THE ULTRASTRUCTURE OF SENILE PLAQUES

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The exact nature of the senile plaque remains obscure despite the wide range of histochemical procedures that have been employed in its study. Senile plaques in Alzheimer's disease and in senile dementia are indistinguishable histologically. They are argyrophilic, periodic acid-Schiff (PAS) positive, and birefringent after staining with Congo red, as recently reviewed in detail by Margolis.[1] The birefringence of senile plaques after staining with Congo red and their positive response to other stains for amyloid has added confusion to the general problem of their pathogenesis. Indeed, it has even been disputed as to whether senile plaques are of endocellular or extracellular origin, as well as whether they are derived from neuronal, microglial or neuroglial cells. Recently we have had the opportunity to examine by electron microscopy senile plaques in a biopsy from the brain of a patient with Alzheimer's disease. It is the purpose in this paper to describe the submicroscopic structure of the senile plaque.

MATERIAL AND METHODS

A small frontal craniotomy was performed under local anesthesia. A biopsy was made of cortex with underlying white matter by sharp dissection. Part of this tissue was fixed in 10 per cent buffered formalin for light microscopy. A Bielschowsky silver impregnation was performed on frozen sections, and hematoxylin and eosin, PAS and Bodian's silver stains were carried out on paraffin sections. The remainder of the specimen was rapidly cut into blocks less than 1 mm. in diameter and fixed in Dalton's osmium chromate solution for 1 hour. Dehydration was by graded increments of ethanol, after which the tissue was embedded in Epon. Sections approximately 1 μ thick were cut with glass knives in a Porter-Blum microtome and viewed by phase microscopy to determine the site of senile plaques and their orientation to recognizable adjacent structures such as blood vessels. Contiguous ultrathin sections were mounted on uncoated copper grids and stained with lead acetate. It thus was possible to locate the previously identified plaque electron microscopically by its geographic relationships to other structures. Multiple blocks were examined in this manner by utilizing phase microscopy for precise localization and orientation of the plaques. Sections were examined with an RCA EMU-3F electron microscope.

RESULTS

Sections impregnated by the Bielschowsky silver method revealed numerous senile plaques crowded together in the cortex (Fig. 1).

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Neurons often contained the twisted strands of argyrophilic material characteristic of the neurofibrillary change of Alzheimer (Fig. 2). Senile plaques also were stained by the Bodian and PAS techniques.

In electron micrographs the neurons contained large amounts of lipofuscin pigment, and in many neurons, neurofibrils were excessively prominent (Fig. 3). These bundles of coarse fibrils arranged in parallel array pushed aside the granule-studded endoplasmic reticulum of Nissl substance. Neurofibrillary change was not limited to the cell body, but sometimes completely filled neuronal processes and even extended into axons, dendrites or terminal endings filled with synaptic vesicles. These fibrils were not only thicker than usual but were also more numerous.

Senile plaques were readily identified with the electron microscope. They were composed of a mass of twisted, interwoven, enlarged and abnormal neurite processes (Figs. 4 and 5). An occasional myelinated axon of roughly normal structure was present, as were rare glial cells. The major constituents within the plaque were neuronal processes, not cells *per se*. Processes were distinctly distended so that their diameter was considerably increased in relation to that of usual neuronal processes within the cortical neuropil. In longitudinal sections (Fig. 5) focal narrowing with globular or fusiform swellings were evident, suggesting that these were beaded fibers of the type often referred to as "torpedo." The swollen neurites were packed with a variety of bodies. Some (Fig. 4) were of indistinct outline, with an amorphous, smudged internal structure. This type of fiber was more common at the margin of a plaque. Some contained neurofibrils and dense, irregularly rounded inclusions. Most of the globular neurite processes were packed with round to ovoid dense bodies (Fig. 5) of variable size. Some of these had the configuration of mitochondria with internal cristae, and others had either lamellar internal structure or were homogeneous. Interspersed among these dense bodies in many processes were microvesicles of the type seen in synaptic endings (Fig. 6). Indeed, synaptic endings on dendrites were occasionally enlarged and filled with abnormally large and dense vesicles (Fig. 7).

Capillaries near or within the plaques (Figs. 8 and 9) consistently had a thick basement membrane. Sometimes the basement membrane not only was thickened but was tortuous. In some vessels the capillary lumen was narrowed.

Discussion

Theories concerning the structural components of senile plaques have been both numerous and diverse. McMenemey[2] has summed up current opinions in stating: "The origin of plaques has been variously ac-

credited to disease of the nerve cells, axis cylinders, all types of glial cells and their processes, and even to the deposition of abnormal products of metabolism; there is perhaps something to be said for all of them."

By electron microscopy the senile plaque is seen to be an ill-defined tangled skein of abnormal unmyelinated neuronal processes: thickened axis cylinders, abnormal dendritic processes, degenerating dendritic processes, abnormal *boutons terminaux,* and neuronal processes packed with thickened neurofibrils. These findings are in keeping with the concepts of del Rio Hortega [3] and of Soniat.[4] Rothschild,[5] although he supported the view that plaques were formed in intercellular ground substance, drew attention to the fact that they were most numerous in areas rich in unmyelinated nerve fibers. Critchley [6] also pointed out that there was no adequate explanation as to why the cerebral cortex should be the site of plaque formation almost exclusively, although he believed that their rare occurrence in the molecular layers of the white matter made a neuronal origin unlikely.

In our material numerous globular and fusiform swellings of neuronal processes were crowded with dense bodies, some of which were recognizable as altered mitochondria. Thick neurofibrils and synaptic vesicles were also present within these varicose degenerating neurites. Whether the small dense structures merely represented a phase of degeneration of mitochondria or whether they were a reflection of a combination of degenerative and regenerative changes in the neurite as well is as yet unknown. The presence of synaptic vesicles, normal and abnormal, within many of the globular processes was an indication of their neuronal origin and pointed to their terminal nature. Alternatively, it might indicate an accumulation of synaptic vesicles at a more proximal level. However, elsewhere in the biopsy specimen, abnormal and enlarged synaptic endings were present on dendrites (Fig. 7).

The possibility that senile plaques are due to an alteration in the ground substance of an unknown type, as proposed by Creutzfeld and Metz [7] and by Rothschild,[5] has become somewhat untenable because of the paucity of extracellular space in the cortex demonstrated by electron microscopy. Although there undoubtedly are variants in the degenerative processes leading to senile plaques, we have no basis on which to confirm Goodman's [8] suggestion that they are formed from the liberation of nerve cell content into the stroma. On the contrary, the plasma membranes surrounding the bulbous neurites were surprisingly intact.

The question of a relationship between amyloid and the senile plaque has been raised repeatedly.[1] It is now possible to relate the specific staining of the senile plaque for amyloid and its birefringence after ex-

posure to Congo red to the ultrastructural components. Recently a number of investigators[9,10] have demonstrated that amyloid actually is an oriented fibrillar material. Thus, it is not surprising that the parallel array of fibrils in neurons and their processes in Alzheimer's disease and in senile dementia might react in a similar manner. It is well known that such a parallel orientation of fibrils or lamellas will lead to birefringence in a variety of tissues. Thus, with the fibrillar ultrastructure of amyloid, its staining reactions and birefringence in all probability are less specific than previously believed. Direct morphologic comparison of the fibrils of amyloid and those of Alzheimer's change reveals that the fibrils in Alzheimer's disease are coarser.

Summary

Senile plaques have been examined by electron microscopy in a biopsy specimen from the frontal cortex in a patient with Alzheimer's disease. They are composed of a twisted mass of varicose neuronal processes that contain numerous small dense abnormal mitochondria, dense bodies and microvesicles. Some are filled with parallel arrays of thick neurofilaments. Capillaries have thickened basement membranes and sometimes have narrowed lumens. The parallel arrays of thickened neurofilaments are believed responsible for the positive stains for amyloid and for the birefringence of senile plaques after exposure to Congo red.

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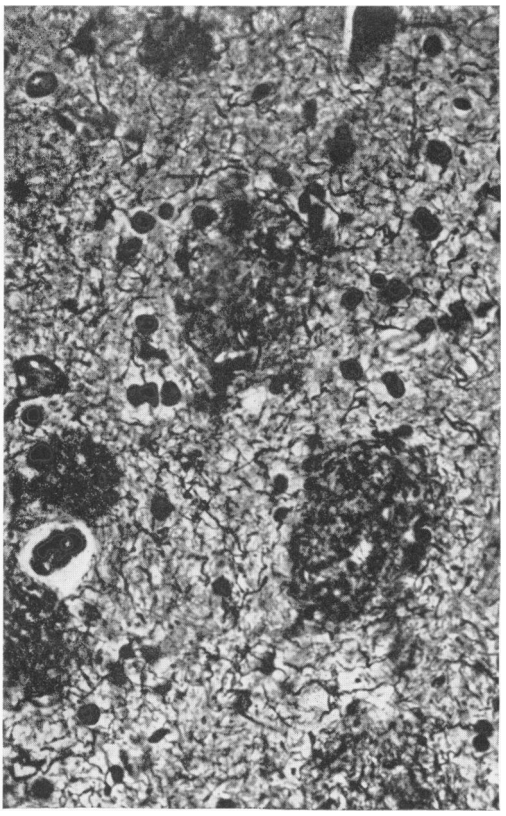
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Light micrograph to demonstrate the configuration and distribution of senile plaques in the cortex. Frozen section. Bielschowsky stain. × 300.

FIG. 2. A cortical neuron. There is a dense skein of silver-impregnated neurofibrillary material. Frozen section, Bielschowsky stain. × 800.

FIG. 3. Electron micrograph of a neuron with Alzheimer's neurofibrillary change, probably comparable to that seen in Figure 2. Nucleus, at the upper left; nucleolus, N. Dense aggregates of lipofuscin are present in the cytoplasm. At lower left arrow are thick neurofibrils arranged in parallel array. At the other arrow (upper right) a similar group of neurofibrils has been cut in cross section. × 20,000.



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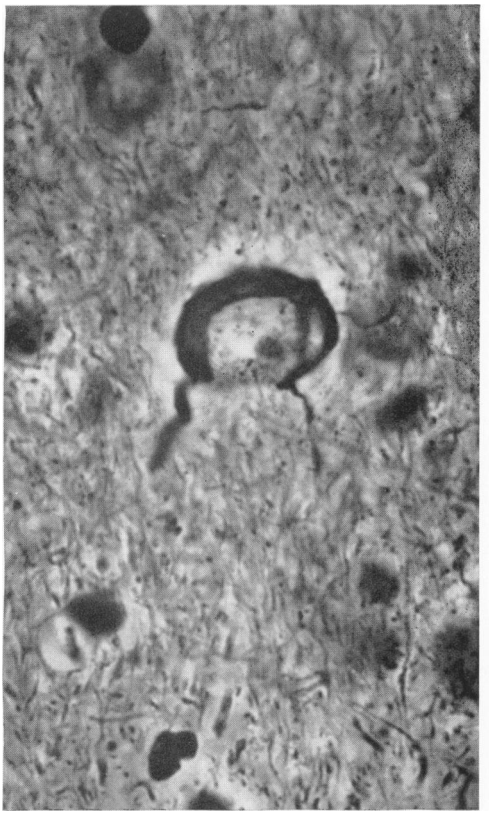

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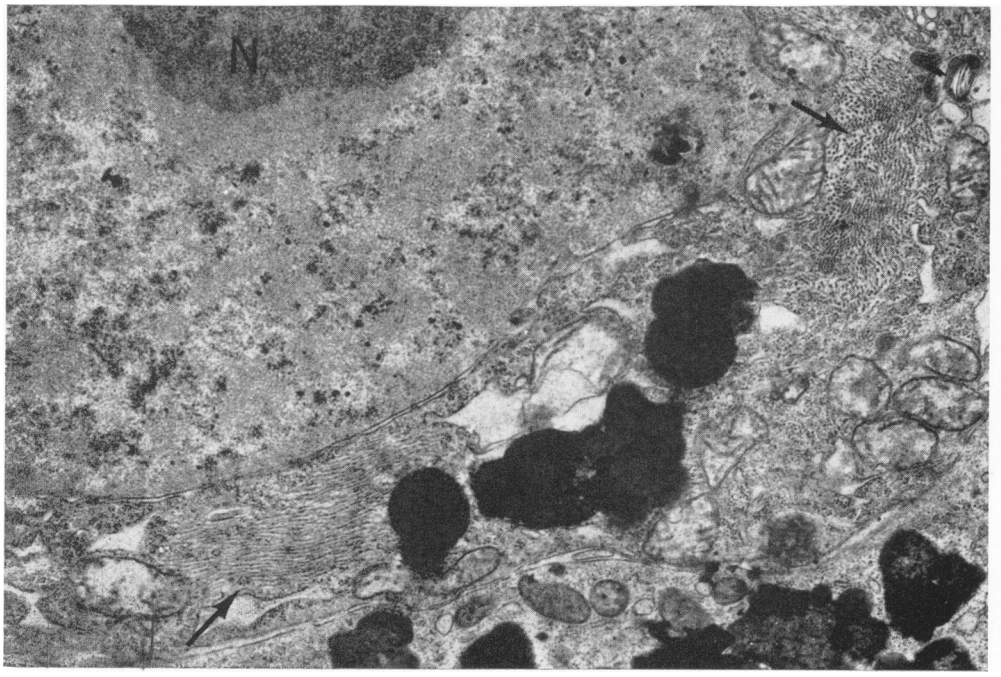

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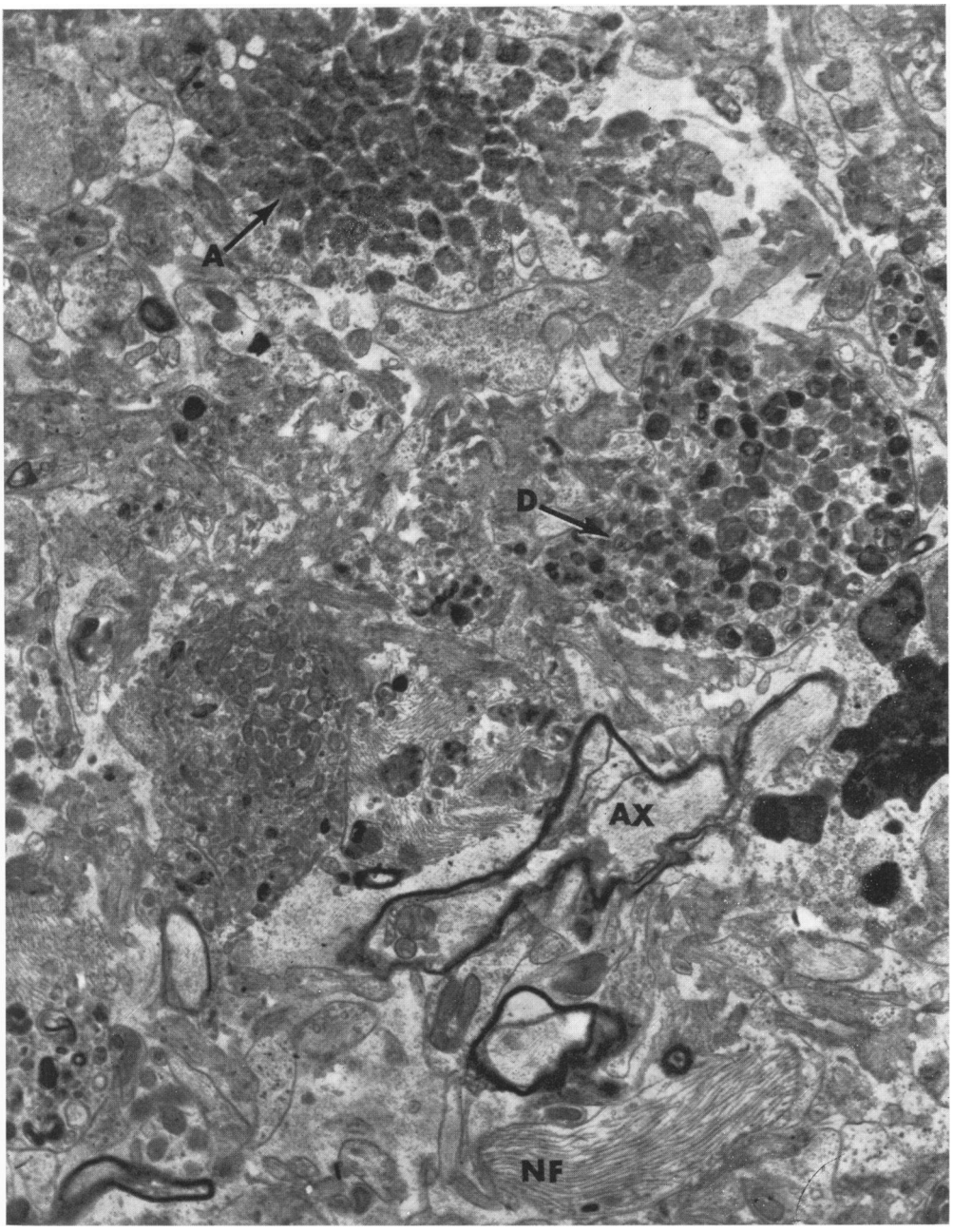
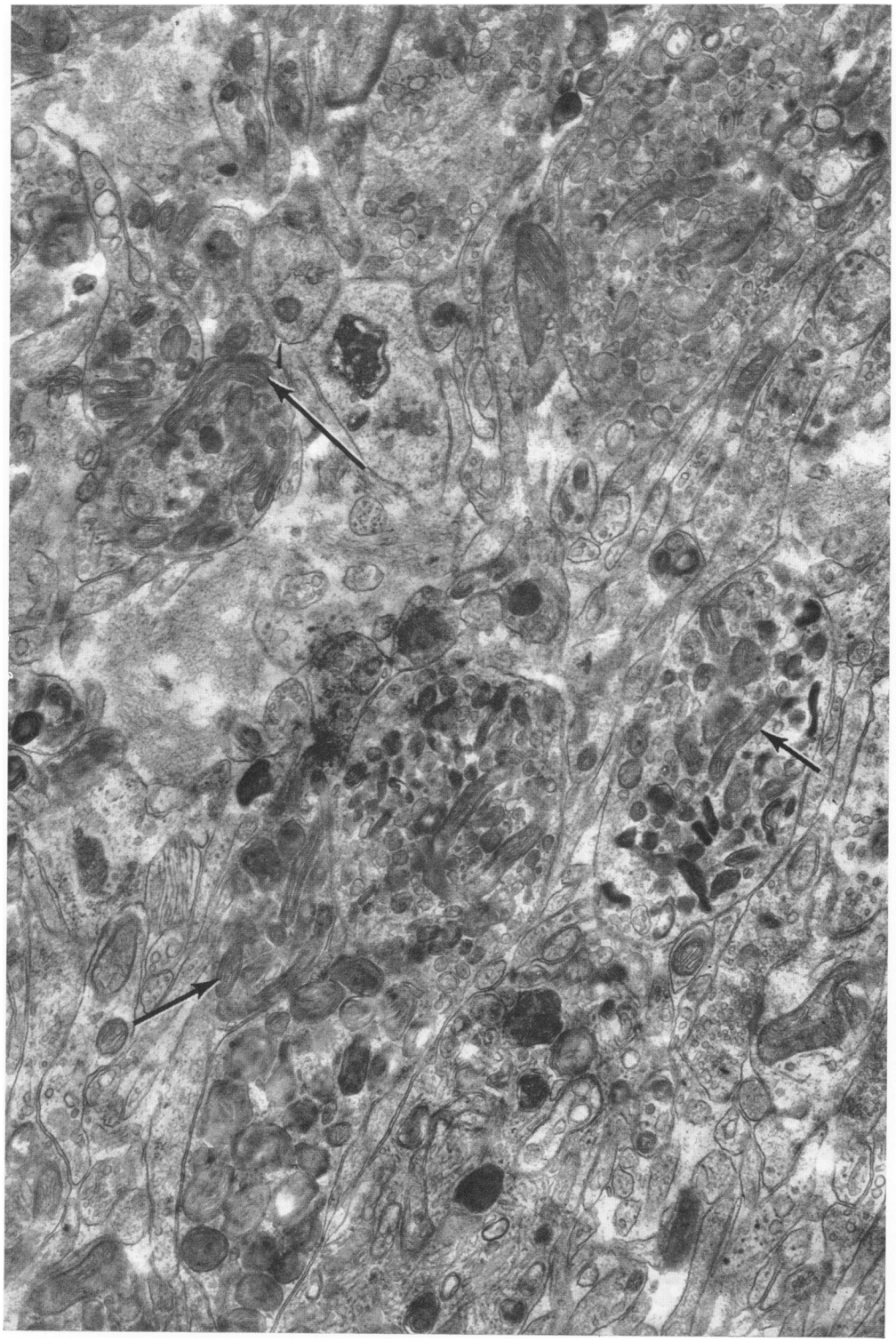


FIG. 4. Part of a senile plaque. Numerous distended neuronal processes fill the field. At the upper center (A) is a process containing poorly outlined amorphous inclusions. At the right (D) another process is filled with dense inclusions. Mitochondria are rare and indistinct. A myelinated axon (AX) is evident. At the bottom of the figure a neuronal process is filled with thick neurofibrils. × 12,000.

FIG. 5. Another senile plaque shows a variety of globoid neuronal processes. The resemblance of some of the inclusions to recognizable mitochondria is distinct (arrows). Small dense, or larger lamellar or even amorphous inclusions are also present. Interspersed among the inclusions in some of the processes are vesicles of the type usually seen in synaptic endings. × 20,000.



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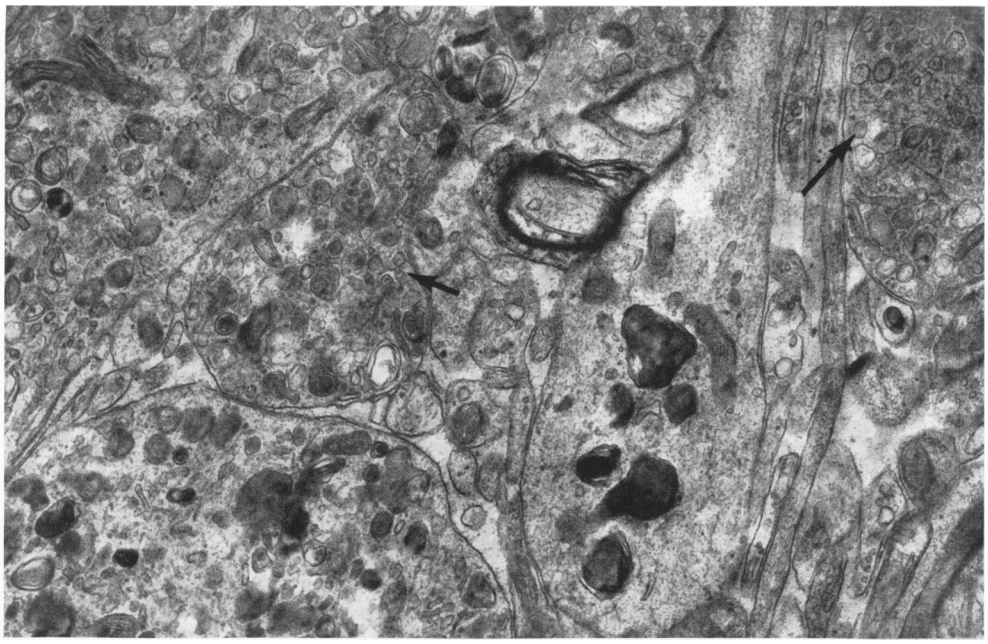

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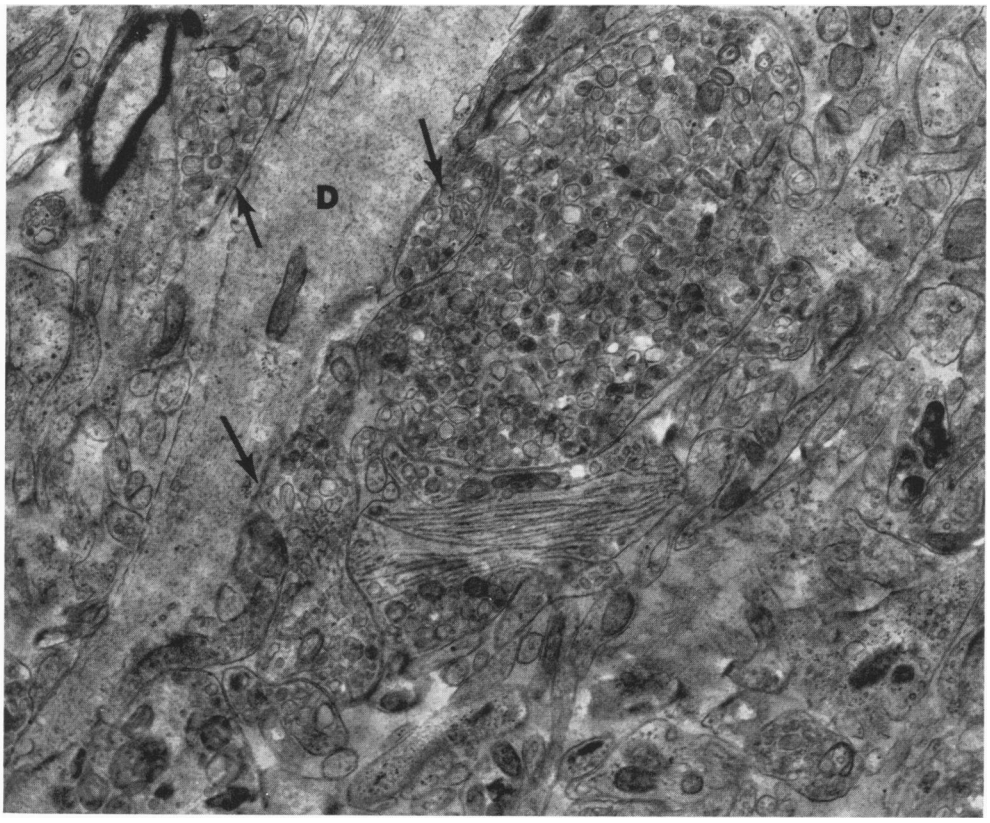

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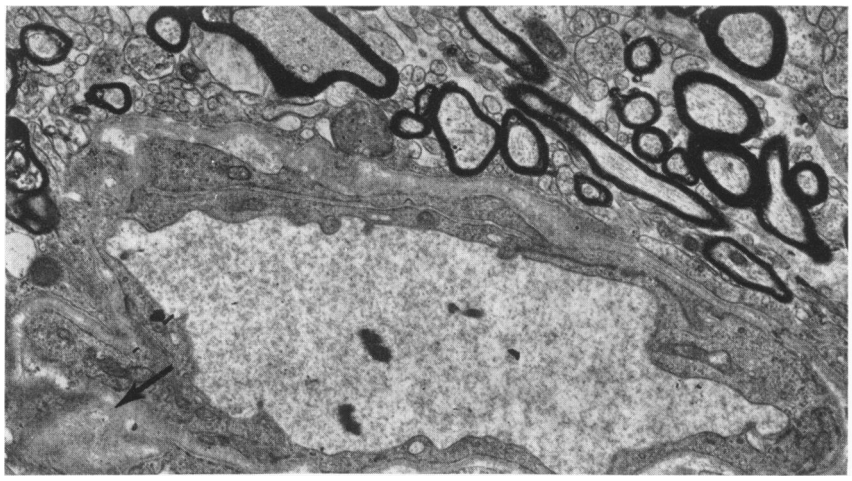

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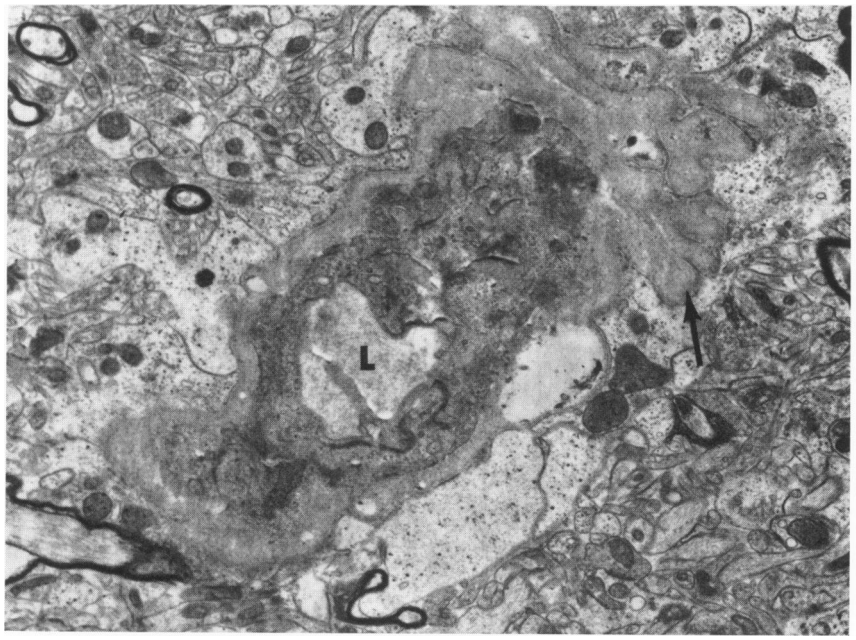

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FIG. 6. Part of a senile plaque shows synaptic vesicles (arrows) more distinctly. In other neurite processes dense inclusions and altered mitochondria are more prominent than vesicles. × 20,000.

FIG. 7. A region slightly outside of a senile plaque. A dendrite (D) extends across the field. Several synaptic endings, *boutons terminaux* (arrows), are in contact with the dendrite. Even in this region, slightly distant to the plaque, the synaptic vesicles are abnormal and thick neurofibrils are evident. × 20,000.

FIG. 8. A small capillary in the deep cortical region. Numerous myelinated axons are present. The basement membrane of the capillary is thick, especially at the arrow. × 6,000.

FIG. 9. A small capillary in the neuropil of the cortex. The basement membrane is markedly thickened and tortuous (arrow). The lumen (L) is narrowed. × 6,000.