ELECTRON MICROSCOPY OF THE SOMATIC SENSORY CORTEX OF THE CAT

III. THE FINE STRUCTURE OF LAYERS III TO VI

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[Plates 21 to 30]

CONTENTS

Variations in the fine structure of layers III to VI of the somatic sensory cortex have been described. Layers III and IV may be readily distinguished from one another and from layers V and VI, but within the latter two layers there is such a slow gradient of change that no clear-cut line of junction can be drawn between them. Layer III is characterized by the presence of many large apical dendrites ascending vertically through it from pyramidal cells in all layers to reach layer I. In parallel with these are many small unmyelinated axons which contain flattened synaptic vesicles and terminate on transversely orientated dendrites in symmetrical synaptic complexes. The remainder of the neuropil is filled by large numbers of dendritic spines receiving axon terminals which contain spherical vesicles and which terminate asymmetrically.

In layer IV there is a marked increase in the number of small myelinated axons ascending from below and ramifying within it. Embedded in the neuropil among these are many small non-pyramidal neurons whose somata and small, irregular dendrites are covered in axon terminals. Also present, and particularly concentrated at the junction with layer III, is a meshwork of fine unmyelinated axons which contain flattened vesicles and terminate in an *en passant* manner as symmetrical type synapses. Most of these axons are orientated transversely. A larger axon terminal which ends in asymmetrical complexes on small dendritic shafts and spines and which may be the terminal of thalamo-cortical axons is only found in any quantity in this layer. On descending into layers V and VI there is a progressive increase in the number of large myelinated fibres and glial cells, and a progressive diminution of neuronal elements, particularly dendritic spines. Some large non-pyramidal cells resembling the smaller ones on layer IV are present in layer VI.

INTRODUCTION

The present study is a continuation of that described in the preceding paper (Jones & Powell 1970*a*). Information about layers III to VI is desirable not only to enable them to be distinguished in experimental brains, but also because they show distinctive differences in light microscopy and may have different connexions. Layer IV, the internal granular layer, for

example, which is highly developed in the sensory areas of the cortex is mainly composed of stellate cells and receives the greater proportion of the terminals of thalamo-cortical fibres (Jones 1968; Jones & Powell 1970 b), while layers III, V and VI are largely formed of pyramidal neurons which are thought to be the main efferent elements of the cortex (Ramón y Cajal 1911; Lorente de Nó 1949).

The material is the same as that described in the preceding paper.

RESULTS

The fine structure of layer III

Layer III can be readily distinguished even in isolated thin sections by the presence of large numbers of large, vertically orientated dendrites running through it in parallel rows (figure 1, plate 21). In sections cut parallel to the surface of the brain these large dendrites all appear in cross-section and give the neuropil a characteristic appearance (figure 3, plate 22). This appearance is reminiscent of the deeper aspect of layer I, but the individual dendrites are generally larger. Many of these large dendrites are seen to be derived from pyramidal neurons in layer III itself, but others can be traced from layers V and VI. Some give off side branches which often bear dendritic spines, but spines are rare on the large dendrites themselves. Many other smaller dendrites are present, and these are orientated transversely, both in a rostro-caudal and medio-lateral direction. The majority have regular outlines and possess many dendritic spines which receive small dense axon terminals ending in asymmetrical thickenings (Type 1 of Gray 1959) (figure 9, plate 24). Less commonly, other small dendrites are present; these have irregular profiles and are contacted by large numbers of small axon terminals ending in either symmetrical (Type 2 of Gray 1959) or asymmetrical thickenings.

Ascending in parallel beside the large apical dendrites are a few small myelinated axons (figure 2, plate 21). These traverse layer III and extend into layers II and I. Some may have small swellings containing synaptic vesicles at their nodes of Ranvier in layer III (figure 2, plate 21). Rather more common are very small unmyelinated axons which may ascend for long distances beside the apical dendrites and may reach layer II. These axons have multiple en passant terminals usually ending on the transverse rather than on the vertical dendrites (figures 4 to 8, plate 23). These terminations are by means of symmetrical synaptic thickenings and the few vesicles within the en passant endings are commonly flattened, irregular or smaller than those in asymmetrically ending terminals. Other unmyelinated axons with similar en passant terminals and symmetrical membrane thickenings may be seen running transversely within layer III, though they are more common in the deeper part and, overall, their numbers are fewer than in layer IV and in the deep part of layer I.

Lying among the vertically orientated axons and dendrites are many neuronal perikarya which may appear in small groups of 3 or 4. Most can be identified as pyramidal cells on the basis of their shape. Many of these have perikarya which are less wide though longer than the similar pyramidal cells of layer II. Many have obvious axon hillocks and long initial segments. Most of them have only a few axon terminals synapsing upon the perikaryon and proximal parts of their apical and basal dendrites; a few may appear on the initial segment of the axon. Small, round perikarya, which can be identified as those of non-pyramidal neurons, are not obvious, except in the deeper part of this layer where it merges insensibly with layer IV.

Between the dendrites and perikarya, the neuropil is composed of very large numbers of

FIGURE 1. Layer III of the cortex in a section cut perpendicular to the surface. The large apical dendrites (D) ascending in parallel towards layer I are typical of the lamina. A small number of myelinated axons are also present. As, astrocyte. × 4000.

FIGURE 2. A vertically orientated, myelinated axon (A) in layer III with a terminal (T) at a node of Ranvier. The arrows indicate the unwrapping of the myelin sheath. × 10000.
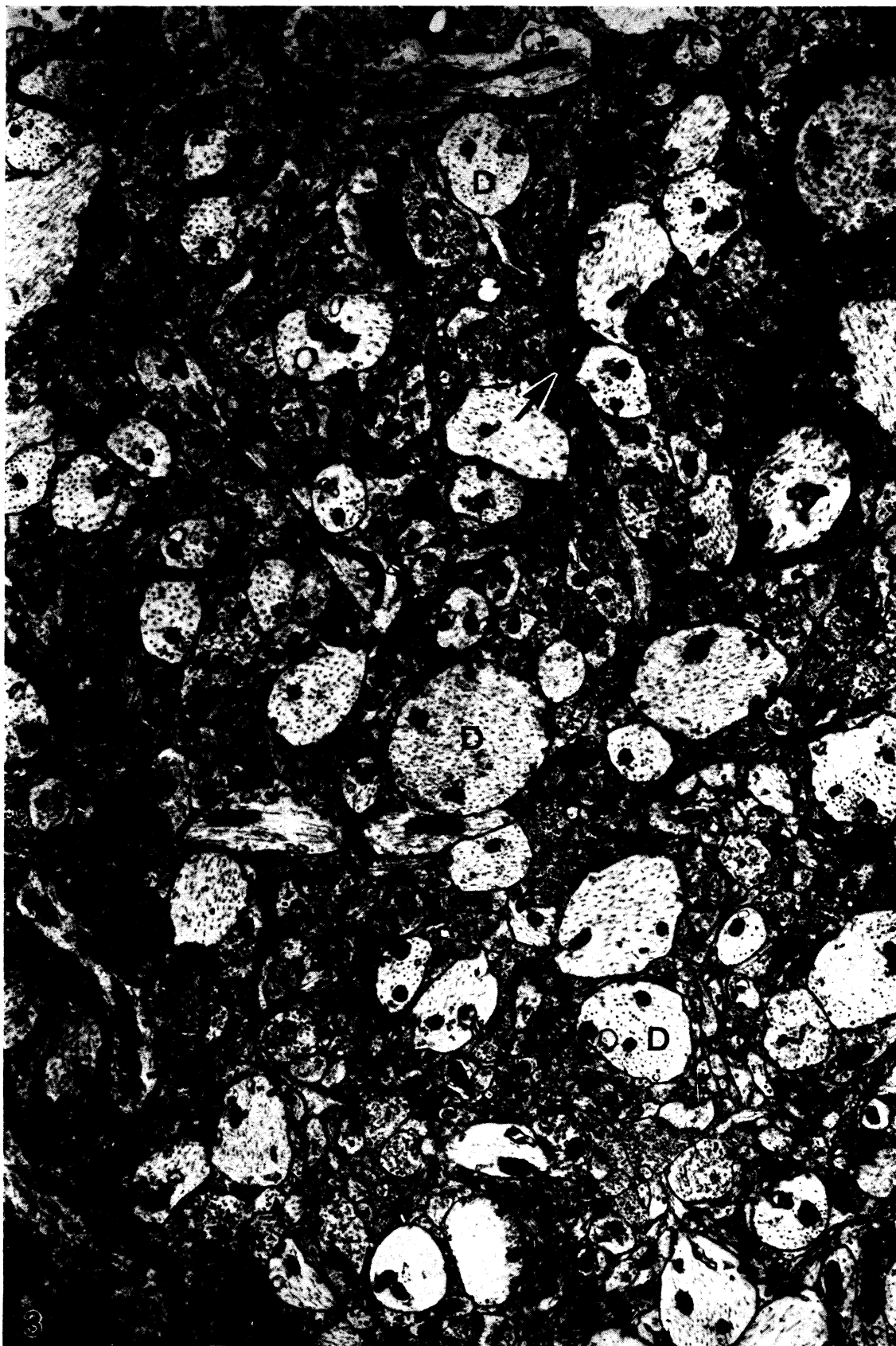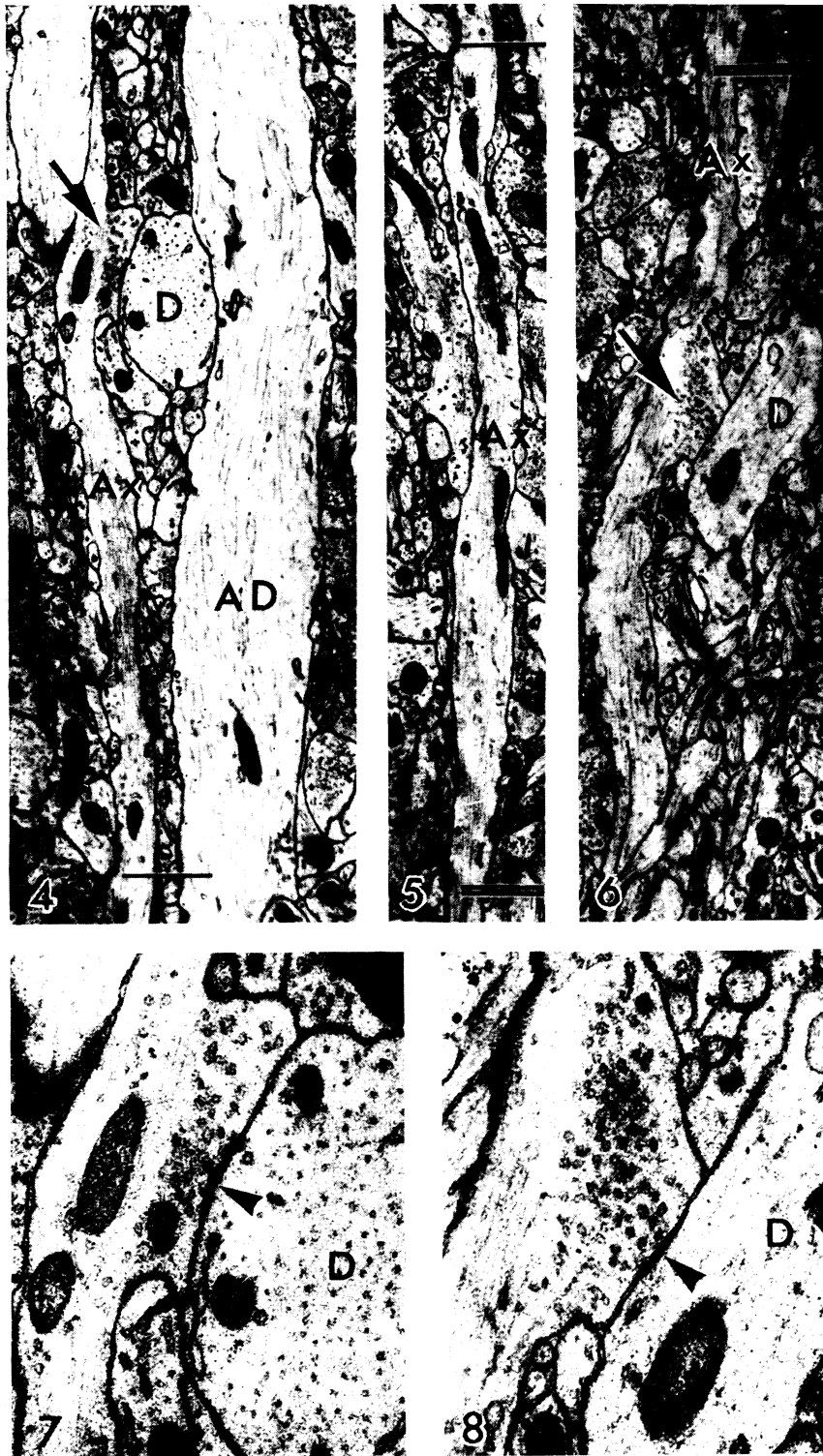
FIGURE 3. The appearance of layer III as seen in a section cut parallel to the surface of the brain. Large numbers of apical dendrites (D) are cut in cross-section and in the intervening neuropil there are many small dendritic spines and axon terminals. Some of the spines (arrow) are seen to arise from the ascending dendrites. × 10 000.

FIGURES 4 to 6. A series of electron micrographs which overlap at the horizontal lines showing an apical dendrite (AD) ascending through layer III accompanied by a thin unmyelinated axon (Ax) which has two *en passant* terminals (arrows) ending on transversely disposed dendrites (D). × 10 000.

FIGURES 7, 8. Views at higher magnification of the terminals shown in figures 4 to 6. Note that the synaptic contact is a symmetrical one and many of the synaptic vesicles are flattened or irregular in shape. D, dendrite. × 28 000.

FIGURE 9. Part of layer III showing the large number of dendritic spines (arrows) which are typical of the layer. Some may be seen to arise from the apical dendrites (AD) ascending through the layer. Small dense axon terminals end on all of the spines. × 20 000.

FIGURE 10. The plexus of small unmyelinated axons which appears at the junction of layers III and IV. Some of these axons are indicated by the arrows. D, dendrite. × 30 000.
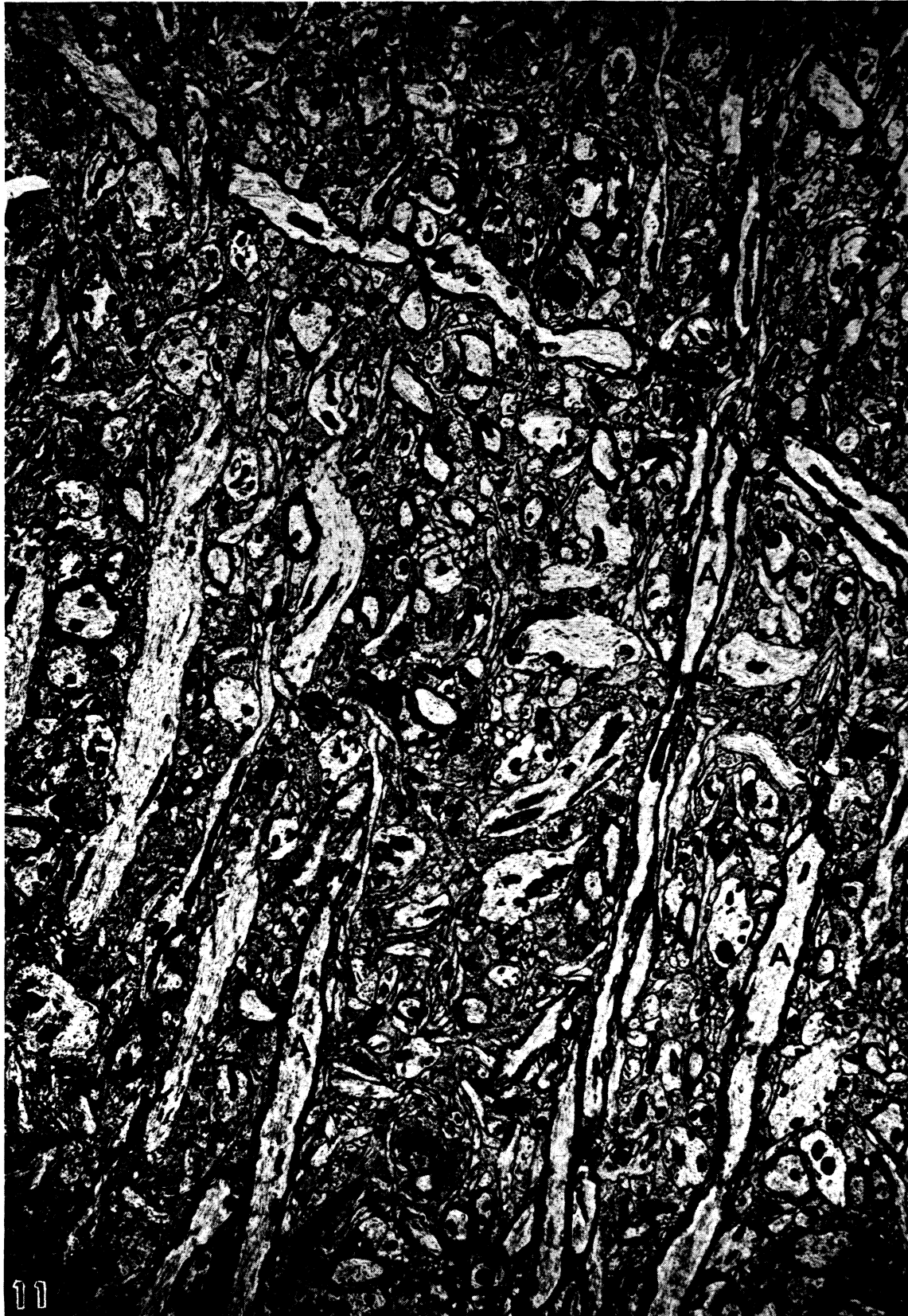
FIGURE 11. The typical appearances of layer IV as seen in a section cut perpendicular to the surface. Many small myelinated axons (A) ascend from deeper layers and appear to break up into more irregularly disposed branches in this layer. × 8000.

FIGURE 12. A part of layer IV as seen in a section cut parallel to the surface. In addition to the myelinated fibres, many small unmyelinated axons are present, some of which (A) give rise to *en passant* axon terminals (arrows). × 13 000.
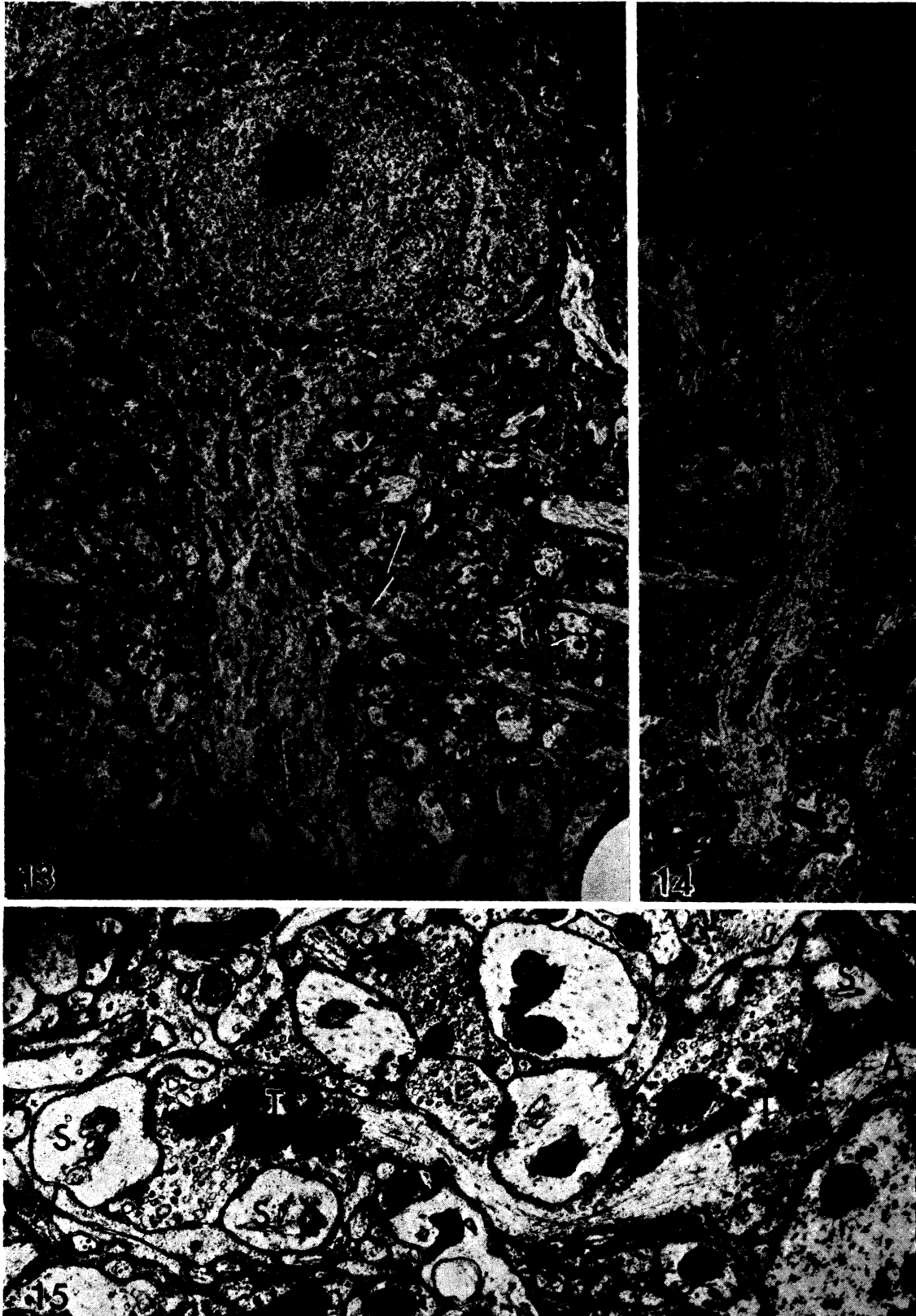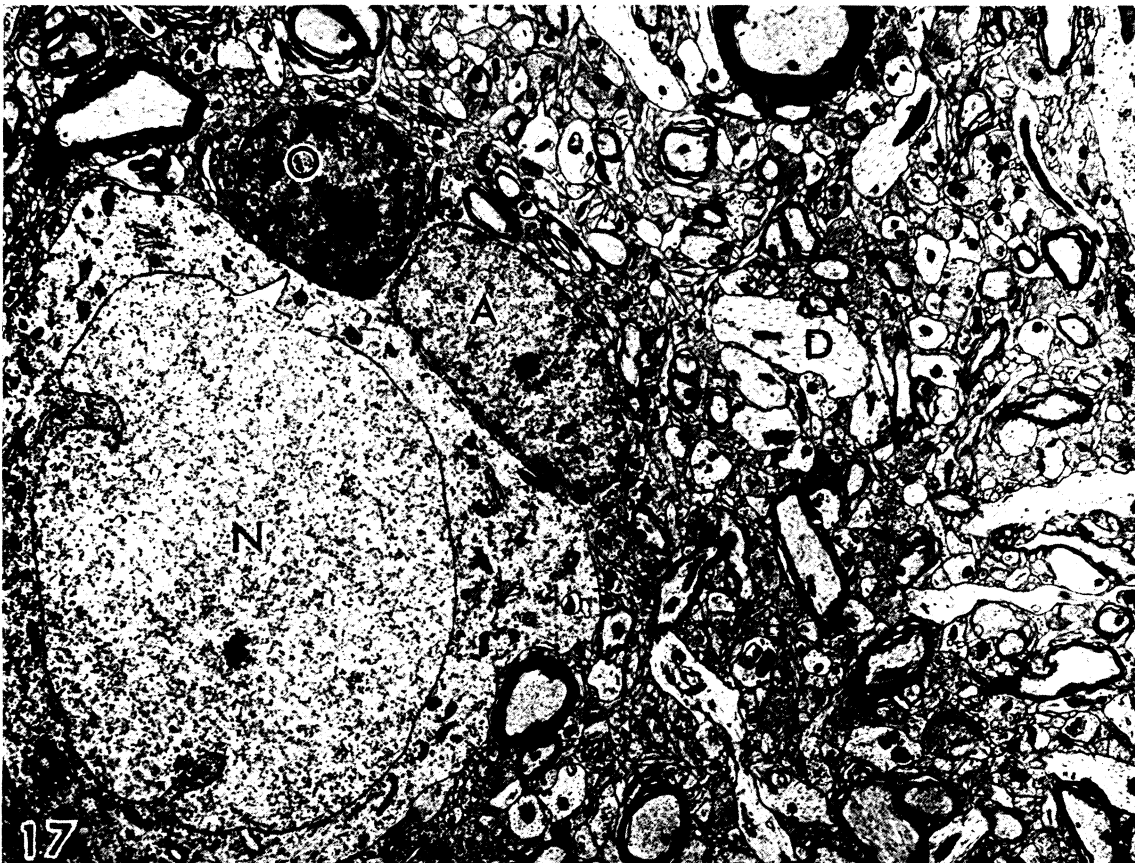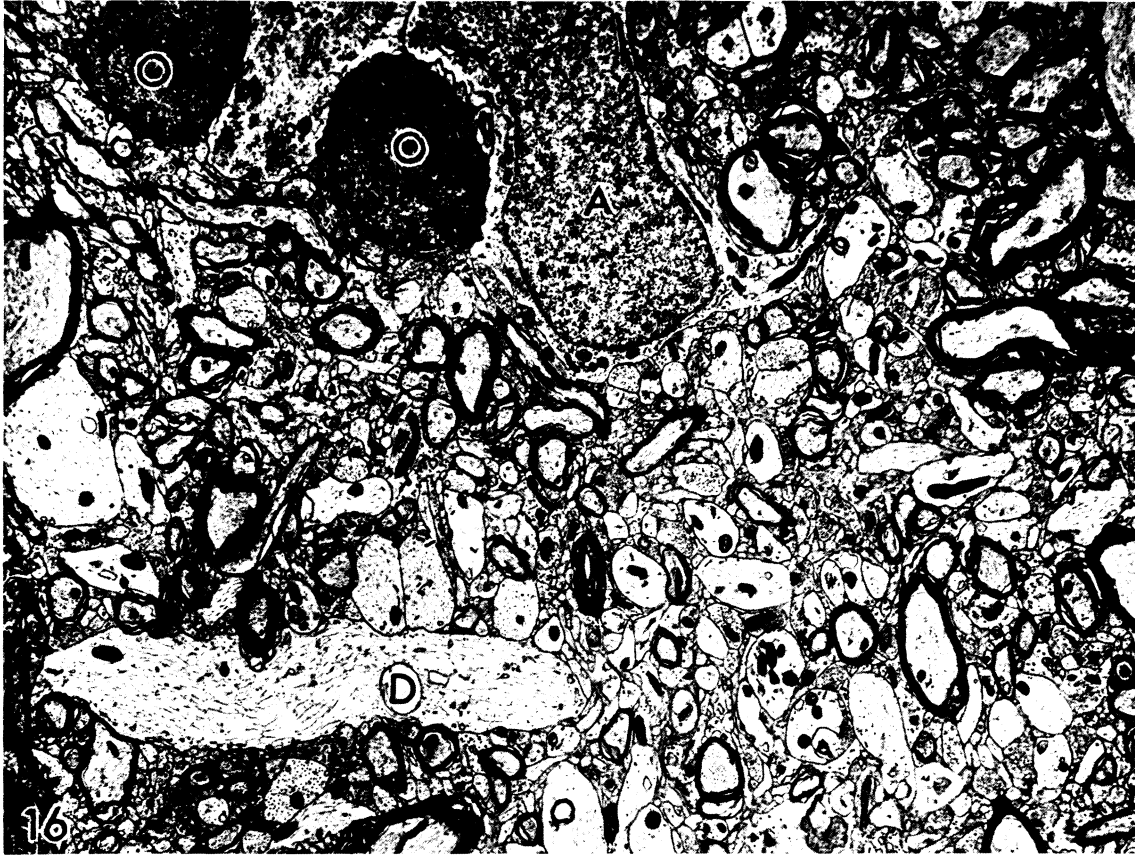
FIGURE 13. The typical neuron (N) of layer IV. This has a round perikaryon and dendrites (D) which branch irregularly and are covered in axon terminals. The smaller branches (arrow) are often beaded. The surface of the brain is towards the top. × 5000.

FIGURE 14. A small dendrite (D) upon which many axon terminals make synaptic contact (arrow heads). Layer IV. × 10000.

FIGURE 15. A type of terminal found frequently in layer IV. The axon (A) enters from the right of the micrograph and expands into two terminals (T) which make synaptic contact with three dendritic spines (S) by means of asymmetrical contacts. × 28000.

FIGURES 16 and 17. The typical appearance of layer V seen in sections cut parallel to the surface. Myelinated fibres of all sizes begin to dominate the neuropil but a number of transversely disposed dendrites (D) are present together with neurons (N) and glial cells. A, astrocyte; O, oligodendrocyte. × 6000.
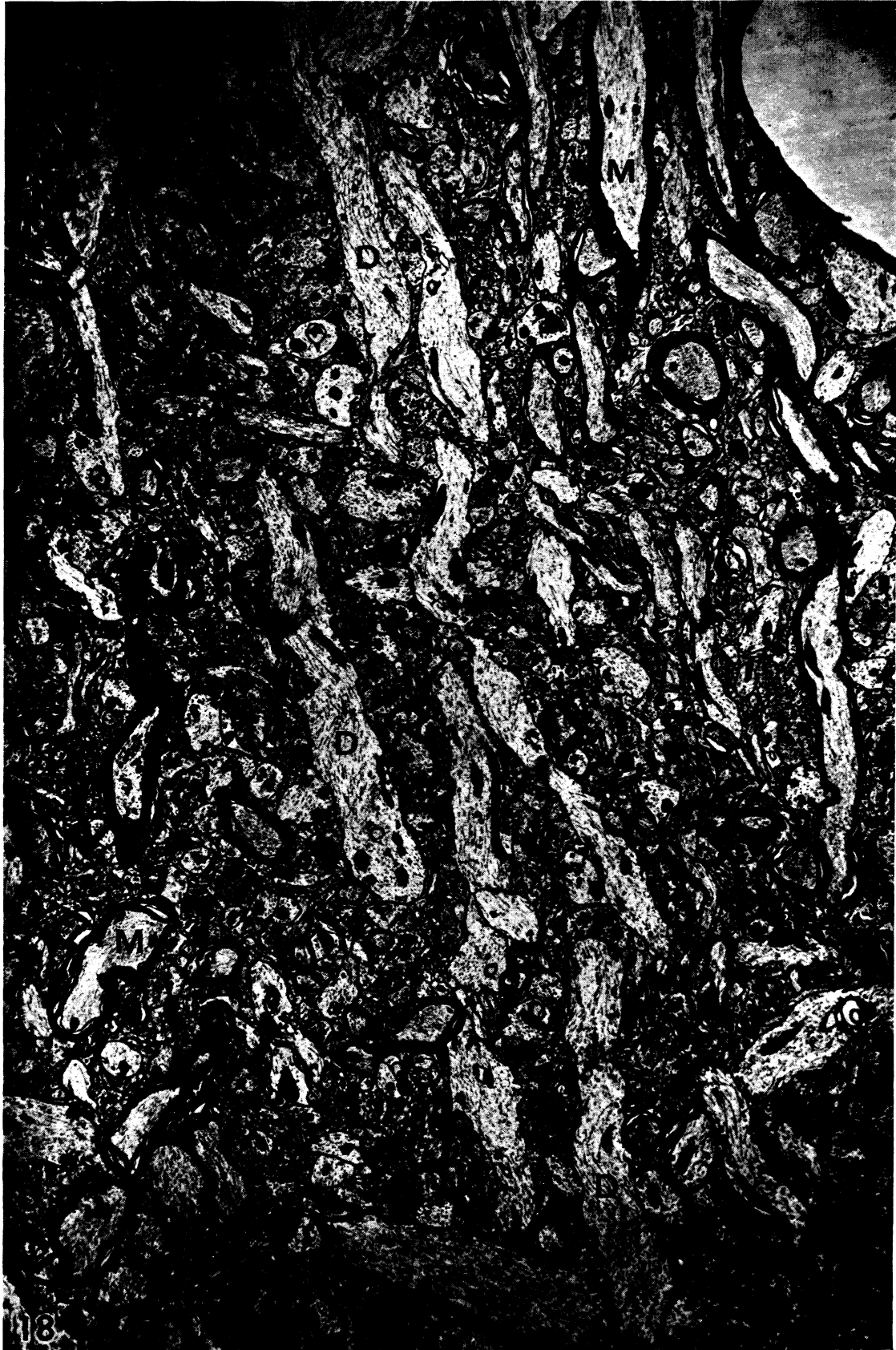
FIGURE 18. Layers V and VI, showing ascending dendrites (D) and large myelinated axons (M). Perpendicular section. × 6000.
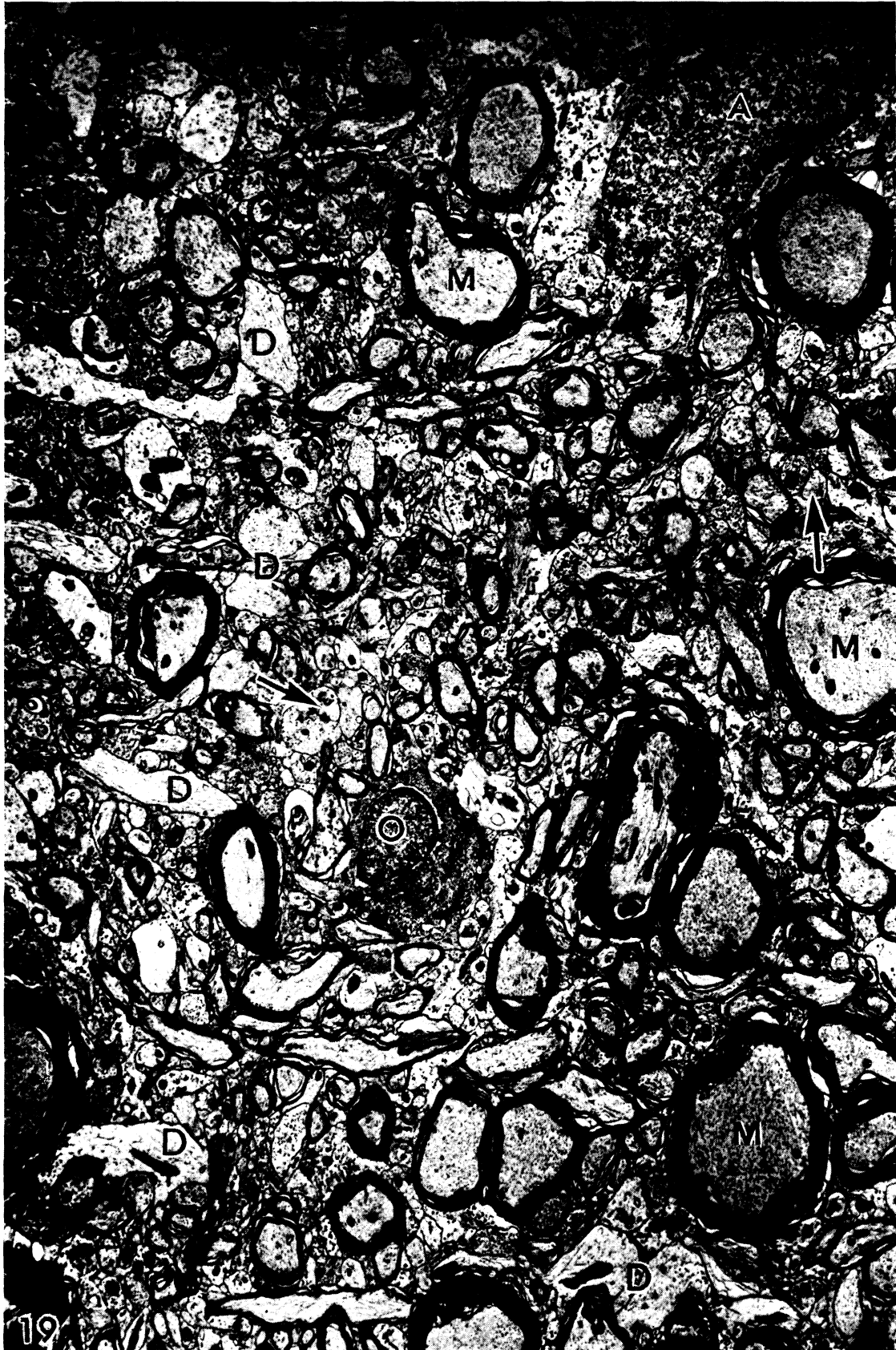
FIGURE 19. The deepest part of layer VI in a section cut parallel to the surface. The dominant features are the presence of very large myelinated axons (M) astrocytes (A) and oligodendrocytes (O), but a few dendrites (D) and an occasional dendritic spine (arrow) are also present. × 6000.

dendritic spines of all sizes and shapes (figure 9, plate 24); each of these receives an axon terminal of the small dense type ending in an asymmetrical type of contact. Many of the spines are seen to be attached to the ascending and transverse dendrites, both large and small, but far more appear unattached in single sections. In a single section of layer III, 137 axon terminals ended on spines, 330 on small dendrites, five on large dendrites and eight on neuronal perikarya.

On passing into the deeper aspect of layer III there is a progressive increase in the number of small myelinated axons, although no large ones are yet seen. A large proportion of these small myelinated fibres are orientated transversely, in contrast to the more superficial part of layer III where most are vertically disposed. Layer III shades insensibly into layer IV, the transition being marked by a gradual increase in the number of small myelinated axons, and there is also a tight plexus of thin unmyelinated axons resembling those seen at the junction of the two parts of layer I (figure 10, plate 24). Oligodendrocytes also become more prominent, most appearing as satellites of the pyramidal neurons.

<center>*The fine structure of layer IV*</center>

Layer IV is distinguished by a number of features. Compared with the more superficial layers, the most striking difference is the appearance of large numbers of medium to large myelinated axons. Many of these are cut longitudinally in sections perpendicular to the surface and are obviously passing up from below (figure 11, plate 25). Several other small myelinated fibres, however, are cut transversely and some obliquely so that there is a dense interlacing plexus in this layer. Scattered in small bundles among these myelinated fibres are large numbers of thin unmyelinated axons which form a dense feltwork close to the junction with layer III. Many of these give rise to *en passant* type synapses with symmetrical membrane thickenings on dendrites in layer IV (figure 12, plate 26). Numerous unmyelinated axons terminate as quite large terminals which have a high concentration of spherical synaptic vesicles, and are intermediate in density between the small dense terminals and the *en passant* terminals of unmyelinated axons. These large terminals have asymmetrical synaptic contacts on both dendritic spines and on small dendritic shafts, some terminals ending on both in the same section. Considerable numbers of these terminals may be presynaptic to several profiles either as a single terminal, as large *en passant* type terminals or as a branching terminal (figure 15, plate 27).

Embedded within the neuropil are large numbers of relatively small neuronal perikarya; some of these resemble small pyramids but the majority are oval (figure 13, plate 27) and have all the characteristics of non-pyramidal cells (Jones & Powell 1970c). Their small, irregular dendrites come off at sharp angles and both the soma and the dendrites are covered in axon terminals (figures 13, 14, plate 27). On the soma the terminals are more lucent than those in the neuropil, make symmetrical synaptic contacts and may have flattened synaptic vesicles. Many of those on the dendrites are similar, but others are dense with spherical vesicles and end in asymmetrical contacts. Isolated small dendrites having an irregular shape and receiving many axon terminals, lie scattered throughout the neuropil (figure 14, plate 27).

Large apical dendrites ascend through layer IV from deeper layers giving off side branches, and both the apical dendrites and side branches have many dendritic spines, although spines do not dominate the neuropil as they do in layers III and I. Oligodendrocytes, and to a lesser extent, astrocytes are common.

The fine structure of layers V and VI

These two layers are difficult to distinguish from each other at the electron microscopic level. Layer V differs from layer IV in that it contains many more myelinated axons, lacks the fine plexus of unmyelinated axons and possesses many large pyramidal neuron perikarya (figures 16 to 18, plates 28 and 29). Similarly, at its deeper aspect, layer VI can be distinguished from the white matter by containing dendrites, neuronal perikarya and axon terminals (figure 19, plate 30). In between, however, there is such a slow gradient of change in passing from layer IV to the white matter that a clear boundary cannot be drawn between layers V and VI. These layers are dominated by myelinated axons of all sizes, the smallest having a total diameter of 0·5 μm and the largest ones of 10 μm. The number of fibres increases progressively on passing deeply within the cortex, and many become arranged in parallel bundles. It is probable that a considerable degree of branching occurs in these layers, for many large myelinated axons (5 to 10 μm) are seen in the deepest parts but those in more superficial parts are slightly thinner. Many of the latter fibres have nodal terminals presynaptic to dendrites or neuronal perikarya. Small dendrites and dendritic spines are uncommon in these two layers, and the spines become even less common in deeper parts, although those encountered may be quite large. Numerous large dendrites are arranged in vertical rows ascending through both layers; most of these are apical dendrites of typical, large pyramidal cells. In addition, some smaller, vertical dendrites are from obviously non-pyramidal cells in both superficial and deep parts. These cells, together with their dendrites, are covered in axon terminals, the majority of which end symmetrically. Such cells may vary considerably in size, the perikarya of some being larger than those of adjacent pyramidal cells while others are as small as those in layer IV.

Many relatively large dendrites arise from the basal aspects of the pyramidal cells and run transversely (figure 16, plate 28). These dendrites possess few dendritic spines and only a very small number of axon terminals make synaptic contact with them. A few smaller and more irregularly shaped dendrites, derived from non-pyramidal cells, are also present. These appear to lack dendritic spines but receive considerable numbers of terminals.

In more superficial parts of layer V, abutting on layer IV many small unmyelinated axons are found; some of these are arranged transversely and make *en passant* synaptic contacts mainly with dendritic shafts by means of symmetrical thickenings. However, there are very few such axons in deeper parts of the two layers.

On passing towards the white matter, glial cells, particularly oligodendrocytes, become more numerous (figures 16 to 19, plates 28 to 30). The latter now frequently appear independent of a neuronal perikaryon and may be seen in clumps of two or more with their plasma membranes separated only by the narrow extracellular cleft.

DISCUSSION

The present observations, together with those of the preceding paper (Jones & Powell 1970 a) show that the laminae of the cerebral cortex can be recognized electron microscopically, and in the case of some layers, such as layer I, there are differences which are sufficiently marked to enable one to subdivide it even further. In addition, the electron microscope has demonstrated differences in the laminae which are not visible with light microscopy, and which are largely expressions of variations in the dendritic and axonal organizations of the layers. The boundaries between adjacent laminae are most clear between layers I and II, but in deeper

layers, particularly in layers V and VI there is a slow gradient of change rather than a clear-cut line of junction. Sections from the middle of layers I to IV and from the deepest part of layer VI could usually be recognized with confidence in blind trials as belonging to the appropriate layer, but sections from layer V and from the middle of layer VI could not be distinguished as belonging to one or the other. Similarly, at the junctions of layers III and IV and of IV and V it would be difficult to state where one layer ended and the other began. The gradient of architectural change which occurs at boundaries between laminae is perhaps analogous to the change which is seen with the light microscope at the margins of architectonic subdivisions of the cortex (Rose 1949).

As well as showing that differences exist at what is essentially the lowest magnification level of the electron microscope, this and the preceding paper (Jones & Powell 1970a) have brought together many of the general aspects of the synaptic organization of the somatic sensory cortex described earlier (Jones & Powell 1970c). It has been found that some significant variations occur in the number and type of synapses at different levels. The superficial part of layer I, for example, contains only terminals which contain spherical vesicles and end in asymmetrical synaptic contacts, while in other layers, although this type of terminal may predominate, large numbers of transversely or vertically orientated unmyelinated axons appear which have terminals containing flattened or pleomorphic vesicles and which end symmetrically. If, as has been postulated (Uchizono 1968; Colonnier 1968) these different types of synapse are respectively excitatory and inhibitory, it would suggest that individual laminae may vary in regard to the relative proportions of excitatory and inhibitory processes taking place within them. Even if these two distinct types of terminal are not the bases of excitatory and inhibitory synapses, if morphological differences are expressions of differences in functional properties, they may still be functionally distinct.

Because many neurons send their dendrites through all layers the differences in the organization of the two types of synapse may indicate to a certain extent a segregation of their effects upon different parts of the dendritic tree. A small proportion of dendritic spines receive symmetrical synapses with flattened vesicles as well as asymmetrical synapses with spherical vesicles (Colonnier 1968; Jones & Powell 1970c), but it is not yet possible to determine whether these spines are located upon specific parts of the apical and basal dendrites of pyramidal neurons. Similarly, although the dendrites of non-pyramidal neurons may be covered in synapses of both types it is not known whether the postulated inhibitory type shows predilection for a specific part of the dendrite.

Of the laminae described in the present paper, lamina IV is perhaps the most distinctive in showing a fine feltwork of small unmyelinated axons many of which contain flattened or pleomorphic synaptic vesicles and terminate symmetrically, together with a high concentration of definitely non-pyramidal neurons. It has been shown that the majority of the thalamo-cortical fibres terminate in this layer (Jones 1968; Jones & Powell 1970b) and, unlike commissural and association fibres, it appears that they may end on both pyramidal and non-pyramidal cells. The proportion ending on the latter is, however, far less than that on pyramidal cells, and thalamo-cortical terminals must constitute a mere fraction of the total synapses upon a single non-pyramidal cell. This would suggest that the small non-pyramidal cell is more than a simple relay, intercalated between the thalamo-cortical fibre and the efferent pyramidal cell, for the majority of the synapses which it receives are derived from other sources. Experimental work (Jones & Powell 1970b) suggests that most of the terminals ending on it are from axons arising

and terminating within the cortex. There would thus appear to be a direct pathway through the cortex—from thalamus to pyramidal cell—and a more indirect one via the non-pyramidal neuron of layer IV which possibly plays back upon the pyramidal cell through an intracortical axon. It has not been possible to determine whether the unmyelinated axons which end in symmetrical synapses are derived from non-pyramidal neurons, but such a possibility seems likely.

Layers V and VI have proved less amenable to analysis, but are of interest in containing many neurons which are undoubtedly non-pyramidal and except for their size have all the characteristics of the smaller stellate neurons of layers II and IV. Probably, they are the spindle and fusiform neurons of light microscopy. They do not seem, however, to receive the terminals of the extrinsic afferents to the somatic sensory cortex so that they may be more concerned in modulating the activity of the large pyramidal neurons of layers V and VI which are generally thought of as the origin of most of the efferent fibres of the cortex. The deepest part of layer VI is also unusual and resembles the superficial part of layer I in having none of the long, thin un-myelinated axons which have flattened vesicles and end in symmetrical contacts. Most of the dendrites in the deeper part of layer VI should be the terminal parts of basal dendrites of pyramidal cells in this layer. The absence of apparently inhibitory synapses from this as well as from the superficial part of layer I where only apical dendritic tips are present, may indicate that the inhibitory effects are not present at the parts of the dendritic tree furthest removed from the soma.

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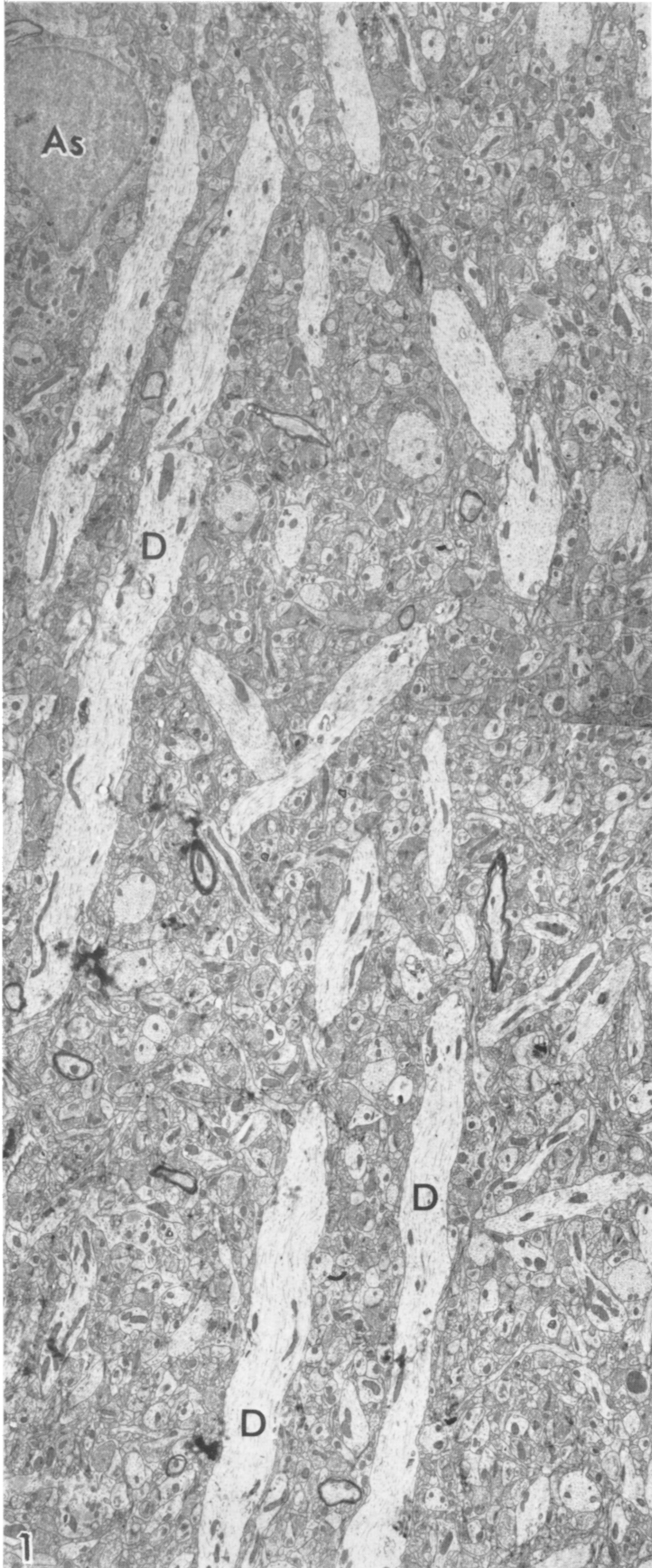
FIGURE 1. Layer III of the cortex in a section cut perpendicular to the surface. The large apical dendrites (D) ascending in parallel towards layer I are typical of the lamina. A small number of myelinated axons are also present. As, astrocyte. × 4000.
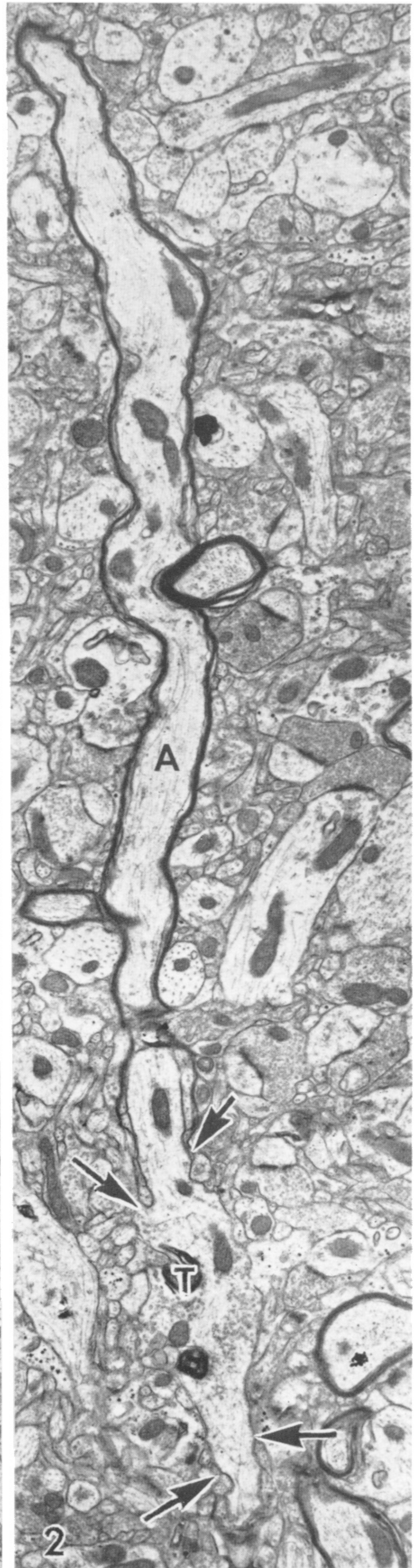
FIGURE 2. A vertically orientated, myelinated axon (A) in layer III with a terminal (T) at a node of Ranvier. The arrows indicate the unwrapping of the myelin sheath. × 10 000.
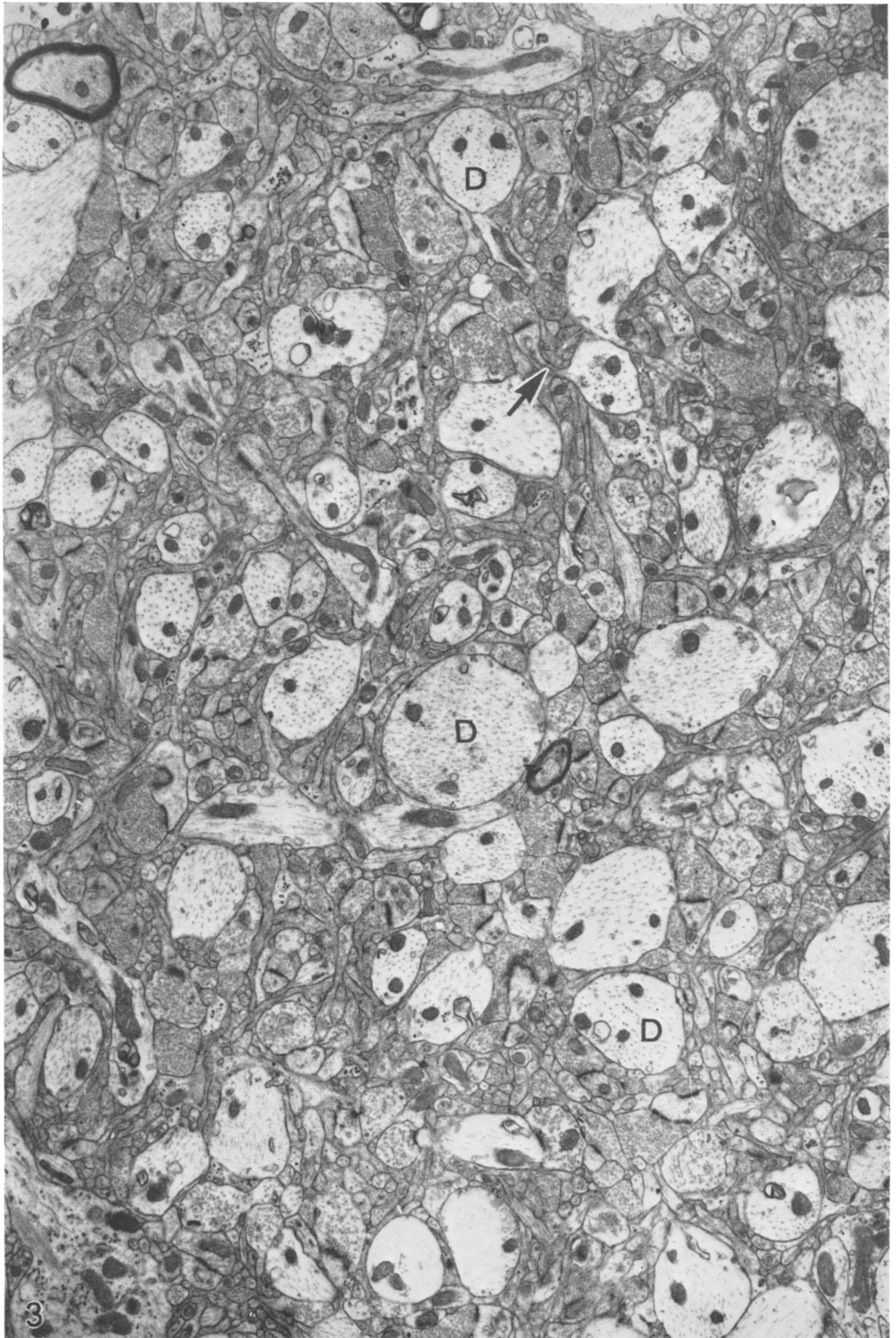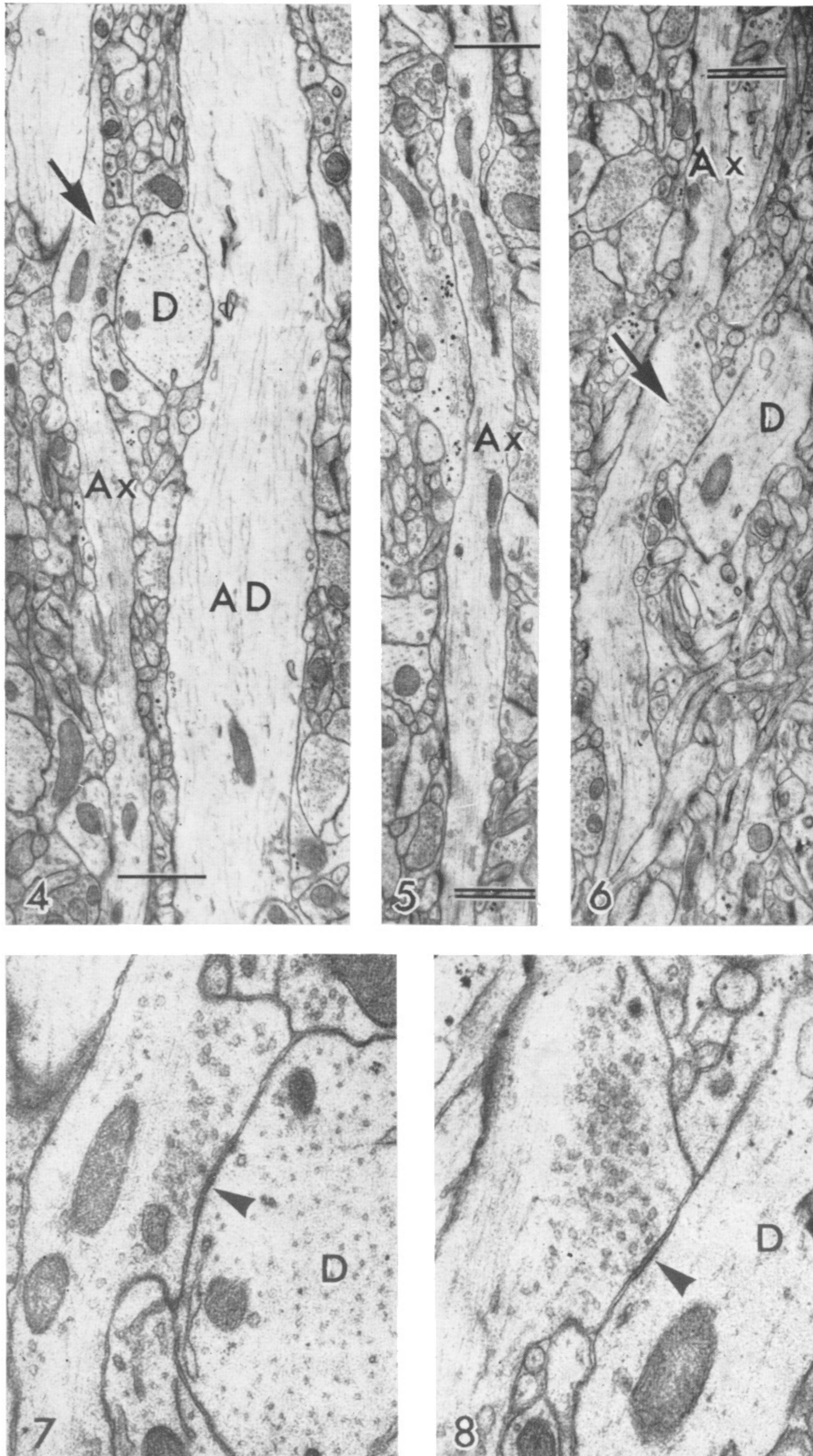
FIGURE 3. The appearance of layer III as seen in a section cut parallel to the surface of the brain. Large numbers of apical dendrites (D) are cut in cross-section and in the intervening neuropil there are many small dendritic spines and axon terminals. Some of the spines (arrow) are seen to arise from the ascending dendrites. × 10 000.

FIGURES 4 to 6. A series of electron micrographs which overlap at the horizontal lines showing an apical dendrite (AD) ascending through layer III accompanied by a thin unmyelinated axon (Ax) which has two *en passant* terminals (arrows) ending on transversely disposed dendrites (D). × 10 000.

FIGURES 7, 8. Views at higher magnification of the terminals shown in figures 4 to 6. Note that the synaptic contact is a symmetrical one and many of the synaptic vesicles are flattened or irregular in shape. D, dendrite. × 28 000.

FIGURE 9. Part of layer III showing the large number of dendritic spines (arrows) which are typical of the layer. Some may be seen to arise from the apical dendrites (AD) ascending through the layer. Small dense axon terminals end on all of the spines. × 20 000.

FIGURE 10. The plexus of small unmyelinated axons which appears at the junction of layers III and IV. Some of these axons are indicated by the arrows. D, dendrite. × 30 000.

FIGURE 11. The typical appearances of layer IV as seen in a section cut perpendicular to the surface. Many small myelinated axons (A) ascend from deeper layers and appear to break up into more irregularly disposed branches in this layer. × 8000.

FIGURE 12. A part of layer IV as seen in a section cut parallel to the surface. In addition to the myelinated fibres, many small unmyelinated axons are present, some of which (A) give rise to *en passant* axon terminals (arrows). × 13 000.
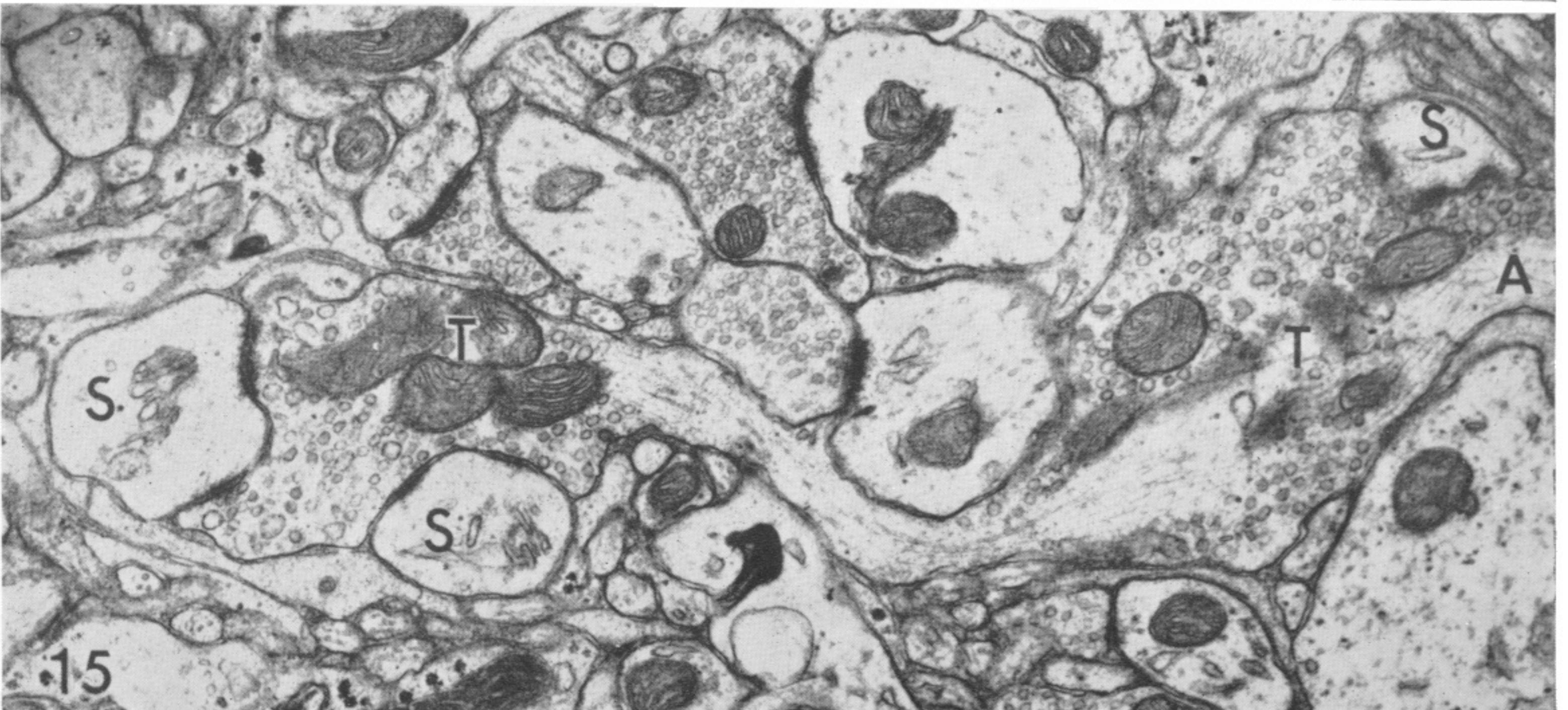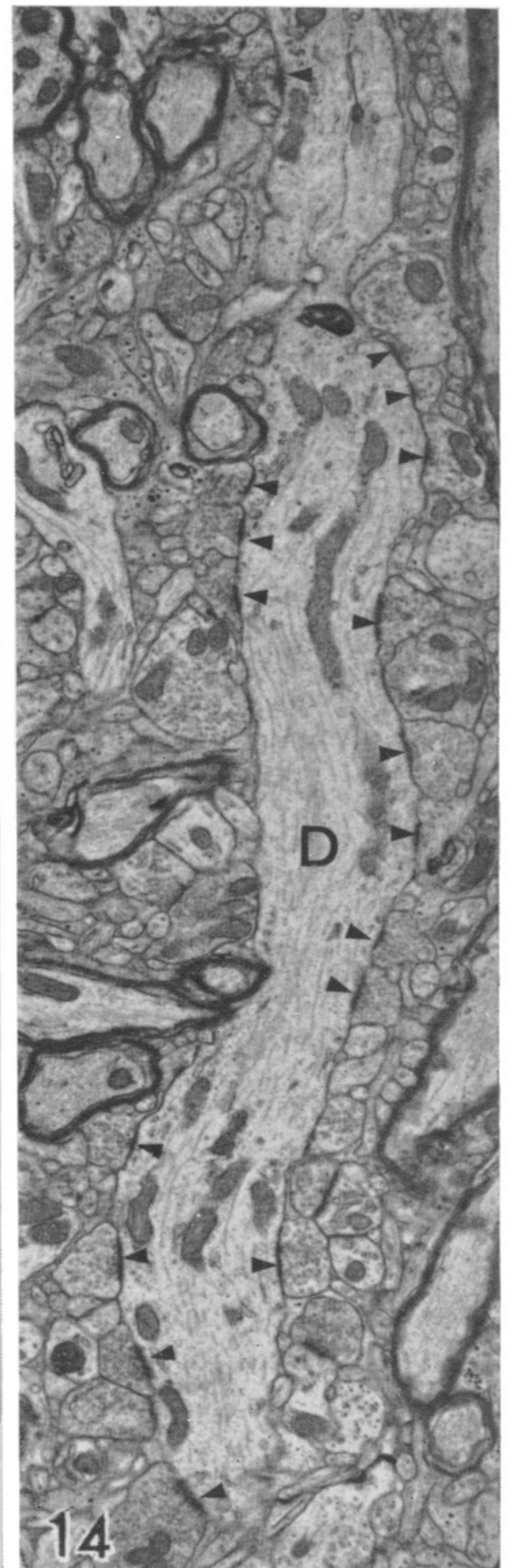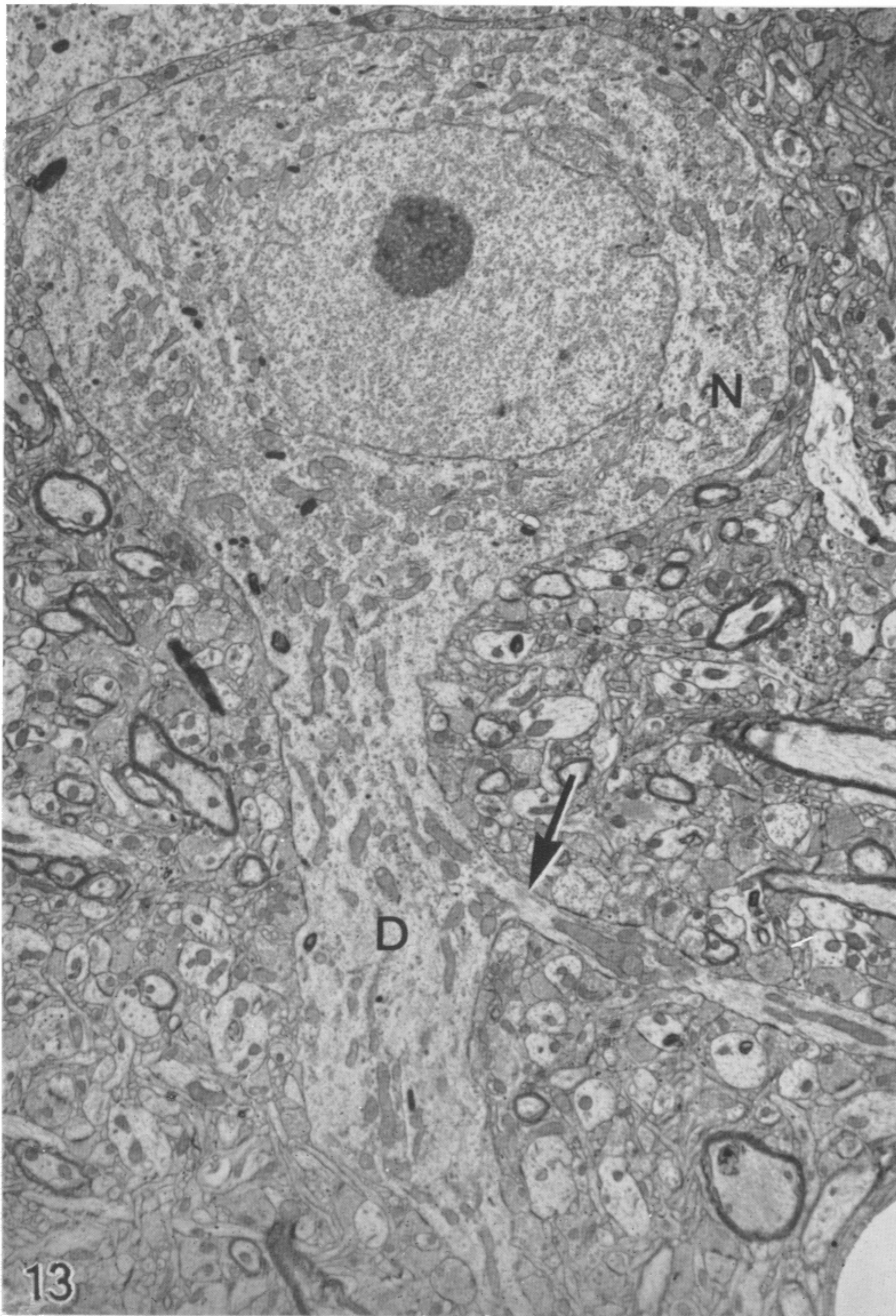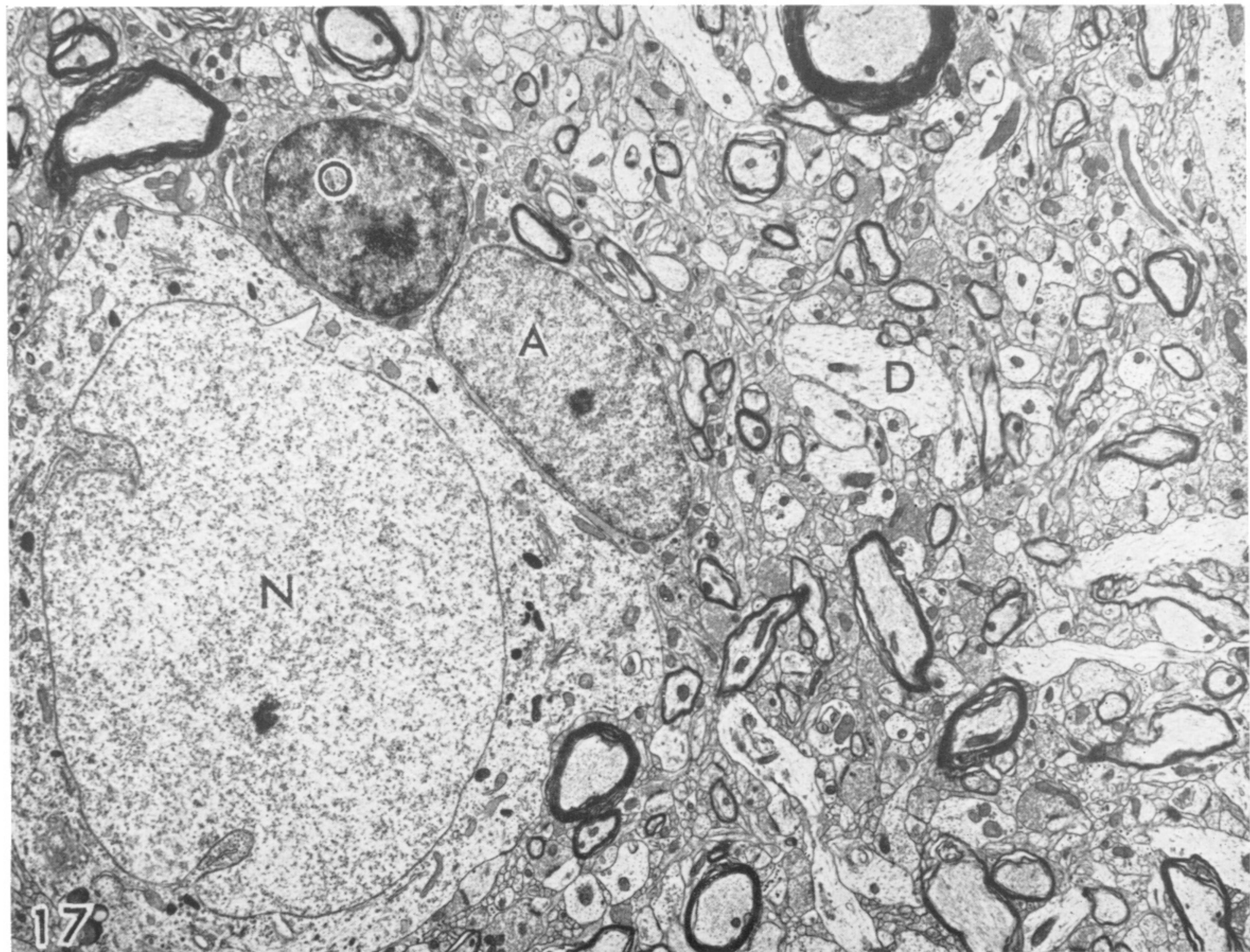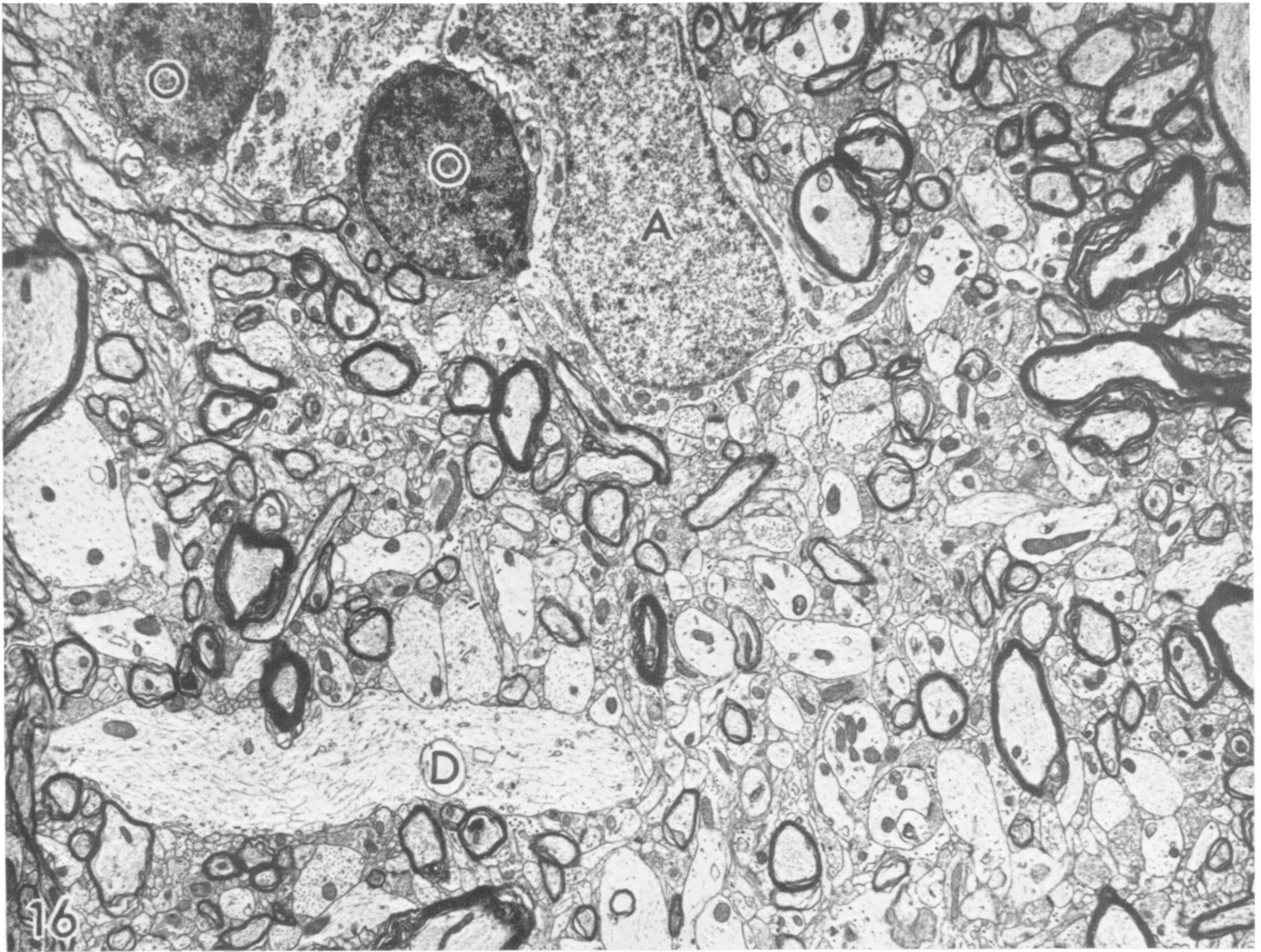
FIGURE 13. The typical neuron (N) of layer IV. This has a round perikaryon and dendrites (D) which branch irregularly and are covered in axon terminals. The smaller branches (arrow) are often beaded. The surface of the brain is towards the top. × 5000.

FIGURE 14. A small dendrite (D) upon which many axon terminals make synaptic contact (arrow heads). Layer IV. × 10000.

FIGURE 15. A type of terminal found frequently in layer IV. The axon (A) enters from the right of the micrograph and expands into two terminals (T) which make synaptic contact with three dendritic spines (S) by means of asymmetrical contacts. × 28000.

FIGURES 16 and 17. The typical appearance of layer V seen in sections cut parallel to the surface. Myelinated fibres of all sizes begin to dominate the neuropil but a number of transversely disposed dendrites (D) are present together with neurons (N) and glial cells. A, astrocyte; O, oligodendrocyte. × 6000.

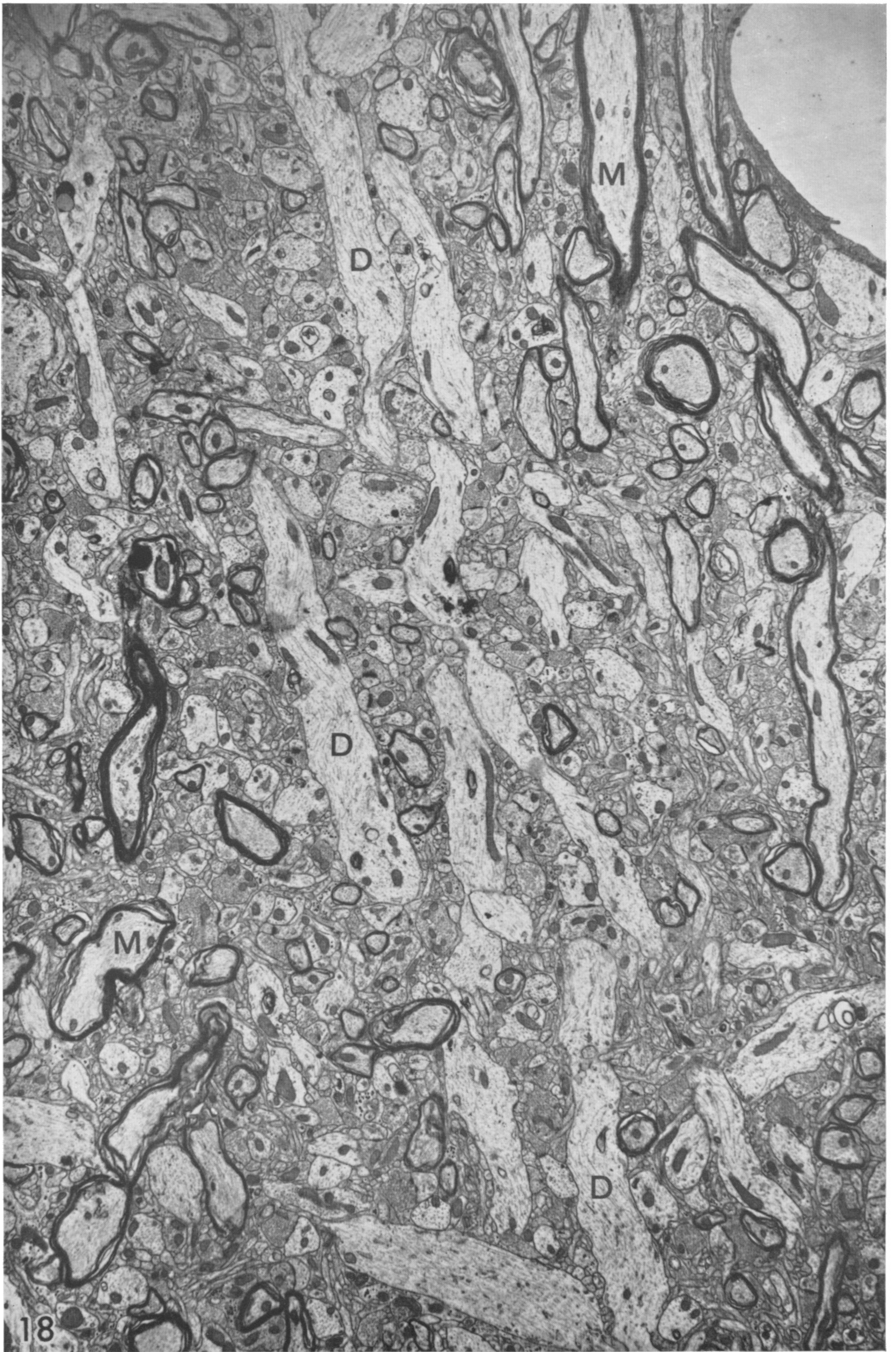FIGURE 18. Layers V and VI, showing ascending dendrites (D) and large myelinated axons (M). Perpendicular section. ×6000.
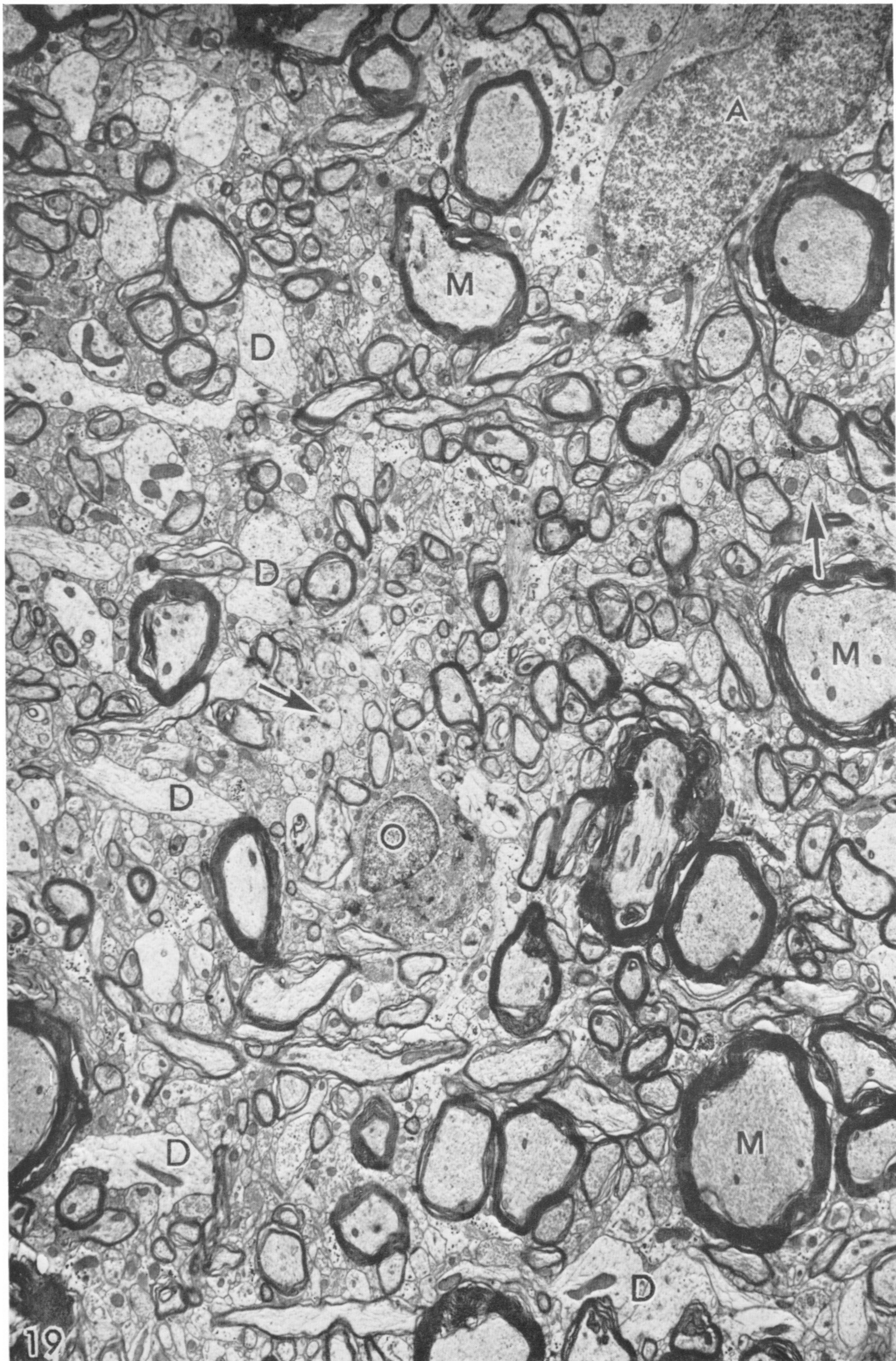
FIGURE 19. The deepest part of layer VI in a section cut parallel to the surface. The dominant features are the presence of very large myelinated axons (M) astrocytes (A) and oligodendrocytes (O), but a few dendrites (D) and an occasional dendritic spine (arrow) are also present. × 6000.