Fine Structure of the Early Changes in the Vestibular Nuclei of the Thiamine-Deficient Rat

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LACK OF THIAMINE (Vitamin B_1) in the diet of several animal species causes well-known neurologic abnormalities, consisting of peripheral nerve degeneration and signs of central nervous system dysfunction. Ataxia is characteristic, with severe inability to maintain or adjust posture; in the later stages, gait is completely impaired and nystagmus appears; finally, there are opisthotonus and death. This picture suggests that disturbed vestibular function plays an important role in the disease. The signs are particularly striking in the pigeon,[1-4] but they are also marked in the rat,[5] dog,[6] and, of course, in man.[7-8]

Neurologic signs appear before lesions can be demonstrated by the usual histologic technics. However, even at this stage, well-localized biochemical changes have been noted by Dreyfus,[9] who found that the activity of transketolase (a thiamine-dependent enzyme) was significantly decreased in the lateral pontine tegmentum, which includes the lateral vestibular nuclei. The abrupt onset of clinical signs coincided with a sudden, severe decrease in the enzymatic activity. Injection of thiamine promptly restored transketolase activity to the level of the presymptomatic stage.

Classic histologic studies by Prickett,[10] Zimmerman,[11] Alexander,[12] Swank and Prados,[3] and others have shown that animals with chronic deficiency of thiamine have symmetrical lesions in the tegmentum of the brain stem involving cranial nerve nuclei. In the rat, the vestibular nuclei, especially the lateral group (Deiter's nuclei) are the ones most affected. The salient features of the lesion in the advanced stage are symmetrical areas of necrosis in Deiter's nuclei, neurons in various stages of degeneration, destruction of all myelinated fibers in the center of the lesion, and glial proliferation. Information about the development of the lesion is scanty and often contradictory.[2,12,13]

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Three ultrastructural studies of the lesion in the brain of thiamine-deficient rats have recently been reported.[14-16] These deal exclusively with glial changes, edema, and the regeneration of myelin; the lateness of neuronal changes was noted; synaptic modifications were not mentioned. Our study confirms the presence of mild glial abnormalities and edema, but emphasizes important changes of neuronal processes, especially axons and presynaptic endings.

Materials and Methods

Albino rats of the Holtzman stain, weighing 100–150 gm., were used. Four groups comprising 12, 16, 12, and 24 rats were studied in succession. The first 3 groups were each divided into equal numbers of controls and test animals; the controls were fed a normal Purina Chow diet ad lib, and the test animals a thiamine-deficient diet.* In the fourth group (24 rats), 12 control animals were fed the thiamine-deficient diet supplemented by 15 μg. of thiamine HCl given subcutaneously at the start of the experiment, with another dose a week later, and twice weekly thereafter; the other 12 were fed the thiamine-deficient diet only. During the first 2 weeks, the weight of all animals increased, confirming the results of Dreyfus and Victor.[5] On about the fifteenth day, the weight of the thiamine-deficient rats leveled for a few days and then decreased progressively, whereas that of the group given supplementary thiamine HCl continued to increase. The latter group and the controls on the normal diet remained asymptomatic and appeared normal.

Acute neurologic manifestations of thiamine deficiency in the rats usually appeared after they had been on the test diet 35–50 days. The standing posture was wide based, and the rats remained immobile; when they did move they walked with great difficulty, and at a slightly later stage they rolled over. Nystagmus was present but disappeared when the disease had progressed to opisthotonic crises. At this time, any change in position elicited opisthotonus. The animals usually died 18–24 hr. after the onset of severe ataxia.

The animals selected for electron microscopic study were in the early stages of the first acute, symptomatic episode of deficiency. They were anesthetized with sodium penotobarbital and perfused in vivo through the aorta via the left ventricle with cold 3% glutaraldehyde in 0.1 M phosphate buffer. The brains were then removed, and the brain stems sectioned coronally at the level of the eighth cranial nerve. Small blocks of tissue (2 mm. or less) were obtained from the lateral pontine tegmentum, which contains the neuronal groups of the lateral vestibular nuclei. The exact site of sampling was verified by light microscopic examination of the remaining tissue and of 1-μ thick sections of the tissue blocks that had been prepared for electron microscopy. The 2-mm. blocks were then subdivided, fixed for 90 min. in OsO_4 in chromate buffer,[17] dehydrated in ethanol, and embedded in Epon or Araldite. The electron microscopes used were the Hitachi HS-7 and the Siemens Elmiskop 1.

Results

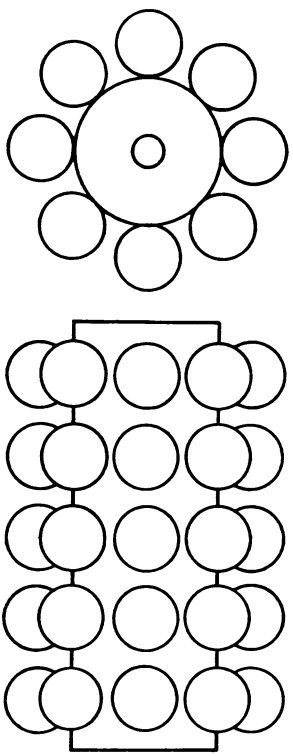
The electron microscopic features reported here are based on tissue obtained from 6 rats with acute thiamine deficiency, 5 normal controls,

* The complete diet (Nutritional Biochemicals Corp.) consisted of (in per cent): vitamin test casein, 18; sucrose, 68; vegetable oil, 10; and U.S.P. salt mixture No. 2, 4. It was fortified with (in gm./100 lb. diet): Vitamin A concentrate (200,000 U./gm.), 4.5; Vitamin D concentrate (400,000 U./gm.), 0.25; alpha tocopherol, 5.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; menadione, 2.25; p-aminobenzoic acid, 5.0; niacin, 4.5; riboflavin, 1.0; pyridoxine HCl, 1.0; calcium pantothenate, 3.0; and in mg./100 lb. diet: biotin, 20; folic acid, 90; and Vitamin B-12, 1.35.

and 4 controls fed the thiamine-deficient diet with subcutaneous supplements of thiamine HCl.

Synapses

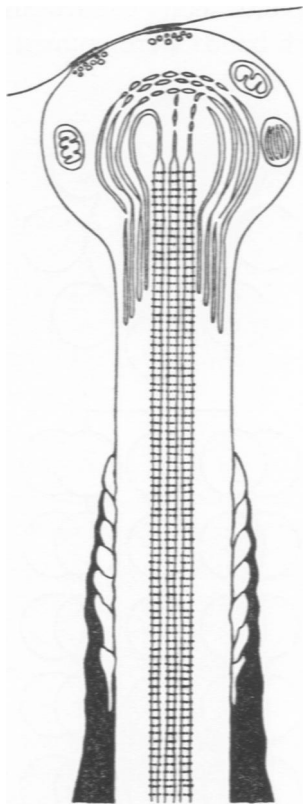
Striking changes were present in the presynaptic endings of the lateral vestibular nuclei of all thiamine-deficient animals; these changes were not found in the controls. Both the axodendritic and axosomatic nerve endings were affected, the former more frequently than the latter. Many neuronal perikarya were surrounded by enlarged boutons. These hypertrophic presynaptic endings were often almost filled with smooth-surfaced endoplasmic reticulum arranged in layers of flattened sacs (Fig. 1). The endings also contained orderly bundles of unusual tubules in an axial position, apparently surrounded by the concentric system of cisternae of smooth-surfaced ergastoplasm (Fig. 2 and 3). The transverse diameter of these particular tubules was 450–490 Å, and there was a fine, solid filament in the center of the lumen (Fig. 3). A characteristic periodicity produced alternate dark and light bands along the length of each tubule (Fig. 4 and Text-fig. 1). The dark bands were approximately 210 Å wide, and



TEXT-FIG. 1. Diagram of banded tubule: a central fiber in cross section at top, and in longitudinal section below.

the center-to-center distance was 240–260 Å; the light bands were 40–50 Å wide. Higher magnification showed that the dark band was a ring made up of particles about 210 Å in diameter and attached to the outer surface of the tubule (Fig. 4, inset). The uneven densities in the lateral view indicated the presence of small spaces between the particles; the appearance in cross section seemed to confirm this. Measurements suggest that 8 such particles form a ring. Text-fig. 1 is a diagrammatic representation of a tubule and its dark, granular bands.

Continuity between these banded tubules and the smooth-surfaced endoplasmic reticulum was evident (Fig. 4). At the junction, the tubular caliber decreased, the periodicity of the bands disappeared, the tubule seemed to merge with the profile of the sac, and the lumens were continuous. The membrane of the sac and the wall of the tubule were approximately 80 Å thick, and correspond to a unit membrane. Text-fig. 2 illustrates the structure of the abnormal nerve ending.



TEXT-FIG. 2. Diagram of banded tubules in the center of a terminal axon, showing their continuity with circumferentially arranged smooth endoplasmic reticulum in the presynaptic bouton.

Most of the classic features of CNS synapses were present in the synaptic junctions of the abnormal endings: [18,19] the electron density of the pre- and postsynaptic membranes was greater than that of adjacent, nonspecialized areas of the cell membrane; the intersynaptic cleft was 200–250 Å wide, with regularly spaced, fine filaments across it; the narrow, irregularly dense zone below the postsynaptic membrane corresponded to the subsynaptic web described by de Robertis [20] (Fig. 1, inset). In the normal endings, there were clusters of vesicles close to the presynaptic membrane; few or no such vesicles were seen in the hypertrophic endings, and when present were not particularly related to the synaptic junction. Usually, the matrix of the hypertrophic ending was dense, in contrast to the translucent sap of the postsynaptic dendrite. A finely granular material, evenly distributed throughout the sac accounted for the density (fig. 1 and 2). The granules were smaller, less regular, and less dense than ribosomes. There were few mitochondria in these endings.

Although the nuclear neuropil contained an abundance of abnormal synaptic bodies, many normal endings were also present in the same area. Both normal and abnormal endings abutted on the surface of a single neuronal soma. Other endings appeared to be degenerating. Dark, small boutons terminaux contained vesicles and increased membranous and tubular profiles barely were distinguishable from the dense matrix. Only the mitochondria were well outlined—because their matrix was of low density and their inner structure well preserved (Fig. 5). Pale, swollen endings were also present; their mitochondria were distorted, with irregular walls and cristae. These endings contained only fragments of tubules in irregularly scattered, widely dispersed, finely granular matrix; synaptic vesicles were rare, and vacuolation was mild (Fig. 6).

Nerve Fibers

A number of abnormalities were found in the axons. Occasional myelinated processes contained orderly bundles of striated tubules with the same features as those in the nerve endings (Fig. 7). Sacs of smooth-surfaced endoplasmic reticulum were much rarer than in the presynaptic endings, but the matrix was dense and granular when abnormal tubules were present. Normal neurofilaments and neurotubules were absent in the affected axons. Irregular tubular and vesicular profiles were occasionally present in the dense axoplasm; in other axons, the axoplasm was dense and vacuolated (Fig. 8) as in wallerian degeneration. Elsewhere, the axoplasm was shrunken and displaced toward one edge, with large vacuoles between the depressed axolemma and the apparently intact myelin sheath (Fig. 7). Focal, fusiform swellings of axons, containing many mitochondria and vesicles, were also seen.

Neurons, Glia, and General Aspects

The large and medium-sized neurons appeared undamaged. The perikarya were rich in ergastoplasm and contained normal organelles. The nuclei and nucleoli were normal. Nerve endings abutting on these neurons were decreased in number, suggesting degeneration of some boutons. The main damage was evident in the nuclear neuropil, which contained hypertrophic axodendritic endings, degenerated axons, and many swollen, clear processes (Fig. 6). Often the nature or origin of these processes could not be established; glial fibrils, glycogen granules, and neurofibrils were absent from the pale matrix which consisted of finely granular or floccular material. Occasionally, they contained tubular profiles, 220 Å in diameter. Although the processes resembled dendrites of the same tissue, there were no synaptic junctions along their surfaces, and they might have been swollen oligodendroglial processes which also contain tubules of this diameter. The extracellular space was mildly increased, especially in the perineuronal areas. Lipid-laden cells were occasionally present in the parenchyma and perivascular spaces. In 2 animals, there was mild fibrous astrocytosis. The oligodendroglial perikarya were normal.

Small, acute, perivascular hemorrhages were present in only 1 rat. The erythrocytes in the perivascular space were intact, and the walls of the vessels were not visibly damaged.

Discussion

Other electron microscopists have emphasized glial changes and edema as the major early effects of thiamine deficiency in the vestibular nuclei of the rat; neuronal changes, in their opinion, are late and occur only with necrosis of the tissue.[14–16] Yonezawa and Iwanami[21] induced thiamine deficiency in tissue cultures of rat and mouse cerebellum and spinal ganglia by adding the antimetabolites oxythiamine and pyrithiamine to the culture media. They learned that high concentrations of either drug caused prompt degeneration of neuronal, glial, and myelin constituents. Chronic changes were found after administration of lower concentrations, and these consisted of bubbling and ballooning of the myelin and swelling of the oligodendroglia. Only light microscopy was utilized in these in-vitro studies; thus, synapses were not considered.

The work reported here shows that in our experimental conditions the most striking tissue changes early in the first acute episode involved axons and presynaptic endings rather than supporting structures. Nerve terminals were affected in 2 ways: degenerative forms of both dark-shrunken and pale-swollen types; and the hypertrophic forms, which were more

characteristic, widespread, and prominent in our material. The latter abnormal forms contained increased amounts of smooth endoplasmic reticulum and bundles of banded tubules.

These hypertrophic endings appeared consistently in 4 successive groups of animals deprived of thiamine, and were absent in the vestibular nuclei of normal animals. They were invariably present when the animal was sacrificed during the acute manifestations in the first clinical bout, but were not found in the same nuclei examined during the preclinical state. Since the symptoms were of a vestibular nature,[3] were well correlated in time with the lesion, and could be readily reversed by the parenteral administration of thiamine HCl, a causal relationship among thiamine deficiency, vestibular lesions, and symptoms may readily be inferred.

Although regional metabolic variations and susceptibilities among neurons are well established, the concept of topographically determined differences among glia is still far from proven. The proposal seems plausible that certain neuronal endings might be particularly susceptible to inadequate thiamine in view of evidence that glycolysis[22] and even protein synthesis[23] may occur at neuronal terminals. The acuity of symptoms and their rapid reversibility also are more characteristic of neuronal than of glial disorder.

That other investigators did not find these synaptic changes suggests that their animals may have been sacrificed either too early (before the symptoms appeared) or too late (during a phase of general tissue reaction). Cultured Purkinje cells and their synapses may not have the same susceptibility to thiamine deficiency as cultured vestibular neurons, let alone the vestibular tissue in vivo.

Banded tubules resembling those found in our experiments have been noted in the "tubular axoplasm" of myelinated axons of the granular cell layer in the cerebellar cortex of the normal rat[24] and in the axons of denervated cerebellar folia in the cat.[25] So far as we know, nerve endings with similar tubules in normal or pathologic material have not been reported. It is a tempting speculation that the nerve terminal changes in the vestibular nuclei of thiamine-deficient rats result from involvement of specific afferent fibers of cerebellar origin. These fibers form part of the cerebellovestibular functional unit, known to be injured in thiamine deficiency. Although the entire afferent neuron may be involved, the changes in the lateral vestibular nuclei demonstrate that hypertrophy occurs almost exclusively in the endings, while degenerative changes predominate in the axons. When vestibulocerebellar fibers are cut, degenerative but not hypertrophic boutons are found.[26]

The presence of focal fusiform swellings, the so-called axonal torpedos, often indicate degenerative changes in axons.[27,28] Vacuolation of the axons, as observed in our material, has been found in other instances in which subacute or chronic axonal damage has been the primary effect of noxious stimuli. Two types of axonal vacuoles can be seen in our electron micrographs. One is the result of multiple intra-axoplasmic vacuoles usually found in axons with abnormally dense and granular axoplasm (Fig. 8); this probably represents primary axoplasmic degeneration. The second type consists of large vacuoles between the axon and the myelin sheath (Fig. 9). This type seem to be formed by swelling of glial cytoplasm in the inner layers of the myelin sheath or by dilation of the extracellular space between the layers of the internal mesaxon.

Degenerative, regenerative, reactive, and dystrophic axons have recently been described by Lampert,[29] who evoked these changes in the spinal cord of the rat by incising the dorsal columns, by inducing experimental allergic encephalomyelitis, or by feeding the animals a diet deficient in Vitamin E. None of the changes resemble those found in the hypertrophic synaptic endings or in the similarly modified axons of our thiamine-deficient rats.

Degenerative synapses resembling those seen in our material have been found in the acoustic ganglia after destruction of the cochlea,[30] in the undercut cerebral cortex,[31] and in the denervated lateral geniculate body.[32] Hypertrophic synapses of the type described here have not been previously reported. The abnormal synapses found by Gonatas and Goldensohn[33] in the cerebral biopsy of a mentally retarded child did not appear at all like those seen in our animals. Hypertrophy of the nerve terminal occurs after sectioning of the afferent fibers,[34,35] but the enlargement parallels the increase in 100-Å neurofilaments in the presynaptic sacs, and neither smooth membranes nor periodic tubules have been seen in that situation.

The increase in extracellular space in our thiamine-deficient animals might be secondary to degeneration of axons and nerve endings. The possibility that this effect was also, to some extent, due to fixation artifacts cannot be completely excluded. But the intracellular elements were well preserved, and in similarly fixed control material the normal mimimal extracellular space was present. A certain degree of cellular swelling was also noted in some dendrites and glial processes. It has been suggested that the primary abnormality in thiamine deficiency is a change of the blood-brain barrier, giving rise to early swelling and edema.[16] Sponginess of the affected area is a constant feature of the lesions of Wernicke's disease. This aspect is probably accounted for by intracellular and extracellular edema, as well as by actual loss of tissue elements.

Opinions vary about the way in which lesions develop in experimental thiamine deficiency. Alexander [12] favored the theory that primary vascular damage leads to secondary degeneration of parenchymal elements. Others believe that neuronal damage is the first lesion.[2] Dreyfus [9] has suggested that oligodendroglia are possibly the main cellular element to be affected by thiamine deficiency, based on his observation that transketolase activity, which is normally high in myelinated regions, is significantly reduced in the vestibular area in thiamine deficiency. On the basis of our study, we believe certain conclusions to be justified: early changes occur in nerve fibers and nerve endings of the lateral vestibular nuclei; primary myelin abnormalities do not occur; neuronal and oligodendroglial perikarya are not affected at an early stage except for mild intracellular edema in the latter; extracellular edema and phagocytosis are mild and are perhaps related to the degree of degeneration of nerve fibers and terminals in the nuclear neuropil; the capillary walls remain intact.

Summary

The effect of thiamine deficiency on the fine structure of the lateral vestibular nucleus was investigated. Material from 9 control rats and 6 thiamine-deficient rats with acute central nervous system symptoms were studied with the electron microscope. Early and unusual changes were found in the nerve terminals and in the axons of the nucleus. Hypertrophy with proliferation of membranes and periodically banded tubular structures characterized the changes in the nerve endings. Another abnormality was mild edema, both in and between cells. Phagocytosis was related to the degree of degeneration of nerve terminals and nerve fibers, and was not marked. No indication of early damage of the neuronal perikarya of the lateral vestibular nucleus was found, nor were there vascular changes.

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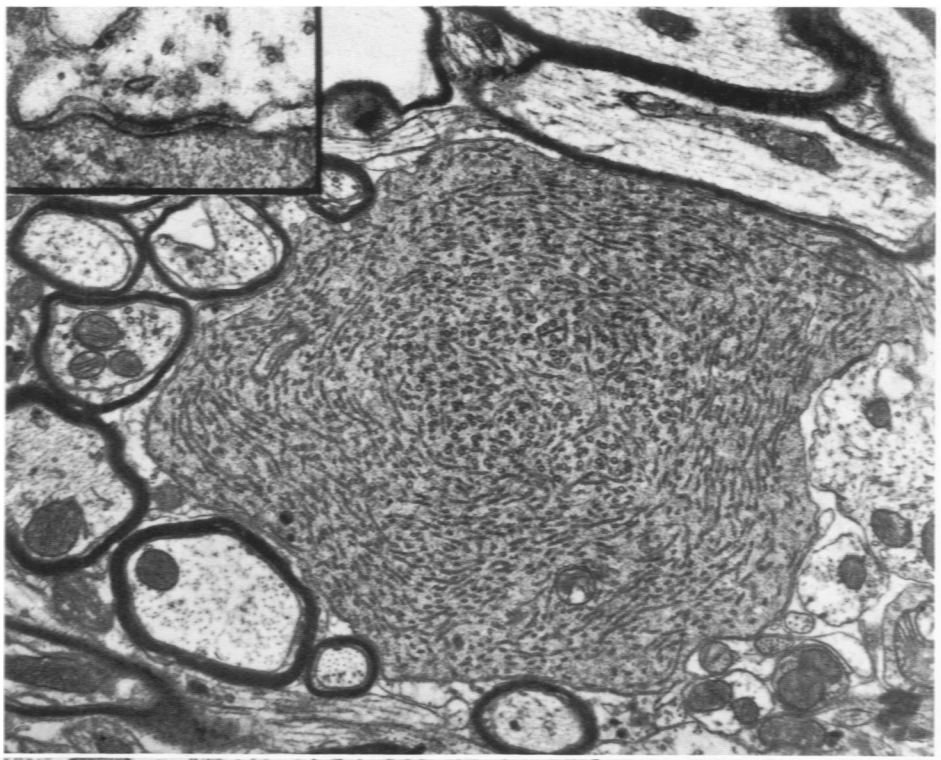
[*Illustrations follow*]

Legends for Figures

Fig. 1. Markedly hypertrophied presynaptic bouton contains central tubules in cross section surrounded by concentric, smooth endoplasmic reticulum. × 13,000. **Inset:** Higher magnification of synaptic membranes at right in Fig. 1. Suggestion of subsynaptic web is apparent above. × 50,000.

Fig. 2. Similar hypertrophic bouton with banded tubules arranged at center, surrounded by smooth endoplasmic reticulum in a dense matrix. × 19,000.



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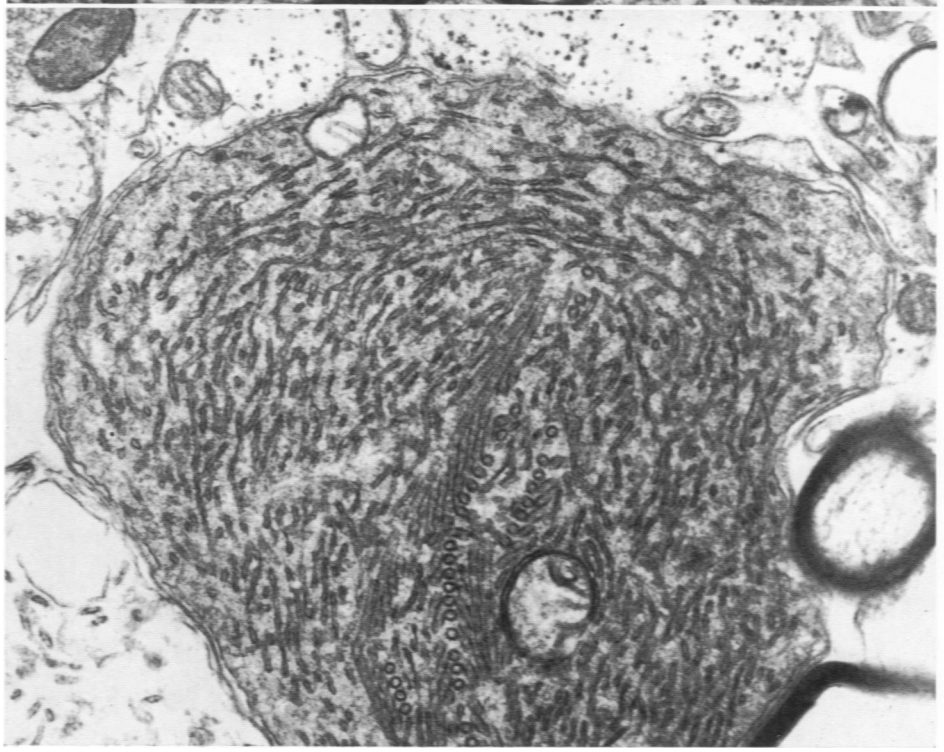

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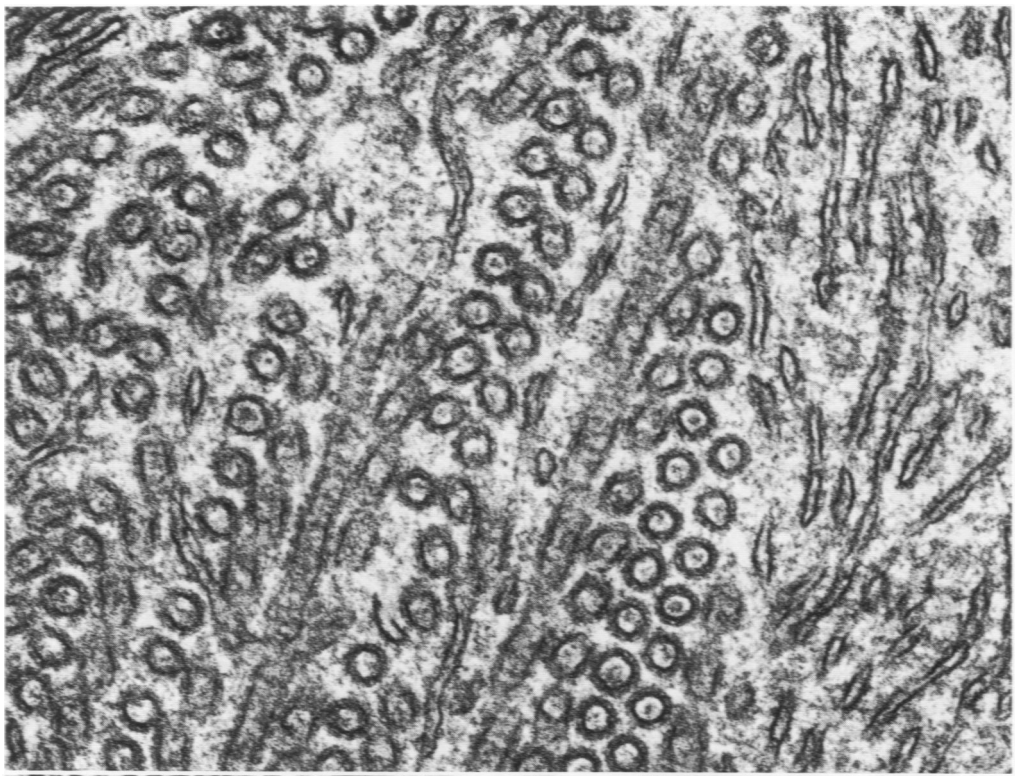
Fig. 3. Banded tubules are seen at higher magnification alternating with those in cross section. Granularity of bands is apparent, as is the difference between the tubules and the adjacent endoplasmic reticulum. The central filament is visible. × 16,000.

Fig. 4. Longitudinal section of banded tubules which are in continuity (at upper left) with smooth endoplasmic reticulum. × 43,000. **Inset:** High magnification of banded tubule. × 76,000.



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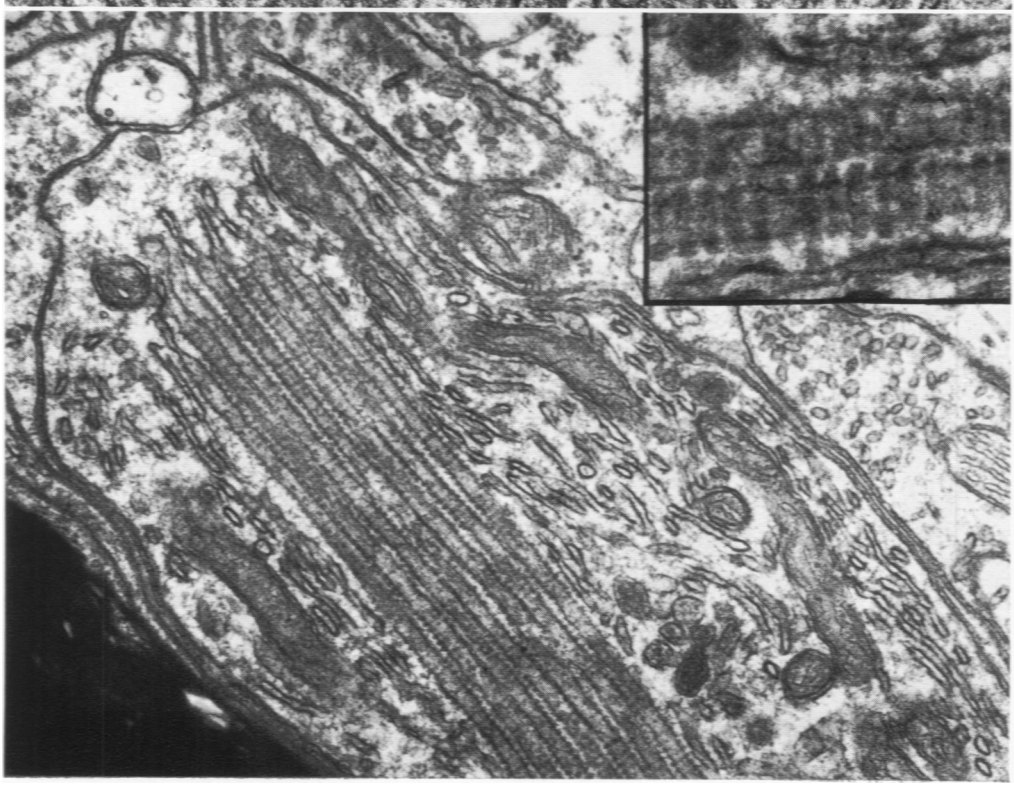

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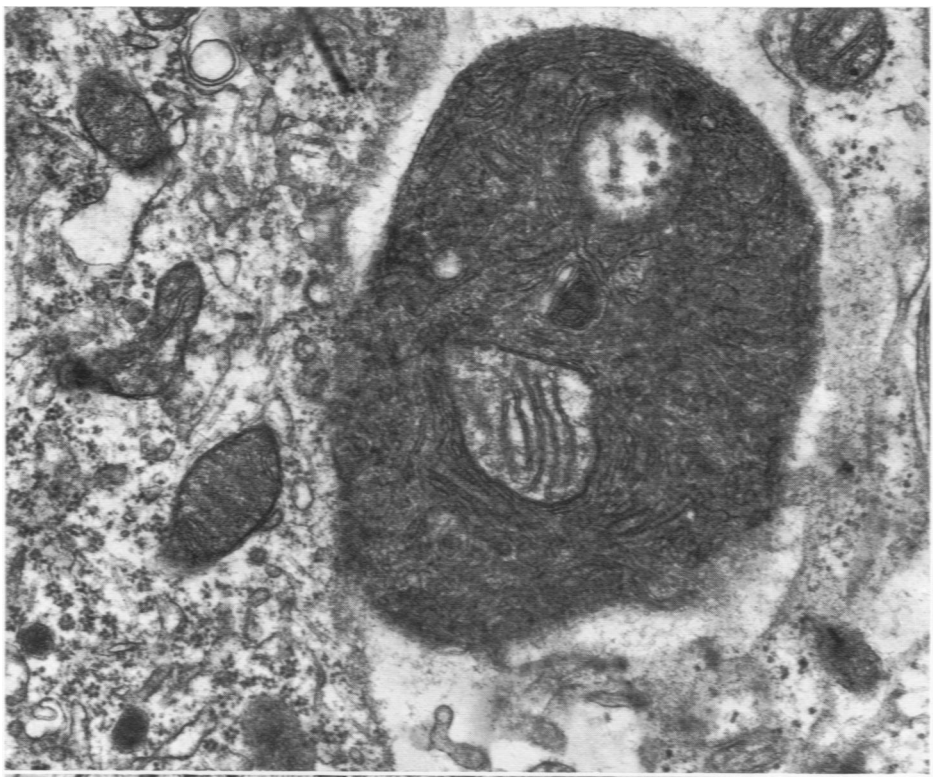
Fig. 5. Degenerating bouton of axosomatic type with very dense matrix and swollen mitochondria. Not specific for thiamine deficiency. × 26,000.

Fig. 6. Degenerating and dilated presynaptic bouton of the en passant axodendritic type. Matrix is light and mitochondria are swollen. Not specific for thiamine deficiency. × 16,000.



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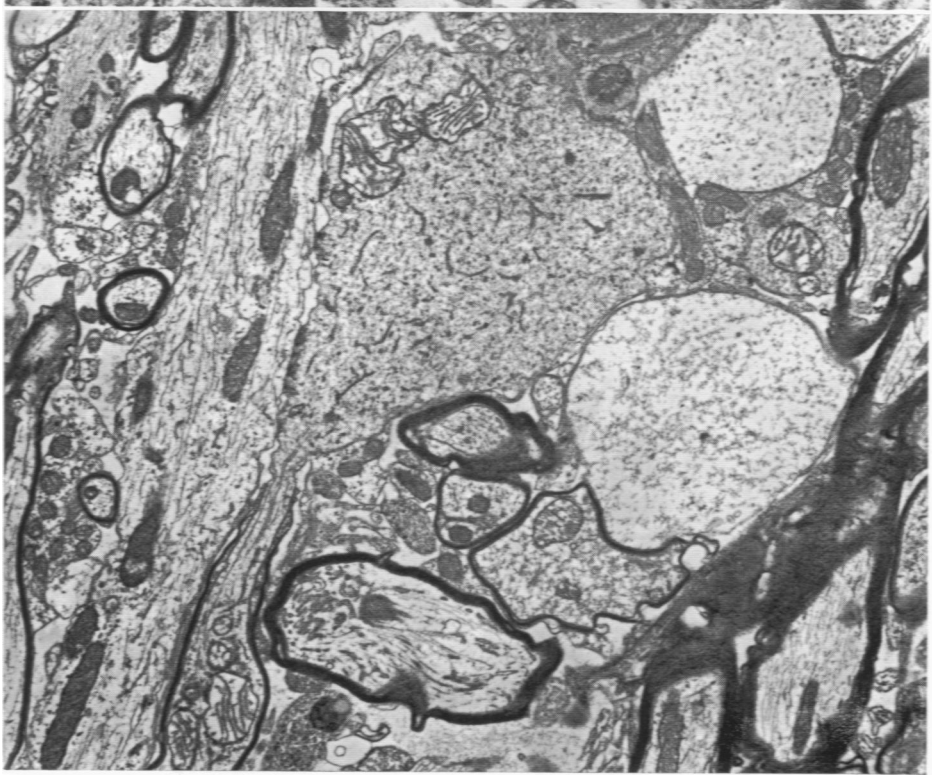

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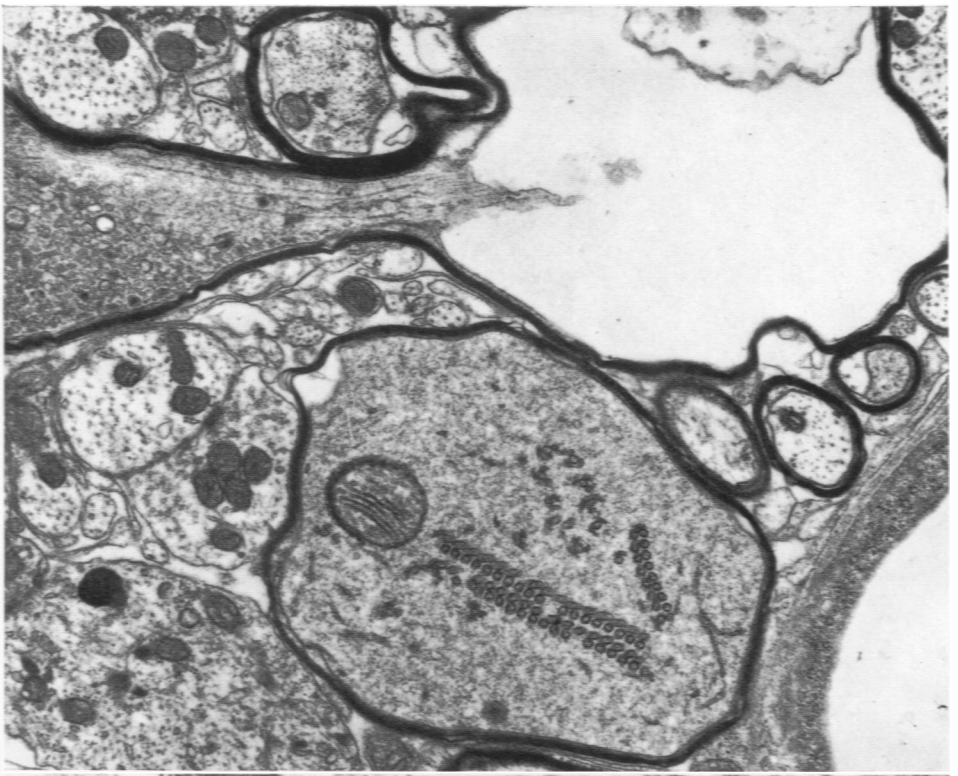

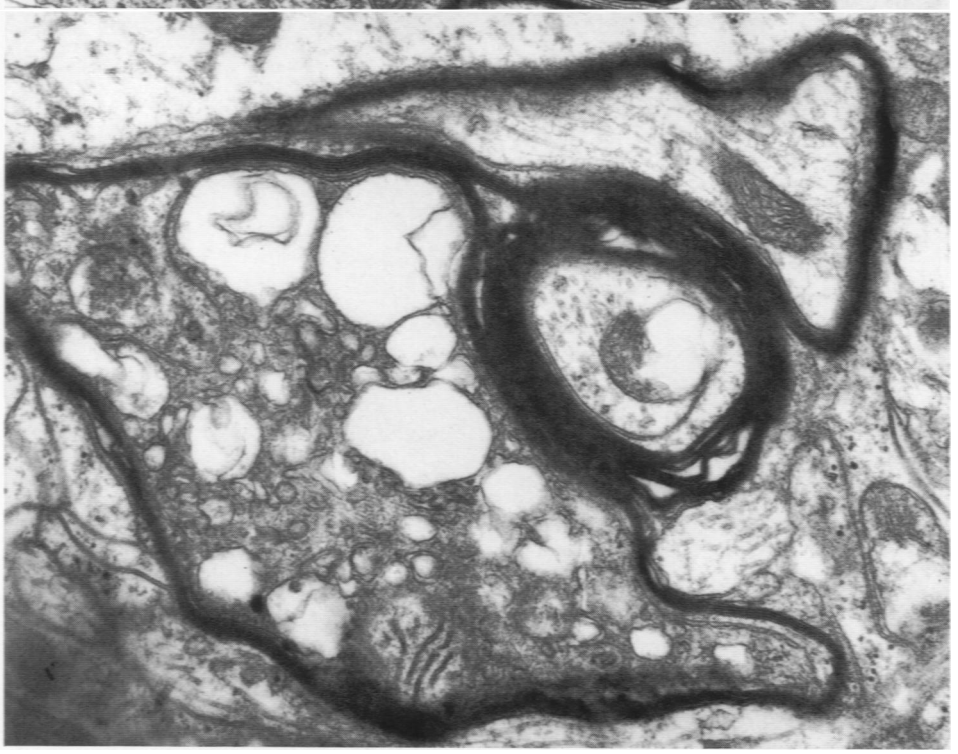

Fig. 7. Axon with thin myelin sheath, moderately dense axoplasm, and central array of banded tubules is seen at bottom, while axon with large extracytoplasmic vacuole and numerous small dense bodies is found at top left. × 16,000. **Fig. 8.** Irregular myelin sheath filled with degenerating axoplasm corresponding to early wallerian degeneration. × 22,000.