ULTRASTRUCTURAL STUDIES OF INH-INDUCED NEUROPATHY IN RATS

I. Early Axonal Changes

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Functional disturbances of peripheral nerve have often been observed during the therapeutic use of isonicotinic acid hydrazide (INH, Isoniazid). These observations have led to light microscopic investigations in rats in which an INH-induced neuropathy was manifested.[1] In general, studies of peripheral nerves have been limited by the resolving power of the light microscope and by the inadequacy of histologic techniques to simultaneously demonstrate the axon, its myelin sheath and Schwann cells. Electron microscopy has obviated these difficulties, presenting a means of studying the close relationship of these structures during pathologic alterations.

The condition of peripheral nerves most extensively studied with the electron microscope has been wallerian degeneration.[2–7] These investigations have confirmed the classic view of a primary axonal change. The few electron microscopic studies of general disorders affecting the peripheral nerves have indicated different pathologic processes in nerve fiber breakdown. Investigation of experimental diphtheritic neuropathy (neuritis) in guinea pigs has shown the Schwann cell membrane system to be the primary target,[8] and the study of a peripheral nerve in a case of metachromatic leukodystrophy has shown the primary alteration to be an abnormal accumulation of lipids in the Schwann cell cytoplasm.[9] A peripheral nerve biopsy in a case with Guillain-Barré syndrome has revealed widespread disruptive changes of the axon and myelin sheath.[10]

INH-induced neuropathy of the rat provides an opportunity for further investigation of the pathologic interrelationship of axon, myelin sheath and Schwann cell. The present paper deals with both phase and electron microscopy in the acute stages of this experimental neuropathy.

This work was partially supported by United States Public Health Service Grant 5482 and Special Fellowship in Neuropathology (BT-794), National Institute of Neurological Diseases and Blindness.

Accepted for publication, March 24, 1964.

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Material and Methods

Twenty female Sprague-Dawley rats weighing 250 to 400 gm. were anesthetized with ether, and fed approximately 350 mg. Isoniazid per kg. daily by oral intubation. Food and water were given *ad libitum*. Groups of 2 to 3 rats were sacrificed on days 6, 7, 8, 9, 10, 13 and 16. Normal rats served as controls. Portions of sciatic nerve were removed from anesthetized rats and immediately placed both in 1 per cent buffered osmium tetroxide at 4° C.[11] for electron microscopy and 10 per cent formalin for light microscopy. The osmic acid fixation was continued for 4 hours, the specimens were dehydrated through the alcohol series, cut into approximately 1 mm. sections, and embedded in Epon and in methacrylate in which 10 per cent divinyl benzol had been added.[12] Sections for phase contrast microscopy were cut from selected blocks with a Porter-Blum microtome. Thin sections for electron microscopy were cut with glass knives and deposited on formvar-covered copper grids. Portions of the tissue were stained with lead hydroxide.[13] A Siemens Elmiskop I electron microscope operated at 80 kv. with 50 μ molybdenum apertures in the objective was used. Initial magnifications ranged from 2,000 to 25,000 with subsequent photographic enlargement.

Frozen sections of the formalin-fixed material were stained with hematoxylin and eosin (H and E), Schroeder's stain for myelin,[14] and Szatmari's stain[15] for axis cylinders.

Results

Clinical Observations

During the period of treatment the experimental animals evidenced a progressive diminution of motor activity, increasing somnolence and a roughening and dirtying of their coats. The severity of these changes was quite variable, with several animals showing minimal changes. During the second week of treatment, rhinitis and diarrhea were occasionally seen, and a few jerking seizures were observed 2 to 4 hours after INH feeding. Some animals displayed an arched posture and sagging of the hind toes; a definite paralysis, however, was never observed.

Light Microscopy

The first detectable histologic alterations were demonstrated by silver impregnation of axis cylinders (Fig. 1). These included focal fragmentation of the cylinder and occasional varicosity formation. Consecutive sections stained for myelin and by H and E showed no alteration of myelin structure and neither infiltration nor proliferation of cellular elements. Advanced lesions affected the myelin sheath and cellular elements, but these were never observed unless prior destruction of central axons had occurred.

The severity of sciatic nerve lesions in the treated rats did not correspond to the duration of INH treatment or to the degree of clinical neurologic deficit observed. A single rat sacrificed after 8 days of INH administration showed no lesion, while another animal, given INH for

9 days and with minimal neurologic signs, exhibited marked nerve alteration involving the axon, the myelin sheath and the cellular elements.

Phase Contrast Microscopy

In longitudinal nerve sections from control animals, the axoplasm demonstrated wavy linear structures within a smooth and uniform background. Such features as Schmidt-Lantermann fissures, nodes of Ranvier, and myelin ovoids in the Schwann cell cytoplasm and axoplasm could be followed in serial sections.

The earliest detectable nerve fiber change appeared as a replacement of the normal wavy axoplasmic linear structures by a granular material with a tendency toward clumping. This was often interspersed upon a background of decreased density (Fig. 2). These features were encountered in consecutive serial sections. The myelin sheaths surrounding altered axons often appeared to be entirely normal. In other instances, however, there was marked disruption of the myelin structure, with collapse of the cylindrical myelin sheath and the formation of irregular globules (Fig. 2). It should be emphasized that the myelin changes were never seen without disruption of the related axoplasm.

Electron Microscopy

The axoplasm of myelinated and non-myelinated fibers in control rats consisted of fine, longitudinally oriented neurofibrils with occasional interspersed vesicular profiles and longitudinally directed mitochondria. The majority of axons in rats given INH showed this pattern. Others, however, exhibited disappearance of the fine filamentous ground substance and the appearance of an irregular granular substance of moderate density with a tendency to clump and to adhere to adjacent structures (vesicles, mitochondria, and axon-Schwann cell membranes; Figs. 3 and 4). Non-myelinated fibers failed to show a similar axoplasmic change. On the other hand, a moderately dense, irregular, clumped granular material was occasionally seen within Schwann cell cytoplasm containing non-myelinated nerve fibers.

Swollen axonal mitochondria with a partial distortion of their internal structure were occasionally seen within a normal axoplasmic ground substance (Fig. 5). The mitochondrial content was often of decreased density although external double membranes and distorted internal cristae were frequently distinguished. An accumulation of altered mitochondria occasionally appeared in perinodal regions (Fig. 6). Membrane-bound pockets of axoplasmic material could be found in this region, and small mitochondria, vesicles and dense bodies often accumulated within and around the perinodal pockets (Figs. 7 and 8). Large

double membrane-bound sacs were occasionally located in the neighborhood of swollen mitochondria (Fig. 9), and small distorted vesicles sometimes appeared on the outer edges of these sacs. The content of the sacs consisted of closely packed, uniform, coarse granules with minimal density following osmium fixation. A marked increase of density was noted after lead staining in methacrylate-embedded tissue. Swollen and partially distorted mitochondria also appeared within the granular and clumped axoplasmic substance. Mitochondria and small vesicles were observed more frequently, however, within normal axoplasm.

The myelin sheath and the Schwann cell appeared intact during the early phases of axonal alteration. Protrusions or loops of myelin sheath extended into the Schwann cell cytoplasm and perinodal axoplasm. These features, however, were of equal frequency in the controls and have been described as a normal variation in the peripheral nerve of the guinea pig.[16]

Discussion

Early in INH-induced neuropathy in rats there were severe axoplasmic changes without a concomitant alteration of the surrounding myelin sheath or Schwann cell. This feature was also suggested by phase contrast microscopy. The changes were qualitatively similar to those in peripheral nerve fibers during the earliest stages of wallerian degeneration following transection or crushing injury.[3,7] It has been suggested that the early appearance of a granular and clumped axoplasmic material in wallerian degeneration represents a progressive fragmentation of neurofilaments following changes in hydration, pH and the ionic milieu of the axoplasm.[3] Experiments on neurofilaments *in vitro* have shown a transformation of the filaments into globular proteins.[17]

Definite axoplasmic changes were not seen in non-myelinated fibers. The only evidence of a pathologic alteration here was the occasional occurrence of a circumscribed accumulation of granular and clumped material within the cytoplasm of Schwann cells containing non-myelinated fibers. This might suggest a relative resistance of non-myelinated fibers to INH-induced neuropathy. These fibers have been reported to be relatively resistant to wallerian degeneration.[18–23] However, it is also possible that the breakdown and disappearance of axoplasmic material in the fibers is a rapid process and consequently may elude electron microscopic investigation.

The occurrence of mitochondrial swelling prior to recognizable axoplasmic clumping may have some significance. These mitochondrial changes have been noted in many different conditions and have found various interpretations.[24] Vial has suggested that the swelling results

from a change in water and ionic content.[3] The relative accumulation in perinodal axoplasm probably reflects the natural preponderance of mitochondria in this region. An extreme swelling of the organelles might give a false impression of an increase in number.

An accumulation of small mitochondria and dense bodies was noted in perinodal axoplasmic pockets. Similar accumulations have been described in the proximal and distal axon stumps following wallerian degeneration.[7,25] These changes have been considered reactive to injured axoplasm. The occurrence of dense bodies among numerous small mitochondria in peripheral nerves,[7] in altered central nervous system fibers,[26] and in degenerating axon terminals[27] has been considered indicative of mitochondrial degeneration. The presence of reactive changes in the perinodal region may reflect a special vulnerability of this area.

The occurrence of large double membrane-bound sacs in axoplasm among normal-appearing neurofilaments has not been described in normal or altered myelinated fibers. The similarities of structural features, the occurrence of intermediate forms and the presence of sacs in the neighborhood of altered mitochondria suggest that they arise from swollen mitochondria. The uniform and coarsely granular content of the sacs increased markedly in density with lead staining. Similar cytoplasmic particles, also exhibiting an increased contrast following lead treatment, have been interpreted as representing glycogen in liver and muscle cells.[28] An accumulation of glycogen within altered mitochondria is unusual; such an interpretation must await a biochemical confirmation.

An experimentally induced primary axonal degenerative neuropathy may be compared with other electron microscopic studies of peripheral nerve disorders. In a case of acute polyradiculoneuritis of the Guillain-Barré type, Finean and Woolf[10] demonstrated a disruptive pattern with extensive axonal changes; the primacy of these alterations remains obscure, however, because of the concomitance of marked axoplasmic and myelin changes. Webster's observations[9] in a case of metachromatic leukodystrophy illustrated a different process, an accumulation of foreign lipid material within Schwann cell cytoplasm. The initial ultrastructural changes observed in experimental diphtheritic neuritis in guinea pigs were interpreted as representing focal disruption of the Schwann cell membranous system.[8] This continuous system includes the surface membrane, the mesaxons, the myelin sheath and the axon-Schwann cell membrane. Following extensive demyelination, secondary axonal degeneration was also noted.

Biochemical investigations[29–33] and therapeutic observations[34–36] have shown that certain actions of INH can be blocked and reversed

by pyridoxine administration. The INH-induced peripheral neuropathy of the rat can also be inhibited by pyridoxal.[37] The interaction of INH and pyridoxine metabolism has been thought to result in a pyridoxine deficiency.[33] It is of interest that human pyridoxine deficiency has been associated with peripheral neuropathy.[38,39] Thus, it seems possible that the primary axonal degeneration in INH-induced neuropathy in rats represents a deficiency disorder. The possibility of a more direct toxic action of INH on the metabolism of nerve fibers cannot, however, be excluded.

Summary

The early changes in INH-induced peripheral neuropathy of rats was investigated by light, phase and contrast microscopy. A primary axonal degeneration was demonstrated. This was manifested electron microscopically by a swelling of axonal mitochondria and coarse granular disruption of the axoplasmic ground substance. In addition, unusual double membrane-bound sacs and perinodal accumulations of small mitochondria and dense bodies were encountered within the axoplasm.

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We would like to acknowledge the capable technical assistance of Misses Susanne Luh, Brunhilde Friedrich, Christa Stark and Ingrid Reichel.

LEGENDS FOR FIGURES

FIG. 1. Rat sciatic nerve. INH, 6 days. Focal fragmentation and varicosity formation appear in the axis cylinders. Szatmari stain.[15] × 300.

FIG. 2. INH, 13 days. Longitudinal section of sciatic nerve. There is granular change and clumping of the axoplasm (x). The surrounding myelin sheath (m) appears intact and includes a Schmidt-Lantermann incisure (bottom). Collapse of other myelin sheaths around altered axoplasm is also evident (right). Phase contrast microscopy. × 1,700.

FIG. 3. INH, 8 days. Cross section of a nerve fiber. An altered granular axoplasm (X) exhibits a tendency toward clumping. MS, myelin sheath; S, Schwann cell. × 6,000.

FIG. 4. INH, 7 days. Longitudinal section. Altered axoplasm (X) may be compared with the normal (A) in an adjacent nerve fiber. Myelin sheaths (MS) appear normal. × 5,000.

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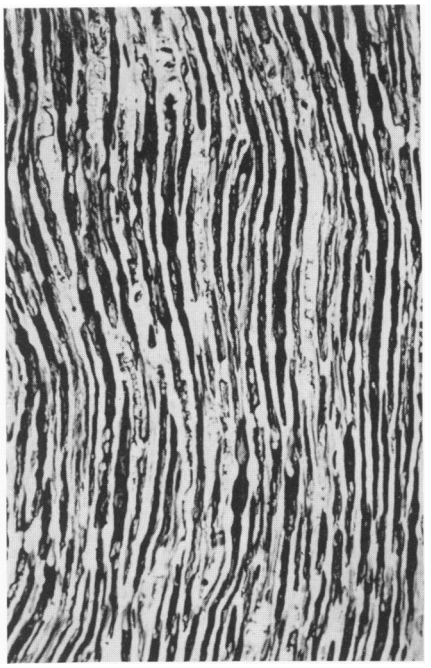

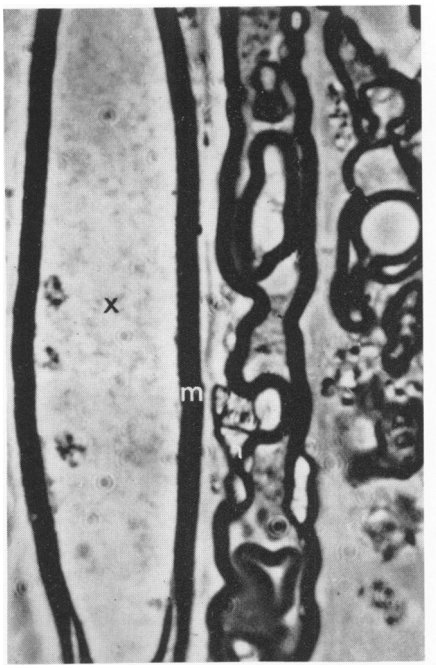

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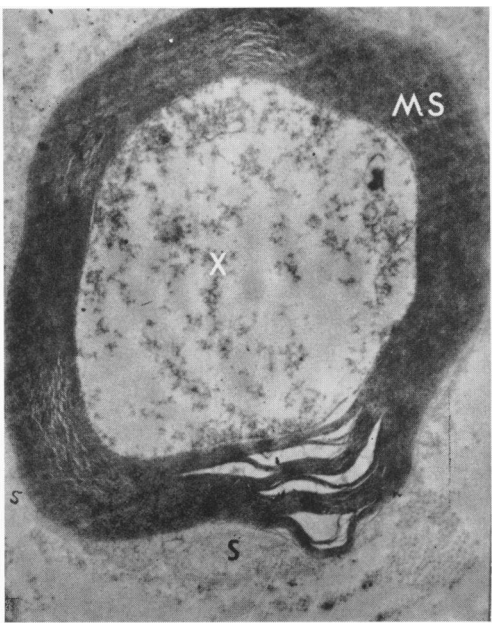

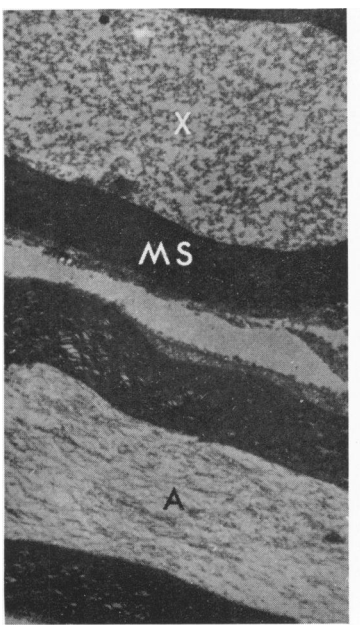

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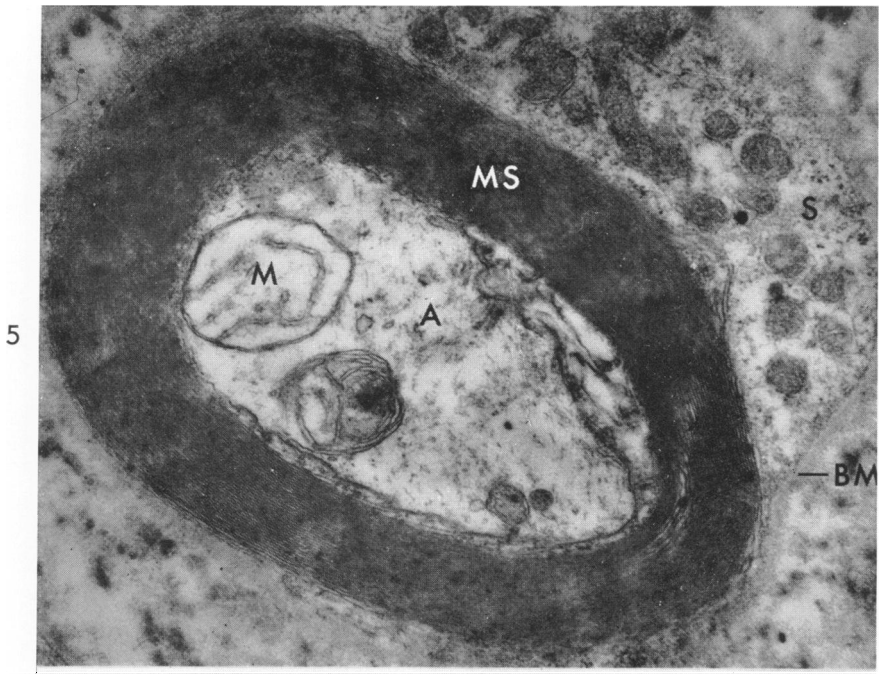

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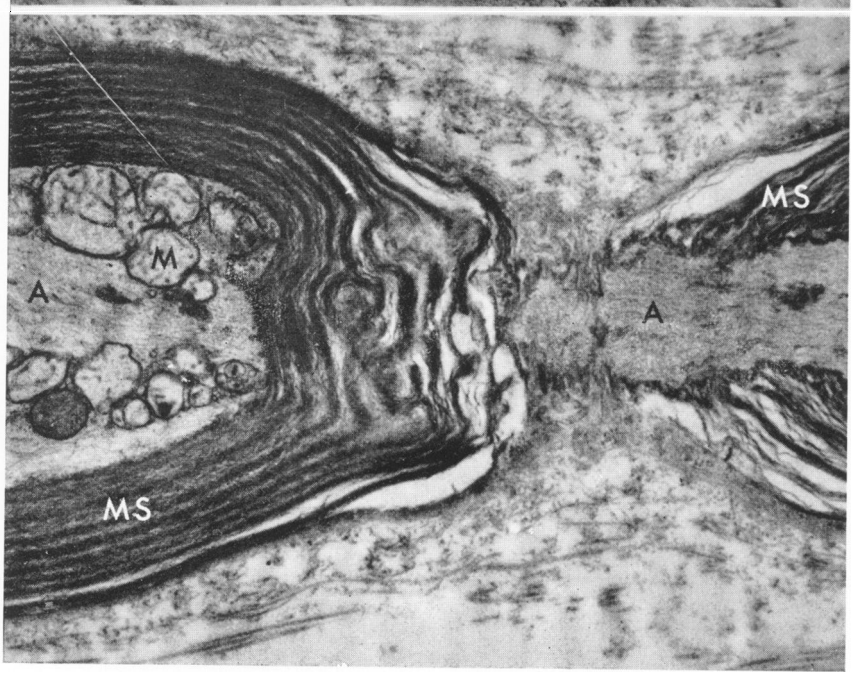

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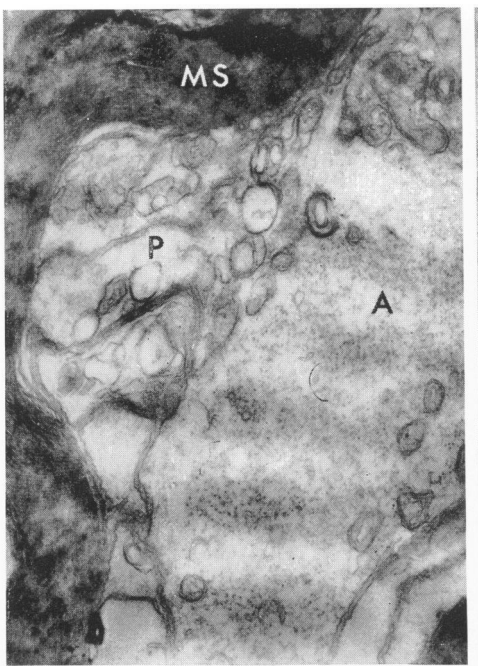
FIG. 5. INH, 12 days. Cross section of a nerve fiber. Swollen axonal mitochondria are shown (M). MS, myelin sheath; A, axoplasmic ground substance; S, Schwann cell cytoplasm; BM, basement membrane. × 33,000.

FIG. 6. INH, 12 days. Swollen mitochondria (M) accumulate in the nodal axoplasm (A). A longitudinal section of a nodal termination of myelin sheath (MS) may also be seen. × 12,000.



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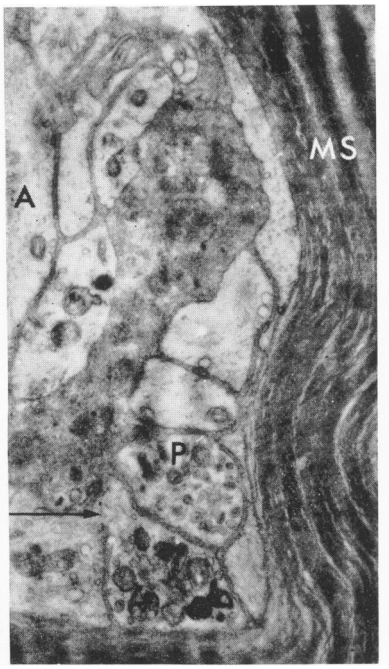

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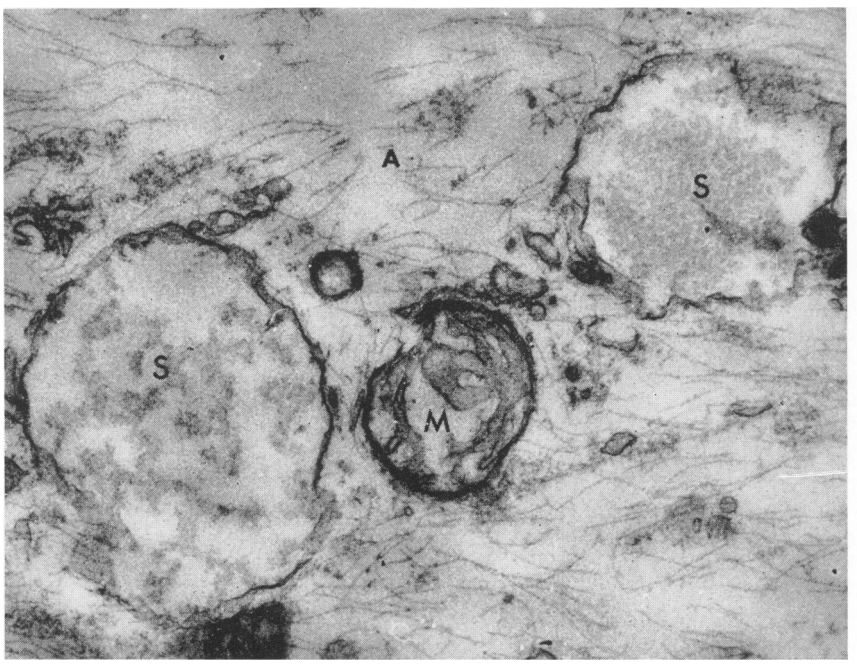

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FIGS. 7 and 8. INH, 6 days. Small mitochondria, vesicles and dense bodies have accumulated within the perinodal pockets (P) of the axoplasm. There is continuity of these pockets with central axoplasm (arrow). MS, myelin sheath; A, axoplasm. × 20,000.

FIG. 9. INH, 8 days. Double membrane-bound sacs (S) contain uniform coarse granules of mild density. These occur within normal axoplasmic ground substance (A) and in the vicinity of swollen mitochondria (M). Epon embedding, unstained. × 30,000.