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Synaptic vesicles in freeze-etched electric tissue of *Torpedo*

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[Plates 65 and 66]

The electric organs of the elasmobranch, *Torpedo*, are composed of stacks of hexagonal cells, called electroplaques, each of which is several millimetres in diameter, but only 5 to 10 μm thick. The ventral surface of each cell is known to be richly innervated by cholinergic nerve terminals (Feldberg & Fessard 1942; Sheridan 1965; Grundfest 1967; Israël & Gautron 1969). We have taken advantage of the high density of innervation of this tissue, and of the fact that

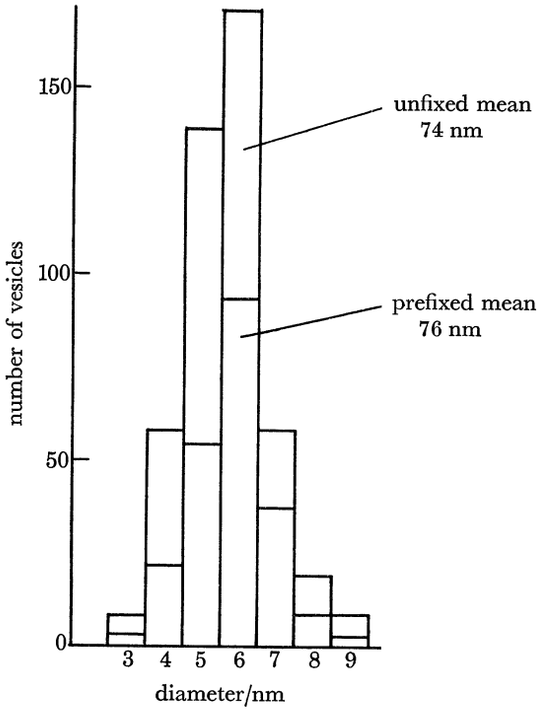

Figure 2. Histogram of vesicle diameters.

freeze-etching readily reveals membrane surfaces (Moor & Mühlethaler 1963), to examine contacts between the synaptic vesicles and the presynaptic membranes of these nerve terminals (Nickel & Potter 1970).

Figure 1 shows a low-power electron micrograph of a replica of parts of several electroplaques; the plaques are seen edge-on. Nerve terminals appear as cylinders which follow the ventral surfaces of the electroplaques, lying between the postsynaptic membrane, with its

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characteristic arcuate infolds, and Schwann cell processes. The organization and simplicity of the tissue are such that the orientation of the synaptic cleft, and the identity of the pre- and postsynaptic membranes, can generally be established regardless of the direction of the fracture plane through the tissue.

In cross-fractured terminals (e.g. figures 1, 3 and 4, plates 65 and 66), variable numbers of spherical, uncoated vesicles were seen, which were usually distributed towards the synaptic cleft. A histogram of the diameters of 464 vesicles (figure 2) showed a single population and a mean vesicle diameter of 75 nm. This distribution and range (30 to 120 nm) of vesicle sizes, and their mean diameter, correspond closely to that obtained with thin sections (Sheridan 1965; Sheridan, Whittaker & Israël 1966; Israël & Gautron 1969; E. Nickel, unpublished). In addition, it is known that a large fraction of the acetylcholine in these electric organs can be isolated in a single population of vesicles having this size range (Sheridan *et al.* 1966; Israël, Gautron & Lesbats 1968; Potter & Nickel 1970). Thus although the intraterminal vesicles of *Torpedo* electric nerves are larger than those seen in mammalian nerves, it may be concluded that they are synaptic vesicles. No other vesicles have been seen in these terminals.

In tissues which had been prefixed before freeze-etching, vesicles were frequently seen to touch the presynaptic membrane (e.g. figure 3), and often to underlie bulges in it (cf. Nickel & Potter 1970), but not to open through the membrane. In contrast, with unfixed tissues, cross-fractures of the synaptic cleft (figure 4) and views of the presynaptic membrane from both sides (figures 5 and 6) showed vesicles fused to the membrane, and open to the synaptic cleft. Such fused vesicles were not seen in association with parts of the nerve terminal membrane facing Schwann cells.

Open vesicles have only been observed in unfixed tissue and this must mean either that they are lost readily during prefixation, or that they are produced without it (e.g. from the effect of adding 25 % glycerol to the tissue medium in order to prevent the formation of ice crystals during tissue freezing). This question may be resolved by freezing techniques which do not require either fixation or glycerol; at present, however, the vesicle–membrane contacts shown in figures 4 to 6 will be considered as a natural phenomenon. The morphology of fused vesicles does not distinguish exocytosis from pinocytosis. Other evidence, however, has indicated that similar views of fused vesicles in muscle capillaries represent different stages in a pinocytotic process (Nickel & Grieshaber 1969). Since acetylcholine is stored in vesicles in *Torpedo* electric organs, and since this neurotransmitter has been found to be released in quantal packets wherever its release has been examined in detail (Katz 1969), we consider that the present micrographs of fused vesicles are most consistent with an exocytotic process.

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FIGURE 1. Survey view of prefixed tissue. (Magn. × 15000.)

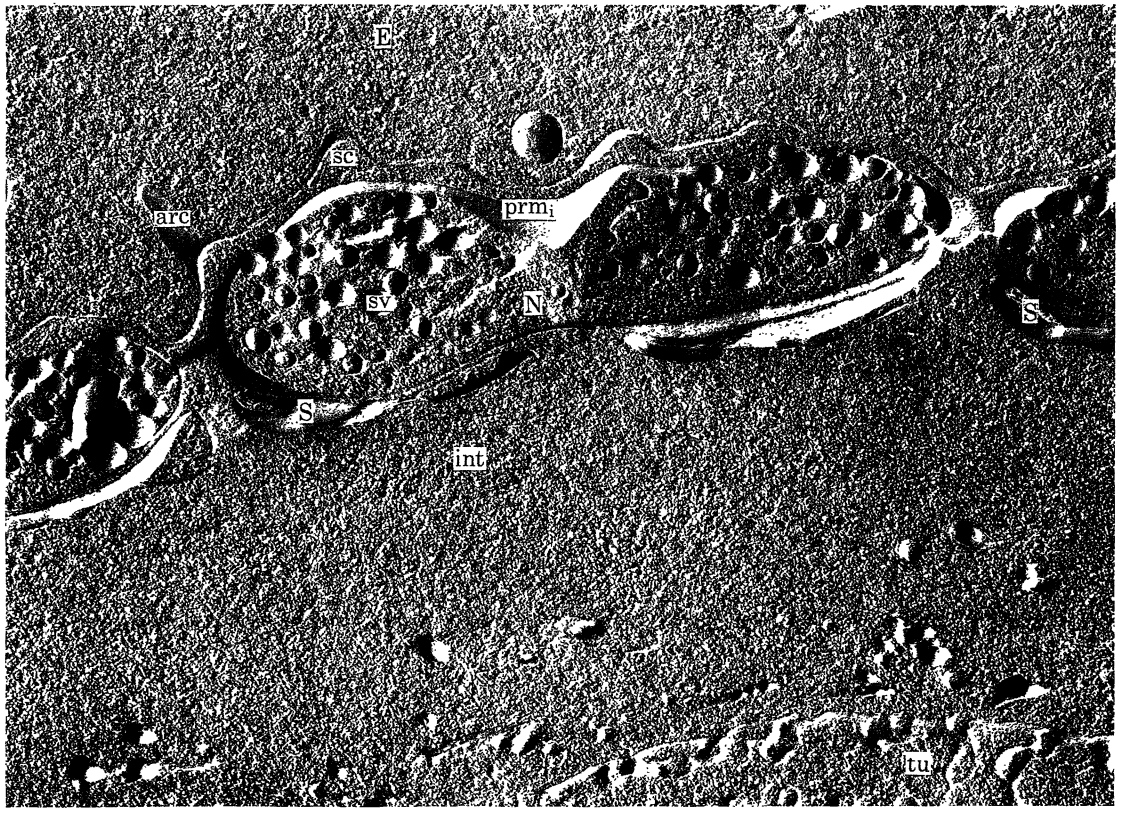

FIGURE 3. Synaptic vesicles in prefixed tissue, showing various vesicle-membrane contacts without formation of orifices. (Magn. × 66000.)

Abbreviations for all figures: E, electroplaque; N, nerve terminal; S, Schwann cell process; arc, arcuate infold of the postsynaptic membrane; int, interstitial space; pom, postsynaptic membrane; prm, presynaptic membrane; sc, synaptic cleft; sv, synaptic vesicle; tu, tubular network of the dorsal surface of electroplaque. (Subscripts i and o indicate views of the cell membrane from the inner and outer sides, respectively.)

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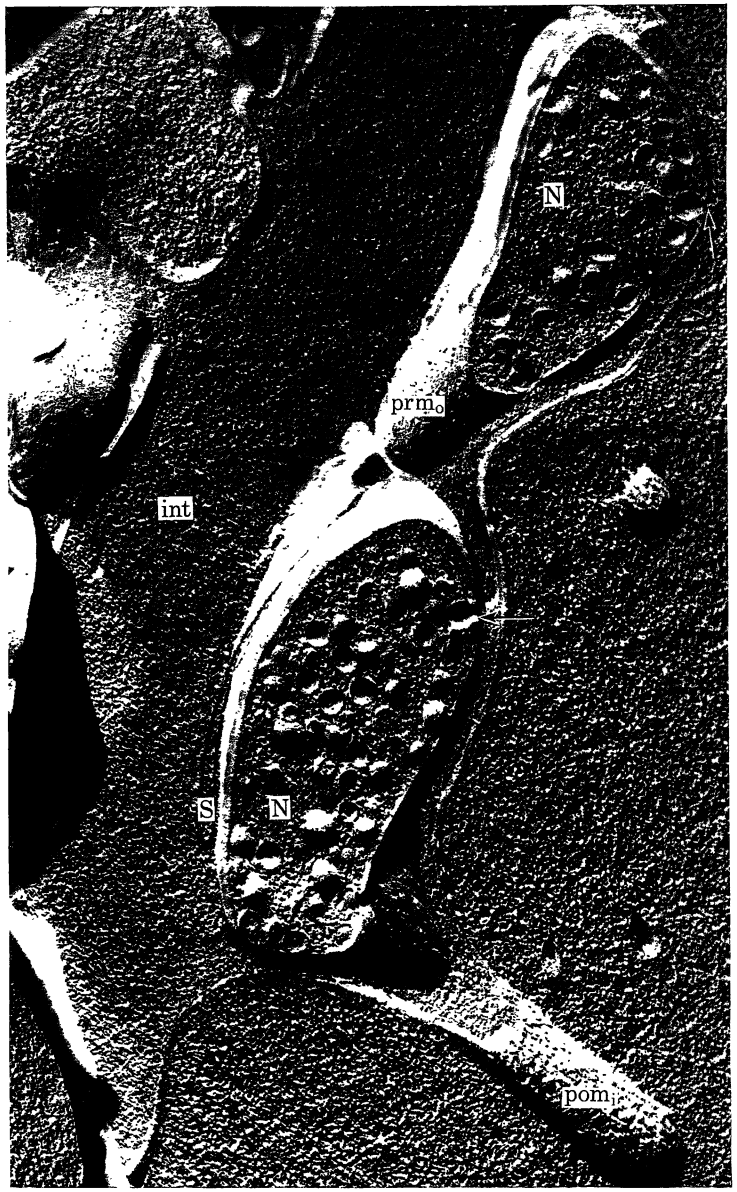

FIGURE 4. Synaptic vesicles fused to the presynaptic membrane and open to the synaptic cleft (arrows) (magn × 64000). This and the next two figures are reprinted from Nickel & Potter (1970) with permission from the publishers of *Brain research*.

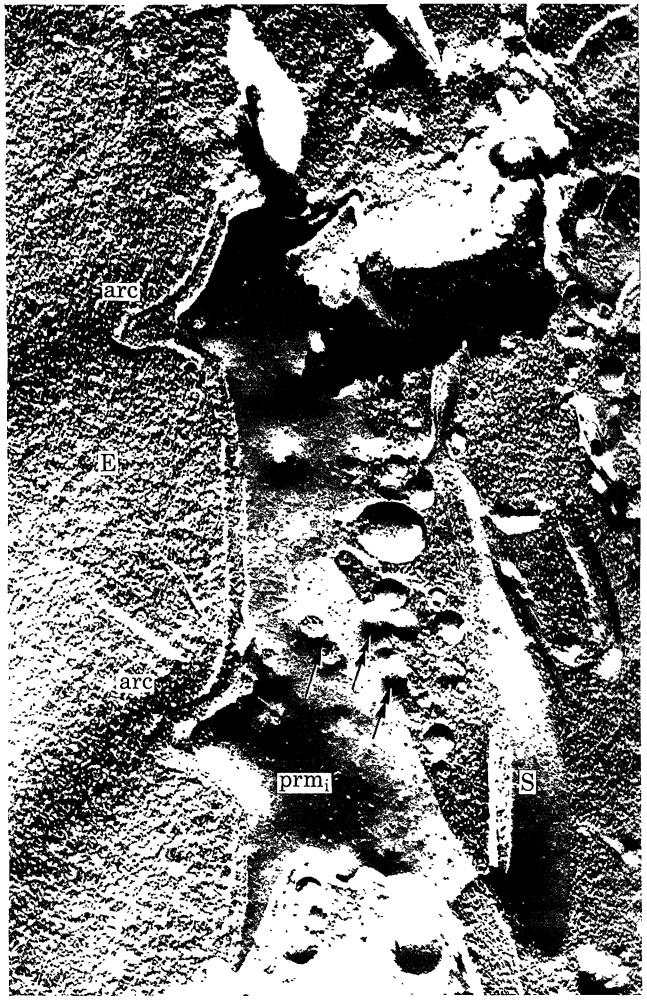

FIGURE 5. Synaptic vesicles fused to the presynaptic membrane, as seen from an intracellular view (magn. × 74000). Here the fracture plane first follows the inside of the presynaptic membrane, past fractured vesicle necks (simple arrows) through which the synaptic cleft can be seen, and then splits the neuroplasm. Two fused vesicles (double-headed arrows) are seen at both depths.

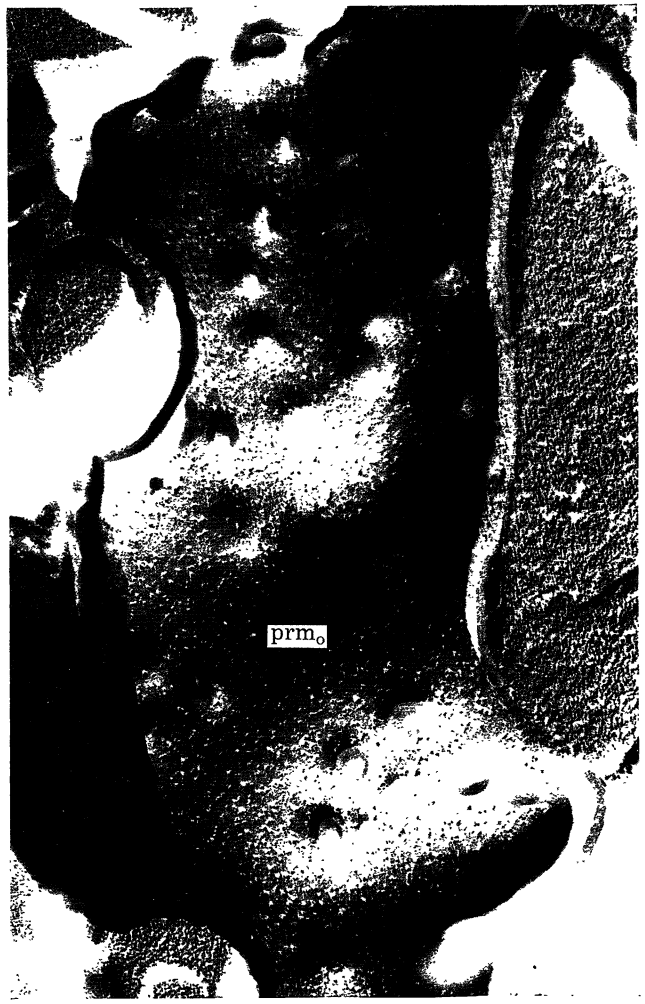

FIGURE 6. Synaptic vesicles fused to the presynaptic membrane, as seen from the synaptic cleft (magn. × 74000). The open necks of fused vesicles appear as pits or invaginations in the presynaptic membrane.

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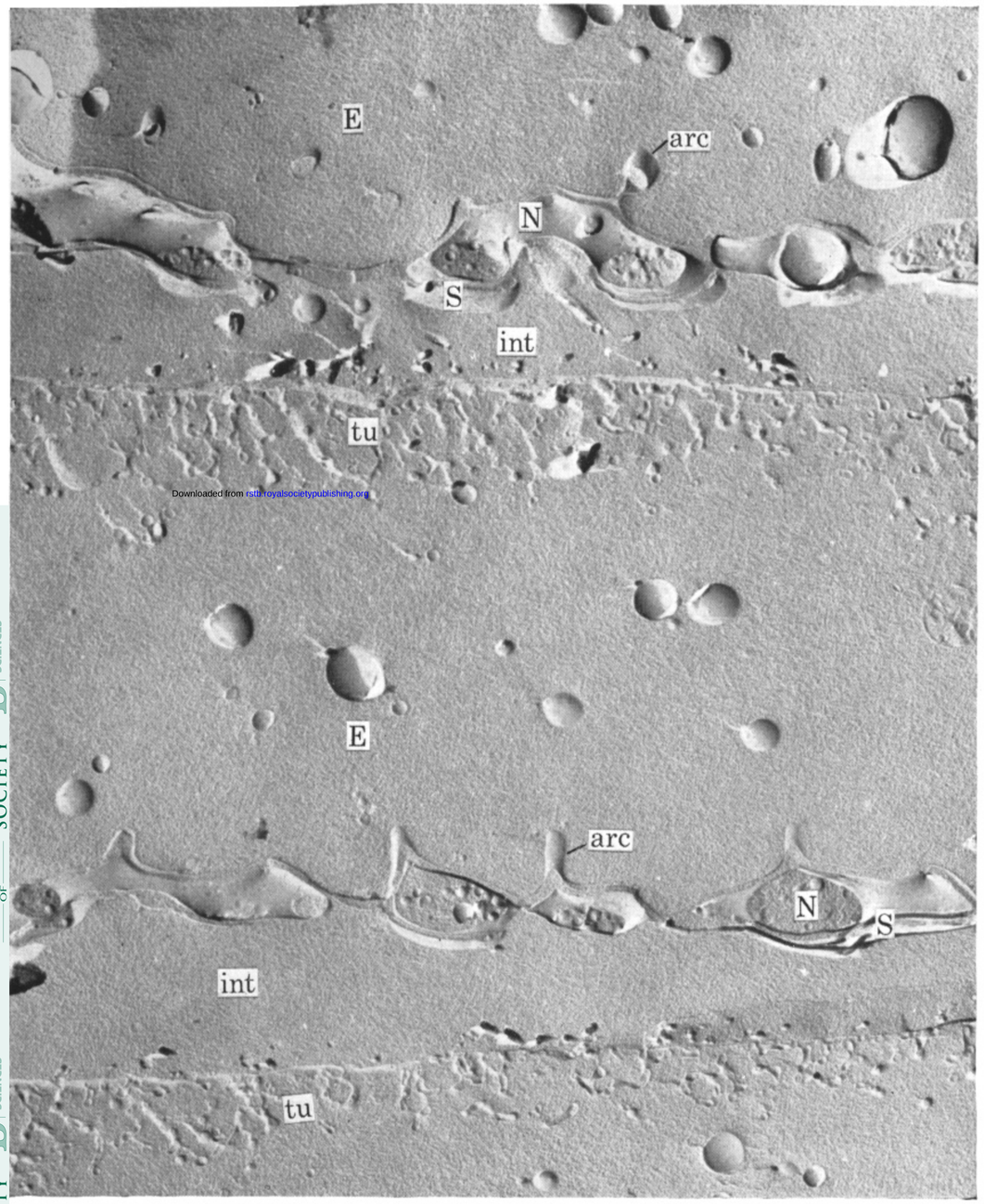

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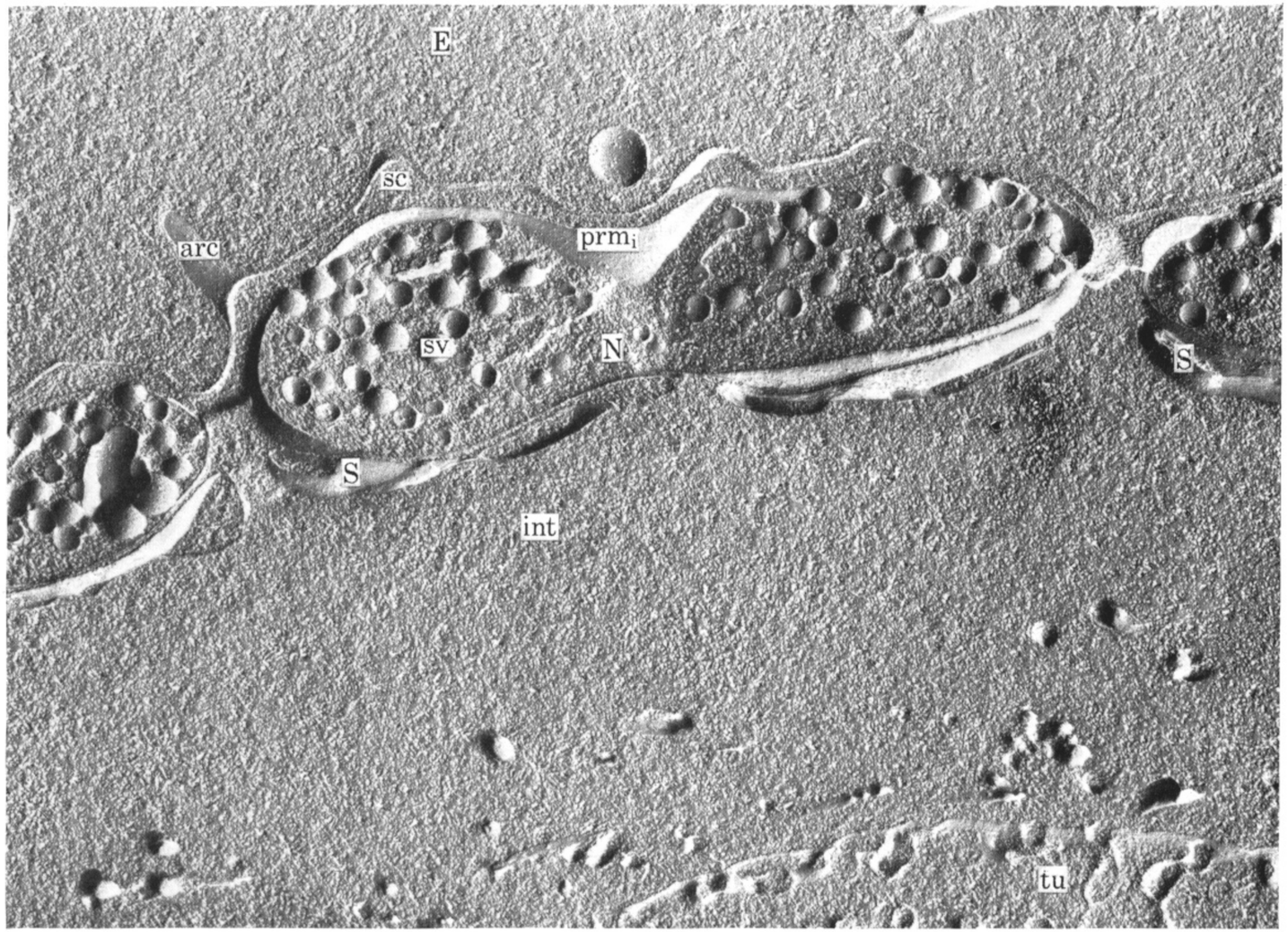

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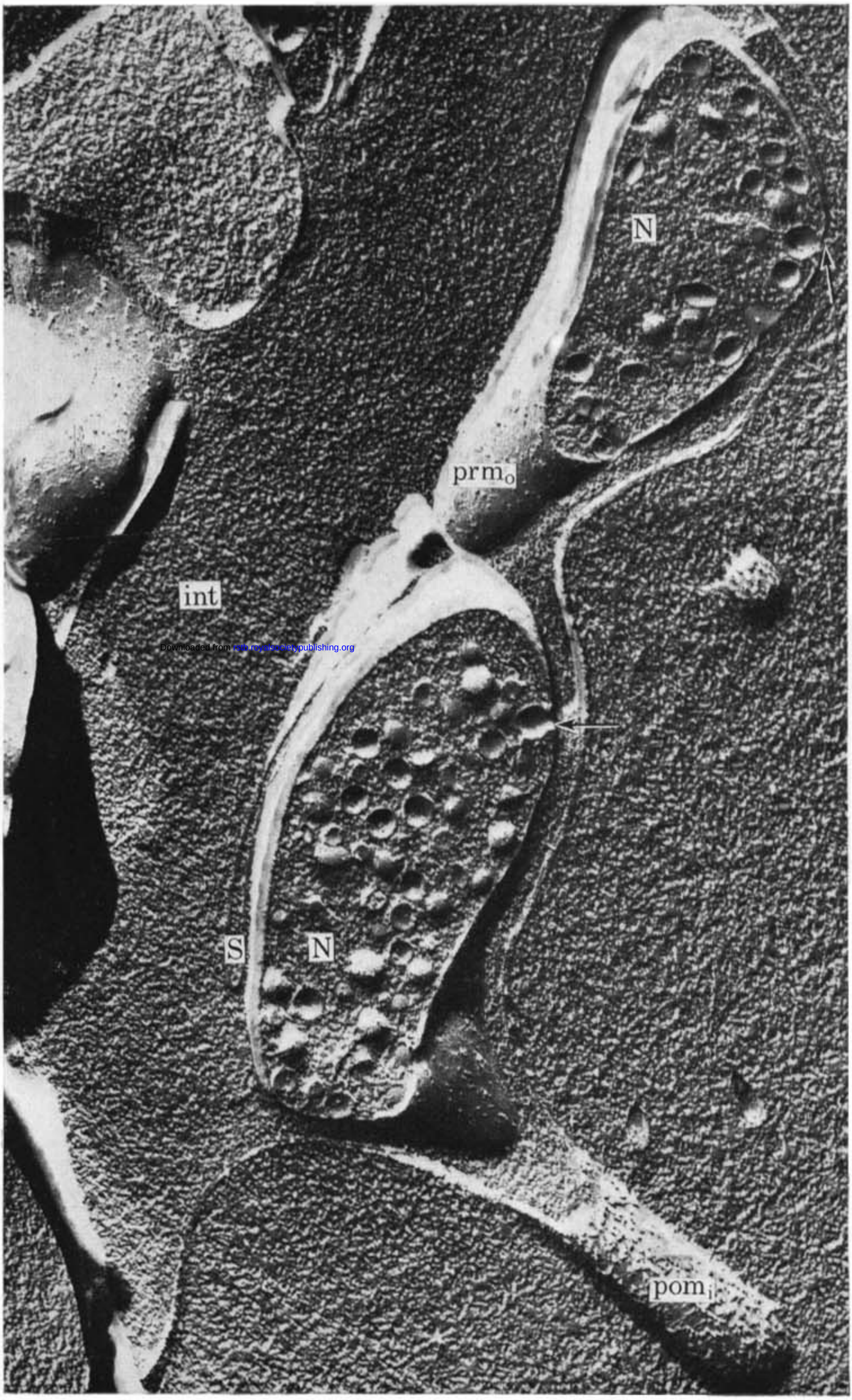

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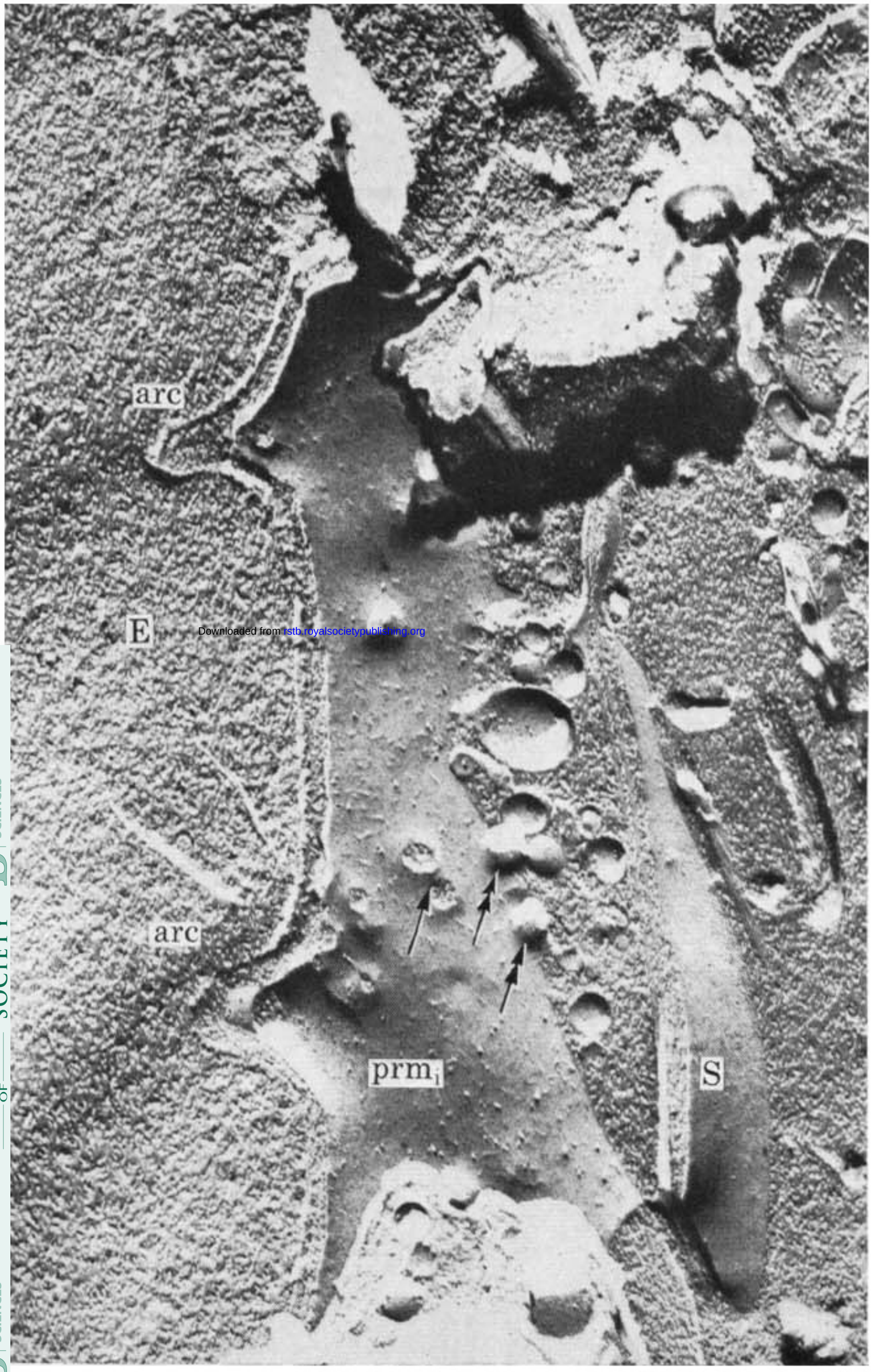

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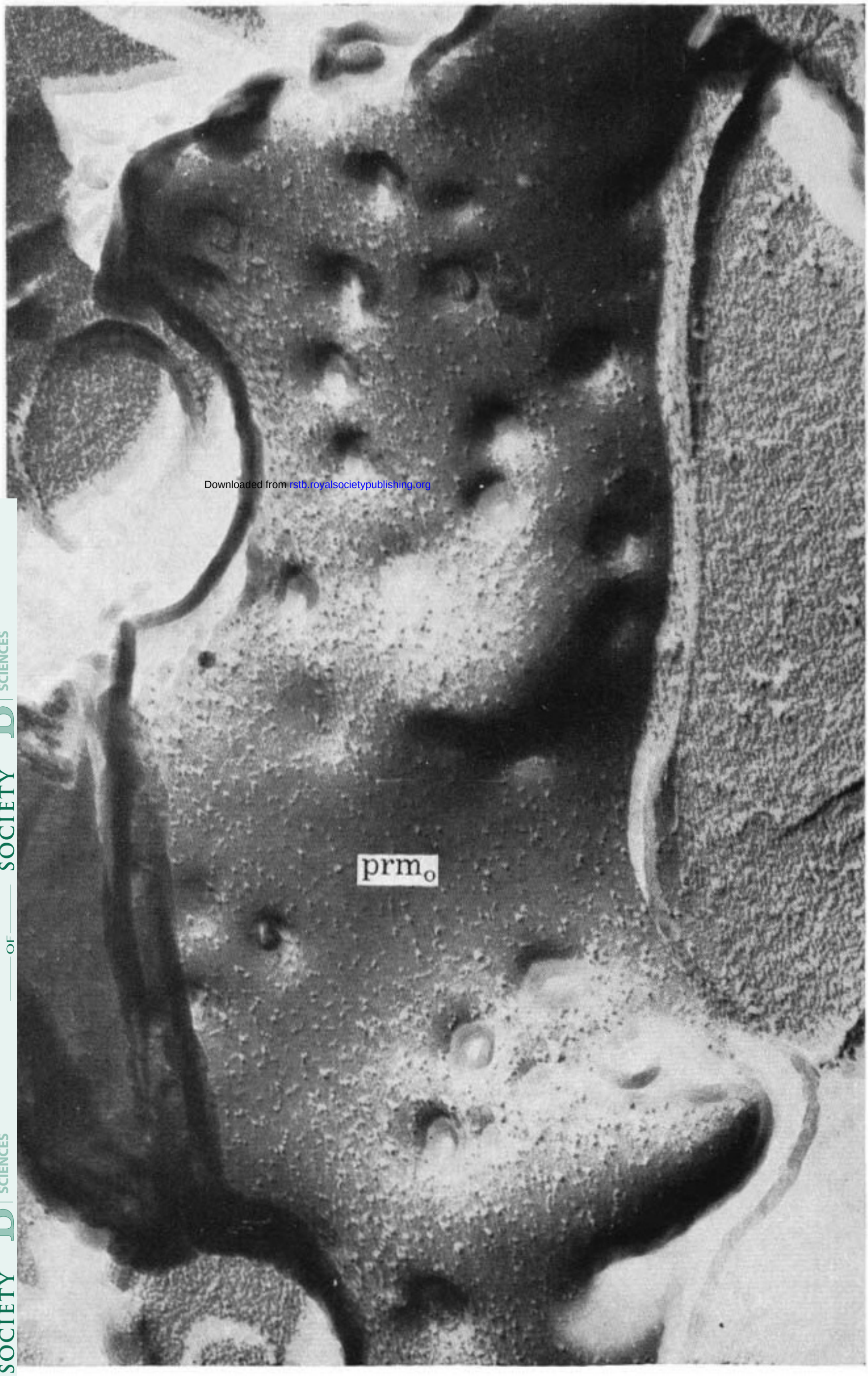

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