

ELECTRON MICROSCOPY OF ULTRA-THIN SECTIONS OF LEPROMATOUS PERIPHERAL NERVES

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The submicroscopic structure of peripheral nerves are now being revealed by many investigators (1, 2, 3, 8) using the high resolving power of the electron microscope. Stimulated by this recent progress in neurology, we have made an electron microscope study of ultra-thin sections of leprous peripheral nerves.

In the present article we report on such a study of sections of great auricular nerves taken from two cases of lepromatous leprosy. In this investigation we were able to elucidate some aspects of the leprous nerve lesions which cannot be observed by the light microscope.

MATERIALS AND METHODS

CASE 1.—M.O., a 36-year-old woman, who had many lepromatous nodules on her face and four extremities, strongly positive for *M. leprae*. The lepromin reaction was negative. The left great auricular nerve was markedly enlarged. After a month of DDS treatment (intramuscular injections, 200 mgm. a week), we extirpated that nerve for biopsy and electron microscope study. The nerve as removed was 3 mm. in diameter.

CASE 2.—Y.Y., a 40-year-old mechanic, who had depigmented macules on the nape of the neck. The right great auricular nerve was moderately enlarged. The histopathologic examination of this nerve revealed lepromatous changes, numbers of lepra cells being observed. The material for the electron microscope study was taken before the commencement of sulfone treatment.

For the histopathologic study of these nerves, frozen sections were examined after Giemsa staining, Ehrlich's myelin-sheath staining, and carbol-fuchsin-hematoxylin staining for the leprosy bacilli.

For the electron microscopy, small chips were fixed in 1 per cent phosphate-buffered osmium tetroxide (pH 7.2), 30 hours in refrigerator. After washing in distilled water, the specimens were dehydrated in alcohol and embedded in a 6:4 mixture of n-butyl and methyl methacrylate. Ultra-thin sections were made with the Shimadzu microtome. The electron microscopes used were the Akashi Tronscope and the Hitachi Hu 10 model.

HISTOPATHOLOGIC FINDINGS

The histopathologic findings of the extirpated auricular nerves with the light microscope are as follows:

Case 1: In almost all of the nerve fibers the axon and myelin sheath have disappeared, and only Schwann cells, endoneurial sheath cells and lepra cells in the endoneurial spaces are observed. Bacilli are found in degenerated nerve fibers but, because of the limited resolving power of the light microscope, the details of the morphological relationship between the bacilli and the nerve fibers could not be made out clearly.

Case 2: Bacilli are found in the endoneurial lepra cells. They also seem to be located in Schwann cells, but their localization inside nerve fibers cannot be clearly observed. In most of the nerve fibers, the axon and myelin sheath have disappeared.

INTERPRETATION OF THE ELECTRON MICROGRAPHS

Structure of the peripheral nerve fiber.—At the beginning of the description of our electron micrographs of these lepromatous peripheral nerves, we think it well to explain the structure of nerve fibers as revealed by the electron microscope, thus to provide the basis of analysis of the leprosy lesions. Fig. 1 shows two myelinated nerve fibers of Case 2 which are surrounded by one Schwann cell. Although slight degeneration of axons and myelin sheaths is observed in these fibers, this picture shows clearly the fundamental structure of the myelinated nerve fibers. The mesaxon, or the outer "surface connecting membrane" of Robertson (8), connects the Schwann-cell plasma membrane and the outer layer of the compact myelin lamellae. Inside the axon, neurofilaments and mitochondria are seen. The Schwann-cell nucleus has a homogeneous nucleoplasm and a double nuclear membrane. The Schwann-cell cytoplasm contains mitochondria and microsomes. The cytoplasm is limited by the Schwann-cell plasma membrane. Outside this plasma membrane, the basement membrane of the Schwann cell is observed. The basement membrane is somewhat broader than the plasma membrane. Outside the basement membrane, transverse sections of neurilemmal collagen fibers are seen.

Observations of the nerve of Case 1.—The longitudinal section of a nerve fiber of the nerve of Case 1 is shown in Fig. 2. In the axon of this fiber, two longitudinally-sectioned bacilli are seen, lying side by side. Vermicular degeneration of the axoplasm is observed in the axon, a change which seems to be the first step of the destruction of the axoplasm. The myelin sheath of this fiber is well preserved. This picture seems to provide definitive proof of the affinity between axon and *M. leprae*.

In Fig. 3 there are two nerve fibers, the upper one of which has its axon and myelin sheath, while the lower one has lost both of those structures. The remaining Schwann-cell cytoplasm forms the syncytial ribbon which is called the "cord of Büngner" (4). The Schwann cell of the Büngner cord stage has less electron-dense cytoplasm than the normal Schwann cell cytoplasm of the intact nerve fiber. Bacilli were found in the cytoplasm of Schwann cells of the Büngner cord, but not in the normal Schwann cell of the intact nerve fibers.

The endoneurial space is occupied by lepra cells, and these cells are not different in appearance from the lepra cells of the skin.

On the whole, the structure of the cords of Büngner is well preserved in the lepromatous lesion of this particular nerve. The neurilemmal collagen fibers have proliferated in this case.

Observations of the nerve of Case 2.—The structure of the biopsied

nerve of Case 2 is quite similar to that of Case 1. Most of the nerve fibers show destruction of axon and myelin sheath, and the remaining Schwann cells form the cords of Büngner which contain numbers of leprosy bacilli. Bacilli are also seen in the lepra cells of the endoneurial space (Figs. 4 and 5). In the lepra cells the bacilli are frequently wrapped in opaque droplets (Fig. 5), as will be described elsewhere (10).

The interesting finding in this nerve was regeneration of axon and myelin sheath in the nerve lesion. The regenerating axon has a relatively small diameter (about 1 μ). A mesaxon connects the regenerating axon and the surface membrane of the Schwann cell. (Figs. 6, 7 and 8.) In some cases the mesaxon show distinct elongation and spiral wrapping around the regenerating axon. This spiral wrapping of the mesaxon around the regenerating axon is the beginning of myelin sheath regeneration (Fig. 6). It is interesting that Schwann cells (of the Büngner cord stage) which contain numbers of bacilli still have the ability to infold the regenerating axon into their cytoplasm and play an important role in the regeneration of the myelin sheath around the newly regenerating axon (Figs. 6 and 8). The mechanism of the myelin regeneration is quite the same as that of the myelinogenesis in the embryonic nerve.

DISCUSSION

Because of the recent advancement of electron microscopy, knowledge of the detailed structure of peripheral nerve fibers has changed considerably from the ideas of them that were gained of them from light microscopy. For one thing, Geren (3) has found that in the embryonic nerve the lamellar system of the myelin sheath is formed by the spiral infolding of the surface membrane of the Schwann cell around the axon. Terry and Harkin (9) studied the regeneration of the myelin sheath following Wallerian degeneration, and they have found that the myelin sheath is regenerated by the same mechanism as that of the myelinogenesis of the embryonic nerve. These findings have provided us the basis of the interpretation of our electron micrographs of lepromatous peripheral nerve lesions.

As is shown in Fig. 2, leprosy bacilli may be found in the axon. They are also found in the Schwann cells of the Büngner cord stage, and the fact that the axon and myelin sheath have already disappeared in those cells suggests the previous destruction of the axon by the invasion of bacilli. The axon and its myelin sheath are digested in the cytoplasm of Schwann cells in the course of Wallerian degeneration, but leprosy bacilli resist the digestion and consequently remain in the Schwann cells which form the cords of Büngner.

The leprosy bacillus seems to enter into the nerve axon by an unknown way. But, since the bacilli are believed to have no ability of moving by themselves, it is more probable that their entrance into the axon is ascribable to some phagocytic activity of the axon itself. Recent tissue-

culture studies of the spinal ganglia have revealed pinocytosis and a tropistic reaction of the growth cones of the axons (6, 7). From this finding we believe it probable that the growth cones of the regenerating axons catch leprosy bacilli and engulf them into the axoplasm. In the pinocytosis of the growth cones of axons, the vacuoles move in centripetal direction in the axon. This finding suggests the presence of a centripetal stream of axoplasm which would transport the engulfed bacilli toward the spinal ganglion cells.

Regenerating axons in leprosy nerve lesions were observed by Mitsuda (5) with the light microscope, but in the present study we could definitely identify the regenerating axons by the presence of the mesaxon which is sometimes wrapped loosely around the small regenerating axon. Mitsuda also observed bacilli in the regenerating axons. We have not, unfortunately, made the same observation in our electron micrographs, but we can fully subscribe to Mitsuda's observation. The presence of bacilli in regenerating axons seems to support the proposition of the phagocytic activity of the growth cones of the regenerating axons.

Based on our analysis of our electron micrographs, we conclude that in the nerve leprosy bacilli are phagocytosed by the axon and not by the Schwann cells. If Schwann cells were actively to phagocytose bacilli, then we would find bacilli in the cytoplasm of Schwann cells which surround normal fibers, as well as in the Schwann cells which—empty of axon and myelin sheath—constitute the Büngner's cords. In this study we could not find bacilli in the Schwann cells around normal fibers. There remains the possibility that the bacilli in the Schwann cells of the Büngner cord had been actively phagocytosed by those cells. In that case, however, a mesaxon-like structure might be seen connecting the phagocytosed bacilli and the surface membrane of the Schwann cells, seeing that that cell membrane is very strong and can be elongated remarkably as observed during myelin regeneration. As it is, we have never seen such a structure in our electron micrographs. We are therefore inclined to believe that the bacilli in the Schwann cells of the Büngner cord were not phagocytosed by those cells. We think, however, that it is too early as yet to reach any conclusion on this matter.

The morphological characteristics of the lepra cells in the endoneurial space were the same as those of the lepra cells of the skin. The pressure of these lepra cells in the endoneurial spaces may be another cause of the Wallerian degeneration of nerve fibers in lepromatous nerve lesions, but these lepra cells do not break the structure of the cord of Büngner in lepromatous nerve lesions; and this is a favorable condition for the regeneration of axons.

SUMMARY

1. Ultra-thin sections of the great auricular nerves taken from two cases of lepromatous leprosy have been examined with the electron microscope.

2. Leprosy bacilli were found in the nerve-fiber axon, in Schwann cells of the cord of Bünchner stage, and in lepra cells located in the endoneurium.

3. Regenerating axons and myelin sheaths were definitely identified in the lepomatous nerve lesions.

4. Based on the analysis of our electron micrographs of lepomatous nerve lesions, it would seem that leprosy bacilli are phagocytized by the axon and not by the Schwann cell.

RESUMEN

1. Cortes ultradelgados de los nervios auriculares mayores obtenidos de dos casos de lepra lepomatosa fueron examinados con el electronmicroscopio.

2. Se descubrieron bacilos leproso en el cilindro-eje de la fibra nerviosa, las células de Schwann de la médula del período de Bünchner y en las células leprosas localizadas en el endoneurio.

3. En las lesiones lepomatosas de los nervios, se identificaron definitivamente cilindros-ejes y vainas de mielina en vías de regeneración.

4. A base del análisis de las electronmicrografías de las lesiones lepomatosas de los nervios, parece que los bacilos leproso son fagocitados por el cilindro-eje no por la célula de Schwann.

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DESCRIPTION OF PLATE

PLATE 20

FIG. 1. Transverse section of two myelinated nerve fibers in the great auricular nerve of Case 2. The axon and myelin sheath show beginning degeneration. Magnification, X 28,000 (slightly reduced).

Key: *a*, axon; *b*, myelin sheath; *c*, Schwann cell nucleus; *d*, mitochondria; *e*, mesaxon; *f*, Schwann cell plasma membrane; *g*, basement membrane of Schwann cell; *h*, transverse section of neurilemmal collagen fibers.

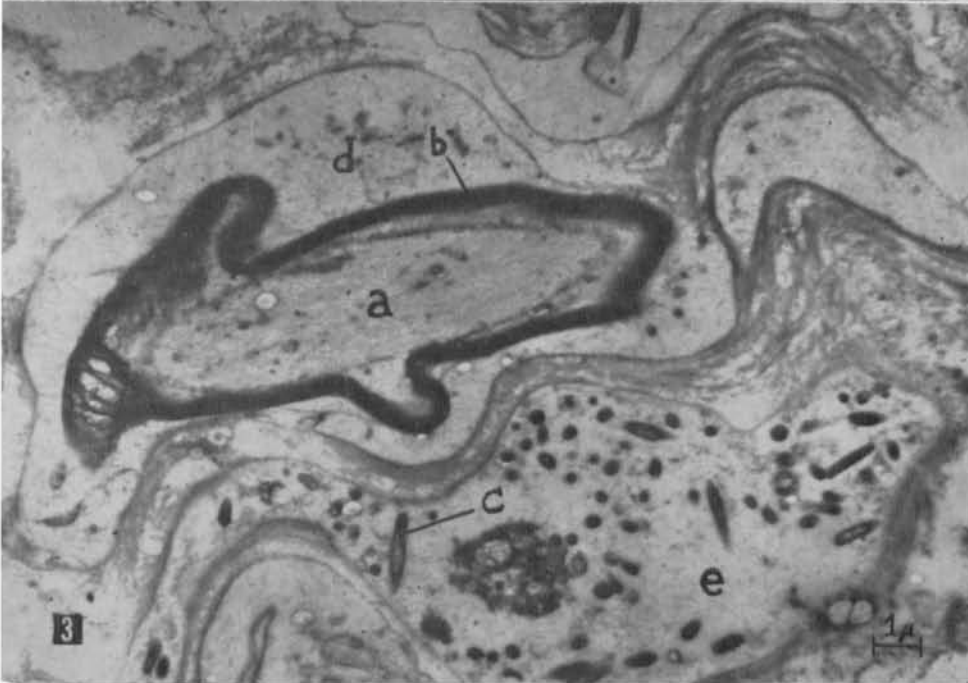
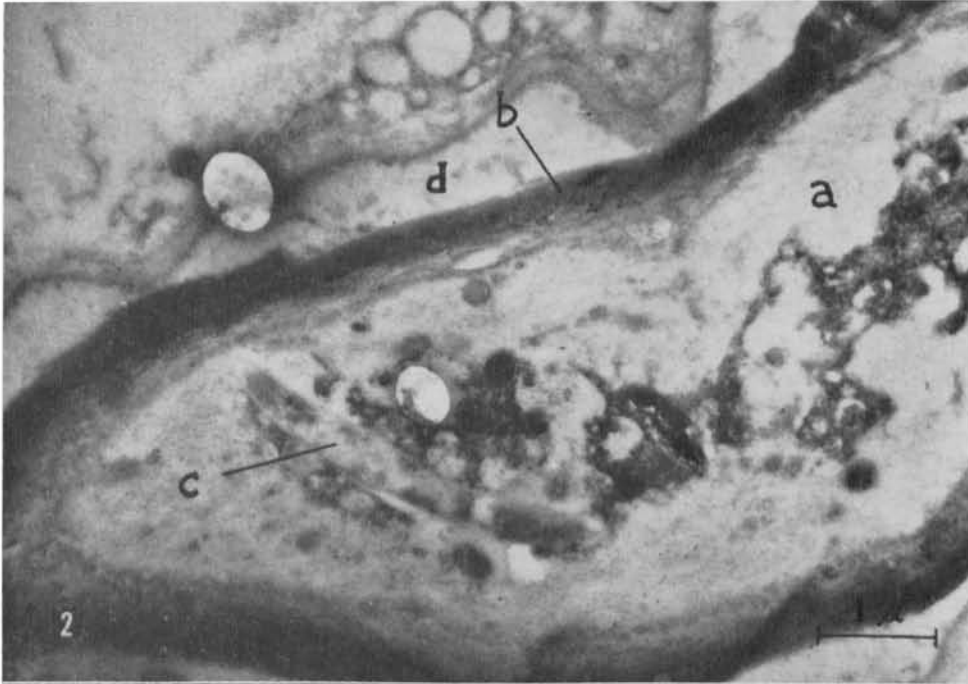


PLATE 21

PLATE 22

FIG. 4. Transverse section of the biopsied nerve of Case 2. Leprosy bacilli are seen inside endoneurial lepra cells. Magnification, X 13,500 (slightly reduced).

Key: *a*, axon; *b*, myelin sheath; *c*, enlarged Schwann cell cytoplasm; *d*, endoneurial lepra cell; *e*, leprosy bacilli; *f*, opaque droplet; *g*, Schwann cell of the cord of Büngner stage.

FIG. 5. Transverse section of the nerve of Case 2. In the cytoplasm of the Schwann cell of the cord of Büngner stage, bacilli lie naked in the cytoplasm, while in the cytoplasm of the lepra cell bacilli lie in side-by-side arrangement wrapped in an opaque droplet. Magnification, X 23,000 (slightly reduced).

Key: *a*, Schwann cell of the cord of Büngner stage; *b*, endoneurial lepra cell; *c*, leprosy bacilli; *d*, opaque droplet; *e*, mitochondria.

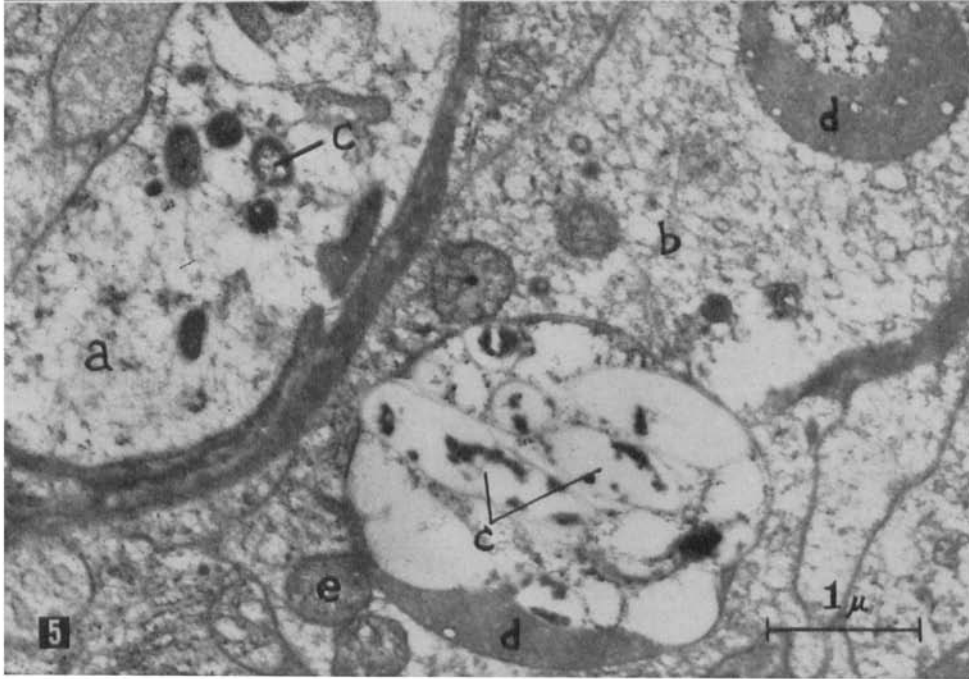
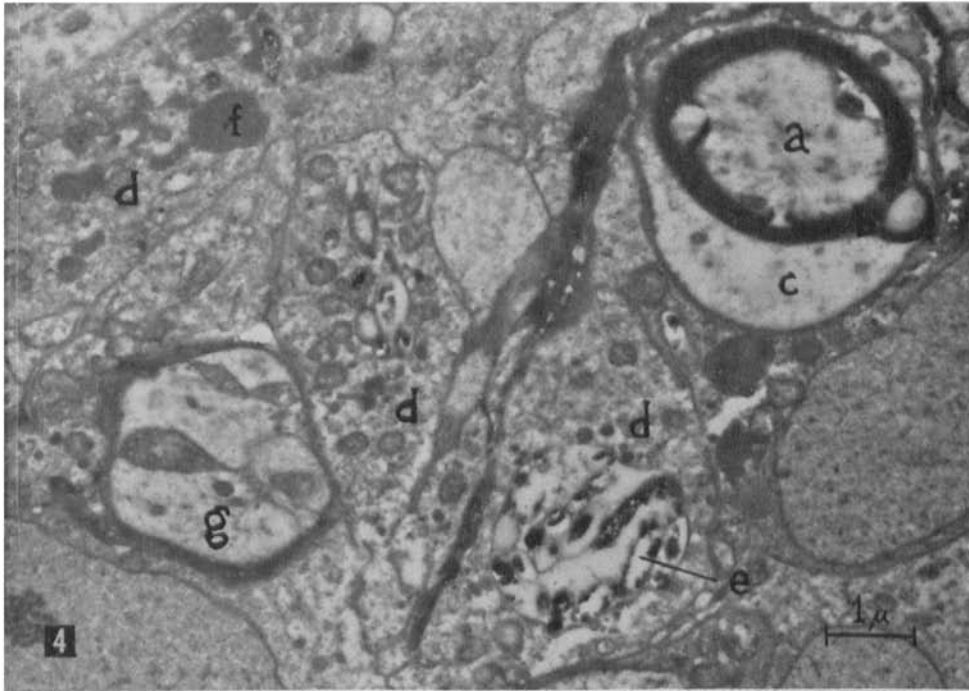


PLATE 22

PLATE 23

FIG. 6. Regenerating axons and the beginning of myelin regeneration are observed in this electron micrograph (Case 2 nerve). Magnification, X 25,000 (slightly reduced).

Key: *a*, regenerating axon; *(b)*, Schwann cell of the cord of Büngner stage; *c*, leprosy bacillus; *d*, mesaxon; *e*, beginning of the myelin regeneration.

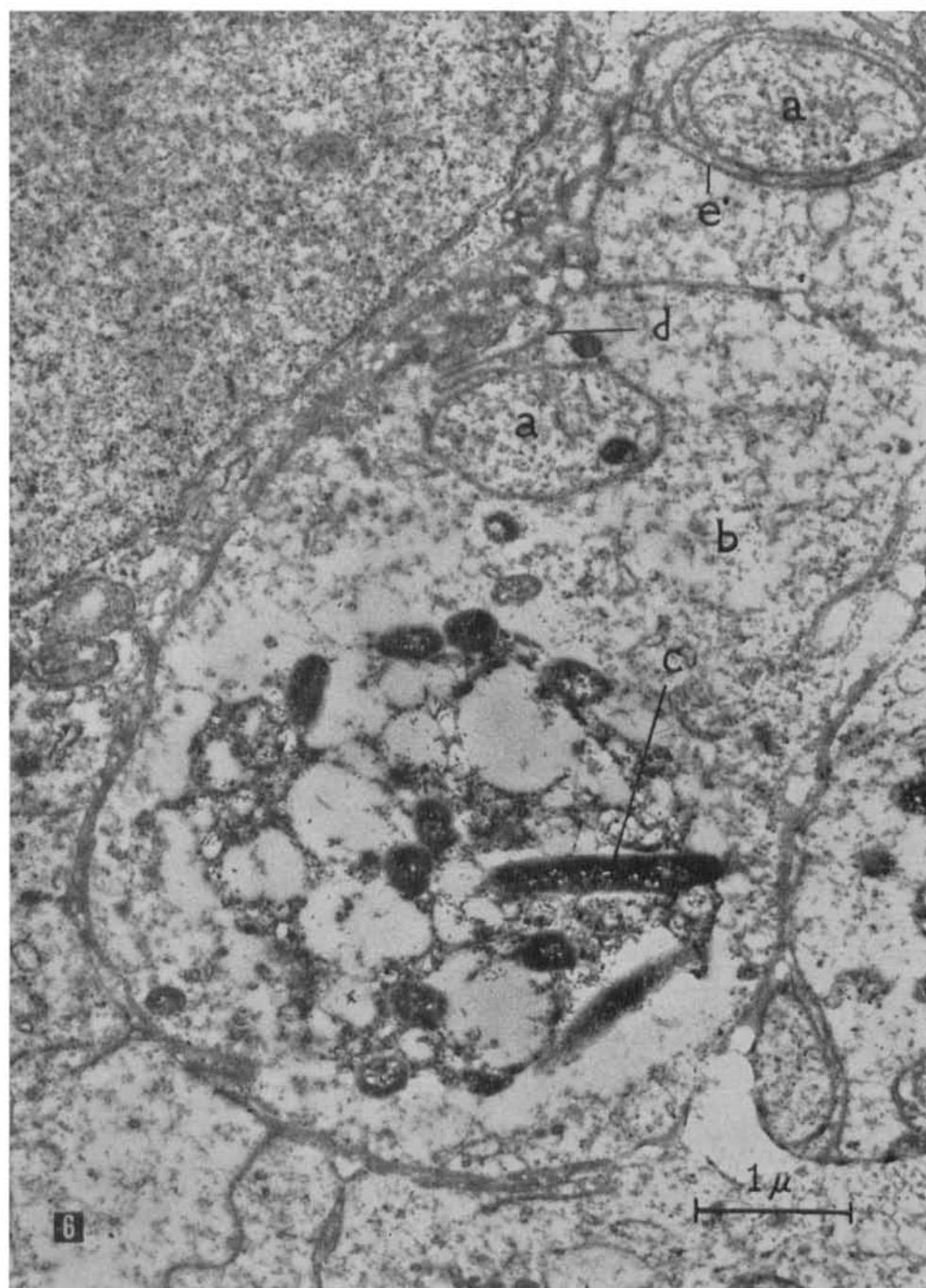


PLATE 23

PLATE 24

FIG. 7. Transverse section of the Schwann cells of the cord of Büngner stage. Magnification, X 10,000 (slightly reduced).

Key: *a*, regenerating axon; *b*, nucleus of a Schwann cell of the cord of Büngner; *c*, Schwann cell of the cord of Büngner stage.

FIG. 8. Similar to Fig. 7, higher magnification. Magnification, X 22,000 (slightly reduced).

Key: *a*, regenerating axon; *b*, Schwann cell of the cord of Büngner stage; *c*, leprosy bacillus; *d*, mesaxon.

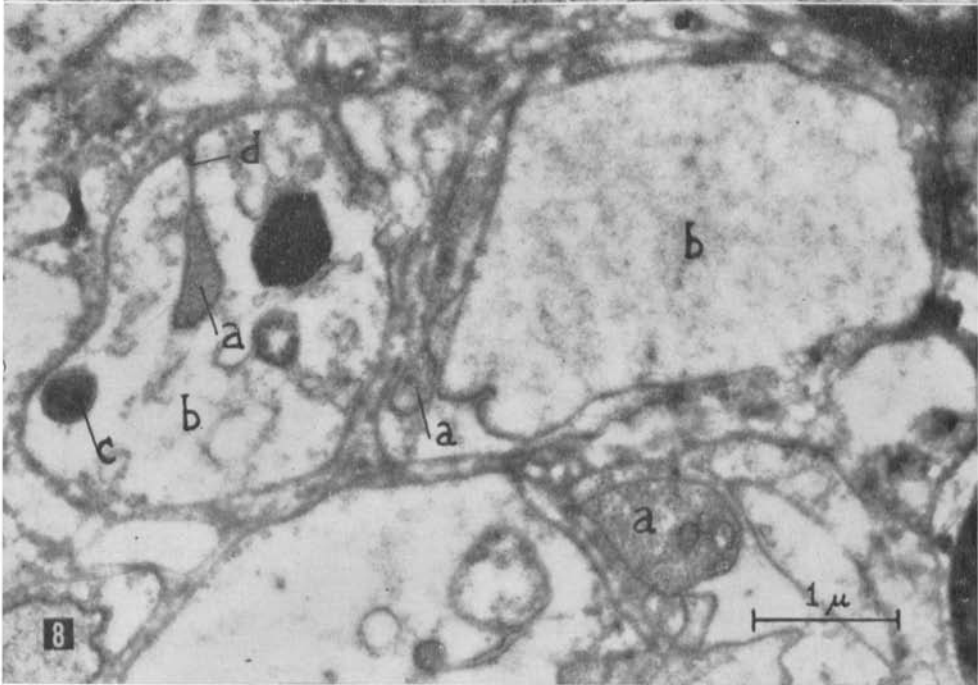
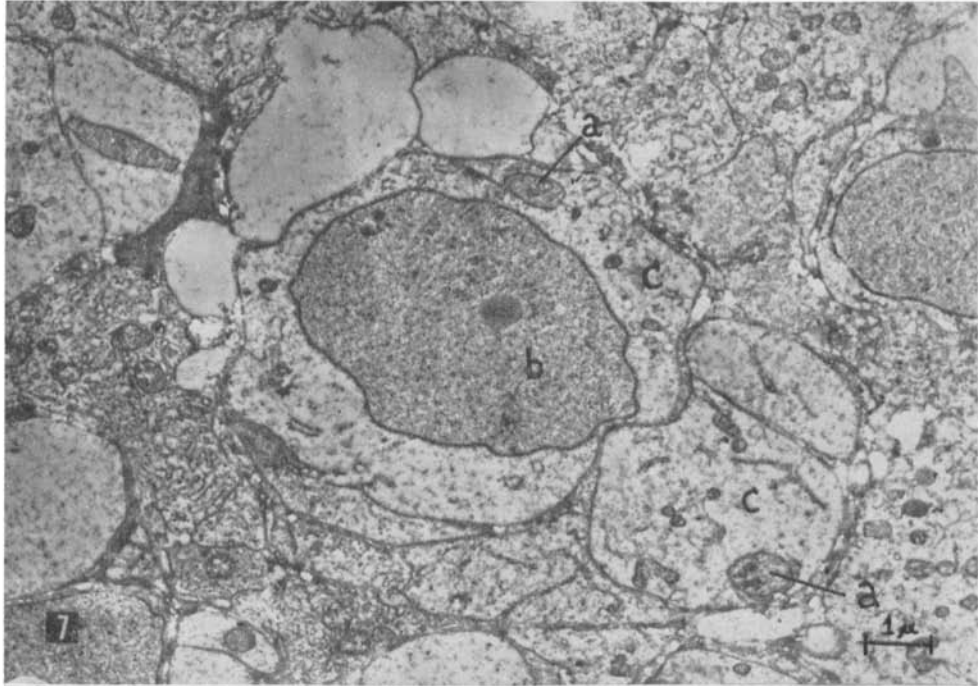


PLATE 24