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

II. THE STRUCTURE AND INNERVATION OF THE PARS DISTALIS AT DIFFERENT STAGES OF THE LIFE-CYCLE

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A number of distinct cell types may be recognized in the pituitary of the eel at the level of ultrastructure by reason of the specificity of the size and electron-density of the granules they contain. The size of the granules and changes in the different cell types at different stages of the life-cycle permit a tentative identification in terms of function.

The pars distalis of the eel pituitary receives the greater part of its innervation from the nucleus lateralis tuberis by Type B neurosecretory fibres (Knowles 1965*a*), which do not stain with the so-called neurosecretory stains, but which nevertheless contain elementary neurosecretory vesicles. Type A, or classical, neurosecretory innervation is also present and seems to be of special importance at certain stages of the life-cycle. The possible function of these two forms of neurosecretory innervation is discussed.

The relationship between the intrinsic endocrine cells of the pars distalis and their neurosecretory innervation is fundamentally similar, at the level of ultrastructure, to that of the neuro-intermediate lobe. There are no direct contacts between the neurosecretory fibres and the intrinsic endocrine cells, but the proximity of the fibre terminals (*ca.* 2000 to 4000 Å) to endocrine cells indicates a functional relationship between these two elements of the pituitary of the eel.

1. Introduction

Experimental evidence has indicated that the hypothalamus exerts a control of function of the pituitary pars distalis of mammals (Harris 1955), though no actual penetration of the pars distalis by neurosecretory fibres has been observed in this group. In the lower vertebrates, however, neurosecretory fibres from the hypothalamus invade the pars distalis of fishes (see Dodd 1963). Da Lage (1955), using the light microscope, found in *Hippocampus* that some fibres which stained with 'neurosecretory' stains appeared to make

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contact with gonadotrophic cells. Follenius & Porte (1962) showed that at the level of ultrastructure there is a close relation between neurosecretory fibres and cells of the pars distalis in two teleost species (*Lebistes reticulatus* and *Perca fluviatilis*). The fibres which they studied contained granules, ranging in diameter from 1000 to 2000 Å, and so correspond to Type A neurosecretory fibres (Knowles 1965*a*).

There have been indications also, at the level of light microscopy, that not only 'classical' neurosecretory fibres (i.e. those stainable by Gomori, alcian blue or aldehyde fuchsin), but also other neurosecretory elements may be concerned in the control of pars distalis function in fishes (Stahl & Leray 1962; Billenstien 1963). The fibres described resemble Type B fibres (Knowles 1965*a*) in that they do not stain with the 'neurosecretory' stains, though they do apparently contain secretory material stainable by other methods. It has, moreover, been shown that changes in the amount of this stainable material can be related to the sexual cycle (Stahl & Leray 1962; Billenstien 1963).

There are therefore indications that neurosecretory control of pars distalis function in teleost fishes may be affected by at least two different kinds of neurosecretory fibre, which resemble the Type A and Type B fibres already described in the neuro-intermediate lobe of the elasmobranch pituitary (Knowles 1965*b*). The origin and course of these fibres and the precise relationship between their terminals and cells of the pars distalis has however not yet been studied at the level of ultrastructure.

It has been suggested by various authors, based on light microscope observations, that fibres originating in the nucleus lateralis tuberis innervate the pars distalis of the teleost pituitary (Bargmann 1953; Stutinsky 1953; Bern & Knowles 1966). It has also been shown with the electron microscope (Mellinger 1963; Follenius 1963) that neurons of the nucleus lateris tuberis in some elasmobranch and teleost fishes contain electron dense granules with a diameter of less than 1000 Å, which thus resemble Type B neurosecretory fibres (Knowles 1965*a*). The destination of these fibres was, however, not ascertained, and it is therefore not yet known whether it is they which innervate the pars distalis and represent those which have been seen with the light microscope. Type B fibres have not as yet been demonstrated in the pars distalis of any vertebrate pituitary at the level of ultrastructure.

The eel offers certain advantages for a study of the neurosecretory innervation of the pars distalis because: (*a*) it has been shown that classical neurosecretory material is present in the pars distalis (Bargmann 1953) and that this region also receives a rich innervation from the nucleus lateralis tuberis (Stutinsky 1953); (*b*) the function of many of the cell types has been affirmed by experiment (Olivereau 1965) and supported by observations of these cells at different stages of the life-cycle (Knowles & Vollrath 1966*a*); (*c*) distinct phases in the life-cycle of the eel *Anguilla* are marked by changes in metabolism and sexual maturity known to be under pituitary control.

In the preceding paper (Knowles & Vollrath 1966*b*), the relationship between neurosecretory fibres and cells of the neuro-intermediate lobe of the eel pituitary has been demonstrated. In this present account the relationship between Types A and B neurosecretory fibres of hypothalamic origin and the different cell types in the pars distalis of the eel pituitary at different stages of the life-cycle, is described.

2. Materials and methods

Golden and silver eels were obtained from Birmingham, Downtown and Tewkesbury (see preceding paper). From the hundred eels used for the study of the neuro-intermediate lobe fourteen pituitaries were selected for study of the pars distalis with the electron microscope, and thirty-eight for study by histological methods. In all ten golden eels and four silver eels were studied with the electron microscope and thirty-four golden eels and four silver eels by the following methods: (*a*) Herlant's tetrachrome stain (Herlant 1960); (*b*) Billenstien (Billenstien 1963); (*c*) lead haematoxylin (MacConaill 1947); (*d*) alcian blue–*PAS*–orange G (Herlant 1960); (*e*) aldehyde fuchsin; (*f*) the Bargmann modification of Gomori's method (Pearse 1960); (*g*) Holmes's axon stain (Holmes 1942); (*h*) Van Giesons's method; (*i*) azan; and (*j*) haematoxylin–eosin.

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.

3. General morphology of the pars distalis

Some accounts of the general morphology of the eel pituitary have already been given (Bargmann 1953; Stutinsky 1953; von Hagen 1936; Bernardi 1948; Evans 1940; Olivereau 1965). A distinction between a rostral pars distalis and a proximal pars distalis may be clearly seen (see figure 4, plate 59). These two regions correspond to the pro-adenohypophysis and meso-adenohypophysis in the nomenclature proposed by Pickford & Atz (1957) and used by Dodd (1963) and others. The proximal pars distalis corresponds to the Übergangsteil of some of the earlier workers. The nomenclature adopted in this paper is the one recommended by the Division of Comparative Endocrinology of the American Society of Zoologists (see Gorbman 1965).

(*a*) *Proximal pars distalis*

The greater part of the proximal pars distalis is occupied by cells which stain intensely with orange G. It has been suggested that these cells, which are especially abundant in the dorsal part, are the somatotropic (*STH*) cells of the pituitary (Olivereau 1965).

Smaller cells which stain a purple colour with *PAS*–alcian blue border the intervascular spaces which extend vertically through the proximal pars distalis (figure 1); these cells stain less intensely than the thyrotropic (*TSH*) cells. They are restricted to the sides of the intervascular channels in the proximal pars distalis especially in the ventral part of the gland. Olivereau & Herlant (1960) have attributed to these cells a gonadotropic (*GTH*) function, since they altered following injection of prolan. Knowles & Vollrath (1966*a*) have shown that their appearance and frequency at different stages of the life-cycle accords with this view.

(*b*) *Rostral pars distalis*

The greater part of the rostral pars distalis is occupied by cells grouped in spherical or elongate follicles or rosettes (figure 1). Von Hagen (1936) has shown that in the young elver these cells are all acidophil, but that at metamorphosis a few cells in each follicle

become basophilic. A number of basophilic cells are also found lying in groups outside the follicles in the adult eel, bordering the intervascular spaces. These cells are also stainable purple by alcian blue-*PAS*; Olivereau (1965) has presented experimental evidence for a possible *TSH* function of these cells. The acidophil cells of the rosettes stain yellow with orange G: they have been termed prolactin cells (Olivereau 1965).

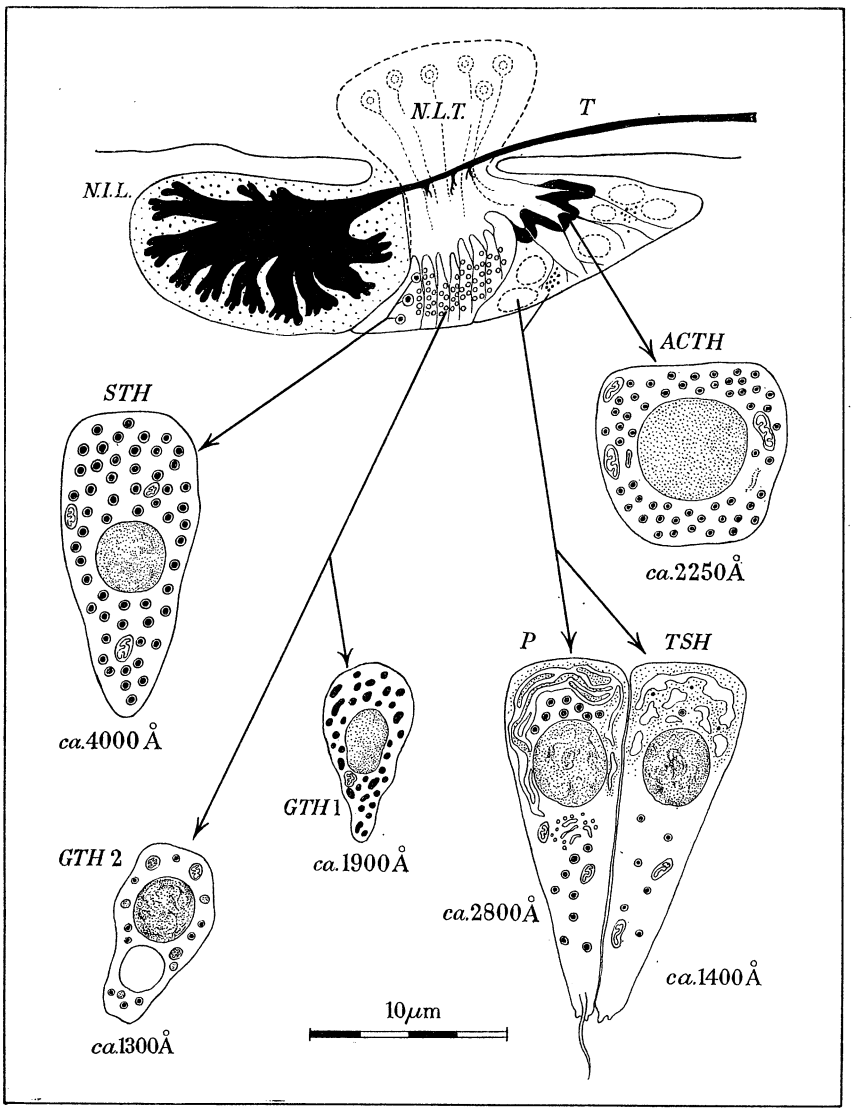


FIGURE 1. Cell types in the pars distalis of the pituitary of *Anguilla* and their position in relation to the neurosecretory innervation. The cells may be distinguished by their dimensions and by the size of the granules they contain; also by their localization in the gland. Their functional designation (*ACTH*, GTH_1, GTH_2, *P*, *STH* and *TSH*) is discussed in the text. *N.I.L.*, Neurointermediate lobe; *N.L.T.*, nucleus lateralis tuberis; *T*, tract from nucleus preopticus.

The dorsal region of the rostral pars distalis contains many cells which can be distinguished by the lead haematoxylin technique (MacConaill 1947; Olivereau 1964). These cells border the nerve tracts which invade the rostral pars distalis. Olivereau & Ball (1964) have presented experimental evidence for their corticotropic (*ACTH*) function.

Nerve fibre tracts, tapering distally, penetrate the two anterior lobes of the pituitary. They extend more deeply into the proximal pars distalis than the rostral pars distalis, and more deeply in the lateral region of the pars distalis than in the centre of this lobe. The nerve fibre tracts stain with silver stains and a few fibres seem to stain also with the neurosecretory stains (Gomori, alcian blue) especially in the more mature animals; in one animal large *PAS*-positive colloid masses of unknown significance were seen in the tracts (figure 5, plate 59).

4. Ultrastructure of the intrinsic endocrine cells

The differences between the ultrastructure of the cell types in *Anguilla* and *Conger* were few and slight and so these two forms are not treated separately except where such differences occur.

(*a*) *Proximal pars distalis*

Under the electron microscope three clearly distinct cell types may be identified in the proximal pars distalis by reasons of their size and also the size of the inclusions they contain.

(1) STH *cells* (Plates 60 and 61).

These cells measuring 15 μm by 6 μm are found in all parts of the proximal pars distalis, but are especially abundant in areas close to the neurosecretory nerve tracts.

They appear filled by spherical or slightly oval membrane-bound vesicles *ca.* 4000 Å in diameter. No clear indication of endoplasmic reticulum or Golgi apparatus can be seen and mitochondria are small and few in number in *Anguilla*. In *Conger* there were more indications of cytomembrane systems (figure 6, plate 60), and the vesicles were sometimes less electron-dense and more elongate than in the golden eel.

(2) GTH *cells* (Plates 60 and 61, figure 30, plate 71).

Two smaller cell types measuring about 10 μm by 5 μm, are found, especially in the more ventral regions of the proximal pars distalis, bordering the intervascular channels (figure 7, plate 60). One cell type, *GTH* 1, contains electron-dense vesicles *ca.* 1900 Å in diameter; many of these vesicles are elongate (figure 1). The other cell type (*GTH* 2) contains relatively few small spherical, only slightly electron-dense vesicles, each *ca.* 1300 Å in diameter and occasional larger vesicles. Endoplasmic reticulum and Golgi apparatus are not obvious in these cells; mitochondria are small, though frequent.

At present it is not possible to attribute distinct specific functions to the two cell types distinguishable by electron microscopy, but a comparison between the inclusions of these cells and those of follicle-stimulating hormone (*FSH*) cells and luteotropic (*LH*) cells of higher vertebrates suggests that the *GTH* 2 cell type is more likely to have *FSH* action; it is therefore interesting that changes in this cell type take place immediately before migration to breeding grounds (figure 30, plate 71). In the present studies it was found that large vacuoles appeared in this cell type in more mature eels; large vacuoles were not found in younger eels, though a few small vacuoles were occasionally seen.

The vacuoles were not bounded by membranes (see figure 30, plate 71); it seems possible that they may indicate aggregations of saturated lipid material which was

dissolved in the reagents used during the preparation of the pituitary for electron microscopy. Inclusions have been described in basophil cells of the proximal pars distalis of the carp by Kurosumi, Kobayashi & Watanabe (1963), and Legait & Legait (1958).

(*b*) *Rostral pars distalis*

In the rostral pars distalis three cell types can be distinguished.

(1) ACTH *cells* (Plates 62 and 63)

Sections through the dorsal portion of the rostral pars distalis (see figure 1) contain *ACTH* cells arranged in two or three layers, bordering the projections of the nerve tracts. These cells stain with lead haematoxylin and under the light microscope have the appearance already described by Olivereau (1964).

Under the electron microscope each *ACTH* cell is cuboid in shape and about 9 μm by 10 μm (figure 1 and figure 10, plate 62). The cytoplasm which is moderately electron-dense contains numerous fine vesicles or microtubules. The endoplasmic reticulum is diffuse and not prominent, and a Golgi area is rarely seen. The nucleus is relatively large, measuring 5·5 μm in diameter.

Typically the cytoplasm of the *ACTH* cells is evenly packed with electron-dense granules measuring 2000 to 2500 Å in diameter. In *Conger* these granules are sometimes elongate (figure 12, plate 63). In *Anguilla* they are generally spherical or subovate (figure 13, plate 63).

(2) *Follicle cells* (Plates 64, 65, 66 and 67)

The greater part of the rostral pars distalis consists of cells grouped in spherical or elongate follicles. Olivereau (1965) and von Hagen (1936) have remarked that each follicle contains two types of cell, distinguished by their staining reactions. At the level of ultrastructure these two cell types may be distinguished by the size and density of the granules which they contain.

(*a*) '*Prolactin*' *cells*. The more abundant cell type which has tentatively been identified as a 'prolactin' cell measures approximately 6 μm by 19 μm in *Conger* and *Anguilla*. The 'prolactin' cells are cone-shaped (see figure 1); their narrow extremity, which borders a central space of the follicle, was ciliated (figure 17, plate 65, and figure 21, plate 67. The prolactin cells contain vesicles *ca.* 2800 Å in diameter in *Anguilla* and 3500 Å in *Conger*. The vesicles are spherical in *Anguilla* but sometimes elongate in *Conger*. The electron density is sometimes uniform and sometimes paler at the centre and darker at the periphery.

The endoplasmic reticulum is regular and prominent and lies at the periphery of the rosette, close to an intervascular channel (figure 15, plate 64).

Secretory vesicles are more abundant in the endoplasmic reticulum and Golgi areas of the cell than near the centre of the follicle. The Golgi zone lies on the other side of the nucleus, near the centre of the follicle (figure 20, plate 67). Throughout the cell mitochondria are present, though more abundant in the lower part of the cell. They were larger in *Conger* than in *Anguilla*, especially in one individual (plate 64).

(*b*) TSH *cells*. The *TSH* cells are clearly distinguishable from 'prolactin' cells by the smaller vesicles (*ca.* 1400 Å) they contain. The endoplasmic reticulum is diffuse, with

wide cisternae (figure 19, plate 66). Many of the *TSH* cells did not seem to be contained in follicles (e.g. figure 19, plate 66), though some evidently were (plate 65). The *TSH* cells appear to border the intervascular spaces, whether in follicles, or not (figure 18, plate 66). The majority of the *TSH* cells lie in the dorsal region of the rostral pars distalis and therefore close to the neurosecretory tracts.

The *TSH* cells of mature silver eels, caught in the autumn on their way to the sea, showed an extremely prominent endoplasmic reticulum and a greater concentration of secretory vesicles (figure 28, plate 70). It is interesting to compare these observations with those of Knowles & Vollrath (1966*a*), who found that eels caught in the sea at Naples in the spring had abundant and prominent *TSH* cells.

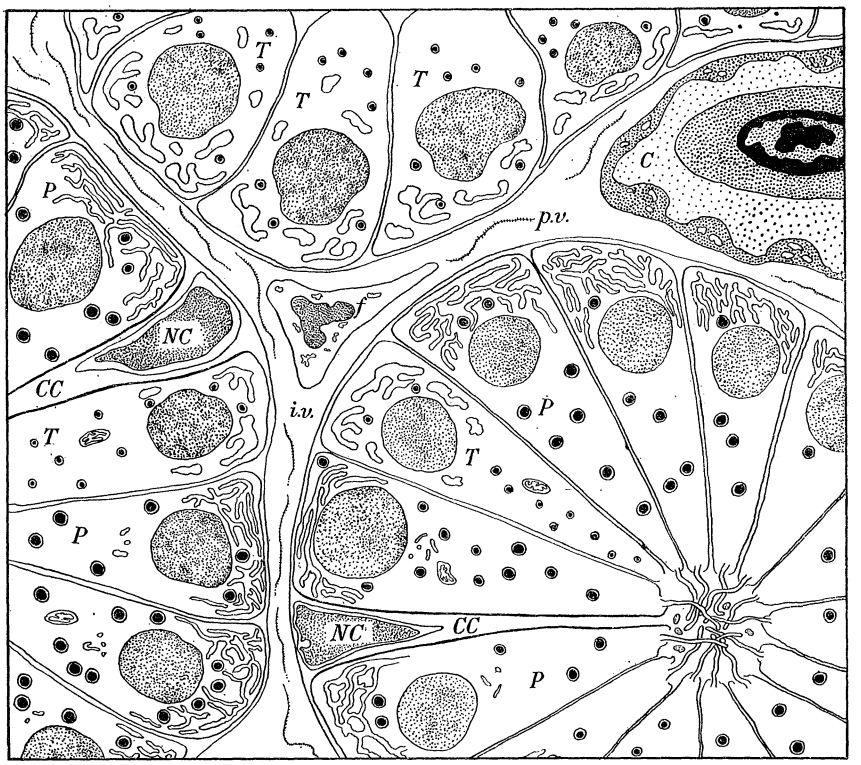


FIGURE 2. A small portion of the rostral pars distalis, showing the relationship between intrinsic cells and extravascular spaces. *C*, Capillary; *CC*, connecting canal; *f*, fibroblast; *i.v.*, intervascular channel; *NC*, neck cell; *P*, 'prolactin' cells; *p.v.*, perivascular space; *T*, thyrotroph.

Besides the secretory cells a few small cells with little cytoplasm may be seen in each follicle. They are here termed neck-cells because they lie in a special relation to fine canals (figure 18, plate 66) which extend from the intervascular channel to the centre of the follicle (see figure 2). They border the intervascular channel (see figure 15, plate 64). Their function is not known, but it is suggested that an alteration in size of these cells could open or close the fine canals they adjoin and thus permit or impede the passage of material to or from the centre of the follicles. Thus a regulation of the activity of the follicle cells could be affected if the neck cells responded to substances in the blood-stream, or its tonicity. In this connexion it is interesting to note that Pickford, Robertson & Sawyer (1965) have suggested that the prolactin-like hormone of *Fundulus* plays an important part in the osmotic regulation of this fish.

5. Innervation of the pars distalis

Under the electron microscope it is possible to see that the nerve fibre tracts which penetrate the two anterior lobes of the pituitary do not make direct contact with the intrinsic endocrine cells but are separated from them by extravascular spaces (Knowles & Vollrath 1966*b*). It would appear that the fibre tracts, covered by a basement membrane sheath (figure 3), extend into the extravascular system and apparently discharge their products into the perivascular and intervascular spaces; clusters of vesicles of synaptic size range, and an electron density of portions of the fibre membranes at the surface,

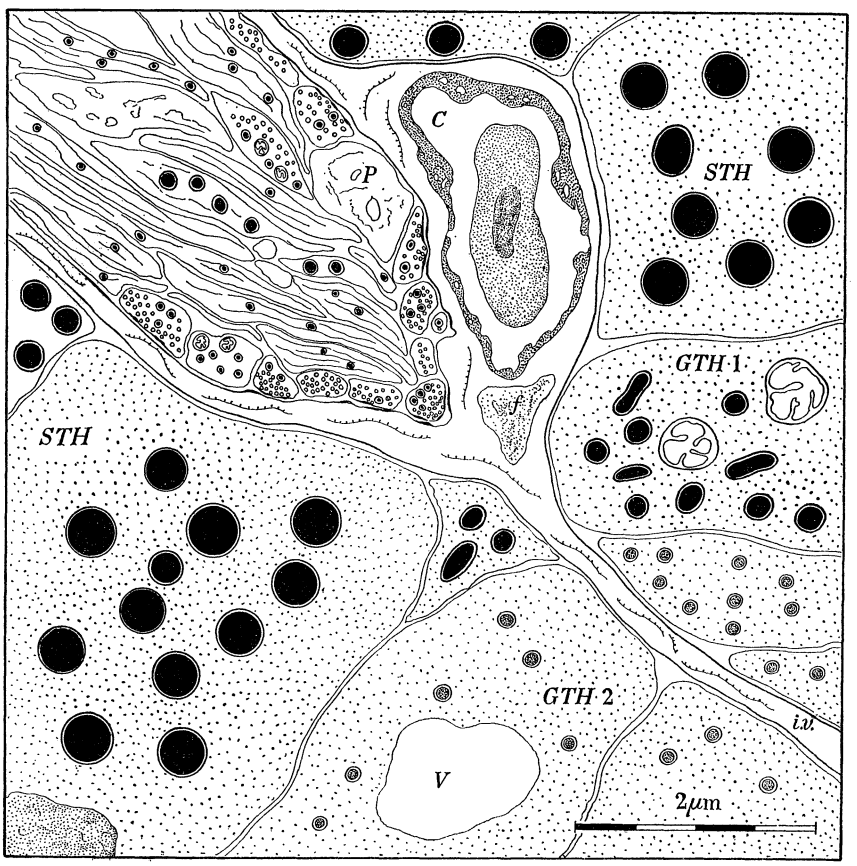


Figure 3. The relationship between neurosecretory fibre terminals, intrinsic endocrine cells and the blood system in the proximal pars distalis of the eel pituitary. (*GTH* 1, *GTH* 2, *STH*, Intrinsic endocrine cells; *i.v.*, intervascular channel; *v*, vacuole. *f*, fibroblast.

indicate possible points of hormone release (figure 11, plate 62, and plate 63). It is worthy of note that in some cases their terminals are separated from the intrinsic cells by a distance less than 3000 Å (plate 63). The general relationship between the intrinsic cells and their neurosecretory innervation (figure 3) appears basically similar to that already described in the preceding paper (p. 320) for the neuro-intermediate lobe.

The greater portion of the tracts did not stain with the 'neurosecretory' stains, yet under the electron microscope it may be observed that most of the fibres in these tracts contain membrane-bound vesicles with an electron-dense centre approximately 700 Å in diameter. These vesicles closely resemble the B type vesicles described in an elasmobranch

pituitary (Knowles 1965*b*). Meurling (1963) has shown that fibres which do not stain with 'neurosecretory' stains are present in the elasmobranch pituitary. Arguments have been presented why they should be termed neurosecretory (Bern & Knowles 1966).

Some fibres in the nerve tracts penetrating the two anterior lobes of the pituitary contain spherical membrane-bound vesicles with electron-dense contents *ca.* 1400 Å in diameter (Type A 3). These correspond in appearance to the typical elementary neurosecretory vesicles described in many neurosecretory systems (i.e. Type A fibres) and it seems probable that it is these fibres which stain with the neurosecretory stains.

The frequency of these Type A fibres appears to vary with the maturity of the animal, for they appeared to be more abundant in silver eels than in younger eels, both under the electron microscope and in alcian blue preparations (figures 27 and 29, plates 70 and 71).

Pituicytes resembling those in the neuro-intermediate lobe (preceding paper, p. 320) were very rare and there were no indications of a central canal in the distal portions of the nerve tracts. Synapses between Type A or Type B fibres and pituicytes were not detected; only a few possible contacts of uncertain significance were seen.

Under the light microscope it could be seen that most of the nerve fibres which invade the pars distalis originate in the nucleus lateralis tuberis (figure 1). The electron microscope observations accord with this finding. Vesicles resembling Type B vesicles were observed in perikarya of this nucleus (figure 24, plate 69). B type fibres were also present, and some appeared to make synaptic contacts either with dendrites (figure 25, plate 69) or other fibres (figure 26, plate 69).

The nucleus preopticus also was examined with the electron microscope. Elementary neurosecretory vesicles of Type A were found in perikarya, but as yet no clear distinction of Type A vesicles of different sizes (Type A_1, A_2, A_3) have been detected in the nucleus preopticus. Many Type B fibres were also present in this region, though perikarya containing such vesicles were not found. The origin and destination of these Type B fibres has not yet been studied. Their presence in the nucleus preopticus region indicates the interesting possibility that this region and the nucleus lateralis tuberis may be linked by Type B fibres.

6. Discussion

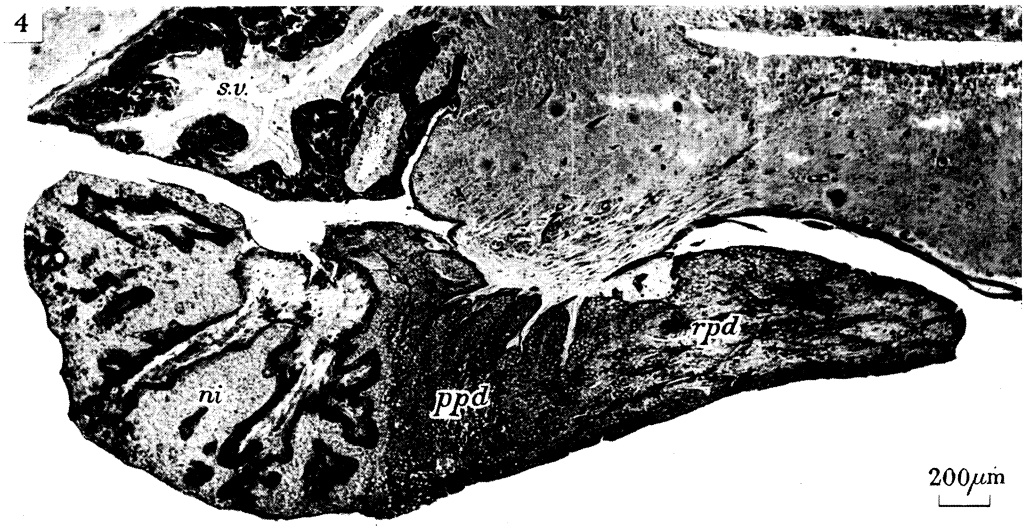
The classification of cell types in the proximal and rostral pars distalis of the eel pituitary by staining reactions and experimental procedures at the level of light microscopy (Olivereau 1965; Olivereau & Herlant 1960), has been supported by the use of the electron microscope. At the level of ultrastructure different cell types may be distinguished by their dimensions and by the size of their membrane-bound inclusions. These granules show a consistency of size in each cell type, possibly indicating that the size of a membrane-bound secretory droplet may bear a direct relation to the chemical composition of its contents. If this is so an identification of cell types in the pituitary at the level of ultrastructure may have certain advantages over histological or histochemical methods, for these may detect different products in a single cell type and so give the illusion of different cell types, whereas the electron microscope may reveal the final product of synthesis and so enable a clearer distinction to be made between different cell types.

In particular a study of the ultrastructure of the eel pituitary allows a clear distinction to be made between two types of cells which occupy the position in the gland where it has been suggested that gonadotrophs are present (Olivereau & Herlant 1960). It has been suggested by many authors that two types of gonadotroph are present in the fish pituitary (see Stahl 1963), but until the present studies, this had not been confirmed at the level of ultrastructure. In *Anguilla*, however, studies in ultrastructure correlated to stages in the life-cycle indicate the probable presence of two types of gonadotroph and show that one type, with features resembling *FSH* cells of higher vertebrates, shows changes as the animals migrate to their breeding grounds. The absence of change in the other type suggests a possibility that this type plays a part in the later phases of reproduction in the eel.

The regular arrangement of the different cell types in the eel pituitary has been noted by workers using the light microscope (see Olivereau 1965). Under the electron microscope the symmetry of this arrangement is even more striking. The reputed *ACTH* cells are separated from the neurosecretory tracts by only a narrow intervascular channel containing connective tissue: the reputed *GTH*, *TSH* and prolactin cells lie farther from the neurosecretory tracts, but border the continuation of the intervascular channel, into which the neurosecretory fibres apparently discharge their products. Thus neurosecretory innervation of *ACTH* function would seem to be more immediate than that of *GTH*, *TSH* or 'prolactin' function since for stimulation of these latter cells products released from the neurosecretory fibre terminals would have to travel a greater distance in the extravascular system. It is not yet clear whether this distinction is a functional one, or whether the different distances of the different cell types from their neurosecretory innervation has a morphological basis only. The factors which lead to the regular arrangement of the different cell types have not yet been studied; the extremely close proximity of the *ACTH* cells to the neurosecretory elements, and the grouping of *TSH* and *GTH* cells close to the intervascular channels indicate a possibility that the position of the cell types may be chemotactically determined during development, and that the differentiation of the *ACTH* cells depends on a proximity to the nervous system. It is interesting to note that Follenius & Porte (1962) have remarked that during development the first acidophil cells of the proximal pars distalis of *Lebistes* and *Perca* to differentiate are adjacent to a basement membrane with which neurosecretory fibres are in contact even at this stage of development; they have suggested that neurosecretory material may influence the differentiation of cells of the pars distalis. The importance of neurosecretory pathways in the differentiation of the adenohypophysis in amphibians has also been demonstrated (Voitkevich 1965).

Studies with the light microscope have shown that the relative numbers of the different cell types of the pars distalis alter during the life-cycle (Knowles & Vollrath 1966*a*); the origin of new cells has not been determined. Follenius (1966) suggests that they may be formed from undifferentiated tissues; a possible transformation of 'prolactin' cells to *TSH* cells must also be considered in view of the observations by von Hagen (1936).

The great abundance of *TSH* cells in the seawater eel taken in the spring (Knowles & Vollrath 1966*a*) may be a seasonal change or might be correlated to a sea water environment. The studies in ultrastructure of the silver eel pituitary however show that *TSH* cell activity appears high in eels still in fresh water, and it appears more likely therefore that



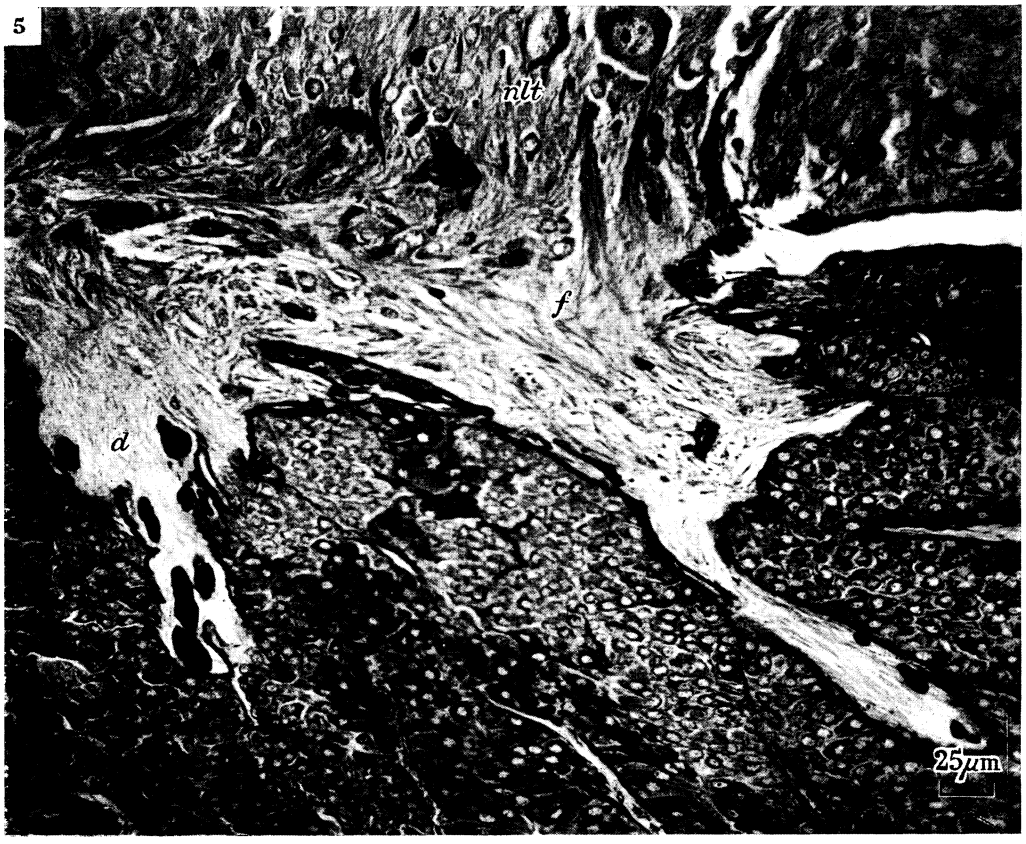


FIGURE 4. An optical micrograph of a lateral longitudinal section of the pituitary of *Anguilla*. *ni*, neuro-intermediate lobe; *ppd*, proximal pars distalis; *rpd*, rostral pars distalis. *s.v.*, saccus vasculosus. (Staining: alcian blue–*PAS*–orange G.)

FIGURE 5. An optical micrograph showing the penetration of the anterior lobes by fibres (*f*) originating in the nucleus lateralis tuberis (*nlt*). *d*, *PAS*-positive droplets.

(*Facing p.* 338)

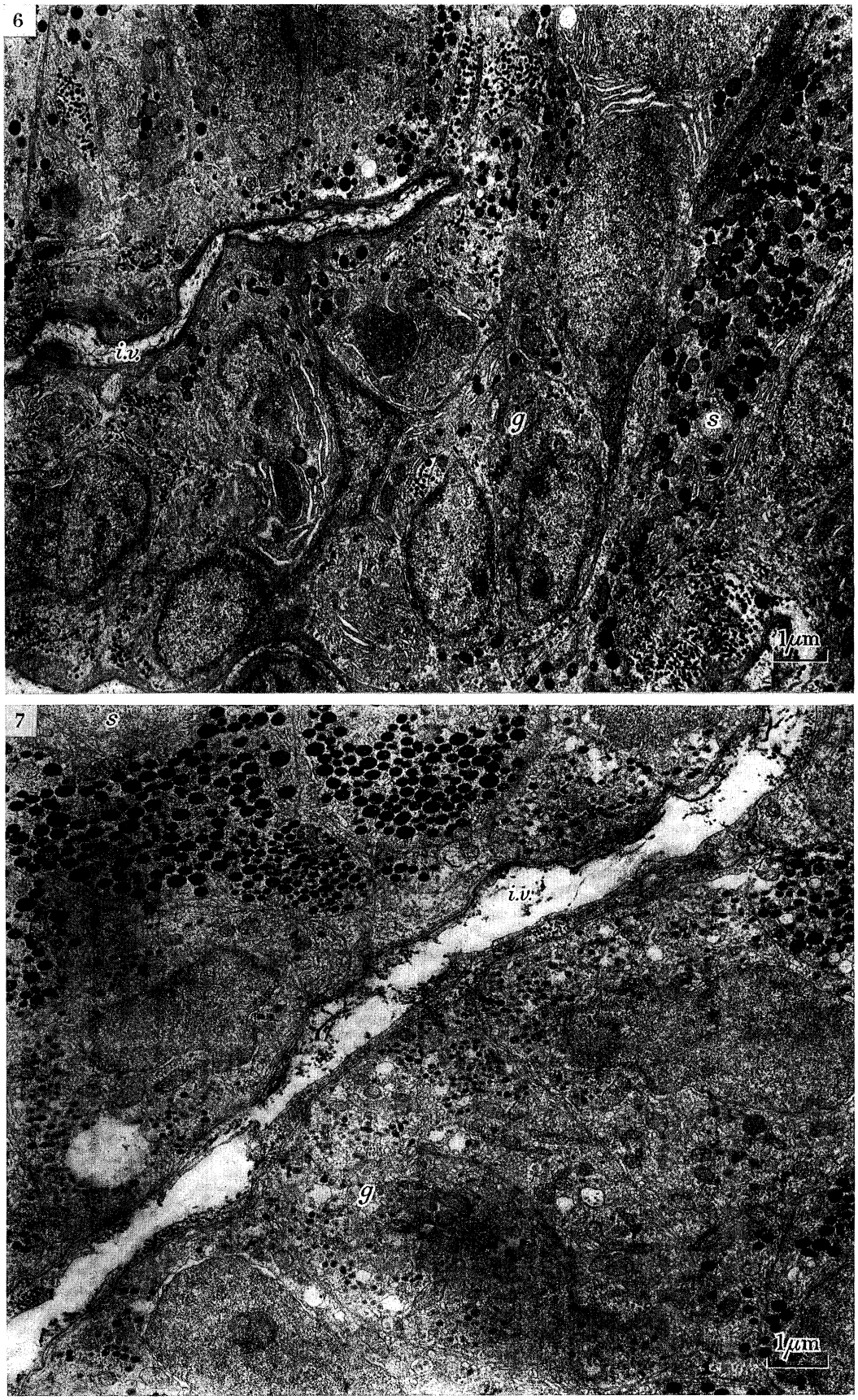


FIGURE 6. A survey picture of the proximal pars distalis of *Conger* pituitary. *g*, *GTH* cells; *s*, *STH* cells; *i.v.*, intervascular channel.

FIGURE 7. As figure 6 but the pituitary of *Anguilla*. *g*, GTH_2 cells; *i.v.*, intervascular channel; *s*, *STH* cells.

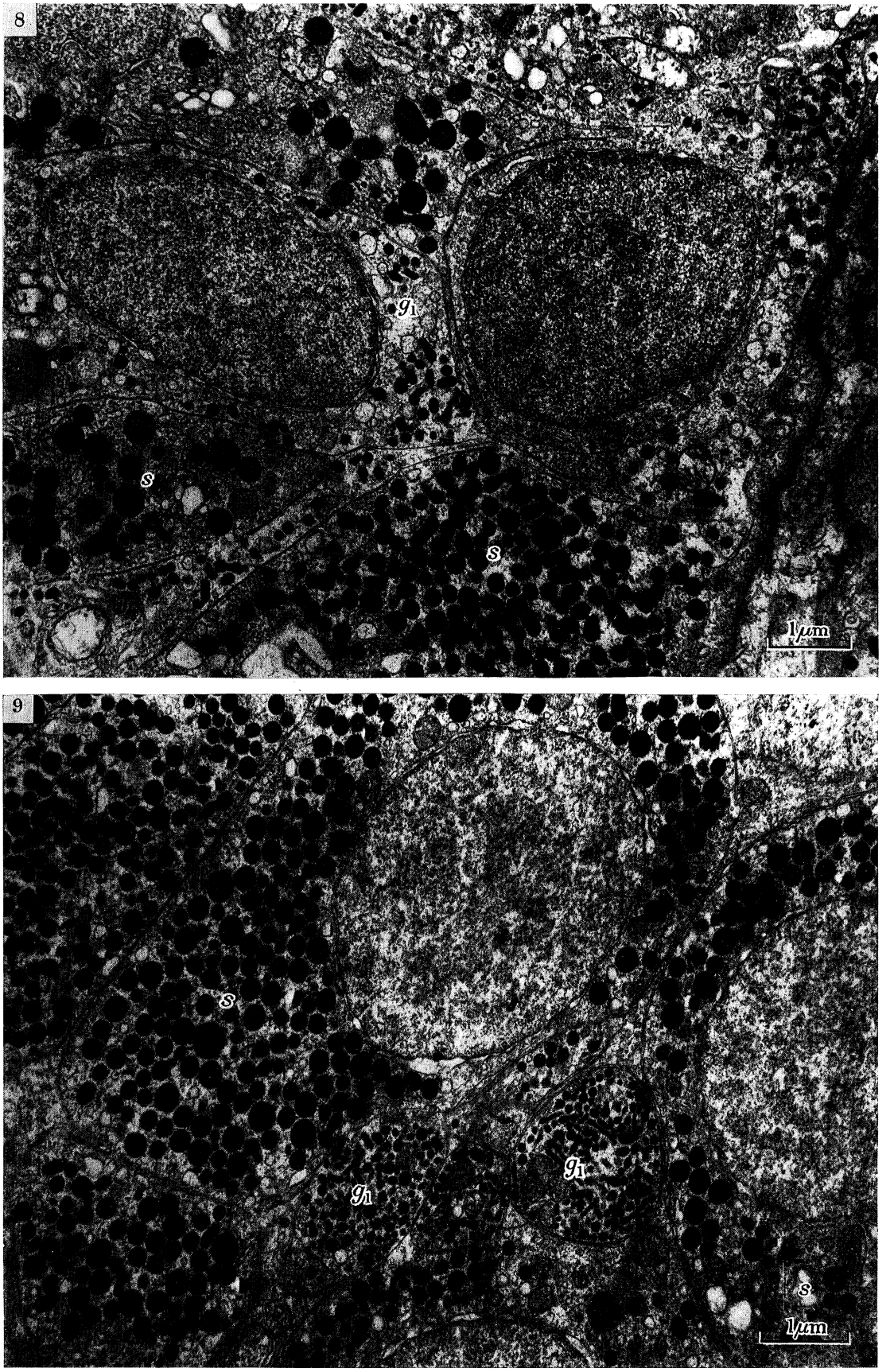


FIGURE 8. Cells in the proximal pars distalis of the pituitary of *Conger*. g_1, GTH_1 cell; *s*, *STH* cell.
FIGURE 9. Cells in the proximal pars distalis of the pituitary of *Anguilla*. g_1, GTH_1 cells; *s*, *STH* cell.

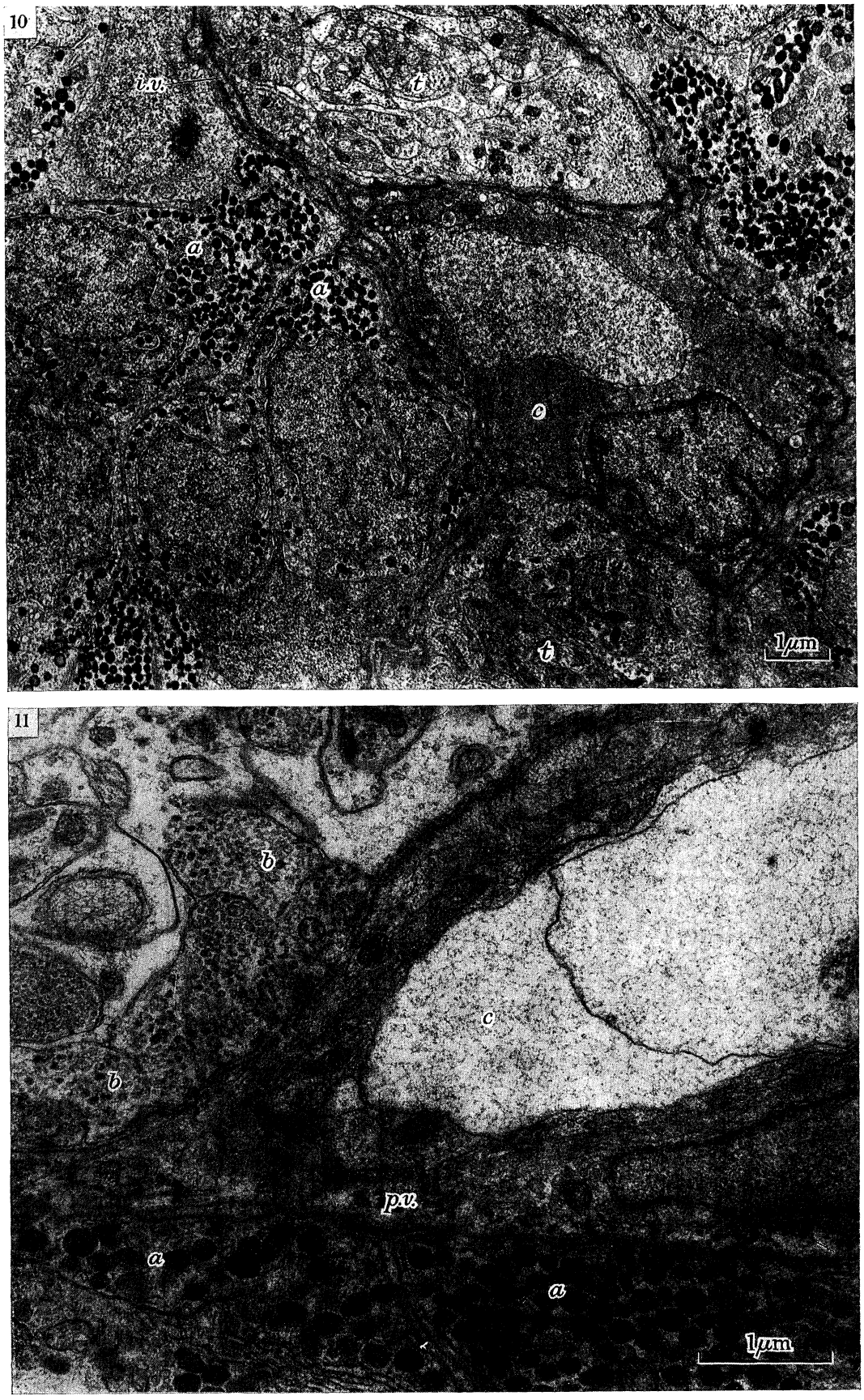


FIGURE 10. A survey picture of a portion of the rostral pars distalis of the pituitary of *Conger*. *a*, *ACTH* cells; *c*, capillary. *i.v.*, intervascular channel; *t*, tract.

FIGURE 11. The relationship in *Anguilla* between Type B fibres (*b*), a capillary (*c*), the perivascular space (*p.v.*) and *ACTH* cells (*a*).

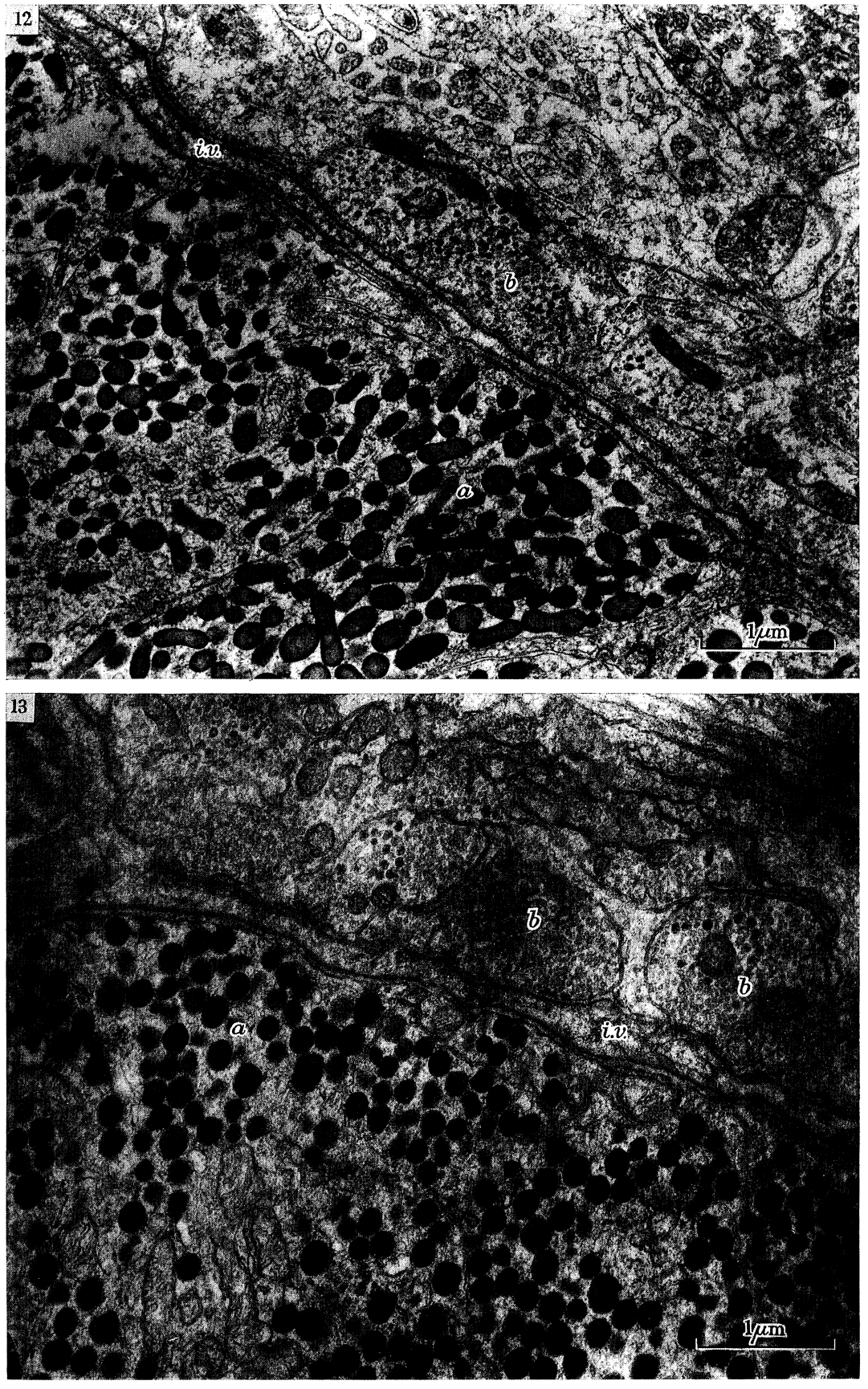
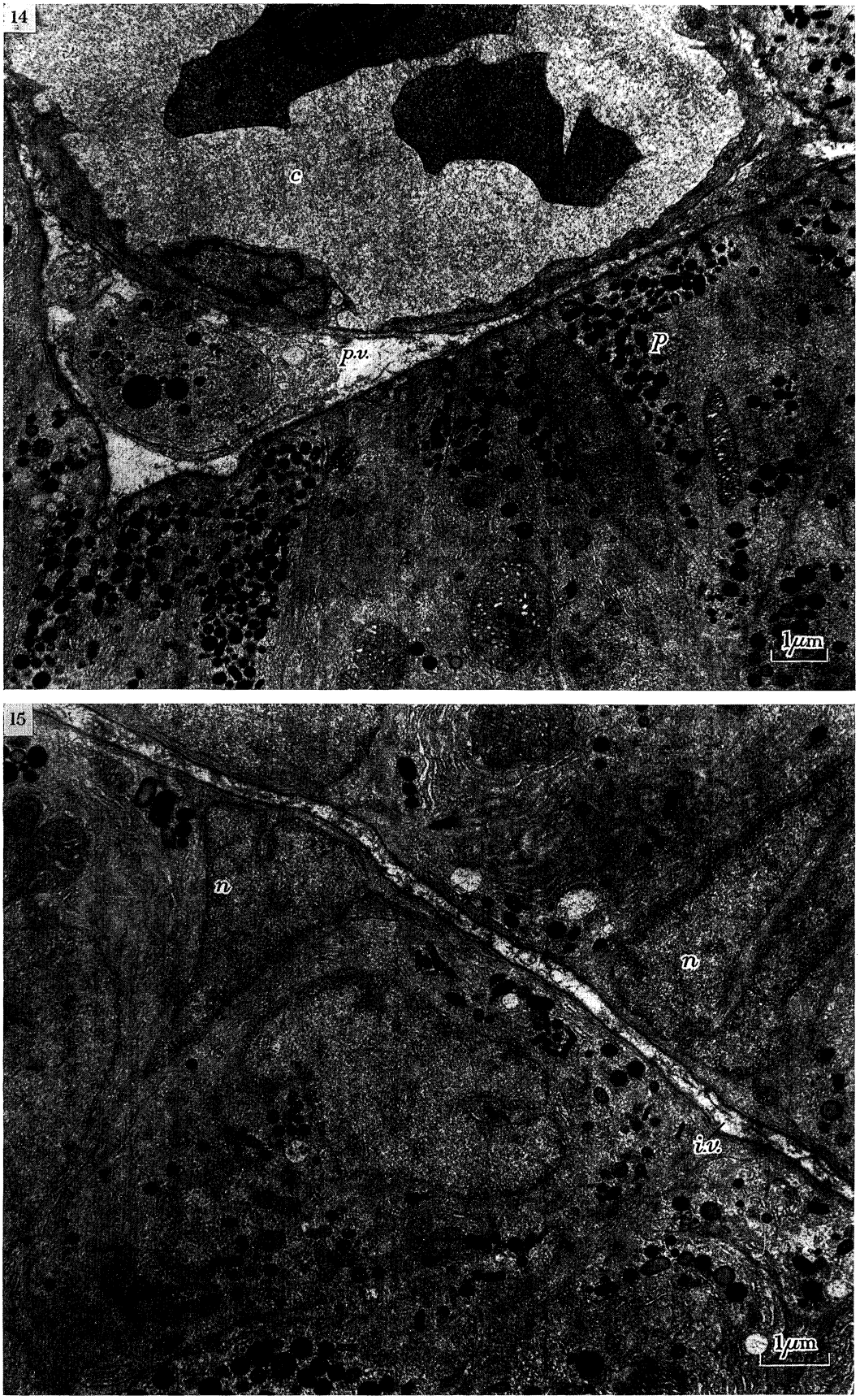


FIGURE 12. The relationship between the neurosecretory innervation and *ACTH* cells in *Conger*. *a*, *ACTH* cell; *b*, Type B fibre; *i.v.*, intervascular channel.

FIGURE 13. As figure 12, but in the pituitary of *Anguilla*. (Lettering as in figure 12.)



FIGURES 14 AND 15. Sections through the periphery of follicles of the rostral pars distalis of the *Conger* pituitary. *c*, Capillary; *i.v.*, intervascular channel; *n*, neck cell; *p*, 'prolactin' cell; *p.v.*, perivascular space.

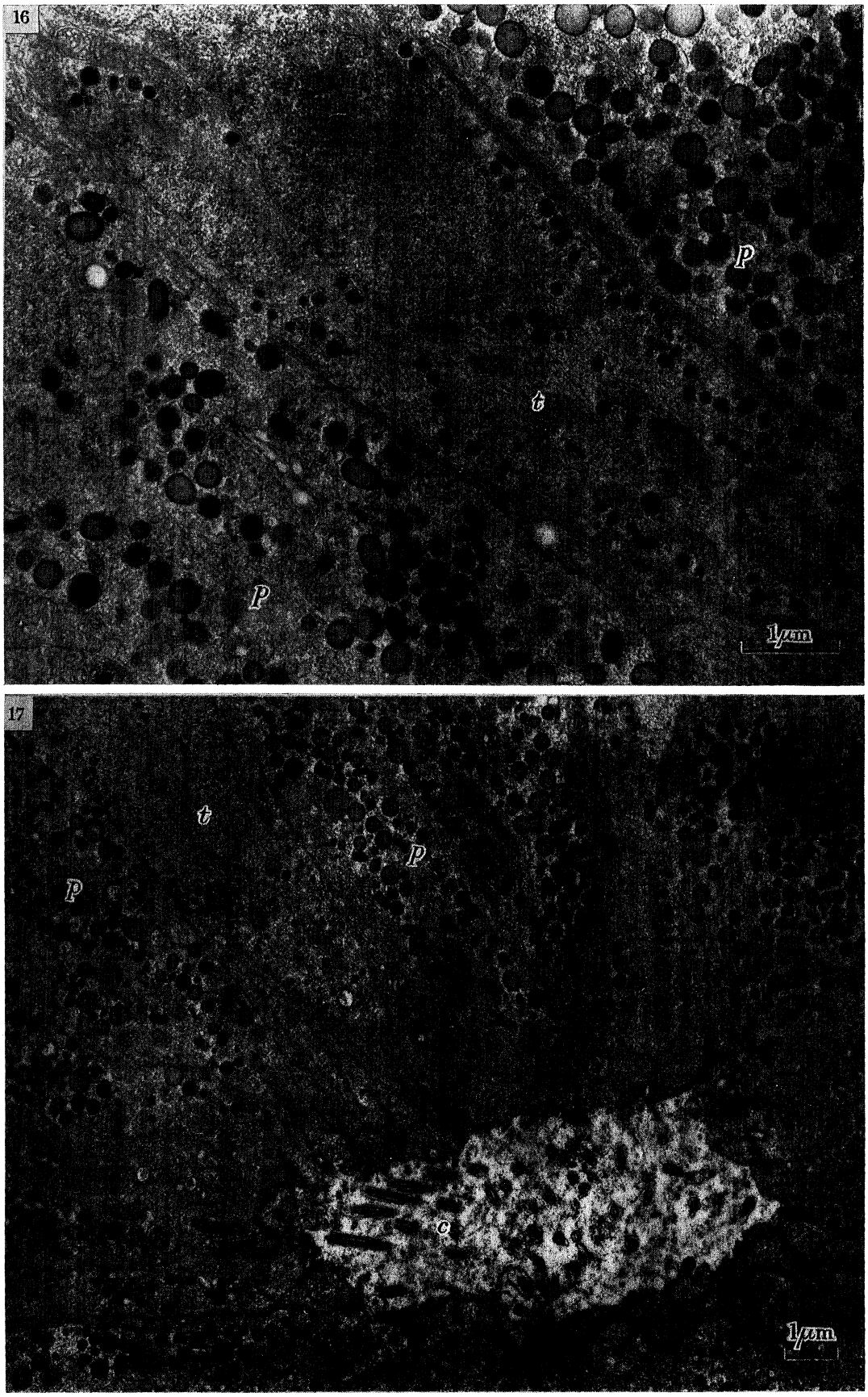
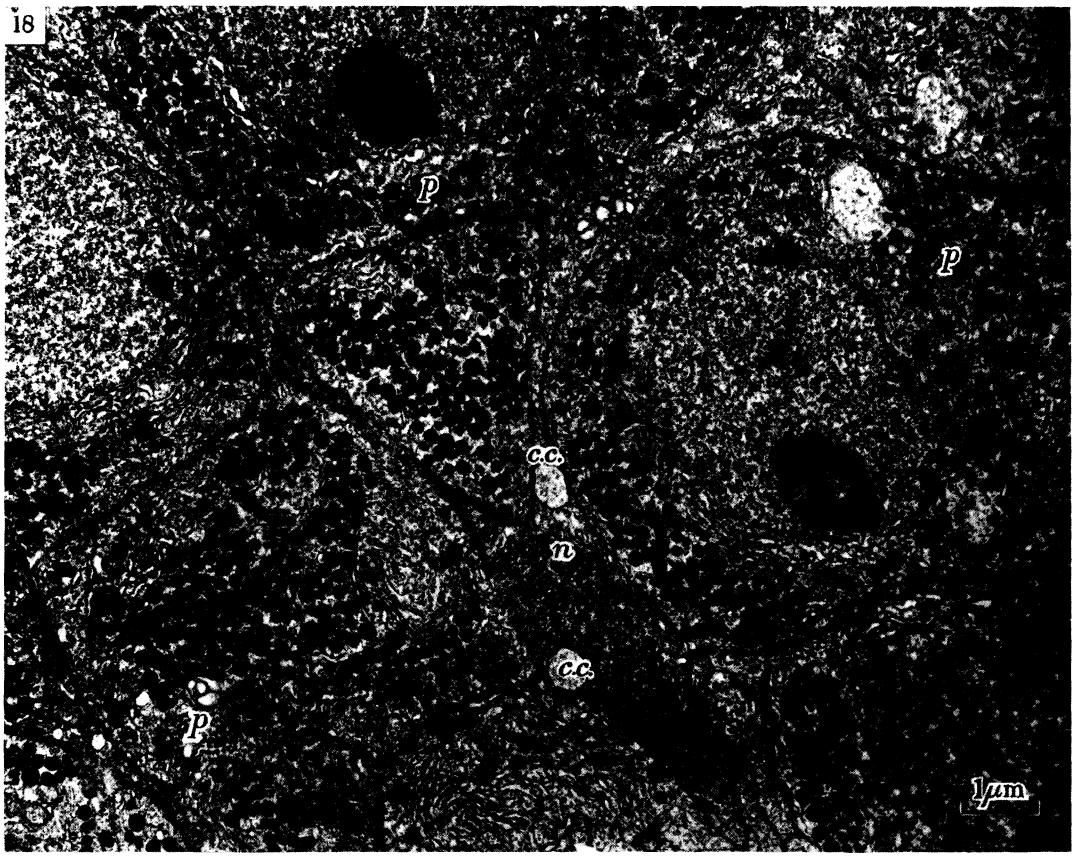


FIGURE 16. A section of a portion of a follicle of the rostral pars distalis of the pituitary of *Conger*. A prolongation of a *TSH* cell (*t*) lies between two 'prolactin' cells (*p*).

FIGURE 17. The central region of the follicle shown at figure 16. Lettering as in figure 16. *c*, Cilia.



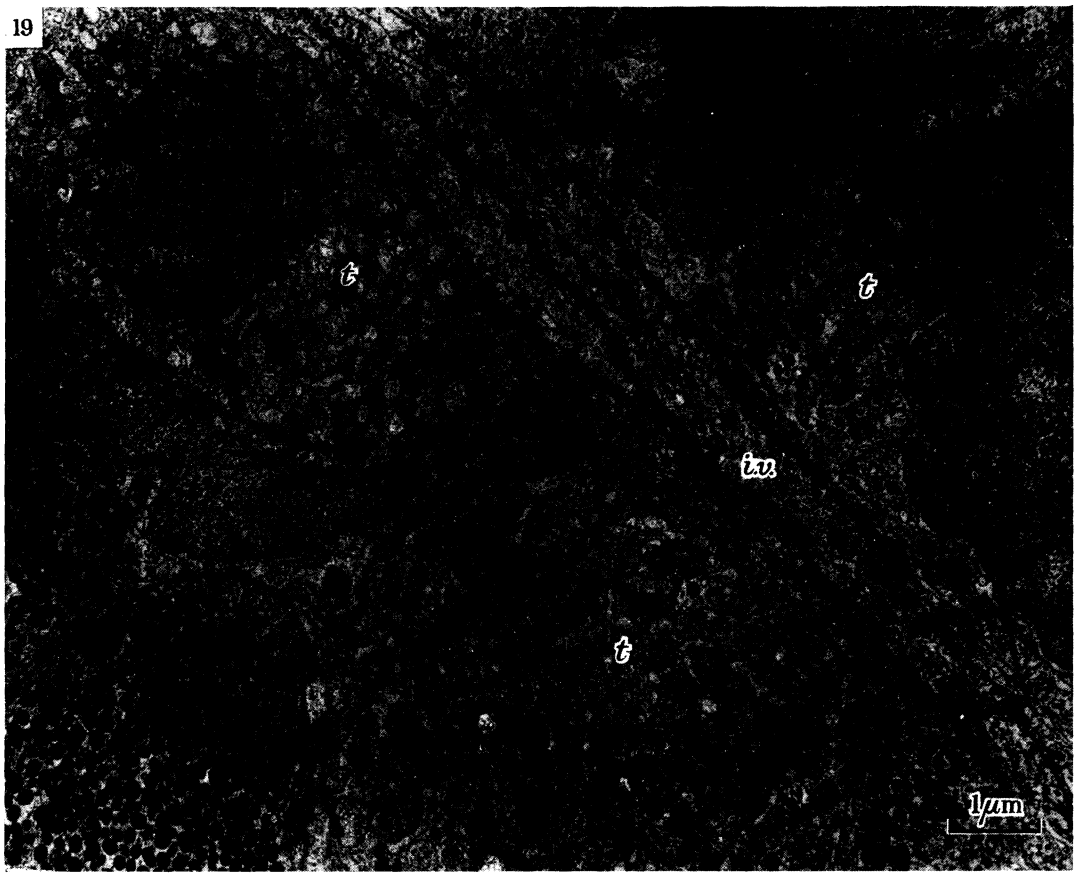


FIGURE 18. A follicle of the rostral pars distalis of the pituitary of *Anguilla*, cut tangentially close to its surface. *c.c.*, Connecting canal; *n*, neck cell; *p*, 'prolactin' cell.

FIGURE 19. *TSH* cells (*t*) at the periphery of the follicle, bordering the intervascular channel (*i.v.*).

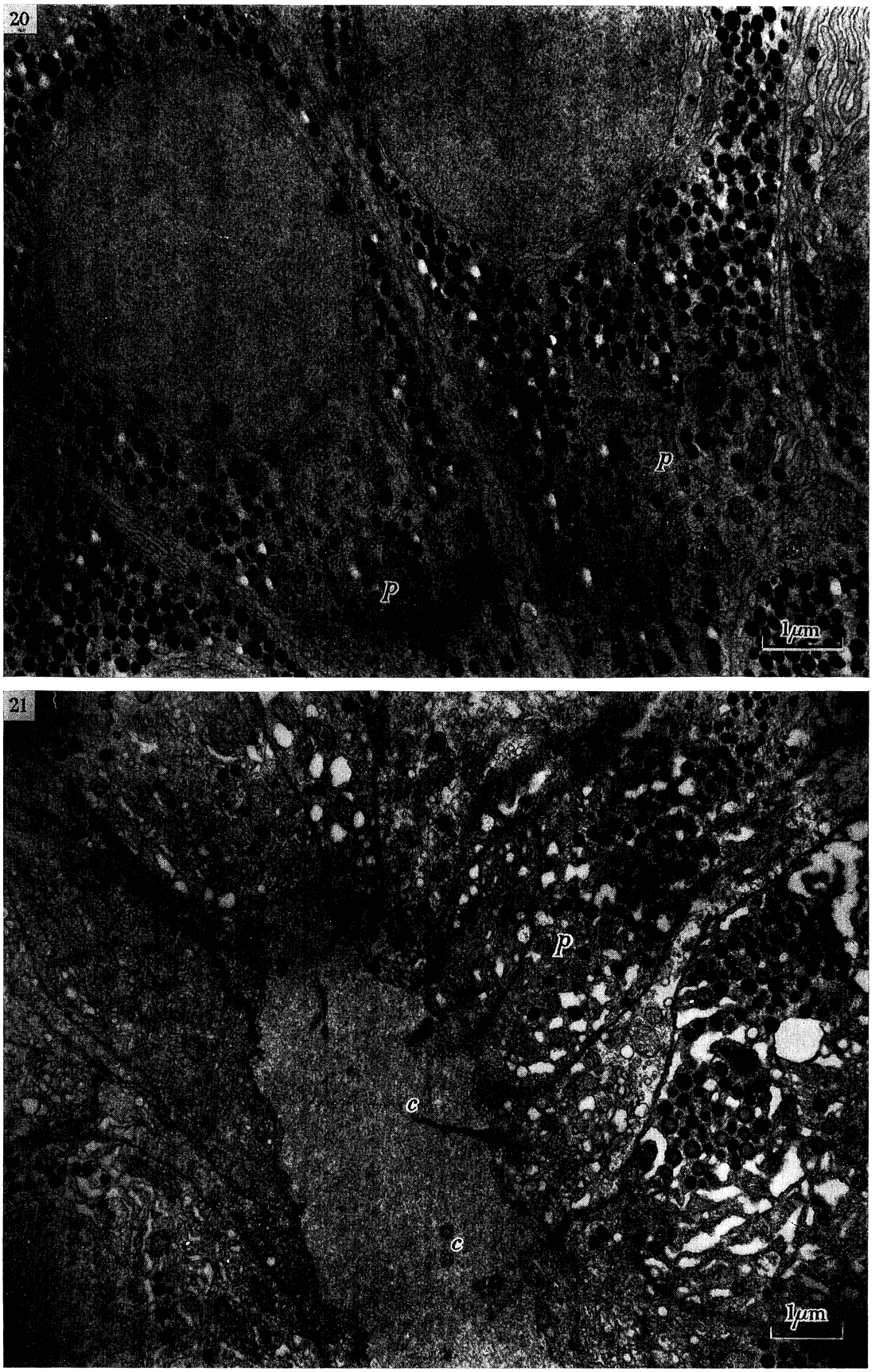
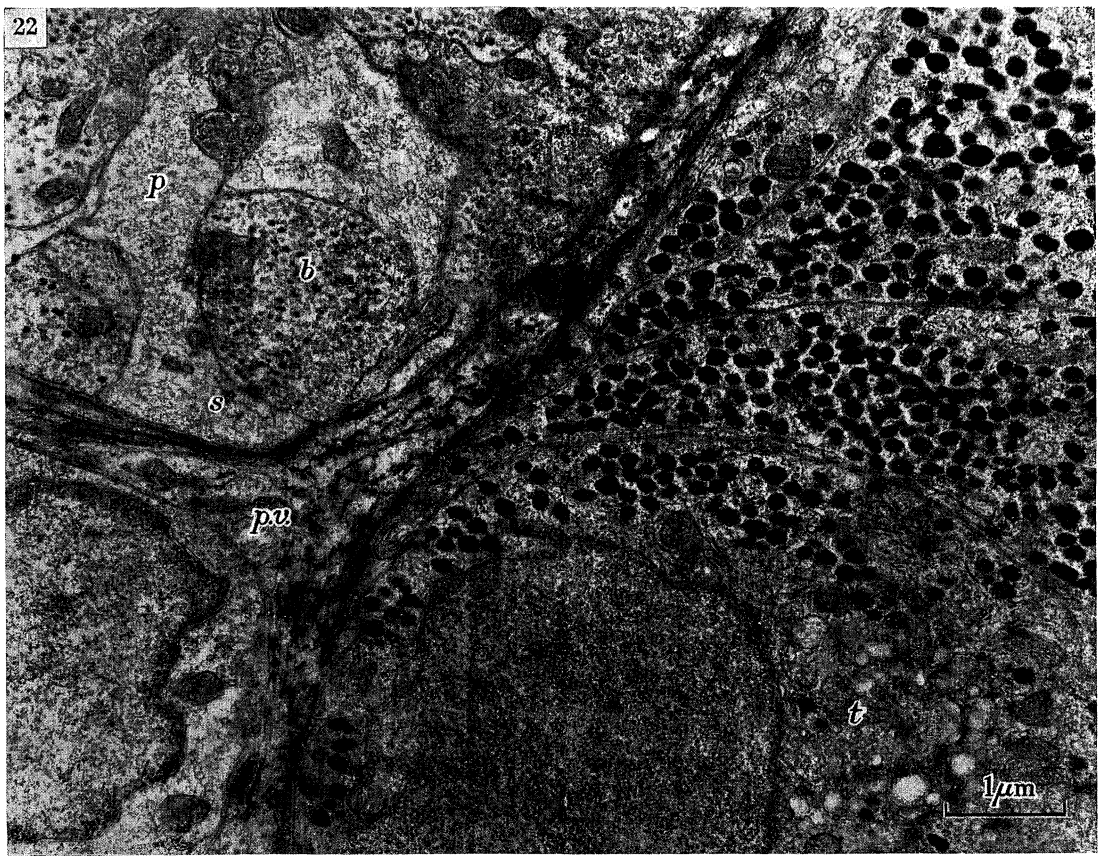


FIGURE 20. A section through a small portion of a follicle in the rostral pars distalis of the pituitary of *Anguilla*, showing 'prolactin' cells (*p*).

FIGURE 21. As figure 20 but at the centre of the follicle, *c*, Cilia; *p*, 'prolactin' cell.



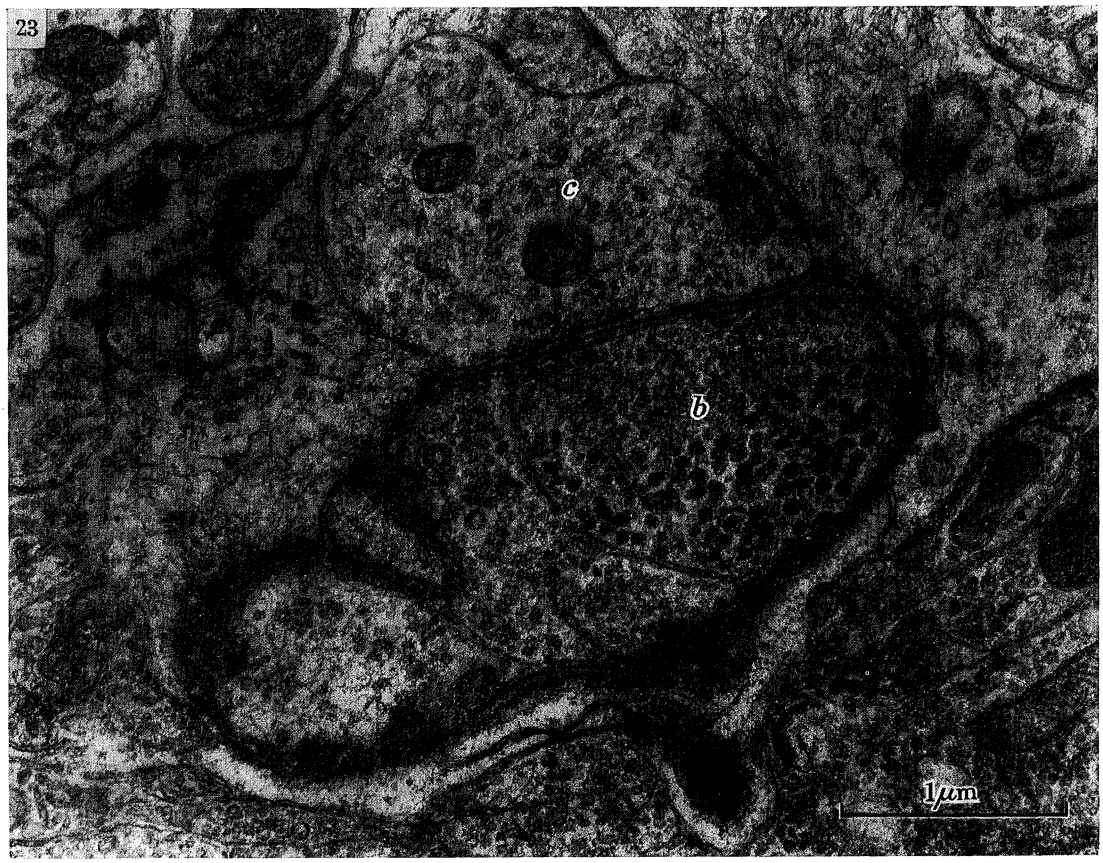


FIGURE 22. Neurosecretory tract, containing Type B fibres (*b*) and pituicytes (*p*) in proximity to a *TSH* cell (*t*) in the rostral pars distalis of the pituitary of *Anguilla*. *p.v.*, Perivascular space; *s*, possible synaptic junction.

FIGURE 23. Type B fibre terminal (*b*) in close association with another cellular element (*c*) possibly a pituicyte, in the tract leading to the rostral pars distalis of *Anguilla*.

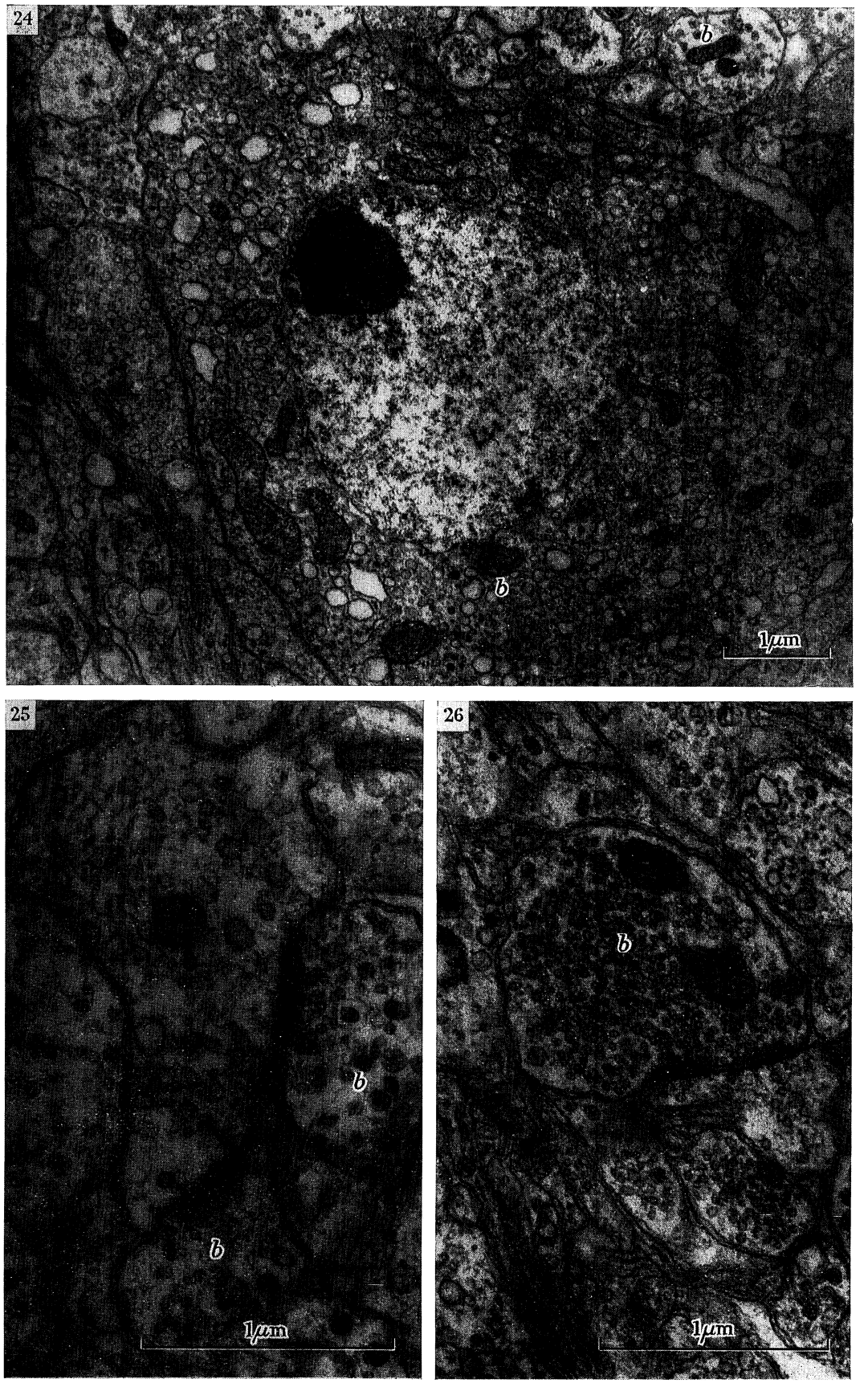
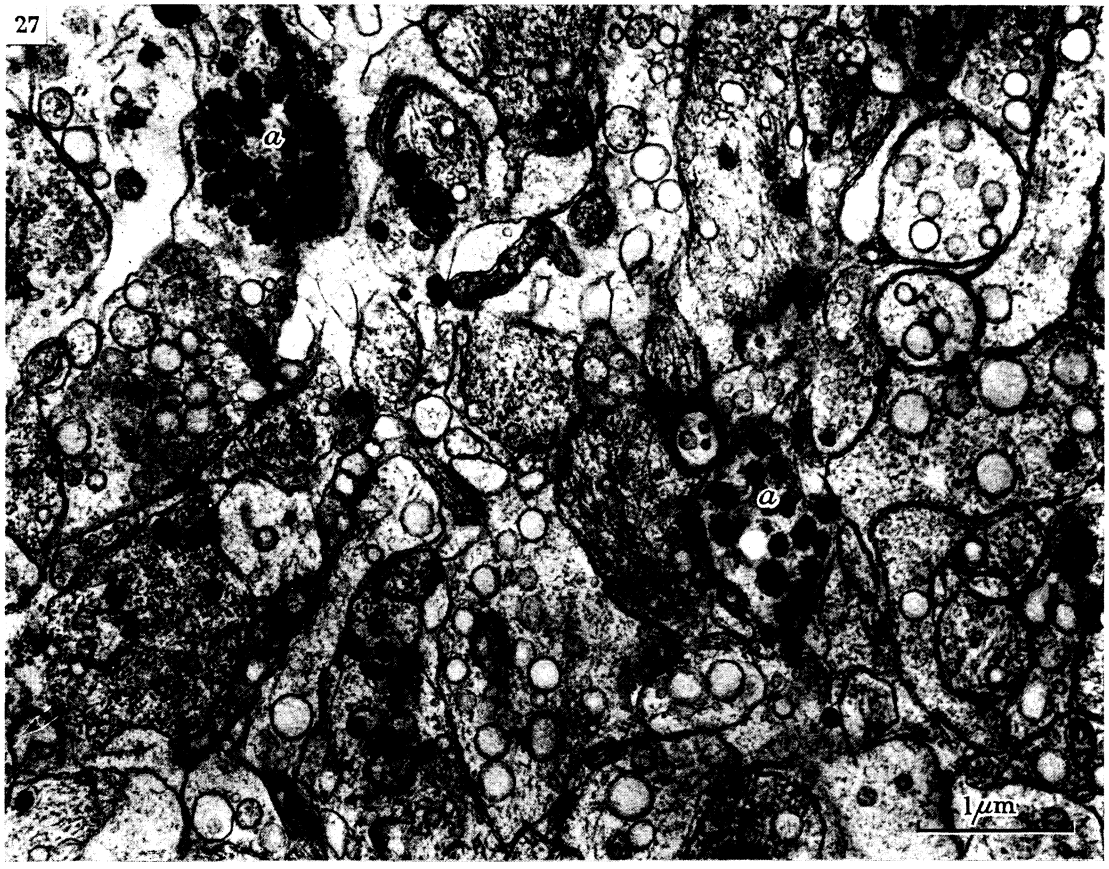


FIGURE 24. A perikaryon and fibres in the nucleus lateralis tuberis, showing Type B vesicles (*b*).
FIGURE 25. Probable axo-dendritic synapse, involving Type B fibres (*b*) in the nucleus lateralis tuberis.
FIGURE 26. Probable axo-axonal synapse involving Type B fibres (*b*) in the nucleus lateralis tuberis.



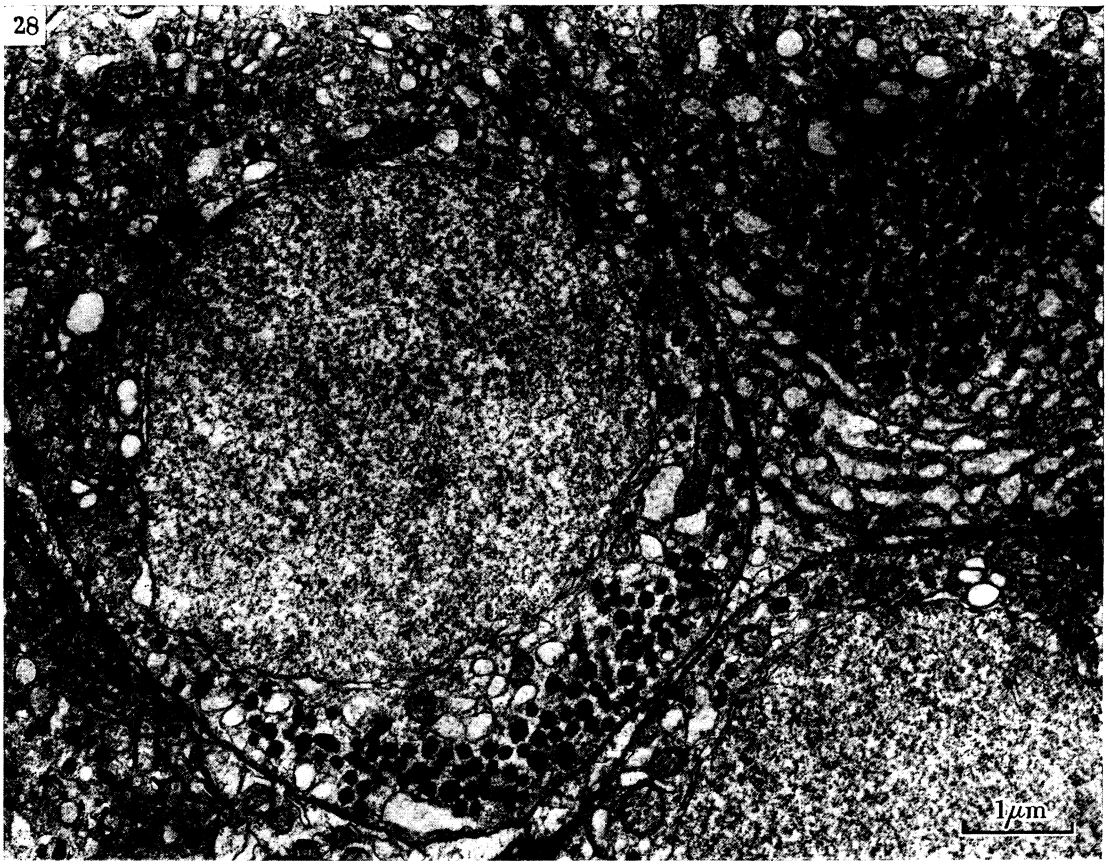
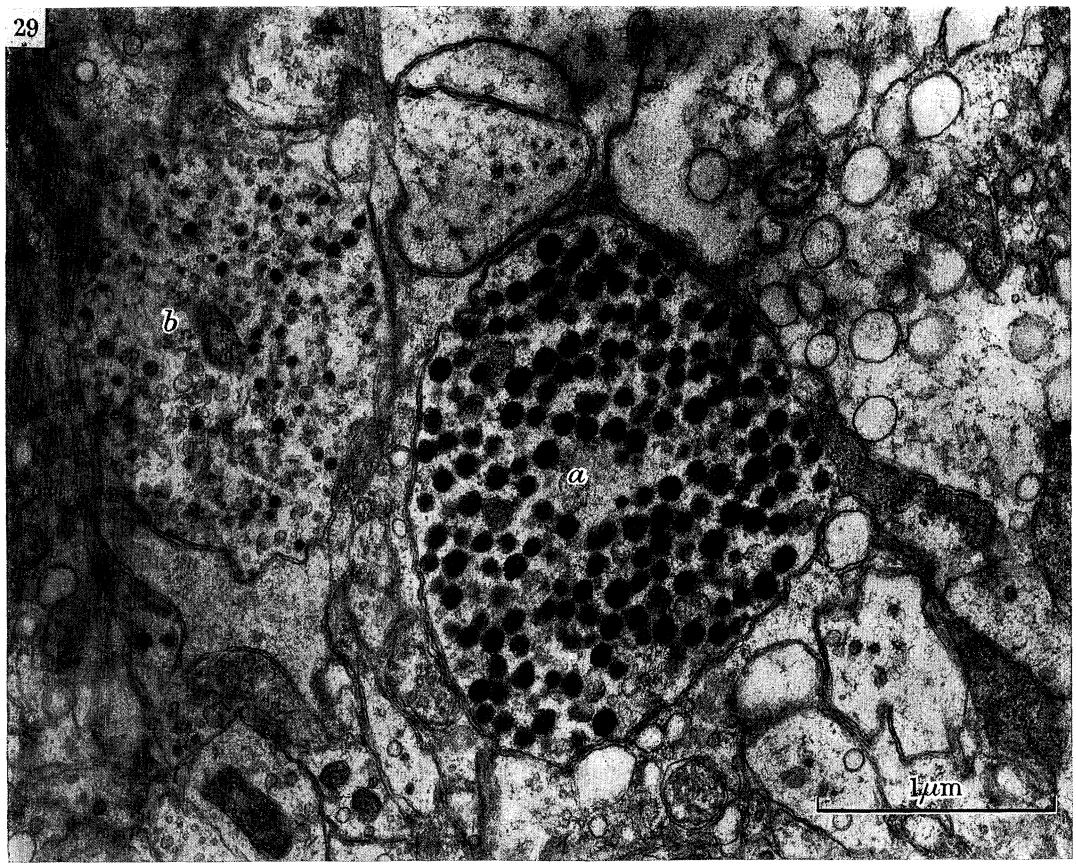


FIGURE 27. Type A fibres (*a*) in the tract leading to the rostral pars distalis of a silver eel (*Anguilla*).
FIGURE 28. *TSH* cells in the rostral pars distalis of a silver eel (*Anguilla*).



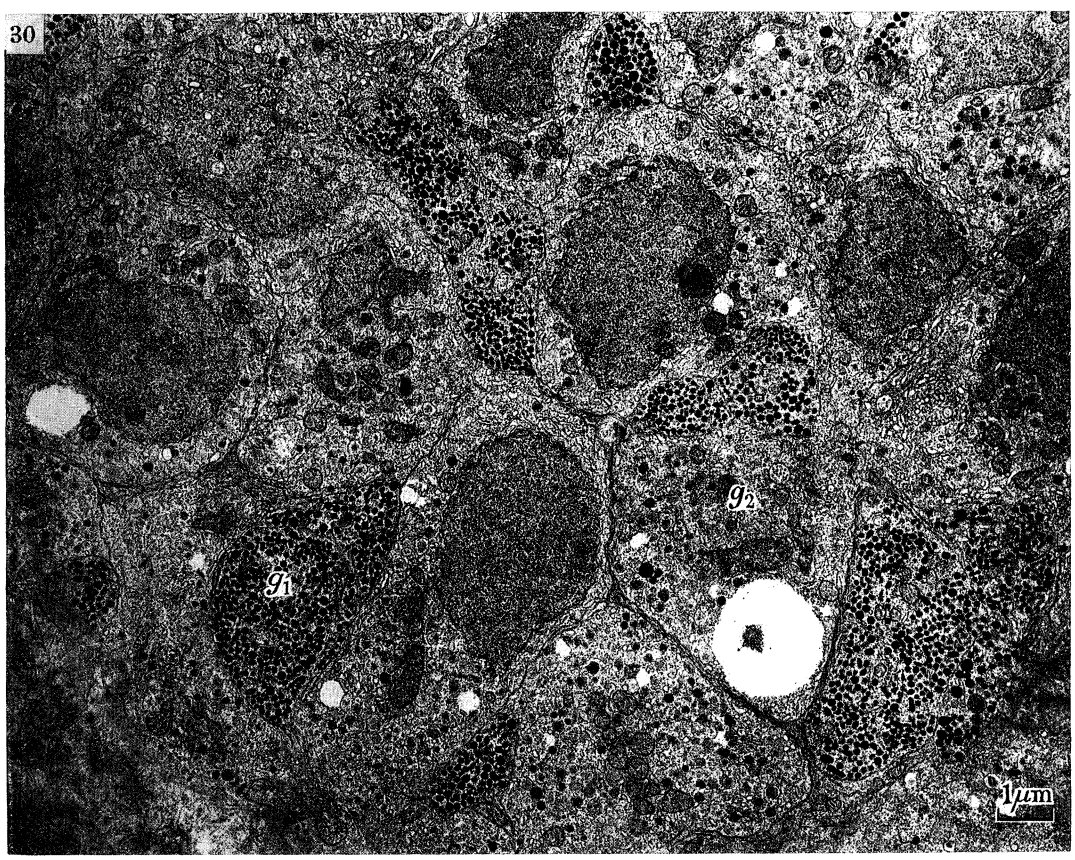


FIGURE 29. A portion of the neurosecretory tract leading to the proximal pars distalis of a silver eel (*Anguilla*). Both Type A (*a*) and Type B (*b*) fibres are present.

FIGURE 30. Cells of the proximal pars distalis of a silver eel. Two cell types, believed to be gonadotrophs (g_1, g_2) can be distinguished.

changes in the *TSH* cells may be related to changes in metabolism accompanying metamorphosis and migration.

It is interesting to compare the size of the granules in the different cell types with that of granules in pituitary cells of higher vertebrates. The *STH* cells in the eel contain granules of *ca.* 4000 Å; those of *STH* cells in the rat and mouse are *ca.* 3500 Å to 4000 Å (Barnes 1963; Herlant 1963). The granules of the *GTH* 2 cells in the eel are *ca.* 1300 Å in diameter, those in the *FSH* cells of the mouse are *ca.* 1500 to 2000 Å in diameter (Barnes 1963); these granules are relatively electron-lucent in both species. The granules in the *GTH* 1 cells were fairly uniform in size in the eel (*ca.* 1900 Å), but precise measurements were difficult as these granules are often elongate in form. In the mouse pituitary *LH* cells contain granules of a fairly wide size-range, 750 to 3000 Å (Barnes 1963), 1000 Å to 2000 Å (Herlant 1963). The diameter of granules in the *ACTH* cells of the eel is 2250 Å. This figure is in accordance with the mean figure for the diameter of granules in the *ACTH* cells of the rat which has been given as 2250 Å (Herlant 1963). The granules in the *TSH* cells of the eel are *ca.* 1400 Å in diameter; those in *TSH* cells of the rat have been given as 1400 to 1500 Å (Herlant 1963). It may be seen therefore that there is a close correspondence between the granule-size in these pituitary cells in both fishes and mammals.

The only cells which did not show a close correspondence were the 'prolactin' cells, which contained granules *ca.* 2800 Å in diameter in the eel, but *ca.* 3500 to 6000 Å in the rat (Herlant 1963). It is perhaps relevant to note that Nicoll & Bern (1964) could not find typical prolactin activity in fish pituitaries.

The general relationship between the neurosecretory tracts, the surrounding extravascular spaces, and the intrinsic endocrine cells of the pars distalis in the eel resembles morphologically the median eminence-portal system-adenohypophysis system of higher vertebrates. Wingstrand (1959) has suggested that the neurosecretory tracts within the fish adenohypophysis represent the median eminence of higher vertebrates. In the eel these penetrations are considerable and might be said to constitute a distal median eminence, and the surrounding extravascular spaces a distal portal system.

It is interesting to note the close morphological similarities between the system of extravascular spaces in the three lobes of the eel pituitary. These spaces could provide channels of communication through which neurosecretory hormones might influence the intrinsic endocrine cells, and their products might be discharged into the blood-stream.

It is not easy to determine the precise origin of all the neurosecretory fibres which invade the pars distalis of the eel pituitary, but most of the Type B fibres appeared to originate in the nucleus lateralis tuberis. Billenstien (1963), Stahl & Leray (1962), and Szabó & Molnar (1965) have shown that stainable material in the nucleus lateralis tuberis is associated with reproduction in certain teleost fishes. Recently Dierickx (1965) has shown that the aldehyde fuchsin-negative fibres in the pars distalis of *Rana temporaria*, believed to be associated with gonadotrophic activity, originate in the area periventricularis of the pars ventralis tuberis.

The presence of Type B vesicles in the perikarya in the nucleus lateralis tuberis and in the neurosecretory fibre terminals in the pars distalis of the eel pituitary accords with these observations and indications that this nucleus plays an important part in the regulation of adenohypophysial function.

It is not possible to postulate on the present evidence a precise function for the B type fibres in the adenohypophysis of the eel, but it should be noted that in the dogfish *Scylliorhinus* B type fibres were associated morphologically with the storage and release pole of the intrinsic cells of the neuro-intermediate lobe, thus indicating a control of hormone release (Knowles 1965*b*).

Type A vesicles were only abundant in fibre tracts leading to the pars distalis at certain stages in the life-cycle, but the significance of this is not clear as we do not know whether their detectable presence indicates activity, or inactivity, of the fibres in which they are contained. There is some reason to believe that an active fibre might have fewer electron-dense vesicles. Palay (1957) found that experimental procedures which caused antidiuretic hormone (*ADH*) release led also to a loss of electron-dense vesicles in the neurohypophysis. Experiments on *Scylliorhinus* (Mellinger 1963) and studies in ultrastructure (Knowles 1965*b*) of the *Scylliorhinus* pituitary indicate that Type A fibres may inhibit *MSH* synthesis in intrinsic cells of the neuro-intermediate lobe. It is possible that Type A fibres may also inhibit activity in cells of the pars distalis of the eel. If so the presence of Type A vesicles at certain stages in the life-cycle may indicate a diminution or cessation of inhibitory activity. It is interesting to note that Type A vesicles and Gomori-positive material are only present in abundance at stages in which *GTH* and *TSH* activity is pronounced. These observations could support a view that the absence of Type A vesicles or Gomori-positive or alcian-blue-positive material in the tracts leading to the pars distalis of the eel may indicate a steady release of neurosecretory material with minimal storage, as might be expected if the function of Type A innervation of the pars distalis is that of sustained inhibition of intrinsic endocrine cells. The possibility however that some Type A fibres may be excitatory cannot be excluded.

No clear indication of function of the pituicytes in the pars distalis could be observed. The pituicytes in the pars distalis were however very few in number compared with those of the neuro-intermediate lobe, and showed no evident ependymal characteristics like those of the neuro-intermediate lobe; neither were extensions of the infundibular recess present (cf. preceding paper). The possibility that Type A fibres leading to the pars distalis may make functional contact with ependyma near the base of the pituitary stalk or at a higher level cannot be excluded. In this connexion it is interesting to note that Leatherland, Budtz & Dodd (1966) have described an area of the hypothalamo-hypophysial tract close to the base of the pituitary stalk of the eel, which they have designated the critical area, where an apparent discontinuity of the tract could be observed in many animals. Schiebler & Hartmann (1963) also noted a decrease of neurosecretory material in the distal part of the tract in the eel and suggested that a release of neurosecretory material might take place before the tract reaches the hypophysis. As yet however there is no direct evidence for a feedback system involving ependymal elements such as has been suggested for the neuro-intermediate lobe (preceding paper), though it might be logical to look for a similar system for the pars distalis in view of the very close resemblances in the neurosecretory innervation of the three lobes of the eel pituitary.

This and the preceding paper form part of a programme of research on neurosecretion in fishes supported by the North Atlantic Treaty Organization. We are indebted to the

Science Committee of NATO for their generous aid, and to the University of Würzburg, Germany, for permitting one of us (Dr Vollrath) a year's leave of absence to participate in this programme.

The electron micrographs were taken on a Siemens EMI, and a Zeiss EM9, both provided by The Medical Research Council.

Some material was obtained at the Stazione Zoologica Naples, Italy. We are grateful to the Director, Dr Peter Dohrn, and to members of his staff.

We are indebted to Mr W. Pardoe who gave expert help in the preparation of the illustrations and Mr H. Penn (of the Aeon Laboratories) who assisted in the preparations of the micrographs. Mr J. Armstrong of the Department of Anatomy, University College London, kindly carried out his specific staining method for bouton mitochondria on some specimens.

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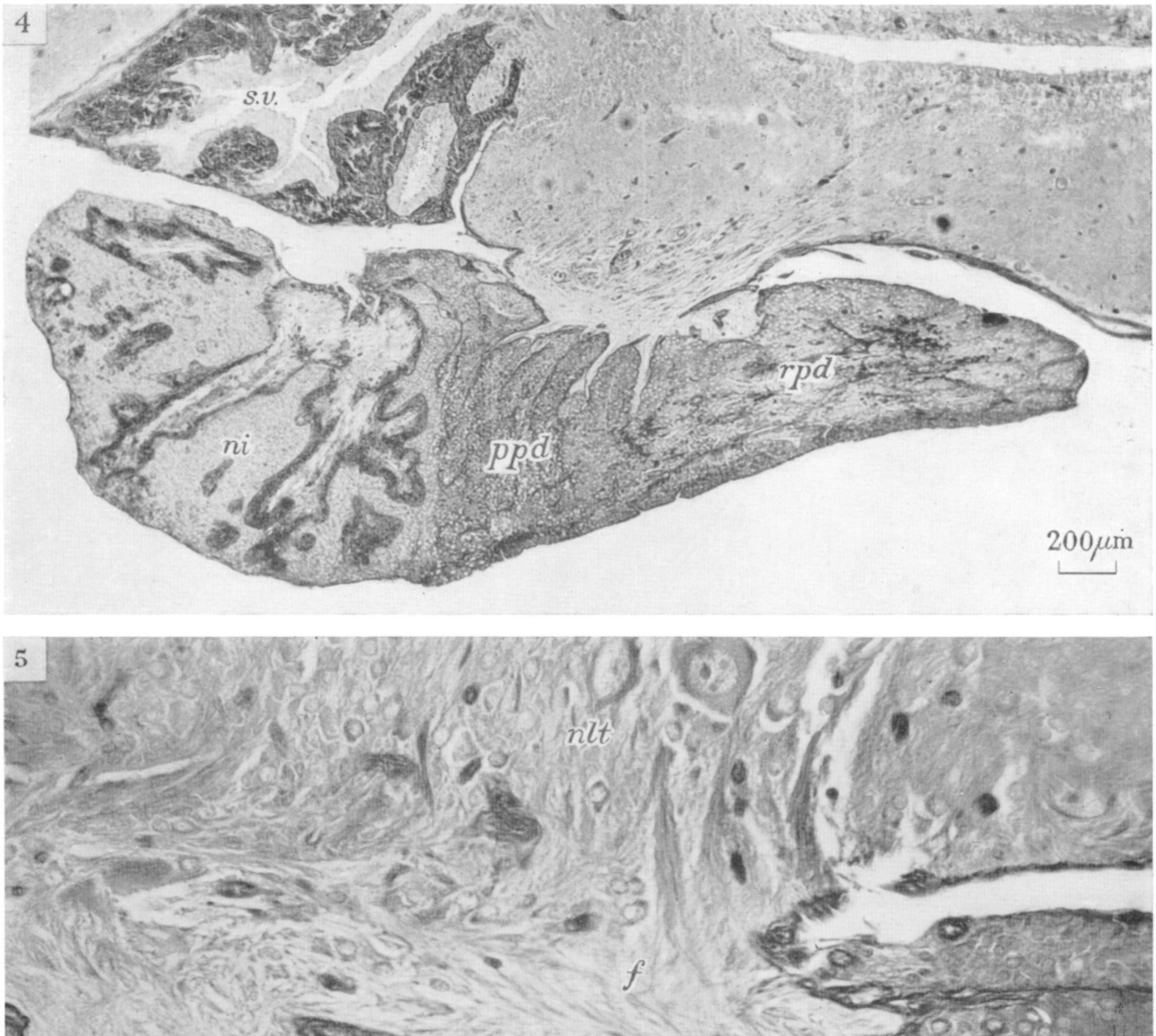
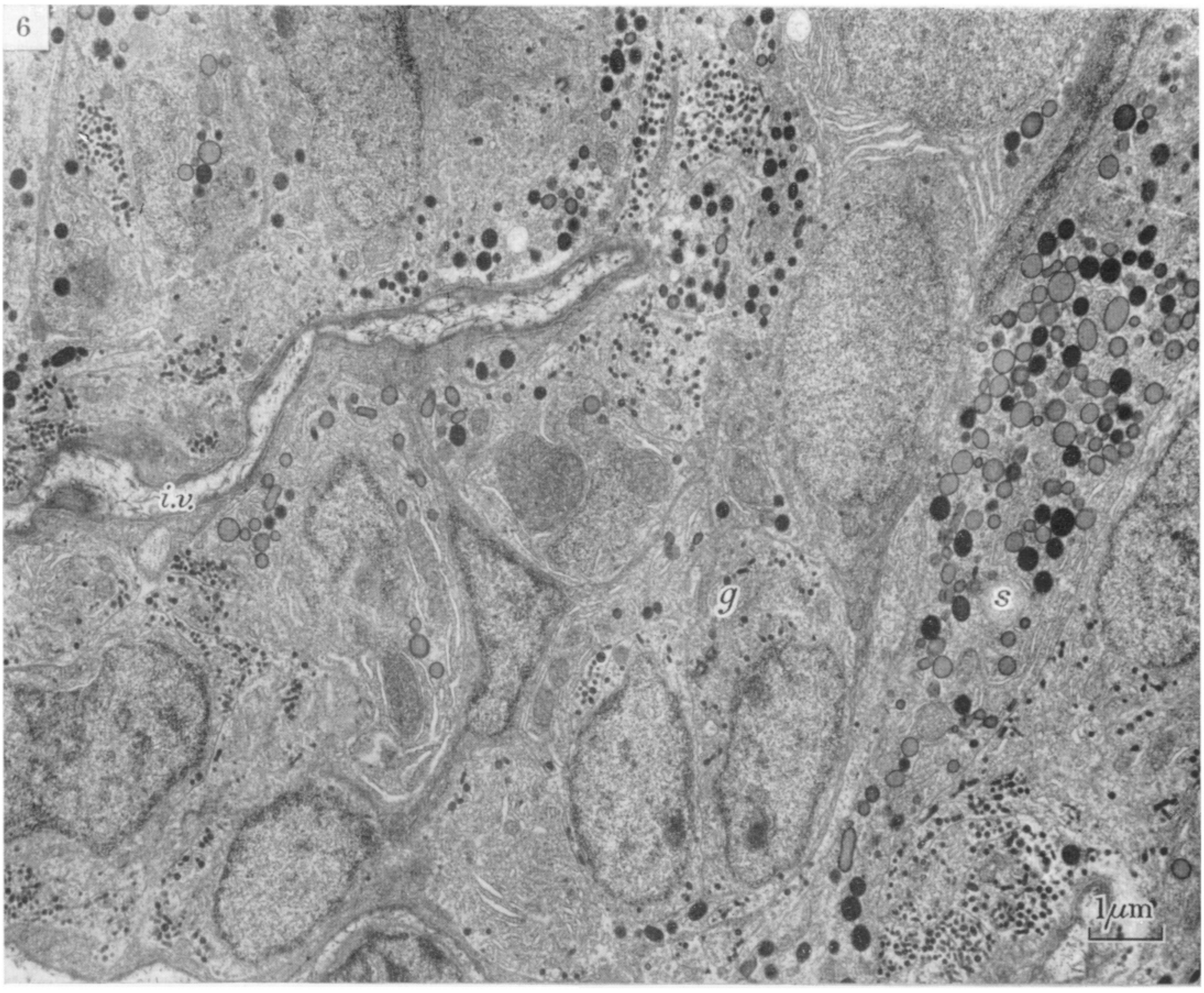


FIGURE 4. An optical micrograph of a lateral longitudinal section of the pituitary of *Anguilla*. *ni*, neuro-intermediate lobe; *ppd*, proximal pars distalis; *rpd*, rostral pars distalis. *s.v.*, saccus vasculosus. (Staining: alcian blue–*PAS*–orange G.)

FIGURE 5. An optical micrograph showing the penetration of the anterior lobes by fibres (*f*) originating in the nucleus lateralis tuberis (*nlt*). *d*, *PAS*-positive droplets.



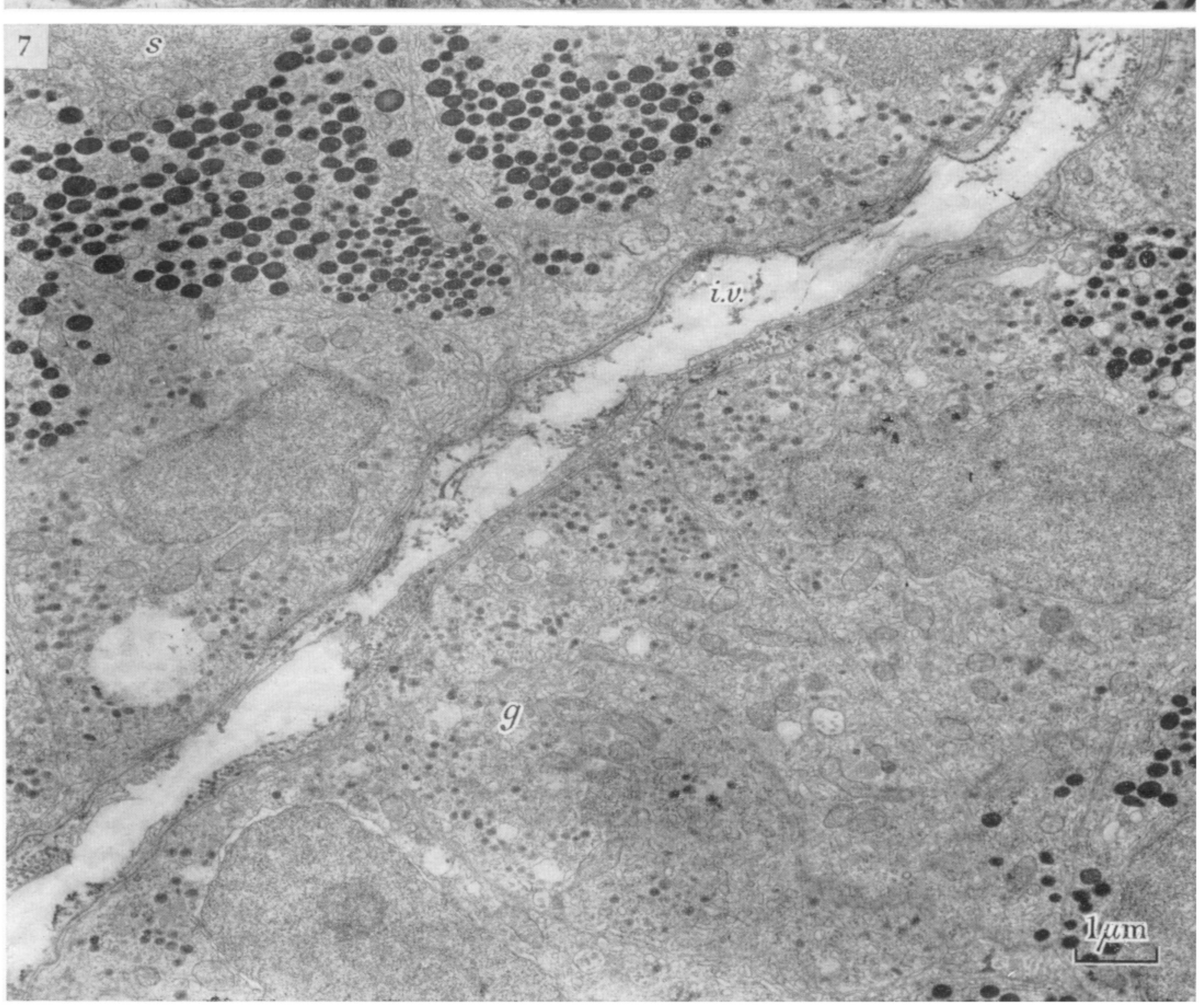
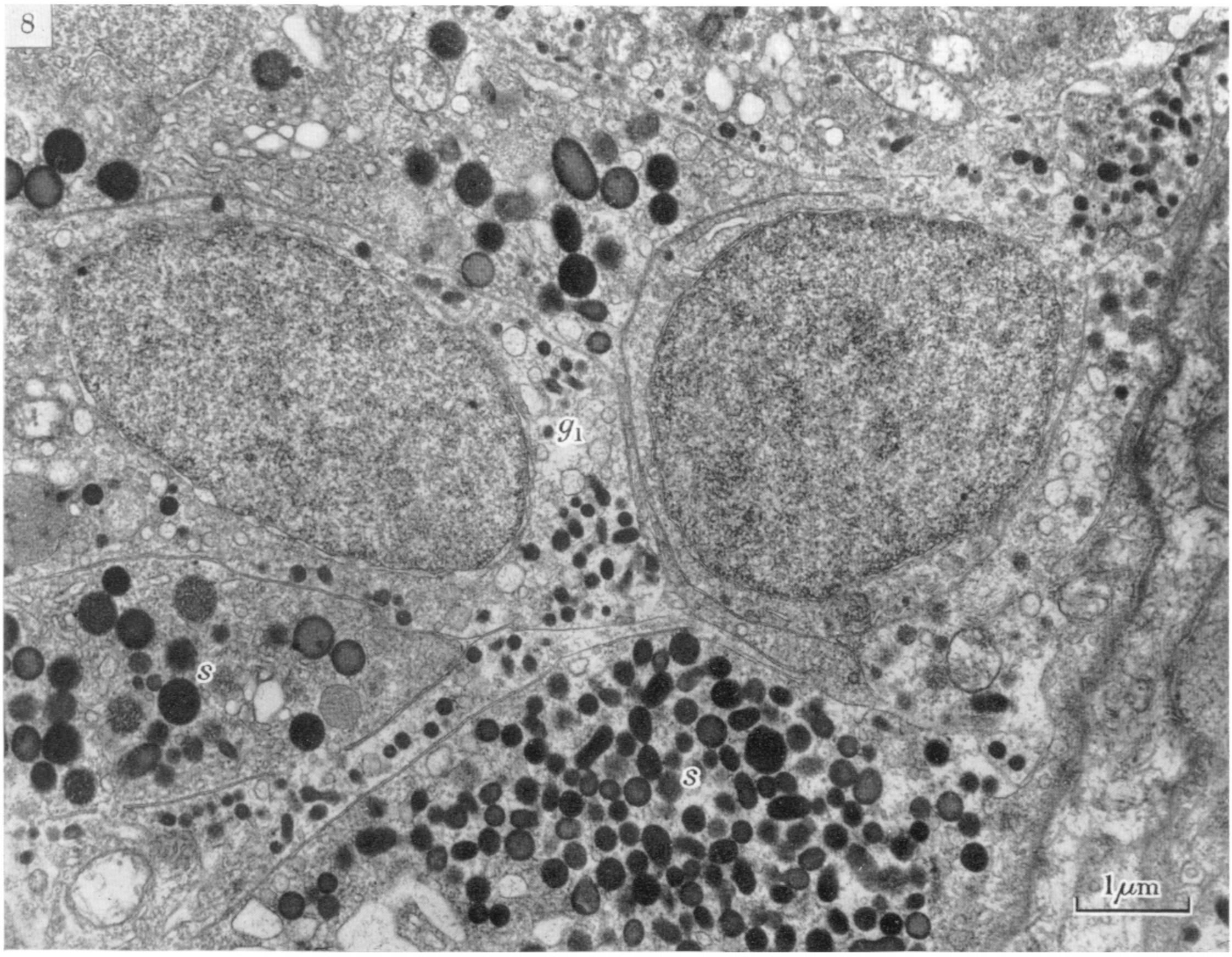


FIGURE 6. A survey picture of the proximal pars distalis of *Conger* pituitary. *g*, *GTH* cells; *s*, *STH* cells; *i.v.*, intervascular channel.

FIGURE 7. As figure 6 but the pituitary of *Anguilla*. *g*, GTH_2 cells; *i.v.*, intervascular channel; *s*, *STH* cells.

8



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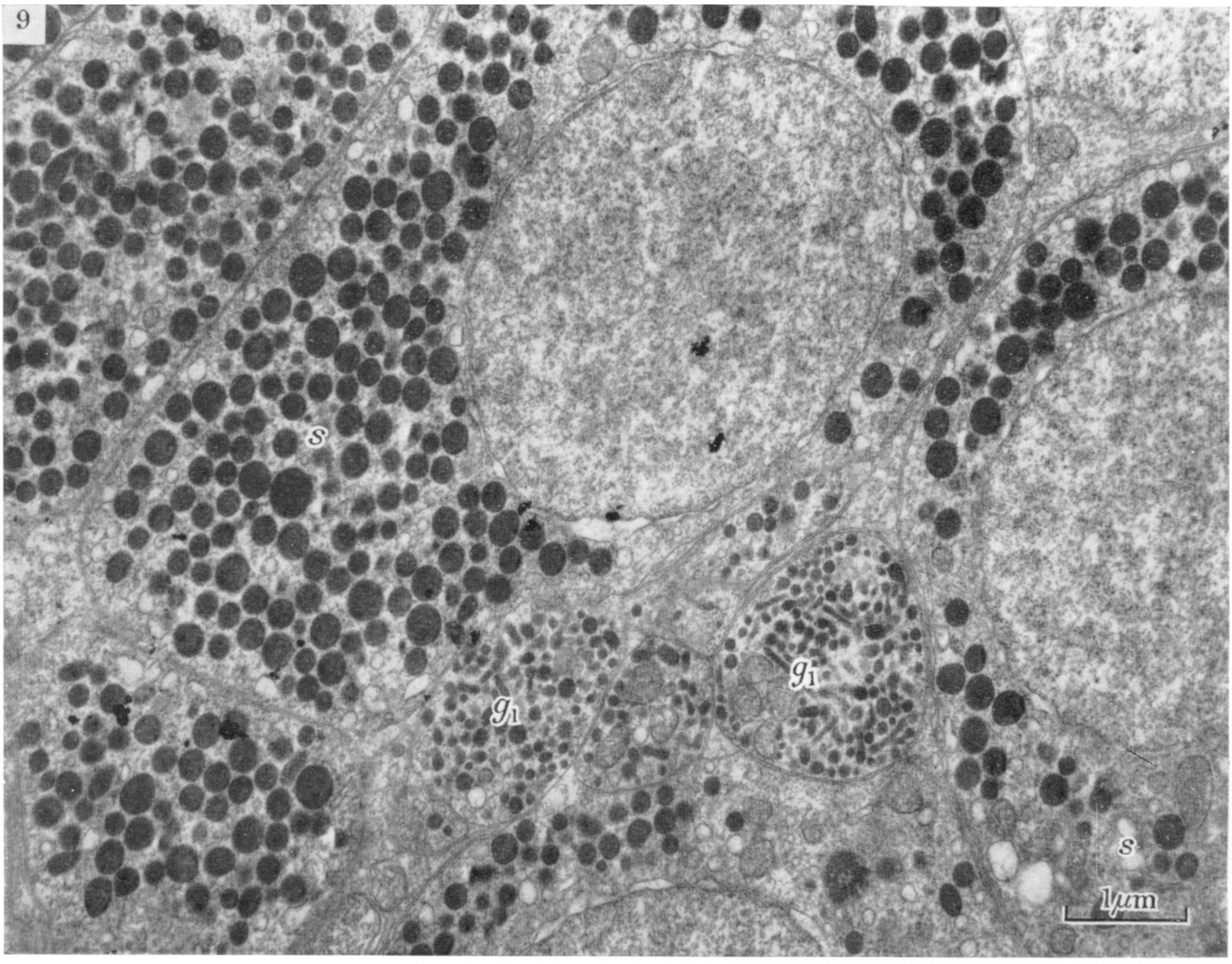
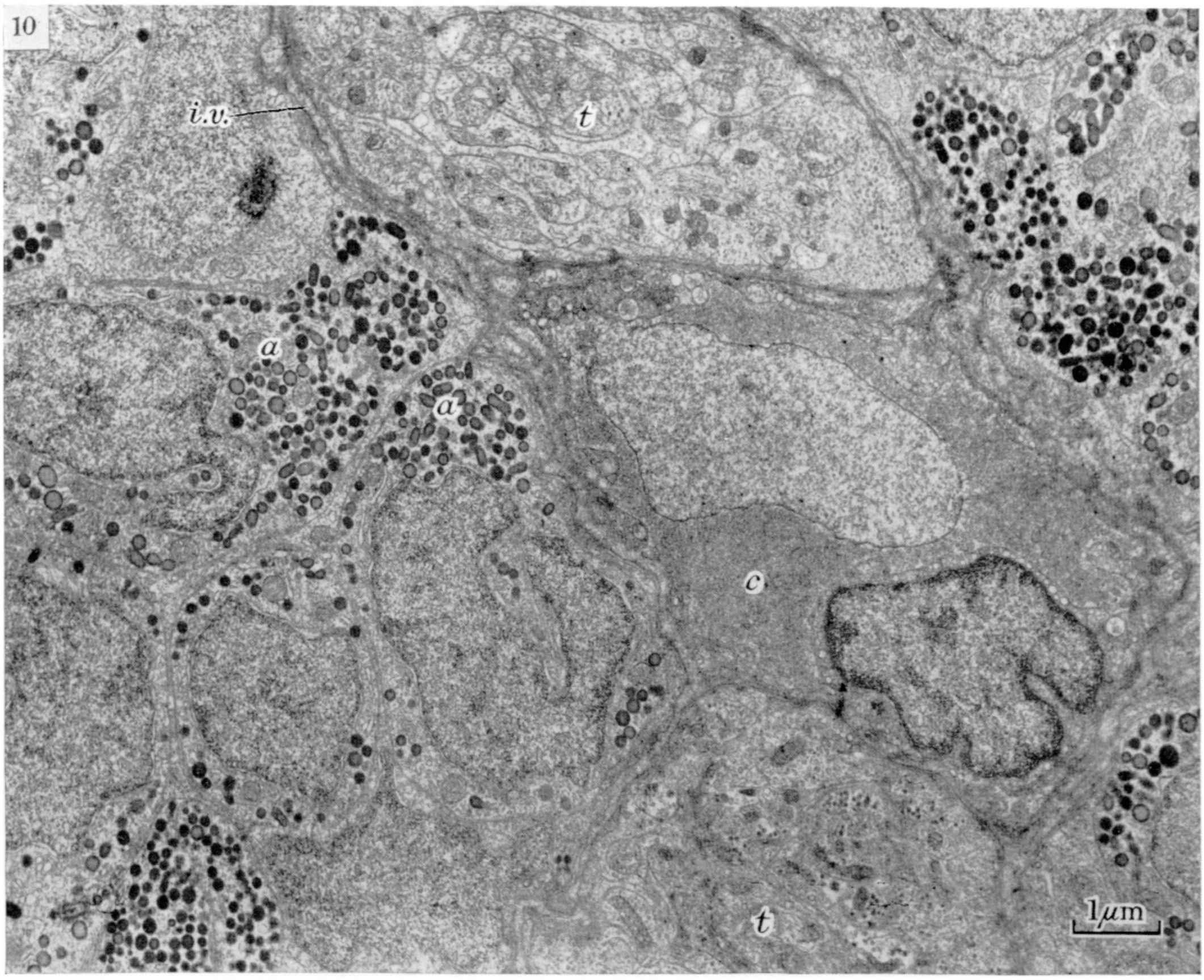


FIGURE 8. Cells in the proximal pars distalis of the pituitary of *Conger*. g_1, GTH_1 cell; *s*, *STH* cell.
FIGURE 9. Cells in the proximal pars distalis of the pituitary of *Anguilla*. g_1, GTH_1 cells; *s*, *STH* cell.



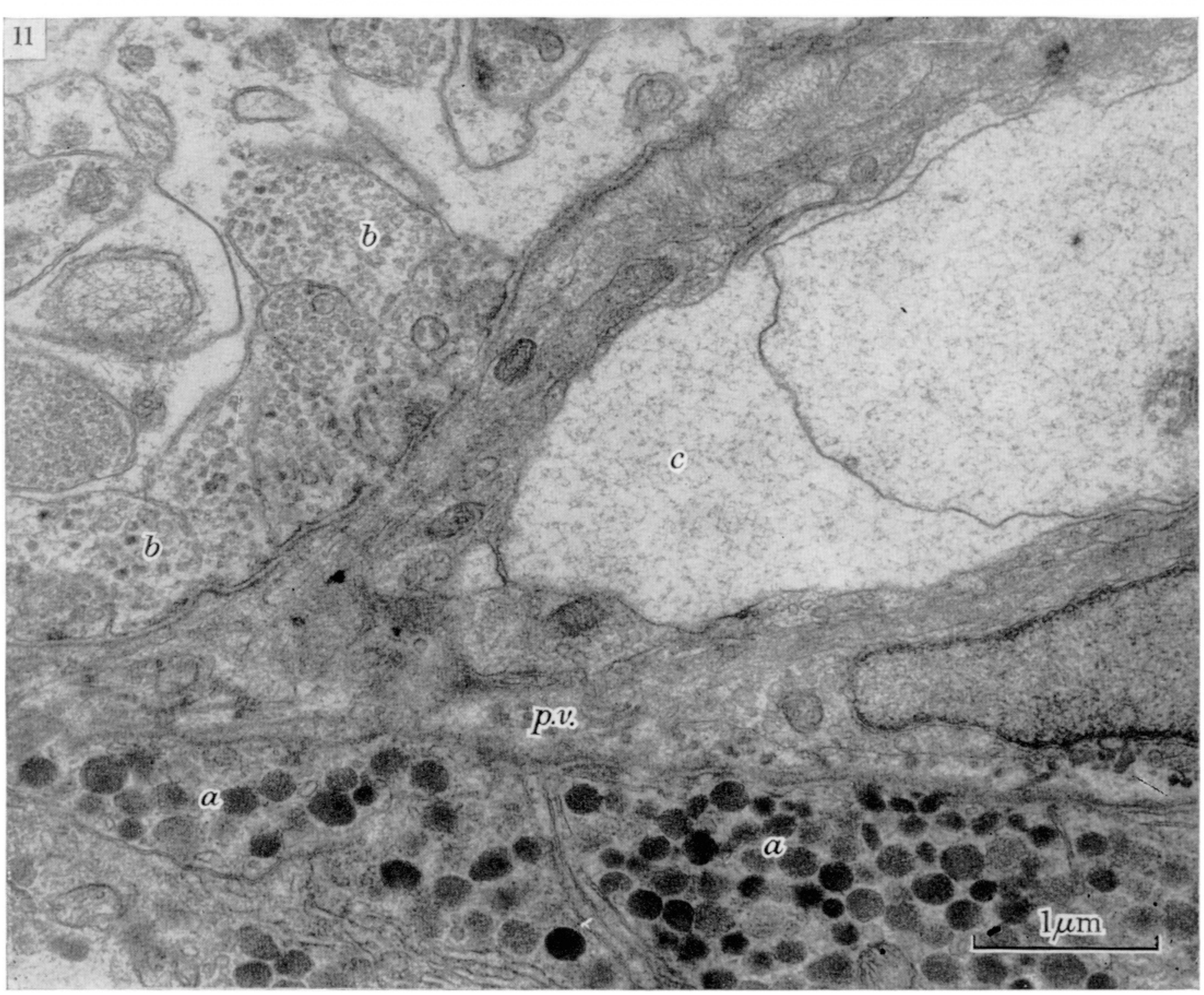
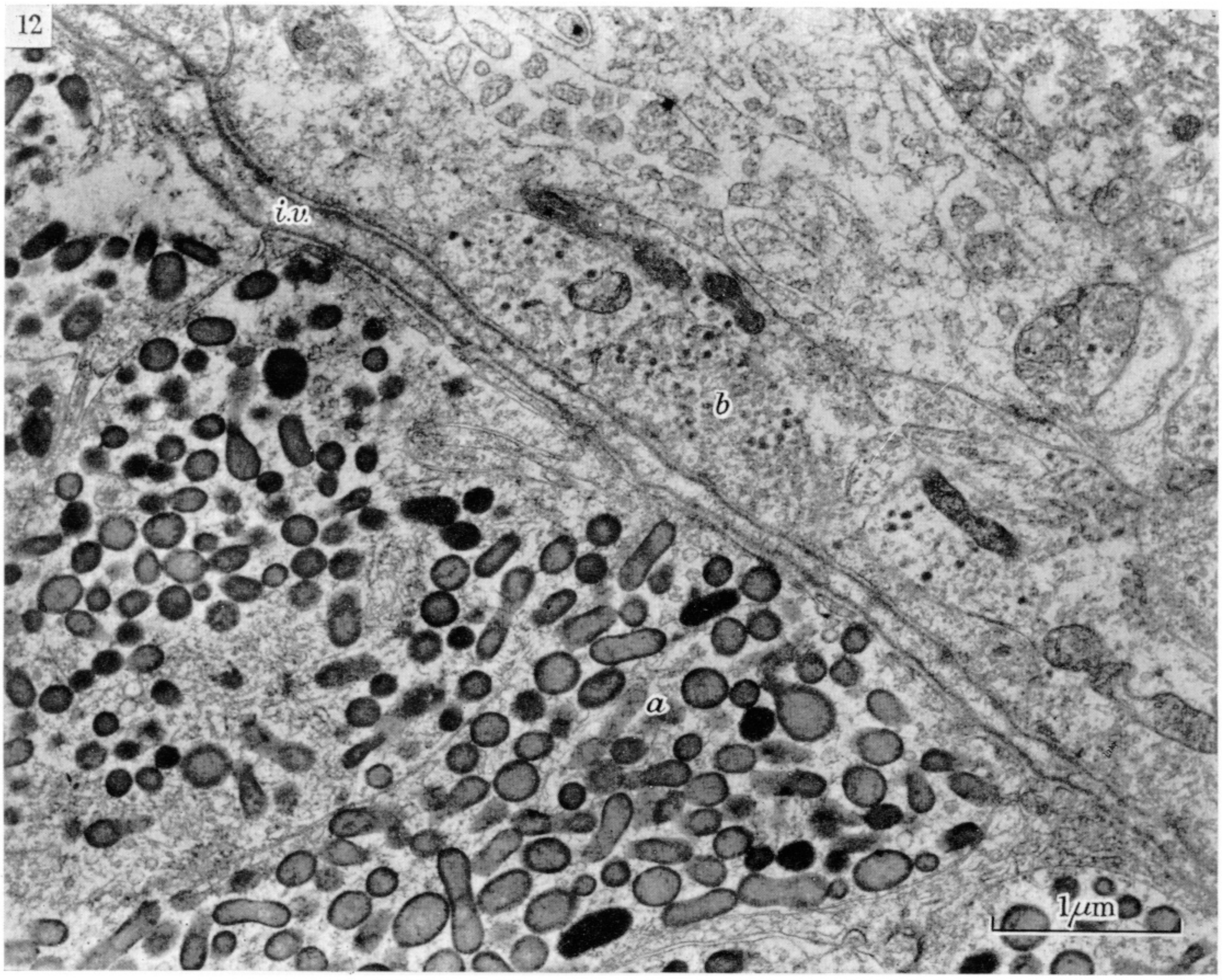


FIGURE 10. A survey picture of a portion of the rostral pars distalis of the pituitary of *Conger*. *a*, *ACTH* cells; *c*, capillary. *i.v.*, intervascular channel; *t*, tract.

FIGURE 11. The relationship in *Anguilla* between Type B fibres (*b*), a capillary (*c*), the perivascular space (*p.v.*) and *ACTH* cells (*a*).

12



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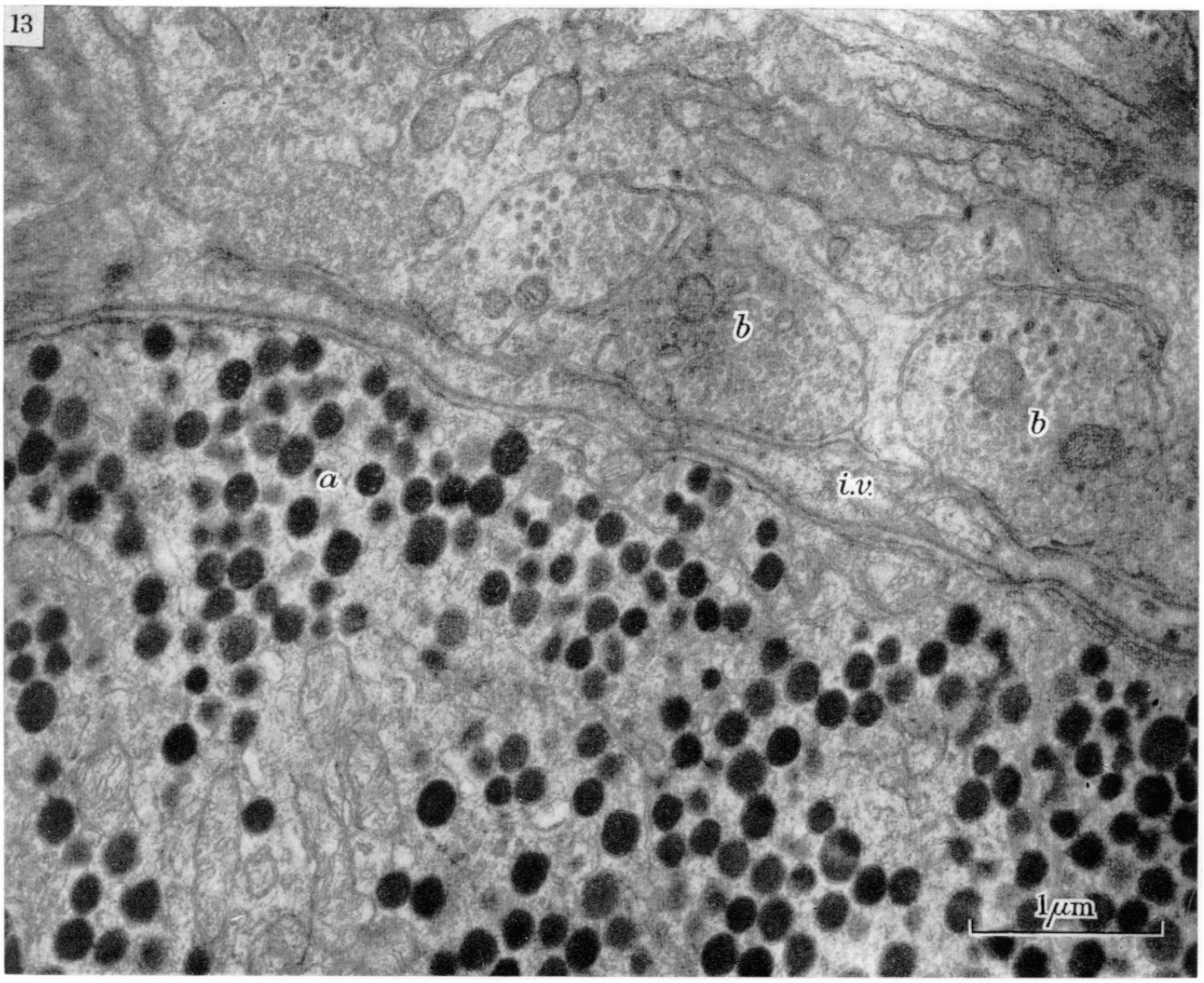
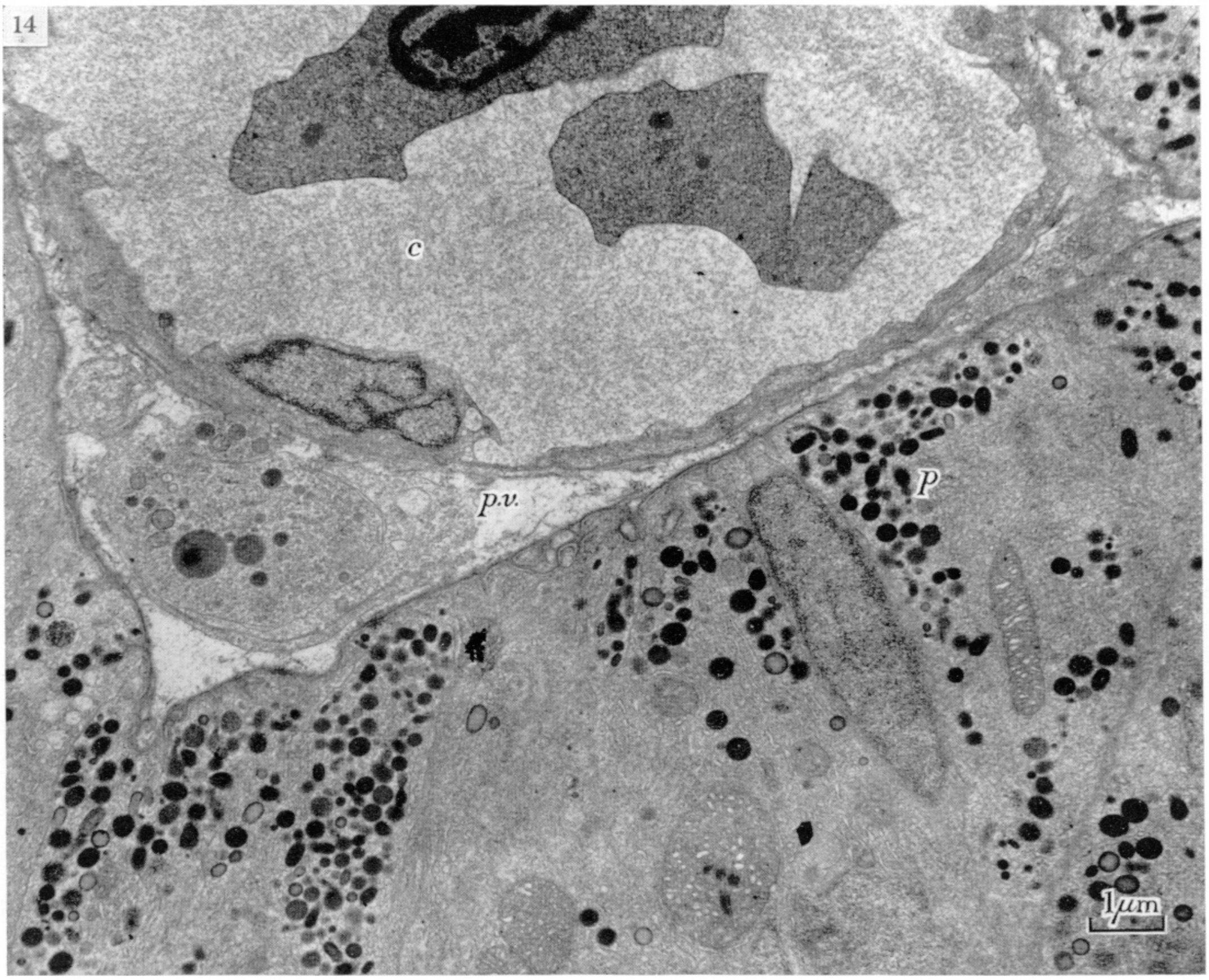


FIGURE 12. The relationship between the neurosecretory innervation and *ACTH* cells in *Conger*. *a*, *ACTH* cell; *b*, Type B fibre; *i.v.*, intervascular channel.

FIGURE 13. As figure 12, but in the pituitary of *Anguilla*. (Lettering as in figure 12.)

14



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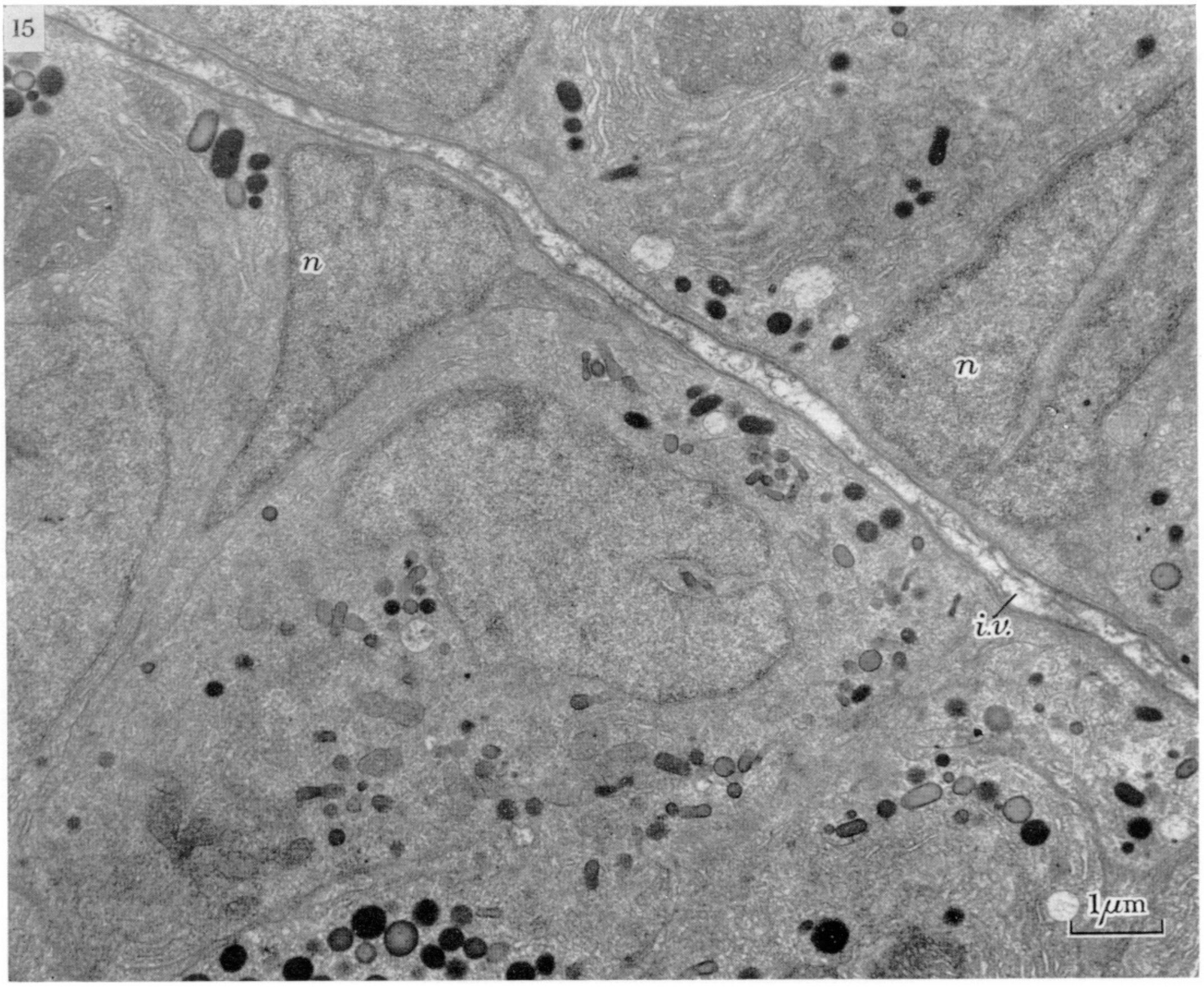
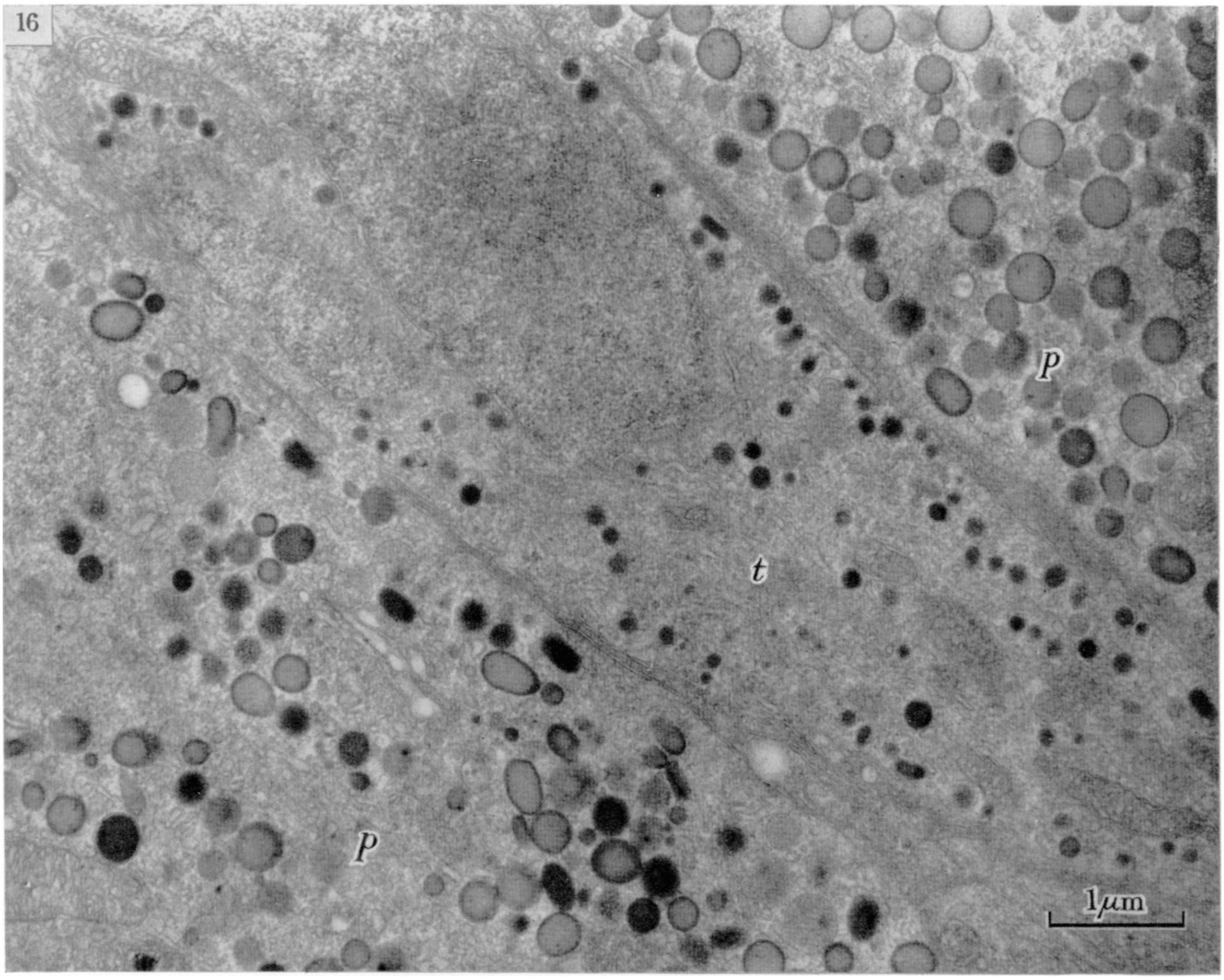


FIGURES 14 AND 15. Sections through the periphery of follicles of the rostral pars distalis of the *Conger* pituitary. *c*, Capillary; *i.v.*, intervascular channel; *n*, neck cell; *p*, 'prolactin' cell; *p.v.*, perivascular space.



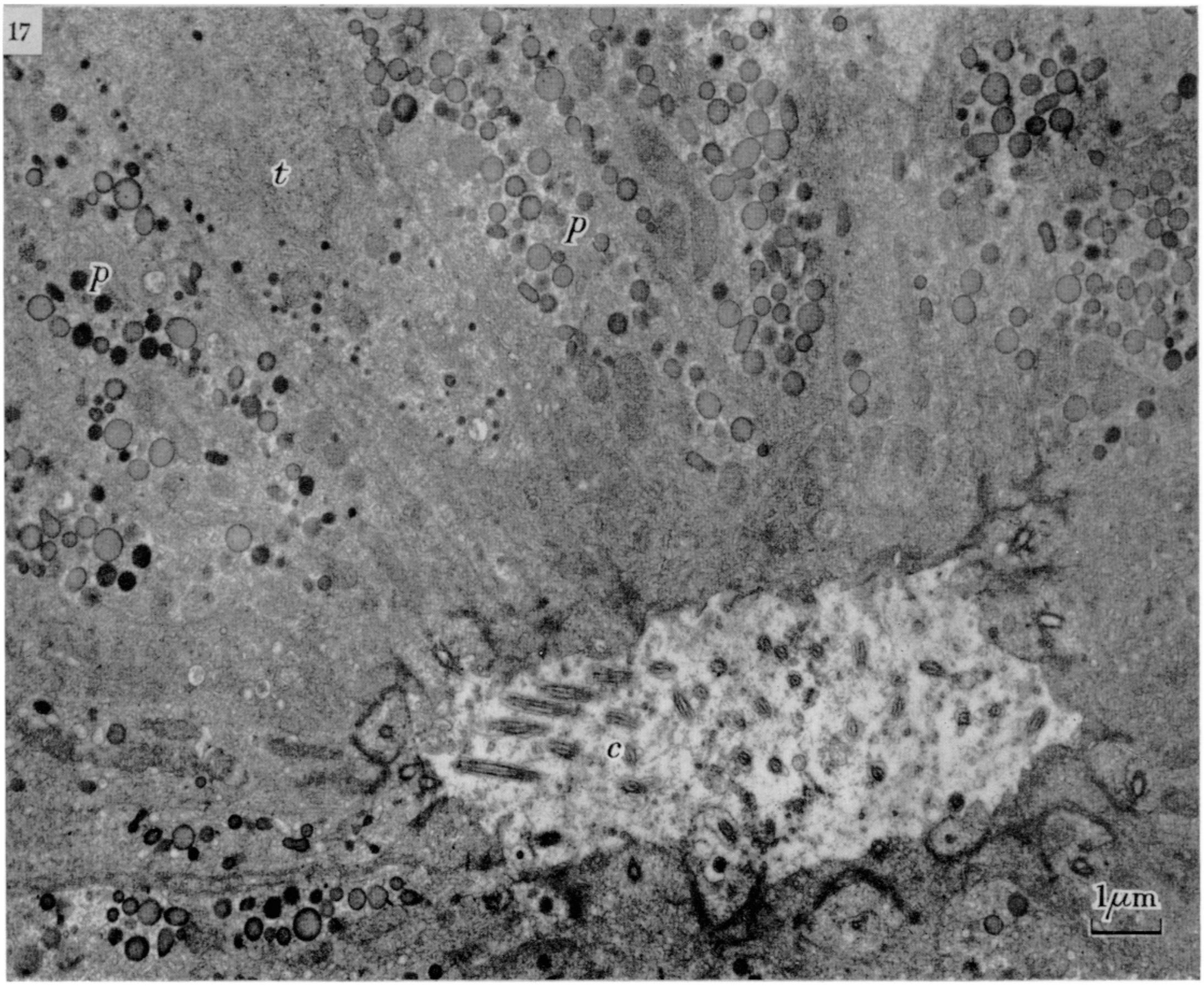
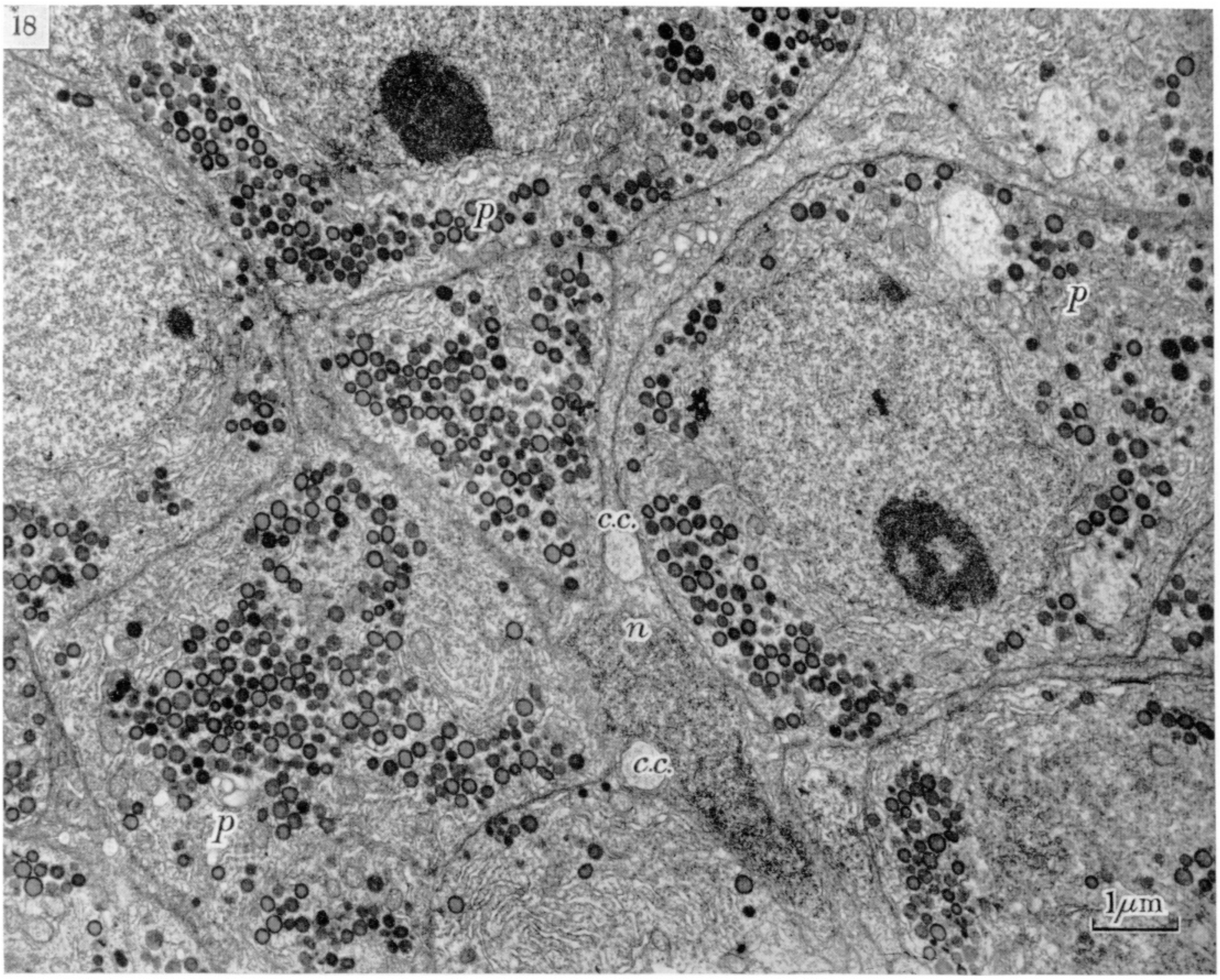


FIGURE 16. A section of a portion of a follicle of the rostral pars distalis of the pituitary of *Conger*. A prolongation of a *TSH* cell (*t*) lies between two 'prolactin' cells (*p*).

FIGURE 17. The central region of the follicle shown at figure 16. Lettering as in figure 16. *c*, Cilia.



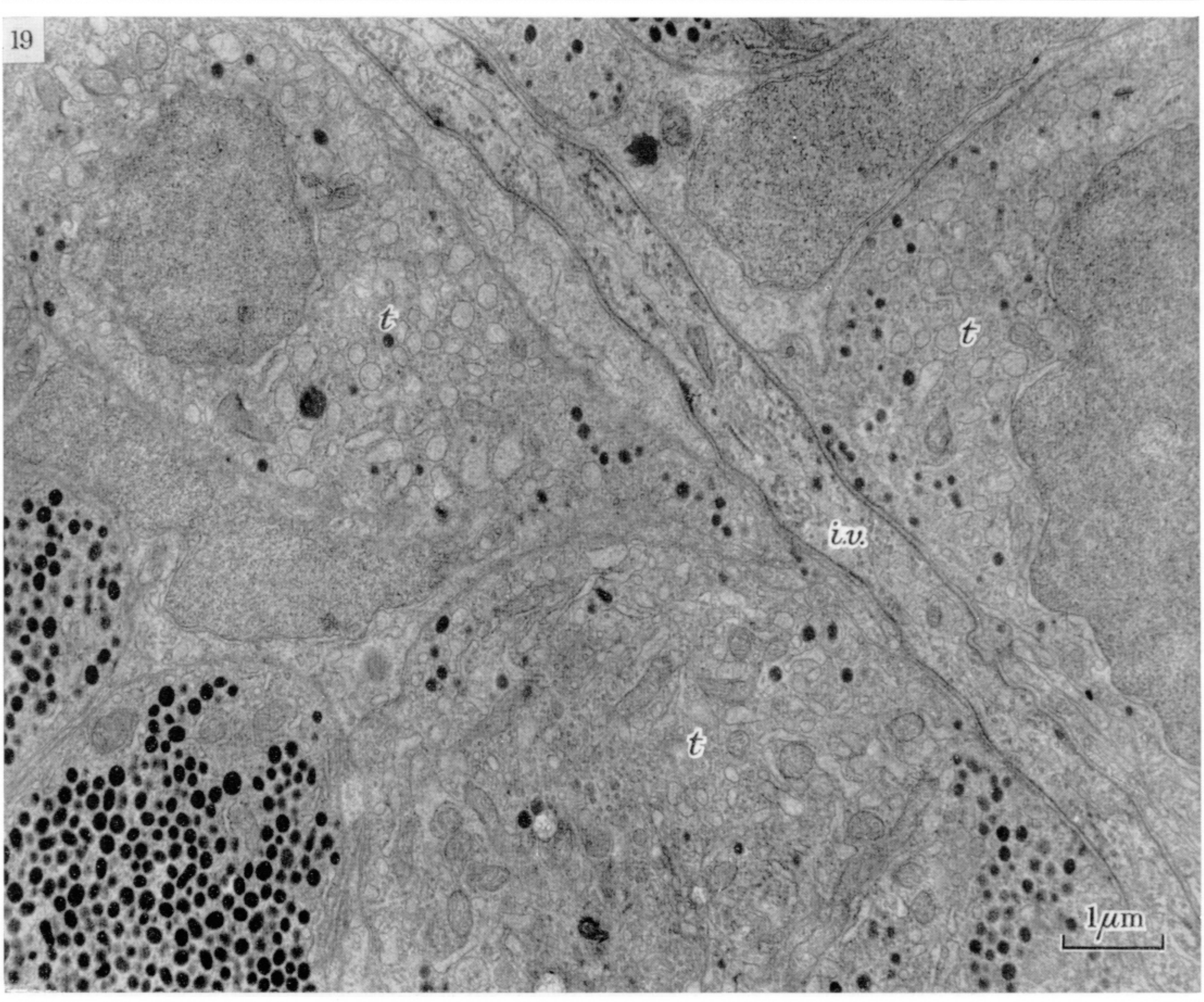
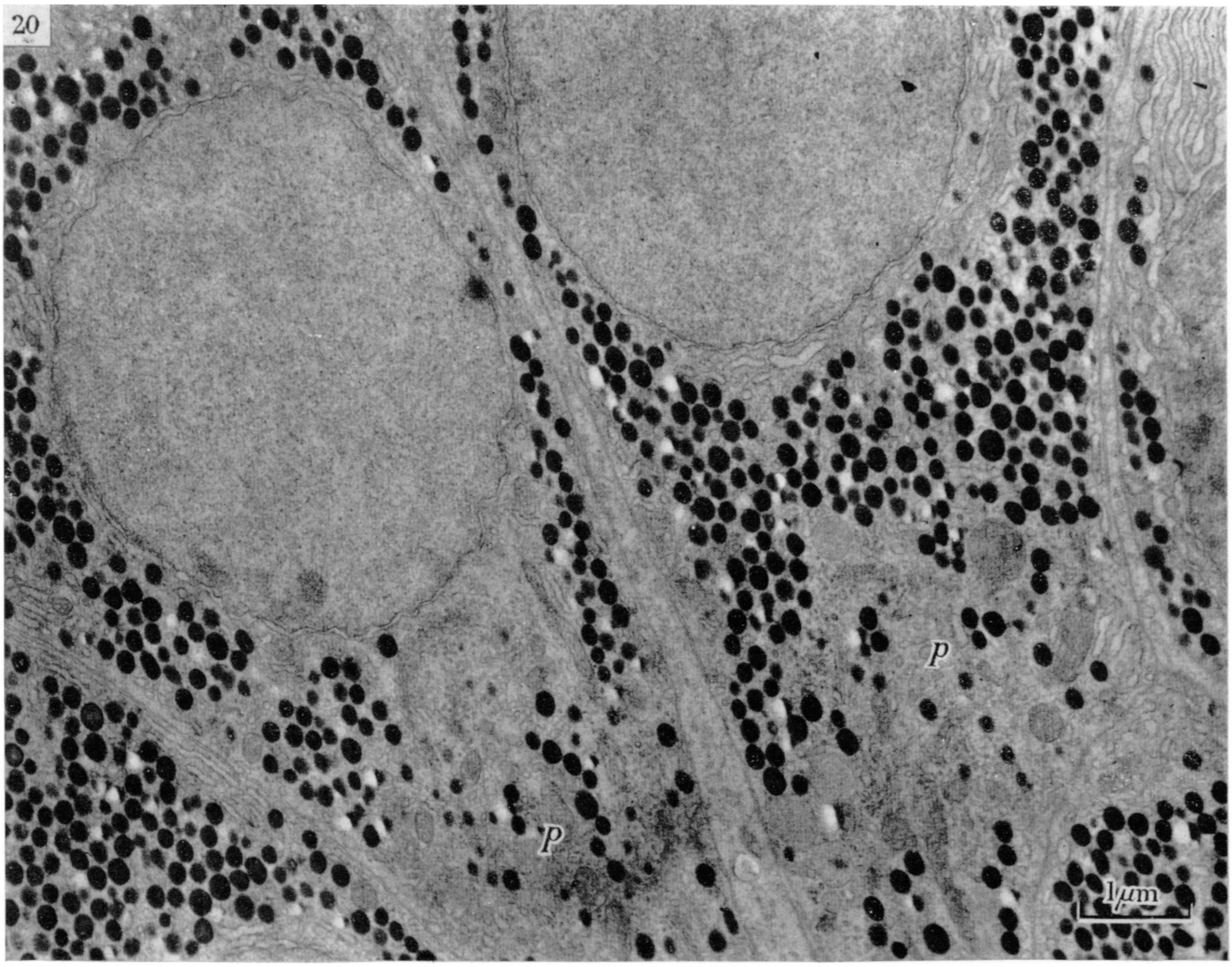


FIGURE 18. A follicle of the rostral pars distalis of the pituitary of *Anguilla*, cut tangentially close to its surface. *c.c.*, Connecting canal; *n*, neck cell; *p*, 'prolactin' cell.

FIGURE 19. *TSH* cells (*t*) at the periphery of the follicle, bordering the intervascular channel (*i.v.*).



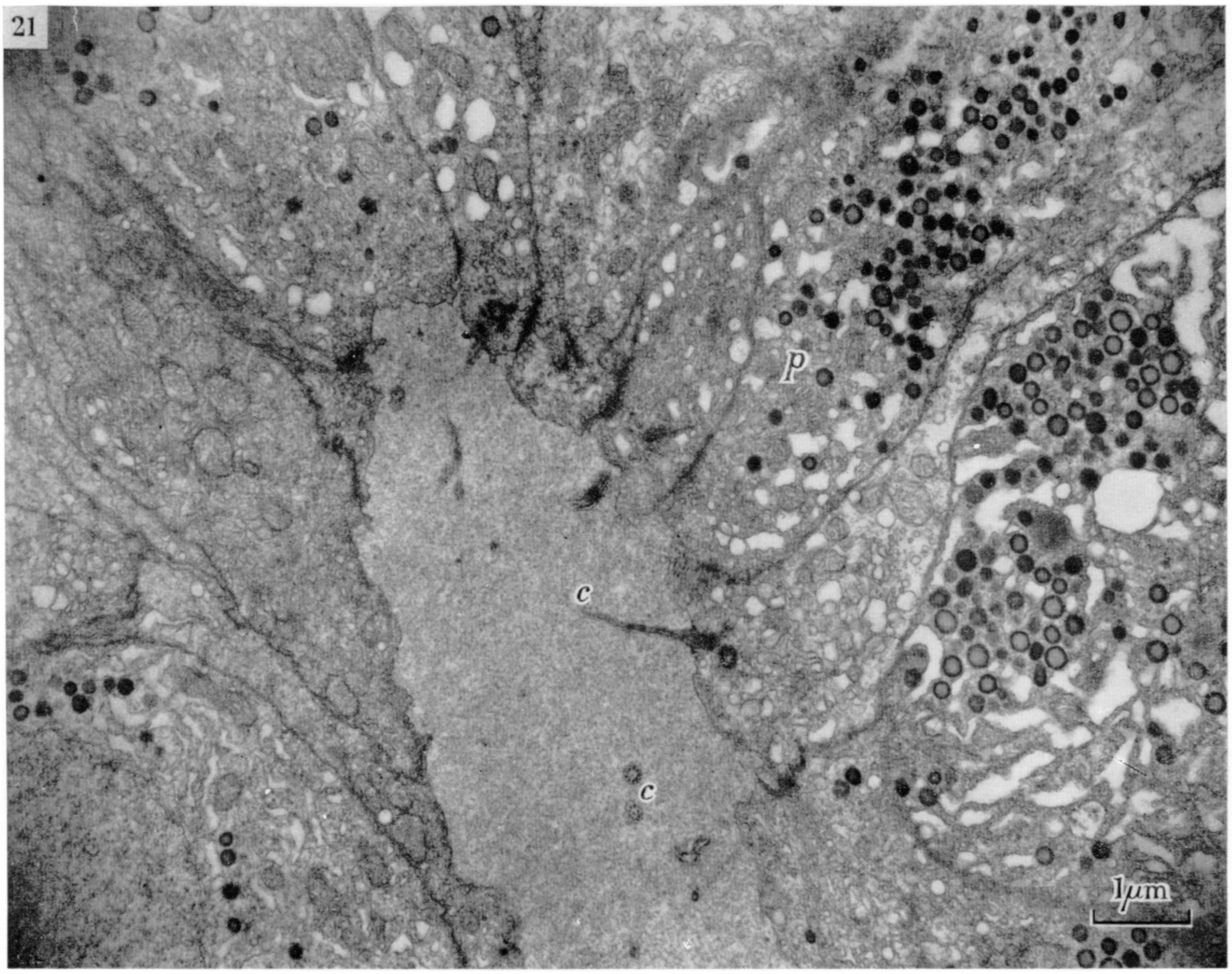


FIGURE 20. A section through a small portion of a follicle in the rostral pars distalis of the pituitary of *Anguilla*, showing 'prolactin' cells (*p*).

FIGURE 21. As figure 20 but at the centre of the follicle, *c*, Cilia; *p*, 'prolactin' cell.

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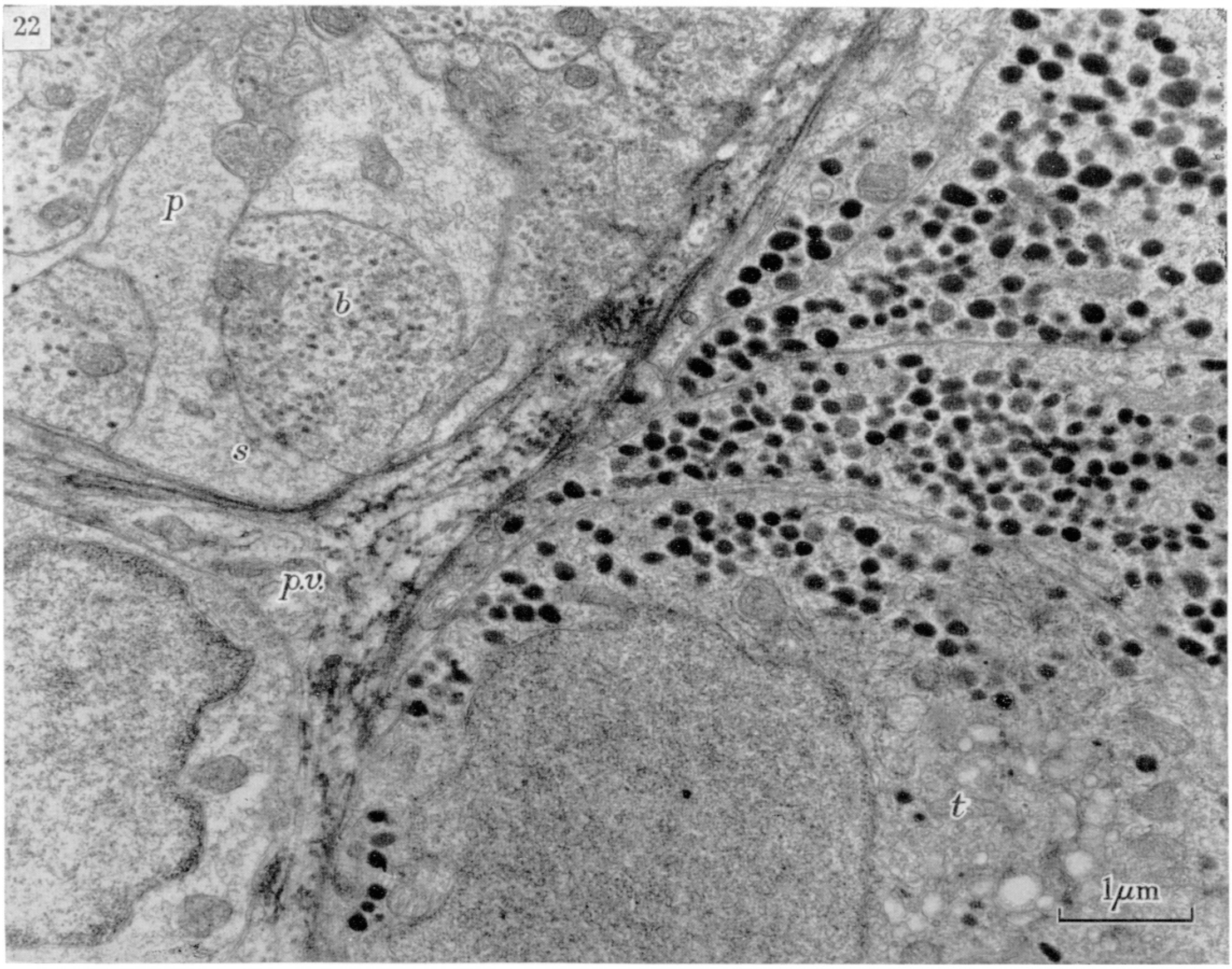

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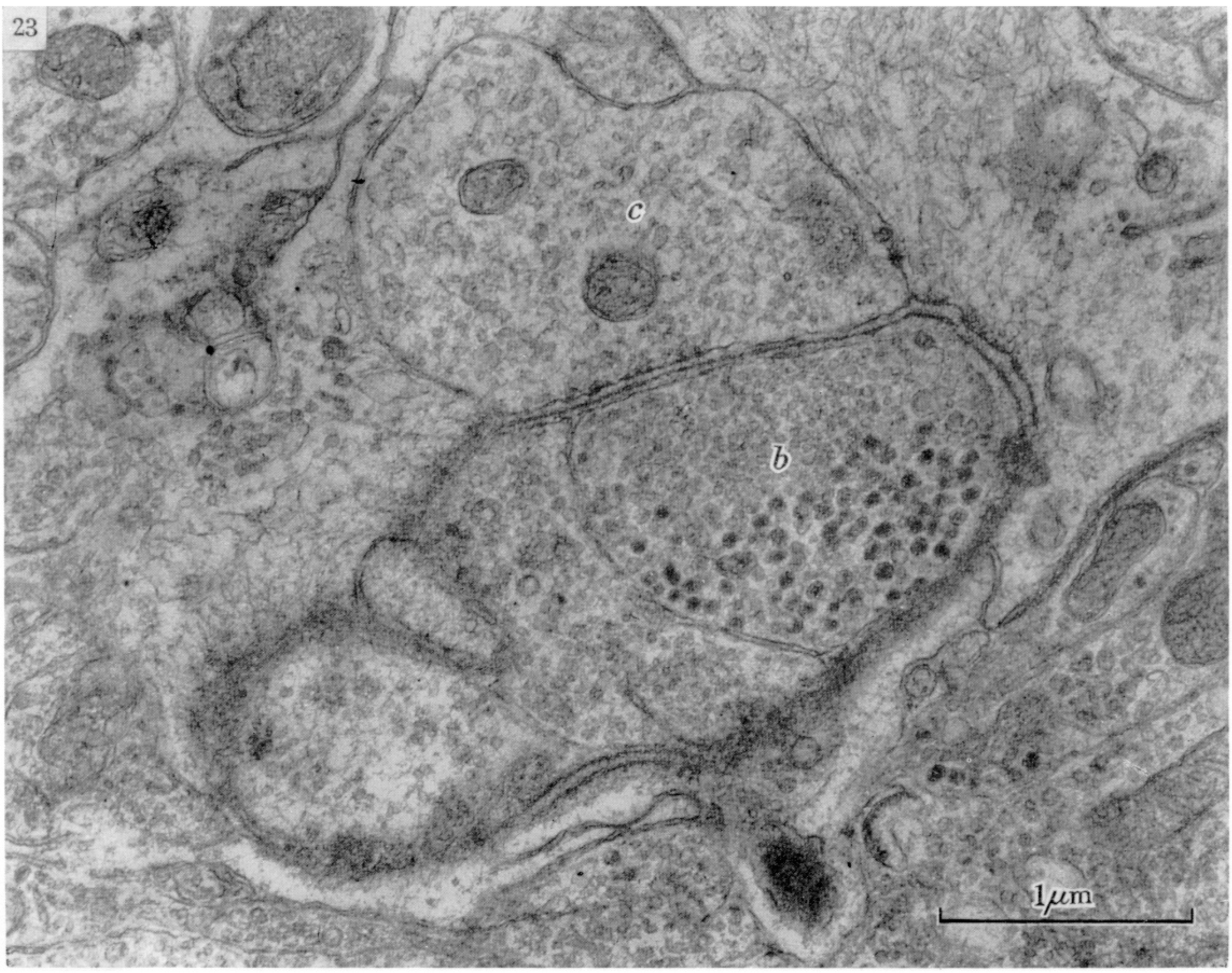
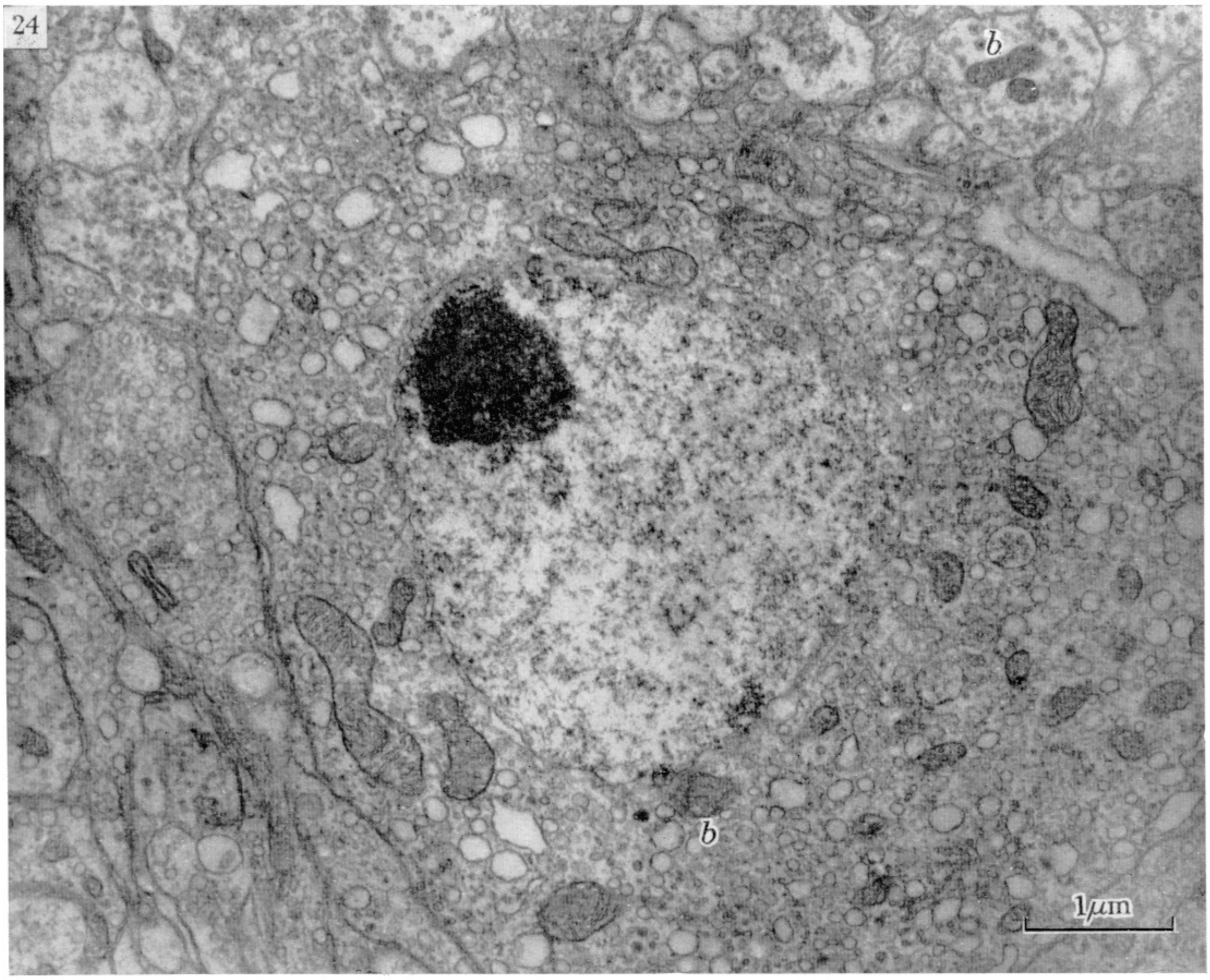
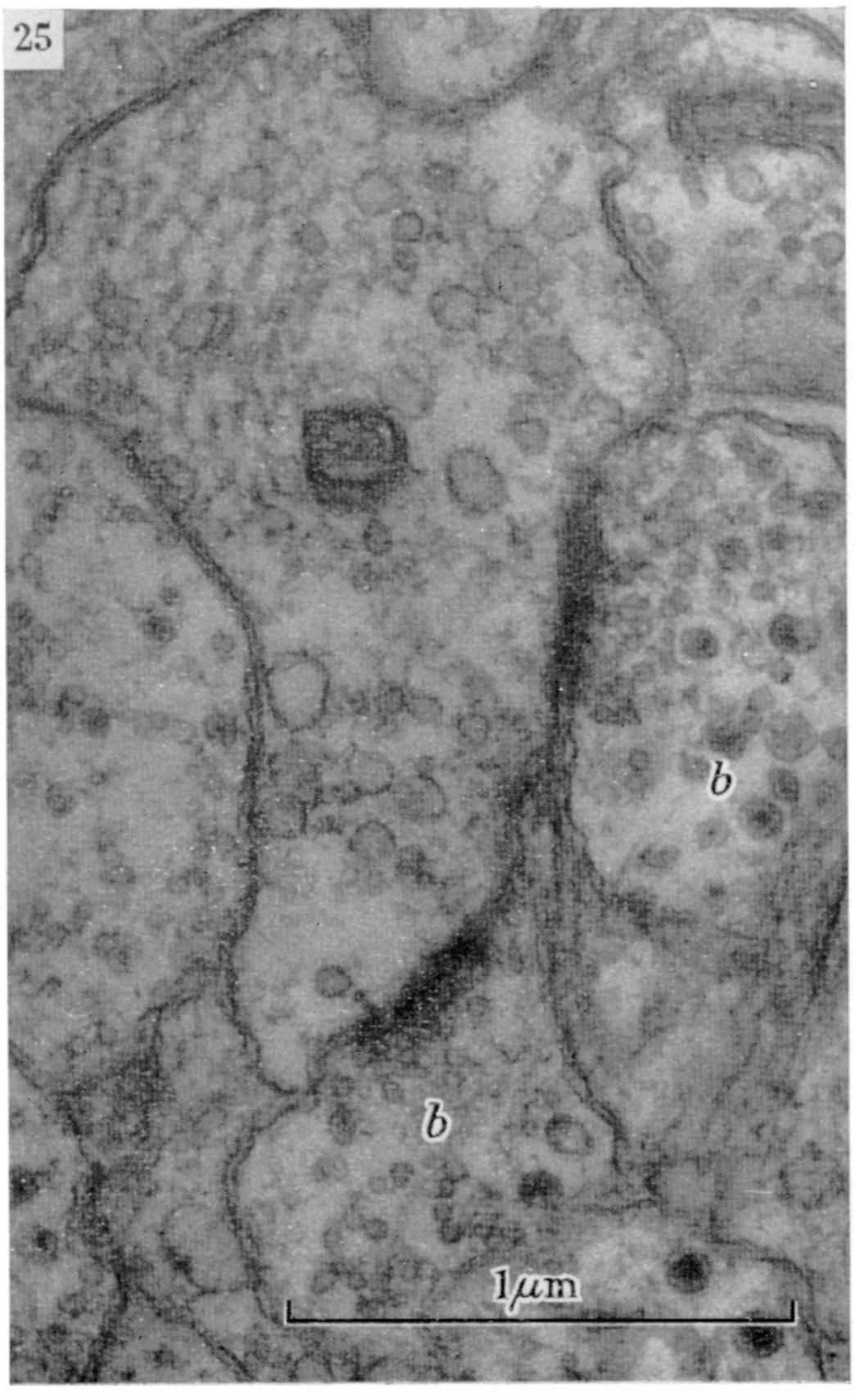


FIGURE 22. Neurosecretory tract, containing Type B fibres (*b*) and pituicytes (*p*) in proximity to a *TSH* cell (*t*) in the rostral pars distalis of the pituitary of *Anguilla*. *p.v.*, Perivascular space; *s*, possible synaptic junction.

FIGURE 23. Type B fibre terminal (*b*) in close association with another cellular element (*c*) possibly a pituicyte, in the tract leading to the rostral pars distalis of *Anguilla*.





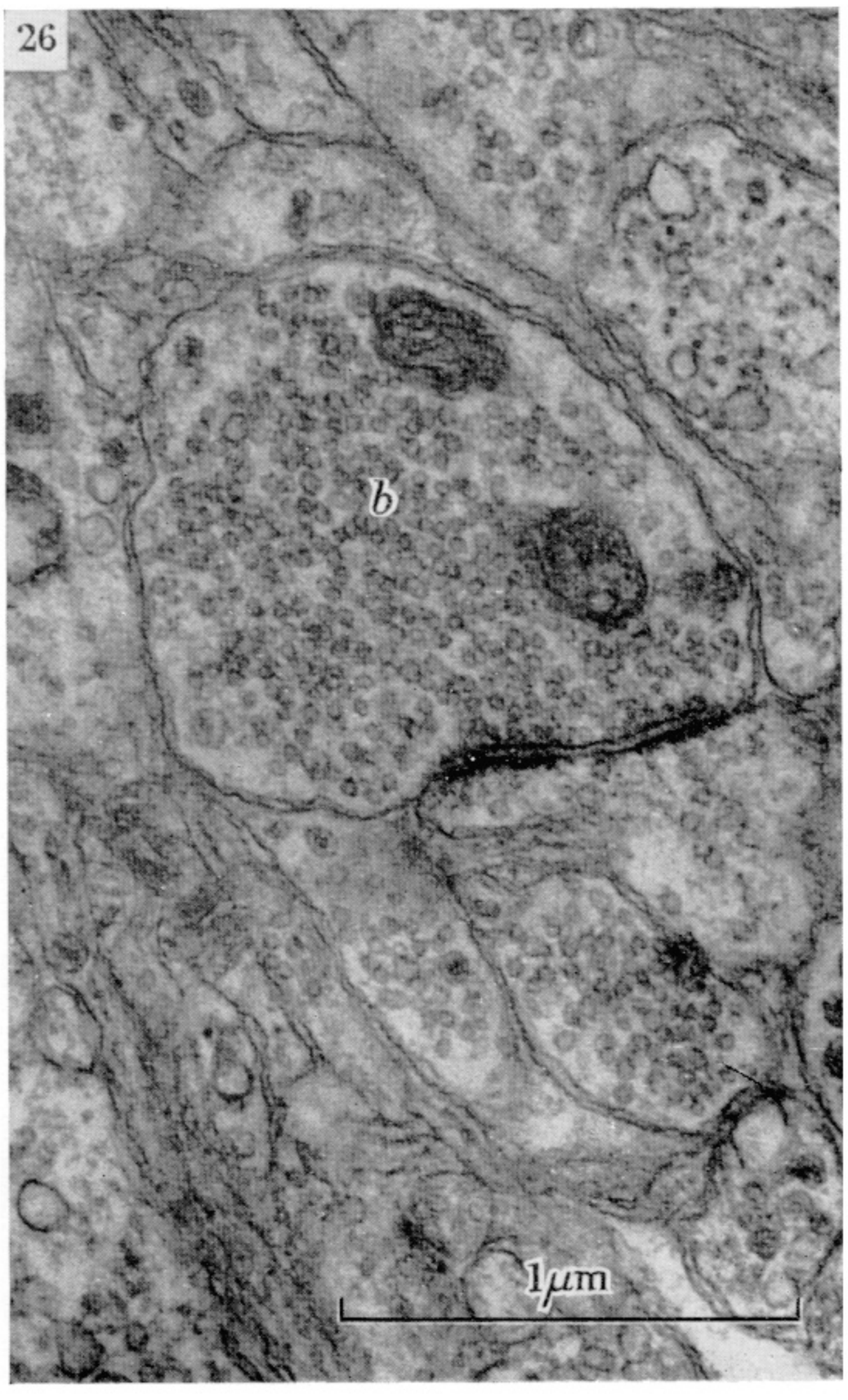
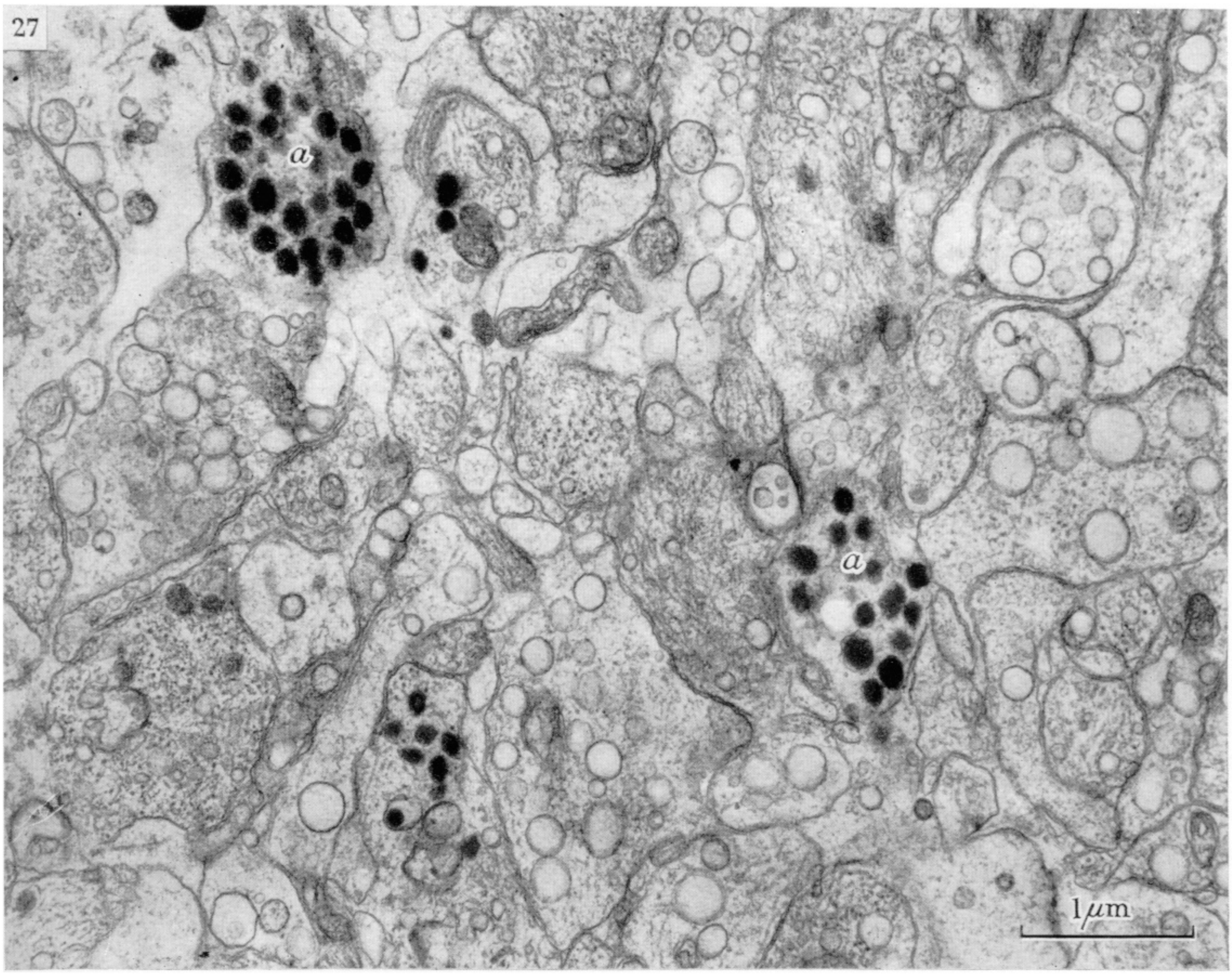


FIGURE 24. A perikaryon and fibres in the nucleus lateralis tuberis, showing Type B vesicles (*b*).
FIGURE 25. Probable axo-dendritic synapse, involving Type B fibres (*b*) in the nucleus lateralis tuberis.
FIGURE 26. Probable axo-axonal synapse involving Type B fibres (*b*) in the nucleus lateralis tuberis.



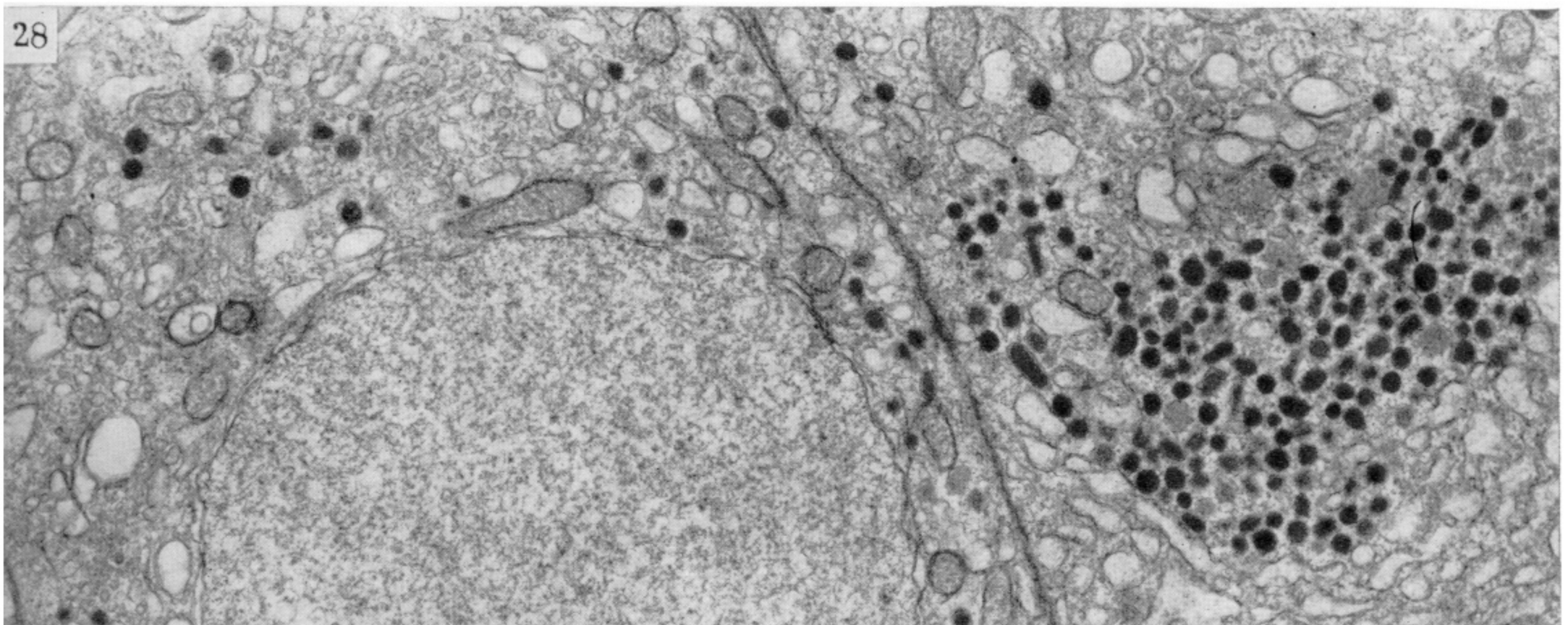


FIGURE 27. Type A fibres (*a*) in the tract leading to the rostral pars distalis of a silver eel (*Anguilla*).
FIGURE 28. *TSH* cells in the rostral pars distalis of a silver eel (*Anguilla*).

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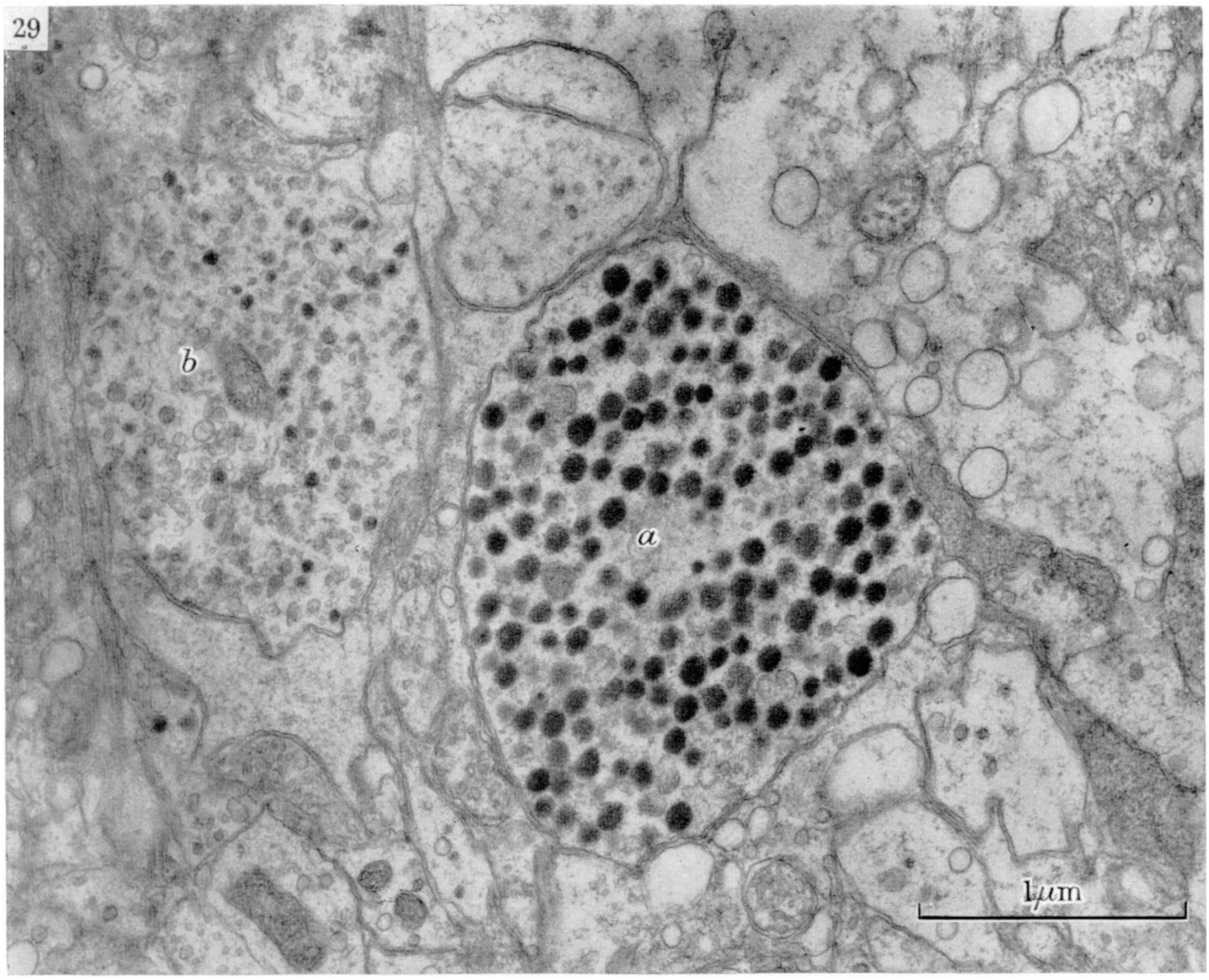

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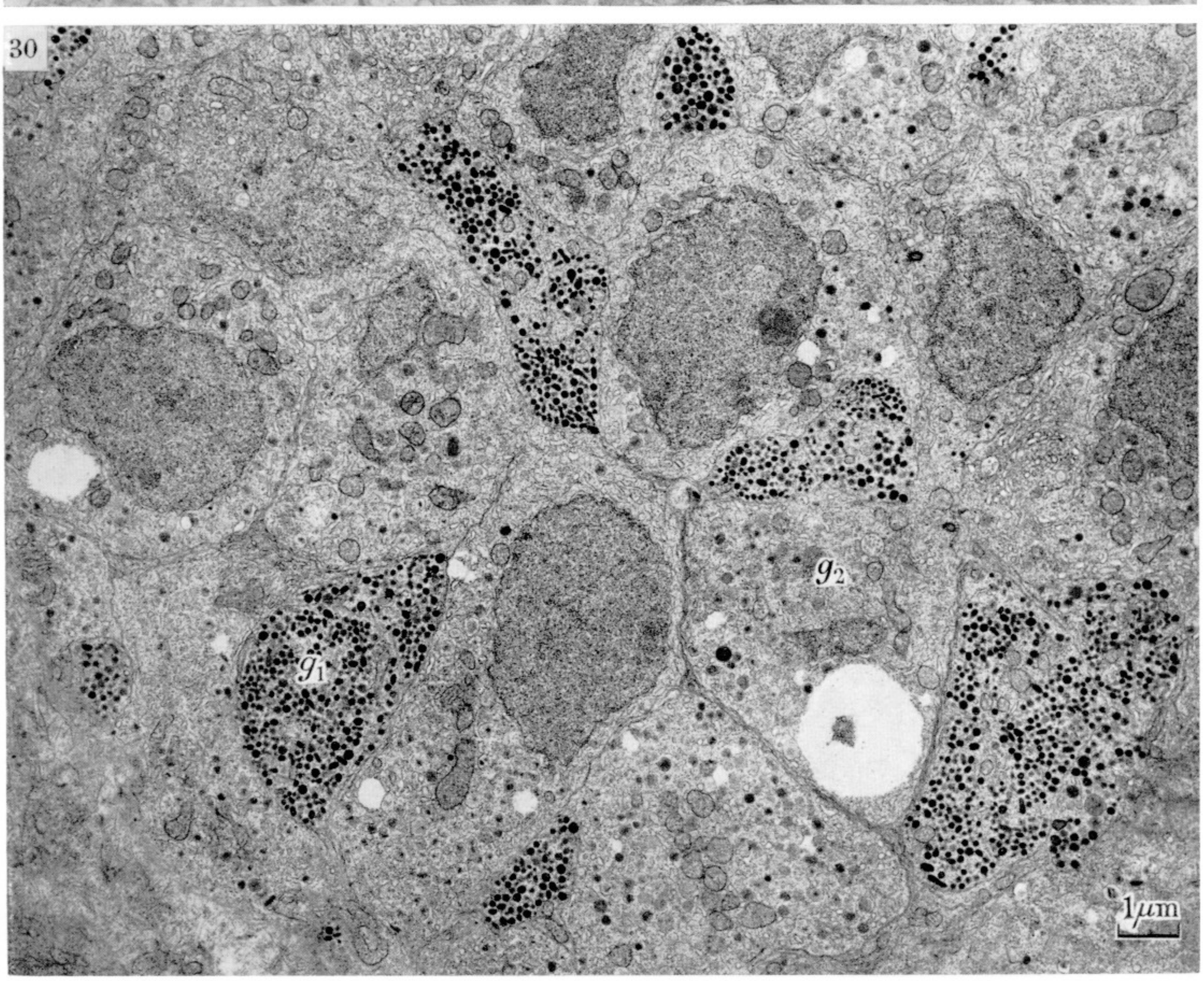

FIGURE 29. A portion of the neurosecretory tract leading to the proximal pars distalis of a silver eel (*Anguilla*). Both Type A (*a*) and Type B (*b*) fibres are present.

FIGURE 30. Cells of the proximal pars distalis of a silver eel. Two cell types, believed to be gonadotrophs (g_1, g_2) can be distinguished.