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Histologic examination of peripheral nerves elongated by tissue expanders

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SUMMARY. **Histological changes induced by the slow stretch of peripheral nerves using a tissue expander were studied using a rat sciatic model. Tissue expanders were adapted to elongate the sciatic nerve *in vivo*. Nerves elongated over an eight week period using this method achieved an average increase in length of 88%.**

Light microscopic examination revealed that individual axons were separated but each axon and the myelin sheaths were stained well. Electron microscopic examination showed that the convoluted contour of the axons appeared and some loss of myelin was also observed, but the intraneural cytoskeleton elements were kept intact.

Soft tissue expansion was introduced by Neumann in 1957.[1] Since then, many reports have described the use of tissue expanders in skin and soft tissue expansion. A few reports have described the use of tissue expanders for elongation of the peripheral nerves.[2,3]

Manders *et al.*[2] and Mackinnon[3] have suggested the possible use of tissue expansion for the elongation of the peripheral nerves. Recently Milner,[4] and Milner and Wilkins[5] have reported the changes observed in peripheral nerves elongated with tissue expanders using electrophysiological and histological techniques. This study was designed to examine the histological changes in the elongated peripheral nerves using both light and electron microscopic techniques.

Materials and methods

Design of the tissue expander

15 cc Silastic® tissue expanders were custom-made

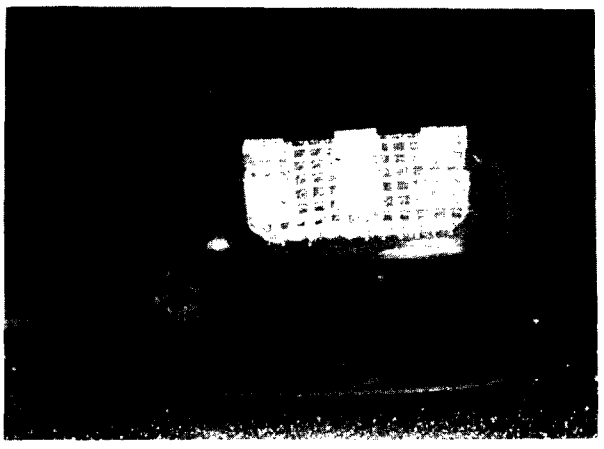


Fig. 1

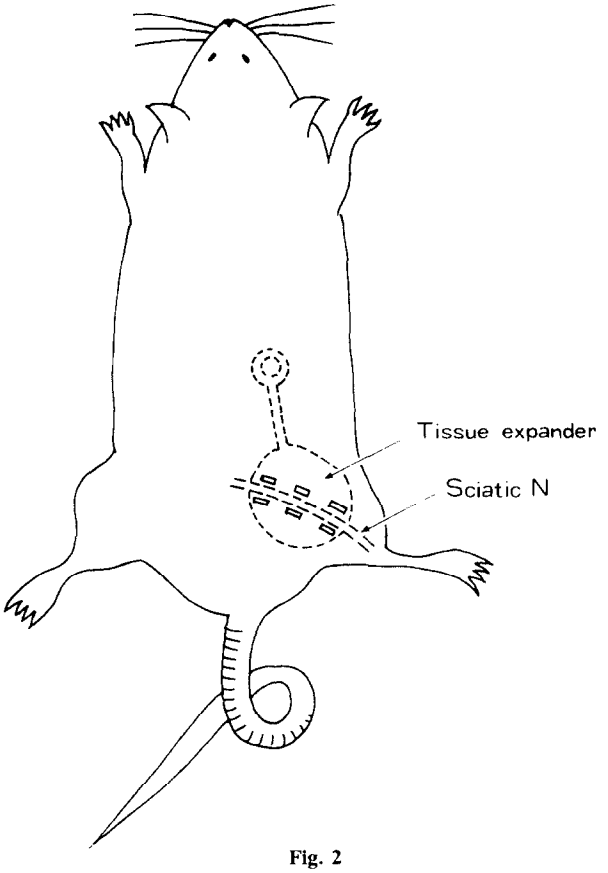


Fig. 2

Figure 1—Close up view of tissue expander. Note the three built-in grooves on the top of the expander to keep the nerve centred over the device. **Figure 2**—Schematic drawing of the experimental model.

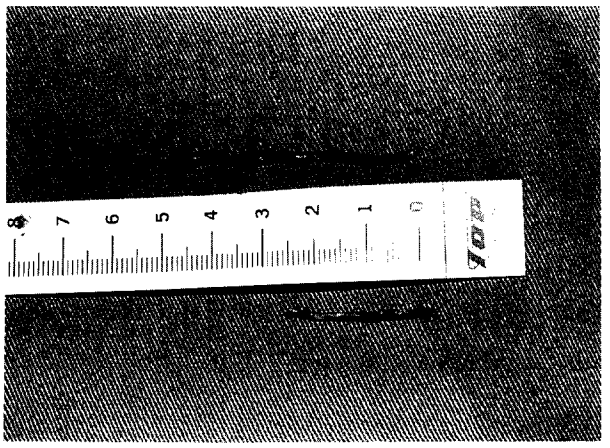


Fig. 3

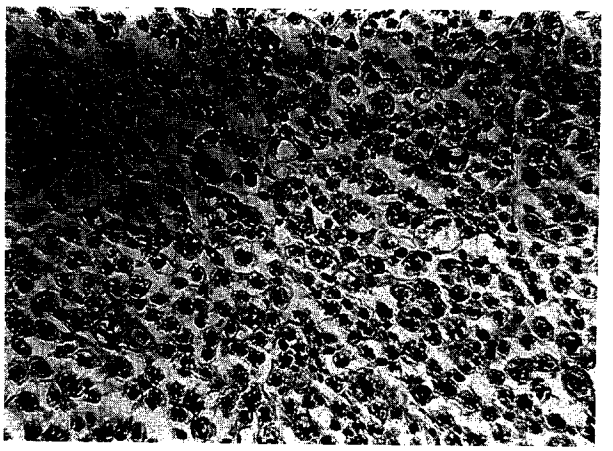
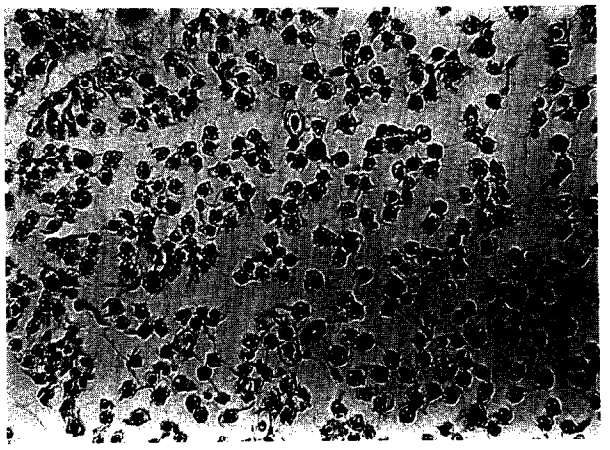


Fig. 4



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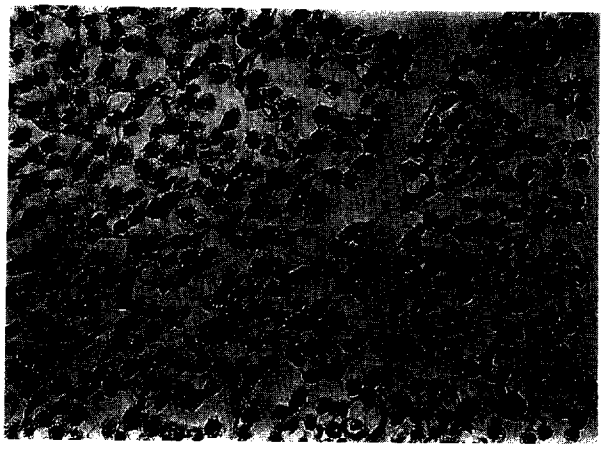

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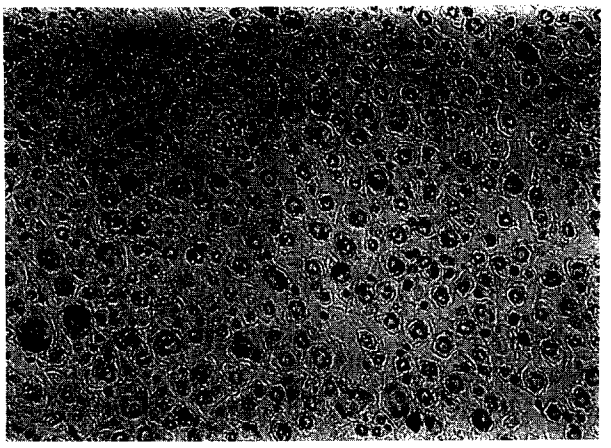

Fig. 7

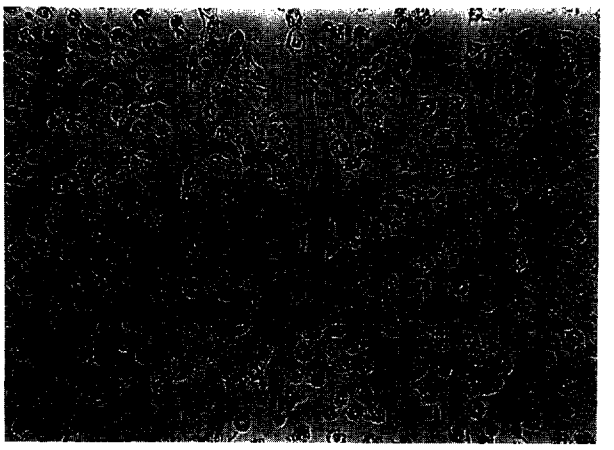

Fig. 8

Figure 3—Typical appearance of the nerve after removal of the expander. The nerve was elongated to almost double the length of the control nerve (below). **Figure 4**—Bodian's stain of the proximal segment of the elongated nerve (×400). Each axon stains equally well. **Figure 5**—Bodian's stain of the middle segment of the elongated nerve (×400). Individual axons are spaced further apart from each other due to swelling of the epineurium. Staining is still consistent. **Figure 6**—Bodian's stain of the distal segment of the elongated nerve (×400). The appearance is nearly identical to that of the middle segment. **Figure 7**—Klüver-Barrera's stain of the proximal segment of the elongated nerve (×400). The myelin density is high in the proximal segment. **Figure 8**—Klüver-Barrera's stain of the middle segment of the elongated nerve (×400). The individual axons are spaced further apart from each other and the myelin density is lower.

with three built-in grooves along their superior surface to keep the nerve centred over the device. An injection port was connected by Silastic tubing to the expander (Fig. 1).

Experimental method

Studies were carried out on 20 male, Wistar-derived rats weighing 200 to 300 g. The rats were anaesthetised using intra-peritoneal injections of sodium pentobarbital. The sciatic nerve was exposed over a distance of 25 mm and two 10-0 nylon sutures were placed in the

Fig. 9

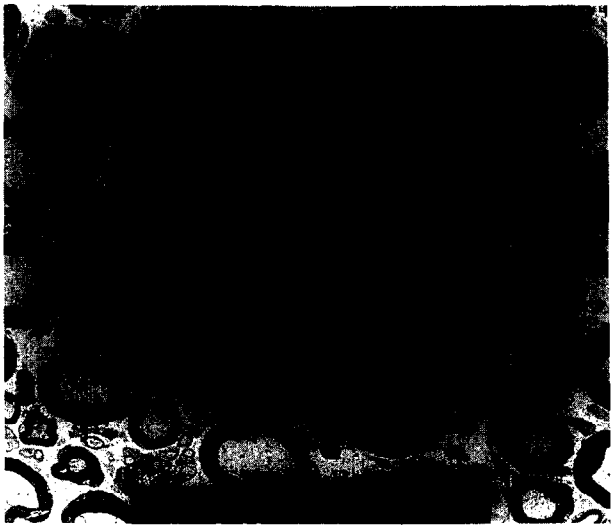

Fig. 10

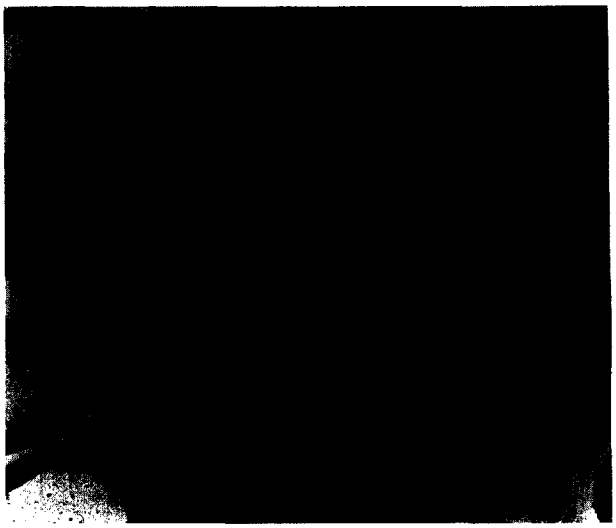

Fig. 11

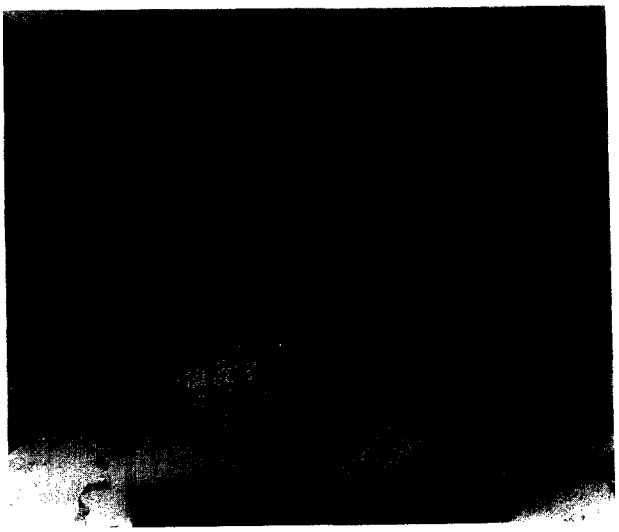

Fig. 12

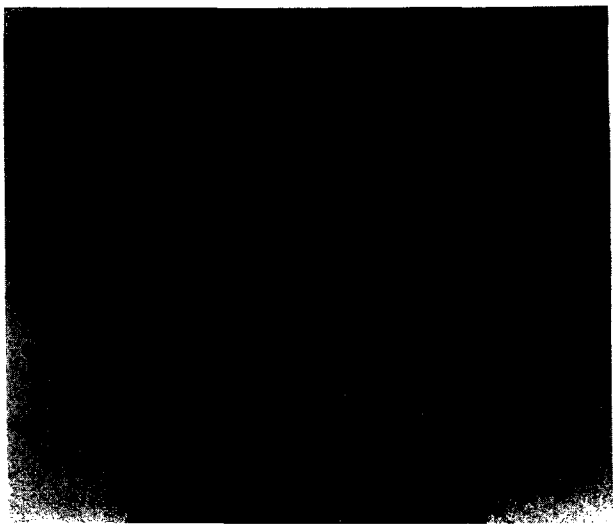

Fig. 13

Fig. 14

Figure 9—Klüver-Barrera's stain of the distal segment of the elongated nerve (× 400). Changes such as vacuolation and segmental demyelination are noted. **Figure 10**—Electron microscopic finding, normal control nerve (× 1500). The cross section of each axon is nearly circular. **Figure 11**—Electron microscopic finding, proximal segment of the elongated nerve (× 1500). The appearance is almost identical to the normal control. **Figure 12**—Electron microscopic finding, middle segment of the elongated nerve (× 1500). Note the convoluted contour of the axons. **Figure 13**—Electron microscopic finding, middle segment of the elongated nerve (× 3500). The intraneural cytoskeleton elements such as neurofilament and tubulin are intact. **Figure 14**—Electron microscopic finding, distal segment of the elongated nerve (× 1500). Some loss of myelin indicating segmental demyelination is observed.

epineurium a measured 25 mm apart using an operating microscope. A tissue expander was then placed under the sciatic nerve. A subcutaneous tunnel was created on the animal's back to accommodate the tubing and injection reservoir (Fig. 2).

Inflation of the expander under the sciatic nerve was accomplished by three separate percutaneous injections of 5 cc normal saline under general anaesthesia. The expander was inflated to a total volume of 15 cc. Injections were performed every 2 weeks at days 14, 28, and 42. The expander was left fully inflated for 2 more weeks. The rats were anaesthetised again with an intra-peritoneal injection of sodium pentobarbital. The sciatic nerve was exposed, and the expander was deflated and removed. The distance between the epineurial marker sutures was measured as soon as possible and in most cases it took two minutes to measure. The sciatic nerve was then removed for histological examination. Three specimens were harvested from each sciatic nerve. Segments of nerve overlying the proximal, middle and distal parts of the tissue expander were examined.

Histological examination

Light microscopic examination of the proximal, middle and distal segments of the elongated sciatic nerve was performed. The nerves were fixed in 10% formalin. Fixed nerve specimens were embedded in paraffin blocks. Transverse sections were then prepared with Bodian's and Klüver-Barrera's stains.

Electron microscopic examination of each segment of the elongated sciatic nerve was also performed. Frozen transverse sections were fixed with 5% glutaraldehyde. Fixed sections were then dehydrated through a graded alcohol series and embedded in Epone. Ultra thin sections were then stained with uranyl acetate and lead citrate.

Results

Following removal of the tissue expanders, the sciatic nerves were found to have significantly increased in length. Elongation of the nerve between marker sutures ranged from 16 to 24 mm. An average of 22 mm was observed. The original nerve length was designed to be 25 mm. Thus, a mean elongation of 88% was observed in the 20 rats studied (Fig. 3).

Bodian's stain

This stain was used to characterise changes in the axons of the elongated nerves. Individual axons were separated by a greater distance than that observed in the normal controls. No differences were observed on Bodian's staining between the three specimens harvested from the proximal, middle and distal segments of the elongated nerves (Figs 4, 5, 6).

Klüver-Barrera's stain

This stain was used to evaluate the myelin sheath of the elongated nerves. The myelin sheath was stained in each of the proximal, middle and distal segments of the nerves, but the myelin density was found to be much higher in the proximal segment. Staining was noted to be weaker in the middle and distal segments of the elongated nerves. Some changes such as vacuolation and segmental demyelination were identified in the middle and distal segments (Figs 7, 8, 9).

Electron microscopic findings

Neural cytoskeleton elements such as neurofilament and tubulin were intact in all segments and no differences were noted when compared with the normal controls. The architecture of the myelin sheaths was, however, altered in the middle and distal segments of the elongated nerves. The myelin sheaths of the normal controls and the proximal segment of the elongated nerves were circular in cross-section (Figs 10, 11). This was in contrast to the middle and distal segments, where the myelin sheaths were convoluted (Figs 12, 13).

Segmental demyelination was also observed in these segments (Fig. 14).

Discussion

We have observed that patients undergoing soft tissue expansion did not develop cutaneous anaesthesia. This led to the conclusion that the cutaneous nerves had elongated, by means of stretch or growth, to allow for continued sensation. A similar effect was observed with motor nerves. Clinical experience has shown that rapid expansion of a nerve, as is seen with a rapidly expanding haematoma, will result in the loss of neural function. By contrast, the slow expansion of a peripheral nerve can be carried out without any measurable loss of nerve function. This has led to the suggestion that peripheral nerves could be safely expanded.

The question has arisen, however, as to what kinds of changes are to be found in the elongated nerves. This study was designed to observe only the histological changes occurring in the elongated peripheral nerves. Functional evaluation of elongated nerves using electrophysiological techniques was not performed.

In this study 8 weeks were required to complete the nerve elongation. Milner[4] has reported three elongation patterns: rapid, intermediate and slow. The slowest group in Milner's study completed nerve elongation in only 17 days. The method described herein could, therefore, be labelled "super slow". By employing this method, the peripheral nerves have been elongated to almost double their original length. This result was surprising and went beyond our expectations. Mechanical disruption of elongated nerves was not observed in any of the animals studied, even when increases in nerve length of up to 100% were achieved. This is probably because the tensile forces were distributed more and more slowly and

uniformly over the expanding portion of the nerves. This is in contrast to the distribution of forces operational in the case of the acutely stretched nerve. The histological changes observed after the super slow elongation of the peripheral nerve were few. Light microscopic examination revealed normal axons along the nerve length. Some changes such as vacuolation and segmental demyelination were, however, recognised in the middle and distal segments of the elongated nerves. The individual axons were noted to be spaced further apart from each other due to swelling of the endoneurium. These findings are in agreement with the study of Milner and Wilkins.[5]

Electron microscopic findings showed that intraneural cytoskeleton elements such as neurofilament and tubulin remained intact. On the other hand, changes in the myelin sheath were recognised in some axons. Twisted shape axons were noted everywhere and we think this is due to the swelling of endoneurium. Once the swelling of endoneurium occurred in the elongated nerves, the axons were also pressed and finally the figures of the axons changed from circular to convoluted.

Of course, some loss of myelin indicating segmental demyelination represents the beginning of nerve degeneration, but the more important finding is that the intraneural cytoskeletons such as neurofilament and tubulin remained intact. This is significant, because these elements are thought to play an important role in normal nerve functioning.

We recognise that the nerves are very durable when confronted with chronic tensile forces. The stretch limit was probably beyond our expectations. The results of this histological study suggest that the super-slow expansion of peripheral nerves using a tissue expander will allow the elongation of nerves to nearly double their original length, without a significant loss of function.

Although significant data were obtained in this study, questions regarding the use of this method must still be resolved. The technical matters that require further study include: the limits of elongation, the optimum size of the expanders, the rapidity of expansion and the approximate interval between injections. Studies of various sizes of nerves being expanded at different speeds are also required.

A study of the functional capacity of peripheral nerves elongated by the "super-slow" tissue expander technique should be performed to confirm the results of this histological study.

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