ON THE PRESENCE OF CENTRIPETAL FIBRES IN THE SUPERIOR MESENTERIC NERVES OF THE RABBIT

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On the basis of certain histological and physiological investigations it has been suggested that some of the fibres in the nerve bundles running in the intestinal mesenteries of the rabbit and the cat are centripetal in direction, having their parent cells somewhere in the intestinal wall, and forming synapses in the local mesenteric sympathetic ganglia. Kuntz & Saccomanno (1944) noted that, after sectioning of neurovascular bundles supplying the small and large intestines in the cat, some fibres remained intact in the nerves distal to the interruption. Kuntz (1938) also observed that there was some evidence of degeneration in the proximal segments of those nerves. In addition, he carried out histological studies on the coeliac ganglion (1938) and the inferior mesenteric ganglion (1940) of the cat, and demonstrated that some terminal arborizations in the ganglia remained after interruption of their central connexions.

More recently Brown & Pascoe (1952) have presented physiological evidence of reflex activity in the local mesenteric sympathetic ganglia in the rabbit. The inferior mesenteric ganglion with the ascending mesenteric nerve attached to its cranial pole was removed from the rabbit and placed in Locke's solution in a recording bath. They found that stimulation of the ascending mesenteric nerve produced an impulse that travelled to the inferior mesenteric ganglion. This was followed after a short interval by an outcoming impulse along the nerve. The latter was abolished by painting the ganglion with nicotine and by adding D-tubocurarine chloride to the bathing solution. The ingoing impulse was conducted at a rate of 0·25 m./sec. at 20° C., the outgoing one at 0·45 m./sec. These results suggest the presence of fibres ending in the ganglion, and originating in some region peripheral to the ganglion.

Also in 1952, Job & Lundberg obtained similar results in the inferior mesenteric ganglion of the cat by stimulating the hypogastric nerve after complete degeneration of the preganglionic spinal roots of the ganglion.

It is not known where those fibres proceeding to the inferior mesenteric ganglion via the ascending mesenteric nerve have their cell bodies. It has been suggested by McLennan & Pascoe (1954) that perhaps they are situated in the walls of the colon.

It is the purpose of the present investigation to discover if there are centripetally running fibres in the nerve bundles of the small gut mesentery in the rabbit, having their cell bodies situated in the periphery, and if there are, what percentage of the total fibre count they form. To establish this some of the neurovascular bundles in the mesentery of rabbits have been divided between ligatures and the animals allowed to survive for a period of 3 weeks, so that nerve fibres disconnected from their cell bodies degenerate fully. The neurovascular bundle distal to the transection was then removed and examined histologically for surviving nerve fibres.

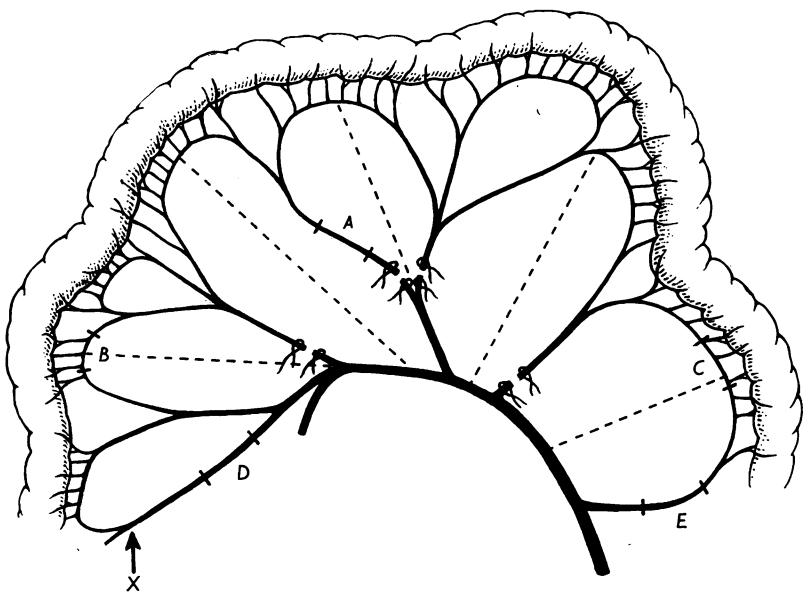
METHODS

Experimental procedure

The operations were performed on adult rabbits of various breeds. The anaesthesia employed was intravenous sodium pentobarbitone (nembutal) supplemented with ether.

Operation

In the first series of animals one to four adjacent neurovascular bundles in the small gut mesentery were doubly ligated near to the main trunk of the superior mesenteric artery, and divided between the ligatures. The cut ends of the bundles were tied together loosely in six animals to prevent excessive retraction, and to help in identification at autopsy. In four animals (4521, 4522, 4537 and 4539) this step was omitted. In addition, the mesentery on either side of each bundle was slit in its entire depth, leaving intact the distal loops of communication between adjacent main branches (Text-fig. 1). By this means a section of mesentery and its contained blood vessels and nerves was 'isolated' except for the communicating loops on either side (B and C in Text-fig. 1) and the length of small intestine supplied by the divided vessels.



Text-fig. 1. Key: A, distal segments; B, C, marginal segments; D, E, control segments; X, site of division of neurovascular bundles in second experiment. – – –, slit in mesentery; ——, neurovascular bundles.

In two animals (4384 and 4393) the main vagal trunks were divided at the cardia in addition to the above procedure.

When four adjacent branches were interrupted, the affected length of small intestine became slightly cyanotic. Division of less than four branches produced no such change.

The post-operative course in nine of the animals was smooth, and they showed

no apparent intestinal upset. Rabbit 4497 made a normal immediate recovery, but from the fifteenth day would not eat. Autopsy was performed on the sixteenth day, and it was then found that the segment of small intestine whose neurovascular bundles had been interrupted was markedly constricted and white. Elsewhere the intestine appeared normal macroscopically. It was not examined histologically. In this animal only two adjacent branches were divided.

The remaining rabbits were allowed to survive for 21 days to allow complete degeneration of those nerve fibres isolated from their cell bodies.

In a second series of animals three adjacent neurovascular bundles were divided between ligatures placed distally (X in Text-fig. 1). The ligatures were placed proximal to the distal loop of communication to ensure an adequate blood supply to the intestine. The mesentery on either side of the bundles was slit as in the first series. The cut ends of the bundles were tied loosely together.

These animals were left for 21 days before autopsy was carried out.

Autopsy

Fairly dense adhesions were present in the mesentery of nearly every animal, but the neurovascular bundles were dissected clear and identified without difficulty. Where the cut ends of the bundles had been tied together there was a bridge of adhesions between them. In those that had been left free, one or more distal lengths had retracted up to the intestine, and were altogether free of adhesions.

First series of rabbits. Short lengths of the neurovascular bundles distal to the division (A in Text-fig. 1) were excised, threaded through spinal cord previously obtained from the rabbit, and retained in a slightly stretched condition on a cardboard frame. Similar lengths were taken from intact neurovascular bundles and from the communicating loop on either side of the operation field (B and C in Text-fig. 1), and treated in the same manner.

The distal segments of mesentery that had retracted in rabbits 4521, 4522, 4537 and 4539 were selected for biopsy as it was possible that regenerating nerve fibres might have bridged the gap between the tied ends of the divided bundles. Some of these adhesions have been examined and none of them showed continuity of nerve fibres between the proximal and distal segments.

Second series of rabbits. Short lengths of bundles proximal to the division were excised, and treated similarly.

Staining

The bundles, together with the spinal cord were fixed in alcohol ammonia solution, stained by Ranson's pyridine silver method (Ranson & Davenport, 1931) and at the completion of the staining process both the cord and its contained bundle were embedded in paraffin and sectioned transversely as near 1–2μ thickness as was possible. It was found that in sections thicker than 3–4μ the nerve fibres were difficult to count accurately.

RESULTS

Histological findings

Full counts were carried out on all the specimens. Most of them contained a number of nerve bundles—varying from one to nine. The results of these counts are shown in Table 1 (first series) and Table 3 (second series).

The number of nerve fibres per unit area was also calculated by the following method:

Each bundle counted was projected on to a ground glass screen at a magnification of 1000, and traced on to tracing paper. The tracings were then cut out and weighed on a microbalance. Unit weight was taken to be the weight of 1 cm.^{2} of tracing paper. The results of these calculations are also shown in Tables 1 and 3.

Table 1. *Total counts and counts per unit area in first series of rabbits*

		Normals		Distal segments		Marginals	
Rabbit	Operation	Total no.	Per unit area	Total no.	Per unit area	Total no.	Per unit area
4348	Four adjacent branches divided proximally	287*	155	297	61	134	91
4384	One branch divided proximally + division of vagi at cardia	532*	222	99	45	116	92
4393	One branch divided proximally + division of vagi at cardia	1421	132	199	72	295	46
4394	Two adjacent branches divided proximally	1459	154	610	52	231	27
4497	Two adjacent branches divided proximally	1337	221	349	39	78	89
4521	Four adjacent branches divided proximally	1338	169	704	67	469	55
4522	Four adjacent branches divided proximally	1599	291	138	69	30	228
4530	Four adjacent branches divided proximally	1063	148	543	47	63	217
4537	Four adjacent branches divided proximally	2264	145	744	92	48	143
4539	Four adjacent branches divided proximally	4144	161	613	87	76	217

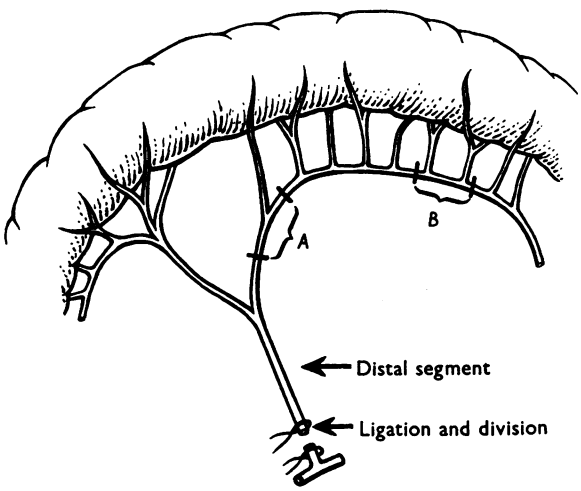
Normals: mean per unit area 174, standard deviation of mean 22.
Distal segments: mean per unit area 63, standard deviation of mean 6.
* These normal segments were taken distal to the division of a main branch of the superior mesenteric artery.

A study of Table 1 indicates a wide variation in the number of fibres in the control specimens, but the counts per unit area are more constant—132–291 with a mean of 174 (standard deviation of mean 22). It is interesting to note that in the normal nerve bundles, the individual nerve fibres are not very closely packed together. Some of the variation in this respect may be due to shrinkage or oedema of the specimens produced during the fixation and staining process. One or two of the nerve bundles examined show a clear zone round the periphery where the fibres had retracted away from the perineurium. In these cases the tracing followed the perineurium.

In making counts some difficulty was encountered, particularly in the control and marginal specimens where two or more nerve fibres were very close to one another. It was not always easy to tell whether there were two to three fibres present, or whether one was looking at a large single deposit of silver. This difficulty was obviated largely by using sufficiently thin sections, and it was found essential for accuracy to use only sections cut at less than 3–4 μ for counting purposes. It is not easy to prepare good sections at this thickness, and many of the nerve bundles tended to break up. Where this had occurred, closely adjacent thicker sections were

used for the calculations of the area. Occasionally one or more nerve bundles were cut obliquely. Usually recutting in a different plane corrected this error.

The total counts in the distal segments range from 99 to 744, and the counts per unit area from 39 to 92 with a mean of 63 (standard deviation of mean 6). In other words, the remaining intact fibres represent approximately 30 % of the total present in the control segments. These fibres are not evenly scattered through the individual bundles. Some of the latter contain many fibres and are apparently little different from the controls. On the whole, however, the decrease in density is obvious on examination. The majority of the intact fibres are of small calibre—probably 1 μ or less in diameter.



Text-fig. 2. Diagram to give possible explanation of varying counts per unit area in marginal bundles.

It is possible that some of those fibres remaining in the distal segments could be accounted for by nerve fibres entering from either side through the marginal nerves. The total counts of the marginal bundles are therefore of more significance than the counts per unit area. Table 1 shows that in only two animals (4384 and 4393) do the nerve fibres in the two marginal bundles outnumber those remaining in the distal segment. In all other rabbits it is clear that they cannot account for all the nerve fibres remaining unless one postulates branching of the axons in the marginal bundles.

Both the total counts and the count per unit area vary much more in the marginal nerves than they do in the control and distal segments. Some of the bundles have the appearance of normal nerves, and others of distal segments, and it seems that the appearance is dependent upon the exact site chosen for biopsy. When the segment lies close to the operation field (A in Text-fig. 2) the nerves are similar to the distal segments. Further away (B in Text-fig. 2) they look like the controls.

As far as was possible the segments of the marginal neurovascular bundles chosen for study were equidistant from the adjacent normal and divided neurovascular bundles (Text-fig. 2).

It seems, therefore, that the majority of the nerve fibres running in the marginal bundles travel with the short terminal branches of the vessels into the intestine, and do not retrace their path to pass centrally in an adjacent main neurovascular bundle. Presumably some of these fibres remaining in the marginal nerves that have the appearance of the distal segments are similar fibres to the intact ones in the distal segments. The counts per unit area of the marginal nerves are probably not accurate, as most of the bundles are very small.

Table 2 shows the total counts and the counts per unit area of normal marginal bundles.

The results obtained in the second series of rabbits are shown in Table 3. The counts per unit area vary between 68 and 156, with a mean of 100 (standard deviation of mean 8).

Table 2. *Normal marginal bundles*

Rabbit	Total No.	Per unit area
4825(A)	420	82
4825(B)	452	81
4825(C)	251	88
4831(A)	117	135
4831(B)	108	208

Table 3. *Total counts and counts per unit area in proximal segments following distal division*

Rabbit	Operation	Total count	Per unit area
4760	Three adjacent branches divided distally	1545	78
4761	Three adjacent branches divided distally	1538(A)	93
		1459(B)	95
4829	Three adjacent branches divided distally	2638	156
4843	Three adjacent branches divided distally	1502	113
4844	Three adjacent branches divided distally	2223(A)	94
		1402(B)	105
4858	Three adjacent branches divided distally	2044(A)	84
		1532(B)	119
4863	Three adjacent branches divided distally	2579	68

Mean per unit area 100, standard deviation of mean 8.

Confirmation of the quantitative changes in fibre count using the second series of rabbits is not ideal as the difference to be expected is only in the region of 30%, and the experimental error is probably greater than 10%. Some individual counts per unit area in the distal segments of the first series, and in the specimen of the second series of animals show an overlap, although the mean figures per unit area are of significant difference.

An attempt was made to demonstrate degeneration in the proximal segment following distal divisions of the neurovascular bundles. Various silver stain techniques were used on specimens taken from 1 to 15 days following operation. In none of these was there unequivocal evidence of degeneration. In order to test the efficiency of the methods employed distal segments were studied after either proximal division or excision of the superior ganglion on the left side. Again, no firm evidence of degeneration was obtained, and it appears that the techniques used were not capable of staining the degeneration granules of such fine nerve fibres. Certainly

the fibres remaining in the distal segment after proximal division are unmyelinated and of small diameter.

Using Nauta's silver stain (1954), degeneration granules of the preganglionic fibres in the greater splanchnic nerves and of the preganglionic supply to the superior cervical ganglion have been shown, but an attempt to show degeneration in post-ganglionic fibres using this stain has failed.

In a third series of rabbits the more peripheral parts of the mesentery were examined for outlying ganglion cells. Two rabbits were used (4877 and 4878), and in each rabbit six lengths of neurovascular bundles were excised from the marginal loops, and six from the proximal ends of the main neurovascular bundles. Each bundle was held in a cardboard frame in a slightly stretched condition, and was fixed in Bouin's solution for 48 hr. The specimens were then dehydrated, cleared, embedded in wax, and 5μ thick serial longitudinal sections cut. The sections were stained with haematoxylin and eosin on the slides. One ganglion cell was discovered in one of the proximal bundles in rabbit 4877, and possibly one in a distal segment. No ganglion cells were seen in the nerve bundles removed from the second rabbit.

DISCUSSION

The intact fibres in the distal segments apparently have their cell bodies in the wall of the gut. There is an alternative possibility that some of these fibres arise from ganglion cells situated in the mesentery distal to the cut. Recently Kuntz & Jacob (1955) have carried out an investigation on the periarterial extension of coeliac and mesenteric plexuses in the rat, cat and human. They found some ganglion cells along the mesenteric arteries and their main branches. The present investigation shows clearly that nearly all these fibres arise within the gut itself and not in ganglia cells in the peripheral part of the mesentery. It remains to be discovered whether they are processes of cells in the intrinsic plexuses.

The termination of the fine centripetal fibres is not known. It is possible that they form part of a local reflex mechanism with synaptic connexions in the superior mesenteric ganglion, and Kuntz (1938) has presented some anatomical evidence that this is the case. If they do so, such a local reflex mechanism must be of great importance as these fibres constitute no less than 30% of the total number of nerve fibres running in the branches of the superior mesenteric nerve.

As already outlined, there is physiological evidence that reflexes can occur through the decentralized abdominal sympathetic ganglia. Kuntz & Saccomanno (1944) carried out both acute and chronic experiments on the inferior mesenteric ganglion of the cat. In the acute experiments the central preganglionic inflow into the ganglion was interrupted by extirpating the spinal cord below the level of the cervical cord, and dividing the vagi at the cardia. In the chronic experiments, they divided the lumbar sympathetic trunk bilaterally, the hypogastric nerves and the coeliac roots, and allowed an interval of 7 days before proceeding with the experiment. In both the acute and chronic experiments they found that the intestino-intestinal reflex persisted, i.e. after the large gut was divided leaving its two segments connected only by mesentery, inhibition of the proximal segment occurred on raising the intra-luminal pressure in the distal segment. They also observed that faradic

stimulation of the nerves supplying the distal segment of intestine produced inhibition of the proximal segment of gut. They carried out similar experiments on the small intestine.

It is difficult to be certain in all cases that the central preganglionic inflow into the mesenteric ganglia is completely interrupted, and it would be additional evidence of local reflexes through the ganglia if the intestinal response was abolished by subsequent removal of the ganglia.

It cannot be assumed that the nerve fibres described in this paper are similar to those described by Brown & Pascoe (1952) in the ascending mesenteric nerve, but the likelihood is that they are. The peripheral preganglionic fibres described by these authors were of small diameter judged by their velocity of conduction (0·25 m/sec.), and they apparently had their cell bodies somewhere in the peripheral distribution of the ascending nerve, and probably in the walls of the colon. McLennan & Pascoe (1954) attempted to determine the origin of these fibres, and they demonstrated that they remained intact after chronic decentralization of the inferior mesenteric ganglion, bilateral splanchnicotomy, bilateral vagotomy, and bilateral abdominal sympathectomy. Following solar ganglionectomy degeneration of some of them may occur.

In the present series, bilateral vagotomy was carried out on two of the rabbits. Although this is not sufficient to base any firm views on, the likelihood of the nerve fibres described being of vagal origin is remote, and in these two animals, no significant difference in total counts or counts per unit area was discovered.

It would appear that those nerve fibres with their cell bodies situated apparently in the intestine are similar to those described by Kuntz (1940) in the colonic neurovascular bundles. Their function is not known, but it is suggested that perhaps they are of importance in the intersegmental activity of the intestines.

SUMMARY

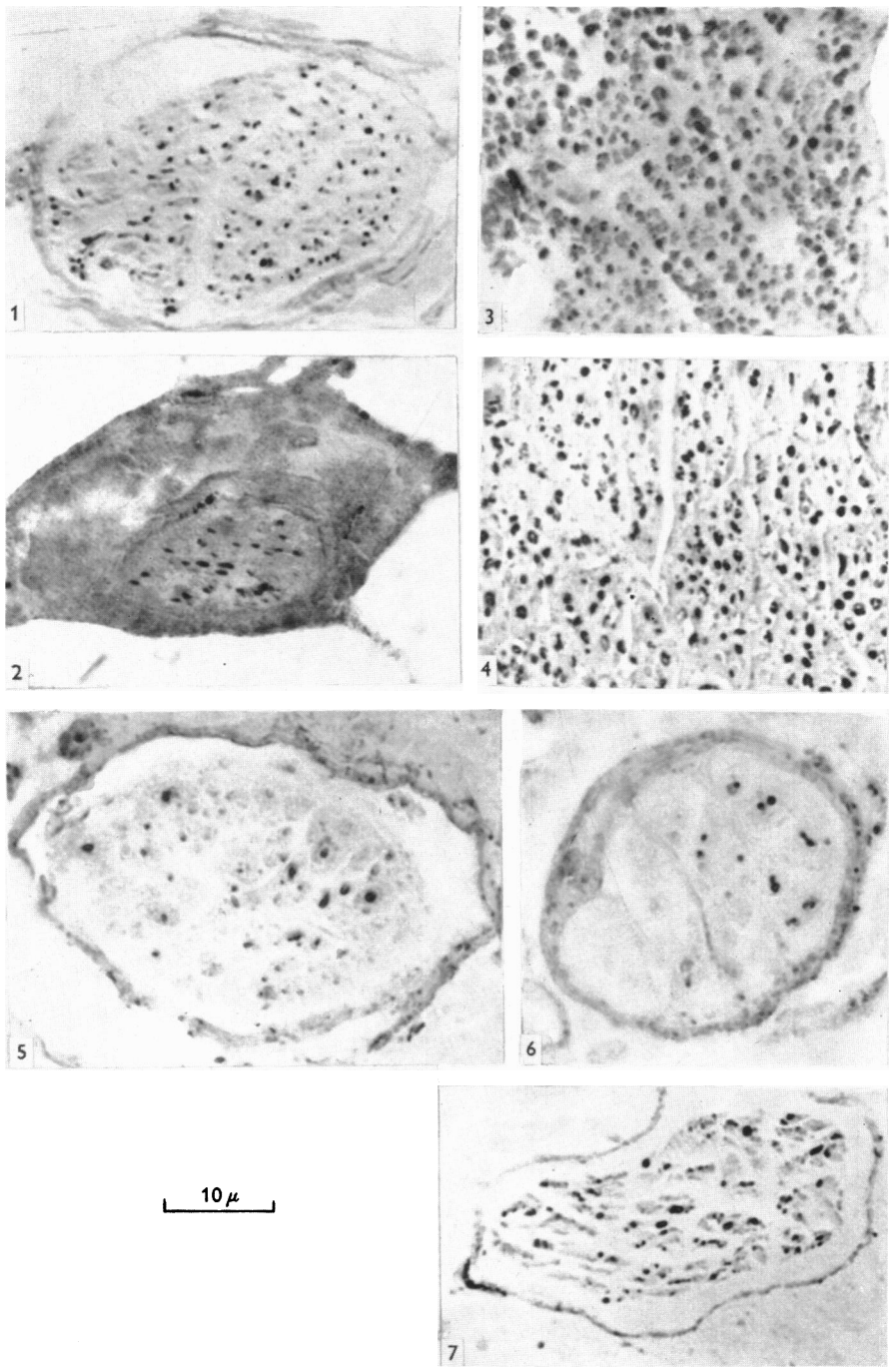
1. Following division of neurovascular bundles in the small gut mesentery of the rabbit, and allowing a period of 21 days for degeneration to occur, some nerve fibres remain intact distal to the interruption.

2. Counts per unit area show that these fibres represent 30 % of the fibres present in the normal superior mesenteric neurovascular bundles.

3. It is suggested that these fibres have their cell bodies situated in the gut wall, and that they are proceeding centripetally to the local sympathetic ganglia to effect synapse there.

4. Physiological evidence of local reflex activity through these ganglia is discussed.

I wish to thank Prof. J. Z. Young, and Dr D. H. L. Evans for their helpful criticism and advice, Miss Pamela Hines for her technical assistance, and Mr J. Armstrong for taking the photographs.



ROSS—Centripetal fibres in superior mesenteric nerves of rabbit

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EXPLANATION OF PLATE

Figs. 1 and 2. Transverse sections of distal segments of nerves following division of superior mesenteric neurovascular bundles proximally. The nerve fibres remaining are the centripetal ones referred to in the text.

Fig. 3. Transverse sections of a normal nerve bundle in the mesentery of the small intestine. The nerve fibres are not closely packed.

Fig. 4. Transverse section of a proximal segment of nerve bundle following division of a superior mesenteric neurovascular bundle distally. The fine centripetal nerve fibres have degenerated.

Figs. 5 and 6. Transverse sections of marginal nerve bundles. The first section contains more nerve fibres per unit area than the second, and is more comparable with a normal marginal bundle. It was taken from a marginal loop near to an intact main neurovascular bundle. The second one was taken close to a divided main neurovascular bundle.

Fig. 7. A normal marginal nerve bundle.