Ultrastructural Cytochemistry of Hatching Gland Cells in *Xenopus* Embryos in Relation to the Hatching Process

By

Norio Yoshizaki

Zoological Institute, Hokkaido University

(*With 4 Plates*)

In a previous study (Yoshizaki, 1973), a description was given of hatching gland cells (HGCs) which are cytologically and topographically unique among the epidermal cells of *Xenopus* tailbud embryos. The possible participation of these cells in the production and the secretion of the hatching enzyme was suggested on the basis of their observed ultrastructures which are similar to those found in the proteinous gland cells (*cf.*, Kurosumi, 1961). In a more recent analysis of the hatching process in the same material, Carroll and Hedrick (1974) presented direct evidence for the secretion of enzyme with protease activity. Consequently, more precise information on the activities of HGC is important for a fuller understanding of the hatching mechanism from both biochemical and morphological points of view. The present study was undertaken to obtain information with the aid of ultrastructural cytochemistry.

Material and Methods

The material used in this study was the South African clawed toad, *Xenopus laevis*. Fertilized eggs were obtained according to the artificial insemination method of Wolf and Hedrick (1971). Embryos at stages 24 to 46 (*cf.*, Nieuwkoop and Faber, 1956) were freed from their envelopes, and fixed for electron microscopic study with 5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2). Since the hatching gland cells are most abundantly distributed in the anterior dorsal part of an embryo (*cf.*, Yoshizaki, 1973), the tissues containing this part were isolated in the fixative and allowed to stand in it for 3 h at 4°C. After fixation the specimens were washed thoroughly in 6 changes, each for 15 min, of 0.1M cacodylate buffer, and submitted to the subsequent treatments.

Ultrastructural localization of carbohydrates was detected by periodic acid-chromic acid-silver methenamine (PA-CrA-Silver) method, after the method of Rambourg and Leblond (1967). This method principally consists of an oxidation of 1,2-glycol and α-amino alcohol groups by periodic acid and chromic acid, followed by staining with silver methenamine. The glutaraldehyde-fixed specimens were dehydrated and embedded in Epon 812 (Luft, 1961). Thin sections were placed on uncoated stainless-steel grids to stain

with PA-CrA-Silver. Control runs were made by treating the sections with silver methenamine without previous oxidations. To discriminate glycogen from other polysaccharides, the fixed specimens were treated, before embedding in Epon 812, with saliva for 90 min at 37°C. For comparison of the results obtained by PA-CrA-Silver method with those by PAS method for light microscopic study, the embryos were fixed with Bouin's solution, sectioned by the paraffin method and stained with the PAS method. Both methods gave essentially the same results.

For detecting acid phosphatase activity on ultrathin sections, the glutaraldehyde-fixed specimens were incubated for 1 h at 37°C in the Gomori's substrate mixture (Gomori, 1952). After incubation, specimens were washed with 0.1M cacodylate buffered 1% OsO_4 for 3 h at 4°C. They were then dehydrated, embedded in Epon 812, sectioned and stained with 2% uranyl acetate. The incubation of the specimens in the mixtures without either β-sodium glycerophosphate or lead nitrate served as controls.

The sections were viewed with a Hitachi HS-7 electron microscope at the initial magnifications of 2,000 to 10,000.

Observations

Carbohydrates

Figs. 1–4 show the hatching gland cells (HGCs) from the stage 34 embryos as stained with PA-CrA-Silver method. Among the organelles in HGC, the apical granules stain most strongly with this method. Besides those localized in the apical region, the granules scattered in the subapical region also stain deeply with silver. Well developed Golgi complexes were seen in the supranuclear region (Figs. 2 and 3), where the silver precipitates were observable in the intralamellar matrixes and vesicles. These observations support my previous conclusion (Yoshizaki, 1973) that the apical granules are formed in the Golgi complexes. The saliva treatment did not affect the stainability of the above mentioned organelles. Glycogen particles were present throughout the ground cytoplasm, although their amount was fairly variable among different HGCs even in the same embryo (Fig. 4). This observed variability is likely to be a reflection of the difference in the activity and/or maturity of HGCs. The apical granules and the Golgi complexes in HGC of the pre-hatching stages always stained with silver. At the post-hatching stage (stage 46), however, silver staining of the intralamellar matrix of the residual Golgi complex was considerably reduced, although undischarged apical granules were well stained as in the preceding stages. Glycogen particles were almost undetectable in HGC at stage 46.

The carbohydrate components were also detected in the common epidermal cells (CECs), and were confined to the mucous vesicles and the Golgi lamellae of the cells, irrespective of the pre- or post-hatching stages (Fig. 1). Glycogen particles were meager both in CECs and the cilia cells (CCs), in comparison with glycogen particles in HGCs.

Acid Phosphatase

Fig. 5 shows the ultrastructural localization of acid phosphatase activity in HGC at the hatching stage (stage 36). The lead phosphate reaction product is localized exclusively in the Golgi lamellae and the Golgi associated granules. No activity was detectable in other organelles or in the ground cytoplasm. The reaction was also negative in the apical granules which are characteristic of HGC.

Examinations of HGC in embryos at stage 34 or earlier revealed that the acid phosphatase activity is entirely negative throughout the cell, even in the Golgi complexes. In contrast, the enzyme activity was discernible in the Golgi complexes of HGC throughout the post-hatching stages, although this activity diminished with the gradual degeneration of the Golgi complexes (*cf.*, Fig. 8). These observations, together with those on the carbohydrate localization as described in the preceding section, strongly indicate that the function of the Golgi complexes changes in HGC at the hatching stage.

Besides the Golgi complexes, the acid phosphatase activity was frequently observable in the membrane-bounded bodies (Figs. 6 and 7) which are present in the basal half of HGC at stage 39 and older ones. Fig. 6 shows 2 types of membrane-bounded bodies: one (Ly) shows the acid phosphatase activity, and the other (Ph), negative to the enzyme reaction, is characterized by the presence of several cytoplasmic elements and double membranes delimiting the body. The former may well be referred to as lysosome, whereas the latter as phagosome (*cf.*, de Duve and Wattiaux, 1966). The section of HGC from the stage 42 embryo (Fig. 7) demonstrated the membrane-bounded bodies (MB) which had the characteristics of both the lysosome and phagosome. At stage 46, the membrane-bounded bodies significantly increase in size so as to occupy about one third of the cell. At this stage the acid phosphatase activity is again detected (Fig. 8). However, the reaction is usually weak, and this is probably due to dilution within such a large-sized body.

Discussion

Previous electron microscopic observations of *Xenopus* HGC (Yoshizaki, 1973) were interpreted to show that the hatching enzyme is included in the apically localized, membrane-limited granules. Ultrastructural examinations for localization of carbohydrates as described in this paper provide substantial evidence for the previously proposed view that the apical granules, characteristic of HGC, are formed in the Golgi complexes. It is difficult, however, to explain the significance of the observed carbohydrate localization in relation to the function of HGC during the hatching process, since the carbohydrate moiety itself does not provide a measure for the presence of enzyme molecules. A possible explanation might be that the hatching enzyme is present in the granules forming a protein-polysaccharide complex. However, further studies will be necessary either by using more direct cytochemical demonstration of proteolytic activity in the granules, or by

determining the activities of the granules isolated from the cellular homogenate in order to clarify this point.

According to Rambourg (1971) the PA-CrA-Silver method in carbohydrate cytochemistry stains the same structures as the classical PAS method. This was also found in the epidermal cells of *Xenopus* embryos. Thus staining with PAS method at the light microscopic level may well be used as a good marker to determine if the apical granules are present in the morphologically defined HGC.

An acid phosphatase is the most commonly used cytochemical marker for lysosomal enzymes (Ericsson, 1969). Recently there have been a number of reports indicating that lysosomal bodies occur in the cells under the process of a physiological remodeling, *e.g.*, in rat pituitary mammotrophs (Smith and Farquhar, 1966) and degenerating notochordal cells in amphibians (Fox, 1973). The stage of the first appearance and the change in the localization of acid phosphatase activity, as observed in this study, could be interpreted in the same context: the lysosomes that originated from the Golgi complex fuse with large membrane-bounded bodies to participate in the digestion or destruction of several cytoplasmic elements. Although the final fate of HGC after stage 46 was not determined in the present study, an important aspect of the present cytochemical approach to HGC is that a change in the activity of the Golgi complexes occurs at the hatching stage. This change will be the cause of the later degeneration of the whole cell.

Summary

In an attempt to determine the cellular activities of the hatching gland cell (HGC) in relation to the hatching process of *Xenopus laevis*, the localization of carbohydrates and acid phosphatase was studied at the electron microscopic level. It was found that in HGC of embryos at the pre-hatching stages, carbohydrates, other than glycogen, occur in the apical granules and the Golgi complexes. In the post-hatching stages, the carbohydrate reaction diminished from the Golgi complexes. The acid phosphatase activity was not detectable in HGC at the pre-hatching stages, but was found in the granules which were formed in the Golgi complexes at the hatching and the post-hatching stages. In the post-hatching stages, these granules, presumably lysosomes, became fused with membrane-bounded bodies, and seem to participate in the degradation of cytoplasmic elements of HGC. These results indicate that at the hatching stage, an important change occurs in HGC, particularly in the Golgi complexes.

The author wishes to offer his sincere thanks to Dr. Ch. Katagiri for his kind guidance throughout the course of this study and reading through the manuscript. Further the author is also grateful to Professor T. Aoto for his keen interest and advice in this study.

References

Carroll, E. J., Jr. and J. L. Hedrick 1974. Hatching in the toad *Xenopus laevis*: morphological events and evidence for a hatching enzyme. Develop. Biol., in press.

de Duve, C. and R. Wattiaux 1966. Functions of lysosomes. Ann. Rev. Physiol. **28**: 435–492.

Ericsson, J. L. E. 1969. Mechanism of cellular autophagy. In *Lysosomes in Biology and Pathology* (Edited by J.T. Dingle and H.B. Fell). Vol. 2, pp. 345–394. North-Holland Pub., Amsterdam.

Fox, H. 1973. Degeneration of the tail notochord of *Rana temporaria* at metamorphic climax. Examination by electron microscopy. Z. Zellforsch. **138**: 371–386.

Gomori, G. 1952. Microscopic histochemistry. Principles and practice. p. 189. Chicago Univ. Press, Chicago.

Kurosumi, K. 1961. Electron microscopic analysis of the secretion mechanism. Intern. Rev. Cytol. **11**: 1–124.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**: 409–414.

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

Rambourg, A. 1971. Morphological and histochemical aspects of glycoproteins at the surface of animal cells. Intern. Rev. Cytol. **31**: 57–114.

———— and C. P. Leblond 1967. Electron microscope observations on the carbohydrate-rich cell coat present at the surface of cells in the rat. J. Cell Biol. **32**: 27–53.

Smith, R. E. and K. G. Farquhar 1966. Lysosomes function in the regulation of the secretory process in cells of the anterior pituitary gland. *Ibid.* **31**: 319–347.

Wolf, D. P. and J. L. Hedrick 1971. A molecular approach to fertilization. II. Viability and artificial fertilization of *Xenopus laevis* gametes. Develop. Biol. **25**: 347–359.

Yoshizaki, N. 1973. Ultrastructure of the hatching gland cells in the South African clawed toad, *Xenopus laevis*. J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. **18**: 469–480.

Explanation of Plates XXIII-XXVI

Abbreviations:

AG, apical granule	MB, membrane-bounded body
CEC, common epidermal cell	Mf, microfilament
g, glycogen particle	Mt, mitochondrion
G, Golgi complex	MV, mucous vesicle
GL, Golgi lamella	N, nucleus
HGC, hatching gland cell	P, pigment granule
L, lipid droplet	Ph, phagosome
Ly, lysosome	

Fig. 1. Apical part of HGC from the stage 34 embryo, stained with PA-CrA-Silver method after treatment with saliva, showing the apical granules (AG). For glycogen, compare with Fig. 4. Common epidermal cell (CEC) with mucous vesicles (MV) to the right. ×12,000.

Figs. 2 and 3. Golgi complexes in the supranuclear region of HGC from the stage 34 embryo, showing the carbohydrate reaction in intralamellar matrixes and terminal blebs. Fig. 2. ×19,000; Fig. 3. ×24,000.

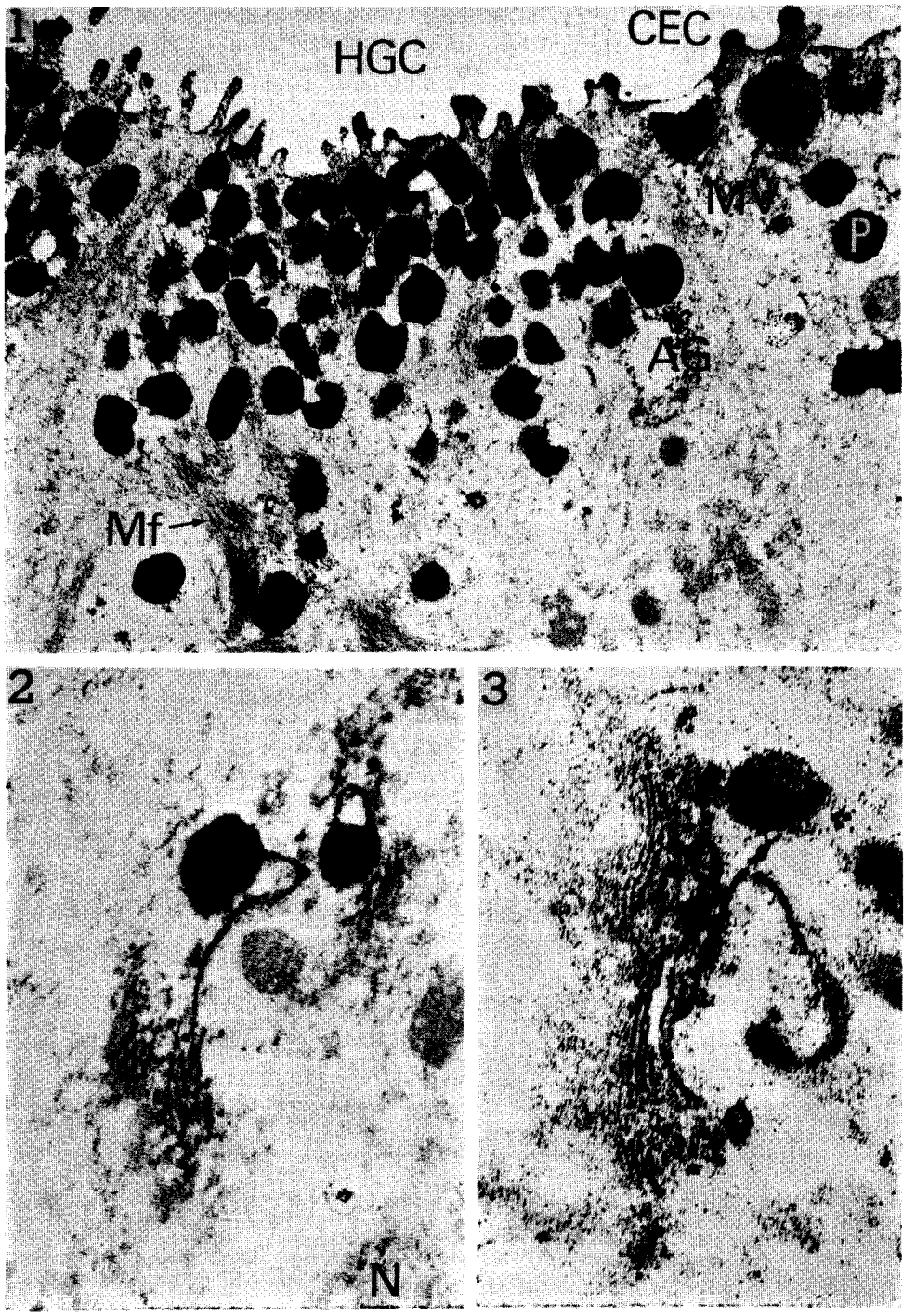
Fig. 4. Apical part of HGC from the stage 34 embryo, showing variable amounts of glycogen particles (g) present in the ground cytoplasm. Mitochondria (Mt) remain unstained. PA-CrA-Silver staining. ×6,800.

Fig. 5. Supranuclear region of HGC from the stage 36 embryo, showing acid phosphatase activity localized in lysosomes (Ly) and Golgi lamellae (GL). ×21,000.

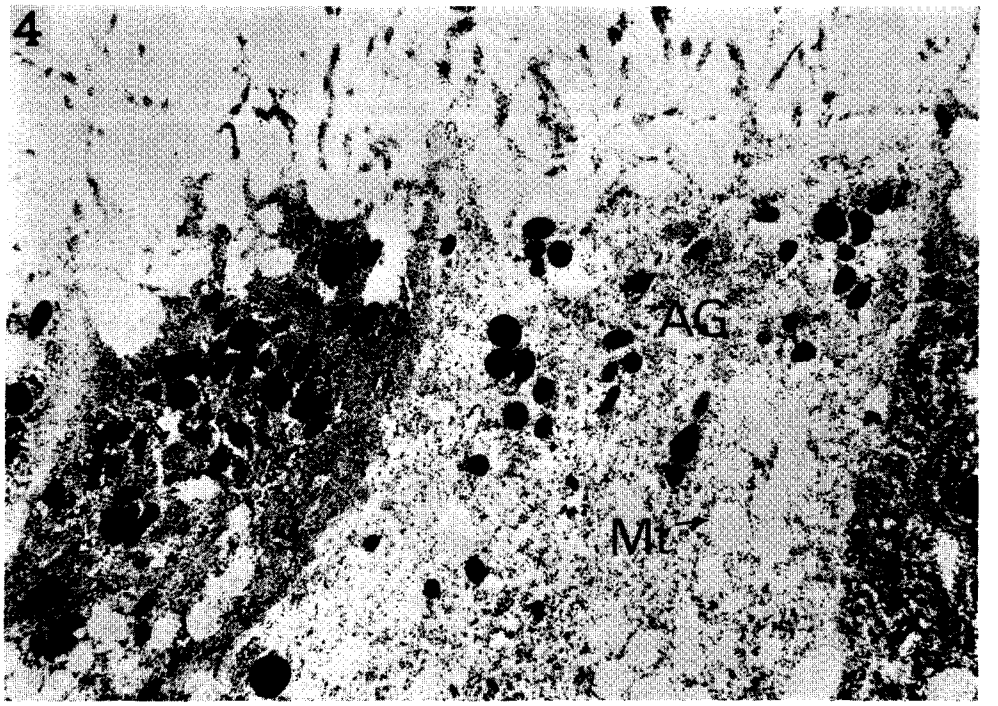
Fig. 6. Basal cytoplasm of HGC in the stage 39 embryo, showing acid phosphatase activity in lysosome (Ly). Note a large phagosome (Ph) without enzyme activity. ×17,500.

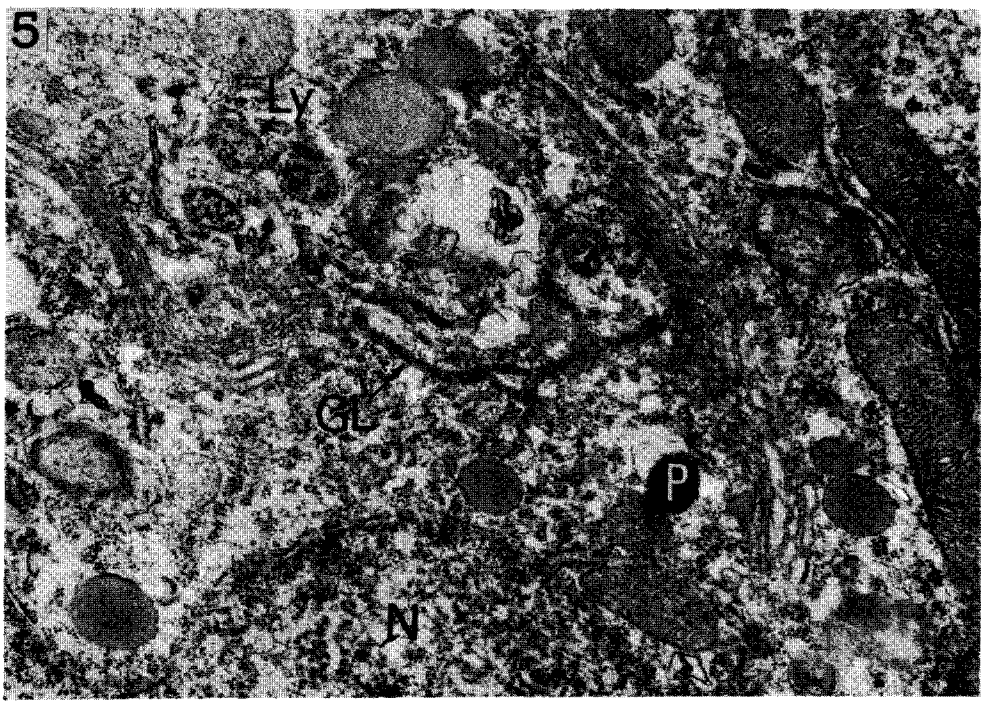
Fig. 7. Basal cytoplasm of HGC at stage 42, showing acid phosphatase activity in several membrane-bounded bodies (MB). ×16,000.

Fig. 8. HGC from the stage 46 embryo, showing the acid phosphatase activity dispersed in an extremely large, membrane-bounded body (MB). Common epidermal cell (CEC) to the left. ×3,800.

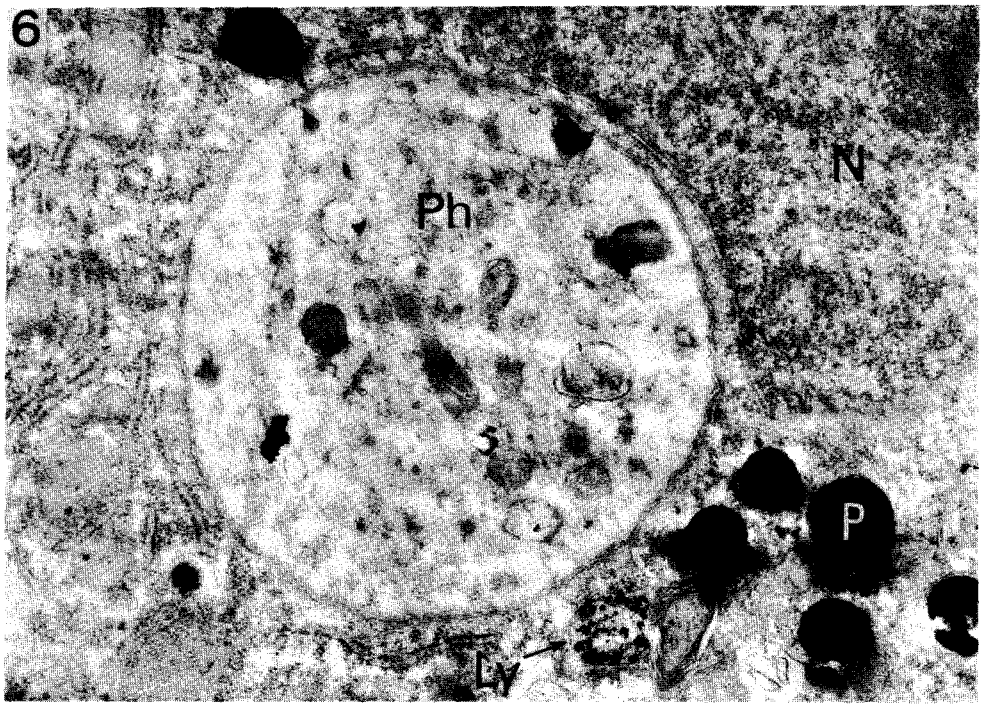


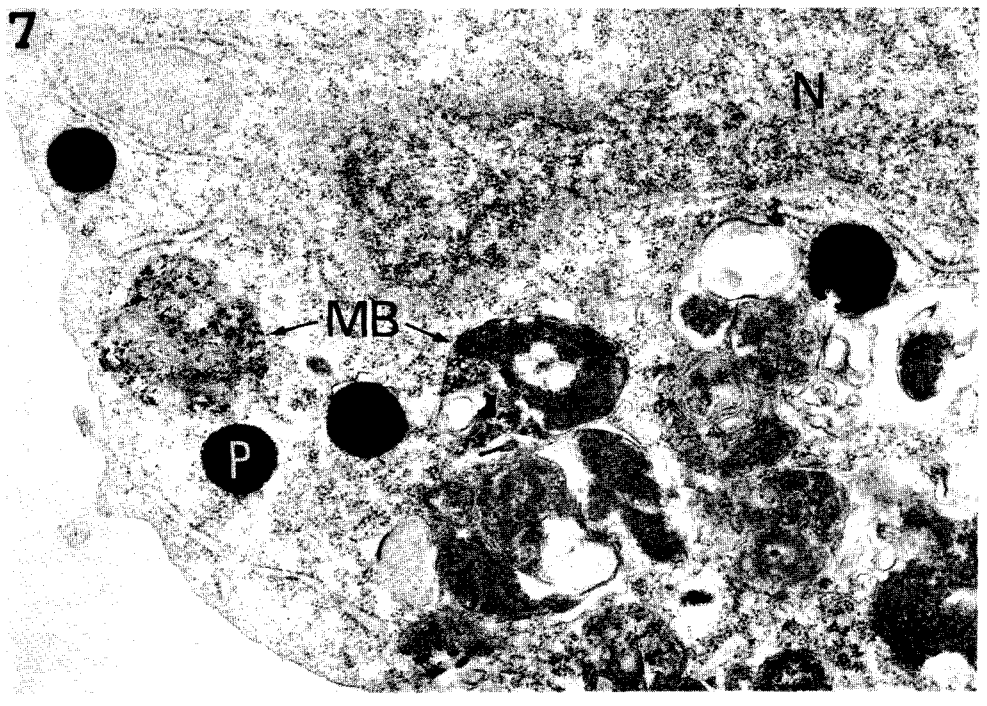
N. Yoshizaki: Ultrastructural Cytochemistry of HGC in Xenopus



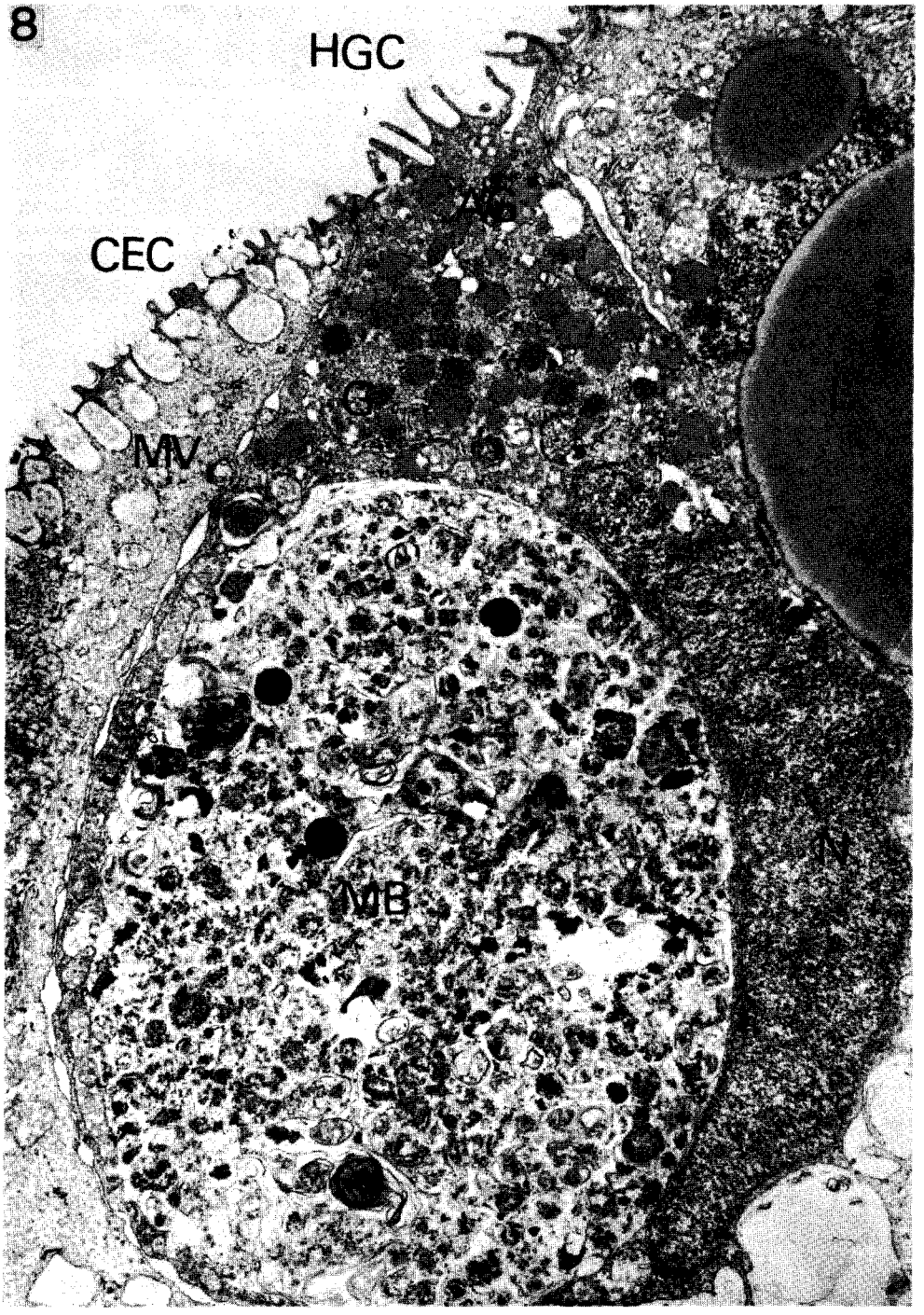


N. Yoshizaki: Ultrastructural Cytochemistry of HGC in Xenopus





N. Yoshizaki: Ultrastructural Cytochemistry of HGC in Xenopus



N. Yoshizaki: Ultrastructural Cytochemistry of HGC in Xenopus