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Electron Microscopic Study of Centripetal Demyelination of Cutaneous Nerves Following Skin Granuloma and Mechanical Injury[1]

Tamotsu Imaeda[2]

The "traumatic degeneration" of peripheral nerves has been described by Ramón y Cajal ([16]) as representing the alteration of both myelin sheath and axon in the central stump of a damaged nerve, in contrast to Wallerian degeneration in the distal part of the same nerve. Distal nerve degeneration and subsequent regeneration after mechanical injuries of peripheral nerve bundles have been well-documented by electron microcroscopy ([2, 3, 5, 9, 10, 11, 12, 15, 17, 18, 19]). However, little information on the ultrastructure of traumatic degeneration in myelinated peripheral nerves is available at present ([7]).

Our previous electron microscopic studies demonstrated that Wallerian degeneration occurs in cutaneous nerves as a result of either mechanical injury of the skin, or granulomatous lesions in the dermis ([13, 14]). Our interest is now focused on the centripetal changes of cutaneous nerves under the same experimental conditions. Events observed in the proximal part of nerves, which are located far from the skin lesions, represent a peculiar demyelination.

In leprosy skin lesions, infiltration of macrophages, their derivatives (lepra cells and epithelioid cells) and leukocytes may cause alteration of cutaneous nerves, possibly as a result of the locally increased mechanical pressure. However, the direct invasion of leprosy bacilli or the manifestation of delayed hypersensitivity in mesenchymal cells of nerve bundles may also provoke cutaneous nerve changes. These possibilities make it difficult to understand the causative mechanisms involved in ultrastructural alterations of these nerves in various types of leprosy skin lesions ([6]).

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2 Tamotsu Imaeda, M.D., Associate Professor, Department of Microbiology, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, 100 Bergen Street, Newark, New Jersey 07103.

The purpose of the present paper, together with our previous reports ([13, 14]), is to provide basic information regarding cutaneous nerve ultrastructures affected by localized pressure, thus facilitating the interpretation of cutaneous nerve alterations in leprosy skin lesions.

MATERIALS AND METHODS

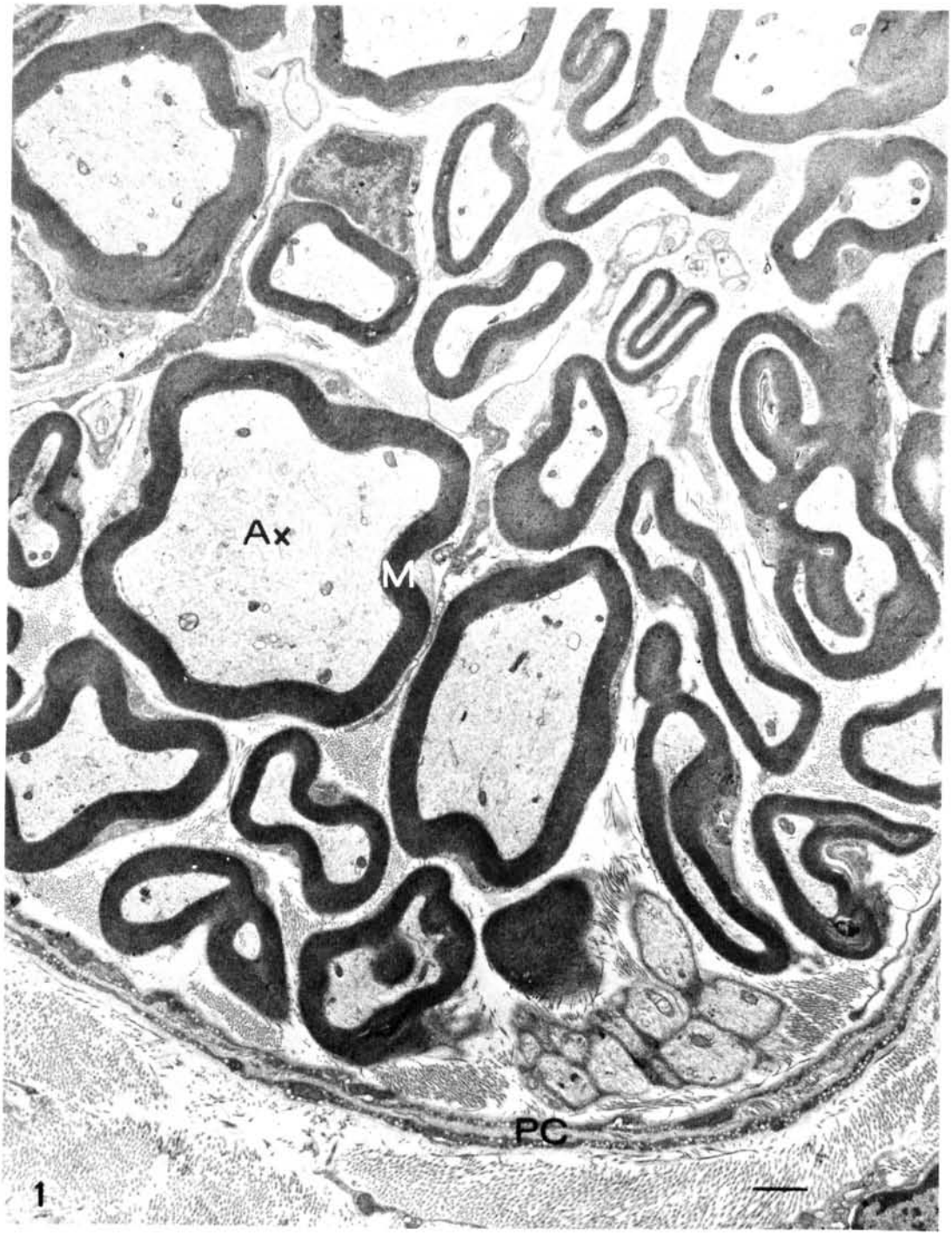
Tips of right earlobes of 30 mice were subjected to a mechanical injury (crushing) with hemostatic forceps. A second group of 30 mice was injected in the tip of the right earlobe with 0.03 ml of complete Freund's adjuvant (Difco) containing 2 mg/ml of heat-killed *Mycobacterium tuberculosis* H37Ra, for granuloma formation.

An approximate 2 mm segment of apparently intact right earlobe roots, at a minimum distance of 3 mm from the border of skin lesions produced either by mechanical injury or by injected mycobacteria, of two mice, one from each experimental group, were taken daily for two weeks and at day thirty. Mechanically injured or granulomatous regions were also biopsied in order to confirm the occurrence of Wallerian degeneration in distal cutaneous nerves. Biopsies of the left earlobe roots were used as controls.

The tissues were immediately immersed in glutaraldehyde, cut into small pieces and postfixed with osmium tetroxide as described previously ([13, 14]). After dehydration with acetones, the tissue bits were embedded in Araldite and examined with a Hitachi HU-11B electron imcroscope after double staining with lead citrate and uranyl acetate.

RESULTS

Cutaneous nerve bundles in the earlobe tip lesions caused mechanically or by granuloma formed with Freund's adjuvant show Wallerian degeneraton. This is character-



Bars indicate 1μ unless otherwise indicated.

FIG. 1. A large nerve bundle proximal to the skin lesion. No significant changes can be seen at the second day following mechanical injury. Ax: axon, M: myelin sheath, PC: perineural cell. X 7,500.

ized by infiltration of both macrophages and leukocytes into the nerve bundles, degeneration of both axon and Schwann cells, shrinkage or degeneration of perineural cells and disintegration of myelin sheath ([13, 14]). Unmyelinated fibers also show degeneration of both axon and Schwann cells ([13, 14]). On the other hand, cutaneous nerves located in the roots of damaged earlobes are devoid of these changes throughout the examined periods, although significant alterations occur in myelinated fibers.

The initial sign of ultrastructural changes in proximal cutaneous nerves is noted as early as the third day in the mechanically injured earlobes. In the case of granuloma it appears after the seventh day of injection. This delayed initiation of nerve alteration may be explained by the fact that Wallerian degeneration begins later in the distal nerves involved in granuloma as contrasted with that of mechanical injury ([14]). However, all the events observed in nerve elements are almost the same in both mechancially injured and granulomatous earlobes. Therefore, the description is made following the sequence of nerve alterations, regardless of the causative damages inflicted at the distal part of cutaneous nerves.

As seen in Figure 1 which shows no ultrastructural changes at the second day after mechanical injury, fixation and embedding methods employed in the present study do not cause any significant artifact in cutaneous nerve elements.

The first alteration in nerve structures is noted at the node of Ranvier. Namely, the myelin at the node shows a vesicular appearance around large fibers (Fig. 2), whereas the small fibers appear to be unaffected (Fig. 3).

Later, abrupt rupture of the myelin sheath takes place, but its lamellar structure remains intact (Fig. 4). This rupture results in the formation of partially denuded axons (Figs. 5, 6, and 7). Ruptured myelin invaginates into the axon, and consequently the myelin appears to be surrounded by axon, depending on the sectioning angle (Figs. 5 and 7). In longitudinal sections, the ruptured end of myelin turns centripetally and fuses to the other part of the myelin (Fig. 6). Due to the rupture of the myelin sheath, fragmentation of myelin occurs (Fig. 7) as one of the characteristic ultrastructural patterns at this stage. In some sections, the ruptured myelin sheath is detached from the large axon and lies within the apparently intact Schwann cell (Fig. 8).

At this stage, a significant swelling of the axon is noted (Fig. 7), as observed in sympathetic nerves proximal to the constriction ([7]). In addition, complete septation is sometimes observed, especially in transverse section (Fig. 7). This pattern may reflect the formation of growth cones in the distal part of the same fiber ([13]). The presence of myelin inside the septal space is explained by the sectioning angle that has passed through the partial invagination of myelin into the axon as seen in Figure 5.

Throughout the demyelination process described above, Schwann cells show a slight shrinkage (Figs. 5 and 7), thus differing from the distal part where Schwann cells undergo degeneration resulting in the formation of ruffled basement membrane ([13, 14]).

When the regeneration process becomes apparent in the distal nerve fibers, at about one week after mechanical injury and about two weeks in the case of granuloma, a remyelination occurs around the partially denuded axon (Fig. 9). This is characterized by several folded membranes of Schwann cell surrounding a large axon. Furthermore, the basement membrane is always attached peripherally to the Schwann cell surface. An elongated mesaxon around the axon has not been observed in the regenerating fibers proximal to the injured skin.

When the regeneration of all nerve elements is almost complete at the distal part, which occurs about the second week after mechanical injury, the myelinated fibers at the proximal part of skin are virtually normal in appearance. In the case of granulomatous lesions, however, the degenerative changes described above persist for at least 30 days.

Throughout the present examination, no

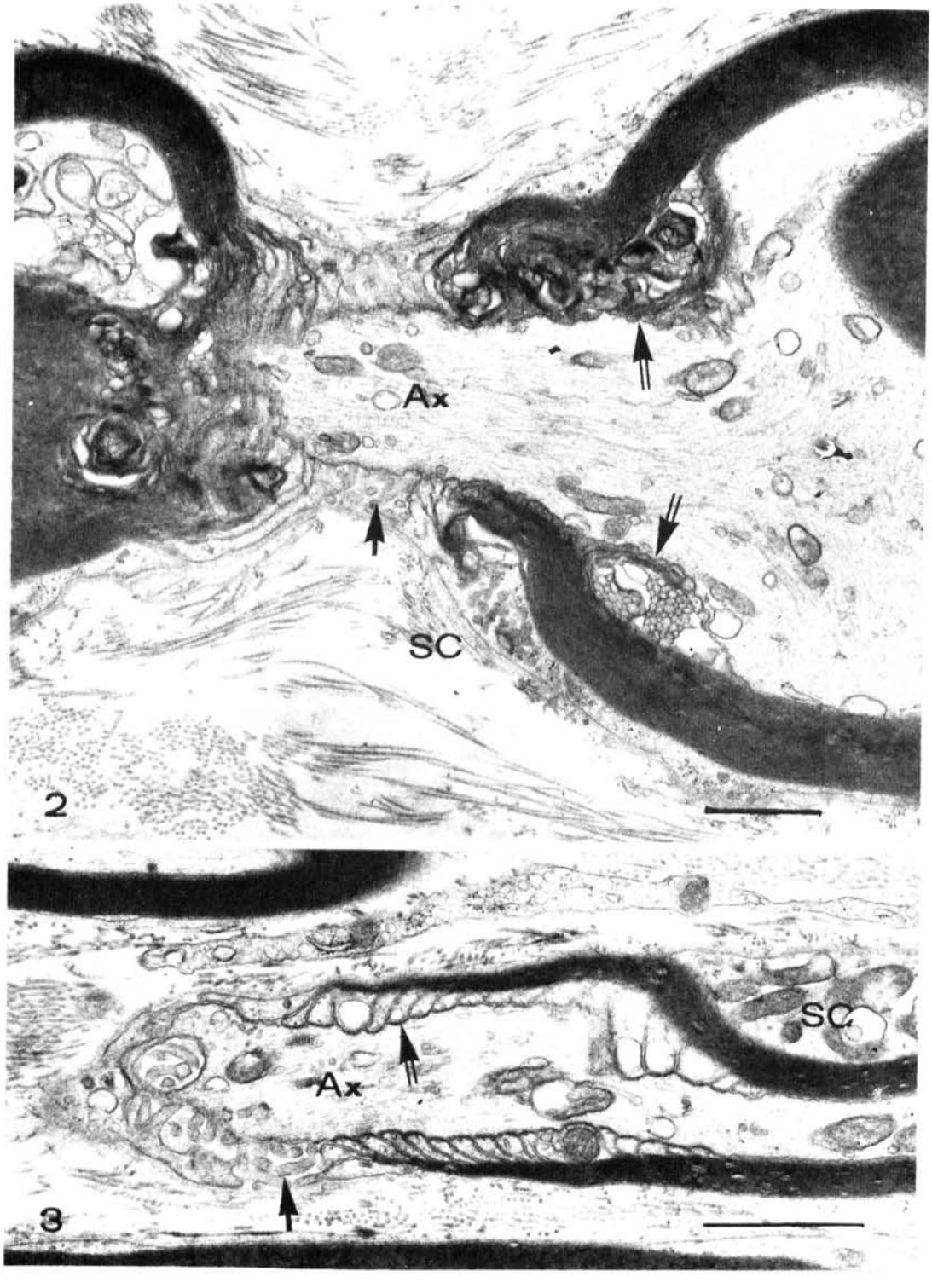


FIG. 2. A node of Ranvier of the large fiber. The juxtanodal myelin shows a vesicular appearance (double arrows), although both myelin sheath (M) and axon (Ax) appear to be intact. An arrow indicates the juxtanodal processes of Schwann cell (SC). Three days after mechanical injury. X 17,000.

FIG. 3. A node of Ranvier of the small fiber at the proximal nerve bundle, which has been found in the same biopsy as shown in Figure 2. Note the intact appearance of juxatanodal myelin structures (double arrow), myelin sheath (M), axon (Ax) and juxtanodal processes of Schwann cell (arrow). SC: Schwann cell. X 24,000.

appreciable changes have been observed in unmyelinated fibers of the proximal cutaneous nerves.

DISCUSSION

Classical observations have demonstrated that "traumatic degeneration" sometimes two or more millimeters in length and located centripetally from the wound ([16]), occurs at the proximal nerve fibers. This degeneration, characterized by the formation of myelin spheres and the swelling of axons, is said to be the immediate consequence of the physical injury affecting the distal part.

In the present study, significant ultrastructural changes are evident in cutaneous nerve bundles at least 3 mm centripetally from local lesions caused by mechanical injury or by granuloma formation. However, these alterations lack all of the characteristic patterns observed in the distal nerves ([13, 14]).

The process of demyelination may be initiated by the vesicular alteration of the juxtanodal myelin sheath of large fibers. Such ultrastructural pattern has not been observed either in control animals or in small fibers (Fig. 3), eliminating the possibility of artifacts during the tissue preparation. It is presumed that juxtanodal myelin is readily influenced by the change of axonal volume or movement following its peripheral degeneration. In fact, the swelling of axons is seen in large axons in the proximal nerve.

It is noteworthy that the myelin alteration takes place only in large nerve fibers. These large fibers may innervate the damaged skin directly, whereas small fibers seem not to reach the skin lesions. Therefore, the electron microscopic images observed in large fibers are consistent with the view that the proximal demyelination

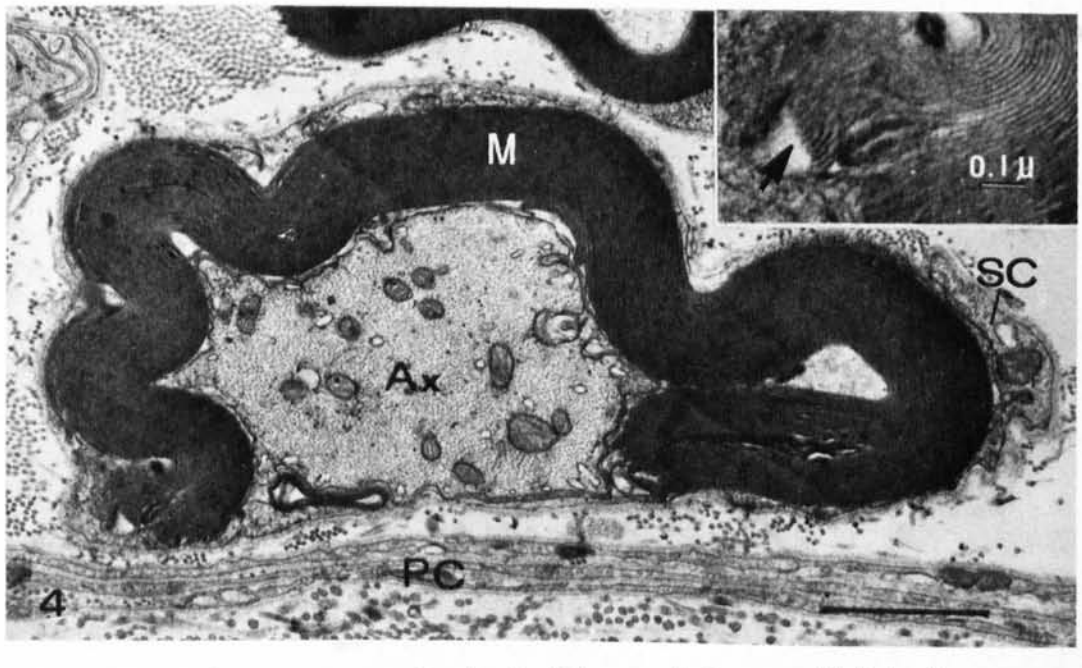


FIG. 4. A discontinuous myelin sheath (M) around the axon (Ax) in the nerve bundle proximal to the mechanically injured skin, at the 4th day. The inset shows the abrupt ending (arrow) of a myelin sheath. SC: Schwann cell, PC: perineural cell. X 28,000. Inset X 64,000.

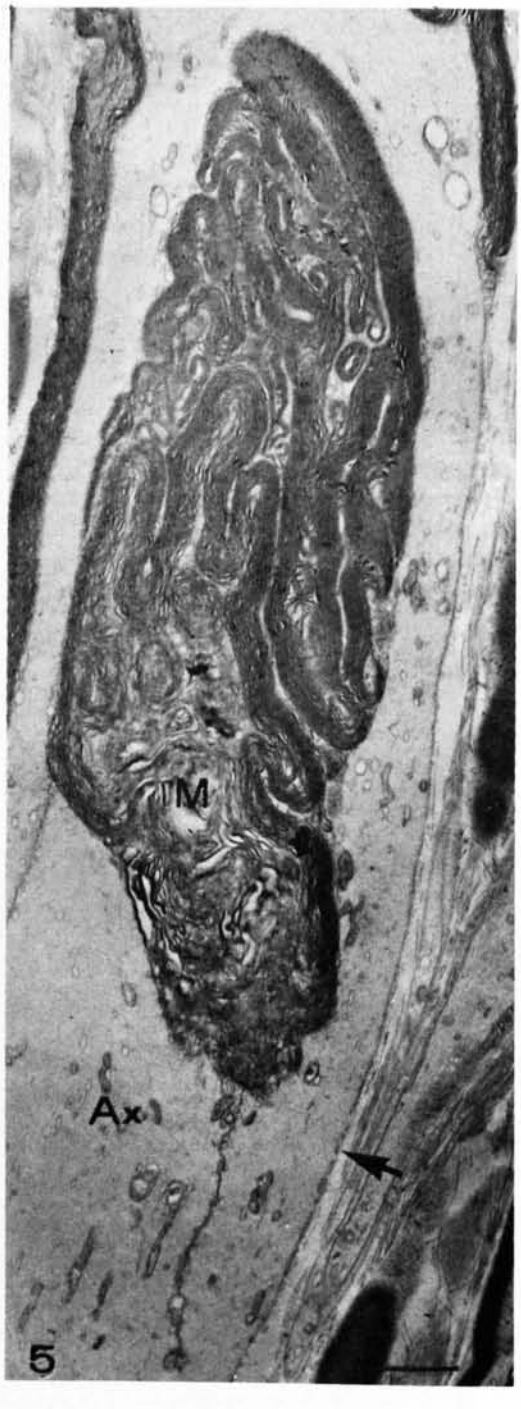


FIG. 5. A partially denuded axon (arrow) in the proximal nerve bundle 4 days after mechanical injury. Note that the axon (Ax) is directly exposed to the basement membrane. An agglutination of myelin (M) is surrounded by the axon. X 12,000.

process may reflect the immediate consequence of distal changes in the corresponding peripheral part of the same fibers.

The rupture of apparently intact myelin sheaths followed by the detachment of myelin from the axon is also characteristic of the proximal nerve alterations. This finding is very similar to that of neuritis caused by diphtheric toxin ([19]), although the causative mechanism seems to be different.

It must be emphasized that the Schwann cell does not show any significant degeneration at the proximal nerves, but it shrinks after demyelination resulting in the partial exposure of axon directly to the surrounding space. Furthermore, the discharge of segmented myelin from Schwann cells, which is typical of the distal nerve degeneration ([13, 14]), is not observed in the proximal nerve bundles. Possibly, myelin fragments formed in the proximal nerves may be disintegrated inside the Schwann cells which have not been damaged during the demyelination process.

Lampert and Cressman ([8]) and Ramón y Cajal ([16]) reported that collateral branches are formed at the node of Ranvier in proximal nerves during regeneration of the spinal cord and the sciatic nerves. Failure to verify this finding in the present study may be explained in terms of the distance between directly damaged nerves and proximal regions examined since axons of the latter are not involved in the new innervation.

Remyelination is initiated by the extension of the Schwann cell processes around the axon, as observed in the central nervous system ([1, 4]). Since the remyelination as a result of a spiral turn of a mesaxon ([13, 14, 17]) has not been found in the proximal nerves, it is reasonable to believe that the extended processes of Schwann cell overlap each other forming a new myelin around the axon.

SUMMARY

Traumatic degeneration in cutaneous nerves proximal to skin wounds caused either by mechanical injury or granuloma in ears of mice were examined with the electron microscope. Regardless of causa-

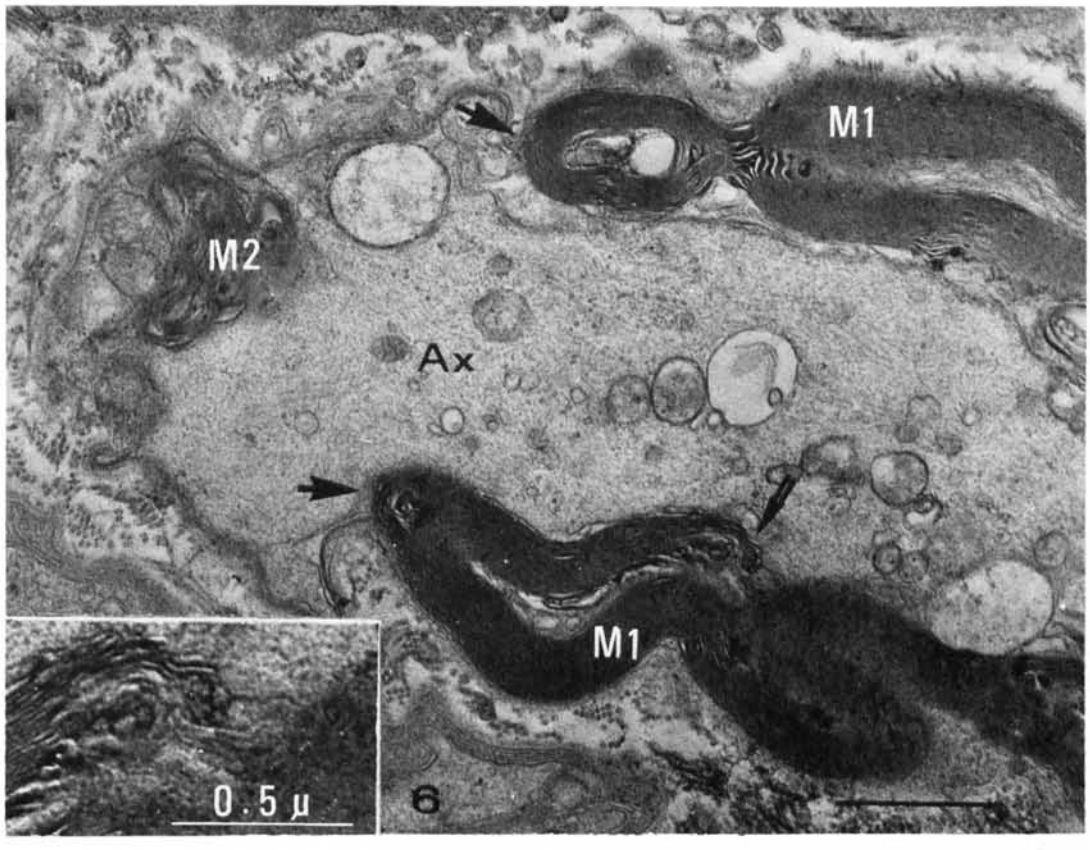


FIG. 6. An irregularly ruptured myelin sheath (M) turns centripetally (arrows) fusing with the other part (double arrow) of sheath, 5 days after mechanical injury. The inset shows a larger magnification of the fusion of the ruptured myelin. Ax: axon, M2: myelin fragment. X 21,000. Inset X 48,000.

tive damages inflicted in the distal skin, the events observed in the proximal nerve bundles lack all the characteristic features of distal nerve degeneration, such as degeneration of both axon and Schwann cell, infiltration of leukocytes and macrophages, and perineural alterations.

The proximal nerve alterations are only evident in the large myelinated fibers. The initial sign of demyelination is represented by vesiculation of myelin lamellae at the node of Ranvier. Later, rupture of the myelin sheath occurs, resulting in the formation of partially demyelinated axon in the intact Schwann cell. Discharge of fragmented myelin from the Schwann cell, however, has not been observed and the myelin remains inside the Schwann cell.

During remyelination, elongated mesaxons around the axon have not been observed, suggesting that the remyelination process in the proximal nerves may not result from the spiral turn of a mesaxon.

It is concluded that this centripetal demyelination may reflect the immediate ultrastructural response of the proximal nerves to the degeneration in the distal part.

RESUMEN

Se examinaron con el microscopio electrónico las degeneraciones traumáticas en nervios cutáneos proximales a heridas de la piel producidas en orejas de ratones, ya sea por injuria mecánica o por granuloma. A pesar de los daños causativos infligidos a la piel distal, los hallazgos observados en los paquetes nerviosos proximales carecen de todos los rasgos característicos de la degeneración nerviosa distal, tales como degeneración del

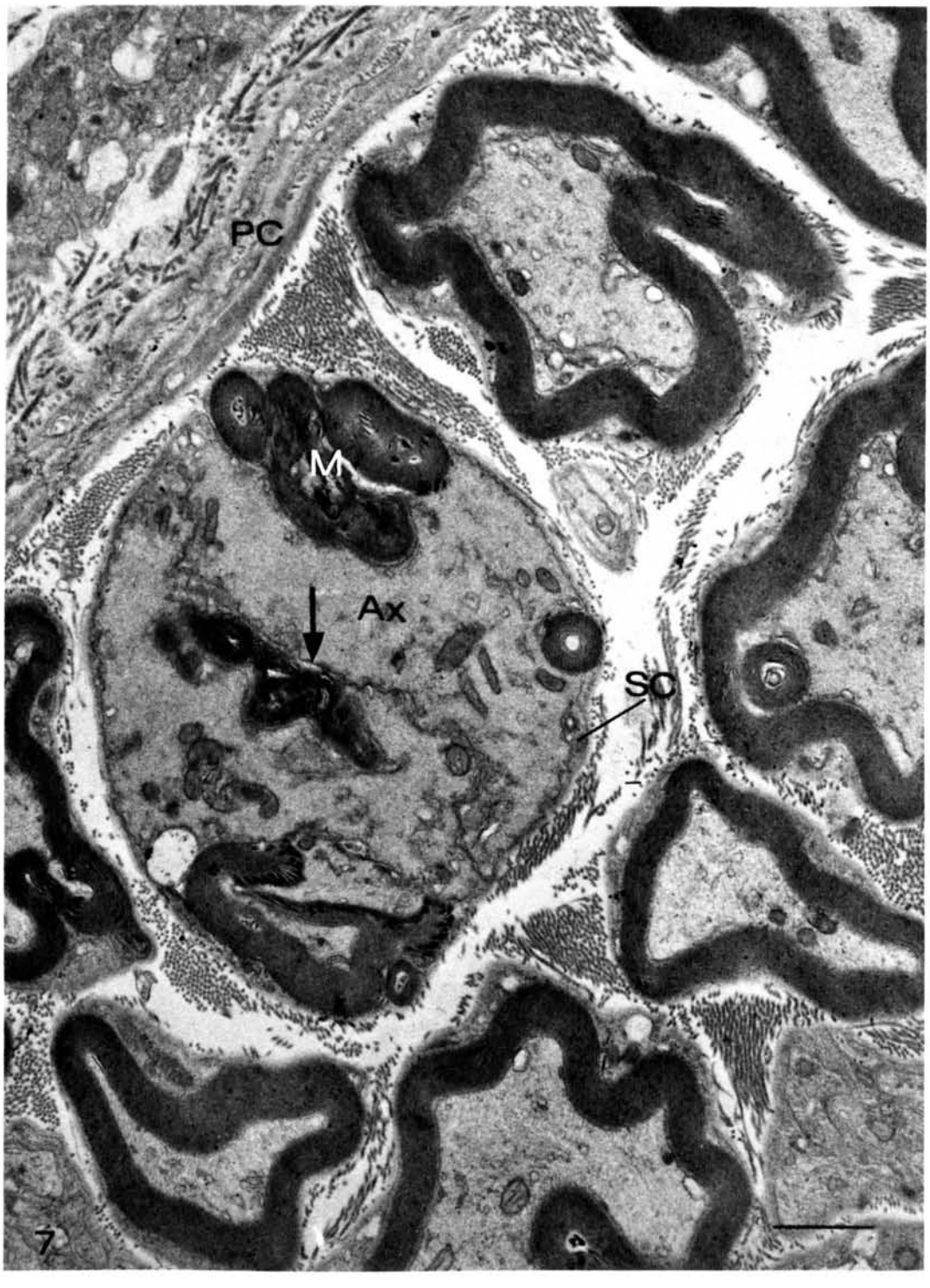


FIG. 7. A large nerve bundle proximal to the granuloma lesion, 14 days after Freund's adjuvant injection. A part of the swollen axon (Ax) containing slightly increased axoplasmic organelles is exposed directly to the Schwann cell basement membrane. The myelin sheath is fragmented into several myelin agglutinates (M) which still maintain the lamellar structure. Note the complete septation of the axon in which myelin fragments are included (arrow). Also note that this demyelination occurs only in the large fiber. SC: Schwann cell. PC: perineural cell. X 15.000.

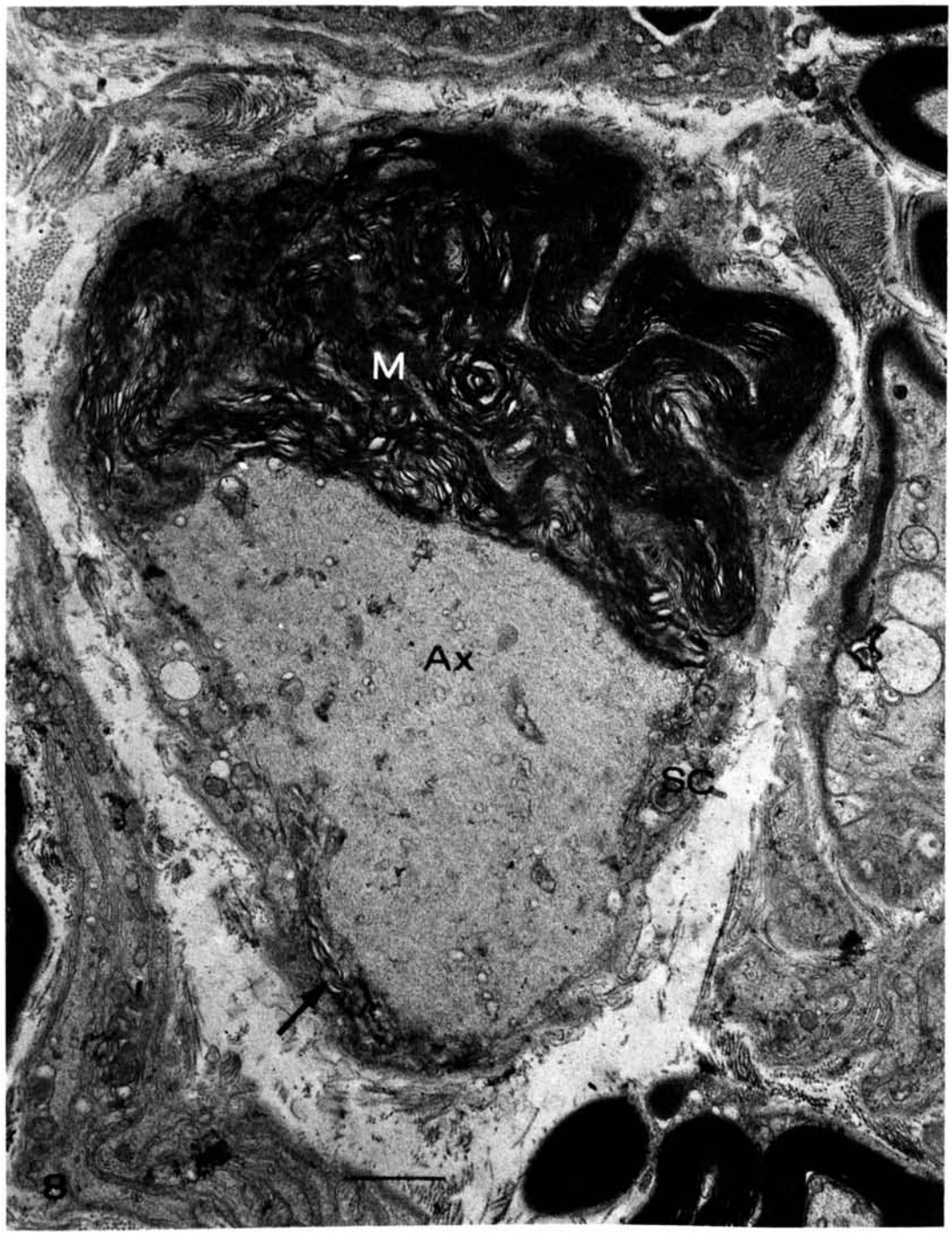


FIG. 8. Myelin (M) detached from the axon (Ax) is accumulated in the Schwann cell cytoplasm (SC). Small myelin debris (arrow) still attaches to the axon, 5 days after mechanical injury. X 15,000.

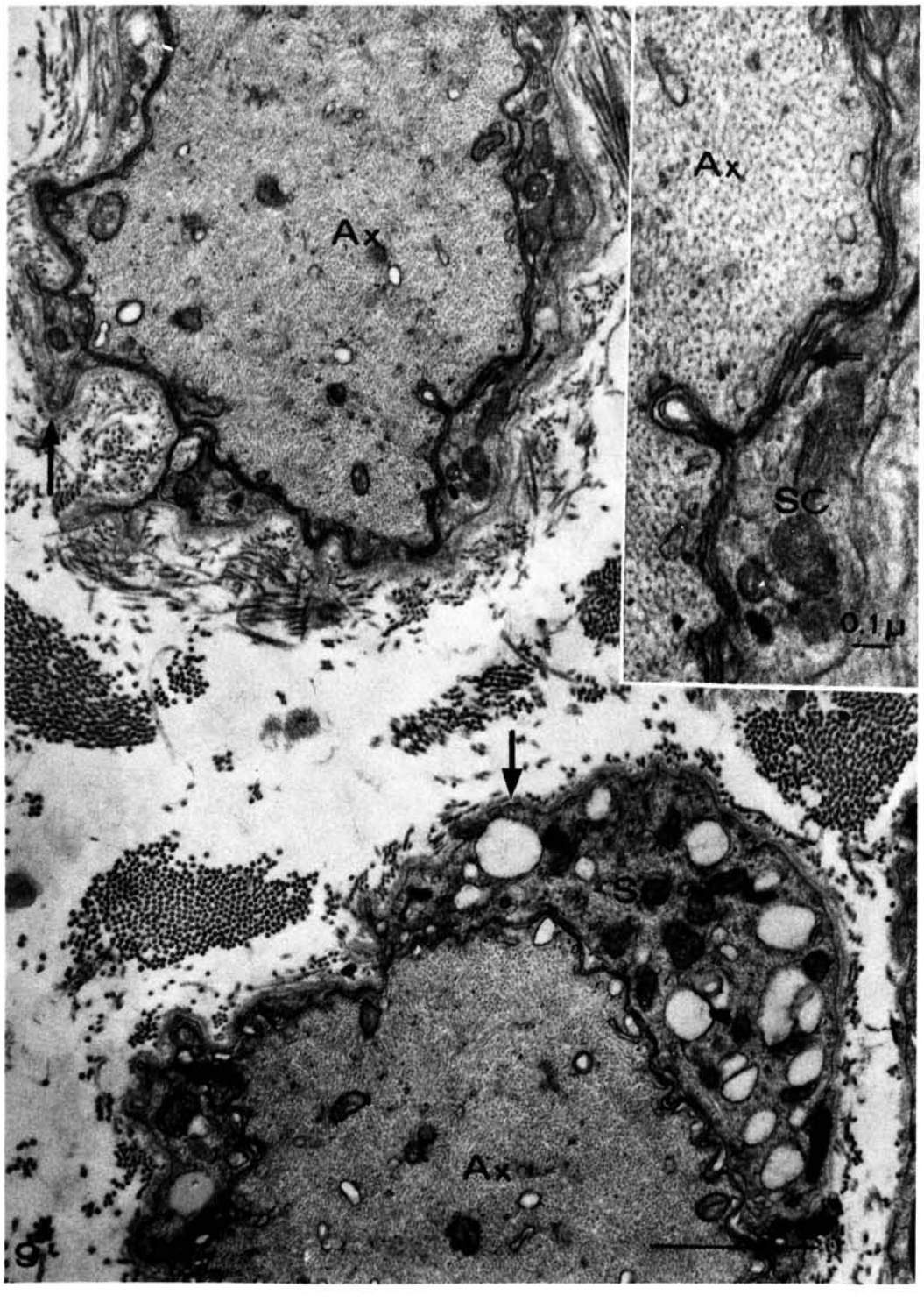


FIG. 9. The beginning of remyelination around demyelinated large axons (Ax). In the proximal nerve bundle 13 days after mechanical injury. Note that basement membranes (arrows) attach to the surface of Schwann cells (SC). The inset shows a larger magnification of the axon-Schwann cell connection. Several membranes (double arrow) derived from the Schwann cell processes surround the axon. X 24,000. Inset X 56,000.

axón o de las células de Schwann, infiltración de linfocitos y macrófagos y alteraciones perineurales.

Las alteraciones nerviosas proximales se evidencian solamente en las fibras mielínicas grandes. El signo inicial de desmielinización está representado por vesiculación de las lamelas de mielina en los nódulos de Ranvier. Posteriormente, se rompe la vaina de mielina, lo que da origen a un axón parcialmente desmielinizado, en una célula de Schwann intacta. Sin embargo, no se ha observado la salida de mielina fragmentada desde la célula de Schwann, y la mielina permanece dentro de la célula de Schwann.

No se han observado mesaxones alargados durante la remielinización, sugiriendo que el proceso de remielinización en los nervios proximales puede no originarse en una vuelta en espiral de un mesoaxón.

Se concluye que esta desmielinización centrípeta puede reflejar la respuesta inmediata ultraestructural de los nervios proximales a la degeneración en la parte distal.

RÉSUMÉ

On a procédé à l'examen au microscope électronique de la dégénérescence traumatique produite dans des nerfs cutanés proches de blessures de la peau causées, soit par des moyens mécaniques, ou par un granulôme, chez la souris. Nonobstant les lésions immédiates infligées à la peau distale, les phénomènes observés dans les faisceaux nerveux proximaux, ne présentent aucune des caractéristiques de la dégénéresence nerveuse distale, telle que dégénérescence conjointe de l' axone et de la cellule de Schwann, infiltration par des leucocytes et des macrophages, ou altérations périnerveuses.

Les altérations des nerfs proximaux ne sont observées que dans les grandes fibres myélinisées. Le signe initial de démyélinisation est représenté par une vésiculation des lamelles de myéline au niveau du nodule de Ranvier. Ultérieurement, il se produit une rupture de la gaine myélinique, qui entraine la formation d'un axone partiellement démyélinisé entouré d'une cellule de Schwann intacte. Une libération de fragments de myéline à partir de la cellule de Schwann n'a toutefois pas été observée; la myéline reste à l'intérieur de la cellule de Schwann.

Au cours de la rémyélinisation, on n'a pas observé de mesaxones allongés autour de l'axone. Ceci suggère que le processus de remyélinisation dans les nerfs proximaux, ne résultent pas d'un enroulement spirale d'un mésaxone.

On en conclut que la démyélinisation centripète peut constituer la réponse immédiate, au niveau de l'ultrastructure, des nerfs proximaux, à la suite d'une dégénérescence dans leur partie distale.

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