ALTERED SYNAPTIC TERMINALS IN CORTEX NEAR TUMOR

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In a series of seven patients, surgical samples of cortex were taken at a short distance from an expanding glial tumor. This material was studied by electron microscopy in an attempt to determine the effect of mechanical pressure on brain parenchyma, with particular reference to synapses. No gross damage was apparent; edema was either absent or minimal. Two conspicuous changes in presynaptic terminals characterized the material and are the subject of this report. In addition, the material from three patients contained senile plaques which were considered to be unrelated to pressure but to correlate with the age of the patients.

MATERIALS AND METHODS

Specimens were taken from cortex excised along with tumors during surgical intervention. The tumors included five glioblastomas and two low-grade astrocytomas; they were located in frontal, temporal, and parietal lobes. Glioblastoma patients ranged in age from 56 to 72 years; the patients with astrocytomas were 44 and 48 years old, respectively. Cortical samples were removed at varying distances from the tumor—the average distance about 1 cm. No blocks were included that showed any discernible evidence of the tumor itself when viewed with the electron microscope. For purposes of comparison, cortex from the vicinity of an intracerebral hematoma in a patient 75 years old was also examined.

Samples were fixed by immersion for 10 minutes in cold 10% formalin prepared from paraformaldehyde[1] and buffered with Millonig's phosphate buffer,[2] followed by immersion for 1 hr. in cold 1% osmium tetroxide also in Millonig's buffer. They were dehydrated by passage through a graded series of alcohols, stained with uranyl acetate in absolute alcohol, and embedded in Vestopal. Sections were stained with Millonig's lead tartrate stain[3] and examined in a Philips EM 200 electron microscope.

RESULTS

Little evidence of wallerian degeneration with its characteristic synaptic changes was encountered in this survey. Two types of reaction after section of axons have been reported in the literature. One of these

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is the accumulation of neurofilaments in the presynaptic terminal.[4–10] In the present study no filamentous boutons such as those described—for example in the avian tectum—were seen. The second type of reaction is the occurrence of some or all of the following: granulation of the presynaptic cytoplasm, packing of vesicles, darkening of mitochondria, and subsequent engulfment of both pre- and postsynaptic elements by glial cells.[11–16] This spectrum of changes was observed to a limited extent in a sample of cortex from the vicinity of an intracerebral hematoma during the present investigation and even less frequently in the samples of cortex near the tumor. When these phenomena occurred, they duplicated changes described in experimental animals. The relative sparsity of wallerian degeneration effects is readily explainable on the basis of the timing involved. In experimental transection of axons the terminals are affected within 1–2 weeks, whereas here surgery took place 17 days postinjury in the case of the hematoma patient, and after 6 or more months' duration of symptoms for the tumor patients. The two major synaptic alterations which did occur seemed not to be related to wallerian degeneration.

Enlarged Terminals

The first alteration of presynaptic terminals seen in this material occurred in abundance. These endings were enlarged 2–10 times their normal size. They contained, besides synaptic vesicles and mitochondria, a variable number of tubular profiles and irregular branching cisterns appearing to be part of a continuous system. A slightly modified presynaptic terminal is seen in Fig. 1. This example contains tubular elements and a dense body. The increased extracellular space in this case is exceptional; most of such endings were situated in otherwise normal fields. The larger terminals had proportionally greater accumulations of the branching cisternae. Such an ending is illustrated in Fig. 2. Synaptic vesicles were reduced in number; they tended to be retained longest near the synaptic cleft. Dendritic endings were generally unchanged; however, in one instance branching cisternae were seen in both pre- and postsynaptic cytoplasm, and similar changes occasionally showed in myelinated axons.

This transformation may be recognized as virtually identical with that described by Gonatas and others in two cases of mental retardation[17,18] and in Alzheimer's disease.[19,20] The endings in the present material differed from those previously reported only in that forms with branching cisternae predominated and, conversely, fewer with fibrillar or tubular contents were seen. No examples of the multilamellar cytoplasmic bodies (MCB) described in Alzheimer's disease[20] were noted,

although somewhat similar dense concentric and linear lamellated figures occurred in presynaptic terminals of normal size (Fig. 3 and 4). These authors suggested two alternative explanations for the presence of enlarged altered terminals: (1) that they are causally related to the symptoms of the patient (seizures, retardation), or (2) that they represent a ubiquitous and nonspecific aspect of degeneration. The present data suggest that this range of alterations in presynaptic endings does indeed indicate a ubiquitous and nonspecific response, but that it may represent a vulnerability of the synapse to a variety of insults rather than solely to axonal injury. Degeneration of terminals is compatible with all of the circumstances under which altered terminals have so far been described, including mental deterioration from age and disease and, in this instance, physical pressure.

Spiral Lamellae

The second alteration consisted of conspicuous concentric or spiral lamellae with a periodic substructure within the cytoplasm of the presynaptic terminal. Whereas the enlarged endings just discussed were of common occurrence, these lamellar whorls were quite rare. Structures of this kind are shown in Fig. 5–8. The lamellae do not form a true spiral as myelin does, but instead occur in a random mixture of circular and spiral layers, terminating blindly. In Fig. 5 four lamellae spiral as a group. The radial spacing was fairly regular at about 360 Å. A scalloped pattern with a period of approximately 180 Å sometimes appeared along one side of each lamella. Most presynaptic endings bearing these figures were slightly enlarged and contained synaptic vesicles of normal size and spacing, although the total number of vesicles was often reduced. They were situated close to the whorls both externally and internally, and even in intermediate gaps in the pattern. Mitochondria usually appeared normal as did the synaptic junctions. A few endings showed dense bodies (Fig. 5), and occasional branching cisternae were present. Spiral structures occurred deep in the interior of the process or, equally often, immediately adjacent to the plasmalemma. In one instance (Fig. 6) a single lamella extended from a centrally situated spiral along the plasma membrane of the terminal. Whorls were sometimes seen in cytoplasm that could not be identified as to type. The only elongated example encountered in the material is shown in Fig. 7.

Details of the substructure of a lamellated whorl is shown in Fig. 8. In this presynaptic terminal the plane of the section passes in such a way that the array is seen in various aspects. At the left of the figure the lamellae are cut at right angles and form continuous lines as in the previous figures. These merge into imperfect hexagonal arrays, the latter

into paired lines spaced 140 Å apart and set at an angle to the plane of the lamellae, and then into dots, also about 140 Å apart.

It seems likely that this structure is essentially a whorled multiple version of the hexagonal arrays seen as single lamellae in septate desmosomes by Locke[21] and by others[22,23] and in the synaptic cleft striations observed by Robertson in the Mauthner cell synapses of the goldfish,[24] and that all of these correspond to the artificially produced hexagonal arrays in lipid-water systems studied by Stoeckenius.[25] Red blood cell membranes treated with saponin[26] and cholesterol monolayers treated with saponin and formalin[27,28] have also been shown to produce such figures. The dimensions reported in all of these structures have varied over a wide range. All hexagonal arrays are thought to consist of cylinders oriented similarly so that various banded patterns appear when the array is viewed from different directions. Comparison with X-ray diffraction data[29] supports the view that these assemblies represent the true configuration of the material and not an artifactually produced one.

Virtually all of the banded material reported in biological material has been seen in conjunction with plasmalemma or other normal membrane. The present figures do not involve normal membrane but are located within the cytoplasm itself, and may correspond rather closely to the arrays produced in vitro as a lipid-water interface. Whether they represent an ephemeral physiologic process or a stage in the degradation of lipid, possibly including saponification, cannot be determined at this time. The whorled figures noted by Cancilla and Zimmerman in tumor cells[30] may perhaps be the counterpart of these and, likewise, represent a stage of lipid transformation as these authors suggest.

Senile Plaques

Two of the brain specimens from tumor patients contained occasional senile plaques; the patients were 60 and 72 years of age. Cortex from the 75-year-old patient with a hematoma also showed these plaques. Their presence was assumed to be correlated with the advanced age of the patients rather than with the presence of tumor. Unusual synapses of the two types under discussion occurred in comparable numbers in patients with and those without senile plaques.

The plaques (Fig. 9) were smaller in extent than those described in Alzheimer's presenile dementia.[31–33] They were characterized by the presence of extracellular deposits of fine fibrils similar to amyloid, and many cytoplasmic profiles containing dense bodies, together with mitochondria or a variety of bodies of medium density. These areas differed from plaques of Alzheimer's presenile dementia only in their limited

extent already mentioned, in a greater admixture of normal elements throughout, and in the absence of excessive numbers or abnormal forms of neurofilaments. Dendrites with swollen mitochondria were present as were astrocytic processes containing glycogen.

Summary

Samples of human cortex from the immediate vicinity of glial tumors were examined with the electron microscope. Two alterations in presynaptic endings were observed. Enlarged terminals containing branching cisternae were common. A second infrequent modification was the presence of spiral or concentric lamellae with a periodic substructure within the presynaptic terminals. Evidence of wallerian degeneration was limited. Some of the specimens contained senile plaques correlated with advanced age.

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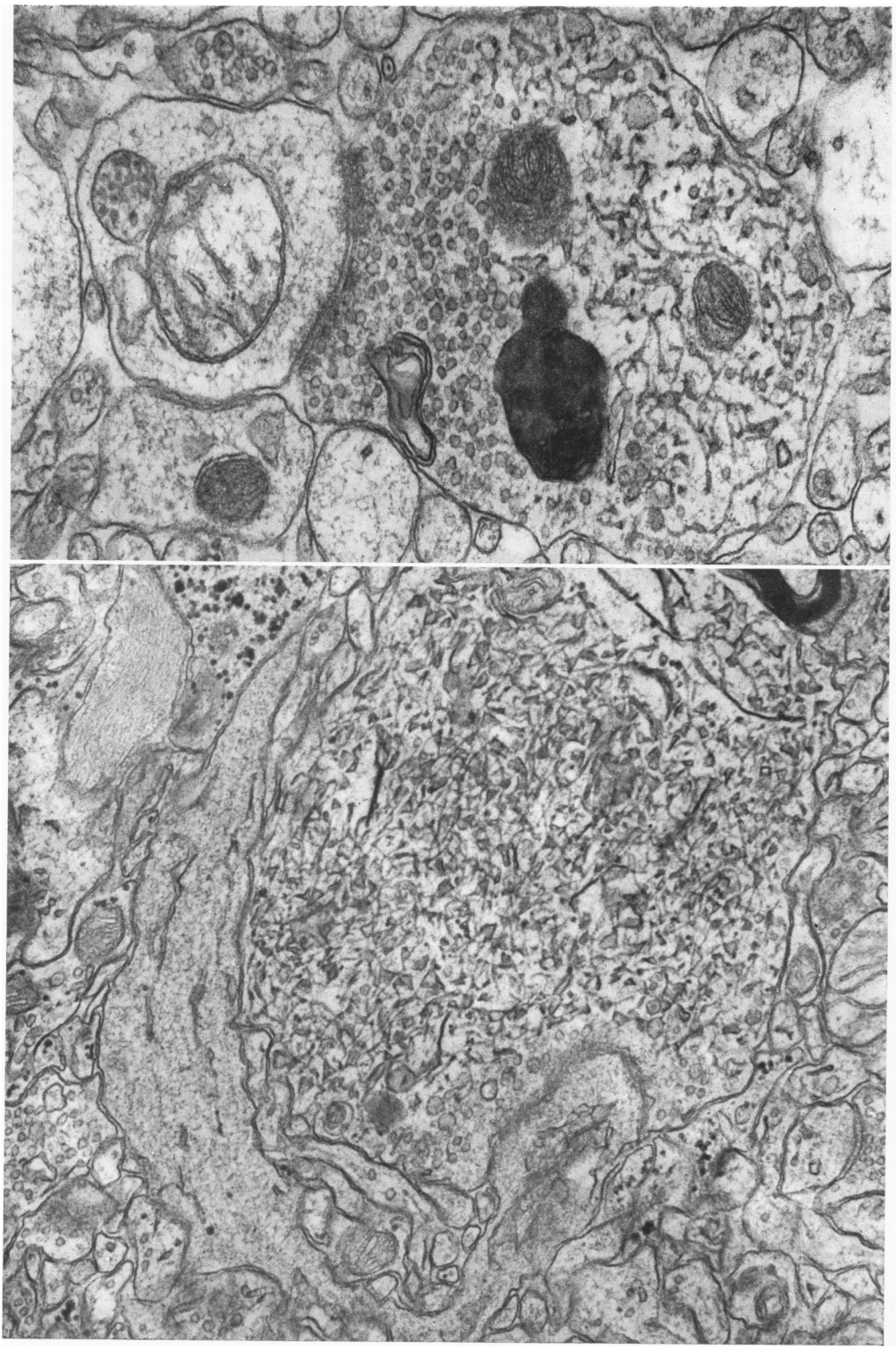
[*Illustrations follow*]

Legends for Figures

Fig. 1. Moderately enlarged presynaptic terminal showing network of tubular elements, a dense body, an irregular membranous inclusion, as well as normal components. Dendritic terminal contains swollen mitochondrion and multivesicular body. Increased extracellular space is exceptional in this material. × 37,000.

Fig. 2. Greatly modified presynaptic terminal filled with branching cisternal profiles. Synaptic vesicles are reduced in number and remain only in region of synaptic junction. × 22,500.



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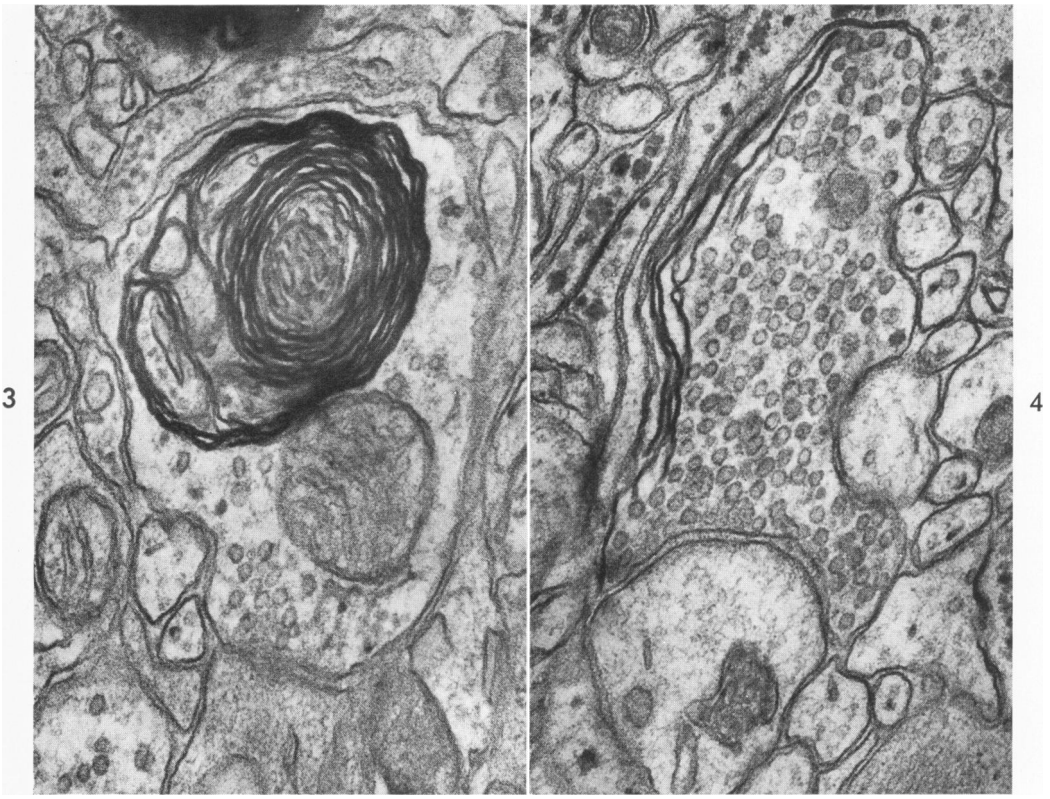

FIG. 3. Presynaptic terminal of normal size containing a dense membranous concentric inclusion. × 40,000.

FIG. 4. Presynaptic terminal of normal size containing dense linear membranous elements. × 45,000.

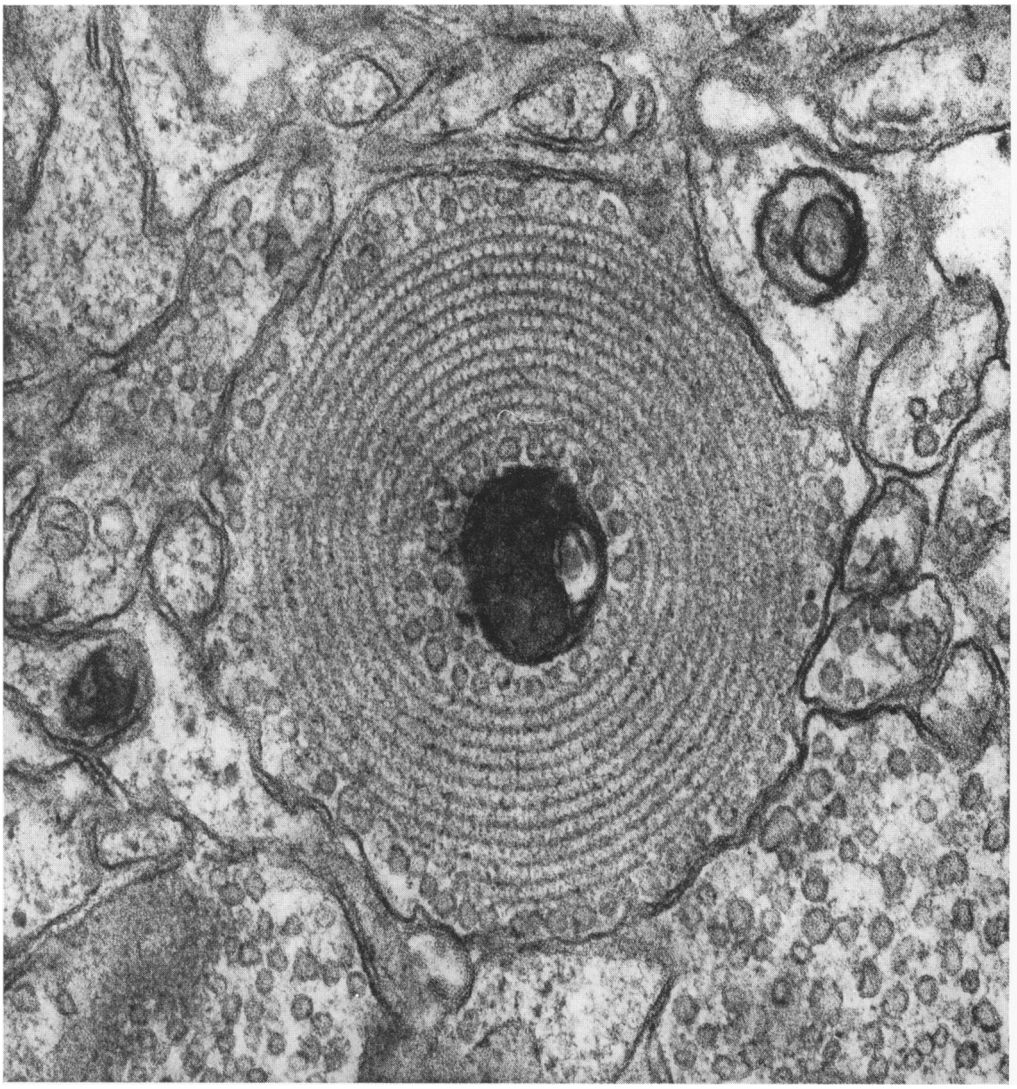

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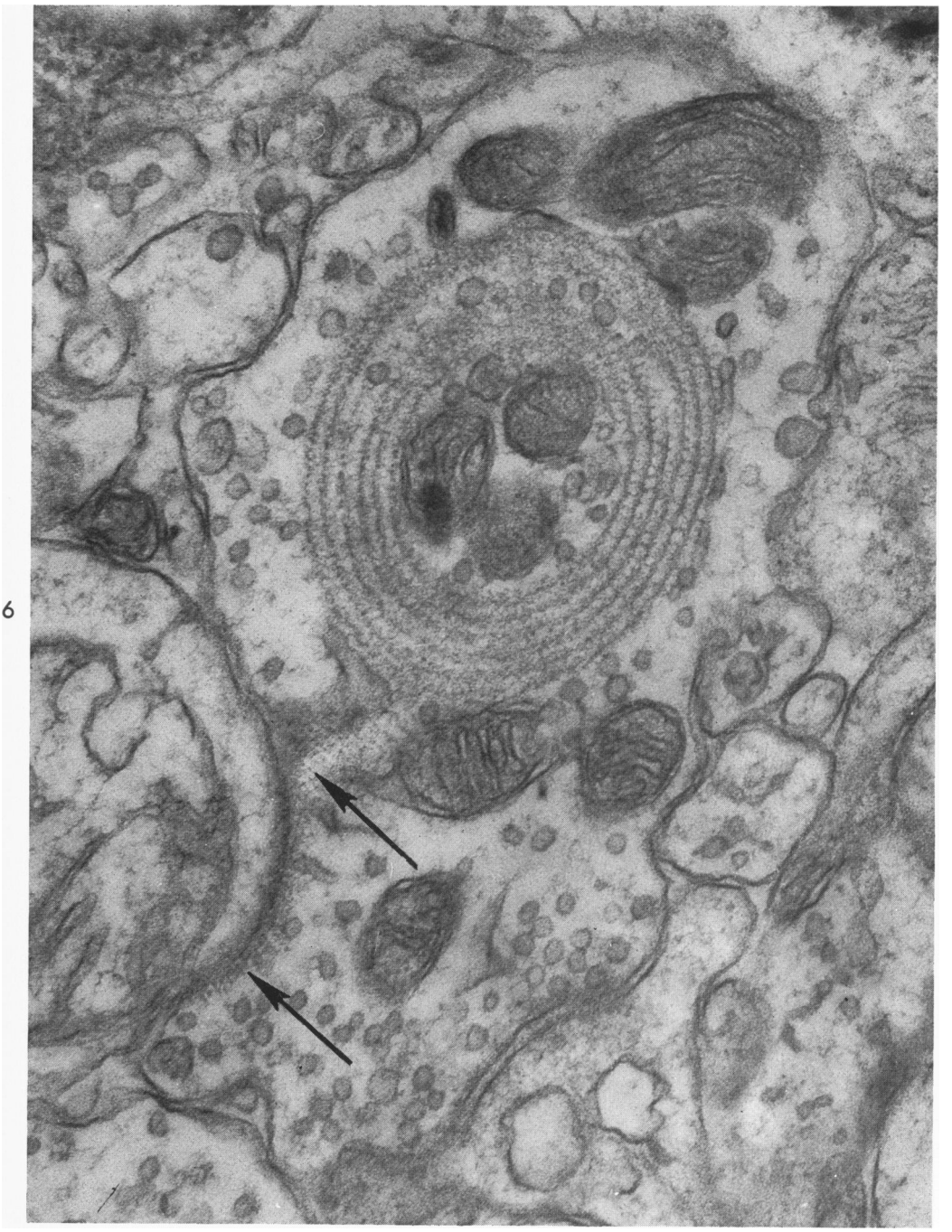
FIG. 5. Spiral figure in presynaptic terminal. It is composed of lamellae having a radial spacing of about 360 Å and a secondary pattern with a periodicity of about 180 Å. The figure is not a single continuous spiral but four lamellae spiralling together. Synaptic vesicles appear both peripherally and centrally and a large dense body occupies middle of figure. × 60,000.



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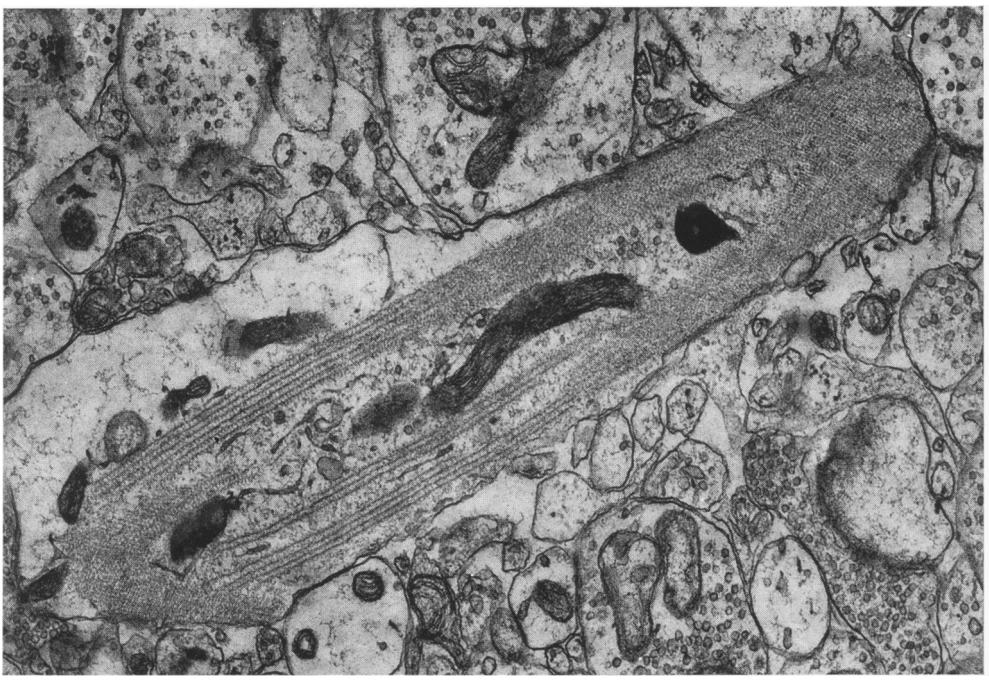

FIG. 6. Whorl in a presynaptic terminal similar to that in Fig. 5. Arrows indicate single row of striations extending from spiral to plasma membrane suggesting a lamella turned in a different plane (compare with Fig. 8). × 55,000.

FIG. 7. Only elongated figure seen in this material. Most whorls seemed to be sections of an oval structure. × 22,500.

FIG. 8. Spiral lamellar figure. Plane of section passes through curvature of whorl in such a way as to demonstrate the substructural pattern viewed in various aspects. At left, lamellae are cut at right angles and appear as parallel lines with 360 Å spacing as in Fig. 5; these merge into an imperfect hexagonal array, then into paired lines 140 Å apart, and those into similarly spaced dots (at right). × 50,000.

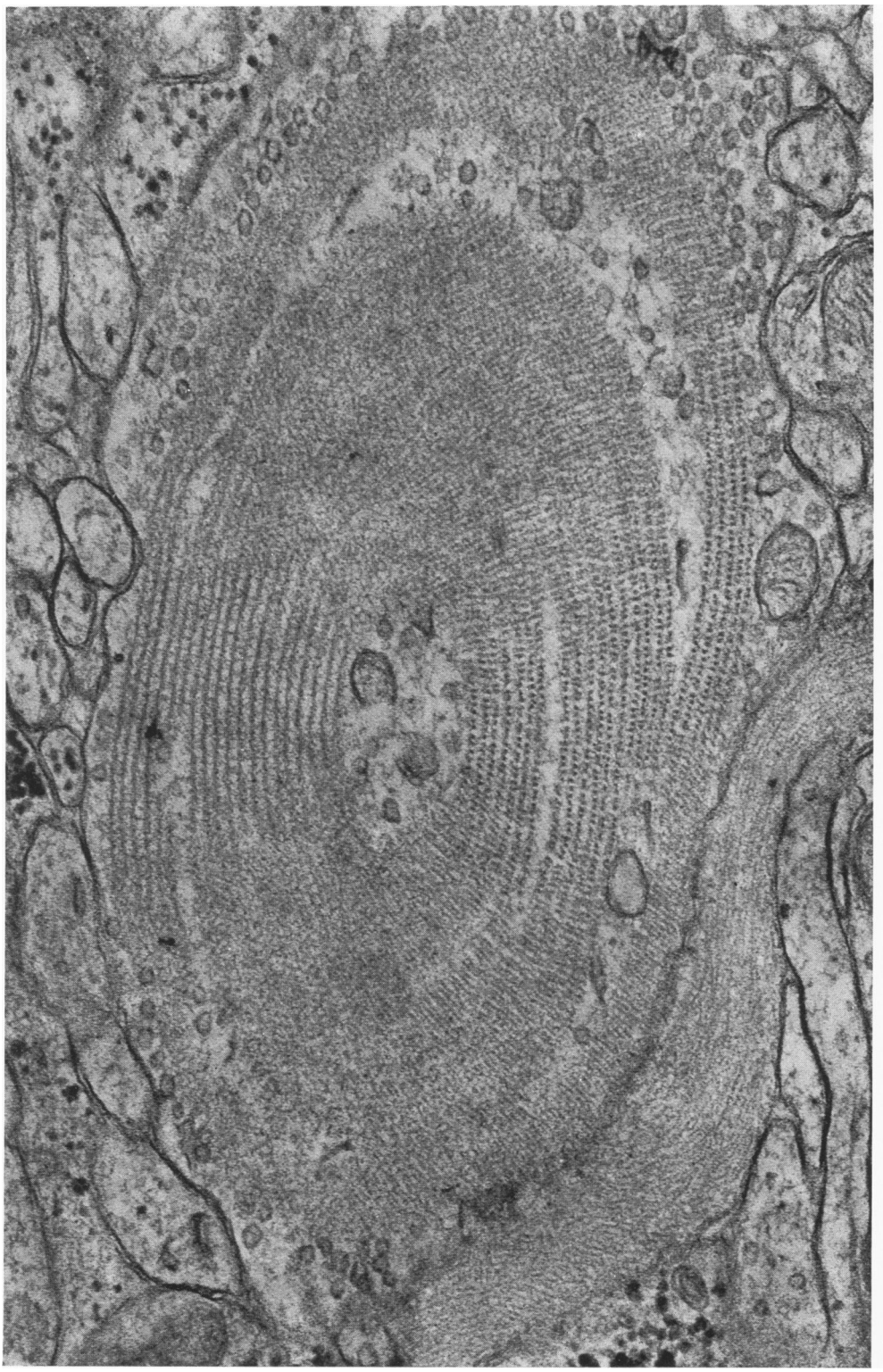

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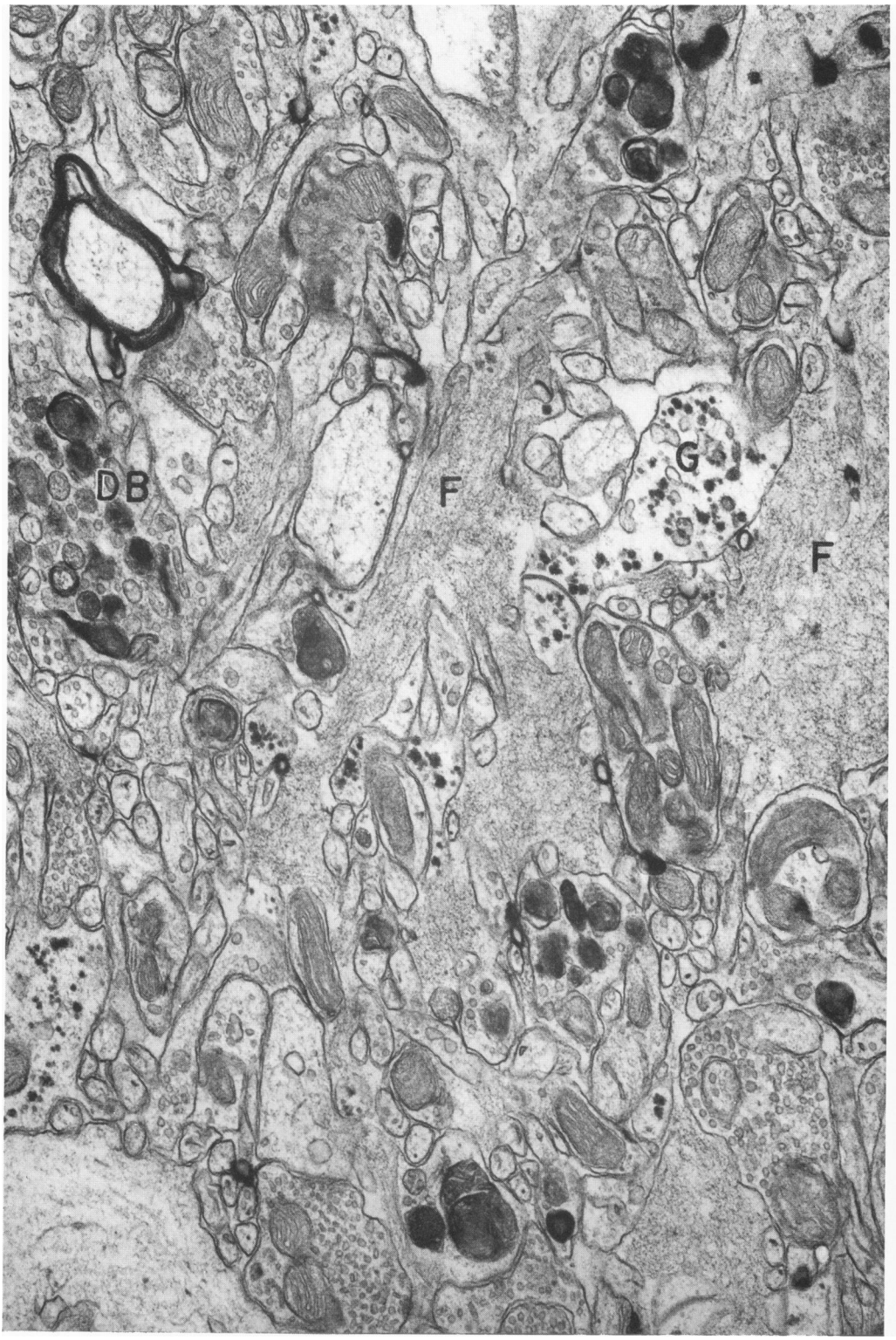
FIG. 9. Part of senile plaque. F, extracellular deposit of fibrillar material similar to amyloid; DB, dense bodies; G, astrocytic process containing glycogen. Normal cell processes and synaptic terminals are intermingled with abnormal components. × 28,000.


DB
F
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F