NEUROSECRETORY INNERVATION OF THE PITUITARY OF THE EELS *ANGUILLA* AND *CONGER*

I. THE STRUCTURE AND ULTRASTRUCTURE OF THE NEURO-INTERMEDIATE LOBE UNDER NORMAL AND EXPERIMENTAL CONDITIONS

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Three kinds of neurosecretory fibre (Types A_1, A_2 and B) are present in the neural component of the neuro-intermediate lobe of the eel pituitary. These fibres do not in the main make any direct contact with the pars intermedia cells, but they are separated by only a narrow extravascular channel, into which both elements discharge their products.

Type A neurosecretory fibres do, however, make direct synaptic contact with pituicytes which resemble ependyma and surround finger-like extensions of the infundibular recess. That these contacts are functional is indicated by the fact that their frequency is related to changes in the environment. When eels are placed on an illuminated white background the synaptic junctions between Type A_2 neurosecretory fibres and pituicytes are very frequent. Similar synaptic junctions between A_1 fibres and pituicytes were only found in animals which had been recently transferred from fresh water to sea water.

A possibility that the pituicytes play some part in a feed-back from the pituitary to the hypothalamus is discussed.

1. Introduction

Within recent years the theory of neurosecretion has undergone some modification (Scharrer 1965; Knowles 1965*a*; Bern & Knowles 1966; Knowles & Bern 1966). Findings with the electron microscope have shown that the neurohaemal concept (Knowles & Carlisle 1956) is not an exclusive criterion for the assessment of neurosecretory activity. According to the neurohaemal concept neurosecretory neurons engage in hormonogenesis

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and discharge their products directly into the blood-stream. Recently, however, it has been shown that some neurosecretory axons directly innervate other endocrine tissues. This form of neurosecretory activity has been studied in some detail in the corpora allata of insects (Scharrer 1964) and the neuro-intermediate lobe of an elasmobranch pituitary (Knowles 1965*b*).

Experimental work on elasmobranchs has shown that colour change in these fishes is regulated by a neurosecretory control of melanophore-dispersing hormone (*MSH*) production and release (Dodd 1963; Mellinger 1963) and in *Scylliorhinus* neurosecretory fibres make apparent synaptic contracts with intrinsic endocrine cells of the neuro-intermediate lobe (Knowles 1965*b*). Such a correlation between structure and function at the level of ultrastructure has not yet been demonstrated in teleosts, though Bargmann & Knoop (1960) have shown that the neuro-intermediate lobes of many species are penetrated by neurosecretory fibres.

The neurosecretory innervation of the pituitary of the eel *Anguilla anguilla* L. has been studied with the light microscope (Bargmann 1953; Stutinsky 1953), and there has also been an attempt to correlate the amount of neurosecretory material with experimental conditions. Alterations in salinity of the water were followed by detectable changes in the Gomori-positive material in the neural component of the neuro-intermediate lobe and in the nucleus preopticus (Schiebler & Hartmann 1963).

There are indications that the colour-change of the eel is under a predominantly hormonal control (Waring 1963), but as yet no study has been made of the neurosecretory innervation of the pituitary in relation to colour change in the eel.

A close morphological association between neurosecretory fibres and pituicytes has been reported in *Scylliorhinus* (Knowles 1965*b*), and other fishes (Bargmann & Knoop 1960; Follenius & Porte 1962), but as yet no indications of a functional relationship have been proved.

There are therefore many indications that neurosecretory fibres may make direct contacts with cells in the pituitaries of fishes, though our knowledge of the functional significance of these contacts in teleosts is slight. The present studies on *Anguilla anguilla* L. and *Conger conger* have been designed to further our understanding of the structural and functional relationships between the cellular elements of the pituitary of the eel and its neurosecretory fibres. This paper relates to this problem in the neuro-intermediate lobe. The innervation of the pars distalis in *Anguilla* and *Conger* will form the subject of the following paper, Part II.

2. Materials and methods

A hundred eels (*Anguilla anguilla* L.) were used for the study of the pituitary and were examined by various histological methods and by electron microscopy.

For comparison four conger eels (measuring 41 to 60 cm), captured in the Bay of Naples in April 1965, were studied, two by the alcian blue–*PAS*–orange G method after fixation in Helly's fluid, and two by electron microscopy.

For a preliminary study of the structure and ultrastructure of the pituitary of *Anguilla* thirty-four eels were obtained from the Birmingham fish market during October and November 1964, imported from Ireland, Denmark and Holland. These eels were golden

in colour and were between 35 and 64 cm in length. Nineteen eel pituitaries, either with or without the brain, were fixed in Bouin's fluid, 10 % formalin or Zenker, sectioned at 5 to 7 μm frontally, horizontally or sagittally, and stained by the following methods, haematoxylin and eosin, azan, Van Gieson, Herlant's tetrachrome stain (Herlant 1960), Billenstien (Billenstien 1963), lead haematoxylin (MacConaill 1947), alcian blue–*PAS*–orange G (Herlant 1960), aldehyde fuchsin, the Bargmann modification of Gomori's method (Pearse 1960), Holmes's axon stain (Holmes 1942) and the Armstrong–Richardson–Young (1956) method for bouton mitochondria. Six brains and pituitaries were stained by the Braak technique (Braak 1962). Nine pituitaries were examined with the electron microscope.

All the subsequent studies were carried out on freshly caught eels examined either soon after capture or after some weeks in complete darkness. Eight silver eels from 46 to 64 cm were obtained from the River Avon at Downtown, Wiltshire, caught on their way to the sea during December 1964. The remainder were caught in the River Severn, near Tewkesbury from January 1965 onwards.

The colour-change experiments were carried out on thirty eels 35 to 64 cm in length. Twenty-six of these were transferred from darkness to black or white tanks 40 cm in depth, for 4 h, and were illuminated from above by a 60 W bulb at a distance of 30 cm from the surface of the water; they were kept in continuously running fresh water, the temperature of which (9 °C) did not vary by more than 2 deg C during the experiments. In order to avoid possible diurnal fluctuations the experiments were carried out between 10 a.m. and 2 p.m. G.M.T.

For comparison with the background experiments two eels were taken directly from some weeks in darkness; two others after 5 days and nights on a white illuminated background.

The melanophore index (see Waring 1963) of each animal was recorded after the colour-change experiments.

In the colour-change experiments fourteen brains and pituitaries were examined by the alcian blue–*PAS*–orange G method, ten by the Braak technique and six by electron microscopy.

In combined sea-water and colour-change experiments twenty eels measuring from 32 to 80 cm were taken from darkness and placed in black and white illuminated tanks, nine in a black tank and eleven in a white tank. Instead of running fresh water however aerated sea water was used. In two series of experiments the animals were transferred directly from fresh water to pure sea water for 4 h and 15 min respectively. In another experiment the animals were placed successively for periods of 1 h each in 25, 50, 75 % and pure sea water.

The brains and pituitaries of seven eels used in these experiments were examined after staining by alcian blue–*PAS*–orange G; seven were used for the Braak technique; six were examined with the electron microscope.

All the specimens for electron microscopy were fixed in osmium tetroxide, buffered at 7·6 pH, for 3 to 4 h. After washing and dehydration, during which the specimens were stained with phosphotungstic acid (see Knowles 1964), the tissues were embedded in Vestopal and cut on a Porter–Blum microtome, using glass knives. Sections were mounted on carbon-coated grids and examined with a Siemens Elmiskop I and a Zeiss EM9.

Some additional contrast was obtained in some cases by staining the sections on grids with uranyl acetate.

In the following studies of the eel pituitary the measurements of the secretory granules were made as follows: (*a*) those granules with a sharply defined surrounding membrane were chosen, as being more likely to represent median than tangential sections; (*b*) the largest granules were selected as being more likely to represent the final, rather than intermediate, stages of granule formation.

3. General morphology of the neuro-intermediate lobe and its innervation

Three distinct lobes can be distinguished in the pituitary of the eel (figures 6 and 9, plate 49). In the young eel, or elver, the pars nervosa and pars intermedia form two distinct lobes (Knowles & Vollrath 1966): in the adult eel, however, the neurosecretory nerve tracts appear to penetrate deeply the intrinsic endocrine tissue of the pars intermedia and thereby constitute a neuro-intermediate lobe (figure 1 *a* and figure 9, plate 49). On closer examination, however, in *Anguilla* a narrow *PAS*-positive zone may be seen to separate the neural and intermediate components in both elvers and adults, except at a few points only.

The intrinsic endocrine cells of the neuro-intermediate lobe lie grouped round the neurosecretory tracts, with their long axes towards the tracts. They are separated from the neurosecretory tracts by the *PAS*-positive zone into which they apparently discharge their products (figures 2 and 3, and figures 13, plate 51, and 17, plate 53).

The blood supply of the neuro-intermediate lobe was not studied in detail in the present survey, though it was noted that relatively wide capillaries extended in a dorso-ventral direction at regular intervals (figure 3); these capillaries apparently lay in the *PAS*-positive zone.

Under the electron microscope the *PAS*-positive zone separating the intrinsic cells from the neurosecretory fibres is approximately 2000 to 4000 Å in width (figure 2, and figure 19, plate 54). This comprises a central space, containing collagen fibres and fibroblasts, bounded by walls 300 to 400 Å thick. These walls are especially electron-dense on the side bordering the connective tissue. Sometimes this space contains also an amorphous ground-substance (figures 17, plate 53, and 18, plate 54), but sometimes this is not apparent. It has been pointed out that ground-substances of this kind may alter in comparison, sometimes permitting a free flow of substances (Wassermann 1965). The term basement membrane has been used to denote this boundary between the neural and intrinsic endocrine components of the pituitary in teleosts (Follenius & Porte 1962). This, however, would not seem to be an accurate description of a structure which in the eel consists essentially of two membranes bordering a space supported by collagen fibres. It is suggested that this space might more properly be called an intervascular channel, since capillaries lie in out-pocketings of it, at intervals, and that its regions surrounding capillaries be termed perivascular spaces. The basement membrane and endothelial lining of the capillaries appeared in the main to be continuous and few pores between the capillaries and perivascular spaces were seen.

It may be seen therefore that a continuous system of extravascular channels lies between

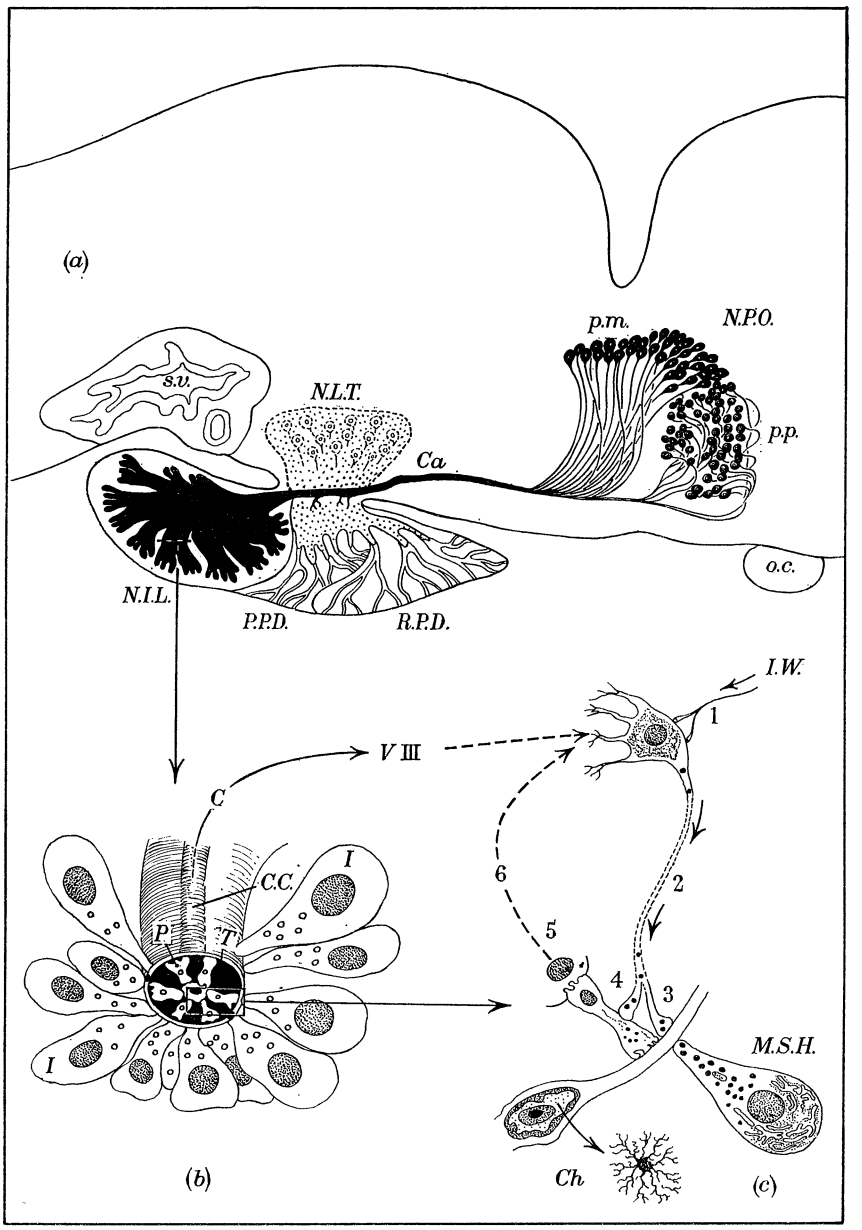


FIGURE 1. The neurosecretory innervation of the pituitary of *Anguilla anguilla* L.

(*a*). The origin and distribution of neurosecretory fibre tracts. The nucleus preopticus-hypophysial tract was drawn from specimens prepared by the Braak technique. *N.I.L.*, Neuro-intermediate lobe; *N.L.T.*, nucleus lateralis tuberis; *N.P.O.*, nucleus preopticus (*p.m.*, pars magnocellularis; *p.p.*, pars parvocellularis). *O.C.*, optic chiasma; *P.P.D.*, proximal pars distalis; *R.P.D.*, rostral pars distalis; *S.V.*, saccus vasculosus; *Ca*, critical area of Leatherland, Budtz & Dodd (1966).

(*b*). A semi-diagrammatic view of a single neurosecretory fibre tract and surrounding intermedia cells (see also figure 3). *C*, Arrow denoting continuity between central canal of tract and third ventricle of brain. *C.C.*, Central canal; *I*, pars intermedia cells; *P*, pituicyte; *T*, neurosecretory fibre tract; *V* III, third ventricle of brain.

(*c*). A diagram to illustrate the hypothesis that pituicytes of an ependymal nature participate in a feed-back mechanism from the distal to the proximal parts of the preoptico-hypophysial neurosecretory system (for details see text). According to this theory when the eel is placed on an illuminated white background (*I.W.*) a stimulus to the nucleus preopticus (1) is transmitted along an axon (2) and causes changes denoting release at an intervascular channel (3) and at the surface of a pituicyte (4). It is postulated that the material released into the intervascular channel affects M.S.H. secretion and thus colour-change, and that simultaneously the pituicyte releases a substance (5), which travels in the cerebrospinal fluid (6) to dendrites of the perikarya of the nucleus preopticus, which project into the third ventricle. *Ch*, Chromatophore, beginning to contract.

the neural and intermediate components of the neurointermediate lobe and may act as a link between these two components as well as a communication with these elements and the blood system (figure 3).

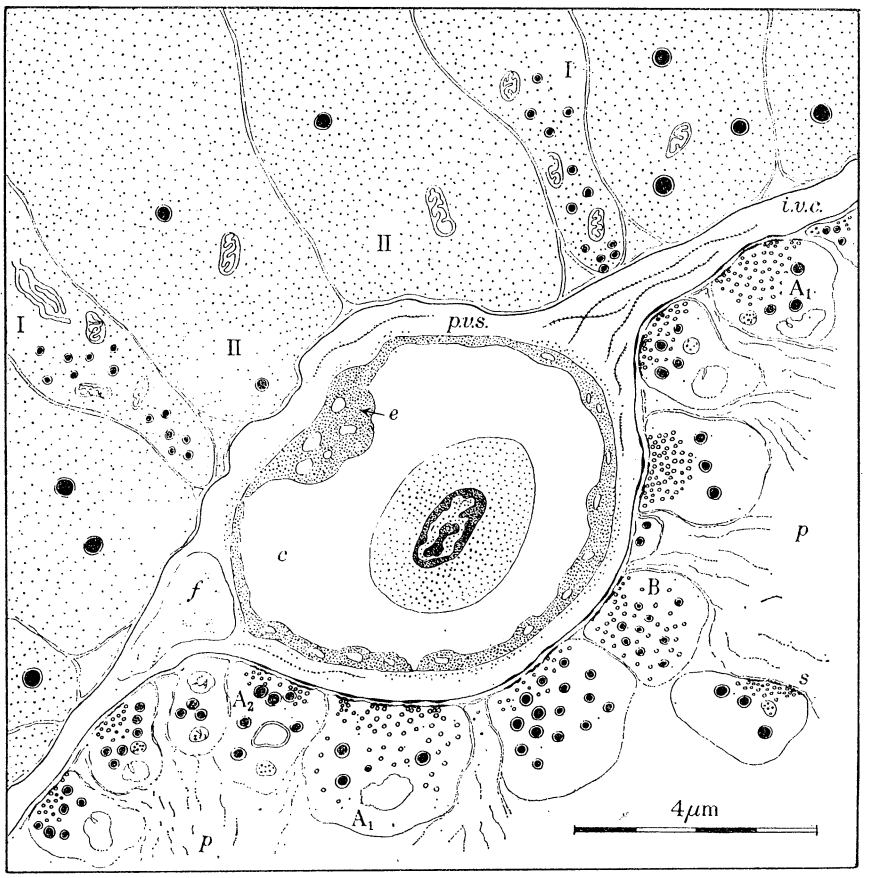


FIGURE 2. The relationship between neurosecretory fibre terminals, intrinsic endocrine cells and the blood stream in the neuro-intermediate lobe of the eel pituitary (see also figure 3). I, II, The two intrinsic cell types. A_1, A_2, two varieties of Type A fibre; B, Type B fibre; *c*, capillary lumen; *e*, endothelium; *f*, fibroblast; *i.v.c.*, intervascular channel; *p*, pituicyte; *p.v.s.*, perivascular space; *s*, synapse.

The greater part of the neurosecretory tracts was Gomori-positive and alcian blue-positive (figure 9, plate 49), but some cells which were not stained by either technique could be seen in the tracts. Some of these cells surround a central canal which appears to be continuous with the ventricles of the brain; others lie singly or in irregular groups; these cells are termed pituicytes (see also Evans 1940; Bargmann 1953).

By means of the Braak technique it is possible to demonstrate *in situ* the hypothalamo-hypophysial system of the eel (figure 8, plate 49). A number of different tracts, evidently originating in different parts of the preoptic nuclei, merge to form a single tract, which terminates in a pars nervosa. In the present studies at least three distinct tracts were observed (figure 1). Some others also have been indicated by Leatherland, Budtz & Dodd (1966).

4. Intrinsic endocrine cells

(*a*) *Anguilla anguilla* L.

Some of the intrinsic cells are spherical or oval and lie close to the intervascular channel; others are elongate or pear-shaped. Sometimes the portion containing the nucleus and endoplasmic reticulum may lie some distance from the intervascular channel, yet be connected to it by a prolongation of the cell. Some of the intrinsic cells, especially those in the peripheral region of the gland, stain by the *PAS* method (figure 26, plate 57). Most of the cells, however, are not *PAS*-positive, but stain with lead haematoxylin. In some cases only the prolongations of the cells are stained by these stains, and the pole farthest from the intervascular channels remains unstained.

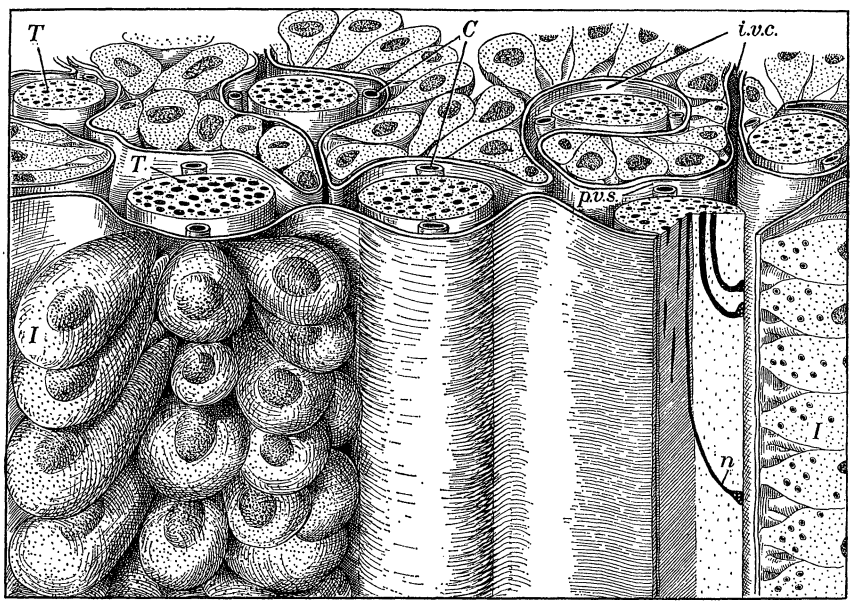


Figure 3. A diagrammatic representation of a small portion of the neuro-intermediate lobe, showing the relationship between the neurosecretory fibre tracts (*T*), the intervascular channels (*i.v.c.*) and the perivascular spaces (*p.v.s.*). A small portion on the right has been cut away to show the relationship between the terminals of the neurosecretory fibres (*n*) and the intrinsic cells (*I*), which are separated by an intervascular channel. *C*, *Capillary*.

The cytoplasm of the lead haematoxylin-positive cells contains granules which stain, but no granulation can be observed in the *PAS*-positive cells under the light microscope. The intensity of the staining varies. There are, moreover, some cells which lie distinct from those radiating from the tracts which did not appear to stain; it is not possible to be sure whether these cells represent a distinct cell type or whether they are the types previously mentioned, cut through the unstained pole of the cell. It is not possible also to distinguish with certainty whether a *PAS*-positive cell may not contain some lead haematoxylin-positive granules, and therefore be stained by both methods.

The *PAS*-positive staining is saliva-resistant, indicating substances other than glycogen. A small *PAS*-positive inclusion was frequently noticed, close to the nucleus, in the intrinsic cells. It is not possible to decide whether the *PAS*-positive and lead haematoxylin-positive cells represent two distinct cell types, or one cell type at different secretory stages.

Under the electron microscope, however, at least two types of cell can be clearly distinguished (figure 4). Type I generally lies close to the intervascular space, and is oval in shape, measuring approximately 5·5 μm by 7 to 10 μm; the nucleus is relatively large and the nucleolus is prominent. The amount of endoplasmic reticulumis small and diffusely scattered in the cell. The membrane-bound electron-dense granules in the cytoplasm vary in size but did not exceed a diameter of 2000 Å and are mainly 1800 Å or less.

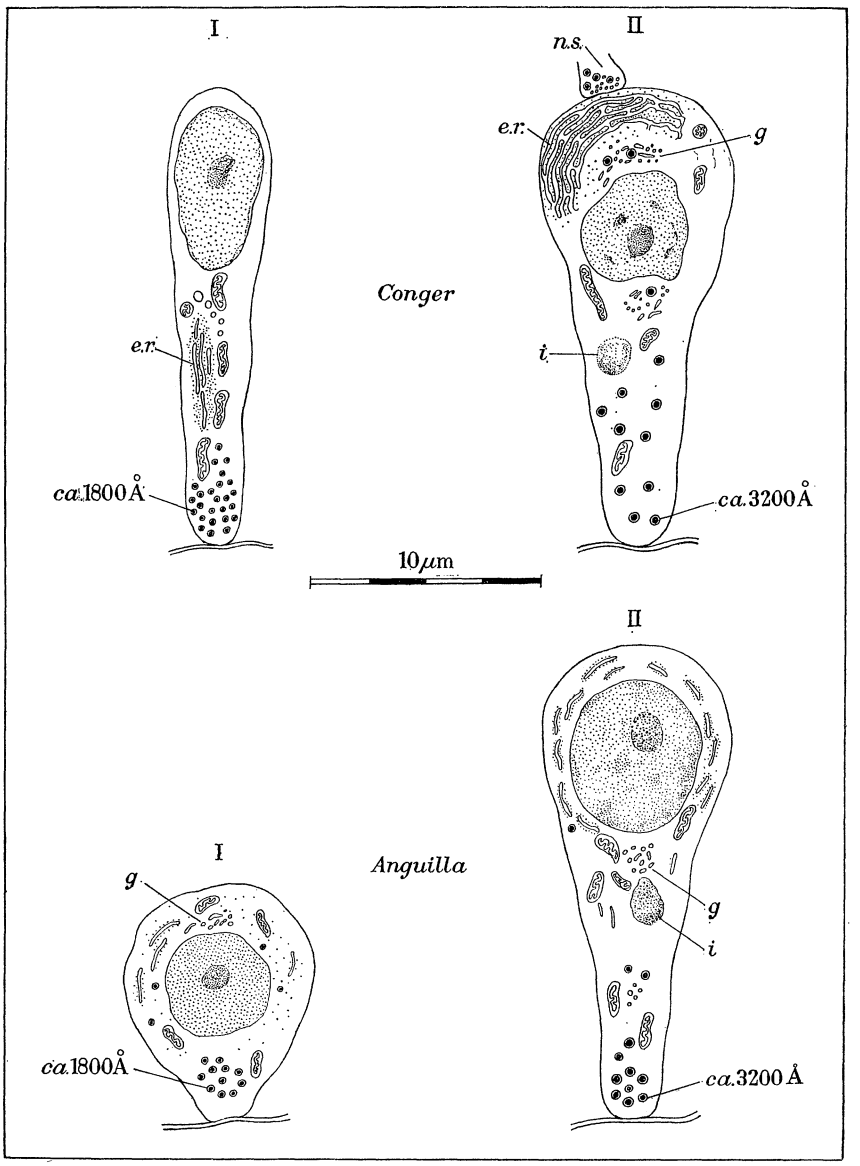


FIGURE 4. A diagram to show the differences between the two cell types present in the neurointermediate lobes of the pituitaries of *Conger* and *Anguilla*. *e.r.*, Endoplasmic reticulum; *g*, Golgi zone; *i*, inclusions; *ns*, neurosecretory fibre terminal.

Type II cells are larger and more elongate than Type I. They measure approximately 7 μm in width near the nucleus; their length is as much as 30 μm. An extensive endoplasmic reticulum is present in the region furthest from the intervascular space (figure 16, plate 53). Electron dense membrane-bound granules, measuring 2700 to 3600 Å are often

abundant in Type II cells. A much larger inclusion, measuring approximately 1 μm, is often present; this inclusion is approximately spherical and membrane-bound; often it contains electron-dense material. Cells resembling Type II cells, but with few electron-dense granules, were often seen in both *Anguilla* and *Conger* (figure 12, plate 51). In these cells the Golgi apparatus is very prominent, and within it signs of vesicle formation are evident. Many mitochondria lie near the Golgi apparatus, and the endoplasmic reticulum is abundant and regular in arrangement. The features would be consistent with the view that synthetic activity is great in this type of cell. It is suggested that this cell type and the Type II cell already described may represent the same cell type at different stages of synthesis and storage.

In addition a third type of cell which did not resemble either Type I or Type II was occasionally seen. This cell closely resembled one of the three cell types described by Ziegler (1963) in the pars intermedia of the rat pituitary and Holmes & Kiernan (1964) in the hedgehog; its nucleus was small and its cytoplasm dense.

(*b*) *Conger conger*

Like *Anguilla*, the neuro-intermediate lobe of the conger pituitary contains both *PAS*-positive and lead haematoxylin-positive cells, and under the electron microscope two types of cell can be distinguished. Type I measures about 3·5 μm in width and has a variable length, sometimes 20 μm or greater (figure 12, plate 51).

Type I cells contain electron-dense vesicles of many sizes. Some are irregular in form and only slightly electron-dense. Those which are spherical, uniformly electron-dense and bounded by a clearly defined outer membrane are approximately 1800 Å in diameter.

The endoplasmic reticulum of the Type I cell in *Conger* runs parallel to the long axis of the cell and mostly lies in the region between the nucleus and the intervascular space (see figure 12, plate 51); ribosomes are present. Many elongate mitochondria lie close to the endoplasmic reticulum.

The Golgi apparatus can be seen but is not always prominent. It appears to lie in the region further from the intervascular space.

Many of the features of Type II cells of the conger's pituitary resemble those found in *Anguilla*. They contain membrane-bound electron-dense vesicles measuring *ca.* 3,200 in diameter. An endoplasmic reticulum of regular form (plate 52) almost fills that pole of the cell furthest from the intervascular space. The Golgi apparatus is prominent.

5. Ultrastructure of the nerve tracts in the neuro-intermediate lobe

A section through a finger-like projection of the pars nervosa shows that neurosecretory fibres of three types are present (figure 2 and figure 18, plate 54). Two of these fibre types contain spherical membrane-bound vesicles with electron-dense contents which entirely fill the bounding membrane. Fibres with vesicles of this type are termed Type A fibres (see Knowles 1960, 1962, 1965*a*, *b*), and the vesicles Type A vesicles.

Measurement of the Type A vesicles shows that two size classes can be recognized. Granules measuring 1600 to 1800 Å are found in some fibres, here termed A_1 fibres. Granules measuring 1200 Å characterize another fibre type, here termed Type A_2.

In contrast to these fibres, which are abundant (plate 54), a few scattered fibres contain comparatively small vesicles of irregular form. The electron-dense contents of these vesicles measures approximately 600 to 800 Å in diameter and does not fill the bounding membrane; their electron density is less than that of Type A vesicles. The fibres containing them are here referred to as Type B fibres (see Knowles 1960, 1965*a*). The Type A and Type B fibres of *Anguilla* and *Conger* are similar. The general characteristics of the neurosecretory fibres (i.e. neurofibrillae and mitochondria) are typical of those of fishes (cf. Bargmann & Knoop 1960; Knowles 1965*b*; Follenius & Porte 1962; Lederis 1964); a few were myelinated, but most were not. Some multilamellate bodies (Knowles 1962; Holmes 1964; Lederis 1964) were present in *Anguilla*.

At the surface of the tract an abundance of neurosecretory fibre terminals of all three fibres types apparently discharge into the intervascular and perivascular space and capillaries (figures 2 and 3, and figure 11, plate 50, and figure 19, plate 54). These fibre terminals have the following characteristics: (*a*) many small electron-lucent vesicles approximately 500 Å in diameter; (*b*) an apparent breakdown of the elementary neurosecretory vesicles, accompanied by a loss of electron density; (*c*) in some cases electron-dense bars along the membrane bordering the perivascular space or capillary.

Pituicytes lie either singly (figure 18, plate 54), or in groups surrounding central canals (figure 25, plate 57, and figure 27, plate 58. The latter have many characteristics of ependymal cells, among others desmosomes, finger-like projections, and cilia (figure 25, plate 57 and figure 27, plate 58); these cells extend from the central canal to the surface of the tract, and lie between the neurosecretory fibres (plate 54). The cytoplasm of the pituicytes is either vacuolated and electron-dense or contains considerable numbers of fibrillae (figure 20, plate 55) and is relatively electron-lucent. Occasionally an endoplasmic reticulum and Golgi apparatus are present as well as membrane-bound inclusions, measuring up to 4000 Å in diameter (figure 25, plate 57). Characteristically the nucleus is deeply indented.

The central canal is sometimes filled by colloid which can be stained by *PAS* and is slightly electron-dense after osmium fixation.

6. Relation between neurosecretory fibres and intrinsic cells

(*a*) *Endocrine cells*

Most of the neurosecretory fibres in the neuro-intermediate lobe of *Anguilla* are not in direct contact with the intrinsic endocrine cells. Their terminals border the intervascular space which separates the neural and intermediate components of the lobe (figure 2). At some points the intervascular space is not present and here a few neurosecretory fibres of Type A_2 are found between the intrinsic endocrine cells. No fibre terminals in contact with the endocrine cells were seen in *Anguilla*.

In contrast in *Conger* only small areas of the intervascular space separate the neural and intermediate components and there is a considerable intermingling of neurosecretory fibres and intrinsic endocrine cells (figure 10, plate 50, and figure 14, plate 52). There are, moreover, indications that at least some fibres make secretomotor junctions with the endocrine cells, characterized by clusters of vesicles of synaptic vesicle size-range.

(*b*) *Pituicytes*

In both *Anguilla* and *Conger* secretomotor junctions between neurosecretory fibres (Type A_1 and A_2) and pituicytes (plates 55 and 56) are seen. A preliminary account of these in *Anguilla* has been given (Knowles & Vollrath 1965*a*). These junctions have the following characteristics of synapses: (1) At these points the neurosecretory fibres contain large numbers of electron-lucent vesicles measuring approximately 500 Å in diameter; these vesicles are either clustered in the centre of the fibre or along that margin of the fibre in juxtaposition to the pituicyte (figure 21, plate 55, and figure 22, plate 56). In the latter case they are surrounded by an area of electron density. (2) The fibre membrane

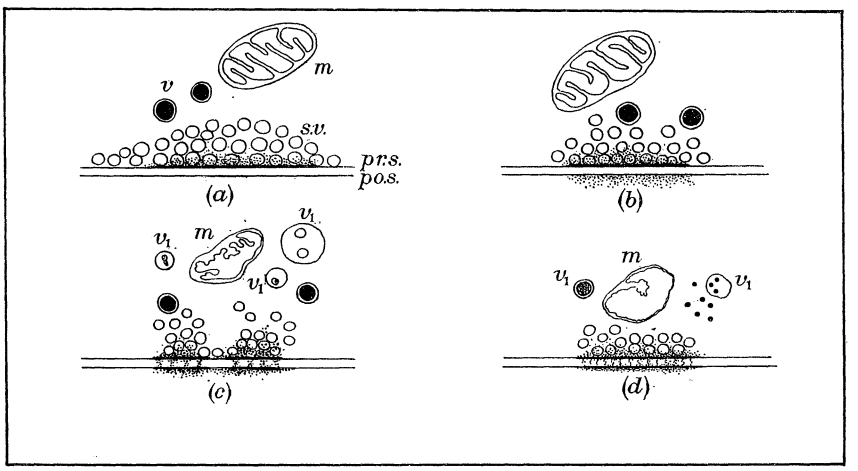


FIGURE 5. Diagrams of different forms of synaptic junctions between neurosecretory fibre terminals and pituicytes observed in animals under experimental conditions (see also plates 55 and 56). *m*, Mitochondrion; *po.s.*, post-synaptic membrane; *pr.s.*, pre-synaptic membrane; *s.v.*, 'synaptic' vesicles; *v*, neurosecretory vesicle; V_1, neurosecretory vesicles in the process of fragmentation.

often appears thickened and electron-dense; in some instances this electron-dense thickening extends to form a bar approximately 2300 Å in length. Sometimes, but not invariably, the membrane of the pituicyte shows corresponding electron-dense thickening opposite that of the neurosecretory fibre (see Knowles & Vollrath, 1965*a*). (3) Fine electron-dense strands have also been observed between the two membranes. The features listed above are not always present in each synapse (see figure 5). The possible functional significance of this will be discussed later.

There are no evident signs of a widening of the intercellular space between the membrane of the cells at these secretomotor junctions.

The elementary neurosecretory vesicles near these junctions are often less electron-dense than those found elsewhere (figure 20, plate 55, and figure 24, plate 56) and resemble those seen in the terminals of the fibres, bordering the extravascular spaces. Within these vesicles an internal structure can be seen and there are indications that these vesicles rupture to release small particles.

In order to determine whether the junctions between neurosecretory fibres and pituicytes have any of the biochemical qualities associated with synapses elsewhere in the

central nervous system the method described by Armstrong, Richardson & Young (1956) for the specific staining of bouton mitochondria was employed. By the use of this technique it is possible to show that a positive reaction is given by the nerve terminals adjoining the extravascular spaces and also at points which lie deeper in the tracts, close to the central pituicytes. The points which stain by the Armstrong–Richardson–Young technique correspond to regions where synaptic junctions are observed under the electron microscope; it is also interesting to record that this technique specific for bouton mitochondria does not stain the mitochondria of the intrinsic cells.

7. Neuro-intermediate lobe under experimental conditions

(*a*) *Colour-change experiments*

The neurosecretory system of *Anguilla* under various conditions of illumination and background was examined by the Braak technique. The melanophore index (M.I.) of each animal was recorded in order to confirm that the conditions of illumination and background used evoked the normal colour-changes (see Waring 1963). On the illuminated black background the M.I. ranged from 3 to 5; on the white background from 1 to 3. No striking differences in the amount of material stained by the Braak technique could be observed in the pituitaries of eels whether taken from darkness or on illuminated white or black backgrounds. On the other hand, the amount of Braak-positive material in the nucleus preopticus of animals which had been maintained on illuminated white or black backgrounds for some hours seemed to be less than in animals taken from darkness. There was also apparently less material in the tract in the experimental animals, and moreover some indications that the animals on white backgrounds had less than those on black backgrounds.

The amount of alcian blue-positive material in the nucleus preopticus, tract, and pituitary of corresponding animals did not always correspond with the density of staining after the Braak technique. Once again no differences in the amount of material in the pituitary could be observed, but, unlike the results of the Braak method, the amount of alcian blue-positive material in the nucleus preopticus and the tract did not differ greatly between experimental animals on illuminated white or black backgrounds.

On the other hand, the *PAS* method applied to the neuro-intermediate lobe demonstrated striking differences between the pituitaries of animals maintained on illuminated white and black backgrounds (figure 26, plate 57, and figure 28, plate 58). A greater number of *PAS*-positive cells were found in animals which had been kept on black backgrounds than in those kept on white backgrounds, though it was not possible to confirm the differences in the cell types seen under the electron microscope. The *PAS* method also revealed differences in the amount of stainable colloid in the central canals, lined by pituicytes, in the neurosecretory tracts (plate 57 and 58). More colloid material was present in animals kept on white backgrounds than in those kept on black backgrounds.

Other differences also were observed at the level of ultrastructure. The presence of synaptic contacts between neurosecretory fibres and pituicytes has already been mentioned. The frequency of these contacts between A_2 fibres (i.e. those containing vesicles *ca.* 1200 Å in diameter) and pituicytes was greater in animals kept on illuminated white backgrounds

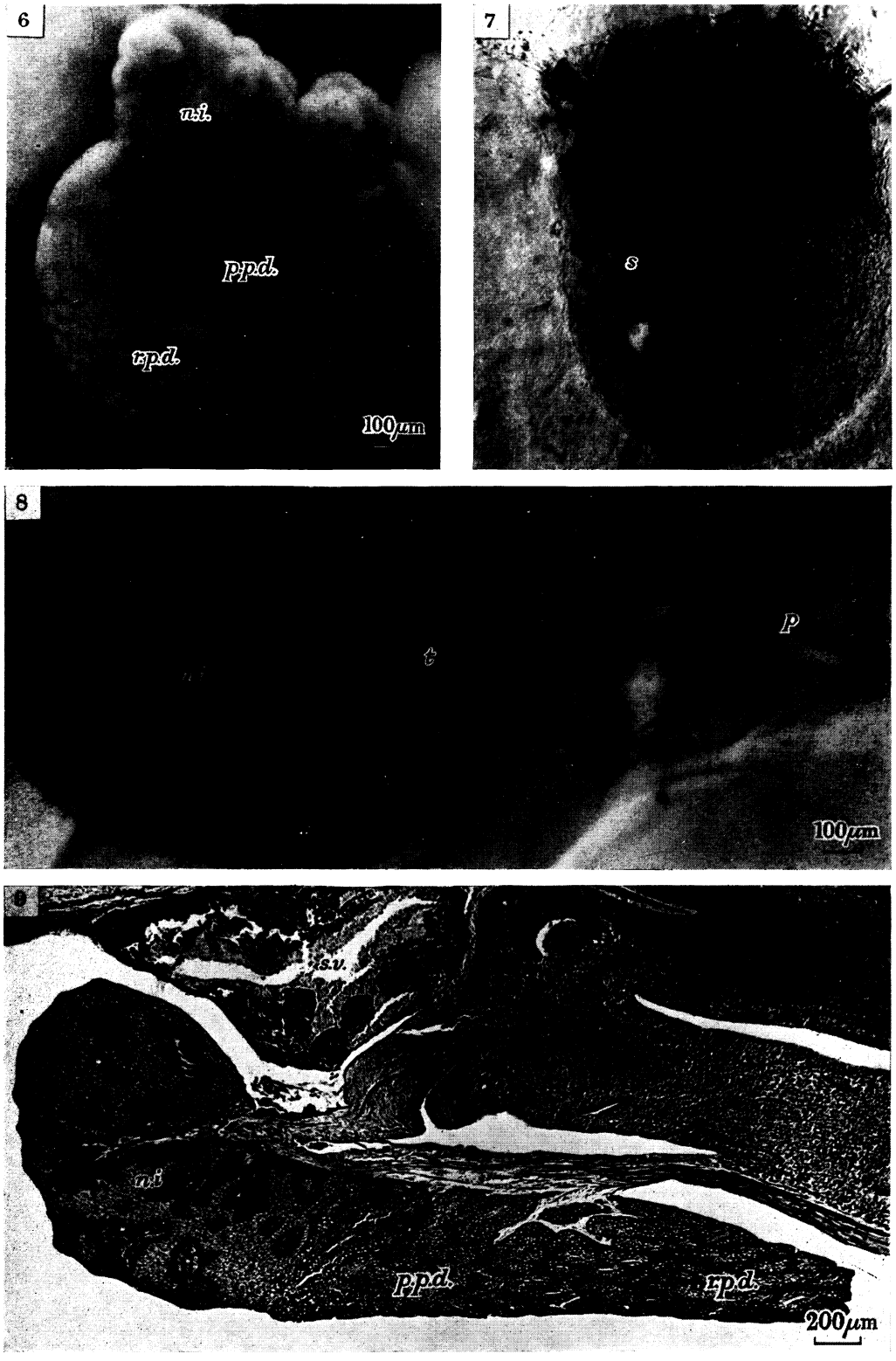


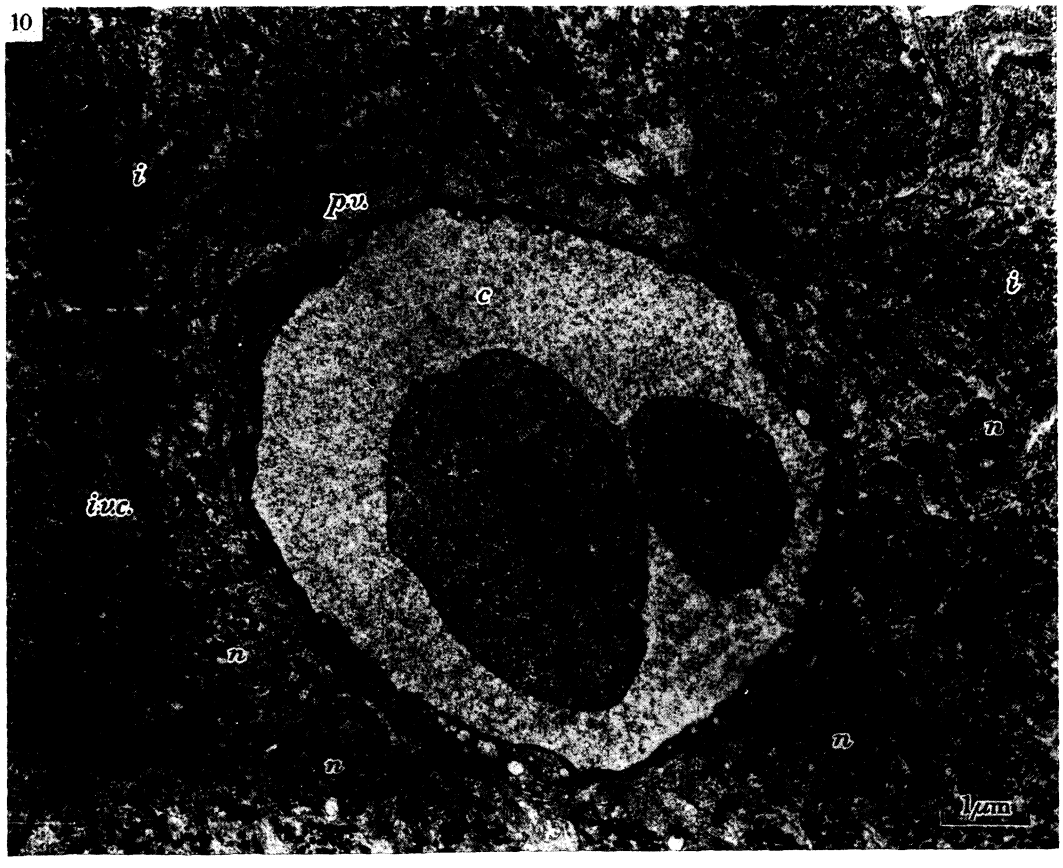
FIGURE 6. The pituitary of *Anguilla*, viewed from below. *n.i.*, Neuro-intermediate lobe; *p.p.d.*, proximal pars distalis; *r.p.d.*, rostral pars distalis.

FIGURE 7. As figure 6 but stained by the Braak technique to demonstrate the neurosecretory tracts in the neuro-intermediate lobe. The dark spot (*s*) is not neurosecretory material but a portion of the saccus vasculosus, lying in the brain below.

FIGURE 8. The hypothalamo-hypophysial neurosecretory system of *Anguilla*, stained by the Braak technique. *n.i.*, neuro-intermediate lobe; *p*, preoptic nuclei; *t*, tract.

FIGURE 9. A medial sagittal section through the pituitary of *Anguilla*, stained by alcian blue–*PAS* orange G. Lettering as in previous figures. (*s.v.*, saccus vasculosus.)

(*Facing p.* 322)



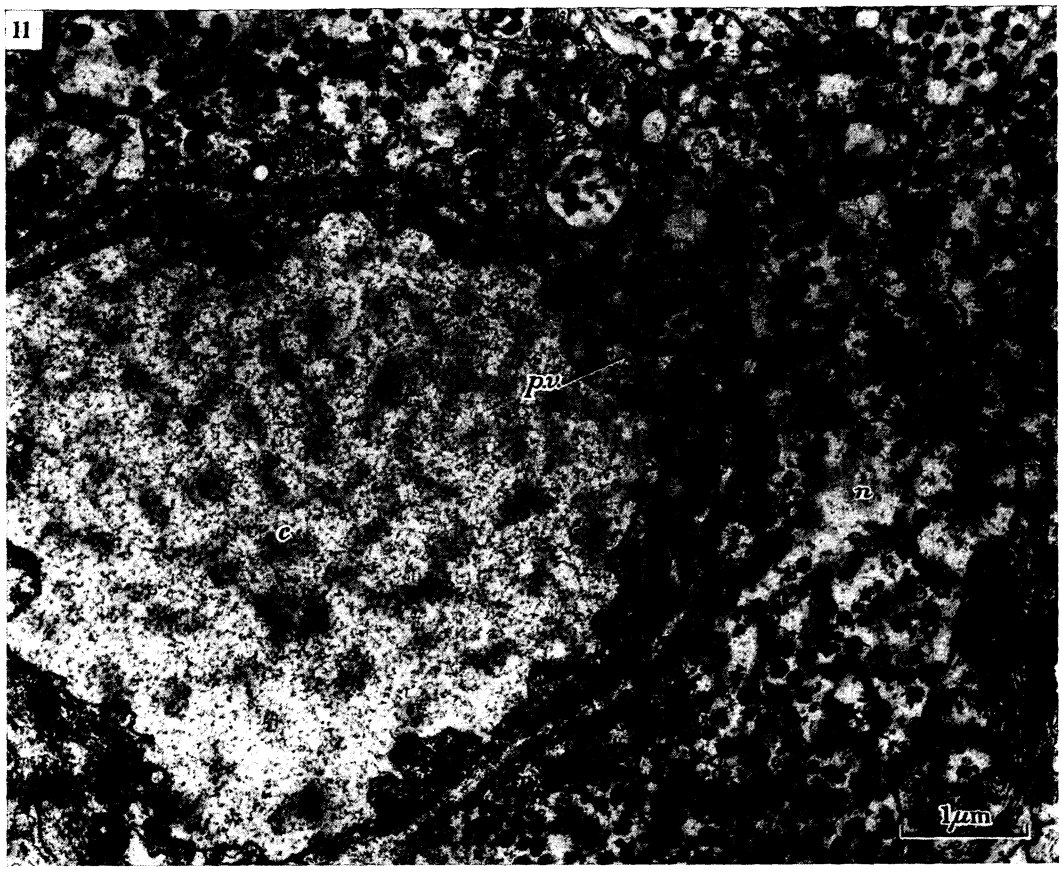


FIGURE 10. The relationship between neurosecretory fibres (*n*), a capillary (*c*), the intervascular channel (*i.v.c.*), the perivascular space (*p.v.*) and intrinsic cells (*i*) in the neuro-intermediate lobe of the conger pituitary.

FIGURE 11. As figure 10, but the pituitary of *Anguilla*.

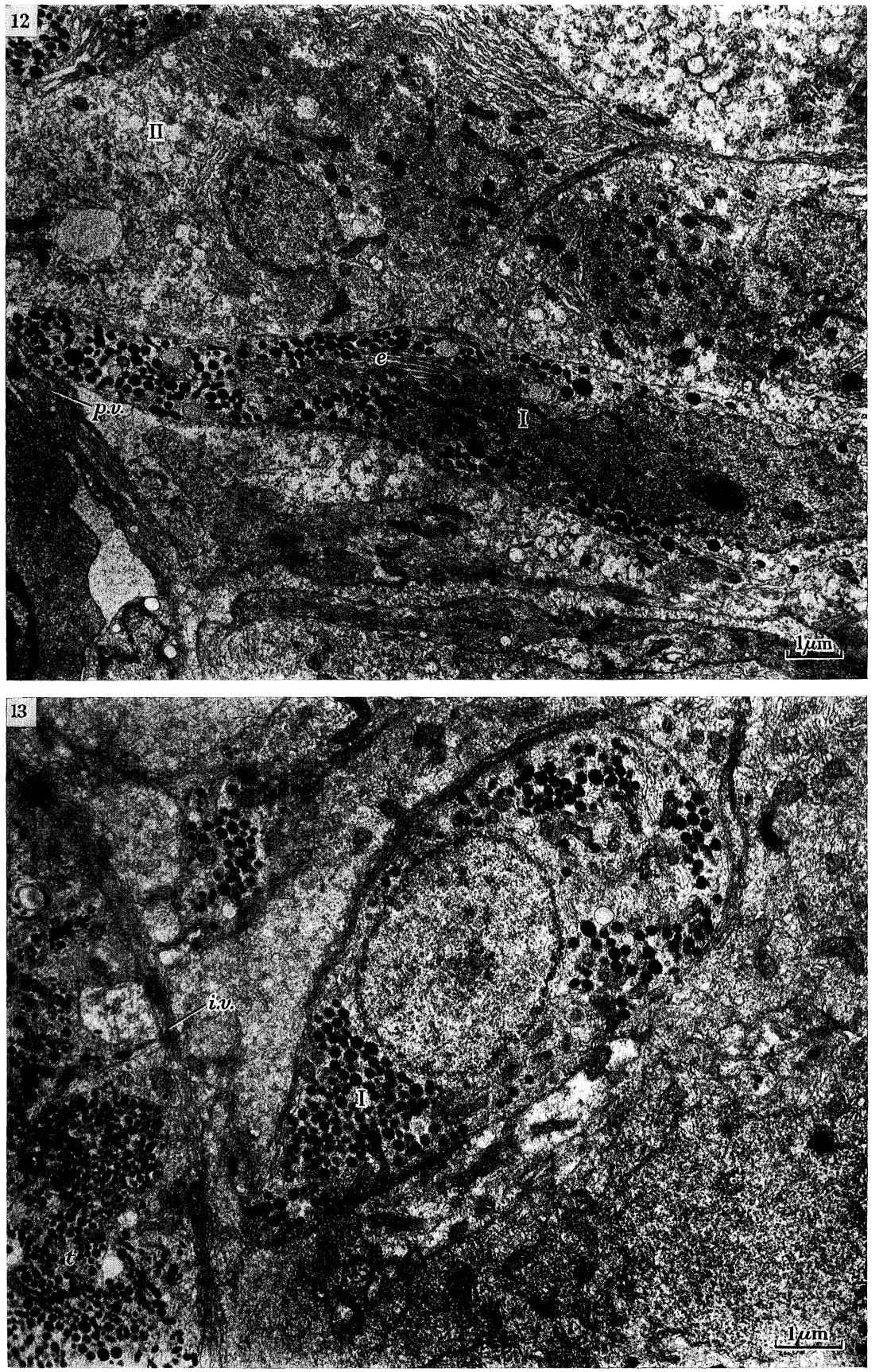


FIGURE 12. Intrinsic cells of the neuro-intermediate lobe of the pituitary of *Conger*. Type I (I) and Type II (II) cells are shown. *e*, Endoplasmic reticulum; *p.v.*, perivascular space.

FIGURE 13. Type I intrinsic cell of the neuro-intermediate lobe of the pituitary of *Anguilla*. *i.v.*, intervascular channel; *t*, neurosecretory tract.

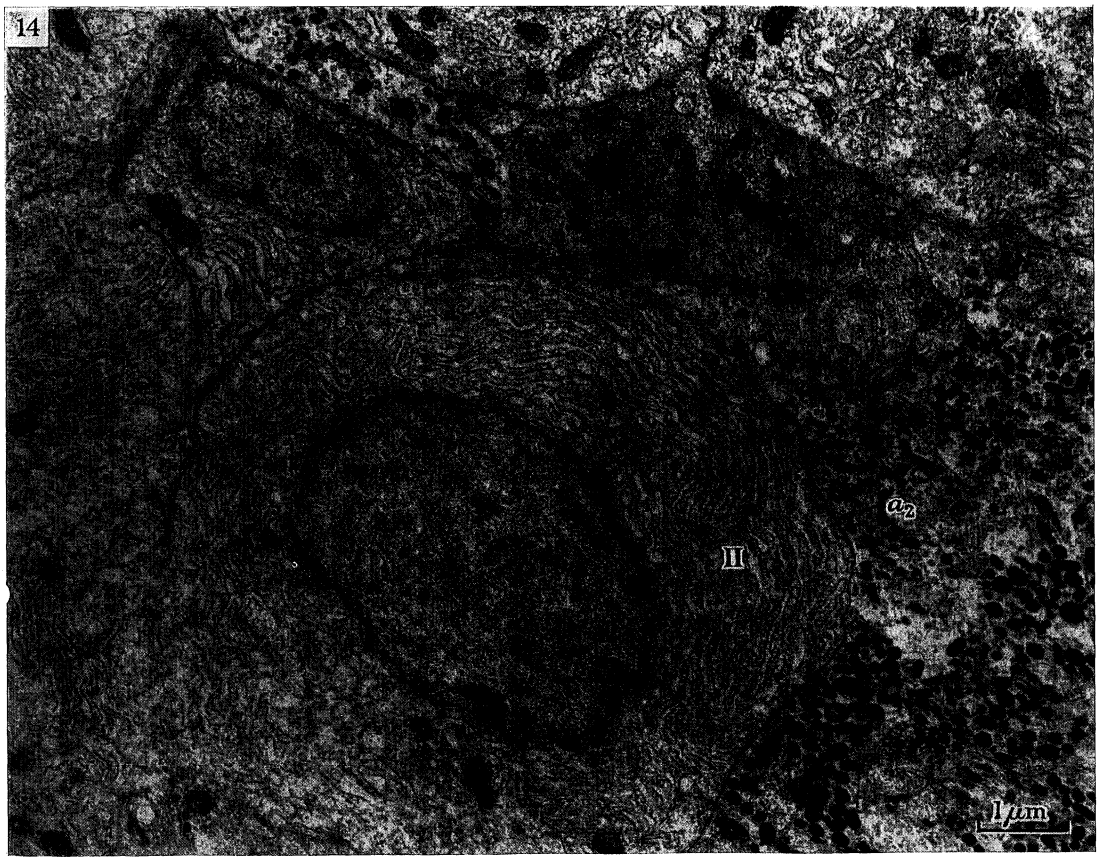


FIGURE 14. The relationship between Type II cells and neurosecretory fibres in *Conger*: II, Type II cell: a_2, Type A_2 fibre containing elementary granules and also small vesicles of synaptic vesicle size-range.

FIGURE 15. A transverse section through a Type II cell of *Conger*. *e*, endoplasmic reticulum; *g*, Golgi zone.

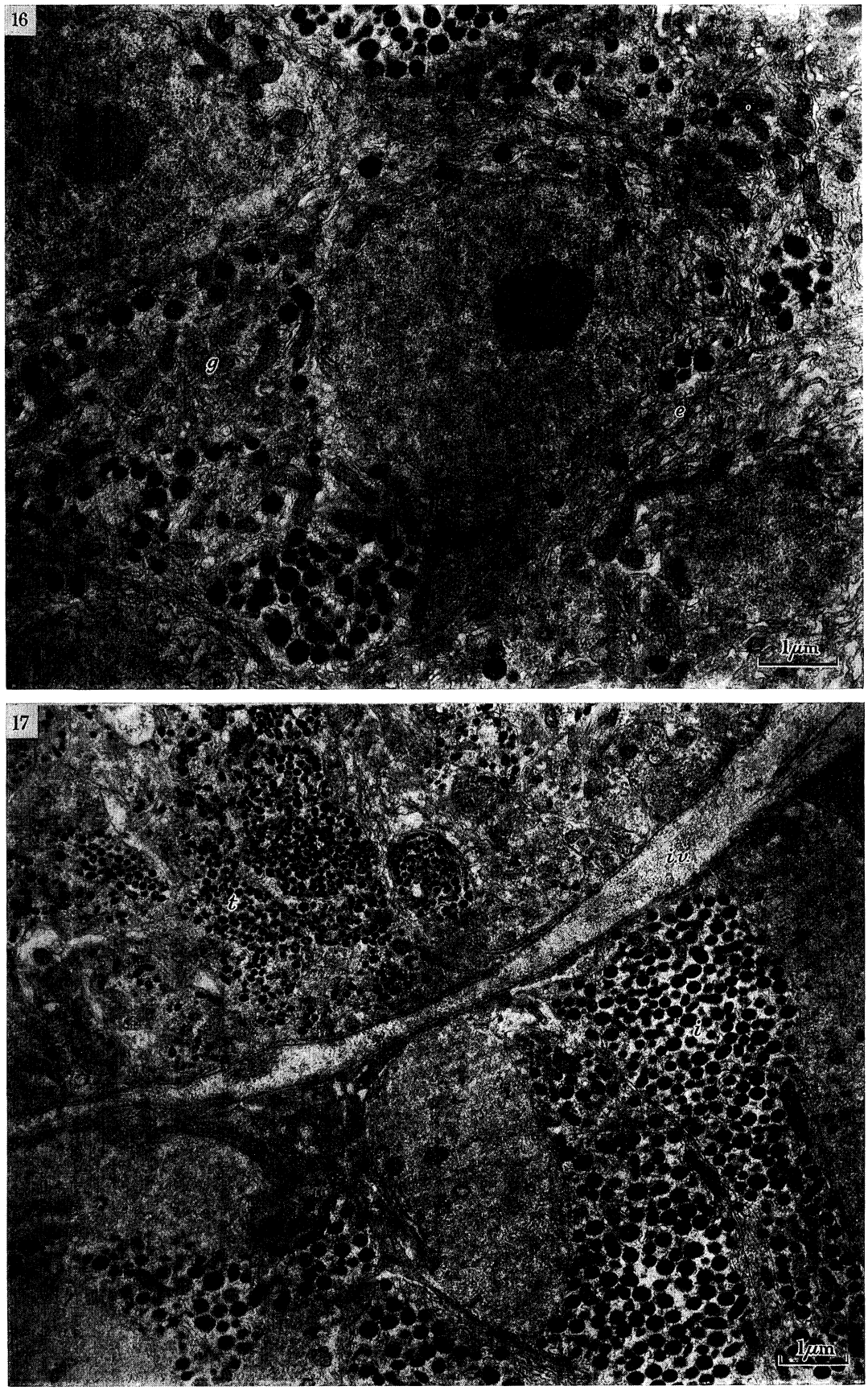


FIGURE 16. A Type II cell from the neuro-intermediate lobe of the pituitary of *Anguilla*. *e*, endoplasmic reticulum; *g*, Golgi zone.

FIGURE 17. The relation between the intrinsic cells (*i*), the intervascular channel (*i.v.*) and the neurosecretory tract (*t*) of *Anguilla*.

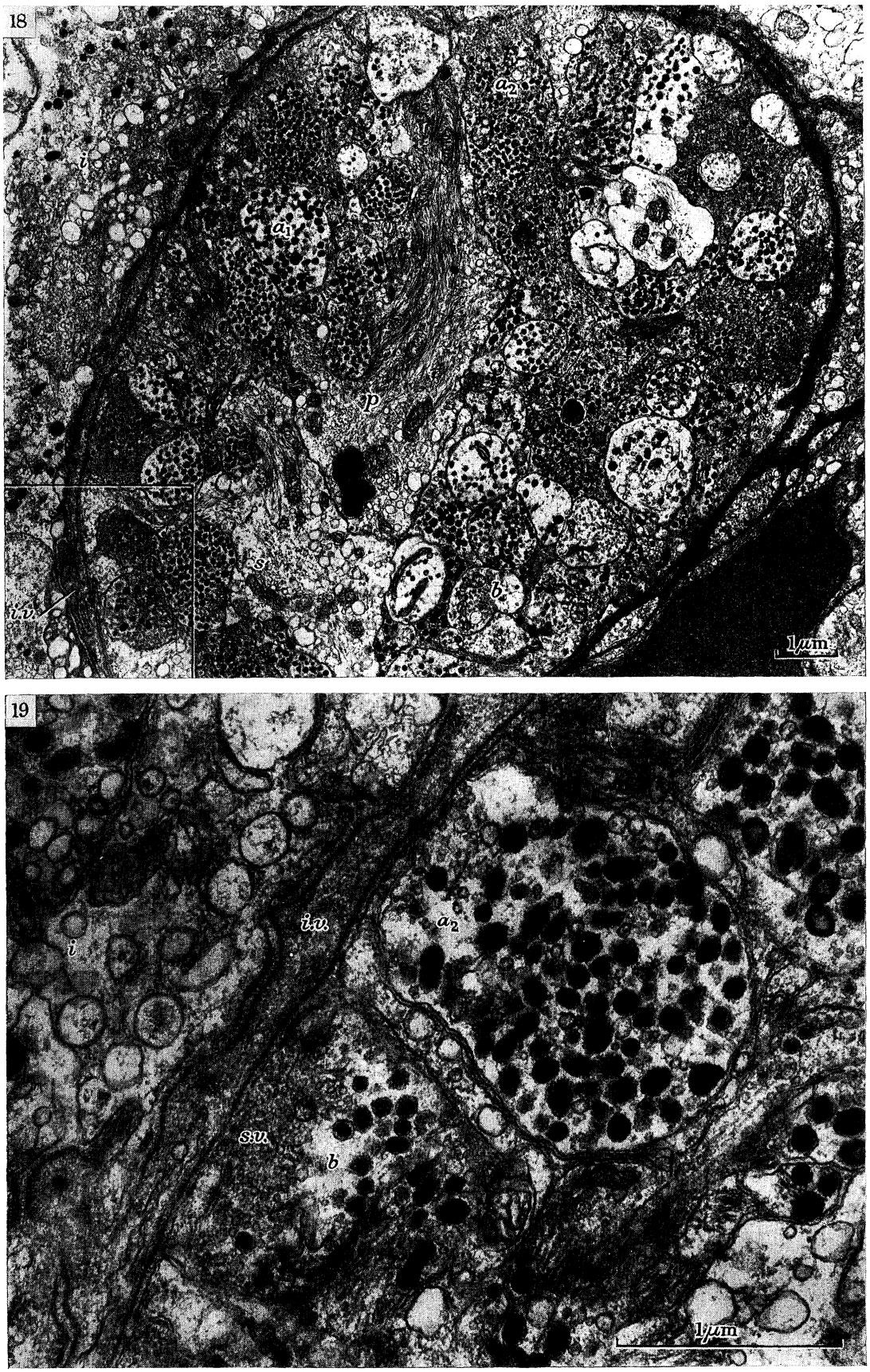
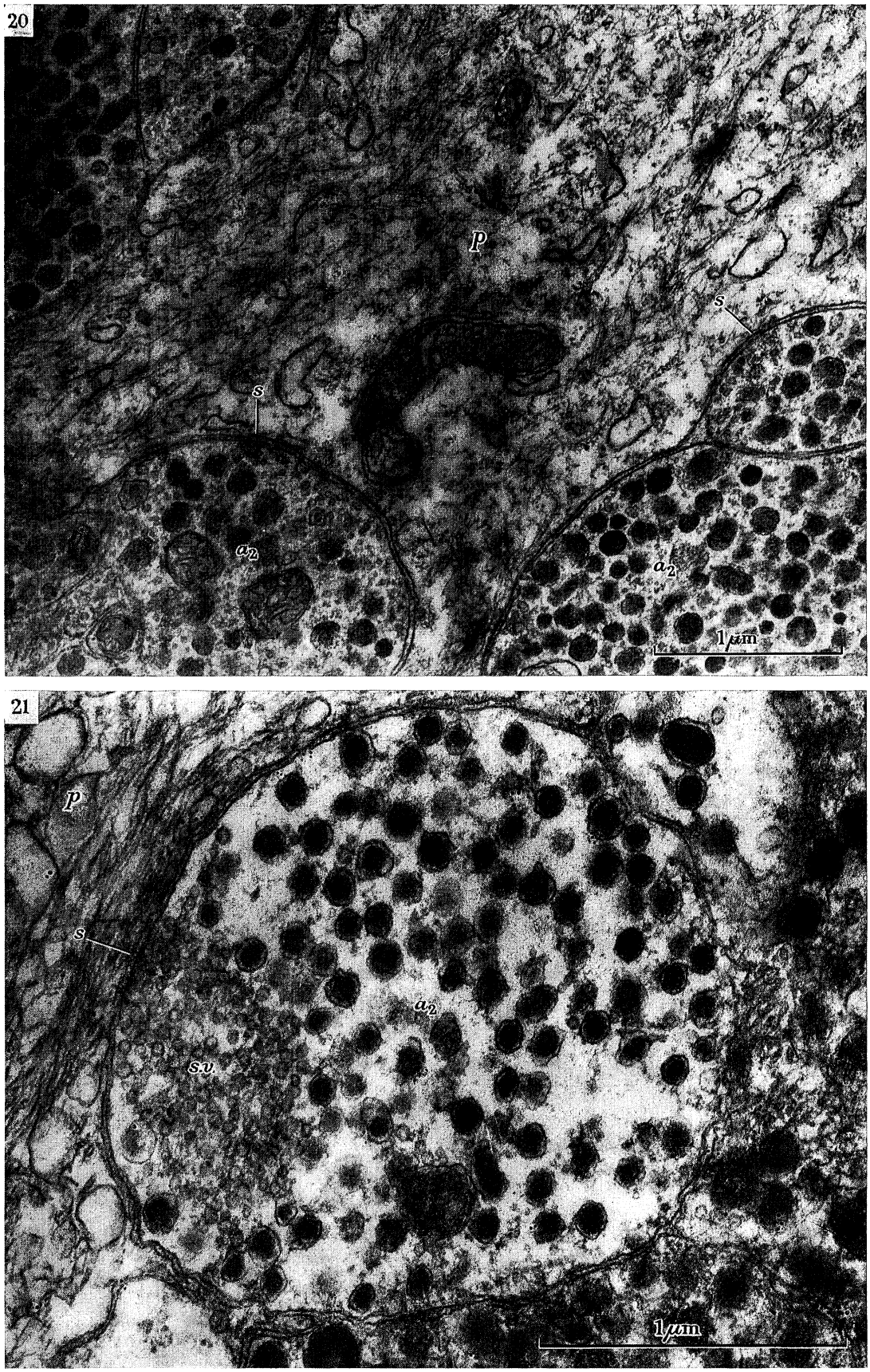


FIGURE 18. A transverse section through a projection of a neurosecretory tract of the neuro-intermediate lobe of *Anguilla*. a_1, a_2, Type A fibres; *b*, Type B fibre; *i*, intermedia cell; *i.v.*, intervascular channel; *p*, pituicyte; a synaptic area is shown at the bottom left corner and in figure 19.

FIGURE 19. The terminals of Type A_2 and Type B fibres in *Anguilla*. The area shown corresponds to the region outlined in figure 18, though from a different specimen. Lettering as in figure 18. *s.v.*: 'synaptic' vesicles.



FIGURES 20, 21. The relation between Type A_2 neurosecretory fibres (a_2) and a pituicyte (p) in *Anguilla*, *s*, synapse; *s.v.*, 'synaptic' vesicles.

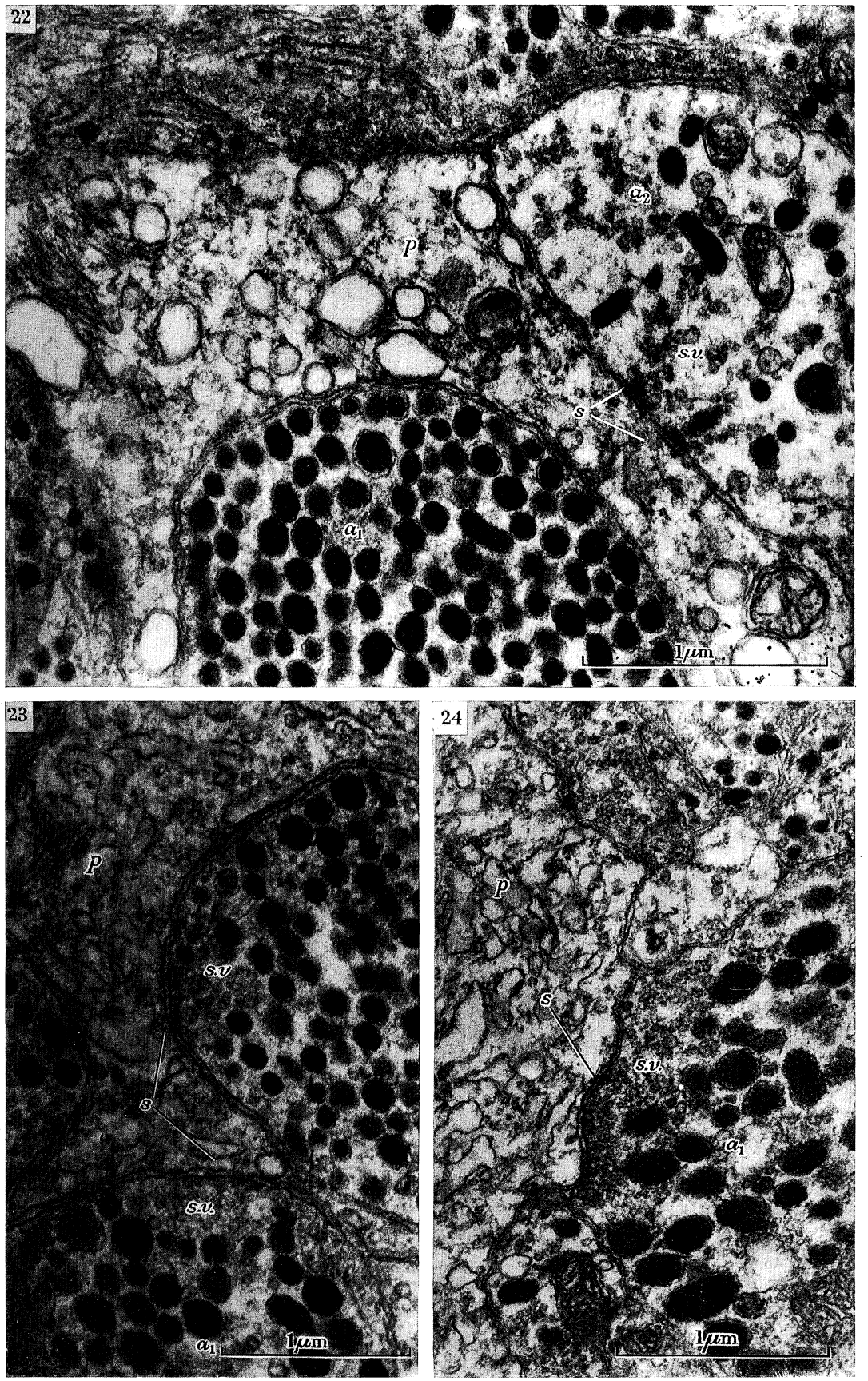


FIGURE 22. The relationship between neurosecretory fibres and pituicytes in an eel (*Anguilla*) kept in fresh water on an illuminated white background. a_1, a_2, Type A fibres; *p*, pituicyte; *s*, synapse; *s.v.*, 'synaptic' vesicles.

FIGURE 23. As figure 22 but from an eel maintained on a white background in sea water. Lettering as in figure 22. Synapses between A_1 fibres and a pituicyte.

FIGURE 24. As figure 23 but from an eel on a black background. Lettering as in figure 22.

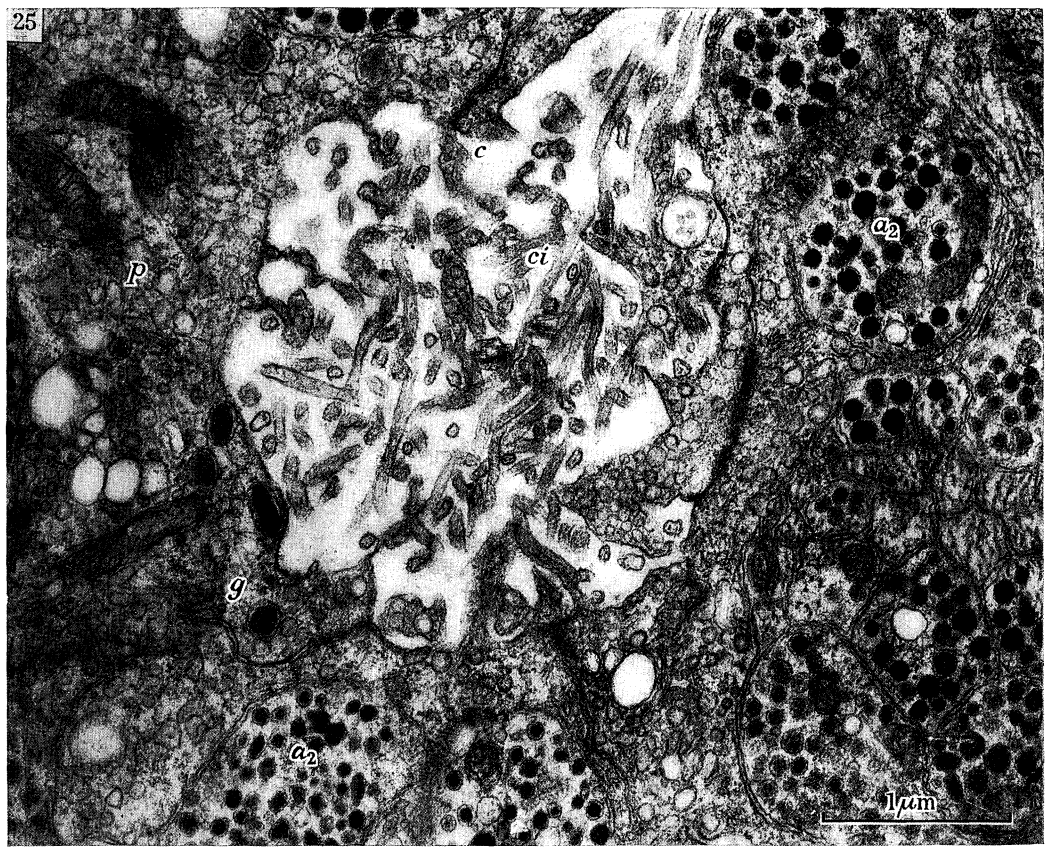


FIGURE 25. An electron micrograph of the central canal in a neurosecretory tract of an eel kept on an illuminated black background. a_2, Type A_2 fibres; *c*, central canal; *ci*, cilia; *g*, granule in pituicyte; *p*, pituicyte.

FIGURE 26. An optical micrograph of a portion of the neuro-intermediate lobe of an eel kept on an illuminated black background. *i*, Intermedia cells; *n*, neurosecretory tract; *p*, pituicytes. (Staining by alcian blue–*PAS*–orange G.) Note the many *PAS*-positive pars intermedia cells.

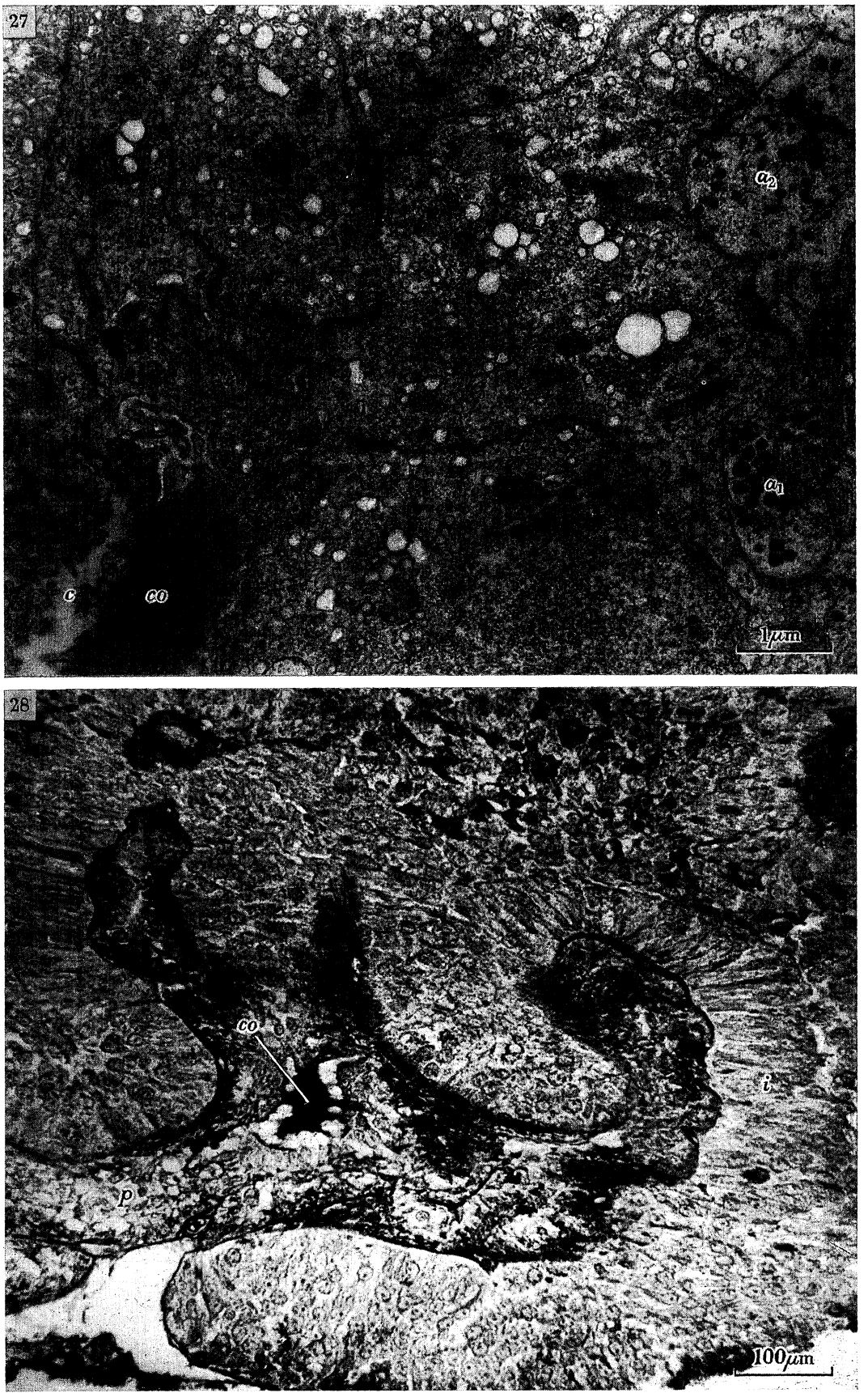


FIGURE 27. An electron micrograph of the central canal in a neurosecretory tract of an eel kept on an illuminated white background. Lettering as in figure 25. a_1, a_2, A_1 and A_2 fibres; *co*, colloid.

FIGURE 28. An optical micrograph of a portion of the neuro-intermediate lobe of an eel kept on an illuminated white background. Staining and lettering as in figure 26. Note the absence of *PAS*-positive pars intermedia cells. *co*, Colloid.

that in those kept on black backgrounds (plate 55, and figure 22, plate 56). In the white background animals synapses were invariably found in quantity: In the black background animals they were rare. These synaptic contacts did not seem to be evenly distributed throughout the neuro-intermediate lobe; there appeared to be more in those regions of the tract where the pituicytes are regularly arranged around a central canal.

(*b*) *Salinity experiments*

Eels were compared on illuminated black and white backgrounds after transference from fresh water to sea water. The results, using histological methods, were the same as those obtained when eels were kept in fresh water only, namely an increase in the number of *PAS*-positive cells on a black background, and an increase of *PAS*-positive colloid in the central canals on a white background.

Under the electron microscope a greater frequency of synapses between A_2 fibres and pituicytes was found in eels on a white background, as in the freshwater experiments. In addition, a number of synapses between A_1 fibres (i.e. with vesicles *ca.* 1700 Å in diameter) and pituicytes were seen in all the animals transferred to sea water (figures 23 and 24, plate 56). Synapses of this kind were extremely rare in animals kept in fresh water; only one synapse of this kind was found in all the freshwater animals examined.

8. Discussion

It has been suggested that two fundamentally different types of neurosecretory fibres, designated as Type A and Type B, may be present in the vertebrate pituitary (Knowles 1965*b*). The present studies on the eel support this view.

The majority of the neurosecretory fibres in the neuro-intermediate lobe of the eel pituitary contain vesicles larger than 1000 Å in diameter, and are therefore described as Type A (Knowles 1965*a*). Two Type A fibres have been found in the eel neurohypophysis with vesicles measuring *ca.* 1200 Å and *ca.* 1700 Å respectively. It is interesting to compare these findings with those of Lederis (1964) who found two fibre types in the trout neurohypophysis containing vesicles *ca.* 1380 and 1850 Å and of Follenius (1963) who found vesicles of *ca.* 1475 Å in the nucleus preopticus of the trout, and vesicles of *ca.* 1295 Å in the perch.

The findings in higher vertebrates (Holmes 1966) also would accord with a view that at least two kinds of Type A fibre are a consistent feature of the neural lobe of the pituitary, and may bear a relation to the usual presence of at least two peptide hormones in this neurohaemal organ.

Fibres with vesicles smaller in diameter than 1000 Å are also present in the neuro-intermediate lobe of the eel pituitary. Morphologically these fibres resemble the Type B fibres which have been described in the pituitaries of an elasmobranch (Knowles 1965*b*) and an amphibian (Cohen 1964). It has been suggested that Type B fibres also may be a usual component of the vertebrate neural lobe (Knowles 1965*b*). Their presence in the eel pituitary supports this view.

In the dogfish a functional relationship between the neurosecretory fibres and the intrinsic endocrine cells of the neuro-intermediate lobe was clearly indicated by the

presence of direct secretomotor junctions between Types A and B fibres and the intrinsic endocrine cells (Knowles 1965*b*). In the present studies no direct contacts between Type B fibres and endocrine cells were seen and contacts between Type A fibres and intrinsic cells were infrequent in *Conger* and very rare in *Anguilla*. A study of the structure and ultrastructure of the eel pituitary does however give some indications that the neurosecretory innervation of the neuro-intermediate lobe is concerned in regulation of intrinsic endocrine function of the intermedia cells.

During development the small area of contact between the neural and intermediate lobe in the elver becomes transformed into a deeply interdigitated area, of a kind which would facilitate interaction between the neural and intermediate tissues (Knowles & Vollrath 1966). Moreover, the terminations of the neurosecretory fibres are evenly distributed over the area of contact between the neural and intermediate lobe and no preferential distribution in respect of blood vessels could be seen, as might be expected if the primary action of the neurosecretory fibres were a discharge of hormones into the systemic circulation. Instead the even distribution of the neurosecretory fibre terminals in close contiguity to the intrinsic endocrine cells and separated from these by a space of only 4000 Å in width or less, indicates a neurosecretory control of pars intermedia function.

The colour-change experiments also support a view that a neurosecretory innervation regulates function of the pars intermedia in the eel, since changes in the environment evoked change in both neurosecretory fibres and intrinsic intermedia cells.

The salinity experiments did not give any clear indication of the precise role of the neuro-intermediate lobe of the pituitary in the control of water balance in the eel. The occurrence of synapses between A_1 fibres and pituicytes after transference to sea water but not in the freshwater animals suggested that the A_1 fibres might be concerned in osmotic control and be active following an increased salinity of the environment. It is therefore interesting to note that Lederis (1964) found that the arginine-vasotocin content of the trout pituitary fell by 50 % after 2 h in sea water and that this was accompanied by a depletion of osmiophilic material from the pituitary, Schiebler & Hartmann (1963) have remarked on differences in the amount of Gomori-positive material in the neuro-intermediate lobes of pituitaries collected from eels in fresh water or sea water; those in sea water contained less stainable material. There are therefore some indications that the neuro-intermediate lobe might be involved in osmotic regulation as an eel passes from fresh water to sea water. It may however not be the only controlling mechanism for osmotic regulation. It has been shown that hypophysectomized eels can withstand changes of salinity (Fontaine, Callamand & Olivereau 1949).

The present studies did not give any indication that intermedia cells might be concerned in water balance, a suggestion which has been made in respect of the mammalian pituitary (Legait 1964). There were, however, indications that three cell types were present in the pars intermedia of the eel pituitary. Stahl (1958) described two cell types in the Mugilidae and suggested that both might be concerned in colour change, one producing a melanocyte-dispersing hormone and the other a melanocyte-concentrating hormone. The possibility however that one of the three cell types in the pars intermedia of the eel may be involved in osmotic control must be taken into account. Ziegler (1963) describes three cell types in

the pituitary of the rat and claims that one of these increased in number after dehydration of the animals.

In the present account cellular elements in the neural components of the pars intermedia have been termed pituicytes. It is questionable whether some of these cells should be designated as ependymal cells, since they have many of the features of ependyma and also line a cavity which is an extension of the infundibular recess. It was, however, not possible to discern any difference between these cells and others which did not appear to border the cavity. Moreover, Bargmann (1953) described all these cells as pituicytes and so to avoid confusion this terminology has been retained. The demonstration of a functional relationship between neurosecretory fibres and cells of ependymal characteristics is however of interest in view of the recent findings by Hagedoorn (1965) of changes in ependymal cells of the third ventricle correlated to neurosecretory activity in the mammal *Mephitis mephitis*. Löfgren (1959, 1960) showed that in the rat the infundibular cavity is partly lined by ependymal cells which are completely different from the cells which cover the rest of the third ventricle. The same observation was made by Stahl & Leray (1962) in a number of teleost fishes. The present studies of the ultra-structure of the eel pituitary, by demonstrating for the first time functional contacts between neurosecretory fibres and ependymal cells indicate strongly that ependymal or other glial elements may play an important role in the process of neurosecretion, either by modulating the firing of neurosecretory axons or by providing a feedback of information to the preoptic nucleus (see Knowles & Vollrath 1965*a*, *b*).

The nature of the junctions between neurosecretory fibres and pituicytes is evidently of great interest. Morphologically these resemble synapses, but it is interesting to note that they did not show all precisely the same features. The range of variation may indicate that the differences represent stages in the formation of transient synapses, and this suggestion is supported by the fact that synapses were not observed in all the animals studied, but appeared to be evoked by changes in the environment (i.e. illumination, background and salinity).

This concept of transient synapses is an unusual one, and it may be that the functional junctions observed differ fundamentally from synapses in the central nervous system. There is insufficient evidence as yet to decide whether a transmitter agent is released and stimulates the pituicyte to activity, or whether the neurosecretory hormone is transported across the junction and thence through the pituicyte into the cerebrospinal fluid. Nishioka, Bern & Mewaldt (1964) considered this second possibility in a bird in which they showed synaptic-like vesicles adjacent to ependymal cells (see also Smoller 1965).

In the present studies there is some reason to believe that a stimulus from a neurosecretory fibre may promote synthesis of a secretory product in a pituicyte, since the conditions of illumination and background which appear to promote synaptic junctions between neurosecretory fibres and pituicytes also lead to the appearance of vesicles larger than neurosecretory vesicles in the pituicytes. Under these conditions *PAS*-positive material was abundant in the central canals, and it seems likely that this material is released from the pituicyte into the cerebro-spinal-fluid (C.S.F.). One may therefore inquire as to the possible significance of a secretion of material from the pituicytes into the C.S.F. It has been shown by Stutinsky (1953) that dendrites of the preoptic nuclei cells project into the

ventricles of the eel brain and it is evident that material released from the pituicytes could travel in the C.S.F. and influence the action of cells in the preoptic nucleus.

All the component parts of a possible system to provide feedback of information from the pituitary to the hypothalamus are therefore present (figure 1). This concept of feedback of information from the distal to the proximal end of a neurosecretory system is a new one. It is evident that a possible role of ependymal elements in communication between different parts of the nervous system would be of considerable theoretical interest.

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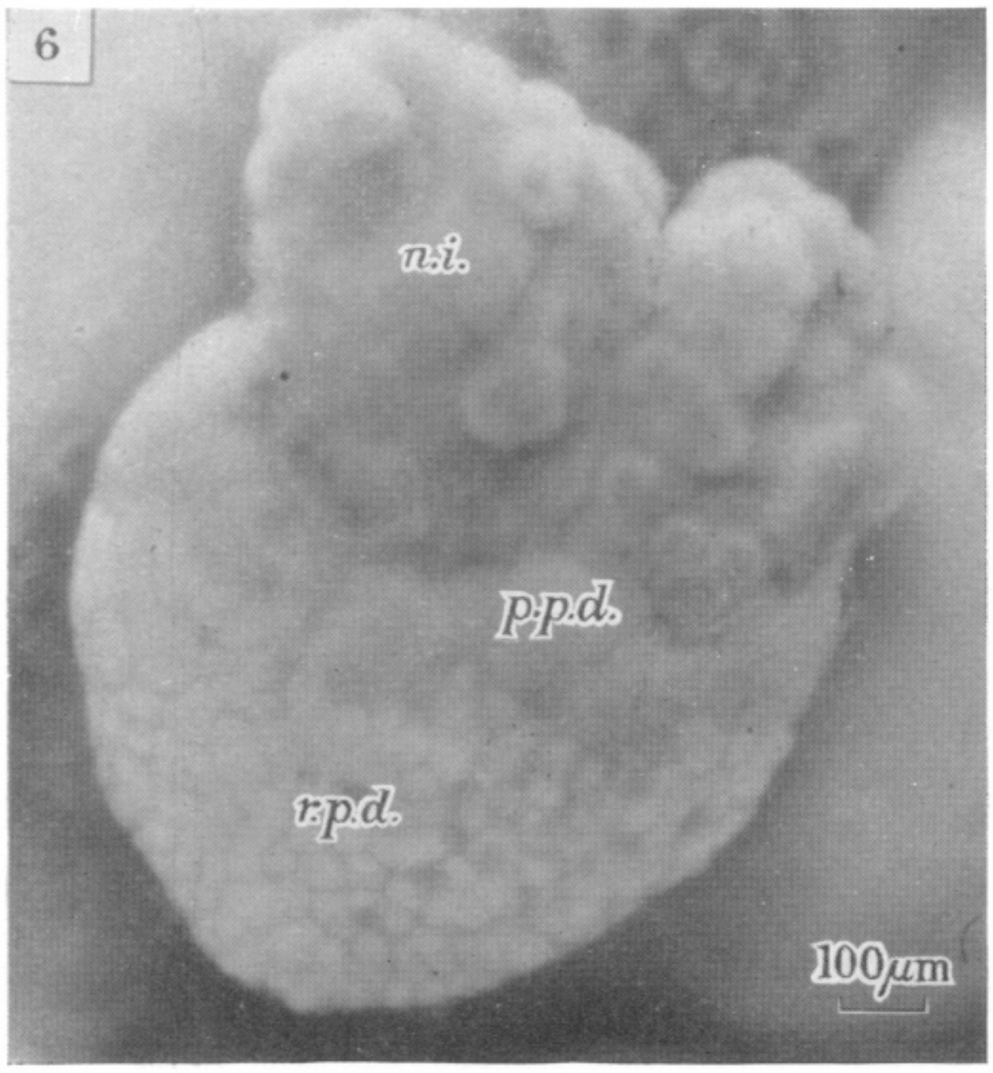

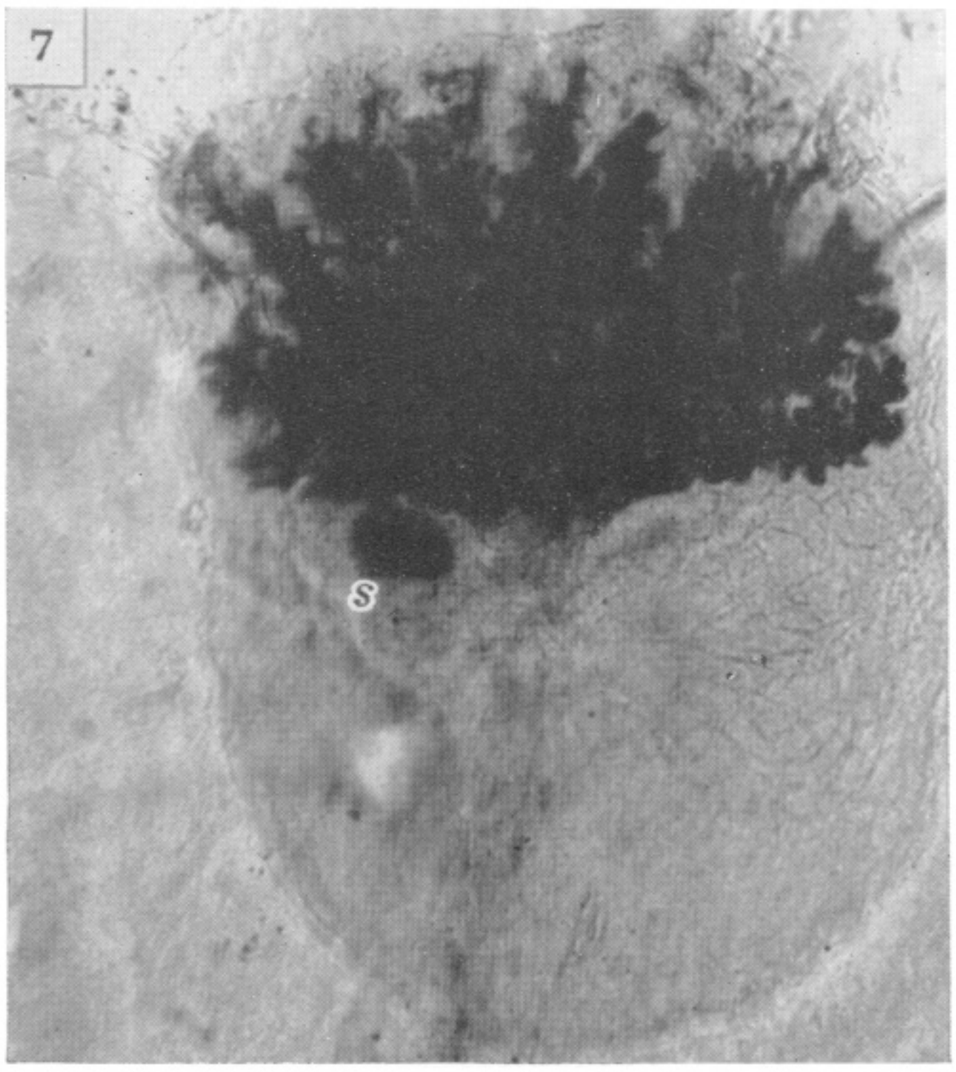

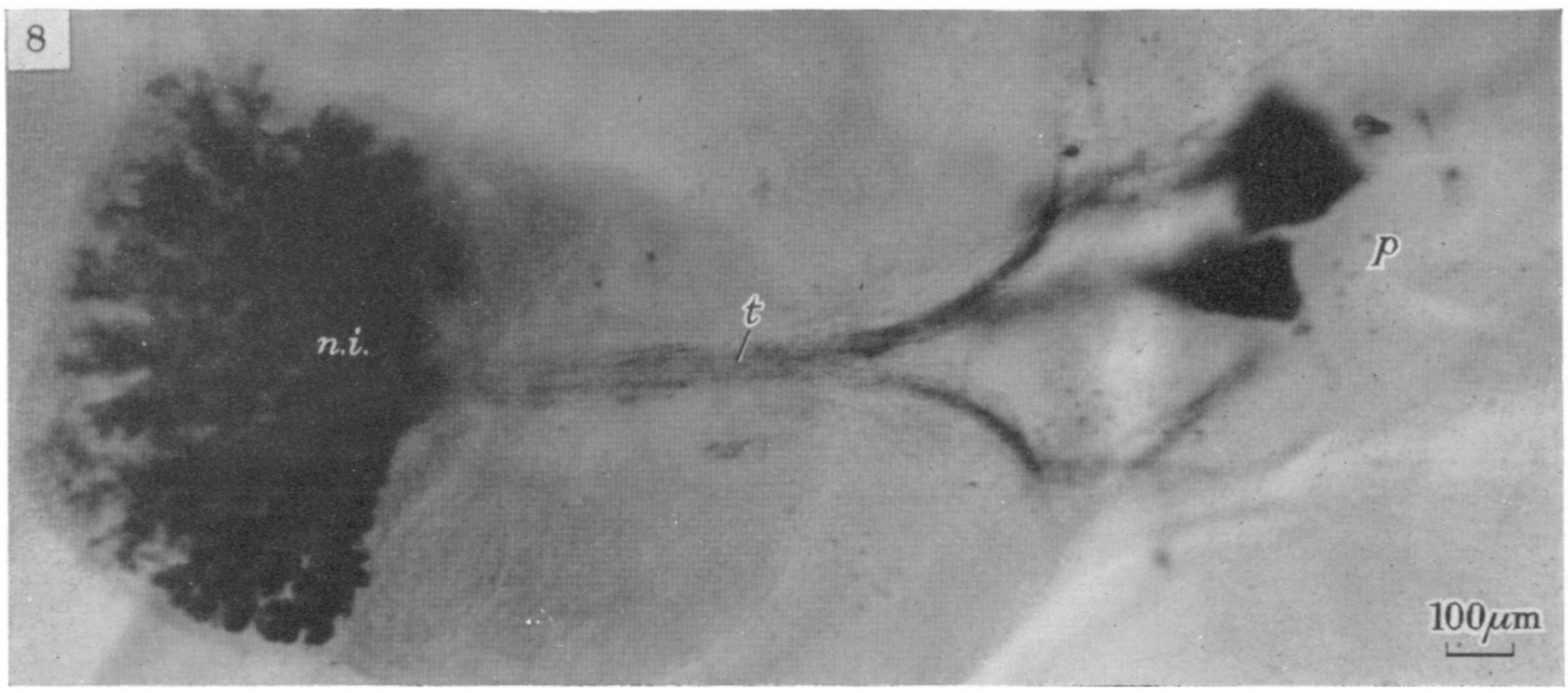

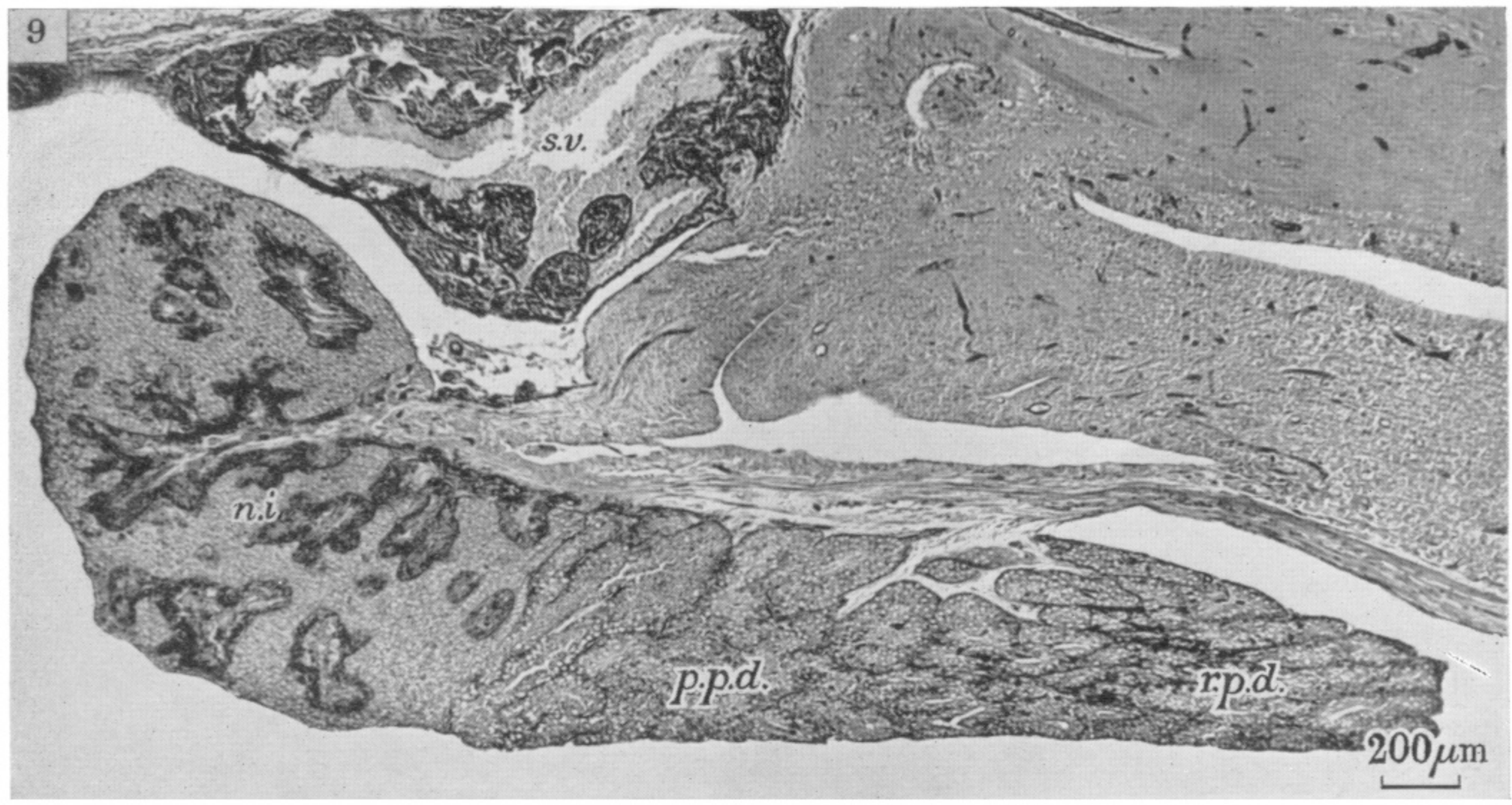

FIGURE 6. The pituitary of *Anguilla*, viewed from below. *n.i.*, Neuro-intermediate lobe; *p.p.d.*, proximal pars distalis; *r.p.d.*, rostral pars distalis.

FIGURE 7. As figure 6 but stained by the Braak technique to demonstrate the neurosecretory tracts in the neuro-intermediate lobe. The dark spot (*s*) is not neurosecretory material but a portion of the saccus vasculosus, lying in the brain below.

FIGURE 8. The hypothalamo-hypophysial neurosecretory system of *Anguilla*, stained by the Braak technique. *n.i.*, neuro-intermediate lobe; *p*, preoptic nuclei; *t*, tract.

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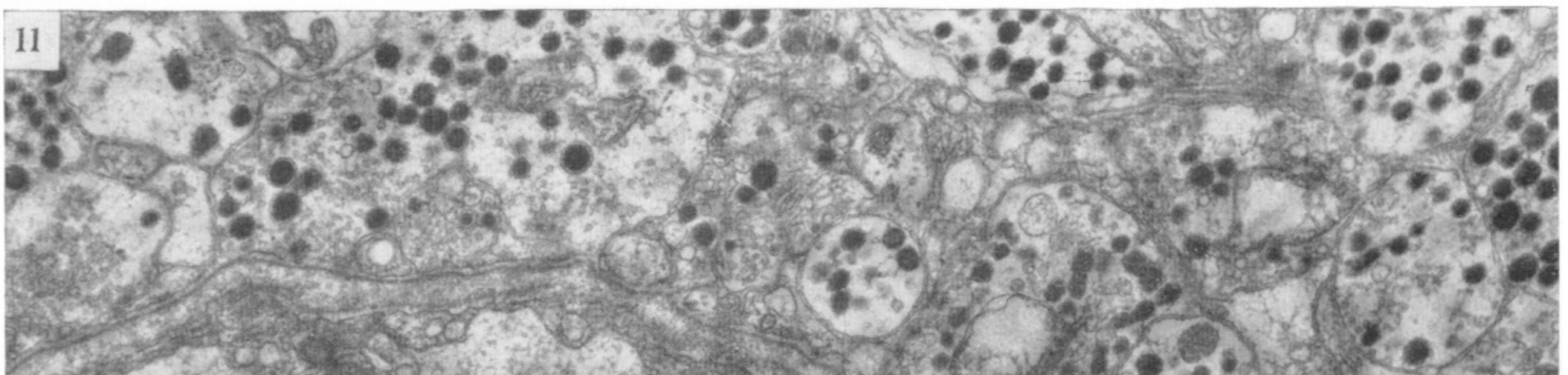

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FIGURE 11. As figure 10, but the pituitary of *Anguilla*.

12

II

e

p.v.

I

1μm

13

i.v.

I

t

1μm

FIGURE 12. Intrinsic cells of the neuro-intermediate lobe of the pituitary of *Conger*. Type I (I) and Type II (II) cells are shown. *e*, Endoplasmic reticulum; *p.v.*, perivascular space.

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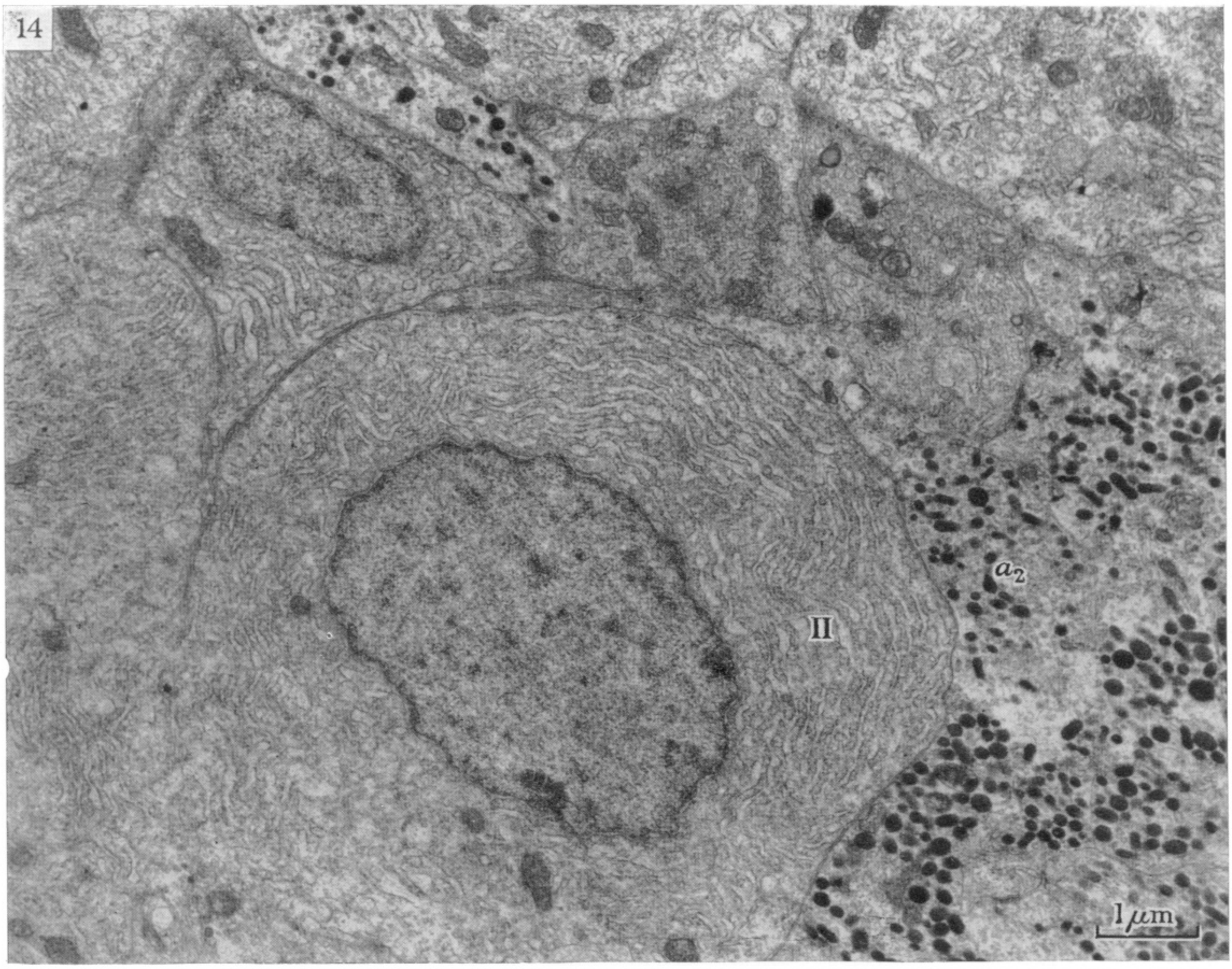

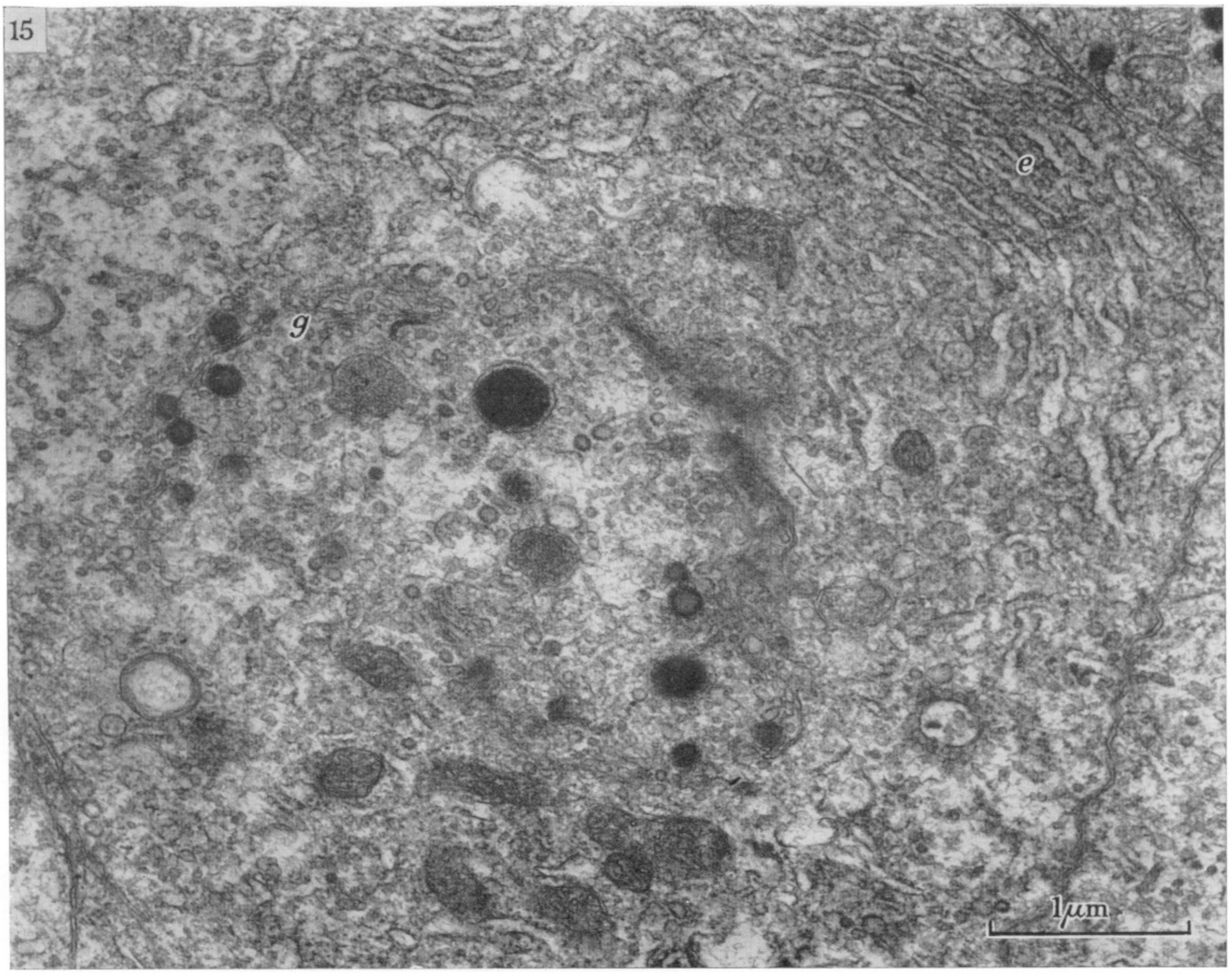

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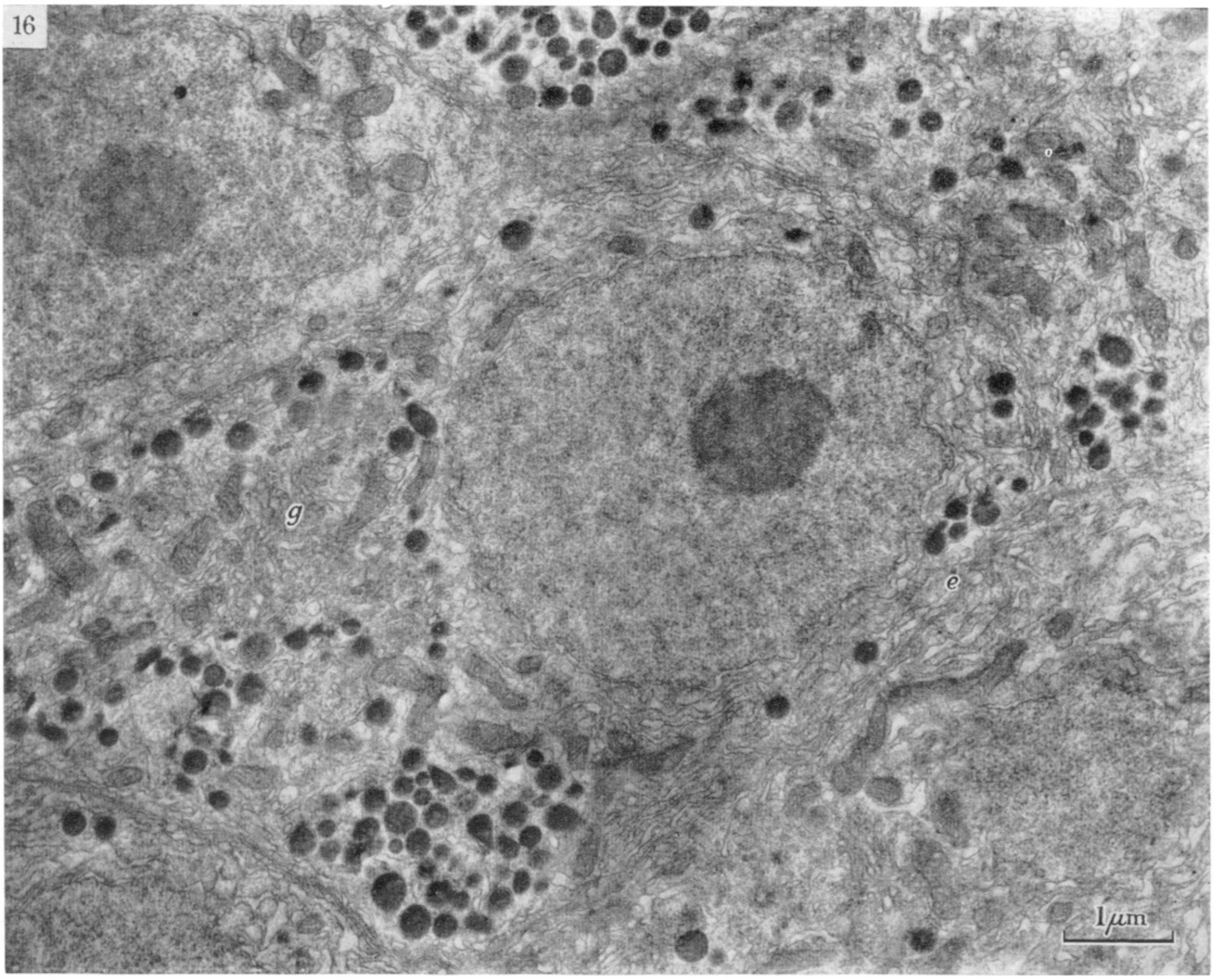

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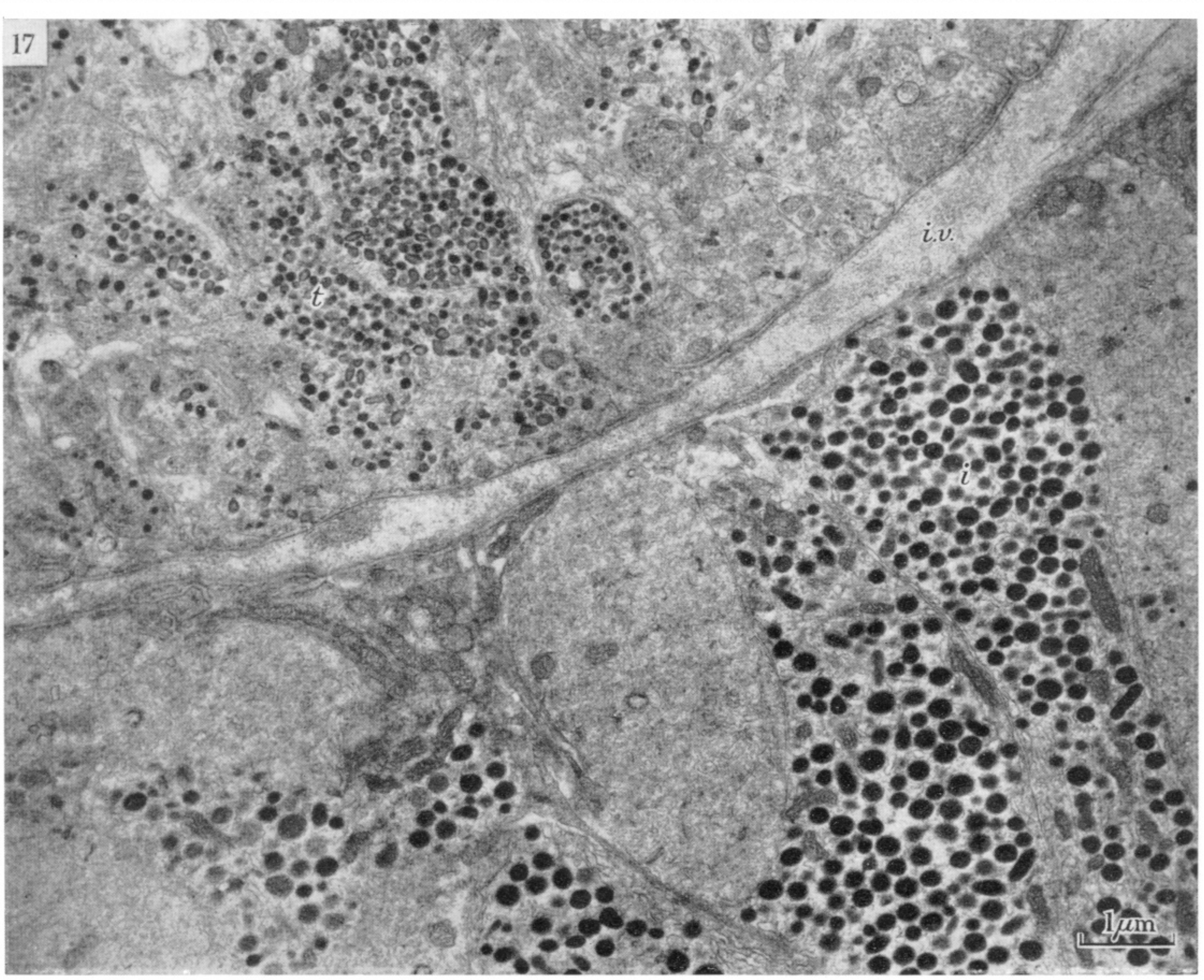
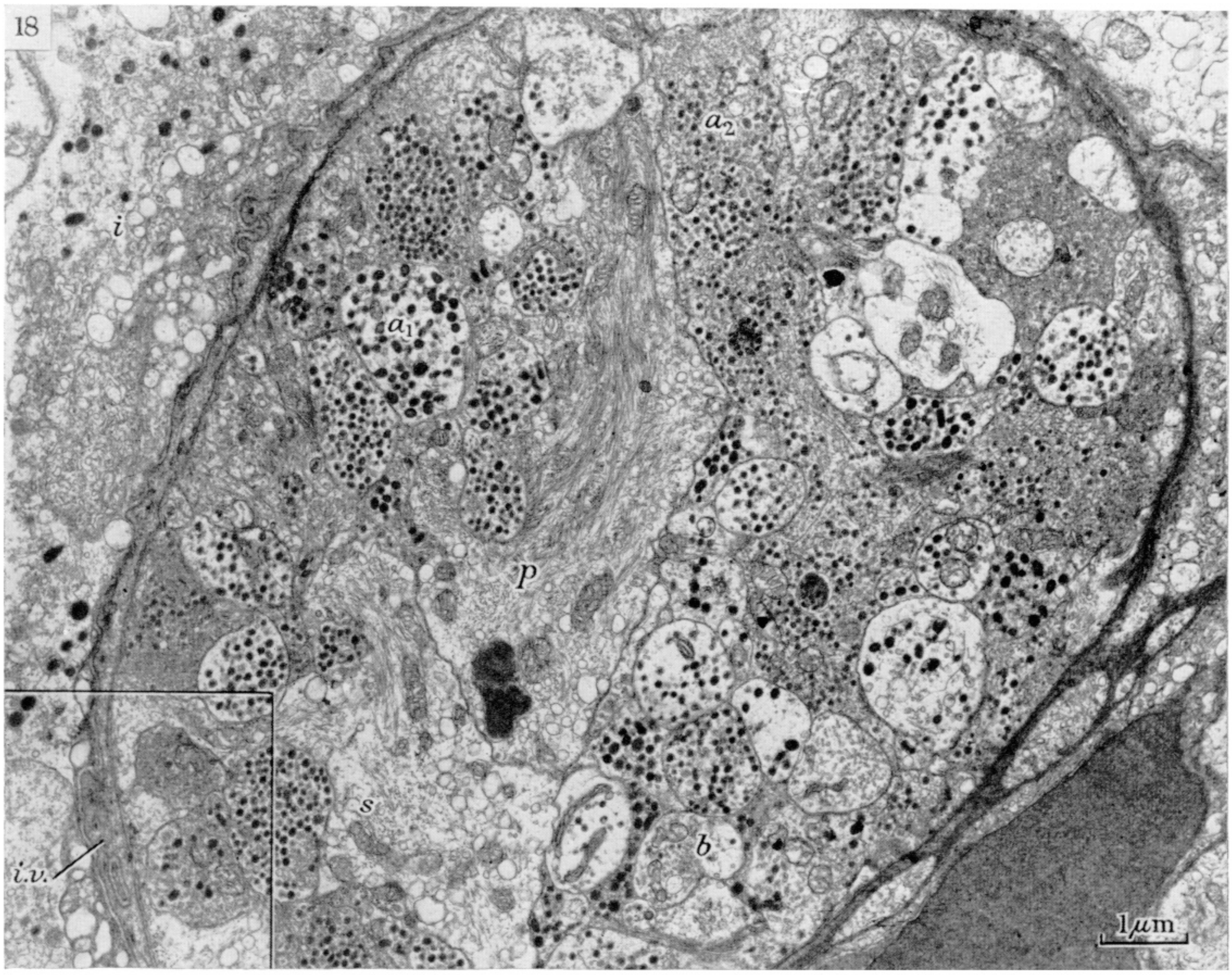


FIGURE 16. A Type II cell from the neuro-intermediate lobe of the pituitary of *Anguilla*. *e*, endoplasmic reticulum; *g*, Golgi zone.

FIGURE 17. The relation between the intrinsic cells (*i*), the intervascular channel (*i.v.*) and the neurosecretory tract (*t*) of *Anguilla*.

18



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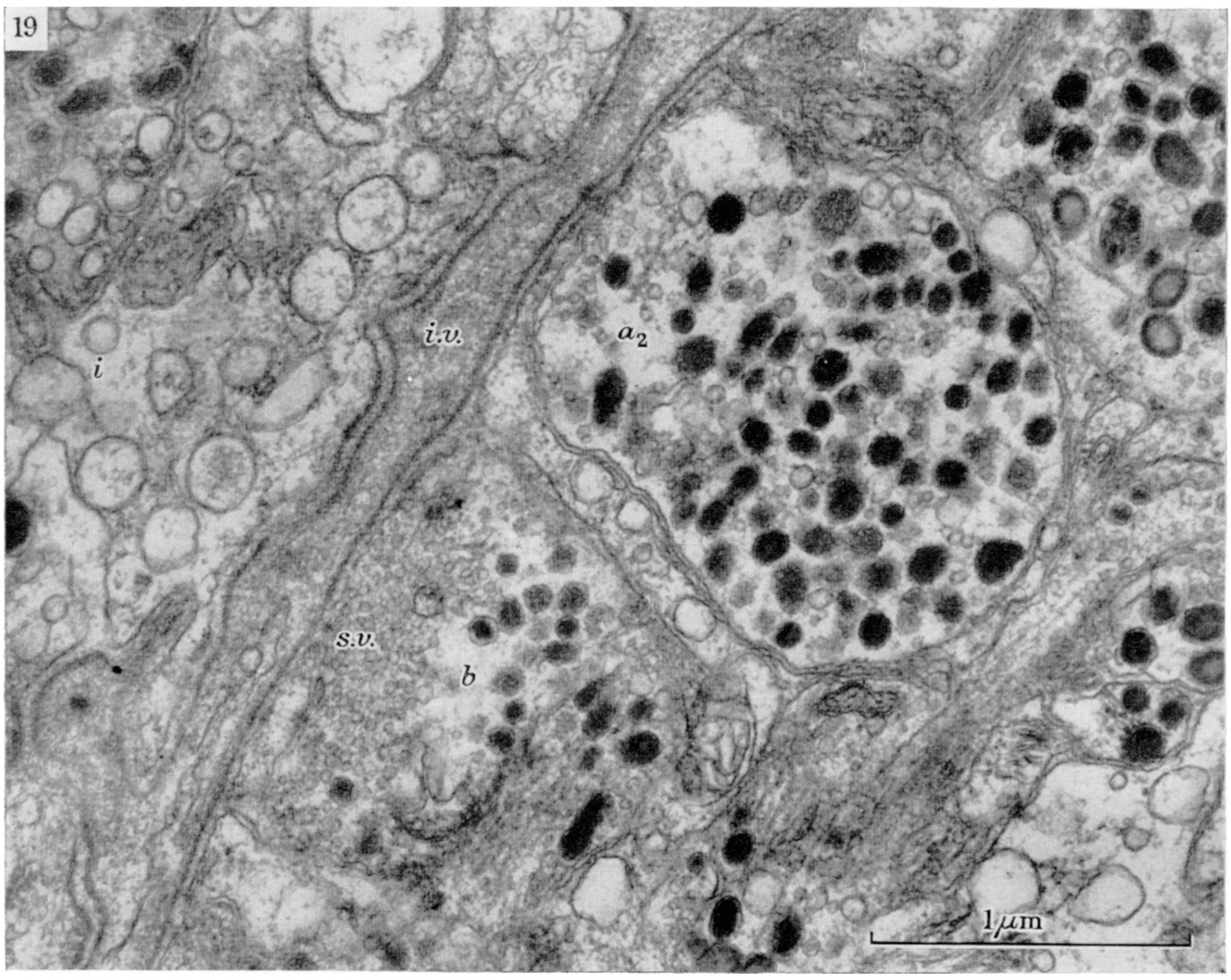
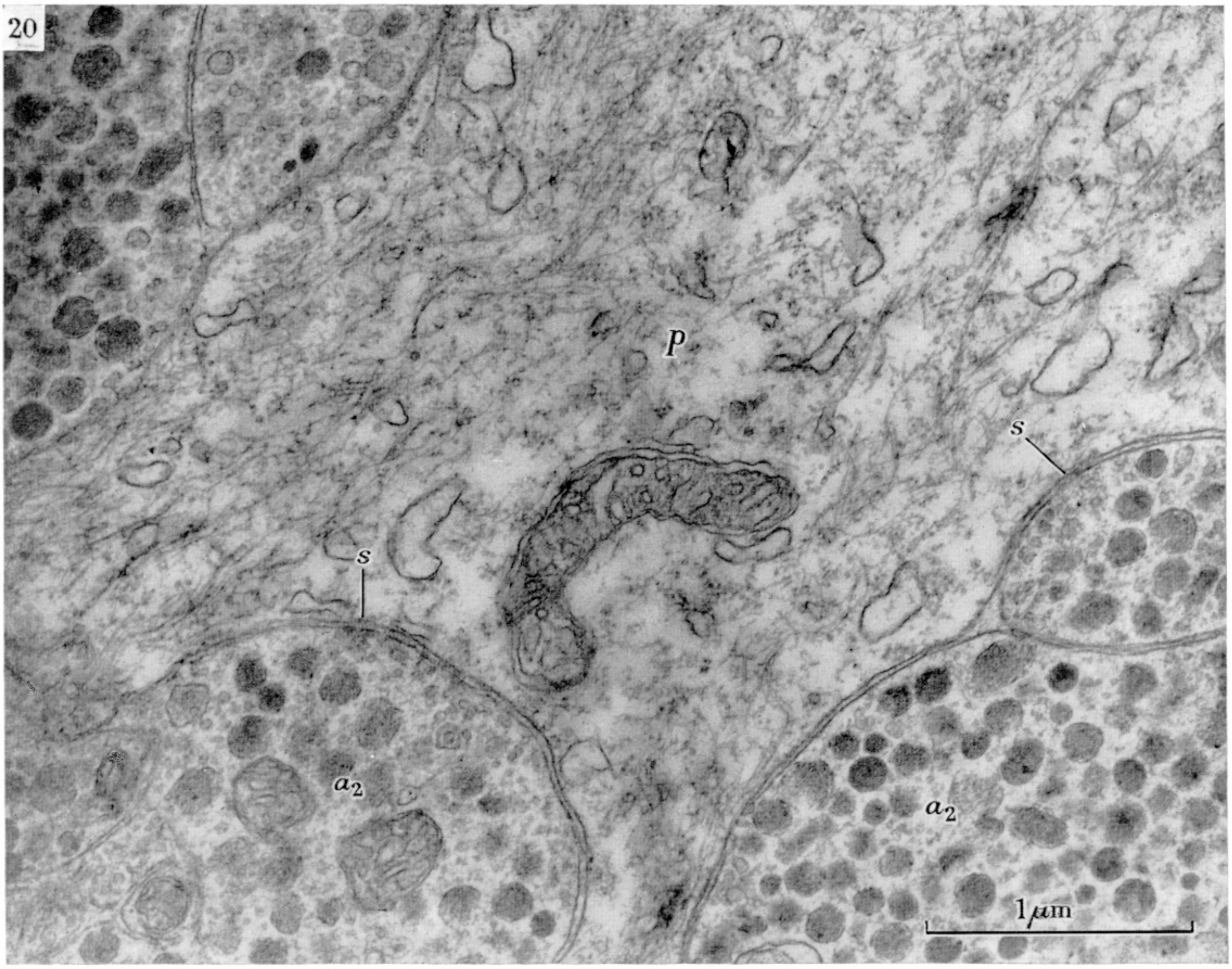


FIGURE 18. A transverse section through a projection of a neurosecretory tract of the neuro-intermediate lobe of *Anguilla*. a_1, a_2, Type A fibres; *b*, Type B fibre; *i*, intermedia cell; *i.v.*, intervascular channel; *p*, pituicyte; a synaptic area is shown at the bottom left corner and in figure 19.

FIGURE 19. The terminals of Type A_2 and Type B fibres in *Anguilla*. The area shown corresponds to the region outlined in figure 18, though from a different specimen. Lettering as in figure 18. *s.v.*: 'synaptic' vesicles.

20



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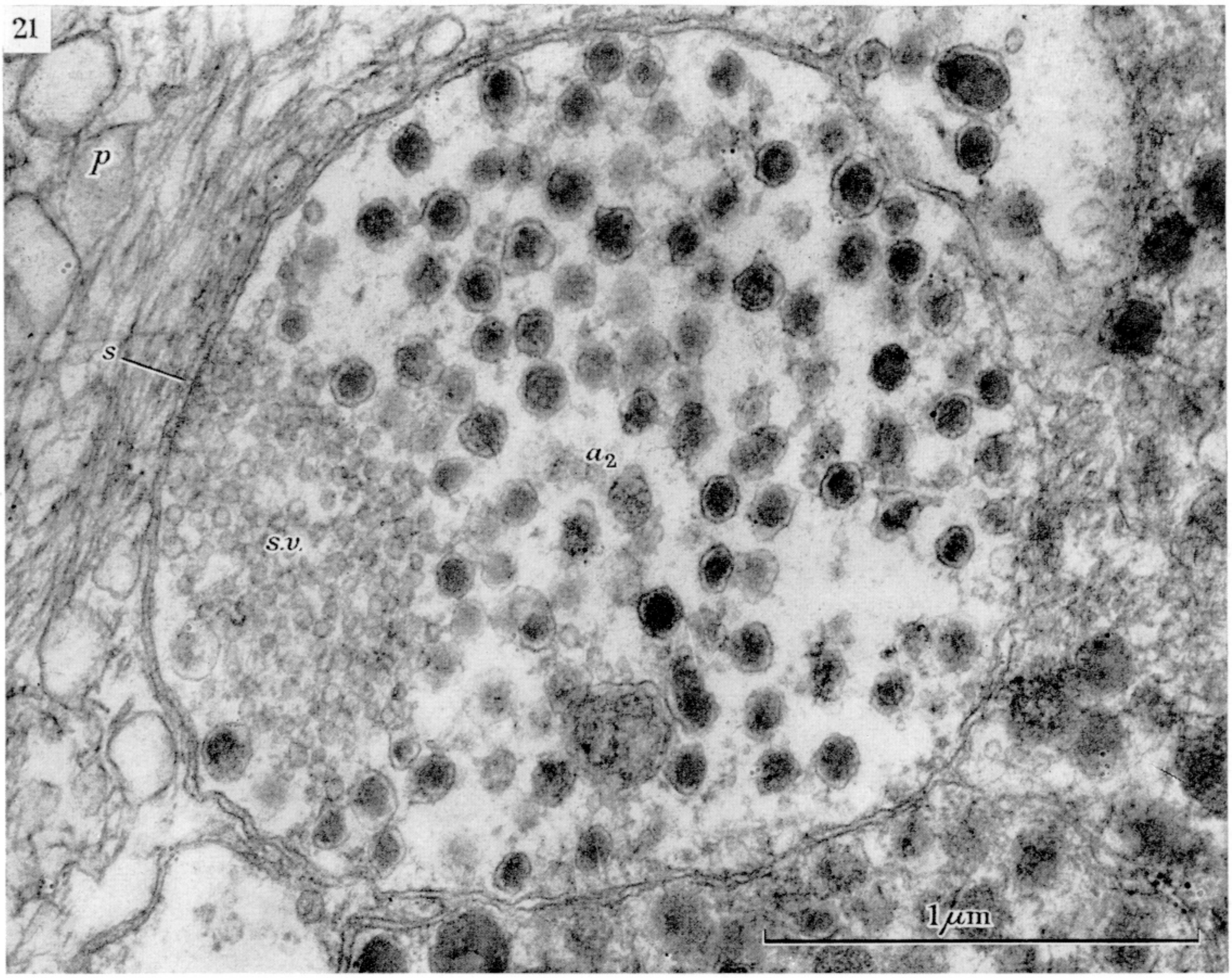
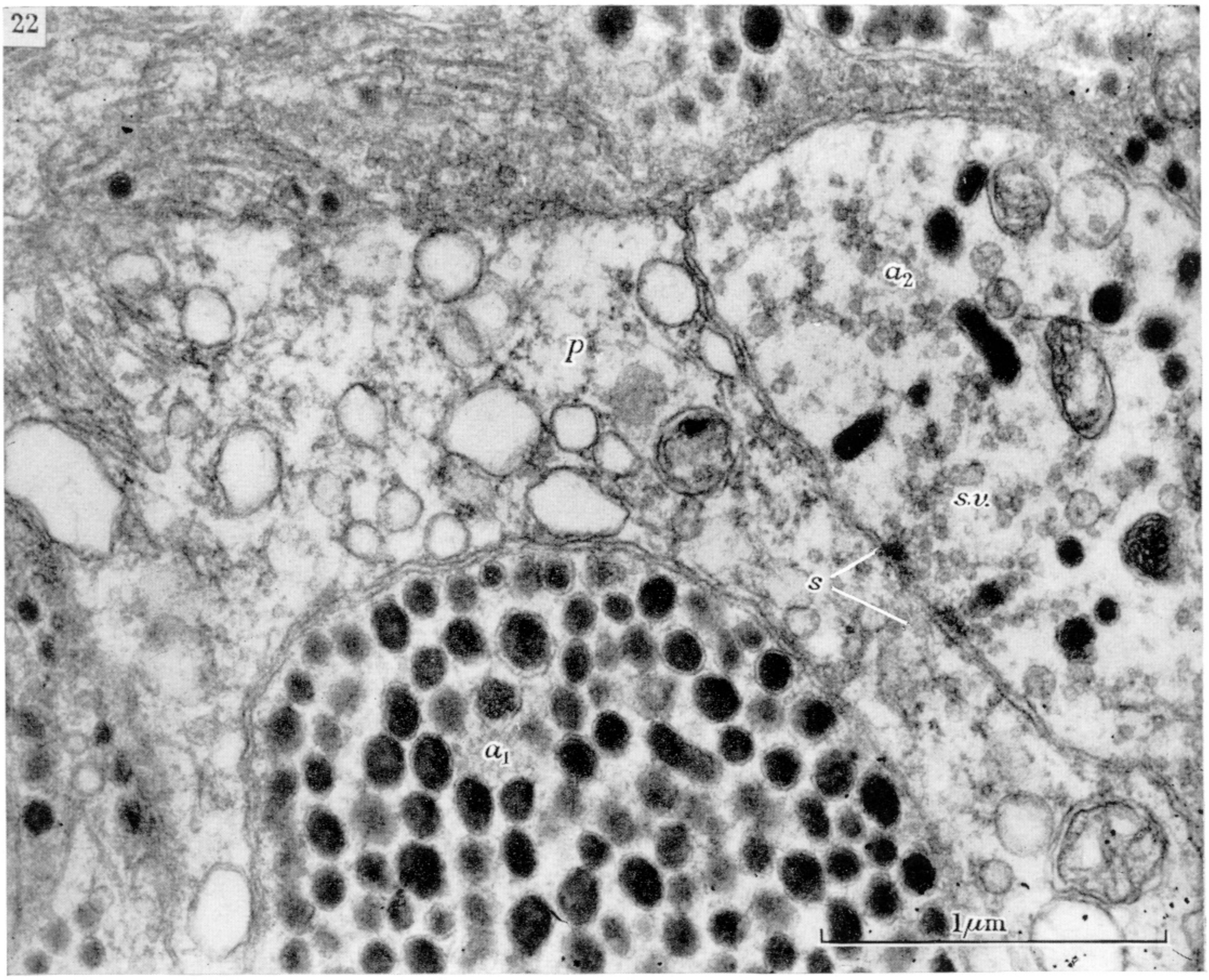


FIGURES 20, 21. The relation between Type A_2 neurosecretory fibres (a_2) and a pituicyte (p) in *Anguilla*, *s*, synapse; *s.v.*, 'synaptic' vesicles.

22



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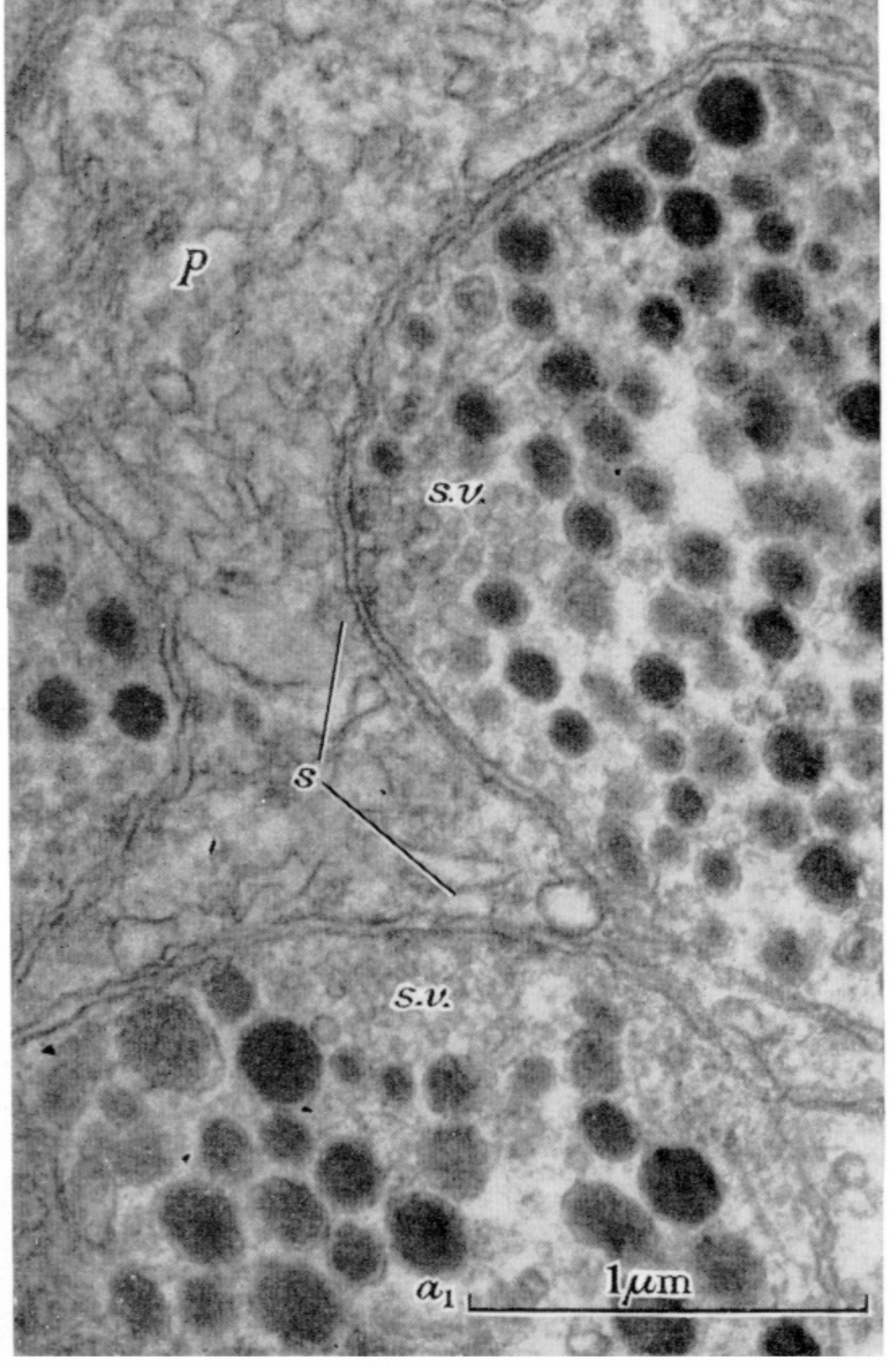

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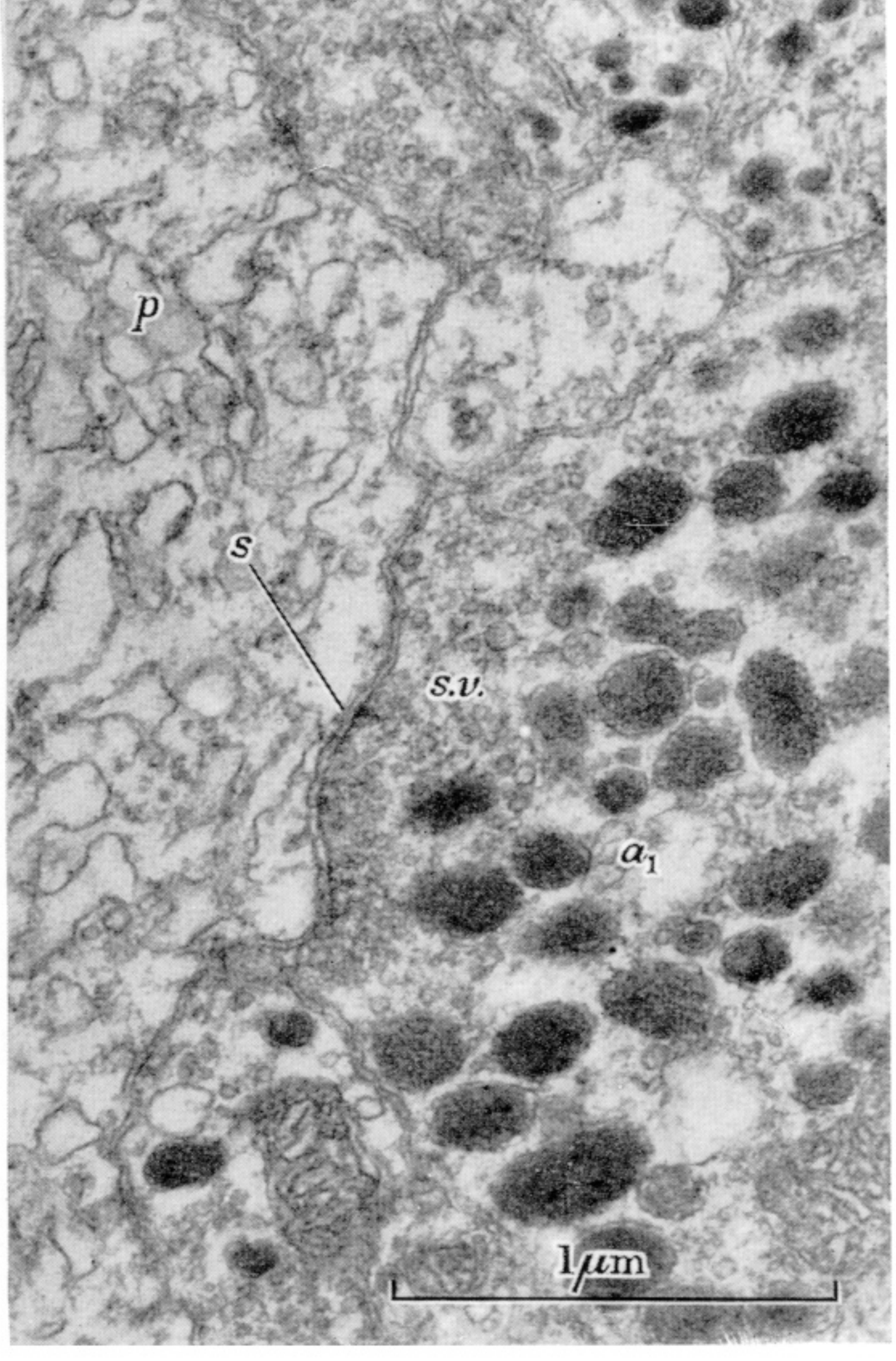
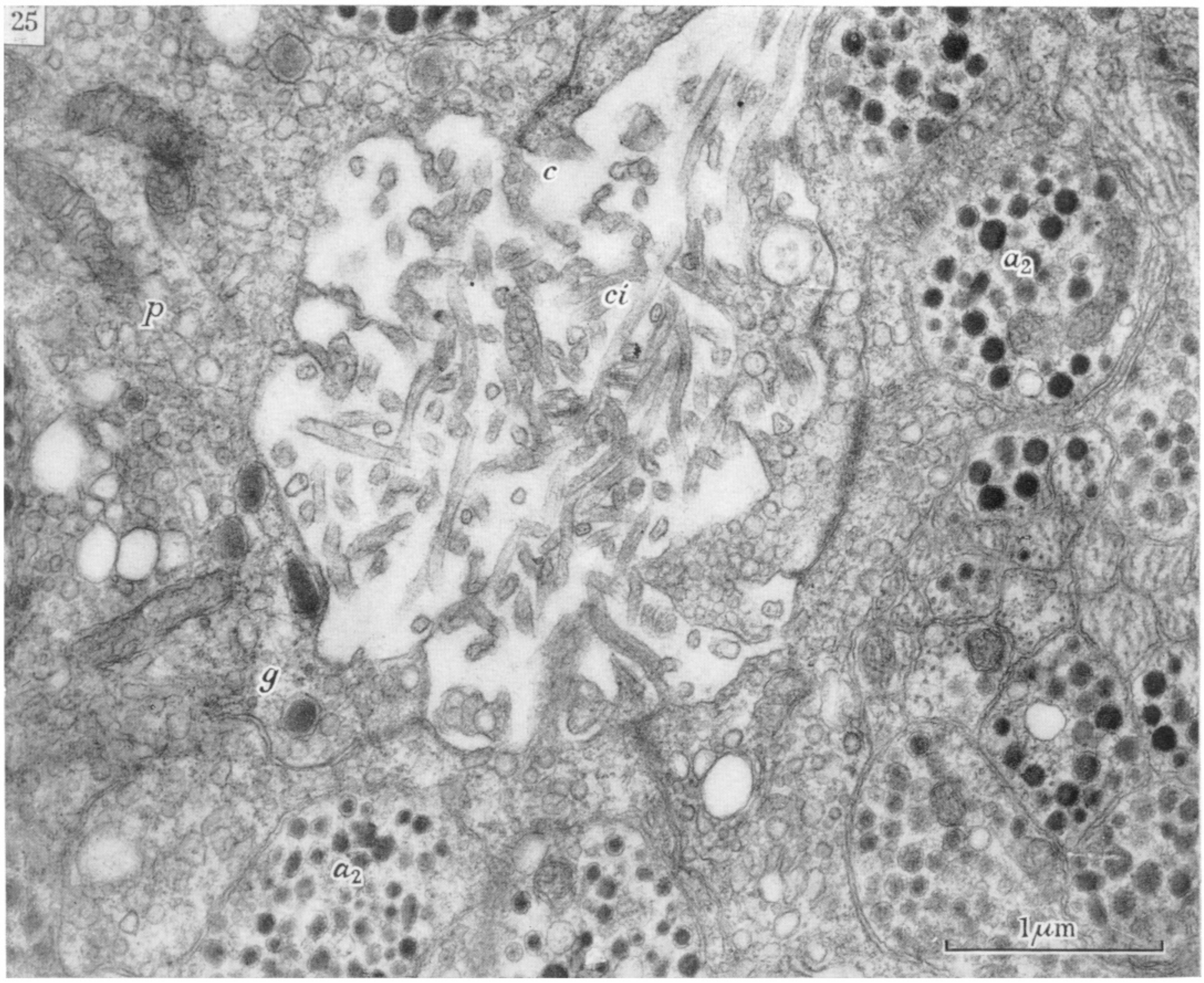


FIGURE 22. The relationship between neurosecretory fibres and pituicytes in an eel (*Anguilla*) kept in fresh water on an illuminated white background. a_1, a_2, Type A fibres; *p*, pituicyte; *s*, synapse; *s.v.*, 'synaptic' vesicles.

FIGURE 23. As figure 22 but from an eel maintained on a white background in sea water. Lettering as in figure 22. Synapses between A_1 fibres and a pituicyte.

FIGURE 24. As figure 23 but from an eel on a black background. Lettering as in figure 22.



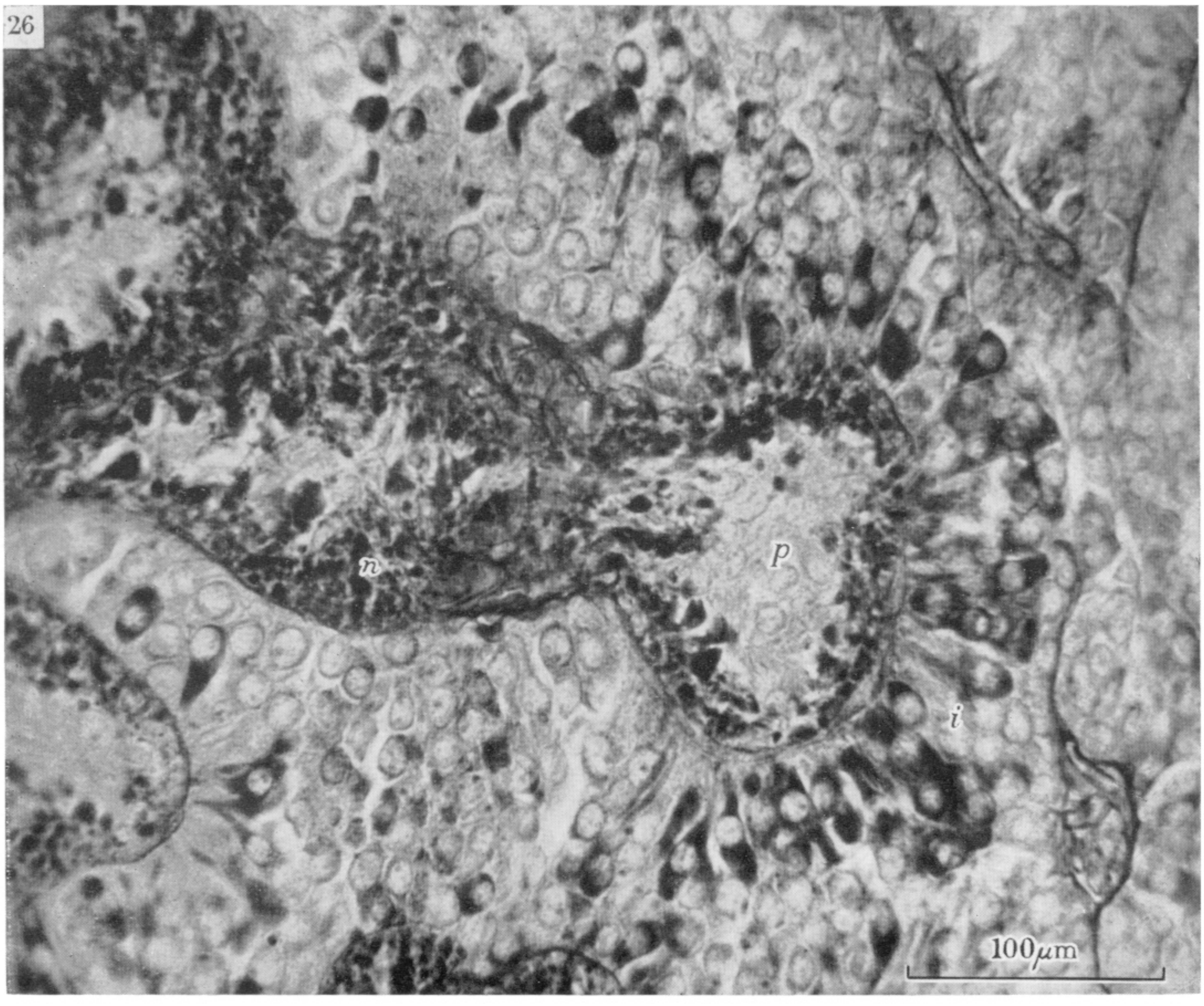
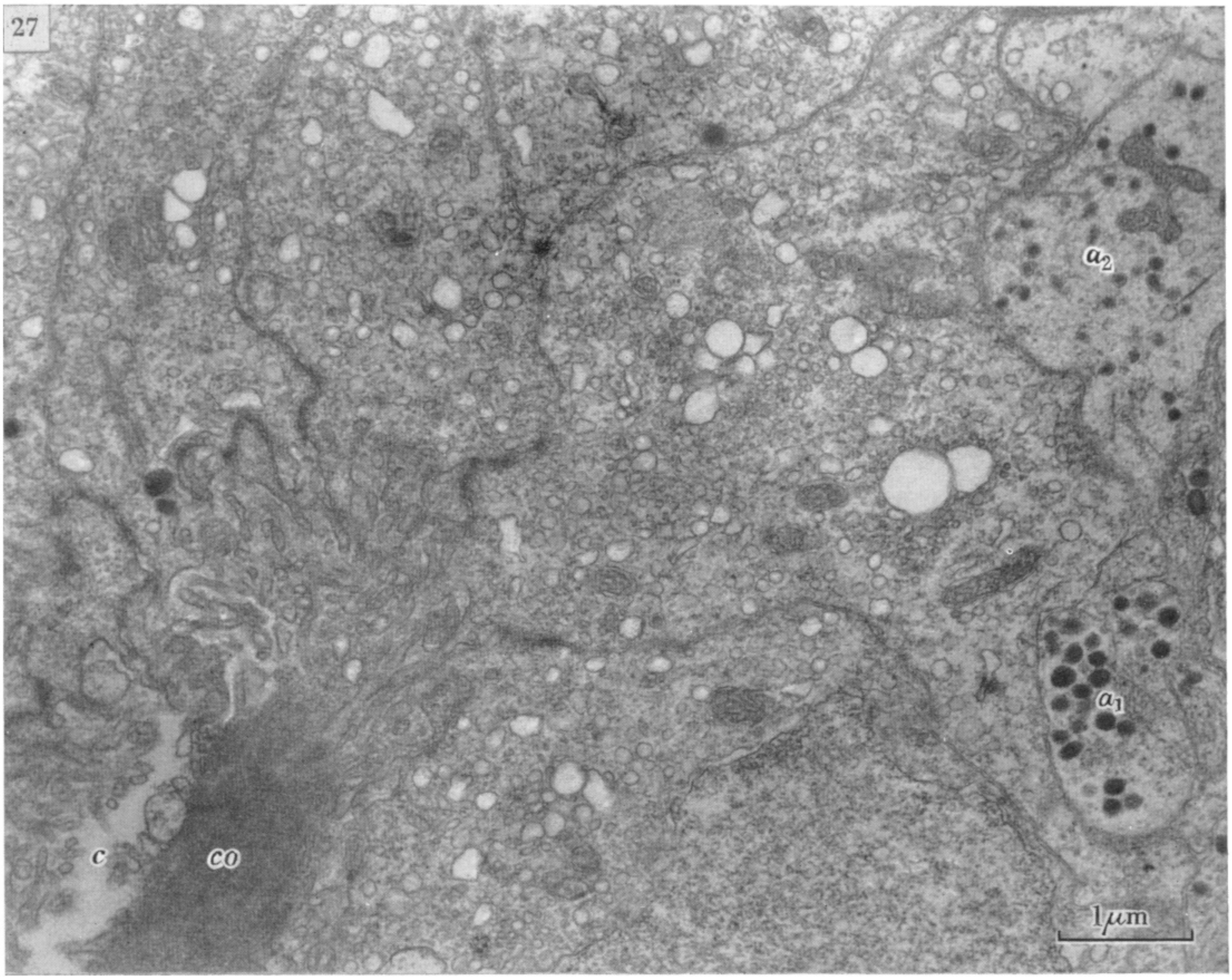


FIGURE 25. An electron micrograph of the central canal in a neurosecretory tract of an eel kept on an illuminated black background. a_2, Type A_2 fibres; *c*, central canal; *ci*, cilia; *g*, granule in pituicyte; *p*, pituicyte.

FIGURE 26. An optical micrograph of a portion of the neuro-intermediate lobe of an eel kept on an illuminated black background. *i*, Intermedia cells; *n*, neurosecretory tract; *p*, pituicytes. (Staining by alcian blue–*PAS*–orange G.) Note the many *PAS*-positive pars intermedia cells.



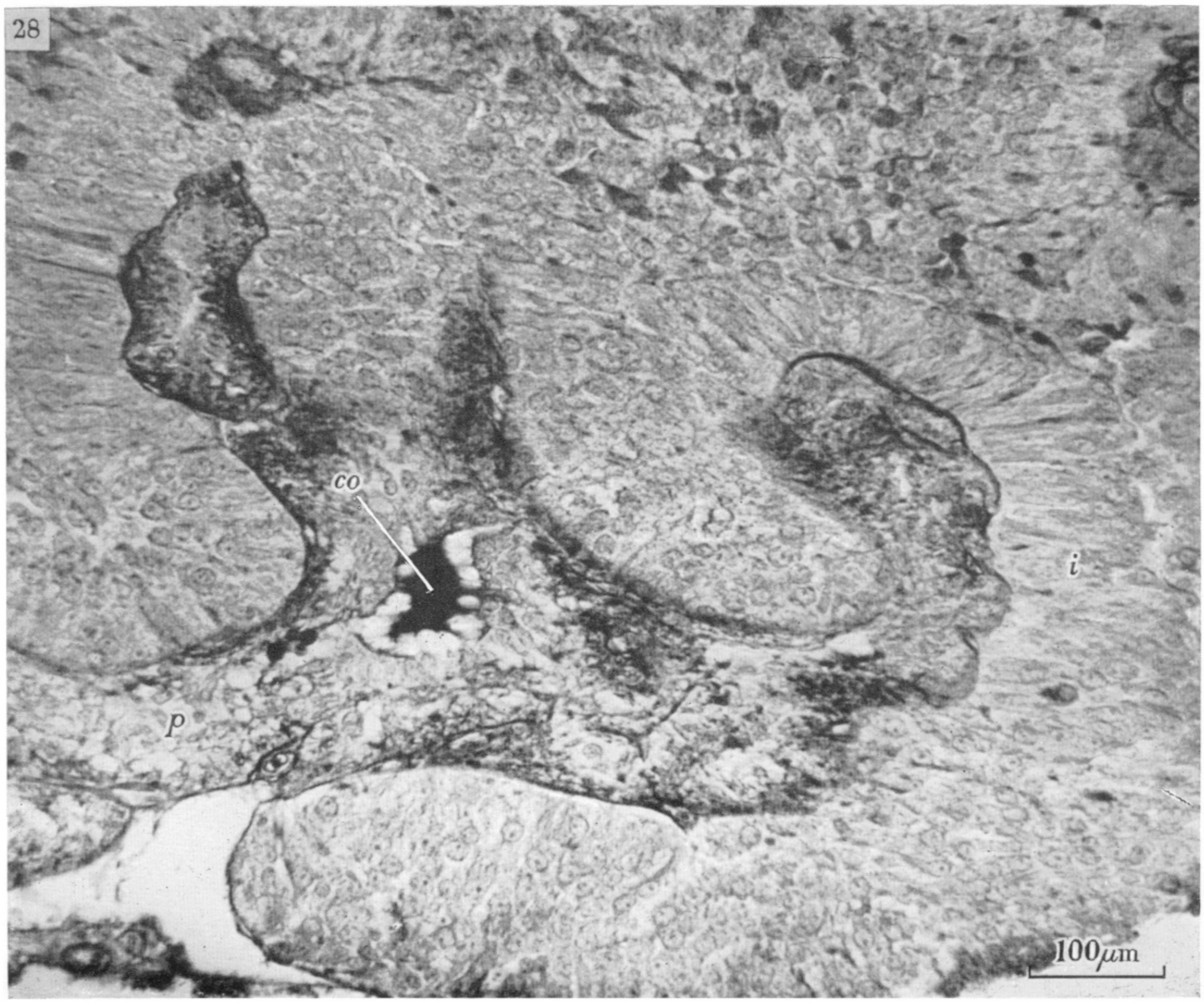


FIGURE 27. An electron micrograph of the central canal in a neurosecretory tract of an eel kept on an illuminated white background. Lettering as in figure 25. a_1, a_2, A_1 and A_2 fibres; *co*, colloid.

FIGURE 28. An optical micrograph of a portion of the neuro-intermediate lobe of an eel kept on an illuminated white background. Staining and lettering as in figure 26. Note the absence of *PAS*-positive pars intermedia cells. *co*, Colloid.