Phil. Trans. Roy. Soc. Lond. B **257**, 29–43 (1970) [29]
Printed in Great Britain

AN ELECTRON MICROSCOPIC STUDY OF TERMINAL DEGENERATION IN THE NEOCORTEX OF THE CAT

By E. G. JONES and T. P. S. POWELL
Department of Human Anatomy, University of Oxford

(*Communicated by G. W. Harris, F.R.S.—*
Received 10 *March* 1969—*Revised* 15 *April* 1969)

[Plates 31 to 40]

CONTENTS

The nature and immediate postoperative course of experimental degeneration of axon terminals have been studied in the somatic sensory cortex. The first somatic sensory area was examined at intervals of 2 to 6 days following lesions in the thalamus, opposite cortex or ipsilateral second somatic sensory area. There is a characteristic sequence of degenerative changes which affects the terminals of each of the afferent fibre systems studied. This commences as a simple, though marked, increase in electron density of the axoplasm with no loss of synaptic vesicles and little alteration in the size or shape of the terminal. Following this, there is a progressive loss of vesicles and disruption of the mitochondria with shrinkage of the terminal and its compression, invasion and fragmentation by astroglial processes. There is evidence that many fragments are phagocytosed by the invading astroglia but a thin sliver always remains attached at the synaptic contact zone. Within the range of survival periods used, no changes affect the synaptic region nor the postsynaptic profile and if the latter is a dendritic spine, it is not detached from the parent dendrite. Changes in degenerating axons are similar, except that the largest thalamo-cortical fibres show a stage of neurofilamentous hyperplasia.

In the cortex at a distance from the lesion only smaller astrocytic processes are involved in breaking down the degenerating products; close to a lesion, however, all astrocytic processes and perikarya become involved and many atypical glial cells which are difficult to classify as astrocytes or oligodendrocytes become visible; the vascular pericytes also display large heterogeneous dense bodies and other inclusions.

Introduction

With the increasing application of the electron microscope to the study of terminal degeneration in the central nervous system, it has become obvious that the nature and the rate of degeneration vary both in different species and in different parts of the same brain. Axon terminals undergo one of two types of degeneration when their parent axon is divided: the first, which is characterized by a marked proliferation of neurofilaments, is the less common but has been demonstrated in diverse sites in the mammal (Colonnier & Guillery 1964; Smith &

Rasmussen 1965; Mugnaini, Walberg & Brodal 1967; Ralston 1968) and also in birds (Gray & Hamlyn 1962; Dowling & Cowan 1966). In the second and more common type, the degenerating terminal shrinks and becomes markedly electron dense with loss of synaptic vesicles and fragmentation of mitochondria. This type of degeneration has now been demonstrated in many parts of the nervous system (Colonnier & Gray 1962; Walberg 1964, 1965; Colonnier 1964; Westrum 1966; Szentágothai, Hámori & Tömböl 1966; Mugnaini & Walberg 1967; Laatsch & Cowan 1967; Ralston 1968; Jones & Powell 1970). While this second type is widespread, it shows a marked variation in the rate at which it occurs in different sites. In the cerebral cortex (Westrum 1966) and dorsal horn of the spinal cord (Ralston 1968; Heimer & Wall 1968) it may be cyclical and prolonged, whereas in other sites such as the substantia gelatinosa (Heimer & Wall 1968) and thalamus (Jones & Powell 1970) it may be sudden and rapid, commencing and being virtually completed in a single day. These observations point to the need for further studies of the degenerative process in other sites to form the basis for future experimental work, and in this paper we describe some of our observations on the neocortex of the cerebral hemisphere. Such an investigation was a necessary prerequisite for the study of the laminar pattern of termination of the extrinsic afferents to the somatic sensory cortex described in the following paper (Jones & Powell 1970*a*), in which many of the features of the degeneration are also illustrated.

Material and methods

This study is based upon experiments carried out in 13 adult cats. All the material is taken from the first somatic sensory area (SI) of the cerebral cortex. In 12 of the animals a lesion was placed in the cortex or thalamus in order to interrupt selectively commissural, ipsilateral associational, and thalamo-cortical fibres passing to SI; in the remaining animal the hindlimb subdivision of SI was isolated from the rest of the cortex by a series of knife cuts in order to interrupt all extrinsic and many of the intrinsic connexions. Further details of the lesions are given in the following paper. The animals were killed after 2, 4, 5 or 6 days by perfusion under Nembutal anaesthesia: the body temperature was first lowered to approximately 25 °C and after opening the thorax, 1 ml of 0·5 % heparin and 1 ml of a vasodilator (1 % sodium nitrite) were injected into the blood stream via the left venticle. A cannula was inserted into the ascending aorta and the blood quickly washed out with approximately 100 ml of a balanced salt solution and this was followed by a fixative mixture consisting of 1 % glutaraldehyde and 4 % formaldehyde

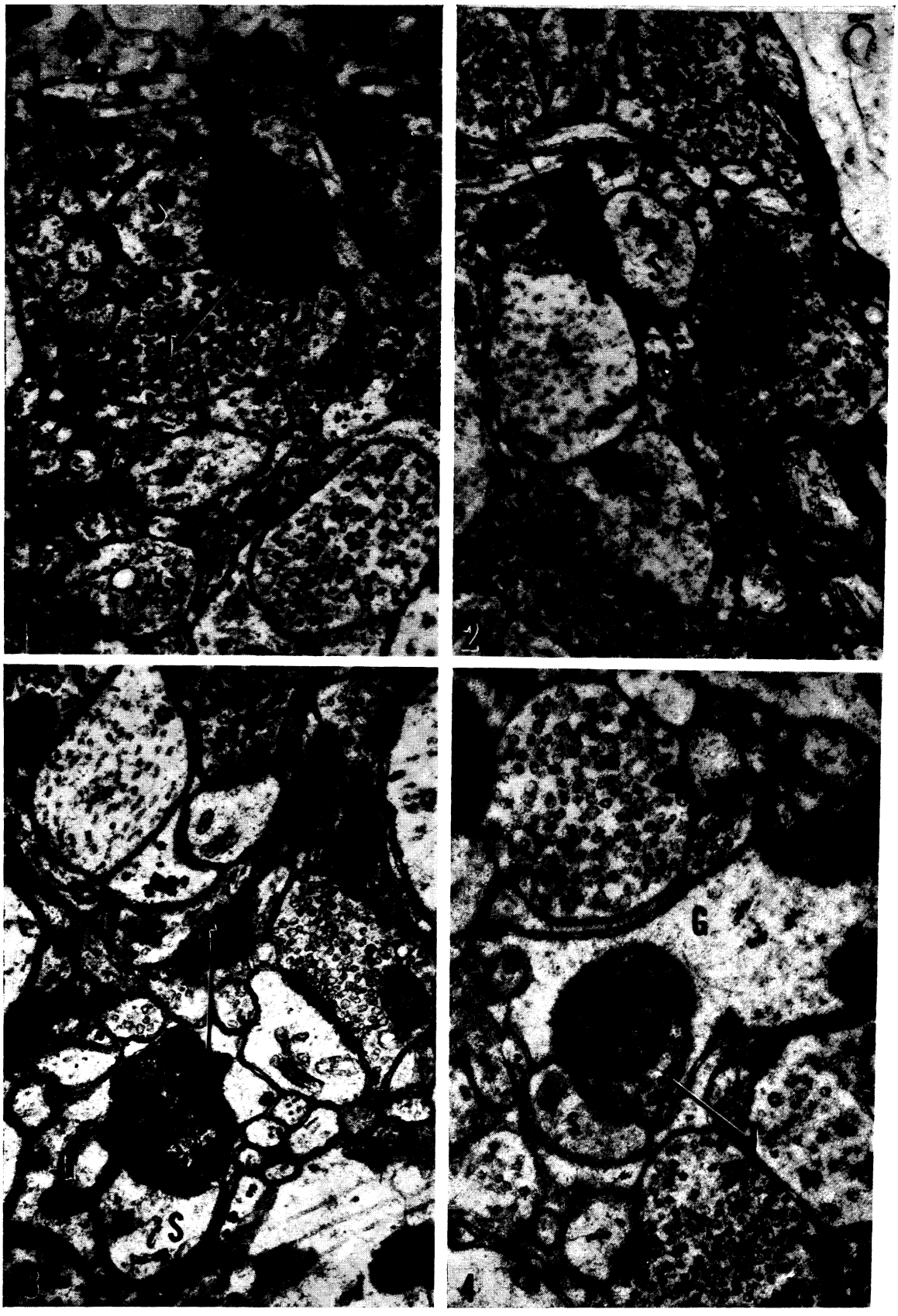
Description of plate 31

Figure 1. A terminal (T) of an association fibre ending by means of a double, asymmetrical synaptic contact on a dendritic spine (S). This represents the earliest stage of degeneration, the only obvious change being an increased density of the background axoplasm. Lead citrate and uranyl acetate stain; × 30000.

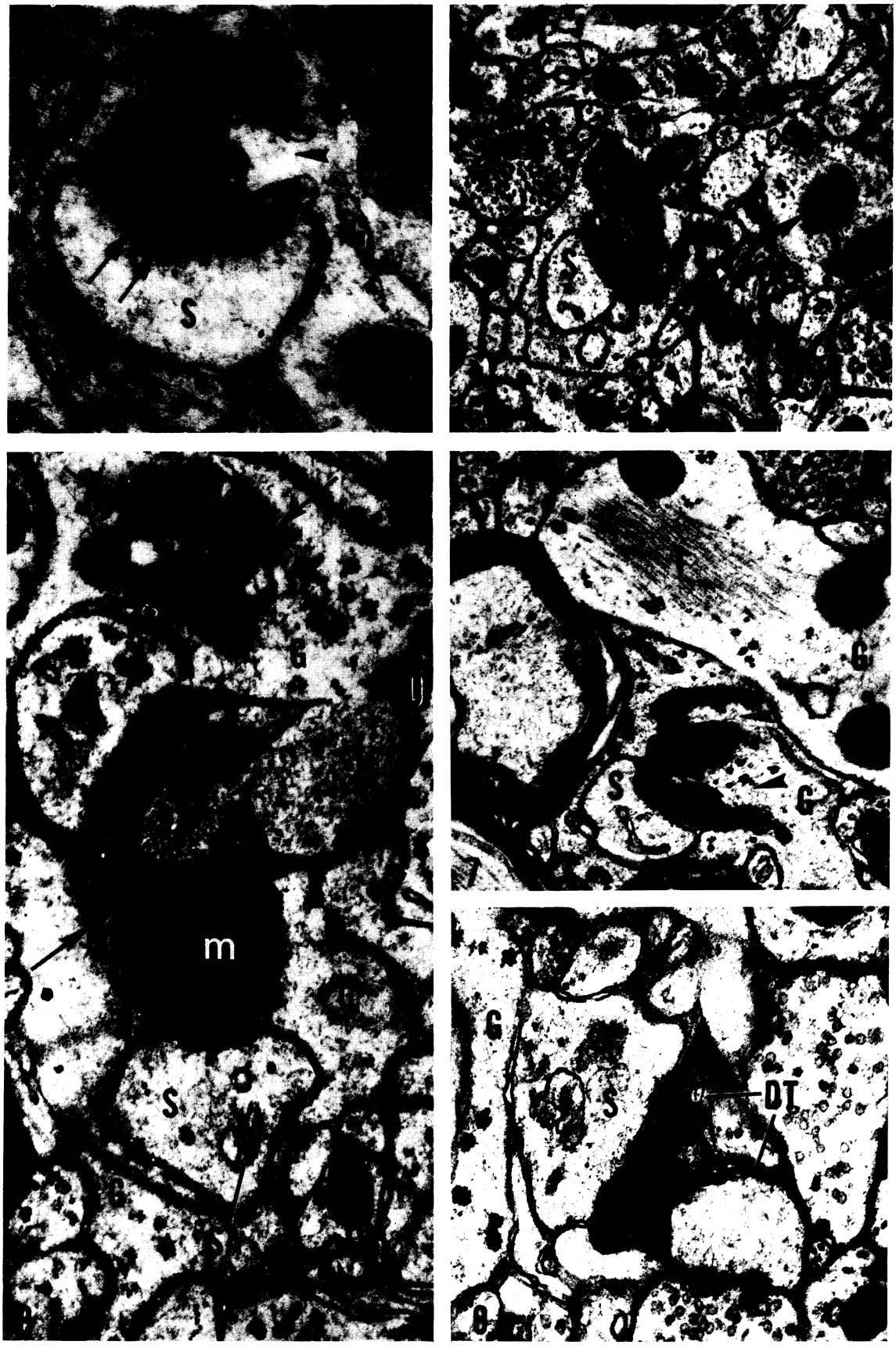
Figure 2. A degenerating thalamo-cortical axon terminal (T) ending on a dendritic spine (S). The terminal is shrunken and distorted but synaptic vesicles are still present. Lead citrate and uranyl acetate; × 29000.

Figure 3. A degenerating commissural axon terminal (T) ending on a dendritic spine (S). The terminal is shrunken and has an irregular outline. A few synaptic vesicles persist but the most obvious components of the terminal are two swollen mitochondria. Small astroglial processes now lie adjacent to the degenerating terminal (G). Lead citrate and uranyl acetate; × 36000.

Figure 4. A degenerating commissural axon terminal from which the synaptic vesicles have disappeared and which consists of little more than a dense bag containing a swollen mitochondrion. The terminal is enfolded by a large astroglial process (G) containing a few glycogen granules. Lead citrate; × 36000.



(*Facing p.* 30)


S
G
m
S
G
S
G
S
DT

made up in a standard 0.1 M phosphate buffer containing added calcium chloride (pH 7.4). Small blocks of tissue taken from the first somatic sensory cortex were postfixed in osmium tetroxide and embedded in Araldite. Thin sections were stained on the grid with lead citrate (Reynolds 1963) or with lead citrate and uranyl acetate (Watson 1958). Many blocks prepared in the same manner but taken from normal brains were available for comparison.

Results

When an afferent pathway to the first somatic sensory cortex is interrupted by an experimental lesion, the terminals of its constituent axons undergo a succession of characteristic degenerative changes which may be interpreted as early, intermediate and late depending upon the presence or absence of synaptic vesicles, the degree of shrinkage of the terminal and the relationships of reactive astroglial processes to it. The results may thus be presented in an orderly sequence as though tracing the stages of degeneration in a single terminal. The stages of terminal degeneration in S I are identical following lesions of the ipsilateral second somatic sensory area (S II), the contralateral somatic sensory cortex or the ventroposterior nucleus of the thalamus, and in the first instance no distinction will be drawn between the nature or rate of degeneration in the three fibre systems concerned.

Degenerative changes in axon terminals

The earliest recognizable degenerative change occurring in axon terminals in the somatic sensory cortex is a marked increase in the electron density of the axoplasm with little change in the size or shape of the affected terminal and, as far as can be ascertained, no loss of synaptic vesicles (figure 1, plate 31). Degenerating axon terminals having this appearance are seen at all the survival periods used but are rather uncommon. More common is a similar form in which the ending displays a marked increase in electron density with no apparent loss of synaptic vesicles but it is slightly shrunken and distorted as though compressed by adjacent (normal)

Description of plate 32

Figure 5. A degenerating commissural axon terminal ending on a dendritic spine by means of an asymmetrical synaptic contact (double arrows) and invaded from the side (arrow head) by a tongue of astroglial cytoplasm (G). Lead citrate and uranyl acetate; × 113000.

Figure 6. A degenerating commissural axon terminal ending on a dendritic spine. Both the terminal and the spine are surrounded by astroglial processes which are beginning to invade (arrow heads) the terminal. Note the dense body (arrow) in the glia. Lead citrate; × 28000.

Figure 7. A degenerating thalamo-cortical axon terminal invaded by tongues of astroglial cytoplasm (arrow heads). The dense granules in the astroglial processes (G) are particles of glycogen. One of the astroglial processes also contains a bundle of filaments (F). Lead citrate; × 30000.

Figure 8. A degenerating thalamo-cortical axon terminal ending on two dendritic spines (S), one of which contains an obvious spine apparatus (SA). The terminal is invaded by a single glial tongue which seems to be dividing it in two. The lower part of the terminal contains a swollen mitochondrion (*m*) and a multivesicular body (double arrow); attached to it (U) is a narrow thread of degenerating axoplasm which may be the terminal unmyelinated segment of the parent axon. Note the irregular inclusion (arrows) contained within the astroglia at the top of the micrograph. Lead citrate; × 50000.

Figure 9. A degenerating axon terminal (DT) caused by undercutting the cortex. The terminal is at a relatively late stage of degeneration but is unusual in not being surrounded by astroglial processes. There are, however, such processes in the vicinity (G) and these contain large glycogen granules. Lead citrate and uranyl acetate; × 44000.

profiles; the synaptic vesicles are packed tightly together and are frequently flattened (figure 2, plate 31). When mitochondria are seen in these early degenerating terminals they appear normal. There is usually little evidence of a glial reaction in the immediate vicinity of the degenerating terminal in these early stages. No terminals display any evidence of neurofilamentous hyperplasia.

In the next stage, the dense terminal is further shrunken and has an irregular, crenellated outline contrasting with the smoothly rounded contours of most of the adjoining normal profiles (figure 3, plate 31). The most obvious features at this stage are the loss of synaptic vesicles—only occasional ones remaining scattered throughout the very dense degenerating axoplasm—together with changes in the mitochondria. The latter appear considerably swollen and their matrix is less dense than the surrounding axoplasm so that they assume a somewhat 'glassy' appearance. The double limiting membrane of the mitochondrion remains intact but the cristae seem to be fragmenting, many losing their attachments to the inner membrane and small ellipsoidal fragments are seen scattered throughout the mitochondrial matrix (figure 3, plate 31). Usually by this stage there is some evidence of glial reaction in the vicinity of the degenerating terminal: swollen astrocytic processes containing glycogen granules are seen in close relation to the terminal and its postsynaptic profile and may bound the former on three sides (figures 3, 4, plate 31). Some degenerating terminals at this stage have become very shrunken, but their mitochondria remain swollen so that the whole profile is little more than a very dense bag containing a slightly paler mitochondrion (figure 4, plate 31).

Subsequent changes in the degenerating terminal consist of fragmentation and dissolution of the mitochondria, continued shrinkage of the terminal which, throughout, remains extremely electron dense, and progressive invasion of the terminal by reactive astroglial processes. Shrunken degenerating terminals containing disrupted mitochondria but with little or no associated glial reaction are occasionally seen (figure 9, plate 32), but more commonly the shrinkage of the terminal and dissolution of the mitochondria is accompanied by disruption and fragmentation of the terminal by the invading astroglia. The thickened astroglial processes surround the degenerating terminal, and if the latter contacts a dendritic spine, this too is commonly partially enveloped (figures 5 to 14, plates 32 and 33), but no glia ever invades the synaptic cleft. Disruption of the terminal by the reactive astroglia involves both compression of the terminal and its invagination by glial cytoplasm. Single degenerating terminal profiles may be invaded by one or more tongues of glial cytoplasm, either from directly opposite the synaptic

---

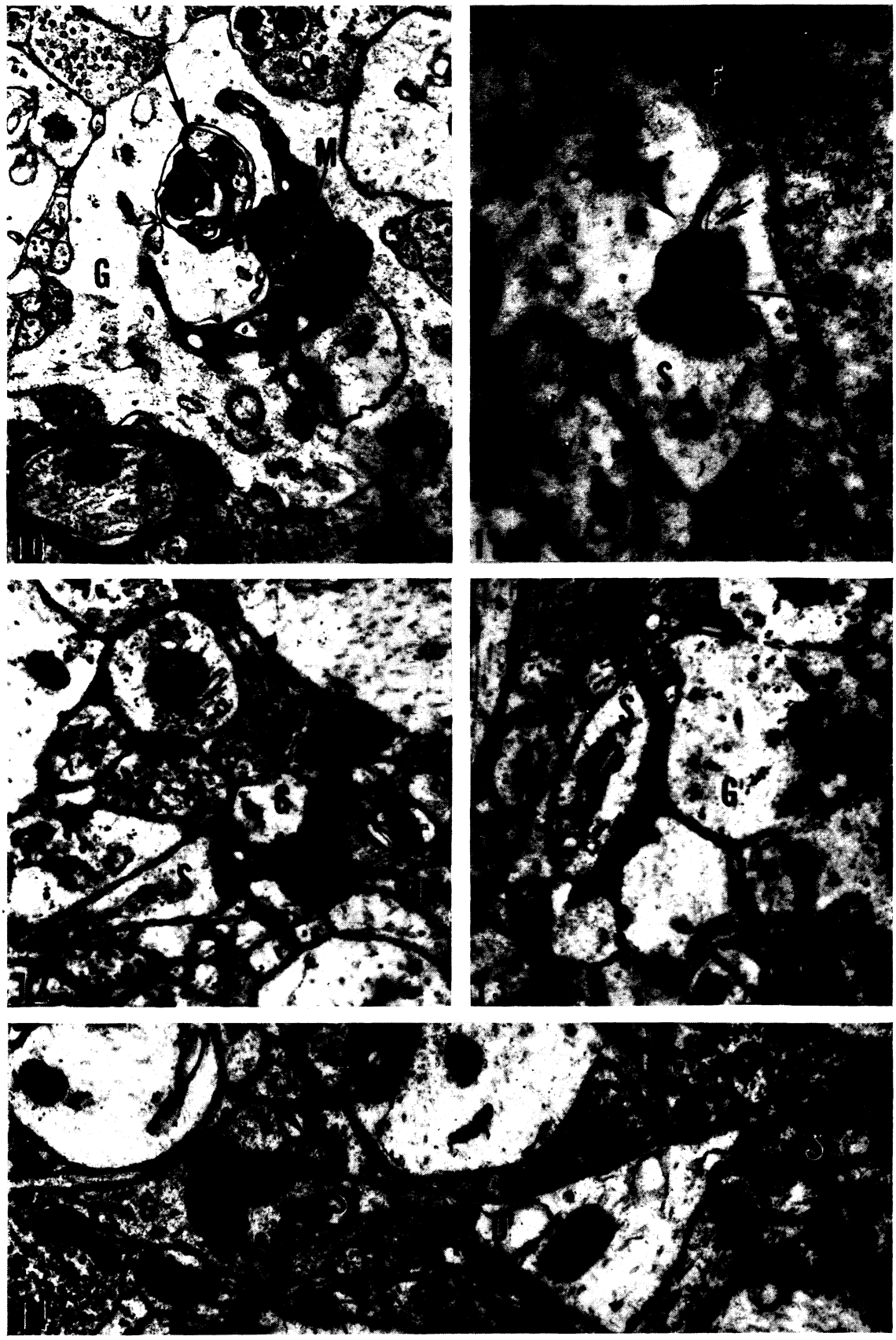
Description of plate 33

Figure 10. A degenerating thalamo-cortical axon terminal much distorted by the invading astroglia and containing a fragmented mitochondrion (M). Note that the astroglial cytoplasm surrounds both the terminal and the postsynaptic profile and contains two membrane-bound inclusions (arrows). Lead citrate; × 30000.

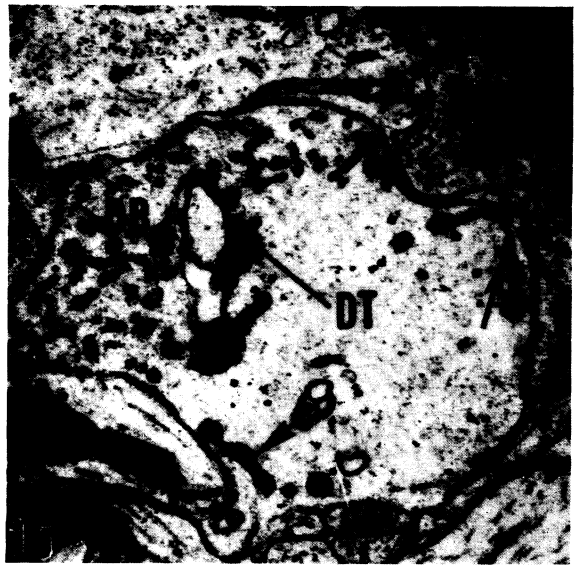
Figure 11. A degenerating commissural axon terminal ending on a dendritic spine (S) which contains a spine apparatus. The terminal is enveloped by the soma of an astrocyte (G) and is compressed (arrows) into a bell-shaped form. N, Nucleus; F, filaments; M, swollen mitochondrion. Lead citrate; × 38000.

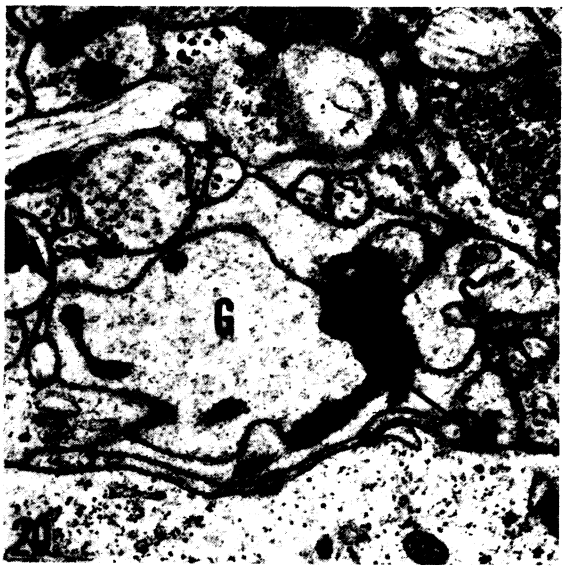
Figures 12 and 13. Degenerating commissural axon terminals ending on dendritic spines and compressed into irregular shapes by the enveloping astroglial processes. Note in each case the elongated 'tail' (arrows). Figure 12: lead citrate and uranyl acetate; Figure 13: lead citrate; both × 29000.

Figure 14. A dendrite (D) to which are attached two spines (S). The spine on the left receives a degenerating commissural axon terminal and both the terminal and the spine are enveloped by astroglial cytoplasm (G), but the spine remains attached to its pedicle. Note the short unmyelinated terminal axon segment (arrow). The right hand spine receives a normal axon terminal. Lead citrate and uranyl acetate; × 30000.



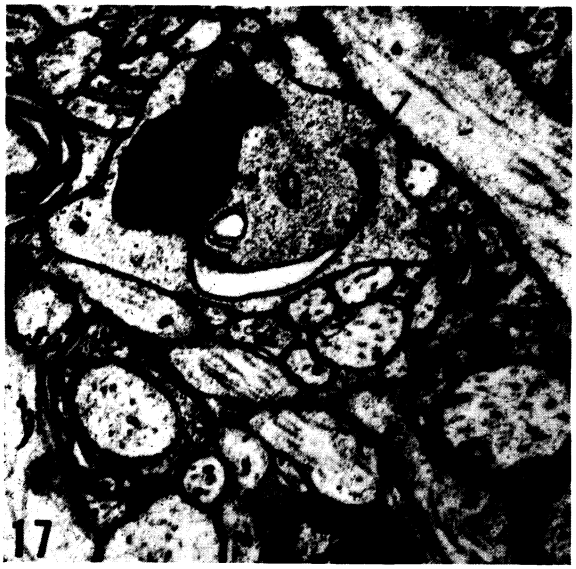
(*Facing p.* 32)

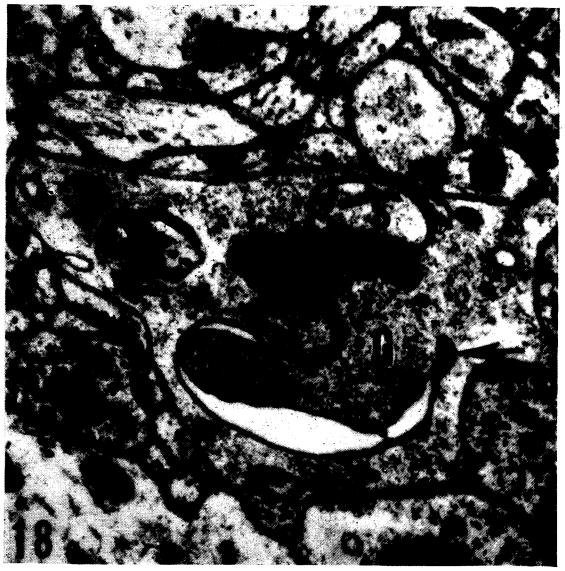

DT
P


G


DT
16


G


17


18

FIGURES 15, 16. Serial sections of a degenerating thalamo-cortical axon terminal (DT) completely surrounded by astroglial cytoplasm which contains small dense bodies (DB) resembling lysosomes and larger ones (arrows) which may be broken-off fragments of the degenerating terminal. Lead citrate and uranyl acetate; × 26 000.

FIGURES 17, 18. Two degenerating thalamo-cortical axon terminals surrounded by astroglial cytoplasm which is so disposed as to suggest that the terminal unmyelinated segment of the parent axon has been thrown into a sinuous shape (arrow). Lead citrate and uranyl acetate; × 31 000.

FIGURE 19. A degenerating unmyelinated axon fragment (U) surrounded by an astroglial process (G). The folding of the glial process is indicated by the arrow heads. Note in figures 17–19 that the glial cell has lost its large glycogen particles and has become finely granular. Lead citrate; × 20 000.

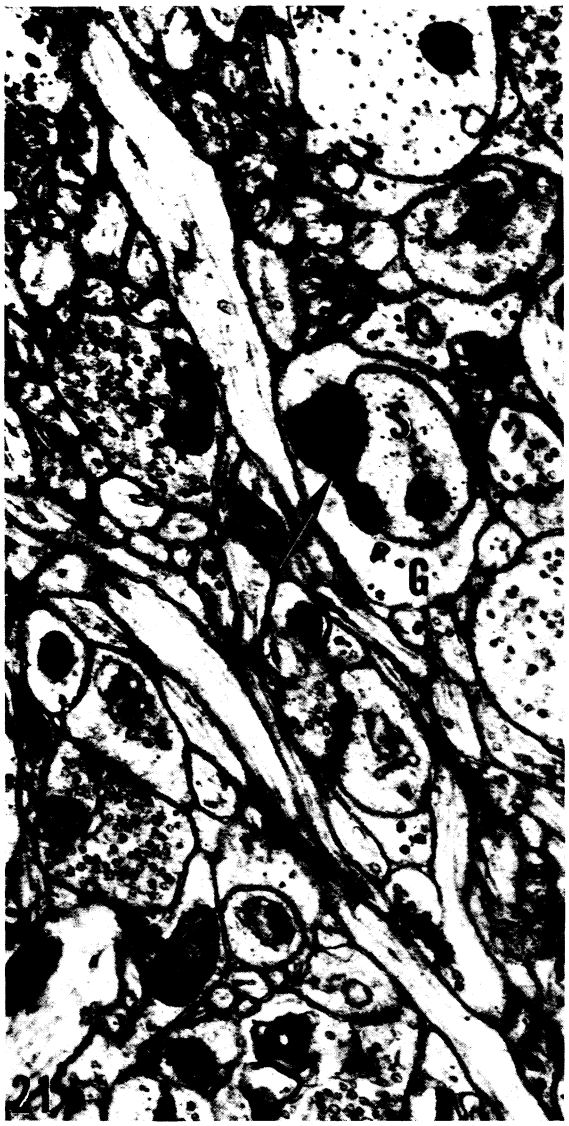
FIGURE 20. A degenerating thalamo-cortical axon terminal (DT) ending on a dendritic spine (S) and intimately related to reduplicated (arrow heads) folds of astroglial cytoplasm (G) which also contain several dense fragments resembling portions of the degenerating terminal. Lead citrate and uranyl acetate; × 25 000.

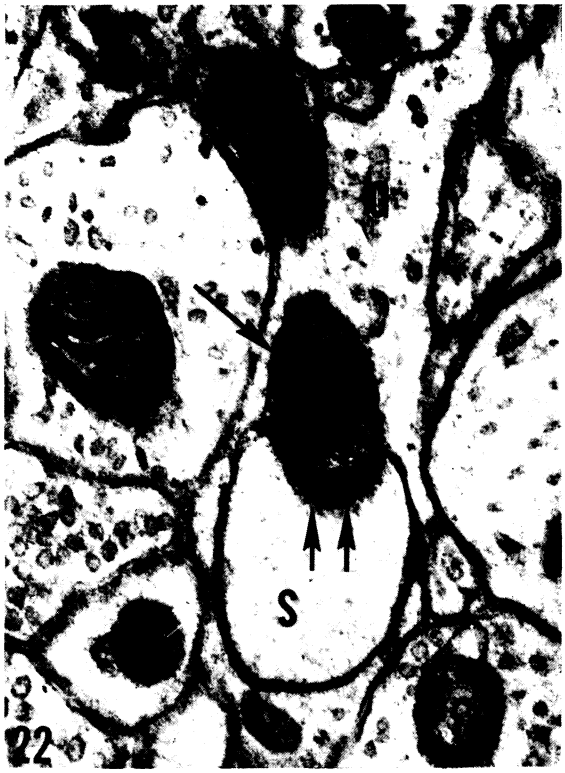
FIGURE 21. A degenerating commissural axon terminal (arrow) at a late stage of degeneration and ending on a dendritic spine (S). Both the terminal and the spine are surrounded by an astroglial process (G). Lead citrate; × 30 000.

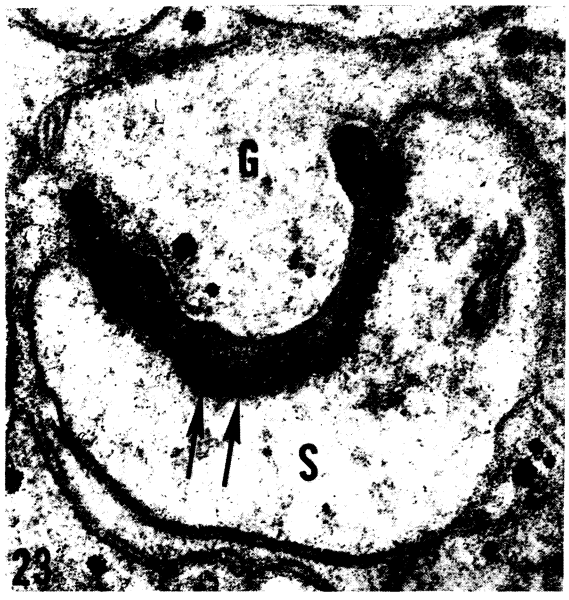
FIGURE 22. A degenerating commissural axon terminal (arrow) ending on a dendritic spine; both the spine and the terminal are surrounded by an astroglial process. The terminal at this late stage of degeneration is greatly shrunken, though it still contains a mitochondrion. The asymmetrical synaptic contact is indicated by the double arrows. Lead citrate and uranyl acetate; × 49 000.

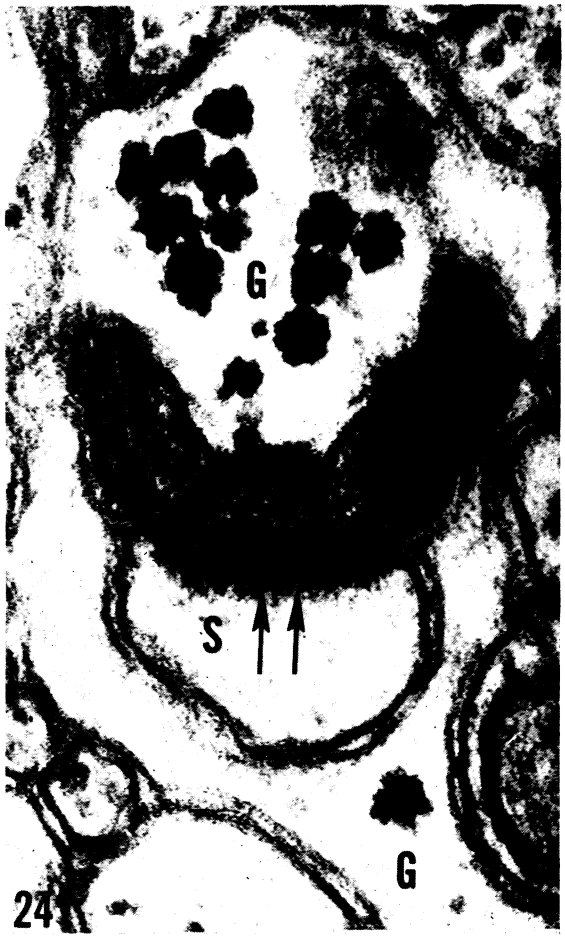
FIGURE 23. A commissural axon terminal at the latest stage of degeneration encountered in the present study. The terminal is reduced to little more than a narrow sliver attached at the synaptic thickening (double arrows). Lead citrate and uranyl acetate; × 84 000.

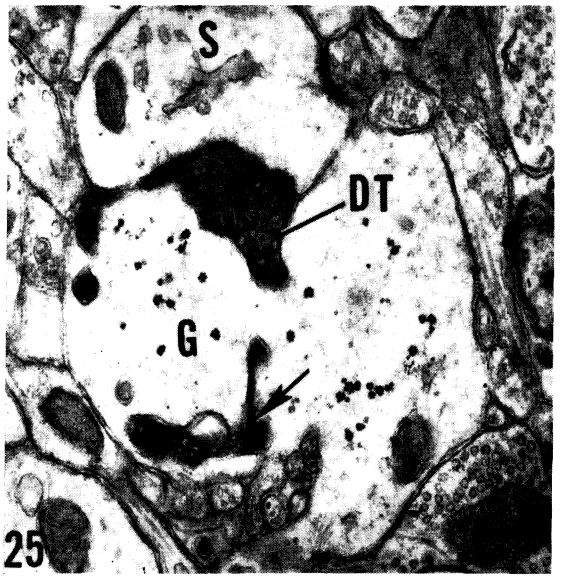
FIGURE 24. A late degenerating commissural axon terminal homogeneously dense, much reduced in size and deeply invaded by an astroglial process in which the glycogen reaction is especially marked. Note that the terminal and the spine (S) with which it makes synaptic contact (double arrows) are surrounded by the glial process but that the plasma membranes of all three remain separate and no glia invades the synaptic cleft. Lead citrate and uranyl acetate; × 150 000.

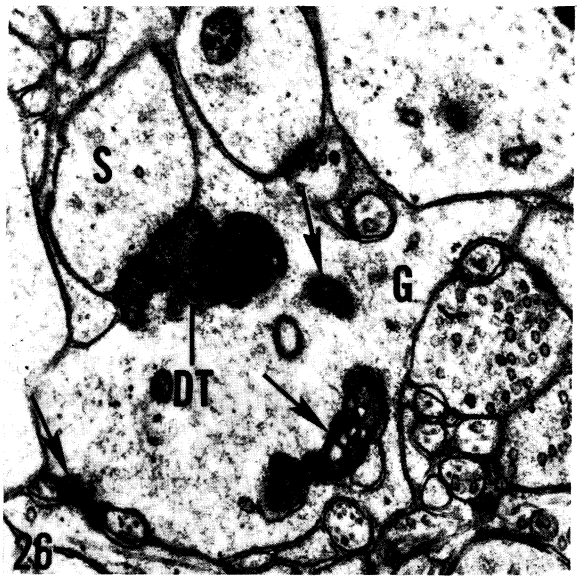

G
21

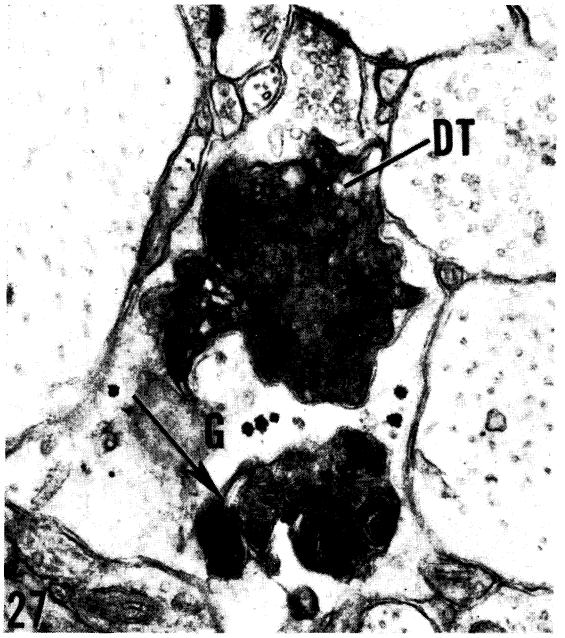

S
22

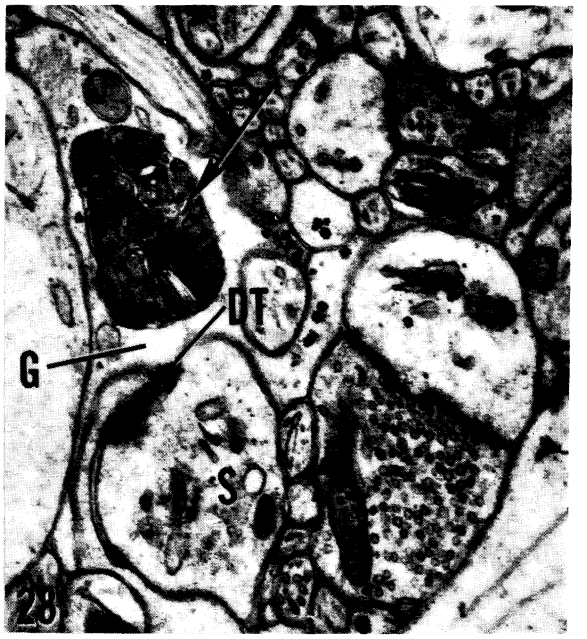

G
S
23

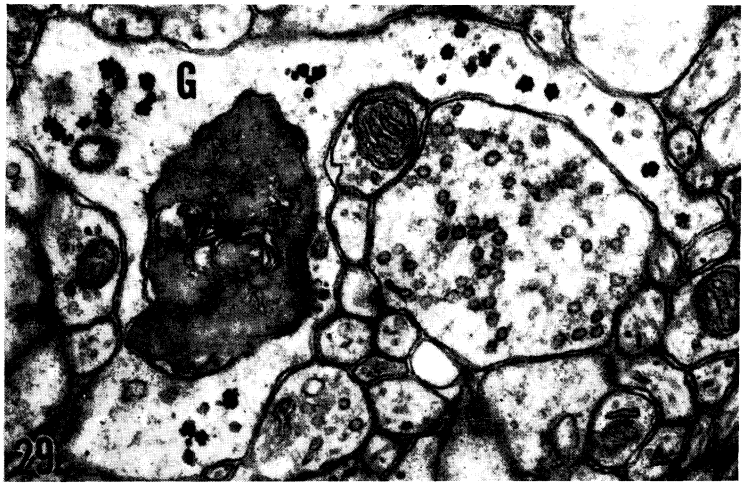

G
S
G
24


S
DT
G
25


S
G
DT
26


DT
G
27


DT
G
28


G
29

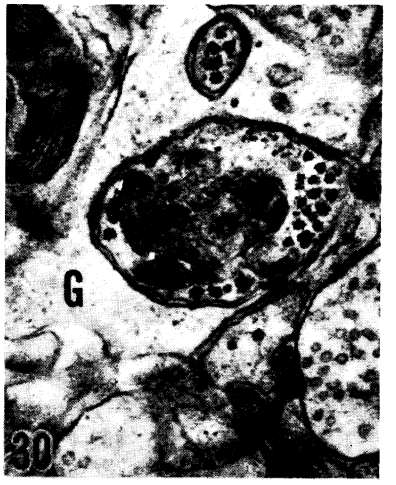

G
30

FIGURE 25. A degenerating commissural axon terminal (DT) ending on a dendritic spine (S) and surrounded by an astroglial process (G) which contains glycogen granules and other dense bodies (arrow) which may be portions of the same terminal. Lead citrate; × 31 000.

FIGURE 26. A degenerating thalamo-cortical axon terminal. Cf. with figure 25. Lead citrate; × 37 000.

FIGURE 27. A degenerating association fibre terminal (DT) surrounded by an astroglial process which also contains a large membrane-bound dense body consisting of granules and whorled membranes. Lead citrate and uranyl acetate; × 38 000.

FIGURE 28. A degenerating commissural axon terminal (DT) reduced to a very small fragment attached to the postsynaptic dendritic spine (S). Associated with the terminal is an astroglial process containing a very large dense body similar to that shown in figure 27. Lead citrate and uranyl acetate; × 33 000.

FIGURE 29. A membrane-bound dense body containing irregular membranous lamellae lying within an astroglial process in which the glycogen reaction is marked. Lead citrate; × 42 000.

FIGURE 30. Two membrane-bound complexes each containing glycogen granules and one a whorled dense body, lying within the cytoplasm of an astroglial process (G). Lead citrate; × 29 000.

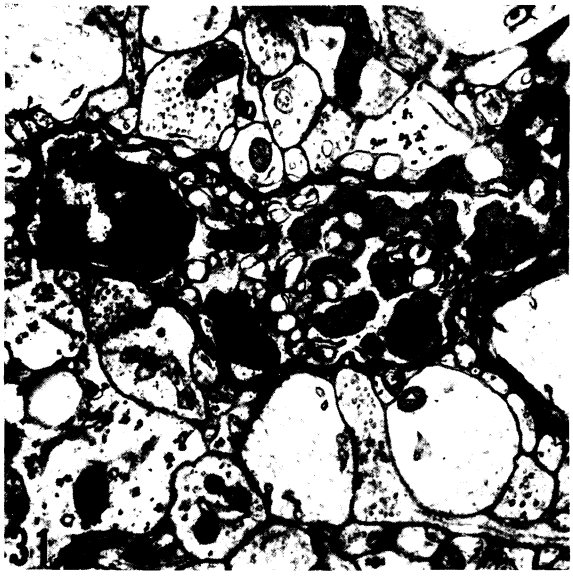
FIGURE 31. A degenerating association fibre showing fragmentation and vacuolation of the axoplasm but little or no change in the myelin sheath. Lead citrate and uranyl acetate; × 19000.

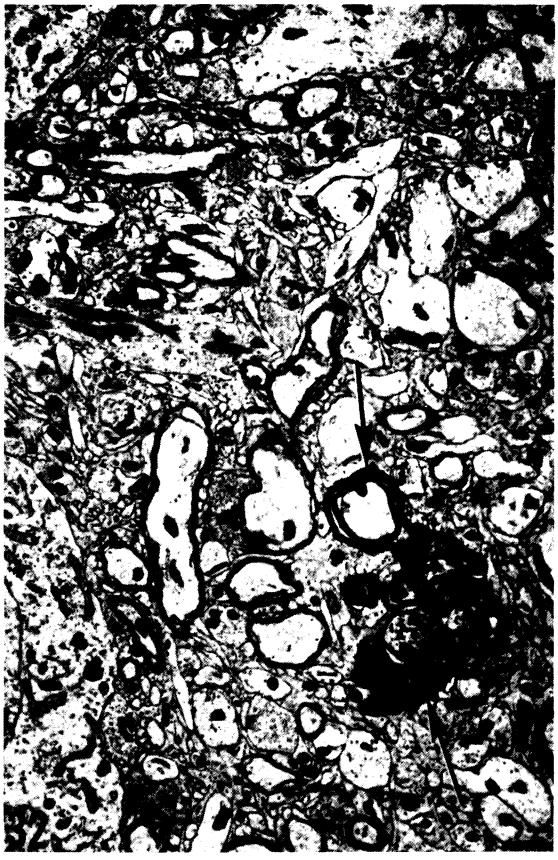
FIGURE 32. A degenerating association fibre showing fragmentation of the axoplasm and folding and reduplication (upper arrow) of the myelin sheath. Lead citrate; × 6000.

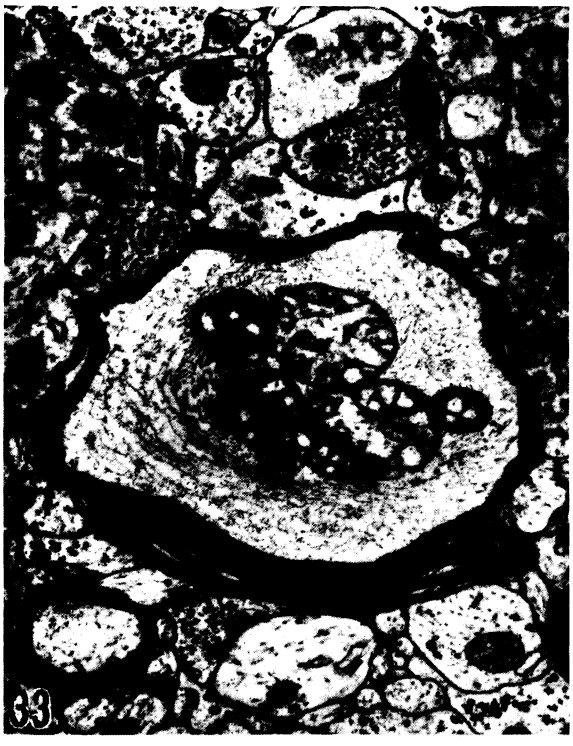
FIGURE 33. A large degenerating thalamo-cortical fibre showing swollen and distorted mitochondria and neurofilamentous hyperplasia. Lead citrate and uranyl acetate; × 17000.

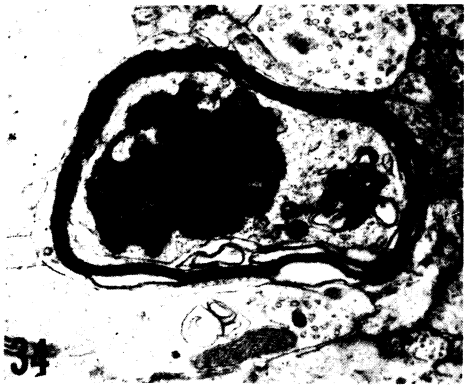
FIGURE 34. A degenerating commissural fibre showing shrinkage of the degenerating axoplasm away from the myelin sheath. Lead citrate; × 20000.

FIGURE 35. A degenerating thalamo-cortical fibre showing the beading of the dense degenerating axoplasm which also contains a swollen mitochondrion (M). Lead citrate; × 33000.









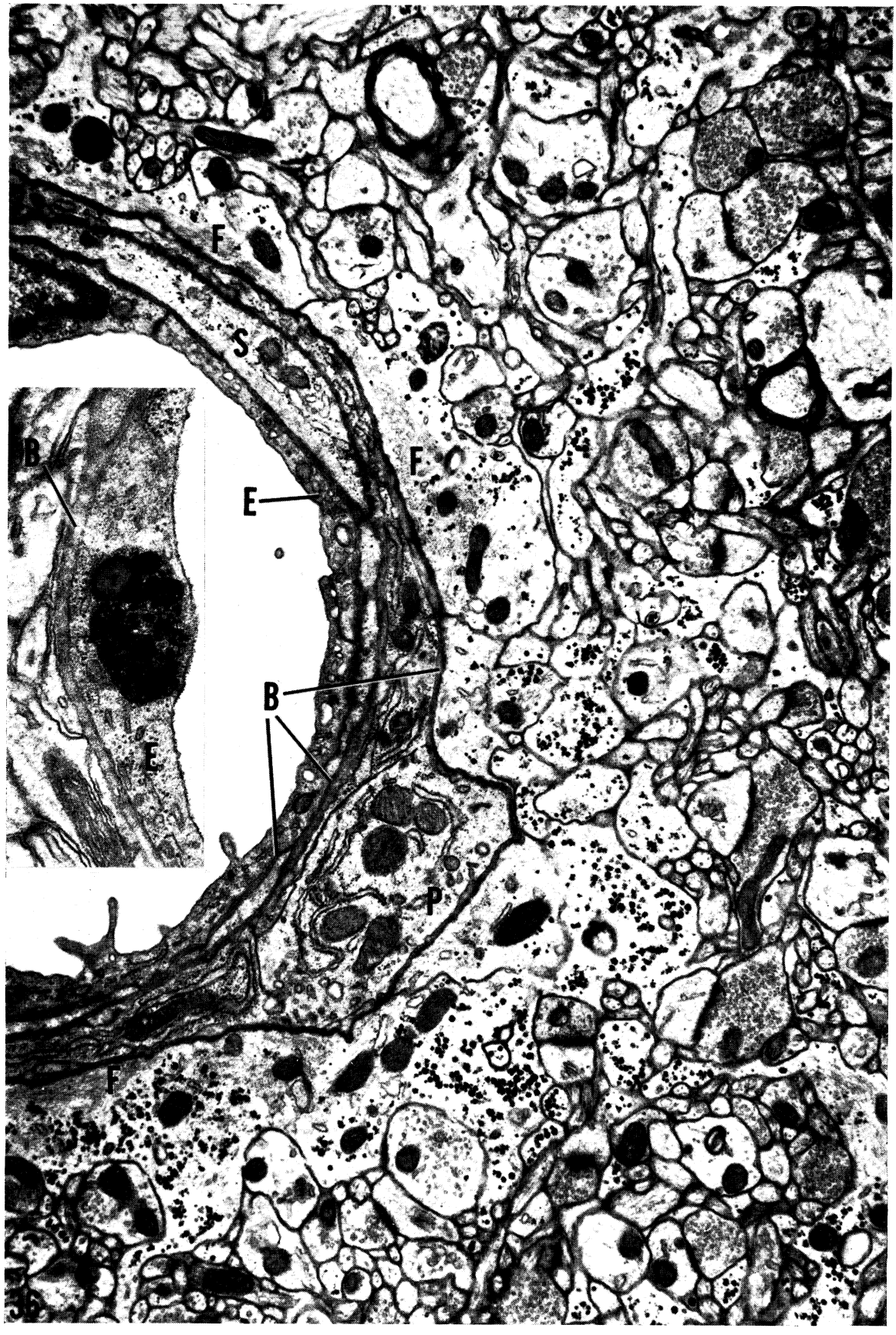

F
S
B
E
F
E
B
P
F

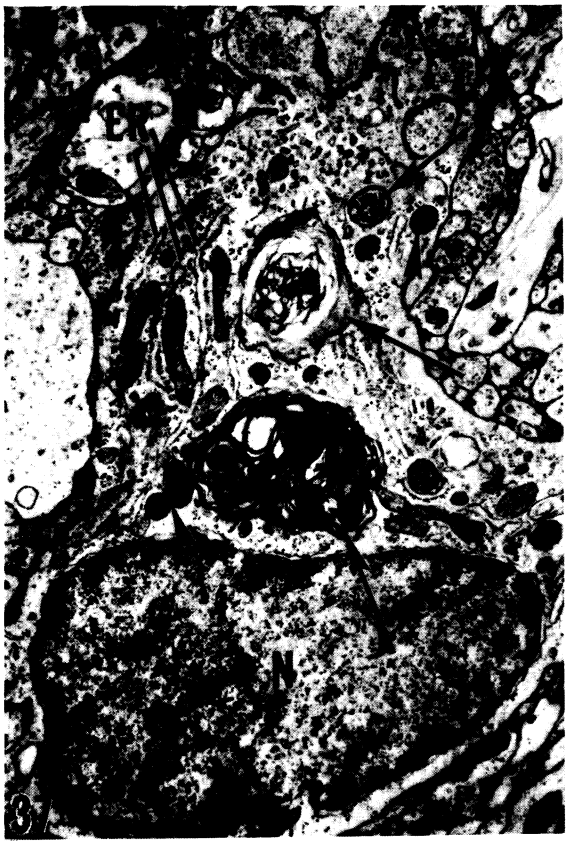
FIGURE 36. Showing a small blood vessel in the cortex of SI five days after a lesion in SII. The perivascular foot processes of the astrocytes are obviously swollen and contain large numbers of glycogen granules and filaments (F). P, pericyte; B, basement membrane; E, endothelium; S, smooth muscle cell. Lead citrate and uranyl acetate; × 18000. *Inset*: a granular and vacuolated dense body in the endothelium of a cortical capillary following a lesion in the thalamus. Lead citrate; × 37000.

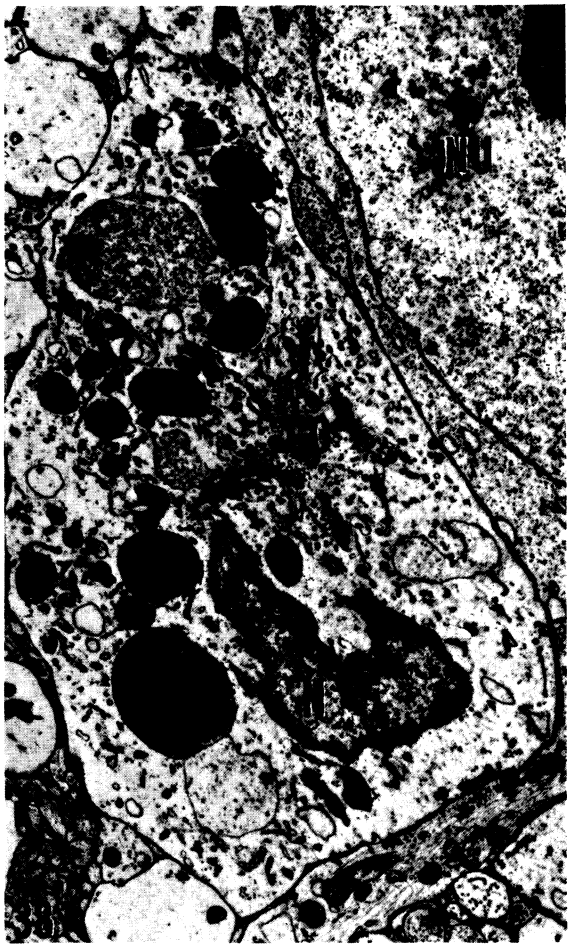
FIGURE 37. An unusual type of glial cell from the cortex of SI near a lesion in SII. This cell contains elongated cisternae of granular endoplasmic reticulum (ER), small dense bodies (arrow heads), several forms of larger inclusion (arrows) and a nucleus resembling that of an oligodendrocyte. Lead citrate and uranyl acetate; $\times 13000$.

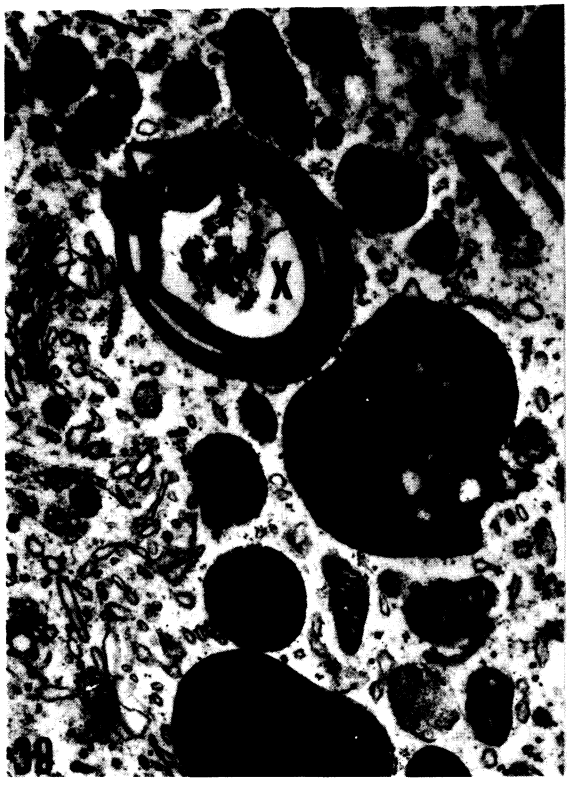
FIGURE 38. A common form of glial cell in the cortex which was isolated by a series of knife cuts. In this cell many forms of dense body are seen and there is a large amount of Golgi apparatus (GO) but endoplasmic reticulum is not prominent. The nucleus (N) resembles that of an oligodendrocyte. NU; nucleus of a neuron. Lead citrate and uranyl acetate; $\times 12000$.

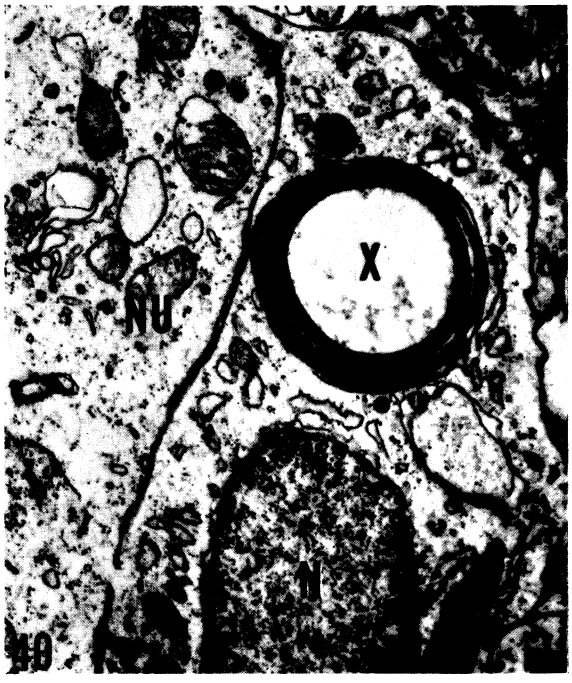
FIGURE 39. Part of the cytoplasm of an astrocyte in the cortex of SI following a lesion in the ipsilateral SII. Dense bodies, Golgi membranes and a membrane-bound myelin figure (X) resembling an engulfed portion of the sheath of a degenerating axon, are present. Lead citrate and uranyl acetate; $\times 31000$.

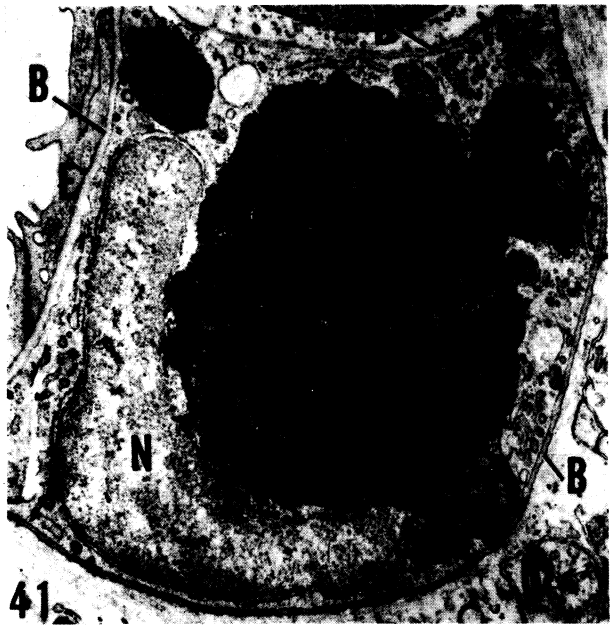
FIGURE 40. Part of an astrocyte in SI after a lesion in the opposite cortex; this cell contains a few small dense bodies and a large membrane-bound myelin figure (X). Lead citrate and uranyl acetate; $\times 20000$.

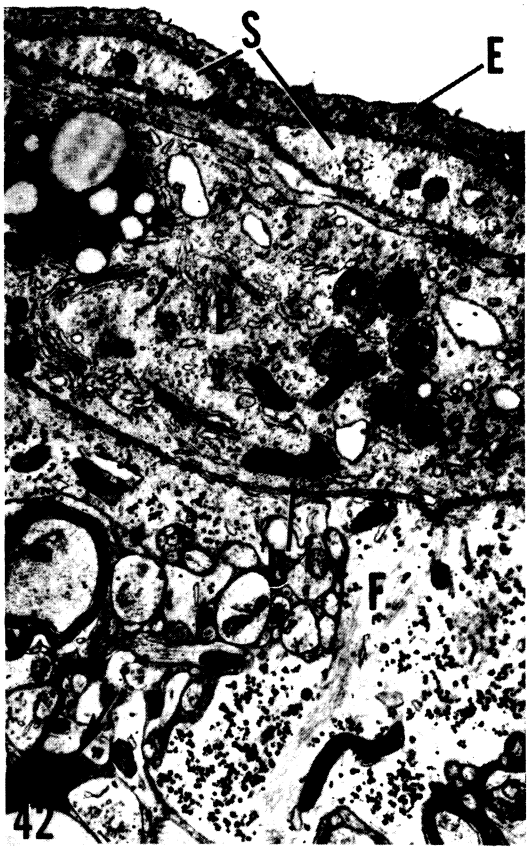

ER
N


NU

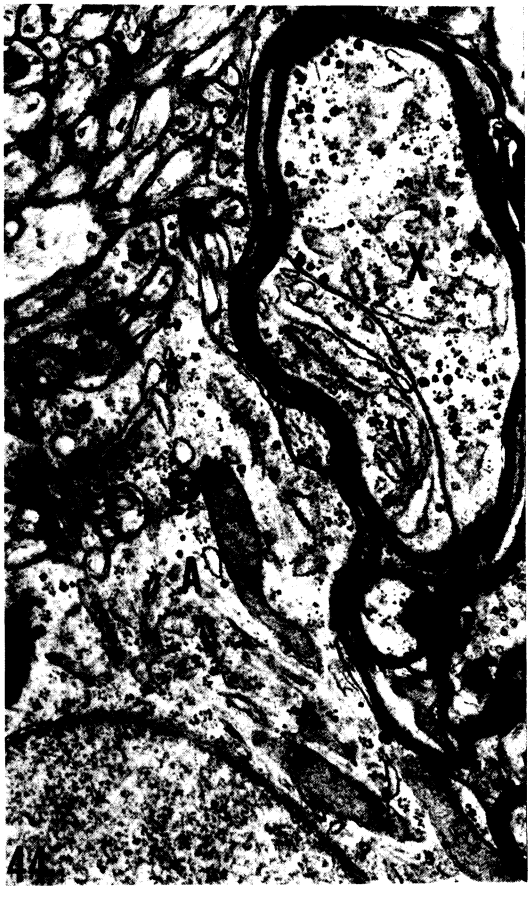

X


X
NU


B
N
B
41


S
E
F
42


NA
43


X
A
44

contact zone (figures 6, 7, plate 32) or from the side, parallel to the contact zone (figures 5, 8, plate 32). The profile may, therefore, assume a very irregular appearance (figures 5 to 9, plate 32), often giving the impression of being cut in two by the glial tongues. In other cases, the terminal is compressed from the sides so that in a single section it may appear as a dense bulb attached at the synaptic thickening and with a thin 'tail' of degenerating axoplasm arising from its surface opposite the point of contact (figures 11 to 13, plate 33). Frequently this arrangement can assume the extreme form illustrated in figure 13. It is to be emphasized that while tongues of astrocytic cytoplasm may deeply invaginate the degenerating terminal, the plasma membranes of the terminal and of the astrocytic process are still quite distinctly separate. At these intermediate stages there is as yet no evidence of absorption within the glial cytoplasm of the degenerating fragments.

Of some interest is the relationship of the preterminal unmyelinated segments of degenerating axons to the invading astroglia. Almost without exception, when these preterminal segments are seen attached to a shrunken degenerating terminal, they assume a sinuous outline, compressed and invaginated at intervals by tongues of astrocytic cytoplasm; the latter may at times be reduplicated (figures 15 to 18, plate 34) and often gives the impression of spiralling about the degenerating unmyelinated segment. Adjacent tongues or lamellae of the reactive astrocyte may have retracted from each other leaving a gap from which a portion of the degenerating preterminal segment has disappeared.

The later stages of the degenerative process are characterized by progressive shrinkage of the part of the dense terminal which remains attached at the synaptic thickening. The degenerating profile may contain distorted, though now shrunken mitochondria but, more commonly, only irregular fragments of disrupted mitochondrial membranes and cristae are seen (figure 10, plate 33); ultimately, all inclusions disappear from the homogeneously dense matrix, leaving a thin sliver of dense material attached at the synaptic thickening and bounded on all sides except at the synaptic contact zone by reactive astroglial processes (figures 23, 24, plate 35). Though it may be considerably shrunken in most dimensions, the degenerating fragment always remains at least as wide as the postsynaptic thickening at which it is attached. Throughout the whole process of degeneration and at all the survival periods used, no obvious changes occurred in the postsynaptic thickening and the remainder of the postsynaptic element always appeared unaffected.

Frequently at these later stages there is evidence that portions of a degenerating terminal which had previously been isolated by glial tongues from the part attached at the point of

---

Description of plate 40

Figure 41. A large vacuolated dense body in the cytoplasm of a vascular pericyte adjacent to a cortical lesion. B, basement membrane; E, endothelium; N, nucleus. Lead citrate; × 15000.

Figure 42. Part of the wall of a small blood vessel in the cortex at the edge of a lesion. The pericyte (P) contains Golgi membranes, vacuoles, and dense bodies some of which are vacuolated. Note the very swollen astroglial foot processes containing glycogen and filaments (F) and the dense material (arrow) in the extracellular space. S, smooth muscle cells. Lead citrate and uranyl acetate; × 14000

Figure 43. The subpial astroglial lamellae of a part of SI close to a lesion in SII. The surface of the brain is towards the top. The glial lamellae are swollen and contain many glycogen granules and filaments (F). A small degenerating axon (arrow) is present just beneath the surface; cf. the normal axon (NA). O: part of an oligodendrocyte. Lead citrate; × 17000.

Figure 44. Part of an astrocyte (A) lying immediately adjacent to a degenerating myelinated axon (on the right) and apparently sending processes into the reduplicated part of the myelin sheath (X). Lead citrate; × 21000.

synaptic contact have become separated and engulfed in the astroglial cytoplasm. Here they are bounded by a single membrane and commonly assume a rounded shape and may be seen to contain irregularly whorled membranous lamellae. Some may be intimately associated with a cluster of glycogen granules (figures 25 to 30, plate 36). They are visible at all the survival periods used but are especially common in the animals which were killed after 4 to 6 days.

The degenerative process appears to be a cyclical one, both early and late stages of terminal degeneration occurring at all survival periods within the range of 2 to 6 days. There were, however, a few minor variations in that in animals killed after two days far fewer degenerating terminals are seen and although both early and late types are present, the former seem to be in the majority. Early and late stages appear with equal frequency at 4- to 6-day survival periods in animals in which commissural and thalamo-cortical fibres have been interrupted but in those in which ipsilateral association fibres passing from SII to SI have been damaged most endings display early and intermediate rather than late changes. Even here, however, a few terminals in very late stages of degeneration are also present.

Degenerative changes in myelinated axons

Many obviously degenerating myelinated axons are seen in SI. Within the limits of the survival times used, all obvious degenerative changes affect the axon itself; while the myelin sheath is in many cases considerably distorted, there is no dissolution or fragmentation of the myelin nor loss of its characteristic lamellar organization. The changes affecting the degenerating myelinated axons resemble those observed during degeneration of their terminals. There is a marked increase in the electron density of the axoplasm and within this dense matrix mitochondria are embedded. It is not possible to be sure whether neurotubules and neurofilaments disappear or are merely obscured by the extreme density of the background axoplasm. In many instances the mitochondria are swollen with fragmented cristae and occupy most of the cross-sectional area of the axon (figure 35, plate 37); in others they appear shrunken and distorted and assume the 'glassy' appearance described in some of the degenerating terminals. In some instances when seen in cross-section, the degenerating axon fills its myelin sheath, but in others it may be shrunken away from the inner myelin lamella (figure 34, plate 37) and lies embedded in a cytoplasmic component resembling that of a typical oligodendrocyte. It is possible that this cytoplasm is derived from the inner myelin lamellae, as in these cases the myelin sheath often appears rather thinner than would be expected in a normal axon of equivalent diameter. When seen in longitudinal section it is obvious that these two appearances visible in cross-section are but different aspects of the same process, for the degenerating axon is irregularly distorted and often beaded, consisting of swollen parts which fill the sheath and narrow connecting strands which do not. These changes appear at all survival periods and are the only type present at the 2-day period. At the later survival times, many axons appear to be in a rather more advanced state of degeneration; in some, particularly those of small diameter, this is manifested by vacuolation of the dense degenerating axoplasm. In fibres of larger diameter, there is more frequently a fine fragmentation of the degenerating axon (figures 31, 32, plate 37). This fragmentation may be combined with vacuolation of some of the particles and is occasionally accompanied by reduplication of the myelin sheath, which may come to surround a space which is usually invaded by astroglial processes (figure 44, plate 40). In other instances the products of degeneration may disappear completely from a segment of the myelin sheath, the walls of which then collapse upon one another.

In blocks from two different brains, each with a lesion in the ventroposterior nucleus of the thalamus and after a post-operative survival of 5 days, a small number of very large myelinated profiles were observed and could be traced for considerable distances in serial sections. Some of these contained a flocculent cytoplasm and one or more very swollen mitochondria resembling those present in the obviously degenerating smaller myelinated fibres. The others contained a marked aggregation of neurofilaments (figure 33, plate 37) and many swollen, distorted mitochondria. Profiles such as these were never seen in normal brains nor in brains with lesions in the cortex, and it seems probable that they represent degeneration of the largest thalamo-cortical fibres.

Glial reactions

In the presence of axonal degeneration in the cortex, the astrocytes present undergo a reactive change which is especially obvious close to a lesion, as for example in the part of SI adjoining a lesion in SII. In addition to those astroglial processes in the immediate vicinity of degenerating axons or terminals, the majority of the astroglial processes present (especially those forming the stacked lamellae beneath the pia mater and the foot processes applied to the basement membrane of capillaries) show swelling and intense glycogen accumulation (figure 36, plate 38, figures 42, 43, plate 40). On approaching the very edge of a lesion or after undercutting the cortex, virtually all the astrocytic processes and even their parent cell somata share this reaction, giving sections viewed at low power a speckled appearance. Within individual processes the glycogen accumulation may be very heavy and many of the granules may be very large or fused to form rosettes.

In parts of SI more remote from lesions in SII and in SI following lesions in the thalamus or opposite cortex, the glial reaction is similar though far less intense. An occasional, perivascular foot process may show enlargement and glycogen deposition, but in general the reaction is confined to those astroglial processes in the immediate vicinity of a degenerating axon or terminal. Several of the features displayed by these processes, notably their mode of invasion of the degenerating terminal have already been alluded to but a more complete description may be given at this point. By the time the degenerating terminals have reached the stage of shrinkage and compression with much reduction in their complement of synaptic vesicles, most, if not all, have become enveloped by one or more swollen, glycogen-containing astrocytic processes. Apart from glycogen and occasional mitochondria, these processes may contain no other organelles in their clear cytoplasm. Some may display a few short cisternae of rough-surfaced endoplasmic reticulum, a multivescular body and a few irregular tubules. Only a very small number contain bundles of glial filaments although these are very common in astrocytic cell bodies, in the perivascular foot processes, and in the subpial lamellae (figure 36, plate 38, figure 43, plate 40). Infrequently, an astrocyte cell soma may fold itself over a degenerating terminal (figure 11, plate 33) but more commonly only the smaller processes do so. In folding around a degenerating terminal, the astroglial process may also come to enfold the dendritic spine with which the terminal makes synaptic contact, but glial cytoplasm never invades the synaptic cleft and within the range of survival periods studied, there is no evidence that dendritic spines are actually separated by the glia from their attachments to their parent dendrite. In suitable single sections and in series of sections, the pedicle of the spine is always seen to be intact, at least up to the seventh postoperative day (figure 14, plate 33).

As degeneration advances and the terminal is invaded and broken up by the reactive astroglia, so other organelles appear in the astroglial cytoplasm: some of these additional

inclusions resemble the degenerating terminal and as they are surrounded by a single membrane only, they would appear to be fragments which have become broken off and taken into the glial cytoplasm. Further inclusions are small, membrane-bound, dense bodies resembling primary lysosomes (figures 15, 16, plate 34) while additional ones are elongated, tubular structures some of which contain dense material and others of which are clear. Some of the glycogen particles, especially those associated with an engulfed fragment, may at this stage become membrane-bound (figure 30, plate 36; figure 37, plate 39). At this point, in some instances, the astroglial process may assume a finely granular appearance and the large glycogen granules disappear; whether the finer granules represent broken down glycogen particles or some other inclusion cannot be determined (figures 15 to 20, plate 34).

In SI of brains in which lesions were placed in the thalamus or in the opposite cortex and in which there could have been no direct damage to the region under study, two main types of glial cell, conforming closely to the typical astrocyte and oligodendrocyte described in normal material (Mugnaini & Walberg 1964; Maxwell & Kruger 1965*a*; Vaughn & Peters 1968) are present. Within the present range of survival periods, only the astrocyte appears to be directly involved in breaking down degenerating axons and their terminals. Apart from their involvement in invading and engulfing degenerating terminals and axon fragments, astrocytic processes may be found filling spaces in the reduplicated myelin sheaths of degenerating axons (figure 44, plate 40). Many astrocytic foot processes and cell somata may be found to contain lamellated bodies and myelin figures some of which resemble very closely engulfed degenerating fibres. Other membrane- and non-membrane-bound dense bodies may also be present. Other types of glial cell which are occasionally present in normal brains are also found. In some instances these resemble the 'lighter' oligodendrocyte of Kruger & Maxwell (1966), while the appearance of others more closely resembles the 'third' type of glial cell described by Vaughn & Peters (1968). That these may play some part in the degenerative process is indicated by the presence in their cytoplasm of membrane- and non-membrane-bound dense bodies, some of them lamellated, and also of large partially vacuolated osmiophilic bodies resembling certain forms of lysosome.

In SI adjacent to a lesion in SII, or after undercutting the cortex, typical astrocytes and oligodendrocytes are still present, the former showing swelling and dense concentrations of glycogen granules as described above. However, many more atypical types which are difficult to classify are also found. Many of these resemble oligodendrocytes in having a dense nucleus with clumped chromatin material but in every case the cytoplasm is pale and in some respects resembles that of an astrocyte. In regard to their contained organelles, however, these cells resemble neither astrocytes nor oligodendrocytes. Some have elongated, sinuous cisternae of granular endoplasmic reticulum while others may contain many very short rough-surfaced cisternae (figures 37, 40, plate 39). Further types may possess very little granular endoplasmic reticulum but instead show a massive accumulation of Golgi apparatus and free-lying smooth cisternae (figures 38, 39, plate 39). All have numerous, membrane-bound bodies of varying size and electron density (figures 37 to 40, plate 39); many contain figures resembling very closely the engulfed myelin sheaths of degenerating axons, while a number may possess distorted dense fragments resembling degenerating axon or terminal fragments and surrounded by a single membrane. None of these atypical cells has free glycogen particles in its cytoplasm but a few may display a membrane-bound group of such granules (figure 37, plate 39).

Certain of the changes appear to affect the vascular pericytes which are situated within a

splitting of the basement membranes of cerebral capillaries. Following lesions in the thalamus or opposite cortex as well as after lesions of the ipsilateral SII, many pericytes in SI are swollen when compared with the normal and contain increased amounts of Golgi apparatus, together with membrane-bound bodies of variable size and electron density which resemble lysosomes. But the most striking feature is the very common appearance of large osmiophilic complexes (figures 41, 42, plate 40); these are bounded by a single membrane and consist of large numbers of dense granules of varying sizes among which are distributed large and small clear vacuoles. In certain respects these resemble the lipofuscin granules occasionally seen in neurons in the normal brain but are far larger and far more numerous. They are also far more granular and more highly vacuolated and their closest resemblance is to the dense vacuolated lytic bodies shown by Smith & Farquhar (1966) in the mammotrophic cells of the anterior pituitary during resorption of secretory granules. Generally in the experimental brains these large profiles are restricted to the pericytes but smaller ones are occasionally encountered in endothelial cells and in glial cells. Close to a lesion, however, some large ones are also found in astrocytes and oligodendrocytes.

'Dark dendrites' and 'dark cells'

Very rarely, in otherwise well-fixed normal and experimental material, a neuronal perikaryon, dendrite or dendritic spine is seen which appears normal in all respects except in having a very dense cytoplasmic matrix. Such dark processes may also be shrunken and distorted and show vacuolation of their contained mitochondria. Two reports (Mugnaini 1965; Vaughn & Peters 1968) have suggested that these are not normal tissue components but are induced by some defect during processing. Evidence to support this was forthcoming in the present study in which at the commencement of a perfusion, a glass cannula broke and obstructed the ascending aorta so that only a very limited quantity of fixative reached the brain. In this brain, otherwise discarded for the purposes of the investigation, large numbers of very dense and shrunken neurons were present, though fixation of adjoining elements was moderately good. Some of these dense profiles received degenerating thalamo-cortical axon terminals, but it should be emphasized that in no other case in a well-fixed brain was such a change observed in the profile postsynaptic to a degenerating terminal.

Discussion

The present study has dealt only with those changes which occur in degenerating axons and their terminals within the cerebral cortex in the immediate postoperative period of 2 to 6 days. This provides a necessary basis for experimental investigations such as the one described in the following paper, but it is to be emphasized that more extensive studies will be required before our understanding of the process of terminal degeneration is complete. An obvious extension of the present investigation would be to follow the degenerative changes over much longer survival periods, particularly to determine what changes occur in the synaptic contact zone and whether the post-synaptic profile is affected in any way.

Perhaps the most significant feature of the degenerative process in the cerebral cortex is that axon terminals, even those of the same fibre system appear to degenerate in a cyclical manner rather than simultaneously. Terminals in early and late stages of degeneration are seen at all the survival periods studied irrespective of whether commissural, associational or thalamo-cortical fibres have been interrupted. There is, however, a definite trend in the process in that

two days after operation early degenerating terminals are in the majority, whereas at 4, 5 and 6 days more than 50 % are in the later stages of degeneration. The full duration of this cyclical process can only be determined by further investigation, but Westrum (1966), in an abstract, notes that some degenerating axon terminals appear in the prepyriform cortex of the rat up to 12 weeks after removal of the olfactory bulb. Similarly, in the inferior olive Walberg (1965) found that some degenerating terminals remain as long as 112 days after a lesion is placed in the cerebral cortex. On the present evidence alone, it is obvious that for terminal degeneration to be completed in the somatic sensory cortex a far longer period of time is required than in certain other sites. In the ventroposterior and medial and lateral geniculate nuclei of the cat, for example, degeneration of cortico-thalamic axon terminals only becomes obvious on the fourth postoperative day, but at that time almost without exception the terminals are at a very late stage of degeneration (Jones & Powell 1970). Degeneration of optic tract terminals in the lateral geniculate nucleus of the cat (Szentágothai *et al.* 1966) and of dorsal root terminals in the substantia gelatinosa (Heimer & Wall 1968) also appears to be a rapid process, in each case being largely complete by the fourth day. Thus, for experimental electron microscopic studies in the thalamus and substantia gelatinosa survival period is critical, whereas in the cortex, dorsal horn (Heimer & Wall 1968; Ralston 1968) and apparently in other sites as well (e.g. Mugnaini *et al.* 1967) a wider range of survival periods is permitted. The findings in the thalamus and substantia gelatinosa indicate that in electron microscopic studies of any region of the brain it is necessary to establish in the first instance the nature and, particularly, the time course of terminal degeneration within it.

One possible factor in causing terminal degeneration in one site to be cyclical and prolonged while that in another is abrupt and rapid, is the degree of collateral branching of afferent axons to the part of the brain under consideration. While cortico-thalamic fibres seem to branch a good deal (Tömböl 1967; Guillery 1967; Ramón y Cajal 1911), they are of universally small diameter (Jones & Powell 1970) and this would also seem to be true of dorsal root fibres terminating in the substantia gelatinosa (Ranson 1913; Ramón y Cajal 1909). On the other hand, in the cortex, thalamo-cortical and association fibres at least, branch widely; the parent axons are large and they divide into branches of variable diameter. Whether thinner fibres degenerate at a slower or faster rate than thicker ones is at present an unresolved question. Van Crevel & Verhaart (1963*a*, *b*) considered on the basis of light microscopic observations that in certain tracts thin fibres degenerate more slowly than thick ones. By contrast, Mugnaini *et al.* (1967) note that thinner primary vestibular fibres to Deiter's nucleus degenerate more rapidly than thick ones and a similar rate of degeneration would be in keeping with the rapidity of degeneration in the substantia gelatinosa and thalamus where the dorsal root and cortical afferents respectively are very thin.

The type of degeneration observed in the cortex resembles very closely that first described by Colonnier & Gray (1962) in the cerebral cortex and subsequently found in many parts of the brain by other workers. None of the degenerating terminals exhibited a stage of neurofilamentous hyperplasia; this is a stage of degeneration in the terminals of optic tract fibres in the lateral geniculate nucleus of the monkey (Colonnier & Guillery 1964) and cat (Szentágothai *et al.* 1966). It has also been demonstrated in the optic tectum of the chick (Gray & Hamlyn 1962) and rat (Lund 1967), in the terminals of the olivo-cochlear bundle (Smith & Rasmussen 1965), in the terminals of centrifugal fibres to the pigeon retina (Dowling & Cowan 1966), in Deiter's nucleus (Mugnaini & Walberg 1967), and in certain laminae of the dorsal horn (Ralston 1968). In many

of these cases the neurofilamentous hyperplasia is followed by shrinkage and increased electron density of the terminal, so that the later stages resemble the dense type of degeneration described in the present and in numerous other investigations. Several possibilities have been advanced to account for this massive increase of neurofilaments in some terminals in response to division of the parent axon (see Mugnaini & Walberg 1967); one which does not seem to have been considered is that it may be closely related to the size of the terminal. It is significant that all the terminals which display this type of degenerative reaction are very large in normal material and in some of the sites mentioned may normally contain a few neurofilaments. Only the largest axon terminals in the somatic sensory cortex contain neurofilaments (Jones & Powell 1970*b*) and these do not seem to be the terminals of the three types of fibre studied in the present material, for these can be seen to be quite small from their appearances at the earliest stages of degeneration. Similarly, in those pathways in which the terminals do not show neurofilamentous hyperplasia, the terminals are small. A necessary prerequisite for neurofilamentous degeneration may, therefore, be a large terminal normally containing a few neurofilaments. It seems significant that some degree of neurofilamentous hyperplasia was noted in the largest degenerating thalamo-cortical axons in the present study, for large axons should normally contain more neurofilaments than smaller ones. Against this hypothesis however, is the observation of Guillery (1965) that in the normal lateral geniculate nucleus of the monkey the 'giant terminals rarely show more than one or two small bundles of neurofilaments', and also the general conclusion of this author that the largest terminals do not contain the most fibrils.

It is apparent that in the early stages of glial invasion, degenerating axon terminals are both compressed and invaginated by the glial cytoplasm. It is probable that the glia also tends to wrap around the ending and especially around the terminal, unmyelinated part of the axon, as evidenced by the reduplicated tongues of astroglia seen in many instances. The sinuosity of the degenerating terminal segment is so characteristic and so common—not only in the cortex but also in the caudate nucleus and olfactory bulb (J. M. Kemp & J. L. Price, personal communications)—that it seems to indicate some constant mechanism. We have only observed (small) dilated extracellular spaces in relation to the tongues of glial cytoplasm enveloping these sinuous degenerating terminal axon segments. The impression is gained that these represent points at which the glia have retracted from the degenerating axon during fixation. Mugnaini *et al.* (1967) have described increased extracellular space formation about degenerating terminals as a stage preceding the glial invasion in Deiter's nucleus but this has not been found in the present material. The reactive astroglial processes must, therefore, make their way towards the degenerating terminal by displacing other, normal profiles. This possibly accounts for the early compression of the degenerating terminal in the stages before glial envelopment.

Fragmentation of the degenerating terminal is obviously one of the main features of the degenerative process and from the intimate association of the astrocytic cytoplasm, it would appear that the astrocytes play a significant part in the dissolution of the products of degeneration. The astrocytic processes completely overlap the degenerating profile but the actual fragmentation appears to be carried out by mechanisms extrinsic to the astroglial cytoplasm, because the fragments remain separated from the glial cytoplasm by their own plasma membranes and by that of the astrocyte. As degeneration advances, however, many quite large dense fragments, frequently containing irregular stacks of membranous lamellae and denser granules, appear within the astrocytic process and are surrounded by a single membrane which suggests that they have now become cell inclusions. Their presence within the astroglial processes which

in the same section are intimately related to the portion of the terminal remaining attached to the postsynaptic profile, or to degenerating axon fragments, leaves little doubt that they are phagocytosed particles. They may perhaps, therefore, be considered as secondary lysosomes. This is supported by the fact that at the stage of maximal fragmentation, the enveloping glia show an aggregation of inclusions resembling primary lysosomes.

The fate of the part of the terminal remaining attached at the synaptic contact zone is unknown but up to 6 days after operation there is no indication of its becoming detached from it postsynaptic profile. Westrum (1966) has made similar observations in the prepyriform cortex of the rat, finding that many remnants of degenerating terminals remain attached for as long as 12 weeks. However, even after 7 days Westrum notes that some of the degenerating terminals are displaced by glia, although the postsynaptic element remains intact. Displacement of the attached part of the degenerating terminal by glia has not been observed in the present material. Colonnier (1964) considered that after undercutting the neocortex many of the dendritic spines which received degenerating terminals were phagocytosed, together with the terminal, by reactive glial processes. In the present investigation, many spines and their attached degenerating fragments were seen to be surrounded by glia but their plasma membranes were always distinct from that of the glia and in serial sections and in favourable single sections, the spines retained their attachments to the pedicle connecting them to the parent dendritic shaft. Up to 6 days at least, there is no evidence that the dendritic spines are detached from the parent dendrite. Westrum (cited by Scheibel & Schiebel 1968) has found no evidence for removal of spines by glia in the prepyriform cortex up to 135 days after excision of the olfactory bulb. Recent light microscopic studies using the Golgi technique have shown that following deafferentation or sensory deprivation, many dendritic spines in the cerebral cortex fail to impregnate (Globus & Scheibel 1967*a*, *b*; Valverde 1967, 1968; Valverde & Estéban 1968). This has usually been taken to indicate that these spines have disappeared. However, the failure of a neuronal element to impregnate is insufficient evidence for claiming that this element is lost. In the present investigation, no recognizable changes affected the postsynaptic thickening nor the postsynaptic profile as a whole; whether the use of longer survival periods would show any transneuronal changes must remain an open question. The brief report of Westrum (1966) would suggest that no changes of this nature occur in the prepyriform cortex.

Damage to the cortex or the presence of degeneration within it is always accompanied by swelling of astroglial processes and a marked increase in the number of glycogen granules which they contain. The glycogen may be identified as such by its close similarity to that originally identified in electron micrographs by Revel, Napolitano & Fawcett (1960) and subsequently shown in many parts of the central nervous system (see Maxwell & Kruger 1965*a*; Laatsch & Cowan 1967). Normal astrocytes frequently contain an occasional glycogen particle, but the difference between the normal complement of such granules and that which appears in response to local injury or to the presence of degeneration induced by distant lesions, is dramatic. The degree of astroglial swelling and the intensity of the glycogen accumulation is to a large extent dependent upon the proximity to the lesion, and local oedema, herniation of the exposed parts of the brain, a low grade local inflammatory response, and the presence in the vicinity of debris from the lesion, all seem likely factors to be considered. These observations indicate that in any experimental study designed to elucidate the part played by the neuroglia in reabsorbing degenerating axons and their terminals, the lesion and the region under investigation should be divorced from one another by as large a tract of normal brain as possible. Furthermore, the

region to be investigated should not be exposed at any time during the operative interference.

The oligodendrocytes appear to play little part in the process at the survival periods used, although some transitional cells which are difficult to classify as astrocytes or oligodendrocytes do appear and commonly contain myelin figures and other dense bodies. It is possible that these are the 'lighter' oligodendrocytes of Kruger & Maxwell (1966) or the 'third' glial cell of Vaughn & Peters (1968). In general, at the survival periods used in the present study the myelin sheaths of degenerating axons, though commonly collapsed and distorted, are rarely fragmented and none lose their characteristic periodicity. At later stages, changes would be expected in these and it would be interesting to know whether these are accompanied by changes in the oligodendrocytes which form the myelin sheath (see Bunge 1968) or whether astrocytes or some other type of cell commence phagocytosing them. Adjacent to a lesion, astrocytes are predominantly involved in attacking the degenerating axons or terminals, but large numbers of glial cells which are impossible to classify as astrocytes or oligodendrocytes also appear and seem to take part in engulfing degenerating fragments. As few of these contain filaments or glycogen, except as membrane-bound clusters, it is likely that they are not astrocytes, but in their lack of cytoplasmic density and absence of tubules and free ribosomal rosettes they bear few resemblances to the oligodendrocyte of the normal cortex. A final evaluation must, therefore, await a more extensive study.

Another element which appears to react to the presence of degeneration, is the vascular pericyte and by far the most distinctive change in it is the appearance of one or more very large membrane-bound complexes consisting of considerable numbers of osmiophilic granules of all sizes interspersed with large and small vacuoles containing translucent material. These inclusions are found occasionally in normal pericytes and have been described in normal oligodendrocytes (Mugnaini & Walberg 1964) but are never so large nor so common as in the presence of degeneration; adjacent to a lesion similar inclusions may also be found in oligodendrocytes, astrocytes and even in capillary endothelium. In some respects these inclusions resemble the lipofuscin granules which are found in neurons of normal animals and which increase in number with age (Samorajski, Ordy & Keefe 1965; Samorajski, Ordy & Rady-Reimer 1968), but they resemble more closely the vacuolated dense bodies which appear in the kidney (Miller & Palade 1964) and anterior pituitary (Smith & Farquhar 1966) during resorption of extraneous protein or of unreleased secretory products and which have been shown to possess lytic activity. If they are identical, then they should be considered as lysosomes. Whether they represent breakdown products of degeneration which are being passed on to the blood stream cannot be determined, but their constant appearance in the pericytes suggests that the lining of the cerebral capillaries is by no means inert during degeneration. Maxwell & Kruger (1965*b*) have indicated that following damage caused by ionizing radiation, pericytes in the walls of capillaries in the cerebral cortex of the rat break out of the confining basement membrane and invade the neuropil. This was not observed in the present study although, conceivably, some of the atypical glial cells described in relation to a lesion could represent escaped pericytes.

This work was supported by grants from the Medical and Science Research Councils and was done during the tenure of a Nuffield Dominions Demonstratorship by E. G. J. on leave from the University of Otago, New Zealand.

References

Bunge, R. P. 1968 Glial cells and the central myelin sheath. *Physiol. Rev.* **48**, 197–251.

Colonnier, M. 1964 Experimental degeneration in the cerebral cortex. *J. Anat., Lond.* **98**, 47–53.

Colonnier, M. & Gray, E. G. 1962 Degeneration in the cerebral cortex. In *Electron microscopy* (ed. E. S. Breese) p. U 3. New York: Academic Press.

Colonnier, M. & Guillery, R. W. 1964 Synaptic organization in the lateral geniculate nucleus of the monkey. *Z. Zellforsch.* **62**, 333–355.

Crevel, H. van & Verhaart, W. J. C. 1963*a* The rate of secondary degeneration in the central nervous system. I. The pyramidal tract of the cat. *J. Anat., Lond.* **97**, 429–450.

Crevel, H. van & Verhaart, W. J. C. 1963*b* The rate of secondary degeneration in the central nervous system. II. The optic nerve of the cat. *J. Anat. Lond.* **97**, 451–464.

Dowling, J. E. & Cowan, W. M. 1966 An electron microscopic study of normal and degenerating centrifugal fiber terminals in the pigeon retina. *Z. Zellforsch.* **71**, 14–28.

Globus, A. & Scheibel, A. B. 1967*a* Synaptic loci on parietal cortical neurons: terminations of corpus callosum fibers. *Science, N.Y.* **156**, 1127–1129.

Globus, A. & Scheibel, A. B. 1967*b* Synaptic loci on visual cortical neurons of the rabbit: the specific afferent radiation. *Exptl Neurol.* **18**, 116–131.

Gray, E. G. & Hamlyn, L. H. 1962 Electron microscopy of experimental degeneration in the avian optic tectum. *J. Anat. Lond.* **96**, 309–316.

Guillery, R. W. 1965 Some electron microscopical observations of degenerative changes in central nervous synapses. In *Progress in brain research*, vol. 14 (eds. M. Singer and J. P. Schadé), pp. 57–76. Amsterdam: Elsevier.

Guillery, R. W. 1967 Patterns of fiber degeneration in the dorsal lateral geniculate nucleus of the cat following lesions in the visual cortex. *J. Comp. Neurol.* **130**, 197–222.

Heimer, L. & Wall, P. D. 1968 The dorsal root distribution to the substantia gelatinosa of the rat with a note on the distribution in the cat. *Exptl Brain Res.* **6**, 89–99.

Jones, E. G. & Powell, T. P. S. 1970 An electron microscopic study of the mode of termination of cortico-thalamic fibres within the thalamic relay nuclei of the cat. *Proc. Roy. Soc. Lond.* B **172**, 173–185.

Jones, E. G. & Powell, T. P. S. 1970*a* An electron microscopic study of the laminar pattern and mode of termination of the afferent fibre pathways in the somatic sensory cortex of the cat. *Phil. Trans. Roy. Soc. Lond.* B **257**, 45–62.

Jones, E. G. & Powell, T. P. S. 1970*b* Electron microscopy of the somatic sensory cortex of the cat. I. Cell types and synaptic organization. *Phil. Trans. Roy. Soc. Lond.* B **257**, 1–11.

Kruger, L. & Maxwell, D. S. 1966 Electron microscopy of oligodendrocytes in normal rat cerebrum. *Am. J. Anat.* **118**, 391–409.

Laatsch, R. H. & Cowan, W. M. 1967 Electron microscopic studies of the dentate gyrus of the rat. II. Degeneration of commissural afferents. *J. Comp. Neurol.* **130**, 241–262.

Lund, R. D. 1967 Ultrastructural investigation of the termination of optic nerve fibers in the superior colliculus of the rat. *Anat. Rec.* **157**, 281.

Maxwell, D. S. & Kruger, L. 1965*a* The fine structure of astrocytes in the cerebral cortex and their response to focal injury produced by heavy ionizing particles. *J. Cell Biol.* **25**, 141–157.

Maxwell, D. S. & Kruger, L. 1965*b* Small blood vessels and the origin of phagocytes in the rat cerebral cortex following heavy particle irradiation. *Exptl Neurol.* **12**, 33–54.

Miller, F. & Palade, G. E. 1964 Lytic activities in renal protein absorption droplets. An electron microscopical cytochemical study. *J. Cell Biol.* **23**, 519–552.

Mugnaini, E. 1965 'Dark cells' in electron micrographs from the central nervous system of vertebrates. *J. Ultrastruct. Res.* **12**, 235–236.

Mugnaini, E. & Walberg, F. 1964 Ultrastructure of neuroglia. *Ergebn. Anat. EntwGesch.* **37**, 193–236.

Mugnaini, E. & Walberg, F. 1967 An experimental electron microscopic study on the mode of termination of cerebellar cortico-vestibular fibers in the cat lateral vestibular nucleus (Deiter's nucleus). *Exptl Brain Res.* **4**, 212–236.

Mugnaini, E., Walberg, F. & Brodal, A. 1967 Mode of termination of primary vestibular fibres in the lateral vestibular nucleus. An experimental electron microscopic study in the cat. *Exptl Brain Res.* **4**, 187–211.

Ralston, H. J. 1968 Dorsal root projections to dorsal horn neurons in the cat spinal cord. *J. comp. Neurol.* **132**, 303–329.

Ramón y Cajal, S. 1909–1911 *Histologie du système Nerveux de l'Homme et des Vertébrés*, 2 vols. Paris: Maloine.

Ranson, S. W. 1913 The course within the spinal cord of the non-medullated fibers of the dorsal roots: a study of Lissauer's tract in the cat. *J. Comp. Neurol.* **23**, 259–281.

Revel, J. P., Napolitano, L. & Fawcett, D. W. 1960 Identification of glycogen in electron micrographs of thin tissue sections. *J. biophys. biochem. Cytol.* **8**, 575–589.

Reynolds, E. S. 1963 The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

Samorajski, T., Ordy, J. M. & Keefe, J. R. 1965 The fine structure of lipofuscin age pigment in the nervous system of aged mice. *J. Cell biol.* **26**, 779–795.

Samorajski, T., Ordy, J. M. & Rady-Reimer, P. 1968 Lipofuscin pigment accumulation in the nervous system of ageing mice. *Anat. Rec.* **160**, 555–574.

Scheibel, M. E. & Schiebel, A. B. 1968 On the nature of dendritic spines—report of a workshop. *Communications in behavioural biology*, Part A, **1**, 231–265.

Smith, C. A. & Rasmussen, G. L. 1965 Degeneration in the efferent nerve endings in the cochlea after axonal section. *J. Cell Biol.* **26**, 63–77.

Smith, R. E. & Farquhar, M. G. 1966 Lysosome function in the regulation of the secretory process in cells of anterior pituitary gland. *J. Cell Biol.* **31**, 319–347.

Szentágothai, J., Hámori, J. & Tömböl, T. 1966 Degeneration and electron microscope analysis of the synaptic glomeruli in the lateral geniculate body. *Exptl Brain Res.* **2**, 283–301.

Tömböl, T. 1967 Short neurons and their synaptic relations in the specific thalamic nuclei. *Brain Res.* **3**, 307–326.

Valverde, F. 1967 Apical dendritic spines of the visual cortex and light deprivation in the mouse. *Exptl Brain Res.* **3**, 337–352.

Valverde, F. 1968 Structural changes in the area striata of the mouse after enucleation. *Exptl Brain Res.* **5**, 274–292.

Valverde, F. & Estéban, M. E. 1968 Peristriate cortex of mouse: location and effects of enucleation on the number of dendritic spines. *Brain Res.* **9**, 145–148.

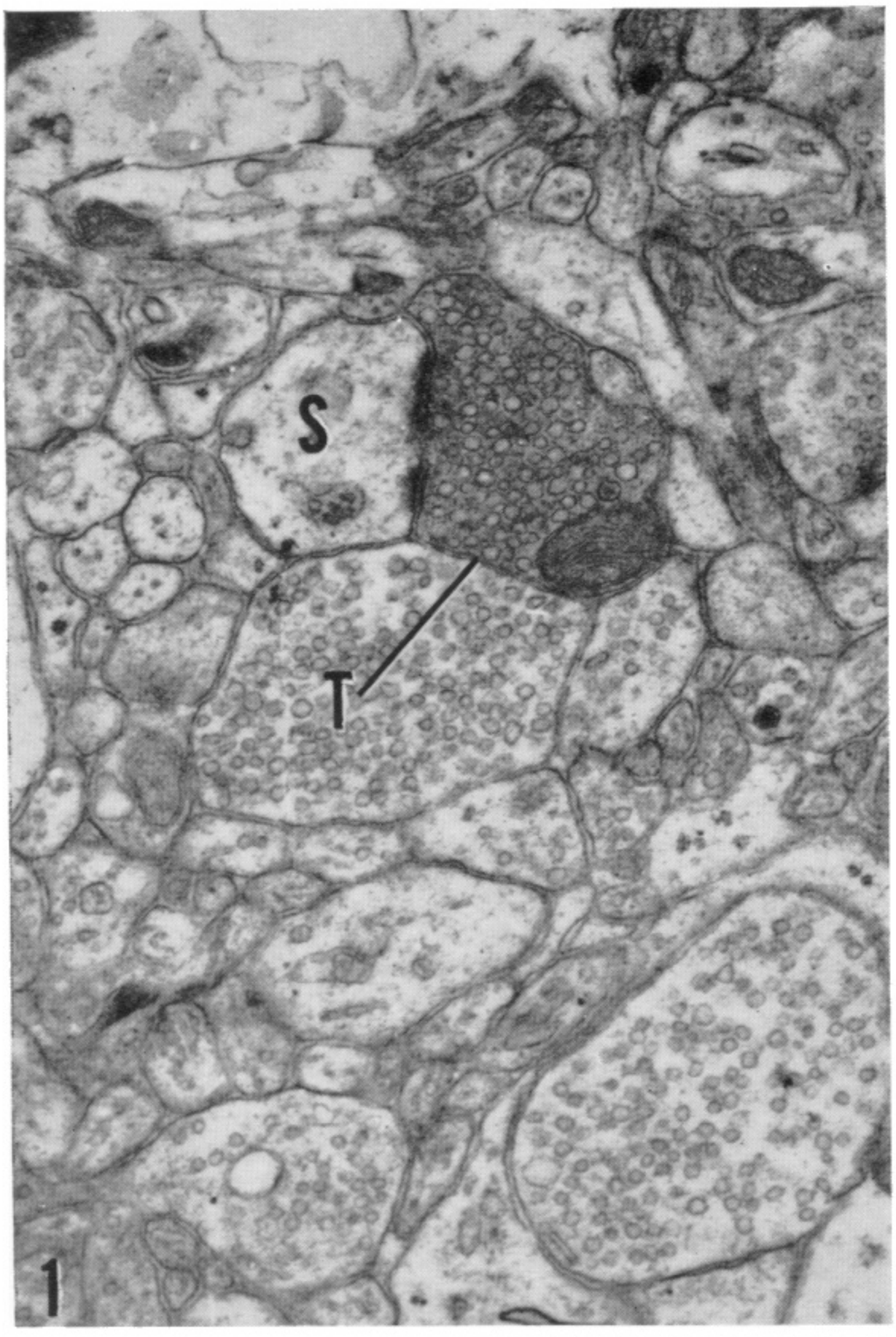
Vaughn, J. E. & Peters, A. 1968 A third neuroglial cell type. An electron microscopic study. *J. comp. Neurol.* **133**, 269–287.

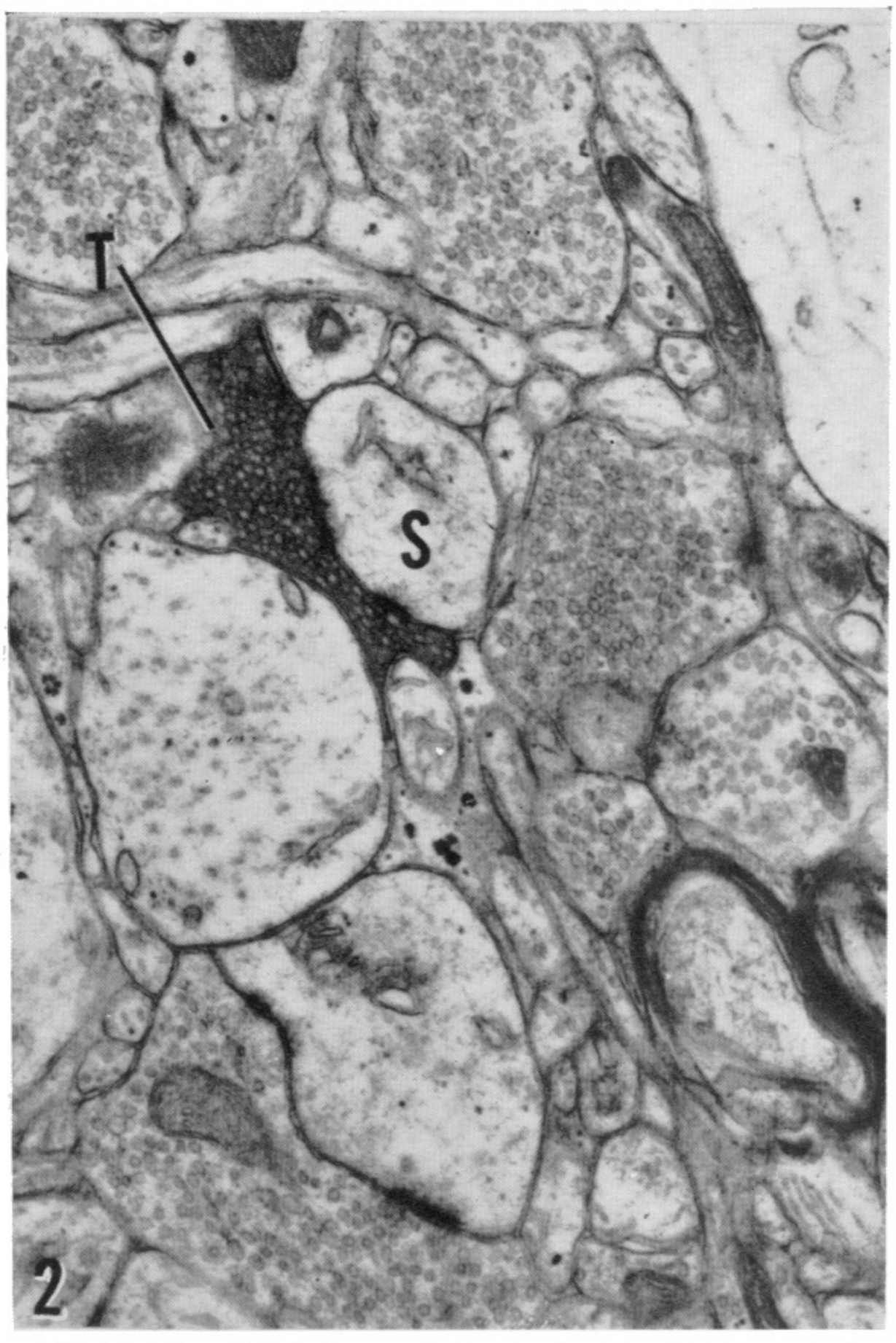
Walberg, F. 1964 The early changes in degenerating boutons and the problem of agyrophilia. Light and electron microscopic observations. *J. comp. Neurol.* **122**, 113–123.

Walberg, F. 1965 An electron microscopic study of terminal degeneration in the inferior olive of the cat. *J. comp. Neurol.* **125**, 205–222.

Watson, M. L. 1958 Staining of tissue sections for electron microscopy with heavy metals. *J. biophys. biochem. Cytol.* **4**, 475–478.

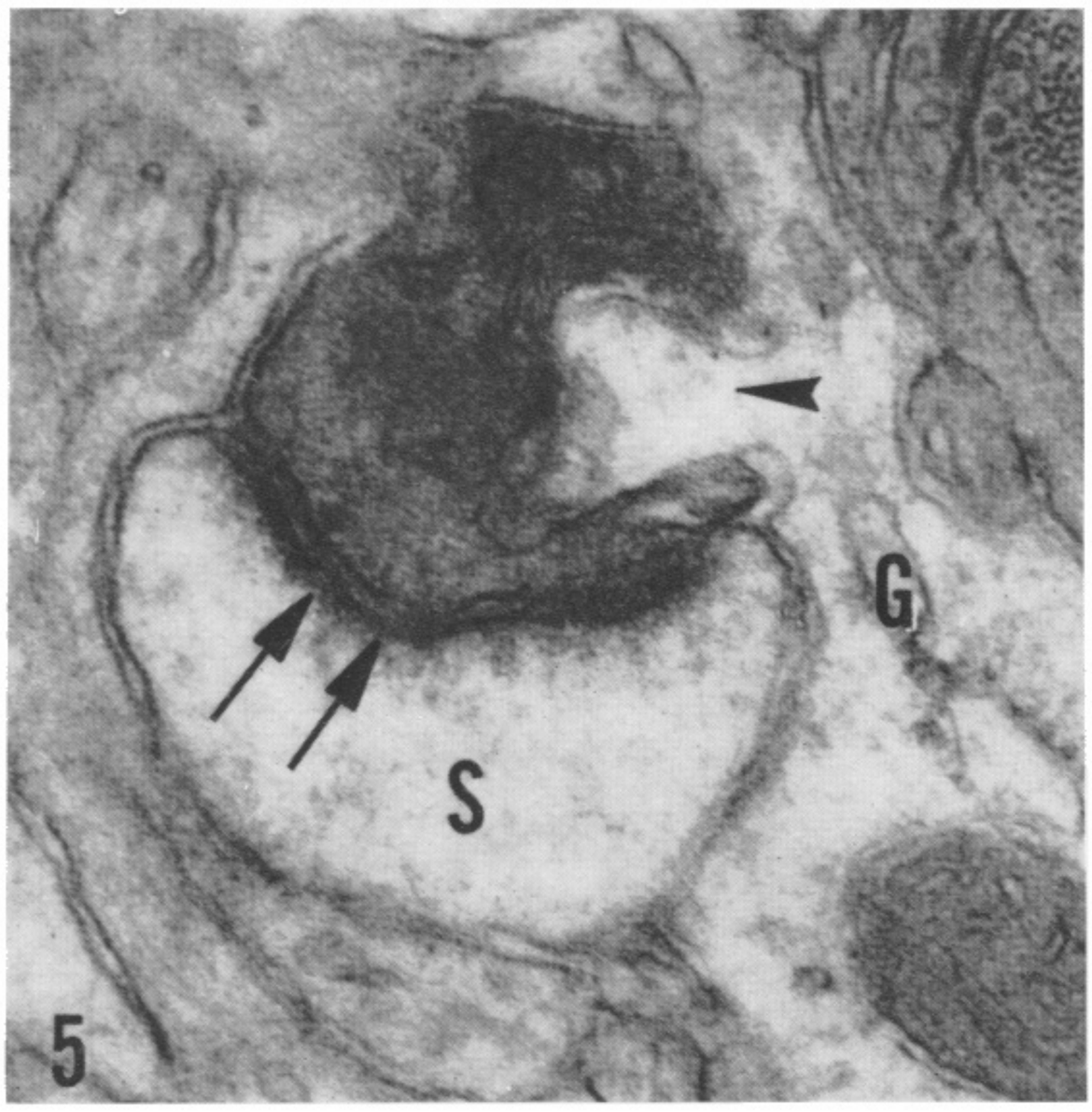
Westrum, L. E. 1966 Electron microscopy of degeneration in the prepyriform cortex. *J. Anat., Lond.* **100**, 683–685.


S
T
1

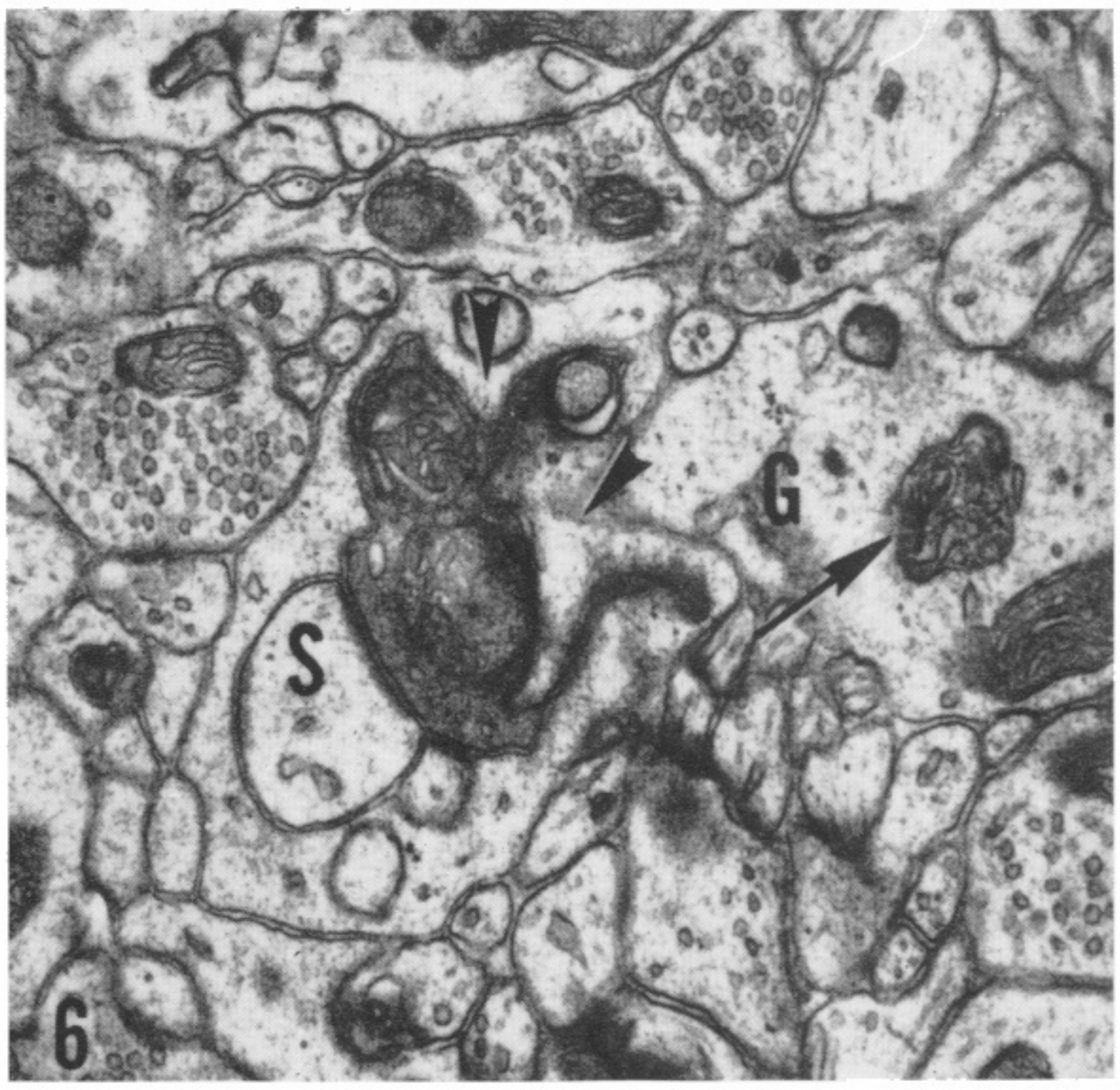

T
S
2

T
G
S
3

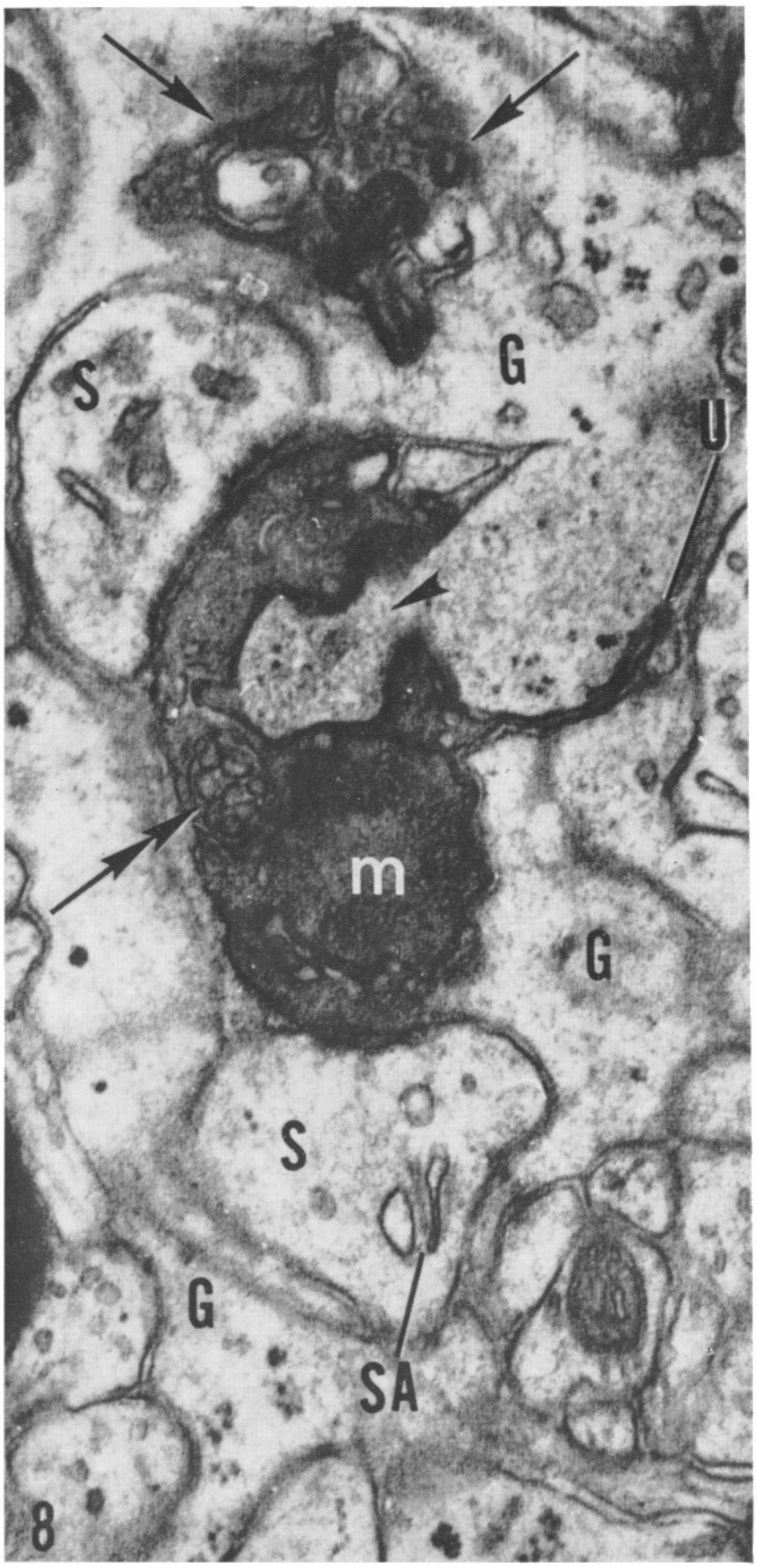
G
S
T
4



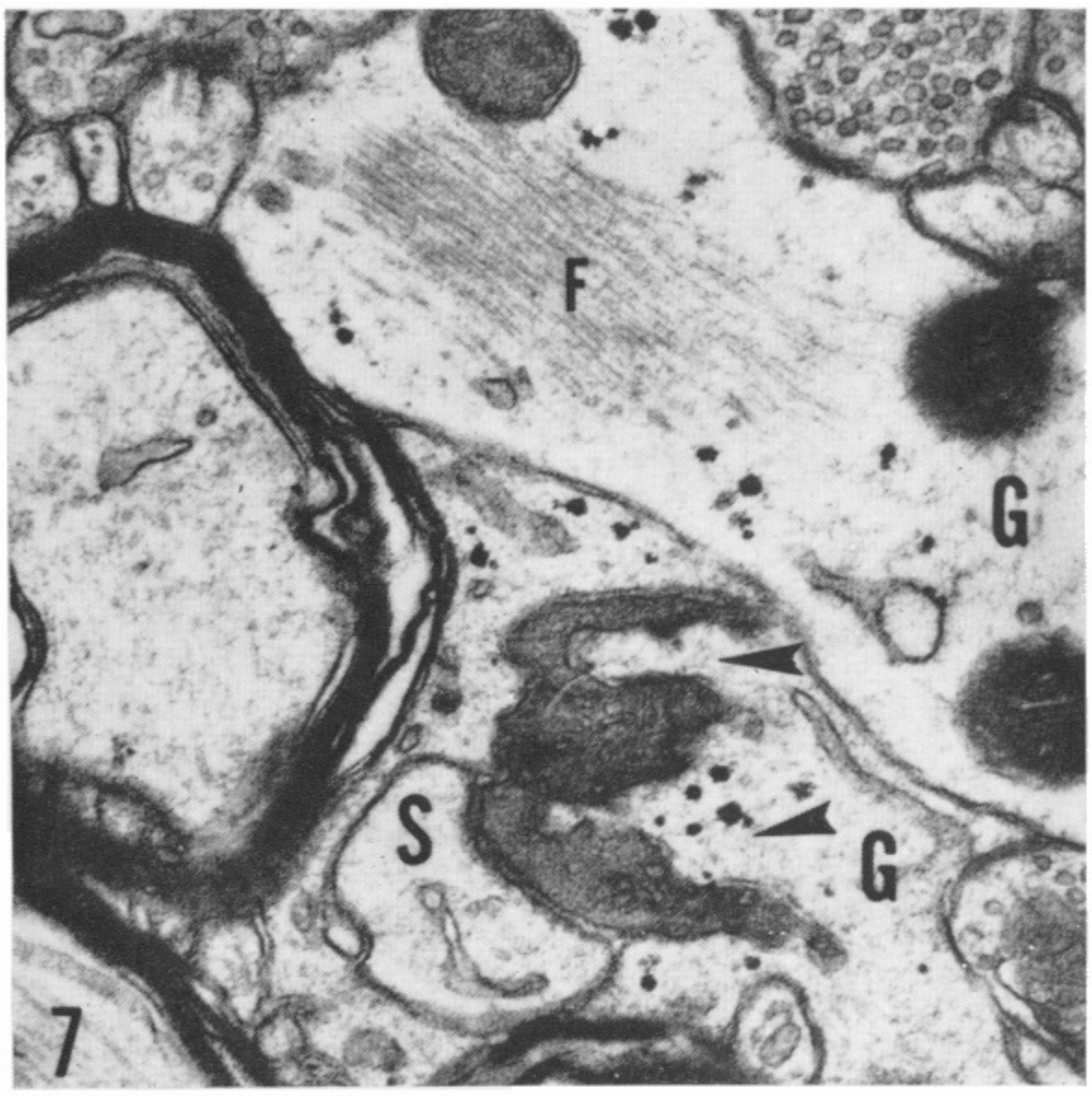
5



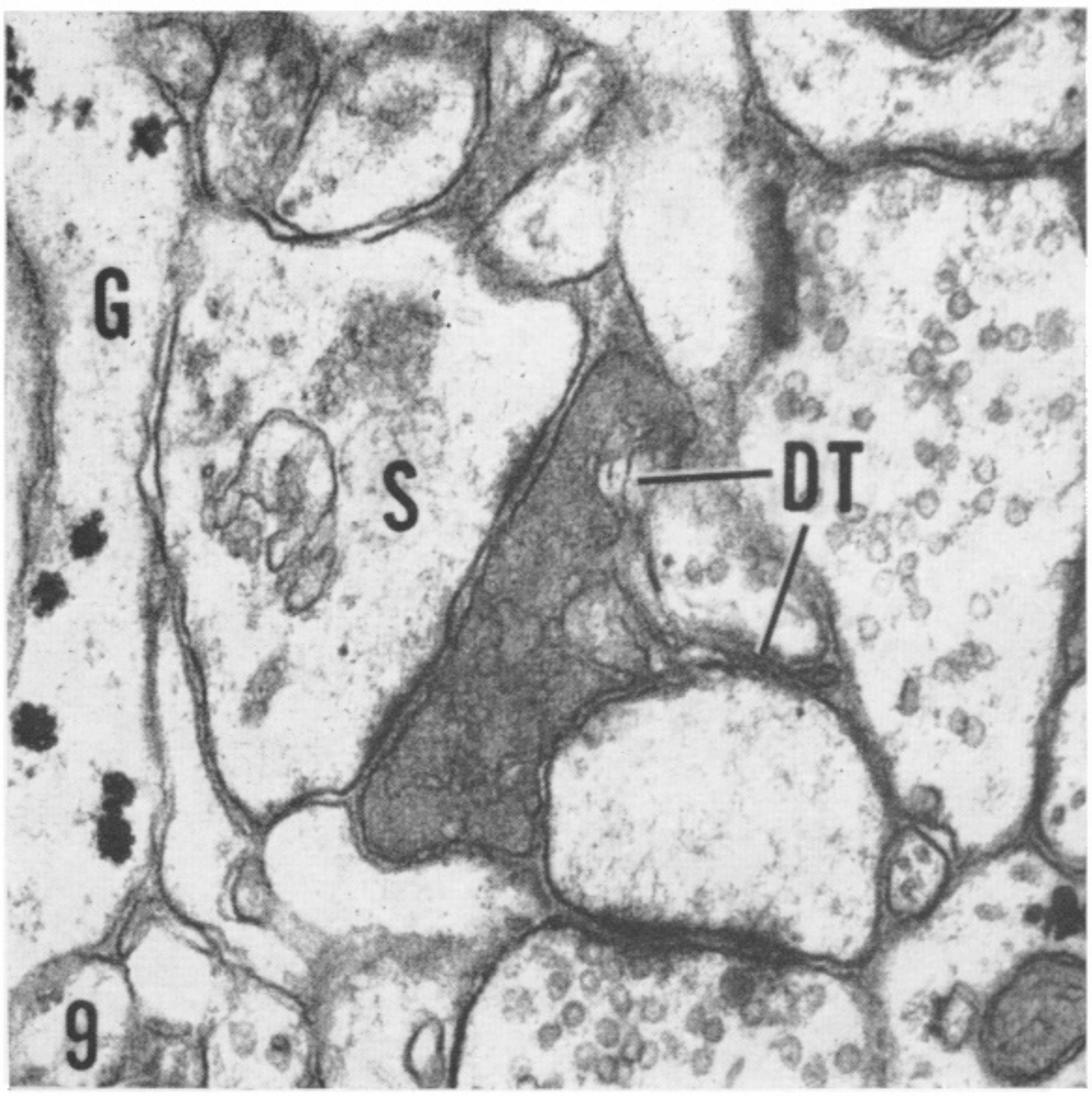
6



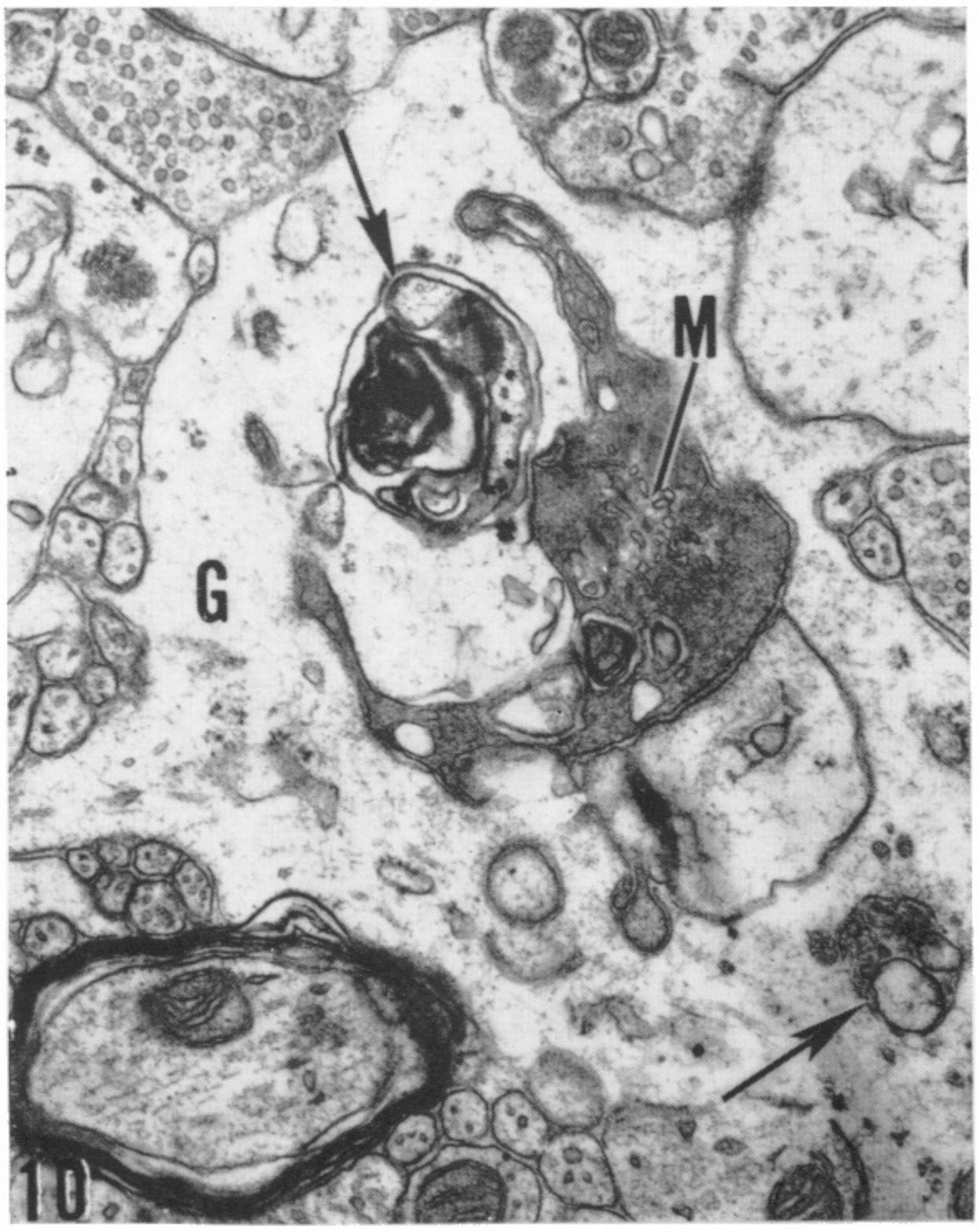
8



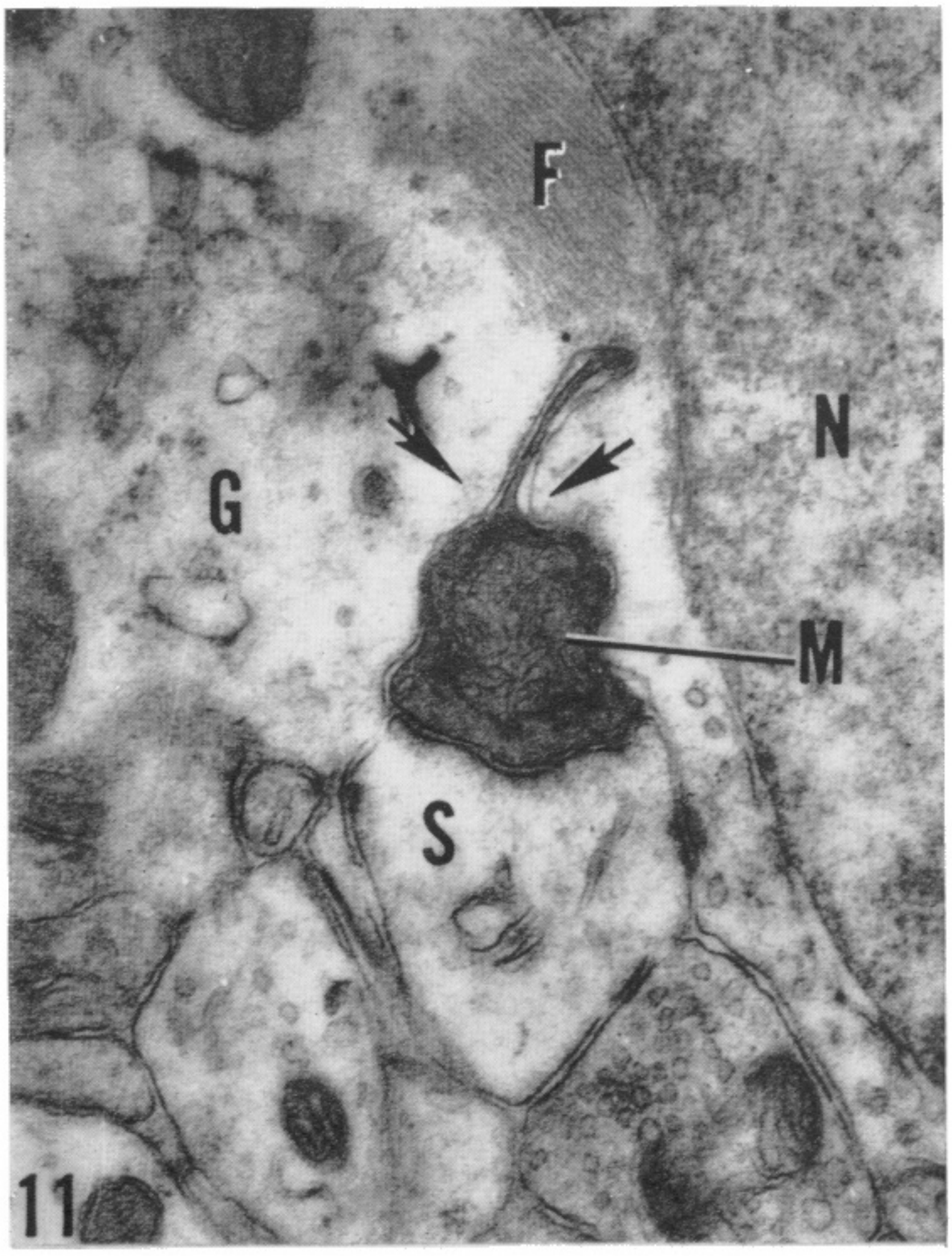
7



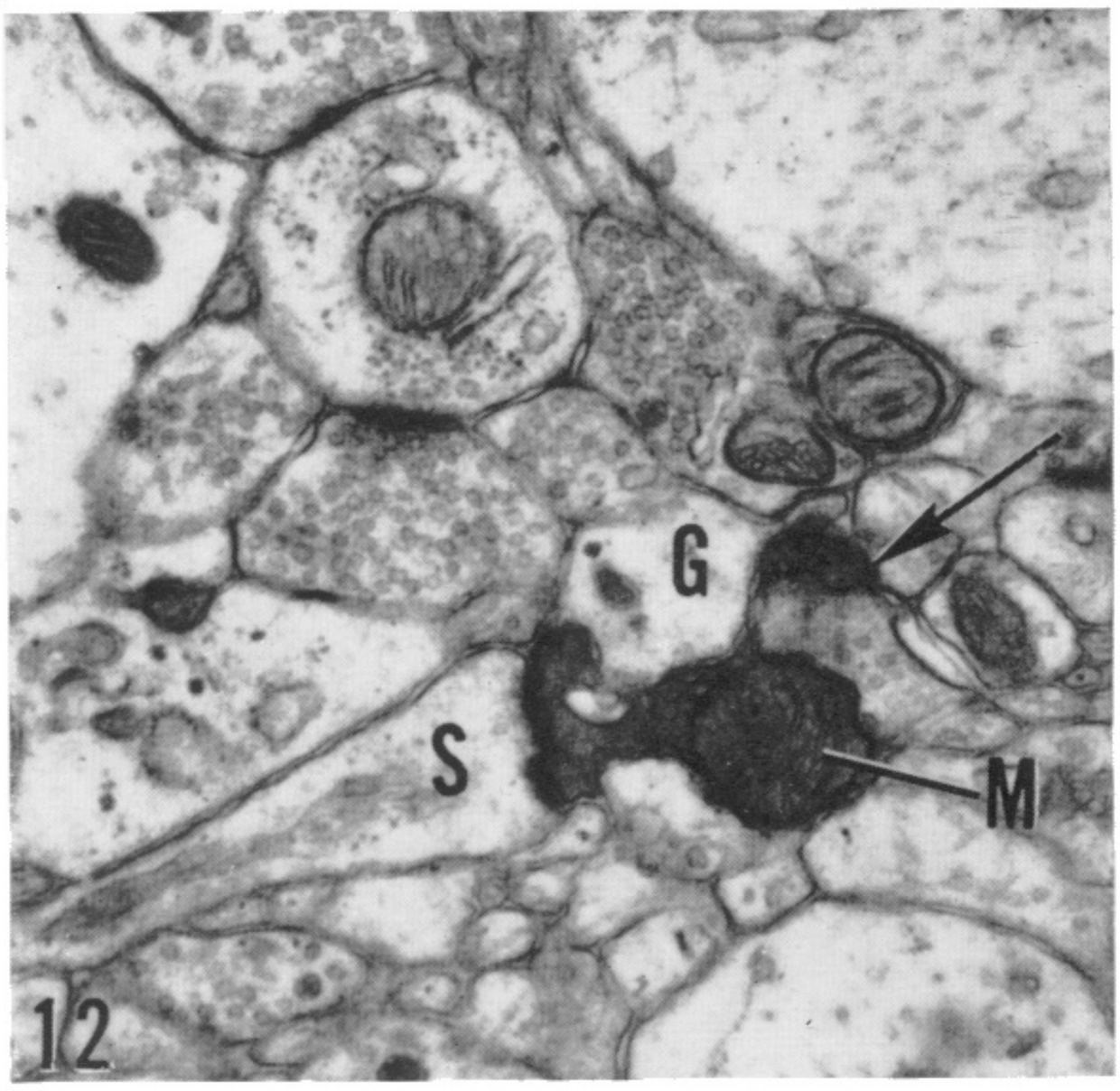
9



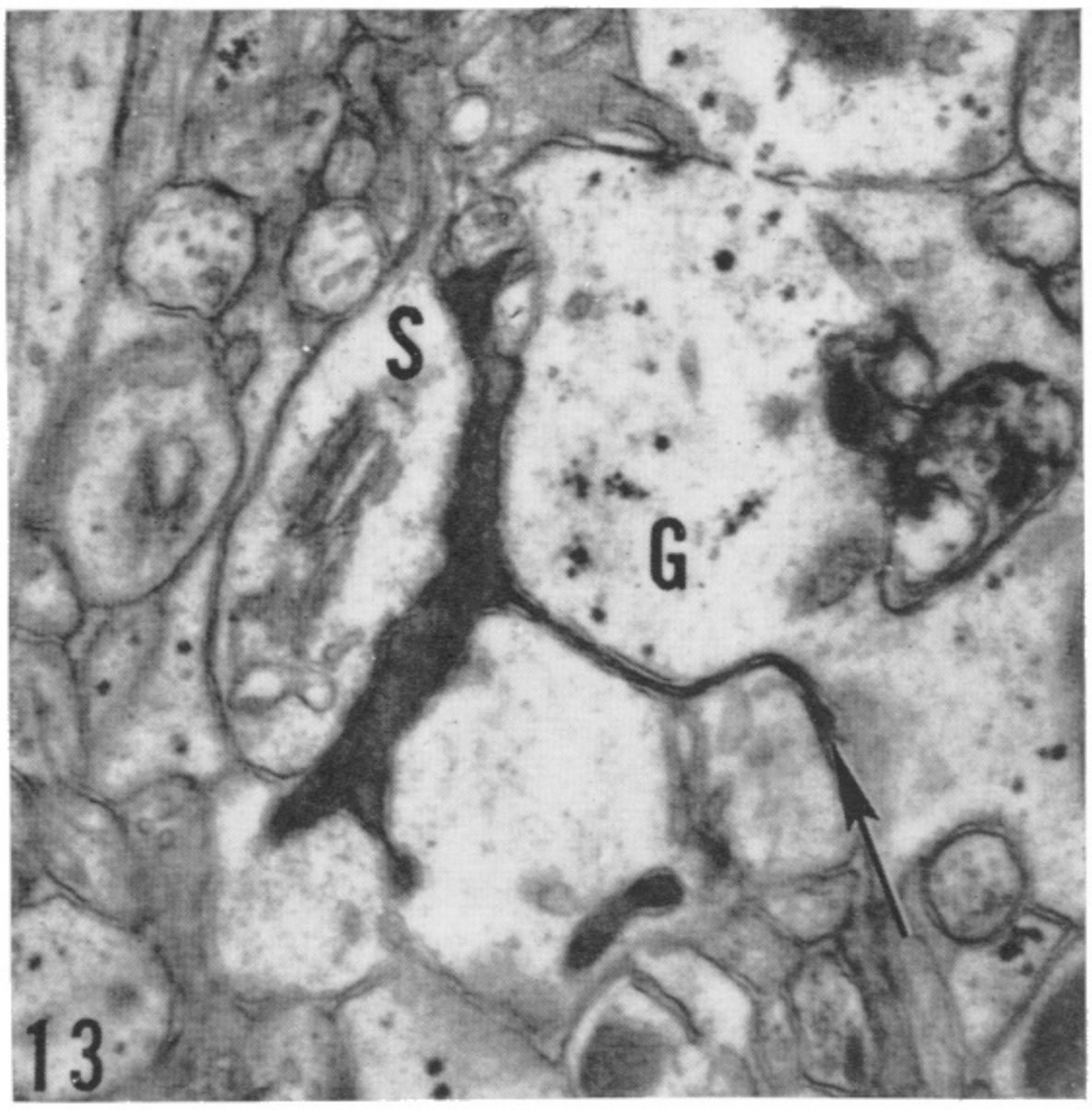
10



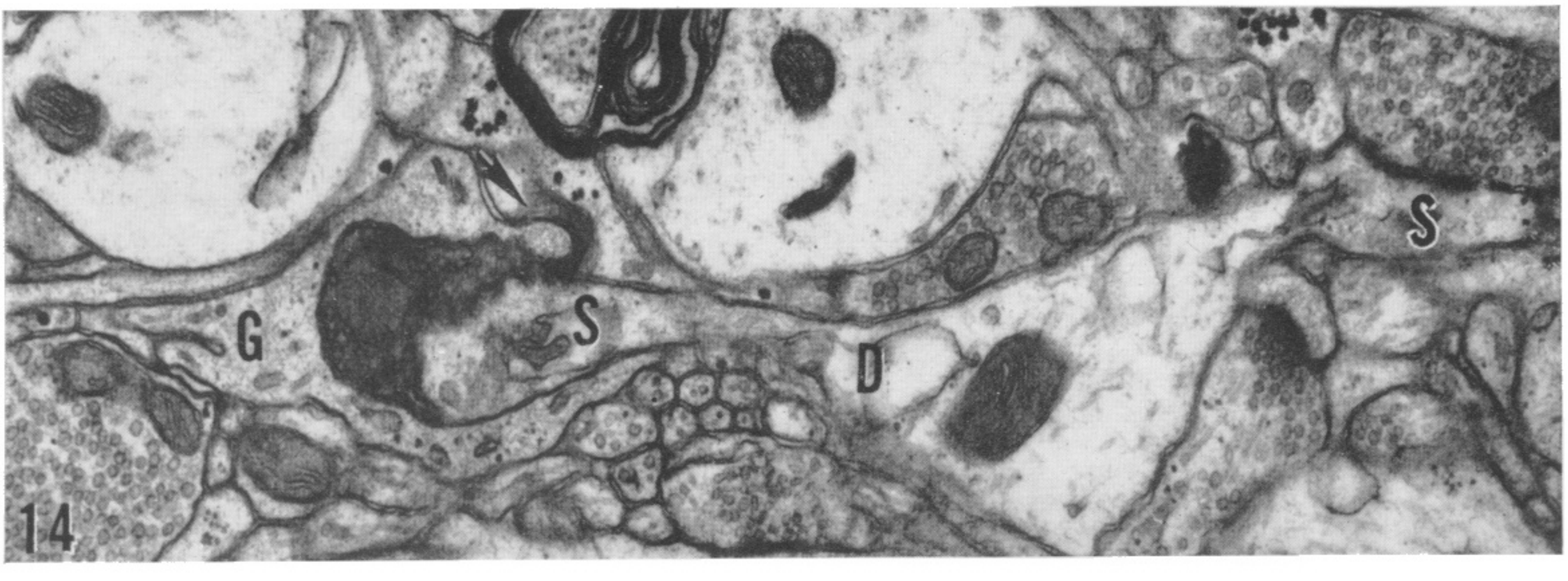
11



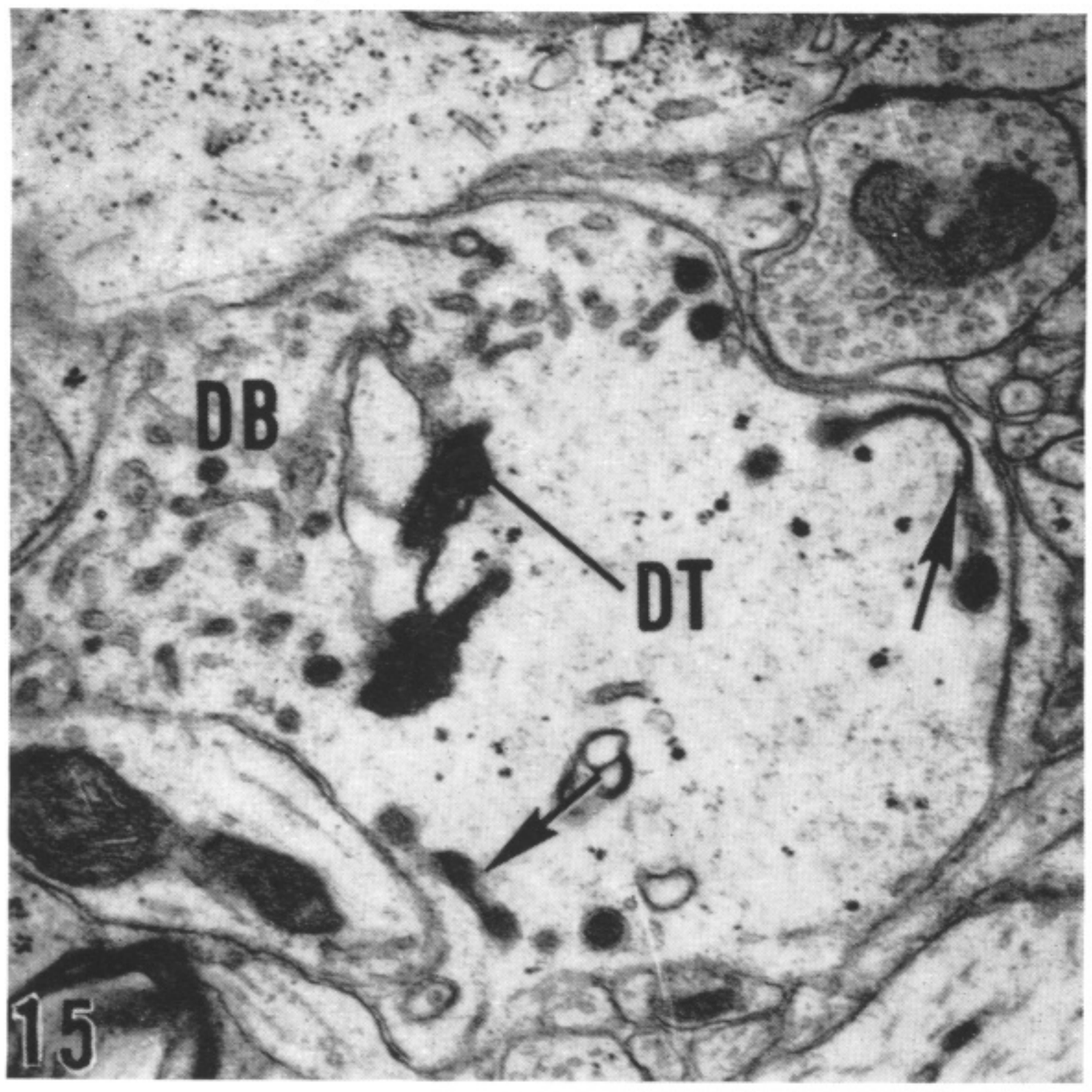
12

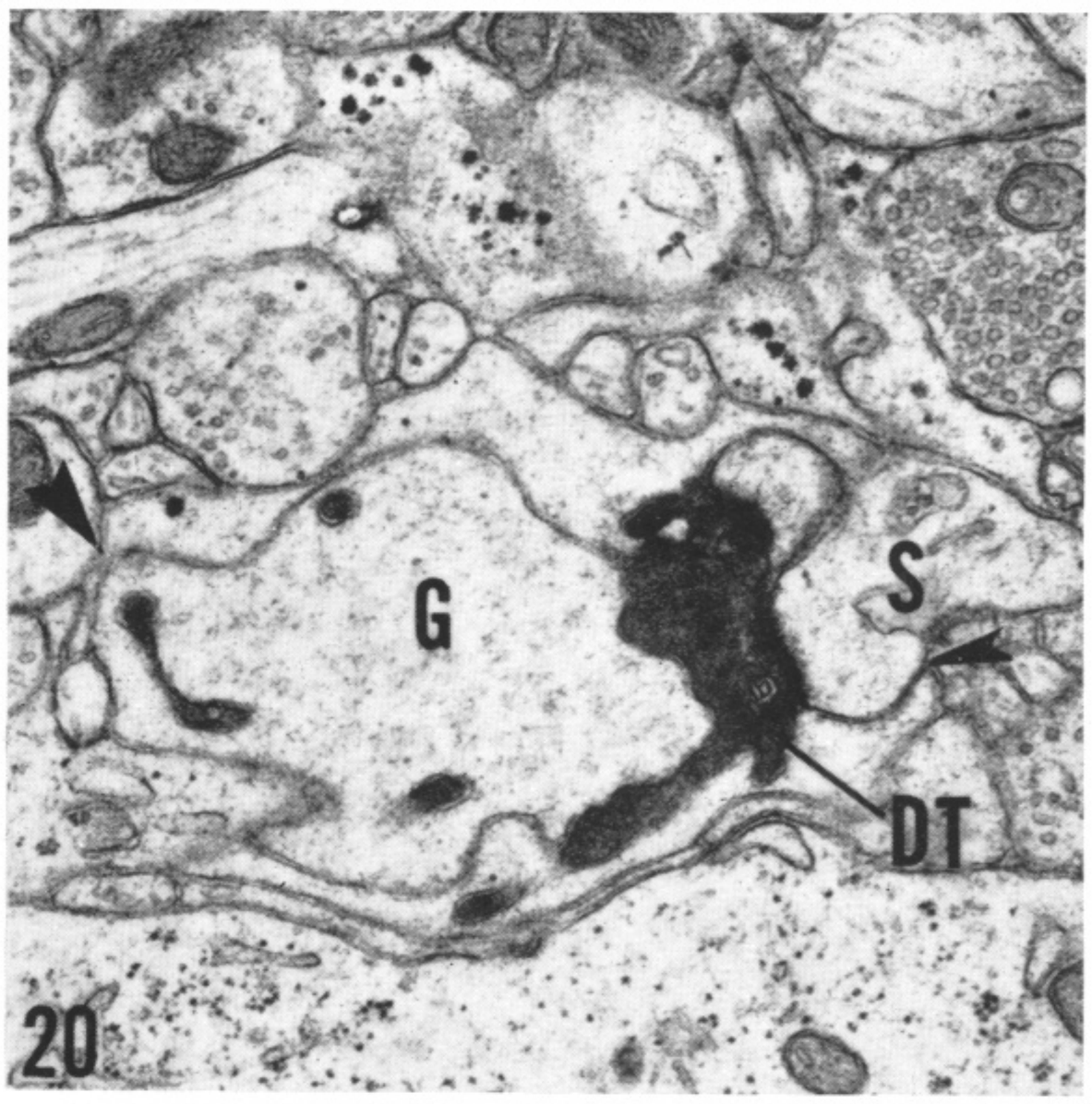


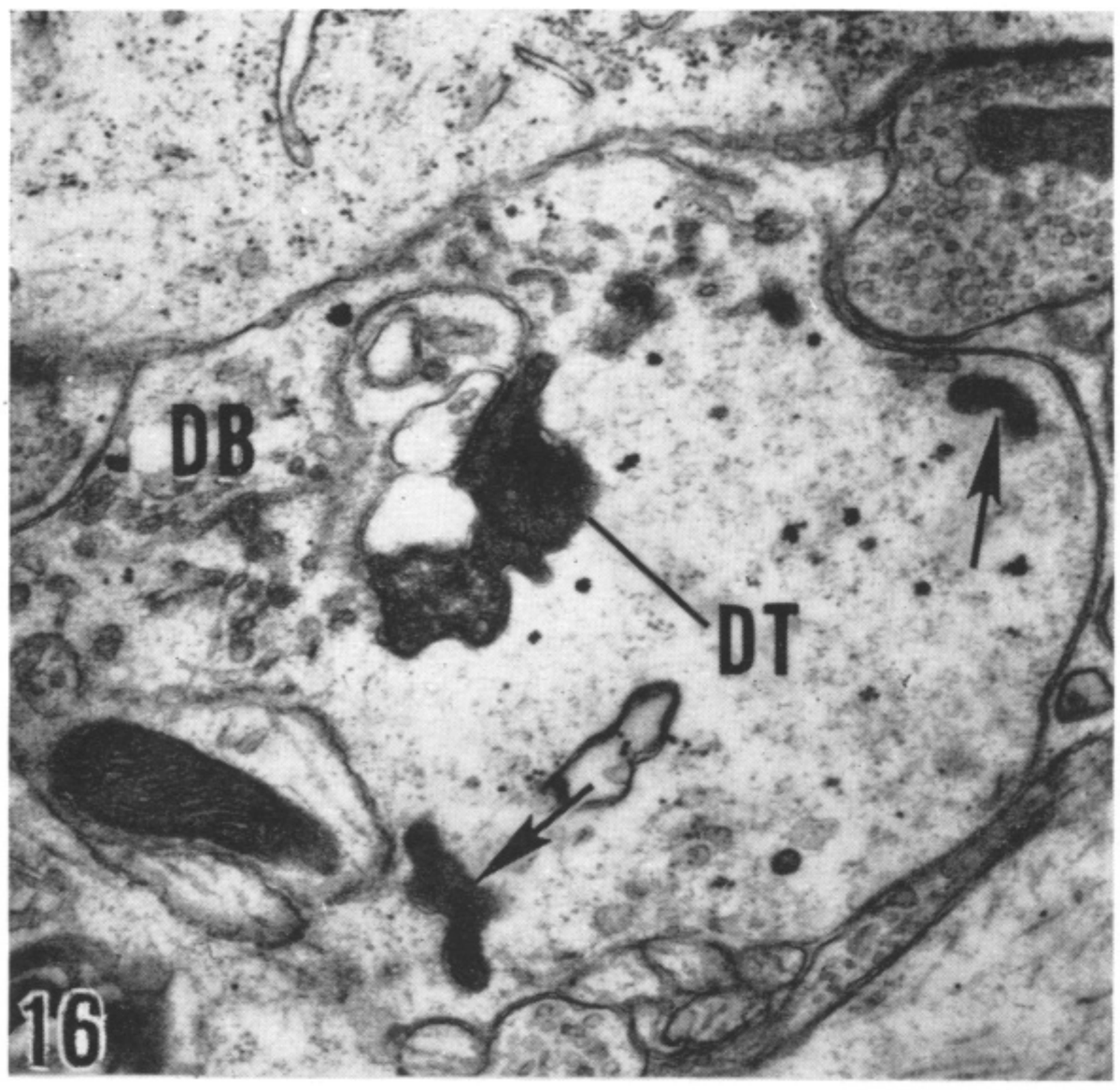
13

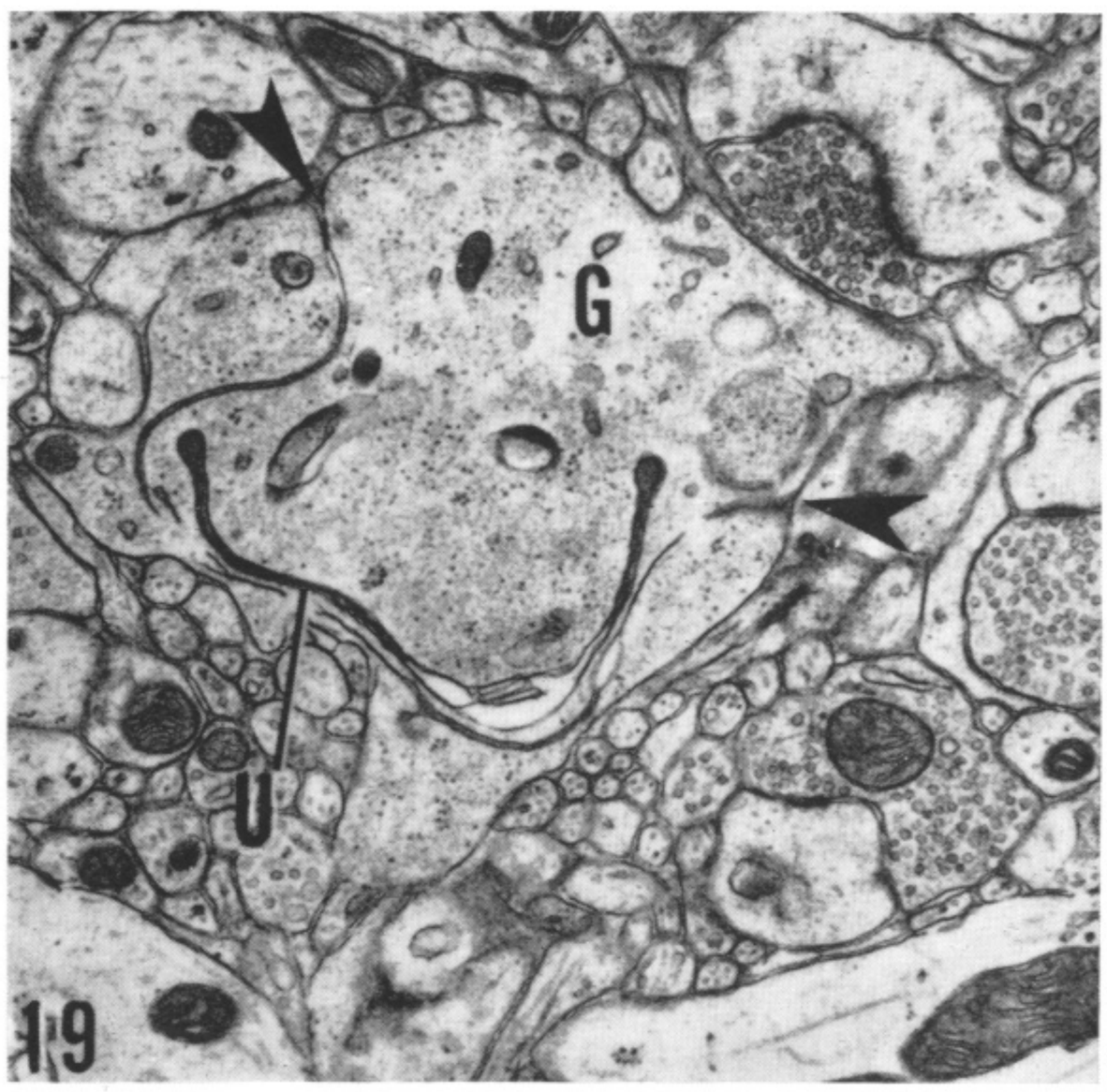


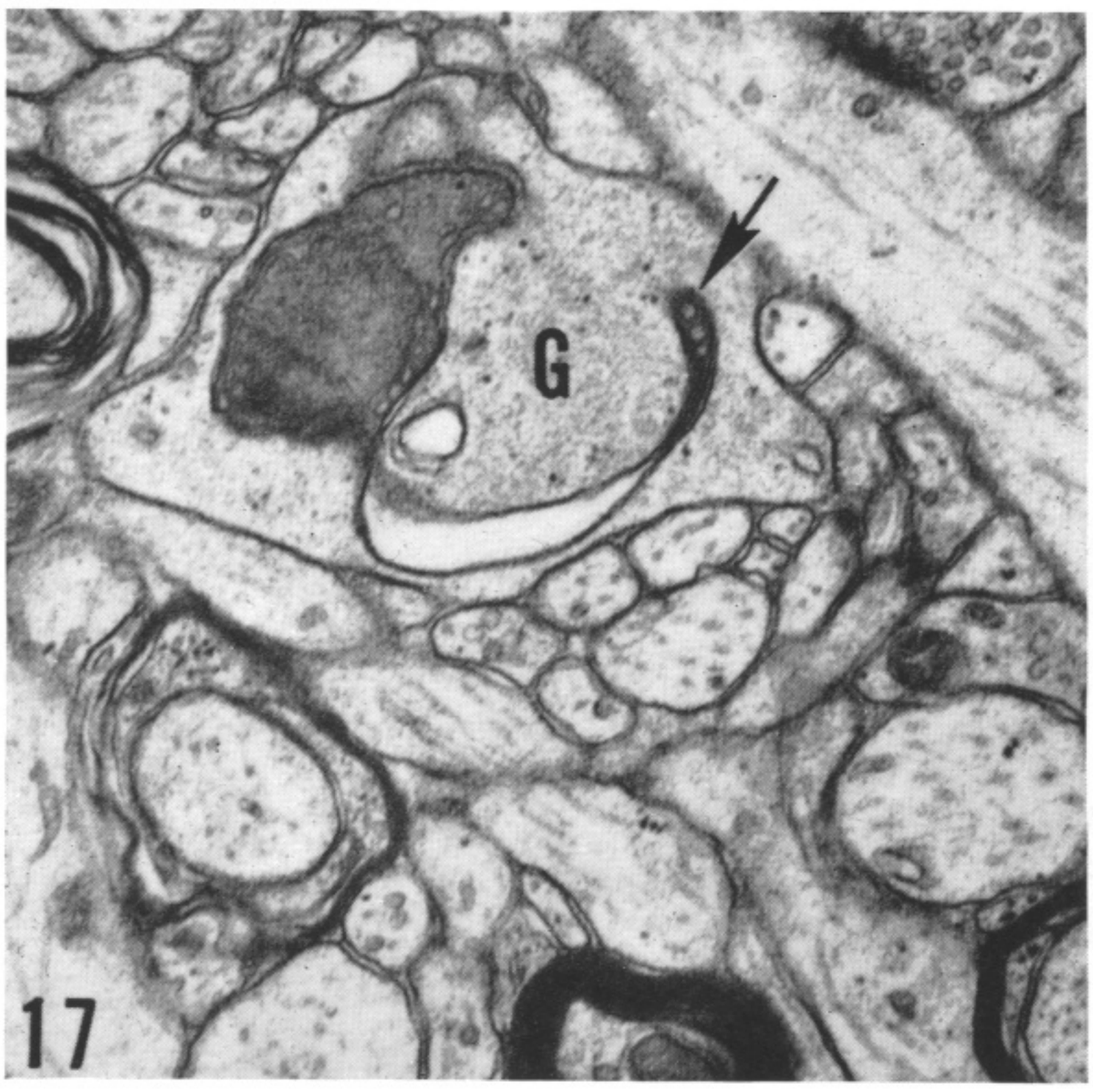
14

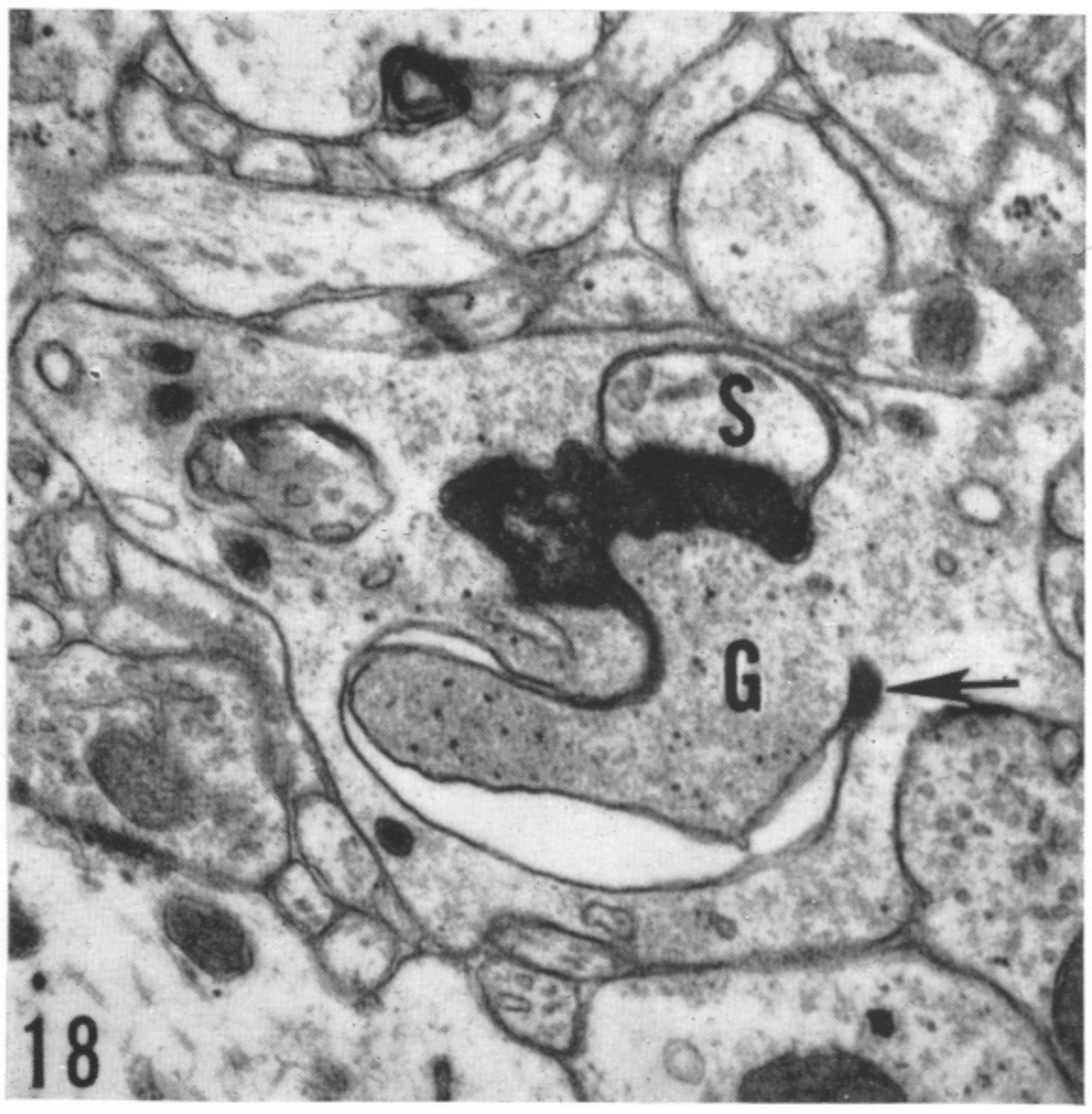












FIGURES 15, 16. Serial sections of a degenerating thalamo-cortical axon terminal (DT) completely surrounded by astroglial cytoplasm which contains small dense bodies (DB) resembling lysosomes and larger ones (arrows) which may be broken-off fragments of the degenerating terminal. Lead citrate and uranyl acetate; × 26000.

FIGURES 17, 18. Two degenerating thalamo-cortical axon terminals surrounded by astroglial cytoplasm which is so disposed as to suggest that the terminal unmyelinated segment of the parent axon has been thrown into a sinuous shape (arrow). Lead citrate and uranyl acetate; × 31000.

FIGURE 19. A degenerating unmyelinated axon fragment (U) surrounded by an astroglial process (G). The folding of the glial process is indicated by the arrow heads. Note in figures 17–19 that the glial cell has lost its large glycogen particles and has become finely granular. Lead citrate; × 20000.

FIGURE 20. A degenerating thalamo-cortical axon terminal (DT) ending on a dendritic spine (S) and intimately related to reduplicated (arrow heads) folds of astroglial cytoplasm (G) which also contain several dense fragments resembling portions of the degenerating terminal. Lead citrate and uranyl acetate; × 25000.

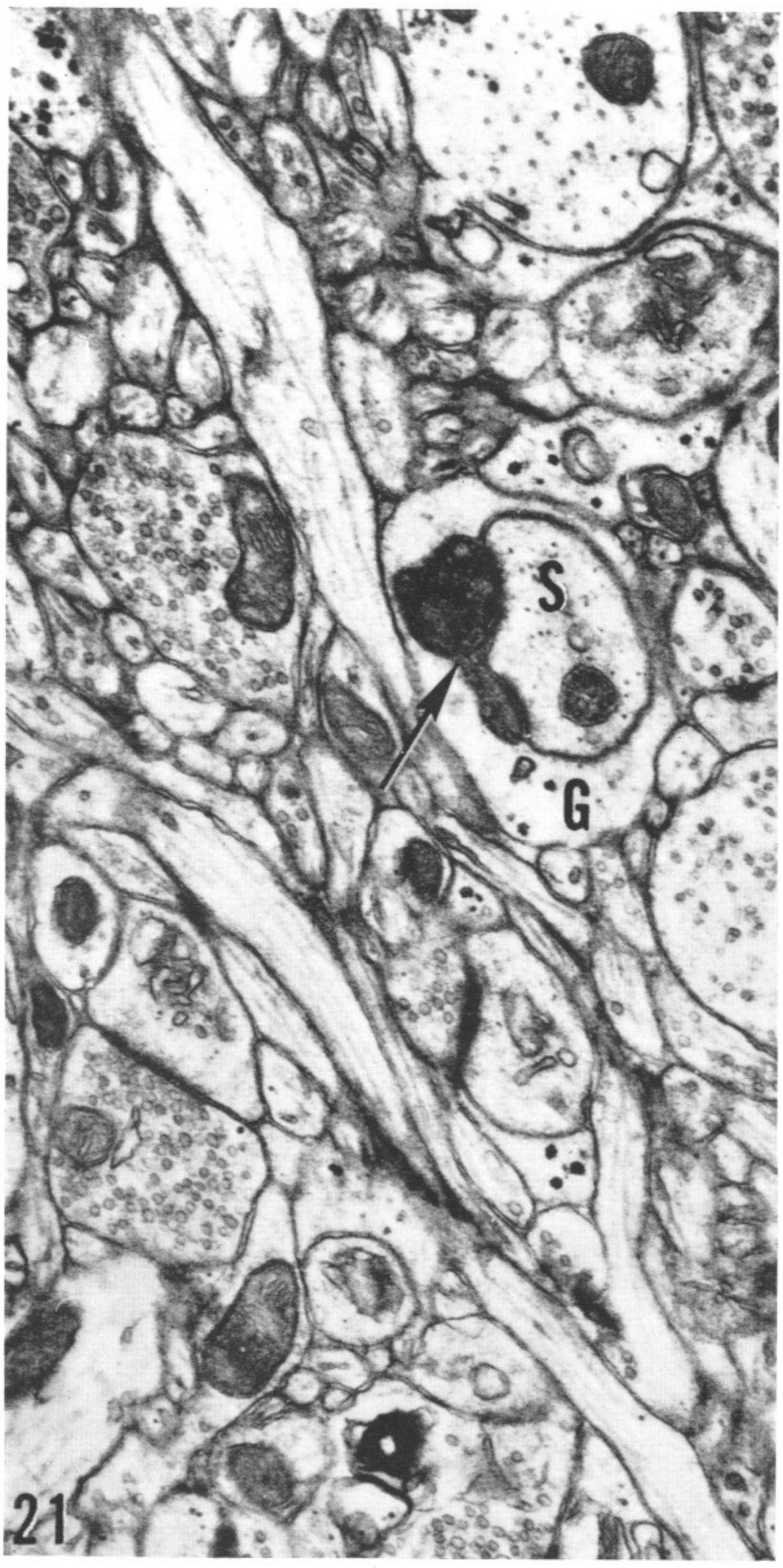


FIGURE 21. A degenerating commissural axon terminal (arrow) at a late stage of degeneration and ending on a dendritic spine (S). Both the terminal and the spine are surrounded by an astroglial process (G). Lead citrate; × 30000.

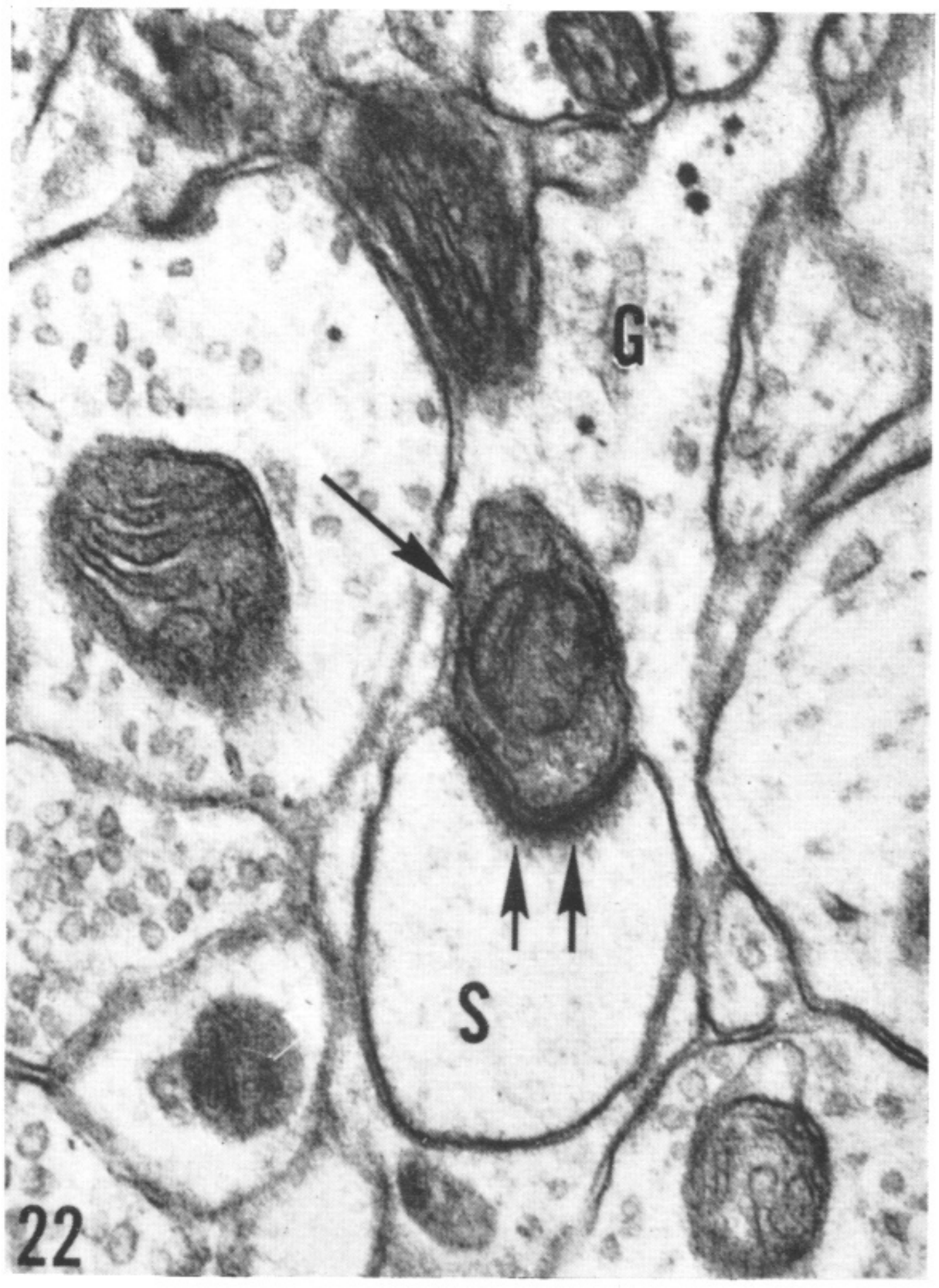


FIGURE 22. A degenerating commissural axon terminal (arrow) ending on a dendritic spine; both the spine and the terminal are surrounded by an astroglial process. The terminal at this late stage of degeneration is greatly shrunken, though it still contains a mitochondrion. The asymmetrical synaptic contact is indicated by the double arrows. Lead citrate and uranyl acetate; × 49000.

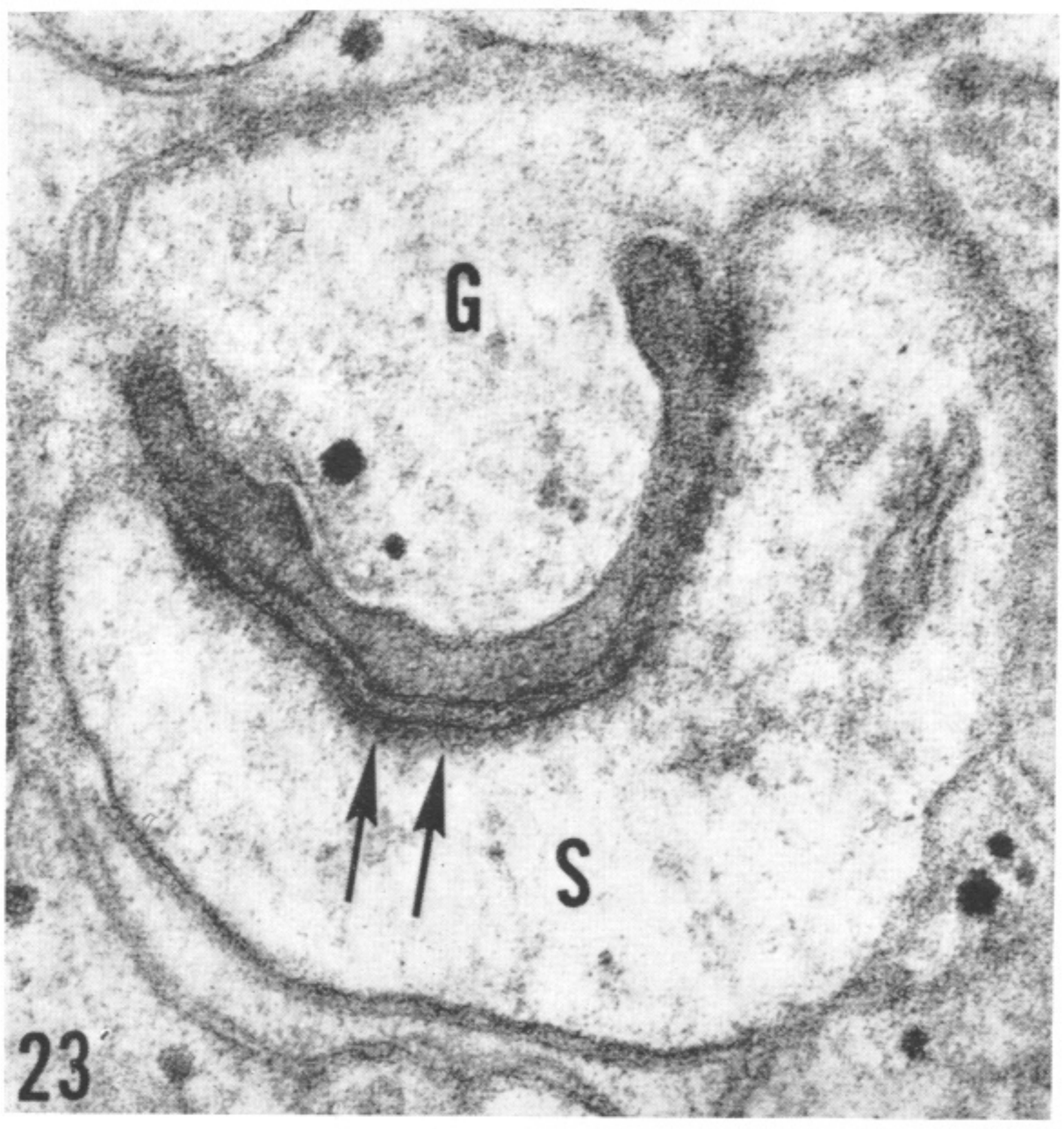


FIGURE 23. A commissural axon terminal at the latest stage of degeneration encountered in the present study. The terminal is reduced to little more than a narrow sliver attached at the synaptic thickening (double arrows). Lead citrate and uranyl acetate; × 84000.

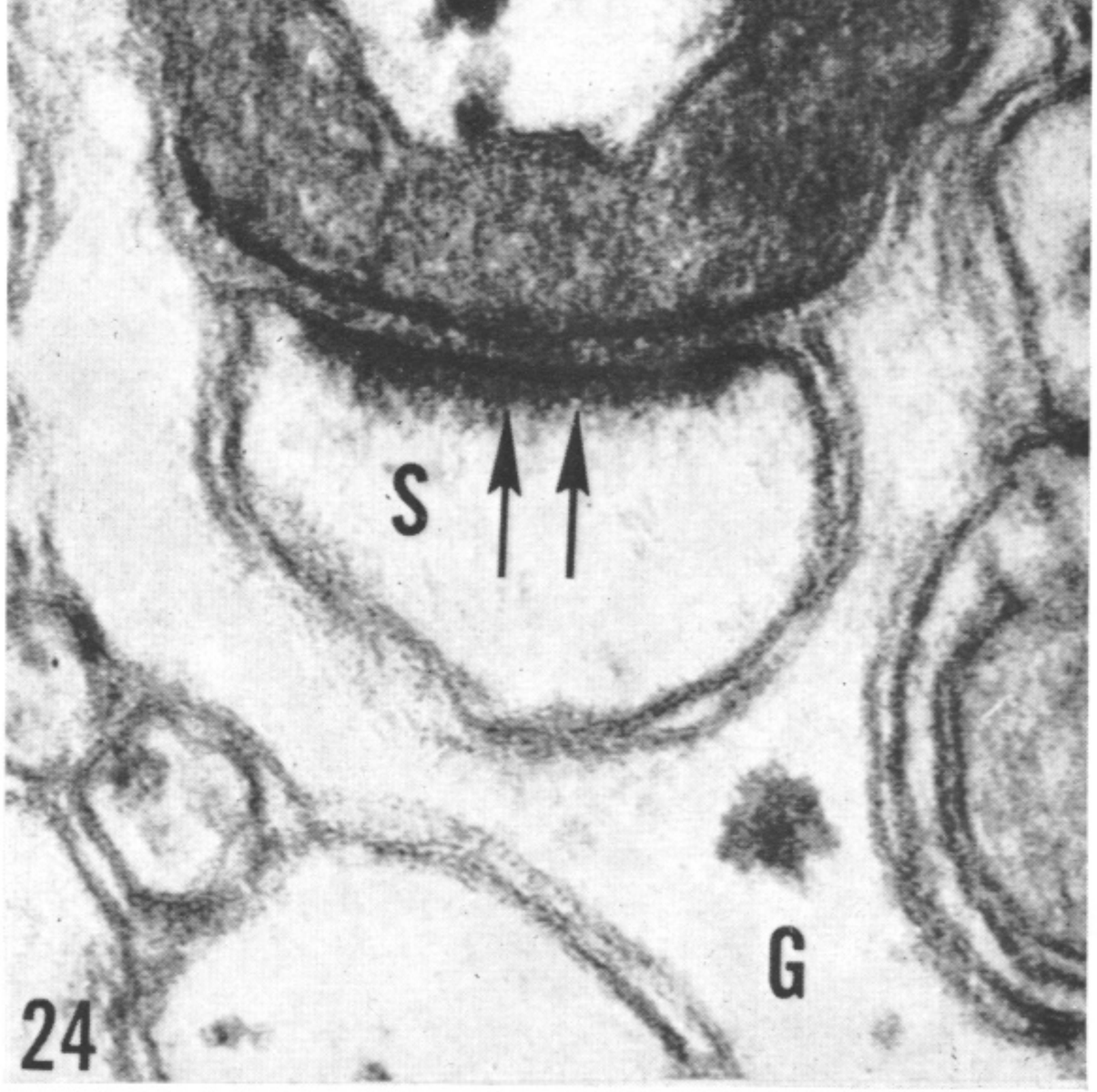
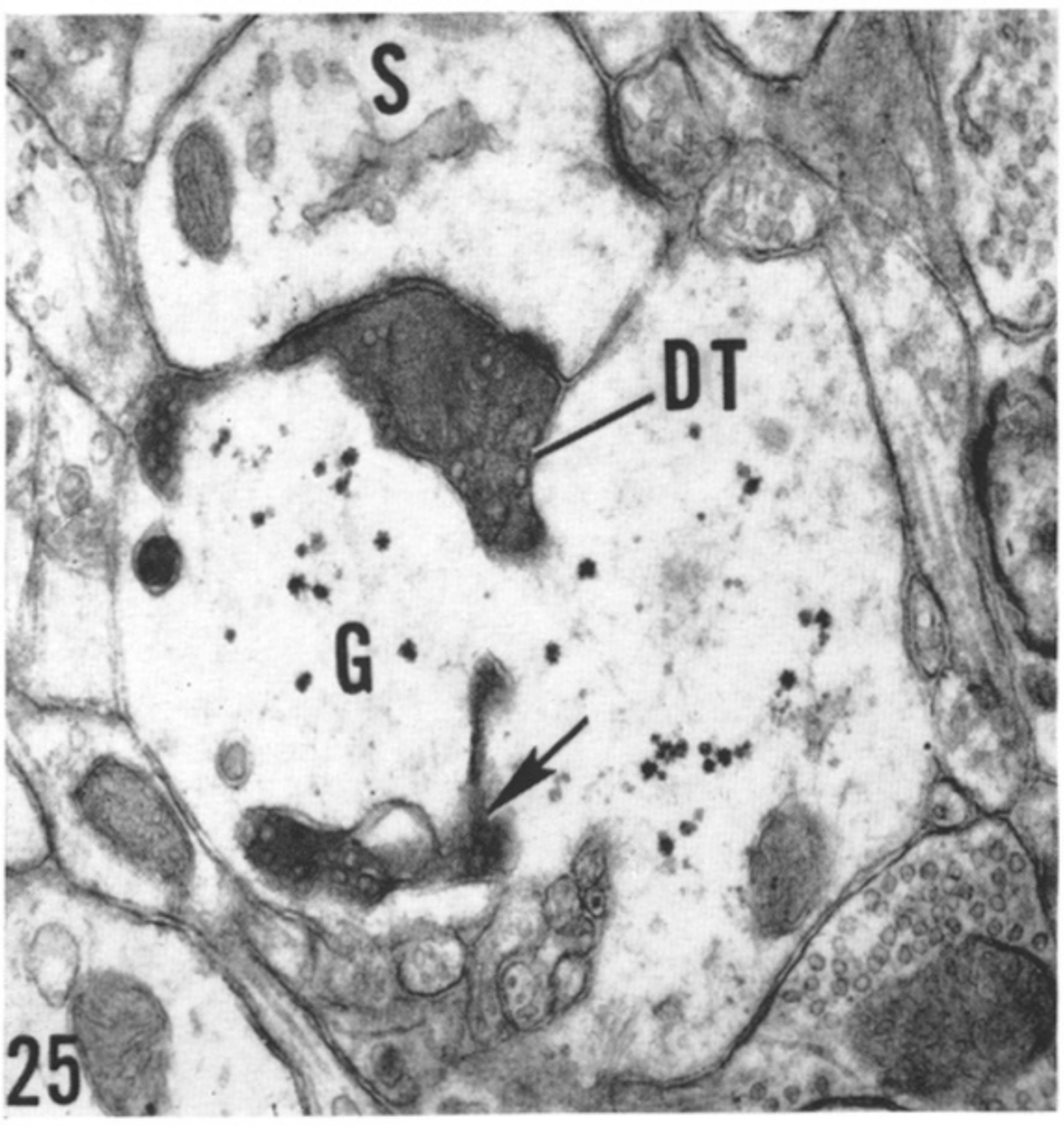
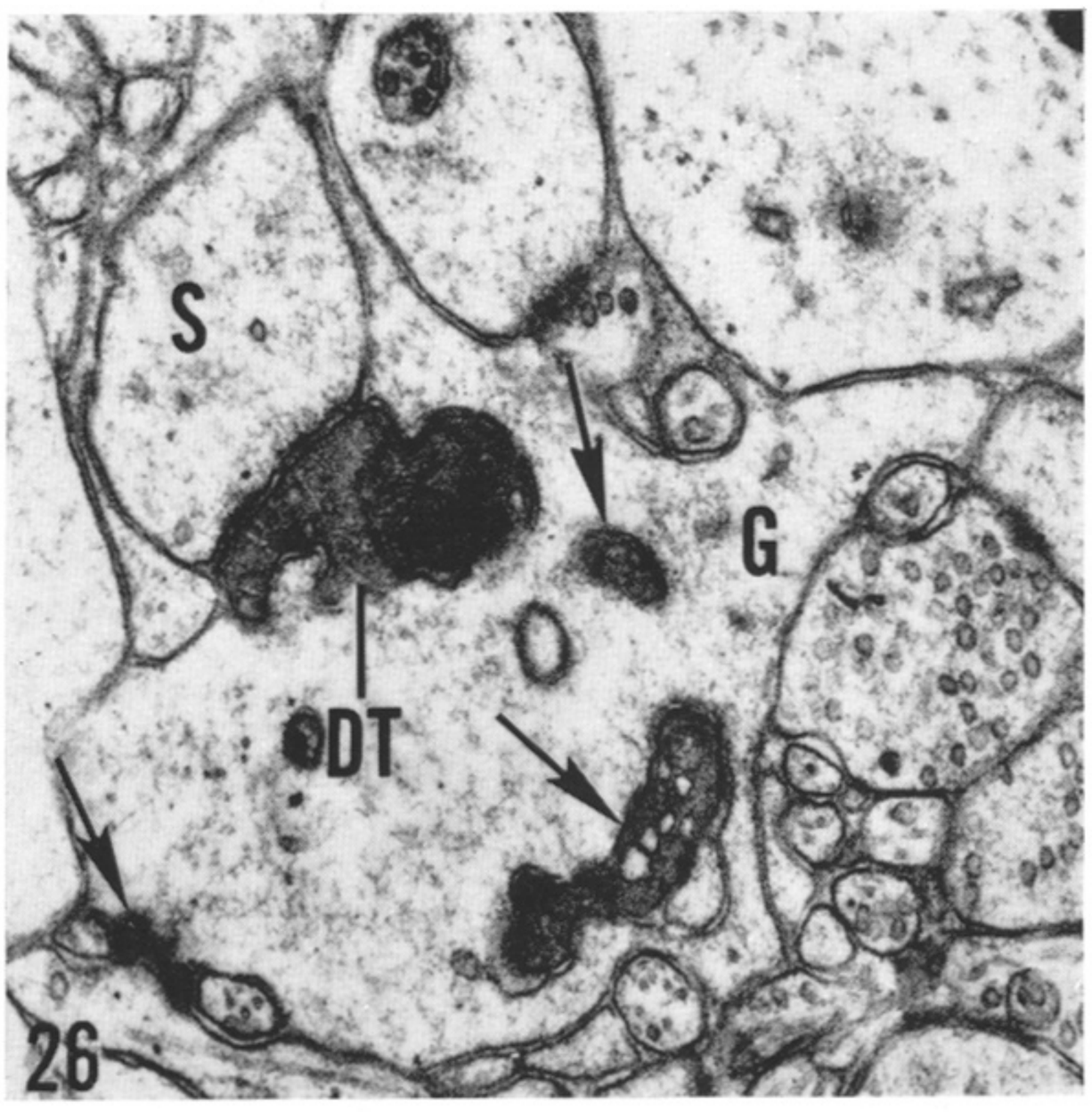
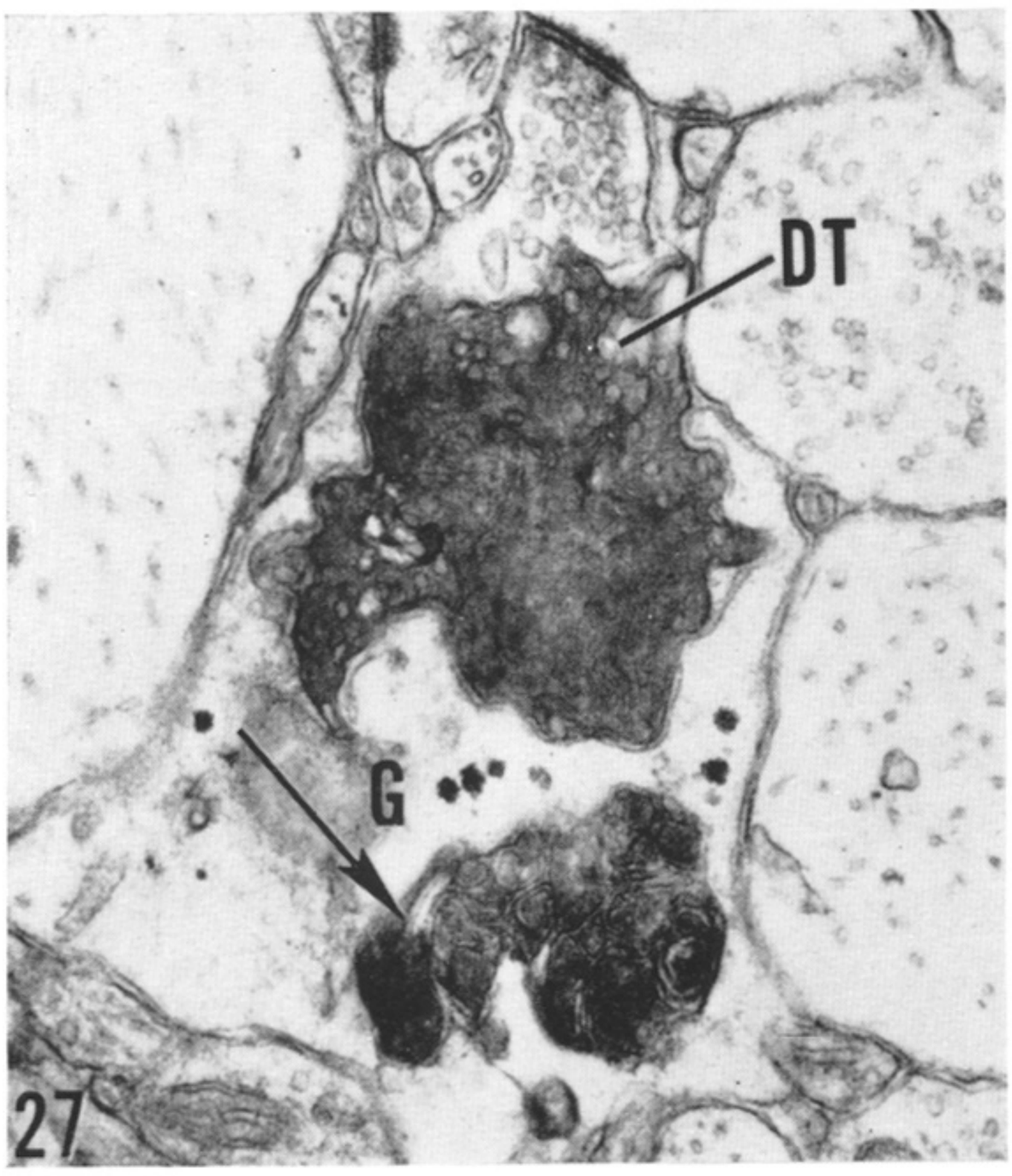
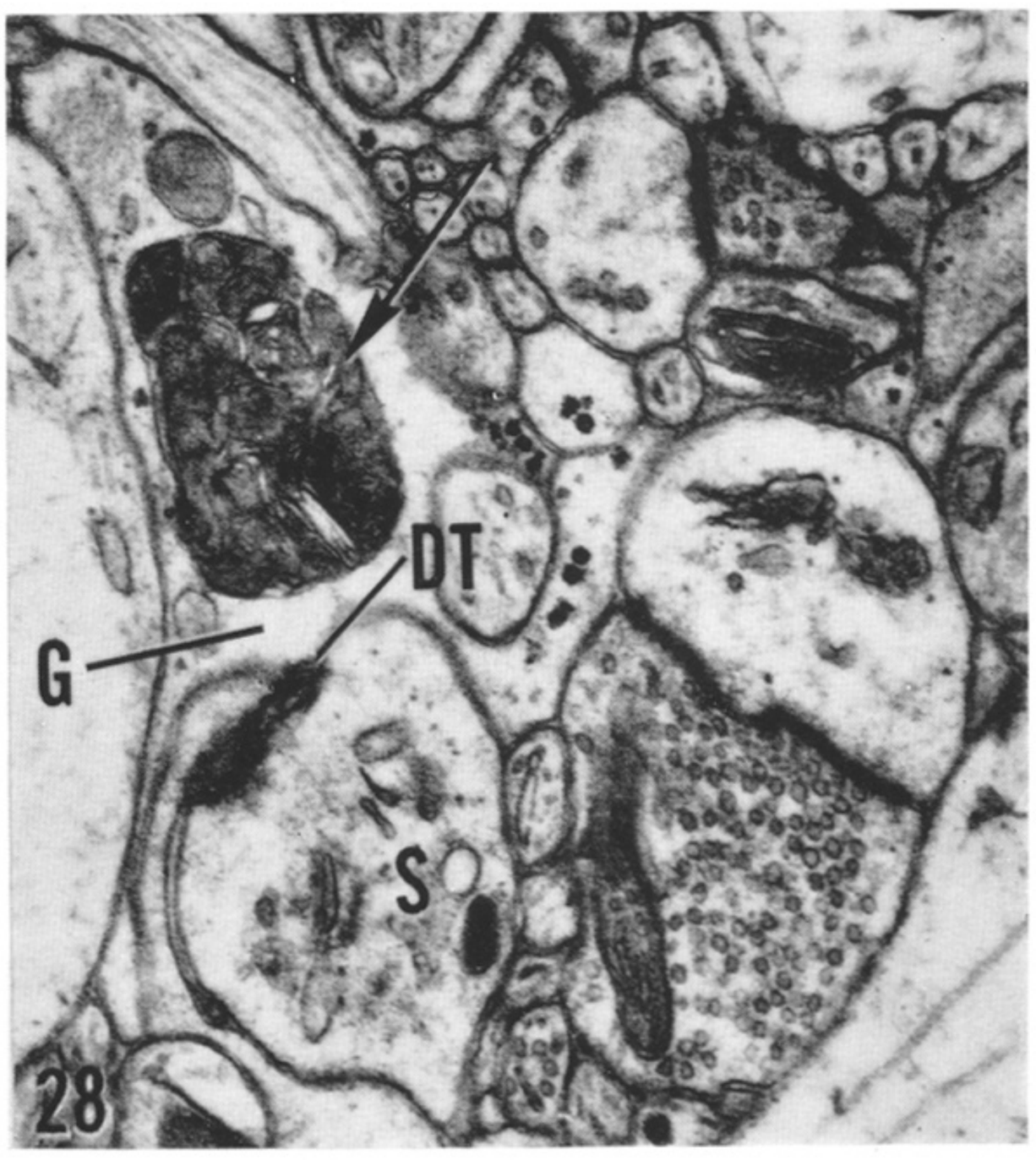


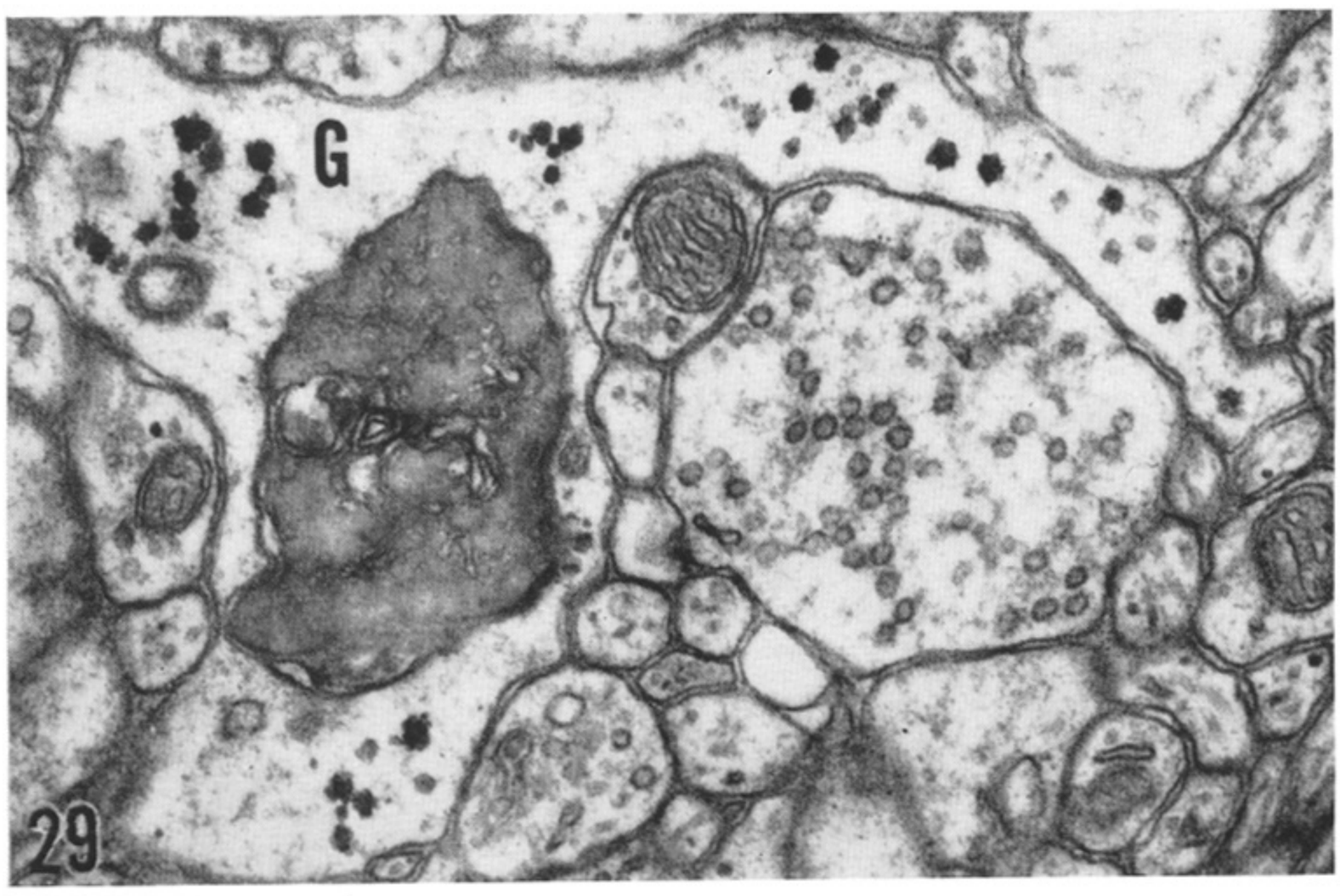
FIGURE 24. A late degenerating commissural axon terminal homogeneously dense, much reduced in size and deeply invaded by an astroglial process in which the glycogen reaction is especially marked. Note that the terminal and the spine (S) with which it makes synaptic contact (double arrows) are surrounded by the glial process but that the plasma membranes of all three remain separate and no glia invades the synaptic cleft. Lead citrate and uranyl acetate; × 150000.











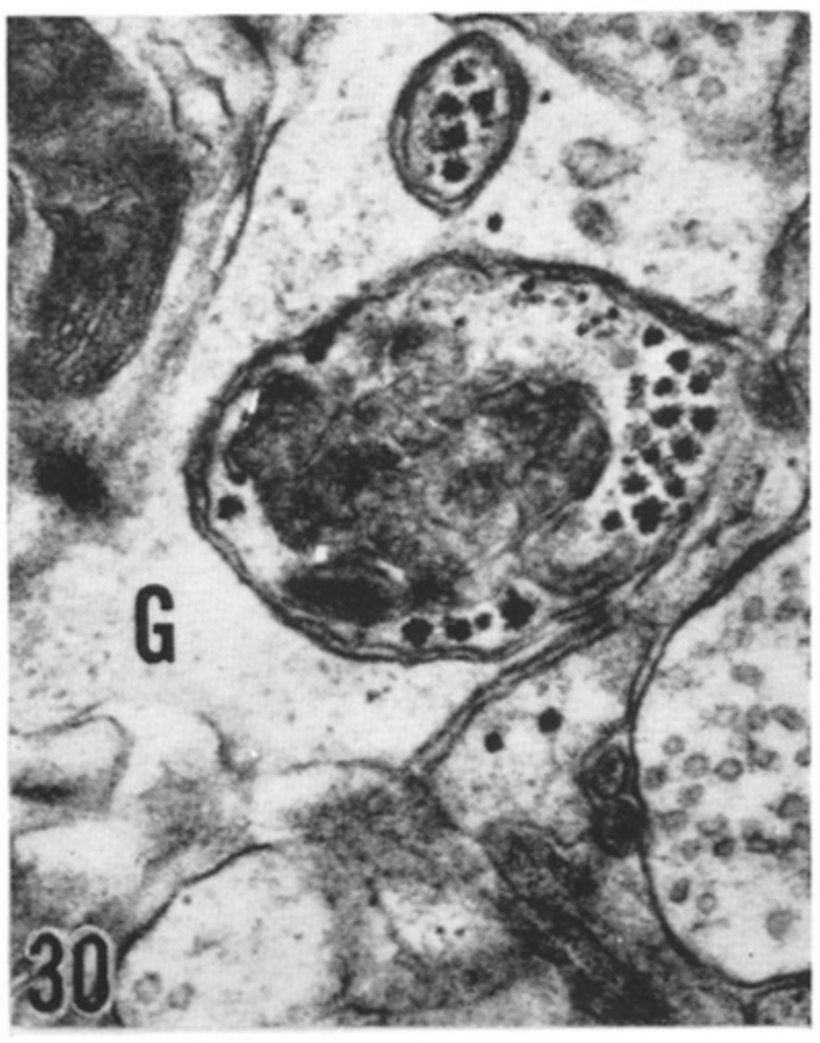


FIGURE 25. A degenerating commissural axon terminal (DT) ending on a dendritic spine (S) and surrounded by an astroglial process (G) which contains glycogen granules and other dense bodies (arrow) which may be portions of the same terminal. Lead citrate; × 31 000.

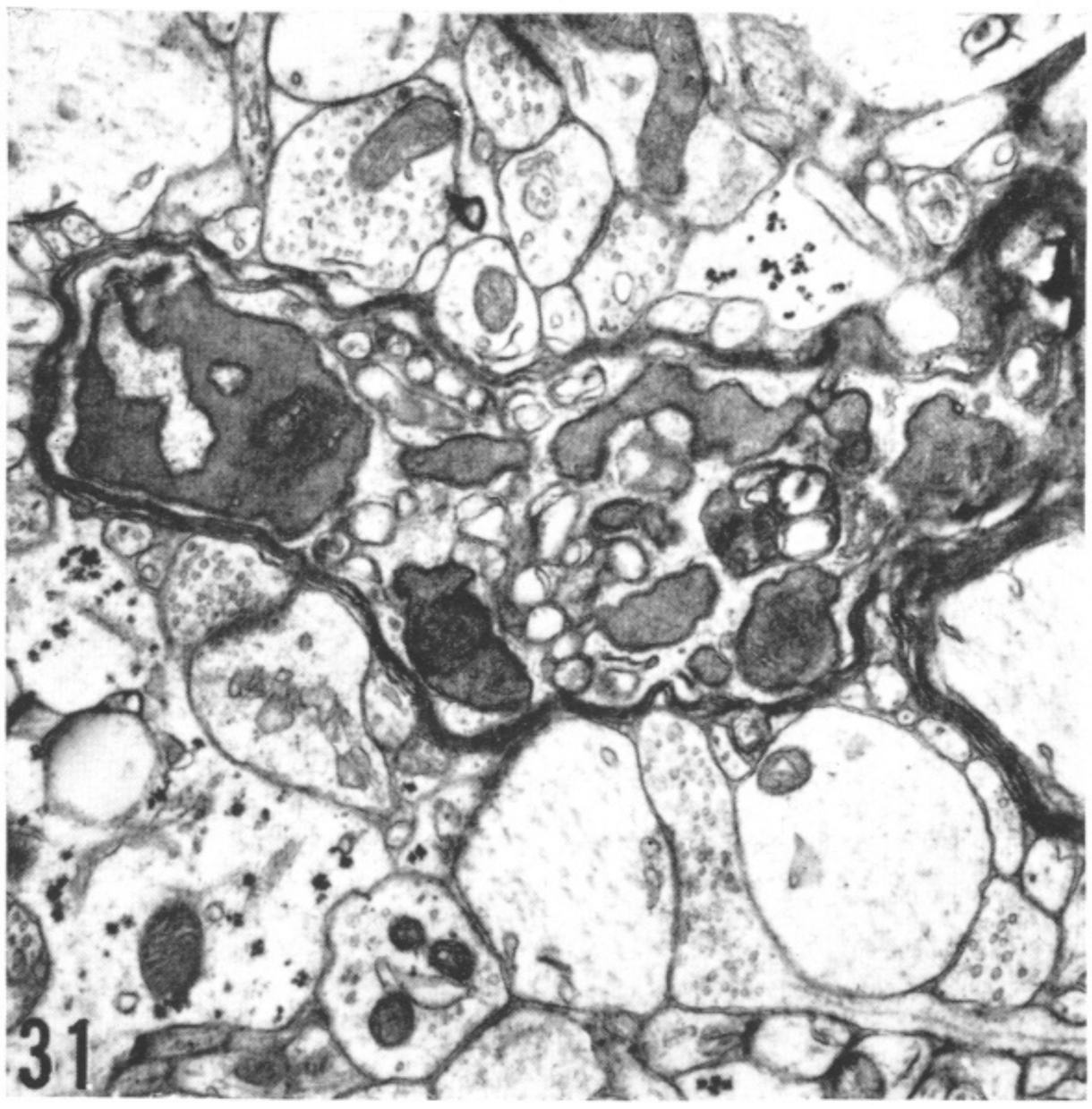
FIGURE 26. A degenerating thalamo-cortical axon terminal. Cf. with figure 25. Lead citrate; × 37 000.

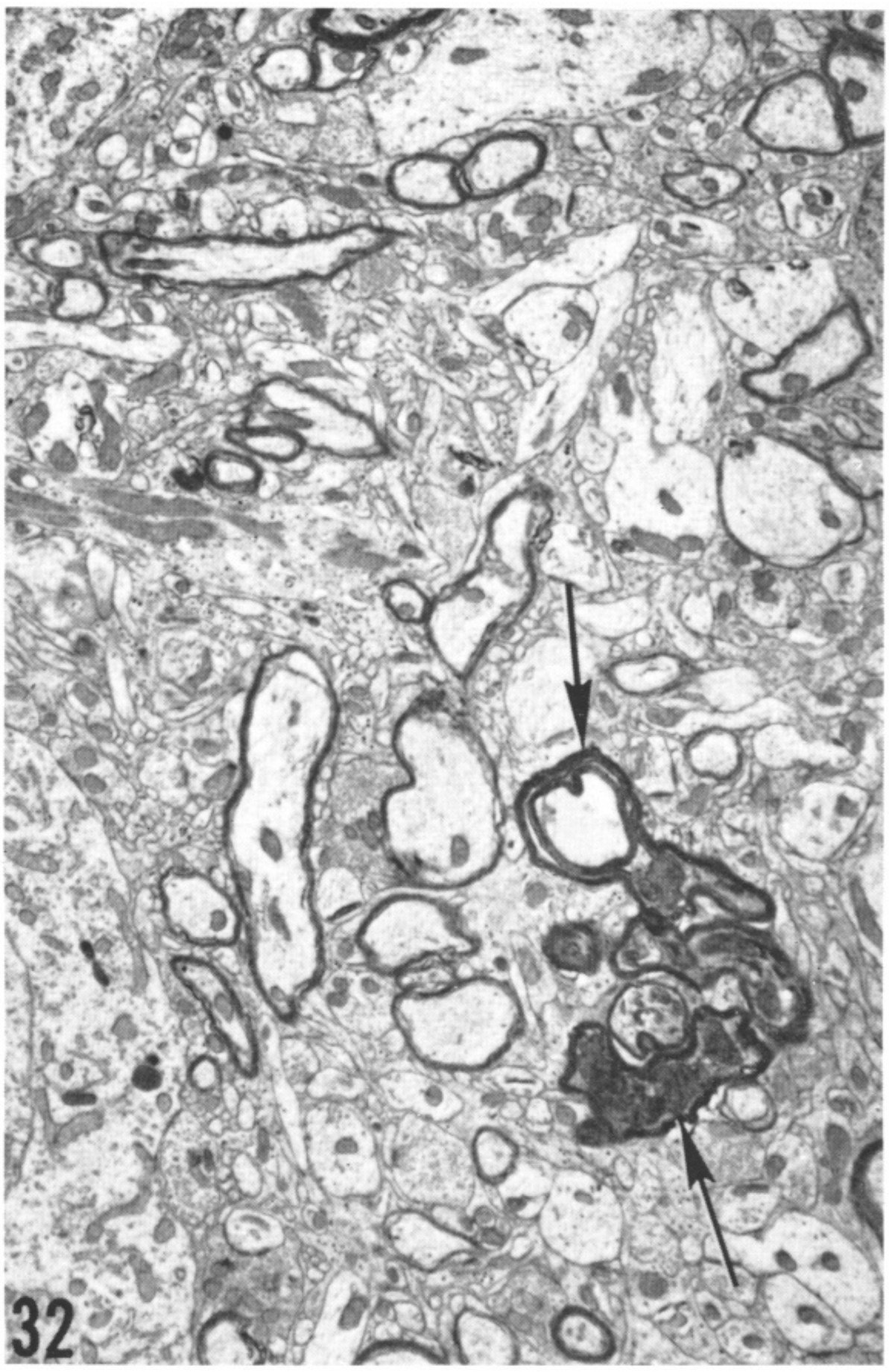
FIGURE 27. A degenerating association fibre terminal (DT) surrounded by an astroglial process which also contains a large membrane-bound dense body consisting of granules and whorled membranes. Lead citrate and uranyl acetate; × 38 000.

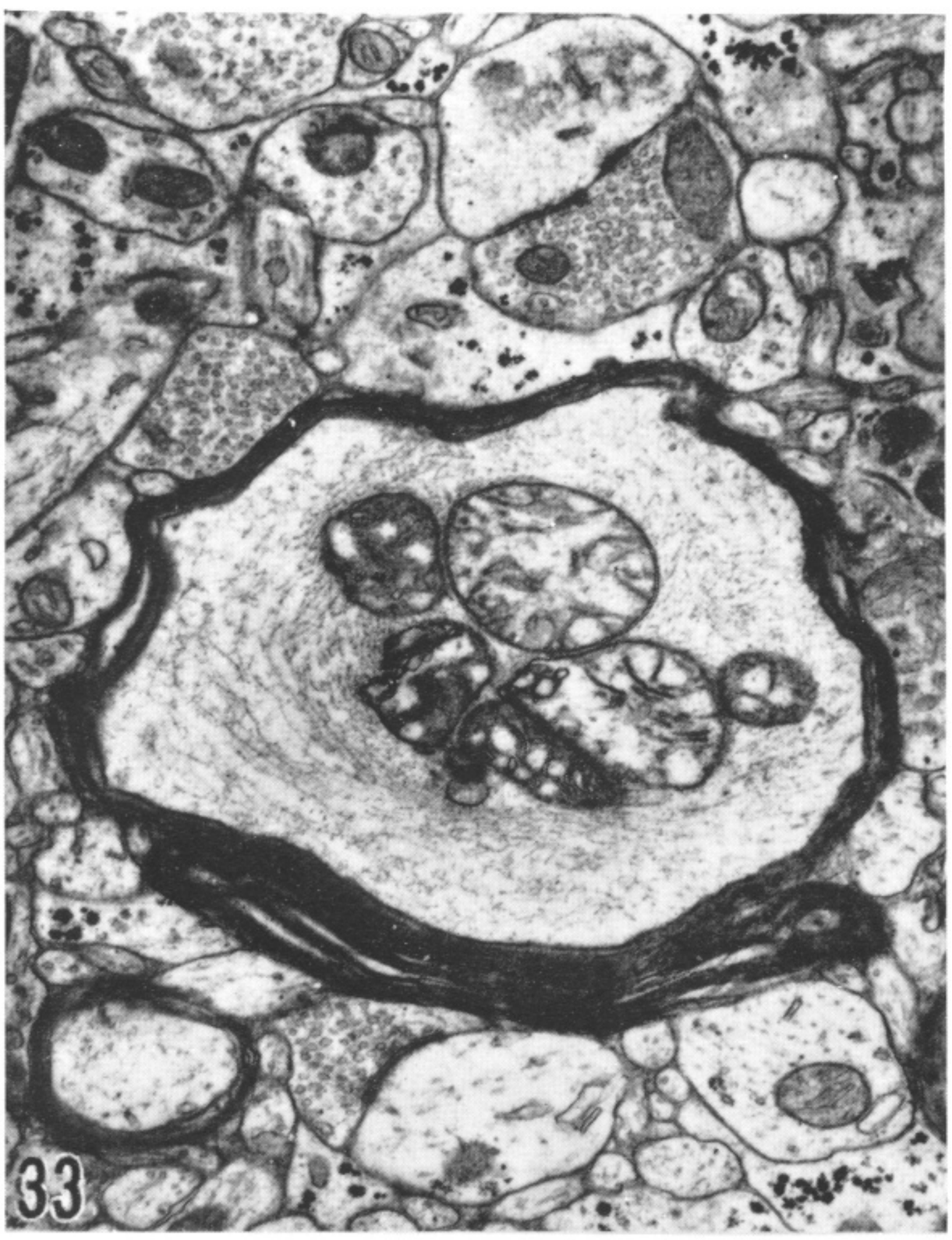
FIGURE 28. A degenerating commissural axon terminal (DT) reduced to a very small fragment attached to the postsynaptic dendritic spine (S). Associated with the terminal is an astroglial process containing a very large dense body similar to that shown in figure 27. Lead citrate and uranyl acetate; × 33 000.

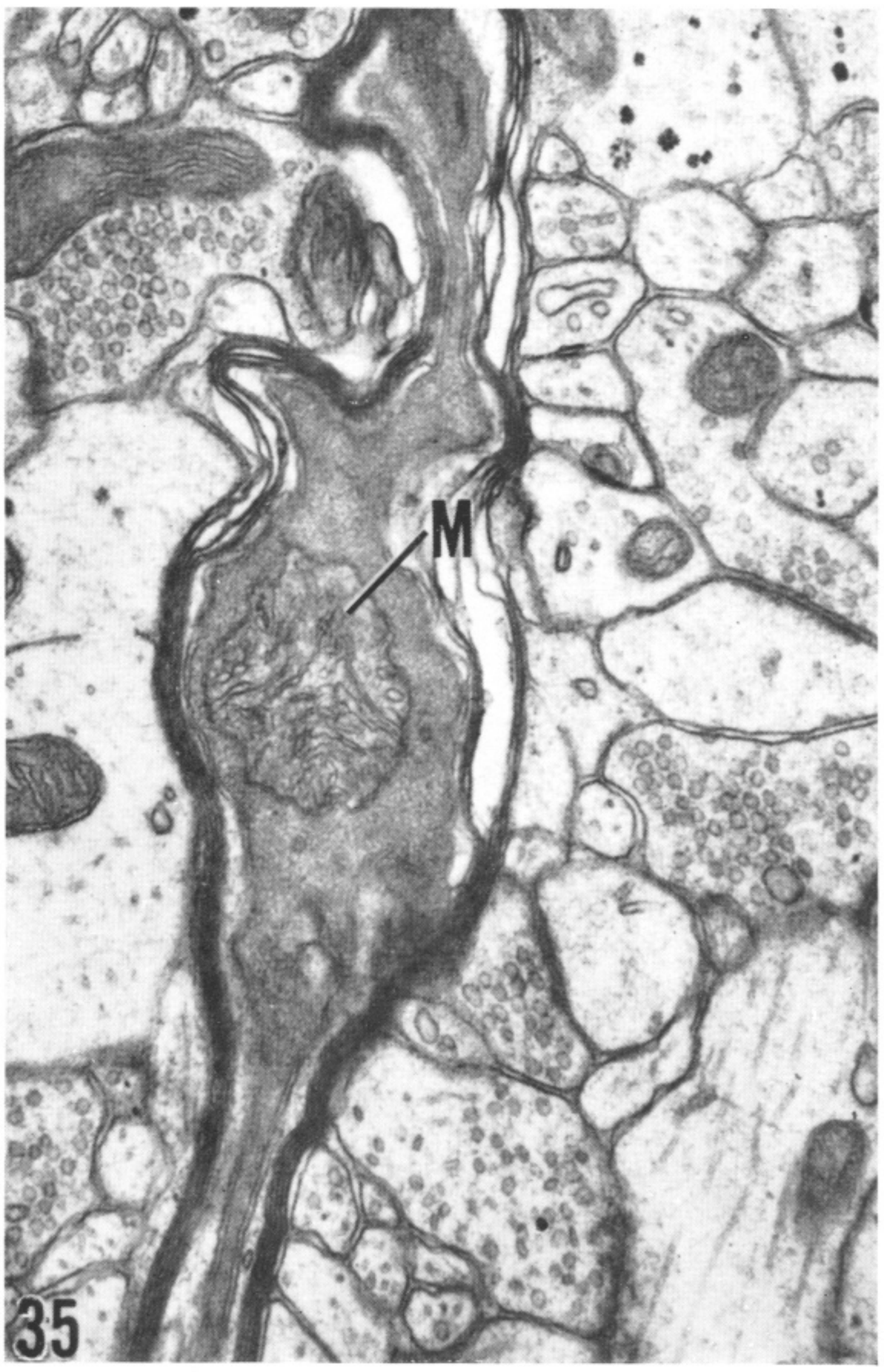
FIGURE 29. A membrane-bound dense body containing irregular membranous lamellae lying within an astroglial process in which the glycogen reaction is marked. Lead citrate; × 42 000.

FIGURE 30. Two membrane-bound complexes each containing glycogen granules and one a whorled dense body, lying within the cytoplasm of an astroglial process (G). Lead citrate; × 29 000.









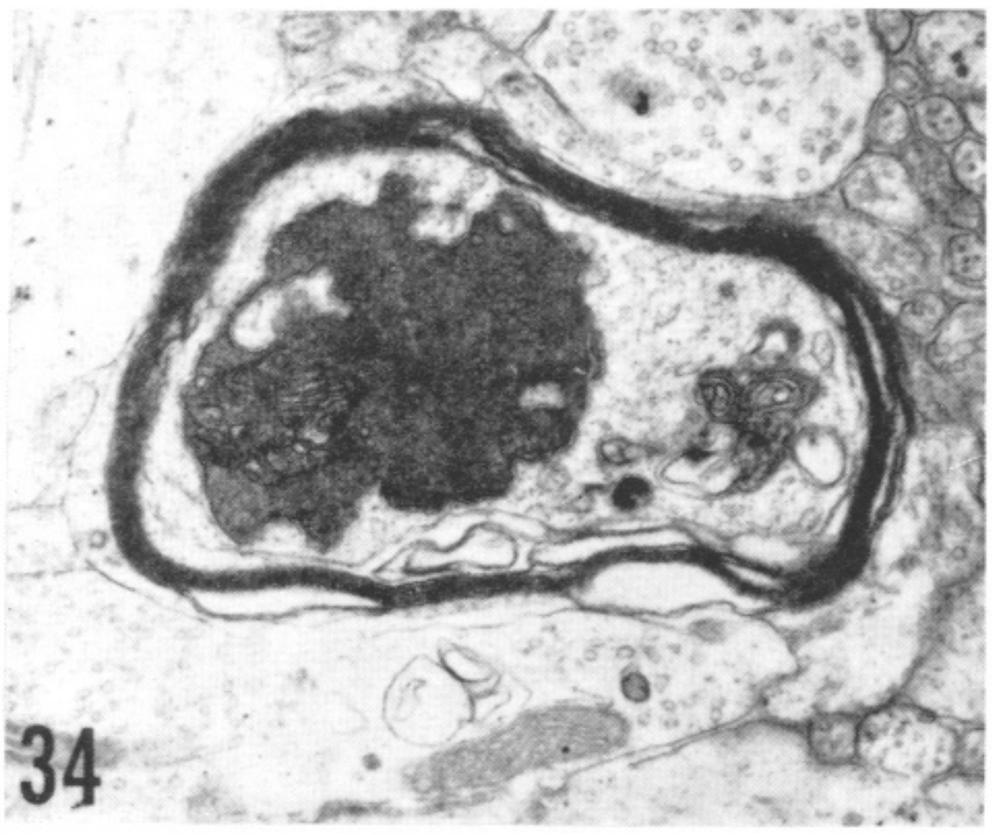


FIGURE 31. A degenerating association fibre showing fragmentation and vacuolation of the axoplasm but little or no change in the myelin sheath. Lead citrate and uranyl acetate; × 19000.

FIGURE 32. A degenerating association fibre showing fragmentation of the axoplasm and folding and reduplication (upper arrow) of the myelin sheath. Lead citrate; × 6000.

FIGURE 33. A large degenerating thalamo-cortical fibre showing swollen and distorted mitochondria and neurofilamentous hyperplasia. Lead citrate and uranyl acetate; × 17000.

FIGURE 34. A degenerating commissural fibre showing shrinkage of the degenerating axoplasm away from the myelin sheath. Lead citrate; × 20000.

FIGURE 35. A degenerating thalamo-cortical fibre showing the beading of the dense degenerating axoplasm which also contains a swollen mitochondrion (M). Lead citrate; × 33000.

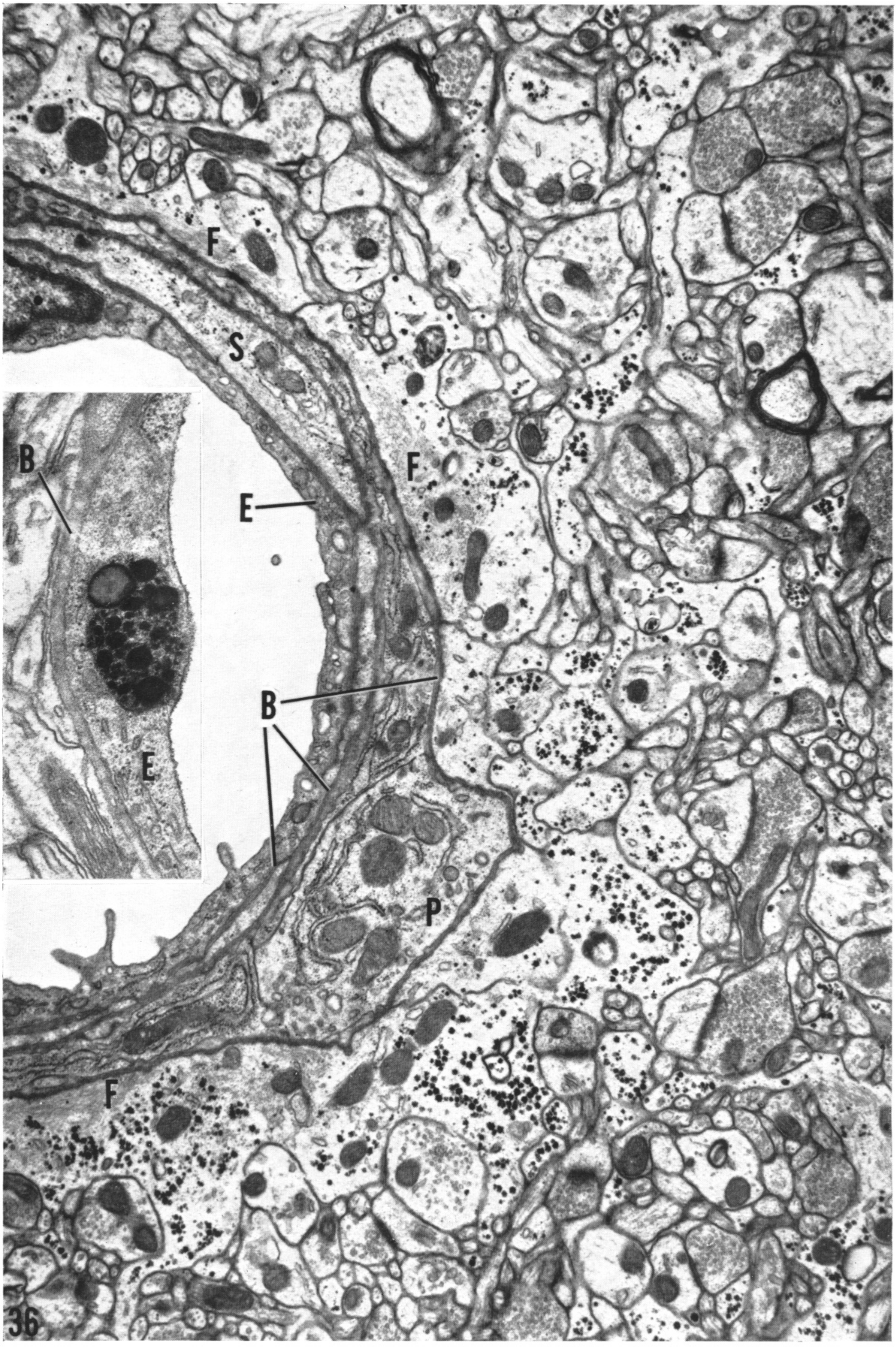
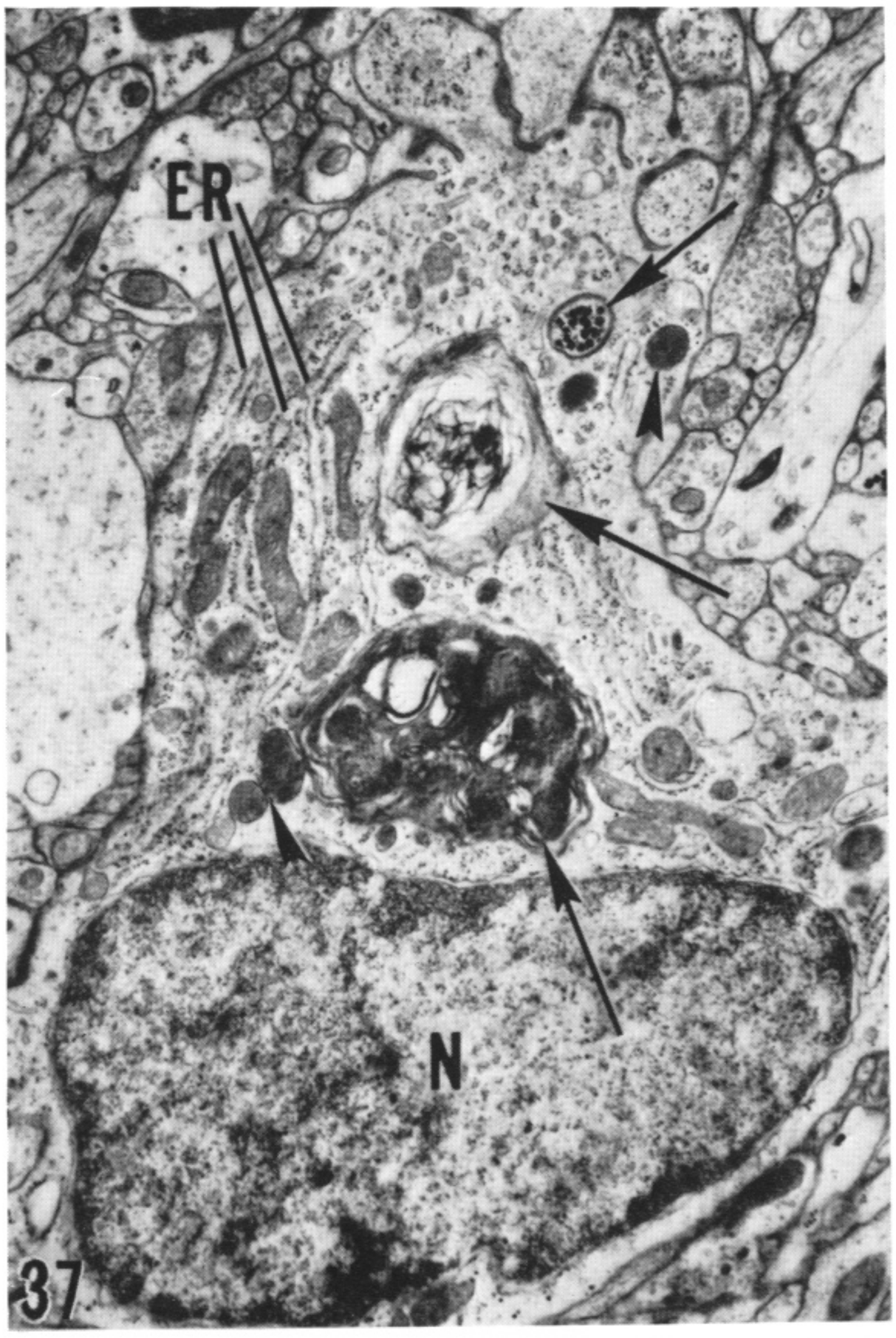
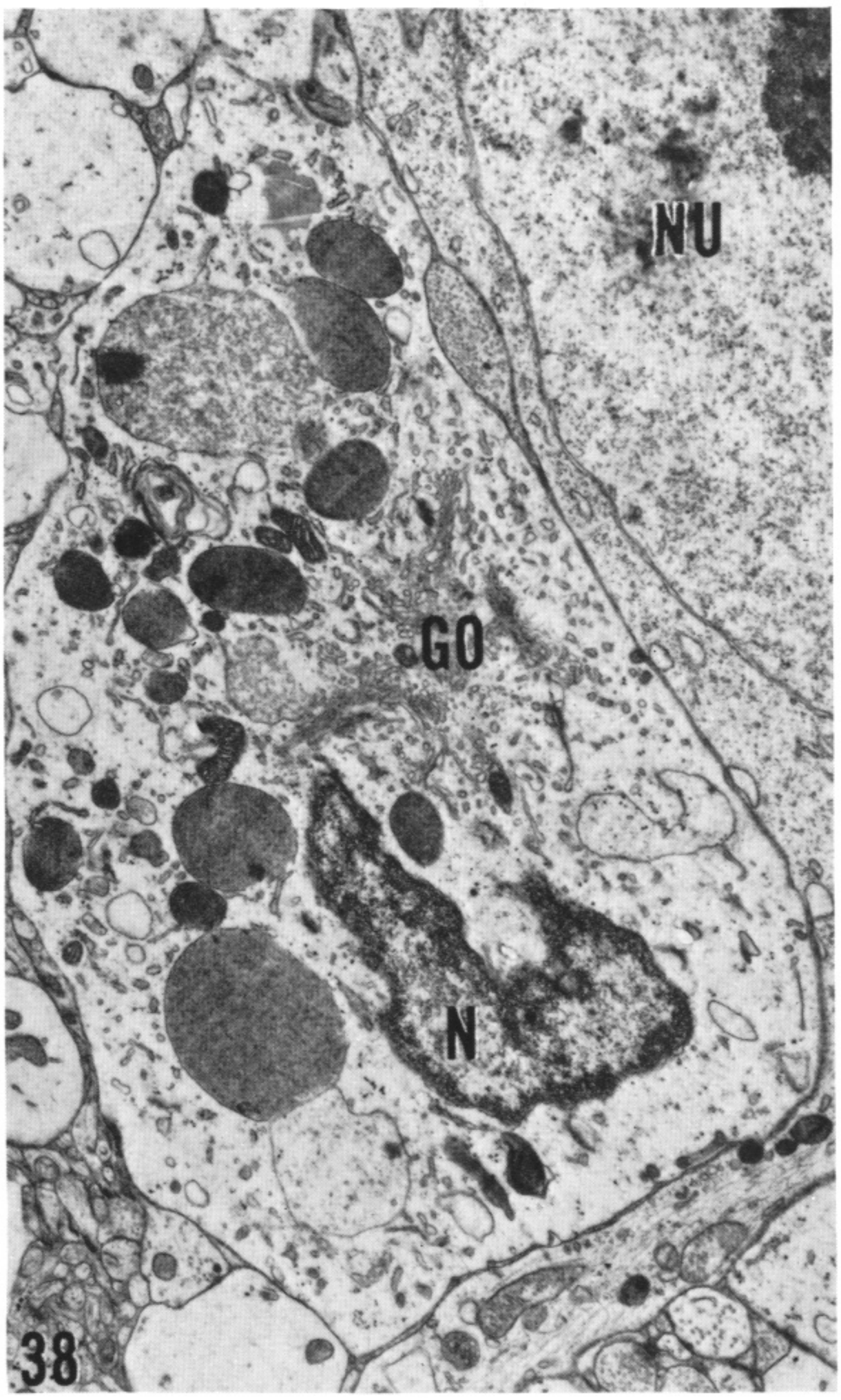


FIGURE 36. Showing a small blood vessel in the cortex of SI five days after a lesion in SII. The perivascular foot processes of the astrocytes are obviously swollen and contain large numbers of glycogen granules and filaments (F). P, pericyte; B, basement membrane; E, endothelium; S, smooth muscle cell. Lead citrate and uranyl acetate; × 18000. *Inset*: a granular and vacuolated dense body in the endothelium of a cortical capillary following a lesion in the thalamus. Lead citrate; × 37000.





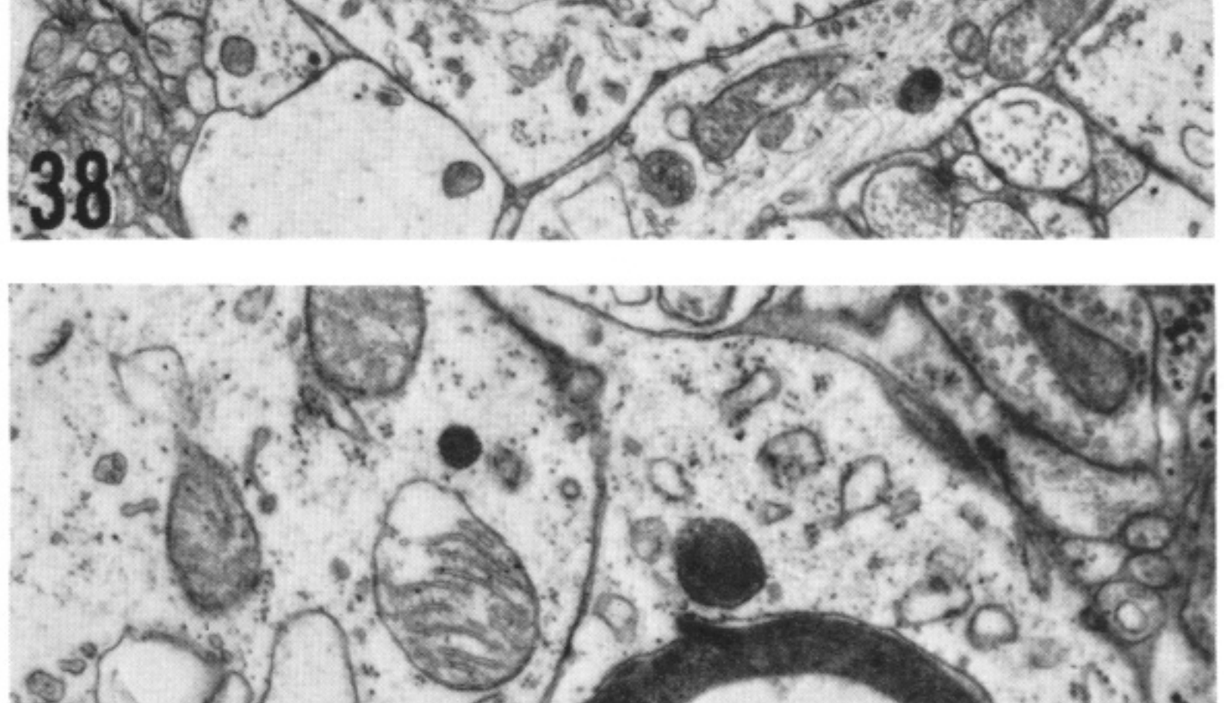
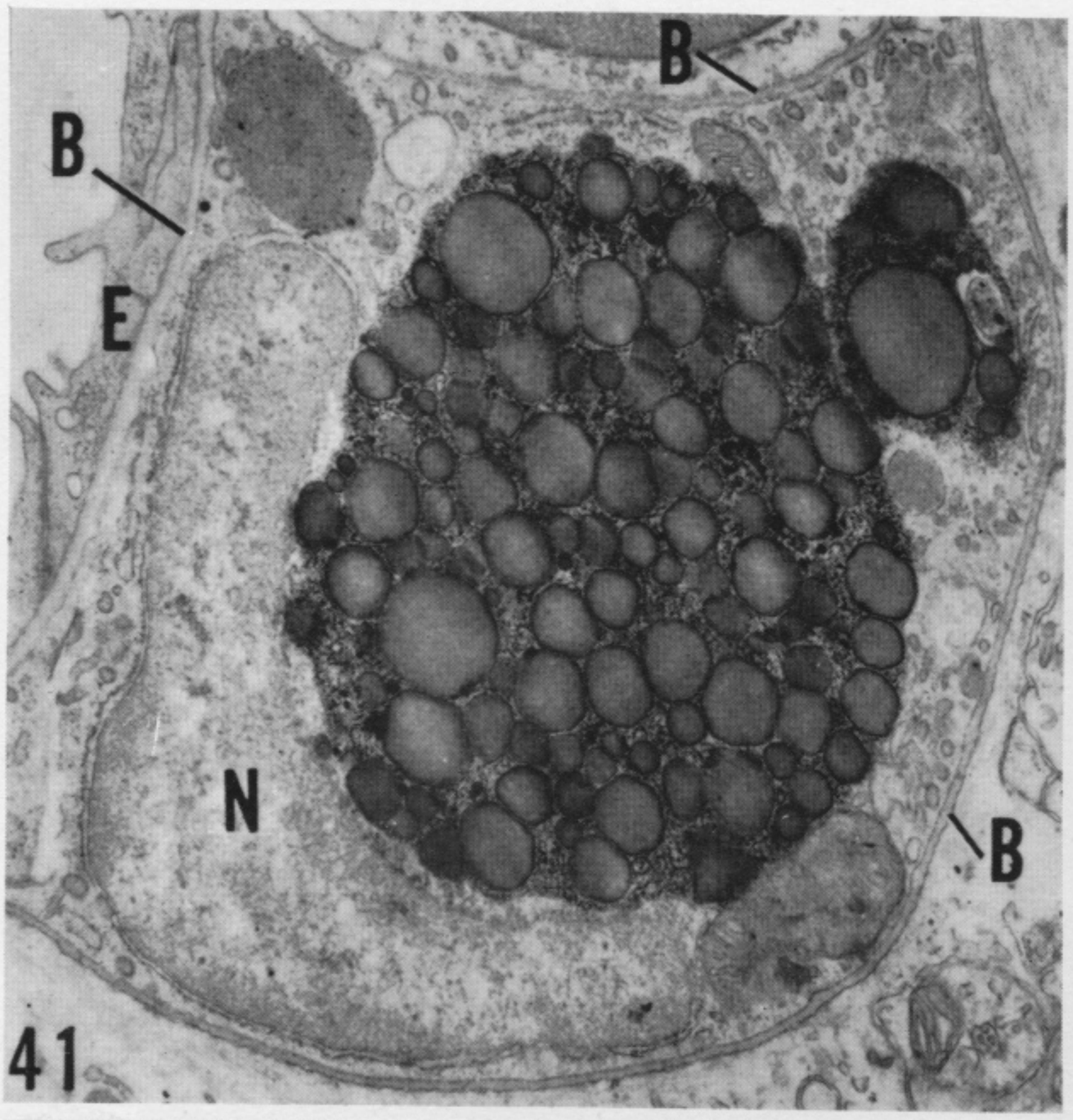


FIGURE 37. An unusual type of glial cell from the cortex of SI near a lesion in SII. This cell contains elongated cisternae of granular endoplasmic reticulum (ER), small dense bodies (arrow heads), several forms of larger inclusion (arrows) and a nucleus resembling that of an oligodendrocyte. Lead citrate and uranyl acetate; × 13000.

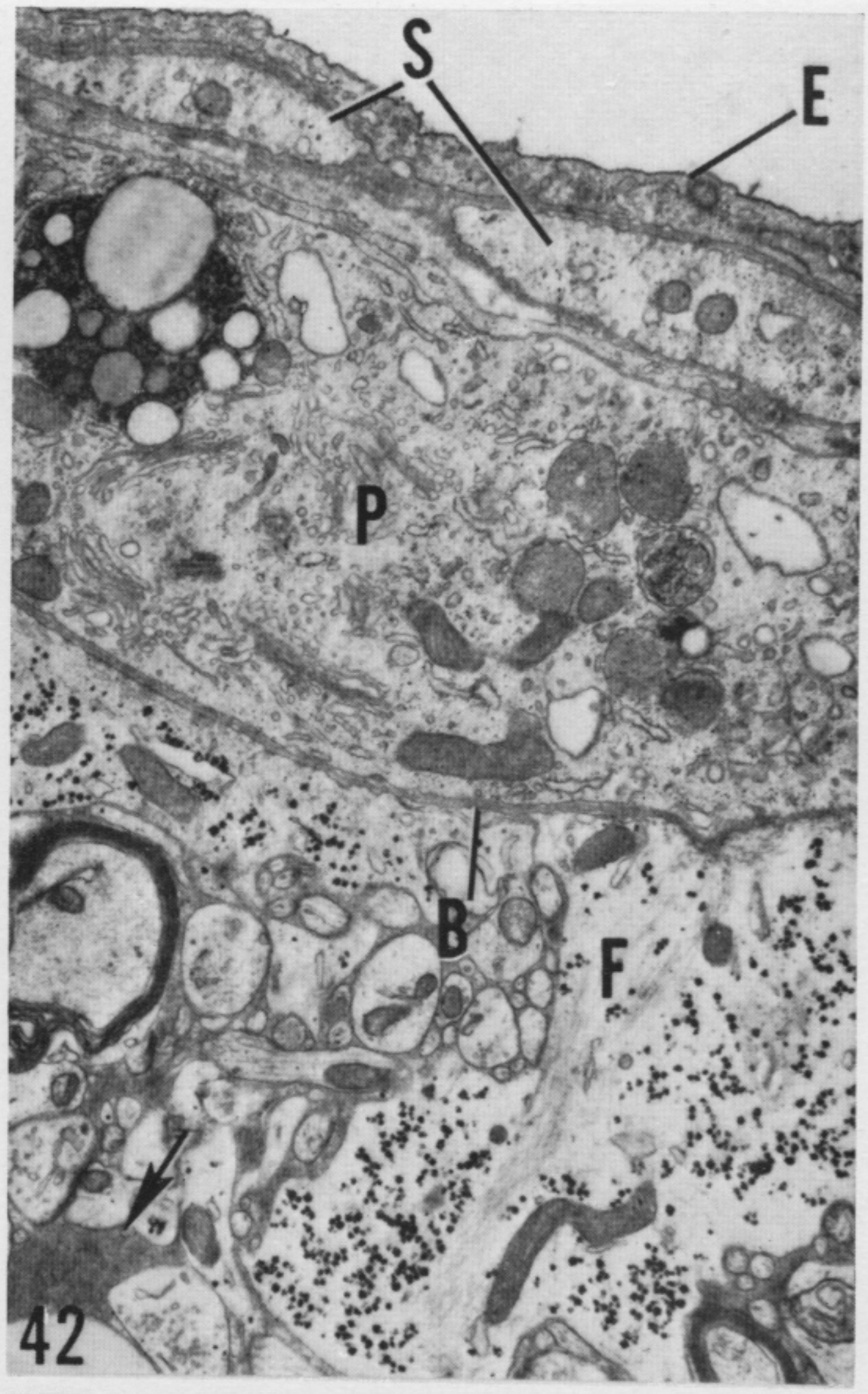
FIGURE 38. A common form of glial cell in the cortex which was isolated by a series of knife cuts. In this cell many forms of dense body are seen and there is a large amount of Golgi apparatus (GO) but endoplasmic reticulum is not prominent. The nucleus (N) resembles that of an oligodendrocyte. NU; nucleus of a neuron. Lead citrate and uranyl acetate; × 12000.

FIGURE 39. Part of the cytoplasm of an astrocyte in the cortex of SI following a lesion in the ipsilateral SII. Dense bodies, Golgi membranes and a membrane-bound myelin figure (X) resembling an engulfed portion of the sheath of a degenerating axon, are present. Lead citrate and uranyl acetate; × 31000.

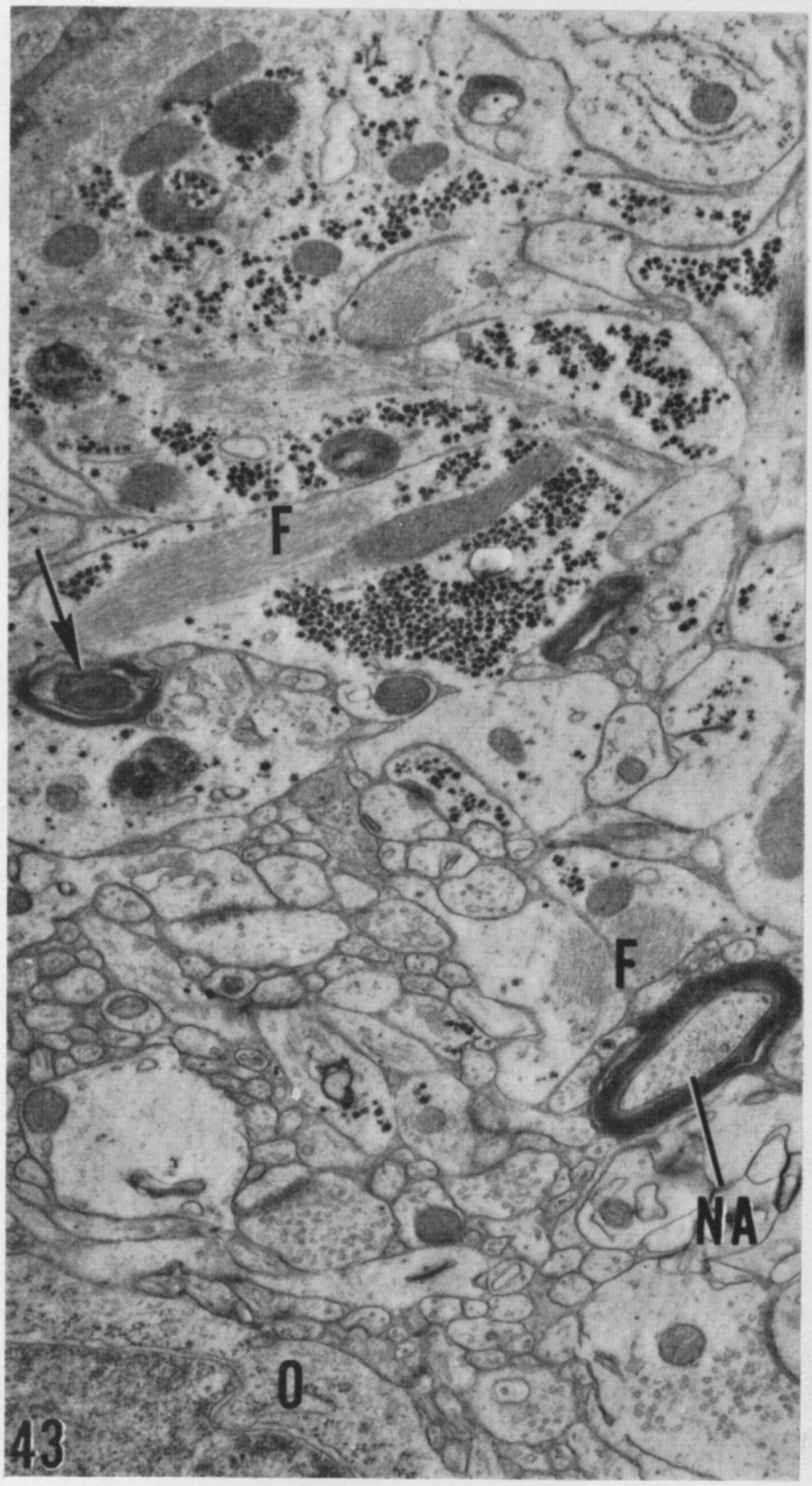
FIGURE 40. Part of an astrocyte in SI after a lesion in the opposite cortex; this cell contains a few small dense bodies and a large membrane-bound myelin figure (X). Lead citrate and uranyl acetate; × 20000.



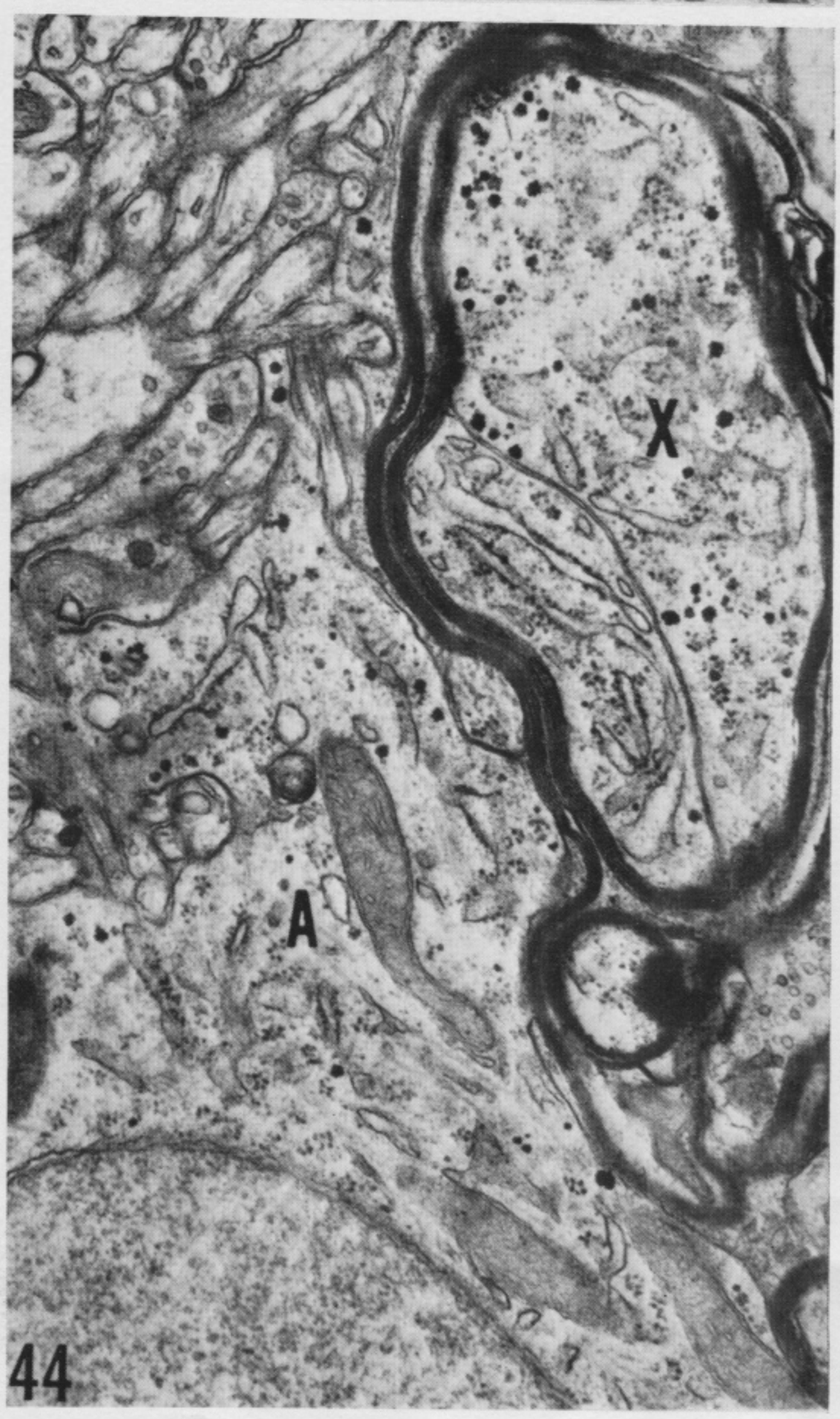
41



42



43



44