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Freeze-fracturing of presynaptic membranes in the central nervous system

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[Plates 67 and 68]

That synaptic vesicles with a diameter of about 50 nm could be demonstrated by the freeze-fracturing technique was an important contribution to the knowledge of synaptic fine structure (Moor, Pfenninger & Akert 1969; Akert, Moor, Pfenninger & Sandri 1969). The finding of spheric profiles (figures 1, 3*a*, *c*) in rapidly frozen tissue renders an artefactual origin of vesicles very unlikely. However, the chief advantage of freeze-fracturing lies in the visualization of membrane surfaces which are of special interest in presynaptic nerve terminals.

The present micrographs were obtained from freeze-fractured monkey, cat, and rat spinal cord, cerebral cortex, and subfornical organ as well as from pigeon optic tectum. Special attention was paid to areas in which large pieces of membranes remained attached to the surfaces of nerve cells (figures 1, 3*a*). This situation suggests a special type of affinity between the two elements, and the association of one of the membranes with clusters of synaptic vesicles indicates that presynaptic membranes may be involved.

The cytoplasmic side of the membrane shows aggregations of small protuberances (figure 1) which sometimes have a crater-like structure (figure 3*a*), and which are seen as 'pits' from the opposite side (figure 2). These 'humps' (internal surface) or 'pits' (external surface) resemble the exo- and endocytotic openings in the plasmalemma of endothelium (figure 3*b*) and in extrasynaptic sites of nerve cells (figure 3*c*). However, they have an outer diameter of less than 20 nm and therefore have not much more than half the size of pinocytotic pores. As they seem to occur in presynaptic membranes exclusively, they may be called '*synaptopores*'. Morphometric analysis suggests that the synaptopore distribution (figure 2) reflects the hexagonal arrangement of synaptic vesicles on the presynaptic membrane shown in electronmicroscopic sections when special contrast methods are applied (Akert *et al.* 1969).

Hence a close relationship between the synaptopores and the transmitter vesicles may be inferred suggesting that the synaptopores are *attachment sites* for the latter. For a number of reasons, it seems unlikely that the synaptic vesicles fuse with the cytoplasmic membranes to release their content in the same way as the plasmalemmal vesicles do (Palade & Bruns 1968). The assumption of a temporary contact (tight or gap junction?) between vesicle and plasmalemma seems more consistent with both physiological and morphological observations.

References (Pfenninger *et al.*)

Akert, K., Moor, H., Pfenninger, K. & Sandri, C. 1969 Contribution of new impregnation methods and freeze-etching to the problem of synaptic fine structure. In *Mechanisms of synaptic transmission* (ed. K. Akert and P. Waser), pp. 223–240. Progr. Brain Res. vol. 31. Amsterdam: Elsevier.

Moor, H., Pfenninger, K. & Akert, K. 1969 Synaptic vesicles in electron micrographs of freeze-etched nerve terminals. *Science, N.Y.* **164**, 1405–1407.

Palade, G. E. & Bruns, R. R. 1968 Structural modulations of plasmalemmal vesicles. *J. Cell Biol.* **37**, 633–649.

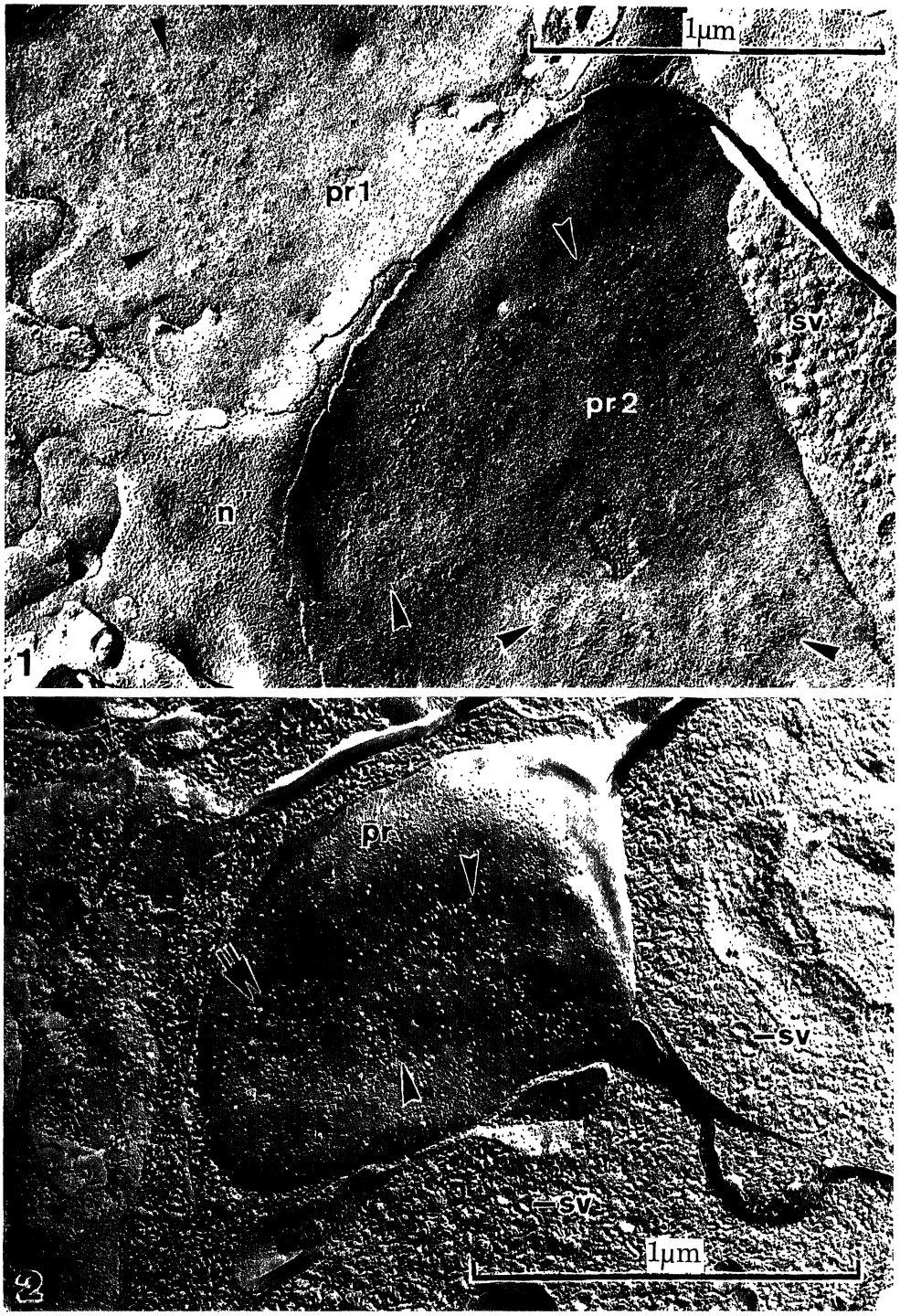

FIGURE 1. Cytoplasmic side of two membrane fragments exposing the presynaptic area (pr1, pr2). They are attached to another nerve element (n) and one of them (pr2) is associated with a large number of synaptic vesicles (sv). Note the clusters of small protuberances, called 'synaptopores' (arrows), which are more clearly recognized in pr1. Cat, spinal cord anterior horn. Glutaraldehyde fixation. (Primary magnification: ×20000.)

FIGURE 2. External side of presynaptic membrane (pr). Synaptopores appear as pits (arrows). At left, they form a more or less complete hexagonal pattern (double arrow). Neighbouring presynaptic bags are filled with synaptic vesicles (sv). Cat, spinal cord anterior horn. Glutaraldehyde fixation. (Primary magnification: ×20000.)

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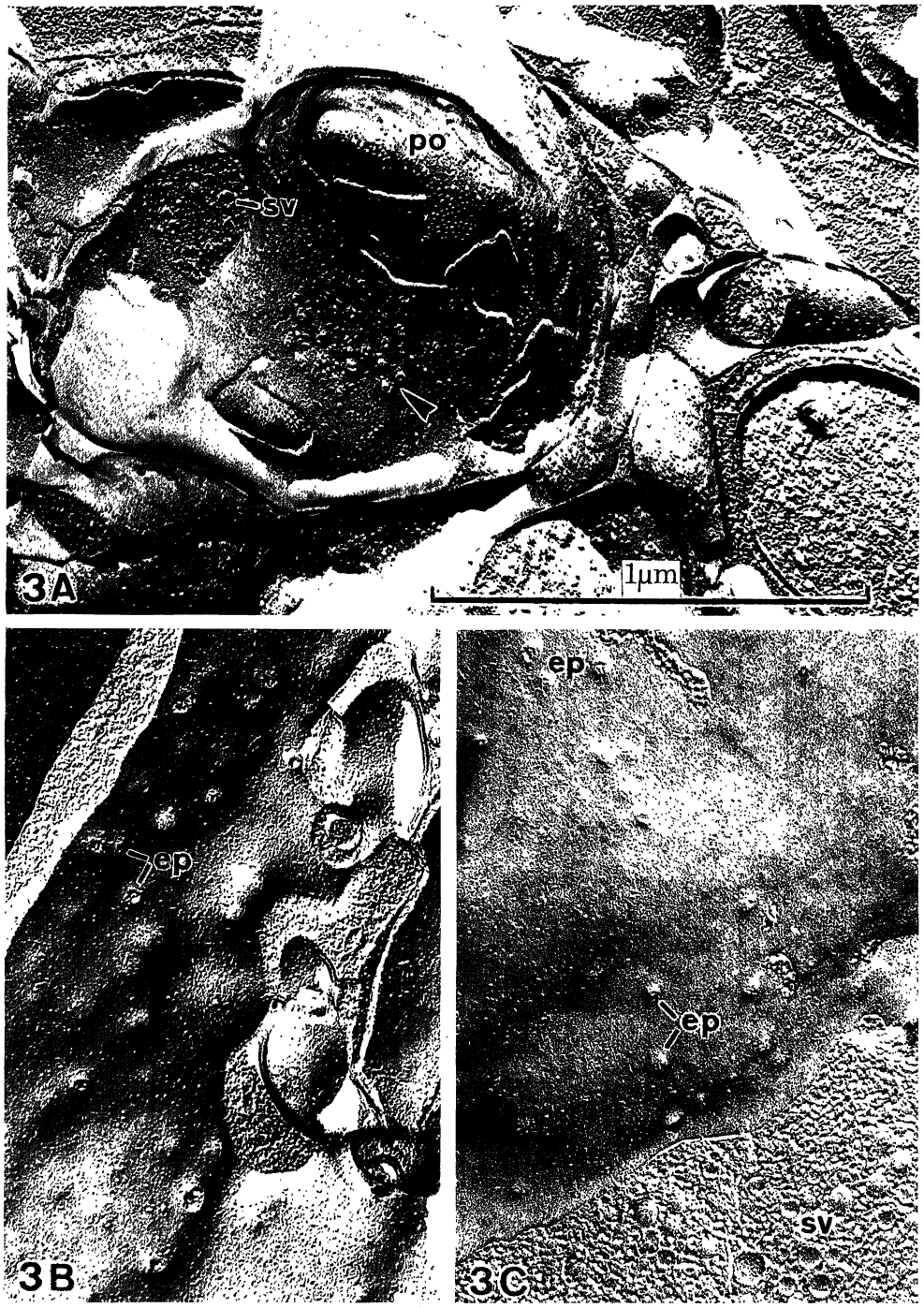

FIGURE 3. Comparison between synaptopores and the fusion sites of plasmalemmal vesicles. All electronmicrographs are reproduced at identical magnification (primary magnification ×20000). Note that the synaptopores (figure 3*a*) have only about half the size of exo/endocytotic pores (figure 3*b* and 3*c*) and that they are clustered in well defined areas. (*a*) Cytoplasmic side of presynaptic membrane in pigeon optic tectum. A small island of cytoplasm with synaptic vesicles (sv) remained attached. Arrow points at synaptopores, po = postsynaptic element. (*b*) Capillary endothelium of cat subfornical organ. The cytoplasmic membrane surface contains a number of stomata (ep) representing fusion (exocytosis) or fission (endocytosis) sites of plasmalemmal vesicles. (*c*) Nerve terminal of cat subfornical organ. The cytoplasmic surface of the plasmalemma contains a number of exo/endocytotic stomata (ep). Fragments of cytoplasm with synaptic vesicles (sv) are attached to the membrane.

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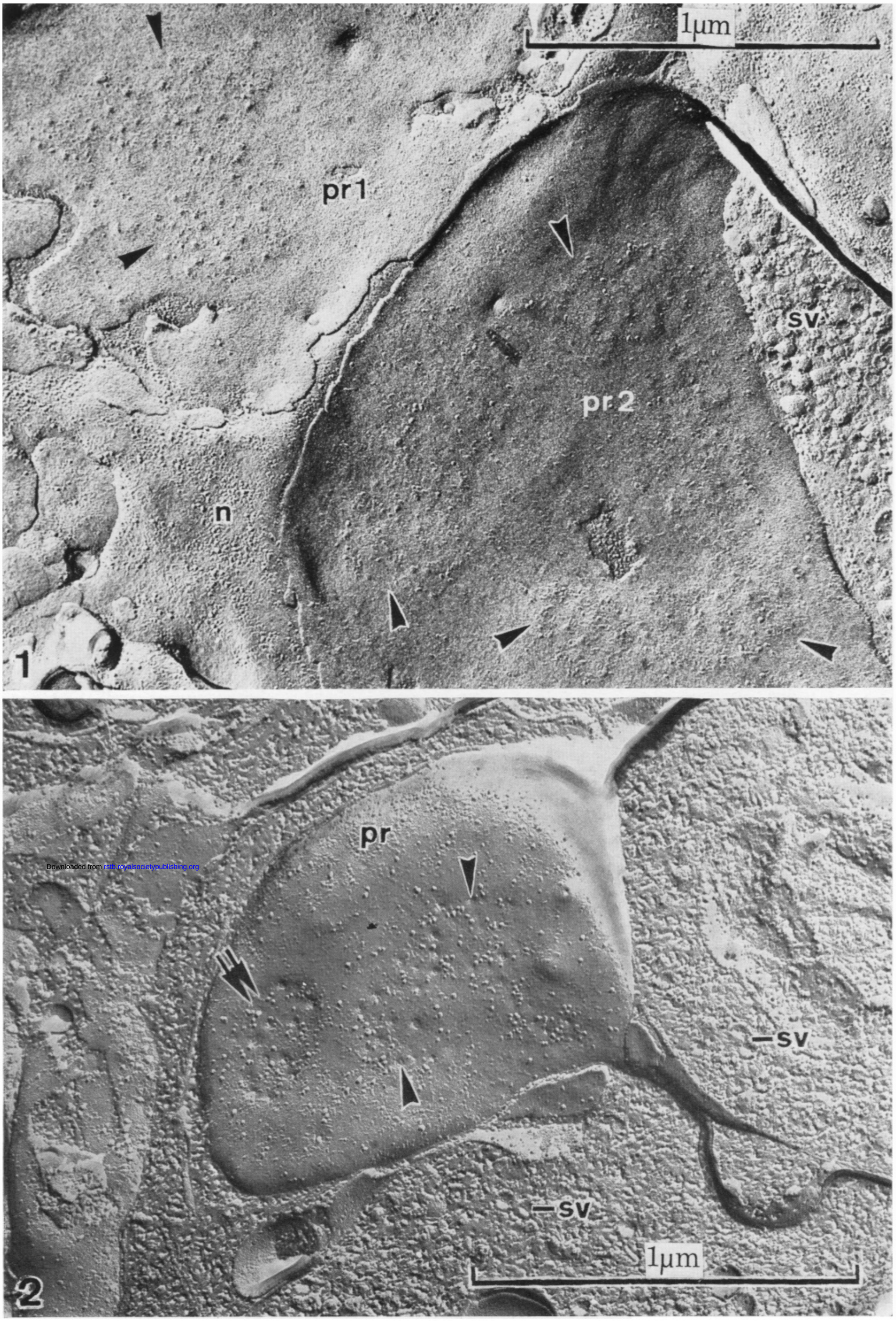

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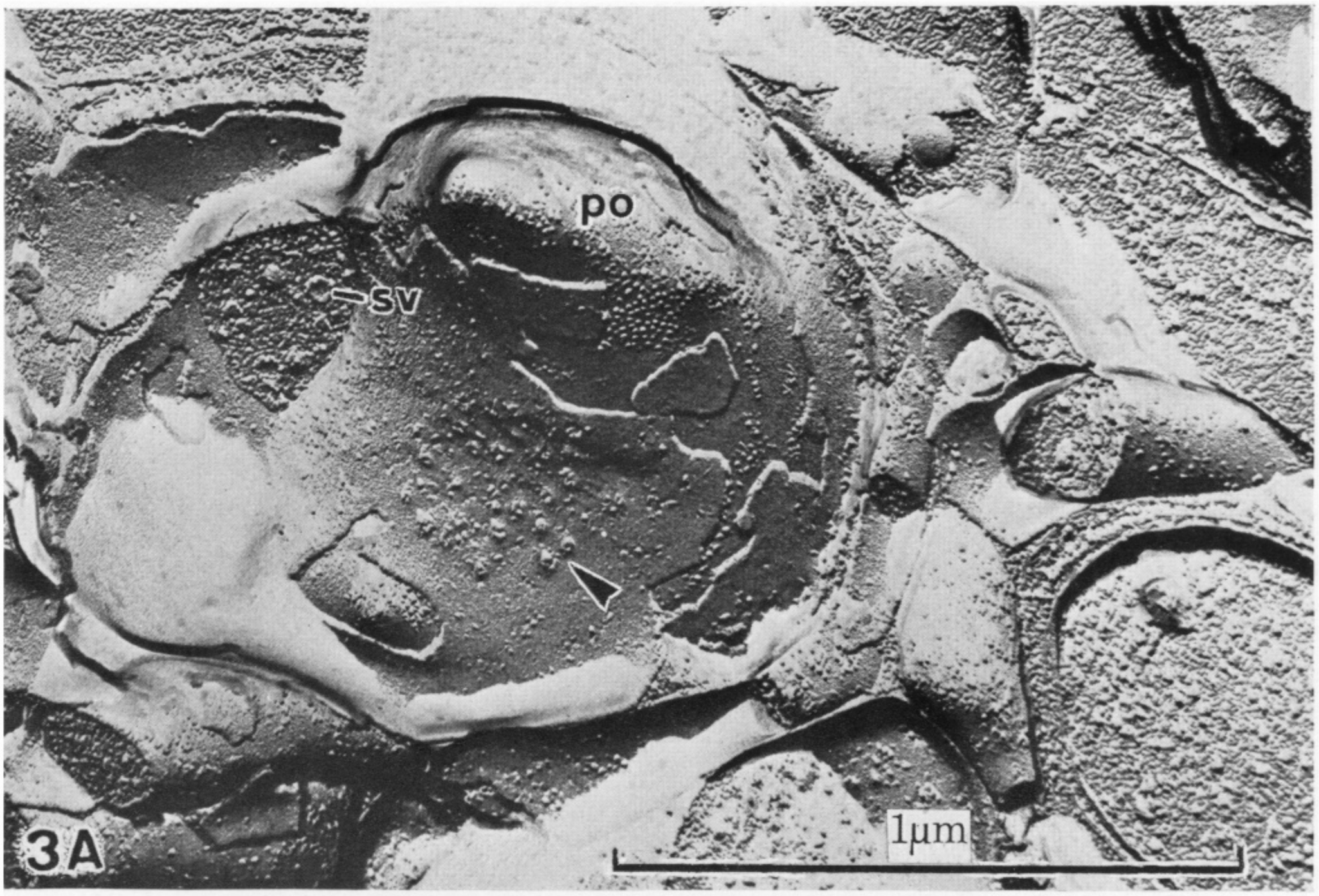

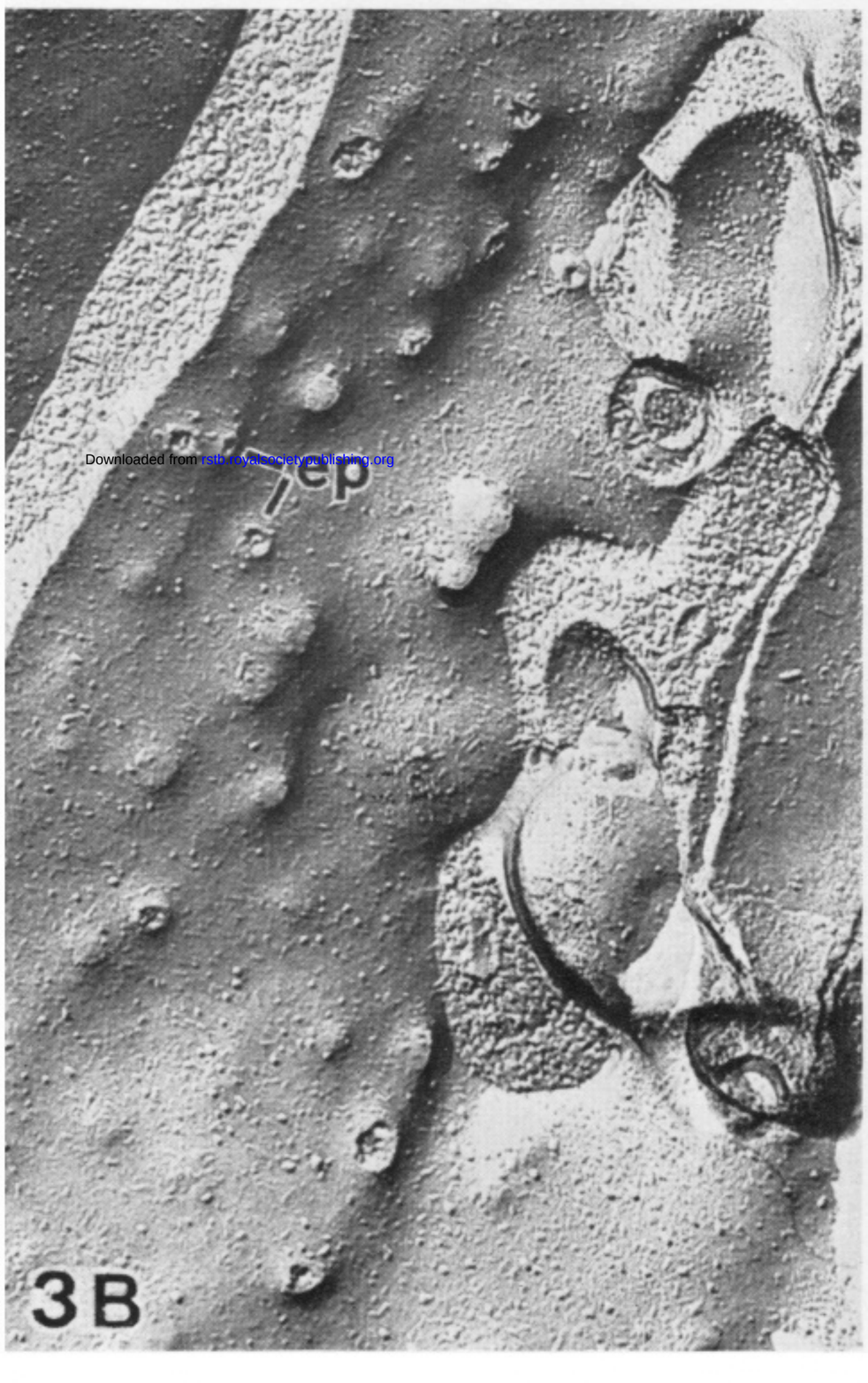

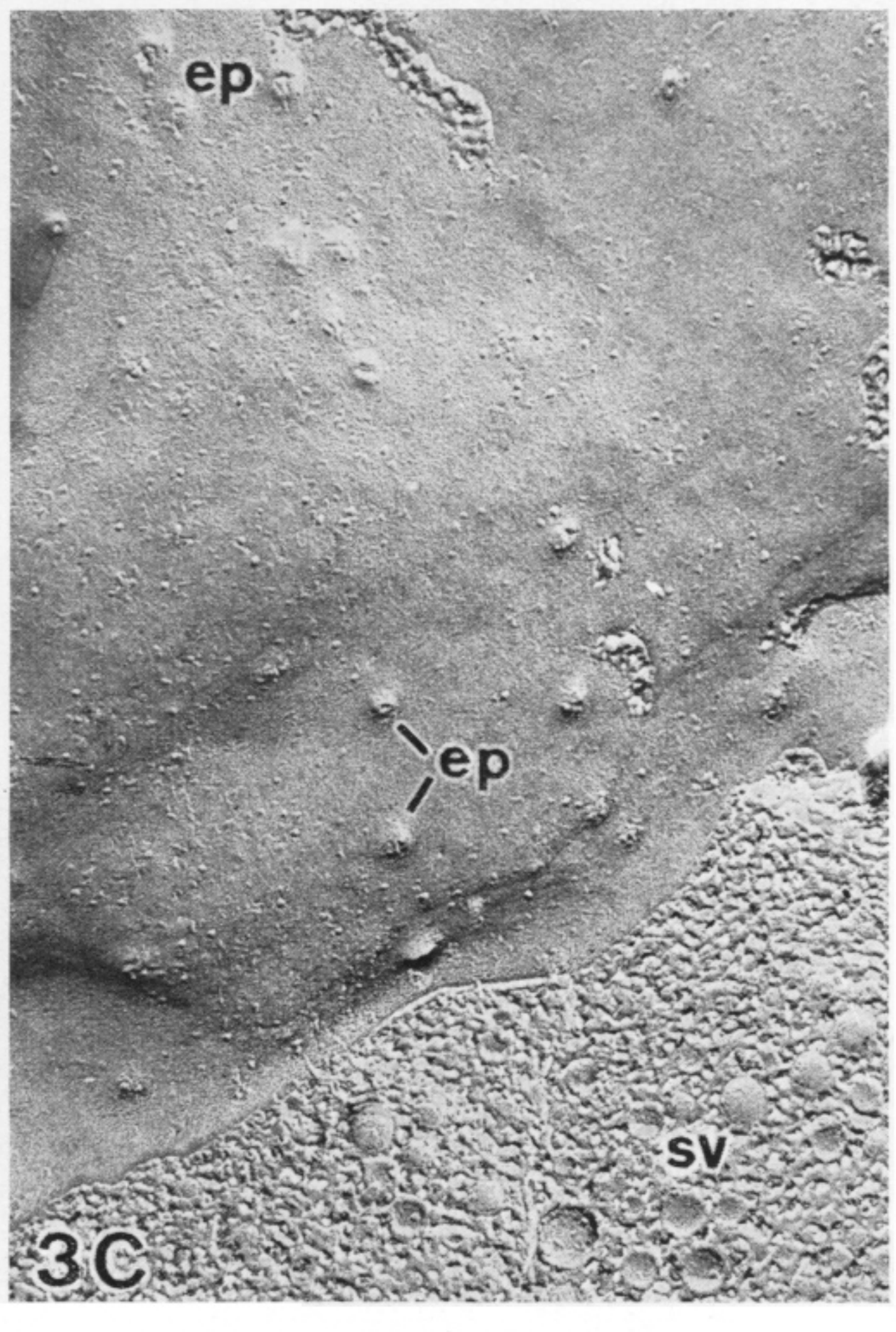

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