

ELECTRON MICROSCOPY OF THE SOMATIC SENSORY CORTEX OF THE CAT

I. CELL TYPES AND SYNAPTIC ORGANIZATION

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Two main types of neuron may be distinguished electron microscopically in the somatic sensory cortex. Pyramidal neurons have a characteristically triangular perikaryon with a high content of ribonucleo-protein consisting mainly of free ribosomes; the nucleus usually shows a single small indentation. Non-pyramidal neurons, which may be large or small, have a higher concentration of all intracytoplasmic organelles and particularly of long cisternae of rough-surfaced endoplasmic reticulum forming Nissl bodies. The nucleus is often deeply indented and crenellated. The two cell types differ also in the nature of their dendritic ramifications and particularly in their synaptic relationships. The majority of axon terminals ending on pyramidal neurons contact dendritic spines and relatively few end on the shafts of dendrites or on the perikaryon. Synapses on spines are typically of the type in which the synaptic thickenings are asymmetrical and the synaptic vesicles spherical. Such synapses, even when they occur on the shafts of pyramidal cell dendrites, are usually associated with a 'spine apparatus'. Most of the few synapses on the dendritic shafts and somata of pyramidal cells are associated with symmetrical membrane contacts and small, flattened or pleomorphic vesicles. Terminals of this type are commonly *en passant* endings of long, thin unmyelinated axons oriented vertically or transversely within the cortex. The somata and the usually irregular dendrites of non-pyramidal neurons are typically covered in axon terminals most of which contain flattened vesicles and end in symmetrical complexes, but a few may contain spherical vesicles and end asymmetrically. The axon hillocks and initial segments of both types of cell are post-synaptic to axon terminals containing small, flattened vesicles and ending symmetrically.

INTRODUCTION

The first indication that synapses in the central nervous system may be divided into two general categories, those with symmetrical and those with asymmetrical contact zones was derived from studies on the cerebral cortex by Gray (1959). Since then it has become apparent that these two types may have important functional implications, possibly forming one of the morphological

features of inhibitory and excitatory synapses. This correlation was postulated by Eccles (1964) and Raviola & Raviola (1967), and more recently Colonnier (1968) has shown that in the visual cortex axon terminals ending in asymmetrical membrane thickenings usually have synaptic vesicles which are spherical in aldehyde-fixed material, whereas those ending in symmetrical contacts more commonly have flattened vesicles. Colonnier has shown, in addition, that the two types of synapse have specific distributions upon different parts of the pyramidal and non-pyramidal neurons.

The present study is in many respects comparable to that of Colonnier, but in a different functional area of the cortex. It has been more concerned, however, with those features of the normal somatic sensory cortex which are of assistance in interpreting concurrent experimental degeneration studies (Jones & Powell 1970), designed to elucidate the site and mode of termination of afferent fibre systems within this cortical area. In dealing with very thin sections it is usually difficult to determine the type of neuron which a degenerating presynaptic terminal is contacting, as the postsynaptic profile is only rarely seen in continuity with the parent cell soma. If a study of the normal cortex could show that the processes of different types of neuron can be recognized even in isolation the problem could, to some extent, be resolved. Similarly, a correlation of the findings in the normal and experimental material might show that the terminal ramifications of different fibre systems could be identified in the normal cortex, and, if so, it would be important to ascertain whether their synaptic terminals show distinct differences.

MATERIAL AND METHODS

The material was taken from the first somatic sensory area of 12 adult cats. The animals were anaesthetized with Nembutal and tissue respiration reduced to a minimum by gradually lowering the body temperature to approximately 25 °C. The thorax was quickly opened and 2.0 ml of an equal parts mixture of 0.5 % heparin and 1 % sodium nitrite were injected into the blood in the left ventricle. With a cannula inserted in the ascending aorta the blood was washed out with a balanced salt solution and this was followed by a cooled solution of fixative made up of 1 % glutaraldehyde and 4 % paraformaldehyde in a 0.1 M phosphate buffer containing added calcium chloride and at a pH of 7.4.

Small cubes of tissue were removed from all topographic subdivisions of the first somatic sensory area. As far as possible they were selected from the summits of gyri so that sections could later be obtained which passed perpendicularly through the full thickness of the cortex with the cortical layers disposed parallel to the pia mater. The cubes were further trimmed to give rectangular blocks approximately 1 mm square on their pial surface and about 2.5 to 3 mm in their long axis. This is a sufficient length to pass through all cortical layers and include a little of the subjacent white matter. These blocks were postfixed in osmium tetroxide and embedded in Araldite. A 'thick' 0.5 to 1 μ m section was cut from the long face of the block and stained for light microscopy by the method of Richardson, Jarett & Finke (1960). In this section the layers of the cortex could be recognized quite readily and the block was then trimmed accordingly to give thin sections from known laminae. These were stained on the grid with lead citrate (Reynolds 1963) or with lead citrate and uranyl acetate (Watson 1958) and were examined in a Siemens Elmiskop 1B or a Philips E.M. 200 electron microscope.

Some additional blocks from the brains used in this study and others from brains fixed in the same manner were prepared for light microscopy by a Golgi-Kopsch method similar to that

described by Colonnier (1964). Examination of this Golgi-impregnated material was of assistance in certain instances in interpreting the electron microscopic findings.

RESULTS

The cell types. Neurons

The results obtained with the Golgi method will not be presented in detail, and for the purposes of the present paper it is sufficient to note that the neurons of the somatic sensory cortex fall into the general categories of pyramidal and non-pyramidal (Sholl 1956). Pyramidal neurons occur in all layers except layer I and are especially concentrated in layers II, III and V. The somata give off apical dendrites which ascend through all supervening layers, giving off a small number of side branches, to end finally as a spray of branches in layer I, and basal dendrites which do not extend beyond the layer containing the cell soma. The branches of these apical and basal dendrites, and these dendrites themselves at a variable distance from the soma, have many dendritic spines. Non-pyramidal neurons have dendrites which have few or no dendritic spines. They include stellate cells which are concentrated in layers II and IV of the cortex and whose dendrites do not extend greatly beyond the layer in which the soma lies, and fusiform and spindle cells whose dendrites may ascend and descend through several layers.

Under the electron microscope, the perikarya of pyramidal and non-pyramidal cells are readily distinguished (figures 1 to 3, plates 1 and 2). When cut in sections perpendicular to the surface, the perikarya of pyramidal neurons usually have a triangular shape with the apex directed towards the surface. The apex tapers gradually to become the thick apical dendrite and in many cases this is sectioned over a considerable part of its length. Basal dendrites are smaller and cut less frequently in continuity with the perikaryon. Most pyramidal cells rarely possess more than two stem basal dendrites, and an axon hillock giving rise to a straight initial axon segment is commonly seen between them. The perikarya of pyramidal neurons vary considerably in size, and this tends to increase with depth except in the case of layer IV where the few pyramidal cells have small perikarya. The internal structure of the pyramidal cell is constant throughout the cortex and resembles in most respects that of neurons in other parts of the brain (figures 1 and 3, plates 1 and 2). The large nucleus commonly has a small indentation of its membranes and sections taken through the edge of such nuclei suggest that, although narrow and never penetrating deeply into the nucleus, this indentation runs along one of its sides. In the cytoplasm rosettes of free ribosomes are especially prominent. Rough-surfaced endoplasmic reticulum is also widespread, though not densely concentrated, normally appearing as one or two short cisternae at regular intervals. Only rarely, at the bases of apical dendrites of the largest pyramidal cells, does the rough surfaced endoplasmic reticulum form large clusters which could be called Nissl bodies. Other organelles, typical of all neurons, are also present, including Golgi membranes, mitochondria, neurotubules, neurofilaments, various forms of dense body and rarely a cilium. Most of these organelles continue for varying distances into the apical and basal dendrites, though their concentration rapidly diminishes. A large number of neurotubules orientated in the long axis is the dominant feature of these dendrites, but there is usually a zone of increased concentration of endoplasmic reticulum, free and attached ribosomes and mitochondria at points where a stem dendrite divides into branches (figure 4, plate 3). The majority of the organelles also continue to a reduced extent into the axon hillock and initial segment of the axon (figure 3, plate 2).

In all layers in which they occur the perikarya of pyramidal neurons may appear singly or in small groups. In the latter case the plasma membranes of adjacent perikarya may be in direct apposition, and separated only by the usual extracellular space. Commonly at such points of contact one or both of the neurons will contain a multilaminar subsurface complex (figure 35, plate 10). This consists of a stack of three or more short cisternae, flattened over most of their length so that their two membranes are in contact, but expanding at their ends. The outer cisterna is in contact with the plasma membrane of the cell, while the inner one is dilated and has ribosomes attached to its deep surface. At times this inner cisterna is in contact with the rough surfaced endoplasmic reticulum in the vicinity and even with the perinuclear space. Simpler multilaminar stacks consisting of only one or two cisternae are often seen where a large astroglial process is in contact with a neuronal perikaryon (figures 31 and 33, plate 10). There was no indication, however, of the presence of tight junctions at any of these regions of contact.

The apical and basal dendrites usually do not branch close to the parent soma and have no spines between the soma and the first branch. The first branches of both apical and basal dendrites are often smaller in diameter than the parent dendrite but never markedly so, and sometimes the division may be a dichotomous one. Dendritic spines appear with increasing frequency beyond the first branch. Few or no axon terminals make synaptic contact with the proximal portions of apical and basal dendrites nor with the perikaryon (figures 1, 3 and 7, plates 1, 2 and 4).

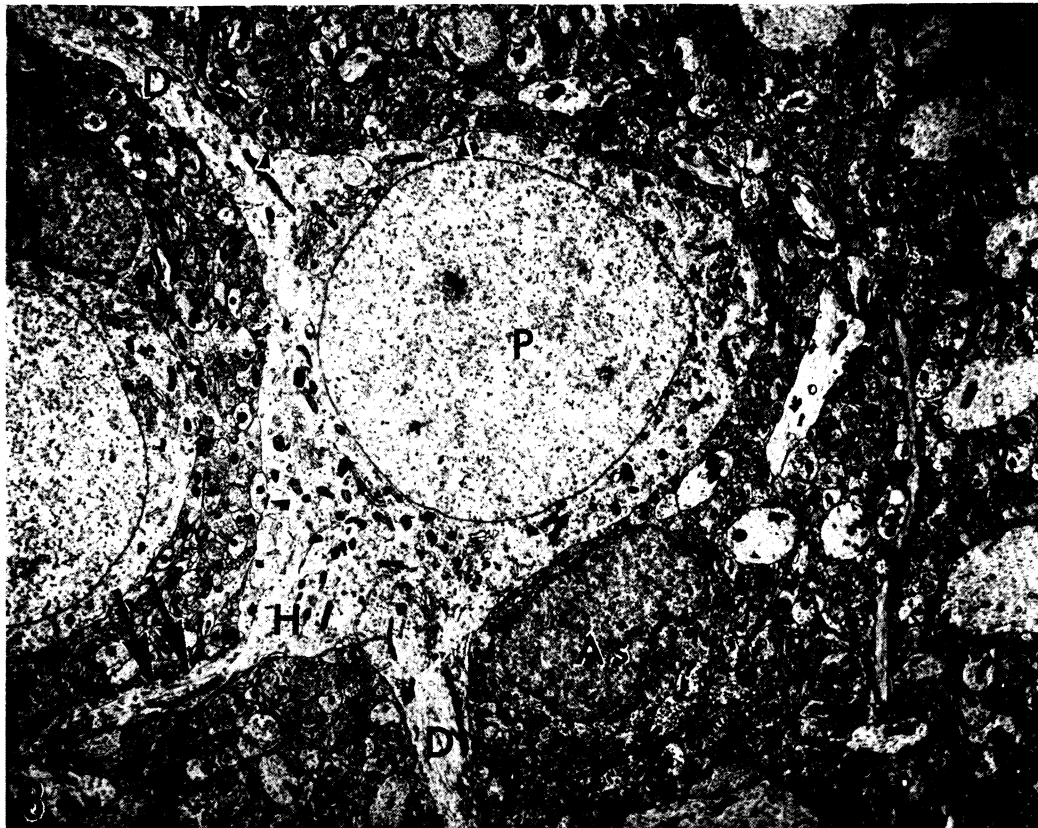
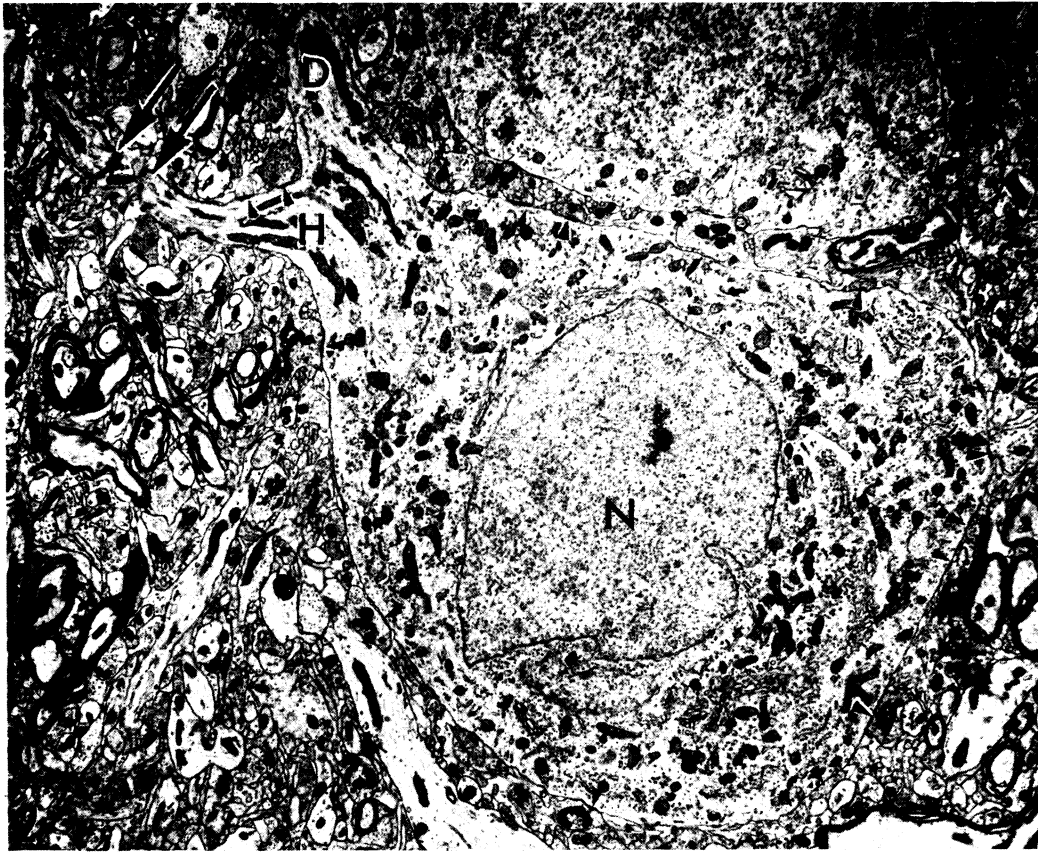
Other neuronal perikarya are frankly non-pyramidal in shape and possess certain other distinctive features (figures 1 and 2, plates 1 and 2). Such cells are found most commonly in layers II, IV and VI, to a lesser extent in layers III and V, and, though rare, are the only type present in layer I. Their greatest concentration is in layer IV where they form 60 to 80 % of the cells present. Their perikarya are round or oval rather than triangular in single sections cut perpendicular to the plane of a lamina. Apart from their different shape, they stand out from the pyramidal cells because of their higher concentration of intracytoplasmic organelles, particularly mitochondria, Golgi apparatus and rough-surfaced endoplasmic reticulum. In contrast to the pyramidal cell the rough-surfaced endoplasmic reticulum usually appears as groups of 4 or more long cisternae, forming obvious Nissl bodies in 3 or more parts of a profile. These cisternae may be continuous with each other and with the perinuclear space. The nucleus is large, as in the pyramidal cell, but commonly the nuclear membranes are irregular and may show multiple deep and wide invaginations. The smaller cells give off several dendrites which, relative to the size of the perikaryon, are much smaller than the apical and basal dendrites of the pyramidal cell and may be beaded. Even in the larger non-pyramidal cells there is a sharp demarcation between the dendrites and the cell soma, rather than a gradual termination. Commonly they branch very close to the soma and there is often a very marked difference in the size of the branches. The small side branches are usually irregular in shape or beaded, which is in accord with their appearance in Golgi material (Ramón y Cajal 1911). Few of the dendrites have spines. The axon hillock is small and the initial segment of the axon is often curved (figure 2, plate 2). The cell soma is frequently covered in axon terminals as also are the proximal portions of the dendrites and particularly their smaller branches (figures 1, 2, 5, 6 and 8, plates 1 to 4).

The glial cells

The glial cells of the somatic sensory cortex resemble those described in many other parts of the central nervous system (Mugnaini & Walberg 1964; Maxwell & Kruger 1965; Kruger &



FIGURE 1. Two pyramidal (P) and non-pyramidal neuron (N) from layer II of the cortex. Note the greater concentration of cytoplasmic organelles in the latter, particularly the aggregations of rough-surfaced cisternae (arrows). There are many axon terminals (arrow heads) on the perikaryon of the non-pyramidal cell but few (large arrow) on the pyramidal cells. The surface of the brain is towards the left. $\times 9000$.



FIGURES 2, 3. A non-pyramidal neuron (N) from layer V of the cortex and a pyramidal neuron (P) from layer II. In each case the surface of the brain is towards the right. Both neurons are sectioned through the axon hillock (H) and the initial segment (arrows) of their axon. Note the high concentration of organelles in the non-pyramidal neuron and their relative paucity in the pyramidal neuron. In addition, the non-pyramidal neuron is covered in synapses (arrow heads) while only a few terminals end on the soma of the pyramidal cell. D, dendrite; As, astrocyte. Figure 2, $\times 7000$; figure 3, $\times 5000$.

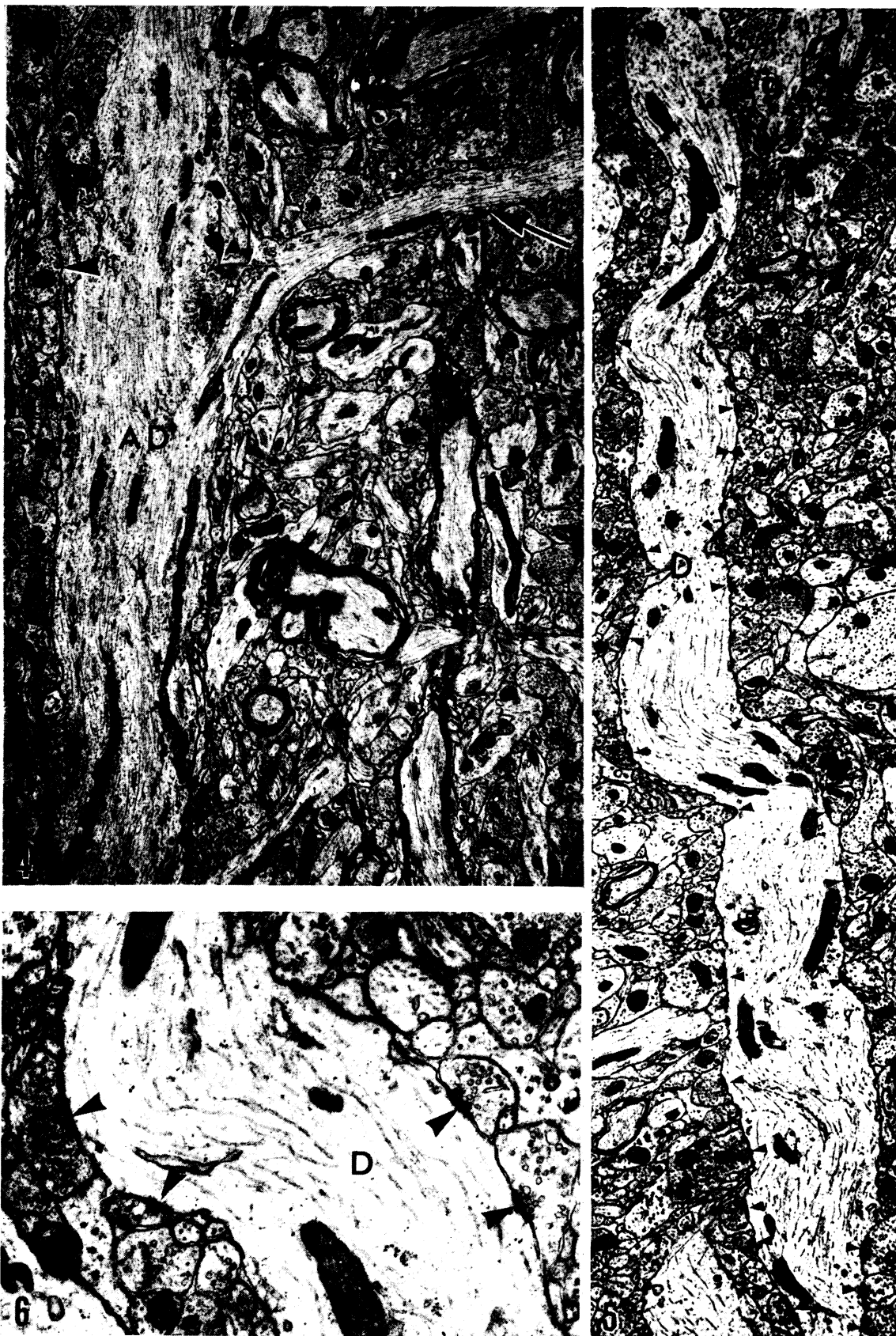


FIGURE 4. Apical dendrite (AD) of a pyramidal neuron in layer V at its first branch. Note the accumulation of ribosomes at the point of branching (double arrows) and the spine (arrow) attached to the branch. There is only one obvious synapse on the shaft of the apical dendrite (arrow head). $\times 12000$.

FIGURE 5. Large vertically oriented dendrite (D) of a non-pyramidal neuron from layer V of the cortex; this dendrite is covered in axon terminals (arrow heads), many of which end asymmetrically (figure 6). Figure 5, $\times 12000$; figure 6, $\times 19000$.

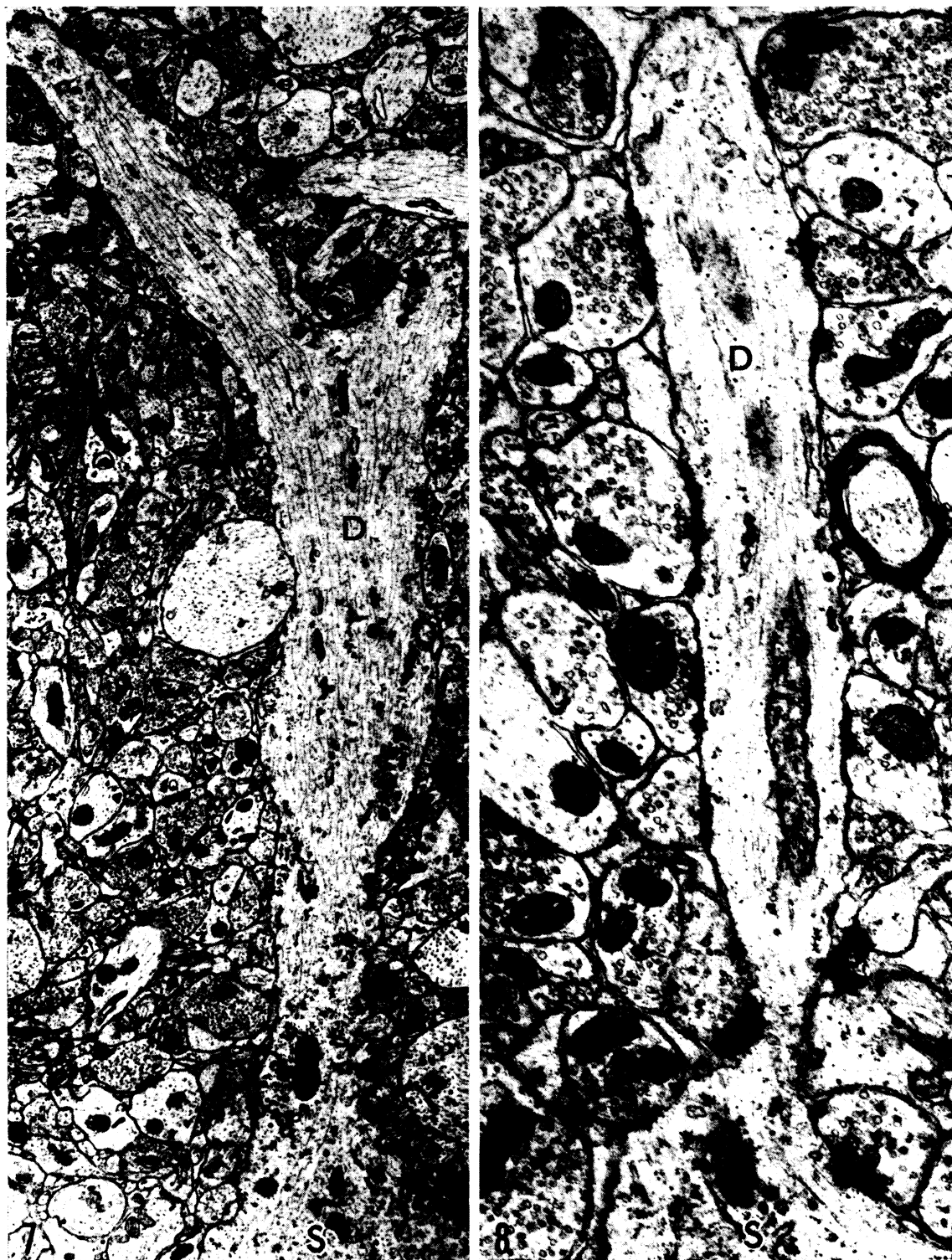


FIGURE 7. Basal dendrite (D) of a pyramidal neuron from layer III, at its point origin from the perikaryon (S). The proximal portion of this dendrite is typically devoid of axon terminals. $\times 10000$.

FIGURE 8. Dendrite (D) of a non-pyramidal cell at its point of origin from the perikaryon (S). Note that this dendrite receives many axon terminals right up to its point of origin. $\times 21000$.

Maxwell 1966; Vaughn & Peters 1968). Astrocytes and oligodendrocytes may be distinguished from one another by the characteristic differences in their nuclei, cytoplasm and content of cytoplasmic organelles. Rarely, a third type of glial cell is seen. This has a relatively dense nucleus, resembling that of an oligodendrocyte, but the cytoplasm is lucent like that of an astrocyte. The cytoplasm is different from both, however, in containing large numbers of long parallel cisternae of endoplasmic reticulum to which a few ribosomes are attached.

The synaptic types

The main types of synapses present in the visual cortex have been fully described by Gray (1959) and Colonnier (1968) and as those in the somatic sensory cortex are, in most respects, similar it is not necessary to give a comprehensive description. Although there is a certain gradation between them, two main types of synaptic contact may be discerned. In both types the pre- and postsynaptic membranes lie parallel to one another and show an increased density. In one type, however, there is a region of increased electron density attached and subjacent to the postsynaptic membrane (figures 9 and 12 to 23, plates 5 to 7) while in the second this is absent (figures 10, 11, and 24 to 34, plates 5, 8 to 10). Following Colonnier (1968) these will be termed 'asymmetrical' and 'symmetrical' respectively. Contacts of both types almost invariably contain a localized aggregation of synaptic vesicles immediately subjacent to the membrane of the axon terminal and this is considered the presynaptic side of the complex. Consistently, as in the visual cortex (Colonnier 1968), the presynaptic element of an asymmetrical synapse contains spherical synaptic vesicles (figures 9, 12 to 23, plates 5 to 7) while that associated with a symmetrical membrane thickening usually contains smaller vesicles some of which are oval ('flattened') or irregular (figures 10, 11, 24 to 34, plates 5, 8 to 10) in shape. In addition, it is noticeable in this material that the majority of axon terminals containing spherical vesicles and terminating in asymmetrical synaptic contacts, no matter what their size, are darker than those with flattened vesicles and symmetrical contacts. This darkening is due partly to an increased density of the background axoplasm and partly to a greater concentration of vesicles. The terminals ending in asymmetrical contacts are usually full of vesicles whereas in those ending as symmetrical contacts the vesicles are commonly fewer and clustered to one side near the synaptic thickening.

The postsynaptic surfaces

Axon terminals are encountered with varying degrees of frequency upon the following types of postsynaptic profile: dendritic spines, dendritic shafts, neuronal perikarya, axon hillocks and initial segments. Axo-spinous contacts are in the majority in the somatic sensory cortex, although more are seen in layers I to IV than in layers V and VI. Every spine receives one dense terminal containing spherical vesicles and terminating in an asymmetrical synaptic contact on the dilated portion of the spine proper (figures 9, 12 to 23, plates 5 to 7). The contact may be single or multiple (usually double). In a small part of one section in which positively-identifiable asymmetrical membrane thickenings on spines were counted, out of a total of 117, 75 were single and the remainder multiple. In all except the smallest spines there is some evidence of a spine apparatus (Gray 1959) close to this contact. A few spines also receive a second, asymmetrical synapse from a further dense terminal. This may occur on the pedicle of the spine or upon the parent dendrite close to the point of attachment of the pedicle (figures 20 and 21, plate 7), and, in the latter case, an additional spine apparatus is commonly present in the shaft beneath the synapse (figures 13, 20 to 22, plates 6 and 7). In addition to the constant asymmetrical

synapse, between 10 and 20% of the spines encountered also received a pale axon terminal containing flattened vesicles and ending as a symmetrical contact on the spine of its pedicle. When this type of synapse appears on the shaft it is not associated with a spine apparatus. Overall, the shafts of small spine-bearing dendrites receive few axon terminals and are never completely surrounded by them; symmetrical and asymmetrical contacts appear with approximately equal frequency. Both types usually have only single points of contact. The larger proximal portions of the apical and basal dendrites of pyramidal cells, close to the soma, receive only rare axon terminals (figures 4 and 27, plates 3 and 8); these are almost invariably pale, end symmetrically and have flattened or irregular vesicles. Other large dendrites arising from neuronal perikarya which have been classified as non-pyramidal, and their smaller, beaded side branches may be covered in axon terminals many of which end symmetrically (figures 5, 6, 8, 24 and 25, plates 3, 4 and 8) and contain flattened or pleomorphic vesicles; though in the case of the smaller branches some may be dense with asymmetrical contacts and spherical vesicles. Moreover, on this type of dendrite, intermediate or difficult-to-classify forms are met with more frequently. Many small dendritic profiles, both regular and irregular, which in single thin sections are isolated from their perikaryon of origin, are covered in axon terminals. None of these bear dendritic spines and most are concentrated in layers II and IV though a few appear in all layers.

Most neuronal perikarya receive axo-somatic synapses, although the numbers vary. Single profiles of pyramidal neurons usually have no more than one to six terminals, and all are pale with symmetrical thickenings and flattened or pleomorphic vesicles (figures 1, 3, 31 to 34, plates 1, 2 and 10). Many of these terminals are bilobed or irregular in shape and may have more than one point of synaptic contact. The perikarya of non-pyramidal cells are often covered in axon terminals, mostly of the pale type, with flattened vesicles and single symmetrical contacts, but an occasional dense terminal ending asymmetrically may be encountered.

Pale terminals with symmetrical membrane thickenings occur on the axon hillock of both pyramidal and non-pyramidal cells (figure 2, plate 2), sometimes even surrounding the origin of the axon. Similar terminals—often very large numbers of them—end on the initial segments of pyramidal cell axons, but are less frequently encountered on those of non-pyramidal cells.

The axonal ramifications

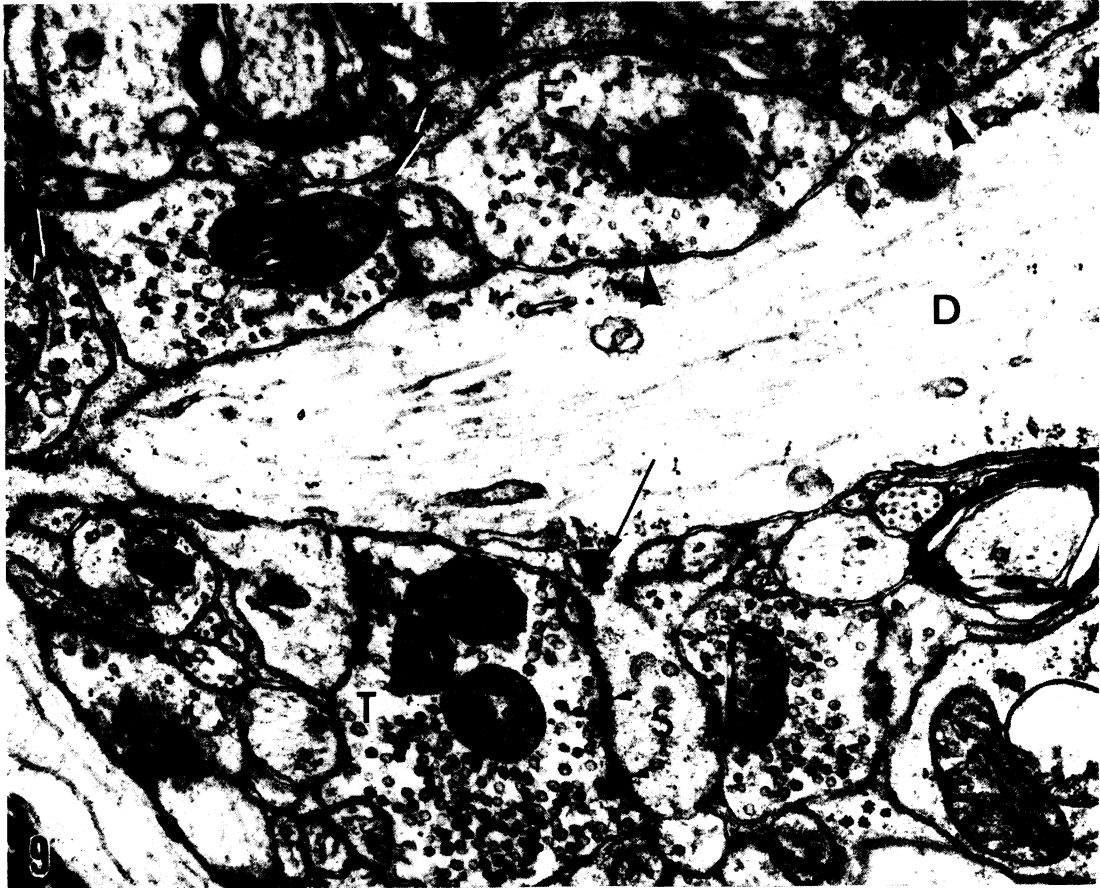
It is apparent that the dense axon terminals which have spherical vesicles and end in asymmetrical synaptic contacts are endings of either myelinated or unmyelinated axons and are

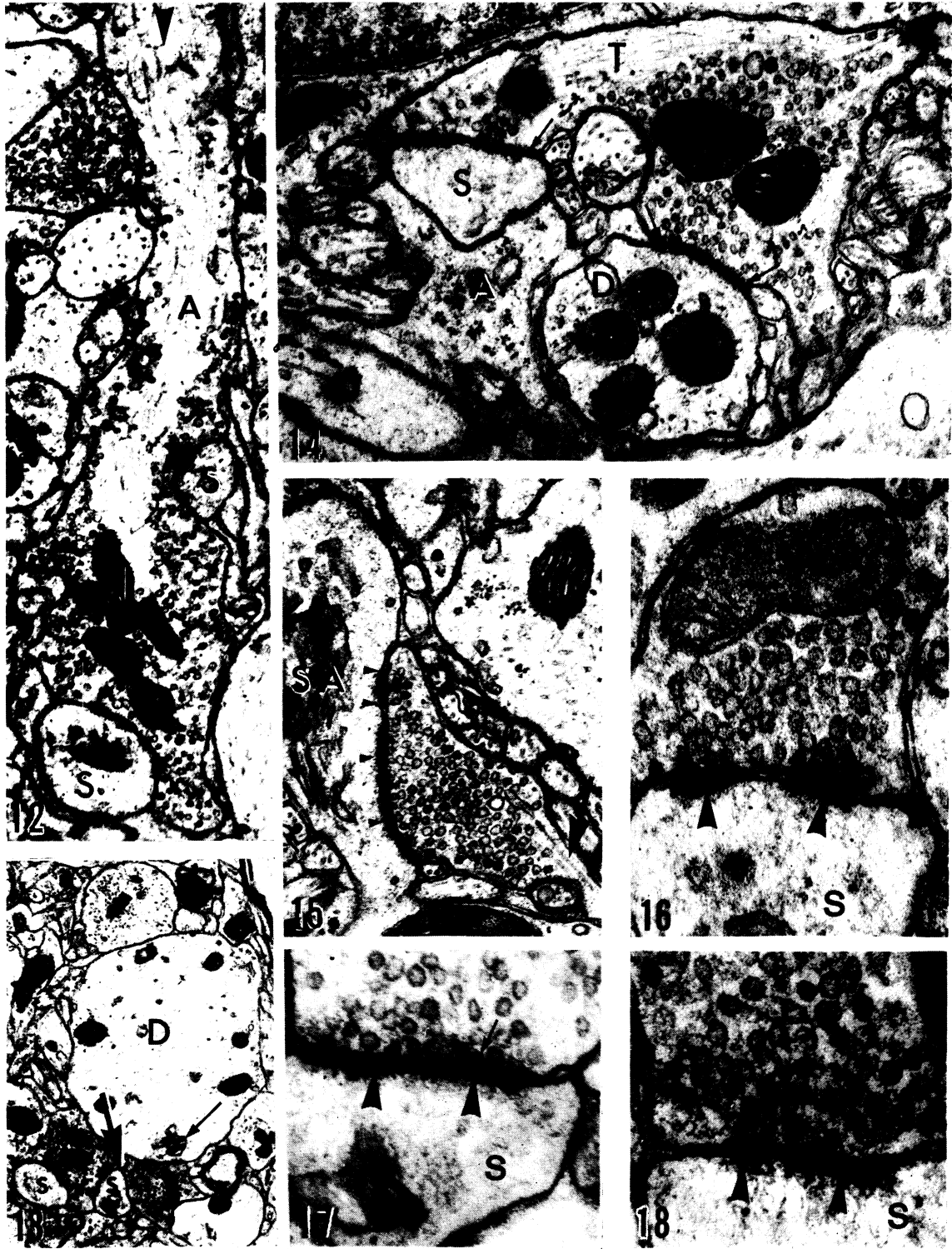
DESCRIPTION OF PLATE 5

FIGURE 9. A large pyramidal cell dendrite (D) giving rise to a spine (S). An axon terminal (T) containing spherical synaptic vesicles ends on the spine by means of an asymmetrical thickening (small arrow heads). The shaft of the dendrite receives two axon terminals (F) which contain a high proportion of small flattened vesicles and end symmetrically (large arrow heads). Two of these terminals are seen to be derived from the same unmyelinated axon (large arrows). The small arrow indicates the spine apparatus at the base of the dendritic spine. $\times 36000$.

FIGURE 10. Two dendrites (D) with which a number of axon terminals containing flattened synaptic vesicles make synaptic contact. Note that in the case of the dendrite on the right, the terminals are derived from a common unmyelinated axon (A). The synaptic contacts are symmetrical. $\times 30000$.

FIGURE 11. Three axon terminals (T) containing flattened or pleomorphic vesicles. Two of these make a symmetrical synaptic contact (arrow heads) with the same dendrite (D). Note the terminal (arrow) containing larger, spherical vesicles. $\times 45000$.





either true terminals or terminals *en passant*. Within this broad classification two main types may be discerned. Throughout the cortex many small dense terminals are seen; these are always filled with synaptic vesicles and probably form the greatest proportion of all terminals in the cortex (figures 9, 15 to 23, plates 5 to 7). They usually end axo-spinously, but some may be seen terminating on shafts of small dendrites and rarely upon the somata of 'non-pyramidal' cells. They are best studied in the superficial one quarter or so of layer I, for here virtually all terminals are of this type. In this position they are true terminal or *en passant* endings of very fine unmyelinated fibres which form an interlacing tangential plexus at the junction of the superficial and deep parts of layer I and which do not seem to branch. A similar unmyelinated tangential plexus is present at the junction of layers III and IV, and in this region large numbers of this small type of terminal are also present. Elsewhere in the cortex identical small dense terminals are seen, but most seem to be true end bulbs rather than *en passant* terminals.

In layer IV and adjacent parts of layers III and V, and to a lesser extent in the deep part of layer I, but not in other regions, a larger dense type of terminal is seen. This has spherical vesicles and ends in asymmetrical contacts mainly on dendritic spines but also to some extent on adjacent dendritic shafts (figures 12 to 14, plate 6). This terminal is derived from a myelinated axon of medium diameter which swells to become the terminal on losing its myelin sheath. This large terminal is usually packed with vesicles and may have contacts on 4 or more adjacent spinous or dendritic profiles. Frequently it narrows again to become a relatively large unmyelinated axon which after a short distance re-expands as a second large terminal with multiple synaptic contacts. Sometimes, the unmyelinated segment may branch to give rise to two terminals. Some of the largest terminals of this type may have a central core of neurotubules and neurofilaments.

The less dense axon terminals with flattened or pleomorphic synaptic vesicles and symmetrical synaptic contacts are largely *en passant* terminals of very long, thin unmyelinated axons which, when seen in continuity, are almost invariably orientated *vertically or transversely* (figures 10, 11, 24, 28 to 30, plates 5, 8 and 9). In this manner they may traverse several laminae of the cortex or remain within a single lamina. Axons of this type, although followed through several serial sections, have only rarely been traced to a myelinated parent fibre. The majority end on

DESCRIPTION OF PLATE 6

FIGURE 12. A large axon terminal of the type typically associated with layer IV. The terminal axon (A) enters from above (arrow head) and expands to terminate asymmetrically on two dendritic spines (S). $\times 29\,000$.

FIGURE 13. Similar terminal (arrow) to that shown in figure 12, terminating on two spines (S) and upon the shaft of an adjacent dendrite (D). Note the spine apparatus (small arrow) beneath the synapse on the shaft. $\times 15\,000$.

FIGURE 14. Similar terminal (T) to that shown in figures 12 and 13 bifurcating to terminate on a spine (S) and a dendrite (D). The small arrows indicate the synaptic contact zone. $\times 36\,000$.

FIGURE 15. Terminal segment of an axon (arrow) expanding to form a terminal which ends asymmetrically (arrow heads) on a dendritic spine containing spine apparatus (SA). $\times 39\,000$.

FIGURE 16. A typical axo-spinous synapse. The terminal contains spherical vesicles and the double synaptic contact (arrow heads) is asymmetrical. S, spine. $\times 97\,000$.

FIGURE 17. An axo-spinous synapse in which there is a single point of synaptic contact. The small arrow indicates an invagination of the presynaptic membrane. S, spine. $\times 68\,000$.

FIGURE 18. Typical asymmetrical axo-spinous synapse with spherical vesicles and a single point of synaptic contact. S, spine. $\times 89\,000$.

dendritic shafts of all sizes but a few terminate on dendritic spines. Their *en passant* terminals can be swellings of the axon or merely a part of the thin fibre itself. It is possible that many of the axon terminals on all somata and initial segments of axons are also derived from axons of this type. However, in a relatively large number of cases axo-somatic terminals with symmetrical contacts and flattened vesicles have been seen to arise from unmyelinated terminal segments of thin myelinated axons or as terminals directly from nodes of Ranvier of small myelinated fibres (figure 30, plate 8). Most of these fibres are orientated vertically. Synapses from nodes of Ranvier are quite common; when the synaptic contact is seen it is usually of the symmetrical type, but the vesicles may be either flattened or spherical. Terminals of this type probably account for the rare occurrence of an isolated large lucent terminal profile containing few vesicles and a central core of neurofilaments and neurotubules.

DISCUSSION

The present results on the somatic sensory cortex confirm those of Colonnier (1968) on the visual cortex and indicate that there is a fundamental similarity on the structural organization of these two sensory areas. Colonnier's observation that pyramidal and stellate neurons may be recognized both on the basis of their overall appearance and on the distribution and type of the synapses upon their soma is borne out. It also appears that in the deeper layers of the cortex other large cells, probably corresponding to the spindle and fusiform neurons of light microscopy, have features making them more similar to the stellate than to the pyramidal cell. This is of interest in view of the fact that the axons of stellate, spindle and fusiform neurons are usually considered to remain within the cortex, whereas those of pyramidal cells leave it (see Globus & Schiebel 1967*a*). The peculiar structure and synaptic arrangements on the non-pyramidal cell may, therefore, be related to their function as the intrinsic interneuronal elements of the cortex. Their high concentration of intracytoplasmic organelles indicates that the content of such inclusions in a neuron is not entirely dependent upon the amount of axoplasm but possibly upon other factors as well. Although the ribosomal content of a pyramidal neuron is probably as high as that of a non-pyramidal cell, most of this is composed of free ribosomes, and there are only

DESCRIPTION OF PLATE 7

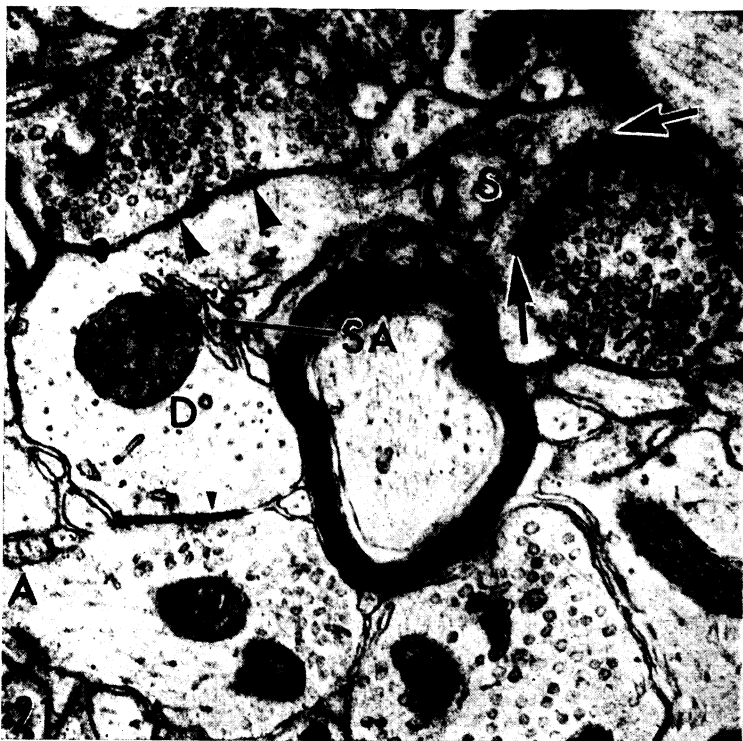
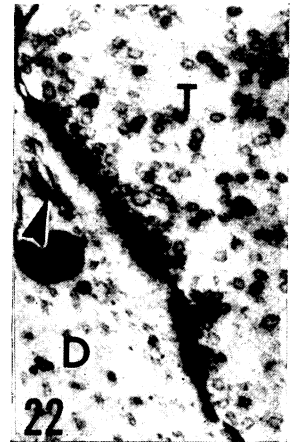
FIGURE 19. A small dendrite (D) giving rise to a dendritic spine (S). A terminal containing spherical vesicles (E) ends asymmetrically (small arrow heads) on the spine while another terminal (T) containing smaller pleomorphic vesicles ends symmetrically on the shaft of the dendrite (large arrow head). The latter terminal is an *en passant* terminal of a small axon (A) at the point at which it loses its myelin sheath (arrows). $\times 27\,000$.

FIGURE 20. A small dendrite (D) giving rise to a large spine (S) which contains a mitochondrion (M). Axon terminals (T) make a symmetrical synaptic contacts with both the spine and the dendrite and beneath each there is a small spine apparatus (arrow). $\times 28\,000$.

FIGURE 21. A dendrite (D) containing spine apparatus (SA) at the base of a spine (S). The spine receives an axon terminal (arrows), but as it is sectioned obliquely the synaptic region cannot be clearly identified. However, a terminal containing spherical vesicles ends on the base of the spine (large arrow heads) and an unmyelinated axon (A) expands to give rise to a terminal containing small flattened vesicles which end symmetrically upon the shaft (small arrow heads). $\times 40\,000$.

FIGURE 22. A large terminal ending in an asymmetrical thickening upon the shaft of a dendrite (D). Note the small spine apparatus beneath one of the synaptic contact zones. $\times 41\,000$.

FIGURE 23. A small dendrite (D) giving rise to a spine (S). Axon terminals (arrows) end on both the spine and the shaft. $\times 15\,000$.



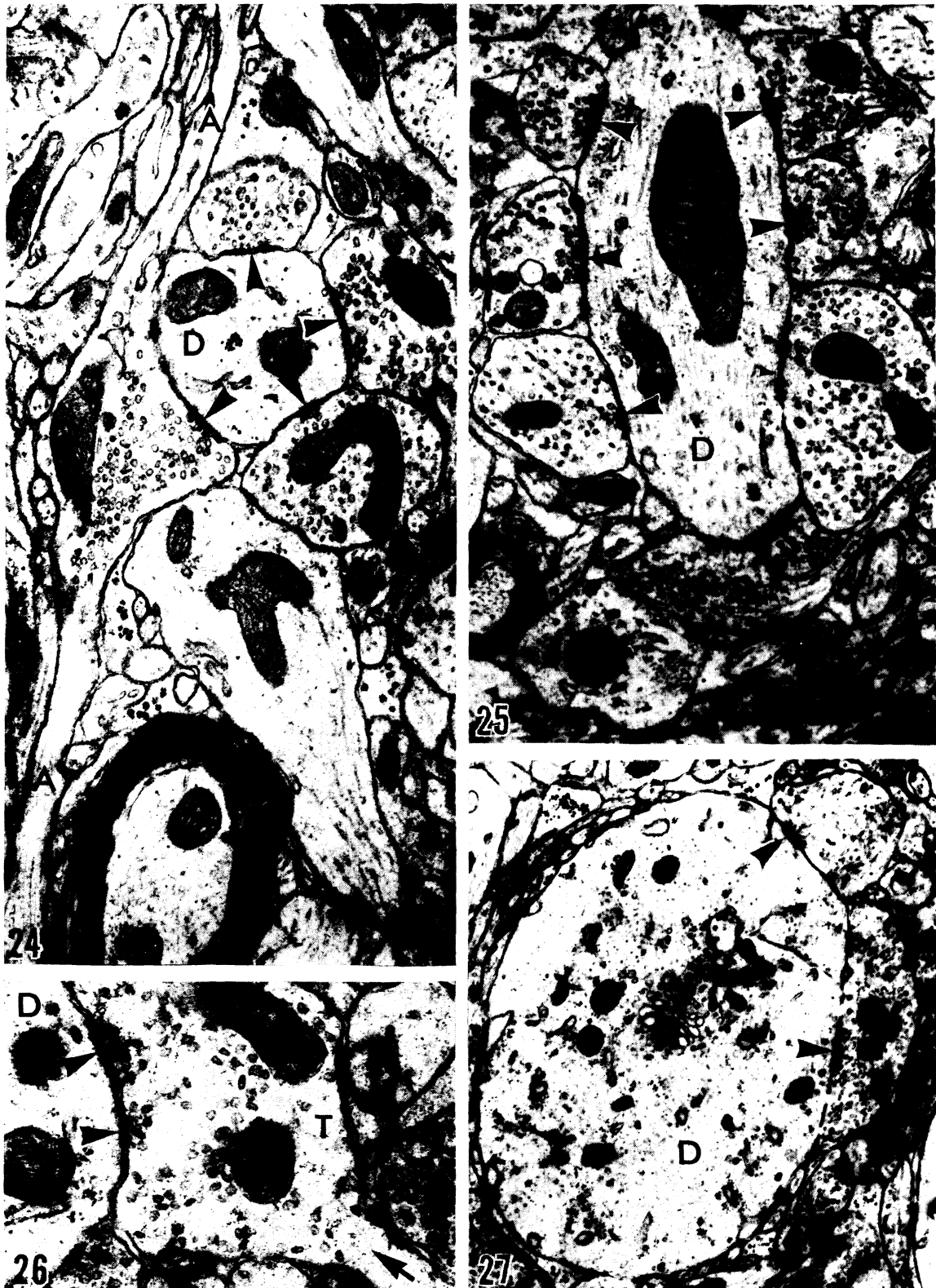


FIGURE 24. A small dendrite (D) surrounded by axon terminals which end in a symmetrical manner (arrow heads). One is an *en passant* terminal of a small unmyelinated axon (A). $\times 30\,000$.

FIGURE 25. A dendrite (D) surrounded by axon terminals, most of which can be recognised to make definite synaptic contacts (large arrow heads) but one zone of opposed membranes (small arrow heads) is not associated with an aggregation of synaptic vesicles. $\times 28\,000$.

FIGURE 26. Terminal portion of an unmyelinated axon (arrow) expanding to form a typical axon terminal (T) containing small flattened vesicles and making symmetrical synaptic contact (arrow heads) with the shaft of a dendrite (D). $\times 40\,000$.

FIGURE 27. The proximal part of an apical dendrite (D) close to its origin from the pyramidal cell soma. Much of the plasma membrane is free of synaptic contacts, only two terminals (arrow heads) being present. These make symmetrical synaptic contacts. $\times 16\,000$.

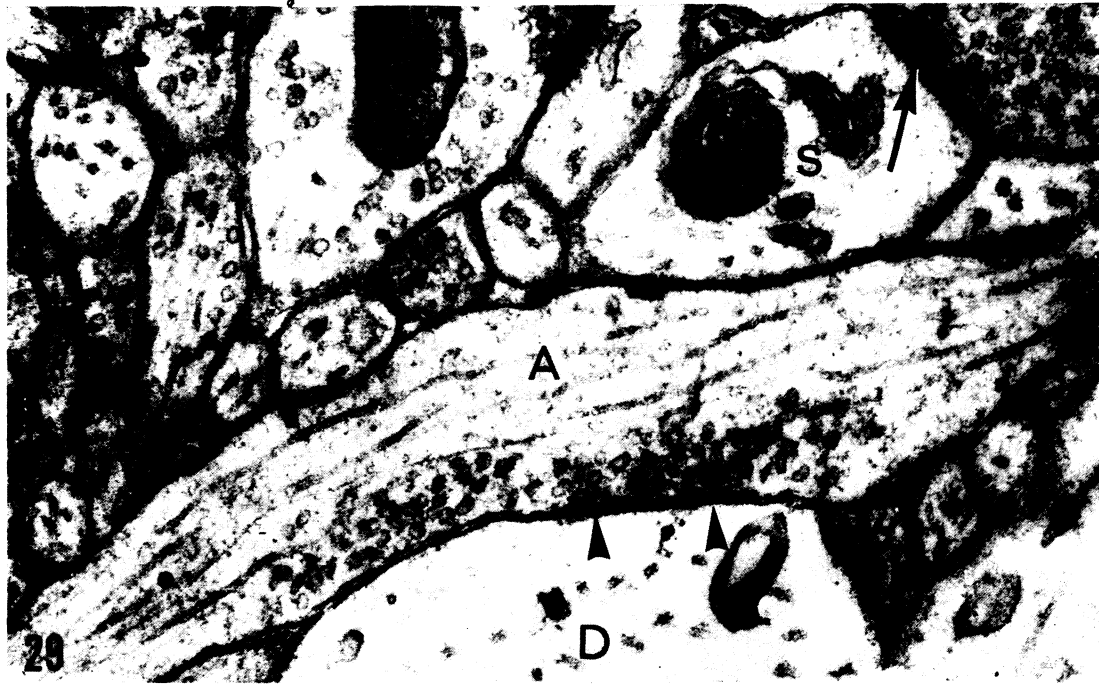
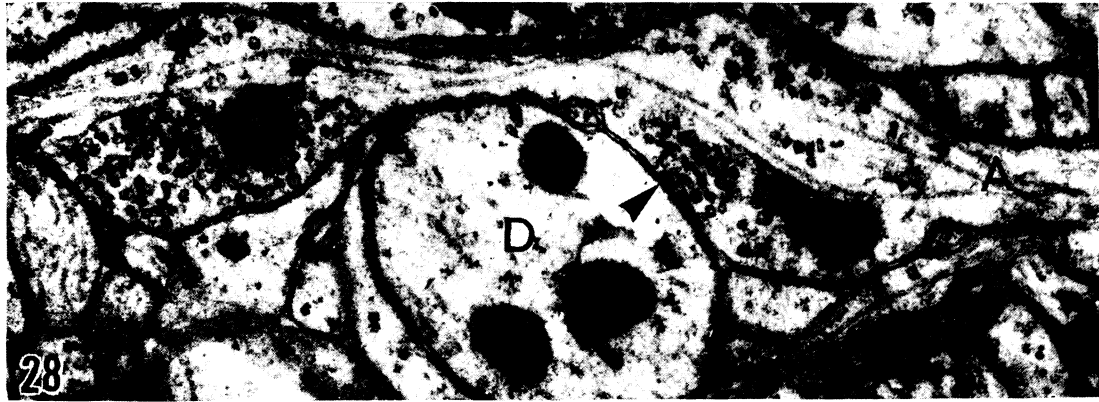


FIGURE 28. Unmyelinated axon (A) expanding at intervals to form two *en passant* terminals one of which ends symmetrically (arrow head) upon a dendrite (D). Note that many of the synaptic vesicles are flattened or irregular in shape. $\times 35000$.

FIGURE 29. Unmyelinated axon (A) containing flattened synaptic vesicles and ending in a symmetrical synaptic contact (arrow heads) upon a dendrite (D). Compare this synaptic complex with the asymmetrical one on the dendritic spine (S) above (arrow). $\times 50000$.

FIGURE 30. A terminal (T) at a node of Ranvier (arrows). The terminal contains flattened vesicles and ends in a symmetrical contact (arrow heads) on the soma of a neuron (N). $\times 30000$.



rarely stacks of rough-surfaced endoplasmic reticulum of the type usually considered to be the equivalent of the Nissl body of light microscopy. The mode of branching of their dendrites, the absence of dendritic spines and particularly the large number of synapses on both somata and dendrites serves further to make the non-pyramidal neurons distinctive entities. It has been known for many years that the stellate neurons are especially numerous in the main sensory areas of the cerebral cortex, and it has usually been assumed that they represent the recipient cell for the heavy thalamic projection to these areas (Ramón y Cajal 1911; Lorente de No 1949). Recently, however, it has become apparent that the vast majority of extrinsic afferents to the visual (Globus & Schiebel 1967*b, c*; Valverde 1967, 1968; Valverde & Estéban 1968) and somatic (Jones & Powell 1970) cortices terminate upon pyramidal neurons, so that most of the synapses on non-pyramidal cells must be derived from axons intrinsic to the cortex.

The synaptic types in the somatic sensory cortex resemble very closely the asymmetrical (Type 1) and symmetrical (Type 2) contacts originally described in the visual cortex by Gray (1959) and more recently in aldehyde-fixed material by Colonnier (1968). Colonnier has pointed out that these two types do not represent two extremes of a continuum and the present observations confirms that, in general, most synapses can be placed in one or other category. On rare occasions an intermediate type, difficult to classify as either symmetrical or asymmetrical, is seen, particularly on the dendrites of non-pyramidal neurons; the exact significance of this is uncertain. The synaptic vesicles of terminals ending in symmetrical synaptic contacts are usually smaller than those associated with asymmetrical synaptic contacts and are commonly flattened or pleomorphic rather than spherical; in this respect, they are again similar to those described in the cerebellum (Uchizono 1965) and in the visual cortex (Colonnier 1968). Whether the symmetrical contacts with flattened vesicles are the morphological basis of inhibitory synapses as postulated in the cerebellum and spinal cord is still a matter of debate (Walberg 1968), but it may be noted that they are the only type on the somata of pyramidal cells, and the vast majority of synapses on the somata of non-pyramidal neurons are also of this type. It seems probable that all terminals with flattened vesicles and symmetrical contacts are derived from axons intrinsic to the cortex as interruption of extrinsic afferent fibres causes degeneration only of terminals which end asymmetrically (Jones & Powell 1970). Furthermore, the relative absence of degenerating terminals which end symmetrically even when a single topographic subdivision of the somatic sensory cortex is isolated with interruption of all extrinsic afferent

DESCRIPTION OF PLATE 10

FIGURE 31. Two axon terminals (T) containing flattened synaptic vesicles and ending in symmetrical contacts upon the perikaryon of a neuron (N). The terminal axon segment (A) of one of the terminals is visible. Note the subsurface complex (large arrows) between the two synapses and beneath an astroglial process (small arrows). $\times 48000$.

FIGURE 32. Unmyelinated axon (A) expanding as a terminal (T) which contains flattened vesicles and ends on the soma of a neuron (N) in a symmetrical contact (arrow heads). $\times 48000$.

FIGURE 33. Large subsurface complex in the perikaryon of a neuron (N). The arrows indicate the plasma membrane of the cell while the four cisternae making up the complex are indicated by arrow heads. Three of the cisternae have fused membranes except at their ends. The deepest is expanded and both it and the dilated ends of the other cisternae are associated with free and attached ribosomes. As, astrocytic processes. $\times 87000$.

FIGURE 34. Two axon terminals (T) arising from a common axon (arrow) and ending axo-somatically (arrow heads). The synaptic contacts are symmetrical. N, soma of neuron. $\times 30000$.

FIGURE 35. Two opposed junctional complexes (arrow heads) beneath the plasma membranes of two immediately adjacent neurons (N). The narrow intercellular cleft is indicated by the small arrows. $\times 38000$.

fibres suggests that such axons have a local origin and relatively short course. Whether they are terminals of the axons of non-pyramidal cells cannot be determined.

Many of the symmetrical synapses are *en passant* endings of thin unmyelinated fibres which may be additional evidence for a local origin, for, over the greater part of their intracortical course the extrinsic afferent fibres are myelinated (Jones & Powell 1970). The thin unmyelinated axons either ascend vertically through the cortex or spread transversely within a single lamina, and as the basic effect of the incoming thalamo-cortical fibres seems to be to activate a narrow column of cells extending through all layers of the cortex (Mountcastle 1957; Powell & Mountcastle 1959; Hubel & Wiesel 1962, 1965) the functions of the unmyelinated intracortical fibres may be to mediate local effects both within and on each side of such columns.

Of the terminals which possess spherical vesicles and end in asymmetrical synaptic contacts and which, on the basis of present conjecture, may be excitatory (see Walberg 1968) two main types are obvious in normal material. Throughout the cortex there are large numbers of small, relatively dense terminals which are derived from short unmyelinated axon segments; most end on dendritic spines, but a few terminate on the shafts of dendrites of both pyramidal and non-pyramidal cells, and, rarely a few are seen on the somata of the latter. Some of these must be the terminals of extrinsic afferents but as large numbers remain even after undercutting of the cortex, many more must be derived from axons intrinsic to the cortex. The second type, which appears in any quantity only in layer IV and in adjacent parts of layers III and V, and to a lesser extent in layer I, is larger but less dense and is derived from an axon of medium diameter which loses its myelin sheath close to the terminal. A single terminal of this type may end on both spines and shafts of dendrites. There seem to be good grounds for interpreting these terminals as those of thalamo-cortical fibres, because an experimental study has shown that degenerating thalamo-cortical axon terminals appear only in the sites mentioned, and end in groups on both spines and dendrites by means of asymmetrical synaptic contacts (Jones & Powell 1970).

The vast majority of synapses of the asymmetrical type occur on pyramidal cells but show a preferential distribution. None appear on the perikaryon, nor between the perikaryon and the point of first branching of the apical or basal dendrites. At these sites the perikaryon or dendrite is largely devoid of synaptic contacts, and when terminals do appear they are of the postulated inhibitory type. Beyond the first branch, however, many asymmetrical synapses are seen and most are on dendritic spines, although a few may appear on the shaft near the point of attachment of a spine. At both sites such asymmetrical synapses are usually associated with a spine apparatus, suggesting that this organelle may be in some way concerned in activity at these suggested excitatory synapses. In contrast to the dendrites of non-pyramidal cells those of pyramidal cells are never covered in synapses (see also Colonnier 1968), and those with symmetrical contacts and flattened vesicles occur less frequently. However, the association of a dendritic spine receiving an asymmetrical contact on the spine proper and either a symmetrical or a further asymmetrical synapse at the base of its pedicle seems to suggest some functional relationship, and is possibly an indication of an integrative process occurring at the level of the spine.

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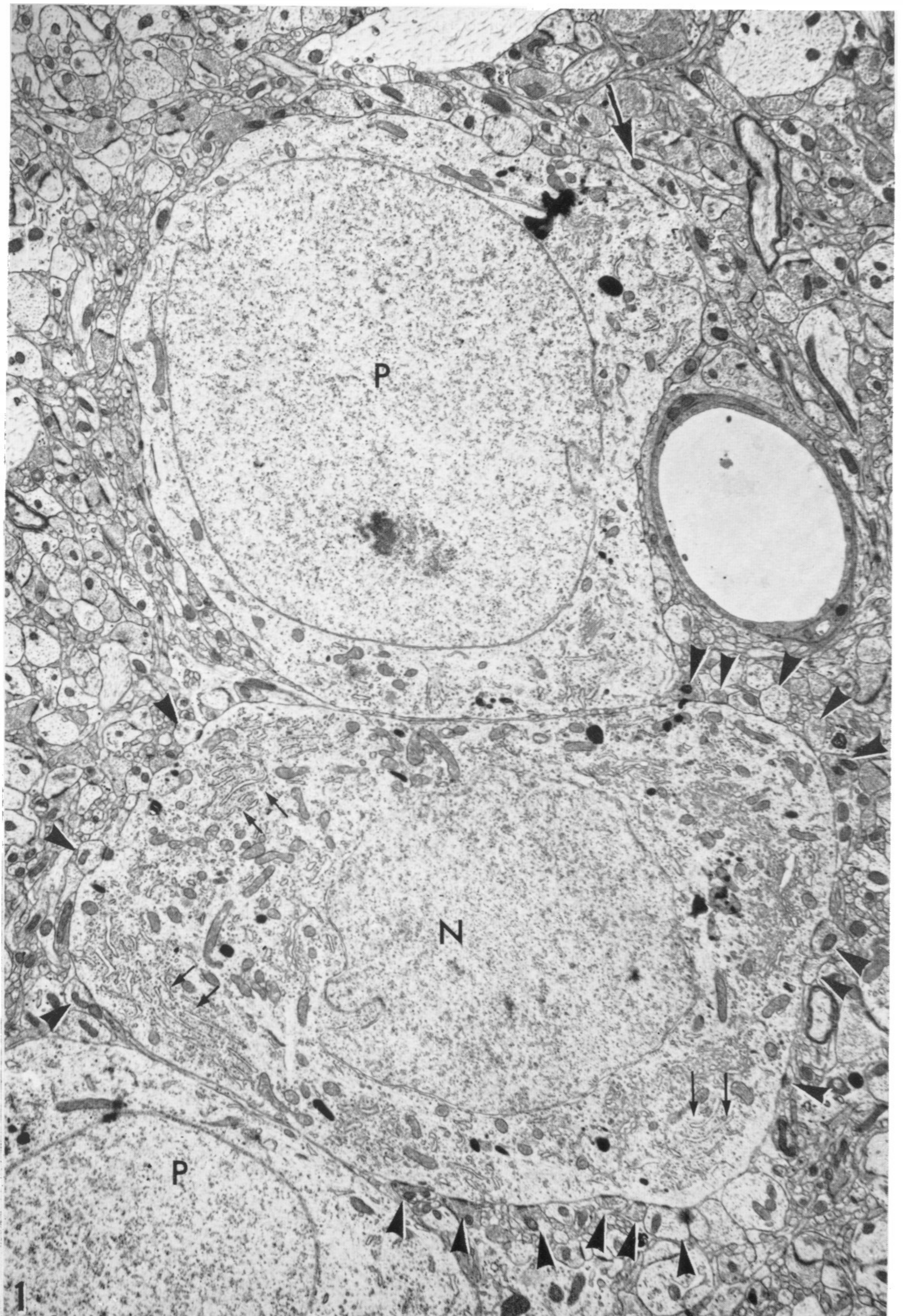
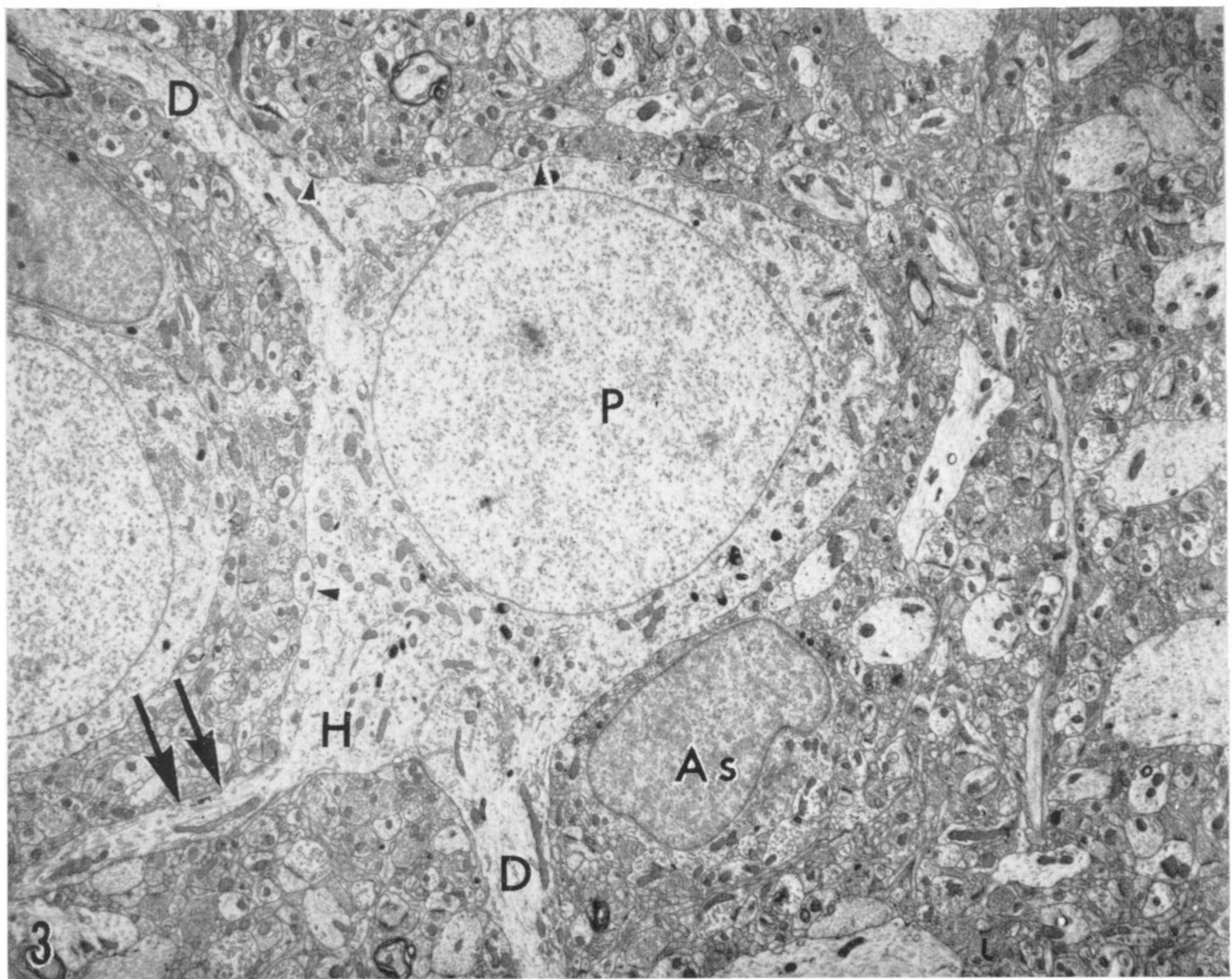
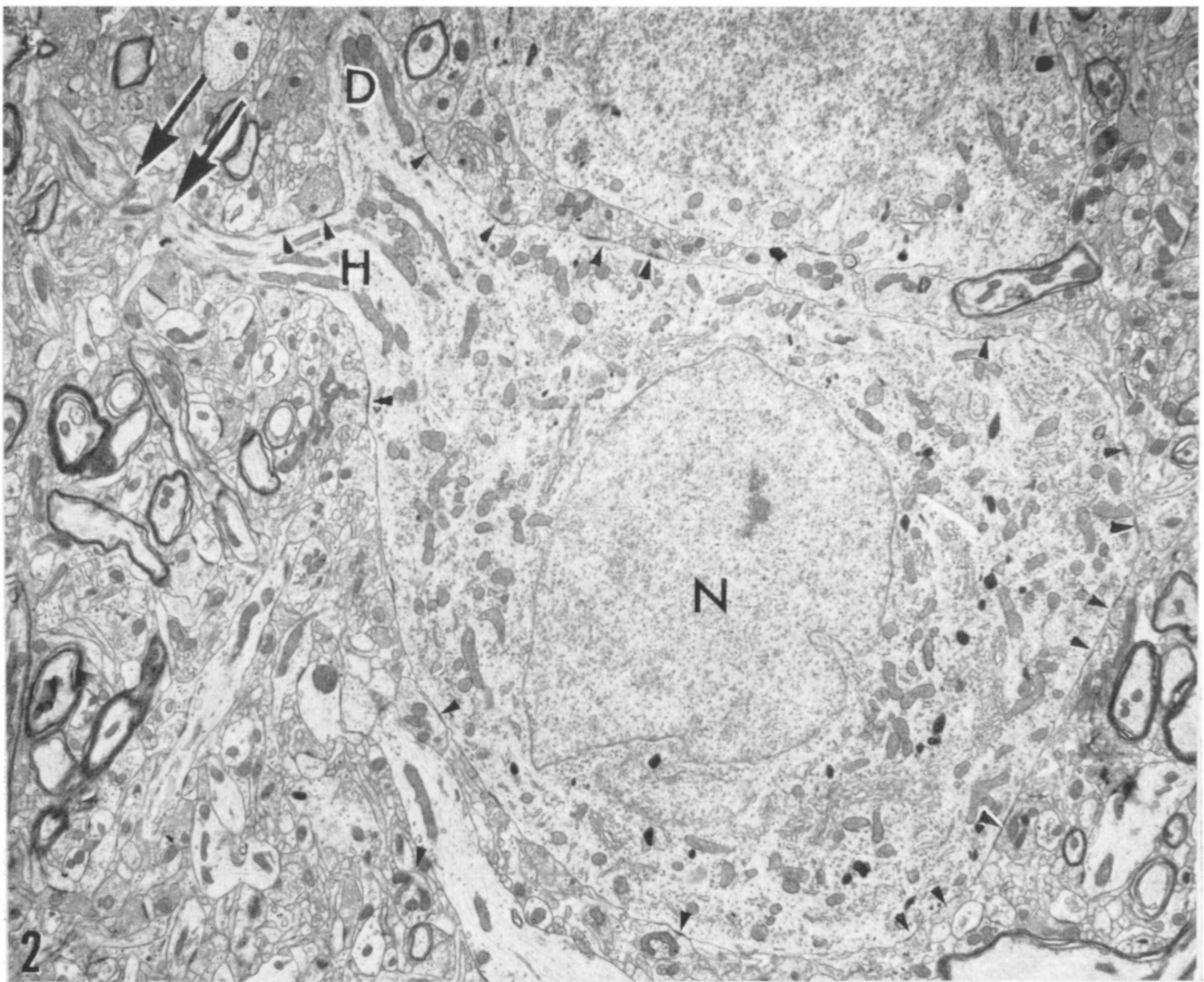


FIGURE 1. Two pyramidal (P) and non-pyramidal neuron (N) from layer II of the cortex. Note the greater concentration of cytoplasmic organelles in the latter, particularly the aggregations of rough-surfaced cisternae (arrows). There are many axon terminals (arrow heads) on the perikaryon of the non-pyramidal cell but few (large arrow) on the pyramidal cells. The surface of the brain is towards the left. $\times 9000$.



FIGURES 2, 3. A non-pyramidal neuron (N) from layer V of the cortex and a pyramidal neuron (P) from layer II. In each case the surface of the brain is towards the right. Both neurons are sectioned through the axon hillock (H) and the initial segment (arrows) of their axon. Note the high concentration of organelles in the non-pyramidal neuron and their relative paucity in the pyramidal neuron. In addition, the non-pyramidal neuron is covered in synapses (arrow heads) while only a few terminals end on the soma of the pyramidal cell. D, dendrite; As, astrocyte. Figure 2, $\times 7000$; figure 3, $\times 5000$.

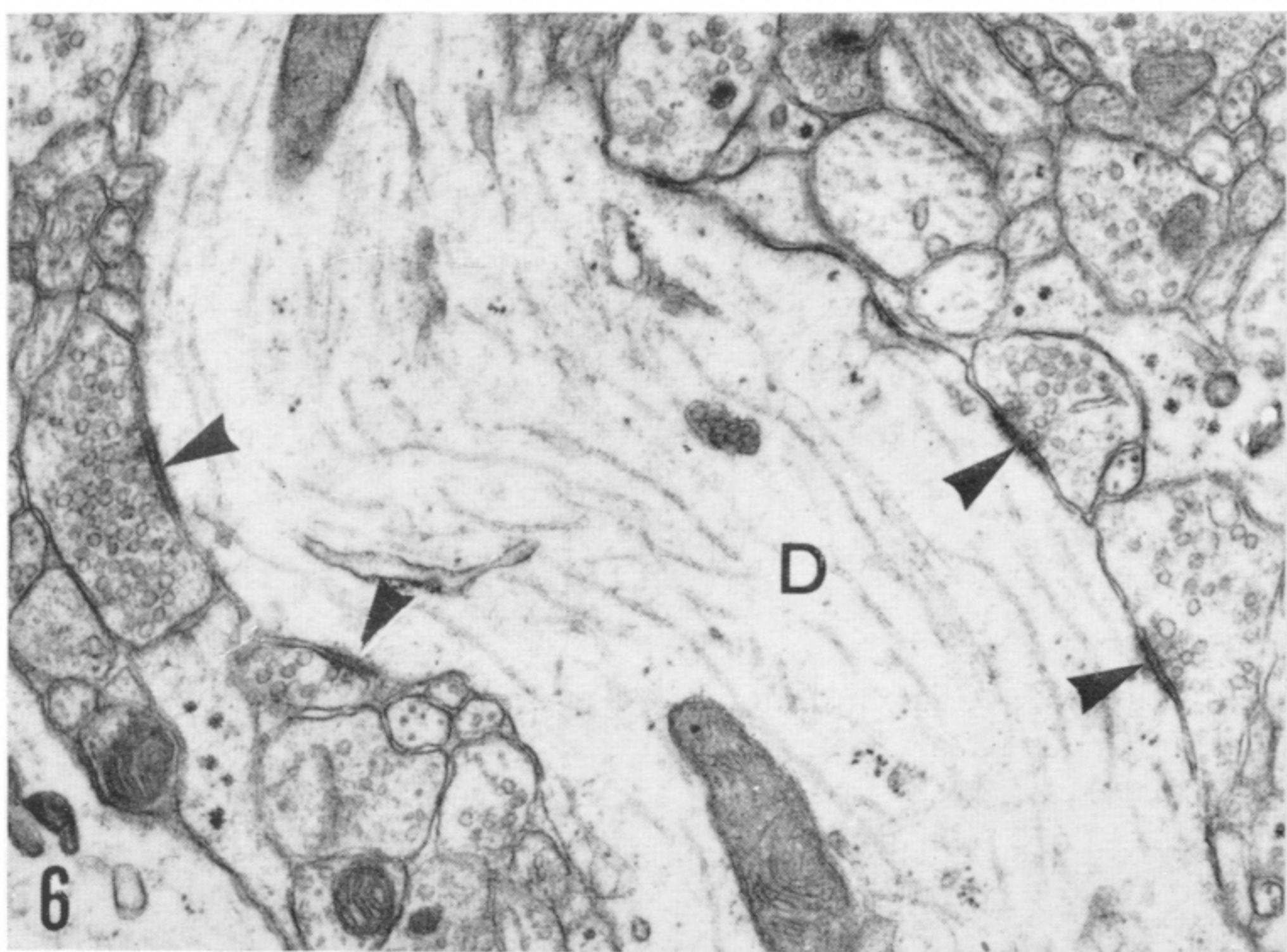
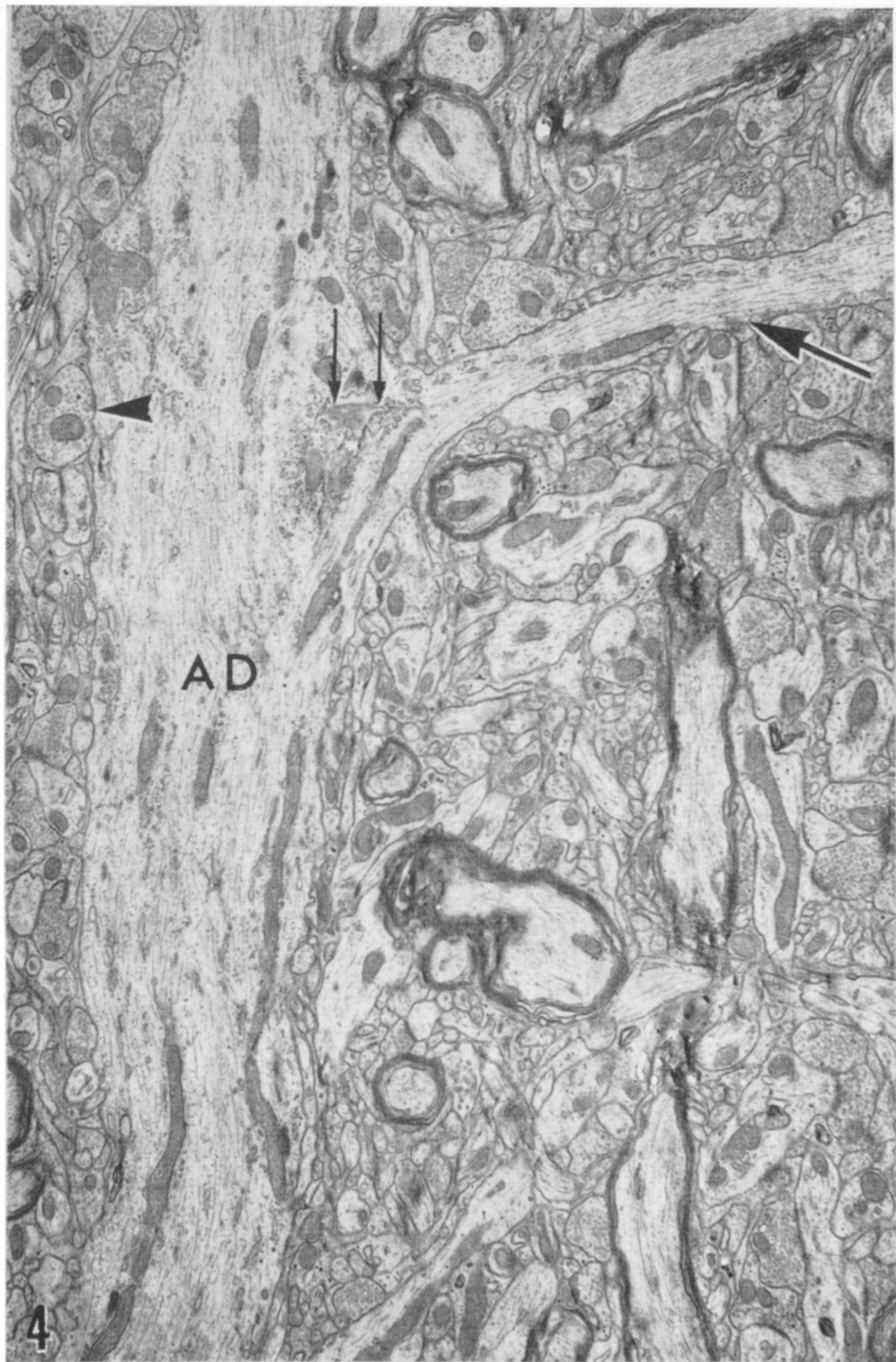


FIGURE 4. Apical dendrite (AD) of a pyramidal neuron in layer V at its first branch. Note the accumulation of ribosomes at the point of branching (double arrows) and the spine (arrow) attached to the branch. There is only one obvious synapse on the shaft of the apical dendrite (arrow head). $\times 12000$.

FIGURE 5. Large vertically oriented dendrite (D) of a non-pyramidal neuron from layer V of the cortex; this dendrite is covered in axon terminals (arrow heads), many of which end asymmetrically (figure 6). Figure 5, $\times 12000$; figure 6, $\times 19000$.

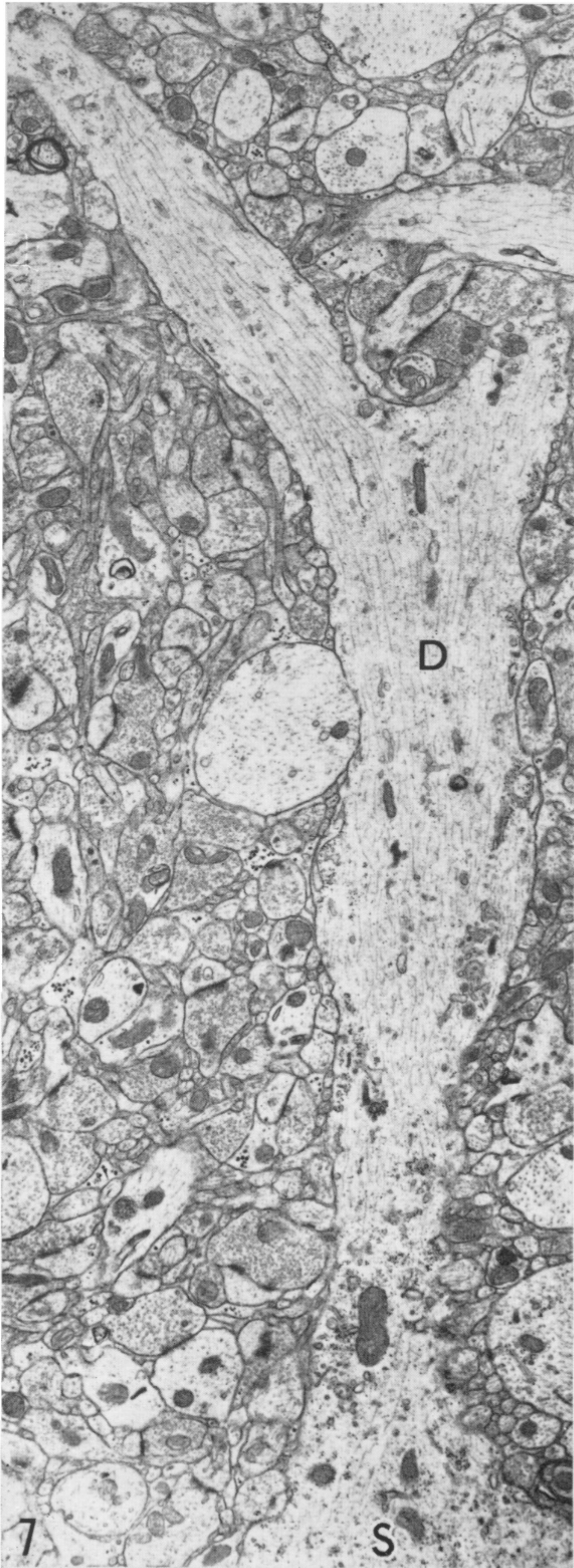
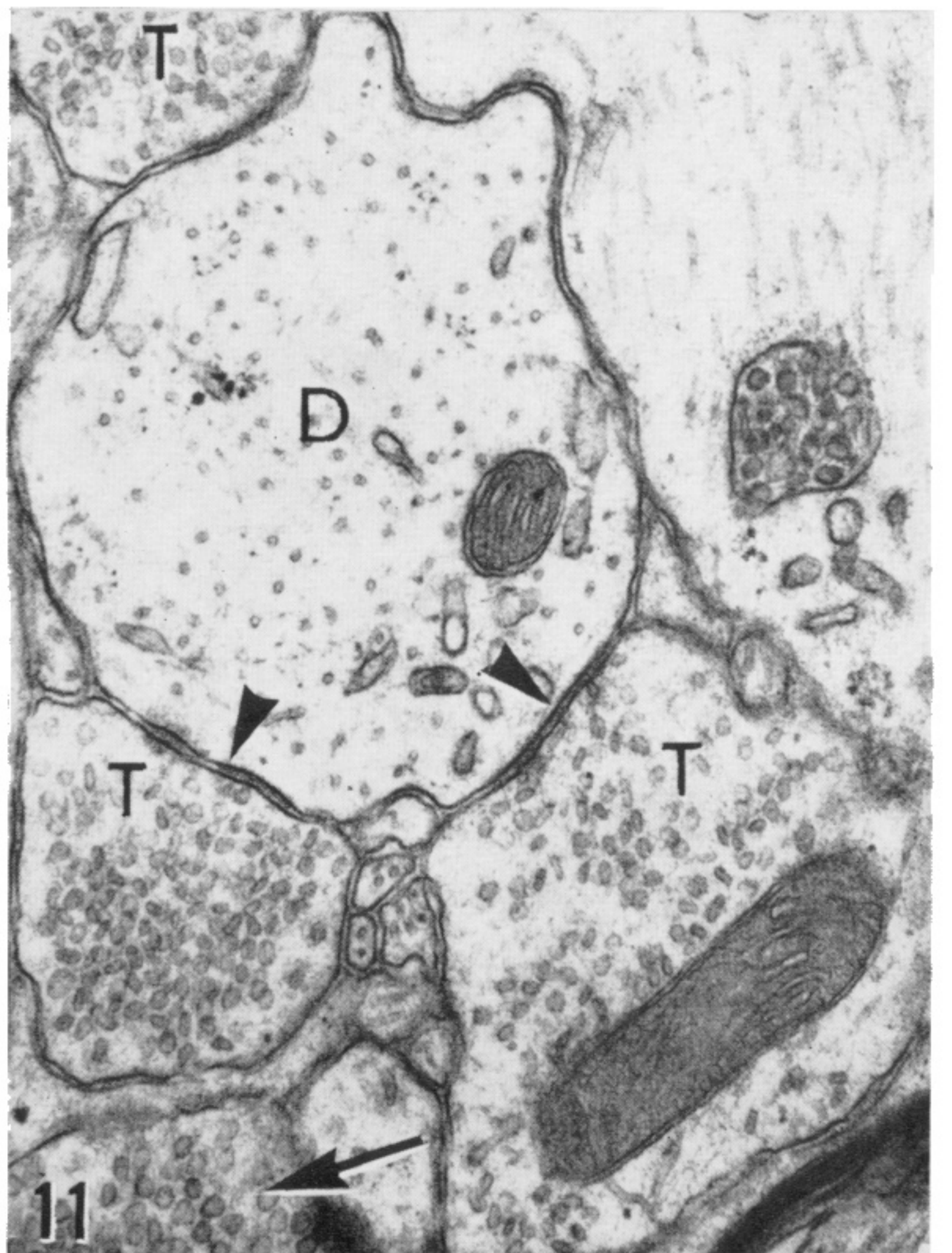
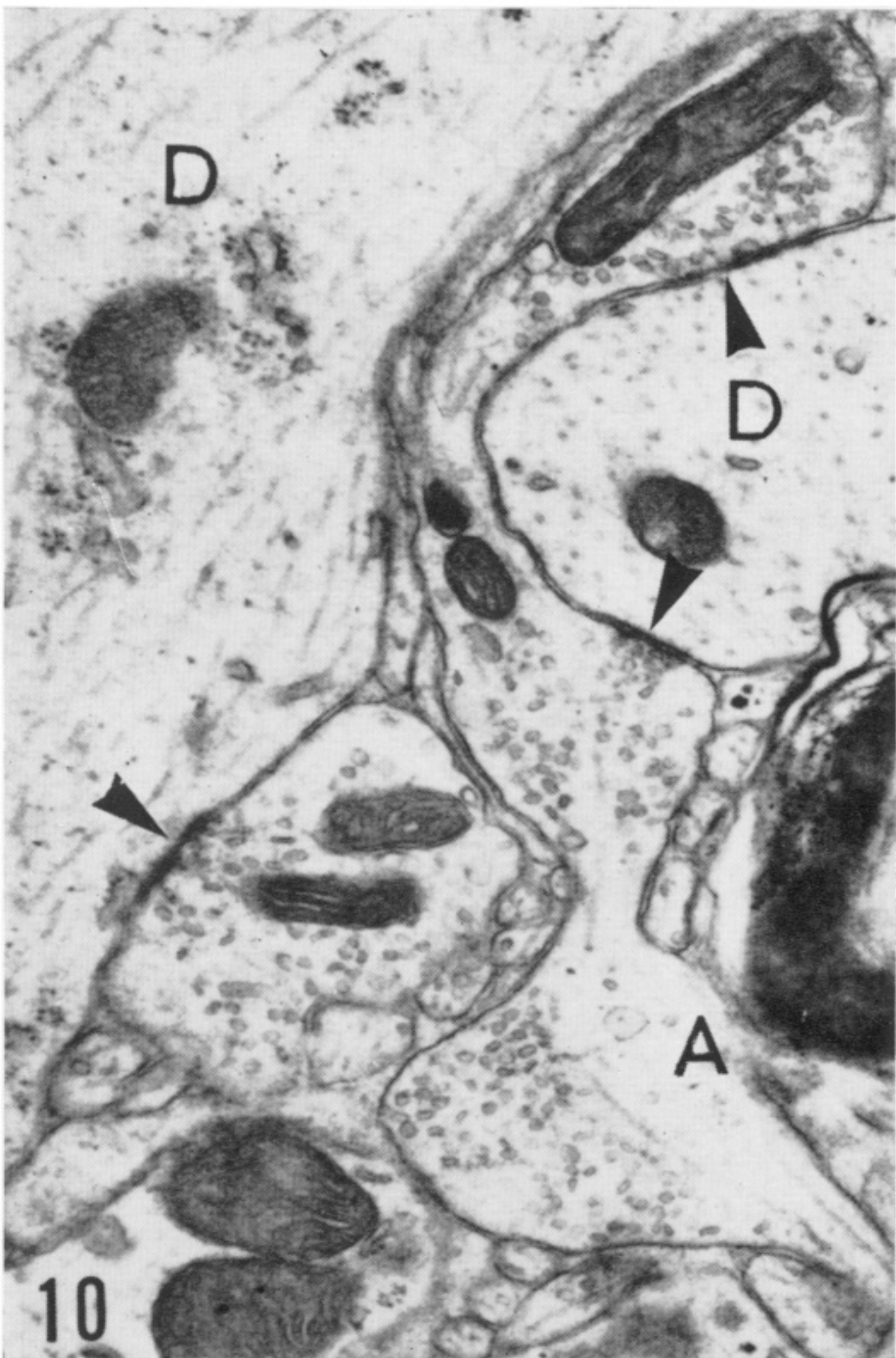
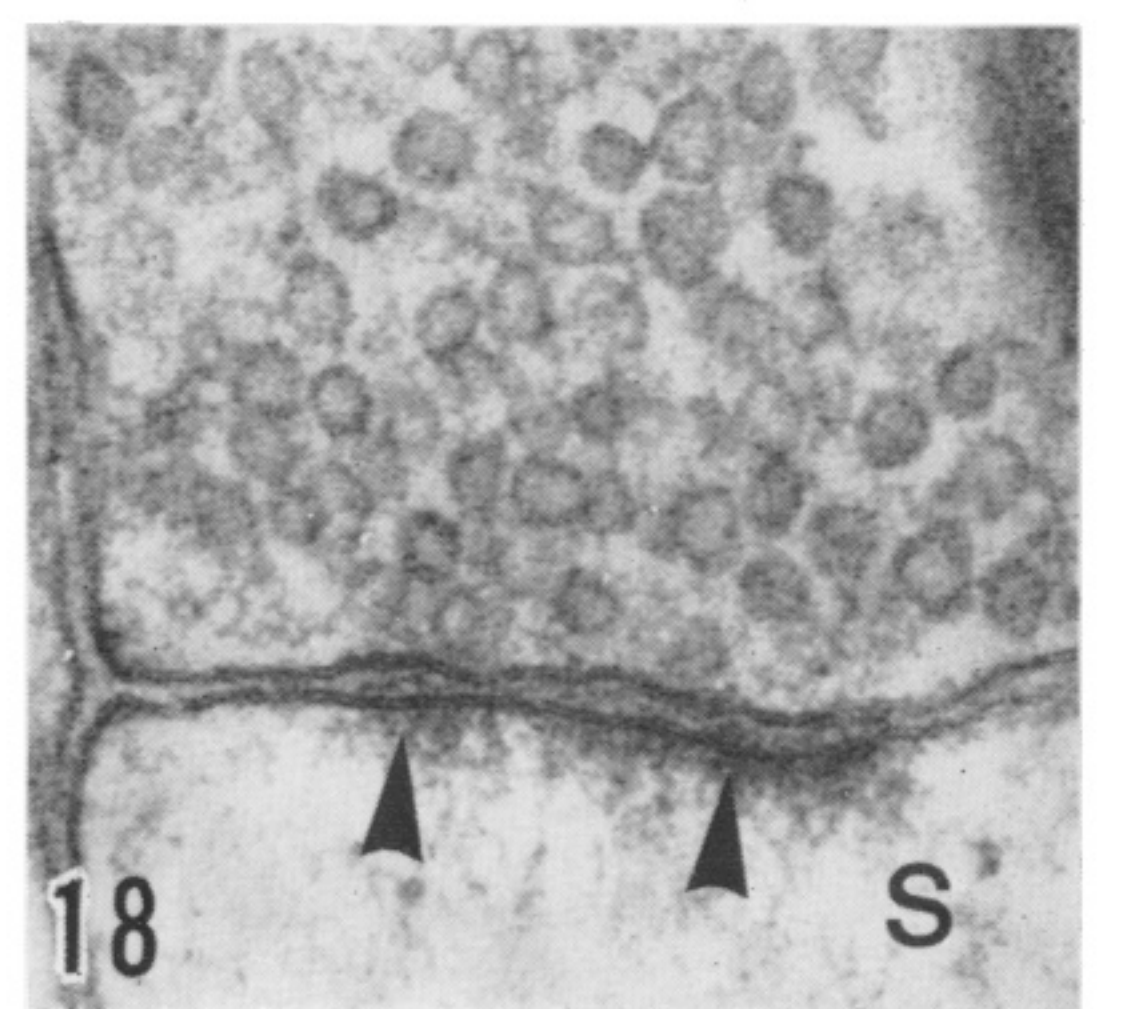
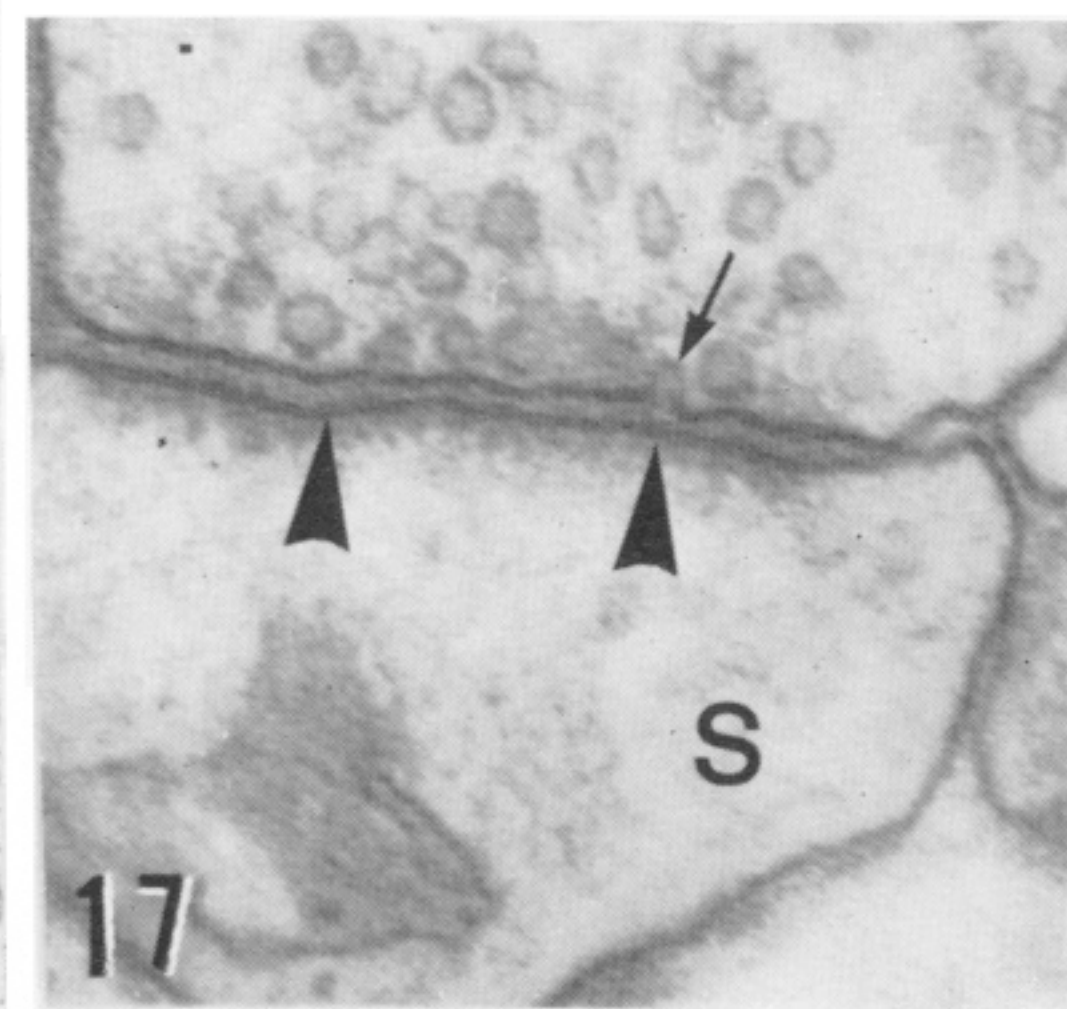
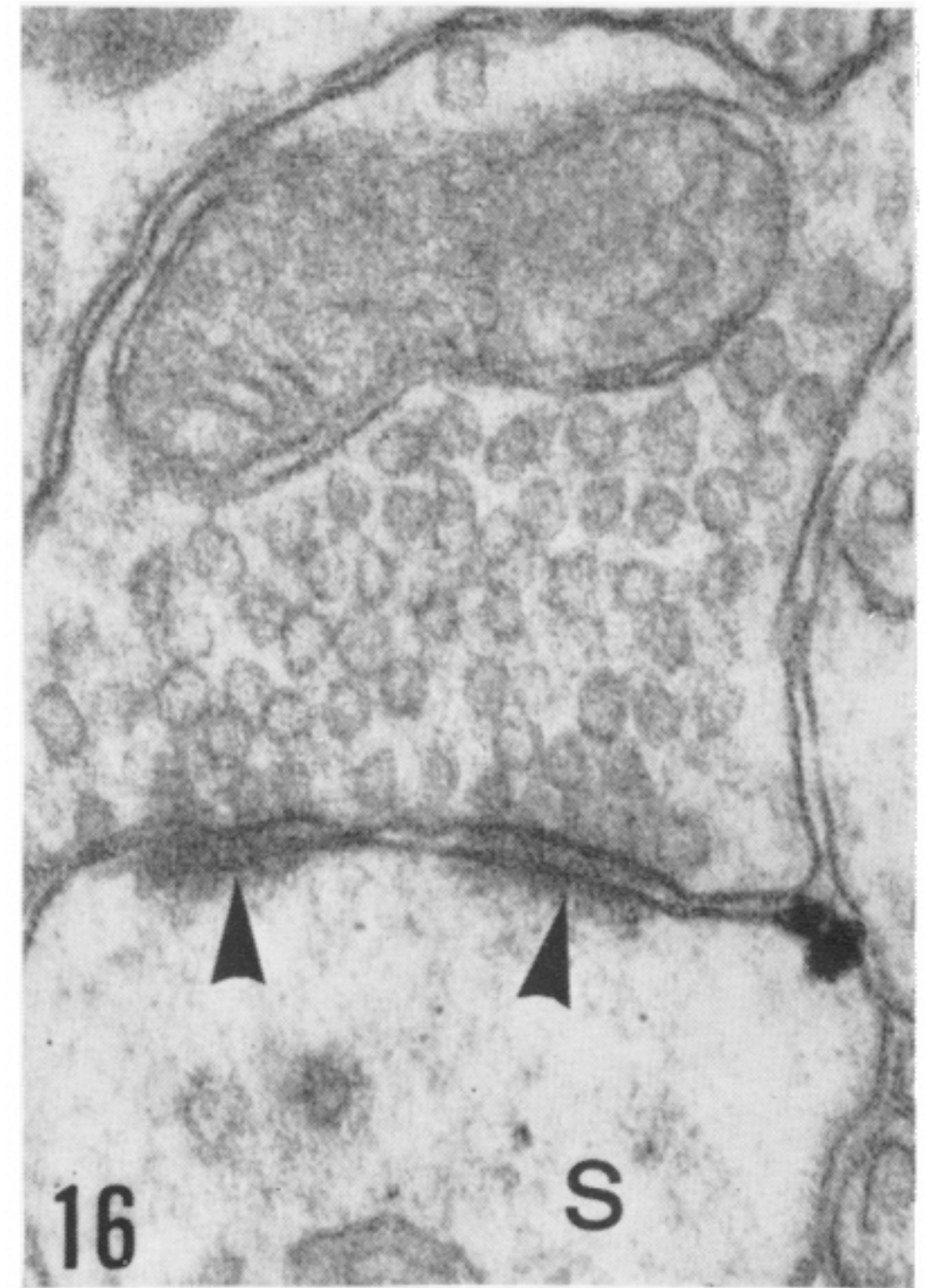
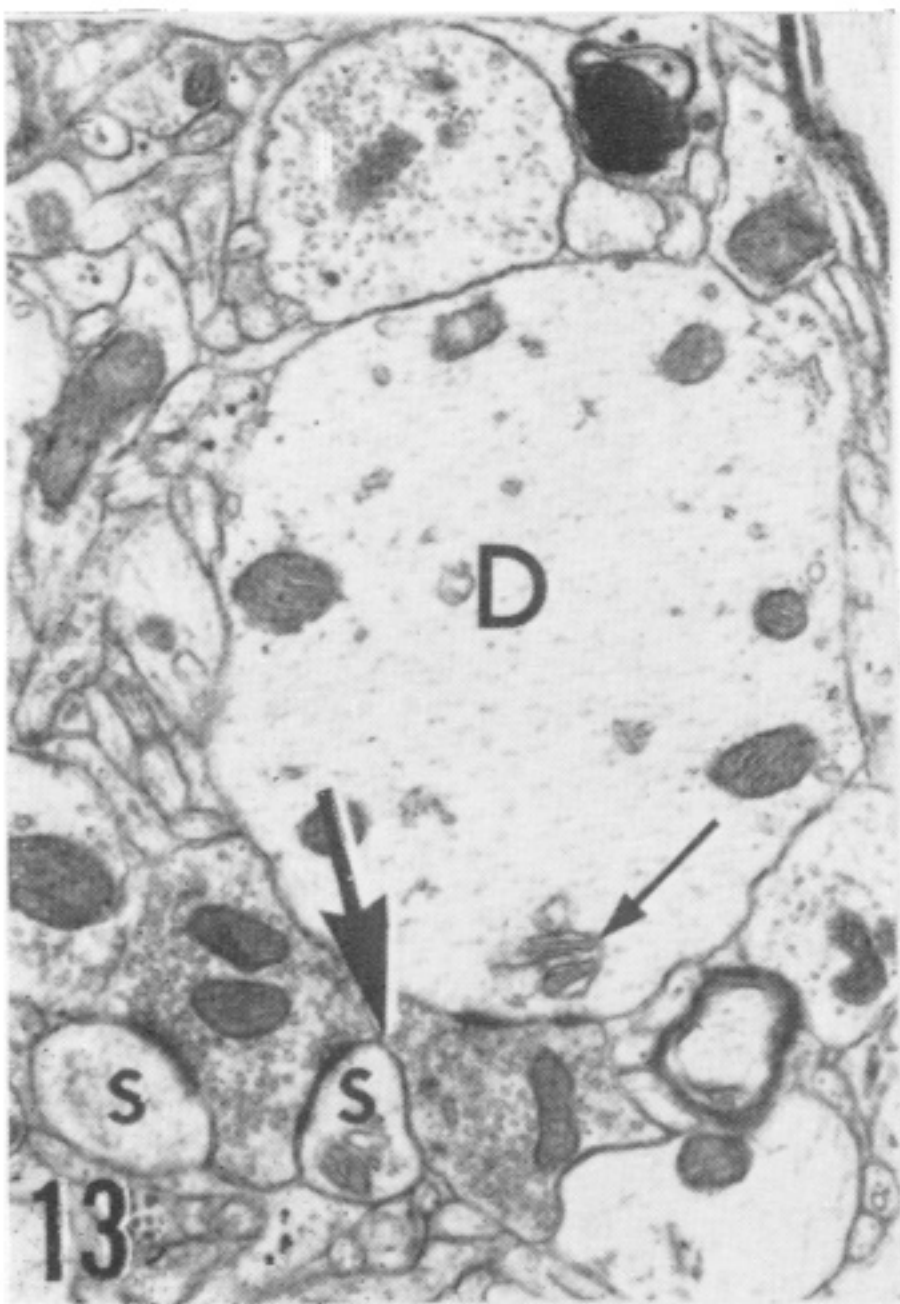
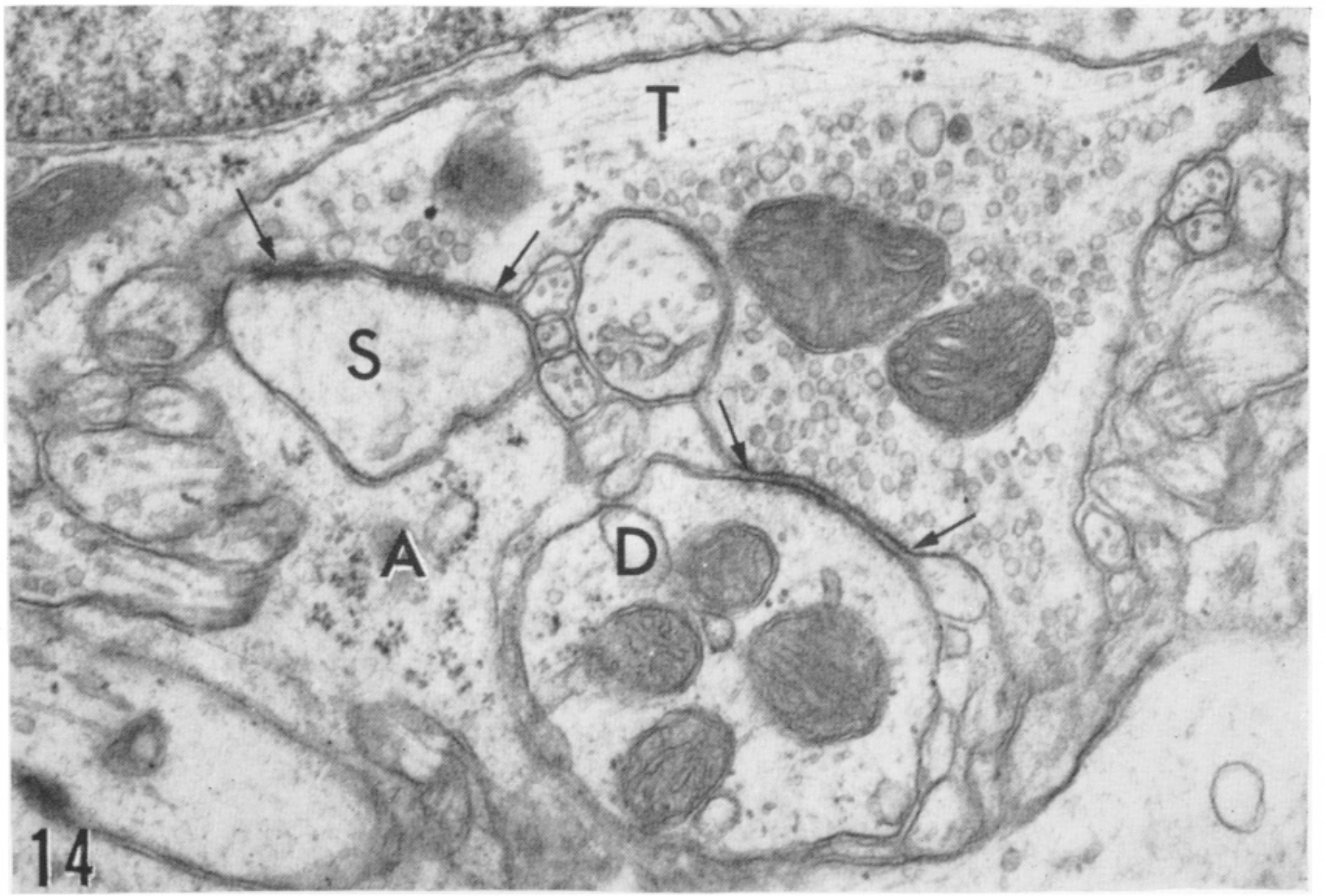
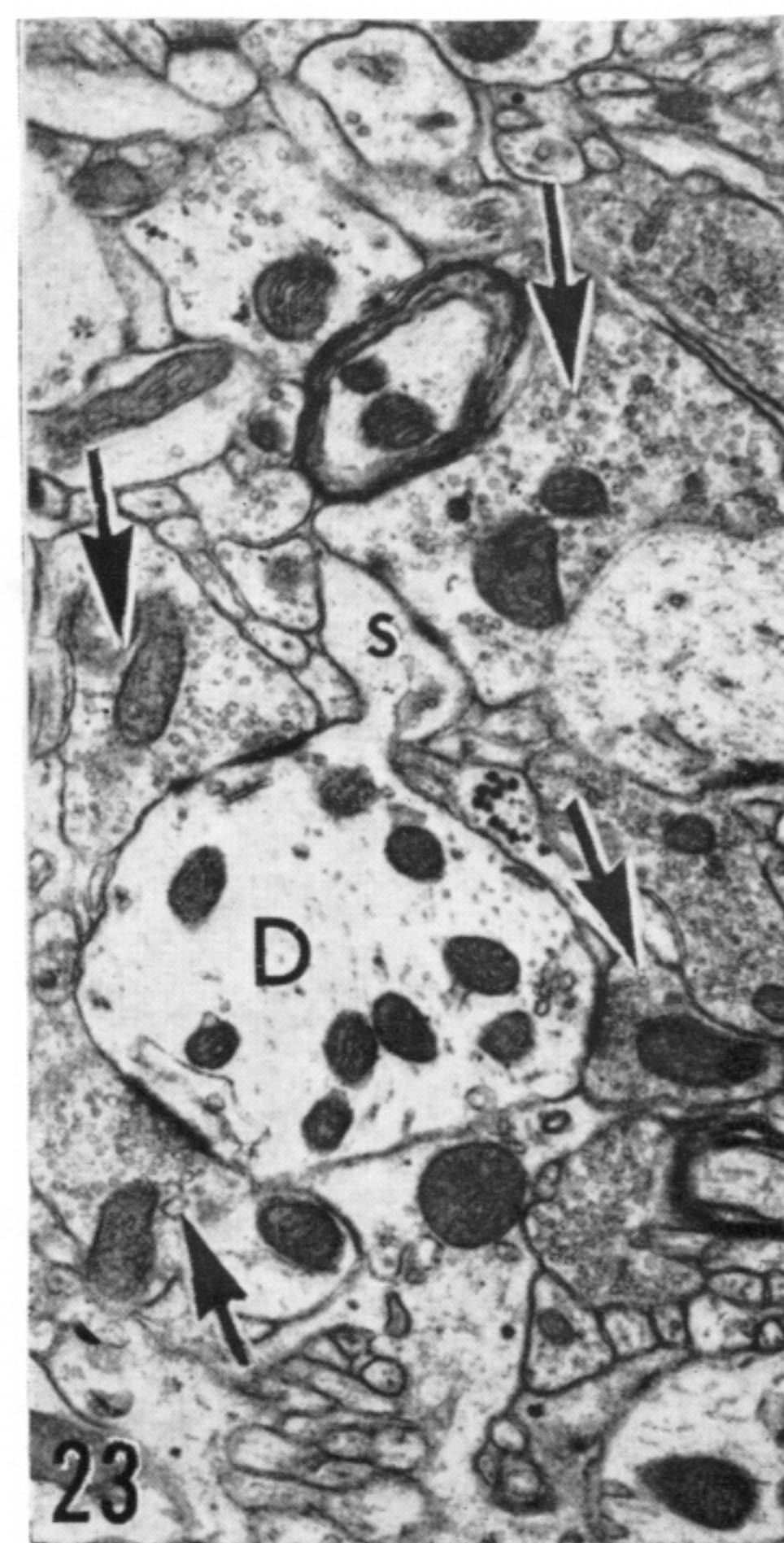
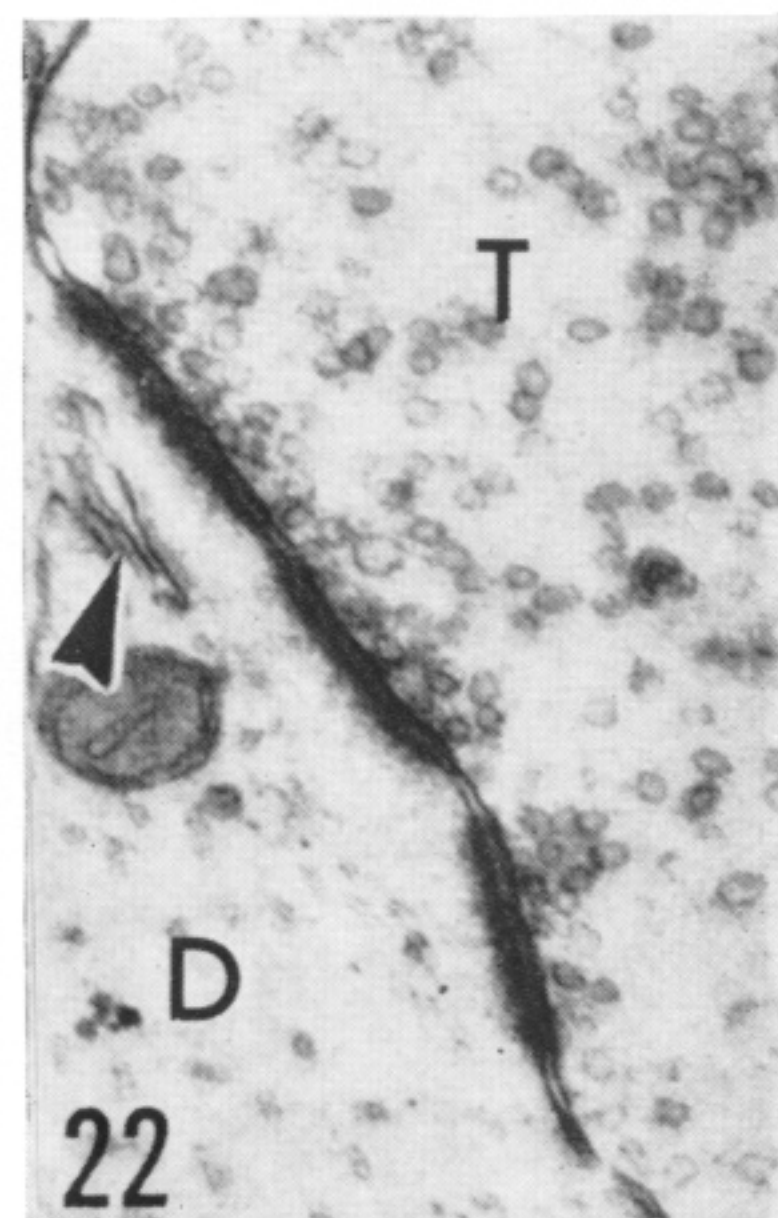
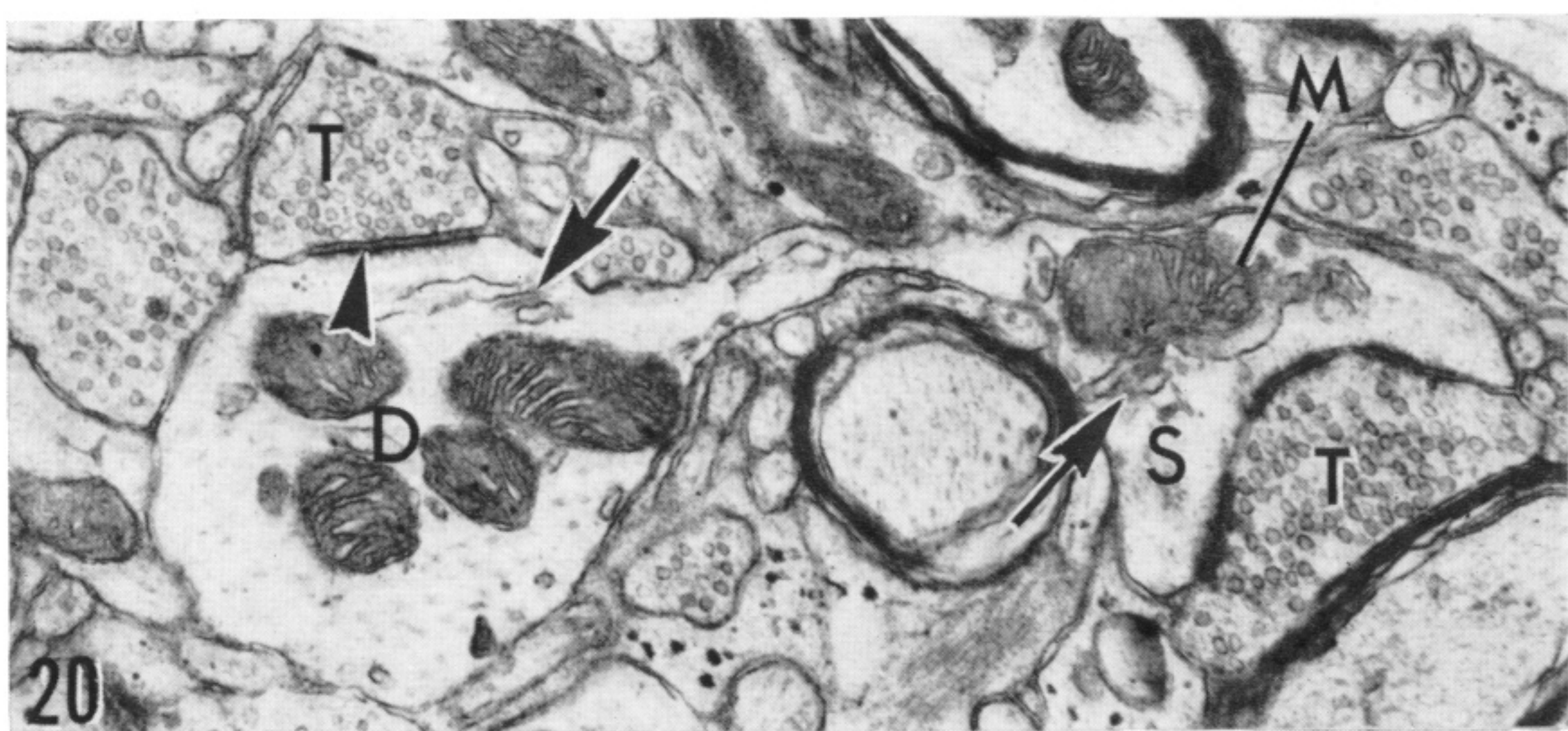
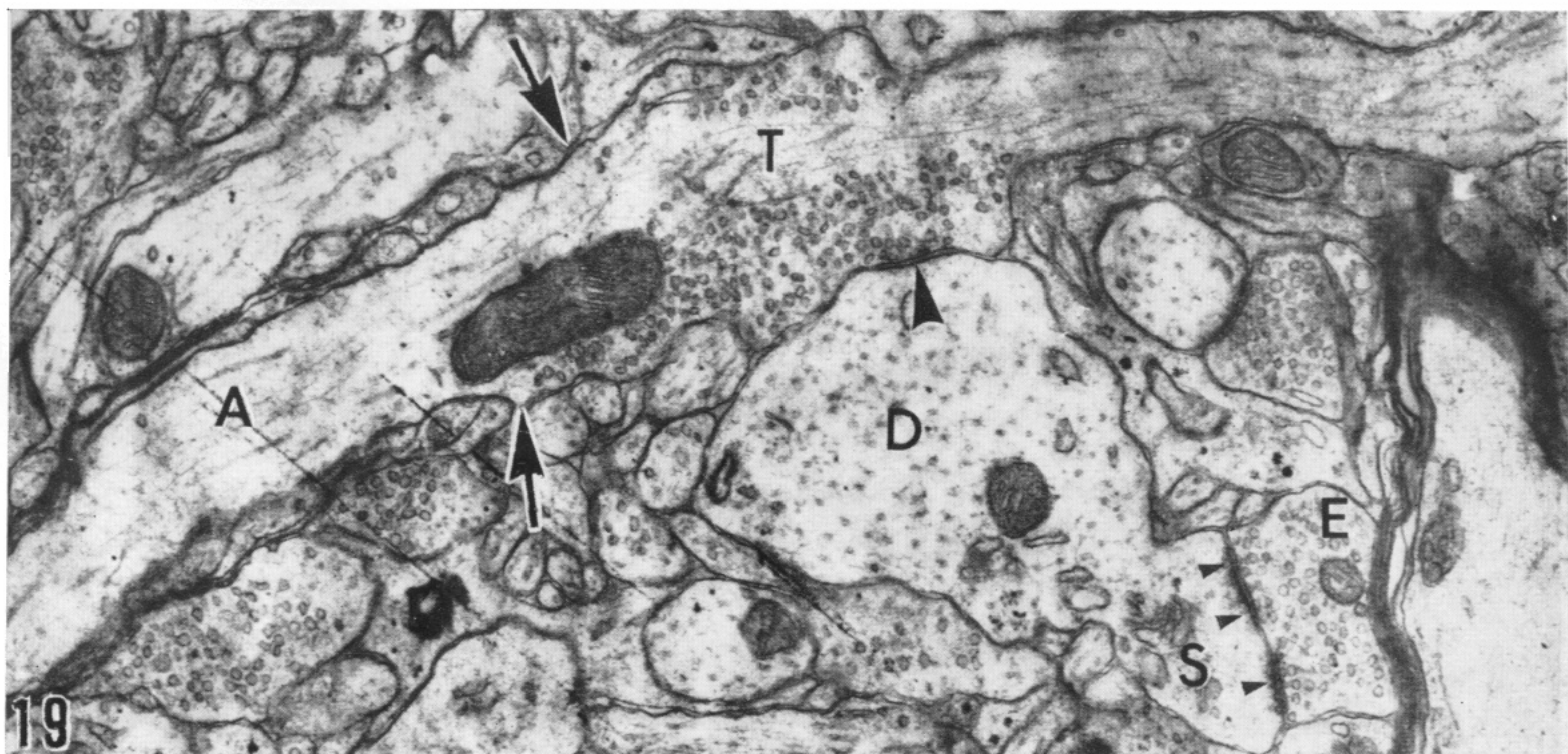


FIGURE 7. Basal dendrite (D) of a pyramidal neuron from layer III, at its point of origin from the perikaryon (S). The proximal portion of this dendrite is typically devoid of axon terminals. $\times 10000$.

FIGURE 8. Dendrite (D) of a non-pyramidal cell at its point of origin from the perikaryon (S). Note that this dendrite receives many axon terminals right up to its point of origin. $\times 21000$.







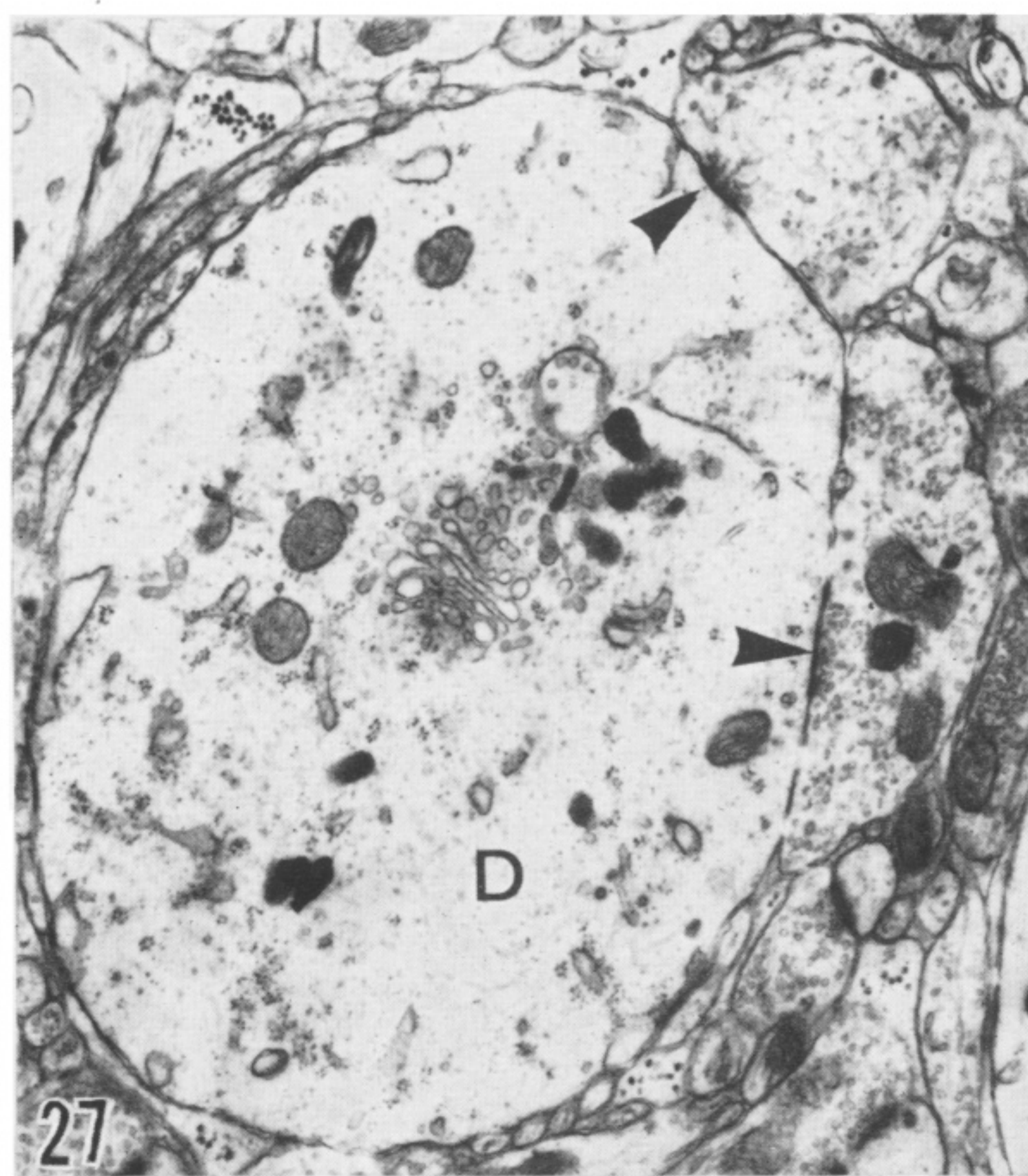
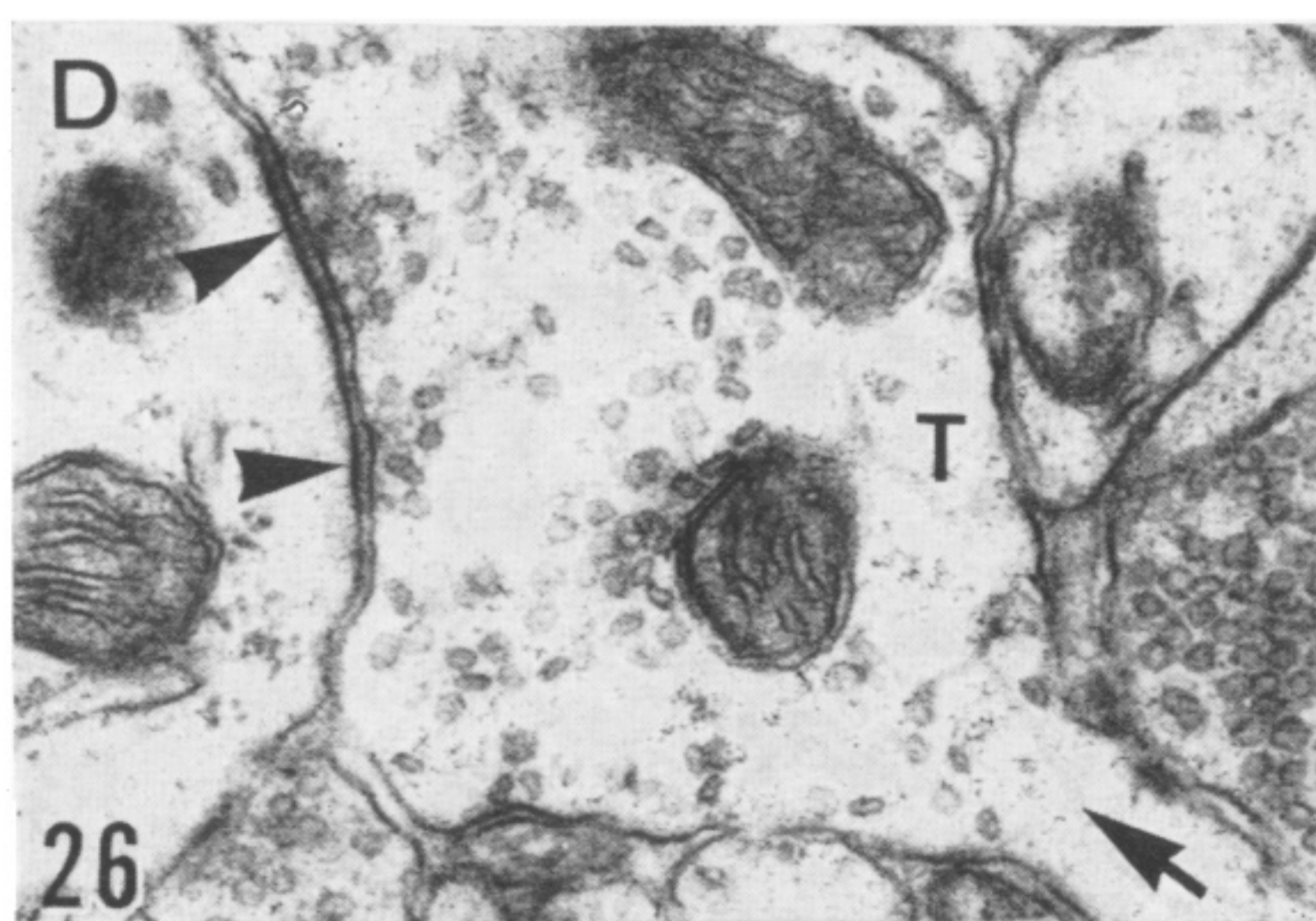
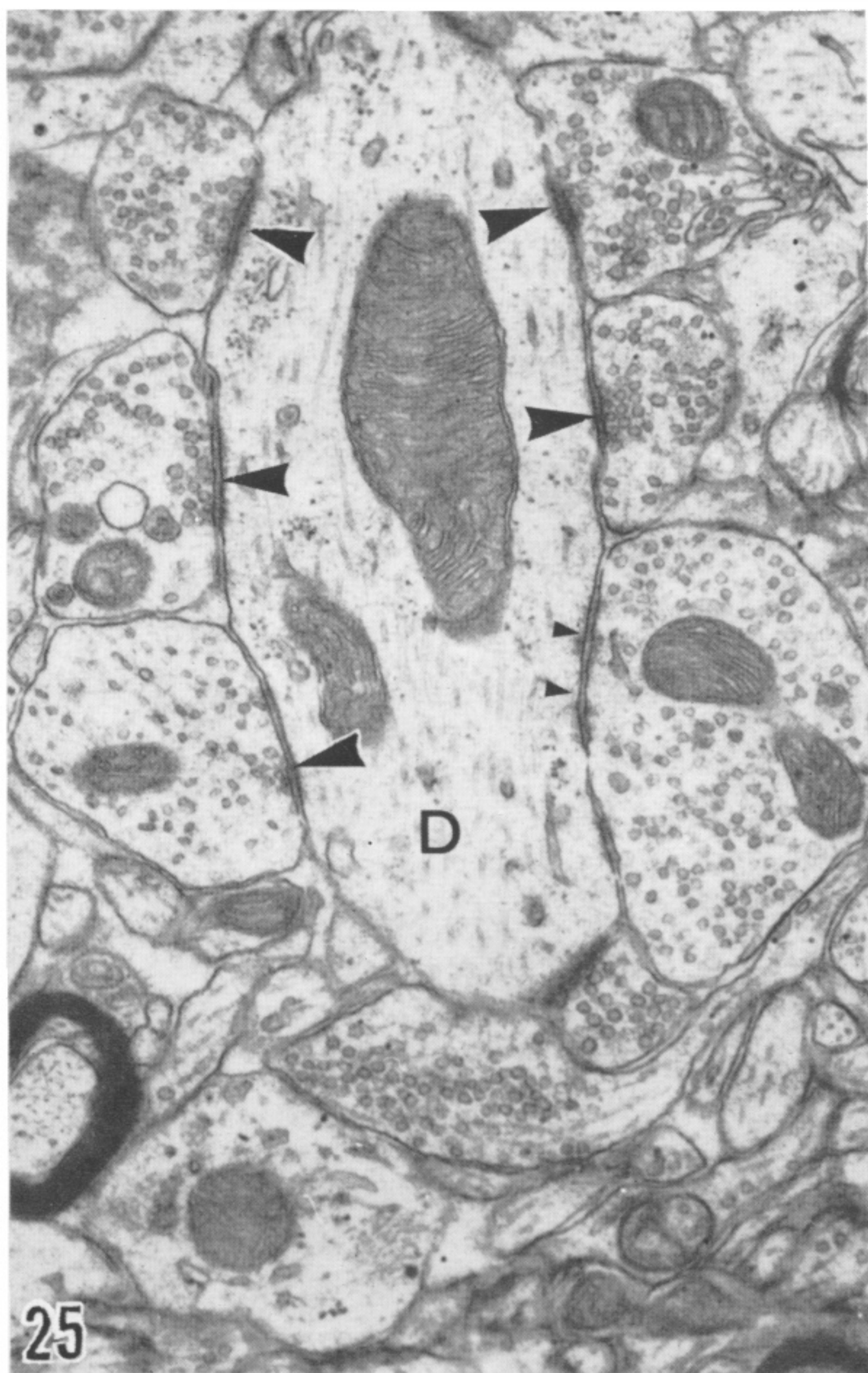


FIGURE 24. A small dendrite (D) surrounded by axon terminals which end in a symmetrical manner (arrow heads). One is an *en passant* terminal of a small unmyelinated axon (A). $\times 30\,000$.

FIGURE 25. A dendrite (D) surrounded by axon terminals, most of which can be recognised to make definite synaptic contacts (large arrow heads) but one zone of opposed membranes (small arrow heads) is not associated with an aggregation of synaptic vesicles. $\times 28\,000$.

FIGURE 26. Terminal portion of an unmyelinated axon (arrow) expanding to form a typical axon terminal (T) containing small flattened vesicles and making symmetrical synaptic contact (arrow heads) with the shaft of a dendrite (D). $\times 40\,000$.

FIGURE 27. The proximal part of an apical dendrite (D) close to its origin from the pyramidal cell soma. Much of the plasma membrane is free of synaptic contacts, only two terminals (arrow heads) being present. These make symmetrical synaptic contacts. $\times 16\,000$.

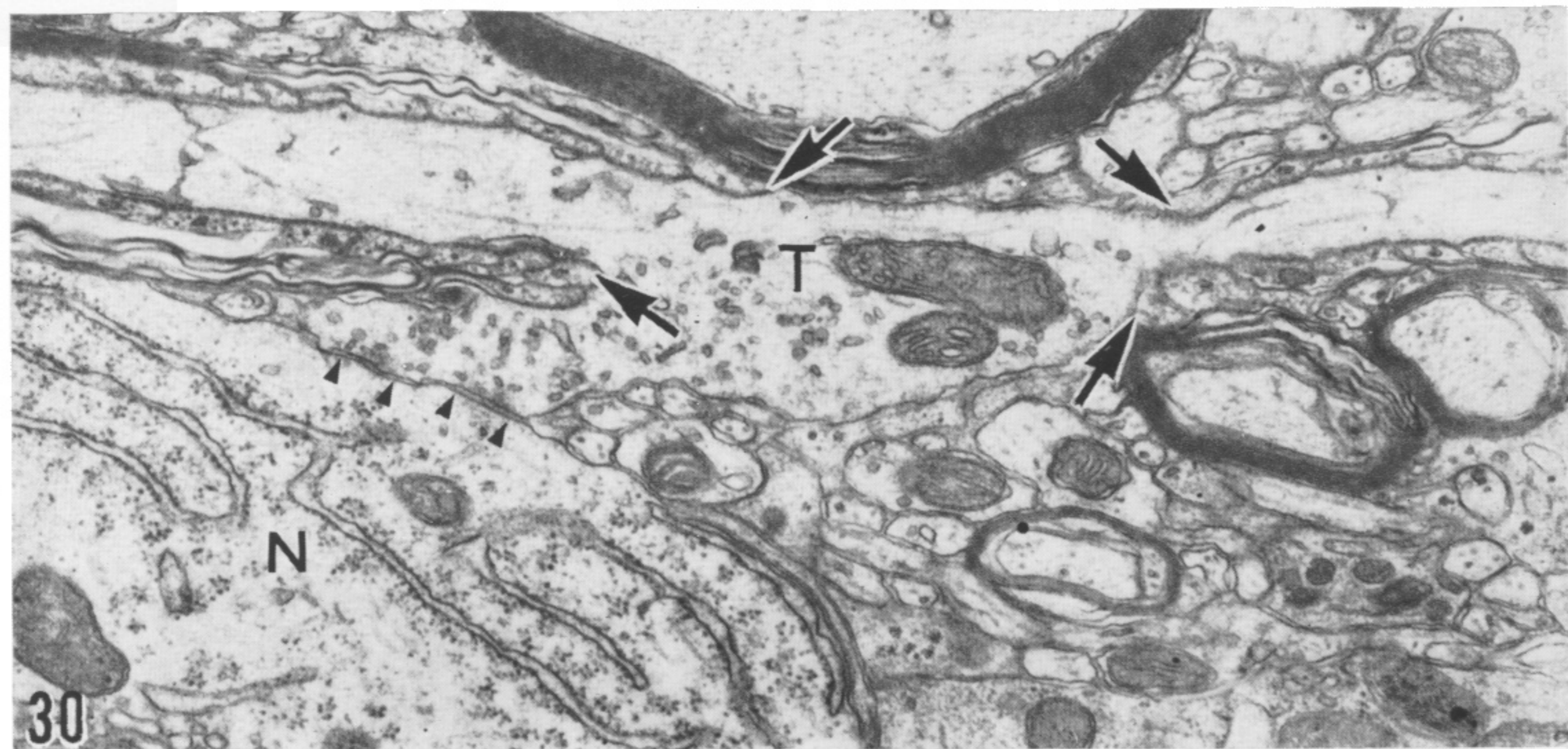
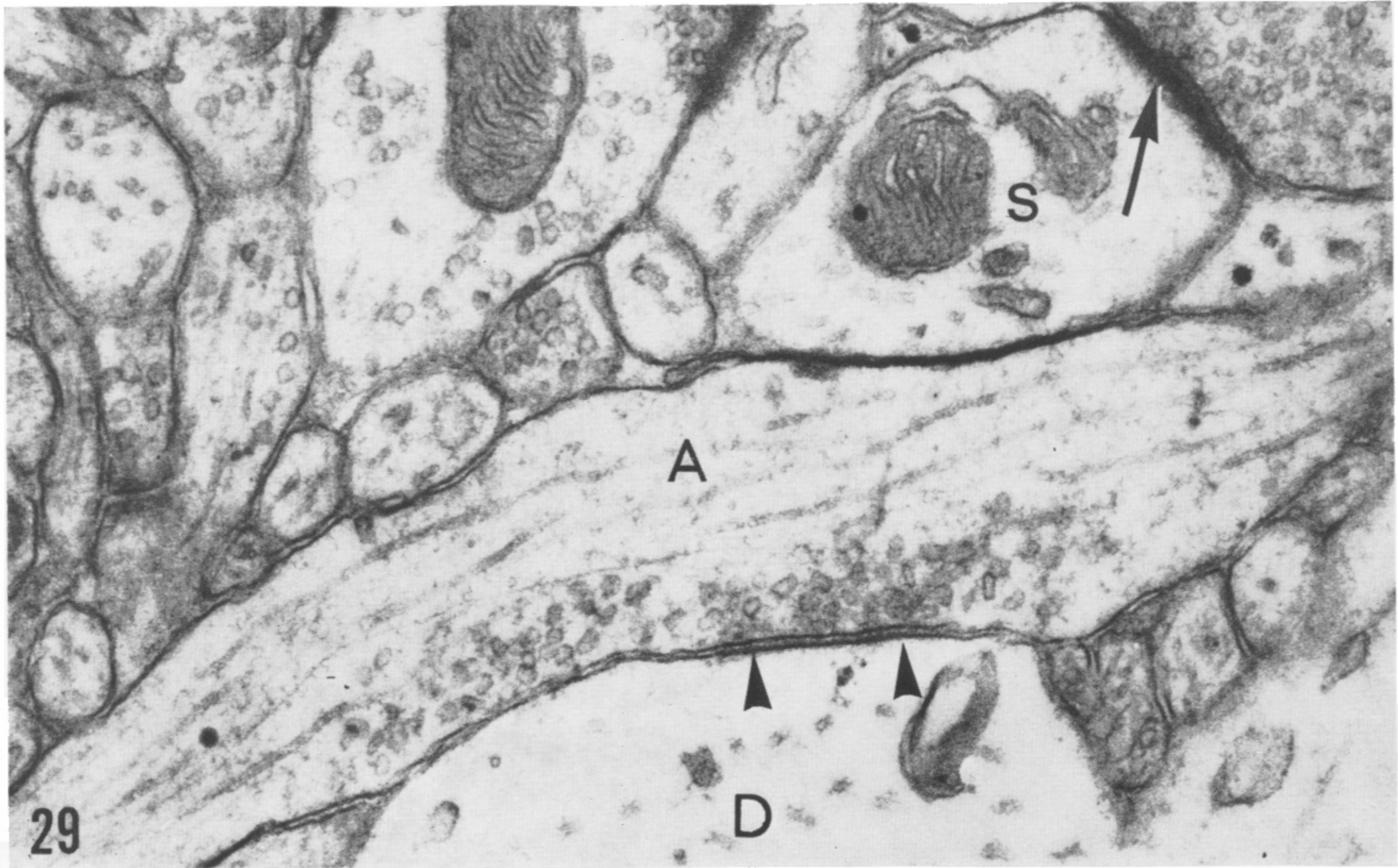
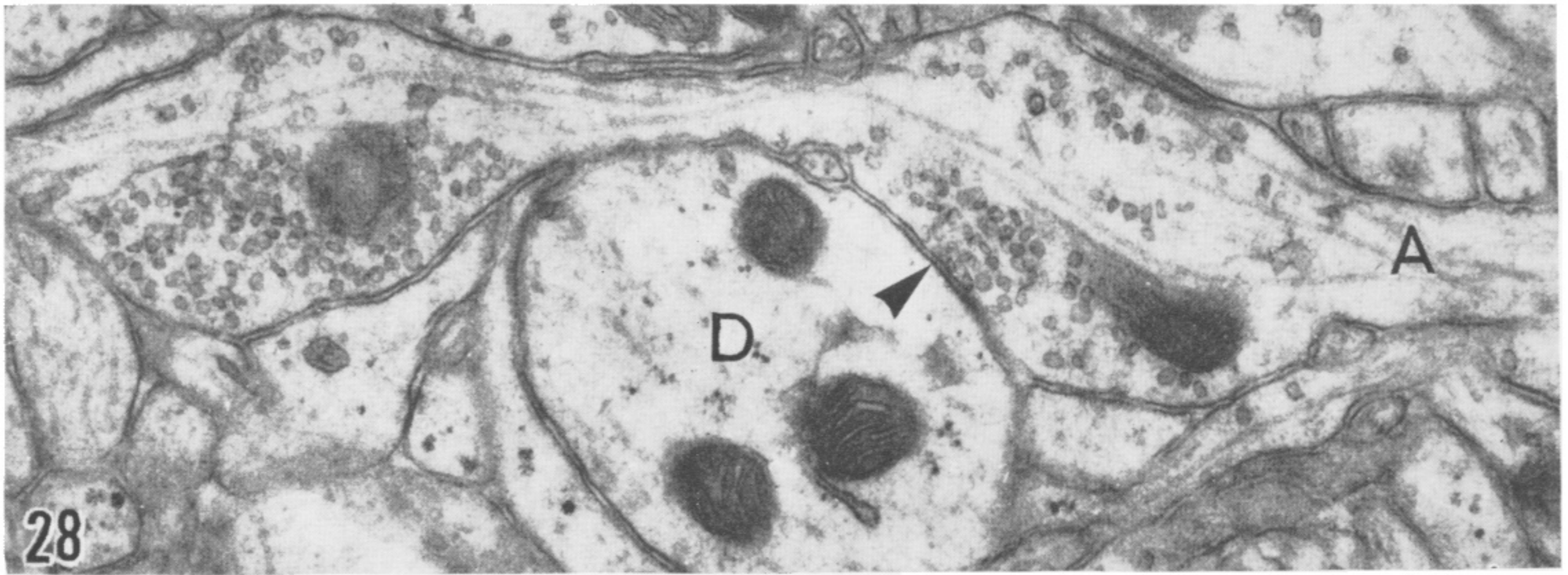


FIGURE 28. Unmyelinated axon (A) expanding at intervals to form two *en passant* terminals one of which ends symmetrically (arrow head) upon a dendrite (D). Note that many of the synaptic vesicles are flattened or irregular in shape. $\times 35\,000$.

FIGURE 29. Unmyelinated axon (A) containing flattened synaptic vesicles and ending in a symmetrical synaptic contact (arrow heads) upon a dendrite (D). Compare this synaptic complex with the asymmetrical one on the dendritic spine (S) above (arrow). $\times 50\,000$.

FIGURE 30. A terminal (T) at a node of Ranvier (arrows). The terminal contains flattened vesicles and ends in a symmetrical contact (arrow heads) on the soma of a neuron (N). $\times 30\,000$.

