PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ultrastructural Data on the Cytology and Cytochemistry of the Autonomic Nervous System

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Ultrastructural data on the cytology and cytochemistry of the autonomic nervous system

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[Plates 54 and 55]

Our demonstration deals with two topics: the first one concerns the so-called large granular vesicles (l.g.v.) or large dense-core vesicles in the nerve fibres and cells of the autonomic nervous system; the second one is related with the cytochemistry of noradrenaline in the sympathetic adrenergic neurons.

All the reported observations were made on rat tissues. The l.g.v. measure 70 to 120 nm in diameter; the centre of such vesicles contains a more or less dense granule. This aspect is seen after fixation with osmium tetroxide as well as after fixation with glutaraldehyde or by a mixture of formaldehyde and glutaraldehyde, followed by osmium tetroxide. In the last case, the granule appears generally more electron-dense than after osmium tetroxide alone. The l.g.v. are widely distributed in the different types of fibres of the autonomic nervous system. They were first described in axon terminals of the preganglionic cholinergic fibres (Taxi 1961) and later they were observed in adrenergic peripheral nerve fibres, where they are associated with small granular vesicles (s.g.v.); these s.g.v. have a similar size to the classical agranular synaptic vesicles. No matter which fixative is used, the central granule of s.g.v. is always extremely dense.

The two kinds of granular vesicles (l.g.v. versus s.g.v.) differ also in their behaviour under the action of reserpine (5 mg/kg i.p.). The dense granules of s.g.v. are depleted (Pellegrino de Iraldi & de Robertis 1961), whereas those of l.g.v. remain practically unaffected (figure 1), in both cholinergic and in adrenergic nerve fibres (Taxi 1965).

Vesicles morphologically indistinguishable from l.g.v. were also described in a special type of cell of the superior cervical ganglion of the rat: the chromaffin-like cells, first observed by Siegrist, de Ribaupierre, Dolivo & Rouiller (1966). In these cells, l.g.v. are numerous, and they are mostly localized beneath the plasma membrane. Furthermore, there are specialized zones of the plasma membrane of the cell body or of their processes, where a row or a cluster of l.g.v., sometimes mixed with s.g.v., are associated with a membrane 'thickening' (Williams 1967; Matthews & Raisman 1969). Sometimes, empty vesicles of the same size as l.g.v. are observed attached to the membrane, and they may be interpreted as the morphological feature of a release process of the dense contents of the l.g.v. (figure 2). Of course, such pictures are not as conclusive as the images of exocytosis, which, until now, have not been found either in the chromaffin-like cells or in the adrenergic or cholinergic peripheral nerve fibres of the rat.

The other set of micrographs illustrates a contribution of high resolution autoradiography to the cytochemistry of noradrenaline. Cytochemical evidences of two pools of noradrenaline in the sympathetic neurons were presented in rats injected with [^{3}H]noradrenaline. As it was pointed out first by Wolfe, Potter, Richardson & Axelrod (1962), one pool of [^{3}H]noradrenaline is related to the s.g.v. and localized mainly in the peripheral region of the nerve fibres. This pool is very sensitive to reserpine, and no labelling is detectable a few hours after reserpine

injection. On the other hand, this pool seems to be protected against the action of monoamine oxidases: the labelling is nearly the same with or without pretreatment with amon oamine oxidase inhibitor.

The other pool may be visualized independently of the presence of s.g.v. It corresponds, for instance, to the labelling of the perikaryon of sympathetic neurons in the cervical superior ganglion. In this case, there is no obvious correlation between the silver grains of the autoradiographic reaction and any discrete organelle of the cytoplasm. Nevertheless, it is hard to assume that a soluble component like [^{3}H]noradrenaline can be maintained in the cytoplasm after the electron microscope preparatory procedures, if it is not bound to some component of the 'background' (= hyaloplasm). Unlike the first pool, this second pool seems largely sensitive to monoamine oxidases, and relatively insensitive to reserpine. In fact, the labelling of the perikarya is poor when the injection of [^{3}H]noradrenaline was not preceded by the treatment with a monoamine oxidase inhibitor (catron, 5 mg/kg i.p.), whereas the autoradiographic reaction is nearly the same in presence or in absence of reserpine.

In the peripheral adrenergic nerve fibres a reserpine-resistant pool, not correlated with s.g.v. may be recognized in animals treated with successively reserpine and, some hours later, with an inhibitor of monoamine oxidase, before the injection of [^{3}H]noradrenaline. Apparently, this second pool is smaller than the s.g.v.-bound one, but precise quantitative estimations are not feasible by this technique. Illustrations of this second pool may be found in earlier papers (Taxi 1969; Taxi & Droz 1969).

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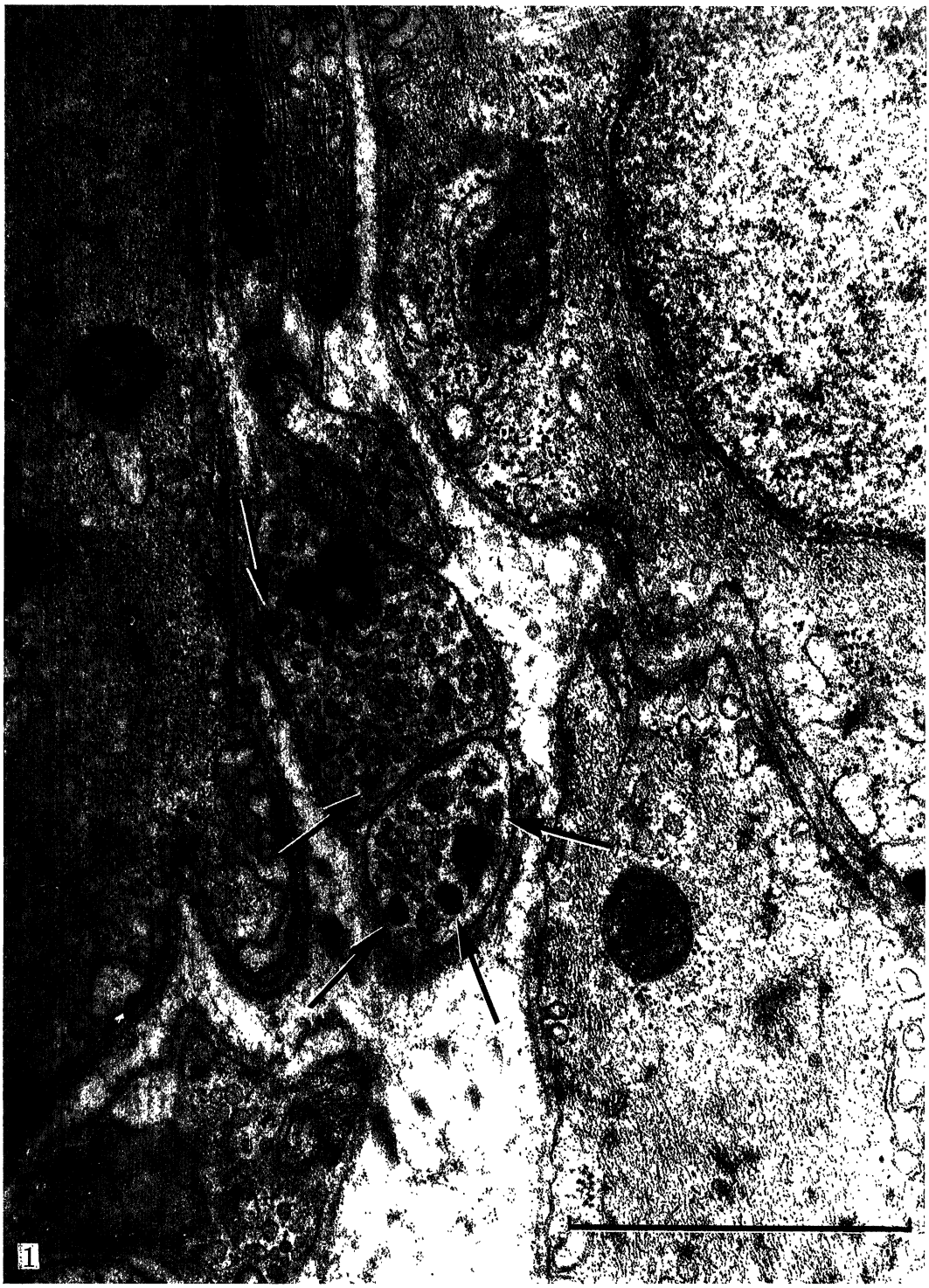

FIGURE 1. Rat vas deferens. (Magn. × 51000.) The fixation was performed 24 h after an injection of reserpine (5 mg/kg i.p.). In the nerve fibres, the small vesicles are all empty, while l.g.v. (arrows) remain normal.

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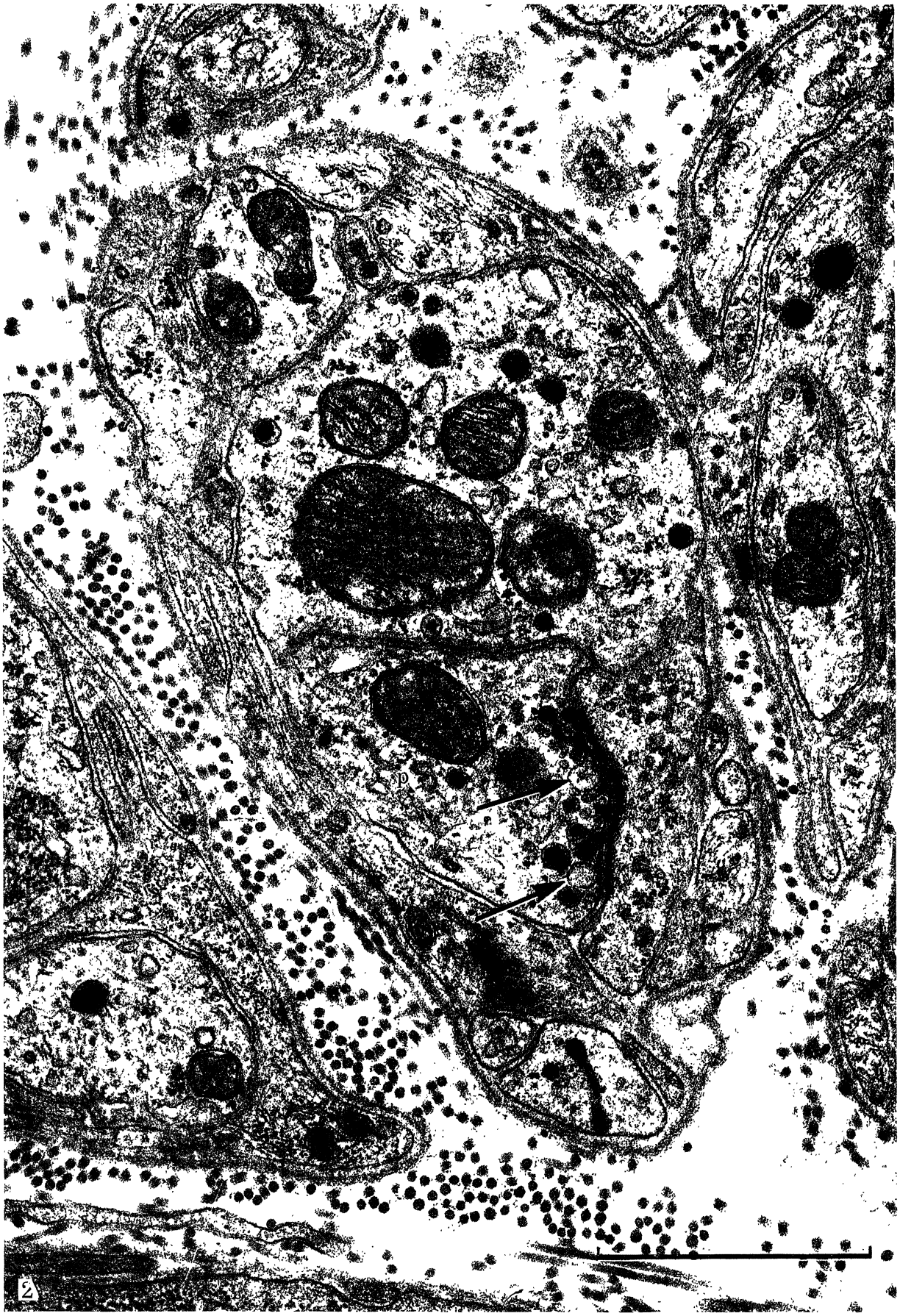

FIGURE 2. Rat superior cervical ganglion. (Magn. ×48000.) A process of a chromaffin-like cell (p) shows a synaptoid zone, characterized by membrane thickenings of the process, and of another one, probably a dendrite. Several l.g.v. are closely associated with this membrane specialization. Among them two vesicles, closely attached to the membrane (arrows), appear empty. Such a picture suggests that these vesicles have released their contents in this position.

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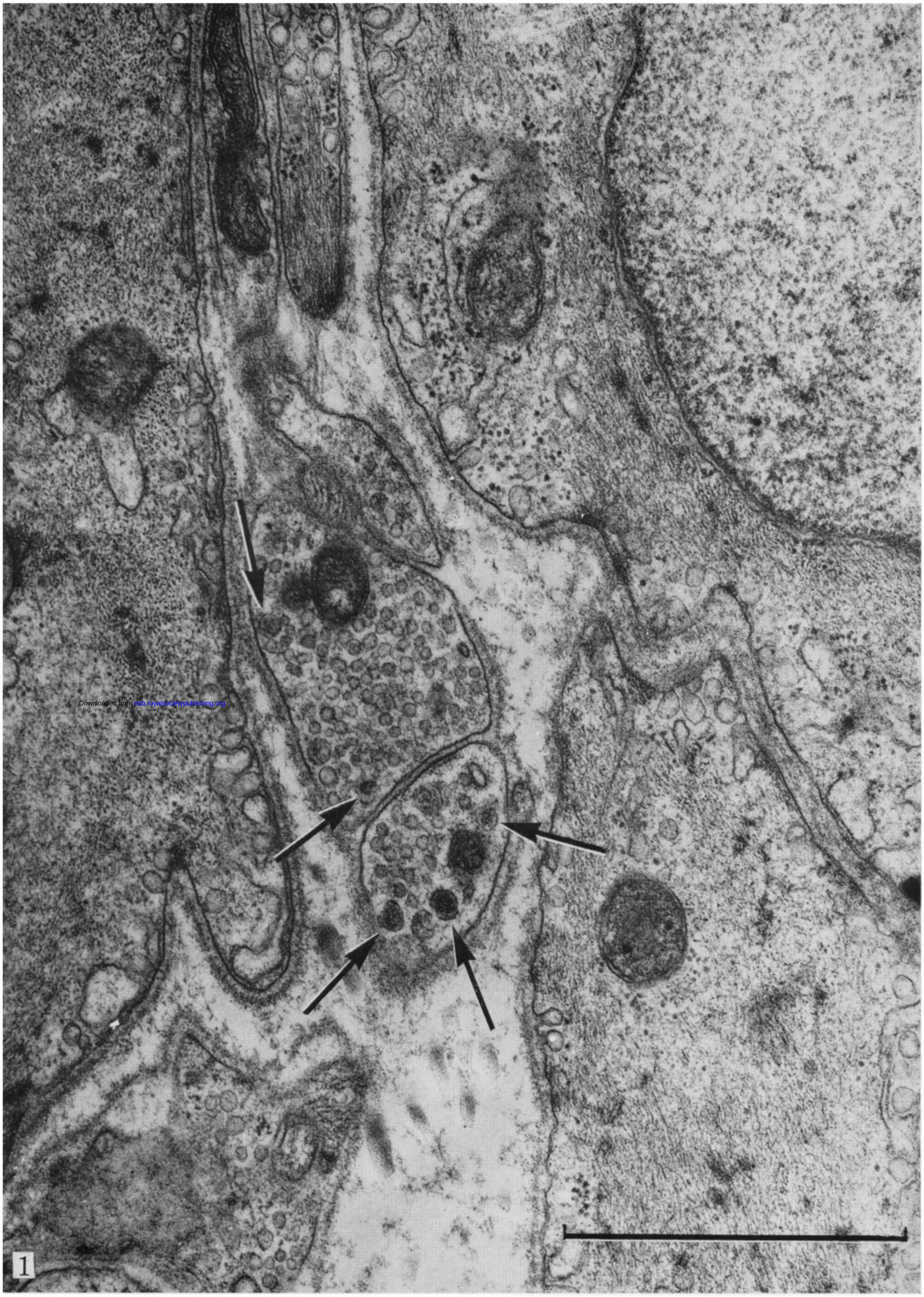

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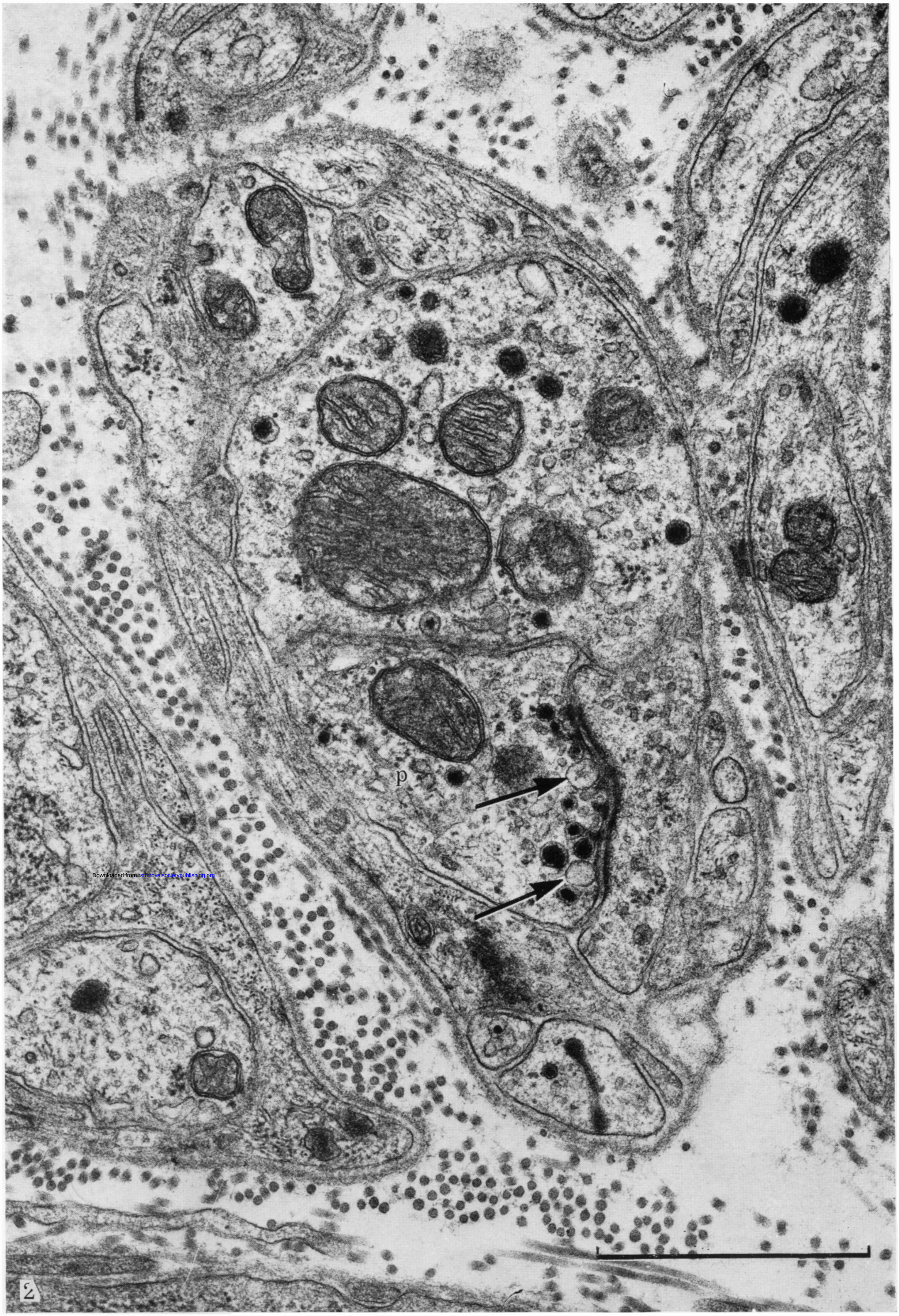

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