CONCENTRIC LAMINATION OF GLIAL PROCESSES IN OLIGODENDROGLIOMAS

DAVID M. ROBERTSON, M.D., and F. STEPHEN VOGEL, M.D.

From the Department of Pathology, Duke University Medical Center, Durham, North Carolina. Dr. Robertson's present address is the Department of Pathology, Queen's University, Kingston, Ontario, Canada

ABSTRACT

Tissues were obtained by open biopsy of a series of human intracranial neoplasms, fixed in Veronal-buffered osmium tetroxide, and embedded in Vestopal-W. In two instances in which specimens were obtained from oligodendrogliomas in regions where the tumor had infiltrated but not entirely destroyed cortical tissues, glial processes were found to be arranged in a highly organized laminar fashion. This feature was not observed in two additional oligodendrogliomas nor in other types of intracerebral neoplasms. Three types of laminar structures were recognized: (*a*) perikaryal sheaths composed of several layers of overlapping or concentrically orientated glial processes, (*b*) layers of longitudinally orientated glial processes along the outer aspect of myelinated axons, and (*c*) small laminated figures composed of several concentrically disposed glial processes. Spirally constituted lamellar systems were not demonstrated. These findings indicate that under certain circumstances glial cells have the capacity to form sheaths and sheath-like structures by concentric lamination of several processes, rather than by spiraling of a single process.

INTRODUCTION

It is generally recognized that myelination in the central nervous system occurs in relation to multilayered periaxonal sheaths of glial cytoplasm, but the mechanism by which the sheaths are formed and encircle the axon has not been determined. A principal difficulty in this respect is the technical problem of obtaining for electron microscopy a well preserved sheath in which the disposition and relationship of the very thin myelin lamellae may be visualized.

In two oligodendrogliomas occurring in human beings, areas of cortex infiltrated by neoplastic cells were examined by electron microscopy. It was observed that glial processes were highly organized in complex sheaths and sheath-like structures, the layers of which were considerably less attenuated than those of compact myelin, thus permitting demonstration of certain features of their organization and formation.

MATERIALS AND METHODS

This study was part of a larger project in which portions of twenty-five human intracranial neoplasms have been examined. Four of these were intracerebral oligodendrogliomas; in three, blocks were taken from regions of cerebral cortex infiltrated by tumor, and two of the latter exhibited the findings to be described herein.

Portions of tissue were obtained by open biopsy, placed immediately in cold Veronal-buffered 2 per cent osmium tetroxide, and rapidly cut into small fragments which were placed in fresh fixative for 1 to 2 hours. The tissue was then dehydrated in graded alcohols, infiltrated with styrene followed by Vestopal-W, and embedded in Vestopal-W, using

the technique recently described by Kurtz (6). Thin sections were cut on a Servall Porter-Blum microtome, mounted unsupported on 300-mesh copper grids, stained for 1 to 4 hours with 2 per cent aqueous uranyl acetate, and examined using a Hitachi HU-11 electron microscope.

Two-micron sections from Vestopal blocks, stained with thionine-azure B (7), were used for orientation. Frozen and paraffin sections were made for diagnostic purposes and to study the distribution of the tumor in relation to surrounding cerebral tissue.

OBSERVATIONS

The structures to be described were found in two oligodendrogliomas in areas of cortex infiltrated by tumor cells. They were not seen in the more central areas of the same tumors in which preexistent neural tissue had been destroyed, nor were they found in two additional oligodendrogliomas nor in several examples of astrocytoma and glioblastoma multiforme.

By light microscopy (Fig. 1) there was obliteration of normal cortical architecture by numerous small, fairly uniform cells with spherical, deeply staining nuclei and moderately abundant cytoplasm which, in routine paraffin sections, appeared relatively clear, producing the "box" effect generally considered significant in the histological identification of oligodendrogliomas (8, 9). Scattered among the tumor cells were a few surviving neurons which were often hyperchromatic and contained abundant lipochrome. There was a definite tendency for tumor cells to aggregate around the neurons. Scattered myelinated axons were also present.

By electron microscopy it was found that, in many areas, glial processes were arranged in a highly organized fashion. This could be recognized to a limited extent by light microscopy of Vestopal-embedded material (Figs. 2, 3), but was not demonstrated in paraffin sections. For descriptive purposes, these glial structures will be divided into three types, although they are probably closely related:

1) Pericelullar Lamination of Processes

The majority of the ensheathed cells were identified as neurons by virtue of their size, abundant endoplasmic reticulum with large cisternae, and numerous lipochrome granules. Occasionally both nucleus and cytoplasm were very dense, corresponding to light microscopic findings of cell shrinkage and hyperchromasia with nuclear pyknosis. The features of some of the smaller ensheathed cells did not permit positive identification, and, although their structure was often suggestive of their being small neurons, a glial origin could not be excluded in other instances.

Surrounding or partially encircling these cells was a series of glial processes, some of which originated in a glial cell in the plane of section

FIGURE 1

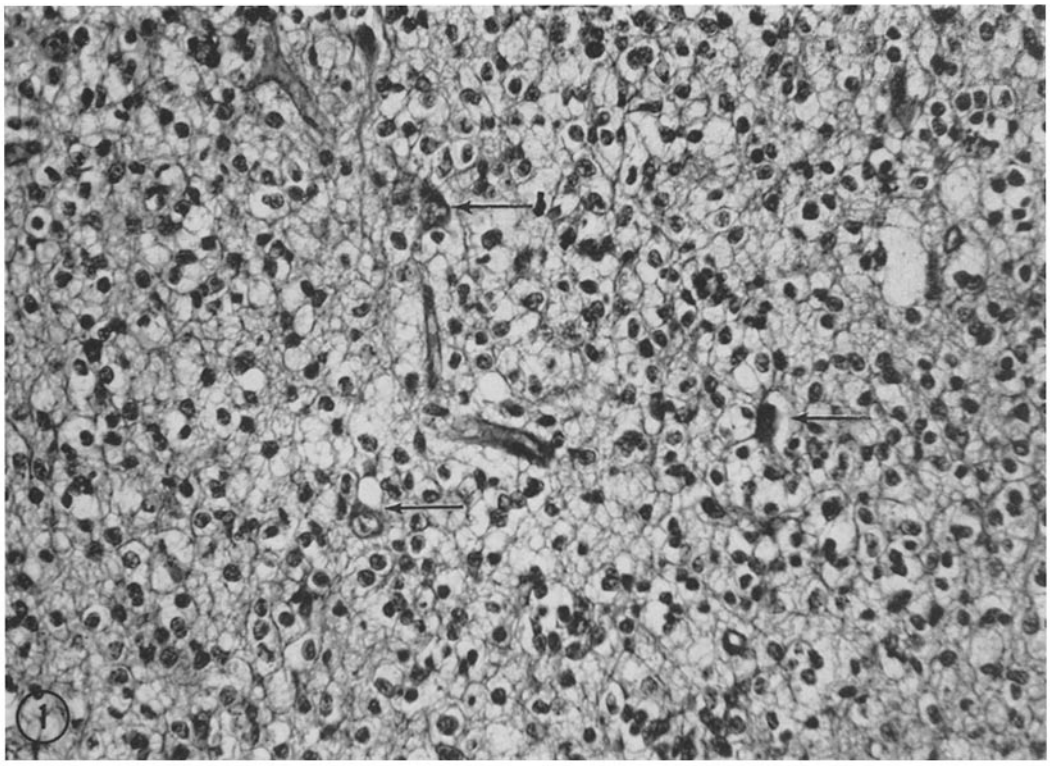
From a portion of cerebral cortex heavily infiltrated by an oligodendroglioma. The tumor cells have dense spherical nuclei and abundant relatively clear cytoplasm. A few hyperchromatic pyramidal neurons are present among the tumor cells (arrows). Paraffin, PAS. × 400.

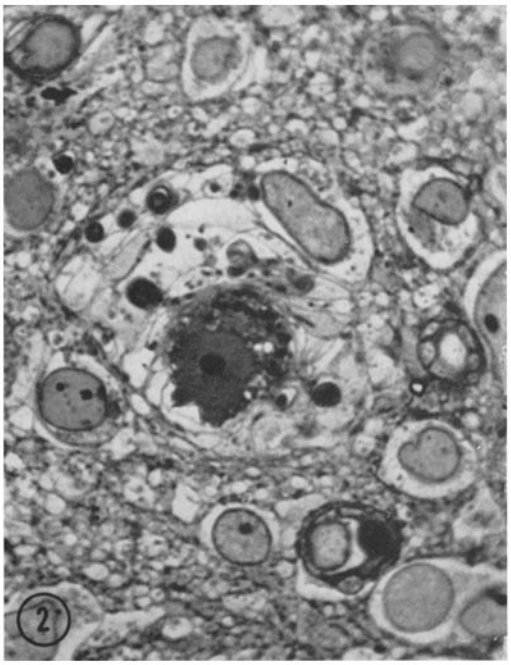
FIGURE 2

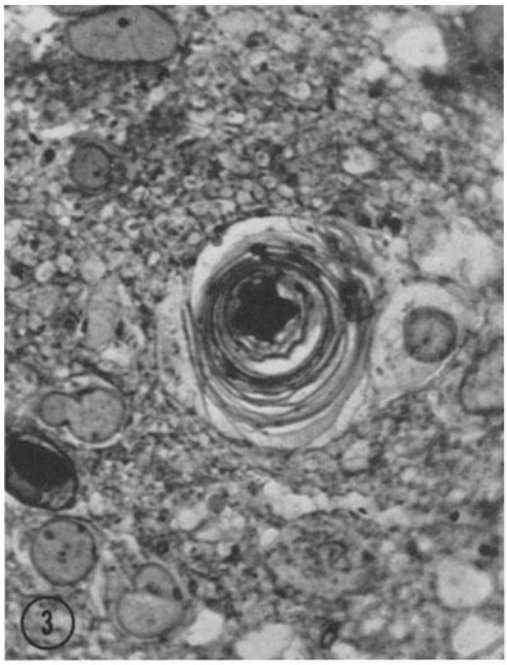
From an area of cortex comparable to that shown in Fig. 1. In the center is a neuron with spherical nucleus, prominent nucleolus, and dense cytoplasm containing lipochrome pigment (black granules). A series of lightly stained overlapping processes form an eccentric sheath around the neuron. Within the sheath are several small dark spherical bodies which correspond in size and position to the small concentric laminated bodies seen by electron microscopy. The surrounding tissue contains several glial cells, the cytoplasmic density of which is similar to that of the sheath. Vestopal, thionine-azure B. × 1450.

FIGURE 3

A laminated sheath surrounds an irregular dense mass of uncertain origin. Several shorter processes and a large glial cell are present on the outer aspect of the sheath. Vestopal, thionine-azure B. × 1450.







(Fig. 4). The innermost processes were, in general, regularly arranged and exhibited considerable attenuation of their cytoplasm, while the more external layers contained more cytoplasm and were more randomly distributed (Fig. 5). Between adjacent processes there were narrow extracellular spaces of relatively constant width, bounded on either side by osmiophilic cell membranes; in regions where the cytoplasm became greatly attenuated, the sheaths usually appeared as a series of closely spaced lines alternately separated from one another by the extracellular spaces and attenuated cytoplasm of the processes (Fig. 6).

While some cells were ensheathed by many overlapping elongated glial processes, which either encircled the cell or covered a portion of its circumference (Fig. 4), others appeared in the plane of section to be completely surrounded by a series of circular cytoplasmic lamellae clearly arranged in a concentric rather than spiral fashion (Fig. 7). Outside of the complete lamellae, one or more incomplete layers composed of several shorter processes were commonly present (Fig. 7). Occasional sheaths contained as many as twenty to twenty-five lamellae (Fig. 8).

Occasionally several sharply localized constrictions, bridged by poorly defined osmiophilic material, were present along the processes (Fig. 7).

2) *Lamination of Glial Process in Relation to Myelin Sheaths (Fig. 10)*

A few large myelinated axons persisted in the involved cortex; the myelin sheaths were irregular in contour and partially disrupted. In longitudinal sections, parallel processes were distributed along the outer aspect of some of the sheaths. The inner processes were again greatly attenuated and closely applied to the sheath, while the outer ones had abundant cytoplasm with mitochondria and other cytoplasmic organelles. As in the case of the perikaryal sheaths, the inner layers were continuous over long distances, while outer layers were made up of several shorter processes more randomly distributed. No opportunity was presented in this material to study these lamellated processes surrounding a myelinated axon cut in cross-section.

3) *Small Laminated Figures with Indeterminate Nidus*

These were very numerous throughout sections of both tumors. Although they had no distinctive or consistent association with other structures they were often located in or near the outer layers of perikaryal sheaths. They were identified by light microscopy of Vestopal-embedded tissue as small dense spheres (Fig. 2).

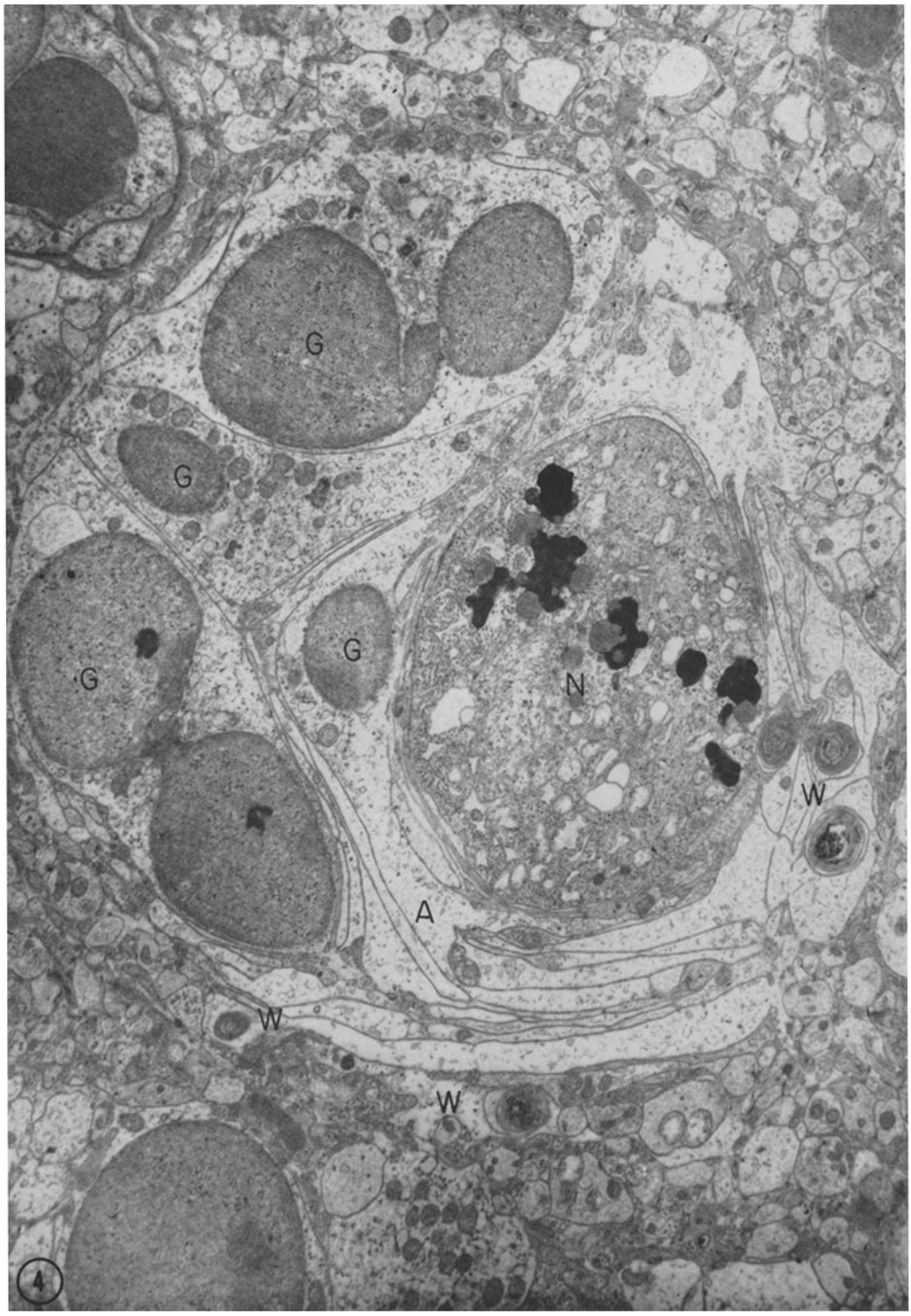
Although the centers of these figures were clearly of cytoplasmic origin, it was not possible to determine their source. Some possessed cytoplasmic organelles (Fig. 9) but others were relatively structureless (Fig. 11). Glial cytoplasmic processes were arranged as a series of lamellae around the centers, generally with greater attenuation of the inner layers.

Most of the figures were formed of three to fifteen circular concentric layers, related externally to a series of partially encircling processes (Fig. 12). In other instances there was a hilum containing a cytoplasmic process entering the figure to become continuous with one of the inner layers; the more external lamellae were reflected at the hilum (Fig. 13). The latter were clearly continuous with glial processes in the surrounding tissue.

When several figures were closely associated, a process that formed a lamella of one often surrounded or partially overlapped a second, forming, in effect, a larger laminated figure with two centres. Occasionally a single process surrounded as many as four or five small laminated structures (Fig. 11).

FIGURE 4

The cytoplasm of a neuron (*N*) is recognized by virtue of its abundant RNA, prominent ergastoplasm, and the presence of lipofuscin granules. Surrounding it is a sheath composed in part of the cell bodies of four glial cells (*G*), and in part by a series of large, somewhat irregularly arranged cytoplasmic processes. At *A*, one process branches to enclose a group of processes. Process *A* is clearly continuous with the cell body which contains a tangentially sectioned nucleus. Several small laminated figures (*W*) are located within or adjacent to the sheath. The surrounding neuropil has retained its compact structure. × 7,000.


G
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G
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N
W
A
W
W
4

DISCUSSION

These specimens, derived from human cerebral cortex infiltrated by oligodendrogliomas, contained several morphological types of highly organized lamellae formed of glial processes. The deeper regions of the same tumors were devoid of these structures, suggesting that they require pre-existing neuronal elements for their formation, and that they are formed rapidly and are subsequently destroyed by compact tumor invasion. It was not possible to determine with certainty whether the structures in question were formed by the tumor cells themselves or hyperplastic glia reactive to the invading neoplasm; however, since they were not found in other types of glial neoplasms, some of long duration, the latter seems less likely.

The lamellae described herein were sufficiently large to provide visual evidence that they were derived from several processes arranged in an interdigitating or concentric fashion rather than from a single spirally orientated process.

There is a suggestion that the long perikaryal cytoplasmic lamellae may have arisen as a result of rearrangement and fusion of processes; the outer layers of the sheaths contained many short, somewhat randomly arranged processes while the inner layers tended to be more orderly and extended for considerable distances, often completely encircling the cell. Some lamellae exhibited a series of localized constrictions which perhaps represent sites of fusion.

Many of the small laminated figures were formed internally of complete, circular lamellae which were partially enclosed externally by one or more processes disposed about the circumference, suggesting that additional circular layers could result from circumferential extension and fusion of the tips of the outer processes. Others were formed, not of circular lamellae, but of processes which entered at a hilum and extended into the surrounding tissue. This raises the possibility that many, if not all, of the small laminated figures were similarly constituted and only occasionally did the plane of section pass through the hilum.

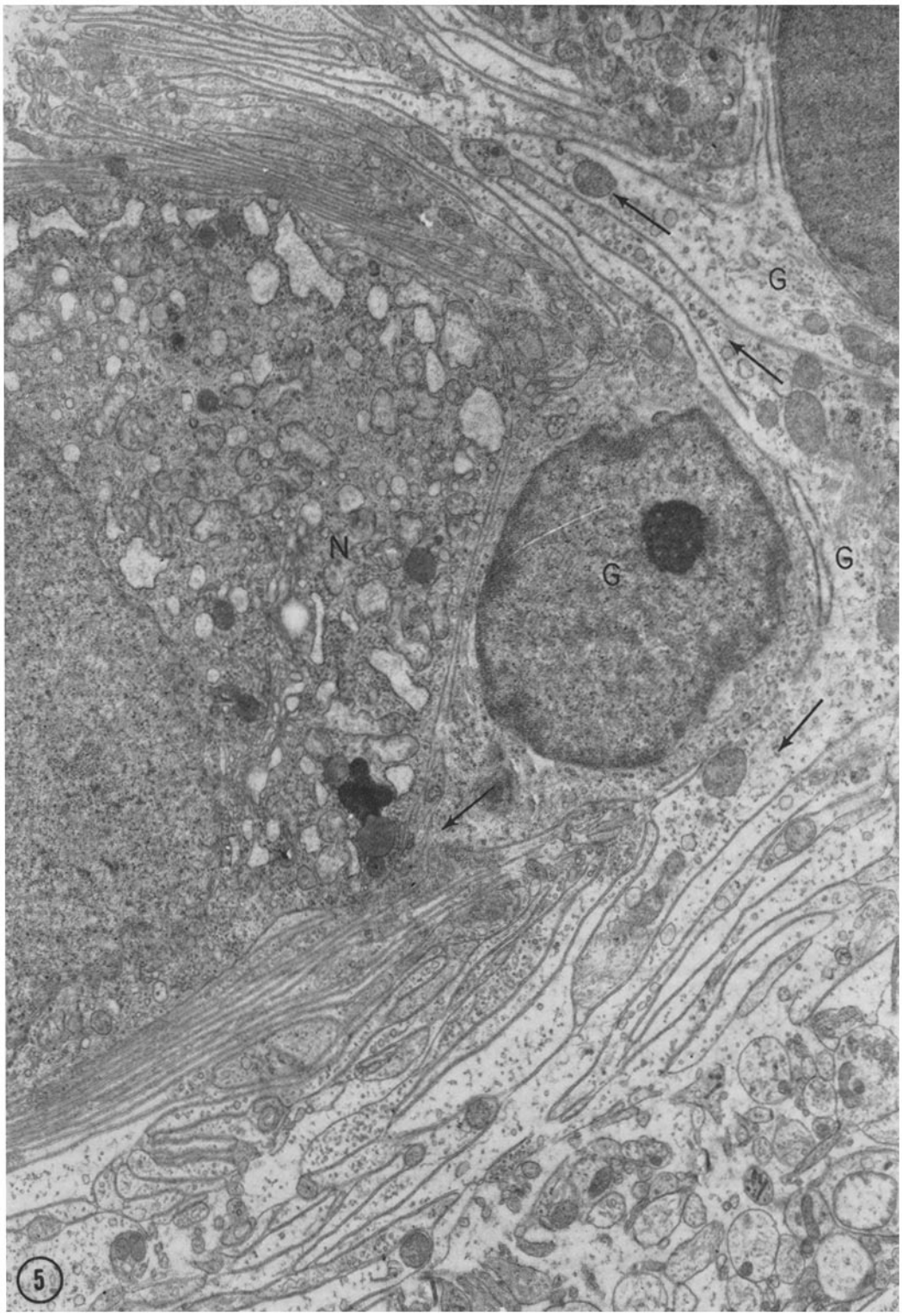
Scharenberg (10) demonstrated by means of silver impregnation of cerebral cortex infiltrated by oligodendrogliomas that neoplastic glial cells developed highly branched processes which formed networks about neurons and axons. It was noted that while these "satellite systems" were commonly seen in oligodendrogliomas they were restricted to small foci within the grey matter. Seemingly these findings correspond to those under present consideration. Luse (11) found "folding and duplication of the plasma membrane" in a glioblastoma multiforme, but the illustration (Fig. 10, *op. cit.*) does not make it clear whether this is actually lamella formation.

Certain morphological similarities exist between the perikaryal sheaths described herein and Schwann cell sheaths about neurons in the eighth nerve ganglia as described in goldfish (12) and rats (13). In the latter, complex perikaryal sheaths were formed of many overlapping processes originating from adjacent Schwann cells. The processes were not entirely regular in arrangement but often ended randomly within the sheath; some reversed direction; others branched, and a few seemingly fused with one another. Variable degrees of cytoplasmic attenuation were manifest and many formed segments of true compact myelin. Since the two types of sheath, compact myelin and lamellated cytoplasmic processes, were closely related, the term "loose myelin" was applied to the latter. The perikaryal lamellar sheaths described herein displayed many of these features, a major difference being that there was no evidence of compact myelin formation even in regions where cytoplasmic processes were greatly attenuated. Concentric perikaryal sheaths such as occurred in tumors under consideration were not described in the eighth nerve ganglia.

It would be of interest to know what relationship, if any, the sheaths described herein have to myelin. Initial studies suggested that myelin sheaths were formed by layering and fusion of several glial processes (1), or by formation of membranes within the cytoplasm of a single glial cell (3). More recent work has indicated that the laminated sheath is formed by the spiraling of a

FIGURE 5

A neuron (*N*) is ensheathed by numerous overlapping processes, some of which originate in the three glial cell bodies (*G*) in the plane of section (arrows). The processes nearer the neuron are attenuated and quite regularly arranged, while those further out are wider, shorter, and more randomly disposed. × 10,000.


G
N
G
G
5

single glial process about an axon in a manner analogous to that occurring in the peripheral nervous system (4, 5, 14). There is no doubt that in early myelinization simple spirals of one or two turns have been demonstrated. Although in fully developed sheaths the mesaxons may be seen and counts of lamellae in various sectors of a sheath are often, but not always, compatible with a spiral arrangement, it has not been technically possible to actually trace the attenuated layers of myelin around their entire circumference to determine unequivocally whether a continuous spiral is actually present. Recently Ross *et al.* (2) found in tissue cultures that some of the myelin lamellae were formed by spiraling of a single process, but others by the apposition of additional glial processes, indicating that the actual mechanisms of myelinization may be relatively complex. On the other hand, findings in tissue culture may not be entirely applicable to the normal brain.

Since the sheaths described herein were devoid of myelin there is no direct evidence that they have any relation to the process of myelinization. However, it seems reasonable to suspect that the remarkable tendency of glial cells in these tumors to form concentric sheaths could be a reflection of the capacities of their progenitors.

Laminated perikaryal glial sheaths have not, so far as we are aware, been described in normal cerebral cortex. It is of interest that Schultz and Pease (15) found that in resolving stab wounds of rat cortex thin myelin sheaths appeared around the bodies of reactive glial cells. Since the sheaths found in the tumors described herein were principally around neurons and did not contain demonstrable myelin, the relationship between the two is uncertain. It would seem, however, that the formation of perikaryal sheaths may be a distinctive pathological reaction worthy of further study.

These findings demonstrate that glial cells have the capacity to form, under certain circumstances, concentrically orientated sheaths and laminated structures derived from several glial processes. The significance of the observations in relation to formation of sheaths in the normal brain, and in terms of pathological reactions, is unknown.

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FIGURE 6

Detail from the upper portion of Fig. 5. The cell membrane enclosing each process appears as a single dense line; between adjacent processes is a narrow, relatively uniform extracellular space. Near the neuron (*N*), where the processes are very narrow (*1*), the width of the process is similar to that of the extracellular space, creating the appearance of a series of equally spaced lines; however, several of the processes can be followed to more expanded portions. Some processes appear as flat lamellae (*2*) and others as oval profiles between flattened lamellae (*3*). × 20,000.

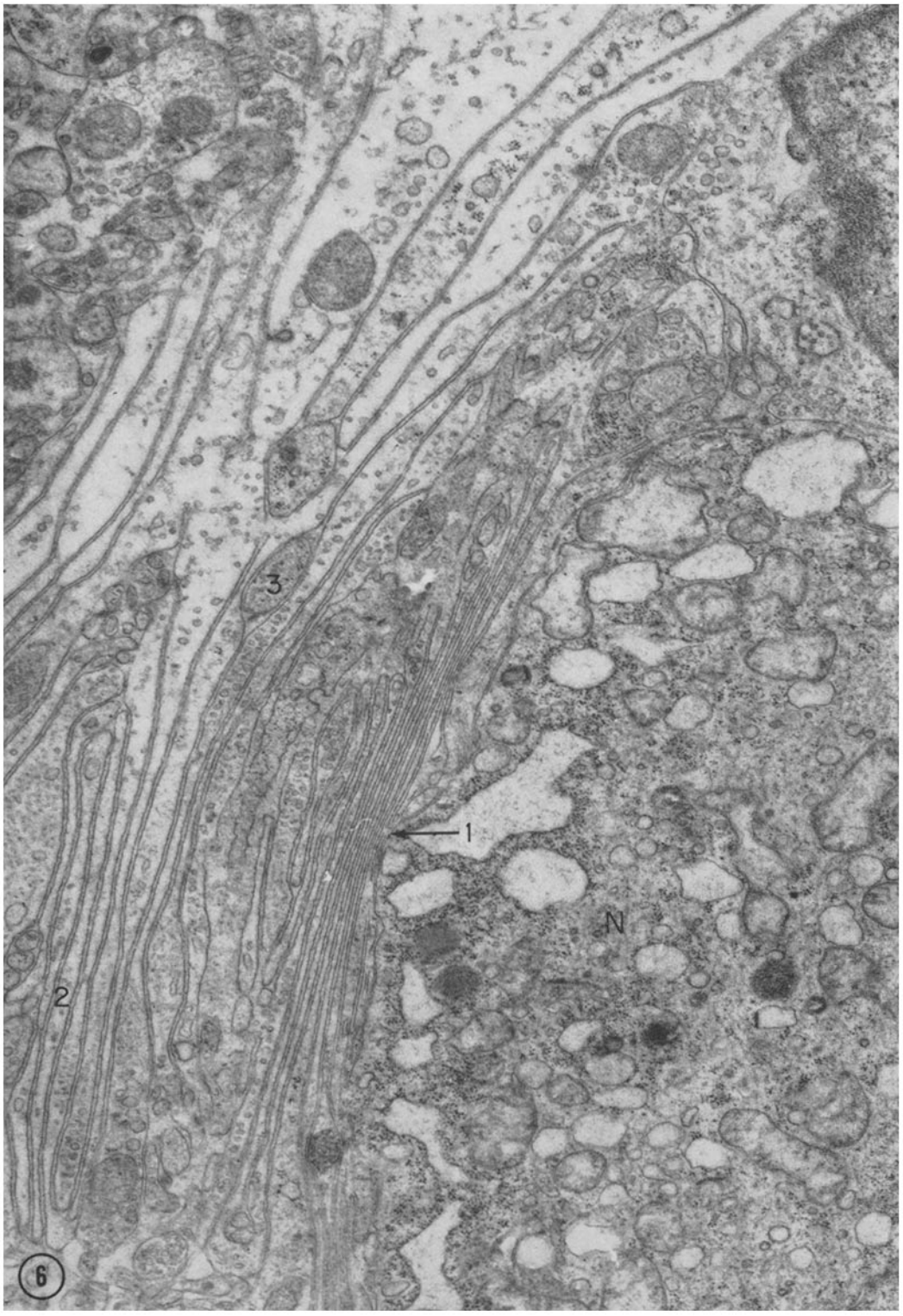

3
1
N
2
6

FIGURE 7

This cell is surrounded by four concentric layers of cytoplasm, each of which forms a continuous lamella in the plane of section. In the upper part of the figure, several elongated processes are applied to the surface of the outermost complete lamella. A partial sheath is present in relation to the cell in the lower right corner. The neuropil is disrupted, and contains numerous very smal glial processes. × 12,000.

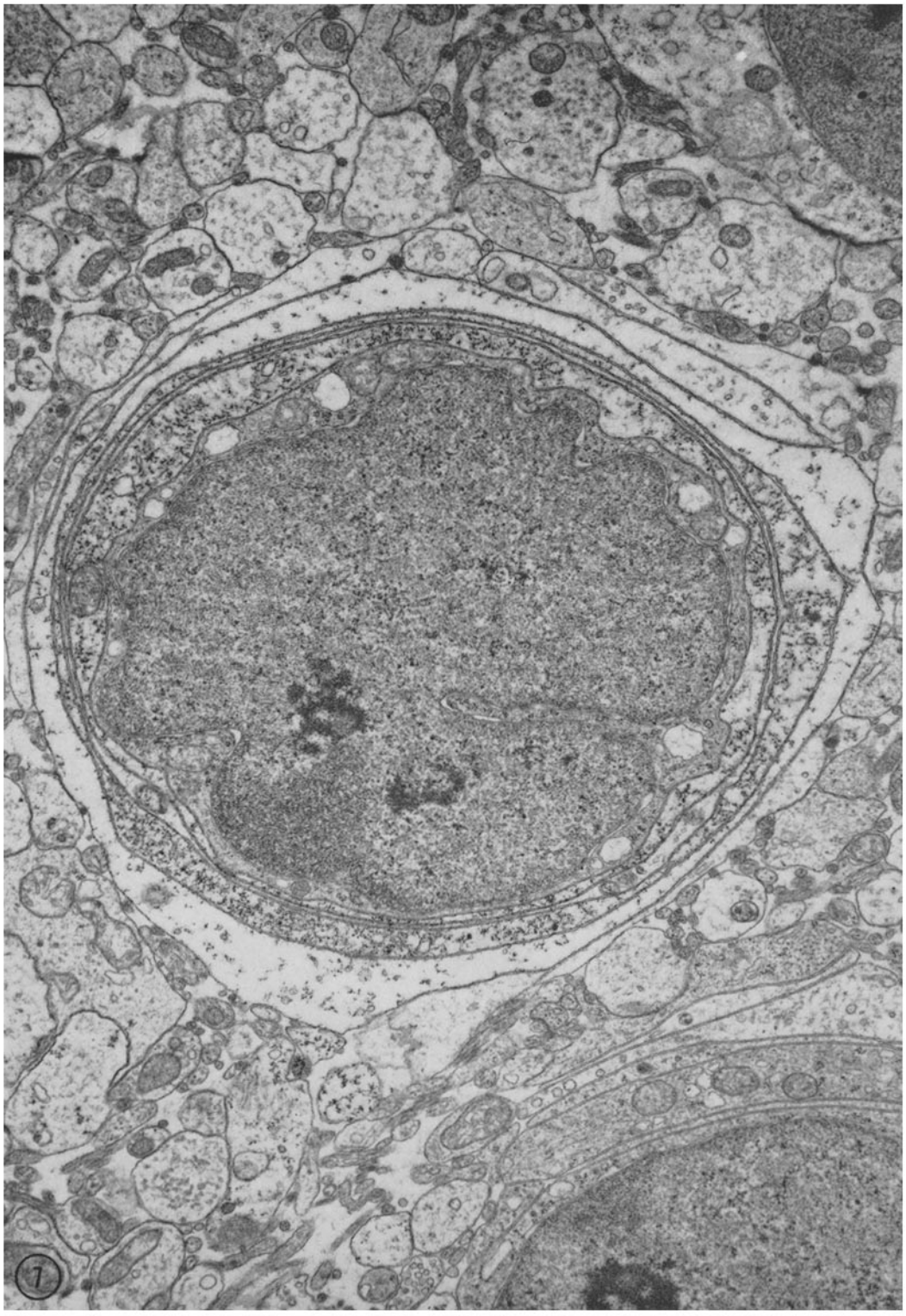
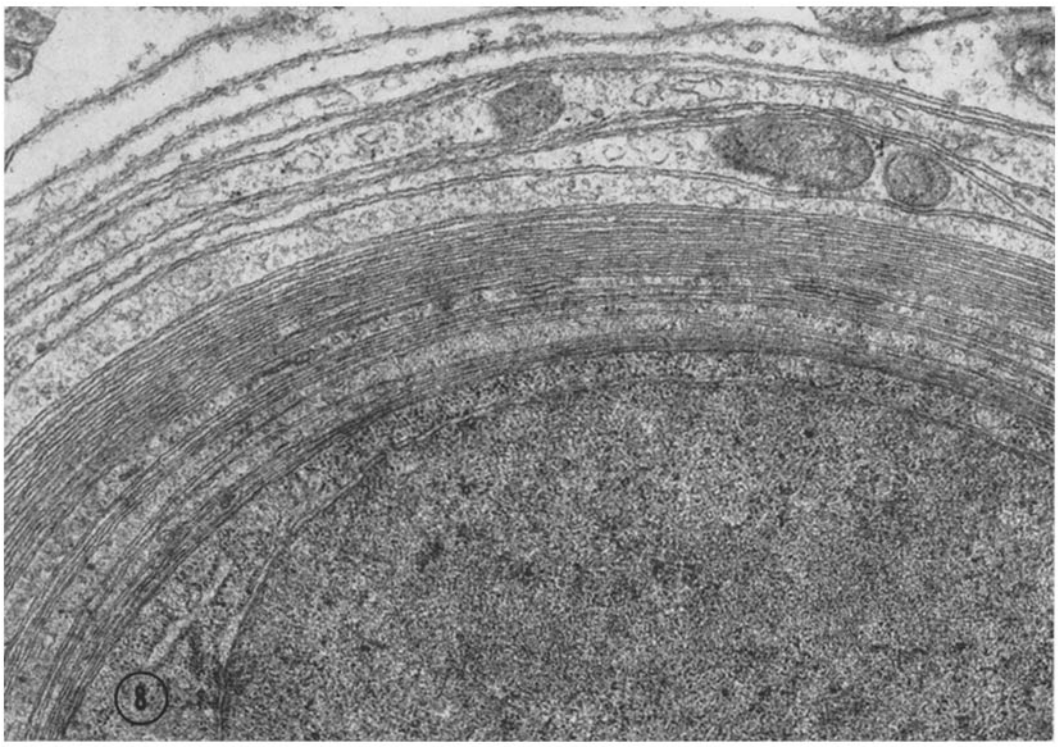


FIGURE 8

A segment of a perikaryal sheath composed of about twenty-five lamellae. Many of the layers have become very compact to form the appearance of a series of relatively evenly spaced lines. × 25,000.

FIGURE 9

A small laminated figure, the outer layers of which have been torn away. The "core" is formed of cytoplasm which contains a mitochondrion, several vacuoles, and numerous small granules; the central region of these figures was seldom this well preserved. There are five concentrically oriented cytoplasmic lamellae around the core, separated from one another by extracellular spaces which are well maintained externally but irregularly narrowed internally. × 41,000.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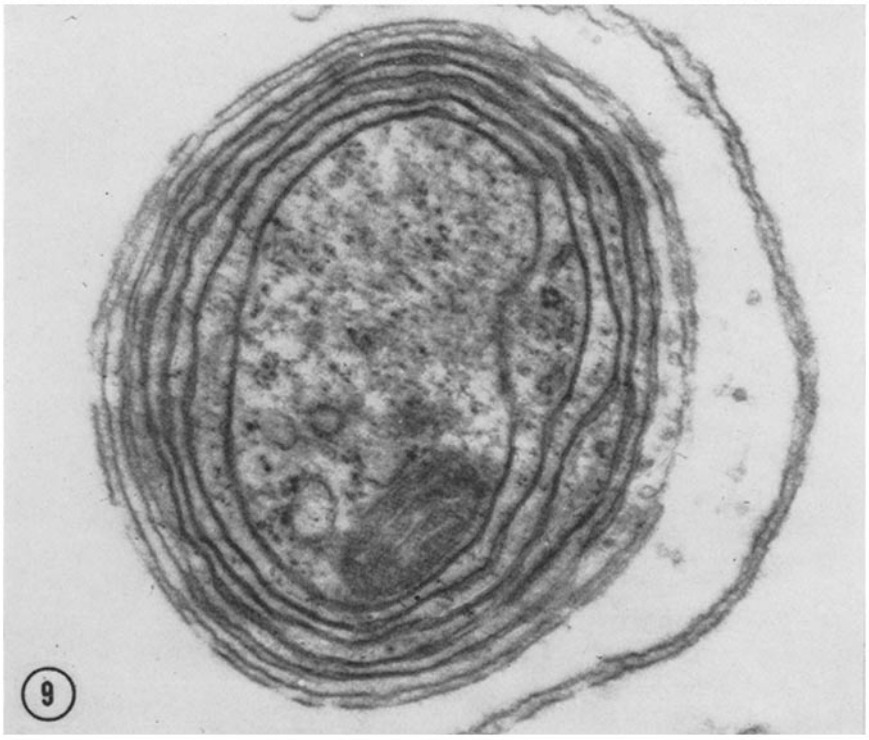
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FIGURE 10

Many longitudinally arranged processes are applied to the outer aspect of a partially fragmented myelin sheath. The inner processes tend to be greatly attenuated, the spacing approaching that of the lamellae of compact myelin (see inset). The outer processes are larger and less regularly disposed. × 29,000; inset, × 50,000.

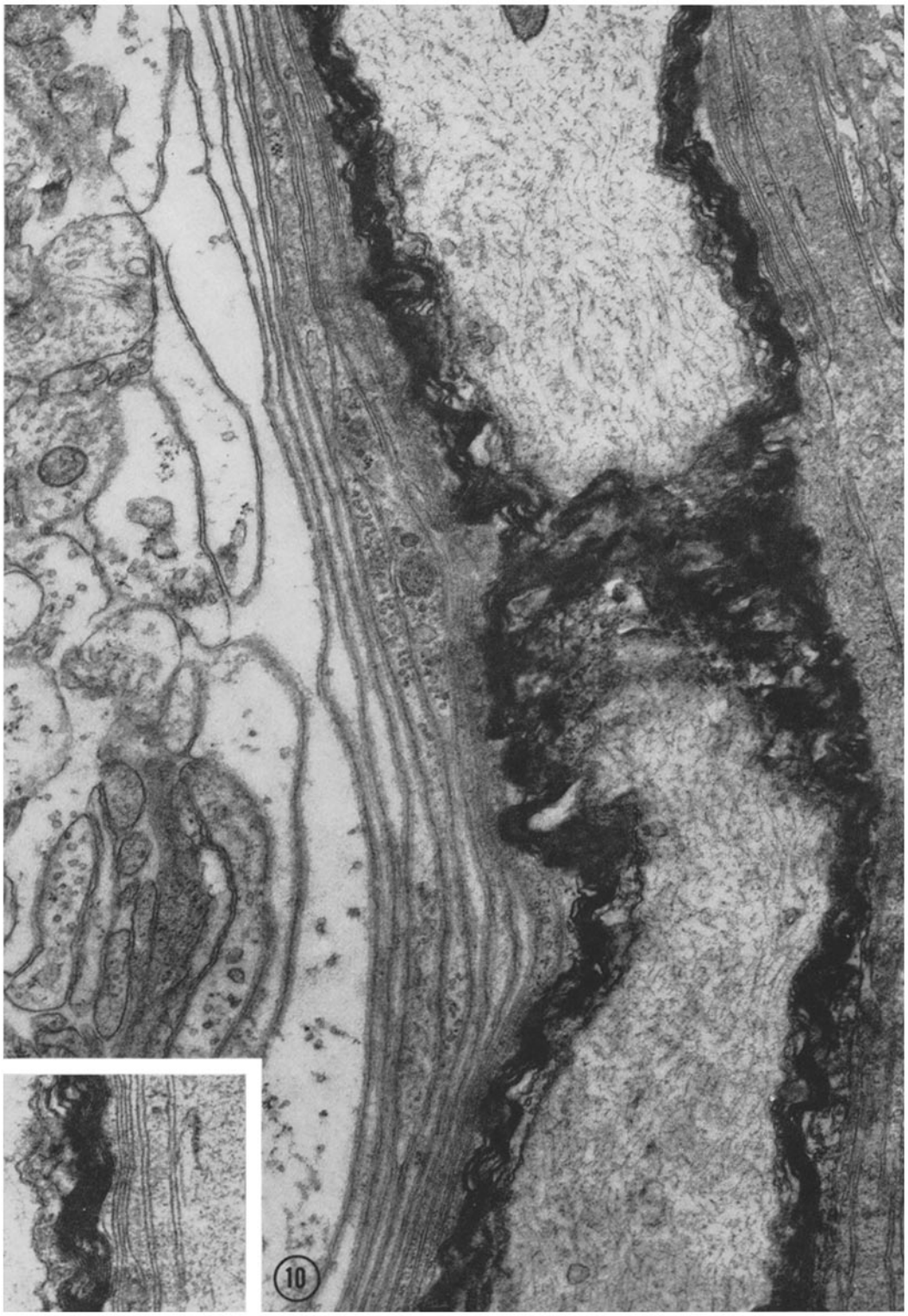
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FIGURE 11

Several small laminated figures, which were located near the edge of a perikaryal sheath. Laminated figure *A* has a large "core" with several mitochondria; the second and ninth lamellae are broad, while the remainder are attenuated. Process *1*, which forms the outermost complete concentric layer of figure *A*, also partially encircles figure *B*. Process *2* encloses two laminated figures, *C* and *D*. Process *3* encases these and three adjacent small figures, and forms a hook-like figure to end at the arrow. There is one crescentic lamella in figure *D*. Many lamellae in several of the figures can be seen to be concentric in arrangement. × 22,000.

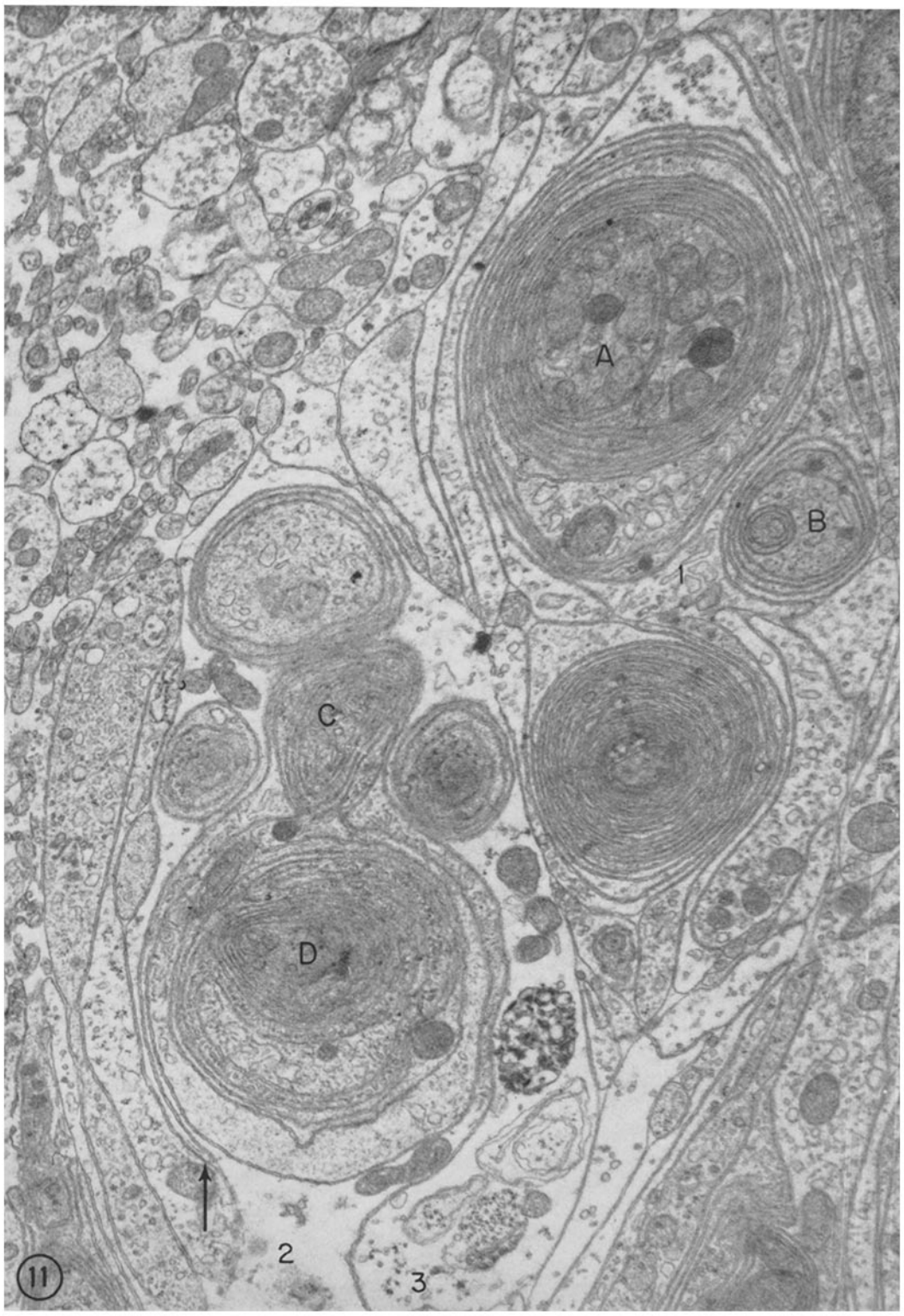
A
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FIGURE 12

Detail from Fig. 13; the concentric orientation of several lamellae in both laminated figures is apparent. In the larger figure, process *1* forms the outermost complete lamella. Process *2* encircles three-fourths of the circumference; process *3*, about one-half, and processes *4* and *5* from smaller arcs. This configuration strongly suggests that additional layers are formed by encircling processes, and that the concentricity of the layers is the result of fusion of the ends of the processes. × 44,000.

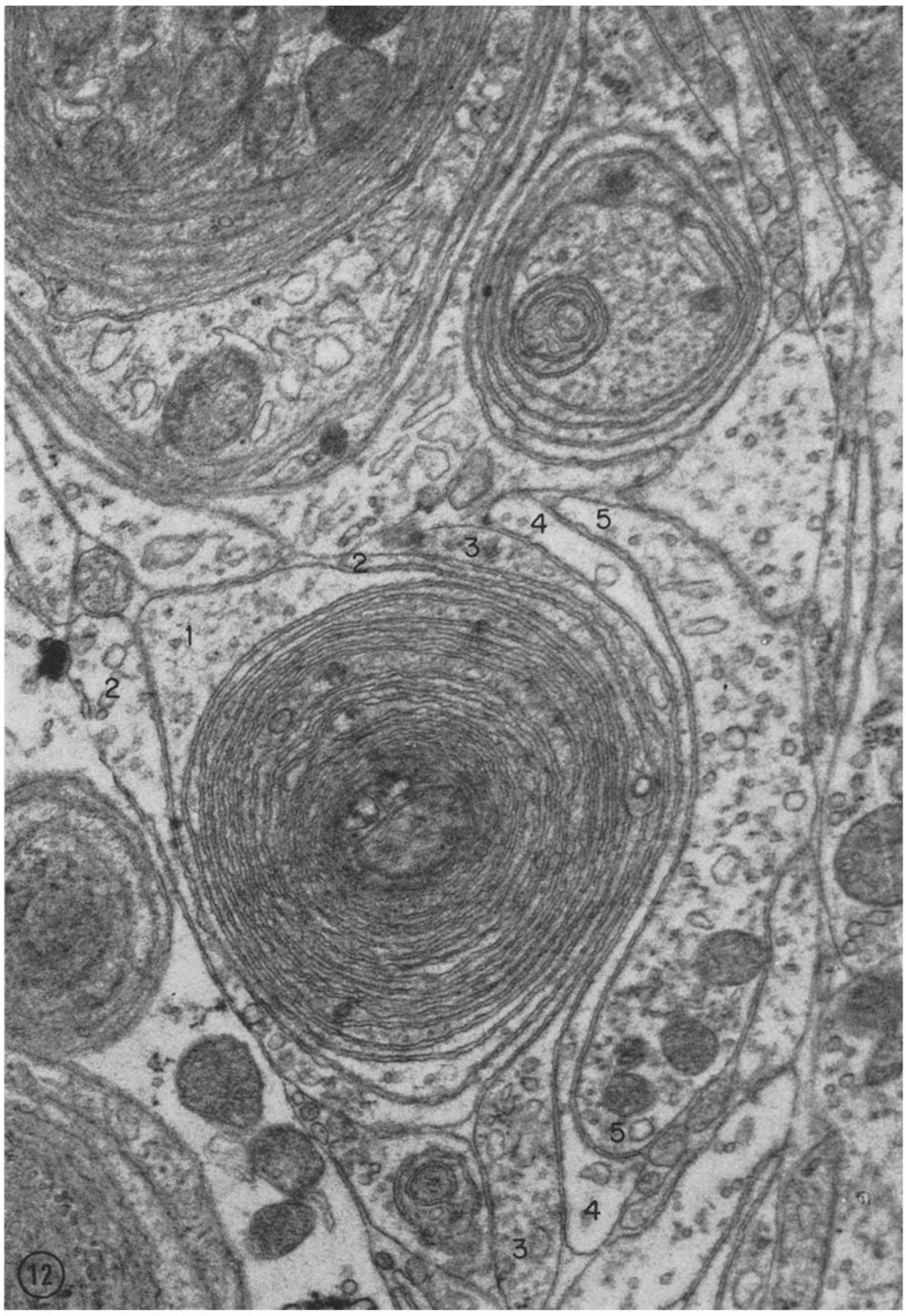
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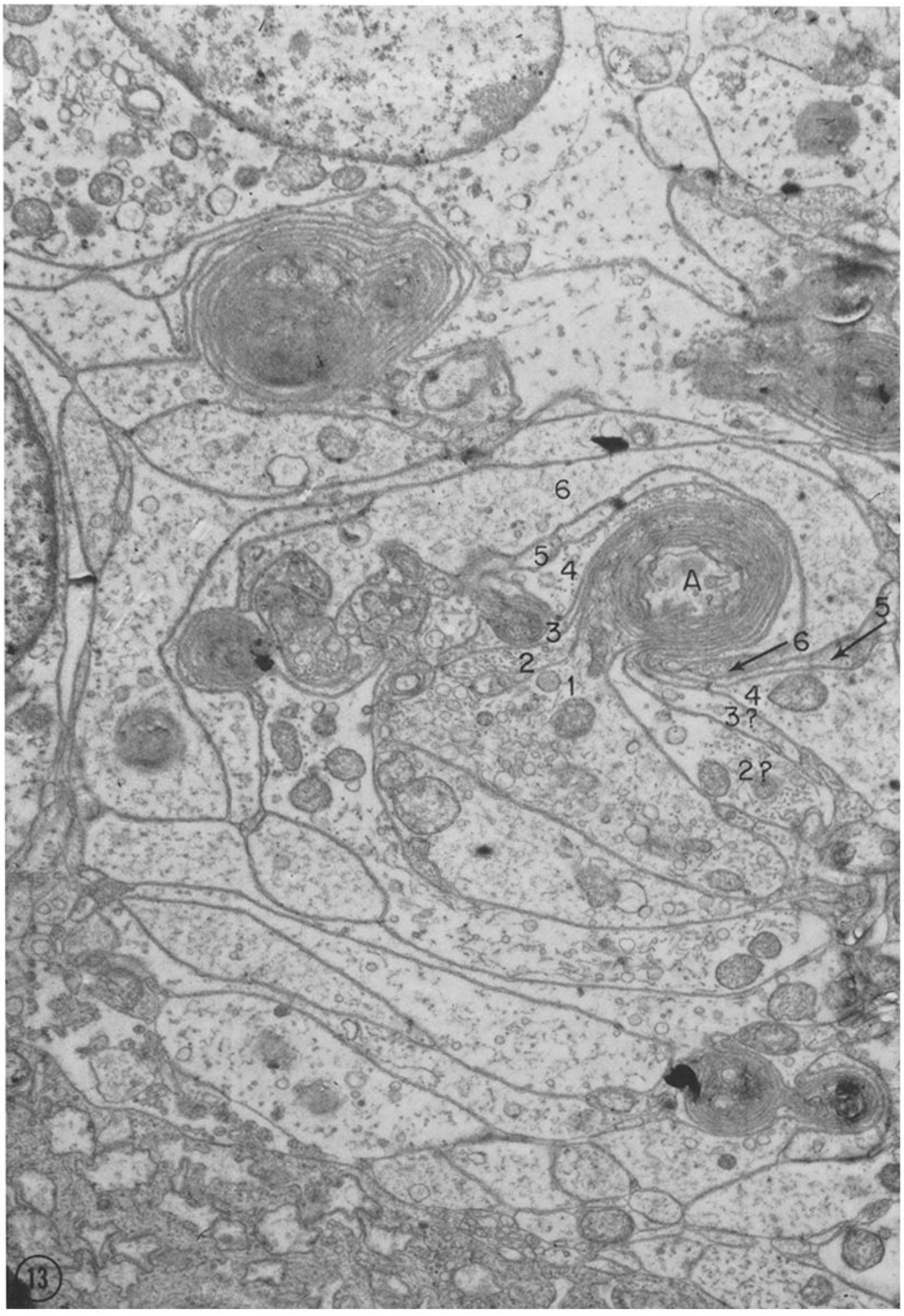
FIGURE 13

Several small laminated figures are disposed in relation to a neuron (lower left) and two glial cells. Laminated figure *A* has about four indistinct inner laminae surrounded by a layer derived from process *1*. Processes *2* to *5* appear to partially encircle the figure forming a hilum about process *1*, and overlap the end of process *6* as they bend back sharply at one side of the hilum. Processes *4*, *5*, and *6* can be readily traced; those labelled *2* and *3* are obliquely cut in one region, making it impossible to prove that they encircle the figure, although it is reasonable to suppose that they do so. × 11,000.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