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Immunohistochemical Localization of LH-Producing Cells in the Adenohypophysis of the Japanese Quail, *Coturnix coturnix japonica*

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Summary. The cells that produce luteinizing hormone (LH) in the adenohypophysis of the Japanese quail were identified immunohistochemically using anti-chicken LH serum and horseradish peroxidase-labeled goat anti-rabbit gamma globulin serum. The LH cells are localized in the caudal lobe of the pars distalis. They are elongate in shape and are polarized toward the sinusoids, especially in their active states. Alterations in size of LH cells are directly related to changes in circulating LH levels as induced by castration or photostimulation. The LH cells identified immunohistochemically were only stained by alcian blue with periodic acid-Schiff (PAS), alcian blue and orange G.

PAS-positive gonadotropic cells in the cephalic lobe were stained immunohistochemically only slightly if at all using anti-chicken LH serum and consequently may be FSH producing cells. In the cephalic lobe another type of basophilic cell was stained with alcian blue. These cells were also stained immunohistochemically with anti-chicken LH serum. These cells may possibly be identified as TSH cells due to the characteristics of the anti-chicken LH serum used in this study which cross react with LH and TSH but only slightly with FSH, and also on the basis of previous light and electron microscopic studies.

Key words: Adenohypophysis – Japanese quail – Immunohistochemistry – LH producing cells.

Introduction

Since the adenohypophysis secretes hormones essential for controlling many other endocrine phenomena, cellular sources of these hormones in the gland

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have been of great interest. In avian species, light and electron microscopic studies revealed several cell types in the adenohypophysis. However, there is still debate about the identification of each cell type, especially in the case of the basophilic cells (Tixier-Vidal and Follett, 1973).

Recently, two papers dealing with the ultrastructure of the adenohypophysis of the Japanese quail appeared (Mikami et al., 1975; Wada, 1975). These workers showed that two different gonadotropic cells are present in the cephalic and caudal lobes. However, before final agreement can be reached on the function of each cell, convincing evidence such as immunohistochemical localization must be obtained.

Immunohistochemical methods to identify tissue antigens have been developed using enzyme-labeled antibodies (Nakane and Pierce, 1966; 1967); the horseradish peroxidase labeled anti-gamma globulin method has been especially successful in identifying each cell type of the rat anterior pituitary (Nakane, 1968; 1970). Avian luteinizing hormone (LH) has been purified and used to raise an antiserum (Follett et al., 1972). We have had the opportunity to employ immunohistochemistry to the adenohypophysis of the Japanese quail using this anti-chicken LH serum. The purposes of this study were 1) to ascertain the cellular source of avian LH in the adenohypophysis by means of immunohistochemistry, 2) to seek correlation between immunohistochemical staining characteristics and physiological conditions and 3) to correlate the cell identified immunohistochemically with the cell types observed with histochemical staining. To measure LH concentration in serum, radioimmunoassay was performed according to Follett et al. (1972).

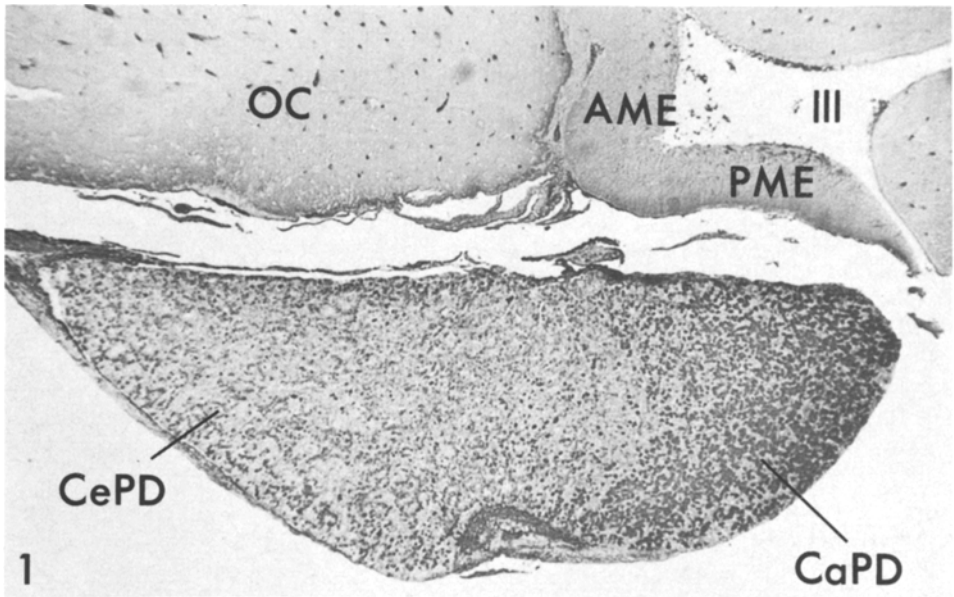
Material and Methods

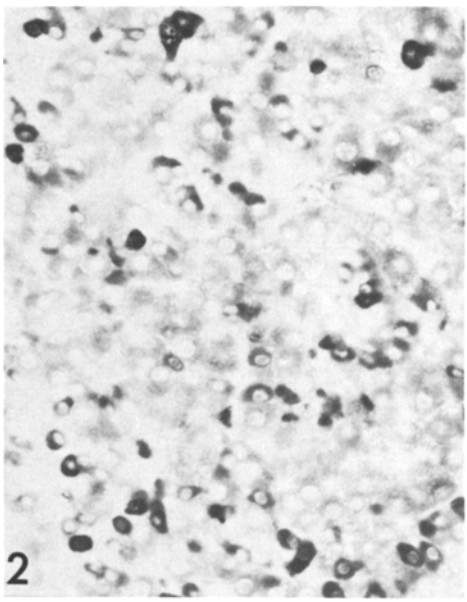
Male Japanese quail *(Coturnix coturnix japonica)* were obtained from a commercial source at the age of 3 weeks and kept under a regime of 8-hour days (light on from 0800 to 1 600) for 2 weeks before the beginning of the experiment. They were divided into 3 groups of 4 birds each. Group 1 was castrated and transferred to 16-hour days (light on from 0800 to 2 400). Group 2 was intact and transferred to the long days. Group 3 was also intact and remained under short days. After 2 weeks, birds were killed by decapitation. For radioimmunoassay of LH, blood was collected in test tubes and allowed to clot at room temperature. Sera were taken after centrifugation for 30 min at $1000 \times g$ and stored at $-20°$ C until assay.

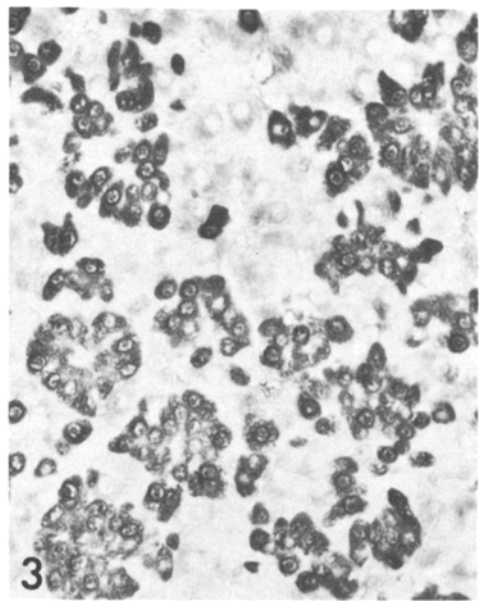
Immediately after taking the blood samples, adenohypophyses with some residual hypothalamic tissue were dissected out and fixed in Bouin's fixative. After embedding the tissue in paraffin, serial sagittal sections were made at 6 μm. The sections were deparaffinized and washed in tap water, then rinsed in 0.01 M phosphate buffered salin (PBS) at pH 7.5.

The immunohistochemical staining procedures were as follows: 1) sections were first incubated with anti-chicken LH serum (3027/2) diluted 1:100 for 1 h at room temperature, 2) washed with PBS, 3) incubated with goat anti-rabbit gamma globulin serum conjugated with horseradish peroxidase diluted 1:2 for 30 min, 4) washed again with PBS, and 5) finally the labeled peroxidase was visualized by incubation with 3, 3′-diaminobenzidine tetrahydrochloride.

Fig. 1. Paramedian sagittal section of the adenohypophysis and ventral hypothalamus from a castrated male Japanese quail stained immunohistochemically with anti-chicken LH serum. The pars distalis has drifted anteriorly and the pars nervosa was removed during fixation. LH producing cells are localized in the caudal lobe of the pars distalis. In the cephalic lobe the scattered positively







stained cells are TSH cells. *AME* anterior median eminence; *CaPD* caudal lobe of the pars distalis; *CePD* cephalic lobe of the pars distalis; *OC* optic chiasma; *PME* posterior median eminence; *III* third ventricle. ×60

Fig. 2. Higher magnification of the central portion of the cephalic lobe of the pars distalis from a castrated male Japanese quail stained immunohistochemically. Positively stained cells are TSH cells. ×400

Fig. 3. Higher magnification of the caudal lobe of the pars distalis from a castrated male Japanese quail after staining immunohistochemically. LH cells are stained positively in the cytoplasm. ×400

For control observation, the following steps were substituted according to normal immunohistochemical procedures: 1) normal rabbit serum was substituted for anti-chicken LH serum, 2) goat anti-rabbit gamma globulin serum was eliminated or normal goat serum was substituted for goat anti-rabbit gamma globulin serum, or 3) horseradish peroxidase was substituted for horseradish peroxidase labeled anti-gamma globulin serum.

Several sections from each bird kept for histochemical staining were stained with periodic acid-Schiff (PAS), alcian blue and orange G (PAS-AB-OG) (Herlant, 1960) for comparison with the result of immunohistochemistry. In order to correlate immunohistochemical and histochemical staining, the selected sections stained immunohistochemically were photographed and the microscopic coordinates of each photographic field were noted. After dismounting and decoloration by oxidation with sulfuric permanganate, the sections were restained with PAS-AB-OG.

LH in serum was determined by a slight modification of the radioimmunoassay described by Follett et al. (1972). LH concentration in serum is expressed as ng equivalent of chicken LH IRC-2/ml.

Anti-chicken LH serum and standard LH were a generous gift from Dr. B.K. Follett (University College of North Wales, U.K.). The anti-rabbit gamma globulin serum was raised in our laboratory. The conjugation of horseradish peroxidase (Sigma, type VI) to the antibody was done with glutaraldehyde according to the method of Avrameas (1969).

Observations

Histochemical staining indicates that there are three types of basophilic cells in the quail adenohypophysis (Tixier-Vidal et al., 1968; Mikami et al., 1975; Wada, 1975). PAS-AB positive cells are present in the cephalic lobe and two different types of AB positive cells are present in the cephalic and caudal lobes, respectively.

Immunohistochemical staining using anti-chicken LH serum revealed the presence of two types of positively stained cells. One is found in the caudal lobe (Figs. 1 and 3) and the other in the cephalic lobe (Figs. 1 and 2). In control sections there was no positive staining distinct from the background.

To correlate the histochemical and immunohistochemical results, immunohistochemically stained sections were destained and restained with PAS-AB-OG. Both types of immunohistochemically stained cells in the cephalic and caudal

Fig. 4. Caudal lobe of the pars distalis from a castrated male Japanese quail stained immunohistochemically with anti-chicken LH serum. LH cells are stained. ×650

Fig. 5. The section illustrated in Figure 4 was destained and restained with PAS-AB-OG. All the LH cells in Figure 4 are stained with AB. The other cell type may be the STH (growth hormone) cell. ×650

Fig. 6. Peripheral region of the cephalic lobe of the pars distalis from a castrated male Japanese quail stained immunohistochemically with anti-chicken LH serum. Stained cells are TSH cells. Arrows indicate the same cells in Figure 7. ×650

Fig. 7. The section illustrated in Figure 6 was destained and then restained with PAS-AB-OG. TSH cells are stained with AB, and PAS-AB positive cells (FSH cells, arrows) are stained immunohistochemically only slightly if at all in Figure 6. ×650

Fig. 8. Caudal lobe of the pars distalis from an intact photostimulated bird stained immunohistochemically. ×650

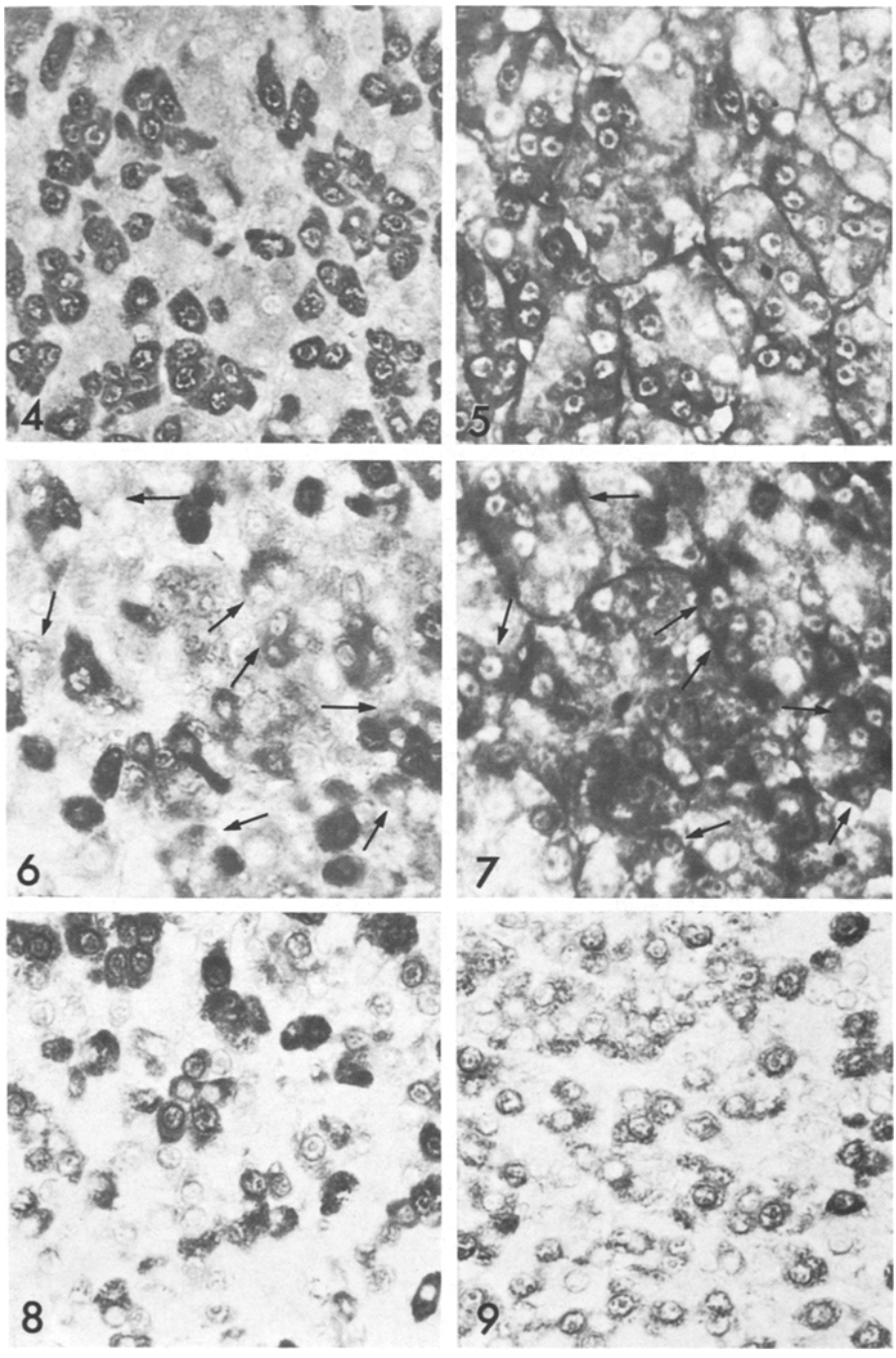


Fig. 9. Caudal lobe of the pars distalis from an intact non-photostimulated bird stained immunohistochemically with anti-chicken LH serum. ×650

Table 1. Body weight, serum LH and area of cloacal protrusion at autopsy in male Japanese quail after experimental manipulation

Groups	Number of birds	Body weight at sacrifice (g)	LH content (ng/ml serum)	Cloacal protrusion (mm^2/100 g BW)
Castrated and photostimulated	4	91.5 ± 3.38[a]	11.85 ± 5.960	32.8 ± 5.17
Intact and photostimulated	4	99.0 ± 3.24	4.92 ± 1.135	222.1 ± 19.01
Intact and non-photostimulated	4	111.5 ± 2.50	0.64 ± 0.159	30.0 ± 2.11

[a] Mean ± standard error

* Significant ($p < 0.05$), ** highly significant ($p < 0.01$) by paired t-test

lobes were stained with AB (Figs. 4–7). However, PAS-AB positive cells in the cephalic lobe appeared to be stained immunohistochemically only slightly if at all (Figs. 6 and 7, arrows).

Serum LH levels in each group are summarized in Table 1. The LH levels in castrated and photostimulated birds were elevated much more (although not significant statistically due to great individual variations) than those of intact photostimulated birds, in which the LH levels were also elevated compared to intact non-photostimulated birds. Cells showing a positive LH immunohistochemical reaction in the caudal lobe also varied in size and stainability under different physiological conditions (Figs. 4, 8 and 9). These cells have more cytoplasm and stain intensively in the castrated and photostimulated birds (Fig. 4) compared to intact photostimulated birds (Fig. 8). Immunohistochemical positive cells in the caudal lobe of intact non-photostimulated birds are stained only weakly (Fig. 9).

Discussion

In a previous electron microscopic study of the adenohypophysis of the Japanese quail (Wada, 1975), two types of gonadotropic cells were found and one, present in the cephalic lobe, was PAS-AB positive and the other, in the caudal lobe, was AB positive. From present observations, it is clear that of the two types of gonadotropic cells in the quail adenohypophysis, AB positive cells in the caudal lobe are LH-producing cells. Thus, in the avian adenohypophysis, at least in this species, LH cells are localized in the caudal lobe of the pars distalis. FSH producing cells may consequently be localized in the cephalic lobe of the pars distalis.

It should be noted that even though there was a slight ultrastructural difference between two types of gonadotropic cells, these cells vacuolated and became similar cells ultrastructurally after castration (Mikami et al., 1975). This raised a difficulty to distinguish these cells. However, it is also apparent that PAS positive gonadotropic cells, which are designated as beta cells by Tixier-Vidal

et al. (1968), are distributed only in the cephalic lobe and that these cells are not immunohistochemically positive to anti-chicken LH serum whereas AB positive cells in the caudal lobe are stained immunohistochemically with anti-chicken LH serum. It is not unreasonable to assume that FSH and LH cells turn to ultrastructurally similar cells after castration.

In this connection, there is some difficulty in identifying LH and TSH cells immunohistochemically using this anti-chicken LH serum. There are two types that show a positive reaction to anti-chicken LH serum. As shown in Figures 1–3, there are some differences in appearance and distribution of the two cell types. Immunohistochemically positive cells in the caudal lobe are distributed evenly throughout the caudal lobe but those in the cephalic lobe are scattered and found more frequently in its periphery. After destaining and restaining with PAS-AB-OG, both types appear AB positive. However, a previous study of electron microscopy on the quail adenohypophysis (Wada, 1975) indicated that the AB positive cells in the cephalic lobe and those in the caudal lobe are quite different in their fine structure. So the AB positive cells in the cephalic lobe and those in the caudal lobe must be of different types; the former being TSH cells and the latter LH cells, because the AB positive cells in the caudal lobe responded to LH-releasing hormone and because the same cell type in this study showed a good correlation with immunohistochemical stainability and with serum levels of LH in different physiological conditions. This similarity of two cell types might be due to the similarity of chemical characteristics of TSH and LH molecules (see Papkoff, 1972) and might have led to a confusion of designation of basophilic cell types; Tixier-Vidal et al. (1968) concluded that AB positive cells in both the cephalic and caudal lobes are TSH cells. In fact, LH and TSH are difficult to separate in chromatographic procedures, and the anti-chicken LH serum used in this study cross-reacts with TSH as well as with LH (Follett et al., 1972).

The present study confirms the general conclusion of Tixier-Vidal and her associate that LH cells are present in the caudal lobe of the pars distalis in avian species. To obtain more clear results on distribution and localization of each basophilic cell type, it will be necessary to obtain pure samples of FSH and TSH and to raise antibodies of each that do not cross with these hormones.

The immunohistochemical staining of LH producing cells in the caudal lobe in different physiological conditions is consistent with the levels of circulating LH measured in those states. LH secretion is photostimulated presumably through LH-releasing hormone and photostimulation may also induce synthesis of LH in the cells immunohistochemically identified in this study.

These results suggest that there may be different routes for stimulation of FSH and LH secretion via the basal infundibular region, median eminence, and pars distalis in avian species. This hypothesis was suggested previously by several investigators on the basis of observations of two morphologically distinct parts of the median eminence that give rise to two non-intermingling portal vessel systems (Vitums et al., 1964; Sharp and Follett, 1969), on the basis of results of experiments with lesions, implants and stimulation (Stetson, 1972a, b; Wada, 1974; Davies and Follett, 1974).

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