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Electron microscopy of presynaptic organelles of the spinal cord

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INTRODUCTION

The central nervous system remains so far largely unexplored with the electron microscope. Palay (1956, 1958) and others have described the basic morphology of the mammalian synapse, and Luse (1956) and Wyckoff & Young (1956) were the first to report on synapses of the mammalian spinal cord. More recently Gray & Guillery (1961) described neurofilamentous rings in the presynaptic cytoplasm of cat cord. The author has been making a comparative study with the electron microscope of synapses of the mammalian cerebral cortex (1959 *a,b,c*, 1961 *a*), cerebellar cortex (1961 *b*) (see also Palay, 1961) and spinal cord (1962 *a,b*). The present work on the spinal cord describes dense structures organized in a regular pattern on the cytoplasmic surface of the presynaptic membrane, which might well provide a key to synaptic function. In addition, further details of presynaptic organelles are described.

METHODS

Small portions of the lower thoracic and upper lumbar region of adult rat and cat spinal cord were removed under ether anaesthesia, cut into slices less than 0·5 mm. thick and fixed in osmium tetroxide in saline at pH 7·4 for 3–6 hr. The slices were further cut into fragments when first placed in the fixative. Ethanol was used for dehydration, followed by staining in 1% phosphotungstic acid in absolute ethanol and embedding in Araldite (see Gray, 1959*c*, for details). Only a small proportion of the pieces were well-fixed and it was usually impossible to decide from which region of the cord the piece was taken, because of its minute size and unknown orientation.

OBSERVATIONS

Pattern of dense structures on cytoplasmic surface of presynaptic membrane

In synapses of the mammalian spinal cord as of the cerebral and cerebellar cortices, avian optic tectum and lizard brain, the thickened region of the presynaptic membrane frequently shows a series of dense structures projecting from it into the presynaptic cytoplasm in an organized pattern. Pl. 1, fig. 1, shows a presynaptic process of cat spinal cord. When the synaptic membranes are seen in sections cut normal to their surfaces, the dense projections (*dp*) can be seen lying along the presynaptic membrane about 1000 Å. apart. They have an irregular, spiky, appearance and extend about 800 Å. into the presynaptic cytoplasm between the aggregated synaptic vesicles (see also Pl. 4, fig. 8). Frequently, when the plane of section is such that the presynaptic membrane is parallel to and enclosed within the thickness of the section (600–900 Å.), these dense structures can be seen to be arranged in a

roughly hexagonal pattern (Pl. 1, fig. 2). The spacing is about 1000 Å. (centre to centre) corresponding with that mentioned above. In this plane the projections appear as aggregations of dense particles with vague radiating spikes. Their true three-dimensional nature is not understood at present and high resolution studies are in progress.

Axo-axonic synapses

In the central nervous system presynaptic processes are commonly seen with their surface membranes closely apposed. Only the usual 200 Å. gap intervenes and no glial process separates them. Usually no specialized thickenings or density increases are situated along the region of apposition, the thickenings only occurring in the region of apposition with dendrite or perikaryon.

In the cord, however, small vesicle containing processes can sometimes be seen in apposition with larger vesicle containing processes and the regions of apposition show specialization. These large processes are in turn seen to be apposed to dendrites with thickenings at the apposition regions. The relationship between the first two processes might well constitute an axo-axonic synapse. This has already been described briefly (Gray, 1962*a*). A further example is described here since it shows certain features in more detail.

A large vesicle-filled process (Pl. 2, fig. 3), containing several mitochondria, occupies the centre of the figure. Because of the vesicles, it is assumed to be an axonal presynaptic process (see Discussion). It is in contact with other processes, presumably dendrites, at the thickenings *j* and *k*. The row of dense projections, described above, shows clearly along the presynaptic membrane at *k*. Yet another apposition region is seen at *l*, where the dense projections form the characteristic regular pattern observable when they lie in the plane of section (see above). Hence this is a large presynaptic process apparently making three axo-dendritic contacts, not an unusual occurrence in the spinal cord.

However this large process is itself contacted by a smaller process (*p*) containing mitochondria and characteristic vesicles and so presumably it also is an axonal process. At the region of apposition it clearly shows a row of the dense structures projecting into its cytoplasm at the point of apposition (*o*) and the membranes appear here slightly more dense than elsewhere but they show no pronounced thickening (compare a typical axo-dendritic contact, Pl. 1, fig. 1). This configuration could represent an axo-axonic synapse (see Discussion).

Mitochondria related to axo-dendritic attachment plaques

Not infrequently in presynaptic processes of the spinal cord, mitochondria (m_1) can be observed in a special relationship with membrane thickenings (*ap*) (Pl. 3, figs. 5–7). A dense zone of material lies between the mitochondrion and the membrane thickening and it bears two cross-striations. The first (s_1) is denser and narrower than the second (s_2). The cristae mitochondriales often appear to radiate inwards preferentially from the border opposite the dense zone. In these examples, the contacts are axo-dendritic. Occasionally similar relationships are seen in axo-somatic contacts (Pl. 2, fig. 4).

These membrane thickenings are assumed to be axo-dendritic attachment plaques

and not synaptic thickenings, since they are not related to presynaptic aggregations of vesicles nor does the thickening on the post-synaptic side appear consistently denser and wider than the presynaptic thickening (see Gray, 1961*a*). The two types can be compared in fig. 6, where a synaptic thickening (*mt*) occurs alongside an attachment plaque (*ap*) in an axo-dendritic contact. Pl. 3, fig. 7, shows a similar arrangement, but in this case the presynaptic density of the attachment plaque is longer than the post-synaptic one. Here, incidentally, the synaptic thickening shows a row of subsynaptic (dendritic) particles (*sp*) already described elsewhere (Gray, 1962*b*).

Presynaptic 'solid' particles, smaller than synaptic vesicles

Occasionally presynaptic processes in the spinal cord (Pl. 4, fig. 8) contain clusters of small dense granulae 150–200 Å. in diameter, together with synaptic vesicles and other presynaptic organelles. Pl. 4, fig. 8, is an extreme case where there are numerous granules and few synaptic vesicles. In other examples small areas of granules could be observed between more extensive zones of vesicles. Unlike the vesicles, they have not been seen preferentially aggregated near the membrane thickenings.

Terminal myelin at synapses

Only very occasionally can a myelinated axon leading to a presynaptic process in turn contacting a post-synaptic process, be seen in the plane of section. Previous examples have been described in the cerebral cortex by Gray (1959*c*) and in the cerebellar cortex (Palay, 1961; Gray, 1961*b*).

Here a presynaptic process from the spinal cord (Pl. 5, fig. 9) is shown in the centre of the figure. It contains several mitochondria, and synaptic vesicles and contacts a dendrite, the apposed membranes showing thickenings (*mt*) at three points. The termination of the myelin sheath (*my*) is seen above; the lamellae peel off just as they do at a node of Ranvier (compare Uzman & Nogueira-Graf, 1957; Robertson, 1959). Engström & Wersäll (1958) have described a similar arrangement in the ear. The neck of axoplasm adjoining the presynaptic process contains a few tubules and neurofilaments and the terminal myelinated part of the axon contains mitochondria. From light microscopy it has long been suspected that in some instances the myelin may approach very closely to the synaptic membranes. In this case the distance is about 2μ.

DISCUSSION

Observation of a series of regularly arranged dense structures projecting from the synaptic membrane into the presynaptic cytoplasm depends on the use of phosphotungstic acid as a stain. The structures are not clearly seen in unstained osmium-fixed material, nor have they been reported by workers on the CNS who have used stains other than PTA. They were first seen and illustrated in the cerebral cortex (Gray, 1959*c*, pl. 2, fig. 8, pl. 4, figs. 12, 14), but at that time they were thought simply to be vesicles nearest the presynaptic membrane, showing increased density perhaps because of deflation after liberation of transmitter substance. Subsequent observations, especially on spinal cord synapses, where the structures are well developed and easily seen in various planes of sections, indicate that the projections

are more likely to be specializations of the presynaptic membrane. They show a marked regular spacing and have not at all the appearance of collapsed vesicles.

The synaptic vesicles are generally supposed to be the storage particles for transmitter substance, but it is now clear that the organelles contained in the presynaptic process are complex, and the projecting structures, rather than the vesicles, may be involved in the production of packets of transmitter (though there is no direct evidence for this). An alternative theory is that the vesicles contain the transmitter substance and the dense projections are functional 'pores' in the presynaptic membrane, perhaps containing receptors to which the vesicles adhere after random collision, ready for a membrane depolarization.

One of the main problems at present in synaptic morphology is to decide whether the membrane thickenings are in fact the transmission regions. Generally only the presynaptic side contains the vesicles, but in sites where they are also present in the post-synaptic process it may well be that the synaptic thickening and in particular, the 'presynaptic' regular projections, are more rigid criteria for the direction of transmission. The axonal contacts on the taste buds, which contain vesicles according to De Lorenzo (1958), are at present being examined from this point of view. In this situation there are apparently no efferents to complicate matters.

Differences between attachment plaques (desmosomes) and the synaptic thickenings have been pointed out previously (Gray, 1959*c*, 1961 *a*,*b*,). The regular structures on the thickening of the presynaptic membrane constitute another difference: they cannot be observed in PTA-stained desmosomes. Singer & Salpeter (1961), however, have described regular arrangements of groups of attachment plaques (bobbins) in the basal region of tadpole skin. These mark the converging points of bundles of intracellular fibrils (bodies of Ebert). Although the spacing, size and shape of the bobbins are unlike the regular projections at the synapse, structural relationships cannot be altogether excluded and a detailed comparison is in progress.

It was surprising to find axo-dendritic attachment plaques in the mammalian spinal cord. They have not so far been observed in the cerebral cortex, although they do occur in the cerebellar cortex, where however they are dendro-dendritic (Gray, 1961*b*). Even more surprising was the presence, in the cord, of complex dense zones apparently connecting a plaque with a presynaptic mitochondrion. Such a relationship has apparently never been previously described, either in nervous or non-nervous tissue. Since the plaques are linked to an energy system, they may have an important function in membrane transport rather than simply for mechanical attachment, at least in this situation.

The presynaptic 'solid' particles, 150–200 Å. in diameter, mentioned above, are similar to those described by Taxi (1961). He observed that they were often located near presynaptic vesicles containing dense bodies. Although such vesicles can sometimes be seen in spinal cord synapses, they have not so far been seen related in position to the solid particles. Similar particles have occasionally been seen in spinal cord dendrites so they may not be specifically presynaptic organelles. From their size it is possible that they are glycogen granules (Luft, 1956).

The observation of apparent axo-axonal contacts in the spinal cord, described above and by Gray (1962*a*), calls for further and more extensive investigation. If the specialized regions between apposed boutons are in fact transmission points then

the system fits the morphological requirements proposed by Eccles (1961) for presynaptic inhibition (see Frank & Fuortes, 1957). This is a mechanism, where it is postulated that the transmitter substance from one bouton depolarizes the membrane of a second adjacent bouton so that the transmitter released from the second bouton is decreased in amount, thus reducing the effect on the post-synaptic process. Such an inhibitory mechanism would be extremely efficient, according to Katz (1962) since the transmitter output would be very sensitive to small modulations of the axon spike. Vesicle-bearing processes with specializations between their apposed membranes are a common feature in the retina (Kidd, 1961, 1962) and Kidd has in fact postulated that presynaptic inhibition might be the mechanism involved. No such contacts have yet been observed in the cerebral cortex, where possibly the predominant inhibitory mechanism involves hyperpolarizing inhibitory substances acting directly on post-synaptic processes.

SUMMARY

1. Dense structures, organized in a regular pattern on the cytoplasmic surface of the presynaptic membrane are a conspicuous feature of central nervous synapses.

2. Some presynaptic processes of the spinal cord contain mitochondria related to presumed attachment plaques by zones of dense material.

3. Apparent axo-axonic synapses are described, which might represent the morphological basis for presynaptic inhibition.

4. Other presynaptic structures described are 'solid' particles amongst the synaptic vesicles, and also the region of termination of the myelin sheath.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Synapse of spinal cord showing regularly arranged dense projections in presynaptic processes.

Fig. 2. Dense projections sectioned in a plane at right angles to that of fig. 1.

PLATE 2

Fig. 3. A large presynaptic process contacted by a small vesicle-containing process (*p*).

Fig. 4. Presynaptic mitochondrion related to attachment plaques by dense zone.

PLATE 3

Figs. 5–7. Presynaptic mitochondria related to attachment plaques by dense zones.

PLATE 4

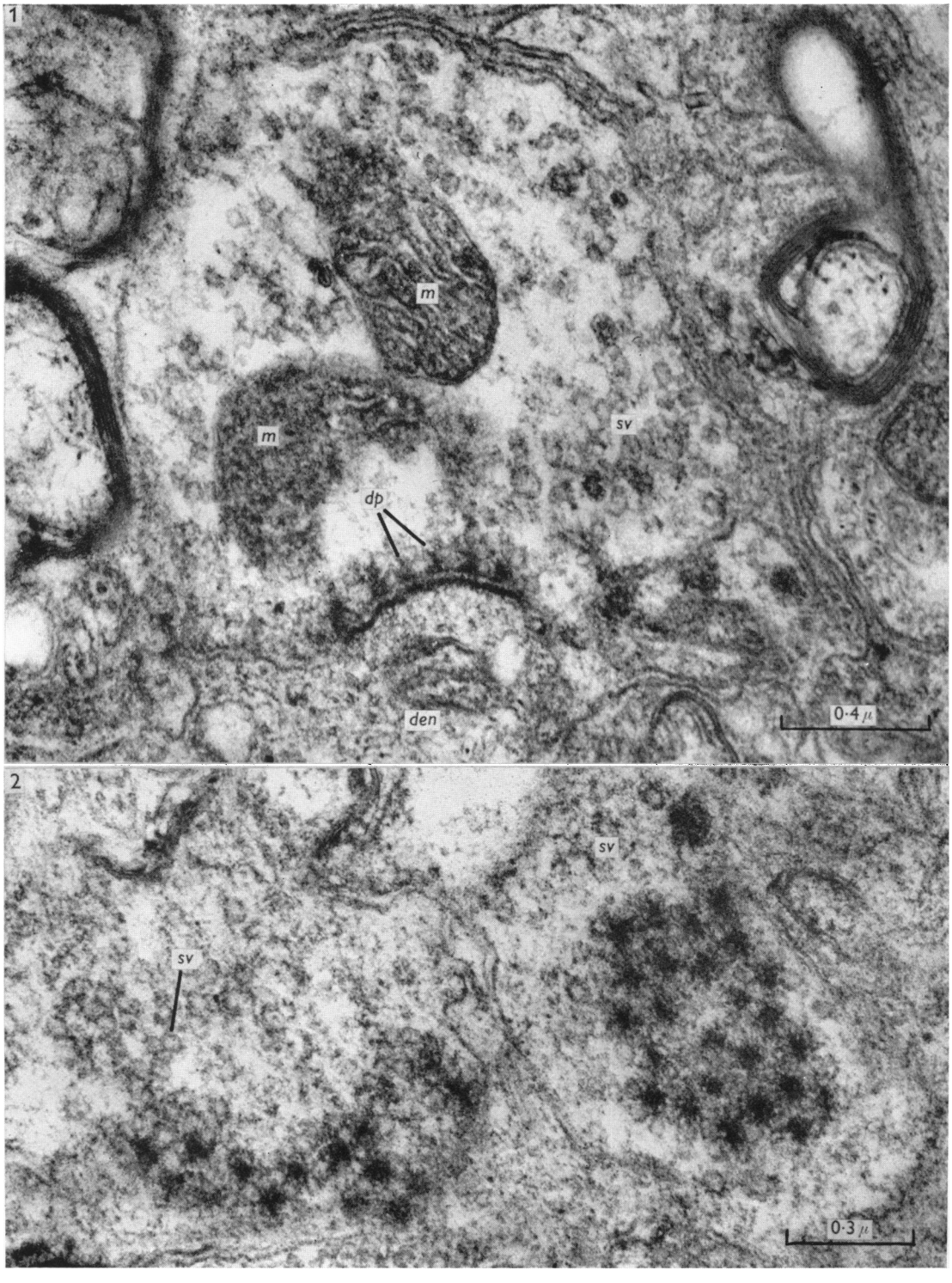
Fig. 8. Presynaptic process of spinal cord containing 'solid' particles.

PLATE 5

Fig. 9. Presynaptic process of spinal cord showing termination of myelin sheath.

KEY TO LETTERING

ap	attachment plaque	*my*	myelin
den	dendrite	*nf*	neurofilaments
dp	presynaptic dense projections	*o*	specialized region of axo-axonal contact
gf	glial fibrils	*p*	postulated process responsible for pre-synaptic inhibition
j	axo-dendritic synaptic thickening		
k	axo-dendritic synaptic thickening	*s*	striations in dense zone
l	presynaptic dense projections	*sp*	subsynaptic particles
m	mitochondrion	*sv*	synaptic vesicles
mt	membrane thickening	*tu*	tubules



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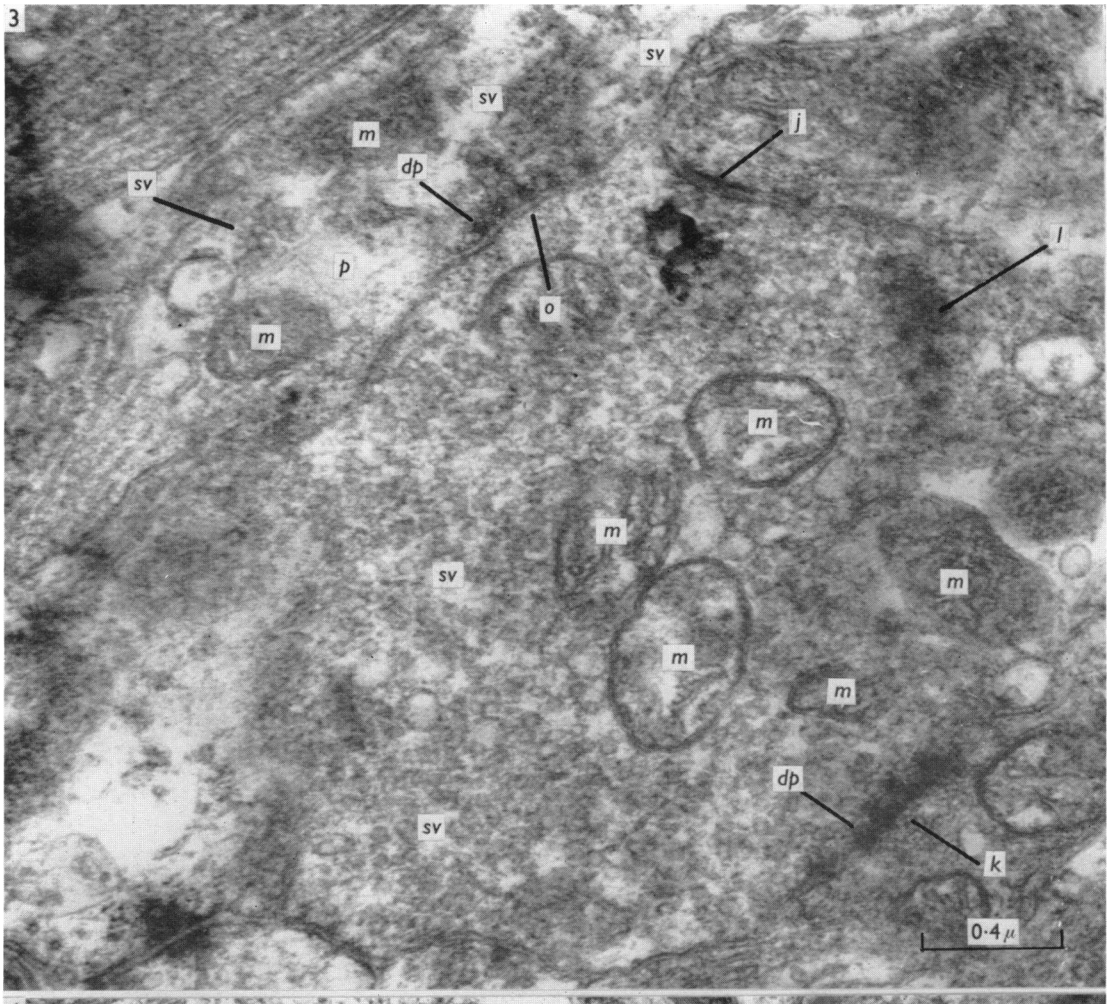
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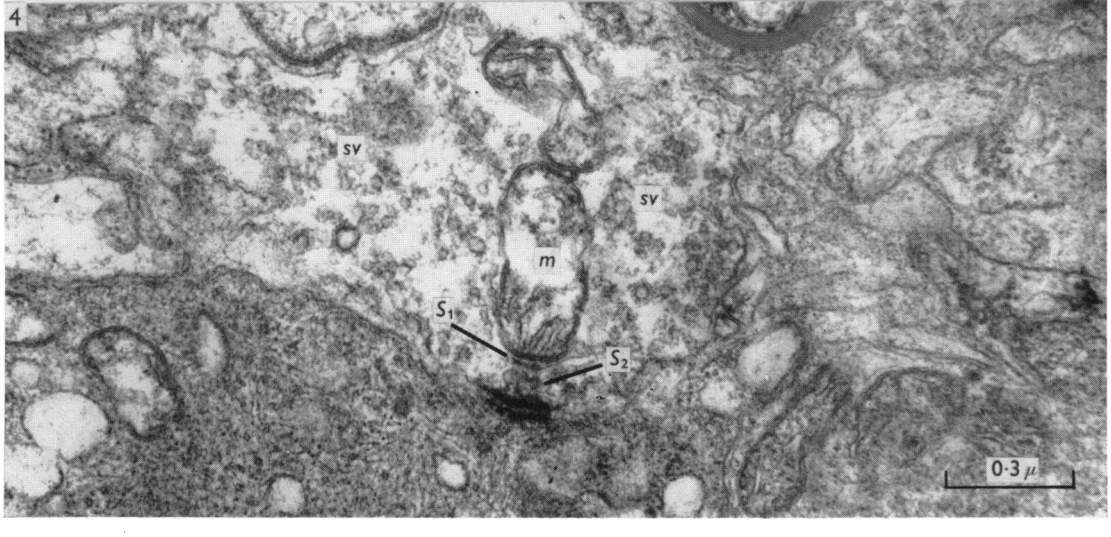
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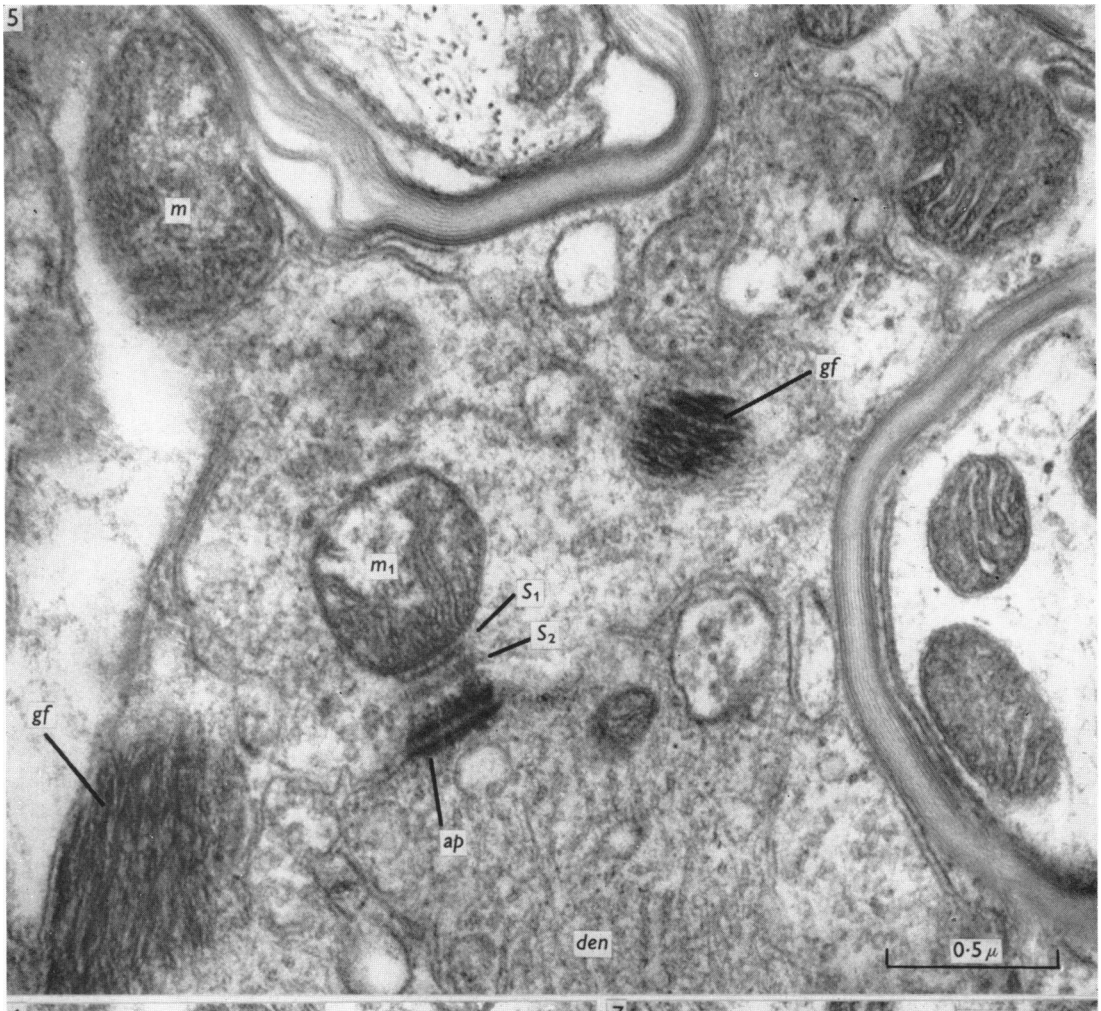
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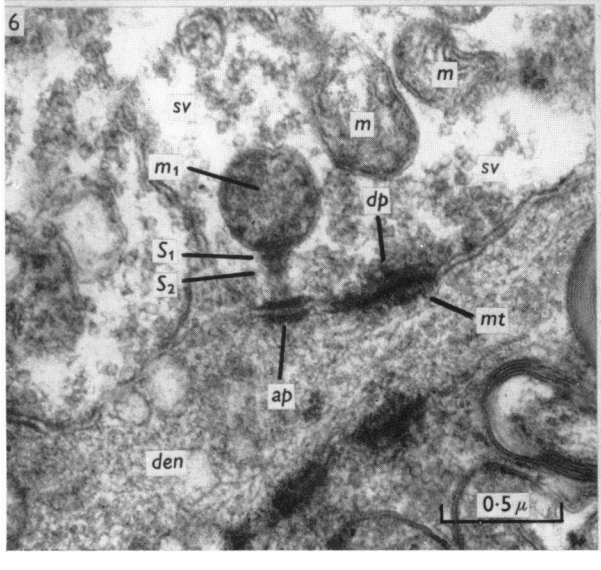
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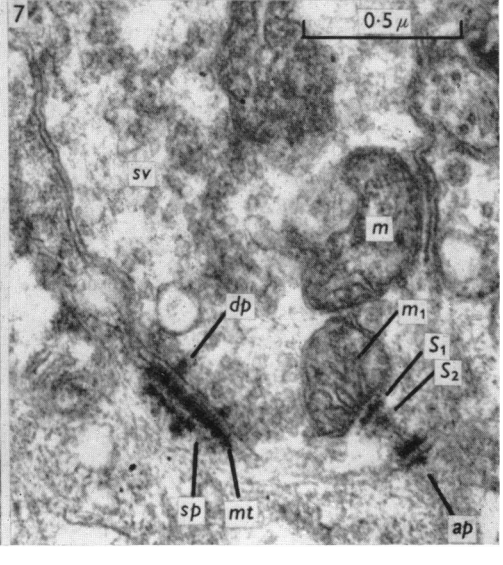
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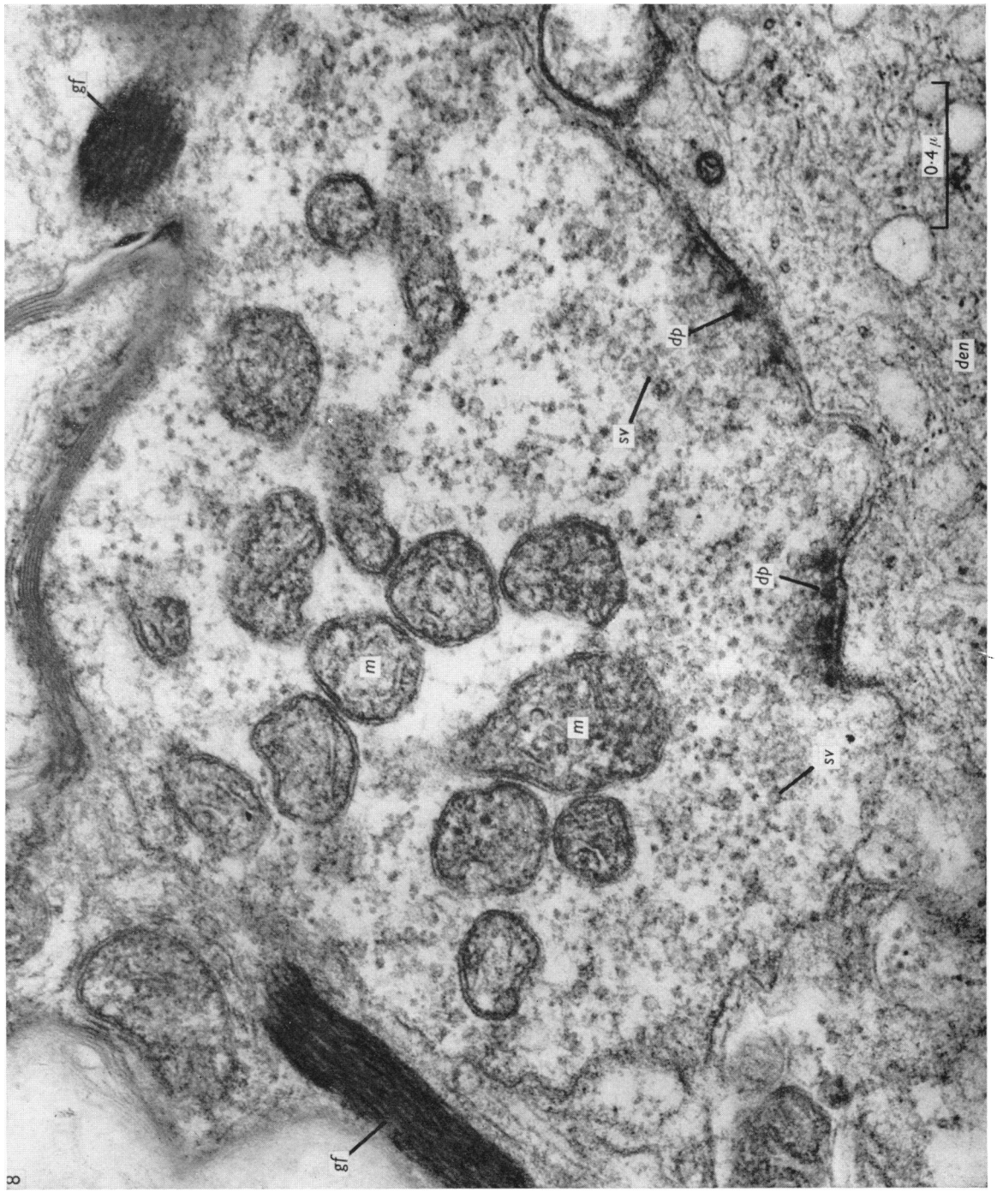

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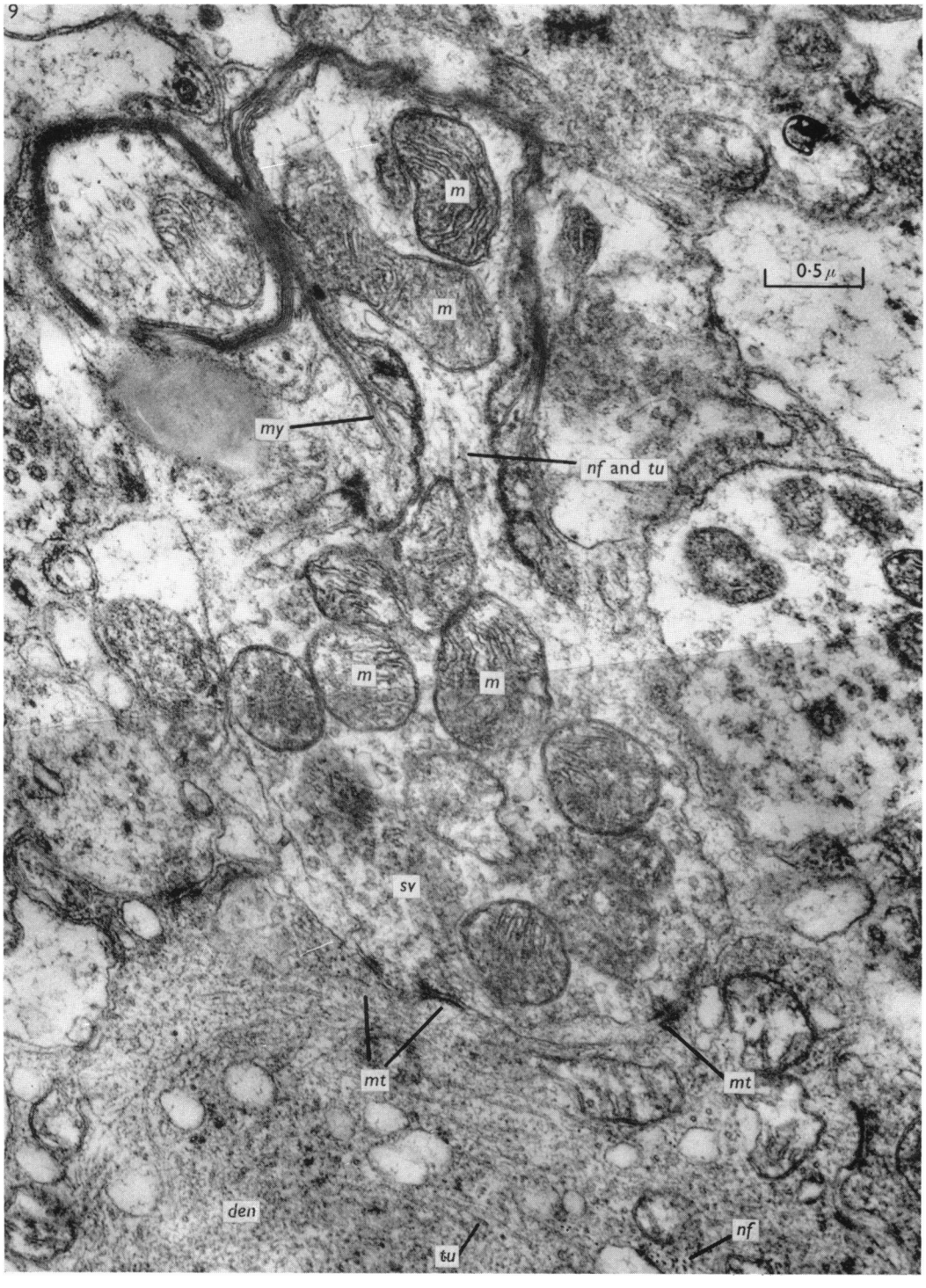
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