Electron Microscopic Studies on the Anterior Pituitary in Larvae of *Xenopus laevis*[1)]

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(*With 2 Plates and 1 Table*)

Identification of the cell types in the anterior lobe of the pituitary has been a subject of continual enquiry in light microscopy. The application of electron microscopy to this problem has recently opened a new field, in which each cell type has been classified on the basis of the size, shape and electron density of the secretory granules.

The ultrastructure of the amphibian pituitary has been studied by many investigators (Cardell, 1964a; Doerr-Schott, 1965a, b; Iturriza, 1964). However, these enquiries concentrate on the adult gland and little research has been done on the larval one.

Since South African clawed toads (*Xenopus laevis*) can easily be raised in laboratories and their pituitary is satisfactory for cytological works, there have been many reports on the larval gland of this species at the light microscopic level (Cordier, 1948, 1953; Saxén *et al.*, 1957; Saxén, 1958; Kerr, 1966). These studies indicate that marked changes occur in the larval pituitary during metamorphosis. Therefore, it would be interesting to follow the ultrastructure of the larval gland. The present study was carried out with *Xenopus* larvae in the prometamorphic stage in an attempt to differentiate the cell types in the anterior pituitary.

Materials and Methods: The larvae of *Xenopus laevis* Daudin used in this investigation were obtained by ovulation and fertilization induced by injection of chorionic gonadotropin. They were kept in aquaria (19–24°C) and fed on canned mixtures of strained vegetables and liver. Developmental stages were determined according to Nieuwkoop and Faber (1956).

For electron microscopy, ten larvae at stages 53–55 (prometamorphosis) were sacrificed by decapitation and their pituitaries were fixed in 1% OsO_4 containing sucrose (0.06 g/ml) and buffered to pH 7.2–7.4 with veronal acetate for 2 hr at 0–4°C. The tissues were dehydrated through a graded series of cold ethanol and embedded in a mixture of n-butyl methacrylate and styrene (1: 1) to which 1–1.5% benzoyl peroxide was added.

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Thin sections were obtained with a Porter-Blum microtome. They were stained with uranyl acetate followed by lead hydroxide (Millonig, 1961) and examined in a Hitachi HS-7 electron microscope. At the same time, thick sections were cut from the same block; these were placed in chloroform for varying times and stained with Heidenhain's Azan, Mallory's trichrome stain or Gabe's aldehyde fuchsin. But most dyes, except acid fuchsin of Mallory, failed to stain the sections.

All the secretory granules with clear contours found in a given cell type were measured under a dissecting microscope by reading the scales placed directly on the electron micrographs magnified 10,000 times. Then their average diameters and standard errors were calculated.

Paraffin sections were stained with Heidenhain's Azan, PAS or Gabe's aldehyde fuchsin in order to study the distribution of cells.

Results

Based on the distribution of cells and the size of their secretory granules, the following four types of glandular cells could be recognized in the pars distalis of the *Xenopus* larvae in the prometamorphic stage.

I. *Alpha-cells.* This type of cell was characterized by secretory granules varying from 120 to 300 mμ in diameter (average $\pm$ standard error, 206.8$\pm$3.1 mμ). In general, they had elongated contours (Fig. 1). The endoplasmic reticulum (ER) was rather vesicular in shape in a cell containing well developed Golgi complex (Fig. 2), or tubular in shape in the one containing less prominent Golgi complex (Fig. 3). The cells with tubular ER were far more abundant in number. The lamellae of the ER were usually arranged parallel to the nuclear envelope (Fig. 3). The Golgi complex was composed mainly of flattened cisternae. In this zone, granules with "loose-fitting" membranes were frequently encountered (Fig. 2).

II. *Delta-cells.* The cells contained secretory granules whose diameters varied between 80 and 180 mμ (115.3$\pm$2.1 mμ). These cells were concentrated in the central region of the gland. Their contours, in contrast to the alpha-cells, were rather round (Fig. 1). Both the endoplasmic reticulum and the Golgi complex contained in this type appeared to be in more dilated forms than in the alpha-cells.

III. *Gamma-cells.* The identification of these cells could be readily made owing to their specific distribution in the gland. Most of them were restricted to the region adjacent to the median eminence (Fig. 4). Their secretory granules were relatively small, ranging from 70 to 150 mμ in diameter (96.3$\pm$2.7 mμ). The granules larger than 120 mμ in diameter were very few. The endoplasmic reticulum was usually vesicular in form.

IV. *Chromophobes.* Almost no secretory granules were found in these cells. The cells were located throughout the gland but tended to aggregate in the antero-rostral region where they were intermingled with the gamma-cells. Their contours varied from a rounded to an irregular shape. In the irregular-shaped

ones, the cytoplasmic processes were observed projecting between neighboring chromophils (Fig. 1).

The condition of organella also varied considerably from cell to cell. In some cells, only the Golgi complex and mitochondria were observed in their relatively pale cytoplasm. In other cells, in addition to the afore-mentioned organella, either the tubular or the vesicular endoplasmic reticulum was seen (Figs. 5 and 6). In general, the mitochondria in these cells were larger and had fewer cristae than those in chromophilic cells (Figs. 4 and 5).

Discussion

Comparison of the secretory granules in respective cell types of the larval pituitary with those of adult glands (Table 1) indicates that in the former the granules are considerably smaller in size than those in the latter. It may be concluded, therefore, that the secretory granules in the anterior pituitary become in general larger as the animals grow.

Table 1. The size of secretory granules of various cell types in the larval and adult pituitary of *Xenopus laevis*

	Alpha-cells	Beta-cells	Gamma-cells	Delta-cells	Reference
Larva (prometa-morphic)	120-300 mμ (206.8±3.1mμ)*	absent	70-150mμ (96.3±2.7mμ)	80-180mμ (115.3±2.1mμ)	The present paper
Adult	300-450mμ	100-900mμ	100-220mμ	120-250mμ	Doerr-Schott (1965b)

* Mean±standard error.

Two different forms of endoplasmic reticulum were found in the alpha-cells, namely, the tubular and vesicular ones. However, since the ER has been shown to vary its shape according to the physiological condition (salamanders, Cardell, 1964b; rats, Herlant, 1964; Rinehart and Farquhar, 1964), the shape of the ER can hardly be employed as a criterion for identifying cell types in the anterior pituitary.

In light microscopy, the relatively small granules in the gamma-cells have been known to show poor affinity to basic dyes such as aniline blue so that some previous workers passed over these cells in the larval pituitary. In fact, they have only recently been described by Kerr (1966). They are believed to be responsible for the production of interstitial cell stimulating hormone (ICSH) in *Xenopus* (Kerr, 1965) as well as in *Rana* (van Oordt, 1961). The present electron microscopic study clearly revealed the presence of the gamma-cells in the larval pituitary in the prometamorphic stage.

It is still premature to say anything definitely about the significance of the chromophobes. In the present study, they were found to show variations in

appearance and cytoplasmic inclusions, suggesting that they are variable in these points according to physiological conditions. Further studies are in progress to clarify the significance of the chromophobes.

Summary

Based on the size of the secretory granules, the following four types of cells were identified in the anterior lobe of the pituitary gland in the larval *Xenopus* in the prometamorphic stage.

The alpha-cells contained secretory granules with an average diameter of 206.8 mμ. The delta-cells had secretory granules with a mean diameter of 115.3 mμ and the gamma-cells were characterized by relatively small granules with a mean diameter of 96.3 mμ. Chromophobes were found to contain almost no secretory granules.

These results were discussed in relation to the data obtained previously by other investigators.

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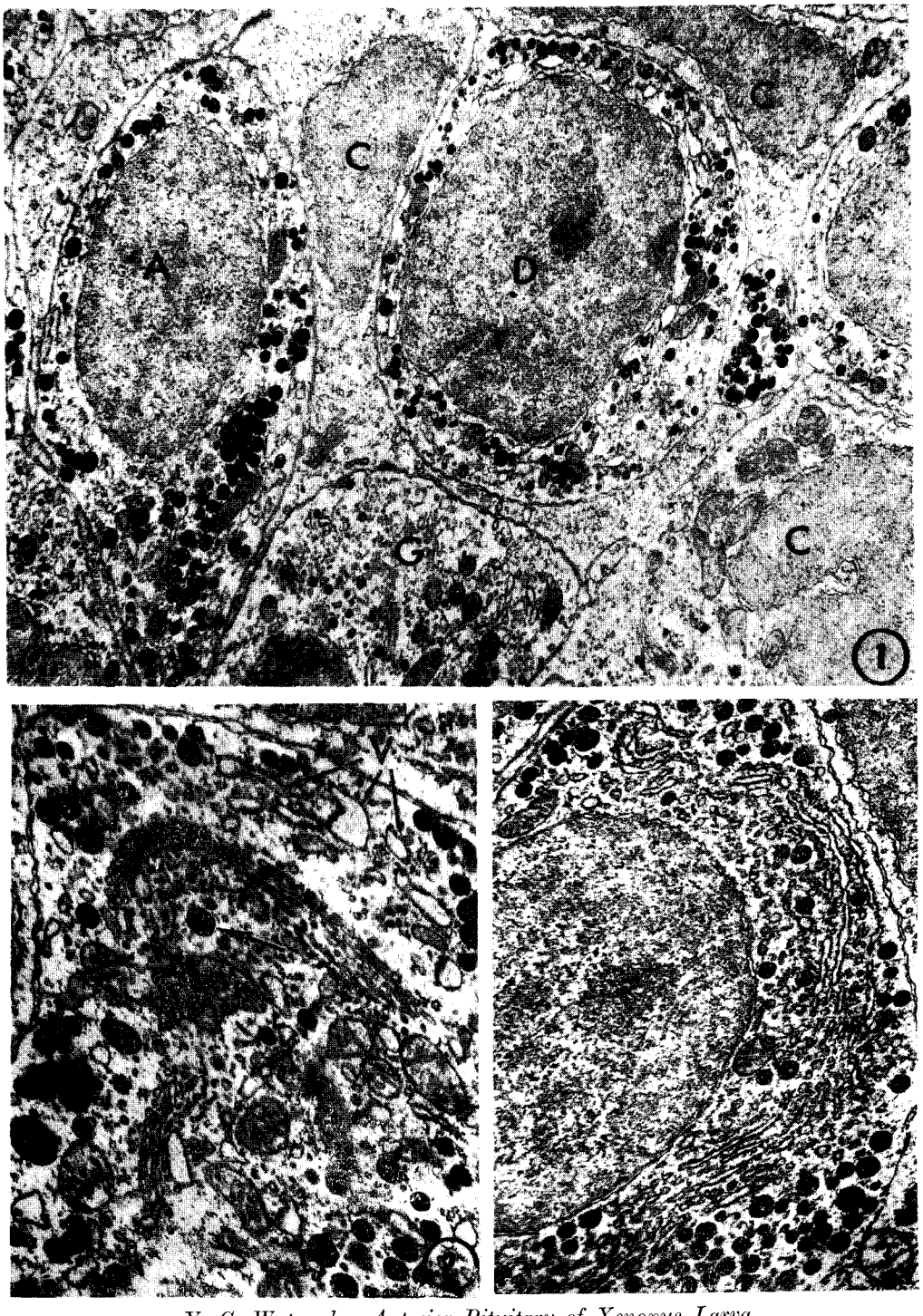

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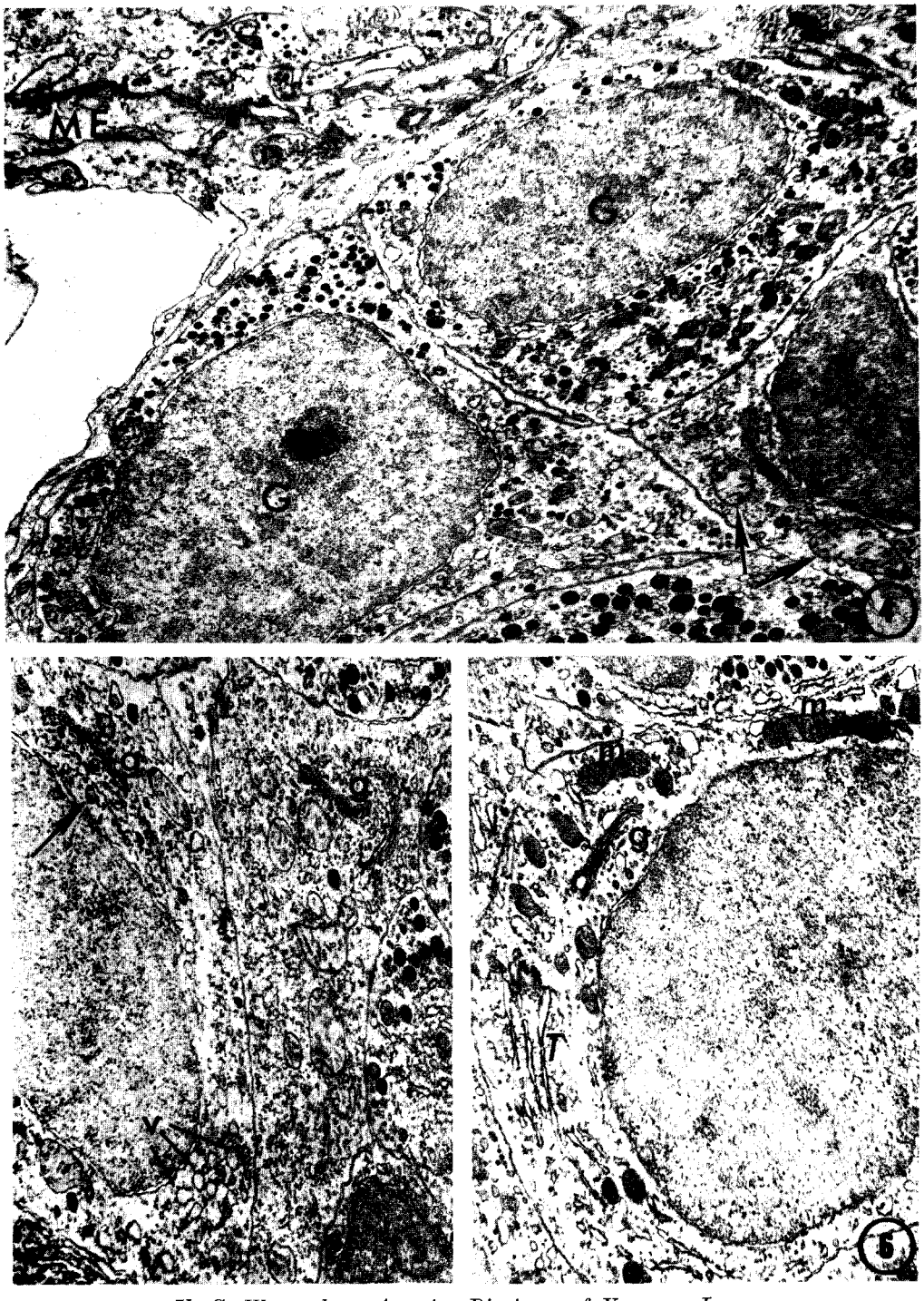

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Explanation of Plates I-II

Plate I

Fig. 1. Electron micrograph showing the alpha- (A), delta-(D) gamma-cell (G) and irregular-shaped chromophobes (C). Note the different-sized secretory granules in the various cell types. × 12,000.

Fig. 2. Golgi zone of alpha-cell. A granule with "loose-fitting" membrane (arrow) is seen. Numerous mitochondria are concentrated in and around this area. In this cell ER is vesicular in shape (V). × 21,000.

Fig. 3. Cytoplasm of alpha-cell with the characteristic large secretory granules and well-developed parallel arrays of the rough-ER. × 13,000.

Plate II

Fig. 4. Gamma-cells (G) located in the antero-rostral region just posterior to the median eminence (ME). Large mitochondria (arrows) of chromophobe (C) are also observed. × 15,000.

Fig. 5. Chromophobes containing the vesicular ER (v), large mitochondria and Golgi complex (g). In upper left, a small granule with relatively large halo is observed (arrow). × 12,000.

Fig. 6. Part of a relatively large chromophobe showing the tubular ER (T), Golgi complex (g) and mitochondria (m) with rather dense matrix. × 12,000.