cell and/or its axons. Single myelinated axons and clusters of non-myelinated axons were present in the normal nerve as anatomically separate elements and there were no specific topographical or topological relationships between the different axon/Schwann cell elements in the endoneurium at the anatomical level at which the nerve was examined.

In the proximal stump of a divided nerve the Schwann cells and their associated axons are arranged in a pattern different from that found in normal peripheral nerves of the same age, in that a variable proportion of the Schwann cells and their axons were associated in "groups". In the earliest nerve segments in which these "groups" were present, they could be divided into two classes:

Type I "Groups". Single myelinated axons which had one or more non-myelinated axons associated with their Schwann cell. The myelinated axons possessed approximately the same number of myelin lamellae as were present encircling axons of a similar diameter in normal nerves from animals of the same age. The non-myelinated axons were applied to the surface of the Schwann cells immediately beneath the basal lamina (see Fig. 10, Morris *et al.*, 1972c).

Type II "Groups". Comprised non-myelinated axons only (see Morris *et al.*, 1972a; Fig. 13). They were distinguished from clusters of "simple" non-myelina-

ted axons on the basis of four structural criteria not all of which were visible in every type II group identified. The criteria were:

1. Shape—in transverse section type II groups were approximately circular.

2. Relationship of the axons to the Schwann cell—in those type II groups containing more than 10 axons they were not enveloped by the Schwann cell cytoplasm in the conventional way but were present in clusters none of which was individually enveloped by the Schwann cell cytoplasm. Consequently, some of the axons were not in contact with the Schwann cell at all, at least in some planes of section. This is an arrangement similar to that seen in developing peripheral nerves (Peters and Muir, 1959; Robertson, 1962; Gamble and Breathnach, 1965; Gamble, 1966; Allt, 1969).

3. Axon size—axons with a diameter of up to 6.0 μm were present.

4. Basal lamina—a proportion of the type II groups possessed either a basal lamina which had a serpentine configuration (Morris *et al.*, 1972a), or, as well as having a basal lamina closely applied to the surface of the Schwann cell and axons, had another envelope of basal lamina which loosely surrounded the entire group.

Within these constraints there was an entire spectrum of configurations for type II "groups". The extent of this spectrum is best defined by a description of the polar forms but it must be understood that this description applies only to type II "groups" seen in the first seven days after nerve section.

1. Type II "groups" which were composed of a Schwann cell associated with up to 80 small axons (less than 1.5 μm in diameter) and which satisfied criteria 1 and 2.

2. Type II "groups" in which the only axonal element was a single large (up to 6.0 μm in diameter) axon. This form of type II "group" had either a serpentine basal lamina, or a close fitting basal lamina round the Schwann cell with a second loose envelope of basal lamina associated with it. Such groups generally satisfied criteria 3 and 4 (Fig. 1).

The general nature of the spectrum between these two polar forms was of a progressive reduction in the diameter of the largest axon in the "group" with the rise in the number of axons per "group". Hence in "groups" containing one or two small axons and a large axon, the large axon retained its diameter of up to 6.0 μm (Fig. 2). In groups containing a larger number of axons, the maximum diameter of the largest axon was smaller so that there was less disparity in size between the largest axon and the smaller axons. Towards the opposite pole of the spectrum where each "group" contained a large number of axons no single axon was conspicuously larger than any of the others.

Segments which contained many axon/Schwann cell complexes identified as type II "groups" also contained other clusters of up to 100 non-myelinated axons but which did not conform to the criteria laid down for the identification of type II "groups" (Fig. 3). These clusters were always irregular in shape and were not usually associated with more than 3 Schwann cells. The relationship of the axons to the Schwann cells was such that single invaginations of the Schwann cell surface membrane enclosed up to four non-myelinated axons. Clusters of non-myelinated axons of this kind were observed in all the nerve segments examined between the sixth day and the sixth week after nerve section.

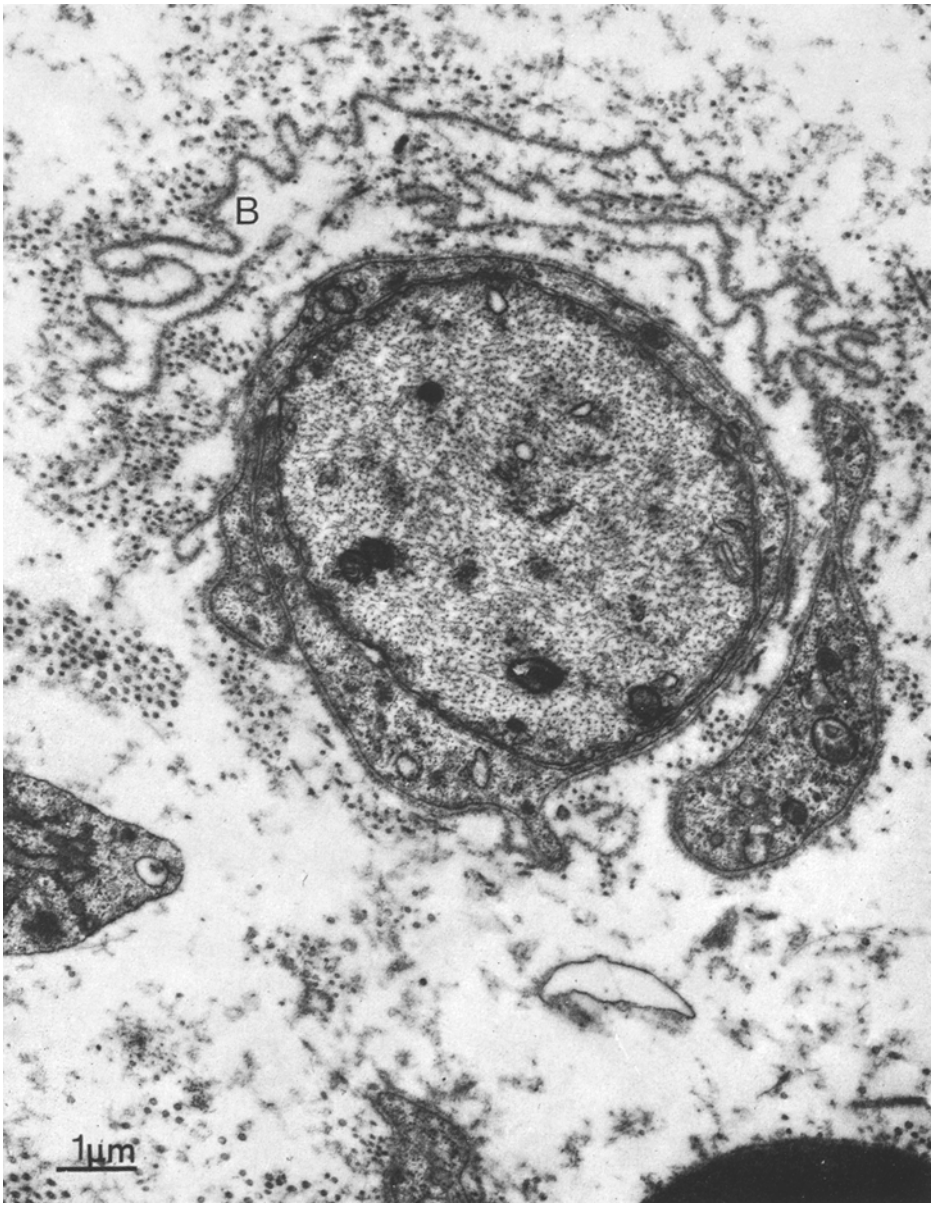

Fig. 1. Segment "A", 10 days. A type II "regenerating unit" (group) comprising a single large axon associated with an extra discontinuous envelope of basal lamina (*B*). This could also be an example of segmental demyelination or a section through an abnormal node of Ranvier rather than the outgrowth from a degenerated myelinated axon

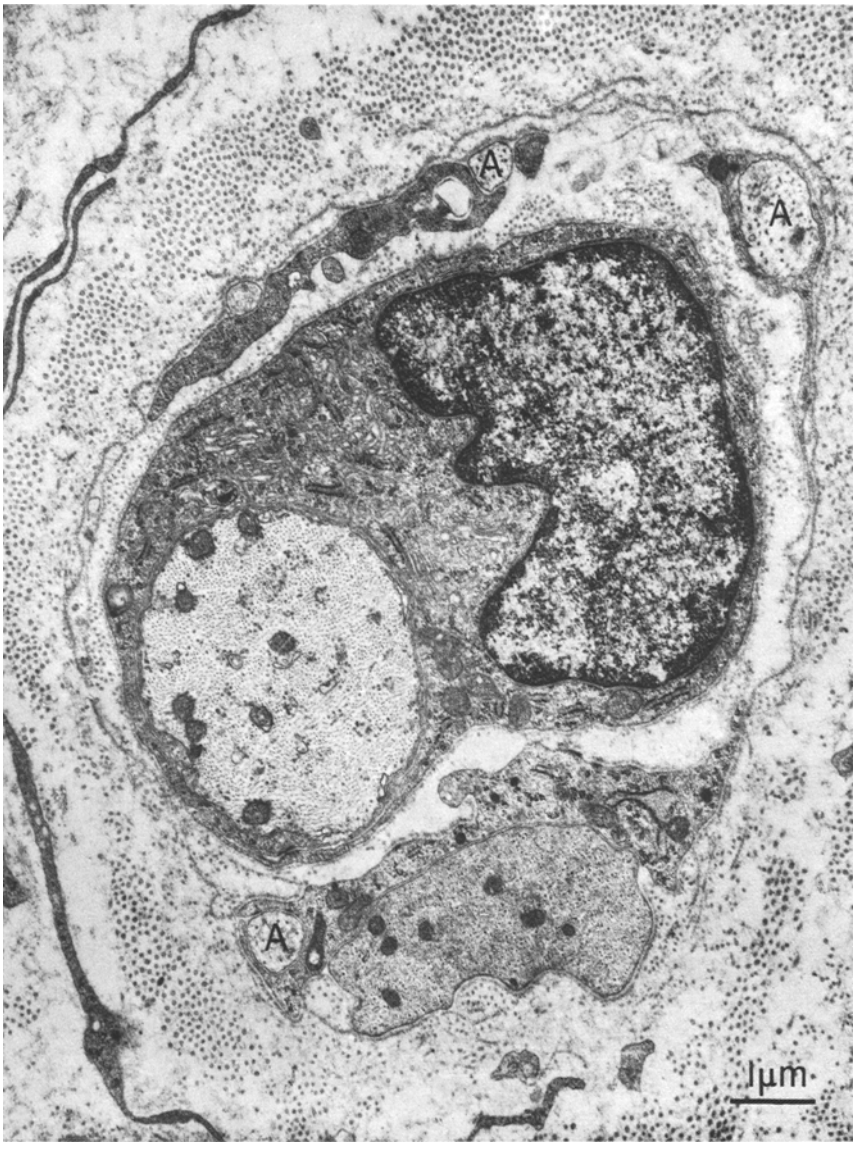

Fig. 2. Segment "A", 8 days. A type II "regenerating unit" (group) in which a single large non-myelinated axon is associated with three small non-myelinated axons (*A*), and an extra envelope of basal lamina. The length of the mesaxon of the large axon suggests that it might be in the initial stages of remyelination. The cytoplasm of the Schwann cell associated with this axon contains only small amounts of rough surfaced endoplasmic reticulum with pale staining material in the cisternae (cf. Fig. 9). Collagen fibrils are present between the two basal lamina envelopes. They are smaller in diameter than most of the fibrils outside the basal lamina

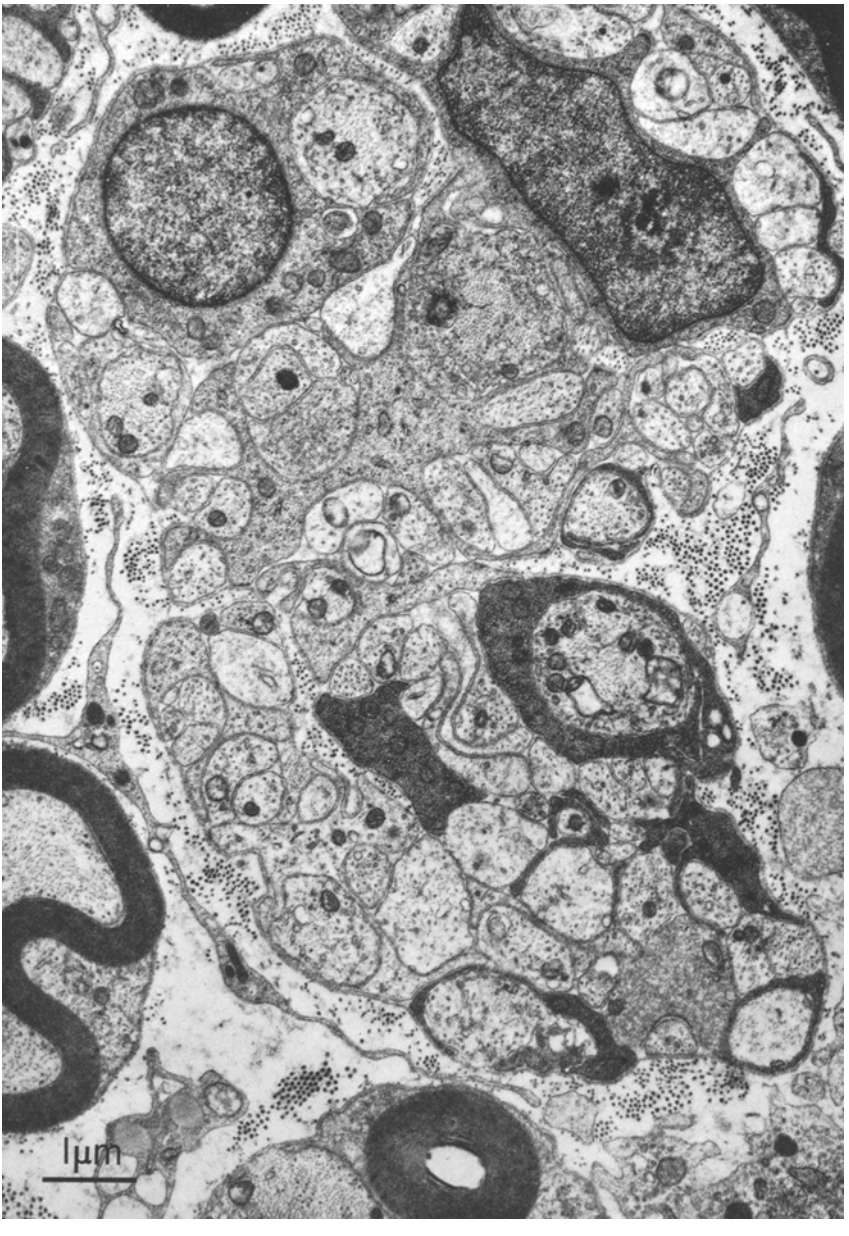

Fig. 3. Segment "A", 8 days. A cluster of non-myelinated axons and their Schwann cells. The axons in this illustration are separated into small bundles each in a separate invagination of the Schwann cell surface membrane and the whole complex is irregular in shape. This is in contrast to the type II "regenerating unit" in Fig. 13, in Morris *et al.*, 1972a

When observed in temporally sequential series of segments all the type I and type II "groups" containing two or more axons appeared to undergo the same general kind of morphological change. Differences among individual groups were of degree and timing rather than deviations from the general pattern of change. To simplify the description, the changes found in type II "groups" which contained more than 10 axons and in which all the axons were of approximately the same diameter will be enumerated. Except where otherwise indicated, the description may be taken to refer to all "groups" which contained two or more axons.

Morphological Changes in Type II "Groups"

In the earliest segments in which they were observed all the axons in each "group" when seen in transverse section, were related to a single Schwann cell and the whole was surrounded by a continuous basal lamina. In segments taken from nerves at a later time after their division the number of Schwann cells in a transverse section of a single type II "group" had risen to as many as six. Moreover, in general, "groups" contained fewer axons. Consequently, the average number of axons per Schwann cell was reduced. In accordance with the rise in the number of Schwann cells per group mitotic figures were observed relatively frequently, for example, up to four mitotic figures have been counted in a single complete section of a sciatic nerve. This is in sharp contrast to the almost complete absence of mitotic figures in Schwann cells in transverse sections of normal nerves in animals of the same age. Segments taken later in time contained "groups" in which Schwann cells were associated with single axons (Fig. 4). Small numbers of myelin lamellae were commonly seen around these axons.

In addition to this, the "groups" found in segments taken later in time had a less compact structure. In this respect, the earliest observable changes were the appearance of gaps between individual Schwann cells. Subsequently, groups were found which were sub-divided into anatomically separated axon/Schwann cell elements (Fig. 4). When the interval between the opposing surfaces of separated Schwann cells was small they did not invariably possess a basal lamina (Fig. 5, inset), but a basal lamina was invariably present on the surfaces of widely separated Schwann cells (Fig. 5). In some instances, "groups" contained cells which did not possess a basal lamina even when individual cellular elements of the "group" were widely separated (Fig. 4) (vide infra).

In segments in which there was separation of the axon/Schwann cell elements within the "groups", collagen fibrils were seen in the intercellular spaces (Fig. 4). When the "groups" were partly separated into their constituent axon/Schwann cell elements a basal lamina was invariably present surrounding the entire "group". In some instances it was intact (Fig. 4), but in others there were breaks in its continuity (Fig. 5). In later segments where the axon/Schwann cell elements were completely separated from each other (when viewed in transverse section), only isolated lengths of a basal lamina were seen at intervals around the "group". In segments in which there was a large number of "groups" the whole nerve trunk appeared to have its axons collected into a series of circumscribed clusters (Fig. 6).

The configurations of the type II "groups" described above were not invariably found in particular nerve segments at fixed intervals after nerve section. However,

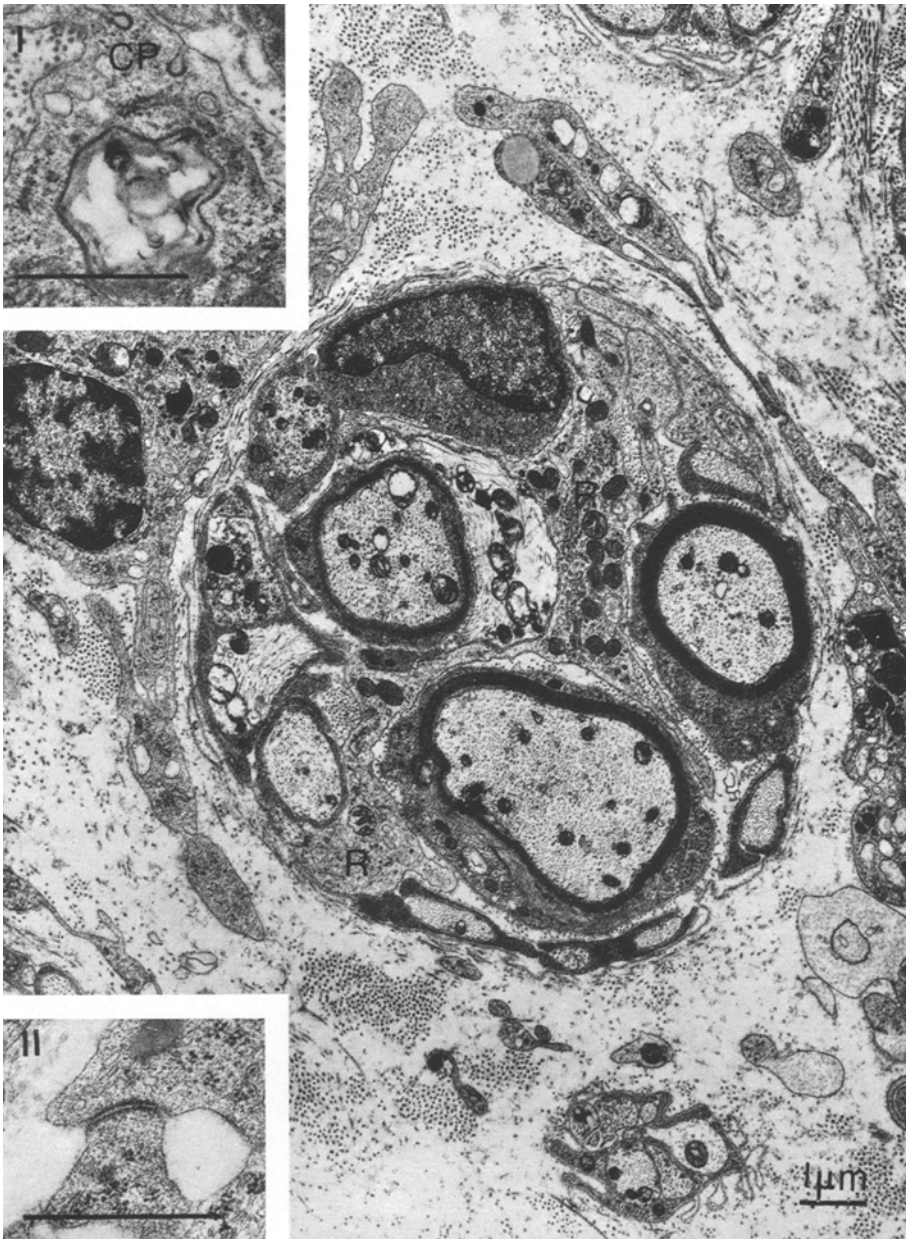

Fig. 4. Segment "A", 8 days. A type II "regenerating unit" (group) at an early stage in its development when there are only clefts between the Schwann cells and the entire "regenerating unit" is surrounded by an almost complete basal lamina. One of the cells (*R*) is only tangentially associated with the axons and does not have a basal lamina, despite being widely separated from the other cells. Inset I: A lamellated osmiophilic structure and two coated pits (*CP*) in an "atypical" cell. Scale = 1 μm. Inset II: A "tight junction" between two "atypical" cells, or different parts of the same cell. Scale = 1 μm

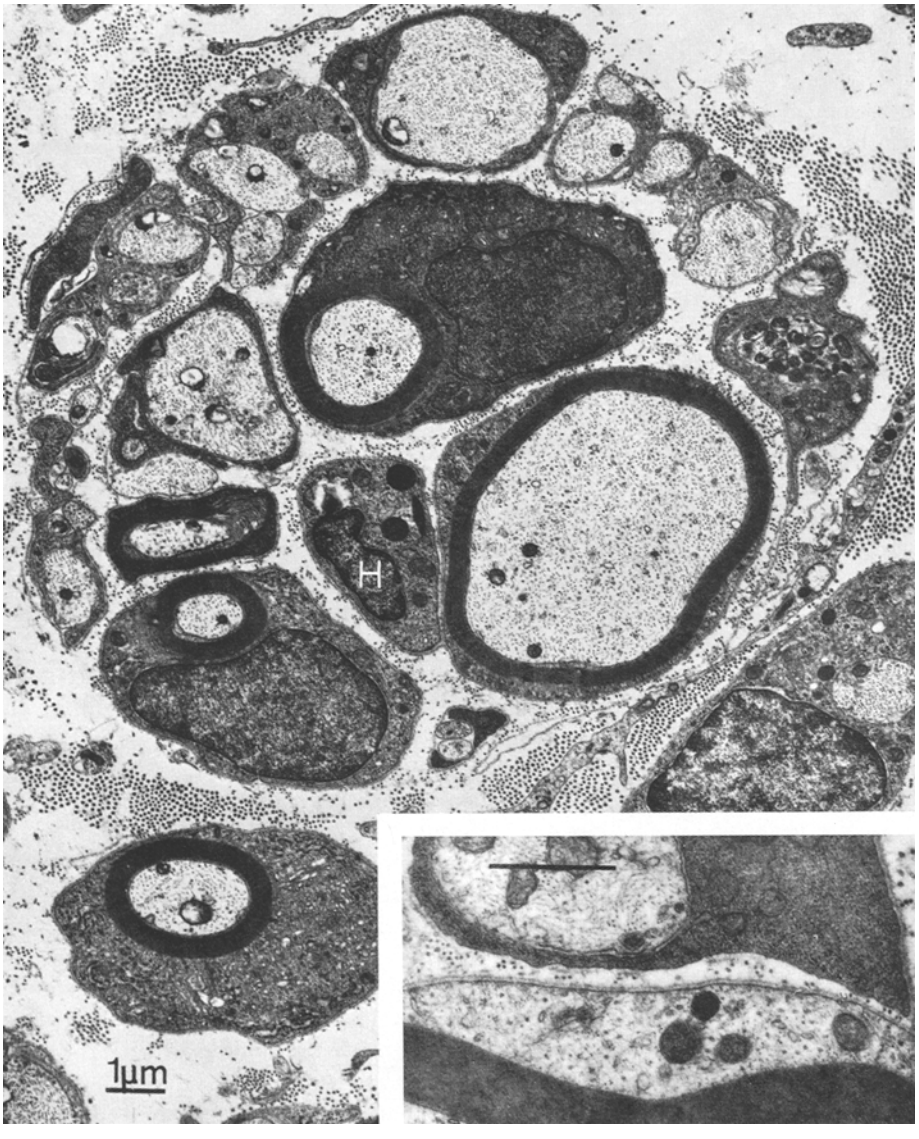

Fig. 5. Segment "A", 14 days. A type II "regenerating unit" (group) in which 5 of the 30 axons are myelinated. A fragmentary basal lamina can still be seen surrounding the entire "regenerating unit". One of the cellular profiles (*H*) possesses a basal lamina but is not associated with any axons and also contains two densely staining inclusions. Inset: Part of a type I "regenerating unit" in which separation of the "satellite" Schwann cell from the Schwann cell of the myelinated axon has not been accompanied by the formation of a complete basal lamina. Collagen fibrils can be seen in the gap between the two Schwann cells. Scale = 1 µm

myelinated axons were seen in type II "groups" as early as the seventh day after nerve section, and by the tenth day nearly all "groups" possessed at least one, and frequently several myelinated axons (Fig. 5). Separation of the axon/Schwann cell elements of the "groups" followed a roughly similar time course and by the tenth day the "tight" structure characteristic of "groups" in the first six days had been superseded by the more dispersed arrangement. Illustrative of the amount of variation in the appearance of "groups" with time was an observation made in segment 18d "A". In this segment there was a "tight" structured group in which one of the axons possessed three myelin lamellae, (a configuration commonly observed on the seventh day after nerve section) whilst another "group" in the same grid square had a dispersed configuration and contained five myelinated axons each of which possessed between 20 and 24 myelin lamellae.

In all segments taken between the tenth and twenty-ninth days after nerve section in which "groups" were distinguishable the most developed form was that in which the anatomically separate axon/Schwann cell elements of the "group" were clearly distinguishable as a circumscribed cluster, usually with a fragmentary basal lamina surrounding the whole. On the twenty-ninth and subsequent days individual "groups" could not be distinguished because of the extensive compartmentation (see Morris *et al.*, 1972d) in all segments of the proximal stump.

The numbers and size of the axons in the type II "groups" varied very widely but with time the average number of axons per group diminished. From an initial complement of up to 80 axons, the number had decreased to a maximum of about 40 by the time that myelinated axons were first observed and at the latest time at which "groups" could be distinguished to between 1 and 20 axons. Between the third and eighth days after nerve section, in "groups" which contained the largest numbers of small non-myelinated axons approximately 50% were less than 0.5 μm in diameter (188 out of 328). In later segments in which "groups" were present as separate axon/Schwann cell elements, only a small proportion of the axons were less than 0.5 μm in diameter.

On the eighth and subsequent days after nerve section myelinated axons were present in nearly all "groups". However, not all the axons in "groups" which contained myelinated axons were myelinated, and in the earliest segments in which myelinated axons were present in the type II "groups" only one, or at most two, of the axons were myelinated. In segments removed at a later date type II "groups" were found containing as many as 12 myelinated axons. Generally, the proportion of non-myelinated to myelinated axons in "groups" decreased with time but non-myelinated axons were present in many "groups" as late as the twenty-ninth day after nerve section, the latest time at which separate "groups" could be distinguished.

Type II "groups" which were composed of single large non-myelinated axons appeared to undergo less complex changes than those described in the foregoing paragraphs. Whereas in the first six days after nerve section large single non-myelinated axons were present in the nerve segments, after this time some single axons of this size class were present which possessed a few myelin rings (Fig. 7). These axons possessed fewer myelin rings than were present around axons of a similar diameter in sections from normal nerves in animals of the same age, or axons of a similar calibre which were part of the initial complement of a type I

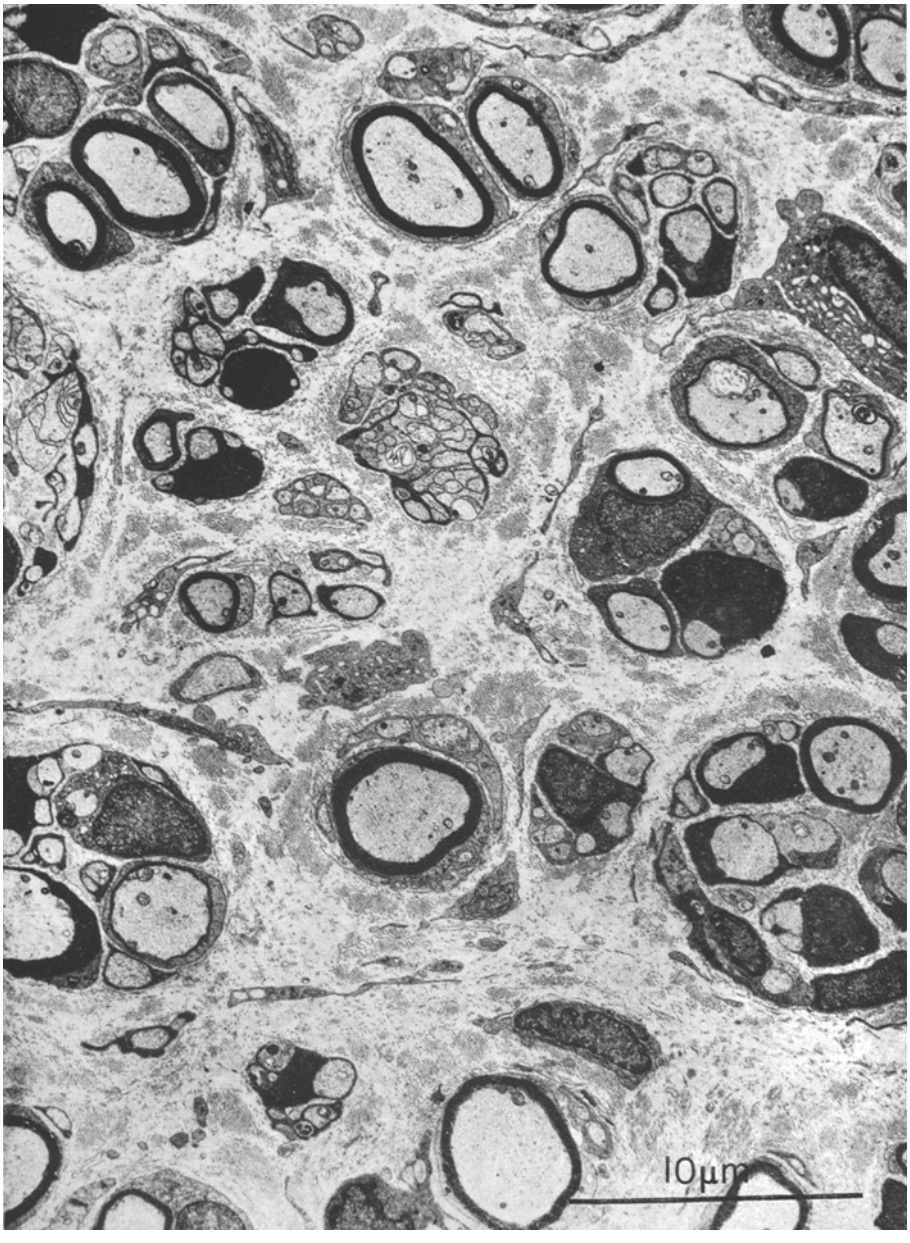

Fig. 6. Segment "B", 18 days. A low magnification electron micrograph of a nerve in which most of the axons are present as components of "regenerating units" (groups)

"group", or around myelinated axons in the same segments which were not components of either type I or type II "groups". In later segments, up to the twenty-ninth day after nerve section, the number of myelin rings around these large single myelinated axons became progressively larger, but at this time they did not

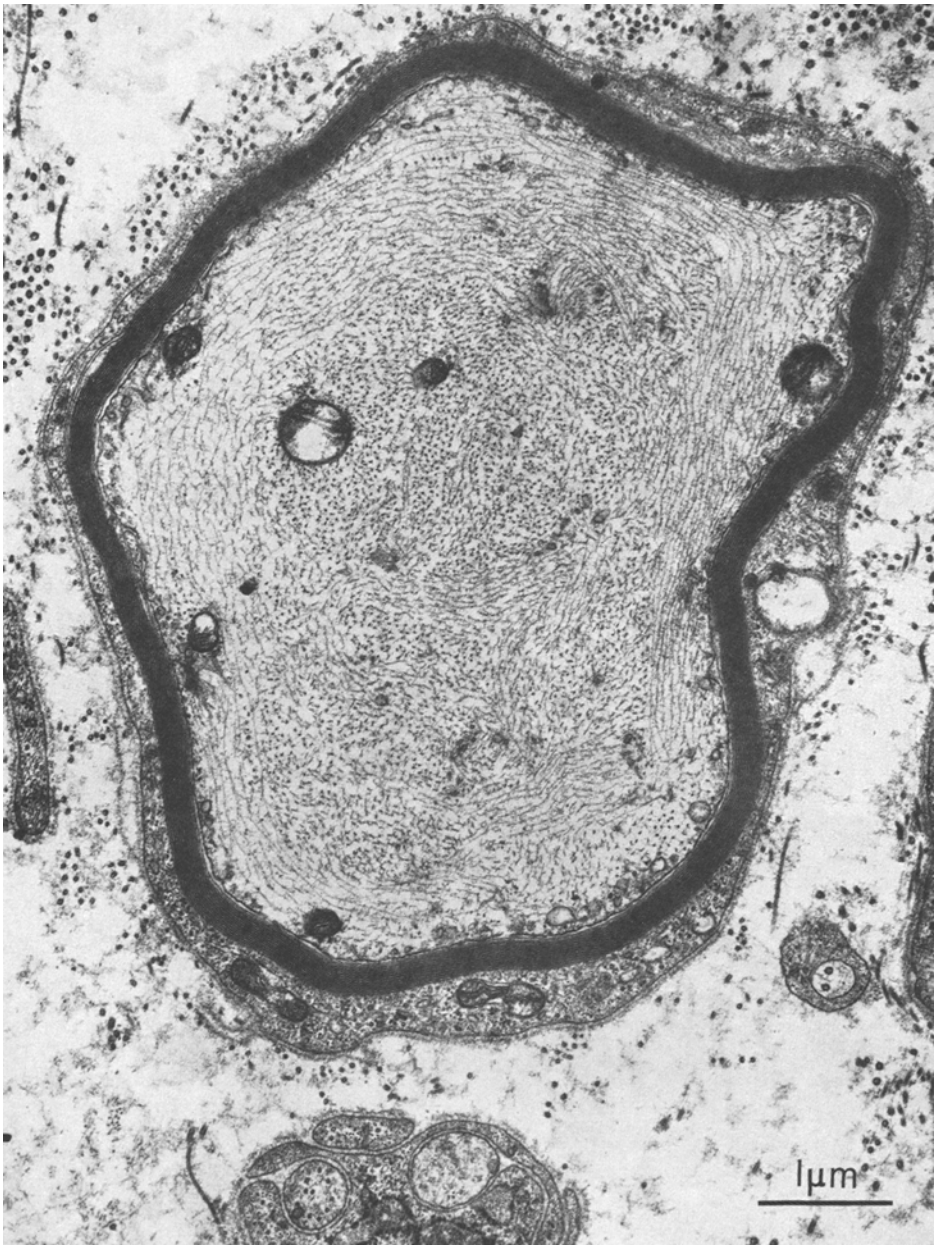

Fig. 7. Segment "A", 10 days. A single axon with a thin myelin sheath. The neurofilaments in the periphery of this axon deviate noticably from the longitudinal

possess a similar number of myelin rings as myelinated axons of the same calibre in a normal nerve.

The occurrence and distribution of the type I and type II "groups" will now be analysed on a more quantitative basis.

Table 2. *Distribution and occurrence of 'regenerating units'*

+ = five or less 'regenerating units' (groups) per grid square. ++ = more than 5 'regenerating units' (groups) per grid square. − = no 'regenerating units' present. O = no data available. C.P. = 'regenerating units' (groups) not distinguishable because of compartmentation.

Segment	24h[a]	36h	48h	60h	72h	4d	5d	6d	7d	8d	10d	11d	12d
A	O	−	−	−	−	−	−	−	++	+	++	+	+
B	−	−	+	−	−	+	+	++	++	++	++	++	++
C	−	+	++	++	++	++	++	++	++	++	++	O	++

Segment	13d	14d	16d	18d	20d	23d	26d	28d	29d	6wk	8wk	10wk	12wk
A	+	++	++	++	++	+	C.P.	+	++	C.P.	C.P.	−	C.P.
B	++	C.P.	++	++	++	+	C.P.	++	++	C.P.	C.P.	C.P.	C.P.
C	C.P.	C.P.	C.P.	C.P.	++	++	C.P.	C.P.	C.P.	C.P.	C.P.	C.P.	C.P.

[a] h = hours, d = days, wk = weeks.

Occurrence and Distribution of the Type I and Type II "Groups"

The distribution and appearance in time of the type I and type II "groups" is shown in Table 2. They were first seen, at a frequency of less than 5 "groups"/grid square, 36 hours after nerve section in segment "C" but 48 hours after nerve section, they were frequent in this segment. In segment "B" in the first five days after nerve section they were present only in the segments taken, 2, 4 and 5 days after nerve section and at a frequency of less than 5 "groups"/grid square, but on the sixth day they were frequent in this segment. In the segments "A" taken 8, 11, 12, 13, 23 and 28 days after nerve section the frequency of occurrence of "groups" did not rise above 5 "groups"/grid square and in the segment "A" taken 10 weeks after nerve section no "groups", either type I or type II, were identifiable. On the sixth and subsequent days after nerve section, all the segments "B", with the exception of the segment taken 23 days after nerve section contained more than 5 "groups"/grid square. All the segments "C" which could be assessed subsequent to 48 hours after nerve section contained more than 5 "groups"/grid square. The designation C.P. in the table indicates that the number of "groups" in the segment was not countable by reason of "Compartmentation" (see Morris *et al.*, 1972d) which was present in the segments so designated.

The variation in the number, proportion and type of "groups" in segment "A" is illustrated in Table 3. The segments chosen for inclusion in this table display the full range of variation experienced in this segment. Of those segments not included in this table, segments 12d "A" and 28d "A" were both similar in appearance to segment 8d "A", and segment 23d "A" was not considered because it showed some signs of mechanical disruption. The counts were made by considering each undegenerated myelinated axon and each type I and type II "group" as single "neural elements". In this way the number of axons in each group did not

Table 3. *Composition of selected segments "A"—arranged in order of increasing degeneration*

N.E. = 'Neural element'—i.e. a single undegenerated myelinated axon or a single complete 'regenerating unit' (groups). R.U. = 'Regenerating unit' (types I and II).

Segment "A"	Total no. N.E.'s	Total no. R.U.'s	No. of type I R.U.'s	No. of type II R.U.'s	Total no. R.U.'s as % of total no. N.E.'s	Type II R.U.'s as % of total no. R.U.'s	Type II R.U.'s as % of total no. N.E.'s
13d[a]	807	9	0	9	1.1%	100%	1.1%
11d	1398	39	3	36	2.8%	92%	2.5%
8d	798	36	9	27	4.5%	75%	3.4%
20d	606	76	14	62	12%	82%	10%
16d	434	96	23	73	22%	76%	17%
18d	547	128	22	106	23%	83%	20%
7d	422	123	33	90	29%	73%	21%
29d	493	157	40	117	32%	74%	24%
14d	492	208	68	140	42%	66%	28%
10d	399	226	75	151	56%	66%	37%

[a] d = days.

Table 4. *'Neural element' and 'regenerating unit' density in selected segments "A"*

N.E. = 'Neural element'—i.e. a single undegenerated myelinated axon or a single complete 'regenerating unit' (groups). R.U. = 'Regenerating unit' (types I and II).

Segment "A"	Total no. N.E.'s	Mean no. N.E.'s/ gd. sq.	Total no. R.U.'s	Mean no. R.U.'s/ gd. sq.
13d[a]	807	134	9	1.5
11d	1398	100	39	2.8
8d	798	100	36	4.5
20d	606	101	76	13
16d	434	72	96	16
18d	547	91	128	21
7d	422	70	123	21
29d	493	82	157	26
14d	492	82	208	35
10d	399	80	226	45
Normal	1021	102	—	—
Normal	915	114	—	—

[a] d = days.

influence the number of "neural elements". The proportion of "groups" to "neural elements" in segment "A" varied very widely in nerve segments taken from the same location in different nerves which had been subject to a similar surgical procedure. Also noticeable is a tendency for the proportion of type I to type II "groups" to rise with increases in the proportion of both types of "group" to the total number of "neural elements".

As illustrated in Table 4 there is also a tendency for the number of "neural elements" per grid square to fall with increases in the proportion of "groups" in

Table 5. *Distribution of 'Regenerating units' in two selected segments "A"*

N.E. = 'Neural element'—i.e. a single undegenerated myelinated axon or complete 'regenerating unit' (groups). R.U. = 'Regenerating unit' (types I and II).

Segment "A"	N.E.'s	R.U.'s	type I R.U.'s	type II R.U.'s	R.U.'s as % of N.E.'s	Type II R.U.'s as % of R.U.'s	Type II R.U.'s as % of N.E.'s
8d[a]	96	5	2	3	5%	60%	3%
	95	3	1	2	3%	66%	2%
	89	3	0	3	3%	100%	3%
	119	3	1	2	3%	66%	3%
	102	8	2	6	8%	75%	6%
	98	2	0	2	2%	100%	2%
	104	8	1	7	8%	87%	7%
	95	4	2	2	4%	50%	2%
	798	36	9	27	4.5%	75%	3.4%
14d	75	37	13	24	49%	65%	32%
	74	38	12	26	51%	68%	35%
	74	30	8	22	40%	74%	30%
	90	39	10	29	43%	74%	32%
	78	36	16	20	46%	55%	25%
	101	28	9	19	28%	68%	19%
	492	208	68	140	42%	66%	28%
Normal	82	—					
	126	—					
	77	—					
	108	—					
	91	—					
	113	—					
	108	—					
	116	—					
	115	—					
	85	—					
Total = 1021							

[a] d = days.

the segments. It is evident that the density of neural elements did not change until the proportion of "groups" rose above 10%.

Table 5 shows the variation in the proportions of "neural elements", and type I and type II "groups" in two segments "A" which were selected as examples of segments with a low and high density of "groups". Each horizontal line of figures was obtained from one grid square which was completely occupied by endoneurium, and grid squares from near the perineurium and near the centre of the nerve were included. As a comparison, the number of neural elements in ten grid squares from a normal nerve of the same age is included. There is comparatively little variation in the number of neural elements in each grid square of

either the experimental nerves or the normal nerve. Also illustrated is the uniformity in different grid squares from the same segment of the proportion of "groups" to "neural elements" and of the proportion of type I to type II "groups" across the nerve. This shows that there are no major non-uniformities in the distribution of either "groups" with respect to "neural elements" or of type I to type II "groups", at least when analysed in numbers of this size.

Schwann Cells Associated with Type I and Type II "Groups"

In those "groups" which were seen in the first six days after nerve section, which were all in segments "B" and "C", the single Schwann cell associated with the axons often contained membranous debris, pale staining globular inclusions and had finger-like processes at the periphery of the cell (see Fig. 12, Morris *et al.*, 1972a). After the sixth day following nerve section, very few Schwann cells in the "groups" were observed to contain any of these elements. However, even during the first six days not all the Schwann cells associated with "groups" possessed these inclusions.

The Schwann cells in the proximal stump, particularly in the first three weeks after nerve section, were less regular in shape than in normal nerve. Examples were seen in which there was a definite projection, which often contained the nucleus, in the outline of the Schwann cell (Fig. 8).

Starting on the seventh day after nerve section alveolate vesicles (Palay, 1963) and/or a dense material in the cisternae of the rough surfaced endoplasmic reticulum were seen in some of the Schwann cells of the proximal stump (cf. Figs. 2 and 9 and inset). Although alveolate vesicles were very occasionally seen before this time, the dense material was never seen either before the seventh day in the proximal stump or in normal nerves. The spatial distribution of these two features coincided with the distribution of the "groups". When there were many "groups" in segment "A", alveolate vesicles and/or the dense material were present in some of the Schwann cells in this segment, but if "groups" were infrequent, the appearances in question were confined to segments "B" and "C" where "groups" were common. It should be emphasised however that either or both features were not present in all the Schwann cells of an affected segment. These ultrastructural features were most frequently seen between seven and ten days after nerve section. After this time they were less conspicuous and by the eighteenth day the Schwann cells in segments "B" and "C" closely resembled those in segment "A".

In those segments "A" in which there were few "groups" there were no discernable cytoplasmic changes peculiar to this segment. However, in some Schwann cells of all segments of nerves taken after the seventh day following nerve section the cytoplasm contained uniformly dense inclusions (Fig. 5, see also Morris *et al.*, 1972d, Fig. 4). Other inclusions seen in some Schwann cells at this time were lamellar structures (Fig. 8 inset) which were irregular in shape with pale banding but which were not π granules (Thomas and Slatford, 1964; Tomonaga and Sluga, 1970). Both the dense inclusions and the lamellar structures were present in the cytoplasm of Schwann cells in normal nerves.

Four cilia (Fig. 9, inset) on Schwann cells were observed during this investigation. Like those reported by Grillo and Palay (1963) in the autonomic nervous

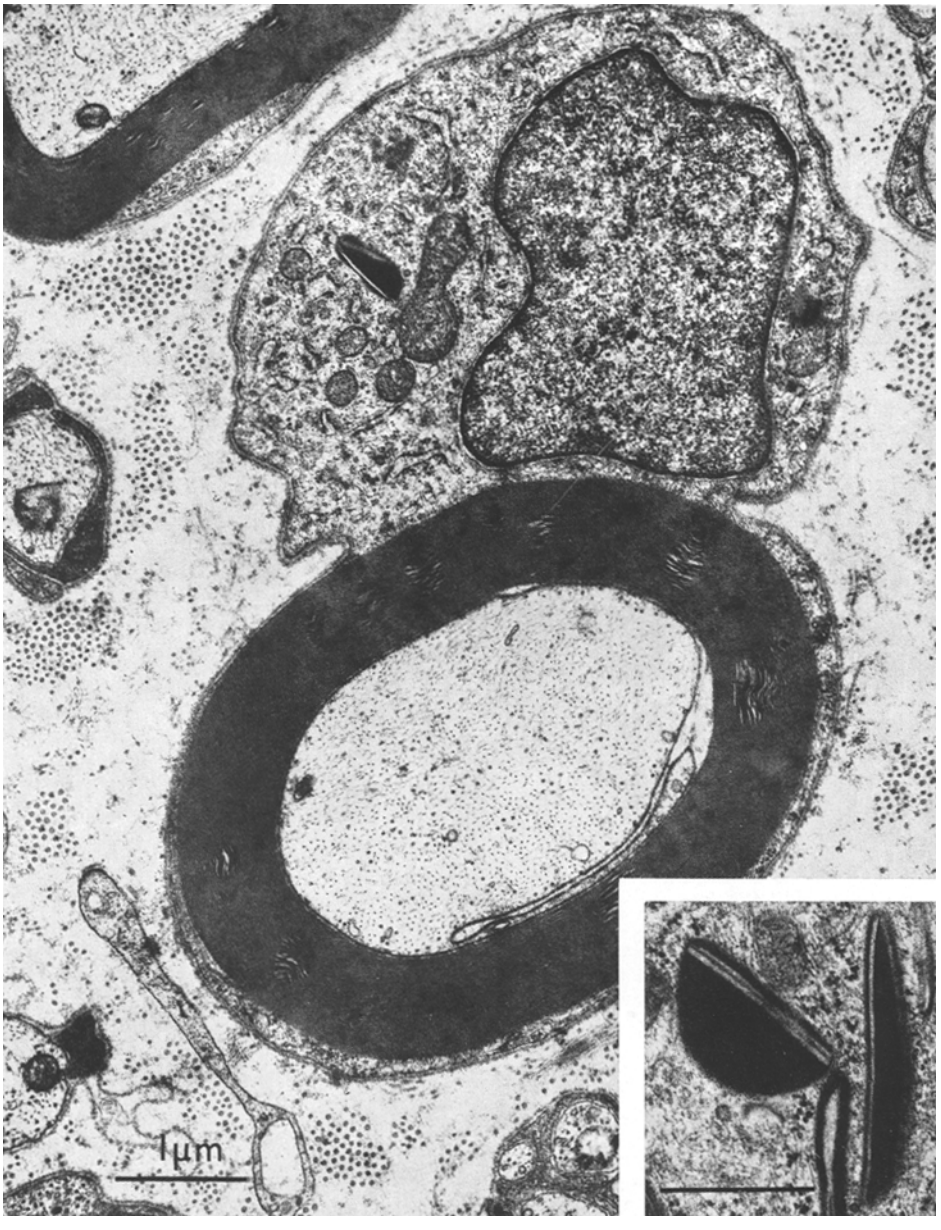

Fig. 8. Segment "B", 18 days. A myelinated axon in which the cytoplasm and nucleus of the associated Schwann cell are concentrated almost entirely on one side of the axon. The Schwann cell cytoplasm contains a lamellated body. Inset: Higher magnification of 2 lamellated bodies. Scale = 0.5 μm

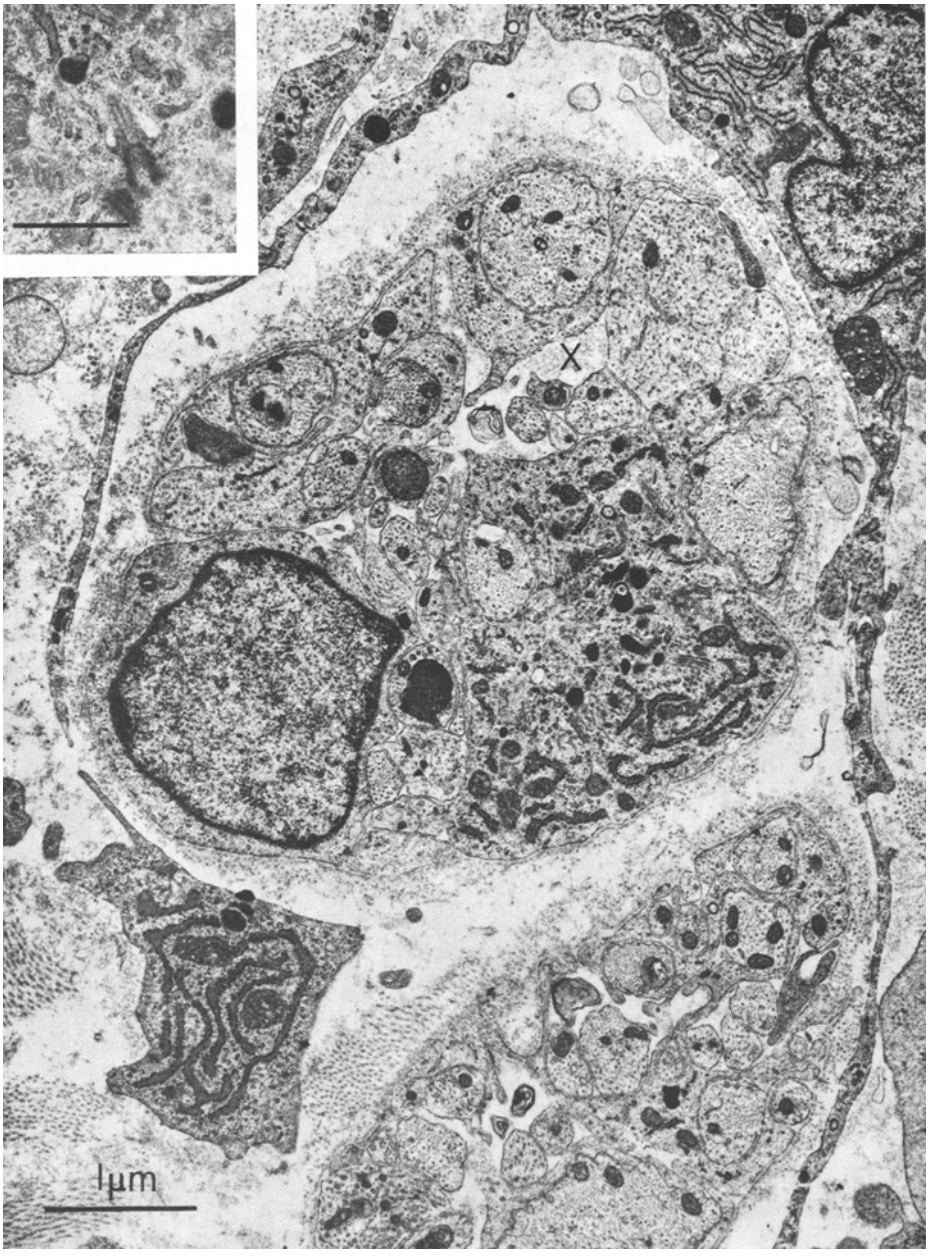

Fig. 9. Segment "A", 7 days. A type II "regenerating unit" (group) at an early stage in its evolution where there are only clefts between the Schwann cells (*X*). The cytoplasm of the Schwann cell(s) contains rough surfaced endoplasmic reticulum of which the cisternae contain a densely staining material. Inset: Part of the cytoplasm of another similar Schwann cell showing the base of a cilium, two dense membrane bound bodies and an alveolate vesicle. Scale = 1 μm

system of the adult rat they were all on Schwann cells associated with non-myelinated axons and were lying in a deep cleft in the Schwann cell cytoplasm. However, unlike the cilia observed by these workers, cilia in the proximal stump had a fibrillar configuration of $9 \times 2 + 0$, having no central apparatus. The classical motile cilium (Fawcett, 1961) has a fibrillar configuration of $9 \times 2 + 2$ central filaments, while those reported by Grillo and Palay (1963) were of a $9 \times 2 + 1$ configuration, having a large central tubule-like structure. In other regions of the mammalian nervous system cilia have been seen with a configuration of $9 \times 2 + 0$ at the base, which become $8 \times 2 + 1$ further from the root of the cilium (Dahl, 1963; Tani and Ametani, 1970), and cilia of a $9 \times 2 + 0$ arrangement have been identified in fibroblasts and smooth muscle cells of the chicken (Sorokin, 1962). In the proximal stump, all the Schwann cells possessing cilia were found in segments containing many "groups", and none were seen in normal nerve, but the numbers were so small that no conclusions can be drawn from this.

Other Cells Associated with Type I and Type II "Groups"

These cells can be divided into two classes, those possessing a basal lamina but which were not associated with axons, and those which did not possess a basal lamina.

The cells which had a basal lamina but which were not associated with axons could not, by definition, be Schwann cells, since the definition of a Schwann cell requires that it be associated with axons as well as a basal lamina. The ultrastructural features of these cells were, however, very similar to Schwann cells and their cytoplasm was often seen to contain dense granules and/or lamellar structures similar to those found in Schwann cells and transformed Schwann cells (Morris *et al.*, 1972a). The basal lamina envelope with which they were surrounded was often continuous with basal lamina around neighbouring indubitable Schwann cells (Fig. 5). Some of the cells of this configuration were observed to be in process of mitosis at the time of their fixation. The timing of the appearance of these cells was such that, though never frequent (not more than 10 in a complete transverse section of a sciatic nerve), they were most often seen in the second and third weeks after nerve section.

The cells associated with "groups" which did not possess a basal lamina exhibited a very wide spectrum of configuration and ultrastructure. They were first seen 5 days after nerve section when the "groups" were still in a relatively "tight" configuration and were usually found in the centre of the group. Similar cells were present in all segments taken at a later time in which the separate "groups" could be distinguished (i.e. segments that did not exhibit "compartmentation"—see Morris *et al.*, 1972d). When associated with "tight groups" these cells were often in contact with axons (Fig. 4). The relationship between these cells and axons was not, however, similar to the typical relationship between Schwann cells and axons, the contact between the two being tangential rather than enveloping (Fig. 4). In later segments where the "groups" were in a dispersed configuration these "atypical" cells were not usually in contact with either the axons or the Schwann cells of the unit. The ultrastructure of these cells was so variable that it is impossible to generalize about their appearance, especially since they were not frequently seen (less than 10% of 'groups' had a cell associated with them).

However, more than 50% of the cells of this type contained either membranous material in vacuoles in the cytoplasm and/or dense bodies similar to those seen in Schwann cells and transformed Schwann cells (Morris *et al.*, 1972a) (Fig. 4, inset I). Some, not always the same cells that contained the membranous material, possessed the dense material in the cisternae of the rough surfaced endoplasmic reticulum and/or the alveolate vesicles. Coated pits were also observed. This dense material in the cisternae of the rough surfaced endoplasmic reticulum and the alveolate vesicles, was not invariably present in the Schwann cells of the groups containing the "atypical" cell. The density of cytoplasmic staining ranged from very pale to very dense and in outline the cells often exhibited a few finger-like processes at the periphery of the cell. A further variant of this class of cell displayed fenestrations in the peripheral cytoplasm which were reminiscent of those seen at the periphery of endoneurial "macrophages" (Morris *et al.*, 1972a). Such cells were also seen to contain globular inclusions similar to those seen in Schwann cells and transformed Schwann cells which were degrading myelin (Morris *et al.*, 1972a).

Finally, a "group" was observed in which there was a "tight junction" either between two parts of the same "atypical" cell or between two different but very similar "atypical" cells (Fig. 4, inset II). This was the only occasion on which a "tight junction" of this kind was observed and also the only occasion on which there was a possibility that two "atypical" cells were present in the same plane of section in one "group".

Collagen Fibrils Associated with Type I and Type II "Groups"

During the first five days after nerve section the collagen fibrils in the proximal stump of the peripheral nerve were disorganised (Morris *et al.*, 1972a), with many bundles and individual collagen fibrils running obliquely across the endoneurium. In later segments in which "groups" were present the collagen fibrils were seen in relation to the units forming a "halo" of fibrils around each "group". During the first five days after nerve section this "polarisation" of collagen fibrils around the "groups" was not conspicuous, but in later sections many collagen fibrils were seen around each "group" (Fig. 5). The collagen fibrils in this location usually, but not invariably, ran longitudinally in the nerve. As well as being seen around the outside of the "groups" collagen fibrils were frequently seen between the Schwann cells within the "groups" (Fig. 4). They were seen in this situation when there was a very small separation between the Schwann cells of a "group" and sometimes when basal lamina was visible on only one of the closely separated Schwann cells (Fig. 5 inset). Collagen fibrils were also frequently seen between the Schwann cells of a "group" in which the basal lamina surrounding the entire "group" was intact, at least in the plane of section being viewed. As with the collagen fibrils around the outside of the "group", the collagen fibrils between the Schwann cells of a "group" usually, but not invariably, were in a longitudinal orientation. Both these collagen fibrils and those closely adjacent to the "outside" of the "group" were smaller, and more variable in calibre, than the endoneurial collagen fibrils in the normal nerve, having an average diameter of about 30 nm in contrast to the 50 nm which is the calibre of endoneurial collagen fibres in the normal nerve (Thomas and Jones, 1967). The collagen fibrils more distant from

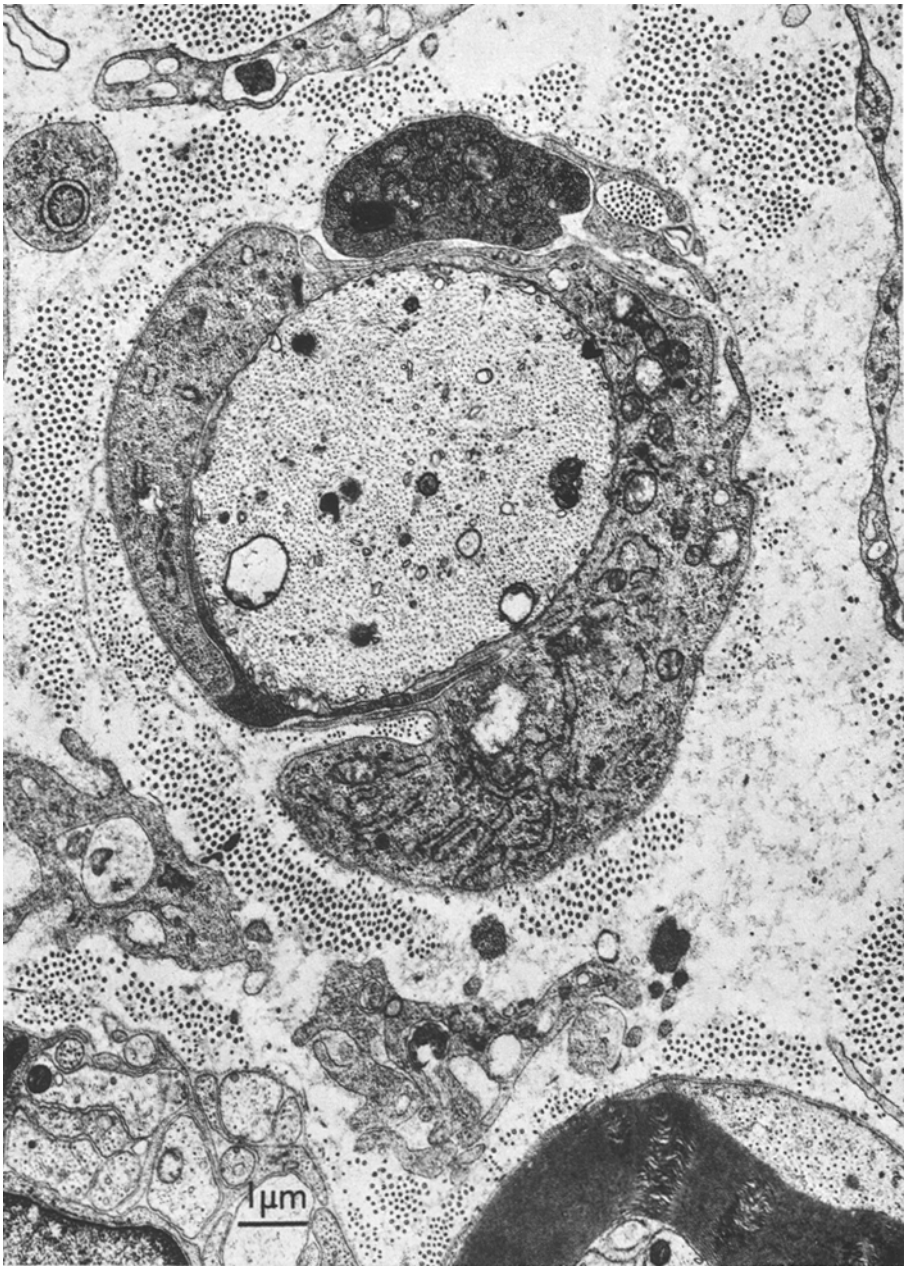

Fig. 10. Segment "A", 10 days. A single large non-myelinated axon which is associated with Schwann cell processes of two markedly different staining densities. This could be a section through an abnormal node of Ranvier. Also noticable in this electron micrograph is the gradation in diameter of the collagen fibrils which are smaller in diameter closer to the Schwann cell

the "group" however, had a diameter of 50 nm and were entirely typical of endoneurial collagen fibrils in the normal nerve (Fig. 10). The variability of the calibre of the collagen fibrils in the divided nerve was most noticeable in the first three weeks after nerve section.

Discussion

In the literature concerning regeneration of peripheral nerves there are many references to the presence of myelinated and non-myelinated axons collected together into clusters (Ranvier, 1878; Ramon y Cajal, 1905; Ranson, 1912; Nageotte, 1922; Holmes and Young, 1942; Evans and Murray, 1956; Thomas, 1968). Schroeder (1968) described what he called "hyperneurotisation" of Bungner bands with multiple myelinated sprouts in an experimental isoniazid neuropathy, while Ochoa and Mair (1969a, b) illustrate what they call "clusters" in normal human sural nerves, which are similar to the type II "groups" in the proximal stump of divided nerves, and which were considered by them to indicate regenerative activity. In a recent paper, Thomas (1970) also illustrates structures similar to type II "groups" which were present in the distal part of a peripheral nerve ten months after nine repeated crush injuries to the nerve. He attributed their development to the multiple sprouting of crushed axons together with a subdivision of the Bungner bands. The earlier literature concerning these "groups" attributes their development to the re-innervation of bands of Bungner by several axons, and in relation to this concept Ochoa and Mair (1969b) remark; "It is reasonable to suppose that collateral branching from an original fibre gives rise to clusters before the branches separate widely from the parent fibre. The presence of clusters in normal nerves could be interpreted as residual foetal structures due to incomplete separation of the myelinated and non-myelinated fibres which before myelination shared the same Schwann cell, but the increase in clusters with age indicates that most of them result from degeneration." There is also the possibility, most recently raised by King and Thomas (1970), of an association of myelinated and non-myelinated axons during regeneration. This follows the work of Evans and Murray (1953, 1954) who showed that regenerating non-myelinated axons could be guided by myelinated axons. It is plain therefore that at the present time some uncertainty exists as to whether these "groups" are the sprouts from a single axon or whether they are aggregates of separate myelinated and non-myelinated axons.

Evidently then the first problem which must be considered in relation to the types I and II "groups" is their origin. The first axon/Schwann cell complex which is identifiable as a type II "group" is the same axon/Schwann cell complex that was used to illustrate the end point of the traumatic degeneration of myelin (see Fig. 13, Morris *et al.*, 1972a). From this stage, the development of the type II "groups" can be followed in a temporal series of nerve segments. As the illustrations in this and the previous paper demonstrate there is an unbroken series from a single myelinated axon to the collection of myelinated and non-myelinated axons which comprise a fully developed type II "group" as far as it can be followed in the divided nerve. Further transformations, if any, are obscured in the divided nerve by the development of "compartmentation" (see Morris *et al.*, 1972d) after the twenty-ninth day following nerve section.

The natural conclusion which derives from the ability to trace a complete and coherent series of developmental stages from a single myelinated axon to a developed type II "group" is that all the axons in the type II "group" are branches from the original myelinated axon. There are two features of the developing "groups" which lend credence to this contention.

1. Using light microscopy Ramon y Cajal (1928) described the production of terminal and collateral sprouts in the proximal stump of a divided nerve. The collaterals he described ran along the same course as the nerve fibre from which they were derived. Clusters of terminal sprouts were also described. By electron microscopy in the proximal stump of a divided nerve we have observed myelin sheaths containing more than one axon (see Fig. 5, 6, in Morris *et al.*, 1972c) and a branch at a node of Ranvier which remained adherent to the external surface of the Schwann cell on the distal side of the node (see Fig. 17, in Morris *et al.*, 1972c), as well as the type I "groups" which, in transverse section have axons applied to the external surface of Schwann cells containing myelinated axons. On the basis of these observations it therefore seems likely that the type I "groups" consist of a myelinated axon and its collaterals which have remained associated with the same Schwann cell. If this evidence is accepted then the non-myelinated axons in each type I "group" are branches of the myelinated axon. The presence in the type II "groups" of myelinated axons after the seventh day following nerve section requires that those components which are myelinated be derived from myelinated axons. The similarities in appearance and development between the type I and the type II "groups" make it reasonable to suppose that the type II "groups" are composed of the terminal sprouts and/or collaterals of parent myelinated axons which have degenerated. If this conception of their origin is correct then each type II "group" will consist of the sprouts produced by a single myelinated axon.

2. A second feature of the type I and type II "groups" which supports the hypothesis that they are developed from a single axon is the persistence of an envelope of basal lamina around the entire "group". The degeneration of a myelinated axon may reasonably be expected to leave behind a cylinder of basal lamina (see Fig. 14, Morris *et al.*, 1972a). This envelope will then contain the terminal sprouts and/or collaterals of the degenerated axon. This interpretation will account for the persistence of an envelope of basal lamina surrounding the entire "group" even when it has separated into its constituent axon/Schwann cell elements. Possible objections to this interpretation of the origin of the type II "groups" are that the axons which comprise such groups may either be branches of non-myelinated axons, or be the result of a chance association of terminal sprouts and/or collaterals from more than one axon. The possibility that the type II "groups" are composed of non-myelinated axons is decisively refuted by the presence of myelinated axons in the overwhelming majority of axon/Schwann cell clusters identified as type II "groups" when examined after the tenth day following nerve section. As far as the possibility that the axons in the groups are derived from different sources is concerned, myelinated axons are often present in the type II "groups" before they have completely separated into their constituent axon/Schwann cell elements. In this configuration there is an intact basal lamina surrounding the entire "group" and at this time myelination is often seen in two or more axons in the same "group". If the axons in the group are

derived from more than one parent axon it becomes necessary for sprouts from divers axons to associate within a single envelope of basal lamina. Direct ultrastructural evidence for or against such an association would be extremely difficult to acquire, but we have never observed axon/Schwann cell elements in a configuration that could be interpreted as a stage in the association of elements derived from different original axons. Consequently we think that such an explanation for the observed configuration of the type II "groups" is very unlikely.

The last problem concerning the origin of the "groups" relates to the single large axons which constituted one pole of the spectrum of the type II "groups". Axons of this configuration possessing high concentrations of axoplasmic organelles are discussed by Morris *et al.* (1972c), and it is thought probable that some at least of them are terminal regenerating sprouts of degenerated myelinated axons. In longitudinal sections such axons have a markedly non-uniform distribution of axoplasmic organelles. Thus, a reasonable interpretation of the axons of this configuration which have a normal axoplasmic appearance and are identified as type II "groups" is that they are also regenerating terminal sprouts of myelinated axons but sectioned across a part of the axon where the concentration of axoplasmic organelles is normal. The only real alternatives to this interpretation are either that they are myelinated axons which have been subject to segmental demyelination, or that they are sections through abnormal nodes of Ranvier in which the Schwann cells have lost the "villous" processes which characterize them at nodes (Robertson, 1957, 1959, 1960; Landon and Williams, 1963). In any other than a divided nerve the possibility of segmental demyelination would have to be very seriously entertained and would probably be the interpretation of choice for these structures. Even in a divided nerve segmental demyelination cannot be positively excluded, particularly when these structures are seen in segment "A" since this segment is located between three and six internodes from the site of division of the nerve. However, there do not appear to be any reports in the literature of segmental demyelination in the proximal stump of divided nerves in the first 14 days after section. In view of this and the presence of similar structures in segment "C", where, being within two internodes of the site of division of the nerve, their presence is very unlikely to be due to segmental demyelination, we consider the most reasonable interpretation of the occurrence of these structures is that they are single terminal sprouts of degenerated myelinated axons. Some of them may be abnormal nodes of Ranvier, particularly those which are surrounded by Schwann cell processes of two markedly differing densities (Fig. 10), but the existence of large single thinly myelinated axons (see Fig. 7), effectively demonstrates that this cannot be the explanation of all such structures. What is more certain is that those type II "groups" which contain a single large non-myelinated axon and one or two small non-myelinated axons (Fig. 2) represent the production of multiple terminal sprouts and/or collaterals from a degenerated myelinated axon rather than either segmental demyelination or a section through an abnormal node of Ranvier.

Thus one pole of the spectrum of configurations exhibited by the type II "groups" is probably regeneration which is occurring by means of a single terminal sprout. The most straightforward interpretation of the rest of the spectrum is that it illustrates an increase in the number of collateral and/or terminal sprouts

produced by the degenerated axons. This interpretation is entirely compatible with the observations made by light microscopy on the regeneration of myelinated axons (Ranson, 1912; Ramon y Cajal, 1928).

In view of the observations discussed above we believe that in each "group" all the axons are derived from a single myelinated axon and that this applies to both type I and type II "groups". Thus, each "group" may be said to be the anatomical expression of the regenerative activities of a single myelinated axon. For this reason we think it would be appropriate to call both types of "group" "regenerating units".

A consequence of the derivation of the type II "regenerating units" from single myelinated axons is that the presence in a nerve segment of such a unit implies that the myelinated axon from which it developed had undergone retrograde degeneration. Thus, inspection of Table 2 immediately indicates that retrograde degeneration of myelinated axons continues for at least seven days after nerve section. This must be so because type II "regenerating units" do not occur in this segment until the seventh day. A further consequence of their derivation is that the number of type II "regenerating units" in a segment is a direct measure of the number of myelinated axons which have undergone retrograde degeneration.

Clearly, for the purpose of assessing the amount of degeneration in myelinated axons it is critical that the type II "regenerating units" should be distinguishable both from clusters of non-myelinated axons and from type I "regenerating units". The only stage when confusion is likely to arise between type II "regenerating units" and non-myelinated axons is just after the formation of the type II units when none of the axons are myelinated. Shortly after this time the type II units can be unequivocally identified by the presence of myelinated axons within them. However, in the first segments in which they were seen, as well as the archetypal type II "regenerating units" without myelinated axons, there were other clusters of non-myelinated axons. The ultrastructural characteristics of these other clusters and the fact that they did not seem to undergo any particular structural development during the course of the first month after nerve section suggests very strongly that they are non-myelinated axons rather than an atypical form of type II "regenerating unit". If this interpretation is correct then the appearance of non-myelinated axons in the proximal stump of a divided nerve contrasts very strongly with the appearance of the type II "regenerating units". The only general exception to this is in segment "C" between 24 and 60 hours after nerve section where the distinction cannot be made with certainty (see Morris *et al.*, 1972c). However, the description given in the observations applies only to the typical members of each class of axon cluster. There are a few clusters of non-myelinated axons which have a configuration intermediate between the early type II "regenerating units" and the clusters of non-myelinated axons. These intermediate forms cannot reasonably be assigned to either group. However, they comprise such a small proportion of the total number of positively identifiable type II "regenerating units" (less than 2%), that we do not consider that their presence introduces an unacceptable error into the estimates of degeneration. As regards the distinction between the type II and type I "regenerating units", in the first month after nerve section, which includes all the time being considered

in this paper, this never presents any problems. During this time the number of myelin lamellae around the axons in the type II "regenerating units" is smaller than the number of lamellae which surround axons of a similar diameter in normal nerves. Hence, axons which have been remyelinated can always be distinguished from axons which have not degenerated by the relative thinness of their myelin. In sum therefore, except in a very few cases, the identification of the type II "regenerating units" is quite unequivocal, and the assessment of the number of axons which have degenerated in a given segment correspondingly reliable.

The amount and variation of retrograde degeneration of axons in segment "A" is illustrated in Table 2. It is indicated by the number of type II "regenerating units" expressed as a percentage of the total number of "neural elements" in a segment. As can be seen from this table it varies between 1.1% and 37% of the axons in segment "A". In the context of the retrograde extent of this degeneration, there is an irreducible minimum of uncertainty as to the precise location of individual sections from different segments in relation to the site of division of the nerve. It was not possible, more than four or five days after the initial nerve section, to establish the site of division of the nerve to an accuracy greater than 0.5 mm. Also, each segment when obtained was approximately 1.5 mm long and during trimming of the block preparatory to section cutting at least 0.5 mm of the segment was removed. This was done deliberately, both to cut more in the centre of the segment and reduce the error in locating the section in relation to the cut end of the nerve, and to remove any part of the segment which might have been damaged during preparation. Hence, since during preparation the proximal and distal ends of the segment cannot be distinguished, there is, in any given segment, taking this uncertainty with the error in location of the site of division of the nerve, an uncertainty of approximately 1.0 mm in the location of individual sections with respect to the site of division of the nerve. Thus, the sections from segments "A" considered in Table 2 are located 3.75 ± 0.5 mm from the site of division of the nerve. This uncertainty must be borne in mind when considering the variation in the amount of degeneration in this segment.

Apropos the estimates of the amount of degeneration in segment "A", a caveat must be entered regarding the possibility of retrograde rather than orthograde growth of the type II "regenerating units". Clearly, if there is an appreciable number of type II units growing in this manner then the estimates of the percentage degeneration cannot be other than wildly spurious. There is one piece of evidence that suggests that retrograde growth may occur. This is the seemingly paradoxical finding that as the number of "regenerating units" in the segments is reduced the proportion of type II "regenerating units" rises, rather than falls as might be expected. On the other hand the possibility of retrograde growth is discounted by the presence of basal lamina, which probably surrounded the original myelinated axon, encircling the type II "regenerating units". The presence of this lamina is incompatible with retrograde growth and suggests that the type II "regenerating units" are growing in an orthograde mode.

As mentioned a somewhat unexpected result which emerges from the data in Table 3 is the increase in the proportion of type II to type I "regenerating units" with decreasing numbers of units in each segment. This is not an exact relationship but nevertheless there does appear to be a correlation between the

two phenomena. If all "regenerating units" have the same longitudinal configuration the expected pattern proceeding distally along a myelinated axon in the proximal stump would be: firstly, a myelinated axon, next, having produced collateral sprouts from its undegenerated part, a type I "regenerating unit", and finally, distal to the part of the original axon that had degenerated, a type II "regenerating unit". This concept of their longitudinal structure would generate an increasing proportion of type I "regenerating units" in nerve segments with progressively fewer units; an expectation which is confounded by the figures.

At least three general possibilities can be distinguished as possible explanations for this finding. They are: 1. sporadic segmental demyelination for several millimetres proximal to the site of division; 2. the presence of two populations of axons in the proximal stump which react in different ways to division; 3. the action of two influences on a single population of axons. There are at present insufficient data available to indicate which, if any, of these possibilities is the correct one.

Another matter which must obtrude into any discussion of the development of "regenerating units" is the question of the origin and nature of the cells which were not Schwann cells but which were associated with a proportion of the "regenerating units". In the case of those cells which were surrounded by a basal lamina, it seems highly probable that it is entirely a matter of definition that they cannot be classified as Schwann cells. Since, by definition, Schwann cells must be associated with axons and the cells in question were not, they cannot, with any justification, be called Schwann cells. Nevertheless, they have all the other ultrastructural characteristics of Schwann cells, and, as described, the basal lamina which surrounds them is often continuous with basal lamina surrounding indubitable Schwann cells. Some of these cells were seen to be fixed while in mitosis and it is noticeable that Schwann cells seen in this configuration rarely had more than one or two axons associated with them. It is possible that the cells inside the "regenerating units" which possessed a basal lamina but no axons were all "Schwann cells" which had entirely divested themselves of their axons and which were just about to start mitosis, were in process of mitosis or had just completed mitosis. However, at present it is not possible to adduce any decisive evidence for this proposal.

As regards the cells associated with "regenerating units" which did not possess a basal lamina, two general possibilities may be distinguished in any consideration of their origin. Either they originate from within the "regenerating units", or they enter the units from the "outside". If they originate from cells already present in the "regenerating units" then, under the hypothesis for the origin of the "regenerating units" which has been advanced in this report, they must be derived from Schwann cells. If they enter the "regenerating units" from the "outside", it is probable that they would have to be derived from more than one cell type since they have such a wide spectrum of appearance, possessing as they do some of the characteristics of Schwann cells, transformed Schwann cells (Morris *et al.*, 1972a), endoneurial "macrophages" (Morris *et al.*, 1972a) and endoneurial fibroblasts (see Morris *et al.*, 1972d), without being positively or consistently identifiable as any one of these cell types.

An observation which could support a Schwann cell origin for these "atypical" cells is the presence of membranous debris in approximately 50% of the cells in

question. It has been established elsewhere (Morris *et al.*, 1972a) that the major part of the traumatic degeneration of myelin in the proximal stump of a divided nerve is accomplished by Schwann cells and transformed Schwann cells. This being so, the finding of membranous material (which may or may not be myelin debris) in some of these cells at least maintains the credibility of a hypothesis by which the cells are derived from Schwann cells.

Another observation which may be interpreted as evidence for the origin of these "atypical" cells from cells already present in the "regenerating units" is that they were seen in units at a very early stage in their development, when they still had a "tight" structure. Entry of a cell into a "regenerating unit" in this configuration might require that the entering cell first penetrate the basal lamina which surrounds the whole unit at this stage of its development and then insinuates itself between the packed axons and Schwann cells of the unit. We have never seen any cells similar to these "atypical" cells either partly covered by basal lamina or in a relationship to a "regenerating unit" which could be interpreted as entering or leaving the unit. This is not a wholly convincing argument since it is clearly not necessary for the "atypical" cell to enter the "regenerating unit" from a lateral direction and it would be quite possible to argue that these cells enter the units at distal ends and then move proximally to generate the observations enumerated above.

Two other observations which may have a bearing on this question are the similarity of the fenestrations in the cytoplasm of one or two of these "atypical" cells to the fenestrations at the periphery of endoneurial "macrophages", and the presence in one of these endoneurial "macrophage-like" cells of globular inclusions similar to those seen in Schwann cells digesting myelin. This particular cell was seen in segment 10d "A". However, since the origin of endoneurial "macrophages" is, as yet, obscure (Morris *et al.*, 1972a) this cannot be said to be evidence as to the cells or origin of the "atypical" cells in the "regenerating units". The other observation was of a "tight junction" either between two parts of the same "atypical" cell or between two separate "atypical" cells. In the vicinity of the nerve the only place where similar "tight junctions" have been observed is between cells in the perineurium and between endoneurial capillary endothelial cells. However, similar junctions have also been observed between epineurial cells which are located in a perineurial situation in relation to Schwann cells and axons found outside the normal perineurium in a divided peripheral nerve. They have also been observed between mesodermal fibroblasts (Devis and James, 1964). Hence both these observations could be said to be equivocal in their implications concerning the origins of the cells on which they were made.

On the basis of the evidence enumerated in the preceding paragraphs it is clearly not possible to arrive at any firm conclusions about the origins of the "atypical" cells associated with "regenerating units", though it seems to us most likely that they are derived from Schwann cells (see also Morris *et al.*, 1972d).

For references, see Part IV (Vol. 124, pp. 165–203, 1972).

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