

THE DEPOSITION OF COLLAGEN IN RELATION  
TO SCHWANN CELL BASEMENT MEMBRANE DURING  
PERIPHERAL NERVE REGENERATION

P. K. THOMAS. From the Institute of Neurology, London, England, and the Department of Pathology, McGill University, Montreal, Canada

The basement membranes surrounding the Schwann cells of peripheral myelinated nerve fibers form uninterrupted tubes ensheathing the fibers, being continuous across the nodes of Ranvier (9, 20). During Wallerian degeneration, the basement membranes persist and within the spaces enclosed by them (the "Schwann tubes") the Schwann cells proliferate concurrently with the removal of the degenerating axon and myelin. Longitudinal columns of cells result, constituting the "bands of Büngner." These changes have been described both in nerve roots (11, 12) and in the peripheral nerve trunk (19).

Nathaniel and Pease (13) have demonstrated that during degeneration after a localized crush injury to the dorsal roots, in the rat, the Schwann cells become surrounded by new basement membrane and collagen fibrils, which form within the original Schwann tubes. The present communication confirms this finding for rabbit peripheral nerve and reports a relationship between the formation of new basement membrane and collagen deposition.

MATERIAL AND METHODS

The observations were made on the nerve to the medial head of the gastrocnemius muscle of adult male albino rabbits. The rabbits were anesthetized with pentobarbital sodium and ether, and the nerves exposed under aseptic conditions. The nerves were cut approximately 3 cm above the muscle without complete severance of the epineurium, so that the divided ends were separated by a short gap. The animals were allowed to survive for periods of 7, 10, 14, 21, 28, and 35 days before biopsy, one animal being examined for each survival time. At biopsy, the animals were again anesthetized with pentobarbital sodium and ether, and the nerves were exposed and fixed *in situ* for 5 minutes in 1 per cent osmium tetroxide in mammalian Ringer solution buffered at pH 7.4 with Veronal-acetate. Short lengths of nerve were then removed from the distal stumps about 2 cm below the point of section and fixed for a further 4 hours at 4°C. Following dehydration in graded concentrations of ethanol, they were stained for 4 hours by immersion in 1 per cent phosphotungstic acid in absolute ethanol and then embedded in Araldite. Transverse and longitudinal sections of the nerves were obtained with a Porter-

Blum microtome and examined with a Siemens Elmiskop 1 or an RCA EMU 3E.

#### OBSERVATIONS

The changes in the nerves examined between 7 and 21 days after section were similar to those reported in an earlier paper on the alterations in the endoneurial sheaths of peripheral myelinated nerve fibers during Wallerian degeneration (19), and therefore they will not be described in detail. At 7 days after operation, the basement membranes that had surrounded the Schwann cells in the intact nerve were still present. In the myelinated nerve fibers, the myelin had broken up into large ovoids, usually containing axon debris, and were surrounded by Schwann cells and their processes. Smaller droplets of myelin were also present, either in membrane-bounded vacuoles within the cytoplasm of Schwann cells or free in the extracellular space within the Schwann tubes. In the region of the ovoids, the basement membranes generally remained expanded, retaining a circular outline in transverse section. Between the ovoids, the Schwann tubes had frequently collapsed and the basement membranes were irregularly folded, this being related to the reduction in the volume of the contents of the tubes. This is illustrated in Fig. 1, where several Schwann cell processes are present within the space bounded by the folded basement membrane, together with material derived from the degenerating axon and myelin. Longitudinal sections through the nerve revealed that the maximal amount of folding occurred between the ovoids of large myelinated fibers. The situation in this nerve, which contains relatively few non-myelinated nerve fibers, thus differs from that in the degenerating nerve roots examined by Nathaniel and Pease (13), where the greatest amount of folding occurred in the basement membranes that had

surrounded Schwann cells associated with non-myelinated axons. In the present investigation, it proved difficult to distinguish, in transverse sections of the nerve, between degenerating Remak fibers and degenerating myelinated nerve fibers in which myelin remains did not happen to be present at that particular level.

Between 10 and 21 days after operation, progressive removal of the myelin and axon debris was evident, although whether this was accomplished by Schwann cells or by macrophages that had entered the Schwann tubes is as yet uncertain. This was accompanied by proliferation of the Schwann cells, which occurred within the confines of the persisting Schwann cell basement membranes. Where the basement membranes had collapsed, the folding remained, so that Schwann tubes with highly irregular outlines in transverse section were frequently encountered.

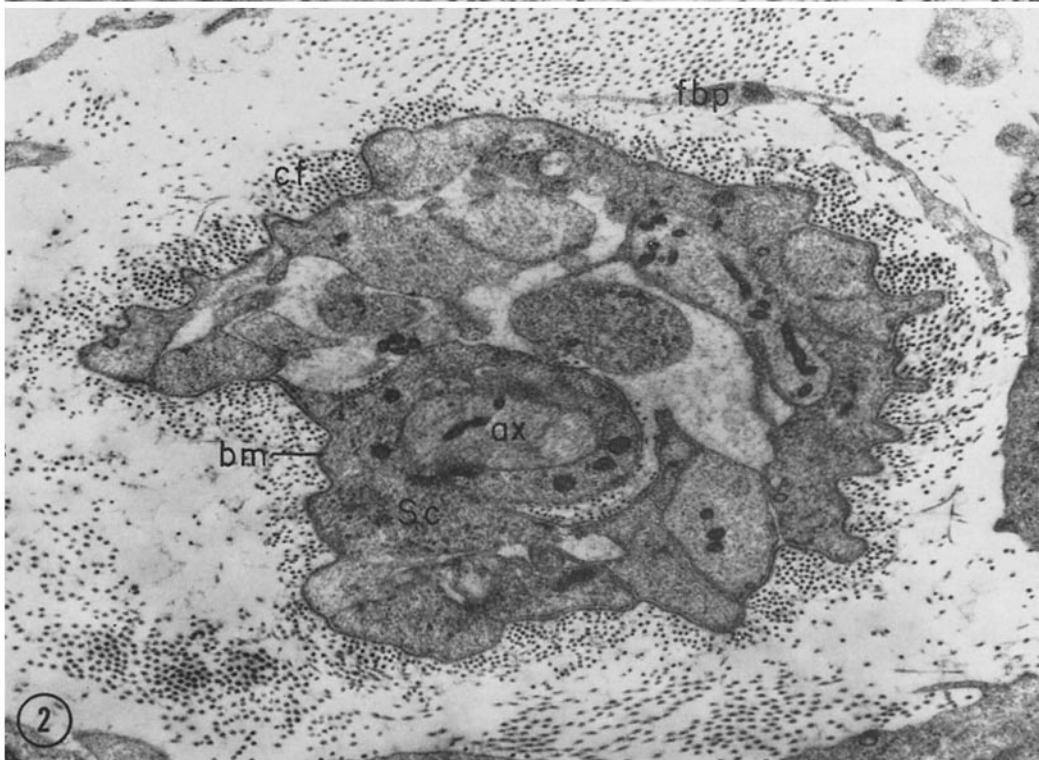
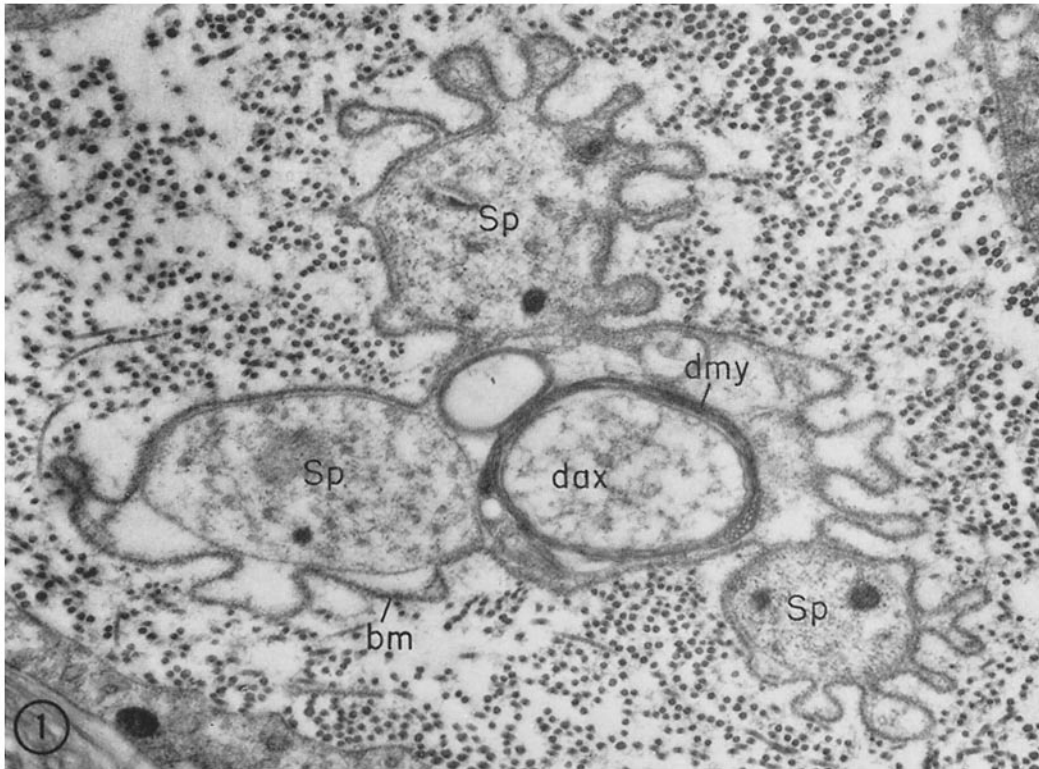
In the material examined at 28 and 35 days after nerve section, numerous axons were present within the Schwann tubes, although, as noted by Glimstedt and Wohlfart (7), differentiation between axons and Schwann cell processes was not always easy except where mesaxons or myelin had formed. Many of the basement membranes that had surrounded the Schwann cells in the uninjured nerve were still intact, enclosing proliferated Schwann cells and cells containing myelin debris and multiple vacuoles. The latter were usually situated centrally and were surrounded by the Schwann cells and their processes.

In relation to some of the proliferated Schwann cells, new basement membrane had formed, this having taken place within the spaces bounded by the original basement membranes. It had a normal relationship to the Schwann cell membrane, being separated from it by a gap of approximately 250 Å. In Figs. 2 and 3, it will be seen that new basement membrane has formed at two

---

FIGURE 1 Transverse section through Schwann tube 7 days after severance of nerve. The basement membrane (*bm*) that surrounded the Schwann cell is folded and encloses several cell processes (*Sp*) probably derived from Schwann cells, together with degenerating myelin (*dmy*) and axonal material (*dax*).  $\times 27,000$ .

FIGURE 2 Portion of transverse section through nerve 28 days after severance. A Schwann tube showing early reinnervation is surrounded by a zone of collagen fibrils (*cf*) and fibroblast processes (*fbp*) external to the basement membrane (*bm*) of the original nerve fiber. New basement membrane and collagen fibrils have formed within the Schwann tube in relation to a Schwann cell (*Sc*) containing an axon (*ax*).  $\times 17,000$ .



sites. At both sites it consists, in transverse section through the nerve, of short lengths with free ends. These appearances cannot therefore be explained in terms of sections through inpocketings of the original basement membrane.

Collagen fibrils had also formed within the Schwann tubes. It was observed that they displayed a strong tendency to be localized in relation to the newly formed basement membrane, usually with a longitudinal orientation. Thus, on the left of Fig. 3, a row of nine collagen fibrils is seen in transverse section, arranged immediately external to a short length of newly formed basement membrane. On the right of the figure, a more extensive length of basement membrane has been formed and is also associated with collagen fibrils. Both the newly formed basement membrane and collagen were generally seen in relation to Schwann cells containing an axon, as is the situation in Figs. 2 and 3.

In other Schwann tubes, the proliferated Schwann cells were completely surrounded by basement membrane. The peripherally placed cells were usually bordered by part of the original basement membrane on their external aspects, this being continuous with newly formed basement membrane on their inner aspects. Collagen fibrils had accumulated between the individual Schwann cells within the Schwann tubes, and this was accompanied by the disappearance of the portions of the original basement membrane intervening between the peripherally placed cells. In Fig. 4, partial separation of the Schwann cells that had formerly comprised a single Büngner band has taken place; a more complete separation is evident in Fig. 5.

In the specimens taken at 14 to 35 days after operation, there was an obvious increase in the number of endoneurial fibroblasts, although quantitative estimates of their numbers in comparison with the normal have not yet been made. An example is shown in Fig. 6. These cells were recog-

nizable by the absence of a basement membrane, the possession of an abundant endoplasmic reticulum, and the presence of multiple elongated cell processes encircling the Schwann tubes and extending longitudinally in the nerve. The endoplasmic reticulum was frequently irregularly dilated and contained fine granular or fibrillar material. Large smooth-walled vacuoles were also present within the cytoplasm, as were small bundles of fine filaments. These features correspond to those described as characterizing fibroblasts in the normal peripheral nerve (18) and elsewhere (16).

#### DISCUSSION

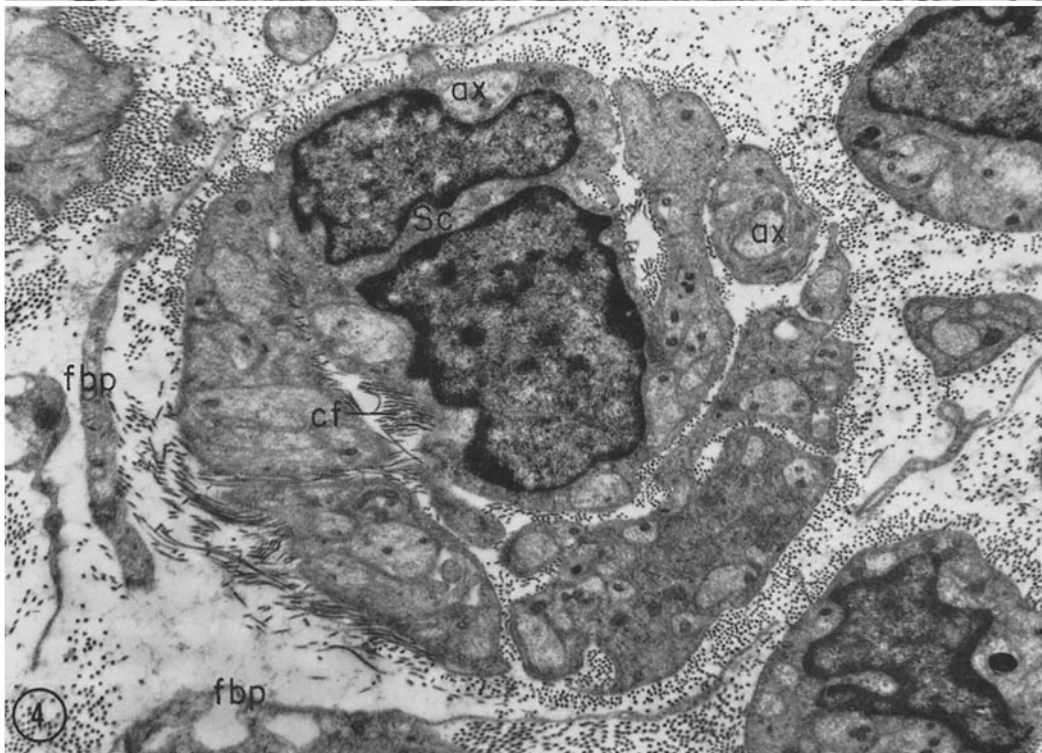
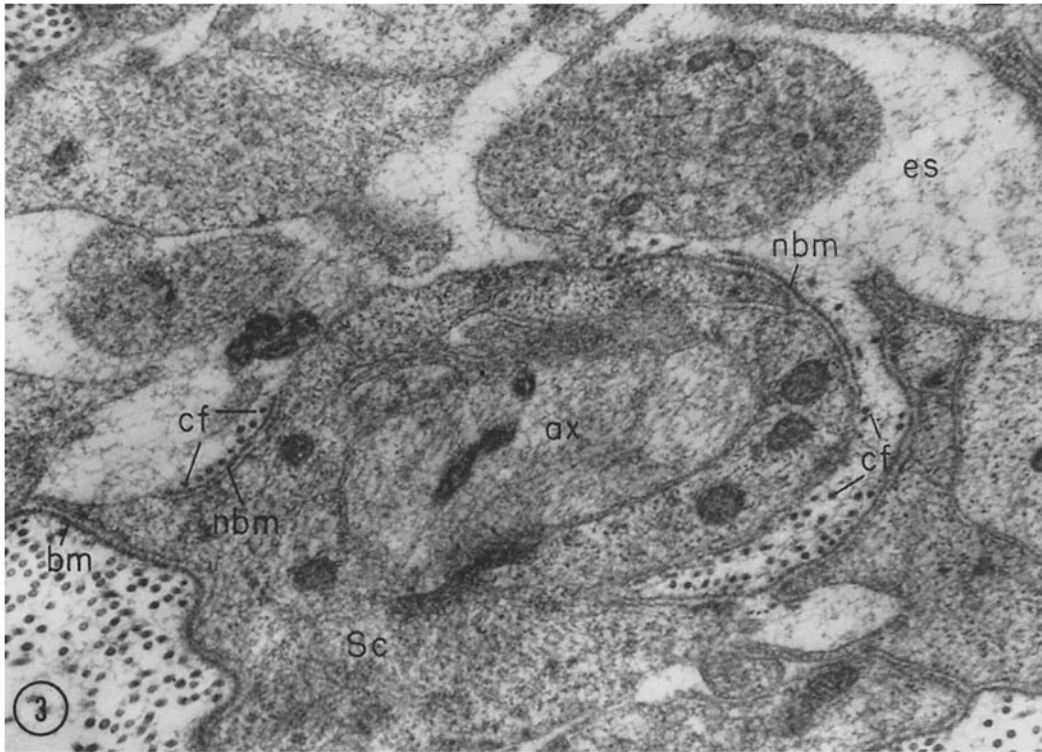
Current views as to the mechanism of collagen formation envisage the release by fibroblasts of monomeric collagen (tropocollagen) into the extracellular space where it then aggregates into fibrils (8). Autoradiographic studies on collagen synthesis in cartilage by Revel and Hay (15) have indicated that soluble collagen probably accumulates initially in the granular endoplasmic reticulum of the chondrocytes and then in large vacuoles in the Golgi zone. Sheldon and Kimball (17) were able to indentify collagen within Golgi vacuoles of chondrocytes. The mechanism of release of the collagen from the cells is still uncertain. Although it has been suggested that intracellular fibrils may be released into the extracellular space by disintegration of the plasma membrane (see *e.g.*, reference 3), this now seems unlikely (15, 16). It is more probable that soluble monomeric collagen is released from secretory vacuoles at the cell surface, possibly by fusion of the wall of the vacuole with the plasma membrane (15).

To the morphologist, the factors involved in the determination of the site of deposition of the collagen fibrils are of considerable interest, but so far progress in this direction has largely been speculative. Soluble collagen may diffuse some

---

FIGURE 3 Detail from Fig. 2, showing newly formed basement membrane (*nbm*) and associated collagen fibrils (*cf*) present within the intratubal extracellular space (*es*). A portion of the original basement membrane (*bm*) is also shown.  $\times 37,000$ .

FIGURE 4 Transverse section through a reinnervated Schwann tube, 28 days after severance of nerve, with several Schwann cells (*Sc*) associated with unmyelinated axons (*ax*). The Schwann cells have become partially separated by the intratubal formation of collagen fibrils (*cf*).  $\times 12,000$ .



distance from the cells of origin before becoming precipitated as fibrils (15), although Porter and Pappas (14) have described, in tissue culture, the deposition of collagen fibrils in close relationship with the cell surface of fibroblasts. It is known that certain mucopolysaccharides, such as chondroitin sulphate, greatly accelerate the polymerization of collagen *in vitro* (see *e.g.*, reference 21). Although fibroblasts may secrete mucopolysaccharide (5), there is as yet no clear evidence that this contributes to the normal formation of collagen. However, as discussed by Revel and Hay (15), it is certainly possible that cells determine the site of precipitation of collagen fibrils by controlling the chemical composition of the extracellular connective tissue space.

The present observations have revealed a selective deposition of collagen fibrils in relation to the newly formed basement membrane of Schwann cells, the outer surface of which shows material extending irregularly into the connective tissue space similar in appearance to the material comprising the basement membrane itself. The newly formed basement membrane therefore appears to provide an environment conducive to collagen polymerization, although the predominantly longitudinal orientation of the collagen fibrils requires explanation. It could be suggested that the new basement membrane is produced by the Schwann cells in response to the presence of collagen, but this seems less likely in view of the fact that the collagen first appears in close proximity to the new basement membrane and not more diffusely throughout the Schwann tubes.

The suggestion that Schwann cells are capable of producing collagen has been advanced several times in the past (see *e.g.*, reference 10), and in recent years this view has been taken by Causey and Barton (2) and Barton (1). This possibility was

also raised by Nathaniel and Pease (13) from observations on regenerating dorsal roots in the rat, because of their finding that endoneurial fibroblasts were rare except in the immediate proximity of capillaries. Their suggestion is difficult to evaluate, however, as Gamble (6), who also examined regenerating dorsal roots in the rat, reported the presence of large numbers of fibroblasts.

In the nerves examined in the present investigation, numerous fibroblasts were encountered in the endoneurial connective tissue between the Schwann tubes. It has to be considered, therefore, whether these cells could be the source of the soluble precursors for the intratubal collagen fibrils, the actual polymerization taking place locally within the Schwann tubes. This would necessitate the passage of unpolymerized collagen through the persisting basement membranes of the original Schwann cells. Although there is no direct evidence on this question, it seems possible that this could occur, since, as was pointed out by Nathaniel and Pease (13), quite large molecules have been shown to cross basement membranes (4). Nevertheless, whatever the origin of the collagen precursor substances, the results reported here lend support to the view expressed by Nathaniel and Pease that Schwann cells are involved in the local polymerization of collagen during nerve regeneration.

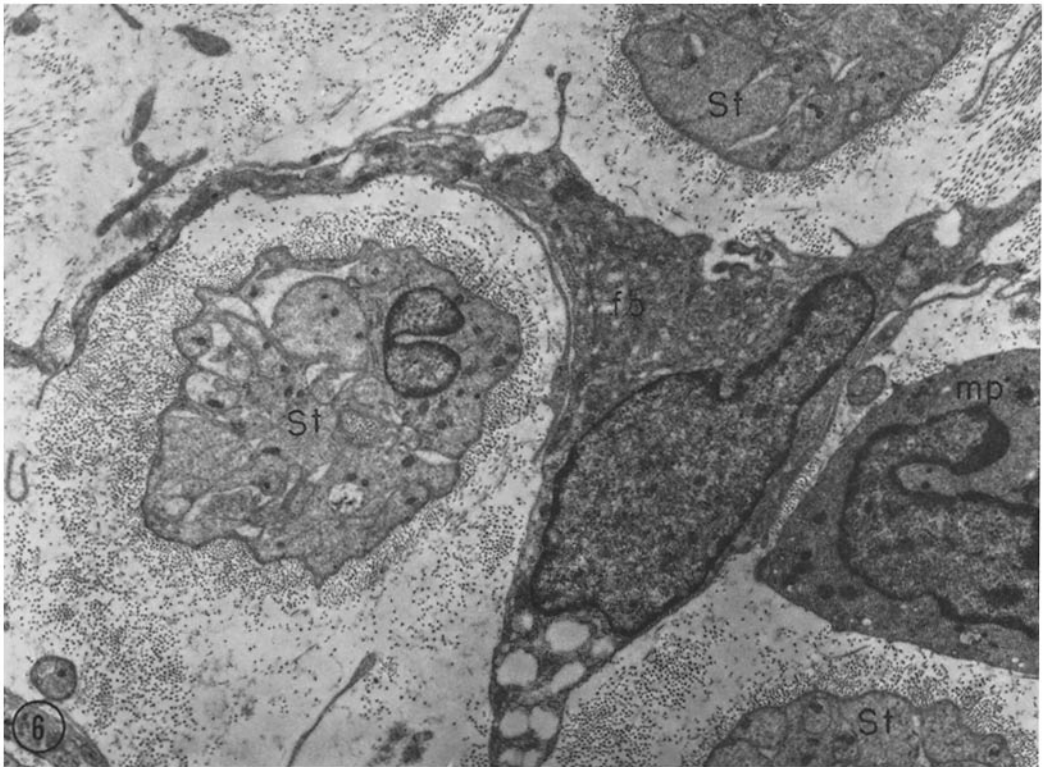
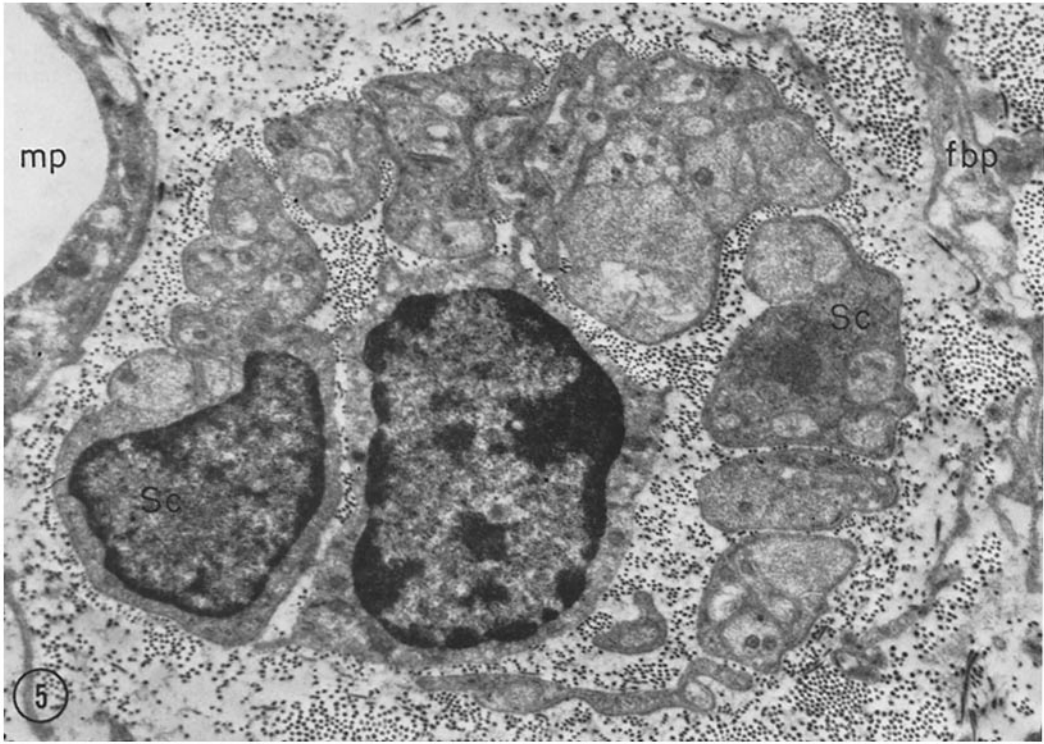
I wish to thank Miss Nora Shepard, Miss Jean Slatford, and Mr. Robert Ng for technical assistance, and Dr. R. D. Harkness, Dr. D. W. James, and Dr. H. Sheldon for helpful discussion. A personal grant from the Polio Research Fund is gratefully acknowledged, as is the provision of generous facilities in the Electron Microscope Laboratory at Maida Vale Hospital by Dr. W. H. McMenemy and Dr. M. Kidd.  
*Received for publication, January 15, 1964.*

---

FIGURE 5 Transverse section taken 35 days after nerve transection of a group of Schwann cells (*Sc*) that had initially comprised a single Büngner band, these having become separated by intratubal collagen formation. Endoneurial fibroblast processes (*fbp*) and a portion of a macrophage (*mp*) are also seen. The nature of the centrally placed cell, which does not possess a basement membrane, is uncertain.  $\times 14,000$ .

FIGURE 6 Portion of transverse section through nerve, 28 days after severance, showing an endoneurial fibroblast (*fb*) with a number of elongated processes extending between adjacent Schwann tubes (*St*). The cell on the extreme right of the figure is probably a macrophage (*mp*).  $\times 3,300$ .





## BIBLIOGRAPHY

1. BARTON, A. A., An electron microscope study of degeneration and regeneration of nerve, *Brain*, 1962, **85**, 799.
2. CAUSEY, G., and BARTON, A. A., The cellular content of the endoneurium of peripheral nerve, *Brain*, 1959, **82**, 594.
3. CHAPMAN, J. A., Morphological and chemical studies of collagen formation. I. The fine structure of guinea pig granulomata, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 639.
4. FARQUHAR, M., and PALADE, G. E., Segregation of ferritin in glomerular protein absorption droplets, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 297.
5. FITTON-JACKSON, S., Structural problems associated with the formation of collagen fibrils *in vivo*, in *Connective Tissue*, a Symposium of the Council for International Organization of Medical Science, London, Blackwell Scientific Publications, 1957, 77.
6. GAMBLE, H. J., Comparative electron-microscope observations on the connective tissues of a peripheral nerve and a spinal nerve root in the rat, *J. Anat., London*, 1964, **98**, 17.
7. GLIMSTEDT, G., and WOHLFART, G., Electron microscope studies of peripheral nerve regeneration, *Lunds Univ. Arsskr.*, 1960, N.F., Avd. 2, **56**, 16.
8. GROSS, J., LAPIERE, C. M., and TANZER, M. L., Organization and disorganization of extracellular substances: the collagen system, in *Cytodifferentiation and Macromolecular Synthesis*, (M. Locke, editor), New York, Academic Press, Inc., 1963, 175.
9. HESS, A., and LANSING, A. I., The fine structure of peripheral nerve fibers, *Anat. Rec.*, 1953, **117**, 175.
10. MASSON, P., Experimental and spontaneous Schwannomas (peripheral gliomas), I, *Am. J. Path.*, 1932, **8**, 367.
11. NATHANIEL, E. J. H., and PEASE, D. C., Degenerative changes in rat dorsal roots during Wallerian degeneration, *J. Ultrastruct. Research*, 1963, **9**, 511.
12. NATHANIEL, E. J. H., and PEASE, D. C., Regenerative changes in rat dorsal roots following Wallerian degeneration, *J. Ultrastruct. Research*, 1963, **9**, 533.
13. NATHANIEL, E. J. H., and PEASE, D. C., Collagen and basement membrane formation by Schwann cells during nerve regeneration, *J. Ultrastruct. Research*, 1963, **9**, 550.
14. PORTER, K. R., and PAPPAS, G. D., Collagen formation by fibroblasts of the chick embryo dermis, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 153.
15. REVEL, J. P., and HAY, E. D., An autoradiographic and electron microscopic study of collagen synthesis in differentiating cartilage, *Z. Zellforsch.*, 1963, **61**, 110.
16. ROSS, R., and BENDITT, E. P., Wound healing and collagen formation. I. Sequential changes in components of guinea pig skin wounds observed by the electron microscope, *J. Cell Biol.*, 1961, **11**, 677.
17. SHELDON, H., and KIMBALL, F. B., Studies on cartilage. III. The occurrence of collagen within vacuoles of the Golgi apparatus, *J. Cell Biol.*, 1962, **12**, 599.
18. THOMAS, P. K., The connective tissue of peripheral nerve: an electron microscope study, *J. Anat., London*, 1963, **97**, 35.
19. THOMAS, P. K., Changes in the endoneurial sheaths of peripheral myelinated nerve fibres during Wallerian degeneration, *J. Anat., London*, 1964, **98**, 175.
20. UZMAN, B. G., and NOGUEIRA-GRAF, G., Electron microscope studies of the formation of nodes of Ranvier in mouse sciatic nerves, *J. Biophysic. and Biochem. Cytol.*, 1959, **3**, 589.
21. WOOD, G. C., The formation of fibrils from collagen solutions. 3. Effect of chondroitin sulphate and some other naturally occurring polyanions on the rate of formation, *Biochem. J.*, 1960, **75**, 605.