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# THE SYNAPTIC ORGANIZATION OF THE CAUDATE NUCLEUS

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[Plates 62 to 66]

The synaptic organization of the caudate nucleus appears to be homogeneous with no specialized groupings of axon terminals and postsynaptic profiles. The nerve terminals in the caudate nucleus fall into two size groups, one about 1 $\mu$m in diameter and the other about 5 $\mu$m diameter. The smaller size group, which comprises the majority of terminals, may be subdivided into three varieties on the basis of vesicle morphology and the type of membrane thickening. Most contain round, 45 nm diameter vesicles and are associated with asymmetrical membrane thickenings. Others contain 48 nm diameter, polymorphic vesicles, which become flat in material washed in cacodylate buffer, and are associated with symmetrical membrane thickenings. A few other terminals with symmetrical membrane thickenings contain flat 42 nm vesicles. The large terminals contain round 45 nm diameter vesicles and have asymmetrical membrane thickenings. Axon terminals with asymmetrical membrane thickenings are found most frequently in contact with dendritic spines, but also with dendritic shafts and cell somata. Terminals with symmetrical membrane thickenings contact dendritic shafts and cell somata, and occasionally dendritic spines with which a terminal with asymmetrical thickenings is also making contact. The two types of terminal with symmetrical contact regions also form synapses onto the initial segments of axons. It is probable that such contact regions are invariably associated with cisternal organs in the initial segments. Serial synapses are found occasionally.

## Introduction

The general appearance of the caudate nucleus and the cell types present, as seen with the electron microscope and in Golgi impregnated material, have been described in the preceding paper. The present study is a continuation of the earlier one and is concerned with the details of the synaptic organization of the same part of the brain. In particular, attention has been concentrated upon those aspects which could help in the interpretation of the findings of the experimental investigations on the termination of the afferent fibres to the striatum. It is known that synapses may differ significantly in certain features of their morphology, such as the type of membrane thickening (Gray 1959; Colonnier 1968), the size of the axon terminal, and the shape and size of the synaptic vesicles; in addition, there may be a preferential distribution of certain types of synapse upon different parts of the neuron. As in the preceding paper, a number of observations made with the electron microscope have been confirmed and extended by examination, with the light microscope, of similar material impregnated with the Golgi method.

## Material and methods

The material and methods are mainly those used in the preceding paper on the cell types. In addition some of the blocks taken for the electron microscopic study were washed in cacodylate buffer in 10 % sucrose instead of phosphate buffer in 10 % sucrose. Two animals were perfused with the paraformaldehyde-glutaraldehyde mixture with the osmolarity raised from 1800 mosmoles to about 2000 mosmoles by the addition of sucrose.

## Results

The high concentration of non-myelinated fibres is one of the characteristic features of the caudate nucleus. They are smaller than most of the myelinated fibres being between 0.3 and 0.5 $\mu$m in diameter (figures 2 and 3, plate 62). Isolated finer non-myelinated axons, which may be less than 0.1 $\mu$m in diameter, are also present, and in some small areas of the nucleus, appear to be concentrated to form a dense interlacing network (figure 3, plate 62); some of these fibres branch. It is not possible to identify the source of these fibres in normal material and it is probable that some, arising from the intrinsic cells, never have a myelin sheath. However, some myelinated fibres have been seen losing their myelin sheath and subsequently travelling distances of over 10 $\mu$m and forming several terminals.

In the aldehyde-fixed material used exclusively in this study, it has been found that the caudate nucleus contains several types of terminal, and they can be clearly divided into two groups on the basis of their size: while most endings are about 1 $\mu$m in diameter a few are about 5 times larger (figures 4 and 6, plates 63 and 64).

The cytoplasm of the smaller endings is a little darker than that of most dendrites and the synaptic vesicles are usually distributed throughout the terminal, and though there is some increase in their density near the synaptic thickening there is no evidence of the clumping associated with ischaemia (Williams & Grossman 1968). They usually contain one or two mitochondria. The form and size of the vesicles and the type of membrane thickening can be used to characterize the terminals more precisely into three varieties. Most of the endings contain rounded vesicles of about 45 nm (figures 5, 6, and 8, plates 63, 64 and 65). The synaptic thickenings show a marked asymmetry in that the thickening associated with the postsynaptic region is greater than that on the presynaptic side (Colonnier 1968) though the latter may have presynaptic dense projections (Gray 1963; Pfenninger, Sandri, Akert & Eugster 1969); this type of membrane thickening is equivalent to the Type I of Gray (1959). The postsynaptic thickening is usually continuous, and is less obvious in cell somata and dendrites than in spines; in the latter the membrane thickening may also be interrupted, and the unthickened piece of the membrane of the spine sometimes forms a peg which protrudes for a variable distance into the terminal. Two other varieties of small terminal are associated with pre- and postsynaptic thickenings of about equal density, forming a symmetrical synaptic region (Colonnier 1968) and correspond to Type II of Gray (1959) (figures 5 and 8, plates 63 and 65). In most of these endings, in material washed in phosphate buffer, the vesicles are rounded or polymorphic and about 48 nm in diameter and so larger than those associated with asymmetrical membrane thickenings (figure 8, plate 65). The rarest type of ending has vesicles of less than 42 nm (mean diameter) and many of them are flattened (figure 5, plate 63); the number of vesicles in these terminals is often lower than in the other types of small terminals and occasionally there are only a few in the region of the synapse, the rest of the terminal being empty. Any of these terminals may contain one or two dense cored vesicles, approximately 100 nm in diameter (figure 6, plate 64). The relative numbers of the three types of small terminal are shown in table 1.

The vesicles in the two types of endings associated with symmetrical membrane thickenings appear to be similar to the large polymorphic and small flat vesicles described by Bodian (1970) in the spinal cord of the monkey. This similarity is increased by the response of the larger vesicles to washing in cacodylate buffer, instead of phosphate buffer, prior to osmification.


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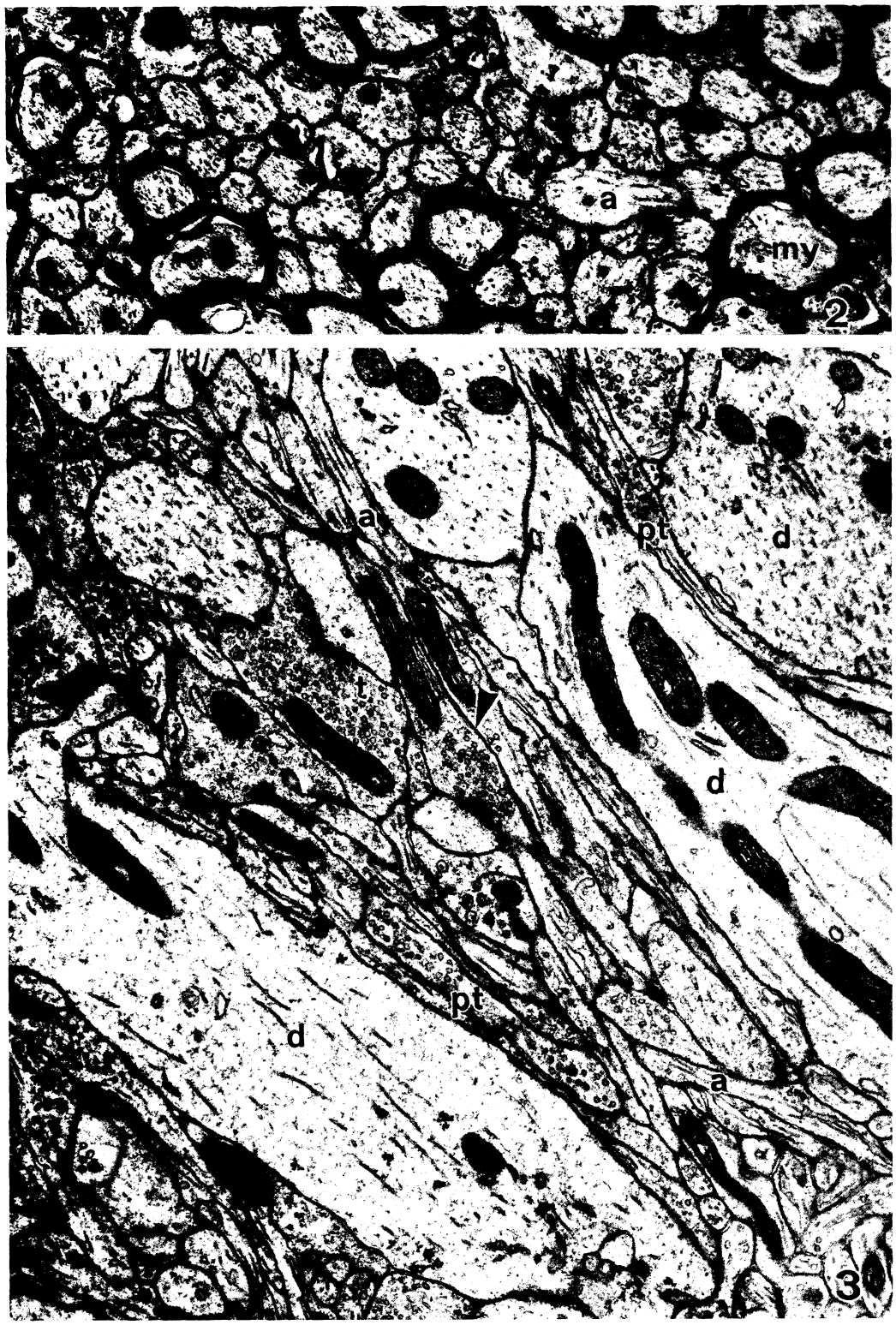


FIGURE 2. An area of myelinated (my) and nonmyelinated (a) nerve fibres. × 16000.

FIGURE 3. Group of fine nonmyelinated axons (a), preterminals (pt) and terminals (t) between and on either side of the two dendrites (d). Note the terminal *en passant* (arrow head). × 20000.

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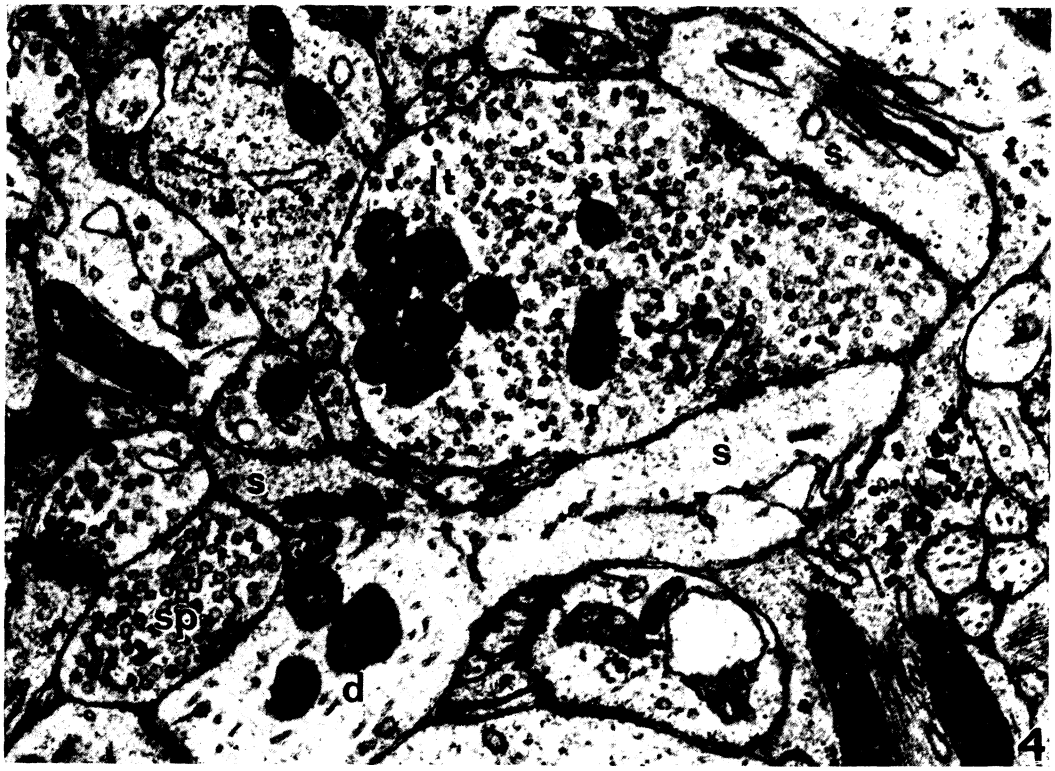


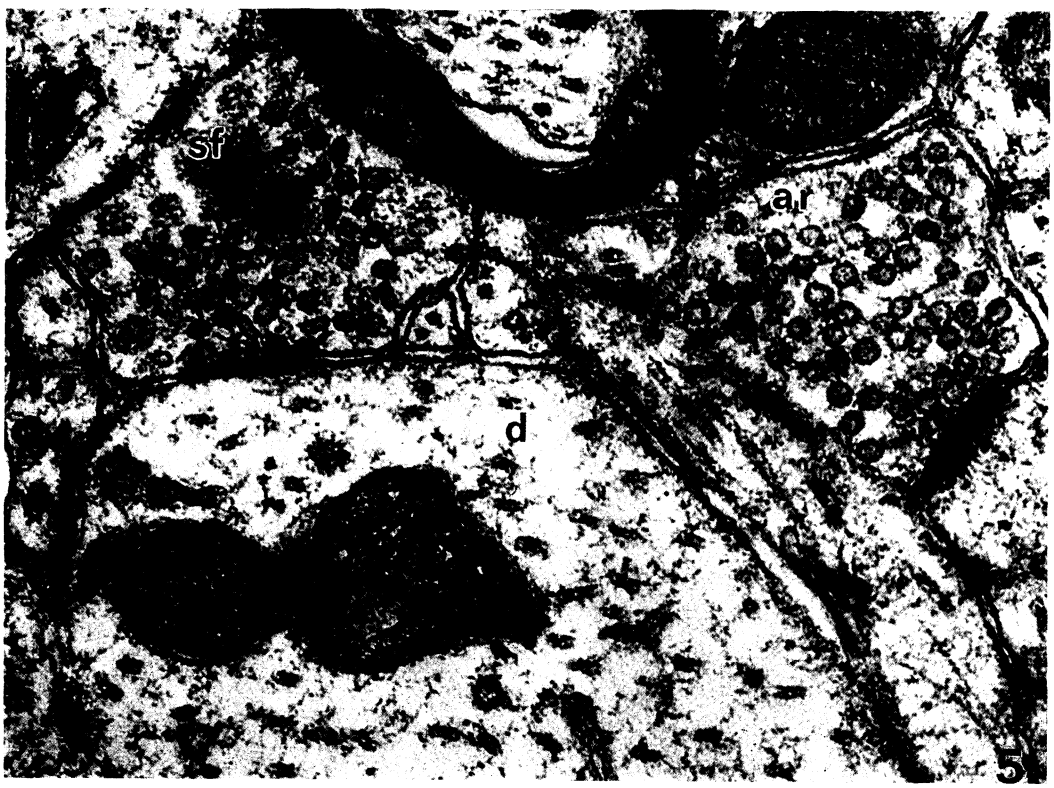


FIGURE 4. A dendrite (d) with a symmetrical synaptic contact from a small terminal with large vesicles (sp). Two spines (s) arise from the dendrite; one receives a contact with asymmetrical membrane thickenings from a large terminal (lt) which also contacts another spine (s). × 30 000.

FIGURE 5. Terminal containing small flat vesicles (sf) forming a contact with symmetrical membrane thickenings on a dendrite (d). Compare the size of these vesicles with those in the adjacent terminal (ar) which forms an asymmetrical synaptic contact. × 86 000.


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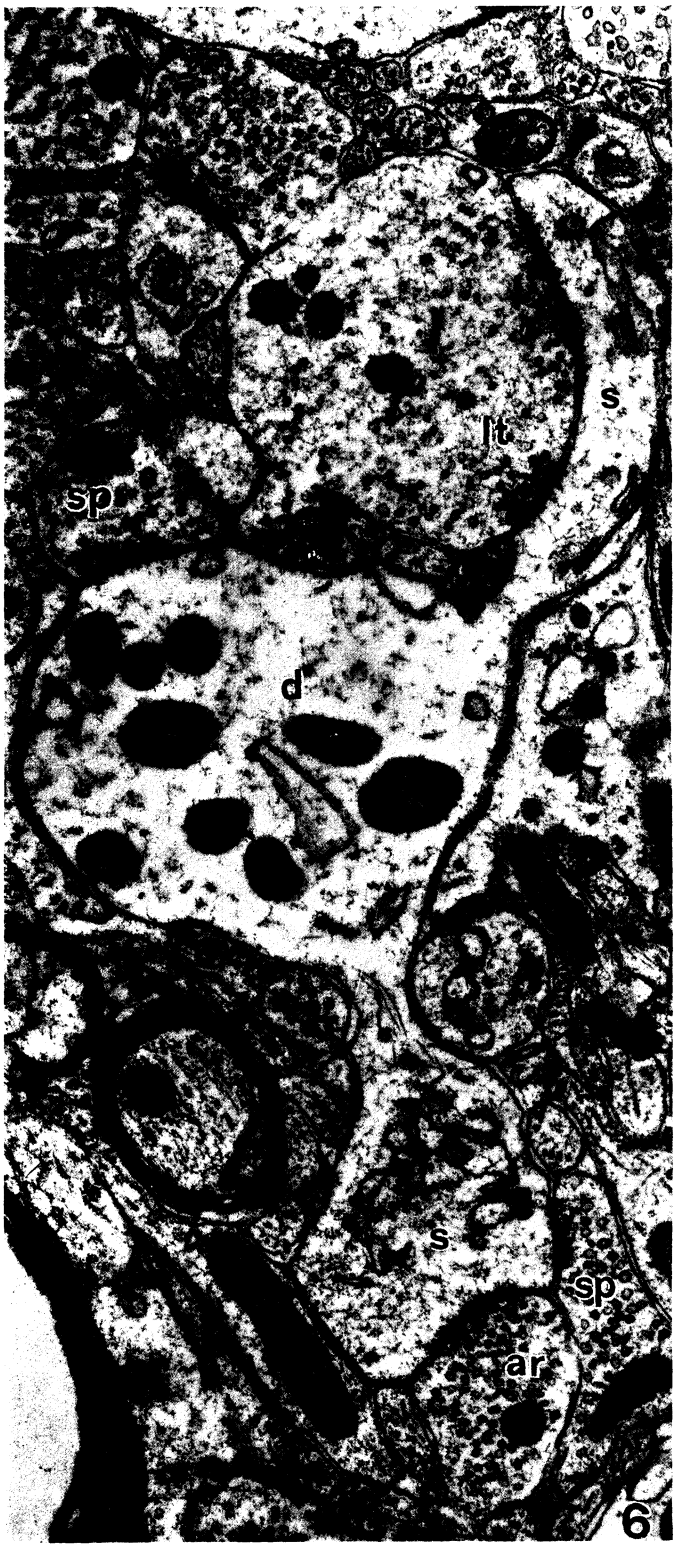



FIGURE 6. The peripheral dendrite (d) of a medium spiny cell with two spines (s). Small terminals with symmetrical membrane thickenings (sp) and large vesicles contact the dendrite and one spine which also receives a small terminal with asymmetrical membrane thickenings (ar). The second spine has a synaptic contact with a large terminal (lt). × 28 000.

FIGURE 7. The initial segment of an axon (is) with a small branch (b) and a cisternal organ (co) which extends beneath two synaptic contacts. One terminal (sp) contains large polymorphic vesicles and the second (sf) small flat vesicles. Note the alveolate vesicles (barred arrows). × 18 000.


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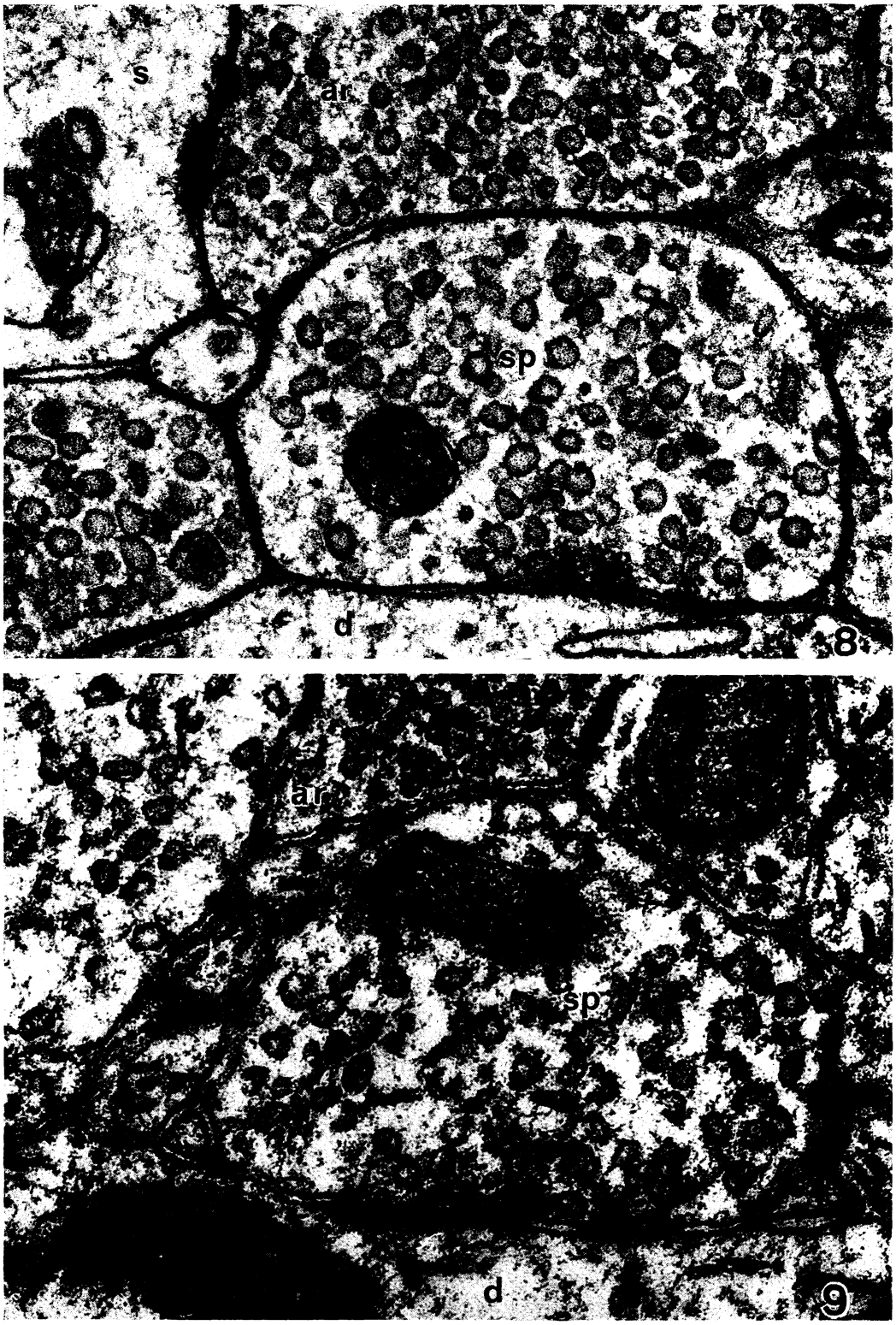


FIGURE 8. Terminal with large polymorphic vesicles (sp) forming a synapse with symmetrical membrane thickenings onto a dendrite (d). Compare the size of the vesicles with those in the terminal above (ar) which has an asymmetrical contact region. × 88 000.

FIGURE 9. Terminal with a symmetrical contact region containing large vesicles (sp) after treatment with cacodylate buffer. Compare these vesicles with the round vesicles in the adjacent pre-terminal above (ar). × 88 000.


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Bodian reports that this procedure produced flattening of a large proportion of the larger vesicles in the monkey spinal cord and a similar effect has been found in the caudate nucleus of the cat (figure 9, plate 65). The cacodylate treatment had no effect on the rounded vesicles in the terminals with asymmetrical membrane thickenings nor apparently on the flat vesicles in the symmetrical endings. It has also been reported that increase in the osmolarity of the perfusing fluid produces selective flattening of the vesicles in terminals with symmetrical membrane thickenings (R. W. Guillery, personal communication) and the effect of adding sucrose to the perfusion fluid was investigated. The osmolarity of the formaldehyde–glutaraldehyde mixture which has been used routinely in this investigation is about 1800 mosmoles and in order to

TABLE 1. DISTRIBUTION OF TYPES OF TERMINAL ON SPINES AND SEGMENTS

| type of terminal | proportion of total terminals (%) | proportion of terminal types on spines (%) | proportion of terminal types on dendrites (%) |
|---|---|---|---|
| asymmetrical round vesicles | 85 | 84 | 16 |
| symmetrical large vesicles | 6 | 6 | 94 |
| symmetrical small flat vesicles | 4 | 38 | 62 |
| unidentified | 5 | — | — |

Total number of terminals counted: 1933.
Terminals with asymmetrical membrane thickenings have not been separated into the large and small types as some of the small endings may be part of large ones.

determine the effect of raising the osmolarity it was increased to 2000 mosmoles. In material fixed with the mixture of higher osmolarity the larger vesicles in the terminals with symmetrical membrane thickenings were flattened, but the degree of change was less marked and less specific than with the cacodylate treatment as the vesicles in the terminals with asymmetrical thickenings also tended to become a little flat; furthermore, there was an increase in the extracellular space of the tissue. It has recently been reported (Valdivia 1970) that the critical factor in flattening vesicles with solutions of high osmolarity is the increase in the osmolarity of the buffer rather than the addition of sucrose to the perfusate, and this finding may explain the comparative lack of specificity in these results.

The other main variety of terminal, which may reach 5 $\mu$m in diameter, often appears paler than the small terminals as the synaptic vesicles are more widely dispersed or grouped near the synaptic complexes leaving part of the profile free of vesicles (figures 4 and 6, plates 63 and 64). These endings contain many mitochondrial profiles, often as many as eight or nine. The membrane thickenings in the synaptic complex are asymmetrical and the vesicles are about 45 nm in diameter and rounded; dense cored vesicles of about 100 nm diameter are also present. These terminals are probably more common than at first appears for unless the section passes through the wide part of the terminals or contains many mitochondria they are not easily differentiated from the small terminals. Any of the types of terminals may be *en passant*, and such endings can be identified most often by the neurotubules passing through the vesicle filled enlargement. Sometimes lengths of axon can be seen joining the dilated regions.

The most common form of synaptic relationship is between a small terminal with asymmetrical membrane thickenings and a spine (table 1). There is usually only one terminal of this type ending on a spine; in cases where a second terminal does make synaptic contact it is more frequently a type forming a symmetrical synapse and is usually but not always closer to the dendritic shaft than the asymmetrical contact (figure 6, plate 64). Axodendritic asymmetrical

synapses are relatively infrequent on all types of dendrite (table 1) except the varicose forms. On the latter they are the commonest form of synaptic contact and their concentration tends to be higher on the swollen portion of the dendritic shaft. There are also relatively few axosomatic asymmetrical contacts, but they terminate on all varieties of neuron and though these are not shown in the table they probably represent about 20 % of the terminals onto cell bodies. The number of axosomatic contacts of any type, however, is low and about 40 % of the profiles of cell somata have no synapses. Of the endings forming symmetrical synaptic contacts more contain the large polymorphic vesicles, and most of these terminate on dendrites and cell bodies of all varieties. Terminals containing small flattened vesicles also end on dendrites and cell bodies of all types, but a significant number also end on spines (table 1).

Only symmetrical terminals are present on axon initial segments and may be either of the large polymorphic vesicle (figures 11 to 13, plate 66), or the small flattened vesicle type (figure 14, plate 66), though there are fewer of the latter, and both kinds of symmetrical terminal may be present on one initial segment (figure 7, plate 64). The concentration of synaptic contacts on initial segments is high and in one series through part of an initial segment 15 $\mu$m long, close to the cell body, nine such contacts were seen. It has not been possible to ascertain whether or not there is a concentration difference in the number of synapses along the length of the initial segment as has been reported in the cerebral cortex (Jones & Powell 1969). Serial sections through about 30 synaptic contacts have shown that a very high proportion are associated with the cisternal organ, and it seems possible that such a relationship is invariable (figures 7 and 11 to 14, plates 64 and 66). In the few cases where this relationship was not observed sections were not available through the whole length of the cisternae, but as the synapse may lie over only part of the organ the finding is inconclusive (figures 12 and 13, plate 66). Other examples occurred where the cisternae lay near the surface of a profile being sectioned tangentially so that identification of synaptic contacts was impossible. In situations where the cisternal organ lies within a bulge which also receives a synapse the complex becomes very similar to a sessile dendritic spine (figure 14, plate 66) (Westrum 1970). The cisternal organ may also lie beneath a projection which receives a synapse or has one immediately adjacent and here the cisternae appear too large to fit into the spine (figure 11, plate 66). A third relationship which has been seen is one in which there does not seem to be any projection from the initial segment and the cisternal organ lies in the main part of the profile with the synapse occurring over some part of the length of the cisternae (figures 7, 12 and 13, plates 64 and 66).

Alveolate vesicles (Palay 1963) are also a common feature of initial segments. They are present in the cytoplasm at any point along their length and also opening on to the plasma membrane where they appear to be in continuity with the extracellular space (figure 11, plate 66).

Quite often any of the types of small terminals forms synapses with more than one postsynaptic process. The commonest of these combinations is with two spines and the spines can arise from different dendrites. Other processes receiving a common terminal may be a spine and a dendrite, a spine and a cell body, a cell body and a dendrite or the initial segment of an axon and a different cell body. Terminals *en passant* also join any of these processes but have not been seen lying in parallel with a dendrite and only very rarely form adjacent synapses onto a cell soma.

Large terminals most frequently synapse onto dendritic spines, and several spines may be associated with one such ending. Very rarely a large terminal contacts a dendrite.

A very uncommon relationship is one in which a terminal forms an asymmetrical synapse with a vesicle-containing profile (figure 10, plate 66). The vesicles in the second profile are

usually larger than those in the first, but whether they are similar to the large polymorphic vesicles is not known and their response to cacodylate has not been observed. Occasionally, however, the second profile contains small flat vesicles. Either type of second terminal may synapse onto a further structure, and in two cases this has been identified as the dendrite of a medium spiny cell and a varicose dendrite respectively. The second synapse in all cases seen has been symmetrical.

## Discussion

This study has shown that the synaptic organization of the caudate nucleus is as homogeneous as the distribution of the constituent neurons and their processes for there are no specialized groups of axon terminals and postsynaptic processes. This homogeneity is emphasized by the finding that all types of small axon terminal contact all possible varieties of dendrite and cell body (figure 1) and, with one exception, that of the varicose dendrite, there is no obvious difference in the proportion of terminals on these two major parts of the neuron. Whether or not the separation of cells into six types on morphological grounds is valid, when they appear to receive a similar range of terminals, is open to question. However, it is probable that some variation in the ratios of the types of terminal on the different cells is present, but this is not readily shown by electron microscopy which allows only a very small portion of the cell to be

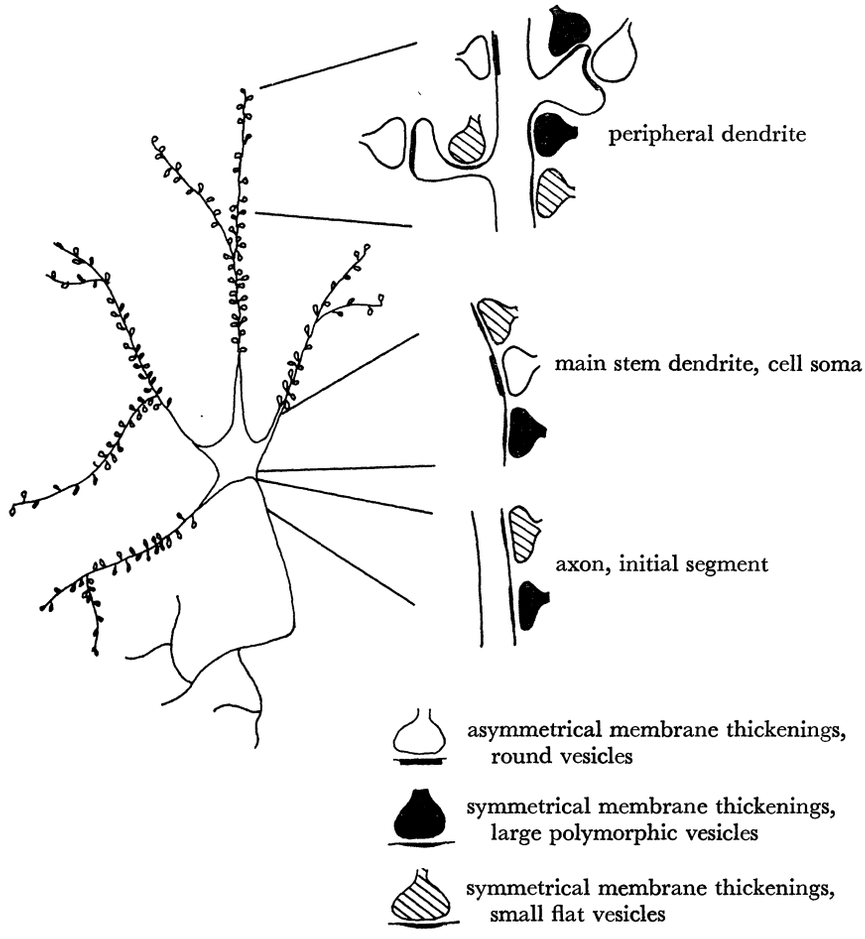


Figure 1. Schematic figure to show the distribution of the different types of axon terminal on the medium spiny cell of the caudate nucleus.

studied at any one time. One difference in the distribution of the varieties of terminal on different cell types does occur. Study of the Golgi impregnated material has shown that only one cell type has a large number of spines, the rest having few or none. Since spines invariably receive a terminal associated with asymmetrical membrane thickenings, the number of terminals of this type will be less on cells with few spines, as the majority of terminals in contact with dendritic shafts end with symmetrical membrane thickenings. The one exception to this latter observation is the varicose dendrite, mentioned above, where terminals with asymmetrical membrane thickenings, at the synapses onto dendrites, predominate.

Rows of synaptic knobs, such as those seen in the globus pallidus (Fox, Hillman, Siegesmund & Sether 1966; Adinolfi 1969; Kemp 1970) are not present in the caudate nucleus (Mori 1965, 1966; Adinolfi & Pappas 1968; Kemp 1968). Golgi impregnation of the nucleus complements this finding for axons rarely lie in parallel with dendrites or cell bodies but cross from one dendrite to another of the same or different cells. This observation, together with the widespread branching of the incoming fibres (Cajal 1911), suggests that a single afferent fibre may have a very wide sphere of influence, and this distribution could be the basis for the considerable overlap of the projections from the adjoining areas of the cerebral cortex found in the monkey (Kemp & Powell 1970).

The dense-cored vesicles which are present in a large proportion of the terminals are of about 100 nm diameter. Though the caudate nucleus has a high concentration of dopamine (Hornykiewicz 1963) it seems unlikely that these particular vesicles are associated with catecholamines as they are often present in the glial processes as well as nerve cell bodies and may be present in regions of the brain where no catecholamine has been demonstrated (Sotelo & Palay 1970). Permanganate fixation, which is usually employed to demonstrate catecholamine containing vesicles, has not been used in this study but numerous dense-cored vesicles have been shown in the nerve terminals in the striatum using this method (Hökfelt 1967).

Symmetrical synapses in the caudate nucleus are associated with two different varieties of synaptic vesicle. The flattened vesicle, which is smaller than the round vesicle associated with asymmetrical membrane thickenings, is similar to those described in other regions of the nervous system (Uchizono 1965, 1967; Walberg 1966; Bodian 1966). The other type differs from the first in that they are larger and polymorphic in material washed in phosphate buffer. The interesting feature of these large vesicles is that they become flattened if the tissue is washed in cacodylate buffer and are thus very similar to the large type of vesicles described by Bodian (1970) in the spinal cord of the monkey. It seems highly probable that the flattening of vesicles

## Description of plate 66

Figure 10. A serial synapse. The first terminal ($t_1$) synapses with asymmetrical membrane thickenings onto a structure containing larger vesicles ($t_2$) which in turn contacts a third profile which contains no vesicles. The second synaptic region has symmetrical membrane thickenings (arrow). × 45000.

Figure 11. A terminal with large vesicles (sp) in contact with an initial segment (is). The adjacent protuberance has floccular cytoplasm and a cisternal organ lies under the symmetrical contact region. Note the alveolate vesicles (barred arrows). × 48000.

Figure 12. An initial segment (is) which contains two cisternal organs (co) the lower of which is associated with a synaptic contact (sp). × 46000.

Figure 13. Four serial sections beyond figure 12. Both cisternal organs are still present (co) but the synaptic contact (sp) is associated with the upper and not the lower cisternal organ. × 46000.

Figure 14. A cisternal organ (co) lying within a protuberance on an initial segment. The terminal on to the bulge contains small flat vesicles (sf). × 48000.


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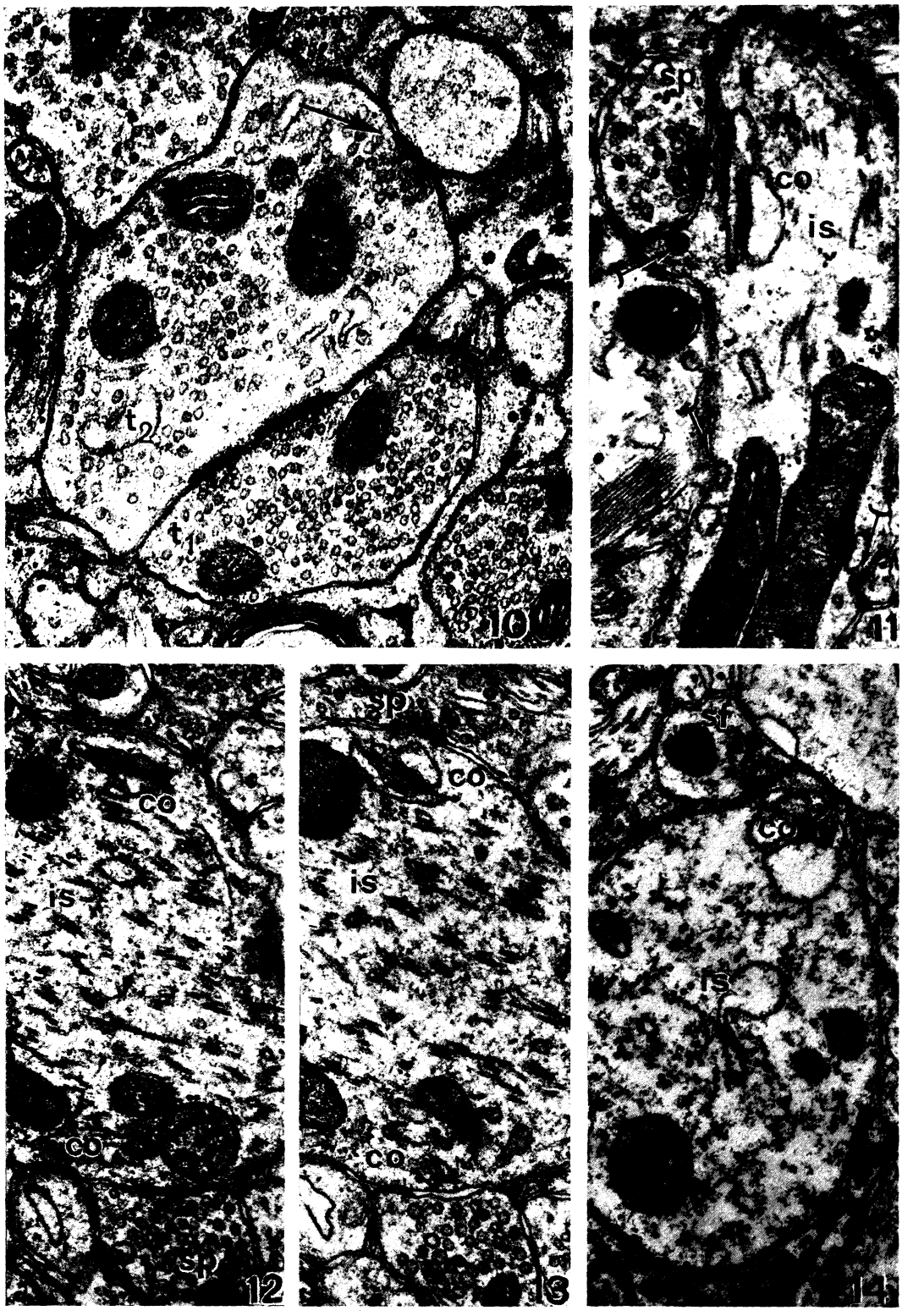


FIGURES 10 to 14. For legends see facing page.

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is an artefact since it appears only in aldehyde-fixed material (see Walberg 1968); the fact that in both the cord and the caudate nucleus it is the large vesicles associated with symmetrical thickenings that are affected by cacodylate buffer may be significant. The flattening of a particular type of vesicle with cacodylate buffer suggests that these vesicles, though less susceptible than some to the influence of aldehydes, are more so than those associated with asymmetrical membrane thickenings and lends support to the idea that the effect is artefactual. The influence of increasing the osmolarity of the perfusing medium is additional support for the hypothesis that vesicle shape is dependent upon the imposed conditions. That flattened vesicles are found more readily in relatively superficial parts of the brain such as the olfactory bulb (Price 1968), cerebral cortex (Colonnier 1968) and cerebellum (Uchizono 1965) rather than in deeply situated regions suggests that the speed of penetration of the fixative and the blood supply to the region may also play a part in the effect. However, in many respects this point is of academic interest and its significance lies in its great practical value since it allows certain types of terminal to be more precisely defined.

Hirata (1966) found that 25 % of the endings in the caudate nucleus contain flattened vesicles. This number is greater than that seen in our material even if terminals containing vesicles flattened by cacodylate buffer treatment are included, making a total of 10 % with flattened vesicles. The difference may be due to a slight difference in the composition of the fixative or in the procedure following the initial fixation, but may also be due to sampling error in either case. It has been our experience that a very large sample is required from material studied with the electron microscope in order to obtain consistent quantitative data.

It has been suggested that flattened vesicles are related to inhibitory processes in several regions of the nervous system (Uchizono 1965; Larramendi, Fickenscher & Lemkey-Johnston 1967; see also Gray 1969). A direct correlation is not possible in the caudate nucleus for the cells from which a particular response is being recorded cannot be identified. It is interesting, however, that excitatory and inhibitory responses can be recorded within the nucleus with microelectrodes after stimulation of the cerebral cortex (Rocha-Miranda 1965; Sedgwick & Williams 1967; Vernon, Hull, Bernardi & Buchwald 1969), thalamus (Purpura & Malliani 1967; Vernon *et al.* 1969) and substantia nigra (Frigyesi & Purpura 1967; Connor 1968). The responses are of varying latencies and in general the short latency response is excitation or facilitation of spontaneous firing or firing induced by application of homocysteic acid. The second, long latency response, suggestive of a multisynaptic pathway, is usually inhibitory. These observations may be correlated with the finding that afferent fibres to the caudate nucleus all terminate with asymmetrical membrane thickenings associated with round vesicles (Kemp 1968) while terminals with symmetrical membrane thickenings and flat or polymorphic vesicles are all terminals of the intrinsic cells of the caudate nucleus (following paper, Kemp & Powell 1971).

In the caudate nucleus it is not uncommon to find spines which receive both asymmetrical and symmetrical terminals so that this region represents a site of integration of information from different sources. Diamond, Gray & Yasargil (1970) suggest that the significance of the spine may be to isolate its synaptic region from other synapses on the neuron, and if this is so this part of the neuron may represent the first stage of dendritic integration, the second occurring on the peripheral parts of the dendrite while the initial segment of the axon is a final site. Synaptic contacts onto the initial segment are common in the caudate nucleus, and although it is our impression from the examination of serial sections that initial segments of all neurons in the caudate nucleus have such synapses upon them it is not possible to be definite on this




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point without a fuller study. Though only symmetrical contacts have been seen on this part of the cell (Jones & Powell 1969; Westrum 1970) terminals forming the synapses may contain either large polymorphic or small flat vesicles. This indicates that the synaptic influence is from two types of cell and that the integration here is also relatively complex. A larger study might reveal that initial segments arising from the various cell types might show differences in the proportions of the two kinds of endings contacting them or in the number of synaptic contacts which they receive.

From the observations in the present material, with the use of serial sections, it seems probable that the cisternal organ is present in the initial segments of most neurons of the caudate nucleus and also that it is invariably related to a synapse. This relationship to a synapse, together with the fact that spines are present on the dendrites of the majority of neurons in this nucleus may be considered to support the suggestion that cisternal organs are present in initial segments of neurons which have dendritic spines with spine apparatus (Peters, Proskauer & Kaiserman-Abramof 1968; Westrum 1970). The possibility of an association between the cisternal organ and the spine apparatus is based upon the similarity in their structure and upon the finding of cisternal organs in pyramidal neurons of the cerebral cortex which also have a large number of dendritic spines with well developed spine apparatus. Before firmer conclusions can be drawn and it is possible to speculate usefully about the functional significance of cisternal organs, however, certain points about their distribution will require clarification. Although cisternal organs have been described in the initial segments of neurons in the cortex of the cerebral hemisphere (Peters *et al.* 1968; Jones & Powell 1969; Westrum 1970), and now in the striatum they appear to be absent from neurons in other parts of the central nervous system (see Peters *et al.* 1968; Conradi 1969; Westrum 1970; Price & Powell 1970), only some of which do not have dendritic spines. In their study of pyramidal neurons of the neocortex Peters *et al.* (1968) considered that the cisternal organ was absent in the majority of profiles of initial segments, whereas in the same site (Jones & Powell 1969) and in olfactory cortex (Westrum 1970) it has been seen frequently. There is also the statement of Peters *et al.* (1968) that there is no apparent relation between the site of the cisternae and the position of a synapse although the present findings and those of Westrum (1970) indicate that they are frequently, at least, associated. Whether or not these discrepancies are genuine or are only apparent and due to the lack of study of initial segments with serial sections cannot be stated. It would appear, therefore, that there is a need to determine, as conclusively as possible with the use of serial sections, whether the cisternal organ is present in the initial segments of neurons from only certain parts of the nervous system and whether it is constantly present in neurons of a particular type; the relationship of the cisternae to synapses on the initial segments and to the presence of dendritic spines and spine apparatus in the same neuron. Is it possible, for example, that the cisternal organ is present only in initial segments which have synapses upon them (as is suggested by the absence of both cisternae and synapses in motoneurons of the spinal cord (Conradi 1969)) and in mitral cells of the olfactory bulb (Price & Powell 1970), or only in neurons which have an appreciable number of dendritic spines and well developed spine apparatus; and is the dense material of the cisternal organ stained by bismuth iodide or phosphotungstic acid as is that of the spine apparatus (Gray & Willis 1970; Adinolfi 1971)?

The alveolate vesicles which are a common feature of the axon initial segment (Conradi 1969; Price & Powell 1970) are also seen in relatively large numbers close to the Golgi apparatus in the perikarya of neurons. They are also occasionally present in dendrites and dendritic

spines. The large number of alveolate vesicles in initial segments is interesting in view of the recent suggestion that they may be associated with synaptic vesicles (Kanaseki & Kadota 1969; Gray & Willis 1970). That many of them apparently open onto the plasma membrane is also worth noting although the significance is unknown. In view of the hypothesis recently proposed by Gray & Willis (1970) that the shell of complex (or alveolate) vesicles in axon terminals contributes to the formation of the presynaptic dense projections it is tempting to speculate that the relative frequency of alveolate vesicles in the initial segments (Conradi 1969; Price & Powell 1970) and nodes of Ranvier (Andres 1965; Karlsson 1967) may be related to the dense undercoating of the plasma membrane at these two sites.

The rarity of serial synapses in the caudate nucleus suggests that the processes of one of the least common cells is involved. There is, however, no evidence to suggest which of the cells might be implicated though the small cell is the rarest. The final process in the complex has been identified in only two cases, and in one has been a varicose dendrite and in the other the dendrite of a medium spiny cell; other examples suggest that the final process may be a spine. In view of this evidence the element of the complex which is least common in the nucleus is probably one or both of the presynaptic processes. It is not possible to identify these positively as axons or dendrites. Dendrites containing vesicles have been described in a number of regions of the central nervous system; they are common, for example, in the superior colliculus (Lund 1969) and in the olfactory bulb (Price & Powell 1970) and Ralston & Herman (1969) consider that some of the vesicle-containing structures in the thalamus are dendrites. The only positive method of solving the problem in the caudate nucleus would be to take serial sections through the structure back to the cell soma.

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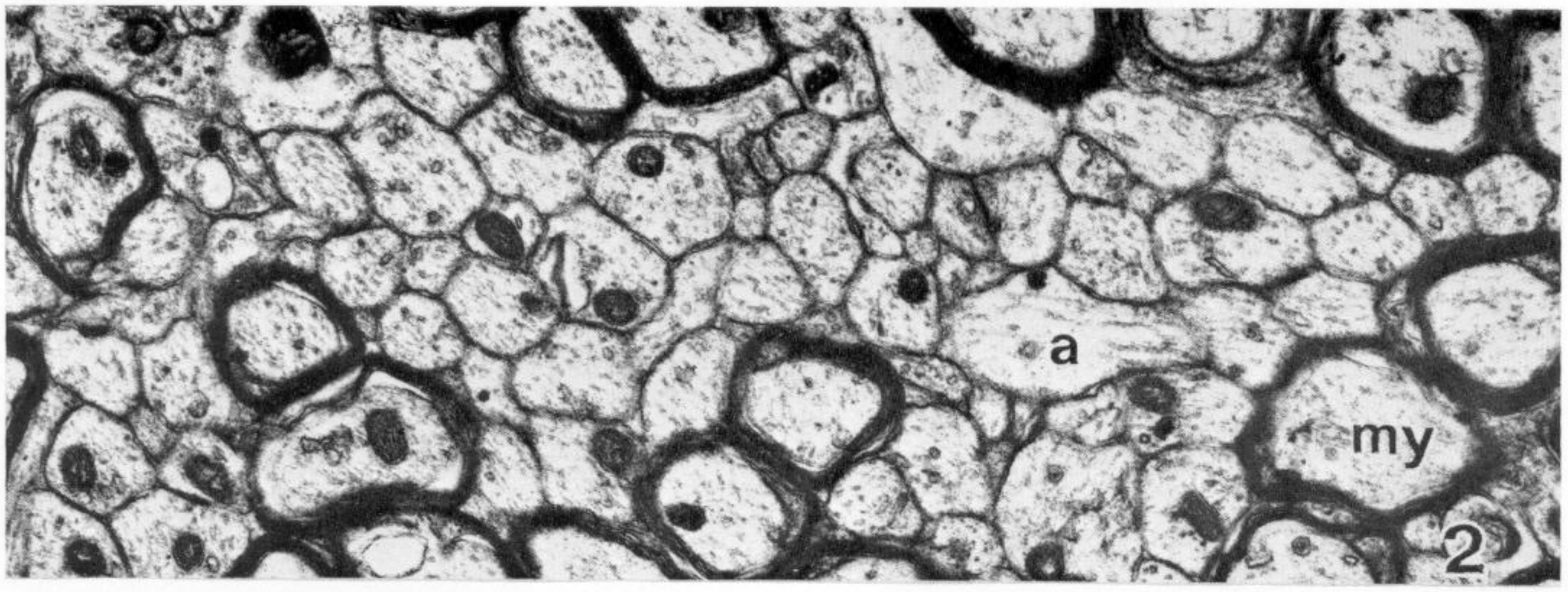


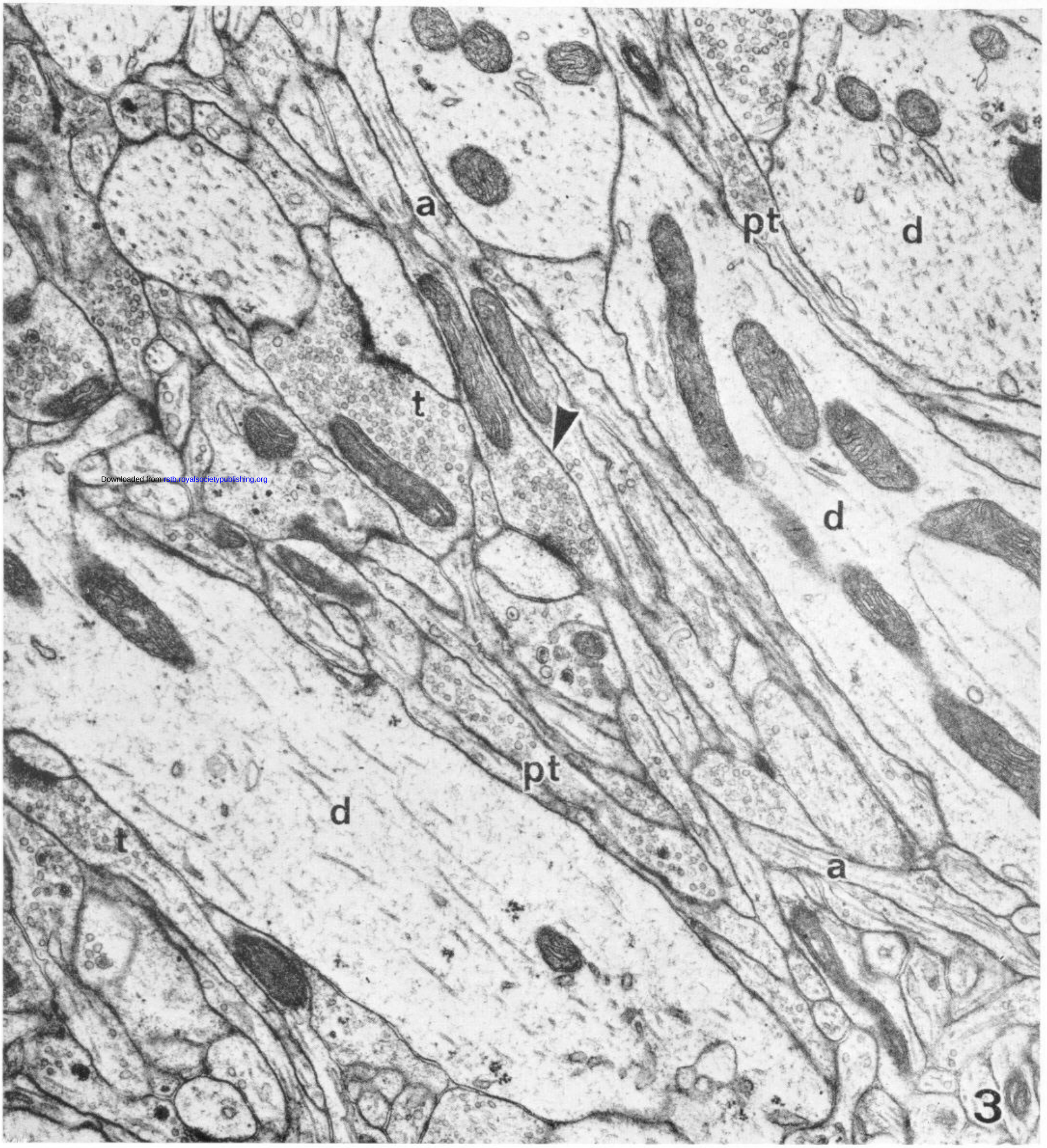






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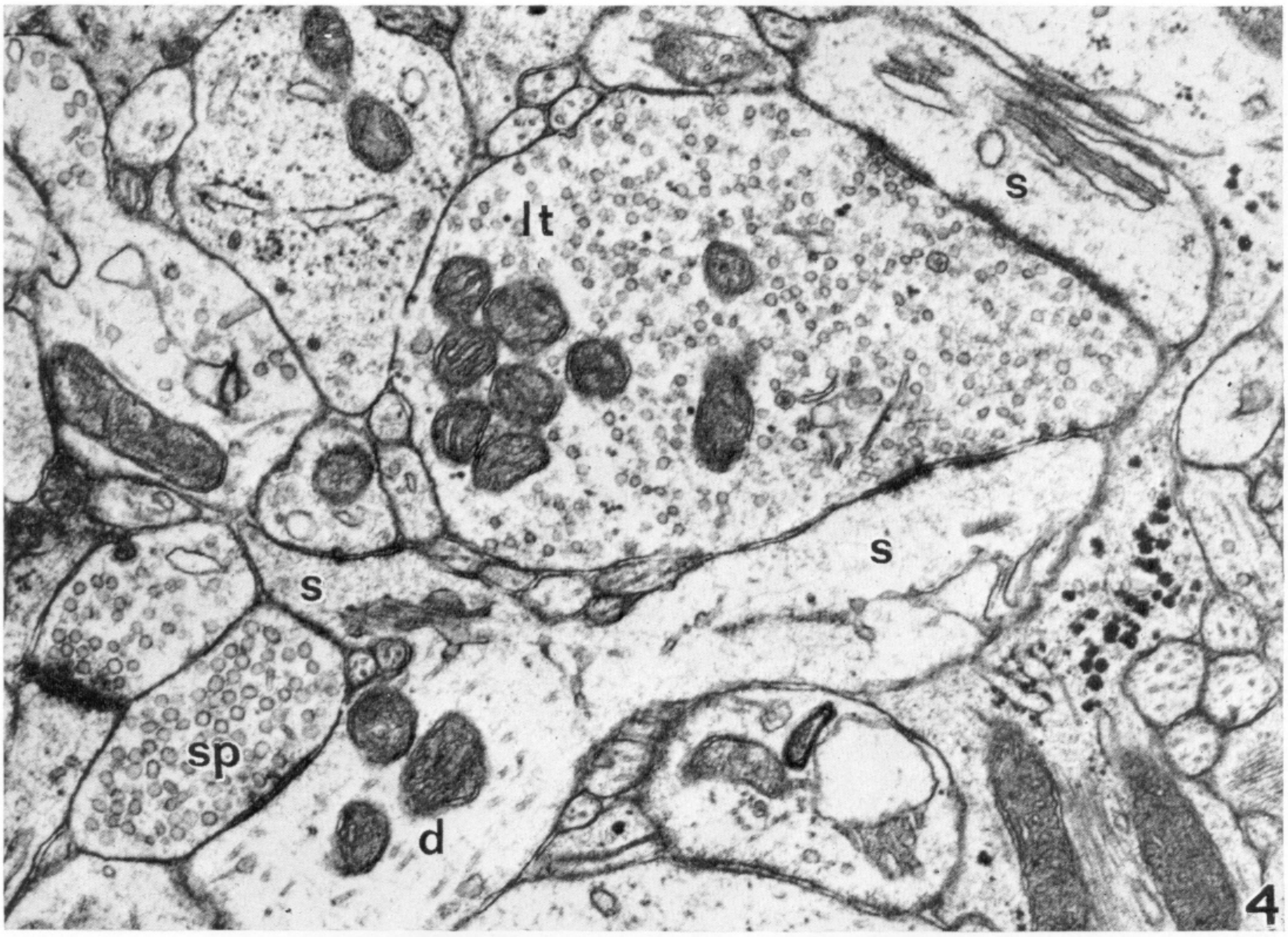
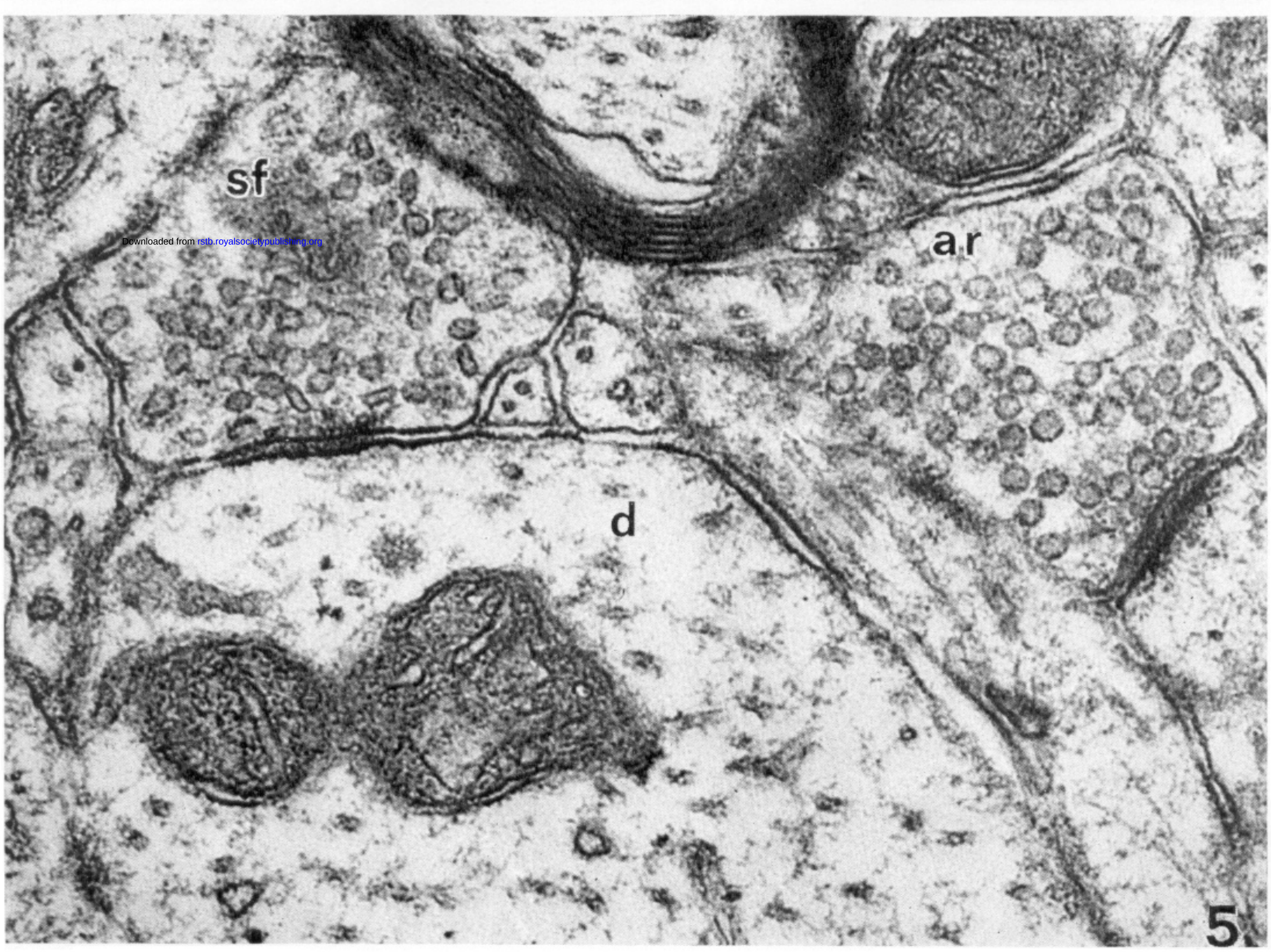
IGURE 2. An area of myelinated (my) and nonmyelinated (a) nerve fibres. × 16000.

IGURE 3. Group of fine nonmyelinated axons (a), preterminals (pt) and terminals (t) between and on either side of the two dendrites (d). Note the terminal *en passant* (arrow head). × 20000.

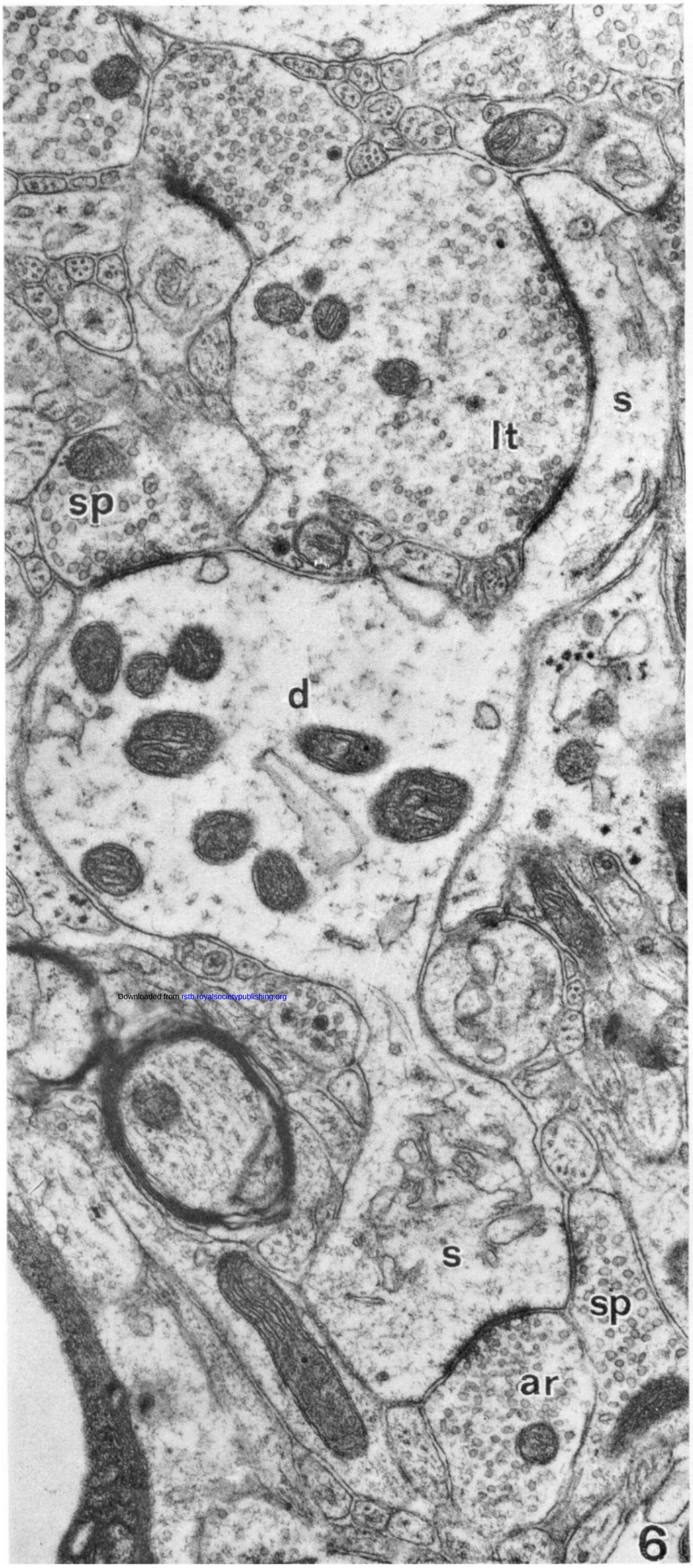





RE 4. A dendrite (d) with a symmetrical synaptic contact from a small terminal with large vesicles (sp). Two spines (s) arise from the dendrite; one receives a contact with asymmetrical membrane thickenings from a large terminal (lt) which also contacts another spine (s). × 30 000.

IGURE 5. Terminal containing small flat vesicles (sf) forming a contact with symmetrical membrane thickenings on a dendrite (d). Compare the size of these vesicles with those in the adjacent terminal (ar) which forms an asymmetrical synaptic contact. × 86 000.



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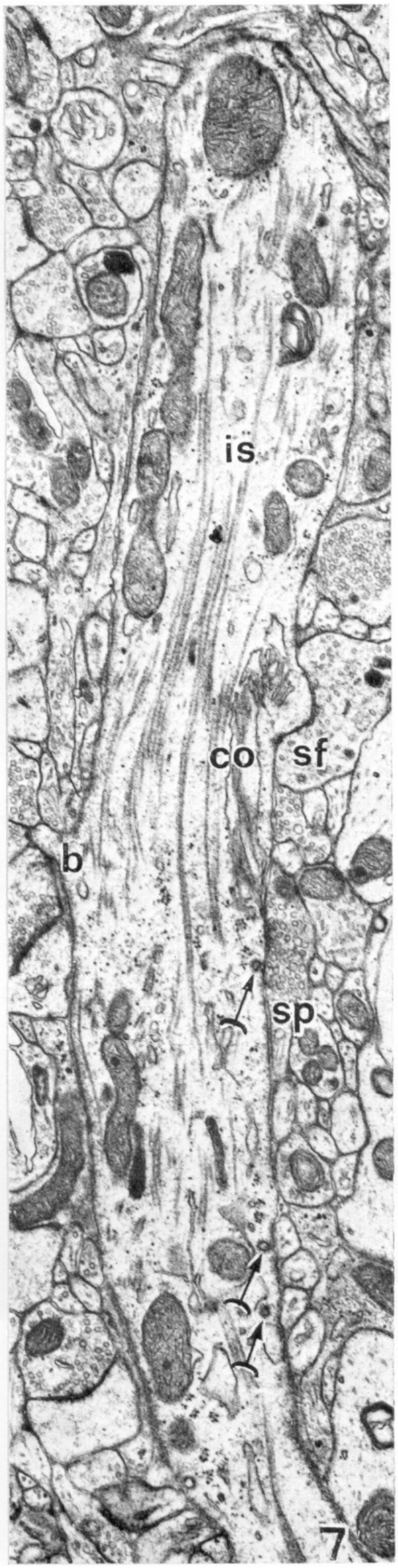


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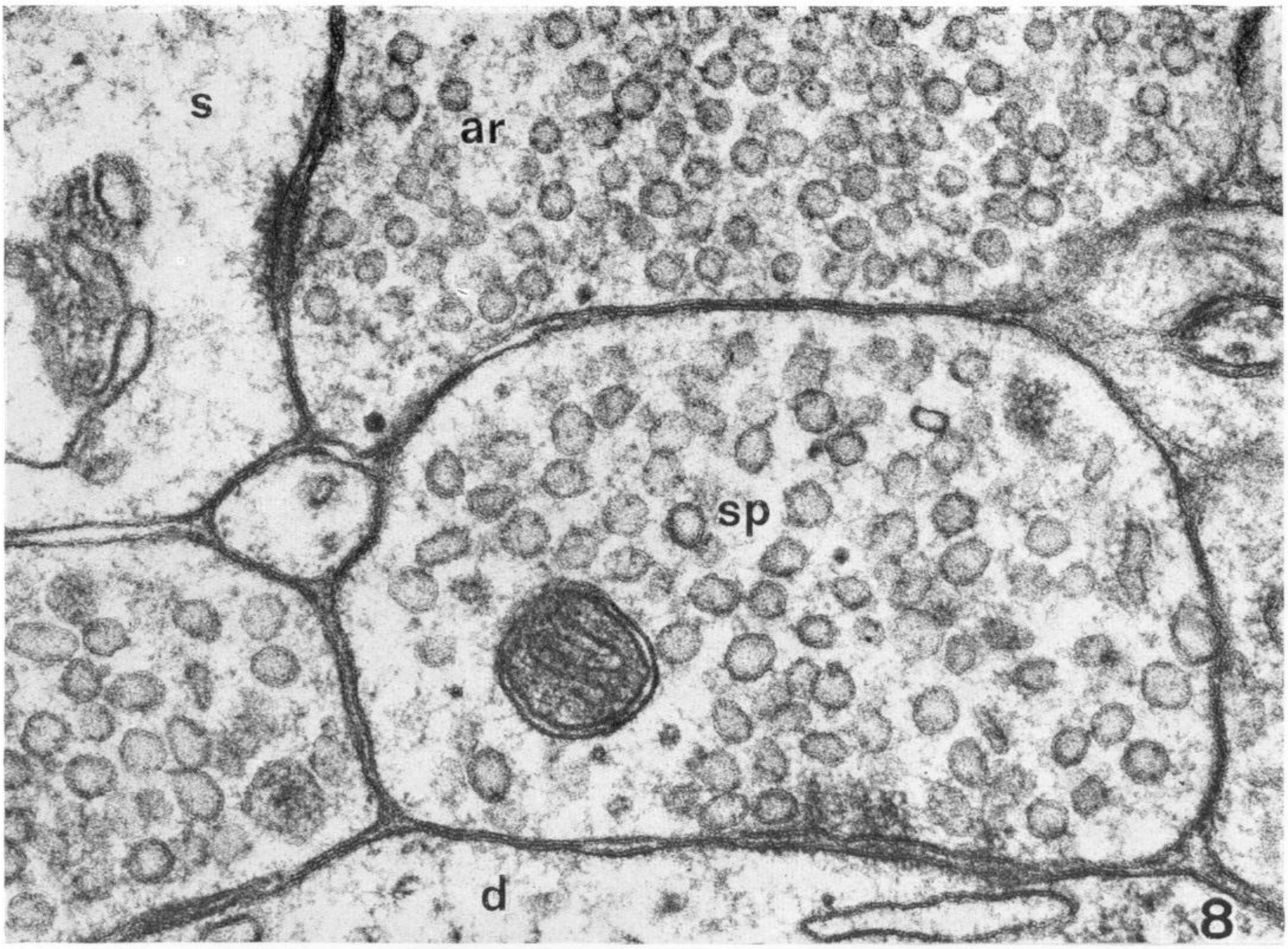
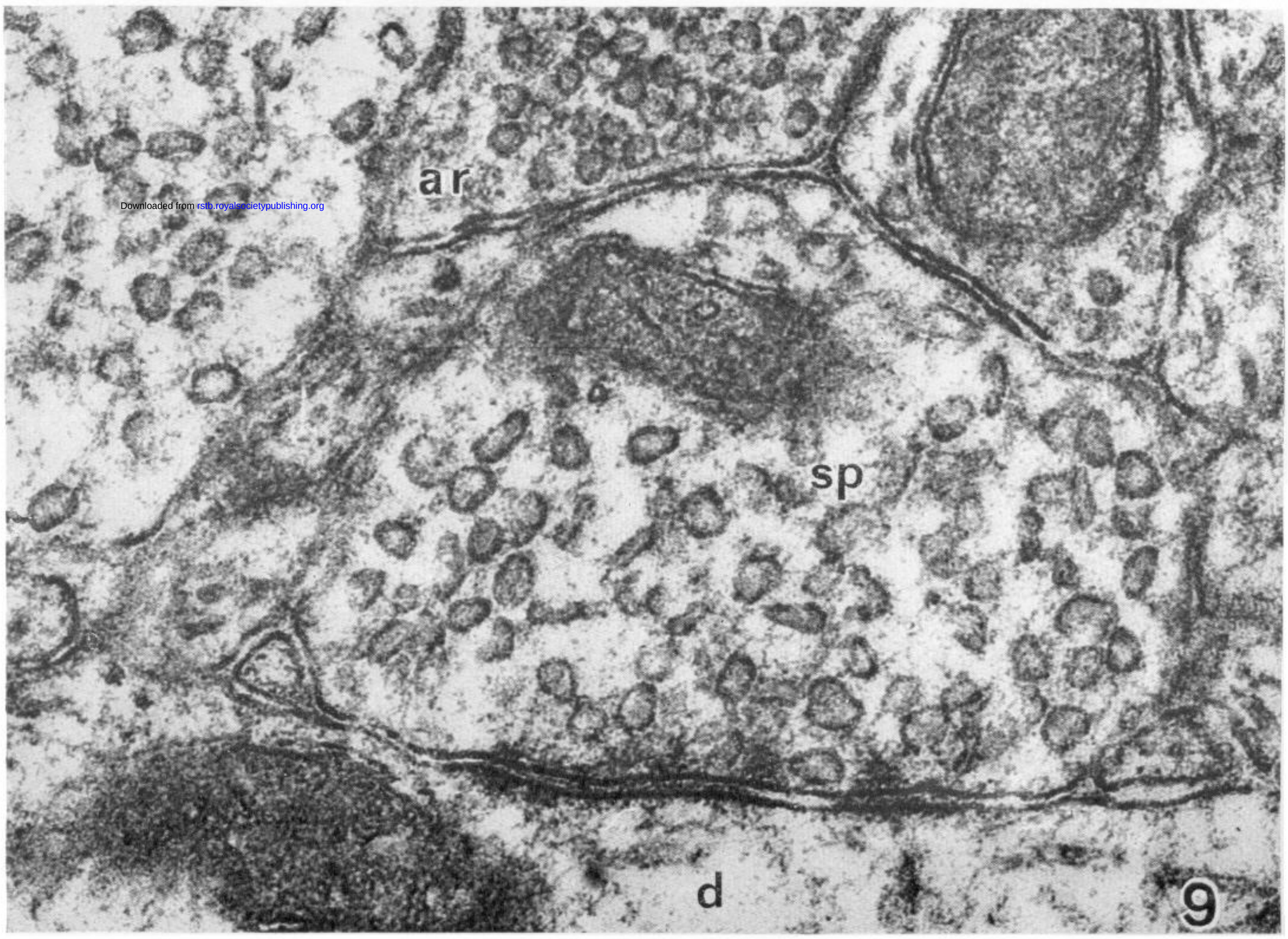
FIGURE 6. The peripheral dendrite (d) of a medium spiny cell with two spines (s). Small terminals with symmetrical membrane thickenings (sp) and large vesicles contact the dendrite and one spine which also receives a small terminal with asymmetrical membrane thickenings (ar). The second spine has a synaptic contact with a large terminal (lt). $\times 28\,000$.

FIGURE 7. The initial segment of an axon (is) with a small branch (b) and a cisternal organ (co) which extends beneath two synaptic contacts. One terminal (sp) contains large polymorphic vesicles and the second (sf) small flat vesicles. Note the alveolate vesicles (barred arrows). $\times 18\,000$.



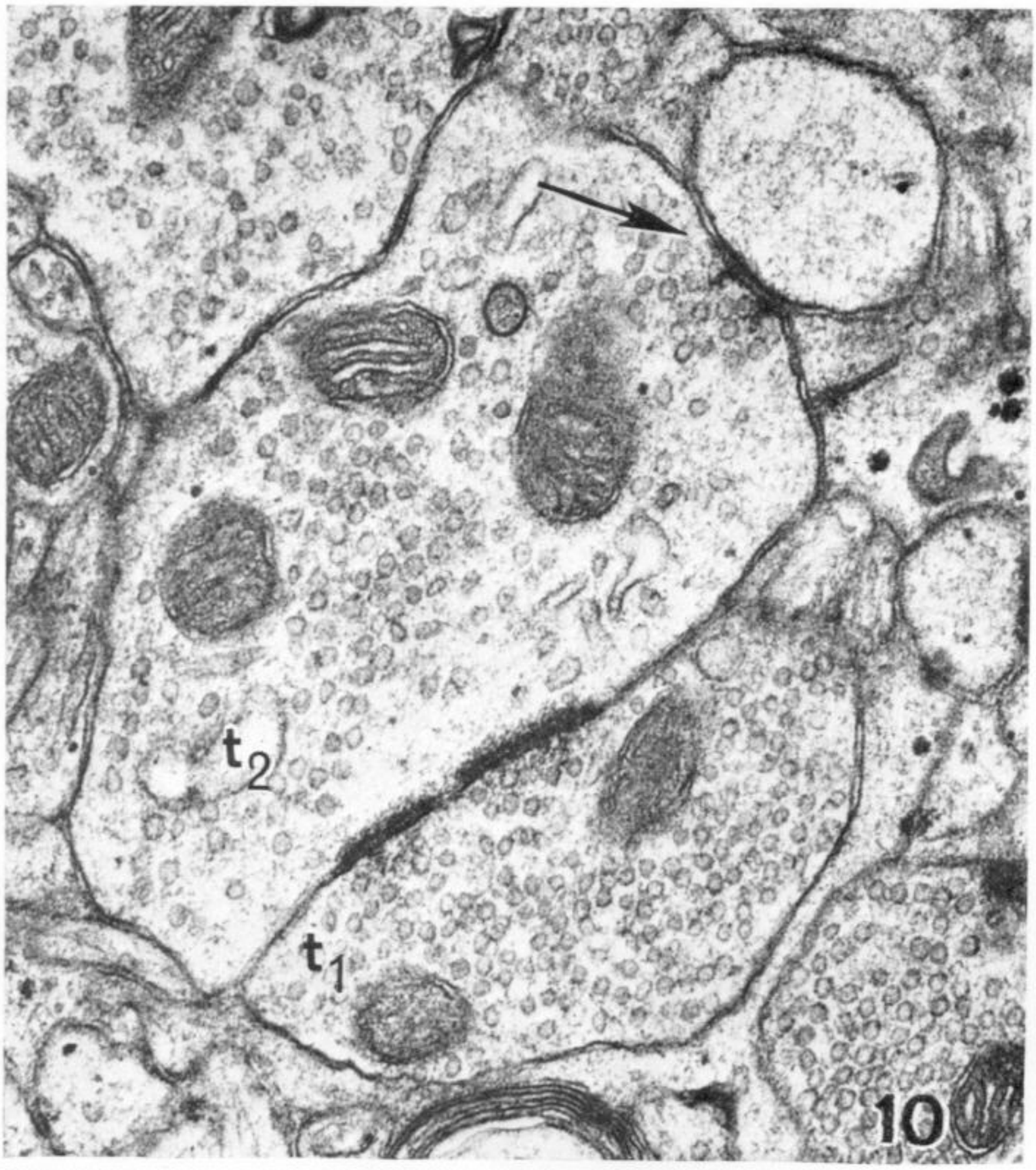



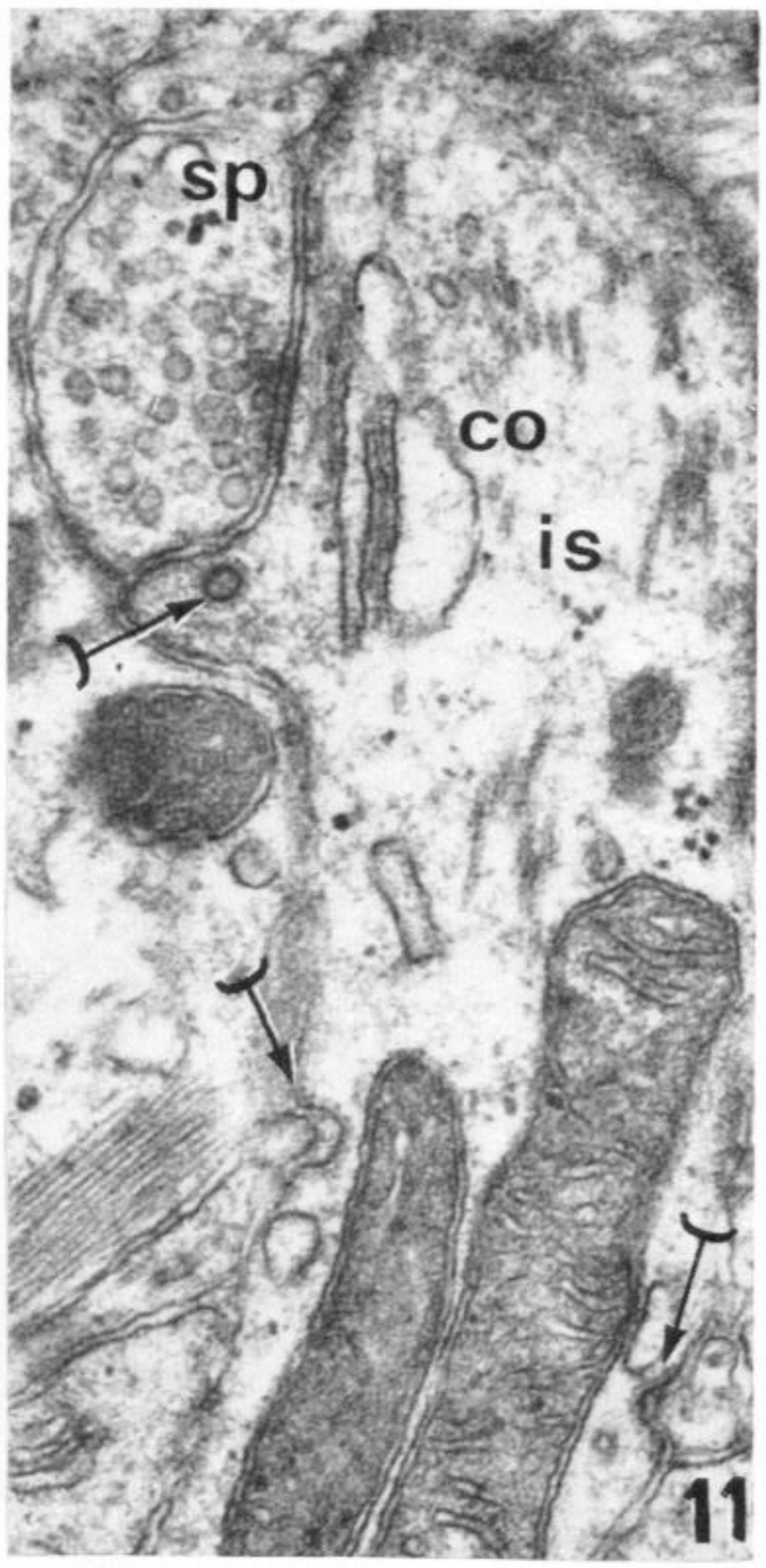




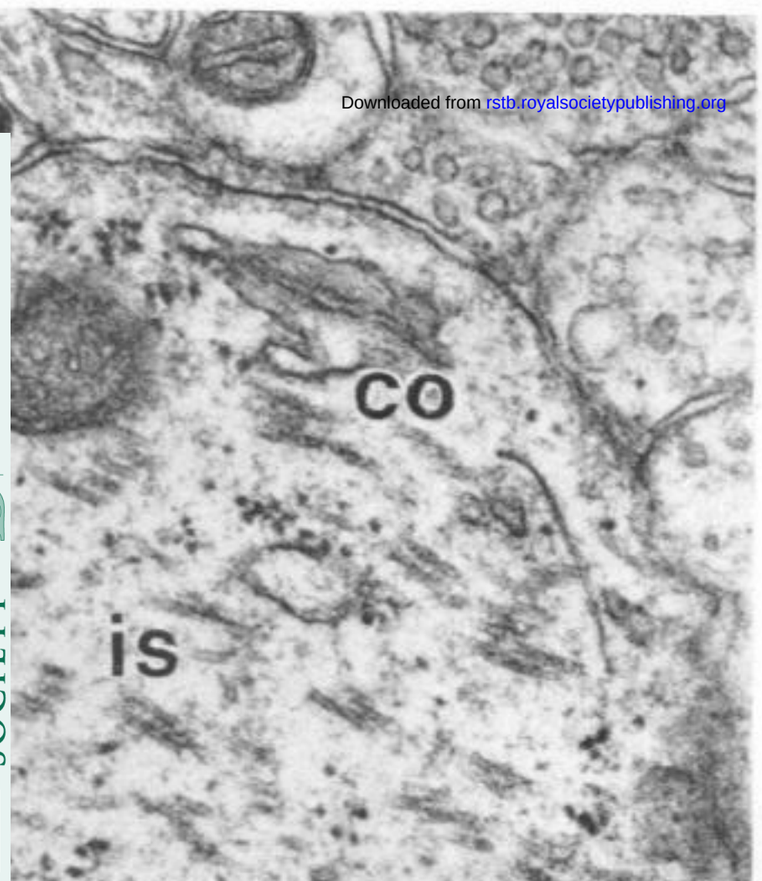

IGURE 8. Terminal with large polymorphic vesicles (sp) forming a synapse with symmetrical membrane thickenings onto a dendrite (d). Compare the size of the vesicles with those in the terminal above (ar) which has an asymmetrical contact region. × 88000.

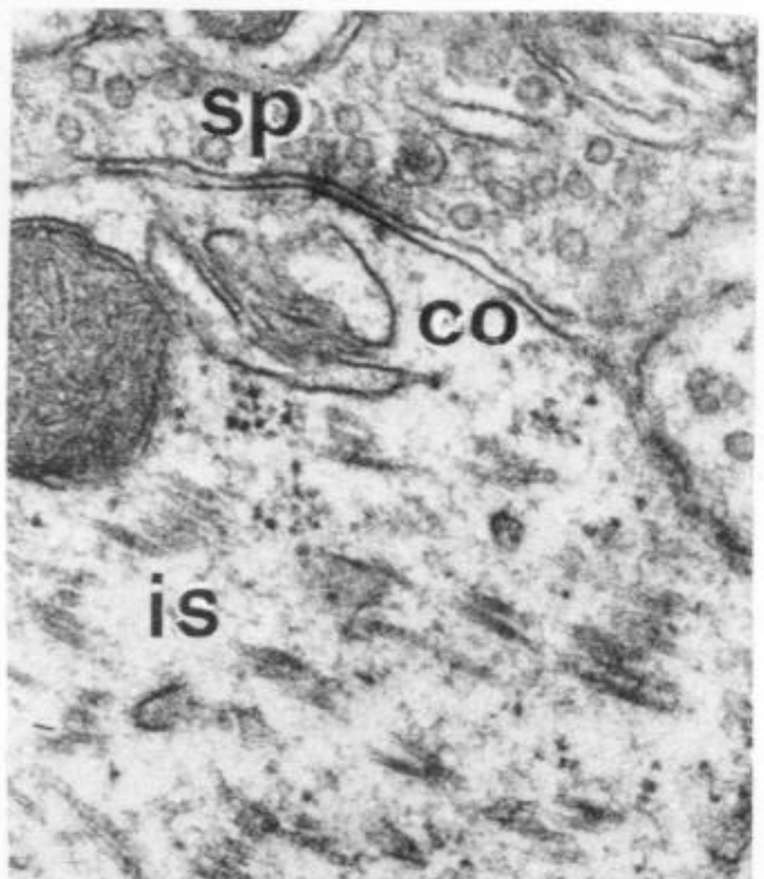
IGURE 9. Terminal with a symmetrical contact region containing large vesicles (sp) after treatment with cacodylate buffer. Compare these vesicles with the round vesicles in the adjacent pre-terminal above (ar). × 88000.

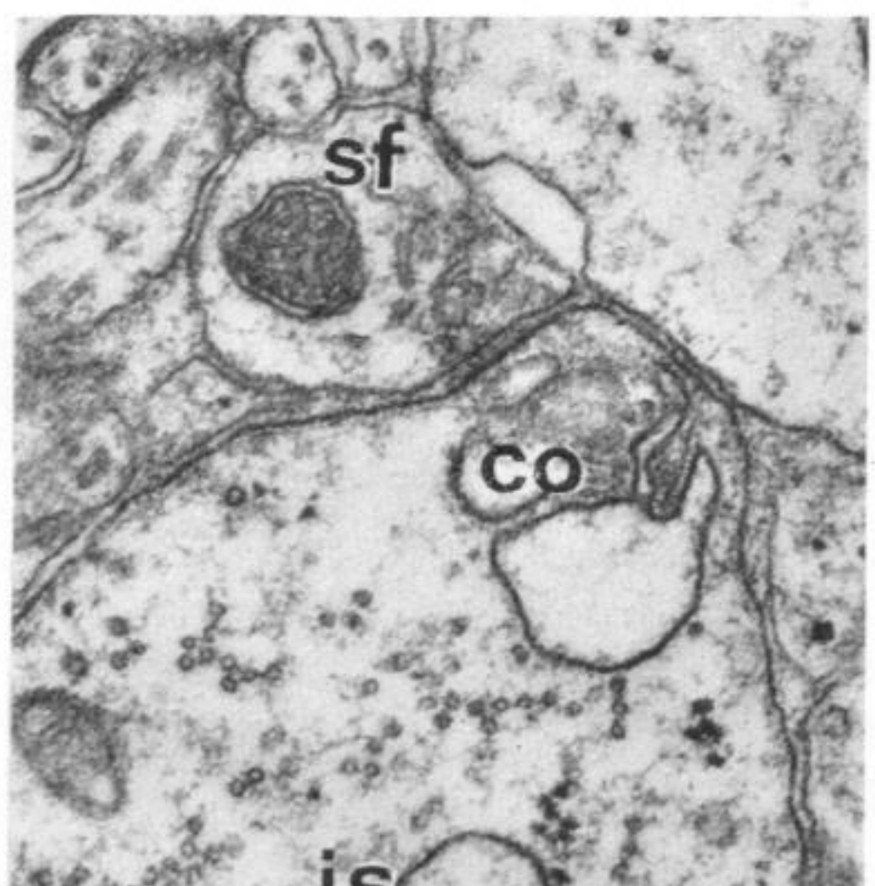







FIGURES 10 to 14. For legends see facing page.