HISTOLOGY AND FINE STRUCTURE OF THE PRE-OPTIC NUCLEUS AND HYPOTHALAMIC TRACTS OF THE EUROPEAN EEL *ANGUILLA ANGUILLA* L.

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[Plates 48 to 57]

CONTENTS

The pre-optic nucleus and hypothalamic tracts of intact and hypophysectomized specimens of the European eel *Anguilla anguilla* L. have been studied *in situ* and by optical and electron microscopy. The *in situ* technique reveals a hitherto unsuspected degree of segregation of the neurosecretory axons which form up to five discrete tracts having separate origins and following distinct paths before converging, at the level of the anterior margin of the pituitary, to form a median tract.

The structure of the pre-optic neurons, as revealed by several different techniques, is described and it is shown that their synthetic poles, identified by a prominent cap of endoplasmic reticulum, are precisely orientated towards the third ventricle and are separated from it by, at most, two or three layers of ependymal cells. Electron microscopy shows that the secretory products lie mainly in the axonal ends of the cells though in Bouin-fixed, wax-embedded material the entire perikaryon stains with neurosecretory dyes and this, and their proximity to the third ventricle, gives the impression that they secrete into the latter, as well as centripetally. This may well be so, but from the work described below it seems more likely that these neurons receive nutrients, or stimuli, or both, from the third ventricle.

Two types of pre-optic neurons, separable by structural features as well as by the size of the elementary

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granules they contain have been identified; these probably give rise to two of the fibre types identified in the neurohypophysis of the eel by Knowles & Vollrath.

Aggregations of neurosecretion, common in the fish pre-optic nucleus, and also, much rarer, colloid vesicles, are described and discussed.

INTRODUCTION

In recent years the focus of research interest and effort in the field of the hypothalamo-neurohypophysial complex has tended to shift from the pituitary gland to the hypothalamus. This change can be traced to two main discoveries: that of Bargmann (1949) who showed that the pre-optic nucleus, its axonal tracts and the neurohypophysis form an integrated functional complex, and that of Harris (1948) who showed that the pars distalis of the mammalian pituitary is served by a portal vascular system of which the primary plexus has a special relationship with at least some of the neurosecretory axons of the hypothalamo-neurohypophysial complex.

Some of the earliest work on the secretory neurons of the diencephalon was carried out on fish (Herrick 1891; Kappers 1906), and Charlton (1932) described the pre-optic nucleus in no fewer than 125 species of bony fishes. Furthermore, the pioneer research that led to the unequivocal demonstration that the hypothalamo-neurohypophysial complex is a functional unit was also largely carried out on fish (Scharrer 1928, 1930, 1932*a*, *b*, 1941; Bargmann 1949, 1953). This early work, as well as more recent research, has been reviewed by Dodd & Kerr (1963).

The hypothalamo-neurohypophysial complex of bony fishes, as of all other vertebrates, consists of nuclei composed of secretory neurons, bordering the pre-optic recess of the third ventricle, and their axons which traverse the hypothalamus in tracts and end in the neurohypophysis. In anamniotes there is one pair of hypothalamic nuclei, whereas in amniotes there are two pairs. In all fishes other than cyclostomes the neurohypophysis interdigitates more or less intimately with the pars intermedia of the pituitary to form the neuro-intermediate lobe.

Prior to 1960 all structural studies on the hypothalamo-neurohypophysial complex of fishes were at the level of the optical microscope using traditional histological fixatives like Bouin's fluid, and paraffin embedding, and the activity of the system was assessed by reference to its affinity for so-called neurosecretory dyes. Knowles & Vollrath (1966) have recently studied the neurohypophysis of the eels *Anguilla* and *Conger* by electron microscopy and have made important advances. *Inter alia* their structural studies have enabled them to make important suggestions as to the functioning of the complex, including the possible incorporation in it of a feedback mechanism. The present paper complements this work by providing the first ultrastructural studies on the eel pre-optic nucleus and tracts.

Since the work of Palay (1960) on the pre-optic nucleus of the goldfish, *Carassius auratus*, the fine structure of this nucleus has been investigated in several other fish species (*Salmo irideus*, Lederis 1962, 1964; Follenius 1963: *Perca fluviatilis*, Follenius & Porte 1962; Follenius 1963: *Gadus morrhua*, *Cottus bubalis*, Lederis 1962: *Zoarces viviparus*, Oztan 1966).

The pre-optic neurons are basically similar in structure in all the fish examined, being large cells, probably the largest in the brain, with large, usually irregular, nuclei, and a prominent array of well-ordered lamellae constituting the main part of the endoplasmic reticulum at one pole of the cell, this merging into irregularly arranged ergastoplasmic tubules and vesicles at the level of the nucleus. Organelles such as mitochondria and Golgi bodies are present, but vary in number and at least four types of secretory inclusions have been described, including the elementary granules which are believed to be hormone-containing structures (Lederis 1964).

But whereas in the neurohypophysis two types of axon have been identified, containing elementary granules of two different sizes (Lederis 1964; Knowles & Vollrath 1966), two types of pre-optic neurons have not previously been identified. However, it may be noted that if, as seems likely, the two types of axons, called Type A 1 and A 2 by Knowles & Vollrath (1966) arise from neurons in the pre-optic nucleus, then two types of neurons, differing at least in respect of the sizes of the elementary granules they produce, must be present, and evidence is adduced later, which has already been briefly reported (Leatherland & Dodd 1967), to show that this is in fact the case in the eel.

The work reported below deals with the structure and ultrastructure of the pre-optic nucleus and its hypothalamic tracts in *Anguilla anguilla* and is intended to complement that of Knowles & Vollrath (1966) on the neurohypophysis and pars intermedia in the same species.

MATERIALS AND METHODS

Animals

Eels were obtained in large numbers throughout the year from a variety of sources. Some were netted, either in the River Severn and its tributaries or in Lincolnshire rivers in the neighbourhood of Boston; others were caught in various rivers and becks in Lancashire by electric fishing methods, and these consisted of a mixture of yellow and silver eels, the ratio yellow:silver falling as the year progressed. Some were fixed at the time of capture, others were transported to Leeds and kept in large fibre-glass aquaria in running tapwater and allowed to acclimatize for at least 2 weeks before use.

Histology

(*a*) *The* in situ *staining technique* (Braak 1962; Leatherland, Budtz & Dodd 1966).

Brains, with pituitary gland attached, are fixed in Bouin's fluid, oxidized in performic acid and stained in Gabe's aldehyde fuchsin for 2 to 4 h. After differentiation and dehydration they are cleared in methyl benzoate and stored in methyl salicylate. Photographs of brains prepared in this way are shown in figures 1 and 2, plate 48.

(*b*) *Optical microscopy*

Eels were killed by decapitation and the brains and pituitary glands exposed to Bouin's fluid within 2 min and subsequently for 8 to 24 h. Serial sections were cut at 5 to 10 μm in transverse and sagittal planes. Several stains were used singly and in combination, including: alcian blue (AB); periodic acid Schiff (PAS); orange G (OG); aldehyde thionine (AT); naphthol yellow (NY), and Gabe's aldehyde fuchsin (AF).

(*c*) *Electron microscopy*

Tissues were fixed by perfusion of cold buffered glutaraldehyde solution (1 part 25 % glutaraldehyde in 3 parts 2.14 % sodium cacodylate solution in distilled water; pH 7.2 to 7.4) into the dorsal aorta near the origin of the circulus cephalicus. The whole brain was then removed from the skull and immersed in cold glutaraldehyde solution after which the regions required for examination were removed by a sharp scalpel. These were then post-fixed for 1 h in 1 % osmium tetroxide solution in 0.1 M sodium cacodylate buffer (pH 7.4 to 7.6), rinsed in the buffer solution, dehydrated in increasing concentrations of ethanol and embedded in Shell epikote resin (Epon).

Sections were cut with glass knives on a Cambridge ultramicrotome (A. F. Huxley pattern), mounted on uncoated copper grids, stained with uranyl acetate and/or lead acetate, and examined in an AEI EM 6B electron microscope.

(*d*) *Optical microscopy of material fixed and embedded for electron microscopy*

Epon-embedded material was cut at 0.5 to 1.0 μm, heat-mounted on slides, stained in Azur II at 70 °C, mounted in immersion oil and examined in the optical microscope.

(*e*) *Hypophysectomy*

Eels were hypophysectomized by the method of Waring (1940).

RESULTS

1. *The* in situ *technique* (figures 1, 2, plate 48)

(*a*) *Pre-optic nucleus*

A detailed study based on this technique has already been reported (Leatherland *et al.* 1966). It clearly demonstrates that the pre-optic nuclei are thin sheets of neurons lying one on either side of the hypothalamus immediately below the ependymal layer of the recessus pre-opticus of the third ventricle. Each nucleus has the form of an inverted 'L' and is characteristically composed of two regions; the pars magnocellularis is the predominantly horizontal component and is composed of large neurons of diameter 31.8 ± 8.3 μm*. It projects backwards so that its posterior tip lies under the subcommissural organ and consists of a sheet of loosely packed neurons, 1 to 3 cells thick. The pars parvocellularis forms the major part of the vertical component of the PON; its cells have a diameter of 19.4 ± 4.9 μm, are closely packed, and form a layer 5 or 6 cells thick.

(*b*) *Hypothalamic axonal tracts*

The axons of the pre-optic neurons join up to give well-defined tracts on each side which follow distinct paths through the hypothalamus and converge to form a single median tract at the level of the anterior margin of the pituitary gland (figures 1, 2, plate 48; 22, plate 57). In some cases stainability of the tracts ends in this region, called the subterminal region by Leatherland *et al.* (1966), but usually a stainable median tract continues beyond this point to end as the neurohypophysis in the neuro-intermediate lobe (meta-adenohypophysis) of the pituitary. The tracts anterior to the subterminal region contain neurosecretion in the form of typical Herring bodies, whereas the median tract is characterized by the presence of larger cylindrical aggregations of neurosecretion (figure 22).

Leatherland *et al.* (1966) have shown that in the eel, one hypothalamic tract originates in the pars magnocellularis, one from the anterior face of the pars parvocellularis and up to three others from the posterior face of the latter. There is some variation in the degree to which these three tracts are separated, but their identities are distinct in a sufficient number of preparations to give support to the view that they may well be separate tracts. Examination of hypothalamic tracts in the electron microscope shows that they contain packages of elementary granules, each enclosed in a limiting membrane and separated from each other by mitochondria, neurofibrillae

* All dimensions are expressed as means ± standard deviations.

and structureless axoplasm. However, the elementary granules in the axons of the neurohypophysis are no longer enclosed in membranes and this is also true of the granules that accumulate at the end of the pituitary stalk after hypophysectomy.

2. *Optical microscopy of Bouin-fixed material embedded in wax*

After fixation in Bouin's fluid and embedding in wax the neurons of both parts of the PON, although their affinity for the so-called neurosecretory dyes is variable, usually take up some stain and frequently the reaction is intense. The cells are rounded or pyriform in section, they have a large nucleus with 2 nucleoli, and the cytoplasm contains chromophilic granules and clear vesicles of a considerable range of sizes (figures 3 to 5, plate 49). The cells are usually unipolar and they stain with a more or less uniform intensity. The polarization of these cells is striking, the pole opposite the axon hillock, called the dendritic pole by some previous workers, always having close contact with the ependymal cells lining the III ventricle. In the antero-ventral region of the pars parvocellularis the dendritic poles of many of the neurons are directly exposed to the cerebro-spinal fluid and some actually protrude into the III ventricle (figures 4, 5, plate 49). Such neurons have been described by several previous workers (Stutinsky 1953; Leatherland *et al.* 1966; Sterba & Weiss 1967), and although protrusion may be a fixation artifact, due to shrinkage of the surrounding tissues, this seems unlikely. As figures 4 and 5, plate 49, show, a series, ranging in degree from mere contact of a neuron with the CSF to more or less complete immersion of the cell body in it, can usually be found. The significance of this phenomenon is discussed later. Neurons of the dorsal part of the pars parvocellularis and those of the pars magnocellularis are usually separated from the third ventricle by two or three layers of ependymal cells (figure 3, plate 49). The close proximity of the neurons to the third ventricle and their frequently intense and uniform stainability has led to the suggestion by several authors that they are, in fact, secreting into the third ventricle. It will be seen below that the superior fixation resulting from the use of glutaraldehyde and osmium tetroxide, and embedding in Epon suggests a different interpretation, at least in the eel.

It is difficult to identify the precise nature of the various cellular inclusions, including the chromophilic granules and clear vesicles, though the former may be aggregations of neurosecretory granules. However, caution is necessary since Bouin-fixed neurons of the PON never show identifiable endoplasmic reticulum such as is always prominent in glutaraldehyde-osmium tetroxide-fixed neurons, and the conclusion is inescapable that Bouin's fluid produces fixation artifacts which render identification of cell contents virtually impossible. (This has been verified by examining, in the electron microscope Bouin-fixed tissue subsequently treated with osmium tetroxide. In particular, the intracellular membranes and their contents take up the neurosecretory dyes and this has given rise to the view that the pre-optic neurons secrete into the third ventricle as well as into the neurohypophysis. That this is unlikely to be the case in the eel, is suggested by examination of the osmic acid-fixed material described below.

3. *Optical microscopy of the glutaraldehyde-osmic acid fixed material embedded in Epon*

Cellular detail seems excellently preserved in this material and the precision of the polarization of the PON cells is striking. In all cases the polar cap of endoplasmic reticulum (*ER*), presumably the synthetic pole of the cells, is readily recognizable and lies adjacent to the cavity of the III ventricle usually separated from it by a few ependymal cells (figures 7 to 9, plate 50) but occasionally reaching the cavity (figure 6, plate 49). The ER contains few or no

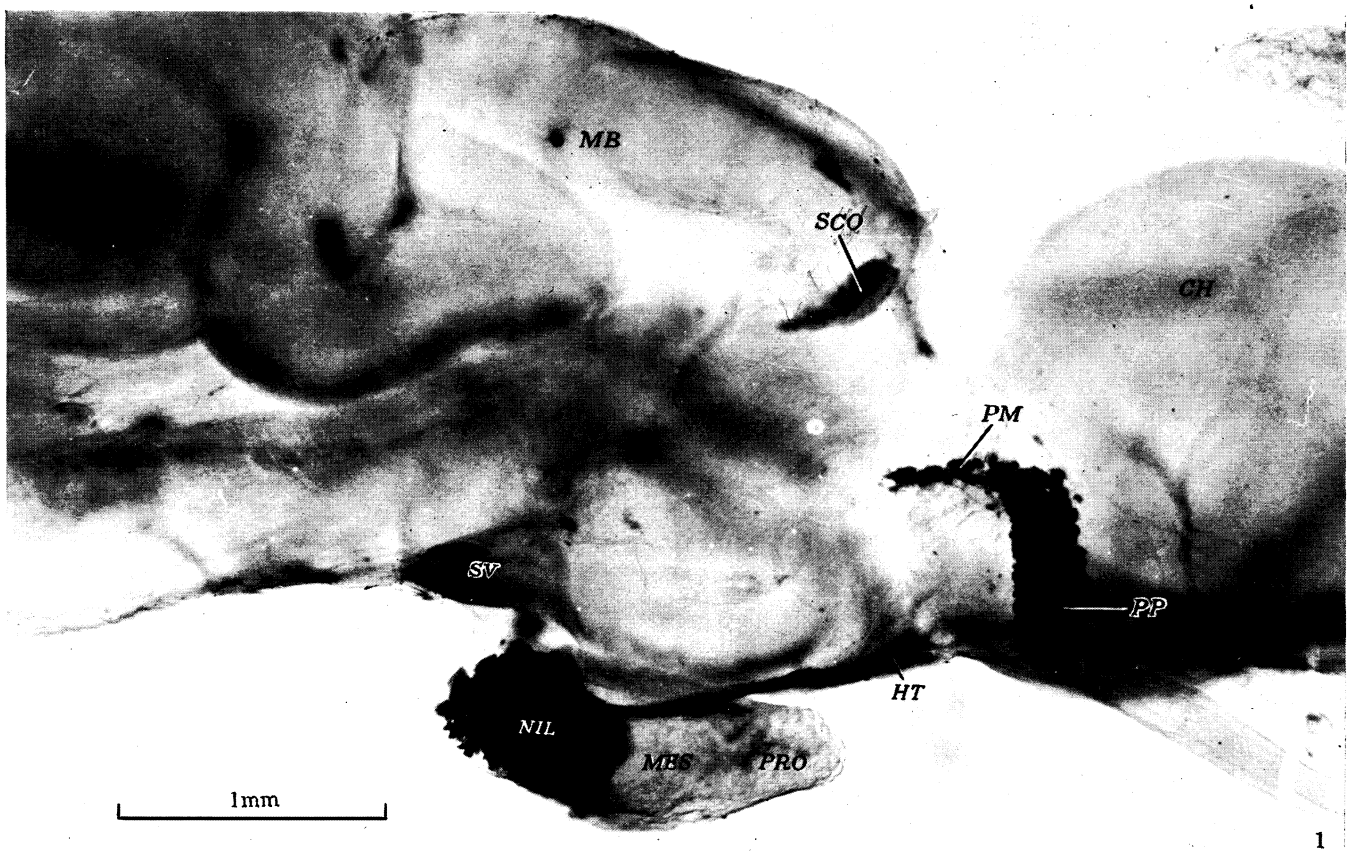
stainable inclusions and cells prepared and stained in this way give no support to the theory that they are secreting into the cerebrospinal fluid. The nucleus is large and more or less homogeneous in staining reaction and it usually contains two prominent nucleoli. The perinuclear cytoplasm contains densely staining granules of a range of sizes, and large vesicles many of which are completely chromophobic. The rich vascularization of the PON region is a prominent feature in these preparations.

4. *Electron microscopy*

The structures already described can be seen in more detail in the electron micrographs (figures 10 to 15, plates 51 to 54). The endoplasmic reticulum forms a prominent cap of regularly arranged lamellae which may contain large spherical inclusions of low electron density; granular inclusions are scarce or absent in this region. The cisternae are either narrow and parallel-sided or irregularly swollen and, as will be seen, this constitutes one of the points of difference between the two neuronal cell types that can be distinguished. The perinuclear cytoplasm contains inclusions of several kinds of which the most prominent are the so-called elementary granules. These are membrane-bounded, electron dense and spherical and range in size between 1600 and 2200 Å; they frequently occur in 'nests' which are probably the product of a single Golgi apparatus; their formation by typical Golgi apparatus is illustrated in figure 11, plate 51. Occasional larger electron dense bodies (up to 20000 Å) are also found (figure 14, plate 53), as are large vesicles (up to 45000 Å) ranging widely in electron density. Some of these are uniformly granular and others contain scattered polyribosome-like structures which suggests their implication in protein synthesis and/or metabolism. Some lie in the endoplasmic reticulum (figure 13, plate 52) and others are free in the perinuclear cytoplasm (figures 10, 14, plates 51 and 53). Electron-lucent vesicles are also found; figure 11, plate 51 illustrates their production by Golgi apparatus. The perinuclear cytoplasm contains, in addition, spherical inclusions of a range of sizes, including polyribosomes, irregularly arranged ergastoplasmic tubules, Golgi apparatus and mitochondria.

'*Dark*' *and* '*Light*' *cell types* (figures 7 to 10 and 15, plates 50, 51 and 54)

Two, easily distinguishable, neuronal cell types are present in both regions of the PON. The physiological significance of the differences is not clear at the moment and 'dark' and 'light' seem appropriate terms to distinguish between them in view of the greater electron density of both cytoplasm and nucleus of the former, especially after uranyl acetate staining. Although these two cell types are found in both pars magnocellularis and pars parvocellularis the dark cell type is more prevalent in the former and the light cells more common in the ventral part of the pars parvocellularis. Apart from the difference in electron density the basic structure and the inclusions are in general similar in the two types of cell though there are significant differences. In the dark cells the polar cap of endoplasmic reticulum consists of lamellae separated by irregular and swollen cisternae, 400 to 6000 Å across, whereas in the light cells the cisternae are narrow and parallel-sided and bounded by membranes that are no more than 400 to 2000 Å apart. A more important difference concerns the sizes of the elementary granules; the mean diameter of 72 of the largest granules from 6 light cells was 1627 ± 31 Å whereas for 52 granules from dark cells it was 2150 ± 30 Å. These sizes correspond with the granule sizes encountered in the two types of axonal endings in the neurohypophysis and the difference between them is highly significant ($P < 0.001$). Other differences between the two



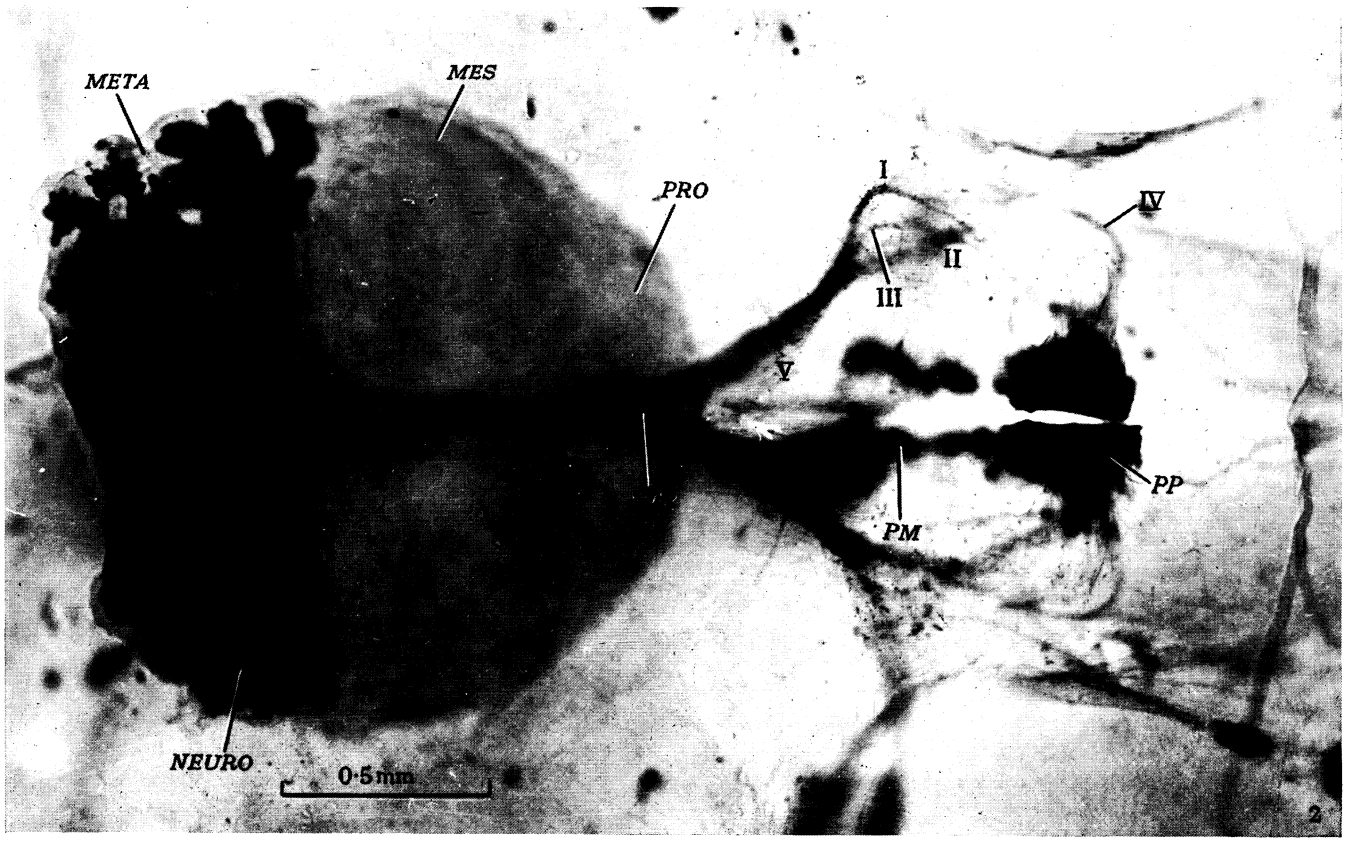


FIGURE 1. *In situ* preparation of the hypothalamo-neurohypophysial-complex of *Anguilla*, lateral view. (Stained AF.)

FIGURE 2. *In situ* preparation of the hypothalamo-neurohypophysial-complex of *Anguilla*, ventral view. (Stained AF.)

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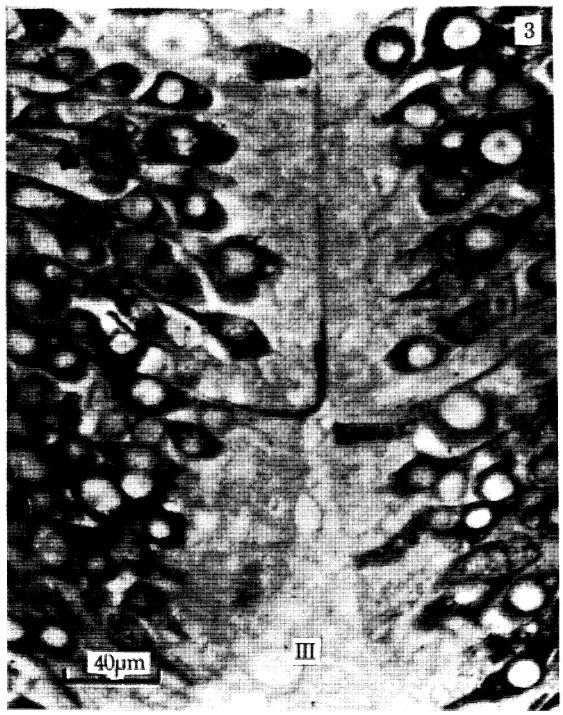
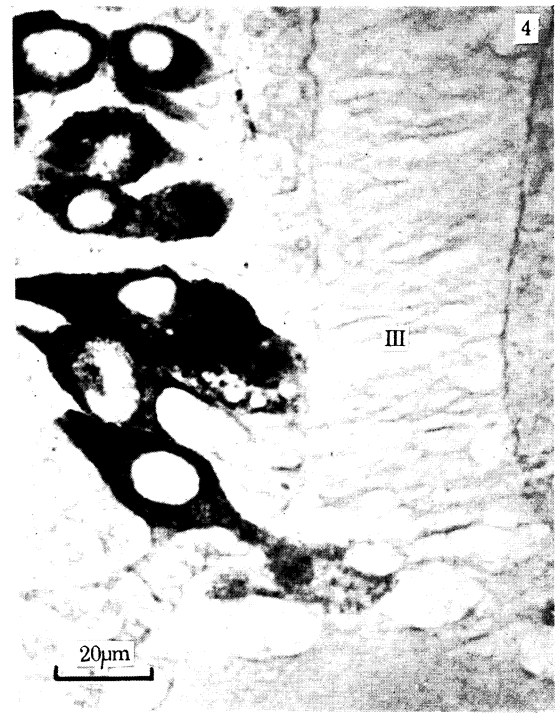
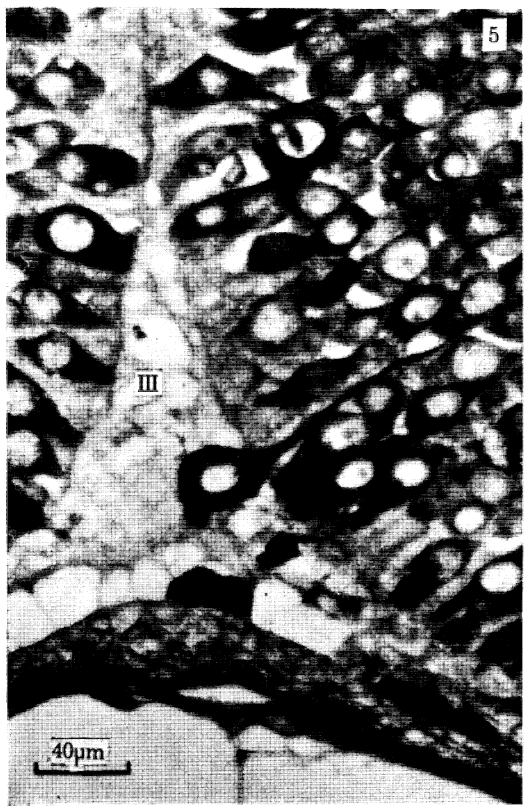
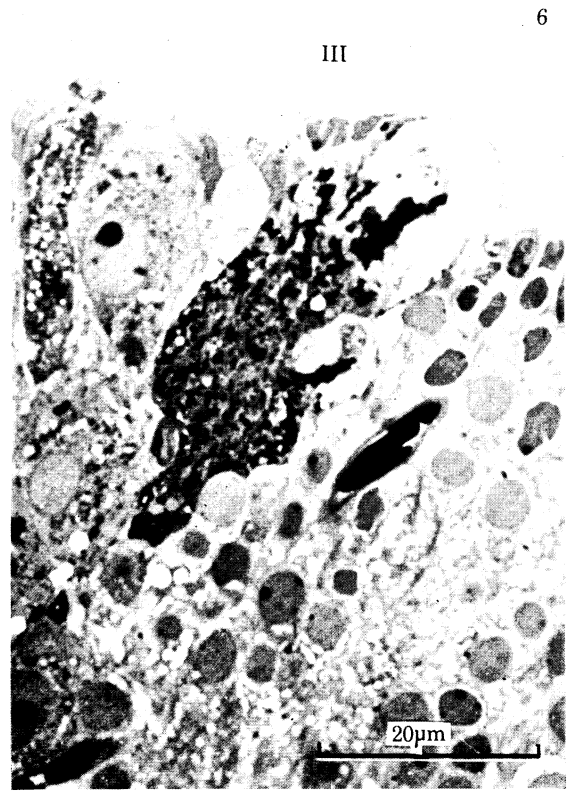




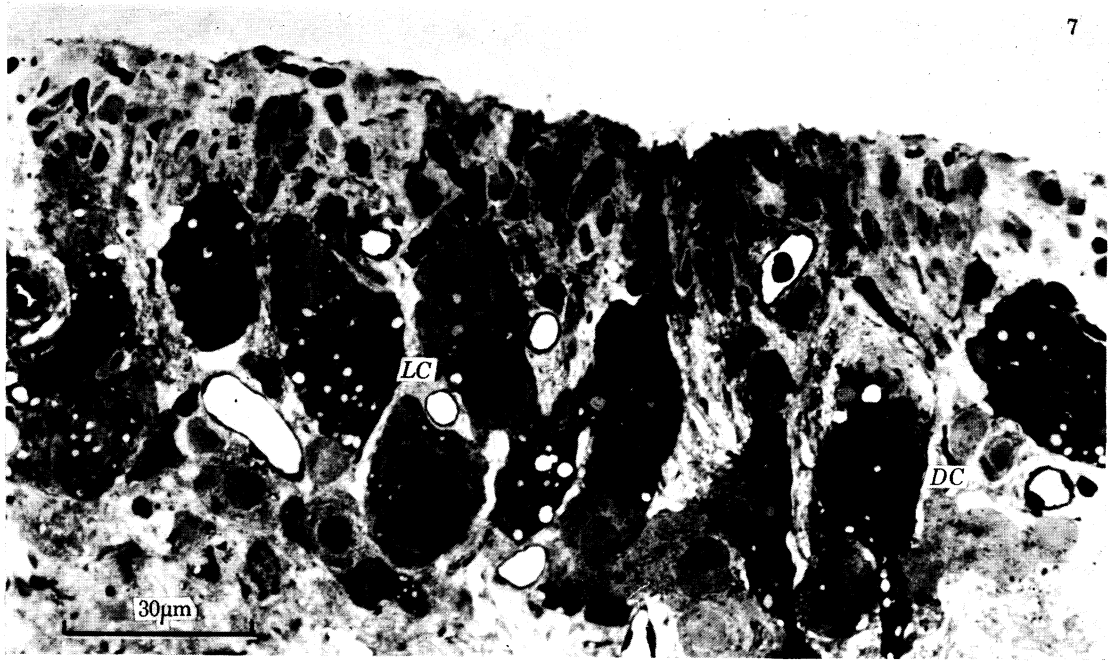


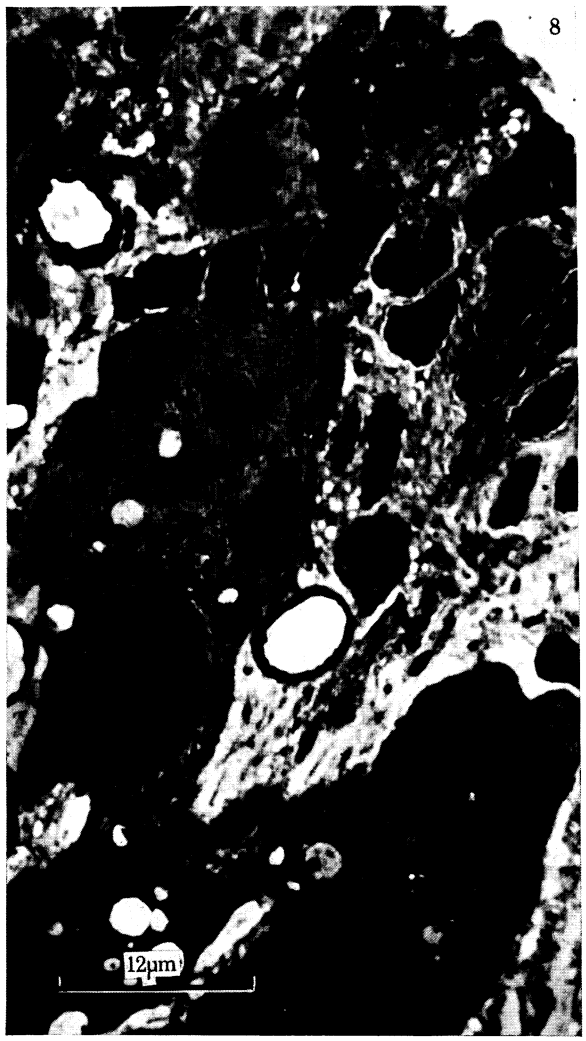


FIGURES 3 to 5. Transverse sections of *Anguilla* PON showing polarization of neurosecretory neurons and contacts between neurons and third ventricle (III).

FIGURE 6. *ER* pole of neuron in contact with cerebro-spinal fluid. Note also axonal pole of neuron, ependymal cells and spongy appearance of glial cells.

(FIGURES 3 to 5. Bouin-fixed, wax-embedded, stained AT–PAS–NY. FIGURE 6. Glutaraldehyde-osmium tetroxide-fixed, Epon embedded, stained Azur II; optical photomicrograph.)





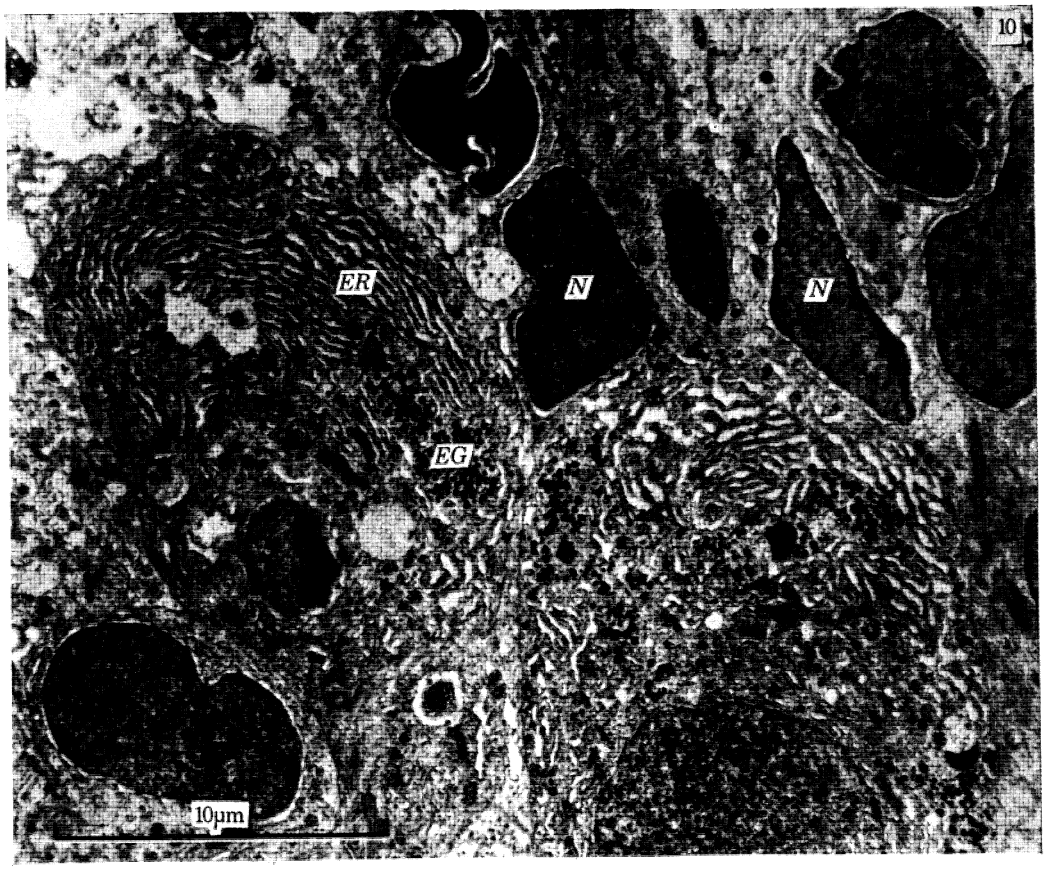
Relationship between neurons of pre-optic nucleus, ependymal cells and third ventricle.

FIGURE 7. 'Dark' (*DC*) and 'light' (*LC*) neurons showing polarization of ER regions towards third ventricle. Note rich vascularization and 'spongy' appearance of surrounding glial cells and prominent nuclei of ependymal cells.

FIGURE 8. Details of two 'light' cells and associated ependyma.

FIGURE 9. Details of 'dark' cells. Note rich vascularization, and difference between *ER* of cells in figures 8 and 9.

(FIGURES 7 to 9. Glutaraldehyde-osmium tetroxide fixed, Epon-embedded, stained Azur II; optical photomicrographs.)



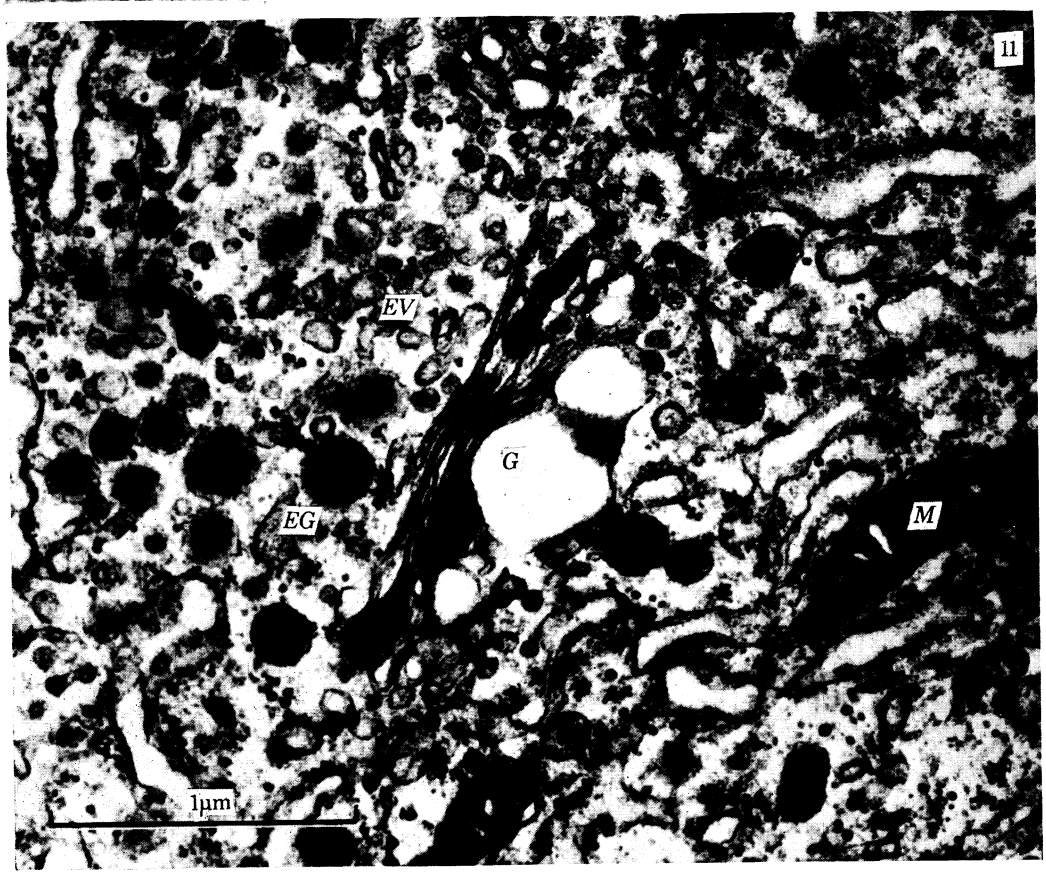
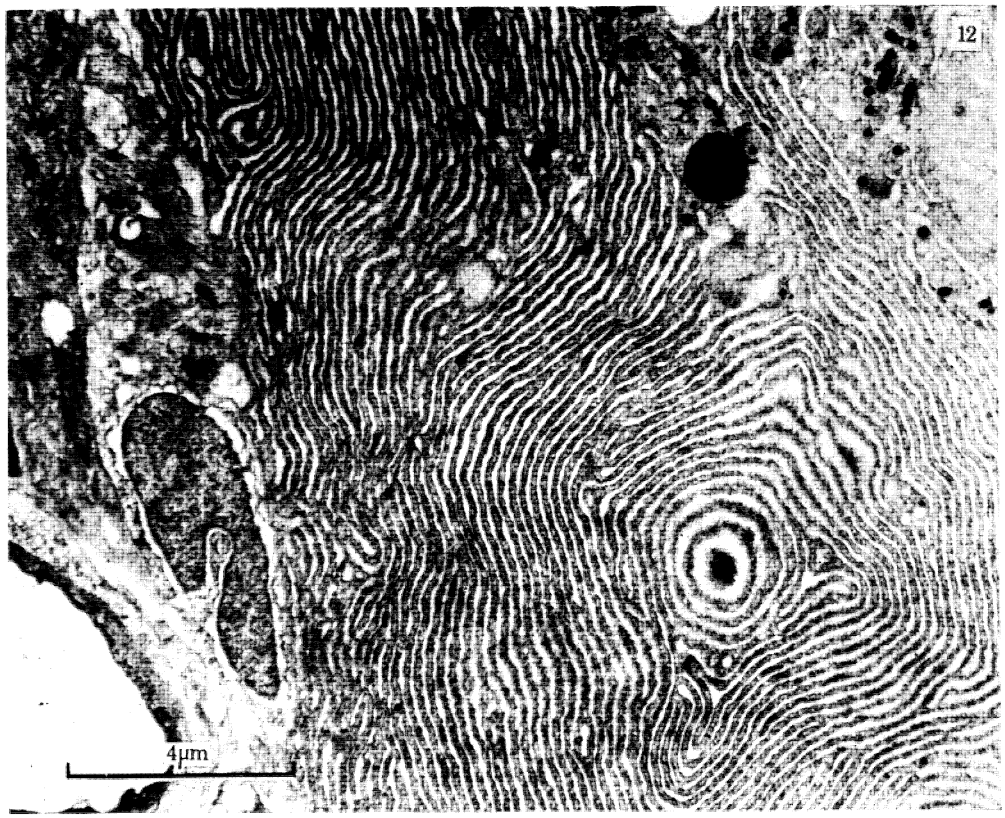


FIGURE 10. Parts of two 'light' cells of the PON showing their close relationship with ependymal cells. (Stained uranyl acetate.) *EG*, elementary granules; *ER*, endoplasmic reticulum; *N*, nuclei of ependymal cells.

FIGURE 11. Perinuclear cytoplasm of a neuron of the PON. *G*, Golgi apparatus; *EG*, elementary granules; *M*, mitochondria; *EV*, ergastoplasmic vesicles. (Stained uranyl acetate.)



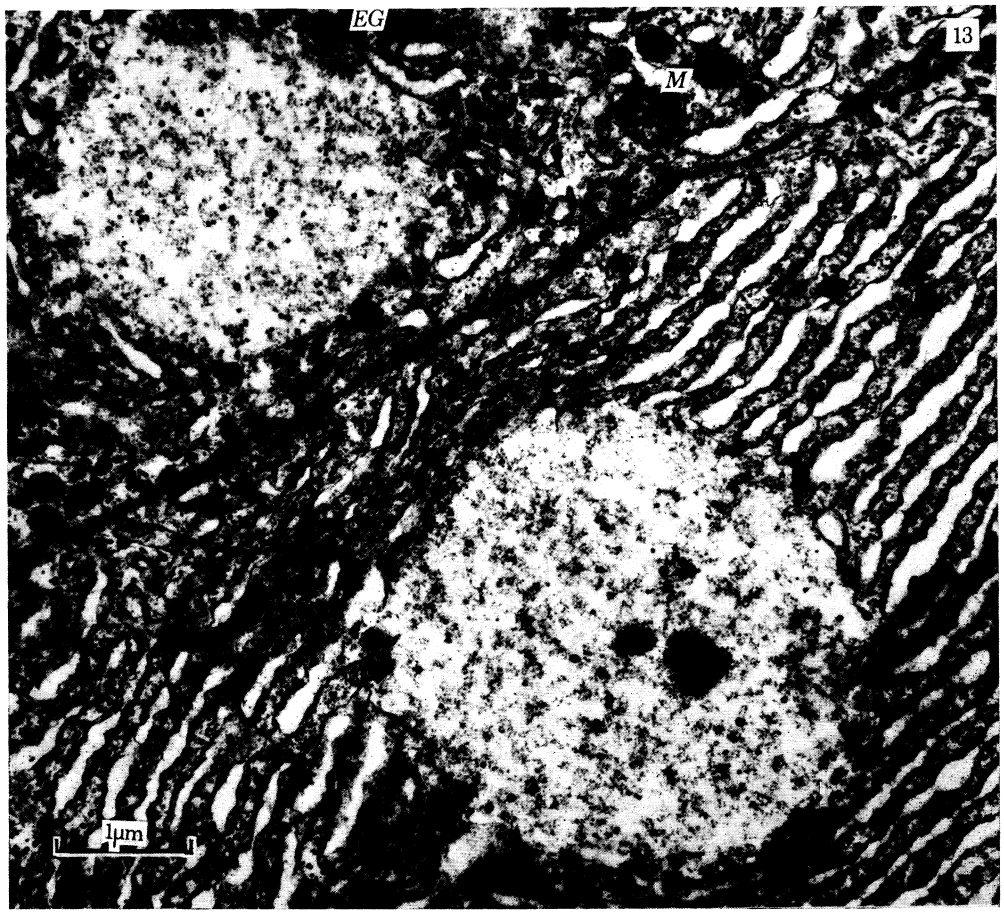


FIGURE 12. Endoplasmic reticulum of a 'light' cell of the pars magnocellularis.

FIGURE 13. Large vesicular inclusions in endoplasmic reticulum of two adjoining neurons. Ribosomes, elementary granules (*EG*) and mitochondria (*M*) also present.

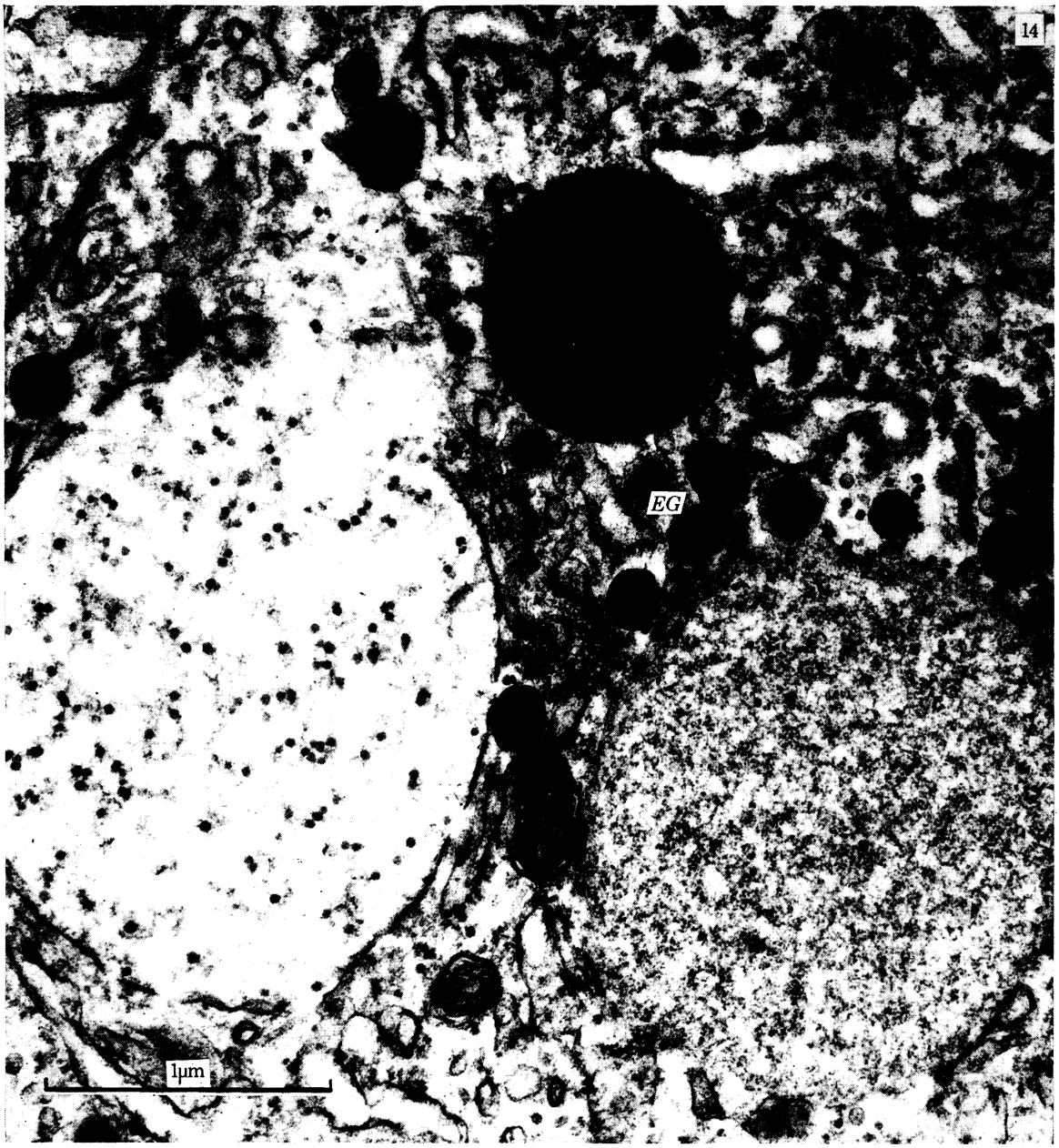


FIGURE 14. Perinuclear region of a neuron containing vesicular and granular cytoplasmic inclusions, including elementary granules (*EG*). (Stained lead acetate.)

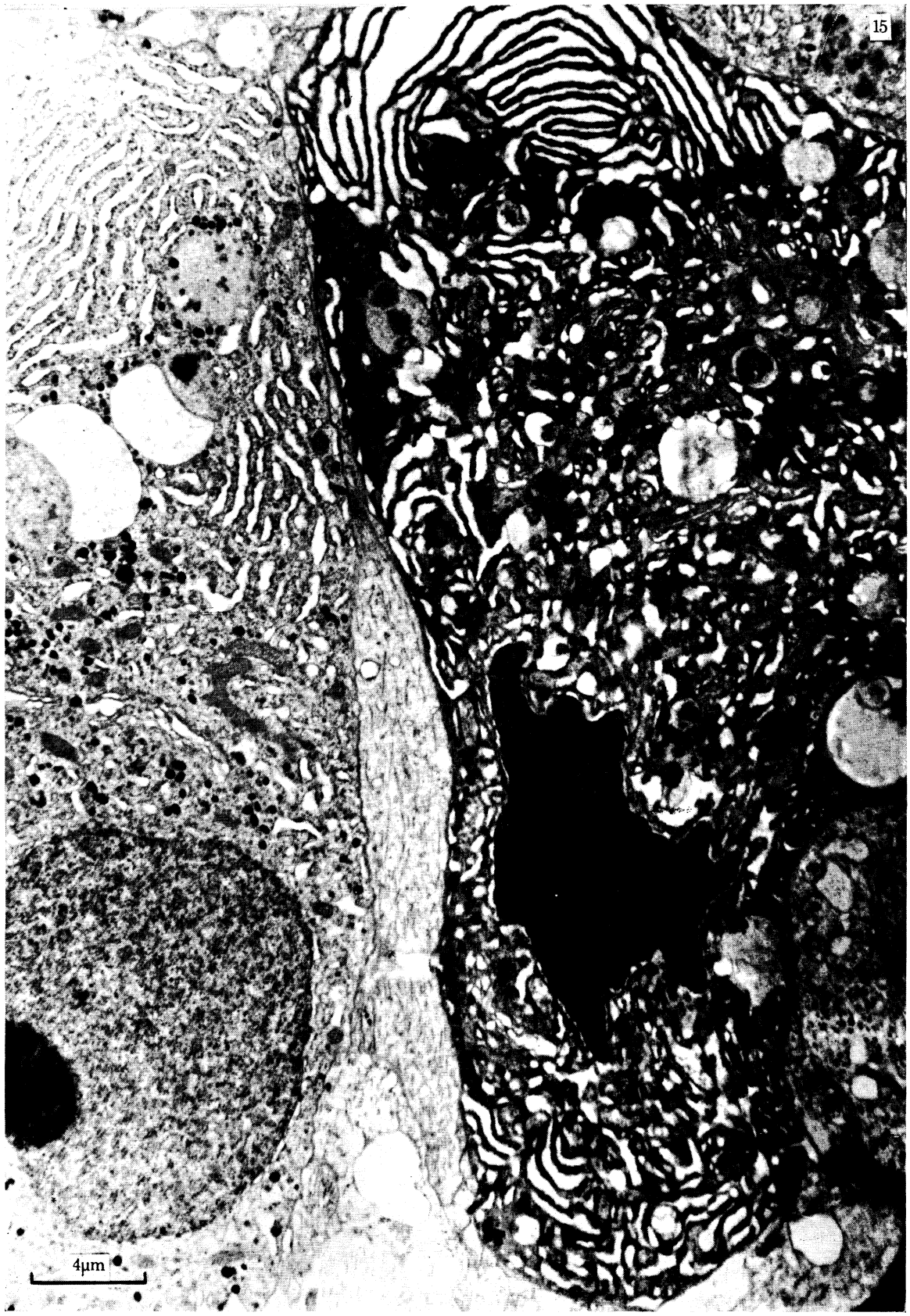


FIGURE 15. 'Dark' and 'light' neurons adjoining. Both contain elementary granules and secretory vesicles of different kinds. Inter-lamellar spaces of endoplasmic reticulum of 'dark' cells characteristically swollen and nucleus deeply invaginated. (Stained uranyl acetate.)

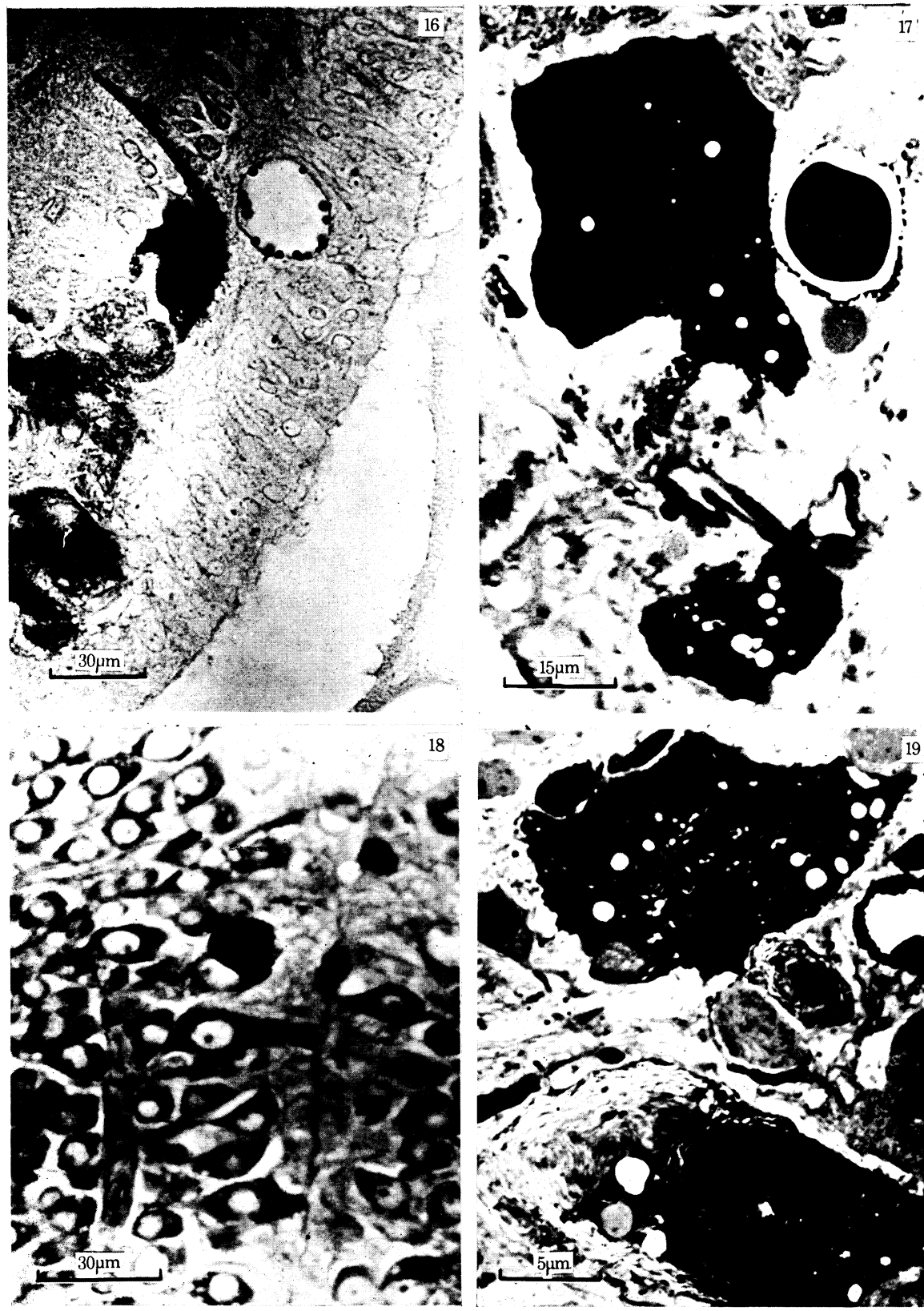


FIGURE 16. Colloid vesicle embedded in ependyma. Contents, apart from peripheral granules, chromophobic. Wax-embedded, stained AT–PAS–NY. (Compare with figure 21, plate 56.)

FIGURE 17. Aggregations of neurosecretion in the PON, containing chromophobic vesicles. Epon-embedded, cut at 1 μm, stained Azur II. (Compare with figure 20, plate 56.)

FIGURE 18. Densely staining neurosecretory neuron which may be a stage in the development of the aggregations shown in figure 17. Wax-embedded, stained AT–PAS–NY.

FIGURE 19. As figure 18, but Epon-embedded, cut at 1 μm and stained Azur II.

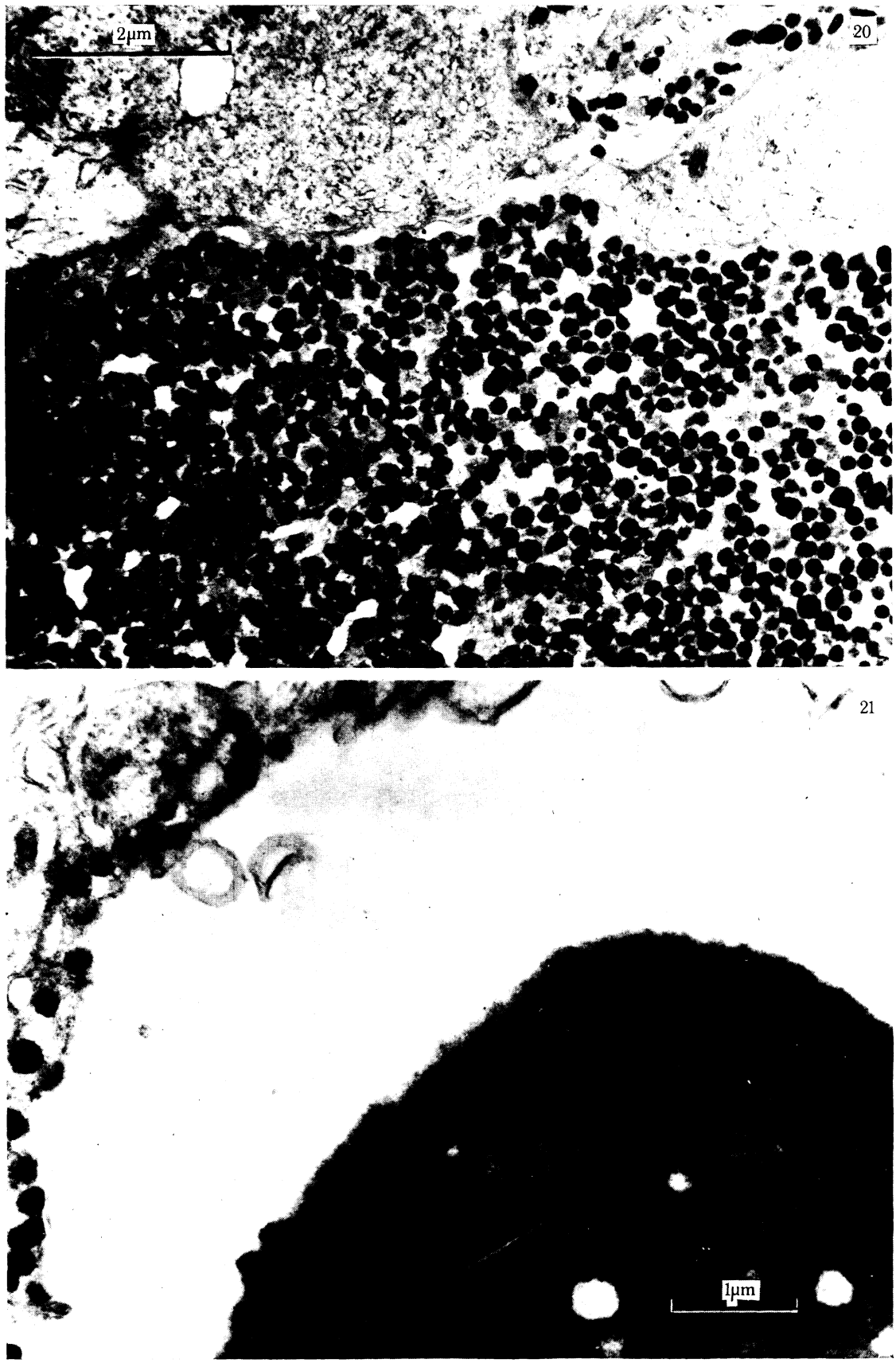
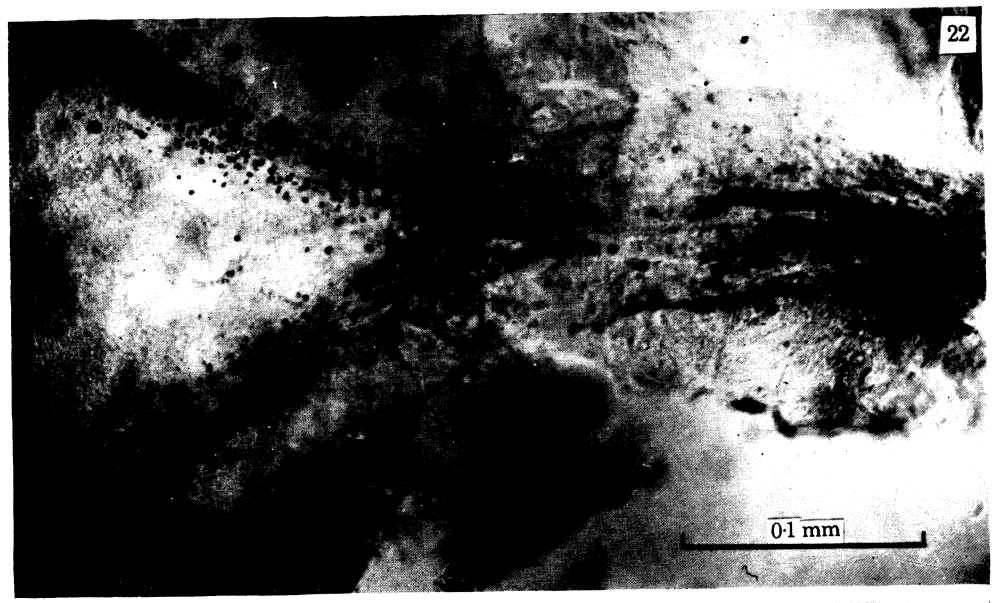
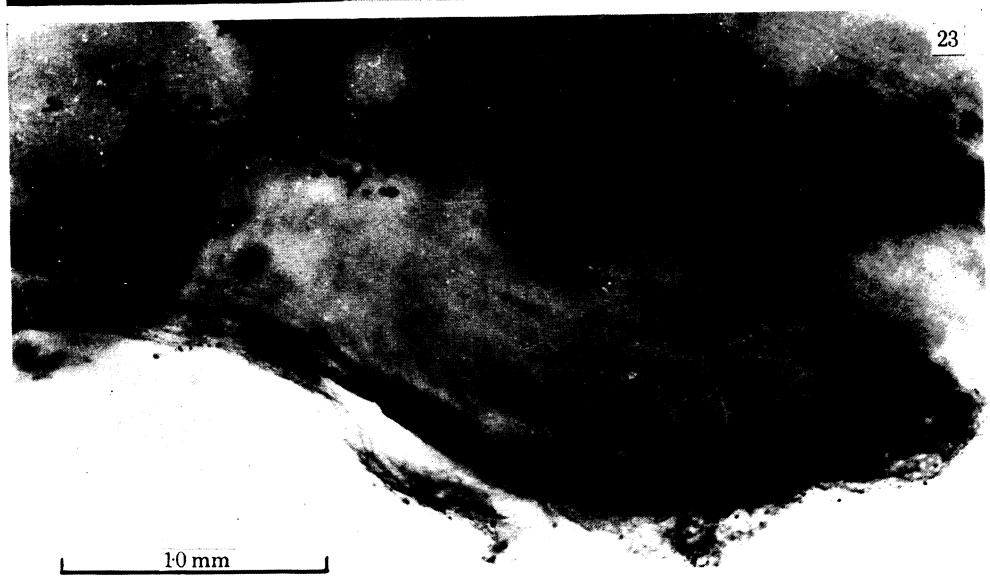
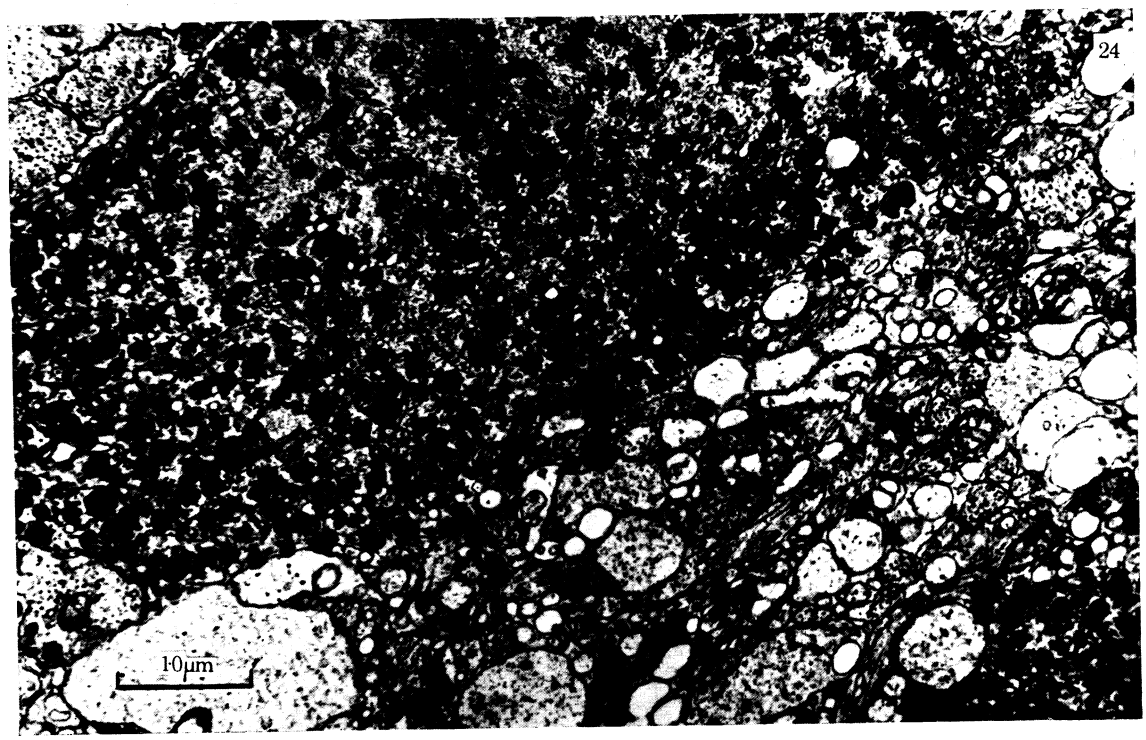


FIGURE 20. Electron micrograph of periphery of neurosecretory aggregation seen in figure 17, plate 55. Note surrounding nervous tissue. (Stained uranyl acetate.)

FIGURE 21. Electron micrograph of periphery of colloid vesicle similar to that shown in figure 16, plate 55 (Stained uranyl acetate.)







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cell types concern the nucleus and the number and range of cellular inclusions. In the light cells the nuclear membrane is smooth and even, whereas it is frequently deeply invaginated in the dark cells. Furthermore, the latter usually contain more cytoplasmic inclusions and for this reason we at first assumed that the different appearances of the two cell types merely reflected different physiological states of a single neuronal species. However, the fact that they contain elementary granules of two different sizes, corresponding closely to those present in two types of axonal ending in the neurohypophysis, and the fact that there are no neurons intermediate in appearance suggest that they are functionally different. It may be noted that the method used to select elementary granules for size measurement does not enable us to say whether the cells contain elementary granules of more than one type.

Aggregations of neurosecretion

More or less extensive droplets of colloid-like material which stain homogeneously with the so-called neurosecretory dyes occur in some 28 % of the pre-optic nuclei examined by the *in situ* technique (Leatherland *et al.* 1966). Such colloid droplets have also been reported by Stutinsky (1953) and by Schiebler & Hartmann (1963). Some, at least, appear to be extracellular aggregations and occur in equal numbers in both regions of the PON. They do not seem to be related to any particular physiological condition of the animals or to season of the year, and their stainability is not correlated with that of the PON. Optical microscope studies of osmium tetroxide-fixed material (figures 17, 19, plate 55) show that the vesicles are strongly Azur II-positive and they contain several large completely chromophobic regions, scattered in a tightly packed mass of granules. No cell outlines are visible and the electron microscope picture (figure 20, plate 56) is consistent with the view that they are extracellular aggregations of granules of different sizes and electron densities, most of which appear to be elementary granules.

Colloid vesicles (figures 16, plate 55; and 21, plate 56).

These rarely seen structures lie between neurons of the PON. In Bouin-fixed, wax-embedded sections they appear as membrane-bound vesicles containing shrunken amorphous 'colloid' strongly positive to Azur II stain, and a peripheral chromophobic zone. Deeply stained isolated droplets are often seen attached to the membrane. In the electron microscope (figure 21) the central 'colloid' is predominantly amorphous but contains scattered electron-lucent regions. The peripheral isolated droplets are seen as electron-dense granules similar in size and appearance to elementary granules. Unlike the aggregations of neurosecretion the colloid vesicles are not stained by neurosecretory dyes; this, and the fact they they occur infrequently, distinguishes them from the aggregations.

DESCRIPTION OF PLATE 57

FIGURE 22. *In situ* preparation of converging neurosecretory tracts and subterminal region (ventral aspect). Note that appearance of secretion changes in this region. Pituitary to the right. (Stained AF.)

FIGURE 23. *In situ* preparation of hypothalamo-neurohypophysial system of *Anguilla* after hypophysectomy (lateral aspect). Dense mass of neurosecretion has collected at cut end of pituitary stalk. (Stained AF.)

FIGURE 24. Electron micrograph of mass of neurosecretion similar to that shown in figure 23. Elementary granules, vesicles of various sizes, mitochondria and 'normal' nervous tissue can be seen. (Stained lead acetate.)

5. *The pre-optic nucleus and tracts in hypophysectomized eels* (figures 23, 24, plate 57)

(*a*) *The pre-optic nucleus*

There were no observable changes in either the histological or ultrastructural appearance of the PON in hypophysectomized eels for periods up to 14 days after hypophysectomy (figure 23).

(*b*) *The hypothalamic tracts*

No apparent changes in either the content or distribution of stainable neurosecretory material, nor changes in fine structure are evident in the hypophysial axons in the hypothalamus of hypophysectomized eels.

An accumulation of stainable neurosecretory material is seen at the cut end of the pituitary stalk as early as 5 h after surgical intervention. The accumulation increases in size and density of stainability in eels killed, 1 to 3, 6 and 14 days after hypophysectomy. In *in situ* preparations and in the light microscope the accumulation appears as a dense homogeneous mass of material that is AF-, AB- and AT-positive (figure 23). Fine structural studies show closely packed neurosecretory and 'ordinary' nerve fibres, the former containing granules of different sizes and electron densities, together with free granules, neurofibrillae, and mitochondria in an amorphous matrix of medium electron density (figure 24).

DISCUSSION

Optical microscopy of sectioned material, usually after Bouin fixation and treatment with so-called neurosecretory dyes, is the technique that has been most frequently used in structural studies of the hypothalamo-neurohypophysial complex and it is now widely recognized that this approach is unreliable as an index of function (Lederis 1964; Bern 1967). If we accept that glutaraldehyde-osmium tetroxide fixation preserves cellular detail better than the traditional fixatives used in optical microscopy, and this seems reasonable in view of the results obtained in the electron microscope, then figures 6, plate 49 and 7 to 9, plate 50, above, reflect cellular structure more accurately than figures 3 to 5, plate 49. The former show a strikingly large polar cap of endoplasmic reticulum at the 'dendritic' pole of the cell, whereas no such region is identifiable in the former. Furthermore, the neurons are markedly polarized and it is the endoplasmic reticulum that invariably adjoins the III ventricle. If we further suppose, as is generally accepted, that this is the cellular organelle concerned in the first steps of intracellular synthesis, then it seems more reasonable to suggest that the pre-optic neurons are *receiving* material from the cerebrospinal fluid rather than secreting into it, though it is possible that both processes are taking place. Most previous workers have held that the neurons secrete into the third ventricle and the process has come to be called 'neurocrinie' and 'hydrencephalokrinie'. Stutinsky (1953) and Sterba & Weiss (1967) have reviewed the earlier literature on this subject, and it must be admitted that the many illustrations in the literature, especially those of Sterba & Weiss (1967) and also figures 3 to 5, plate 49, are most persuasive; but there is a case for reconsidering this interpretation in the eel. The suggestion that active uptake of materials from the cerebrospinal fluid by the neurons of the pre-optic nucleus is taking place receives support from our own work on the intracisternal injection of ^{35}S-cysteine and ^{14}C-serine in the eel (Leatherland & Dodd 1969) and it would provide a mechanism for the proposal of Knowles & Vollrath (1966) that functional control of the activity of the hypothalamo-neurohypophysial-

complex of eels incorporates a 'feed-back' link between the pituicytes of the neurohypophysis and the cells of the pre-optic nucleus.

Fine-structural studies have shown that a considerable variety of inclusions are present in the pre-optic neurons and there is an urgent need to investigate their nature and functions. Lederis (1962) and Sachs (1963) have shown, by differential centrifugation and bioassay, that some at least of the elementary granules contain hormone, but the nature and functions of most of the other cytoplasmic inclusions is not yet established.

The demonstration that there are two types of neurons in the pre-optic nucleus is significant. It has been known for some time that there are at least three types of fibre in the eel neurohypophysis called by Knowles & Vollrath (1966) A 1, A 2 and B respectively. The A-type fibres react with the neurosecretory dyes, but it is possible to distinguish between them since the elementary granules they contain are different in size. In view of this it seems to follow that there must be two distinct neuronal cell types containing stainable neurosecretion in the pre-optic nucleus of the eel since both A-type fibres originate in this nucleus. This conclusion is borne out by the findings reported above. Whether or not the neurons are vasotocinergic and ichthyotocinergic respectively remains to be investigated, but the existence of two cell types would permit the separate control of each and more easily account for the reports in the literature (Lederis 1964; Sawyer & Pickford 1963) that there is differential release of the two hormones in response to different stimuli.

Aggregations of neurosecretion, and colloid vesicles or similar structures, have been widely reported in the PON of fishes (Stutinsky 1953; Schiebler & Hartmann 1963), though their function, indeed their precise nature, remains obscure. Stutinsky reported intra-axonal colloid masses along the lengths of the hypophysial axons of the eel though we have not encountered these. The smaller aggregations have the appearance of neurons packed to capacity with stainable granules and in these cells the large chromophobic nucleus is easily visible. But the larger masses appear to be intercellular, as stated by Schiebler & Hartmann (1963) who found them in *Tinca vulgaris*. It seems possible that these are formed by the breakdown of neurons of the type just considered or possibly by release of neurosecretion from abnormally short or broken axons. It is impossible to say whether or not these aggregations play any part in the physiology of the pre-optic nucleus or are merely aberrant. Their widespread occurrence argues against the latter view and seems to indicate that they may be significant, though their function is at present obscure.

The colloid vesicles may represent the residues that remain after the elementary granules have been released from the aggregations of neurosecretion. In most cases a few elementary granules remain at the periphery of the vesicles, the latter containing electron-dense amorphous material which seems to have undergone shrinkage. If this explanation of the genesis of the vesicles is correct it is still not clear what happens to the elementary granules since even though the regions of the aggregations and vesicles are always richly vascularized it is unlikely that the elementary granules can pass, without alteration, into the blood system. Some of our preparations suggest that they may be discharged, presumably intercellularly, into the III ventricle.

The studies of Leatherland *et al.* (1966), extended in the present work, have shown a degree of subdivision of origin and morphology of the hypothalamic tracts that was unsuspected and which could form the basis of a subdivision of function and control. The discovery of the two types of neurons and the subterminal region, in which some of the axons appear to end,

reinforces the suggestion of a division of labour as do the discoveries of Lederis (1964) and Sawyer & Pickford (1963) that the hormones can be differentially depleted.

A good deal more is known about the structure of the hypothalamo-neurohypophysial complex of fishes than about its function; systemic functions have been particularly difficult to demonstrate (Dodd, Perks & Dodd 1966). The close relationship between the neurohypophysis and the meta-adenohypophysis in these animals is suggestive of a functional relationship between the two, and Knowles & Vollrath (1966) have recently investigated this possibility. They have shown that some of the neurosecretory axons of the neurohypophysis end on blood vessels and there is evidence from their work that these are important in colour change in the eel. Others end on pituicytes that line the ramifications of the third ventricle within the neuro-intermediate lobe and these appear to control secretory activity of the pituicytes. The products of secretion enter the cerebro-spinal fluid and come to bathe the area of the pre-optic nucleus. Knowles & Vollrath (1966) believe that they may in some way influence the activity of the pre-optic neurons and thus mediate a feed-back mechanism. In this context we have shown that the pre-optic neurons are so situated that their secretory poles are in almost direct contact with the CSF and the suggestion that they have a sensory role as well as a secretory one has been made several times since Verney (1947) showed that the neurons of the hypothalamic neurosecretory nuclei of the dog are sensitive to osmotic changes in the blood. This work has been reviewed by Dierickx (1962) and Smoller (1965). Dierickx (1962) suggests that the 'periventricular network of dendrites' which originates from the neurosecretory neurons in *Rana temporaria* respond to osmotic changes in the cerebo-spinal fluid. He did not find a close relationship between these dendrites and blood vessels, and does not support Verney's view that the neuronal cell bodies respond to osmotic changes in the blood, though, as we have shown, their relationships with the third ventricle indicate that they may well be able to respond to osmotic changes in the cerebro-spinal fluid which would, presumably, accompany such changes in the blood. On the other hand, Smoller (1956) describes dendritic processes of neurosecretory neurons in *Hyla regilla* that are in contact with the cerebro-spinal fluid and each of which carries a large cilium. These bulbous expansions also contain all the organelles of the perikaryon and Smoller believes them to be secretory as well as sensory. But she makes the important reservation that morphological studies alone will not reveal the function of these 'dendrites'. In the eel, the juxta-ependymal regions of the pre-optic neurons have none of the morphological characteristics associated with the more sophisticated types of receptors, yet it is still possible that they have a sensory role, as osmoreceptors or photoreceptors. This possibility was envisaged by Bern (1967) for the 'dendrite-like' processes of the hypothalamic neurosecretory cells of lower vertebrates and it certainly merits further investigation.

The effects of hypophysectomy on the pre-optic neurons and their tracts in the eel have been studied in the optical microscope by Stutinsky (1953), who found that stainability of the neurons persisted unchanged for at least 45 days after removal of the pituitary; indeed the axons stained more intensely than in the controls and a mass of stainable material collected and persisted at the cut end of the pituitary stalk. These results have been confirmed above and it has been shown that the neurosecretory material that collects at the cut end of the stalk contains masses of elementary granules in addition to other components. We found no evidence to indicate that a neural lobe was being reconstituted within the aggregation of neurosecretion (Sathyanesan & Gorbman 1965); this may have been due to the fact that our observations stopped 14 days after hypophysectomy.

We are indebted to Sir Francis Knowles, F.R.S., for much stimulating discussion of this work which forms part of a programme of research on neurosecretion in fishes supported by the North Atlantic Treaty Organisation. Financial support was provided by the Science Committee of N.A.T.O., and a Research Studentship by the Science Research Council.

ABBREVIATIONS USED ON PLATES

CH	cerebral hemispheres	*PM*	pars magnocellularis
HT	hypothalamic tracts	*PP*	pars parvocellularis
MES	meso-adenohypophysis	*PRO*	pro-adenohypophysis
META	meta-adenohypophysis	*SCO*	subcommissural organ
MB	mid-brain	*STR*	subterminal region
NEURO	neurohypophysis	*SV*	saccus vasculosus
NIL	neuro-intermediate lobe	I-V	hypothalamic tracts

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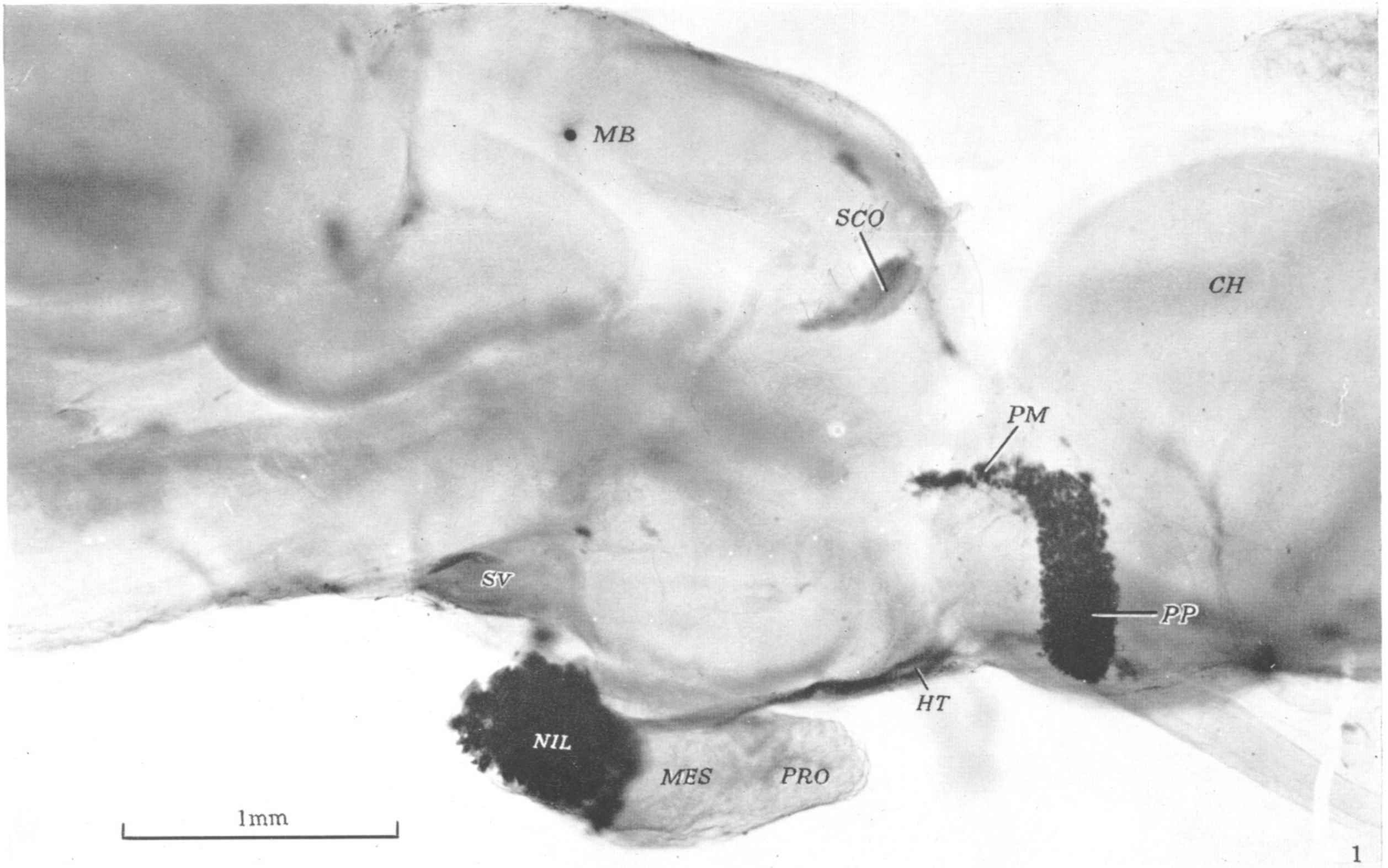

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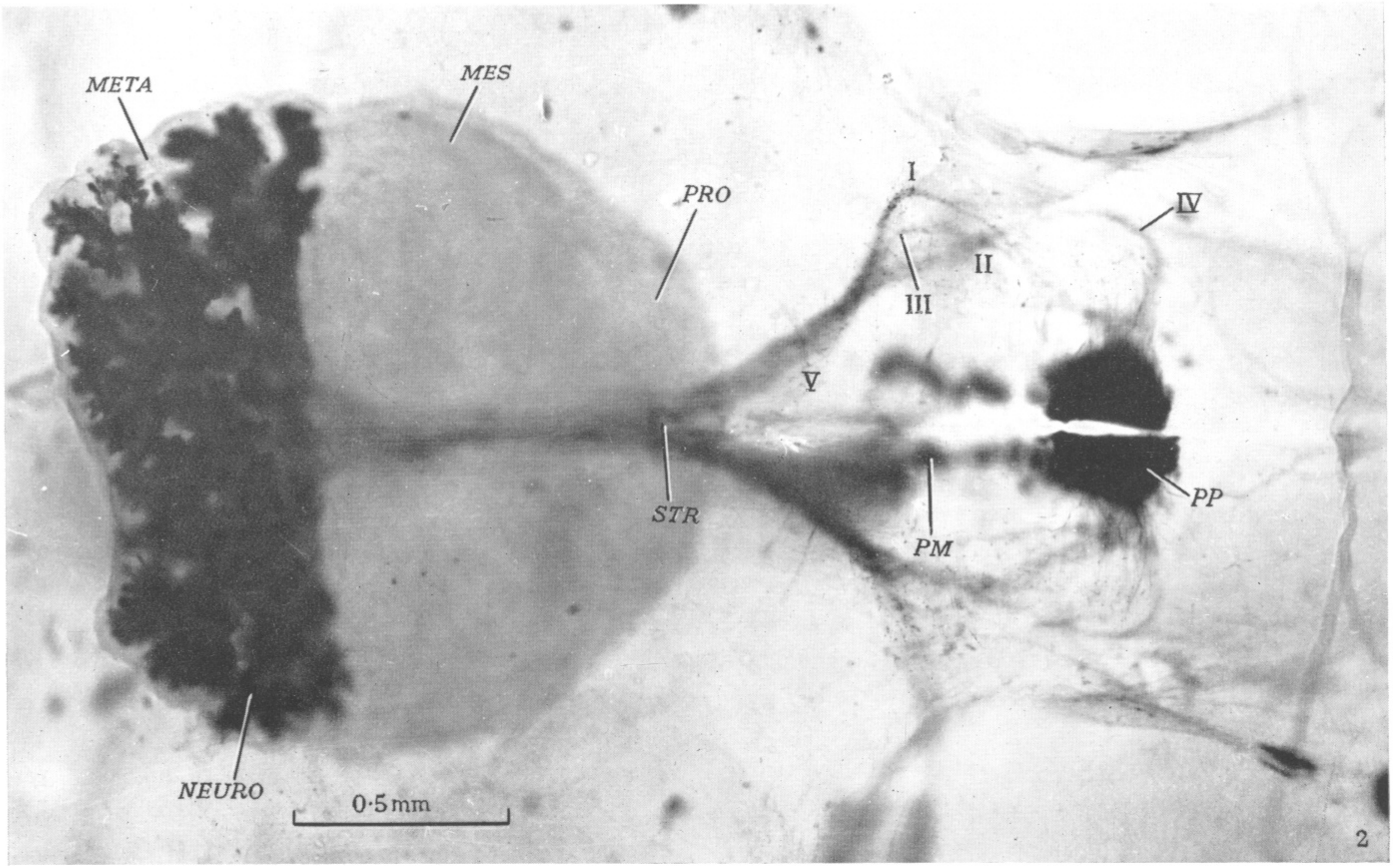
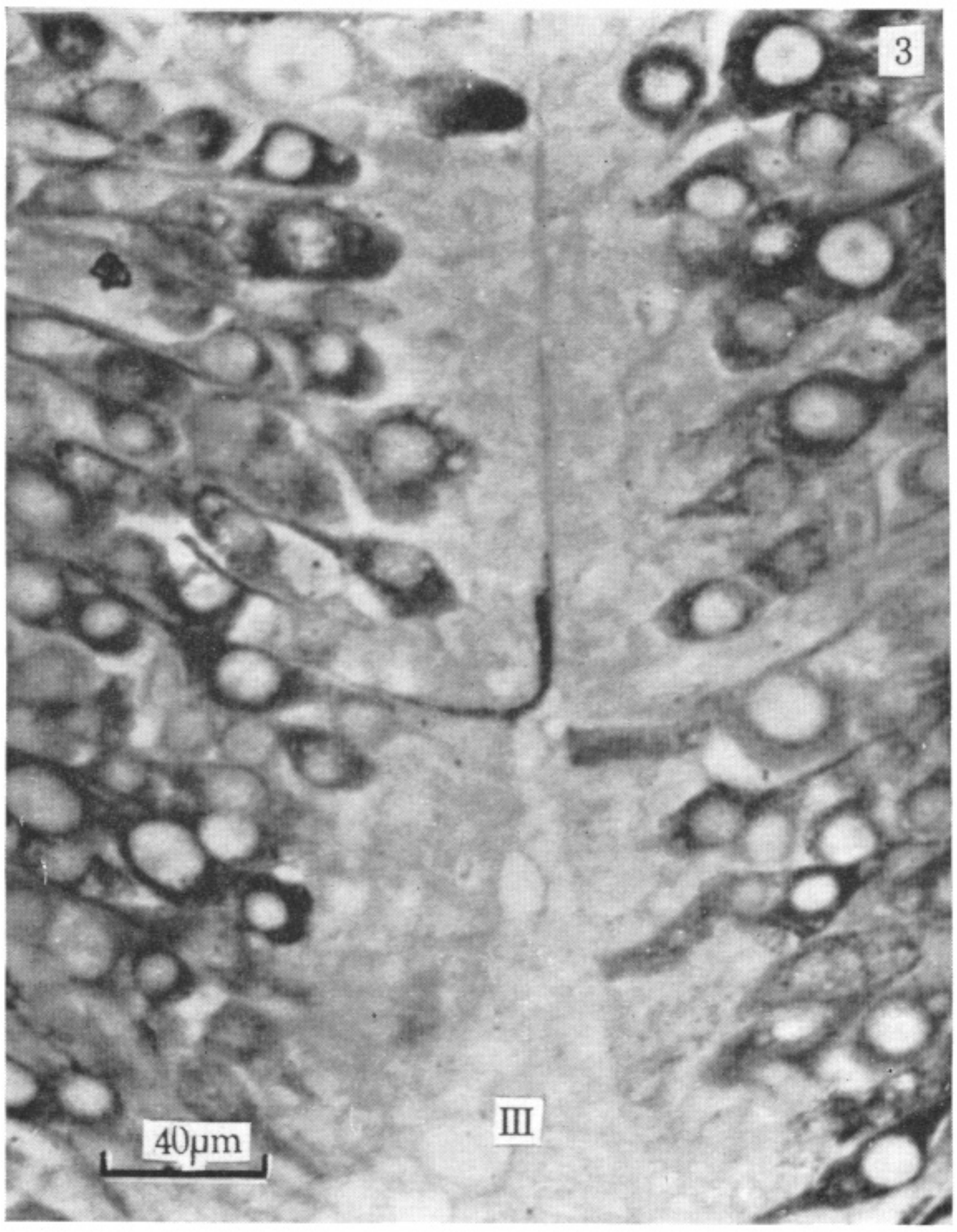
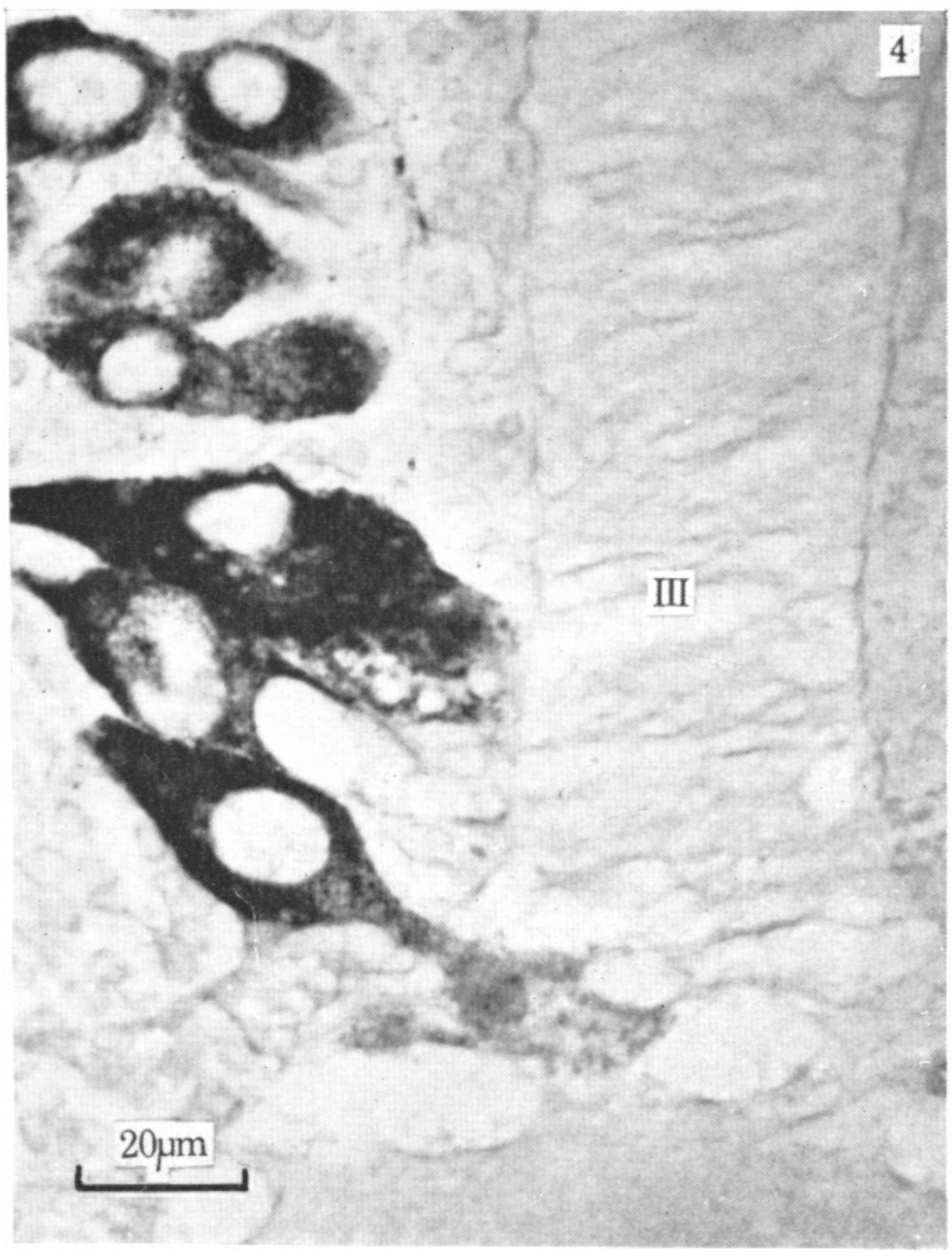


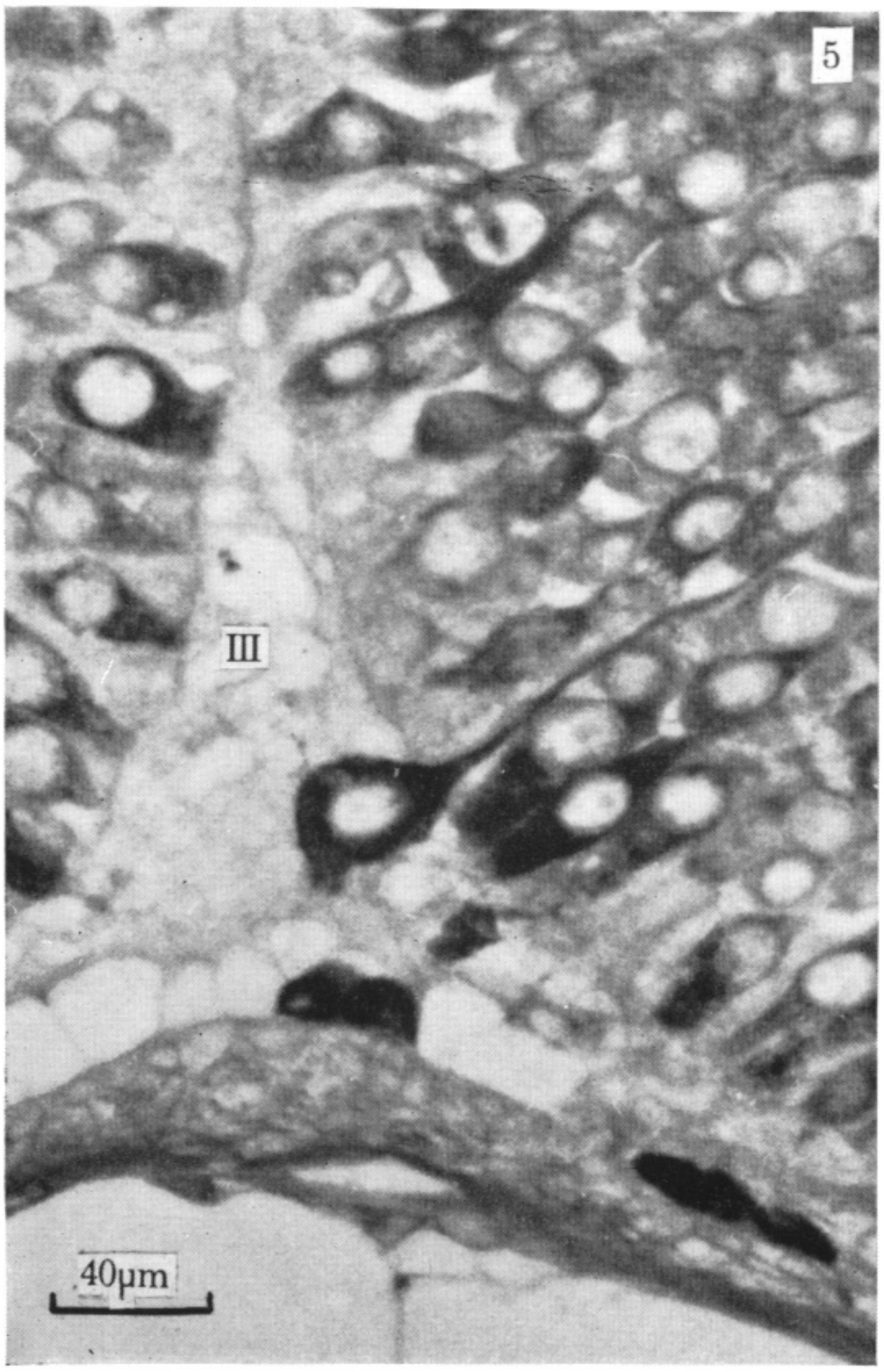
2

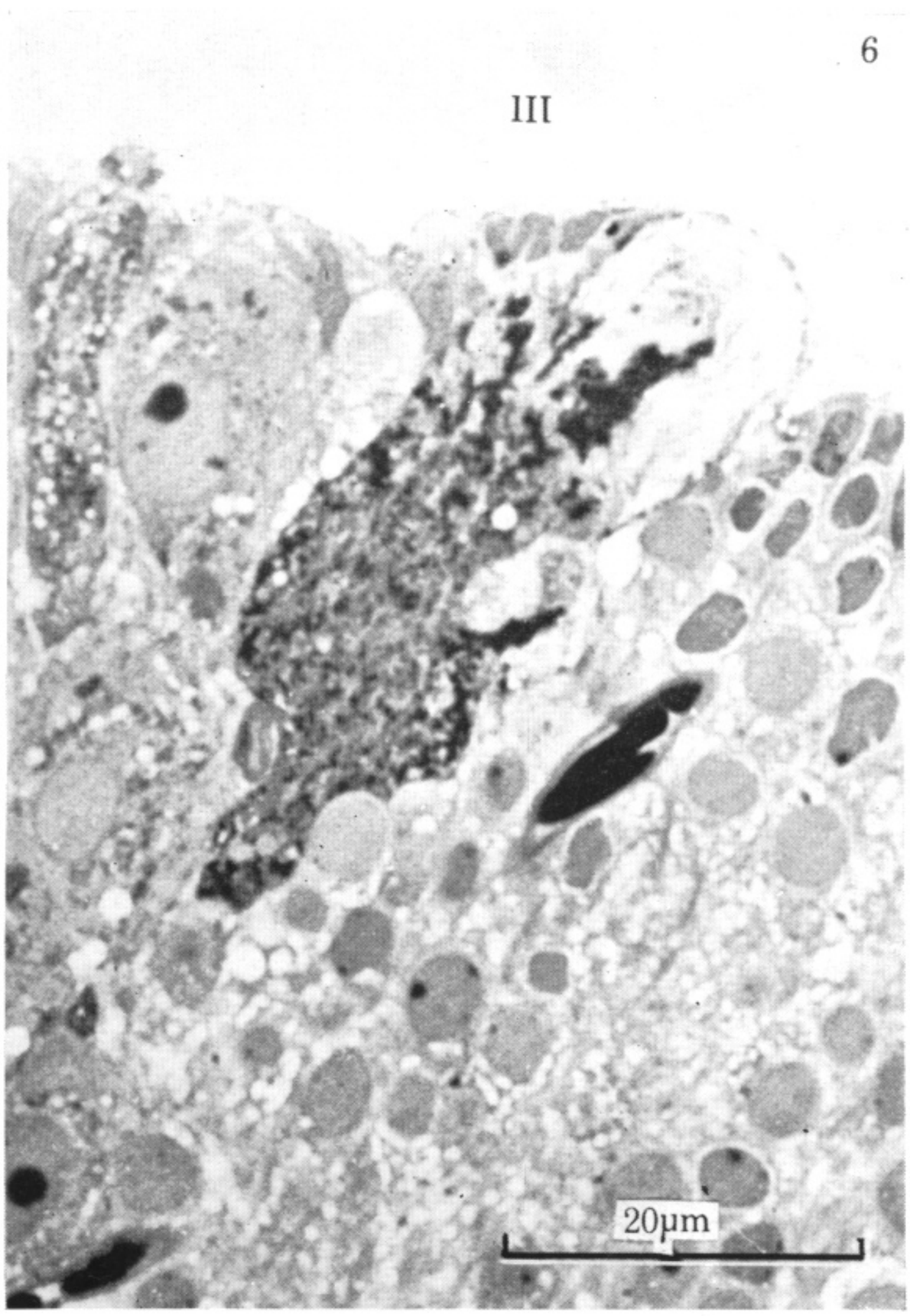
FIGURE 1. *In situ* preparation of the hypothalamo-neurohypophysial-complex of *Anguilla*, lateral view. (Stained AF.)

FIGURE 2. *In situ* preparation of the hypothalamo-neurohypophysial-complex of *Anguilla*, ventral view. (Stained AF.)





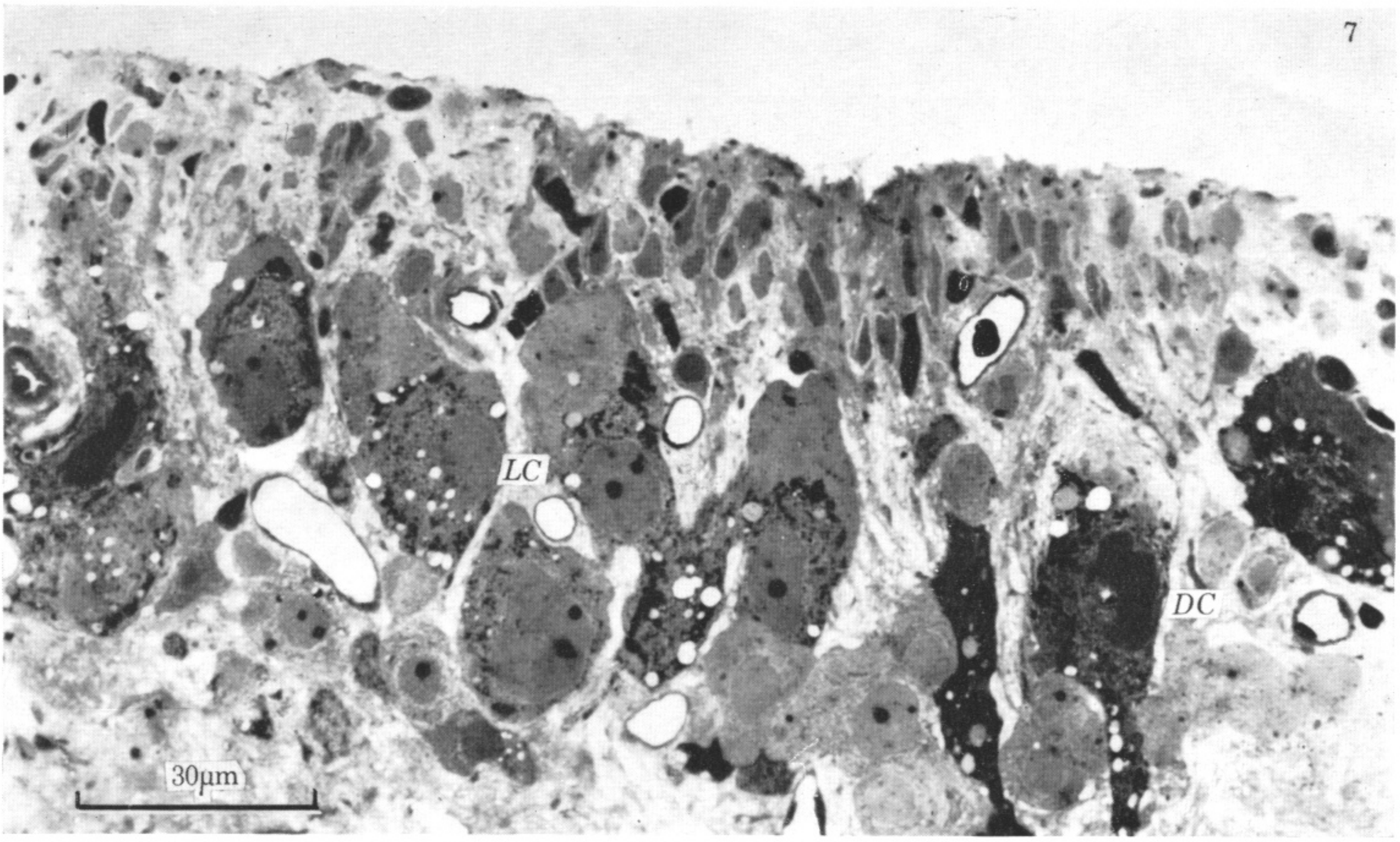


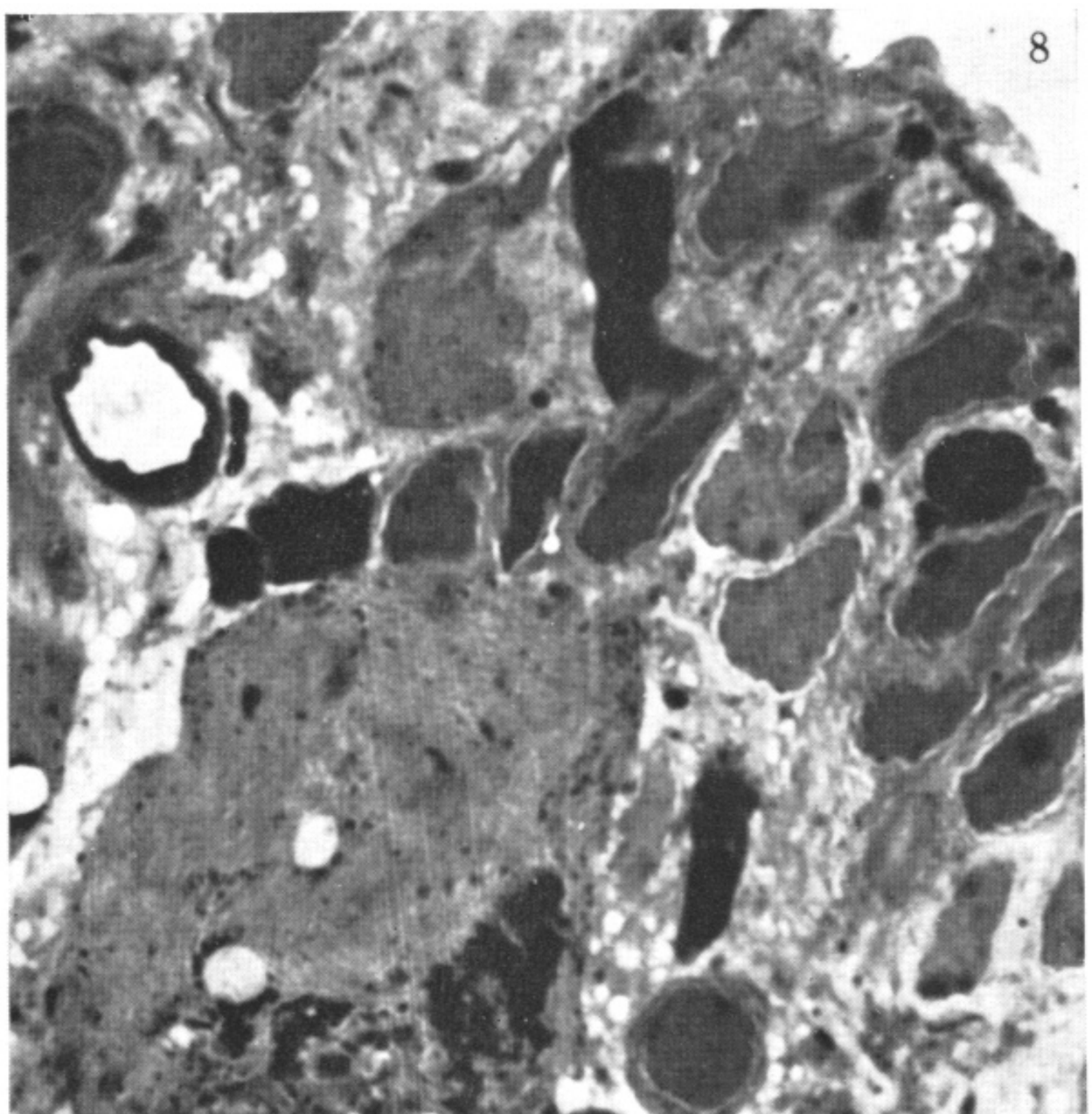


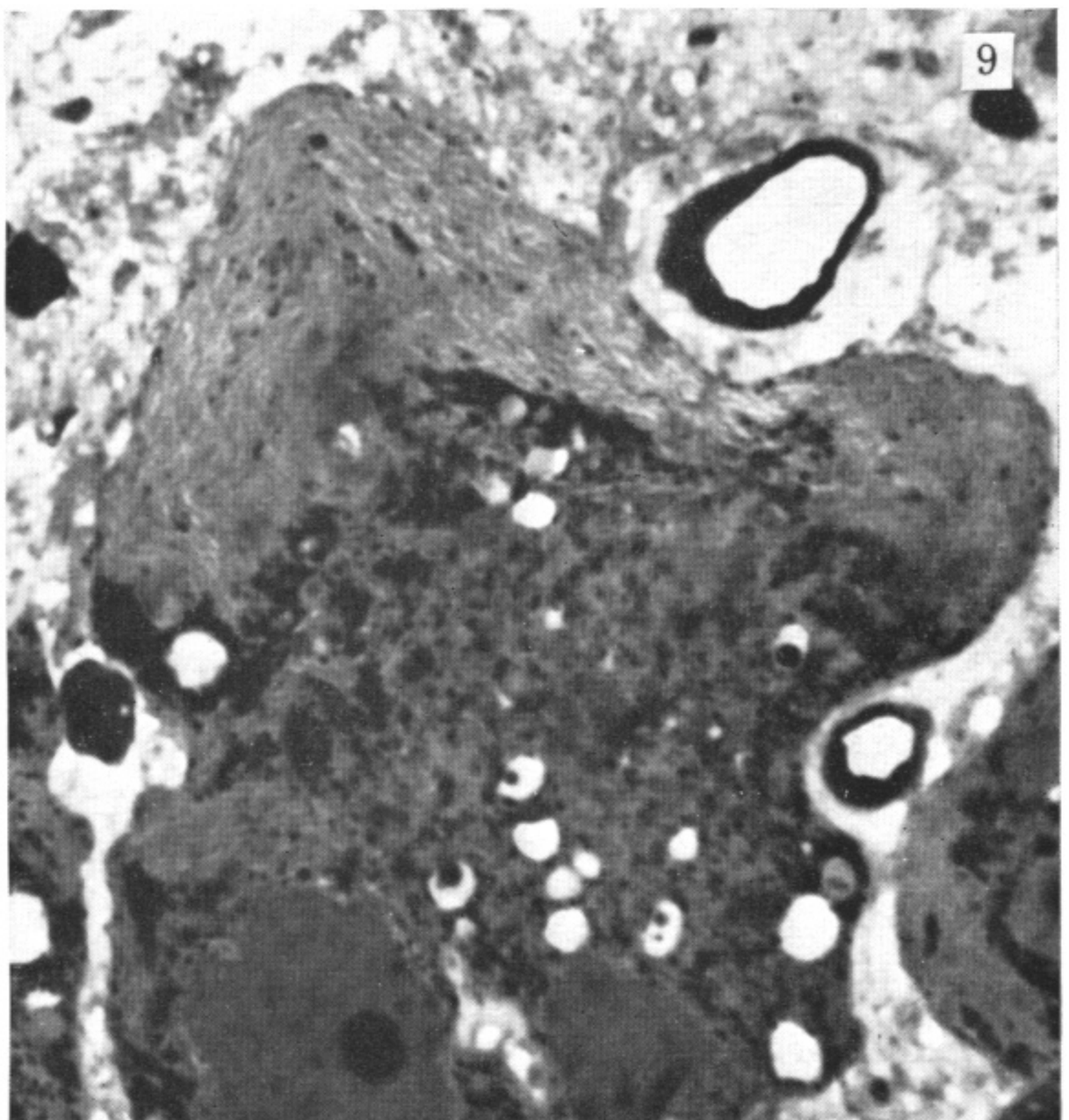
FIGURES 3 to 5. Transverse sections of *Anguilla* PON showing polarization of neurosecretory neurons and contacts between neurons and third ventricle (III).

FIGURE 6. *ER* pole of neuron in contact with cerebro-spinal fluid. Note also axonal pole of neuron, ependymal cells and spongy appearance of glial cells.

(FIGURES 3 to 5. Bouin-fixed, wax-embedded, stained AT–PAS–NY. FIGURE 6. Glutaraldehyde-osmium tetroxide-fixed, Epon embedded, stained Azur II; optical photomicrograph.)







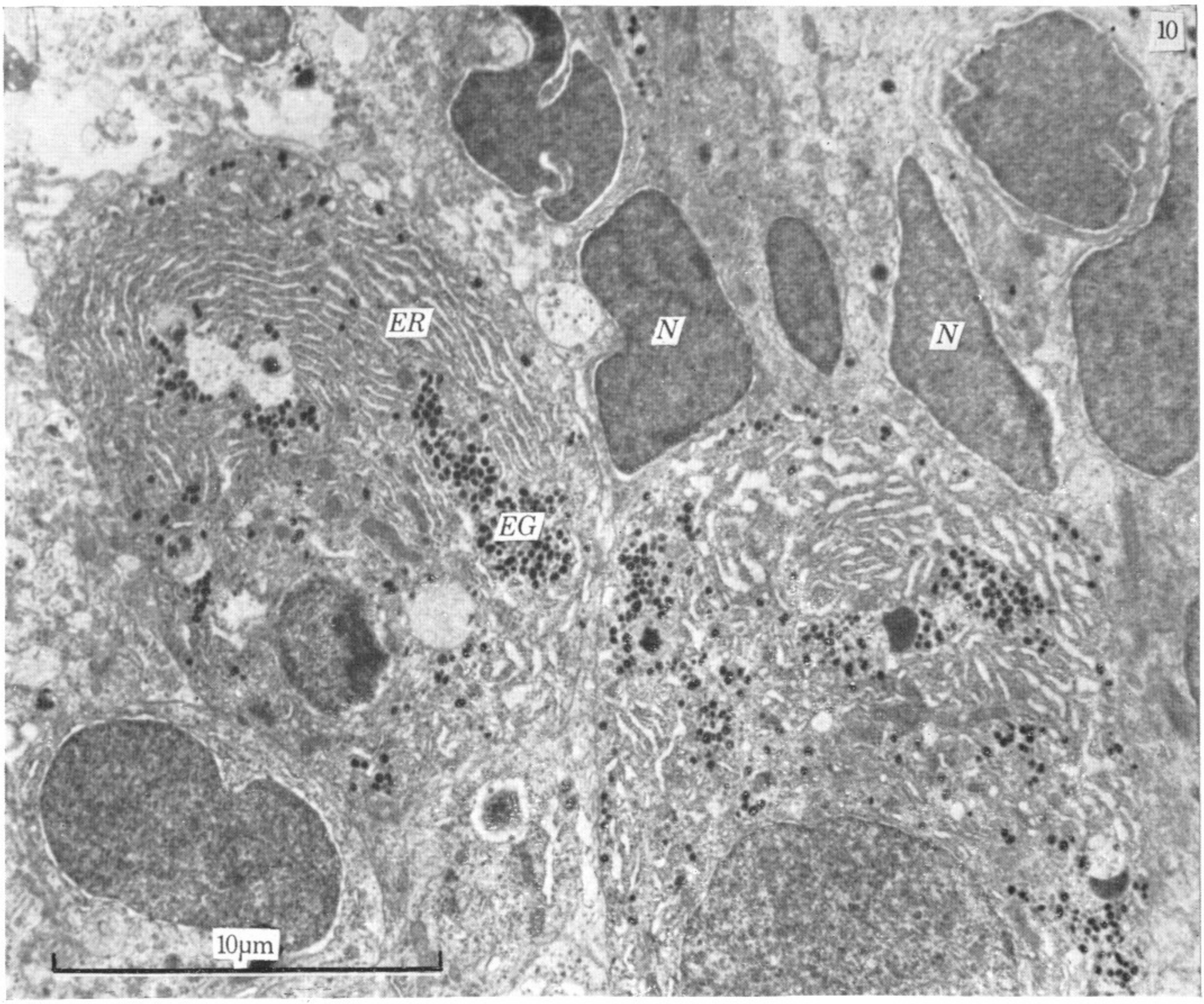
Relationship between neurons of pre-optic nucleus, ependymal cells and third ventricle.

FIGURE 7. 'Dark' (*DC*) and 'light' (*LC*) neurons showing polarization of ER regions towards third ventricle. Note rich vascularization and 'spongy' appearance of surrounding glial cells and prominent nuclei of ependymal cells.

FIGURE 8. Details of two 'light' cells and associated ependyma.

FIGURE 9. Details of 'dark' cells. Note rich vascularization, and difference between *ER* of cells in figures 8 and 9.

(FIGURES 7 to 9. Glutaraldehyde-osmium tetroxide fixed, Epon-embedded, stained Azur II; optical photomicrographs.)



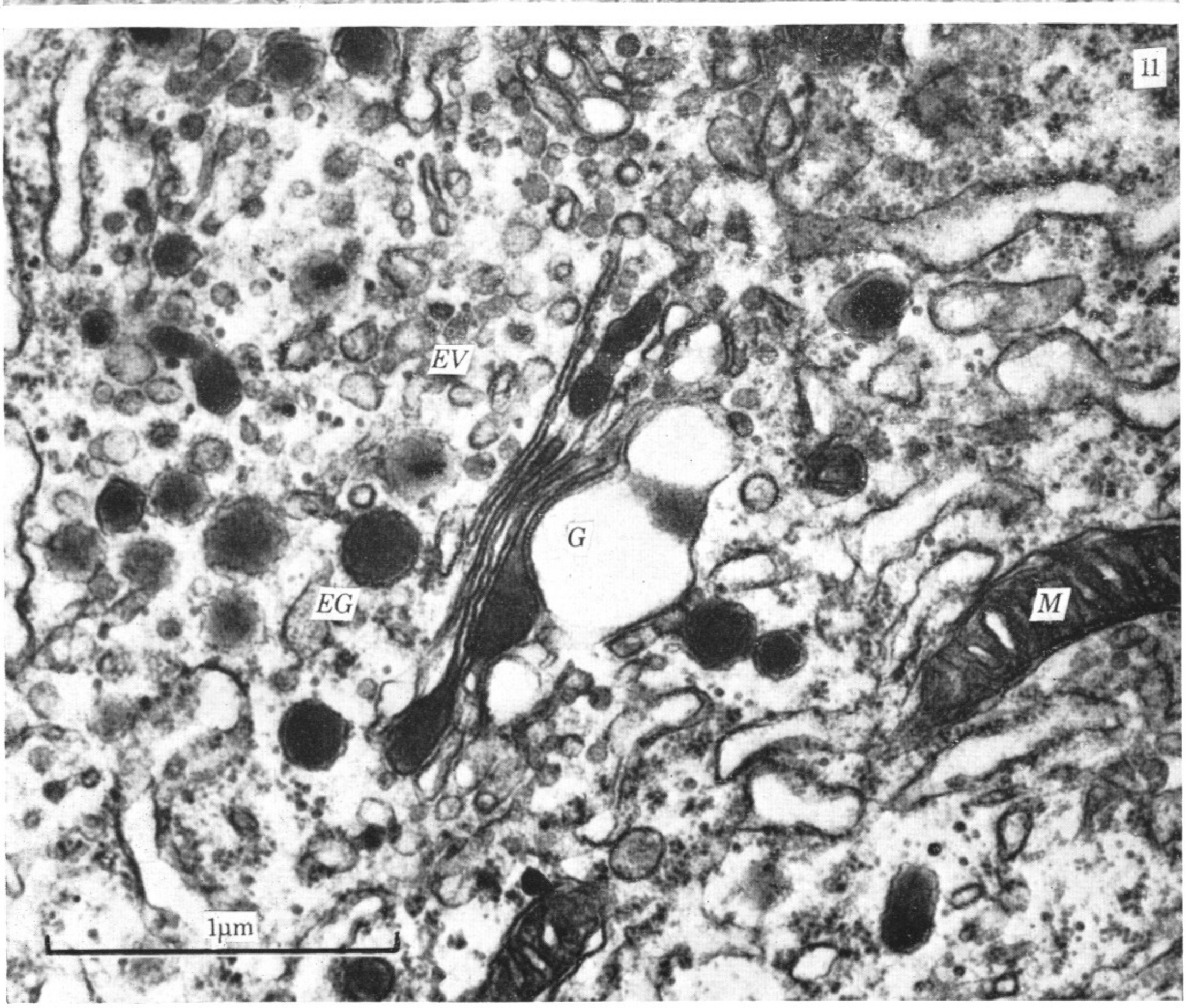
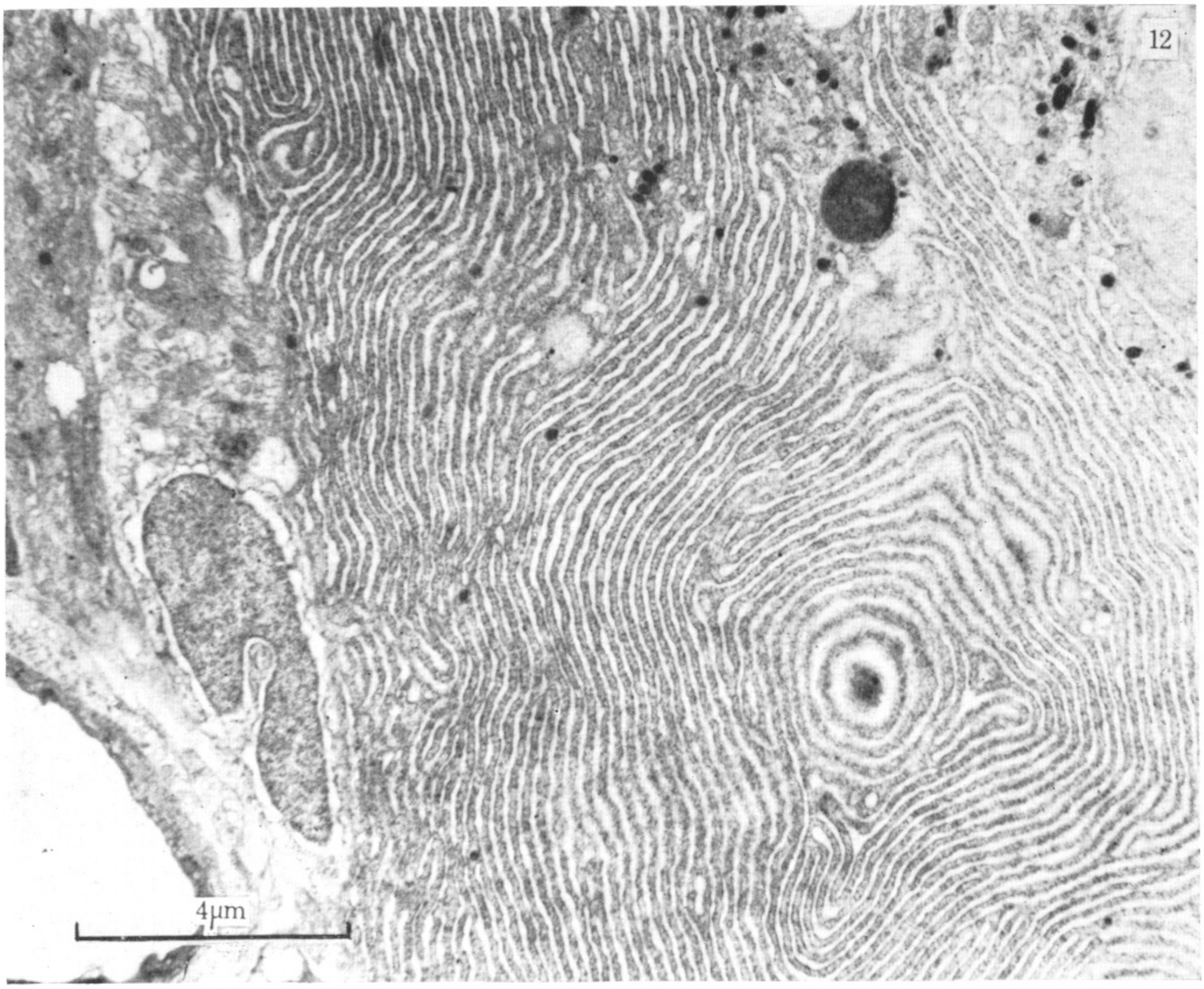


FIGURE 10. Parts of two 'light' cells of the PON showing their close relationship with ependymal cells. (Stained uranyl acetate.) *EG*, elementary granules; *ER*, endoplasmic reticulum; *N*, nuclei of ependymal cells.

FIGURE 11. Perinuclear cytoplasm of a neuron of the PON. *G*, Golgi apparatus; *EG*, elementary granules; *M*, mitochondria; *EV*, ergastoplasmic vesicles. (Stained uranyl acetate.)



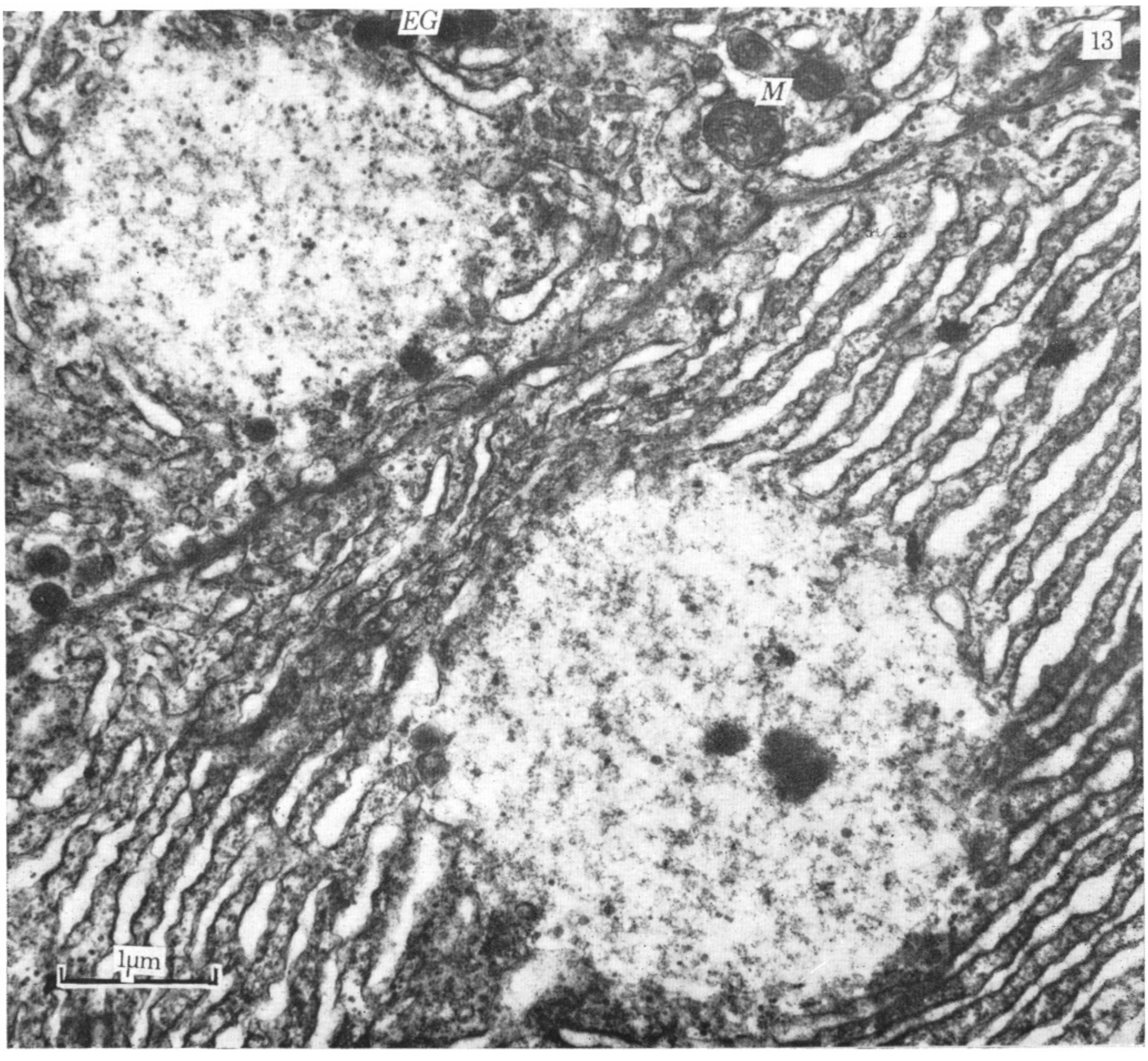


FIGURE 12. Endoplasmic reticulum of a 'light' cell of the pars magnocellularis.

FIGURE 13. Large vesicular inclusions in endoplasmic reticulum of two adjoining neurons. Ribosomes, elementary granules (*EG*) and mitochondria (*M*) also present.

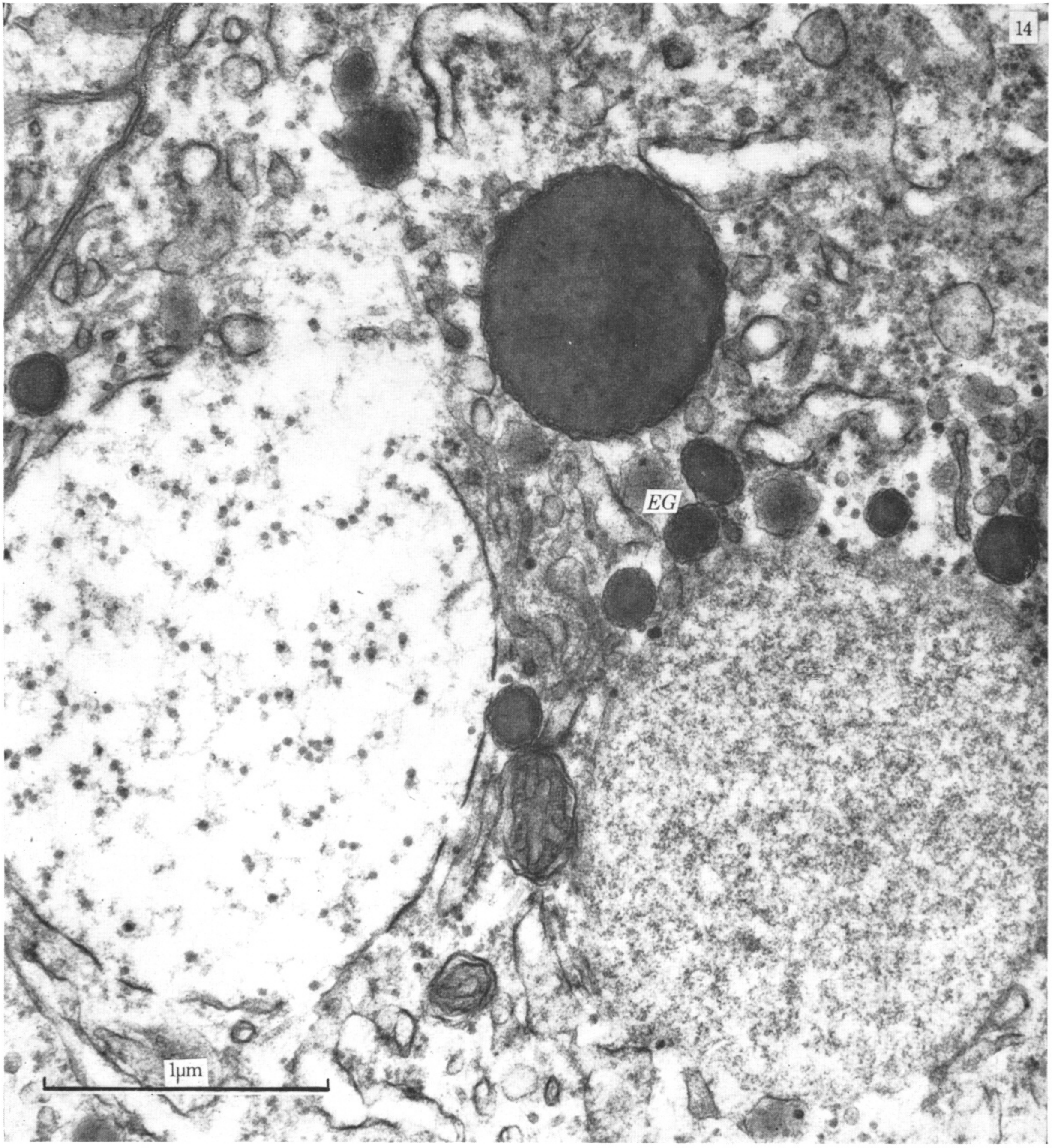


FIGURE 14. Perinuclear region of a neuron containing vesicular and granular cytoplasmic inclusions, including elementary granules (*EG*). (Stained lead acetate.)

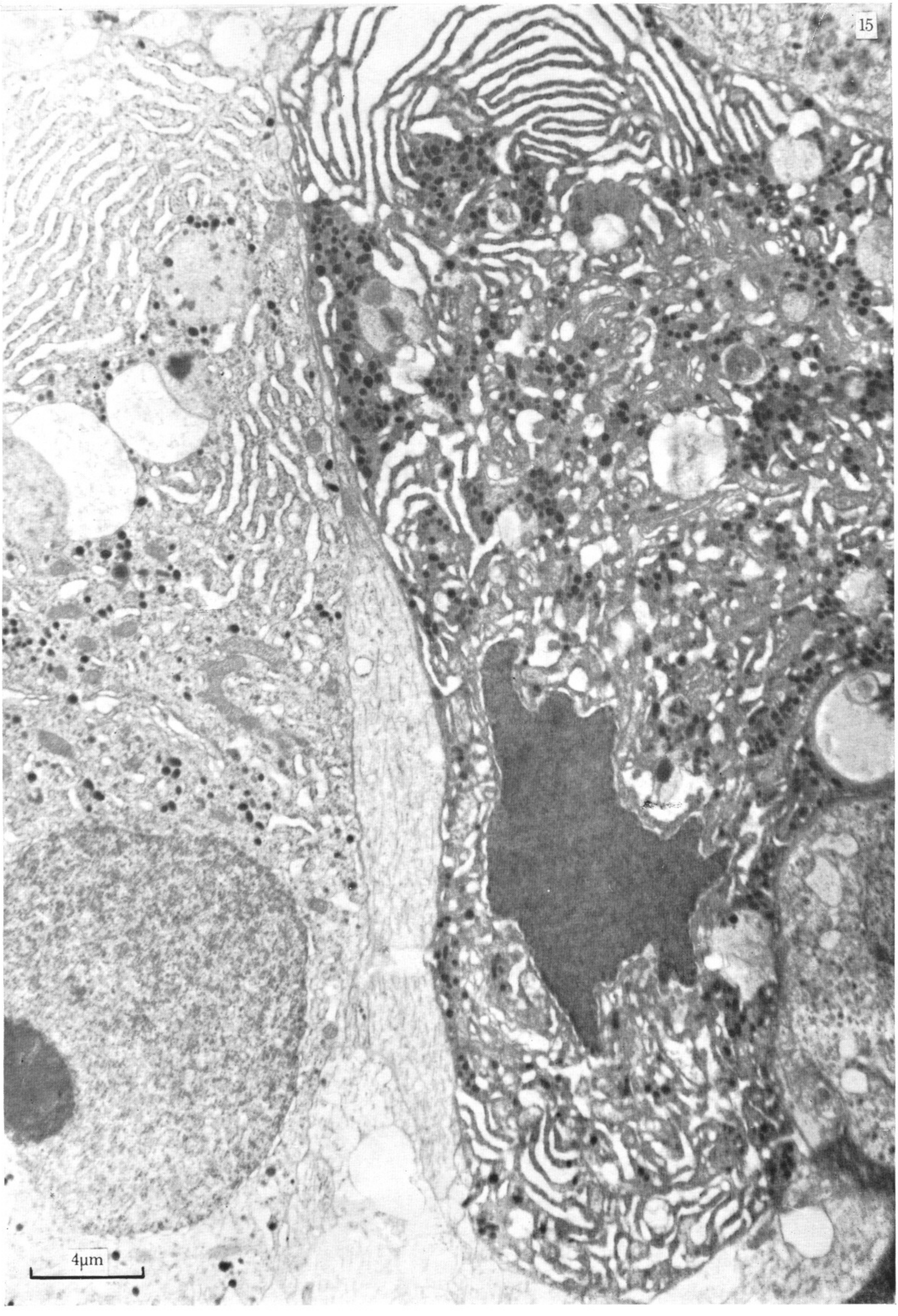
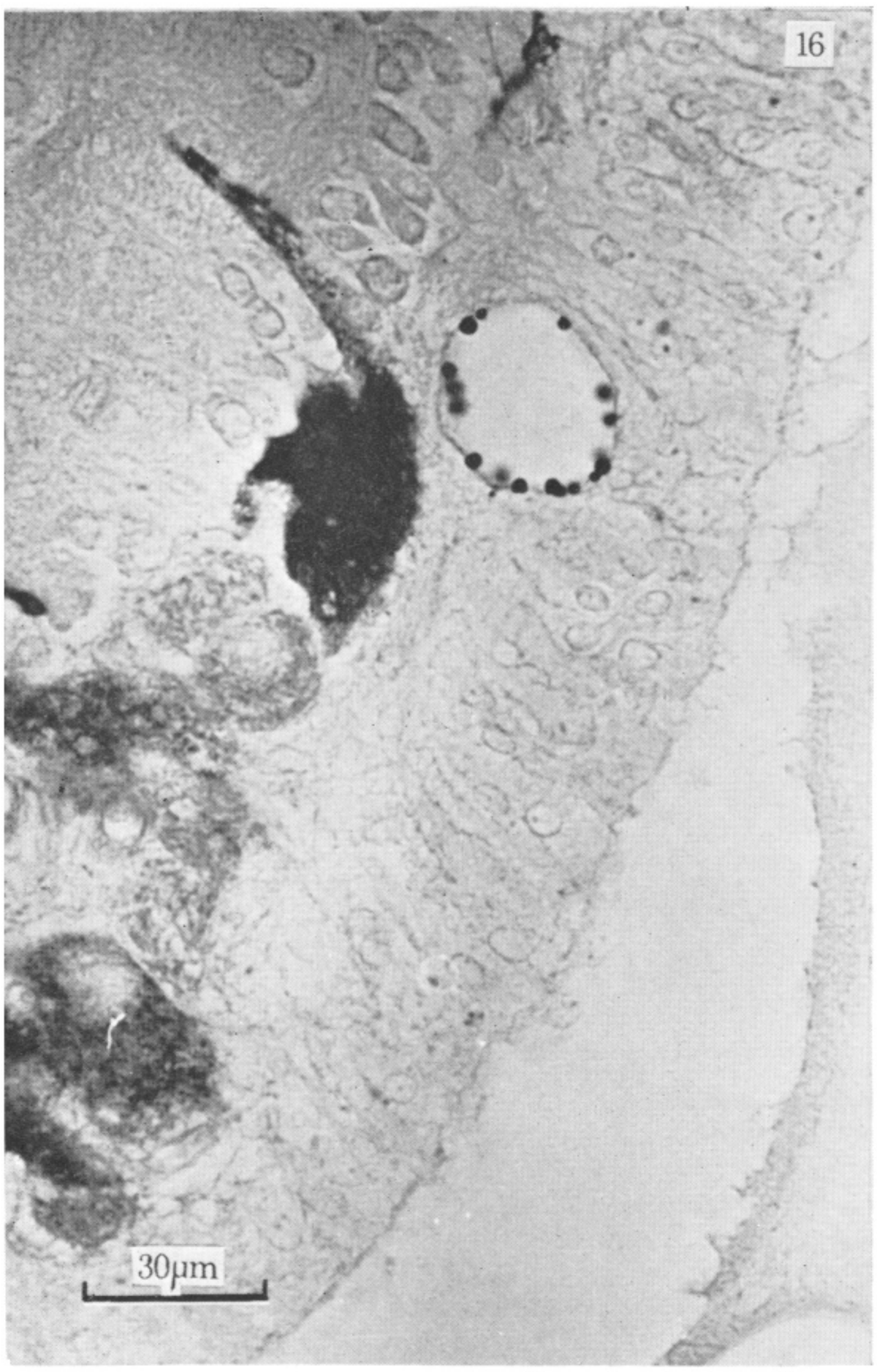
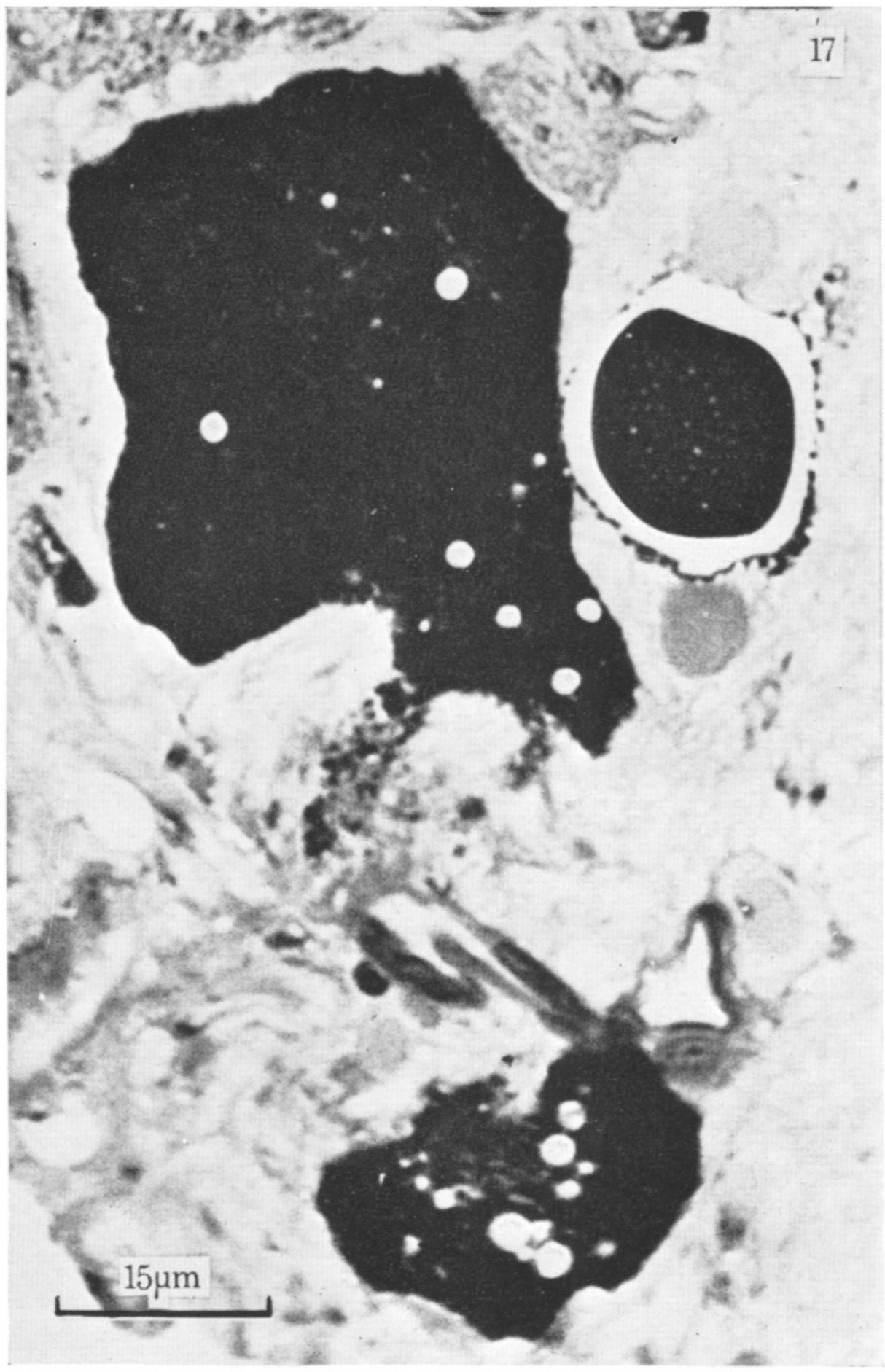
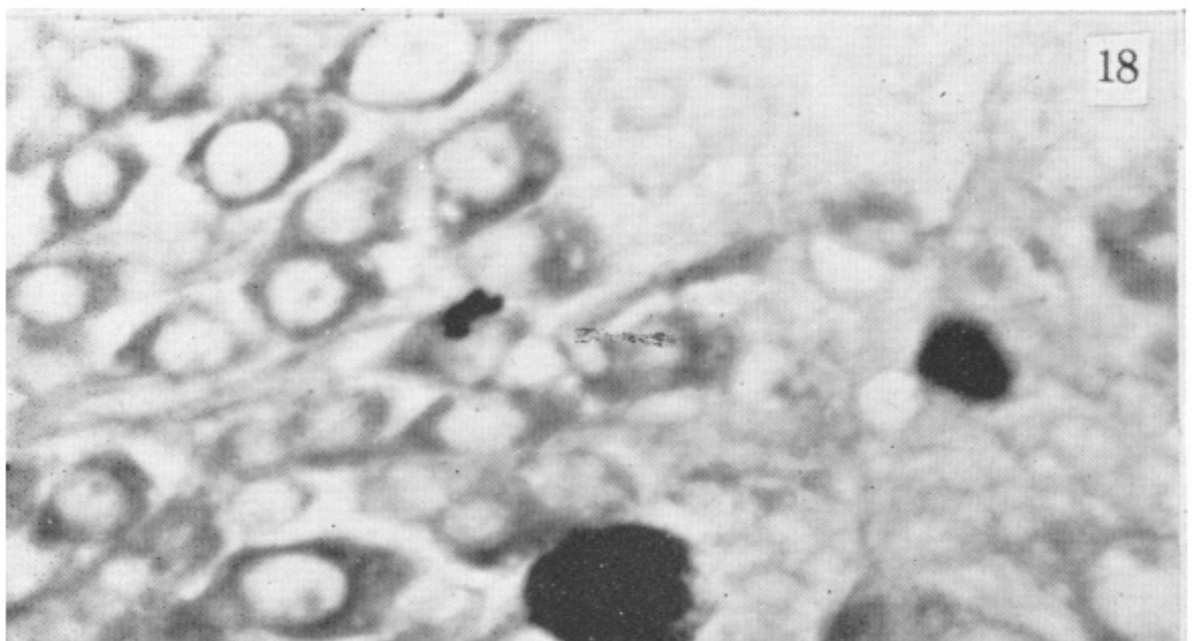


FIGURE 15. 'Dark' and 'light' neurons adjoining. Both contain elementary granules and secretory vesicles of different kinds. Inter-lamellar spaces of endoplasmic reticulum of 'dark' cells characteristically swollen and nucleus deeply invaginated. (Stained uranyl acetate.)







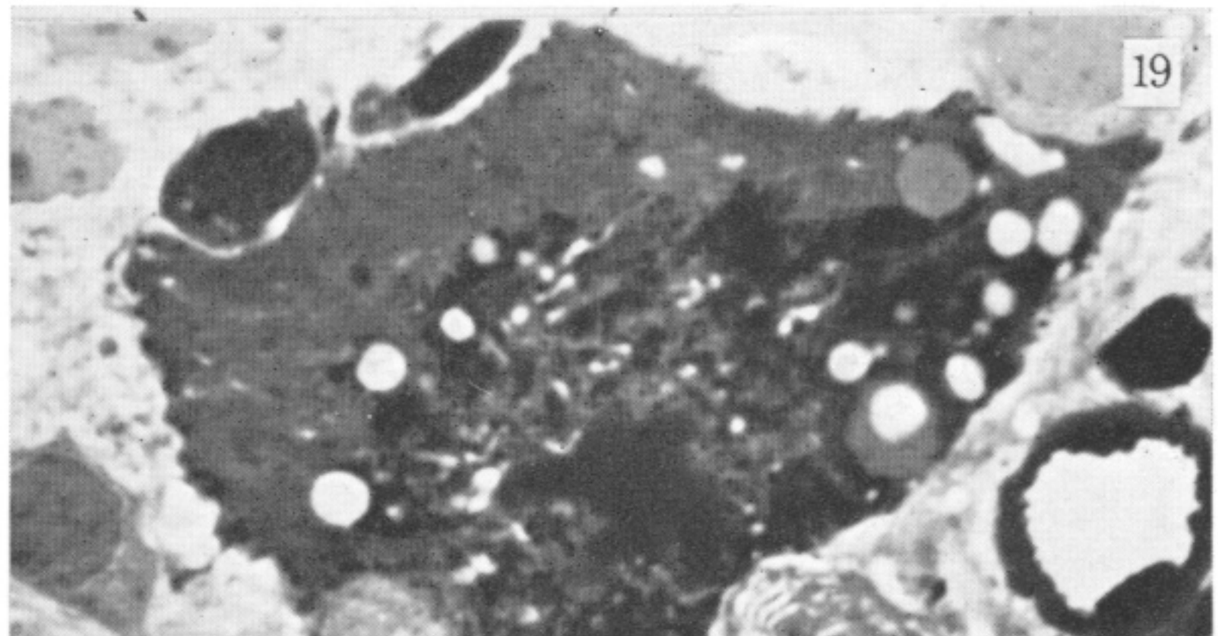


FIGURE 16. Colloid vesicle embedded in ependyma. Contents, apart from peripheral granules, chromophobic. Wax-embedded, stained AT–PAS–NY. (Compare with figure 21, plate 56.)

FIGURE 17. Aggregations of neurosecretion in the PON, containing chromophobic vesicles. Epon-embedded, cut at 1 μm, stained Azur II. (Compare with figure 20, plate 56.)

FIGURE 18. Densely staining neurosecretory neuron which may be a stage in the development of the aggregations shown in figure 17. Wax-embedded, stained AT–PAS–NY.

FIGURE 19. As figure 18, but Epon-embedded, cut at 1 μm and stained Azur II.

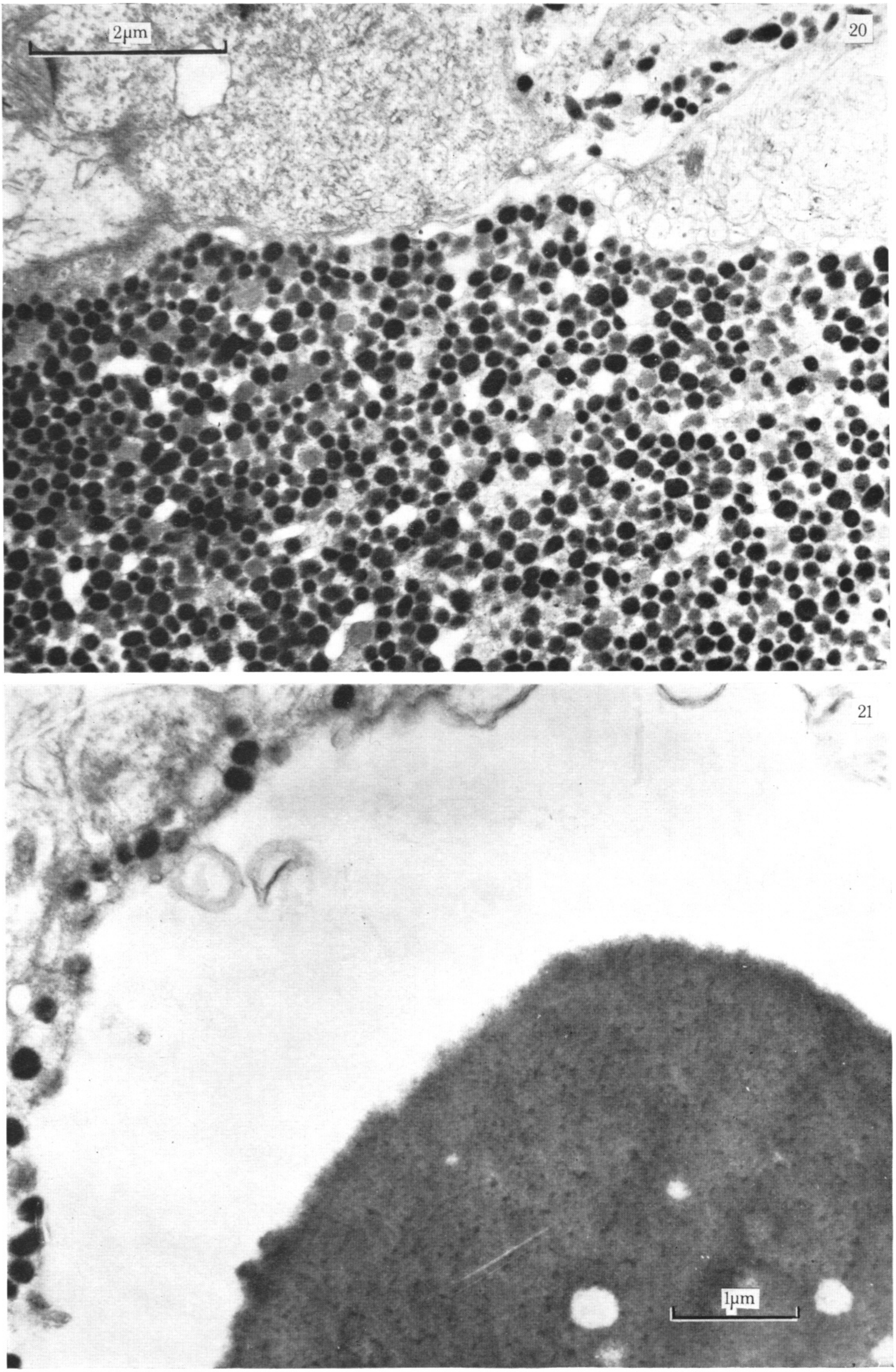
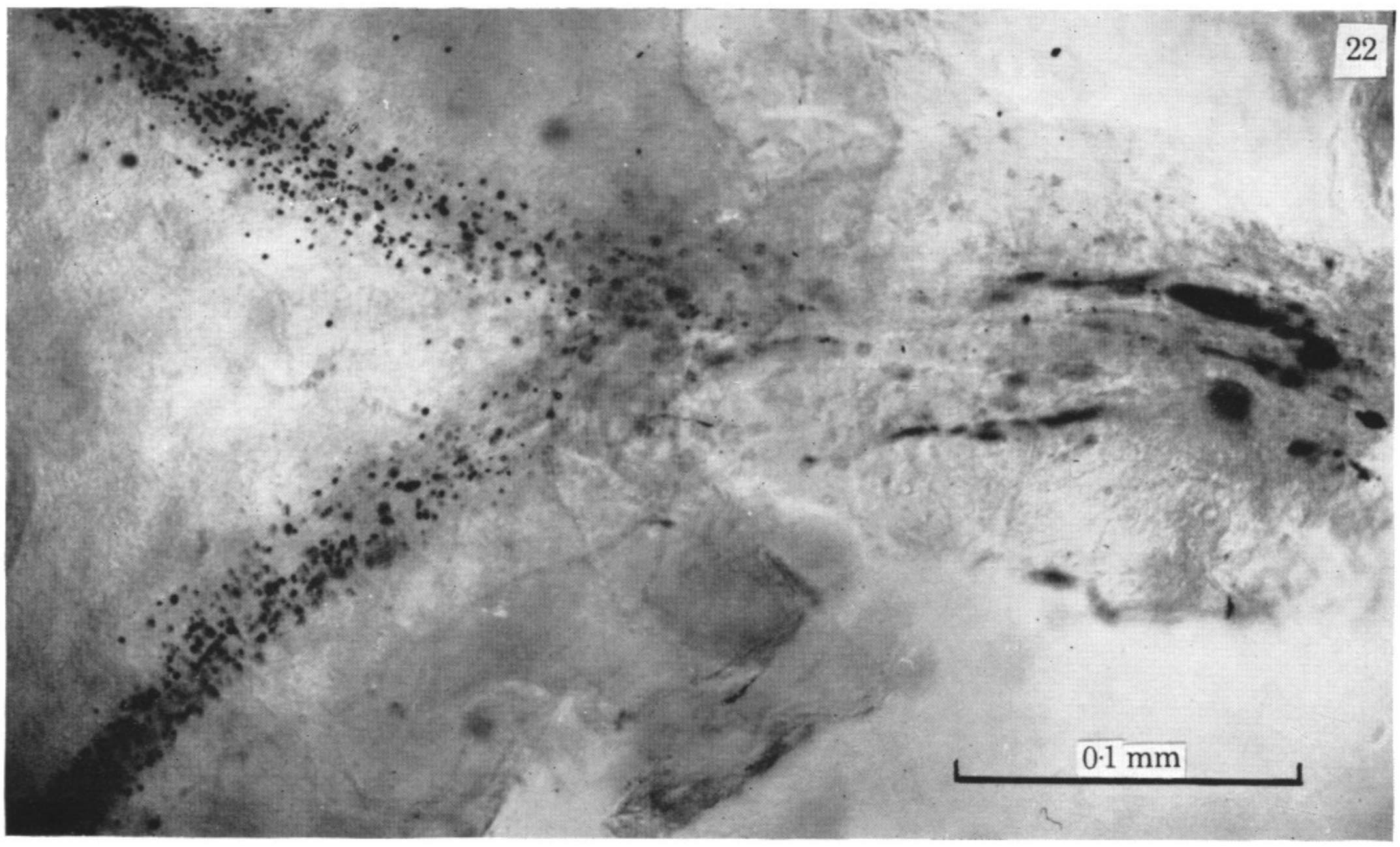


FIGURE 20. Electron micrograph of periphery of neurosecretory aggregation seen in figure 17, plate 55. Note surrounding nervous tissue. (Stained uranyl acetate.)

FIGURE 21. Electron micrograph of periphery of colloid vesicle similar to that shown in figure 16, plate 55 (Stained uranyl acetate.)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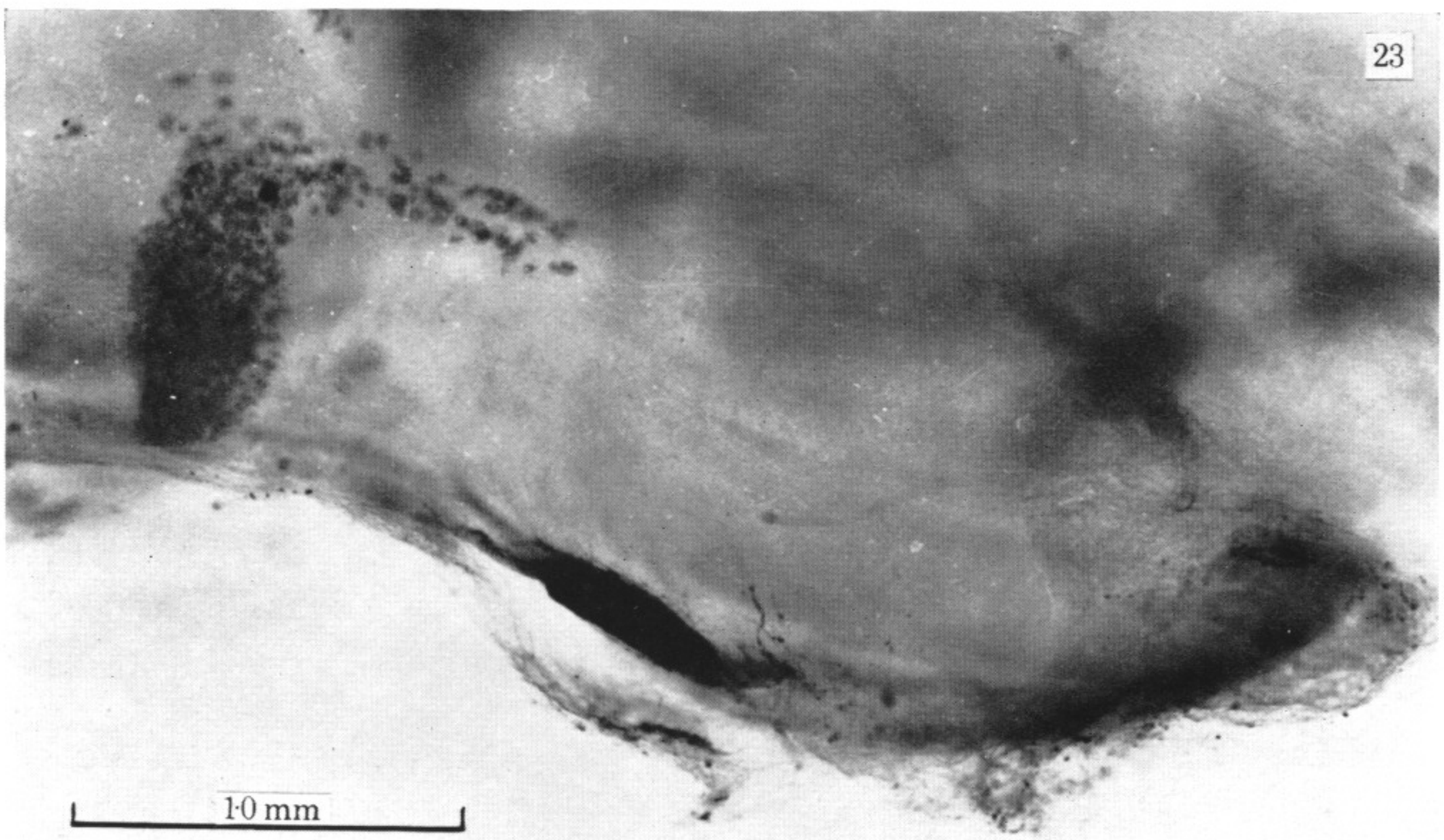
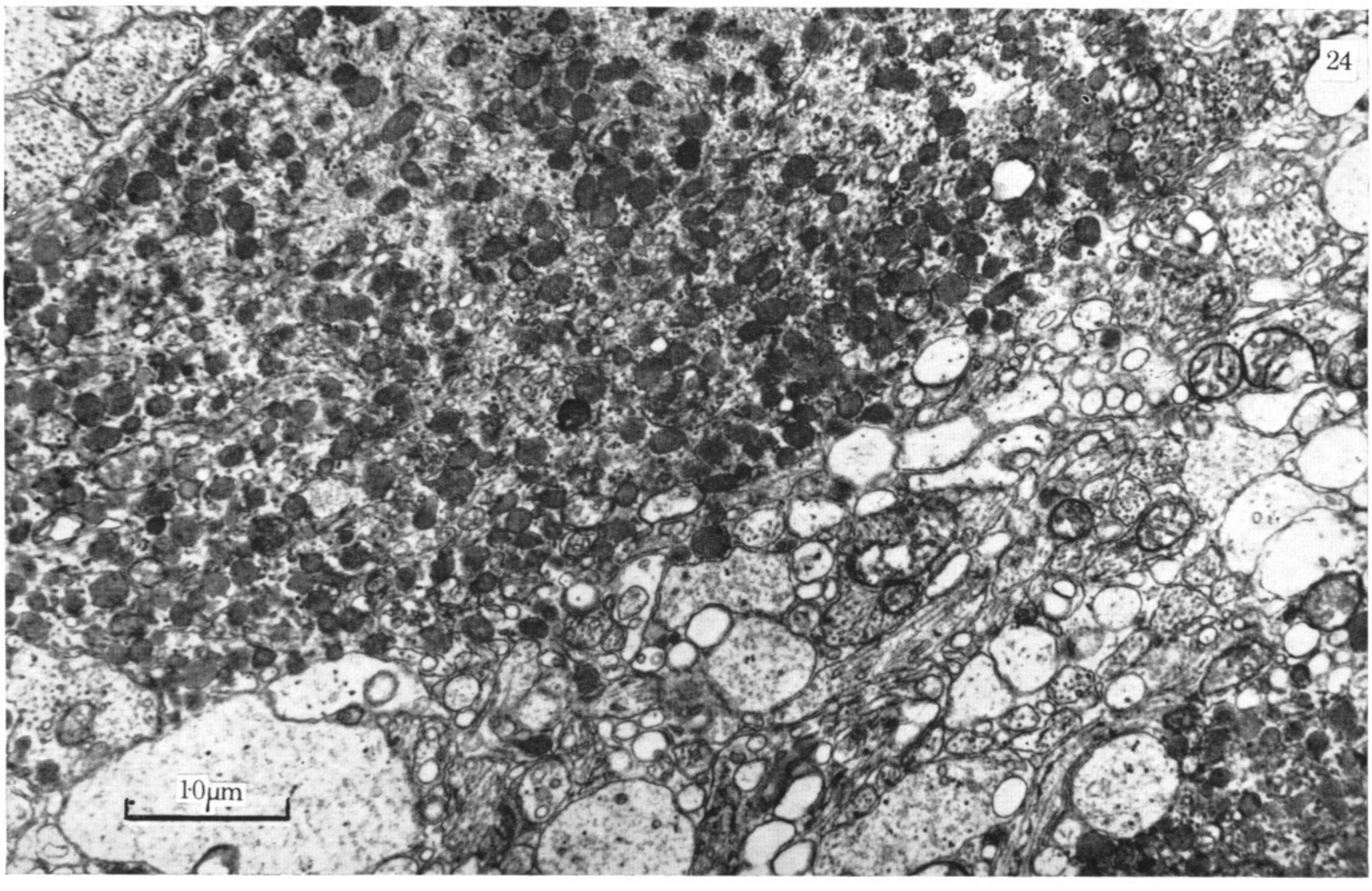

23
1·0 mm


24
1·0μm