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# Experimental Study on Orientation of Regenerating Fibers in the Severed Peripheral Nerve*)

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## ABSTRACT

The objective of this study was to ascertain how the orientation of regenerating fibers of the proximal stump of severed peripheral nerves is determined at time of growth. In other words, a gap was intentionally created between the both stumps of severed peripheral nerves to determine whether regenerating fibers are guided in the direction of the distal stump or if they grow randomly. The following results were obtained.

1) Regenerating fibers sprouting from the proximal stump definitely extend in the direction of the distal stump.
2) Even when the distal stump is replaced with a nerve fragment, the regenerating fibers grow in that direction.

These results are completely different from those of Weiss and Taylor, and thus the conclusion was reached that the possibility the orientation of regenerating fibers is determined by chemotaxis cannot be ruled out.

## INTRODUCTION

Recovery of severed peripheral nerve is not adequate merely with the distal extension of nerve fibers, it becomes meaningful only when functional recovery of muscle and sensory end organs has been achieved. The prognosis of neurorrhaphy of peripheral nerves in clinical cases is not satisfactory despite the introduction of microsurgical techniques and development of various suture techniques.

The funicular suture technique which is becoming a routine procedure aims at accurately coaptating the corresponding funiculi of the severed stumps to prevent misdirection so that they can grow accurately in their respective governing areas[11,16]. However, as the funicular pattern changes even at short distances, the indications for this procedure is for only fresh clean cut cases. Further, in order to accurately coaptate the respective funiculi, it is necessary to strip the epineurium and separate each of the funiculi. Such procedures are traumatic and may disrupt the delicate perineurial barrier.

It can be said that we have exhausted means of technical improvement such as methods of suture, and enhancement of treatment results have reached their limit. However, on the other hand, in some clinical cases in whom the peripheral nerve has been severed it has been noted on exposure several months later that both ends of the nerve have spontaneously become connected, and that the tissue achieving the connection consists of regenerating fibers. Thus, on the one hand, we have cases who show spontaneous connection, while on the other hand, there are cases in whom regeneration is poor despite the best of efforts. Therefore, in light of these facts it is considered that the mechanism of regenerating sprouting axons from the proximal stump should be re-studied from its very basis once again to improve the results. Especially, the fact whether or not the orientation of the regenerating fibers when ex-

*) 越智光夫：末梢神経損傷における再生線維の方向性に関する実験的研究

tending is affected by the distal stump, is a major problem which has not yet been elucidated. There are two theories, the neurotropism theory and the contact guidance theory, regarding factors which determine the orientation of regenerating nerves. Both theories have long histories, but it is still not possible to decide upon one of the two, and the matter is still being debated.

The neurotropism theory assumes that some kind of chemical substance which guides regenerating fibers is released from the distal stump[3,4]. On the other hand, Weiss[18,19] after performing a series of experiments, established the contact guidance theory which is contrary to the above. Under his theory, the regenerating fibers begin to extend, not induced by chemical substances, but by surface contact with solid substances and extend at random. Although detailed follow-up tests have not been performed, the latter theory tends to have gained greater support.

Therefore, I prepared an experimental model whereby a gap was intentionally created between the two ends of severed peripheral nerves so as to facilitate the observation of the orientation of fibers growing out of the proximal stump. I would like to report on this in vivo phenomenon.

## MATERIALS AND METHODS

In this study, a silicone cross chamber which consists of four channels was used to observe the sprouting of regenerating fibers from the proximal to distal stumps. The channels make it easy to quantitate the regenerating fibers within the channels and check the orientation of the axons.

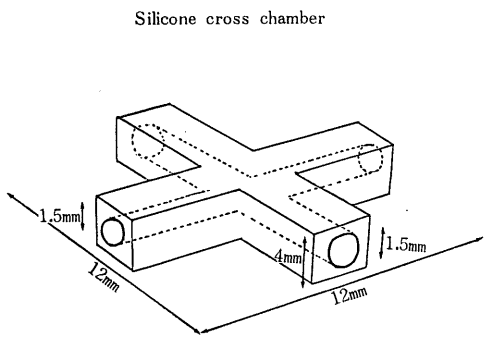



**Fig. 1.** Silicone cross chamber: An approximately 12×12mm square block was cut into a cross-shape and tunnels (channels) 12 mm in length and 1.5 mm in diameter were bored.

The silicone cross chamber was made by boring into a silicone block, four 1.5 mm diameter tunnels which converge at direct angle to one another. Each tunnel was cut off at a length of 6 mm from the point of convergence, and the external was trimmed so as to make an approximately 4 mm×4 mm shaped square pillar (Fig. 1). Thus, this silicone cross chamber has four channels of equal length which cross at right angle to one another A silicone block was used becau e the channel will not collapse within the body, it is easy to shape and its foreign body reaction is small.

The common peroneal nerve of male Wistar rats weighing about 300 g was amputated and the proximal end was inserted about 2 mm into one of the cross channels and fastened in place by one stitch (10-0 nylon) under a microscope and immobilized. The following five groups were used for study involving the three remaining channels.

Group 1 (No distal stump): No procedures were applied to the remaining three channels and all ends were left open. In order to exclude effects of the distal cut end, the remaining peripheral nerve trunk of the common peroneal nerve was resected and the fascia that it penetrates was closed by suture.

Group 2 (Distal stump in direct alignment with proximal stump): The distal end of the nerve was inserted 2 mm into the channel directly opposing that into which the proximal end had been entered, and immobilized. Thus, the gap between the two stumps was about 8 mm.

Group 3 (Distal stump placed at right angle to proximal stump): The distal end was placed into a channel at a 90° angle to that of the proximal stump and immobilized.

Group 4 (Nerve fragment placed at right angle to proximal stump): In lieu of placing the distal stump into the channel crossing at 90° to that holding the proximal stump as in the case of Group 3, a nerve fragment about 1 cm length was free grafted and immobilized. The remanining peripheral nerve trunk was resected when possible, and the fascia it penetrates was sutured.

Group 5 (Distal stumps of common preoneal nerve and sural nerve at right angles to pro-

Methods

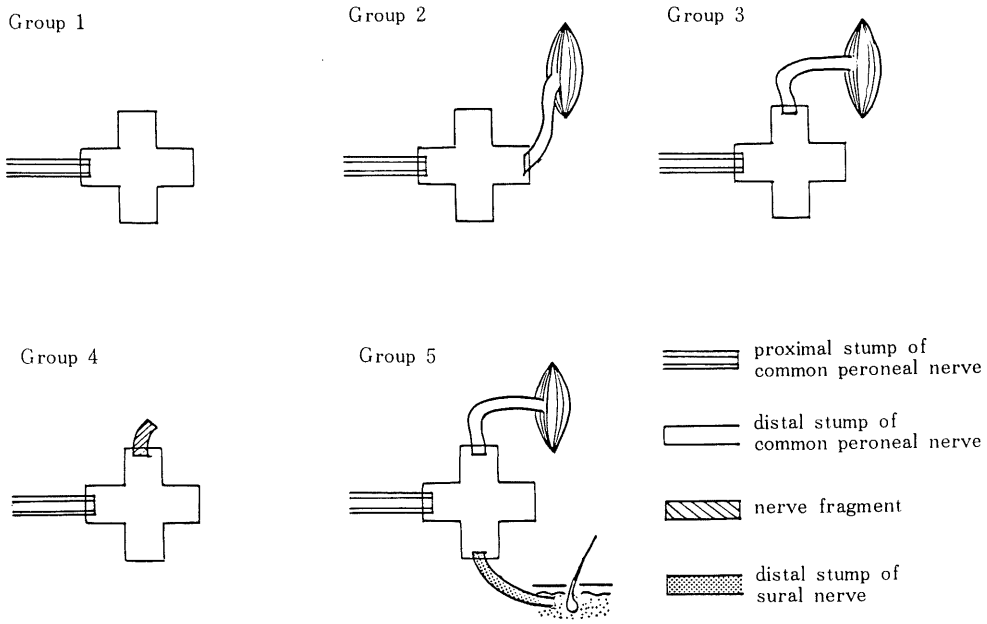



**Fig. 2.** Schematic drawing of the methods used for each experimental group: In all groups the proximal stump of the common peroneal nerve was inserted into one channel of the silicone cross chamber.
Group 1 : No distal stump.
Group 2 : Distal and proximal stumps in direct alignment.
Group 3 : Distal and proximal stumps at right angle.
Group 4 : Nerve fragment (1 cm) placed at right angle to proximal stump.
Group 5 : Distal stumps of the common peroneal and sural nerves at right angles to proximal stump.

ximal stump): The distal stump of the common peroneal nerve was inserted into one of the channels at 90° to the proximal stump and the distal stump of the sural nerve was inserted likewise into the corresponding channel, and both nerves were immobilized. The objective for establishing Group 5 was to determine whether the motor nerves could locate the motor fiber Schwann tubes and the sensory could locate the sensory fiber Schwann tubes (Fig. 2).

The chambers of the 5 groups were opened one month and two months after surgery. The chambers in Group 3 only were also opened two weeks after surgery, and the regeneration during the fairly early stage was observed. Prior to removing the specimens from the chambers, the proximal stumps of the common peroneal nerve were given an electric stimulation and the evoked potential from the anterior tibial muscle was recorded. The number of regenerated myelinated fibers which extended into the respective channels from the proximal stump were quantitatively assessed histologically, at the site of 2 mm from the channel crossing in the groups one and two months after the operation. That is, the myelinated fibers of total transverse sections were counted and the pattern of each group was determined based on the number of regenerated fibers by direction. Further, the state of early regenerating fibers was observed under electron microscope (EM) two weeks after operation. That is, specimens were taken from four channels and from the common peroneal nerve proximal to the chamber. The specimens were fixed with buffered glutaraldehyde and osmium tetroxide, and then embedded in Epon. Total transverse sections were stained and examined under light microscope and electron microscope.

## RESULTS

The new grown tissue extending into the chamber from the proximal stump differed markedly by channel. That is, in certain directions there was very good regeneration, the external circumference showed epineurium-like tissue, containing numerous myelinated and unmyelinated axons, perineurium-like tissue, Schwann cells and vasculature basically, there was also tissue considered to be nerve trunk. While in another direction, hardly any myelinated or unmyelinated fibers and Schwann cells could be seen, and regeneration from the proximal stump was very poor. Thus, the pattern of the regenerated fibers that extended into the channels demonstrated characteristic differences by group.

It was possible to record good evoked potential from the anterior tibial muscle by stimulating the proximal nerve in Groups 2, 3 and 5, two months after operation. However, it was not possible to elicit evoked muscle potential in Group 1 and 4. Fig. 3 shows the evoked muscle potential of Group 3, two months after operation. It was demonstrated electrophysiologically that the regenerated fibers from the proximal stump extended to the anterior tibial muscle, the target organ. The macroscopic and histological findings in the respective groups are presented hereunder.

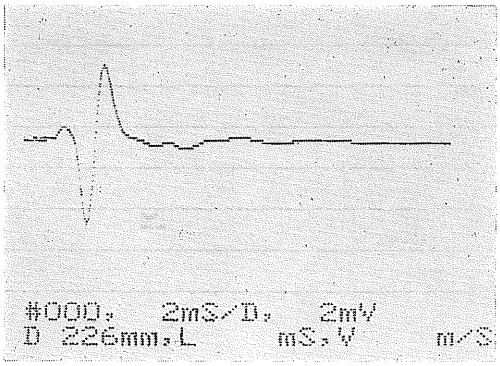



**Fig. 3.** Evoked potential of the anterior tibial muscle following electrical stimulation of the proximal stump of the common peroneal nerve two-months after surgery: This demonstrates electrophysiologically that the regenerating fibers from the proximal stump have reached the target organ and function recovery has been achieved.

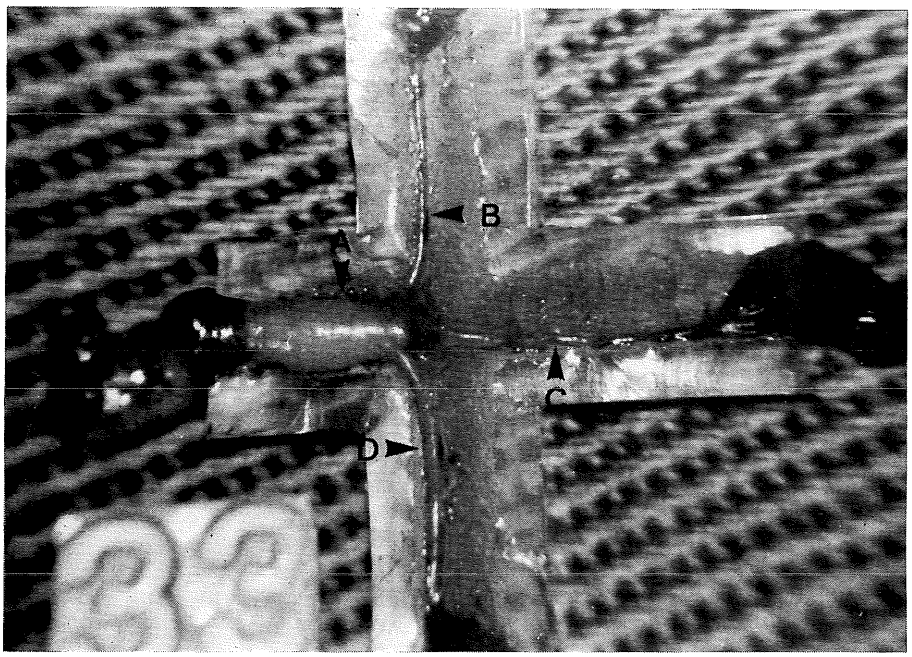



**Fig. 4.** State of nerve of Group 1 within a chamber opened after elapse of two months: The proximal stump was inserted into the left channel. Diameter of new tissue is large up to the junction, but growth in the three open-end directions is poor. A, B, C and D show the nerve within the channels at 2 mm respectively from the junction.

Group 1 (No distal stump): The new tissue from the proximal stump was comparatively large up to the junction, but after branching off into three directions they abruptly became thin and terminated (Fig. 4). As shown in Fig. 4, the 2 mm sites from the junction were named A, B, C and D, and the myelinated fibers at A, 2 mm to the proximal side of the junction, numbered 591 (one month, and 520, 1123 and 955 (two months), while at 4 mm distally at point B, the numbers (one month) were 0, and (two months) 58, 12 and 119, at C, 11 (one month), and 166, 36 and 3 (two months), and at D, 0 (one month), and 177, 19, and 37 (two months), indicating a sharp decrease distal to the junction (Table 1). From these findings it can be seen that growth of the regenerated fibers is poor, and that no specific findings regarding orientation could be observed. And the number of regenerating fibers was few at the outlet port of the channel.

Group 2 (Distal stump in direct alignment with proximal stump): The proximal and distal stumps were linked with thick new tissue, but the area close to the proximal and distal stumps were generally large, but tended to become smaller near the junction. However, the empty channels without nerves showed only a small amount of new tissue growth. When the site 2 mm to the proximal stump side from the junction is signified A, the total number of myelinated fibers observed at A was 1602 (one month), and 1569, 2143 and 1735 (two months). Almost all of the myelinated fibers grew out straight towards the distal stump, and at the opposing cite C they numbered 1099 (one month), and 2008, 1704 and 883 (two months). However, the sprouting towards the two empty channels were very few, being less than 100 in all four rats (Table 1).

Group 3 (Distal stump placed at right angle to proximal stump): At 1 and 2 months after surgery, the proximal and distal stumps were linked with thick new tissue, whe.e there were very little growth in the direction of the two empty channels. Fig. 5 shows 1 case two months after surgery. The arrows indicate sites A, B, C and D, that is, at points 2 mm respectively from the junction, the number of myelinated fibers observed at A was 821 (one

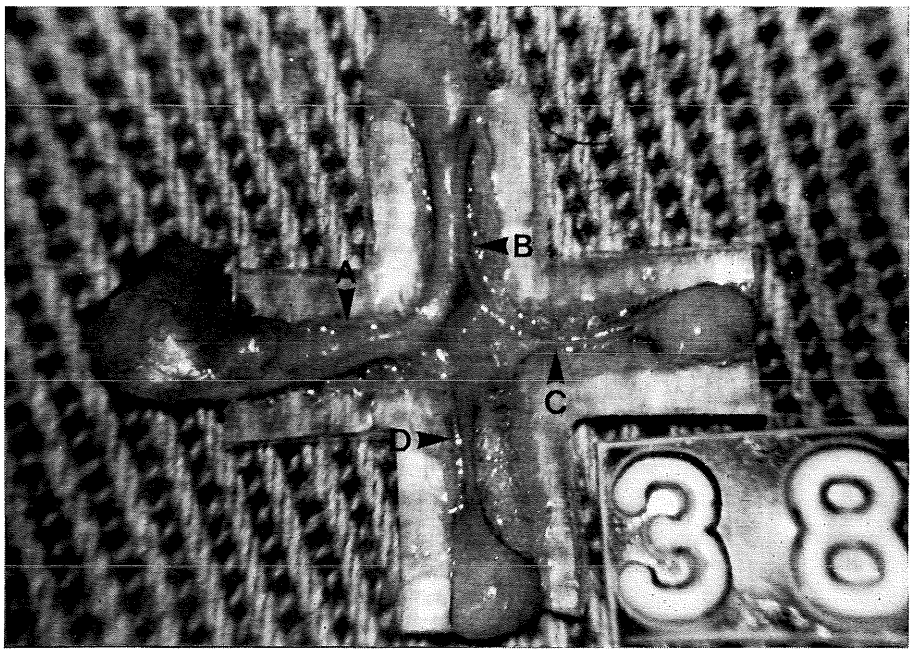



**Fig. 5.** State of nerves of Group 3 within a chamber opened after two months: The proximal stump was inserted into the left channel and the distal stump was placed in the upper channel. New tissue grew between the proximal and distal stumps strongly linking the two, but growth in the two open-end directions was poor. A, B, C and D in this figure correspond to the sites in Fig. 4.

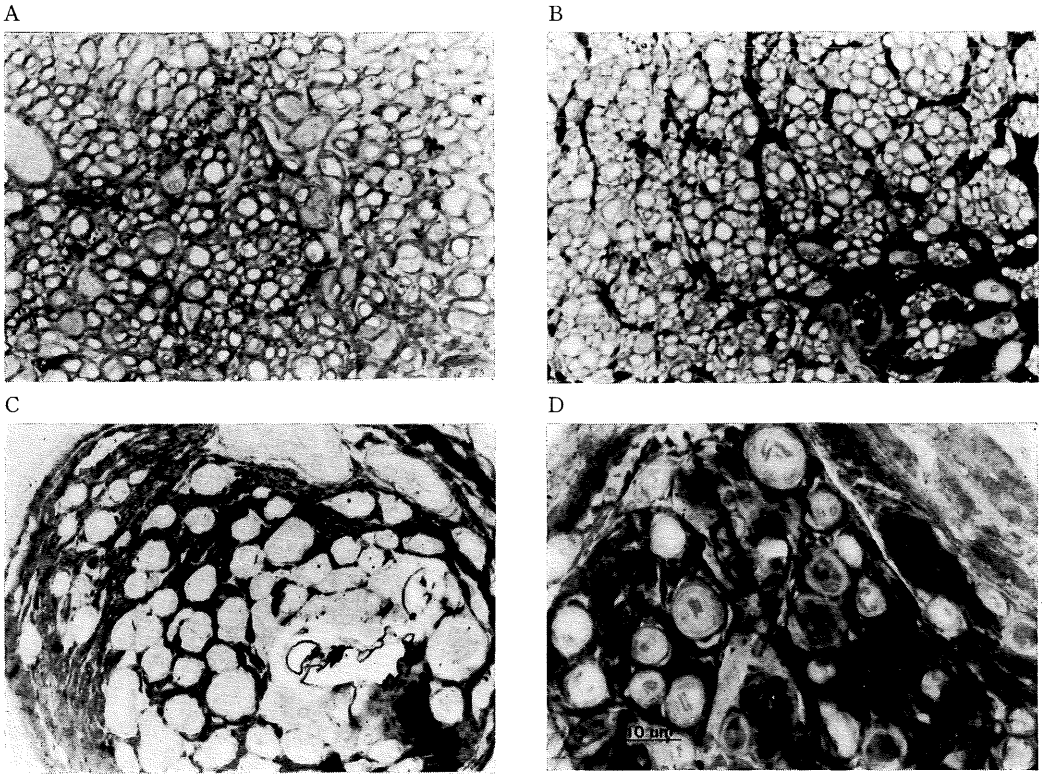



**Fig. 6.** Light microscope images at sites A, B, C and D of the regenerating fibers extending into the chamber shown in Fig. 5: A is the site 2 mm on the proximal stump side of the junction (above left), B is the site 2 mm on the distal stump side of the junction (above right), C is a site directly opposite to A (below left), and D is a site directly opposite to B (below right).
Good nerve regeneration can be observed at A and B, but at the open ends C and D, only scar tissue centering around the new vessel can be observed. (Tolouidine blue stain × 400).

month), and 1821, 1261 and 1864 (two months); at B it was 1018 (one month), and 2317, 713 and 1040 (two months); at C it was 18 (one month), 6, 122 and 171 (two months), and at D it was 128 (one month), and 19, 2 and 6 (two months). Thus, both sites of A and B showed good nerve regeneration (Table 1). In other words, the regenerating fibers from the proximal stump change their direction 90° and extend towards the channel in which the distal stump has been inserted. The light microcope images of cross-sections at A, B, C and D of the case in Fig. 5 are as shown in Fig. 6. The EM image is as shown in Fig. 7, indicating that there is definite directional specificity of the regenerating fibers.

The one and two months postoperative states have been described above, but a report will also be made on the condition two weeks after surgery. Grossly, the new grown tissue is not as firm as that one and two months after the operation, and presented a yellowish fibrin-like appearance and was soft like jelly. The connection between both nerve stumps which had been inserted into the channels and the new grown tissue could be disrupted with comparatively little strength. Hardly difference in the diameter of the new grown tissue could be observed, and there were no findings indicative of the new tissue extending in any particular direction being especially large. Histological review showed that although no regenerative fibers could be observed in empty channels, regenerated fibers could be seen extending into channels in the direction of distal stump which had been inserted. The regenerating fibers could be seen in small volume only in the approximately central portion of the channel, indicating that regenerating fibers do not extend along the channel walls. Fig. 8 shows regen-

**Table 1.** Number of myelinated fibers at sites A, B, C and D: A is 2 mm to the proximal stump side, and the figures underlined indicate the direction in which the distal stump or nerve fragment was inserted. AC and BD are at right angle to one another.

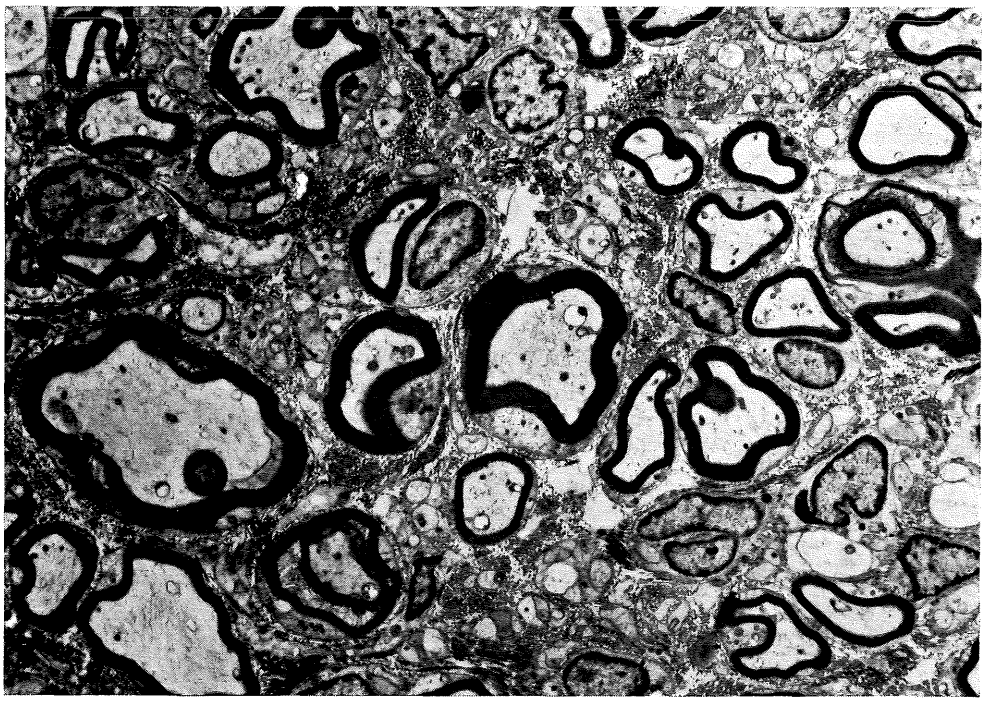
| | A | B | C | D |
|---|---|---|---|---|
| Group 1 | proximal stump channel | | | |
| | 1 M 591 | 0 | 11 | 0 |
| | 2 M 520 | 58 | 166 | 177 |
| | 1123 | 12 | 36 | 19 |
| | 955 | 119 | 3 | 37 |
| Group 2 | proximal stump channel | | distal stump channel | |
| | 1 M 1602 | 9 | 1099 | 2 |
| | 2 M 1569 | 11 | 2008 | 7 |
| | 2143 | 15 | 1704 | 83 |
| | 1735 | 35 | 883 | 20 |
| Group 3 | proximal stump channel | distal stump channel | | |
| | 1 M 821 | 1018 | 18 | 128 |
| | 2 M 1821 | 2317 | 6 | 19 |
| | 1261 | 713 | 122 | 2 |
| | 1864 | 1040 | 171 | 6 |
| Group 4 | proximal stump channel | free nerve fragment channel | | |
| | 1 M 1399 | 677 | 7 | 4 |
| | 2 M 1356 | 1196 | 125 | 79 |
| | 1821 | 1137 | 13 | 3 |
| | 812 | 702 | 9 | 3 |
| Group 5 | proximal stump channel | distal stump of common peroneal nerve channel | | distal stump of sural nerve channel |
| | 1 M 692 | 519 | 26 | 279 |
| | 2 M 1012 | 1395 | 12 | 329 |
| | 863 | 1422 | 56 | 468 |
| | 1215 | 1161 | 23 | 382 |

erated fibers in the central portion of the channel at the site 2 mm towards the distal stump side of the junction in 1 case two weeks after operation.

Group 4 (Nerve fragment placed at right angle to proximal stump): There was thick new growing tissue connecting the proximal stump and nerve fragment as in the case of Group 3, but the new tissue extending into empty channels was of small diameter (Fig. 9). Measurement of the myelinated fibers showed that almost all regenerating fibers from the proximal stump were directed towards the grafted nerve (Table 1). The histological pictures of A, B, C and D are as shown in Fig. 10 and 11. There were few regenerating fibers at C and D in the two empty channels, and the degree of maturation was poor.

Group 5 (Distal stumps of common peroneal nerve and sural nerve at right angles to proximal stump): The new growing tissue within the chamber firmly links the proximal stump with the respective distal stumps, but that extending towards the empty channel is small in diameter. Our findings of the diameters of the new growing tissue extending towards the distal stump of the common peroneal nerve and that of the sural nerve showed that the tissue growing

A



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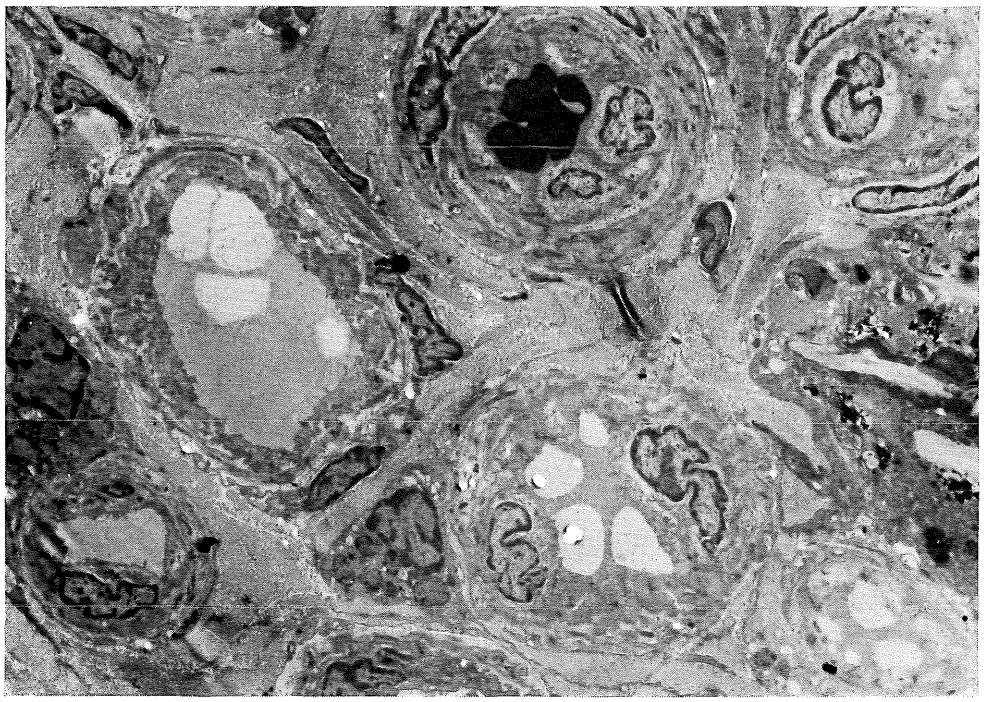
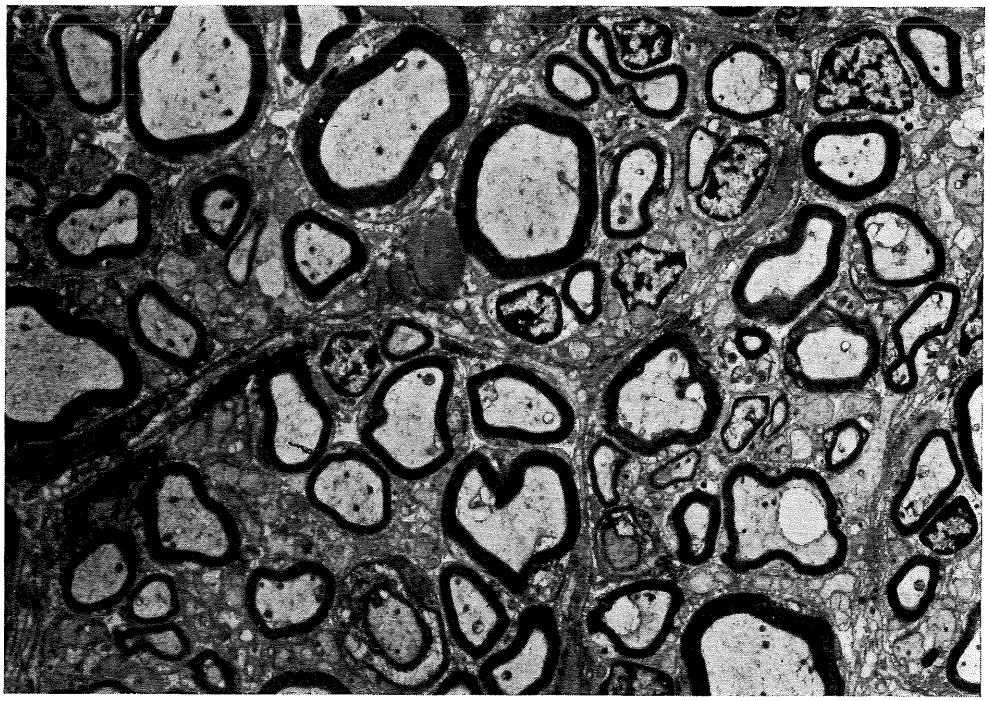


**Fig. 7.** EM images of sites A (above lelt), B (above right), C (below left) and D (below right) in Fig. 6: Many myelinated and non-myelinated fibers, Schwann cells and perineurium which divides these into a number of compartments can be observed. This state is called compartmentation, and is fundamentally considered to be the peripheral nerve trunk. However, hardly any regenerating fibers can be seen in C and D (Uranyl acetate and lead citrate stain × 2000).

B



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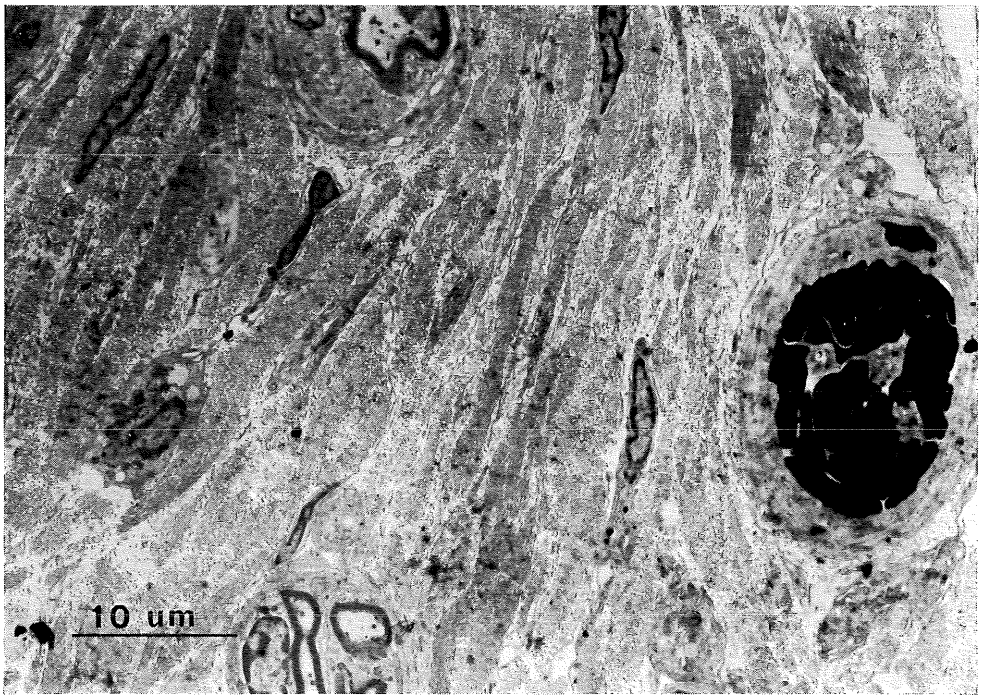

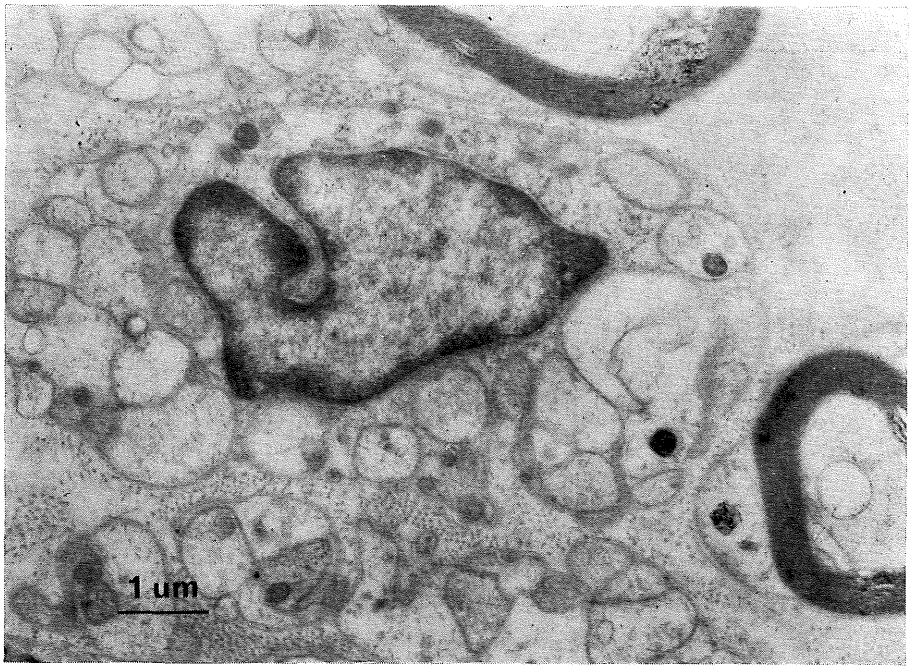


**Fig. 8.** EM image of the site 2 mm towards the distal stump side of the junction two weeks after surgery: Although immature, myelinated fibers can be seen in the center and many unmyelinated fibers are noted around the Schwann cells. (Uranyl acetate and lead citrate stain × 5000).

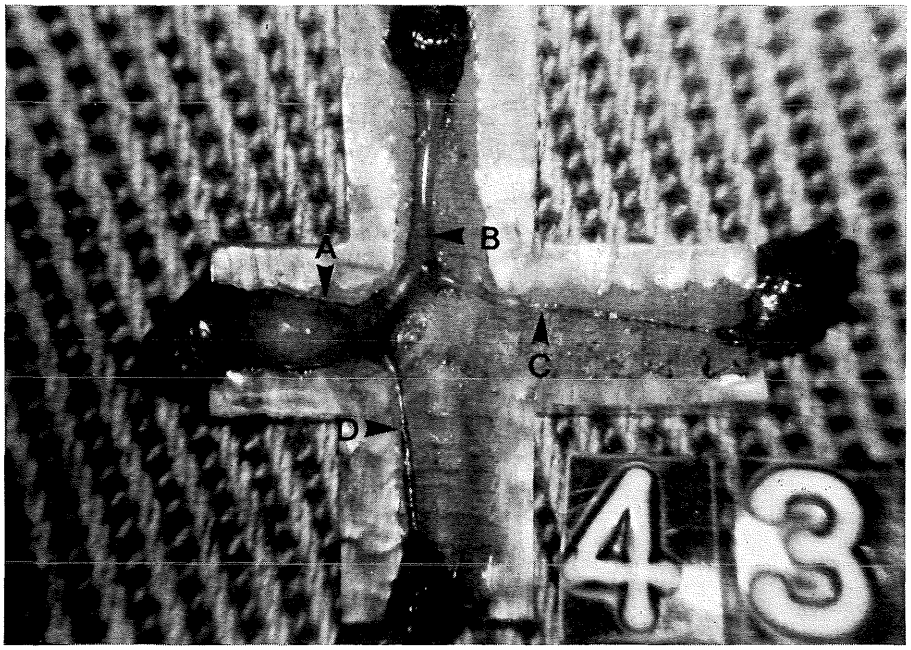


**Fig. 9.** State of nerves of Group 4 within a chamber opened after two moths: The proximal stump was inserted into the left channel and a grafted nurve fragment (1 cm) was placed in the upper channel. New tissue grew between the proximal stump and nerve fragment strongly linking the two, but growth in the two open-end directions was poor. A, B, C and D in this figure correspond to the sites in Fig. 4.

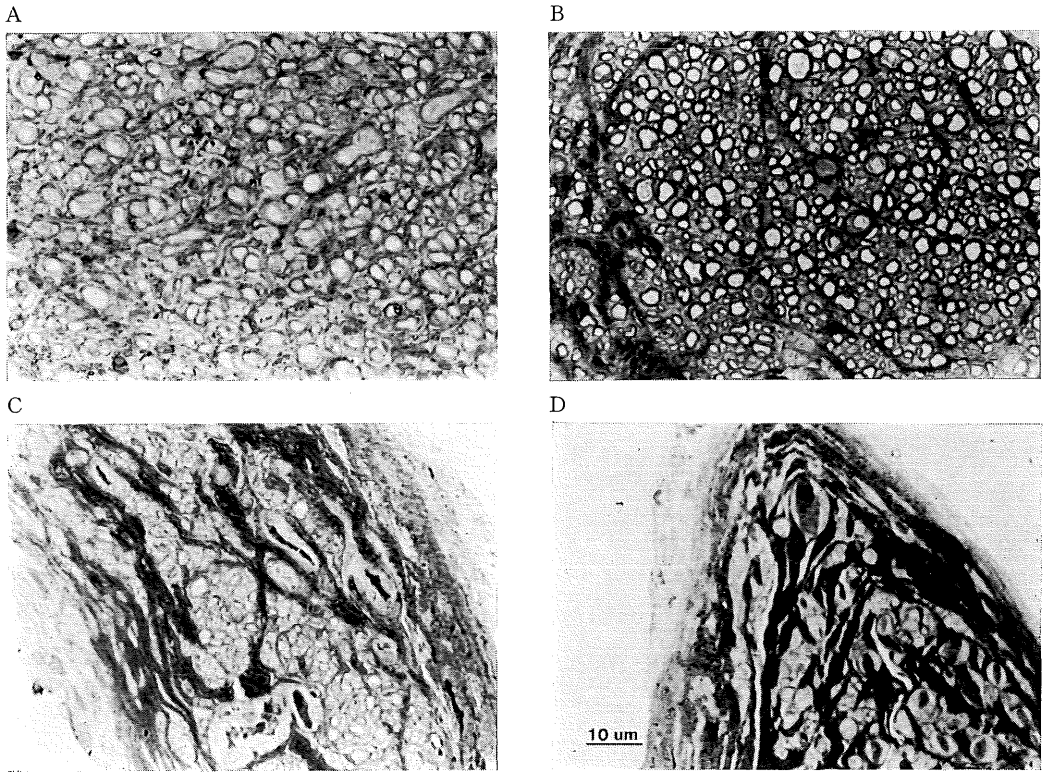


**Fig. 10.** Light microscope images at sites A, B, C and D of regenerating fibers extending into the chamber shown in Fig. 9: A is a site 2 mm on the proximal stump side of the junction (above left), B is a site 2 mm on the nerve fragment side of the junction (above right), C is a site directly opposite to A (below left) and D is a site directly opposite to B (below right). Good nerve regeneration can be observed at A and B, but at open-ends C and D regeneration is very poor. (Tolouidine-blue stain × 400).

towards the common peroneal nerve was larger. The number of myelinated fibers are as shown in Table 1. Those extending from the proximal stump counted at site A, 2 mm from the junction, showed that at the first month 692 were observed and 1012, 863 and 1215 at the second month, those extending from the distal stump of the ommon peroneal nerve at site B numbered 519 at the first month, and 1395, 1422 and 1161 at the second month, those from the distal stump of the sural nerve at site D numbered 279 at the first month, and 329, 468 and 382 at the second month, while for the open ended channel at site C, it was 26 at the first month, and 12, 59 and 23 at the second month. In other words, the regenerating fibers from the proximal stump separate and extend towards the two distal stump, and it was observed that the ratio of fibers to the common peroneal nerve outnumbered those extending towards the sural nerve by more than two-fold. Fig. 12 shows the state of fiber regeneration in 1 case two months after surgery.

Fig. 13 shows schematic drawings of regenerating fibers extending into channels are practically all oriented towards those in which distal nerve stumps or nerve fragments have been placed, while in empty channels only very poor regeneration could be seen. It was also found that the roles played by nerve fragments and distal stumps were about the same in determining the direction and growth of regenerating fibers, as there was only a slight difference between both in the number of myelinated fibers.

## DISCUSSION

In Experiment 1 where neither the distal nerve stump nor nerve fragment was inserted, there were few regenerating fibers and the dis-

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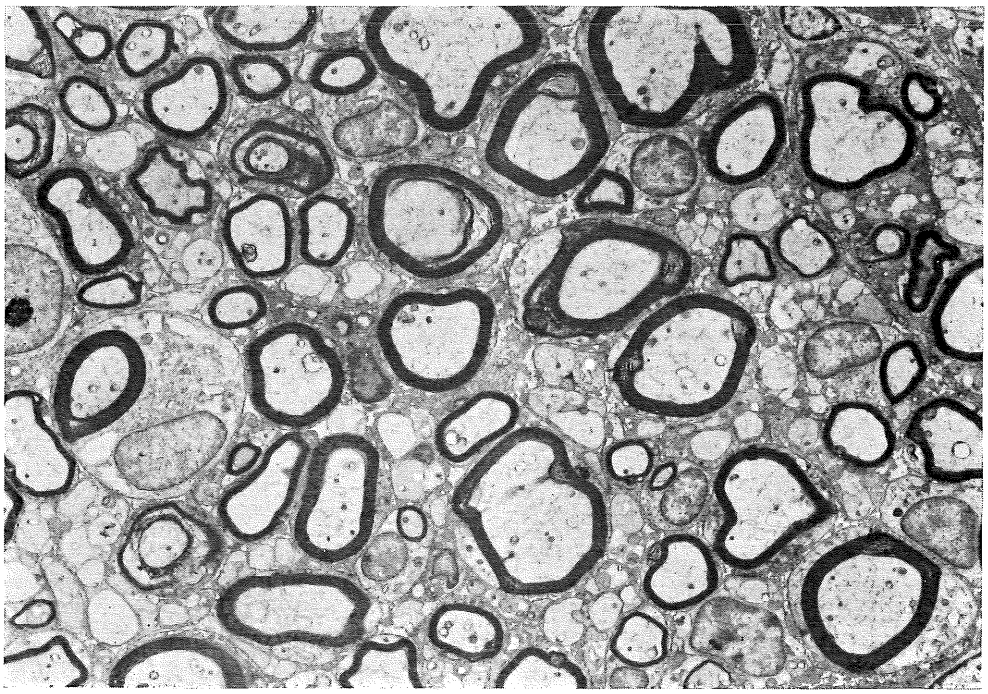

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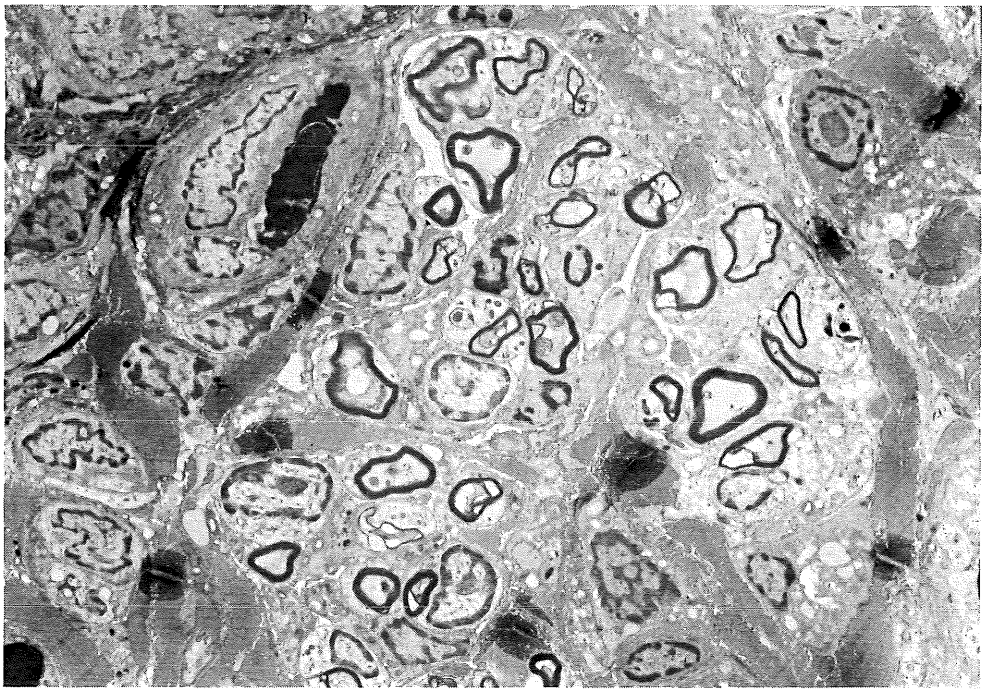
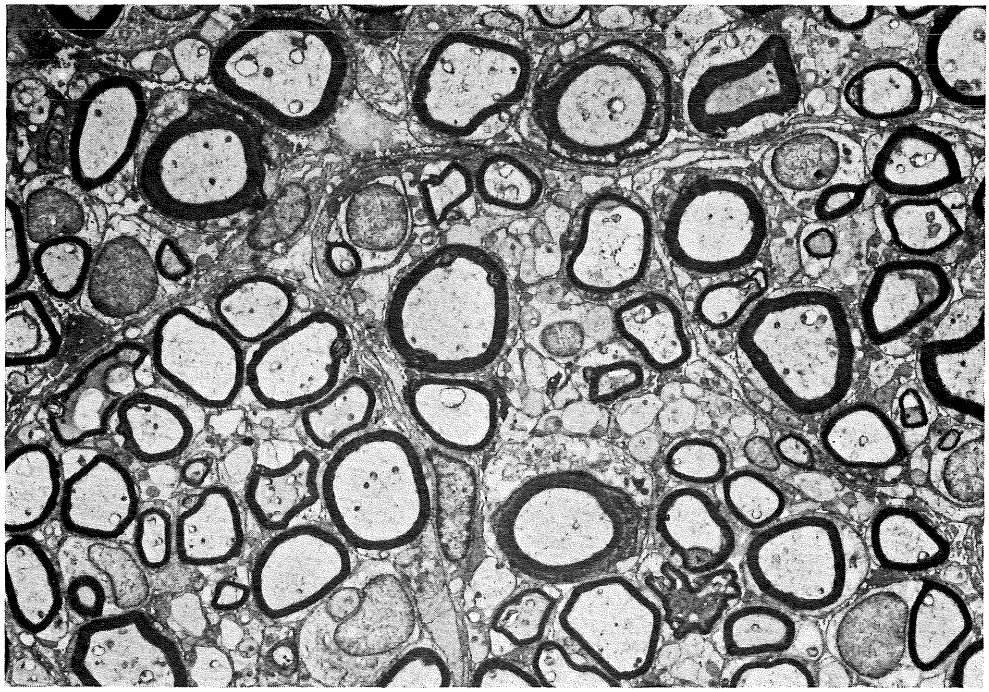


**Fig. 11.** EM images of sites A (above left), B (above right), C (below left) and D (below right) in Fig. 10: Very good regenerating fibers are observed in A and B as in the case of the distal stump inserted rats in Group 3. On the other hand, although regenerating fibers can be observed in C and D, the number is small and the diameter of the myelinated fibers is small and growth is poor. (Uranylacetate and lead citrate stain × 2000).

B



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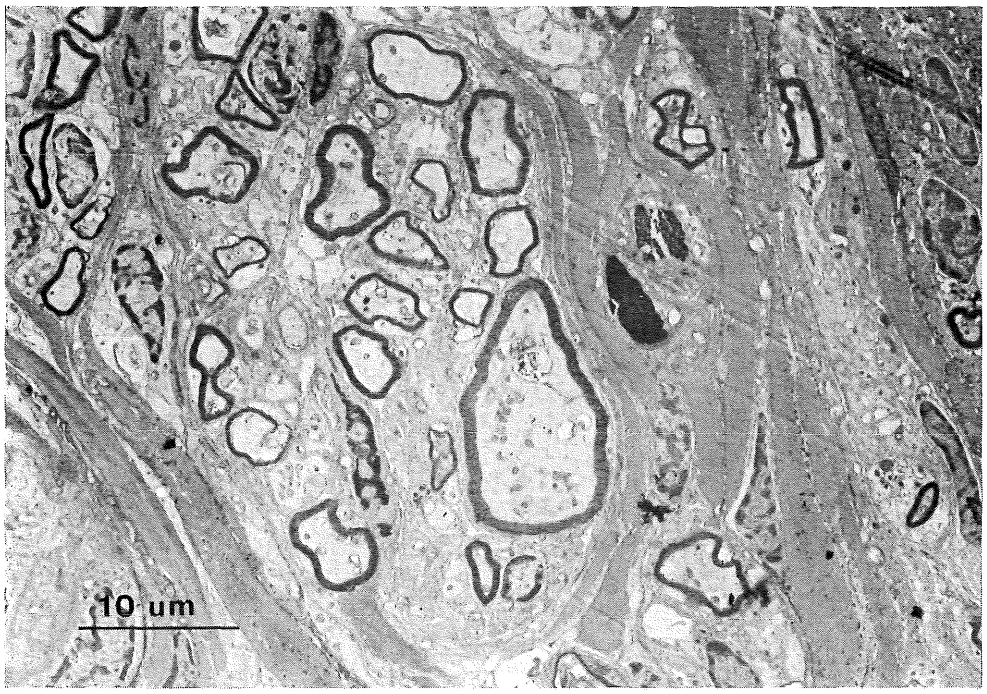

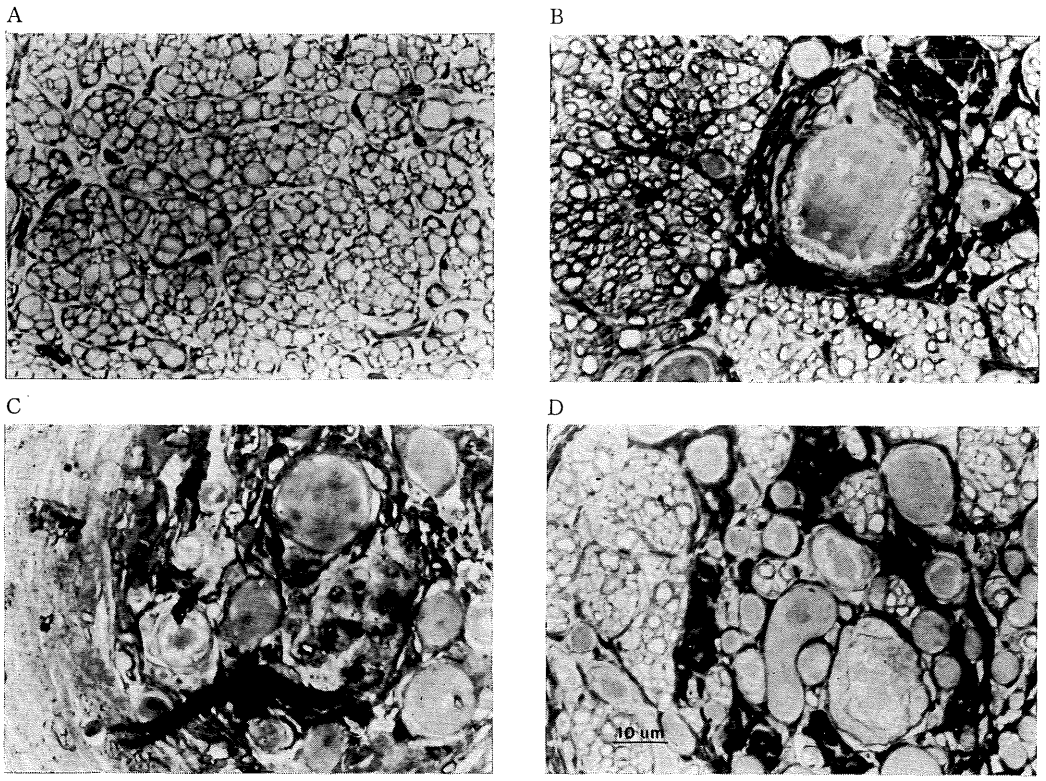


**Fig. 12.** Light microscope images of sites A, B, C and D of Group 5 in the chamber opened at two months after surgery: A (above left) is a site 2 mm on the proximal stump side of the junction, B (above right) is a site 2 mm on the distal stump side of the common peroneal nerve side of the junction, C (below left) is a site directly opposite to A, and the channel was open-ended without any nerve, D (below right) is a site 2 mm on the distal stump side of the sural nerve side. Good regenerating fibers can be observed at A, B and D, there are hardly any regenerating nerves at C which is open-ended. (Tolouidine blue stain × 400).

tance of growth was short, with very few showing growths beyond the silicone cross chamber. Further, the fibers extended distally in almost equal proportions into the 3 channels. In other words, no specific orientation of the regenerating fibers could be observed as they extended distally within the space in the silicone block.

When the distal stump is inserted into a silicone cross chamber which has such characteristics, extension of the regenerating nerve will be promoted in that direction, and it is obvious from my experiments in Groups 2, 3 and 5 that connection of both nerve ends will be achieved. In other words, we were able to verify through our in vivo experiment that the distal stump possesses some important factor which controls the orientation of the regenerating fibers. This important observation was also reported recently by Lundborg et al. (1981)[9,10], in which they used a squareformed mesothelial chamber. Although the material used to enable observation of both ends of the severed nerve and the methodology used by the author differed completely from theirs, the phenomenon observed was essentially the same.

With regards to study of the direction of the outgrowing nerve fibers, there is the well-known in-vivo work of Weiss and Taylor (1944)[5]. They used an artery tube as the chamber for the severed nerve and studied the orientation of the regenerating fibers. They negated the neurotropism on the basis of their results, and upholding the report of Harrison (1914)[7] that the regenerating fibers require some solid support, they established the contact guidance theory. Their results differed completely from mine, in that the regenerating fibers which sprouted from the proximal stump were not

Results

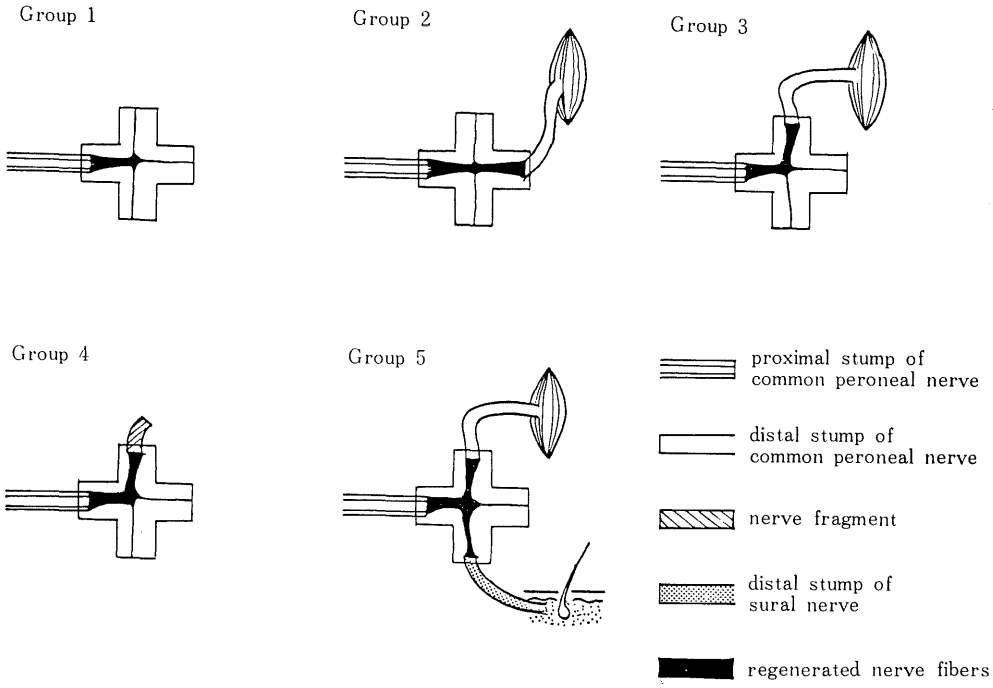


**Fig. 13.** Schematic illustrations of the regenerating fibers in the chambers of each group: Good regenerating fiber growth from the proximal stump can be seen in the channels in which a distal stump or nerve fragment was inserted but growth into the open-ended channel is very poor.

attracted by the degenerated nerve, but grew similarly towards a channel without the nerve as well as that with the nerve. In other words, the regenerating fibers grew at random along the vessel wall. However, in their experiment, they grafted a Y-shaped bifurcation of the common iliac artery to another rat to serve as a chamber to observe the orientation of the regenerating fiber. Thus, it is assumed that they were unable to observe the important role played by the degenerated nerve. That is, it is at present common knowledge to consider that using the artery of another rat as the chamber would pose a grave immunological problem upon the regenerating fibers. Lundborg[10] used a mesothelial tube and observed outgrowing of regenerating fibers from the proximal stump through the center of the tube, and reported that the fibers did not grow along parts of the tube walls, which agrees to my observations as described in connection with the two week cases of Group 2.

Nakai[12,13] cultured the spinal ganglion taken from 2 to 4 month old fetuses and 7 to 14 day old chick embryos, and studied the dynamics of the growing nerve fibers. His results indicated that the filopodia in many instances creeps along some structure, but as it often moves freely and frequently changes its course, it is difficult to consider that the growth cone merely advances mechanically along rails that have been laid. It can be conjectured that the growth cone advances not because it is controlled only by the contact guidance, but because it probably has an autonomous selection capacity.

As is evident from my results, the regenerating fibers do extend some distance in the direction of the open channels, and thus it was demonstrated that they can grow for some distance by self-construction even in the absence of effects of the distal stump and nerve fragment. In other words, the regenerating fibers can grow somewhat without being affected by the distal stump etc, if the circumstances of the surrounding are favorable, and it is obvious

that they extend under some predetermined rule, which may be contact guidance. However, the essential points are as follows. That is, if the distal stump or nerve fragment is within a distance range whereby it can exert effects, the regenerating fibers will sprout and grow a distance range whereby it can exert effects, towards the distal stump or nerve fragment, and establish good organization. In other words, the distal stump and nerve fragment have effects powerful enough to override whatever rule there is that governs the regenerating fibers when there is no distal stump, and guide the fibers in their direction. This implies that the tip of the cone possesses the capacity to make selection and sense affecting factors, and thus can make subtle changes in its behavior. It is assumed these results explain the difference in regenerating fiber orientation, organization and maturation between cases with and without the distal stump or nerve fragment.

Weiss advanced the pioneer fiber theory in which he states the peripheral nerve extends distally. That is, some of the fibers among the sprouting regenerating fibers extend along the Schwann tube as pioneer fibers and communicate with the target organ, after which they acquire a trophic factor, and guide other regenerating fibers in that direction. To confirm this, the experiment of Group 4 was carried out in which a nerve fragment only was inserted. Findings differing definitely from those of the distal stump inserted group (Group 3) could not be observed. In other words, this suggests that the muscles and sensory end organs governed by the common peroneal nerve, have as target organs hardly any important roles in orientation. Further, in some of the two week cases in Group 3, the orientation of regenerating fibers was already determined. At such an early time, it is obvious that the evoked muscle potential was not recorded, and the pioneer fiber and the target organs such as governing muscles and sensory organs are not united.

Nerve Growth Factor is trophic towards sensory neurons in vitro, but it is well known it has no effects on motor neurons[8]. On the other hand, in vivo Nerve Growth Factor is transported its lifetime in sensory neurons[14,15]. However, there are no Nerve Growth Factors in motor neurons. Goedert[5] et al. demonstrated the biological importance of retrograde axonal transport of Nerve Growth Factor in sensory neurons in newborn and adult rats using neuropeptide substance P as a biological marker. In view of the above, if regenerating fibers are guided and extend by chemical substances from the distal stump under the neurotropism theory, it is likely that the chemical substance would differ for motor fibers and sensory fibers. In other words, the possibility that the chemical substance for sensory fibers would have no effects on motor fibers cannot be denied. The aim of Experiment 5 was to ascertain whether the sensory fibers were capable of only searching for the sensory fiber Schwann tubes, and the motor fibers were capable of only searching for the motor fiber Schwann tubes. Practically the common peroneal nerve mostly consists of motor fibers, while the sural nerve has no motor fibers. If the above fibers have the above capabilities, theoretically, practically most of the fibers should be oriented towards the distal stump of the common peroneal nerve which was involved in motor prior to severance because the common peroneal nerve was used as the proximal stump. As there were only 4 rats, no definitive statement can be made, but many fibers were oriented towards the distal stump of the sural nerve, indicating that whether the stump originally consisted of sensory fibers or motor fibers did not have great effects on the orientation of regenerating fibers. It seems that the ratio of regenerating fibers may depend on the ratio between the diameter of the common peroneal nerve and that of the sural nerve.

The experimental results have demonstrated in vivo that regenerating fibers from the proximal stump of severed peripheral nerves have special characteristics for seeking out the distal stump following complicated pathways if necessary, and growth in that direction is promoted so that both stumps can be reunited. From these results, it can be readily assumed that the severed distal stump surface per se plays a key role. Forssman[4] and Cajal[3] presume some kind of neurotrophic substance is released from the degenerating fiber which serves to guide the regenerating fibers. This is an old hypothesis, but there have been yet no reports which positively affirm it. The findings of my experiment can be readily explained by the

mechanism of the neurotrophism, but I have not been able to directly prove that a neurotrophic factor is being released.

It is felt two hypotheses can be advanced. The first being that regenerating fibers are guided in that direction due to some unidentified neurotrophic factor, and the other is that the Schwann cells per se are released from the distal stump or nerve fragment and provide orientation to the regenerating nerves. As to neurotrophic factors, Nerve Growth Factor is well known at present, and according to the experiment of Gundersen et al.[6], the dorsal root axons are chemotaxic to Nerve Growth Factor.

It was demonstrated that they possess such characteristics as attempting to extend in the direction of higher concentration. It is yet unknown as to whether such trophic factors are released also in vivo. If they are being released, it is considered the source for releasing such factors is located within tissue structure of the peripheral nerve trunk. This experiment also does not, however, positively confirm that this neurotrophic factor is being conveyed by retrograde axonal transport after transection. The results of the experiments shown here, indicate that it may be well assumed that such factors are already stored in the peripheral nerve trunk. Some scientists feel that the source is the Schwann cell or the degenerated myeline[1,2,21].

Another hypothesis is that Schwann cells per se emerge from the distal stump or nerve fragment and establish contact with the regenerating fibers by which they are guided to the distal stump or nerve fragment. Observation of the channel crossing in the two-week rats of Group 3 showed that there were more Schwann cells in B where the distal stump had been inserted than in C or D in which no nerves had been inserted and no axons were seen. However, it is difficult to conceive that the Schwann cells will emerge several mm from the distal stump Thomas[17] reporded on his EM observations of the distal stump of the peripheral nerve, and pointed out that Schwann cells do in fact emerge from the distal stump. However, it was only for a limited distance, and not as great a distance as in my experimental model. Thus, it seems to be considered that even if there is emerging of Schwann cells, it is not so great as to affect the orientation of the regenerating fibers. However, the above possibility can not be denied because the condition which surrounded the distal stump in my experiment is different from that in Thomas' observation.

The theoretical background as to whether or not the results obtained in my experiment are due to neurotropism will require further study, but my findings were definitely different from those of Weiss and Taylor[20]. The regenerating fibers from the proximal stump do possess a characteristic of extending towards the distal stump, and it is felt that it is possible for spontaneous connection of the severed peripheral nerves occasionally encountered in clinical cases to actually occur.

From some time in the past it had been assumed that there was a special hormone in the body, and with advancement in techniques for isolation and extraction, it has been demonstrated that it actually exists and plays an important role. This historical finding gives us hope that it will become possible in the future to isolate and extract minute volumes of in vivo neurotrophic factors. And when it becomes possible to use such factors in vivo, great strides can be expected in the clinical recovery of the severed peripheral nerve.

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