J. Anat. (1974), **117**, 3, pp. 517–529
With 4 figures
Printed in Great Britain

Regeneration of peripheral unmyelinated nerves. Fate of the axonal sprouts which develop after injury

GARTH M. BRAY AND ALBERT J. AGUAYO

Division of Neurology, Montreal General Hospital and Department of Neurology and Neurosurgery, McGill University, Montreal, Canada

(*Accepted* 8 *March* 1974)

INTRODUCTION

Ultrastructural reactions of unmyelinated fibres to injury have been investigated in several pre- and postganglionic sympathetic nerves. Rapid degeneration and regeneration have been observed in such postganglionic sympathetic nerves as the rabbit anterior mesenteric nerve (Bray, Peyronnard & Aguayo, 1972; Aguayo, Peyronnard & Bray, 1973) and the rat carotid nerves (Matthews, 1973). Similarly, Dyck and Hopkins (1972) observed that axons in the predominantly preganglionic rat cervical sympathetic trunk degenerated within 3 days of crush injury and showed regenerative sprouting by 5 days after injury.

One of the most striking features of unmyelinated nerves regenerating after crush injury or transection is the presence of increased numbers of small axons per Schwann cell (Dyck & Hopkins, 1972; Bray *et al.* 1972; Aguayo, Peyronnard & Bray, 1973). Furthermore, in nerves such as the rat cervical sympathetic trunk, where total axonal populations can be studied quantitatively, there is an absolute increase in the number of these small axons ('sprouts') per whole nerve transverse section two weeks after crush injury (Bray, Aguayo & Martin, 1973). The object of the present study was to determine the fate of these axonal sprouts during later stages of regeneration. Thus, rat cervical sympathetic trunks were studied by quantitative ultrastructural techniques at intervals up to 6 months after crush injury.

MATERIALS AND METHODS

Sprague–Dawley rats of either sex weighing 250–300 g were anaesthetized with pentobarbital (50 mg/kg body weight). The left cervical sympathetic trunk was crushed for one minute with jewellers' forceps at a level approximately 10 mm below the superior cervical ganglion. One, 3 and 6 months later, two animals at each time interval were reanaesthetized with pentobarbital and systemically perfused with fixative (2·25% glutaraldehyde and 0·75% paraformaldehyde in 0·1 M Sorensen's phosphate buffer). Both left and right carotid sheaths were dissected from the upper mediastinum to the carotid bifurcation, immersed in chilled fixative and processed for embedding in Epon-812. Prior to polymerization, each carotid sheath was cut into segments 2 mm in length and embedded for transverse sectioning. Cervical sympathetic trunks were identified by phase microscopy in 1 μm thick sections

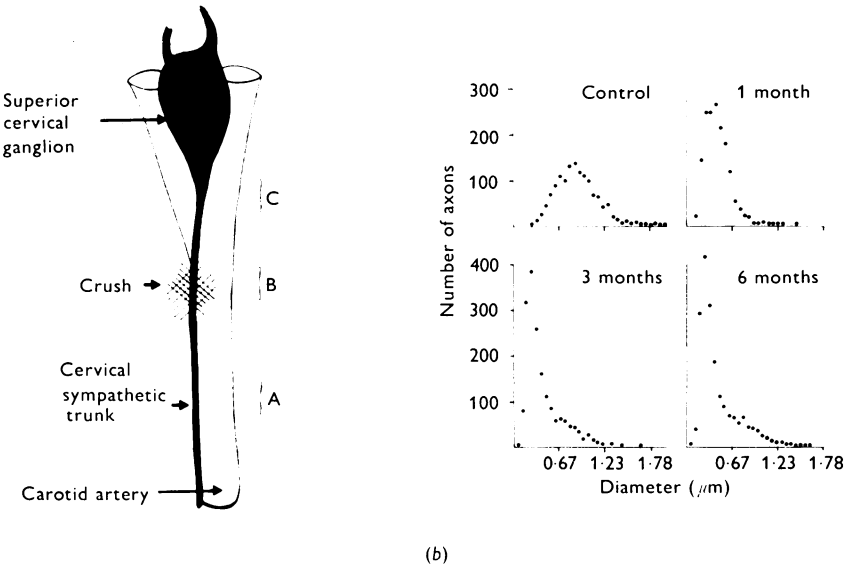

Fig. 1. (*a*) Diagram of a rat cervical sympathetic trunk adjacent to the carotid artery. The superior cervical ganglion is situated at the level of the carotid bifurcation. Unmyelinated axons were studied by quantitative ultrastructural techniques at 3 levels: A, 6–8 mm proximal to crush injury; B, at the level of crush; C, 6–8 mm distal to crush. (*b*) Representative size–frequency histograms for axonal diameters in the distal segments of control and regenerating nerves examined 1, 3 and 6 months after crush injury.

stained with paraphenylenediamine. Ultrathin sections of cervical sympathetic trunks were mounted on copper grids, stained with lead citrate and examined with a Siemens Elmiskop I-A.

For quantitative studies, injured (left) cervical sympathetic trunks were examined at the level of crush as well as 6–8 mm distal and 6–8 mm proximal to the level of injury (Fig. 1*a*). Uninjured (right) cervical sympathetic trunks were studied at levels corresponding to the proximal and distal levels of the injured nerves. In addition, uninjured right and left cervical sympathetic trunks from seven rats were examined to determine the extent of variation in axon and Schwann cell unit populations between both nerves in the same animal. Complete montages of each cervical sympathetic trunk in cross-section were prepared from overlapping electron micrographs printed at a final magnification of $\times 5000$. These montages were used to determine total numbers of Schwann cell units (Aguayo & Bray, 1974) per whole transverse section. Montages of approximately one-third of each nerve, printed at a final magnification of $\times 10000$, were also prepared; these were used to determine axonal diameters and the numbers of axons per Schwann cell unit.

Diameters of 1000 unmyelinated axons were measured with a Zeiss particle analyser and the following parameters calculated for each nerve level: median axonal diameter, the mean diameter of the 20 largest axons and the proportion of regenerating axons with diameters greater than 0·90 μm. The latter two parameters were calculated to permit approximate comparisons with previous studies of regenerating myelinated fibres. Cragg & Thomas (1964) used mean diameters of the 20 largest myelinated

fibres to assess axonal maturation. Gutmann & Sanders (1943) evaluated axonal maturation by determining the proportion of myelinated fibres with diameters greater than 8 μm, which was approximately the 80th percentile for their control nerves; in the present study of unmyelinated nerve fibres, 0·90 μm represented the 80th percentile for control axonal diameters.

Total numbers of unmyelinated axons per whole transverse section were calculated by multiplying the total number of Schwann cell units counted for the whole nerve, by the mean number of axons per unit, determined for a portion of the nerve. The error of the method was estimated as follows: complete montages of three nerves were prepared at both $\times 5000$ and $\times 10000$; the total number of unmyelinated axons was determined by two methods: A, counting all axons at $\times 10000$ and B, counting all Schwann cell units at $\times 5000$ and determining the ratio of axons to Schwann cell units for a portion of the nerve at $\times 10000$. The following results were obtained for these three nerves:

	Total axonal counts		
Nerve	Method A	Method B	Error (%)
628LK	3621	3432	5·2
629RI	2576	2522	2·0
800RC	4190	4410	5·3

Consequently, method *B* was judged to be sufficiently accurate for the present quantitative study.

In some rats a small branch of the vagus nerve joins the cervical sympathetic trunk (Bray & Aguayo, unpublished observations). However, two characteristics of this branch permitted its exclusion from quantitative determinations of unmyelinated axons in the cervical sympathetic trunk in the present study. First, it contains a greater proportion of myelinated axons than the cervical sympathetic trunk. Secondly, its fibres are descending while those of the cervical sympathetic trunk are ascending; presumably axons in this branch of the vagus nerve degenerated below the level of injury while those in the cervical sympathetic trunk degenerated above the level of injury.

RESULTS

Normal cervical sympathetic trunks

The rat cervical sympathetic trunk is a unifascicular nerve containing preganglionic autonomic axons. It extends from the middle cervical ganglion to the superior cervical ganglion – a distance of approximately 20 mm in adult animals. Most axons in rat cervical sympathetic trunks are unmyelinated (Dyck & Hopkins, 1972; Aguayo, Martin & Bray, 1972). In cross-section electron micrographs these axons are arranged within Schwann cell units, which may be defined as basal lamina-enclosed Schwann cell processes containing one or more axons (Aguayo & Bray, 1974).

Table 1 summarizes quantitative data for uninjured left and right cervical sympathetic trunks from seven adult rats. In five of the seven animals, the total number of axons per transverse section was remarkably similar for both nerves from the same animal, but in two (animals 917 and 944) the differences were fairly marked.

Table 1. *Quantitative data for uninjured right and left cervical sympathetic trunks from adult rats*

(Total number of unmyelinated axons and Schwann cell units were counted in cross-section electron micrograph montages printed at ×5000; axonal diameters were determined for a minimum of 1000 axons from non-overlapping electron micrographs printed at ×10000. *Myelinated fibres expressed as % of total number axons – unmyelinated plus myelinated.)

A. *Numbers of axons and Schwann cell units*

		Unmyelinated fibres				Myelinated fibres	
		Axons		Schwann			
Animal	Side	Total	L/R	cell units	Axons/unit	Total	%*
917	L	4383	1·39	848	5·2	55	1·3
	R	3151		978	3·2	55	1·7
918	L	5107	0·99	780	6·6	81	1·6
	R	5166		772	6·6	117	2·2
919	L	5536	0·99	923	6·0	36	0·6
	R	5615		1085	5·2	70	1·2
920	L	7465	0·99	963	7·7	29	0·4
	R	7533		1344	5·4	46	0·6
942	L	3426	1·01	975	3·5	79	2·3
	R	3396		1128	3·0	140	4·0
943	L	4251	1·03	1175	3·6	138	3·2
	R	4133		1256	3·2	183	4·2
944	L	5115	1·26	1036	4·9	129	2·5
	R	4062		1132	3·6	143	3·5
Mean ± s.d.	L	5040 ± 1278		957 ± 128	5·4 ± 1·5		
	R	4722 ± 1522		1106 ± 198	4·3 ± 1·4		

B. *Axonal diameters*

Animal	Side	Median	Mean	s.d.	% > 0·90 μm	Mean of 20 largest
917	L	0·62	0·72	0·23	8·4	1·22
	R	0·61	0·64	0·20	11·4	1·31
918	L	0·60	0·63	0·19	9·8	1·25
	R	0·65	0·63	0·17	8·0	1·12
919	L	0·61	0·64	0·19	9·5	1·33
	R	0·65	0·68	0·19	13·3	1·28
920	L	0·62	0·66	0·22	13·6	1·28
	R	0·63	0·67	0·19	13·1	1·22
942	L	0·63	0·66	0·21	12·8	1·22
	R	0·62	0·65	0·19	10·7	1·26
943	L	0·60	0·60	0·18	8·6	1·18
	R	0·70	0·72	0·21	19·4	1·36
944	L	0·66	0·68	0·20	13·1	1·22
	R	0·63	0·65	0·20	10·7	1·23
Mean ± s.d.	L	0·62 ± 0·02			10·8 ± 2·3	1·24 ± 0·05
	R	0·64 ± 0·03			12·4 ± 3·6	1·25 ± 0·08

However, taking the group as a whole, the differences between the two sides for any of the parameters used were not statistically significant ($P > 0·05$). Thus, in quantitative studies of these nerves, it seemed reasonable to use the uninjured cervical sympathetic trunks as controls for the injured nerves on the opposite side.

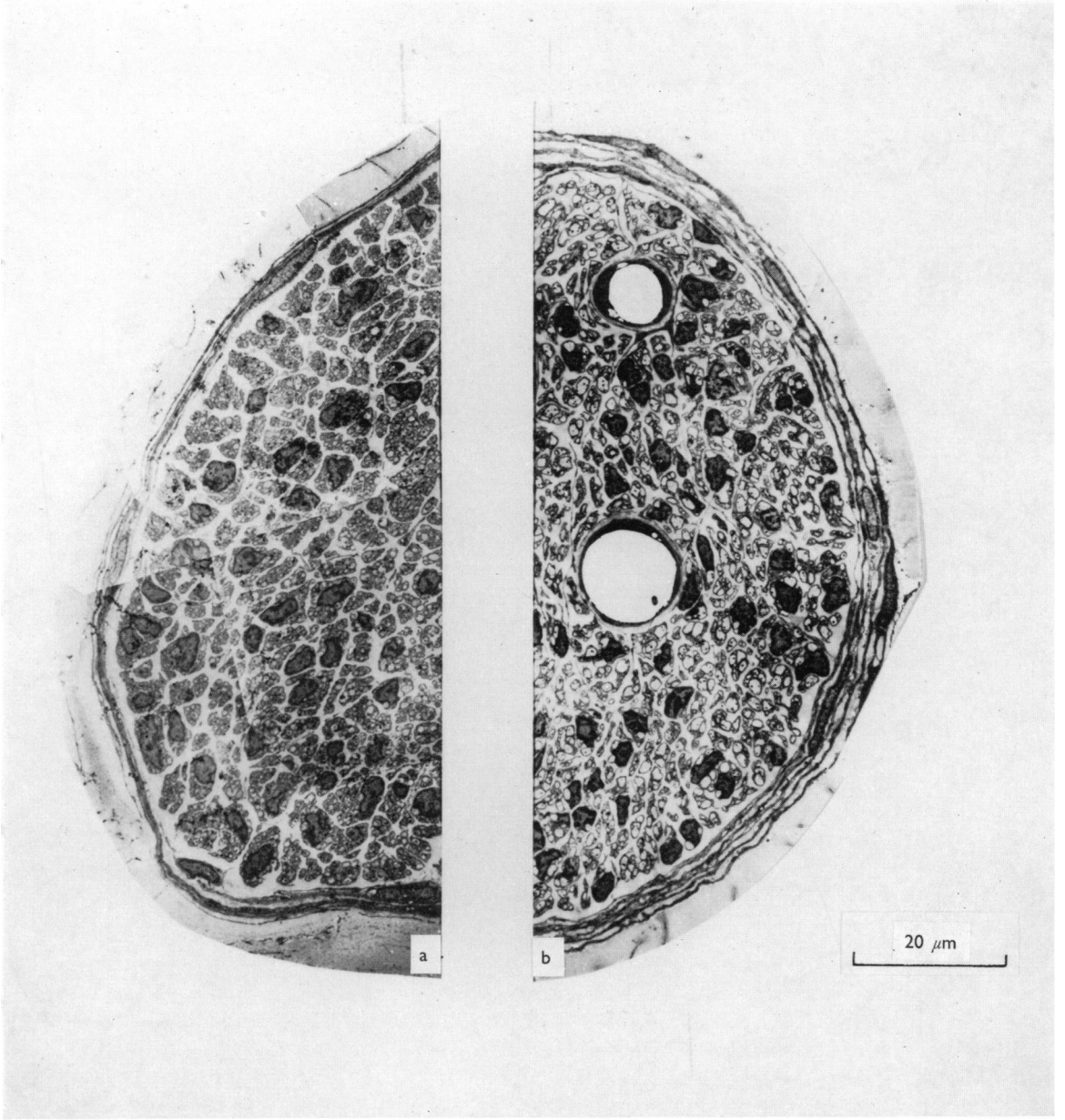

Fig. 2. Transverse section electron micrographic montages of portions of two rat cervical sympathetic trunks distal to the level of crush injury. (*a*) One month after crush injury there are numerous small axons per Schwann cell unit. (*b*) Six months after injury there are fewer axons per Schwann cell unit and axons with diameters approximately normal are present. Increased numbers of Schwann cell nuclei are present in both nerves.

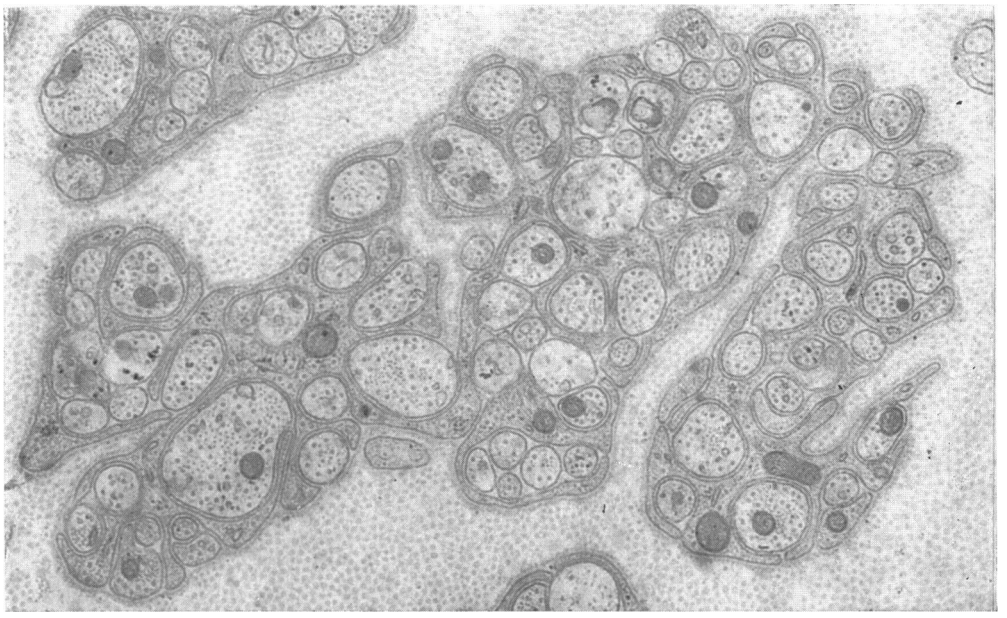

Fig. 3. A single axon-Schwann cell unit from the distal segment of a rat cervical sympathetic trunk one month after crush injury. Although there are increased numbers of small axons, most are surrounded by Schwann cell cytoplasm which shows few reactive changes. Electron micrograph, × 20000.

Regenerating cervical sympathetic trunks

(*a*) *Distal to crush injury*

One month after crush, most Schwann cell units contained many small axons and most of these axons were individually surrounded by Schwann cell cytoplasm (Figs. 2*a* and 3). The Schwann cell cytoplasm contained increased numbers of ribosomes and mitochondria as compared with normal controls, as well as occasional dense, amorphous inclusions which presumably were the remnants of degenerating axons. Three months after injury Schwann cell units contained fewer axons than at one month. Although many of these axons were abnormally small, most units also contained one or more axons whose diameters were approximately normal (Fig. 4*a*). At this stage reactive changes were less prominent in Schwann cell cytoplasm. However, Schwann cells frequently had elongated cytoplasmic processes and occasionally surrounded bundles of collagen ('collagen pockets'). These appearances were still present six months after injury (Fig. 4*b*).

Fig. 4. (*a*), (*b*) Distal segments of regenerating cervical sympathetic trunks 3 months (*a*) and 6 months (*b*) after crush injury. Schwann cell units are irregular in outline and contain fewer axons than were present one month after injury. Occasional Schwann cell processes surround bundles of collagen. Although many axons are abnormally small, most Schwann cell units contain at least one axon of approximately normal diameter. (*c*), (*d*) Proximal segments of regenerating rat cervical sympathetic trunks 1 month (*c*) and 6 months (*d*) after injury. Axonal diameters are uniformly reduced in size 1 month after injury; by 6 months their diameters are more variable and resemble the distal segments of these nerves. Electron micrographs, × 11000.

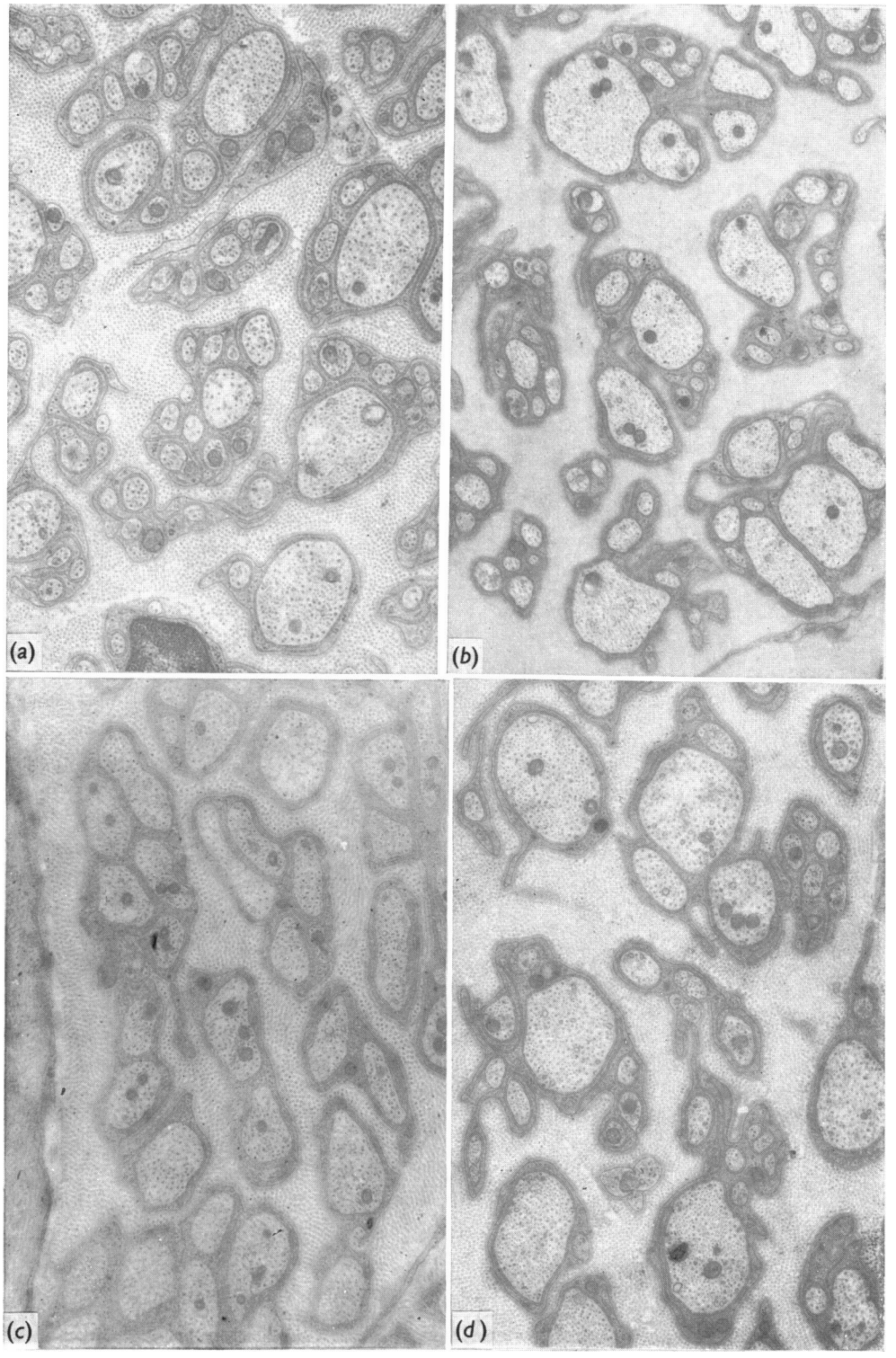
(a)
(b)
(c)
(d)

Table 2. *Quantitative data for rat cervical sympathetic trunks crushed on the left side* 1, 3 *or* 6 *months prior to sacrifice*

(Two animals were studied at each time interval. For control data, right cervical sympathetic trunks from the same animals were examined at corresponding levels.)

Months after crush	Regenerating			Control	
	Proximal	Crush	Distal	Proximal	Distal
	A. Unmyelinated axons per whole transverse section				
1	4725	10922	13562	3227	4646
	5480	5608	18654	4190	3569
3	4632	3659	9777	4018	4600
	4225	3827	6100	3496	2341
6	4528	4830	3866	4559	3600
	2264	3068	1854	3151	2522
	B. Schwann cell units per whole transverse section				
1	1350	984	832	978	968
	1096	701	836	900	830
3	1219	642	897	837	1150
	1006	890	509	908	899
6	924	966	500	970	943
	566	520	530	553	615
	C. Axons per Schwann cell unit				
1	3·5	11·1	16·3	3·3	4·8
	5·0	8·0	22·6	4·7	4·3
3	3·8	5·7	10·9	4·8	4·0
	4·2	4·3	6·8	3·3	4·6
6	4·9	5·0	7·2	4·7	4·1
	4·1	5·9	3·5	5·7	4·1

Table 2 summarizes the quantitative data pertaining to these nerves. One month after crush injury there were approximately four times as many axons per whole transverse section distal to the level of injury as there were in control nerves. The ratio of axons per Schwann cell unit was increased proportionately, because the number of Schwann cell units per whole transverse section did not change appreciably after injury. At later intervals after injury the number of axons per Schwann cell unit and the total number of axons per whole transverse section decreased so that by 6 months after injury both these measurements were within the normal range.

Median axonal diameters, which were reduced to less than 0·4 μm 1 month after cervical sympathetic trunk injury, remained at this size 3 and 6 months after injury (Table 3) and the peak of the axonal diameter–frequency histograms was persistently shifted to the left (Fig. 1*b*). However, between 1 and 6 months after injury, there was an increase in the proportion of unmyelinated axons with diameters which were approximately normal (Table 3). This increase was most strikingly demonstrated by the mean diameters of the 20 largest axons which had increased to over 90% of normal 6 months after injury. The proportions of axons with diameters greater than 0·90 μm also increased from less than 1% 1 month after injury to approximately 10% by 6 months.

Table 3. *Axonal maturation in regenerating unmyelinated nerve fibres*

(Axonal diameters were determined for the proximal and distal portions of injured (L) and control (R) cervical sympathetic trunks from adult rats. * Because satisfactory sections were not available for the control nerve from rat no. 800, the injured nerve from rat no. 800 was compared with the control nerve from rat no. 799.)

		Axonal diameters							
		Median			Mean of the 20 largest axons			Proportion > 0·90 μm	
Months after injury	Animal number	L	R	L × 100 / R (%)	L	R	L × 100 / R (%)	L (%)	R (%)
		6–8 mm distal to crush							
1	506	0·35	0·66	53	0·78	1·40	56	0·1	17·9
	507	0·33	0·72	46	0·88	1·44	61	0·7	23·1
3	543	0·33	0·73	45	1·08	1·50	72	3·5	28·7
	544	0·37	0·69	54	1·30	1·29	100	5·9	18·3
6	628	0·31	0·64	48	1·15	1·24	93	6·1	11·1
	629	0·40	0·68	59	1·37	1·43	96	12·0	19·8
		6–8 mm proximal to crush							
1	799	0·44	0·70	63	1·08	1·38	78	3·0	22·9
	800	0·44	—	63*	0·96	—	70*	1·0	—
3	543	0·35	0·64	55	1·00	1·20	83	2·0	12·4
	544	0·29	0·58	50	1·20	1·14	105	3·5	7·7
6	628	0·37	0·66	56	1·37	1·31	105	9·5	15·4
	629	0·42	0·80	53	1·20	1·56	77	5·5	38·0

(*b*) *At the level of injury*

Ultrastructural changes in Schwann cells and axons were basically similar at the level of the crush injury and 6–8 mm distal to this level. However, adjacent to the level of injury, the perineurium was thickened and occasional unmyelinated fibres were segregated into small fascicles. Quantitative studies (Table 3) demonstrated that changes at the level of injury paralleled those which occurred more distally in the cervical sympathetic trunk. However, the increase in the number of axons per whole transverse section and the mean number of axons per Schwann cell unit was less than in the distal part of the nerve; compared with control nerves there were twice as many axons at the level of injury and four times as many 6–8 mm distal to the level of crush.

(*c*) *Proximal to crush injury*

Six to eight mm proximal to the level of crush injury, median axonal diameters were reduced to less than 0·50 μm by 1 month after injury (Table 3). Axonal diameters appeared to be affected uniformly and groups of axonal sprouts were not observed (Fig. 4*c*). In addition the total number of unmyelinated axons and the ratio of axons per Schwann cell unit were not increased in the proximal nerve segments (Table 3).

Axon–Schwann cell units were even more abnormal in the proximal segments of

these unmyelinated nerves by 3 and 6 months after injury; each unit contained one or more axons with normal diameters as well as several small axons (Fig. 4*d*). Although median axonal diameters remained small at both 3 and 6 months after injury there was a gradual increase in the number of axons with diameters which were approximately normal. Mean diameters of the 20 largest axons from these proximal nerve segments increased to approximately 90% of normal and the proportion of axons with diameters greater than 0·90 μm increased to about 10% of the total (as compared with normal 10–20%) by 6 months after injury (Table 3).

DISCUSSION

During regeneration of unmyelinated nerve fibres, four stages can be recognized: axonal sprouting, longitudinal growth, loss of redundant sprouts, and axonal maturation. In addition, important retrograde axonal changes also occur.

Axonal sprouting begins during the first 2 days after axonal interruption (Dyck & Hopkins, 1972; Bray *et al.* 1972; Aguayo *et al.* 1973) and leads to increased numbers of small axons distal to the level of injury. Two weeks after injury there is a sixfold increase in the number of axons in distal segments of crushed cervical sympathetic trunks (Bray *et al.* 1973). The present study indicates that, 1 month after crush injury, there is still a fourfold increase in the axonal population distal to the level of crush. Because the axonal population was only twice normal at the level of injury in the same nerves, and similar to normal 6–8 mm proximal to injury, it must be assumed that repeated branching occurs beyond the level of injury as in myelinated fibres.

Longitudinal growth of regenerating unmyelinated axons was previously demonstrated by anatomic methods (Evans & Murray, 1954), by the recovery of functions such as the response of cat nictitating membranes (Murray & Thompson, 1957) and intragastric pressures (Evans & Murray, 1954) to direct nerve stimulation, and by the ability of regenerating rat cervical sympathetic trunks to propagate compound action potentials (Hopkins, 1970; Hopkins & Lambert, 1972). These studies have indicated average regeneration rates of 1–2 mm per day in unmyelinated nerve fibres. Similar rates of regeneration have also been determined for myelinated fibres (Gutmann, Guttman, Medawar & Young, 1942).

Loss of redundant axonal sprouts in cervical sympathetic trunks regenerating after crush injury has been documented quantitatively in the present study. Total populations of unmyelinated axons, as well as ratios of axons per Schwann cell unit, declined from values 4 times normal 1 month after injury to values within the normal range 6 months after injury. This loss is presumably due to atrophy and resorption of axons which have failed to make terminal connexions; loss of unmyelinated axons has also been observed during normal development of rat cervical sympathetic trunks (Aguayo, Terry & Bray, 1973).

Axonal maturation is the process leading to restoration of normal diameters in regenerating nerves (Guth, 1956). Previous studies have indicated that myelinated fibres regenerating after crush injury eventually achieve normal diameters. Mean axonal diameters and proportions of large fibres were normal in distal segments of rabbit peroneal nerves by 250 days after injury and proximal segments of the same

nerves were normal by 200 days (Gutmann & Sanders, 1943). Studying the same nerve, Cragg & Thomas (1964) demonstrated that mean diameters of the 20 largest myelinated fibres were 90% of normal both distal and proximal to the level of crush 1 year after injury. These measurements of axonal maturation in myelinated fibres can be compared with similar measurements for regenerating unmyelinated nerve fibres from the present study (Table 3). Although mean diameters of the 20 largest fibres achieved similar relative values for myelinated and unmyelinated fibres, other parameters of axonal regeneration, such as median axonal diameters and the proportion of axons with diameters above the 80th percentile for control nerves, were much lower for the regenerating unmyelinated fibres. Thus, although shorter regeneration times were examined in the present investigation, it appears likely that maturation of axonal diameters is less rapid in rat cervical sympathetic trunks regenerating after crush injury.

Median diameters of regenerating axons in rat cervical sympathetic trunks remained below normal because of the persistence of numerous axons with exceedingly small diameters. Excessive numbers of small unmyelinated axons have also been observed in cutaneous nerves 20 weeks after injury (Orgel, Aguayo & Williams, 1972). In regenerating cervical sympathetic trunks this population of small axons could account for the persistent dispersal of the compound action potential observed by Hopkins & Lambert (1972) On the other hand, the population of larger axons, although proportionately small, must be responsible for the normal conduction velocity of the first peak of the compound action potential by two months after crush injury.

It could not be determined in the present study why so many axons in the cervical sympathetic trunk remain small after crush injury. Although axonal maturation failed to occur in both myelinated (Aitken, 1949) and unmyelinated fibres (Aguayo, Peyronnard & Bray, 1973) when longitudinal growth and the formation of terminal connexions were prevented, longitudinal growth was not deliberately impeded in the present study. Thus, it is unlikely that all axons with small diameters represent regenerating sprouts which have failed to make terminal connexions. Another possible explanation for the persistence of small axons in regenerating cervical sympathetic trunks after 6 months is that longer intervals are required for maturation of these unmyelinated fibres. Certainly in myelinated fibres a greater degree of axonal maturation was observed one year after injury (Gutmann & Sanders, 1943; Cragg & Thomas, 1964). It is possible, therefore, that a larger proportion of regenerating unmyelinated nerve fibres would achieve normal diameters if studied after similar intervals.

Retrograde changes. Persistence of axons with small diameters proximal to crush injury also appears to be characteristic of regenerating unmyelinated nerve fibres. The present study has demonstrated that median axonal diameters in the proximal segments of rat cervical sympathetic trunks were approximately 60% of normal 1 month after crush injury. The reduction of axonal diameters at this proximal level was probably due to retrograde atrophy rather than axonal sprouting because increased axon–Schwann cell unit ratios, characteristic of regenerative sprouting (Aguayo, Peyronnard, Martin & Bray, 1973), were not observed 1 month after injury. Furthermore, there was no indication at the level of crush that significant numbers of these small axons could represent axons regenerating in the wrong direction. Median axonal diameters remained small 3 and 6 months after injury and although

the proportion of large unmyelinated axons had increased, there were also increased numbers of small axons. In myelinated nerves, on the other hand, definite retrograde atrophy follows crush injury, but axonal diameters eventually return to normal in the proximal nerve segments (Gutmann & Sanders, 1943; Cragg & Thomas, 1961; Aitken & Thomas, 1962; Cragg & Thomas, 1964).

Retrograde degeneration of axons and their cell bodies may be a significant factor contributing to the limited axonal maturation which occurs in regenerating cervical sympathetic trunks. Lewis, Jones, Breathnach & Navaratnam (1972) have demonstrated that, following axonal interruption, retrograde neuronal degeneration is more extensive in autonomic nerves than in somatic nerves. Therefore, in the present experiments, it is possible that some neurons in the intermedio-lateral cell columns of the spinal cord were affected by irreversible retrograde changes leading to a reduced number of neurons and proximal axons being available for regenerative axonal sprouting. However, the total number of axons distal to the level of injury was approximately normal by 6 months after crush, so that, if neurons had been lost, each surviving neuron must have been supporting an increased number of axonal branches. Conceivably there is a critical limit to the axonal volume or membrane surface area which can be sustained by each remaining neuron, and if so the increased number of axonal branches per neuron must cause this limit to be reached at axonal diameters less than normal. Thus one may speculate that unmyelinated axons in regenerating cervical sympathetic trunks remain small because the metabolic capacity of their nerve cell bodies to support larger axonal volumes or surface areas is exceeded. But it must be stressed that no direct evidence for these speculations has been presented, and in any case, the course of axonal maturation was not followed beyond 6 months.

SUMMARY

Cervical sympathetic trunks from adult rats were studied by quantitative ultrastructural techniques at intervals between 1 and 6 months after crush injury. *Distal* to the injury axonal sprouts, previously demonstrated to develop shortly after injury, progressively decreased in number. Although median axonal diameters remained at approximately one-half of normal throughout the 6 month period studied, there was a gradual increase in the proportion of axons with larger diameters. *Proximal* to the level of injury median axonal diameters were reduced 1 month after injury, presumably because of retrograde atrophy. The proportion of proximal axons with larger diameters was also increased 3 and 6 months after injury.

Thus during regeneration of crushed unmyelinated nerve fibres there was a gradual decline in the number of axonal sprouts distal to the level of injury, retrograde atrophy, and eventual maturation of a proportion of axons both proximal and distal to the level of injury. However, a large population of axons with abnormally small diameters persisted for at least 6 months.

The technical assistance of M. Attiwell, N. Browarska, W. Downey, S. Harrington, A. Liberman and J. Trecarten is acknowledged with gratitude. This project was supported by grants from the Medical Research Council and the Muscular Dystrophy Association of Canada.

REFERENCES

AGUAYO, A. J., MARTIN, J. B. & BRAY, G. M. (1972). Effects of nerve growth factor antiserum on peripheral unmyelinated nerve fibers. *Acta Neuropathologica* **20**, 288–298.

AGUAYO, A. J., PEYRONNARD, J. M. & BRAY, G. M. (1973). A quantitative ultrastructural study of regeneration from isolated proximal stumps of transected unmyelinated nerves. *Journal of Neuropathology and Experimental Neurology* **32**, 256–270.

AGUAYO, A. J., PEYRONNARD, J. M., MARTIN, J. B. & BRAY, G. M. (1973). Responses of unmyelinated nerves to axonal transection and neuronal loss. In *New Developments in Electromyography and Clinical Neurophysiology* (Ed. J. Desmedt), **2**, pp. 166–173. Basel: Karger.

AGUAYO, A. J., TERRY, L. C. & BRAY, G. M. (1973). Spontaneous loss of axons in sympathetic unmyelinated nerve fibers of the rat during development. *Brain Research* **54**, 360–364.

AGUAYO, A. J. & BRAY, G. M. (1974). Experimental pathology of unmyelinated nerve fibers. In *Peripheral Neuropathy* (Ed. P. J. Dyck, P. K. Thomas and E. H. Lambert). Philadelphia: W. B. Saunders.

AITKEN, J. T. (1949). The effects of peripheral connections on the maturation of regenerating nerve fibers. *Journal of Anatomy* **83**, 32–43.

AITKEN, J. T. & THOMAS, P. K. (1962). Retrograde changes in fiber size following nerve section. *Journal of Anatomy* **96**, 121–129.

BRAY, G. M., AGUAYO, A. J. & MARTIN, J. B. (1973). Immunosympathectomy: late effects on the composition of rat cervical sympathetic trunks and influence on axonal regeneration after crush. *Acta Neuropathologica* **26**, 345–354.

BRAY, G. M., PEYRONNARD, J. M. & AGUAYO, A. J. (1972). Reactions of unmyelinated nerve fibers to injury – An ultrastructural study. *Brain Research* **42**, 297–309.

CRAGG, B. G. & THOMAS, P. K. (1961). Changes in conduction velocity and fibre size proximal to peripheral nerve lesions. *Journal of Physiology* **157**, 315–327.

CRAGG, B. G. & THOMAS, P. K. (1964). The conduction velocity of regenerated peripheral nerve fibres. *Journal of Physiology* **171**, 164–175.

DYCK, P. J. & HOPKINS, A. P. (1972). Electron microscopic observations on degeneration and regeneration of unmyelinated nerve fibres. *Brain* **95**, 223–234.

EVANS, D. H. L. & MURRAY, J. G. (1954). Regeneration of non-medullated nerve fibres. *Journal of Anatomy* **88**, 465–480.

GUTH, L. (1956). Regeneration in the mammalian peripheral nervous system. *Physiological Reviews* **36**, 441–478.

GUTMANN, E. & SANDERS, F. K. (1943). Recovery of fibre numbers and diameters in the regeneration of peripheral nerves. *Journal of Physiology* **101**, 489–518.

GUTMANN, E., GUTTMANN, L., MEDAWAR, P. B. & YOUNG, J. Z. (1942). The rate of regeneration of nerve. *Journal of Experimental Biology* **19**, 14–44.

HOPKINS, A. P. (1970). Conduction in regenerating unmyelinated fibres. *The Physiologist* **13**, 224.

HOPKINS, A. P. & LAMBERT, E. H. (1972). Conduction in regenerating unmyelinated fibres. *Brain* **95**, 213–222.

LEWIS, P. R., JONES, P. B., BREATHNACH, S. M. & NAVARATNAM, V. (1972). Regenerative capacity of visceral preganglionic neurones. *Nature New Biology* **236**, 181–182.

MATTHEWS, M. R. (1973). An ultrastructural study of axonal changes following constriction of postganglionic branches of the superior cervical ganglion in the rat. *Philosophical Transactions of the Royal Society* **264**, 479–508.

MURRAY, J. G. & THOMPSON, J. W. (1957). The occurrence and function of collateral sprouting in the sympathetic nervous system of the cat. *Journal of Physiology* **135**, 133–163.

ORGEL, M., AGUAYO, A. J. & WILLIAMS, H. B. (1972). Sensory nerve regeneration: an experimental study of skin grafts in the rabbit. *Journal of Anatomy* **111**, 121–135.