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SOME OBSERVATIONS ON NEUROSECRETORY INNERVATION  
IN THE PITUITARY GLAND OF THE MEDAKA,  
*ORYZIAS LATIPES*

Seiichi KASUGA\* and HIROYA TAKAHASHI\*

It is well known that, in the pituitary gland of teleost fishes, the neurohypophysial tissue invades deeply and complicatedly into the adenohypophysis, making an outstanding difference from the gland of tetrapods (Kobayashi and Matsui, 1969). This morphological characteristic of the teleost pituitary offers some interesting problems about the structural connection between adenohypophysial glandular cells and neurohypophysial elements that are believed to mediate neurosecretory control of adenohypophysial activities in response to various extrinsic and intrinsic stimuli (Jørgensen and Larsen, 1967).

In tetrapod only pars intermedia of the pituitary has been electron microscopically evidenced to receive neurosecretory innervation from neighbouring pars nervosa (Etkin, 1967). In teleost fishes, on the other hand, not only the cells of the meta-adenohypophysis (pars intermedia) but those of the pro- and meso-adenohypophysis have also been observed to reveal quite an intimate connection including synaptoid contact with neurosecretory axons (cf. Jørgensen, 1968). Studying the fine structure of the neurohypophysis of the medaka, Oota (1963) noticed the neurosecretory axon endings in direct contact with the adenohypophysis, though he did not mention about the detailed features of such a connection between both pituitary components. Information about the fact of the glandular cells related to the innervated axons seems, however, to be still insufficient to establish the actual mode of neurosecretory control over adenohypophysial secretion in fishes.

In view of the obvious sensitiveness of gonad maturation, ovulation and spawning to environmental changes, especially those of photoperiod (Egami, 1954; Yoshioka, 1962, 1963, 1966), the medaka, *Oryzias latipes*, seems to be a material suited for investigating the mechanism of possible neurosecretory control of the gonadotropic function of the pituitary. As one of the results of the work directed to elucidate such a mechanism, this report deals mainly with ultrastructural findings of neurosecretory axons found in the pituitary gland of the medaka, with special reference to the mode of innervation into the adenohypophysis.

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### Material and methods

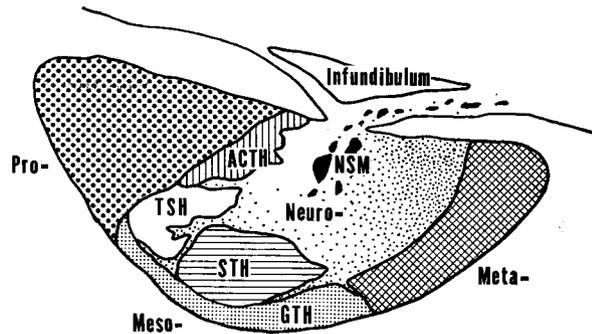
Adult female medaka, *Oryzias latipes*, of the wild type, ranging from 2.6 to 3.8 cm in body length, were used as a material. The fish were collected from a pond in Yunokawa in the suburbs of Hakodate, and were subsequently reared in an outdoor pond of the Faculty of Fisheries, Hokkaido University, under natural conditions of temperature and light. Samplings of the fish from the stock pond were done for one year from September 1968 on.

For light microscopy, the head of the fish was fixed *in toto* for 24 hours in Heidenhain's Susa and embedded in paraffin. Serial sections of 6  $\mu$  in thickness were cut frontally or sagittally, and were stained with Gomori-Halmi's aldehyde fuchsin (AF, Halmi, 1952), Gomori's chrome alum hematoxylin and phloxine (CH-P), Mallory's Azan, or periodic acid-Schiff reagent (PAS).

For electron microscopical studies the pituitary gland attached to the hypothalamus was quickly dissected out from the head under a binocular dissecting microscope immediately after the decapitation. Some of the materials were fixed for 2 hours in 1% osmium tetroxide buffered at pH 7.4 with 0.1 M Millonig's phosphate buffer at 0-4°C. Others were fixed for 1 hour in 3.5% glutaraldehyde buffered at pH 7.4 with phosphate buffer, rinsed for 30 minutes in the same buffer and postfixed for 1 hour with the osmium tetroxide solution. The fixed specimens were successively dehydrated through a graded series of ethanol and embedded in Epon-epoxy resin mixture (Luft, 1961). Sections were cut with glass knives on a Porter-Blum microtome and doubly stained with uranyl acetate saturated in 50% ethanol and lead citrate (Reynolds, 1963). A Hitachi HS-7 electron microscope was used for the examination of the sections.

### Observations

Light microscopically, axons loaded with Gomori-positive neurosecretory materials enter into the neurohypophysis through the posterior wall of the infundibular recess, forming large Herring bodies near the infundibular stalk. The accumulation of Gomori-positive materials is prominent especially in the posterior region of the neurohypophysis. Numerous pituicytes are seen scattering through the neurohypophysis, with a tendency to gather as a distinct cluster in the posterior zone of the organ (Fig. 3). The interdigitational histoarchitecture is distinctly displayed between the neurohypophysis and the meso- and meta-adenohypophysis, for the distribution of Gomori-positive materials is clearly traced among the cells of the meso- and meta-adenohypophysis (Figs. 1 and 2). Among



Text-fig. 1. A schematic illustration of the pituitary gland of the medaka in a median sagittal plane, indicating the location of various glandular cell types. *ACTH*, adrenocorticotrophic cells; *GTH*, gonadotrophic cells; *STH*, somatotrophic cells; *TSH*, thyrotrophic cells; *Pro-*, pro-adenohypophysis; *Meso-*, meso-adenohypophysis; *Meta-*, meta-adenohypophysis; *Neuro-*, neurohypophysis; *NSM*, neurosecretory material.

the cells of the pro-adenohypophysis, however, no Gomori-positive materials are detectable by light microscopy.

Adenohypophysis is divided into three zones, namely, pro-, meso-, and meta-adenohypophysis (Text-fig. 1). Pro-adenohypophysis which occupies the anterior one third of the pituitary consists chiefly of one type of acidophilic cells, probably of prolactin cells in nature. Sometimes chromophobic cells are observable around blood capillaries, which are numerous encountered, and in the peripheral region of this zone.

Meso-adenohypophysis, which is situated in the middle region of the adenohypophysis, includes four types of cells, two of them being basophilic cells. Basophilic cells of one type with folded nuclei are seen clustered along the ventral margin of the meso-adenohypophysis, with Gomori-positive materials accumulated above the cluster. These cells alter their cytological characters in definite harmony with the reproductive cycle of the fish (Kasuga, unpublished), and are designated as gonadotrophic cells (*GTH*-cells). Basophilic cells of another type, surrounded by neurohypophysial tissue, are present in the anterior region of the meso-adenohypophysis. These cells are possibly thyrotrophic in nature (*TSH*-cells), because the cells show specific hypertrophy following treatment of the fish with thiourea (Kasuga, unpublished). The remaining two types of the meso-adenohypophysial cells are acidophilic, one existing in the central region and another in the anterior region adjacent to the pro-adenohypophysis. The former is well stained with azocarmine G, orange G or phloxine, while the latter is stainable weakly only with azocarmine G. On the basis of their topographic position in the meso-adenohypophysis, they are presumed to be somatotrophic (*STH*)

and adrenocorticotropic (ACTH) cells, respectively.

Meta-adenohypophysis is located in the posterior part of the pituitary and, light microscopically, only one type of glandular cells was discernible in this zone with the stainings employed in this study.

Electron microscopically, numerous neurosecretory axons are found to run as bundles in the central region of the neurohypophysis, being surrounded by pituicytes. The axons are all of the unmyelinated type, and are distinguishable into three types in terms of secretory granules contained in them. The first type has granules of 1,400–1,800 Å, the second one those of about 1,200 Å and the third one those of 850–1,150 Å in size. In the following descriptions, these three types will be termed as types A<sub>1</sub>, A<sub>2</sub> and B, respectively, according to the classification of the neurosecretory fibers in the fish pituitary made by Knowles and Vollrath (1966 a, b).

It is probable that a certain nerve fiber with no neurosecretory function exists besides the neurosecretory axons in the neurohypophysis, as reported by Oota (1963) in the medaka. However, the present observation failed to confirm the existence of such an ordinary axon in the neurohypophysis, for an uneven and rather sparse distribution of the secretory granules in some of the axon endings seems to take the feature of ordinary nerve endings in a certain plane of the ultra-thin sections.

In neurosecretory axons there occur mitochondria and neurotubules of about 200 Å in width along with secretory granules. Besides, some vacuoles were occasionally observed in the axons of the specimens treated with glutaraldehyde prior to OsO<sub>4</sub> fixation. On many occasions a huge number of neurosecretory granules are restrictedly deposited in an axon, resulting in its bulbous distension which may correspond to the so-called Herring body (Fig. 4). The neurosecretory granules which pack the large Herring bodies are exclusively of 1,400–1,800 Å in size (type A<sub>1</sub> granules).

Neurosecretory axons are observed to terminate on the pericapillary space and its ramifications in the adenohypophysial tissue and even directly on glandular cells and pituicytes. These endings are bulbous in contour and are usually packed with numerous neurosecretory granules, small vesicles of about 500 Å in diameter regarded as synaptic vesicles, and mitochondria, but containing no neurotubules and neurofilaments. Synaptic vesicles are generally assembled to form a cluster along the thick and electron dense presynaptic membrane of an axon ending and are concurrently dispersed in the ending. Neurosecretory granules in axon endings generally show various degrees of electron density in their contents, on many occasions, with the appearance of electron lucent vesicles which are slightly larger in size than electron dense secretory granules. Sometimes the limiting membranes of the electron lucent vesicles appear to be broken.

Bordering on the blood capillaries in the neurohypophysis all of the three types of axons terminate with typical bulbous endings, of which the axons of type  $A_1$  are dominant in distribution. In the neurohypophysis of the medaka, the space between the nerve ending and the endothelium is from 750 to 1,500 Å in width including the basement membrane, being much narrower than that observed in other vertebrates (Fig. 5). A similar case has been pointed out in *Lebistes reticulatus* by Follenius and Porte (1962).

Two types of pituicytes are ultrastructurally discernible in the neurohypophysis. Pituicytes of the first type project their cytoplasmic processes complicatedly among nerve fibers (Fig. 6). Sometimes they are gathered as a cluster in the posterior region of the neurohypophysis, with desmosomes connecting them with one another. In the center of the cluster there is a lumen which resembles in feature the central canal found in the neurohypophysis of the eel (Knowles and Vollrath, 1965 a, b, 1966 a). Microvilli and cilia which lack the central filament protrude into this lumen (Fig. 7). On these pituicytes neurosecretory axons of types  $A_1$  and B end with a distinct synaptoid contact (Figs. 8 and 9). Pituicytes of the second type mostly exist near the meso-adenohypophysial cells, often being closely adjacent to the latter, and project their processes toward the blood capillary (Figs. 10 and 11). No synaptoid contact was demonstrated between these pituicytes and axons of any type, so far as the writers observed.

Glandular cells in the pro-adenohypophysis include numerous electron dense granules of about 2,500 Å in diameter (Fig. 12). The granules are mostly spherical in shape but are oval in some ones. Besides a few lamellae of rough endoplasmic reticulum surrounding the nucleus, Golgi complex and other usual cytoplasmic inclusions, these cells contain mitochondria of a peculiar type which are lacking in the usual arrangement of the mitochondrial crests and are sometimes fairly large in size (Fig. 13). Furthermore it is noticed that they are surrounded by cells of possible supporting nature which extend cytoplasmic processes toward the blood capillary. In these supporting cells numerous mitochondria are assembled in the cytoplasm adjacent to the pericapillary space (Fig. 12).

Neurosecretory axons of type B penetrate deeply into the pro-adenohypophysis, repeatedly making a direct synaptoid contact with glandular cells. The axons appear singly among the cells, and each seems to keep a distinct synapse on the respective cell. Axons of the other types are not detected in the pro-adenohypophysis. The pericapillary space in this zone is wider than that found in the neurohypophysis and is nearly devoid of ramification. No axon endings of any type are detectable to terminate on the pericapillary space.

Meso-adenohypophysial cells designated as ACTH-cells are provided with small round secretory granules of about 1,700 Å in diameter. These granules are various in electron density and are dispersed uniformly in the cytoplasm which

contains well-developed Golgi apparatus, sparse rough endoplasmic reticulum and free ribosomes. Surrounding the ACTH-cells there occur numerous type B axons, cytoplasmic processes of pituicytes of the first type, and thin ramifications of the pericapillary space. Besides, type A<sub>2</sub> axons are rarely noticed around the cells. Type B axons run in direct contact with the ACTH-cells. As shown in Fig. 15, some of these axons attached to an ACTH-cell bear a definite synaptoid contact with the same cell. Type B axons end on the ramification of the pericapillary space adjacent to ACTH-cells, too. Axons of another type, probably of type A<sub>1</sub>, which contain electron lucent vesicles of 1,300 Å in size together with moderately dense granules of similar size, also terminate on the ramification. Glandular cells regarded as TSH-cells are compactly packed with large secretory granules, about 3,300 Å in diameter, of uniform and high electron density. These cells are characterized by the existence of the endoplasmic reticulum which appears as parallel lamellae surrounding the nucleus. On the TSH-cells axons of types A<sub>2</sub> and B end directly to make a synaptoid contact. Furthermore, axons of both types often terminate on the ramifications of the pericapillary space around TSH-cells. It was remarked that, as shown in Fig. 16, a pinocytotic pit was noticeable on the plasma membrane of a TSH-cell near the region of synapse between the cells and a type A<sub>2</sub> axon ending.

Angular TSH-cells have moderately electron dense granules of 2,000-2,500 Å in size. These granules are vesicular in feature and are present abundantly in the peripheral region of the cytoplasm along with elongated mitochondria, Golgi apparatus, and a few lysosomal dense bodies. On TSH-cells, type B axon endings are frequently found to make a synaptoid contact. Sometimes a small cup-like concave of the plasma membrane of the cell is encountered near the region of the synapse (Fig. 17). GTH-cells are irregular in shape and are charged with secretory granules ranging from 2,500 to 2,000 Å in size, lysosomal dense bodies, and rough endoplasmic reticulum of vesicular appearance. The secretory granules are sometimes seen to be of dumbbell shape of about 6,000 Å in length. In addition, large globules of about 8,000 Å in diameter are occasionally encountered intermingling with the secretory granules. Bundles of type A<sub>1</sub> and type B axons are frequently found permeating among GTH-cells (Fig. 18). Besides, type B axons make a synaptoid contact with these cells. Type A<sub>1</sub> and type B axons are found to end also on the ramified pericapillary spaces surrounding these cells. In the meta-adenohypophysis, two types of glandular cells, one with granules of about 3,000 Å and another with spherical or elongated granules of less than 2,500 Å in diameter, are noticeable. A few large granules similar in size to those of the former cell are also seen in the latter cell. Glia-like supporting cells of elongated shape are also encountered projecting their cytoplasmic processes

among the glandular cells. Type A<sub>1</sub> axons, which have neurosecretory granules of 1,500 Å in size, end on the glandular cells of the first type, but not on those of the second type so far as the present observation is concerned. On the other hand, type B axon endings make a synaptoid contact with both types of the glandular cells. Occasionally more than two axon endings with synaptoid structure terminate on a single cell. As shown in Fig. 19, a small cup-like concave of the plasma membrane of glandular cell of the second type is in some cases recognized as in the case in TSH-cells. Moreover a secretory granule of low electron density existed in contact with the plasma membrane, as if the contents of the granule were being released into the intercellular space (Fig. 20).

### Discussion

In recent years, electron microscopic studies on the pituitary gland of teleost fishes have accumulated the evidence that neurosecretory nerves may regulate the adenohypophysial functions either by making direct contact on the glandular cells or by acting through diffusible principles discharged into perivascular spaces and blood capillaries (Jørgensen, 1968; Perks, 1969). In some fishes such as *Perca fluviatilis* (Follenius and Porte, 1962), *Anguilla anguilla*, *Conger conger* (Knowles and Vollrath, 1966 b), *Tinca tinca* (Vollrath, 1967) and *Oncorhynchus nerka* (Nagahama and Yamamoto, 1969), adenohypophysial cells are generally separated from the neurohypophysial tissue by the basement membrane, which may prevent the innervation of neurosecretory axons into the adenohypophysis. On the other hand, in fishes such as *Lebistes reticulatus* (Follenius, 1965), *Gasterosteus aculeatus* (Follenius, 1967), *Hippocampus guttulatus* and *H. kuda* (Knowles, Vollrath and Nishioka, 1967; Vollrath, 1967), neurosecretory axons have been shown to enter into the adenohypophysis and make a synaptoid contact with glandular cells. In the latter case, however, adenohypophysial cell types which are directly connected with axon terminals have been mentioned obscurely in the previous literatures.

In the medaka, *Oryzias latipes*, Oota (1963) described only briefly that many neurosecretory axon endings were in direct contact with the adenohypophysis. From the present observations, it may be safe to conclude that neurosecretory axons of the neurohypophysis establish quite an intimate connection with all the seven types of adenohypophysial cells, viz., prolactin-, ACTH-, STH-, TSH-, GTH- cells and two types of meta-adenohypophysial ones. Especially, type B axon endings were evidenced to make a distinct synaptoid contact with every one of these cell types. A similar case of direct connection between type B axons and all the different adenohypophysial cell types has been reported quite recently by Zambrano (1970) in *Gillichthys mirabilis*.

In the pro-adenohypophysis of the medaka, only type B axons innervate with frequent synapse on the presumed prolactin cells. This zone is further characterized by the presence of the pericapillary space which is wider than that in the other zones of the pituitary and is lacking in ramifications, and by the existence of peculiar supporting cells which appear to separate the glandular cells from the vascular channel. Moreover no neurosecretory terminals are found bordering on the pericapillary space, exhibiting a striking contrast to features seen in the other zones. Sage (1968) suggested the probable lack of hypothalamic control over prolactin cells in *Xiphophorus* hybrid in view of their autonomous capacity to produce and release the hormone in *in vitro* condition. On the contrary, Ball *et al.* (1965) revealed the possibility of an inhibitory hypothalamic control of prolactin secretion in *Poecilia formosa* with ectopic pituitary homograft. Functional significance of the above-mentioned ultrastructural characteristics in the pro-adenohypophysis of the medaka remains to be clarified by further studies. However, it is at least recognizable that a certain activity of the pro-adenohypophysial cells of the fish may be under some neurosecretory regulation.

All the four types of meso-adenohypophysial cells have a direct connection with type B axons, as described before. In the meso-adenohypophysis of the medaka, there appear narrow ramifications of the pericapillary space, which permeate complicatedly among cell groups. Axons of types B and A<sub>2</sub> are repeatedly observed to terminate on these ramifications, being located closely adjacent to glandular cells. In addition, it is worthy to note that both type B and type A<sub>2</sub> axons terminate on STH-cells with evident synaptoid structures. A similar synaptoid connection is demonstrated between meta-adenohypophysial cells of the first type and types B and A<sub>1</sub> axon endings. These facts may provide an additional support for the concept of "dual neurosecretory control" of adenohypophysial function proposed first by Knowles (1965). Basing on the observations upon the intermediate lobe of the dogfish pituitary, he suggested that the axons with neurosecretory granules larger than 1,000 Å in size may control the hormone synthesis probably by peptide principle whereas those with granules less than 1,000 Å may regulate their hormone release probably by monoamine substance. Later he and his collaborators extended the concept to the regulation mechanism of teleost adenohypophysial activities (Knowles *et al.*, 1967).

It has become increasingly conceivable that the granules in types A<sub>1</sub> and A<sub>2</sub> axons may contain neurosecretory principles of polypeptide nature while those in type B axons may charge those of monoamine nature (Kobayashi and Matsui, 1969). In this context, mention must be made of the fact that many type B axon endings are noticed to abut on ACTH-cells, often having a synaptoid contact with the cells. This fact may imply that these axons may greatly influence the ACTH-cells which are thus capacitated to execute prompt discharge of the hormone in

case of need. Furthermore, it is interesting to note that small cup-like concaves of the plasma membrane of TSH-cells, and of meta-adenohypophysial cells as well, are encountered near the point of the synapse with type B axons. In addition a secretory granule exists in contact with the plasma membrane of meta-adenohypophysial cells, as if the contents of the granule were being released.

A synaptoid contact of type A<sub>1</sub> neurosecretory axons with pituicyte was reported by Knowles and Vollrath (1965 a, b) in *Anguilla anguilla*. In the medaka both types A<sub>1</sub> and B axons make synaptoid contact with pituicytes of the first type. This seems to indicate that the pituicyte may be controlled in its function by the neurosecretory nerve system, though the functional role played by the pituicyte in relation to neurosecretory phenomena remains at present quite open to question.

### Summary

In the medaka, *Oryzias latipes*, adenohypophysial cells keep ultrastructurally quite intimate connections with the neurohypophysial tissue. Three types of neurosecretory axons are discernible in the neurohypophysis. The first type contains neurosecretory granules of 1,400–1,800 Å (type A<sub>1</sub>), the second those of about 1,200 Å (type A<sub>2</sub>) and the third those of 850–1,150 Å (type B) in size. In the neurohypophysis, the three types of axons terminate on the pericapillary space and its ramifications, type A<sub>1</sub> axons being predominant in distribution. Two types of pituicytes are detected ultrastructurally. With one type of the cells, axons of types A<sub>1</sub> and B make a contact in the synaptoid fashion.

Type B axons make a synaptoid contact with all of the seven types of pituitary glandular cells, namely, prolactin-, ACTH-, TSH-, STH-, GTH- cells and two types of meta-adenohypophysial glandular cells. In the pro-adenohypophysis, only type B axons invade and make a synaptoid contact with the glandular cells. No axon endings appear to end on the pericapillary space in this zone. In the meso-adenohypophysis, axon endings of types A<sub>2</sub> and B are located bordering on the pericapillary space and its ramifications among cell groups, with distinct synaptoid structure. Furthermore, axons of both types make a synaptoid contact with STH-cells. In the meta-adenohypophysis, axons of type A<sub>1</sub> innervate deeply, sometimes having a synaptoid connection with glandular cells. Some of these facts seem to provide an additional support for the mechanism of dual neurosecretory control of secretory activity in meso- and meta-adenohypophysial cells in teleost.

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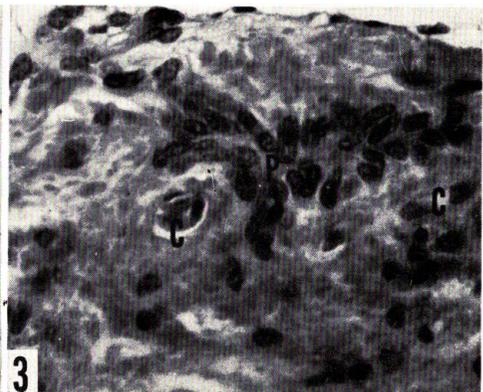
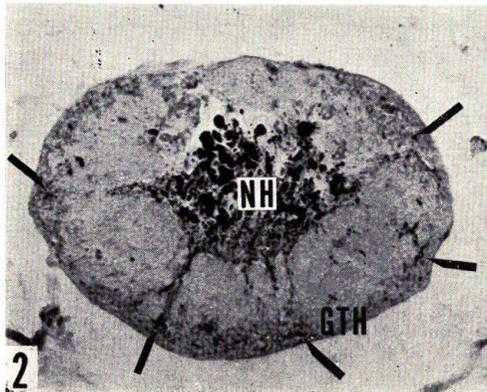
## Explanation of Plates

### PLATE I

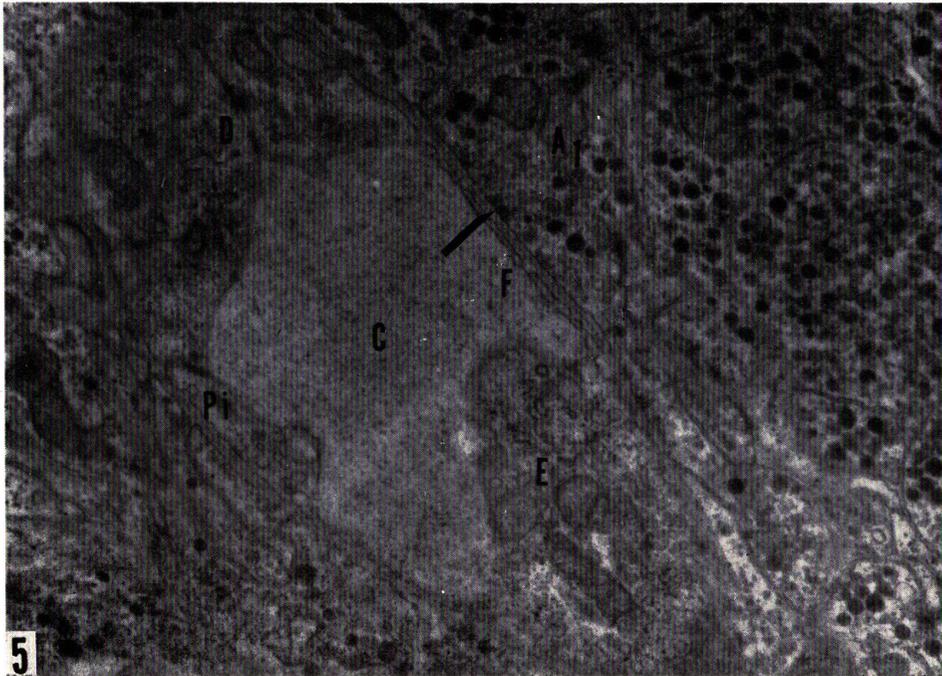
Fig. 1. A median sagittal section through the pituitary gland of a mature female medaka. AF,  $\times 350$ .

Fig. 2. A frontal section through the meso-adenohypophysis, revealing a neurohypophysial tissue (*NH*) loaded with AF-positive neurosecretory materials invading the meso-adenohypophysis deeply. AF-positive materials (arrows) are also seen accumulated among GTH-cells (*GTH*) distributed on the periphery of the meso-adenohypophysis. AF,  $\times 170$ .

Fig. 3. Posterior region of the neurohypophysis, indicating the presence of a cluster of pituicytes (*P*). *C*, blood capillary. CH-P,  $\times 610$ .



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## PLATE II

Fig. 4. A part of type  $A_1$  neurosecretory axon (*HB*) distended by the tremendous accumulation of secretory granules, which may correspond to light microscopic Herring body. *C*, blood capillary; *P*, pituicyte.  $\text{OsO}_4$ ,  $\times 5,000$ .

Fig. 5. Ending of type  $A_1$  axon (arrow) abutting on the pericapillary space in the neurohypophysis. *A*<sub>1</sub>, type  $A_1$  axon; *C*, blood capillary; *D*, desmosome; *E*, endothelial cell; *F*, fenestration in the endothelial wall; *Pi*, pinocytosis of the endothelial cell.  $\text{OsO}_4$ ,  $\times 15,000$ .

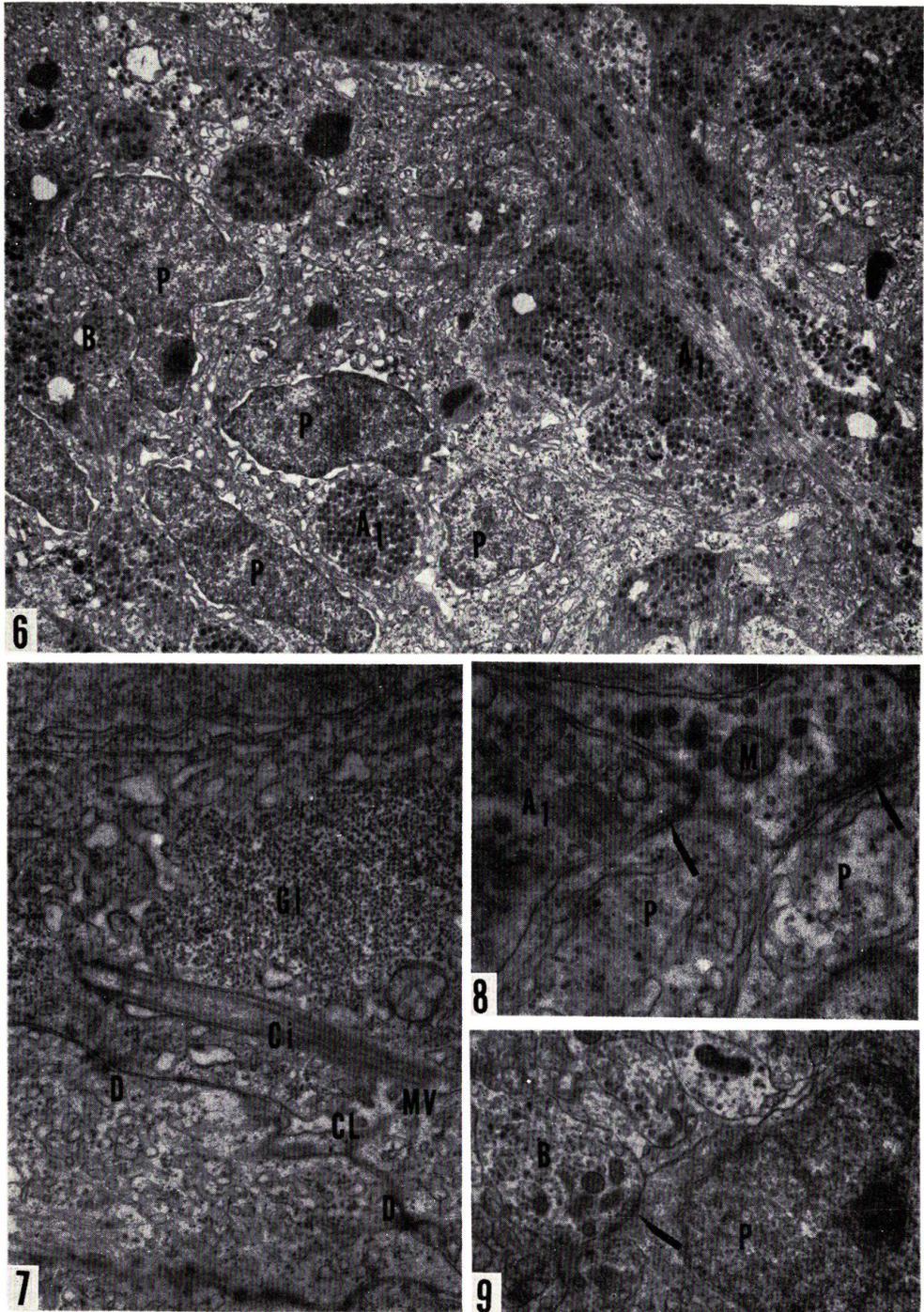
### PLATE III

Fig. 6. Fine structural make-up of the neurohypophysis.  $A_1$ , type  $A_1$  axons;  $B$ , type B axon;  $P$ , pituicytes of the first type. Glutaraldehyde- $OsO_4$ ,  $\times 5,000$ .

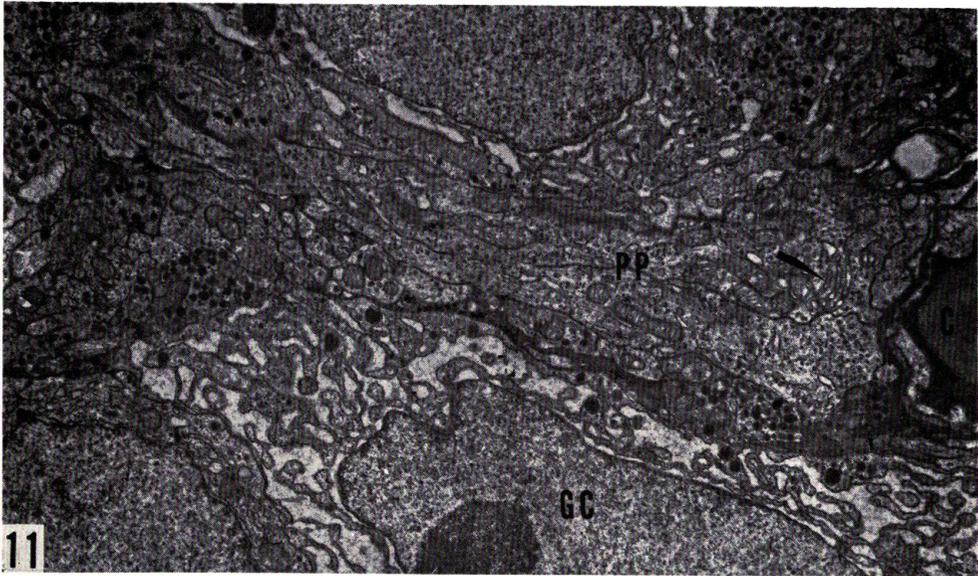
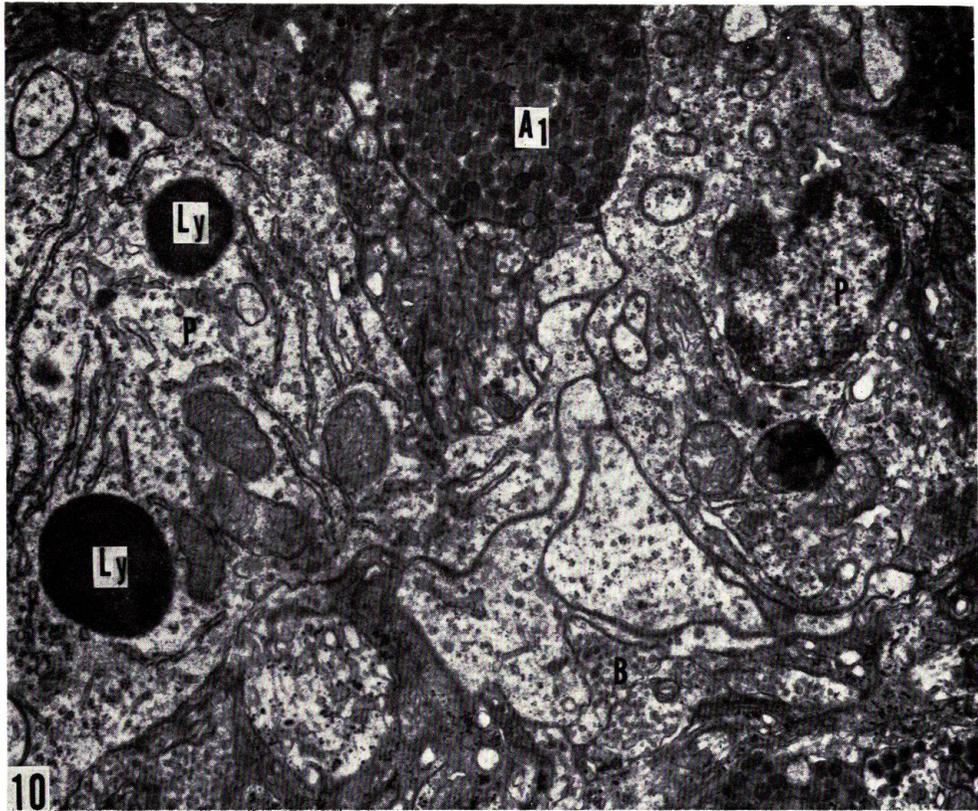
Fig. 7. Central lumen ( $CL$ ) in a cluster of pituicytes.  $Ci$ , cilium;  $D$ , desmosome;  $Gl$ , glycogen granules;  $MV$ , microvilli.  $OsO_4$ ,  $\times 18,000$ .

Fig. 8. Synapses (arrows) of type  $A_1$  axon endings ( $A_1$ ) with pituicyte ( $P$ ) of the first type.  $M$ , mitochondrion.  $OsO_4$ ,  $\times 30,000$

Fig. 9. Synapse (arrow) of type B axon ( $B$ ) with pituicyte ( $P$ ) of the first type.  $OsO_4$ ,  $\times 9,000$ .



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## PLATE IV

Fig. 10. Pituitocytes of the second type (*P*) and associated neurosecretory axons. *A*<sub>1</sub>, type A<sub>1</sub> axon; *B*, type B axon; *Ly*, lysosomal dense body. Glutaraldehyde-OsO<sub>4</sub>, ×15,000.

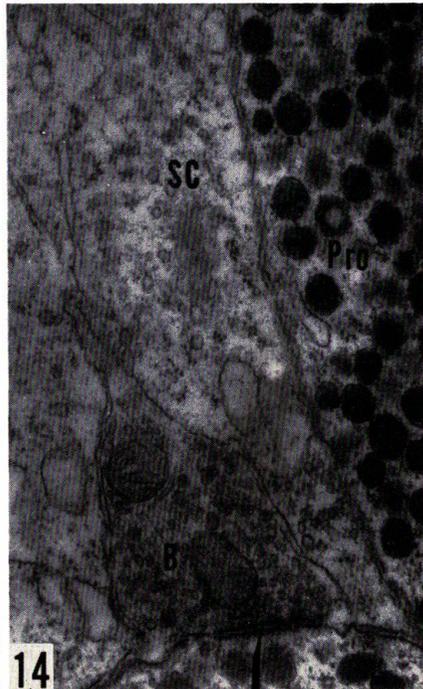
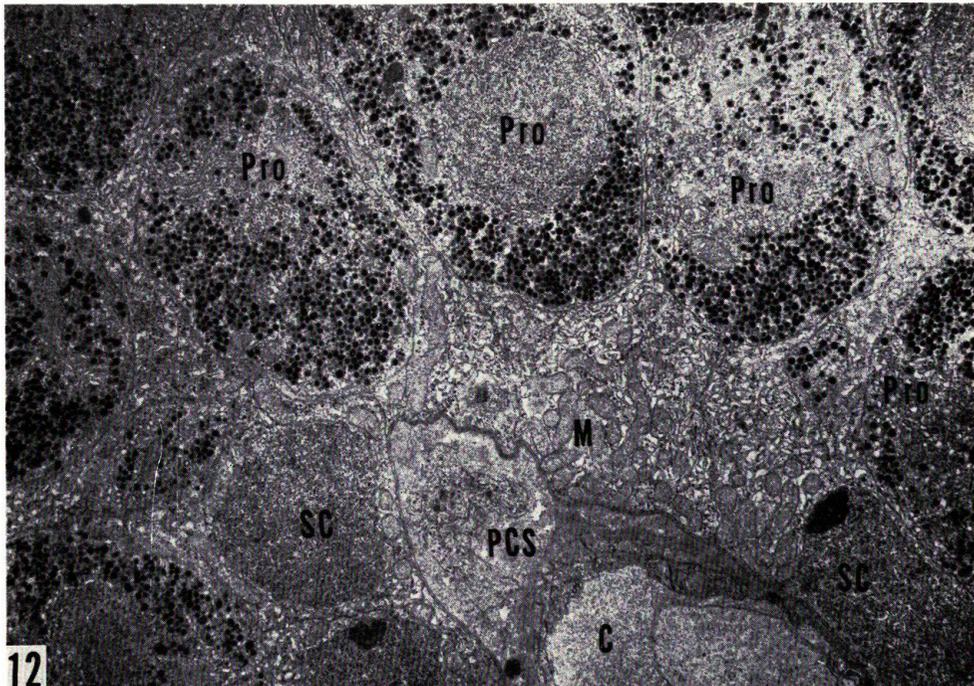
Fig. 11. Processes (*PP*) of pituitocytes of the second type extending to the blood capillary (*C*). An arrow indicates an annulated lamellae-like complex in the process. Type of glandular cell (*GC*) is uncertain, though probably being either TSH or GTH in nature. Glutaraldehyde-OsO<sub>4</sub>, ×7,500.

## PLATE V

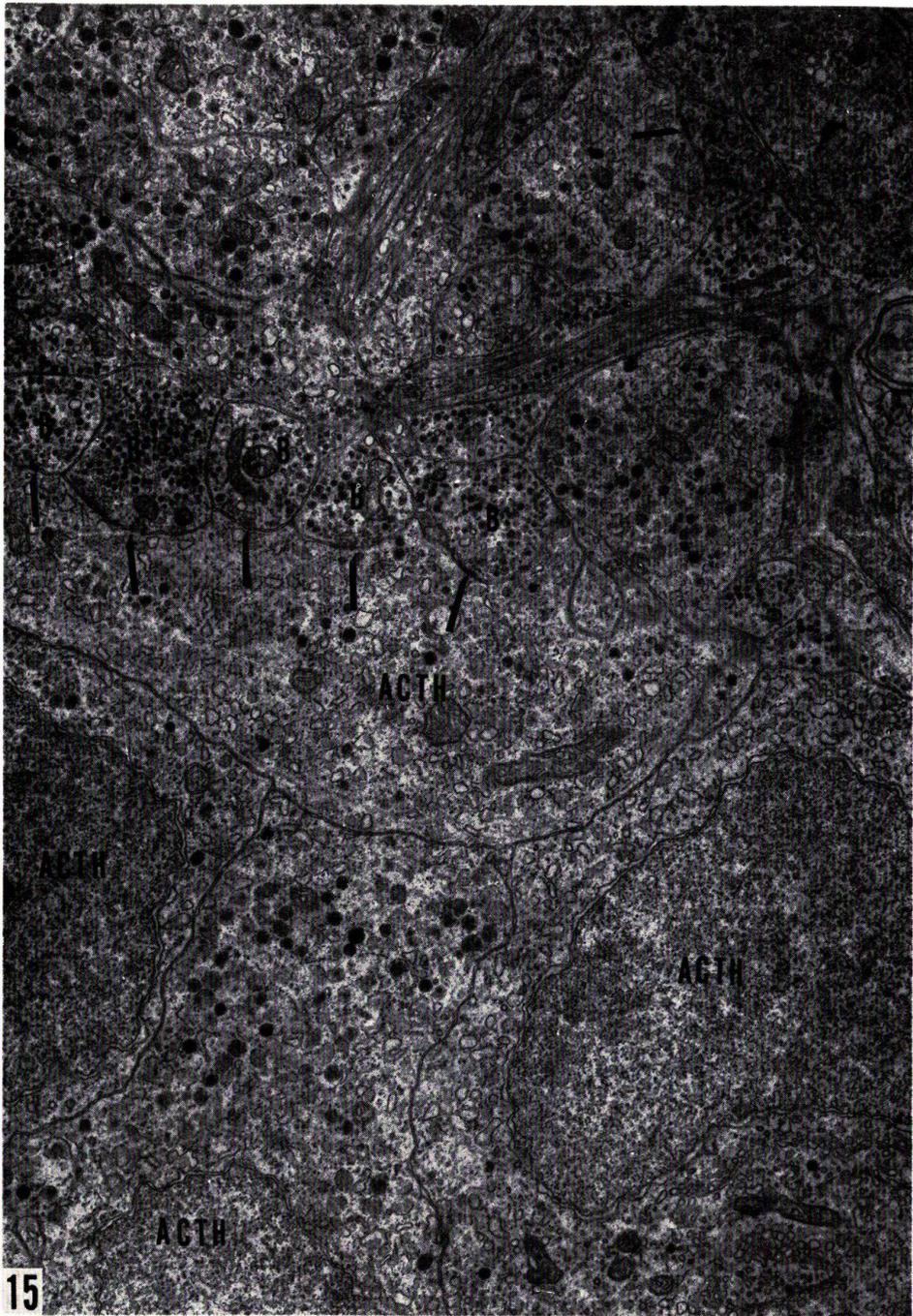
Fig. 12. Pro-adenohypophysial glandular cell (prolactin cells, *Pro*) separated from the blood capillary (*C*) by glia-like supporting cells (*SC*) with numerous mitochondria (*M*) in the cytoplasm facing on the pericapillary space (*PCS*).  $\text{OsO}_4$ ,  $\times 4,500$ .

Fig. 13. A large vacuolated mitochondria (*M*) in a prolactin cell (*Pro*). Type B axon (*B*) makes a synaptoid contact (arrow) with the cell. *SC*, supporting cell. Glutaraldehyde- $\text{OsO}_4$ ,  $\times 9,000$ .

Fig. 14. Synapse of type B axon (*B*) with the prolactin cell (*Pro*). *SC*, supporting cell.  $\text{OsO}_4$ ,  $\times 30,000$ .



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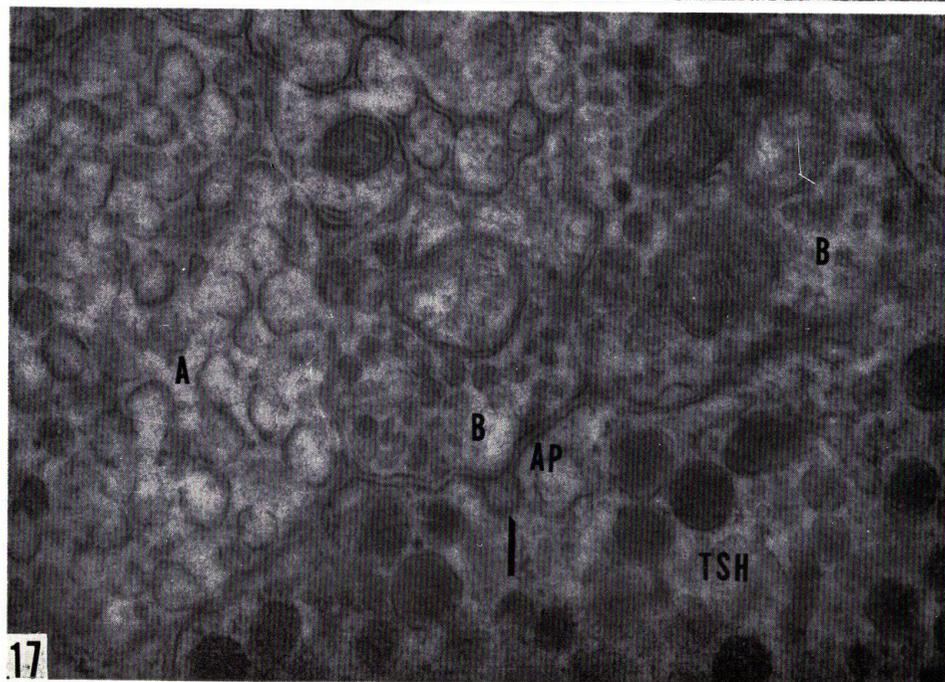
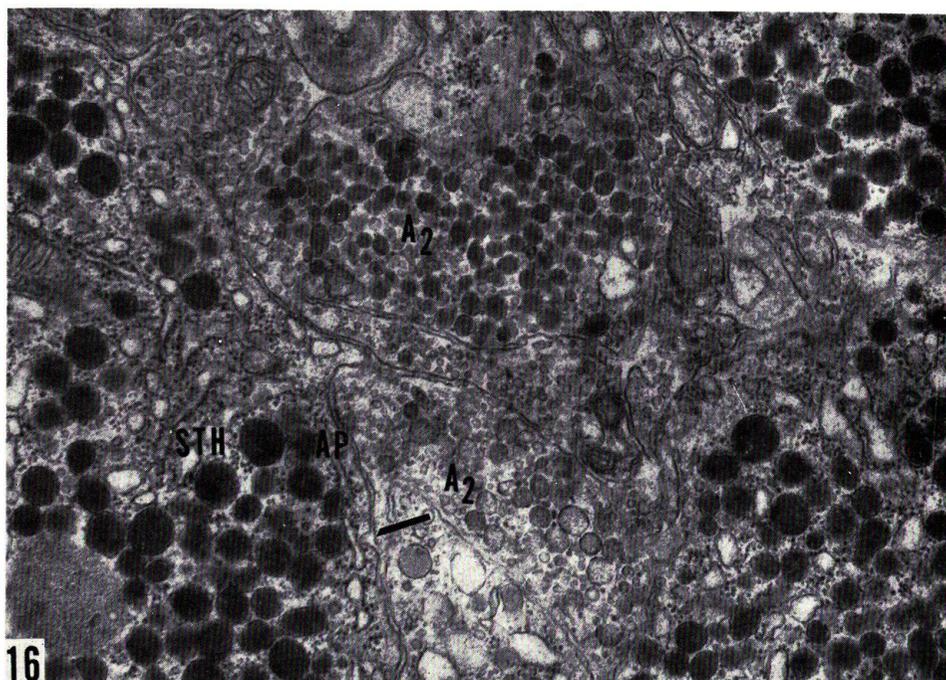
PLATE VI

Fig. 15. Endings of type B axons (*B*) making a synaptoid contact (arrows) with a single ACTH-cell (*ACTH*). OsO<sub>4</sub>, ×7,500.

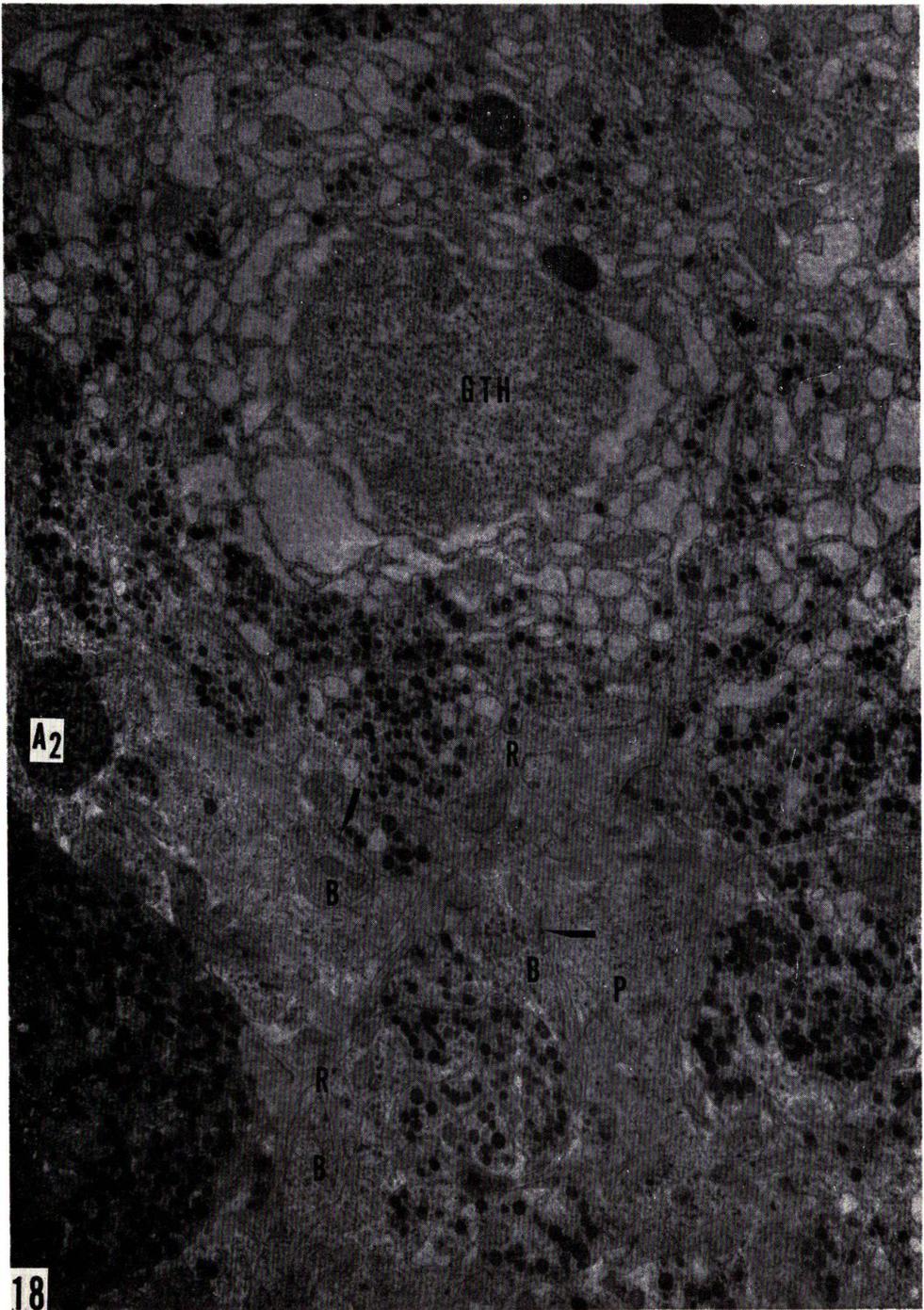
## PLATE VII

Fig. 16. Type A<sub>2</sub> axon (*A*<sub>2</sub>) making a synaptoid contact with STH-cell (*STH*). A small pit (arrow) is seen in the plasma membrane of the cell in the neighbourhood of the active point (*AP*) of synapse. OsO<sub>4</sub>, ×25,000.

Fig. 17. Type B axons (*B*) having direct contact with TSH-cell (*TSH*). A cup-like concave (arrow) is seen on the plasma membrane of the TSH-cell in the neighbourhood of the active point (*AP*) of the synapse. Type A axon (*A*) containing electron lucent granules is also present closely abutting on the cell. OsO<sub>4</sub>, ×40,000.



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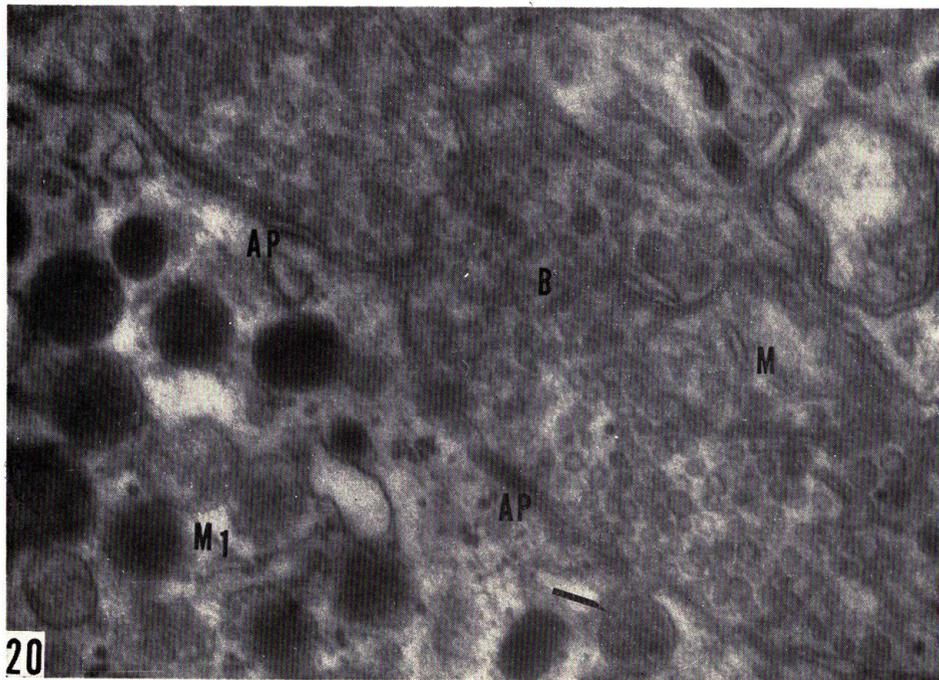
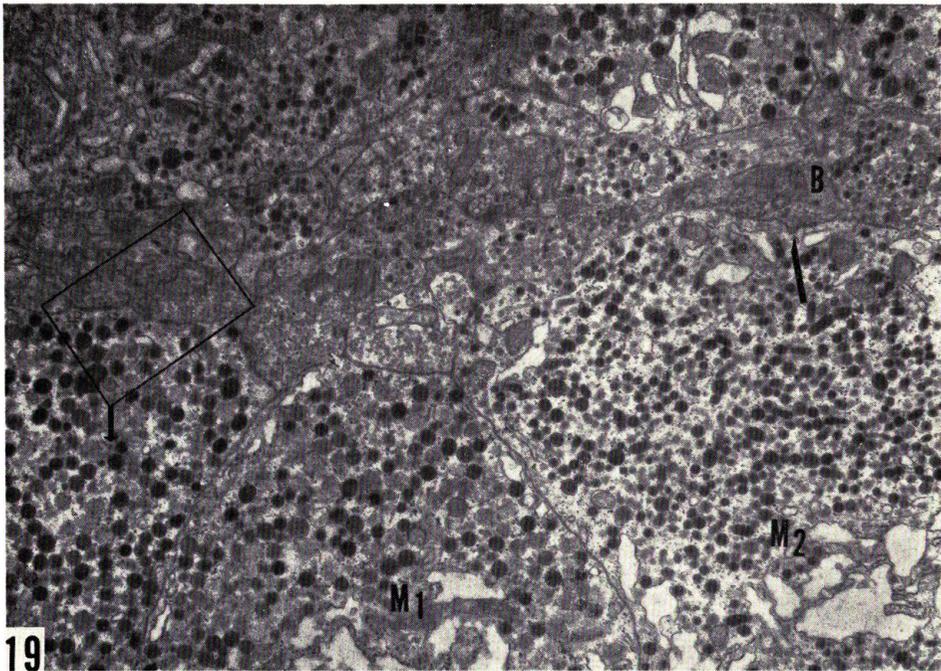
## PLATE VIII

Fig. 18. Type B axon endings (*B*) with a synaptoid apparatus ending on the GTH-cell (*GTH*) and on the ramification of the pericapillary space (*R*), respectively. *A*<sub>2</sub>, type A<sub>2</sub> axon; *P*, pituicyte. OsO<sub>4</sub>, ×12,000.

## PLATE IX

Fig. 19. Bundle of type B axons (*B*) invading among the two types of meta-adenohypophysial glandular cells ( $M_1$  and  $M_2$ ), some of the axons bearing the synaptic connection with the cells. An arrow indicates a cup-like concave of the plasma membrane of the cell of the second type ( $M_2$ ) in the neighbourhood of the active point of the synapse. Glutaraldehyde-OsO<sub>4</sub>,  $\times 7,500$ .

Fig. 20. Synaptic endings of type B axons (*B*) on the meta-adenohypophysial glandular cell of the first type ( $M_1$ ). Limiting membrane of the secretory granule (arrow) in the cell is found connecting with the plasma membrane near the active point (*AP*) of the synapse. The area taken in this figure corresponds to the region squarely outlined in Fig. 19. *M*, mitochondrion. Glutaraldehyde-OsO<sub>4</sub>,  $\times 50,000$ .



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