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Cell Types in the Adenohypophysis of the Japanese Quail and Effects of Injection of Luteinizing Hormone-Releasing Hormone

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Summary. The cells of the adenohypophysis of the Japanese quail were studied by both light and electron microscopy after exposure to long photoperiods or injection of luteinizing hormone-releasing hormone (LRH). Six cell types were identified in the adenohypophysis by examining alternate thick and thin sections by light and electron microscopy.

In the cephalic lobe, there are four types of glandular cells. They are the prolactin cells, ACTH cells, TSH cells, and gonadotropic cells (FSH ?). In the caudal lobe, there are two types of cells, STH cells and gonadotropic cells (LH ?).

After exposure to long daily photoperiods, gonadotropic cells in both lobes were strongly activated. They became larger and accumulated many granules. ACTH cells became vacuolated; granules were sparse.

Synthetic LRH injection (10 μg/0.2 ml/day) for 10 days to the non-photostimulated quail stimulated certain numbers of the gonadotropic cells in the both lobes, although the response of the cells was less than that induced by photostimulation. No response was seen in the other cell types.

Key words: Adenohypophysis — Japanese quail — Cell types — LRH injection — Light and electron microscopy.

Introduction

Many investigations both in birds and mammals, have been centered on the hypothalamic median eminence, the adenohypophysis and the functional relation between them (see for review, Kobayashi and Wada, 1973). In avian species the adenohypophysis has been studied by several investigators (see for review, Tixier-Vidal and Follett, 1973), but there are still some discrepancies about the number and function of the adenohypophysial cell types.

Most of the investigations have employed tinctorial and cytochemical methods; differentiation of the cell types was based on their tinctorial properties (Tixier-Vidal, 1963; Gourdji, 1965; Matsuo *et al.*, 1968; Tixier-Vidal *et al.*, 1968). Recently, electron microscopy has been employed on the adenohypophysis of several avian species: the domestic fowl (Mikami, 1958; Payne, 1965), domestic mallard (Tixier-Vidal, 1965), the pigeon (Tixier-Vidal and Assenmacher, 1966), the White-crowned Sparrow (Mikami *et al.*, 1969, 1973), and the Japanese quail

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(Tixier-Vidal *et al.*, 1972). These observations made it possible to identify cell types on the basis of the number and size of secretory granules and profiles of cell organelles.

In those investigations, however, the identifications of the cell types by electron and light microscopy have not always been adequately correlated. In this paper, I have attempted to identify each cell type both by electron microscopy and light microscopy.

Material and Methods

Male Japanese quail (*Coturnix coturnix japonica*) were obtained from a commercial source at the age of 3 weeks and kept under a short daily photoperiod of 8L 16D (lights on from 0800 to 1600) for 2 or 3 weeks before the start of the experiment. Then they were divided into three groups, each of six birds. Group 1 was transferred to a long daily photoperiod of 16L 8D (lights on from 0800 to 2400). Groups 2 and 3 remained under the short daily photoperiod. The birds of Group 3 received daily (ca. 1400) single subcutaneous injection of synthetic luteinizing hormone-releasing hormone (LRH) with LH releasing activity (Daiich Seiyaku Co. Ltd., Lot. 5-T) at a dose of 10 μg in 0.2 ml saline containing 1.5% gelatine. Group 1 and 2 were injected with vehicle only. All experimental birds were kept at 25 ± 3 °C and given commercial quail food and water *ad libitum*.

The birds received ten injections. On the day after the last injection, all were killed by decapitation. The adenohypophyses were removed immediately after decapitation and weighed on torsion balance. They were fixed in ice-cold solution of 1% osmium tetroxide in phosphate buffer (pH 7.6) for 2 hours. The tissues were then washed with phosphate buffer, dehydrated through an ethanol series and propylene oxide, and embedded in Epon 812. Thin sections were cut with a glass knife on a JUM-5B ultramicrotome (Japan Electron Optics Laboratory, Ltd.), stained with uranyl acetate followed by lead citrate, and examined with a JEM-T8 electron microscope (Japane Electron Optics Laboratory, Ltd.).

For light microscopy, thick sections (about 1 μm) adjacent to the thin sections were cut and used for tinctorial and cytochemical methods. For the tinctorial and cytochemical methods, epoxy resin was removed from the thick sections according to the method of Mikami and Tanimura (1968), and the sections were stained with periodic acid-Schiff (PAS), alcian blue, and orange G (PAS-AB-OG) (Herlant, 1960) without permanganate oxidation or with Herlant's tetrachrome (Herlant, 1960). Testes, adrenals, and thyroids were removed after decapitation and weighed.

Results

Adenohypophysial Cells of Photostimulated and Nonphotostimulated Birds

Although the staining of some cell types was weak in the thick sections made from Epon embedded blocks, six cell types were differentiated by their tinctorial and cytochemical properties. Electron microscopic examination of the thin sections next to the thick sections also revealed six cell types. For avoidance of further confusion in nomenclature, presumptive functional designations of each cell type are employed based on their properties and fine structure (see Discussion).

Cephalic Gonadotropic (GTH) Cells. In the cephalic lobe, there are PAS-positive basophilic cells that also stained with alcian blue with PAS-AB-OG stain; they appear red violet. With Herlant's tetrachrome method, their cytoplasm is blue with fine granules. Electron microscopically these cells contain many spherical, uniformly electron-dense granules with diameter of 150–300 nm (sometimes up to 500 nm) (Fig. 1). The rough endoplasmic reticulum is scattered through the cytoplasm, and dilated in active cells which show deep violet in the thick sections.

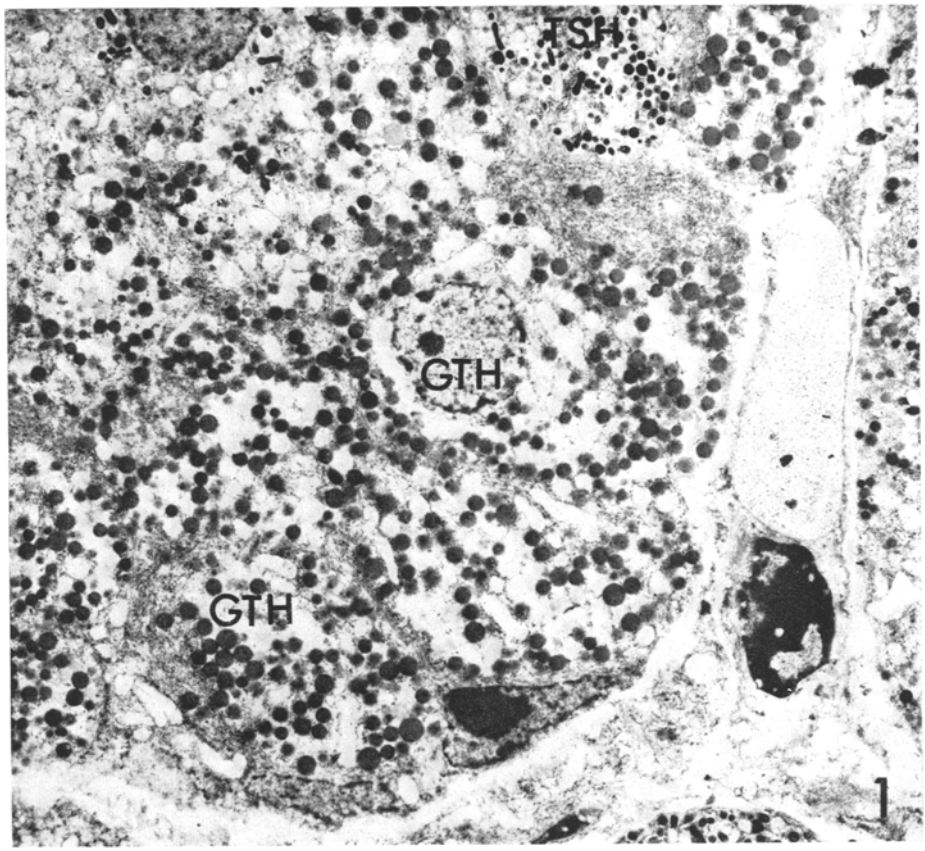

Fig. 1. Electron micrograph from a section of the cephalic lobe of the pars distalis of a photostimulated Japanese quail. *GTH*, cephalic gonadotropic cells; *TSH*, thyrotropic cells; ×6500

In the adenohypophysis of short-day birds, these cells are round and rather chromophobic, but some show PAS-positive reaction. Under the electron microscope, these cells have a smooth surface and a smaller number of granules (Fig. 4).

Thyrotropic (TSH) Cells. In the cephalic lobe, there are other basophilic cells which become pale blue in color after application of PAS-AB-OG staining technique and are blue with Herlant's tetrachrome method. Electron microscopic observations show that these cells contain polymorphic, electron-dense granules of 150–250 nm in diameter (Fig. 2). The rough endoplasmic reticulum is more or less dilated. These cells in the adenohypophyses of non-photostimulated birds have no affinity to any stain, but general cytoplasmic profiles under electron microscope are similar to those of the photostimulated cells of this type.

Caudal Gonadotropic (GTH) Cells. In the caudal lobe, there is a single type of basophilic cell which can be stained with alcian blue and gives very weak PAS-positive reaction; therefore, these cells appear pale blue in color with PAS-AB-OG stain. They show fine blue granules in the cytoplasm with Herlant's tetrachrome

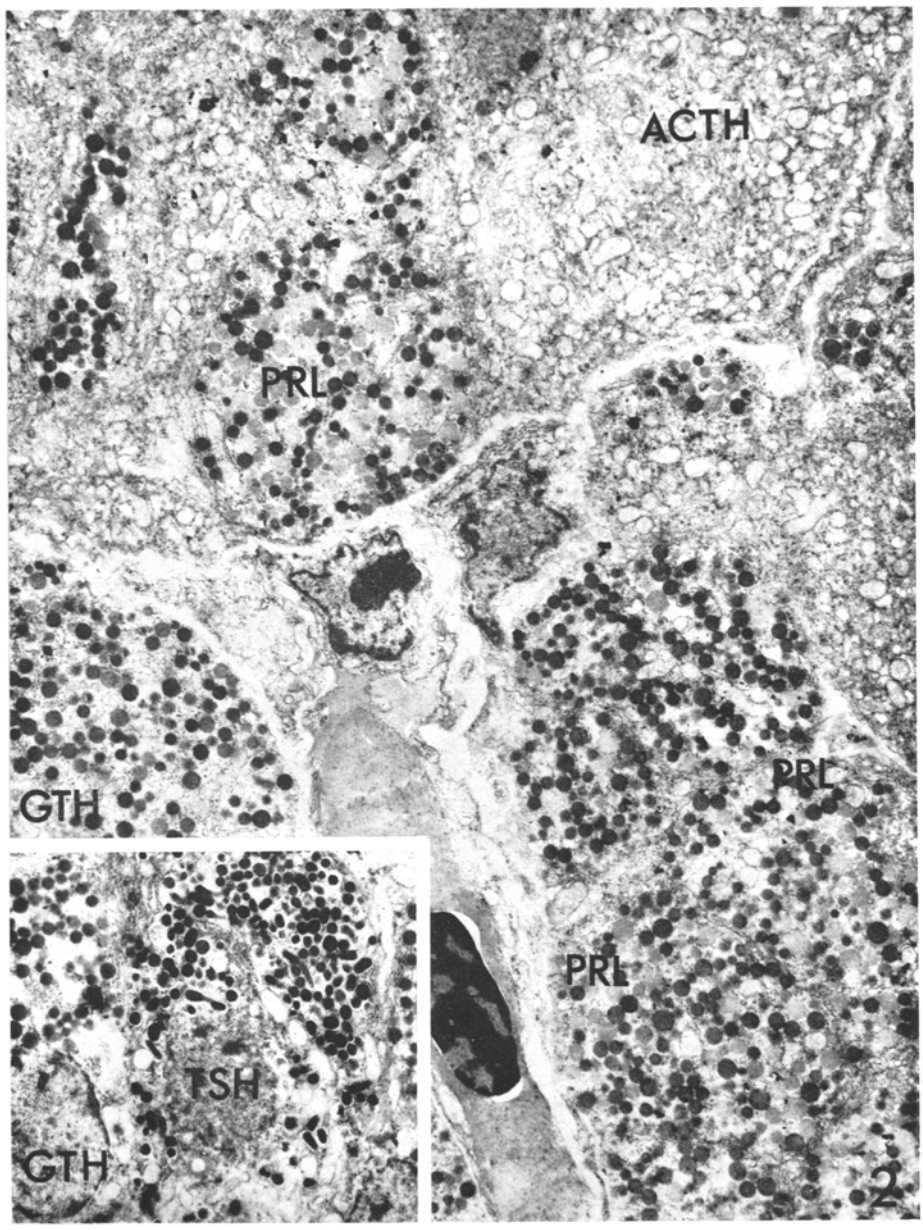

Fig. 2. Electron micrograph of the cephalic lobe of the pars distalis from a photostimulated Japanese quail. Inset shows a TSH cell. *GTH*, cephalic gonadotropic cells; *TSH*, thyrotropic cells; *PRL*, prolactin cells; *ACTH*, corticotropic cells. ×6500

method. The cells are elongated in shape and the nuclei are eccentric, especially when stimulated (Fig. 3). The granules observed under the electron microscope are variable in electron density and have diameters ranging from 200–300 nm

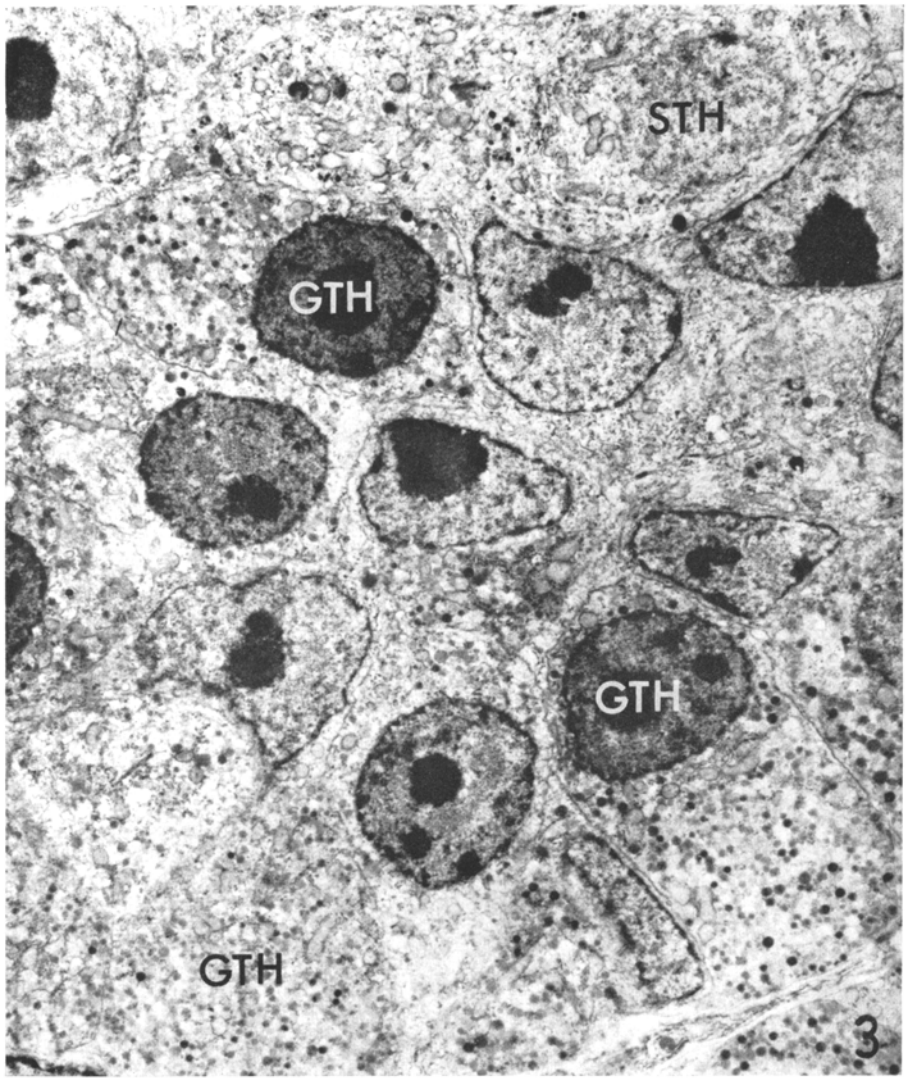

Fig. 3. Electron micrograph from a section of the caudal lobe of the pars distalis of a photostimulated Japanese quail. *GTH*, caudal gonadotropic cells; *STH*, somatotropic cells. ×6500

(sometimes up to 400 nm). The endoplasmic reticulum is well developed with many attached ribosomes. In the short-day birds, the stainability of these cells is weak, and the cell size is small. Electron microscopically, granules are less electron dense than those of photostimulated birds.

Somatotropic (STH) Cells. In the caudal lobe, there is a cell type that is rather chromophobic with PAS-AB-OG and Herlant's tetrachrome method. The cells are round in shape and large in volume. These cells are evident with electron-dense granules of 100–150 nm in diameter. The granules are distributed in the

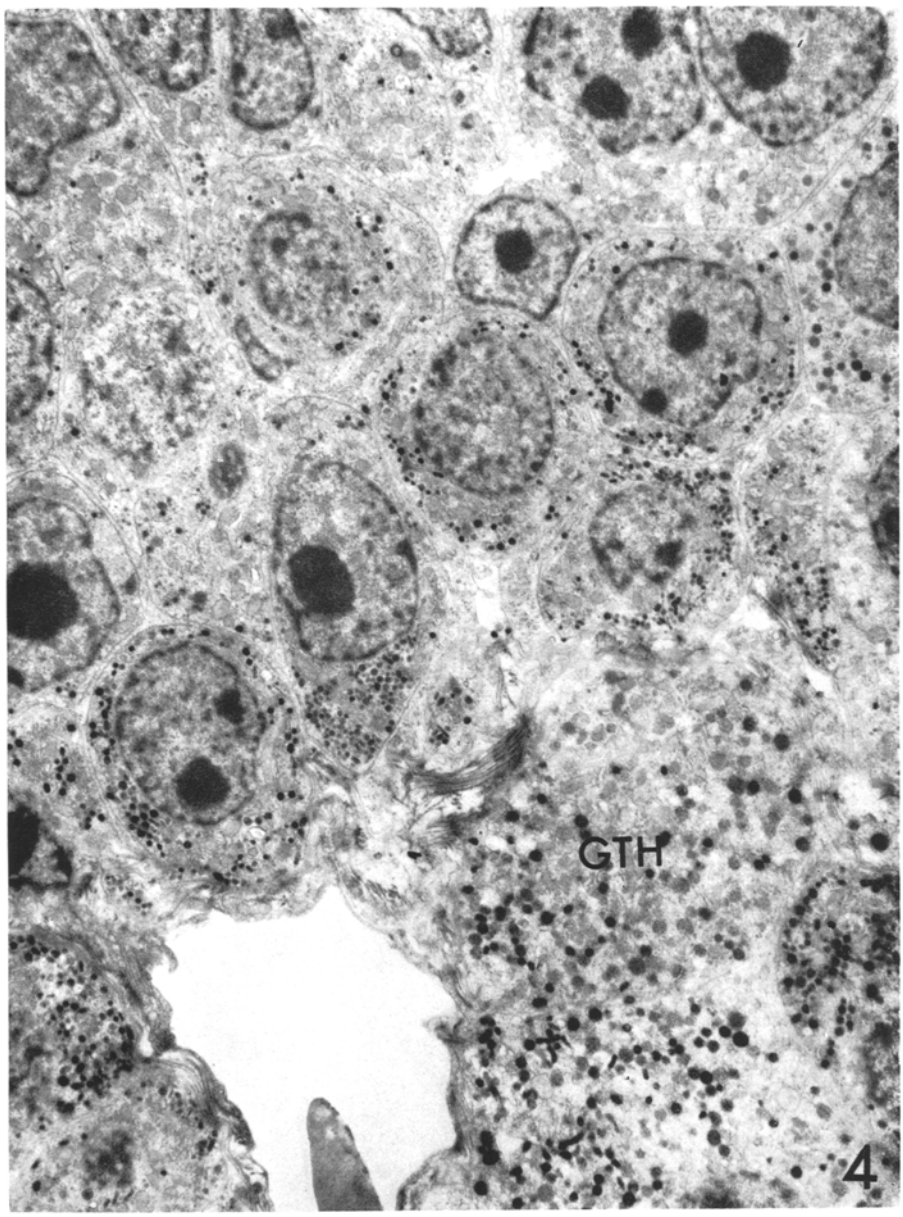

Fig. 4. Electron micrograph of the cephalic lobe of the pars distalis of a non-photostimulated Japanese quail. All the cells except for those in the lower seemed to be inactive. *GTH*, cephalic gonadotropic cells. Other cells are difficult to identify. ×6500

peripheral region of the cells (Fig. 3). The endoplasmic reticulum is rather vesicular. Large electron-dense bodies (perhaps lysosomes) are also seen. However, in non-photostimulated birds these cells contain more and larger granules (up to 200 nm), which are evenly distributed through the cytoplasm in some cases.

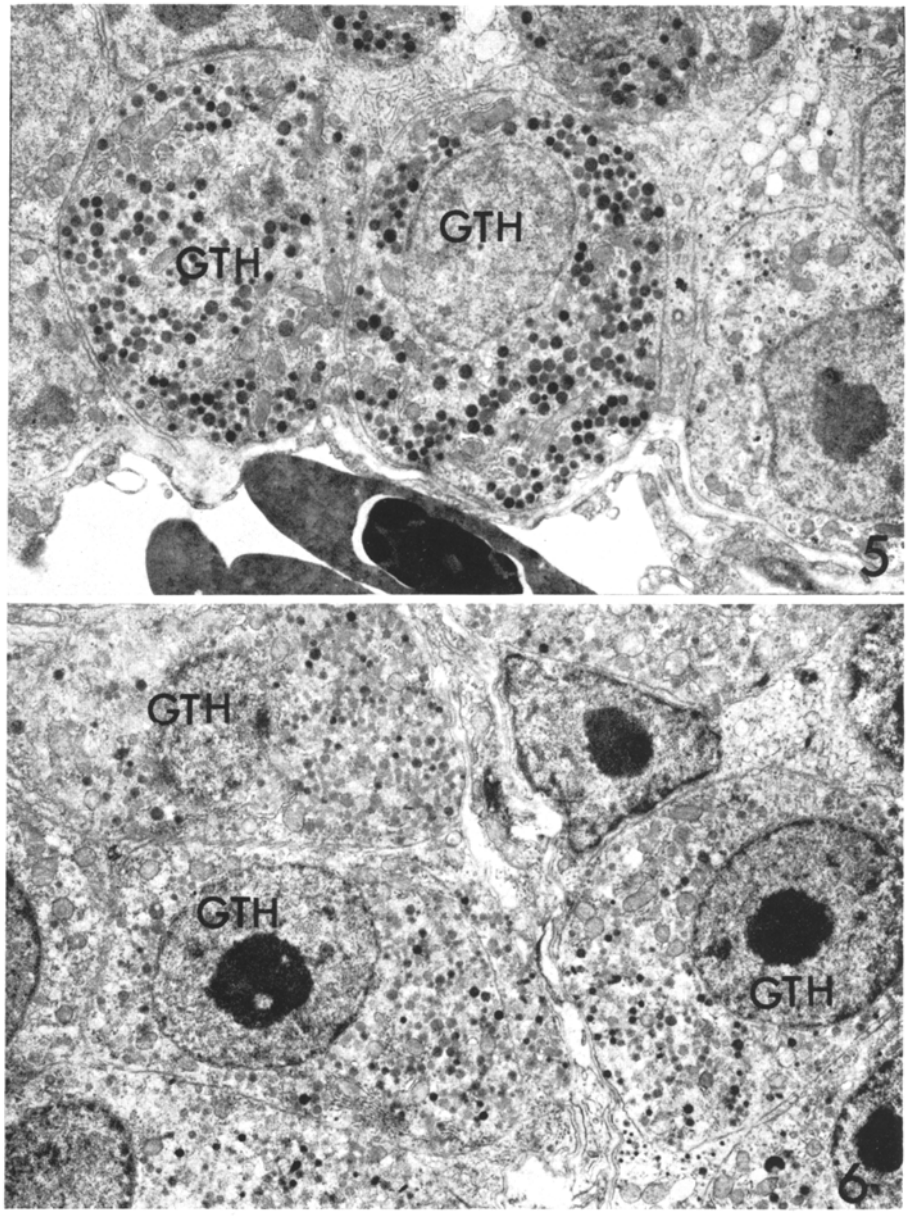

Fig. 5. Electron micrograph of the cephalic lobe of the pars distalis of a non-photostimulated Japanese quail treated with synthetic LRH for 10 days. Cephalic gonadotropic cells (*GTH*) are activated. ×6500

Fig. 6. Electron micrograph of the caudal lobe of the pars distalis of a non-photostimulated bird treated with synthetic LRH for 10 days. Caudal gonadotropic cells (*GTH*) are activated. ×6500

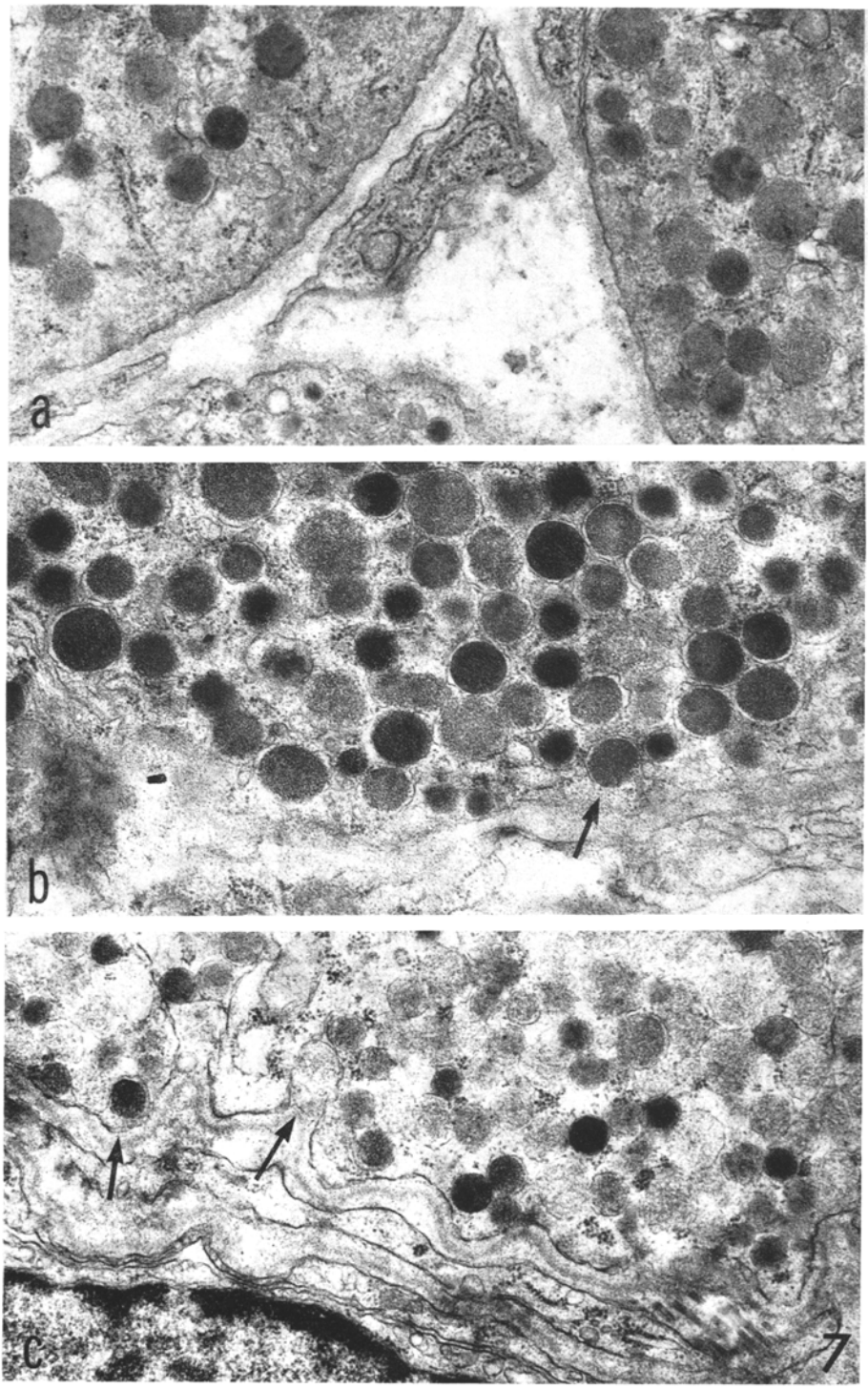
a
b
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Table 1. Effect of synthetic LRH on weights of endocrine organs in the Japanese quail

Group	Number of birds	Body weight (g)	Organ weight (mg/100 g BW)			
			Adeno-hypo-physis	testes	Adrenal glands	Thyroid glands
I. Long-day (16L8D)	6	103 ± 0.7[a]	1.38 ± 0.09	532.4 ± 25.4	8.73 ± 0.65	5.94 ± 1.04
II. Short-day (8L16D)	6	103 ± 1.4	0.77 ± 0.03[b]	17.0 ± 2.5[b]	7.93 ± 0.65	9.59 ± 1.16[c]
III. Short-day injected with LRH (8L16D)	6	102 ± 2.6	0.94 ± 0.06[b, d]	22.3 ± 5.4[b]	8.04 ± 0.33	8.24 ± 0.64

[a] Mean $\pm$ standard error.
[b] Highly significant ($p < 0.01$) compared with group I.
[c] Significant ($p < 0.05$) compared with group I.
[d] Significant ($p < 0.05$) compared with group II.

Prolactin (PRL) Cells. In the cephalic lobe there are acidophilic cells that are faintly pink with Herlant's tetrachrome method. These cells contain relatively large granules (diameter ranging from 300 up to 500 nm) (Fig. 2). The endoplasmic reticulum is well developed.

Corticotropic (ACTH) Cells. In the cephalic lobe, there are cells that are chromophobic to both PAS-AB-OG and Herlant's tetrachrome. They contain very few electron-dense granules of about 100–150 nm in diameter (Fig. 2). The endoplasmic reticulum appears to form large vesicles. However, the cells of this type in non-photostimulated birds contain more granules than those of the photostimulated birds.

Adenohypophysial Cells of the Nonphotostimulated Birds after LRH Injection

Although both cephalic and caudal gonadotropic cells of the saline-injected birds under short days did not tend to take stain, these cells become chromophilic after 10 daily injections of LRH at a dose of 10 μg. Electron microscopy revealed that both types became larger and more granular than those of the saline-injected birds (Figs. 4—6). Also the surfaces of these cells became undulated. Granules appeared close to the cell surface and extrusion of granules was often seen (Fig. 7). The changes induced by LRH were similar to those aroused by photostimulation, although the stainability and granulation of the

Fig. 7a—c. Higher magnification of gonadotropic cells. (a) Cephalic gonadotropic cells of a non-photostimulated Japanese quail treated with vehicle only. Note that the cell surface is smooth. (b) Cephalic gonadotropic cell of a non-photostimulated bird treated with synthetic LRH for 10 days. The cell surface is undulated and a granule is expelled (arrow). (c) Caudal gonadotropic cell of a non-photostimulated bird injected with synthetic LRH for 10 days. Arrows indicate the extrusion of granules. ×31500

cells were not as strong compared with those of photostimulated birds. After LRH injection the weight of the adenohypophysis increased compared to that of non-photostimulated control birds (Table 1). The testicular weight of LRH-injected birds was no different than that of non-photostimulated ones. No significant changes occurred in the other cell types after LRH injection.

Discussion

Judging from tinctorial properties, it seems that cephalic gonadotropic cells, which are PAS-positive and AB-positive, and blue in color with Herlant s tetrachrome method, correspond to the beta cell of Tixier-Vidal *et al.* (1968). The profiles of these cells resemble in general the beta cells of the Japanese quail (Tixier-Vidal *et al.*, 1972) and of the Pekin duck (Tixier-Vidal and Assenmacher, 1966) and also the Type A gonadotropic cell (Mikami *et al.*, 1969) and GTH (FSH ?) cell of Mikami *et al.* (1973) in the White-crowned Sparrow. These cells appear to be gonadotropic cells since they respond to LRH injection. They are likely the FSH-producing cells, since they are distributed in the cephalic lobe, in which Brasch and Betz (1971) found most FSH activity in the chick.

TSH cells in this study are similar to the delta cell of Tixier-Vidal *et al.* (1968) in the Japanese quail, although they are somewhat different from the delta cell of Tixier-Vidal *et al.* (1972) in terms of the profile of granules. This may be due to difference of physiological condition. They are basophilic and exclusively located in the cephalic lobe. They did not respond to LRH. Considering the observation that TSH activity is found only in the cephalic lobe in the chicken (Brasch and Betz, 1971), these could be TSH cells.

Caudal gonadotropic cells may not be the same as the delta cells that have been described in the caudal lobe and thought to be TSH cells by Tixier-Vidal *et al.* (1968) who described AB-positive cells in both cephalic and caudal lobes as delta cells. However, electron microscopy in the present study shows that AB-positive cells in the caudal lobe are different, especially in size and shape of granules, from those in the cephalic lobe. Therefore they are probably not TSH cells, but rather could be LH cells, because they respond to LRH injection. This suggestion is also supported by the fact that LH activity in the chick is mostly present in the caudal lobe (Brasch and Betz, 1971). Moreover LH-producing cells have now been localized in the caudal lobe immunohistochemically using anti-avian LH serum (Wada and Asai, unpublished).

Presumptive STH cells are the same as the alpha cells (STH cells) of Tixier-Vidal *et al.* (1968) judging from their location. Under the electron microscope, they are somewhat different from somatotropic cells of Mikami *et al.* (1969, 1973), especially in the size of the granules. This may be ascribed to differences of species, age or physiological condition; the Japanese quails used in this study were about one month old and still growing.

The erythrosinophilic cells of the cephalic lobe are the same as the eta cells of Tixier-Vidal *et al.* (1968). These are most probably prolactin cells.

The presumptive ACTH cells in this study are the same as the epsilon cells (Tixier-Vidal *et al.*, 1968) because of their chromophobic nature and similar location in the cephalic lobe. Under the electron microscope, these cells resemble

ACTH cells of the duck after metopirone treatment (Tixier-Vidal, 1965) and ACTH cells of White-crowned Sparrows after adrenalectomy (Mikami *et al.*, 1969).

The possibility that the cephalic lobe secretes FSH and the caudal lobe secretes LH fits well with the hypothesis that the anterior and posterior median eminence have separate functions in gonadotropic function. It has also been shown that capillaries covering the anterior median eminence drain the cephalic lobe and those covering the posterior median emienence drain the caudal lobe via separate portal vessel systems (Vitums *et al.*, 1964). More recently, two separate regions in the hypothalamus were reported by Stetson (1972a, b) and Wada (1974); the anterior infundibular nucleus complex which controls FSH secretion and the posterior one which controls LH secretion.

Mammalian LRH can induce premature ovulation in the domestic fowl (van Tienhoven and Schally, 1972) and LH release detected by radioimmunoassay (Furr *et al.*, 1973). LH-releasing factor is suggested to be a substance similar to mammalian LRH (Jackson, 1972a, b; Jaffcoate *et al.*, 1974). In this experiment, using male Japanese quail, synthetic LRH induced activation of gonadotropic cells in both lobes, presumably FSH and LH producing cells. In the rat LRH can maintain the activities of FSH and LH cells (Debeljuk *et al.*, 1973) and LRH has also FRH property (see Schally *et al.*, 1972, 1973). However, the testes of the quail receiving LRH did not show any growth. This could be due to the dose of LRH employed.

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