EVIDENCE FOR A DUAL CONTROL, BY NEUROSECRETION, OF HORMONE SYNTHESIS AND HORMONE RELEASE IN THE PITUITARY OF THE DOGFISH *SCYLLIORHINUS STELLARIS*

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The intrinsic secretory cells of the neuro-intermediate lobes of the pituitary of the elasmobranch fish *Scylliorhinus stellaris* belong to two main categories (1) central cells which do not demonstrate any marked polarity of their cytoplasmic organelles and show no clear indications of being under neurosecretory control, and (2) peripheral cells which are polarized along an axis from a synthetic pole to a storage and release pole. Neurosecretory fibres make intimate contact with the two poles of these peripheral cells.

The neurosecretory fibres may be classed as type A or type B by the size and appearance of the elementary neurosecretory vesicles they contain. These two fibres innervate the synthetic and release poles of the intrinsic cells respectively by direct contact of their terminals to form secretomotor junctions.

It seems likely that there is a dual control of synthesis and release of a melanophore-dispersing hormone (*MSH*). The possibility that type A fibres inhibit *MSH* synthesis by a peptide substance with oxytocic action, and that *MSH* release is controlled by amine production by type B fibres is discussed.

I. Introduction

De Robertis (1962) has suggested that all neurons should be termed neurosecretory. Certainly secretion is in fact a property of neurons. A distinction can however be made between neurons which make direct synaptic connexions with other cells and neurons which liberate hormones into the blood stream, via a neurohaemal organ (Knowles & Carlisle 1956; Bern 1963).

Typically those neurons which make synaptic connexions are either cholinergic or adrenergic. The former contain electron-lucent vesicles measuring 300 to 400 Å in mean diameter (Seite 1961); the adrenergic type are characterized by electron-dense granules

of about 200 Å in diameter, in aggregations of variable size measuring some 500 to 2000 Å in diameter (Von Euler 1963). Neurons with these characteristics have been termed neurohumoral.

The term neurosecretion was originally used to denote neurons which did not appear to make synaptic connexions with effector cells, and which contained considerable quantities of material stainable by normal histological methods, which was released into the blood stream (Scharrer & Scharrer 1954). This secretory material was shown experimentally to be hormonal and to be contained in membrane-bound vesicles ranging in size from 1000 to 3000 Å. Almost all such neurons were selectively stained by the use of the Gomori chrome-alum-haematoxylin method, by paraldehyde fuchsin or by the performic acid-alcian blue technique.

The distinction based on morphology, between neurohumoral and neurosecretory activity, may be convenient and, indeed, represent a fundamental distinction in terms of function, but it has been criticized in points of detail. De Robertis (1962), for example, has pointed out that some adrenergic neurons, and other neurons with similar morphological characteristics, secrete into the blood stream. Bern (1962) has remarked that the staining properties of so-called neurosecretory cells may differ, and moreover that there is one area in which the distinction between neurosecretory and neurohumoral phenomena may be difficult to make, namely the direct innervation of other endocrine tissues by neurons which by all other criteria resemble typical neurosecretory cells (Bern 1963). Such neurons appear to make direct contact with endocrine cells of the corpus allatum of insects (Schultz 1960), and with the pars intermedia cells of the pituitary of elasmobranchs (Scharrer 1952; Van de Kamer & Verhagen 1955; Mellinger 1963*b*), amphibians (Dawson 1953) and mammals (Kurosumi, Matsuzawa & Shibasaki 1961). Neurosecretory fibres may even invade the meso-adenohyphysis (proximal pars distalis) in teleosts (see Dodd 1963), and appear to make contact with specific cells believed to be gonadotrophs (Da Lage 1955).

On purely morphological grounds therefore the dividing line between neurosecretion and neurohumoral function is an indistinct one. A typical neurohumoral activity is based on direct synaptic contact, but some neurosecretory neurons appear to make some kind of synaptic connexion with other cells of the endocrine system. Ultimately the criteria used to distinguish neurosecretory from neurohumoral phenomena may be functional and biochemical, possibly accompanied by differences between mechanisms of hormone release either at cell-to-cell junctions or into the blood stream. A comparison between a neurohumoral synapse and a secretomotor junction between a neurosecretory fibre terminal and another endocrine cell would therefore seem to be of considerable importance. A detailed study of the ultrastructure or neurophysiology of such a junction has however not yet been reported.

In vertebrates the greatest degree of penetration of an endocrine tissue by neurosecretory fibres is found in the pituitary gland of elasmobranch fishes. Indeed the neural and hypophysial components are intermingled in some species to such an extent that they constitute a neuro-intermediate lobe (meta-adenohypophysis). The degree of intermingling is not the same in all species (Meurling 1962, 1963). In *Scyllium* (Scharrer 1952), *Raja*, *Dasyatis* and *Torpedo* (Bargmann 1955) cell cords of the intermediate lobe are penetrated by broad bundles of neurosecretory nerve fibres. In *Squalus* the two lobes, neural and

intermediate remain distinct, but nerves of two kinds, one which stains with the Gomori chrome-alum haematoxylin technique and the other not, enter the intermediate lobe (Meurling 1962). In *Etmopterus spinax* also the two lobes are distinct, but nerves of two kinds enter the intermediate lobe (Meurling 1963).

It is evident therefore that the innervation of the elasmobranch intermediate lobe is a complex one, comprising different forms of innervation, but our knowledge of the significance of the different neurons is slight. In the absence of precise knowledge of the ultrastructure it has not been possible to decide whether the different nerves innervate distinct intrinsic cell types, or whether possibly some of the neuronal elements are concerned in the control of release from neurosecretory axonal end bulbs, a suggestion which has been discussed by Bern (1962) and envisaged earlier by Hild (1951), Bargmann (1954) and Scharrer & Scharrer (1954). On theoretical grounds Waring (1963) has suggested that colour changes of elasmobranch fishes might depend either on an inactivation of metabolic activity of hormone-producing cells, or on prevention of release of their stored product. As yet however no morphological basis for such a suggestion has been adduced.

The genus *Scylliorhinus* has been the object of many investigations into the structure and function of the neuro-intermediate lobe. Its normal histology has been investigated by Della Corte and others (see Della Corte 1961). A general account of its ultrastructure has been given by Mellinger (1963*b*). These studies have shown that neurosecretory fibres invade the lobe and make contact with its intrinsic cells. Biochemical methods have resulted in the extraction of a melanophore-dispersing principle and also of one or more oxytocin-like substances (Perks & Dodd 1963; Heller 1964). The nature of its control by neurosecretory fibres from the hypothalamus has been studied experimentally by Perks & Dodd (1960) and by Mellinger (1963*a*). The normal colour change of this genus has been extensively studied (see Waring 1963). The species *Scylliorhinus stellaris* therefore appeared suitable for an electron microscopic study of the neuro-intermediate lobe of the pituitary, in order to determine the precise relation between its intrinsic secretory cells and the neurosecretory and other fibres which appear to innervate them. It was hoped that such an investigation might contribute to a better understanding of the mechanism of control of endocrine tissues by neurosecretory nerves.

2. Materials and methods

Young and mature male and female individuals of the species *Scylliorhinus stellaris* were obtained fresh from the Bay of Naples. They were killed by decapitation, and the pituitary dissected as soon after decapitation as possible, generally within two minutes. Small fragments of the neuro-intermediate lobe were placed in an osmic-acid fixative which had been stabilized by means of sodium acetate-veronal buffer to pH 7·6 and rendered approximately iso-osmotic with the blood of the animals.

Fixation took place at 4 °C for 3 h. After washing and dehydration, during which the specimens were stained with phosphotungstic acid (see Knowles 1964), the tissues were embedded in Vestopal W and cut on a Porter–Blum microtome, using diamond or glass knives. Sections were mounted on carbon-coated grids and examined with a Siemens Elmiskop I. Some additional contrast was obtained in some cases by staining the sections on grids with uranyl acetate.

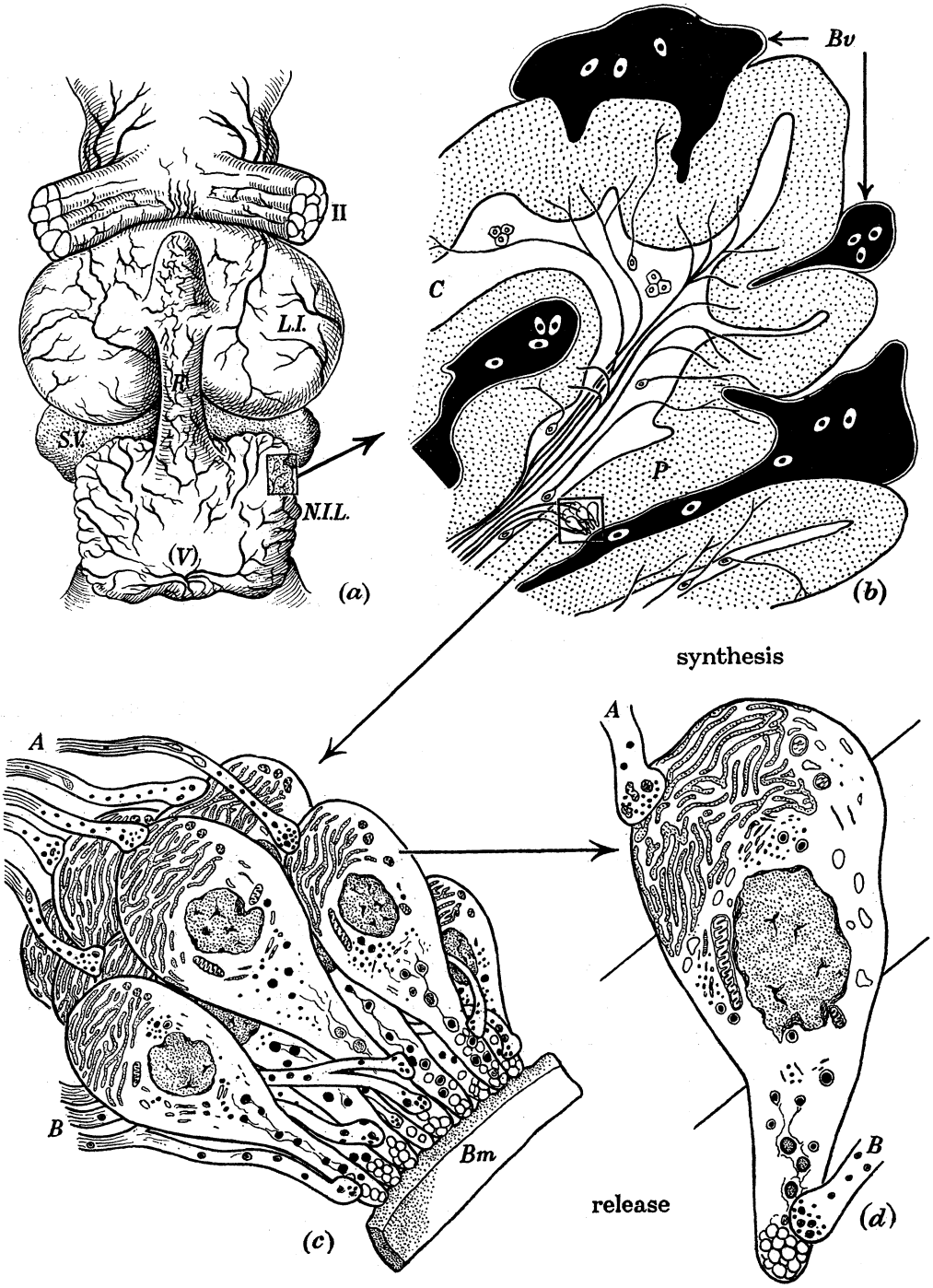

FIGURE 1. THE DISTRIBUTION OF INTRINSIC CELLS AND NEUROSECRETORY FIBRES IN THE NEURO-INTERMEDIATE LOBE OF THE PITUITARY OF *SCYLLIORHINUS STELLARIS*.

(*a*) A ventral view of the pituitary and associated structures (II: the base of the optic nerve. *L.I.*: Inferior lobe of the hypothalamus. *R*: rostral lobe. *S.V.*: saccus vasculosus. *N.I.L.*: Neuro-intermediate lobe.) *V*: position of attachment of the ventral lobe.

(*b*) A diagram of a small portion of a neuro-intermediate lobe, showing the relationship of blood vessels (*Bv*), intrinsic cells of the peripheral layer (*P*) and nervous and other elements in the central region (*C*).

(*c*) A few peripheral intrinsic cells and their innervation by type *A* and type *B* neurosecretory fibres (*Bm*: basement membrane).

(*d*) A single peripheral intrinsic cell, showing the distinct regions of hormone synthesis, hormone assemblage and transformation, and hormone storage and release. This and the preceding figure are semi-diagrammatic and the relative sizes of some of the cell components have been altered slightly for the sake of clarity.

A comparable series of neuro-intermediate lobe material was prepared for light microscopy and stained by the use of the Bargmann modification of the Gomori chrome alum-haematoxylin method. Comparisons were made between this material and the electron micrographs.

3. Morphology of the neuro-intermediate lobes

The neuro-intermediate part of the pituitary gland of *Scylliorhinus* consists of two paired lobes, collectively termed the meta-adenohypophysis (Dodd 1963), which lie on either side of a central region which extends anteriorally to form a rostral lobe (figure 1) and ventrally to form a ventral lobe; these two regions comprise the pro- and meso-adenohypophysis respectively. No unequivocal function has yet been assigned to the rostral lobe, but Dodd (1960*a*), Dodd, Evennett & Goddard (1960) have shown that atrophy of the gonads follows removal of the ventral lobe, and Dent & Dodd (1961) and Mellinger (1963*b*) have adduced some evidence that the ventral lobe is the site of a thyroid-stimulating hormone (*TSH*). The neuro-intermediate lobes contain a melanophore-dispersing hormone, *MSH* (Dodd 1960).

The two neuro-intermediate lobes of *Scylliorhinus stellaris* are lobulated. Under the dissecting microscope each lobule is seen to be further subdivided into cords (figure 1 (*b*)), each of which comprises a central portion consisting of loosely packed cells and neurosecretory fibres surrounded by a peripheral area 1 to 6 cells thick.

Under the electron microscope a considerable amount of secretory material could be demonstrated in the peripheral cells, and these appeared to form the principal endocrine tissue of the neuro-intermediate lobe. The peripheral secretory cells exhibit a marked polarity. That region of the cells which contains secretory material extends towards blood vessels and terminates in close juxtaposition to the capillary walls (figure 1 (*c*)).

The origin of the blood vessels which bear so close a relation to the secretory cells of the neuro-intermediate lobe was not determined in the present studies. The blood supply of this area has however been described in the closely related species *Scylliorhinus caniculus* by Meurling (1960) and by Mellinger (1963*b*) who have shown the presence of a definite portal system. The median hypothalamic floor is modified to form a region resembling a median eminence, covered with capillaries of the primary plexus and with an attachment of the anterior or rostral lobe (mesoadenohypophysis) to its median surface (figure 1 (*a*)).

Mellinger (1963*b*) however considers that paired lateral veins which appear to drain many parts of the mid-brain form the main blood supply to the neuro-intermediate lobe and that the portal system to the neuro-intermediate lobe of *Scylliorhinus caniculus* is an embryological rudiment. The arterial supply is small (Meurling 1960).

4. Intrinsic endocrine cells

During preliminary studies the activity of the intrinsic cells of the neuro-intermediate lobe appeared to be greater in mature female fish than in immature females or males. This difference has been reported also by Castigli (1937) in *Scylliorhinus stellaris* and by other workers for other elasmobranchs (see Della Corte 1961). A number of male fish however contained very active secretory cells in their neuro-intermediate lobes, as denoted by the

size and complexity of the endoplasmic reticulum, and the evidence of sexual differences was not consistent. It is possible that differences in the secretory activity of neuro-intermediate lobe cells may be correlated to the conditions under which animals are kept. Cohen (1964) has observed that endoplasmic reticulum formation in the pars intermedia of *Xenopus* varies according to conditions of illumination and the shade of the background on which animals are placed.

The cells of the peripheral regions of the neuro-intermediate lobe appeared to differ from those of more central regions, both in structure and in innervation; the two cell types will therefore be treated separately.

(*a*) *Peripheral cells*

Each peripheral endocrine cell is ovoid and elongate, with one end extending in the direction of the surface of the gland and thus towards the wall of the nearest blood vessel. The length of this extension is related to the distance between the nucleus of any cell and the surface of the gland. Those cells in the deeper layers of the peripheral part of the gland have longer processes than those close to the surface (figure 1 (*c*)). The proportions of the peripheral secretory cells therefore vary, but in the main measure approximately 7 to 10 μm at their widest point and 20 μm or more in length. So far as can be ascertained each cell in the peripheral region of the gland extends to the surface and so its secretory products may be discharged directly into the blood stream.

A basement membrane, approximately 0·4 μm in width, separates the ends of the secretory cells from the blood stream.

The peripheral secretory cells, thus orientated in the direction of the blood, demonstrate a striking and consistent polarity in the distribution of their constituent organelles. It is possible to recognize an area of hormone synthesis characterized by an abundant endoplasmic reticulum, one or more regions of hormone assemblage or transformation as denoted by tubules and lamellae of the Golgi apparatus and a region of hormone storage and release in which membrane-bound secretory droplets accumulate (figure 1 *d*). This latter region occupies the prolongation of the cell towards the blood stream and represents approximately a third of its volume. The Golgi zones lie close to the nucleus and together with it comprise approximately one third of the cell. The remaining third contains the endoplasmic reticulum, which is thus at the pole of the cell most distant from the blood stream and nearest to the centre of each lobule (figure 1 (*b*), (*c*)).

The polarity of the peripheral cells, and the relationship of this polarity to the blood stream is so striking and consistent that, for the sake of convenience in this account, sections passing through the main axis of the cell, i.e. parallel to the plane of polarity, will be designated as longitudinal sections (e.g. figure 3, plate 48). Sections along a plane at right angles to the main axis of the cell will be termed transverse sections (e.g. figure 5, plate 49 and figure 8, plate 50).

The endoplasmic reticulum

Figure 4, plate 48, shows the endoplasmic reticulum in longitudinal section, whereas figure 5, plate 49 shows it in transverse section. A consideration of these two planes of section makes it possible to discern a plane of polarity at right angles to the main axis of

the cell, since the endoplasmic reticulum does not extend throughout the apical pole of the cell but stops short, leaving about a quarter of the apical pole free of membranes of the endoplasmic reticulum. This clear area contains fine microtubules and mitochondria (figure 5, plate 49). An occasional mitochondrion was found within the endoplasmic reticulum complex but generally mitochondria lay beside, but not within, the endoplasmic reticulum. The mitochondria in this area of the cell were approximately spherical, 0·3 μm in diameter; their shape contrasts sharply with those in the central portion of the cell, which are elongate (figure 4, plate 48).

The endoplasmic reticulum of the peripheral secretory cells contains continuous flattened cisternae separated from one another by cytoplasm which contains ribosomes. The cisternae of the endoplasmic reticulum did not appear to contain any appreciable amounts of electron-dense material in sections stained by osmium, phosphotungstic acid or uranyl acetate, used either singly or in combination. Fine strands of material may be observed but no granules or droplets. The outermost cisternae lie close beneath the surface membrane of the cells, but are separated from it by a layer of ribosome-containing cytoplasm (see figure 5, plate 49). The only exception to this condition was observed at points where the terminals of neurosecretory fibres made contact with the peripheral secretory cells (figure 18, plate 55); here the cisternae seemed to be separated from the cytoplasm of the neurons by membranes only.

Golgi regions

A transverse or oblique section through a cell close to the nucleus passes through a Golgi region and also some prolongations of the cisternae of the endoplasmic reticulum (figure 8, plate 50). The level at which such a section is taken is indicated by the condition of the cisternae of the endoplasmic reticulum. As has been observed these are flattened, parallel and continuous near the apex of the cell, whether in transverse or longitudinal sections (plate 48, and figure 6, plate 49). At slightly lower levels they continue to appear thus in section. At still lower levels however they appear tubular and discontinuous in transverse sections (figure 8, plate 50).

Mitochondria of the Golgi region are elongate and lie along the main axis of the cell (figures 3 & 4, plate 48, figure 6, plate 49). They were often observed in close juxtaposition to the nucleus, apparently in continuity with the outermost membrane enclosing the perinuclear space. The nucleoplasm was especially electron-dense close to this point.

The tubules and lamellae of the Golgi apparatus were typical in form and it is evident that electron-dense material assembles within these tubules and cisternae to form spherical masses (figures 6 and 7, plates 49 and 50, and figure 10, plate 51).

The exact relationship of the Golgi apparatus to the cisternae of the endoplasmic reticulum, and whether any continuity occurs, could not be determined with confidence. It is however perhaps noteworthy that at certain points where the Golgi lamellae and the cisternae of the endoplasmic reticulum approached one another an apparent interruption of the lamellae and an extension of these lamellae towards the endoplasmic reticulum could be observed (figure 7, plate 50, figure 9, plate 51). At these points osmiophilic material was present.

Cilia

A number of electron micrographs of the Golgi region of peripheral intrinsic cells revealed the presence of banded fibrils, generally lying parallel to tubules of the Golgi apparatus (figure 10, plate 51). The periodicity of banding on these fibrils was 700 Å in the majority of fibres (80%), though in some cases the distance between successive bands was less, and ranged down to 530 Å. The appearance of these fibres and the periodicity of banding differed from collagen fibres found in the basement membrane region at the surface of the intrinsic cells, and it appears more likely that they should be interpreted as ciliary rootlets (Fawcett 1961). The occasional presence of a basal body or centriole near these banded fibres (figure 10, plate 51) lends further support to their identification as ciliary rootlets.

Cilia were found within and among peripheral secretory cells. Few distal portions of cilia were observed, but in these the number of filaments appeared to be atypical, with nine outer filaments but no central axial filament.

Modified cilia have been detected in the third ventricle of the fish brain by Bargmann & Knoop (1955), who suggested that these modified cilia may have a secretory function. The distribution and function of cilia in the neuro-intermediate lobe of *Scylliorhinus* was not studied critically; there were some indications that these cilia were directed inwards, to lie in the central spaces of the cords of the neuro-intermediate lobe.

The secretory pole

That region of the cell furthest from the endoplasmic reticulum typically contained osmiophilic droplets, microtubules and a few relatively small mitochondria.

The osmiophilic droplets were in the form of spherical globules, ranging in size from 3000 to 13000 Å. These observations accord with the results obtained by Della Corte (1961) who considered that the globules he observed under the optical microscope arose from granules by simple enlargement, but contrast with the views of Mellinger (1963*b*) who suggests that globule formation might be a sign of degeneration and that the globules described by Della Corte were indications of cellular death. Since Mellinger studied *Scylliorhinus caniculus* and Della Corte studied *Scylliorhinus stellaris* it is possible that interspecific differences exist. The present micrographs however give clear indication that large droplet formation may be observed in an activity secreting cell.

The smaller droplets are uniformly electron-dense, whereas the largest droplets or globules were electron-dense at the periphery but more electron-lucent elsewhere (figure 12, plate 52). The micrographs indicate a possibility that, while accumulation of osmiophilic material may take place in the Golgi zone, further accumulation of non-osmiophilic material may continue after the secretory droplets have passed beyond the Golgi region.

Some sections through the secretory poles of cells showed secretory droplets lying within relatively large and irregular lacunae, bounded by smooth membranes, which they did not fill, the remainder of the space being occupied by material which was slightly more electron-dense than the cytoplasm surrounding these lacunae (figure 11, plate 52), but less electron-dense than the droplets within. This condition too would accord with the suggestion that electron-dense droplets may gather non-osmiophilic material.

The terminal region of the secretory pole of a typical peripheral secretory cell contained osmiophilic droplets and globules resembling those found in the more central part of the

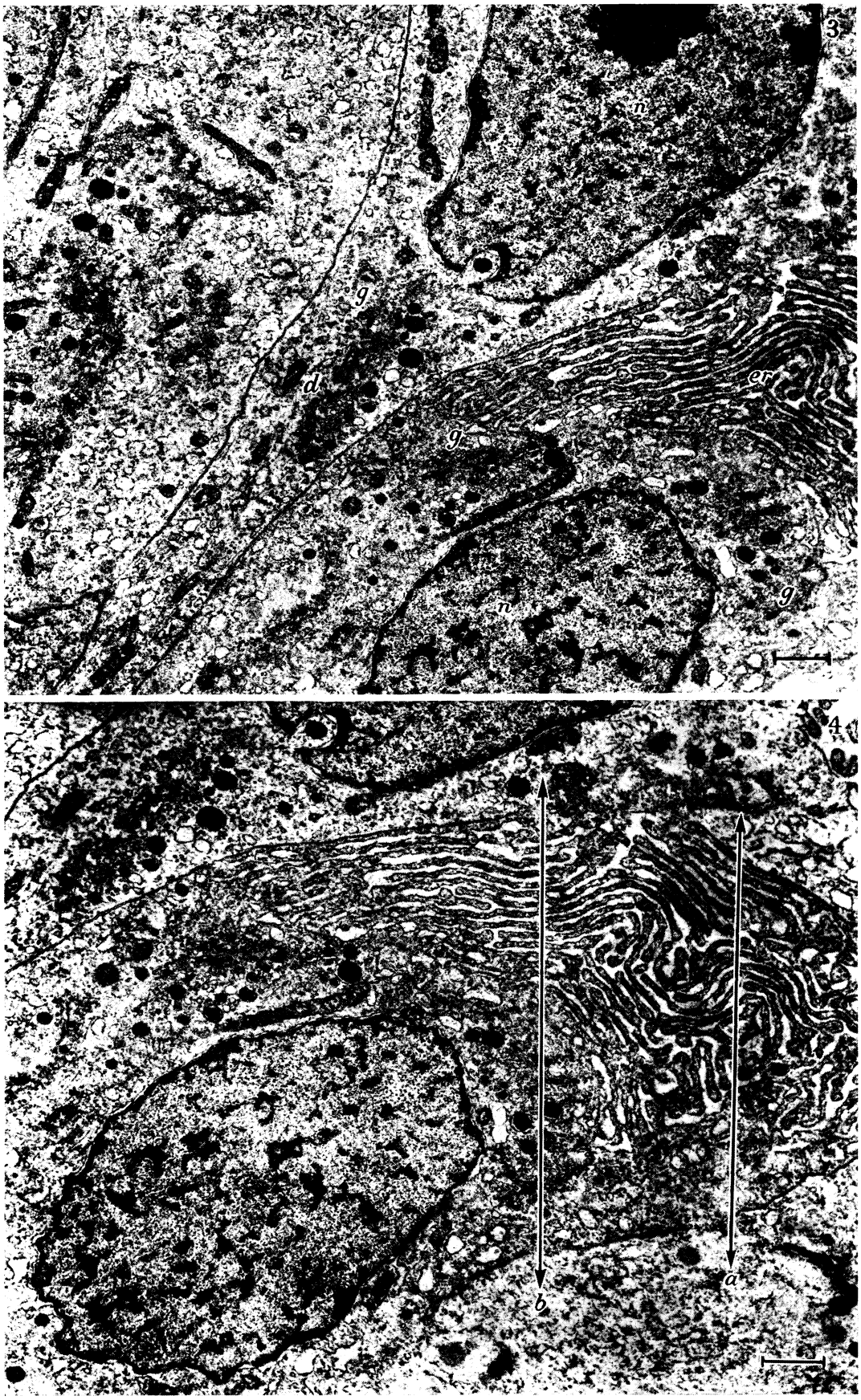

FIGURE 3. A section through the peripheral region of the neuro-intermediate lobe, showing intrinsic cells cut in longitudinal section. *d*, distal prolongation; *er*, endoplasmic reticulum; *g*, Golgi zone; *n*, nucleus.

FIGURE 4. As figure 3, but showing more of the secretory pole of the lower cell. The vertical lines *a* and *b* denote the planes of section of figure 5 and 8 respectively.

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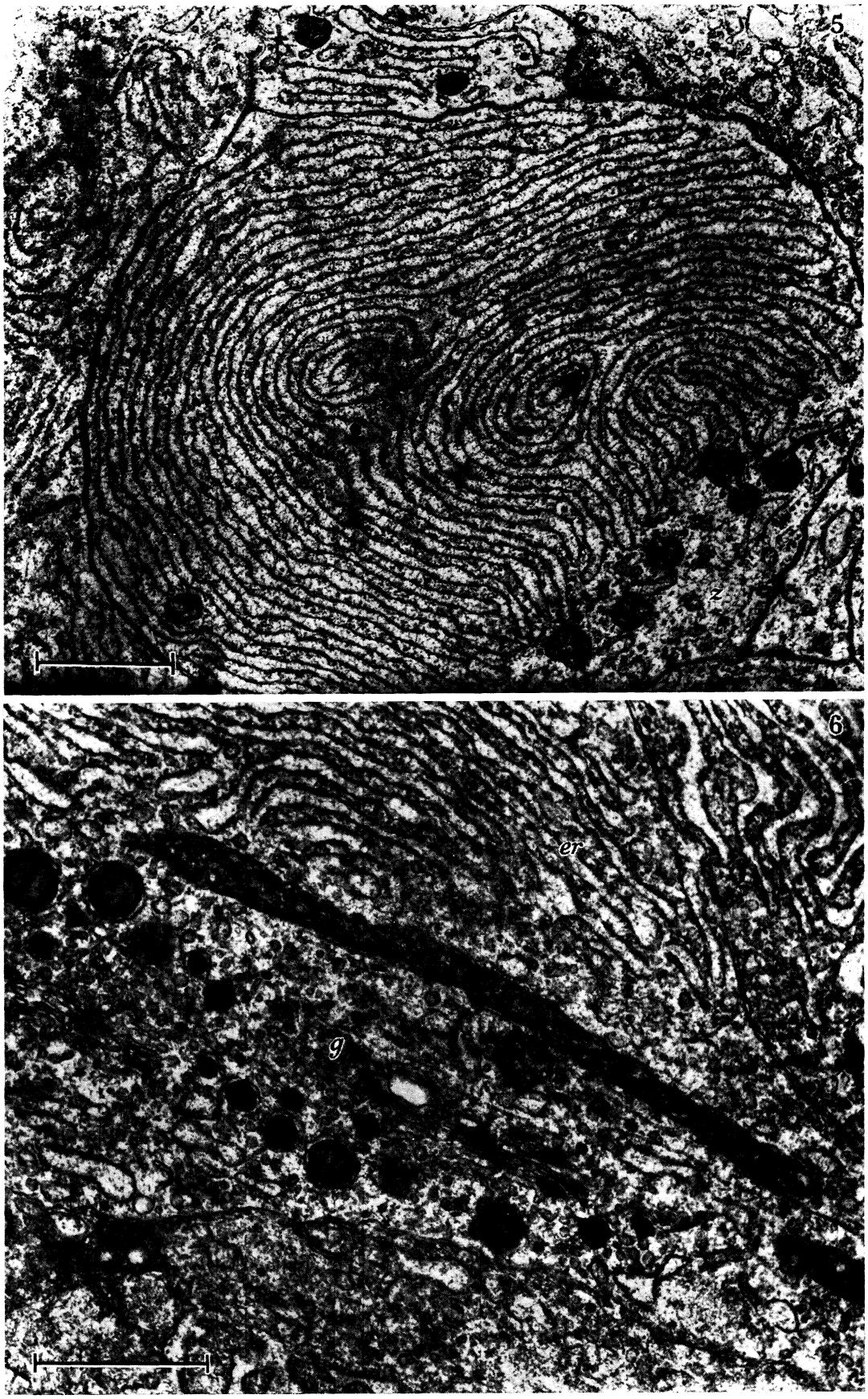


FIGURE 5. A transverse section through the secretory pole of a peripheral intrinsic secretory cell. *z*, zone containing mitochondria and fibrillae, but no endoplasmic reticulum.

FIGURE 6. A longitudinal section through a Golgi zone of an intrinsic cell, showing the elongate type of mitochondrion characteristic of this region. *g*, Golgi tubules and vesicles; *er*, endoplasmic reticulum.

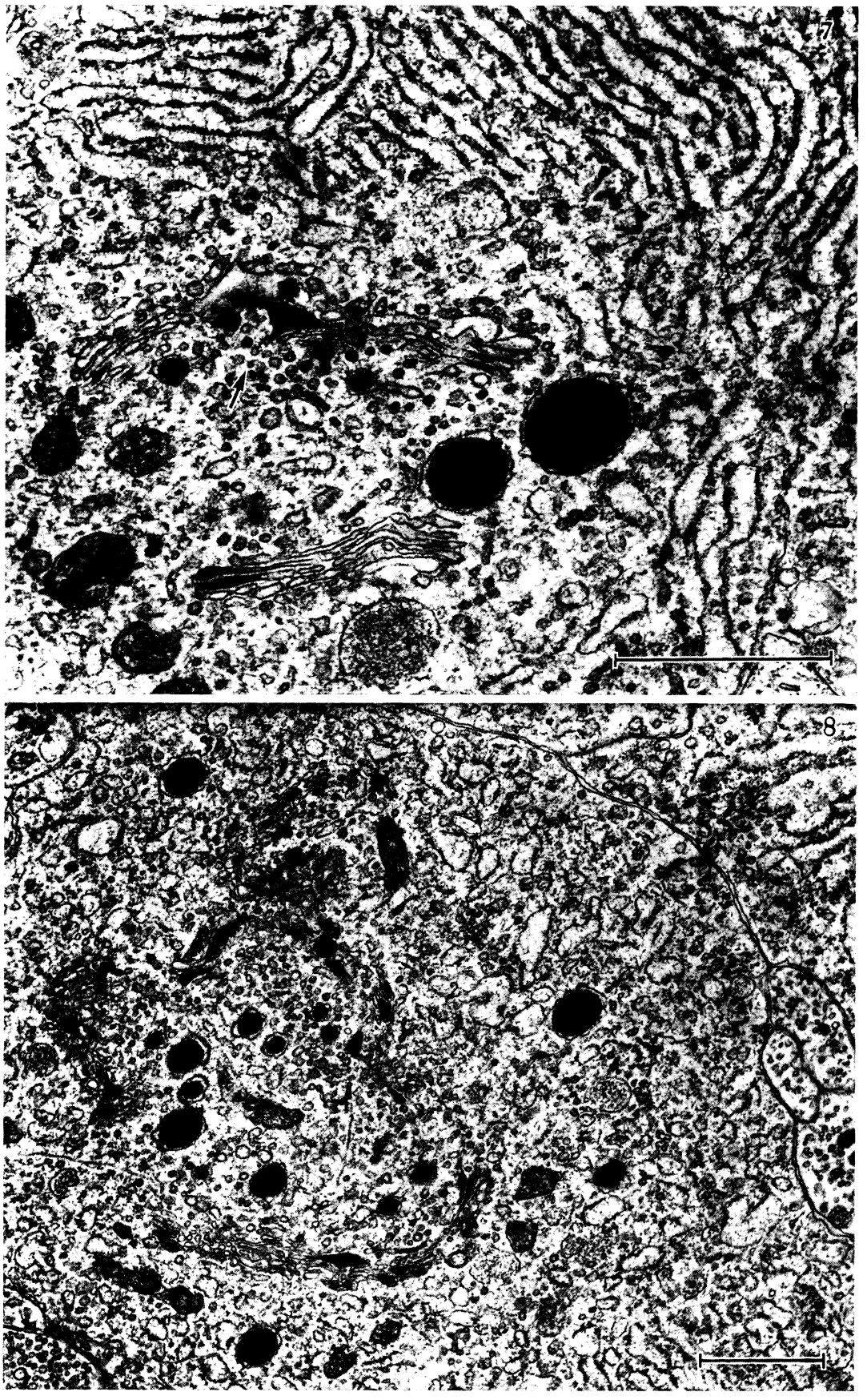


FIGURE 7. An oblique section through a Golgi zone of an intrinsic cell. An accumulation of electron-dense material to form spherical droplets is shown; an arrow points to a region, close to the endoplasmic reticulum, which shows an irregular form, and apparent discontinuity of the Golgi lamellae (see also figure 9, plate 51).

FIGURE 8. A transverse section through a Golgi zone in the central region of an intrinsic cell (see figure 4, plate 48). At this level cisternae of the endoplasmic reticulum are discontinuous and tubular. Fine fibrillae are found in the Golgi zone.

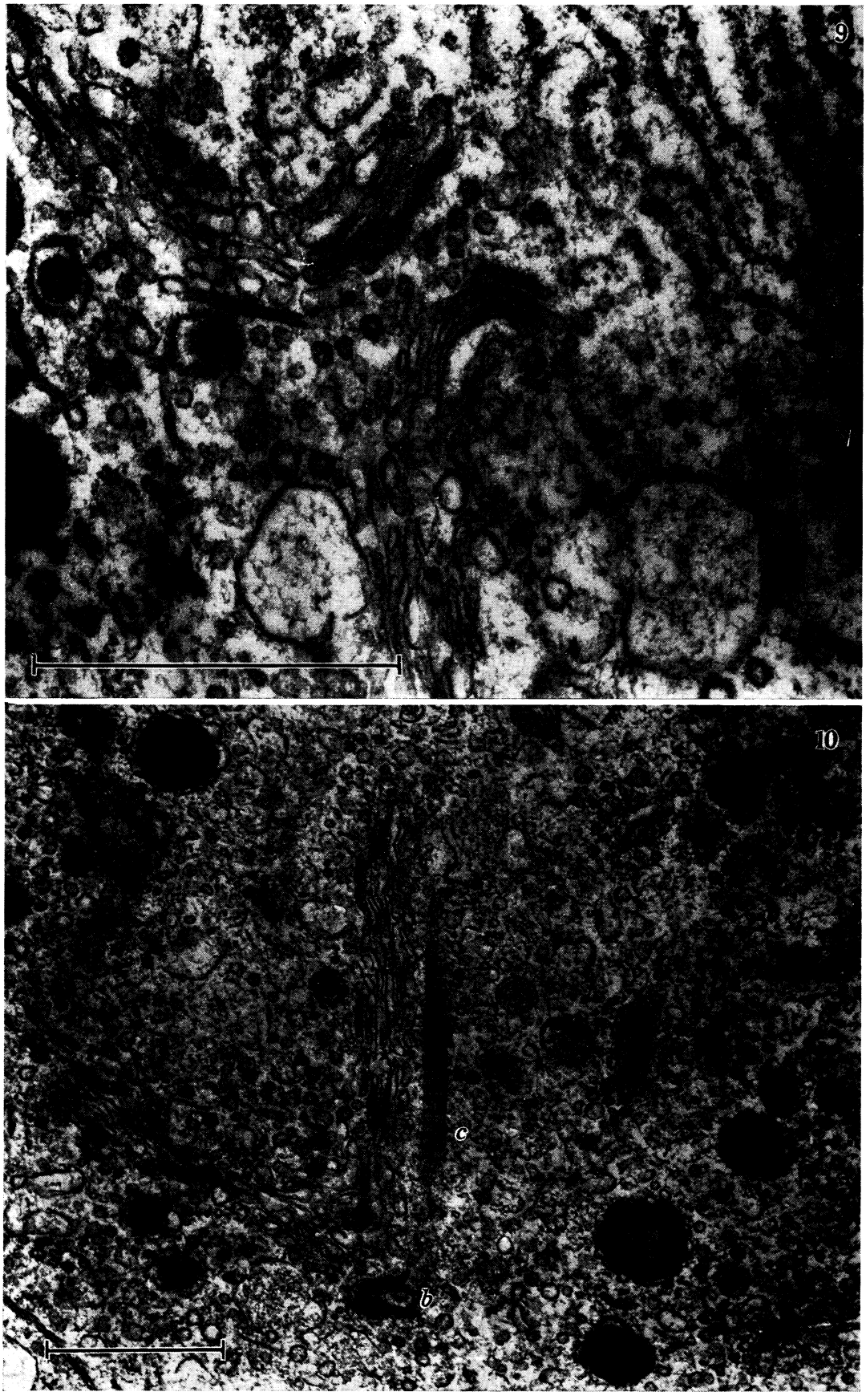


FIGURE 9. A longitudinal section through the region in which the Golgi lamellae and the endoplasmic reticulum are in close approximation to one another. At this point the Golgi lamellae are recurved and filled with electron-dense material.

FIGURE 10. A ciliary rootlet and its associated centriole or basal body, lying in the Golgi region of an intrinsic secretory cell. *b*, basal body; *c*, ciliary rootlet.

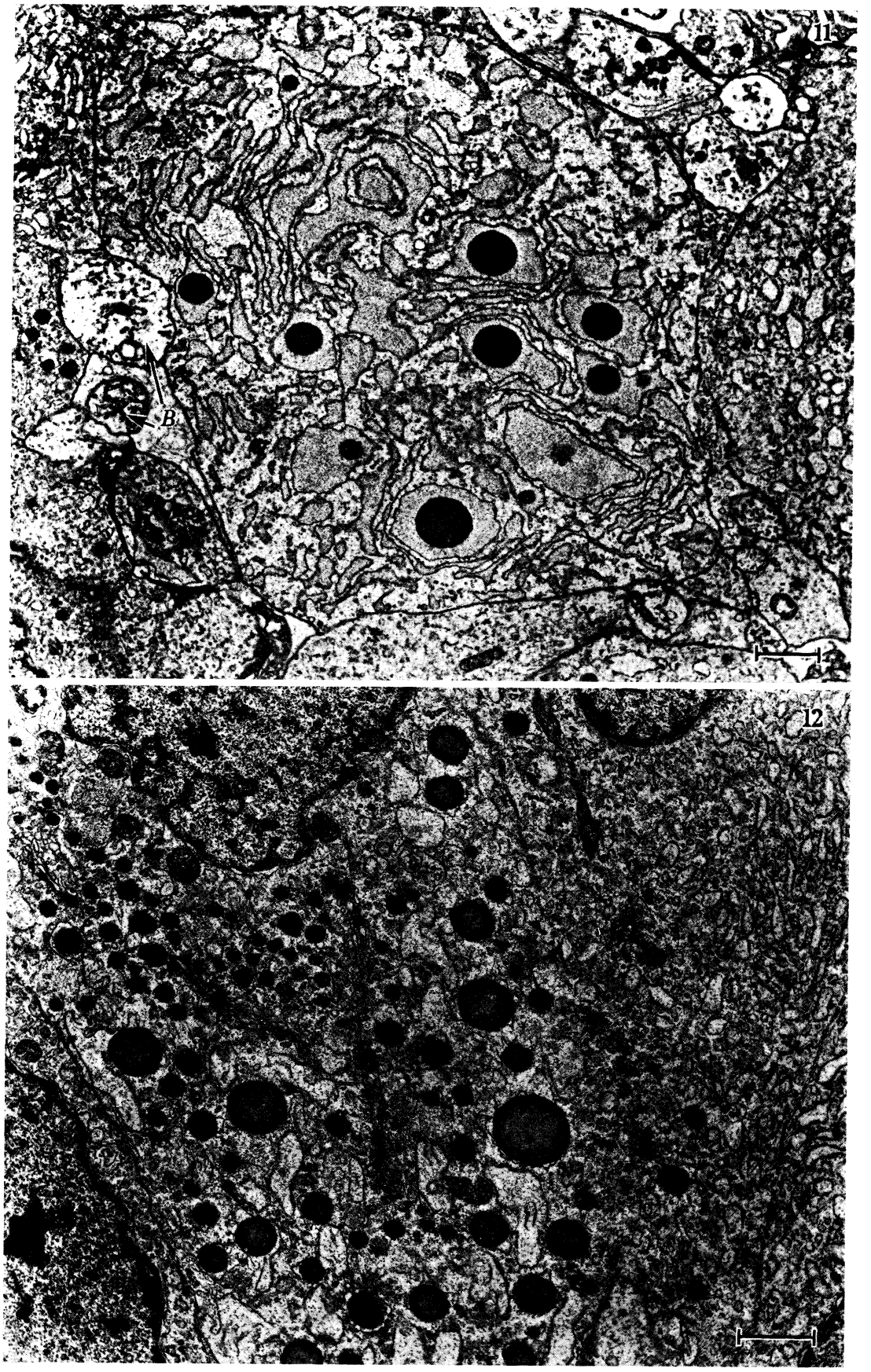


FIGURE 11. A transverse section through a distal prolongation of an intrinsic secretory cell. Globules of secretory material lie within lacunae, which contain also fine granular particles. *B*, type B fibres.

FIGURE 12. A longitudinal section through the proximal part of the storage and release end of an intrinsic cell. In this cell no fine granular material surrounds the electron-dense globules.

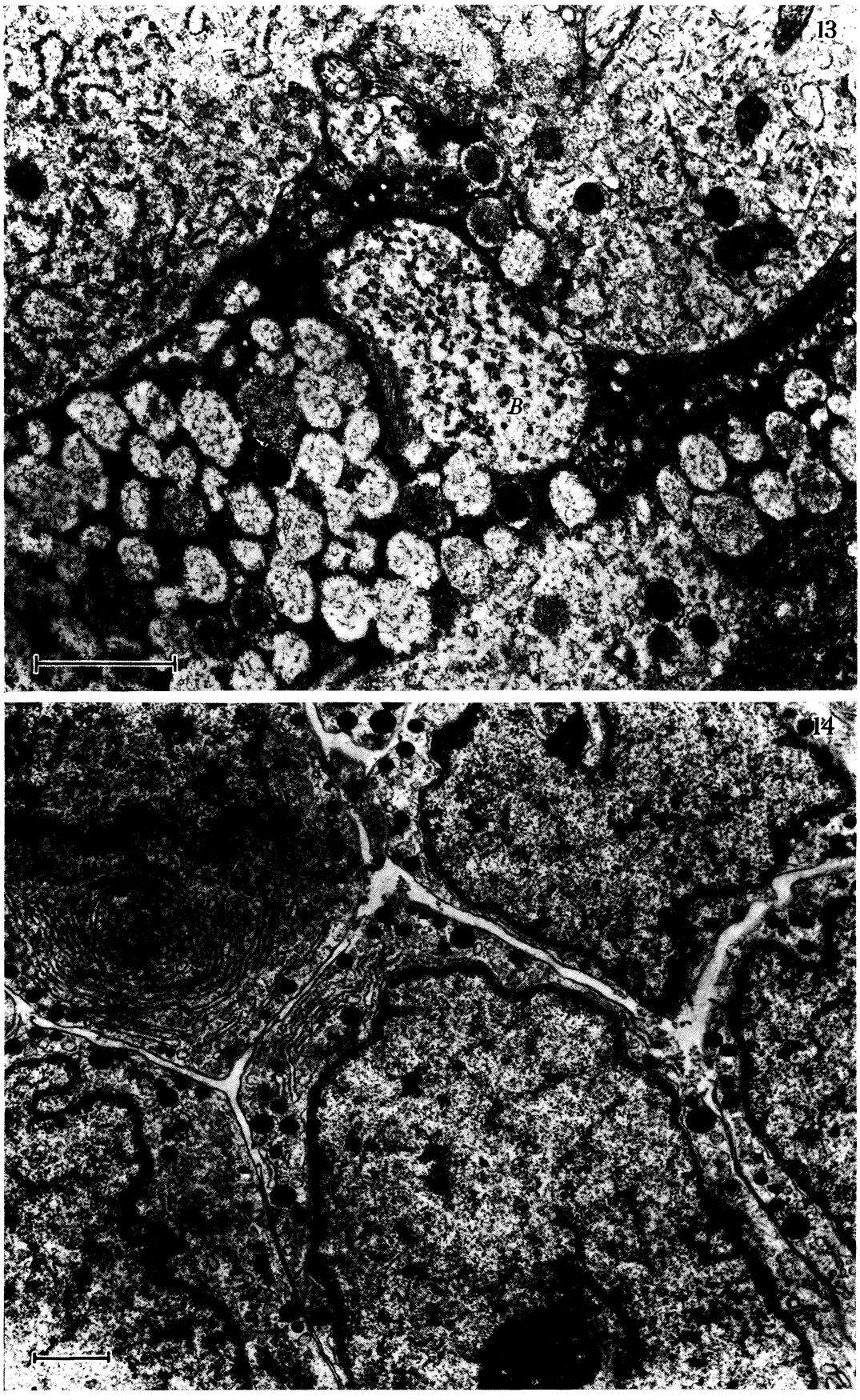


FIGURE 13. A longitudinal section through a distal prolongation of a peripheral intrinsic cell, close to its termination. At this point many vesicles, containing varying amounts of electron-dense material, are found.

(*B*, a type B fibre cut in transverse section. The abundance of small 'empty' vesicles indicates that the plane of section passes through the termination of a type B fibre invaginating the storage and release pole of an intrinsic cell.)

FIGURE 14. A section through cells in the central region of a cord of the neuro-intermediate lobe.

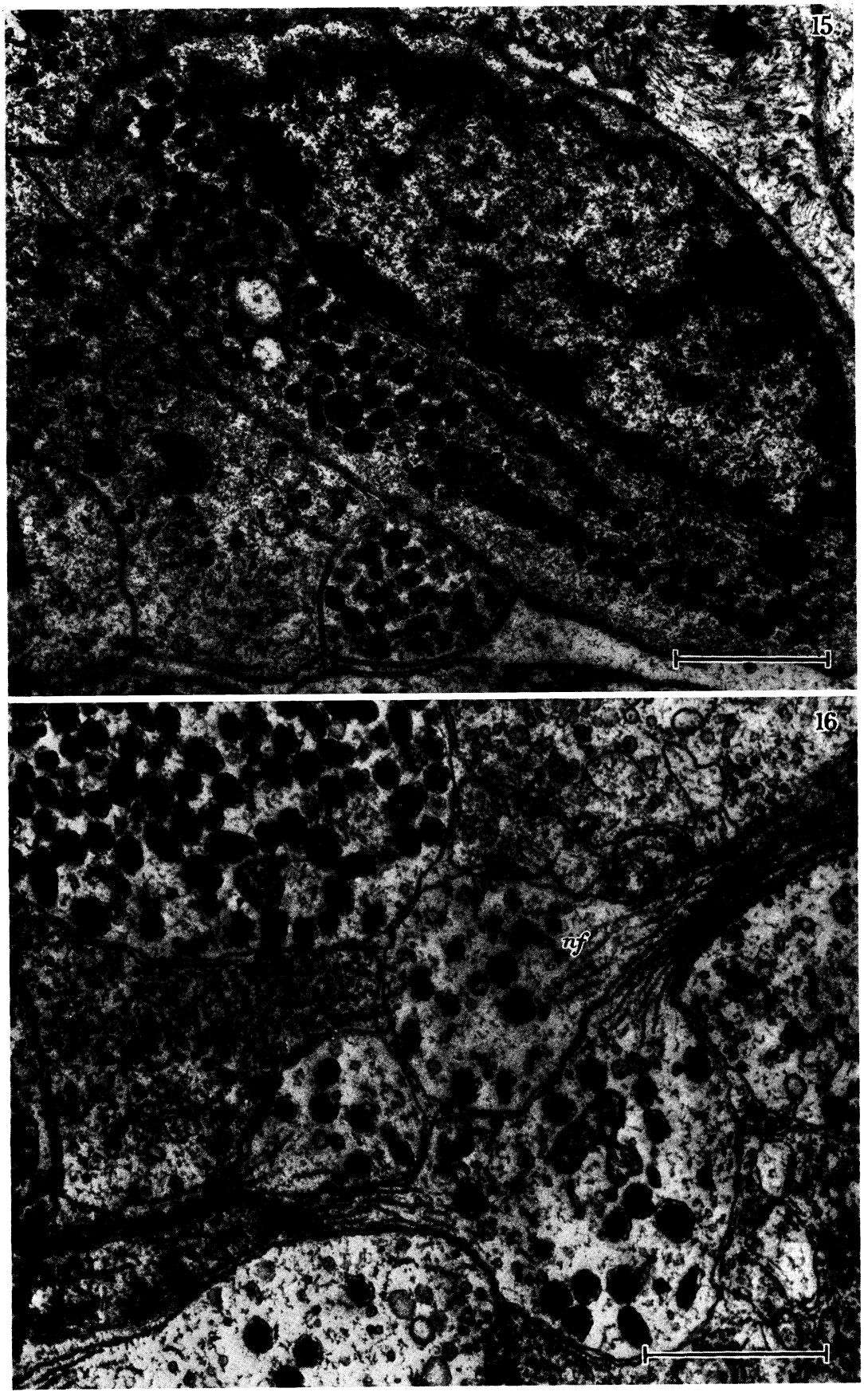
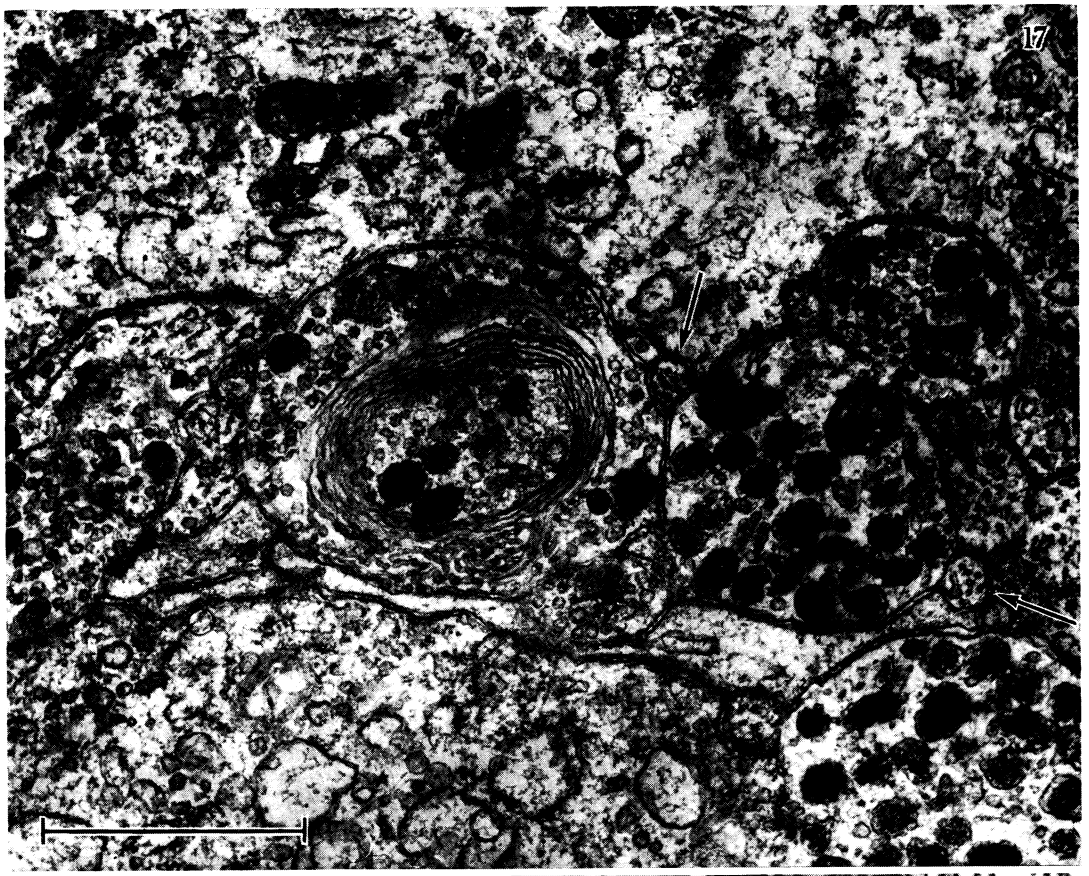


FIGURE 15. A section through a cell in the neuro-intermediate lobe. The inclusions in this cell are identical in size and form to those in the axons of type A (see succeeding figures).

FIGURE 16. Type A fibres in longitudinal and transverse sections, showing large and small vesicles and neurofibrillae (*nf*).



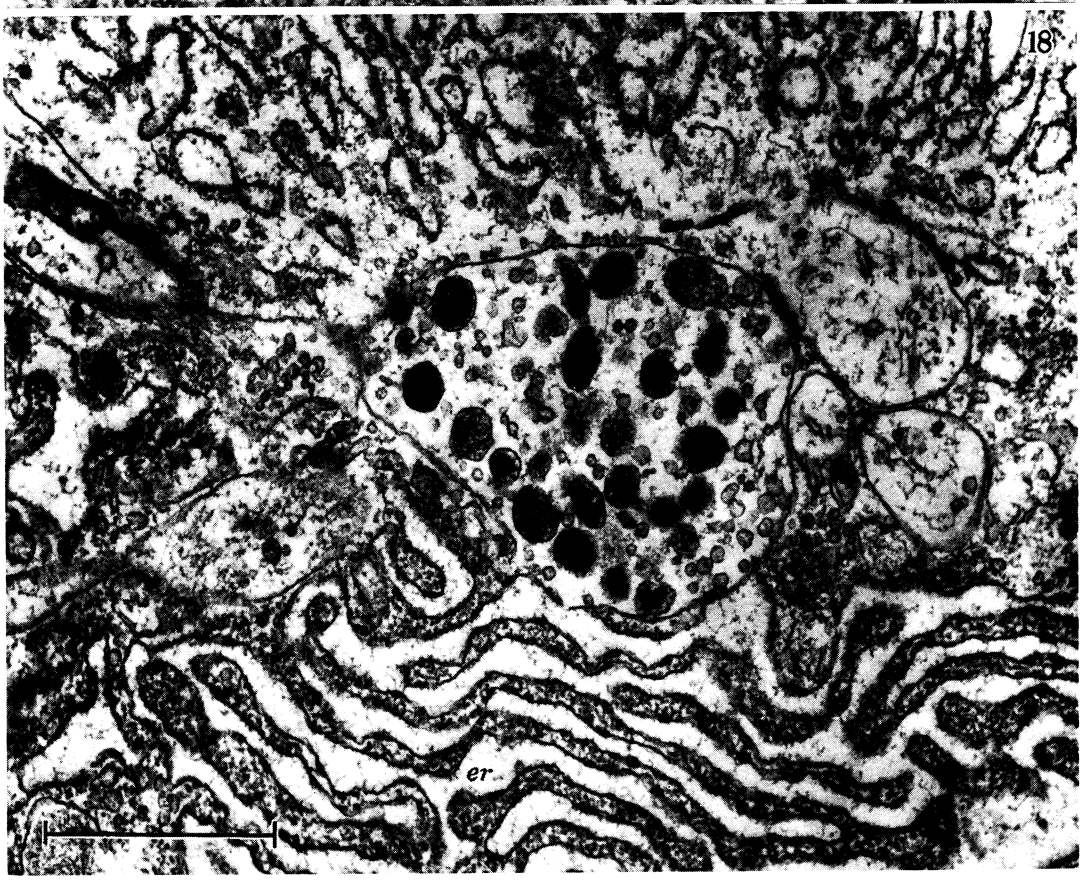


FIGURE 17. Transverse sections through type A fibres, one of which contains a multilamellate body, continuous with tubules in the axoplasm. The arrows point at fibres which contain neurofibrillae, but no evident secretory material.

FIGURE 18. A secretomotor junction at the terminal of a type A fibre in contiguity with secretory poles of two intrinsic peripheral secretory cells (*er*, endoplasmic reticulum).

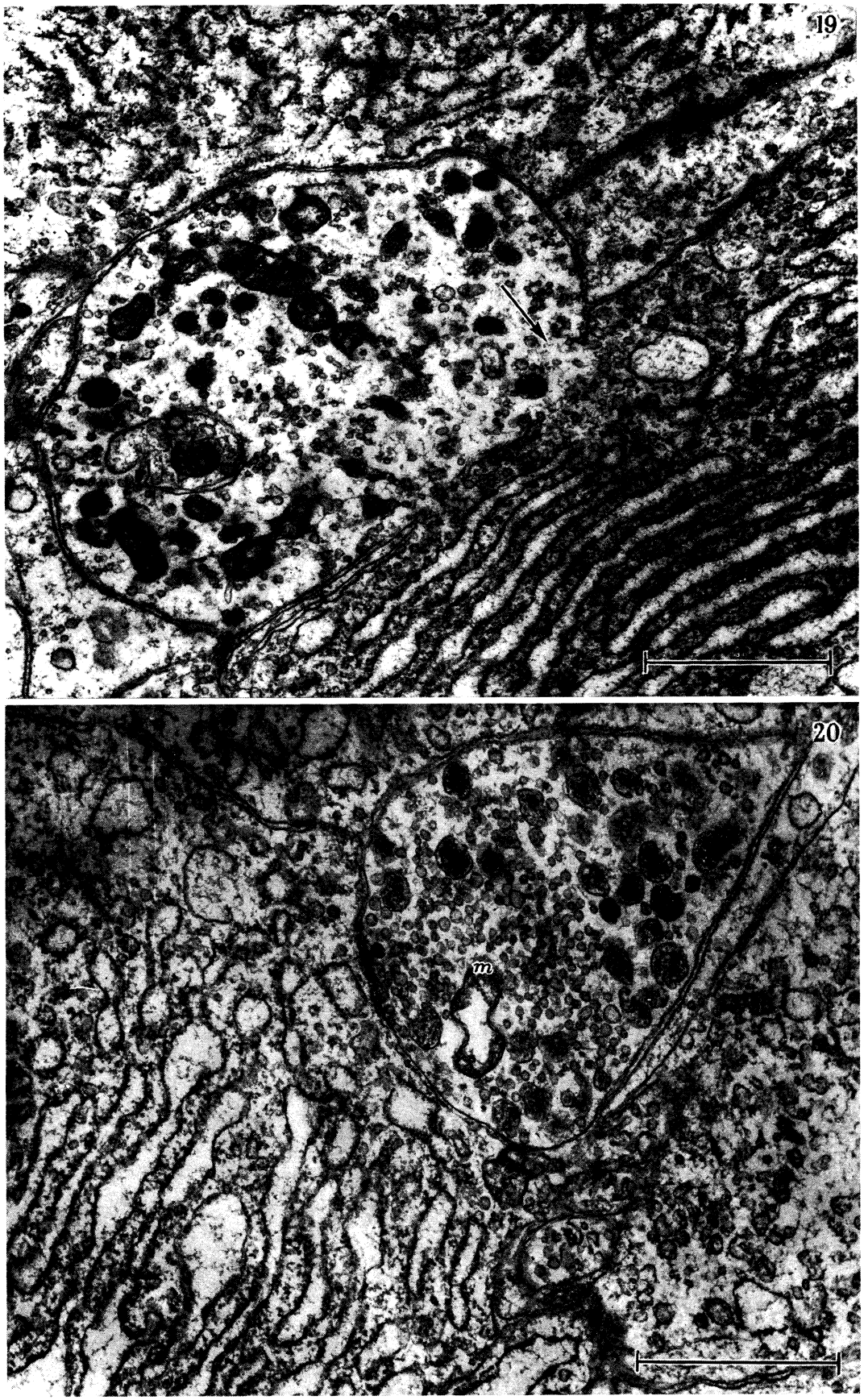


FIGURE 19. As figure 18, plate 55, but showing elementary neurosecretory vesicles of less electron density, and a greater number of small electron-lucent vesicles. The arrow points at one of the areas where the intervening membranes between the neurosecretory fibre and the intrinsic cell appear to have broken down. Near these points small electron-lucent vesicles, like those in the axon, are found in the cytoplasm of the endoplasmic reticulum (see also figure 22, plate 57).

FIGURE 20. As the two preceding figures but showing indications that the small electron-lucent vesicles may arise by fragmentation of larger electron-dense vesicles. (*m*, mitochondrion).

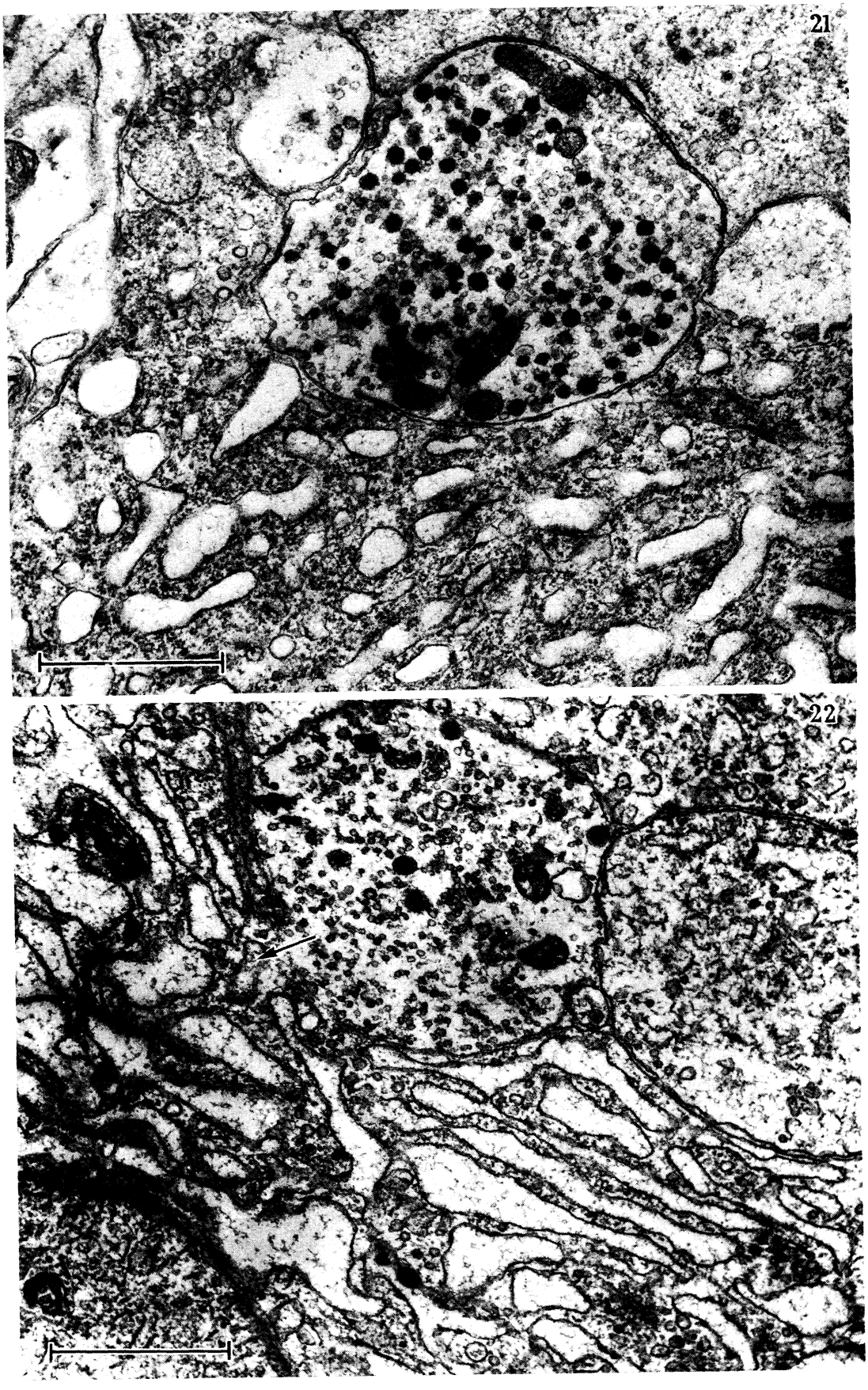


FIGURE 21. A neurosecretory fibre (in close association with the secretory pole of an intrinsic cell) which resembles the type A fibres of preceding figures except that its vesicles are smaller; this type of fibre is designated type 'A'[2] in the text.

FIGURE 22. A type A^2 fibre termination. This figure should be compared with figure 19, plate 56. The arrow points at a region where the cytoplasm of the fibre and that of the cell appear to be continuous.

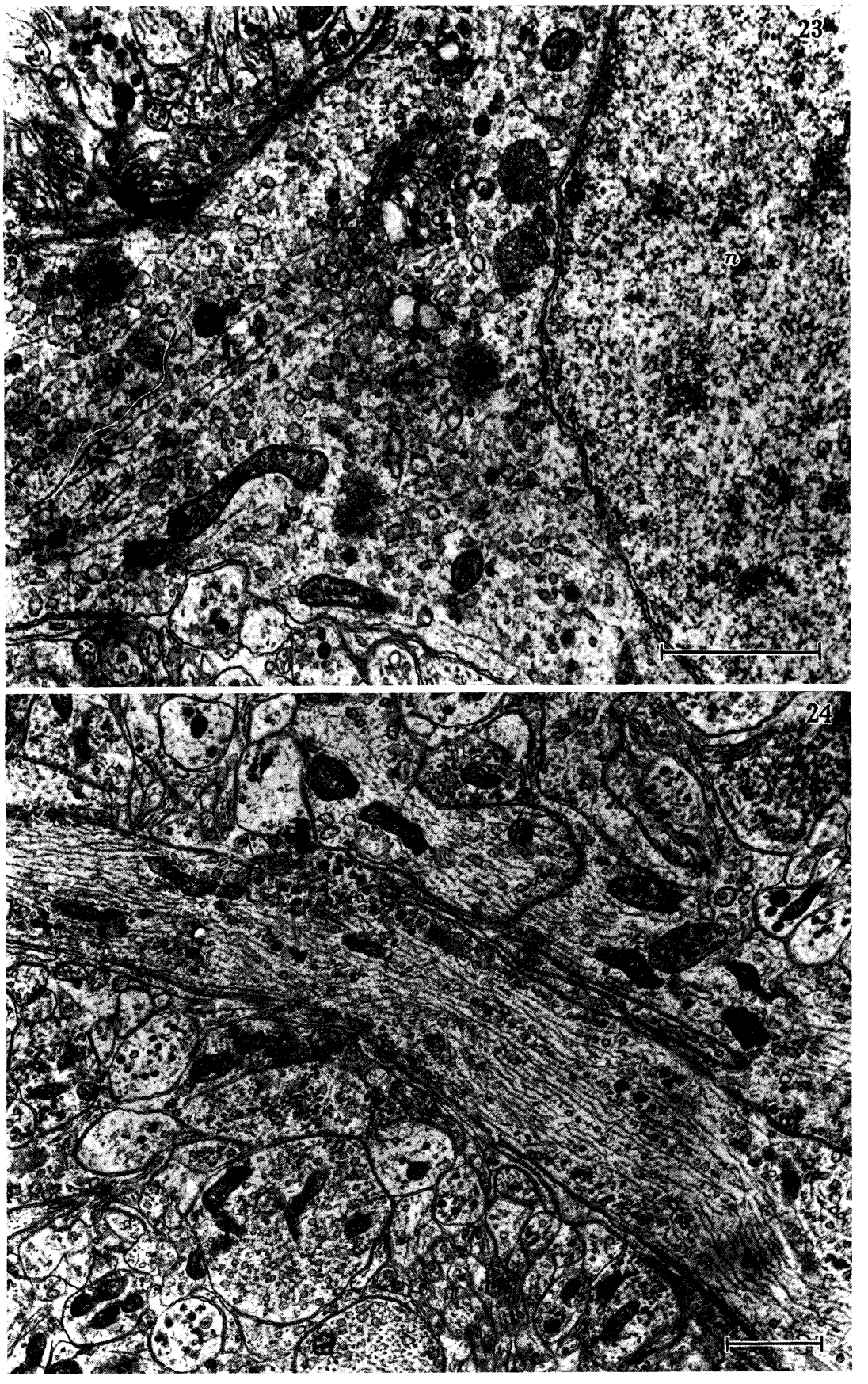


FIGURE 23. A portion of a B-type perikaryon, in the area of the axon hillock, showing B-type secretory vesicles, neurofibrillae and other cytoplasmic inclusions (*n*, nucleus).

FIGURE 24. B-type axon fibres in transverse and longitudinal section.

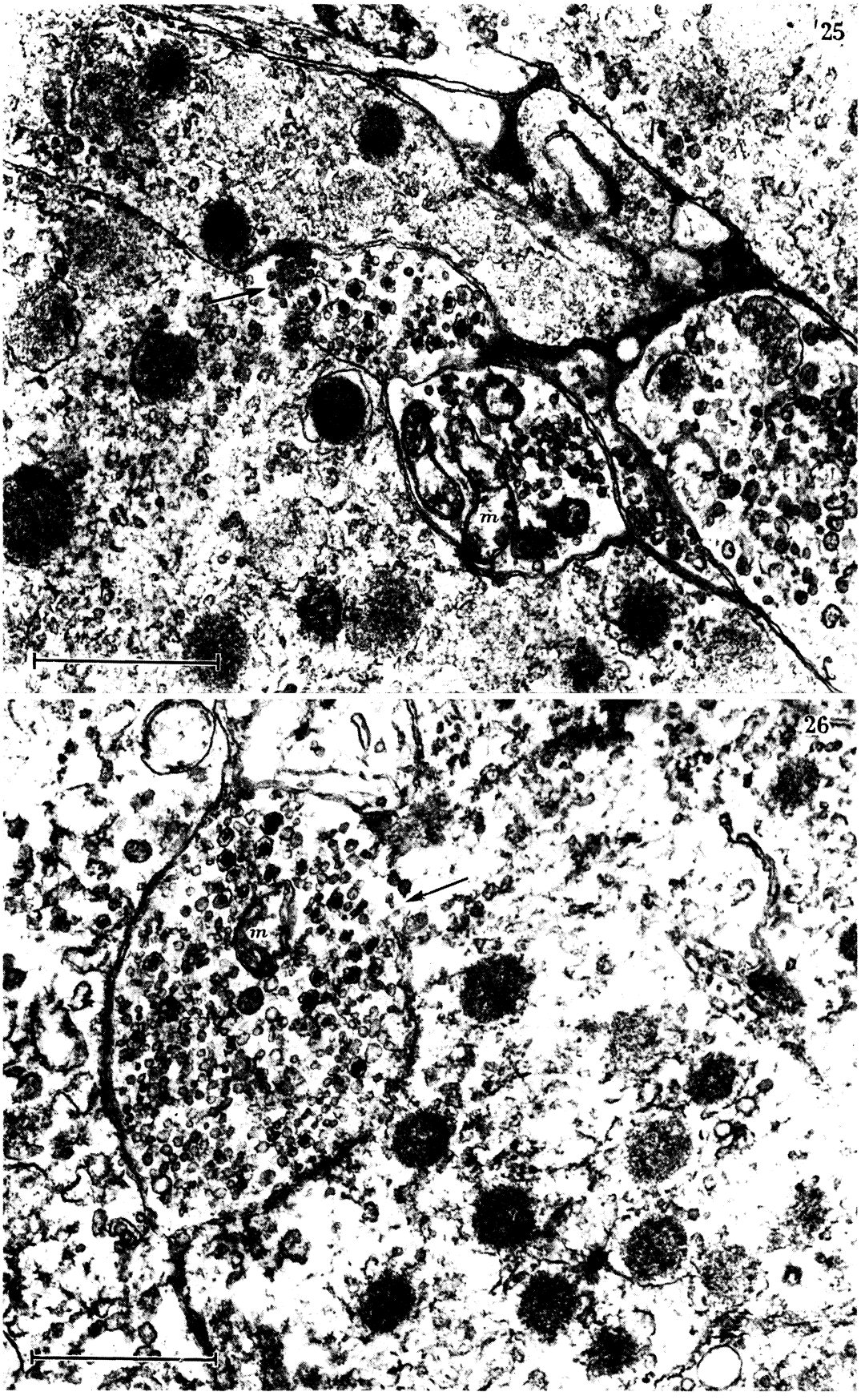


FIGURE 25. A secretomotor junction of B-type fibre terminals adjacent to the secretory and release poles of two peripheral secretory cells. An arrow points to an accumulation of small vesicles at a point where membranes between the fibre and one secretory cell are not apparent (*m*, mitochondrion).

FIGURE 26. As figure 25. The arrow points to a region where no membranes separate the fibre and the secretory cell. As in the preceding figure, membranes surrounding the electron-dense droplets in the intrinsic secretory cell are either fragmentary or absent.

cell, and also vesicles of similar size, either devoid of osmiophilic contents or with varying degrees of osmiophilia (figure 13, plate 53). The range of osmiophilia was such as to suggest that a loss of osmiophilic material precedes or accompanies hormone release.

(*b*) *Central cells*

The position of the peripheral secretory cells is such that, although tightly packed, each appears to have an extension that terminates at the surface of a blood vessel, separated from the blood stream only by a basement membrane. In contrast the cells in the central portion of each lobule of the neuro-intermediate lobe are not in evident direct proximity to the blood stream and are loosely packed, with distinct intercellular spaces (figure 14, plate 53) into which it is possible that their secretory products are discharged.

Unlike the peripheral cells the central cells do not demonstrate any marked polarity, especially in respect of their contained secretory droplets. These resembled those of the peripheral cells in their general appearance and were of the same general dimensions as those in the main body of the peripheral cells, i.e. 3000 Å. It may be remarked however that the larger droplets, about 13000 Å in diameter, which were sometimes observed in the prolongations of the peripheral cells (see figures 11 and 12, plate 52) were not observed in the central cells during the present study. This may be correlated with the fact that the central cells, unlike the peripheral cells, do not appear to have any special storage region for their secretory products. Instead these may be seen as spherical droplets evenly distributed round the periphery of each cell.

Some slight degree of polarity of the central cells is provided by the position of the endoplasmic reticulum, which lay at one end of the cell (figure 14, plate 53). This endoplasmic reticulum generally occupied a smaller proportion of the cell than that of the peripheral cells and was also relatively less compact and regular in form.

In comparison with the peripheral cells the mitochondria of the central cells were very small and few in number and the Golgi apparatus too was smaller and often indistinct. The ciliary roots found in association with the Golgi apparatus of the peripheral cells were not detected in the central cells.

Deep indentations of the nuclei were less frequent in the central cells than in the peripheral cells, but were nevertheless observed.

Empty secretory vesicles (i.e. those devoid of osmiophilic contents) were not observed in the central cells, in contrast to the prolongations of the peripheral cells (see figure 13, plate 53).

Differences in form between central and peripheral cells do not necessarily denote differences in hormone content but may represent merely a different mode of action due to the relative positions of these cells. It is evident that secretory material is stored in considerable amounts in the peripheral cells (plate 52) and that a sudden and considerable release may also take place (figure 13, plate 53). If one may accept the widely held view that these cells produce *MSH* it follows that this mode of action might bring about swift and striking colour change of the animal. In contrast no signs of considerable hormone storage or release were observed in the central cells, suggesting that if these also manufacture *MSH* they may be involved not in sudden and considerable colour changes but rather in maintaining a background level of melanocyte-stimulating hormone and thus a tonic stimulus to the melanophores.

The differences in structure of the central and peripheral cells may thus be explained in terms of different modes of action of cells of the same endocrine type. Such a hypothesis would be in accord with the view of Della Corte (1961) who by using normal histological methods discerned cells of different type, as distinguished by the size of their inclusions. Della Corte however preferred to regard these cells as fundamentally similar since their tinctorial reactions were the same, and considered them to be cells of one type, but with different forms of stages of secretion.

Intrinsic secretory cells appeared to form but one element of the central cords of the neuro-intermediate lobe. In addition glial elements and perikarya and fibres of neurosecretory cells were observed. A few possible secretomotor junctions between neurosecretory fibres and glial elements were seen [c.f. Knowles & Vollrath 1965].

5. Innervation of the neuro-intermediate lobes

The central regions of the lobules of the neuro-intermediate lobes contain neurosecretory fibres, which form a central mass stainable by the Gomori chrome-alum haematoxylin method. Gomori-positive axons also extend into the peripheral zones of the lobules (figure 1), but appear to stop short of the surface.

Under the electron microscope two kinds of neurosecretory fibres could be distinguished by the size and appearance of the secretory inclusions they contain. These two fibres bear some general resemblance to the two fibre types which have been described in another neurosecretory region, the pericardial organ of the crustacean *Squilla mantis* (Knowles 1962, 1964), and which were designated as type A and type B fibres. Accordingly in the present account the two fibre types will be also termed A and B fibres in view of the general resemblance to those of *Squilla*, though without *necessarily* implying any precise relation in terms of function.

(*a*) *Type A neurosecretory fibres*

Fibres, which are here termed type A fibres, contained considerable numbers of spherical or ovoid inclusions which had a mean diameter of 1500 Å with a maximal diameter of 1800 Å. It is suggested that the maximal figure gives the most accurate impression of the size of these vesicles, since a mean diameter, even of the larger vesicles, must result from measurements of some profiles which represent sections tangential to the vesicles. These inclusions were bounded by an outer membrane which was almost completely filled by the electron-dense contents of each vesicle (plates 54, 55). Under higher magnifications a small clear zone could be seen to separate each electron-dense mass from its bounding membrane (e.g. figure 16, plate 54), yet nevertheless the outlines of the membrane and its electron-dense contents were approximately similar and regular in form.

The axons of type A neurosecretory elements contained neurofibrillae or microtubules approximately 200 Å in diameter. These fibrillae were found in proximal regions, i.e. those parts of the fibres in the central regions of the lobules of the neuro-intermediate lobes. Such fibrillae resemble elements which have been described in neurosecretory fibres in other species. There were, in addition, some larger tubules approximately 600 Å in diameter, which in this respect resemble tubules in the axons of type A fibres in *Squilla mantis* (Knowles 1962). Aggregations of such tubules to form multilamellate bodies were sometimes,

but rarely, observed in the present study (figure 17, plate 55). Nevertheless the presence of such concentrically arranged tubules in neurosecretory axons in such a wide range of animals as crustaceans (Knowles 1962, 1964), mammals (Holmes & Kiernan 1964) and an elasmobranch fish indicates that formations of this type may play an essential part in normal neurosecretion.

Mellinger (1963*b*) has drawn attention to the fact that fibres which stain by Gomori chrome-alum-haematoxylin or performic acid-alcian blue techniques are rarely found extending to the surface of the neuro-intermediate lobes of *Scylliorhinus caniculus*. The arrangement of such fibres in *Scylliorhinus stellaris* appears to be similar. Neither by the use of normal histological techniques nor by using the electron microscope could clear evidence of the termination of neurosecretory fibres on the walls of blood vessels in the neuro-intermediate lobe be obtained. A possible functional significance of this finding will be discussed later.

On the other hand clear and striking evidence of the termination of type A fibres on the peripheral secretory cells of the neuro-intermediate lobe could be obtained (figure 18, plate 55 and plates 56, 57). Comparable terminals were not observed in relation to the central secretory cells.

The criteria used to determine whether a given micrograph depicted a fibre termination, or a more proximal region of a fibre were as follows: (*a*) there has been general agreement that the preterminal and terminal portions of neurosecretory fibres do not, unlike more proximal regions, contain neurofibrillae or micro-tubules. (*b*) An abundance of vesicles of approximately 500 Å in diameter is a characteristic of neurosecretory fibre terminations. They have been termed synaptic vesicles, but it has been pointed out that morphological descriptions which carry physiological implications must be used with care (Holmes & Knowles 1960; Knowles 1962). (*c*) Mitochondria are found throughout neurosecretory neurons (see plate 54) but are especially abundant at the terminals. In the present studies mitochondria with an 'empty' appearance were a typical feature of terminations of both type A and type B fibres (figure 20, plate 56 and figure 26, plate 59). (*d*) It has been shown that electrical stimulation of some neurosecretory fibres results in a loss of density of the elementary neurosecretory vesicles at the terminals, followed by the appearance of smaller vesicles within each elementary neurosecretory vesicle and the ultimate disintegration of the larger vesicles to set free the smaller ones (Knowles 1963). It is therefore interesting to note that type A fibres which by other criteria appeared to be fibre terminals showed features similar to those which had been elicited in other neurosecretory fibre terminals by electrical stimulation (figure 18 plate 55, and plate 56).

The examination of specimens stained by the Gomori technique, under the optical microscope, suggested a possibility that type A fibres innervated, either directly or by diffusion, the secretory poles of the intrinsic peripheral cells. Electron micrographs extend this view by making it clear that most if not all the type A fibres form intimate secretomotor junctions with the synthetic poles of the peripheral cells (figure 1). Many terminals of type A fibres making intimate contact with the region of endoplasmic reticulum of peripheral cells were observed: in contrast no indubitable type A fibre terminals were found elsewhere. It seems likely therefore that type A fibres are concerned in the control of *MSH* synthesis.

Mellinger (1963*b*) has reported the presence of two kinds of fibre originating in the preoptic nucleus of *Scylliorhinus caniculus*: one contained vesicles of approximately 1300 Å in diameter, and appeared to terminate in the median eminence; the other contained vesicles of approximately 1800 Å in diameter and terminated in contact with cells of the neuro-intermediate lobe.

There were some indications of the presence of two kinds of type A fibre in the present studies. One, with vesicles of diameter 1800 Å resembled that described by Mellinger, but fibres containing vesicles of a size range of 1300 Å were not observed in the neuro-intermediate lobe of *Scylliorhinus stellaris*. A few fibres with inclusions of a mean diameter of 1000 Å were noted and termed A2 fibres (plate 57). Because of their rarity it was not easy to exclude the possibility that the smaller vesicles were merely vesicles of 1800 Å diameter cut at a tangent, thus seeming smaller. In the case of that fibre depicted at figure 21, plate 57, however such a possibility seems unlikely in view of the uniformity of size of the vesicles. Moreover the endoplasmic reticulum of the cell with which this fibre made contact differed in proportions and appearance from the endoplasmic reticulum of a typical intrinsic cell of the neuro-intermediate lobe; the cytoplasm appeared denser, containing more ribosomes and the lacunae were wider and shorter. An occasional cell of this type was detected in the neuro-intermediate lobe. Possibly they correspond to a few atypical cells noted by Della Corte (1961), using conventional histological methods.

(*b*) *Type B neurosecretory neurons*

When the peripheral regions of the neuro-intermediate cords were examined in precise longitudinal section no fibres stained by the Gomori *CAH* method could be detected close to the surface. On the other hand the electron microscope revealed fibres with electron-dense inclusions which appeared to terminate on the distal prolongations of the intrinsic secretory cells close to the point where they discharged their contents into the blood stream (figures 1(*c*), (*d*)). These fibres will be termed type B fibres in the present account. The spatial relationship between these type B fibres and the peripheral intrinsic cells indicates that they control hormone release. The secretomotor junctions which they make with the peripheral cells lie in the region of hormone storage and release (figure 1, and plate 59).

Type B fibres resembled type A fibres in the dimensions of their terminals (approximately 2 μm) though the diameter of their more proximal regions was sometimes slightly smaller (compare figure 24, plate 58, and figure 16, plate 54). The size and appearance of their contained inclusions was very different from those of type A fibres. The maximal diameter of type A vesicles was 1800 Å and most of this was occupied by electron-dense material. The diameter of type B vesicles was difficult to determine since the membrane was often irregular in outline. The electron-dense part of a type B vesicle, however was considerably smaller than that of a type A vesicle, with a mean diameter of 600 Å. At the perikaryon (figure 23, plate 58) or in the proximal portion of the fibre (figure 24, plate 58), the electron-dense contents almost filled the spherical bounding membrane of the vesicle, but at or near the terminals the bounding membrane was larger, and irregular in outline and a clear electron-lucent region lay between the electron-dense contents and the surface (figures 25 and 26, plate 59).

Type B fibres therefore differ from type A fibres morphologically by reason of the very different size and appearance of the vesicles they contain. It is possible, indeed probable, that the irregularity of type B vesicles may be an artifact of fixation, but it is clear that type A and type B vesicles react differently to the same fixative and that their different appearance after fixation represents a fundamental dissimilarity.

Mitochondria of irregular form, with large central vacuoles, were observed in both type A and type B fibre terminals, but were more frequent in type B terminals. Small electron-lucent vesicles of a mean diameter of 500 Å were found in approximately equal concentrations in both type A and type B fibres.

A transverse section through the distal regions of a number of intrinsic cells indicated the possibility that a single type B fibre terminal might innervate more than one intrinsic cell. There were similar, though less clear, indications in respect of type A fibres.

Perikarya of type B fibres were frequently found in the central region of the neuro-intermediate cords (figure 23, plate 58) either singly or in clusters to form small nuclei. In contrast, perikarya of type A fibres in the neuro-intermediate lobe were very rare indeed (only two, one of which is depicted at figure 15, plate 54, were found in many hundreds of micrographs).

(*c*) *Non-neurosecretory nerve fibres*

A distinction between neurosecretory neurons and conventional motor or sensory neurons is difficult to make on the basis of ultrastructure alone, for both contain neurofibrillae in their proximal regions and both contain vesicles of synaptic vesicle size-range at their terminals. The main criterion is therefore whether or not elementary neurosecretory vesicles are present, and this criterion may be objected to on the grounds that a neurosecretory fibre which had recently discharged its hormone content might not have these.

There were however fibres (see figure 17, plate 55) which lay either in groups among type A fibres, or sometimes singly, which contained neurofibrillae of typical size range (150 to 200 Å) but no other evident components. These fibres which could by no criteria be termed neurosecretory were very considerably smaller than either type A or type B fibres. Indeed two of those shown at figure 17, plate 55 had each a diameter of 2000 Å i.e. little greater than a type A neurosecretory vesicle. It is therefore clear that these fibres could not represent type A fibres without neurosecretory vesicles. It is more difficult to be certain they cannot be 'empty' type B fibres, but they are considerably smaller than any fibres in which type B vesicles were found and on the basis of the available evidence there is no reason to identify these small fibres as neurosecretory fibres.

6. Secretomotor junctions of neurosecretory fibres and intrinsic endocrine cells

Some of the criteria used to determine whether a profile of a neurosecretory fibre represents a fibre terminal have already been listed, namely the absence of neurofibrillae, an abundance of small vesicles, atypical mitochondria and a loss of electron density of the elementary neurosecretory vesicles. In addition, fibres with these features were often oval rather than circular in outline, and the cell membranes separating the axoplasm of the fibre from the cytoplasm of the intrinsic secretory cell differed from those found elsewhere. These profiles were therefore interpreted as secretomotor junctions.

Various degrees of fragmentation of the cell membrane of the intrinsic secretory cell and the membrane of the neurosecretory fibre were observed at secretomotor junctions. In some the membrane of the axon was intact but that of the intrinsic cell was interrupted, as

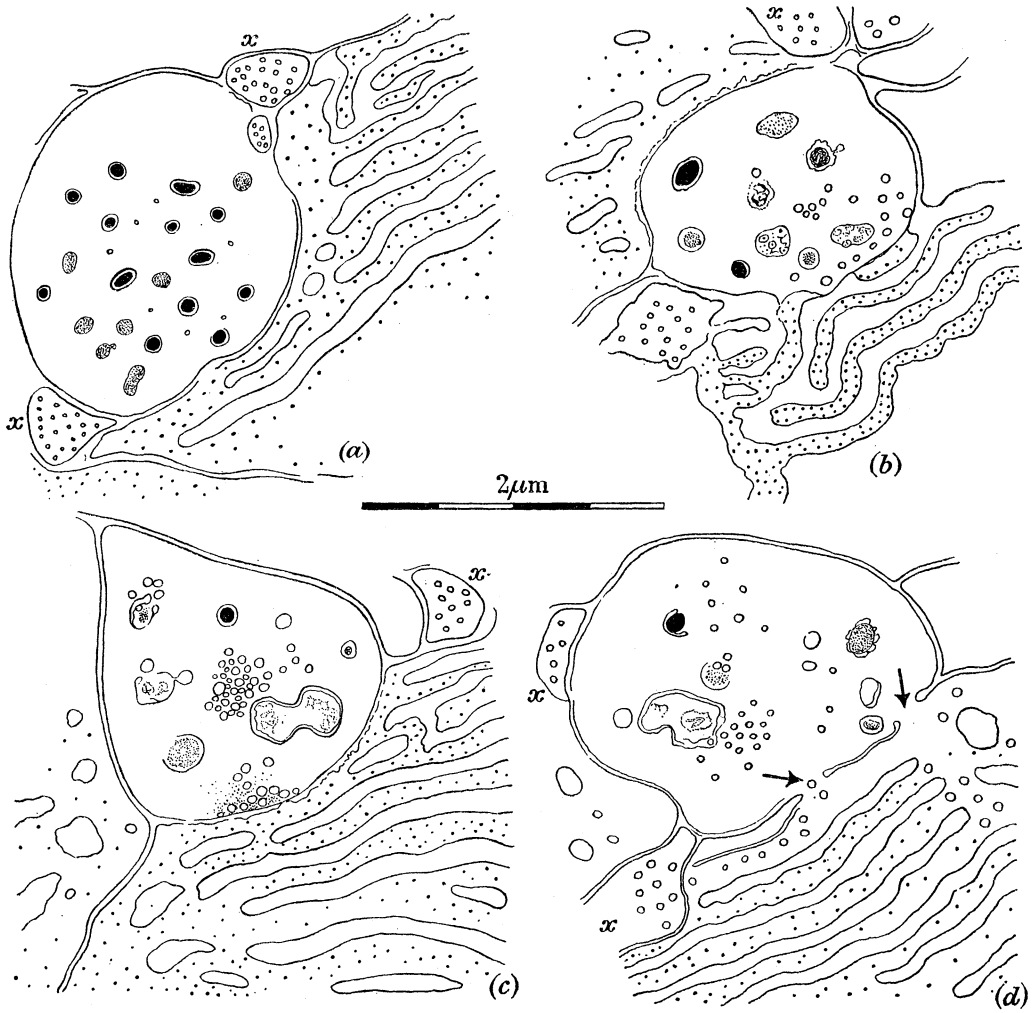


FIGURE 2. STAGES OF VESICLE BREAKDOWN AT THE TERMINALS OF TYPE A NEUROSECRETORY FIBRES

(*a*) At this stage there are many uniformly dense spherical or sub-ovate electron-dense vesicles. A certain number of vesicles are slightly less regular in form and less electron-dense. Few small vesicles are present.

(*b*) A reduction in the number of electron-dense vesicles is accompanied by an increase in the number of larger, irregularly shaped and more electron-lucent vesicles. In some of these smaller vesicles may be observed.

(*c*) Clear indication of the breakdown of elementary neurosecretory vesicles could be seen in some profiles. The smaller vesicles which resulted from this process frequently aggregated in the centre of the fibres. Clusters of vesicles of synaptic vesicle size-range were present, close to that portion of the cell membrane in close contact with the synthetic pole of the peripheral intrinsic cell of the neuro-intermediate lobe (see figure 20, plate 56).

(*d*) Some secretomotor junctions contained few, if any, electron dense vesicles but many small electron-lucent vesicles. A breakdown of cell-membranes at the junction was often apparent and there were indications that small vesicles had passed through the pores thus formed. (The possibility that these 'pores' are artifacts is discussed in the text.)

though by large pores (figure 21, plate 57). In others both axon and intrinsic cell membranes were interrupted at certain points along their length, so that there was apparent cytoplasmic continuity between the axoplasm and the cytoplasm of the intrinsic cell (figure 19, plate 56). It is arguable that the gaps in membranes observed at the secretomotor junctions represent fixation artifacts, but the fact that they appear in sections in which membranes elsewhere are well-preserved (e.g. figure 19) suggests that the membranes at secretomotor junctions differ in some way from membranes elsewhere in the cells concerned. Moreover small vesicles similar in size and appearance to those in axon terminals were observed in the outermost layers of endoplasmic reticulum nearby (figure 19), as might be expected if membrane breakdown occurred at the secretomotor junction.

The elementary neurosecretory vesicles of type A at secretomotor junctions were very different from those found in more proximal regions. They were larger, less electron-dense and within them smaller vesicles could be observed. Various intermediate stages could be seen, ranging from spherical electron-dense vesicles to irregularly shaped electron-lucent vesicles containing smaller vesicles (figure 2). These profiles are consistent with a view that hormone release from type A fibres is accompanied by the fragmentation of elementary neurosecretory vesicles (Knowles 1963).

Various authors have pointed to the presence of small clusters of 'synaptic' vesicles at neurosecretory fibre terminals (e.g. Oota 1963*a*), and it has been suggested that these play a part in hormone release (De Robertis 1962). Clusters of small vesicles of synaptic vesicle size-range were found at secretomotor junctions of both type A and type B fibres (e.g. figure 20, plate 56) but there were no clear indications of their origin or function. Histochemical studies are needed to determine whether cholinesterase activity occurs at secretomotor junctions between neurosecretory fibres and other endocrine cells.

7. Discussion

A study of the ultrastructure of the pituitary of *Scylliorhinus* emphasizes the difficulty of defining neurosecretion by morphological criteria alone, a difficulty already envisaged at the Third International Symposium on Neurosecretion (see De Robertis 1962; Scharrer 1962).

At this symposium Scharrer remarked that 'neurosecretory cells do not form synapses with other neurons or effector organs. Their axons end at blood spaces into which they release the secretory material'. This is certainly true of most neurosecretory systems, but in the pituitary of elasmobranch fishes there are fibres which do not appear to liberate their products into the blood stream though by other criteria they might be judged to be neurosecretory. It is pertinent therefore to enquire whether these fibres do exhibit neurosecretion.

Various studies on *Scylliorhinus caniculus* and on other closely related selachians have pointed to the importance of that system of neurons which begins in the nucleus preopticus, where its cell-bodies are located, and continues as fibres containing stainable secretory material. These fibres pass down the infundibular stem and terminate in the neuro-intermediate lobe. This system, as described by Scharrer (1952) and Mazzi (1952) in *Scylliorhinus stellaris* and by Bargmann (1955) and Mellinger (1963*b*) in *Scylliorhinus caniculus* appears to be represented in the present studies by those fibres which have been termed

A fibres; these fibres contain vesicles which in their size, *ca.* 1800 Å resemble those of the hypothalamo-hypophysial neurosecretory system of amniotes; their tinctorial affinities, using the Bargmann modification of the Gomori *CAH* staining technique are also similar. Moreover the distribution of these fibres may be correlated to oxytocic activity (Perks & Dodd 1960). In phylogeny they seem to be homologous with the hypothalamic-hypophysial tracts of tetrapods (Dodd 1963). They therefore are neurosecretory by all the generally accepted criteria save one, namely that their products appear to act locally, by diffusion, not at a distance after transport in the blood. Since however in other respects they resemble generally accepted neurosecretory systems it is considered that they should be termed neurosecretory.

It is less easy to classify the type B fibres as neurosecretory. The main justification for so doing is that they contain appreciable numbers of membrane-bound vesicles, though each has an overall diameter of 1000 Å or less. In general appearance however under the electron microscope they resemble type A fibres. They moreover resemble type A fibres in that they directly innervate the intrinsic endocrine cells of the neuro-intermediate lobe. In some other respects, however, notably their distribution and tinctorial affinities, they differ from type A fibres. It seems likely that type B fibres, unlike type A fibres, do not originate in the preoptic nucleus; the presence of small nuclei of type B perikaya in the neuro-intermediate lobe shows that this type of neurosecretory fibre may sometimes have a peripheral origin. The possibility however that most of the B fibres in the neuro-intermediate lobe originate in the brain cannot be excluded. Mellinger (1963*b*) describes a system of fibres originating in the nucleus lateralis tuberis, with many of the characteristics of type B fibres; the size of their electron-dense vesicles was less than 1000 Å and a clear electron-lucent halo between the electron-dense contents and the membrane of each vesicle was evident; they were Gomori *CAH* negative and alcian blue negative. Mellinger suggested that some of these type B fibres might terminate in the median eminence region, but he envisaged also the possibility that others might enter the neuro-intermediate lobe. Meurling (1963) has shown that Gomori positive fibres and also fibres which do not stain with the Gomori technique may be demonstrated in the pituitary of elasmobranch fishes.

It is interesting to compare also the observations of Billenstien (1963) on the brook trout *Salvelinus fontinalis*. She demonstrates a system of fibres which originate in the nucleus lateralis tuberis and terminate in the more dorsal regions of the pars intermedia; these were not stained by the Gomori *CAH* or aldehyde fuchsin methods but could be shown by acid fuchsin or azocarmine. In contrast the fibres originating in the preoptic nucleus were Gomori *CAH* and *AF* positive and predominated in the more ventral and posterior regions of the pars intermedia.

Fibres of type B dimensions and appearance have been demonstrated in the hilar region of the infundibulum in a bullfrog (Oota & Kobayashi 1963), in a turtle (Oota 1963*a*) and in a mouse (Oota 1963*b*); the vesicles, which had a mean diameter of approximately 900 Å, were found in the median eminence of the turtle and the mouse but not apparently in the bullfrog.

Fibres of the B type which may be detected by silver staining or under the electron microscope after osmium fixation, but which do not stain by the Gomori *CAH* or performic alcian blue methods, have been demonstrated in the pars intermedia of the pituitary gland

of *Xenopus* (Cohen 1964). The vesicles which are present in the pars intermedia of the rat pituitary gland (Kurosumi *et al.* 1962) also appear to be of the B type insofar as a clear electron-lucent halo surrounds the dense osmiophilic material in each vesicle. Type B fibres therefore seem to be present in the pars intermedia and the median eminence at many levels of the vertebrate series. In some instances they appear to liberate their products into the blood stream. In other situations they seem to make direct secretomotor junctions with endocrine cells.

Type B fibres in the pars intermedia and median eminence have been described by most authors as neurosecretory, though they do not agree with all the criteria which have been used to define neurosecretory systems (Bern 1962, 1963). They do nevertheless share two important features in common with other systems which have been termed neurosecretory.

(*a*) They contain membrane-bound electron-dense vesicles greater in size than synaptic vesicles.

(*b*) They are concerned in endocrine regulation. It has been suggested (Bern & Knowles 1965) that this latter criterion, that neurosecretory systems exercise endocrine control either by releasing hormones into the blood stream or by controlling other endocrine systems is a fundamental one; using this criterion both type A and type B fibres in the pituitary of *Scylliorhinus* are neurosecretory, though neither liberate secretory material into the blood stream, and type B fibres do not stain with the Gomori technique.

The morphological differences in the type A and type B fibres may indicate significant chemical dissimilarities; their tinctorial affinities would support this view. Scharrer (1962) remarked that evidence points to the stainability of both peptide hormonal material and a carrier protein in hypothalamo-hypophysial neurosecretory systems, and suggested that this is presumably due to the presence of the same amino-acids in both carrier protein and octapeptide. Type B fibres of the neuro-intermediate lobe do not, however, stain with Gomori *CAH* methods, though they may be detected by the use of silver stains or at the level of ultrastructure after osmium impregnation.

The chemical characterization of type B vesicles would be of great interest. There is as yet no evidence, direct or indirect, which has a bearing on this problem in elasmobranch fishes. There are however indications in the neurosecretory systems in other groups that vesicles of type B appearance and size may be associated with catechol or other biologically active amines. Pellegrino De Iraldi, Duggan & De Robertis (1963) showed that vesicles with an outer diameter of 1300 Å and a clear space between an outer bounding membrane and an inner, smaller, electron-dense granule were associated with a high concentration of catechol amine material. Carlson, Falck & Hillarp (1962) showed that monoamines, mainly of the catechol amine type were accumulated in the median eminence of the mouse and the rat. Recently Fuxe (1964) has extended these observations by demonstrating monoamines in the median eminence and the infundibular stem of a number of mammal species. The distribution of these amines accords with the distribution of type B fibres as demonstrated by electron microscopy (Oota 1963*a*, *b*; Oota & Kobayashi 1963). Mention should also be made of two types of fibre, type A and type B, in the pericardial organs of crustaceans; thus far only a peptide hormone and 5, 6-dihydroxytryptamine have been extracted from these organs (Carlisle 1964) and there are indications that the type A fibres may be associated with the peptide hormones (Knowles 1964). There is therefore some evidence,

largely inferential, which indicates a possibility that type A fibres which are Gomori *CAH* positive and contain electron-dense granules of a diameter of 1500 to 1800 Å may be engaged in peptide hormone production whereas type B fibres which are Gomori negative and contain vesicles with electron-dense contents of a diameter of 1000 Å or less may manufacture and release catechol—or other aromatic amines. It has been suggested however that until an exact determination of the chemical nature of type B fibres and inclusions has been made the terms peptide and non-peptide neurosecretion might be used to denote fibres with type A and type B characteristics (Knowles 1965).

Dodd (1963) has remarked that an intimate relationship between the hypothalamic neurosecretory system and the intermediate lobes exists in all the lower vertebrates which exhibit hormonally-controlled colour change. Indeed he has suggested that the original function of the neurohypophysial system was to act as a link between the eyes and that part of the pituitary which contains chromactivating hormones.

The results of experiments have pointed to an inhibitory control of synthesis of *MSH* by some form of nervous control originating in the hypothalamus. Mellinger (1963*a*, *b*) showed that background responses in *Scylliorhinus caniculus* were abolished by severance of the pituitary stalk, and that animals so treated darkened maximally and that the cells of the neuro-intermediate lobe manifested a great activity of the endoplasmic reticulum. Comparable experiments on amphibian colour change have shown that section of the fibre tracts leading from the hypothalamus to the infundibular process was followed by an increased activity of the *MSH*-producing cells of the pars intermedia (Etkin 1962).

Biochemical analysis of extracts of elasmobranch neuro-intermediate lobes have shown that they contain appreciable quantities of *MSH* and one or more substances with oxytocic activity (Heller 1964). There is no reason to suppose that the *MSH* is synthesized elsewhere than in the intrinsic cells of the neuro-intermediate lobe. The oxytocin analogue is not apparently manufactured in the neuro-intermediate lobe, but in the hypothalamus. Perks & Dodd (1960) found oxytocic activity in extracts of the preoptic nucleus and neuro-intermediate lobe of *Scylliorhinus*, but showed that after section of the hypothalamo-hypophysial tract the oxytocic activity of the neuro-intermediate lobe decreased and finally disappeared.

Interruption of the hypothalamo-hypophysial tract therefore brings about two very evident changes in the hormone content of the neuro-intermediate lobe—a *decrease* in oxytocic content and an *increase* in *MSH* content. Electron micrographs demonstrate a very intimate relationship between neurosecretory fibres of the hypothalamo-hypophysial tract and the endoplasmic reticulum region of intrinsic secretory cells of the neuro-intermediate lobe and provide no clear evidence that neurosecretory fibres in the neuro-intermediate lobe discharge their products into the blood stream. These facts, taken together, indicate a possibility that a substance with oxytocic properties is manufactured in the hypothalamus, passed along axon fibres to the neuro-intermediate lobe and that it there inhibits synthesis of *MSH* by the intrinsic cells of that lobe. Whether the hormone released from the type A fibres intervenes directly as an inhibitor of hormone synthesis or excites the production of an inhibitor has yet to be determined. The evidence however points to the action of type A fibres as being a tonic rather than a phasic one, for which a relatively large and slowly destroyed peptide molecule might be suitable (see Welsh 1955).

Experimental evidence (Mellinger 1963*a*, *b*) indicates that not only *MSH* synthesis but also *MSH* release may be under an inhibitory control in *Scylliorhinus*. The distribution of type B fibres indicates that they are concerned in the regulation of *MSH* release. A temporal distinction may be made between the relatively slow and continuous process of synthesis and the more rapid and possibly discontinuous activity of hormone release. It is interesting to note that Carlisle (1964) has remarked that the heart-stimulating peptide hormones and amines released from neurosecretory nerve endings in the pericardium of crustaceans differ in their speed of action and the duration of the responses which they evoke; a quick short response is produced by 5 . 6–dihydroxytryptamine, a slow long response by one or more polypeptides.

Koelle (1959) has pointed to the basic similarities between nerve cells and neurosecretory elements and remarks 'The chief differences between the two systems are spatial—the interposition of a few hundred ångströms or the circulatory system between the sites of release and action of the transmitters—and temporal where the extremes can be calculated in terms of milliseconds or days'. It now seems evident that the spatial distinction is not an essential one. Fibres of both type A and type B appear to make secretomotor junctions with intrinsic cells of the neuro-intermediate lobe in *Scylliorhinus*. Blood-borne hormones would not seem to be a necessary criterion for neurosecretion.

The distinction between neurosecretory and non-neurosecretory phenomena on a temporal basis however seems to be a useful one. Neurosecretory neurons manufacture and store appreciable quantities of secretory material, and are therefore suitable for prolonged stimulation of their target organs without fatigue. It has been suggested that this tonic activity may be an important functional feature of all neurosecretory systems (Knowles 1955) and one in which they differ from the more phasic cholinergic systems.

Within this group of neurosecretory systems a further distinction on a temporal basis may be considered. The distribution of neurosecretory fibres in the neuro-intermediate lobe of the pituitary of *Scylliorhinus* indicates a possible distinction between peptide hormone-containing fibres engaged in the long-term control of hormone synthesis, possibly to be measured in hours, and another type of neurosecretory system, chemically and structurally different, controlling the more transitory activity of hormone release, possibly to be measured in seconds or minutes.

Hitherto the first form of neurosecretion has attracted the attention of most investigators who have studied the pituitary of the higher vertebrates. Holmes & Zuckerman (1962) have however remarked that neurons which are not neurosecretory in their affinity for the neurosecretory stains may be detected in the neural process of some mammals. It would be interesting to know whether these neurons resemble the type B neurons of the present study and whether they too are concerned in the regulation of hormone release from some part of the adenohypophysis.

Studies on the ultrastructure of the dogfish pituitary may be seen therefore to have a direct bearing on two important controversies, namely the definition of neurosecretion and to what extent the adenohypophysis of vertebrates is under a neurosecretory control (see Schreiber 1963). It is evident that in primitive vertebrates at least one part of the adenohypophysis may be under a precise neurosecretory control, which regulates hormone synthesis and hormone release. The electron microscope studies moreover demonstrate

that, at the level of ultrastructure, more than one type of neurosecretion may be envisaged and that neurosecretory fibres may in some instances make direct secretomotor junctions with their target organs.

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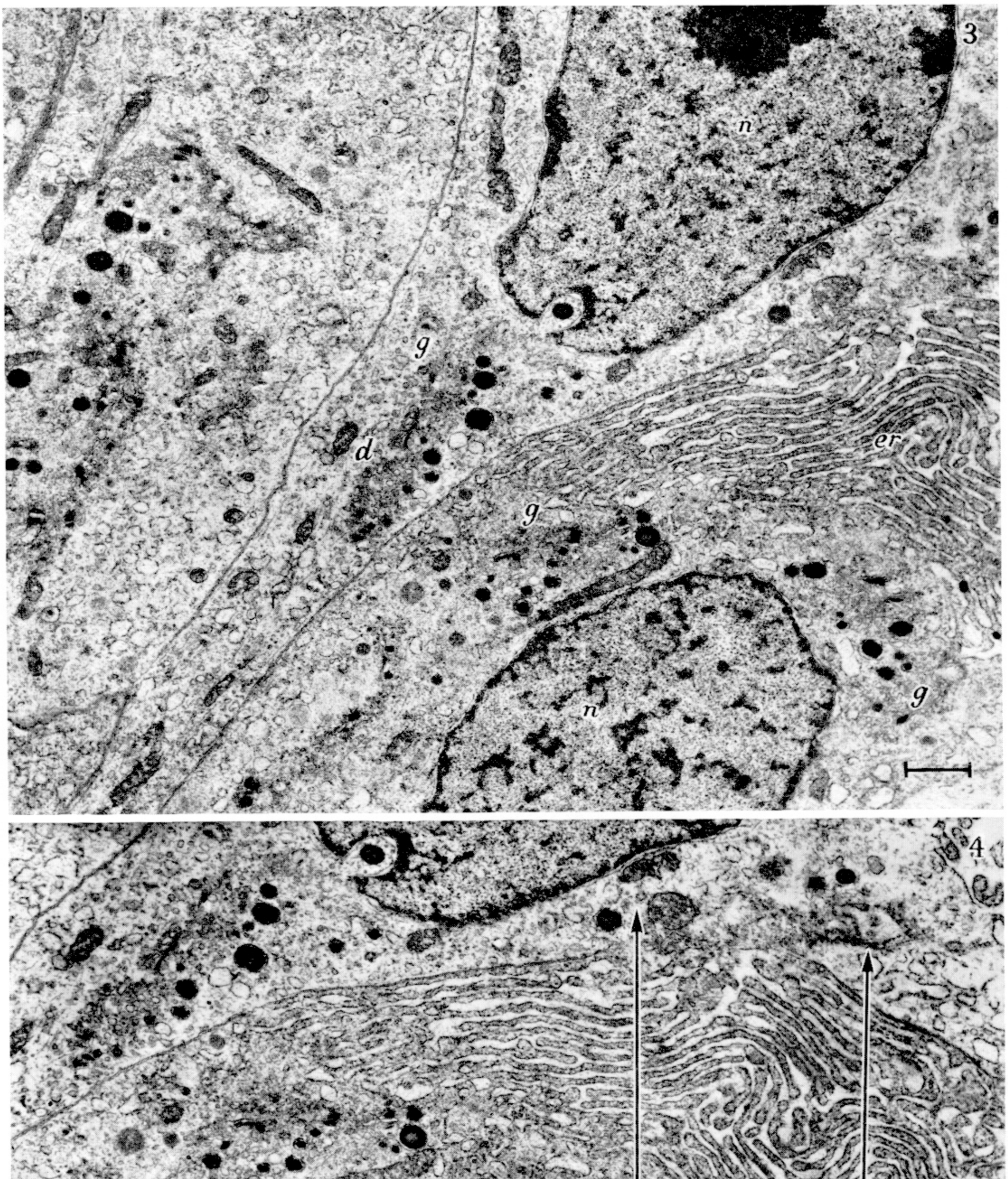
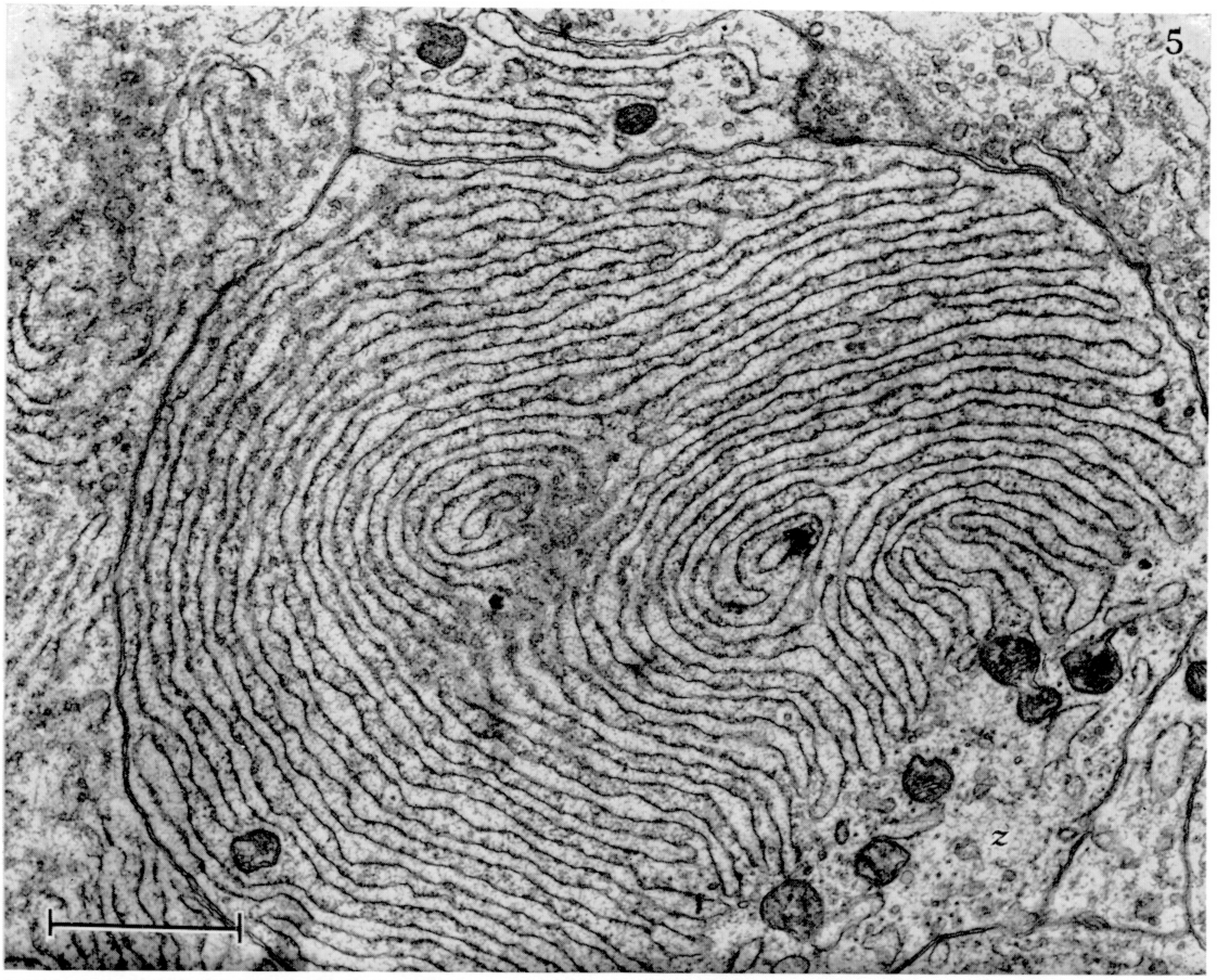


FIGURE 3. A section through the peripheral region of the neuro-intermediate lobe, showing intrinsic cells cut in longitudinal section. *d*, distal prolongation; *er*, endoplasmic reticulum; *g*, Golgi zone; *n*, nucleus.

FIGURE 4. As figure 3, but showing more of the secretory pole of the lower cell. The vertical lines *a* and *b* denote the planes of section of figure 5 and 8 respectively.



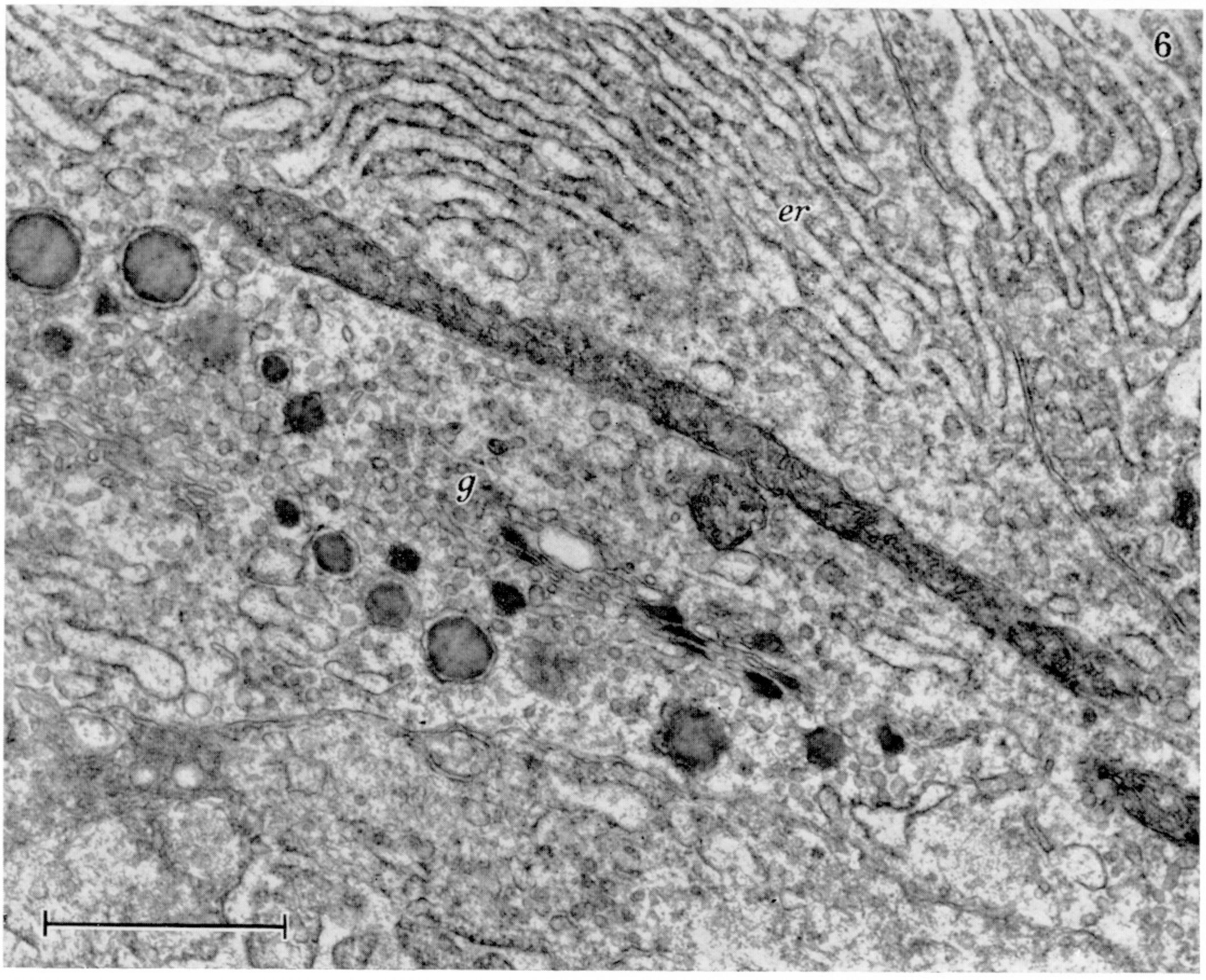
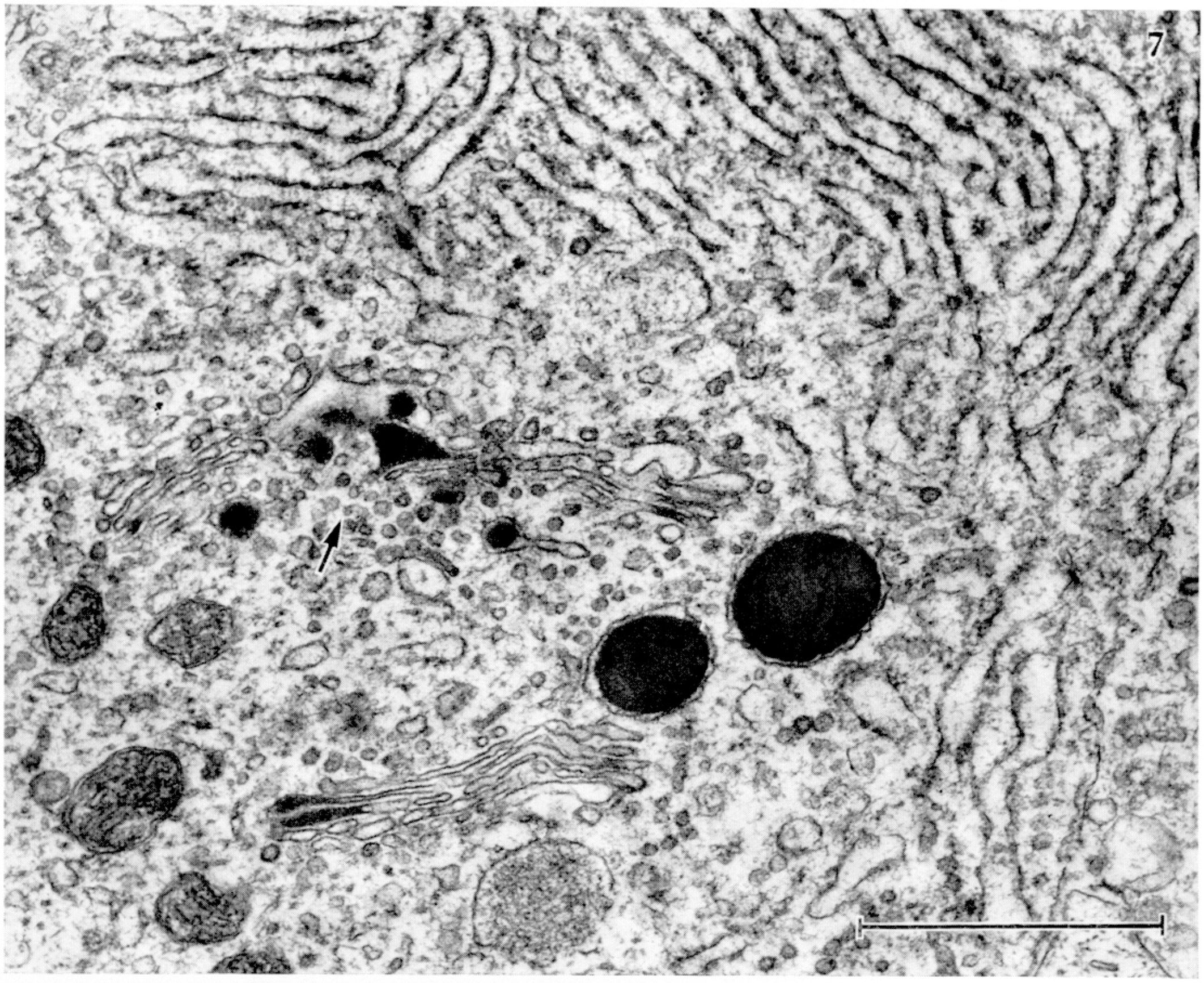


FIGURE 5. A transverse section through the secretory pole of a peripheral intrinsic secretory cell. *z*, zone containing mitochondria and fibrillae, but no endoplasmic reticulum.

FIGURE 6. A longitudinal section through a Golgi zone of an intrinsic cell, showing the elongate type of mitochondrion characteristic of this region. *g*, Golgi tubules and vesicles; *er*, endoplasmic reticulum.



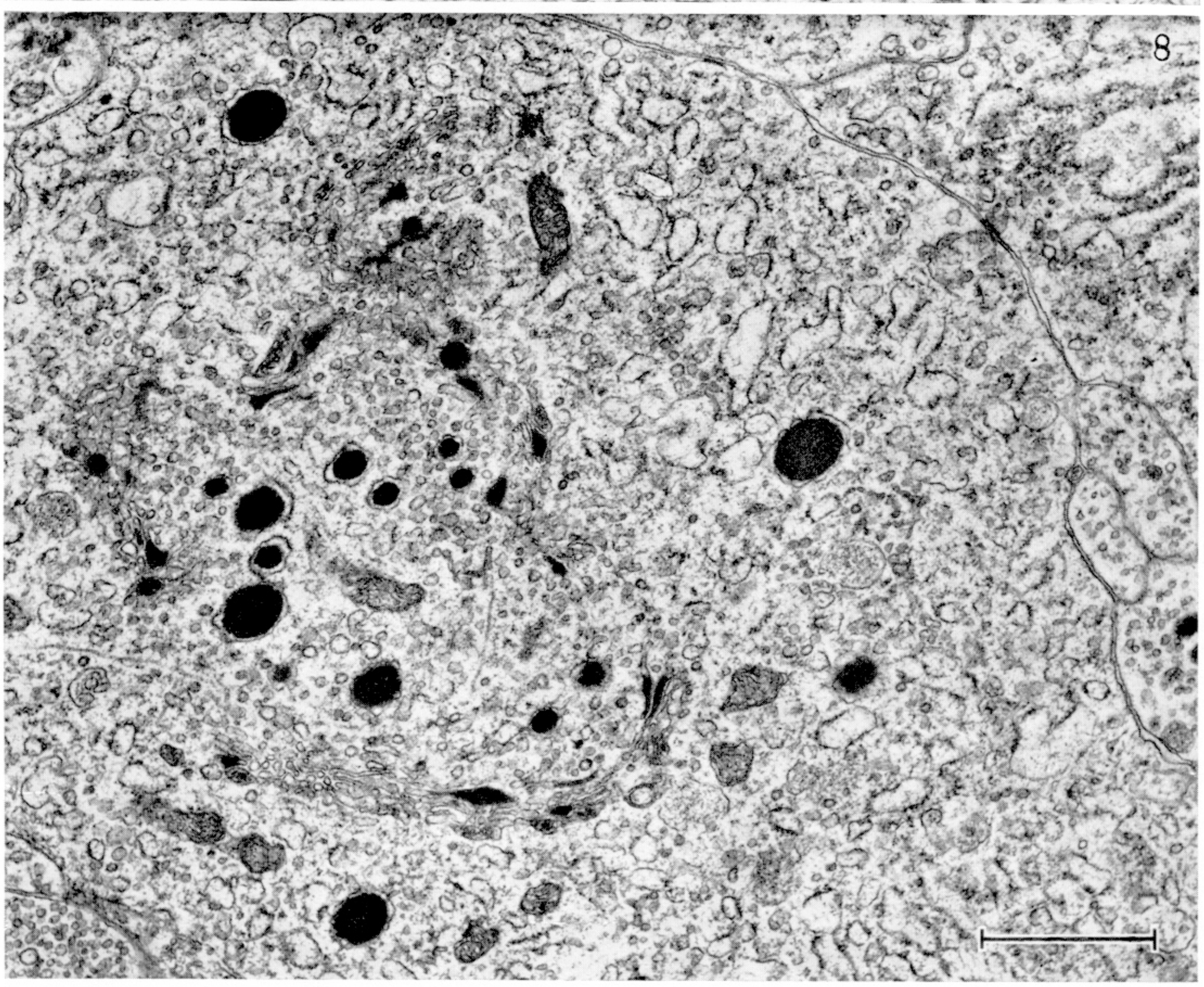
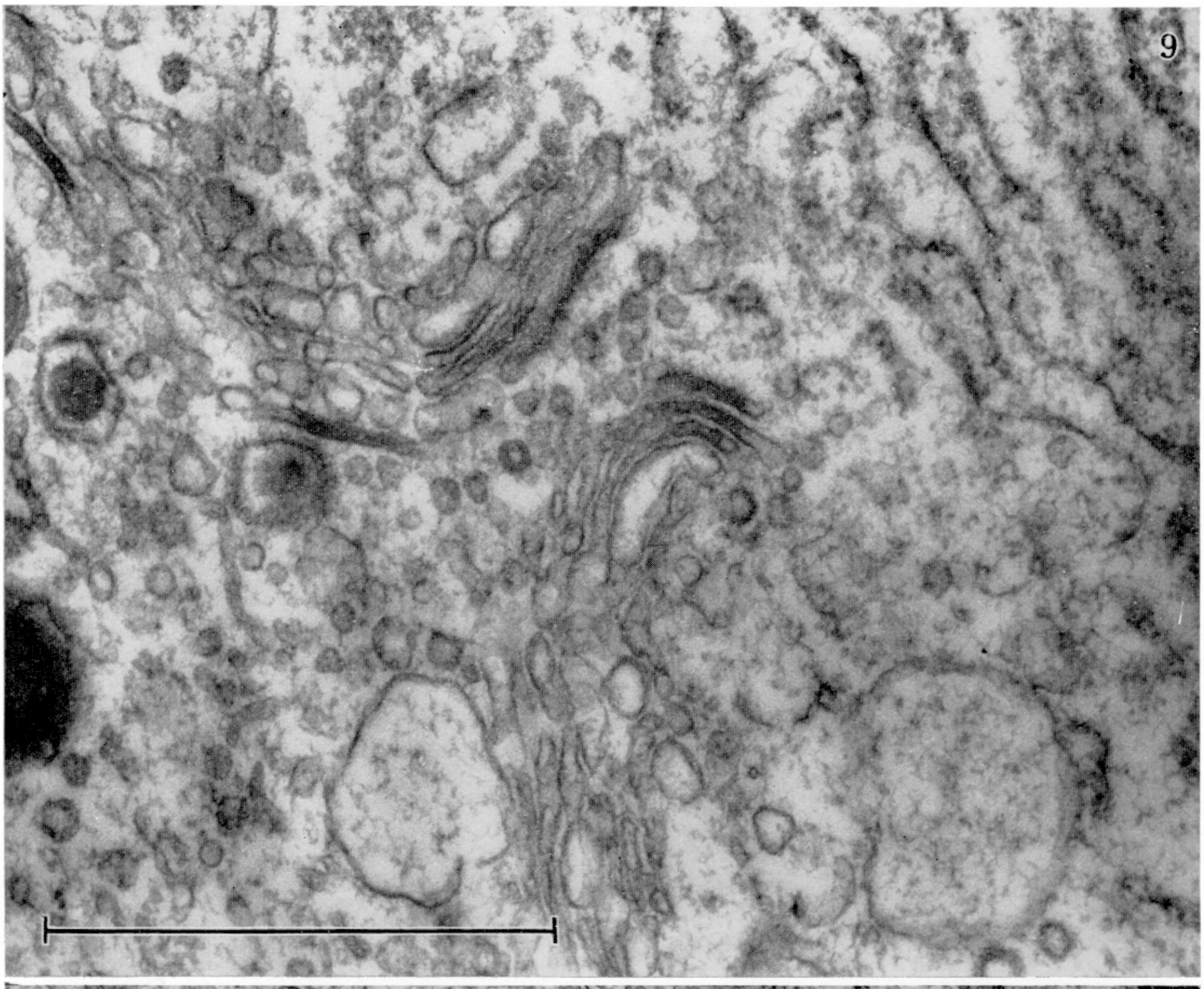


FIGURE 7. An oblique section through a Golgi zone of an intrinsic cell. An accumulation of electron-dense material to form spherical droplets is shown; an arrow points to a region, close to the endoplasmic reticulum, which shows an irregular form, and apparent discontinuity of the Golgi lamellae (see also figure 9, plate 51).

FIGURE 8. A transverse section through a Golgi zone in the central region of an intrinsic cell (see figure 4, plate 48). At this level cisternae of the endoplasmic reticulum are discontinuous and tubular. Fine fibrillae are found in the Golgi zone.



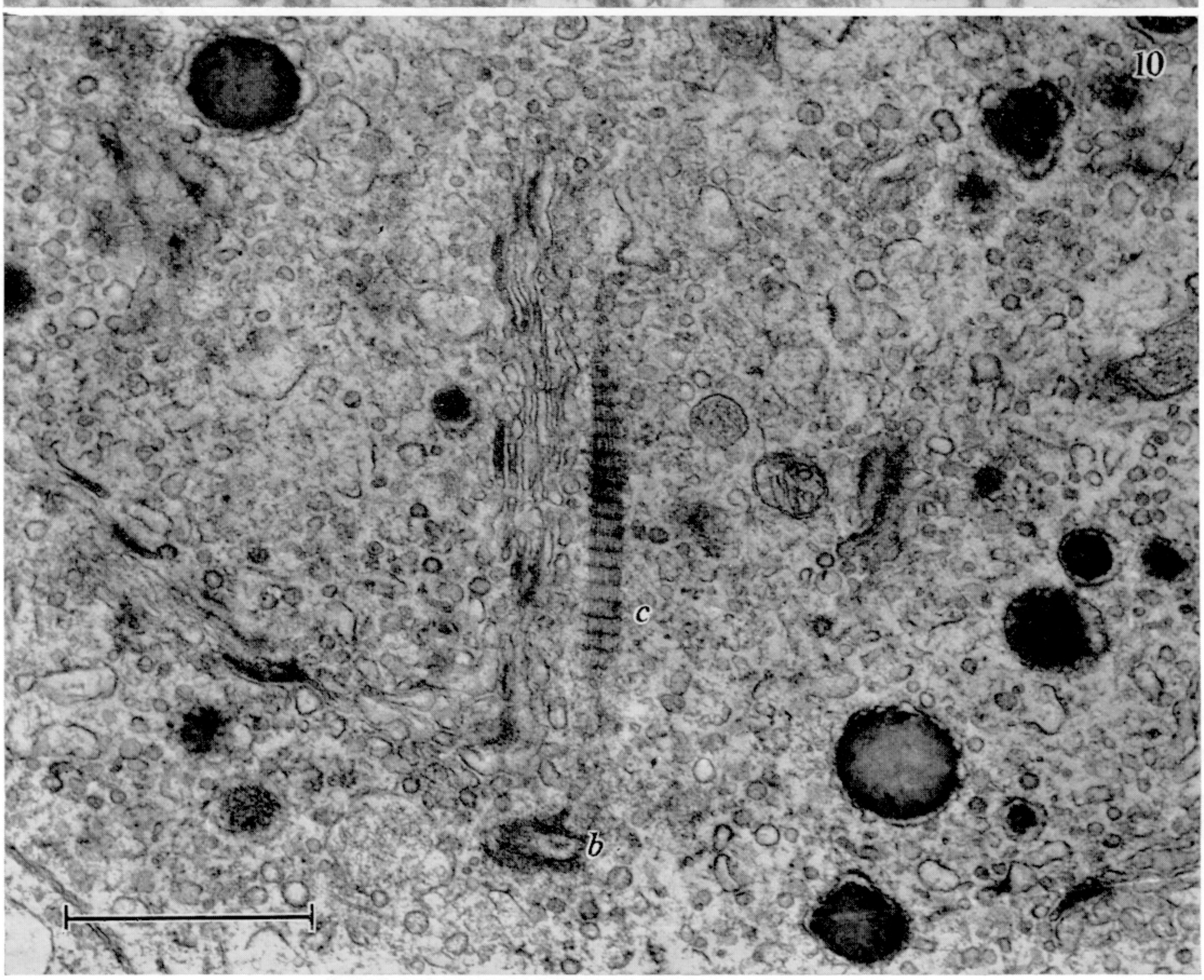
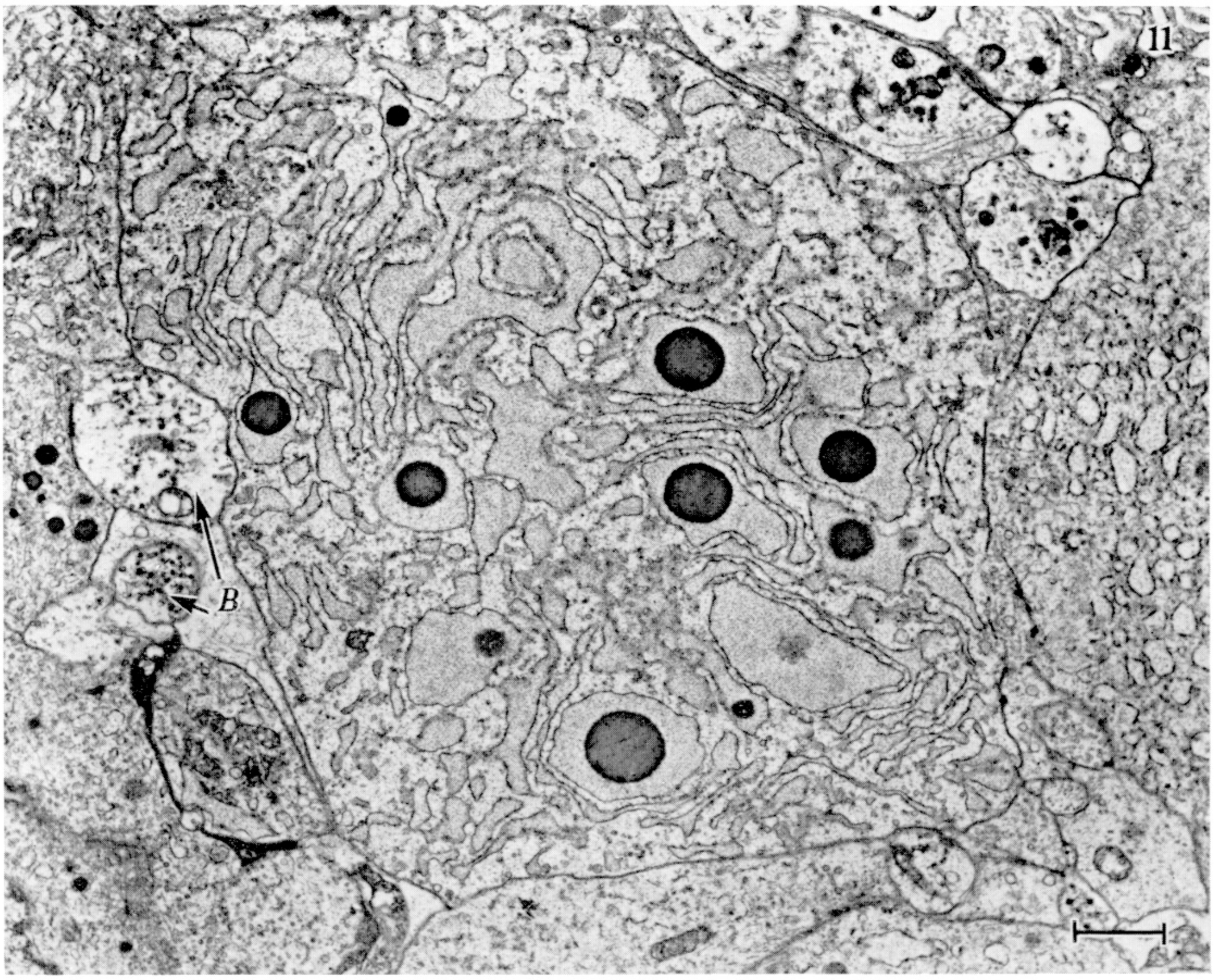


FIGURE 9. A longitudinal section through the region in which the Golgi lamellae and the endoplasmic reticulum are in close approximation to one another. At this point the Golgi lamellae are recurved and filled with electron-dense material.

FIGURE 10. A ciliary rootlet and its associated centriole or basal body, lying in the Golgi region of an intrinsic secretory cell. *b*, basal body; *c*, ciliary rootlet.



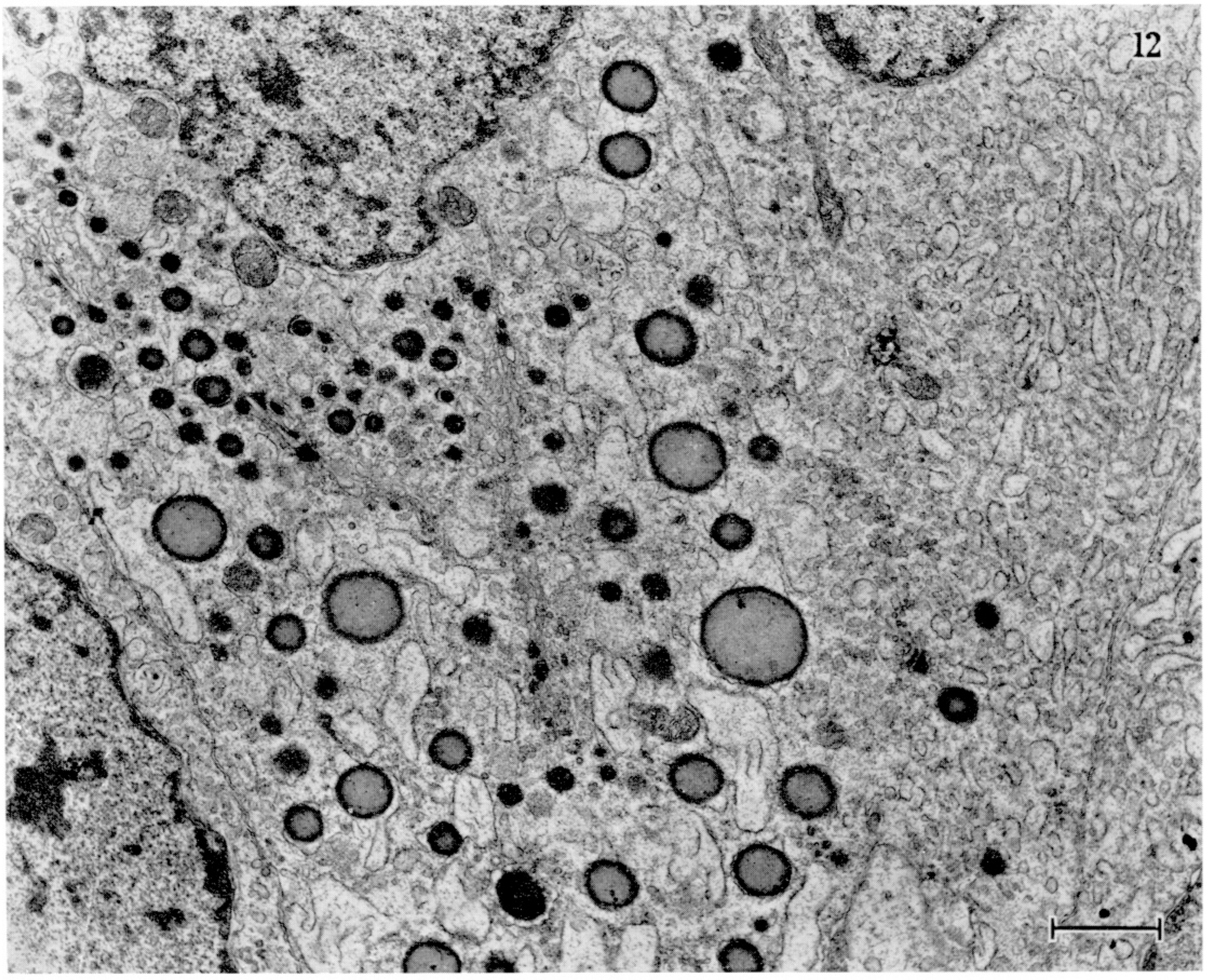
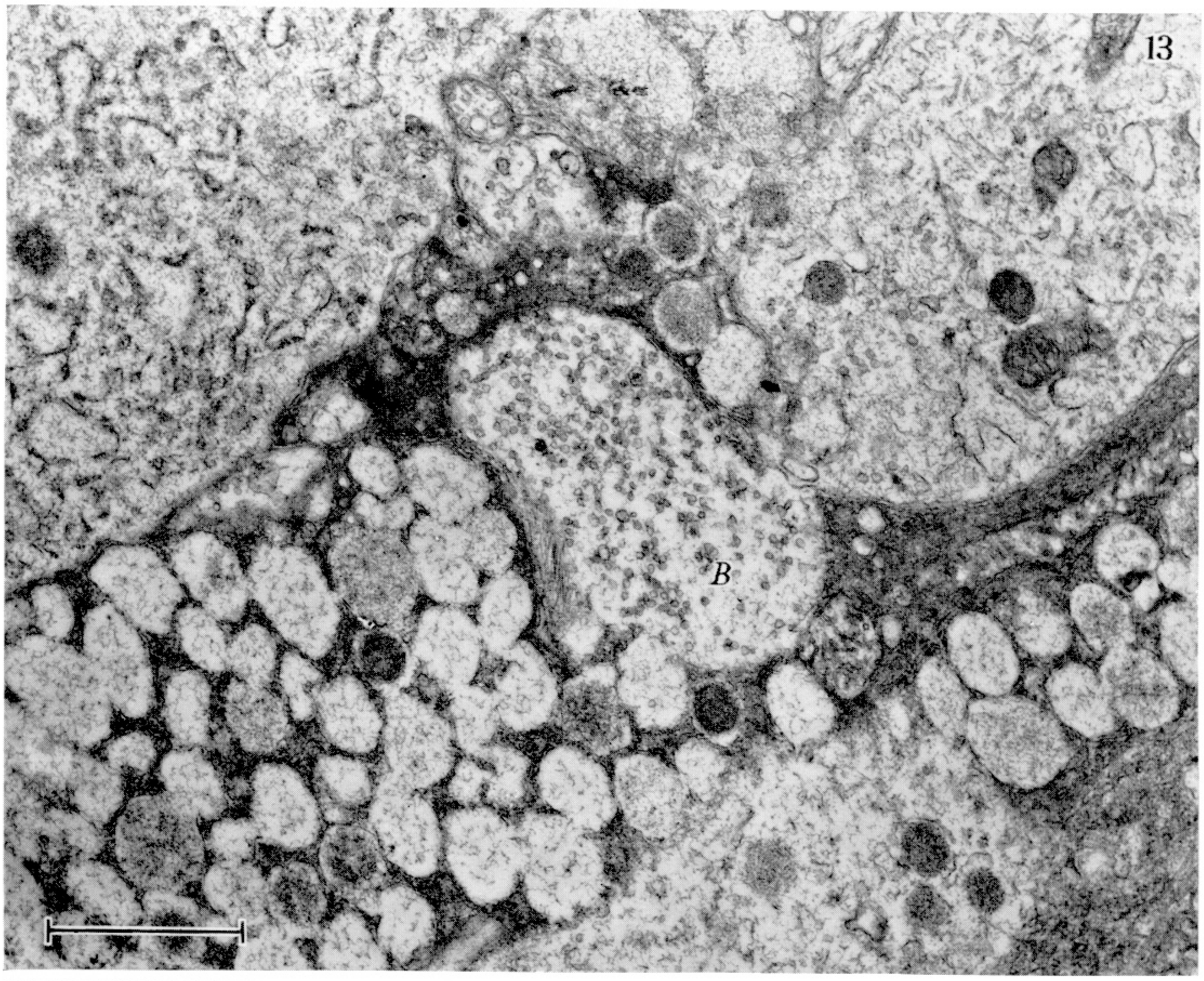


FIGURE 11. A transverse section through a distal prolongation of an intrinsic secretory cell. Globules of secretory material lie within lacunae, which contain also fine granular particles. *B*, type B fibres.

FIGURE 12. A longitudinal section through the proximal part of the storage and release end of an intrinsic cell. In this cell no fine granular material surrounds the electron-dense globules.



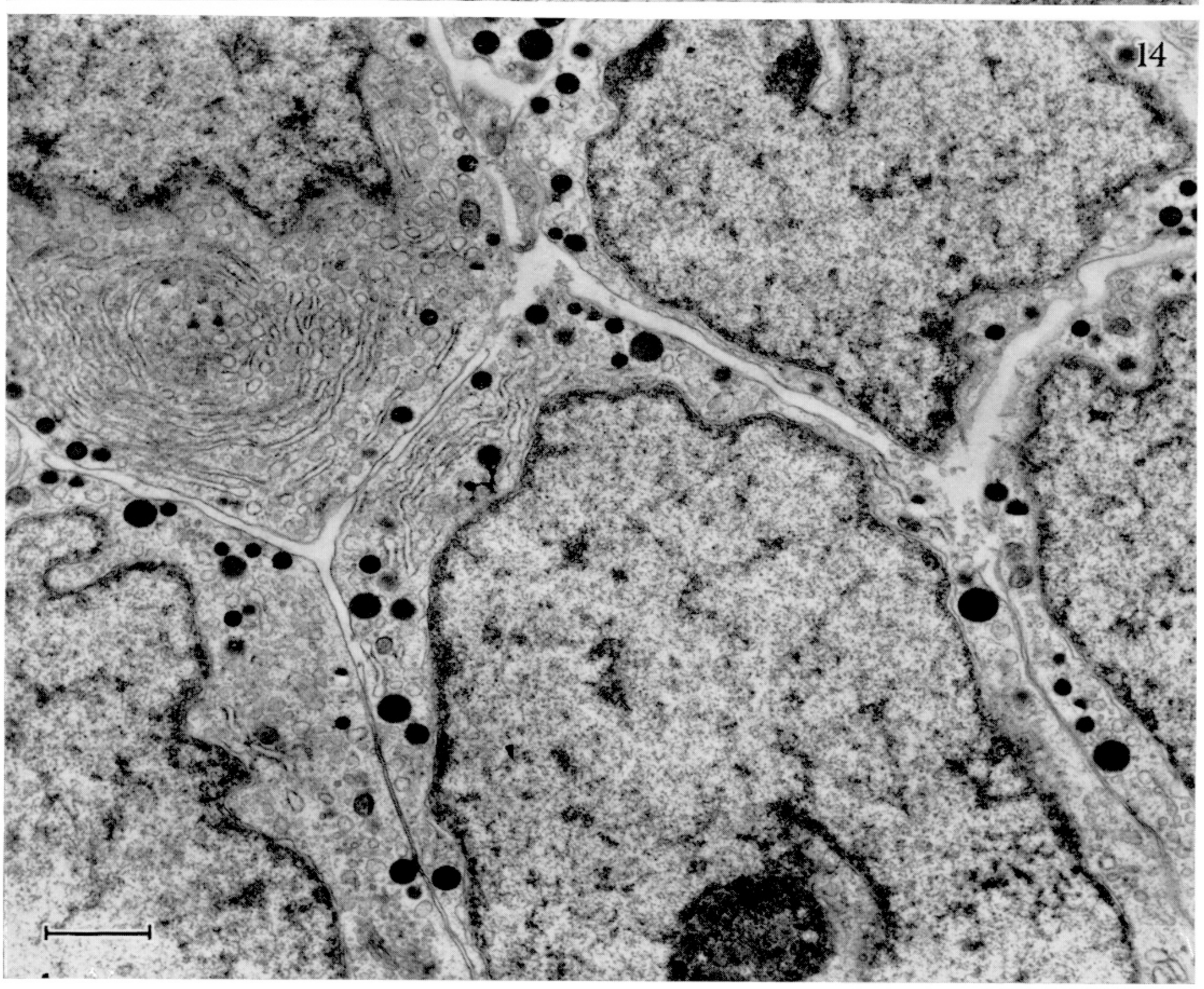


FIGURE 13. A longitudinal section through a distal prolongation of a peripheral intrinsic cell, close to its termination. At this point many vesicles, containing varying amounts of electron-dense material, are found.

(*B*, a type B fibre cut in transverse section. The abundance of small 'empty' vesicles indicates that the plane of section passes through the termination of a type B fibre invaginating the storage and release pole of an intrinsic cell.)

FIGURE 14. A section through cells in the central region of a cord of the neuro-intermediate lobe.

15

16

nf

FIGURE 15. A section through a cell in the neuro-intermediate lobe. The inclusions in this cell are identical in size and form to those in the axons of type A (see succeeding figures).

FIGURE 16. Type A fibres in longitudinal and transverse sections, showing large and small vesicles and neurofibrillae (*nf*).

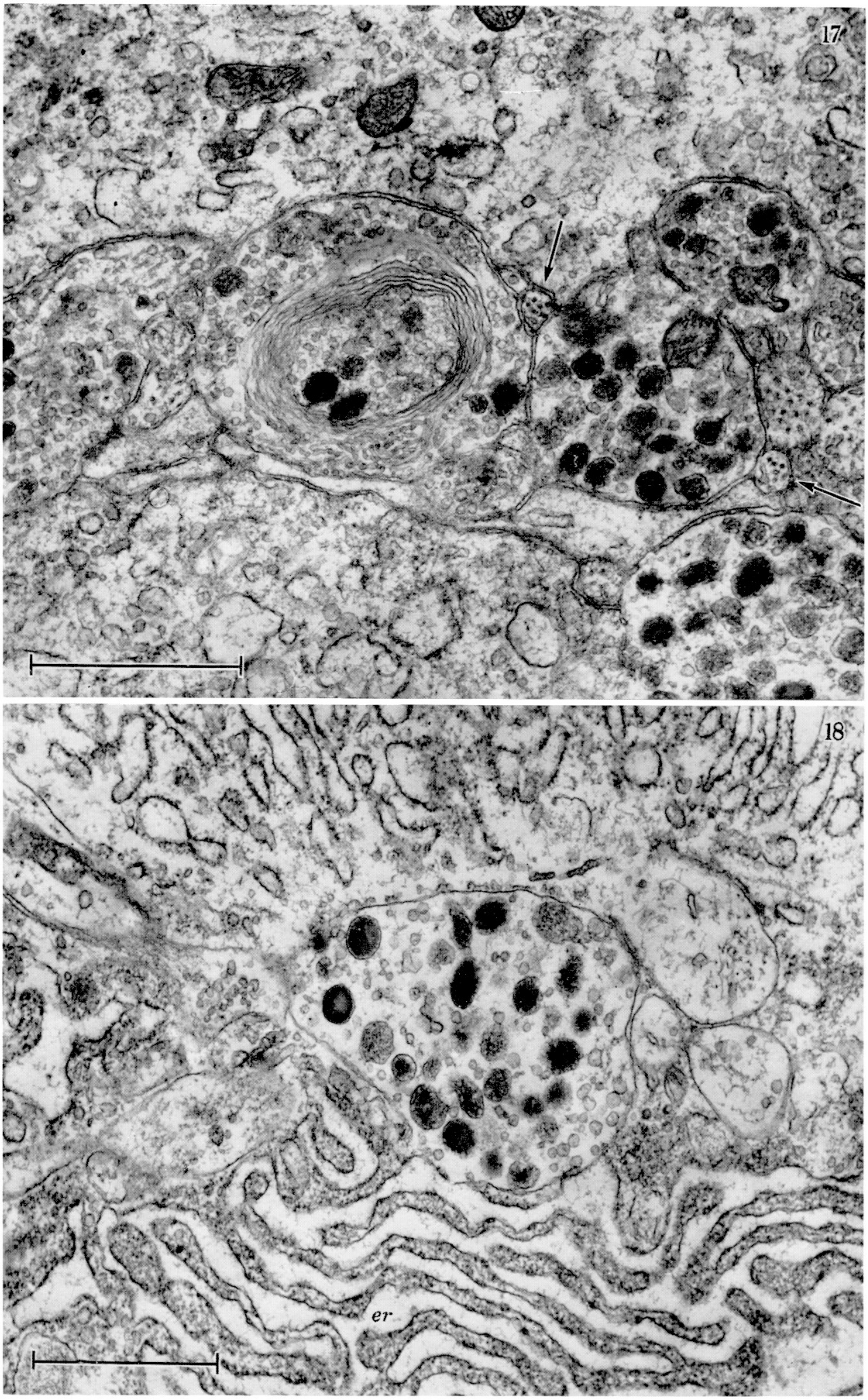
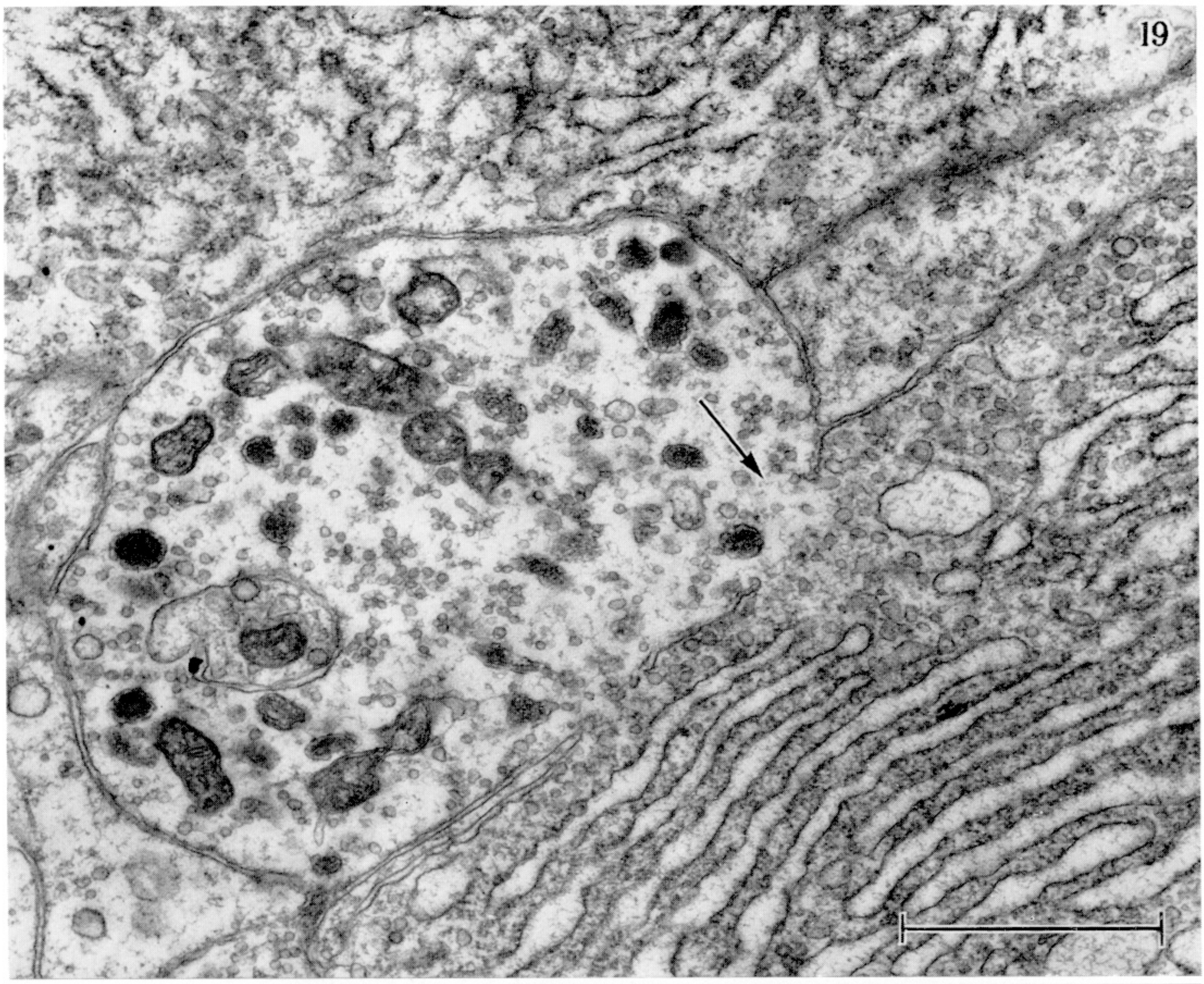


FIGURE 17. Transverse sections through type A fibres, one of which contains a multilamellate body, continuous with tubules in the axoplasm. The arrows point at fibres which contain neurofibrillae, but no evident secretory material.

FIGURE 18. A secretomotor junction at the terminal of a type A fibre in contiguity with secretory poles of two intrinsic peripheral secretory cells (*er*, endoplasmic reticulum).



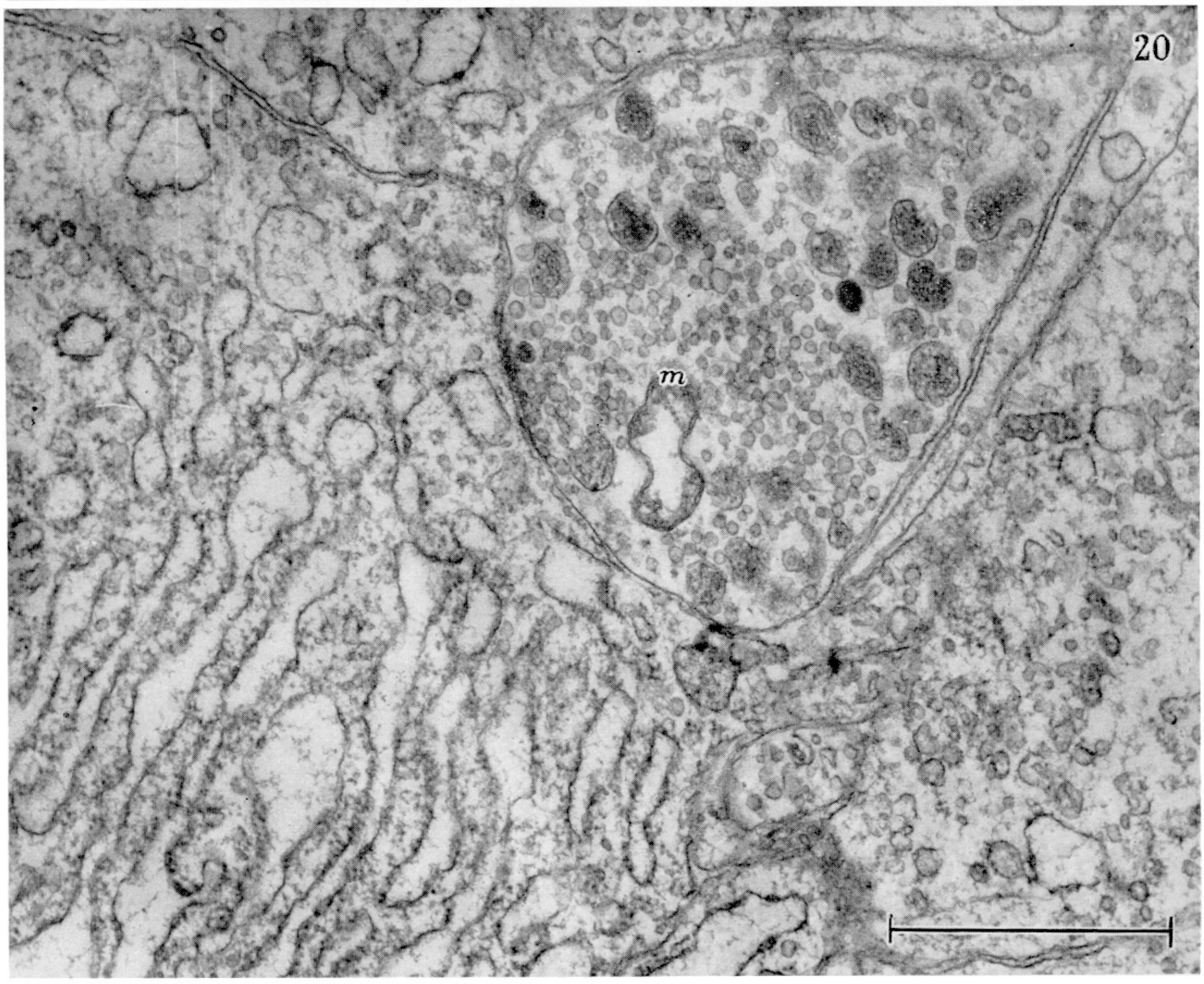
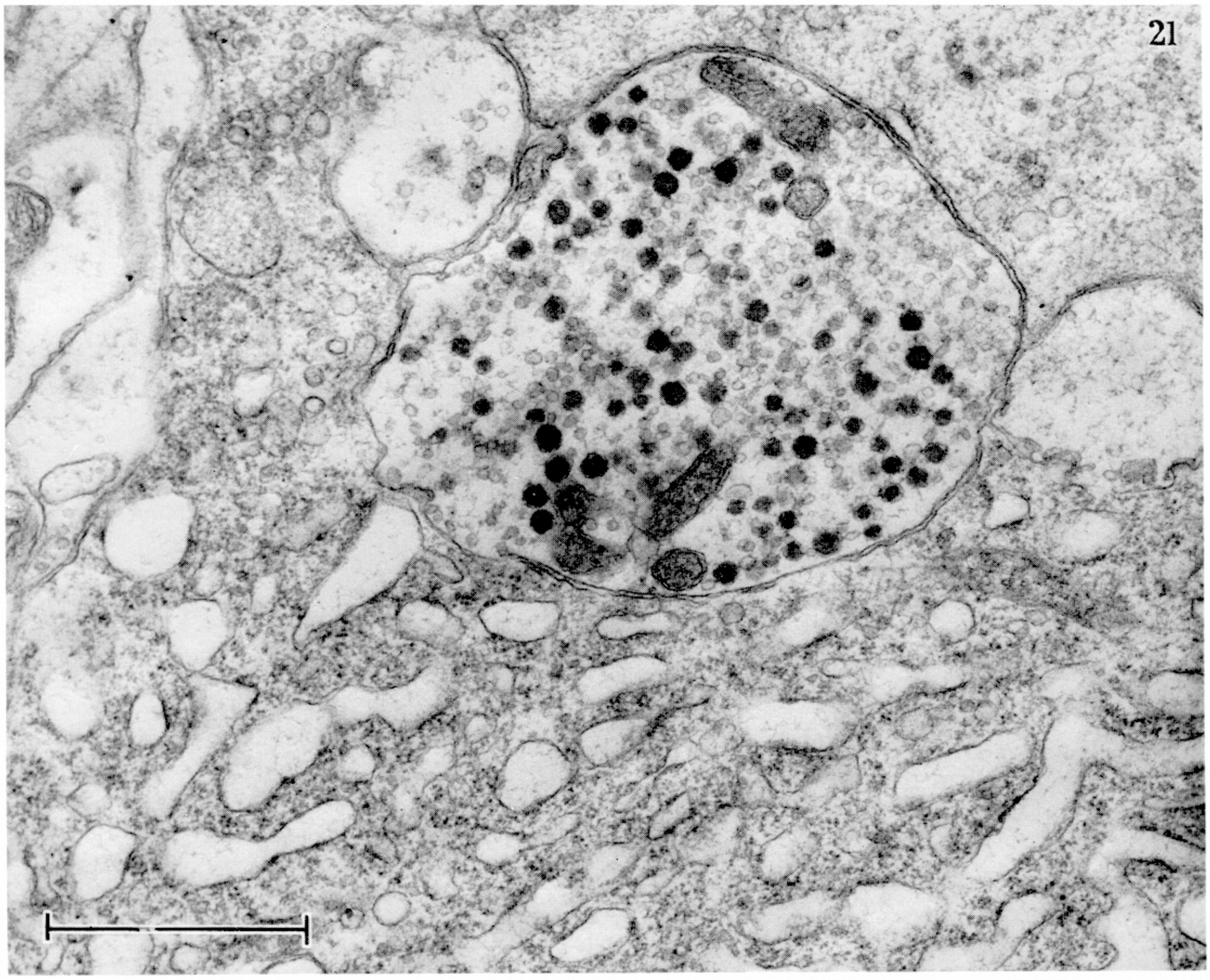


FIGURE 19. As figure 18, plate 55, but showing elementary neurosecretory vesicles of less electron density, and a greater number of small electron-lucent vesicles. The arrow points at one of the areas where the intervening membranes between the neurosecretory fibre and the intrinsic cell appear to have broken down. Near these points small electron-lucent vesicles, like those in the axon, are found in the cytoplasm of the endoplasmic reticulum (see also figure 22, plate 57).

FIGURE 20. As the two preceding figures but showing indications that the small electron-lucent vesicles may arise by fragmentation of larger electron-dense vesicles. (*m*, mitochondrion).



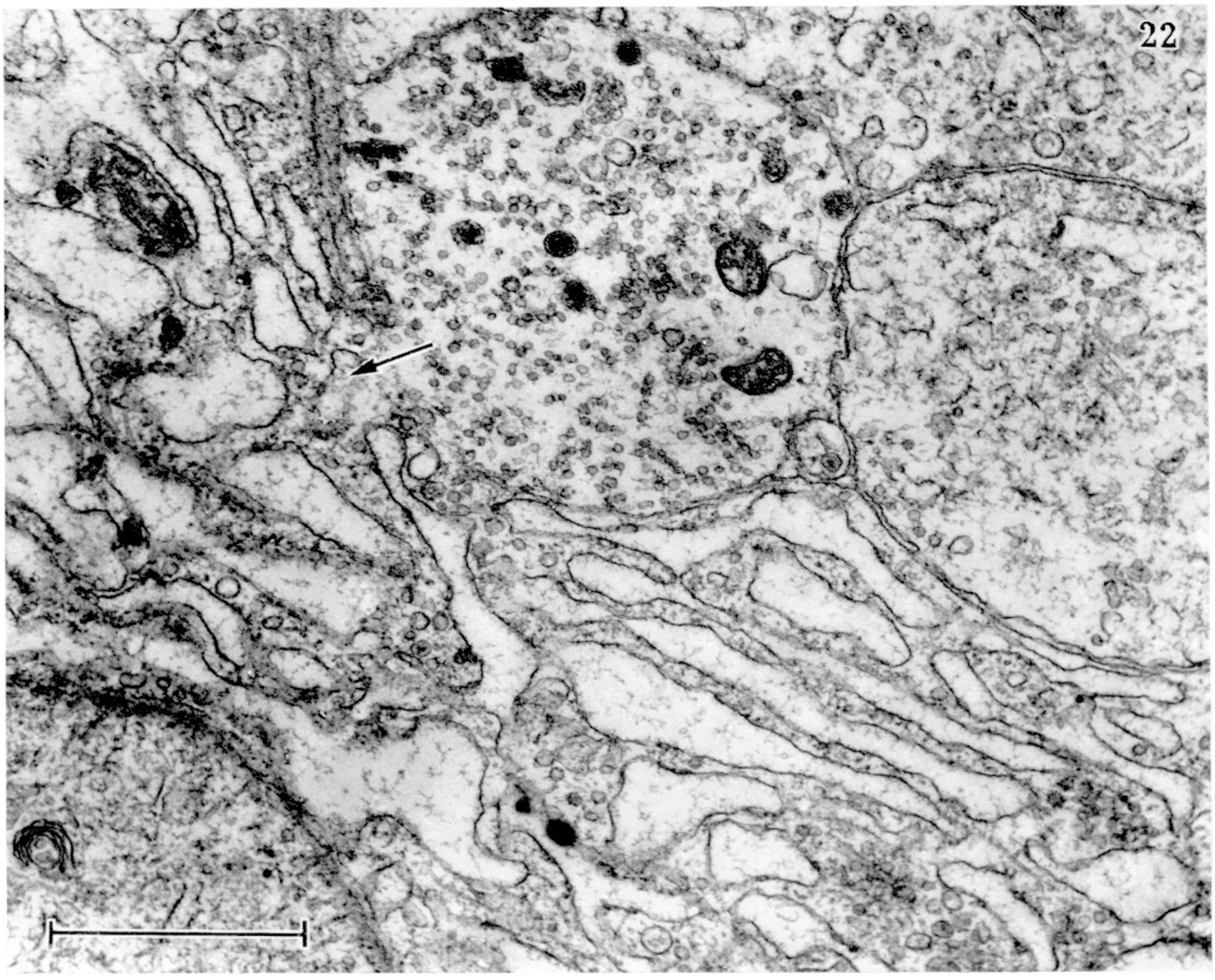
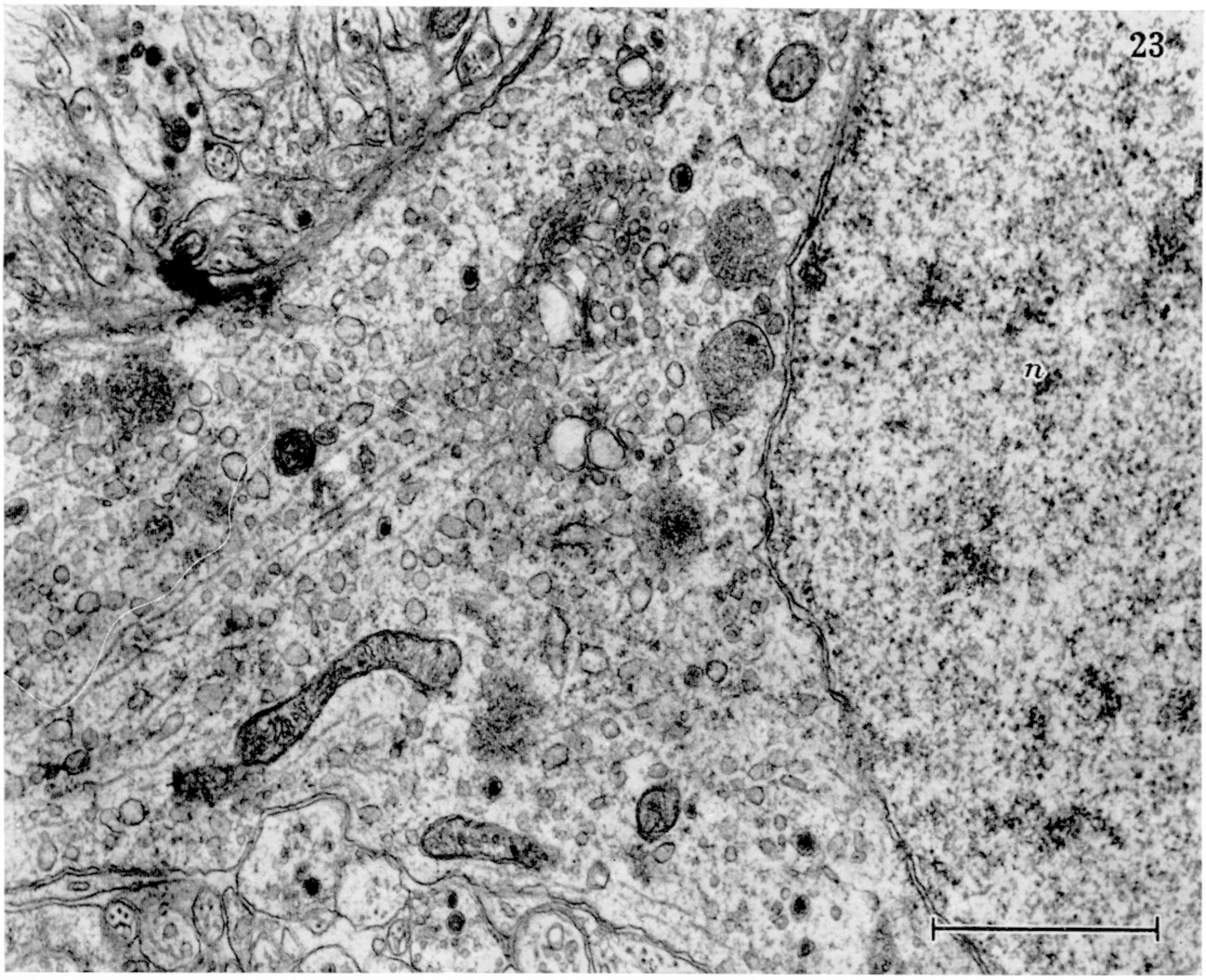


FIGURE 21. A neurosecretory fibre (in close association with the secretory pole of an intrinsic cell) which resembles the type A fibres of preceding figures except that its vesicles are smaller; this type of fibre is designated type 'A'[2] in the text.

FIGURE 22. A type A^2 fibre termination. This figure should be compared with figure 19, plate 56. The arrow points at a region where the cytoplasm of the fibre and that of the cell appear to be continuous.



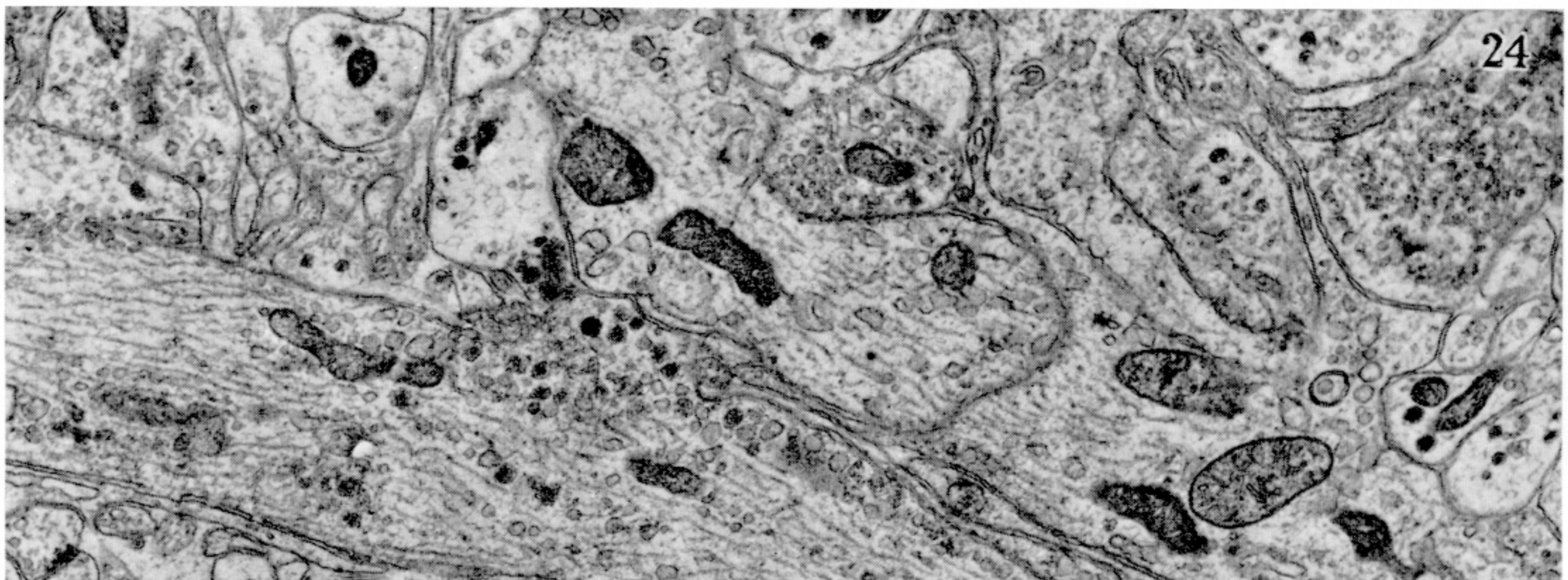
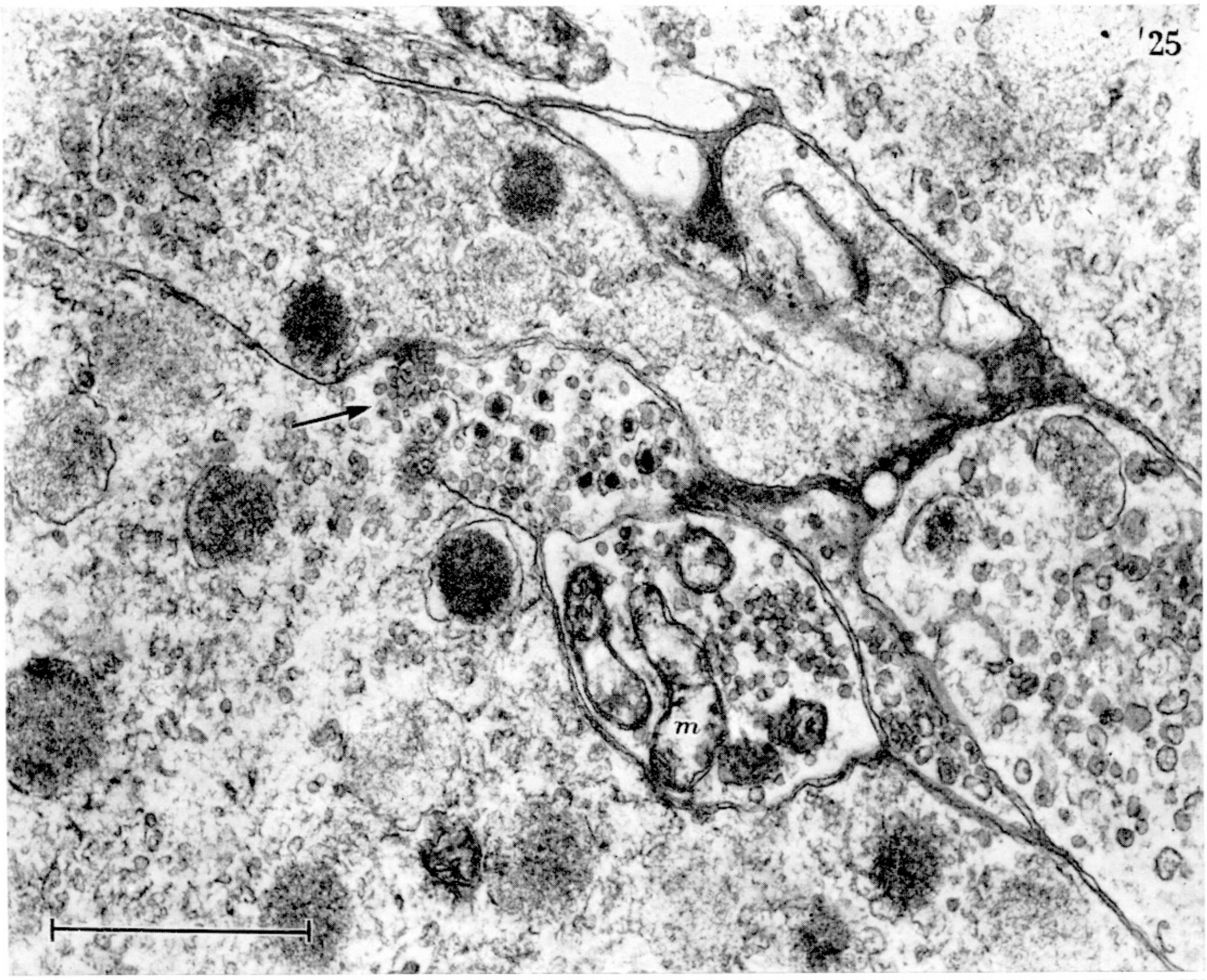


FIGURE 23. A portion of a B-type perikaryon, in the area of the axon hillock, showing B-type secretory vesicles, neurofibrillae and other cytoplasmic inclusions (*n*, nucleus).

FIGURE 24. B-type axon fibres in transverse and longitudinal section.



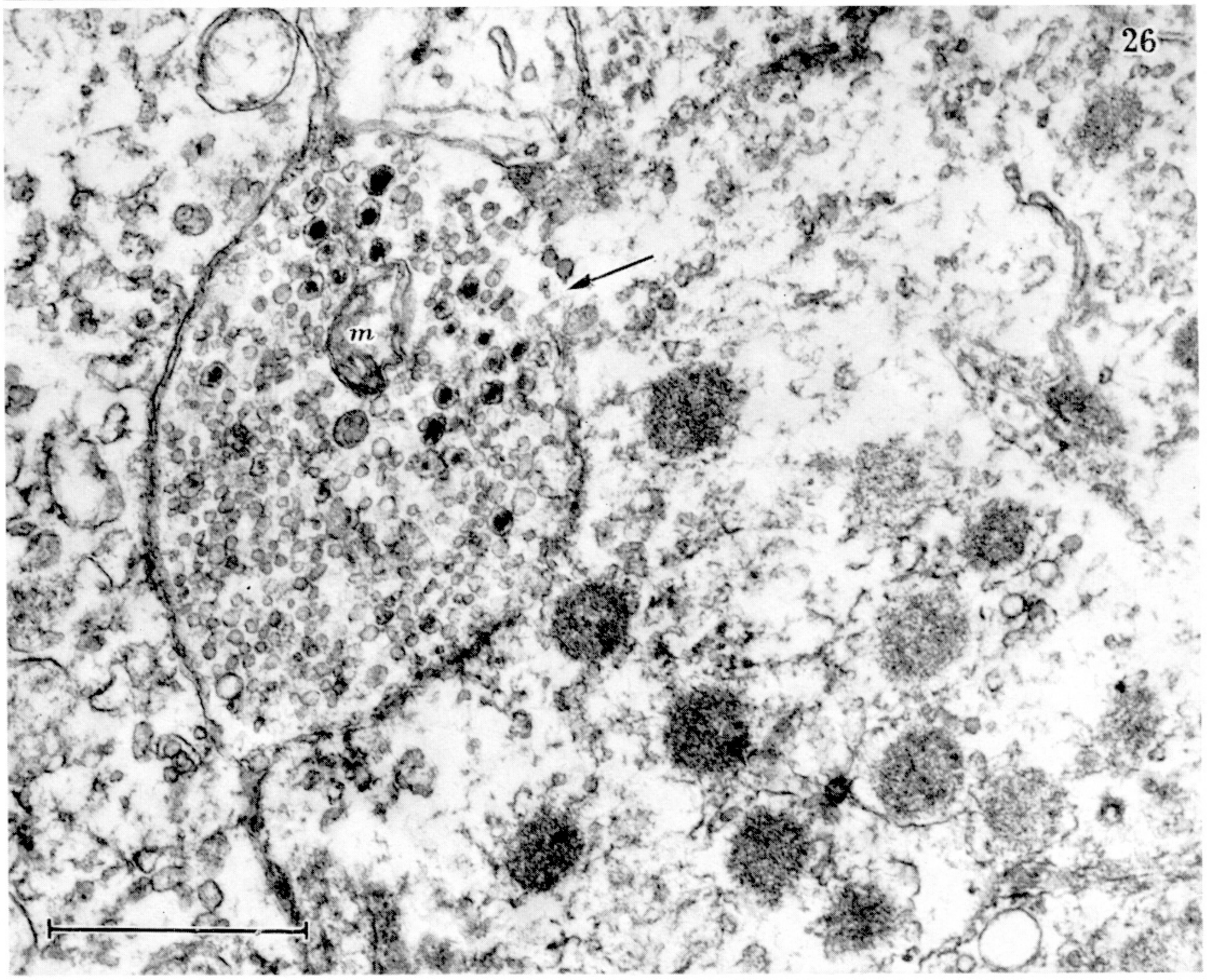


FIGURE 25. A secretomotor junction of B-type fibre terminals adjacent to the secretory and release poles of two peripheral secretory cells. An arrow points to an accumulation of small vesicles at a point where membranes between the fibre and one secretory cell are not apparent (*m*, mitochondrion).

FIGURE 26. As figure 25. The arrow points to a region where no membranes separate the fibre and the secretory cell. As in the preceding figure, membranes surrounding the electron-dense droplets in the intrinsic secretory cell are either fragmentary or absent.