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Fine Structure of Noradrenaline Storage Vesicles in Nerve Terminals of the Rat Vas Deferens

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Fine structure of noradrenaline storage vesicles in nerve terminals of the rat vas deferens

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[Plates 56 to 60]

Most of our knowledge of storage and release mechanisms for catecholamines comes from work on the adrenal medulla (see N. & A. G. Kirshner, p. 279). There are important functional differences between the adrenal medullary cells and sympathetic neurons: in the latter the synthetic apparatus of the cell and the site of release are widely separated and re-uptake of released transmitter plays an important role in the maintenance of transmitter stores. At the ultrastructural level there is evidence for only a single population of catecholamine storage particles in each type of chromaffin cell in the adrenal medulla, whereas sympathetic neurons contain two kinds of catecholamine storage particles, whose distribution throughout the neuron is very different. It is not yet possible to explain the structural differences in terms of the functional differences. The finding that large dense-cored vesicles travel down the axon (see P. Banks & K. B. Helle, p. 305; B. G. Livett *et al.*, p. 359; A. Dahlström, p .325) suggests that they may be at least partly made in the cell body and play a role in supplying the terminal with some constituents synthesized in the cell body; whereas the finding that the small dense-cored vesicles are located almost exclusively in nerve terminals (Geffen & Ostberg 1969; Fillenz 1970) suggests that they are concerned with release, which presumably occurs mainly, if not exclusively, in this part of the neuron.

In the present study Woods's (1969) fixative has been used to re-examine the vesicles in the terminal axons of the smooth muscle coat of the normal rat vas deferens. The possible functional implications of the ultrastructural appearances are discussed.

Results and discussion

The nervous elements in the smooth muscle coat of the rat vas deferens consist almost entirely of terminal axons: the non-terminal axons, running in bundles at the outer edge of the smooth muscle coat, are characterized by being parallel-sided, by having a Schwann cell envelope, containing neurotubules and only very few vesicles. The terminal axons are characterized by periodic axon expansions which have been called varicosities; these axon expansions contain few, if any neurotubules; in the rat vas deferens they are mostly free of Schwann cell cytoplasm and they contain accumulations of vesicles. The varicosities have variable relations to the smooth muscle cells: sometimes they invaginate the smooth muscle cell, being separated from it by a 20 nm neuromuscular cleft, whereas at other times there is a distance of 300 to 400 nm between a varicosity and the nearest smooth muscle cell.

Varicosities show considerable variation in the number of vesicles they contain: (figure 1, plate 56) some are closely packed with vesicles, others are only sparsely supplied with vesicles, and some are virtually empty. This is not dependent on their proximity to the smooth muscle cell, or the plane of section, since some large diameter axon profiles invaginating smooth muscle

cells have been found to contain very few vesicles. In the few cases where it was possible to observe a number of consecutive varicosities belonging to the same axon terminal, the degree of vesicle crowding was found to be similar in the varicosities belonging to the same terminal, but very different in adjacent varicosities belonging to different axon terminals. This further confirms that the number of vesicles in a varicosity is not an artefact of the plane of section, but a characteristic of the terminal, possibly related to its functional history. There is no evidence in the varicosities of the vesicle clustering at the axon membrane so characteristic of the neuromuscular junction and synapses of the c.n.s.; however, accumulations of vesicles against the axon membrane are seen occasionally in parallel-sided axons, enveloped by Schwann cells which appear to be intermediate between non-terminal and terminal axons (to be called preterminal axons) (figure 2).

Vesicle types

Whereas most authors describe small agranular, small dense-cored and large dense-cored vesicles in sympathetic terminal fibres, with the use of acrylic aldehyde in sodium dichromate (Woods 1969) as a fixative, the small agranular vesicles virtually disappear. That they do not represent a separate population of vesicles but rather vesicles which have lost their core in the course of fixation is also suggested by the finding of Tranzer & Thoenen (1967) that after incubation with catecholamines no agranular vesicles are seen even in glutaraldehyde-fixed material. In axon varicosities of rat vas deferens fixed with acrylic aldehyde in sodium dichromate three kinds of vesicles are seen: small dense-cored vesicles, large dense-cored vesicles and flattened or elongated vesicles.

Small dense-cored vesicles

These have an average diameter of 45 nm but show a considerable variation in the size and electron-density of their core. At one extreme is the vesicle with a densely staining black core which fills the vesicle except for a relatively clear peripheral zone 20 nm in width. At the other extreme is the vesicle with no clearly defined core, but granular contents which show a certain degree of clumping. There are numerous vesicles with small, grey rather than black cores which are either central or eccentric in position.

The use of acrylic aldehyde in sodium dichromate without postosmication shows the cores of the vesicles but not their membranes. The great variation in size and electron-density of cores is even more striking (figure 3). The granular substructure of the vesicle also becomes evident. Size and electron-density of the core tend to increase and decrease together.

There is evidence that exogenous catecholamines are taken up into nerve terminals and a proportion at least are stored in vesicles (Potter & Axelrod 1963; Descarries & Droz 1970). Tranzer & Thoenen (1968) have shown that after incubation of a tissue with noradrenaline and 5-hydroxy-dopamine, the core size and electron-density of small dense-cored vesicles was greatly increased. The parallel depletion of noradrenaline stores and the degranulation of small

Description of plate 56

Figure 1. Two varicosities from normal rat vas deferens showing variation in vesicle crowding. Acrylic aldehyde in sodium dichromate–osmium tetroxide fixation, sodium ferricyanide block staining, lead citrate section staining, for all figures except figure 3. Calibration 0.1 μm.

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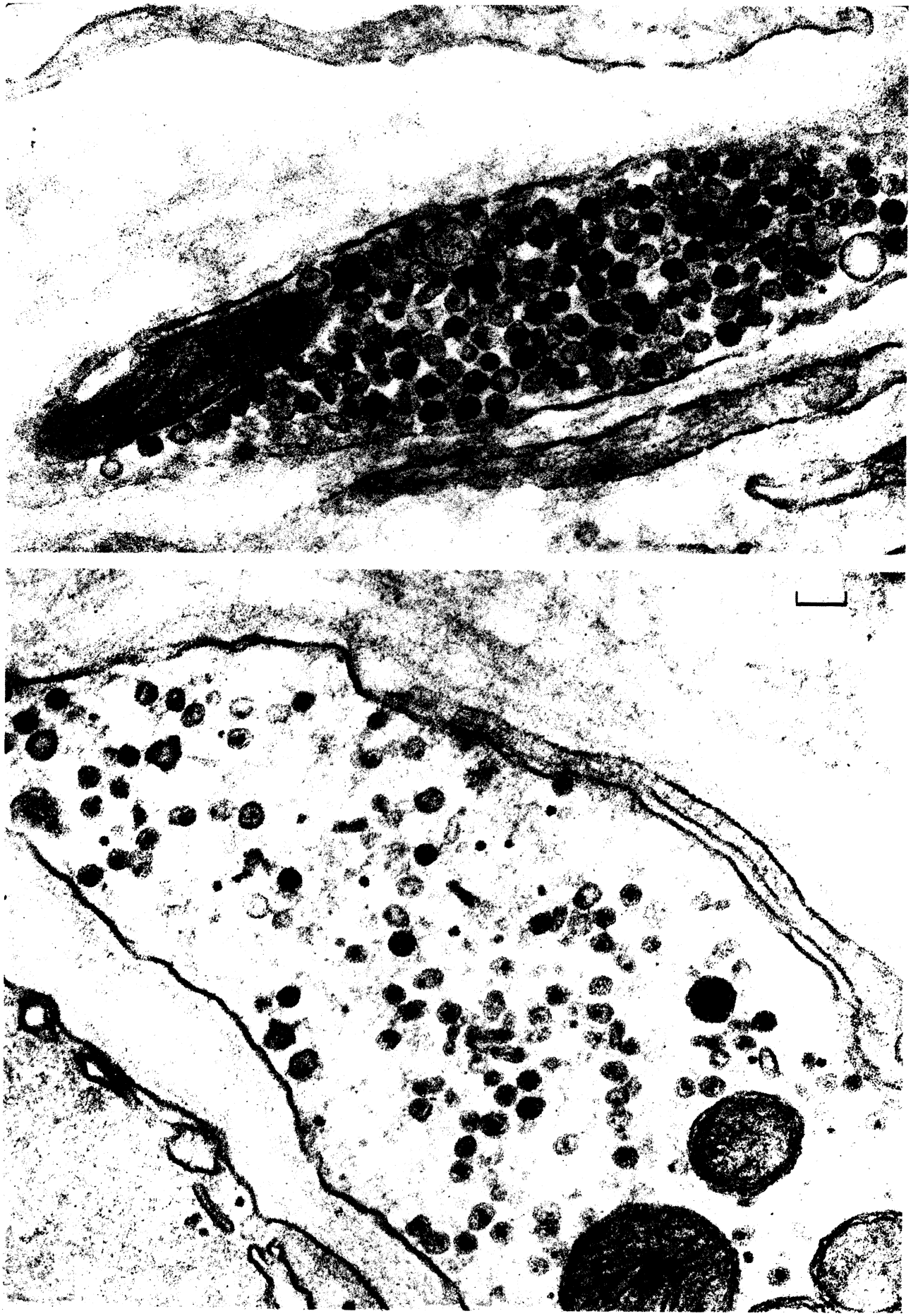

FIGURE 1. For legend see facing page.

(*Facing p.* 320)

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FIGURE 2. Axon in a nerve bundle showing accumulation of small dense-cored vesicles. Calibration 0.2 μm.

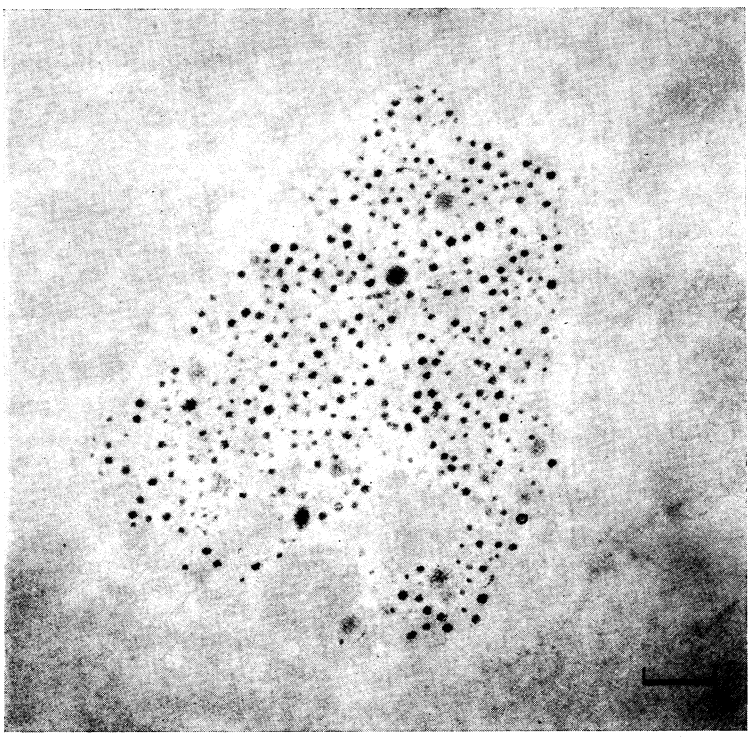

FIGURE 3. Varicosity, fixed with acrylic aldehyde in sodium dichromate without postosmication, showing cores of vesicles only. Lead staining. Calibration 0.2 μm.

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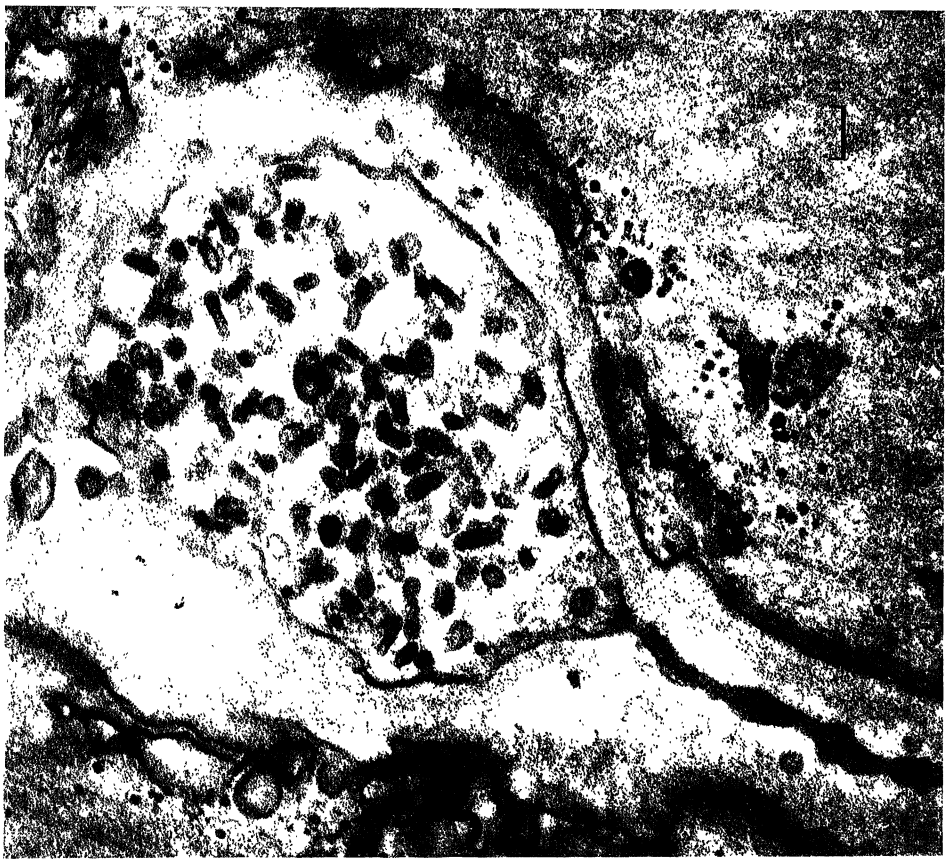

FIGURE 5. Varicosity from normal rat vas deferens containing a majority of flattened vesicles. Calibration 0.1 μm.

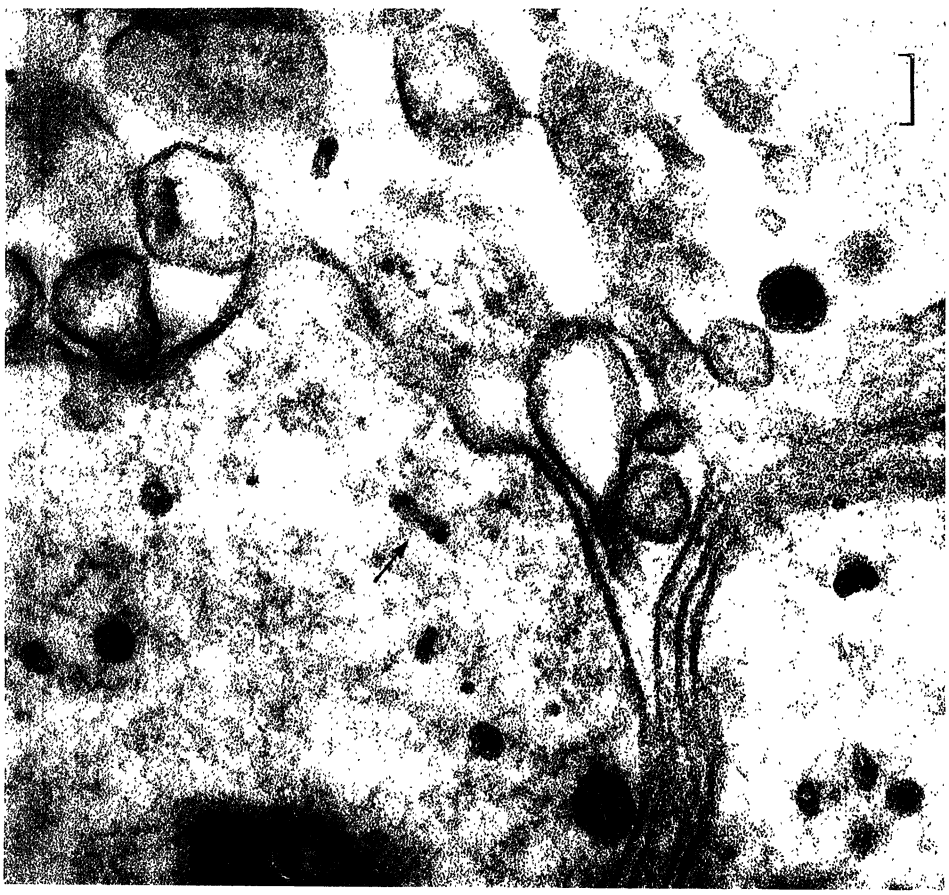

FIGURE 4. Varicosity containing elongated vesicle with two dense cores ↑. Calibration 0.1 μm.

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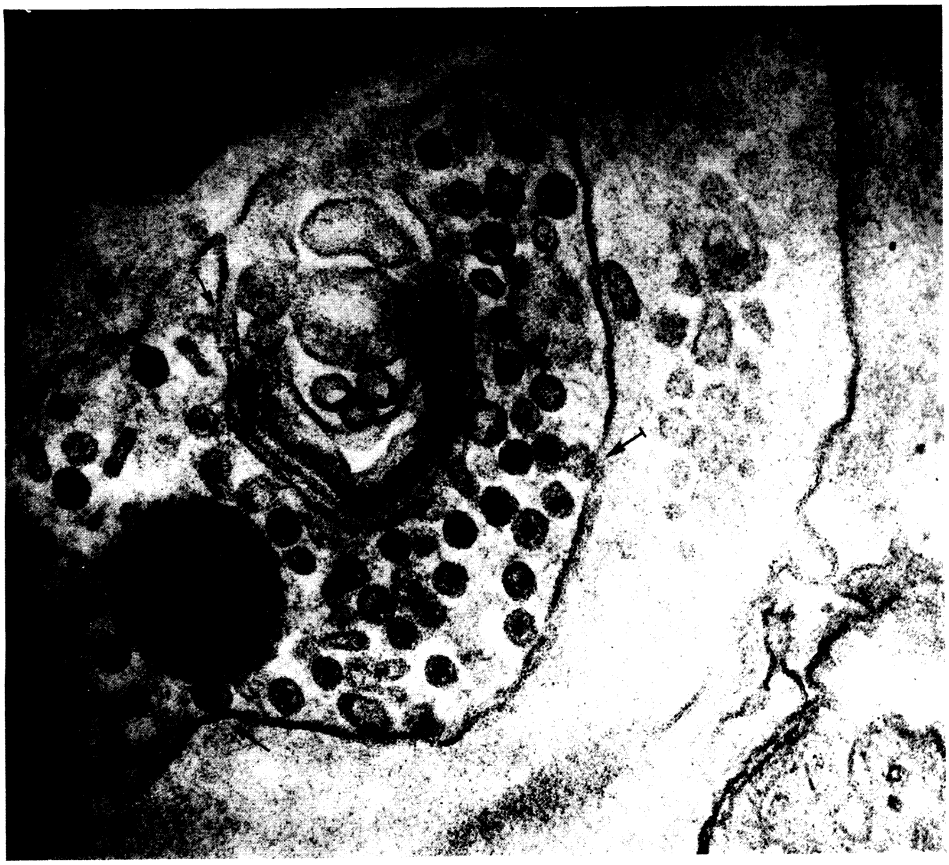

FIGURE 7. Fusion of membrane of small vesicles (cored at ↑ and empty at ↑) with varicosity membrane. Calibration 0.1 μm.

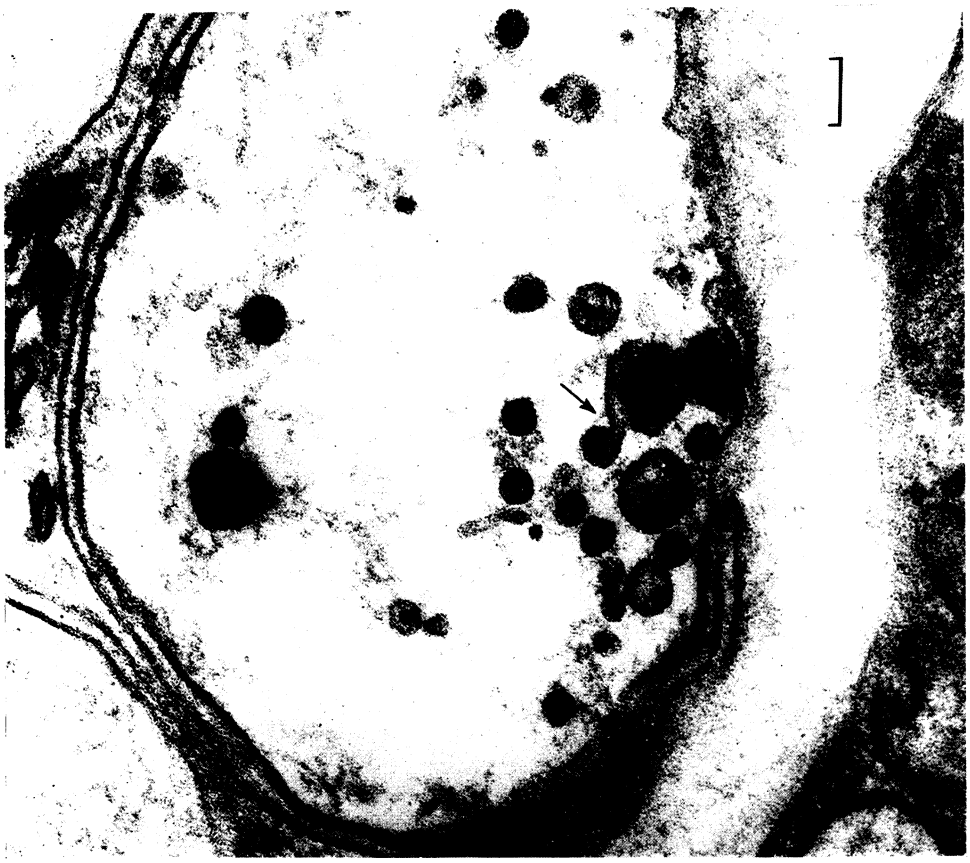

FIGURE 6. Large dense-cored vesicle with a process suggesting the pinching-off of a small vesicle ↑. Calibration 0.1 μm.

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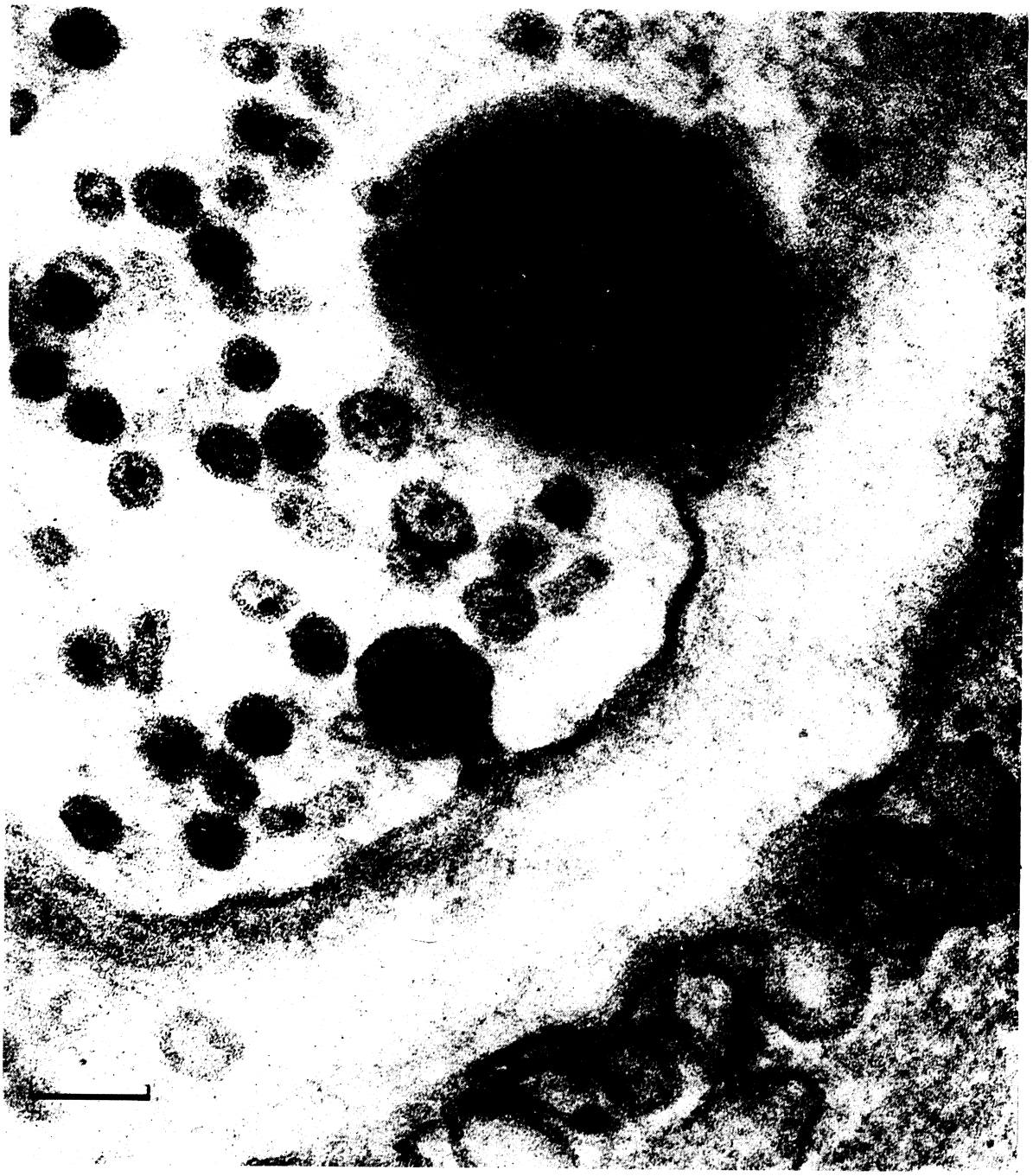

FIGURE 8. Fusion of membranes of large vesicle and axon varicosity. Calibration 0.1 μm.

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dense-cored vesicles by reserpine (van Orden, Bensch & Giarman 1967) was among the earliest evidence for implicating the small dense-cored vesicles in noradrenaline storage in nerve terminals. There is therefore considerable justification for equating core size with catecholamine content in small dense-cored vesicles. This means that the ultrastructural appearance suggests that small dense-cored vesicles show a wide range of catecholamine content. This could explain the width of the low density noradrenaline peak on the sucrose density gradient (Bisby & Fillenz 1970). This could be due either to partial emptying of vesicles during release, or to gradual filling of vesicles.

Besides variation in crowding of vesicles in different varicosities, they also show variation in the size distribution of the cores: in some most of the vesicles have large dark cores, in others the vesicles have small, pale cores; again the distribution is such that fixation artefacts can be excluded and that the distribution of core size is likely to be a reflexion of the functional state of the axon terminal.

Elongated vesicles

These do not appear to be a separate population but a variety of small dense-cored vesicles; they are elongated or occasionally dumb-bell shaped and usually contain a small core at one end. Occasionally dumb-bell shaped vesicles, with two small punctate cores at each end, have been seen (figure 4). Vesicles with large, electron-dense cores are always circular in profile, never flattened. It would appear, then, that the flattened vesicles which from the appearance of their cores one may conclude have a low catecholamine content, have certain special properties which cause them to assume a flattened appearance as a result of fixation (cf. Bodian 1970). Or alternatively that the flattened appearance is not an effect of fixation but that the dumb-bell shaped vesicles represent division and thus new formation of small dense-cored vesicles. Conversely, the flattened vesicles could represent those which have recently discharged their contents. In the parallel sided preterminal axons containing small dense-cored vesicles referred to above, elongated vesicles were prominent. This seems to favour the hypothesis that they represent newly forming small dense-cored vesicles.

Although elongated vesicles usually constitute a small proportion of the total population, this is not always the case: in one case where three consecutive varicosities of the same terminal appeared in the one section, two of the varicosities contained mostly flattened vesicles (figure 5) and the third had no vesicles.

Large dense-cored vesicles

The large dense-cored vesicles have an average diameter of 85 nm and their occurrence in varicosities of rat vas deferens is sporadic: many sections of varicosities show no large dense-cored vesicles, whereas in others there may be 2 to 3. Vesicle counts in a large number of varicosities have shown large dense-cored vesicles to be about 4 % of the total. Similar figures for the percentage of large dense-cored vesicles in rat vas deferens have been obtained by Farrell (1968).

The core of large dense-cored vesicles shows little variation in size, but considerable variation in density. Tranzer & Thoenen (1968) presented histochemical evidence for the storage of catecholamines in large as well as small dense-cored vesicles; this has now been confirmed by the isolation of two populations of noradrenaline storage particles by subcellular fractionation and the electronmicroscopic identification of the high density particles with the large dense-cored vesicles seen in tissue sections (Bisby & Fillenz 1970). Calculations from the noradrenaline distribution on sucrose density gradients suggest that the mean noradrenaline concentration in

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large dense-cored vesicles is lower than in small dense-cored vesicles (M. A. Bisby & M. Fillenz in preparation). Tranzer & Thoenen (1968) showed that degranulation of large dense-cored vesicles after reserpine could be demonstrated in non-osmicated material; osmication revealed a core in large dense-cored vesicles which presumably represents something other than catecholamine. In preliminary experiments with A. D. Smith and M. A. Bisby we have obtained evidence that both small and large dense-cored vesicles contain dopamine β-hydroxylase (DBH), but that the ratio of DBH to noradrenaline is greater for the large than for the small dense-cored vesicles. The core of the large dense-cored vesicle could therefore be made up of soluble DBH in addition to catecholamines as well as other substances. It is therefore not possible to relate electron-density to catecholamine content, as in the small dense-cored vesicles.

The fact that both large and small dense-cored vesicles contain DBH suggests that they are related and allows for the possibility that one could be derived from the other. Figure 6 shows an appearance which suggests the pinching off of a small vesicle from a large dense-cored vesicle.

Evidence of exocytosis

There is both biochemical and morphological evidence for release of adrenal catecholamines by exocytosis. The evidence that the same mechanism operates at nerve terminals is less complete and there is no indication which population of vesicles contributes to release. Stimulation of nerve terminals causes depletion of the vesicular store of noradrenaline (Chang & Chang 1965; M. A. Bisby & M. Fillenz, unpublished observations; M. Fillenz & P. Howe, unpublished observations): release of DBH and chromogranin as well as noradrenaline occurs into the perfusate (A. D. Smith, this volume, p. 363). The amount of protein relative to catecholamines released from nerve terminals is much lower than that released from adrenal medullary cells. This could be due to release from two pools of catecholamines, one of which consists mainly of catecholamines and is deficient in soluble proteins.

Fusion of the vesicle membranes with the axon membrane is only rarely seen in nerve terminals in normal tissues. Figure 7 shows an axon varicosity with such membrane fusion involving small dense-cored vesicles and empty vesicles (? after loss of its contents) and figure 8 shows fusion of the membrane of a large dense-cored vesicle with the axon membrane. This, then, could provide supporting morphological evidence for release of noradrenaline from both large and small dense-cored vesicles. In view of the relative proportion of large and small dense-cored vesicles, the major contribution is likely to come from the latter, but in organs such as the cat's spleen, where large dense-cored vesicles are around 20 % of the total, they could be making a significant contribution to release. The ratio of soluble proteins to catecholamines released from different organs on nerve stimulation could therefore vary according to the ratio of the two vesicle populations.

Conclusion

The variations in small dense-cored vesicle number, size and electron density of their core, and in the proportion of elongated vesicles in varicosities of a single terminal could be important indices of its functional state. Small dense-cored vesicles appear to have a wide range of catecholamine content. Their origin is not known, but they could be formed by budding from large vesicles and by division of small ones. Both large and small vesicles show fusion with the axon membrane and so would appear to be capable of exocytosis.

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References (Fillenz)

Bisby, M. A. & Fillenz, M. 1970 *J. Physiol.* **210**, 49–50*P*.
Bodian, D. 1970 *J. Cell. Biol* **44**, 115–124.
Chang, C. C. & Chang, J. C. 1965 *Br. J. Pharmac. Chemother.* **25**, 758–762.
Descarries, L. & Droz, B. 1970 *J. Cell Biol.* **44**, 385–399.
Farrell, K. E. 1968 *Nature, Lond.* **217**, 279–281.
Fillenz, M. 1970 *Proc. Roy. Soc. Lond.* B **174**, 459–468.
Geffen, L. B. & Ostberg, A. 1969 *J. Physiol.* **204**, 583–592.
Potter, L. J. & Axelrod, J. 1963 *J. Pharmac. exp. Ther.* **142**, 291–298.
Tranzer, J. P. & Thoenen, H. 1967 *Experientia* **23**, 123–124.
Tranzer, J. P. & Thoenen, H. 1968 *Experientia* **24**, 484–486.
van Orden III, L. S., Bensch, K. G. & Giarman, N. J. 1967 *J. Pharmac. exp. Ther.* **155**, 428–439.
Woods, R. I. 1969 *J. Physiol.* **203**, 35–36*P*.

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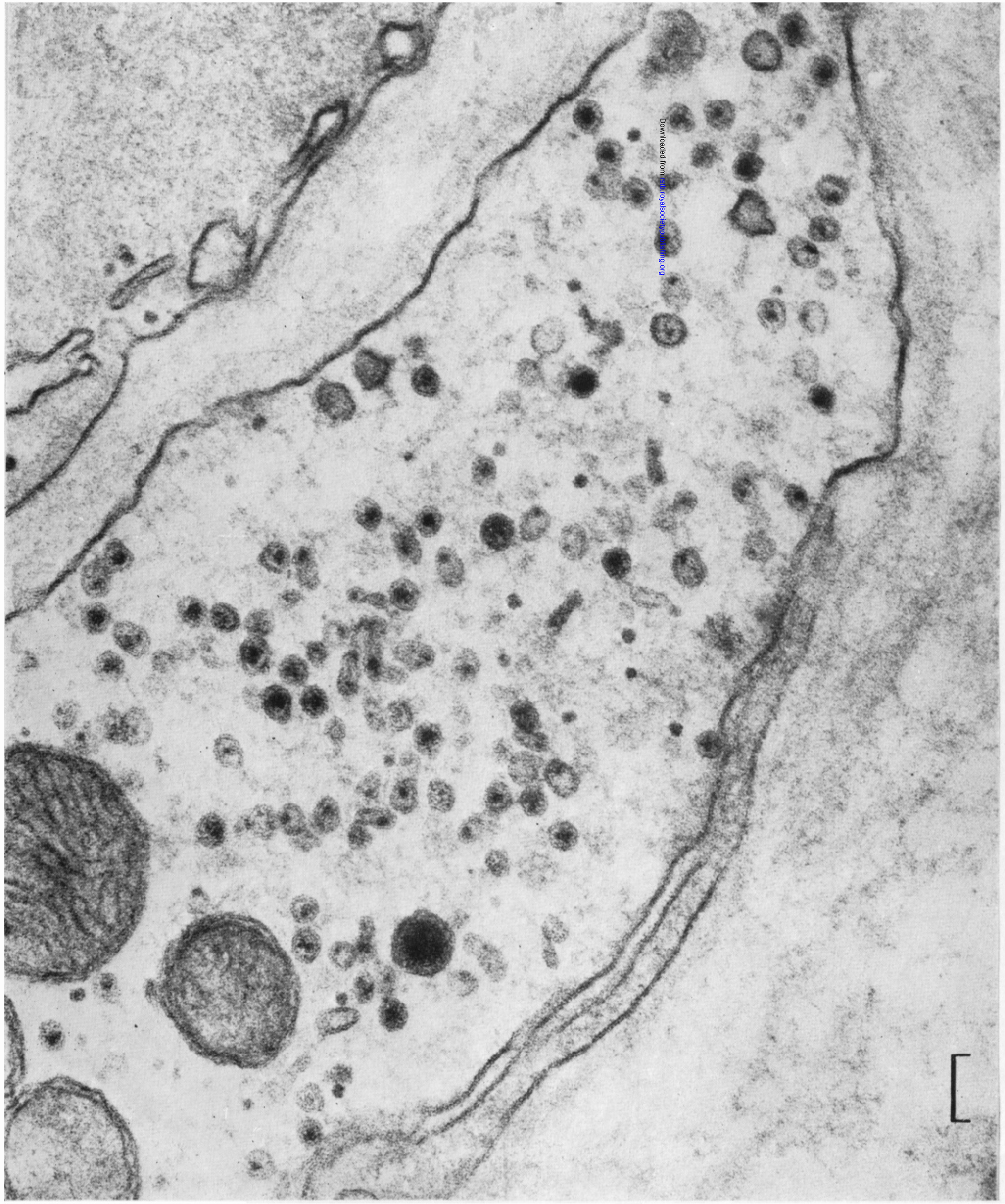

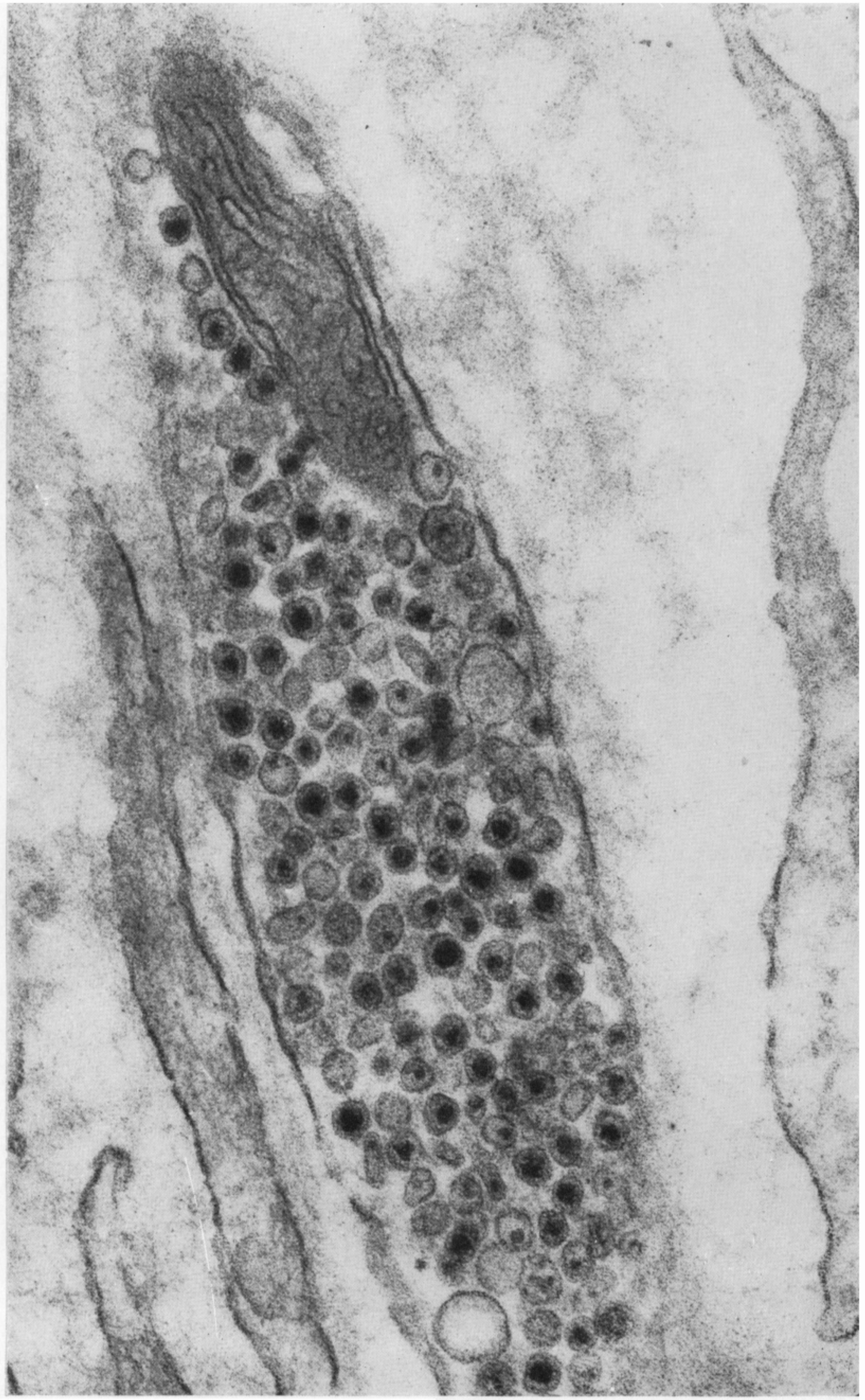

FIGURE 1. For legend see facing page.

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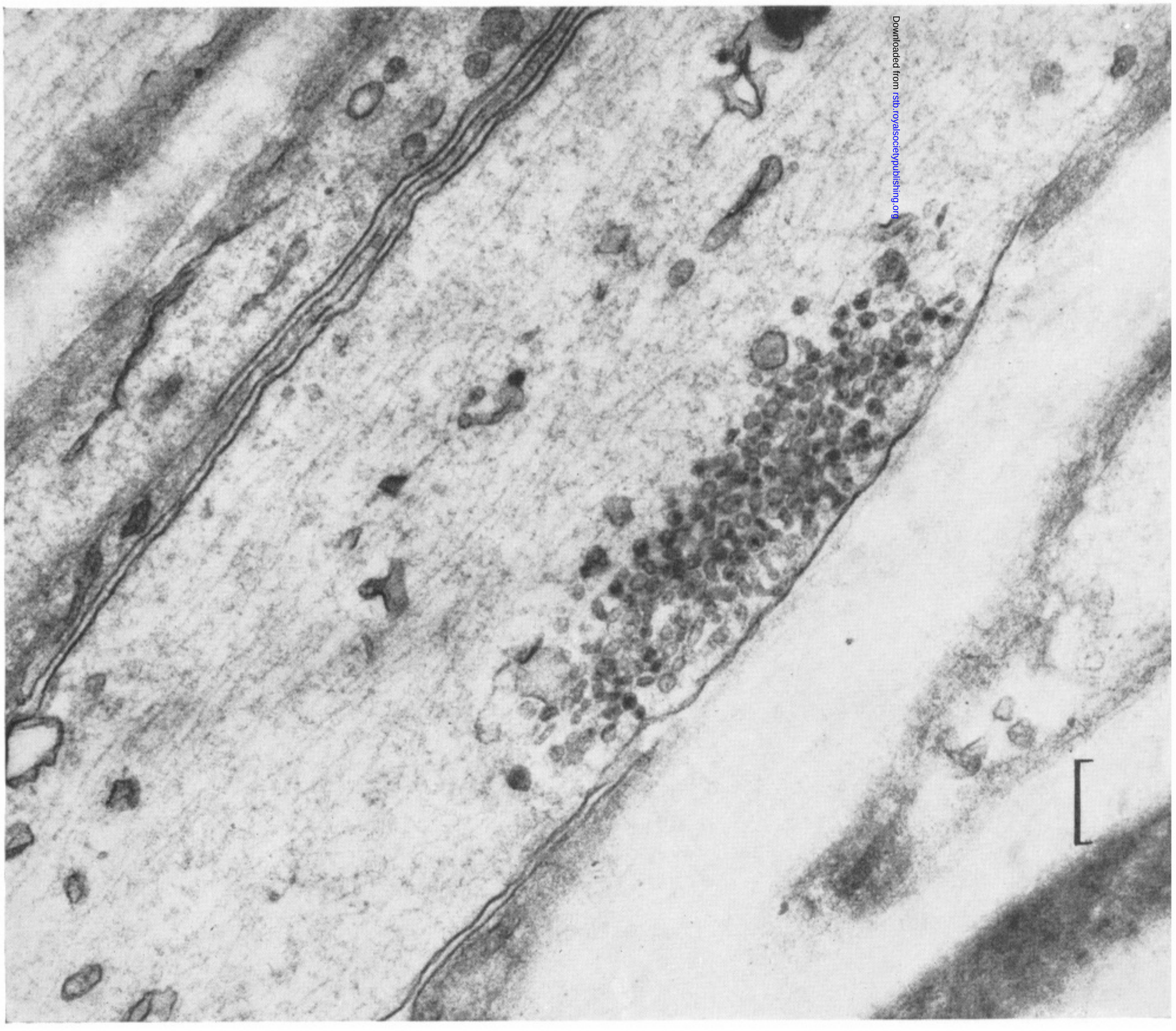

FIGURE 2. Axon in a nerve bundle showing accumulation of small dense-cored vesicles. Calibration 0.2 μm.

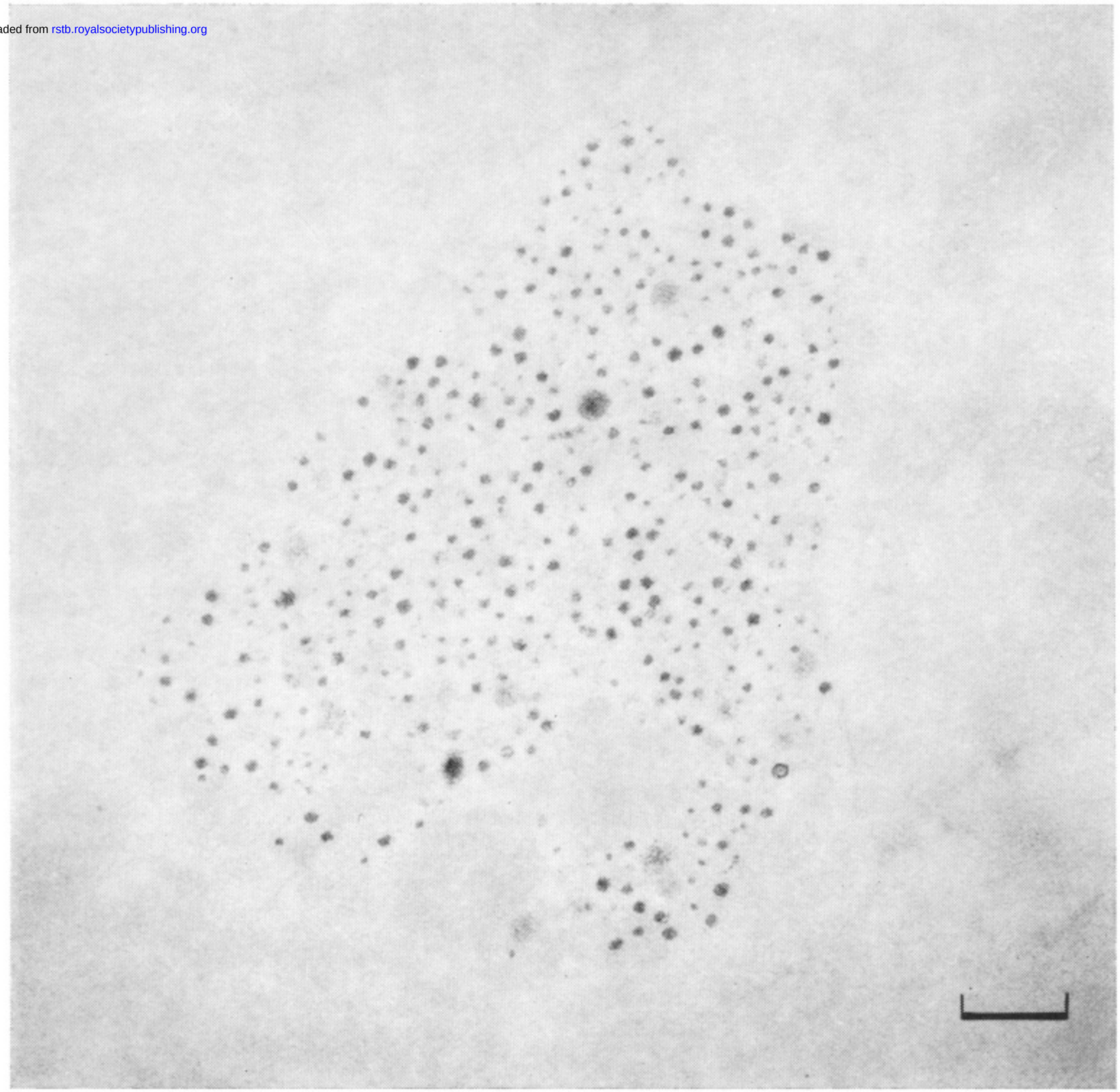

FIGURE 3. Varicosity, fixed with acrylic aldehyde in sodium dichromate without postosmication, showing cores of vesicles only. Lead staining. Calibration 0.2 μm.

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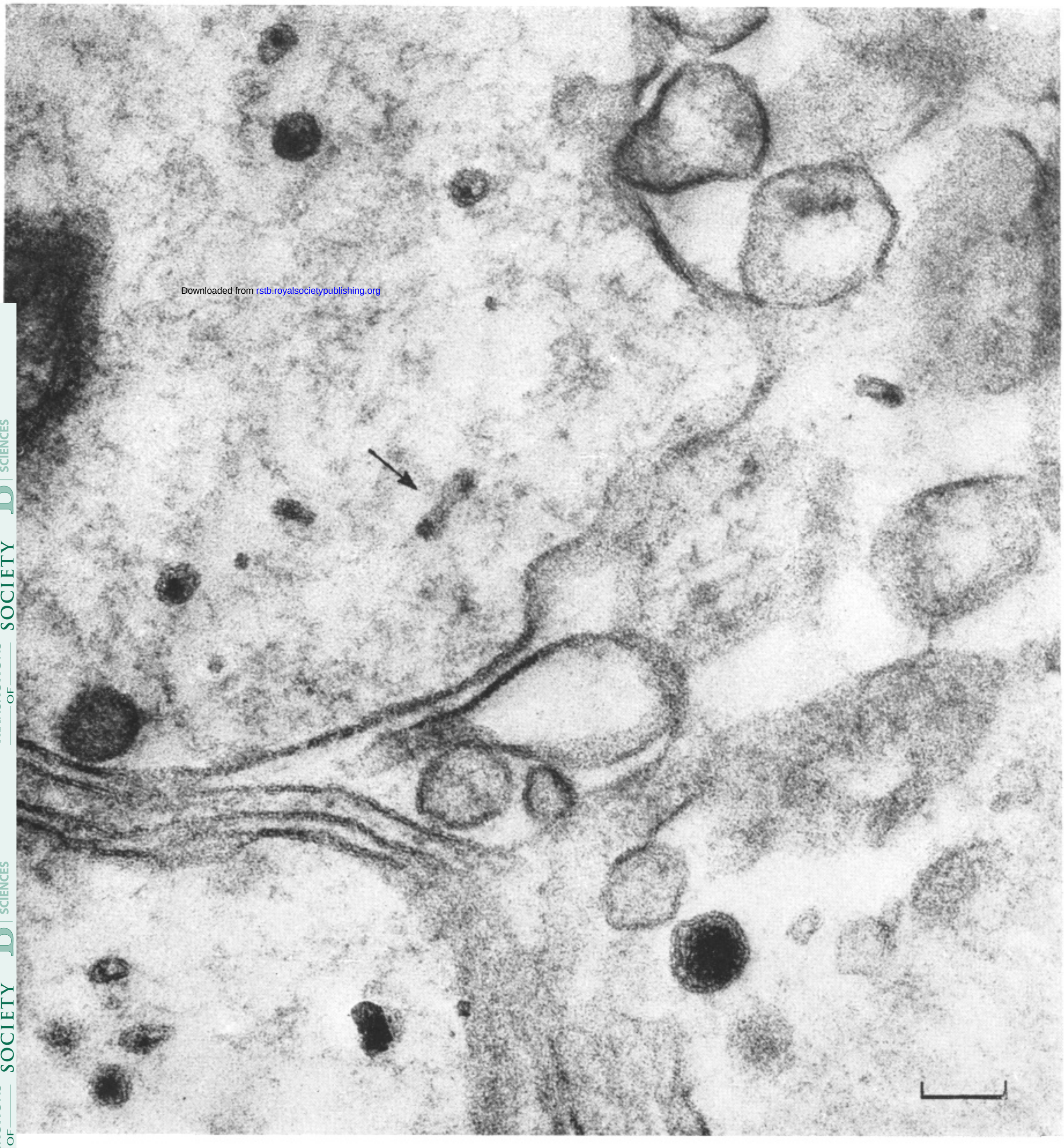
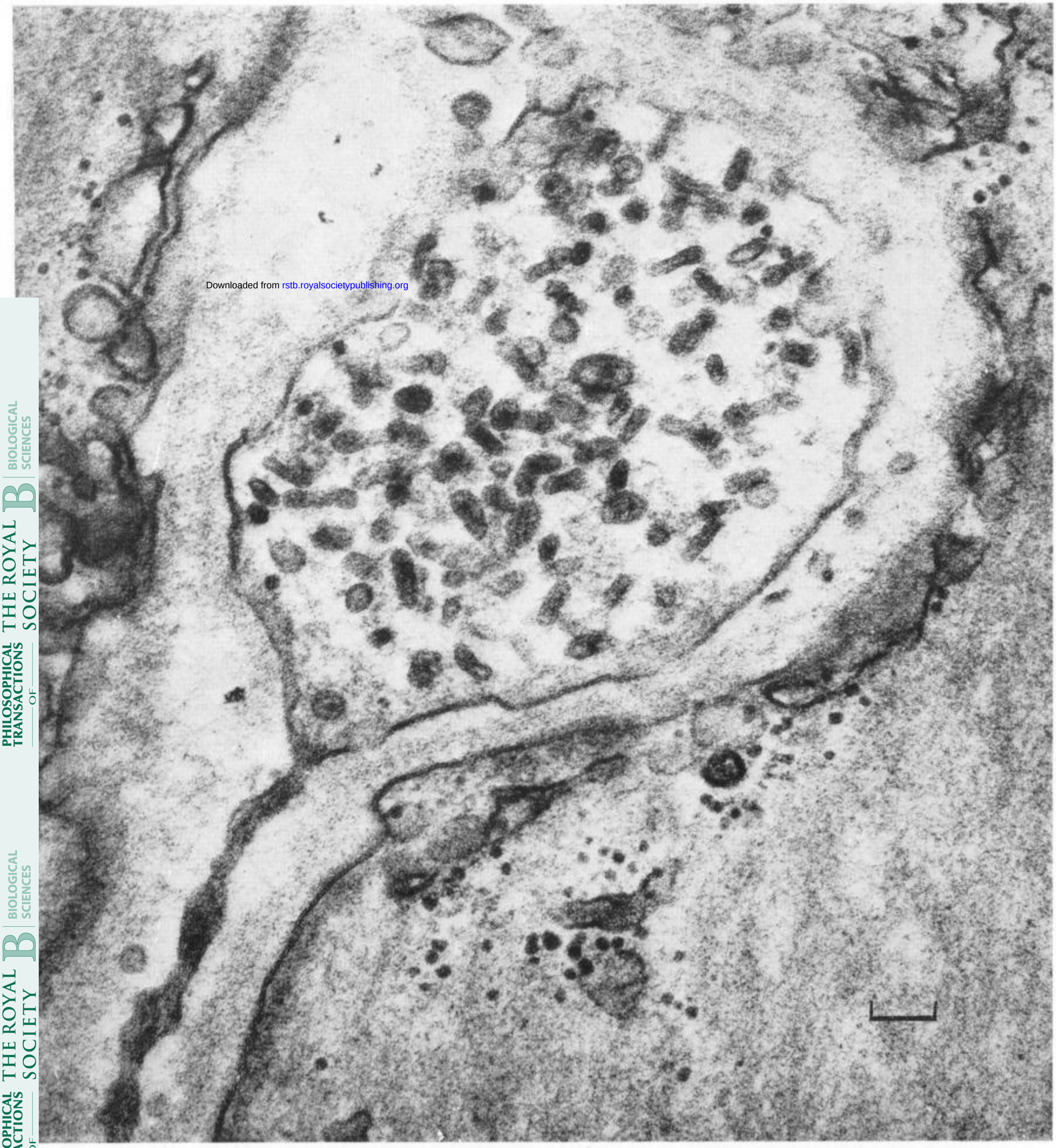


FIGURE 4. Varicosity containing elongated vesicle with two dense cores ↑. Calibration 0.1 μm.



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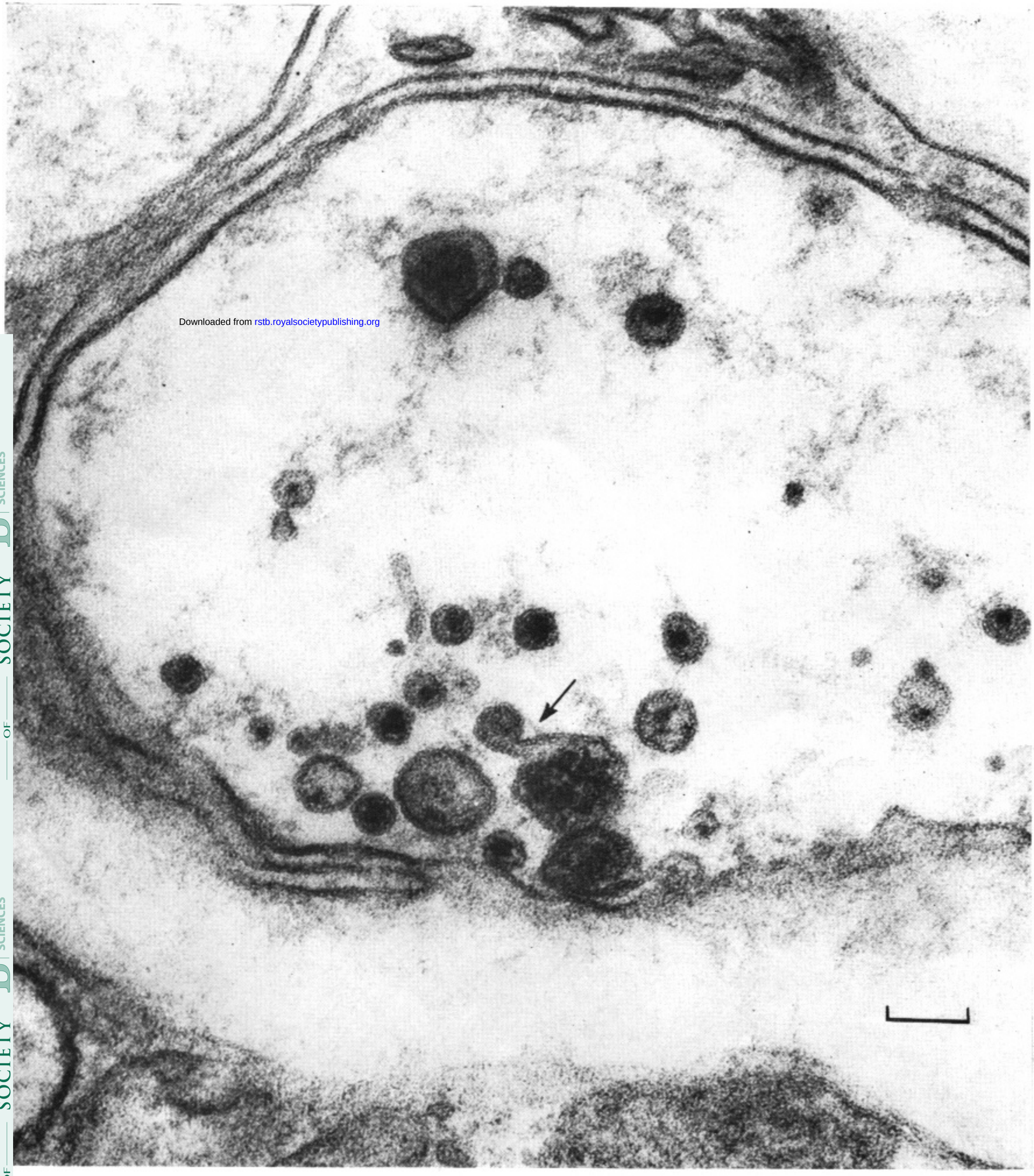

FIGURE 6. Large dense-cored vesicle with a process suggesting the pinching-off of a small vesicle ↑. Calibration 0.1 μm.

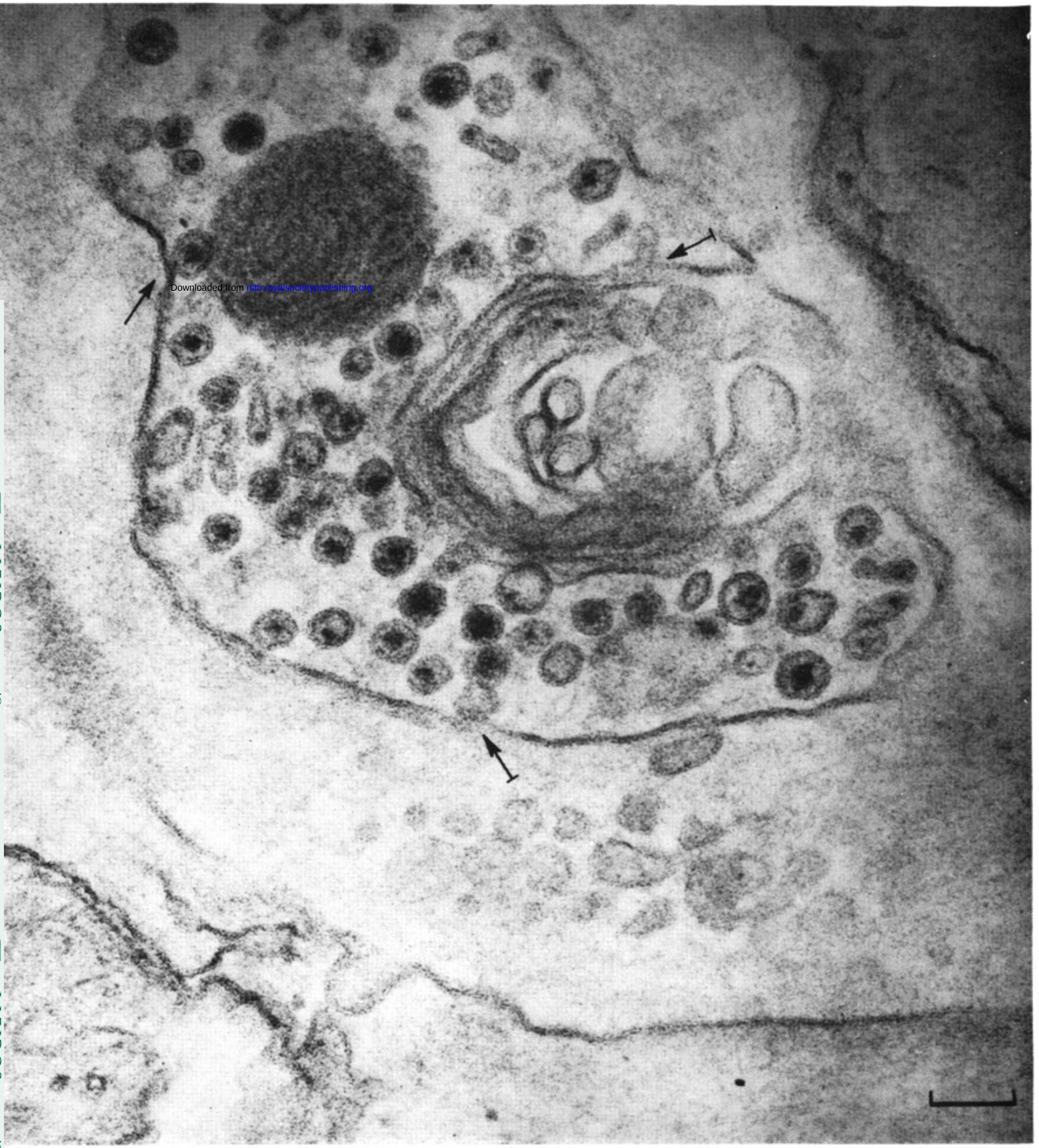

FIGURE 7. Fusion of membrane of small vesicles (cored at ↑ and empty at ↕) with varicosity membrane. Calibration 0.1 μm.

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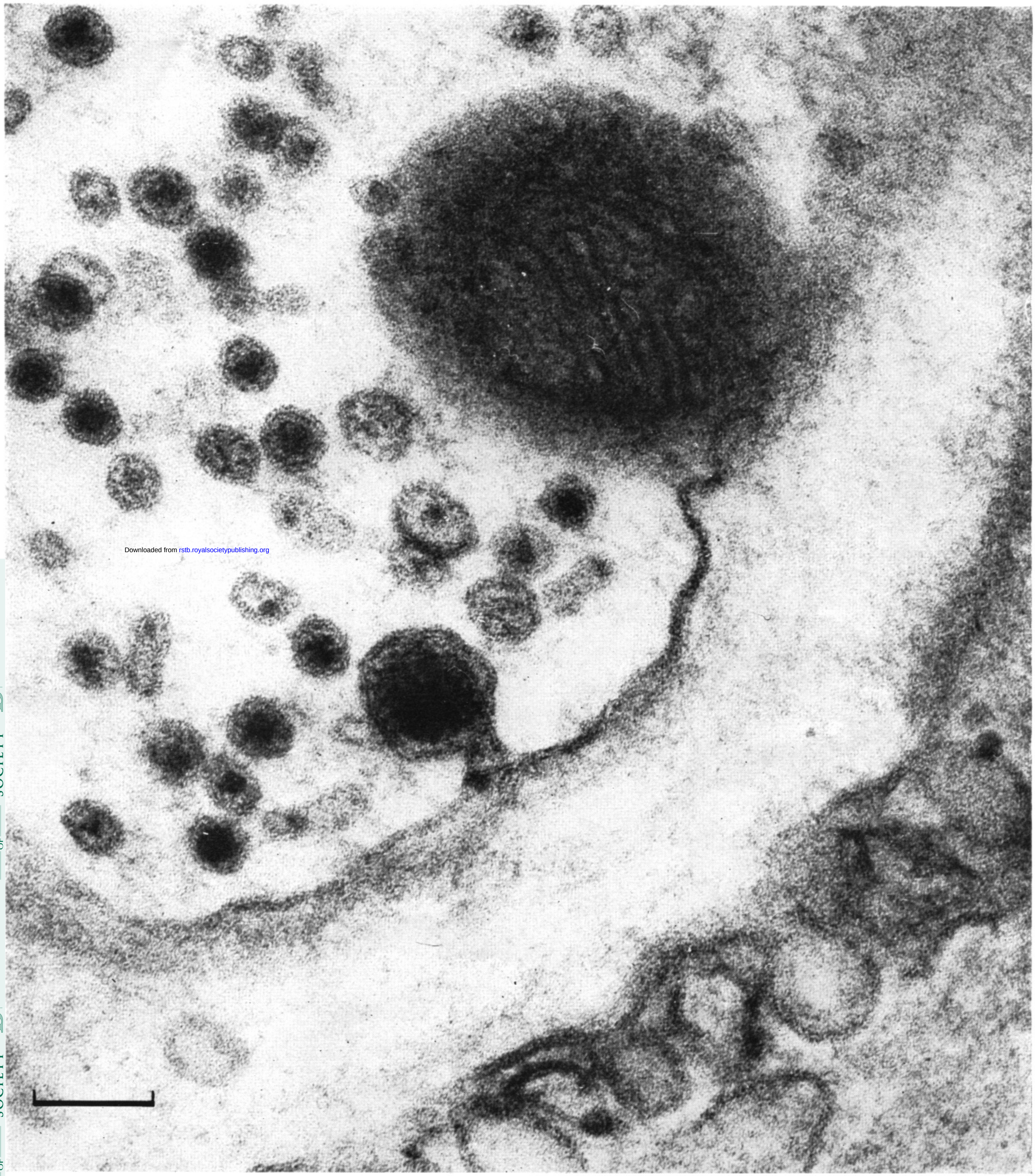

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FIGURE 8. Fusion of membranes of large vesicle and axon varicosity. Calibration 0.1 μm.