HOKKAIDO UNIVERSITY

Title	Ultrastructural Observations on the Thyrotrope in Metamorphosing Xenopus laevis (With 5 Plates)
Author(s)	WATANABE, Yūichi G.
Citation	北海道大學理學部紀要, 18(1), 1-8
Issue Date	1971-10
Doc URL	https://hdl.handle.net/2115/27511
Type	departmental bulletin paper
File Information	18(1)_P1-8.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Ultrastructural Observations on the Thyrotrope in Metamorphosing *Xenopus laevis*[1)]

By

Yûichi G. Watanabe[2)]

Zoological Institute, Hokkaido University

(With 5 Plates)

The pars distalis of metamorphosing amphibians has attracted the attention of many investigators since it became known that most active TSH secretion from the hypophysis takes place during metamorphic climax of the animals. With the purpose to obtain morphological evidence of the changes in the glandular cells during this period, a number of cytological works have been made at the light microscopic level on this gland (Allen *et al.*, 1929; D'Angelo, 1940, 1941; Cordier, 1953; Saxén, 1958; Saxén *et al.*, 1957; Kerr, 1966; Goos *et al.*, 1968). Most of these workers described intensity of tinctorial affinity of the glandular cells in relation to the state of their secretory activity. However, a definite correlationship between the two parameters has never been established. Moreover, the observations were sometimes contradictory even in the same species; for instance, Saxén *et al.* (1957) described a change of tinctorial affinity in the thyrotrope of metamorphosing *Xenopus*, whereas Kerr (1966) could not find any cytological change of the same cell.

In view of inconsistency of the data in light microscopy, a more detailed observation by electron microscopy seemed desirable. At present there are but a little ultrastructural studies made on the pars distalis of metamorphosing amphibians (Watanabe, 1966; Dent and Gupta, 1967; Doerr-Schott, 1968), which have mainly dealt with the purpose to classify the cell types. The present study is concerned with ultrastructural changes of the thyrotropes of *Xenopus* during metamorphosis.

Materials and Methods

Fertilized eggs of *Xenopus laevis* were obtained through injection of chorionic gonadotropin. The larvae were maintained at about 20°C and fed boiled alfalfa powder.

1) Contribution No. 910 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo Japan.

2) Present address: Department of Anatomy, School of Medicine, Tokushima University, Tokushima, Japan.

Three animals at stages 56, 58, 60, 62, 64 and 66 (stages of Nieuwkoop and Faber, 1956), respectively, were selected and sacrificed by decapitation. For convenience' sake, the period between stages 56–58 will be described as early climax, stages 60–62 mid-climax and stages 64–66 late climax, respectively, in the present paper. The hypophyses were removed and fixed at 4°C in buffered 1% OsO_4 containing sucrose for 2 hours. The tissues were then dehydrated through a cold ethanol series and embedded in styrene-methacrylate resin (Kushida, 1961). Thin sections were cut with a Porter-Blum microtome and mounted on formval-coated copper grids. After double staining of uranyl acetate and lead hydroxide (Millonig, 1961), these were examined in a Hitachi HS-7 electron microscope.

For light microscopy, paraffin sections of the pars distalis were stained with PAS, Heidenhain's Azan and Gabe's Aldehyde Fuchsin (AF).

Observations

Identification of the thyrotrope

In light microscopy, it has been generally agreed that in *Xenopus* the delta basophils are responsible for thyrotropin secretion (Kerr, 1966; Streb, 1967; Goos *et al.*, 1968). Ultrastructurally, classification of the glandular cells is usually made on the basis of the size of their secretory granules. The cells that contain medium-sized granules of 115.3 mμ in an average diameter have been identified as the delta-thyrotrope in prometamorphic *Xenopus* (Watanabe, 1966). However, the size of elementary granules cannot be used as a sole criterion for identification of this cell throughout metamorphosis because of the scarcity of ordinary electron dense secretory granules during metamorphic climax.

In the present study, therefore, identification of the thyrotrope was carried out by comparing the data obtained by the light microscope with those obtained by the electron microscope. In light microscopy three types of chromophils have been known to be present in the pars distalis of *Xenopus* larva (Kerr, 1966; Goos *et al.*, 1968). The gamma-cells are concentrated in the rostral part around the median eminence, while the acidophils and delta-thyrotropes are widely distributed through the rest part of the gland. Of the latter two cell types, the acidophils are known to become smaller in size during metamorphic climax by reducing the volume of their cytoplasm (Cordier, 1953; Saxén *et al.*, 1957; Kerr, 1966), thus making the thyrotrope more conspicuous. From the reason described above, there seemed to remain no doubt in identifying large-sized hypertrophied cells as the thyrotropes.

Ultrastructure of the thyrotrope

The feature of cytoplasmic constituents of the thyrotrope varied from cell to cell even within a gland. Therefore, more than two cells were shown to present the general picture of thyrotropes at each stage.

Early climax (*Stages 56–58*). Some cells were found to contain electron dense granules distributed only sparsely among abundant ER vesicles (Fig. 1). In others,

either granulation was lacking (Fig. 2) or only ill-defined granules of very low electron density were recognized (Fig. 3). These "pale cells" were much larger in number than the granulated cells, so that it was not rare to see that most part of a low power electron micrograph was occupied by such cells.

In contrast to the scarcity of secretory granules, membrane vesicles of varied size (20–500 mμ in diameter) were distributed abundantly throughout the cytoplasm (Figs. 1–3). Some of the vesicles were studded with ribosome particles on their outer surface, the rough-surfaced ER. There were many veiscles which showed an intermediate condition between the rough- and smooth-surfaced ER. Frequently incomplete vesicles, mostly semicircular in shape, were seen in the cytoplasm (Fig. 4, inset).

The size of the Golgi complex was variable in different cells. Around the Golgi zone, cored vesicles were observed (Fig. 4). They were characterized by a wide space between the dense core and the surrounding membrane, the structures having never been seen in the corresponding glandular cells of the adult. Mitochondria were usually small in size, and some of them were so electron dense that they were hardly distinguished from secretory granules (Fig. 2).

Mid-climax (*Stages 60–62*). The cells containing secretory granules increased in number during this period. The morphology of the granules was variable from cell to cell or even within a cell (Figs. 5 and 6). They varied from small dense spherules to larger hemispheres with lower electron density. There could also be seen a flocculent material. It was frequently accompanied by fragmented membranes (Fig. 5). Various intermediate forms between these structures were present throughout the cytoplasm.

The pale cells containing few secretory granules were seen during this period, but they were rather small in number at stage 62. In these cells the Golgi complex was well developed, but the cored vesicles were not as many as in stages 56–58, or early climax.

In addition, some thyrotropes contained conspicuous electron dense bodies measuring up to 800 mμ in diameter (Fig. 7). These were distinguishable from the ordinary secretory granule on the basis of their large size and higher electron density. There were seen few cells that possessed both the dense body and the secretory granules at a time.

Late climax (*Stages 64–66*). In general, the cytoplasmic organelles were poorly developed, and the Golgi complex, in particular, was hard to see in many thyrotropes during this period.

The dense bodies became extremely large in size, measuring in some more than 2 μ in diameter. Most of them became less electron dense, and within them round or irregularly shaped dense masses were included frequently (Figs. 8 and 9).

By light microscopy the dense bodies were stained strongly with AF (Fig. 9, inset), but almost unstained with PAS or aniline blue after Heidenhain's Azan stain. The thyrotrope that contains these structures was greatly variable in number among animals of the same age.

During late climax, especially at stage 66, the thyrotropes containing sparse to moderate amount of granules appeared to increase in number (Fig. 10). Unlike the previous period, in which secretory granules were predominantly less electron dense, most granules looked compact. The granules were many, whereas the cytoplasmic organelles were less prominent in these cells. Both the size and the number of ER vesicles were inconsistent among different cells.

Discussion

There have been many light microscopic studies on the pars distalis of the hypophysis in metamorphosing amphibians (Allen *et al.*, 1929; D'Angelo, 1940, 1941; Cordier, 1953; Saxén, 1958; Saxén *et al.*, 1957; Kerr, 1966; van Oordt, 1966; Goos *et al.*, 1968; Streb, 1968). These authors have described an increase in number of the basophils, the supposed thyrotropes, during metamorphosis of the animals. In *Xenopus*, the thyrotrope increased greatly in number between stage 56 and 60 (Watanabe, 1969), suggesting that differentiation and/or multiplicatoin of this cell take place actively during this period. At the electron microscopic level, a considerable number of thyrotropes possessed only a few compact secretory granules during the same period. Of these "pale" cells, some contained exclusively cell organelles in their rich cytoplasm (Fig. 2), thereby they are presumed to be newly differentiated thyrotropes. While other cells contained less electron dense veiscles (Fig. 3), cored vesicles (Fig. 4) or fragments of membranes (Figs. 4, inset). These pictures may be taken as to imply that during earlier climax most thyrotropes do not accumulate compact secretory granules as in usual manner. Since data are available indicating that relatively large amount of thyrotropin is released during this period (Saxén *et al.*, 1957; Hanaoka, 1966), the granule storage phase of these cells might be omitted, or, if any, it may be very rapidly passed over and this may explain why electron dense granules are absent in some thyrotropes.

During early climax, cored vesicles were observed frequently around the Golgi area of some thyrotropes. It has generally been accepted that condensation of secretory product is attained within the Golgi cisternae and "condensing vacuoles" (for review, see Beams and Kessel, 1968). Therefore, the characteristic wide space between the dense core and its surrounding membrane of the cored vesicle may be explained as a feature resulted from insufficient condensation of secretory material.

As to the releasing mechanism of secretory granules, Farquhar (1961) described that in the rat hypophysis granules are discharged by the so-called "reversed pinocytosis". A similar mechanism has been postulated in the autotransplanted hypophysis of the newt (Masur, 1969). In anurans, on the other hand, Iturriza (1964) and Doerr-Schott (1962) observed granule dissolution within the cytoplasm. The present investigation revealed that in the thyrotropes of *Xenopus* secretory granules lost their electron density and clear contour and that, at the same time, fragmentation of the limiting membrane occurred. However, it

cannot be excluded that such granule rupture results from bad fixation, as was suggested by Heath (1969). The releasing mechanism of granules from the basophils remains to be clarified by future work.

At stage 60 and later, large dense bodies were observed in the thyrotropes. The structures stained strongly with AF, as is the case of the ordinary secretory granule, but they failed to stain with PAS or aniline blue. These findings suggest that the dense body has something in common with secretory granules though chemical nature differs between the two structures. Further studies are needed to elucidate the functional significance of the dense body.

It has generally been agreed that cells with few granulation at the electron microscopic level fail to stain with various dyes employed in light microscopy. In the present ultrastructural study, some thyrotropes during the earlier climax stage were found to contain few electron dense secretory granules. These cells were so large in size that they were unmistakably distinguished from other types of cells. By light microscopy, however, no chromophobes in corresponding size were present in the pars distalis of the animal in the same age. Therefore, it may be safe to conclude that there is no correlation between the tinctorial affinity of some thyrotropes and their electron-opaque granules in metamorphosing *Xenopus* larva.

Summary

The thyrotrope in the pars distalis of *Xenopus laevis* was investigated with special reference to its ultrastructural changes during metamorphosis.

From stage 56 to 58, or during early metamorphic climax, only small number of secretory granules were accumulated in the thyrotrope. During this period many cells contained well-developed cytoplasmic organelles and few electron dense granules. These cells were interpreted as the newly differentiated thyrotropes possessing not much storage of granules yet.

Between stages 60–62, or during metamorphic mid-climax, less electron dense granules were the main cytoplasmic inclusions. They seemed to reflect dissolution of secretory granules within cytoplasm. At the same time, cells containing electron dense bodies in variable size appeared occasionally. The size of the dense body and the number of cells possessing it apparently increased up to the end of metamorphosis. The moderately granulated thyrotropes were found to take place more frequently in the pars distalis of the animals during stages 64–66, or at late metamorphic climax.

Acknowledgments

The writer wishes to express his cordial thanks to Professor T. Aoto for his kind advice and careful revision of the manuscript. He is also indebted to Professor S. Daikoku, who has given support in many ways to the completion of the present study.

References

Allen, B. M., E. D. Torreblanca and J. A. Benjamin 1929. A study upon the histogenesis of the pars anterior of the hypophysis of *Bufo* during metamorphosis. Anat. Rec. **44**: 208–209.

Beams, H. W. and R. G. Kessel 1968. The Golgi apparatus: Structure and function. Int. Rev. Cytol. **23**: 209–276.

Cordier, R. 1953. L'hypophyse de *Xenopus*. Interprétation histophysiologique. Ann. Soc. Roy. Zool. Belg. **84**: 5–16.

D'Angelo, S. A. 1940. The morphology of the pituitary and thyroid gland of several species of Anura during different phases of metamorphosis. Anat. Rec. Suppl. **76**: 17.

———— 1941. An analysis of the morphology of the pitutiary and thyroid glands in amphibian metamorphosis. Am. J. Anat. **69**: 407–437.

Dent, J. N. and B. L. Gupta 1967. Ultrastructural observations on the developmental cytology of the pitutiary gland in the spotted newt. Gen. Comp. Endocrinol. **8**: 273–288.

Doerr-Schott, J. 1962. Evolution des cellules gonadotropes β au cours du cycle annuel chez la Grenouille rousse *Rana temporaria* L. Etude au microscope électronique; Observations histochimiques et cytophysiologiques. Gen. Comp. Endocrinol. **2**: 541–550.

———— 1968. Dévelopment de l'hypophyse de *Rana temporaria* L. Etude au microscope électronique. Z. Zellforsch. **90**: 616–645.

Farquhar, M. G. 1961. Origin and fate of secretory granules in cells of the anterior pituitary gland. Trans. N.Y. Acad. Sci. **23**: 346–352.

Goos, H. J. Th., A.-M. de Knecht and J. de Vries 1968. Hypothalamic neurosecretion and metamorphosis in *Xenopus laevis*. I. The effect of propylthiouracil. Z. Zellforsch. **86**: 384–392.

Hanaoka, Y. 1966. Uptake of ^{131}I by the thyroid gland during metamorphosis in *Xenopus laevis*. J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. **16**: 106–112.

Heath, E. 1970. Cytology of the pars anterior of the bovine adenohypophysis. Am. J. Anat. **127**: 131–158.

Iturriza, F. C. 1964. An electron microscopic study of the toad pars distalis. Gen. Comp. Endocrinol. **4**: 225–232.

Kerr, T. 1966. The development of the pitutiary in *Xenopus laevis* Daudin. Gen. Comp. Endocrinol. 6: 303–311.

Kushida, H. 1961. A styrene-methacrylate resin embedding method for ultrathin sectioning. J. Electronmicr. **10**: 16–19.

Masur, S. K. 1969. Fine structure of the autotransplanted pituitary in the red eft, *Notophthalamus viridescens*. Gen. Comp. Endocrinol. **12**: 12–32.

Millonig, G. 1961. A modified procedure for lead staining of thin sections. J. Biophys. Biochem. Cytol. Suppl. **11**: 736–739.

Nieuwkoop, P. D. and J. Faber 1956. Normal table of *Xenopus laevis* (Daudin). North-Holland Publ. Co., Amsterdam, 1956.

Saxén, L. 1958. The onset of thyroid activity in relation to the cytodifferentiation of the anterior pitutiary. Acta Anat. **32**: 87–100.

————, E. Saxén, S. Toivonen and K. Salimäki 1957. The anterior pituitary and the thyroid function during normal and abnormal development of the frog. Ann. Zool. Soc. "Vanamo" **18**: 1–44.

Streb, M. 1967. Experimentelle Untersuchungen über die Beziehung zwischen

Schilddrüse und Hypophyse während der Larvalentwicklung und Metamorphose von *Xenopus laevis* Daudin. Z. Zellforsch. **82**: 407–433.

van Oordt, P.G.W.J. 1966. Changes in the pituitary of the common toad, *Bufo bufo*, during metamorphosis, and the identification of the thyrotropic cells. Z. Zellforsch. **75**: 47–56.

Watanabe, Y.G. 1966. Electron microscopic studies on the anterior pituitary in larvae of *Xenopus laevis*. J. Fac. Sci. Hokkaido Univ. Ser. VI, **16**: 85–89.

———— 1969. On the number of thyrotrophs in *Xenopus laevis* during metamorphosis. Annot. Zool. Japon. **42**: 63–67.

Explanation of Plates I-V

Plate I

Fig. 1. A survey electron micrograph of thyrotropes from a larva at stage 58. Electron dense secretory granules are contained only sparsely. On the contrary, largesized ER vesicles are abundant. ×11,600.

Fig. 2. A thyrotrope at stage 58, showing rich cytoplasm with no electron dense granules. Both ER vesicles and mitochondria are considerably smaller in size than those in the cells shown in Figure 1. ×10,400.

Plate II

Fig. 3. Stage 56. The cytoplasm contains number of less dense granules and membrane vesicles. Mitochondria (arrows) are small and have dense matrix. ×10,400.

Fig. 4. Well-developed Golgi complex from a thyrotrope at stage 56. In the center of the figure characteristic cored vesicles are seen. Note a wide space between the surrounding membrane and the dense core. ×15,500.

Inset; Semicircular membranes in the larva at stage 56 (arrows). ×22,000.

Plate III

Fig. 5. A thyrotrope of a larva at stage 60. Secretory granules of this cell are greatly variable in size and in electron density. Among them flocculent substances with fragmented membranes are frequently observed (arrows). The Golgi complex and mitochondria are not seen. ×11,100.

Fig. 6. Another thyrotrope at stage 60. Most secretory granules of this cell lose their electron density and their contour is ill-defined. The cell organelles are only poorly developed except those sparsely contained ER vesicles. ×12,600.

Plate IV

Fig. 7. Thyrotropes of an animal at stage 62, showing dense bodies contained in the cytoplasm. ×8,900.

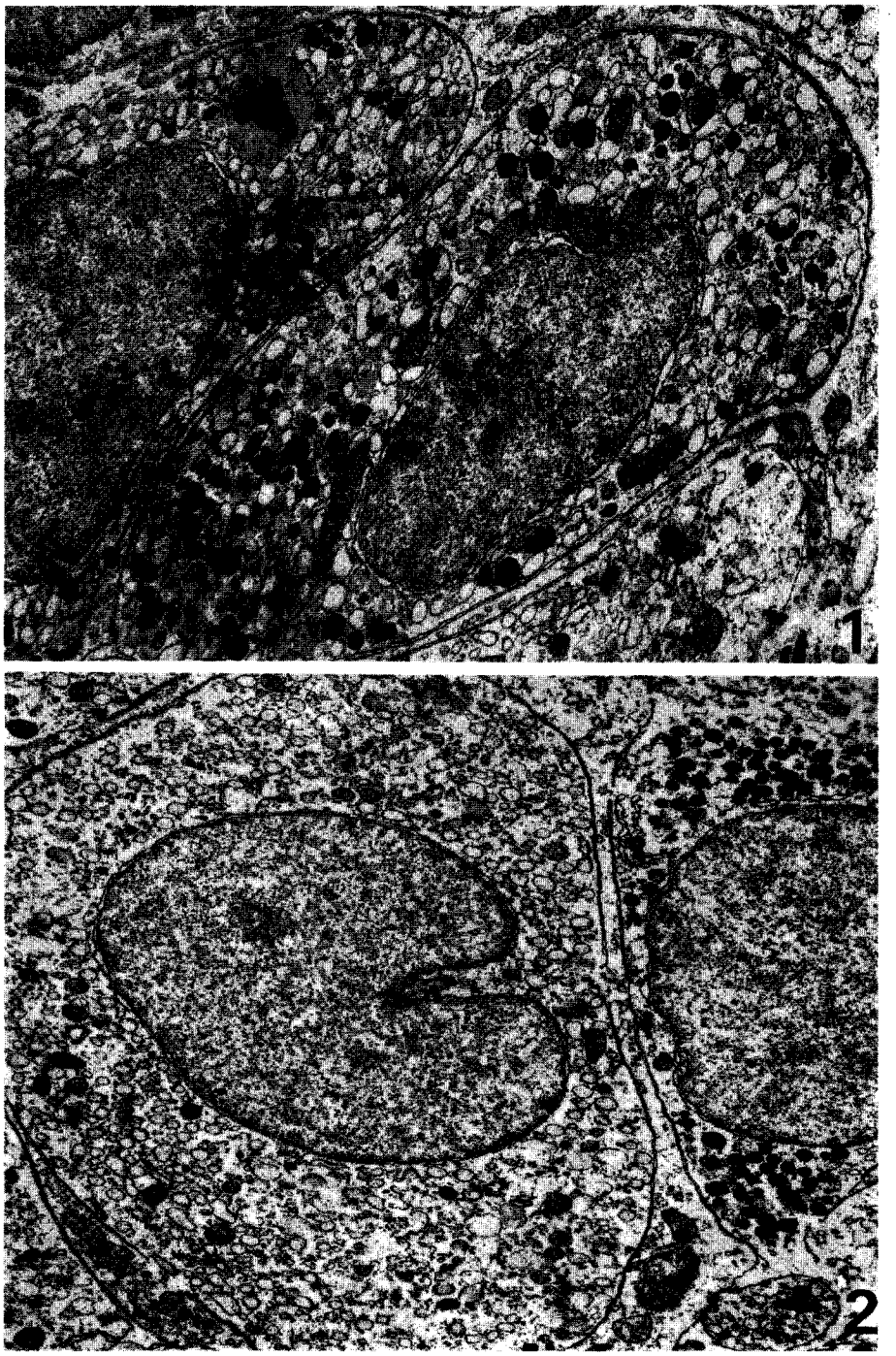
Fig. 8. A large thyrotrope at stage 64. It contains a relatively small number of dense bodies in the remarkably rich cytoplasm. The dense body (arrow) includes a round, denser core. ER vesicles are sparse. ×9,600.

Plate V

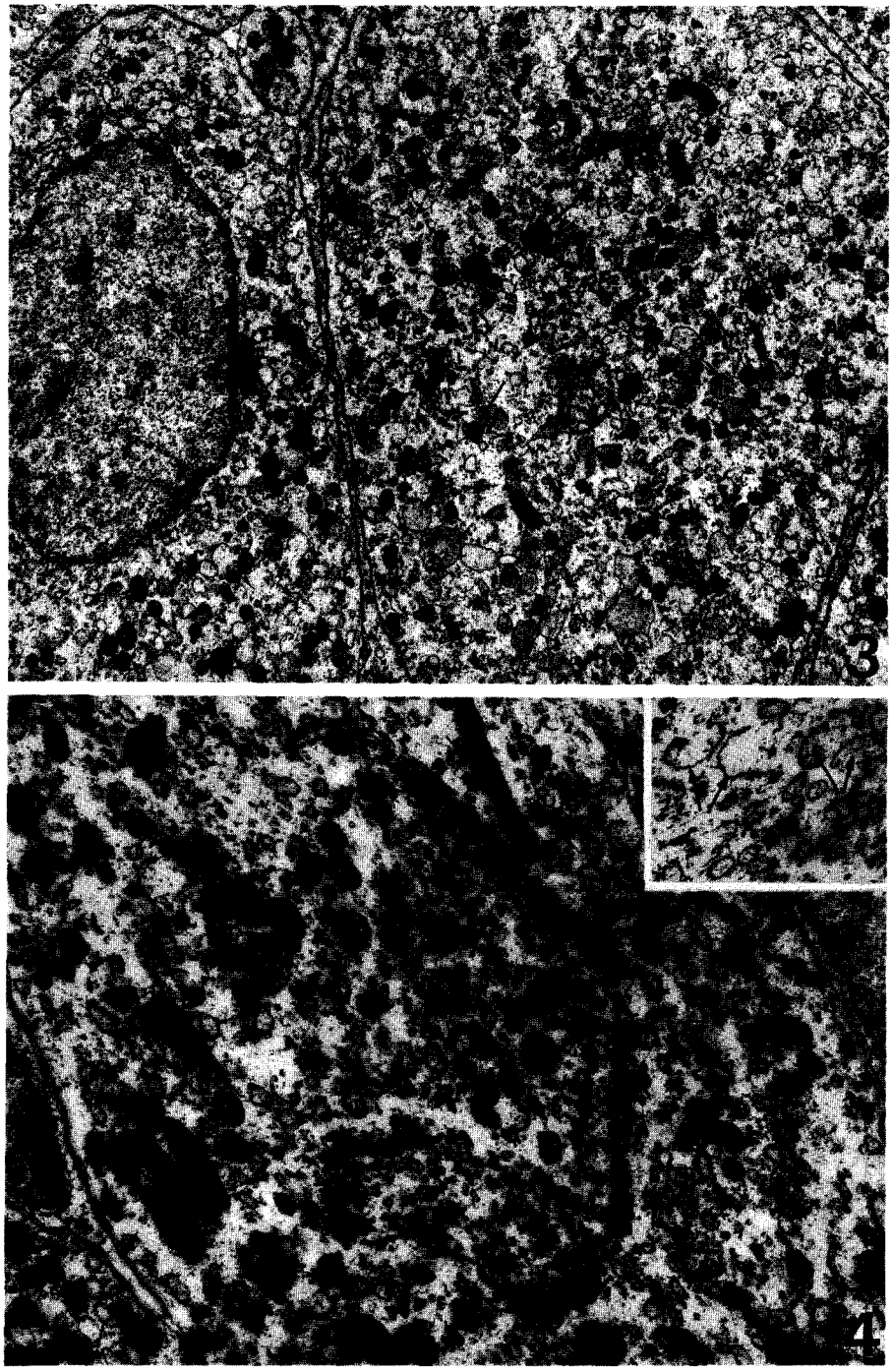
Fig. 9. A thyrotrope at stage 66, showing many dense bodies of different electron densities. Some of them contain round or irregular denser masses. ×15,200.

Inset; Photomicrograph of the pars distalis in an animal of the same age. Those AF-positive structures (arrows) seem to correspond to the dense bodies observed at the electron microscopic level. Gabe's AF. ×1,270.

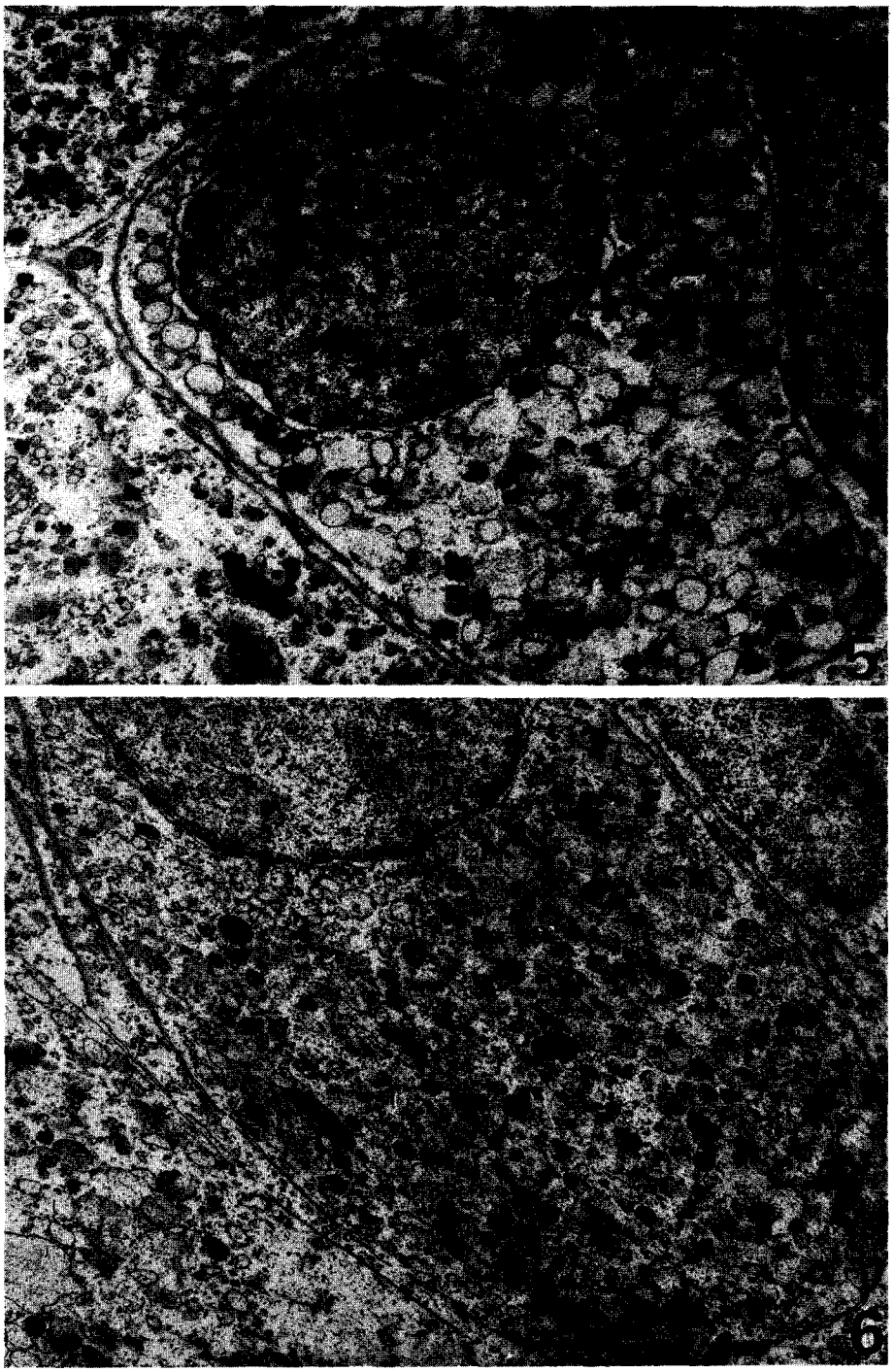
Fig. 10. A thyrotrope at stage 66, indicating moderate number of compact secretory granules. The Golgi complex is not very conspicuous (arrow). ×9,600.



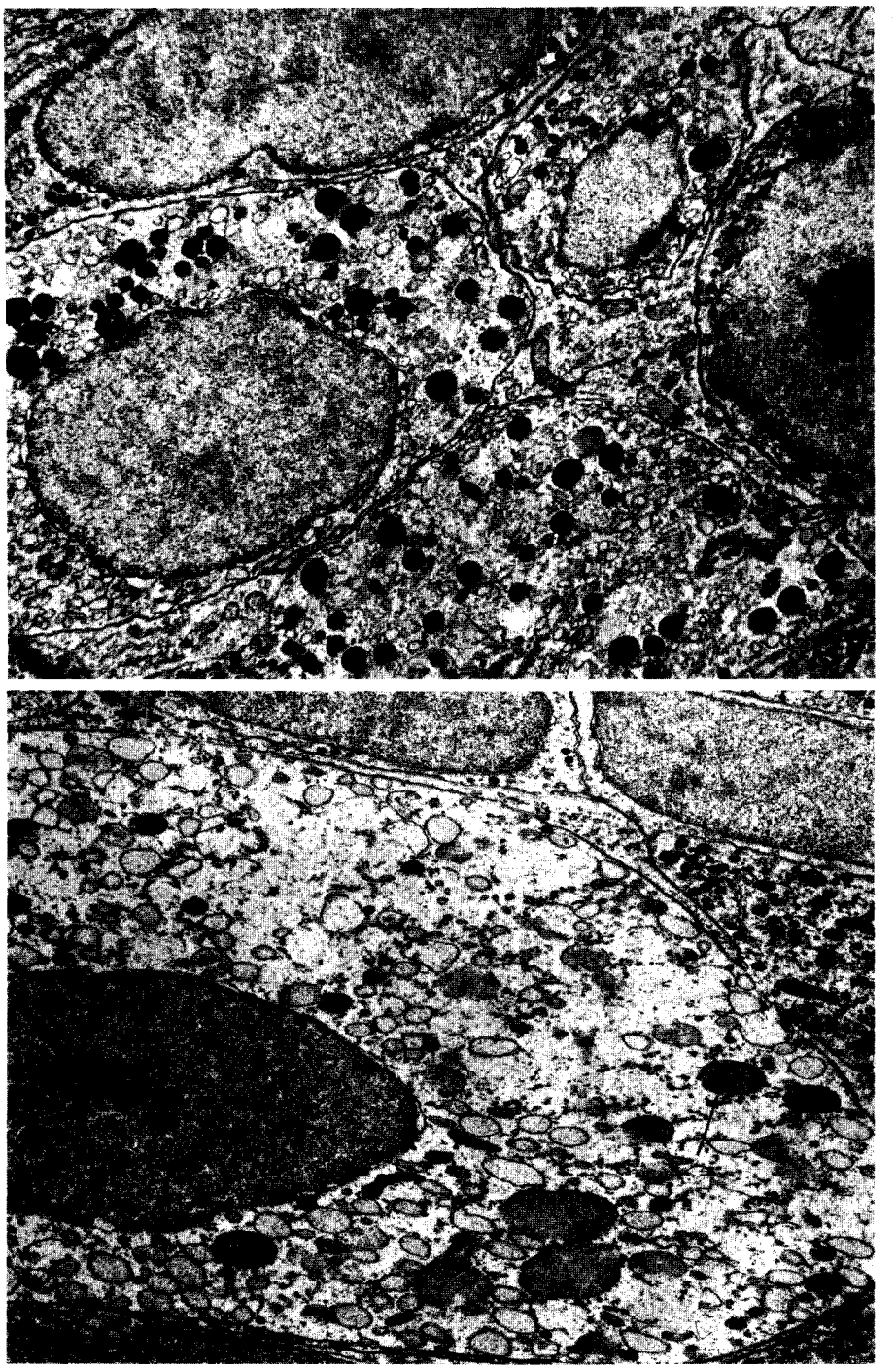
Y. G. Watanabe: Thyrotrope in Metamorphosing Xenopus



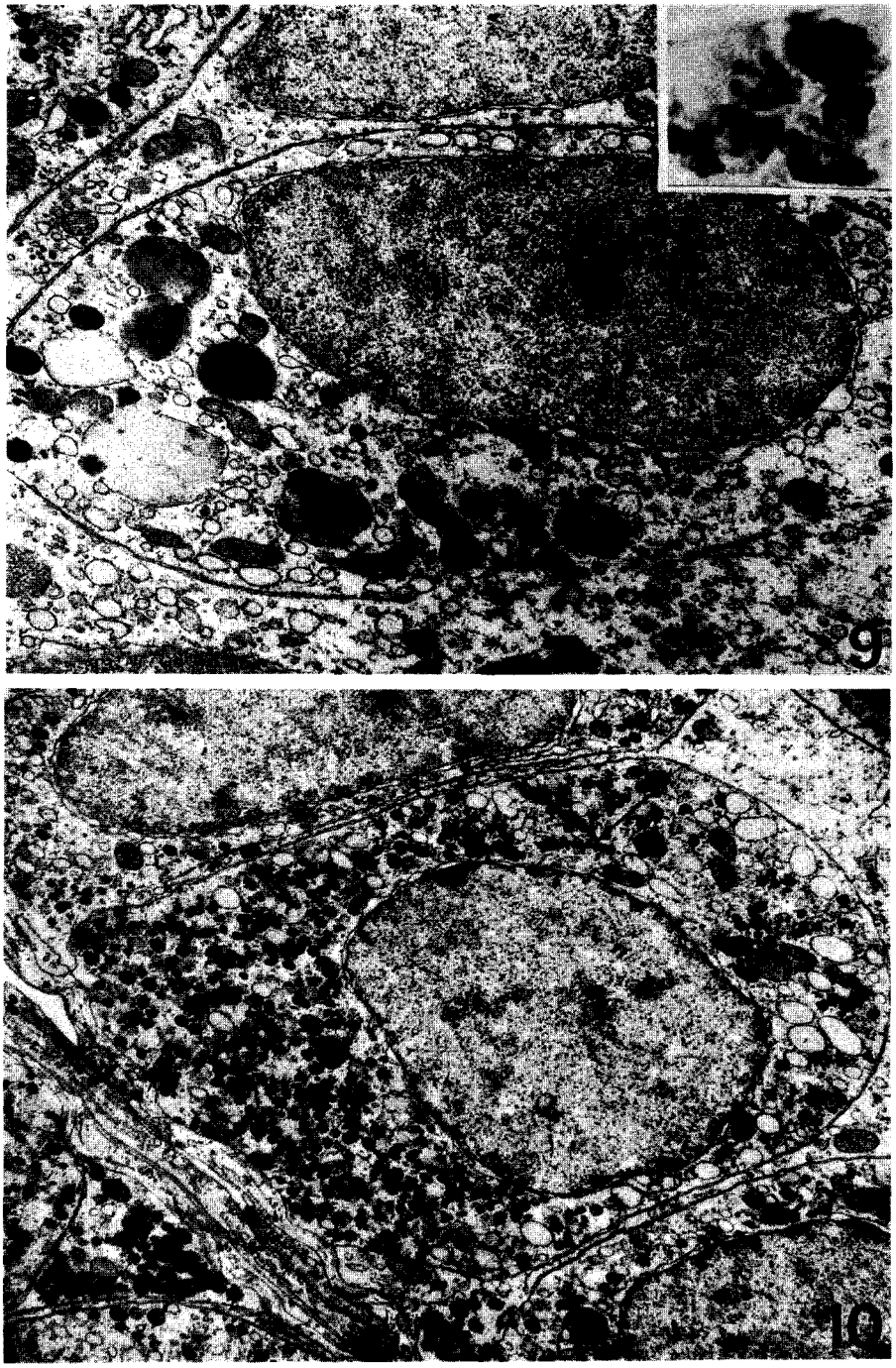
Y. G. Watanabe: *Thyrotrope in Metamorphosing Xenopus*



Y. G. Watanabe: *Thyrotrope in Metamorphosing Xenopus*



Y. G. Watanabe: *Thyrotrope in Metamorphosing Xenopus*



Y. G. Watanabe: *Thyrotrope in Metamorphosing Xenopus*