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THE OCCURRENCE AND FUNCTION OF COLLATERAL SPROUTING IN THE SYMPATHETIC NERVOUS SYSTEM OF THE CAT

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It is now established that surviving axons in incompletely denervated striated muscle extend their existing fields of innervation by a process of collateral sprouting, thereby accounting for the functional recovery which occurs (for review see Edds, 1953). A similar process also occurs in skin partly deprived of its sensory nerve supply (Weddell, Guttmann & Gutmann, 1941). Although the phenomenon has not been directly observed in the sympathetic nervous system, there is evidence of remarkable recovery of function after incomplete denervation (Simeone, Cannon & Rosenblueth, 1938; Geohegan & Aidar, 1942).

In the present work, partial denervation of the superior cervical ganglion in the cat was performed by severing rami communicantes from their respective thoracic nerves. Subsequently, sprouts arise within the ganglion from the remaining intact preganglionic fibres and form synapses with ganglion cells denervated by the preliminary operation. This spatial increment in the field of innervation of the remaining preganglionic fibres results in an almost complete functional recovery of the ganglion even when as many as 90% of the fibres have been divided. Experiments have been performed so as to correlate the histological and functional changes within the ganglion, including the time course of the sprouting process and its effect on the acetylcholine release, electrical and pharmacological responses. It seems most likely that under similar conditions post-ganglionic fibres also respond by collateral sprouting. The factors responsible for the efficiency of the sprouting process in the sympathetic nervous system are discussed. A preliminary account of this work has already been published (Murray & Thompson, 1956).

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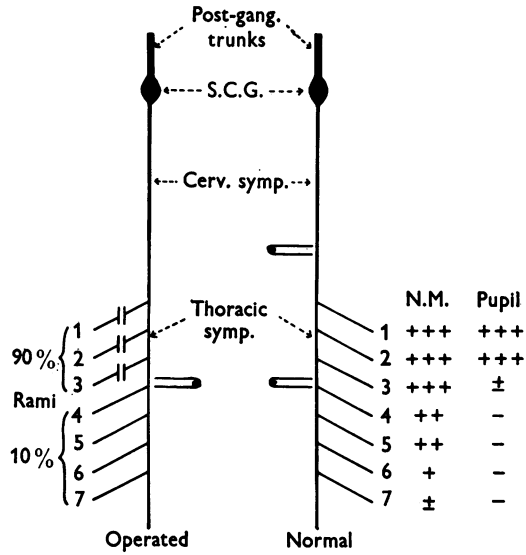
METHODS

Adult cats were used throughout the experiments. All preliminary operations were carried out with full aseptic precautions under pentobarbitone sodium anaesthesia (Nembutal, Abbott Laboratories, 40 mg/kg intraperitoneally). The chest was opened under artificial positive pressure respiration. The superior cervical ganglion on the right side was partially denervated by severing the first, second and third rami communicantes from their respective thoracic nerves (T1, T2 and T3) to the stellate ganglion and thoracic sympathetic chain. Both white and grey rami were divided, and in order to minimize the possibility of regeneration at these sites the divided ends were separated by at least a centimetre.



Text-fig. 1. Diagram of the experimental lesions of the nerves, levels at which they were stimulated, and the origins of some of the preganglionic fibres.

Subsequent experiments, performed at varying intervals after the preliminary operation, were carried out in most cases under ether followed by chloralose anaesthesia (80 mg/kg); the remainder, under pentobarbitone sodium (40 mg/kg), showed no significant difference when the results were compared. With the animal on artificial respiration the chest was opened and the thoracic sympathetic trunk divided caudad to T5 and the rami communicantes to T4 and T5 cut. The sympathetic chain below T3 was then mobilized and placed on electrodes so that all fibres contributing to the sympathetic chain by T4 and thoracic nerves caudad to that level were stimulated. Electrodes were placed at corresponding positions on the partially denervated and normal sides (Text-fig. 1); the nerves were immersed in liquid paraffin and precautions were taken to prevent stimulus spread. The cervical sympathetic trunks were later divided and the cranial ends stimulated. Platinum wire electrodes were used, carrying supramaximal square wave stimuli of 0·5 msec duration at frequencies from 1–100 c/s.

In some animals intermediate recordings were made between the preliminary operation and the final experiment. Under pentobarbitone sodium anaesthesia and with aseptic precautions, about 3 cm of cervical sympathetic trunk was dissected free on the operated and control sides. The cervical sympathetic trunks were stimulated in continuity, with sterile electrodes. The wounds were then sutured, and after varying survival periods the animals were killed. By this means

observations were made at different times in the same animal on the return of function of the partially denervated ganglion.

The responses of the superior cervical ganglia to electrical stimulation applied simultaneously to the preganglionic trunks on the two sides were studied. Kymograph recordings of the contractions of both nictitating membranes with levers at 6½ times magnification were obtained. By means of small springs attached to the levers any shortening of the nictitating membranes was made to act against the springs. Considerably better discrimination between small differences in contraction of the nictitating membranes was obtained by this method than by isotonic levers. Before each experiment the levers were calibrated and the springs adjusted so that equal tensions gave equal heights on the kymograph recordings on the two sides. It was found that by loading the nictitating membranes on the two sides with an initial 4·5–4·8 g tension, satisfactory responses were obtained. The stimulating circuits on the two sides were independent.

The size of the pupils on the normal and operated sides was measured before and after preganglionic nerve stimulation. Under standard bright light conditions the pupils on both sides took up a narrow elliptical form. Any change in the narrow diameter was measured by means of callipers.

Simultaneous tracings were made of the contractions of the nictitating membranes to a series of doses of different drugs injected into the femoral vein. Doses of adrenaline hydrochloride, noradrenaline hydrochloride, acetylcholine chloride, hexamethonium iodide, nicotine tartrate, tetramethyl-ammonium iodide and eserine sulphate are given in terms of these salts. In addition, to avoid systemic effects, some of the drugs were given by retrograde injection into the carotid artery, the method used being similar to that described by Paton & Perry (1953), with the exception that a self-sealing rubber and glass cannula was used in place of a remote control 'bulldog'.

Ganglion action potentials were recorded in response to stimulation of the preganglionic fibres in the chest and in the neck with single maximal volleys on the normal and operated sides. The exposure of the ganglion and the method of recording were similar to those described by Paton & Perry (1953), the only major difference being the position of the ganglionic electrode; this was placed round the middle of the body of the ganglion instead of the upper pole since this was found to diminish the stimulus artifact.

Perfusion of normal and partially denervated ganglia was performed by the technique described by Kibjakow (1933), Feldberg & Gaddum (1934) and F. C. MacIntosh (unpublished), using Locke's solution containing 2×10^{-6} eserine. The ACh in the perfusate was assayed on the blood pressure of an eviscerated eserinized cat or on the prostigminized, morphinized ileum of the guinea-pig (W. D. M. Paton, personal communication).

After completion of the functional tests the superior cervical ganglia on the normal and operated sides, together with lengths of cervical trunks, were removed for histological examination. One part of each cervical trunk was stained by the pyridine-silver method described by Ranson & Davenport (1931) which stains all axons, both myelinated and unmyelinated. The other part was stained by the Weigert technique which stains myelinated fibres only. Counts of the total number of axons present in pyridine silver sections were made with a binocular microscope at a magnification of 1200 diameters. A graticule was placed in one eyepiece and the counts made section by section until the whole area of the nerve was counted. For study of myelinated fibres, the Weigert sections were photographed at a magnification of 750 directly on bromide paper and the fibres counted and their outside diameters measured. Details of the above methods of staining and counting are described by Evans & Murray (1954). The ganglia were fixed in a solution containing 10% formalin and 2% pyridine for a minimum of 14 days. Each specimen was cut into as many sections of 30 μ as possible. All suitable sections were stained by the Bielschowsky silver impregnation technique (Pl. 1, fig. 1).

RESULTS

Number, size, function and origin of preganglionic fibres to the superior cervical ganglion

The total number of axons remaining in the cervical sympathetic trunks of three cats, after the first three rami had been divided and 20, 49 and 50 days allowed for fibres to degenerate, were 1179, 1448 and 1377. Of these 635, 582 and 542 were myelinated fibres, practically all about 2μ or less in diameter; the remainder were unmyelinated. Foley & DuBois (1940) have shown that the total number of axons in the normal cervical sympathetic trunk of the cat is about 7000 (7407 ± 1250 (8)) of which almost 50% are myelinated (3690 ± 846 (8)) and the rest unmyelinated. The sizes of the myelinated fibres are approximately as follows: 5% about 6μ in diameter, 50% about 4μ and the remainder about 2μ or less (De Castro, 1951). The results suggest that the first three rami contribute about 80–90% of the fibres in the cervical sympathetic trunk and the remaining rami from T4, 5, 6, and 7 (Langley, 1900; De Castro, 1951) contribute only about 10–20% of the total. It is clear that the large fibres are contained in the first three rami. In none of the specimens counted was there a significant vagal or aortic nerve fascicle within the sympathetic trunk (Foley, 1945; Agostoni, Chinnock, Daly & Murray, 1957) and the discrete fascicles from the superior cervical ganglion (Foley, 1945) were excluded from the counts. However, post-ganglionic axons, predominantly unmyelinated, some projecting caudally from the superior cervical ganglion, others passing cranially, form about 10% of the total number of fibres in the normal cervical sympathetic trunk (Foley, 1945) and cannot be distinguished in the present counts from preganglionic fibres. This fact would tend to lower the estimated number of preganglionic fibres contributed by the rami caudad to T3 and suggests that the true figure is about 10% or slightly less of the total number of preganglionic fibres.

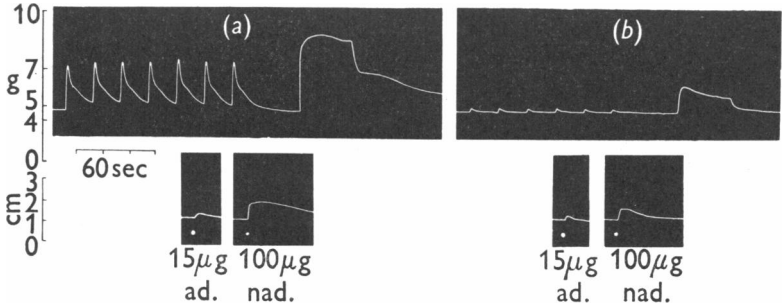
By using different thresholds of stimulation the findings of Bishop & Heinbecker (1932) were confirmed. They showed that the larger fibres in the cervical sympathetic activate ganglion cells which are distributed to the nictitating membrane and pupil, whereas the smaller fibres are concerned with vasomotor and pilomotor functions as well. It is known that preganglionic fibres of these size groupings are normally in functional relationship with their own groups of ganglion cells (Eccles, 1935).

By stimulating individual rami in the chest and recording the magnitude of the nictitating membrane contractions, it was shown that the great majority of preganglionic fibres for the nictitating membrane arise from T1, T2 and T3, about equally from each, to a much less extent from T4 and T5 and very few from T6 and T7 (Text-fig. 1). Indeed, stimulation of T1, T2, or T3 individually gave a greater response than T4, T5, T6 and T7 together. The pupillo-dilator fibres arise mainly from T1 and T2 and to a much less extent

from T3. In no case was there dilatation of the pupil on stimulation caudad to T3. No significant difference in the distribution or function of preganglionic fibres from the thoracic nerves was found on the right as compared with the left side. These results agree largely with the findings of Langley (1900) and De Castro (1951).

Responses of the partially denervated ganglion

The results in this section were obtained in the late stages, 4 weeks or longer after partial denervation of the superior cervical ganglion on one side by section of the first three rami communicantes. Text-fig. 2 shows the responses of the nictitating membranes (*a*) on the right side, on which the rami had been divided 49 days previously, and (*b*) on the left (control) side. Approximately



Text-fig. 2. The responses of the nictitating membranes on (*a*) the operated side, on which rami T1–3 had been divided 49 days previously, and (*b*) the normal side. T4–7 on both sides stimulated simultaneously. The contractions of the nictitating membranes on the two sides to I.V. adrenaline and noradrenaline are also shown. [In all kymograph recordings, the smaller responses are due to 4 maximal stimuli at 1 c/s repeated every min and the larger at 10 c/s continuously for 1 or 2 min. Throughout the recordings, a scale in g of the tensions exerted by the nictitating membranes on the two sides is given. A scale in cm is also given to indicate the magnification of the responses. Time scale throughout 60 sec.]

equal numbers of preganglionic fibres were stimulated with electrodes placed at corresponding positions on the thoracic trunks just caudad to T3 on the two sides (Text-fig. 1). By this means the preganglionic fibres from T4–7, or about 10% of the total number of fibres to the cervical trunks, were stimulated on the two sides. The responses of the nictitating membrane on the partially denervated side were several times greater than those on the normal side.

The increased responses are not due to a peripheral sensitization of the effector organ on the operated side, since the simultaneously recorded contractions of the nictitating membranes on the two sides to I.V. adrenaline or noradrenaline gave practically equal results (Text-fig. 2). The comparison was made at various dose levels and parallel and coincidental dose-response curves were obtained in the majority of cases. Stimulation of the post-ganglionic trunks on the two sides gave practically equal contractions of the nictitating membranes.

It may be concluded that some mechanism within the superior cervical ganglion itself is responsible for the increased responses. This agrees with the findings of Simeone *et al.* (1938).

An important indication of the mechanism responsible for the increased effects of the partially denervated ganglion was obtained when it was observed that the pupil dilated fully on stimulation of the operated side caudad to T3. In contrast, there was no dilatation of the pupil on similar stimulation on the control side, since the pupillo-dilator fibres normally arise from T1, T2 and to a less extent T3. This indicates that the remaining intact preganglionic fibres on the partially denervated side now activate post-synaptic elements with which they are not normally in contact.

There are several possible means which might interact one with another whereby such a process could occur. It may be that the ganglion cells within the superior cervical ganglion have become hypersensitive to ACh released within the ganglion. In the late stages this is not so. The simultaneously recorded contractions of the nictitating membranes on the operated and normal sides show practically equal responses to a series of small I.V. doses of nicotine (0·01–0·1 mg/kg) or TMA (0·01–0·1 mg/kg). Furthermore, intra-arterial injections of ACh, nicotine, or TMA into the carotid arteries on the normal and partially denervated sides produce practically equal contractions of the nictitating membranes on the two sides. The doses of ACh injected I.A. included those at the lower end of the dose response curve (1–10μg).

A second possible explanation would be that the ACh released by stimulation of 10% of preganglionic fibres is more effective within a partially denervated ganglion than in a normal ganglion. It might be that the ACh diffuses more readily or is less effectively broken down. Thirdly, it might be that the remaining intact preganglionic fibres on the partially denervated side have increased their field of innervation anatomically by means of collateral sprouting. The experiments to be described throw light on these possibilities.

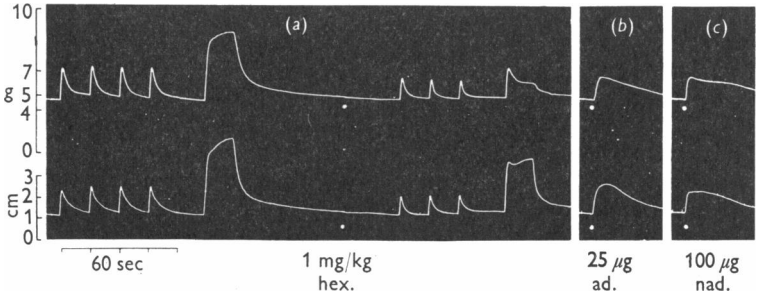
Time course of return of function of the partially denervated ganglion

In order to assess the degree of return of function of the ganglion at varying times after section of the first three rami, the responses on stimulation of the thoracic trunk caudad to T3 on the operated side were compared with those on stimulation of the whole of the cervical sympathetic on the normal side (Text-fig. 1). The results obtained could be grouped into early, intermediate and late stages after denervation.

Text-fig. 3 shows the responses of the nictitating membrane on the operated side 28 days after division of the first three rami and on the normal side. They are practically identical, although the number of preganglionic fibres stimulated on the operated side is only about 10% of that on the normal side. There was no evidence of hypersensitivity of the nictitating membrane on the partially

denervated side to I.V. adrenaline or noradrenaline. Stimulation of the post-ganglionic trunks on the two sides gave practically equal results. The pupil on the operated side dilated as fully as on the normal side and showed no fatigue on continuous stimulation at 10 c/s. Similar results were obtained in all the animals tested in the late stages at 28, 38, 40, 49, 50 and 130 days respectively after section of the rami. They indicate that at 4 weeks or later after operation there is a complete return of function.

In contrast to the results in the late stages, a typical example of the responses of the nictitating membrane in the early stages (4–5 days) is shown in Text-fig. 4. The size of the contractions on the partially denervated side, although slightly greater than those on stimulation of T4–7 on the normal side, is still appreciably less than on stimulation of the whole of the cervical sympathetic

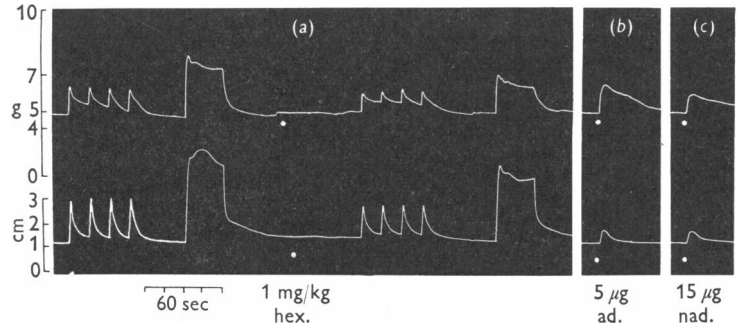


Text-fig. 3. Simultaneously recorded responses of nictitating membranes: upper, operated side (rami T1–3 divided 28 days previously); lower, normal side. (*a*) T4–7 on operated side and whole of cervical sympathetic trunk on normal side stimulated—effect of hexamethonium 1 mg/kg I.V.; responses to I.V. adrenaline (*b*) and noradrenaline (*c*).

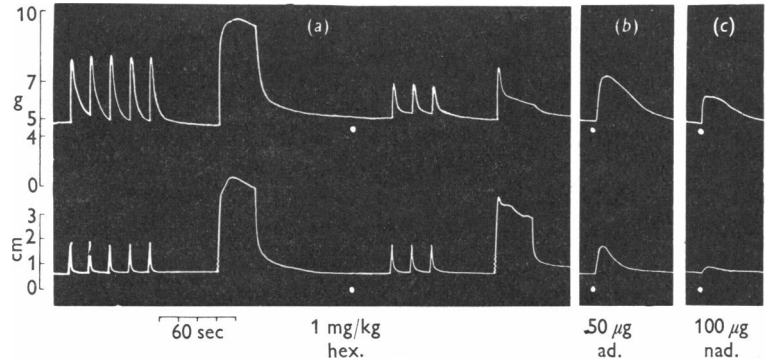
on the normal side. There is at this stage some increased sensitivity of the nictitating membrane on the operated side to adrenaline and noradrenaline (Text-fig. 4). In none of the animals at this early stage was there dilatation of the pupil on stimulation of the operated side. The results were obtained in one animal at 4 days and in three at 5 days after operation. In two of the animals the cervical sympathetic trunk was stimulated in continuity under sterile conditions on the operated and normal sides. After completion of the functional tests, the operation site was resutured and final experiments performed 28 and 49 days later. This permitted observations to be made in the same animals at the early and late stages.

Between the contrasting results of the early and late stages, there was an intermediate group occurring at 10, 14, 15, 18 and 20 days after partial denervation. Text-fig. 5 shows the response of the nictitating membrane at 18 days after operation. The contractions of the nictitating membrane were considerably larger than those on the normal side, despite the fact that only 10% of the number of preganglionic fibres were stimulated on the operated

side as compared with the normal side. The increase in size of contractions was particularly marked at the slower rate of stimulation. At this intermediate stage there was a marked hypersensitivity of the nictitating membrane to I.V. adrenaline or noradrenaline. Furthermore, stimulation of the post-ganglionic trunk on the operated side gave increased responses of the nictitating membrane, especially at the slower rate of stimulation, as compared with the normal



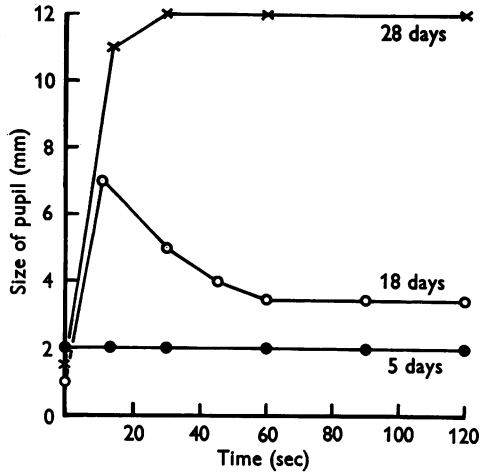
Text-fig. 4. Simultaneously recorded responses of nictitating membranes: upper, operated side (rami T1–3 divided 5 days previously); lower, normal side. (*a*) T4–7 on operated side and whole of cervical sympathetic trunk on normal side stimulated—effect of hexamethonium 1 mg/kg I.V.; responses to I.V. adrenaline (*b*), and noradrenaline (*c*).



Text-fig. 5. Simultaneously recorded responses of nictitating membranes: upper, operated side (rami T1–3 divided 18 days previously); lower, normal side. (*a*) T4–7 on operated side and whole of cervical sympathetic trunk on normal side stimulated; effect of hexamethonium 1 mg/kg I.V.; responses to I.V. adrenaline (*b*), and noradrenaline (*c*).

side. Another distinctive feature of the intermediate stage was partial dilatation of the pupil on stimulation of T4–7 on the operated side. At 10 days after operation the pupil dilated from a resting diameter of 2 to 4 mm, whereas at 18 days the pupil dilated from 1 to 7 mm. In both cases the response showed fatigue, the size decreasing fairly rapidly after 10–20 sec on continuous stimulation at 10 c/s for 2 min (Text-fig. 6).

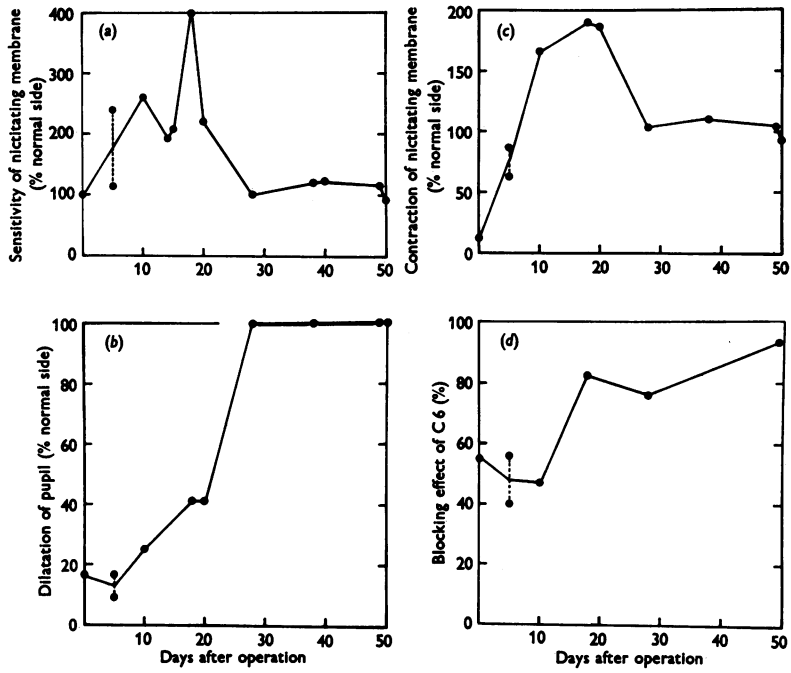
Text-fig. 7 *a* shows the degree of increased sensitivity of the nictitating membrane to 100μg of noradrenaline I.V. by expressing the size of the contraction on the operated side as a percentage of the simultaneously recorded response on the normal side, at varying times after partial denervation. In the early stages there is some degree of hypersensitivity of the nictitating membrane on the operated side. This hypersensitivity reaches a maximum within the third week after operation, but by 4 weeks or later has almost disappeared.



Text-fig. 6. Size of pupil at different times after operation on continuous stimulation of T4–7 at 10 c/s; rami T1–3 divided 5, 18 and 28 days previously.

Text-fig. 6 gives typical examples of dilatation of the pupil on stimulation of T4–7 on the operated side at early, intermediate and late stages. Text-fig. 7 *b* shows the time course of increase in dilatation of the pupil on stimulating the operated side. This pupillary response is a convenient measure of the process whereby the remaining preganglionic fibres progressively activate ganglion cells with which they previously did not form synapses. It is difficult to see how a time course such as this could result from a more 'efficient' diffusion of ACh within the ganglion resulting from partial denervation. Nor is it likely that a lowered level of cholinesterase plays a significant part in the eventual return of function of a partially denervated ganglion. Sawyer & Hollinshead (1945) and Koelle (1950) have shown that the activity of the true or specific cholinesterase in the superior cervical ganglion of the cat falls maximally within 10 days of a lesion of the preganglionic trunk. Text-fig. 7 *c* shows that there is an increase with time in the magnitude of contraction of the nictitating membrane on the operated side on stimulation of T4–7. In the late stages, the responses on stimulation of the remaining 10% of preganglionic fibres equal those on stimulation of the whole of the cervical trunk on the normal side. The

distinctive feature, however, is that at the intermediate stage the responses on the operated side are much greater than those on the normal side.



Text-fig. 7. Time course of recovery of function after section of rami T1–3; (*a*) the degree of sensitivity of the nictitating membrane to noradrenaline 100 μg I.V. expressed as the size of the contraction on the operated side as % of that on the normal; (*b*) the size of the pupil on the operated side 30 sec after beginning continuous stimulation of T4–7 at 10 c/s expressed as % of that on the normal side on stimulation of the whole of the cervical trunk; (*c*) the size of the contraction of the nictitating membrane on the operated side on stimulation of T4–7 at 1 c/s for 4 sec expressed as % of that on stimulation of the whole of the cervical trunk on the normal side; (*d*) the relative efficiency of the blocking effect of hexamethonium by plotting $100 - X$, where

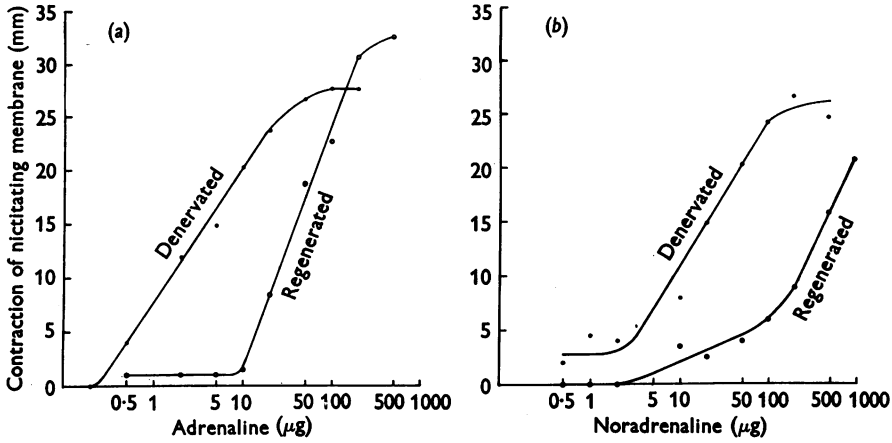
$$X = \frac{\text{height of nictitating membrane contraction after } C^6}{\text{height of nictitating membrane contraction before } C^6} \times 100.$$

The height of the nictitating membrane on the operated side was measured 1 min after beginning continuous stimulation of T4–7 at 10 c/s and on the normal side on stimulation of the whole of the cervical trunk (points joined by broken line represent results obtained from two different experiments).

Hypersensitivity of smooth muscle and ganglion cells

Simeone (1937) has shown that with re-innervation of the superior cervical ganglion after a lesion of the cervical sympathetic trunk in the cat, there is a concomitant disappearance of the hypersensitivity of the nictitating membrane on that side. In order to find out whether hypersensitivity of the denervated nictitating membrane persists if re-innervation is prevented, the following experiment was performed. On the right side, the cervical sympathetic trunk

was divided, but the two ends were held in close apposition by means of clotted plasma (Seddon & Medawar, 1942). On the left side, the nictitating membrane was denervated as completely as possible by excising the stellate and superior cervical ganglia. The nodose ganglion and a length of vagus were also removed since sympathetic fibres may travel by that route (Agostoni *et al.* 1957). Stimulation of the cervical sympathetic trunks showed that regeneration was complete on the right side, but there was none on the left. Text-fig. 8 gives the dose-response curves of the nictitating membranes to I.V. adrenaline



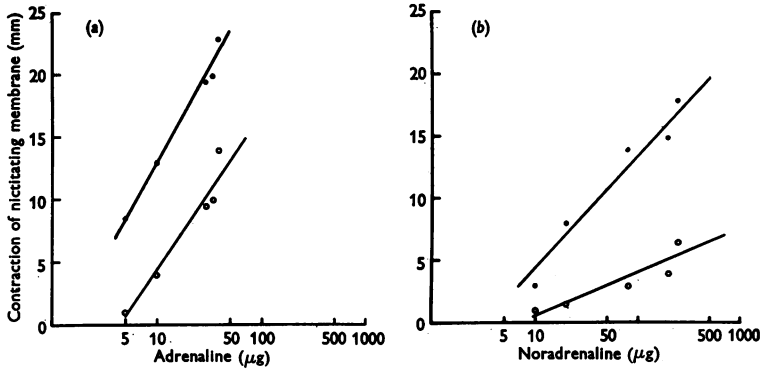
Text-fig. 8. Dose-response curves of contractions of nictitating membranes to I.V. doses of (*a*) adrenaline, and (*b*) noradrenaline. Seventy days previously, right nictitating membrane completely denervated, ●; and left preganglionic trunk divided, but allowed to regenerate, ○.

and noradrenaline 70 days after operation. Comparison with dose-response curves from other animals confirms that hypersensitivity of the nictitating membrane disappears on re-innervation of the ganglion, but remains, apparently indefinitely, if re-innervation is prevented. In our experiments there was no demonstrable diminution in hypersensitivity of the nictitating membrane even 290 days after complete denervation.

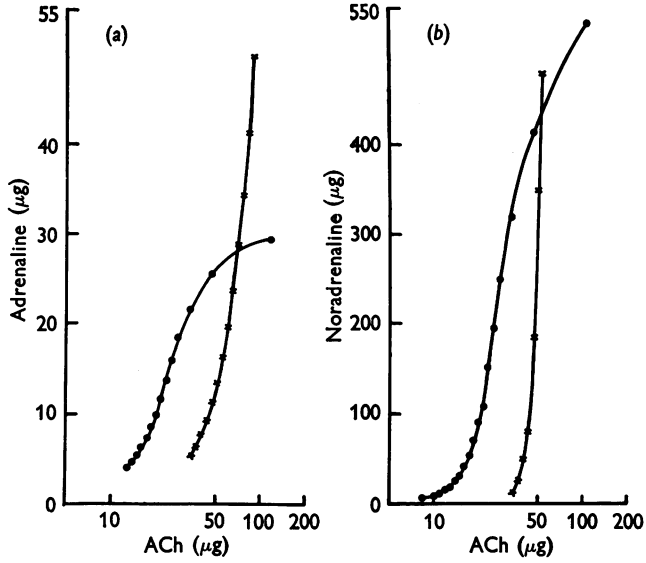
The disappearance of hypersensitivity of the nictitating membrane in the late stages after partial denervation is not due to the outgrowth of axons from the divided rami T1–3. Comparison of the numbers of fibres in the cervical trunk 10 cm from the divided rami 20 days after section, i.e. before axon tips could have grown as far as that (Butson, 1950) and at 49 and 50 days after section, indicates that there is little or no regeneration of axons from the divided rami to the superior cervical ganglion within the times tested.

Although there is no evidence of hypersensitivity of ganglion cells in the late stages after partial denervation, this is not so at the intermediate stage. Text-fig. 9 shows the dose-response curves to I.V. adrenaline and noradrenaline of the nictitating membranes in a cat in which the first three rami on the right

side had been divided 15 days previously. In the same animal, dose-response curves of the nictitating membranes to I.A. ACh acting on the right and left ganglia were also obtained. From these two sets of data, the doses of I.V. adrenaline or noradrenaline and I.A. ACh which gave equal contractions of the nictitating membranes on the normal and operated sides were plotted (Text-fig. 10). They show that when correction has been made for peripheral hypersensitivity, the ganglion cells on the operated side require less ACh than those



Text-fig. 9. Dose-response curves of contractions of nictitating membranes to I.V. doses of (*a*) adrenaline and (*b*) noradrenaline: rami T 1–3 divided 15 days previously on right side (●); left side control (○).

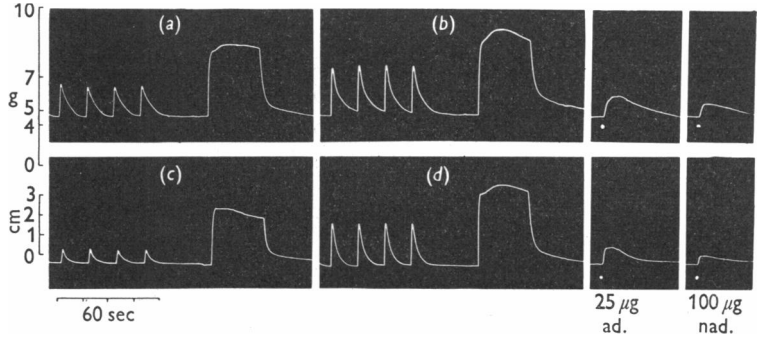


Text-fig. 10. Graphs showing the doses of I.A. ACh and I.V. adrenaline (*a*) and noradrenaline (*b*) required to produce equal contractions of the nictitating membrane: same animal as in Fig. 9; right side, T 1–3 cut 15 days previously (●), left side control (×).

on the normal side for a given effect. However, we were unable to demonstrate any hypersensitivity of the ganglion cells on the operated side to I.A. TMA. Eserine, 25μg I.A. on the partially denervated side, further increased the sensitivity of the ganglion cells to I.A. ACh but not to TMA. It may be that a lowered cholinesterase level at the intermediate stage plays a part in the hypersensitivity of ganglion cells to ACh.

Effect of varying the degree of denervation of the ganglion

In three animals, only the first and second rami communicantes were divided. 42, 53 and 55 days later experiments similar to those after division of T1–3 were carried out. The number of fibres remaining in the cervical trunk on the operated side of one animal was 3147. Of these, 1248 were myelinated, a considerable number being about 4μ in diameter and a few as large as 6μ. Thus about 40% of the preganglionic fibres to the superior cervical ganglion remained after division of T1 and T2 and some of these were of the larger myelinated type.



Text-fig. 11. Responses of nictitating membranes: upper, operated side (rami T1–2 divided 55 days previously), lower, normal side. (*a*) Thoracic sympathetic trunk caudad to T3, and (*b*) caudad to T2 stimulated; (*c*) trunk caudad to T2, and (*d*) whole cervical trunk stimulated: responses to I.V. adrenaline and noradrenaline also shown.

Text-fig. 11 gives the results 55 days after section of T1 and T2. Stimulation of the thoracic trunk caudad to T3 on the operated side, i.e. 10% of preganglionic fibres, gave smaller responses than stimulation caudad to T2, i.e. 40% of preganglionic fibres. The latter gave almost identical results when compared with stimulation of the whole of the cervical trunk on the normal side. Thus the responses to stimulation of 10% of the fibres is greater after 90% (T1–3) of the total number have been divided than after 60% (T1–2). There was no demonstrable hypersensitivity of the nictitating membrane or ganglion cells on the operated side. In all animals stimulation caudad to T2 on the operated side produced full dilatation of the pupil as compared with stimulation of the cervical trunk on the normal side. In one animal full

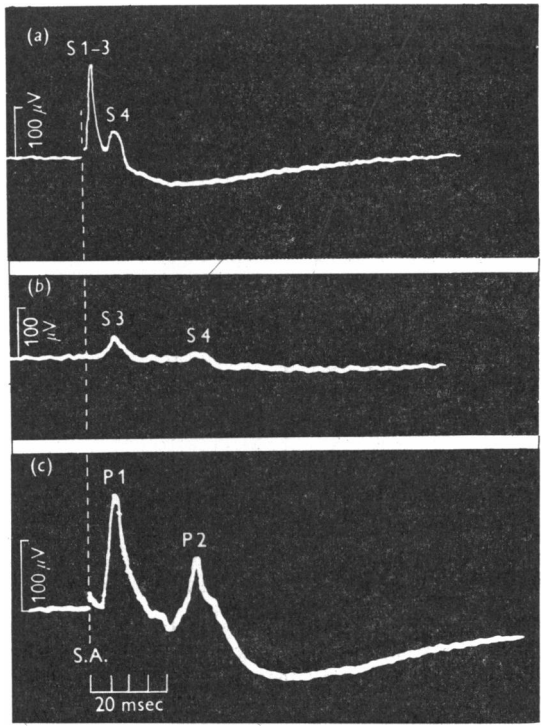
dilatation of the pupil was obtained on stimulation caudad to T3 on the operated side, but in the other two there was only partial dilatation. In none of these instances was there fatigue of the dilated pupil on continuous stimulation for 2 min at 10 c/s.

The results indicate that there is a relatively greater improvement in return of function the greater the degree of original denervation, and is in keeping with the results in partially denervated muscle (Van Harreveld, 1945; Weiss & Edds, 1946). However, there appears to be a limit to the process. In one animal, T1–5 were divided on one side. Fifty-five days later functional tests were carried out and the number and size of fibres remaining in the cervical trunk on the operated side counted. The number of axons was 788, of which only 82 were myelinated, all 2μ or less in diameter. These counts suggest that the number of preganglionic fibres arising from T6 and T7 is probably about 1% of the total number of preganglionic fibres in a normal cervical trunk. Stimulation of the operated side caudad to T5 produced considerably greater effects than similar stimulation on the normal side, but appreciably smaller responses than stimulation of the whole of the normal cervical trunk. In addition, there remained a marked hypersensitivity of the nictitating membrane on the operated side to I.V. adrenaline and noradrenaline. The pupil on the operated side dilated from 2 to 7 mm at 5 sec after the beginning of continuous stimulation at 10 c/s, but decreased to 5 mm at 10 sec afterwards and remained at 5 mm for the rest of the 120 sec stimulation.

Electrical recordings

Text-fig. 12*a* shows the action potential recorded from a normal superior cervical ganglion on stimulation of the preganglionic cervical sympathetic trunk, including the slow negative and positive after-potentials as described by Eccles (1935). The S1 wave takes off from the stimulus artifact and immediately merges into S2 and S3, to be followed by the smaller and separate S4, which leads into the slow negative and positive after-potentials. The S2 and S3 spikes are not separate in this recording; calculation of the conduction velocities shows that the second discrete spike is due to S4 fibres so that the first spike is presumably due to fibres of S2 and S3, whilst the rising limb up to the point of inflexion represents the S1 component. In comparison, Text-fig. 12*b* shows the action potential of the same ganglion obtained on stimulation of T4–7 in the chest. As a result of activating only 10% of the preganglionic fibres the action potential is considerably smaller, consisting of two small spikes followed by a slow positive after-potential. In the example shown, the first spike (about 20% height of normal S2), occurs after a latency of 20 msec (measured on faster sweeps) to be followed by the second and smaller spike with a latency of 80 msec. The nerve fibres belonging to the first group of cells had a lower threshold than those belonging to the second group. Furthermore,

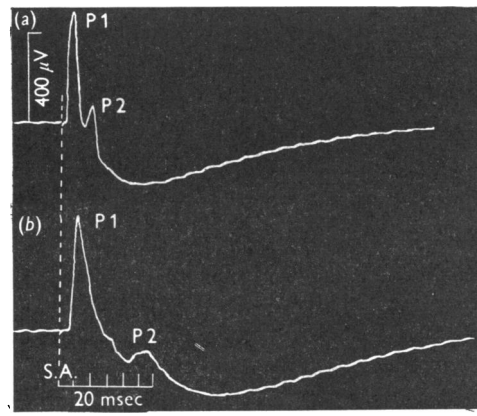
measurement of the conduction velocities (by recording from the preganglionic trunk just below the ganglion) showed that the faster group of fibres had a velocity of 8–10 m/sec, whilst impulses in the slower group travelled at 0·6–1·0 m/sec. These fibres can therefore be identified with the S3 and S4 groups of Eccles (1935).



Text-fig. 12. Action potentials recorded from superior cervical ganglion. (*a*) Normal ganglion; stimulation of cervical trunk, showing spikes followed by slow after-potentials. (*b*) Normal ganglion; stimulation of rami T4–7 only, showing two spikes S3 and S4 followed by after-positivity; (*c*) partially denervated ganglion, T1–3 cut 70 days previously; stimulation T4–7 showing two spikes P1 and P2 followed by after-positivity. Note that S3 and S4 in (*b*) and P1 and P2 in (*c*) coincide. Time scale 20 msec. S.A. = stimulus artifact.

Similar experiments were repeated in cats in which T1–3 had been divided at least 70 days previously. At similar amplification, stimulation of T4–7 in the chest now produced a ganglion action potential of a magnitude comparable with that obtained from a normal ganglion on stimulation of the entire preganglionic cervical sympathetic trunk (Text-fig. 12*c*). It differed, however, in possessing only two spikes, which we may call P1 and P2, the second being smaller than the first. In the particular experiment shown in Text-fig. 12*c*, P1 had a latency of 12 msec, and P2 of 80 msec (measured on faster sweep speed recordings). These values are much greater than those occurring with the

normal action potential on stimulation of the preganglionic cervical sympathetic in the neck (cf. Text-fig. 12*a*), but are comparable with the latencies of the much reduced action potential obtained on stimulation of T4–7 in the chest of a normal cat. Indeed, it is evident that the two spikes in Text-fig. 12 (*b* and *c*) almost exactly coincide. Furthermore, the conduction velocities for the two groups of fibres initiating the P1 and P2 spikes were measured and found to be 10·6 and 1·75 m/sec respectively. These values are of the same order as the S3 and S4 groups, previously mentioned. It appeared that the two remaining groups of fibres had, by the process of collateral sprouting, re-innervated the majority of denervated ganglion cells, reorganizing them into two new groups.



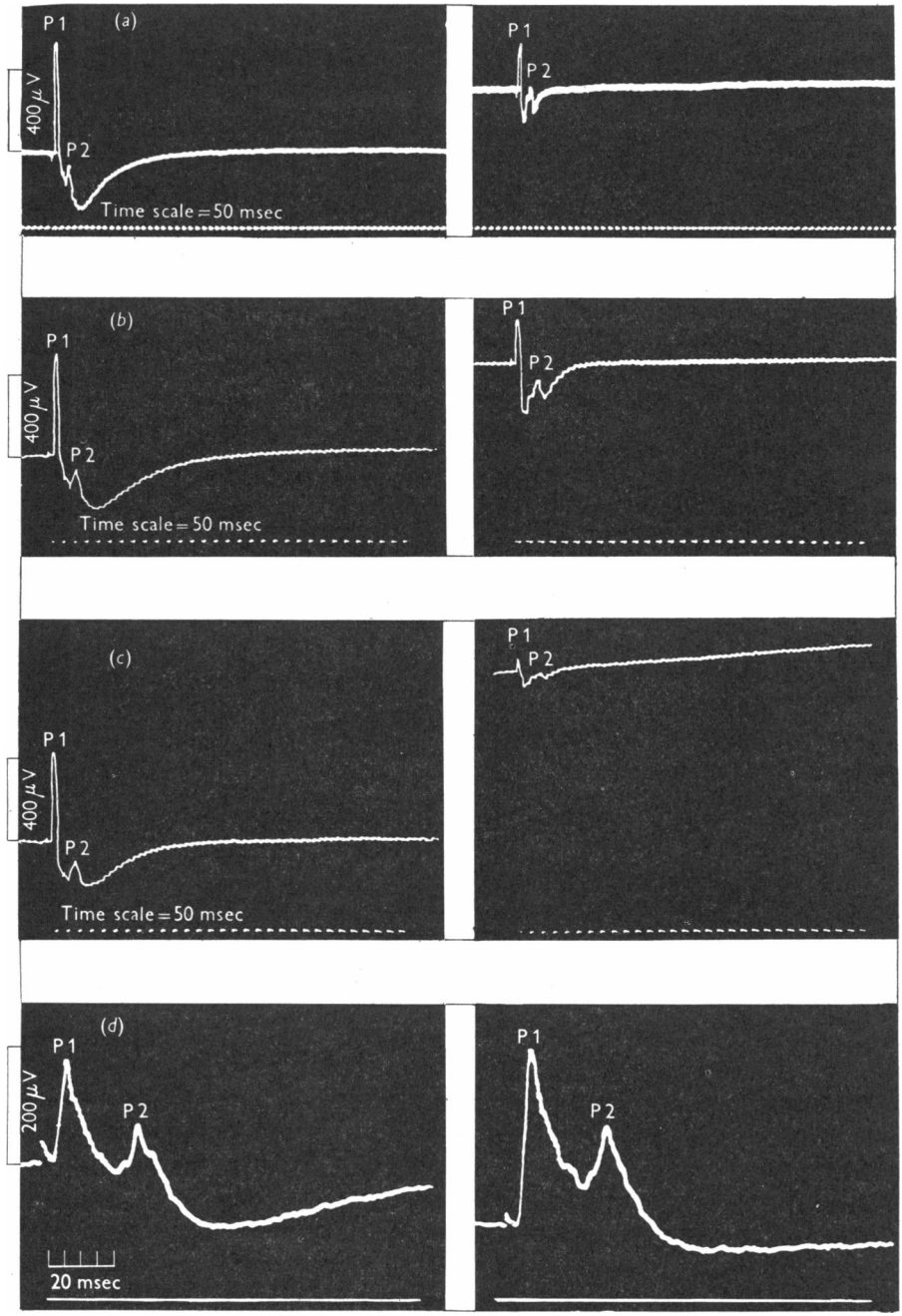
Text-fig. 13. Action potentials of superior cervical ganglion, T1–3 cut 87 days previously. (*a*) Stimulating electrodes on cervical trunk, 4·25 cm from ganglion; (*b*) stimulating electrodes on T4–7, 14 cm from ganglion. Note increase in latency for both P1 and P2 spikes when electrodes moved away from superior cervical ganglion. Time scale 20 msec. S.A. = stimulus artifact.

Thus, those re-innervated by fibres from the S3 group formed the first spike P1 and those by fibres from the S4 group formed the second spike P2. That the two spikes represent the activity of two groups of ganglion cells innervated by two different groups of fibres is shown from the following results. First, the spikes appear at entirely different thresholds, P1 always at a lower threshold than P2. Secondly, the latency for each spike agrees well with the known conduction velocities of the S3 and S4 groups of fibres. Thirdly, both the latencies and the distance between the two spikes depends critically on the distance of the stimulating electrodes from the ganglion (Text-fig. 13). Thus, if the stimulating electrodes are moved on to the cervical trunk, P1 and P2 now approximate, although the magnitude of the action potential does not alter from that obtained on stimulation of T4–7 in the chest. Calculation shows that the reduction of the latency for each spike is accounted for by the reduction of the conduction distance.

As in the normal ganglion, ACh 100μg I.A. produces a short-lasting depolarization equal in magnitude to about half the initial spike height (Text-fig. 14*a*). It is transient and is accompanied by characteristic changes in the action potential. Both spikes are partially abolished and there is an alteration in shape due mainly to the marked reduction in the after-positivity. Likewise, nicotine 50μg I.A. (Text-fig. 14*b*) and tetramethylammonium 100μg I.A. (Text-fig. 14*c*) produce a depolarization of the ganglion equal to about $1\frac{1}{2}$ and $2\frac{1}{2}$ times the initial spike height respectively, accompanied to a greater or less degree by the same changes in the shape of the action potential as occurs after ACh (Text-fig. 14 *a*). A tetanus of 75 c/s lasting for 10 sec and delivered to the fibres from T4–7 in the chest produces a post-tetanic potentiation with increase in the heights of P1 and P2 together with diminution of the after-positivity lasting from 2 to 5 min (Text-fig. 14*d*). It is evident that there are no significant differences, in the behaviour of the electrical responses, between a partially denervated ganglion in the late stages and a normal ganglion.

The action of I.V. hexamethonium on both normal and partially denervated ganglia (in the late stages) was determined by measuring the depression in height of the S1–3 and S4 spikes in the former case and the height of the P1 and P2 spikes in the latter. The results are shown graphically in Text-fig. 15, using hexamethonium 1 mg/kg I.V. in each instance. In the normal ganglion, the S4 spike is depressed to a relatively greater degree than the S1–3 spike; the degree of block is about twice as much in the case of the S4 spike. In contrast, in a partially denervated ganglion, the P1 and P2 spikes are about equally depressed and the magnitude of synaptic block for a given dose of hexamethonium is much greater than that seen in the normal ganglion.

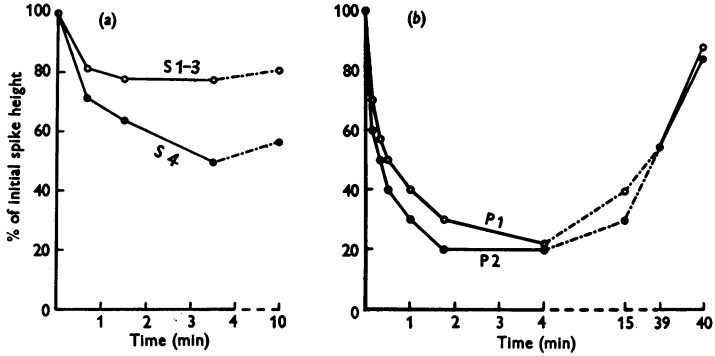
Release of acetylcholine. The normal superior cervical ganglion was perfused with eserinized Locke's solution and the effluent assayed for ACh. Before stimulation of the sympathetic chain, the effluent contained no detectable ACh (certainly less than 1 ng/ml.). In two normal cats the thoracic sympathetic chain just caudad to T3 was stimulated for 4 min at a rate of 10 c/s, producing a contraction of the nictitating membrane and causing the appearance of 3·5 and 2·7 ng ACh/ml. in the effluent, with a total output of 15·9 and 16·8μg respectively. Similar stimulation of the whole of the cervical sympathetic trunk on the same side caused a much more vigorous contraction of the nictitating membrane with the appearance of 13 and 8 ng ACh/ml. in the effluent with a total output of 56·5 and 64·8 ng respectively. In three cats, in which the 1st, 2nd and 3rd rami communicantes had been divided for 21, 68 and 100 days respectively, the amounts of ACh released from the superior cervical ganglion on the operated sides were obtained. In each case thoracic trunks caudad to T3 were stimulated for 4 min at 10 c/s and contractions of the membranes on the operated side practically equalled those on stimulation of the whole of the cervical sympathetic on the normal side. In the first cat



Text-fig. 14. Upper records, action potentials from superior cervical ganglion, T1–3 cut 87 days previously: lower records, arbitrary base line; an increase in the distance between the arbitrary base line and the action potential record represents the degree of depolarization of the ganglion. Left before, right after; (*a*) Acetylcholine 100 μg I.A. showing depolarization of ganglion and accompanying depression of P1 and P2 spikes and alteration in after-positivity; (*b*) nicotine 50 μg I.A. and (*c*) tetramethylammonium 100 μg I.A. showing similar effects. (*d*) Effects of a tetanus 75 c/s for 10 sec (T1–3 cut 70 days previously); note increase in height of P1 and P2 with reduction in after-positivity and hyperpolarization of ganglion.

stimulation caused the appearance of 7·4 ng ACh/ml. in the effluent with a total output of 37·5 ng, in the second there was 5·5 ng with a total output of 36 ng and in the third 8·4 ng with a total output of 35·7 ng.

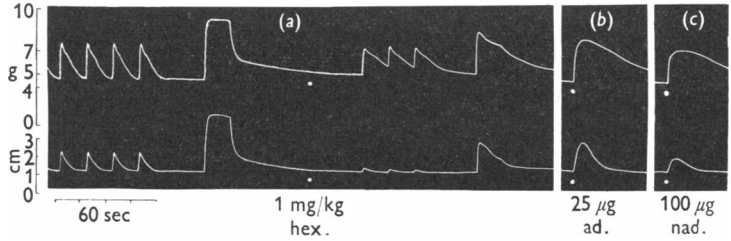
The results indicate that the amount of ACh released in a partially denervated ganglion is about twice as great as compared with a normal ganglion, when approximately equal numbers, i.e. 10% of the total preganglionic fibres, are stimulated. Secondly, the increase in amount of ACh released at the earlier stage of 21 days is as great as at the later stages of 68 and 100 days. Thirdly, although the responses of the nictitating membrane on stimulation of 10% of fibres on the operated side are as great as those on stimulation of 100% of fibres on the normal side, the amount of ACh released in the operated ganglion is only about 60% of that in the normal ganglion.



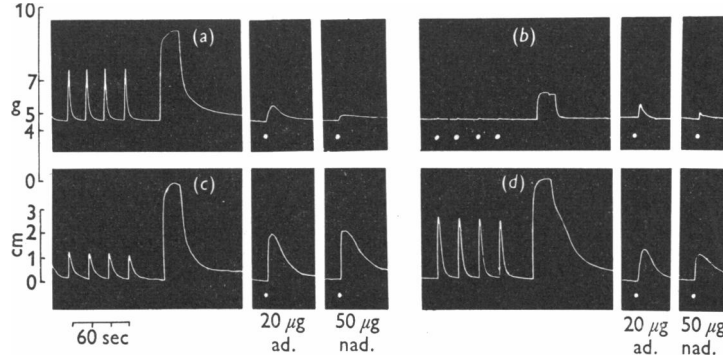
Text-fig. 15. Graphs showing the effects at varying times after 1 mg/kg hexamethonium I.V. on ganglion action potentials: (*a*) normal side, stimulating cervical trunk; (*b*) operated side (T1–3 cut 70 days previously), stimulating T4–7. Effects measured as % depression of initial spike heights of (*a*) S1–3 and S4 and (*b*) P1 and P2.

Effect of hexamethonium

Text-figs. 3–5 and 16 illustrate the blocking effects of hexamethonium on both normal and partially denervated ganglia at 5, 10, 18 and 28 days after operation. Text-fig. 7(*d*) gives the relative efficiency of the blocking effect of hexamethonium on the partially denervated side at different times after operation. In the late group of animals, hexamethonium blocked the ganglion on the partially denervated side much more efficiently than on the normal side (Text-fig. 3). The results in the earlier stages are somewhat different. At 5 and 10 days after operation hexamethonium is relatively inefficient in blocking ganglionic transmission (Text-figs. 4 and 16), whilst in the later stages, 18 and 20 days after operation, the efficiency increases (Text-fig. 5). Text-fig. 7 (*a*, *b* and *d*) shows that the time course of the blocking effect of hexamethonium approximately parallels that of the functional completion of sprouts and is inversely proportional to that of the degree of hypersensitivity of the effector organs.



Text-fig. 16. Simultaneously recorded responses of nictitating membranes. Upper, operated side (rami T1–3 divided 10 days previously); lower, normal side. (*a*) T4–7 on operated side and whole of cervical sympathetic trunk on normal side stimulated—effect of hexamethonium 1 mg/kg I.V.; responses to I.V. adrenaline (*b*), and noradrenaline (*c*).



Text-fig. 17. Responses of the nictitating membranes on the normal and operated sides (90% of the post-ganglionic fibres divided) to stimulation of cervical sympathetic trunks and to I.V. adrenaline and noradrenaline. (*a*) Normal side—responses did not significantly differ at any stage; (*b*) operated side immediately after operation, (*c*) 11 days later, and (*d*) 70 days later.

Partial denervation of the post-ganglionic trunk

Text-fig. 17 shows the responses of the nictitating membrane on the left normal side on stimulation of the cervical sympathetic in continuity under sterile conditions and also to I.V. adrenaline and noradrenaline. After dissection of the right post-ganglionic trunk the responses equalled those on the normal left side, indicating that the post-ganglionic trunk had not been damaged by the dissection. The post-ganglionic trunk of the superior cervical ganglion is composed of two larger and one or two smaller fascicles (Langley, 1900). The two large fascicles on the right side were then divided leaving only the small one, composing about 10% of the whole bulk of the post-ganglionic trunk. The divided proximal and distal ends were ligated and separated by a distance of at least 1 cm. Text-fig. 17 shows the responses of the membrane immediately afterwards to stimulation of the cervical trunk and to I.V. adrenaline and noradrenaline. At slow rates there is practically no response,

and at faster rates only a small response. After completion of the functional tests, the wound was sutured. Twelve days later, the right and left cervical trunks were again stimulated in continuity under sterile conditions. At this stage there was an increase in the responses to stimulation and a marked increase to adrenaline and noradrenaline on the operated side (Text-fig. 17). At a terminal experiment 70 days after the original section of the post-ganglionic trunk, the response of the nictitating membrane on the operated side had markedly increased, especially at the slower rate of stimulation, and was greater than on the opposite control side. However, the responses on the operated side to I.V. adrenaline and noradrenaline had decreased as compared with 12 days after the operation, although still greater than the original responses. Throughout the three periods at which the nictitating membrane responses were recorded the results on the left normal side showed no significant differences.

The return of function of the nictitating membrane 70 days after partial division of the post-ganglionic trunk and the concurrent decrease in hypersensitivity to adrenaline and noradrenaline is unlikely to be due to growth of axons from the site of division to the denervated end organs. With the precautions taken, the observations of Tuckett (1895), Langley (1900) and Machida (1929) suggest that regeneration, should it occur, would take much longer than 70 days. This was confirmed by an experiment in another cat in which the post-ganglionic trunk was completely divided on one side and the proximal and distal ends ligated leaving a gap of at least 1 cm. Stimulation of the cervical trunk 72 days later produced no response of the nictitating membrane or pupil. The responses of the nictitating membrane to adrenaline and noradrenaline remained as great as at 12 days after the original operation.

The evidence indicates that after partial denervation of the post-ganglionic trunk the return of function is due to a regenerative process and that this is most likely to be effected by collateral sprouting of the remaining intact post-ganglionic fibres.

Histological findings

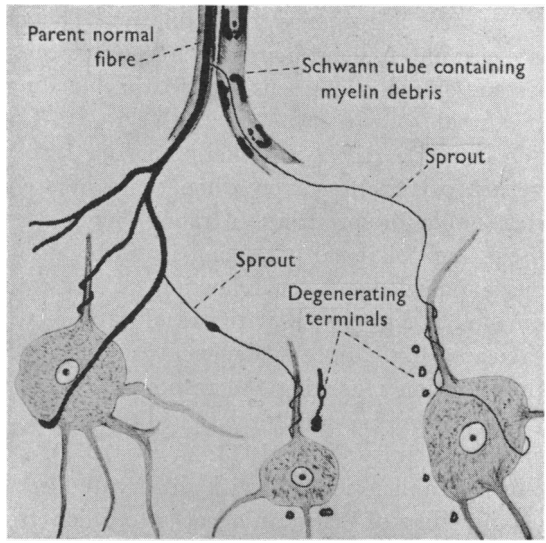
The preganglionic trunks 1 cm or more caudad to the superior cervical ganglia were examined. Five days after division of rami T 1–3 a relatively small number of remaining intact preganglionic fibres could be seen, but the great majority of Schwann tubes were filled with the debris of degenerating axons. Pl. 1, fig. 2*a*, shows a single normal preganglionic fibre with a Schwann cell nucleus and the axon surrounded by the faintly stained Schwann cell cytoplasm. Pl. 1, fig. 2*b*, shows a Schwann tube distended with the debris of a degenerating axon. In some instances a small collateral sprout could be observed within the same Schwann tube as the larger parent axon (Pl. 1, fig. 2*c*). These sprouts frequently grew into adjoining tubes still containing the debris of a previously divided axon and presumably were guided along them (Weiss, 1941). In a few instances the point of sprouting could be identified (Pl. 1,

fig. 3). In preganglionic trunks it was not possible to trace the sprouts to their terminations to see whether they came into close apposition with ganglion cells or not. Although sprouts occurred in this situation, there was a considerably larger number within the ganglion itself.

Within the operated ganglion in the early stages, e.g. 5 days after section, numerous fragments of preganglionic terminations in the process of breaking up could be identified. Pl. 2, fig. 7, shows the typical rings and segments. These appearances were found throughout the ganglion indicating that the fibres arising from T 1–3 were distributed widely within the ganglion. In addition, it was shown that the intact fibres arising from T 4–7, although mostly distributed to the caudal portion of the ganglion, were also present in the cranial half in considerable numbers. Pl. 2, fig. 6, shows a collateral sprout which has arisen from a remaining intact preganglionic fibre within the ganglion. It has grown into an adjacent Schwann tube still containing the debris of a divided axon and has subsequently been guided down the tube. The sprout itself divides into further sprouts and the point of division of one is shown in Pl. 2, fig. 6. The typical bulbous appearance of very early regenerating nerve fibres can be seen on one of the sprouts. In Pl. 2, fig. 6 (bottom right), there is a very fine sprout running along the edge of a tube still containing debris. These sprouts can be traced under the microscope and come into close apposition with adjoining ganglion cells and their dendrites. Pl. 2, fig. 8, shows tiny collateral sprouts coming into close apposition with the cell body of the same ganglion cell at a different focus as in Pl. 2, fig. 7. They are some of the terminations of the sprouts shown in Pl. 2, fig. 6. At 5 days after division of T 1–3 there are side by side degenerating terminal preganglionic fibres and regenerating collateral sprouts. In the early stages, 5–10 days after operation, the tiny collateral sprouts are very numerous and widely distributed throughout the ganglion, coming into close apposition with the majority of cells within the ganglion. They arise from preganglionic fibres within the ganglion at more proximal levels where Schwann tubes are still present and at more peripheral levels after the Schwann cells have merged into their homologues, the glial cells (Text-fig. 18). In the present study, it was clear that approximation of preganglionic terminations to dendrites occurred much more frequently and extensively than to cell bodies, both in normal ganglia and in those in which sprouting had taken place. Pl. 2, fig. 9, shows a sprout terminating on a dendrite of a ganglion cell 10 days after partial denervation.

The number of very fine preganglionic terminations is greatest during the period from 5 to 10 days after partial denervation, and although their number declines thereafter, the density of preganglionic endings appears to remain about the same. By the 4th–8th week the size of many of the preganglionic endings resembles that seen normally (Pl. 1, figs. 4, 5). These observations suggest that most, if not all, of the new sprouts are formed soon after opera-

tion, and subsequently undergo growth and maturation. So far as can be seen from the sections, the great majority of cells within the ganglion are re-innervated by sprouting from the remaining 10% of preganglionic fibres. However, 4 weeks or later after operation there is still a larger proportion of small preganglionic terminations in a partially denervated than in a normal ganglion.



Text-fig. 18. Diagram of an area of superior cervical ganglion 5 days after section of rami T1–3, showing two sprouts, arising from a normal parent preganglionic fibre and typical fragments of degenerating preganglionic terminals.

DISCUSSION

The present observations show that after 90% of the preganglionic fibres to the superior cervical ganglion have been divided, collateral sprouts arise from the remaining intact preganglionic fibres. Histologically, sprouts appear within 5 days of denervation and come into close association with the majority of ganglion cells denervated by the operation. Subsequently they undergo maturation and this is practically complete by 4–8 weeks. Ganglion action-potential recordings indicate that stimulation of the remaining 10% of preganglionic fibres cause discharges from a much larger number of post-synaptic elements than are normally activated by them. This increased field of innervation of the remaining preganglionic fibres on the operated side results in much greater responses of the nictitating membrane and, as evidenced by dilatation of the pupil, activation of ganglion cells with which they previously did not form synapses. In the late stages, hypersensitivity of

the effector organs or ganglion cells plays little or no part in the increased responses. Providing 10% of the preganglionic fibres are available for sprouting, the return of function of the ganglion is for most purposes complete in 4 weeks. However, ganglia in which sprouting has occurred are deficient in two respects. First, only about 60% of the amount of ACh released on stimulation of the normal ganglion is found on the partially denervated side. The results indicate, however, that a preganglionic fibre can double the amount of ACh it releases in response to stimulation. The greater number of terminations may account for the increase. Secondly, the ganglion on the operated side becomes much more sensitive to the blocking effect of hexamethonium than normal.

The outstanding feature in the sympathetic nervous system is the remarkably complete return of function even when there are relatively few preganglionic fibres available for sprouting. Although much work has been done on the stimulus causing collateral sprouting in limb muscles (reviewed by Edds, 1953), precise knowledge is still lacking. Edds concludes that the sprouting phenomenon is a response of the residual fibre to the action of a humoral agent released by adjacent degenerating nerve fibres. There is, however, more information regarding the response. The number of sprouts arising from any parent fibre is directly related to the number of degenerating fibres in the immediate vicinity (Edds, 1953; Morris, 1953). For the sprouts to function, they must establish contact with denervated end-plates and this depends on the availability of Schwann sheaths to guide growing sprouts to these end-plates (Morris, 1953). Because of the compact nature of a ganglion, there will be an extensive intermingling of intact and degenerating nerve fibres. This is particularly so after division of rami T 1–3 since it has been shown that intact and degenerating fibres are widely distributed within the ganglion and not located in separate areas. In contrast to muscle, where the Schwann sheaths leading to motor end-plates are relatively discrete, the adventitial cells within the ganglion which guide regenerating nerve fibres to ganglion cells form a syncytium (De Castro, 1951; Causey & Hoffman, 1955). It appears likely that in a ganglion, in contrast to a muscle, there would be a greater number of sprouts formed and they would have greater success in reaching end organs. Thirdly, because of the close apposition of ganglion cells and parent fibres, sprouts would have a much shorter course than in muscle. This may be a determining factor in the number of sprouts that can be supported by a nerve fibre. Lastly, the target area for the sprouts to make functional connexion may be relatively large. The superior cervical ganglion contains many of the largest ganglion cells in the sympathetic system, some of the perikarya measuring more than 30–40μ in diameter and the dendrites extend in many directions to a greater extent than the cell diameter (De Castro, 1932). Sholl (1956) has shown that in the central nervous system the surface area of

the perikaryon is only about 10% of that of dendrites and a relationship of similar order may exist in sympathetic ganglia. As far as is known, the synapse in a ganglion is formed by the close approximation of preganglionic terminations with cells and in particular their processes (De Castro, 1951; Causey & Hoffman, 1955).

Of some importance is a consideration of the factors which might limit the re-innervation of a ganglion by sprouts. One factor would be the capacity of a parent fibre to produce a large number of sprouts. It has been calculated that growing sprouts constitute less than 1% of the volume of the whole neurone, but axoplasm drawn into them may be available only locally and this may place some limitation on the numbers (Edds, 1953). Nerve cells which have supported new branches for several months have enlarged perikarya with normal patterns of Nissl substance (Cavanaugh, 1951) and the axons hypertrophy (Edds, 1949). Such a mechanism may permit a small number of axons supporting a large number of new branches to mature, although the process may take many months (Edds, 1953). In the present experiments, with 1% of preganglionic fibres left intact, there was only partial recovery even 55 days after operation.

It is clear that re-innervation of a ganglion by sprouts is accompanied by a decrease in hypersensitivity both of effector organs and of ganglion cells. At a certain stage in the recovery process, the balance between function of sprouts and persisting hypersensitivity is such that their effects summate to produce an overshoot in response of the effector organ. The degree and time course of this phenomenon will depend on the rate and extent of the re-innervation. It is known that the overshoot in size of the pupil during regeneration of post-ganglionic fibres is greater in degree and duration than with preganglionic fibres (Machida, 1929). This may be explained by the fact that re-innervation by post-ganglionic fibres takes a longer time and is less complete than after a comparable lesion of preganglionic fibres (Langley, 1897; Lee, 1929; Machida, 1929; Brücke, 1931), probably owing to the great dispersal of end organs to be re-innervated and the many and lengthy pathways by which the fibres can be guided. In addition, post-ganglionic denervation results in a greater degree of hypersensitivity of the effector organ than does preganglionic denervation (Cannon & Rosenblueth, 1949). The present experiments suggest that the overshoot in responses of effector organs is more prolonged as the result of collateral sprouting of post-ganglionic as compared with preganglionic fibres.

Since collateral sprouting occurs in man (Coërs, 1956), it is of interest to examine the possible relationship to surgical sympathectomy. Preganglionic sympathectomy of the upper limb leaves the 1st thoracic ramus and cervico-thoracic ganglion as a possible source for collateral sprouting (Geohegan & Aidar, 1942). Even after ganglionectomy, escape routes may be provided by intermediate ganglia and their aberrant pre- and post-ganglionic fibres (Skoog,

1947; Boyd & Munro, 1949). When return of function occurs, it may be slight and take up to a year or more to develop (Simmons & Sheehan, 1939; Smithwick, 1940; Barcroft & Hamilton, 1948*a*, *b*; Haxton, 1954), suggesting that if collateral sprouting is involved, the escape fibres are few in number. In contrast, recovery after thoraco-lumbar sympathectomy may develop in a few weeks and in this situation there is known to be a considerable number of escape fibres (Boyd & Monro, 1949; Monro, 1954). Sprouts may re-innervate any effector organ of the same type, i.e. cholinergic or adrenergic, irrespective of the original functional connexions of the pre- or post-ganglionic fibres, and this may lead to bizarre results. In the present experiments complete preganglionic denervation of the superior cervical ganglion was followed by sprouting of the vagal efferent fibres in the adjacent nodose ganglion. The sprouts partly re-innervated the superior cervical ganglion by way of the existing communications. As a result, nictitating membrane contractions on the operated side could be produced by vascular and gastric reflexes passing up the contralateral vagus. An example of the bizarre phenomenon in man may be gustatory sweating, which sometimes occurs after sympathectomy of the upper arm (Haxton, 1948). Exaggerated reflex responses of sweating in the face may be due to summation of the effects of partial re-innervation by sprouting from adjacent nerves and persisting hypersensitivity of the sweat glands (Wilson, 1936). Furthermore, it is now evident that caution is needed when performing animal experiments. As has been shown, collateral sprouting can occur very rapidly and reliance cannot be placed on this type of regeneration not having taken place within the times of the experiments. Failure to appreciate this fact may lead to erroneous results.

The concept of the nervous system as a static structure is undergoing radical changes. The extension of sprouts from intact nerve fibres into zones of sensory loss in skin, motor loss in muscle, and in the sympathetic nervous system are excellent illustrations of the lability of nervous tissue. Recently, histological and functional evidence has been obtained that collateral sprouting occurs in the spinal cord of cat and monkey (Liu & Chambers, 1956; McCouch, Austin & Liu, 1956). It appears that sprouting in response to trauma and other causes (Coërs, 1956) is a general phenomenon in the nervous system. The functional efficiency will vary in different situations depending to some extent on the factors discussed above.

SUMMARY

1. In cats, the superior cervical ganglion on one side was partially denervated by severing the rami communicantes from the first, second and third thoracic nerves; this causes about 90% of the preganglionic fibres to degenerate; the residual 10% consist chiefly of fibres 2μ or less in diameter.

2. Stimulation of the remaining 10% of fibres initially produces a very small contraction of the nictitating membrane. Four to eight weeks later the re-

sponses have markedly increased and equal those on stimulation of the whole of the cervical trunk on the normal side.

3. Dilatation of the pupil on stimulation of the remaining fibres showed that they now form synapses with ganglion cells not previously activated by them.

4. Hypersensitivity of the effector organ or ganglion cells plays little or no part in the eventual recovery of function, although it may be present in the early stages. The return of function coincides with the disappearance of the hypersensitivity.

5. Histological examination of partially denervated ganglia showed that collateral sprouts arise from the remaining intact preganglionic fibres and come into close apposition with the majority of ganglion cells denervated by the preliminary operation.

6. Experiments recording ganglion action potentials confirmed that the majority of cells within a partially denervated ganglion are now activated by stimulation of the remaining 10% of preganglionic fibres, although in a normal ganglion only a small proportion of cells are in synaptic connexion with these fibres. Although the temporal relationships of the ganglion action potential are considerably altered, its responses to repetitive excitation or to drugs is qualitatively normal.

7. Perfusion of a partially denervated ganglion showed that stimulation of the remaining 10% of preganglionic fibres released twice as much acetylcholine as stimulation of a similar number of fibres to a normal ganglion. Nevertheless, the total amount of acetylcholine released in a partially denervated ganglion was only about 60% of that on stimulation of all the preganglionic fibres on the normal side.

8. The partially denervated ganglion becomes more sensitive to the blocking effect of hexamethonium than a normal ganglion.

9. Post-ganglionic fibres also appear to respond to partial denervation by collateral sprouting.

10. The possible factors responsible for the efficiency of the sprouting process in the sympathetic nervous system are discussed.

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EXPLANATION OF PLATES

All are longitudinal sections of the superior cervical ganglia or the preganglionic trunks stained by the Bielschowsky method.

PLATE 1

Fig. 1. A low-power view of the superior cervical ganglion, 5 days after division of rami T1–3: small sections of the preganglionic (upper) and post-ganglionic trunks (lower) are shown.

Fig. 2. Sections of the preganglionic trunk 1 cm caudad to the superior cervical ganglion 5 days after division of rami T1–3: *a*, a single intact preganglionic fibre with a Schwann cell nucleus and faintly stained, the Schwann cell cytoplasm around the axon; *b*, a Schwann tube filled with the debris of a degenerating axon; *c*, a single intact preganglionic fibre with an adjacent Schwann tube filled with debris; between the two is a fine collateral sprout which has arisen from the intact axon.

Fig. 3. A section of the preganglionic trunk (as in fig. 2) showing a few remaining intact small preganglionic fibres, the majority of tubes containing faintly stained debris. In the middle is a very fine collateral sprout (slightly out of focus) arising from an intact axon; the sprout grows into an adjacent tube containing debris. Bottom, a Schwann cell nucleus in the process of mitosis.

Fig. 4. A ganglion cell and preganglionic fibres in a normal superior cervical ganglion.

Fig. 5. Same cell as in fig. 4 focused so that the preganglionic terminations in close apposition with the cell surface can be seen.

PLATE 2

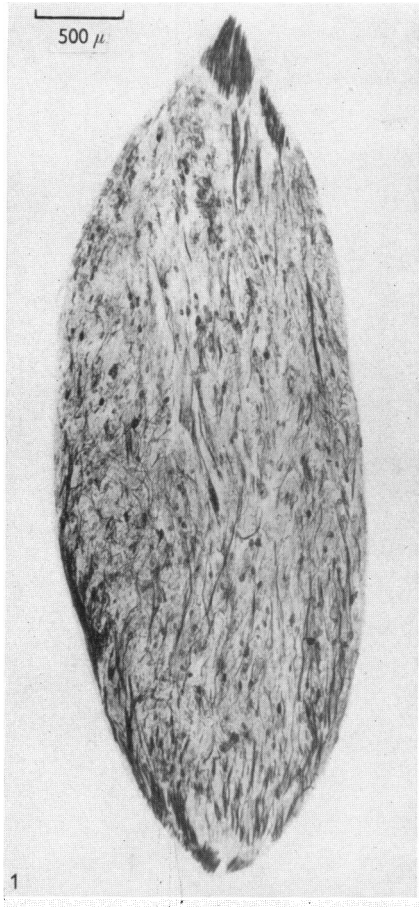
Fig. 6. Section within a ganglion 5 days after division of rami T1–3. A sprout has arisen more proximally from a parent intact preganglionic axon, grown into an adjacent Schwann tube still containing debris (top left hand) and been guided down the tube. A Schwann cell nucleus in the tube can be recognized. The sprout in turn divides into further sprouts and the point of division of one can be seen. On one sprout (bottom right) a bulbous appearance typical of a very early regenerating fibre can be seen. Bottom right is a Schwann tube filled with debris, a tiny new sprout running alongside.

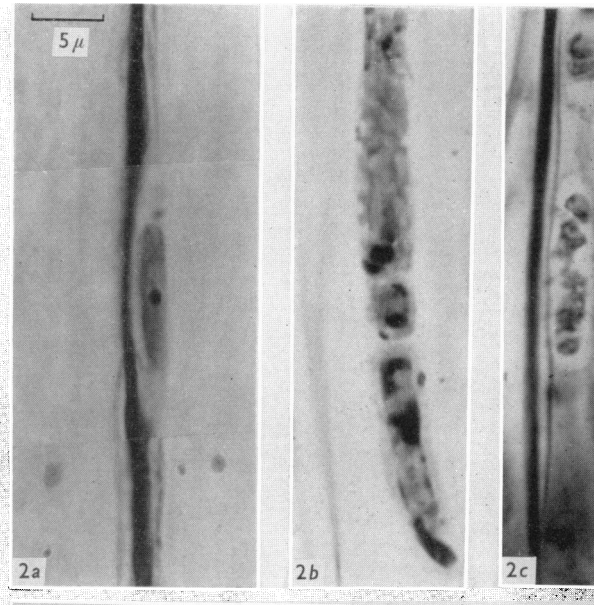
Fig. 7. A ganglion cell adjacent to the area shown in fig. 6 with nucleus, nucleolus and dendrites. Top left hand is a fragment of a preganglionic termination with a rounded mass of axoplasm in the process of degeneration. Several ring-like appearances, typical of degenerating fine preganglionic terminations, can be seen.

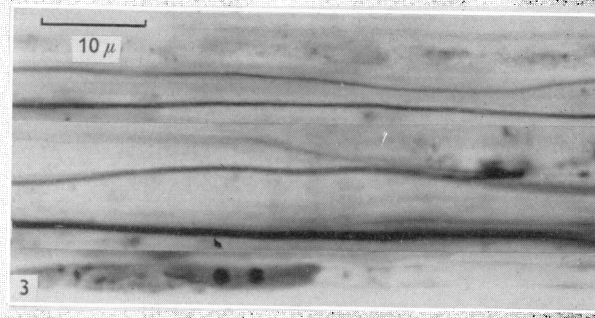
Fig. 8. Same cell as in fig. 7 at a slightly different focus showing tiny sprouts, some coming into close apposition with the cell surface, and dendrites.

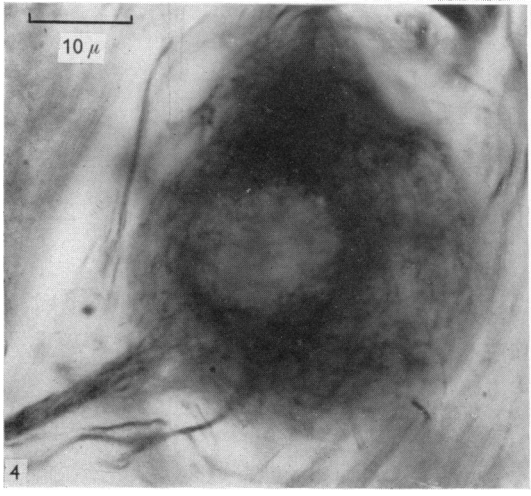
Fig. 9. Part of a ganglion cell (right) with a dendrite (left) 10 days after section of rami T1–3; a sprout which could be traced from a preganglionic parent fibre is seen spiralling round the dendrite.

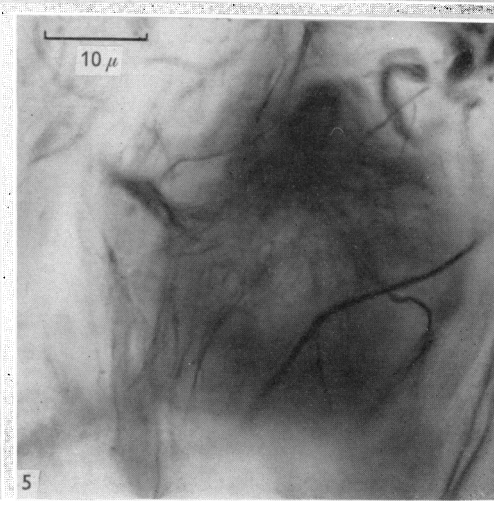
Fig. 10. A Schwann tube filled with debris of a degenerating axon; the nucleolus of a Schwann cell nucleus shown right. Rami T1–3 had been divided 10 days previously. Two fine sprouts are seen running along within the same tube.











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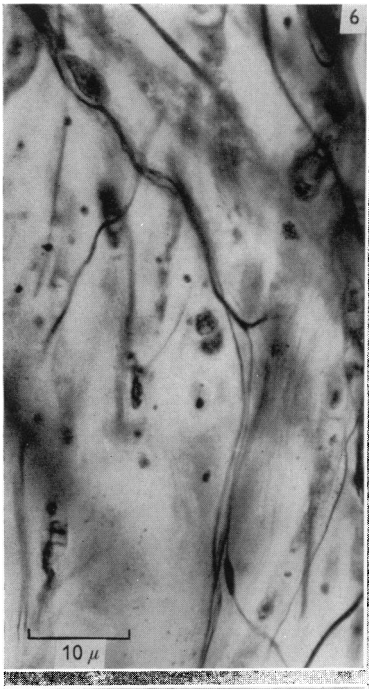
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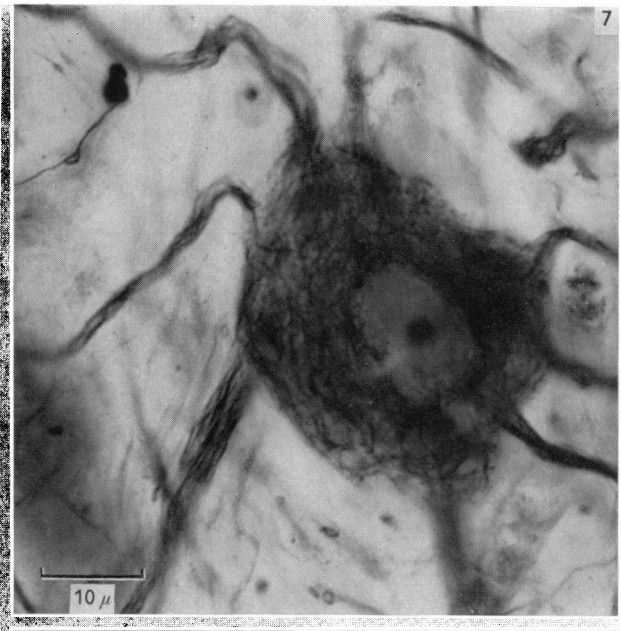
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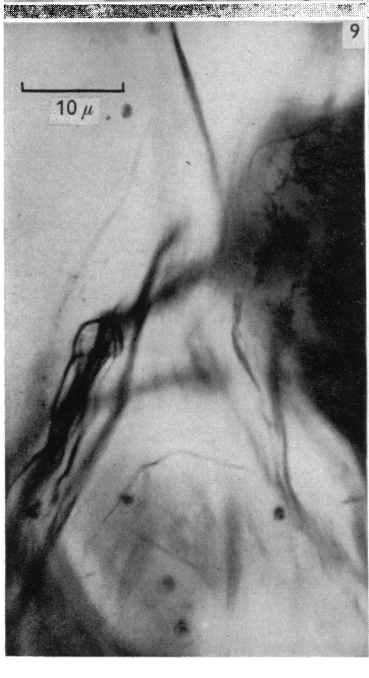
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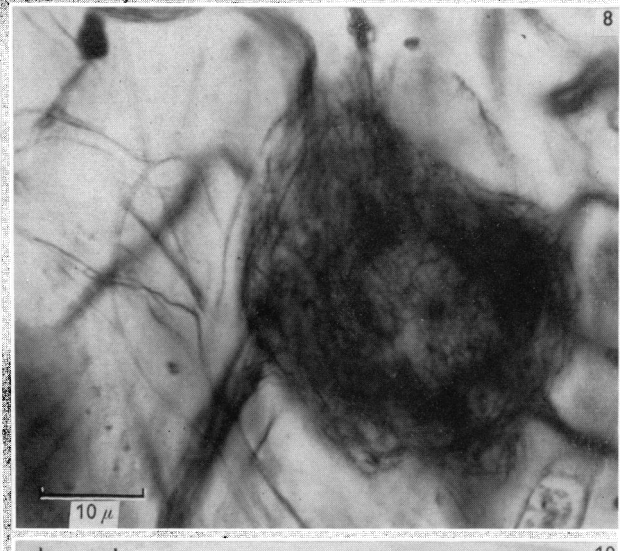
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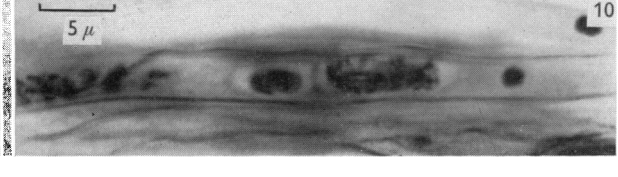
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