REGENERATION OF FIBRE DIAMETER AFTER CROSS-UNIONS OF VISCERAL AND SOMATIC NERVES

By S. A. SIMPSON and J. Z. YOUNG, *Department of Zoology and Comparative Anatomy, Oxford*

1. INTRODUCTION

In a normal nerve each function is subserved by fibres of a particular range of size. It is not clear to what extent functional efficiency is dependent on fibre diameter, but it seems certain that gross abnormalities following degeneration and regeneration, such as a great reduction in the size of the somatic motor fibres, must produce a corresponding functional derangement, even if the connexions are re-established correctly. Gutmann & Sanders (1943) have shown that after nerve suture the normal bimodal distribution of fibre sizes in the peroneal nerve of the rabbit is not restored, even after one year's regeneration. However, if the nerve is crushed, interrupting the axons but not the tubes in which they run, the bimodal distribution again begins to become apparent after about 200 days.

It is therefore important to discover the factors controlling the magnitude of fibre diameter attained after regeneration. Two obvious possibilities, which formed the basis of the present experiments, are: (1) that the diameter of each parent axon in the central stump determines that of the new fibre in the peripheral stump; (2) that the fibre diameter is determined by the size of the connective tissue tubes remaining in the peripheral stump after degeneration. Nageotte and Guyon (1918) and Sanders & Young (1944) have already shown by another method that there is some peripheral factor which affects the diameter of regenerated axons. They utilized the fact that the sciatic nerve contains motor, sensory, and sympathetic fibres, lying together in its upper portion, but disposed separately in its lower portion. After section and suture in the upper levels, the subsequent fibre connexions become mixed, so that fibres of all sizes grow down into branches some of which contained large, others only small, tubes. Yet after regeneration the fibres were found to be larger in the motor (large tube) than in the cutaneous (small tube) branches.

This condition could be attributed to restriction by the small tubes of the diameter attained by fibres growing within them. It was therefore attempted to settle the question by the more direct experiment of joining nerves containing fibres of different sizes. The necessary conditions were obtained by making unions between somatic and visceral (pre- and post-ganglionic) trunks. This results in the formation of very unusual terminal fibre connexions (see Langley & Anderson, 1904). It thereby became apparent that a further factor to be considered is that the diameter reached by a regenerating fibre is greatly influenced by the connexion which it effects peripherally. Somatic motor fibres which gain connexion with muscles become much larger than those which fail to make such connexion. Since this very important and interesting functional factor is operative it is evident that the possible constricting influence exercised by the tubes of the peripheral stump can be shown only by comparing nerves of different tube sizes but having comparable terminal connexions. Such comparison is possible in the present series of experiments, and shows that the restrictive effect of the peripheral tubes is marked only when these are very small in comparison with the size of the fibres in the central stump.

2. METHODS

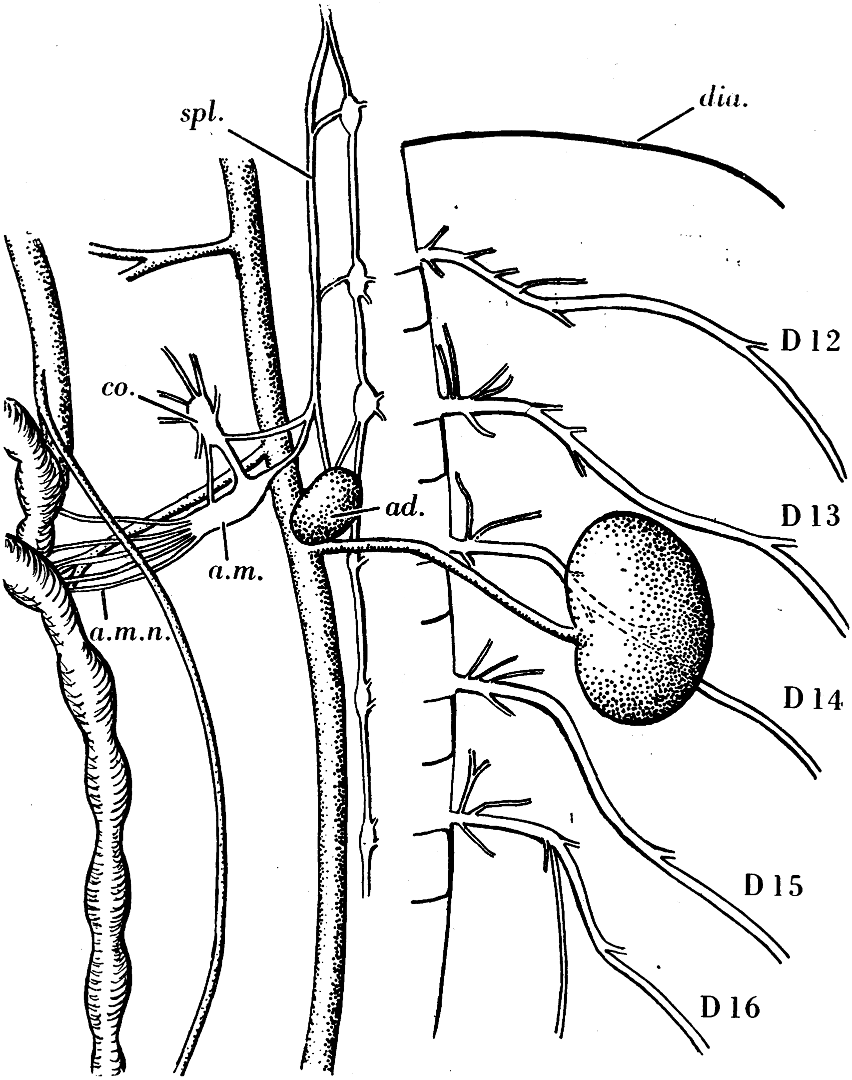
The ideal method of testing the influence of central and of peripheral stumps on the size of regenerated fibres would be to join nerves containing, respectively, only large and only small fibres. Unfortunately, though there are nerves which approach this condition, all mammalian nerves with large fibres contain also some small ones. The following nerves in the rabbit, however, provide a close approach to the ideal conditions for such cross-unions:

(1) The anterior mesenteric nerves, composed almost wholly of small non-medullated fibres.

(2) The great splanchnic nerve, composed mainly of small medullated fibres.

(3) The ventral rami of the post-thoracic spinal nerves, containing fibres of all sizes from the largest downwards, distributed bimodally.

Details of the composition of these nerves are discussed later. Their anatomical situation provides very many advantages for the making of cross-unions. Text-fig. 1 shows diagrammatically the arrangement of the solar plexus and neighbouring structures in the rabbit. The abdominal ganglia are highly variable: often there are separate coeliac and anterior mesenteric ganglia, as shown, but these may be almost fused, or a third ganglion may occur on the great splanchnic nerve. Notwithstanding such variations, the anterior mesenteric nerves form a sufficiently definite entity to be used in cross-unions either as central or as peripheral stumps. The nerves never form a single bundle, but consist rather of a set of nearly parallel strands, running along the posterior aspect of the anterior mesenteric artery for a centimetre or more before breaking up into smaller branches. These strands therefore provide a most convenient source of non-medullated fibres, containing only a very few small medullated fibres. They are readily accessible at operation. If cut as far as possible from the ganglion they can be isolated as a definite trunk some millimetres long for use as a central stump. A cut made immediately distal to the anatomical ganglion isolates a trunk which can be used as a peripheral stump, although a few nerve cells are likely to be left peripheral to the cut, because of the diffuse nature of the ganglion.



Text-fig. 1. Diagram of solar plexus and related nerves in the rabbit. D12–16 are the post-thoracic somatic nerves. The great splanchnic nerve (*spl.*) divides into branches to the adrenal (*ad.*) and to the coeliac (*co.*) and anterior mesenteric (*a.m.*) ganglia. From the latter proceed the anterior mesenteric nerves (*a.m.n.*). *dia.* shows the position of the diaphragm.

The great splanchnic nerve leaves the sympathetic chain above the diaphragm and with practice can usually be found easily just behind that muscle. Thence it runs, as a single or double strand, backwards for as much as 2 cm. before dividing into branches to the ganglia of the solar plexus. A satisfactory length of nerve is therefore available for suturing, but a serious handicap is its very small diameter, making it difficult to handle after being moved.

The ventral rami of the post-thoracic nerves (D12–D15) were used as a source of large fibres. Their correct naming presents difficulties: in the rabbit D12 is the last intercostal nerve; the first three lumbar nerves usually do not communicate to form a plexus, and the genito-femoral nerve arises from D16. Nerves D13 and D14 may therefore be called the anterior and posterior iliohypogastrics, and D15 the ilio-inguinal. However, attempts to adopt the nomenclature of human anatomy serve only to obscure the essential similarity of all these nerves. Each passes between the psoas muscles, giving branches thereto, then runs for a considerable distance across the ventral surface of m. obliquus and divides into two branches which penetrate and innervate the obliqui, finally emerging to innervate the belly skin.

The stretch of nerve across the mm. obliqui is easily isolated and is long enough to be joined to the anterior mesenteric or splanchnic nerves. Though convenient in this respect these post-intercostal nerves have the disadvantage that they give off small branches as they pass over the muscles, and the number of fibres in the nerves therefore differs slightly at different levels. There is also some possibility of anomalous results from escape of fibres from these branches after their severance.

Operative conditions and post-operative health of the animals employed provided no serious obstacles. Large animals in good health were used, irrespective of race or sex. Some were lost prematurely, but this was to be expected in such prolonged experiments, and there was little evidence of internal disturbance resulting from operation.

The selected nerves were cut and mobilized for a sufficient length and then joined by application of concentrated cockerel plasma (Young & Medawar, 1940). Without this plasma the experiments would have been difficult or impossible, since the nerve trunks are too small to allow of stitching without considerable interference: with the plasma good apposition was secured in most cases.

To investigate the apposition the actual site of union was always removed at autopsy, fixed and sectioned. The junctions were classified according to an arbitrary scale: 1 was used for a good junction, with minimal separation of the stumps; 2 indicated some separation, and hence a slight narrowing or waist; 3 was a bad union, with a definite gap of a millimetre or more. Control of conditions at the junction is most important in such experiments. Counts of the numbers and sizes of nerve fibres are significant only when considered in relation to the state of the union above them. Ideally, a detailed investigation should have been made of the variability introduced by this factor alone, but such a course would have meant a quite impracticable number of experiments of each type.

In most of the experiments several cross-unions were made in the one animal. Thus in rabbit 811, D12 was sutured into the peripheral stump of the great splanchnic, D13 into the anterior mesenteric, and D14, after severance, into its own peripheral stump. This procedure was economical of material and rendered comparison more reliable. Each piece of nerve fixed was identified by a small letter: thus 811*l* was the somatic nerve 10 mm. below the D14 union, and 811*j* a piece 2 cm. lower. (All pieces can be identified by reference to Table 1.)

A possible source of serious error is the invasion of the peripheral stump by fibres from other nerves severed during the operation. Powers of regeneration seem to be especially high in this part of the body (perhaps because of the high temperature), and in several experiments it was found that adventitious fibres had joined the peripheral stump from considerable distances. The most potent source of such invasion was the branches of the post-thoracic nerves to the psoas muscles (Text-fig. 1), especially as the point of operative union often lay directly upon these muscles, which may have been incised during nerve mobilization and have thus come to contain cut nerve ends. In some instances invasion of fibres had certainly occurred from such a source. Possible false conclusions from such connexions have therefore been carefully considered in each case, and the sections of the line of union examined with this in view. In future experiments of this type, wrapping, or some other form of nerve shielding, seems highly desirable.

The great length of somatic nerve necessarily mobilized to effect connexion with the splanchnic or anterior mesenteric nerves produced some complications. The long line of scarred muscle which resulted often brought about adhesions to the liver or stomach, so that post-experimental dissection was difficult. Within the nerve itself the mobilization delayed removal of the products of degeneration, so that numerous myelin remains were found at 100 and some even at 200 days after operation (Pl. 4, fig. 21). In this respect the condition of the nerves approached that of free nerve grafts. In some of the experiments actual grafts were made of one type of nerve into another, and the effects of long mobilization in direct unions were therefore an advantage in making the grafts more directly comparable with the cases of direct union.

All histological material was removed alive from the anaesthetized animal and stretched carefully on pieces of card. For counting of medullated fibres the nerves were fixed in Flemming's chrome-osmium-acetic mixture, embedded in paraffin through cedarwood oil, sectioned transversely at 4 μ, and stained with the Weigert method used by Gutmann & Sanders (1943). For study of non-medullated

Table 1

			Central stump																
			Somatic						Splanchnic						Anterior mesenteric				
		1	2	3	4	5	6	7	2	3	4	5	6	7	2	3	5	6	7
		Days of regeneration	Animal and nerve	Union	Level	No. of fibres	Area medullated %	% of medullated area >6 μ	Animal and nerve	Union	Level	No. of fibres	Area medullated %	% of medullated area >6 μ	Animal and nerve	Union	No. of fibres	Area medullated %	% of medullated area >6 μ
		—	999*c*	Normal nerve	—	2663	61·5	91	999*b*	Normal nerve	—	2192	48·2	4	732*a*	Normal nerve	14	0·1	0
		100	842*c*	Central stump	—	2180	38·6	65	—	—	—	—	—	—	—	—	—	—	—
Peripheral stump	Somatic	100	847*d*	1	Near	2256	33·3	59	959*f*	2	Near	1218	17·7	42	731*c*	1	11	0·1	0
			847*e*	—	Far	1487	16·7	27	959*h*	—	Far	1136	10·9	45	—	—	—	—	—
			841*a*	3	Near	1116	9·5	44	—	—	—	—	—	—	834*a*	2	19	0·1	0
			853*h*	2	Graft	2245	20·0	52	—	—	—	—	—	—	—	—	—	—	—
			859*b*	1	Graft	2795	28·4	59	—	—	—	—	—	—	854*a*	2	634	5·1	45
			842*a*	1	Graft	5158	26·6	28	—	—	—	—	—	—	959*a*	2	Many, not counted		
		200	811*l*	1	Near	3733	49·3	73	—	—	—	—	—	—	835*a*	2	43	0·3	0
			811*j*	—	Far	3240	46·5	72	—	—	—	—	—	—	958*a*	2	0	0	0
	Splanchnic	100	833*c*	2	Near	1306	14·0	40	854*e*	—	Near	350	6·6	5	848*g*	2	0	0	0
			859*f*	1–2	Graft	1493	22·8	64	—	—	—	—	—	—	—	—	—	—	—
			859*g*	—	Graft	1239	17·3	54	—	—	—	—	—	—	—	—	—	—	—
			853*c*	—	Graft	1914	20·0	59	—	—	—	—	—	—	—	—	—	—	—
		200	811*f*	1	Near	1433	41·0	65	—	—	—	—	—	—	—	—	—	—	—
	Anterior mesenteric	100	735*b*	2	Near	1160	4·1	20	841*d*	2	Near	69	0·1	0	853*m*	2	18	0·1	0
			833*a*	1	Near	Not counted	—	—	—	—	—	—	—	—	—	—	—	—	—
		200	811*b*	2	Near	2173	16·2	25	—	—	—	—	—	—	—	—	—	—	—

fibres, and usually for the site of union, Bodian's method was used, with alcohol-formol-acetic fixation.

3. COUNTING TECHNIQUE

It being surprisingly easy to draw false conclusions by simple visual or qualitative judgements of degree of medullation, resort was therefore made to counting and measurement. In most instances this was done by making direct photographs of the nerves by projection through the microscope on to bromide paper at a magnification of × 750 (Sanders & Young, 1944). In a comparison of fibre sizes it is important that the degree of unavoidable error should be constant. This was ensured by the use of (1) standard histological technique, (2) repeated checking of magnification, (3) standard counting technique. In most cases not all the fibres in a nerve were measured, the sampling method used by Gutmann & Sanders (1943) being adopted. Fibres were referred to groups by their outside diameters, as in that method. Where fibres were not round, exact measurements were made of the longest diameter of the fibre and of that at right angles to the longest, the mean of the two measurements being taken. In the case of crenated fibres the diameter was measured and then 10 % of this was added (Duncan, 1934). Sampling technique was not used where the density of fibres appeared uneven; in such instances photographs of the whole nerve were taken, and all the fibres measured.

4. METHOD OF EXPRESSING RESULTS

Some difficulty is occasioned by the fact that it is not possible to test directly the effect of union of fibres of one size with those of another, because all the nerves used contain some range of fibre size. A means of expressing this range is therefore necessary, both in the normal nerves and in the results of cross-unions. Histograms or cumulative percentage curves of frequency of fibres of various diameters give good visual demonstration of the facts, but for numerical treatment we have used expressions involving the squares of the diameters (see Gutmann & Sanders, 1943). The general problem of the investigation may be stated as an attempt to ascertain to what extent the cross-sectional area presented by the tubes into which nerve fibres are growing affects the diameter which those fibres attain. We have therefore, for each of the normal nerves, calculated the area represented by fibres of various diameters. Since, however, the nerves differ widely in total area, it is necessary to divide the figures thus obtained by the total areas of the nerves, giving an *area factor*, or proportion of the total area which is occupied by fibres. Thus a normal somatic nerve (999*c*) has an area of fibre of 184,000 μ^2 and a total area of 299,000 μ^2, giving an area factor of 61·5 %. The great splanchnic (999*b*) with fibre area of 19,200 μ^2 and total area of 39,600 μ^2 has an area factor of 48·2 %.

Since the difference between the splanchnic and somatic nerves is, broadly, that the latter contain numerous fibres greater than 6 μ, we have also calculated the area occupied by fibres greater than this diameter. Thus in the above normal somatic nerve the fibres greater than 6 μ give an area of 166,000 μ^2, whereas for the great splanchnic nerve the figure is only 720 μ^2. In other words, for a given area the two nerves are occupied by similar areas of medullated fibre, but in the case of the somatic nerve the large fibres make up 91 %, and in the splanchnic nerve 4 % only of the medullated area.

5. NORMAL COMPOSITION OF THE NERVES USED

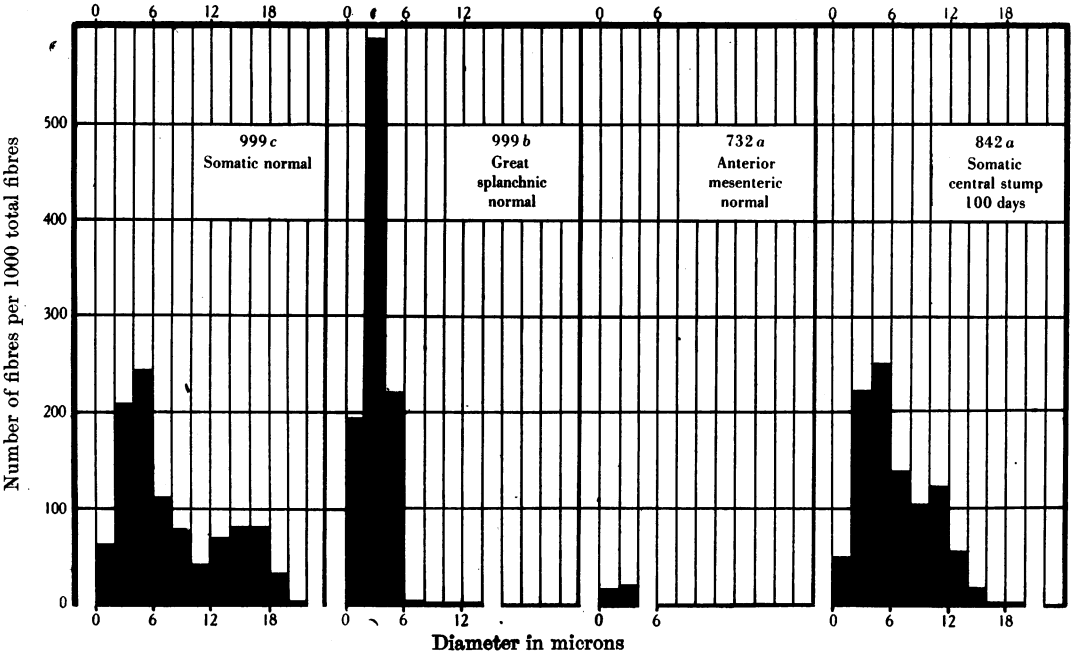
(1) *Nerves with large medullated fibres.* The ventral rami of the post-thoracic nerves contain a very high proportion of large fibres (Pl. 1, fig. 1). Text-figs. 2 and 4 show a histogram and a cumulative percentage curve of the distribution of fibres in the nerve (999*c*) at the middle of D 12. This nerve contained 2663 medullated fibres with a sharply bimodal distribution similar to that found in other motor nerves. The group of large fibres constitutes a considerably greater proportion of the total than in the mixed region of the peroneal nerve recently investigated by Gutmann & Sanders (1943). They found 17·6 % of fibres of diameter greater than 8 μ, whereas the present post-thoracic nerves manifest 37·6 %. The area factor measure, already explained, shows that of the 61·5 % of the total nerve area occupied by medullated fibres 91 % is occupied by fibres of diameter greater than 6 μ. Moreover, the largest fibres present in these post-thoracic nerves are larger than those in the peroneal nerve.

We have not attempted to study the change in number of fibres along the course of any one nerve, nor to compare D 12 with the other lumbar nerves used. The four nerves do not differ greatly in diameter, but they decrease slightly in size craniocaudally. For purposes of experiment it would have been more satisfactory to use nerves containing large fibres only, but none such are known, and the lumbar nerves chosen, besides being conveniently situated, approach the ideal by reason of their high large-fibre content.

(2) *Nerves with small medullated fibres.* The great splanchnic nerve of the rabbit (Pl. 1, fig. 2) consists mainly of medullated fibres smaller than 6 μ in diameter. Nerve 999*b* manifested two trunks at the level studied, and these contained 2192 fibres distributed as shown in the histogram and cumulative percentage curves (Text-figs. 2, 4). The proportion (48·2 %) of the nerve area occupied by medullated fibres approaches that of a normal

somatic nerve, but 4 % only of this area is composed of fibres larger than 6 μ. A similar composition with few large fibres has been noted by Bishop & Heinbecker (1930) and figured by Otuka (1940). The large medullated fibres are peculiar in having exceptionally thick sheaths.

(3) *Non-medullated nerves.* The anterior mesenteric nerves (Pl. 1, fig. 3) are a series of bundles composed almost wholly of non-medullated fibres, amongst which are scattered a very few very small medullated fibres (Text-fig. 2). Thus, in nerve 732*a* there were fourteen such fibres occupying less than 0·1 % of the total area of the nerve. The average diameter of these fibres was 3·9 μ, and none was larger than 6 μ.



Text-fig. 2. Histograms of composition of normal nerves, and of a central stump.

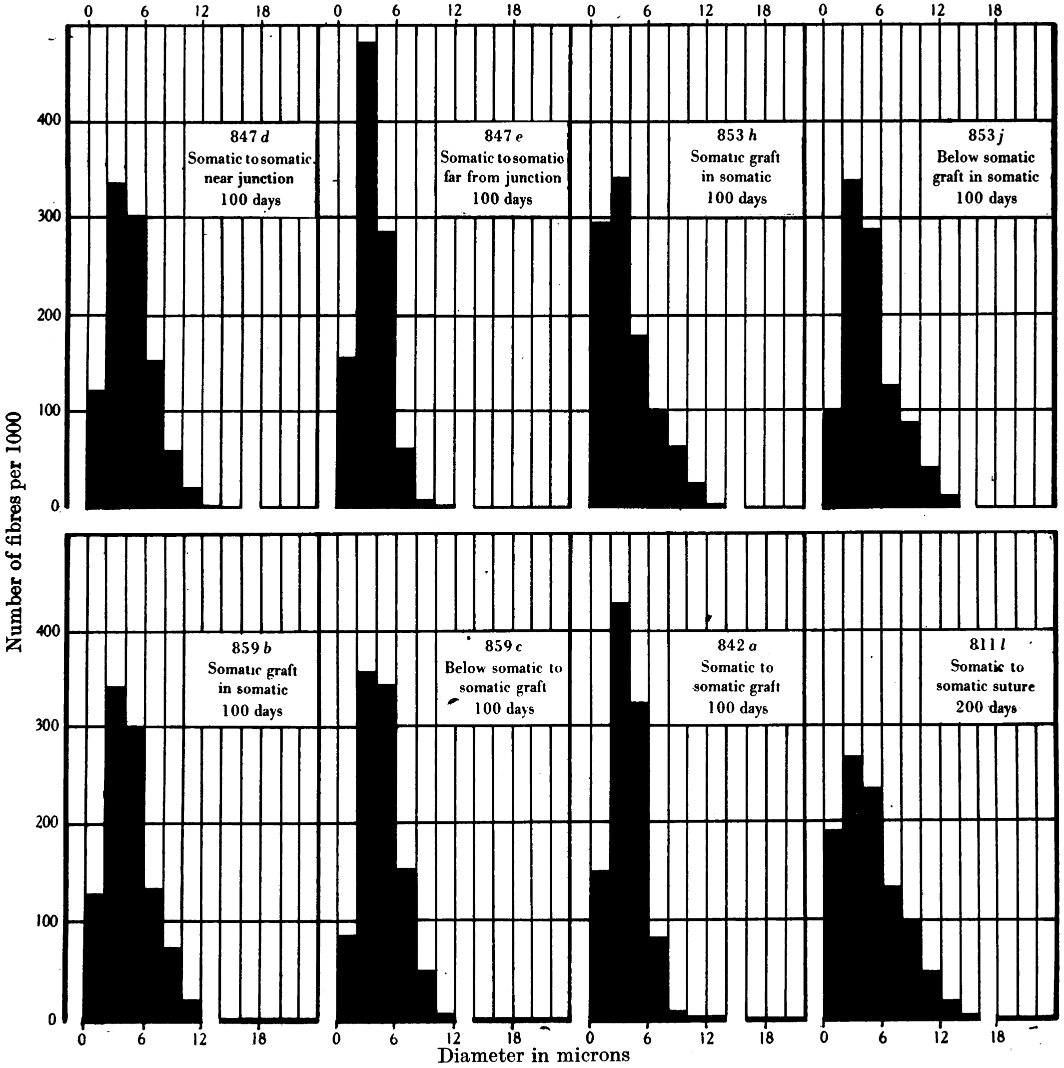
6. DIRECT UNION OF SOMATIC NERVES

Before drawing conclusions about cross-unions it is essential to discover the precise fibre composition appearing after the suture of one lumbar nerve into another. In making the cross-unions, long stretches of nerve are freed from their surrounding tissue, a procedure inevitably resulting in considerable damage to the nerve, both from its handling and from interference with its blood supply. A similar nerve mobilization was therefore performed in most of the control sutures, the nerves generally being united with their neighbours.

The nerve 847*d* (D 13) was cut close to its exit from the intervertebral foramen, turned backwards and united with the central end of D 14, cut at some distance from its foramen: 100 days later a good union was found (Pl. 1, fig. 5), and histological examination confirmed that, despite some criss-crossing, there was no marked separation of the stumps. The peripheral stump (Pl. 1, fig. 4) 10 mm. below the union contained 2256 fibres: i.e. about the number in a normal nerve. The proportion of the nerve area (33·3 %) occupied by medullated fibres was, however, much below normal, and of this 59 % only was contributed by fibres of larger diameter than 6 μ. The largest fibre was 16 μ only in diameter, compared with the normal 22 μ. No bimodality appeared in the distribution (Text-fig. 3) and the peak of the small-fibre group was at 2–4 μ, instead of the normal 4–6 μ.

A second count made some 2 cm. distally on the same peripheral stump (847*e*) (Text-fig. 3) showed a somewhat smaller number of fibres, the result probably of loss of branches rather than of failure of medullation. However, the proportion (16·7 %) of the nerve occupied by medullated fibres and the proportion (27 %) of this contributed by fibres greater than 6 μ were both much smaller than at a point immediately below the suture, the conclusion being that increase of diameter and medullation were proceeding progressively along the distal trunk (see Gutmann & Sanders, 1943).

This case shows that 100 days after suture medullation may be still far from complete. Later stages can be studied in 811*l*, where D14 was cut in such a way as to leave the two stumps held together by the epineurium, thus minimizing retraction. (This procedure favours regeneration in that the disturbances due to mobilization are avoided.) The nerves were removed 200 days later: longitudinal sections of the point of injury showed no marked separation of the stumps (Pl. 2, fig. 7): 10 mm. below this level (811*l*) there were 3733 medullated fibres distributed as shown in Text-figs. 3 and 4, and Pl. 1, fig. 6. The distribution shows no trace of bimodality, but the proportion of nerve area occupied by medullated fibres is greater than at 100 days after suture, and the proportion (73 %) of this area occupied by fibres greater than 6 μ is also greater, though it remains less than the corresponding proportion in a normal nerve. The largest fibres present remain smaller than those in a normal nerve. At 2 cm. below the injury (811*j*) the total fibre area and the area of fibres greater than 6 μ diameter are little less than at the more proximal level. If the diameters of the fibres are still increasing the gradient along the nerve is less steep than at the 100-days stage.



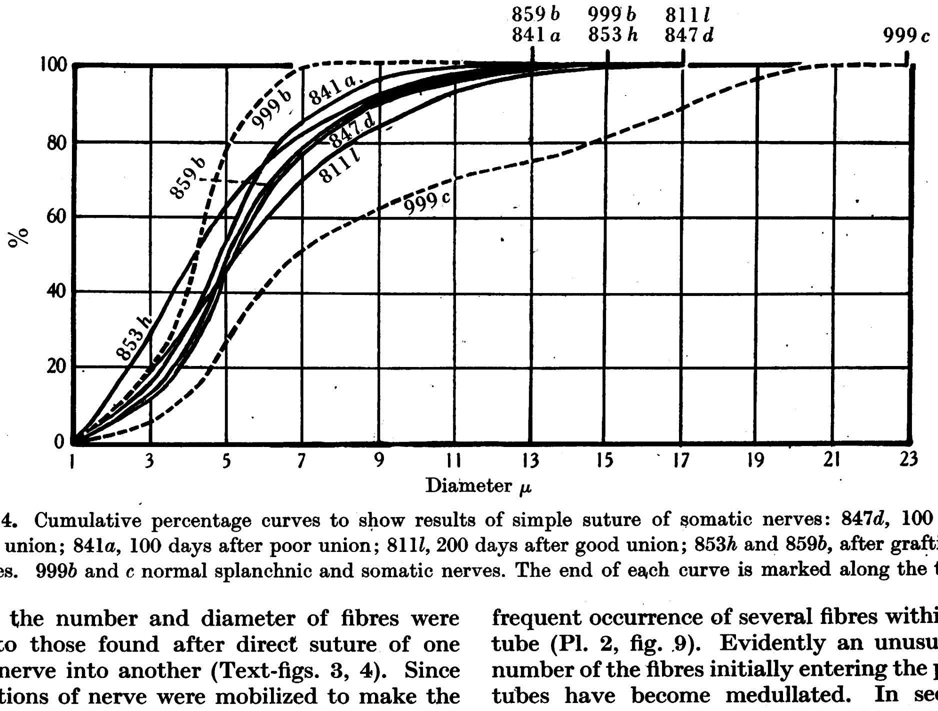
Text-fig. 3. Histograms of peripheral stumps after various direct unions between somatic central and peripheral stumps. Except where stated all counts taken 10 mm. below the union.

In a further case of suture, D12 as central stump was joined to D13, but sections of the region of union examined after 100 days (841*a*) showed considerable separation: it was not surprising therefore to find only 1116 fibres, occupying 9·5 % of the nerve area, with 44 % only of the medullated area occupied by fibres larger than 6 μ (Text-fig. 4): 2 cm. distally there were still fewer and smaller fibres. This case shows how a bad union may affect the degree of medullation.

Some of the cross-unions to be discussed later were made by taking free grafts of nerves. As a control in three cases grafts were made from one lumbar nerve into another. In two of these cases (853*h* and 859*b*) in which 2 cm. of D15 was grafted to D14, the number and diameter of fibres were similar to those found after direct suture of one lumbar nerve into another (Text-figs. 3, 4). Since long sections of nerve were mobilized to make the sutures it was to be expected that they would behave much like grafts.

The third graft, 842*a* (Pl. 2, fig. 9), was a piece of D13 joined proximally to D12 but distally to a piece of great splanchnic, whose distal end, in turn, was joined to the anterior mesenteric, the ganglion being removed. When examined 100 days later, this series of grafts showed a progressive reduction, passing peripherally, in the number and size of medullated fibres. Many such fibres occurred in the grafted piece of somatic nerve, some in the splanchnic nerve, but very few in the anterior mesenteric nerve. It was noteworthy that all the fibres in the grafted somatic nerve were small and very numerous: the total of 5158 is more than twice that found in the central stump itself, 842*c* (Pl. 2, fig. 8, and Text-fig. 2), which contained 2180 fibres of the somewhat reduced diameter characteristic of central stumps (Gutmann & Sanders, 1943).

It is well known that much branching of fibres occurs after severance of a nerve, and that many new fibres penetrate each of the tubes of a peripheral stump (Cajal, 1928; Holmes & Young, 1942). However, in previous investigations it has been found that the number of medullated fibres is not greater in the peripheral than in the central stump, unless the latter is the smaller (Dogliotti, 1933; Aird & Naffziger, 1939). In the present case the characteristic features are the smallness of fibres (occupying 26·6 % only of the nerve, of which 28 % only is contributed by fibres greater than 6 μ), and the frequent occurrence of several fibres within a single tube (Pl. 2, fig. 9). Evidently an unusually large number of the fibres initially entering the peripheral tubes have become medullated. In seeking the reason for the difference between this case and that of the other somatic grafts the most obvious explanatory factor is the mode of peripheral connexion made by the fibres. In this case alone among the somatic peripheral stumps studied were the fibres unable to reach their normal end-organs. Recently it has been found (Sanders & Young, 1945) that, when the axons of the peroneal nerve are interrupted by crushing high up, while lower down the nerve is cut so that the outgrowing fibres cannot reach their end-organs, a phenomenon occurs exactly comparable to the one under discussion: many small fibres are found in the tubes between the crushed point and the neuroma which forms at the severed end of the nerve. Weiss & Taylor (1944) have also shown that connection with the periphery increases the size of regenerating fibres.



Text-fig. 4. Cumulative percentage curves to show results of simple suture of somatic nerves: 847*d*, 100 days after good union; 841*a*, 100 days after poor union; 811*l*, 200 days after good union; 853*h* and 859*b*, after grafting somatic nerves. 999*b* and *c* normal splanchnic and somatic nerves. The end of each curve is marked along the top line.

A most important fact is thus revealed, that when regenerating fibres succeed in making peripheral contact they increase in diameter throughout their whole length, at the expense of others in the same tube. The forces which produce this effect are still unknown, but they must constitute some of the most important of those controlling regeneration.

Summary of effects of direct union of somatic nerves

The results give a reasonably consistent picture of the progress of medullation after simple union or after grafting of somatic nerve, which is summarized in the cumulative percentage curves of Text-fig. 4. The curves are approximately similar in the various regenerated nerves and show that the proportion of the larger fibres present is less than that in a normal somatic nerve but much greater than that in a normal splanchnic nerve. If union has been good and connexion made with the periphery, 100 days after operation the largest fibres 10 mm. below the union may be some 16 μ in diameter and about 60 % of the total medullated fibre area made up by fibres greater than 6 μ: there is a considerable decline in diameter passing peripherally along the nerve. However, 200 days after the operation the fibres are both more numerous and larger, more than 70 % of the area of medullation being made up by fibres above 6 μ, and the decline of fibre diameter along the distal stump being less steep: the nerve remains subnormal in condition, in particular without any reappearance of bimodal distribution.

7. DIRECT UNION OF SPLANCHNIC NERVE

Before assessing the results of cross-unions it is desirable to know the fibre composition resulting in the peripheral stump after the great splanchnic nerve has been cut and the ends rejoined. The nerve being small, the operation is difficult, and we obtained but one satisfactory experiment, 854*e*, in which the nerve was first cut almost completely and the remaining strand then crushed thoroughly. This procedure should have provided optimal conditions for regeneration, but unfortunately no longitudinal sections were prepared to show conditions at the point of injury. However, sections of the peripheral stump showed the presence, 100 days after operation, of 350 medullated fibres occupying 6·6 % of the total area of the nerve: only 5 % of this medullated area was made up of fibres greater than 6 μ, whilst the largest fibre in the nerve was 10 μ only in diameter (Pl. 2, fig. 10). From the uncertain nature of the union in this case we cannot be sure that it represents a typical result of simple great splanchnic regeneration, but it suggests that, as would be expected, small fibres only are regenerated.

8. DIRECT UNION OF ANTERIOR MESENTERIC NERVES

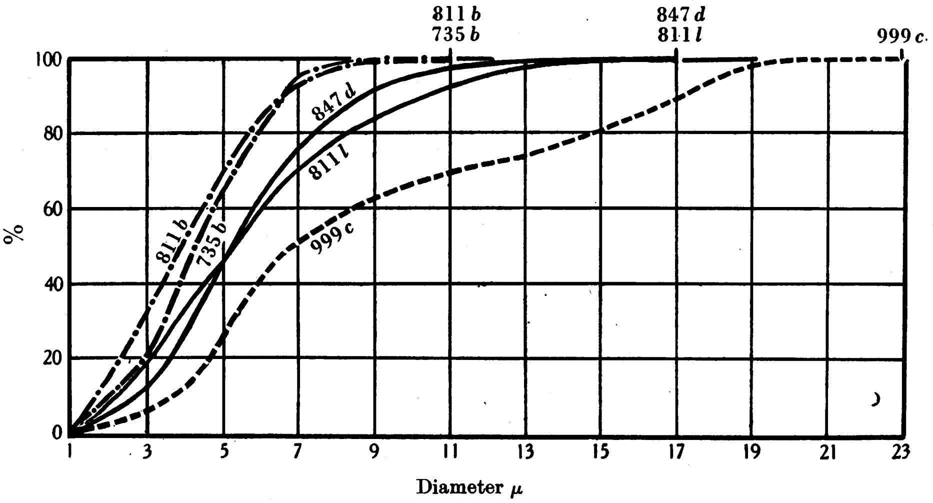
The anterior mesenteric nerves are such irregular bundles that it was decided not to attempt to sever them completely and to make a union. Instead, a single cut was made across the greater part of the bundle, just distal to the ganglion. No great retraction occurred, and when the site was examined 100 days later there was no obvious swelling or other sign of disturbance. Longitudinal sections showed that the cut had been made through the distal part of the ganglion, leaving a few nerve cells on its peripheral side. This, together with the fact that not all of the bundles were severed, means that some of the fibres had not undergone any interruption, although undoubtedly the majority had done so. The cross-sections of the distal stump 853*m* (Pl. 2, fig. 11), stained by Weigert's technique, showed bundles very similar to those of the normal anterior mesenteric nerves, containing in all eighteen medullated fibres, occupying less than 0·1 % of the area of the nerve. The average diameter of these fibres was 1·5 μ only and none was larger than 6 μ.

9. UNION OF LARGE CENTRAL FIBRES WITH SMALL PERIPHERAL TUBES

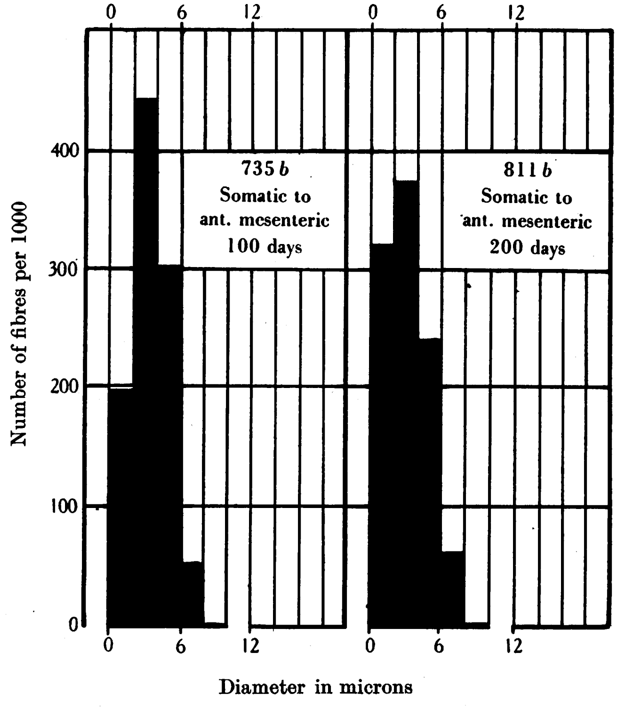
(1) *Union of somatic with anterior mesenteric nerves.* This most interesting union was made successfully in three cases, all of which agree in demonstrating that medullated fibres can be produced in a non-medullated post-ganglionic nerve, but that such medullated fibres are smaller than those formed when large fibres grow into large tubes.

In 735*b* the anterior mesenteric nerves were cut distal to the ganglion, and sutured to the mobilized central end of D12. The ganglion itself was left in place, and sections of the material removed 100 days later showed the somatic nerve running past a large mass of nerve cells into a region of mixed nerve cells and bundles, from which the main anterior mesenteric nerves arose. There was slight separation of the stumps, varying for the different bundles: some were so close to the central stump as to constitute a group 1 union, others showed a separation classed as a group 2 union.

The bundles in the peripheral stump were well filled with small medullated fibres (Pl. 3, fig. 14). In all the bundles included in the cross-section there were 1160 fibres, but these were mostly small and occupied 4·1 % only of the nerve area: moreover, few of them were larger than 6 μ, representing 20 % only of the total medullated area (Text-figs. 5, 6). Since there are normally very few medullated fibres in this nerve it is evident that nearly all those found therein had formed in Schwann tubes previously devoid of them. Presumably the myelin is formed in the usual manner in conjunction with Schwann



Text-fig. 5. Cumulative percentage curves to show small diameter attained in the peripheral stump by fibres following suture of somatic into anterior mesenteric nerves: 735*b*, after 100 days; 811*b*, after 200 days. 847*d* and 811*l* show peripheral stumps 100 and 200 days after direct somatic-somatic suture. 999*c*, normal somatic nerve.



Text-fig. 6. Histograms of distribution of medullated fibres in anterior mesenteric nerves after union with somatic nerves.

cells. The most likely supposition is that the 'cells of Remak' of the typical non-medullated fibre, presumably of similar origin to Schwann cells, when supplied, *inter alia*, with axons of suitable diameter, can assist in myelin formation. Another possibility is the migration of the Schwann cells from the central stump, but this is very unlikely because the stretch of medullated nerve examined was at least 1 cm. long, and Schwann cells are probably not very actively migratory when in contact with an axon.

Rabbit 833*a* showed a similar state of affairs when examined 100 days after suture. In this specimen the anterior mesenteric nerves were cut farther from the ganglion, which was almost completely removed. Longitudinal sections confirmed excellent union with the anterior mesenteric bundles (Pl. 2, fig. 12), which were full of medullated fibres. The bundles became curled in fixation and no satisfactory transverse sections could be obtained for counting; their general composition, however, was evidently very similar to that in 735*b*, none of the fibres being larger than 6 μ. The union was here so good that the small size of the new fibres cannot be ascribed to any difficulty in crossing from one stump to the other.

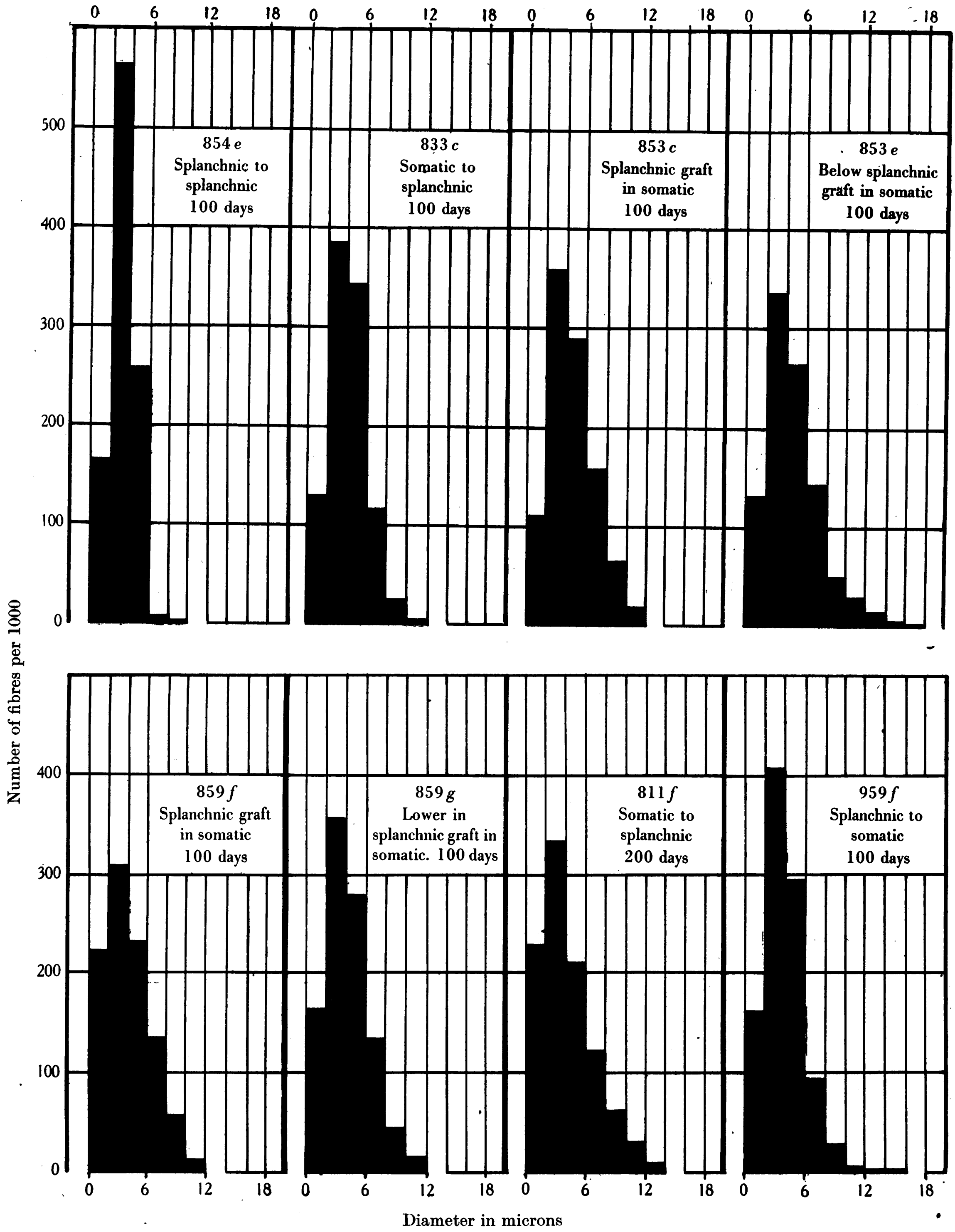
These two instances show that the large fibres can produce medullated fibres when joined with the tubes of even very small non-medullated nerves. The restriction on diameter increase persists during the second 3 months after union. Nerve 811*b* was examined 200 days after a successful union of D13 with the anterior mesenteric nerves, the ganglion being turned aside. The union was not quite so close as in 833*a*, and was graded as 2: the bundles in the peripheral stump contained a total of 2173 small medullated fibres, little larger than those encountered after 100 days' regeneration. They occupied 16·2 % of the nerve area, and only 25 % of this was made up of fibres larger than 6 μ, the largest fibres being 10 μ only in diameter. These figures may be compared with those for the direct suture of somatic with somatic nerve (811*l*) and of somatic with splanchnic (811*f*), made in the same animal (Text-fig. 9). In seeking the factor responsible for this limitation of diameter it must be remembered that in the cross-unions of somatic into visceral nerves the fibres effect no normal functional connexions at the periphery, and that there is evidence that, in the absence of such connexions, the rate of increase of fibre diameter is low. The greater size of the fibres in 811*l* than in the other two nerves may be partly due to this factor. However, the somatic fibres in the anterior mesenteric, 811*b*, are smaller than those in the splanchnic, 811*f*, though in neither of these nerves have they normal peripheral connexions. Similarly, they are smaller in 735*b*, an anterior mesenteric peripheral stump, than in 833*c* or 842*a*, splanchnic and somatic, which have also received somatic fibres for 100 days without permitting these to reach normal peripheral connexions. There is thus clear evidence that the small size of the tubes in the anterior mesenteric nerves exercises a restrictive influence on the increase in diameter of regenerating fibres within them, irrespective of the terminal connexions.

(2) *Union of somatic with splanchnic nerves.* This cross-union was made in one animal leaving the ganglion attached to the lower end of the splanchnic nerve, and in another animal after partial ganglionectomy. In two further animals, pieces of splanchnic nerve were used as grafts in a somatic nerve, thus allowing the regenerating fibres to establish normal peripheral connexions after passing through the graft.

In 833*c*, nerve D13 was joined to the splanchnic, and the main solar plexus ganglia were removed: union was found to be moderate, grade 2 (Pl. 3, fig. 17). The central stump being larger than the peripheral, some of the fibres emerging from the former made accessory bundles extending a short distance along the latter. The peripheral stump contained 1306 medullated fibres, occupying 14·0 % of the nerve area: the largest fibre was 12 μ only in diameter, and the area of those greater than 6 μ was 40 % only of the total medullated area (Text-figs. 7, 8, and Pl. 3, fig. 16). It was not possible to determine the peripheral connexions of these fibres; presumably most ended blindly at the lower cut end of the splanchnic nerve, whilst others perhaps reached the adrenal. Evidently the somatic fibres had produced in the splanchnic nerve new fibres considerably larger than those normally resident there. Though these new fibres were not nearly so large as those found in a somatic peripheral stump connected with the periphery (847*d*) they were only little smaller than the fibres found in the somatic stump 842*a*, which was not allowed to make connexion with the normal end-organs.

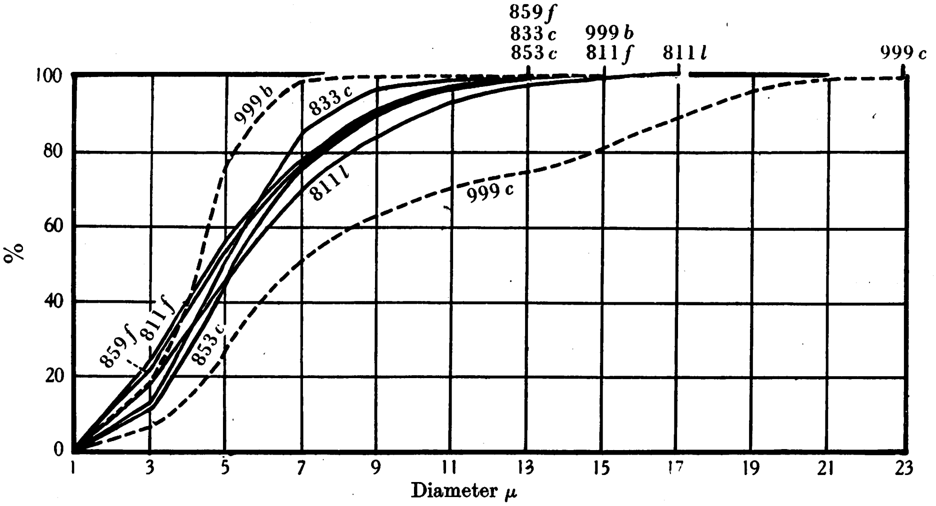
Examination was made of 811*f* 200 days after a very good union of D12 with the splanchnic nerve, the ganglion being left intact: 1433 medullated fibres (considerably larger than those of 833*c*) were present, occupying 41·0 % of the nerve; 65 % of the medullated area was made up of fibres larger than 6 μ, the largest being 14 μ. The connexions made by these fibres in the solar plexus and the adrenal remain uncertain. The fact that the fibres were smaller than those in the somatic peripheral stump 811*l* (see Text-fig. 9) may have been due partly to the influence of the peripheral connexions, partly to the constricting influence of the small splanchnic tubes.

The two instances of grafted great splanchnic nerve show very clearly that large fibres can be formed in even small tubes. The more successful was 859*f*, placed in D13: apposition was good at the upper junction for most of the bundles, and the union was classed as 1–2. The great majority of the



Text-fig. 7. Histograms of peripheral stumps 10 mm. below cross-unions involving the great splanchnic nerves.

fibres ran within the graft, but a few bundles extended some distance alongside it: such accessory bundles became scarcer distally, and few extended the whole 15 mm. length of the graft. Counts made at two levels in the graft showed 1493 and 1239 fibres respectively with nerve areas 22·8 and 17·3 %, 64 and 54 % being made up by fibres greater than 6 μ. (The distributions are shown in Text-figs. 7 and 10.) Comparison with the figures for 859*b*, in the same animal, where a piece of somatic nerve was used as the graft, shows no very marked differences. Moreover, the fibres are considerably larger than those of 833*c*, a piece of splanchnic nerve containing somatic fibres not connected with their normal end-organs. Evidently therefore the tubes of 859*f* have become dilated under the influence of the large somatic fibres having normal peripheral connexions. This result was confirmed in the second grafted piece of great splanchnic nerve, 853*c*, though in this case the closeness of the union was not studied. The figures in Table 1 and in Pl. 3, fig. 18, show that the fibres present in this graft were similar to those in piece of somatic nerve (853*h*) used as a graft in the same animal (Text-fig. 10).



Text-fig. 8. Cumulative percentage curves to show distribution of fibres 10 mm. below union of somatic into splanchnic nerves. 833*c*, 100 days after the union. 859*f* and 853*c*, 100 days after grafting pieces of great splanchnic into a somatic nerve. 811*f*, 200 days after union. 999*b* and *c*, normal splanchnic and somatic nerves for comparison.

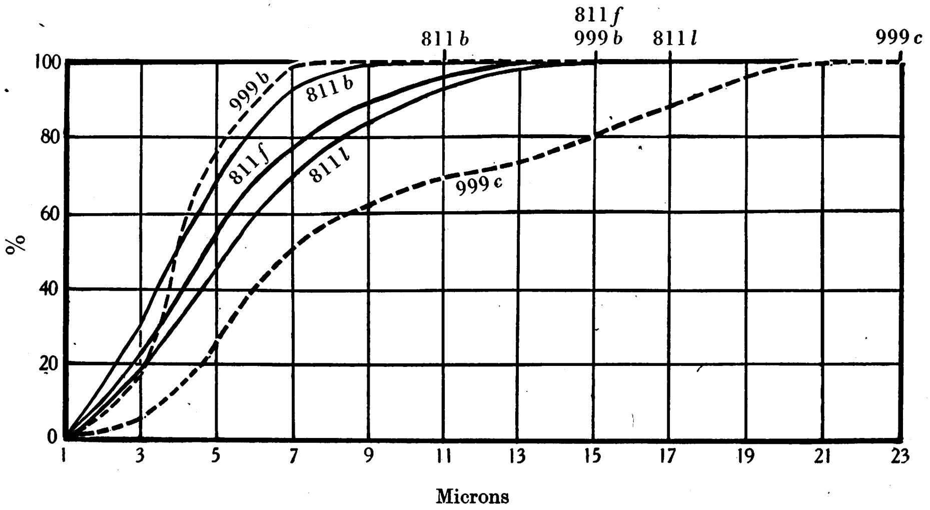
(3) *Union of splanchnic with anterior mesenteric nerves.* The one experiment (841*d*) in which this union was made produced sixty-nine small medullated fibres in the peripheral stump after 100 days: i.e. a number definitely in excess of that normally found in the anterior mesenteric, but much less than that obtaining in the splanchnic. The occurrence of fewer medullated fibres after this experiment than after innervation of the anterior mesenteric by a somatic central stump shows the influence of the central stump upon regeneration. On the other hand, the fewness of medullated fibres compared with the number produced in 854*e* by innervation by the splanchnic of its own peripheral stump shows again the restrictive effect of the peripheral tubes, although it must be remembered that the terminal connexions are also different in the two cases.

10. SMALL FIBRES GROWING INTO LARGE TUBES

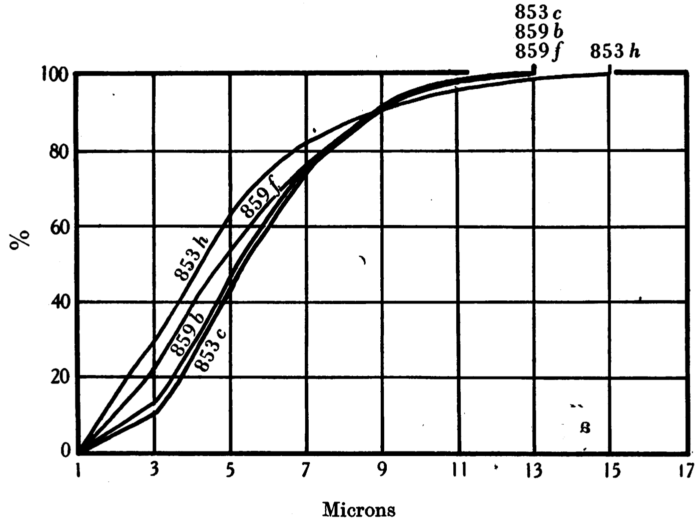
(1) *Union of great splanchnic with a somatic nerve.* The actual operation was not difficult, but a serious source of error arose from the point of union lying on the psoas muscles, which contain bundles of nerve fibres. The peripheral stump being larger than the central, there was consequent opportunity for complicating fibres from extraneous somatic nerves to invade the union. In one case such invasion certainly took place and the experiment was disregarded.

In a second case, 959*f*, examined after 100 days, a good union of splanchnic central and somatic peripheral stumps had been made and undoubtedly most of the fibres in the latter came from the former. However, muscle containing nerve fibres surrounded the point of union: some of these fibres were proceeding from the somatic nerve to the muscle, but the possibility that others were passing in the opposite direction could not be excluded.

Transverse sections (Pl. 4, fig. 20) showed larger fibres in the peripheral than in the central stump. The former contained, 10 mm. below the junction,



Text-fig. 9. Cumulative percentage curves of distribution of fibre sizes 10 mm. below union of somatic nerves into anterior mesenteric (811*b*), great splanchnic (811*f*) and somatic nerves (811*l*) 200 days after operation. 999*b* and *c*, normal splanchnic and somatic nerves for comparison.

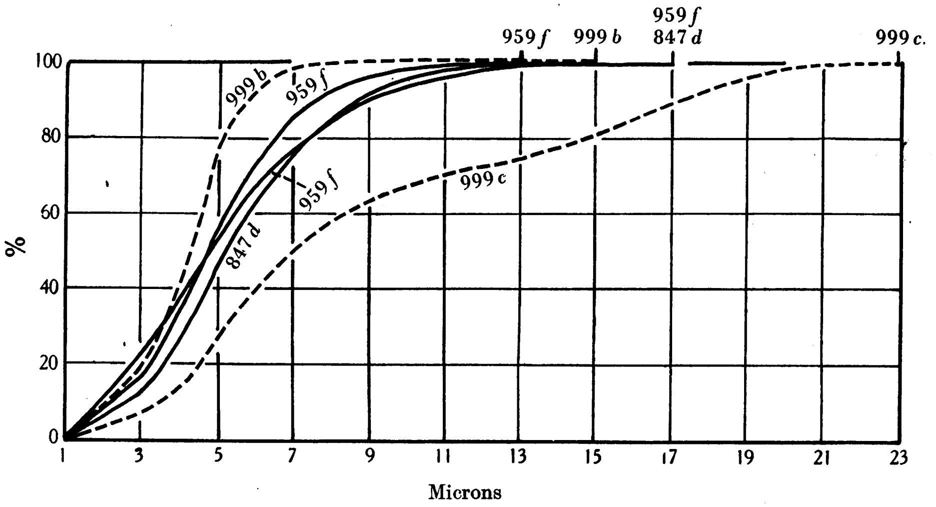


Text-fig. 10. Cumulative percentage curves of distribution of fibres in grafts 853*h* and 859*b* of somatic into somatic nerves, 853*c* and 859*f* of splanchnic into somatic nerves. 100 days after operation. No clear effect of tube size on regeneration is apparent.

1218 fibres, having a myelin area representing 17·7 % of the total nerve area, 42 % of this myelin area being made up by fibres larger than 6 μ. More distally (959*h*) the figures were not greatly different. The central stump fibres were not counted and measured, but appeared to be, if anything, smaller than those in a normal splanchnic nerve. It seemed therefore that many of the fibres in the peripheral stump were larger than their central parent axons (cf. 999*b* and 959*f* in Text-fig. 11), a conclusion to be accepted with reserve until its confirmation by future experiments in which all possibility of extraneous fibre invasion has been excluded.

In this experiment electrical or mechanical stimulation of the nerve, either above or below the union, caused contraction of the body-wall muscles. The threshold was higher above the union, as would be expected *ceteris paribus* if the fibres there were smaller. This result agrees with that of Langley & Anderson (1904), who recorded contraction of the thyro-arytenoid muscle after suture of the preganglionic fibres of the cervical sympathetic into the peripheral stump of the recurrent laryngeal nerve, and of the sterno-mastoid muscle after a similar suture of the cervical sympathetic into the spinal accessory nerve.

A further case, in which the diameter of fibres appeared to be greater in the peripheral than in the central stump, was revealed by measurements made of the peripheral stump below the graft of a piece of great splanchnic nerve into a somatic nerve. In Text-fig. 7 the histogram of 853*e*, from below the graft, shows fibres larger than those in 853*c* within it. Here again, however, the possibility of invasion of the lower junction by somatic fibres cannot be altogether excluded.



Text-fig. 11. Cumulative percentage curves of distribution of fibres after union of splanchnic with somatic (959*f*), somatic with splanchnic (859*f*) and somatic with somatic nerves (847*d*). All after 100 days. 999*b* and *c* normal splanchnic and somatic nerves.

(2) *Union of anterior mesenteric with a somatic nerve.* This union was made very easily. The anterior mesenteric nerves were cut as far distally as possible, freed with the distal part of the ganglion, and turned laterally to meet the mobilized distal stump of a somatic nerve. The apposed nerve ends were remarkably well united by plasma. The danger of invasion by extraneous fibres was minimized by effecting the operative union at some distance from the psoas musculature.

In 731*c*, the anterior mesenteric ganglion was almost isolated during the operation, with consequent severance of most of the fibres to it from the splanchnic nerves: union between the anterior mesenteric nerves and D 13 was very close. Examined 100 days after union, the peripheral stump contained eleven very small medullated fibres only, representing less than 0·1 % of the area of the whole nerve. Yet successful union was proved by the presence of very many non-medullated axons in each of the tubes of the peripheral stump.

An essentially similar result after 100 days was given by 834*a*, despite a union not so satisfactory: the peripheral stump contained nineteen small medullated, and very many non-medullated, fibres (Pl. 4, fig. 21).

In two further cases (854*a*, 959*a*) the union had made contact with the traumatized muscles and medullated fibres were found, in one case as a single bundle, in the other scattered throughout the peripheral stump (see Table 1).

Anterior mesenteric fibres made to grow into somatic nerves were in two animals left to regenerate for 200 days: in one of these animals very few, and in the other no, medullated fibres appeared, yet the unions were moderately well made and non-medullated fibres were abundant in the peripheral stumps of both (Pl. 4, fig. 23). These fibres seemed slightly larger than those seen after 100 days, but they are too variable to confirm this impression by measurement. Certainly fibres of the anterior mesenteric nerve are not able to increase in diameter even when made to grow into large tubes: they remain as numerous small fibres mostly in contact with the walls of the host tubes.

In all these experiments the nerves were stimulated electrically and mechanically before fixation. Only in the cases in which invasion from somatic motor fibres had occurred (854*a*, 959*a*) did this produce contraction of the mm. obliqui. The inability of the abundant non-medullated post-ganglionic fibres present in the other nerves to establish functional connexion with the muscle fibres agrees with the finding of Langley & Anderson (1904) that post-ganglionic cervical sympathetic fibres after union with the hypoglossal nerve were unable to cause contraction of the tongue. Unfortunately, these experiments are insufficient of themselves to determine whether this failure to establish muscle connexion is due to some qualitative difference between different fibre types or simply to the very small size of the post-ganglionic fibres.

(3) *Union of anterior mesenteric with great splanchnic.* In one experiment (848*g*) the anterior mesenteric fibres were made to grow into the great splanchnic nerve, thus making a circle in which the processes of the ganglion cells returned to those cells. As in the last experiment, the anterior mesenteric fibres grew out abundantly but failed to become medullated. The endings made by the fibres when in contact with their own cell bodies have not yet been studied.

DISCUSSION

The results here submitted show that the control of fibre diameter in a regenerating nerve is exercised by at least three factors: (1) the diameter of the central fibres, (2) the nature of the terminal fibre connexions made, (3) the size of the peripheral tubes. It is possible that a fourth factor, not investigated here, is the length of nerve to be regenerated. The influence of the central stump is well shown by the fact that somatic fibres growing into post-ganglionic trunks, previously non-medullated, can produce therein medullated fibres. Further, the larger somatic fibres are able, when growing into a splanchnic nerve, to produce in it fibres larger than are normally present, even without effecting typical peripheral connexions: this result is similar to one obtained by Langley & Anderson (1904), who found after union of cervical somatic nerves with the superior cervical sympathetic trunk that the latter came to contain fibres larger than are normally found therein.

The converse effect of the central stump is shown by the inability of splanchnic nerve fibres to make, in the large tubes of a somatic nerve, fibres equivalent in size to those produced by regenerating somatic fibres (Text-fig. 11). An even more striking but comparable phenomenon is the inability of the post-ganglionic fibres of the anterior mesenteric ganglion to produce medullated fibres when growing into the large tubes of a somatic nerve. The difference in behaviour between the processes of sympathetic cells and those of somatic sensory and motor cells may be associated with the multiplicity of branches of the sympathetic cell, or with some qualitative difference of the axoplasm.

That the peripheral connexion exerts an influence on the diameter of the fibres in the regenerating nerve appears clearly from the present experiments. If a somatic nerve is sutured and also cut peripherally so that the regenerating fibres cannot reach their end-organs the fibres in that nerve will be more numerous and smaller than after simple suture. In such a nerve with an obstructed lower end many small fibres occur in each tube and evidently an unusually large number of the initial invading sprouts become medullated. The unions between somatic central and splanchnic peripheral stumps provide a very satisfactory confirmation of this effect. Where the somatic fibres grow directly into the splanchnic nerve, left *in situ*, they produce fibres smaller than those found when a piece of splanchnic nerve is used as a graft in a somatic nerve, the fibres in the latter case being able to reach normal peripheral endings. Very similar effects of the peripheral influence on medullation have been found in the nerves of the hind limb (Sanders & Young, 1945). It remains to discover how this influence is exerted.

Since the terminal connexions affect the diameters attained by regenerating fibres it is possible to assess the degree of restriction exercised by the small-sized peripheral tubes only by comparing nerves which end similarly. Although the experiments were not planned with the possibility of a terminal influence in mind, they provided several suitably comparable cases, which demonstrate that the small tubes of the anterior mesenteric nerves restrict the growth of somatic fibres within them. There is therefore good evidence that very small tubes, such as those of a non-medullated nerve, restrict the growth of regenerating fibres. On the contrary, the pieces of splanchnic nerve used as grafts in a somatic nerve, in such a way that the fibres were enabled to reach the periphery, came to contain fibres nearly, or quite, as large as those occurring in pieces of somatic nerve similarly grafted. The restrictive influence of moderate-sized tubes is therefore not severe. Though further evidence on this point is desirable, the indications are that, in man, a piece of cutaneous nerve used as a graft in a motor nerve would not unduly restrict the growth of the motor fibres. Any restrictive influence might also be of practical importance in connexion with the shrinkage of the tubes of a peripheral stump left for long without innervation. Holmes & Young (1942) and Sanders & Young (1944) found evidence of delay in medullation under such conditions; possibly the extra collagen developed in the tube walls limits their extensibility. The tubes in the present experiments were refilled within a few weeks of degeneration.

The limiting effect of small tubes on regeneration is shown by the thinness of the myelin developed. All measurements so far given have represented the outside diameter of fibres. However, comparison of Pl. 1, fig. 6 with Pl. 2, fig. 15 and Pl. 3, fig. 19, shows that when large fibres inflate small tubes they produce a myelin sheath which is thinner than that of a fibre of similar *total* diameter produced in a larger tube. We have not yet made measurements to study this effect further, or to discover whether terminal connexion affects myelin thickness. Clearly such inquiry is of the greatest importance in analysing the restrictive influence of the tube, and in elucidating the nature of the myelin sheath and the factors controlling its thickness.

SUMMARY

1. The diameter reached by regenerating nerve fibres depends on their effecting connexion with their end-organs. Nerves without terminal connexions become filled with many and small nerve fibres.

2. When fibres grow out from somatic nerves into the non-medullated anterior mesenteric nerves, medullated fibres are formed in the latter. Such fibres are, however, of smaller diameter and have thinner myelin than those formed after union of a somatic with a somatic or a splanchnic nerve.

3. When a somatic nerve, containing large medullated fibres, is united with the splanchnic nerve, containing small medullated fibres, the new fibres produced in the splanchnic nerve are of diameter similar to that produced in a somatic peripheral stump with similar terminal connexions. The large central fibres can therefore inflate the smaller peripheral tubes.

4. The size of new fibres is therefore controlled partly by the size of their parent fibres, but very small Schwann tubes in the peripheral stump have a restrictive influence on fibre growth.

5. The non-medullated fibres of the anterior mesenteric nerves do not produce medullated fibres when made to grow into the Schwann tubes of a somatic nerve.

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EXPLANATION OF PLATES

PLATE 1

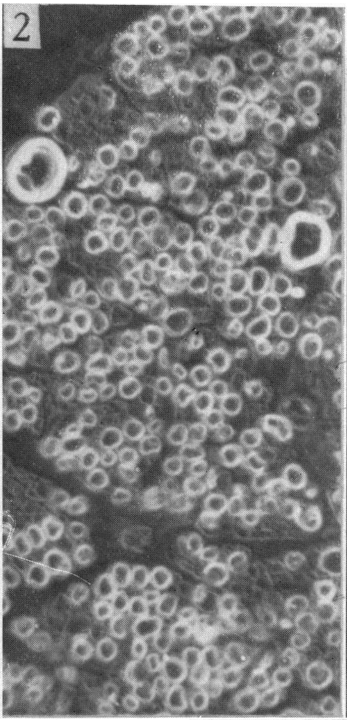
Fig. 1. Portion of normal somatic nerve (iliohypogastric, D13) of rabbit, showing large and small fibres (999*c*). This and all the similar figures are of material fixed in Flemming's fluid and stained with Weigert. The photographs are negatives, made by direct projection on to bromide paper, all at a magnification of × 750.

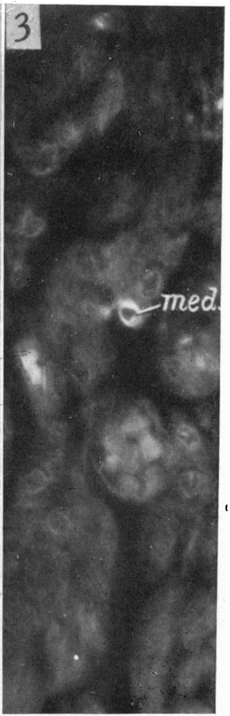
Fig. 2. Normal great splanchnic, mostly small fibres (999*b*).

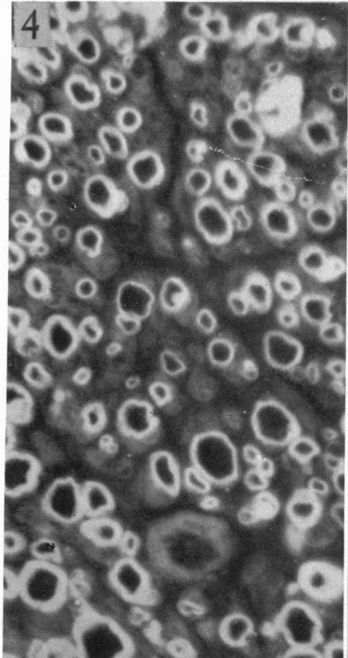
Fig. 3. Normal anterior mesenteric (732*a*). Only one very small medullated fibre (*med.*) is present.

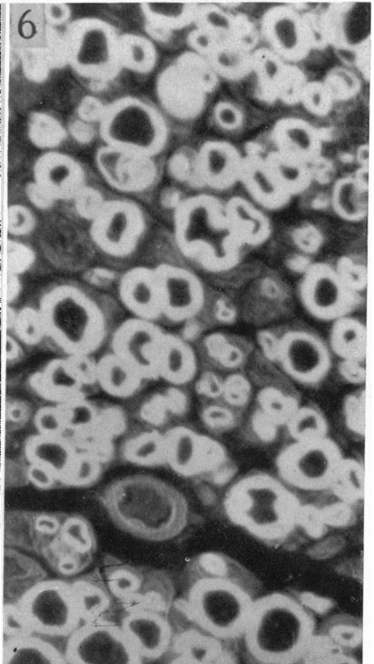
Fig. 4. Somatic nerve 100 days after simple union with its own central stump (847*d*). Except where stated all figures after suture are of transverse sections 10 mm. below the union.

Fig. 5. Longitudinal section of simple somatic union after 100 days (847*d*). In this and all the figures of unions the central stump is above.

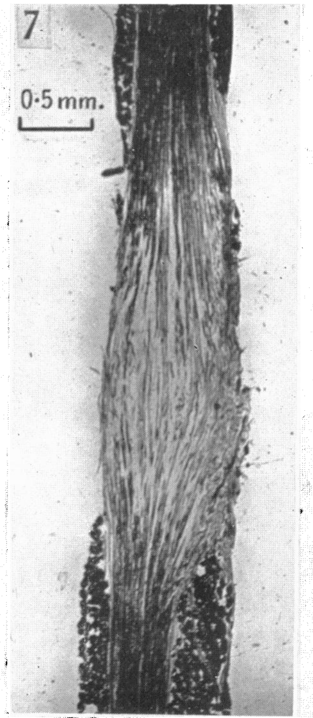


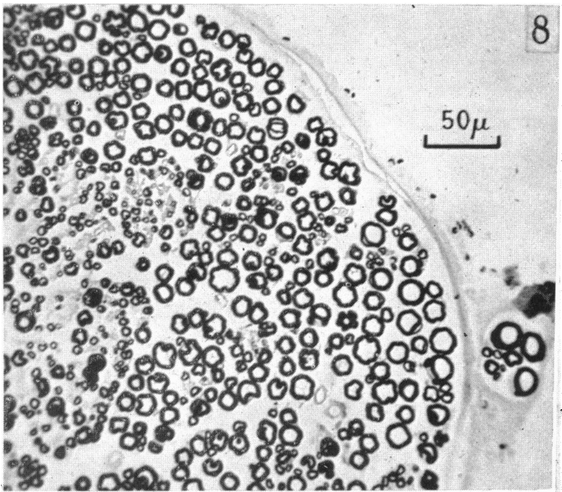


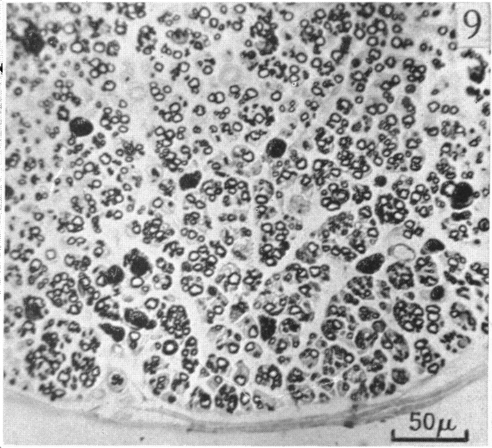


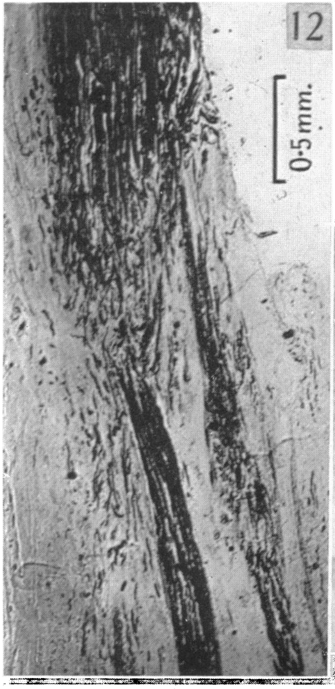


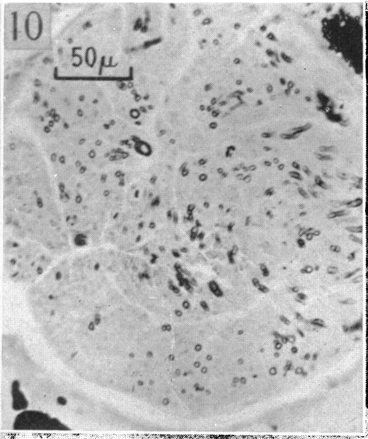
SIMPSON AND YOUNG—REGENERATION OF VISCERAL AND SOMATIC NERVES

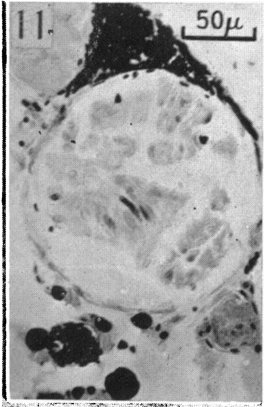












SIMPSON AND YOUNG—REGENERATION OF VISCERAL AND SOMATIC NERVES

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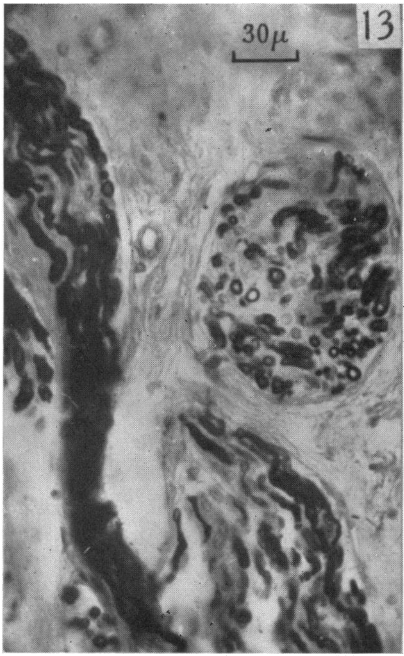

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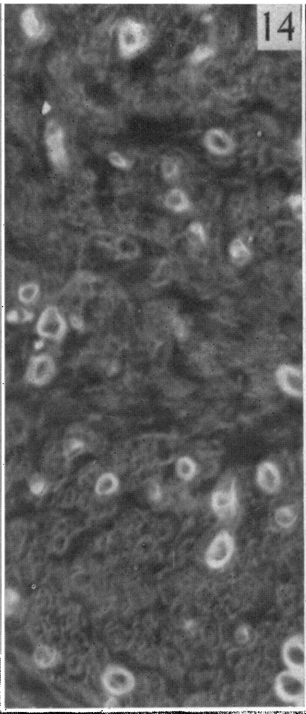

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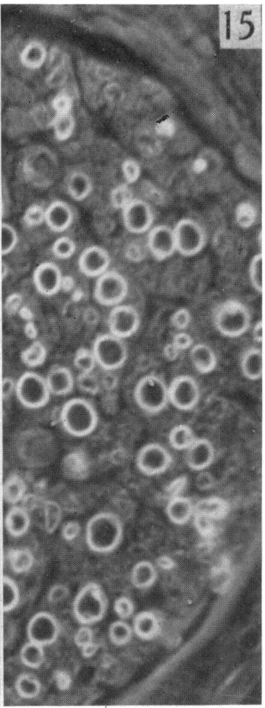

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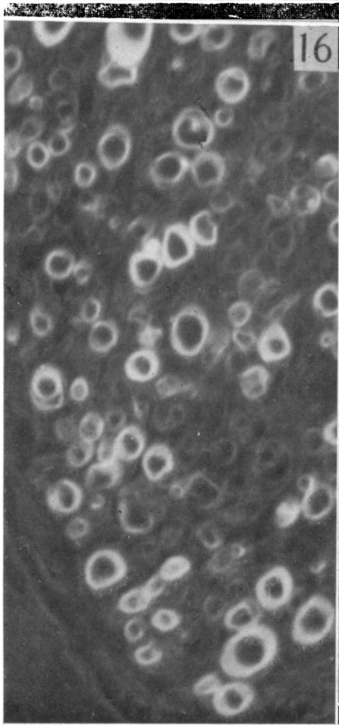

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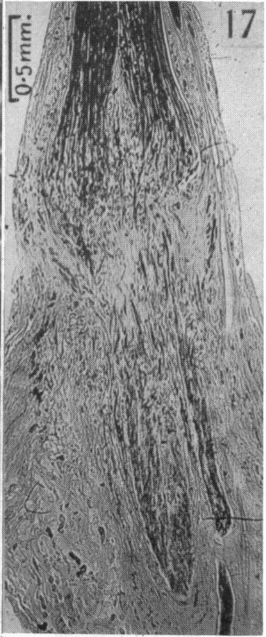

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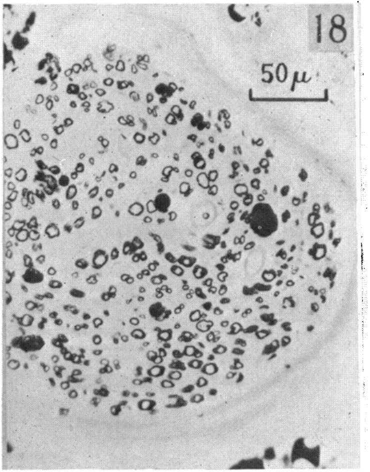

SIMPSON AND YOUNG—REGENERATION OF VISCERAL AND SOMATIC NERVES

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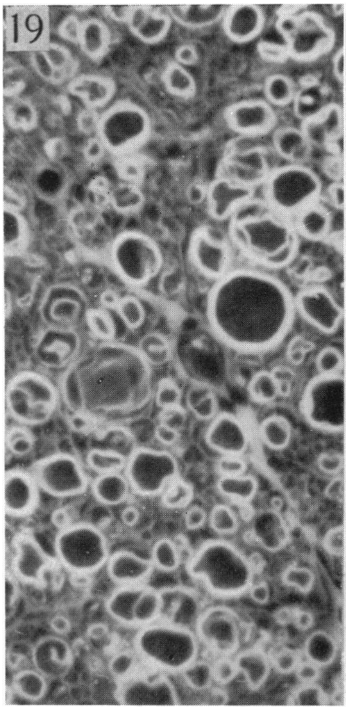

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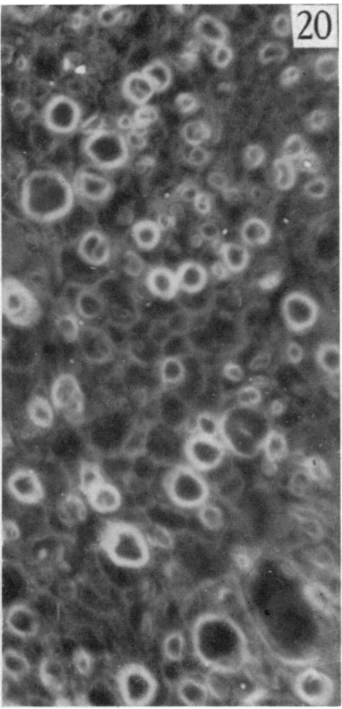

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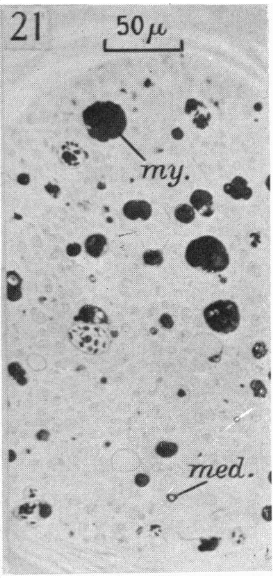

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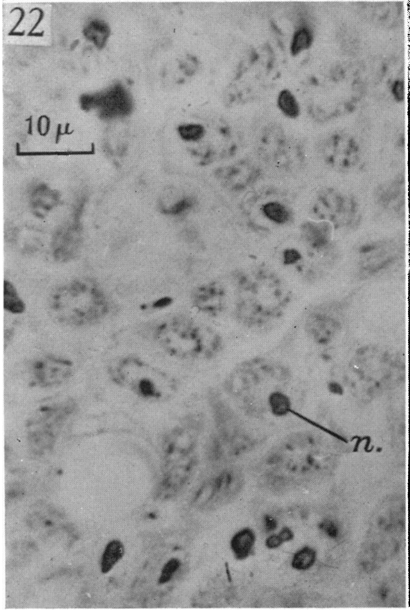

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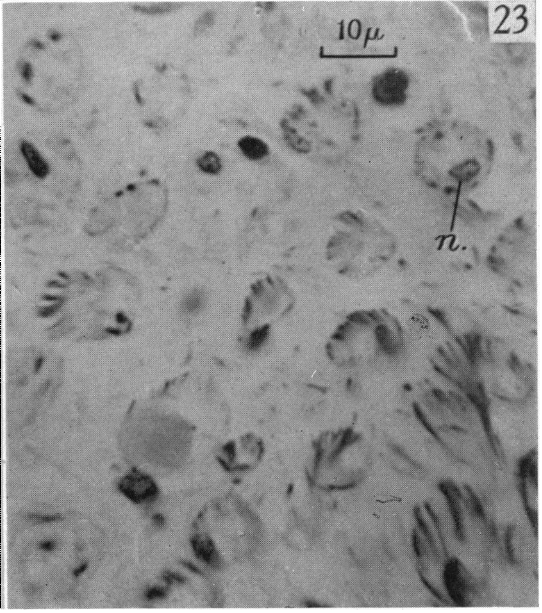

SIMPSON AND YOUNG—REGENERATION OF VISCERAL AND SOMATIC NERVES

Fig. 6. Somatic nerve 200 days after simple union with its own central stump (811*l*).

PLATE 2

Fig. 7. Longitudinal section of simple somatic union 200 days (811*l*).

Fig. 8. Central stump of somatic nerve 100 days after grafting to a piece of somatic nerve (842*a*).

Fig. 9. Portion of one somatic nerve grafted into another (100 days) (842*a*). Note very large number of fibres, often several in each tube. This is an unusual case in that there are more fibres in the peripheral than in the central stump.

Fig. 10. Splanchnic nerve 100 days after union with its own central stump (854*e*). Composition similar to that of normal nerve.

Fig. 11. Anterior mesenteric nerve 100 days after union with its own central stump (853*m*). Composition similar to that of normal nerve, containing only a few, small, medullated fibres.

Fig. 12. Low-power view of same slide as Pl. 3, fig. 13, to show union of a somatic nerve with the bundles of the anterior mesenteric, and medullated fibres in the latter (833*a*).

PLATE 3

Fig. 13. Anterior mesenteric innervated for 100 days by a somatic nerve (833*a*). Numerous small medullated fibres seen in transverse section and longitudinal section.

Fig. 14. Anterior mesenteric innervated for 100 days by a somatic nerve (735*b*). Many small medullated fibres present.

Fig. 15. Anterior mesenteric innervated for 200 days by a somatic nerve (811*b*). Fibres more numerous than after 100 days but not much larger.

Fig. 16. Splanchnic nerve innervated for 100 days by a somatic nerve (833*c*).

Fig. 17. Longitudinal section of union of somatic and splanchnic nerves (833*c*).

Fig. 18. Splanchnic nerve innervated for 100 days by a somatic nerve (853*c*).

PLATE 4

Fig. 19. Splanchnic nerve innervated for 200 days by a somatic nerve (811*f*).

Fig. 20. Somatic nerve innervated for 100 days by a splanchnic (959*f*). The fibres are larger than those of the splanchnic, but the possibility of invasion from the muscles must be borne in mind.

Fig. 21. Somatic nerve innervated for 100 days by anterior mesenteric (834*a*). At most a very few possible medullated fibres (*med.*). Numerous myelin remains (*my.*).

Fig. 22. Somatic nerve innervated for 100 days by anterior mesenteric (834*d*). Bodian stain showing unmedullated nerve fibres in the Schwann tubes. *n*, Schwann cell nucleus.

Fig. 23. Somatic nerve innervated for 200 days by anterior mesenteric (958*a*). Bodian stain. The unmedullated fibres are perhaps slightly larger than 100 days after union.