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FINE STRUCTURE OF THE GLANDULAR CELLS IN THE  
ADENOHYPHYSIS OF THE KOKANEE  
*ONCORHYNCHUS NERKA*\*

Yoshitaka NAGAHAMA\*\* and Kiichiro YAMAMOTO\*\*

The morphology of the pituitary gland of the teleost fishes has already been studied by many investigators with the light microscope and knowledge about the organ at the level of the light microscope has been accumulated pretty well (Pickford and Atz, 1957). As the component cells of the pituitary are very small in size, however, the definite identification of every hormone-secretory cell was difficult by the light microscope. Recently, several researchers have studied the fine structure of the component cells of the pituitary gland with the aid of electron microscope, viz. the fine structure of some hormone-secretory cells has been demonstrated by Kurosumi *et al.* (1963) in the carp, Öztan (1966) in the blenny fish, Knowles and Vollrath (1966) in the eel, Dharmamba and Nishioka (1968) in *Tilapia* and Nagahama and Yamamoto (1969) in the goldfish. However, the results thus obtained are conflicting and are too meagre to get good understanding about every hormone-secretory cell of fish pituitary.

As for the pituitary gland of the salmonid fishes, a considerable number of studies with the light microscope have already been made by many authors such as Olivereau and Ridgway (1962), Robertson and Wexler (1962a, b), van Overbeeke and McBride (1967) and Fagerlund *et al.* (1968), while no study on the fine structure of salmon pituitary has yet been reported except a brief report of Follenius (1963) and so the study of fine structure of hormone-secretory cells is almost incomplete as yet. Thus, it is very interesting to study kokanee pituitary with electron microscope so as to know the fine structure of every hormone-secretory cell.

Before proceeding further, we wish to express our hearty thanks to Mr. Tadaei Nakayama, the director of Hokkaido Salmon Hatchery and Mr. Toshiro Terao, the Research Fellow of the Hokkaido Salmon Hatchery for the facility they have extended in the collection of the material.

**Materials and methods**

The kokanee, the landlocked form of *Oncorhynchus nerka*, from 4 months to 2

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years in age and in various stages of sexual maturation, were used as material. They were composed of 0-year, one year and two year old group, and were kept separately in each outdoor pond of Chitose Branch of Hokkaido Salmon Hatchery. Samplings were made in June, October and November of 1968.

For the light microscopical observations, pituitary glands were fixed for about 24 hours with Zenker-formol's and Bouin-Holland-Sublimate's solution. Then, they were cut at 5-7 micra in thickness by the usual paraffin method, and stained by the various techniques such as Heidenhain's azan, Halmi's aldehyde fuchsin-light green-orange G and MacConaill's lead hematoxylin method (MacConaill, 1947).

For the electron microscopical observations, the organs were removed and their small pieces were usually immersed in Millonig's solutions for two hours. But some materials were placed in 6.25% glutaraldehyde in 0.05M phosphate buffer for one hour and then immersed in Millonig's solutions for two hours. After fixation, the tissues were dehydrated by the quick cold ethanol method with two changes of propylene oxide, and then embedded in Epon Epoxy resin mixture. Thick sections of about 1 micron for light microscopy were cut with glass knives on a Porter-Blum microtome and stained with Richardson *et al.*'s method (1961). Thin sections were cut by the same method at a thickness of about 500 to 800Å, stained with uranyl acetate and Karnovsky's lead method or Reynolds' method, and examined with a Hitachi HS-7 electron microscope.

## Results

The pituitary gland of the kokanee consists of four regions, i.e. pro-adenohypophysis, meso-adenohypophysis, meta-adenohypophysis and neurohypophysis. In midsagittal sections, the pro-adenohypophysis occupies a large part of the anterior portion of the gland and is quite distinct from the other regions. Most of the cells in this area are acidophilic, columnar and are arranged in the form of follicles. The remaining cells of this area are observed to form a palisade-like layer located at the ventromedial edge of the pro-adenohypophysis. The meso-adenohypophysis comprises most part of the central pituitary. This lobe consists of columns of cells, separated by the small amounts of sparse connective tissue. Three kinds of cells, i.e. basophils, acidophils and chromophobes are recognized in this region. In immature fish, however, this lobe develops poorly and occupies only a small part of the pituitary. The meta-adenohypophysis occupies the half of the pituitary and is rich in the plexus of blood vessels. In midsagittal sections of the immature fish, the neurohypophysis is located in the uppermost part of the pituitary and occupies a half of the organ. The branching lobe of the neurohypophysis which invades into pro- and meso-adenohypophysis includes only a trace of

A-F positive material, while one which invades into meta-adenohypophysis is embedded with a plenty of A-F positive material.

#### Pro-adenohypophysis

a) *Prolactin cell*: Erythrosinophil eta cells in fish pituitary are judged to be prolactin-producing cells (Ball, 1965; Emmart *et al.*, 1966). These cells are found occupying the most part of pro-adenohypophysis in kokanee. They are stained with azocarmine G or light green but they have no affinity to the lead-hematoxylin stain. These cells are columnar in shape, measure 10–20  $\mu$  in long axis and are arranged in the form of follicles (Fig. 1). The follicles usually have a spherical lumen which frequently contains a material positive to aldehyde fuchsin and aniline blue. The nucleus is situated in the opposite side of the lumen and generally contains one or two acidophilic nucleoli. The basal cytoplasm is stained more deeply with the acid dyes than the apical cytoplasm of the cell.

Electron microscopical observations have made clear that the prolactin cells encircling the lumen are joined with each other by the tight junction and desmosome (Fig. 6). Very short microvilli and cilia showing a typical 9+2 fibril pattern are extended into this lumen (Fig. 5). Many membrane-bound secretory granules are found in the cytoplasm (Fig. 4). They are high in electron density and measure 200–300  $m\mu$  in diameter, usually round in shape but rarely of irregular outline. Well developed rough-surfaced endoplasmic reticula lie in the outer part of the cytoplasm (Fig. 7). They usually appear as parallel lamellae and it is difficult to find out their dilated cisternae. Some well developed Golgi apparatus are observed to be distributed in the apical cytoplasm close to the nucleus. Mitochondria, moderate in number and short or long rod-shaped, are found to be distributed sparsely in the cytoplasm. Their cristae are moderate in number, but no mitochondrial granules are found within the organelles. A few lysosomes, round, oval or polygonal in shape, were also encountered in the cells.

b) *Corticotroph*: In midsagittal sections, corticotrophs are observed at the ventromedial edge of the pro-adenohypophysis as a palisade-like layer composed of 2 or 3 rows of cells. These cells are usually oriented towards the branches of neurohypophysis. The remarkable characteristic of these cells is to be stained strongly purple by MacConaill's lead hematoxylin (Fig. 2). The cells are round or elliptical in shape and measure 10–12  $\mu$  in diameter. The nucleus showing often irregular contour is usually located in the central portion of this cell.

According to the electron microscopic observations, the most striking features of the corticotrophs lie in the characters of secretory granules (Fig. 8). The granules measuring 150–250  $m\mu$  in dimension are distributed thoroughly in the cytoplasm and exhibit various electron densities. Their limiting membranes are often separated widely from the granular core (Fig. 9). Golgi apparatus develop moderately, consisting of a few flattened sacs, many vesicles clustering at the both

ends of the sacs, and several vacuoles. The rough-surfaced endoplasmic reticulum also develops moderately and is distributed throughout the cytoplasm. The profiles of the reticulum appear as vacuoles or slightly dilated cisternae. Mitochondria are few in number and are usually round or rod-like in shape, and have poorly developed cristae.

#### Meso-adenohypophysis

c) *Somatotroph*: In immature kokanee somatotrophs occupy most part of the ventral area of the meso-adenohypophysis. They are generally round, oval or polygonal in shape, and are about  $10\ \mu$  in diameter. When stained by azan method, the cytoplasm of the cells is stained strongly by azocarmine G (azan). The nucleus, round, oval or polygonal in shape, is situated in the central portion of the cells, and contains one acidophilic nucleolus.

Electron microscopical observations reveal that somatotrophs include many membrane-bound secretory granules ranging from 200 to  $350\ m\mu$  in diameter, which are high in electron density and are round in shape (Fig. 10). The rough-surfaced endoplasmic reticulum in general appears as well-developed parallel lamellae around the nucleus, and sometimes as dilated cisternae. Golgi apparatus consist of a few sacs, many vesicles and vacuoles. The secretory granules at the beginning of formation are observed in the dilated sacs of the Golgi apparatus (Fig. 11). Mitochondria are moderately developed. They are round or short rod-like in shape, and have poorly developed cristae.

d) *Thyrotroph*: Thyrotrophs are stained weakly blue with aniline blue (azan), and they are also PAS- and aldehyde fuchsin-positive. These cells are generally elongated or polyhedral in shape, measure  $10\text{--}12\ \mu$  in dimension, are small in number and found to be situated singly in the dorsal part of the meso-adenohypophysis. The nucleus is situated in the central part of the cells and contains one or two acidophilic nucleoli.

In electron micrographs, the thyrotrophs are lighter in appearance than the other cells. Their cytoplasm, however, is evenly filled with spherical secretory granules of a small size about  $100\text{--}200\ m\mu$  and of moderate electron density (Fig. 12). The granules are the smallest among those found in all glandular cells. No limiting membrane is recognized around the granules. The rough-surfaced endoplasmic reticulum develops poorly and appears as parallel lamellae or the dilated cisternae. The Golgi apparatus consists of flattened sacs and small vesicles. The secretory granules at the beginning of formation are often observed near Golgi apparatus. Mitochondria are usually globular but sometimes long rod-like. Mitochondrial cristae are poorly developed and no intramitochondrial granules were detected within them.

e) *Gonadotroph*: In immature kokanee the meso-adenohypophysis is relatively small in size due to poor development of gonadotrophs, while in mature

fish this lobe becomes larger and occupies most part of the meso-adenohypophysis due to the increase of the size and number of gonadotrophs. In general, the gonadotrophs of the kokanee are located in the central and the ventral part of the meso-adenohypophysis. They are variable in shape but mostly are round or elliptical and measure about  $15\ \mu$  in diameter (Fig. 3). A comparatively small number of fairly large granules are found in the basophilic cytoplasm. The granules are strongly PAS-positive, and stained with aniline blue (azan), and aldehyde fuchsin (A-F). From these characteristics it is easy to distinguish the gonadotrophs from the other glandular cells. The large nucleus is located in a corner of the cell.

Observations with the electron microscope detected two kinds of secretory granules present in the gonadotrophs, i.e. many high electron-dense granules ranging from  $300\text{--}500\ m\mu$  in diameter and a few low electron-dense granules of about  $3\ \mu$  in the largest (Fig. 13). The large granules correspond to the fairly large PAS positive granules demonstrated by the light microscope. Well developed endoplasmic reticulum of rough-surfaced type is seen generally as small vesicles or tubules, sometimes as dilated vacuoles. Golgi apparatus develops moderately, and sometimes dilated cisternae of agranular type appear in the field. Rod-like mitochondria are observed distributed evenly in the cytoplasm.

*Relationship among glandular cells, neurohypophysis and capillaries.*

Light microscopical observations revealed that a very thin membranous layer stained strongly blue by azan staining method separates glandular cells from neurohypophysis. Fig. 14 shows the surface of contact between glandular cells and neurohypophysis. From the figure it is clear that the glandular cells do not contact directly with neurohypophysis but they usually touch a thin layer of fibroblastic cells which confront neurohypophysis across a basement membrane.

Most of the pituitary capillaries are lined by a single layer of the endothelial cells (Fig. 15). The endothelial cells of the capillary are flat in shape and irregular in outline. A relatively small amount of the cytoplasm of the cells contains a few mitochondria, rough-surfaced endoplasmic reticulum and free-ribosomes. Fenestrae of about  $600\text{--}800\ \text{\AA}$  in diameter can be often identified in the endothelial cells of the capillary. A fenestra is bridged by a single-layered membrane which separates the blood cavity from the pericapillary cavity. Outside to the pericapillary cavity, a kind of cells are located. The cells are enclosed on their inner side with a basement membrane and on the other side they are often in direct contact with glandular cells. These cells are fibroblastic in nature as they contain many fibrous elements and are often surrounded with collagen fibres.

### Discussion

At the level of the light microscope, classification of pituitary glandular cell in the salmonid fishes has been performed by staining reactions and mor-

phological changes during sexual maturation and spawning (Olivereau and Ridgway, 1962; Robertson and Wexler, 1962 a, b; van Overbeeke and McBride, 1967). Moreover, recently, Fagerlund *et al.* (1968) identified the corticotropic cells in sockeye salmon and rainbow trout on the basis of the response of the cell to metopirone treatment. As a result of these investigations, it was cleared that the pro-adenohypophysis contains two kinds of glandular cells, i.e. the prolactin producing cell and the corticotroph, and the meso-adenohypophysis contains three kinds of glandular cells, i.e. the somatotroph, the thyrotroph and the gonadotroph.

At the level of the light microscope, the prolactin cell of the kokanee can be identified from other glandular cell by being columnar in shape and by their arrangement in the form of follicles. The similar follicular arrangement of the prolactin cells has been demonstrated in some kinds of fishes such as the eel and the salmonid fishes (Honma, 1960; Kawamoto, 1967). The cells contain many membrane-bound secretory granules, being 200–300  $m\mu$  in diameter, high in electron density, and mostly round or rarely irregular polygonal in shape. These characteristics in granules agree with those of secretory granules in the same type cells of *Tilapia mossambica* (Dharmamba and Nishioka, 1968) and the eel (Knowles and Vollrath, 1966; Hopkins and Baker, 1968). However, they are different from mammotrophs of mammals, which contain the secretory granules of irregular elliptical shape and of large size (Kurosumi, 1968; Dekker, 1968). Moreover, another characteristic of prolactin cells of the kokanee is that they are joined strongly by the tight junction and desmosome with each other, and to possess some cilia and very short microvilli on its surface facing to the follicular lumen. The cilia show a 9+2 fibril pattern and are different from cilia in mouse anterior pituitary, which show a 9+0 fibril pattern (Barnes, 1961).

The corticotrophs of the kokanee are characterized by being stained strongly by MacConaill's lead hematoxylin as already shown by Fagerlund *et al.* (1968). The cells contain characteristic granules showing various electron densities and a dimension of 150–250  $m\mu$ . The limiting membranes of the granules are often separated widely from the central cores. Thus, the corticotrophs of the kokanee clearly differ from the ACTH cells of *Anguilla* whose cytoplasm is evenly packed with electron-dense granules measuring 2000 to 2500 Å in diameter (Knowles and Vollrath, 1966). On the other hand, the corticotrophs of rat include small secretory granules which are of about 150–200  $m\mu$  and show a vesicular form with a dark central core (Kurosumi and Kobayashi, 1966). Thus, the corticotrophs of rat resemble the same type cells in the kokanee.

The somatotrophs are stained strongly by acid dyes, and is round, oval or polygonal in shape. They include many membrane-bound secretory granules ranging from 200 to 350  $m\mu$  in dimension, which are high in electron density and are generally round in shape. These morphological features of kokanee somato-

trophs are very similar not only to those of the goldfish as described by Yamamoto and Nagahama (1969), but also to STH cells of higher vertebrates (Kurosumi, 1968).

The thyrotrophs of the kokanee are stained weak blue with azan staining, are small in number and found situated singly in the dorsal part of the meso-adenohypophysis. These light microscopical characteristics are very similar to those of *Poecilia* (Sage, 1967) and the goldfish (Nagahama and Yamamoto, 1969). The thyrotrophs of the kokanee are clearly distinguishable in fine structure from other glandular cells by the presence of small secretory granules ranging from 100–200  $\mu$  in diameter as in the case of *Anguilla* and *Conger* (Knowles and Vollrath, 1966) and the goldfish (Nagahama and Yamamoto, 1969). In *Zoarcetes viviparus*, however, Öztan (1966) found the thyrotrophs containing the secretory granules of 400  $\mu$ .

The gonadotrophs of the kokanee contain a comparatively small number of fairly large granules in the basophilic cytoplasm and they show a marked change in close relation to reproductive cycle. These findings agree with the results obtained by Robertson and Wexler (1962 a, b) and van Overbeeke and McBride (1967). The electron microscopical observations revealed that they hold the large granules of about 3  $\mu$  and small granules of 300 to 500  $\mu$  in the cytoplasm. Therefore, the gonadotrophs of the kokanee agree well with those demonstrated in many kinds of fishes such as the goldfish (Scruggs, 1951), the carp and the eel (Kurosumi *et al.*, 1963) etc. On the contrary, two types of gonadotrophs as reported in mammalian pituitary gland (Kurosumi, 1968), are discerned in the adenohypophysis of *Zoarcetes viviparus* by Öztan (1966) having only one sort of secretory granules measuring lesser than 250  $\mu$ . Furthermore, Olivereau and Herlant (1960) and Olivereau and Ridgway (1962) described two types of gonadotrophs present in the adenohypophysis of the eel and salmon, though the fine structure of these gonadotrophs was not reported. In order to clarify these controversial problems it seems urgent to study the morphological changes of the gonadotrophs in detail during the maturation periods and in experimental conditions.

The glandular cells in the pro- and meso-adenohypophysis are not attached directly to the neurohypophysis but the former confront usually a thin layer of connective tissue which is in contact with the neurohypophysis across the basement membrane. The same type of contact between the glandular cells and the neurohypophysis has been already demonstrated in the eel and conger (Knowles and Vollrath, 1966).

No report concerning the fine structure of the capillary in fish pituitary has been published. In the present fish the capillaries are lined by a layer of endothelial cells which are flat in shape and irregular in outline. Outside the layer of

endothelial cells, wide cavity is observed, i.e. pericapillary cavity. At some places in the layer of endothelial cells, the fenestrae of about 600–800 Å are revealed, which separate the blood cavity from the pericapillary cavity by a single-layered membrane. A kind of cells of fibroblastic nature enclose the pericapillary cavity. These cells are in direct contact with the glandular cells. The fine structure of capillaries in the pituitary of the kokanee, therefore, agree well with those found in mammals (Fujita, 1969), except a direct contact of the fibroblastic cells to the glandular cells.

### Summary

In the pro- and meso-adenohypophysis of the kokanee (*Oncorhynchus nerka*), five glandular cells have been revealed on the level of light- and electron microscopy.

The prolactin producing cells are columnar in shape and arranged in follicles. They contain the secretory granules measuring from 200 to 300  $m\mu$  in diameter, which are round in shape but rarely irregular. Moreover, they are adjoined strongly each other by the tight junctions and desmosomes and possess some cilia and microvilli on its surface facing to the follicular lumen. The corticotrophs are stained specifically with lead-hematoxylin and they are found in the ventromedial edge of the pro-adenohypophysis and form a palisade-like layer composed of two or three rows of cells. The secretory granules of the cells measure 150–250  $m\mu$  in diameter and show various electron densities, and their limiting membranes are often widely separated from the granular cores. The somatotrophs are stained strongly by acid dyes, and is round, oval or polygonal in shape. These cells include many membrane-bound secretory granules ranging from 200 to 350  $m\mu$  in diameter, which are high in electron density and are round in shape. The thyrotrophs are stained weak blue with azan staining, are small in number and found situated singly in dorsal part of the meso-adenohypophysis. They contain many small secretory granules ranging from 100–200  $m\mu$ . The gonadotrophs are characterized by the presence of two kinds of granules, the larger granules from 500 to 3000  $m\mu$  and the smaller ones ranging from 300 to 500  $m\mu$  in diameter.

The glandular cells in the pro- and meso-adenohypophysis are not directly in contact with neurohypophysis but they touch a thin layer of fibroblastic cells which confront neurohypophysis across a basement membrane.

The endothelial cells have some fenestrae of about 600–800 Å in diameter, each fenestra being bridged by a single-layered membrane separating a blood cavity from the surrounding pericapillary cavity. A kind of cells of fibroblastic nature are situated outside to the pericapillary cavity and they are in direct contact with the glandular cells.

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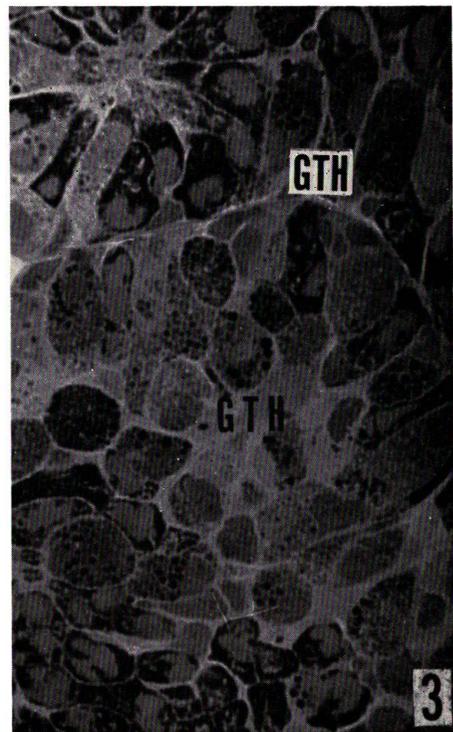
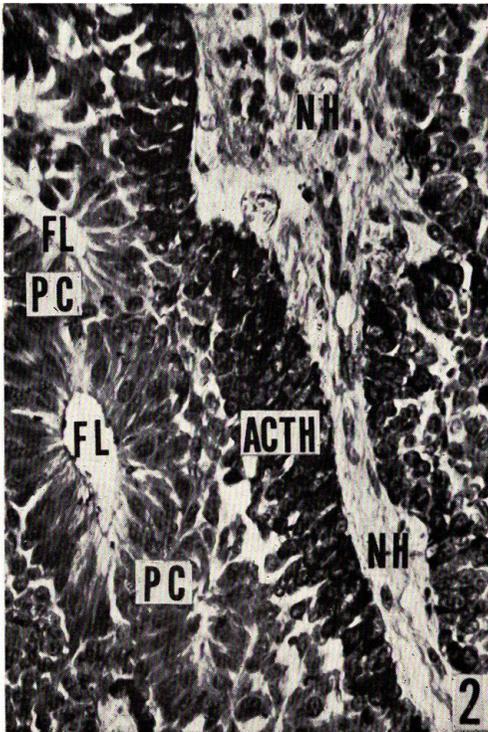
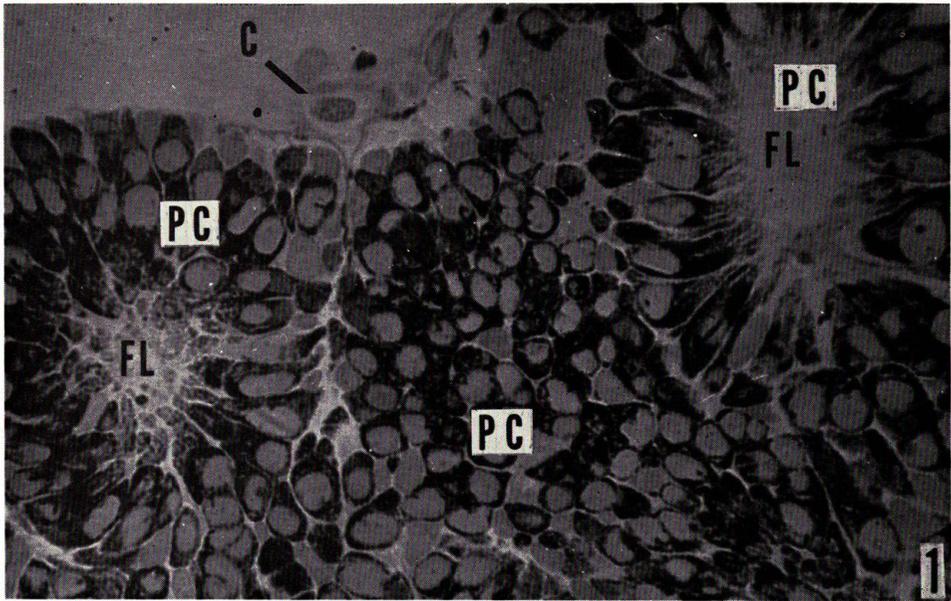


## PLATE I

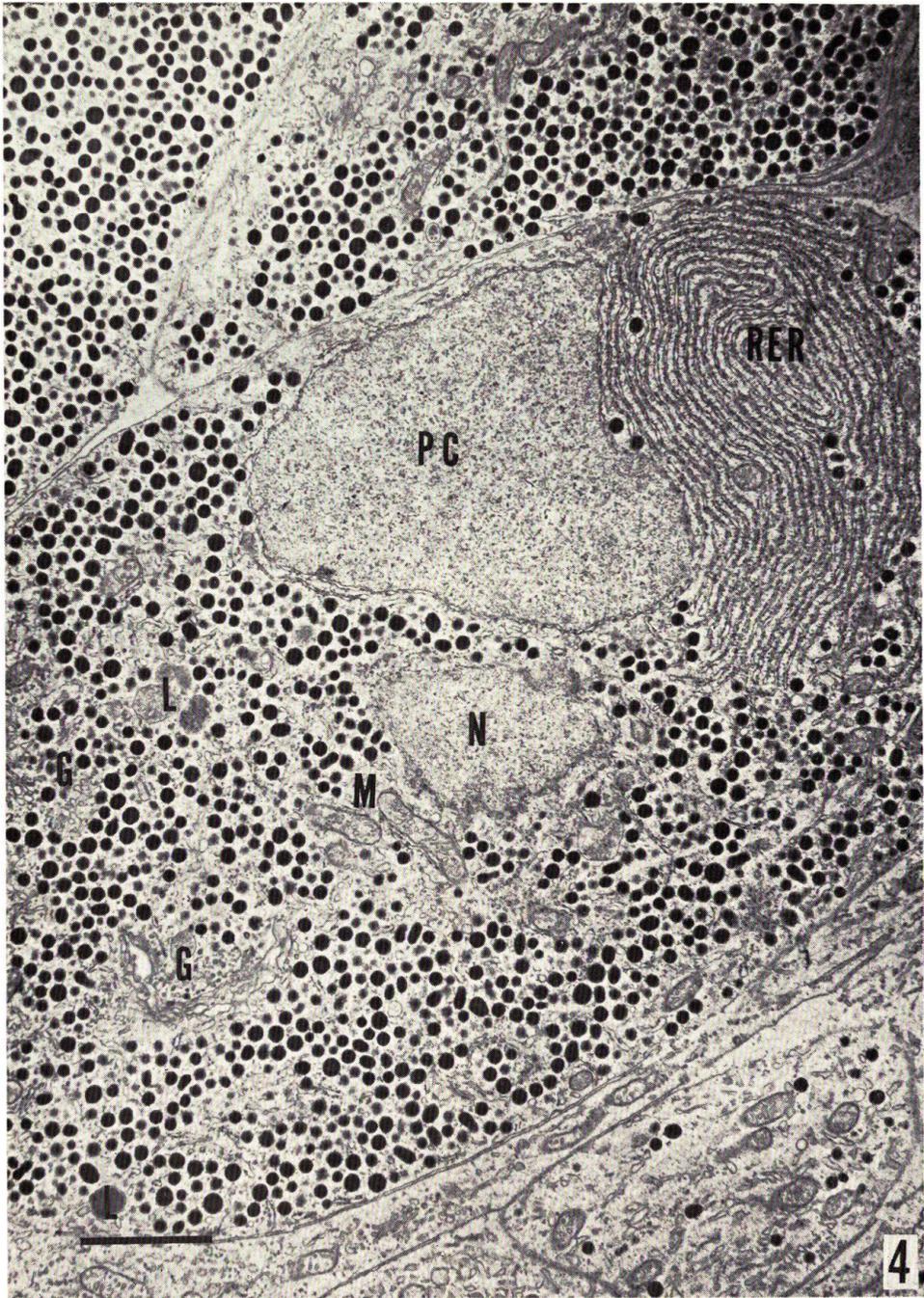
Fig. 1. Prolactin cells showing a follicular arrangement in the pro-adenohypophysis of the kokanee pituitary. C, capillary. PC, prolactin producing cells. FL, follicular lumen. Azur II-methylene blue.  $\times 540$ .

Fig. 2. A midsagittal section of the kokanee pituitary. The palisade layer of lead-hematoxylin-positive corticotrophs (ACTH) is seen between the lead-hematoxylin-negative prolactin producing cells (PC) and a branch of neurohypophysis (NH). FL, follicular lumen. Lead-hematoxylin.  $\times 630$ .

Fig. 3. Part of the meso-adenohypophysis in a sexually mature kokanee. Note many gonadotrophs (GTH) containing a few large granules and many small granules in their cytoplasm. Azur II-methylene blue.  $\times 680$ .



Y. NAGAHAMA & K. YAMAMOTO : Adenohypophysis of the kokanee



Y. NAGAHAMA & K. YAMAMOTO : Adenohypophysis of the kokanee

## PLATE II

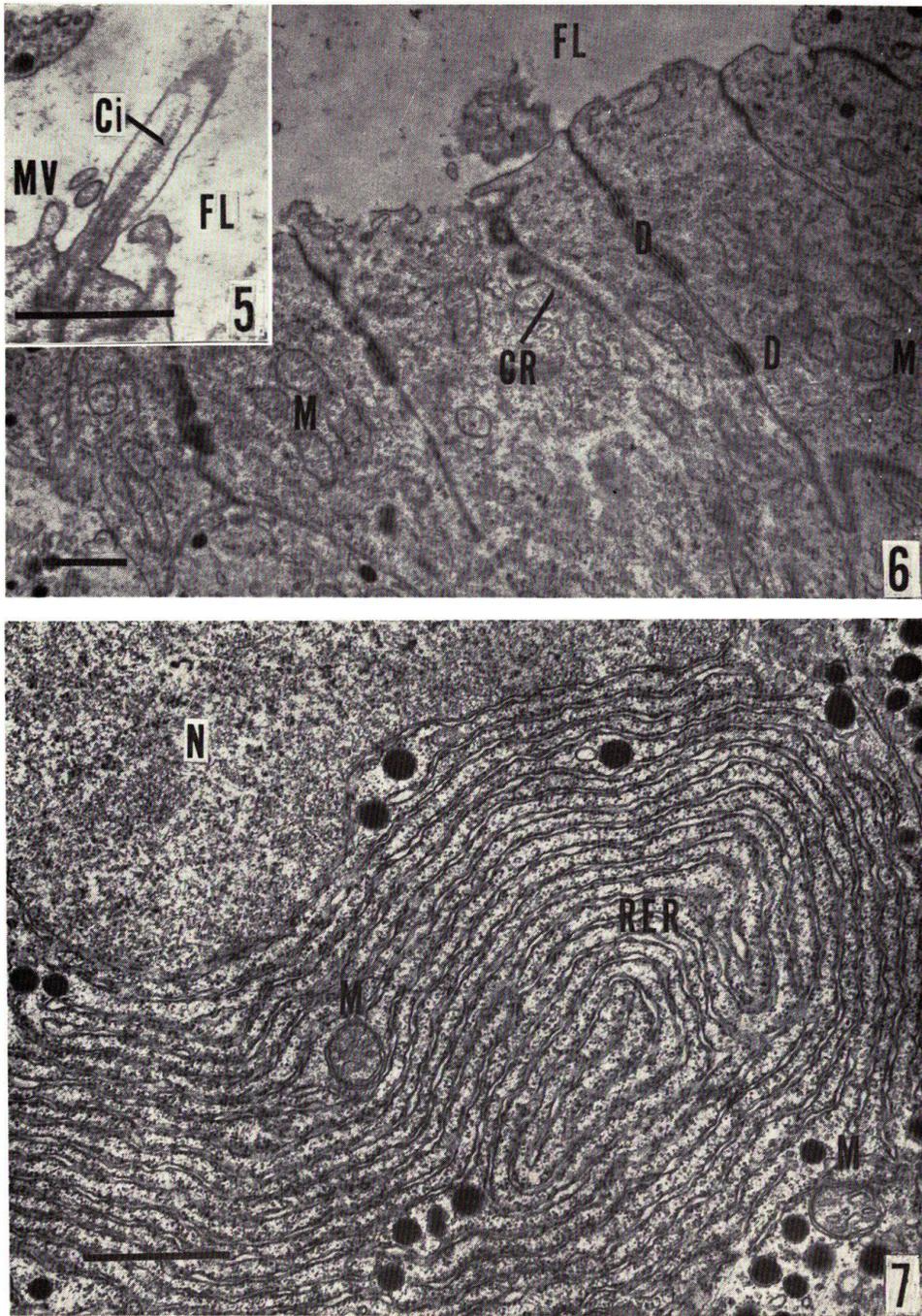
Fig. 4. Prolactin producing cells (PC) of the kokanee pituitary. The rough endoplasmic reticulum (RER) and Golgi apparatus (G) are well developed. A moderate number of mitochondria (M) and lysosomes (L) are observed. N, nucleus.  $\times 8,400$ .

### PLATE III

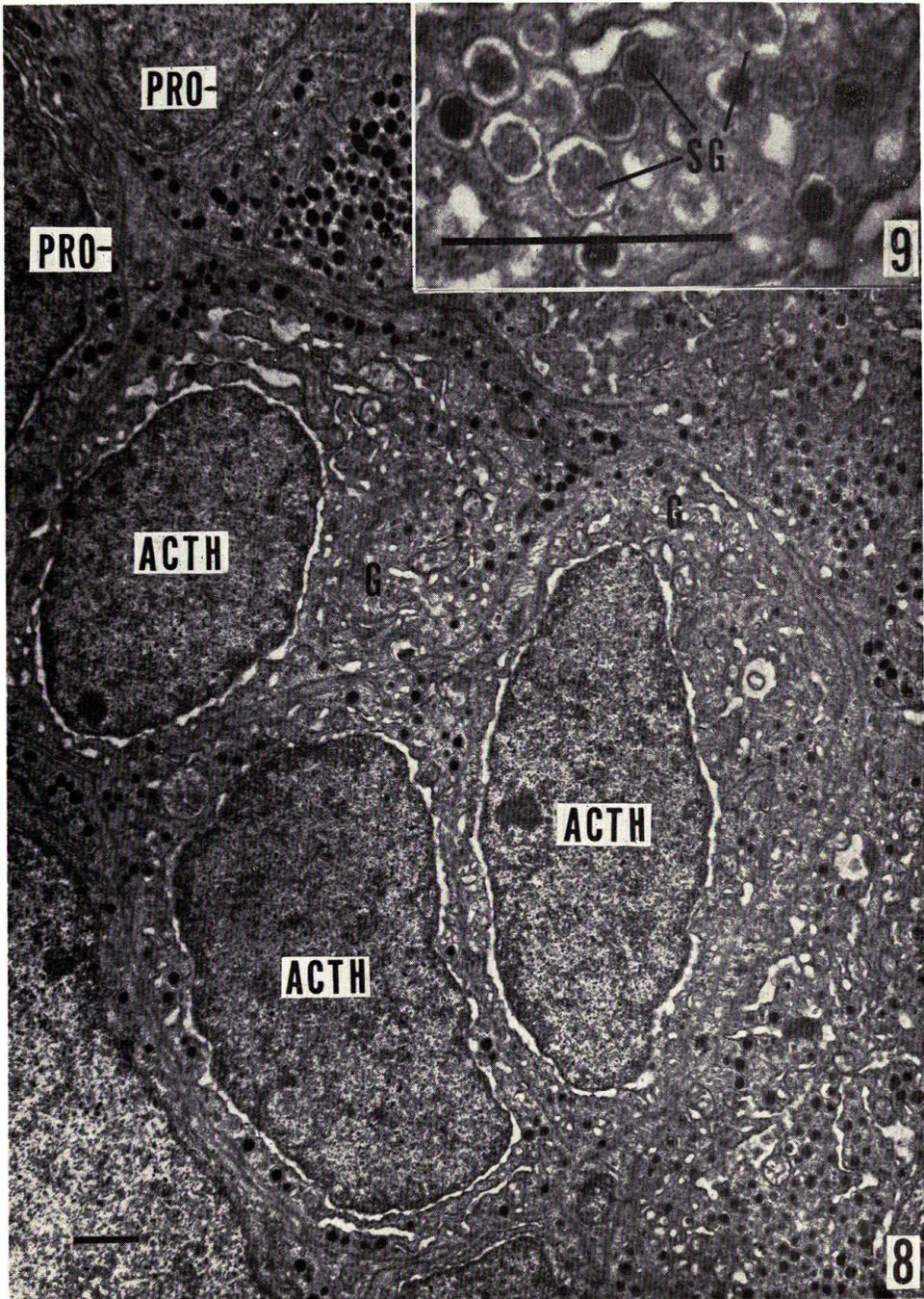
Fig. 5. Longitudinal section of a cilium (Ci) observed in the prolactin producing cells of the kokanee pituitary. Microvilli (MV) are also extended into the follicular lumen (FL).  $\times 22,000$ .

Fig. 6. Follicular lumen (FL) sides of the prolactin producing cells in a kokanee pituitary. They are joined with each cilium root (CR). D, desmosome. M, mitochondrion.  $\times 11,200$ .

Fig. 7. Part of a prolactin cell of the kokanee pituitary. Notice the well developed rough endoplasmic reticulum (RER). M, mitochondrion.  $\times 20,000$ .



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#### PLATE IV

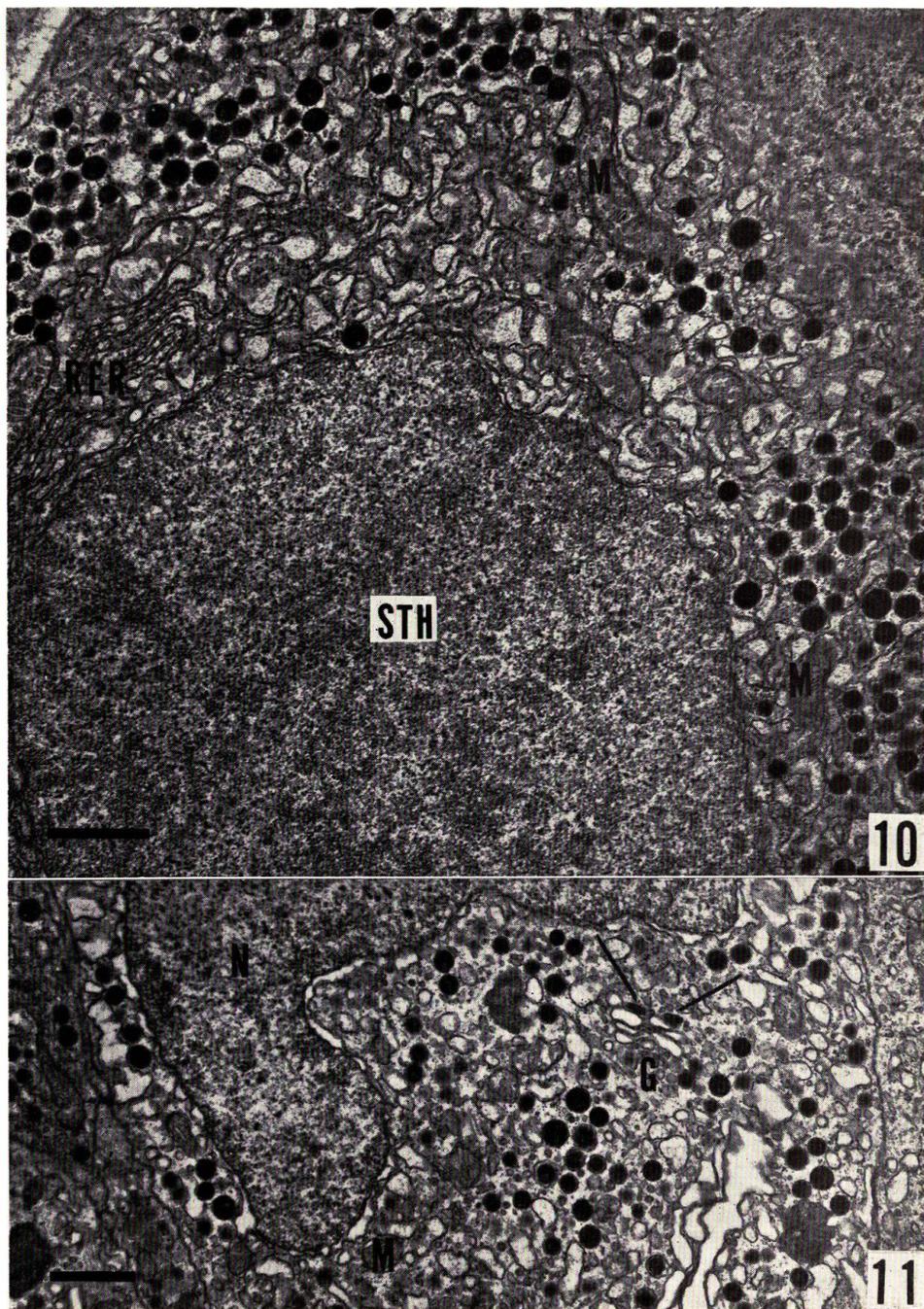
Fig. 8. Corticotrophs (ACTH) of the kokanee pituitary. The limiting membranes of the secretory granules are often widely separated from their contents. M, mitochondrion. G, Golgi apparatus. RER, rough endoplasmic reticulum.  $\times 9,000$ .

Fig. 9. Part of a corticotroph of the kokanee pituitary. The secretory granules (SG) show various electron densities, and their limiting membranes are widely separated from the cores.  $\times 40,000$ .

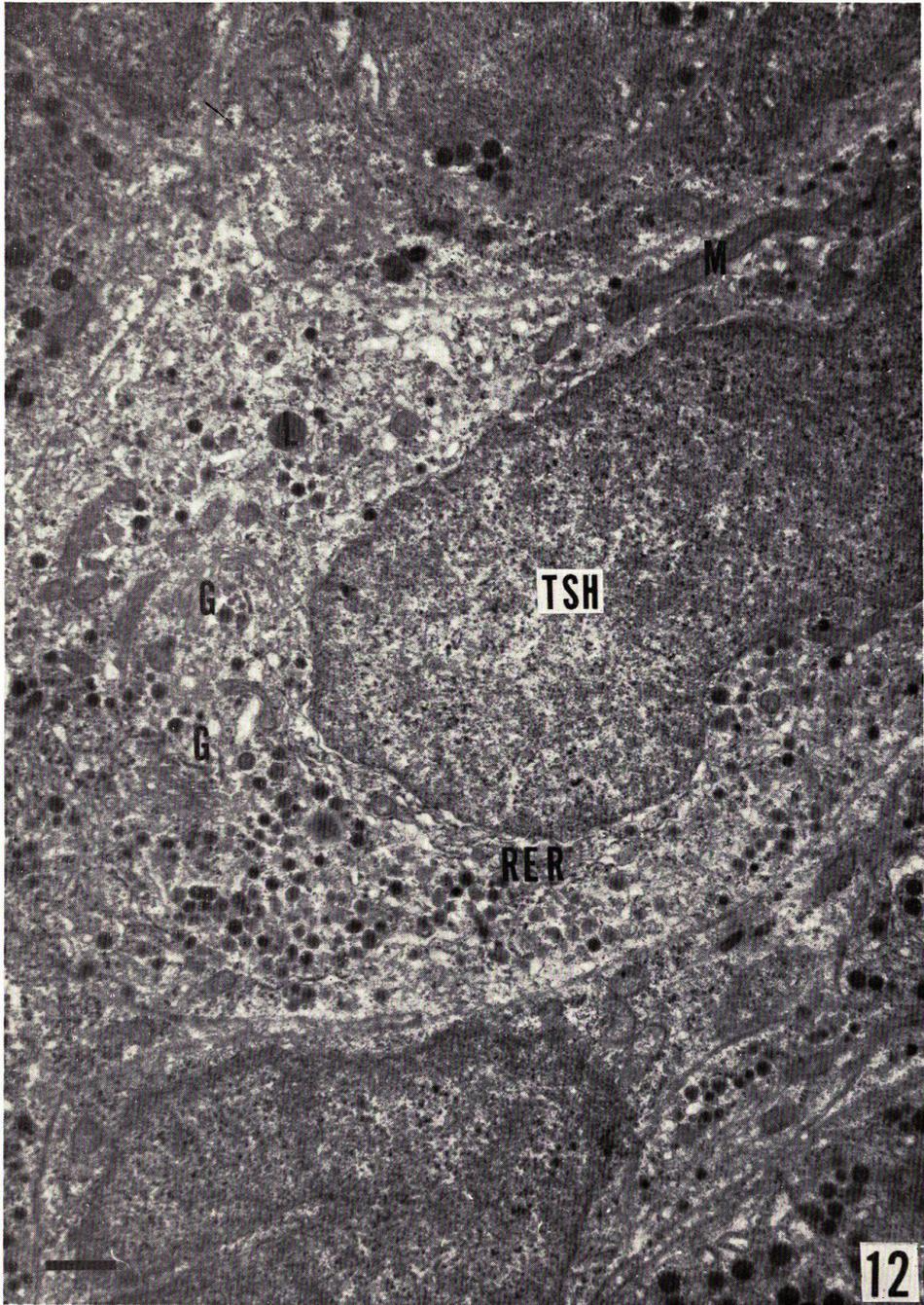
## PLATE V

Fig. 10. Part of a somatotroph (STH) of the kokanee pituitary. The well developed rough endoplasmic reticulum (RER) and moderate numbers of mitochondria (M) are observed.  $\times 13,800$ .

Fig. 11. Part of a somatotroph of the kokanee pituitary. The well developed Golgi apparatus (G) are observed. The formation of the secretory granules is recognized in the dilated sacs of the Golgi apparatus (arrow). M, mitochondrion. N, nucleus.  $\times 12,000$ .



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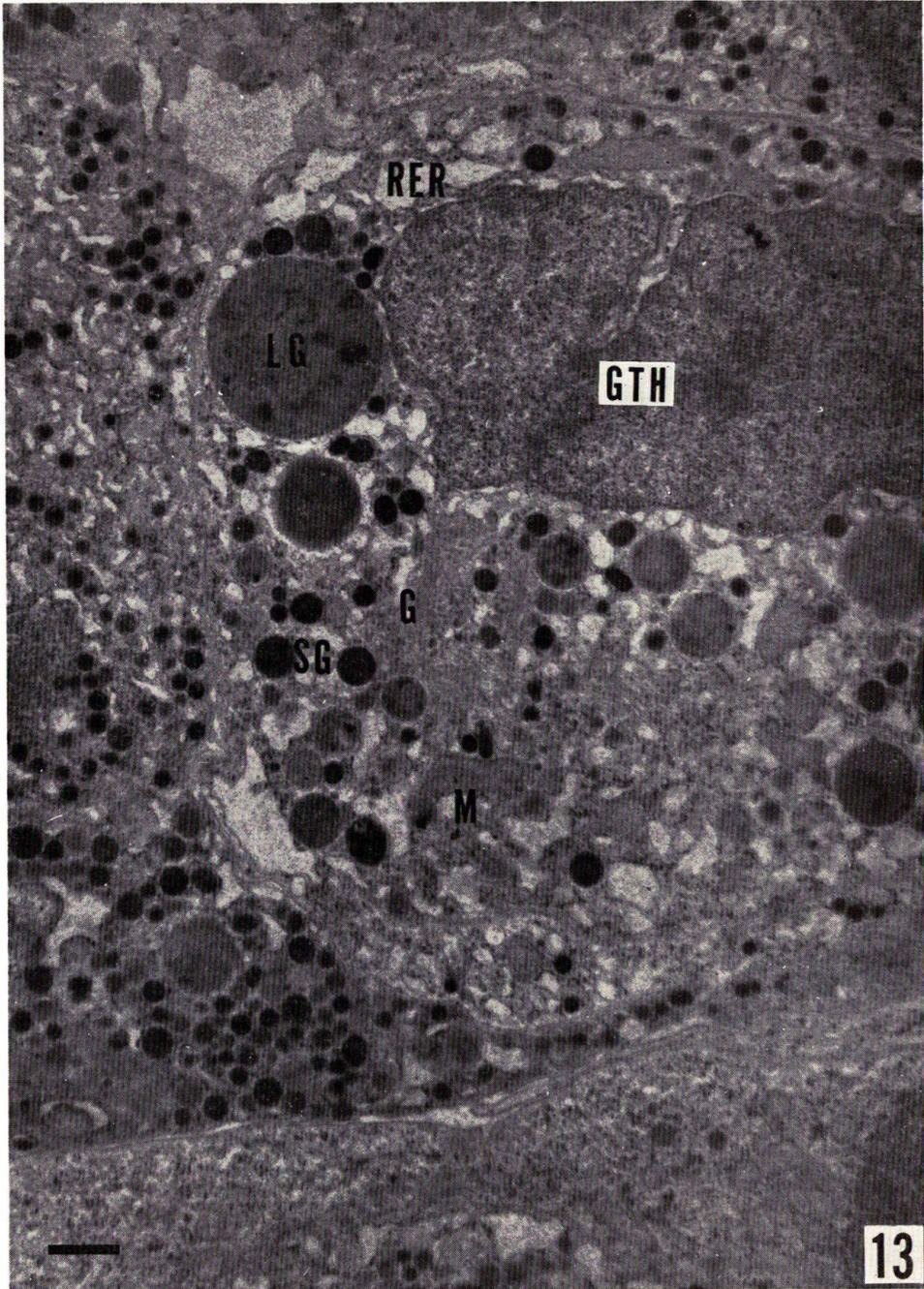
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## PLATE VI

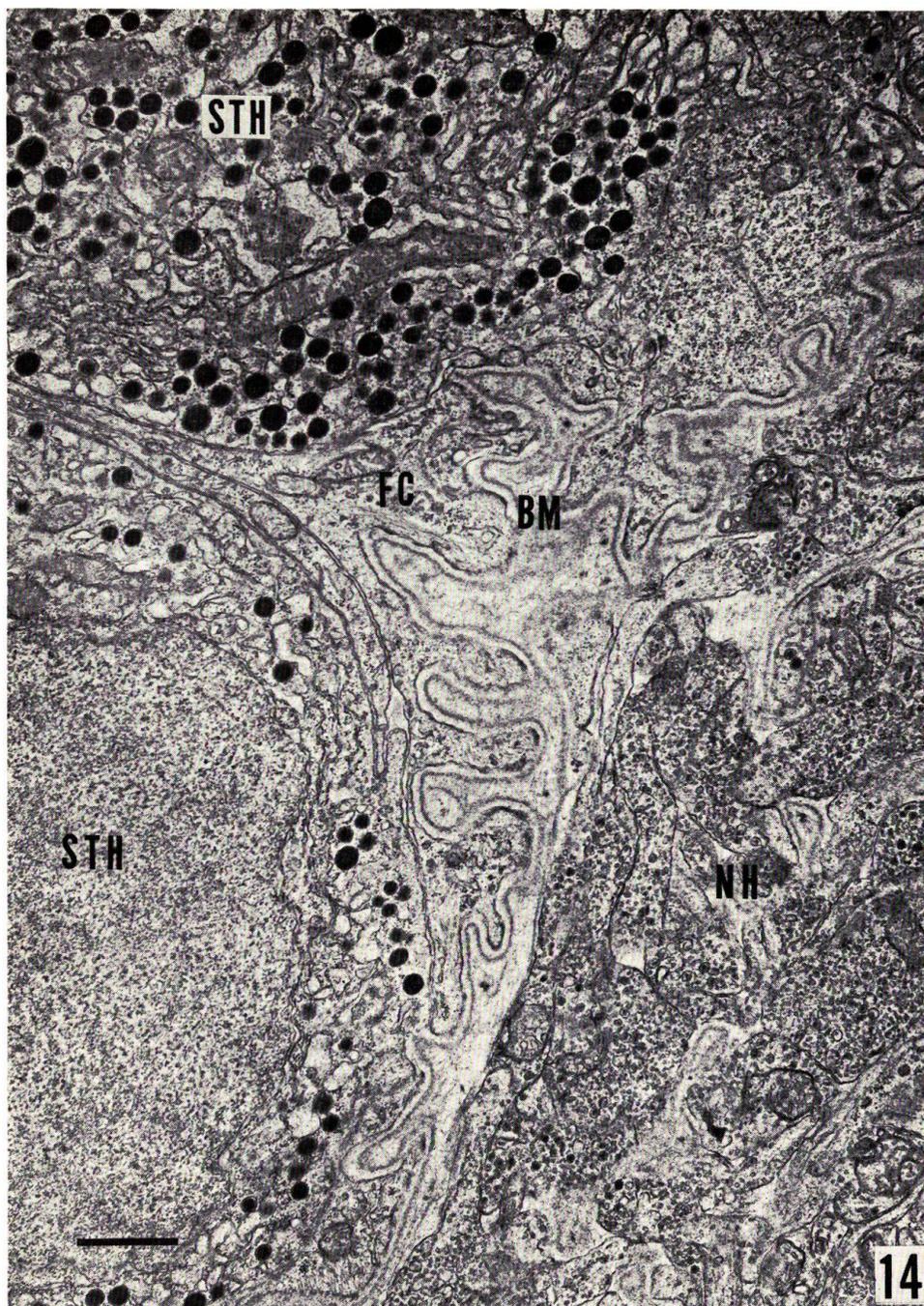
Fig. 12. Thyrotroph (TSH) of the kokanee pituitary. The cells are evenly filled with spherical small secretory granules. M, mitochondrion. G, Golgi apparatus. RER, rough endoplasmic reticulum. L, Lysosome.  $\times 9,000$ .

## PLATE VII

Fig. 13. Gonadotrophs (GTH) of the mature kokanee pituitary. Notice the large, low electron-dense granules (LG) and the small high electron-dense granules (SG) in the cytoplasm. M, mitochondrion. G, Golgi apparatus. RER, rough endoplasmic reticulum.  $\times 9,000$ .



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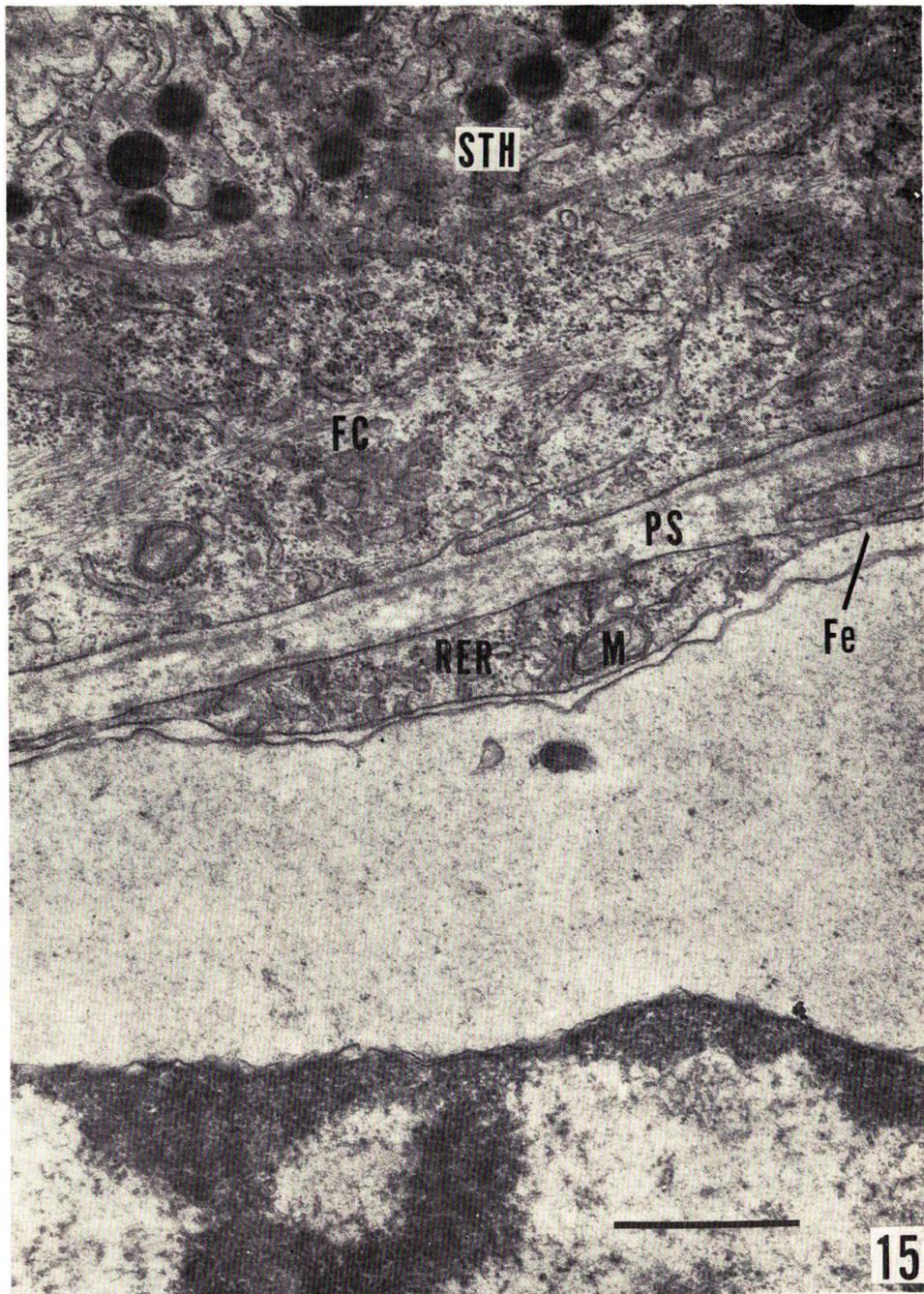
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## PLATE VIII

Fig. 14. Relationship between the neurohypophysis (NH) and somatotrophs (STH) in the kokanee pituitary. They are separated by a tissue which is differentiated into a basement membrane (BM) and a thin layer of fibroblastic cells (FC).  $\times 13,800$ .

## PLATE IX

Fig. 15. Somatotroph (STH) and a capillary of the kokanee pituitary. A fenestrae (Fe) can be observed in a layer of endothelial cells. RER, rough endoplasmic reticulum. PS, pericapillary space. FC, fibroblastic cells. M, mitochondrion.  $\times 27,000$ .



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